

**An immunoproteomic approach  
for identification of *Cryptococcus neoformans* proteins  
recognized by murine and human antibodies**

Der Fakultät für Lebenswissenschaften

der Universität Leipzig

eingereichte

DISSERTATION

zur Erlangung des akademischen Grades

*Doctor rerum naturalium*

*Dr. rer. nat.*

vorgelegt

von Frau Master of Science Anne Elisabeth Greßler

geboren am 05.03.1993 in Naumburg an der Saale

Leipzig, den 26. November 2021



Die vorliegende Dissertationsschrift wurde am Institut für Immunologie der Veterinärmedizinischen Fakultät, Universität Leipzig, unter der Leitung von Herrn Prof. Dr. med. vet. Gottfried Alber angefertigt.

Die vorliegende Arbeit umfasst die folgenden wissenschaftlichen Veröffentlichungen:

Firacative C\*, Gressler AE\*, Schubert K, Schulze B, Müller U, Brombacher F, von Bergen M, Alber G: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection. *Sci Rep.* 2018 Feb 8;8(1):2681. doi: 10.1038/s41598-018-21039-z.

\* geteilte Erstautor:innenschaft

Gressler AE, Volke D, Firacative C, Schnabel CL, Müller U, Krizsan A, Schulze-Richter B, Brock M, Brombacher F, Escandón P, Hoffmann R, Alber G: Identification of Disease-Associated Cryptococcal Proteins Reactive With Serum IgG From Cryptococcal Meningitis Patients. *Front Immunol.* 2021 Jul 23;12:709695. doi: 10.3389/fimmu.2021.709695. eCollection 2021.

This thesis was conducted and written at the Institute of Immunology at the Faculty of Veterinary Medicine, Leipzig University under the supervision of Prof. Dr. med. vet. Gottfried Alber.

The thesis contains the following peer-reviewed scientific publications:

Firacative C\*, Gressler AE\*, Schubert K, Schulze B, Müller U, Brombacher F, von Bergen M, Alber G: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection. *Sci Rep.* 2018 Feb 8;8(1):2681. doi: 10.1038/s41598-018-21039-z.

\* shared first authorship

Gressler AE, Volke D, Firacative C, Schnabel CL, Müller U, Krizsan A, Schulze-Richter B, Brock M, Brombacher F, Escandón P, Hoffmann R, Alber G: Identification of Disease-Associated Cryptococcal Proteins Reactive With Serum IgG From Cryptococcal Meningitis Patients. *Front Immunol.* 2021 Jul 23;12:709695. doi: 10.3389/fimmu.2021.709695. eCollection 2021.

# Table of Contents

Abbreviations .....	VI
Bibliographische Darstellung .....	VII
1 Introduction .....	1
1.1 <i>Cryptococcus neoformans</i> – an environmental fungal pathogen .....	1
1.1.1 Immunocompromised patients are at risk for cryptococcal disease .....	1
1.2 The role of cell-mediated immune response against <i>C. neoformans</i> .....	2
1.2.1 T helper type 1 responses are protective in cryptococcal infection .....	2
1.2.2 T helper type 2-mediated immune responses are detrimental in cryptococcal infection...	2
1.2.3 The cryptococcal capsule induces a Th2 shift .....	3
1.2.4 Progressing HIV-infection is associated with an increasing Th2 bias.....	4
1.3 The role of humoral immune response in <i>C. neoformans</i> infection.....	4
1.3.1 Deficiencies in B cells or immunoglobulins predispose for development of systemic cryptococcosis .....	4
1.3.2 Anti-cryptococcal antibodies are ubiquitously present in human sera .....	5
1.3.3 Anti-cryptococcal antibodies support innate immune cell function .....	5
1.3.4 Direct actions of anti-cryptococcal antibodies .....	6
1.3.5 Antibodies as tools for identification of immunoreactive fungal proteins suitable for development of vaccines .....	7
1.4 Aim of the study .....	8
1.4.1 Publication 1: Identification of T helper (Th)1- and Th2-associated antigens of <i>Cryptococcus neoformans</i> in a murine model of pulmonary infection <sup>142</sup> .....	8
1.4.2 Publication 2: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients <sup>143</sup> .....	9
1.5 References.....	10
2 First publication: Identification of T helper (Th)1- and Th2-associated antigens of <i>Cryptococcus neoformans</i> in a murine model of pulmonary infection .....	18
3 Second publication: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients.....	33

4	Summary .....	53
4.1	References.....	58
5	Zusammenfassung.....	60
5.1	Literatur .....	65
	Author Contribution Statement .....	VIII
	Supplementary documents of the first publication: Firacative and Gressler <i>et al.</i> 2018.....	XII
	Supplementary Figure 1 .....	XII
	Supplementary Figure 2 .....	XIII
	Supplementary Figure 3 .....	XIV
	Supplementary Figure 4 .....	XVI
	Supplementary documents of the second publication: Gressler <i>et al.</i> 2021 .....	XVII
	Supplementary Figure 1 .....	XVII
	Supplementary Figure 2 .....	XVIII
	Supplementary Figure 3 .....	XIX
	Supplementary Figure 4 .....	XX
	Supplementary Figure 5 .....	XXI
	Supplementary Figure 6 .....	XXII
	Supplementary Table 1.....	XXIII
	Supplementary Table 2.....	XXV
	Supplementary Table 3.....	XXV
	Supplementary Table 4.....	XXVI
	Supplementary Table 5.....	XLI
	Supplementary Table 6.....	LXXXII
	Supplementary Table 7.....	LXXXIII
	Acknowledgements .....	LXXXIV
	Curriculum Vitae .....	LXXXV
	List of publications and presentations .....	LXXXVII
	Selbstständigkeitserklärung .....	LXXXIX

# Abbreviations

AIDS	acquired immunodeficiency syndrome
CD	cluster of differentiation
CFU	colony-forming units
CM	cryptococcal meningitis
<i>C. neoformans</i>	<i>Cryptococcus neoformans</i>
<i>C. gattii</i>	<i>Cryptococcus gattii</i>
CPS	capsular polysaccharides
GXM	glucuronoxylomannan
HIV	human immunodeficiency virus
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IL-4R	interleukin 4 receptor
IL-4R $\alpha$	interleukin 4 receptor alpha chain
iNOS	inducible nitric oxide synthase
mAb	monoclonal antibody
mRNA	messenger ribonucleic acid
OVA	ovalbumin
PBMCs	peripheral blood mononuclear cells
Rag1 protein	recombination activating gene 1 protein
SCID	severe combined immunodeficiency
Th cell	T helper cell
TLR	toll-like receptor
TNF	tumor necrosis factor
WT	wild type
YM1	chitinase 3-like 3 protein

# Bibliographische Darstellung

Anne Elisabeth Greßler

**An immunoproteomic approach for identification of *Cryptococcus neoformans* proteins recognized by murine and human antibodies**

Fakultät für Lebenswissenschaften

Universität Leipzig

*Dissertation*

156 Seiten, 241 Literaturangaben, 20 Abbildungen, 12 Tabellen

---

The opportunistic fungal pathogen *Cryptococcus neoformans* (*C. neoformans*) causes the systemic disease cryptococcosis, mainly affecting immunocompromised persons such as AIDS patients. Infection with *C. neoformans* occurs through inhalation of spores or yeasts. Systemic disease is established through dissemination to the bloodstream and fungal growth in the brain leading to the main manifestation as cryptococcal meningitis (CM). Development of an anti-cryptococcal vaccine is of great importance due to high mortality rates even in patients receiving antifungal therapy and therefore research has focused on identification of cryptococcal proteins suitable as vaccine candidates. This thesis focuses on the identification of cryptococcal proteins recognized by murine and human serum antibodies, therefore possessing immunoreactive properties beneficial for vaccine development. In the first study, sera from naïve and *C. neoformans*-infected mice were analyzed. Serological analysis revealed increased total serum concentrations of Th2-associated IgG1 antibodies, but not Th1-associated IgG2a antibodies. Additionally, titers of anti-cryptococcal IgG1 antibodies strongly increased upon cryptococcal infection. Using immunoproteomic analysis, cryptococcal protein spots specifically reactive with murine IgG2a or IgG1 antibodies were identified. The proteins contained in those spots are therefore associated with protective Th1 or detrimental Th2 responses rendering them interesting candidates for future research. The second study focuses on identification of cryptococcal proteins recognized by human antibodies from sera of Colombian HIV-positive and HIV-negative CM patients, and healthy individuals. We detected an increase in titers of anti-cryptococcal IgG, but not IgM antibodies in sera from HIV-negative CM patients. Immunoproteomic analysis revealed several cryptococcal protein spots preferentially recognized by sera from CM patients or healthy individuals. Proteins contained in those spots were identified and recombinantly expressed. Subsequent quantification of serum IgG immunoreactivity revealed twelve disease-associated proteins, defined by significantly stronger reactivity with sera from CM patients compared to healthy individuals. Some of these proteins could be suitable candidates for development of an anti-cryptococcal vaccine based on lack of homology to human proteins. Additionally, several cryptococcal proteins identified using murine or human sera are involved in cryptococcal virulence or survival, rendering them excellent candidates for targeting by new antifungal agents.

# 1 Introduction

## 1.1 *Cryptococcus neoformans* – an environmental fungal pathogen

The fungus *Cryptococcus neoformans* (*C. neoformans*) is an opportunistic pathogen causing the fatal systemic disease cryptococcosis<sup>1</sup>. Infection with *C. neoformans* occurs through the inhalation of spores and yeast cells<sup>2</sup>. Cryptococcal cells or spores have been detected in several environmental sources such as bird droppings<sup>3,4</sup>, house dust<sup>5</sup>, trees<sup>6</sup>, and decaying wood<sup>7,8</sup> across the globe. Therefore, exposure to the fungus is very likely ubiquitous. However, development of severe cryptococcal disease, mostly manifesting as cryptococcal meningitis (CM)<sup>9</sup>, mainly occurs in persons with impaired cell-mediated immunity<sup>10,11</sup>. Rajasingham et al., 2017, estimated a total of 223,100 global cases of cryptococcal meningitis resulting in 181,100 annual deaths in 2014<sup>12</sup>, emphasizing the importance of research on novel therapeutic and prophylactic approaches for anti-cryptococcal therapy. Currently, a licensed vaccine against *C. neoformans* is not available<sup>13</sup>.

### 1.1.1 Immunocompromised patients are at risk for cryptococcal disease

The majority of patients suffering from systemic cryptococcosis are human immunodeficiency virus (HIV)-positive patients with acquired immunodeficiency syndrome (AIDS), accounting for 80-95% of all cases<sup>9,11,14</sup>. Especially at risk are those patients with a CD4<sup>+</sup> (cluster of differentiation 4-positive) T helper (Th) cell count of less than 100 cells per microliter not receiving anti-retroviral therapy<sup>12</sup>. Without anti-retroviral therapy, independently of an antifungal therapy with fluconazole, mortality rates were as high as 100% among AIDS patients with CM in Zambia in 2001<sup>15</sup>. Since then, establishment of cryptococcal antigen screening methods<sup>16</sup> and anti-retroviral therapy<sup>12</sup> have reduced AIDS-related deaths. However, cryptococcal meningitis was still estimated to cause 15% of AIDS-related deaths globally in 2014 especially in low- and mid-income countries, with Sub-Saharan Africa bearing the greatest burden of this disease (75% of world-wide CM deaths)<sup>12</sup>. Estimated one year mortality rates differ strongly between high-income countries (20% for patients in care, 45% for patients not in care) and low-income countries (70% for patients in care, 100% for patients not in care)<sup>12</sup>.

Other persons at risk for CM are individuals immunosuppressed due to medical treatment. The main group of those patients are organ transplant recipients receiving immunosuppressing drugs and cancer patients receiving immunocompromising chemotherapy<sup>17</sup>. Additionally, patients treated with steroids, suffering from diabetes mellitus, renal insufficiency or cirrhosis are at risk for developing cryptococcosis<sup>17</sup>. Interestingly, pulmonary infections are more common within non-HIV cryptococcosis patients (40% of all cases) compared to HIV-infected patients (10% of all cases)<sup>17</sup>.



## 1.2 The role of cell-mediated immune response against *C. neoformans*

The immune response against *C. neoformans* is critically orchestrated by T helper cells, with Th type 1 responses being associated with protection against fatal outcome of disease and Th2 responses associated with disseminated disease and immunopathology<sup>18</sup>.

### 1.2.1 T helper type 1 responses are protective in cryptococcal infection

The different effects of Th1 and Th2 responses on anti-cryptococcal defense were first observed based on cytokine expression in response to infection of mice with *C. neoformans* strains exhibiting different virulence potential. Infection with weakly virulent strains was associated with rapid clearance of fungi from the lungs, an increased production of Th1 cytokines interleukin (IL)-2 and interferon (IFN)- $\gamma$ , and absent or low production of the Th2 cytokine IL-4 in spleen cells<sup>19</sup> or lung leukocytes<sup>20</sup> cultivated *ex vivo* or, concentration of the respective cytokines in BAL fluid<sup>21</sup>. In contrast, mice infected with highly virulent strains, resulting in high fungal burden in the lung and dissemination to the brain, showed increased concentrations of IL-4, but low concentration of IFN- $\gamma$  in BAL fluid<sup>21</sup> or in supernatants of lung leukocytes cultivated *ex vivo*<sup>20</sup>.

Intratracheal *C. neoformans* infection of IFN- $\gamma$  knockout mice resulted in increased pulmonary fungal burden and a switch from chronic to progressive cryptococcal infection, demonstrating the importance of IFN- $\gamma$  for defense against cryptococcal infection<sup>22</sup>. Impaired fungal clearance was associated with higher concentrations of Th2 cytokines produced by isolated lung leukocytes of IFN- $\gamma$ -deficient mice compared to WT mice<sup>22</sup>. The generation of a *C. neoformans* strain expressing murine IFN- $\gamma$  (strain H99- $\gamma$ ) from the serotype A wild type strain H99 furthermore established the protective effect of this cytokine during cryptococcosis<sup>23</sup>. Mice infected with *C. neoformans* H99- $\gamma$  showed lower lung fungal burden, higher concentration of Th1 and inflammatory cytokines and lower concentration of Th2 cytokines in lung homogenates, compared to mice infected with the wild type strain, and completely resolved the infection<sup>23,24</sup>. Interestingly, after resolving the primary H99- $\gamma$  infection, mice showed significantly increased resistance to a subsequent challenge with the otherwise lethal strain H99<sup>23,25</sup>. However, protection was abrogated in knockout mice deficient in T cells and production of Th1-related cytokines, but was still present in mice lacking B cells or the receptor for IL-4 (IL-4R)<sup>25</sup>. Similarly, mice showed increased survival and decreased fungal burden when infected with a *C. neoformans* H99 strain genetically modified to express the pro-inflammatory cytokine murine tumor necrosis factor (TNF)- $\alpha$ , which reduced the development of a pulmonary Th2 cytokine bias during cryptococcal infection<sup>26</sup>.

### 1.2.2 T helper type 2-mediated immune responses are detrimental in cryptococcal infection

Detrimental influence of Th2 responses for the outcome of cryptococcal infections has been demonstrated by various studies using Th2 cytokine-deficient mice. Infection of IL-4/IL-13-deficient mice with *C. neoformans* serotype A strain H99 resulted in enhanced clearance of fungi from the lungs and increased levels of IFN- $\gamma$  compared to BALB/c wild type (WT) animals, but ultimately infection

was still fatal<sup>27</sup>. In contrast, other studies using the *C. neoformans* serotype D strain 1841 for infection of IL-13-deficient or IL-4R  $\alpha$  chain (IL-4R $\alpha$ )-deficient mice demonstrated increased survival or complete resistance, respectively, after infection<sup>28,29</sup>.

Critical players in the control of *C. neoformans* are classically activated macrophages, characterized by production of inducible nitric oxide synthase (iNOS), which are able to phagocytose and kill cryptococcal cells<sup>22,30</sup> (reviewed in <sup>31,32</sup>). In contrast, alternatively activated macrophages expressing arginase and chitinase 3-like 3 enzyme (YM1), fail to control cryptococcal infection<sup>22,27,28,30,33</sup> (reviewed in <sup>31,32</sup>). Polarized activation of macrophages critically depends on cytokine signaling. IFN- $\gamma$ -deficient mice infected with *C. neoformans* showed morphologically larger lung macrophages containing numerous intracellular cryptococci and YM1 crystals, as well as decreased mRNA expression of iNOS but increased mRNA expression of arginase in lung homogenates<sup>22</sup>. Consistent with that, infection with *C. neoformans* strain H99- $\gamma$  resulting in enhanced survival of infected mice, was characterized by classical macrophage activation in contrast to infection with a *C. neoformans* WT strain leading to pronounced alternative activation of macrophages<sup>24</sup>. Macrophages in *C. neoformans*-infected mice deficient in Th2-associated cytokines were biased towards classical activation with decreased expression of arginase, YM1 or CD206 and higher iNOS expression, ultimately resulting in improved cryptococcal killing and protection of mice from cryptococcal infection<sup>28,29,34</sup>. Underlining the role of IL-4 for development of alternatively activated macrophages and detrimental immune responses, knockout of the IL-4R $\alpha$  specifically on lung macrophages led to significantly improved survival of mice, although the pulmonary Th2 phenotype (immune cell composition, cytokine production) was similar to mice heterozygous for IL-4R $\alpha$  <sup>35</sup>. This demonstrates a central role of macrophage polarization for the course and outcome of cryptococcal infection.

### 1.2.3 The cryptococcal capsule induces a Th2 shift

A major virulence factor of *C. neoformans* is the polysaccharide capsule consisting mainly of glucuronoxylomannan (GXM) and to a lesser extent of galactoxylomannan and few mannoproteins<sup>36</sup>. Four different serotypes of *C. neoformans* and the closely related species *Cryptococcus gattii* (*C. gattii*) were defined based on reactivity of the capsule with rabbit polyclonal sera<sup>36</sup>. Distribution of serotypes causing cryptococcosis differs between regions, with serotype A accounting for 95% of all cases in a study from Colombia<sup>37</sup>, and 92% of all cases in a study from India<sup>38</sup>. A study from France found 61% of all cases to be caused by *C. neoformans* serotype A strains and 19% by serotype D strains<sup>39</sup>.

Interestingly, *C. neoformans* is able to induce a non-protective Th2-biased immune response through proteins like laccase<sup>40</sup>, urease<sup>33</sup>, and its polysaccharide capsule<sup>41</sup>. Cryptococcal capsular polysaccharides (CPS) were demonstrated to induce immunological unresponsiveness in mice towards subsequent vaccination with CPS in Freund's adjuvant<sup>42,43</sup>. Furthermore, fungicidal activity of alveolar macrophages was higher against acapsular compared to encapsulated cryptococcal cells, accompanied by inhibition of TNF- $\alpha$  production<sup>44,45</sup>, and purified CPS induced expression of immunosuppressive

cytokine IL-10 in human monocytes<sup>46</sup>. CPS reduced lymphocyte proliferation in response to *C. neoformans* stimulation, likely due to reduced phagocytosis of *C. neoformans* by human peripheral blood mononuclear cells (PBMCs) in the presence of capsule or addition of CPS, which could be reversed by incubation with an anti-capsular monoclonal antibody (mAb), but not blocking of IL-10<sup>47</sup>. Similarly, incubation with encapsulated cryptococci reduced phagocytosis and IL-12 production of human monocytes, as well as IFN- $\gamma$  production by PBMCs, although the effects could be reversed by the addition of anti-IL-10 mAb in this study<sup>48</sup>. Finally, co-cultivation of murine CD4<sup>+</sup> splenocytes with antigen-presenting spleen adherent cells and CPS resulted in increased production of Th2 cytokine IL-4 as well as immunosuppressive cytokine IL-10, but not IFN- $\gamma$  or TNF- $\alpha$  compared to medium control, demonstrating that CPS could directly induce a Th2-type cytokine pattern<sup>41</sup>.

#### **1.2.4 Progressing HIV-infection is associated with an increasing Th2 bias**

The importance of T helper, and especially T helper type 1 immune responses against *C. neoformans*, is further demonstrated by the fact that disseminated cryptococcosis mainly affects HIV-positive patients with severe immunosuppression<sup>9</sup>. Importantly, a hallmark in the development of AIDS is an increasing imbalance between Th1 and Th2 type responses, with a shift towards Th2 type immune responses with progressing disease<sup>49</sup>. This is marked by loss of IL-2 and IFN- $\gamma$  production and simultaneous increase in IL-4 and IL-10 production when human PBMCs were stimulated with influenza A virus or phytohemagglutinin, respectively<sup>49</sup>.

### **1.3 The role of humoral immune response in *C. neoformans* infection**

Defense against systemic cryptococcal disease has historically been associated with cell-mediated immunity as mainly patients with T cell deficiencies, most prominently AIDS patients, are affected by cryptococcosis. However, several studies indicate a critical role for B cells and more specifically, anti-cryptococcal antibodies in protection against *C. neoformans*.

#### **1.3.1 Deficiencies in B cells or immunoglobulins predispose for development of systemic cryptococcosis**

A role for humoral immunity in anti-cryptococcal defense in humans is supported by the identification of humoral immunity defects like immunoglobulin (Ig)G-deficiencies, mostly IgG-, IgG2- or IgG4-deficiency, as a risk factor for systemic cryptococcal disease caused by *C. neoformans*<sup>50-53</sup>, and *C. gattii*<sup>54</sup>. Confirmatively, human X-linked hyper-IgM syndrome, characterized by decreased levels of serum IgG, IgA, IgE, but elevated levels of serum IgM<sup>55</sup>, has been identified as a risk factor for the development of cryptococcal meningitis or other forms of disseminated cryptococcosis<sup>56-59</sup>. Interestingly, a study published in 2018 found reduced percentage of B cells, memory B cells, and more specific, IgM-producing memory B cells in PBMCs of otherwise non-immunocompromised CM patients, linking B cells to protection against CM<sup>60</sup>. The percentages of IgM-expressing B cells in PBMCs were also lower in HIV-positive patients with history of cryptococcosis compared to HIV-

positive patients without previous cryptococcal disease and healthy control persons<sup>61</sup>. Mice lacking B cells<sup>62,63</sup>, or B-1a cells<sup>64</sup> showed increased fungal organ burden and decreased survival upon cryptococcal infection. Adoptive transfer of lymphocytes from B cell-deficient mice immunized through sublethal infection with *C. neoformans* into severe combined immunodeficiency (SCID) mice resulted in significantly weaker protection against *C. neoformans* challenge infection compared to mice receiving lymphocytes from immunized B cell-sufficient mice<sup>65</sup>. Similarly, adoptive transfer of naïve B cells to recombination activating gene 1 (Rag1)-deficient mice before cryptococcal infection decreased fungal dissemination to the brain<sup>66</sup>. Additionally, mice lacking secreted IgM antibodies showed decreased survival upon pulmonary *C. neoformans* infection, accompanied by higher blood and brain fungal burden<sup>67</sup>. However, in a model of systemic *C. neoformans* infection, absence of serum IgM had a beneficial effect reflected by increased survival<sup>68</sup>. Taken together, the majority of these studies argues for a role of B cell-mediated immunity in protection during *C. neoformans* infection.

### 1.3.2 Anti-cryptococcal antibodies are ubiquitously present in human sera

Anti-cryptococcal antibodies directed against cryptococcal capsular polysaccharides<sup>61,69–77</sup>, mannoproteins<sup>78</sup>, and cryptococcal proteins<sup>79–83</sup> have been ubiquitously detected in human sera, independently of predisposing HIV-infection or a previous history of cryptococcosis. There are two leading hypotheses regarding the origin of those antibodies: latent infection with *C. neoformans* or permanent exposure to the fungus, triggering constant antibody production. Constant exposure to the fungus is quite likely, as *C. neoformans* was detected in several environmental reservoirs<sup>5–8</sup>, most prominently bird droppings<sup>3,4</sup>. Evidence for latent or dormant infection was provided by several publications demonstrating the reactivation of a previous, asymptomatic cryptococcal infection for *C. neoformans*<sup>84–86</sup>, as well as *C. gattii*<sup>87</sup> (reviewed in<sup>88</sup>). Another explanation to ubiquitous presence of anti-cryptococcal antibodies, at least directed against CPS, is cross-reactivity with antibodies produced in response to other fungi, as the structure of the cell wall polysaccharides is conserved in many fungi<sup>89</sup>.

### 1.3.3 Anti-cryptococcal antibodies support innate immune cell function

*C. neoformans* can be killed by different innate immune cells, such as alveolar macrophages<sup>44,90</sup>, dendritic cells<sup>91</sup>, natural killer cells<sup>92,93</sup>, and neutrophils<sup>44</sup>. Phagocytosis of cryptococcal cells is critical for killing by macrophages and dendritic cells<sup>94</sup>. Antibodies were shown to critically influence the effectivity of the phagocytic process. Early studies of *C. neoformans*-phagocyte interaction postulated killing of cryptococcal cells by human and murine PBMCs, monocytes or macrophages only in the presence of anti-cryptococcal antibodies<sup>95–98</sup>. Similar observations were made for murine and human NK cells, which did not inhibit growth of *C. neoformans* in the absence of anti-cryptococcal antibodies<sup>99,100</sup>. A murine monoclonal antibody (mAb 18B7) against CPS also enhanced the antifungal activity of neutrophils from AIDS patients<sup>101</sup>.

Anti-cryptococcal antibodies were found to opsonize cryptococcal cells, thereby promoting phagocytosis by macrophages and binding of dendritic cells to *C. neoformans*<sup>102,103</sup>. Additionally, anti-

capsular mAbs enhance complement activity<sup>104,105</sup>, or act as opsonins in complement-deficient mice, promoting survival of *C. neoformans*-infected mice<sup>106</sup>, or reducing lung fungal burden<sup>107</sup>.

Interestingly, protection conferred by anti-capsular murine mAbs was dependent on the antibody isotype. Administration of the mAbs E1 (IgG1)<sup>108</sup>, 17E12 (IgG1)<sup>109</sup>, 2H1 (IgG1)<sup>110,111</sup>, 12A1 (IgM)<sup>112,113</sup> before or after infection with *C. neoformans* prolonged survival of mice and/or enhanced phagocytosis of cryptococcal cells. In contrast, administration of mAbs 4H3 (IgG3)<sup>109</sup>, 3E5 (IgG3)<sup>109</sup>, and 13F1 (IgM)<sup>112,113</sup>, did not prolong survival and/or enhance phagocytosis. Isotype switching of non-protective mAbs from IgG3 to IgG1 (mAb 3E5)<sup>114</sup> or IgG2b (mAb 4H3)<sup>115</sup>, resulted in protection of mice from fatal cryptococcosis or enhanced phagocytosis (mAb 2D10 (IgM to IgG1, IgG2a or IgG2b switch))<sup>111</sup>. However, protection was dependent on both the fungal strain used<sup>116</sup>, as well as the mouse genotype<sup>117</sup>. The mAb 3E5 isotype IgG1 was protective in C57BL/6J and A/JCr mice, whereas the IgG3 switch variant was nonprotective<sup>117</sup>. In contrast, mAb 3E5 (IgG1) was non-protective in 129/Sv as well as C57BL/6x129/Sv mice, but showed significant protection as IgG3 switch variant in C57BL/6x129/Sv mice, indicating that the efficacy of the antibody isotype against *C. neoformans* is dependent on the genetic background of the host<sup>117</sup>. Additionally, distinct functions of Fc receptors, expressed on immune cell subsets and binding the fragment crystallizable region also known as constant region of antibodies, influence the outcome of infection, as the IgG1 mAb 3E5 showed no opsonizing capacity and the protective effect was ablated in FcR $\gamma$ -deficient mice<sup>118</sup>. However, the IgG3 3E5 switch variant was generally non-protective, but increased phagocytosis in macrophages from FcR $\gamma$ -deficient mice although with no influence on fungal colony-forming units (CFU)<sup>118</sup>. Furthermore, several studies showed an influence of isotype change on differences in fine specificity, and kinetics of antibody-antigen interactions<sup>119,120</sup>. In conclusion, both antigen-specificity and the isotype play a role for protective antibody effector functions.

#### 1.3.4 Direct actions of anti-cryptococcal antibodies

One important function of antifungal antibodies in cryptococcal infection is the binding and therefore neutralization of immunosuppressive CPS. Administration of IgG1 mAb 2H1 before intravenous infection of mice or rats with *C. neoformans* led to reduced serum GXM levels, reduction of tissue polysaccharide and improved fungal clearance<sup>121,122</sup>. *C. neoformans* is capable of biofilm formation resulting in increased fungal resistance, which was prevented by GXM-binding mAbs 18B7 (IgG1) and 12A1 (IgM)<sup>123</sup>. Human IgM antibodies were also effective in inhibiting titan cell formation, another virulence trait of *C. neoformans* which prevents phagocytosis<sup>124</sup>. Interestingly, a study published in 2010 found a direct influence of antibody-binding to the capsule on the gene expression of cryptococcal cells<sup>125</sup>. Binding of protective mAb 18B7 led to upregulation of genes encoding proteins involved in fatty acid synthesis and changes in lipid metabolism, resulting in increased susceptibility to the antifungal agent amphotericin B<sup>125</sup>. In contrast, binding of non-protective mAbs (12A1, 13F1) had only moderate effects on gene expression<sup>125</sup>. Synergism of the mAb 2H1 and amphotericin B treatment was

also found in a murine model of systemic cryptococcosis<sup>126</sup>. Unfortunately, despite promising results in murine studies, a phase I evaluation of mAb 18B7 application in HIV-positive patients successfully treated for CM revealed poor cerebrospinal fluid penetration<sup>127</sup>, and development of this mAb for usage in anti-cryptococcal therapy of humans was not continued.

### **1.3.5 Antibodies as tools for identification of immunoreactive fungal proteins suitable for development of vaccines**

Anti-cryptococcal antibodies bind different fungal components, including capsular polysaccharides<sup>61,69-77</sup>, but also cryptococcal proteins<sup>79-83</sup>. However, only a few studies aimed at the identification of the respective proteins bound by those antibodies, although cryptococcal proteins showing immunoreactivity could potentially be used as vaccine candidates. The need for immunoreactive cryptococcal proteins for development of a vaccine is further strengthened by the fact that CPS are not only immunosuppressive<sup>42,43</sup>, but also poorly immunogenic<sup>36,128</sup>. Nevertheless, studies using conjugate vaccines consisting of GXM coupled to tetanus toxoid prolonged survival of *C. neoformans*-infected mice<sup>129,130</sup>. Unfortunately, both, protective but also non-protective antibodies were induced upon vaccination with GXM-tetanus toxoid conjugates and therefore, a GXM-tetanus toxoid-based vaccine was not developed further<sup>131,132</sup>.

Thus, cryptococcal proteins are more promising candidates than cryptococcal polysaccharides for development of an anti-cryptococcal vaccine. Previous studies focused on the identification of cryptococcal proteins immunoreactive with sera from *C. neoformans*- and *C. gattii*-infected mice<sup>133-135</sup>, *C. gattii*-infected koalas<sup>136</sup>, or *C. gattii*-infected humans<sup>137</sup> using two-dimensional gel electrophoresis and immunoblotting. Two of these studies aimed to identify potentially protective cryptococcal proteins, either based on stronger reactivity with sera from mice immunized with *C. neoformans* strain H99- $\gamma$  and thus protected against fatal cryptococcal disease<sup>133</sup>, or based on presence of proteins in Th1-inducing cryptococcal protein fractions out of which individual protein spots also showed stronger reactivity with sera from H99- $\gamma$  immunized mice<sup>134</sup>. A similar approach was used for identification of potentially protective *C. gattii* proteins exhibiting stronger reactivity with sera from mice protectively immunized with *C. gattii* protein fractions<sup>135</sup>. One study focused on the identification of cryptococcal proteins contained in spots showing IgG binding with sera from human patients infected with *C. gattii*<sup>137</sup>. However, immunoreactivity of the proteins contained in immunoreactive spots was not confirmed through subsequent recombinant protein expression in any of these studies. Therefore, the proteins identified in those studies can only be designated as potentially immunoreactive cryptococcal proteins.

However, the general approach of using immunoreactive proteins as vaccine antigen candidates has been demonstrated to mediate protection against cryptococcal infection. Packaging of proteins previously demonstrated to be immunoreactive into glucan particles and subsequent application via subcutaneous injection for immunization of mice conferred significant protection against challenge with the highly virulent *C. neoformans* serotype A strain KN99<sup>138,139</sup>. Importantly, this method of antigen

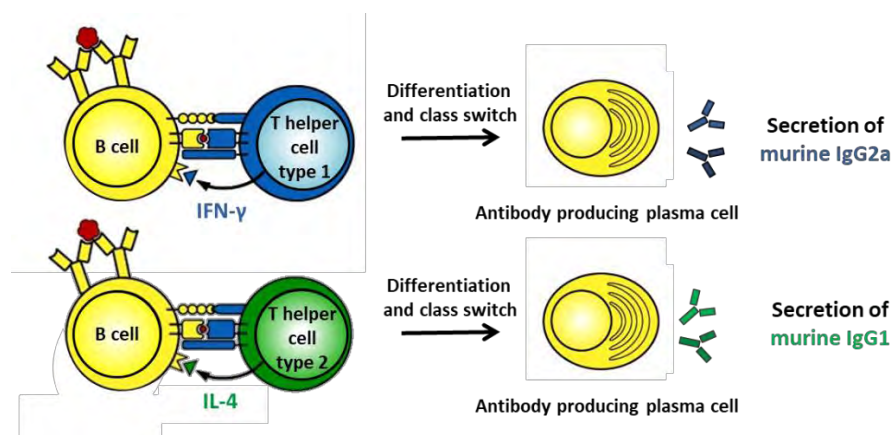
application was successfully tested using various proteins in different mouse genotypes, emphasizing the efficacy of the immunization strategy<sup>138,139</sup>. Protective effects in murine cryptococcal infection models were also observed when immunizing mice with cytosolic proteins of capsular cryptococci contained in fibrin microspheres<sup>140</sup>, or alkaline protein extracts of acapsular cryptococci loaded into glucan particles<sup>141</sup>.

#### 1.4 Aim of the study

We aimed to identify immunoreactive cryptococcal proteins reactive with a) murine (Publication 1: Firacative and Gressler *et al.* 2018)<sup>142</sup> or b) human (Publication 2: Gressler *et al.* 2021)<sup>143</sup> sera, to identify proteins potentially suitable for development of an anti-cryptococcal vaccine or for targeting by antifungal agents.

##### 1.4.1 Publication 1: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection<sup>142</sup>

The approach of this publication is based on the established link between certain cytokines and the production of distinct antibody isotypes. Cytokines induce transcription of specific constant region encoding genes through binding of germline promotor elements<sup>144</sup>. Even though cytokines do not directly induce the switching process, the transcriptional induction or suppression precedes the switching to the same isotype after B cell activation<sup>145</sup>. Incubation of murine B cells with IL-12 or IFN- $\gamma$  has been associated with the production of antibodies of the isotypes IgG2a, IgG2b and IgG3<sup>146-149</sup>. In contrast, IL-4 stimulates production of murine IgG1 and IgE<sup>147-149</sup>, in accordance with the finding, that the germline promoters for the  $\gamma$ 1 and  $\epsilon$  antibody chain have binding sites for the IL-4-induced transcriptional activator Stat6 (signal inducer and activator of transcription 6)<sup>144</sup>. The mechanisms is illustrated in Figure 1.



**Figure 1: Class-switch reaction of B cells is influenced by T helper (Th) cell cytokines.** B cells need antigen-dependent stimulation as well as T cell co-stimulation through membrane-bound receptors and cytokines for differentiation and maturation. Upon maturation, switching to production of a distinct isotype takes place (class switch). In mice, the Th1 cytokine IFN- $\gamma$  promotes switching to production of IgG2a (besides IgG2b and IgG3), whereas the Th2 cytokine IL-4 stimulates production of IgG1 (besides IgE)<sup>146-149</sup>. Figure adapted from Figure 9-11 Immunobiology, 7ed. (© Garland Science 2008)<sup>150</sup>.

Therefore, we aimed to identify proteins preferentially bound by IFN- $\gamma$ -stimulated IgG2a antibodies, therefore associated with protective Th1 responses, and proteins bound by IL-4-promoted IgG1

antibodies, thus associated with detrimental Th2 responses. Increasing titers of *C. neoformans*-specific IgG1 antibodies and a shift of the total serum IgG2a to IgG1 ratio towards IgG1 have previously been linked to progressing cryptococcal disease in mice<sup>28,29</sup>. Our approach is further supported by the fact that previous studies on the fungal pathogen *Candida albicans* linked IgG2a production with protective immune responses<sup>151,152</sup>. In a proteomics approach for identification of immunoreactive *Candida albicans* spots, sera from mice immunized with a weakly virulent *Candida albicans* strain and subsequently protected against lethal infection contained fungus-specific antibodies mainly of the isotype IgG2a whereas susceptible mice produced antibodies of different isotypes against *Candida albicans* proteins<sup>151,152</sup>. Connection of IgG1 antibodies with detrimental Th2 responses and IgG2a or IgG2b antibodies with protective Th1 responses has been furthermore established in several non-fungal infection models<sup>153,154</sup>. BALB/c WT mice infected with *Rodentibacter pneumotropicus* showed increased lung bacterial burden and a polarization of IgG isotypes towards IgG1, whereas C57BL/6 mice showed lower bacterial burden and increased levels of Th1-associated IgG2b antibodies<sup>153</sup>. Similarly, IL-12-deficient mice infected with *Leishmania major* did not only show increased parasite burden compared to BALB/c WT mice, but also reduction of pathogen-specific IgG2a antibodies<sup>154</sup>. Features of experimental asthma in ovalbumin (OVA)-sensitized mice, classically connected to Th2 responses, were improved by application of synthetic toll-like receptor (TLR)-3 or TLR-7 ligands, also resulting in decreased titers of OVA-specific IgG1 for both ligands, and increased titers of OVA-specific IgG2a when TLR-7 was activated<sup>155</sup>.

Based on the established connection between Th responses and immunoglobulin isotypes, we aimed to identify cryptococcal proteins immunoreactive with either IgG2a or IgG1 antibodies, which are therefore associated with protective Th1 or detrimental Th2 responses, respectively. Th1-associated proteins could be used for development of an anti-cryptococcal vaccine, whereas Th2-associated proteins are potential targets for new antifungal substances.

#### **1.4.2 Publication 2: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients<sup>143</sup>**

The second publication focuses on identification of cryptococcal proteins immunoreactive with human sera from patients with cryptococcal meningitis and healthy individuals, all living in Colombia. This is the first study to investigate the cryptococcal protein targets of human anti-cryptococcal IgG antibodies using an immunoproteomic approach. In contrast to other studies, we also applied recombinant protein expression of potentially immunoreactive proteins to confirm reactivity with human sera. Using this approach, we aimed to identify cryptococcal proteins showing significantly stronger reactivity with serum IgG from CM patients compared to healthy individuals, as those can be considered disease-associated proteins. These proteins are potentially implicated in virulence, but are also very interesting research objects because of their potential for vaccine development, as they are immunogenic even in patients with severely impaired immune responses like AIDS patients.



## 1.5 References

1. Kwon-Chung, K. J. *et al.*: *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of Cryptococcosis. *Cold Spring Harb Perspect Med* 4(7), 1–27 (2014).
2. Velagapudi, R., Hsueh, Y.-P., Geunes-Boyer, S., Wright, J. R. & Heitman, J.: Spores as infectious propagules of *Cryptococcus neoformans*. *Infect Immun* 77(10), 4345–4355 (2009).
3. Soltani, M., Bayat, M., Hashemi, S. J., Zia, M. & Pestechian, N.: Isolation of *Cryptococcus neoformans* and other opportunistic fungi from pigeon droppings. *J Res Med Sci* 181, 56–60 (2013).
4. Zarrin, M., Jorfi, M., Amirrajab, N. & Rostami, M.: Isolation of *Cryptococcus neoformans* from pigeon droppings in Ahwaz, Iran. *Turk J Med Sci* 40(2), 313–316 (2010).
5. Brito-Santos, F. *et al.*: Indoor dust as a source of virulent strains of the agents of cryptococcosis in the Rio Negro micro-region of the Brazilian Amazon. *Microorganisms* 8(5), 682 (2020).
6. Gugnani, H. C. *et al.*: Isolation of *Cryptococcus gattii* and *Cryptococcus neoformans* var. *grubii* from the flowers and bark of Eucalyptus trees in India. *Med Mycol* 43(6), 565–569 (2005).
7. Lazera, M. S. *et al.*: Possible primary ecological niche of *Cryptococcus neoformans*. *Med Mycol* 38(5), 379–383 (2000).
8. Grover, N., Nawange, S. R., Naidu, J., Singh, S. M. & Sharma, A.: Ecological niche of *Cryptococcus neoformans* var. *grubii* and *Cryptococcus gattii* in decaying wood of trunk hollows of living trees in Jabalpur City of Central India. *Mycopathologia* 164(4), 159–170 (2007).
9. Aguiar, P. A. *et al.*: The epidemiology of cryptococcosis and the characterization of *Cryptococcus neoformans* isolated in a Brazilian University Hospital. *Rev Inst Med Trop São Paulo* 59, e13 (2017).
10. Chang, C. C. *et al.*: HIV and co-infections. *Immunological reviews* 2541, 114–142 (2013).
11. Sloan, D. J. & Parris, V.: Cryptococcal meningitis: epidemiology and therapeutic options. *Clin Epidemiol* 6, 169–182 (2014).
12. Rajasingham, R. *et al.*: Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 17(8), 873–881 (2017).
13. Ueno, K., Yanagihara, N., Shimizu, K. & Miyazaki, Y.: Vaccines and protective immune memory against cryptococcosis. *Biol Pharm Bull* 43(2), 230–239 (2020).
14. Pyrgos, V., Seitz, A. E., Steiner, C. A., Prevots, D. R. & Williamson, P. R.: Epidemiology of cryptococcal meningitis in the US: 1997-2009. *PLoS one* 8(2), e56269 (2013).
15. Mwaba, P. *et al.*: Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. *Postgrad Med J* 77(914), 769–773 (2001).
16. Kaplan, J. E. *et al.*: Cryptococcal antigen screening and early antifungal treatment to prevent cryptococcal meningitis: A review of the literature. *J Acquir Immune Defic Syndr* 68 Suppl 3, S331–339 (2015).
17. Pappas, P. G.: Cryptococcal infections in non-HIV-infected patients. *Trans Am Clin Climatol Assoc* 124, 61–79 (2013).
18. Gibson, J. F. & Johnston, S. A.: Immunity to *Cryptococcus neoformans* and *C. gattii* during cryptococcosis. *Fungal Genet Biol* 78, 76–86 (2015).
19. Blackstock, R., Buchanan, K. L., Adesina, A. M. & Murphy, J. W.: Differential regulation of immune responses by highly and weakly virulent *Cryptococcus neoformans* isolates. *Infect Immun* 67(7), 3601–3609 (1999).
20. Jain, A. V. *et al.*: Th2 but not Th1 immune bias results in altered lung functions in a murine model of pulmonary *Cryptococcus neoformans* infection. *Infect Immun* 77(12), 5389–5399 (2009).
21. Abe, K. *et al.*: Th1-Th2 cytokine kinetics in the bronchoalveolar lavage fluid of mice infected with *Cryptococcus neoformans* of different virulences. *Microbiol Immunol* 44(10), 849–855 (2000).
22. Arora, S. *et al.*: Role of IFN-gamma in regulating T2 immunity and the development of alternatively activated macrophages during allergic bronchopulmonary mycosis. *J Immunol* 174(10), 6346–6356 (2005).

23. Wormley, F. L., Perfect, J. R., Steele, C. & Cox, G. M.: Protection against cryptococcosis by using a murine gamma interferon-producing *Cryptococcus neoformans* strain. *Infect Immun* 75(3), 1453–1462 (2007).
24. Hardison, S. E. *et al.*: Pulmonary infection with an interferon-gamma-producing *Cryptococcus neoformans* strain results in classical macrophage activation and protection. *Am J Pathol* 176(2), 774–785 (2010).
25. Wozniak, K. L. *et al.*: Insights into the mechanisms of protective immunity against *Cryptococcus neoformans* infection using a mouse model of pulmonary cryptococcosis. *PloS one* 4(9), e6854 (2009).
26. Fa, Z. *et al.*: TNF- $\alpha$ -producing *Cryptococcus neoformans* exerts protective effects on host defenses in murine pulmonary cryptococcosis. *Front Immunol* 10, 1725 (2019).
27. Zhang, Y. *et al.*: Robust Th1 and Th17 immunity supports pulmonary clearance but cannot prevent systemic dissemination of highly virulent *Cryptococcus neoformans* H99. *Am J Pathol* 175(6), 2489–2500 (2009).
28. Müller, U. *et al.*: IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages and allergic inflammation during pulmonary infection of mice with *Cryptococcus neoformans*. *J Immunol* 179(8), 5367–5377 (2007).
29. Müller, U. *et al.*: A gene-dosage effect for interleukin-4 receptor alpha-chain expression has an impact on Th2-mediated allergic inflammation during bronchopulmonary mycosis. *J Infect Dis* 198(11), 1714–1721 (2008).
30. Davis, M. J. *et al.*: Macrophage M1/M2 polarization dynamically adapts to changes in cytokine microenvironments in *Cryptococcus neoformans* infection. *mBio* 4(3), e00264-13 (2013).
31. Leopold Wager, C. M., Hole, C. R., Wozniak, K. L. & Wormley, F. L.: Cryptococcus and phagocytes: complex interactions that influence disease outcome. *Frontiers in microbiology* 7, 105 (2016).
32. Nelson, B. N., Hawkins, A. N. & Wozniak, K. L.: Pulmonary macrophage and dendritic cell responses to *Cryptococcus neoformans*. *Frontiers in cellular and infection microbiology* 10, 37 (2020).
33. Osterholzer, J. J. *et al.*: Cryptococcal urease promotes the accumulation of immature dendritic cells and a non-protective T2 immune response within the lung. *Am J Pathol* 174(3), 932–943 (2009).
34. Stenzel, W. *et al.*: IL-4/IL-13-dependent alternative activation of macrophages but not microglial cells is associated with uncontrolled cerebral cryptococcosis. *Am J Pathol* 174(2), 486–496 (2009).
35. Müller, U. *et al.*: Abrogation of IL-4 receptor- $\alpha$ -dependent alternatively activated macrophages is sufficient to confer resistance against pulmonary cryptococcosis despite an ongoing T(h)2 response. *Int Immunol* 25(8), 459–470 (2013).
36. Zaragoza, O. *et al.*: The capsule of the fungal pathogen *Cryptococcus neoformans*. *Adv Appl Microbiol* 68, 133–216 (2009).
37. Ordóñez, N. & Castañeda, E.: Varieties and serotypes of *Cryptococcus neoformans* clinical isolates in Colombia. *Rev Iberoam Micol* 18(3), 128–130 (2001).
38. Banerjee, U., Datta, K. & Casadevall, A.: Serotype distribution of *Cryptococcus neoformans* in patients in a tertiary care center in India. *Med Mycol* 42(2), 181–186 (2004).
39. Desnos-Ollivier, M., Patel, S., Raoux-Barbot, D., Heitman, J. & Dromer, F.: Cryptococcosis serotypes impact outcome and provide evidence of *Cryptococcus neoformans* speciation. *mBio* 6(3), e00311 (2015).
40. Qiu, Y. *et al.*: Immune modulation mediated by cryptococcal laccase promotes pulmonary growth and brain dissemination of virulent *Cryptococcus neoformans* in mice. *PloS one* 7(10), e47853 (2012).
41. Almeida, G. M., Andrade, R. M. & Bento, C. A.: The capsular polysaccharides of *Cryptococcus neoformans* activate normal CD4(+) T cells in a dominant Th2 pattern. *J Immunol* 167(10), 5845–5851 (2001).
42. Murphy, J. W. & Cozad, G. C.: Immunological unresponsiveness induced by cryptococcal capsular polysaccharide assayed by the hemolytic plaque technique. *Infect Immun* 5(6), 896–901 (1972).
43. Kozel, T. R., Gulley, W. F. & Cazin Jr., J.: Immune response to *Cryptococcus neoformans* soluble polysaccharide: Immunological unresponsiveness. *Infect Immun* 18(3), 701–707 (1977).
44. Vecchiarelli, A. *et al.*: Encapsulation of *Cryptococcus neoformans* regulates fungicidal activity and the antigen presentation process in human alveolar macrophages. *Clin Exp Immunol* 98(2), 217–223 (1994).

45. Vecchiarelli, A. *et al.*: Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1 beta secretion from human monocytes. *Infect Immun* 63(8), 2919–2923 (1995).
46. Vecchiarelli, A. *et al.*: Purified capsular polysaccharide of *Cryptococcus neoformans* induces interleukin-10 secretion by human monocytes. *Infect Immun* 64(7), 2846–2849 (1996).
47. Syme, R. M., Bruno, T. F., Kozel, T. R. & Mody, C. H.: The capsule of *Cryptococcus neoformans* reduces T-lymphocyte proliferation by reducing phagocytosis, which can be restored with anticapsular antibody. *Infect Immun* 67(9), 4620–4627 (1999).
48. Retini, C. *et al.*: Interdependency of interleukin-10 and interleukin-12 in regulation of T-cell differentiation and effector function of monocytes in response to stimulation with *Cryptococcus neoformans*. *Infection and immunity* 69(10), 6064–6073 (2001).
49. Clerici, M. & Shearer, G. M.: A TH1-->TH2 switch is a critical step in the etiology of HIV infection. *Immunol Today* 14(3), 107–111 (1993).
50. Gupta, S., Ellis, M., Cesario, T., Ruhling, M. & Vayuvegula, B.: Disseminated cryptococcal infection in a patient with hypogammaglobulinemia and normal T cell functions. *Am J Med* 82(1), 129–131 (1987).
51. Wahab, J. A., Hanifah, M. J. & Choo, K. E.: Bruton's agammaglobulinaemia in a child presenting with cryptococcal empyema thoracis and periauricular pyogenic abscess. *Singapore Med J* 36(6), 686–689 (1995).
52. Kaushik, A., Rao, H., Tengson, R., Rezk, A. & Kest, H.: Disseminated cryptococcosis with immunoglobulin G subclass deficiency. *Pediatr Int* 61(2), 198–199 (2019).
53. Wienemann, T. *et al.*: Cryptococcal meningoencephalitis in an IgG2-deficient patient with multiple sclerosis on fingolimod therapy for more than five years - case report. *BMC Neurol* 20(1), 158 (2020).
54. Marr, K. A., Datta, K., Pirofski, L.-A. & Barnes, R.: *Cryptococcus gattii* infection in healthy hosts: a sentinel for subclinical immunodeficiency? *Clin Infect Dis* 54(1), 153–154 (2012).
55. Winkelstein, J. A. *et al.*: The X-linked hyper-IgM syndrome: Clinical and immunologic features of 79 patients. *Medicine* 82(6), 373–384 (2003).
56. Tabone, M.-D. *et al.*: Disseminated lymphonodular cryptococcosis in a child with X-linked hyper-IgM immunodeficiency. *Pediatr Infect Dis J* 13(1), 77–79 (1994).
57. Jo, E.-K. *et al.*: X-linked Hyper-IgM syndrome associated with *Cryptosporidium parvum* and *Cryptococcus neoformans* infections: the first case with molecular diagnosis in Korea. *J Korean Med Sci* 17(1), 116–120 (2002).
58. Górgolas, M. de *et al.*: Cryptococcal meningitis in a patient with X-linked hyper-IgM1 syndrome. *Scand J Infect Dis* 37(6-7), 526–528 (2005).
59. Guo, L.-Y. *et al.*: Characteristics and outcomes of cryptococcal meningitis in HIV seronegative children in Beijing, China, 2002-2013. *BMC Infect Dis* 16(1), 635 (2016).
60. Rohatgi, S. *et al.*: Antibody and B cell subset perturbations in human immunodeficiency virus-uninfected patients with cryptococcosis. *Open Forum Infect Dis* 5(1), ofx255 (2018).
61. Subramaniam, K. *et al.*: IgM(+) memory B cell expression predicts HIV-associated cryptococcosis status. *J Infect Dis* 199(2), 244–251 (2009).
62. Rivera, J., Zaragoza, O. & Casadevall, A.: Antibody-mediated protection against *Cryptococcus neoformans* pulmonary infection is dependent on B cells. *Infect Immun* 73(2), 1141–1150 (2005).
63. Marquis, G., Montplaisir, S., Pelletier, M., Mosseau, S. & Auger, P.: Genetic resistance to murine cryptococcosis: Increased susceptibility in the CBA/N XID mutant strain of mice. *Infect Immun* 53(1), 282–287 (1985).
64. Szymczak, W. A. *et al.*: X-linked immunodeficient mice exhibit enhanced susceptibility to *Cryptococcus neoformans* infection. *mBio* 4(4), e00265-13 (2013).
65. Aguirre, K. M. & Johnson, L. L.: A role for B cells in resistance to *Cryptococcus neoformans* in mice. *Infect Immun* 65(2), 525–530 (1997).
66. Dufaud, C., Rivera, J., Rohatgi, S. & Pirofski, L.-A.: Naïve B cells reduce fungal dissemination in *Cryptococcus neoformans* infected Rag1<sup>-/-</sup> mice. *Virulence* 9(1), 173–184 (2018).

67. Subramaniam, K. S. *et al.*: The absence of serum IgM enhances the susceptibility of mice to pulmonary challenge with *Cryptococcus neoformans*. *J Immunol* 184(10), 5755–5767 (2010).
68. Subramaniam, K. S., Datta, K., Marks, M. S. & Pirofski, L.-A.: Improved survival of mice deficient in secretory immunoglobulin M following systemic infection with *Cryptococcus neoformans*. *Infect Immun* 78(1), 441–452 (2010).
69. Dromer, F., Aucouturier, P., Clauvel, J. P., Saimot, G. & Yeni, P.: *Cryptococcus neoformans* antibody levels in patients with AIDS. *Scand J Infect Dis* 20(3), 283–285 (1988).
70. Houpt, D. C., Pfrommer, G. S., Young, B. J., Larson, T. A. & Kozel, T. R.: Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. *Infect Immun* 62(7), 2857–2864 (1994).
71. Deshaw, M. & Pirofski, L.-A.: Antibodies to the *Cryptococcus neoformans* capsular glucuronoxylomannan are ubiquitous in serum from HIV+ and HIV– individuals. *Clin Exp Immunol* 99(3), 425–432 (1995).
72. Dromer, F., Denning, D. W., Stevens, D. A., Noble, A. & Hamilton, J. R.: Anti-*Cryptococcus neoformans* antibodies during cryptococcosis in patients with the acquired immunodeficiency syndrome. *Serodiag Immun Inf D* 7(4), 181–188 (1995).
73. Abadi, J. & Pirofski, L.-A.: Antibodies reactive with the cryptococcal capsular polysaccharide glucuronoxylomannan are present in sera from children with and without human immunodeficiency virus infection. *J Infect Dis* 180(3), 915–919 (1999).
74. Fleuridor, R., Lyles, R. H. & Pirofski, L.: Quantitative and qualitative differences in the serum antibody profiles of human immunodeficiency virus-infected persons with and without *Cryptococcus neoformans* meningitis. *J Infect Dis* 180(5), 1526–1535 (1999).
75. Subramaniam, K., French, N. & Pirofski, L.-A.: *Cryptococcus neoformans*-reactive and total immunoglobulin profiles of human immunodeficiency virus-infected and uninfected Ugandans. *Clin Diagn Lab Immunol* 12(10), 1168–1176 (2005).
76. Jalali, Z., Ng, L., Singh, N. & Pirofski, L.-A.: Antibody response to *Cryptococcus neoformans* capsular polysaccharide glucuronoxylomannan in patients after solid-organ transplantation. *Clin Vaccine Immunol* 13(7), 740–746 (2006).
77. Rohatgi, S. *et al.*: Fc gamma receptor 3A polymorphism and risk for HIV-associated cryptococcal disease. *mBio* 4(5), e00573-13 (2013).
78. Pitzurra, L., Perito, S., Baldelli, F., Bistoni, F. & Vecchiarelli, A.: Humoral response against *Cryptococcus neoformans* mannoprotein antigens in HIV-infected patients. *Clin Exp Immunol* 133(1), 91–96 (2003).
79. Chen, L.-C., Goldman, D. L., Doering, T. L., Pirofski, L.-A. & Casadevall, A.: Antibody response to *Cryptococcus neoformans* proteins in rodents and humans. *Infect Immun* 67(5), 2218–2224 (1999).
80. Goldman, D. L. *et al.*: Serologic evidence for *Cryptococcus neoformans* infection in early childhood. *Pediatrics* 107(5), E66 (2001).
81. Davis, J. *et al.*: Serologic evidence for regional differences in pediatric cryptococcal infection. *Pediatr Infect Dis J* 26(6), 549–551 (2007).
82. Saha, D. C., Xess, I., Zeng, W. Y. & Goldman, D. L.: Antibody responses to *Cryptococcus neoformans* in Indian patients with cryptococcosis. *Med Mycol* 46(5), 457–463 (2008).
83. Chai, H. C. & Tay, S. T.: Detection of IgM and IgG antibodies to *Cryptococcus neoformans* proteins in blood donors and HIV patients with active cryptococcosis. *Mycoses* 52(2), 166–170 (2009).
84. Spitzer, E. D., Spitzer, S. G., Freundlich, L. F. & Casadevall, A.: Persistence of initial infection in recurrent *Cryptococcus neoformans* meningitis. *Lancet* 341(8845), 595–596 (1993).
85. Garcia-Hermoso, D., Janbon, G. & Dromer, F.: Epidemiological evidence for dormant *Cryptococcus neoformans* infection. *J Clin Microbiol* 37(10), 3204–3209 (1999).
86. Saha, D. C. *et al.*: Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. *Clin Vaccine Immunol* 14(12), 1550–1554 (2007).
87. Hagen, F., van Assen, S., Luijckx, G. J., Boekhout, T. & Kampinga, G. A.: Activated dormant *Cryptococcus gattii* infection in a Dutch tourist who visited Vancouver Island (Canada): a molecular epidemiological approach. *Med Mycol* 48(3), 528–531 (2010).

88. Alanio, A.: Dormancy in *Cryptococcus neoformans*: 60 years of accumulating evidence. *J Clin Invest* 130(7), 3353–3360 (2020).
89. Rodrigues, M. L., Nimrichter, L., Cordero, R. J. & Casadevall, A.: Fungal polysaccharides: biological activity beyond the usual structural properties. *Front Microbiol* 2, 171 (2011).
90. Liu, L., Tewari, R. P. & Williamson, P. R.: Laccase protects *Cryptococcus neoformans* from antifungal activity of alveolar macrophages. *Infect Immun* 67(11), 6034–6039 (1999).
91. Wozniak, K. L. & Levitz, S. M.: *Cryptococcus neoformans* enters the endolysosomal pathway of dendritic cells and is killed by lysosomal components. *Infect Immun* 76(10), 4764–4771 (2008).
92. Marr, K. J. *et al.*: *Cryptococcus neoformans* directly stimulates perforin production and rearms NK cells for enhanced anticryptococcal microbicidal activity. *Infect Immun* 77(6), 2436–2446 (2009).
93. Wiseman, J. C., Ma, L. L., Marr, K. J., Jones, G. J. & Mody, C. H.: Perforin-dependent cryptococcal microbicidal activity in NK cells requires PI3K-dependent ERK1/2 signaling. *Journal of immunology (Baltimore, Md. : 1950)* 178(10), 6456–6464 (2007).
94. Rohatgi, S. & Pirofski, L.-A.: Host immunity to *Cryptococcus neoformans*. *Future Microbiol* 10(4), 565–581 (2015).
95. Diamond, R. D.: Antibody-dependent killing of *Cryptococcus neoformans* by human peripheral blood mononuclear cells. *Nature* 247(5437), 148–150 (1974).
96. Diamond, R. D. & Allison, A. C.: Nature of the effector cells responsible for antibody-dependent cell-mediated killing of *Cryptococcus neoformans*. *Infect Immun* 14(3), 716–720 (1976).
97. Kozel, T. R. & Follette, J. L.: Opsonization of encapsulated *Cryptococcus neoformans* by specific anticapsular antibody. *Infect Immun* 31(3), 978–984 (1981).
98. Mukherjee, S., Feldmesser, M. & Casadevall, A.: J774 murine macrophage-like cell interactions with *Cryptococcus neoformans* in the presence and absence of opsonins. *J Infect Dis* 173(5), 1222–1231 (1996).
99. Nabavi, N. & Murphy, J. W.: Antibody-dependent natural killer cell-mediated growth inhibition of *Cryptococcus neoformans*. *Infect Immun* 51(2), 556–562 (1986).
100. Miller, M. F., Mitchell, T. G., Storkus, W. J. & Dawson, J. R.: Human natural killer cells do not inhibit growth of *Cryptococcus neoformans* in the absence of antibody. *Infect Immun* 58(3), 639–645 (1990).
101. Monari, C. *et al.*: Antibody to capsular polysaccharide enhances the function of neutrophils from patients with AIDS against *Cryptococcus neoformans*. *AIDS* 13(6), 653–660 (1999).
102. Netski, D. & Kozel, T. R.: Fc-dependent and Fc-independent opsonization of *Cryptococcus neoformans* by anticapsular monoclonal antibodies: importance of epitope specificity. *Infect Immun* 70(6), 2812–2819 (2002).
103. Kelly, R. M., Chen, J., Yauch, L. E. & Levitz, S. M.: Opsonic requirements for dendritic cell-mediated responses to *Cryptococcus neoformans*. *Infect Immun* 73, 592–598 (2005).
104. Kozel, T. R., de Jong, Bouke C. H., Grinsell, M. M., MacGill, R. S. & Wall, K. K.: Characterization of anticapsular monoclonal antibodies that regulate activation of the complement system by the *Cryptococcus neoformans* capsule. *Infect Immun* 66(4), 1538–1546 (1998).
105. Casadevall, A. *et al.*: Characterization of a murine monoclonal antibody to *Cryptococcus neoformans* polysaccharide that is a candidate for human therapeutic studies. *Infect Immun* 42(6), 1437–1446 (1998).
106. Shapiro, S. *et al.*: Immunoglobulin G monoclonal antibodies to *Cryptococcus neoformans* protect mice deficient in complement component C3. *Infection and immunity* 70(5), 2598–2604 (2002).
107. Dromer, F., Perronne, C., Barge, J., Vilde, J. L. & Yeni, P.: Role of IgG and complement component C5 in the initial course of experimental cryptococcosis. *Clin Exp Immunol* 78(3), 412–417 (1989).
108. Dromer, F., Charreire, J., Contrepolis, A., Carbon, C. & Yeni, P.: Protection of mice against experimental cryptococcosis by anti-*Cryptococcus neoformans* monoclonal antibody. *Infect Immun* 55(3), 749–752 (1987).
109. Mukherjee, J., Scharff, M. D. & Casadevall, A.: Protective murine monoclonal antibodies to *Cryptococcus neoformans*. *Infect Immun* 60(11), 4534–4541 (1992).

110. Feldmesser, M., Mednick, A. & Casadevall, A.: Antibody-mediated protection in murine *Cryptococcus neoformans* infection is associated with pleiotropic effects on cytokine and leukocyte responses. *Infection and immunity* 70(3), 1571–1580 (2002).
111. Mukherjee, S., Lee, S. C. & Casadevall, A.: Antibodies to *Cryptococcus neoformans* glucuronoxylomannan enhance antifungal activity of murine macrophages. *Infect Immun* 63(2), 573–579 (1995).
112. Mukherjee, J., Nussbaum, G., Scharff, M. D. & Casadevall, A.: Protective and nonprotective monoclonal antibodies to *Cryptococcus neoformans* originating from one B cell. *J Exp Med* 181, 405–409 (1995).
113. Tabora, C. P. & Casadevall, A.: Immunoglobulin M efficacy against *Cryptococcus neoformans*: mechanism, dose dependence, and prozone-like effects in passive protection experiments. *Journal of immunology (Baltimore, Md. : 1950)* 166(3), 2100–2107 (2001).
114. Yuan, R., Casadevall, A., Spira, G. & Scharff, M. D.: Isotype switching from IgG3 to IgG1 converts a nonprotective murine antibody to *Cryptococcus neoformans* into a protective antibody. *J Immunol* 154(4), 1810–1816 (1995).
115. Yuan, R. *et al.*: Isotype switching increases efficacy of antibody protection against *Cryptococcus neoformans* infection in mice. *Infect Immun* 66(3), 1057–1062 (1998).
116. Mukherjee, J., Scharff, M. D. & Casadevall, A.: Variable efficacy of passive antibody administration against diverse *Cryptococcus neoformans* strains. *Infect Immun* 63(9), 3353–3359 (1995).
117. Rivera, J. & Casadevall, A.: Mouse genetic background is a major determinant of isotype-related differences for antibody-mediated protective efficacy against *Cryptococcus neoformans*. *Journal of immunology (Baltimore, Md. : 1950)* 174(12), 8017–8026 (2005).
118. Yuan, R., Clynes, R., Oh, J., Ravetch, J. V. & Scharff, M. D.: Antibody-mediated modulation of *Cryptococcus neoformans* infection is dependent on distinct Fc receptor functions and IgG subclasses. *The Journal of experimental medicine* 187(4), 641–648 (1998).
119. Torres, M., May, R., Scharff, M. D. & Casadevall, A.: Variable-region-identical antibodies differing in isotype demonstrate differences in fine specificity and idiotype. *Journal of immunology (Baltimore, Md. : 1950)* 174(4), 2132–2142 (2005).
120. Torres, M., Fernández-Fuentes, N., Fiser, A. & Casadevall, A.: The immunoglobulin heavy chain constant region affects kinetic and thermodynamic parameters of antibody variable region interactions with antigen. *J Biol Chem* 282(18), 13917–13927 (2007).
121. Mukherjee, S., Lee, S., Mukherjee, J., Scharff, M. D. & Casadevall, A.: Monoclonal antibodies to *Cryptococcus neoformans* capsular polysaccharide modify the course of intravenous infection in mice. *Infect Immun* 62(3), 1079–1088 (1994).
122. Goldman, D. L., Lee, S. C. & Casadevall, A.: Tissue localization of *Cryptococcus neoformans* glucuronoxylomannan in the presence and absence of specific antibody. *Infect Immun* 63(9), 3448–3453 (1995).
123. Martinez, L. R. & Casadevall, A.: Specific antibody can prevent fungal biofilm formation and this effect correlates with protective efficacy. *Infect Immun* 73(10), 6350–6362 (2005).
124. Trevijano-Contador, N. *et al.*: Human IgM inhibits the formation of titan-like cells in *Cryptococcus neoformans*. *Infect Immun* 88(4), e00046-20 (2020).
125. McClelland, E. E., Nicola, A. M., Prados-Rosales, R. & Casadevall, A.: Ab binding alters gene expression in *Cryptococcus neoformans* and directly modulates fungal metabolism. *The Journal of clinical investigation* 120(4), 1355–1361 (2010).
126. Mukherjee, J., Zuckier, L. S., Scharff, M. D. & Casadevall, A.: Therapeutic efficacy of monoclonal antibodies to *Cryptococcus neoformans* glucuronoxylomannan alone and in combination with amphotericin B. *Antimicrob Agents Chemother* 38(3), 580–587 (1994).
127. Larsen, R. A. *et al.*: Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. *Antimicrobial agents and chemotherapy* 49(3), 952–958 (2005).
128. Armstrong-James, D. *et al.*: Immunotherapeutic approaches to treatment of fungal diseases. *Lancet Infect Dis* 17(12), 393–402 (2017).

129. Mukherjee, J., Casadevall, A. & Scharff, M. D.: Molecular characterization of the humoral responses to *Cryptococcus neoformans* infection and glucuronoxylomannan-tetanus toxoid conjugate immunization. *J Exp Med* 177(4), 1105–1116 (1993).
130. Devi, S. J.: Preclinical efficacy of a glucuronoxylomannan-tetanus toxoid conjugate vaccine of *Cryptococcus neoformans* in a murine model. *Vaccine* 14(9), 841–844 (1996).
131. Casadevall, A. & Pirofski, L.-A.: Insights into mechanisms of antibody-mediated immunity from studies with *Cryptococcus neoformans*. *Curr Mol Med* 5(4), 421–433 (2005).
132. Datta, K. & Pirofski, L.-A.: Towards a vaccine for *Cryptococcus neoformans*: principles and caveats. *FEMS yeast research* 6(4), 525–536 (2006).
133. Young, M., Macias, S., Thomas, D. & Wormley, F. L.: A proteomic-based approach for the identification of immunodominant *Cryptococcus neoformans* proteins. *Proteomics* 9(9), 2578–2588 (2009).
134. Chaturvedi, A. K., Weintraub, S. T., Lopez-Ribot, J. L. & Wormley, F. L.: Identification and characterization of *Cryptococcus neoformans* protein fractions that induce protective immune responses. *Proteomics* 13(23-24), 3429–3441 (2013).
135. Chaturvedi, A. K. *et al.*: Vaccine-mediated immune responses to experimental pulmonary *Cryptococcus gattii* infection in mice. *PLoS One* 9(8), e104316 (2014).
136. Jobbins, S. E. *et al.*: Immunoproteomic approach to elucidating the pathogenesis of cryptococcosis caused by *Cryptococcus gattii*. *J Proteome Res* 9(8), 3832–3841 (2010).
137. Martins, L. M. *et al.*: Immunoproteomics and immunoinformatics analysis of *Cryptococcus gattii* : novel candidate antigens for diagnosis. *Future Microbiol* 8(4), 549–563 (2013).
138. Specht, C. A. *et al.*: Vaccination with recombinant cryptococcus proteins in glucan particles protects mice against cryptococcosis in a manner dependent upon mouse strain and cryptococcal species. *mBio* 8(6), e01872-17 (2017).
139. Hester, M. M. *et al.*: Protection of mice against experimental cryptococcosis using glucan particle-based vaccines containing novel recombinant antigens. *Vaccine* 38(3), 620–626 (2020).
140. Khan, A. A., Jabeen, M., Chauhan, A. & Owais, M.: Vaccine potential of cytosolic proteins loaded fibrin microspheres of *Cryptococcus neoformans* in BALB/c mice. *J Drug Target* 20(5), 453–466 (2012).
141. Specht, C. A. *et al.*: Protection against experimental cryptococcosis following vaccination with glucan particles containing *Cryptococcus* alkaline extracts. *mBio* 6(6), 165rv113 (2015).
142. Firacative, C. *et al.*: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection. *Sci Rep* 81, 2681 (2018).
143. Gressler, A. E. *et al.*: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients. *Front Immunol* 12, 709695 (2021).
144. Stavnezer, J., Guikema, J. E. & Schrader, C. E.: Mechanism and regulation of class switch recombination. *Annu Rev Immunol* 26, 261–292 (2008).
145. Stavnezer, J.: Immunoglobulin class switching. *Curr Opin Immunol* 8(2), 199–205 (1996).
146. Germann, T. *et al.*: Interleukin-12 profoundly up-regulates the synthesis of antigen-specific complement-fixing IgG2a, IgG2b and IgG3 antibody subclasses in vivo. *Eur J Immunol* 25(3), 823–829 (1995).
147. Snapper, C. M. & Paul, W. E.: Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 236(4804), 944–947 (1987).
148. Snapper, C. M. & Mond, J. J.: Towards a comprehensive view of immunoglobulin class switching. *Immunol Today* 141, 15–17 (1993).
149. Finkelman, F. D. *et al.*: Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol* 8, 303–333 (1990).
150. Murphy, K. M. *et al.* *Janeway's Immunobiology*. 7th ed. (Garland Science, New York, NY, USA, 2008).
151. Fernández-Arenas, E., Molero, G., Nombela, C., Diez-Orejas, R. & Gil, C.: Contribution of the antibodies response induced by a low virulent *Candida albicans* strain in protection against systemic candidiasis. *Proteomics* 4(4), 1204–1215 (2004).

152. Fernández-Arenas, E., Molero, G., Nombela, C., Diez-Orejas, R. & Gil, C.: Low virulent strains of *Candida albicans*: unravelling the antigens for a future vaccine. *Proteomics* 4(10), 3007–3020 (2004).
153. Fornefett, J. *et al.*: Comparative analysis of humoral immune responses and pathologies of BALB/c and C57BL/6 wildtype mice experimentally infected with a highly virulent *Rodentibacter pneumotropicus* (*Pasteurella pneumotropica*) strain. *BMC Microbiol* 181, 45 (2018).
154. Mattner, F., Di Padova, K. & Alber, G.: Interleukin-12 is indispensable for protective immunity against *Leishmania major*. *Infect Immun* 65(11), 4378–4383 (1997).
155. Sel, S. *et al.*: Immunomodulatory effects of viral TLR ligands on experimental asthma depend on the additive effects of IL-12 and IL-10. *Journal of immunology (Baltimore, Md. : 1950)* 178(12), 7805–7813 (2007).



## 2 First publication: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection

SCIENTIFIC REPORTS 

OPEN

### Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection

Received: 2 October 2017

Accepted: 29 January 2018

Published online: 08 February 2018

Carolina Firacative<sup>1,2,5</sup>, A. Elisabeth Gressler<sup>1</sup>, Kristin Schubert<sup>3</sup>, Bianca Schulze<sup>1,7</sup>, Uwe Müller<sup>1</sup>, Frank Brombacher<sup>4</sup>, Martin von Bergen<sup>3,5</sup> & Gottfried Alber<sup>1</sup>

Carolina Firacative and A. Elisabeth Gressler contributed equally to this work.

#### Highlights:

- Concentrations of the Th2-associated immunoglobulin IgG1, but not the Th1-associated antibody IgG2a increased upon cryptococcal infection in mice of different genotypes
- Titers of anti-cryptococcal IgG1 and IgG2a antibodies increased over the course of cryptococcal infection, but only titers of anti-cryptococcal IgG1 antibodies and concentrations of total serum IgG1 were correlated, arguing for a disease-dependent induction of the Th2-associated isotype IgG1 during cryptococcal infection
- Immunoproteomic analysis revealed several cryptococcal protein spots bound by IgG1 and IgG2a antibodies from murine sera
- Some cryptococcal proteins were identified exclusively in IgG1- or IgG2a-reactive spots, rendering them Th1- or Th2-associated proteins

# Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection

Carolina Firacative<sup>1,2,6</sup>, A. Elisabeth Gressler<sup>1</sup>, Kristin Schubert<sup>3</sup>, Bianca Schulze<sup>1,7</sup>, Uwe Müller<sup>1</sup>, Frank Brombacher<sup>4</sup>, Martin von Bergen<sup>3,5</sup> & Gottfried Alber<sup>1</sup>

*Cryptococcosis*, caused by *Cryptococcus neoformans*, has been demonstrated to be controlled by T helper (Th)1 cells while Th2 cells are associated with fungal growth and dissemination. Although cryptococcal immunoreactive protein antigens were previously identified, their association with Th1 or Th2 immune responses was not provided. In mice, Th1-dependent IFN- $\gamma$  induces the production of IgG2a, whereas the Th2 cytokine IL-4 stimulates the expression of IgG1 rendering each isotype an indicator of the underlying Th cell response. Therefore, we performed an immunoproteomic study that distinguishes Th1- and Th2-associated antigens by their reactivity with Th1-dependent IgG2a or Th2-dependent IgG1 antibodies in sera from *C. neoformans*-infected wild-type mice. We additionally analysed sera from Th2-prone IL-12-deficient and Th1-prone IL-4R $\alpha$ -deficient mice extending the results found in wild-type mice. In total, ten, four, and three protein antigens associated with IgG1, IgG2a, or both isotypes, respectively, were identified. Th2-associated antigens represent promising candidates for development of immunotherapy regimens, whereas Th1-associated antigens may serve as candidates for vaccine development. In conclusion, this study points to intrinsic immunomodulatory effects of fungal antigens on the process of Th cell differentiation based on the identification of cryptococcal protein antigens specifically associated with Th1 or Th2 responses throughout mice of different genotypes.

*Cryptococcus neoformans*, an encapsulated basidiomycetous yeast, is the main etiological agent of cryptococcosis, a systemic and potentially fatal fungal infection. *C. neoformans* is ubiquitously present in the environment, especially in pigeon guano, which is the main known ecological niche of this pathogen<sup>1,2</sup>. Pulmonary infection with *C. neoformans* usually occurs through inhalation of infectious spores or desiccated yeasts from the environment establishing a normally latent, asymptomatic or minimally symptomatic disease in immunocompetent individuals<sup>1-3</sup>, although the risk to develop chronic allergic diseases such as asthma, has been shown to be enhanced in rats and BALB/c mice experimentally infected with *C. neoformans*<sup>4,5</sup>. In contrast, in immunocompromised persons such as AIDS patients, solid organ transplant recipients, or patients receiving exogenous immunosuppression,

<sup>1</sup>Institute of Immunology/Molecular Pathogenesis, Centre for Biotechnology and Biomedicine, College of Veterinary Medicine, University of Leipzig, Leipzig, Germany. <sup>2</sup>Group of Microbiology, National Institute of Health, Bogota, Colombia. <sup>3</sup>Department of Molecular Systems Biology, Helmholtz-Centre for Environmental Research, Leipzig, Germany. <sup>4</sup>International Centre for Genetic Engineering & Biotechnology (ICGEB), Cape Town component, South Africa and University of Cape Town, Division Immunology, Institute of Infectious Diseases and Molecular Medicine (IDM) & South African Medical Research Council (SAMRC), Cape Town, South Africa. <sup>5</sup>Institute of Biochemistry, Faculty of Biosciences, Pharmacy and Psychology, University of Leipzig, Leipzig, Germany. <sup>6</sup>Present address: School of Medicine and Health Sciences, Universidad del Rosario, Bogota, Colombia. <sup>7</sup>Present address: Research Group Microbial Immunology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute (HKI), Jena, Germany. Carolina Firacative and A. Elisabeth Gressler contributed equally to this work. Correspondence and requests for materials should be addressed to G.A. (email: alber@vetmed.uni-leipzig.de)

unresolved or untreated pulmonary cryptococcosis may lead to dissemination affecting the central nervous system (CNS) and causing meningitis or meningoencephalitis with a high mortality rate<sup>1,6</sup>. With about a quarter of a million individuals affected with cryptococcal meningitis per year and over 180,000 attributable annual deaths, this fungal infection is still responsible for 15% of all AIDS-associated mortalities<sup>7</sup>.

It is well known that the main host defence mechanism to resolve cryptococcosis is cell-mediated immunity by suppressing the growth of the yeasts in the lungs, which impedes dissemination to the CNS<sup>8</sup>. While T helper (Th)1 cells play a central role in induction of a protective immune response against cryptococcal infection, Th2 cells producing interleukin (IL)-4, IL-13, and IL-5, are detrimental in infection with *C. neoformans*<sup>9,10</sup>. Interestingly, *C. neoformans* is able to subvert immunoprotection by suppressing cellular immune response and through induction of humoral Th2 cell mediated immunity, resulting in a permissive environment for cryptococcal growth, characterized by IL-4-dependent immunoglobulin (Ig)E production, IL-13 dependent mucus production by goblet cells, IL-5-dependent eosinophilia, and functional pulmonary impairment, which are also features typically described in asthma<sup>2,4,5,11</sup>. Despite the benefits of available antifungal drugs, the emergence of drug-resistant fungal strains and several side-effects resulting from long term medication and toxicity, limit their use<sup>12</sup>. Therefore, adjunctive immunotherapy together with antifungal treatment is a promising option for the future<sup>11</sup>. The identification of cryptococcal protein antigens is of great interest for the development of an antifungal vaccine<sup>11</sup>. Particularly the discrimination between antigens that induce protective cell-mediated immunity responses to cryptococcal infection and antigenic compounds that are detrimental in cryptococcosis could contribute to the identification of vaccine candidates and targets for specific immunotherapy, respectively, which may help to reduce fungal burden, preventing the spread of the yeast from the lungs and increase the survival rate of the patients<sup>11</sup>.

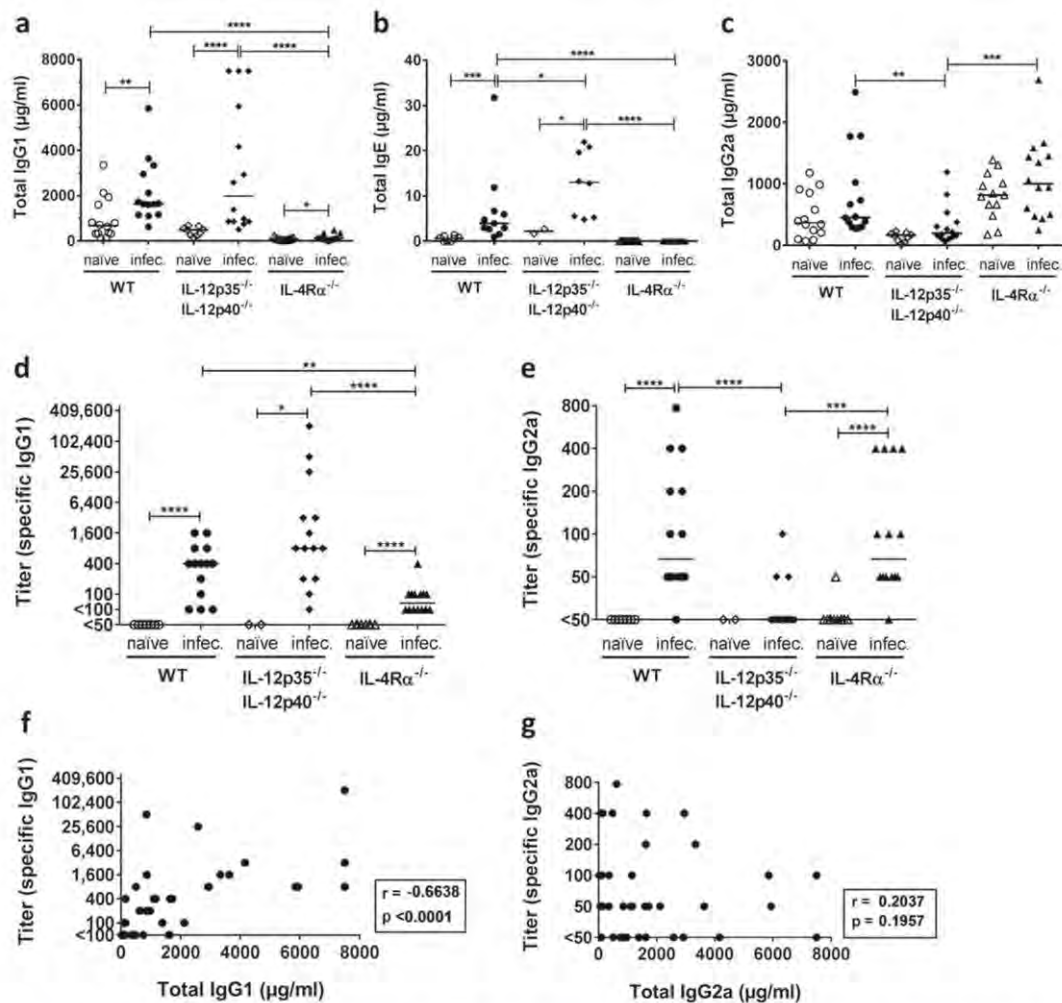
Previous studies have aimed to identify protective cryptococcal proteins and protein fractions by their reactivity with antibodies produced by mice immunized with a murine IFN- $\gamma$ -expressing *C. neoformans* strain (H99 $\gamma$ ) that were in consequence protected against a subsequent challenge infection with a *C. neoformans* wild-type strain<sup>13,14</sup>. In another study, immunoreactive proteins of *C. gattii*, the closest related species to *C. neoformans* and the second most common etiological agent of cryptococcosis, have been identified using antibodies from sera of naturally infected koalas<sup>15</sup>. However, those studies lack the specific discrimination between immunoprotective and immunopathologic properties of the antigen that are associated with either a Th1 or a Th2 response. We decided to use a proteomic approach involving two-dimensional (2D) gel electrophoresis and subsequent immunoblot, for the identification of Th1- and Th2-associated cryptococcal antigens based on the linkage of class switching in B cells with production of distinct Th cell cytokines<sup>16</sup>. Several murine studies firmly established that IL-4 regulates B cells for secretion of IgG1 antibodies, whereas interferon- $\gamma$  stimulates the expression of IgG2a antibodies rendering either isotype an indicator of the underlying Th2 or Th1 response in mice<sup>16–18</sup>. Therefore, we chose to identify Th1- and Th2-associated antigens of *C. neoformans* by their reactivity specifically with either Th1-dependent IgG2a or Th2-dependent IgG1 from sera of infected wild-type and gene-deficient mice that lack either Th1 or Th2 responses. The proteomic approach allows separating the cellular proteins and the identification of immunoglobulins binding specific antigens<sup>19</sup>. Using this technique, distinct Th2- and Th1-associated fungal proteins were identified which are likely to play a role in shaping the Th cell response to *C. neoformans* and may be used for development of anti-fungal immunotherapy or vaccination regimens.

## Results

### Pulmonary infection with *C. neoformans* leads to dominant production of Th2-dependent IgG1 and IgE.

In a BALB/c model of intranasal cryptococcal infection, IgG1/IgE and IgG2a have been shown to be valid indicators for preferential Th2 and Th1 responses, respectively<sup>5,20</sup>. Wild-type mice are susceptible to infection with the *C. neoformans* strain 1841<sup>5,20</sup>, while IL-4R $\alpha$ -deficient mice, characterized by a dominant Th1 and Th17 response, are resistant to pulmonary cryptococcal infection<sup>20</sup>. In contrast, IL-12p35- and IL-12p40-deficient mice do succumb significantly earlier to intranasal infection (unpublished data) accompanied by a stronger Th2 response similarly as previously published for intravenously infected IL-12-deficient mice<sup>21</sup>. Sera from wild-type and knock-out mouse lines were used in order to rigorously distinguish specific Th1- and Th2-associated cryptococcal antigens. Levels of total IgG1 and IgE increased significantly in mice of all genotypes after infection with *C. neoformans*, except for IgE levels in IL-4R $\alpha$ -deficient mice, which are unable to produce IgE<sup>20</sup>, underlining the overall Th2-biased immune response to this pathogen (Fig. 1a,b). In contrast, IgG2a levels were not influenced by cryptococcal infection (Fig. 1c). Comparison of total immunoglobulin levels revealed an increased Th2 response for IL-12p35- and IL-12p40-deficient mice, evidenced by the higher levels of total IgE in comparison with wild-type mice and comparable levels of IgG1 (Fig. 1a,b). Opposite, infected IL-4R $\alpha$ -deficient mice showed a marked diminution in their Th2-associated B cell response, with no production of IgE and notably lower levels of total IgG1 compared to wild-type mice (Fig. 1a,b), consistent with previously published experiments<sup>20</sup>. Among all three groups of infected mice, production of total Th1-dependent IgG2a was determined to be lower in IL-12-deficient mice, while IL-4R $\alpha$ -deficient mice produced the highest levels of this IgG isotype, although without statistical significance (Fig. 1c). When comparing the total levels of IgG1 and IgG2a in sera from infected IL-12- and IL-4R $\alpha$ -deficient mice, a significant negative correlation between IgG1 and IgG2a was found ( $r = -0.5077$ ,  $p = 0.0058^{**}$ ), confirming the opposite Th phenotypes in the selected mutant mice, while in sera from infected wild-type mice, there was no correlation between the two IgG isotypes (data not shown).

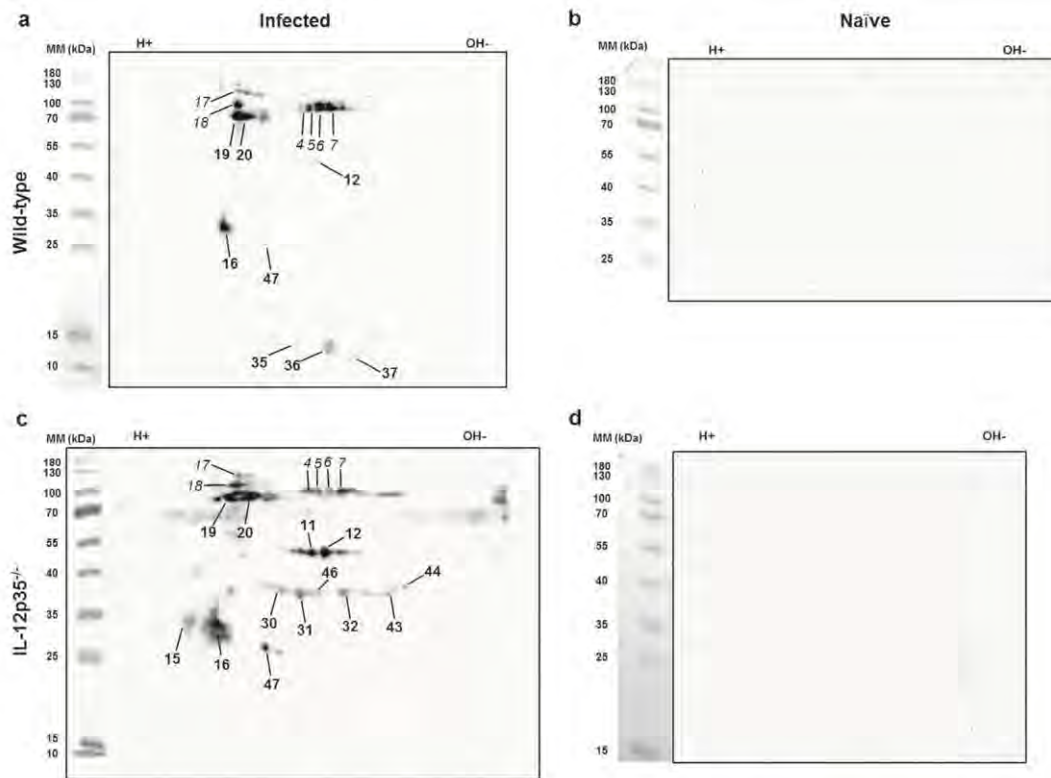
After quantification of total immunoglobulin concentrations, the titers of *C. neoformans*-specific IgG1 and IgG2a antibodies were determined, using a cryptococcal antigen-specific enzyme-linked immunosorbent assay (ELISA). Compared to wild-type and IL-12-deficient mice, titers for specific IgG1 antibodies against *C. neoformans* were lower in infected IL-4R $\alpha$ -deficient mice (Fig. 1d), while infected IL-12-deficient mice showed reduced levels of specific IgG2a antibodies compared to infected wild-type and IL-4R $\alpha$ -deficient mice (Fig. 1e). Overall, titers for *C. neoformans*-specific IgG1 antibodies reached higher values in comparison to *C. neoformans*-specific IgG2a titers. Furthermore, titers of *C. neoformans*-specific IgG1 and IgG2a showed a significant increase upon



**Figure 1.** Total and *C. neoformans*-specific levels of Th2-dependent IgG1 and IgE predominate in sera from susceptible wild-type (WT) and IL-12-deficient mice with pulmonary cryptococcosis. Total IgG1 (a) and IgE (b) levels increased significantly after infection with *C. neoformans* for most genotypes, whereas IgG2a levels (c) were not influenced by *C. neoformans*. Compared to wild-type mice, IL-4R $\alpha$ -deficient mice had markedly lower levels of immunoglobulin (Ig)G1 (a), no production of IgE (b) and similar levels of total immunoglobulin (Ig)G2a (c). Opposite, IL-12-deficient mice showed similar IgG1 and significantly higher IgE levels (a,b), while reduced levels of IgG2a (c), compared to wild-type mice. Titers of *C. neoformans*-specific IgG2a (d) and IgG1 (e) antibodies in infected (infect.) mice reflected the distribution observed for total immunoglobulin levels. *C. neoformans*-specific IgG1 and IgG2a antibodies were absent in sera of naïve mice. A strong correlation was observed between total and *C. neoformans*-specific IgG1 levels (f) but not between total and *C. neoformans*-specific IgG2a levels (g) in infected mice of all genotypes. Each spot represents a serum sample of an individual mouse (2 to 14 animals per group from at least two independent experiments) with the line indicating the median. Serum samples were obtained from mice intranasally infected with 500 colony forming units of *C. neoformans* strain 1841 after about 56 days post infection. Statistical significance determined by Mann-Whitney U test is shown as following: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , and \*\*\*\* $p \leq 0.0001$ . Correlation was determined by nonparametric Spearman's correlation test.

cryptococcal infection for all genotypes except for IgG2a detected in sera of IL-12-deficient mice (Fig. 1d,e). *C. neoformans*-specific IgG1 and IgG2a antibodies were absent in sera from naïve mice of all genotypes (Fig. 1d,e). A strong correlation was found between total and specific levels of IgG1 ( $r = -0.6638$ , \*\*\*\* $p < 0.0001$ , Fig. 1f) but not between total and specific levels of IgG2a ( $r = 0.2037$ ,  $p = 0.1957$ , Fig. 1g) in sera from infected mice of all genotypes, indicating an influence of *C. neoformans* on the production of IgG1 but not IgG2a antibodies. We did not determine *C. neoformans*-specific IgE titers in serum samples, as these are expected to be very low according to a previous study<sup>22</sup>. In conclusion, we could confirm the previously observed Th2-tilted immune response on the level of both, total and specific IgG1 and IgG2a antibodies upon *C. neoformans* infection.

**Proteomic analysis reveals cryptococcal antigens specifically reactive with IgG1 or IgG2a antibodies.** After determining the titers for specific IgG1 and IgG2a antibodies against *C. neoformans* proteins, representative serum samples from each group of infected mice, five from wild-type and two from gene-deficient

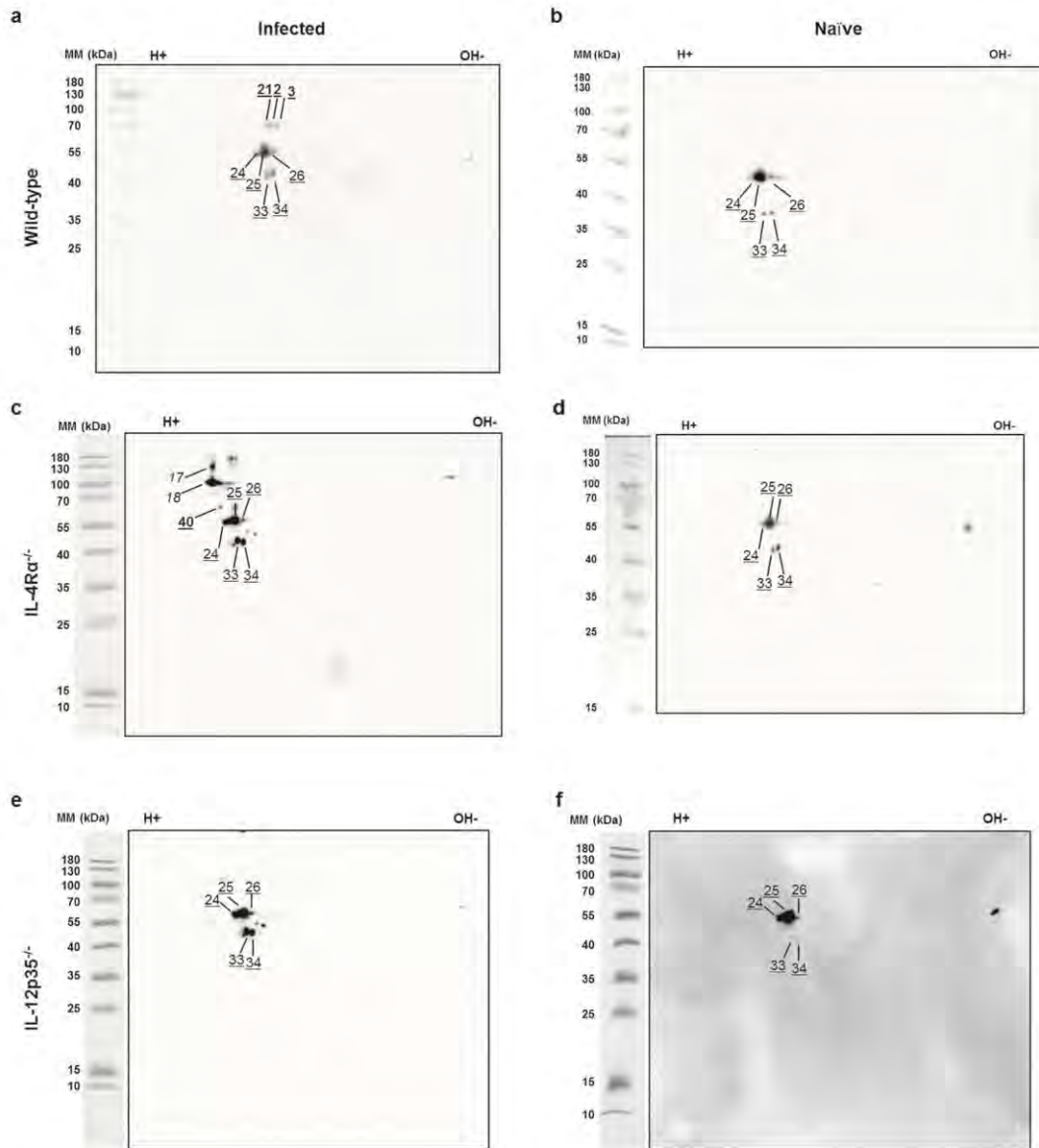


**Figure 2.** IgG1-immunoreactive proteins from *Cryptococcus neoformans* were detected with sera from representative infected but not naïve wild-type and IL-12-deficient mice. Whole cell proteins of *C. neoformans* strain 1841 separated by 2D electrophoresis were transferred to nitrocellulose membranes, which were thereafter incubated with sera from infected and naïve wild-type and gene-deficient mice diluted 1:1,000. IgG1-immunoreactive proteins were detected using sera from an infected wild-type (a), a naïve wild-type (b), an infected IL-12-deficient (c), and a naïve IL-12-deficient (d) mouse. Protein abundance as shown in the Coomassie staining did not correlate with the strength of the immunoreactive signal (Fig. 4). Only the spots that could be mapped on Coomassie-stained gels were numbered in the blot images. Bold numbers indicate strictly IgG1-reactive proteins while italic numbers mark proteins reactive with both, IgG1 and IgG2a antibodies. Images were cropped to improve clarity. Full-length blots without numbered protein spots are presented in Supplementary Fig. 2. Abbreviation: MM = molecular mass.

mice, were tested by immunoblot analyses following one-dimensional (1D) gel electrophoresis. Serum samples that showed high titers of *C. neoformans*-specific antibodies and titers around the median value were chosen. Additionally, serum samples from naïve mice (one per genotype) were included. As expected, detection of IgG1-reactive proteins after 1D sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of cryptococcal proteins and subsequent immunoblotting revealed several bands following incubation with sera from infected wild-type mice (Supplementary Fig. S1). No bands were visible after incubation of the membrane with sera from naïve wild-type mice, naïve IL-12-deficient mice and both, infected and naïve IL-4R $\alpha$ -deficient mice (Supplementary Fig. S1). IL-12p35-deficient mice produced strong IgG1 responses, indicated by an increased number of IgG1-reactive protein bands visible after incubation with sera (Supplementary Fig. S1).

Analysis of IgG2a-reactive proteins revealed one band after incubation of the membrane with sera from naïve mice of all genotypes, indicating nonspecific reactivity with cryptococcal proteins (Supplementary Fig. S1). As expected, incubation with sera from infected mice of all genotypes resulted in additional protein bands, demonstrating that IgG2a antibodies recognized several *C. neoformans* proteins.

To achieve sufficient resolution for identification of individual *C. neoformans* proteins, 2D gel electrophoresis and subsequent immunoblot experiments were performed. We decided to analyse sera from five wild-type, four IL-12-deficient and four IL-4R $\alpha$ -deficient mice. Only immunoreactive spots, which could be accurately mapped on the corresponding Coomassie-stained SDS gels were taken into account for further analysis. Representative immunoblots of individual mice, which display most but not all immunoreactive spots observed in a total of four to five mice, are shown in Figures 2 and 3. Nine IgG1-immunoreactive protein spots were detected using sera from infected wild-type mice (Fig. 2a, bold numbers). When using sera from infected IL-12p35-deficient mice, seven additional IgG1-immunoreactive protein spots were identified (Fig. 2c, bold numbers). Importantly, as already indicated by 1D gel analysis (Supplementary Fig. S1), no proteins were found to be IgG1-immunoreactive with sera from naïve wild-type and naïve IL-12p35-deficient mice (Fig. 2b,d). Most IgG1-immunoreactive spots could be identified in several individual mice of different genotypes (Fig. 5). Sera from IL-4R $\alpha$ -deficient mice were not tested for IgG1-reactive antigens, as no immunoreactive bands were detected after 1D analysis (Supplementary



**Figure 3.** IgG2a-immunoreactive proteins from *Cryptococcus neoformans* were detected with sera from representative infected and naïve wild-type, IL-4R $\alpha$ -deficient, and IL-12-deficient mice. Whole cell proteins of *C. neoformans* strain 1841 separated by 2D electrophoresis were transferred to nitrocellulose membranes, which were thereafter incubated with sera from infected and naïve wild-type and IL-4R $\alpha$ -deficient mice diluted 1:1,000. IgG2a-immunoreactive proteins were detected using sera from an infected (a) and naïve (b) wild-type mouse, an infected (c) and naïve (d) IL-4R $\alpha$ -deficient mouse, and an infected (e) and naïve (f) IL-12-deficient mouse. Protein abundance as shown in the Coomassie staining did not correlate with the strength of the immunoreactive signal (Fig. 4). Only the spots that could be mapped on Coomassie-stained gels were numbered in the blot images. Bold, underlined numbers mark IgG2a-reactive, *C. neoformans*-specific spots, while light, underlined numbers indicate IgG2a-reactive but *C. neoformans*-unspecific spots. Italic numbers mark spots reactive with both IgG1 and IgG2a antibodies. Images were cropped to improve clarity. Full-length blots without numbered protein spots are presented in Supplementary Fig. 3. Abbreviation: MM = molecular mass.

Fig. S1). Nine protein spots were determined to be exclusively IgG2a-immunoreactive (Fig. 3a,c,e), from which five were considered IgG2a-immunoreactive but not *C. neoformans*-specific, as they also reacted with sera from naïve wild-type, IL-4R $\alpha$ -deficient, and IL-12-deficient mice (Fig. 3b,d,f, light, underlined numbers). Three protein spots were identified as infection-specific when using serum from an infected wild-type mouse (Fig. 3a, bold, underlined numbers), which were not detectable using sera from IL-12-deficient mice (Fig. 3e). In contrast, incubation with serum from infected IL-4R $\alpha$ -deficient mice resulted in one additional spot (Fig. 3c). Notably, in contrast to IgG1-reactive proteins, infection-specific IgG2a-reactive spots only occurred in single animals without a high consistency (Fig. 5). To our surprise, only one protein spot (#18) was both IgG1- and IgG2a-immunoreactive in sera from infected wild-type mice. Few spots (#4–7, #17) were exclusively IgG1-immunoreactive in sera from infected wild-type mice (Fig. 2a,c, italic numbers), but showed reactivity with IgG2a when using serum from

Protein (MW; UniProt ID)	Number of isoforms (spot #)	Immunological characteristics previously reported <sup>a,b,c</sup>	Ref.
14-3-3 protein, putative (29.0 kDa; Q5K8Z6)	1 (#16)	<sup>a</sup> Recognized as an antigen in mice infected with <i>C. neoformans</i> H99 $\gamma$ and in <i>C. gattii</i> infection in humans and koalas. <sup>c</sup> Antibodies against this protein are induced in the course of the natural infection of schistosomiasis.	13,15,26,52
Elongation factor 1-beta (24.4 kDa; Q5KKD1)	1 (#15)	<sup>a</sup> Recognized as an antigen in patients with cryptococcosis caused by <i>C. gattii</i> and as a non-specific antigen in <i>C. gattii</i> infection in koalas.	25,26
Expressed protein Q5K7Y6 (14.9 kDa; Q5K7Y6)	1 (#35)	Not reported to date	
Glyceraldehyde-3-phosphate dehydrogenase (36,308 kDa; J9VRH1)	1 (#44)	<sup>a</sup> Recognized as an antigen in patients with cryptococcosis caused by <i>C. gattii</i> . <sup>b</sup> Immunoreactive protein identified in mice inoculated with <i>Candida albicans</i> and in sera from patients with paracoccidioidomycosis. <sup>b</sup> This protein appeared not to be a suitable target for the development of immunotherapeutic strategies against candidiasis, despite being an immunodominant component that induces antibody response against <i>C. albicans</i> . <sup>b</sup> The most abundant allergen from <i>Aspergillus fumigatus</i> in human sera. <sup>c</sup> Common immunogenic antigen among <i>Eimeria</i> species. This protein was evaluated in form of DNA vaccine, which induced effective protection against single and mixed infection of these species. <sup>c</sup> Antibodies against this protein are induced in infection of schistosomiasis. This protein is considered a target of protective immunity in humans against <i>Schistosoma mansoni</i> and <i>S. haematobium</i> .	13,36,53-57
Hsp71-like protein (69.6 kDa; J9VZ70)	1 (#19)	<sup>a</sup> 70-kD Hsp family from <i>C. neoformans</i> described as a major target molecule of the humoral response in mice. <sup>a</sup> Hsp70 recognized as an antigen in mice infected with <i>C. neoformans</i> H99 $\gamma$ and <i>C. gattii</i> . <sup>a</sup> Hsp70 identified as immunodominant protein in mice immunized with <i>C. gattii</i> cell wall and cytoplasmic protein preparations.	12-15,23,26,37,58
Hsp72-like protein (69.513; J9VU43)	1 (#20)	<sup>a</sup> 70-kD Hsp family from <i>C. neoformans</i> described as a major target molecule of the humoral immune response in mice.	57
Phosphopyruvate hydratase (Enolase) (47.7 kDa; Q5KLA7)	2 (#11, #12)	<sup>a</sup> Recognized as an antigen in mice infected with <i>C. neoformans</i> H99 $\gamma$ and in <i>C. gattii</i> infection in humans and koalas. <sup>a</sup> Immunodominant protein identified in mice immunized with <i>C. gattii</i> cell wall and cytoplasmic protein preparations. <sup>b</sup> Stimulates protective IgG2a in sera from vaccinated mice with systemic candidiasis. <sup>b</sup> A major antigen of fungal infection ( <i>A. fumigatus</i> and <i>C. albicans</i> )	13-15,17,26,37,59
Thioredoxin-dependent peroxide reductase (21.6 kDa; Q5KEB3)	1 (#47)	Not reported to date	13
Transaldolase (35.3 kDa; Q5K952)	4 (#30-32, #43, #46)	<sup>a</sup> Recognized as an antigen in mice infected with <i>C. neoformans</i> H99 $\gamma$ . <sup>a</sup> Immunodominant protein identified in mice immunized with <i>C. gattii</i> cell wall protein preparations. <sup>b</sup> Identified as an allergen of <i>Fusarium proliferatum</i> and <i>Cladosporium</i> and <i>Penicillium</i> species.	13,15,23,60
Uncharacterized protein J9W025 (14.8 kDa; J9W025)	2 (#36, #37)	Not reported to date.	

**Table 1.** IgG1-immunoreactive proteins from *Cryptococcus neoformans*. Ten cryptococcal immunogenic proteins were identified to react specifically with IgG1 antibodies from mice infected with *C. neoformans*. MW: Molecular weight; UniProt ID: Identification number in the UniProt database. <sup>a</sup>Described in proteomics studies of cryptococcosis, <sup>b</sup>other mycoses, and <sup>c</sup>other infections.

infected IL-4R $\alpha$ -deficient mice (Fig. 3c, (spots #4–7 not shown)). In conclusion, this suggests that the host genotype in some cases (i.e. under strong Th cell polarizing conditions) affects the regulation of the Th-dependent isotype, but, in the wild-type host, fungal antigens significantly determine the Th cell phenotype. Immunoreactivity of the different proteins spots, seen by the intensity on the membrane, did not correlate with the abundance of the proteins in the microorganism, as evidenced by Coomassie staining of the proteins in the gel (Fig. 4). This indicates that the given protein abundance does not influence the degree of immunoreactivity.

Mass spectra analysis of the 31 immunoreactive protein spots (Fig. 4) led to the identification of 17 unique cryptococcal proteins, as different isoforms of the same protein were identified in more than one spot. The frequency of these proteins in *C. neoformans*-infected mice of different genotypes is displayed in Fig. 5. Ten cryptococcal proteins were determined to be exclusively Th2-associated antigens as they reacted specifically with IgG1 antibodies (Table 1, Fig. 5), while only three proteins were exclusively immunoreactive with Th1-dependent IgG2a among the *C. neoformans*-specific proteins (Table 2, Fig. 5). From the five spots that were not *C. neoformans*-specific, but also exclusively IgG2a-reactive, one protein was identified (Table 2, Fig. 5). Identification of the six protein spots reactive with both, IgG1 and IgG2a antibodies (#4–7, #17, #18) revealed three immunoreactive proteins (Table 3, Fig. 5). Tables 1–3 give an overview of the immunological characteristics of these proteins or family of proteins as reported in previous studies of fungal infections including cryptococcosis. The proteins identified herein have various functions in cellular metabolism, growth as well as stress resistance and virulence. Eight out of the 17 identified proteins were previously identified in immunoproteomic studies on immunoreactive antigens of *C. neoformans* and *C. gattii*<sup>13–15,23</sup>. Taken together, using a proteomic approach we could

Protein (MW; UniProt ID)	Number of isoforms (spot #)	Immunological characteristics previously reported <sup>a,b</sup>	Ref.
<b><i>C. neoformans</i>-specific</b>			
Hsp60-like protein (61.4 kDa; J9VJ21)	1 (#40)	<sup>b</sup> Vaccination with recombinant Hsp60 protects mice against a lethal intravenous inoculum of <i>Histoplasma capsulatum</i> yeast cells. <sup>b</sup> IgG1 and IgG2a monoclonal antibodies significantly prolonged the survival of mice infected with <i>H. capsulatum</i> .	40,43
Phosphoglucosyltransferase (60.5 kDa; J9W313)	2 (#2, #21)	<sup>b</sup> Recognized as an antigen in <i>A. fumigatus</i> .	39
Pyruvate decarboxylase (67.6 kDa; J9VTH3)	1 (#3)	<sup>b</sup> Stimulates protective IgG2a in sera from vaccinated mice with systemic candidiasis. <sup>b</sup> Recognized as an antigen of <i>Aspergillus fumigatus</i> .	30,33
<b><i>C. neoformans</i>-unspecific</b>			
ATP synthase subunit beta (58.7 kDa; J9VPP7)	5 (#24–26, #33–34)	<sup>a</sup> Recognized as an antigen in mice infected with <i>C. neoformans</i> H99 $\gamma$ , an antigen in humans infected with <i>C. gattii</i> and as a non-specific antigen in <i>C. gattii</i> infection in koalas.	14,15,26

**Table 2.** IgG2a-immunoreactive proteins from *Cryptococcus neoformans*. Four cryptococcal immunogenic proteins were identified to react specifically with IgG2a antibodies. Three of them were determined as *C. neoformans*-specific and one was regarded as *C. neoformans*-unspecific, as it reacted strongly with sera from naive wild type, IL-12- and IL-4R $\alpha$ -deficient mice. MW: Molecular weight; UniProt ID: Identification number in the UniProt database infec.: infected. <sup>a</sup>Described in proteomics studies of cryptococcosis and <sup>b</sup>other mycoses.

identify ten IgG1- and three IgG2a-reactive *C. neoformans*-specific antigens using sera from infected wild-type and gene-deficient mice, which can be associated with either Th2- or Th1-mediated immune responses.

## Discussion

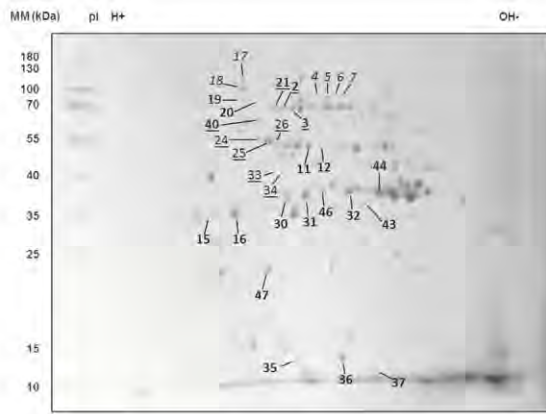
Cryptococcosis remains one of the prominent infectious diseases in both, industrialized and developing countries. Even though overall outcome of antifungal therapy is effective, the rates of morbidity, mortality and relapse episodes among cryptococcosis patients continue to be remarkably high<sup>7</sup>. The search for alternative treatments for this mycosis and the prevention of cryptococcal dissemination by immunotherapy or vaccination is therefore of significant importance. Previously, attempts have been made to establish a protective vaccine against cryptococcosis by using capsular polysaccharides for immunization of mice, which turned out to elicit immunological unresponsiveness<sup>24</sup>. This could be overcome by linking cryptococcal polysaccharides to carrier proteins (i.e. using conjugate vaccines). In contrast to carbohydrate antigens, immunoreactive protein antigens are capable of eliciting direct T cell-dependent responses<sup>25</sup>, which is critical for the control of cryptococcal infection. In contrast to previous studies identifying immunoreactive cryptococcal antigens<sup>13–15,26</sup>, the immunoproteomic approach utilized in the present study is the first of its kind for the discrimination of cryptococcal Th2- and Th1-associated antigens seen by their reactivity with antibodies of the murine isotypes IgG1 or IgG2a, respectively. We confirmed the previously observed capacity of the fungus to induce a biased Th2 response in BALB/c mice<sup>4,5</sup> demonstrated by the significantly increased levels of total IgG1 and IgE levels upon infection with *C. neoformans* and higher levels of IgG1 than IgG2a antibodies specific for cryptococcal antigens. We also identified a larger number of IgG1-reactive *C. neoformans*-specific antigens, which also occurred with enhanced consistency throughout different animals in comparison to IgG2a-reactive *C. neoformans*-specific antigens.

The induction of Th2-tilted immune responses by *C. neoformans* has been associated with cell wall and capsular components such as chitin and glucuronoxylomannan<sup>27–29</sup>. In addition to these carbohydrate factors, several proteins have been identified, such as (i) Pik1, Rub1 and Ena1, which deletion resulted in a decreased Th2-response upon infection<sup>30</sup>, (ii) laccase and urease, which promoted Th2 polarization<sup>31,32</sup>, or (iii) Ssa1, that was shown to promote macrophage M2 skewing during the afferent phase of the immune response against *C. neoformans*<sup>33</sup>. From these immunomodulatory proteins, the Hsp70 protein Ssa1 (annotated as Hsp71-like protein) was also identified in our study.

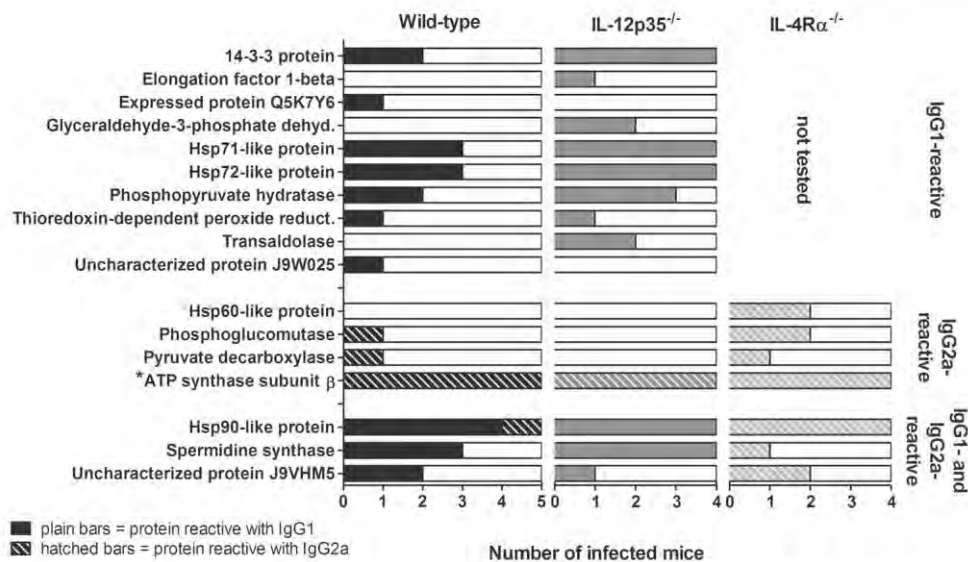
Interestingly, we were able to identify distinct Th1- and Th2-associated cryptococcal antigens throughout mice of different genotypes, which seemingly contrasts the immunological paradigm that the process of Th cell differentiation is mainly influenced by the surrounding cytokine milieu rather than the immunogenic antigen<sup>34</sup>. As the differentiation of T cells occurs after the interaction with antigen-presenting cells (APCs)<sup>35</sup>, distinct antigens may influence APCs to produce certain cytokines driving either Th1- or Th2-differentiation. It is conceivable that cellular vs. secreted cryptococcal proteins could target different APCs. However, presently it is not clear which of the identified proteins are either cell-associated or secreted. Other parameters influencing Th cell differentiation are the dose and binding strength of the antigen to the T-cell receptor resulting in different strength of T-cell receptor signalling and therefore distinct activation of downstream signals and transcription factors<sup>34,36</sup>. At this point it remains unclear how the identified Th1- and Th2-associated immunoreactive fungal antigens exert their influence on immune cells, but we hypothesize distinct direct interactions with APCs during the process of T cell differentiation. Future experiments therefore will include direct stimulation of APCs and T cells with the identified recombinantly expressed Th1- or Th2-associated *C. neoformans* antigens and furthermore the recombinant antigens will be used *in vivo* for immunization of mice.

IgG1-specific antigens are promising targets for specific immunotherapies addressed to restrain Th2-type responses, which are associated with exacerbation of disease, by skewing the Th cell differentiation towards a protective Th1 response. The IgG1-immunoreactive antigens identified in our study include proteins that are essential for growth and virulence of *C. neoformans* as they are involved in metabolism, oxidative stress,





**Figure 4.** Protein profile of *Cryptococcus neoformans* with indicated immunoreactive protein spots. Whole cell proteins of *C. neoformans* strain 1841 were separated by isoelectric point and molecular weight. After 2D gel electrophoresis, gels were stained with Coomassie Brilliant Blue G250. Numbered spots in the stained gel represent all antigenic proteins that were identified in this study. Bold non-underlined and bold underlined numbers indicate IgG1- and IgG2a-immunoreactive proteins, respectively. The spots in italic were reactive with both isotypes as shown in Figures 2 and 3. Light underlined numbers indicate IgG2a-immunoreactive proteins, which were not specific for *C. neoformans*. Abbreviation: MM = molecular mass.



**Figure 5.** Frequency of cryptococcal proteins, reactive with IgG1, IgG2a, or with IgG1 and IgG2a antibodies in *Cryptococcus neoformans*-infected mice of different genotypes. Immunoreactive cryptococcal proteins were identified using sera from infected mice of different genotypes. Proteins were grouped according to their reactivity with IgG1, IgG2a, or with IgG1 and IgG2a antibodies. The isotype which showed reactivity in the individual animal is indicated by plain bars (IgG1) or hatched bars (IgG2a). Three IgG2a-reactive proteins reacted exclusively with sera from infected mice, while one protein also showed reactivity with sera from naïve mice (marked with an asterisk (\*), see also Fig. 3). Sera from IL-4Rα-deficient mice were not tested by 2D analysis for IgG1-reactive proteins, as no immunoreactive protein bands for this isotype could be detected when investigating these sera in 1D analysis (Supplementary Fig. S1). Sera from infected animals were taken from at least three independent experiments in late infection state (at least 56 days post infection). Abbreviations: Glyceraldehyde-3-phosphate dehyd. = Glyceraldehyde-3-phosphate dehydrogenase; Thioredoxin-dependent peroxide reduct. = Thioredoxin-dependent peroxide reductase.

protein synthesis, and to maintain cell wall integrity (Table 1, Fig. 5). From those, six proteins, phosphopyruvate hydratase (enolase), elongation factor 1-β, 14-3-3 protein, Hsp71-like protein (Ssa1), transaldolase and glyceraldehyde-3-phosphate dehydrogenase, have been previously reported to be immunogenic in *C. neoformans* and its sibling species *C. gattii*<sup>13-15,23,26</sup>, although their association with Th phenotypes remained unclear in these studies. Nevertheless, this supports the immunodominant nature of these proteins and their role in inducing a Th-dependent antibody response, therefore rendering them excellent candidates for future experiments. Surprisingly, Hsp71-like protein (Ssa1) is among those proteins reactive only with Th2-dependent IgG1 antibodies, although Ssa1 has been reported to influence the immune response to *C. neoformans* during the afferent

Protein (MW; UniProt ID)	Number of isoforms (spot #)	Immunological characteristics previously reported <sup>a,b</sup>	Ref.
Hsp90-like protein (79.2 kDa; J9VVA4)	1 (#18)	<sup>a</sup> Hsp90 recognized as an antigen in mice infected with <i>C. neoformans</i> H99. <sup>b</sup> A major antigen of <i>A. fumigatus</i> .	13,43 <sup>a</sup>
Spermidine synthase, putative (82.4 kDa; Q5KEA8)	4 (#4–7)	Not reported to date.	
Uncharacterized protein J9VHM5 (73.0 kDa; J9VHM5)	1 (#17)	Not reported to date.	

**Table 3.** *Cryptococcus neoformans* proteins immunoreactive with both, IgG1 and IgG2a antibodies. Three cryptococcal immunogenic proteins were identified to react with both IgG1 and IgG2a antibodies from mice infected with *C. neoformans*. Immunological characteristics of the proteins or protein family compared to previous studies of fungal infections, including cryptococcosis, are given. MW: Molecular weight. UniProt ID: Identification number in the UniProt database. <sup>a</sup>Described in proteomics studies of cryptococcosis, and <sup>b</sup>other mycoses.

phase, but not during the efferent phase, eliciting no influence on adaptive immune response<sup>33</sup>. Additionally to antigens previously identified, this is the first time that Hsp72-like protein, a member of the highly immunogenic Hsp70 family<sup>37</sup>, thioredoxin-dependent peroxide reductase and two uncharacterized proteins (expressed protein Q5K7Y6, uncharacterized protein J9W025) are recognized as immunoreactive antigens in cryptococcal species. The identification of Th2-associated pathogenic proteins is of major therapeutic interest as a recent study could show that infection with a *C. neoformans* mutant strain lacking three chitin deacetylases and therefore chitosan, a component of the fungal cell wall and virulence factor, led to the development of a predominant Th1-type response and as a consequence to robust protective immunity if challenged with a *C. neoformans* wild-type strain<sup>38</sup>. Similarly, infection of mice with *C. neoformans* mutants characterized by a decreased Th2 bias after deletion of the respective genes, resulted not only in a prolonged survival of the animals but also in a predominant Th1-mediated immune response and decreased dissemination to the CNS, although in these cases prolonged immunity was not tested<sup>30,31</sup>. The proteins identified in our study may therefore serve as targets for the generation of *C. neoformans* knock-out mutants that could be used for similar vaccination-challenge experiments.

Three *C. neoformans*-specific antigens were found to be associated with a Th1 response as they reacted specifically with IgG2a antibodies (Table 2, Fig. 5). The protein phosphoglucomutase has been described so far only in *Aspergillus fumigatus* as an antigen expressed during invasive aspergillosis<sup>39</sup>. Vaccination with recombinant Hsp60-like protein has been associated with an improved course of disease in murine *Histoplasma capsulatum* infections<sup>40</sup>, underlining the potential protective influence of Hsp60-like protein also witnessed by its association with Th1-dependent IgG2a antibodies. Pyruvate decarboxylase is, to our knowledge, newly recognized as a fungal antigen. Further studies are necessary to test these IgG2a-reactive antigens in vaccination approaches for induction of a protective Th1 immune response. The identified IgG2a-reactive but *C. neoformans*-unspecific antigen ATP synthase subunit  $\beta$  could also be of particular interest for future studies, as this protein could be cross-reactive with different fungal species. Given the fact that this antigen was exclusively IgG2a-reactive and reactive with sera from all mice tested, indicating an immunodominant role, this protein could represent an excellent candidate for a protective vaccine against *C. neoformans* and potentially other fungal species. Previous studies demonstrated that immunization using protein fractions of *C. neoformans* and *C. gattii* prolongs the survival of mice against pulmonary cryptococcal infection<sup>14,23</sup>, but it has not been possible to elicit long lasting and effective protection. This suggests that a future vaccine should consist of fungal antigens selected for association with a protective Th1-response, rather than whole protein preparations.

Three proteins, Hsp90-like protein, spermidine synthase, and an uncharacterized protein (J9VHM5), were recognized by both IgG1 and IgG2a antibodies (Table 3, Fig. 5). Hsp90 has been identified as major immunogenic antigen not only in *C. neoformans*<sup>13,14</sup> but also in *A. fumigatus*<sup>39</sup>. Reactivity with both isotypes could depend on a high fungal or microbial immunogenicity as evidenced by the high number of individual mice recognizing these antigens. Our study is the first to compare cryptococcal antigens recognized by sera of individual mice in contrast to other studies, which used pooled sera for their investigations. We found that several immunoreactive proteins, especially IgG1- or IgG1- and IgG2a-reactive proteins were observed with a high consistency throughout sera of individual mice. A likely explanation for this observation is the uniform major histocompatibility complex (MHC) haplotype (H-2<sup>d</sup>) of BALB/c inbred mice used in this study. Other immunoproteomic studies also used BALB/c mice<sup>13,14</sup> for their experiments, which resulted in the identification of a number of identical antigens, underlining the importance of the MHC haplotype for antigen recognition by Th cells and development of antimicrobial antibodies. A recent immunization/challenge study proposed to combine multiple protein antigens in light of a critical role of MHC-II haplotype diversity for protection<sup>41</sup>.

We chose to identify immunoreactive cryptococcal antigens using sera from mice infected with *C. neoformans* for at least 56 days to mimic a prolonged interaction of the fungus with the immune system, as it occurs within the human population. We do not expect a different pattern of immunoreactive proteins in earlier stages of infection, as hallmarks of a Th2-polarisation, like IL-13 and IL-5 production as well as expression of GATA3 in Th cells are present in wild-type mice infected with *C. neoformans* already on day 21 post infection (dpi)<sup>42</sup>. Furthermore, there was no obvious influence of the susceptibility and fungal burden on the pattern of immunoreactive proteins identified, as we could observe several proteins recognized by sera from mice of all genotypes despite their underlying predominant immune response, different courses of disease and fungal burden in the lung.

Although most of the proteins identified in this study are associated with cytoplasmic functions, it is known that proteins like 14-3-3 protein, heat shock proteins, pyruvate decarboxylase, and phosphopyruvate hydratase (enolase) can be found in the cell wall of fungi<sup>43,44</sup>. The protein export mechanisms of these proteins may serve to

promote microbial interaction with the host to stimulate an immune response. As previously reported in other studies, no mannoproteins were found to be immunoreactive with either IgG1 or IgG2a antibodies<sup>14</sup>, indicating that the method used for protein extraction in this study may underrepresent these scarce proteins or other immunoreactive proteins<sup>15</sup>.

To conclude, our study resulted in the identification of a significant number of antigens that are associated with Th2-dependent IgG1 antibodies and potentially may serve for fungus-specific immunotherapy strategies. In addition, selected antigens reactive with Th1-dependent IgG2a can be used for protective immunization experiments. Besides, some of the identified Th1- or Th2-associated serological antigen-specific responses may have the potential to be used as diagnostic markers to monitor the prognosis or antifungal treatment response of patients with cryptococcosis. At the same time, the finding of distinct IgG1- and IgG2a-immunoreactive fungal proteins provides molecular candidates to study immunomodulatory mechanisms of fungal antigens during the process of Th cell differentiation.

## Materials and Methods

**Sera collection.** Serum samples, obtained after at least 56 dpi from wild-type and gene-deficient adult female BALB/c mice (H-2<sup>d</sup>) previously infected by nasal inhalation with a single inoculum of 500 colony forming units of *C. neoformans* strain 1841 (serotype D) yeasts<sup>21</sup>, were utilized through the study. Sera from infected immunocompetent wild-type mice, which have shown to develop a strong Th2 response with high levels of IgE<sup>5,22</sup>, were tested. In addition, sera from infected IL-12-deficient mice (IL-12p35<sup>-/-</sup> and IL-12p40<sup>-/-</sup>), which present a strong Th2 biased immune response upon pulmonary infection with *C. neoformans*<sup>21</sup> were included. Sera from infected IL-4R $\alpha$ -deficient mice (IL-4R $\alpha$ <sup>-/-</sup>), which show a reduced Th2-immune response in pulmonary cryptococcosis<sup>20,46</sup> were also tested to enlighten the cryptococcal specific isotype production in a Th1 driven environment. All BALB/c wild-type mice succumbed to intranasal infection starting at 70 dpi (median survival time 74 dpi, unpublished data). In contrast, death of IL-12p35<sup>-/-</sup> and IL-12p40<sup>-/-</sup> mice started at significantly earlier time points (median survival time 52 dpi, unpublished data), whereas all IL-4R $\alpha$ -deficient mice survived the pulmonary cryptococcal infection, but maintained detectable levels of cryptococcal cells in their lungs<sup>20</sup>. As negative controls sera from naïve mice of all three genotypes were used. Per group, 14 infected and 9–14 naïve mice from at least two different infection experiments were analysed. The mice were maintained under specific pathogen-free conditions, according to the guidelines authorized by the Animal Care and Usage Committee of the “Landesdirektion Sachsen” ([www.lids.sachsen.de](http://www.lids.sachsen.de), Chemnitz, Germany) with food and water *ad libitum*. All infection experiments were carried out in accordance with the guidelines of the Committee of the “Landesdirektion Sachsen” according to the approved protocols with numbers 24-9168.11-TVV 5/01 and 24-9168.11 TVV 15/05.

**Protein extraction.** *C. neoformans* strain 1841 was recovered from 10% fetal calf serum stocks stored at  $-80^{\circ}\text{C}$  and grown for 48 h in Sabouraud dextrose agar medium while shaking gently at  $30^{\circ}\text{C}$ . For ELISA, yeast cells were harvested by centrifugation and washed twice with 250 mM sucrose. After washing, yeast pellets were resuspended in lysis buffer containing 10 mM Tris/HCl pH 7.5 supplemented with 5 mM EDTA and 1x protease-inhibitor cocktail (Roche, Basel, Switzerland). Thereafter, the suspension was transferred into a lysis-tube containing a mix of 0.1 mm glass beads together with 1.4 mm ceramic beads (PEQLAB, Erlangen, Germany) and cells were lysed by homogenization in the Peqlab-homogenizer at  $4^{\circ}\text{C}$  (Precellys<sup>®</sup> 24). The suspension was centrifuged twice transferring every time only the supernatant. The protein concentration was estimated using the Bradford reagent (Carl Roth, Karlsruhe, Germany) and samples were stored at  $-30^{\circ}\text{C}$ .

For one- and two-dimensional (1D and 2D) gel electrophoresis, some modifications were done to the protein extraction methodology in order to increase and maintain the solubility of the proteins. Yeast cells were harvested as previously mentioned and after washing, in addition to the lysis buffer, the pellets were mixed with an equal volume of a solution containing 8% CHAPS and 100 mM DTT. This suspension was disposed into a mortar and cells were frozen with liquid nitrogen and homogenized with a pestle twice. The homogenates were centrifuged and the protein suspensions were recovered. Protein content was estimated using the Bradford reagent (Carl Roth, Karlsruhe, Germany). Finally, proteins were precipitated overnight at  $-20^{\circ}\text{C}$  with 100% TCA (final concentration of 10% w/v) and washed three times with cold acetone to remove impurities or interfering substances. Pellet samples were kept at  $-30^{\circ}\text{C}$  until further analyses.

**Immunoglobulin isotyping.** Total levels of Th2-dependent IgG1 and IgE and Th1-dependent IgG2a were determined in mice sera as previously described<sup>5</sup>. Briefly, 96 well round bottom plates were coated overnight at  $4^{\circ}\text{C}$  with goat anti-mouse-IgG1, -IgE or -IgG2a, respectively (SouthernBiotech, Birmingham, AL, USA) in carbonate buffer. The plates were washed once with phosphate buffered saline (PBS) containing 0.05% Tween-20 (PBST) and blocked with PBS containing 0.5% BSA and 0.1% gelatine for 1 h at room temperature. Mouse IgG1, IgE, and IgG2a (SouthernBiotech, Birmingham, AL, USA), were used as standards, respectively. Sera were diluted in blocking buffer containing 0.05% Tween-20 up to 1:25,000 for IgG1 and IgG2a and up to 1:90 for IgE. The plates were incubated with the serum samples for 1.5 h at room temperature and washed three times with PBST. Detection was done with goat antibodies labelled with horseradish peroxidase (HRP) and specific for mouse IgG1, IgE, and IgG2a, respectively (SouthernBiotech, Birmingham, AL, USA), diluted 1:4,000. After 2 h incubation, the plates were washed four times with PBST and developed with 3,3',5,5'-tetramethylbenzidine (KPL, Gaithersburg, MD, USA). Immediately after the wells with the higher concentration of the standard antibody reached an OD of 1.3 at 650/480 nm, developing of the plates was stopped by adding 1M H<sub>3</sub>PO<sub>4</sub>. A final reading of the plates was done at 450/630 nm and the concentration of each immunoglobulin isotype was calculated per serum sample<sup>17</sup>.

Additionally, titers of *C. neoformans*-specific IgG1 and IgG2a antibodies were determined for all serum samples as previously described<sup>47</sup>, with some modifications. ELISA plates were coated overnight with 0.5 µg of *C. neoformans* 1841 protein extract per well. Blocking was done with 5% skim milk dissolved in PBS (SM). Sera from infected mice were diluted in SM containing 0.05% Tween-20 (SMT) starting from 1:100 up to 1:409,600 for IgG1, due to expected higher titers, and starting from 1:50 up to 1:25,600 for IgG2a. Sera from naïve mice were diluted in SMT starting from 1:50 up to 1:25,600 for both isotypes. Detection was done with goat anti-mouse IgG1, human ads-HRP or goat anti-mouse IgG2a, human ads-HRP, respectively (SouthernBiotech, Birmingham, AL, USA). Development of the plates was done with 3,3',5,5'-tetramethylbenzidine (KPL, Gaithersburg, MD, USA) for 45 min at room temperature and stopped with H<sub>3</sub>PO<sub>4</sub> prior to OD determination at 450/630 nm<sup>47</sup>. The titer of *C. neoformans*-specific immunoglobulins was defined as the highest dilution at which the OD still showed a linear reduction. ELISA experiments to determine *C. neoformans*-specific IgE titers were not carried out, as previous studies of our group indicate that the expected concentration of *C. neoformans*-specific IgE is very low<sup>22</sup>. For all ELISA experiments, wells incubated without serum samples but with all other reagents were used as blanks. All experiments were done in technical duplicates.

**One-dimensional electrophoresis and immunoblot analysis.** In order to assess the reactivity of serum IgG1 and IgG2a antibodies against specific cryptococcal proteins, one-dimensional (1D) SDS-PAGE and western blot were performed, according to methods previously described<sup>48,49</sup>. Briefly, *C. neoformans* protein pellets were dissolved in PBS to a final concentration of 1 mg/ml, mixed with the same volume of 2x Lämmli buffer and heated for 5 min at 95 °C. In each well of a 12.5% acrylamide gel 10 µg of protein were applied. Proteins were separated in Tris-glycine-SDS running buffer using the Owl™ Dual-Gel Vertical Electrophoresis Systems P8DS equipment (ThermoFisher Scientific, Waltham, MA, USA). For immunological detection, the separated proteins were transferred onto a nitrocellulose membrane by electroblotting using the Mini Trans-Blot equipment (BioRad, Hercules, CA, USA). After blotting, membranes were blocked overnight at 4 °C with 5% skim milk dissolved in distilled water (blocking buffer). Subsequently, membranes were incubated for 3 h at room temperature with sera from infected and naïve mice, respectively, diluted 1:1,000 in blocking buffer containing 0.1% Tween-20. Membranes were washed with PBST and incubated 1 h at room temperature with 1:4,000 goat anti-mouse IgG1 or goat anti-mouse IgG2a antibodies coupled to HRP (SouthernBiotech, Birmingham, AL, USA) to detect specific IgG1 or IgG2a antibodies, respectively, which bind to one or more *C. neoformans* proteins. Development of the membranes was done with SuperSignal® West Pico Chemiluminescent Substrate (ThermoFisher Scientific, Waltham, MA, USA).

**Two-dimensional electrophoresis.** Sera with *C. neoformans* specific immunoglobulin levels near to the median values of all samples of a genotype, in order to guarantee representative results for all samples, were further analysed by 2D gel electrophoresis. Additionally, serum samples with high titers of specific antibodies for *C. neoformans* were also investigated by 2D gel electrophoresis to see if sera with high titers are reactive with an increased number of *C. neoformans* proteins. In total, five serum samples from infected wild-type mice and four sera from infected mice of each gene-deficient mouse strain were studied. From naïve mice, one serum sample per genotype was included.

Per gel, a pellet of 100 µg of *C. neoformans* proteins was resuspended in 125 µl of rehydration buffer (7M urea, 2M thiourea, 4% CHAPS, 50 mM DTT, 1% BioLyte® (BioRad, Hercules, CA, USA), 0.001% bromophenol blue) and applied onto an IPG strip (BlueStrips 3–10 NL/7 cm, SERVA, Heidelberg, Germany). Strips were rehydrated for 6 h at room temperature and proteins were focused overnight using the PROTEAN IEF cell (BioRad, Hercules, CA, USA) under the following conditions: active rehydration, 50 V for 6 h; Step 1, 150 V, rapid ramp for 1 h; Step 2, 300 V, rapid ramp for 1 h; Step 3, 1,000 V, linear ramp for 1 h; Step 4, 3,000 V, linear ramp for 2 h; Step 5, 3,000 V, rapid ramp for 2 h; and Step 6, 500 V for 12 h. Following isoelectric focusing, strips were soaked twice in equilibration buffer containing 6M urea, 2% SDS, 50 mM Tris/HCl pH 8.8 and 20% glycerol for 15 min. For the first equilibration step 2% DTT was added to the equilibration buffer and for a second equilibration step 2.5% iodoacetamide was added to the equilibration buffer. After equilibration, strips were soaked briefly in Tris-glycine-SDS running buffer and placed separately on a 12.5% acrylamide SDS gel. Proteins were separated in a second dimension in the Owl™ Dual-Gel Vertical Electrophoresis Systems P8DS equipment (ThermoFisher Scientific, Waltham, MA, USA). Proteins in the gels were stained with Coomassie Brilliant Blue G250 dissolved in 10% acetic acid and 50% methanol and subsequently destained with a solution containing only 10% acetic acid and 50% methanol followed by washing with water, or alternatively transferred onto a nitrocellulose membrane for further detection of immunoreactive proteins, as described above. Membranes were incubated with sera from infected mice diluted 1:1,000 in blocking buffer containing 0.1% Tween-20. Sera from naïve mice diluted 1:500 were also tested.

**Identification of proteins by mass spectrometry.** Spots of interest were mapped by overlaying Ponceau-stained nitrocellulose membranes, immunoblots and Coomassie-stained gels. Mapping was carried out with the software Delta2D (DECODON, Greifswald, Germany). The spots were excised manually from Coomassie-stained gels and digested *in situ* with trypsin. As described previously<sup>50</sup>, the resulting peptides were eluted out of the gel, concentrated by vacuum centrifugation, and analysed using a hybrid mass spectrometer (QExactive HF, ThermoFisher Scientific, Waltham, MA, USA) equipped with a chip-based electrospray device (TriVersa NanoMate, Advion) and coupled to a nano-ultra-performance liquid chromatography system (Dionex UltiMate 3000 RS, ThermoFisher Scientific, Waltham, MA, USA). A mass spectra (MS) database search was conducted using the MaxQuant software (version 1.4.1.2)<sup>51</sup> against a concentrated UniProt database, which contains all reviewed and unreviewed *C. neoformans* proteins (crytococcusneoformans.uniprot.fasta). For the search, the following parameters were included: trypsin digestion, up to two missed cleavages, fixed modifications:

carbamidomethylation as well as oxidation and the following variable modifications: first search peptide tolerance of 10 ppm, FTMS/MS/MS match tolerance of 10 ppm, a minimum of two peptides/protein, including at least one unique protein.

**Statistical analysis.** Mann-Whitney U test was performed to determine the significance of the differences in the total level of immunoglobulins between wild-type, IL-12-deficient and IL-4R $\alpha$ -deficient mice according with the ELISA results, as the data did not show a Gaussian distribution. Data are presented as individual points and medians. A nonparametric Spearman's correlation test was done to determine the strength and direction of association between total and specific levels of IgG1 and IgG2a. The degree of significance was annotated as following: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ . GraphPad PRISM v7 software was used for statistical analyses (GraphPad Software, La Jolla, CA, USA).

**Data availability statement.** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## References

1. Kwon-Chung, K. J. *et al.* Cryptococcus neoformans and Cryptococcus gattii, the etiologic agents of cryptococcosis. *Cold Spring Harb. Perspect. Med.* **4**, a019760 (2014).
2. Goldman, D. L. *et al.* Serologic evidence for Cryptococcus neoformans infection in early childhood. *Pediatrics*. **107**, E66 (2001).
3. Saha, D. C. *et al.* Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. *Clin. Vaccine Immunol.* **14**, 1550–1554 (2007).
4. Goldman, D. L., Davis, J., Bommarito, F., Shao, X. & Casadevall, A. Enhanced allergic inflammation and airway responsiveness in rats with chronic Cryptococcus neoformans infection. Potential role for fungal pulmonary infection in the pathogenesis of asthma. *J. Infect. Dis.* **193**, 1178–1186 (2006).
5. Müller, U. *et al.* IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages and allergic inflammation during pulmonary infection of mice with Cryptococcus neoformans. *J. Immunol.* **179**, 5367–5377 (2007).
6. Pappas, P. G. Cryptococcal infections in non-HIV-infected patients. *Trans. Am. Clin. Climatol. Assoc.* **124**, 61–79 (2013).
7. Rajasingham, R. *et al.* Global burden of disease of HIV-associated cryptococcal meningitis. An updated analysis. *Lancet Infect. Dis.* (2017).
8. Huffnagle, G. B., Yates, J. L. & Lipscomb, M. F. Immunity to a pulmonary Cryptococcus neoformans infection requires both CD4+ and CD8+ T cells. *J. Exp. Med.* **173**, 793–800 (1991).
9. Price, M. S. & Perfect, J. R. Host defenses against cryptococcosis. *Immunol. Invest.* **40**, 786–808 (2011).
10. Müller, U. *et al.* Lack of IL-4 receptor expression on T helper cells reduces T helper 2 cell polyfunctionality and confers resistance in allergic bronchopulmonary mycosis. *Mucosal Immunol.* **5**, 299–310 (2012).
11. Gullo, F. P. *et al.* Cryptococcosis: Epidemiology, fungal resistance, and new alternatives for treatment. *Eur. J. Clin. Microbiol. Infect. Dis.* **32**, 1377–1391 (2013).
12. Lewis, R. E. Current concepts in antifungal pharmacology. *Mayo Clin. Proc.* **86**, 805–817 (2011).
13. Young, M., Macias, S., Thomas, D. & Wormley, F. L. Jr. A proteomic-based approach for the identification of immunodominant Cryptococcus neoformans proteins. *Proteomics*. **9**, 2578–2588 (2009).
14. Chaturvedi, A. K., Weintraub, S. T., Lopez-Ribot, J. L. & Wormley, F. L. Jr. Identification and characterization of Cryptococcus neoformans protein fractions that induce protective immune responses. *Proteomics*. **13**, 3429–3441 (2013).
15. Jobbins, S. E. *et al.* Immunoproteomic approach to elucidating the pathogenesis of cryptococcosis caused by Cryptococcus gattii. *J. Proteome Res.* **9**, 3832–3841 (2010).
16. Snapper, C. M. & Paul, W. E. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science*. **236**, 944–947 (1987).
17. Germann, T. *et al.* Interleukin-12/T cell stimulating factor, a cytokine with multiple effects on T helper type 1 (Th1) but not on Th2 cells. *Eur. J. Immunol.* **23**, 1762–1770 (1993).
18. Stevens, T. L. *et al.* Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature*. **334**, 255–258 (1988).
19. Benndorf, D. *et al.* Identification of spore allergens from the indoor mould Aspergillus versicolor. *Allergy*. **63**, 454–460 (2008).
20. Müller, U. *et al.* A gene-dosage effect for interleukin-4 receptor alpha-chain expression has an impact on Th2-mediated allergic inflammation during bronchopulmonary mycosis. *J. Infect. Dis.* **198**, 1714–1721 (2008).
21. Decken, K. *et al.* Interleukin-12 is essential for a protective Th1 response in mice infected with Cryptococcus neoformans. *Infect. Immun.* **66**, 4994–5000 (1998).
22. Piehler, D. *et al.* Eosinophils contribute to IL-4 production and shape the T-helper cytokine profile and inflammatory response in pulmonary cryptococcosis. *Am. J. Pathol.* **179**, 733–744 (2011).
23. Chaturvedi, A. K. *et al.* Vaccine-mediated immune responses to experimental pulmonary Cryptococcus gattii infection in mice. *PLoS One*. **9**, e104316 (2014).
24. Kozel, T. R., Gulley, W. F. & Cazin, J. Jr. Immune response to Cryptococcus neoformans soluble polysaccharide: immunological unresponsiveness. *Infect. Immun.* **18**, 701–707 (1977).
25. Pirofski, L. A. Polysaccharides, mimotopes and vaccines for fungal and encapsulated pathogens. *Trends Microbiol.* **9**, 445–451 (2001).
26. Martins, L. M. S. *et al.* Immunoproteomics and immunoinformatics analysis of Cryptococcus gattii. Novel candidate antigens for diagnosis. *Future Microbiol.* **8**, 549–563 (2013).
27. Wiesner, D. L. *et al.* Chitin recognition via chitotriosidase promotes pathologic type-2 helper T cell responses to cryptococcal infection. *PLoS Pathog.* **11**, e1004701 (2015).
28. Almeida, G. M., Andrade, R. M. & Bento, C. A. M. The Capsular Polysaccharides of Cryptococcus neoformans Activate Normal CD4+ T Cells in a Dominant Th2 Pattern. *J. Immunol.* **167**, 5845–5851 (2001).
29. Shoham, S., Huang, C., Chen, J. M., Golenbock, D. T. & Levitz, S. M. Toll-like receptor 4 mediates intracellular signaling without TNF- $\alpha$  release in response to Cryptococcus neoformans polysaccharide capsule. *J. Immunol.* **166**, 4620–4626 (2001).
30. He, X. *et al.* Virulence factors identified by Cryptococcus neoformans mutant screen differentially modulate lung immune responses and brain dissemination. *Am. J. Pathol.* **181**, 1356–1366 (2012).
31. Qiu, Y. *et al.* Immune modulation mediated by cryptococcal laccase promotes pulmonary growth and brain dissemination of virulent Cryptococcus neoformans in mice. *PLoS One*. **7**, e47853 (2012).
32. Osterholzer, J. J. *et al.* Cryptococcal urease promotes the accumulation of immature dendritic cells and a non-protective T2 immune response within the lung. *Am. J. Pathol.* **174**, 932–943 (2009).

33. Eastman, A. J. *et al.* Cryptococcal heat shock protein 70 homolog Ssa1 contributes to pulmonary expansion of *Cryptococcus neoformans* during the afferent phase of the immune response by promoting macrophage M2 polarization. *J. Immunol.* **194**, 5999–6010 (2015).
34. Zhu, J., Yamane, H. & Paul, W. E. Differentiation of effector CD4 T cell populations (\*). *Annu. Rev. Immunol.* **28**, 445–489 (2010).
35. Chen, L. & Flies, D. B. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat. Rev. Immunol.* **13**, 227–242 (2013).
36. Jin, B., Sun, T., Yu, X.-H., Yang, Y.-X. & Yeo, A. E. T. The effects of TLR activation on T-cell development and differentiation. *Clin. Dev. Immunol.* **2012**, 836485 (2012).
37. Kakeya, H. *et al.* Heat shock protein 70 (hsp70) as a major target of the antibody response in patients with pulmonary cryptococcosis. *Clin. Exp. Immunol.* **115**, 485–490 (1999).
38. Upadhyay, R. *et al.* Induction of Protective Immunity to Cryptococcal Infection in Mice by a Heat-Killed, Chitosan-Deficient Strain of *Cryptococcus neoformans*. *MBio.* **7** (2016).
39. Denizkus, N. *et al.* Fungal antigens expressed during invasive aspergillosis. *Infect. Immun.* **73**, 4704–4713 (2005).
40. Gomez, F. J., Allendoerfer, R. & Deepe, G. S. Jr. Vaccination with recombinant heat shock protein 60 from *Histoplasma capsulatum* protects mice against pulmonary histoplasmosis. *Infect. Immun.* **63**, 2587–2595 (1995).
41. Specht, C. A. *et al.* Vaccination with Recombinant *Cryptococcus* Proteins in Glucan Particles Protects Mice against Cryptococcosis in a Manner Dependent upon Mouse Strain and Cryptococcal Species. *MBio.* **8** (2017).
42. Piehler, D. *et al.* The IL-33 receptor (ST2) regulates early IL-13 production in fungus-induced allergic airway inflammation. *Mucosal Immunol.* **9**, 937–949 (2016).
43. Guimaraes, A. J., Frases, S., Gomez, F. J., Zancoppe-Oliveira, R. M. & Nosanchuk, J. D. Monoclonal antibodies to heat shock protein 60 alter the pathogenesis of *Histoplasma capsulatum*. *Infect. Immun.* **77**, 1357–1367 (2009).
44. Nombela, C., Gil, C. & Chaffin, W. L. Non-conventional protein secretion in yeast. *Trends Microbiol.* **14**, 15–21 (2006).
45. Pietrella, D. *et al.* Role of mannoprotein in induction and regulation of immunity to *Cryptococcus neoformans*. *Infect. Immun.* **69**, 2808–2814 (2001).
46. Stenzel, W. *et al.* IL-4/IL-13-dependent alternative activation of macrophages but not microglial cells is associated with uncontrolled cerebral cryptococcosis. *Am. J. Pathol.* **174**, 486–496 (2009).
47. Grahner, A., Müller, U., von Buttlar, H., Treudler, R. & Alber, G. Analysis of asthma patients for cryptococcal seroreactivity in an urban German area. *Med. Mycol.* **53**, 576–586 (2015).
48. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227**, 680–685 (1970).
49. Burnette, W. N. “Western blotting”. Electrophoretic transfer of proteins from sodium dodecyl sulfate–polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* **112**, 195–203 (1981).
50. Tamm, J. M. *et al.* Identification of new potential allergens from Nile perch (*Lates niloticus*) and cod (*Gadus morhua*). *J. Invest. Allergol. Clin. Immunol.* **23**, 159–167 (2013).
51. Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* **26**, 1367–1372 (2008).
52. Schechtman, D., Tarrab-Hazdai, R. & Arnon, R. The 14-3-3 protein as a vaccine candidate against schistosomiasis. *Parasite Immunol.* **23**, 213–217 (2001).
53. da Fonseca, C. A. *et al.* Two-dimensional electrophoresis and characterization of antigens from *Paracoccidioides brasiliensis*. *Microbes Infect.* **3**, 535–542 (2001).
54. Gil, M. L., Dagan, S., Eren, R. & Gozalbo, D. Evaluation of the usefulness of anti-glyceraldehyde-3-phosphate dehydrogenase antibodies as a treatment for invasive candidiasis in a murine model. *Antonie Van Leeuwenhoek.* **89**, 345–350 (2006).
55. Pitarch, A. *et al.* Analysis of the serologic response to systemic *Candida albicans* infection in a murine model. *Proteomics.* **1**, 550–559 (2001).
56. Tian, L. *et al.* Protective Efficacy of Coccidial Common Antigen Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) against Challenge with Three *Eimeria* Species. *Front Microbiol.* **8**, 1245 (2017).
57. El Ridi, R., Shoemaker, C. B., Farouk, F., El Sherif, N. H. & Afifi, A. Human T- and B-cell responses to *Schistosoma mansoni* recombinant glyceraldehyde 3-phosphate dehydrogenase correlate with resistance to reinfection with *S. mansoni* or *Schistosoma haematobium* after chemotherapy. *Infect. Immun.* **69**, 237–244 (2001).
58. Kakeya, H. *et al.* A 77-kilodalton protein of *Cryptococcus neoformans*, a member of the heat shock protein 70 family, is a major antigen detected in the sera of mice with pulmonary cryptococcosis. *Infect. Immun.* **65**, 1653–1658 (1997).
59. Fernandez-Arenas, E., Molero, G., Nombela, C., Diez-Orejas, R. & Gil, C. Contribution of the antibodies response induced by a low virulent *Candida albicans* strain in protection against systemic candidiasis. *Proteomics.* **4**, 1204–1215 (2004).
60. Chou, H. *et al.* The transaldolase, a novel allergen of *Fusarium proliferatum*, demonstrates IgE cross-reactivity with its human analogue. *PLoS One.* **9**, e103488 (2014).
61. Chou, H. *et al.* Transaldolases are novel and immunoglobulin E cross-reacting fungal allergens. *Clin. Exp. Allergy.* **41**, 739–749 (2011).

## Acknowledgements

We thank Dr. Daniela Volke for her valuable advices for 2D gel electrophoresis and Maj Schuster for support in preparing samples for mass spectrometry. We are grateful to Prof. Alexander Dalpke for critical reading of the manuscript. We thank Prof. Uwe Völker and Dr. Leif Steil for support in the mapping of immunoreactive proteins. C.F. and A.E.G. were funded by non-profit organizations, i.e. C.F. was supported by a Georg Forster Research Fellowship from the Alexander von Humboldt Foundation, Germany, for Postdoctoral Researchers. A.E.G. was supported by a doctoral stipend from the Foundation of the German Economy (Stiftung der Deutschen Wirtschaft) and by funds from the Ph.D. program (“Doktorandenförderplatz”) of the University of Leipzig, Leipzig, Germany. We acknowledge support from the German Research Foundation (DFG) and Leipzig University within the program of Open Access Publishing.

## Author Contributions

C.F., B.S., M.v.B., and G.A. designed the study; C.F., A.E.G., K.S., B.S., and U.M. performed experiments; C.F., A.E.G., K.S., and U.M. analysed data; K.S., U.M., F.B., and M.v.B. provided key reagents; C.F., A.E.G., U.M., and G.A. wrote the paper; C.F. and A.E.G. prepared the figures; all authors reviewed the results and approved the final version of the manuscript.

## Additional Information

**Supplementary information** accompanies this paper at <https://doi.org/10.1038/s41598-018-21039-z>.

**Competing Interests:** The authors declare no competing interests.

**Publisher's note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2018

### 3 Second publication: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients



ORIGINAL RESEARCH  
published: 23 July 2021  
doi: 10.3389/fimmu.2021.709695



## Identification of Disease-Associated Cryptococcal Proteins Reactive With Serum IgG From Cryptococcal Meningitis Patients

A. Elisabeth Gressler<sup>1\*</sup>, Daniela Volke<sup>2</sup>, Carolina Firacative<sup>3</sup>, Christiane L. Schnabel<sup>1</sup>, Uwe Müller<sup>1</sup>, Andor Krizsan<sup>2</sup>, Bianca Schulze-Richter<sup>1</sup>, Matthias Brock<sup>4</sup>, Frank Brombacher<sup>5</sup>, Patricia Escandón<sup>6</sup>, Ralf Hoffmann<sup>2</sup> and Gottfried Alber<sup>1\*</sup>

**Keywords:** *Cryptococcus neoformans*, immunoproteomics, cryptococcal meningitis, humoral immunity, human samples, fungal infection.

#### Highlights:

- Human sera from Colombian HIV-positive and HIV-negative cryptococcal meningitis (CM) patients and healthy individuals were analyzed
- Total serum IgM concentrations were lower in sera from CM patients
- Anti-cryptococcal IgG antibody titers were higher in HIV-negative, but not HIV-positive CM patients compared to healthy individuals
- Production of anti-cryptococcal IgG, but not IgM antibodies was induced in response to cryptococcal infection of wild type and IL-4R $\alpha$ -deficient mice
- Immunoproteomic analysis of human sera revealed several IgG-reactive cryptococcal protein spots recognized with different intensity by sera from individual experimental groups
- Recombinant protein expression and quantification of immunoreactivity of individual proteins revealed disease-associated cryptococcal proteins, defined by significantly stronger immunoreactivity with sera from CM patients compared to healthy individuals





# Identification of Disease-Associated Cryptococcal Proteins Reactive With Serum IgG From Cryptococcal Meningitis Patients

A. Elisabeth Gressler<sup>1\*</sup>, Daniela Volke<sup>2</sup>, Carolina Firacative<sup>3</sup>, Christiane L. Schnabel<sup>1</sup>, Uwe Müller<sup>1</sup>, Andor Krizsan<sup>2</sup>, Bianca Schulze-Richter<sup>1</sup>, Matthias Brock<sup>4</sup>, Frank Brombacher<sup>5</sup>, Patricia Escandón<sup>6</sup>, Ralf Hoffmann<sup>2</sup> and Gottfried Alber<sup>1\*</sup>

<sup>1</sup> Institute of Immunology, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany, <sup>2</sup> Institute of Bioanalytical Chemistry, Leipzig University, Leipzig, Germany, <sup>3</sup> Studies in Translational Microbiology and Emerging Diseases (MICROS) Research Group, School of Medicine and Health Sciences, Universidad del Rosario, Bogota, Colombia, <sup>4</sup> Fungal Genetics and Biology Group, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom, <sup>5</sup> International Centre for Genetic Engineering and Biotechnology (ICGEB), Cape Town Component, Cape Town, South Africa, <sup>6</sup> Microbiology Group, Instituto Nacional de Salud, Bogota, Colombia

## OPEN ACCESS

### Edited by:

Heinrich Kerner,  
University of Tasmania, Australia

### Reviewed by:

Sunil Joshi,  
University of Miami, United States  
Xiaorong Lin,  
University of Georgia, United States

### \*Correspondence:

Gottfried Alber  
alber@rz.uni-leipzig.de  
A. Elisabeth Gressler  
elisabeth.gressler@uni-leipzig.de

### Specialty section:

This article was submitted to  
Microbial Immunology,  
a section of the journal  
Frontiers in Immunology

**Received:** 14 May 2021

**Accepted:** 21 June 2021

**Published:** 23 July 2021

### Citation:

Gressler AE, Volke D, Firacative C, Schnabel CL, Müller U, Krizsan A, Schulze-Richter B, Brock M, Brombacher F, Escandón P, Hoffmann R and Alber G (2021) Identification of Disease-Associated Cryptococcal Proteins Reactive With Serum IgG From Cryptococcal Meningitis Patients. *Front. Immunol.* 12:709695. doi: 10.3389/fimmu.2021.709695

*Cryptococcus neoformans*, an opportunistic fungal pathogen ubiquitously present in the environment, causes cryptococcal meningitis (CM) mainly in immunocompromised patients, such as AIDS patients. We aimed to identify disease-associated cryptococcal protein antigens targeted by the human humoral immune response. Therefore, we used sera from Colombian CM patients, with or without HIV infection, and from healthy individuals living in the same region. Serological analysis revealed increased titers of anti-cryptococcal IgG in HIV-negative CM patients, but not HIV-positive CM patients, compared to healthy controls. In contrast, titers of anti-cryptococcal IgM were not affected by CM. Furthermore, we detected pre-existing IgG and IgM antibodies even in sera from healthy individuals. The observed induction of anti-cryptococcal IgG but not IgM during CM was supported by analysis of sera from *C. neoformans*-infected mice. Stronger increase in IgG was found in wild type mice with high lung fungal burden compared to IL-4R $\alpha$ -deficient mice showing low lung fungal burden. To identify the proteins targeted by human anti-cryptococcal IgG antibodies, we applied a quantitative 2D immunoproteome approach identifying cryptococcal protein spots preferentially recognized by sera from CM patients or healthy individuals followed by mass spectrometry analysis. Twenty-three cryptococcal proteins were recombinantly expressed and confirmed to be immunoreactive with human sera. Fourteen of them were newly described as immunoreactive proteins. Twelve proteins were classified as disease-associated antigens, based on significantly stronger immunoreactivity with sera from CM patients compared to healthy individuals. The proteins identified in our screen significantly expand the pool of cryptococcal proteins with potential for (i) development of novel anti-cryptococcal agents based on implications in cryptococcal virulence or survival, or (ii) development of an anti-cryptococcal vaccine, as several candidates lack homology

to human proteins and are localized extracellularly. Furthermore, this study defines pre-existing anti-cryptococcal immunoreactivity in healthy individuals at a molecular level, identifying target antigens recognized by sera from healthy control persons.

**Keywords:** *Cryptococcus neoformans*, immunoproteomics, cryptococcal meningitis, humoral immunity, human samples, fungal infection

## INTRODUCTION

*Cryptococcus neoformans*, an encapsulated opportunistic fungal pathogen, is the main agent causing cryptococcosis, a fatal systemic disease (1). Cryptococcal infection occurs through inhalation of spores ubiquitously present in birds' droppings (2–4), house dust (5) and decaying wood (6). However, the most common clinical manifestation of cryptococcosis is not pulmonary, but disseminated disease manifesting mostly as cryptococcal meningitis (CM) (7).

The main risk factor for development of systemic cryptococcal disease is impaired cell-mediated immunity (8), typically occurring in HIV-positive patients with AIDS. These patients account for 80–95% of all cases (7, 8), but individuals receiving immunosuppressive drugs are also at risk (8). Cases of CM have also been reported in immunocompetent persons (9), and in persons with increased M2 polarization of brain macrophages (10). Additionally, several case reports describe CM or other forms of disseminated cryptococcal disease in persons with humoral immunity defects like immunoglobulin (Ig)G-deficiencies (11–14) or X-linked hyper-IgM syndrome, which is characterized by reduced IgG and IgA serum levels and normal or elevated serum IgM (15–18). CM has also been found in patients with reduced percentage of IgM-producing memory B cells (19), indicating the contribution of humoral immunity in anti-cryptococcal defense for control of *C. neoformans*.

Although both, absolute case number and mortality rates of CM, decreased in recent years due to facilitated diagnosis through serum cryptococcal antigen screening and treatment of underlying HIV infection with anti-retroviral therapy, CM remains a severe health issue especially in low-income and middle-income countries, causing an estimated number of 181,000 deaths per year in the world (20, 21). Additionally, access to antifungals is limited in most low-income countries and when available, antifungal agents cause severe side-effects (22). Therefore, the treatment of fungal disease using immunotherapeutic approaches or the design of anti-fungal vaccines is gaining increased attention (23–26).

Cell-mediated immunity seems critical for cryptococcal clearance (27, 28). Nevertheless, several studies indicate importance of humoral immunity for protection from infection (29, 30). In addition to defects in B cells and antibody-mediated immunity constituting a risk factor for cryptococcal disease in humans (11–17), mice lacking B-1a B cells (31, 32), or soluble IgM antibodies (33) showed significantly higher fungal burden and decreased survival upon cryptococcal challenge. Anti-cryptococcal antibodies produced by humans (34, 35) and

mice (36–39) were shown to act as opsonins and promote phagocytosis and killing of cryptococcal cells *in vitro*. Consequently, several studies using cryptococcal antigenic compounds, with or without additional adjuvants, for vaccination of mice or rats elicited an antibody-mediated response protective against subsequent cryptococcal challenge (24, 40–46). Therefore, targeting the antibody-mediated response appears as a promising approach for prevention and treatment of CM.

Various studies demonstrated the ubiquitous presence of antibodies in human sera directed against cryptococcal capsular polysaccharides (47–56), mannoproteins (57), and cryptococcal proteins (58–62), regardless of predisposing HIV infection or even previous history of cryptococcal disease. However, the *C. neoformans* proteins targeted by these human antibodies have not been identified so far. Previous studies focused on identification of proteins that are immunoreactive with sera from mice (45, 63, 64), and koalas (65), or immunoreactive proteins from *Cryptococcus gattii* targeted by murine (46) or human antibodies (66). Those studies identified proteins contained in immunoreactive cryptococcal protein spots, but did not confirm immunoreactivity of the identified proteins by subsequent recombinant expression.

Therefore, we aimed to identify immunoreactive *C. neoformans* proteins using human sera from Colombian CM patients with or without underlying HIV infection and sera from healthy individuals (controls) living in Colombia to guarantee similar environmental exposure to *C. neoformans*. We were able to define IgG as the predominant isotype mounted in a disease-specific manner against *C. neoformans*. Using a quantitative immunoproteomic approach based on 2-dimensional gel electrophoresis and subsequent recombinant expression, we identified disease-associated cryptococcal proteins, defined by significantly stronger reactivity with sera from CM patients compared to healthy individuals. Our study therefore critically expands the pool of immunoreactive cryptococcal protein antigens associated with CM. Some of these proteins are promising targets for (i) anti-cryptococcal chemotherapy or (ii) development of an anti-cryptococcal, or even pan-fungal, vaccine.

## MATERIAL AND METHODS

### Patients and Sera

Sera were obtained from HIV-positive ( $CD4^+$  T cells <250 cells/ $\mu$ L) and HIV-negative Colombian cryptococcal meningitis (CM) patients at the time of diagnosis. Diagnosis was secured by positive culture from cerebrospinal fluid for *C. neoformans* and

visualization of the encapsulated yeasts, stained with Indian ink, by direct microscopy. Detection of cryptococcal antigen (CrAg) in serum samples or cerebrospinal fluid by latex agglutination system (CALAS<sup>®</sup>) was carried out after diagnosis. The following clinical data was collected: age, sex, HIV status, CD4 count (for HIV-positive patients). Apart from the immunosuppression with HIV, other risk factors were also noted as underlying conditions. Sera from healthy Colombian individuals without cryptococcosis or any other mycosis were used as controls. Serum samples were collected in hospitals from different states of Colombia and sent to the Microbiology Group of the National Institute of Health, Bogota, Colombia, as part of the surveillance program for cryptococcosis. These samples belong to the sera collection of the Microbiology Group and were collected between 1990 and 2014. Informed consent was obtained from the patients prior to investigation. Human samples were used with approval from the Technical Committee of Research (CTIN) and the Ethical Committee for Research (CEIN) of the National Institute of Health, Bogota, Colombia, Memorandum No 3000-12829 of 2015. Sera from healthy controls were obtained with the approval of the ethical committee of Corporación para Investigaciones Biológicas (CIB) and Hospital La Maria IRB Number 7250 in Medellin, Colombia. An informed consent form was signed by all people enrolled in the study. All clinical information from the participants in the study was anonymized.

### Mouse Experiments

Wild type (WT) and IL-4R $\alpha$ -deficient (IL-4R $\alpha$ <sup>-/-</sup>) Balb/cJ (67) adult female mice were infected intranasally with 500 colony forming units (CFU) of *C. neoformans* strain 1841 (serotype D) yeasts. Cryptococcal cells were prepared before infection as previously described (68). For determination of fungal burden in the lung, mice were sacrificed at different time points, lungs were homogenized and plated on Sabouraud dextrose agar. Colonies were counted after two days of incubation at 30°C and number of CFU per lung were calculated. Serum samples were obtained at the endpoint of the experiment for measurement of anti-cryptococcal Ig titers. The mice were maintained under specific pathogen-free conditions, according to the guidelines authorized by the Animal Care and Usage Committee of the “Landesdirektion Sachsen” (www.lids.sachsen.de, Chemnitz, Germany) with food and water *ad libitum*. All infection experiments were carried out in accordance with the guidelines of the Animal Care and Use Committee of the “Landesdirektion Sachsen” according to the approved protocols with numbers 24-9168.11-15/05 and 24-9168 TVV 16/09.

### Flow Cytometry Measurement of *C. neoformans*-Specific Immunoglobulins

*C. neoformans* serotype A strain H99 cells were recovered from 10% skim milk stocks stored at -80°C and washed once with phosphate buffered saline (PBS). From cell suspension, 2x10<sup>5</sup> (human IgG analysis) or 5x10<sup>5</sup> (human IgM analysis, murine IgM and IgG analysis) cryptococcal cells were incubated with human serum samples diluted 1:10 in FACS buffer (3% FCS,

0.1% NaN<sub>3</sub> in PBS) for 30 min at 4°C. Cells were washed once with FACS buffer and once with PBS and incubated with Fixable Viability Dye eFluor<sup>™</sup> 780 (1:500 in PBS; ThermoFisher Scientific, Waltham, MA, USA) for 20 min at 4°C. Afterwards, cells were washed twice with FACS buffer and incubated for 30 min at 4°C with secondary antibodies from SouthernBiotech (Birmingham, AL, USA) labeled with FITC, diluted as indicated in FACS buffer: anti-human IgG-FITC (1:1,000; Cat. No. 2040-02), anti-human IgM-FITC (1:500; Cat. No. 2020-02), goat-anti mouse IgG-FITC, human adsorbed (1:500; Cat. No. 1030-02), or goat anti-mouse IgM-FITC (1:500; Cat. No. 1021-02). Thereafter, cells were washed three times with FACS buffer and 2% paraformaldehyde was added for fixation for 20 min at 4°C. Cells were washed once and resuspended in FACS buffer. Measurement of median fluorescent intensity of 10,000 events was performed using a BD LSRFortessa<sup>™</sup> (Becton Dickinson, Franklin Lakes, NJ, USA). Analysis was carried out using FlowJo v10 (BD Life Sciences, Ashland, OR, USA). Gating strategy is shown in **Supplementary Figure 1A**.

For verification of *C. neoformans* specificity of the signal, human sera were incubated with 2x10<sup>5</sup> (IgG)/5x10<sup>5</sup> (IgM) *C. neoformans* H99 or *Candida albicans* SC5314 cells for 30 min at 4°C prior to detection of anti-cryptococcal antibodies. Sera were separated from the cells by centrifugation and pre-absorbed sera were transferred to *C. neoformans* H99 cells, followed by incubation for another 30 min at 4°C as described above. The resulting post-absorption values were calculated as percent of the MFI signal without pre-absorption to determine “quenchable sera”.

### Isolation of Cryptococcal Proteins

*C. neoformans* H99 (serotype A) and 1841 (serotype D) cells were recovered from 10% skim milk stocks stored at -80°C and grown independently for 48 h in Sabouraud dextrose broth while shaking (80 rpm) at 30°C. Cells were harvested by centrifugation and washed twice with 250 mM sucrose. The pellet was resuspended in lysis buffer [5 mM Tris/HCl pH 7.5, 2.5 mM EDTA, 0.5X protease inhibitor cocktail (Roche, Basel, Switzerland)] additionally containing 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS, Cat. No. 1479, Carl Roth, Karlsruhe, Germany) and 50 mM dithiothreitol (DTT, Cat. No. 6908, Carl Roth, Germany). The cell suspension was transferred into a mortar, frozen with liquid nitrogen and homogenized with a pestle twice. Afterwards, homogenates were centrifuged and supernatant was recovered. Protein content was estimated using Bradford reagent (Carl Roth, Karlsruhe, Germany). Proteins were precipitated with 10% trichloroacetic acid over night at -20°C and centrifuged. After removal of the supernatant, the pellet was washed three times with ice-cold acetone and air-dried. The protein pellet was dissolved in a solution containing 7 M urea, 2 M thiourea, and 4% CHAPS and protein content was estimated using Bradford reagent (Carl Roth, Germany).

### Isolation of Cryptococcal Capsular Polysaccharide

*C. neoformans* H99 and 1841 cells were recovered from 10% skim milk stocks stored at -80°C and grown independently for

four days in Sabouraud dextrose broth while shaking (80 rpm) at 30°C. Cell supernatant was harvested by centrifugation and sodium acetate was added slowly up to a concentration of 10% w/v while stirring. Polysaccharide precipitation was performed by addition of 2.5 volumes of 99.5% ethanol and incubation for three days at room temperature (RT). After removal of the supernatant by centrifugation, polysaccharides were air dried and dissolved in deionized H<sub>2</sub>O. Polysaccharide concentration was measured by the method of Dubois (69).

### ELISA Analysis of Human and Murine Samples

Concentration of IgG and IgM antibodies in human and murine sera were determined by ELISA analysis. Briefly, 96 well round bottom plates were coated overnight at 4°C with the following antibodies from SouthernBiotech (AL, USA) diluted in carbonate buffer: goat anti-human IgG, mouse adsorbed (0.5 µg/mL, Cat. No. 2044-01), goat anti-human IgM (1 µg/mL, Cat. No. 2020-01), goat anti-mouse IgG, human adsorbed (2.5 µg/mL, Cat. No. 1030-01), goat anti-mouse IgM (2.5 µg/mL, Cat. No. 1021-01). The plates were washed once with PBS containing 0.05% Tween-20 (PBS-T) and blocked with PBS containing 0.5% BSA and 0.1% gelatin (RT, 1.5 h). The following Igs from SouthernBiotech (AL, USA) were used as standards: Human IgG (starting dilution 0.25 µg/mL, Cat. No. 0150-01), human IgM (starting dilution 1 µg/mL, Cat. No. 0158L-01), mouse IgG (starting dilution 0.5 µg/mL, Cat. No. 0107-01) and mouse IgM (starting dilution 1 µg/mL, Cat. No. 0101-01). Sera were diluted in blocking buffer containing 0.05% Tween-20 and incubated for 2 h at RT, followed by washing with PBS-T. Afterwards, plates were incubated with HRP-labelled secondary antibodies from SouthernBiotech (AL, USA) specific for human IgG (Cat. No. 2040-05), human IgM (Cat. No. 2020-05), mouse IgG (Cat. No. 1030-05) or mouse IgM (Cat. No. 1021-05) diluted 1:5000, or 1:4000 for quantification of human Igs or murine Igs, respectively. After 2 h incubation, the plates were washed four times with PBST and developed with 3,3',5,5'-tetramethylbenzidine (Kirkegaard & Perry Lab Inc (KPL), Gaithersburg, MD, USA). Colorimetric reaction was stopped when OD<sub>650/480</sub> of the first standard dilution reached a value of 1.3 with 1 M H<sub>3</sub>PO<sub>4</sub> and OD<sub>450/630</sub> was measured using the SpectraMAX 340PC Photometer and SoftMaxPro (v5.0) software (Molecular Devices, San José, CA; USA) was used for calculation of immunoglobulin concentration.

For determination of *C. neoformans*-specific antibody titers, 96 well plates were coated over night at 4°C with proteins or polysaccharides (10 µg/mL in carbonate buffer), respectively. Plates were washed once with PBS-T and blocked with 5% skim milk dissolved in PBS for one hour at RT. Serum samples were diluted 1:400 up to 1:409,600 (IgG) or 1:100 up to 1:102,400 (IgM) in serum diluent (5% skim milk dissolved in PBS containing 0.1% Tween-20) for human sera samples or 1:100 up to 1:102,400 (IgG and IgM) for analysis of mouse sera and incubated for two hours at RT. Next, plates were washed five times with PBS-T and incubated with secondary antibody from SouthernBiotech (Birmingham, AL, USA), diluted 1:5,000 in serum diluent (goat anti-human IgG-HRP (Cat. No. 2040-05); goat anti-human IgM-HRP (Cat. No. 2020-05); goat anti-mouse

IgG-HRP (Cat. No. 1030-05), goat anti-mouse IgM-HRP, (Cat. No. 1021-05) for 2 hours at RT. Plates were washed five times with PBS-T and developed using 3,3',5,5'-tetramethylbenzidine (KPL, MD, USA). Reaction was stopped by adding 1 M H<sub>3</sub>PO<sub>4</sub> after 15 min (IgG, human samples), 20 min (IgM, human samples) or 30 min (IgG and IgM, murine samples) for detection. OD<sub>450/630</sub> was measured using SpectraMAX 340PC Photometer and SoftMaxPro (v5.0) software (Molecular Devices, CA; USA). The titer of *C. neoformans*-reactive antibodies was defined as the highest dilution with an OD>0.1 after subtraction of serum background signal determined in wells without protein coating.

### Two-Dimensional (2D) Gel Electrophoresis and Immunoproteome Analysis

Protein solution containing *C. neoformans* H99 proteins purified by TCA precipitation was supplemented with DTT (50 mM), 1% BioLyte<sup>®</sup> (BioRad, Hercules, CA, USA) and 0.001% bromophenol blue. SERVA IPG BlueStrips (3-10 NL, SERVA, Heidelberg, Germany) were rehydrated at RT with 100 µg of protein for six hours. Proteins were focused overnight using the PROTEAN IEF cell (BioRad, CA, USA) under the following conditions: active rehydration, 50 V for 6 h; Step 1, 150 V, rapid ramp for 1 h; Step 2, 300 V, rapid ramp for 1 h; Step 3, 1,000 V, linear ramp for 1 h; Step 4, 3,000 V, linear ramp for 2 h; Step 5, 3,000 V, rapid ramp for 2 h; and Step 6, 500 V for 12 h. Following isoelectric focusing, strips were soaked two times in equilibration solution (6 M urea, 2% sodium dodecyl sulfate (SDS), 50 mM Tris/HCl pH 8.8, 20% glycerol) and 1.) 2% DTT (Cat. No. 6908, Carl Roth, Germany) or 2.) 5% iodoacetamide (Cat. No. I6125, Sigma-Aldrich, St. Louis, MO, USA) for 15 min each. After equilibration, proteins were separated on acrylamide SDS gels containing 0.5% 2,2,2-trichloroethanol (TCE, Sigma-Aldrich, MO, USA) using the Owl<sup>™</sup> Dual-Gel Vertical Electrophoresis Systems P8DS equipment (ThermoFisher Scientific, MA, USA). Proteins in the gels were stained with Coomassie Brilliant Blue G250 for cutting and digestion of protein spots. For detection of immunoreactive proteins fluorescent TCE staining was UV-activated for 1 min (ChemiDoc MP, BioRad, CA, USA) for protein visualization and proteins were transferred from the gel onto a nitrocellulose membrane by electroblotting in tank blots using the Mini Trans-Blot equipment (BioRad, CA, USA). After blotting, membranes were blocked with 1x BlueBlock PF blocking buffer for 1.5 h (Serva, Germany). Membranes were incubated with pooled human serum (4°C, overnight), diluted 1:1,000 in 1x BlueBlock PF. Membranes were washed three times with Tris-buffered saline (TBS), containing 0.05% Tween 20 (TBS-T) and incubated (RT, 1 h) with goat anti-human IgG-AlexaFluor<sup>®</sup> 647 (SouthernBiotech Cat. No. 2040-31, AL, USA) diluted 1:2,500 in BlueBlock PF, followed by three washing steps with TBS-T. Total cryptococcal proteins stained with TCE were imaged by fluorescence in the stain free channel. Immunoreactive proteins were detected subsequently in the Cy5 channel using the ChemiDoc MP device (BioRad, CA, USA). Delta2D 4.8 software (DECODON, Greifswald, Germany) was used for quantification and analysis of

immunoreactive protein spots, as well as mapping of the immunoreactive spots on the corresponding Coomassie-stained gel for mass spectrometry analysis. Analysis in Delta2D was carried out as follows: Spot boundaries were detected on a fused image, created from the protein spot patterns of all blots. Spots, detected at similar intensities among different experiments were considered in further analysis. Spot boundaries were transferred onto immunoblot signal images for quantification. Statistical analysis of immunoblot signals of serum sub-pools from HIV-positive CM patients, HIV-negative CM patients and healthy control patients was carried out using the T-test implemented in Delta2D relying on using the following parameters: Test design: between-subjects, used Welch approximation, alpha (overall threshold p-value): 0.01, p-values based on permutation, all permutations used: true, number of permutations per spot: 924, significance determined by standard Bonferroni correction, HCL: complete linkage, Euclidean Distance.

### In-Gel Digest and nRPC-ESI-MS/MS-TWIMS

Gel spots from the 2D gels and bands of the expressed proteins were excised with the ExQuest™ Spot Cutter (Bio-Rad Laboratories, Hercules, California, USA) and transferred into 0.5 mL reaction tubes. Gel pieces were washed three times (5 min, 100 µL 30% (v/v) acetonitrile in 50 mmol/L ammonium bicarbonate), dehydrated with acetonitrile (5 min, 100 µL), rehydrated with a mixture of 2 µL trypsin solution (Serva, Germany, 50 ng/µL in 3 mmol/L aqueous ammonium bicarbonate) and 18 µL of 3 mmol/L aqueous ammonium bicarbonate. After incubation (37°C, 4 h), supernatants were transferred to new 0.5 mL reaction tubes. Remaining gel pieces were washed once with 60% (v/v) aqueous acetonitrile containing 0.1% (v/v) formic acid and acetonitrile (20 µL per tube, RT, 5 min). Supernatants were transferred to the corresponding reaction tube and dried (60°C, 1 h) in a vacuum concentrator 5301 (Eppendorf Vertrieb Deutschland GmbH, Hamburg, Germany). The dried digests were dissolved in a mixture of 1.5 µL of acetonitrile containing 0.1% (v/v) formic acid (eluent B) and 48.5 µL of 0.1% aqueous formic acid (eluent A) and separated on a nanoACQUITY Ultra Performance LC™ (Waters Corp., Manchester, UK) system coupled online to a Q-TOF SYNAPT G2-Si instrument (Waters Corp., UK). Peptides were trapped on a nanoACQUITY Symmetry C18-column, internal diameter (ID) 180 µm, length 2 cm, particle diameter 5 µm, flow rate of 5 µL/min (3% eluent B, 6 min) on a C18-BEH 130 column (ID 75 µm, length 10 cm, particle diameter 1.7 µm; 35°C) at a flow rate of 0.3 µL/min using linear gradient from 3% to 40% eluent B in 18.5 min. The nanoESI source was equipped with a PicoTip Emitter (New Objective, Littleton, US) at a spray voltage of 3 kV, sampling cone was 30 V, source offset 80 V, source temperature 100°C, cone gas flow 20 L/h, and nanoflow gas pressure 0.2 bar. Mass spectra were recorded in positive ion mode using a high-definition data-dependent acquisition approach (HD-DDA) for top 6 ions as described before (70). LC-MS/MS raw files were processed with the Mascot search engine (Version 2.7; Matrix Science Ltd., Waters, UK) using the

following parameters Swissprot protein database (loaded 4<sup>th</sup> November 2019), NCBI Cryptococcus (loaded on 21<sup>th</sup> June 2018; 335 811 sequences), enzyme trypsin, 2 miss cleavage sides, as fixed modification cysteine carbamidomethylation (+57.022 Da), as variable modification methionine oxidation (+15.9949 Da), 20 ppm peptide tolerance and 0.08 Da fragment tolerance. Error tolerant was selected for MS/MS search. Proteins identified by at least three peptides and a protein score  $\geq 50$  were considered as confident.

### RNA Isolation and cDNA Synthesis

*C. neoformans* H99 (serotype A) and JEC21 (serotype D) cells were cultured in Sabouraud dextrose broth for 16 h at 30°C shaking at 80 rpm. Cells were washed once with PBS and transferred into a mortar and cells were frozen with liquid nitrogen and homogenized with a pestle twice. For the second freezing step, 500 µL NucleoZOL reagent (Macherey-Nagel, Düren, Germany) was added per  $1 \times 10^7$  cells. RNA extraction was performed according to the manufacturer's protocol, except precipitation of RNA with isopropanol was performed for 2 h at -20°C. RNA was re-suspended in RNase-free water and concentration was determined using NanoDrop (ThermoScientific, MA, USA). cDNA synthesis was performed with High-Capacity cDNA Reverse Transcription Kit (Cat. No. 4368814, ThermoFisher, MA, USA) according to the manufacturer's protocol, except only Oligo-dT primers were used.

### Recombinant Protein Expression

Genes of interest were amplified from *C. neoformans* H99 cDNA by polymerase chain reaction (PCR) using Phusion™ High-Fidelity DNA Polymerase (Cat. No. F530S, ThermoFisher, MA, USA). Primers are listed in **Supplementary Table 1**. PCR products and the target vector pET28a+ were purified using NucleoSpin® PCR and gel clean-up kit (Cat. No. 740609, Macherey-Nagel, Germany) or the QIAprep® Spin Miniprep Kit (Cat. No. 27106, Qiagen, Venlo, Netherlands), respectively. PCR products and the vector were digested for 16 h at 37°C in CutSmart buffer (New England Biolabs (NEB), Ipswich, MA, USA) with the respective restriction enzymes. The following New England Biolabs (MA, USA) enzymes were used: *NdeI* (R0111S), *NotI* (R3189S), *BamHI*-HF (R3136S), and *HindIII*-HF (R3104S). Digested vector was additionally dephosphorylated using Quick CIP (M0525S, NEB; MA, USA) according to the manufacturers protocol. Digested products were again purified with NucleoSpin® PCR and gel clean-up kit (Macherey-Nagel, Germany). Ligation was performed using the Quick Ligation™ Kit (Cat. No. M2200, NEB, MA, USA) according to the manufacturer's instructions. Chemically competent *Escherichia coli* (*E. coli*) DH5 $\alpha$  cells were transformed with insert-containing plasmids by the addition of plasmid DNA into the cell suspension followed by 30 min incubation on ice. Next, cells were heat-shocked for 1 min at 42°C and again incubated for 2 min on ice. Cells were cultivated for 1 h at 37°C shaking at 200 rpm in Lysogeny broth (LB) medium (10 g/L NaCl, 5 g/L yeast extract, 10 g/L pepton/L) and plated on LB-medium agar plates,

containing 30 µg/mL kanamycin. Colonies were checked for inserts in a PCR reaction using DreamTaq DNA Polymerase (Cat. No. EP0702, ThermoFisher, MA, USA). Plasmids of *E. coli* DH5α colonies positive for insertion of the respective gene were isolated with QIAprep® Spin Miniprep Kit (Cat. No. 27106, Qiagen, Netherlands) and used for transformation of *E. coli* strain Rosetta pLys using the transformation protocol mentioned above, except transformed cells were cultivated on LB agar containing 30 µg/mL kanamycin and 34 µg/mL chloramphenicol. For expression of recombinant proteins, a pre-culture of *E. coli* strain Rosetta pLys cells containing *C. neoformans* genes was cultivated o.n. in LB medium containing kanamycin and chloramphenicol at 37°C, 200 rpm. 1 mL of the culture was added into fresh LB medium (with kanamycin and chloramphenicol) and grown until OD<sub>600</sub> reached a value of 0.5. IPTG was added to achieve a concentration of 1mM IPTG in the culture and cells were further cultivated for 2 h at 37°C, 200 rpm. For one protein, CP\_02943, a concentration of 0.5 mM IPTG was used and cells were cultivated for 4 h at 30°C for induction of protein expression. Concentration of *E. coli* cells was determined by counting before addition of IPTG and after induction of protein expression and 1x10<sup>9</sup> cells were harvested, centrifuged, resuspended in Lämmli buffer (0.125 M Tris-HCl pH 6.75, 20% glycerol, 2.5% SDS, 10% 2-β-mercaptoethanol, 0.05% bromophenol blue) and boiled at 95°C for 15 min.

### Recombinant Production and Purification of Hsp71-Like Protein and Phosphoglucosyltransferase From *C. neoformans* JEC21

The genes coding for the Hsp71-like protein and the phosphoglucosyltransferase from *C. neoformans* serotype D strain JEC21 were amplified from cDNA using Phusion polymerase (Thermo). The primer used for amplification are listed in **Supplementary Table 1**. DNA fragments were introduced into expression plasmids by *in vitro* recombination using the InFusion HD cloning kit (Cat. No. 638920, Clontech/Takara, Saint-Germain-en-Laye, France) whereby the gene of the Hsp71-like protein was cloned into an *Nco*I restricted SM-X-URA plasmid (71) containing a sequence coding for an N-terminal Strep-tag and the phosphoglucosyltransferase gene into a *Bam*HI/*Not*I restricted modified pET43.1H6 plasmid introducing an N-terminal His-tag (72). All plasmids were initially amplified in *E. coli* DH5-α. For expression and purification of the phosphoglucosyltransferase the pET-plasmid was transferred into *E. coli* BL21(DE3) Rosetta2 cells and expression was performed in Overnight Express Instant TB Medium (Cat. No. 71491, Novagen, Sigma-Aldrich, MO, USA). Cells were disrupted by sonication in 50 mM Tris/HCl buffer pH 8.0 with 150 mM NaCl (buffer A) and the cell-free extract was applied to a Ni-NTA Agarose gravity-flow column (2 ml bed volume, Qiagen). After a stringency wash with 20 mM imidazole in buffer A, the protein was eluted in the presence of 200 mM imidazole in buffer A and concentrated and desalted against buffer A using Amicon-Ultra centrifugal filter units with a 30 kDa cut-off (Merck, Darmstadt, Germany). The SM-X plasmid containing the Hsp71-like coding

gene was used for transformation of an *Aspergillus niger* negative ATNT16 expression platform strain (71). Gene expression was induced by growth of transformants in *Aspergillus* minimal medium in the presence of 15 µg/ml doxycycline (73). Mycelium was ground to a fine powder under liquid nitrogen and resuspended in buffer A. The protein was purified to homogeneity from cell-free extracts *via* Strep-tag purification using a Strep-tactin Sepharose gravity-flow column (2 ml bed volume) as described in the manufacturer's protocol (IBA Lifesciences). Homogeneity of purified proteins was analysed by SDS-PAGE on a NuPAGE 4-12% Bis-Tris gels in a MES-buffered running system (ThermoFisher, MA, USA). Purified proteins were shock-frozen in liquid nitrogen and lyophilised for storage upon use.

### Quantification of Immunogenicity of Recombinant Proteins

Crude protein extracts from *E. coli* containing recombinant *C. neoformans* proteins were separated using SDS-PAGE [Owl™ Dual-Gel Vertical Electrophoresis Systems P8DS equipment (ThermoFisher Scientific, MA, USA)]. Gels contained 0.5% 2,2,2-trichloroethanol (Sigma-Aldrich, MO, USA) for staining of proteins. For protein visualization TCE-staining was UV-activated for 1 min (ChemiDoc MP, BioRad, CA, USA). Proteins were transferred from the gel onto a nitrocellulose membrane by electroblotting in tank blots using the Mini Trans-Blot equipment (BioRad, CA, USA). After blotting, membranes were blocked with 1x BlueBlock PF blocking buffer for 1.5 h (Serva, Germany) and incubated with pooled human serum (4°C, overnight), diluted 1:1,000 in 1x BlueBlock PF. Membranes were washed three times with Tris-buffered saline (TBS), containing 0.05% Tween 20 (TBS-T) and incubated (RT, 1 h) with goat anti-human IgG-AlexaFluor® 647 (SouthernBiotech Cat. No. 2040-31, AL, USA) diluted 1:2,500 in BlueBlock PF, followed by three washing steps with TBS-T. Total *E. coli* proteins stained with TCE were imaged by fluorescence in the stain free channel. Immunoreactive proteins were detected subsequently in the Cy5 channel using the ChemiDoc MP device (BioRad, CA, USA). Signal intensities recorded for *E. coli* proteins (fluorescence, stain free channel) and immunoreactive signals (fluorescence, Cy5 channel) were quantified with the Image Lab 6.0.1 software (BioRad) using the "Volume tool". The background signal, defined as signal intensity in the *E. coli* protein sample before induction of protein expression, was subtracted from the signal in the sample after induction of protein expression with IPTG. Finally, ratios of the immunoreactive signal (fluorescence, Cy5 channel) divided by the protein signal on the membrane (fluorescence, stain free channel) were calculated. Three different exposure times were analyzed per experiment. For further visualization of loading pattern and immunoreactivity quantification see **Figure 4A** and **Supplementary Figure 5**.

### Statistical Analysis

Mann-Whitney U test was used for statistical analysis of data from flow cytometry, ELISA, and quantification of

immunoreactivity of recombinant cryptococcal proteins, as the data did not show a Gaussian distribution (tested by Kolmogorov–Smirnov test, D’Agostino and Pearson omnibus normality test, and Shapiro–Wilk normality test). Flow cytometry and ELISA data are presented as individual dots and medians. Data from quantification of the immunoreactivity of recombinant cryptococcal proteins is depicted as median and range. The degree of significance was annotated as following: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ . GraphPad PRISM v7 software was used for statistical analyses (GraphPad Software, La Jolla, CA, USA). Statistical analysis of the immunoproteome data was carried out as described in the 2D analysis section.

## RESULTS

### Cryptococcal Meningitis Is Accompanied by an Increase in Anti-Cryptococcal IgG, but Not IgM Antibodies, in HIV-Negative CM Patients

We aimed to identify disease-associated proteins of *C. neoformans* that are targeted by human antibodies. Therefore, we first characterized serum samples from a Colombian cohort to (i) confirm *C. neoformans* specificity of antibodies contained in the sera, and (ii) determine the dominant antibody isotype of the human anti-cryptococcal serum antibodies. The sample collection consisted of sera from HIV-positive ( $CD4^+$  T cells  $<250$  cells/ $\mu$ L,  $n=28$ ) and HIV-negative ( $n=16$ ) Colombian cryptococcal meningitis (CM) patients as well as healthy Colombian blood donors ( $n=15$ ) (Table 1). All CM patients were diagnosed to be infected with a serotype A *C. neoformans* strain. Besides cancer or corticosteroid treatment, for most of the HIV-negative patients diagnosed with CM the

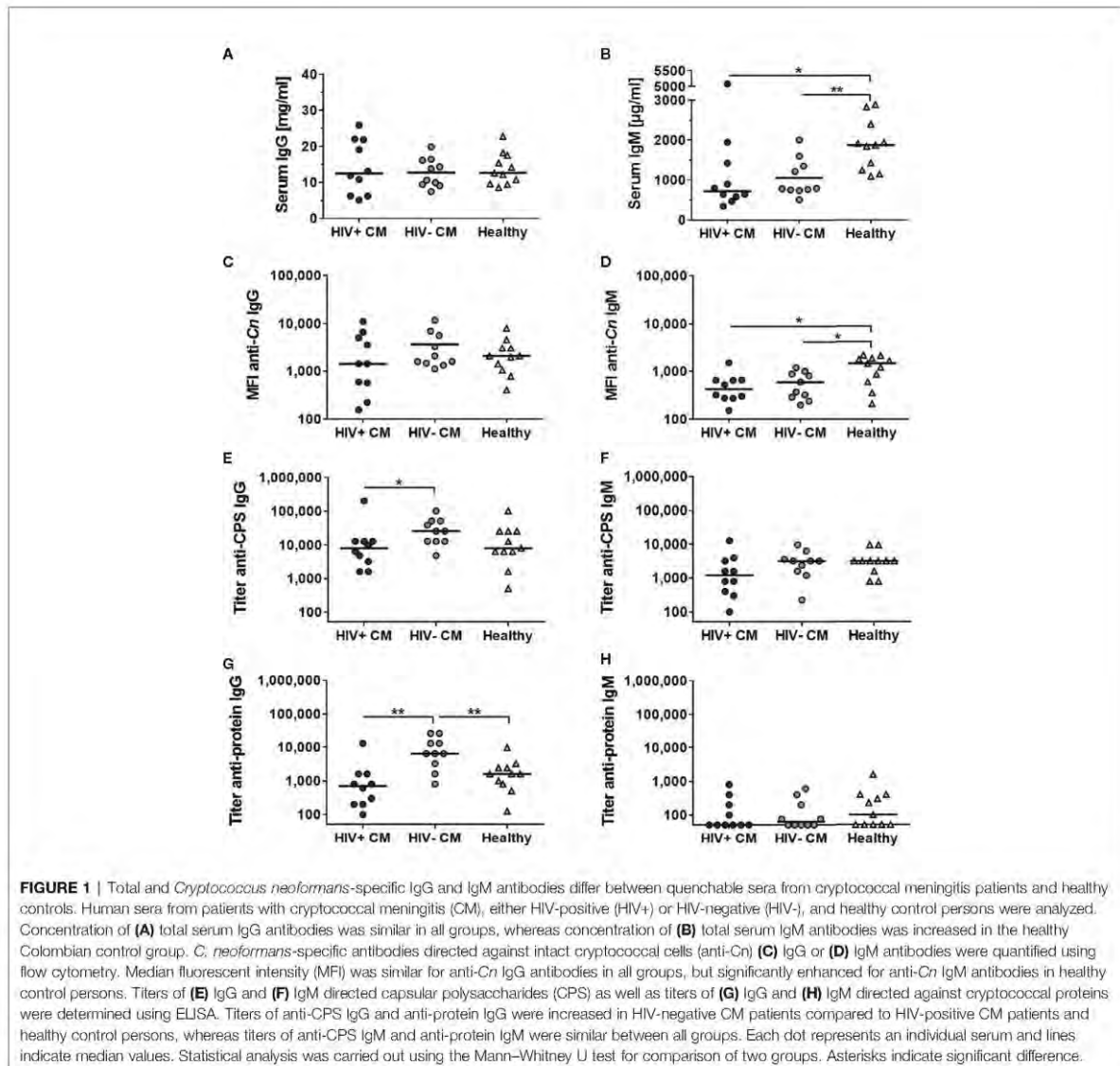
underlying risk factor(s) could not be identified (termed “unknown”, Table 1).

We determined *C. neoformans* specificity of the antibodies contained in the individual sera of the collection, using a flow cytometry-based assay for quenching (74). Each individual serum was pre-absorbed with *C. neoformans* H99 (serotype A) or *Candida albicans* SC5314 cells prior to detection of IgG and IgM antibodies directed against intact cryptococcal cells (anti-*Cn* IgG and anti-*Cn* IgM). Sera that showed a significantly stronger reduction of median fluorescent intensity (MFI) for anti-*Cn* IgG and anti-*Cn* IgM when pre-absorbed with *C. neoformans* cells compared to *C. albicans* cells, were selected for further analysis (Supplementary Figure 1 and Table 1). In these quenchable sera, we quantified total serum IgG and IgM concentrations (Figures 1A, B) and levels of anti-cryptococcal antibodies directed against (i) intact cryptococci (anti-*Cn* Igs) by flow cytometry (Figures 1C, D) or titers of anti-cryptococcal antibodies against (ii) purified capsular polysaccharides (CPS, anti-CPS Igs, Figures 1E, F), and (iii) cryptococcal proteins (anti-protein Igs, Figures 1G, H) by ELISA analysis. Total serum IgG levels were similar for all groups investigated (Figure 1A). Interestingly, levels of total serum IgM and anti-*Cn* IgM directed against intact fungal cells were significantly increased in healthy control persons, while similar among CM patients (Figures 1B, D). Anti-cryptococcal IgG and IgM antibodies were detected in sera of all groups, even in healthy controls at surprisingly high levels (Figures 1C–G). HIV-negative CM patients had higher anti-CPS IgG (Figure 1E) and anti-protein IgG (Figure 1G) titers compared to HIV-positive CM patients (CPS, proteins) and healthy control persons (proteins). This difference was also observed as a statistically non-significant trend for anti-*Cn* IgG directed against intact fungal cells (Figure 1C). In contrast, titers of anti-CPS (Figure 1F) and anti-protein IgM (Figure 1H) were similar for all groups, independent of HIV status or CM.

TABLE 1 | Collection of Colombian sera from cryptococcal meningitis patients and healthy persons.

Group	HIV status	Selection	Number of sera	Age (years)	Gender	Risk factor
Cryptococcal meningitis patients	Positive	Total sera	28	20-51; median 31	Female (n=7) Male (n=21)	HIV infection, $CD4^+$ T cells $<250$ cells/ $\mu$ L.
		Quenchable sera	10	24-45; median 32	Female (n=4) Male (n=6)	HIV infection, $CD4^+$ T cells $<250$ cells/ $\mu$ L.
	Negative	Total sera	16	7-72; median 42	Female (n=6) Male (n=10)	Corticosteroids: n=2; Cancer: n=3; ND: n=4; unknown: n=7.
		Quenchable sera	10	29-67; median 35	Female (n=4) Male (n=6)	Corticosteroids: n=1; Cancer: n=3; ND: n=1; unknown: n=5.
Healthy controls	Negative	Total sera	15	19-60; median 28	Female (n=8) Male (n=7)	none
		Quenchable sera	11	19-60; median 28	Female (n=6) Male (n=5)	none

Cryptococcosis patients used in this study were diagnosed with cryptococcal meningitis and were HIV-positive or HIV-negative. Control sera were derived from healthy blood donors living in Colombia. Quenchable sera were selected according to *Cryptococcus neoformans* specificity of the antibody signal determined by pre-absorption experiments using flow cytometry (Supplementary Figure 1). ND, not defined; n, Number of sera.



Therefore, serological data from human serum samples point towards increased production of anti-cryptococcal IgG, but not IgM antibodies, in response to cryptococcal infection. However, this was not detectable in HIV-positive CM patients, potentially due to severe immunosuppression caused by the underlying HIV infection.

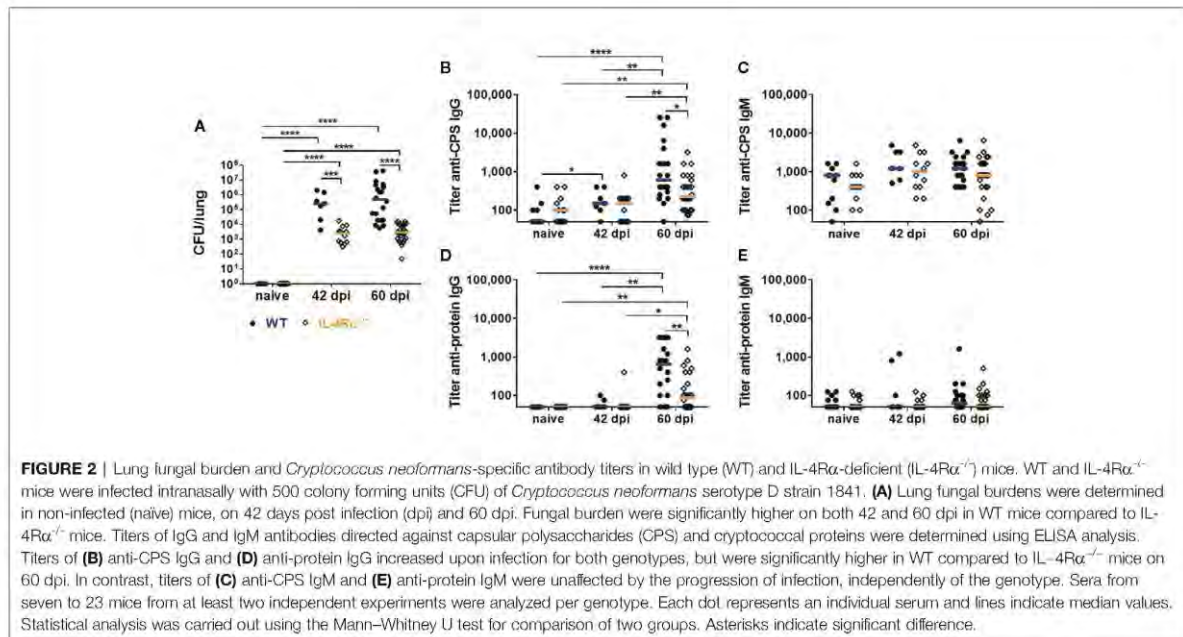
### Cryptococcal Infection Results in Increased Anti-Cryptococcal IgG but Unaltered IgM Levels in a Murine Model of Pulmonary Infection

We decided to further investigate the nature of the humoral anti-cryptococcal immune response regarding (i) the dominant

isotype of anti-cryptococcal antibodies, (ii) the influence of antigen dose, represented by fungal burden in the lung, and (iii) potential differences in the humoral immune response provoked by latent pulmonary infection or active systemic disease. Therefore, we used wild type (WT) Balb/c mice which are susceptible to disseminated cryptococcal infection, and IL-4R $\alpha$ -deficient (IL-4R $\alpha$ <sup>-/-</sup>) Balb/c mice which do not succumb to cryptococcal infection, but develop a latent pulmonary infection (75), after intranasal infection with the *C. neoformans* serotype D strain 1841.

As previously described (75), lung fungal burdens in IL-4R $\alpha$ <sup>-/-</sup> mice were significantly lower on 42 days post infection (dpi) and 60 dpi, compared to WT mice (Figure 2A).





Total serum IgG and IgM concentrations increased during the course of infection, independently of the mouse genotype (**Supplementary Figures 2A, B**). Titers of anti-cryptococcal CPS and anti-cryptococcal protein IgG and IgM antibodies were measured using ELISA analysis. Anti-cryptococcal IgG and IgM directed against the intact fungal organism (anti-*Cn* Igs) were quantified using flow cytometry. Levels of anti-cryptococcal IgG directed against each antigenic compound investigated, increased after pulmonary infection for both genotypes compared to naïve mice (**Figures 2B, D** and **Supplementary Figure 2C**). Interestingly, the increase in anti-CPS (**Figure 2B**) and anti-protein (**Figure 2D**) IgG titers was significantly higher in WT mice compared to IL-4R $\alpha$ <sup>-/-</sup> mice at 60 dpi (**Figures 2B, D**), but levels of anti-*Cn* IgG directed against intact cryptococcal cells were similar between both groups at all time points (**Supplementary Figure 2C**). However, titers of anti-CPS and anti-protein IgG correlated positively with lung fungal burden in WT (anti-protein IgG:  $r=0.5759$ ,  $p=0.0017$ ; anti-CPS IgG:  $r=0.4296$ ,  $p=0.0253$ ), but not in IL-4R $\alpha$ <sup>-/-</sup> mice (**Supplementary Table 2**). We therefore conclude that latent pulmonary infection present in IL-4R $\alpha$ <sup>-/-</sup> mice (75) triggers intermediate production of anti-cryptococcal IgG. In contrast, wild type mice developing disseminated cryptococcal disease (75) show further increased production of anti-cryptococcal IgG driven by increased antigen load.

In contrast to anti-cryptococcal IgG levels, anti-cryptococcal CPS and protein IgM titers were unaffected by progression of the infection (**Figures 2C, E**), with exception of elevated anti-*Cn* IgM levels in WT and IL-4R $\alpha$ <sup>-/-</sup> mice on day 42 post infection (**Supplementary Figure 2D**). This discrepancy might result from applying different methods for determination of anti-cryptococcal antibody levels [directed against isolated capsular

material (ELISA) vs. intact fungal capsule (FACS)]. Surprisingly, levels of anti-*Cn* IgM antibodies directed against intact *C. neoformans* cells in WT mice were inversely correlated with lung fungal burden ( $r=-0.4747$ ,  $p=0.0123$ ), suggesting a decreased production of anti-*Cn* IgM antibodies with increasing fungal burden. Remarkably, anti-CPS IgM (**Figure 2C**) as well as anti-*Cn* IgM (**Supplementary Figure 2D**) antibodies were also measurable in naïve mice of both genotypes, pointing to the possibility of cross-reactivity of these antibodies with polysaccharides from other fungi, which may also be reflected in the anti-cryptococcal IgM levels measured using human serum samples (**Figures 1D, F**).

In conclusion, analysis of murine antibodies demonstrated an increase of anti-cryptococcal IgG in a lung fungal burden-dependent manner in response to cryptococcal infection, whereas this was not the case for IgM antibodies. The anti-cryptococcal IgG response was more pronounced in wild type mice, developing systemic cryptococcal disease (75), compared to IL-4R $\alpha$ <sup>-/-</sup> mice, that exhibit latent pulmonary infection (75).

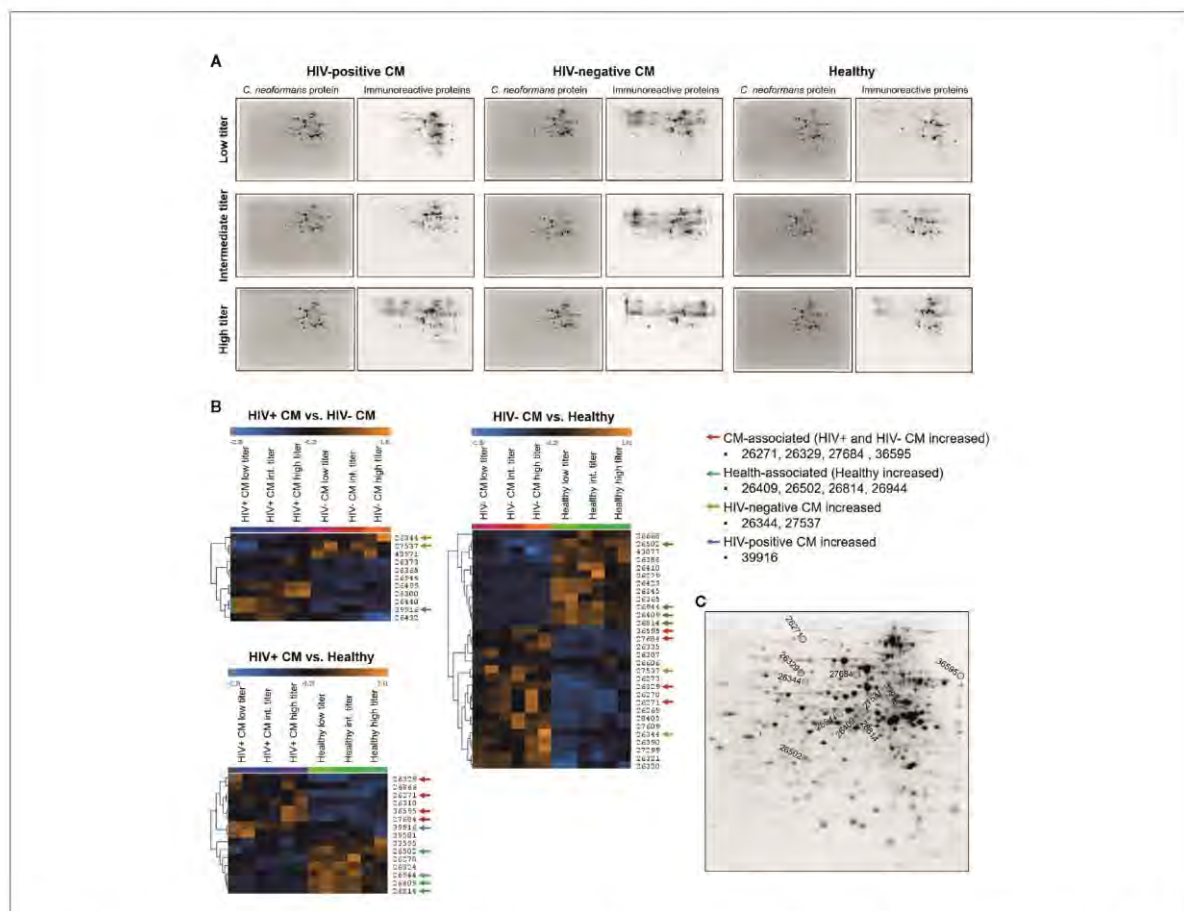
### Immunoproteome Analysis Reveals Several Disease-Associated Cryptococcal Proteins

Quantification of anti-cryptococcal antibodies in human and murine sera revealed IgG to be the predominant isotype induced in response to cryptococcal disease. Therefore, we decided to identify the targets of the human anti-cryptococcal protein IgG antibodies using two-dimensional (2D) gel electrophoresis and immunoblotting. Cryptococcal proteins were separated by 2D gel electrophoresis, transferred onto nitrocellulose membranes and incubated with human sera previously defined as “quenchable” sera (**Table 1**). We created serum pools for each group (HIV+

CM patients, HIV- CM patients, healthy control persons), based on the anti-protein IgG titers of the sera (low titer, intermediate titer, high titer) to facilitate detection of all proteins recognized by individual sera (**Supplementary Table 3**). Representative images of fluorescent signals of total cryptococcal proteins and immunoreactive proteins (bound by serum IgG) are shown in **Figure 3A** for all groups. Quantification with Delta 2D-software (DECODON) and subsequent statistical analysis revealed four CM-associated spots (red arrows), significantly stronger recognized by sera from both, HIV-positive and HIV-negative, CM patients, compared to healthy individuals (**Figure 3B**).

Additionally, four spots were recognized with significantly higher intensity by healthy individuals (health-associated, green arrows). Two spots were strongly recognized by HIV-negative CM patients (yellow arrows) and one spot showed the highest reactivity with sera from HIV-positive CM patients (blue arrows). The respective spots are marked in a representative 2D gel image (**Figure 3C**).

Sample analysis using reverse phase chromatography coupled on-line via an electrospray ionization source to a mass spectrometer revealed a total of 143 proteins contained in these eleven spots of interest (**Supplementary Table 4**). Proteins were chosen for recombinant expression that were



**FIGURE 3** | Two-dimensional (2D) analysis of the immunoproteome recognized by cryptococcal meningitis (CM) patients and healthy individuals revealed infection- and health-associated spots. *Cryptococcus neoformans* proteins (strain H99) were separated by 2D gel electrophoresis and transferred onto nitrocellulose membranes. **(A)** Representative blot images of total cryptococcal proteins (stained with UV-activated 2,2,2-trichloroethanol (TCE)) and immunoreactive protein spots bound by serum IgG (detected using AF-647 labeled secondary antibody) are shown. The contrast and brightness of the images were adjusted for publication and do not reflect actual signal intensities. **(B)** Heat maps of immunoreactive protein spots' fluorescence signals recognized with different intensities by sera from different groups ( $p < 0.01$ ). Spots were either (i) CM-associated (red arrows) – significantly stronger reactivity with CM patient sera (HIV-positive and HIV-negative) compared to healthy individuals, (ii) health-associated (green arrows) – significantly stronger reactivity with sera from healthy individuals compared to CM patients (HIV-positive and HIV-negative). Three spots were found that showed higher reactivity of (iii) HIV-negative sera (yellow arrows) or (iv) HIV-positive sera (blue arrows) compared to the respective other two groups. Immunoreactivity per sub-pool was determined in duplicates. Spot identifiers (row numbers) were automatically created by Delta 2D software. Analysis parameters: Test design: between-subjects, used Welch approximation, alpha: 0.01, p-values based on permutation, all permutations used: true, number of permutations per spot: 924, significance determined by standard Bonferroni correction, HCL: complete linkage, Euclidean Distance. **(C)** Spots of interest were highlighted in a 2D gel image. Proteins were stained with UV-activated TCE. Int., Intermediate.

preferentially present in (i) CM-associated spots, (ii) health-associated spots, or (iii) abundant in both types of spots, indicating potential immunodominance. We expressed those proteins recombinantly to verify specific IgG-mediated recognition by human sera. cDNA sequences from *C. neoformans* strain H99 of twenty-three proteins were amplified (**Supplementary Figure 3**), cloned into pET28a+ vectors and used for transformations of *E. coli* cells. Detailed information on the proteins is listed in **Table 2**, including homology to proteins in humans and proteins of pathogenic fungi capable of causing systemic disease (*Aspergillus fumigatus*, *Histoplasma capsulatum*, *C. albicans*, and *Pneumocystis carinii*) (detailed information on homology analysis in **Supplementary Table 5**). Additionally, predicted protein function, previously reported presence in immunoreactive cryptococcal spots and evidence for extracellular localization is listed. For confirmation of successful recombinant protein expression, *E. coli* protein lysates were separated using SDS-PAGE and production of the desired proteins was confirmed by mass spectrometry of the respective protein band and staining of N- and C-terminal His-Tag on immunoblots (**Supplementary Figure 4** and **Supplementary Table 6**).

For quantification of recombinant protein immunoreactivity, *E. coli* proteins were separated by SDS-PAGE, blotted onto nitrocellulose membranes and incubated with pools of sera from HIV-positive CM patients, HIV-negative CM patients, or healthy control persons. Based on similar 2D immunoproteome analysis results (**Figure 3B**) obtained for all three serum sub-pools of each group (low, intermediate, and high titer, **Supplementary Table 2**), all sera of each respective group (HIV+CM, HIV-CM, Healthy) were pooled for quantifying the immunoreactivity of the recombinant proteins. Quantification of immunoreactivity was performed as shown in **Figure 4A**: *E. coli* samples before induction of recombinant protein expression and after induction of recombinant protein expression using IPTG were loaded side by side. For serum incubation, the blots were split in three parts and incubated with the indicated serum pools. For calculation, background signal intensity before induction of protein expression (grey boxes) was subtracted from signal intensity after induction of protein expression (black boxes). Finally, the immunoreactive signal intensity (fluorescence, Cy5 channel) was divided by the signal intensity of *E. coli* protein (fluorescence, stain free channel), thereby normalizing the immunoreactivity onto the protein loading. Representative blots for all proteins are shown in **Supplementary Figure 5**. Using this approach, twenty-three proteins were confirmed to be immunoreactive with human sera (**Figure 4** and **Supplementary Figure 6**). Most proteins showed reactivity with sera from HIV-positive and HIV-negative CM patients, but also with sera from healthy individuals.

Our screen revealed twelve disease-associated cryptococcal proteins, as the recombinant proteins showed significantly stronger reactivity with serum IgG from CM patients compared to healthy control persons (**Figures 4B–M**). Two proteins, extracellular elastolytic metalloprotease and glucose-methanol-choline oxidoreductase, showed similar reactivity in both HIV-

positive and HIV-negative CM patients (**Figures 4D, E**). The remaining ten proteins were recognized significantly stronger by serum IgG from HIV-positive CM patients compared to HIV-negative CM patients (**Figures 4B, C, F–M**). Eleven recombinant cryptococcal proteins were proven to be immunoreactive with human sera, although with different recognition patterns: Five proteins were preferentially immunoreactive with sera from HIV-positive CM patients (**Supplementary Figures 6A–E**). Four proteins showed decreased reactivity with sera from HIV-negative CM patients (**Supplementary Figures 6F–I**), and two proteins were recognized with similar intensities by all three groups (**Supplementary Figures 6J, K**). Among all recombinant proteins, four proteins showed remarkably strong immunoreactivity with sera of all groups (Ratio value >2 for all groups). These proteins, GTP-binding protein ypt1 (**Figure 4G**), Hsp71-like protein (**Supplementary Figure 6B**), Hsp72-like protein (**Supplementary Figure 6J**) and ketol-acid reductoisomerase (**Supplementary Figure 6I**), could therefore represent immunodominant proteins. Two additionally produced recombinant proteins derived from the gene sequence of serotype D strain *C. neoformans* JEC21, Hsp71-like protein purified from a heterologous expression in *Aspergillus niger* and purified phosphoglucosyltransferase expressed in *E. coli*, were also proven to be immunoreactive with sera from CM patients and healthy individuals (data not shown), although we did not perform quantification of immunoreactivity for these proteins. Both proteins show very high homology to their corresponding homologue in the serotype A strain H99 (**Supplementary Table 7**). This indicates cross-reactivity between corresponding proteins from different *C. neoformans* serotypes.

## DISCUSSION

In this study, we characterized the humoral immune response in HIV-positive and HIV-negative CM patients as well as healthy individuals regarding (i) the quantity of anti-cryptococcal IgG and IgM antibodies to identify the dominant isotype in anti-cryptococcal humoral immunity, and (ii) the target proteins of the human humoral immune response against *C. neoformans* to identify disease-associated cryptococcal proteins.

Our study revealed IgG to be the dominant isotype induced in response to CM. This was reflected by increased titers of anti-cryptococcal protein and CPS IgG in HIV-negative CM patients compared to HIV-positive CM patients and healthy individuals, although not reaching statistical significance for anti-CPS IgG (HIV-negative CM patients compared to healthy group). Similarly, previous studies showed increased anti-glucuronoxylomannan (GXM) titers in HIV-negative CM patients compared to healthy individuals (19) or HIV-positive cryptococcosis patients (61). However, in our study similar titers of anti-cryptococcal IgG antibody were detected in HIV-positive CM patients and healthy individuals, confirming previous reports (55, 58, 62). We hypothesize, that severely immunosuppressed AIDS patients (CD4<sup>+</sup> T cell count <250 cells/ $\mu$ L) are not able to mount a

**TABLE 2 |** Recombinant cryptococcal proteins immunoreactive with IgG from human serum samples.

Protein information Name	MW [kDa]	Accession no.	Homology					Predicted function				Described as immuno-reactive	Extra-cellular appearance
			Hu.	Af.	Hc.	Ca.	Pc.	EC description	EC no.	GO Term Name	GO ID		
26S proteasome regulatory subunit N8	38,70	AFR92184	x	x	x	x	x	Protein-serine/threonine phosphatase	3.1.3.16	No data available			
Chlorophyll synthesis pathway protein BchC	38,00	AFR97763	x	x	x	x		L-riditol 2-dehydrogenase	1.1.1.14	oxidation-reduction process	0055114		
<b>Cytoplasmic protein CNAG_02943</b>	68,78	AFR93749		x	x	x	x	No data available		No data available			Extracellular vesicle (76)
<b>Deoxyuridine 5'-triphosphate nucleotidohydrolase</b>	73,83	AFR94562	x	x	x	x	x	Histone acetyltransferase	2.3.1.48	dUMP biosynthetic process	0006226		
<b>Extracellular elastinolytic metalloproteinase*</b>	91,72	AFR97484		x				Metalloendo-peptidases	3.4.24.-	Metalloendo-peptidase activity	0004222		Secretory signal peptide*
<b>Glucose-methanol-choline oxidoreductase</b>	65,32	AFR94515	x	x	x			Choline dehydrogenase	1.1.99.1	oxidation-reduction process	0055114		
Glutamate dehydrogenase (NADP)	49,19	AFR97782	x	x	x	x		Glutamate dehydrogenase (NADP(+))	1.4.1.4	cellular amino acid metabolic process	0006520	(45)	Extracellular vesicle (76)
<b>Glycerol-3-phosphate dehydrogenase (NAD (+))</b>	37,80	AFR92257	x	x	x	x		Glycerol-3-phosphate dehydrogenase (NAD (+))	1.1.1.8	carbohydrate metabolic process	0005975		
<b>GTP-binding protein ypt1</b>	22,61	AFR94332	x	x	x	x	x	Small monomeric GTPase	3.6.5.2	GTPase activity	0003924		
<b>Heat shock 70kDa protein 4</b>	85,69	AFR98435	x	x	x	x	x	No data available		ATP binding	0005524		
Hsp71-like protein	69,57	AFR97929	x	x	x	x	x	Non-chaperonin molecular chaperone ATPase	3.6.4.10	ATP binding	0005524	(45, 46, 63, 64)	Extracellular vesicle (76)
Hsp72-like protein	69,51	AFR97952	x	x	x	x	x	Non-chaperonin molecular chaperone ATPase	3.6.4.10	ATP binding	0005524	(64)	
<b>Hsp75-like protein</b>	67,13	AFR92468	x	x	x	x	x	Non-chaperonin molecular chaperone ATPase	3.6.4.10	ATP binding	0005524	(45, 65)	Extracellular vesicle (76)
Hypothetical protein CNAG_05236	52,24	AFR94491		x	x	x		Fumarate hydratase	4.2.1.2	No data available			
<b>Hypothetical protein CNAG_06113</b>	36,61	AFR98337		x	x	x		No data available		RNA binding	0003723		Extracellular vesicle (76)
Hypothetical protein CNAG_06946	39,13	AFR94883	x	x	x	x	x	No data available		No data available			
Ketol-acid reductoisomerase, mitochondrial	44,34	AFR96043		x	x	x		Ketol-acid reductoisomerase (NADP(+))	1.1.1.86	branched-chain amino acid biosynthetic process	0009082	(65, 66)	Extracellular vesicle (76)
Mannose-1-phosphate guanylyltransferase	39,95	AFR98009	x	x	x	x	x	Mannose-1-phosphate guanylyltransferase	2.7.7.13	GDP-mannose biosynthetic process	0009298	(66)	
<b>Phosphoglucomutase</b>	60,54	AFR98550	x	x	x	x	x	Phosphoglucomutase	5.4.2.10	carbohydrate metabolic process	0005975	(64)	
Pyruvate decarboxylase	67,61	AFR97558		x	x	x		Pyruvate decarboxylase	4.1.1.1	mitochondrion	0005739	(64)	Extracellular vesicle (76)

(Continued)

TABLE 2 | Continued

Protein information Name	MW [kDa]	Accession no.	Homology					Predicted function				Described as immuno- reactive	Extra- cellular appearance
			Hu.	Af.	Hc.	Ca.	Pc.	EC description	EC no.	GO Term Name	GO ID		
<b>Transaldolase</b>	35,29	AFR98178	x	x	x	x		Transaldolase	2.2.1.2	carbohydrate metabolic process	0005975	(46, 63, 64)	
Transketolase	74,33	AFR95182	x	x	x	x	x	Transketolase	2.2.1.1	transketolase activity	0004802		
<b>Urease accessory protein UreG</b>	33,63	AFR92807		x	x			No data available		nitrogen compound metabolic process	0006807		

Twenty-three cryptococcal proteins were recombinantly expressed in *Escherichia coli*. Production of the desired protein was confirmed using mass spectrometry. All proteins were proven to be immunoreactive with human sera. Proteins printed in bold were classified as disease-associated cryptococcal proteins, as they showed significantly stronger reactivity with sera from cryptococcal meningitis patients compared to healthy individuals on Western Blots. Presence of a secretory signal peptide was checked with SignalP-5.0\*. Detailed information on homology to human proteins (Hu.), or homologous proteins in fungal pathogens causing systemic infections such as *Aspergillus fumigatus* (Af.), *Histoplasma capsulatum* (Hc.), *Candida albicans* (Ca.), and *Pneumocystis carinii* (Pc.) are listed in **Supplementary Table 4**. Predicted function was collected from the database FungiDB<sup>8</sup> which uses orthology to predict gene function. Enzyme Commission (EC) numbers (no.), classifying enzymes based on the chemical reactions they catalyze and corresponding descriptions are listed. Additionally, Gene Ontology (GO) Term Names, describing predicted biological processes, cellular localization, or molecular functions the protein may be involved with and corresponding GO identifier (GO ID) are included. Information on previous description of the respective protein to be contained in immunoreactive spots as well as evidence for extracellular appearance is listed in the table.

\*<http://www.cbs.dtu.dk/services/SignalP/>.

<sup>8</sup><https://fungidb.org/fungidb/app>.

proper immune response to cryptococcal infection, and, therefore, no increase in anti-cryptococcal IgG titers is detectable in these patients. However, some studies measured increased anti-GXM IgG titers in HIV-positive patients with or without cryptococcal infection compared to HIV-negative individuals (49, 50, 52, 53, 56). This might be caused by underlying HIV infection leading to increased levels of serum IgG in general (77–79). In the sera used here, however, total serum IgG concentrations were similar in all groups (**Figure 1A**). Corroborating the data from HIV-negative CM patients, cryptococcal infection of WT and IL-4R $\alpha^{-/-}$  mice led to an increase in anti-cryptococcal IgG, but not IgM production, consistent with a previously published study (80). Interestingly, titers of anti-cryptococcal IgG were significantly higher in WT mice, developing disseminated cryptococcal disease and high fungal burden in the lung (75), compared to IL-4R $\alpha^{-/-}$  mice, exhibiting a latent pulmonary cryptococcal infection without overt disease (75).

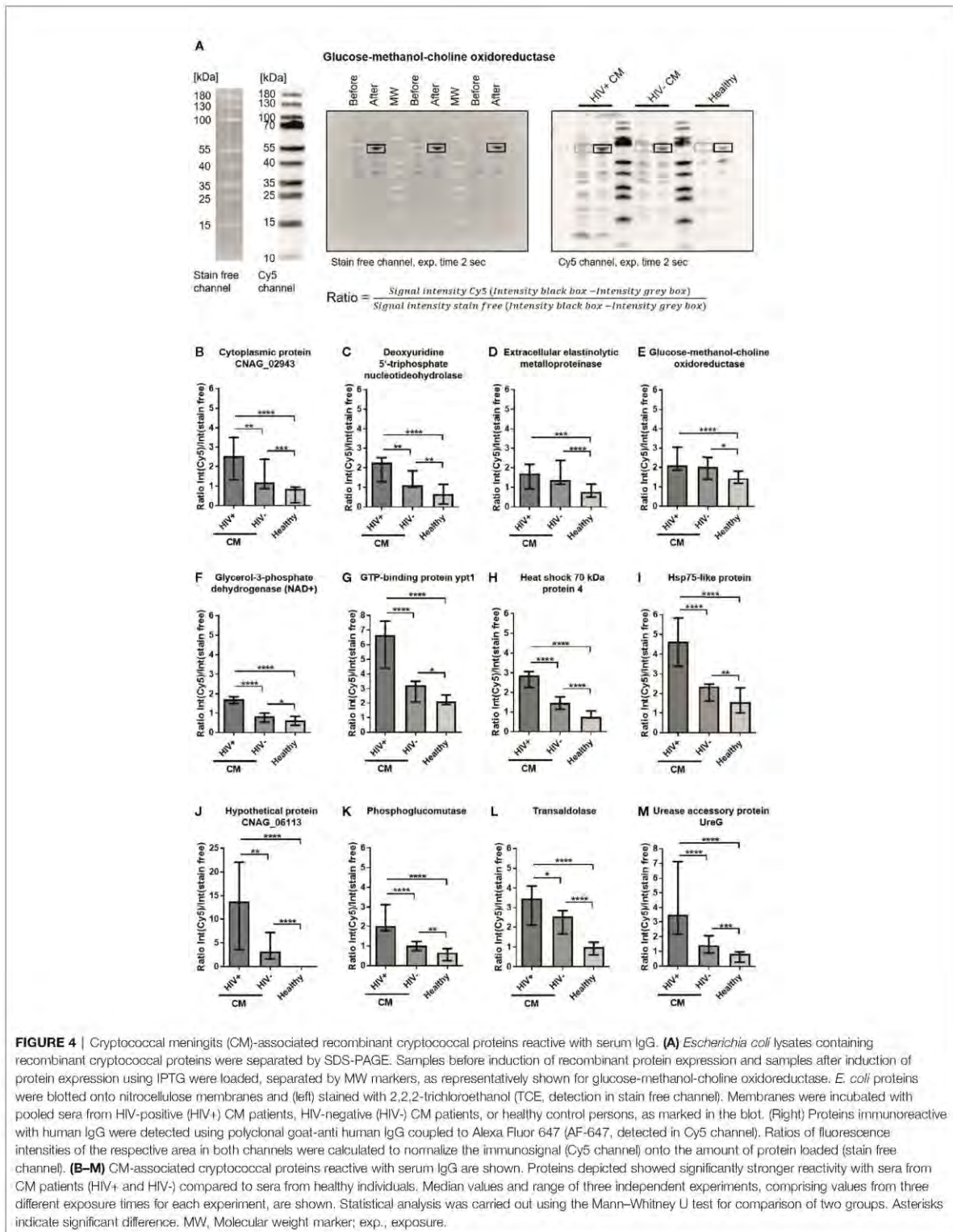
Remarkably, anti-cryptococcal IgM and IgG antibodies were detected in considerably high frequencies even in sera from healthy human individuals, which we categorize as “pre-existing” antibodies. This finding is in accordance with previously published results, demonstrating the presence of antibodies directed against cryptococcal CPS (33, 47–54, 56), cryptococcal proteins (58–62) or mannoproteins (57) in human sera, independent of cryptococcal disease or the HIV status.

We therefore hypothesize that latent pulmonary infection, as observed in IL-4R $\alpha^{-/-}$  mice (75) and hypothesized in healthy individuals evidenced by reactivation of dormant cryptococcal infection (81, 82), is sufficient to trigger basal production of anti-cryptococcal IgG and IgM antibodies. Alternatively, environmental exposure of humans to cryptococcal cells detectable in different reservoirs (3–6) could trigger the observed production of anti-

cryptococcal Igs. In contrast, dissemination of *C. neoformans* leading to systemic cryptococcal disease, as present in WT mice (75) and CM patients, leads to an increase in anti-cryptococcal IgG, but not IgM antibodies. However, in CM patients suffering from severe immunosuppression (CD4<sup>+</sup> T cells <250 cells/ $\mu$ L), production of anti-cryptococcal IgG antibodies in response to infection may be impaired. Anti-cryptococcal IgM antibodies ubiquitously present in the sera analyzed in this study are believed to mainly target polysaccharides of the cell wall, which are conserved structures (83) ubiquitously present on fungal organisms colonizing mice and humans (84). Therefore, those antibodies may be cross-reactive with different fungal species and could be non-specific for *C. neoformans*, as evidenced by the presence of anti-CPS IgM titers in naïve mice in our study and additional studies (33, 80, 85), and thus could potentially be produced in response to colonization by commensal fungi.

Interestingly, total serum IgM levels were found to be decreased in HIV-positive and HIV-negative CM patients compared to healthy individuals, which has also been observed by McGowan et al. in 2006 (77), although other studies demonstrated increased serum IgM in HIV-positive patients compared to healthy individuals (52, 53, 78). Anti-Cn IgM antibodies directed against intact cryptococcal cells were also present at significantly lower levels in CM patients compared to healthy control persons. Interestingly, previous studies showed lower percentage of IgM-expressing memory B cells compared to healthy individuals in both HIV-positive (55) and HIV-negative (19) cryptococcosis patients, proposing a decreased proportion of IgM-expressing memory B cells as a risk factor for cryptococcal disease, indicating a role of IgM antibodies in defense against *C. neoformans*.

We aimed to identify cryptococcal proteins targeted by human serum IgG antibodies, as previous studies demonstrated



protection of mice from lethal challenge with *C. neoformans* after immunization with protein fractions immunoreactive with mouse sera (45, 46), or proteins with immunogenic properties packaged into glucan particles (86, 87). Additionally, we aimed to identify disease-specific proteins, exclusively recognized by sera from CM patients, indicating a role in cryptococcal pathogenesis and therefore rendering them potential targets of anti-cryptococcal therapy.

Immunoreactivity of twenty-three cryptococcal proteins contained in immunoreactive protein spots was verified by recombinant expression and subsequent incubation with human sera. Most proteins showed reactivity with sera of all groups (HIV-positive and HIV-negative CM patients, as well as healthy individuals). Surprisingly, immunoreactivity of most recombinant proteins was highest with sera from HIV-positive CM patients, in contrast to anti-cryptococcal IgG titers in those patients, which were similar to titers in healthy individuals and higher in HIV-negative CM patients. Recognition of major immunogenic antigens by anti-cryptococcal antibodies may be facilitated when specific proteins are used in contrast to crude antigenic mixtures utilized for ELISA analysis, containing limited amounts of specific antigens.

Twelve proteins were demonstrated to be disease-associated, as they were significantly stronger recognized by sera from CM patients compared to healthy persons. Four of those proteins are especially interesting candidates for vaccine development, as they do not show homology to any known human protein, but to proteins from other pathogenic fungi. Those four proteins are extracellular elastinolytic metalloprotease, cytoplasmic protein CNAG\_02943, hypothetical protein HP\_06113, and urease accessory protein UreG.

Extracellular elastinolytic metalloproteinase is critical for crossing of the blood-brain barrier and therefore required for establishment of fungal disease in the central nervous system. This was demonstrated using an *mpr1Δ* *C. neoformans* strain, with *mpr1* being the serotype D homologue of the elastinolytic metalloprotease identified in our study (88, 89). Extracellular elastinolytic metalloprotease is an especially interesting target protein for development of anti-cryptococcal treatment options, as inhibition of Mpr1 by natural product inhibitors prevented cryptococcal cells from crossing the blood brain barrier in an *in vitro* transwell model (90). Extracellular elastinolytic metalloproteinase contains a secretory signal peptide making it also an attractive candidate for vaccine development. Indeed, vaccination with recombinant extracellular elastinolytic metalloprotease (Mep1) contained in glucan particles led to significantly prolonged survival of C57BL/6 mice when challenged orotracheally into the lung with *C. neoformans* H99 (87).

Two disease-associated proteins with unknown functions, cytoplasmic protein CNAG\_02943 and hypothetical protein CNAG\_06113, predicted to possess RNA-binding capacity, have been identified in our screen. Interestingly, both proteins were detected in extracellular vesicles of *C. neoformans*, implicating them in cryptococcal virulence (76) and also rendering them interesting vaccine candidates.

Urease accessory protein UreG is required for activation of apourease (91), an enzyme crucial for cryptococcal virulence, as urease hydrolyzes urea for usage as nitrogen source and leading to increased local pH interfering with host function (92). UreG mediates activation of apourease by incorporation of Ni<sup>2+</sup> ions, and was shown to be critical for brain invasion, as a mutant strain lacking *ure7*, encoding UreG in *C. neoformans*, led to significantly reduced fungal burden in brains of C57BL/6 mice infected intravenously with *ure7Δ* or wild type H99 cryptococcal cells (91). Therefore, urease accessory protein UreG is a promising target for anti-cryptococcal chemotherapeutics.

We identified eight additional disease-associated cryptococcal proteins. All those proteins possess homologs in *Homo sapiens*, although with varying sequence similarity (for further information see **Supplementary Table 4**). Some of these proteins may nonetheless represent interesting targets for development of anti-cryptococcal therapy, based on their roles in cryptococcal metabolism, virulence or survival.

Phosphoglucosyltransferase, transaldolase, and glycerol-3-phosphate dehydrogenase (NAD<sup>+</sup>) are confirmed or predicted to be central metabolic enzymes involved in carbohydrate metabolism (93). Two of them were previously described to be contained in *C. neoformans* protein spots immunoreactive with sera from mice infected with *C. neoformans* strain 1841 (phosphoglucosyltransferase, transaldolase) (64), or sera from mice immunized with the *C. neoformans* strain H99γ, engineered to produce murine IFN-γ, or immunized with *C. gattii* protein fractions (transaldolase) (46, 63). Additionally, phosphoglucosyltransferase derived from the genetic sequence of cryptococcal serotype D strain JEC21 was also recognized by human sera, indicating serotype-independent recognition. Interestingly, transaldolase is implicated in virulence of *C. neoformans* as it possesses the capability to bind heparin (94) and plasminogen (95) and shows increased expression in response to nitric oxide stress (96). Another disease-associated protein identified in our screen, the enzyme glucose-methanol-choline oxidoreductase, was shown to be upregulated in *C. gattii* under iron deprivation, implicating a role in iron acquisition which is critical for cryptococcal pathogenesis (97). The homologues of the disease-associated protein GTP-binding protein ypt1 in *C. albicans* (98), and *Saccharomyces cerevisiae* (99, 100) are critical for intracellular vesicle traffic and cell survival, as null mutants of *S. cerevisiae* are not viable (101).

Four *C. neoformans* heat shock proteins of the Hsp70 family were proven to be immunoreactive in our study. This confirms previous studies, revealing Hsp70 proteins to be contained in cryptococcal protein spots reactive with sera from patients with pulmonary cryptococcosis (102), mice immunized with the *C. neoformans* strain H99γ (45, 63), mice intratracheally infected with *C. neoformans* strain YC-11 (serotype A) (103), or 1841 (serotype D) (64), mice immunized with *C. gattii* protein fractions (46), and koalas infected with *C. gattii* (65). Two Hsp70 proteins identified to be immunoreactive in our study, Hsp71-like protein and Hsp75-like protein, were also detected in extracellular vesicles of *C. neoformans* (76). Furthermore, anti-Hsp70 antibodies, directed against cryptococcal Hsp70 protein recombinantly

produced in *E. coli*, revealed that Hsp70 proteins are located at the fungal surface (104). This indicates extracellular presence of Hsp70 proteins rendering them interesting antigens for vaccine development. Furthermore, the heat shock protein Ssa1, corresponding to Hsp71-like protein in our study, is implicated in fungal virulence by influencing the immune response towards M2 macrophage polarization during early infection in a pulmonary mouse infection model (105). In our study, Hsp75-like protein and Hsp70 protein 4 were demonstrated to be disease-associated, whereas Hsp71-like and Hsp72-like proteins were preferentially recognized by HIV-positive, but not HIV-negative patients compared to healthy individuals. Additionally, we could demonstrate serotype-independent recognition of Hsp71-like protein by human sera. Overall, proteins of the Hsp70 family showed particularly strong immunoreactivity with all sera, emphasizing their immunodominant role previously described.

In conclusion, we identify several disease-associated cryptococcal protein antigens based on their preferential reactivity with human sera from HIV-positive and HIV-negative CM patients. Some of these proteins are interesting candidates for future research on anti-cryptococcal chemotherapy or development of an anti-cryptococcal vaccine. Several proteins are implicated in cryptococcal virulence or fungal metabolism and survival and could therefore be targeted by anti-fungal agents. One disease-associated protein identified in our screen, extracellular elastinolytic protease, was already successfully inhibited by natural products in an *in vitro* transwell model, decreasing cryptococcal virulence (90). Therefore, targeting other virulence-associated proteins using similar approaches could be beneficial. Potential candidate antigens for development of anti-cryptococcal vaccines should be located on the cell surface or presented extracellularly to facilitate antibody-mediated neutralization and recognition by antigen-presenting cells. Extracellular location is evident for seven immunoreactive proteins identified in our screen, rendering them promising candidates for a vaccination approach. Furthermore, some of these proteins did not show homology to human proteins, and are therefore excellent targets for further development of an anti-cryptococcal vaccine, as this minimizes the risk of autoimmune responses (26). Additionally, all disease-associated proteins identified in our screen possess homologous proteins in other fungal pathogens, rendering them potential targets for development of pan-fungal vaccines (26).

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

## REFERENCES

1. Kwon-Chung KJ, Fraser JA, Doering TL, Wang Z, Janbon G, Idnurm A, et al. *Cryptococcus neoformans* and *Cryptococcus gattii*, the Etiologic Agents of Cryptococcosis. *Cold Spring Harb Perspect Med* (2014) 4:1–27. doi: 10.1101/cshperspect.a019760

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Technical Committee of Research (CTIN) and the Ethical Committee for Research (CEIN) of the National Institute of Health, Bogota, Colombia; Ethical committee of Corporación para Investigaciones Biológicas (CIB) and Hospital La Maria IRB Number 7250 in Medellin, Colombia. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

AEG designed experiments, performed experiments and wrote the manuscript. DV, UM, and MB designed and performed experiments. CF designed the experiments and provided serum samples. CS and BS-R developed methods. FB, PE, and RH provided mice, serum samples and key reagents. GA conceptualized the project and wrote the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

AEG was supported by funds from the Ph.D. program (“Doktorandenförderplatz”) of the University of Leipzig, Leipzig, Germany. CF was supported by a Georg Forster Research Fellowship for postdoctoral researchers from the Alexander von Humboldt Foundation. Equipment and software for mass spectrometry analysis was funded by the European Fund for Regional Structure Development (grant number 100193542) and the Deutsche Forschungsgemeinschaft (grant number INST 268/387-1). We acknowledge support from Leipzig University for Open Access Publishing.

## ACKNOWLEDGMENTS

The authors thank Antje Keim from the group of Prof. Dr. Norbert Sträter (Structural analysis of biopolymers, University of Leipzig, Leipzig, Germany) for assistance in production of chemically competent *Escherichia coli* cells.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2021.709695/full#supplementary-material>

2. Negroni R, Arechavala AI. Pathogenesis of Cryptococcosis in Humans. In: SK Singh, editor. *Human Emerging and Re-emerging Infections: Bacterial & Mycotic Infections*. Hoboken, New Jersey, United States: John Wiley & Sons (2016). p. 915–27. doi: 10.1002/9781118644843.ch49
3. Soltani M, Bayat M, Hashemi SJ, Zia M, Pestechian N. Isolation of *Cryptococcus neoformans* and Other Opportunistic Fungi From Pigeon Droppings. *J Res Med Sci* (2013) 18:56–60.



4. Zarrin M, Jorfi M, Amirrajab N, Rostami M. Isolation of *Cryptococcus neoformans* From Pigeon Droppings in Ahwaz, Iran. *Turk J Med Sci* (2010) 40:313–6. doi: 10.3906/sag-0808-10
5. Brito-Santos F, Trilles L, Firacative C, Wanke B, Carvalho-Costa FA, Nishikawa MM, et al. Indoor Dust as a Source of Virulent Strains of the Agents of Cryptococcosis in the Rio Negro Micro-Region of the Brazilian Amazon. *Microorganisms* (2020) 8:682. doi: 10.3390/microorganisms8050682
6. Lazera MS, Salmato Cavalcanti MA, Londero AT, Trilles L, Nishikawa MM, Wanke B. Possible Primary Ecological Niche of *Cryptococcus neoformans*. *Med Mycol* (2000) 38:379–83. doi: 10.1080/mmy.38.5.379.383
7. Aguiar PA, Pedrosa RD, Borges AS, Moreira TD, Araújo LB, Röder DV. The Epidemiology of Cryptococcosis and the Characterization of *Cryptococcus neoformans* Isolated in a Brazilian University Hospital. *Rev Inst Med Trop Sao Paulo* (2017) 59:e13. doi: 10.1590/S1678-9946201759013
8. Sloan DJ, Parris V. Cryptococcal Meningitis: Epidemiology and Therapeutic Options. *Clin Epidemiol* (2014) 6:169–82. doi: 10.2147/CLEP.S38850
9. Bollam R, Yassin M, Phan T. Disseminated Cryptococcosis in an Immunocompetent Patient. *Respir Med Case Rep* (2020) 30:101034. doi: 10.1016/j.rmcr.2020.101034
10. Panackal AA, Wuest SC, Lin Y-C, Wu T, Zhang N, Kosa P, et al. Paradoxical Immune Responses in Non-HIV Cryptococcal Meningitis. *PLoS Pathog* (2015) 11:e1004884. doi: 10.1371/journal.ppat.1004884
11. Gupta S, Ellis M, Cesario T, Rühling M, Vayuvugula B. Disseminated Cryptococcal Infection in a Patient With Hypogammaglobulinemia and Normal T Cell Functions. *Am J Med* (1987) 82:129–31. doi: 10.1016/0002-9343(87)90388-3
12. Kaushik A, Rao H, Tengson R, Rezk A, Kest H. Disseminated Cryptococcosis With Immunoglobulin G Subclass Deficiency. *Pediatr Int* (2019) 61:198–9. doi: 10.1111/ped.13741
13. Wienemann T, Müller A-K, MacKenzie C, Bieler C, Weyers V, Aktas O, et al. Cryptococcal Meningoencephalitis in an IgG2-Deficient Patient With Multiple Sclerosis on Fingolimod Therapy for More Than Five Years - Case Report. *BMC Neurol* (2020) 20:158. doi: 10.1186/s12883-020-01741-0
14. Wahab JA, Hanifah MJ, Choo KE. Bruton's Agammaglobulinemia in a Child Presenting With Cryptococcal Empyema Thoracis and Periauricular Pyogenic Abscess. *Singapore Med J* (1995) 36:686–9.
15. Tabone M-D, Leverger G, Landman J, Aznar C, Boccon-Gibod L, Lasfargues G. Disseminated Lymphonodular Cryptococcosis in a Child With X-Linked hyper-IgM Immunodeficiency. *Pediatr Infect Dis J* (1994) 13:77–9. doi: 10.1097/00006454-199401000-00020
16. Górgolas M, Erice A, Gil A, Gutiérrez J, Rivas P, Hernando C, et al. Cryptococcal Meningitis in a Patient With X-Linked Hyper-IgM1 Syndrome. *Scand J Infect Dis* (2005) 37:526–8. doi: 10.1080/00365540510036570
17. Guo L-Y, Liu L-L, Liu Y, Chen T-M, Li S-Y, Yang Y-H, et al. Characteristics and Outcomes of Cryptococcal Meningitis in HIV Seronegative Children in Beijing, China, 2002–2013. *BMC Infect Dis* (2016) 16:635. doi: 10.1186/s12879-016-1964-6
18. Jo E-K, Kim H-S, Lee MY, Iseki M, Lee J-H, Song C-H, et al. X-Linked Hyper-IgM Syndrome Associated With *Cryptosporidium parvum* and *Cryptococcus neoformans* Infections: The First Case With Molecular Diagnosis in Korea. *J Korean Med Sci* (2002) 17:116–20. doi: 10.3346/jkms.2002.17.1.116
19. Rohatgi S, Nakouzi A, Carreño LJ, Slosar-Cheah M, Kuniholm MH, Wang T, et al. Antibody and B Cell Subset Perturbations in Human Immunodeficiency Virus-Uninfected Patients With Cryptococcosis. *Open Forum Infect Dis* (2018) 5:ofx255. doi: 10.1093/ofid/ofx255
20. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global Burden of Disease of HIV-associated Cryptococcal Meningitis: An Updated Analysis. *Lancet Infect Dis* (2017) 17:873–81. doi: 10.1016/S1473-3099(17)30243-8
21. Kaplan JE, Vallabhaneni S, Smith RM, Chideya-Chihota S, Chebab J, Park BJ. Cryptococcal Antigen Screening and Early Antifungal Treatment to Prevent Cryptococcal Meningitis: A Review of the Literature. *J Acq Imm Def* (2015) 68:331–9. doi: 10.1097/QAI.0000000000000484
22. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. *N Engl J Med* (2018) 378:1004–17. doi: 10.1056/NEJMoa1710922
23. Armstrong-James D, Brown GD, Netea MG, Zelante T, Gresnigt MS, van de Veerdonk, et al. Immunotherapeutic Approaches to Treatment of Fungal Diseases. *Lancet Infect Dis* (2017) 17:393–402. doi: 10.1016/S1473-3099(17)30442-5
24. Ueno K, Yanagihara N, Shimizu K, Miyazaki Y. Vaccines and Protective Immune Memory Against Cryptococcosis. *Biol Pharm Bull* (2020) 43:230–9. doi: 10.1248/bpb.b19-00841
25. Santos E, Levitz SM. Fungal Vaccines and Immunotherapeutics. *Cold Spring Harb Perspect Med* (2014) 4:a019711. doi: 10.1101/cshperspect.a019711
26. Oliveira LV, Wang R, Specht CA, Levitz SM. Vaccines for Human Fungal Diseases: Close But Still a Long Way to Go. *NPJ Vaccines* (2021) 6:33. doi: 10.1038/s41541-021-00294-8
27. Gibson JF, Johnston SA. Immunity to *Cryptococcus neoformans* and *C. gattii* During Cryptococcosis. *Fungal Genet Biol* (2015) 78:76–86. doi: 10.1016/j.fgb.2014.11.006
28. Rohatgi S, Pirofski L-A. Host Immunity to *Cryptococcus neoformans*. *Future Microbiol* (2015) 10:565–81. doi: 10.2217/fmb.14.132
29. Trevijano-Contador N, Pirofski L. Antibody Immunity and Natural Resistance to Cryptococcosis. *Curr Trop Med Rep* (2019) 6:50–4. doi: 10.1007/s40475-019-00174-1
30. Mukaremera L, Nielsen K. Adaptive Immunity to *Cryptococcus neoformans* Infections. *J Fungi (Basel)* (2017) 3:64. doi: 10.3390/jof3040064
31. Szymczak WA, Davis MJ, Lundy SK, Dufaud C, Olszewski M, Pirofski L-A. X-Linked Immunodeficient Mice Exhibit Enhanced Susceptibility to *Cryptococcus neoformans* Infection. *mBio* (2013) 4:e00265–13. doi: 10.1128/mBio.00265-13
32. Marquis G, Montplaisir S, Pelletier M, Mosseau S, Auger P. Genetic Resistance to Murine Cryptococcosis: Increased Susceptibility in the CBA/N Xid Mutant Strain of Mice. *Infect Immun* (1985) 47:282–7. doi: 10.1128/iai.47.1.282-287.1985
33. Subramaniam KS, Datta K, Quintero E, Manix C, Marks MS, Pirofski L-A. The Absence of Serum IgM Enhances the Susceptibility of Mice to Pulmonary Challenge With *Cryptococcus neoformans*. *J Immunol* (2010) 184:5755–67. doi: 10.4049/jimmunol.0901638
34. Zhong Z, Pirofski L-A. Opsonization of *Cryptococcus neoformans* by Human Anticryptococcal Glucuronoxylomannan Antibodies. *Infect Immun* (1996) 64:3446–50. doi: 10.1128/iai.64.9.3446-3450.1996
35. Rodrigues MI, Travassos LR, Miranda KR, Franzen AJ, Rozental S, Souza W, et al. Human Antibodies Against a Purified Glucosylceramide From *Cryptococcus neoformans* Inhibit Cell Budding and Fungal Growth. *Infect Immun* (2000) 68:7049–60. doi: 10.1128/IAI.68.12.7049-7060.2000
36. Nabavi N, Murphy JW. Antibody-Dependent Natural Killer Cell-Mediated Growth Inhibition of *Cryptococcus neoformans*. *Infect Immun* (1986) 51:556–62. doi: 10.1128/iai.51.2.556-562.1986
37. Netski D, Kozel TR. Fc-Dependent and Fc-Independent Opsonization of *Cryptococcus neoformans* by Anticapsular Monoclonal Antibodies: Importance of Epitope Specificity. *Infect Immun* (2002) 70:2812–9. doi: 10.1128/iai.70.6.2812-2819.2002
38. Mukherjee S, Lee SC, Casadevall A. Antibodies to *Cryptococcus neoformans* Glucuronoxylomannan Enhance Antifungal Activity of Murine Macrophages. *Infect Immun* (1995) 63:573–9. doi: 10.1128/iai.63.2.573-579.1995
39. Dromer F, Perronne C, Barge J, Vilde JL, Yeni P. Role of IgG and Complement Component C5 in the Initial Course of Experimental Cryptococcosis. *Clin Exp Immunol* (1989) 78:412–7.
40. Gadebusch HH. Specific Degradation of *Cryptococcus neoformans* 3723 Capsular Polysaccharide by a Microbial Enzyme: III. Antibody Stimulation by Partially Decapsulated Cells. *J Infect Dis* (1960) 107:406–9. doi: 10.1093/infdis/107.3.406
41. Gadebusch HH. Immunization Against *Cryptococcus neoformans* by Capsular Polysaccharide. *Nature* (1963) 199:710. doi: 10.1038/199710a0
42. Devi SJ. Preclinical Efficacy of a Glucuronoxylomannan-Tetanus Toxoid Conjugate Vaccine of *Cryptococcus neoformans* in a Murine Model. *Vaccine* (1996) 14:841–4. doi: 10.1016/0264-410X(95)00256-Z
43. Fleuridor R, Lees A, Pirofski L. A Cryptococcal Capsular Polysaccharide Mimotope Prolongs the Survival of Mice With *Cryptococcus neoformans* Infection. *J Immunol* (2001) 166:1087–96. doi: 10.4049/jimmunol.166.2.1087

44. Maitta RW, Datta K, Lees A, Belouski SS, Pirofski L-A. Immunogenicity and Efficacy of *Cryptococcus neoformans* Capsular Polysaccharide Glucuronoxylomannan Peptide Mimotope-Protein Conjugates in Human Immunoglobulin Transgenic Mice. *Infect Immun* (2004) 72:196–208. doi: 10.1128/iai.72.1.196-208.2004
45. Chaturvedi AK, Weintraub ST, Lopez-Ribot JL, Wormley FL. Identification and Characterization of *Cryptococcus neoformans* Protein Fractions That Induce Protective Immune Responses. *Proteomics* (2013) 13:3429–41. doi: 10.1002/pmic.201300213
46. Chaturvedi AK, Hameed RS, Wozniak KL, Hole CR, Leopold Wager CM, Weintraub ST, et al. Vaccine-Mediated Immune Responses to Experimental Pulmonary *Cryptococcus gattii* Infection in Mice. *PLoS One* (2014) 9:e104316. doi: 10.1371/journal.pone.0104316
47. Dromer F, Aucouturier P, Clauvel JP, Saimot G, Yeni P. *Cryptococcus neoformans* Antibody Levels in Patients With AIDS. *Scand J Infect Dis* (1988) 20:283–5. doi: 10.3109/00365548809032452
48. Houpt DC, Pfrommer GS, Young BJ, Larson TA, Kozel TR. Occurrences, Immunoglobulin Classes, and Biological Activities of Antibodies in Normal Human Serum That Are Reactive With *Cryptococcus neoformans* Glucuronoxylomannan. *Infect Immun* (1994) 62:2857–64. doi: 10.1128/iai.62.7.2857-2864.1994
49. Deshaw M, Pirofski L-A. Antibodies to the *Cryptococcus neoformans* Capsular Glucuronoxylomannan are Ubiquitous in Serum From HIV+ and HIV- Individuals. *Clin Exp Immunol* (1995) 99:425–32. doi: 10.1111/j.1365-2249.1995.tb05568.x
50. Dromer F, Denning DW, Stevens DA, Noble A, Hamilton JR. Anti-*Cryptococcus neoformans* Antibodies During Cryptococcosis in Patients With the Acquired Immunodeficiency Syndrome. *Serodiag Immun Inf D* (1995) 7:181–8. doi: 10.1016/0888-0786(96)87297-0
51. Abadi J, Pirofski L-A. Antibodies Reactive With the Cryptococcal Capsular Polysaccharide Glucuronoxylomannan Are Present in Sera From Children With and Without Human Immunodeficiency Virus Infection. *J Infect Dis* (1999) 180:915–9. doi: 10.1086/314953
52. Fleuridor R, Lyles RH, Pirofski L. Quantitative and Qualitative Differences in the Serum Antibody Profiles of Human Immunodeficiency Virus-Infected Persons With and Without *Cryptococcus neoformans* Meningitis. *J Infect Dis* (1999) 180:1526–35. doi: 10.1086/315102
53. Subramaniam K, French N, Pirofski L-A. *Cryptococcus neoformans*-Reactive and Total Immunoglobulin Profiles of Human Immunodeficiency Virus-Infected and Uninfected Ugandans. *Clin Diagn Lab Immunol* (2005) 12:1168–76. doi: 10.1128/CDLI.12.10.1168-1176.2005
54. Jalali Z, Ng L, Singh N, Pirofski L-A. Antibody Response to *Cryptococcus neoformans* Capsular Polysaccharide Glucuronoxylomannan in Patients After Solid-Organ Transplantation. *Clin Vaccine Immunol* (2006) 13:740–6. doi: 10.1128/CVI.00139-06
55. Subramaniam K, Metzger B, Hanau LH, Guh A, Rucker L, Badri S, et al. IgM (+) Memory B Cell Expression Predicts HIV-associated Cryptococcosis Status. *J Infect Dis* (2009) 200:244–51. doi: 10.1086/599318
56. Rohatgi S, Gohil S, Kuniholm MH, Schultz H, Dufaud C, Armour KL, et al. Fc Gamma Receptor 3A Polymorphism and Risk for HIV-associated Cryptococcal Disease. *mBio* (2013) 4:e00573–13. doi: 10.1128/mBio.00573-13
57. Pitzurra L, Perito S, Baldelli F, Bistoni F, Vecchiarelli A. Humoral Response Against *Cryptococcus neoformans* Mannoprotein Antigens in HIV-Infected Patients. *Clin Exp Immunol* (2003) 133:91–6. doi: 10.1046/j.1365-2249.2003.02181.x
58. Chen L-C, Goldman DL, Doering TL, Pirofski L-A, Casadevall A. Antibody Response to *Cryptococcus neoformans* Proteins in Rodents and Humans. *Infect Immun* (1999) 67:2218–24. doi: 10.1128/IAI.67.5.2218-2224.1999
59. Goldman DL, Khine H, Abadi J, Lindenberg DJ, Pirofski L-A, Ramata N, et al. Goldman Et al. - 2001 - Serologic Evidence for *Cryptococcus neoformans* Inf. *Pediatrics* (2001) 107:1–6. doi: 10.1542/peds.107.5.e66
60. Davis J, Yong Zheng W, Glatman-Freedman A, Navoa JA, Pagcatipunan MR, Lessin H, et al. Serologic Evidence for Regional Differences in Pediatric Cryptococcal Infection. *Pediatr Infect Dis J* (2007) 26:549–51. doi: 10.1097/INF.0b013e318047e073
61. Saha DC, Xess I, Zeng WY, Goldman DL. Antibody Responses to *Cryptococcus neoformans* in Indian Patients With Cryptococcosis. *Med Mycol* (2008) 46:457–63. doi: 10.1080/13693780801961345
62. Chai HC, Tay ST. Detection of IgM and IgG Antibodies to *Cryptococcus neoformans* Proteins in Blood Donors and HIV Patients With Active Cryptococcosis. *Mycoses* (2009) 52:166–70. doi: 10.1111/j.1439-0507.2008.01549.x
63. Young M, Macias S, Thomas D, Wormley FL. A Proteomic-Based Approach for the Identification of Immunodominant *Cryptococcus neoformans* Proteins. *Proteomics* (2009) 9:2578–88. doi: 10.1002/pmic.200800713
64. Firacative C, Gressler AE, Schubert K, Schulze B, Müller U, Brombacher F, et al. Identification of T Helper (Th)1- and Th2-associated Antigens of *Cryptococcus neoformans* in a Murine Model of Pulmonary Infection. *Sci Rep* (2018) 8:2681. doi: 10.1038/s41598-018-21039-z
65. Jobbins SE, Hill CJ, D'Souza-Basseal JM, Padula MP, Herbert BR, Krockenberger MB. Immunoproteomic Approach to Elucidating the Pathogenesis of Cryptococcosis Caused by *Cryptococcus gattii*. *J Proteome Res* (2010) 9:3832–41. doi: 10.1021/pr100028t
66. Martins LM, Andrade HM, Vainstein MH, Wanke B, Schrank A, Balaguez CB, et al. Immunoproteomics and Immunoinformatics Analysis of *Cryptococcus gattii* Novel Candidate Antigens for Diagnosis. *Future Microbiol* (2013) 8:549–63. doi: 10.2217/fmb.13.22
67. Mohrs M, Ledermann B, Köhler G, Dorfmueller A, Gessner A, Brombacher F. Differences Between IL-4- and IL-4 Receptor  $\alpha$ -Deficient Mice in Chronic Leishmaniasis Reveal a Protective Role for IL-13 Receptor Signaling. *J Immunol* (1999) 162:7302–8.
68. Müller U, Stenzel W, Köhler G, Werner C, Polte T, Hansen G, et al. IL-13 Induces Disease-Promoting Type 2 Cytokines, Alternatively Activated Macrophages and Allergic Inflammation During Pulmonary Infection of Mice With *Cryptococcus neoformans*. *J Immunol* (2007) 179:5367–77. doi: 10.4049/jimmunol.179.8.5367
69. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric Method for Determination of Sugars and Related Substances. *Anal Chem* (1956) 28:350–6. doi: 10.1021/ac60111a017
70. Rojas Echeverri JC, Milkovska-Stamenova S, Hoffmann R. A Workflow Towards the Reproducible Identification and Quantitation of Protein Carbonylation Sites in Human Plasma. *Antioxid (Basel)* (2021) 10:369. doi: 10.3390/antiox10030369
71. Geib E, Baldeweg F, Doerfer M, Nett M, Brock M. Cross-Chemistry Leads to Product Diversity From Atromentin Synthetases in *Aspergillus niger*. *Cell Chem Biol* (2019) 26:223–34. doi: 10.1016/j.chembiol.2018.10.021
72. Fleck CB, Brock M. *Aspergillus fumigatus* Catalytic Glucokinase and Hexokinase: Expression Analysis and Importance for Germination, Growth, and Conidiation. *Eukaryot Cell* (2010) 9:1120–35. doi: 10.1128/EC.00362-09
73. Geib E, Brock M. ATNT: An Enhanced System for Expression of Polycistronic Secondary Metabolite Gene Clusters in *Aspergillus niger*. *Fungal Biol Biotechnol* (2017) 4:13. doi: 10.1186/s40694-017-0042-1
74. Grahner A, Müller U, Buttlar Hv, Treudler R, Alber G. Analysis of Asthma Patients for Cryptococcal Seroreactivity in an Urban German Area. *Med Mycol* (2015) 53:576–86. doi: 10.1093/mmy/myv024
75. Müller U, Stenzel W, Köhler G, Polte T, Blessing M, Mann A, et al. A Gene-Dosage Effect for Interleukin-4 Receptor Alpha-Chain Expression has an Impact on Th2-Mediated Allergic Inflammation During Bronchopulmonary Mycosis. *J Infect Dis* (2008) 198:1714–21. doi: 10.1086/593068
76. Rodrigues ML, Nakayasu ES, Oliveira DL, Nimrichter L, Nosanchuk JD, Almeida IC, et al. Extracellular Vesicles Produced by *Cryptococcus neoformans* Contain Protein Components Associated With Virulence. *Eukaryot Cell* (2008) 7:58–67. doi: 10.1128/EC.00370-07
77. McGowan JP, Shah SS, Small CB, Klein RS, Schnipper SM, Chang CJ, et al. Relationship of Serum Immunoglobulin and IgG Subclass Levels to Race, Ethnicity and Behavioral Characteristics in HIV Infection. *Med Sci Monit* (2006) 12:CR11–6.
78. Lugada ES, Mermin J, Asjo B, Kaharuzza F, Downing R, Langeland N, et al. Immunoglobulin Levels Amongst Persons With and Without Human Immunodeficiency Virus Type 1 Infection in Uganda and Norway. *Scand J Infect Dis* (2004) 59:203–8. doi: 10.1111/j.0300-9475.2004.01376.x
79. Shirai A, Cosentino M, Leitman-Klinman SF, Klinman DM. Human Immunodeficiency Virus Infection Induces Both Polyclonal and Virus-Specific B Cell Activation. *J Clin Invest* (1992) 89:561–6. doi: 10.1172/JCI115621

80. Casadevall A, Scharff MD. The Mouse Antibody Response to Infection With *Cryptococcus neoformans*: VH and VL Usage in Polysaccharide Binding Antibodies. *J Exp Med* (1991) 174:151–60. doi: 10.1084/jem.174.1.151
81. Garcia-Hermoso D, Janbon G, Dromer F. Epidemiological Evidence for Dormant *Cryptococcus neoformans* Infection. *J Clin Microbiol* (1999) 37:3204–9. doi: 10.1128/JCM.37.10.3204-3209.1999
82. Hagen F, van Assen S, Luijckx GJ, Boekhout T, Kampinga GA. Activated Dormant *Cryptococcus gattii* Infection in a Dutch Tourist Who Visited Vancouver Island (Canada): A Molecular Epidemiological Approach. *Med Mycol* (2010) 48:528–31. doi: 10.3109/13693780903300319
83. Rodrigues ML, Nimrichter L, Cordero RJ, Casadevall A. Fungal Polysaccharides: Biological Activity Beyond the Usual Structural Properties. *Front Microbiol* (2011) 2:171. doi: 10.3389/fmicb.2011.00171
84. Underhill DM, Iliiev ID. The Mycobiota: Interactions Between Commensal Fungi and the Host Immune System. *Nat Rev Immunol* (2014) 14:405–16. doi: 10.1038/nri3684
85. Dufaud C, Rivera J, Rohatgi S, Pirofski L-A. Naive B Cells Reduce Fungal Dissemination in *Cryptococcus neoformans* Infected Rag1<sup>-/-</sup> Mice. *Virulence* (2018) 9:173–84. doi: 10.1080/21505594.2017.1370529
86. Specht CA, Lee CK, Huang H, Hester MM, Liu J, Luckie BA, et al. Vaccination With Recombinant *Cryptococcus* Proteins in Glucan Particles Protects Mice Against Cryptococcosis in a Manner Dependent Upon Mouse Strain and Cryptococcal Species. *mBio* (2017) 8:e01872–17. doi: 10.1128/mBio.01872-17
87. Hester MM, Lee CK, Abraham A, Khoshkenar P, Ostroff GR, Levitz SM, et al. Protection of Mice Against Experimental Cryptococcosis Using Glucan Particle-Based Vaccines Containing Novel Recombinant Antigens. *Vaccine* (2020) 38:620–6. doi: 10.1016/j.vaccine.2019.10.051
88. Vu K, Tham R, Uhrig JP, Thompson GR, Na Pombeyra S, Jamklang M, et al. Invasion of the Central Nervous System by *Cryptococcus neoformans* Requires a Secreted Fungal Metalloprotease. *mBio* (2014) 5:e01101–14. doi: 10.1128/mBio.01101-14
89. Eigenheer RA, Jin Lee Y, Blumwald E, Phinney BS, Gelli A. Extracellular Glycosylphosphatidylinositol-Anchored Mannoproteins and Proteases of *Cryptococcus neoformans*. *FEMS Yeast Res* (2007) 7:499–510. doi: 10.1111/j.1567-1364.2006.00198.x
90. Aaron PA, Vu K, Gelli A. An Antivirulence Approach for Preventing *Cryptococcus neoformans* From Crossing the Blood-Brain Barrier Via Novel Natural Product Inhibitors of a Fungal Metalloprotease. *mBio* (2020) 11:e01249–20. doi: 10.1128/mBio.01249-20
91. Singh A, Panting RJ, Varma A, Saijo T, Waldron KJ, Jong A, et al. Factors Required for Activation of Urease as a Virulence Determinant in *Cryptococcus neoformans*. *mBio* (2013) 4:e00220–13. doi: 10.1128/mBio.00220-13
92. Rutherford JC. The Emerging Role of Urease as a General Microbial Virulence Factor. *PLoS Pathog* (2014) 10:e1004062. doi: 10.1371/journal.ppat.1004062.g001
93. Almeida F, Wolf JM, Casadevall A. Virulence-Associated Enzymes of *Cryptococcus neoformans*. *Eukaryot Cell* (2015) 14:1173–85. doi: 10.1128/EC.00103-15
94. Ikeda R, Ichikawa T, Tsukiji Y-K, Kawamura K, Kikuchi A, Ishida Y-I, et al. Identification of Heparin-Binding Proteins on the Cell Surface of *Cryptococcus neoformans*. *Med Mycol* (2018) 59(3):E47–52. doi: 10.3314/mmj.18-00001
95. Stie J, Bruni G, Fox D. Surface-Associated Plasminogen Binding of *Cryptococcus neoformans* Promotes Extracellular Matrix Invasion. *PLoS One* (2009) 4:e5780. doi: 10.1371/journal.pone.0005780
96. Missall TA, Pusateri ME, Donlin MJ, Chambers KT, Corbett JA, Lodge JK. Posttranslational, Translational, and Transcriptional Responses to Nitric Oxide Stress in *Cryptococcus neoformans*: Implications for Virulence. *Eukaryot Cell* (2006) 5:518–29. doi: 10.1128/EC.5.3.518-529.2006
97. Crestani J, Carvalho PC, Han X, Seixas A, Broetto L, Fischer JD, et al. Proteomic Profiling of the Influence of Iron Availability on *Cryptococcus gattii*. *J Proteome Res* (2012) 11:189–205. doi: 10.1021/pr2005296
98. Lee SA, Mao Y, Zhang Z, Wong B. Overexpression of a Dominant-Negative Allele of YPT1 Inhibits Growth and Aspartyl Protease Secretion in *Candida albicans*. *Microbiology* (2001) 147:1961–70. doi: 10.1099/00221287-147-7-1961
99. Segev N, Mulholland J, Botstein D. The Yeast Gtp-Binding YPT1 Protein and a Mammalian Counterpart Are Associated With the Secretion Machinery. *Cell* (1988) 52:915–24. doi: 10.1016/0092-8674(88)90433-3
100. Bacon RA, Salminen A, Ruohola H, Novick P, Ferro-Novick S. The GTP-binding Protein YPT1 Is Required for Transport in Vitro: The Golgi Apparatus Is Defective in Ypt1 Mutants. *J Cell Biol* (1989) 109:1015–22. doi: 10.1083/jcb.109.3.1015
101. Segev N, Botstein D. The Ras-Like Yeast Ypt1 Gene is Itself Essential for Growth, Sporulation, and Starvation Response. *Mol Cell Biol* (1987) 7:2367–77. doi: 10.1128/MCB.7.7.2367
102. Kakeya H, Udono H, Maesaki S, Sasaki E, Kawamura S, Hossain MA, et al. Heat Shock Protein 70 (hsp70) as a Major Target of the Antibody Response in Patients With Pulmonary Cryptococcosis. *Clin Exp Immunol* (1999) 115:485–90. doi: 10.1046/j.1365-2249.1999.00821.x
103. Kakeya H, Udono H, Ikuno N, Yamamoto Y, Mitsutake K, Miyazaki T, et al. A 77-Kilodalton Protein of *Cryptococcus neoformans*, a Member of the Heat Shock Protein 70 Family, is a Major Antigen Detected in the Sera of Mice With Pulmonary Cryptococcosis. *Infect Immun* (1997) 5:1653–8. doi: 10.1128/iai.65.5.1653-1658.1997
104. Silveira CP, Piffer AC, Kmetzsch L, Fonseca FL, Soares DA, Staats CC, et al. The Heat Shock Protein (Hsp) 70 of *Cryptococcus neoformans* Is Associated With the Fungal Cell Surface and Influences the Interaction Between Yeast and Host Cells. *Fungal Genet Biol* (2013) 60:53–63. doi: 10.1016/j.fgb.2013.08.005
105. Eastman AJ, He X, Qiu Y, Davis MJ, Vedula P, Lyons DM, et al. Cryptococcal Heat Shock Protein 70 Homolog Ssa1 Contributes to Pulmonary Expansion of *Cryptococcus neoformans* During the Afferent Phase of the Immune Response by Promoting Macrophage M2 Polarization. *J Immunol* (2015) 194:5999–6010. doi: 10.4049/jimmunol.1402719

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Gressler, Volke, Firacative, Schnabel, Müller, Krizsan, Schulze-Richter, Brock, Brombacher, Escandón, Hoffmann and Alber. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

## 4 Summary

Cryptococcal meningitis is a fatal systemic disease caused by the opportunistic fungal pathogen *C. neoformans* leading to an estimated number of 180,000 deaths per year<sup>1</sup>. This disease mainly affects immunocompromised patients, mostly AIDS patients<sup>2-4</sup>. Besides the search for novel antifungal compounds, research of the past years focused on the development of an anti-cryptococcal vaccine to mediate protection against severe disease. However, to date only a limited number of immunogenic fungal protein antigens have been identified which could potentially be used for vaccine development.

The two studies presented in this thesis demonstrate the applicability of an immunoproteomic approach for the identification of immunoreactive cryptococcal proteins potentially suitable for vaccine development. We used two-dimensional gel electrophoresis to separate cryptococcal proteins, Western blotting, and subsequent incubation with murine or human sera for identification of immunoreactive cryptococcal proteins. Furthermore, the composition of total serum and *C. neoformans*-specific antibodies of different isotypes contained in the sera was quantified using ELISA analysis.

### **Mice of different genotypes develop different disease phenotypes after cryptococcal infection and show predominant production of Th2-associated IgG1 antibodies.**

The first study used sera from *C. neoformans*-infected mice of different genotypes which develop different courses of disease. BALB/c wild type (WT) mice intranasally infected with *C. neoformans* succumb to the infection after a median time of ten weeks<sup>5,6</sup>, whereas IL-12-deficient mice, showing impaired T helper (Th) type 1 responses, succumb significantly earlier to the infection compared to WT mice<sup>7</sup>. In contrast, IL-4R $\alpha$ -deficient mice, with an impaired ability to form Th2 responses, are resistant to severe disease and develop a latent pulmonary infection<sup>6</sup>. This highlights the importance of immune response polarization towards a Th1 bias, favoring fungal clearance, in contrast to a Th2 bias promoting fungal growth and dissemination. Interestingly, the production of different antibody isotypes has been linked to distinct cytokines. The Th2 cytokine IL-4 favors production of IgG1 antibodies and the Th1 cytokine IFN- $\gamma$  is associated with IgG2a production<sup>8-11</sup>. We used this connection to identify cryptococcal proteins contained in spots reactive with either IgG2a or IgG1 antibodies, which are therefore associated with protective Th1 or detrimental Th2 responses, respectively.

The total serum concentrations of Th2-associated IgG1 antibodies were significantly higher in *C. neoformans*-infected mice compared to naïve mice for all genotypes, although total serum IgG1 antibody concentrations were significantly lower in IL-4R $\alpha$ -deficient mice compared to WT and IL-12-deficient mice, representing the Th2-dependency of IgG1 induction. In contrast, total serum concentrations of Th1-associated IgG2a antibodies were similar in naïve and infected mice and therefore unaffected by the infection. Nevertheless, there were genotype-dependent differences, as Th1-impaired IL-12-deficient mice had lower total serum IgG2a concentrations compared to the other two mouse strains. IgG1 and IgG2a antibodies directed against cryptococcal proteins were not detectable in sera of

naïve mice of all genotypes using ELISA analysis. Anti-cryptococcal serum IgG1 titers increased strongly upon infection in WT and IL-12-deficient mice, whereas titers in sera from IL-4R $\alpha$ -deficient mice showed only a moderate, but nevertheless significant increase, again reflecting genotype-dependent differences. Furthermore, we found a significant correlation between increasing total serum IgG1 concentrations and titers of anti-cryptococcal IgG1 antibodies. Therefore, we hypothesize that the observed increase of Th2-associated IgG1 antibodies is triggered by cryptococcal infection. In contrast, anti-cryptococcal IgG2a titers were only moderately, but still significantly increased upon infection in WT and IL-4R $\alpha$ -deficient mice, but not IL-12-deficient mice, reflecting the impaired production of IgG2a antibodies in IL-12-deficient mice. Furthermore, total serum IgG2a concentrations and anti-cryptococcal IgG2a titers were not correlated. All in all, analysis of antifungal antibodies reflects the Th2- and not Th1-biased immune response observed upon cryptococcal infection.

**Two-dimensional immunoproteomic analysis using murine sera leads to identification of cryptococcal proteins contained in Th1-associated IgG2a-reactive, or Th2-associated IgG1-reactive protein spots.**

For the identification of immunoreactive protein antigens, cryptococcal proteins were separated by two-dimensional gel electrophoresis, blotted on nitrocellulose membranes, and incubated with sera from individual mice of different genotypes. Anti-cryptococcal IgG1 or IgG2a antibodies were detected using isotype-specific secondary antibodies and spot patterns were compared between naïve and infected mice, as well as between mice from different genotypes. Cryptococcal protein spots reactive with anti-cryptococcal IgG1 antibodies were not detected in sera from naïve mice but were present when membranes were incubated with sera from infected WT and IL-12-deficient mice. Interestingly, sera from IL-12-deficient mice reacted with an increased number of cryptococcal protein spots compared to WT mice. Analysis of IgG2a antibodies revealed cryptococcal protein spots reactive with serum IgG2a from naïve mice of all genotypes. Incubation of cryptococcal proteins with sera from infected IL-12-deficient mice resulted in the same spot pattern as for naïve mice. In contrast, sera from infected WT and IL-4R $\alpha$ -deficient mice recognized additional protein spots, with the highest number of spots observed after incubation with sera from IL-4R $\alpha$ -deficient mice.

Mass spectrometry analysis of all immunoreactive spots was carried out and the most abundant protein of each spot was selected as a potentially immunoreactive protein. Ten proteins were exclusively present in IgG1-reactive spots and four proteins were only identified in IgG2a-reactive spots, out of which one protein was identified in the five spots also recognized by sera from naïve mice, indicating potential cross-reactivity with other fungal proteins. Three proteins were found in both IgG1- and IgG2a-reactive spots. Eight out of seventeen of the proteins identified in the immunoreactive spots of our study were previously described to be contained in immunoreactive cryptococcal protein spots by other publications<sup>12-16</sup>, strengthening the probability of immunogenic properties. Furthermore, several of the identified proteins were described to be implicated in cryptococcal virulence, rendering them potential targets for anti-cryptococcal agents.

**Serologic analysis of human sera reveals increased titers of anti-cryptococcal IgG, but not IgM antibodies in sera from HIV-negative cryptococcal meningitis (CM) patients, but not HIV-positive CM patients compared to healthy individuals.**

The second study focuses on the identification of cryptococcal proteins immunoreactive with human sera from cryptococcal meningitis (CM) patients and healthy individuals. We used sera from HIV-positive CM patients (CD4<sup>+</sup> T cells <250 cells/ $\mu$ L), HIV-negative CM patients and healthy individuals, all living in Colombia in order to guarantee similar environmental microbial exposure, also to *C. neoformans*. We aimed to determine proteins preferentially recognized by sera from CM patients, rendering them disease-associated proteins. These proteins are of interest because of potential implications in virulence, but also because of their potential for vaccine development, as they are immunogenic even in patients with severely impaired immune responses.

Previous publications produced mixed results regarding the main isotype produced in response to cryptococcal infection. Additionally, in contrast to anti-cryptococcal capsular polysaccharide (CPS) titers<sup>17–29</sup>, the quantification of anti-cryptococcal protein antibodies often relied on semi-quantitative methods like counting of bands on Western blots for individual sera<sup>23,24,30–34</sup>. Therefore, we decided to quantify the concentrations of total serum antibodies and titers of *C. neoformans*-specific antibodies of the isotypes IgG and IgM in human sera. The total serum IgG concentrations were similar in sera from all three groups. In contrast, total serum IgM concentrations were lower in sera from CM patients, independently of their HIV status, compared to healthy individuals. Interestingly, previous publications found decreased proportions of IgM-expressing memory B cells in both, HIV-positive and HIV-negative cryptococcosis patients compared to healthy individuals<sup>25,27</sup>. However, titers of anti-cryptococcal IgM antibodies directed against cryptococcal proteins or CPS were similar for all groups. In contrast, anti-protein and anti-CPS IgG titers were increased in sera from HIV-negative CM patients, but not HIV-positive CM patients, compared to healthy individuals. We hypothesize that the severely immunocompromised HIV-positive CM patients are not able to generate a strong immune response towards *C. neoformans* and are therefore lacking the increase in anti-cryptococcal IgG titers observed for HIV-negative CM patients in response to cryptococcal infection.

**Induction of anti-cryptococcal IgG, but not IgM antibody production during cryptococcal infection is confirmed by analysis of murine sera.**

To further investigate the dominant isotype produced in response to *C. neoformans* infection, we analyzed sera from naïve and intranasally infected BALB/c WT mice, susceptible to disseminated cryptococcal disease, and IL-4R $\alpha$ -deficient mice, developing a latent pulmonary cryptococcal infection. Over the course of infection, WT mice showed significantly higher fungal burden in the lungs compared to IL-4R $\alpha$ -deficient mice. IgM antibodies directed against cryptococcal proteins and CPS were detected at similar titers over the course of infection, although concentrations of total serum IgM antibodies were increased by day 60 post infection for both genotypes. Anti-CPS IgM antibodies were even present in naïve mice, indicating cross-reactivity with polysaccharides from other microbes. In contrast, titers of anti-cryptococcal protein and CPS IgG antibodies as well as total serum IgG concentrations increased

over the course of infection for mice of both genotypes. Interestingly, anti-cryptococcal IgG titers were significantly higher in WT mice compared to IL-4R $\alpha$ -deficient mice 60 days post infection. Furthermore, titers of anti-cryptococcal IgG antibodies were positively correlated with lung fungal burden in WT mice, but not in IL-4R $\alpha$ -deficient mice. Based on the serological data from human CM patients and experimentally infected mice, we therefore hypothesize that production of IgG, but not IgM antibodies is induced during cryptococcal infection and that the increase of anti-cryptococcal IgG titers is triggered by high fungal burden and disseminated disease.

**Two-dimensional immunoproteomic analysis of sera from Colombian CM patients and healthy individuals leads to the identification of disease-associated immunoreactive cryptococcal proteins.**

Two-dimensional immunoproteomic analysis was applied to identify immunoreactive cryptococcal protein spots. *C. neoformans* proteins were separated by two-dimensional gel electrophoresis and blotted on nitrocellulose membranes. Human sera were combined in subpools for each experimental group and used for membrane incubation. Whole cryptococcal proteins as well as immunoreactive protein spots were detected using fluorescence-based protein detection and fluorophore-coupled anti-human IgG antibodies to allow for quantification. Binding of IgG to protein spots was compared between the three groups (HIV-positive and HIV-negative CM patients, healthy control persons) using the software Delta2D (DECODON). Briefly, fluorescence intensity in each immunoreactive spot was quantified, normalized to the protein loading, and statistically analyzed with Delta2D. The analysis revealed several CM-associated spots, showing increased reactivity with sera from HIV-positive and HIV-negative CM patients compared to healthy individuals, but also spots exhibiting stronger reactivity with sera from healthy individuals, HIV-positive, or HIV-negative CM patients compared to the other respective groups. Mass spectrometry analysis of those spots of interest resulted in the identification of 143 cryptococcal proteins. Twenty-three proteins were selected for recombinant expression in *Escherichia coli* based on their abundance in several spots of interest, previously described potential immunoreactivity, or implication in cryptococcal virulence. The identity of the recombinantly produced proteins was confirmed using mass spectrometry. Quantification of immunoreactivity of the recombinant proteins was achieved by analysis of IgG-binding to the respective proteins contained in *Escherichia coli* protein lysates on Western blots and normalization of the immunoreactive signal by protein loading. Immunoreactivity of all 23 cryptococcal proteins could be confirmed with sera from both cryptococcal meningitis patients and healthy individuals. Fourteen of these proteins are newly described as immunoreactive protein antigens, whereas nine proteins were previously reported to be contained in spots immunoreactive with sera from *C. neoformans*-infected mice<sup>12-14,35</sup>, or koalas<sup>15</sup> and humans<sup>16</sup> infected with the closely related fungal pathogen *Cryptococcus gattii*. However, this is the first study to confirm immunoreactivity of those proteins using recombinant expression and subsequent incubation with sera. Quantification of the immunoreactivity revealed a disease-associated recognition pattern for twelve of the 23 proteins, defined by significantly stronger reactivity with sera from CM patients independently of their HIV status compared to healthy individuals. The identified proteins

are very interesting candidates for future research as they were recognized even by sera from severely immunocompromised AIDS patients. Three of those proteins show favorable properties for usage as vaccine candidates like extracellular appearance<sup>36</sup> or existence of a secretory signal peptide, and no significant homology to human proteins. Supportively, one protein identified in our analysis, extracellular elastinolytic metalloprotease, was already included in a murine anti-cryptococcal vaccination study and was demonstrated to increase survival of *C. neoformans*-infected C57BL/6 WT mice<sup>37</sup>. Furthermore, six proteins described as disease-associated proteins in our study are implicated in cryptococcal virulence, survival or metabolism and could therefore be targeted by antifungal drugs. Interestingly, the inhibition of the previously mentioned extracellular elastinolytic metalloproteinase prevented crossing of cryptococcal cells through the blood brain barrier in an *in vitro* transwell model<sup>38</sup>, strengthening our hypothesis that targeting disease-associated proteins could be utilized for anti-cryptococcal therapy.

### Conclusions

This thesis demonstrates the power of immunoproteomic approaches for the identification of immunoreactive cryptococcal proteins with excellent potential for development of an anti-cryptococcal vaccine or new antifungal therapy strategies.

Proteins identified in the first study<sup>35</sup> are contained in immunoreactive spots associated with protective Th1 or detrimental Th2 responses. Five of these proteins were recombinantly expressed in the second study<sup>39</sup> and immunoreactivity was confirmed. Two of those proteins showed stronger reactivity with sera from HIV-positive and HIV-negative CM patients compared to healthy individuals, whereas three proteins showed stronger reactivity with sera from HIV-positive, but not HIV-negative CM patients compared to healthy individuals. Reactivity of the proteins with antibodies from different species also highlights their strong immunogenicity and underlines the power of the immunoproteomic approach. The second study<sup>39</sup> led to the identification of 23 cryptococcal proteins showing confirmed immunoreactivity with human sera from cryptococcal meningitis patients but also healthy individuals, emphasizing their immunogenic potential. Fourteen of these proteins are newly described as immunoreactive cryptococcal proteins. Quantification of the immunoreactivity revealed a disease-associated recognition pattern for twelve proteins, characterized by significantly higher immunoreactivity with sera from CM patients compared to healthy individuals. Some of the identified proteins are potential vaccine candidates based on their immunogenic properties and lack of homology to human proteins. Additionally, some proteins are involved in fungal virulence or survival and are therefore interesting targets for new antifungal agents.

Previous studies showed promising results when using recombinant cryptococcal proteins for vaccination of mice<sup>37,40</sup>. Therefore, the immunoreactive cryptococcal proteins identified in this thesis are very promising candidates for future research and ultimately for the development of an anti-cryptococcal vaccine.



## 4.1 References

1. Rajasingham, R. *et al.*: Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 17(8), 873–881 (2017).
2. Aguiar, P. A. *et al.*: The epidemiology of cryptococcosis and the characterization of *Cryptococcus neoformans* isolated in a Brazilian University Hospital. *Rev Inst Med Trop São Paulo* 59, e13 (2017).
3. Pyrgos, V., Seitz, A. E., Steiner, C. A., Prevots, D. R. & Williamson, P. R.: Epidemiology of cryptococcal meningitis in the US: 1997-2009. *PLoS one* 8(2), e56269 (2013).
4. Sloan, D. J. & Parris, V.: Cryptococcal meningitis: epidemiology and therapeutic options. *Clin Epidemiol* 6, 169–182 (2014).
5. Müller, U. *et al.*: IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages and allergic inflammation during pulmonary infection of mice with *Cryptococcus neoformans*. *J Immunol* 179(8), 5367–5377 (2007).
6. Müller, U. *et al.*: A gene-dosage effect for interleukin-4 receptor alpha-chain expression has an impact on Th2-mediated allergic inflammation during bronchopulmonary mycosis. *J Infect Dis* 198(11), 1714–1721 (2008).
7. Decken, K. *et al.*: Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. *Infect Immun* 66(10), 4994–5000 (1998).
8. Germann, T. *et al.*: Interleukin-12 profoundly up-regulates the synthesis of antigen-specific complement-fixing IgG2a, IgG2b and IgG3 antibody subclasses in vivo. *Eur J Immunol* 25(3), 823–829 (1995).
9. Snapper, C. M. & Paul, W. E.: Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 236(4804), 944–947 (1987).
10. Snapper, C. M. & Mond, J. J.: Towards a comprehensive view of immunoglobulin class switching. *Immunol Today* 14(1), 15–17 (1993).
11. Finkelman, F. D. *et al.*: Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol* 8, 303–333 (1990).
12. Young, M., Macias, S., Thomas, D. & Wormley, F. L.: A proteomic-based approach for the identification of immunodominant *Cryptococcus neoformans* proteins. *Proteomics* 9(9), 2578–2588 (2009).
13. Chaturvedi, A. K., Weintraub, S. T., Lopez-Ribot, J. L. & Wormley, F. L.: Identification and characterization of *Cryptococcus neoformans* protein fractions that induce protective immune responses. *Proteomics* 13(23-24), 3429–3441 (2013).
14. Chaturvedi, A. K. *et al.*: Vaccine-mediated immune responses to experimental pulmonary *Cryptococcus gattii* infection in mice. *PLoS One* 9(8), e104316 (2014).
15. Jobbins, S. E. *et al.*: Immunoproteomic approach to elucidating the pathogenesis of cryptococcosis caused by *Cryptococcus gattii*. *J Proteome Res* 9(8), 3832–3841 (2010).
16. Martins, L. M. *et al.*: Immunoproteomics and immunoinformatics analysis of *Cryptococcus gattii* : novel candidate antigens for diagnosis. *Future Microbiol* 8(4), 549–563 (2013).
17. Dromer, F., Denning, D. W., Stevens, D. A., Noble, A. & Hamilton, J. R.: Anti-*Cryptococcus neoformans* antibodies during cryptococcosis in patients with the acquired immunodeficiency syndrome. *Serodiag Immun Inf D* 7(4), 181–188 (1995).
18. Houpt, D. C., Pfrommer, G. S., Young, B. J., Larson, T. A. & Kozel, T. R.: Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. *Infect Immun* 62(7), 2857–2864 (1994).
19. Deshaw, M. & Pirofski, L.-A.: Antibodies to the *Cryptococcus neoformans* capsular glucuronoxylomannan are ubiquitous in serum from HIV+ and HIV- individuals. *Clin Exp Immunol* 99(3), 425–432 (1995).
20. Abadi, J. & Pirofski, L.-A.: Antibodies reactive with the cryptococcal capsular polysaccharide glucuronoxylomannan are present in sera from children with and without human immunodeficiency virus infection. *J Infect Dis* 180(3), 915–919 (1999).
21. Subramaniam, K., French, N. & Pirofski, L.-A.: *Cryptococcus neoformans*-reactive and total immunoglobulin profiles of human immunodeficiency virus-infected and uninfected Ugandans. *Clin Diagn Lab Immunol* 12(10), 1168–1176 (2005).

22. Jalali, Z., Ng, L., Singh, N. & Pirofski, L.-A.: Antibody response to *Cryptococcus neoformans* capsular polysaccharide glucuronoxylomannan in patients after solid-organ transplantation. *Clin Vaccine Immunol* 13(7), 740–746 (2006).
23. Davis, J. *et al.*: Serologic evidence for regional differences in pediatric cryptococcal infection. *Pediatr Infect Dis J* 26(6), 549–551 (2007).
24. Saha, D. C., Xess, I., Zeng, W. Y. & Goldman, D. L.: Antibody responses to *Cryptococcus neoformans* in Indian patients with cryptococcosis. *Med Mycol* 46(5), 457–463 (2008).
25. Subramaniam, K. *et al.*: IgM(+) memory B cell expression predicts HIV-associated cryptococcosis status. *J Infect Dis* 200(2), 244–251 (2009).
26. Rohatgi, S. *et al.*: Fc gamma receptor 3A polymorphism and risk for HIV-associated cryptococcal disease. *mBio* 4(5), e00573-13 (2013).
27. Rohatgi, S. *et al.*: Antibody and B cell subset perturbations in human immunodeficiency virus-uninfected patients with cryptococcosis. *Open Forum Infect Dis* 51, ofx255 (2018).
28. Hlupeni, A. *et al.*: Antibody responses in HIV-infected patients with advanced immunosuppression and asymptomatic cryptococcal antigenemia. *Open forum infectious diseases* 61, ofy333 (2019).
29. Dromer, F., Aucouturier, P., Clauvel, J. P., Saimot, G. & Yeni, P.: *Cryptococcus neoformans* antibody levels in patients with AIDS. *Scand J Infect Dis* 20(3), 283–285 (1988).
30. Lamura, L. *et al.*: Analysis of antibody response to *Cryptococcus neoformans* in five patients with AIDS and cryptococcosis by immunoblotting. *J Infect* 401, 64–68 (2000).
31. Goldman, D. L. *et al.*: Serologic evidence for *Cryptococcus neoformans* infection in early childhood. *Pediatrics* 107(5), E66 (2001).
32. Saha, D. C. *et al.*: Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. *Clin Vaccine Immunol* 14(12), 1550–1554 (2007).
33. Chai, H. C. & Tay, S. T.: Detection of IgM and IgG antibodies to *Cryptococcus neoformans* proteins in blood donors and HIV patients with active cryptococcosis. *Mycoses* 52(2), 166–170 (2009).
34. Chen, L.-C., Goldman, D. L., Doering, T. L., Pirofski, L.-A. & Casadevall, A.: Antibody response to *Cryptococcus neoformans* proteins in rodents and humans. *Infect Immun* 67(5), 2218–2224 (1999).
35. Firacative, C. *et al.*: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection. *Sci Rep* 81, 2681 (2018).
36. Rodrigues, M. L. *et al.*: Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. *Eukaryot Cell* 71, 58–67 (2008).
37. Hester, M. M. *et al.*: Protection of mice against experimental cryptococcosis using glucan particle-based vaccines containing novel recombinant antigens. *Vaccine* 38(3), 620–626 (2020).
38. Aaron, P. A., Vu, K. & Gelli, A.: An antivirulence approach for preventing *Cryptococcus neoformans* from crossing the blood-brain barrier via novel natural product inhibitors of a fungal metalloprotease. *mBio* 11, e01249-20 (2020).
39. Gressler, A. E. *et al.*: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients. *Front Immunol* 12, 709695 (2021).
40. Specht, C. A. *et al.*: Vaccination with recombinant cryptococcus proteins in glucan particles protects mice against cryptococcosis in a manner dependent upon mouse strain and cryptococcal species. *mBio* 8(6), e01872-17 (2017).

## 5 Zusammenfassung

Der opportunistisch pathogene Pilz *Cryptococcus neoformans* ist der hauptsächliche Erreger der lebensbedrohlichen Kryptokokkenmeningitis, welche jährlich etwa 180.000 Todesfälle verursacht<sup>1</sup>. Die Krankheit betrifft hauptsächlich immunsupprimierte Personen, insbesondere AIDS-Patient:innen<sup>2-4</sup>. Zusätzlich zur Erforschung neuer anti-fungaler Substanzen ist daher die Entwicklung einer gegen *C. neoformans* wirksamen Vakzine seit mehreren Jahren ein Schwerpunkt der Kryptokokkenforschung. Jedoch wurden bisher nur wenige immunogene Pilzproteine identifiziert, die grundsätzlich für die Entwicklung einer Vakzine geeignet sind.

Die beiden in dieser Dissertation vorgestellten Arbeiten verdeutlichen die Anwendbarkeit von immunproteomischen Verfahren zur Identifizierung von immunreaktiven Kryptokokkenproteinen, welche grundsätzlich als Vakzin-Antigene genutzt werden können. Hierfür wurden Proteine mittels zweidimensionaler (2D-) Gelelektrophorese aufgetrennt, auf Nitrozellulosemembranen übertragen und für die Identifikation immunreaktiver Proteine mit murinen oder humanen Seren inkubiert. Des Weiteren wurden die absoluten Konzentrationen von Antikörpern sowie die Titer anti-fungaler Antikörper bestimmt.

### **Mäuse verschiedener Genotypen zeigen unterschiedliche Krankheitsverläufe nach Infektion mit *C. neoformans* und eine vorherrschende Produktion von Th2-assoziierten IgG1-Antikörpern.**

In der ersten Publikation wurden Seren von *C. neoformans*-infizierten Mäusen verschiedener Genotypen, welche nach der Infektion unterschiedliche Krankheitsverläufe ausbilden, verwendet. Intranasal mit *C. neoformans* infizierte BALB/c Wildtyp-Mäuse (WT-Mäuse) versterben nach durchschnittlich zehn Wochen an der disseminierten Erkrankung<sup>5,6</sup>, während Interleukin (IL)-12-defiziente Mäuse, welche in der Ausbildung von T-Helferzell (Th)-Antworten des Typs 1 beeinträchtigt sind, signifikant früher versterben<sup>7</sup>. Im Gegensatz dazu sind IL-4-Rezeptor  $\alpha$  (IL-4R $\alpha$ )-defiziente Mäuse, welche keine funktionalen Th2-Antworten entwickeln, resistent gegenüber der lebensbedrohlichen Erkrankung und entwickeln eine latente, pulmonale Infektionsform<sup>6</sup>. Diese Ergebnisse verdeutlichen den protektiven Einfluss von Th1-Immunantworten sowie den schädlichen Einfluss einer Th2-Immupolarisierung im Rahmen der Kryptokokkose. Interessanterweise wird die Produktion verschiedener Antikörperisotypen durch unterschiedliche Zytokine begünstigt. Während das Th2-Zytokin IL-4 die Produktion von IgG1-Antikörpern induziert, ist das Th1-Zytokin mit der Produktion von IgG2a-Antikörpern assoziiert<sup>8-11</sup>. Die erste Publikation nutzt diese Verknüpfung von Th-Zytokinen und Antikörperisotypen. Es wurden Kryptokokkenproteine identifiziert, welche von IgG1- oder IgG2a-Antikörpern gebunden werden und daher mit protektiven Th1- oder pathologischen Th2-Antworten assoziiert sind.

Die Konzentrationen von Serumantikörpern wurden mittels ELISA bestimmt. Die Infektion mit *C. neoformans* führte zu einem Anstieg der Serumkonzentration von IgG1-Antikörpern in allen

Mausstämmen, wobei die Konzentration in IL-4R $\alpha$ -defizienten Mäusen signifikant niedriger als in WT- und IL-12-defizienten Mäusen war. Dies verdeutlicht die Th2-Abhängigkeit der IgG1-Induktion. Im Gegensatz dazu konnten ähnliche IgG2a-Serumkonzentrationen in naiven und infizierten Mäusen aller Genotypen gemessen werden. Genotyp-spezifisch wiesen Th1-defiziente IL-12-Knockout-Mäuse niedrigere IgG2a-Serumkonzentrationen als Mäuse anderer Genotypen auf. *C. neoformans*-spezifische IgG1- und IgG2a-Antikörper konnten in Seren von naiven Tieren nicht detektiert werden. Die Titer von anti-Kryptokokken IgG1-Antikörpern stiegen nach der *C. neoformans*-Infektion stark an, wobei der Anstieg in Seren von WT- und IL-12-defizienten Mäusen stärker war als bei IL-4R $\alpha$ -defizienten Mäusen. Des Weiteren konnte eine signifikante Korrelation zwischen ansteigenden IgG1-Serumkonzentrationen und anti-Kryptokokken IgG1-Titern festgestellt werden. Der Konzentrationsanstieg von Th2-assoziierten IgG1-Antikörpern könnte daher durch die Infektion mit *C. neoformans* verursacht worden sein. Die Titer von anti-Kryptokokken IgG2a-Antikörpern waren in infizierten WT- und IL-4R $\alpha$ -defizienten Mäusen nur moderat, jedoch trotzdem signifikant erhöht, was bei IL-12-defizienten Mäusen nicht der Fall war. Zusätzlich ergab sich keine Korrelation zwischen IgG2a-Serumkonzentrationen und anti-Kryptokokken IgG2a-Titern. Die vorherrschende Induktion des Th2-assoziierten Isotyps IgG1 bestätigt die in der Literatur postulierte Tendenz zur Ausprägung von Th2-Antworten während der Kryptokokkeninfektion.

**Die 2D-Immunproteomanalyse von murinen Seren führt zur Identifizierung von Kryptokokkenproteinen, welche in Th1-assoziierten IgG2a-reaktiven oder Th2-assoziierten IgG1-reaktiven Protein-Spots enthalten sind.**

Zur Identifizierung von immunreaktiven *C. neoformans*-Antigenen wurden Kryptokokkenproteine mittels 2D-Gelelektrophorese aufgetrennt, auf Nitrozellulosemembranen übertragen und mit Seren individueller Mäuse inkubiert. Die Detektion von gebundenen anti-Kryptokokken IgG1- oder IgG2a-Antikörpern erfolgte mittels Isotyp-spezifischer Sekundärantikörper und die reaktiven Protein-Spots wurden verglichen. Es konnten keine IgG1-reaktiven Protein-Spots nach Inkubation mit Seren naiver Mäuse detektiert werden. Seren infizierter WT- und IL-12-defizienter Mäuse enthielten jedoch IgG1-Antikörper, welche an Kryptokokkenprotein-Spots banden, wobei die meisten immunreaktiven Protein-Spots unter Verwendung von Seren IL-12-defizienter Mäuse detektiert wurden. Im Gegensatz zur Analyse von IgG1-Antikörpern konnten nach Inkubation von Kryptokokkenproteinen mit Seren naiver Mäuse IgG2a-reaktive Protein-Spots beobachtet werden. Diese Protein-Spots wurden auch von Seren infizierter IL-12-defizienter Mäuse gebunden. Nach Inkubation mit Seren von infizierten WT- und IL-4R $\alpha$ -defizienten Mäusen konnte eine höhere Anzahl von IgG2a-reaktiven Spots detektiert werden, wobei hier Seren von IL-4R $\alpha$ -defizienten Mäusen die meisten Kryptokokkenprotein-Spots erkannten.

Massenspektrometrie wurde eingesetzt, um in den immunreaktiven Protein-Spots enthaltene Proteine zu identifizieren, wobei das am häufigsten im jeweiligen Spot vorhandene Protein als potentiell immunreaktives Protein postuliert wurde. Zehn Proteine wurden ausschließlich in IgG1-reaktiven Protein-Spots gefunden, während vier Proteine nur in IgG2a-reaktiven Spots vorhanden waren, wovon

ein Protein in den Spots identifiziert wurde, welche auch von Seren naiver Mäuse gebunden wurden. Letzteres Protein könnte kreuzreaktiv mit Proteinen anderer Pilze sein. Drei Proteine wurden in IgG1- und IgG2a-reaktiven Spots identifiziert. Einige der in dieser Studie identifizierten Proteine wurden bereits in früheren Publikationen als potentiell immunreaktive Kryptokokkenproteine beschrieben<sup>12-16</sup>, wodurch die Wahrscheinlichkeit steigt, dass diese Proteine tatsächlich immunogene Eigenschaften besitzen. Des Weiteren sind einige der identifizierten Proteine als Virulenzfaktoren beschrieben und könnten daher Ziele neuer anti-fungaler Substanzen darstellen.

**Die Analyse humaner Seren zeigt eine Erhöhung von anti-Kryptokokken IgG-Titern, jedoch nicht anti-Kryptokokken IgM-Titern in Seren von HIV-negativen, jedoch nicht HIV-positiven Patient:innen mit Kryptokokkenmeningitis im Vergleich zu Seren gesunder Personen.**

Die zweite Publikation beschäftigt sich mit der Identifizierung von Kryptokokkenproteinen welche Immunreaktivität mit humanen Seren zeigen. Hierfür wurden Seren von in Kolumbien lebenden HIV-positiven Kryptokokkenmeningitispatient:innen (KM-Patient:innen) mit einer CD4<sup>+</sup> T-Zellkonzentration von weniger als 250 Zellen pro Mikroliter, HIV-negativen KM-Patient:innen und gesunden Personen analysiert. Es wurde die Identifizierung von Proteinen angestrebt, welche präferierte Reaktivität mit Seren von KM-Patient:innen zeigen, wodurch sie als Krankheits-assoziierte Proteine definiert werden können. Diese Proteine sind von besonderem Interesse, da sie einerseits potentielle Virulenzfaktoren darstellen und andererseits vielversprechende Vakzinkandidaten sind, da sie auch in immunsupprimierten Personen immunogen wirken.

Bisherige Publikationen zeigten unterschiedliche Ergebnisse bezüglich des hauptsächlich im Zuge der Kryptokokkeninfektion produzierten Antikörper-Isotyps. Im Unterschied zu Titern von gegen die Kryptokokkenkapsel gerichteten Antikörpern, welche mittels ELISA quantifiziert wurden<sup>17-29</sup>, wurde die Konzentration von gegen Kryptokokkenproteine gerichteten Antikörpern bisher hauptsächlich durch semiquantitative Methoden bestimmt, wie das Zählen von Banden in Western Blots nach Seruminkubation<sup>24,25,30-34</sup>. Daher wurden in der vorliegenden Studie die Konzentrationen von Serumantikörpern und die Titer von anti-Kryptokokken Immunglobulinen für die Antikörperklassen IgM und IgG in humanen Seren quantifiziert. Die Serumkonzentration von IgG-Antikörpern war in Seren aller Gruppen ähnlich. Im Gegensatz dazu waren IgM-Antikörper in Seren von KM-Patient:innen unabhängig von ihrem HIV-Status in signifikant niedrigerer Konzentration vorhanden. Interessanterweise wiesen HIV-positive und HIV-negative KM-Patient:innen in früheren Studien einen verringerten Anteil von IgM-exprimierenden Gedächtnis-B-Zellen im Vergleich zu gesunden Personen auf<sup>26,28</sup>. Die Quantifizierung von gegen *C. neoformans*-Proteine oder Kapselpolysaccharide (KPS) gerichteten Antikörpern mittels ELISA ergab jedoch ähnliche anti-Kryptokokken IgM Titer in Seren aller Gruppen. Demgegenüber waren die Titer von anti-Kryptokokken IgG-Antikörpern in Seren von HIV-negativen KM-Patient:innen im Vergleich zu Kontrollpersonen, aber auch HIV-positiven KM-Patient:innen, erhöht. Der ausbleibende Anstieg von anti-*C. neoformans* IgG-Titern in HIV-positiven

KM-Patient:innen könnte auf der schweren Immunsuppression dieser Personen beruhen, wodurch keine adäquate Immunreaktion auf die Infektion initiiert werden kann.

**Die erhöhte Produktion von anti-Kryptokokken IgG, aber nicht IgM-Antikörpern während der Kryptokokkeninfektion wird durch die Analyse von murinen Seren bestätigt.**

Zur weiteren Analyse des hauptsächlich durch *C. neoformans* induzierten Isotyps wurden weitere Antikörperbestimmungen in murinen Seren von naiven und intranasal infizierten BALB/c WT-Mäusen, welche eine systemische Kryptokokkose entwickeln, und IL-4R $\alpha$ -defizienten Mäusen, welche eine latente, pulmonale Kryptokokkeninfektion zeigen, durchgeführt. Während der gesamten Infektion zeigten WT-Mäuse eine höhere fungale Lungenlast als IL-4R $\alpha$ -defiziente Mäuse. Anti-Kryptokokken IgM-Antikörper wurden während des gesamten Beobachtungszeitraums in ähnlicher Konzentration in Seren von naiven und infizierten Tieren detektiert, während die Serumkonzentration von IgM-Antikörpern 60 Tage nach der Infektion in beiden Mausstämmen erhöht war. Demgegenüber zeigten sich erhöhte Titer von anti-Kryptokokken IgG-Antikörpern und eine erhöhte IgG-Serumkonzentration mit fortschreitender Infektion in Seren von Mäusen beider Genotypen. Interessanterweise konnten 60 Tage nach der Infektion signifikant höhere anti-*C. neoformans* IgG-Titer in WT-Mäusen, welche eine hohe Pilzlast in der Lunge aufwiesen, als in IL-4R $\alpha$ -defizienten Mäusen, welche eine moderate Lungenpilzlast zeigten, detektiert werden. Des Weiteren waren die anti-*C. neoformans* IgG-Titer in WT-Mäusen mit der Kryptokokkenorganlast der Lunge positiv korreliert, was für IL-4R $\alpha$ -defizienten Mäuse nicht der Fall war. Auf der Grundlage der humanen und murinen serologischen Daten stellen wir daher die Hypothese auf, dass die Produktion von IgG-, aber nicht IgM-Antikörpern im Zuge der Kryptokokkeninfektion induziert und durch hohe Pilzlast und Dissemination verstärkt wird.

**Krankheits-assoziierte Kryptokokkenproteine können durch 2D-Immunproteomanalyse unter Verwendung von Seren kolumbianischer KM-Patient:innen und gesunder Personen identifiziert werden.**

Für die Identifizierung von immunreaktiven Kryptokokkenprotein-Spots wurde eine 2D-Immunproteomanalyse durchgeführt. Es wurden *C. neoformans*-Proteine mittels 2D-Gelelektrophorese aufgetrennt, auf Nitrozellulosemembranen übertragen und mit gepoolten humanen Seren inkubiert. Sowohl die Kryptokokkenproteine als auch die immunreaktiven Proteine, an welche Serum-IgG-Antikörper gebunden haben, wurden mittels fluoreszierender Farbstoffe detektiert, um eine Quantifizierung zu ermöglichen. Die entstehenden Fluoreszenzsignale wurden zwischen den drei Gruppen (HIV-positive und HIV-negative KM-Patient:innen sowie gesunde Personen) mit Hilfe der Software Delta2D (DECODON) verglichen. Hierfür wurde die Fluoreszenzintensität der immunreaktiven Protein-Spots quantifiziert, durch die Proteinbeladung normalisiert und statistisch analysiert. Es konnten Protein-Spots identifiziert werden, welche eine stärkere Reaktivität mit Seren von KM-Patient:innen im Vergleich zu gesunden Personen zeigten, jedoch auch solche Spots, die stärker mit Seren von gesunden Personen, HIV-positiven oder HIV-negativen KM-Patient:innen im Vergleich zu den jeweiligen anderen Gruppen reagierten. Mittels massenspektrometrischer Analyse konnten

143 Proteine in diesen differentiell immunreaktiven Spots identifiziert werden. Dreiundzwanzig Proteine wurden auf Grund von häufigem Auftreten in verschiedenen immunreaktiven Spots, bereits postulierter Immunreaktivität, oder einem potentiellen Einfluss auf die Virulenz des Pilzes für die rekombinante Expression in *Escherichia coli* ausgewählt. Die Identität der rekombinant exprimierten Proteine wurde durch Massenspektrometrie bestätigt. Für die Quantifizierung der Immunreaktivität der rekombinanten Proteine wurde erneut die Bindung von Serum-IgG-Antikörpern mittels Fluoreszenzfarbstoff quantifiziert und über die vorhandene Gesamtproteinmenge normalisiert. Alle 23 Kryptokokkenproteine zeigten Immunreaktivität mit humanen Seren von KM-Patient:innen, aber auch Seren von gesunden Individuen. Vierzehn dieser Proteine wurden bisher nicht als immunreaktive Kryptokokkenproteine beschrieben, während zwölf Proteine in Kryptokokkenprotein-Spots nachgewiesen wurden, welche Reaktivität mit Seren *C. neoformans*-infizierter Mäuse<sup>12-14,35</sup>, oder mit Seren von Koalas<sup>15</sup> und Menschen<sup>16</sup>, die mit dem nah verwandten Pilz *Cryptococcus gattii* infiziert waren, zeigten. Die vorliegende Publikation ist jedoch die erste Studie, welche die tatsächliche Immunreaktivität dieser Proteine durch rekombinante Expression und isolierte Betrachtung bestätigt. Die Quantifizierung der Immunreaktivität ergab ein Krankheits-assoziiertes Erkennungsmuster für zwölf der 23 Kryptokokkenproteine, definiert durch signifikant stärkere Reaktivität des jeweiligen Proteins mit Seren von KM-Patient:innen im Vergleich zu gesunden Personen. Die identifizierten Proteine stellen interessante Kandidaten für zukünftige Studien dar, da sie selbst mit Seren von immunsupprimierten AIDS-Patient:innen starke Reaktivität zeigten. Drei Proteine weisen zusätzlich keine signifikante Homologie mit humanen Proteinen auf und konnten extrazellulär nachgewiesen werden<sup>36</sup>, oder besitzen ein sekretorisches Signalpeptid, was sie zu vielversprechenden Vakzinantigenen macht. Eines dieser Proteine, die extrazelluläre elastinolytische Metalloproteinase, wurde bereits in einer murinen Vakzinstudie verwendet und konnte das Überleben von *C. neoformans*-infizierten C57BL/6 WT-Mäusen signifikant verlängern<sup>37</sup>. Des Weiteren sind sechs der Krankheits-assoziierten Kryptokokkenproteine in das Überleben des Pilzes, die Virulenz und den Metabolismus involviert, weshalb sie als potentielle Angriffspunkte anti-fungaler Therapie dienen könnten. Interessanterweise konnte gezeigt werden, dass nach Inhibierung der erwähnten extrazellulären Metalloproteinase die Fähigkeit von Kryptokokken zur Translokation durch die Blut-Hirn-Schranke in einem *in vitro* Transwell-Modell eingeschränkt war<sup>38</sup>. Dies bestärkt die Hypothese, dass eine Inhibierung der identifizierten Krankheits-assoziierten Proteine in der anti-fungalen Therapie genutzt werden könnte.

### **Schlussfolgerungen**

Die vorgelegte Dissertation demonstriert die Nutzbarkeit immunproteomischer Analysemethoden für die Identifizierung von immunreaktiven Kryptokokkenproteinen. Es konnten Kandidatenantigene mit exzellentem Potential für die Entwicklung prophylaktischer anti-Kryptokokkenvakzinen oder therapeutischer anti-fungaler Wirkstoffe identifiziert werden.

Die in der ersten Studie<sup>35</sup> identifizierten Proteine sind in Protein-Spots enthalten, welche mit protektiven Th1- oder pathologischen Th2-Antworten assoziiert sind. Fünf dieser Proteine wurden im Rahmen der zweiten Studie<sup>39</sup> rekombinant exprimiert und die Immunreaktivität der Proteine konnte bestätigt werden. Zwei dieser Proteine zeigten stärkere Reaktivität mit Seren von HIV-positiven und HIV-negativen KM-Patient:innen im Vergleich zu Seren gesunder Individuen, während die drei anderen Proteine stärkere Reaktivität mit Seren von HIV-positiven KM-Patient:innen als mit Seren von HIV-negativen KM-Patient:innen und gesunden Personen aufwiesen. Die Erkennung der Proteine durch Antikörper verschiedener Spezies lässt auf eine starke Immunogenität schließen und verdeutlicht außerdem das erfolgsversprechende Potential des immunproteomischen Ansatzes für die Identifizierung von immunreaktiven Proteinen.

In der zweiten Studie<sup>39</sup> konnten 23 Proteine identifiziert werden, welche erwiesenermaßen Immunreaktivität mit humanen Seren von KM-Patient:innen aber auch gesunden Personen zeigen, was die Immunogenität der Proteine verdeutlicht. Vierzehn Kryptokokkenproteine wurden zum ersten Mal als immunreaktive Antigene beschrieben. Durch Quantifizierung der Immunreaktivität konnte ein Krankheits-assoziiertes Erkennungsmuster für zwölf Proteine ermittelt werden, definiert durch signifikant stärkere Reaktivität mit Seren von KM-Patient:innen im Vergleich zu gesunden Personen. Das immunogene Potential sowie die Funktion der Proteine oder ihre molekularen Eigenschaften, wie beispielsweise das Nicht-Vorhandensein von Homologie mit humanen Proteinen, markiert einzelne Proteine als Zielproteine für anti-fungale Wirkstoffe oder prädestiniert sie für die Verwendung als Vakzinantigene.

Frühere Studien verwendeten bereits rekombinant hergestellte Kryptokokkenproteine für die Immunisierung von Mäusen und konnten vielversprechende protektive Effekte erzielen<sup>37,40</sup>. Daher stellen die in den Studien der vorliegenden Dissertation identifizierten Proteine aussichtsreiche Kandidaten für zukünftige Forschungen und schlussendlich die Entwicklung einer gegen *C. neoformans* wirksamen Vakzine dar.

## 5.1 Literatur

1. Rajasingham, R. *et al.*: Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 17(8), 873–881 (2017).
2. Aguiar, P. A. *et al.*: The epidemiology of cryptococcosis and the characterization of *Cryptococcus neoformans* isolated in a Brazilian University Hospital. *Rev Inst Med Trop São Paulo* 59, e13 (2017).
3. Pyrgos, V., Seitz, A. E., Steiner, C. A., Prevots, D. R. & Williamson, P. R.: Epidemiology of cryptococcal meningitis in the US: 1997-2009. *PloS one* 8(2), e56269 (2013).
4. Sloan, D. J. & Parris, V.: Cryptococcal meningitis: epidemiology and therapeutic options. *Clin Epidemiol* 6, 169–182 (2014).
5. Müller, U. *et al.*: IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages and allergic inflammation during pulmonary infection of mice with *Cryptococcus neoformans*. *J Immunol* 179(8), 5367–5377 (2007).



6. Müller, U. *et al.*: A gene-dosage effect for interleukin-4 receptor alpha-chain expression has an impact on Th2-mediated allergic inflammation during bronchopulmonary mycosis. *J Infect Dis* 198(11), 1714–1721 (2008).
7. Decken, K. *et al.*: Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. *Infect Immun* 66(10), 4994–5000 (1998).
8. Snapper, C. M. & Paul, W. E.: Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 236(4804), 944–947 (1987).
9. Finkelman, F. D. *et al.*: Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol* 8, 303–333 (1990).
10. Snapper, C. M. & Mond, J. J.: Towards a comprehensive view of immunoglobulin class switching. *Immunol Today* 141, 15–17 (1993).
11. Germann, T. *et al.*: Interleukin-12 profoundly up-regulates the synthesis of antigen-specific complement-fixing IgG2a, IgG2b and IgG3 antibody subclasses in vivo. *Eur J Immunol* 25(3), 823–829 (1995).
12. Young, M., Macias, S., Thomas, D. & Wormley, F. L.: A proteomic-based approach for the identification of immunodominant *Cryptococcus neoformans* proteins. *Proteomics* 9(9), 2578–2588 (2009).
13. Chaturvedi, A. K., Weintraub, S. T., Lopez-Ribot, J. L. & Wormley, F. L.: Identification and characterization of *Cryptococcus neoformans* protein fractions that induce protective immune responses. *Proteomics* 13(23-24), 3429–3441 (2013).
14. Chaturvedi, A. K. *et al.*: Vaccine-mediated immune responses to experimental pulmonary *Cryptococcus gattii* infection in mice. *PLoS One* 9(8), e104316 (2014).
15. Jobbins, S. E. *et al.*: Immunoproteomic approach to elucidating the pathogenesis of cryptococcosis caused by *Cryptococcus gattii*. *J Proteome Res* 9(8), 3832–3841 (2010).
16. Martins, L. M. *et al.*: Immunoproteomics and immunoinformatics analysis of *Cryptococcus gattii* : novel candidate antigens for diagnosis. *Future Microbiol* 8(4), 549–563 (2013).
17. Dromer, F., Aucouturier, P., Clauvel, J. P., Saimot, G. & Yeni, P.: *Cryptococcus neoformans* antibody levels in patients with AIDS. *Scand J Infect Dis* 20(3), 283–285 (1988).
18. Houpt, D. C., Pfrommer, G. S., Young, B. J., Larson, T. A. & Kozel, T. R.: Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. *Infect Immun* 62(7), 2857–2864 (1994).
19. Deshaw, M. & Pirofski, L.-A.: Antibodies to the *Cryptococcus neoformans* capsular glucuronoxylomannan are ubiquitous in serum from HIV+ and HIV- individuals. *Clin Exp Immunol* 99(3), 425–432 (1995).
20. Dromer, F., Denning, D. W., Stevens, D. A., Noble, A. & Hamilton, J. R.: Anti-*Cryptococcus neoformans* antibodies during cryptococcosis in patients with the acquired immunodeficiency syndrome. *Serodiag Immun Inf D* 7(4), 181–188 (1995).
21. Abadi, J. & Pirofski, L.-A.: Antibodies reactive with the cryptococcal capsular polysaccharide glucuronoxylomannan are present in sera from children with and without human immunodeficiency virus infection. *J Infect Dis* 180(3), 915–919 (1999).
22. Subramaniam, K., French, N. & Pirofski, L.-A.: *Cryptococcus neoformans*-reactive and total immunoglobulin profiles of human immunodeficiency virus-infected and uninfected Ugandans. *Clin Diagn Lab Immunol* 12(10), 1168–1176 (2005).
23. Jalali, Z., Ng, L., Singh, N. & Pirofski, L.-A.: Antibody response to *Cryptococcus neoformans* capsular polysaccharide glucuronoxylomannan in patients after solid-organ transplantation. *Clin Vaccine Immunol* 13(7), 740–746 (2006).
24. Davis, J. *et al.*: Serologic evidence for regional differences in pediatric cryptococcal infection. *Pediatr Infect Dis J* 26(6), 549–551 (2007).
25. Saha, D. C., Xess, I., Zeng, W. Y. & Goldman, D. L.: Antibody responses to *Cryptococcus neoformans* in Indian patients with cryptococcosis. *Med Mycol* 46(5), 457–463 (2008).
26. Subramaniam, K. *et al.*: IgM(+) memory B cell expression predicts HIV-associated cryptococcosis status. *J Infect Dis* 200(2), 244–251 (2009).

27. Rohatgi, S. *et al.*: Fc gamma receptor 3A polymorphism and risk for HIV-associated cryptococcal disease. *mBio* 4(5), e00573-13 (2013).
28. Rohatgi, S. *et al.*: Antibody and B cell subset perturbations in human immunodeficiency virus-uninfected patients with cryptococcosis. *Open Forum Infect Dis* 51, ofx255 (2018).
29. Hlupeni, A. *et al.*: Antibody responses in HIV-infected patients with advanced immunosuppression and asymptomatic cryptococcal antigenemia. *Open forum infectious diseases* 61, ofy333 (2019).
30. Chen, L.-C., Goldman, D. L., Doering, T. L., Pirofski, L.-A. & Casadevall, A.: Antibody response to *Cryptococcus neoformans* proteins in rodents and humans. *Infect Immun* 67(5), 2218–2224 (1999).
31. Lamura, L. *et al.*: Analysis of antibody response to *Cryptococcus neoformans* in five patients with AIDS and cryptococcosis by immunoblotting. *J Infect* 401, 64–68 (2000).
32. Goldman, D. L. *et al.*: Serologic evidence for *Cryptococcus neoformans* infection in early childhood. *Pediatrics* 107(5), E66 (2001).
33. Saha, D. C. *et al.*: Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. *Clin Vaccine Immunol* 14(12), 1550–1554 (2007).
34. Chai, H. C. & Tay, S. T.: Detection of IgM and IgG antibodies to *Cryptococcus neoformans* proteins in blood donors and HIV patients with active cryptococcosis. *Mycoses* 52(2), 166–170 (2009).
35. Firacative, C. *et al.*: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection. *Sci Rep* 81, 2681 (2018).
36. Rodrigues, M. L. *et al.*: Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. *Eukaryot Cell* 71, 58–67 (2008).
37. Hester, M. M. *et al.*: Protection of mice against experimental cryptococcosis using glucan particle-based vaccines containing novel recombinant antigens. *Vaccine* 38(3), 620–626 (2020).
38. Aaron, P. A., Vu, K. & Gelli, A.: An antivirulence approach for preventing *Cryptococcus neoformans* from crossing the blood-brain barrier via novel natural product inhibitors of a fungal metalloprotease. *mBio* 11, e01249-20 (2020).
39. Gressler, A. E. *et al.*: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients. *Front Immunol* 12, 709695 (2021).
40. Specht, C. A. *et al.*: Vaccination with recombinant cryptococcus proteins in glucan particles protects mice against cryptococcosis in a manner dependent upon mouse strain and cryptococcal species. *mBio* 8(6), e01872-17 (2017).

# Author Contribution Statement

## Nachweise über die Anteile der Co-Autoren

A. Elisabeth Greßler

**An immunoproteomic approach for identification of *Cryptococcus neoformans* proteins recognized by murine and human antibodies**

---

**Supporting document for contributions of Co-authors to the publication:**

**Title:** Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection

**Journal:** Scientific Reports

**Author's:** Carolina Firacative\*, A. Elisabeth Gressler\*, Kristin Schubert, Bianca Schulze, Uwe Müller, Frank Brombacher, Martin von Bergen & Gottfried Alber  
\* shared first authorship

---

Contribution Carolina Firacative:

- Conception of experiments
- Establishment of protein extraction for two-dimensional gel electrophoresis
- Execution of two-dimensional gel electrophoresis and Western blotting experiments
- Quantification of antibody concentrations using ELISA
- Analysis, processing and interpretation of data
- Writing of the publication

Contribution A. Elisabeth Greßler:

- Execution of two-dimensional gel electrophoresis and Western blotting experiments
- Quantification of antibody concentrations using ELISA
- Analysis, processing and interpretation of data
- Writing of the publication
- Execution of additional experiments for finishing of the revised manuscript (conception of experiments, analysis and re-writing of the manuscript)

Contribution Kristin Schubert:

- Execution of protein identification using mass spectrometry
- Analysis of mass spectrometry data

Contribution Bianca Schulze:

- Contribution to conception of the study
- Conception and execution of experiments

Contribution Uwe Müller:

- Establishment of experiments (ELISA)
- Provided sera of naive and infected mice for analysis
- Support in cultivation of fungi
- Assistance in data analysis and interpretation

Contribution Frank Brombacher:

- Provided IL-4R $\alpha$ - and IL-12-deficient mice

Contribution Martin von Bergen:

- Supply of material expenses for mass spectrometry analysis
- Support in interpretation of mass spectrometry data

Contribution Gottfried Alber:

- Conception of the project
- Supply of material expenses
- Support in data analysis and interpretation
- Writing and revision of the publication



A. Elisabeth Greßler



Gottfried Alber

**Supporting document for contributions of Co-authors to the publication:**

**Title:** Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients

**Journal:** Frontiers in Immunology

**Author's:** A. Elisabeth Gressler, Daniela Volke, Carolina Firacative, Christiane L. Schnabel, Uwe Müller, Andor Krizsan, Bianca Schulze-Richter, Matthias Brock, Frank Brombacher, Patricia Escandón, Ralf Hoffmann & Gottfried Alber

---

Contribution A. Elisabeth Greßler:

- Conception of experiments
- Execution of two-dimensional gel electrophoresis and Western blotting experiments
- Quantification of antibody concentrations using ELISA
- Quantification of antibody levels using flow cytometry
- Establishment and execution of recombinant expression of cryptococcal proteins in *Escherichia coli*
- Analysis, processing and interpretation of data
- Writing of the publication

Contribution Daniela Volke

- Support in execution of two-dimensional gel electrophoresis and establishment of a novel staining strategy
- Execution of protein identification using mass spectrometry
- Analysis of mass spectrometry raw data
- Support in extended analysis of two-dimensional gel electrophoresis and mass spectrometry data

Contribution Carolina Firacative:

- Project idea and support of sera from Colombian cryptococcal meningitis patients and healthy control individuals
- Execution of preliminary experiments
- Support in data analysis

Contribution Christiane L. Schnabel

- Contribution to establishment of a novel staining strategy for two-dimensional immunoproteome analysis
- Support in analysis and interpretation of data

Contribution Uwe Müller:

- Support in analysis and interpretation of data

Contribution Andor Krizsan:

- Support in establishment of recombinant expression of cryptococcal proteins
- Supply of materials for recombinant protein expression

Contribution Bianca Schulze

- Assistance in conception of experiments and establishment of experimental methods
- Support for data analysis

Contribution Matthias Brock

- Assistance in establishment of recombinant expression of cryptococcal proteins
- Production of some recombinant cryptococcal proteins (from *C. neoformans* serotype D strain) in *Escherichia coli* or *Aspergillus fumigatus* for comparison with recombinant proteins produced by A. Elisabeth Greßler (from *C. neoformans* serotype A strain)

Contribution Frank Brombacher:

- Provided IL-4R $\alpha$ -deficient mice

Contribution Patricia Escandón

- Conception of the project
- Provided sera from Colombian cryptococcal meningitis patients and including clinical and diagnostic data

Contribution Ralf Hoffmann:

- Supply of material expenses for mass spectrometry analysis
- Support in interpretation of mass spectrometry data

Contribution Gottfried Alber:

- Conception of the project
- Supply of material expenses
- Support in data analysis and interpretation
- Writing and revision of the publication

  
A. Elisabeth Grebler

  
Gottfried Alber

## Supplementary documents of the first publication: Firacative and Gressler *et al.* 2018

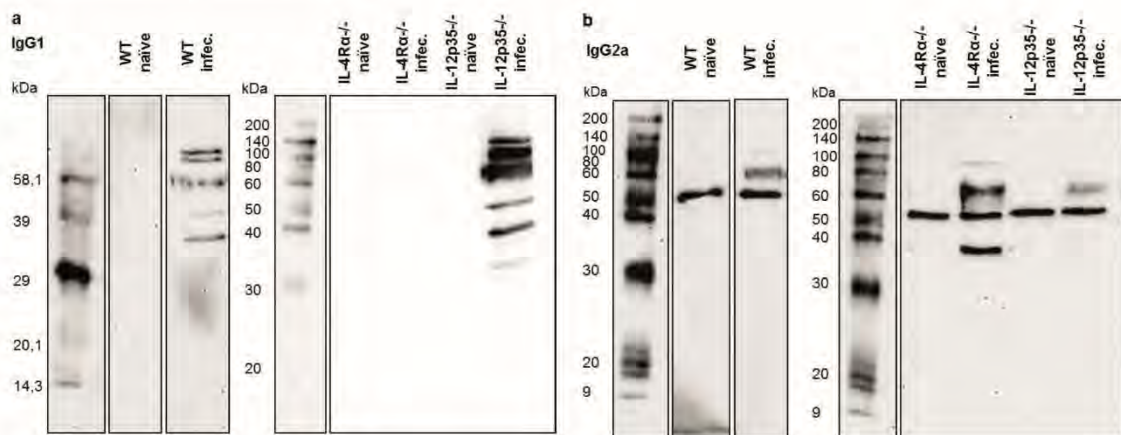
Supplementary information accompanies this paper at <https://doi.org/10.1038/s41598-018-21039-z>.

### Supplementary Figure 1

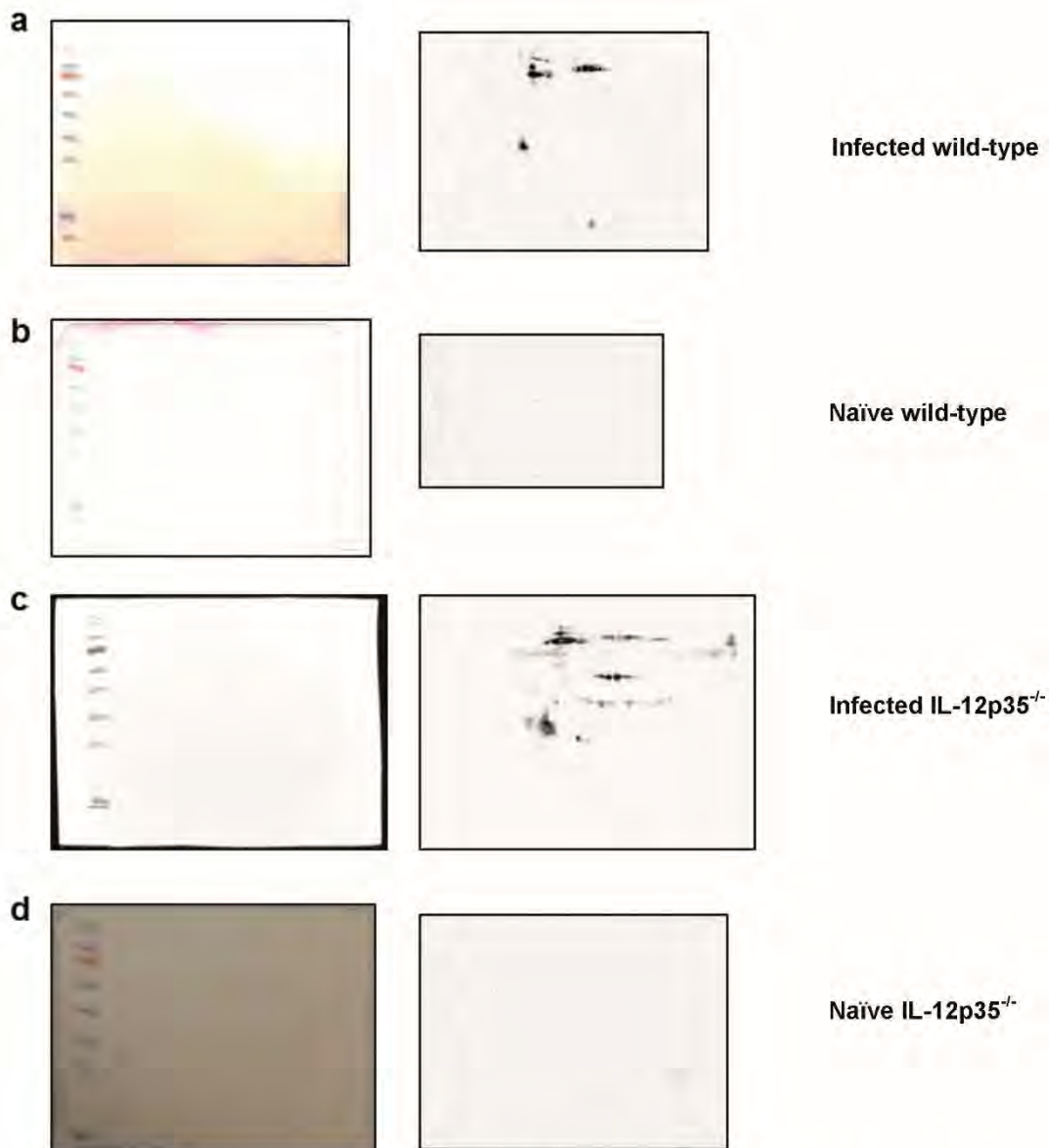
#### Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection

Carolina Firacative, A. Elisabeth Gressler, Kristin Schubert, Bianca Schulze, Uwe Müller, Frank Brombacher, Martin von Bergen, Gottfried Alber

#### Supplementary Information



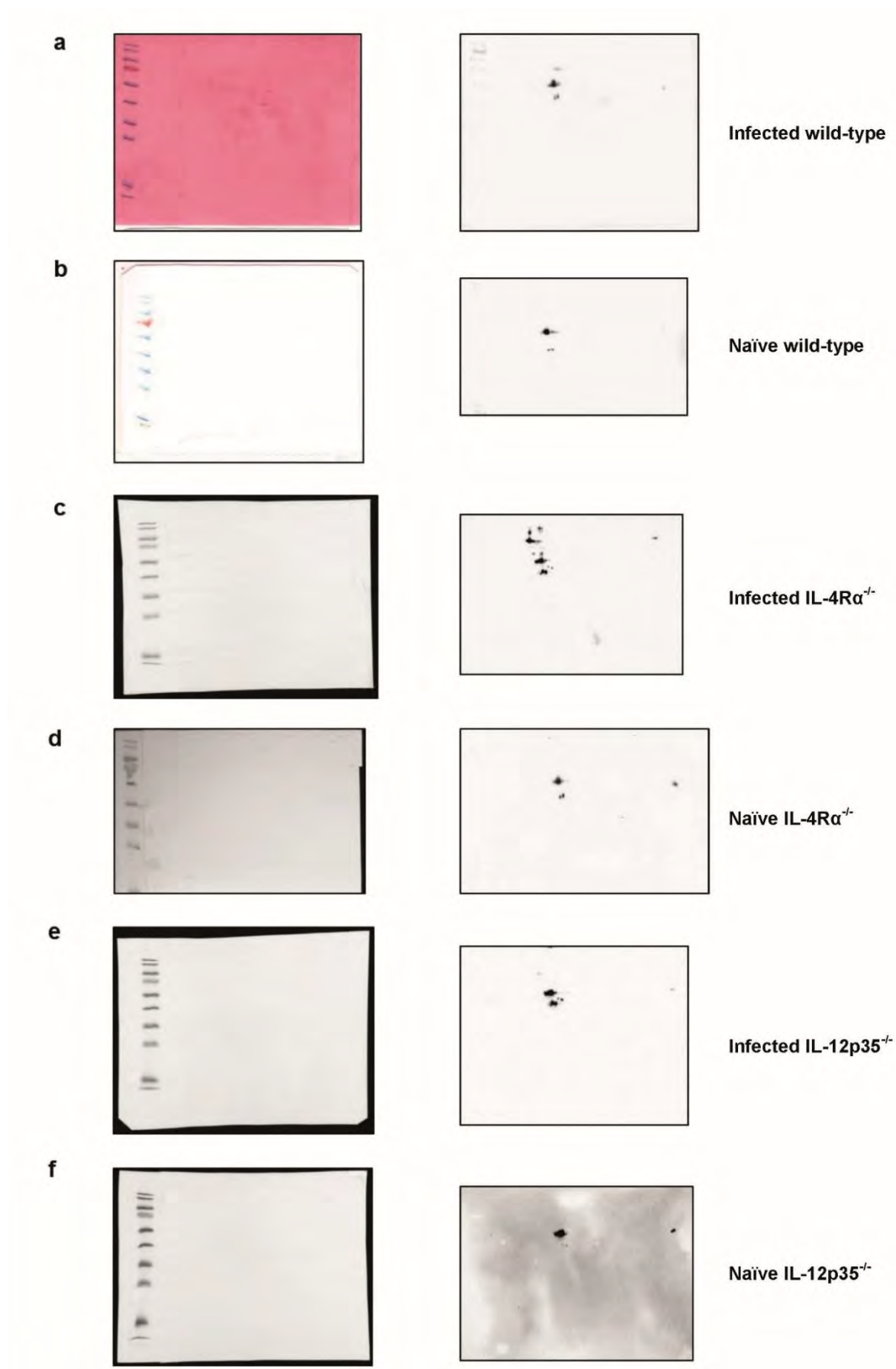
**Supplementary Figure 1: Proteins of *Cryptococcus neoformans* strain 1841 separated by 1D gel electrophoresis react with antibodies from sera of infected and naïve wild-type and gene-deficient mice.** Whole cell proteins of *C. neoformans* strain 1841 were separated by molecular weight for immunoblot analysis. After 1D gel electrophoresis, gels were transferred to nitrocellulose membranes. IgG1-immunoreactive proteins (A) and IgG2a-immunoreactive proteins (B) were detected using HRP-coupled secondary antibodies. Lanes of both membranes were incubated separately with sera from representative naïve and infected (infec.) wild-type (WT), an IL-12p35-deficient and an IL-4R $\alpha$ -deficient (IL-4R $\alpha$ <sup>-/-</sup>) mice. Lanes were also incubated with sera from naïve mice of all genotypes. All sera were diluted 1:1,000. Images were cropped to improve clarity. Full-length blots are presented in Supplementary Figure 4.

**Supplementary Figure 2**

**Supplementary Figure 2: Whole blots from Figure 2.** IgG1-immunoreactive proteins from *Cryptococcus neoformans* detected with sera from representative infected but not naïve wild-type and IL-12-deficient mice. Whole cell proteins of *C. neoformans* strain 1841 separated by 2D electrophoresis were transferred to nitrocellulose membranes, which were thereafter incubated with sera from infected and naïve wild-type and gene-deficient mice diluted 1:1,000. IgG1-immunoreactive proteins were detected using sera from an infected wild-type (a), a naïve wild-type (b), an infected IL-12-deficient (c) and a naïve IL-12-deficient (d) mouse. Protein abundance seen in the Coomassie staining did not correlate with the strength of the immunoreactive signal (Fig. 4). Pictures of the whole membrane were taken with white light to visualize the pre-stained marker that was transferred to the membrane and recolored to grey scale when needed (left images). Immunoreactivity of the proteins with IgG1 was visualized with chemiluminescence (right images). The portion of the membrane with the marker was cropped, adjusted in size and overlapped with the whole membrane visualized with chemiluminescence.

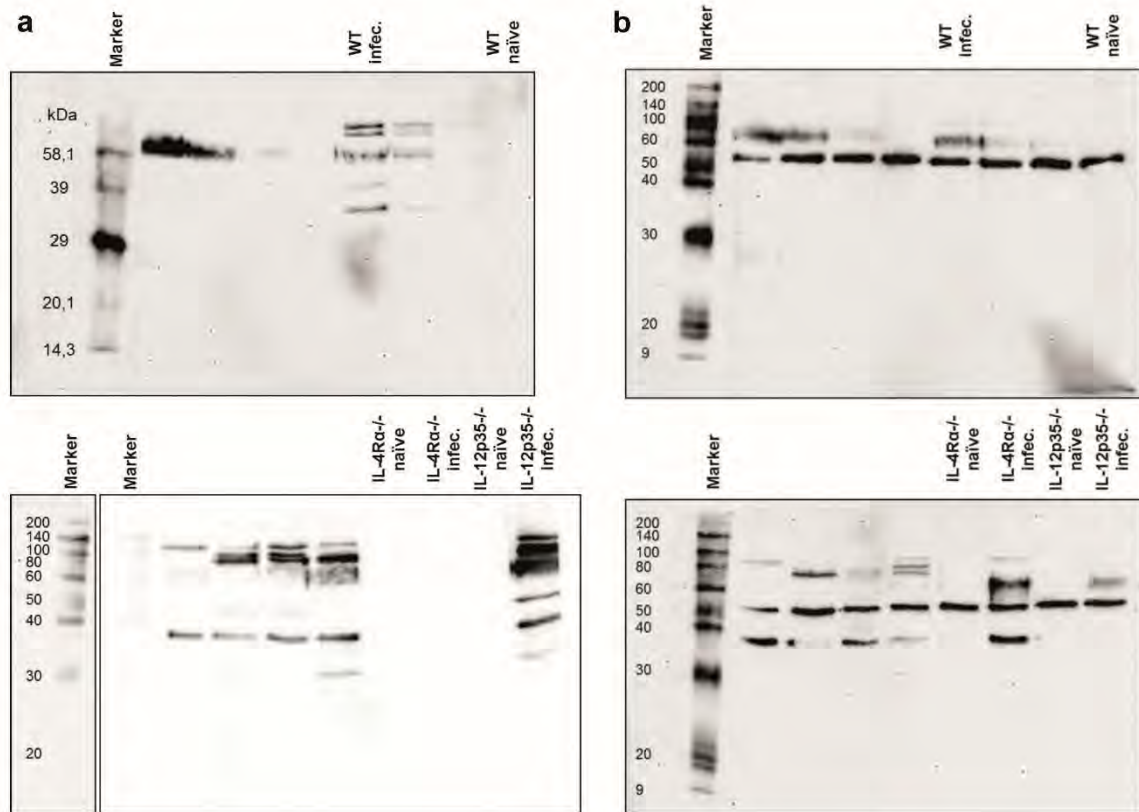


**Supplementary Figure 3**



**Supplementary Figure 3: Whole blots from of Figure 3.** IgG2a-immunoreactive proteins from *Cryptococcus neoformans* detected with sera from representative infected and naïve wild-type and IL-4R $\alpha$ -deficient mice. Whole cell proteins of *C. neoformans* strain 1841 separated by 2D electrophoresis were transferred to nitrocellulose membranes, which were thereafter incubated with sera from infected and naïve wild-type and IL-4R $\alpha$ -deficient mice diluted 1:1,000. IgG2a-immunoreactive proteins were detected using sera from an infected wild-type (a), a naïve wild-type (b), an infected IL-4R $\alpha$ -deficient (c), a naïve IL-4R $\alpha$ -deficient (d), an infected IL-12-deficient (e) and a naïve IL-12-deficient (f) mouse are shown. Protein abundance seen in the Coomassie staining did not correlate with the strength of the immunoreactive signal (Fig. 4). Pictures of the whole membrane were taken with white light to visualize the pre-stained marker that was transferred to the membrane and recolored to grey scale when needed (left images). Immunoreactivity of the proteins with IgG2a was visualized with chemiluminescence (right images). The portion of the membrane with the marker was cropped, adjusted in size and overlapped with the whole membrane visualized with chemiluminescence.

## Supplementary Figure 4



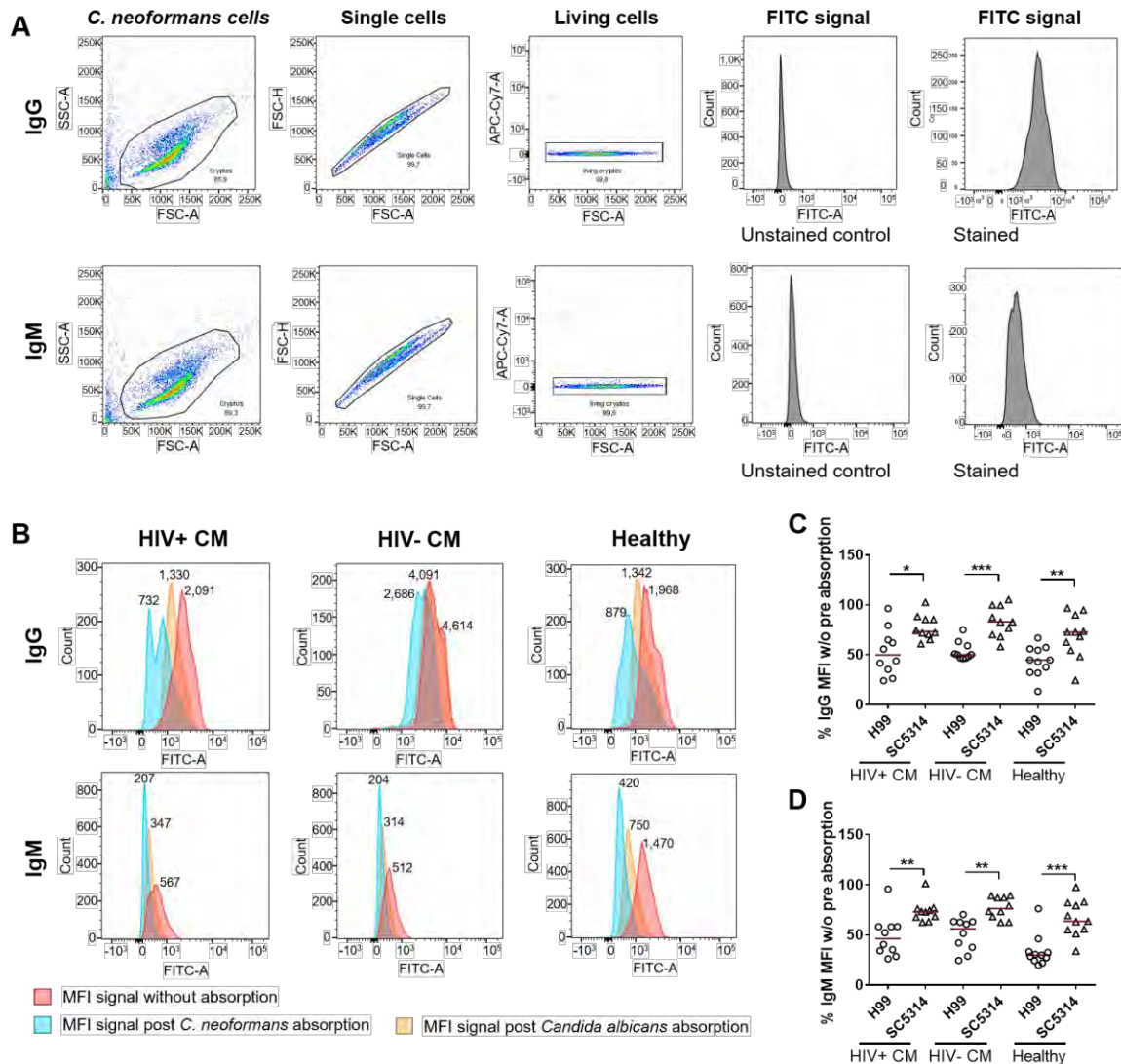
**Supplementary Figure 4: Whole blots from Supplementary Figure 1.** Proteins of *Cryptococcus neoformans* strain 1841 separated by 1D gel electrophoresis react with antibodies from sera of infected and naïve wild-type and gene-deficient mice. Whole cell proteins of *C. neoformans* strain 1841 were separated by molecular weight for immunoblot analysis. After 1D gel electrophoresis, gels were transferred to nitrocellulose membranes. IgG1-immunoreactive proteins (A) and IgG2a-immunoreactive proteins (B) were detected using HRP-coupled secondary antibodies. Lanes of both membranes were incubated separately with sera from representative naïve and infected (infec.) wild-type (WT), an IL-12p35-deficient (IL-12p35<sup>-/-</sup>) and an IL-4R $\alpha$ -deficient (IL-4R $\alpha$ <sup>-/-</sup>) mice. Lanes were also incubated with sera from naïve mice of all genotypes. All sera were diluted 1:1,000.

# Supplementary documents of the second publication: Gressler *et al.* 2021

The Supplementary Material for this article can be found online at:

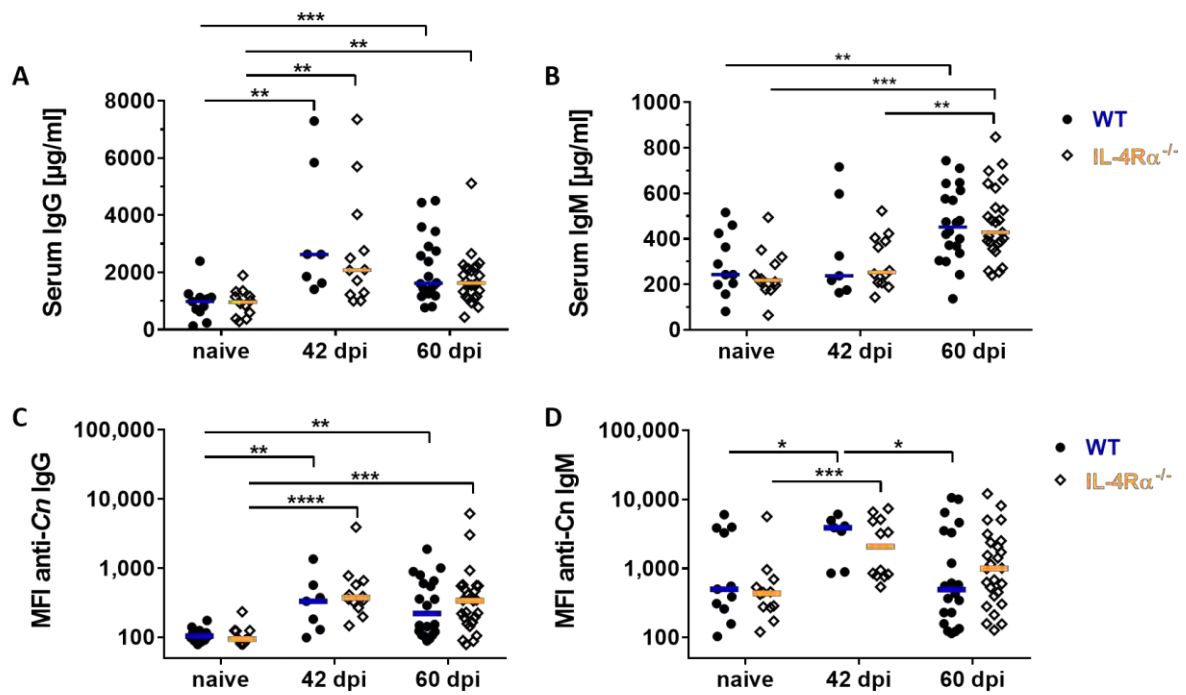
<https://www.frontiersin.org/articles/10.3389/fimmu.2021.709695/full#supplementary-material>.

## Supplementary Figure 1



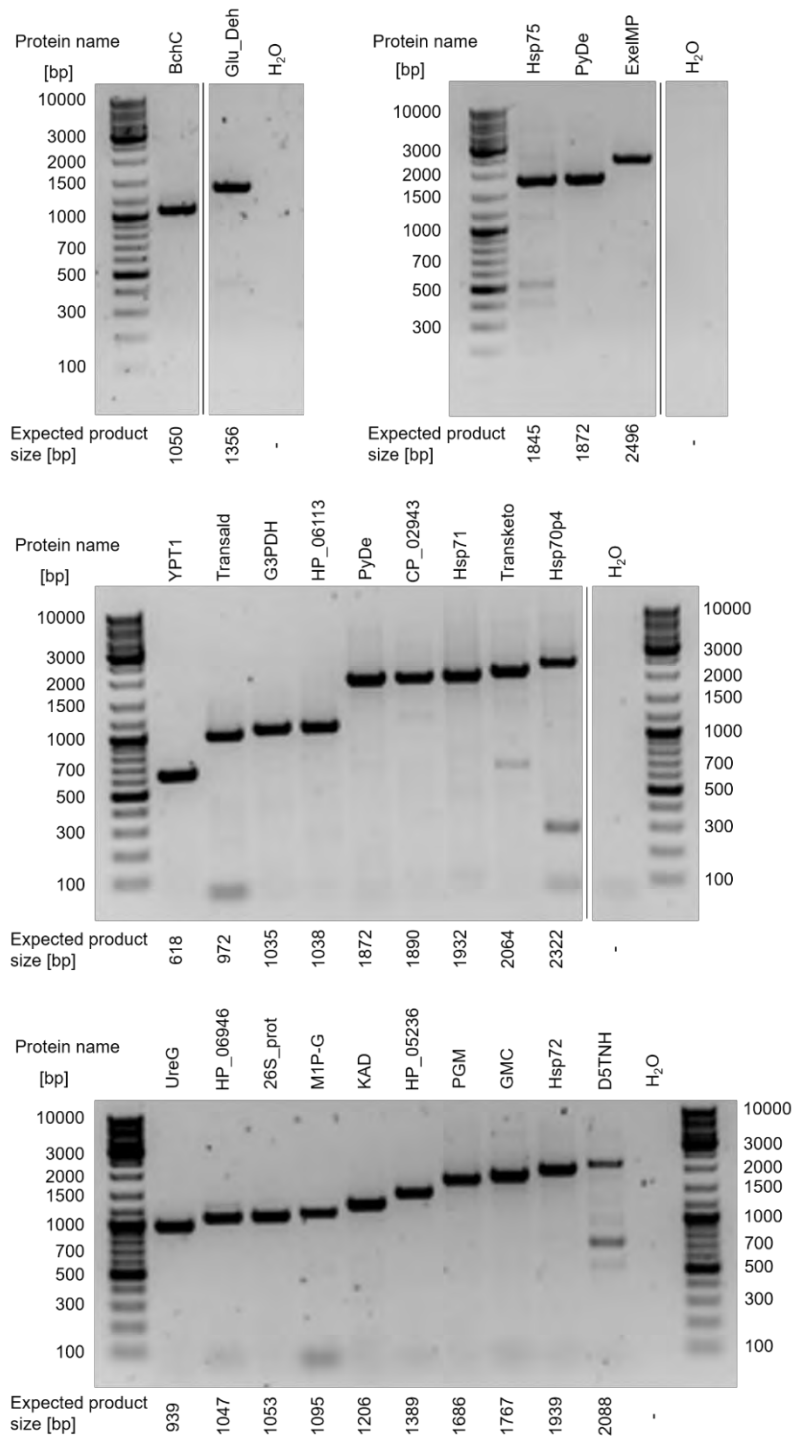
**Supplementary Figure 1: Pre-absorption of human sera with *Cryptococcus neoformans* H99 resulted in significantly stronger median fluorescence intensity (MFI) reduction than pre-absorption with *Candida albicans* SC5314.** Human sera from HIV-positive (HIV+) and HIV-negative (HIV-) cryptococcal meningitis (CM) patients and healthy control persons were used without (w/o) pre-absorption, or after pre-absorption with *C. neoformans* H99 cells or *C. albicans* SC5314 cells. For pre-absorption experiments, pre-absorbed sera were transferred to new *C. neoformans* cells. Quantification of IgG or IgM bound to cryptococcal cells was determined using FITC-labelled secondary antibodies and flow cytometry to quantify the median fluorescence intensity (MFI). **A)** Gating for flow cytometry analysis with representative pseudocolor plots. **B)** Histograms of representative sera of all groups show differences in fluorescence intensities before absorption (red), and after pre-absorption with *C. neoformans* H99 cells (blue) or with *C. albicans* SC5314 cells (orange). MFI is shown. Percentages of the MFI signal w/o pre-absorption compared to MFI signal after H99 and SC5314 absorption for **C)** IgG and **D)** IgM antibodies was depicted for each serum group. Each dot represents an individual serum and lines indicate median values. Statistical analysis was carried out using the Mann–Whitney U test for comparison of two groups. Asterisks indicate significant difference.

**Supplementary Figure 2**



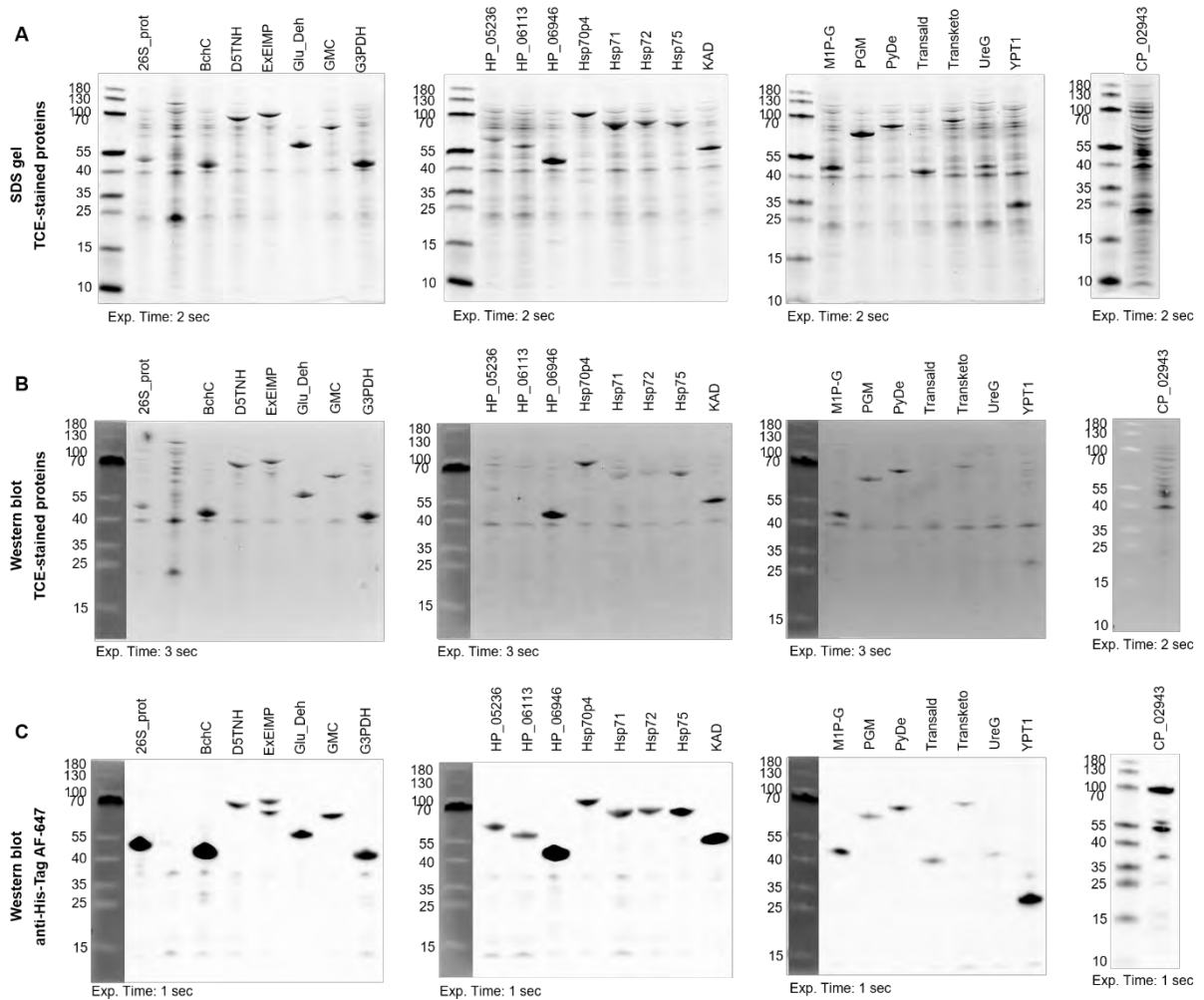
**Supplementary Figure 2: Levels of mouse serum IgG and IgM antibodies, and IgG and IgM antibodies directed against intact cryptococcal cells (anti-Cn Igs) in wild type (WT) and IL-4R $\alpha$ -deficient (IL-4R $\alpha$ <sup>-/-</sup>) mice.** WT and IL-4R $\alpha$ <sup>-/-</sup> mice were infected intranasally with 500 colony forming units (CFU) of *Cryptococcus neoformans* serotype D strain 1841. Total serum **A)** IgG and **B)** IgM levels were increased over the course of cryptococcal infection, independently of the mouse genotype. Anti-Cn **C)** IgG or **D)** IgM antibodies were quantified by incubation of *C. neoformans* 1841 cells with mouse sera and subsequent detection by FITC-labeled secondary antibodies using flow cytometry. Median fluorescence intensity (MFI) was increased for anti-Cn IgG antibodies in both genotypes compared to naïve mice, but similar for anti-Cn IgM antibodies in naïve and infected animals 60 dpi, in contrast to elevated 42 dpi for mice of both genotypes. Sera from seven to 23 mice from at least two independent experiments were analyzed per genotype. Each dot represents an individual serum and lines indicate median values. Statistical analysis was carried out using the Mann–Whitney U test for comparison of two groups. Asterisks indicate significant difference.

**Supplementary Figure 3**



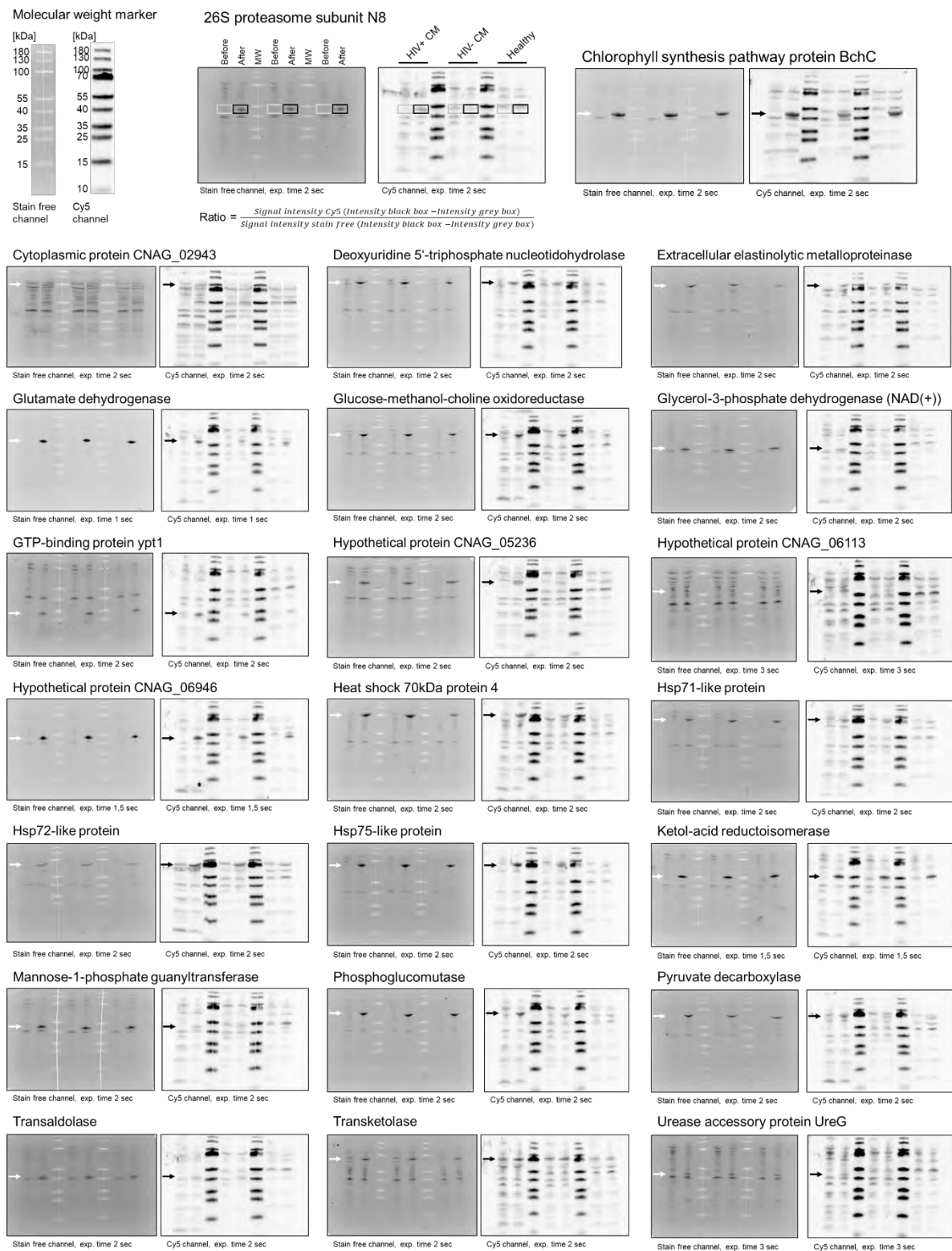
**Supplementary Figure 3: Amplified cDNA sequences of cryptococcal proteins for recombinant expression.** cDNA sequences of cryptococcal proteins for recombinant expression in *Escherichia coli* were amplified using PCR. Primers used for amplification are listed in Table S6. Resulting fragments were separated by agarose gel electrophoresis and the expected product size verified. Protein names: 26S: 26S proteasome subunit N8; BchC: Chlorophyll synthesis pathway protein BchC; CP\_02943: Cytoplasmic protein CNAG\_02943, D5TNH: Deoxyuridine 5'-triphosphate nucleotidohydrolase; ExEIMP: Extracellular elastinolytic metalloproteinase; Glu\_DeH: Glutamate dehydrogenase; GMC: Glucose-methanolcholine oxidoreductase; G3PDH: Glycerol-3-phosphate dehydrogenase (NAD(+)); YPT1: GTP-binding protein ypt1; HP\_05236: Hypothetical protein CNAG\_05236; HP\_06113: Hypothetical protein CNAG\_06113; HP\_06946: Hypothetical protein CNAG\_06946; Hsp70p4: Heat shock 70kDa protein 4; Hsp71: Hsp71-like protein; Hsp72: Hsp72-like protein; Hsp75: Hsp75-like protein; KAD: Ketol-acid reductoisomerase; M1P-G: Mannose-1-phosphate guanyltransferase; PGM: Phosphoglucomutase; PyDe: Pyruvate decarboxylase; Transald: Transaldolase; Transketo: Transketolase; UreG: Urease accessory protein UreG.

**Supplementary Figure 4**



**Supplementary Figure 4: Confirmation of complete expression of recombinant cryptococcal proteins in *Escherichia coli*.** **A)** Crude *E. coli* lysates containing recombinant cryptococcal proteins were separated by SDS-PAGE. Proteins were stained with UV-activated 2,2,2-trichloroethanol (TCE). Identity of the proteins was confirmed by mass spectrometric analysis (Table S5). **B)** Proteins were blotted onto nitrocellulose membranes and **C)** N- and C-terminal His-Tag was detected using an anti-His Tag antibody coupled to Alexa Fluor 647 (AF-647). Protein names: 26S: 26S proteasome subunit N8; BchC: Chlorophyll synthesis pathway protein BchC; CP\_02943: Cytoplasmic protein CNAG\_02943, D5TNH: Deoxyuridine 5'-triphosphate nucleotidohydrolase; ExEIMP: Extracellular elastolytic metalloproteinase; Glu\_Deh: Glutamate dehydrogenase; GMC: Glucose-methanol-choline oxidoreductase; G3PDH: Glycerol-3-phosphate dehydrogenase (NAD(+)); YPT1: GTP-binding protein ypt1; HP\_05236: Hypothetical protein CNAG\_05236; HP\_06113: Hypothetical protein CNAG\_06113; HP\_06946: Hypothetical protein CNAG\_06946; Hsp70p4: Heat shock 70kDa protein 4; Hsp71: Hsp71-like protein; Hsp72: Hsp72-like protein; Hsp75: Hsp75-like protein; KAD: Ketol-acid reductoisomerase; M1P-G: Mannose-1-phosphate guanyltransferase; PGM: Phosphoglucomutase; PyDe: Pyruvate decarboxylase; Transald: Transaldolase; Transketo: Transketolase; UreG: Urease accessory protein UreG.

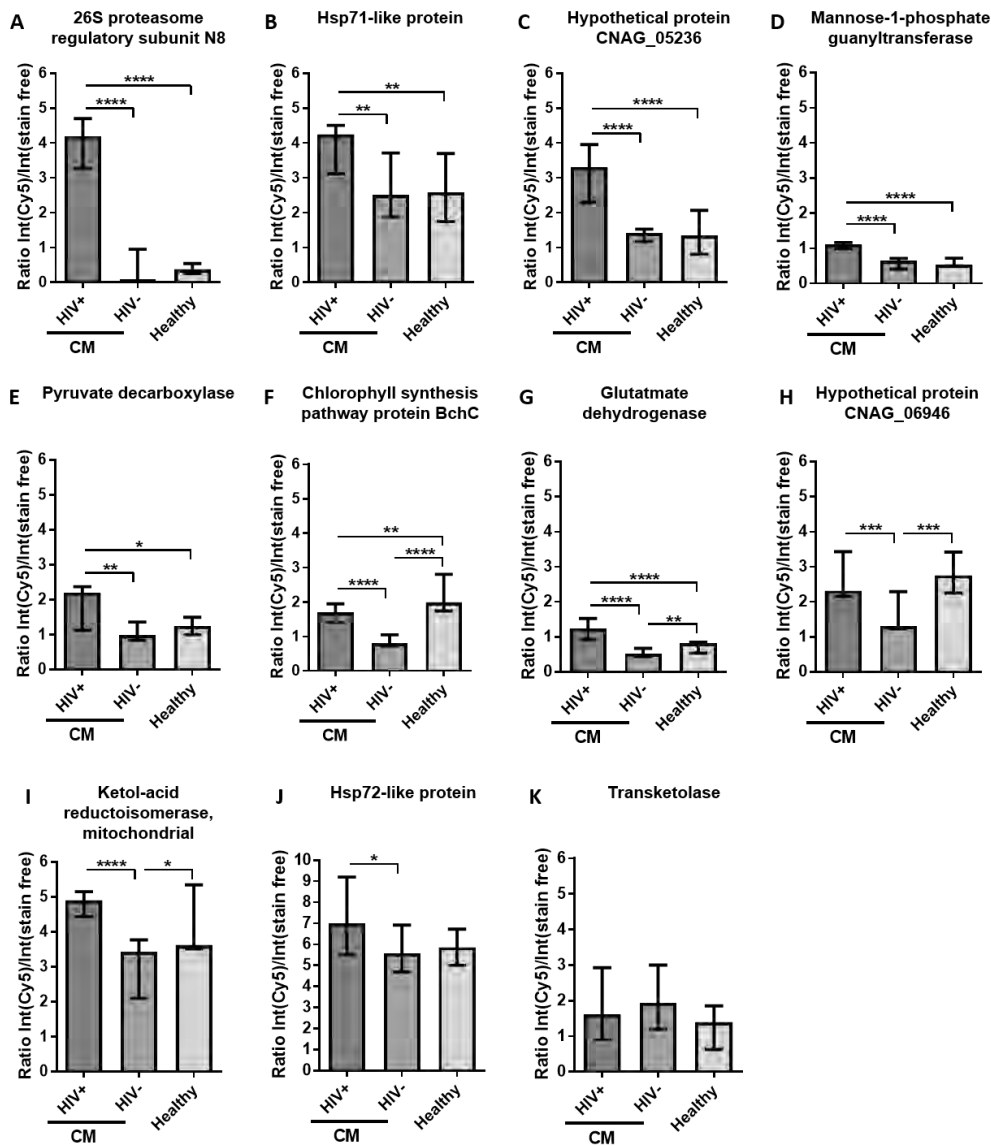
Supplementary Figure 5



**Supplementary Figure 5: Representative blot images for quantification of immunoreactivity of recombinant cryptococcal proteins.** Crude *Escherichia coli* lysates containing recombinant cryptococcal proteins were separated by SDS-PAGE. Samples before induction of recombinant protein expression and samples after induction of protein expression using IPTG were loaded, as representatively marked in the blot from 26S proteasome subunit N8. Proteins were blotted onto nitrocellulose membranes and (left) stained with UV-activated 2,2,2-trichloroethanol (TCE, detection in stain free channel). Membranes were incubated with sera from HIV-positive CM patients, HIV-negative CM patients, or healthy control persons, as representatively marked in the blot from 26S proteasome subunit N8. (Right) Proteins immunoreactive with human IgG were detected using polyclonal goat-anti human IgG coupled to Alexa Fluor 647 (AF-647, detected in Cy5 channel). MW: Molecular weight marker; exp.: exposure.



**Supplementary Figure 6**



**Supplementary Figure 6: Other recombinant proteins of *Cryptococcus neoformans* with different reactivity with human serum IgG.** Cryptococcal proteins recombinantly expressed in *Escherichia coli* were incubated with pooled sera from HIV+ or HIV-negative patients with cryptococcal meningitis (CM) or healthy control persons on Western blots. Whole *E. coli* proteins on the membranes were stained with UV-activated 2,2,2-trichloroethanol and detected in the stain-free channel. Proteins reactive with human IgG were stained using an anti-human IgG-Alexa Fluor 647 antibody and detected in the Cy5 channel. Ratios of intensities of the respective band in both channels were calculated to normalize the immunosignal (Cy5) onto the amount of protein loaded (stain free). Proteins showed **A-E)** significantly stronger reactivity with sera from HIV+ CM patients compared to both other groups, **F-I)** significantly less reactivity with HIV- CM patients compared to both other groups, or **J-K)** were similarly recognized by all three serum pools. Median values and range of three independent experiments, comprising values from three different exposure times for each experiment, are shown. Statistical analysis was carried out using the Mann-Whitney U for comparison of two groups. Asterisks indicate significant difference.

**Supplementary Table 1**

**Supplementary table 1: Primer sequences** used for amplification of cDNA sequences from *C. neoformans* for recombinant protein expression.

Targeted Protein	Serotype / strain	Primer name	Sequence
26S proteasome regulatory subunit N8	serotype A / H99	26S_prot_fwd	TT ACT CAT ATG CCC GGC TTA ACA ACG GCA C
26S proteasome regulatory subunit N8	serotype A / H99	26S_prot_rev	CGT TTT GCGG CCGC CTT TTT CTT CTT CTC TTT CTC CTC T
chlorophyll synthesis pathway protein BehC	serotype A / H99	Chloro_BchC_fwd	GC ACC CAT ATG GTC GCC AAG GAG ATG AAC G
chlorophyll synthesis pathway protein BehC	serotype A / H99	Chloro_BchC_rev	GCT TTT GCG GCC GCG TCC TTG TGT TCG GGC TTG
Cytoplasmic protein CNAG_02943	serotype A / H99	CP_02943_rev	GGC CCT GCGG CCGC CTC CTT TTT GGC TGA TCC AAA GT
Cytoplasmic protein CNAG_02943	serotype A / H99	CP_02943_fwd	TAT TC CAT ATG TCC CAT TTC GAC ACT GTC TCC
deoxyuridine 5~-triphosphate nucleotidohydrolase	serotype A / H99	D5TNH_fwd	TA TTA CAT ATG TCC AGA TTC GTC AGG CCT TC
deoxyuridine 5~-triphosphate nucleotidohydrolase	serotype A / H99	D5TNH_rev	GTA AAA GCGG CCGC AAT CAA GCT CCC AGC AAC ATC
extracellular elastinolytic metalloproteinase	serotype A / H99	Ex_el_MP_fwd	TA TAA CAT ATG CGC TCC TCC GCG CTC AT
extracellular elastinolytic metalloproteinase	serotype A / H99	Ex_el_MP_rev	CGA TAA GCG GCC GCA GCC TTT TTG GAC TCG CAG AC
glucose-methanol-choline oxidoreductase	serotype A / H99	GMC_oxired_fwd	TA TTA CAT ATG GTT CAC GCT GCT ACT CAC C
glucose-methanol-choline oxidoreductase	serotype A / H99	GMC_oxired_rev	AA CCC AAG CTT CTT TGT CTC TTT GTA AAG GTC GG
glutamate dehydrogenase (NADP)	serotype A / H99	Glu_Dehyd_rev	GCA TTT GCG GCC GCC CAC CAG TCA CCC TGT TCG
glutamate dehydrogenase (NADP)	serotype A / H99	Glu_Dehyd_fwd	TA TTA CAT ATG TCC AAC TAC CCC TCT GAG CC
glycerol-3-phosphate dehydrogenase (NAD(+))	serotype A / H99	GPDH_2_fwd	TA TGG CAT ATG GGC AAG GAA AAG GTT GCT GTT
glycerol-3-phosphate dehydrogenase (NAD(+))	serotype A / H99	GPDH_2_rev	GCA AAT GCGG CCGC AAG CCC CTC GGT CAG TTT C
GTP-binding protein ypt1	serotype A / H99	YPT1_rev	ACT AAA GCGG CCGC GCA GCA TCC ACC AGC GGT
GTP-binding protein ypt1	serotype A / H99	YPT1_fwd	TA TAA CAT ATG TCT GCC CCA GAA TAC GAC TAC
heat shock 70kDa protein 4	serotype A / H99	HSP70_P4_rev	GCA TTC GCGG CCGC ATC GAT ATC CAT CTC CTC AAC C
heat shock 70kDa protein 4	serotype A / H99	HSP70_P4_fwd	TC CAA CAT ATG GCC AGT GTC GTC GGT ATT GA
hsp71-like protein	serotype A / H99	HSP71_fwd	TA TTA CAT ATG GTT AAG GCT GTT GGT ATT GAT TTG G
hsp71-like protein	serotype A / H99	HSP71_rev	AAA TGT GCGG CCGC GTC GAC CTC CTC AAC GGA AG
hsp71-like protein	serotype D / JEC21	Hsp71CnIFNTstrep SMx_f	GTT CGA GAA GCC ATG GAT GGT TAA GGC TGT TGG TAT TGA T
hsp71-like protein	serotype D / JEC21	Hsp71CnIFNTstrep SMx_r	ACT GCT GTT ACC ATG GTT AGT CGA CCT CCT CAA CG G
hsp72-like protein	serotype A / H99	HSP72_fwd	TA TTC GGA TCC ATG ACA AAA GCT ATC GGT ATT GAC T

hsp72-like protein	serotype A / H99	HSP72_rev	GCA TTC GCGG CCGC ATC AAC TTC CTC AAC TGA AGG AC
hsp75-like protein	serotype A / H99	HSP75_rev	GCA TTT GCG GCC GCA CGG GCA GAA GCC ATG GC
hsp75-like protein	serotype A / H99	HSP75_fwd	AT TAG CAT ATG TCC GCT GAA GAC GTT TTC GAG
hypothetical protein CNAG_05236	serotype A / H99	HP_05236_fwd	TA TTA GGA TCC ATG TCT ACA ACG ATG GTC CCA G
hypothetical protein CNAG_05236	serotype A / H99	HP_05236_rev	GCA TTC GCGG CCGC ATC ATC GTC ACT TTC ACC ATC ACT
hypothetical protein CNAG_06113	serotype A / H99	HP_06113_fwd	TA TAA CAT ATG TCG GTC GTG TCG AAG AAC CT
hypothetical protein CNAG_06113	serotype A / H99	HP_06113_rev	TTT AAA GCGG CCGC AGC GCC CAA AGC GGG GAA
hypothetical protein CNAG_06946	serotype A / H99	HP_06946_fwd	TA GGA CAT ATG CTG CGC ACA GCT TCA AGA AAC
hypothetical protein CNAG_06946	serotype A / H99	HP_06946_rev	CGA TTA GCGG CCGC CGC CTC AAG TGC CTT CTT TG
ketol-acid reductoisomerase	serotype A / H99	KAD_fwd	TG TTT CAT ATG TCC TTC TCT AGA GCT TCC AGC
ketol-acid reductoisomerase	serotype A / H99	KAD_rev	GCA TTC GCGG CCGC AAG CTC ATC CTT GTT GGC GTC
Mannose-1-phosphate guanyltransferase	serotype A / H99	M1P-G_fwd	TA TGA CAT ATG AAG GCC CTG ATC CTC GTC G
Mannose-1-phosphate guanyltransferase	serotype A / H99	M1P-G_rev	CCA TAC GCGG CCGC CAT AAC AAT ACG GGG CTC AGT G
phosphoglucomutase	serotype A / H99	PGM_fwd	GC TTC CAT ATG TCC AAT ATC ATA ACC GTC AAG ACA
phosphoglucomutase	serotype A / H99	PGM_rev	GCA TTC GCGG CCGC AGT GAT AAC ACT GGG CTT CTC
phosphoglucomutase	serotype D / JEC21	PGM_pET_fwd	CAT CAT CAT AGC GGA TCC ATG TCC GAT ATC GTA ACC GTC A
phosphoglucomutase	serotype D / JEC21	PGM_pET_rev	ATA CAG CTG TGC GGC CGC TTA AGT GAT AAC ACT AGG CTT CTC
pyruvate decarboxylase	serotype A / H99	PyDe_rev	GCA ATT GCGG CCGC GGC CCT GTC GTT GGC TTC
pyruvate decarboxylase	serotype A / H99	PyDe_fwd	TA GGG CAT ATG TCC AGT AAC GAA CAA GTA GCC TT
transaldolase	serotype A / H99	Transald_fwd	CC ACC CAT ATG CCC ACT TCT CTT GAA GCT CTT
transaldolase	serotype A / H99	Transald_rev	GCT TTA GCGG CCGC AGC CTT GAG CTT CTC GAT CAA
transketolase	serotype A / H99	Transketo_fwd	AA TTT CAT ATG GCC AAC TTC TCC AGC AAC GA
transketolase	serotype A / H99	Transketo_rev	CAG CGA GCGG CCGC CTC AGA GAT GTC GTC CAA AGC
urease accessory protein UreG	serotype A / H99	UreG_fwd	TA GTA CAT ATG GCA GTG CCT GCT CAG CCT
urease accessory protein UreG	serotype A / H99	UreG_rev	GCA TTC GCGG CCGC TGC CTT AGC CTT ACC ATT TCC TT

### Supplementary Table 2

**Supplementary table 2: Correlation of lung fungal burden with anti-cryptococcal antibody levels in sera from wild type (WT) and IL-4R $\alpha$ -deficient (IL-4R $\alpha$ <sup>-/-</sup>) mice.** Correlation analysis was performed using non-parametric Spearman’s rank correlation. Number of colony-forming units (CFU) in the lungs of the mice were correlated with titers of anti-protein and anti-CPS IgG and IgM determined by ELISA, or levels of IgG and IgM directed against intact cryptococcal cells (anti-*Cn* IgG, anti-*Cn* IgM). Asterisks indicate significant differences. ns: not significant.

Genotype	Parameter 1	Parameter 2	Spearman’s $\rho$	p value	Summary
WT	Anti-protein IgG	Lung CFU	0.4017	0.0378	*
	Anti-protein IgM	Lung CFU	0.1049	0.6026	ns
	Anti-CPS IgG	Lung CFU	0.4277	0.0261	*
	Anti-CPS IgM	Lung CFU	-0.1579	0.4314	ns
	Anti- <i>Cn</i> IgG	Lung CFU	-0.3755	0.0536	ns
	Anti- <i>Cn</i> IgM	Lung CFU	-0.5364	0.0039	**
IL-4R $\alpha$ <sup>-/-</sup>	Anti-protein IgG	Lung CFU	0.03094	0.8537	ns
	Anti-protein IgM	Lung CFU	0.1229	0.4624	ns
	Anti-CPS IgG	Lung CFU	-0.04810	0.7743	ns
	Anti-CPS IgM	Lung CFU	-0.04391	0.7935	ns
	Anti- <i>Cn</i> IgG	Lung CFU	0.04882	0.7710	ns
	Anti- <i>Cn</i> IgM	Lung CFU	0.06031	0.7191	ns

### Supplementary Table 3

**Supplementary table 3: Sub-pools of sera from CM patients and healthy individuals used for 2D immunoproteome analysis.** Quenchable sera from Colombian HIV-positive (HIV+) and HIV-negative (HIV-) CM patients, as well as healthy control persons were pooled according to their infection status (group) and anti-cryptococcal protein IgG titers (sub-pools) for immunoproteome analysis. CM: cryptococcal meningitis, *Cn*: *Cryptococcus neoformans*. No.: Number.

Group	Sub-pools	Titer anti- <i>Cn</i> protein IgG	No. of sera
HIV+ CM patients	Low titer	1:100 – 1:200	3
	Intermediate titer	1: 300 – 1:800	4
	High titer	1:1600 – 1:12800	3
HIV- CM patients	Low titer	1:800 – 1:3200	3
	Intermediate titer	1:6400 – 1:12800	4
	High titer	1:12800 – 1:25600	3
Healthy control persons	Low titer	1:125 – 1:1000	4
	Intermediate titer	1:1600	3
	High titer	1:2400 – 1:9600	4

Supplementary Table 4

Supplementary table 4: Proteins identified in the eleven spots of interest marked in Figure 3C are listed.

Identification of each protein in the respective spot is marked with an "x".  
Accession number was retrieved from the NCBI protein database: <https://www.ncbi.nlm.nih.gov/protein/>.  
Raw data of mass spectrometry analysis is provided in separate register tabs for each spot.

accession	Protein name	Identification in spot:												
		26271	26329	27684	36555	26344	27537	39916	26409	26502	26814	26944		
AFR94156	2,3-bisphosphoglycerate-independent phosphoglycerate mutase [Cryptococcus neoformans var. grubii H99]			x										
AFR93317	20S proteasome subunit beta 1													
AFR98380	26S protease regulatory subunit 6A-B [Cryptococcus neoformans var. grubii H99]													
AFR92184	26S proteasome regulatory subunit N8 [Cryptococcus neoformans var. grubii H99]													
AFR98890	2-dehydroepiandrosterone 2-reductase													
AFR92474	3-(2'-,5'-biphosphate nucleotidase [Cryptococcus neoformans var. grubii H99]													
AFR94742	3-deoxy-7-phosphoheptulonate synthase [Cryptococcus neoformans var. grubii H99]													
AFR94296	3-oxoacid CoA-transferase [Cryptococcus neoformans var. grubii H99]													
AFR98086	5-methyltetrahydropteroylglutamate-homocysteine S-methyltransferase [Cryptococcus neoformans var. grubii H99]													
AFR94072	6-phosphogluconate dehydrogenase, decarboxylating 1 [Cryptococcus neoformans var. grubii H99]													
AFR98659	acetolactate synthase, small subunit [Cryptococcus neoformans var. grubii H99]													
AFR93774	acetyl-CoA C-acetyltransferase [Cryptococcus neoformans var. grubii H99]													
AFR95008	aconitate hydratase, mitochondrial													
AFR92615	actin [Cryptococcus neoformans var. grubii H99]													
AFR96928	actin binding protein [Cryptococcus neoformans var. grubii H99]													
AFR96040	alanine-tRNA ligase [Cryptococcus neoformans var. grubii H99]													
AFR96761	alcohol dehydrogenase, propanol-preferring [Cryptococcus neoformans var. grubii H99]													
AFR95862	allergen [Cryptococcus neoformans var. grubii H99]													
AFR95964	anthranilate synthase component 1 [Cryptococcus neoformans var. grubii H99]													
AFR93867	argininosuccinate lyase [Cryptococcus neoformans var. grubii H99]													
AFR95199	argininosuccinate synthase [Cryptococcus neoformans var. grubii H99]													
AFR98265	aspartate aminotransferase [Cryptococcus neoformans var. grubii H99]													
AFR92389	aspartate-semialdehyde dehydrogenase [Cryptococcus neoformans var. grubii H99]													
AFR96238	ATP synthase subunit beta, mitochondrial [Cryptococcus neoformans var. grubii H99]													
AFR96168	ATP-dependent RNA helicase DBP2-A [Cryptococcus neoformans var. grubii H99]													
AFR97093	ATP-dependent RNA helicase DBP5 [Cryptococcus neoformans var. grubii H99]													
AFR98608	branched-chain amino acid aminotransferase [Cryptococcus neoformans var. grubii H99]													
AFR95095	branched-chain-amino-acid transaminase [Cryptococcus neoformans var. grubii H99]													
AFR94513	catalase [Cryptococcus neoformans var. grubii H99]													
AFR94509	chaperone regulator [Cryptococcus neoformans var. grubii H99]													
AFR97763	chlorophyll synthesis pathway protein BcHc													
AFR99053	citrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]													
AFR92199	citrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]													
AFR95319	COP9 signalosome complex subunit 1 [Cryptococcus neoformans var. grubii H99]													
AFR93749	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]													
AFR94962	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]													
AFR93365	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]													
AFR93041	dehydrogenase [Cryptococcus neoformans var. grubii H99]													
AFR94562	deoxyuridine 5'-triphosphate nucleotidohydrolase [Cryptococcus neoformans var. grubii H99]													
AFR94390	diphosphomevalonate decarboxylase [Cryptococcus neoformans var. grubii H99]													
AFR95638	D-lactaldehyde dehydrogenase [Cryptococcus neoformans var. grubii H99]													
AFR94027	D-lactate dehydrogenase													
AFR92550	elongation factor 1-gamma [Cryptococcus neoformans var. grubii H99]													
AFR94637	elongation factor 2 [Cryptococcus neoformans var. grubii H99]													
AFR93435	endothelin-converting enzyme [Cryptococcus neoformans var. grubii H99]													
AFR97484	extracellular elastolytic metalloproteinase [Cryptococcus neoformans var. grubii H99]													
AFR95720	fatty acid synthase subunit beta, fungi type [Cryptococcus neoformans var. grubii H99]													
AFR95957	formate dehydrogenase [Cryptococcus neoformans var. grubii H99]													
AFR93031	fructose-bisphosphate aldolase 1													
AFR97862	fumarate hydratase, mitochondrial [Cryptococcus neoformans var. grubii H99]													
AFR98332	G protein beta subunit-like [Cryptococcus neoformans var. grubii H99]													





Spot ID 26271

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
37	2::AFR98435.1	heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	1715	85923	38	58
37	2::XP_012053205.1	heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	1715	85923	38	58
Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
39	2::AFR92468.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	3056	67372	34	59,1
37	2::XP_012046659.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	3056	67372	34	59,1
39	2::AGV14158.1	cytoplasmic protein, variant [Cryptococcus neoformans var. grubii H99]	568	60947	15	38
39	2::XP_012048239.1	cytoplasmic protein, variant [Cryptococcus neoformans var. grubii H99]	568	60947	15	38
39	2::AFR93749.2	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	568	68793	15	33,1
39	2::XP_012047840.1	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	568	68793	15	33,1
39	2::AFR92890.1	methylenetetrahydrofolate reductase [Cryptococcus neoformans var. grubii H99]	173	70413	5	12,2
39	2::XP_012046943.1	methylenetetrahydrofolate reductase [Cryptococcus neoformans var. grubii H99]	173	70413	5	12,2
39	1::ALBU_BOVIN	Serum albumin OS=Bos taurus OX=9913 GN=ALB PE=1 SV=4	96	71244	4	7,4
39	2::AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	74	68079	3	5,5
39	2::XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	74	68079	3	5,5

Spot ID 26329

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
37	2::AFR98550.2	phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	1322	60675	30	74,9
37	2::XP_012053286.1	phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	1322	60675	30	74,9
37	2::AFR98239.1	phosphomevalonate kinase [Cryptococcus neoformans var. grubii H99]	372	59686	10	21,8
37	2::XP_012053077.1	phosphomevalonate kinase [Cryptococcus neoformans var. grubii H99]	372	59686	10	21,8
37	2::AFR95319.1	COP9 signalosome complex subunit 1 [Cryptococcus neoformans var. grubii H99]	254	62428	7	16,5
37	2::XP_012050236.1	COP9 signalosome complex subunit 1 [Cryptococcus neoformans var. grubii H99]	254	62428	7	16,5
37	2::AFR98435.1	heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	248	85923	9	15,4
37	2::XP_012053205.1	heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	248	85923	9	15,4
37	2::AFR97886.1	mitochondrial outer membrane 72k protein [Cryptococcus neoformans var. grubii H99]	232	69862	8	14,7
37	2::XP_012052684.1	mitochondrial outer membrane 72k protein [Cryptococcus neoformans var. grubii H99]	232	69862	8	14,7
37	2::AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	187	68079	5	10,4
37	2::XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	187	68079	5	10,4
37	2::AFR93749.2	cytoplasmic protein, variant [Cryptococcus neoformans var. grubii H99]	162	60947	6	12,4
37	2::XP_012047840.1	cytoplasmic protein, variant [Cryptococcus neoformans var. grubii H99]	162	60947	6	12,4
37	2::AFR92468.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	161	67372	4	8
37	2::XP_012046659.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	161	67372	4	8
37	2::AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	125	69698	3	6,4
37	2::XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	125	69698	3	6,4
37	2::AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	125	69759	3	6,4
37	2::XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	125	69759	3	6,4
37	2::AFR98912.2	hexokinase [Cryptococcus neoformans var. grubii H99]	157	61380	7	16,7
37	2::XP_012053621.1	hexokinase [Cryptococcus neoformans var. grubii H99]	157	61380	7	16,7
37	2::AFR97413.2	protein transporter SEC31 [Cryptococcus neoformans var. grubii H99]	125	154056	4	3,3
37	2::XP_012051953.1	protein transporter SEC31 [Cryptococcus neoformans var. grubii H99]	125	154056	4	3,3
37	2::AFR92196.1	T-complex protein 1 subunit epsilon [Cryptococcus neoformans var. grubii H99]	107	60257	4	7,1
37	2::XP_012046498.1	T-complex protein 1 subunit epsilon [Cryptococcus neoformans var. grubii H99]	107	60257	4	7,1
37	2::AFR94562.2	deoxyuridine 5--triphosphate nucleotidohydrolase [Cryptococcus neoformans var. grubii H99]	106	73956	4	6,9
37	2::XP_012048586.1	deoxyuridine 5--triphosphate nucleotidohydrolase [Cryptococcus neoformans var. grubii H99]	106	73956	4	6,9
37	1::ALBU_BOVIN	Serum albumin OS=Bos taurus OX=9913 GN=ALB PE=1 SV=4	56	71244	3	5,3
Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
39	2::AFR98550.2	phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	602	60675	13	32,8
39	2::XP_012053286.1	phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	602	60675	13	32,8
39	2::AFR92468.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	373	67372	11	22
39	2::XP_012046659.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	373	67372	11	22
39	2::AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	242	69698	8	16,5
39	2::XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	242	69698	8	16,5
39	2::AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	240	69759	7	14,8
39	2::XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	240	69759	7	14,8
39	1::HS71A_BOVIN	Heat shock 70 kDa protein 1A OS=Bos taurus OX=9913 GN=HSPA1A PE=1 SV=2	68	70500	3	5,9
39	1::HS71B_BOVIN	Heat shock 70 kDa protein 1B OS=Bos taurus OX=9913 GN=HSPA1B PE=2 SV=1	68	70470	3	5,9
39	2::AGV14869.1	hexokinase, variant [Cryptococcus neoformans var. grubii H99]	207	61380	6	16,9
39	2::AFR98912.2	hexokinase [Cryptococcus neoformans var. grubii H99]	207	61380	6	16,9
39	2::XP_012053621.1	hexokinase [Cryptococcus neoformans var. grubii H99]	207	61380	6	16,9
39	2::XP_012053803.1	hexokinase, variant [Cryptococcus neoformans var. grubii H99]	207	61380	6	16,9
39	2::AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	74	68079	4	7,9
39	2::XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	74	68079	4	7,9



Spot ID 26344

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
37	2:AFR95502.2	V-type proton ATPase catalytic subunit A [Cryptococcus neoformans var. grubii H99]	406	68545	12	25.7
37	2:XP_012049757.1	V-type proton ATPase catalytic subunit A [Cryptococcus neoformans var. grubii H99]	406	68545	12	25.7
37	2:AFR94027.1	D-lactate dehydrogenase [Cryptococcus neoformans var. grubii H99]	398	58490	11	27.4
37	2:XP_012048058.1	D-lactate dehydrogenase [Cryptococcus neoformans var. grubii H99]	398	58490	11	27.4
37	2:AFR98380.1	26S protease regulatory subunit 6A-B [Cryptococcus neoformans var. grubii H99]	352	52127	14	35.3
37	2:XP_012053170.1	26S protease regulatory subunit 6A-B [Cryptococcus neoformans var. grubii H99]	352	52127	14	35.3
37	2:AFR96040.1	alanine-tRNA ligase [Cryptococcus neoformans var. grubii H99]	243	112034	8	11.1
37	2:XP_012050473.1	alanine-tRNA ligase [Cryptococcus neoformans var. grubii H99]	243	112034	8	11.1
37	2:AFR98550.2	phosphoglucosyltransferase [Cryptococcus neoformans var. grubii H99]	232	60675	6	14.8
37	2:XP_012053288.1	phosphoglucosyltransferase [Cryptococcus neoformans var. grubii H99]	232	60675	6	14.8
37	2:AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	232	68079	7	16.5
37	2:XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	232	68079	7	16.5
37	2:AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	230	69698	7	14.8
37	2:XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	230	69698	7	14.8
37	2:AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	230	69759	7	14.8
37	2:XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	230	69759	7	14.8
37	2:AFR92468.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	187	67372	7	13.5
37	2:XP_012046659.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	187	67372	7	13.5
37	1:ALBU_BOVIN	Serum albumin OS=Bos taurus OX=9913 GN=ALB PE=1 SV=4	193	71244	10	18.8
37	2:AFR97484.2	extracellular elastinolytic metalloproteinase [Cryptococcus neoformans var. grubii H99]	190	92066	4	8.7
37	2:XP_012051981.1	extracellular elastinolytic metalloproteinase [Cryptococcus neoformans var. grubii H99]	190	92066	4	8.7
37	2:AFR95154.1	homocitrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	185	53963	5	12.9
37	2:XP_012049240.1	homocitrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	185	53963	5	12.9
37	2:AFR94491.2	hypothetical protein CNAG_05236 [Cryptococcus neoformans var. grubii H99]	184	52430	6	18
37	2:XP_012048550.1	hypothetical protein CNAG_05236 [Cryptococcus neoformans var. grubii H99]	184	52430	6	18
37	2:AFR92273.1	ubiquitin-activating enzyme E1 [Cryptococcus neoformans var. grubii H99]	176	112989	4	6.2
37	2:XP_012046554.1	ubiquitin-activating enzyme E1 [Cryptococcus neoformans var. grubii H99]	176	112989	4	6.2
37	2:AFR94513.1	catalase [Cryptococcus neoformans var. grubii H99]	149	56090	4	12
37	2:XP_012048806.1	catalase [Cryptococcus neoformans var. grubii H99]	149	56090	4	12
37	2:AFR93378.1	hypothetical protein CNAG_03878 [Cryptococcus neoformans var. grubii H99]	105	48023	3	8.1
37	2:XP_012047524.1	hypothetical protein CNAG_03878 [Cryptococcus neoformans var. grubii H99]	105	48023	3	8.1
37	2:AGV15424.1	galactokinase, variant [Cryptococcus neoformans var. grubii H99]	100	48219	3	8.5
37	2:AFR93446.2	galactokinase [Cryptococcus neoformans var. grubii H99]	100	58992	3	6.9
37	2:XP_012047283.1	galactokinase [Cryptococcus neoformans var. grubii H99]	100	58992	3	6.9
37	2:XP_012047568.1	galactokinase, variant [Cryptococcus neoformans var. grubii H99]	100	48219	3	8.5
37	1:FETA_BOVIN	Alpha-fetoprotein OS=Bos taurus OX=9913 GN=AFP PE=2 SV=1	95	70368	5	9
37	2:AFR97093.2	ATP-dependent RNA helicase DBP5 [Cryptococcus neoformans var. grubii H99]	95	59752	3	7
37	2:XP_012051508.1	ATP-dependent RNA helicase DBP5 [Cryptococcus neoformans var. grubii H99]	95	59752	3	7
37	2:AFR95611.1	nucleolar protein 56 [Cryptococcus neoformans var. grubii H99]	66	63021	3	6.3
37	2:XP_012050017.1	nucleolar protein 56 [Cryptococcus neoformans var. grubii H99]	66	63021	3	6.3

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
39	2:AFR94027.1	D-lactate dehydrogenase [Cryptococcus neoformans var. grubii H99]	303	58490	9	19.9
39	2:XP_012048058.1	D-lactate dehydrogenase [Cryptococcus neoformans var. grubii H99]	303	58490	9	19.9
39	2:AFR95502.2	V-type proton ATPase catalytic subunit A [Cryptococcus neoformans var. grubii H99]	203	68545	7	14.9
39	2:XP_012049757.1	V-type proton ATPase catalytic subunit A [Cryptococcus neoformans var. grubii H99]	203	68545	7	14.9
39	2:AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	195	68079	5	10.4
39	2:XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	195	68079	5	10.4
39	2:AFR96040.1	alanine-tRNA ligase [Cryptococcus neoformans var. grubii H99]	188	112034	4	5.1
39	2:XP_012050473.1	alanine-tRNA ligase [Cryptococcus neoformans var. grubii H99]	188	112034	4	5.1
39	2:AFR94513.1	catalase [Cryptococcus neoformans var. grubii H99]	173	56090	4	15.5
39	2:XP_012048806.1	catalase [Cryptococcus neoformans var. grubii H99]	173	56090	4	15.5
39	2:AFR92273.1	ubiquitin-activating enzyme E1 [Cryptococcus neoformans var. grubii H99]	118	112989	4	6.2
39	2:XP_012046554.1	ubiquitin-activating enzyme E1 [Cryptococcus neoformans var. grubii H99]	118	112989	4	6.2
39	2:AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	117	69759	6	13.1
39	2:AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	117	69698	6	13.1
39	2:XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	117	69759	6	13.1
39	2:XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	117	69698	6	13.1
39	2:AFR92468.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	86	67372	5	13.7
39	2:XP_012046659.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	86	67372	5	13.7
39	2:AFR95155.1	homocitrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	100	53963	4	8.8
39	2:XP_012049240.1	homocitrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	100	53963	4	8.8
39	1:ALBU_BOVIN	Serum albumin OS=Bos taurus OX=9913 GN=ALB PE=1 SV=4	87	71244	3	5.6
39	2:AFR98380.1	26S protease regulatory subunit 6A-B [Cryptococcus neoformans var. grubii H99]	72	52127	4	10.3
39	2:XP_012053170.1	26S protease regulatory subunit 6A-B [Cryptococcus neoformans var. grubii H99]	72	52127	4	10.3

Spot ID 26409

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
37	2:AFR97783.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	2935	38489	21	73,4
37	2:XP_012052598.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	2935	38489	21	73,4
37	2:AFR97782.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	666	49505	13	36,6
37	2:XP_012052614.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	666	49505	13	36,6
37	2:AFR92807.1	urease accessory protein UreG [Cryptococcus neoformans var. grubii H99]	263	33777	7	28,2
37	2:XP_012046883.1	urease accessory protein UreG [Cryptococcus neoformans var. grubii H99]	263	33777	7	28,2
37	1:ACT_CRYNH	Actin OS=Cryptococcus neoformans var. grubii serotype A (strain H99 / ATCC 208821 / CBS 10515 / FGSC 9487) OX=235443 GN=CNAG_00483 PE=3 SV=2	254	42089	5	17,6
37	2:AFR92615.2	actin [Cryptococcus neoformans var. grubii H99]	254	42307	5	17,5
37	2:XP_012046325.1	actin [Cryptococcus neoformans var. grubii H99]	254	42307	5	17,5
37	2:AFR98895.1	guanine nucleotide-binding protein subunit beta-like protein [Cryptococcus neoformans var. grubii H99]	254	34716	9	33,1
37	2:XP_012053792.1	guanine nucleotide-binding protein subunit beta-like protein [Cryptococcus neoformans var. grubii H99]	254	34716	9	33,1
37	2:AFR95986.1	glyceraldehyde-3-phosphate dehydrogenase [Cryptococcus neoformans var. grubii H99]	206	36571	4	22,4
37	2:XP_012050787.1	glyceraldehyde-3-phosphate dehydrogenase [Cryptococcus neoformans var. grubii H99]	206	36571	4	22,4
37	2:AFR95182.1	transketolase [Cryptococcus neoformans var. grubii H99]	129	74685	3	5,1
37	2:XP_012049657.1	transketolase [Cryptococcus neoformans var. grubii H99]	129	74685	3	5,1
37	2:AFR96057.2	hypothetical protein CNAG_05739 [Cryptococcus neoformans var. grubii H99]	128	37455	3	10,9
37	2:XP_012050353.1	hypothetical protein CNAG_05739 [Cryptococcus neoformans var. grubii H99]	128	37455	3	10,9
37	2:AFR96009.2	mannose-1-phosphate guanyltansferase [Cryptococcus neoformans var. grubii H99]	125	40211	4	11,8
37	2:XP_012052460.1	mannose-1-phosphate guanyltansferase [Cryptococcus neoformans var. grubii H99]	125	40211	4	11,8
Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
39	2:AFR97783.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	2328	38489	20	69,6
39	2:XP_012052598.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	2328	38489	20	69,6
39	2:AFR97441.1	homoserine dehydrogenase [Cryptococcus neoformans var. grubii H99]	335	39776	10	32,8
39	2:XP_012052199.1	homoserine dehydrogenase [Cryptococcus neoformans var. grubii H99]	335	39776	10	32,8
39	2:AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	296	69759	8	17,1
39	2:AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	296	69698	8	17,1
39	2:XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	296	69759	8	17,1
39	2:XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	296	69698	8	17,1
39	2:AFR93260.2	hypothetical protein CNAG_03755 [Cryptococcus neoformans var. grubii H99]	200	40158	5	17,4
39	2:XP_012047209.1	hypothetical protein CNAG_03755 [Cryptococcus neoformans var. grubii H99]	200	40158	5	17,4
39	2:AFR98895.1	guanine nucleotide-binding protein subunit beta-like protein [Cryptococcus neoformans var. grubii H99]	181	34716	6	23,9
39	2:XP_012053792.1	guanine nucleotide-binding protein subunit beta-like protein [Cryptococcus neoformans var. grubii H99]	181	34716	6	23,9
39	2:AFR94883.2	hypothetical protein CNAG_06946 [Cryptococcus neoformans var. grubii H99]	174	39281	5	17
39	2:XP_012049004.1	hypothetical protein CNAG_06946 [Cryptococcus neoformans var. grubii H99]	174	39281	5	17
39	2:AFR92474.1	3-(2~),5--bisphosphate nucleotidase [Cryptococcus neoformans var. grubii H99]	153	38891	7	26
39	2:XP_012047050.1	3-(2~),5--bisphosphate nucleotidase [Cryptococcus neoformans var. grubii H99]	153	38891	7	26
39	2:AFR98337.1	hypothetical protein CNAG_06113 [Cryptococcus neoformans var. grubii H99]	140	36583	3	12,8
39	2:XP_012053143.1	hypothetical protein CNAG_06113 [Cryptococcus neoformans var. grubii H99]	140	36583	3	12,8
39	2:AFR98009.2	mannose-1-phosphate guanyltansferase [Cryptococcus neoformans var. grubii H99]	135	40211	5	15,9
39	2:XP_012052460.1	mannose-1-phosphate guanyltansferase [Cryptococcus neoformans var. grubii H99]	135	40211	5	15,9
39	2:AFR96057.2	hypothetical protein CNAG_05739 [Cryptococcus neoformans var. grubii H99]	132	37455	4	13,6
39	2:XP_012050353.1	hypothetical protein CNAG_05739 [Cryptococcus neoformans var. grubii H99]	132	37455	4	13,6
39	2:AFR92257.1	glycerol-3-phosphate dehydrogenase (NAD+) [Cryptococcus neoformans var. grubii H99]	129	38007	3	10,2
39	2:XP_012046543.1	glycerol-3-phosphate dehydrogenase (NAD+) [Cryptococcus neoformans var. grubii H99]	129	38007	3	10,2
39	2:AFR94072.1	6-phosphogluconate dehydrogenase, decarboxylating 1 [Cryptococcus neoformans var. grubii H99]	114	54192	4	9
39	2:XP_012048411.1	6-phosphogluconate dehydrogenase, decarboxylating 1 [Cryptococcus neoformans var. grubii H99]	114	54192	4	9
39	2:AFR92807.1	urease accessory protein UreG [Cryptococcus neoformans var. grubii H99]	115	33777	3	11,2
39	2:XP_012046883.1	urease accessory protein UreG [Cryptococcus neoformans var. grubii H99]	115	33777	3	11,2
39	2:AFR92431.2	phosphopantothencycysteine decarboxylase [Cryptococcus neoformans var. grubii H99]	101	35266	3	11,1
39	2:XP_012046246.1	phosphopantothencycysteine decarboxylase [Cryptococcus neoformans var. grubii H99]	101	35266	3	11,1
39	2:AFR98921.1	succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial [Cryptococcus neoformans var. grubii H99]	85	70454	3	6
39	2:XP_012051649.1	succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial [Cryptococcus neoformans var. grubii H99]	85	70454	3	6
39	2:AFR97782.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	74	49505	3	6
39	2:XP_012052614.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	74	49505	3	6
39	2:AFR95182.1	transketolase [Cryptococcus neoformans var. grubii H99]	58	74685	3	4,7
39	2:XP_012049657.1	transketolase [Cryptococcus neoformans var. grubii H99]	58	74685	3	4,7
39	2:AFR95155.1	homocitrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	57	53963	3	6,3
39	2:XP_012049240.1	homocitrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	57	53963	3	6,3

Spot ID 26502

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
37	2::AFR93876.1	GTP-binding protein ypt2 [Cryptococcus neoformans var. grubii H99]	435	23743	7	51,2
37	2::XP_012048152.1	GTP-binding protein ypt2 [Cryptococcus neoformans var. grubii H99]	435	23743	7	51,2
37	2::AFR94332.1	GTP-binding protein ypt1 [Cryptococcus neoformans var. grubii H99]	191	22771	5	34,6
37	2::XP_012048696.1	GTP-binding protein ypt1 [Cryptococcus neoformans var. grubii H99]	191	22771	5	34,6
37	2::AFR95492.1	uracil phosphoribosyltransferase [Cryptococcus neoformans var. grubii H99]	364	25703	8	49,6
37	2::XP_012050086.1	uracil phosphoribosyltransferase [Cryptococcus neoformans var. grubii H99]	364	25703	8	49,6
37	2::AFR93317.2	20S proteasome subunit beta 1 [Cryptococcus neoformans var. grubii H99]	285	25548	5	23,3
37	2::XP_012047234.1	20S proteasome subunit beta 1 [Cryptococcus neoformans var. grubii H99]	285	25548	5	23,3
37	2::AFR96238.1	ATP synthase subunit beta, mitochondrial [Cryptococcus neoformans var. grubii H99]	238	58688	9	27,6
37	2::XP_012050629.1	ATP synthase subunit beta, mitochondrial [Cryptococcus neoformans var. grubii H99]	238	58688	9	27,6
37	2::AFR96043.1	ketol-acid reductoisomerase, mitochondrial [Cryptococcus neoformans var. grubii H99]	132	44371	3	9,7
37	2::XP_012050476.1	ketol-acid reductoisomerase, mitochondrial [Cryptococcus neoformans var. grubii H99]	132	44371	3	9,7
37	2::AFR97763.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	78	38489	3	8,9
37	2::XP_012052598.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	78	38489	3	8,9
37	2::AFR95498.1	small subunit ribosomal protein S9 [Cryptococcus neoformans var. grubii H99]	59	22281	3	16,1
37	2::XP_012050082.1	small subunit ribosomal protein S9 [Cryptococcus neoformans var. grubii H99]	59	22281	3	16,1

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
39	2::AFR92542.2	hypothetical protein CNAG_00409 [Cryptococcus neoformans var. grubii H99]	234	40637	4	17,9
39	2::XP_012046300.1	hypothetical protein CNAG_00409 [Cryptococcus neoformans var. grubii H99]	234	40637	4	17,9
39	2::AFR92468.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	200	67372	5	12,9
39	2::XP_012046659.1	hsp75-like protein [Cryptococcus neoformans var. grubii H99]	200	67372	5	12,9
39	2::AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	114	69759	4	8,2
39	2::AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	114	69698	4	8,3
39	2::XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	114	69759	4	8,2
39	2::XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	114	69698	4	8,3
39	2::AFR98178.1	transaldolase [Cryptococcus neoformans var. grubii H99]	155	35443	3	12,7
39	2::XP_012052888.1	transaldolase [Cryptococcus neoformans var. grubii H99]	155	35443	3	12,7
39	2::AFR97862.1	fumarate hydratase, mitochondrial [Cryptococcus neoformans var. grubii H99]	152	55201	5	17,8
39	2::XP_012052672.1	fumarate hydratase, mitochondrial [Cryptococcus neoformans var. grubii H99]	152	55201	5	17,8
39	2::AFR94490.1	protein BMH2 [Cryptococcus neoformans var. grubii H99]	147	29090	3	20,3
39	2::XP_012048794.1	protein BMH2 [Cryptococcus neoformans var. grubii H99]	147	29090	3	20,3
39	2::AFR94515.1	glucose-methanol-choline oxidoreductase [Cryptococcus neoformans var. grubii H99]	136	65625	5	10,9
39	2::XP_012048807.1	glucose-methanol-choline oxidoreductase [Cryptococcus neoformans var. grubii H99]	136	65625	5	10,9
39	2::AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	128	68079	4	9,1
39	2::XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	128	68079	4	9,1
39	2::AFR96301.2	large subunit ribosomal protein L7/L12 [Cryptococcus neoformans var. grubii H99]	109	18920	4	46,9
39	2::XP_012050442.1	large subunit ribosomal protein L7/L12 [Cryptococcus neoformans var. grubii H99]	109	18920	4	46,9
39	2::AFR97763.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	75	38489	3	12
39	2::XP_012052598.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	75	38489	3	12



Spot ID 26944

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
37 2::	AFR97763.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	875	38489	17	56,2
37 2::	XP_012052598.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	875	38489	17	56,2
37 2::	AFR98178.1	transaldolase [Cryptococcus neoformans var. grubii H99]	373	35443	9	39
37 2::	XP_012052888.1	transaldolase [Cryptococcus neoformans var. grubii H99]	373	35443	9	39
37 2::	XP_012050476.1	ketol-acid reductoisomerase, mitochondrial [Cryptococcus neoformans var. grubii H99]	295	44371	8	25,9
37 2::	AFR96254.1	UMP-CMP kinase [Cryptococcus neoformans var. grubii H99]	276	30840	8	36,1
37 2::	XP_012050636.1	UMP-CMP kinase [Cryptococcus neoformans var. grubii H99]	276	30840	8	36,1
37 2::	AFR97782.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	246	49505	8	26,6
37 2::	XP_012052614.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	246	49505	8	26,6
37 2::	AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	226	69698	6	13,1
37 2::	XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	226	69698	6	13,1
37 2::	AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	226	69759	6	13,1
37 2::	XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	226	69759	6	13,1
37 2::	AFR94883.2	hypothetical protein CNAG_06946 [Cryptococcus neoformans var. grubii H99]	202	39281	6	21,8
37 2::	XP_012049004.1	hypothetical protein CNAG_06946 [Cryptococcus neoformans var. grubii H99]	202	39281	6	21,8
37 2::	AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	188	68079	6	10,6
37 2::	XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	188	68079	6	10,6
37 2::	AFR98791.1	ribose-phosphate pyrophosphokinase [Cryptococcus neoformans var. grubii H99]	169	39037	5	14,3
37 2::	XP_012053714.1	ribose-phosphate pyrophosphokinase [Cryptococcus neoformans var. grubii H99]	169	39037	5	14,3
37 2::	AFR98332.1	G protein beta subunit-like [Cryptococcus neoformans var. grubii H99]	145	37319	4	18
37 2::	XP_012053137.1	G protein beta subunit-like [Cryptococcus neoformans var. grubii H99]	145	37319	4	18
37 2::	AFR94962.1	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	125	37997	3	11,2
37 2::	XP_012049371.1	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	125	37997	3	11,2
37 2::	AFR97441.1	homoserine dehydrogenase [Cryptococcus neoformans var. grubii H99]	114	39776	6	19,2
37 2::	XP_012052199.1	homoserine dehydrogenase [Cryptococcus neoformans var. grubii H99]	114	39776	6	19,2

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
39 2::	AFR97929.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	1557	69759	20	33,1
39 2::	XP_012052712.1	hsp71-like protein [Cryptococcus neoformans var. grubii H99]	1557	69759	20	33,1
39 2::	AFR97952.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	1293	69698	16	27,7
39 2::	XP_012052733.1	hsp72-like protein [Cryptococcus neoformans var. grubii H99]	1293	69698	16	27,7
39 2::	AFR97763.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	803	38489	15	55,9
39 2::	XP_012052598.1	chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99]	803	38489	15	55,9
39 2::	AFR96043.1	ketol-acid reductoisomerase, mitochondrial [Cryptococcus neoformans var. grubii H99]	355	44371	9	28,9
39 2::	XP_012050476.1	ketol-acid reductoisomerase, mitochondrial [Cryptococcus neoformans var. grubii H99]	355	44371	9	28,9
39 2::	AFR97558.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	339	68079	10	19,6
39 2::	XP_012052124.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	339	68079	10	19,6
39 2::	AFR98178.1	transaldolase [Cryptococcus neoformans var. grubii H99]	320	35443	6	31,3
39 2::	XP_012052888.1	transaldolase [Cryptococcus neoformans var. grubii H99]	320	35443	6	31,3
39 2::	AFR93031.1	fructose-bisphosphate aldolase 1 [Cryptococcus neoformans var. grubii H99]	292	39630	5	23,4
39 2::	XP_012047721.1	fructose-bisphosphate aldolase 1 [Cryptococcus neoformans var. grubii H99]	292	39630	5	23,4
39 2::	AFR94883.2	hypothetical protein CNAG_06946 [Cryptococcus neoformans var. grubii H99]	287	39281	6	20,7
39 2::	XP_012049004.1	hypothetical protein CNAG_06946 [Cryptococcus neoformans var. grubii H99]	287	39281	6	20,7
39 2::	AFR98659.1	acetolactate synthase, small subunit [Cryptococcus neoformans var. grubii H99]	282	39244	5	19,9
39 2::	XP_012053485.1	acetolactate synthase, small subunit [Cryptococcus neoformans var. grubii H99]	282	39244	5	19,9
39 2::	AFR94962.1	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	256	37997	7	29,6
39 2::	XP_012049371.1	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	256	37997	7	29,6
39 2::	AFR94637.2	elongation factor 2 [Cryptococcus neoformans var. grubii H99]	216	93334	6	8,4
39 2::	XP_012048908.1	elongation factor 2 [Cryptococcus neoformans var. grubii H99]	216	93334	6	8,4
39 2::	AGV14286.1	elongation factor 2, variant [Cryptococcus neoformans var. grubii H99]	180	91946	5	7,4
39 2::	XP_012049590.1	elongation factor 2, variant [Cryptococcus neoformans var. grubii H99]	180	91946	5	7,4
39 2::	AFR97782.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	199	49505	5	15,5
39 2::	XP_012052614.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	199	49505	5	15,5
39 2::	AFR93867.1	argininosuccinate lyase [Cryptococcus neoformans var. grubii H99]	154	52598	7	21
39 2::	XP_012048159.1	argininosuccinate lyase [Cryptococcus neoformans var. grubii H99]	154	52598	7	21
39 2::	AFR95182.1	transketolase [Cryptococcus neoformans var. grubii H99]	146	74685	4	8,2
39 2::	XP_012049657.1	transketolase [Cryptococcus neoformans var. grubii H99]	146	74685	4	8,2
39 2::	AFR97441.1	homoserine dehydrogenase [Cryptococcus neoformans var. grubii H99]	143	39776	6	22,1
39 2::	XP_012052199.1	homoserine dehydrogenase [Cryptococcus neoformans var. grubii H99]	143	39776	6	22,1
39 2::	AFR92184.1	26S proteasome regulatory subunit N8 [Cryptococcus neoformans var. grubii H99]	87	38733	3	11,1
39 2::	XP_012046487.1	26S proteasome regulatory subunit N8 [Cryptococcus neoformans var. grubii H99]	87	38733	3	11,1
39 2::	AGV15308.1	glutamine synthetase, variant [Cryptococcus neoformans var. grubii H99]	43	39890	3	12,6
39 2::	AFR92589.1	glutamine synthetase [Cryptococcus neoformans var. grubii H99]	43	39890	3	12,6
39 2::	XP_012046729.1	glutamine synthetase [Cryptococcus neoformans var. grubii H99]	43	39890	3	12,6
39 2::	XP_012046730.1	glutamine synthetase, variant [Cryptococcus neoformans var. grubii H99]	43	39890	3	12,6



39 2::XP_012046704.1	S-adenosylmethionine synthase [Cryptococcus neoformans var. grubii H99]	160	43341	3	10,8
39 2::AFR95034.1	xenobiotic reductase [Cryptococcus neoformans var. grubii H99]	156	41535	4	12,8
39 2::XP_012049323.1	xenobiotic reductase [Cryptococcus neoformans var. grubii H99]	156	41535	4	12,8
39 2::AFR92615.2	actin [Cryptococcus neoformans var. grubii H99]	143	42307	4	15,1
39 2::XP_012046325.1	actin [Cryptococcus neoformans var. grubii H99]	143	42307	4	15,1
39 2::AFR97782.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	139	49505	3	9,3
39 2::XP_012052614.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	139	49505	3	9,3
39 2::AFR98435.1	heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	127	85923	4	9,2
39 2::XP_012053205.1	heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	127	85923	4	9,2
39 2::AFR93954.1	T-complex protein 1 subunit theta [Cryptococcus neoformans var. grubii H99]	125	58614	5	10,1
39 2::XP_012048096.1	T-complex protein 1 subunit theta [Cryptococcus neoformans var. grubii H99]	125	58614	5	10,1
39 2::AFR95182.1	transketolase [Cryptococcus neoformans var. grubii H99]	81	74685	3	6
39 2::XP_012049657.1	transketolase [Cryptococcus neoformans var. grubii H99]	81	74685	3	6
39 2::AFR98009.2	mannose-1-phosphate guanyltransferase [Cryptococcus neoformans var. grubii H99]	79	40211	3	11,8
39 2::XP_012052460.1	mannose-1-phosphate guanyltransferase [Cryptococcus neoformans var. grubii H99]	79	40211	3	11,8
39 2::AFR94380.2	sarcosine oxidase [Cryptococcus neoformans var. grubii H99]	63	49685	4	10,5
39 2::XP_012048509.1	sarcosine oxidase [Cryptococcus neoformans var. grubii H99]	63	49685	4	10,5
39 2::AFR95720.1	fatty acid synthase subunit beta, fungi type [Cryptococcus neoformans var. grubii H99]	59	275733	3	1,4
39 2::XP_012049942.1	fatty acid synthase subunit beta, fungi type [Cryptococcus neoformans var. grubii H99]	59	275733	3	1,4
39 2::XP_012052462.1	pyruvate kinase [Cryptococcus neoformans var. grubii H99]	58	61939	3	5,6
39 2::XP_012052782.1	pyruvate kinase, variant [Cryptococcus neoformans var. grubii H99]	58	57396	3	6
39 2::AGV14792.1	transcription factor C subunit 7, variant [Cryptococcus neoformans var. grubii H99]	55	39081	3	8,2
39 2::AFR98460.2	transcription factor C subunit 7 [Cryptococcus neoformans var. grubii H99]	55	39081	3	8,2
39 2::XP_012053054.1	transcription factor C subunit 7 [Cryptococcus neoformans var. grubii H99]	55	39081	3	8,2
39 2::XP_012053222.1	transcription factor C subunit 7, variant [Cryptococcus neoformans var. grubii H99]	55	39081	3	8,2





Spot ID 36595

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
37	2:AFR96168.2	ATP-dependent RNA helicase DBP2-A [Cryptococcus neoformans var. grubii H99]	566	58016	14	26,4
37	2:XP_012050393.1	ATP-dependent RNA helicase DBP2-A [Cryptococcus neoformans var. grubii H99]	566	58016	14	26,4
37	2:AFR93774.1	acetyl-CoA C-acetyltransferase [Cryptococcus neoformans var. grubii H99]	204	42673	4	20,2
37	2:XP_012048218.1	acetyl-CoA C-acetyltransferase [Cryptococcus neoformans var. grubii H99]	204	42673	4	20,2

Gel	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
39	2:AFR96168.2	ATP-dependent RNA helicase DBP2-A [Cryptococcus neoformans var. grubii H99]	302	58016	10	21,2
39	2:XP_012050393.1	ATP-dependent RNA helicase DBP2-A [Cryptococcus neoformans var. grubii H99]	302	58016	10	21,2
39	2:AFR95169.1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 9 [Cryptococcus neoformans var. grubii H99]	147	45614	4	12,4
39	2:XP_012049223.1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 9 [Cryptococcus neoformans var. grubii H99]	147	45614	4	12,4



39 2::XP_012053141.1	hypothetical protein, variant [Cryptococcus neoformans var. grubii H99]	291	39960	4	16,8
39 2::AFR92550.1	elongation factor 1-gamma [Cryptococcus neoformans var. grubii H99]	284	46734	9	25,5
39 2::XP_012046703.1	elongation factor 1-gamma [Cryptococcus neoformans var. grubii H99]	284	46734	9	25,5
39 2::AFR97747.2	taurine catabolism dioxygenase TauD [Cryptococcus neoformans var. grubii H99]	282	50644	8	24,3
39 2::XP_012052362.1	taurine catabolism dioxygenase TauD [Cryptococcus neoformans var. grubii H99]	282	50644	8	24,3
39 2::AFR92199.1	citrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	242	51137	7	18,1
39 2::XP_012046501.1	citrate synthase, mitochondrial [Cryptococcus neoformans var. grubii H99]	242	51137	7	18,1
39 2::AFR92389.1	aspartate-semialdehyde dehydrogenase [Cryptococcus neoformans var. grubii H99]	241	39669	7	24
39 2::XP_012046616.1	aspartate-semialdehyde dehydrogenase [Cryptococcus neoformans var. grubii H99]	241	39669	7	24
39 2::AFR98608.1	branched-chain amino acid aminotransferase [Cryptococcus neoformans var. grubii H99]	180	47090	6	16,2
39 2::XP_012053446.1	branched-chain amino acid aminotransferase [Cryptococcus neoformans var. grubii H99]	180	47090	6	16,2
39 2::AFR98529.1	hypothetical protein CNAG_06294 [Cryptococcus neoformans var. grubii H99]	156	51491	5	12,3
39 2::XP_012053387.1	hypothetical protein CNAG_06294 [Cryptococcus neoformans var. grubii H99]	156	51491	5	12,3
39 2::AFR95859.1	pyruvate dehydrogenase (acetyl-transferring) E1 component, alpha subunit [Cryptococcus neoformans var. grubii H99]	143	45944	4	10,9
39 2::XP_012050793.1	pyruvate dehydrogenase (acetyl-transferring) E1 component, alpha subunit [Cryptococcus neoformans var. grubii H99]	143	45944	4	10,9
39 2::AFR92228.1	hypothetical protein CNAG_00091 [Cryptococcus neoformans var. grubii H99]	142	52694	4	16,5
39 2::XP_012046521.1	hypothetical protein CNAG_00091 [Cryptococcus neoformans var. grubii H99]	142	52694	4	16,5
39 2::AFR94742.1	3-deoxy-7-phosphoheptulonate synthase [Cryptococcus neoformans var. grubii H99]	139	42380	4	11,7
39 2::XP_012049520.1	3-deoxy-7-phosphoheptulonate synthase [Cryptococcus neoformans var. grubii H99]	139	42380	4	11,7
39 2::AFR98265.1	aspartate aminotransferase [Cryptococcus neoformans var. grubii H99]	135	46108	4	10,9
39 2::XP_012053092.1	aspartate aminotransferase [Cryptococcus neoformans var. grubii H99]	135	46108	4	10,9
39 2::AFR99053.1	chorismate synthase [Cryptococcus neoformans var. grubii H99]	126	45696	4	16,9
39 2::XP_012053886.1	chorismate synthase [Cryptococcus neoformans var. grubii H99]	126	45696	4	16,9
39 2::AFR94166.1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase [Cryptococcus neoformans var. grubii H99]	105	58991	4	15
39 2::XP_012048337.1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase [Cryptococcus neoformans var. grubii H99]	105	58991	4	15
39 2::AFR98073.1	GMP synthase [glutamine-hydrolyzing] [Cryptococcus neoformans var. grubii H99]	102	60245	3	6,6
39 2::XP_012052820.1	GMP synthase [glutamine-hydrolyzing] [Cryptococcus neoformans var. grubii H99]	102	60245	3	6,6
39 2::AFR95182.1	transketolase [Cryptococcus neoformans var. grubii H99]	96	74685	3	5,5
39 2::XP_012049657.1	transketolase [Cryptococcus neoformans var. grubii H99]	96	74685	3	5,5
39 2::AFR94509.1	chaperone regulator [Cryptococcus neoformans var. grubii H99]	96	41144	4	14,1
39 2::XP_012048804.1	chaperone regulator [Cryptococcus neoformans var. grubii H99]	96	41144	4	14,1
39 2::AFR97947.1	glycerol-3-phosphate dehydrogenase (NAD(+)) [Cryptococcus neoformans var. grubii H99]	94	42302	4	9,2
39 2::XP_012052730.1	glycerol-3-phosphate dehydrogenase (NAD(+)) [Cryptococcus neoformans var. grubii H99]	94	42302	4	9,2
39 2::AFR93420.2	isocitrate dehydrogenase, NADP-dependent [Cryptococcus neoformans var. grubii H99]	65	50965	3	8,2
39 2::XP_012047274.1	isocitrate dehydrogenase, NADP-dependent [Cryptococcus neoformans var. grubii H99]	65	50965	3	8,2

**Supplementary Table 5**

**Supplementary table 5: Information on homology of the twenty-three cryptococcal proteins identified to be immunoreactive with human sera**

Homology of immunoreactive cryptococcal proteins to human proteins, *Saccharomyces cerevisiae* proteins, and proteins from pathogenic fungi causing systemic infections was investigated. Individual BLAST results for each homology search are listed in separate register tabs. Homology was determined using the NCBI protein blast tool: [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

No. of register tab	Homology investigated	of
2	Homology to human proteins	all 23 immunoreactive proteins
3	Homology to <i>S. cerevisiae</i> proteins	all 23 immunoreactive proteins
4	Homology to proteins from pathogenic fungi	26S proteasome subunit N8
5	Homology to proteins from pathogenic fungi	Chlorophyll synthesis pathway protein BchC
6	Homology to proteins from pathogenic fungi	Cytoplasmic protein CNAG_02943
7	Homology to proteins from pathogenic fungi	Deoxyuridine 5'-triphosphate nucleotidohydrolase
8	Homology to proteins from pathogenic fungi	Extracellular elastinolytic metalloproteinase
9	Homology to proteins from pathogenic fungi	Glucose-methanol-choline oxidoreductase
10	Homology to proteins from pathogenic fungi	Glutamate dehydrogenase (NADP)
11	Homology to proteins from pathogenic fungi	Glycerol-3-phosphate dehydrogenase (NAD(+))
12	Homology to proteins from pathogenic fungi	GTP-binding protein ypt1
13	Homology to proteins from pathogenic fungi	Heat shock 70kDa protein 4
14	Homology to proteins from pathogenic fungi	Hsp71-like protein
15	Homology to proteins from pathogenic fungi	Hsp72-like protein
16	Homology to proteins from pathogenic fungi	Hsp75-like protein
17	Homology to proteins from pathogenic fungi	Hypothetical protein CNAG_05236
18	Homology to proteins from pathogenic fungi	Hypothetical protein CNAG_06113
19	Homology to proteins from pathogenic fungi	Hypothetical protein CNAG_06946
20	Homology to proteins from pathogenic fungi	Ketol-acid reductoisomerase, mitochondrial
21	Homology to proteins from pathogenic fungi	Mannose-1-phosphate guanyltansferase
22	Homology to proteins from pathogenic fungi	Phosphoglucomutase
23	Homology to proteins from pathogenic fungi	Pyruvate decarboxylase
24	Homology to proteins from pathogenic fungi	Transaldolase
25	Homology to proteins from pathogenic fungi	Transketolase
26	Homology to proteins from pathogenic fungi	Urease accessory protein UreG
27	Homology to proteins from <i>C. neoformans</i> H99	all 23 immunoreactive proteins





Protein Name	Accession	Strain	Gene ID	Gene Name	Gene Type	Gene Description	Gene Length (aa)	% Identity	% Coverage	Reference			
Hypoethelial protein CMAG_06946	AFR94883	2	MG1	homolog [Homo sapiens]			322	97%	1,005-107	49.71%	576	AAI17847.1	
Hypoethelial protein CMAG_06946	AFR94883	3	MG1	evolved precursor [Homo sapiens]			322	97%	2,005-107	49.43%	376	NP_057553.4	
Hypoethelial protein CMAG_06946	AFR94883	4	ResName: full-MG1, exonucleolus; flag; precursor [Homo sapiens]				322	97%	2,005-107	49.43%	376	CB18073.1	
Hypoethelial protein CMAG_06946	AFR94883	5	C3.2a110 protein [Homo sapiens]				246	97%	1,005-78	43.10%	322	AAI11396.2	
Hypoethelial protein CMAG_06946	AFR94883	6	chromosome 12 open reading frame 10, isoform CRA_b [Homo sapiens]				246	97%	2,005-78	43.10%	325	FAW95687.1	
Hypoethelial protein CMAG_06946	AFR94883	7	chromosome 17 open reading frame 10, isoform CRA_d [Homo sapiens]				236	70%	1,005-75	47.56%	261	FAW95690.1	
Hypoethelial protein CMAG_06946	AFR94883	8	C3.2a110 protein [Homo sapiens]				203	61%	4,005-63	46.51%	221	AAI12800.1	
Hypoethelial protein CMAG_06946	AFR94883	9	unnamed protein product [Homo sapiens]				125	37%	5,005-34	54.35%	166	BAG56948.1	
Hypoethelial protein CMAG_06946	AFR94883	10	chromosome 12 open reading frame 10, isoform CRA_c [Homo sapiens]				107	37%	2,005-27	41.04%	132	FAW95689.1	
no homolog in <i>Homo sapiens</i>	AFR94883	0											
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	1	mannose-1-phosphate guanylyltransferase beta isoform 2 [Homo sapiens]				445	100%	6,005-156	58.63%	360	NP_058906.2	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	2	mannose-1-phosphate guanylyltransferase beta isoform 1 [Homo sapiens]				444	100%	1,005-155	58.63%	360	AAI038161.1	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	3	mannose-1-phosphate guanylyltransferase beta isoform 1 [Homo sapiens]				429	100%	2,005-149	54.59%	387	NP_037466.3	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	4	unnamed protein product [Homo sapiens]				429	100%	4,005-149	54.59%	307	BAL14892.1	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	5	GDP-mannose pyrophosphorylase A variant [Homo sapiens]				211	98%	2,005-63	32.85%	420	BAC99687.1	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	6	mannose-1-phosphate guanylyltransferase alpha [Homo sapiens]				209	98%	6,005-63	32.85%	420	NP_001261273.1	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	7	unnamed protein product [Homo sapiens]				209	98%	6,005-63	32.85%	420	BAA91460.1	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	8	unnamed protein product [Homo sapiens]				209	98%	7,005-63	32.85%	420	BAF93560.1	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	9	GDP-mannose pyrophosphorylase A [Homo sapiens]				197	89%	1,005-58	34.28%	399	AAI038157.1	
mannose-1-phosphate guanylyltransferase [Homo sapiens]	AFR94883	10	GDP-mannose pyrophosphorylase A, isoform CRA_c [Homo sapiens]				192	88%	7,005-56	29.96%	473	FAW970759.1	
phosphoglucomutase [Homo sapiens]	AFR94883	1	Crystal structure of wild-type human phosphoglucomutase 1 [Homo sapiens]				634	100%	0.0	56.72%	385	5F9C_A	
phosphoglucomutase [Homo sapiens]	AFR94883	2	unnamed protein product [Homo sapiens]				633	100%	0.0	56.72%	362	BAC935188.1	
phosphoglucomutase [Homo sapiens]	AFR94883	3	phosphoglucomutase-1 isoform 1 [Homo sapiens]				633	100%	0.0	56.72%	362	NP_005242.2	
phosphoglucomutase [Homo sapiens]	AFR94883	4	Crystal structure of the R503Q missense variant of human PG-M1 [Homo sapiens]				631	100%	0.0	56.54%	364	5VGT_B	
phosphoglucomutase [Homo sapiens]	AFR94883	5	Crystal structure of the R515Q missense variant of human PG-M1 [Homo sapiens]				631	100%	0.0	56.54%	385	51WV_A	
phosphoglucomutase [Homo sapiens]	AFR94883	6	Crystal structure of the R422Q missense variant of human PG-M1 [Homo sapiens]				631	100%	0.0	56.54%	385	6U06_A	
phosphoglucomutase [Homo sapiens]	AFR94883	7	PGM1 [Homo sapiens]				631	100%	0.0	56.54%	362	AAA60080.1	
phosphoglucomutase [Homo sapiens]	AFR94883	8	Crystal structure of the G121R mutant of human phosphoglucomutase 1 [Homo sapiens]				630	100%	0.0	56.54%	362	5F9C_A	
phosphoglucomutase [Homo sapiens]	AFR94883	9	Crystal structure of the G291R mutant of human phosphoglucomutase 1 [Homo sapiens]				630	100%	0.0	56.54%	362	AAI167763.2	
no homolog in <i>Homo sapiens</i>	AFR94883	10					630	100%	0.0	56.54%	362	516H_A	
private decarboxylase [Homo sapiens]	AFR94883	1	transaldolase [Homo sapiens]				384	99%	4,005-133	61.18%	337	NP_002746.1	
transaldolase [Homo sapiens]	AFR94883	2	TALDO1 protein [Homo sapiens]				384	99%	4,005-133	61.18%	336	AAI11897.2	
transaldolase [Homo sapiens]	AFR94883	3	transaldolase 1, isoform CRA_c [Homo sapiens]				179	44%	4,005-55	62.07%	154	EAO02379.1	
transaldolase [Homo sapiens]	AFR94883	4	transaldolase [Homo sapiens]				107	24%	2,005-28	66.67%	78	AAI31313.1	
transaldolase [Homo sapiens]	AFR94883	5	transaldolase 1, isoform CRA_b [Homo sapiens]				104	26%	6,005-37	58.10%	86	EAO02378.1	
transaldolase [Homo sapiens]	AFR94883	6	transaldolase 1, isoform CRA_a [Homo sapiens]				86.3	22%	2,005-20	56.94%	72	EAO02377.1	
transaldolase [Homo sapiens]	AFR94883	1	The structure of human Transaldolase [Homo sapiens]				181	181	1,005-47	27.42%	616	3M05_A	
transaldolase [Homo sapiens]	AFR94883	2	transaldolase [Homo sapiens]				180	180	2,005-47	27.42%	623	AAA61222.1	
transaldolase [Homo sapiens]	AFR94883	3	Crystal structure of Human Transaldolase (TCD) [Homo sapiens]				180	18000%	87%	2,005-47	27.42%	616	300Y_A
transaldolase [Homo sapiens]	AFR94883	4	transaldolase isoform 1 [Homo sapiens]				180	180	87%	2,005-47	27.42%	623	NP_001055.1
transaldolase [Homo sapiens]	AFR94883	5	Human transaldolase in covalent complex with donor, ketose D-fructose-6-phosphate [Homo sapiens]				180	180	87%	2,005-47	27.66%	637	4KXV_A
transaldolase [Homo sapiens]	AFR94883	6	transaldolase variant [Homo sapiens]				180	180	87%	3,005-47	27.42%	623	BAC97338.1
transaldolase [Homo sapiens]	AFR94883	7	transaldolase isoform 2 [Homo sapiens]				179	179	87%	4,005-47	27.45%	631	NP_00124857.1
transaldolase [Homo sapiens]	AFR94883	8	Human transaldolase variant T382E [Homo sapiens]				179	179	87%	5,005-47	27.42%	637	6R0J_A
transaldolase [Homo sapiens]	AFR94883	9	Human transaldolase variant E150Q in covalent complex with donor, ketose D-fructose-6-phosphate [Homo sapiens]				179	179	87%	7,005-47	27.50%	637	6IAI3_A
transaldolase [Homo sapiens]	AFR94883	10	unnamed protein product [Homo sapiens]				176	176	87%	4,005-46	27.28%	631	BAC56947.1
no homolog in <i>Homo sapiens</i>	AFR94883	0											

**Homology of immunoreactive cryptococcal proteins to proteins from *S. cerevisiae***

A maximum of 10 hits from the BLAST search is listed for each protein

accession no.	Hit No.	<i>Saccharomyces cerevisiae</i> homology (max. 10 hits)	species	Max score	Total score	Query Cover	E value	% identity	Acc. Len	accession no.2
AFR92184	1	Rpr8p [Saccharomyces cerevisiae YJM1078]	Saccharomyces cerevisiae YJM1078	285	286	87%	4.00E-94	45.57%	338	AJP41711.1
AFR92184	2	Rpr8p [Saccharomyces cerevisiae YJM270]	Saccharomyces cerevisiae YJM270	285	286	87%	4.00E-94	45.57%	338	AUT13617.1
AFR92184	3	Rpr8p [Saccharomyces cerevisiae YJM1089]	Saccharomyces cerevisiae YJM1089	285	285	87%	6.00E-94	45.57%	338	AUT1193.1
AFR92184	4	Rpr8p [Saccharomyces cerevisiae YJM1386]	Saccharomyces cerevisiae YJM1386	285	285	87%	6.00E-94	45.57%	338	AU00007.1
AFR92184	5	hypothetical protein B6NZ3_1804 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	285	285	87%	7.00E-94	45.57%	338	CNH7802.1
AFR92184	6	Rpr8p [Saccharomyces cerevisiae YJM1193]	Saccharomyces cerevisiae YJM1193	285	285	87%	9.00E-94	45.54%	336	AUT1198.1
AFR92184	7	SXZ_G004820.mRNA.1.CDS.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	284	284	84%	2.00E-93	46.37%	336	CAD654886.1
AFR92184	8	hypothetical protein S2EP1_000907.560 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	283	283	87%	4.00E-93	45.54%	336	GES57880.1
AFR92184	9	Rpr8p [Saccharomyces cerevisiae YJM1399]	Saccharomyces cerevisiae YJM1399	283	283	87%	5.00E-93	45.23%	336	AU020552.1
AFR92184	10	proteasome regulatory particle lid subunit Rpr8 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	282	282	87%	8.00E-93	45.26%	338	NP_014909.3
AFR92763	1	hypothetical protein QAA3_5134 [Saccharomyces cerevisiae La (v) Oa23]	Saccharomyces cerevisiae La (v) Oa23	124	124	94%	2.00E-31	28.61%	358	FGA8019.1
AFR92763	2	Xy2p [Saccharomyces cerevisiae YJM1083]	Saccharomyces cerevisiae YJM1083	121	121	93%	1.00E-30	29.07%	356	AV46114.1
AFR92763	3	Xy2p [Saccharomyces cerevisiae YJM1447]	Saccharomyces cerevisiae YJM1447	120	120	93%	3.00E-30	28.86%	356	AV65832.1
AFR92763	4	Xy2p [Saccharomyces cerevisiae YJM1389]	Saccharomyces cerevisiae YJM1389	120	120	93%	3.00E-30	28.86%	356	AV59623.1
AFR92763	5	Xy2p [Saccharomyces cerevisiae YJM1250]	Saccharomyces cerevisiae YJM1250	120	120	93%	3.00E-30	28.86%	356	AV50644.1
AFR92763	6	HNL_G0048600.mRNA.1.CDS.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	121	121	91%	3.00E-30	27.96%	417	CAD6594683.1
AFR92763	7	SXZ_G0027370.mRNA.1.CDS.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	120	120	93%	3.00E-30	28.86%	356	CAD6588042.1
AFR92763	8	D-xylulose reductase Xy2 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	120	120	93%	3.00E-30	28.86%	356	NP_013713.1
AFR92763	9	Xy2p [Saccharomyces cerevisiae YJM401]	Saccharomyces cerevisiae YJM401	120	120	93%	4.00E-30	28.86%	356	AV60955.1
AFR92763	10	Xy2p [Saccharomyces cerevisiae YJM1129]	Saccharomyces cerevisiae YJM1129	120	120	93%	4.00E-30	28.86%	356	AV46606.1
AFR93749	1	Sim1p [Saccharomyces cerevisiae YJM1199]	Saccharomyces cerevisiae YJM1199	162	16200%	62%	2.00E-41	26.23%	686	AIR43671.1
AFR93749	2	Sim1p [Saccharomyces cerevisiae YJM1083]	Saccharomyces cerevisiae YJM1083	162	16200%	62%	2.00E-41	26.23%	686	AIR42901.1
AFR93749	3	Regulator of the glycerol channel 1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	159	15900%	62%	9.00E-41	26.49%	584	DM078644.1
AFR93749	4	Sim1p [Saccharomyces cerevisiae YJM1387]	Saccharomyces cerevisiae YJM1387	159	15900%	62%	2.00E-40	26.49%	620	AIR48556.1
AFR93749	5	XKS2_U_G004940.mRNA.1.CDS.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	159	15900%	62%	2.00E-40	26.73%	688	CAD6602688.1
AFR93749	6	Sim1p [Saccharomyces cerevisiae YJ293]	Saccharomyces cerevisiae YJ293	159	15900%	62%	2.00E-40	26.73%	686	FIL00835.1
AFR93749	7	Sim1p [Saccharomyces cerevisiae YJM1615]	Saccharomyces cerevisiae YJM1615	159	15900%	62%	3.00E-40	26.73%	686	AIR45015.1
AFR93749	8	Sim1p [Saccharomyces cerevisiae YJM1460]	Saccharomyces cerevisiae YJM1460	159	15900%	62%	3.00E-40	26.49%	683	AIR51933.1
AFR93749	9	Sim1p [Saccharomyces cerevisiae VLS]	Saccharomyces cerevisiae VLS	158	15800%	62%	3.00E-40	26.49%	673	FGA84042.1
AFR93749	10	phosphatidylserine 4- $\beta$ -phosphatase-binding protein Slm1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	158	15800%	62%	4.00E-40	26.49%	677	GFF73549.1
AFR94562	1	Crt1p [Saccharomyces cerevisiae YJM113]	Saccharomyces cerevisiae YJM113	382	382	67%	5.00E-125	41.39%	474	FGA7702.1
AFR94562	2	Crt1p [Saccharomyces cerevisiae YJM1190]	Saccharomyces cerevisiae YJM1190	386	386	72%	2.00E-124	39.77%	651	AV47811.1
AFR94562	3	Crt1p [Saccharomyces cerevisiae YJM1078]	Saccharomyces cerevisiae YJM1078	386	386	72%	2.00E-124	39.77%	651	AIP4054.1
AFR94562	4	Crt1p [Saccharomyces cerevisiae YJM1399]	Saccharomyces cerevisiae YJM1399	386	386	72%	3.00E-124	39.77%	651	AV60957.1
AFR94562	5	Crt1p [Saccharomyces cerevisiae YJM1450]	Saccharomyces cerevisiae YJM1450	386	386	72%	3.00E-124	39.77%	651	AV66389.1
AFR94562	6	Crt1p [Saccharomyces cerevisiae YJM681]	Saccharomyces cerevisiae YJM681	386	386	72%	3.00E-124	39.77%	651	AV80882.1
AFR94562	7	Crt1p [Saccharomyces cerevisiae YJM1250]	Saccharomyces cerevisiae YJM1250	386	386	72%	3.00E-124	39.77%	651	AV50946.1
AFR94562	8	Crt1p [Saccharomyces cerevisiae YJM1133]	Saccharomyces cerevisiae YJM1133	386	386	72%	3.00E-124	39.58%	651	AV47823.1
AFR94562	9	Crt1p [Saccharomyces cerevisiae YJM1463]	Saccharomyces cerevisiae YJM1463	386	386	72%	3.00E-124	39.77%	651	AV67848.1
AFR94562	10	Crt1p [Saccharomyces cerevisiae YJM1311]	Saccharomyces cerevisiae YJM1311	385	385	72%	4.00E-124	39.77%	651	AV53781.1
AFR94562	0	no homology in Saccharomyces cerevisiae								
AFR94562	0	no homology in Saccharomyces cerevisiae								
AFR94515	0	no homology in Saccharomyces cerevisiae								
AFR97782	1	Gdh3p [Saccharomyces cerevisiae YJM1389]	Saccharomyces cerevisiae YJM1389	544	544	98%	0.0	61.27%	479	FGA59856.1
AFR97782	2	Gdh3p [Saccharomyces cerevisiae YJM1399]	Saccharomyces cerevisiae YJM1399	543	543	98%	0.0	61.49%	457	AQ096484.1
AFR97782	3	Gdh3p [Saccharomyces cerevisiae YJM70]	Saccharomyces cerevisiae YJM70	543	543	98%	0.0	61.54%	454	AUT18410.1
AFR97782	4	Gdh3p [Saccharomyces cerevisiae YJM1444]	Saccharomyces cerevisiae YJM1444	543	543	98%	0.0	61.27%	457	AQ07146.1
AFR97782	5	Gdh3p [Saccharomyces cerevisiae YJM796]	Saccharomyces cerevisiae YJM796	543	543	98%	0.0	61.27%	479	FGA76133.1
AFR97782	6	SXZ_G0048680.mRNA.1.CDS.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	543	543	98%	0.0	61.27%	457	CAD6594670.1
AFR97782	7	Gdh3p [Saccharomyces cerevisiae YJM1386]	Saccharomyces cerevisiae YJM1386	543	543	98%	0.0	61.27%	457	AQ09549.1
AFR97782	8	glutamate dehydrogenase (NADP+) [GDH3] [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	543	543	98%	0.0	61.27%	457	NP_069339.1
AFR97782	9	Gdh3p [Saccharomyces cerevisiae YJM1385]	Saccharomyces cerevisiae YJM1385	543	543	98%	0.0	61.27%	457	AQ096282.1
AFR97782	10	glutamate dehydrogenase (NADP+) [GDH3] [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	543	543	98%	0.0	61.27%	457	PF1013599.1



glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	1	HN1_G0043370.mRNA.1.CDS.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	377	377	98%	9.00E-110	46.97%	391	CAD6088830.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	2	Gpd1p [Saccharomyces cerevisiae YJM1129]	Saccharomyces cerevisiae YJM1129	326	326	98%	2.00E-109	46.89%	391	AU018468.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	3	glycerol-3-phosphate dehyd rogenase (NAD(+)) GPD1 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	326	326	98%	3.00E-109	46.89%	391	NP_0110262.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	4	Y0U22Wp-like protein [Saccharomyces cerevisiae AW010631]	Saccharomyces cerevisiae AW010631	325	325	98%	4.00E-109	46.89%	388	EDV73249.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	5	Glycerol-3-phosphate dehyd rogenase (NAD(+)) GPD1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	325	325	98%	4.00E-109	46.89%	391	FM122236.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	6	Gpd1p [Saccharomyces cerevisiae YJM1208]	Saccharomyces cerevisiae YJM1208	324	324	98%	1.00E-108	46.69%	391	AU05067.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	7	glycerol-3-phosphate dehyd rogenase [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	317	317	98%	1.00E-105	46.13%	391	AA127375.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	8	Gpd2p [Saccharomyces cerevisiae AW019796]	Saccharomyces cerevisiae AW019796	307	307	98%	9.00E-102	44.09%	392	FGA73087.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	9	Gpd2p [Saccharomyces cerevisiae V13]	Saccharomyces cerevisiae V13	306	306	98%	1.00E-101	43.80%	392	FGA84870.1
glycerol-3-phosphate dehydrogenase (NADH) [Cryptococcus neoformans var. grubi H99]	AFR92257	10	glycerol-3-phosphate dehyd rogenase [Saccharomyces cerevisiae RMI1-1a]	Saccharomyces cerevisiae RMI1-1a	306	306	98%	2.00E-101	43.80%	392	FW080860.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	1	Ypt1p [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	281	281	98%	2.00E-96	67.80%	206	NP_116615.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	2	un-named protein product [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	280	280	98%	4.00E-96	67.80%	206	CAA250366.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	3	Ypt1p [Saccharomyces cerevisiae YJM1388]	Saccharomyces cerevisiae YJM1388	280	280	98%	6.00E-96	67.32%	206	AV23348.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	4	Ypt1p [Saccharomyces cerevisiae YJM1447]	Saccharomyces cerevisiae YJM1447	279	279	98%	9.00E-96	67.32%	206	AV24986.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	5	Structure of doubly prenylated Ypt1-GDI complex [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	279	279	98%	1.00E-95	67.32%	206	28C0_Y
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	6	Ypt1p [Saccharomyces cerevisiae Vn13]	Saccharomyces cerevisiae Vn13	274	274	82%	2.00E-93	77.06%	219	FGA79075.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	7	GppWhp40ubound Ypt1p GTPase [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	271	271	81%	1.00E-92	77.38%	185	Y1Z_N_A
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	8	Ssa4p [Saccharomyces cerevisiae F0ctnB]	Saccharomyces cerevisiae F0ctnB	222	222	99%	4.00E-73	50.98%	215	FGA38852.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	9	Ssa4p [Saccharomyces cerevisiae YM993]	Saccharomyces cerevisiae YM993	222	222	99%	4.00E-73	50.98%	215	AYH75783.1
GTP-binding protein Ypt1 [Cryptococcus neoformans var. grubi H99]	AFR94332	10	Pair family GTPase SEC4 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	222	222	99%	6.00E-73	50.98%	215	NP_116650.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	1	Ssa4p [Saccharomyces cerevisiae YJM1381]	Saccharomyces cerevisiae YJM1381	556	556	84%	0.0	47.72%	659	AW00918.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	2	Ssa4p [Saccharomyces cerevisiae YJM1352]	Saccharomyces cerevisiae YJM1352	555	555	84%	0.0	47.72%	659	AW15101.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	3	Structure of the Hsp110-Hsc70 Nucleotide Exchange Complex [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	555	555	84%	0.0	47.72%	668	3C7N_A
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	4	Ssa4p [Saccharomyces cerevisiae YJM1592]	Saccharomyces cerevisiae YJM1592	555	555	84%	0.0	47.57%	693	AU09495.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	5	adenyl-nucleotide exchange factor SEF1 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	555	555	84%	0.0	47.72%	693	NP_015219.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	6	Ssa4p [Saccharomyces cerevisiae YM4270]	Saccharomyces cerevisiae YM4270	555	555	84%	0.0	47.72%	693	AV076925.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	7	Ssa4p [Saccharomyces cerevisiae YJM1574]	Saccharomyces cerevisiae YJM1574	555	555	84%	0.0	47.72%	693	AU24946.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	8	Ssa4p [Saccharomyces cerevisiae R103]	Saccharomyces cerevisiae R103	555	555	84%	0.0	47.72%	697	FW692723.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	9	adenyl-nucleotide exchange factor SEE1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	555	555	84%	0.0	47.72%	699	FM172630.1
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubi H99]	AFR94835	10	Pair family GTPase SEC4 [Saccharomyces cerevisiae YJM1403]	Saccharomyces cerevisiae YJM1403	554	594	84%	0.0	47.72%	699	FGA08478.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	1	Ssa4p [Saccharomyces cerevisiae YJM1401]	Saccharomyces cerevisiae YJM1401	1011	1011	100%	0.0	78.33%	642	AW012525.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	2	K7_Ssa4p [Saccharomyces cerevisiae Ykpa1 no. 7]	Saccharomyces cerevisiae Ykpa1 no. 7	1009	1009	100%	0.0	78.17%	642	GA224965.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	3	Ssa4p [Saccharomyces cerevisiae YJM1400]	Saccharomyces cerevisiae YJM1400	1009	1009	100%	0.0	78.17%	642	AU044845.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	4	Hsp70 chaperone ssa4 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	1008	1008	100%	0.0	78.17%	642	GHM69001.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	5	Ssa4p [Saccharomyces cerevisiae YJM1385]	Saccharomyces cerevisiae YJM1385	1008	1008	100%	0.0	78.02%	642	AU048484.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	6	hypothetical protein SCEP1_0017002900 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	1007	1007	100%	0.0	78.14%	642	GF66890.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	7	Ssa4p [Saccharomyces cerevisiae YM993]	Saccharomyces cerevisiae YM993	1005	1005	100%	0.0	78.14%	642	AV34706.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	8	SK2_G0001850.mRNA.1.CDS.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	1000	1000	100%	0.0	78.14%	642	CA9654332.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	9	Ssa4p [Saccharomyces cerevisiae YM993]	Saccharomyces cerevisiae YM993	959	959	100%	0.0	78.14%	642	AYH75564.1
hsp71-like protein [Cryptococcus neoformans var. grubi H99]	AFR97929	10	Hsp70 family chaperone Ssa4 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	959	959	100%	0.0	78.14%	642	NP_0110293
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	1	Ssa4p [Saccharomyces cerevisiae YJM1460]	Saccharomyces cerevisiae YJM1460	1028	1028	100%	0.0	79.04%	642	AU054545.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	2	Ssa4p [Saccharomyces cerevisiae YJM1401]	Saccharomyces cerevisiae YJM1401	1026	1026	100%	0.0	78.86%	642	AU051252.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	3	K7_Ssa4p [Saccharomyces cerevisiae Ykpa1 no. 7]	Saccharomyces cerevisiae Ykpa1 no. 7	1025	1025	100%	0.0	78.73%	642	GA224965.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	4	Hsp70 chaperone ssa4 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	1023	1023	100%	0.0	78.73%	642	GHM69001.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	5	hypothetical protein SCEP1_0017002900 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	1023	1023	100%	0.0	78.73%	642	AU049484.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	6	hypothetical protein SCEP1_0017002900 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	1023	1023	100%	0.0	78.73%	642	GF66890.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	7	Ssa4p [Saccharomyces cerevisiae YJM993]	Saccharomyces cerevisiae YJM993	1020	1020	100%	0.0	78.73%	642	AV34706.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	8	Ssa4p [Saccharomyces cerevisiae YJM993]	Saccharomyces cerevisiae YJM993	1009	1009	100%	0.0	78.86%	642	AYH75564.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	9	Ssa4p [Saccharomyces cerevisiae YJM270]	Saccharomyces cerevisiae YJM270	1009	1009	100%	0.0	78.86%	642	AV34706.1
hsp72-like protein, partial [Cryptococcus neoformans var. grubi H99]	AFR97952	10	Ssa4p [Saccharomyces cerevisiae YJM1342]	Saccharomyces cerevisiae YJM1342	1008	1009	100%	0.0	78.86%	642	AU048205.1
hsp75-like protein [Cryptococcus neoformans var. grubi H99]	AFR92468	1	Hsp70 family ATPase S82 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	888	888	99%	0.0	70.10%	613	NP_0114190.1
hsp75-like protein [Cryptococcus neoformans var. grubi H99]	AFR92468	2	S82p [Saccharomyces cerevisiae YM993]	Saccharomyces cerevisiae YM993	888	888	99%	0.0	70.10%	613	AYH76198.1
hsp75-like protein [Cryptococcus neoformans var. grubi H99]	AFR92468	3	S82p [Saccharomyces cerevisiae YJM118]	Saccharomyces cerevisiae YJM118	887	887	99%	0.0	69.93%	613	CA78286.1
hsp75-like protein [Cryptococcus neoformans var. grubi H99]	AFR92468	4	S82p [Saccharomyces cerevisiae YJM978]	Saccharomyces cerevisiae YJM978	887	887	99%	0.0	69.93%	613	AU11670.2
hsp75-like protein [Cryptococcus neoformans var. grubi H99]	AFR92468	5	S82p [Saccharomyces cerevisiae YJM1447]	Saccharomyces cerevisiae YJM1447	884	884	99%	0.0	69.77%	613	AU22897.1

hsp75-like protein [Cryptococcus neoformans var. grubii H99]	AFR92468	6	Ssb2p [Saccharomyces cerevisiae VIM649]	884	884	99%	0.0	69.77%	613	AI1097/0.1
hsp75-like protein [Cryptococcus neoformans var. grubii H99]	AFR92468	7	Hsp70 family A1 P35c S381 [Saccharomyces cerevisiae S288C]	884	884	99%	0.0	69.77%	613	NP_010052.1
hsp75-like protein [Cryptococcus neoformans var. grubii H99]	AFR92468	8	Ssb2p [Saccharomyces cerevisiae VIM1388]	883	883	99%	0.0	69.77%	613	AI074/45.1
hsp75-like protein [Cryptococcus neoformans var. grubii H99]	AFR92468	9	Ssb2p [Saccharomyces cerevisiae VIM1342]	882	882	99%	0.0	69.61%	613	AV195/89.1
hsp75-like protein [Cryptococcus neoformans var. grubii H99]	AFR92468	10	Ssb2p [Saccharomyces cerevisiae VIM1085]	882	882	99%	0.0	69.61%	613	AI1136/7.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	1	XXY51_A_G0019320-mRNA1.CDS.1 [Saccharomyces cerevisiae]	284	284	96%	1.00E-89	36.76%	501	CA0648/899.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	2	hypothetical protein H910_VIM1395/00018 [Saccharomyces cerevisiae VIM1399]	282	282	96%	2.00E-88	35.19%	501	AV103/002.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	3	hypothetical protein H797_VIM1335/00020 [Saccharomyces cerevisiae VIM1336]	281	281	96%	5.00E-88	34.79%	551	AV1191/01.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	4	EM14501-3B_G0016810-mRNA1.CDS.1 [Saccharomyces cerevisiae]	281	281	96%	5.00E-88	34.39%	551	CA0663/108.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	5	hypothetical protein H825_VIM1460/00019 [Saccharomyces cerevisiae VIM1460]	280	280	96%	9.00E-88	34.29%	551	AV198/83.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	6	Cut1p [Saccharomyces cerevisiae S288C]	280	280	96%	9.00E-88	34.29%	551	NP_01036/03.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	7	hypothetical protein SCFP1_00680/01100 [Saccharomyces cerevisiae]	280	280	96%	9.00E-88	34.19%	551	GF57/1007.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	8	SX2_G0012720-mRNA1.CDS.1 [Saccharomyces cerevisiae]	280	280	96%	9.00E-88	34.29%	551	CA0663/08/94.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	9	hypothetical protein H829_VIM1473/00017 [Saccharomyces cerevisiae VIM1479]	280	280	96%	1.00E-87	34.29%	551	AV192/738.1
hypothetical protein CMAG_05236 [Cryptococcus neoformans var. grubii H99]	AFR94491	10	hypothetical protein H80_VIM1526/00146 [Saccharomyces cerevisiae VIM1526]	280	280	96%	1.00E-87	34.29%	551	AV192/337.1
hypothetical protein CMAG_05113 [Cryptococcus neoformans var. grubii H99]	AFR96337	0	no homolog in Saccharomyces cerevisiae	280	280	96%				
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	1	hypothetical protein H49_VIM195/00029 [Saccharomyces cerevisiae VIM195]	272	272	93%	7.00E-89	42.77%	336	AV334/8.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	2	Myp1p [Saccharomyces cerevisiae S288C]	272	272	93%	8.00E-89	42.77%	336	NP_011083.3
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	3	hypothetical protein H788_VIM1248/00029 [Saccharomyces cerevisiae VIM1248]	272	272	93%	8.00E-89	42.77%	336	AV104/92.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	4	hypothetical protein H80_VIM1526/00020 [Saccharomyces cerevisiae VIM1526]	272	272	94%	8.00E-89	43.36%	336	AU156/882.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	5	hypothetical protein H80_VIM1342/00029 [Saccharomyces cerevisiae VIM1342]	272	272	94%	9.00E-89	42.48%	336	AU148/254.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	6	hypothetical protein H75_VIM326/00026 [Saccharomyces cerevisiae VIM326]	272	272	93%	9.00E-89	42.77%	336	AV194/56/1.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	7	hypothetical protein H79_VIM993/00026 [Saccharomyces cerevisiae VIM993]	271	271	94%	2.00E-88	42.48%	336	AH175/713.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	8	EM14501-3B_G002280-mRNA1.CDS.1 [Saccharomyces cerevisiae]	271	271	94%	2.00E-88	42.48%	336	CA0663/182/1.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	9	hypothetical protein H786_VIM1242/00029 [Saccharomyces cerevisiae VIM1242]	271	271	94%	2.00E-88	42.48%	336	CA046/142/9.1
hypothetical protein CMAG_06946 [Cryptococcus neoformans var. grubii H99]	AFR94883	10	hypothetical protein H835_A62 [Saccharomyces cerevisiae]	270	270	92%	2.00E-88	42.73%	326	P107/844.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	1	XXY51_A_G0042000-mRNA1.CDS.1 [Saccharomyces cerevisiae]	339	339	95%	0.0	68.62%	395	CAD663/737.1.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	2	EM14501-3B_G00384/01 [Saccharomyces cerevisiae]	338	338	95%	0.0	68.62%	395	CAD663/630/6.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	3	Inv3p [Saccharomyces cerevisiae VIM195]	336	336	95%	0.0	68.62%	395	AV173/26/1.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	4	hypothetical protein SCFP1_00020/01100 [Saccharomyces cerevisiae]	336	336	95%	0.0	68.62%	395	G165/712/1.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	5	Inv3p [Saccharomyces cerevisiae VIM1419]	336	336	95%	0.0	68.62%	395	AV163/47.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	6	Inv3p [Saccharomyces cerevisiae VIM1089]	336	336	95%	0.0	68.62%	395	AV146/39/1.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	7	ketol-acid reductoisomerase [Saccharomyces cerevisiae S288C]	335	335	95%	0.0	68.37%	395	NP_0134/49.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	8	aerohydrophobic ionomereolactase [Saccharomyces cerevisiae]	335	335	95%	0.0	68.37%	395	AA483/579.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	9	Inv3p [Saccharomyces cerevisiae VIM1335]	335	335	95%	0.0	68.37%	395	AV156/27/1.1
ketol-acid red actinomease, mitochondrial [Cryptococcus neoformans var. grubii H99]	AFR96043	10	Inv3p [Saccharomyces cerevisiae VIM1447]	335	335	95%	0.0	68.37%	395	AV166/89/1.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	1	mannose-1-phosphate guanylyltransferase [Saccharomyces cerevisiae S288C]	487	487	100%	9.00E-173	63.76%	361	NP_0102/28.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	2	Psalp [Saccharomyces cerevisiae VIM1386]	486	486	100%	3.00E-172	63.49%	361	AU199/67/1.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	3	mannose-1-phosphate guanylyltransferase [Saccharomyces cerevisiae]	485	485	100%	4.00E-172	63.49%	361	AA48/67/1.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	4	Psalp [Saccharomyces cerevisiae VIM693]	484	484	100%	1.00E-171	63.49%	361	AV139/48/1.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	5	Psalp [Saccharomyces cerevisiae AVR796]	355	355	68%	2.00E-122	62.24%	253	EG475/78/1.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	6	Psalp [Saccharomyces cerevisiae Fnc168]	353	353	69%	7.00E-122	62.84%	253	EG439/46/2.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	7	Psalp [Saccharomyces cerevisiae VIM195]	353	353	69%	1.00E-121	62.84%	253	EG487/67/1.1
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	AFR98009	8	B4_G0045430-mRNA1.CDS.1 [Saccharomyces cerevisiae]	276	333	62%	8.00E-92	72.11%	227	CA0663/09/143.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	1	Pgm2p [Saccharomyces cerevisiae VIM1244]	641	641	99%	0.0	58.14%	569	A579/87/1.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	2	Pgm2p [Saccharomyces cerevisiae VIM1478]	640	640	99%	0.0	58.14%	569	A587/37/1.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	3	Pgm2p [Saccharomyces cerevisiae VIM1443]	640	640	99%	0.0	58.14%	569	A596/87/1.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	4	phosphoglucomutase PG42 [Saccharomyces cerevisiae S288C]	639	639	99%	0.0	58.14%	569	NP_01382/3.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	5	Pgm2p [Saccharomyces cerevisiae VIM1399]	639	639	99%	0.0	58.14%	569	A589/95/1.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	6	Pgm2p [Saccharomyces cerevisiae fostr8]	639	639	99%	0.0	58.14%	569	EG457/326/1.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	7	Pgm2p [Saccharomyces cerevisiae VIM195]	639	639	99%	0.0	57.97%	569	A562/844.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	8	Pgm2p [Saccharomyces cerevisiae VIM320]	639	639	99%	0.0	57.97%	569	A565/002/1.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	9	InvL_G0019320-mRNA1.CDS.1 [Saccharomyces cerevisiae]	639	639	99%	0.0	57.79%	569	CA0663/09/29.1
phosphoglucomutase [Cryptococcus neoformans var. grubii H99]	AFR95550	10	Pgm2p [Saccharomyces cerevisiae VIM1381]	639	639	99%	0.0	57.97%	569	A588/849.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	1	Pdc1p [Saccharomyces cerevisiae VIM1478]	472	472	99%	7.00E-160	42.30%	513	AV168/03/1.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	2	Pdc1p [Saccharomyces cerevisiae fostr8]	470	470	99%	3.00E-159	42.14%	513	EG457/76/1.1

pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	3	Pdbp1 [Saccharomyces cerevisiae YJM1418]	Saccharomyces cerevisiae YJM1418	463	463	99%	1,00E-156	45.60%	563	AIK95999.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	4	Pdbp2 [Saccharomyces cerevisiae YJM202]	Saccharomyces cerevisiae YJM202	462	462	99%	3,00E-156	45.60%	563	AIK95999.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	5	Pdbp3 [Saccharomyces cerevisiae YJM270]	Saccharomyces cerevisiae YJM270	461	461	99%	1,00E-155	42.46%	563	AV17427.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	6	Pdbp4 [Saccharomyces cerevisiae YJM1388]	Saccharomyces cerevisiae YJM1388	461	461	99%	1,00E-155	47.79%	563	AV159139.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	7	Pdbp5 [Saccharomyces cerevisiae YJM1400]	Saccharomyces cerevisiae YJM1400	461	461	99%	2,00E-155	42.63%	563	AV160489.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	8	Pdbp6 [Saccharomyces cerevisiae YJM693]	Saccharomyces cerevisiae YJM693	460	460	99%	3,00E-155	47.79%	563	AV182392.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	9	Pdbp7 [Saccharomyces cerevisiae YJM1083]	Saccharomyces cerevisiae YJM1083	460	460	99%	3,00E-155	47.63%	563	AV166129.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	AFR97558	10	Pdbp8 [Saccharomyces cerevisiae YJM1402]	Saccharomyces cerevisiae YJM1402	460	460	99%	3,00E-155	42.46%	563	AV161393.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	1	Tal1p [Saccharomyces cerevisiae YJM535]	Saccharomyces cerevisiae YJM535	411	411	99%	7,00E-144	64.91%	335	AV179885.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	2	Tal2p [Saccharomyces cerevisiae YJM1399]	Saccharomyces cerevisiae YJM1399	383	383	99%	9,00E-138	62.22%	335	AV169200.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	3	Tal3p [Saccharomyces cerevisiae YJM1190]	Saccharomyces cerevisiae YJM1190	382	382	99%	1,00E-132	62.22%	335	AV147744.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	4	unnamed protein product [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	382	382	99%	1,00E-132	64.91%	335	CAA34078.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	5	sedheptulose-7-phosphate-1D-glyceraldehyde-3-phosphate transaldolase TAL1 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	382	382	99%	1,00E-132	65.22%	335	NP_013438.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	6	Tal1p [Saccharomyces cerevisiae YJM1304]	Saccharomyces cerevisiae YJM1304	382	382	99%	1,00E-132	65.22%	335	AV152223.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	7	Tal2p [Saccharomyces cerevisiae Foste8B]	Saccharomyces cerevisiae Foste8B	382	382	99%	2,00E-132	62.22%	335	FGA57488.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	8	EM14501-3B_G0039430.mRNA T.CDB.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	380	380	99%	9,00E-132	64.91%	335	CAD0656302.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	9	Nm1p [Saccharomyces cerevisiae F103]	Saccharomyces cerevisiae F103	375	375	98%	9,00E-130	58.26%	333	FW095841.1
transaldolase [Cryptococcus neoformans var. grubii H99]	AFR98178	10	Cystal Structure of TAL2_TFAST [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	374	374	98%	3,00E-129	58.26%	339	3C00_A
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	1	Tk1b [Saccharomyces cerevisiae YJM1250]	Saccharomyces cerevisiae YJM1250	885	885	99%	0,00E+00	62.72%	680	AV114840.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	2	Tk1p [Saccharomyces cerevisiae YJM195]	Saccharomyces cerevisiae YJM195	885	885	99%	0,00E+00	62.57%	680	AV196719.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	3	transketolase [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	884	884	100%	0,00E+00	62.57%	680	GA068916.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	4	Tk1b [Saccharomyces cerevisiae YJM1574]	Saccharomyces cerevisiae YJM1574	884	884	99%	0,00E+00	62.57%	680	AV194726.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	5	transketolase TK1 [Saccharomyces cerevisiae S288C]	Saccharomyces cerevisiae S288C	884	884	99%	0,00E+00	62.57%	680	NP_013399.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	6	YHR074C-pile protein [Saccharomyces cerevisiae ANR1531]	Saccharomyces cerevisiae ANR1531	884	884	99%	0,00E+00	62.37%	680	E0268722.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	7	EM14501-3B_G0019930.mRNA T.CDB.1 [Saccharomyces cerevisiae]	Saccharomyces cerevisiae	884	884	99%	0,00E+00	62.28%	680	CAD0646952.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	8	Tk1b [Saccharomyces cerevisiae Foste8B]	Saccharomyces cerevisiae Foste8B	884	884	98%	0,00E+00	62.79%	691	FGA56378.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	9	Tk1p [Saccharomyces cerevisiae YJM1478]	Saccharomyces cerevisiae YJM1478	883	883	99%	0,00E+00	62.43%	680	AV182612.1
transketolase [Cryptococcus neoformans var. grubii H99]	AFR95182	10	Tk1b [Saccharomyces cerevisiae YJM1202]	Saccharomyces cerevisiae YJM1202	883	883	99%	0,00E+00	62.43%	680	AV112679.1
unase; accessory protein UteG [Cryptococcus neoformans var. grubii H99]	AFR92807	0	no homolog in Saccharomyces cerevisiae								

Homology of 26S proteasome regulatory subunit N8 to proteins from other pathogenic fungi

Job Title	AFR92184.1	26S proteasome regulatory subunit N8 [Cryptococcus neoformans var. grubii H99] ...
RID	7EASZK8H016 Search expires on 04-16 01:39 am	
Program	BLASTP Help	
Database	nr	
Query ID	AFR92184.1	
Description	26S proteasome regulatory subunit N8 [Cryptococcus neoformans var. grubii H99] ...	
Molecule type	amino acid	
Query length	350	

Hit no.	Sequence identifier	Protein Homology	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq ref XP_018224692.1	hypothetical protein T552_03040 [Pneumocystis carinii B80]	Pneumocystis carinii B80	335	335	91%	3,00E-113	48.13%	340	XP_018224692.1
2	Select seq ref XP_750500.1	26S proteasome regulatory particle subunit Rom8 [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	322	322	99%	3,00E-108	48.65%	350	XP_750500.1
3	Select seq gb EHH03813.1	26S proteasome regulatory subunit [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	313	313	86%	2,00E-104	50.62%	362	EHH03813.1
4	Select seq gb KAG5292699.1	26S proteasome regulatory subunit [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	313	313	86%	2,00E-104	50.62%	362	KAG5292699.1
5	Select seq gb EER37256.1	26S proteasome regulatory subunit [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	312	312	86%	4,00E-104	50.62%	362	EER37256.1
6	Select seq gb KGR06355.1	26S proteasome regulatory subunit N8 [Candida albicans P57072]	Candida albicans P57072	287	287	93%	7,00E-95	45.43%	330	KGR06355.1
7	Select seq gb EEO46242.1	26S proteasome regulatory subunit RPN8 [Candida albicans WO-1]	Candida albicans WO-1	286	286	93%	1,00E-94	45.43%	330	EEO46242.1
8	Select seq ref XP_714586.2	proteasome regulatory particle lid subunit [Candida albicans SC5314]	Candida albicans SC5314	286	286	93%	3,00E-94	45.13%	330	XP_714586.2
9	Select seq gb KGO85165.1	26S proteasome regulatory subunit N8 [Candida albicans P94015]	Candida albicans P94015	286	286	93%	3,00E-94	45.43%	330	KGO85165.1
10	Select seq gb KHC34019.1	26S proteasome regulatory subunit N8 [Candida albicans P76067]	Candida albicans P76067	285	285	93%	4,00E-94	45.13%	330	KHC34019.1
11	Select seq gb KHC65044.1	26S proteasome regulatory subunit N8 [Candida albicans P75016]	Candida albicans P75016	283	283	93%	2,00E-93	45.13%	330	KHC65044.1
12	Select seq gb RLP65333.1	hypothetical protein L150_04104 [Candida albicans C85291]	Candida albicans C85291	283	283	93%	3,00E-93	45.13%	330	RLP65333.1
13	Select seq gb KGU26393.1	26S proteasome regulatory subunit N8 [Candida albicans P75063]	Candida albicans P75063	281	281	93%	1,00E-92	44.84%	330	KGU26393.1
14	Select seq gb KAF6060523.1	prothrome IK farnesyltransferase [Candida albicans]	Candida albicans	280	280	82%	2,00E-87	48.00%	727	KAF6060523.1
15	Select seq ref XP_001535965.1	26S proteasome regulatory subunit rpn8 [Histoplasma capsulatum NAml1]	Histoplasma capsulatum NAml1	231	231	86%	2,00E-73	39.06%	307	XP_001535965.1
16	Select seq gb Q5559229.1	26S proteasome regulatory subunit [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	209	209	64%	5,00E-66	48.02%	197	Q5559229.1
17	Select seq gb EER37627.1	translation initiation factor eIF3f [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	72.0	72.0	86%	4,00E-13	25.32%	351	EER37627.1
18	Select seq gb EHH06061.1	translation initiation factor eIF3f [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	71.6	71.6	86%	5,00E-13	25.32%	351	EHH06061.1
19	Select seq gb EGC48818.1	translation initiation factor eIF3f [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	71.6	71.6	86%	5,00E-13	25.32%	351	EGC48818.1
20	Select seq ref XP_001544909.1	eukaryotic translation initiation factor: 3 subunit EIFC [Histoplasma capsulatum NAml1]	Histoplasma capsulatum NAml1	71.6	71.6	86%	5,00E-13	25.32%	347	XP_001544909.1
21	Select seq ref XP_752300.1	eukaryotic translation initiation factor: 3 subunit EIFC, putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	70.1	70.1	83%	2,00E-12	25.75%	345	XP_752300.1
22	Select seq gb EDP51473.1	COP9 signalosome subunit 6 [CsnF], putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	48.5	48.5	55%	2,00E-05	25.45%	399	EDP51473.1
23	Select seq gb KAF4256167.1	hypothetical protein CNMCM8714_003777 [Aspergillus fumigatus]	Aspergillus fumigatus	48.5	48.5	55%	2,00E-05	25.45%	399	KAF4256167.1
24	Select seq ref XP_753908.1	COP9 signalosome subunit 6 [CsnF], putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	48.5	48.5	55%	2,00E-05	25.45%	399	XP_753908.1
25	Select seq gb OXN02854.1	hypothetical protein CDV58_28276 [Aspergillus fumigatus]	Aspergillus fumigatus	48.5	48.5	55%	2,00E-05	25.45%	399	OXN02854.1
26	Select seq gb KAG5290672.1	COP9 complex subunit 6 [Histoplasma capsulatum]	Histoplasma capsulatum	47.0	47.0	81%	7,00E-05	22.55%	406	KAG5290672.1
27	Select seq gb Q5555610.1	COP9 complex subunit 6 [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	45.4	45.4	81%	2,00E-04	22.28%	406	Q5555610.1
28	Select seq gb KAG5301126.1	COP9 complex subunit 6 [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	45.1	45.1	81%	3,00E-04	22.28%	406	KAG5301126.1
29	Select seq ref XP_001542756.1	predicted protein [Histoplasma capsulatum NAml1]	Histoplasma capsulatum NAml1	40.0	40.0	14%	0.001	38.00%	85	XP_001542756.1
30	Select seq gb EHH10359.1	COP9 complex subunit 6 [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	40.8	40.8	14%	0.006	38.00%	428	EHH10359.1
31	Select seq gb EGC44839.1	COP9 signalosome complex subunit [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	40.8	40.8	14%	0.006	38.00%	396	EGC44839.1
32	Select seq gb EER36571.1	COP9 signalosome complex subunit 6 [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	40.4	40.4	14%	0.008	38.00%	396	EER36571.1

Homology of chlorophyll synthesis pathway protein BchC to proteins from other pathogenic fungi

Job Title	Accession	Per. Ident	E value	Acc. Len	Accession			
RID	KAF4255038.1	8.00E-139	55.00%	348	KAF4255038.1			
Program	XP_750211.1	1.00E-137	54.71%	348	XP_750211.1			
Database	KAF4283998.1	4.00E-137	54.71%	348	KAF4283998.1			
nr	FER43395.1	2.00E-135	54.97%	348	FER43395.1			
BLASTP	EEH11347.1	2.00E-135	54.97%	348	EEH11347.1			
AFR97763.1	KAG5289431.1	3.00E-135	54.97%	348	KAG5289431.1			
nr	XP_001541507.1	8.00E-129	53.51%	341	XP_001541507.1			
chlorophyll synthesis pathway protein BchC [Cryptococcus neoformans var. grubii H99] ...	OSS61485.1	6.00E-112	49.85%	351	OSS61485.1			
amino acid	BOY065.2	5.00E-38	31.12%	358	BOY065.2			
349								
Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
hypothetical protein CNMCM8714_004641 [Aspergillus fumigatus]	Aspergillus fumigatus	140	140	93%	1.00E-37	31.56%	368	XP_747006.2
alcohol dehydrogenase, zinc-containing, putative [Aspergillus fumigatus AT293]	Aspergillus fumigatus var. RP-2014	140	140	93%	2.00E-37	31.56%	368	KEY79024.1
hypothetical protein CNMCM8689_006617 [Aspergillus fumigatus]	Aspergillus fumigatus var. RP-2014	137	137	90%	1.00E-36	31.17%	348	KEY82214.1
NADP-dependent mannitol dehydrogenase [Histoplasma capsulatum H143]	Aspergillus fumigatus AT293	137	137	90%	1.00E-36	30.86%	348	XP_746677.1
NADP-dependent mannitol dehydrogenase [Histoplasma capsulatum G186AR]	Aspergillus fumigatus AT293	133	133	86%	7.00E-35	29.55%	383	XP_752468.1
NADP-dependent mannitol dehydrogenase [Histoplasma capsulatum G217B]	Aspergillus fumigatus var. RP-2014	131	131	96%	3.00E-34	29.26%	383	KEY83794.1
hypothetical protein HCAG_03605 [Histoplasma capsulatum Nam1]	Aspergillus fumigatus	121	121	98%	1.00E-30	28.29%	359	OXN21058.1
NADP-dependent mannitol dehydrogenase [Histoplasma capsulatum]	Aspergillus fumigatus	119	119	98%	6.00E-30	28.01%	359	KAF4283945.1
RecName: F-11e:Probable D-xylulose reductase A. AltName: Full=Xylitol dehydrogenase A [Aspergillus fumigatus A1163]	Aspergillus fumigatus	119	119	98%	9.00E-30	28.01%	359	KEY83797.1
sorbitol/xylitol dehydrogenase, putative [Aspergillus fumigatus AT293]	Aspergillus fumigatus var. RP-2014	119	119	98%	1.00E-29	28.53%	356	KAG5289961.1
sorbitol/xylitol dehydrogenase [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus AT293	118	118	98%	2.00E-29	28.01%	359	XP_752467.1
xylitol dehydrogenase [Aspergillus fumigatus AT293]	Aspergillus fumigatus AT293	117	117	94%	4.00E-29	28.24%	356	FER44525.1
xylitol dehydrogenase [Aspergillus fumigatus AT293]	Aspergillus fumigatus AT293	117	117	91%	7.00E-29	28.45%	386	XP_752993.1
xylitol dehydrogenase XdhB [Aspergillus fumigatus AT293]	Aspergillus fumigatus Z5	117	117	91%	7.00E-29	28.45%	386	KMK5585.1
xylitol dehydrogenase XdhB [Aspergillus fumigatus Z5]	Aspergillus fumigatus var. RP-2014	116	116	91%	1.00E-28	28.45%	386	KEY78517.1
Alcohol dehydrogenase GroE5-like domain family protein [Candida albicans]	Candida albicans	109	109	97%	3.00E-26	24.53%	371	KAF6060460.1
hypothetical protein MG7_04208 [Candida albicans P34048]	Candida albicans P34048	109	109	96%	3.00E-26	24.80%	365	KGU23402.1
hypothetical protein MEV_04133 [Candida albicans F60002]	Candida albicans F60002	109	109	96%	3.00E-26	24.80%	365	KHC49512.1
hypothetical protein CAALFM_C502690WA [Candida albicans SC5314]	Candida albicans SC5314	109	109	96%	4.00E-26	24.80%	365	XP_720595.2
hypothetical protein MEO_04155 [Candida albicans P94015]	Candida albicans P94015	107	107	96%	2.00E-25	24.52%	365	KGQ85228.1
hypothetical protein W5Q_04296 [Candida albicans SC5314]	Candida albicans SC5314	107	107	97%	3.00E-25	24.46%	389	KHC75169.1
hypothetical protein CNMCM8812_006395 [Aspergillus fumigatus]	Aspergillus fumigatus	106	106	91%	4.00E-25	28.49%	374	KAF4258330.1
conserved hypothetical protein [Candida albicans WO-1]	Candida albicans WO-1	106	106	96%	5.00E-25	24.25%	365	EQ46306.1
hypothetical protein CNMCM8057_006180 [Aspergillus fumigatus]	Aspergillus fumigatus	106	106	91%	6.00E-25	28.49%	374	KAF4252399.1
hypothetical protein MGO_04183 [Candida albicans P76055]	Candida albicans P76055	104	104	86%	2.00E-24	25.45%	326	KHC33479.1
zinc-binding oxidoreductase, putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	103	103	96%	5.00E-24	27.58%	345	EDP50874.1
hypothetical protein MGO_04191 [Candida albicans P76067]	Candida albicans P76067	102	102	86%	7.00E-24	25.15%	326	KHC33535.1
zinc-binding oxidoreductase, putative [Aspergillus fumigatus AT293]	Aspergillus fumigatus AT293	102	102	96%	1.00E-23	27.58%	345	XP_748310.1
hypothetical protein CNMCM8057_003646 [Aspergillus fumigatus]	Aspergillus fumigatus	102	102	90%	1.00E-23	31.60%	390	KAF4257428.1
alcohol dehydrogenase, propanol-preferring [Candida albicans P75010]	Candida albicans P75010	102	102	96%	1.00E-23	26.83%	349	KHC65564.1
xylitol dehydrogenase [Histoplasma capsulatum]	Histoplasma capsulatum	102	102	85%	1.00E-23	26.33%	401	OSS6248.1
xylitol dehydrogenase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	100	100	85%	5.00E-23	27.30%	315	FEH10825.1
xylitol dehydrogenase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	100	100	85%	7.00E-23	25.88%	402	FEH09761.1
alcohol dehydrogenase, propanol-preferring [Candida albicans P78042]	Candida albicans P78042	100	100	96%	8.00E-23	26.56%	349	KHC80255.1
alcohol dehydrogenase, propanol-preferring [Candida albicans P94015]	Candida albicans P94015	99.4	99.4	96%	1.00E-22	26.56%	349	KGC899001.1

45	Select seq gbl   KGR21974.1	alcohol dehydrogenase, propanol-prefering [Candida albicans P37037]	98.2	98.2	96%	3,00E-22	349	KGR21974.1
46	Select seq ref   XP_001542152.1	hypothetical protein HCAG_02323 [Histoplasma capsulatum NAM1]	98.6	98.6	80%	4,00E-22	392	XP_001542152.1
47	Select seq gbl   Q556207.1	xyliitol dehydrogenase [Histoplasma capsulatum]	95.1	95.1	57%	4,00E-22	217	Q556207.1
48	Select seq gbl   EER38102.1	xyliitol dehydrogenase [Histoplasma capsulatum H143]	98.6	98.6	85%	5,00E-22	402	EER38102.1
49	Select seq gbl   EGC44228.1	xyliitol dehydrogenase [Histoplasma capsulatum H88]	98.2	98.2	85%	5,00E-22	402	EGC44228.1
50	Select seq gbl   KAF4281421.1	hypothetical protein CNMCM8057_008592 [Aspergillus fumigatus]	97.8	97.8	77%	5,00E-22	373	KAF4281421.1
51	Select seq gbl   KAG5300562.1	xyliitol dehydrogenase [Histoplasma capsulatum G217B]	98.2	98.2	85%	7,00E-22	402	KAG5300562.1
52	Select seq gbl   KHC46597.1	alcohol dehydrogenase, propanol-prefering [Candida albicans Ca6]	97.1	97.1	95%	8,00E-22	349	KHC46597.1
53	Select seq ref   XP_1712930.2	hypothetical protein CAALFM_C204480WA [Candida albicans SC5314]	97.1	97.1	96%	8,00E-22	349	XP_1712930.2
54	Select seq ref   XP_001540536.1	hypothetical protein HCAG_04376 [Histoplasma capsulatum NAM1]	94.7	94.7	57%	8,00E-22	231	XP_001540536.1
55	Select seq gbl   KCG063211.1	alcohol dehydrogenase, propanol-prefering [Candida albicans P37005]	97.1	97.1	96%	9,00E-22	349	KCG063211.1
56	Select seq gbl   KGU35046.1	alcohol dehydrogenase, propanol-prefering [Candida albicans P57055]	96.7	96.7	96%	1,00E-21	349	KGU35046.1
57	Select seq gbl   KHC38072.1	alcohol dehydrogenase, propanol-prefering [Candida albicans P76055]	96.3	96.3	96%	2,00E-21	349	KHC38072.1
58	Select seq gbl   KGR12215.1	alcohol dehydrogenase, propanol-prefering [Candida albicans P57072]	95.1	95.1	81%	4,00E-21	349	KGR12215.1
59	Select seq gbl   KAG5288703.1	xyliitol dehydrogenase [Histoplasma capsulatum]	93.2	93.2	81%	8,00E-21	291	KAG5288703.1
60	Select seq gbl   KAF42786804.1	hypothetical protein CNMCM8689_001933 [Aspergillus fumigatus]	93.2	93.2	77%	8,00E-21	286	KAF42786804.1
61	Select seq gbl   OXN22905.1	hypothetical protein CDV57_07821 [Aspergillus fumigatus]	94.7	94.7	90%	9,00E-21	392	OXN22905.1
62	Select seq gbl   KGU32545.1	alcohol dehydrogenase, propanol-prefering [Candida albicans P75063]	94.0	94.0	96%	9,00E-21	349	KGU32545.1
63	Select seq gbl   KAF4259583.1	hypothetical protein CNMCM8714_001626 [Aspergillus fumigatus]	95.9	95.9	77%	1,00E-20	983	KAF4259583.1
64	Select seq gbl   KAF4281930.1	hypothetical protein CNMCM8689_000053 [Aspergillus fumigatus]	95.9	95.9	77%	1,00E-20	983	KAF4281930.1
65	Select seq gbl   KAF4256636.1	hypothetical protein CNMCM8812_007136 [Aspergillus fumigatus]	95.9	95.9	77%	1,00E-20	1007	KAF4256636.1
66	Select seq gbl   OXN23992.1	hypothetical protein CDV57_05960 [Aspergillus fumigatus]	95.9	95.9	77%	1,00E-20	989	OXN23992.1
67	Select seq gbl   KHC39510.1	alcohol dehydrogenase, propanol-prefering [Candida albicans P76067]	93.6	93.6	81%	2,00E-20	349	KHC39510.1
68	Select seq gbl   KAF42605015.1	hypothetical protein CNMCM8057_008389 [Aspergillus fumigatus]	92.0	92.0	77%	2,00E-20	286	KAF42605015.1
69	Select seq gbl   KAF4272374.1	hypothetical protein CNMCM8812_008750 [Aspergillus fumigatus]	91.7	91.7	77%	3,00E-20	271	KAF4272374.1
70	Select seq gbl   EDP53467.1	zinc-containing alcohol dehydrogenase, putative [Aspergillus fumigatus A1163]	92.8	92.8	96%	3,00E-20	350	EDP53467.1
71	Select seq ref   XP_748515.2	zinc-containing alcohol dehydrogenase, putative [Aspergillus fumigatus AT293]	92.8	92.8	96%	3,00E-20	350	XP_748515.2
72	Select seq gbl   KEY79810.1	alcohol dehydrogenase zinc containing [Aspergillus fumigatus var. RP-2014]	92.4	92.4	96%	3,00E-20	350	KEY79810.1
73	Select seq gbl   KAF4290597.1	hypothetical protein CNMCM8686_000878 [Aspergillus fumigatus]	94.7	94.7	77%	3,00E-20	1007	KAF4290597.1
74	Select seq gbl   OXN01887.1	hypothetical protein CDV58_09378 [Aspergillus fumigatus]	94.4	94.4	77%	3,00E-20	974	OXN01887.1
75	Select seq gbl   KEY80906.1	alcohol dehydrogenase [Aspergillus fumigatus var. RP-2014]	92.0	92.0	67%	4,00E-20	338	KEY80906.1
76	Select seq gbl   KAF4275182.1	hypothetical protein CNMCM8812_002682 [Aspergillus fumigatus]	92.4	92.4	96%	4,00E-20	350	KAF4275182.1
77	Select seq gbl   EDP53510.1	alcohol dehydrogenase, putative [Aspergillus fumigatus A1165]	92.0	92.0	67%	4,00E-20	338	EDP53510.1
78	Select seq ref   XP_748470.1	alcohol dehydrogenase, putative [Aspergillus fumigatus AT293]	92.0	92.0	67%	4,00E-20	338	XP_748470.1
79	Select seq gbl   KMK58705.1	alcohol dehydrogenase, putative [Aspergillus fumigatus Z5]	92.0	92.0	69%	1,00E-19	452	KMK58705.1
80	Select seq gbl   KAG5296597.1	alcohol dehydrogenase [Histoplasma capsulatum G217B]	89.7	89.7	97%	6,00E-19	429	KAG5296597.1
81	Select seq gbl   EEH09339.1	alcohol dehydrogenase [Histoplasma capsulatum G186AR]	88.2	88.2	97%	1,00E-18	346	EEH09339.1
82	Select seq ref   XP_755928.2	alcohol dehydrogenase, zinc-containing [Aspergillus fumigatus AT293]	87.8	87.8	91%	1,00E-18	353	XP_755928.2
83	Select seq gbl   KEY83215.1	alcohol dehydrogenase zinc containing [Aspergillus fumigatus var. RP-2014]	87.8	87.8	91%	2,00E-18	353	KEY83215.1
84	Select seq gbl   KAF6065296.1	Alcohol dehydrogenase GroES-like domain family protein [Candida albicans]	87.4	87.4	90%	2,00E-18	329	KAF6065296.1
85	Select seq gbl   Q5550937.1	alcohol dehydrogenase [Histoplasma capsulatum H88]	88.2	88.2	97%	2,00E-18	266	Q5550937.1
86	Select seq gbl   KMK58799.1	glucan 1,4-alpha-glucosidase [Aspergillus fumigatus Z5]	89.0	89.0	73%	2,00E-18	978	KMK58799.1
87	Select seq gbl   EDP55099.1	alcohol dehydrogenase, zinc-containing [Aspergillus fumigatus A1163]	87.4	87.4	91%	2,00E-18	353	EDP55099.1
88	Select seq gbl   EGC49857.1	alcohol dehydrogenase [Histoplasma capsulatum H88]	88.2	88.2	97%	3,00E-18	523	EGC49857.1
89	Select seq gbl   KAF4288160.1	hypothetical protein CNMCM8689_006441 [Aspergillus fumigatus]	86.7	86.7	96%	3,00E-18	325	KAF4288160.1
90	Select seq ref   XP_719434.1	L-iditol 2-dehydrogenase [Candida albicans SC5314]	86.7	86.7	82%	5,00E-18	360	XP_719434.1
91	Select seq gbl   KAF6065294.1	Xyloulose reductase [Candida albicans P60002]	84.7	84.7	65%	6,00E-18	266	KAF6065294.1
92	Select seq gbl   KHC42856.1	D-xylose reductase [Candida albicans P60002]	85.9	85.9	82%	8,00E-18	360	KHC42856.1
93	Select seq gbl   KAF4262005.1	hypothetical protein CNMCM8057_001589 [Aspergillus fumigatus]	85.5	85.5	96%	1,00E-17	356	KAF4262005.1
94	Select seq gbl   KAF4280870.1	hypothetical protein CNMCM8689_001454 [Aspergillus fumigatus]	85.1	85.1	96%	1,00E-17	356	KAF4280870.1
95	Select seq ref   XP_746830.1	alcohol dehydrogenase, putative [Aspergillus fumigatus AT293]	84.3	84.3	30%	2,00E-17	353	XP_746830.1
96	Select seq gbl   EDP48048.1	alcohol dehydrogenase, putative [Aspergillus fumigatus A1165]	84.3	84.3	30%	2,00E-17	353	EDP48048.1
97	Select seq gbl   EEH03657.1	alcohol dehydrogenase [Histoplasma capsulatum G186AR]	84.0	84.0	48%	3,00E-17	327	EEH03657.1
98	Select seq ref   XP_717649.1	alcohol dehydrogenase [Candida albicans SC5314]	84.0	84.0	72%	4,00E-17	348	XP_717649.1
99	Select seq gbl   KGU32026.1	alcohol dehydrogenase 2 [Candida albicans P34048]	82.8	82.8	31%	8,00E-17	348	KGU32026.1
100	Select seq gbl   EER37405.1	alcohol dehydrogenase [Histoplasma capsulatum H143]	82.8	82.8	46%	9,00E-17	341	EER37405.1

Homology of cytoplasmic protein CNAG\_025943 to proteins from other pathogenic fungi

Job Title	Program	Database	Query ID	Description	Molecule type	Query length	Hit No.	Sequence identifier	Protein Homology	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
AFR93749:cytoplasmic protein [Cryptococcus...]	BLASTP	nr	AFR93749.2	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	amino acid	629	1	Select seq gb  Q5551182.1	PH domain-containing protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	235	235	61%	1.00E-66	35.05%	815	Q5551182.1
							2	Select seq gb  KAG5297233.1	PH domain-containing protein [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	235	235	61%	1.00E-66	35.05%	819	KAG5297233.1
							3	Select seq gb  KAG5303584.1	PH domain-containing protein [Histoplasma capsulatum]	Histoplasma capsulatum	235	235	61%	1.00E-66	35.05%	819	KAG5303584.1
							4	Select seq ref  XP_001539841.1	conserved hypothetical protein [Histoplasma capsulatum Nam1]	Histoplasma capsulatum Nam1	226	226	61%	2.00E-63	34.01%	824	XP_001539841.1
							5	Select seq gb  Q5563405.1	PH domain-containing protein [Histoplasma capsulatum]	Histoplasma capsulatum	222	222	61%	1.00E-61	32.85%	845	Q5563405.1
							6	Select seq gb  KEY82384.1	hypothetical protein BA78_4708 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	222	222	76%	1.00E-61	31.54%	848	KEY82384.1
							7	Select seq ref  XP_751107.1	PH domain protein [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	221	221	76%	2.00E-61	31.54%	848	XP_751107.1
							8	Select seq gb  ONX02033.1	hypothetical protein CDV58_09129 [Aspergillus fumigatus]	Aspergillus fumigatus	221	221	76%	2.00E-61	31.54%	848	ONX02033.1
							9	Select seq gb  EDP49797.1	PH domain protein [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	221	221	76%	2.00E-61	31.54%	848	EDP49797.1
							10	Select seq gb  EEH09097.1	PH domain-containing protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	220	220	61%	4.00E-61	32.85%	845	EEH09097.1
							11	Select seq gb  EER44143.1	PH domain-containing protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	220	220	61%	4.00E-61	32.85%	841	EER44143.1
							12	Select seq gb  KEY78406.1	hypothetical protein BA78_3122 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	179	179	60%	3.00E-48	29.49%	538	KEY78406.1
							13	Select seq ref  XP_750682.1	PH domain protein [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	179	179	60%	3.00E-48	29.49%	538	XP_750682.1
							14	Select seq gb  EDP49363.1	PH domain protein [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	179	179	60%	3.00E-48	29.49%	538	EDP49363.1
							15	Select seq gb  KAF4254870.1	hypothetical protein CNMCM8714_004770 [Aspergillus fumigatus]	Aspergillus fumigatus	179	179	60%	3.00E-48	29.49%	538	KAF4254870.1
							16	Select seq ref  XP_018226371.1	hypothetical protein T552_01135 [Pneumocystis carinii B80]	Pneumocystis carinii B80	162	162	62%	4.00E-41	23.79%	776	XP_018226371.1
							17	Select seq gb  KMK63122.1	PH domain-containing protein [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	151	151	60%	1.00E-38	26.99%	507	KMK63122.1
							18	Select seq gb  Q5557933.1	PH domain-containing protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	145	145	54%	2.00E-36	27.09%	500	Q5557933.1
							19	Select seq gb  KAG5294174.1	PH domain-containing protein [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	145	145	54%	2.00E-36	27.09%	500	KAG5294174.1
							20	Select seq gb  EGC49238.1	PH domain-containing protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	144	144	54%	3.00E-36	27.09%	507	EGC49238.1
							21	Select seq gb  KAG5300341.1	PH domain-containing protein [Histoplasma capsulatum]	Histoplasma capsulatum	144	144	60%	4.00E-36	25.77%	500	KAG5300341.1
							22	Select seq ref  XP_001536073.1	predicted protein [Histoplasma capsulatum Nam1]	Histoplasma capsulatum Nam1	144	144	54%	5.00E-36	27.09%	500	XP_001536073.1
							23	Select seq gb  Q5566477.1	PH domain-containing protein [Histoplasma capsulatum]	Histoplasma capsulatum	143	143	54%	1.00E-35	27.09%	528	Q5566477.1
							24	Select seq gb  EEH09556.1	PH domain-containing protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	126	126	48%	9.00E-30	27.24%	528	EEH09556.1
							25	Select seq gb  EER41378.1	PH domain-containing protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	107	107	38%	7.00E-24	27.71%	421	EER41378.1
							26	Select seq ref  XP_710843.2	phosphatidylinositol 4,5-bisphosphate-binding protein [Candida albicans SC5314]	Candida albicans SC5314	106	106	60%	8.00E-23	24.03%	798	XP_710843.2
							27	Select seq gb  KHC64138.1	hypothetical protein MGI_04655 [Candida albicans P75016]	Candida albicans P75016	105	105	60%	1.00E-22	24.03%	794	KHC64138.1
							28	Select seq gb  KGO88441.1	hypothetical protein MGI_04695 [Candida albicans GC75]	Candida albicans GC75	105	105	60%	1.00E-22	24.03%	798	KGO88441.1
							29	Select seq gb  KGU04796.1	hypothetical protein MEO_04653 [Candida albicans P87]	Candida albicans P87	105	105	60%	1.00E-22	24.03%	798	KGU04796.1
							30	Select seq gb  KGU22645.1	hypothetical protein MGT_04677 [Candida albicans P34048]	Candida albicans P34048	105	105	60%	1.00E-22	24.03%	804	KGU22645.1
							31	Select seq gb  KGU25092.1	hypothetical protein MGV_04698 [Candida albicans P75063]	Candida albicans P75063	105	105	60%	1.00E-22	24.03%	794	KGU25092.1
							32	Select seq gb  EEO46795.1	conserved hypothetical protein [Candida albicans WO-1]	Candida albicans WO-1	105	105	60%	1.00E-22	24.03%	794	EEO46795.1
							33	Select seq gb  KGR11052.1	hypothetical protein MGR_04678 [Candida albicans P37037]	Candida albicans P37037	105	105	60%	1.00E-22	24.03%	798	KGR11052.1
							34	Select seq gb  KGO86079.1	hypothetical protein MEO_04697 [Candida albicans P37005]	Candida albicans P37005	105	105	60%	1.00E-22	24.03%	798	KGO86079.1
							35	Select seq gb  KHC33807.1	hypothetical protein W50_04722 [Candida albicans Ca6]	Candida albicans Ca6	105	105	60%	1.00E-22	24.03%	798	KHC33807.1
							36	Select seq gb  KGO84193.1	hypothetical protein MEO_04623 [Candida albicans P94015]	Candida albicans P94015	105	105	60%	1.00E-22	24.03%	798	KGO84193.1
							37	Select seq gb  KGR04949.1	hypothetical protein MGS_04686 [Candida albicans P57072]	Candida albicans P57072	105	105	60%	1.00E-22	24.03%	794	KGR04949.1
							38	Select seq gb  KHC47714.1	hypothetical protein MEG_04598 [Candida albicans P60002]	Candida albicans P60002	105	105	60%	1.00E-22	24.03%	798	KHC47714.1
							39	Select seq gb  KAF6070908.1	PH domain family protein [Candida albicans]	Candida albicans	105	105	61%	2.00E-22	23.58%	788	KAF6070908.1
							40	Select seq gb  RLP65863.1	hypothetical protein LL50_04639 [Candida albicans Ca529L]	Candida albicans Ca529L	104	104	60%	2.00E-22	24.03%	794	RLP65863.1
							41	Select seq gb  KGT66072.1	hypothetical protein MEK_04686 [Candida albicans 12C]	Candida albicans 12C	104	104	60%	3.00E-22	24.03%	675	KGT66072.1

Homology of deoxyuridine 5'-triphosphate nucleotidylhydrolase to proteins from other pathogenic fungi

Job Title AFR94562.deoxyuridine 5'-triphosphate nucleotidylhydrolase  
 RID 7EAGP7NV016 Search expires on 04-16 03:44 am  
 Program BLASTP  
 Database nr  
 Query ID AFR94562.2  
 Description deoxyuridine 5'-triphosphate nucleotidylhydrolase [Cryptococcus neoformans var. grubii H99]  
 Molecule type amino acid  
 Query Length 695

Ht. No.	Sequence identifier	Protein Homology	Species	Max Score	Total Score	Query Cover	Per. Ident.	E value	Acc. Ident.	Accession
1	Select seq gb KAG5289022.1	corinin-like protein crn1 [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	420	420	71%	2,00E-138	43.94%	584	KAG5289022.1
2	Select seq gb KAG4259353.1	hypothetical protein CNMCM8057_002830 [Aspergillus fumigatus]	Aspergillus fumigatus	415	415	63%	5,00E-136	46.64%	603	KAF4259353.1
3	Select seq gb KAG5302390.1	corinin-like protein crn1 [Histoplasma capsulatum]	Histoplasma capsulatum	414	414	71%	5,00E-136	43.54%	583	KAG5302390.1
4	Select seq gb QSS57369.1	corinin-like protein crn1 [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	412	412	71%	3,00E-135	43.65%	584	QSS57369.1
5	Select seq ref XP_735760.1	actin-binding protein, putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	405	405	63%	5,00E-132	45.45%	615	XP_735760.1
6	Select seq gb KAF4258836.1	hypothetical protein CNMCM8714_002024 [Aspergillus fumigatus]	Aspergillus fumigatus	393	393	61%	1,00E-127	46.36%	603	KAF4258836.1
7	Select seq gb KEV76968.1	actin binding protein [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	385	385	61%	2,00E-124	44.76%	609	KEV76968.1
8	Select seq gb QSS61074.1	corinin-like protein crn1 [Histoplasma capsulatum]	Histoplasma capsulatum	377	377	69%	4,00E-122	42.09%	558	QSS61074.1
9	Select seq gb EEH11748.1	corinin-like protein crn1 [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	375	375	71%	3,00E-121	40.92%	557	EEH11748.1
10	Select seq gb FEF95589.1	corinin-like protein crn1 [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	373	373	71%	1,00E-120	41.04%	558	FEF95589.1
11	Select seq ref XP_0193311032.1	Crm1p [Candida albicans SC5314]	Candida albicans SC5314	375	375	64%	3,00E-120	41.25%	633	XP_0193311032.1
12	Select seq gb KAF6070624.1	hypothetical protein FO864_001277 [Candida albicans]	Candida albicans	374	374	64%	4,00E-120	41.25%	620	KAF6070624.1
13	Select seq gb KGR09330.1	hypothetical protein MG5_051333 [Candida albicans P37037]	Candida albicans P37037	373	373	60%	1,00E-119	41.43%	628	KGR09330.1
14	Select seq gb KGG825688.1	hypothetical protein MGL_051148 [Candida albicans GC75]	Candida albicans GC75	370	370	64%	1,00E-118	41.21%	628	KGG825688.1
15	Select seq gb KEQ40983.1	hypothetical protein CAWG_055336 [Candida albicans WO-1]	Candida albicans WO-1	370	370	64%	1,00E-118	41.21%	628	KEQ40983.1
16	Select seq gb KGG083391.1	hypothetical protein MEO_05075 [Candida albicans P94015]	Candida albicans P94015	370	370	64%	1,00E-118	41.21%	638	KGG083391.1
17	Select seq gb KGU23235.1	hypothetical protein MGK_051335 [Candida albicans P57055]	Candida albicans P57055	370	370	64%	2,00E-118	41.21%	638	KGU23235.1
18	Select seq gb KHG20551.1	hypothetical protein MG7_051335 [Candida albicans P34048]	Candida albicans P34048	370	370	64%	2,00E-118	41.21%	643	KHG20551.1
19	Select seq gb KHG68964.1	hypothetical protein MGS_05154 [Candida albicans P78042]	Candida albicans P78042	370	370	64%	2,00E-118	41.21%	643	KHG68964.1
20	Select seq gb RP66274.1	hypothetical protein L150_05052 [Candida albicans C6529L]	Candida albicans C6529L	370	370	57%	3,00E-118	44.10%	633	RP66274.1
21	Select seq gb KHG50874.1	hypothetical protein MGE_05098 [Candida albicans P15010]	Candida albicans P15010	370	370	57%	3,00E-118	44.10%	638	KHG50874.1
22	Select seq gb KGG84493.1	hypothetical protein MEU_051333 [Candida albicans P37005]	Candida albicans P37005	343	343	53%	2,00E-111	46.42%	379	KGG84493.1
23	Select seq gb KAF4258892.1	hypothetical protein CNMCM8057_003033 [Aspergillus fumigatus]	Aspergillus fumigatus	213	213	19%	3,00E-64	73.72%	199	KAF4258892.1
24	Select seq gb KAF4255202.1	hypothetical protein CNMCM8714_004532 [Aspergillus fumigatus]	Aspergillus fumigatus	213	213	19%	3,00E-64	73.72%	199	KAF4255202.1
25	Select seq ref XP_790098.1	dUTPase [Dut], putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	214	214	19%	6,00E-64	73.72%	250	XP_790098.1
26	Select seq gb KEV79008.1	dUTPase Dut putative [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	214	214	19%	8,00E-61	73.72%	250	KEV79008.1
27	Select seq gb EEH11214.1	dUTPase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	198	198	20%	1,00E-58	71.43%	203	EEH11214.1
28	Select seq gb EER43265.1	dUTPase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	197	197	19%	4,00E-58	72.26%	205	EER43265.1
29	Select seq gb QSS61617.1	dUTPase [Histoplasma capsulatum]	Histoplasma capsulatum	197	197	19%	5,00E-58	72.26%	205	QSS61617.1
30	Select seq ref XP_001540936.1	deoxyuridine 5'-triphosphate nucleotidylhydrolase [Histoplasma capsulatum MAm1]	Histoplasma capsulatum MAm1	196	196	19%	6,00E-58	72.26%	173	XP_001540936.1
31	Select seq ref XP_018227251.1	deoxyuridine 5'-triphosphate nucleotidylhydrolase [Pneumocystis carinii B80]	Pneumocystis carinii B80	192	192	20%	7,00E-57	67.63%	148	XP_018227251.1
32	Select seq ref XP_738145.1	bifunctional dUTP/dUTP diphosphatase [Candida albicans SC5314]	Candida albicans SC5314	182	182	20%	4,00E-55	62.68%	159	XP_738145.1
33	Select seq ref XP_001541917.1	conserved hypothetical protein [Histoplasma capsulatum MAm1]	Histoplasma capsulatum MAm1	189	189	32%	7,00E-52	45.91%	485	XP_001541917.1
34	Select seq ref XP_018226396.1	hypothetical protein T552_01159 [Pneumocystis carinii B80]	Pneumocystis carinii B80	585	109	22%	5,00E-08	28.21%	528	XP_018226396.1
35	Select seq ref XP_001542288.1	conserved hypothetical protein [Histoplasma capsulatum MAm1]	Histoplasma capsulatum MAm1	56.2	56.2	21%	3,00E-07	24.71%	484	XP_001542288.1
36	Select seq ref XP_748786.1	vegetative incompatibility WD repeat protein, putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	56.2	159	32%	3,00E-07	26.51%	553	XP_748786.1
37	Select seq gb QSS56796.1	transcription initiation factor TFIID subunit 5 [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	54.7	54.7	26%	1,00E-06	24.86%	748	QSS56796.1
38	Select seq gb KAG5289590.1	transcription initiation factor TFIID subunit 5 [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	54.7	54.7	26%	1,00E-06	24.86%	748	KAG5289590.1
39	Select seq gb KAG5302969.1	transcription initiation factor TFIID subunit 5 [Histoplasma capsulatum]	Histoplasma capsulatum	54.7	54.7	26%	1,00E-06	24.86%	748	KAG5302969.1
40	Select seq gb QSS61642.1	transcription initiation factor TFIID subunit 5 [Histoplasma capsulatum]	Histoplasma capsulatum	54.7	54.7	26%	1,00E-06	24.86%	777	QSS61642.1
41	Select seq gb EER3237.1	transcription initiation factor TFIID subunit 5 [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	54.3	54.3	26%	2,00E-06	25.81%	715	EER3237.1
42	Select seq gb KAF4269372.1	hypothetical protein CNMCM8714_008548 [Aspergillus fumigatus]	Aspergillus fumigatus	54.3	54.3	17%	2,00E-06	22.78%	991	KAF4269372.1
43	Select seq gb KEV75971.1	ribosome biogenesis protein Rbc1 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	53.5	53.5	17%	2,00E-06	22.78%	496	KEV75971.1
44	Select seq ref XP_790920.1	ribosome biogenesis protein (Rbc1), putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	53.5	53.5	17%	2,00E-06	22.78%	496	XP_790920.1
45	Select seq gb EEH11186.1	transcription initiation factor TFIID subunit 5 [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	53.9	53.9	26%	2,00E-06	25.81%	715	EEH11186.1
46	Select seq gb OXN06425.1	hypothetical protein CDV58_05855 [Aspergillus fumigatus]	Aspergillus fumigatus	53.9	53.9	25%	2,00E-06	23.81%	1359	OXN06425.1
47	Select seq ref XP_7494943.1	WD repeat protein [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	53.9	53.9	25%	2,00E-06	23.81%	1359	XP_7494943.1



48	Select seq ref XP_001540910.1	conserved hypothetical protein [Histoplasma capsulatum NAm1]	53.5	53.5	26%	2,00E-06	25.81%	715	XP_001540910.1
49	Select seq gb   OXN23213.1	hypothetical protein CDV57_08193 [Aspergillus fumigatus]	53.9	53.9	25%	2,00E-06	23.81%	1359	OXN23213.1
50	Select seq gb   EDPS5968.1	WD repeat protein [Aspergillus fumigatus A1163]	53.9	53.9	25%	2,00E-06	23.81%	1359	EDPS5968.1
51	Select seq gb   KEV79684.1	hypothetical protein WD repeat protein [Aspergillus fumigatus var. RP-2014]	53.9	53.9	25%	2,00E-06	23.81%	1359	KEV79684.1
52	Select seq ref XP_748808.1	vegetative incompatibility WD repeat protein, putative [Aspergillus fumigatus AF293]	50.8	1.09	25%	1,00E-05	30.52%	376	XP_748808.1
53	Select seq gb   KEV78985.1	transcription initiation factor TFIID subunit [Aspergillus fumigatus var. RP-2014]	51.2	51.2	26%	1,00E-05	26.46%	745	KEV78985.1
54	Select seq ref XP_750057.1	transcription initiation factor TFIID subunit, putative [Aspergillus fumigatus AF293]	51.2	51.2	26%	1,00E-05	26.46%	745	XP_750057.1
55	Select seq gb   KAF4265409.1	hypothetical protein CNMCM8714_006630 [Aspergillus fumigatus]	50.8	50.8	28%	2,00E-05	25.70%	736	KAF4265409.1
56	Select seq gb   KAF4271634.1	hypothetical protein CNMCM8057_0065948 [Aspergillus fumigatus]	50.4	50.4	28%	2,00E-05	25.70%	736	KAF4271634.1
57	Select seq gb   QSS56993.1	stress protein p66 [Histoplasma capsulatum H88]	50.4	50.4	19%	2,00E-05	31.91%	613	QSS56993.1
58	Select seq gb   EEP93197.1	actin cortical patch component [Histoplasma capsulatum H143]	50.4	50.4	19%	2,00E-05	31.91%	622	EEP93197.1
59	Select seq gb   EGC46372.1	stress protein [Histoplasma capsulatum H88]	50.1	50.1	19%	2,00E-05	31.91%	622	EGC46372.1
60	Select seq gb   OXN24919.1	hypothetical protein CDV57_07286 [Aspergillus fumigatus]	48.5	48.5	16%	3,00E-05	31.90%	250	OXN24919.1
61	Select seq gb   EDPA9513.1	wd-repeat protein [Aspergillus fumigatus A1163]	49.7	95.5	33%	5,00E-05	26.45%	1029	EDPA9513.1
62	Select seq gb   KAG5296856.1	WD domain-containing protein, vegetative incompatibility protein HET-E-1 [Histoplasma capsulatum G2178]	49.7	49.7	17%	5,00E-05	26.83%	1650	KAG5296856.1
63	Select seq gb   KAG5302772.1	stress protein p66 [Histoplasma capsulatum]	48.5	48.5	20%	8,00E-05	30.99%	613	KAG5302772.1
64	Select seq gb   EEH11382.1	stress protein p66 [Histoplasma capsulatum G186AR]	48.5	48.5	20%	8,00E-05	30.99%	622	EEH11382.1
65	Select seq gb   KAG5289389.1	stress protein p66 [Histoplasma capsulatum G2178]	48.5	48.5	20%	8,00E-05	30.99%	613	KAG5289389.1
66	Select seq gb   EEH08015.1	WD domain-containing protein [Histoplasma capsulatum G186AR]	48.5	48.5	27%	1,00E-04	28.21%	1445	EEH08015.1
67	Select seq gb   EGC00985.1	conserved hypothetical protein [Histoplasma capsulatum H88]	48.5	48.5	17%	1,00E-04	26.83%	1407	EGC00985.1
68	Select seq gb   QSS52600.1	WD domain-containing protein, vegetative incompatibility protein HET-E-1 [Histoplasma capsulatum H88]	48.5	48.5	17%	1,00E-04	26.83%	1632	QSS52600.1
69	Select seq gb   EEP15011.1	conserved hypothetical protein [Histoplasma capsulatum H143]	48.1	48.1	17%	1,00E-04	26.83%	826	EEP15011.1
70	Select seq gb   OXN09345.1	hypothetical protein CDV58_01338 [Aspergillus fumigatus]	47.0	47.0	20%	1,00E-04	25.17%	336	OXN09345.1
71	Select seq gb   KAF4268300.1	hypothetical protein CNMCM8714_001818 [Aspergillus fumigatus]	47.0	47.0	20%	1,00E-04	25.17%	336	KAF4268300.1
72	Select seq gb   KAG5300702.1	ribosome assembly protein RRB1 [Histoplasma capsulatum G2178]	47.4	47.4	17%	2,00E-04	20.56%	495	KAG5300702.1
73	Select seq gb   EEH09899.1	ribosome assembly protein RRB1 [Histoplasma capsulatum G186AR]	47.4	47.4	17%	2,00E-04	20.56%	495	EEH09899.1
74	Select seq ref XP_018225698.1	hypothetical protein TS52_01863 [Pneumocystis carinii B80]	47.0	47.0	28%	2,00E-04	23.38%	467	XP_018225698.1
75	Select seq gb   OXN09364.1	hypothetical protein CDV58_01343 [Aspergillus fumigatus]	46.2	46.2	27%	4,00E-04	21.32%	436	OXN09364.1
76	Select seq gb   KAG5296404.1	chromatin assembly factor 1 subunit B [Histoplasma capsulatum]	46.2	46.2	28%	4,00E-04	25.58%	775	KAG5296404.1
77	Select seq gb   KAG5295365.1	chromatin assembly factor 1 subunit B [Histoplasma capsulatum G2178]	46.2	46.2	28%	5,00E-04	25.58%	775	KAG5295365.1
78	Select seq gb   QSS50619.1	chromatin assembly factor 1 subunit B [Histoplasma capsulatum H88]	46.2	46.2	28%	5,00E-04	25.58%	775	QSS50619.1
79	Select seq ref XP_748030.1	chromatin assembly factor 1 subunit C, putative [Aspergillus fumigatus AF293]	45.1	45.1	27%	9,00E-04	21.84%	436	XP_748030.1
80	Select seq ref XP_748036.1	WD repeat protein [Aspergillus fumigatus AF293]	43.9	43.9	20%	0.002	26.92%	407	XP_748036.1
81	Select seq gb   KEV75701.1	hypothetical protein WD repeat protein [Aspergillus fumigatus var. RP-2014]	43.5	43.5	20%	0.002	26.92%	407	KEV75701.1
82	Select seq gb   KAF4260553.1	hypothetical protein CNMCM8714_001077 [Aspergillus fumigatus]	41.6	41.6	19%	0.009	25.93%	376	KAF4260553.1
83	Select seq ref XP_018225862.1	guanine nucleotide-binding protein subunit beta-like protein [Pneumocystis carinii B80]	41.2	41.2	18%	0.011	26.47%	312	XP_018225862.1
84	Select seq ref XP_749949.1	ribosome biogenesis protein Rsa4, putative [Aspergillus fumigatus AF293]	40.8	40.8	27%	0.019	25.37%	515	XP_749949.1

Homology of extracellular elastinolytic metalloproteinase to proteins from other pathogenic fungi

Job Title	AFR97484:extracellular elastinolytic metalloproteinase...									
RID	7EAMZNYN013 Search expires on 04-16 01:45 am									
Program	BLASTP									
Database	nr									
Query ID	AFR97484.2									
Description	extracellular elastinolytic metalloproteinase [Cryptococcus neoformans var. grubii H99] ...									
Molecule type	amino acid									
Query Length	831									
Sequence identifier	Protein Homolog									
Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb OXN26486.1	hypothetical protein CDV57_04637 [Aspergillus fumigatus]	Aspergillus fumigatus	382	438	70%	2,00E-121	43.96%	634	OXN26486.1
2	Select seq gb KAF4252216.1	hypothetical protein CNMCM8714_007506 [Aspergillus fumigatus]	Aspergillus fumigatus	382	440	70%	3,00E-121	43.96%	634	KAF4252216.1
3	Select seq ref XP_747506.1	elastinolytic metalloproteinase Mep [Aspergillus fumigatus AT293]	Aspergillus fumigatus AT293	382	440	70%	3,00E-121	43.96%	634	XP_747506.1
4	Select seq emb CAA83015.1	metalloprotease (MEP) [Aspergillus fumigatus]	Aspergillus fumigatus	380	439	70%	9,00E-121	43.96%	634	CAA83015.1
5	Select seq gb AAB07708.1	metalloprotease [Aspergillus fumigatus]	Aspergillus fumigatus	368	427	70%	5,00E-116	43.02%	634	AAB07708.1
6	Select seq pdb 4K90 A	Extracellular metalloproteinase from Aspergillus [Aspergillus fumigatus AT293]	Aspergillus fumigatus AT293	354	354	53%	5,00E-114	47.85%	389	4K90_A
7	Select seq pdb 4K90 B	Extracellular metalloproteinase from Aspergillus [Aspergillus fumigatus AT293]	Aspergillus fumigatus AT293	59.3	59.3	6%	8,00E-09	41.38%	215	4K90_B

Homology of glucose-methanol-choline oxidoreductase to proteins from other pathogenic fungi

Hit No.	Sequence identifier	Protein Homolog	Species	Max. Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb KA1288192.1	hypothetical protein CNVICM8689_006173 [Aspergillus fumigatus]	Aspergillus fumigatus	268	268	96%	3.00E-81	33.84%	576	KA1288192.1
2	Select seq gb EDP53503.1	GMC oxidoreductase, putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	257	257	94%	2.00E-76	33.68%	646	EDP53503.1
3	Select seq ref XP_748478.1	GMC oxidoreductase, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	255	255	94%	1.00E-75	33.33%	646	XP_748478.1
4	Select seq gb KA65295253.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	211	211	96%	7.00E-60	29.03%	604	KA65295253.1
5	Select seq gb KEV78899.1	aryl alcohol dehydrogenase [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	210	210	95%	3.00E-59	29.72%	601	KEV78899.1
6	Select seq gb EEH08424.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	209	209	96%	5.00E-59	29.03%	604	EEH08424.1
7	Select seq ref XP_746395.1	aryl-alcohol dehydrogenase, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	209	209	95%	7.00E-59	29.72%	601	XP_746395.1
8	Select seq gb KMG58320.1	aryl-alcohol dehydrogenase [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	209	209	95%	1.00E-58	29.72%	625	KMG58320.1
9	Select seq ref XP_001544836.1	predicted protein [Histoplasma capsulatum Nam1]	Histoplasma capsulatum Nam1	208	208	96%	1.00E-58	28.87%	604	XP_001544836.1
10	Select seq gb EGC43458.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	205	205	96%	1.00E-57	28.71%	604	EGC43458.1
11	Select seq gb KA65294977.1	glucose-methanol-choline oxidoreductase:GMC oxidoreductase [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	190	190	96%	6.00E-52	28.40%	613	KA65294977.1
12	Select seq gb GSS49367.1	glucose-methanol-choline oxidoreductase:GMC oxidoreductase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	189	189	96%	1.00E-51	27.85%	613	GSS49367.1
13	Select seq gb GSS56658.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	188	188	96%	3.00E-51	28.74%	567	GSS56658.1
14	Select seq gb GSS48722.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	187	187	96%	3.00E-51	28.50%	567	GSS48722.1
15	Select seq gb KA65295252.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	187	187	96%	5.00E-51	28.67%	567	KA65295252.1
16	Select seq gb KA65302285.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	187	187	96%	5.00E-51	28.50%	581	KA65302285.1
17	Select seq gb KA652939536.1	glucose-methanol-choline oxidoreductase:GMC oxidoreductase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	186	186	96%	1.00E-50	28.02%	613	KA652939536.1
18	Select seq gb EEH03916.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	183	183	96%	5.00E-50	28.62%	564	EEH03916.1
19	Select seq gb EER39959.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	178	178	96%	3.00E-48	27.93%	540	EER39959.1
20	Select seq gb EER39780.1	glucose-methanol-choline oxidoreductase:GMC oxidoreductase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	175	175	96%	8.00E-47	27.04%	565	EER39780.1
21	Select seq ref XP_001544930.1	conserved hypothetical protein [Histoplasma capsulatum Nam1]	Histoplasma capsulatum Nam1	174	174	95%	1.00E-46	26.80%	565	XP_001544930.1
22	Select seq gb EGC43381.1	glucose-methanol-choline oxidoreductase:GMC oxidoreductase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	174	174	96%	2.00E-46	26.70%	565	EGC43381.1
23	Select seq gb EDP52931.1	GMC oxidoreductase, putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	166	166	96%	2.00E-43	29.04%	629	EDP52931.1
24	Select seq gb OXN08932.1	hypothetical protein CDV58_02306 [Aspergillus fumigatus]	Aspergillus fumigatus	166	166	96%	2.00E-43	29.04%	629	OXN08932.1
25	Select seq ref XP_754807.1	GMC oxidoreductase, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	165	165	96%	8.00E-43	28.87%	629	XP_754807.1
26	Select seq gb GSS560612.1	glucose-methanol-choline oxidoreductase:GMC oxidoreductase [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	162	162	94%	2.00E-42	26.48%	519	GSS560612.1
27	Select seq gb KA64265156.1	hypothetical protein CNVICM8714_006892 [Aspergillus fumigatus]	Aspergillus fumigatus	163	163	96%	2.00E-42	28.87%	629	KA64265156.1
28	Select seq ref XP_001536349.1	predicted protein [Histoplasma capsulatum Nam1]	Histoplasma capsulatum Nam1	160	160	96%	6.00E-42	26.80%	512	XP_001536349.1
29	Select seq gb OXN26627.1	hypothetical protein CDV57_04928 [Aspergillus fumigatus]	Aspergillus fumigatus	162	162	97%	8.00E-42	26.56%	609	OXN26627.1
30	Select seq gb KA64266416.1	hypothetical protein CNVICM8057_000205 [Aspergillus fumigatus]	Aspergillus fumigatus	161	161	97%	1.00E-41	26.39%	609	KA64266416.1
31	Select seq gb KA64254441.1	hypothetical protein CNVICM8714_006280 [Aspergillus fumigatus]	Aspergillus fumigatus	160	160	97%	2.00E-41	26.27%	609	KA64254441.1
32	Select seq gb KA64277028.1	hypothetical protein CNVICM8689_005130 [Aspergillus fumigatus]	Aspergillus fumigatus	160	160	97%	3.00E-41	26.39%	609	KA64277028.1
33	Select seq gb KEV78504.1	oxidoreductase GMC [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	160	160	96%	3.00E-41	28.48%	629	KEV78504.1
34	Select seq gb KA64273685.1	hypothetical protein CNVICM8812_007184 [Aspergillus fumigatus]	Aspergillus fumigatus	158	158	97%	1.00E-40	26.23%	609	KA64273685.1
35	Select seq ref XP_752502.2	versicolurin B synthase, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	156	156	97%	7.00E-40	26.26%	612	XP_752502.2
36	Select seq gb KEV79769.1	oxidoreductase GMC [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	149	149	51%	2.00E-39	33.12%	308	KEV79769.1
37	Select seq gb OXN25116.1	hypothetical protein CDV57_04795 [Aspergillus fumigatus]	Aspergillus fumigatus	148	148	51%	3.00E-39	33.75%	308	OXN25116.1
38	Select seq gb EDP56370.1	versicolurin B synthase, putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	154	154	97%	3.00E-39	26.43%	611	EDP56370.1
39	Select seq gb KA64256707.1	hypothetical protein CNVICM8714_003376 [Aspergillus fumigatus]	Aspergillus fumigatus	154	154	95%	3.00E-39	26.83%	615	KA64256707.1
40	Select seq gb KEV81089.1	versicolurin B synthase [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	154	154	97%	3.00E-39	26.10%	612	KEV81089.1
41	Select seq gb OXN01777.1	hypothetical protein CDV58_09388 [Aspergillus fumigatus]	Aspergillus fumigatus	148	148	51%	3.00E-39	33.75%	316	OXN01777.1
42	Select seq gb KA64275596.1	hypothetical protein CNVICM8812_000758 [Aspergillus fumigatus]	Aspergillus fumigatus	154	154	95%	4.00E-39	26.83%	615	KA64275596.1
43	Select seq gb EEH08138.1	glucose-methanol-choline oxidoreductase:GMC oxidoreductase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	147	147	87%	4.00E-37	26.65%	520	EEH08138.1
44	Select seq gb KMG61525.1	GMC oxidoreductase [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	147	147	95%	1.00E-36	26.50%	615	KMG61525.1
45	Select seq gb KEV79469.1	oxidoreductase GMC [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	145	145	94%	6.00E-36	26.21%	612	KEV79469.1
46	Select seq ref XP_755835.1	GMC oxidoreductase, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	144	144	94%	1.00E-35	26.21%	632	XP_755835.1
47	Select seq gb EDP55006.1	GMC oxidoreductase, putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	141	141	94%	8.00E-35	26.04%	632	EDP55006.1

48	Select seq emb1 CAE47863.1	vericolin b synthase like protein, putative [Aspergillus fumigatus]	140	98%	4,00E-34	652	CAE47863.1
49	Select seq gb  KA65301772.1	oxidoreductase [Histoplasma capsulatum G2178]	137	95%	9,00E-34	543	KA65301772.1
50	Select seq gb  EE03123.1	oxidoreductase [Histoplasma capsulatum G186AR]	137	95%	1,00E-33	543	EE03123.1
51	Select seq gb  EEF40305.1	oxidoreductase [Histoplasma capsulatum H143]	137	95%	1,00E-33	543	EEF40305.1
52	Select seq ref  XP_747216.1	choline oxidase (Coda), putative [Aspergillus fumigatus AF293]	137	96%	2,00E-33	542	XP_747216.1
53	Select seq gb  OXN03810.1	hypothetical protein CDV58_07504 [Aspergillus fumigatus]	127	39%	3,00E-32	257	OXN03810.1
54	Select seq gb  KA42452523.1	hypothetical protein CNMCM8057_005440 [Aspergillus fumigatus]	127	39%	3,00E-32	265	KA42452523.1
55	Select seq gb  OXN29099.1	hypothetical protein CDV57_07839 [Aspergillus fumigatus]	127	39%	3,00E-32	279	OXN29099.1
56	Select seq ref  XP_001537047.1	hypothetical protein HCAG_08156 [Histoplasma capsulatum Nam1]	132	95%	5,00E-32	543	XP_001537047.1
57	Select seq gb  EEF37452.1	choline dehydrogenase [Histoplasma capsulatum H143]	129	79%	8,00E-31	567	EEF37452.1
58	Select seq gb  KA65293810.1	choline dehydrogenase [Histoplasma capsulatum]	128	79%	1,00E-30	498	KA65293810.1
59	Select seq gb  KM60527.1	GMC oxidoreductase [Aspergillus fumigatus Z5]	128	88%	2,00E-30	578	KM60527.1
60	Select seq gb  EEH03617.1	choline dehydrogenase [Histoplasma capsulatum G186AR]	127	79%	3,00E-30	568	EEH03617.1
61	Select seq gb  EGC47941.1	choline dehydrogenase [Histoplasma capsulatum H88]	126	75%	7,00E-30	567	EGC47941.1
62	Select seq gb  KA4248066.1	hypothetical protein CNMCM8057_007909 [Aspergillus fumigatus]	115	45%	8,00E-28	283	KA4248066.1
63	Select seq gb  KA65290461.1	alcohol oxidase [Histoplasma capsulatum]	118	95%	6,00E-27	604	KA65290461.1
64	Select seq gb  EEH03136.1	alcohol oxidase [Histoplasma capsulatum G186AR]	117	95%	3,00E-26	572	EEH03136.1
65	Select seq gb  OS552715.1	alcohol oxidase [Histoplasma capsulatum H88]	115	96%	3,00E-26	606	OS552715.1
66	Select seq gb  KA65287467.1	alcohol oxidase [Histoplasma capsulatum]	115	96%	5,00E-26	606	KA65287467.1
67	Select seq gb  KEY79463.1	glucose oxidase [Aspergillus fumigatus var. RP-2014]	114	95%	1,00E-25	561	KEY79463.1
68	Select seq gb  KA65296732.1	alcohol oxidase [Histoplasma capsulatum G2178]	113	96%	2,00E-25	606	KA65296732.1
69	Select seq gb  KEY9108.1	oxidoreductase GMC [Aspergillus fumigatus var. RP-2014]	110	73%	3,00E-24	598	KEY9108.1
70	Select seq gb  EEE39022.1	GMC oxidoreductase [Histoplasma capsulatum H143]	110	53%	3,00E-24	679	EEE39022.1
71	Select seq gb  KA65300972.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum G2178]	109	53%	3,00E-24	679	KA65300972.1
72	Select seq gb  EEH10180.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum G186AR]	109	53%	3,00E-24	679	EEH10180.1
73	Select seq gb  EGC40842.1	alcohol oxidase [Histoplasma capsulatum H88]	105	96%	8,00E-23	596	EGC40842.1
74	Select seq gb  EEE37155.1	alcohol oxidase [Histoplasma capsulatum H143]	104	96%	1,00E-22	596	EEE37155.1
75	Select seq gb  OS559059.1	choline dehydrogenase [Histoplasma capsulatum]	102	102	3,00E-22	514	OS559059.1
76	Select seq ref  XP_001544221.1	predicted protein [Histoplasma capsulatum Nam1]	102	60%	6,00E-22	511	XP_001544221.1
77	Select seq gb  KA4255254.1	hypothetical protein CNMCM8714_004517 [Aspergillus fumigatus]	100	52%	3,00E-21	678	KA4255254.1
78	Select seq gb  KA4293619.1	hypothetical protein CNMCM8686_005607 [Aspergillus fumigatus]	100	52%	3,00E-21	678	KA4293619.1
79	Select seq ref  XP_748276.1	choline dehydrogenase, putative [Aspergillus fumigatus AF293]	100	52%	3,00E-21	678	XP_748276.1
80	Select seq gb  EDP50909.1	choline dehydrogenase, putative [Aspergillus fumigatus A1163]	100	52%	4,00E-21	678	EDP50909.1
81	Select seq ref  XP_749312.1	GMC oxidoreductase [Aspergillus fumigatus AF293]	98.2	79%	2,00E-20	599	XP_749312.1
82	Select seq gb  KA4262755.1	hypothetical protein CNMCM8812_004431 [Aspergillus fumigatus]	97.8	79%	2,00E-20	599	KA4262755.1
83	Select seq gb  EDP53800.1	GMC oxidoreductase [Aspergillus fumigatus A1163]	97.8	79%	2,00E-20	599	EDP53800.1
84	Select seq gb  KEY08655.1	choline dehydrogenase [Aspergillus fumigatus var. RP-2014]	97.8	52%	2,00E-20	678	KEY08655.1
85	Select seq gb  EEF42395.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum H143]	94.4	41%	2,00E-20	290	EEF42395.1
86	Select seq gb  KA4259031.1	hypothetical protein CNMCM8057_002935 [Aspergillus fumigatus]	97.1	79%	3,00E-20	599	KA4259031.1
87	Select seq gb  OXN03618.1	hypothetical protein CDV58_07825 [Aspergillus fumigatus]	97.1	79%	3,00E-20	601	OXN03618.1
88	Select seq gb  KA4279020.1	hypothetical protein CNMCM8689_003985 [Aspergillus fumigatus]	96.7	79%	5,00E-20	599	KA4279020.1
89	Select seq gb  EEH04811.1	alcohol oxidase [Histoplasma capsulatum G186AR]	95.1	96%	2,00E-19	596	EEH04811.1
90	Select seq gb  KA4277876.1	hypothetical protein CNMCM8057_002092 [Aspergillus fumigatus]	94.4	51%	3,00E-19	585	KA4277876.1
91	Select seq gb  KA4293081.1	hypothetical protein CNMCM8686_006611 [Aspergillus fumigatus]	94.4	51%	3,00E-19	585	KA4293081.1
92	Select seq gb  KA4287451.1	hypothetical protein CNMCM8689_008763 [Aspergillus fumigatus]	94.0	51%	3,00E-19	585	KA4287451.1
93	Select seq gb  EDP54987.1	glucose oxidase, putative [Aspergillus fumigatus A1163]	84.3	95%	3,00E-16	605	EDP54987.1
94	Select seq gb  OS564825.1	alcohol oxidase [Histoplasma capsulatum]	84.3	53%	3,00E-16	527	OS564825.1
95	Select seq ref  XP_755816.1	glucose oxidase, putative [Aspergillus fumigatus AF293]	82.4	74%	2,00E-15	636	XP_755816.1
96	Select seq gb  OXN07622.1	hypothetical protein CDV58_05009 [Aspergillus fumigatus]	82.4	51%	2,00E-15	605	OXN07622.1
97	Select seq gb  KA4258665.1	hypothetical protein CNMCM8714_002094 [Aspergillus fumigatus]	75.9	51%	2,00E-13	559	KA4258665.1
98	Select seq gb  KM61544.1	glucose oxidase, putative [Aspergillus fumigatus Z5]	70.1	51%	1,00E-11	550	KM61544.1
99	Select seq gb  KA65290869.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum]	61.2	21%	3,00E-10	142	KA65290869.1
100	Select seq gb  EEF42396.1	glucose-methanol-choline oxidoreductase [Histoplasma capsulatum H143]	57.8	7%	6,00E-10	57	EEF42396.1

Homology of glutamate dehydrogenase (NADP) to proteins from other pathogenic fungi

Job Title	AFR97782:glutamate dehydrogenase (NADP) [Cryptococcus 7EAH52N013 Search expires on 04-16 01:45 am]									
Program	BLASTP									
Database	nr									
Query ID	AFR97782.1									
Description	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99] ...									
Molecule type	amino acid									
Query Length	451									
Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E. value	Acc. Len	Accession
1	Select seq ref  XP_752164.1	Glutamate/Lucine/Phenylalanine/Valine dehydrogenase, putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	595	595	100%	0,00E+00	65.94%	458	XP_752164.1
2	Select seq gb  KAG529/588.1	NADP-specific glutamate dehydrogenase [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	566	566	100%	0,00E+00	63.07%	460	KAG529/588.1
3	Select seq gb  EEH08744.1	NADP-specific glutamate dehydrogenase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	566	566	100%	0,00E+00	63.07%	460	EEH08744.1
4	Select seq gb  EER43525.1	NADP-specific glutamate dehydrogenase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	564	564	100%	0,00E+00	62.85%	460	EER43525.1
5	Select seq ref  XP_710311.1	glutamate dehydrogenase (NADP+)[Candida albicans SC5314]	Candida albicans SC5314	556	556	98%	0,00E+00	61.61%	456	XP_710311.1
6	Select seq gb  KGO92993.1	NADP-specific glutamate dehydrogenase [Candida albicans GC75]	Candida albicans GC75	553	553	98%	0,00E+00	61.39%	456	KGO92993.1
7	Select seq gb  KHC52488.1	NADP-specific glutamate dehydrogenase [Candida albicans P37039]	Candida albicans P37039	553	553	98%	0,00E+00	61.39%	456	KHC52488.1
8	Select seq gb  EEQ4915.1	NADP-specific glutamate dehydrogenase [Candida albicans WO-1]	Candida albicans WO-1	553	553	98%	0,00E+00	61.39%	456	EEQ4915.1
9	Select seq gb  QSS63765.1	NADP-specific glutamate dehydrogenase [Histoplasma capsulatum]	Histoplasma capsulatum	443	443	80%	8,00E-154	62.13%	372	QSS63765.1
10	Select seq ref  XP_001540184.1	glutamate dehydrogenase [Histoplasma capsulatum NAra1]	Histoplasma capsulatum NAra1	419	419	76%	6,00E-145	60.45%	351	XP_001540184.1

Homology of glycerol-3-phosphate dehydrogenase (NAD(+)) to proteins from other pathogenic fungi

Job Title AFR92257:glycerol-3-phosphate dehydrogenase...  
 RID 7EAHTM|9016 Search expires on 04-16 01:45 am  
 Program BLASTP  
 Database nr  
 Query ID AFR92257.1  
 Description glycerol-3-phosphate dehydrogenase (NAD (+)) [Cryptococcus neoformans var. grubii H99] ...  
 Molecule type amino acid  
 Query length 344

Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E Value	Acc. Len	Accession
1	Select seq ref XP_714402.1	glycerol-3-phosphate dehydrogenase (NAD(+)) [Candida albicans SC5314]	Candida albicans SC5314	321	321	97%	4,00E-107	47.54%	403	XP_714402.1
2	Select seq gb KGU29074.1	glycerol-3-phosphate dehydrogenase (NAD(+)) [Candida albicans P34048]	Candida albicans P34048	319	319	97%	2,00E-106	47.25%	403	KGU29074.1
3	Select seq ref XP_755159.2	glycerol-3-phosphate dehydrogenase (GtdB), putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	308	308	95%	6,00E-102	44.11%	403	XP_755159.2
4	Select seq gb KGU22640.1	glycerol-3-phosphate dehydrogenase (NAD(+)) [Candida albicans P34048]	Candida albicans P34048	293	293	98%	1,00E-96	44.13%	371	KGU22640.1
5	Select seq ref XP_713824.1	Gpd2p [Candida albicans SC5314]	Candida albicans SC5314	292	292	98%	2,00E-96	44.13%	371	XP_713824.1
6	Select seq gb AAW26270.1	sn-glycerol-3-phosphate dehydrogenase NAD+ [Candida albicans]	Candida albicans	291	291	95%	4,00E-96	44.96%	366	AAW26270.1
7	Select seq gb KGU25742.1	glycerol-3-phosphate dehydrogenase (NAD(+)) [Candida albicans P57055]	Candida albicans P57055	288	288	98%	8,00E-95	43.85%	371	KGU25742.1
8	Select seq gb OKN05819.1	hypothetical protein CDV58_05705 [Aspergillus fumigatus]	Aspergillus fumigatus	288	288	97%	2,00E-94	42.49%	396	OKN05819.1
9	Select seq ref XP_749965.1	glycerol-3-phosphate dehydrogenase (GtdA), putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	288	288	98%	3,00E-94	39.85%	416	XP_749965.1
10	Select seq gb KE77044.1	glycerol-3-phosphate dehydrogenase GtdB [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	287	287	92%	4,00E-94	43.35%	382	KE77044.1
11	Select seq gb KMK62136.1	glycerol-3-phosphate dehydrogenase (GtdB) [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	286	286	97%	8,00E-94	42.49%	396	KMK62136.1
12	Select seq gb KAF4266291.1	hypothetical protein CNMCM8057_000315 [Aspergillus fumigatus]	Aspergillus fumigatus	287	287	96%	1,00E-93	40.00%	414	KAF4266291.1
13	Select seq gb KAF4284878.1	hypothetical protein CNMCM8689_005517 [Aspergillus fumigatus]	Aspergillus fumigatus	290	290	97%	7,00E-91	42.64%	803	KAF4284878.1
14	Select seq gb KAF4270316.1	hypothetical protein CNMCM8714_002270 [Aspergillus fumigatus]	Aspergillus fumigatus	287	287	97%	1,00E-89	42.39%	803	KAF4270316.1
15	Select seq gb KAF4278833.1	hypothetical protein CNMCM8057_008569 [Aspergillus fumigatus]	Aspergillus fumigatus	287	287	97%	1,00E-89	42.39%	803	KAF4278833.1
16	Select seq gb EDP54367.1	glycerol-3-phosphate dehydrogenase (GtdB), putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	273	273	85%	1,00E-88	47.02%	396	EDP54367.1
17	Select seq ref XP_001541170.1	glycerol-3-phosphate dehydrogenase [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	272	272	98%	1,00E-87	39.95%	427	XP_001541170.1
18	Select seq gb KMK57510.1	glycerol-3-phosphate dehydrogenase (GtdA) [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	270	270	98%	2,00E-87	40.00%	396	KMK57510.1
19	Select seq gb EER36879.1	glycerol-3-phosphate dehydrogenase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	273	273	98%	4,00E-87	39.95%	496	EER36879.1
20	Select seq gb EGC49155.1	glycerol-3-phosphate dehydrogenase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	273	273	98%	4,00E-87	39.95%	496	EGC49155.1
21	Select seq gb KAG5288275.1	glycerol-3-phosphate dehydrogenase [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	272	272	98%	5,00E-87	39.95%	496	KAG5288275.1
22	Select seq gb EEH06404.1	glycerol-3-phosphate dehydrogenase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	272	272	98%	6,00E-87	39.95%	496	EEH06404.1

**Homology of GTP-binding protein ypt1 to proteins from other pathogenic fungi**  
 AFR94332.GTP-binding protein ypt1 [Cryptococcus...  
 7EA05BPP013 Search expires on 04-16 01:45 am  
 BLASTP  
 nr  
 AFR94332.1  
**GTP-binding protein ypt1 [Cryptococcus neoformans var. grubii H95] ...**  
 amino acid  
 205

Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq ref XP_747911.2	secretion related GTPase SrgB/Ypt1 [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	331	331	98%	2,00E-116	81.68%	201	XP_747911.2
2	Select seq gb KAG5288058.1	GTP-binding protein ypt1 [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	327	327	98%	6,00E-115	80.65%	201	KAG5288058.1
3	Select seq ref XP_001539336.1	GTP-binding protein ypt1 [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	322	322	97%	9,00E-113	80.50%	204	XP_001539336.1
4	Select seq ref XP_018224969.1	GTP-binding protein ypt1 [Pneumocystis carinii B80]	Pneumocystis carinii B80	322	322	98%	2,00E-112	76.35%	204	XP_018224969.1
5	Select seq gb EHH02945.1	GTP-binding protein ypt1 [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	322	322	97%	2,00E-112	80.50%	205	EHH02945.1
6	Select seq ref XP_721550.1	Rab family GTPase [Candida albicans SC5314]	Candida albicans SC5314	296	296	98%	2,00E-102	71.57%	207	XP_721550.1
7	Select seq gb KAF4261634.1	hypothetical protein CN1MCM8714_000463 [Aspergillus fumigatus]	Aspergillus fumigatus	243	243	77%	4,00E-82	76.73%	159	KAF4261634.1
8	Select seq gb EGC45505.1	GTP-binding protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	239	239	97%	3,00E-80	57.00%	205	EGC45505.1
9	Select seq ref XP_018225729.1	GTP-binding protein ypt2 [Pneumocystis carinii B80]	Pneumocystis carinii B80	239	239	82%	4,00E-80	63.91%	205	XP_018225729.1
10	Select seq ref XP_001537350.1	GTP-binding protein SAS1 [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	238	238	97%	1,00E-79	56.50%	205	XP_001537350.1
11	Select seq ref XP_746667.1	Rab GTPase SrgA, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	235	235	83%	2,00E-78	61.63%	206	XP_746667.1
12	Select seq gb EER44812.1	GTP-binding protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	232	232	95%	3,00E-77	56.63%	204	EER44812.1
13	Select seq gb KAF4254239.1	hypothetical protein CN1MCM8714_005269 [Aspergillus fumigatus]	Aspergillus fumigatus	228	228	82%	8,00E-70	60.95%	740	KAF4254239.1
14	Select seq gb KAF4259146.1	hypothetical protein CN1MCM8057_002887 [Aspergillus fumigatus]	Aspergillus fumigatus	228	228	82%	1,00E-69	60.95%	749	KAF4259146.1
15	Select seq gb KAF4266811.1	hypothetical protein CN1MCM8812_002542 [Aspergillus fumigatus]	Aspergillus fumigatus	228	228	82%	1,00E-69	60.95%	749	KAF4266811.1
16	Select seq gb KEV83193.1	hypothetical protein BA78_2663 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	228	228	82%	1,00E-69	60.95%	749	KEV83193.1
17	Select seq gb KGR01655.1	small GTP-binding protein domain [Candida albicans P57072]	Candida albicans P57072	201	201	83%	9,00E-65	52.91%	210	KGR01655.1
18	Select seq pdb 6O62 A	Crystal structure of Sec4p, a Rab family GTPase from Candida albicans [Candida albicans SC5314]	Candida albicans SC5314	199	199	83%	1,00E-64	52.91%	184	6O62_A
19	Select seq ref XP_718237.1	Rab family GTPase [Candida albicans SC5314]	Candida albicans SC5314	200	200	83%	1,00E-64	52.91%	210	XP_718237.1
20	Select seq ref XP_018225825.1	GTP-binding protein ypt3 [Pneumocystis carinii B80]	Pneumocystis carinii B80	187	187	98%	3,00E-59	47.34%	209	XP_018225825.1
21	Select seq gb KMK57506.1	Ras GTPase Rab11, putative [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	179	179	100%	4,00E-56	43.56%	224	KMK57506.1
22	Select seq gb EEQ43799.1	GTP-binding protein YPT31/YPT8 [Candida albicans WO-1]	Candida albicans WO-1	176	176	98%	5,00E-55	44.02%	219	EEQ43799.1
23	Select seq ref XP_001539394.1	Rab1A [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	175	175	92%	1,00E-54	45.50%	210	XP_001539394.1
24	Select seq gb KEV76294.1	Ras GTPase Rab11 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	173	173	96%	6,00E-54	44.83%	204	KEV76294.1
25	Select seq ref XP_749969.1	Ras GTPase Rab11, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	173	173	96%	6,00E-54	44.83%	204	XP_749969.1
26	Select seq ref XP_710395.2	Rab family GTPase [Candida albicans SC5314]	Candida albicans SC5314	170	170	91%	2,00E-52	45.99%	222	XP_710395.2
27	Select seq gb KEV83157.1	hypothetical protein BA78_8550 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	154	154	84%	3,00E-46	44.44%	217	KEV83157.1
28	Select seq gb KAG5298458.1	GTP-binding protein ypt5 [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	154	154	84%	3,00E-46	44.44%	217	KAG5298458.1
29	Select seq gb KAF4274789.1	hypothetical protein CN1MCM8812_004156 [Aspergillus fumigatus]	Aspergillus fumigatus	152	152	95%	9,00E-46	41.25%	218	KAF4274789.1
30	Select seq gb EHH05080.1	GTP-binding protein ypt5 [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	152	152	84%	2,00E-45	43.85%	217	EHH05080.1
31	Select seq ref XP_752022.1	RAB GTPase Ypt5, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	151	151	84%	3,00E-45	43.85%	218	XP_752022.1
32	Select seq gb EER40504.1	GTP-binding protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	146	146	54%	8,00E-44	67.57%	109	EER40504.1
33	Select seq ref XP_018225995.1	GTP-binding protein ypt4 [Pneumocystis carinii B80]	Pneumocystis carinii B80	148	148	75%	5,00E-44	45.45%	204	XP_018225995.1
34	Select seq gb KAF6072007.1	Ras family protein [Candida albicans]	Candida albicans	145	145	83%	2,00E-43	43.60%	171	KAF6072007.1
35	Select seq ref XP_018227056.1	hypothetical protein T552_00741 [Pneumocystis carinii B80]	Pneumocystis carinii B80	148	148	80%	2,00E-43	43.75%	269	XP_018227056.1
36	Select seq ref XP_750514.2	secretion related GTPase SrgD [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	146	146	82%	5,00E-43	42.94%	231	XP_750514.2
37	Select seq ref XP_018227732.1	GTP-binding protein ypt5 [Pneumocystis carinii B80]	Pneumocystis carinii B80	142	142	92%	1,00E-41	38.22%	204	XP_018227732.1
38	Select seq ref XP_755892.1	RAS small monomeric GTPase Rab6, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	138	138	83%	4,00E-40	39.53%	207	XP_755892.1
39	Select seq gb EDP56100.1	secretion related GTPase SrgD [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	138	138	82%	7,00E-40	40.43%	242	EDP56100.1
40	Select seq gb EHH06834.1	conserved hypothetical protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	137	137	95%	1,00E-39	37.76%	209	EHH06834.1
41	Select seq gb EGC47645.1	conserved hypothetical protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	137	137	78%	1,00E-39	40.95%	209	EGC47645.1
42	Select seq gb KAG5292244.1	GTP-binding protein ypt4 [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	136	136	78%	1,00E-39	40.95%	209	KAG5292244.1
43	Select seq gb KGO81055.1	GTP-binding protein YPT6 [Candida albicans P37005]	Candida albicans P37005	134	134	83%	1,00E-38	39.55%	214	KGO81055.1
44	Select seq gb KGU18126.1	small GTP-binding protein domain [Candida albicans P34048]	Candida albicans P34048	134	134	83%	1,00E-38	39.55%	217	KGU18126.1

45	Select seq ref   XP_713074.1	Rab family GTPase [Candida albicans SC5314]	134	83%	1,00E-38	219	XP_713074.1
46	Select seq gb   KGU01412.1	GTP-binding protein yH1 [Candida albicans L26]	134	83%	1,00E-38	224	KGU01412.1
47	Select seq gb   KGR01028.1	GTP-binding protein yH1 [Candida albicans P78048]	134	83%	1,00E-38	225	KGR01028.1
48	Select seq gb   KGU00888.1	small GTP-binding protein domain [Candida albicans P87]	134	83%	1,00E-38	217	KGU00888.1
49	Select seq gb   EEC43709.1	GTP-binding protein yH1 [Candida albicans WO-1]	134	83%	1,00E-38	218	EEC43709.1
50	Select seq gb   KHC43906.1	small GTP-binding protein domain [Candida albicans P60002]	134	83%	1,00E-38	217	KHC43906.1
51	Select seq gb   KAF6066732.1	GTP-binding protein YPT6 [Candida albicans]	134	83%	2,00E-38	226	KAF6066732.1
52	Select seq gb   KHC43833.1	small GTP-binding protein domain [Candida albicans P60002]	129	81%	2,00E-36	216	KHC43833.1
53	Select seq ref   XP_714216.1	Rab family GTPase [Candida albicans SC5314]	129	81%	2,00E-36	216	XP_714216.1
54	Select seq ref   XP_001538657.1	GTP-binding protein ypt5 [Histoplasma capsulatum NAM1]	124	74%	3,00E-35	181	XP_001538657.1
55	Select seq ref   XP_018224693.1	GTP-binding protein yH1 [Histoplasma capsulatum NAM1]	124	78%	1,00E-34	232	XP_018224693.1
56	Select seq ref   XP_018224693.1	hypothetical protein T552_03041 [Pneumocystis carinii B80]	123	73%	1,00E-34	184	XP_018224693.1
57	Select seq ref   XP_754545.1	RAB GTPase Yps21/Yps1, putative [Aspergillus fumigatus Af293]	124	94%	6,00E-34	262	XP_754545.1
58	Select seq ref   XP_715017.1	Rab family GTPase [Candida albicans SC5314]	122	75%	6,00E-34	221	XP_715017.1
59	Select seq gb   KHC42068.1	small GTP-binding protein domain [Candida albicans P76067]	122	75%	7,00E-34	221	KHC42068.1
60	Select seq gb   OXN03600.1	hypothetical protein CDV58_07064 [Aspergillus fumigatus]	124	82%	7,00E-34	281	OXN03600.1
61	Select seq gb   EEH00663.1	GTPase Rab7 [Histoplasma capsulatum G186AR]	121	78%	9,00E-34	205	EEH00663.1
62	Select seq gb   EER38901.1	vacuolar biogenesis protein [Histoplasma capsulatum H143]	122	78%	4,00E-33	317	EER38901.1
63	Select seq gb   RWMK59837.1	Rab small monomeric GTPase Rab7, putative [Aspergillus fumigatus Z5]	119	78%	8,00E-33	207	RWMK59837.1
64	Select seq ref   XP_018226663.1	hypothetical protein T552_00885 [Pneumocystis carinii B80]	115	78%	2,00E-31	205	XP_018226663.1
65	Select seq ref   XP_753438.1	Rab small monomeric GTPase Rab7, putative [Aspergillus fumigatus G217B]	113	75%	7,00E-31	171	XP_753438.1
66	Select seq gb   EEH04127.1	vacuolar sorting-associated protein [Histoplasma capsulatum G186AR]	116	66%	7,00E-31	285	EEH04127.1
67	Select seq gb   KAG5301471.1	alpha-galactosidase, putative [Aspergillus fumigatus A1163]	114	78%	8,00E-31	201	KAG5301471.1
68	Select seq ref   XP_746678.2	Ras family GTPase [RaB4b], putative [Aspergillus fumigatus Af293]	117	62%	9,00E-31	344	XP_746678.2
69	Select seq ref   XP_001539014.1	GTP-binding protein YPT52 [Histoplasma capsulatum NAM1]	115	66%	1,00E-30	285	XP_001539014.1
70	Select seq gb   KEV82209.1	Ras GTPase RaB4 [Aspergillus fumigatus var. RP-2014]	117	62%	1,00E-30	344	KEV82209.1
71	Select seq gb   RWMK59885.1	secretion-like GTPase SrgD [Aspergillus fumigatus Z5]	115	82%	1,00E-30	291	RWMK59885.1
72	Select seq gb   OXN02106.1	Ras family GTPase [RaB4b], [Aspergillus fumigatus Z5]	112	75%	1,00E-30	172	OXN02106.1
73	Select seq gb   RWMK59899.1	Ras family GTPase [RaB4b], [Aspergillus fumigatus Z5]	117	62%	2,00E-30	371	RWMK59899.1
74	Select seq gb   KAG5289960.1	Ras family GTPase [RaB4b], GTP-binding protein ypt4 [Histoplasma capsulatum G217B]	116	71%	2,00E-30	343	KAG5289960.1
75	Select seq gb   EEH10826.1	conserved hypothetical protein [Histoplasma capsulatum G186AR]	116	71%	2,00E-30	343	EEH10826.1
76	Select seq gb   KAF4257142.1	hypothetical protein CNMCM8714_003101 [Aspergillus fumigatus]	113	75%	3,00E-30	216	KAF4257142.1
77	Select seq ref   XP_714835.1	Rab family GTPase [Candida albicans SC5314]	112	78%	6,00E-30	217	XP_714835.1
78	Select seq gb   EEC47226.1	GTP-binding protein YPT7 [Candida albicans WO-1]	112	78%	6,00E-30	217	EEC47226.1
79	Select seq gb   EEC47226.1	small GTP-binding protein domain [Candida albicans P94015]	112	78%	7,00E-30	217	EEC47226.1
80	Select seq gb   KAG54524.1	conserved hypothetical protein [Histoplasma capsulatum H143]	115	73%	1,00E-29	442	KAG54524.1
81	Select seq gb   EER44524.1	hypothetical protein CNMCM8714_007386 [Aspergillus fumigatus]	112	94%	3,00E-29	283	EER44524.1
82	Select seq gb   KAF4257684.1	hypothetical protein CDV57_08592 [Aspergillus fumigatus]	114	61%	2,00E-28	1039	KAF4257684.1
83	Select seq gb   OXN23303.1	hypothetical protein CDV58_08123 [Aspergillus fumigatus]	113	61%	3,00E-28	1039	OXN23303.1
84	Select seq gb   OXN04057.1	GTP-binding nuclear protein Ran, putative [Aspergillus fumigatus Af293]	105	82%	2,00E-27	215	OXN04057.1
85	Select seq ref   XP_751206.1	GTP-binding nuclear protein GSP1/Ran [Histoplasma capsulatum G186AR]	103	87%	8,00E-27	213	XP_751206.1
86	Select seq gb   EEH09231.1	conserved hypothetical protein [Histoplasma capsulatum G186AR]	103	75%	2,00E-26	239	EEH09231.1
87	Select seq gb   EEH07767.1	conserved hypothetical protein [Histoplasma capsulatum G186AR]	103	75%	2,00E-26	239	EEH07767.1
88	Select seq gb   EER36498.1	GTP-binding nuclear protein GSP1/Ran [Histoplasma capsulatum H143]	102	87%	3,00E-26	213	EER36498.1
89	Select seq gb   KAG5297101.1	Ras family protein [Candida albicans]	101	72%	3,00E-26	169	KAG5297101.1
90	Select seq gb   KAF6063586.1	Ras family protein [Candida albicans]	100	78%	4,00E-26	170	KAF6063586.1
91	Select seq gb   KAF6063586.1	secretion related GTPase SrgD [Aspergillus fumigatus var. RP-2014]	101	56%	5,00E-26	202	KAF6063586.1
92	Select seq gb   KAF4259133.1	hypothetical protein CNMCM8714_001858 [Aspergillus fumigatus]	101	56%	5,00E-26	202	KAF4259133.1
93	Select seq gb   KGU14629.1	small GTP-binding protein domain [Candida albicans P87]	103	77%	6,00E-26	288	KGU14629.1
94	Select seq gb   KGU14629.1	Ras small monomeric GTPase RasB [Aspergillus fumigatus Af293]	101	79%	1,00E-25	239	KGU14629.1
95	Select seq ref   XP_755112.1	RasB [Aspergillus fumigatus]	101	75%	1,00E-25	243	XP_755112.1
96	Select seq gb   AAF94030.1	small GTP-binding protein domain [Candida albicans P60002]	102	77%	1,00E-25	290	AAF94030.1
97	Select seq gb   KHC57248.1	YPT7 homologue [Candida albicans]	102	77%	2,00E-25	288	KHC57248.1
98	Select seq emb   CAA21981.1	Ypt7p [Candida albicans SC5314]	102	77%	2,00E-25	288	CAA21981.1
99	Select seq ref   XP_721474.1	small G-protein Gsp1p [Candida albicans]	102	77%	2,00E-25	288	XP_721474.1
100	Select seq gb   AAF78478.1	small G-protein Gsp1p [Candida albicans]	100	78%	2,00E-25	214	AAF78478.1



Homology of heat shock 70kDa protein 4 to proteins from other pathogenic fungi

Job Title	HT No.	Sequence Identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
RID	1	Select seq ref   XP_752631.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	750	750	84%	0.00E+00	57.27%	714	XP_752631.1
Program	2	Select seq gbl   KAF4259893.1	hypothetical protein CNMCM8714_001456 [Aspergillus fumigatus]	Aspergillus fumigatus	750	750	84%	0.00E+00	57.27%	713	KAF4259893.1
Database	3	Select seq gbl   EDP56497.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	749	749	84%	0.00E+00	57.12%	714	EDP56497.1
Query ID	4	Select seq ref   XP_001543737.1	heat shock protein Hsp88 [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	738	738	83%	0.00E+00	55.35%	717	XP_001543737.1
Description	5	Select seq gbl   OSS53508.1	hsp88-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	738	738	83%	0.00E+00	55.20%	717	OSS53508.1
Molecule type	6	Select seq gbl   KAG5304943.1	hsp88-like protein [Histoplasma capsulatum]	Histoplasma capsulatum	737	737	83%	0.00E+00	55.20%	717	KAG5304943.1
Query Length	7	Select seq ref   XP_018277484.1	hypothetical protein T552_00013 [Pneumocystis carinii B80]	Pneumocystis carinii B80	729	729	85%	0.00E+00	53.53%	724	XP_018277484.1
	8	Select seq gbl   OSS58525.1	hsp88-like protein [Histoplasma capsulatum]	Histoplasma capsulatum	654	654	70%	0.00E+00	57.40%	651	OSS58525.1
	9	Select seq gbl   EGC47336.1	hsp88-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	652	652	83%	0.00E+00	51.63%	664	EGC47336.1
	10	Select seq gbl   EEH06535.1	hsp88-like protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	652	652	83%	0.00E+00	51.63%	664	EEH06535.1
	11	Select seq ref   XP_718458.1	adenyl-nucleotide exchange factor [Candida albicans SC5314]	Candida albicans SC5314	605	605	84%	0.00E+00	47.89%	701	XP_718458.1
	12	Select seq gbl   EEO42590.1	heat shock protein Hsp88 [Candida albicans WO-1]	Candida albicans WO-1	595	595	84%	0.00E+00	47.89%	702	EEO42590.1
	13	Select seq gbl   KGU36186.1	heat shock protein 110kDa [Candida albicans P75063]	Candida albicans P75063	593	593	84%	0.00E+00	47.73%	702	KGU36186.1
	14	Select seq gbl   RUP61900.1	hypothetical protein L150_00590 [Candida albicans Cs529L]	Candida albicans Cs529L	592	592	84%	0.00E+00	47.73%	702	RUP61900.1
	15	Select seq gbl   KAF6063130.1	hypothetical protein FOB64_006136 [Candida albicans]	Candida albicans	550	550	84%	0.00E+00	45.15%	670	KAF6063130.1
	16	Select seq gbl   KAF6063131.1	hypothetical protein FOB64_006136 [Candida albicans]	Candida albicans	462	462	68%	4.00E-154	45.64%	567	KAF6063131.1
	17	Select seq gbl   EEH03794.1	hsp70-like protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	315	315	83%	2.00E-96	33.54%	653	EEH03794.1
	18	Select seq gbl   OSS54271.1	hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	315	315	83%	3.00E-96	33.54%	653	OSS54271.1
	19	Select seq ref   XP_018274954.1	hsp72-like protein [Pneumocystis carinii B80]	Histoplasma capsulatum H88	310	310	84%	1.00E-94	32.08%	645	XP_018274954.1
	20	Select seq gbl   AAD00455.1	heat shock protein 70 [Pneumocystis carinii]	Pneumocystis carinii B80	310	310	84%	1.00E-94	32.08%	645	AAD00455.1
	21	Select seq gbl   EGC48122.1	hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	309	309	83%	3.00E-94	32.83%	639	EGC48122.1
	22	Select seq gbl   AAD09565.1	heat shock protein 70 [Pneumocystis carinii]	Pneumocystis carinii	309	309	84%	3.00E-94	31.93%	647	AAD09565.1
	23	Select seq sp   Q00043.1	RecName: Full=Heat shock 70 kDa protein [Histoplasma capsulatum]	Histoplasma capsulatum	302	302	83%	6.00E-91	32.52%	705	Q00043.1
	24	Select seq ref   XP_750490.1	molecular chaperone Hsp70 [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	295	295	83%	5.00E-89	33.08%	638	XP_750490.1
	25	Select seq gbl   KAF6069522.1	Heat shock protein SSA4 [Candida albicans]	Candida albicans	294	294	83%	1.00E-88	32.32%	639	KAF6069522.1
	26	Select seq gbl   KAF6069523.1	Heat shock protein SSA4 [Candida albicans]	Candida albicans	294	294	83%	2.00E-88	32.32%	642	KAF6069523.1
	27	Select seq gbl   EEO41915.1	heat shock protein SSA4 [Candida albicans WO-1]	Candida albicans WO-1	294	294	83%	2.00E-88	32.32%	643	EEO41915.1
	28	Select seq gbl   KGU30551.1	hsp71-like protein [Candida albicans]	Candida albicans	294	294	83%	2.00E-88	32.32%	647	KGU30551.1
	29	Select seq ref   XP_722186.1	Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans	294	294	83%	2.00E-88	32.32%	656	XP_722186.1
	30	Select seq gbl   AET14827.1	heat shock protein 70 [Candida albicans]	Candida albicans	293	293	83%	5.00E-88	32.32%	656	AET14827.1
	31	Select seq gbl   KGT72617.1	hsp72-like protein [Candida albicans 12C]	Candida albicans 12C	290	290	84%	7.00E-87	31.83%	645	KGT72617.1
	32	Select seq gbl   KGU36014.1	hsp72-like protein [Candida albicans P75063]	Candida albicans P75063	290	290	83%	1.00E-86	32.16%	655	KGU36014.1
	33	Select seq ref   XP_713669.2	Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans SC5314	289	289	84%	1.00E-86	31.68%	645	XP_713669.2
	34	Select seq gbl   EEO42742.1	heat shock protein SSA2 [Candida albicans WO-1]	Candida albicans WO-1	289	289	84%	1.00E-86	31.83%	645	EEO42742.1
	35	Select seq gbl   AAS58470.1	heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	288	288	81%	3.30E-86	33.39%	608	AAS58470.1
	36	Select seq gbl   KGO98820.1	hsp72-like protein [Candida albicans P37005]	Candida albicans P37005	288	288	84%	2.00E-86	31.68%	645	KGO98820.1
	37	Select seq gbl   AC295776.1	heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	281	281	80%	4.00E-84	33.17%	580	AC295776.1
	38	Select seq gbl   KAF6063334.1	Hsp70 family protein [Candida albicans]	Candida albicans	275	275	53%	8.00E-84	38.31%	414	KAF6063334.1
	39	Select seq gbl   KAF6068821.1	Endoplasmic reticulum chaperone BiP [Candida albicans]	Candida albicans	282	282	76%	8.00E-84	31.41%	672	KAF6068821.1
	40	Select seq ref   XP_018224343.1	chaperone DnaK [Pneumocystis carinii B80]	Pneumocystis carinii B80	281	281	84%	1.00E-83	30.35%	655	XP_018224343.1
	41	Select seq gbl   KGR13197.1	chaperone DnaK [Candida albicans P57072]	Candida albicans P57072	282	282	76%	1.00E-83	31.41%	687	KGR13197.1
	42	Select seq ref   XP_719467.2	Hsp70 family ATPase [Candida albicans SC5314]	Candida albicans SC5314	282	282	76%	2.00E-83	31.41%	687	XP_719467.2
	43	Select seq gbl   KGU15991.1	chaperone DnaK [Candida albicans 19F]	Candida albicans 19F	281	281	76%	2.00E-83	31.41%	687	KGU15991.1
	44	Select seq gbl   AAB59248.1	endoplasmic reticulum Hsp70 homolog [Pneumocystis carinii]	Pneumocystis carinii	280	280	84%	3.00E-83	30.35%	655	AAB59248.1
	45	Select seq emb   CA66308.1	heat shock protein 70 [Candida albicans]	Candida albicans	278	278	49%	6.00E-83	39.90%	613	CA66308.1

46	Select seq gb   KHC47150.1	chaperone DnaK [Candida albicans Cs6]	280	280	76%	1,00E-82	31.24%	687	KHC47150.1
47	Select seq sp   P87222.2	Candida albicans WO-1	277	277	49%	2,00E-82	39.63%	613	P87222.2
48	Select seq gb   KGR00935.1	Candida albicans P57072	276	276	49%	4,00E-82	39.63%	613	KGR00935.1
49	Select seq ref   XP_747200.1	Aspergillus fumigatus A1293	276	276	49%	7,00E-82	40.62%	614	XP_747200.1
50	Select seq gb   KHC54832.1	Candida albicans P75010	275	275	49%	9,00E-82	39.37%	613	KHC54832.1
51	Select seq gb   KGT63078.1	Candida albicans 12C	275	275	49%	1,00E-81	39.37%	613	KGT63078.1
52	Select seq ref   XP_716208.1	Hsp70 family ATPase [Candida albicans SC5314]	275	275	49%	1,00E-81	39.37%	613	XP_716208.1
53	Select seq gb   ACZ95783.1	heat shock protein 70 [Candida albicans]	273	273	49%	3,00E-81	39.21%	583	ACZ95783.1
54	Select seq gb   EGC49409.1	DnaK-type molecular chaperone b1pA [Histoplasma capsulatum H88]	275	275	83%	6,00E-81	29.03%	676	EGC49409.1
55	Select seq gb   EEH05682.1	dnaK-type molecular chaperone b1pA [Histoplasma capsulatum G186AR]	275	275	83%	7,00E-81	29.03%	676	EEH05682.1
56	Select seq gb   KAG5294347.1	dnaK-type molecular chaperone b1pA [Histoplasma capsulatum G217B]	274	274	83%	8,00E-81	29.03%	676	KAG5294347.1
57	Select seq gb   QSS566325.1	dnaK-type molecular chaperone b1pA [Histoplasma capsulatum]	274	274	83%	9,00E-81	29.03%	676	QSS566325.1
58	Select seq ref   XP_749594.1	Hsp70 chaperone BIP/Kar2, putative [Aspergillus fumigatus A1293]	273	273	84%	3,00E-80	29.74%	672	XP_749594.1
59	Select seq gb   KAF4256863.1	hypothetical protein CNMCM8057_003921 [Aspergillus fumigatus]	276	276	49%	4,00E-80	40.62%	859	KAF4256863.1
60	Select seq gb   KAF4260162.1	hypothetical protein CNMCM8714_001290 [Aspergillus fumigatus]	276	276	49%	4,00E-80	40.62%	859	KAF4260162.1
61	Select seq ref   XP_001543760.1	heat shock 70 kDa protein [Histoplasma capsulatum NAM1]	270	270	49%	7,00E-80	40.10%	613	XP_001543760.1
62	Select seq gb   KAG5291976.1	heat shock protein SSB1 [Histoplasma capsulatum G217B]	268	268	49%	5,00E-79	39.58%	614	KAG5291976.1
63	Select seq gb   EEH06561.1	heat shock protein SSB1 [Histoplasma capsulatum G186AR]	268	268	49%	5,00E-79	39.58%	613	EEH06561.1
64	Select seq gb   EER40742.1	heat shock protein SSB [Histoplasma capsulatum H143]	268	268	49%	6,00E-79	39.37%	613	EER40742.1
65	Select seq ref   XP_018227313.1	hsp75-like protein [Pneumocystis carinii B80]	264	264	56%	2,00E-77	35.29%	612	XP_018227313.1
66	Select seq ref   XP_001538200.1	heat shock 70 kDa protein C precursor [Histoplasma capsulatum NAM1]	257	257	83%	2,00E-74	28.29%	677	XP_001538200.1
67	Select seq gb   EGC47363.1	conserved hypothetical protein [Histoplasma capsulatum H88]	251	251	49%	7,00E-73	37.80%	599	EGC47363.1
68	Select seq gb   EER37272.1	hsp70-like protein [Histoplasma capsulatum H143]	241	241	71%	1,00E-69	30.80%	569	EER37272.1
69	Select seq ref   XP_001537067.1	heat shock protein SSC1, mitochondrial precursor [Histoplasma capsulatum NAM1]	216	216	57%	1,00E-59	34.08%	676	XP_001537067.1
70	Select seq gb   EEH03103.1	heat shock protein SSC1 [Histoplasma capsulatum G186AR]	216	216	57%	1,00E-59	34.08%	675	EEH03103.1
71	Select seq gb   KAG5301798.1	heat shock protein SSC1 [Histoplasma capsulatum G217B]	216	216	57%	1,00E-59	34.08%	675	KAG5301798.1
72	Select seq gb   EGC48453.1	conserved hypothetical protein [Histoplasma capsulatum H88]	216	216	57%	1,00E-59	34.08%	675	EGC48453.1
73	Select seq gb   EDP54522.1	mitochondrial Hsp70 chaperone [Sc70], putative [Aspergillus fumigatus A1163]	216	216	57%	2,00E-59	33.86%	661	EDP54522.1
74	Select seq gb   KEY79550.1	mitochondrial Hsp70 chaperone [Sc70], putative [Aspergillus fumigatus var. RP-2014]	215	215	57%	3,00E-59	33.86%	684	KEY79550.1
75	Select seq ref   XP_755328.1	mitochondrial Hsp70 chaperone [Sc70], putative [Aspergillus fumigatus A1293]	215	215	57%	3,00E-59	33.86%	685	XP_755328.1
76	Select seq ref   XP_018225788.1	hsp77-like protein [Pneumocystis carinii B80]	212	212	81%	3,00E-58	27.54%	657	XP_018225788.1
77	Select seq gb   KGO89268.1	hsp77-like protein [Candida albicans P94015]	209	209	74%	4,00E-57	29.17%	648	KGO89268.1
78	Select seq gb   EEO47268.1	heat shock protein SSC1, mitochondrial precursor [Candida albicans WO-1]	208	208	74%	5,00E-57	29.17%	648	EEO47268.1
79	Select seq gb   RLP63394.1	hsp77-like protein [Candida albicans Cs529L]	208	208	74%	5,00E-57	29.17%	648	RLP63394.1
80	Select seq gb   QSS59214.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum]	198	198	34%	1,00E-56	40.15%	309	QSS59214.1
81	Select seq ref   XP_713153.1	Hsp70 family ATPase [Candida albicans SC5314]	207	207	74%	1,00E-56	29.26%	648	XP_713153.1
82	Select seq gb   KGO95737.1	hsp77-like protein [Candida albicans P37005]	206	206	74%	2,00E-56	29.26%	648	KGO95737.1
83	Select seq ref   XP_018225450.1	hypothetical protein T553_02387 [Pneumocystis carinii B80]	202	202	85%	1,00E-53	25.10%	904	XP_018225450.1
84	Select seq gb   KAF6063693.1	Hsp70 family protein [Candida albicans]	191	191	63%	4,00E-51	30.22%	608	KAF6063693.1
85	Select seq ref   XP_001544351.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum NAM1]	182	182	31%	6,00E-51	40.24%	611	XP_001544351.1
86	Select seq gb   KAF6063692.1	Hsp70 family protein [Candida albicans]	189	189	63%	9,00E-51	30.22%	543	KAF6063692.1
87	Select seq gb   EEO47168.1	heat shock protein SSC1, mitochondrial precursor [Candida albicans WO-1]	190	190	63%	1,00E-50	30.30%	638	EEO47168.1
88	Select seq gb   RLP66487.1	chaperone DnaK [Candida albicans Cs529L]	190	190	63%	1,00E-50	30.30%	638	RLP66487.1
89	Select seq ref   XP_715228.1	Hsp70 family ATPase [Candida albicans SC5314]	190	190	63%	1,00E-50	30.30%	638	XP_715228.1
90	Select seq gb   KGR07696.1	chaperone DnaK [Candida albicans P37037]	190	190	63%	1,00E-50	30.30%	638	KGR07696.1
91	Select seq gb   KHC47008.1	chaperone DnaK [Candida albicans P37039]	189	189	63%	2,00E-50	30.30%	638	KHC47008.1
92	Select seq gb   KGO83718.1	chaperone DnaK [Candida albicans P37005]	189	189	63%	2,00E-50	30.30%	638	KGO83718.1
93	Select seq gb   KGU20262.1	chaperone DnaK [Candida albicans P34048]	189	189	63%	3,00E-50	30.30%	638	KGU20262.1
94	Select seq gb   KAG82346.1	chaperone DnaK [Candida albicans P94015]	189	189	63%	4,00E-50	30.10%	638	KAG82346.1
95	Select seq gb   AAB34280.1	70 kDa heat shock protein [Candida albicans]	178	178	32%	4,00E-50	40.16%	244	AAB34280.1
96	Select seq gb   KHC30703.1	chaperone DnaK [Candida albicans P76067]	188	188	63%	4,00E-50	30.10%	638	KHC30703.1
97	Select seq gb   EER41540.1	dnaK-type molecular chaperone b1pA [Histoplasma capsulatum H143]	155	155	34%	3,00E-41	33.08%	319	EER41540.1
98	Select seq gb   AAD00456.1	heat shock protein 70B/SSB1 [Pneumocystis carinii]	151	151	26%	5,00E-41	39.13%	207	AAD00456.1
99	Select seq gb   KAF4293845.1	hypothetical protein CNMCM8686_005196 [Aspergillus fumigatus]	164	164	52%	7,00E-41	27.91%	997	KAF4293845.1
100	Select seq gb   KEY79855.1	Hsp70 chaperone Hsp1, Orp150 [Aspergillus fumigatus var. RP-2014]	164	164	52%	7,00E-41	27.91%	996	KEY79855.1

Homology of hsp71-like protein to proteins from other pathogenic fungi

Job Title	Sequence Identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E Value	Acc. Len	Accession
RID	AF197929	hsp71-like protein [Cryptococcus...]	Pneumocystis carinii	1055	1055	99%	0.00E+00	79.17%	647	AAD09565.1
Program	BLASTP	hsp72-like protein [Pneumocystis carinii]	Pneumocystis carinii B80	1053	1053	99%	0.00E+00	79.01%	645	XP_018224954.1
Database	nr	heat shock protein 70 [Pneumocystis carinii]	Pneumocystis carinii	1050	1050	99%	0.00E+00	78.55%	645	AAD00455.1
Query ID	AF197929.1	hsp70-like protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	1041	1041	94%	0.00E+00	82.30%	653	EH03794.1
Description	amino acid	hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	1041	1041	94%	0.00E+00	82.13%	653	CSS54271.1
Molecule type	643	molecular chaperone Hsp70 [Aspergillus fumigatus A7293]	Aspergillus fumigatus A7293	1038	1038	93%	0.00E+00	82.15%	638	XP_750490.1
Query Length		heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	1027	1027	92%	0.00E+00	82.41%	608	AAS58470.1
		heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	1007	1007	90%	0.00E+00	82.96%	580	ACZ95776.1
		RecName: Full=Heat shock 70 kDa protein [Histoplasma capsulatum]	Histoplasma capsulatum	998	998	100%	0.00E+00	77.18%	705	Q00043.1
		hsp72-like protein [Candida albicans 12C]	Candida albicans 12C	998	998	100%	0.00E+00	78.29%	645	KG172617.1
		Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans SC5314	996	996	100%	0.00E+00	78.29%	645	XP_713669.2
		heat shock protein SSA2 [Candida albicans WO-1]	Candida albicans WO-1	996	996	100%	0.00E+00	78.14%	645	EQ42742.1
		hsp72-like protein [Candida albicans P37005]	Candida albicans P37005	995	995	100%	0.00E+00	78.14%	645	KG098820.1
		hsp71-like protein [Candida albicans P75063]	Candida albicans P75063	988	988	93%	0.00E+00	80.86%	655	KGU16014.1
		Hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	983	983	94%	0.00E+00	78.52%	639	EGC48122.1
		Heat shock protein SSA4 [Candida albicans]	Candida albicans	979	979	93%	0.00E+00	81.38%	639	KAF069522.1
		Heat shock protein SSA4 [Candida albicans]	Candida albicans	979	979	93%	0.00E+00	81.38%	642	KAF069523.1
		heat shock protein SSA4 [Candida albicans WO-1]	Candida albicans WO-1	979	979	93%	0.00E+00	81.38%	643	EQ41915.1
		Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans SC5314	979	979	93%	0.00E+00	81.38%	656	XP_722186.1
		hsp71-like protein [Candida albicans P34048]	Candida albicans P34048	979	979	93%	0.00E+00	81.38%	647	KGU10551.1
		heat shock protein 70 [Candida albicans]	Candida albicans	977	977	93%	0.00E+00	81.38%	656	AET14827.1
		hsp70-like protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	894	894	81%	0.00E+00	81.94%	569	ERS37271.1
		DnaK-type molecular chaperone b1pA [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	783	783	95%	0.00E+00	61.53%	676	EGC49409.1
		dnaK-type molecular chaperone b1pA [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	783	783	95%	0.00E+00	61.53%	676	EH05682.1
		dnaK-type molecular chaperone b1pA [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	783	783	95%	0.00E+00	61.36%	676	KAG5294347.1
		dnaK-type molecular chaperone b1pA [Histoplasma capsulatum]	Histoplasma capsulatum	778	778	95%	0.00E+00	61.04%	676	CSS566325.1
		Hsp70 chaperone Bp/Kar2, putative [Aspergillus fumigatus A7293]	Aspergillus fumigatus A7293	767	767	94%	0.00E+00	61.90%	672	XP_749594.1
		Endoplasmic reticulum chaperone BIP [Candida albicans]	Candida albicans	757	757	94%	0.00E+00	60.91%	672	KAF066821.1
		chaperone DnaK [Pneumocystis carinii B80]	Pneumocystis carinii B80	757	757	93%	0.00E+00	60.36%	655	XP_018224343.1
		endoplasmic reticulum HSP70 homolog [Pneumocystis carinii]	Pneumocystis carinii	756	756	93%	0.00E+00	60.20%	655	AAB58248.1
		chaperone DnaK [Candida albicans P57072]	Candida albicans P57072	754	754	93%	0.00E+00	61.08%	687	KGR13197.1
		Hsp70 family ATPase [Candida albicans SC5314]	Candida albicans SC5314	754	754	93%	0.00E+00	61.08%	687	XP_719462.2
		chaperone DnaK [Candida albicans 19F]	Candida albicans 19F	753	753	93%	0.00E+00	60.92%	687	KGU15991.1
		chaperone DnaK [Candida albicans C6b]	Candida albicans C6b	752	752	93%	0.00E+00	61.08%	687	RHC47150.1
		heat shock 70 kDa protein C precursor [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	746	746	95%	0.00E+00	59.16%	677	XP_001338200.1
		Hsp70 family protein [Candida albicans]	Candida albicans	721	721	63%	0.00E+00	85.01%	414	KAF060334.1
		heat shock protein 70 [Candida albicans]	Candida albicans	718	718	85%	0.00E+00	65.40%	613	CAA66308.1
		hsp75-like protein [Candida albicans P57072]	Candida albicans P57072	718	718	85%	0.00E+00	65.40%	613	KGR00935.1
		RecName: Full=Ribosome-associated molecular chaperone SSB1; AltName: Full=Hsp70 chaperone s5b [Candida albicans WO-1]	Candida albicans WO-1	718	718	85%	0.00E+00	65.40%	613	P87222.2
		hsp75-like protein [Candida albicans 12C]	Candida albicans 12C	716	716	85%	0.00E+00	65.22%	613	KG163078.1

41	Select seq ref XP_716208.1	Hsp70 family ATPase [Candida albicans SC5314]	716	716	85%	0.00E+00	613	XP_716208.1
42	Select seq gb KHC54832.1	hsp75-like protein [Candida albicans P75010]	715	715	85%	0.00E+00	613	KHC54832.1
43	Select seq gb AC295783.1	heat shock protein 70 [Candida albicans]	710	710	85%	0.00E+00	583	AC295783.1
44	Select seq ref XP_018227313.1	hsp75-like protein [Pneumocystis carinii B80]	702	702	85%	0.00E+00	583	XP_018227313.1
45	Select seq ref XP_747200.1	Hsp70 chaperone [HscA, putative [Aspergillus fumigatus A293]]	686	686	87%	0.00E+00	614	XP_747200.1
46	Select seq gb KAF4260162.1	hypothetical protein ONMCM8714_001290 [Aspergillus fumigatus]	684	684	87%	0.00E+00	614	KAF4260162.1
47	Select seq gb KAF4256863.1	hypothetical protein ONMCM8057_00392 [Aspergillus fumigatus]	684	684	87%	0.00E+00	614	KAF4256863.1
48	Select seq gb KAG5291976.1	heat shock protein S81 [Histoplasma capsulatum G2178]	679	679	87%	0.00E+00	614	KAG5291976.1
49	Select seq ref XP_001543760.1	heat shock 70 kDa protein [Histoplasma capsulatum Nam1]	676	676	87%	0.00E+00	613	XP_001543760.1
50	Select seq gb EEH06561.1	heat shock protein S81 [Histoplasma capsulatum G186AR]	676	676	87%	0.00E+00	613	EEH06561.1
51	Select seq gb EER40742.1	heat shock protein S81 [Histoplasma capsulatum G186AR]	673	673	87%	0.00E+00	613	EER40742.1
52	Select seq gb EGC47363.1	conserved hypothetical protein [Histoplasma capsulatum H88]	640	640	87%	0.00E+00	599	EGC47363.1
53	Select seq gb EDP54522.1	mitochondrial Hsp70 chaperone [Hsc70, putative [Aspergillus fumigatus A1163]]	577	577	94%	0.00E+00	661	EDP54522.1
54	Select seq ref XP_755328.1	mitochondrial Hsp70 chaperone [Hsc70, putative [Aspergillus fumigatus A293]]	577	577	94%	0.00E+00	685	XP_755328.1
55	Select seq gb KE79550.1	mitochondrial Hsp70 chaperone [Hsc70, putative [Aspergillus fumigatus var. RP-2014]]	577	577	94%	0.00E+00	684	KE79550.1
56	Select seq gb EG48453.1	conserved hypothetical protein [Histoplasma capsulatum H88]	569	569	94%	0.00E+00	675	EG48453.1
57	Select seq gb KAG5301798.1	heat shock protein S5C1 [Histoplasma capsulatum G2178]	569	569	94%	0.00E+00	675	KAG5301798.1
58	Select seq ref XP_001537067.1	heat shock protein S5C1, mitochondrial precursor [Histoplasma capsulatum Nam1]	569	569	94%	0.00E+00	676	XP_001537067.1
59	Select seq gb EEH03103.1	heat shock protein S5C1 [Histoplasma capsulatum G186AR]	566	566	94%	0.00E+00	675	EEH03103.1
60	Select seq ref XP_018225788.1	hsp77-like protein [Pneumocystis carinii B80]	566	566	96%	0.00E+00	657	XP_018225788.1
61	Select seq gb RUF6394.1	hsp77-like protein [Candida albicans Cs529L]	548	548	95%	0.00E+00	648	RUF6394.1
62	Select seq gb KGG89268.1	hsp77-like protein [Candida albicans P94015]	547	547	95%	0.00E+00	648	KGG89268.1
63	Select seq gb EEG47268.1	heat shock protein S5C1, mitochondrial precursor [Candida albicans WO-1]	547	547	95%	0.00E+00	648	EEG47268.1
64	Select seq ref XP_713153.1	Hsp70 family ATPase [Candida albicans SC5314]	544	544	95%	0.00E+00	648	XP_713153.1
65	Select seq gb KGO95737.1	hsp77-like protein [Candida albicans P37005]	544	544	95%	0.00E+00	648	KGO95737.1
66	Select seq gb KAF6063692.1	Hsp70 family protein [Candida albicans]	515	515	81%	8.00E-177	543	KAF6063692.1
67	Select seq gb KAF6063693.1	Hsp70 family protein [Candida albicans]	516	516	81%	4.00E-176	528	KAF6063693.1
68	Select seq gb QSS59214.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum]	492	492	43%	2.00E-171	309	QSS59214.1
69	Select seq gb RUP66487.1	chaperone DnaK [Candida albicans Cs529L]	496	496	90%	6.00E-168	638	RUP66487.1
70	Select seq gb KGG83718.1	chaperone DnaK [Candida albicans P37005]	496	496	90%	6.00E-168	638	KGG83718.1
71	Select seq gb KGO82346.1	chaperone DnaK [Candida albicans P94015]	496	496	90%	1.00E-168	638	KGO82346.1
72	Select seq gb KHC47008.1	chaperone DnaK [Candida albicans P37039]	495	495	90%	8.00E-167	638	KHC47008.1
73	Select seq gb KGR07696.1	chaperone DnaK [Candida albicans P37037]	495	495	90%	2.00E-167	638	KGR07696.1
74	Select seq gb KGU20262.1	chaperone DnaK [Candida albicans P34098]	494	494	90%	2.00E-167	638	KGU20262.1
75	Select seq gb KHK30703.1	chaperone DnaK [Candida albicans P76067]	494	494	90%	3.00E-167	638	KHK30703.1
76	Select seq ref XP_715228.1	Hsp70 family ATPase [Candida albicans SC5314]	494	494	90%	5.00E-167	638	XP_715228.1
77	Select seq gb EEQ47168.1	heat shock protein S5C1, mitochondrial precursor [Candida albicans WO-1]	493	493	90%	6.00E-167	638	EEQ47168.1
78	Select seq ref XP_001544351.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum Nam1]	435	487	62%	6.00E-149	311	XP_001544351.1
79	Select seq gb AAB34280.1	70 kDa heat shock protein [Candida albicans]	421	421	37%	1.00E-144	244	AAB34280.1
80	Select seq gb EER40931.1	heat shock protein S5C1 [Histoplasma capsulatum H143]	366	426	74%	1.00E-118	552	EER40931.1
81	Select seq gb EER41540.1	dnaK-type molecular chaperone b1pA [Histoplasma capsulatum H143]	350	350	39%	1.00E-115	646	EER41540.1
82	Select seq gb AAD00456.1	heat shock protein 70B/S81 [Pneumocystis carinii]	327	327	32%	3.00E-108	207	AAD00456.1
83	Select seq gb QSS53508.1	hsp88-like protein [Histoplasma capsulatum H88]	271	271	93%	3.00E-80	717	QSS53508.1
84	Select seq gb KAG5304943.1	hsp88-like protein [Histoplasma capsulatum]	270	270	93%	3.00E-80	717	KAG5304943.1
85	Select seq ref XP_001543737.1	heat shock protein Hsp88 [Histoplasma capsulatum Nam1]	269	269	93%	1.00E-79	717	XP_001543737.1
86	Select seq gb QSS58525.1	hsp88-like protein [Histoplasma capsulatum]	265	265	75%	6.00E-79	651	QSS58525.1
87	Select seq gb EG47336.1	hsp88-like protein [Histoplasma capsulatum H88]	266	266	75%	6.00E-79	664	EG47336.1
88	Select seq gb EEH06535.1	hsp88-like protein [Histoplasma capsulatum G186AR]	266	266	75%	7.00E-79	664	EEH06535.1
89	Select seq gb KAF4259893.1	hypothetical protein ONMCM8714_001456 [Aspergillus fumigatus]	266	266	93%	2.00E-78	713	KAF4259893.1
90	Select seq ref XP_752611.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus A293]	266	266	93%	2.00E-78	714	XP_752611.1
91	Select seq gb EDP56497.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus A1163]	265	265	93%	4.00E-78	714	EDP56497.1
92	Select seq ref XP_018227484.1	hypothetical protein 1552_0003 [Pneumocystis carinii B80]	261	261	94%	7.00E-77	724	XP_018227484.1
93	Select seq gb AGT21630.1	heat shock protein 70 [Aspergillus fumigatus]	236	236	20%	2.00E-74	130	AGT21630.1
94	Select seq gb EEQ42580.1	heat shock protein Hsp88 [Candida albicans WO-1]	236	236	57%	1.00E-67	702	EEQ42580.1
95	Select seq gb KGU30186.1	heat shock protein 110kDa [Candida albicans P75063]	236	236	57%	1.00E-67	702	KGU30186.1
96	Select seq ref XP_718458.1	adenyl-nucleotide exchange factor [Candida albicans SC5314]	236	236	57%	1.00E-67	701	XP_718458.1
97	Select seq gb RUP61900.1	hypothetical protein L150_00590 [Candida albicans Cs529L]	236	236	57%	2.00E-67	702	RUP61900.1
98	Select seq ref XP_749367.1	Hsp70 chaperone [Hsp], putative [Aspergillus fumigatus A293]	227	227	68%	2.00E-65	570	XP_749367.1
99	Select seq gb KAF6061150.1	Hsp70 family protein [Candida albicans]	225	399	82%	3.00E-65	512	KAF6061150.1
100	Select seq gb KAF6063130.1	hypothetical protein FOB4_006136 [Candida albicans]	229	229	57%	3.00E-65	670	KAF6063130.1

Homology of hsp72-like protein to proteins from other pathogenic fungi

Job Title	AFR97952:hsp72-like protein [Cryptococcus...]
RID	7EAVJIG016 Search expires on 01-16 01:46 am
Program	BLASTP
Database	nr
Query ID	AFR97952.1
Description	<b>hsp72-like protein [Cryptococcus neoformans var. grubii H99]</b>
Molecule type	amino acid
Query length	642

Hit No.	Sequence Identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb AAD09565.1	heat shock protein 70 [Pneumocystis carinii]	Pneumocystis carinii	1054	1054	99%	0.00E+00	79.44%	647	AA009555.1
2	Select seq ref XP_018224954.1	hsp72-like protein [Pneumocystis carinii] (B80)	Pneumocystis carinii (B80)	1053	1053	99%	0.00E+00	79.29%	645	XP_018224954.1
3	Select seq gb AAD00485.1	heat shock protein 70 [Pneumocystis carinii]	Pneumocystis carinii	1050	1050	99%	0.00E+00	78.83%	645	AA000455.1
4	Select seq ref XP_750490.1	molecular chaperone Hsp70 [Aspergillus fumigatus A293]	Aspergillus fumigatus A293	1047	1047	94%	0.00E+00	82.81%	638	XP_750490.1
5	Select seq gb EEHQ3794.1	hsp70-like protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	1045	1045	94%	0.00E+00	82.30%	653	EEHQ3794.1
6	Select seq gb QSS54271.1	hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	1045	1045	94%	0.00E+00	82.13%	653	QSS54271.1
7	Select seq gb AAS58470.1	heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	1036	1036	92%	0.00E+00	83.08%	608	AAS58470.1
8	Select seq gb ACZ95776.1	heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	1017	1017	90%	0.00E+00	83.82%	580	ACZ95776.1
9	Select seq sp Q00043.1	RecName: Full=Heat shock 70 kDa protein [Histoplasma capsulatum]	Histoplasma capsulatum	1001	1001	100%	0.00E+00	76.34%	705	Q00043.1
10	Select seq gb KGT72671.1	hsp72-like protein [Candida albicans 12C]	Candida albicans 12C	999	999	100%	0.00E+00	78.95%	645	KGT72671.1
11	Select seq gb EEQ42742.1	heat shock protein SSA2 [Candida albicans WO-1]	Candida albicans WO-1	998	998	100%	0.00E+00	78.95%	645	EEQ42742.1
12	Select seq ref XP_713669.2	Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans SC5314	998	998	100%	0.00E+00	78.95%	645	XP_713669.2
13	Select seq gb KGO98820.1	hsp72-like protein [Candida albicans P37005]	Candida albicans P37005	998	998	100%	0.00E+00	78.95%	645	KGO98820.1
14	Select seq gb KGLJ8604.1	hsp72-like protein [Candida albicans P75063]	Candida albicans P75063	987	987	94%	0.00E+00	80.69%	655	KGLJ8604.1
15	Select seq gb EGC48122.1	Hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	987	987	94%	0.00E+00	78.52%	639	EGC48122.1
16	Select seq gb KAF069522.1	Heat shock protein SSA4 [Candida albicans]	Candida albicans	982	982	100%	0.00E+00	78.76%	639	KAF069522.1
17	Select seq gb KAF069523.1	Heat shock protein SSA4 [Candida albicans]	Candida albicans	982	982	94%	0.00E+00	81.71%	642	KAF069523.1
18	Select seq gb EEQ41915.1	heat shock protein SSA4 [Candida albicans]	Candida albicans	982	982	94%	0.00E+00	81.71%	643	EEQ41915.1
19	Select seq ref XP_722186.1	Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans SC5314	982	982	94%	0.00E+00	81.71%	656	XP_722186.1
20	Select seq gb KGLJ30551.1	hsp75-like protein [Candida albicans P34048]	Candida albicans P34048	982	982	94%	0.00E+00	81.71%	647	KGLJ30551.1
21	Select seq gb AET14827.1	heat shock protein 70 [Candida albicans]	Candida albicans	980	980	94%	0.00E+00	81.71%	656	AET14827.1
22	Select seq gb EER37272.1	hsp70-like protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	898	898	82%	0.00E+00	82.13%	599	EER37272.1
23	Select seq gb EGC49409.1	DnaK-type molecular chaperone bpa [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	782	782	95%	0.00E+00	61.85%	676	EGC49409.1
24	Select seq gb EEHQ5682.1	dnaK-type molecular chaperone bpa [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	782	782	95%	0.00E+00	61.85%	676	EEHQ5682.1
25	Select seq gb KAG5294347.1	dnaK-type molecular chaperone bpa [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	781	781	95%	0.00E+00	61.69%	676	KAG5294347.1
26	Select seq gb OSS66325.1	dnaK-type molecular chaperone bpa [Histoplasma capsulatum]	Histoplasma capsulatum	776	776	95%	0.00E+00	61.86%	676	OSS66325.1
27	Select seq gb KAF068822.1	Endoplasmic reticulum chaperone BiP [Candida albicans]	Candida albicans	772	772	94%	0.00E+00	61.89%	672	KAF068822.1
28	Select seq ref XP_749594.1	Hsp70 chaperone BiP/Kar2, putative [Aspergillus fumigatus A293]	Aspergillus fumigatus A293	771	771	94%	0.00E+00	62.30%	672	XP_749594.1
29	Select seq ref XP_719462.2	Hsp70 family ATPase [Candida albicans SC5314]	Candida albicans SC5314	767	767	93%	0.00E+00	62.07%	687	XP_719462.2
30	Select seq gb KGR13157.1	chaperone DnaK [Candida albicans P57072]	Candida albicans P57072	767	767	93%	0.00E+00	62.07%	687	KGR13157.1
31	Select seq gb KHG47150.1	chaperone DnaK [Candida albicans Ca6]	Candida albicans Ca6	766	766	93%	0.00E+00	62.07%	687	KHG47150.1
32	Select seq gb KGLJ5991.1	chaperone DnaK [Candida albicans 19F]	Candida albicans 19F	766	766	93%	0.00E+00	61.90%	687	KGLJ5991.1
33	Select seq ref XP_018224343.1	chaperone DnaK [Pneumocystis carinii] (B80)	Pneumocystis carinii (B80)	761	761	94%	0.00E+00	60.86%	655	XP_018224343.1
34	Select seq gb AAB58248.1	endoplasmic reticulum HSP70 homolog [Pneumocystis carinii]	Pneumocystis carinii	759	759	94%	0.00E+00	60.69%	655	AAB58248.1
35	Select seq ref XP_001538200.1	heat shock 70 kDa protein C precursor [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	744	744	95%	0.00E+00	59.49%	677	XP_001538200.1
36	Select seq emb CAA66308.1	heat shock protein 70 [Candida albicans]	Candida albicans	724	724	85%	0.00E+00	65.76%	613	CAA66308.1
37	Select seq gb KGR00935.1	hsp75-like protein [Candida albicans P57072]	Candida albicans P57072	724	724	85%	0.00E+00	65.76%	613	KGR00935.1
38	Select seq sp P87222.2	RecName: Full=Ribosome-associated molecular chaperone SSB1; AltName: Full=Heat shock protein SSB1; AltName: Full=Hsp70 chaperone Ssb [Candida albicans WO-1]	Candida albicans WO-1	723	723	85%	0.00E+00	65.76%	613	P87222.2
39	Select seq gb KAF069334.1	Hsp70 family protein [Candida albicans]	Candida albicans	723	723	63%	0.00E+00	84.77%	414	KAF069334.1
40	Select seq gb KGT63078.1	hsp75-like protein [Candida albicans 12C]	Candida albicans 12C	721	721	85%	0.00E+00	65.58%	613	KGT63078.1

41	Select seq gb   KHCS4832.1	hsp75-like protein [Candida albicans P75010]	721	721	85%	0.00E+00	613	KHCS4832.1
42	Select seq ref   XP_716208.1	Hsp70 family ATPase [Candida albicans SC5314]	721	721	85%	0.00E+00	613	XP_716208.1
43	Select seq gb   AC295783.1	heat shock protein 70 [Candida albicans]	715	715	85%	0.00E+00	583	AC295783.1
44	Select seq gb   XP_018227313.1	hsp75-like protein [Pneumocystis carinii B80]	704	704	91%	0.00E+00	612	XP_018227313.1
45	Select seq gb   XP_747200.1	Hsp70 chaperone [Hsa], putative [Aspergillus fumigatus A7293]	689	689	88%	0.00E+00	614	XP_747200.1
46	Select seq gb   KAF4260162.1	hypothetical protein CNMCM8714_001290 [Aspergillus fumigatus]	689	689	88%	0.00E+00	614	KAF4260162.1
47	Select seq gb   KAF4256863.1	hypothetical protein CNMCM8057_003921 [Aspergillus fumigatus]	688	688	88%	0.00E+00	859	KAF4256863.1
48	Select seq gb   KAG5291976.1	heat shock protein S881 [Histoplasma capsulatum G2178]	687	687	88%	0.00E+00	614	KAG5291976.1
49	Select seq ref   XP_001543760.1	heat shock 70 kDa protein [Histoplasma capsulatum Nam1]	684	684	88%	0.00E+00	613	XP_001543760.1
50	Select seq gb   EEH06561.1	heat shock protein S88 [Histoplasma capsulatum G186AR]	684	684	88%	0.00E+00	613	EEH06561.1
51	Select seq gb   EER40742.1	heat shock protein S88 [Histoplasma capsulatum H143]	682	682	88%	0.00E+00	613	EER40742.1
52	Select seq gb   EGC47363.1	conserved hypothetical protein [Histoplasma capsulatum H88]	644	644	88%	0.00E+00	599	EGC47363.1
53	Select seq ref   XP_755328.1	mitochondrial Hsp70 chaperone (hsc70), putative [Aspergillus fumigatus A7293]	578	578	92%	0.00E+00	685	XP_755328.1
54	Select seq gb   KEV79550.1	mitochondrial Hsp70 chaperone (hsc70), putative [Aspergillus fumigatus var. RP-2014]	578	578	92%	0.00E+00	684	KEV79550.1
55	Select seq gb   EDP54522.1	mitochondrial Hsp70 chaperone (hsc70), putative [Aspergillus fumigatus A1163]	577	577	92%	0.00E+00	661	EDP54522.1
56	Select seq ref   XP_001537067.1	heat shock protein SSC1, mitochondrial precursor [Histoplasma capsulatum Nam1]	570	570	92%	0.00E+00	676	XP_001537067.1
57	Select seq gb   EGC48453.1	conserved hypothetical protein [Histoplasma capsulatum H88]	570	570	92%	0.00E+00	675	EGC48453.1
58	Select seq gb   KAG5301798.1	heat shock protein SSC1 [Histoplasma capsulatum G2178]	570	570	92%	0.00E+00	675	KAG5301798.1
59	Select seq gb   EEH03103.1	heat shock protein SSC1 [Histoplasma capsulatum G186AR]	570	570	92%	0.00E+00	675	EEH03103.1
60	Select seq ref   XP_018225788.1	hsp7-like protein [Pneumocystis carinii B80]	561	561	96%	0.00E+00	657	XP_018225788.1
61	Select seq gb   RLP63394.1	hsp77-like protein [Candida albicans Cas29L]	551	551	95%	0.00E+00	648	RLP63394.1
62	Select seq gb   EEO47268.1	heat shock protein SSC1, mitochondrial precursor [Candida albicans WO-1]	550	550	95%	0.00E+00	648	EEO47268.1
63	Select seq gb   KGC089266.1	chaperone DnaK [Candida albicans P94015]	550	550	95%	0.00E+00	648	KGC089266.1
64	Select seq gb   KAG5301798.1	Hsp70 family ATPase [Candida albicans SC5314]	548	548	95%	0.00E+00	648	KAG5301798.1
65	Select seq gb   KGC095737.1	hsp77-like protein [Candida albicans P37005]	547	547	95%	0.00E+00	648	KGC095737.1
66	Select seq gb   KAF6063692.1	Hsp70 family protein [Candida albicans]	515	515	81%	1.00E-176	543	KAF6063692.1
67	Select seq gb   KAF6063693.1	Hsp70 family protein [Candida albicans]	515	515	81%	5.00E-176	608	KAF6063693.1
68	Select seq gb   QSS59214.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum]	498	498	43%	2.00E-173	309	QSS59214.1
69	Select seq gb   KGC083718.1	chaperone DnaK [Candida albicans P37005]	494	494	90%	3.00E-167	638	KGC083718.1
70	Select seq gb   RLP64687.1	chaperone DnaK [Candida albicans Cas29L]	494	494	90%	5.00E-167	648	RLP64687.1
71	Select seq gb   KGC082346.1	chaperone DnaK [Candida albicans P94015]	493	493	90%	9.00E-167	638	KGC082346.1
72	Select seq gb   KHC47008.1	chaperone DnaK [Candida albicans P37039]	493	493	90%	1.00E-166	638	KHC47008.1
73	Select seq gb   KGU20262.1	chaperone DnaK [Candida albicans P34048]	493	493	90%	1.00E-166	638	KGU20262.1
74	Select seq gb   KGR07696.1	chaperone DnaK [Candida albicans P37037]	493	493	90%	1.00E-166	638	KGR07696.1
75	Select seq gb   EEO47168.1	heat shock protein SSC1, mitochondrial precursor [Candida albicans WO-1]	491	491	90%	4.00E-166	638	EEO47168.1
76	Select seq gb   KHCS0703.1	chaperone DnaK [Candida albicans P76067]	491	491	90%	4.00E-166	638	KHCS0703.1
77	Select seq ref   XP_715228.1	Hsp70 family ATPase [Candida albicans SC5314]	491	491	90%	5.00E-166	638	XP_715228.1
78	Select seq ref   XP_001544351.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum Nam1]	440	486	41%	7.00E-151	311	XP_001544351.1
79	Select seq gb   AAB34280.1	70 kDa heat shock protein [Candida albicans]	426	426	37%	2.00E-146	244	AAB34280.1
80	Select seq gb   EER40331.1	heat shock protein SSC1 [Histoplasma capsulatum H143]	368	429	71%	2.00E-119	552	EER40331.1
81	Select seq gb   EER41540.1	dnaK-type molecular chaperone b1pA [Histoplasma capsulatum H143]	352	352	39%	4.00E-116	319	EER41540.1
82	Select seq gb   IAAD00456.1	heat shock protein 70b/S881 [Pneumocystis carinii]	323	323	32%	9.00E-107	207	IAAD00456.1
83	Select seq gb   QSS53508.1	hsp88-like protein [Histoplasma capsulatum H88]	267	267	93%	5.00E-79	717	QSS53508.1
84	Select seq gb   KAG5304943.1	hsp88-like protein [Histoplasma capsulatum]	267	267	93%	6.00E-79	717	KAG5304943.1
85	Select seq gb   KAF4259893.1	hypothetical protein CNMCM8714_001456 [Aspergillus fumigatus]	267	267	93%	7.00E-79	713	KAF4259893.1
86	Select seq ref   XP_752631.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus A7293]	267	267	93%	7.00E-79	714	XP_752631.1
87	Select seq gb   EOP56097.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus A1163]	266	266	93%	2.00E-78	714	EOP56097.1
88	Select seq ref   XP_001543737.1	heat shock protein Hsp88 [Histoplasma capsulatum Nam1]	265	265	93%	2.00E-78	717	XP_001543737.1
89	Select seq gb   EGC47363.1	hypothetical protein Hsp88 [Histoplasma capsulatum H88]	264	264	75%	3.00E-78	664	EGC47363.1
90	Select seq gb   EEH06535.1	hsp88-like protein [Histoplasma capsulatum G186AR]	263	263	75%	4.00E-78	664	EEH06535.1
91	Select seq gb   QSS38525.1	hsp88-like protein [Histoplasma capsulatum]	263	263	75%	4.00E-78	651	QSS38525.1
92	Select seq ref   XP_018227484.1	hypothetical protein TSS2_00013 [Pneumocystis carinii B80]	263	263	94%	3.00E-77	724	XP_018227484.1
93	Select seq gb   AGT21630.1	heat shock protein 70 [Aspergillus fumigatus]	236	236	20%	4.00E-74	130	AGT21630.1
94	Select seq gb   KGU36186.1	heat shock protein 110kDa [Candida albicans P75063]	241	241	58%	3.00E-69	702	KGU36186.1
95	Select seq gb   EEO42580.1	heat shock protein Hsp88 [Candida albicans WO-1]	240	240	58%	3.00E-69	702	EEO42580.1
96	Select seq ref   XP_718458.1	adenyl-nucleotide exchange factor [Candida albicans SC5314]	240	240	58%	4.00E-69	701	XP_718458.1
97	Select seq gb   RLP61900.1	hypothetical protein L150_00590 [Candida albicans Cas29L]	240	240	58%	5.00E-69	702	RLP61900.1
98	Select seq gb   KAF6063130.1	hypothetical protein F064_006136 [Candida albicans]	234	234	58%	4.00E-67	670	KAF6063130.1
99	Select seq ref   XP_749367.1	Hsp70 chaperone (BIP), putative [Aspergillus fumigatus A7293]	228	228	68%	8.00E-66	570	XP_749367.1
100	Select seq gb   EOP53895.1	Hsp70 chaperone (BIP), putative [Aspergillus fumigatus A1163]	227	227	68%	2.00E-65	570	EOP53895.1

Homology of hsp75-like protein to proteins from other pathogenic fungi

Hit No.	Sequence Identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq ref [XP_018227313.1]	hsp75-like protein [Pneumocystis carinii B80]	Pneumocystis carinii B80	880	880	99%	0.00E+00	69.66%	612	XP_018227313.1
2	Select seq sp [P87222.2]	Full-Heat shock protein SSB1, AltName: Full-Hsp70 chaperone Ssb [Candida albicans WO-1]	Candida albicans WO-1	852	852	99%	0.00E+00	68.46%	613	P87222.2
3	Select seq gb [KGR00935.1]	hsp75-like protein [Candida albicans P57072]	Candida albicans P57072	852	852	99%	0.00E+00	68.46%	613	KGR00935.1
4	Select seq ref [XP_716208.1]	Hsp70 family ATPase [Candida albicans SC5314]	Candida albicans SC5314	852	852	99%	0.00E+00	68.46%	613	XP_716208.1
5	Select seq gb [KGT63078.1]	hsp75-like protein [Candida albicans 12C]	Candida albicans 12C	850	850	99%	0.00E+00	68.30%	613	KGT63078.1
6	Select seq gb [KHC54832.1]	hsp75-like protein [Candida albicans P75010]	Candida albicans P75010	850	850	99%	0.00E+00	68.30%	613	KHC54832.1
7	Select seq emb [CA465308.1]	heat shock protein 70 [Candida albicans]	Candida albicans	849	849	99%	0.00E+00	68.14%	613	CA465308.1
8	Select seq ref [XP_747200.1]	Heat shock protein 70 [Aspergillus fumigatus A293]	Aspergillus fumigatus A293	816	816	99%	0.00E+00	66.01%	614	XP_747200.1
9	Select seq gb [KAF4260162.1]	hypothetical protein CNMCM87.14_001290 [Aspergillus fumigatus]	Aspergillus fumigatus	816	816	99%	0.00E+00	66.01%	614	KAF4260162.1
10	Select seq gb [KAF4256863.1]	hypothetical protein CNMCM8057_003921 [Aspergillus fumigatus]	Aspergillus fumigatus	815	815	99%	0.00E+00	66.01%	614	KAF4256863.1
11	Select seq gb [KAC295783.1]	heat shock protein 70 [Candida albicans]	Candida albicans	815	815	94%	0.00E+00	68.84%	583	KAC295783.1
12	Select seq gb [KAG5291976.1]	heat shock protein SSB1 [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	801	801	99%	0.00E+00	65.20%	614	KAG5291976.1
13	Select seq gb [EEH06561.1]	heat shock protein SSB1 [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	797	797	99%	0.00E+00	65.20%	613	EEH06561.1
14	Select seq ref [XP_001543760.1]	heat shock 70 kDa protein [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	796	796	99%	0.00E+00	65.20%	613	XP_001543760.1
15	Select seq gb [EER40742.1]	heat shock protein SSB [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	795	795	99%	0.00E+00	65.20%	613	EER40742.1
16	Select seq gb [AAD09565.1]	heat shock protein 70 [Pneumocystis carinii]	Pneumocystis carinii	782	782	97%	0.00E+00	62.81%	647	AAD09565.1
17	Select seq ref [XP_018224954.1]	hsp72-like protein [Pneumocystis carinii B80]	Pneumocystis carinii B80	780	780	96%	0.00E+00	62.96%	645	XP_018224954.1
18	Select seq gb [AAD00455.1]	heat shock protein 70 [Pneumocystis carinii]	Pneumocystis carinii	779	779	96%	0.00E+00	62.79%	645	AAD00455.1
19	Select seq gb [EGC47363.1]	conserved hypothetical protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	764	764	99%	0.00E+00	63.73%	599	EGC47363.1
20	Select seq gb [AAS8470.1]	heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	762	762	96%	0.00E+00	62.48%	608	AAS8470.1
21	Select seq ref [XP_750490.1]	molecular chaperone Hsp70 [Aspergillus fumigatus A293]	Aspergillus fumigatus A293	761	761	96%	0.00E+00	62.84%	638	XP_750490.1
22	Select seq gb [EEH03794.1]	hsp70-like protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	758	758	96%	0.00E+00	61.99%	653	EEH03794.1
23	Select seq gb [Q554271.1]	hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	758	758	96%	0.00E+00	61.82%	653	Q554271.1
24	Select seq gb [KGU36014.1]	hsp72-like protein [Candida albicans P75063]	Candida albicans P75063	756	756	90%	0.00E+00	65.71%	655	KGU36014.1
25	Select seq gb [AC295776.1]	heat shock protein 70 [Aspergillus fumigatus]	Aspergillus fumigatus	756	756	90%	0.00E+00	65.34%	580	AC295776.1
26	Select seq gb [KGT72617.1]	hsp72-like protein [Candida albicans 12C]	Candida albicans 12C	756	756	90%	0.00E+00	65.71%	645	KGT72617.1
27	Select seq gb [EEQ42742.1]	heat shock protein S5A2 [Candida albicans WO-1]	Candida albicans WO-1	756	756	90%	0.00E+00	65.71%	645	EEQ42742.1
28	Select seq gb [KQG98820.1]	hsp72-like protein [Candida albicans P37005]	Candida albicans P37005	756	756	90%	0.00E+00	65.71%	645	KQG98820.1
29	Select seq ref [XP_713669.2]	Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans SC5314	756	756	90%	0.00E+00	65.71%	645	XP_713669.2
30	Select seq gb [KAF609522.1]	Heat shock protein S5A4 [Candida albicans]	Candida albicans	755	755	95%	0.00E+00	64.68%	639	KAF609522.1
31	Select seq gb [KAF609523.1]	Heat shock protein S5A4 [Candida albicans]	Candida albicans	755	755	95%	0.00E+00	64.68%	642	KAF609523.1
32	Select seq gb [EEQ41915.1]	heat shock protein S5A4 [Candida albicans WO-1]	Candida albicans WO-1	755	755	95%	0.00E+00	64.68%	643	EEQ41915.1
33	Select seq ref [XP_722186.1]	Hsp70 family chaperone [Candida albicans SC5314]	Candida albicans SC5314	755	755	95%	0.00E+00	64.68%	656	XP_722186.1
34	Select seq gb [KGU30551.1]	hsp72-like protein [Candida albicans P34048]	Candida albicans P34048	755	755	95%	0.00E+00	64.68%	647	KGU30551.1
35	Select seq gb [AET14827.1]	heat shock protein 70 [Candida albicans]	Candida albicans	752	752	95%	0.00E+00	64.51%	656	AET14827.1
36	Select seq sp [Q00043.1]	Heat shock protein 70 kDa protein [Histoplasma capsulatum]	Histoplasma capsulatum	725	725	96%	0.00E+00	60.54%	705	Q00043.1
37	Select seq gb [EGC48122.1]	hsp70-like protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	718	718	96%	0.00E+00	59.63%	639	EGC48122.1
38	Select seq ref [XP_018224343.1]	chaperone Dnak [Pneumocystis carinii B80]	Pneumocystis carinii B80	699	699	99%	0.00E+00	57.63%	655	XP_018224343.1
39	Select seq gb [AAS8248.1]	endoplasmic reticulum HSP70 homolog [Pneumocystis carinii]	Pneumocystis carinii	697	697	95%	0.00E+00	57.46%	655	AAS8248.1
40	Select seq ref [XP_749594.1]	Hsp70 chaperone Bip/Kar2, putative [Aspergillus fumigatus A293]	Aspergillus fumigatus A293	674	674	96%	0.00E+00	56.08%	672	XP_749594.1

41	Select seq gb Q56G325.1	dnk-type molecular chaperone b1pA [Histoplasma capsulatum]	671	671	94%	0.00E+00	56.43%	676	Q56G325.1
42	Select seq gb EGC49409.1	Dnak-type molecular chaperone b1pA [Histoplasma capsulatum H88]	670	670	94%	0.00E+00	56.43%	676	EGC49409.1
43	Select seq gb EEH0582.1	dnk-type molecular chaperone b1pA [Histoplasma capsulatum G186AR]	670	670	94%	0.00E+00	56.43%	676	EEH0582.1
44	Select seq gb KAG5294347.1	Hsp70 family ATPase [Candida albicans P57072]	662	662	97%	0.00E+00	54.24%	687	KAG5294347.1
45	Select seq ref XP_719462.2	chaperone Dnak [Candida albicans P57072]	662	662	97%	0.00E+00	54.24%	687	XP_719462.2
46	Select seq gb KGR13197.1	Endoplasmic reticulum chaperone BIP [Candida albicans]	662	662	97%	0.00E+00	54.24%	687	KGR13197.1
47	Select seq gb KAF606882.1	chaperone Dnak [Candida albicans 19F]	661	661	97%	0.00E+00	54.08%	687	KAF606882.1
48	Select seq gb KGU15094.1	chaperone Dnak [Candida albicans C6f]	660	660	97%	0.00E+00	54.08%	687	KGU15094.1
49	Select seq gb KHC47150.1	heat shock 70 kDa protein C precursor [Histoplasma capsulatum MAm1]	640	640	94%	0.00E+00	54.62%	677	KHC47150.1
50	Select seq ref XP_001538200.1	Hsp70-like protein [Histoplasma capsulatum H143]	621	621	82%	0.00E+00	59.49%	569	XP_001538200.1
51	Select seq gb EER3727.1	Hsp70 family protein [Candida albicans]	618	618	65%	0.00E+00	73.02%	414	EER3727.1
52	Select seq gb KAF606334.1	Hsp70-like protein [Pneumocystis carinii B80]	518	518	99%	1.00E-176	44.86%	657	KAF606334.1
53	Select seq ref XP_01825788.1	mitochondrial Hsp70 chaperone [Sac20, putative [Aspergillus fumigatus A1163]]	510	510	95%	1.00E-173	46.66%	661	XP_01825788.1
54	Select seq gb EOP54522.1	mitochondrial Hsp70 chaperone [Sac70, putative [Aspergillus fumigatus A1163]]	511	511	95%	2.00E-173	46.66%	661	EOP54522.1
55	Select seq ref XP_755328.1	conserved hypothetical protein [Histoplasma capsulatum H88]	508	508	97%	1.00E-172	45.69%	685	XP_755328.1
56	Select seq gb KEV79550.1	heat shock protein S5C1, mitochondrial precursor [Histoplasma capsulatum MAm1]	508	508	97%	2.00E-172	45.69%	675	KEV79550.1
57	Select seq gb EGC48453.1	heat shock protein S5C1 [Histoplasma capsulatum MAm1]	508	508	97%	2.00E-172	45.69%	675	EGC48453.1
58	Select seq ref XP_001537067.1	heat shock protein S5C1 [Histoplasma capsulatum G217B]	508	508	97%	2.00E-172	45.69%	675	XP_001537067.1
59	Select seq gb KAG5301798.1	heat shock protein S5C1 [Histoplasma capsulatum G217B]	508	508	97%	2.00E-172	45.69%	675	KAG5301798.1
60	Select seq gb EFG03103.1	Hsp70 family protein [Candida albicans G186AR]	508	508	97%	4.00E-167	47.58%	648	EFG03103.1
61	Select seq gb KLF63394.1	Hsp70-like protein [Candida albicans Cas29L]	493	493	86%	5.00E-167	47.58%	648	KLF63394.1
62	Select seq gb KQD89268.1	Hsp77-like protein [Candida albicans P94015]	493	493	86%	5.00E-167	47.58%	648	KQD89268.1
63	Select seq ref XP_713153.1	Hsp70 family ATPase [Candida albicans P37005]	493	493	86%	5.00E-167	47.58%	648	XP_713153.1
64	Select seq gb EEQ47268.1	chaperone Dnak [Candida albicans P94015]	493	493	86%	5.00E-167	47.58%	648	EEQ47268.1
65	Select seq gb KAG095737.1	heat shock protein S5C1, mitochondrial precursor [Candida albicans WO-1]	491	491	86%	2.00E-166	47.40%	648	KAG095737.1
66	Select seq gb KAF6063693.1	Hsp77-like protein [Candida albicans P37005]	479	479	94%	5.00E-162	44.71%	608	KAF6063693.1
67	Select seq gb KAF6063692.1	Hsp70 family protein [Candida albicans]	476	476	85%	1.00E-161	47.34%	543	KAF6063692.1
68	Select seq gb KLF66487.1	chaperone Dnak [Candida albicans Cas29L]	452	452	89%	2.00E-151	42.88%	638	KLF66487.1
69	Select seq gb KQD837.8.1	chaperone Dnak [Candida albicans P37005]	452	452	89%	3.00E-151	42.88%	638	KQD837.8.1
70	Select seq gb KQD82346.1	chaperone Dnak [Candida albicans P94015]	452	452	89%	4.00E-151	42.88%	638	KQD82346.1
71	Select seq gb KGR02062.1	chaperone Dnak [Candida albicans P34048]	451	451	89%	8.00E-151	42.53%	638	KGR02062.1
72	Select seq gb KGR07696.1	chaperone Dnak [Candida albicans P37037]	451	451	89%	1.00E-150	42.70%	638	KGR07696.1
73	Select seq gb KHC47008.1	chaperone Dnak [Candida albicans P37039]	451	451	89%	1.00E-150	42.70%	638	KHC47008.1
74	Select seq gb KHC30703.1	chaperone Dnak [Candida albicans P70067]	450	450	89%	2.00E-150	42.70%	638	KHC30703.1
75	Select seq gb EEQ47168.1	heat shock protein S5C1, mitochondrial precursor [Candida albicans WO-1]	449	449	89%	3.00E-150	42.53%	638	EEQ47168.1
76	Select seq ref XP_715228.1	Hsp70 family ATPase [Candida albicans P37005]	449	449	89%	3.00E-150	42.53%	638	XP_715228.1
77	Select seq gb Q5559214.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum]	398	398	42%	7.00E-135	72.87%	309	Q5559214.1
78	Select seq gb AAB34280.1	70 kDa heat shock protein [Candida albicans]	376	376	30%	3.00E-127	75.93%	244	AAB34280.1
79	Select seq gb AAD00456.1	heat shock protein 70B/58B1 [Pneumocystis carinii]	357	357	38%	4.00E-120	82.04%	207	AAD00456.1
80	Select seq ref XP_0015404351.1	heat shock 70 kDa protein 7 [Histoplasma capsulatum MAm1]	360	360	38%	4.00E-120	82.04%	207	XP_0015404351.1
81	Select seq gb EER41540.1	dnk-type molecular chaperone b1pA [Histoplasma capsulatum H143]	331	331	41%	1.00E-108	63.53%	319	EER41540.1
82	Select seq gb EER40331.1	heat shock protein S5C1 [Histoplasma capsulatum H143]	318	318	76%	3.00E-100	41.86%	552	EER40331.1
83	Select seq gb KAF4259893.1	hypothetical protein CNMCM8714.001456 [Aspergillus fumigatus]	270	270	61%	1.00E-80	38.16%	713	KAF4259893.1
84	Select seq ref XP_752631.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus A1293]	270	270	61%	1.00E-80	38.16%	714	XP_752631.1
85	Select seq gb EOP56497.1	Hsp70 chaperone Hsp88 [Aspergillus fumigatus A1163]	270	270	61%	3.00E-80	37.89%	714	EOP56497.1
86	Select seq gb Q5538525.1	Hsp88-like protein [Histoplasma capsulatum]	254	254	75%	3.00E-75	33.40%	651	Q5538525.1
87	Select seq ref XP_001543737.1	Hsp88-like protein [Histoplasma capsulatum MAm1]	255	255	75%	7.00E-75	33.40%	717	XP_001543737.1
88	Select seq gb EGC7336.1	Hsp88-like protein [Histoplasma capsulatum H88]	254	254	75%	8.00E-75	33.40%	664	EGC7336.1
89	Select seq gb EEH06535.1	Hsp88-like protein [Histoplasma capsulatum G186AR]	254	254	75%	1.00E-74	33.40%	664	EEH06535.1
90	Select seq gb Q553508.1	Hsp88-like protein [Histoplasma capsulatum H88]	254	254	75%	1.00E-74	33.40%	717	Q553508.1
91	Select seq gb KAG5304943.1	Hsp88-like protein [Histoplasma capsulatum]	254	254	75%	2.00E-74	33.40%	717	KAG5304943.1
92	Select seq ref XP_018227484.1	hypothetical protein 1552.00013 [Pneumocystis carinii B80]	250	250	69%	6.00E-73	33.49%	724	XP_018227484.1
93	Select seq gb EEQ42580.1	heat shock protein Hsp88 [Candida albicans WO-1]	235	235	60%	2.00E-67	34.30%	702	EEQ42580.1
94	Select seq gb KGU136186.1	heat shock protein 110K0a [Candida albicans P75063]	234	234	60%	2.00E-67	34.30%	702	KGU136186.1
95	Select seq ref XP_718458.1	adenyl-nucleotide exchange factor [Candida albicans SC5314]	234	234	60%	3.00E-67	34.30%	701	XP_718458.1
96	Select seq gb RII161900.1	hypothetical protein L150_00590 [Candida albicans Cas29L]	234	234	60%	6.00E-67	34.30%	702	RII161900.1
97	Select seq gb KAF6063130.1	hypothetical protein FOB64_006136 [Candida albicans]	222	222	60%	4.00E-63	33.95%	670	KAF6063130.1
98	Select seq ref XP_018227150.1	hypothetical protein 1552_00832 [Pneumocystis carinii B80]	202	202	68%	2.00E-56	29.37%	561	XP_018227150.1
99	Select seq ref XP_749867.1	Hsp70 chaperone (BP), putative [Aspergillus fumigatus A1293]	197	197	61%	1.00E-54	31.85%	570	XP_749867.1
100	Select seq gb EOP53895.1	Hsp70 chaperone (BP), putative [Aspergillus fumigatus A1163]	197	197	61%	2.00E-54	31.85%	570	EOP53895.1



Homology of hypothetical protein **CNAG\_05236** to proteins from other pathogenic fungi

Job Title AFR94491\_hypothetical protein CNAG\_05236 [Cryptococcus  
 RID 7EAKBKZ016 Search expires on 04-16 01:46 am  
 Program BLASTP  
 Database nr  
 Query ID AFR94491.2  
 Description hypothetical protein **CNAG\_05236** [Cryptococcus neoformans var. grubii H99] ...  
 Molecule type amino acid  
 Query Length 462

Hit No.	Sequence Identifier	Protein Homology	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb [KAG5297447.1]	DUF2408 superfamily domain-containing protein [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	308	308	98%	8.00E-100	37.20%	442	KAG5297447.1
2	Select seq gb [KAG5303809.1]	DUF2408 superfamily domain-containing protein [Histoplasma capsulatum]	Histoplasma capsulatum	306	306	98%	3.00E-99	37.42%	442	KAG5303809.1
3	Select seq gb [ERR43665.1]	conserved hypothetical protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	304	304	98%	2.00E-98	36.98%	442	ERR43665.1
4	Select seq gb [QSS63630.1]	DUF2408 superfamily domain-containing protein [Histoplasma capsulatum]	Histoplasma capsulatum	301	301	92%	2.00E-97	38.28%	428	QSS63630.1
5	Select seq gb [KAF4254288.1]	hypothetical protein CNMC8057_005288 [Aspergillus fumigatus]	Aspergillus fumigatus	295	295	97%	9.00E-95	35.54%	438	KAF4254288.1
6	Select seq ref [XP_001540065.1]	conserved hypothetical protein [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	275	275	86%	9.00E-88	37.72%	393	XP_001540065.1
7	Select seq gb [KHC55948.1]	hypothetical protein MEW_00952 [Candida albicans P60002]	Candida albicans P60002	221	221	96%	2.00E-64	30.57%	614	KHC55948.1
8	Select seq gb [KGR14209.1]	hypothetical protein MG5_00944 [Candida albicans P57072]	Candida albicans P57072	220	220	96%	3.00E-64	30.45%	616	KGR14209.1
9	Select seq gb [KGG09642.1]	hypothetical protein MEC_00956 [Candida albicans P94015]	Candida albicans P94015	219	219	99%	7.00E-64	30.36%	617	KGG09642.1
10	Select seq gb [KGR02715.1]	hypothetical protein MG1_00957 [Candida albicans GC75]	Candida albicans GC75	219	219	96%	7.00E-64	30.37%	614	KGR02715.1
11	Select seq gb [KHG67596.1]	hypothetical protein MGE_00963 [Candida albicans P75010]	Candida albicans P75010	219	219	96%	7.00E-64	30.37%	615	KHG67596.1
12	Select seq ref [XP_723556.1]	hypothetical protein CAALFM_C109850CA [Candida albicans SC5314]	Candida albicans SC5314	219	219	96%	7.00E-64	30.37%	616	XP_723556.1
13	Select seq gb [KGU36771.1]	hypothetical protein MGK_00942 [Candida albicans P57055]	Candida albicans P57055	219	219	96%	7.00E-64	30.37%	616	KGU36771.1
14	Select seq gb [KAF6062710.1]	hypothetical protein FOB64_005768 [Candida albicans]	Candida albicans	219	219	96%	8.00E-64	30.37%	618	KAF6062710.1
15	Select seq gb [KHC40990.1]	hypothetical protein MGO_00942 [Candida albicans P76055]	Candida albicans P76055	218	218	96%	1.00E-63	30.26%	616	KHC40990.1
16	Select seq gb [KHC41656.1]	hypothetical protein MGO_00944 [Candida albicans P76067]	Candida albicans P76067	218	218	96%	1.00E-63	30.26%	616	KHC41656.1
17	Select seq gb [KGU32173.1]	hypothetical protein MG7_00946 [Candida albicans P34048]	Candida albicans P34048	218	218	96%	1.00E-63	30.37%	616	KGU32173.1
18	Select seq ref [XP_750431.1]	conserved hypothetical protein [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	195	267	97%	4.00E-57	33.61%	387	XP_750431.1
19	Select seq gb [KEY82363.1]	hypothetical protein BA78_1132 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	195	266	97%	5.00E-57	33.61%	387	KEY82363.1
20	Select seq gb [EEH08875.1]	aconitate hydratase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	168	292	98%	4.00E-44	35.25%	1174	EEH08875.1
21	Select seq gb [EEQ42242.1]	conserved hypothetical protein [Candida albicans WO-1]	Candida albicans WO-1	145	145	78%	8.00E-38	27.42%	452	EEQ42242.1

Homology of hypothetical protein **CNAG\_06113** to proteins from other pathogenic fungi

Job Title AFR98337\_hypothetical protein CNAG\_06113 [Cryptococcus  
 RID 7EAKY229016 Search expires on 04-16 01:46 am  
 Program BLASTP  
 Database nr  
 Query ID AFR98337.1  
 Description hypothetical protein **CNAG\_06113** [Cryptococcus neoformans var. grubii H99]  
 Molecule type amino acid  
 Query Length 345

Hit No.	Sequence Identifier	Protein Homology	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb [KAG607240.1]	Guanine nucleotide exchange factor in Golgi transport (t terminal family protein [Candida albicans])	Candida albicans	58.9	58.9	50%	1.00E-08	29.89%	1983	KAG607240.1
2	Select seq gb [KAF4254286.1]	hypothetical protein CNMC8074_005281 [Aspergillus fumigatus]	Aspergillus fumigatus	53.9	53.9	59%	3.00E-07	31.01%	304	KAF4254286.1
3	Select seq gb [KGR10135.1]	hypothetical protein MG5_00923 [Candida albicans P5702]	Candida albicans P5702	50.1	50.1	43%	5.00E-06	32.24%	267	KGR10135.1
4	Select seq ref [XP_754527.1]	telomere and ribosome-associated protein Srm1, putative [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	49.7	49.7	58%	8.00E-06	30.51%	331	XP_754527.1
5	Select seq ref [XP_019330968.1]	hypothetical protein CAALFM_C304810CA [Candida albicans SC5314]	Candida albicans SC5314	49.3	49.3	43%	9.00E-06	32.24%	267	XP_019330968.1
6	Select seq gb [KGO88094.1]	hypothetical protein MLO_D2899 [Candida albicans P94015]	Candida albicans P94015	48.9	48.9	43%	9.00E-06	32.24%	267	KGO88094.1
7	Select seq gb [KAG5295291.1]	elictor protein [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	47.0	47.0	42%	6.00E-05	32.91%	309	KAG5295291.1
8	Select seq gb [EGC45501.1]	elictor protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	47.0	47.0	42%	6.00E-05	32.91%	313	EGC45501.1
9	Select seq gb [EHR42437.1]	elictor protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	47.0	47.0	42%	6.00E-05	32.91%	317	EHR42437.1
10	Select seq gb [EHR08464.1]	elictor protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	46.6	46.6	42%	7.00E-05	32.91%	309	EHR08464.1
11	Select seq gb [DSS50274.1]	elictor protein [Histoplasma capsulatum]	Histoplasma capsulatum	46.2	46.2	42%	1.00E-04	32.91%	309	DSS50274.1
12	Select seq ref [XP_001544870.1]	predicted protein [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	45.4	45.4	42%	2.00E-04	32.91%	350	XP_001544870.1

Homology of hypothetical protein CNAG\_06946 to proteins from other pathogenic fungi

Job Title AFR94883: hypothetical protein CNAG\_06946 [Cryptococcus  
 RID 7EAKWPF8013 Search expires on 04-16 01:46 am  
 Program BLASTP  
 Database nr  
 Query ID AFR94883.2  
 Description hypothetical protein CNAG\_06946 [Cryptococcus neoformans var. grubii H99] ...  
 Molecule type amino acid  
 Query Length 348

Hit No.	Sequence identifier	Protein Homologs	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq ref XP_018226844.1	hypothetical protein T552_01063 [Pneumocystis carinii B80]	Pneumocystis carinii B80	259	299	93%	1,00E-99	47.71%	322	XP_018226844.1
2	Select seq ref XP_752265.2	UPFD160 domain protein MYG1, putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	273	273	95%	8,00E-89	43.56%	364	XP_752265.2
3	Select seq gb KEY81792.1	hypothetical protein UPFD160 MYG1 [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	271	271	95%	5,00E-88	43.68%	364	KEY81792.1
4	Select seq gb Q5554376.1	MYG1 protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	271	271	99%	7,00E-88	43.38%	381	Q5554376.1
5	Select seq gb KAG5292787.1	MYG1 protein [Histoplasma capsulatum G2178]	Histoplasma capsulatum G2178	271	271	99%	1,00E-87	43.83%	377	KAG5292787.1
6	Select seq gb KAG5293520.1	MYG1 protein [Histoplasma capsulatum]	Histoplasma capsulatum	268	268	99%	1,00E-86	43.23%	380	KAG5293520.1
7	Select seq gb EGC48776.1	MYG1 protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	265	265	94%	1,00E-85	43.87%	372	EGC48776.1
8	Select seq gb KGR04195.1	hypothetical protein MG1_00175 [Candida albicans GC75]	Candida albicans GC75	265	265	97%	1,00E-85	42.37%	354	KGR04195.1
9	Select seq gb KGU36379.1	hypothetical protein MG1_00177 [Candida albicans P75063]	Candida albicans P75063	264	264	97%	2,00E-85	42.37%	354	KGU36379.1
10	Select seq gb KGO99315.1	hypothetical protein MEO_00176 [Candida albicans P94015]	Candida albicans P94015	264	264	97%	2,00E-85	42.37%	354	KGO99315.1
11	Select seq gb EEQ2963.1	conserved hypothetical protein [Candida albicans WO-1]	Candida albicans WO-1	263	263	94%	2,00E-85	43.88%	334	EEQ2963.1
12	Select seq gb RLP61499.1	hypothetical protein L150_00180 [Candida albicans Ca529L]	Candida albicans Ca529L	263	263	97%	4,00E-85	42.37%	354	RLP61499.1
13	Select seq gb KHC68367.1	hypothetical protein MGE_00183 [Candida albicans P75010]	Candida albicans P75010	263	263	97%	6,00E-85	42.82%	354	KHC68367.1
14	Select seq gb KGT72381.1	hypothetical protein MEK_00181 [Candida albicans 12C]	Candida albicans 12C	263	263	94%	6,00E-85	43.88%	354	KGT72381.1
15	Select seq gb EEH06020.1	MYG1 protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	263	263	94%	7,00E-85	43.72%	371	EEH06020.1
16	Select seq gb KGR1229.1	hypothetical protein MG3_00227 [Candida albicans P78048]	Candida albicans P78048	262	262	97%	1,00E-84	42.09%	354	KGR1229.1
17	Select seq gb KGU18444.1	hypothetical protein MEM_00186 [Candida albicans L26]	Candida albicans L26	262	262	97%	1,00E-84	42.09%	354	KGU18444.1
18	Select seq gb KHC82974.1	hypothetical protein W5Q_00181 [Candida albicans SC5314]	Candida albicans SC5314	261	261	97%	2,00E-84	42.09%	354	KHC82974.1
19	Select seq gb KGU33575.1	hypothetical protein MG7_00176 [Candida albicans P34048]	Candida albicans P34048	261	261	97%	2,00E-84	42.82%	354	KGU33575.1
20	Select seq gb KHC89903.1	hypothetical protein I503_00183 [Candida albicans SC5314]	Candida albicans SC5314	261	261	97%	3,00E-84	42.09%	354	KHC89903.1
21	Select seq gb KGU19552.1	hypothetical protein MEY_00182 [Candida albicans 19F]	Candida albicans 19F	260	260	97%	5,00E-84	42.09%	354	KGU19552.1
22	Select seq gb KGR23254.1	hypothetical protein MG9_00184 [Candida albicans P37037]	Candida albicans P37037	260	260	97%	6,00E-84	42.09%	354	KGR23254.1
23	Select seq ref XP_712957.2	hypothetical protein CAALFM_C101910WA [Candida albicans SC5314]	Candida albicans SC5314	258	258	94%	5,00E-83	43.58%	354	XP_712957.2
24	Select seq gb EER37668.1	MYG1 protein [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	221	221	83%	2,00E-68	42.55%	371	EER37668.1
25	Select seq gb KAF6070304.1	hypothetical protein FOB64_002380 [Candida albicans]	Candida albicans	216	216	80%	7,00E-68	42.96%	287	KAF6070304.1
26	Select seq gb KAF6070303.1	hypothetical protein FOB64_002380 [Candida albicans]	Candida albicans	182	182	64%	3,00E-55	44.16%	230	KAF6070303.1

Homology of ketol-acid reductoisomerase to proteins from other pathogenic fungi

Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb  KAF4269555.1	hypothetical protein CNMCM8714_008189 [Aspergillus fumigatus]	Aspergillus fumigatus	580	580	97%	0.00E+00	70.93%	508	KAF4269555.1
2	Select seq ref  XP_754177.1	Ketol-acid reductoisomerase [Aspergillus fumigatus AF293]	Aspergillus fumigatus	579	579	97%	0.00E+00	70.93%	508	XP_754177.1
3	Select seq gb  KEY81936.1	ketol acid reductoisomerase [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	579	579	97%	0.00E+00	70.93%	508	KEY81936.1
4	Select seq gb  KMK54237.1	Ketol-acid reductoisomerase [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	578	578	97%	0.00E+00	70.93%	396	KMK54237.1
5	Select seq gb  EER37855.1	ketol-acid reductoisomerase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	566	566	87%	0.00E+00	76.14%	410	EER37855.1
6	Select seq gb  KAG528727.1	ketol-acid reductoisomerase [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	565	565	87%	0.00E+00	76.14%	410	KAG528727.1
7	Select seq gb  EEH03260.1	ketol-acid reductoisomerase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	563	563	87%	0.00E+00	76.14%	410	EEH03260.1
8	Select seq ref  XP_001536226.1	ketol-acid reductoisomerase, mitochondrial precursor [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	554	554	90%	0.00E+00	70.28%	433	XP_001536226.1
9	Select seq gb  Q5561043.1	ketol-acid reductoisomerase [Histoplasma capsulatum]	Histoplasma capsulatum	552	552	87%	0.00E+00	71.47%	433	Q5561043.1
10	Select seq gb  KGU25635.1	ketol-acid reductoisomerase, mitochondrial [Candida albicans P57055]	Candida albicans P57055	548	548	98%	0.00E+00	67.66%	400	KGU25635.1
11	Select seq gb  KGO89779.1	ketol-acid reductoisomerase, mitochondrial [Candida albicans GC75]	Candida albicans GC75	548	548	98%	0.00E+00	67.66%	400	KGO89779.1
12	Select seq gb  KGO84082.1	ketol-acid reductoisomerase, mitochondrial [Candida albicans P94015]	Candida albicans P94015	548	548	98%	0.00E+00	67.66%	400	KGO84082.1
13	Select seq gb  KHC32009.1	ketol-acid reductoisomerase, mitochondrial [Candida albicans P76055]	Candida albicans P76055	546	546	98%	0.00E+00	67.66%	400	KHC32009.1
14	Select seq ref  XP_714297.2	ketol-acid reductoisomerase [Candida albicans SC5314]	Candida albicans SC5314	546	546	98%	0.00E+00	67.66%	400	XP_714297.2

Homology of mannose-1-phosphate guanylyltransferase to proteins from other pathogenic fungi

Job Title AFR98009:mannose-1-phosphate guanylyltransferase...  
 RID 7EAMC24E013 Search expires on 04-16 03:46 am  
 Program BLASTP  
 Database nr  
 Query ID AFR98009.2  
**Description** mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99] ...  
 Molecule type amino acid  
 Query Length 364

Hit No.	Sequence Identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb KAF4277012.1	hypothetical protein	Aspergillus fumigatus	544	544	100%	0.00E+00	71.51%	364	KAF4277012.1
2	Select seq sp QJ43E8.1	ReName: Full=mannose-1-phosphate guanylyltransferase; AltName: Full=GDP-mannose pyrophosphorylase; AITName: Full=GTP-mannose-1-phosphate guanylyltransferase (Aspergillus fumigatus AF293)	Aspergillus fumigatus AF293	544	544	100%	0.00E+00	71.51%	364	QJ43E8.1
3	Select seq gb EDP90516.1	mannose-1-phosphate guanylyltransferase [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	541	541	99%	0.00E+00	71.15%	373	EDP90516.1
4	Select seq ref XP_001544505.1	mannose-1-phosphate guanylyltransferase [Histoplasma capsulatum Nam1]	Histoplasma capsulatum Nam1	541	541	100%	0.00E+00	71.78%	364	XP_001544505.1
5	Select seq gb KMK54868.1	Mannose-1-phosphate guanylyltransferase [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	539	539	95%	0.00E+00	71.27%	585	KMK54868.1
6	Select seq gb EGC43154.1	mannose-1-phosphate guanylyltransferase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	538	538	98%	0.00E+00	72.22%	374	EGC43154.1
7	Select seq gb EEH08109.1	mannose-1-phosphate guanylyltransferase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	538	538	98%	0.00E+00	71.22%	374	EEH08109.1
8	Select seq gb KEI82064.1	mannose-1-phosphate guanylyltransferase [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	535	535	97%	0.00E+00	71.71%	378	KEI82064.1
9	Select seq ref XP_751679.1	mannose-1-phosphate guanylyltransferase [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	535	535	97%	0.00E+00	71.71%	426	XP_751679.1
10	Select seq ref XP_710946.1	mannose-1-phosphate guanylyltransferase [Candida albicans SC5314]	Candida albicans SC5314	522	522	100%	0.00E+00	66.85%	362	XP_710946.1
11	Select seq gb AAC64911.1	putative GDP-mannose pyrophosphorylase [Candida albicans]	Candida albicans	520	520	100%	0.00E+00	66.58%	362	AAC64911.1
12	Select seq gb AAC64912.1	putative GDP-mannose pyrophosphorylase [Candida albicans]	Candida albicans	518	518	100%	0.00E+00	66.30%	362	AAC64912.1
13	Select seq ref XP_018224557.1	mannose-1-phosphate guanylyltransferase [Pneumocystis carinii B80]	Pneumocystis carinii B80	497	497	100%	1.00E-176	65.38%	362	XP_018224557.1
14	Select seq gb EER39809.1	mannose-1-phosphate guanylyltransferase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	443	443	82%	3.00E-156	73.33%	300	EER39809.1
15	Select seq gb EER41439.1	GDP-mannose pyrophosphorylase A [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	182	182	98%	1.00E-52	29.93%	437	EER41439.1
16	Select seq ref XP_018227384.1	hypothetical protein T552_00500 [Pneumocystis carinii B80]	Pneumocystis carinii B80	181	181	96%	2.00E-52	31.08%	415	XP_018227384.1
17	Select seq gb EEH09499.1	GDP-mannose pyrophosphorylase A [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	180	180	98%	6.00E-52	29.69%	437	EEH09499.1
18	Select seq gb Q5566421.1	GDP-mannose pyrophosphorylase A [Histoplasma capsulatum]	Histoplasma capsulatum	180	180	98%	8.00E-52	29.69%	427	Q5566421.1
19	Select seq gb KMK63090.1	GDP-mannose pyrophosphorylase A [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	174	174	97%	1.00E-49	28.88%	437	KMK63090.1
20	Select seq gb KAF4261611.1	hypothetical protein CNMCM8057_001790 [Aspergillus fumigatus]	Aspergillus fumigatus	174	174	97%	4.00E-49	28.88%	489	KAF4261611.1
21	Select seq gb KAF4260758.1	hypothetical protein CNMCM8714_000935 [Aspergillus fumigatus]	Aspergillus fumigatus	174	174	97%	4.00E-49	28.88%	489	KAF4260758.1
22	Select seq ref XP_001538106.1	hypothetical protein HCAG_05711 [Histoplasma capsulatum Nam1]	Histoplasma capsulatum Nam1	154	154	91%	2.00E-41	27.53%	512	XP_001538106.1
23	Select seq gb KEI80934.1	GDP-mannose pyrophosphorylase A [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	149	149	94%	5.00E-40	26.24%	483	KEI80934.1
24	Select seq ref XP_750653.1	GDP-mannose pyrophosphorylase A [Aspergillus fumigatus AF293]	Aspergillus fumigatus AF293	149	149	92%	1.00E-39	26.57%	524	XP_750653.1
25	Select seq gb EDP40331.1	GDP-mannose pyrophosphorylase A [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	122	122	70%	2.00E-30	25.95%	425	EDP40331.1
26	Select seq gb KGG090958.1	mannose-1-phosphate guanylyltransferase [Candida albicans P94015]	Candida albicans P94015	116	116	98%	5.00E-28	23.21%	458	KGG090958.1
27	Select seq gb KGU31713.1	mannose-1-phosphate guanylyltransferase [Candida albicans P34048]	Candida albicans P34048	115	115	98%	1.00E-27	23.21%	458	KGU31713.1
28	Select seq ref XP_722154.1	Paa2p [Candida albicans SC5314]	Candida albicans SC5314	115	115	98%	1.00E-27	23.21%	458	XP_722154.1
29	Select seq gb KGU13544.1	mannose-1-phosphate guanylyltransferase [Candida albicans P87]	Candida albicans P87	115	115	98%	1.00E-27	23.21%	458	KGU13544.1
30	Select seq gb OXN04521.1	hypothetical protein CDV58_06494 [Aspergillus fumigatus]	Aspergillus fumigatus	107	107	67%	3.00E-25	28.72%	362	OXN04521.1
31	Select seq gb KAF4268143.1	hypothetical protein CNMCM8812_001949 [Aspergillus fumigatus]	Aspergillus fumigatus	102	102	56%	2.00E-24	32.89%	251	KAF4268143.1
32	Select seq gb KAF4289442.1	hypothetical protein CNMCM8686_002547 [Aspergillus fumigatus]	Aspergillus fumigatus	102	102	56%	6.00E-24	32.89%	303	KAF4289442.1
33	Select seq gb OXN01852.1	hypothetical protein CDV58_09284 [Aspergillus fumigatus]	Aspergillus fumigatus	67.8	67.8	25%	3.00E-13	30.36%	117	OXN01852.1
34	Select seq gb KGR02592.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans GC75]	Candida albicans GC75	60.8	60.8	98%	3.00E-09	21.20%	732	KGR02592.1
35	Select seq gb KGR16966.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P78048]	Candida albicans P78048	60.8	60.8	98%	4.00E-09	21.20%	732	KGR16966.1
36	Select seq gb KGG97847.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P37005]	Candida albicans P37005	60.8	60.8	98%	4.00E-09	21.20%	732	KGG97847.1
37	Select seq gb KHC83609.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans SC5314]	Candida albicans SC5314	60.8	60.8	98%	4.00E-09	21.20%	732	KHC83609.1
38	Select seq gb KGU13107.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P87]	Candida albicans P87	60.8	60.8	98%	4.00E-09	21.20%	732	KGU13107.1
39	Select seq gb KHC67475.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P75010]	Candida albicans P75010	59.3	59.3	98%	1.00E-08	20.95%	732	KHC67475.1
40	Select seq gb KHC74160.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P75016]	Candida albicans P75016	59.3	59.3	98%	1.00E-08	20.95%	732	KHC74160.1
41	Select seq gb KHC55826.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P60002]	Candida albicans P60002	59.3	59.3	98%	1.00E-08	20.95%	732	KHC55826.1
42	Select seq gb KHC42370.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P76067]	Candida albicans P76067	59.3	59.3	98%	1.00E-08	20.95%	732	KHC42370.1
43	Select seq ref XP_711895.2	translation initiation factor eIF-2B catalytic subunit epsilon [Candida albicans SC5314]	Candida albicans SC5314	59.3	59.3	98%	1.00E-08	20.95%	732	XP_711895.2

44	Select seq gb   KGU17713.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans L26]	Candida albicans L26	59.3	59.3	98%	1,00E-08	20.95%	732	KGU17713.1
45	Select seq gb   KGU90520.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P94015]	Candida albicans P94015	59.3	59.3	98%	1,00E-08	20.95%	731	KGU90520.1
46	Select seq gb   KGU36651.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P57055]	Candida albicans P57055	59.3	59.3	98%	1,00E-08	20.95%	732	KGU36651.1
47	Select seq gb   KHC84263.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P78042]	Candida albicans P78042	59.3	59.3	98%	1,00E-08	20.95%	732	KHC84263.1
48	Select seq gb   EEQ42357.1	translation initiation factor eIF-2B epsilon subunit [Candida albicans WO-1]	Candida albicans WO-1	59.3	59.3	98%	1,00E-08	20.95%	732	EEQ42357.1
49	Select seq gb   KGU34277.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P75063]	Candida albicans P75063	59.3	59.3	98%	1,00E-08	20.95%	732	KGU34277.1
50	Select seq gb   KGR14088.1	translation initiation factor eIF-2B subunit epsilon [Candida albicans P57072]	Candida albicans P57072	59.3	59.3	98%	1,00E-08	20.95%	732	KGR14088.1
51	Select seq gb   EEQ44807.1	predicted protein [Candida albicans WO-1]	Candida albicans WO-1	52.0	52.0	9%	2,00E-08	63.89%	36	EEQ44807.1
52	Select seq gb   KAF6062851.1	eIF4-gamma/eIF5/eIF2-epsilon family protein [Candida albicans]	Candida albicans	54.3	54.3	98%	5,00E-07	20.45%	712	KAF6062851.1
53	Select seq gb   KAF4254824.1	hypothetical protein CNMCM8812_007885 [Aspergillus fumigatus]	Aspergillus fumigatus	39.7	39.7	15%	0.001	28.95%	77	KAF4254824.1

Homology of phosphoglucomutase to proteins from other pathogenic fungi

Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq gb   ECG40807.1	phosphoglucomutase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	702	702	99%	0.00E+00	61.35%	556	EGC40807.1
2	Select seq gb   EEH04778.1	phosphoglucomutase [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	700	700	99%	0.00E+00	61.35%	556	EEH04778.1
3	Select seq gb   KAG5296699.1	phosphoglucomutase [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	699	699	99%	0.00E+00	61.35%	556	KAG5296699.1
4	Select seq ref   XP_001536486.1	phosphoglucomutase [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	696	696	99%	0.00E+00	60.64%	556	XP_001536486.1
5	Select seq ref   XP_715772.2	phosphoglucomutase [Candida albicans SC5314]	Candida albicans SC5314	685	685	99%	0.00E+00	60.28%	560	XP_715772.2
6	Select seq gb   KHC29649.1	phosphoglucomutase [Candida albicans P76055]	Candida albicans P76055	685	685	99%	0.00E+00	60.28%	560	KHC29649.1
7	Select seq gb   RLP66776.1	hypothetical protein L150_05559 [Candida albicans Ca529L]	Candida albicans Ca529L	685	685	99%	0.00E+00	60.28%	560	RLP66776.1
8	Select seq gb   KHC54366.1	phosphoglucomutase [Candida albicans P75010]	Candida albicans P75010	682	682	99%	0.00E+00	60.11%	560	KHC54366.1
9	Select seq gb   KAF4294963.1	hypothetical protein PgmA [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	678	678	99%	0.00E+00	60.04%	555	XP_754438.1
10	Select seq gb   KAF4294963.1	hypothetical protein PgmA [Aspergillus fumigatus]	Aspergillus fumigatus	677	677	99%	0.00E+00	59.79%	555	KAF4294963.1
11	Select seq gb   KEY79481.1	phosphoglucomutase PgmA [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	677	677	99%	0.00E+00	60.04%	555	KEY79481.1
12	Select seq gb   KAF4260782.1	hypothetical protein CNMCM8812_005242 [Aspergillus fumigatus]	Aspergillus fumigatus	643	643	92%	0.00E+00	61.61%	522	KAF4260782.1
13	Select seq gb   EER37117.1	phosphoglucomutase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	632	632	99%	0.00E+00	57.80%	522	EER37117.1
14	Select seq ref   XP_018227010.1	hypothetical protein T552_00697 [Pneumocystis carinii B80]	Pneumocystis carinii B80	587	587	99%	0.00E+00	51.87%	556	XP_018227010.1
15	Select seq gb   KAF6067110.1	Phosphoglucomutase/phosphomannomutase, alpha/beta/alpha domain I family protein [Candida albicans]	Candida albicans	404	404	53%	8.00E-135	64.57%	515	KAF6067110.1
16	Select seq gb   KAF6067109.1	Phosphoglucomutase/phosphomannomutase, alpha/beta/alpha domain III family protein [Candida albicans]	Candida albicans	251	251	41%	2.00E-79	54.01%	230	KAF6067109.1
17	Select seq gb   KHC35499.1	phosphoglucomutase [Candida albicans P76055]	Candida albicans P76055	55.1	55.1	89%	6.00E-07	23.62%	616	KHC35499.1
18	Select seq gb   RLP64423.1	hypothetical protein L150_03169 [Candida albicans Ca529L]	Candida albicans Ca529L	53.9	53.9	89%	1.00E-06	23.62%	616	RLP64423.1
19	Select seq gb   KGU30458.1	phosphoglucomutase [Candida albicans P57055]	Candida albicans P57055	53.9	53.9	89%	1.00E-06	23.62%	616	KGU30458.1
20	Select seq gb   KHC36319.1	phosphoglucomutase [Candida albicans P76067]	Candida albicans P76067	53.9	53.9	89%	1.00E-06	23.62%	616	KHC36319.1
21	Select seq gb   KHC42249.1	phosphoglucomutase [Candida albicans Ca6]	Candida albicans Ca6	53.5	53.5	89%	2.00E-06	22.79%	616	KHC42249.1
22	Select seq gb   KHC7774.1	phosphoglucomutase [Candida albicans P78042]	Candida albicans P78042	53.5	53.5	89%	2.00E-06	23.62%	616	KHC7774.1
23	Select seq ref   XP_719837.1	phosphoribomutase [Candida albicans SC5314]	Candida albicans SC5314	53.1	53.1	89%	2.00E-06	23.53%	616	XP_719837.1
24	Select seq gb   KGO87015.1	phosphoglucomutase [Candida albicans P94015]	Candida albicans P94015	53.1	53.1	89%	2.00E-06	23.62%	616	KGO87015.1
25	Select seq gb   KGR08869.1	phosphoglucomutase [Candida albicans P57072]	Candida albicans P57072	53.1	53.1	89%	2.00E-06	23.62%	616	KGR08869.1
26	Select seq gb   KGU26618.1	phosphoglucomutase [Candida albicans P34048]	Candida albicans P34048	53.1	53.1	89%	2.00E-06	23.62%	616	KGU26618.1
27	Select seq gb   OXN08158.1	hypothetical protein CDV58_03166 [Aspergillus fumigatus]	Aspergillus fumigatus	53.1	53.1	71%	2.00E-06	23.73%	602	OXN08158.1
28	Select seq gb   KGO97726.1	phosphoglucomutase [Candida albicans GC75]	Candida albicans GC75	52.8	52.8	76%	3.00E-06	24.10%	616	KGO97726.1
29	Select seq gb   KHC51489.1	phosphoglucomutase [Candida albicans P60002]	Candida albicans P60002	52.8	52.8	82%	3.00E-06	23.87%	616	KHC51489.1
30	Select seq gb   EEQ44767.1	conserved hypothetical protein [Candida albicans WO-1]	Candida albicans WO-1	52.8	52.8	89%	3.00E-06	22.79%	616	EEQ44767.1
31	Select seq gb   KMK62391.1	phosphoglucomutase [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	52.8	52.8	71%	3.00E-06	23.73%	602	KMK62391.1
32	Select seq gb   KHC63643.1	phosphoglucomutase [Candida albicans P75010]	Candida albicans P75010	52.4	52.4	89%	3.00E-06	23.26%	616	KHC63643.1
33	Select seq gb   KGU08832.1	phosphoglucomutase [Candida albicans P87]	Candida albicans P87	52.4	52.4	89%	4.00E-06	23.62%	616	KGU08832.1
34	Select seq gb   KAF6071210.1	Phosphoglucomutase/phosphomannomutase, alpha/beta/alpha domain I family protein [Candida albicans]	Candida albicans	52.0	52.0	89%	5.00E-06	22.70%	580	KAF6071210.1
35	Select seq gb   EGC44969.1	phosphoglucomutase [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	39.3	39.3	54%	0.042	24.01%	645	EGC44969.1

Homology of pyruvate decarboxylase to proteins from other pathogenic fungi

Job Title	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
AFR97558:pyruvate decarboxylase [Cryptococcus...]	Aspergillus fumigatus A1293	463	463	97%	2,00E-156	42.79%	569	XP_754512.1
7EAMVSV8016 Search expires on 04-16 01:47 am	Aspergillus fumigatus	463	463	97%	2,00E-156	42.79%	570	KAF4261268.1
BLASTP	Aspergillus fumigatus	455	455	97%	5,00E-153	41.87%	573	Q5552791.1
nr	Histoplasma capsulatum H88	454	454	98%	6,00E-153	41.18%	573	KAG5296666.1
AFR97558.1	Histoplasma capsulatum G217B	448	448	97%	2,00E-150	41.87%	573	KAG5287398.1
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99] ...	Histoplasma capsulatum NAM1	442	442	97%	3,00E-148	41.41%	568	XP_001536456.1
amino acid	Candida albicans P75010	441	441	98%	7,00E-148	41.65%	567	KHC600979.1
623	Candida albicans Ca529L	440	440	98%	3,00E-147	41.49%	567	RUP65072.1
Protein Homolog	Candida albicans SC5314	439	439	98%	6,00E-147	41.33%	567	XP_715533.1
pyruvate decarboxylase PdcA, putative [Aspergillus fumigatus A1293]	Candida albicans WO-1	422	422	98%	9,00E-140	38.84%	599	EEQ45293.1
hypothetical protein CNM1CM871.4_000674 [Aspergillus fumigatus]	Candida albicans P87	421	421	98%	1,00E-139	38.92%	599	KGU07648.1
pyruvate decarboxylase [Histoplasma capsulatum H88]	Candida albicans P75010	420	420	98%	4,00E-139	38.84%	599	KHC63088.1
pyruvate decarboxylase [Histoplasma capsulatum G217B]	Candida albicans P94015	420	420	98%	6,00E-139	38.62%	599	KGQ86439.1
pyruvate decarboxylase [Histoplasma capsulatum]	Candida albicans Ca529L	419	419	98%	1,00E-138	38.62%	599	RUP64633.1
pyruvate decarboxylase [Histoplasma capsulatum NAM1]	Candida albicans P75063	417	417	98%	4,00E-138	38.62%	599	KGU28315.1
pyruvate decarboxylase [Candida albicans P75010]	Candida albicans P34048	416	416	98%	1,00E-137	38.46%	599	KGU26041.1
pyruvate decarboxylase [Candida albicans Ca529L]	Candida albicans P37005	416	416	98%	1,00E-137	38.53%	599	KGQ90135.1
indolepyruvate decarboxylase 1 [Candida albicans SC5314]	Candida albicans SC5314	415	415	98%	5,00E-137	38.38%	599	KHC35058.1
Pyruvate decarboxylase [Candida albicans]	Candida albicans P76055	414	414	98%	3,00E-137	38.38%	599	XP_019330908.1
pyruvate decarboxylase isozyme 1 [Candida albicans WO-1]	Candida albicans GC75	414	414	98%	7,00E-137	38.31%	599	KGQ97139.1
hypothetical protein MEQ_03402 [Candida albicans P87]	Candida albicans P76067	414	414	98%	1,00E-136	38.38%	599	KHC35831.1
hypothetical protein MGE_03410 [Candida albicans P75010]	Candida albicans P75016	413	413	98%	2,00E-136	38.31%	599	KHC69208.1
hypothetical protein MEO_03381 [Candida albicans P94015]	Candida albicans Ca6	413	413	98%	2,00E-136	38.31%	599	KHC39025.1
hypothetical protein L150_03387 [Candida albicans Ca529L]	Histoplasma capsulatum H88	413	413	95%	3,00E-136	38.50%	613	Q555255.1
hypothetical protein MGV_03431 [Candida albicans P75063]	Candida albicans P57055	412	412	98%	6,00E-136	38.15%	599	KGU29966.1
hypothetical protein MG7_03416 [Candida albicans P34048]	Histoplasma capsulatum	409	409	98%	1,00E-135	38.93%	546	Q5564891.1
hypothetical protein MEU_03422 [Candida albicans P37005]	Candida albicans P57072	410	410	98%	3,00E-135	38.23%	599	KGR08115.1
Pdc12p [Candida albicans SC5314]	Histoplasma capsulatum G217B	409	409	95%	1,00E-134	37.91%	613	KAG5300802.1
hypothetical protein MGO_03402 [Candida albicans P76055]	Histoplasma capsulatum	408	408	95%	2,00E-134	38.25%	613	KAG5291046.1
hypothetical protein MG1_03434 [Candida albicans GC75]	Histoplasma capsulatum H88	406	406	98%	3,00E-134	38.73%	546	EGC40773.1
hypothetical protein MGQ_03410 [Candida albicans P76067]	Histoplasma capsulatum G186AR	399	399	98%	1,00E-131	38.73%	546	EEH04743.1
hypothetical protein MGL_03399 [Candida albicans P75016]	Candida albicans	404	404	97%	5,00E-129	39.33%	935	KAF6069922.1
hypothetical protein WSO_03444 [Candida albicans Ca6]	Histoplasma capsulatum H143	385	385	93%	7,00E-126	38.36%	569	EEFR37079.1
pyruvate decarboxylase [Histoplasma capsulatum]	Histoplasma capsulatum H88	373	373	95%	8,00E-121	35.83%	592	EGC44480.1
pyruvate decarboxylase [Histoplasma capsulatum G217B]	Histoplasma capsulatum G186AR	368	368	95%	4,00E-119	35.60%	592	EEH10009.1
pyruvate decarboxylase [Histoplasma capsulatum]	Histoplasma capsulatum NAM1	350	350	95%	3,00E-112	36.23%	571	XP_001542400.1
pyruvate decarboxylase [Histoplasma capsulatum H88]	Aspergillus fumigatus A1293	288	288	96%	2,00E-88	32.74%	575	XP_731481.1
Pyruvate decarboxylase domain protein [Candida albicans]	Histoplasma capsulatum	249	249	58%	1,00E-75	38.42%	385	Q5565506.1
pyruvate decarboxylase [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	250	250	76%	5,00E-75	38.42%	497	EEER38847.1
pyruvate decarboxylase dehydrogenase E1 component, alpha subunit [Histoplasma capsulatum H88]								

41	Select seq gb   KGO81086.1	phenylpyruvate decarboxylase [Candida albicans P37005]	238	238	96%	3,00E-69	27.74%	629	KGO81086.1
42	Select seq gb   KHC54712.1	phenylpyruvate decarboxylase [Candida albicans P75010]	238	238	96%	5,00E-69	27.74%	629	KHC54712.1
43	Select seq gb   KGU21147.1	phenylpyruvate decarboxylase [Candida albicans P57055]	238	238	96%	6,00E-69	27.74%	629	KGU21147.1
44	Select seq gb   EEQ43737.1	conserved hypothetical protein [Candida albicans WO-1]	237	237	96%	6,00E-69	27.74%	629	EEQ43737.1
45	Select seq gb   KHC27996.1	phenylpyruvate decarboxylase [Candida albicans P76055]	237	237	96%	7,00E-69	27.74%	629	KHC27996.1
46	Select seq gb   KGU18160.1	phenylpyruvate decarboxylase [Candida albicans P34048]	237	237	96%	7,00E-69	27.74%	629	KGU18160.1
47	Select seq gb   KGO81291.1	phenylpyruvate decarboxylase [Candida albicans GC75]	237	237	95%	7,00E-69	27.74%	629	KGO81291.1
48	Select seq gb   KGO81988.1	phenylpyruvate decarboxylase [Candida albicans P94015]	237	237	96%	8,00E-69	27.74%	629	KGO81988.1
49	Select seq ref   XP_711076.1	phenylpyruvate decarboxylase [Candida albicans SC5314]	237	237	95%	8,00E-69	27.74%	629	XP_711076.1
50	Select seq gb   KGU21554.1	phenylpyruvate decarboxylase [Candida albicans P75063]	237	237	96%	9,00E-69	27.74%	629	KGU21554.1
51	Select seq gb   KGR00811.1	phenylpyruvate decarboxylase [Candida albicans P57072]	236	236	96%	2,00E-68	27.74%	629	KGR00811.1
52	Select seq gb   KHC59906.1	phenylpyruvate decarboxylase [Candida albicans P75016]	236	236	96%	2,00E-68	27.74%	629	KHC59906.1
53	Select seq gb   KAF6066894.1	Thiamine pyrophosphate enzyme, N-terminal TPP binding domain family protein [Candida albicans]	234	234	96%	4,00E-68	27.78%	604	KAF6066894.1
54	Select seq gb   RLP67149.1	hypothetical protein L150_05944 [Candida albicans Cas29L]	235	235	96%	5,00E-68	27.59%	629	RLP67149.1
55	Select seq gb   KAF6066695.1	Thiamine pyrophosphate enzyme, N-terminal TPP binding domain family protein [Candida albicans]	229	229	96%	5,00E-66	27.49%	601	KAF6066695.1
56	Select seq gb   KAF4284148.1	hypothetical protein CNMCM8686_005553 [Aspergillus fumigatus]	194	194	98%	1,00E-53	27.26%	561	KAF4284148.1
57	Select seq ref   XP_753176.1	pyruvate decarboxylase, putative [Aspergillus fumigatus A1293]	194	194	98%	1,00E-53	27.26%	561	XP_753176.1
58	Select seq gb   KAF4277066.1	hypothetical protein CNMCM8689_005046 [Aspergillus fumigatus]	194	194	98%	2,00E-53	27.26%	561	KAF4277066.1
59	Select seq gb   KAF4261643.1	hypothetical protein CNMCM8812_004837 [Aspergillus fumigatus]	189	189	98%	1,00E-51	27.05%	561	KAF4261643.1
60	Select seq gb   KEY84000.1	pyruvate decarboxylase [Aspergillus fumigatus var. RP-2014]	189	189	98%	2,00E-51	27.05%	561	KEY84000.1
61	Select seq gb   KMK62950.1	pyruvate decarboxylase [Aspergillus fumigatus Z5]	188	188	98%	3,00E-51	27.05%	561	KMK62950.1
62	Select seq gb   KAF4268510.1	hypothetical protein CNMCM8714_001417 [Aspergillus fumigatus]	187	187	98%	7,00E-51	27.05%	561	KAF4268510.1
63	Select seq gb   KMK55416.1	pyruvate decarboxylase [Aspergillus fumigatus Z5]	151	288	86%	2,00E-38	43.39%	532	KMK55416.1

Homology of transaldolase to proteins from other pathogenic fungi

Job Title	AFR98178:transaldolase [Cryptococcus neoformans...
RID	7EAN03P2013 Search expires on 04-16 01:47 am
Program	BLASTP
Database	nr
Query ID	AFR98178.1
Description	transaldolase [Cryptococcus neoformans var. grubii H99]
Molecule type	amino acid
Query Length	323
Hit No.	Protein Homolog
1	sedoheptulose-7-phosphate:D-glycerinaldehyde-3-phosphate transaldolase [Candida albicans SC5314]
2	transaldolase [Candida albicans P57072]
3	transaldolase [Aspergillus fumigatus A1293]
4	transaldolase [Histoplasma capsulatum NAM1]
5	transaldolase [Histoplasma capsulatum H143]
6	transaldolase [Histoplasma capsulatum G2178]
7	transaldolase [Histoplasma capsulatum G186AR]
8	transaldolase [Histoplasma capsulatum]

Hit No.	Sequence identifier	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq ref   XP_715147.1	Candida albicans SC5314	407	407	95%	2,00E-142	67.63%	323	XP_715147.1
2	Select seq gb   KGR01844.1	Candida albicans P57072	406	406	95%	4,00E-142	67.63%	323	KGR01844.1
3	Select seq ref   XP_753176.1	Aspergillus fumigatus A1293	397	397	100%	1,00E-138	62.77%	324	XP_753176.1
4	Select seq ref   XP_001543592.1	Histoplasma capsulatum NAM1	392	392	100%	1,00E-136	62.46%	324	XP_001543592.1
5	Select seq gb   EER41273.1	Histoplasma capsulatum H143	392	392	100%	1,00E-136	62.46%	324	EER41273.1
6	Select seq gb   KAG5291776.1	Histoplasma capsulatum G2178	391	391	100%	2,00E-136	62.15%	324	KAG5291776.1
7	Select seq gb   EEH05501.1	Histoplasma capsulatum G186AR	372	372	100%	8,00E-129	60.62%	322	EEH05501.1
8	Select seq gb   Q5558364.1	Histoplasma capsulatum	363	363	95%	4,00E-124	60.90%	396	Q5558364.1



Homology of transketolase to proteins from other pathogenic fungi

Job Title AFR95182:transketolase [Cryptococcus neoformans...  
 RID 7EAN9ZM016 Search expires on 04-16 01:47 am  
 Program BLASTP  
 Database nr  
 Query ID AFR95182.1  
 Description transketolase [Cryptococcus neoformans var. grubii H95]  
 Molecule type amino acid  
 Query Length 687

Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident	E value	Acc. Len	Accession
1	Select seq ref XP_752720.1	transketolase TktA [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	936	936	99%	0,00E+00	65.15%	684	XP_752720.1
2	Select seq gb EEH09436.1	transketolase TktA [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	927	927	99%	0,00E+00	64.43%	685	EEH09436.1
3	Select seq emb CAF32073.1	transketolase, putative [Aspergillus fumigatus]	Aspergillus fumigatus	926	926	99%	0,00E+00	64.39%	689	CAF32073.1
4	Select seq gb EGC49955.1	transketolase TktA [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	924	924	99%	0,00E+00	64.29%	685	EGC49955.1
5	Select seq gb KAG5298561.1	transketolase TktA [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	922	922	99%	0,00E+00	64.14%	685	KAG5298561.1
6	Select seq gb EER43789.1	transketolase TktA [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	920	920	99%	0,00E+00	64.29%	866	EER43789.1
7	Select seq gb KMK62633.1	transketolase TktA [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	896	896	93%	0,00E+00	65.84%	661	KMK62633.1
8	Select seq ref XP_018225687.1	transketolase [Pneumocystis carinii B80]	Pneumocystis carinii B80	882	882	95%	0,00E+00	61.91%	681	XP_018225687.1
9	Select seq ref XP_001539533.1	transketolase 1 [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	853	853	99%	0,00E+00	60.93%	650	XP_001539533.1
10	Select seq ref XP_717648.1	transketolase [Candida albicans SC5314]	Candida albicans SC5314	833	833	98%	0,00E+00	59.73%	677	XP_717648.1
11	Select seq gb KHC40841.1	transketolase 1 [Candida albicans P76055]	Candida albicans P76055	832	832	98%	0,00E+00	59.73%	677	KHC40841.1
12	Select seq gb KHC42342.1	transketolase 1 [Candida albicans P76067]	Candida albicans P76067	832	832	98%	0,00E+00	59.73%	677	KHC42342.1
13	Select seq gb EFQ42384.1	transketolase [Candida albicans WO-1]	Candida albicans WO-1	832	832	98%	0,00E+00	59.73%	677	EFQ42384.1
14	Select seq gb KGR14060.1	transketolase 1 [Candida albicans P57072]	Candida albicans P57072	832	832	98%	0,00E+00	59.73%	677	KGR14060.1
15	Select seq gb KHC47965.1	transketolase 1 [Candida albicans Ca6]	Candida albicans Ca6	832	832	98%	0,00E+00	59.73%	677	KHC47965.1
16	Select seq sp O94039.1	RecName: Full=Transketolase 1; Short=TK 1 [Candida albicans]	Candida albicans	829	829	98%	0,00E+00	59.59%	677	O94039.1
17	Select seq gb QSS63067.1	transketolase TktA [Histoplasma capsulatum]	Histoplasma capsulatum	781	781	85%	0,00E+00	63.90%	666	QSS63067.1
18	Select seq gb OXN28503.1	hypothetical protein CDV57_02755 [Aspergillus fumigatus]	Aspergillus fumigatus	567	567	96%	0,00E+00	43.05%	704	OXN28503.1
19	Select seq gb KAF4279687.1	hypothetical protein CNMCM8057_002553 [Aspergillus fumigatus]	Aspergillus fumigatus	566	566	96%	0,00E+00	43.05%	704	KAF4279687.1
20	Select seq gb KAF4270204.1	hypothetical protein CNMCM8812_001249 [Aspergillus fumigatus]	Aspergillus fumigatus	566	566	96%	0,00E+00	43.05%	704	KAF4270204.1
21	Select seq gb KAF4288021.1	hypothetical protein CNMCM8689_007016 [Aspergillus fumigatus]	Aspergillus fumigatus	566	566	96%	0,00E+00	43.05%	704	KAF4288021.1
22	Select seq gb KAF4261671.1	hypothetical protein CNMCM8714_000444 [Aspergillus fumigatus]	Aspergillus fumigatus	564	564	96%	0,00E+00	43.05%	704	KAF4261671.1
23	Select seq gb KMK58568.1	dihydroxy-acetone synthase, putative [Aspergillus fumigatus Z5]	Aspergillus fumigatus Z5	559	559	96%	0,00E+00	42.42%	714	KMK58568.1
24	Select seq gb EDP53359.1	dihydroxy-acetone synthase, putative [Aspergillus fumigatus A1163]	Aspergillus fumigatus A1163	558	558	96%	0,00E+00	42.42%	714	EDP53359.1
25	Select seq gb KEY82415.1	dihydroxy-acetone synthase [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	556	556	96%	0,00E+00	42.42%	714	KEY82415.1
26	Select seq ref XP_748613.1	dihydroxy-acetone synthase, putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	554	554	96%	0,00E+00	42.27%	714	XP_748613.1
27	Select seq gb KAF6062881.1	Transketolase, thiamine diphosphate binding domain family protein [Candida albicans]	Candida albicans	447	447	51%	3.00E-152	58.26%	364	KAF6062881.1
28	Select seq ref XP_018227397.1	hypothetical protein T552_00513 [Pneumocystis carinii B80]	Pneumocystis carinii B80	42.0	42.0	29%	0.007	24.89%	349	XP_018227397.1

Homology of urease accessory protein UreG to proteins from other pathogenic fungi

Job Title	AFR92807:urease accessory protein UreG [Cryptococcus...]									
RID	7EANFWBN013 Search expires on 04-16 01:47 am									
Program	BLASTP									
Database	nr									
Query ID	AFR92807.1									
Description	urease accessory protein UreG [Cryptococcus neoformans var. grubii H99] ...									
Molecule type	amino acid									
Query length	312									
Hit No.	Sequence identifier	Protein Homolog	Species	Max Score	Total Score	Query Cover	Per. Ident.	E value	Acc. Len	Accession
1	Select seq ref  XP_755621.1	urease accessory protein UreG, putative [Aspergillus fumigatus A1293]	Aspergillus fumigatus A1293	342	342	82%	1,00E-117	65.23%	271	XP_755621.1
2	Select seq gb  KEY75387.1	urease accessory protein UreG [Aspergillus fumigatus var. RP-2014]	Aspergillus fumigatus var. RP-2014	341	341	82%	2,00E-117	65.23%	271	KEY75387.1
3	Select seq gb  EGC46498.1	CobW domain-containing protein [Histoplasma capsulatum H88]	Histoplasma capsulatum H88	339	339	78%	1,00E-116	66.53%	266	EGC46498.1
4	Select seq gb  EEH11515.1	CobW/P47K family protein [Histoplasma capsulatum G186AR]	Histoplasma capsulatum G186AR	338	338	78%	3,00E-116	66.12%	266	EEH11515.1
5	Select seq gb  QSS61315.1	CobW/P47K family protein [Histoplasma capsulatum]	Histoplasma capsulatum	337	337	78%	4,00E-116	66.12%	268	QSS61315.1
6	Select seq ref  XP_001541677.1	urease accessory protein ureG [Histoplasma capsulatum NAM1]	Histoplasma capsulatum NAM1	337	337	78%	7,00E-116	66.12%	266	XP_001541677.1
7	Select seq gb  KAG5289263.1	CobW/P47K family protein [Histoplasma capsulatum G217B]	Histoplasma capsulatum G217B	337	337	78%	9,00E-116	66.12%	266	KAG5289263.1
8	Select seq gb  KAF4258061.1	hypothetical protein CNMCM8714_002546 [Aspergillus fumigatus]	Aspergillus fumigatus	249	249	59%	1,00E-82	65.41%	180	KAF4258061.1
9	Select seq gb  EER39334.1	urease accessory protein ureG [Histoplasma capsulatum H143]	Histoplasma capsulatum H143	241	241	55%	1,00E-79	66.86%	173	EER39334.1

Homology to proteins in *Cryptococcus neoformans* H99

All H99s from the BLAST search is listed for each protein.

Table with columns: accession no., alternative acc. no., hit no., C. neoformans H99 homology (all hits), species, Maxscore, Total score, Query Cover, E value, % Identity, Acc. Len, accession no2. The table lists numerous protein entries and their corresponding homology results in C. neoformans H99, including protein names like 26S proteasome regulatory subunit 1A8, chaperonin 60, and various GTP-binding proteins.



Supplementary Table 6

Supplementary table 6: Confirmation of recombinant protein identity

Cryptococcal proteins were recombinantly expressed in *Escherichia coli*. Identity of the recombinant proteins was confirmed through mass spectrometry analysis of the respective protein band.

cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
26S proteasome regulatory subunit N8 [Cryptococcus neoformans var. grubii H99]	39_24S_prot	2:AFR92184.1	26S proteasome regulatory subunit N8 [Cryptococcus neoformans var. grubii H99]	2831	38753	24	75.1
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
chlorophyll synthesis pathway protein BcHc [Cryptococcus neoformans var. grubii H99]	38_BcHc	2:AFR87763.1	chlorophyll synthesis pathway protein BcHc [Cryptococcus neoformans var. grubii H99]	3194	39489	31	67
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
deoxyuridine 5--triphosphate nucleotidyltransferase [Cryptococcus neoformans var. grubii H99]	74_D57NH	2:AFR94562.2	deoxyuridine 5--triphosphate nucleotidyltransferase [Cryptococcus neoformans var. grubii H99]	2596	73956	39	70.1
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
extracellular elastinolytic metalloproteinase [Cryptococcus neoformans var. grubii H99]	92_EEIMP	2:AFR7494.2	extracellular elastinolytic metalloproteinase [Cryptococcus neoformans var. grubii H99]	1959	92085	34	51.7
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	48_Glu_Deh	2:AFR7792.1	glutamate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	2930	49505	32	58.8
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
glucose-methanol-choline oxidoreductase [Cryptococcus neoformans var. grubii H99]	38_GOC	2:AFR4416.1	glucose-methanol-choline oxidoreductase [Cryptococcus neoformans var. grubii H99]	2928	4516	31	51.6
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
glucose-6-phosphate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	38_G6PDH_2	2:AFR92267.1	glucose-6-phosphate dehydrogenase (NADP) [Cryptococcus neoformans var. grubii H99]	4392	38077	34	67.7
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
hypothetical protein CNAG_05236 [Cryptococcus neoformans var. grubii H99]	52_HP5236	2:AFR94491.2	hypothetical protein CNAG_05236 [Cryptococcus neoformans var. grubii H99]	1749	52430	31	51.9
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
hypothetical protein CNAG_08113 [Cryptococcus neoformans var. grubii H99]	37_HP8113	2:AFR88337.1	hypothetical protein CNAG_08113 [Cryptococcus neoformans var. grubii H99]	1732	36593	25	61.7
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
hypothetical protein CNAG_06846 [Cryptococcus neoformans var. grubii H99]	39_HP6846	2:AFR94893.2	hypothetical protein CNAG_06846 [Cryptococcus neoformans var. grubii H99]	1217	39291	22	50.6
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	86_Hsp70p4	2:AFR98435.1	heat shock 70kDa protein 4 [Cryptococcus neoformans var. grubii H99]	3091	85923	50	85.4
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
has71-like protein [Cryptococcus neoformans var. grubii H99]	70_Hsp71	2:AFR97929.1	has71-like protein [Cryptococcus neoformans var. grubii H99]	5234	89759	57	66.7
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
has72-like protein [Cryptococcus neoformans var. grubii H99]	70_Hsp72	2:AFR97952.1	has72-like protein [Cryptococcus neoformans var. grubii H99]	4633	89888	45	52.6
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
has75-like protein [Cryptococcus neoformans var. grubii H99]	67_Hsp75	2:AFR92468.1	has75-like protein [Cryptococcus neoformans var. grubii H99]	4318	67372	51	63.7
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
leucyl-tRNA synthetase, mitochondrial [Cryptococcus neoformans var. grubii H99]	44_KAD	2:AFR96943.1	leucyl-tRNA synthetase, mitochondrial [Cryptococcus neoformans var. grubii H99]	1958	44371	31	76.3
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	40_M1P-G	2:AFR98009.2	mannose-1-phosphate guanylyltransferase [Cryptococcus neoformans var. grubii H99]	915	40211	14	60.2
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
phosphoglucosylase [Cryptococcus neoformans var. grubii H99]	8_LP-GM	2:AFR96502.2	phosphoglucosylase [Cryptococcus neoformans var. grubii H99]	3059	86675	37	71.9
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	86_P70A	2:AFR94568.1	pyruvate decarboxylase [Cryptococcus neoformans var. grubii H99]	1144	89795	24	69.9
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
ribitol dehydrogenase [Cryptococcus neoformans var. grubii H99]	35_T_riald	2:AFR88178.1	ribitol dehydrogenase [Cryptococcus neoformans var. grubii H99]	1996	35443	29	69.7
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
transketolase [Cryptococcus neoformans var. grubii H99]	74_Transketo	2:AFR95182.1	transketolase [Cryptococcus neoformans var. grubii H99]	1347	74895	30	44.5
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
urease accessory protein UreG [Cryptococcus neoformans var. grubii H99]	34_UreG	2:AFR92807.1	urease accessory protein UreG [Cryptococcus neoformans var. grubii H99]	889	33777	17	56.4
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
GTP-binding protein ypt1 [Cryptococcus neoformans var. grubii H99]	23_YPT1	2:AFR9432.1	GTP-binding protein ypt1 [Cryptococcus neoformans var. grubii H99]	1053	22771	19	92.4
cloned protein	Sample ID	Protein Accession	Protein Description	Protein Score	Protein Mass	Matched Peptides	Protein Coverage
cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	CP_02843	2:AFR93749.2	cytoplasmic protein [Cryptococcus neoformans var. grubii H99]	1959	89793	37	84.1

**Supplementary Table 7**

**Supplementary Table 7: Degree of similarity of the immunoreactive proteins from *C. neoformans* serotype A strain H99 to homologous proteins from the *C. neoformans* serotype D strain JEC21 or *C. gattii* strain WM276 (serotype B).** Comparison of sequence similarity was carried out using the ncbi protein BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Cryptococcus (taxid:5206) was appointed as the organism for search. Acc. No.: Accession number.

<i>C. neoformans</i> strain H99 serotype A		<i>C. neoformans</i> strain JEC21 serotype D						<i>C. gattii</i> strain WM276 serotype B					
Protein	Acc. No.	Acc. No.	Max score	Total score	Query Cover	E value	Percent Identity	Acc. No.	Max score	Total score	Query Cover	E value	Percent Identity
26S proteasome regulatory subunit N8	AFR92184	XP_566467.1	714	714	100%	0.0	99.71%	XP_003191856.1	706	706	100%	0.0	98.29%
chlorophyll synthesis pathway protein BchC	AFR97763	XP_569885.1	704	704	100%	0.0	97.13%	XP_003192587.1	709	709	100%	0.0	97.99%
cytoplasmic protein CNAG_02943	AFR93749	XP_024514313.1	1122	1122	86%	0.0	98.91%	XP_003193066.1	1256	1256	100%	0.0	97.46%
deoxyuridine 5'-triphosphate nucleotidohydrolase	AFR94562	XP_572500.1	1302	1302	100%	0.0	95.11%	XP_003196292.1	830	830	58%	0.0	96.58%
extracellular elastinolytic metalloproteinase	AFR97484	XP_567342.1	1587	1587	100%	0.0	94.10%	XP_003196170.1	1463	1463	100%	0.0	85.80%
glucose-methanol-choline oxidoreductase	AFR94515	XP_567934.1	1204	1204	100%	0.0	98.47%	XP_003196508.1	1171	1171	100%	0.0	94.56%
glutamate dehydrogenase (NADP)	AFR97782	XP_569406.1	923	923	100%	0.0	97.34%	XP_003192625.1	912	912	100%	0.0	96.45%
glycerol-3-phosphate dehydrogenase (NAD(+))	AFR92257	XP_024512024.1	685	685	100%	0.0	95.93%	XP_003191980.1	681	681	100%	0.0	93.90%
GTP-binding protein ypt1	AFR94332	XP_024513861.1	421	421	100%	1.00E-151	99.51%	XP_003193368.1	422	422	100%	4.00E-152	100.00%
heat shock 70kDa protein 4	AFR98435	XP_568283.1	1555	1555	100%	0.0	97.67%	XP_003197358.1	1500	1500	100%	0.0	95.99%
hsp71-like protein	AFR97929	XP_569509.1	1292	1292	100%	0.0	98.44%	XP_003192735.1	1293	1293	100%	0.0	98.29%
hsp72-like protein, partial	AFR97952	XP_569545.1	1295	1295	100%	0.0	98.45%	XP_003192750.1	1286	1286	100%	0.0	97.98%
hsp75-like protein	AFR92468	XP_566757.1	1256	1256	100%	0.0	99.67%	XP_003192118.1	1255	1255	100%	0.0	99.51%
hypothetical protein CNAG_05236	AFR94491	XP_024513811.1	937	937	100%	0.0	99.13%	XP_003196439.1	922	922	100%	0.0	97.84%
hypothetical protein CNAG_06113	AFR98337	XP_568431.1	652	652	100%	0.0	95.42%	XP_003197250.1	468	468	100%	1.00E-165	94.20%
hypothetical protein CNAG_06946	AFR94883	XP_570168.2	715	715	100%	0.0	98.85%	XP_003193750.1	681	681	96%	0.0	97.32%
ketol-acid reductoisomerase	AFR96043	XP_571345.1	825	825	100%	0.0	99.50%	XP_003194784.1	820	820	100%	0.0	98.75%
Mannose-1-phosphate guanyltransferase	AFR98009	XP_569600.1	742	742	100%	0.0	99.18%	XP_003195224.1	206	206	98%	5.00E-62	32.32%
phosphoglucosmutase	AFR98550	XP_568570.1	1131	1131	100%	0.0	98.22%	XP_003197445.1	1098	1098	100%	0.0	94.47%
pyruvate decarboxylase	AFR97558	XP_567475.2	1258	1258	99%	0.0	97.11%	XP_003193906.1	1246	1246	99%	0.0	96.46%
transaldolase	AFR98178	XP_567910.1	640	640	100%	0.0	95.98%	XP_003196531.1	629	629	100%	0.0	93.50%
transketolase	AFR95182	XP_570402.1	1396	1396	100%	0.0	97.53%	XP_003193474.1	1353	1353	100%	0.0	95.34%
urease accessory protein UreG	AFR92807	XP_566996.1	617	617	100%	0.0	95.24%	XP_003191625.1	551	551	100%	0.0	92.99%

# Acknowledgements

I would like to express my deepest appreciation to the many people which contributed to this thesis, directly or indirectly over the past years, and without whom finishing this thesis would have been impossible.

First, I wish to thank Leipzig University for granting me a “Doktorandenförderplatz” and the “Stiftung der Deutschen Wirtschaft” for awarding me with a stipend to fund parts of my PhD thesis. I’m very grateful to Prof. Dr. Thomas M. Magin at the Faculty of Life Sciences, Leipzig University for supervising and assessing my thesis, and Prof. Dr. Michal Olszewski at the University of Michigan Medical School, USA for assessment of this thesis. I would also like to sincerely thank Prof. Dr. Gottfried Alber for the opportunity to work on this project, extensive supervision and both, scientific and personal advice and profound belief in my abilities. Special thanks to PD Dr. Uwe Müller, Dr. Christiane L. Schnabel, Dr. Carolina Firacative and Dr. Bianca Schulze for supervision, helpful advice, and unwavering support throughout my PhD time.

The completion of this dissertation would not have been possible without my cooperation partners for mass spectrometry analysis and establishment of recombinant protein expression. Therefore, I would like to thank Dr. Daniela Volke, Dr. Kristin Schubert, Dr. Andor Krizsan, Dr. Matthias Brock, Prof. Dr. Ralf Hoffmann and Prof. Dr. Martin von Bergen for their support and scientific advice.

Special thanks to my colleagues at the Institute of Immunology at the Faculty of Veterinary Medicine for valuable scientific advice and support: Florian Hohnstein, Maria-Christin Jentsch, Sabrina Lübke, Dr. Friederike Rabiger, Silke Lehnert, Dr. Martina Protschka, Dr. Maria Eschke, Dr. Nicole Schütze, Anett Grohs, Dr. Laura Schneider, Dr. Mahmud Muhsen, and Sophie Öhlmann.

I’m extremely grateful for the emotional support of my friends and family. Throughout my PhD time they provided extensive motivation and lend an ear to any problems. Many thanks to my sister Henriette for technical support on various occasions. Special thanks to all my wonderful friends participating with me in musical projects, like various choirs or band projects. Making music with all of you was the greatest source of my strength, carrying me through all difficulties of this thesis. Thanks also to my flat mates sharing my life over the past years. You have greatly contributed to success of this work. Finally, I want to thank Teresa Martin for unconditional moral and emotional support throughout the last years.

# Curriculum Vitae

---

## Anne Elisabeth Greßler

Kurt-Eisner-Straße 64  
04275 Leipzig, Deutschland  
+49 151 23679696  
egressler@gmx.net

**Date of birth** 05.03.1993  
**Birthplace** Naumburg (Saale), Germany  
**Nationality** German

---

## WORK EXPERIENCE

- 03.2017 – present      **Doctoral researcher**  
Institute of Immunology, College of Veterinary Medicine, University of Leipzig, Germany
- Funded by the stipend program of the University of Leipzig (“Doktorandenförderplatz”) and the “Stiftung der Deutschen Wirtschaft”
  - Thesis title: An immunoproteomic approach for identification of *Cryptococcus neoformans* proteins recognized by murine and human antibodies
- 10.2015 – 02.2016      **Scientific associate (Tutor)**  
10.2014 – 02.2015      Friedrich-Schiller-University Jena, Germany
- Supervision of undergraduates, exam preparation and repetition of lecture material for the lecture “Biochemie I”
- 

## EDUCATION

- 10.2014 – 12.2016      **Master’s degree in Biochemistry**  
Friedrich-Schiller-University Jena, Germany
- Degree: Master of Science (M.Sc.); 1.1
  - Master thesis at the Leibniz Institute for natural product research and infection biology, Hans Knöll Institute Jena
  - Title master thesis: The role of *Candida albicans ECE1* und *EEDI* for colonization of and translocation through the murine gastrointestinal tract
- 10.2011 – 09.2014      **Bachelor’s degree in Biochemistry/Molecular biology**  
Friedrich-Schiller-University Jena, Germany
- Degree: Bachelor of Science (B.Sc.); 1.7
  - Title bachelor thesis: Characterization of a potential target gene of the microRNA miR444 in *Triglochin maritimum*
- 09.2003 – 08.2011      **General qualification for university entrance**  
CJD Christophorusschule Droyßig, Germany
- Degree: 1.6



**PRACTICAL COURSES**

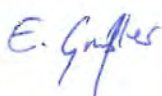
- 09.2015 – 10.2015      Laboratory internship at the institute for Biochemistry and Biophysics
- Friedrich-Schiller-University Jena, Germany
  - Research group of Prof. Thorsten Heinzel
- 08.2015                    Laboratory internship at the Leibniz Institute on Aging (Fritz Lipmann Institute) Jena
- Kaether Research Group
- 03.2014                    Laboratory internship at the University Hospital Jena
- Department of Experimental Nephrology

**FOREIGN LANGUAGE COMPETENCE**

- English                    B2 (general qualification for university entrance)
- French                     A2 (DELF certificate)

**SPECTRUM OF SCIENTIFIC METHODS**

- Animal experiments      • Basic and advanced training course in 11.2018 and basic course in 01.2016
- Extensive participation in mouse experiments during Master's thesis (experimental design, monitoring, dissection, post mortem analysis)
  - Approval of application for an animal experiment during doctoral thesis (Landesdirektion Sachsen, DD24.1-5131/446/37 (TVV35/18))
- 
- Cell biology                • Cultivation and infection of eukaryotic cells under normoxia and hypoxia, cultivation in trans-well systems, transfection
- 
- Proteomics                 • 2D gel electrophoresis, SDS-PAGE, Western blotting, mass spectrometry analysis (sample preparation, data evaluation)
- 
- Flow cytometry            • Isolation and analysis of gastrointestinal and respiratory immune cell populations
- Evaluation of flow cytometry data
- 
- Molecular biology        • ELISA analysis, Cytokine multiplex assay, LDH assay
- DNA and RNA extraction, PCR techniques
  - Cloning, transformation, plasmid isolation and sequencing analysis
  - Recombinant expression of fungal proteins in *Escherichia coli*
- 
- Microbiology              • Cultivation of yeasts and bacteria



Elisabeth Greßler, Leipzig, 04.11.2021

# List of publications and presentations

## Parts of this work were published in a peer-reviewed scientific journal:

Firacative C\*, Gressler AE\*, Schubert K, Schulze B, Müller U, Brombacher F, von Bergen M, Alber G: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection. *Sci Rep.* 2018 Feb 8;8(1):2681. doi: 10.1038/s41598-018-21039-z.

\* shared first authorship

Gressler AE, Volke D, Firacative C, Schnabel CL, Müller U, Krizsan A, Schulze-Richter B, Brock M, Brombacher F, Escandón P, Hoffmann R, Alber G: Identification of Disease-Associated Cryptococcal Proteins Reactive With Serum IgG From Cryptococcal Meningitis Patients. *Front Immunol.* 2021 Jul 23;12:709695. doi: 10.3389/fimmu.2021.709695. eCollection 2021.

## Other publications:

Dunker C, Polke M, Schulze-Richter B, Schubert K, Rudolphi S, Gressler AE, Pawlik T, Prada Salcedo JP, Niemiec MJ, Slesiona-Künzel S, Swidergall M, Martin R, Dandekar T, Jacobsen ID: Rapid proliferation due to better metabolic adaptation results in full virulence of a filament-deficient *Candida albicans* strain. *Nat Commun.* 2021 Jun 23;12(1):3899. doi: 10.1038/s41467-021-24095-8.

## Posters

Gressler AE, Firacative C, Schulze B, Schubert K, Müller U, Brombacher F, von Bergen M, Alber G: T helper cell (Th)1 and Th2-associated antigens in the fungal infection cryptococcosis. 14<sup>th</sup> Research Festival for Life Sciences. 2018. Leipzig, Germany.

Gressler AE: The immunoproteome of *Cryptococcus neoformans* in health and disease. 15<sup>th</sup> German Society for immunology (DGfI) Spring School on Immunology. 2019. Ettal, Germany.

Gressler AE, Schulze B, Volke D, Firacative C, Escandón P, Hoffmann R, Alber G: The immunoproteome of *Cryptococcus neoformans* in health and disease. 15<sup>th</sup> Research Festival for Life Sciences. 2019. Leipzig, Germany.

Gressler AE, Schulze B, Volke D, Müller U, Piehler D, Grahner A, Escandón P, Hoffmann R, Firacative C, Alber G: Surprisingly similar humoral immune response to *Cryptococcus neoformans* in patients with cryptococcal meningitis and in healthy people with presumed environmental exposure. 16<sup>th</sup> Research Festival for Life Sciences. 2020. Leipzig, Germany.

Gressler AE, Schulze B, Volke D, Müller U, Firacative C, Escandón P, Hoffmann R, Alber G: Investigating the humoral immune response against *Cryptococcus neoformans* in patients with cryptococcal meningitis and in healthy persons with presumed environmental exposure. 54<sup>th</sup> Scientific Conference of the German speaking Mycological Society (DMykG) e. V. and 3<sup>rd</sup> International Symposium of the CRC/Transregio FungiNet. 2020. Digital conference.

Gressler AE, Volke D, Firacative C, Schnabel CL, Müller U, Krizsan A, Schulze-Richter B, Brock M, Brombacher F, Escandón P, Hoffmann R, Alber G: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients. 73<sup>rd</sup> Annual Conference of the German Society for Hygiene and Microbiology (DGHM). 2021. Digital conference.

**Oral presentations**

Gressler AE: Immunproteome analysis of a fungal pathogen (mouse and human samples). Doktoranden-Kolloquium des Biotechnologisch-Biomedizinisches Zentrums (BBZ). 2018. Leipzig, Germany.

Gressler AE, Firacative C, Schubert K, Schulze B, Müller U, Brombacher F, von Bergen M, Alber G: Identification of T helper (Th)1- and Th2-associated antigens of *Cryptococcus neoformans* in a murine model of pulmonary infection. Symposium „Infection and immune defense” of the research group infection immunology of the German Society for immunology (DGfI) and German Society for Hygiene and Microbiology (DGHM). 2018. Rothenfels, Germany.

Gressler AE: The humoral immune response against the fungus *Cryptococcus neoformans* in Colombian cryptococcosis patients. Doktoranden-Kolloquium des Biotechnologisch-Biomedizinisches Zentrums (BBZ). 2020. Leipzig, Germany.

Gressler AE, Schulze B, Volke D, Müller U, Piehler D, Grahnert A, Firacative C, Escandón P, Hoffmann R, Alber G: Surprisingly similar humoral immune response to *Cryptococcus neoformans* in patients with cryptococcal meningitis and in healthy people with presumed environmental exposure. 6<sup>th</sup> Joint conference of German Society for Hygiene and Microbiology (DGHM) and the Association for General and Applied Microbiology (VAAM). 2020. Leipzig, Germany.

Gressler AE: Identification of disease-associated cryptococcal proteins targeted by human IgG induced as the dominant isotype during cryptococcal meningitis. Doktoranden-Kolloquium des Biotechnologisch-Biomedizinisches Zentrums (BBZ). 2021. Leipzig, Germany.

Gressler AE, Volke D, Firacative C, Schnabel CL, Müller U, Krizsan A, Schulze-Richter B, Brock M, Brombacher F, Escandón P, Hoffmann R, Alber G: Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients. 55<sup>th</sup> Scientific Conference of the German speaking Mycological Society (DMyG). 2021. Digital Conference.

# Selbstständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Dissertation mit dem Titel „An immunoproteomic approach for identification of *Cryptococcus neoformans* proteins recognized by murine and human antibodies“, selbstständig und ausschließlich unter Verwendung der angegebenen Hilfsmittel und Quellen angefertigt habe. Es wurden keine unzulässigen oder weiteren Hilfsmittel in Anspruch genommen. Anteile Dritter an den in dieser Dissertation aufgeführten Publikationen wurden im entsprechenden Abschnitt „Nachweis über die Anteile der Co-Autoren/Author contribution statement“ offengelegt. Wörtlich oder sinngemäß aus fremden Quellen entnommene Gedanken, Erkenntnisse und Abbildungen wurden als solche kenntlich gemacht.

Ich versichere des Weiteren, dass Dritte von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorliegenden Dissertation stehen. Bei der geistigen Herstellung der Arbeit wurde kein Promotionsberater in Anspruch genommen.

Ich habe mich bisher keinem anderen Promotionsverfahren unterzogen. Die Dissertation wurde weder in der gegenwärtigen noch in einer anderen Fassung an einer wissenschaftlichen Einrichtung mit dem Ziel der Promotion vorgelegt.

Leipzig, \_\_\_\_\_

\_\_\_\_\_  
Elisabeth Greßler