

(43) International Publication Date
21 March 2013 (21.03.2013)(51) International Patent Classification:
C07K 14/37 (2006.01) *C07K 16/14* (2006.01)

1124 West Carson Street, Building N-14, Room 11, Torrance, CA 90502 (US).

(21) International Application Number:
PCT/US2012/055659(74) Agents: **HERBERT, Michael, L.** et al.; Jones Day, 222 East 41st Street, New York, NY 11017-6702 (US).(22) International Filing Date:
14 September 2012 (14.09.2012)

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(25) Filing Language: English

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(26) Publication Language: English

Published:(30) Priority Data:
61/535,257 15 September 2011 (15.09.2011) US

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(71) Applicant (for all designated States except US): **LOS ANGELES BIOMEDICAL RESEARCH INSTITUTE AT HARBOR-UCLA MEDICAL CENTER** [US/US];

1124 West Carson Street, Building N-14, Room 11, Torrance, CA 90502 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **IBRAHIM, Ashraf, S.** [US/US]; 1124 West Carson Street, Building N-14, Room 11, Torrance, CA 90502 (US). **LIU, Mingfu** [CA/US]; 1124 West Carson Street, Building N-14, Room 11, Torrance, CA 90502 (US). **GHEBREMARIAM, Teklegiorgis** [US/US]; 1124 West Carson Street, Building N-14, Room 11, Torrance, CA 90502 (US). **FU, Yue** [US/US]; 1124 West Carson Street, Building N-14, Room 11, Torrance, CA 90502 (US). **EDWARDS, John, E.** [US/US]; 1124 West Carson Street, Building N-14, Room 11, Torrance, CA 90502 (US). **Filler, Scott** [US/US];

(54) Title: IMMUNOTHERAPY AND DIAGNOSIS OF MUCORMYCOSIS USING CotH

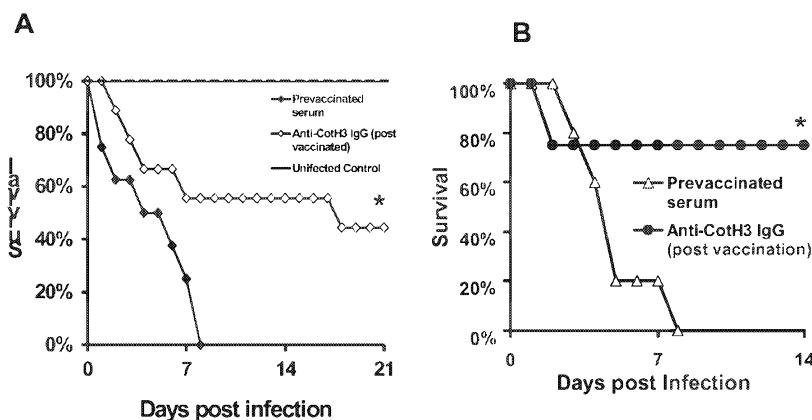


FIGURE 60

(57) Abstract: The invention provided Mucorales CotH polypeptides and encoding nucleic acid molecules. The Mucorales CotH polypeptides and encoding nucleic acids can be advantageously used to diagnose, treat or prevent fungal conditions, in particular mucormycosis.

IMMUNOTHERAPY AND DIAGNOSIS OF MUCORMYCOSIS USING CotH

[0001] This application claims the benefit of priority of United States Provisional application serial No. 61/535,257, filed September 15, 2011, the entire contents of which is incorporated herein by reference.

5 [0002] This invention was made with government support under NIH grant numbers 011671 and 013377 awarded by NIAID. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

10 [0003] The present invention relates generally to compositions and methods for detecting, treating and preventing infectious diseases in a patient, and more specifically to compositions and methods that target specific proteins or nucleic acids unique to fungi that cause mucormycosis.

15 [0004] About 180 of the 250,000 known fungal species are recognized to cause disease (*mycosis*) in man and animal. Some of fungi can establish an infection in all exposed subjects, e.g., the systemic pathogens *Histoplasma capsulatum* and *Coccidioides immitis*. Others, such as *Candida*, *Aspergillus* species and *Zygomycetes* are opportunist pathogens which ordinarily cause disease only in a compromised host. Fungi of the class *Zygomycetes*, order Mucorales, can cause Mucormycosis, a potentially deadly fungal 20 infection in human. Fungi belonging to the order Mucorales are distributed into at least six families, all of which can cause mucormycosis (Ibrahim et al. *Zygomycosis*, p. 241-251, In W. E. Dismukes, P. G. Pappas, and J. D. Sobel (ed.), *Clinical Mycology*, Oxford University Press, New York (2003); Kwon-Chung, K. J., and J. E. Bennett, *Mucormycosis*, p. 524-559, *Medical Mycology*, Lea & Febiger, Philadelphia (1992), and 25 Ribes et al. *Zygomycetes in Human Disease*, *Clin Microbiol Rev* 13:236-301 (2000)). However, fungi belonging to the family *Mucoraceae*, and specifically the species *Rhizopus oryzae* (*Rhizopus arrhizus*), are by far the most common cause of infection (Ribes et al., *supra*). Increasing cases of mucormycosis have been also reported due to 30 infection with *Cunninghamella spp.* in the *Cunninghamellaceae* family (Cohen-Abbo et al., *Clinical Infectious Diseases* 17:173-77 (1993); Kontoyianis et al., *Clinical Infectious Diseases* 18:925-28 (1994); Kwon-Chung et al., *American Journal of Clinical Pathology* 64:544-48 (1975), and Ventura et al., *Cancer* 58:1534-36 (1986)). The remaining four

families of the *Mucorales* order are less frequent causes of disease (Bearer et al., *Journal of Clinical Microbiology* 32:1823-24 (1994); Kamalam and Thambiah, *Sabouraudia* 18:19-20 (1980); Kemna et al., *Journal of Clinical Microbiology* 32:843-45 (1994); Lye et al., *Pathology* 28:364-65 (1996), and Ribes et al., (*supra*)).

5 [0005] The agents of mucormycosis almost uniformly affect immunocompromised hosts (Spellberg et al., *Clin. Microbiol. Rev.* 18:556-69 (2005)). The major risk factors for mucormycosis include uncontrolled diabetes mellitus in ketoacidosis known as diabetes ketoacidosis (DKA), other forms of metabolic acidosis, treatment with corticosteroids, organ or bone marrow transplantation, neutropenia, trauma 10 and burns, malignant hematological disorders, and deferoxamine chelation-therapy in subjects receiving hemodialysis.

10 [0006] Recent reports have demonstrated a striking increase in the number of reported cases of mucormycosis over the last two decades (Gleissner et al., *Leuk. Lymphoma* 45(7):1351-60 (2004)). There has also been an alarming rise in the incidence 15 of mucormycosis at major transplant centers. For example, at the Fred Hutchinson Cancer Center, Marr et al. have described a greater than doubling in the number of cases from 1985-1989 to 1995-1999 (Marr et al., *Clin. Infect. Dis.* 34(7):909-17 (2002)). Similarly, Kontoyiannis et al. have described a greater than doubling in the incidence of 20 mucormycosis in transplant subjects over a similar time-span (Kontoyiannis et al., *Clin. Infect. Dis.* 30(6):851-6 (2000)). Given the increasing prevalence of diabetes, cancer, and organ transplantation in the aging United States population, the rise in incidence of mucormycosis is anticipated to continue unabated for the foreseeable future.

25 [0007] Therefore, there exists a need for compounds and methods that can reduce the risk of mucormycosis pathogenesis and provide effective therapies without adverse effects. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF INVENTION

30 [0008] In accordance with the present invention, there are provided *Mucorales* CotH polypeptides and encoding nucleic acid molecules. The *Mucorales* CotH polypeptides and encoding nucleic acids can be advantageously used to diagnose, treat or prevent fungal conditions, in particular mucormycosis. Furthermore, the *Mucorales* CotH

polypeptides and encoding nucleic acids are useful to generate or screen for agents that can alter Mucorales CotH activity or expression, which can further be used to treat or prevent fungal conditions.

[0009] The invention also provides vectors containing Mucorales CotH nucleic acids, host cells containing such vectors, Mucorales CotH anti-sense nucleic acids and related compositions. The invention additionally provides Mucorales CotH oligonucleotides that can be used to hybridize to or amplify a Mucorales CotH nucleic acid. Anti-Mucorales CotH specific antibodies are also provided. Further provided are kits containing Mucorales CotH nucleic acids or Mucorales CotH specific antibodies.

10 Such kits and reagents can be used to diagnose fungal infection cause by Mucorales organisms. Also provided are pharmaceutical and vaccine compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Figure 1 shows a FAR-western blot of four open reading frames (ORF) predicted to be cell surface proteins.

[0011] Figure 2 shows GRP78 binds to germlings but not to spores of *R. oryzae*.

[0012] Figure 3 shows expression of the four glycosylphosphatidylinositol (GPI) anchored predicted to act as ligands to GRP78.

20 [0013] Figure 4, panels A and B, show expression of CotH genes in *R. oryzae* germlings incubated with endothelial cells.

[0014] Figure 5 shows homology of the 4 putative GPI-anchored proteins to each other and the number of predicted N-and O-glycosylation sites in CotH3.

25 [0015] Figure 6, panel A demonstrates the ability of *R. oryzae* to adhere to human umbilical endothelial cells but not plastic. Further, *Saccharomyces cerevisiae* doesn't adhere to endothelial cells. Panel B, showing *R. oryzae* CotH2 and CotH3 enabling *S. cerevisiae* to bind endothelial cells expressing GRP78.

[0016] Figure 7 shows CotH3 is conserved among various Mucorales including *R. oryzae* 99-880, *R. oryzae* 99-892, *Mucor* sp., *Lichtheimia corymbifera*, *Cunninghamella bertholletiae* and *R. microsporus*.

30 [0017] Figure 8, panels A and B, show *S. cerevisiae* expressing CotH2 or CotH3 adhere to and invades endothelial cells or CHO cells overexpressing GRP78 but not CHO parent cells.

[0018] Figure 9 shows the amino acid sequence (SEQ ID NO. 1) and the nucleic acid coding sequence (SEQ ID NO. 2) of CotH1 from *R. oryzae*.

[0019] Figure 10 shows the amino acid sequence (SEQ ID NO. 3) and the nucleic acid coding sequence (SEQ ID NO. 4) of CotH2 from *R. oryzae*.

5 [0020] Figure 11 shows the amino acid sequence (SEQ ID NO. 5) and the nucleic acid coding sequence (SEQ ID NO. 6) of CotH3 from *R. oryzae*.

[0021] Figure 12 shows the amino acid sequence (SEQ ID NO. 7) and the nucleic acid coding sequence (SEQ ID NO. 8) of RO3G_16295 from *R. oryzae*.

10 [0022] Figure 13 shows detection of CotH3 in sheep's blood spiked with *R. oryzae* by PCR using oligonucleotide primers having the nucleic acid sequence of SEQ ID NO: 33 and SEQ ID NO: 34.

[0023] Figure 14 shows detection of CotH3 in sheep's blood spiked with *Mucor sp.* or *Lichtheimia corymbifera* by PCR using oligonucleotide primers having the nucleic acid sequence of SEQ ID NO: 33 and SEQ ID NO: 34.

15 [0024] Figure 15 shows detection of CotH3 in sheep's blood spiked with *Cunninghamella bertholletiae* or *R. microsporus* by PCR using oligonucleotide primers having the nucleic acid sequence of SEQ ID NO: 33 and SEQ ID NO: 34.

20 [0025] Figure 16 shows no detection of CotH3 in sheep's blood spiked with *Aspergillus fumigates* or *Candida albicans* by PCR using oligonucleotide primers having the nucleic acid sequence of SEQ ID NO: 33 and SEQ ID NO: 34.

[0026] Figure 17 shows the highest homology of any of the CotH predicted proteins (in this case CotH3 [R03G_11882] SEQ ID NO: 5) with an amino acid sequence of a protein from *Stigmatella aurantiaca* (ZP_01460584) (SEQ ID NO: 65).

25 [0027] Figure 18 shows an amino acid sequence alignment between the RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Talaromyces stipitatus* ATCC 10500 (EED23986) protein (SEQ ID NO: 67).

[0028] Figure 19 shows an amino acid sequence alignment between the RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Penicillium marneffei* ATCC 18224 (XP_002144175) protein (SEQ ID NO: 68).

[0029] Figure 20 shows an amino acid sequence alignment between the RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Aspergillus niger* (XP_001392236) protein (SEQ ID NO: 69).

[0030] Figure 21 shows an amino acid sequence alignment between the 5 RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Aspergillus nidulans* (XP_658934) protein (SEQ ID NO: 70).

[0031] Figure 22 shows an amino acid sequence alignment between the RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Ustilago maydis* (XP_760027) protein (SEQ ID NO: 71).

10 [0032] Figure 23 shows an amino acid sequence alignment between the RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Coccidioides immitis* (XP_001243211) protein (SEQ ID NO: 72).

[0033] Figure 24 shows an amino acid sequence alignment between the RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Neurospora crassa* (XP_956792) 15 protein (SEQ ID NO: 73).

[0034] Figure 25 shows an amino acid sequence alignment between the RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Cryptococcus neoformans* (XP_775558) protein (SEQ ID NO: 74).

[0035] Figure 26 shows an amino acid sequence alignment between the 20 RO3G_16295 protein from *R. oryzae* (SEQ ID NO: 66) and *Streptomyces lividans* (EFD65170) protein (SEQ ID NO: 75).

[0036] Figure 27 shows a nucleic acid sequence of CotH3 from *Rhizopus oryzae* 99-880 (including introns from Data base) (SEQ ID NO: 9).

[0037] Figure 28 shows a nucleic acid sequence of CotH3 from *Rhizopus oryzae* 99- 25 880 (exon only from Data base) (SEQ ID NO: 10).

[0038] Figure 29 shows an amino acid sequence of CotH3 from *Rhizopus oryzae* 99-880 (predicted amino acids) (SEQ ID NO: 11).

[0039] Figure 30 shows a nucleic acid sequence of CotH3 from *Rhizopus oryzae* 99-880 (including introns from sequenced data) (SEQ ID NO: 12).

30 [0040] Figure 31 shows a nucleic acid sequence of CotH3 from *Rhizopus oryzae* 99-892 (exons only from Sequenced data) (SEQ ID NO: 13).

[0041] Figure 32 shows a nucleic acid sequence of CotH3 from *Rhizopus oryzae* 99-892 (including intron from Sequenced data) (SEQ ID NO: 14).

[0042] Figure 33 shows the predicted amino acid sequence of CotH3 from *R. oryzae* 99-892 (excluding introns) (SEQ ID NO: 15).

5 [0043] Figure 34 shows the predicted amino acid sequence of CotH3 from *R. oryzae* 99-892 (including introns) (SEQ ID NO: 16).

[0044] Figure 35 shows a nucleic acid sequence of CotH3 sequence from *Mucor* sp. 99-932(exons only from sequenced data) (SEQ ID NO: 17).

[0045] Figure 36 shows the predicted amino acid sequence of CotH3 from *Mucor* 99-932(AA exons only) (SEQ ID NO: 18).

5 [0046] Figure 37 shows a nucleic acid sequence of CotH3 from *Mucor* 99-932 (including introns) (SEQ ID NO: 19).

[0047] Figure 38 shows the predicted amino acid sequence of CotH3 from *Mucor* 99-932(including introns) (SEQ ID NO: 20).

10 [0048] Figure 39 shows a nucleic acid sequence of CotH3 from *Lichtheimia* *corymbifera* (exons only from sequenced data) (SEQ ID NO: 21).

[0049] Figure 40 shows the predicted amino acid sequence of CotH3 from *Lichtheimia* *corymbifera* (exons only from sequenced data) (SEQ ID NO: 22).

[0050] Figure 41 shows a nucleic acid sequence of CotH3 from *Lichtheimia* *corymbifera* (including introns) (SEQ ID NO: 23).

15 [0051] Figure 42 shows the predicted amino acid sequence of CotH3 from *Lichtheimia* *corymbifera* (including introns) (SEQ ID NO: 24).

[0052] Figure 43 shows a nucleic acid sequence of CotH3 sequence from *Cunninghamella bertholletiae* (exons only) (SEQ ID NO: 25).

20 [0053] Figure 44 shows the predicted amino acid sequence of CotH3 from *Cunninghamella bertholletiae* (exons only from sequenced data) (SEQ ID NO: 26).

[0054] Figure 45 shows a nucleic acid sequence of CotH3 from *Cunninghamella bertholletiae* (including introns) (SEQ ID NO: 27).

[0055] Figure 46 shows the predicted amino acid sequence of CotH3 from *Cunninghamella bertholletiae* (including introns from sequenced data) (SEQ ID NO: 28).

25 [0056] Figure 47 shows a nucleic acid sequence of CotH3 from *R. microsporus* (exons only from sequenced data) (SEQ ID NO: 29).

[0057] Figure 48 shows the predicted amino acid sequence of CotH3 from *R. microsporus* (exons only from sequenced data) (SEQ ID NO: 30).

30 [0058] Figure 49 shows a nucleic acid sequence of CotH3 from *R. microsporus* (including introns, has only one intron) (SEQ ID NO: 31).

[0059] Figure 50 shows the predicted amino acid sequence of CotH3 from *R. microsporus* (including introns) (SEQ ID NO: 32).

[0060] Figure 51, panels A and B, show a FAR western blot of *R. oryzae* surface proteins that bound to GRP78 (Panel A) and a dendrogram showing the close identity

between *CotH2* and *CotH3* predicated proteins and the divergence of the *CotH* proteins from the fourth identified ORF widely present in fungi without an identified function (i.e. RO3G_16295) (Panel B).

[0061] Figure 52, panels A-C, show expression of *CotH* genes and RO3G_16295 in response to germination and to host cell interaction. All *CotH* genes were expressed in resting spores but only *CotH3* was expressed in germlings of *R. oryzae* grown in YPD at 37°C (Panel A). Exposure of *R. oryzae* germlings to endothelial cells induced expression of only *CotH2* and *CotH3* genes as determined by RT-PCR (Panel B). Quantification of gene expression of *CotH* genes in *R. oryzae* germlings on endothelial cells by qRT-PCR demonstrated 16 and 4 fold increase in expression of *CotH3* and *CotH2* relative to the non expressed *CotH1*, respectively. RO3G_16295 was not expressed under any of the conditions tested. * $P<0.001$ vs. *CotH1* expression and ** $P<0.001$ vs. *CotH1* and *CotH2* expression, by Wilcoxon Rank Sum test (Panel C). N=9 from three independent experiments.

[0062] Figure 53 shows antibodies raised against peptide GAGKKHNNAKQSWNW (SEQ ID NO: 39) recognized *CotH2* and *CotH3* but not *CotH1* proteins heterologously expressed by *S. cerevisiae*. Peptide was coupled with KLH and used to raise rabbit antibodies commercially. *S. cerevisiae* heterologously expressing *CotH* proteins were stained with the antibodies then counter stained with FITC labeled anti-rabbit goat antibody prior to visualizing the cells with confocal microscopy.

[0063] Figure 54, panels A-C, show endothelial cell surface GRP78 binds to *S. cerevisiae* cells heterologously expressing *CotH2* or *CotH3* but not *CotH1* and *S. cerevisiae* expressing *CotH2* or *CotH3* adhered and invaded endothelial cells or CHO cells overexpressing GRP78 but not *S. cerevisiae* expressing *CotH1* or empty plasmid.

Endothelial cell surface proteins were labeled with NHS-biotin and then extracted with *n*-octyl-β-d-glucopyranoside in PBS containing Ca^{2+} and Mg^{2+} and protease inhibitors. The labeled proteins (250 µg) were incubated with yeast cells (2×10^8), then the unbound proteins were removed by extensive rinsing with PBS containing Ca^{2+} and Mg^{2+} . The membrane proteins that remained bound to the organisms were eluted with 6 M urea, separated on 10% SDS-PAGE, and identified by immunoblotting with anti-GRP78 Ab (Panel A). Adherence and endocytosis (determined by differential fluorescence) assays were carried out using endothelial cells (Panel B), or CHO parent cells or those overexpressing GRP78 (Panel C) split on 12-mm glass coverslips. * $P<0.001$ vs. *S. cerevisiae* expressing empty plasmid or *CotH1* and ** $P<0.001$ vs. *CotH1* and *CotH2*

expression, by Wilcoxon Rank Sum test. N=9 from three independent experiments. Data are expressed as median \pm interquartile range.

[0064] Figure 55, panels A and B, show anti-CotH3 Abs (raised against peptide GAGKKHNNAKQSWNW (SEQ ID NO: 39) block endothelial cell endocytosis of and

5 damage by *R. oryzae*. Adherence and endocytosis (determined by differential fluorescence) assays were carried out using endothelial cells split on 12-mm glass coverslips, while damage was carried out using the 96-well plate ^{51}Cr - release method.

Endothelial cells were incubated with 50 $\mu\text{g}/\text{ml}$ anti-CotH3 or with serum from the same rabbit prior to vaccination (control) for 1 hour prior to addition of *R. oryzae* germlings.

10 Blocking of CotH3 and CotH2 (since the antibodies react to CotH2 proteins) abrogates endocytosis of *R. oryzae* by endothelial cells (data derived from >700 fungal cells interacting with approximately 200 endothelial cells/each group/experiment, with an average of 59% cells being endocytosed in the control) (Panel A) and reduces the ability of the fungus to cause endothelial cell damage (Panel B). * $P < 0.01$ compared with pre-
15 vaccinated serum or with no serum by Wilcoxon rank-sum test. $n = 6$ slides per group from 3 independent experiments for endocytosis, and $n = 6$ wells per group from 2 independent experiments for damage assay. Data are expressed as median \pm interquartile range.

[0065] Figure 56, panels A-C, show RNA-i construct targeting CotH2 and

20 CotH3 inhibits the expression of both genes, reduces CotH2 and CotH3 protein synthesis on the cell surface and has no effect on the growth or the pattern of germination of *R. oryzae*. *R. oryzae* was transformed with an RNA-i construct (pRNAi) targeting *CotH2* and *CotH3* expression or with empty plasmid. Two transformants were shown to have > 80% reduction in the expression of *CotH2* and *CotH3* relative to cells transformed with the
25 empty plasmid as determined by qRT-PCR (Panel A). Flow cytometry testing using anti-*CotH* antibodies demonstrated reduction in cell surface expression of *CotH* proteins on *R. oryzae* cells transformed with the RNA-i construct compared to those transformed with the empty plasmid, wild type cells or negative control (i.e. wild type *R. oryzae* stained with commercial IgG instead of anti-*CotH* antibodies) (Panel B). The two transformants with
30 reduced *CotH2* and *CotH3* expression had similar growth rate (Panel C) as the wild type cells or cells transformed with the empty plasmid.

[0066] Figure 57, panels A-C, show inhibition of *CotH2* and *CotH3* expression compromise the ability of *R. oryzae* to invade and damage endothelial cells and CHO cells overexpressing GRP78. Adherence and endocytosis (determined by differential

fluorescence) assays were carried out using endothelial cells split on 12-mm glass coverslips, while damage was carried out using the 96-well plate ^{51}Cr -release method. *R. oryzae* germlings transformed with the RNA-i construct caused less invasion (Panel A) and damage (Panel B) to endothelial cells when compared to cells transformed with empty 5 plasmid. Transformants with RNA-i targeting *CotH2* and *CotH3* caused equivalent damage to CHO cells overexpressing GRP78 when compared to CHO parent cells. In contrast, *R. oryzae* germlings transformed with the empty plasmid or wild type *R. oryzae* caused significantly more damage to CHO cell overexpressing GRP78 vs. CHO parent 10 cells (Panel C). * $P < 0.005$ compared with empty plasmid, ** $P < 0.01$ vs. wild type or empty plasmid, and ‡ $P < 0.01$ vs. CHO parent cells by Wilcoxon rank-sum test. $n = 6$ slides per group from 3 independent experiments for endocytosis, and $n = 9$ wells per group from 3 independent experiments for damage assay. Data are expressed as median \pm interquartile range.

[0067] Figure 58, panels A-C, show inhibition of *CotH2* and *CotH3* expression 15 attenuates virulence of *R. oryzae* in diabetic ketoacidotic mice. Panel A shows the survival of mice ($n = 10$ for wild type or 9 for RNA-i or empty plasmid transformants) infected intratracheally with one of the three strains. Inhaled inocula were 2.4×10^3 , 2.8×10^3 , and 2.5×10^3 spores, for wild type, empty plasmid, or RNA-i cells, respectively. * $P < 0.003$ vs. wild type or empty plasmid infected mice by log Rank test. Panel B shows the lung 20 and brain fungal burden of diabetic ketoacidotic mice ($n = 9$ per group) infected intratracheally with wild type (1.7×10^3), empty plasmid (3.0×10^3) or RNA-i (3.1×10^3) cells. Mice were sacrificed on day +2 relative to infection and their organs processed for tissue fungal burden using SYBR green assay. Data are expressed as median \pm interquartile range. * $P < 0.001$ compared to wild type or empty plasmid infected mice by 25 Wilcoxon Rank Sum test. Panel C shows *in vivo* expression of *CotH* genes in lungs and brains harvested from mice infected with wild type, empty plasmid or RNA-i construct as determined by qRT-PCR using specific primers to each of the *CotH* genes. Data are expressed as mean \pm SD. * $P < 0.001$ vs. wild type or empty plasmid.

[0068] Figure 59 shows histopathological examination of lungs harvested from 30 diabetic ketoacidotic mice infected with wild type or *R. oryzae* transformed with empty plasmid or RNA-i. Periodic acid Schieff (PAS) stained sections demonstrating extensive hyphal elements (arrows) from organs collected from mice infected with wild type or *R. oryzae* transformed with empty plasmid but not *R. oryzae* transformed with RNA-i construct.

[0069] Figure 60 shows passive immunization with antiCotH antibodies raise against peptide GAGKKHNNAKQSWNW (SEQ ID NO: 39) (A) or peptide MGQTNDGAYRDPTDNNK (SEQ ID NO: 40) (B) protect mice from *R. oryzae* infection. Diabetic ketoacidotic mice were given 1 mg of antiCotH IgG or pre-vaccination serum 5 (control) 2 hr prior to infecting intratracheally with 2.4×10^3 spores of *R. oryzae* 99-880 (wild type). A second dose of the polyclonal antibody or the pre- vaccination serum was given on day +3 relative to infection. * $P < 0.03$ vs. mice receiving pre-vaccination serum.

[0070] Figure 61 shows the specificities of the CotH3 molecular beacon detection of different fungal species. The amplification plot was generated in StepOnePlus 10 Real-Time PCR machine (Applied Biosystems). The x axis is the time from the initiation of amplification; the y axis is the increase in fluorescence (ΔRn); threshold fluorescence is shown as the bold horizontal line (It is equal to the average plus 2 times SD for the water negative control samples). Signals can be amplified from water samples (0.5 ml) spiked with 10^5 spores of *R. oryzae* but not *Candida albicans* or *Aspergillus fumigatus*.

[0071] Figure 62 shows the sensitivity of the CotH3 molecular beacon detection 15 of water samples (0.5 ml) spiked with different inocula of *R. oryzae* 99-880. The amplification plot was generated in StepOnePlus Real-Time PCR machine (Applied Biosystems). The x axis is the time from the initiation of amplification; the y axis is the increase in fluorescence (ΔRn); threshold fluorescence is shown as the bold horizontal line 20 (It is equal to the average plus 2 times SD for the water negative control samples).

[0072] Figure 63 shows the sensitivity and specificity of the CotH3 molecular beacon probe in detection of *Rhizopus oryzae* spores in blood. The amplification plot was generated in StepOnePlus Real-Time PCR machine (Applied Biosystems). The x axis is the time from the initiation of amplification; the y axis is the increase in fluorescence 25 (ΔRn); threshold fluorescence is shown as the bold horizontal line (It is equal to the average plus 2 times SD for the water negative control samples). Blood, is inoculated control. R: *R. oryzae* 99-880 at different inocula (e.g. R10= *R. oryzae* 10 spores used to spike 350 μ l of blood), A: *A. fumigants*, C: *C. albicans* each used to spike 350 μ l of blood at 10^5 cells.

[0073] Figure 64 shows the specificity of the CotH3 molecular beacon probe in 30 detection of *Rhizopus*. The amplification plot was generated in a StepOnePlus Real-Time PCR machine (Applied Biosystems). The x axis is the time from the initiation of amplification; the y axis is the increase in fluorescence (ΔRn); threshold fluorescence is shown as the bold horizontal line (It is equal to the average plus 2 times SD for the water

negative control samples). Blood: uninoculated blood sample; 99-892: *R. oryzae* 99-892; ATCC62417: *R. microspores* ATCC62417; R1000: *R. oryzae* 99-880. All strains were used to spike blood (350 µl) with 10³ spores.

5

DETAILED DESCRIPTION OF THE INVENTION

[0074] The compositions and methods disclosed herein are based, at least in part, on the identification and characterization of cell surface proteins that are uniquely expressed by fungi of the Mucorales order and can facilitate binding of endothelial cells during fungal infections. Mucormycosis, which is mainly caused by *Rhizopus oryzae*, is characterized by angioinvasion and vascular thrombosis. Interactions between Mucorales and endothelial cells is an important factor in establishing a fungal condition. The recently identified Glucose Regulated Protein 78 (GRP78), a novel host receptor that mediates invasion and subsequent damage of human umbilical vein endothelial cells by *R. oryzae* germlings, provides a likely target ligand for *R. oryzae* and other Mucorales species to bind during invasion (Liu et al., *J. Clin. Invest.* 120:1914-24 (2010)).

[0075] In accordance with the present invention, provided are nucleic acids encoding Mucorales CotH polypeptides and other polypeptides disclosed herein, or functional polypeptide fragments thereof.

[0076] As used herein, the term "Mucorales CotH" refers to sub-family members of the CotH family of proteins, wherein the Mucorales CotH proteins include cell surface proteins expressed by fungi in the Mucorales order that are involved in the process of adherence and invasion of host cells, such as endothelial cell. Because Mucorales CotH proteins are unique to Mucorales, the presence or absence of Mucorales CotH nucleic acid or polypeptide or changes in Mucorales CotH nucleic acid or polypeptide expression can serve as a marker for infection by a Mucorales species, for example, mucormycosis. Thus, the invention includes Mucorales CotH nucleic acids and/or polypeptides that can be used for screening for a fungal condition and/or for developing drug candidates for the treatment of a fungal condition.

[0077] The term "functional," when used herein as a modifier of an Mucorales CotH polypeptide, or polypeptide fragment thereof, refers to a polypeptide that exhibits functional characteristics similar to CotH1, CotH2 and CotH3 as disclosed herein. For example, when CotH3 or CotH2 were expressed in *S. cerevisiae*, the *S. cerevisiae* cells adhere to and invade endothelial cells or CHO cells overexpressing GRP78. Therefore, one function of Mucorales CotH is a pro-adherance and/or pro-invasion function. In

another aspect, a functional Murcorales CotH polypeptide or fragment thereof can also include *in vivo* or *in vitro* binding to a GRP78 protein, variant or fragment thereof.

[0078] The nucleic acid molecules described herein are useful for producing invention proteins, when such nucleic acids are incorporated into a variety of protein expression systems known to those of skill in the art. In addition, such nucleic acid molecules or fragments thereof can be labeled with a readily detectable substituent and used as hybridization probes for assaying for the presence and/or amount of an invention Murcorales CotH gene or mRNA transcript in a given sample. The nucleic acid molecules described herein, and fragments thereof, are also useful as primers and/or templates in a PCR reaction for amplifying genes encoding invention proteins described herein.

[0079] The term "nucleic acid", also referred to as polynucleotides, encompasses ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), probes, oligonucleotides, and primers and can be single stranded or double stranded. DNA can be either complementary DNA (cDNA) or genomic DNA, and can represent the sense strand, the anti-sense strand or both. Examples of nucleic acids are RNA, cDNA, or isolated genomic DNA encoding an Murcorales CotH polypeptide. Such nucleic acids include, but are not limited to, nucleic acids comprising substantially the same nucleotide sequence as set forth in 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31. In general, a genomic sequence of the invention includes regulatory regions such as promoters, enhancers, and introns that are outside of the exons encoding a Murcorales CotH but does not include proximal genes that do not encode Murcorales CotH.

[0080] Use of the terms "isolated" and/or "purified" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been produced in such form by the hand of man, and thus are separated from their native *in vivo* cellular environment.

[0081] The term substantially the same nucleotide sequence refers to DNA having sufficient identity to the reference polynucleotide, such that it will hybridize to the reference nucleotide under moderately stringent hybridization conditions. In one embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide sequence encodes substantially the same amino acid sequence as that set forth in any of SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31. In another embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide sequence has at least 65% identity with respect to the reference nucleotide sequence. DNA having substantially the same nucleotide sequence can have at least 65%

identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, or at least 99% identity to the reference nucleotide sequence.

[0082] As used herein, a "modification" of a nucleic acid can also include one or several nucleotide additions, deletions, or substitutions with respect to a reference sequence. A modification of a nucleic acid can include substitutions that do not change the encoded amino acid sequence due to the degeneracy of the genetic code. Such modifications can correspond to variations that are made deliberately, or which occur as mutations during nucleic acid replication.

[0083] Exemplary modifications of the recited Mucorales CotH sequences include sequences that correspond to homologs of other species, including species of the Mucorales order such as *A. corymbifera*, *A. elegans*, *A. rouxii*, *B. circina*, *B. multispora*, *C. brefeldii*, *C. angarensis*, *C. recurvatus*, *D. fulva*, *E. anomalus*, *H. elegans*, *H. assamensis*, *K. cordensis*, *M. amphibiorum*, *P. parasitica*, *P. agaricina*, *P. anomala*, *P. circinans*, *R. endophyticus*, *R. javensis*, *S. umbellata*, *S. megalocarpus*, *T. elegans*, *T. indicae-seudatica*, *Z. californiensis*, *R. azygosporus*, *R. caespitosus*, *R. homothallicus*, *R. oryzae*, *R. microspores*, *R. microsporus* var. *rhizopodiformis*, *R. schipperae*, or any other species of the Mucorales order disclosed herein. The corresponding Mucorales CotH sequences of Mucorales species can be determined by methods known in the art, such as by PCR or by screening genomic, cDNA or expression libraries.

[0084] Another exemplary modification of the invention Mucorales CotH can correspond to splice variant forms of the Mucorales CotH nucleotide sequence. Additionally, a modification of a nucleotide sequence can include one or more non-native nucleotides, having, for example, modifications to the base, the sugar, or the phosphate portion, or having a modified phosphodiester linkage. Such modifications can be advantageous in increasing the stability of the nucleic acid molecule.

[0085] The invention also encompasses nucleic acids which differ from the nucleic acids shown in SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31, but which have the same phenotype. Phenotypically similar nucleic acids are also referred to as functionally equivalent nucleic acids. As used herein, the phrase functionally equivalent nucleic acids encompasses nucleic acids characterized by slight and non-consequential sequence variations that will function in substantially the same manner to produce the same protein product(s) as the nucleic acids disclosed herein. In particular, functionally equivalent nucleic acids encode polypeptides that are the same as those

encoded by the nucleic acids disclosed herein or that have conservative amino acid variations. For example, conservative variations include substitution of a non-polar residue with another non-polar residue, or substitution of a charged residue with a similarly charged residue. These variations include those recognized by skilled artisans as 5 those that do not substantially alter the tertiary structure of the protein.

[0086] Further provided are nucleic acids encoding Mucorales CotH polypeptides that, by virtue of the degeneracy of the genetic code, do not necessarily hybridize to the invention nucleic acids under specified hybridization conditions. As used herein, the term degenerate refers to codons that differ in at least one nucleotide from a 10 reference nucleic acid, but encode the same amino acids as the reference nucleic acid.

Nucleic acids encoding the invention Mucorales CotH polypeptides can be comprised of nucleotides that encode substantially the same amino acid sequence as set forth in SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32.

[0087] In one embodiment, the invention provides an isolated nucleic acid 15 encoding a polypeptide as disclosed herein including a Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof. The invention also provides an isolated nucleic acid encoding a Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof, comprising a nucleic acid selected from: (a) nucleic acid encoding an amino acid sequence set forth in SEQ ID 20 NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32, or (b) nucleic acid that hybridizes to the nucleic acid of (a) under low, moderately or highly stringent conditions, wherein said nucleic acid contiguously encodes biologically active Mucorales CotH 25 polypeptide, or (c) nucleic acid degenerate with respect to either (a) or (b) above, wherein said nucleic acid encodes biologically active Mucorales CotH polypeptide. In one aspect, the nucleic acid of the invention hybridizes under highly stringent conditions.

[0088] Hybridization refers to the binding of complementary strands of nucleic acid, for example, sense:antisense strands or probe:target-nucleic acid to each other through hydrogen bonds, similar to the bonds that naturally occur in chromosomal DNA. Stringency levels used to hybridize a given probe with target-DNA can be readily varied 30 by those of skill in the art.

[0089] The phrase "stringent hybridization" is used herein to refer to conditions under which polynucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of a hybrid is a function of sodium ion concentration and temperature.

Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions.

[0090] As used herein, the phrase "moderately stringent hybridization" refers to conditions that permit target-nucleic acid to bind a complementary nucleic acid. The hybridized nucleic acids will generally have at least about 60% identity, at least about 75% identity, or at least about 85% identity; or at least about 90% identity. Moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42EC, followed by washing in 0.2X SSPE, 0.2% SDS, at 42EC.

[0091] The phrase "highly stringent hybridization" refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65EC, for example, if a hybrid is not stable in 0.018M NaCl at 65EC, it will not be stable under high stringency conditions, as contemplated herein. High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42EC, followed by washing in 0.1X SSPE, and 0.1% SDS at 65EC.

[0092] The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 22EC, followed by washing in 1X SSPE, 0.2% SDS, at 37EC. Denhart's solution contains 1% Ficoll, 1% polyvinylpyrrolidone, and 1% bovine serum albumin (BSA). 20X SSPE (sodium chloride, sodium phosphate, ethylene diamide tetraacetic acid (EDTA)) contains 3M sodium chloride, 0.2M sodium phosphate, and 0.025 M (EDTA). Other suitable moderate stringency and high stringency hybridization buffers and conditions are well known to those of skill in the art and are described, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1989; and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, MD (1999)). Nucleic acids encoding polypeptides hybridize under moderately stringent or high stringency conditions to substantially the entire sequence, or substantial portions, for example, typically at least 15-30 nucleotides of the nucleic acid sequence set forth in SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31.

[0093] The invention also provides a modification of a Mucorales CotH nucleotide sequence that hybridizes to a Mucorales CotH nucleic acid molecule, for example, a nucleic acid molecule set forth in SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31, under moderately stringent conditions. Modifications of 5 Mucorales CotH nucleotide sequences, where the modification has at least 65% identity to a Mucorales CotH nucleotide sequence, are also provided. The invention also provides modification of a Mucorales CotH nucleotide sequence having at least 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, or at least 99% identity. The 10 invention also provides modification of Mucorales CotH nucleotide sequences, wherein the amino acid sequence encoded by the modified nucleic acid has 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, or at least 99% identity to the amino acid sequence set forth in SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 15 32.

[0094] "Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or 20 amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present invention.

[0095] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be 25 determined using software programs known in the art, for example those described in Ausubel et al., *supra*. Preferably, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions 30

= 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein + SPupdate + PIR. Details of these programs can be found at the National Center for Biotechnology Information.

5 Biologically equivalent polynucleotides are those having the specified percent homology and encoding a polypeptide having the same or similar biological activity.

[0096] One means of isolating a nucleic acid encoding a Mucorales CotH polypeptide is to probe a cDNA library or genomic library with a natural or artificially designed nucleic acid probe using methods well known in the art. Nucleic acid probes derived from the Mucorales CotH gene are particularly useful for this purpose. DNA and 10 cDNA molecules that encode Mucorales CotH polypeptides can be used to obtain complementary genomic DNA, cDNA or RNA from any number of Mucorales species sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods well known in the art (see, for example, Sambrook et al., *supra*, 1989; Ausubel et al., *supra*, 1999).

15 [0097] The invention additionally provides a Mucorales CotH oligonucleotide comprising between 15 and 300 contiguous nucleotides of SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31, or the anti-sense strand thereof. As used herein, the term "oligonucleotide" refers to a nucleic acid molecule that includes at least 15 contiguous nucleotides from a reference nucleotide sequence, and can include at least 16, 20 17, 18, 19, 20 or at least 25 contiguous nucleotides, and often includes at least 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, up to 350 contiguous nucleotides from the reference nucleotide sequence. The reference nucleotide sequence can be the sense strand or the anti-sense strand. Accordingly, in one aspect of the inventions, CotH oligonucleotides can comprise the nucleic acid sequence of 25 ATGAAATTATCTATTATCCGCTGCC (SEQ ID NO: 33),
GCTGGGAATATAATTGTCATCGA (SEQ ID NO: 34),
GATGACAATTATATTCCCAGC (SEQ ID NO: 35),
GAGTAGACGTAATTAGATCCAA (SEQ ID NO: 36)
AAACGTACCTGCTGACCGAAC (SEQ ID NO: 37) or oligonucleotide disclosed 30 herein.

[0098] The Mucorales CotH oligonucleotides of the invention that contain at least 15 contiguous nucleotides of a reference Mucorales CotH nucleotide sequence are able to hybridize to Mucorales CotH under moderately stringent hybridization conditions and thus can be advantageously used, for example, as probes to detect Mucorales CotH

DNA or RNA in a sample, and to detect splice variants thereof; as sequencing or PCR primers; as antisense reagents to block transcription of Mucorales CotH RNA in cells; or in other applications known to those skilled in the art in which hybridization to a Mucorales CotH nucleic acid molecule is desirable.

5 [0099] The isolated Mucorales CotH nucleic acid molecules of the invention can be used in a variety of diagnostic and therapeutic applications. For example, the isolated Mucorales CotH nucleic acid molecules of the invention can be used as probes, as described above; as templates for the recombinant expression of Mucorales CotH polypeptides; or in screening assays to identify cellular molecules that bind Mucorales

10 CotH.

[00100] Another useful method for producing a Mucorales CotH nucleic acid molecule of the invention involves amplification of the nucleic acid molecule using PCR and Mucorales CotH oligonucleotides and, optionally, purification of the resulting product by gel electrophoresis. Either PCR or RT-PCR can be used to produce a Mucorales CotH 15 nucleic acid molecule having any desired nucleotide boundaries. Desired modifications to the nucleic acid sequence can also be introduced by choosing an appropriate oligonucleotide primer with one or more additions, deletions or substitutions. Such nucleic acid molecules can be amplified exponentially starting from as little as a single gene or mRNA copy, from any cell, tissue or species of interest.

20 [00101] The invention thus provides methods for detecting Mucorales CotH nucleic acid in a sample. The methods of detecting Mucorales CotH nucleic acid in a sample can be either qualitative or quantitative, as desired. For example, the presence, abundance, integrity or structure of a Mucorales CotH can be determined, as desired, depending on the assay format and the probe used for hybridization or primer pair chosen

25 for application.

[00102] Useful assays for detecting Mucorales CotH nucleic acid based on specific hybridization with an isolated Mucorales CotH nucleic acid molecule are well known in the art and include, for example, *in situ* hybridization, which can be used to detect altered chromosomal location of the nucleic acid molecule, altered gene copy 30 number, and RNA abundance, depending on the assay format used. Other hybridization assays include, for example, Northern blots and RNase protection assays, which can be used to determine the abundance and integrity of different RNA splice variants, and Southern blots, which can be used to determine the copy number and integrity of DNA. A Mucorales CotH hybridization probe can be labeled with any suitable detectable moiety,

such as a radioisotope, fluorochrome, chemiluminescent marker, biotin, or other detectable moiety known in the art that is detectable by analytical methods.

[00103] Useful assays for detecting a Mucorales CotH nucleic acid in a sample based on amplifying a Mucorales CotH nucleic acid with two or more Mucorales CotH oligonucleotides are also well known in the art, and include, for example, qualitative or quantitative polymerase chain reaction (PCR); reverse-transcription PCR (RT-PCR); single strand conformational polymorphism (SSCP) analysis, which can readily identify a single point mutation in DNA based on differences in the secondary structure of single-strand DNA that produce an altered electrophoretic mobility upon non-denaturing gel

10 electrophoresis; and coupled PCR, transcription and translation assays, such as a protein truncation test, in which a mutation in DNA is determined by an altered protein product on an electrophoresis gel. Additionally, the amplified Mucorales CotH nucleic acid can be sequenced to detect mutations and mutational hot-spots, and specific assays for large-scale screening of samples to identify such mutations can be developed.

15 **[00104]** The invention further provides an isolated Mucorales CotH polypeptides, immunogenic fragment thereof, or a functional fragment thereof, encoded by a Mucorales CotH nucleic acid of the invention. For example, the invention provides a polypeptide comprising the same or substantially the same amino acid sequence as set forth in SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32. Also provided is a Mucorales 20 CotH polypeptide encoded by a nucleotide sequence comprising the same or substantially the same nucleotide sequence as set forth in SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31.

25 **[00105]** As employed herein, the term substantially the same amino acid sequence refers to amino acid sequences having at least about 65% identity with respect to the reference amino acid sequence, and retaining comparable functional and biological activity characteristic of the protein defined by the reference amino acid sequence. In one aspect, proteins having substantially the same amino acid sequence will have at least 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, or at least 99% 30 identity. It is recognized, however, that polypeptides, or encoding nucleic acids, containing less than the described levels of sequence identity arising as splice variants or that are modified by conservative amino acid substitutions, or by substitution of degenerate codons are also encompassed within the scope of the present invention.

[00106] Also encompassed by the term Mucorales CotH are functional fragments or polypeptide analogs thereof. The term "functional fragment" refers to a peptide fragment that is a portion of a full length Mucorales CotH protein, provided that the portion has a biological activity, as defined herein, that is characteristic of the corresponding full length protein. For example, in aspect of the invention, the functional fragments of the invention can bind to the GRP78 protein or more specifically the functional fragments of the invention can bind to the GRP78 protein expressed by epithelial cells. Thus, the invention also provides functional fragments of invention Mucorales CotH proteins, which can be identified using the binding and routine methods, such as bioassays described herein.

[00107] As used herein, the term "polypeptide" when used in reference to Mucorales CotH is intended to refer to a peptide or polypeptide of two or more amino acids. The term polypeptide analog includes any polypeptide having an amino acid residue sequence substantially the same as a sequence specifically described herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the ability to functionally mimic a Mucorales CotH as described herein. A "modification" of a Mucorales CotH polypeptide also encompasses conservative substitutions of a Mucorales CotH polypeptide amino acid sequence.

Conservative substitutions of encoded amino acids include, for example, amino acids that belong within the following groups: (1) non-polar amino acids (Gly, Ala, Val, Leu, and Ile); (2) polar neutral amino acids (Cys, Met, Ser, Thr, Asn, and Gln); (3) polar acidic amino acids (Asp and Glu); (4) polar basic amino acids (Lys, Arg and His); and (5) aromatic amino acids (Phe, Trp, Tyr, and His). Other minor modifications are included within Mucorales CotH polypeptides so long as the polypeptide retains some or all of its function as described herein.

[00108] The amino acid length of functional fragments or polypeptide analogs of the present invention can range from about 5 amino acids up to the full-length protein sequence of an invention Mucorales CotH. In certain embodiments, the amino acid lengths include, for example, at least about 10 amino acids, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 250 or more amino acids in length up to the full-length Mucorales CotH protein sequence. The functional fragments can be contiguous amino acid sequences of a

Mucorales CotH polypeptide, including contiguous amino acid sequences of SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32.

[00109] In another embodiment, the invention provides an immunogenic fragment of the Mucorales CotH polypeptides disclosed herein. The immunogenic fragments of the invention can include immunogenic epitopes, which can be identified using experimental methods well known in the art. Additionally, computational modeling can also be used to identify immunogenic epitopes. See, for example, Tong et al. (*Brief Bioinform.* 8(2):96-108 (2006)) and Ponomarenko et al. (2008) "B-cell epitope prediction," in Structural Bioinformatics, Bourne PE and Gu J (eds) Wiley-Liss; 2 edition, pgs. 849-879. Once an epitope bearing reactivity with an antibody raised against the intact protein is identified, the polypeptide can be tested for specificity by amino acid substitution at every position and/or extension at both C and/or N terminal ends. Such epitope bearing polypeptides typically contain at least six to fourteen amino acid residues, and can be produced, for example, by polypeptide synthesis using methods well known in the art or by fragmenting an existing protein. Accordingly, in some aspects of the invention, an immunogenic fragment of the Mucorales CotH polypeptides disclosed herein can include the amino acid sequence GAGKKHNNAKQSWNW (SEQ ID NO: 39) or MGQTNDGAYRDPTDNNK (SEQ ID NO: 40).

[00110] With respect to the molecule used as immunogens pursuant to the present invention, those skilled in the art will recognize that the protein can be truncated or fragmented without losing the essential qualities as an immunogenic vaccine. For example, a protein can be truncated to yield an N-terminal fragment by truncation from the C-terminal end with preservation of the functional properties of the molecule as an immunogenic. Similarly, C-terminal fragments can be generated by truncation from the N-terminal end with preservation of the functional properties of the molecule as an immunogenic. Other modifications in accordance with the teachings and guidance provided herein can be made pursuant to this invention to create other polypeptide functional fragments, immunogenic fragments, variants, analogs or derivatives thereof, to achieve the therapeutically useful properties described herein with the native proteins.

[00111] Accordingly, the term "immunogenic fragment" as it is used herein refers to a portion of a protein that is recognized by a T-cell and/or B-cell antigen receptor. The immunogenic portion generally includes at least 5 amino acid residues, or alternatively at least 6, or alternatively at least 7, or alternatively at least 8, or alternatively at least 9, or alternatively at least 10, or alternatively at least 11, or alternatively at least 12, or

alternatively at least 13, or alternatively at least 14, or alternatively at least 15, or alternatively at least 16, or alternatively at least 17, or alternatively at least 18, or alternatively at least 18, or alternatively at least 19, or alternatively at least 20, or alternatively at least 25, or alternatively at least 30, or alternatively at least 50, or

5 alternatively at least 100 amino acid residues of a CotH polypeptide disclosed herein. Alternatively, the immunogenic portion can include at most 5 amino acid residues, or alternatively at most 6, or alternatively at most 7, or alternatively at most 8, or alternatively at most 9, or alternatively at most 10, or alternatively at most 11, or alternatively at most 12, or alternatively at most 13, or alternatively at most 14, or

10 alternatively at most 15, or alternatively at most 16, or alternatively at most 17, or alternatively at most 18, or alternatively at most 18, or alternatively at most 19, or alternatively at most 20, or alternatively at most 25, or alternatively at most 30, or alternatively at most 50, or alternatively at most 100 amino acid residues of a CotH polypeptide disclosed herein. In some aspects, immunogenic portions can contain a small

15 N-and/or C-terminal fragment (e.g., 5-30 amino acids, preferably 10-25 amino acids).

[00112] A modification of a polypeptide can also include derivatives, analogues and functional mimetics thereof, provided that such polypeptide displays the Mucorales CotH biological activity. For example, derivatives can include chemical modifications of the polypeptide such as alkylation, acylation, carbamylation, iodination, or any

20 modification that derivatizes the polypeptide. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups can be derivatized to form salts, methyl and ethyl esters or other types of esters or

25 hydrazides. Free hydroxyl groups can be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine can be derivatized to form N-im-benzylhistidine. Also included as derivatives or analogues are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids, for example, 4-hydroxyproline, 5-hydroxylysine, 3-methylhistidine,

30 homoserine, ornithine or carboxyglutamate, and can include amino acids that are not linked by peptide bonds. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions of residues, relative to the sequence of a polypeptide whose sequence is shown herein, so long as Mucorales CotH activity is maintained.

[00113] The invention provides an isolated Mucorales CotH polypeptides, immunogenic fragment thereof, or functional fragment thereof. The invention Mucorales CotH polypeptides can be isolated by a variety of methods well-known in the art, for example, recombinant expression systems, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography, and the like. Other well-known methods are described in Deutscher et al., Guide to Protein Purification: Methods in Enzymology, Vol. 182, (Academic Press, (1990)). Alternatively, the isolated polypeptides of the present invention can be obtained using well-known recombinant methods (see, for example, Sambrook et al., *supra*, 1989; Ausubel et al., *supra*, 1999). The methods and conditions for biochemical purification of a polypeptide of the invention can be chosen by those skilled in the art, and purification monitored, for example, by an immunological assay or a functional assay.

[00114] An example of the means for preparing the invention polypeptide(s) is to express nucleic acids encoding Mucorales CotH in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian cell such as an oocyte, or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again using well-known purification methods, so described herein. Invention polypeptides can be isolated directly from cells that have been transformed with expression vectors as described herein.

Recombinantly expressed polypeptides of the invention can also be expressed as fusion proteins with appropriate affinity tags, such as glutathione S transferase (GST) or poly His, and affinity purified. The invention polypeptide, biologically functional fragments, and functional equivalents thereof can also be produced by chemical synthesis. For example, synthetic polypeptides can be produced using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, CA) employing the chemistry provided by the manufacturer.

[00115] The present invention also provides compositions containing an acceptable carrier and any of an isolated, purified Mucorales CotH mature protein or functional polypeptide fragments thereof, alone or in combination with each other. These polypeptides or proteins can be recombinantly derived, chemically synthesized or purified from native sources. As used herein, the term "acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as phosphate buffered saline solution, water and emulsions such as an oil and water emulsion, and various types of wetting agents.

[00116] The invention thus provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound selected from the group consisting of

a Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof as described herein, an antisense nucleic acid as described herein or an anti-Mucorales CotH antibody as described herein. The invention additionally provides a method of treating or preventing mucormycosis in a subject in need thereof by

5 administering a therapeutically effective amount of a pharmaceutical composition containing a pharmaceutically acceptable carrier and a compound selected from the group consisting of a Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof as described herein, an antisense nucleic acid as described herein or an anti-Mucorales CotH antibody as described herein. The invention additionally
10 provides a method of treating or preventing mucormycosis in a subject in need thereof by administering an therapeutically effective amount of a vaccine composition as disclosed herein.

[00117] Also provided are antisense-nucleic acids having a sequence capable of binding specifically with full-length or any portion of an mRNA that encodes Mucorales
15 CotH polypeptides so as to prevent translation of the mRNA. The antisense-nucleic acid can have a sequence capable of binding specifically with any portion of the sequence of the cDNA encoding Mucorales CotH polypeptides. As used herein, the phrase binding specifically encompasses the ability of a nucleic acid sequence to recognize a complementary nucleic acid sequence and to form double-helical segments therewith via
20 the formation of hydrogen bonds between the complementary base pairs. An example of an antisense-nucleic acid is an antisense-nucleic acid comprising chemical analogs of nucleotides.

[00118] The present invention provides means to modulate levels of expression of Mucorales CotH polypeptides by recombinantly expressing Mucorales CotH anti-sense
25 nucleic acids or employing synthetic anti-sense nucleic acid compositions (hereinafter SANC) that inhibit translation of mRNA encoding these polypeptides. Synthetic oligonucleotides, or other antisense-nucleic acid chemical structures designed to recognize and selectively bind to mRNA are constructed to be complementary to full-length or portions of an Mucorales CotH coding strand, including nucleotide sequences set forth in
30 SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31.

[00119] The SANC is designed to be stable in the blood stream for administration to a subject by injection, or in laboratory cell culture conditions. The SANC is designed to be capable of passing through the cell membrane in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SANC, which render it capable of

passing through cell membranes, for example, by designing small, hydrophobic SANC chemical structures, or by virtue of specific transport systems in the cell which recognize and transport the SANC into the cell. In addition, the SANC can be designed for administration only to certain selected cell populations by targeting the SANC to be 5 recognized by specific cellular uptake mechanisms which bind and take up the SANC only within select cell populations. In a particular embodiment the SANC is an antisense oligonucleotide.

[00120] For example, the SANC may be designed to bind to a receptor found only in a certain cell type, as discussed above. The SANC is also designed to recognize and 10 selectively bind to target mRNA sequence, which can correspond to a sequence contained within the sequences shown in SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31. The SANC is designed to inactivate target mRNA sequence by either binding thereto and inducing degradation of the mRNA by, for example, RNase I digestion, or inhibiting translation of mRNA target sequence by interfering with the binding of 15 translation-regulating factors or ribosomes, or inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups which either degrade or chemically modify the target mRNA. SANCs have been shown to be capable of such properties when directed against mRNA targets (see Cohen et al., *TIPS*, 10:435 (1989) and Weintraub, *Sci. American*, January (1990), pp.40).

[00121] Compositions comprising an amount of the antisense-nucleic acid of the 20 invention, effective to reduce expression of Mucorales CotH polypeptides by entering a cell and binding specifically to mRNA encoding Mucorales CotH polypeptides so as to prevent translation and an acceptable hydrophobic carrier capable of passing through a cell membrane are also provided herein. Suitable hydrophobic carriers are described, for 25 example, in U.S. Patent Nos. 5,334,761; 4,889,953; 4,897,355, and the like. The acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.

[00122] Antisense-nucleic acid compositions are useful to inhibit translation of 30 mRNA encoding invention polypeptides. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding Mucorales CotH polypeptides and inhibit translation of mRNA and are useful as compositions to inhibit expression of Mucorales CotH associated genes in a tissue sample or in a subject.

[00123] The invention also provides a method for expression of a Mucorales CotH polypeptide by culturing cells containing a Mucorales CotH nucleic acid under conditions suitable for expression of Mucorales CotH. Thus, there is provided a method for the recombinant production of a Mucorales CotH of the invention by expressing the nucleic acid sequences encoding Mucorales CotH in suitable host cells. Recombinant DNA expression systems that are suitable to produce Mucorales CotH described herein are well-known in the art (see, for example, Ausubel et al., *supra*, 1999). For example, the above-described nucleotide sequences can be incorporated into vectors for further manipulation. As used herein, vector refers to a recombinant DNA or RNA plasmid or virus containing discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof.

[00124] The invention also provides vectors containing the Mucorales CotH nucleic acids of the invention. Suitable expression vectors are well-known in the art and include vectors capable of expressing nucleic acid operatively linked to a regulatory sequence or element such as a promoter region or enhancer region that is capable of regulating expression of such nucleic acid. Appropriate expression vectors include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

[00125] The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a nucleic acid can be introduced into a host cell. The vector can be used for propagation or harboring a nucleic acid or for polypeptide expression of an encoded sequence. A wide variety of vectors are known in the art and include, for example, plasmids, phages and viruses. Exemplary vectors can be found described in, for example, Sambrook et al., *supra*; Ausubel et al., *supra*.

[00126] Promoters or enhancers, depending upon the nature of the regulation, can be constitutive or regulated. The regulatory sequences or regulatory elements are operatively linked to a nucleic acid of the invention such that the physical and functional relationship between the nucleic acid and the regulatory sequence allows transcription of the nucleic acid.

[00127] Suitable vectors for expression in prokaryotic or eukaryotic cells are well known to those skilled in the art (see, for example, Ausubel et al., *supra*, 1999). Vectors useful for expression in eukaryotic cells can include, for example, regulatory elements including the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, Moloney murine leukemia

virus (MMLV) promoter, and the like. The vectors of the invention are useful for subcloning and amplifying a Mucorales CotH nucleic acid molecule and for recombinantly expressing a Mucorales CotH polypeptide. A vector of the invention can include, for example, viral vectors such as a bacteriophage, a baculovirus or a retrovirus; 5 cosmids or plasmids; and, particularly for cloning large nucleic acid molecules, bacterial artificial chromosome vectors (BACs) and yeast artificial chromosome vectors (YACs). Such vectors are commercially available, and their uses are well known in the art. One skilled in the art will know or can readily determine an appropriate promoter for expression in a particular host cell.

10 [00128] The invention additionally provides recombinant cells containing Mucorales CotH nucleic acids of the invention. The recombinant cells are generated by introducing into a host cell a vector containing a Mucorales CotH nucleic acid molecule. The recombinant cells are transduced, transfected or otherwise genetically modified. Exemplary host cells that can be used to express recombinant Mucorales CotH molecules 15 include mammalian primary cells; established mammalian cell lines, such as COS, CHO, HeLa, NIH3T3, HEK 293 and PC12 cells; amphibian cells, such as *Xenopus* embryos and oocytes; and other vertebrate cells. Exemplary host cells also include insect cells such as *Drosophila*, yeast cells such as *Saccharomyces cerevisiae*, *Saccharomyces pombe*, or *Pichia pastoris*, and prokaryotic cells such as *Escherichia coli*.

20 [00129] In one embodiment, the invention provides a vaccine composition having an immunogenic amount of a Mucorales CotH polypeptide, an immunogenic fragment thereof or a variant of the polypeptide. The vaccine composition also can include an adjuvant. The formulation of the vaccine composition of the invention is effective in inducing protective immunity in a subject by stimulating both specific humoral 25 (neutralizing antibodies) and effector cell mediated immune responses against Mucorales CotH polypeptide. The vaccine composition of the invention is also used in the treatment or prophylaxis of fungal infections such as, for example, mucormycosis.

[00130] The vaccine of the present invention will contain an immunoprotective quantity of Mucorales CotH polypeptide antigens and is prepared by methods well known 30 in the art. The preparation of vaccines is generally described in, for example, M. F. Powell and M. J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (1995); A. Robinson, M. Cranage, and M. Hudson, eds., "Vaccine Protocols (Methods in Molecular Medicine)," Humana Press (2003); and D. Ohagan, ed., "Vaccine

Ajuvants: Preparation Methods and Research Protocols (Methods in Molecular Medicine)," Humana Press (2000).

[00131] Mucorales CotH polypeptide, and peptide fragments or variants thereof can include immunogenic epitopes, which can be identified using methods known in the art and described in, for example, Geysen et al. *Proc. Natl. Acad. Sci. USA* 81: 3998 (1984)). Briefly, hundreds of overlapping short peptides, e.g., hexapeptides, can be synthesized covering the entire amino acid sequence of the target polypeptide (i.e., Mucorales CotH). The peptides while still attached to the solid support used for their synthesis are then tested for antigenicity by an ELISA method using a variety of antisera.

10 Antiserum against Mucorales CotH protein can be obtained by known techniques, Kohler and Milstein, *Nature* 256: 495-499 (1975), and can be humanized to reduce antigenicity, see, for example, U.S. Patent No. 5,693,762, or produced in transgenic mice leaving an unarranged human immunoglobulin gene, see, for example, U.S. Patent No. 5,877,397.

15 Once an epitope bearing hexapeptide reactive with antibody raised against the intact protein is identified, the peptide can be further tested for specificity by amino acid substitution at every position and/or extension at both C and/or N terminal ends. Such epitope bearing polypeptides typically contain at least six to fourteen amino acid residues, and can be produced, for example, by polypeptide synthesis using methods well known in the art or by fragmenting an Mucorales CotH polypeptide. With respect to the molecule

20 used as immunogens pursuant to the present invention, those skilled in the art will recognize that the Mucorales CotH polypeptide can be truncated or fragmented without losing the essential qualities as an immunogenic vaccine. For example, Mucorales CotH polypeptide can be truncated to yield an N-terminal fragment by truncation from the C-terminal end with preservation of the functional properties of the molecule as an

25 immunogen. Similarly, C-terminal fragments can be generated by truncation from the N-terminal end with preservation of the functional properties of the molecule as an immunogen. Other modifications in accord with the teachings and guidance provided herein can be made pursuant to this invention to create other Mucorales CotH polypeptide functional fragments, immunogenic fragments, variants, analogs or derivatives thereof, to achieve the therapeutically useful properties described herein with the native protein.

[00132] The vaccine compositions of the invention further contain conventional pharmaceutical carriers. Suitable carriers are well known to those of skill in the art. These vaccine compositions can be prepared in liquid unit dose forms. Other optional components, e.g., pharmaceutical grade stabilizers, buffers, preservatives, excipients and

the like can be readily selected by one of skill in the art. However, the compositions can be lyophilized and reconstituted prior to use. Alternatively, the vaccine compositions can be prepared in any manner appropriate for the chosen mode of administration, e.g., intranasal administration, oral administration, etc. The preparation of a pharmaceutically acceptable vaccine, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

5 [00133] The immunogenicity of the vaccine compositions of the invention can further be enhanced if the vaccine further comprises an adjuvant substance. Various methods of achieving adjuvant effect for the vaccine are known. General principles and methods are detailed in "The Theory and Practical Application of Adjuvants", 1995, 10 Duncan E.S. Stewart-Tull (ed.), John Wiley & Sons Ltd, ISBN 0-471-95170-6, and also in "Vaccines: New Generation Immunological Adjuvants", 1995, Gregoriadis G et al. (eds.), Plenum Press, New York, ISBN 0-306-45283-9, both of which are hereby incorporated by reference herein.

15 [00134] Preferred adjuvants facilitate uptake of the vaccine molecules by antigen presenting cells (APCs), such as dendritic cells, and activate these cells. Non-limiting examples are selected from the group consisting of an immune targeting adjuvant; an immune modulating adjuvant such as a toxin, a cytokine, and a mycobacterial derivative; an oil formulation; a polymer; a micelle forming adjuvant; a saponin; an 20 immunostimulating complex matrix (ISCOM® matrix); a particle; DDA (dimethyldioctadecylammonium bromide); aluminium adjuvants; DNA adjuvants; and an encapsulating adjuvant. Liposome formulations are also known to confer adjuvant effects, and therefore liposome adjuvants are included according to the invention.

25 [00135] In addition to vaccination of subjects susceptible to fungal infections such as mucormycosis, the vaccine compositions of the present invention can be used to treat, immunotherapeutically, subjects suffering from a variety of fungal infections. Accordingly, vaccines that contain one or more of Mucorales CotH polynucleotides, 30 polypeptides and/or antibody compositions described herein in combination with adjuvants, and that act for the purposes of prophylactic or therapeutic use, are also within the scope of the invention. In an embodiment, vaccines of the present invention will induce the body's own immune system to seek out and inhibit Mucorales CotH molecules.

[00136] The term "vaccine", as used herein, refers to a composition that can be administered to an individual to protect the individual against an infectious disease. Vaccines protect against diseases by inducing or increasing an immune response in an

animal against the infectious disease. An exemplary infectious disease amenable to treatment with the vaccines of the invention is mucormycosis. The vaccine-mediated protection can be humoral and/or cell mediated immunity induced in host when a subject is challenged with, for example, Mucorales CotH or an immunogenic portion or fragment thereof.

5 [00137] The term "adjuvant" is intended to mean a composition with the ability to enhance an immune response to an antigen generally by being delivered with the antigen at or near the site of the antigen. Ability to increase an immune response is manifested by an increase in immune mediated protection. Enhancement of humoral immunity can be 10 determined by, for example, an increase in the titer of antibody raised to the antigen. Enhancement of cellular immunity can be measured by, for example, a positive skin test, cytotoxic T-cell assay, ELISPOT assay for IFN-gamma or IL-2. Adjuvants are well known in the art. Exemplary adjuvants include, for example, Freud's complete adjuvant, Freud's incomplete adjuvant, aluminum adjuvants, MF59 and QS21.

15 [00138] The term "treating" or "treatment," as it is used herein is intended to mean an amelioration of a clinical symptom indicative of a fungal condition. Amelioration of a clinical symptom includes, for example, a decrease or reduction in at least one symptom of a fungal condition in a treated individual compared to pretreatment levels or compared to an individual with a fungal condition. The term "treating" also is 20 intended to include the reduction in severity of a pathological condition, a chronic complication or an opportunistic fungal infection which is associated with a fungal condition. Such pathological conditions, chronic complications or opportunistic infections are exemplified below with reference to mucormycosis. Mucormycosis and other such pathological conditions, chronic complications and opportunistic infections also can be 25 found described in, for example, Merck Manual, Sixteenth Edition, 1992, and Spellberg et al., *Clin. Microbiol. Rev.* 18:556-69 (2005).

30 [00139] The term "preventing" or "prevention," as it is used herein is intended to mean a forestalling of a clinical symptom indicative of a fungal condition. Such forestalling includes, for example, the maintenance of normal physiological indicators in an individual at risk of infection by a fungus or fungi prior to the development of overt symptoms of the condition or prior to diagnosis of the condition. Therefore, the term "preventing" includes the prophylactic treatment of individuals to guard them from the occurrence of a fungal condition. Preventing a fungal condition in an individual also is intended to include inhibiting or arresting the development of the fungal condition.

Inhibiting or arresting the development of the condition includes, for example, inhibiting or arresting the occurrence of abnormal physiological indicators or clinical symptoms such as those described above and/or well known in the art. Therefore, effective prevention of a fungal condition would include maintenance of normal body temperature, weight,

5 psychological state as well as lack of lesions or other pathological manifestations in an individual predisposed to a fungal condition. Individuals predisposed to a fungal condition include an individual who is immunocompromised, for example, but not limited to, an individual with AIDS, azotemia, diabetes mellitus, diabetic ketoacidosis, neutropenia, bronchiectasis, emphysema, TB, lymphoma, leukemia, or burns, or an 10 individual undergoing chemotherapy, bone marrow-, stem cell- and/or solid organ transplantation or an individual with a history of susceptibility to a fungal condition. Inhibiting or arresting the development of the condition also includes, for example, inhibiting or arresting the progression of one or more pathological conditions, chronic complications or susceptibility to an opportunistic infection associated with a fungal 15 condition.

[00140] A "subject," "individual" or "patient" is used interchangeably herein, and refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, rats, rabbits, simians, bovines, ovines, porcines, canines, felines, farm animals, sport animals, pets, equines, and primates, particularly humans.

20 **[00141]** The term "fungal condition" as used herein refers to fungal diseases, infection, or colonization including superficial mycoses (i.e., fungal diseases of skin, hair, nail and mucous membranes; for example, ringworm or yeast infection), subcutaneous mycoses (i.e., fungal diseases of subcutaneous tissues, fascia and bone; for example, mycetoma, chromomycosis, or sporotrichosis), and systemic mycoses (i.e., deep-seated 25 fungal infections generally resulting from the inhalation of air-borne spores produced by causal moulds; for example, zygomycosis, aspergillosis, cryptococcosis, candidiasis, histoplasmosis, coccidiomycosis, paracoccidiomycosis, fusariosis (halohyphomycoses), blastomycosis, penicilliosis or sporotrichosis).

[00142] As used herein, the term "zygomycosis" is intended to mean a fungal 30 condition caused by fungi of the class *Zygomycetes*, comprised of the orders Mucorales and Entomophthorales. The Entomophthorales are causes of subcutaneous and mucocutaneous infections known as entomophthoromycosis, which largely afflict immunocompetent hosts in developing countries. Zygomycosis is also referred to as

mucormycosis and the two terms are used interchangeably to refer to similar types of fungal infections.

[00143] As used herein, the term “mucormycosis” is intended to mean a fungal condition caused by fungi of the order Mucorales. Mucormycosis is a life-threatening fungal infection almost uniformly affecting immunocompromised hosts in either developing or industrialized countries. Fungi belonging to the order Mucorales are distributed into at least six families, all of which can cause cutaneous and deep infections. Species belonging to the family Mucoraceae are isolated more frequently from patients with mucormycosis than any other family. Among the Mucoraceae, *Rhizopus oryzae* (*Rhizopus arrhizus*) is a common cause of infection. Other exemplary species of the Mucoraceae family that cause a similar spectrum of infections include, for example, *Rhizopus microsporus* var. *rhizopodiformis*, *Absidia corymbifera*, *Apophysomyces elegans*, *Mucor* species, *Rhizomucor pusillus* and *Cunninghamella* spp (*Cunninghamellaceae* family). Mucormycosis is well known in the art and includes, for example, rhinocerebral mucormycosis, pulmonary mucormycosis, gastrointestinal mucormycosis, disseminated mucormycosis, bone mucormycosis, mediastinum mucormycosis, trachea mucormycosis, kidney mucormycosis, peritoneum mucormycosis, superior vena cava mucormycosis or external otitis mucormycosis.

[00144] Fungi belonging to the order Mucorales are currently distributed into the families of Choanephoraceae; Cunninghamellaceae; Mucoraceae; Mycotyphaceae; Phycomycetaceae; Pilobolaceae; Saksenaeaceae; Syncephalastraceae; and Umbelopsidaceae. Each of these fungi families consists of one or more genera. For example, fungi belonging to the order Mucorales, family Mucoraceae, are further classified into the genera of *Absidia* (e.g., *A. corymbifera*); *Actinomucor* (e.g., *A. elegans*); *Amylomyces* (e.g., *A. rouxii*); *Apophysomyces*; *Backusella* (e.g., *B. circina*); *Benjaminiella* (e.g., *B. multispora*); *Chaetocladium* (e.g., *C. brefeldii*); *Circinella* (e.g., *C. angarensis*); *Cokeromyces* (e.g., *C. recurvatus*); *Dicranophora* (e.g., *D. fulva*); *Ellisomyces* (e.g., *E. anomalus*); *Helicostylum* (e.g., *H. elegans*); *Hyphomucor* (e.g., *H. assamensis*); *Kirkomyces* (e.g., *K. cordensis*); *Mucor* (e.g., *M. amphibiorum*); *Parasitella* (e.g., *P. parasitica*); *Philophora* (e.g., *P. agaricina*); *Pilaira* (e.g., *P. anomala*); *Pirella* (e.g., *P. circinans*); *Rhizomucor* (e.g., *R. endophyticus*); *Rhizopodopsis* (e.g., *R. javensis*); *Rhizopus*; *Sporodiniella* (e.g., *S. umbellata*); *Syzygites* (e.g., *S. megalocarpus*); *Thamnidium* (e.g., *T. elegans*); *Thermomucor* (e.g., *T. indicae-seudaticae*); and *Zygorhynchus* (e.g., *Z. californiensis*). The genus *Rhizopus*, for example, consists of *R.*

azygosporus; R. caespitosus; R. homothallicus; R. oryzae; R. microsporus, R. microsporus var. rhizopodiformis and R. schipperae species.

[00145] The term "immunogenic amount" as used herein refers an effective amount of a particular epitope of a polypeptide of the invention or a fragment or variant thereof that can induce the host immune response against the polypeptide or the infectious agent expressing the polypeptide. This amount is generally in the range of 20 μ g to 10 mg of antigen per dose of vaccine and depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired.

5 The precise amount of immunogen required can be calculated by various methods such as, for example, antibody titration. The term effective amount refers to an amount of a compound or compositions that is sufficient to provide a desired result. Thus, as used to describe a vaccine, an effective amount refers to an amount of a compound or composition (e.g., an antigen) that is sufficient to produce or elicit a protective immune response. An effective amount with respect to an immunological composition is an amount that is 10 sufficient to elicit an immune response, whether or not the response is protective.

[00146] The "therapeutically effective amount" will vary depending on the polypeptide, polynucleotide, antibody, antibody fragment or compositions, the disease and its severity and the age, weight, etc., of the patient to be treated all of which is within the skill of the attending clinician. It is contemplated that a therapeutically effective amount of 20 one or more of a polynucleotide, polypeptide, antibody, antibody fragment or composition described herein will alter a fungal pathogen penetration through and damage of endothelial cells in the patient as compared to the absence of treatment. As such, fungal pathogenesis is decreased. A therapeutically effective amount is distinguishable from an amount having a biological effect (a "biologically effective amount"). A polypeptide, 25 polynucleotide, antibody, antibody fragment or compositions of the present invention may have one or more biological effects *in vitro* or even *in vivo*, such as reducing function of a Mucorales CotH polypeptide. A biological effect, however, may not result in any clinically measurable therapeutically effect as described herein as determined by methods within the skill of the attending clinician.

30 [00147] In one embodiment, nucleic acids encoding the invention Mucorales CotH polypeptides can be delivered into mammalian cells, either *in vivo* or *in vitro* using suitable vectors well-known in the art. Suitable vectors for delivering a Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof to a mammalian cell, include viral vectors such as retroviral vectors, adenovirus, adeno-

associated virus, lentivirus, herpesvirus, as well as non-viral vectors such as plasmid vectors. Such vectors are useful for providing therapeutic or immunogenic amounts of a Mucorales CotH polypeptide (see, for example, U.S. Patent No. 5,399,346, issued March 21, 1995). Delivery of Mucorales CotH polypeptides or nucleic acids therapeutically can 5 be particularly useful when targeted to a muscle cell, bone marrow cell or B-cell, thereby presenting the encoded mucorales CotH polypeptide for development of an immune response. Such presentation is commonly known in the art as a DNA vaccination.

[00148] Viral based systems provide the advantage of being able to introduce relatively high levels of the heterologous nucleic acid into a variety of cells. Suitable viral 10 vectors for introducing invention nucleic acid encoding an Mucorales CotH protein into mammalian cells are well known in the art. These viral vectors include, for example, Herpes simplex virus vectors (Geller et al., *Science*, 241:1667-1669 (1988)); vaccinia virus vectors (Piccini et al., *Meth. Enzymology*, 153:545-563 (1987)); cytomegalovirus vectors (Mocarski et al., in Viral Vectors, Y. Gluzman and S.H. Hughes, Eds., Cold Spring 15 Harbor Laboratory, Cold Spring Harbor, N.Y., 1988, pp. 78-84)); Moloney murine leukemia virus vectors (Danos et al., *Proc. Natl. Acad. Sci. USA*, 85:6460-6464 (1988); Blaese et al., *Science*, 270:475-479 (1995); Onodera et al., *J. Virol.*, 72:1769-1774 (1998)); adenovirus vectors (Berkner, *Biotechniques*, 6:616-626 (1988); Cotten et al., *Proc. Natl. Acad. Sci. USA*, 89:6094-6098 (1992); Graham et al., *Meth. Mol. Biol.*, 7:109-127 (1991); 20 Li et al., *Human Gene Therapy*, 4:403-409 (1993); Zabner et al., *Nature Genetics*, 6:75-83 (1994)); adeno-associated virus vectors (Goldman et al., *Human Gene Therapy*, 10:2261-2268 (1997); Greelish et al., *Nature Med.*, 5:439-443 (1999); Wang et al., *Proc. Natl. Acad. Sci. USA*, 96:3906-3910 (1999); Snyder et al., *Nature Med.*, 5:64-70 (1999); Herzog et al., *Nature Med.*, 5:56-63 (1999)); retrovirus vectors (Donahue et al., *Nature Med.*, 25 4:181-186 (1998); Shackleford et al., *Proc. Natl. Acad. Sci. USA*, 85:9655-9659 (1988); U.S. Patent Nos. 4,405,712, 4,650,764 and 5,252,479, and WIPO publications WO 92/07573, WO 90/06997, WO 89/05345, WO 92/05266 and WO 92/14829; and lentivirus vectors (Kafri et al., *Nature Genetics*, 17:314-317 (1997)).

[00149] Vectors useful for therapeutic administration of a Mucorales CotH polypeptide of nucleic acid can contain a regulatory element that provides tissue specific or inducible expression of an operatively linked nucleic acid. One skilled in the art can readily determine an appropriate tissue-specific promotor or enhancer that allows expression of a Mucorales CotH polypeptide or nucleic acid in a desired tissue or cell. Any of a variety of inducible promoters or enhancers can also be included in the vector for

regulatable expression of a Mucorales CotH polypeptide or nucleic acid. Such inducible systems, include, for example, tetracycline inducible system (Gossen & Bizard, *Proc. Natl. Acad. Sci. USA*, 89:5547-5551 (1992); Gossen et al., *Science*, 268:1766-1769 (1995); Clontech, Palo Alto, CA); metallothionein promoter induced by heavy metals; insect

5 steroid hormone responsive to ecdysone or related steroids such as muristerone (No et al., *Proc. Natl. Acad. Sci. USA*, 93:3346-3351 (1996); Yao et al., *Nature*, 366:476-479 (1993); Invitrogen, Carlsbad, CA); mouse mammary tumor virus (MMTV) induced by steroids such as glucocorticoid and estrogen (Lee et al., *Nature*, 294:228-232 (1981); and heat shock promoters inducible by temperature changes.

10 [00150] An inducible system particularly useful for therapeutic administration utilizes an inducible promotor that can be regulated to deliver a level of therapeutic product in response to a given level of drug administered to an individual and to have little or no expression of the therapeutic product in the absence of the drug. One such system utilizes a Gal4 fusion that is inducible by an antiprogestin such as mifepristone in a

15 modified adenovirus vector (Burien et al., *Proc. Natl. Acad. Sci. USA*, 96:355-360 (1999). Another such inducible system utilizes the drug rapamycin to induce reconstitution of a transcriptional activator containing rapamycin binding domains of FKBP12 and FRAP in an adeno-associated virus vector (Ye et al., *Science*, 283:88-91 (1999)). It is understood that any combination of an inducible system can be combined in any suitable vector,

20 including those disclosed herein. Such a regulatable inducible system is advantageous because the level of expression of the therapeutic product can be controlled by the amount of drug administered to the individual or, if desired, expression of the therapeutic product can be terminated by stopping administration of the drug.

[00151] The invention additionally provides an isolated anti-Mucorales CotH antibody having specific reactivity with a Mucorales CotH polypeptide, an immunogenic fragment thereof, or functional fragment thereof. For example, an anti-Mucorales CotH antibody of the invention can have specific reactivity to a polypeptide having the amino acid sequence GAGKKHNNAKQSWNW (SEQ ID NO: 39) or

25 MGQTNDGAYRDPTDNNK (SEQ ID NO: 40). The anti-Mucorales CotH antibody can be a monoclonal antibody or a polyclonal antibody. The invention further provides cell lines producing monoclonal antibodies having specific reactivity with a Mucorales CotH polypeptide, an immunogenic fragment thereof, or functional fragment thereof.

30 [00152] The invention thus provides antibodies that specifically bind a Mucorales CotH polypeptide. As used herein, the term "antibody" is used in its broadest sense to

include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. With regard to an anti-Mucorales CotH antibody of the invention, the term "antigen" means a native or synthesized Mucorales CotH polypeptide or fragment thereof. An anti-Mucorales CotH antibody, or antigen binding fragment of such an antibody, is characterized by having specific binding activity for a Mucorales CotH polypeptide or a peptide portion thereof of at least about $1 \times 10^5 \text{ M}^{-1}$. Thus, Fab, F(ab')₂, Fd and Fv fragments of an anti-Mucorales CotH antibody, which retain specific binding activity for a Mucorales CotH polypeptide, are included within the definition of an antibody. Specific binding activity of a Mucorales CotH polypeptide can be readily determined by one skilled in the art, for example, by comparing the binding activity of an anti-Mucorales CotH antibody to a Mucorales CotH polypeptide versus a control polypeptide that is not a Mucorales CotH polypeptide. Methods of preparing polyclonal or monoclonal antibodies are well known to those skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988)).

[00153] In addition, antibodies of the invention can be naturally occurring antibodies as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse et al. (Huse et al., *Science* 246:1275-1281 (1989)). These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies are well known to those skilled in the art (Winter and Harris, *Immunol. Today* 14:243-246 (1993); Ward et al., *Nature* 341:544-546 (1989); Harlow and Lane, *supra*, 1988); Hilyard et al., Protein Engineering: A practical approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995)).

[00154] Anti-Mucorales CotH antibodies can be raised using a Mucorales CotH immunogen such as an isolated Mucorales CotH polypeptide having the amino acid sequence of SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32, or an immunogenic fragment thereof, which can be prepared from natural sources or produced recombinantly, or a peptide portion of the Mucorales CotH polypeptide. Such peptide portions of a Mucorales CotH polypeptide are functional antigenic fragments if the

antigenic peptides can be used to generate a Mucorales CotH -specific antibody. A non-immunogenic or weakly immunogenic Mucorales CotH polypeptide or portion thereof can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Accordingly, in some 5 aspects of the invention, an immunogenic fragment of the CotH polypeptides disclosed herein can be conjugated to a carrier molecule, such as, but not limited to KLH or BSA. Various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art (see, for example, Harlow and Lane, *supra*, 1988). An immunogenic Mucorales CotH polypeptide fragment can also be generated by expressing 10 the peptide portion as a fusion protein, for example, to glutathione S transferase (GST), polyHis or the like. Methods for expressing peptide fusions are well known to those skilled in the art (Ausubel et al., *supra*).

[00155] The invention further provides a method for detecting the presence of a Mucorales organism in a sample by contacting a sample with a Mucorales CotH-specific 15 antibody, and detecting the presence of specific binding of the antibody to the sample, thereby detecting the presence of a Mucorales CotH polypeptide in the sample. Mucorales CotH specific antibodies can be used in diagnostic methods and systems to detect the level of Mucorales CotH present in a sample. As used herein, the term "sample" is intended to mean any biological fluid, cell, tissue, organ or portion thereof, 20 that includes or potentially includes Mucorales CotH nucleic acids or polypeptides. The term includes samples present in an individual as well as samples obtained or derived from the individual. For example, a sample can be a histologic section of a specimen obtained by biopsy, or cells that are placed in or adapted to tissue culture. A sample further can be a subcellular fraction or extract, or a crude or substantially pure nucleic acid or protein 25 preparation.

[00156] Mucorales CotH -specific antibodies can also be used for the immunoaffinity or affinity chromatography purification of the invention Mucorales CotH. In addition, methods are contemplated herein for detecting the presence of an invention 30 Mucorales CotH protein in a cell, comprising contacting the cell with an antibody that specifically binds to Mucorales CotH polypeptides under conditions permitting binding of the antibody to the Mucorales CotH polypeptides, detecting the presence of the antibody bound to the Mucorales CotH polypeptide, and thereby detecting the presence of invention polypeptides in a cell. With respect to the detection of such polypeptides, the antibodies can be used for *in vitro* diagnostic or *in vivo* imaging methods.

[00157] Immunological procedures useful for *in vitro* detection of target Mucorales CotH polypeptides in a sample include immunoassays that employ a detectable antibody. Such immunoassays include, for example, immunohistochemistry, immunofluorescence, ELISA assays, radioimmunoassay, FACS analysis, 5 immunoprecipitation, immunoblot analysis, Pandex microfluorimetric assay, agglutination assays, flow cytometry and serum diagnostic assays, which are well known in the art (Harlow and Lane, *supra*, 1988; Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Press (1999)).

[00158] An antibody can be made detectable by various means well known in the 10 art. For example, a detectable marker can be directly attached to the antibody or indirectly attached using, for example, a secondary agent that recognizes the Mucorales CotH specific antibody. Useful markers include, for example, radionucleotides, enzymes, binding proteins such as biotin, fluorogens, chromogens and chemiluminescent labels.

[00159] As used herein, the terms label and indicating means in their various 15 grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal. Any label or indicating means can be linked to invention nucleic acid probes, expressed proteins, polypeptide fragments, or antibody molecules. These atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic 20 chemistry.

[00160] The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturation to form a fluorochrome (dye) that is a useful immunofluorescent tracer. A description of immunofluorescent analytic techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis 25 et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

[00161] In one embodiment, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, and the like. In another embodiment, radioactive elements are employed labeling agents. The linking of a label to a substrate, 30 i.e., labeling of nucleic acid probes, antibodies, polypeptides, and proteins, is well known in the art. For instance, an invention antibody can be labeled by metabolic incorporation of radiolabeled amino acids provided in the culture medium. See, for example, Galfre et al., *Meth. Enzymol.*, 73:3-46 (1981). Conventional means of protein conjugation or coupling by activated functional groups are particularly applicable. See, for example,

Aurameas et al., *Scand. J. Immunol.*, Vol. 8, Suppl. 7:7-23 (1978), Rodwell et al., *Biotech.*, 3:889-894 (1984), and U.S. Patent No. 4,493,795.

[00162] Invention nucleic acids, oligonucleotides, including antisense, vectors containing invention nucleic acids, transformed host cells, polypeptides and combinations thereof, as well as antibodies of the present invention, can be used to screen compounds to determine whether a compound functions as a potential agonist or antagonist of invention polypeptides. These screening assays provide information regarding the function and activity of invention polypeptides, which can lead to the identification and design of compounds that are capable of specific interaction with one or more types of polypeptides, peptides or proteins.

[00163] Thus, the invention provides methods for identifying compounds which bind to Mucorales CotH polypeptides. The invention proteins can be employed in a competitive binding assay. Such an assay can accommodate the rapid screening of a large number of compounds to determine which compounds, if any, are capable of binding to Mucorales CotH polypeptides. Subsequently, more detailed assays can be carried out with those compounds found to bind, to further determine whether such compounds act as modulators, agonists or antagonists of invention Mucorales CotH polypeptides. Compounds that bind to and/or modulate invention Mucorales CotH polypeptides can be used to treat a variety of pathologies mediated by invention Mucorales CotH polypeptides.

[00164] Various binding assays to identify cellular proteins that interact with protein binding domains are known in the art and include, for example, yeast two-hybrid screening assays (see, for example, U.S. Patent Nos. 5,283,173, 5,468,614 and 5,667,973; Ausubel et al., *supra*, 1999; Luban et al., *Curr. Opin. Biotechnol.* 6:59-64 (1995)) and affinity column chromatography methods using cellular extracts. By synthesizing or expressing polypeptide fragments containing various Mucorales CotH sequences or deletions, the Mucorales CotH binding interface can be readily identified.

[00165] In another embodiment of the invention, there is provided a bioassay for identifying compounds which modulate the activity of invention Mucorales CotH polypeptides. According to this method, invention polypeptides are contacted with an "unknown" or test substance, for example, in the presence of a reporter gene construct responsive to a Mucorales CotH signaling pathway, the activity of the polypeptide is monitored subsequent to the contact with the "unknown" or test substance, and those substances which cause the reporter gene construct to be expressed are identified as

functional ligands for Mucorales CotH polypeptides. Such reporter gene assays and systems are well known to those skilled in the art (Ausubel et al., *supra*, 1999). In addition, a reporter gene construct can be generated using the promoter region of Mucorales CotH and screened for compounds that increase or decrease Mucorales CotH gene promoter activity. Such compounds can also be used to alter Mucorales CotH expression.

5 [00166] In accordance with another embodiment of the present invention, transformed host cells that recombinantly express invention polypeptides can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by 10 comparing the Mucorales CotH-mediated response, for example, via reporter gene expression in the presence and absence of test compound, or by comparing the response of test cells or control cells, to the presence of the compound.

15 [00167] As used herein, a compound or a signal that modulates the activity of invention polypeptides refers to a compound or a signal that alters the activity of Mucorales CotH polypeptides so that the activity of the invention polypeptide is different in the presence of the compound or signal than in the absence of the compound or signal. In particular, such compounds or signals include agonists and antagonists. An agonist encompasses a compound or a signal that activates Mucorales CotH protein expression or 20 biological activity. Alternatively, an antagonist includes a compound or signal that interferes with Mucorales CotH expression or biological activity. Typically, the effect of an antagonist is observed as a blocking of agonist-induced protein activation. Antagonists include competitive and non-competitive antagonists.

25 [00168] Assays to identify compounds that modulate Mucorales CotH polypeptide expression can involve detecting a change in Mucorales CotH polypeptide abundance in response to contacting the cell with a compound that modulates Mucorales CotH activity. Assays for detecting changes in polypeptide expression include, for example, immunoassays with Mucorales CotH -specific Mucorales CotH antibodies, such as immunoblotting, immunofluorescence, immunohistochemistry and immunoprecipitation assays, as described above.

30 [00169] As understood by those of skill in the art, assay methods for identifying compounds that modulate Mucorales CotH activity generally require comparison to a control. One type of a "control" is a cell or culture that is treated substantially the same as the test cell or test culture exposed to the compound, with the distinction that the "control" cell or culture is not exposed to the compound. Another type of "control" cell or culture

can be a cell or culture that is identical to the test cells, with the exception that the "control" cells or culture do not express a Mucorales CotH polypeptide. Accordingly, the response of the transfected cell to a compound is compared to the response, or lack thereof, of the "control" cell or culture to the same compound under the same reaction

5 conditions.

[00170] The invention further provides a method for modulating an activity mediated by a Mucorales CotH polypeptide by contacting the Mucorales CotH polypeptide with an effective, modulating amount of an agent that modulates Mucorales CotH activity. The Mucorales CotH activity can be, for example, binding to GRP78.

10 The invention additionally provides a method of modulating the level of adhesion to a cell.

[00171] In some embodiment, the invention provides a method of detecting a Mucorales CotH nucleic acid molecule in a sample. Such methods of the invention can include the steps of contacting a sample with two or more oligonucleotides disclosed herein, amplifying a nucleic acid molecule, and detecting the amplification. It is understood that methods for amplifying a nucleic acid are well known to one of skill in the art, which can be readily selected and applied to the methods of the invention. For example, in some aspects, the amplification is performed using polymerase chain reaction (PCR). In some aspects of the invention, at least one of the two or more oligonucleotides used in the method of the invention includes an oligonucleotide having the nucleic acid sequence of ATGAAATTATCTATTATATCCGCTGCC (SEQ ID NO: 33),

15 GCTGGGAATATAATTGTCATCGA (SEQ ID NO: 34),

GATGACAATTATATTCCCAGC (SEQ ID NO: 35),

GAGTAGACGTAATTAGATCCAA (SEQ ID NO: 36),

20 AACCGTACCTGCTGACCGAATC (SEQ ID NO: 37) or any oligonucleotide disclosed herein.

[00172] The invention further provides a method of diagnosing mucormycosis infection in a subject by detecting the presence of a Mucorales organism in a sample from the patient. The method can include the steps of (a) providing a test sample from the subject; (b) contacting the sample with an agent that can binds a nucleic acid or a polypeptide of the invention under suitable conditions, wherein the conditions allow specific binding of the agent to the nucleic acid or polypeptide; and (c) comparing the amount of the specific binding in the test sample with the amount of specific binding in a control sample, wherein an increased or decreased amount of the specific binding in the test sample as compared to the control sample is diagnostic of mucormycosis infection. In

some aspects of the invention, the agent is selected from the group consisting of an anti-Mucorales CotH antibody or a CotH oligonucleotide as described herein.

[00173] In accordance with another embodiment of the present invention, there are provided diagnostic systems, preferably in kit form, comprising at least one invention nucleic acid or antibody in a suitable packaging material. The diagnostic kits containing nucleic acids are derived from the Mucorales CotH -encoding nucleic acids described herein. In one embodiment, for example, the diagnostic nucleic acids are derived from any of SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31 and can be oligonucleotides of the invention. In some aspects of the invention, at least one oligonucleotide comprises a nucleic acid sequence selected from

ATGAAATTATCTATTATATCCGCTGCC (SEQ ID NO: 33),

GCTGGGAATATAATTGTCATCGA (SEQ ID NO: 34),

GATGACAATTATATTCCCAGC (SEQ ID NO: 35),

GAGTAGACGTAATTAGATCCAA (SEQ ID NO: 36),

AAACGTACCTGCTGACCGAATC (SEQ ID NO: 37) or any oligonucleotide disclosed herein. Invention diagnostic systems are useful for assaying for the presence or absence of nucleic acid encoding Mucorales CotH in either genomic DNA or mRNA.

[00174] A suitable diagnostic system includes at least one invention nucleic acid or antibody, as a separately packaged chemical reagent(s) in an amount sufficient for at least one assay. For a diagnostic kit containing nucleic acid of the invention, the kit will generally contain two or more nucleic acids. When the diagnostic kit is to be used in PCR, the kit will contain at least two oligonucleotides that can serve as primers for PCR. Those of skill in the art can readily incorporate invention nucleic probes and/or primers or invention antibodies into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein. A kit containing a Mucorales CotH antibody can contain a reaction cocktail that provides the proper conditions for performing an assay, for example, an ELISA or other immunoassay, for determining the level of expression of a Mucorales CotH polypeptide in a sample, and can contain control samples that contain known amounts of a Mucorales CotH polypeptide and, if desired, a second antibody specific for the anti-Mucorales CotH antibody.

[00175] The contents of the kit of the invention, for example, Mucorales CotH nucleic acids or antibodies, are contained in packaging material, preferably to provide a sterile, contaminant-free environment. In addition, the packaging material contains instructions indicating how the materials within the kit can be employed both to detect the

presence or absence of a particular Mucorales CotH sequence or Mucorales CotH polypeptide or to diagnose the presence of, or a predisposition for a condition associated with mucormycosis. The instructions for use typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the 5 relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

[00176] It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also provided within the definition of the invention provided herein. Accordingly, the following examples are 10 intended to illustrate but not limit the present invention.

EXAMPLE I
Cell Surface CotH3 Protein Facilitates Binding to Host GRP78 During Fungal Invasion of Endothelial Cells

[00177] Cell wall material was collected from supernatants of protoplasts of *R. oryzae* germlings. *R. oryzae* ligands bound to rGrp78 were isolated by FAR Western blot analysis using anti-Grp78 Ab and identified by MALDI-TOF-MS/MS analysis (Figure 1). Briefly, protein spots of interest were excised and sent to the UCLA W. M. Keck Proteomic Center for identification on a Thermo LTQ-Orbitrap XL mass spectrometer (San Jose, CA) equipped with an Eksigent (Dublin, CA) NanoLiquid chromatography-1D plus system and an Eksigent autosampler. Proteins within the spots were in-gel tryptic digested as described by Shevchenko *et al.* (Shevchenko et al.(1996). Proc Natl Acad Sci U S A 93: 14440-14445.; Shevchenko et al., *Anal. Chem.*, 68(5):850-8 (1996)). The eluted peptides were loaded onto a CVC Microtech (Fontana, CA) 35 mm length, 100 μ m ID C18 pre-Trap column and washed for 10 min with 100% Buffer A (2% acetonitrile 20 containing 0.1% formic acid) at a flow rate of 5 μ l/min. The peptides were separated on a 15 cm New Objective ProteoPep IntegraFrit column (Woburn, MA) using a flow rate of 300 nl/min. The following elution gradient was used: 0-15 min 0-30% Buffer B (98% acetonitrile containing 0.1% formic acid), 15-20min 30-80% Buffer B and 20-22 min 80% Buffer B. The column was then re-equilibrated for 13 min with Buffer A. The eluting 25 analytes were sprayed in positive mode into the LTQ-Orbitrap MS using electrospray ionization voltage of 2300 V, capillary voltage of 45 V, tube lens of 130 V, and capillary temperature of 200°C. Information dependent acquisition was performed where the 6 most intense ions were selected in the *m/z* range of 300-1600 using a 60 K resolution FTMS scan and subjecting them to MS-MS using broadband collision induced

disassociation of normalized collision energy of 35 and LTQ detection. Peaks were excluded from further MS-MS for a period of 60 sec.

[00178] The resulting MS/MS spectra was searched against the *Rhizopus oryzae* 99-880 database

5 (http://www.broadinstitute.org/annotation/genome/rhizopus_oryzae/MultiHome.html) using the Matrix Science MASCOT Daemon search engine (Boston, MA). The following search parameters were used: peptide tolerance: ± 10 ppm, MS/MS tolerance ± 0.3 Da, maximum missed cleavages: 2, fixed modifications: carboxymethyl (C) and variable modifications: deamidization (ND) and oxidation (M). Proteins identified within a
10 particular included those with a minimum of two unique peptides that are ranked as number 1 and with an ion scores with a $p < 0.05$.

[00179] Expression of the putative ligands in *R. oryzae* incubated with endothelial cells was detected by RT-PCR (Figure 4). Interaction of the ligand with GRP78 was confirmed by heterologously expressing the ligand in *Saccharomyces cerevisiae* (Figure 6)
15 and comparing its adherence to and invasion of endothelial cells and CHO cells overexpressing GRP78 to *S. cerevisiae* transformed with empty plasmid (control) (Figure 8)..

[00180] Three of the ORF had homology to CotH family of proteins implicated in spore coat formation from several bacteria:

20

- RO3G_05018, CotH1
- RO3G_08029, CotH2
- RO3G_11882, CotH3

[00181] A fourth ORF (RO3G_16295) is widely present in other pathogenic fungi, but none of these ORFs appear to encode a protein that has an identified function. As
25 sequence comparison between the identified CotH polypeptides and other bacterial CotH proteins shows very little sequence identity (Figure 17 and Table 1).

Table 1. Sequence similarity of *Rhizopus* CotH and bacterial CotH (different sizes)

	Flammeovirga yaeyamensis (ACY02060)	Desulfotomaculum reducens (YP_001112853)	Bacillus amyloliquefaciens (YP_001422883)	Bacillus cereus (ZP_04217292)
RO3G_05018	18.5	13.6	12.9	14.5
RO3G_08029	18.6	15.8	13.8	13.6
RO3G_11882	18	14.1	13	13.6

[00182] RO3G_16295 appears to be a common protein, it's homologues (usually ~25% identity at amino acid) can be seen from many different fungi as well as a few bacteria (Figures 18-26). All these proteins are not characterized. To name a few:

- *Talaromyces stipitatus* ATCC 10500 (EED23986);
- 5 - *Penicillium marneffei* ATCC 18224 (XP_002144175);
- *Aspergillus niger* (XP_001392236);
- *Aspergillus nidulans* (XP_658934);
- *Ustilago maydis* (XP_760027);
- *Coccidioides immitis* (XP_001243211);
- 10 - *Neurospora crassa* (XP_956792);
- *Cryptococcus neoformans* (XP_775558); and
- *Streptomyces lividans* (EFD65170).

[00183] CotH3 and to a lesser extent CotH2 were expressed in *R. oryzae* germlings interacting with human umbilical vein endothelial cells (Figure 4). CotH1 was expressed by *R. oryzae* spores but not germlings interacting with endothelial cells (Figure 3). Although FAR-Western analysis identified the 4th ORF (RO3G_16295), this gene was not expressed by *R. oryzae* germlings interacting with endothelial cells. *S. cerevisiae* expressing CotH3, and to a lesser extent CotH2, specifically bound endothelial cell GRP78 (Figure 6). Heterologous expression of CotH3 in the non-adherent *S. cerevisiae* promoted adherence and subsequent invasion of endothelial cells and CHO cells overexpressing GRP78 (Figure 58). A sequence alignment between CotH3 polypeptides from various Mucorales species show an >90% sequence identity between the various species (Figure 7 and Table 2)

[00184] These results show that *R. oryzae* invasion of endothelial cells is

25 facilitated by CotH3, or CotH2 binding to GRP78.

Table 2. Alignment of Nucleotide (CotH3 and other genera exons only)

CotH 3	R. oryzae 99-880	R. oryzae 99-892	Mucor 99-932	Absidia Corymbifera	Cunninghamella bertholletiae	R. microsporus
R. oryzae 99-880	100%	95.82	96.42 %	99.34 %	97.80 %	99.94
R. oryzae 99-892	95.82	100%	100.00 %	96.53 %	94.51 %	96.47 %
Mucor 99-932	96.42 %	100.00 %	100%	96.53 %	94.51 %	96.47 %
Absidia Corymbifera	99.34 %	96.53 %	96.53 %	100%	97.36 %	99.39 %
Cunninghamella bertholletiae	97.80 %	94.51 %	94.51 %	97.36 %	100%	97.85 %
R. microsporus	99.94	96.47 %	96.47 %	99.39 %	97.85 %	100%

EXAMPLE II**CotH3 and CotH2 are Unique Mucorales Invasins that Bind to Endothelial Cell GRP78.**

R. oryzae and culture conditions

[00185] Several clinical Mucorales isolates were used in the experiments disclosed herein. For example, *R. oryzae* 99-880 and *Mucor* sp. 99-932 were isolated from brain samples, whereas *R. oryzae* 99-892 and *Rhizopus* sp 99-1150 were isolated from lungs samples of infected patients (samples were obtained from the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio). *Cunninghamella bertholletiae* 182 is also a clinical isolate, which was a kind gift from Dr. Tomas Walsh (NIH).

Lichtheimia corymbifera is also a clinical isolate obtained from the DEFEAT Mucor clinical study (Spellberg et al. (2102), J Antimicorbiol Chemother 67(3):715-22)

[00186] Mucorales were grown on potato dextrose agar (PDA, BD Diagnostic) plates for 3-5 days at 37°C, while *A. fumigatus* and *C. albicans* were grown on Sabouraud dextrose agar (SDA) plates for 2 weeks and 48 h at 37°C, respectively. The sporangiospores were collected in endotoxin free Dulbecco's phosphate buffered saline (PBS) containing 0.01% Tween 80, washed with PBS, and counted with a hemocytometer to prepare the final inocula. For *C. albicans*, blastospores were collected in PBS after growing the organisms in YPD medium [1% yeast extract (Difco Laboratories), 2% bacto-peptone (Difco) and 2% glucose (Sigma)] at 30°C for overnight. To form germlings, spores were incubated in liquid YPD medium at 37°C with shaking for 1-3 h based on the assay under study. Germlings were washed twice with RPMI 1640 without glutamine (Irvine Scientific) for all assays used except for isolating the endothelial cell receptor experiments in which the germlings were washed twice with PBS (plus Ca²⁺ and Mg²⁺).

Heterologous expression of CotH genes in *S. cerevisiae*

[00187] The entire ORF of *CotH1*, *CotH2*, and *CotH3* were PCR amplified from cDNA extracted from *R. oryzae* spores grown on PDA plates by using Phusion high fidelity PCR Kit (New England Biolabs) and the primers listed in Table 3. The pESC-LEU yeast dual expression vector (Stratagene) was used to clone and express these genes under the Gal1 promoter. The vector was digested with *BamHI* and *Sall*. PCR amplified inserts from each of the CotH genes were cloned into pESC-LEU by using In-Fusion 2.0 Dry-Down PCR Cloning

Kit, per the manufacturer's instructions (Clontech Laboratories). The generated yeast expression vectors were independently transformed into yeast strain LL-20 by the polyethylene glycol-LiOAc method, and transformants were screened on the solid synthetic dextrose minimal medium lacking leucine. *S. cerevisiae* transformed with the empty plasmid served as control.

Table 3. Primers used in this study

Primer Name	Primer Sequence (SEQ ID NOS 41-64, respectively, in order of appearance)	Reaction/Use
Coth1-F	AAAAAAACCCCGGATCCTATGAAATCCCTACTTTGTTGATTTC	RT-PCR and to clone Coth1 in to expression vector pESC-L ^{eu}
Coth1-R	TCTGTTCCATGTCGACCTAGAAGAAAGGCAAATAAGTGC	RT-PCR and to clone Coth2 in to expression vector
Coth2-F	AAAAAACCCCGGATCCTATGAAATTACTCACTATAGTATCCCTCT	RT-PCR to clone Coth3 in to expression vector
Coth2-R	TCTGTTCCATGTCGACTTAAAGATAAGCAGTGGCAACTAAAG	RT-PCR to clone Coth3 in to expression vector
Coth3-F	AAAAAACCCCGGATCCTATGAAATTATCTATTATCCGCTTGCC	RT-PCR to clone Coth3 in to expression vector
Coth3-R	TCTGTTCCATGTCGACTTAAAGATAAGCAGTGGCTAAAGCG	Detection in mucorales
Ligand#4-F R03G 16295	AAAAAACCCCGGATCCTATGATTGCTACCCCTTTGAAA	RT-PCR
Ligand#4-R R03G 16295	TCTGTTCCATGTCGACTTAAAGAAATAAGAATGTTGCAGC	RT-PCR
Coth3-F-ORF	ATGAAATTATCTATTATCCGCTGCC	Detection IN OTHER MUCORALS1.9Kb
Coth3-R-ORF	TTAGAAATACAAGGAGAGCTAAAGCG	Detection IN OTHER MUCORALS1.9Kb
RNAi-cothF-F	GCATGCTAGAACAGAAAGAAAGTTGATCGTTC	RNAi-forward
RNAi-ccohF-R	GTACGACGTTCACGAATCTGTGTTAGG	RNAi-forward
RNAi-I-F	CCGGCGGGACGTTCACGAATCTGTGTTAGG	RNAi-Reverse
RNAi-I-R	GCTAGCAGAACAGAAAGAAAGTTGATCGTTC	RNAi-Reverse
Coth1-F	CAAACAAATGATGGGGCCTA	qRT-PCR for Expression
Coth1-R	CGTTTGTGTTCAAGATTACACCA	qRTPCR for Expression
Coth2-F	CCTAATAAGGACAACGGCAAACG	qRT-PCR for Expression
Coth2-R	TGGCAATGGCTGTGTTATC	qRT-PCR for Expression
Coth3-F	GCCAATCCTAATGGTGAAGC	qRT-PCR for Expression
Coth3-R	CATGAAACGGTCGAGATCAA	qRT-PCR for Expression

RO Actin-F	AGCTCCCTTGTAAACCCCAAGT	qRT-PCR for Expression
RO Actin-R	ACGACCAAGGGCATACAAAGG	qRT-PCR for Expression
RO 18sRNA-F	GCGGATTCGGCATGGCC	qRTPCR for CFU
RO 18sRNA-R	CCATGATAGGGCAGAAAATCG	qRTPCR for CFU

Anti-CotH antibody production and cell surface localization

[00188] Rabbit polyclonal antibodies were raised against two peptides predicted to be antigenic. The peptides GAGKKHNNAKQSWNW (SEQ ID NO: 39), and MGQTNDGAYRDPTDNNK (SEQ ID NO: 40) were coupled with KLH and used to commercially vaccinate rabbits (ProMab Biotechnologies Inc., Richmond, CA). Purified IgG from the vaccinated rabbits were used to detect cell surface localization of CotH proteins on *S. cerevisiae* and on *R. oryzae* interacting with endothelial cells (Liu et al., *J. Clin. Invest.*, 120:1914-1924 (2010)).

[00189] For localizing the CotH proteins to the cell surface of *S. cerevisiae*, blastospores expressing individual CotH genes were incubated first with the anti-CotH IgG at 1:50, followed by fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG at 1:100. The stained cells were imaged with Leica confocal microscope and the entire yeast cells were visualized with differential interference contrast (DIC).

[00190] For detecting the expression of the CotH proteins on *R. oryzae*, spores were germinated in YPD for 3 hours at 37°C. Germlings were stained with the anti-CotH IgG at 1:50, followed by FITC-labeled goat anti-rabbit IgG at 1:100. A FACSCaliber (Becton Dickinson) instrument equipped with an argon laser emitting at 488 nm was used for flow cytometric analysis. Fluorescence emission was read with a 515/40 bandpass filter. Fluorescence data were collected with logarithmic amplifiers. The mean fluorescence intensities of 10⁴ events were calculated using the CELLQUEST software.

Endothelial cells and Chinese Hamster Ovary (CHO) cells

[00191] Endothelial cells were collected from umbilical vein endothelial cells by the method of Jaffe et al. (Jaffe et al., *J. Clin. Invest.* 52:2745-2756 (1973)). The cells were harvested by using collagenase and were grown in M-199 (Gibco BRL) enriched with 10% fetal bovine serum, 10% defined bovine calf serum, L-glutamine, penicillin, and streptomycin (all from Gemini Bio-Products, CA). Second-passage cells were grown to confluence in 96-well tissue culture plates (Costar, Van Nuys, CA) on fibronectin (BD Biosciences). All incubations were in 5% CO₂ at 37°C. The reagents were tested for endotoxin using a chromogenic limulus amebocyte lysate assay (BioWhittaker, Inc., Walkersville, MD), and the endotoxin concentrations were less than 0.01 IU/ml. Endothelial cell collection was approved

by Institutional Review Board at Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center. CHO cell line C.1 which was derived from parental DHFR-deficient CHO cells engineered to overexpress GRP78s were kind gifts of Dr. Randall Kaufman (Morris et al., *J. Biol. Chem.*, 272:4327-4334 (1997); Reddy et al., *J. Biol. Chem.*, 278:20915-20924 (2003)).

Extraction of endothelial cell membrane proteins

[00192] Endothelial cell membrane proteins were extracted according to the method of Isberg and Leong (Isberg and Leong, *Cell* 60:861-871 (1990)). Briefly, confluent endothelial cells in 100-mm diameter tissue culture dishes were rinsed twice with warm DPBS containing Ca^{2+} and Mg^{2+} (PBS-CM) and then incubated with Ez-Link Sulfo-NHS-LS Biotin (0.5 mg/ml, Pierce) in PBS-CM for 12 min at 37°C in 5% CO_2 . The cells were then rinsed extensively with cold PBS-CM and scraped from the tissue culture dishes. The endothelial cells were collected by centrifugation at 500 $\times g$ for 5 min at 4°C and then lysed by incubation for 20 min on ice in PBS-CM containing 5.8% n-octyl- β -D-glucopyranoside (w/v) (Cal BioChem) and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 $\mu\text{g}/\text{ml}$ pepstatin A, 1 $\mu\text{g}/\text{ml}$ leupeptin, and 1 $\mu\text{g}/\text{ml}$ aprotinin) (Sigma). The cell debris was removed by centrifugation at 5000 $\times g$ for 5 min at 4°C. The supernatant was collected and centrifuged at 100,000 $\times g$ for 1 h at 4°C. The concentration of the endothelial cell proteins in the resulting supernatant was determined using Bradford method (Bio-Rad).

RNA interference of CotH2/CotH3

[00193] Previously described RNA interference (RNAi) technology (Ibrahim et al., *Mol. Microbiol.*, 77:587-604 (2010)) was utilized to inhibit the expression of CotH2 and CotH3 in *R. oryzae*. A 450 bp fragment commonly shared between CotH2 and CotH3 ORF was PCR amplified and cloned as an inverted repeat under control of the *Rhizopus* expression vector pPdcA-Ex (Mertens et al., *Archives of microbiology* 186:41-50 (2006)). Additionally, an intron from the *Rhizopus* pdcA gene (Skory, *Curr. Microbiol.*, 47: 59-64 (2003)) was included between repeat to serve as a linker for stabilization of the intended dsRNA structure (Nakayashiki et al., *Fungal Genet. Biol.*, 42:275-283 (2005); Wesley et al., *Plant J.*, 27:581-590 (2001)). The generated plasmid was transformed into *R. oryzae* *pyrF* mutant using the

biolistic delivery system (Skory, *Mol. Genet. Genomics* 268: 397-406 (2002)) (BioRad) and transformants were selected on minimal medium lacking uracil.

Binding of GRP78 by *S. cerevisiae* expressing CotH.

[00194] *S. cerevisiae* cells (8×10^8) expressing CotH1, CotH2, CotH3, or empty plasmid were incubated for 1 h on ice with 250 μ g of biotin-labeled endothelial cell surface proteins in PBS-CM plus 1.5% n-octyl- β -D-glucopyranoside and protease inhibitors. The unbound endothelial cell proteins were washed away by three rinses with this buffer. The endothelial cell proteins that remained bound to the fungal cells were eluted twice with 6M urea (Fluka) and the supernatant was combined and concentrated to appropriate volume with a Microcon centrifugal filter (10,000 MWCO, Millipore). The proteins were then separated on 10% SDS-PAGE, and transferred to PVDF-plus membranes (GE Water& Process Technologies). The membrane was then treated with Western Blocking Reagent (Roche) and probed with a rabbit anti-GRP78 antibody (Abcam) followed with secondary antibodies of HRP-conjugated goat anti-rabbit IgG (Pierce), respectively. After incubation with SuperSignal West Dura Extended Duration Substrate (Pierce), the signals were detected using a CCD camera.

Interactions of fungi with endothelial or CHO cells

[00195] The number of organisms endocytosed by endothelial cells or CHO cells was determined using a modification of a previously described differential fluorescence assay (Ibrahim et al., *Infect. Immun.*, 63:4368-4374 (1995)). Briefly, 12-mm glass coverslips in a 24-well cell culture plate were coated with fibronectin for at least 4 hrs, and seeded with endothelial or CHO cells until confluence. After washing twice with prewarmed HBSS, the cells were then infected with 10^5 cells of *S. cerevisiae* expressing CotH or *R. oryzae* in RPMI 1640 medium that has been germinated for 1 h. Following incubation for 3 h, the cells were fixed in 3% paraformaldehyde and the cells were stained with 1% Uvitex (a kind gift from Jay Isharani, Ciba-Geigy, Greensboro, N.C.) for 1 hr, which specifically binds to the chitin of fungal cell wall. After washing 3 times with PBS, the coverslips were mounted on a glass slide with a drop of ProLong Gold antifade reagent (Molecular Probes) and sealed with nail polish. The total number of cell associated organisms (i.e. germlings adhering to monolayer)

was determined by phase-contrast microscopy. The same field was examined by epifluorescence microscopy, and the number of uninternalized germlings (which were brightly fluorescent) was determined. The number of endocytosed organisms was calculated by subtracting the number of fluorescent organisms from the total number of visible organisms. At least 400 organisms were counted in 20-40 different fields on each slide. Two slides per arm were used for each experiment and the experiment was performed in triplicate on different days.

[00196] *R. oryzae*-induced endothelial or CHO cell damage was quantified by using a chromium (^{51}Cr) release assay (Ibrahim et al., *J. Infect. Dis.*, 198:1083-1090 (2008)). Briefly, endothelial cells or CHO cells grown in 96-well tissue culture plates containing detachable wells were incubated with 1 μCi per well of $\text{Na}_2^{51}\text{CrO}_4$ (ICN, Irvine, CA) in M-199 medium (for endothelial cells) or Alpha minimum Eagle's medium (for CHO cells) for 16 h. On the day of the experiment, the unincorporated ^{51}Cr was aspirated, and the wells were washed twice with warmed Hanks' balanced salt solution (Irvine Scientific, Irvine, CA). Cells were infected with fungal germlings (1.5×10^5 germinated for 1 h) suspended in 150 μl of RPMI 1640 medium (Irvine Scientific) supplemented with glutamine. Spontaneous ^{51}Cr release was determined by incubating endothelial or CHO cells in RPMI 1640 medium supplemented with glutamine without *R. oryzae*. After 3 h of incubation at 37°C in a 5% CO_2 incubator, 50% of the medium was aspirated from each well and transferred to glass tubes, and the cells were manually detached and placed into another set of tubes. The amount of ^{51}Cr in the aspirate and the detached well was determined by gamma counting. The total amount of ^{51}Cr incorporated by endothelial cells in each well equaled the sum of radioactive counts per minute of the aspirated medium plus the radioactive counts of the corresponding detached wells. After the data were corrected for variations in the amount of tracer incorporated in each well, the percentage of specific endothelial cell release of ^{51}Cr was calculated by the following formula: $[(\text{experimental release} \times 2) - (\text{spontaneous release} \times 2)] / [\text{total incorporation} - (\text{spontaneous release} \times 2)]$. Each experimental condition was tested at least in triplicate and the experiment repeated at least once.

[00197] For antibody blocking of adherence, endocytosis, or damage caused by *R. oryzae*, the assays were carried out as described above except for incubating endothelial cells with 50 μg of anti-CotH antibodies (purified IgG) or with serum obtained from the same

rabbit prior to vaccination with CotH3 peptide predicted to be antigenic for 1 h prior to adding *R. oryzae* germlings.

In vivo virulence studies

[00198] For *in vivo* studies, ICR male mice (≥ 20 g) (Taconic Farms) were rendered DKA with a single i.p. injection of 190 mg/kg streptozotocin in 0.2 ml citrate buffer 10 days prior to fungal challenge (Ibrahim et al., *Antimicrob. Agents Chemother.*, 47:3343-3344 (2003)). Glycosuria and ketonuria were confirmed in all mice 7 days after streptozotocin treatment. Diabetic ketoacidotic mice were infected with fungal spores by intratracheal route after sedating the mice with ketamine (66 mg/kg) and xylazine (4.8 mg/kg) with a target inoculum of 2.5×10^5 spores. To confirm the inoculum, the lungs from three mice that were sacrificed immediately following inoculation, were homogenized in PBS and quantitatively cultured on PDA plates containing 0.1% triton and colonies were counted following a 24 h incubation period at 37°C. The primary efficacy endpoint was time to moribundity. In some experiments, as a secondary endpoint, fungal burden in the lungs and brains (primary target organs) was determined on day +2 post infection by qPCR assay as previously described (Ibrahim et al., *Antimicrob. Agents Chemother.*, 49:721-727 (2005)). Values were expressed as \log_{10} spore equivalent/g of tissue. Histopathological examination was carried out on sections of the harvested organs after fixing in 10% zinc formalin. The fixed organs were embedded in paraffin, and 5 mm sections were stained with hematoxylin and eosin (H&E) or Periodic acid-Schiff stains to detect *R. oryzae* hyphae (Ibrahim et al., *J. Clin. Invest.*, 117:2649-2657 (2007)).

[00199] For *in vivo* expression of the CotH genes, lungs and brains collected from mice 48 h post infection intratracheally with wild-type *R. oryzae*, or transformants with empty plasmid or with RNA-i construct were flash frozen in liquid nitrogen and process for RNA extraction using a Tri Reagent solution (Ambion). Reverse transcription was performed with RETROscript (Ambion) using primers listed in Table 3. For quantitative RT-PCR, SYBR green assays were performed. Constitutively expressed *ACT1* was used as a control for all reactions. Calculations and statistical analyses were performed using ABI PRISM 7000 Sequence Detection System User Bulletin 2 (Applied Biosystems).

Passive immunization

[00200] To detect if antibodies against CotH proteins protect mice from mucormycosis, diabetic ketoacidotic mice were immunized with 1 mg of rabbit purified anti-CotH IgG raised against GAGKKHNNAKQSWNW (SEQ ID NO: 39) or MGQTNDGAYRDPTDNNK (SEQ ID NO: 40) by intraperitoneal injection 2 hours prior to infecting the mice intratracheally as outlined above. Control mice were infected similarly but received a similar dose from the same rabbit prior to vaccinating with the CotH3 peptide. Three days post infection a repeated dose of the antibody or the control serum (prior to vaccination) was introduced. The primary efficacy endpoint was time to moribundity.

Statistical analysis

[00201] Differences in CotH expression and fungi-endothelial cell interactions were compared by the non-parametric Wilcoxon Rank Sum test. The non-parametric log-rank test was used to determine differences in survival times. Comparisons with *P* values of <0.05 were considered significant.

Results

Isolation of Putative *R. oryzae* Ligand(s) That Bind to Endothelial Cell GRP78.

[00202] To identify the *R. oryzae* ligand that binds to endothelial cell GRP78, cell wall material from supernatants of protoplasts of *R. oryzae* germlings were collected (Michielse et al., *Mol. Genet. Genomics*, 271:499-510 (2004)). Incubating protoplasts in the presence of an osmotic stabilizer (e.g. sorbitol) enables regeneration of the cell wall, and during regeneration cell wall constituents are released into the supernatant (Pitarch et al., *Mol. Cell. Proteomics*, 5:79-96 (2006); Pitarch et al., *Electrophoresis*, 20:1001-1010 (1999)). After a 2 h incubation period, (Michielse et al., *Mol. Genet. Genomics*, 271:499-510 (2004)) protoplasts were pelleted and the supernatant was sterilized in the presence of protease inhibitors. The supernatant was concentrated and protein concentration was measured. Negative control samples were processed similarly with the exception of absence of protoplasts. FAR Western blot analysis (Wu et al., *Nat. Protoc.*, 2:3278-3284 (2007)) using recombinant human Grp78 and anti-Grp78 Ab revealed the presence of 4 bands collected from the supernatant of *R. oryzae* protoplasts that bound to Grp78p (Figure 51A). These

bands were excised for protein identification by MALDI-TOF-MS/MS analysis. Only 4 ORFs predicted to be cell surface proteins were identified with GPI anchor sequence at the c-terminus, signal peptides at the N-terminus and multiple predicted N- and O-glycosylation sites. Three of the ORF (i.e. RO3G_05018, RO3G_08029, and RO3G_11882) had limited homology of 17% at the amino acid level to CotH family of proteins implicated in spore coat formation from several bacteria (Giorno et al., *J. Bacteriol.*, 189:691-705 (2007); Naclerio et al., *J. Bacteriol.*, 178:6407 (1996)). These were named CotH1 (RO3G_05018), CotH2 (RO3G_08029) and CotH3 (RO3G_11882). The fourth ORF RO3G_16295 is widely present in many fungi and some bacteria without an identified function.

CotH2 and CotH3 are closely related to each other with 77% identity at the amino acid level, while CotH1 is more distantly related (Figure 51b). The fourth ORF had an overall identity of 10% to the three CotH proteins at the amino acid level. Upon searching the *R. oryzae* (*delema*) 99-880 genome data base, two more related ORFs were found (66% homology at the amino acid level) and were predicted to encode GPI-anchored proteins. These two ORFs (RO3G_09276; and RO3G_01139) had distant homology to CotH1, CotH2, CotH3 proteins (20-24% at the amino acid level). These ORFs were named CotH4 and CotH5, respectively.

[00203] The possibility of the presence of this family of genes in other *Mucorales* known to cause human mucormycosis was also examined. Using primers that span the entire CotH3 ORF (1.9 kb), bands were amplified from clinical isolates including *R. oryzae* 99-892, *Mucor* sp. 99-932, *Lichtheimia corymbifera*, *Cunninghamella bertholletiae*, and *Rhizomucor*. Sequence analysis of these PCR-amplified bands revealed more than 90% identity at the nucleotide and predicted amino acid level with *R. oryzae* 99-880 CotH3. Collectively, these studies show the uniqueness of CotH family of genes to agents of mucormycosis.

CotH2 and CotH3 are Expressed During Interaction of *R. oryzae* with Endothelial Cells.

[00204] Based on the results disclosed herein, it was hypothesizes that, if any of the isolated proteins represented a fungal ligand to GRP78, then the proteins must be expressed during *R. oryzae* interaction with endothelial cells. Since *R. oryzae* binds endothelial cell GRP78 while in germlings, the expression of these four ORFs in spores or germlings were studied. All CotH genes were expressed in the spore form while only CotH3 was expressed in germlings of *R. oryzae*. Importantly, when *R. oryzae* germlings were incubated with

endothelial cells, both CotH2 and CotH3 were expressed (Figure 52B) with CotH3 having 16 fold and 4 fold increase compared to CotH1 and CotH2, respectively (Figure 52C). Finally, the fourth ORF RO3G_16295 was not expressed *R. oryzae* spores or germlings (Fig. 52A) or in *R. oryzae* germlings interacting with endothelial cells (Fig 52B). These results showed that CotH3 and to a lesser extent CotH2 are putative candidates for interacting with GRP78 during invasion of human cells.

S. cerevisiae Cells Expressing CotH2 or CotH3 Bound GRP78 and Adhered to and Invaded Endothelial Cells and CHO cells Overexpressing GRP78.

[00205] To study the role of CotH1, CotH2 and CotH3 in interacting with the GRP78 receptor, CotH2 or CotH3 were heterologously expressed in the non-adherent non invading *S. cerevisiae*. The transformed yeast cells were tested for their ability to specifically bind endothelial cell GRP78. Antibodies raised against two CotH3 peptides predicted to be antigenic and surface expressed (GAGKKHNNAKQSWNW (SEQ ID NO: 39), and MGQTNDGAYRDPTDNNK (SEQ ID NO: 40)) recognized *S. cerevisiae* expressing CotH3, and to a lesser extent CotH2, but not cells expressing CotH1 (Figure 53). *S. cerevisiae* cells expressing CotH3 primarily bound GRP78 from endothelial cell membrane protein extracts. CotH2 expressing yeast cells also bound GRP78 from the same extract but *S. cerevisiae* expressing CotH1 (Figure 54A). These results indicated that CotH3, and to lesser extent CotH2, interact with endothelial cell GRP78 during invasion of *R. oryzae* of the endothelium. To confirm this hypothesis, the ability of the transformed yeast cells to adhere to and invade endothelial cells *in vitro* was examined.

[00206] Compared to empty plasmid, *S. cerevisiae* expressing CotH1 had no enhancement in adherence to or endocytosis (invasion) of endothelial cells. In contrast, yeast cells expressing CotH2 or CotH3 had multiple fold increase in adherence to and invasion of endothelial cells compared to *S. cerevisiae* expressing CotH1 or those transformed with empty plasmid (Figure 54B). Importantly, cells expressing CotH3 had significantly higher ability to adhere to and invade endothelial cells compared to yeast cells expressing CotH2. To examine if this enhanced adherence to and invasion of endothelial cells was due to interactions with GRP78, the ability of *S. cerevisiae* expressing CotH1, CotH2, CotH3 or empty plasmid to adhere to and invade parent CHO cells were compared to CHO cells overexpressing GRP78

(Morris et al., *J. Biol. Chem.*, 272:4327-4334 (1997); Reddy et al., *J. Biol. Chem.*, 278:20915-20924 (2003)).

[00207] Only yeast cells expressing CotH2 or CotH3 had significant enhancement in their adhering to and invading CHO cells overexpressing GRP78 (Figure 54C). Yeast cells expressing CotH1, CotH2 or CotH3 demonstrated no increased ability to bind to and invade parent CHO cells. Collectively, these data show that CotH3, and to a lesser extent CotH2, represents an adhesin/invasin of *R. oryzae* during interacting with endothelial cell GRP78.

CotH3 Protein is a *R. oryzae* Invasin.

[00208] Because endocytosis of the fungus was previously shown to be a prerequisite for *R. oryzae* to cause endothelial cell damage, (Ibrahim et al., *Infect. Immun.* 73:778-783 (2005); Liu et al., *J. Clin. Invest.*, 120:1914-1924 (2010)) blocking the function or expression of CotH3 to protect endothelial cells from *R. oryzae*-induced endocytosis and subsequent damage was investigated. Endocytosis, but not adherence, of *R. oryzae* germlings was abrogated by addition of rabbit anti-CotH3 polyclonal antibodies, but not pre-immune serum collected from the same animal (Figure 55A). The damage to endothelial cells caused by *R. oryzae* germlings was reduced by >40% using anti-CotH3 antibodies (Figure 55B)

[00209] To complement the antibody blocking studies, suppression of CotH3 and CotH2 expression was investigated to determine their impact on adherence, endocytosis, and endothelial cell damage. Using a previously described RNA-i method (Ibrahim et al., *Mol. Microbiol.*, 77:587-604 (2010)), a ~400 bp fragment was used to suppress both genes in one construct. CotH2 and CotH3 expression in two clones of *R. oryzae* *pyrf* mutant (Skory and Ibrahim, *Curr. Genet.* 52:23-33 (2007)) transformed with the RNA-i construct harboring PyrF as a selection marker (i.e. Trans 2 and Trans 6) were almost entirely abrogated compared to *R. oryzae* *pyrf* mutant transformed with empty plasmid (Figure 56A).

[00210] Next, the cell surface expression of CotH2 and CotH3 on the constructed mutants was assayed by flow cytometry using anti-CotH3 polyclonal antibodies as described herein. *R. oryzae* transformed with the RNA-i construct expressed less cell surface CotH2 and CotH3 proteins compared to wild-type or *R. oryzae* transformed with the empty plasmid (Figure 56B). These RNA-i transformants had no difference in growth rate, cell size or germination when compared to the wild-type or empty plasmid transformed cells (Figure

56C). However, the reduction of *R. oryzae* cell surface expression of CotH2 and CotH3 resulted in significant reduction of endothelial cell endocytosis of *R. oryzae* germlings and subsequent endothelial cell damage (Fig 57A and 57B). These results show that CotH3 and CotH2 are required for maximal invasion of endothelial cells by *R. oryzae*.

[00211] To further demonstrate that CotH3 and CotH2 function as invasins via binding to GRP78, the ability of *R. oryzae* germlings with CotH3 and CotH2 RNA-i construct to cause damage to CHO cells overexpressing GRP78 or parent CHO cells (which do not overexpress GRP78) were compared to *R. oryzae* transformed with the empty plasmid or wild type *R. oryzae* cells (Morris et al., *J. Biol. Chem.*, 272:4327-4334 (1997); Reddy et al., *J. Biol. Chem.*, 278:20915-20924 (2003)). As previously shown (Liu et al., *J. Clin. Invest.*, 120:1914-1924 (2010)), wild type *R. oryzae* caused considerably more damage to CHO cells overexpressing GRP78 when compared to CHO parent cells. These results were further confirmed by a similar pattern of cell damage caused by *R. oryzae* germlings transformed with the empty plasmid. In contrast, CHO cells overexpressing GRP78 and CHO parent cells were equally susceptible to damage caused by *R. oryzae* germlings with reduced cell surface expression of CotH3 and CotH2 (Figure 57C). Collectively, these results indicate that CotH3 and CotH2 are cell surface proteins that mediate invasion (endocytosis) of endothelial cells via binding to GRP78.

CotH2 and CotH3 are Required for Full Virulence of *R. oryzae* *in vivo*.

[00212] Because CotH3 and CotH2 function as invasins of endothelial cells, it was hypothesized that these two genes are critical determinants of virulence. To test this hypothesis, the virulence of *R. oryzae* with reduced cell surface expression of CotH2 and CotH3 was compared to wild-type or to *R. oryzae* transformed with empty plasmid using an intratracheally infected diabetic ketoacidotic mouse model. Despite the initial infection being initiated by inoculating the lungs in this model, the infection hematogenously disseminates to other target organs such as the brain. Empty plasmid harboring cells were as virulent as wild type *R. oryzae* cells (median survival time of 3 vs. 4 days of the wild type and the empty plasmid infected mice, respectively, $P=0.33$). In contrast, mice infected with the RNAi-transformant had attenuated virulence, which was shown by a 10 day median survival time and 1/3 of the mice surviving the lethal infection ($P=0.003$) (Figure 58A). Additionally, mice

infected with the RNA-i transformant had significantly less fungal burden in the lungs and brains (primary and secondary target organs) when compared to the same organs recovered from mice infected with wild type cells or those infected with the empty plasmid transformant (Figure 58B).

[00213] To further demonstrate that the attenuated virulence observed with mice infected with *R. oryzae* transformed with the RNA-i construct was due to actual inhibition of CotH2 and CotH3, the pattern of *in vivo* expression of these genes was assessed in fungal hyphae recovered from the mouse target organs. CotH1 was not expressed in mice infected with wild type *R. oryzae*, or *R. oryzae* transformed with the empty plasmid or RNA-i constructs. In contrast, CotH2 showed a four fold and two fold increase in expression in the lungs and brains of mice infected with either the wild type *R. oryzae* or *R. oryzae* transformed with the empty plamid, receptively (Figure 58C) compared to CotH1. Additionally, CotH3 had significantly higher expression than CotH2 in the lungs, but not brains, of mice infected with wild type the empty plasmid transformant. Finally, fungal cells recovered from mice infected with *R. oryzae* transformed with the RNA-i construct had no expression of any of the CotH genes (Figure 58C). These results indicate that CotH2 and CotH3 are expressed *in vivo* and the reduced virulence in mice infected with *R. oryzae* transformed with the RNA-i is due to a lack of expression of any of the CotH genes.

[00214] To compare the severity of infection, histopathological examination of mice organs infected with the three different strains was conducted. Lungs harvested from mice infected with *R. oryzae* transformed with RNA-i construct had normal histology compared with lungs taken from mice infected with the wild type or *R. oryzae* transformed with the empty plasmid, which had an abundance of fungal abscesses characterized by phagocyte infiltration and substantial edema (Figure 59).

Anti-CotH3p antibodies protect diabetic ketoacidotic mice from *R. oryzae* infection.

[00215] Because the above data showed that CotH2 and CotH3 proteins act as invasins to mammalian cells *in vitro* and because CotH2 and Cot3 were required for full virulence of *R. oryzae* in the hematogenously disseminated murine model infection initiated by intratracheal inoculation, the use of anti-CotH3 and CotH2 antibodies raised against peptide GAGKKHNNAKQSWNW (SEQ ID NO: 39) or peptide

MGQTNDGAYRDPTDNNK (SEQ ID NO: 40) were investigated for their protective affect against the disease (antibodies raised against these 2 peptides recognized *S. cerevisiae* expressing either CotH2 or CotH3 proteins). 1 mg of the polyclonal antibodies was administered to diabetic ketoacidotic mice two hours prior to and three days post infecting intratracheally with *R. oryzae* spores. Mice receiving the anti-CotH2 and anti-CotH3 rabbit IgG had a significantly enhanced survival time compared to mice receiving pre-vaccination serum from the same rabbit. Survival at day 21 post infection was 44% for the mice receiving antibodies raised against peptide GAGKKHNNAKQSWNW (SEQ ID NO: 39) vs. 0% survival for the mice receiving the control pre-vaccination IgG (Figure 60A). Further, Survival at day 14 post infection was 75% for mice receiving antibodies raised against peptide MGQTNDGAYRDPTDNNK (SEQ ID NO: 40) vs. 0% survival for mice receiving the control pre-vaccination IgG (Figure 60B). These results demonstrate that antibodies targeting CotH proteins can be used to treat mucormycosis.

EXAMPLE III Diagnostic Methods for Detecting Mucormycosis

[00216] A series of experiments were performed to determine the detection capability of Nucleic Acid Sequence-Based Amplification (NASBA). A NASBA primer pair was designed to amplify a 127 bp CotH3 using *Rhizopus oryzae* total RNA as a template.

CotH3 forward primer 5'- GATGACAATTATATTCCCAGC-3' (SEQ ID NO: 35),
CotH3 reverse primer 5' - GAGTAGACGTAATTAGATCCAA-3' (SEQ ID NO: 36),
Molecular beacon probe: 5'-CGCGATCAAACGTACCTGCTGACCGAATCGATCGCG-3'
(SEQ ID NO: 38)

[00217] RNAs from *Aspergillus fumigatus*, *Candida albicans*, and *Rhizopus oryzae* were isolated using an RNeasy® Plant Mini Kit (Qiagen) according to manufacturer's instructions. Total RNA isolated from four different *R. oryzae* spores (10, 100, 1000, 10000 spores, respectively) were added to the NASBA reactions.

[00218] To test the specificity of the molecular beacon for *Rhizopus spp.*, RNAs isolated from *C. albicans* and *A. fumigatus* were used as controls. 300 ng total RNA was added to each reaction. NASBA reactions were performed with NucliSENS EasyQ Basic kit

v2 (bioMerieux bv, Boxtel, NL) according to manufacturer's instructions. In brief, The NASBA reaction volume was 20 μ l (per reaction) in MicroAmp[®] 96-Well Reaction Plate (Applied Biosystems) and contained 5.4 μ l of sterile water, 0.4 μ l of each primer, 0.2 μ l probe, 4 μ l of 5 x NASBA buffer. Then 5 μ l of purified RNA (300 ng) or water (when preparing no template controls) was added to the premix. Reaction mixtures were subsequently incubated at 65 °C for 5 min, cooled down to 41 °C for 5 min, after which 5 μ l of enzyme mix from the NucliSENS EasyQ Basic kit v2 was added. This mix consisted of containing T7 RNA polymerase, AMV-RT (avian myeloblastosis virus reverse transcriptase), RNase H, and BSA (bovine serum albumin). Reactions were incubated at 41 °C for 90 min. The fluorescence signal was measured with StepOnePlus Real-Time PCR machine (Applied Biosystems).

[00219] Using the NASBA amplification assay described above, the CotH3 molecular beacon primers/probe showed differential detection of fungal species, i.e. specificity for *R. oryzae*. Application products from samples spiked with *R. oryzae* readily show amplification, whereas amplification products from samples spiked with *A. fumigatus* or *C. albicans* were not detected (Figure 61).

[00220] The CotH3 molecular beacon primers/probe showed highly sensitive detection of *R. oryzae*. Fungal spores were germinated in YPD broth for 3 hours at 37°C shaker. 10 μ l of samples containing 10 to 10⁵ of germlings were aliquoted into 250 μ l of sheep blood. Total RNA was isolated from the spiked blood samples with RNeasy Plant Mini Kit (Qiagen) and eluted in 30 μ l elution buffer. Five microliter of the total RNA was added to each NASBA reaction. Application products were detected in all samples, include samples inoculated with only 10 germlings (Figure 62).

[00221] The CotH3 molecular beacon primers showed not only highly sensitive detection of *R. oryzae*, but also a robust specificity. Fresh *R. oryzae*, *A. fumigatus* and *C. albicans* spores were collected, counted and aliquoted into 10 μ l of YPD broth each. 350 μ l of sheep blood was added into each tube and incubated for 24 hours at 37°C shaker. Total RNA was isolated with RNeasy Plant Mini Kit (Qiagen) and eluted in 30 μ l in elution buffer. Five microliter of the RNA was added to each NASBA reaction. No amplification products were detected in samples inoculated with 10⁶ spores of *A. fumigatus* or *C. albicans*, whereas each sample inoculated with 10 to 10⁴ spores of *R. oryzae* were detectable (Figure 63).

[00222] The CotH3 molecular beacon primers/probe showed detection of multiple *Rhizopus* species. Fresh *R. oryzae* (99-880 and 99-892 isolates) and *R. microsporus* spores (1000 spores each) were aliquoted into 10 µl of YPD broth. 350 µl of sheep blood was added into each tube and incubated for 24 hours at 37°C shaker. Total RNA was isolated with RNeasy Plant Mini Kit (Qiagen) and eluted in 30 µl in elution buffer. Five microliter of the RNA was added to each NASBA reaction. Not only were samples containing *R. oryzae* 99-892 spores detectable, but samples containing *R. oryzae* 99-880 (R1000) and *R. microspores* (ATCC62417) showed amplification (Figure 64). These results show that primers designed from CotH genes of *R. oryzae* can detect other strains of *R. oryzae* as well as other species of *Rhizopus* confirming the conserved nature of CotH genes among Mucorales.

[00223] Throughout this application various publications have been referenced. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains. Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

1. An isolated nucleic acid encoding a Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof.
2. An isolated nucleic acid encoding a Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof, comprising a nucleic acid selected from:
 - (a) nucleic acid encoding an amino acid sequence set forth in SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32, or
 - (b) nucleic acid that hybridizes to the nucleic acid of (a) under moderately stringent conditions, wherein said nucleic acid contiguously encodes biologically active Mucorales CotH polypeptide, or
 - (c) nucleic acid degenerate with respect to either (a) or (b) above, wherein said nucleic acid encodes biologically active Mucorales CotH polypeptide.
3. The nucleic acid of claim 2, wherein said nucleic acid hybridizes under highly stringent conditions.
4. The nucleic acid of claim 2, wherein said amino acid sequence comprises least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to the amino acids sequence set forth in SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32.
5. The nucleic acid of claim 2, wherein said amino acid sequence consists of the amino acid sequence set forth in SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32, or a modification thereof.
6. The nucleic acid of claim 2, wherein said nucleic acid is cDNA.
7. A vector containing the nucleic acid of claim 2.
8. A recombinant cell containing the nucleic acid of claim 2.

9. A CotH oligonucleotide, comprising between 15 and 300 contiguous nucleotides of SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31 or the anti-sense strand thereof.

10. The CotH oligonucleotide of claim 9, wherein oligonucleotide comprises the nucleic acid sequence of ATGAAATTATCTATTATATCCGCTGCC (SEQ ID NO: 33), GCTGGGAATATAATTGTCATCGA (SEQ ID NO: 34), GATGACAATTATATTCCCAGC (SEQ ID NO: 35), GAGTAGACGTAATTAGATCCAA (SEQ ID NO: 36) or AACGTACCTGCTGACCGAATC (SEQ ID NO: 37).

11. An antisense-nucleic acid capable of specifically binding to mRNA encoded by said nucleic acid according to claim 2.

12. A kit for detecting the presence of a Mucorales CotH nucleic acid sequence comprising at least one oligonucleotide according to claim 9.

13. The kit of claim 12, wherein said at least one oligonucleotide comprises the nucleic acid sequence selected from ATGAAATTATCTATTATATCCGCTGCC (SEQ ID NO: 33), GCTGGGAATATAATTGTCATCGA (SEQ ID NO: 34), GATGACAATTATATTCCCAGC (SEQ ID NO: 35), GAGTAGACGTAATTAGATCCAA (SEQ ID NO: 36) or AACGTACCTGCTGACCGAATC (SEQ ID NO: 37).

14. An isolated Mucorales CotH polypeptide, an immunogenic fragment thereof, or a functional fragment thereof.

15. The Mucorales CotH polypeptide of claim 14, wherein said polypeptide comprises at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to the amino acids sequence set forth in SEQ ID NOS: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32.

16. The Mucorales CotH polypeptide of claim 15, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 1, 3, 5, 11, 15, 16, 18, 20, 22, 24, 26, 28, 30 or 32.

17. The Mucorales CotH polypeptide of claim 14, wherein said functional fragment binds to GRP78.

18. The Mucorales CotH polypeptide of claim 17, wherein said functional fragment binds to GRP78 expressed by endothelial cells.
19. The Mucorales CotH polypeptide of claim 14, wherein said polypeptide is encoded by a nucleotide sequence comprising least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to the nucleic acid sequence as set forth in SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31.
20. The Mucorales CotH polypeptide of claim 14, wherein said polypeptide is encoded by a nucleotide sequence comprising the nucleic acid sequence set forth in SEQ ID NOS: 2, 4, 6, 9, 10, 12-14, 17, 19, 21, 23, 25, 27, 29 or 31.
21. The Mucorales CotH polypeptide of claim 14, wherein said immunogenic fragment consists essentially of the amino acid sequence GAGKKHNNAKQSWNW (SEQ ID NO: 39) or MGQTNDGAYRDPTDNNK (SEQ ID NO: 40).
22. The Mucorales CotH polypeptide claim 21, wherein said immunogenic fragment is conjugated to a carrier protein.
23. The Mucorales CotH polypeptide claim 22, wherein said polypeptide said carrier protein is keyhole limpet hemocyanin (KLH).
24. A method for expression of a Mucorales CotH polypeptide, said method comprising culturing cells of claim 8 under conditions suitable for expression of said Mucorales CotH.
25. An isolated anti-Mucorales CotH antibody having specific reactivity with a Mucorales CotH polypeptide according to claim 14.
26. The antibody according to claim 25, wherein said antibody is a monoclonal antibody.
27. A cell line producing the monoclonal antibody of claim 26.
28. The antibody according to claim 25, wherein said antibody is a polyclonal antibody.

29. The antibody according to claim 25, wherein said antibody has specific reactivity to a polypeptide consisting essentially of the amino acid sequence GAGKKHNNAKQSWNW (SEQ ID NO: 39) or MGQTNDGAYRDPTDNNK (SEQ ID NO: 40).

30. A composition comprising an amount of the antisense-nucleic acid according to claim 11 effective to inhibit expression of a Mucorales CotH polypeptide and an acceptable carrier capable of delivering said antisense-nucleic acid to a cell.

31. A method for identifying nucleic acids encoding a Mucorales CotH polypeptide, comprising contacting a sample containing nucleic acids with one or more oligonucleotides according to claim 9, wherein said contacting is effected under highly stringent hybridization conditions, and identifying a nucleic acid that hybridizes to said oligonucleotide.

32. A method of detecting a Mucorales CotH nucleic acid molecule in a sample, comprising contacting said sample with two or more oligonucleotides of claim 9, amplifying a nucleic acid molecule, and detecting said amplification.

33. The method of claim 32, wherein said amplification is performed using polymerase chain reaction.

34. The method of claim 32, wherein at least one of said two or more oligonucleotides comprise the nucleic acid sequence of ATGAAATTATCTATTATATCCGCTGCC (SEQ ID NO: 33), GCTGGGAATATAATTGTCATCGA (SEQ ID NO: 34), GATGACAATTATATTCCCAGC (SEQ ID NO: 35), GAGTAGACGTAATTAGATCCAA (SEQ ID NO: 36) or AAACGTACCTGCTGACCGAATC (SEQ ID NO: 37).

35. A method for detecting the presence of a Mucorales organism in a sample, comprising contacting a sample with an antibody according to claim 25, and detecting the presence of specific binding of said antibody to said sample, thereby detecting the presence of Mucorales organism in said sample.

36. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound selected from the group consisting of the Mucorales CotH polypeptide,

immunogenic fragment thereof, or functional fragment thereof of claim 14, the antisense-nucleic acid of claim 11 or the anti-Mucorales CotH antibody of claim 25.

37. A vaccine composition for immunization of a subject against a fungal condition, comprising an immunogenic amount of a Mucorales CotH polypeptide, or an immunogenic fragment of said polypeptide, and a pharmaceutically acceptable carrier.

38. The vaccine composition of claim 37, further comprising an adjuvant.

39. A method of treating or preventing mucormycosis in a subject in need thereof comprising administering an therapeutically effective amount of a pharmaceutical composition of claim 36 or a vaccine composition of claim 37.

40. A method of diagnosing mucormycosis infection in a subject, comprising the steps of:

(a) providing a test sample from the subject;

(b) contacting said sample with an agent that can binds said nucleic acid of claim 1 or said Mucorales CotH polypeptide of claim 14 under suitable conditions, wherein said conditions allow specific binding of said agent to said nucleic acid or polypeptide; and

(c) comparing the amount of said specific binding in said test sample with the amount of specific binding in a control sample, wherein an increased or decreased amount of said specific binding in said test sample as compared to said control sample is diagnostic of mucormycosis infection.

41. The method of claim 40, wherein said agent is selected from the group consisting of the anti-Mucorales CotH antibody of claim 25 or the CotH oligonucleotide of claim 9.

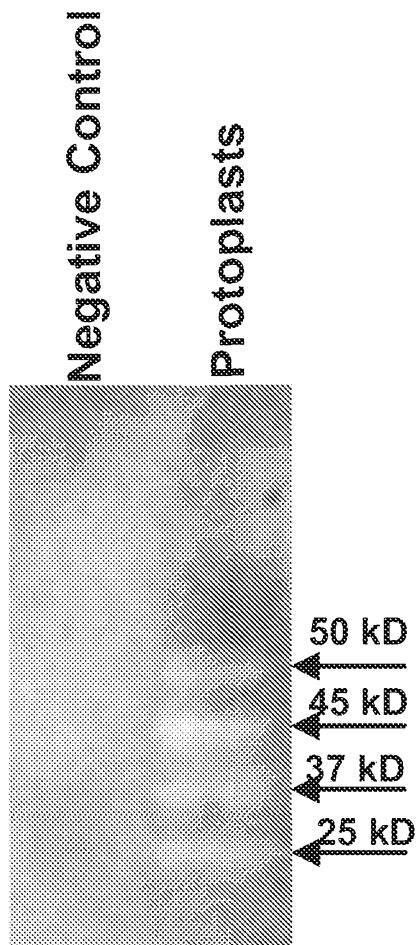


FIGURE 1

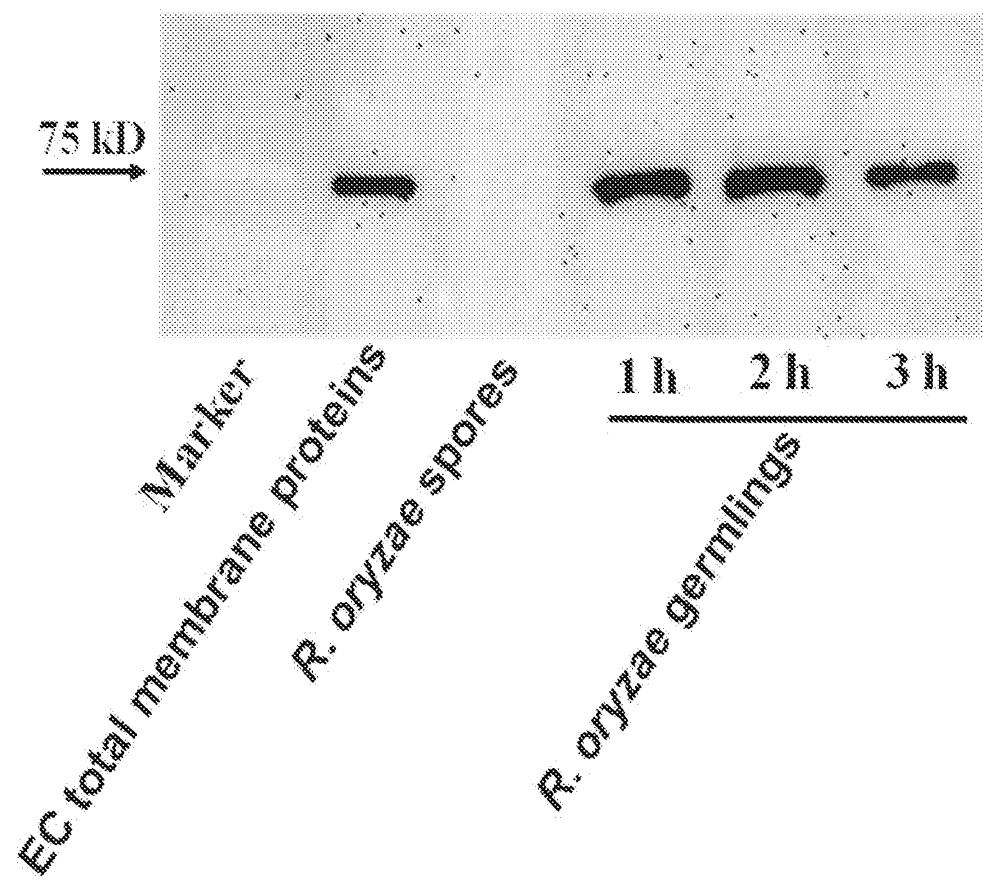


FIGURE 2

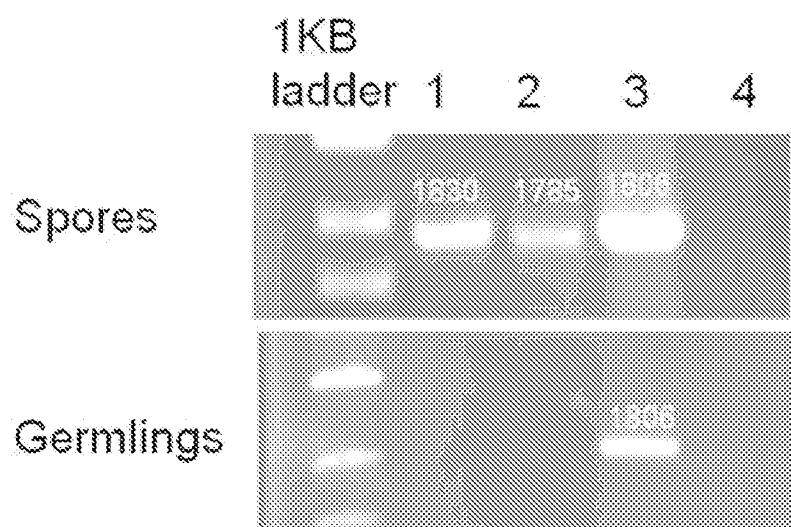


FIGURE 3

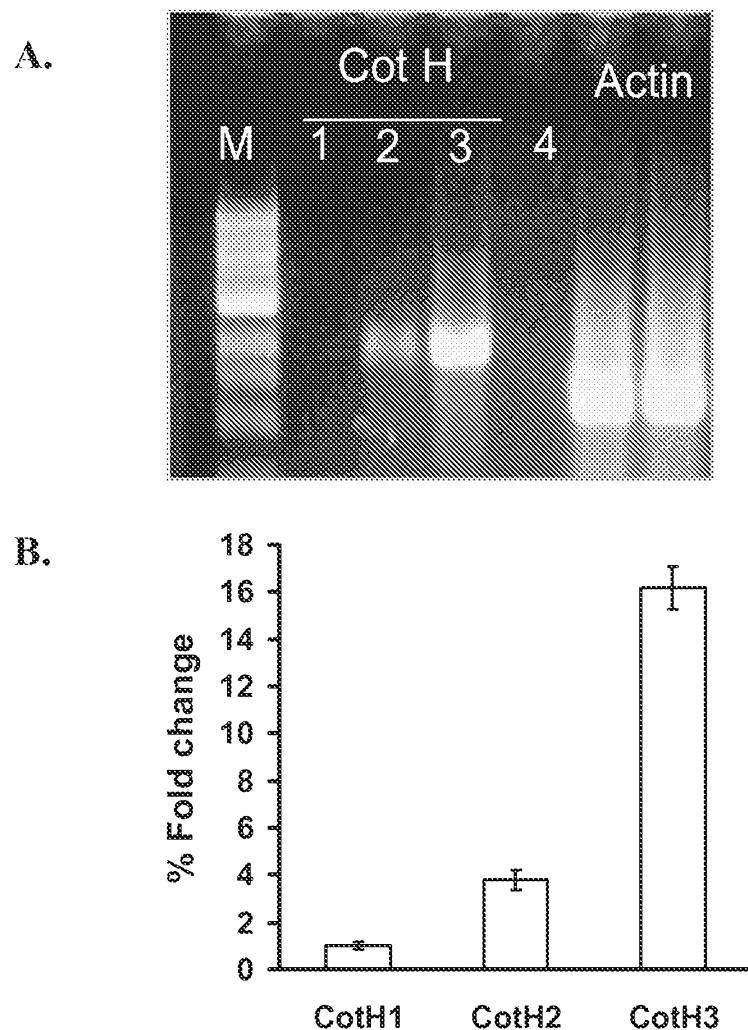
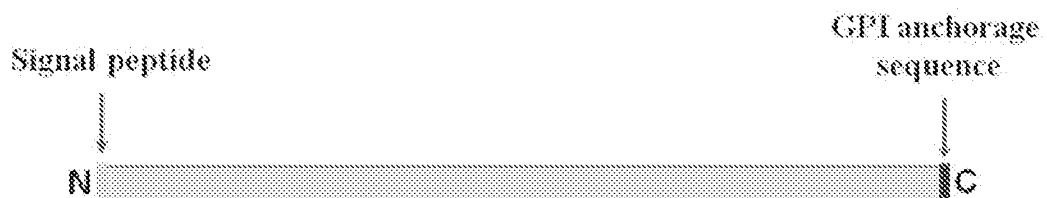


FIGURE 4



18 putative glycosylation sites with 10-N-linked and 8-O-linked sites.

	CotH1	CotH2	CotH3	RO3G_16295
CotH1	46%	46%	10%	
CotH2		78%	10%	
CotH3			9%	
RO3G_16295				

FIGURE 5

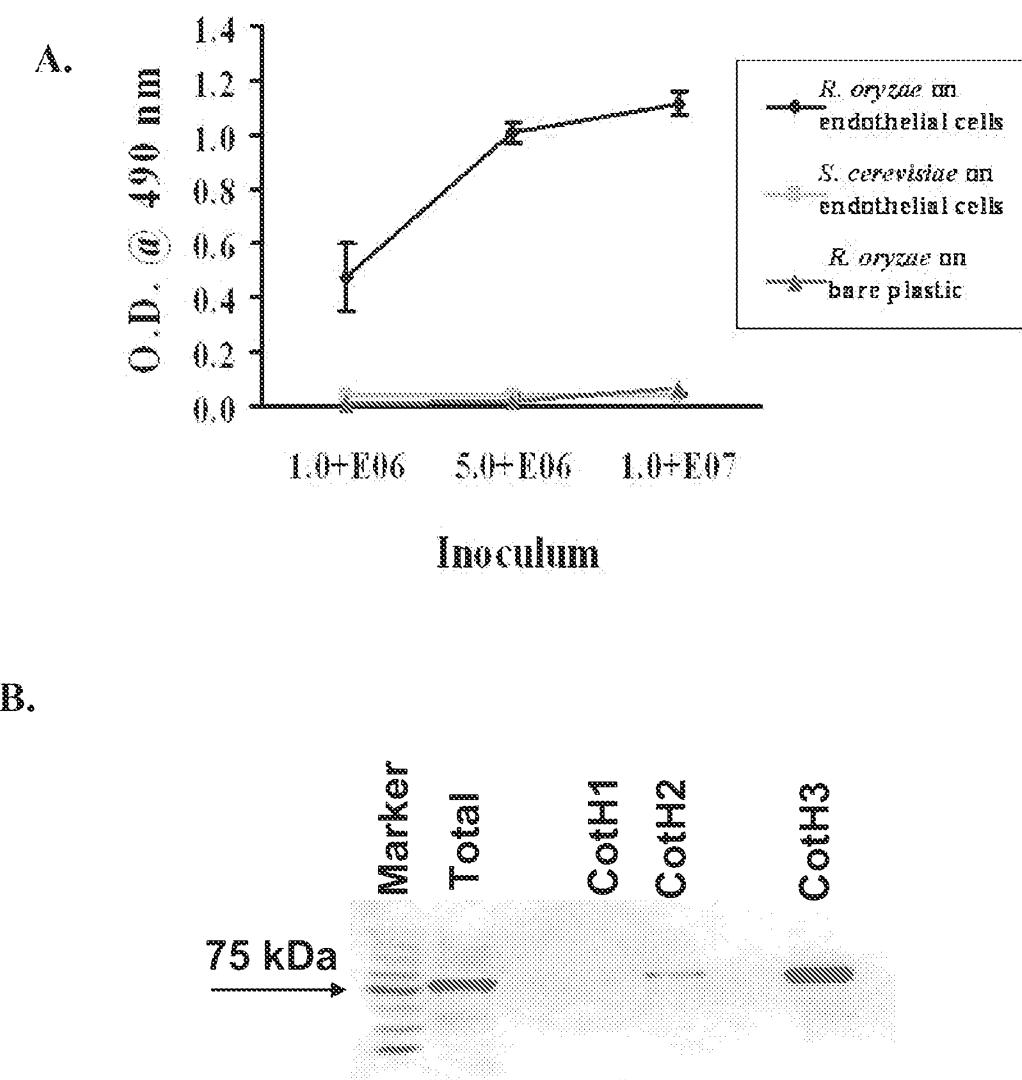
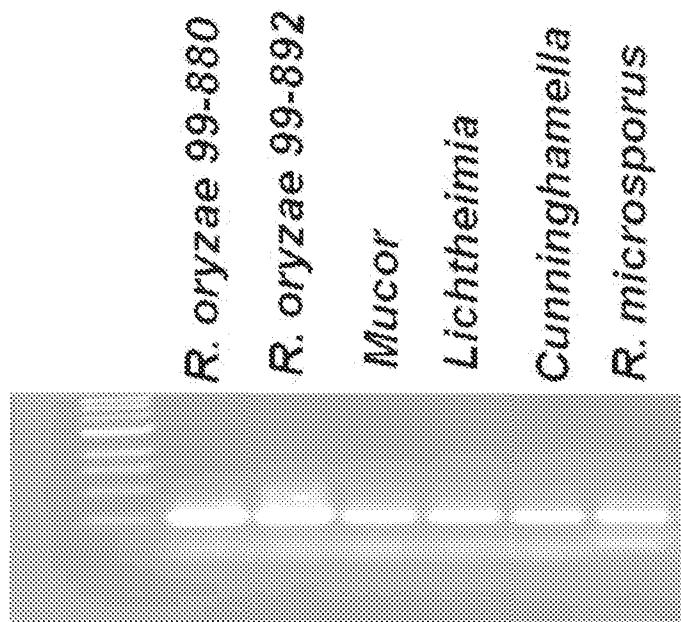


FIGURE 6



> 90% homology at the
nucleotide and amino acid levels

FIGURE 7

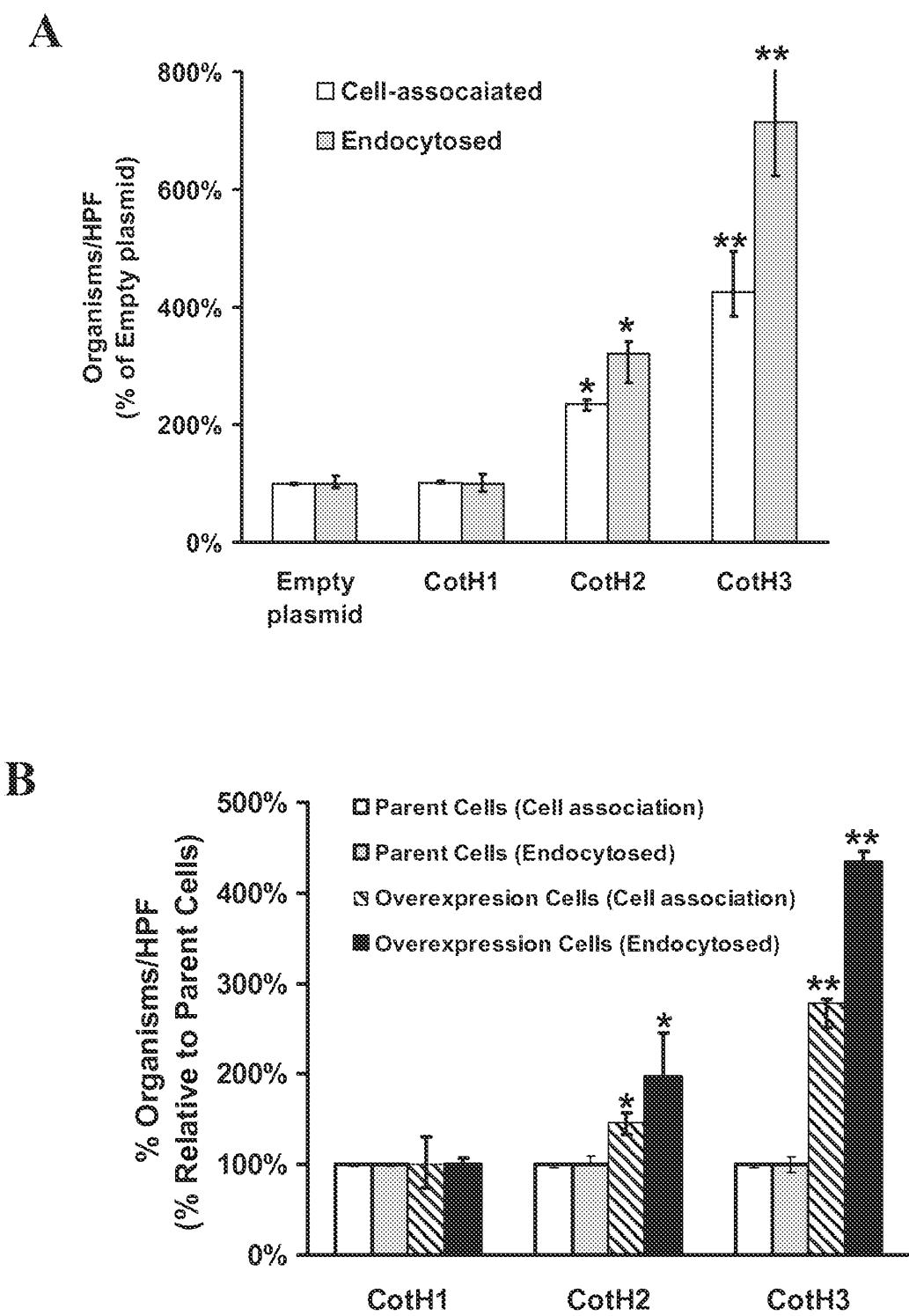


FIGURE 8

>Coth1 (R03G_05018) (mw=68.77)

MKSLLFVVVFIFLTYYAKVSFKVIAPDAKNRVHVNINGVLVELKASDPDVYVTGFAELKHGQSY
 NYVVDGNAEPFKRLLNGSSTKNEFFNRPVTYATNIPELPSILTEGSWTRGDTSNPIWDSNYVPSIF
 VTGNPREMNELIENVKKNTYKTKITFIGPETINTFEGCTLGLHKPGRKHNDAKQSWIWLPEGQFM
 ANRNWFKIRHMEEDPTQLREKLYADILRKMGTYANEANMVRFFINKEGMGIFNMLDDVIMYSYINA
 MFYHGDTPEQLGGLYDGASGASFNFPGDFDSFIPNVESPLDQDAIEPFSKAFTSIDFLEDEQVKTI
 GKYFDYDQFLRFMVMFLTGDWDGYWQEQTNDGAYIDINDHNKIYLGQDFDATFGVNLEQKREFV
 NVSYTEPKLFPGGVLINRLLQNPGVKKTFENYLKITVQEIEFNNATLGPYVTARHEFLAPDLQWDR
 SIKQRSPGNIFGWTFEQTYENLFEGVTAPGKNSGGADWGLLEWAKEKAVKSYLSSEAADAATV
 TQVPEAPGTDGTPSESTAWPHANTRFRQAEASNTHKIGTSSPSNFIVKIKQGTVSSSSSIKRTPCI
 LPLVILASTLFASFF*

Coding sequence (exons only) :1830nt

ATGAAATCCCTACTTTGTTATTCATCTTTAACACACATATGCCGCTAAAGTATCATT
 AAAGTTATCGCTCTGATGCTAAAAACAGAGTTCATGTCAATATCAATGGAGTACTTGTGAAATT
 AAGGCCAGTGATCCAGATGTTCTACTACACCGGTTTGCTGAACAAAGCATGGACAAAGTTAT
 AATTACGTTGTCGATGGAAATGCAGAGCCATTCAAACGTCTATTGAATGGCTCTTCTACTAAAAAC
 GAGTTCTCAATCGACCTGTAACCTACGCTACCAACATTCCCGAGCTACCCAGCATTCTACTGAA
 GGTAGCTGGACTCGTGGAGATACGAGTAATCCTATTGGGATAGTAACATGTCCCTCTATCTT
 GTCACTGGAAATCCAAGGGAAATGAATGAATTATTGAGAATGTGAAAAGAACACATATAAAACA
 AAAATCACTTTATTGGACCTGAGACTATCAACACATTGAGGTTGTACCCCTGGACTTCATAAA
 CCTGGCGTAAACATAATGATGCTAAACAATCTGGATATGGCTCTCCCTGAAGGTCAATTATG
 GCGAATCGAAATTGGTTAAGATTGACATATGGAAGAACATCCTACACAACATTCTGAAAGCTT
 TATGCAGATATCCTTCGAAAGATGGGACCTATGCGAATGAGGCCAATATGGTCGATTCTTATA
 AACAAAGGAAGGCATGGGTATCTTAATATGTTGGACGATGTTATTATGTATTCTTATTAATGCC
 ATGTTTACACGGTGATACTCCTGAACAGCTCGGTGGCTTACGACGGTGCCTCTGGTGCTCA
 TTCAATTTCTGGTGAATTGATAGCTTCATCCCGAATGCGAACATCCCCGCTTGACCAAGATGCT
 ATCGAGCCTTCTAAAGCCTTACTAGTATTGACTTTGGAAAGACGAAACAGGTCAAGACGATC
 GGAAAGTATTGATTATGACCAATTCTCGTTCATGGTCATGGAGTTCTACCGGTGACTGG
 GATGGCTACTGGCAAGAGCAAACAAATGATGGGCCTATTGATATCAACGACCACAAACAAATT
 TATTACTTGGGCAGGATTGACGCCACCTTGGTGTAAATCTGAACAAAAACGAGAGTTGTC
 AATGTGTCTTAACTGAATACCCAAACTGTTCTGGAGGTGTTGATCAACAGACTTCTCAA
 AACCCAGGTGTCAAGAAAACGTTGAAAACATCTGAAAATCACAGTACAAGAGATCTCAACAAAC
 GCCACGCTGGCCCCATGTCAGTCCTGCCACGAATTCTTGCTCCAGATCTCAGTGGATCGT
 TCGATAAAACACGGCTCTGGAAATATCTTGGTGGACGTTGAGCAGACATATGAAAATCTA
 TTTGAAGGGTGTACTGCTCTGGAAAAAAACTCGGGTGGTGTGATTGGGGTCTTCTTGAATGGGTG
 GCTGCAAAGGAAAAGCAGTCAAAAGTTACCTCAGTTGCTGAAGCCGCTGATGCTGCCACTGTT
 ACGCAAGTACCAAGAGCTCTGGTACAGATGGCACTCCTCCGAATCAACTGCCTGGCCTCATGCC
 AATACAAGGTTAGACAAGCCGAAGCTTCTAATACTCATAAAATAGGCACCTCATCGCCTCTAAT
 TTTATTGTTAAATCAAGCAAGGTACTGTGTATCTATCTAAAGAACCCCATGTATT
 CTACCTCTGTTATCTTG GCTAGCACTTTATTGCCTCTTCTTAG

FIGURE 9

>CotH2 (RO3G_08029) (mw=65.26)

MKLSLTIVSSSFLVAIAHAASVQFNLIAPSATDVKVSVNGQQVALTASDPNVPYFTGSAEVGGTEE
 SFERSLAGITNSTFNDYFNRPTVYANLPQLPWIENDPQWTRKGKKAEIFDDNYIPSVFFHGDDSQ
 VQDLVKNVPKDKVTGTLFIGSNYVHSFANVSEGIHGAGKKHNNAKQSWKWTLSGTDTMGNRNFK
 LRHMEEDPTQIRERLYADILHAMGTYANETTMVRLFINGQGFGTFNMLDDITEFSYINAMFYGGNP
 PATLGPLFDGASGADFIYHPGNLDGYSSWKPNKDNAMEGEYEAFDPLCKAWNETDYTDNTAIANFE
 KMFDEHFLRFMVIEYLTAHWDGYWMQNTNDGAYRDPSENWKYFLDQDFDATFGVNLDVPENKDF
 ISVSYKDFPSRYPAGVMANGLLQNADKKAKFEQYLTETVRVLFNNVTLNRLVLAHNFLSPDLEWD
 RSIVQQSPGTNFGWTFEQTSQNLWQGVSAAPNNNGGAEWGLVEYIAAKSQAMAKEFNITIVSEPVG
 PPAANGTATSTNDGGNTHTAAGESKPASSSESSGSKIASQSVSGASRSAVSTVLLGVTALVATAIF
 *

Coding sequence (exons only) :1785nt

ATGAAATTATCACTCACTATAGTATCCTCTTCATTTAGTAGCCATTGCACATGCTGCTTCAGTA
 CAATTCAATTAAATTGCTCCAAGTCCAACGTGATGTCAAAGTATCTGTAATGGACAGCAGGTTGCC
 CTTACAGCTTCAGACCCAATGTGCCTATTTCACCGGATCTGCTGAAGTTGGTGGAACTGAAGAA
 AGCTTGAGCGTTCTCGCTGGTATTACAAACTCCACATTCAATGATTTTATAACCGTCCTGTT
 ACTTACGCTAACCTCCTCAATTACCATGCCAATTGAAAATGATCCACAAATGGACTCGCAAAGGA
 AAGAAAGCTGAAATATTGACGACAACATACATCCCCTCCGTATTCTTCACGGTGATGATAGCCAA
 GTCCAAGATTGGTAAAAATGTGCCAAAGACAAAGTTACTGGCACCTGACTTCATTGGTCC
 AATTACGTTCATTCTTCGCAAATGTCTCCTTGGATTACATGGTGAGGAAAGAACACAACAT
 GCAAAGCAATCCTGGAAGTGGACCTTGTCTGGTACTGATACTATGGCAACCGTAACCATTAAAG
 CTTCGTCATATGGAAGAAGATCCTACACAGATCCGTGACGTCTTATGCTGATATATTGATGCT
 ATGGGAACCTATGCCAATGAGACCACTATGGTCCGATTGTTATTACGGTCAAGGTTTGGTACC
 TTCAACATGCTAGACGATATTACTGAATTTCATCAATGCCATGTTCTATGGTGGTAATCCT
 CCTGCTACTTTAGGACCTCTATTGATGGTGCAAGCGGTGCAGACTTATTACCATCCTGGTAAT
 CTCGATGGTTATTCCCTTGGAAACCTAATAAGGACAACGCAAACGGTGAAGGCTATGAGGCCTT
 GATCCTCTATGTAAGGCTTGGAACGAAACCGACTATACCGATAACACAGCCATTGCCAACTTGAA
 AAAATGTTGATACCGAGCACTTTACGATTACGTGATTGAATATCTAACTGCTCACTGGGAT
 GGTTATTGGATGGGACAGACCAACGATGGTGCTTACGTGACCCAAAGTGACAATAACAAGTGGTAC
 TTTTGGATCAAGATTGATGCCACATTGGTCAATTGGACGTTCTGAGAATAAAAGACTTT
 ATCAGTGTCTCCTACAAGGATTCCCACCTCGTTACCTGCTGGTGTATGGCAATGGTCTCTTA
 CAGAATGCTGATAAAAAGCCAAGTTGAAACAGTACTGACTGAAACTGTTCGCGTCTGTTCAAT
 AATGTCACTTGACTAATCGTGTCTGGCTATCCACAACTTCCTCTCCTGATCTGAATGGGAT
 CGATCCATCGTCAACAGTCGCCCTGGTACTAATTGGATGGACCTTGAGCAAACCTCTCAAAAC
 TTATGGCAAGGGTGTCTCAGCCCCAAATAACAAACGGAGGTGGTGCTGAGTGGGGCTTGGTGAATAT
 ATCGCAGAAAATCCCAAGCCATGGCTAAGGAATTAAATACACTATTGCTCTGAACCTGTAGGT
 CCTCCTGCTGCTAATGGAACGTGCAACTCTACTAATGATGGTGGTAACACTCATACCGCTGCCGGA
 GAAAGTAAGCCTGCCTCAAGTTCTGAATCTCCGGTTCGAAAATTGCTTCTCAAAGCGTATCAGGT
 GCTCCCGTTCTGCTGTATCTACCGTCTTATTAGGTGTTACAGCTTAGTTGCACTGCTATCTT
 TAA

FIGURE 10

>CotH3 (RO3G_11882) (mw=65.77)

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
 YVAGGTEESFDRSLDGITNSTLNDFYNRPVYANLPQLPWPIEKDPQWTRSGSKADIFDDNYIPS
 FFHGDDSQVNQVVKNVPADRIISGTLFIGSNYVYSFQNVSFGIHAGKKHNNAKQSWNWILSGSDT
 MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGTYANDATMVRLFINNQGFGTFNMLDDITQFSYIN
 AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNEAYEALDPLCKAWNNETTYTD
 NTAIANFEKMFDFLDRFMRFMVEYLTADWDGYWMGQTNDGAYRDPTDNNKWKYFLDQDFDGTFGVNL
 AAPEGNAFLDVSYKDFPSRYPGAVMINNLLQNADKKATFEKYLTERVRLFNNVTLTNRVLALHNF
 LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGVTAAPNNNGGAAFGLVEYIAAKAQAVAKEFNI
 SIVSQPVGPPSANGTTAAAPAPAAGNSTGKGGNQSISSSASSNKTSAQSTSGASRSKTAPIVLAIS
 ALALLVF*

Coding sequence (exons only) : 1806nt

ATGAAATTATCTATTATCCGCTGCCTTTAGTGGCTATTACACACGCTGCTCAATAAGTT
 AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
 GCTCAGATGCAAATGTCCTTATTCACTGGTCAGCTGAAGTTGGTGCTCAAAGACATACAAA
 TATGTTGCAGGTGGAACAGAAAGAAAGTTGATCGTTCTCTGATGGAATCACAAACTCAACACTT
 AATGATTTTATAACCGCCCCGTCACTTATGCTAACCTCCTCAATTACCTGGCCAATTGAAAAA
 GACCCCTCAGTGGACTCGTTCTGGAAAGCAAAGCCGACATTTCGATGACAATTATATTCCCAGCGTT
 TTTTCCACGGAGATGACAGTCAAGTCAAATGTGGTTAAAACGTACCTGCTGACCGAATCAGT
 GGTACACTGACCTTATTGGATCTAACGTCTACTCTTCCAGAATGTCTCATTGGTATTAC
 GGTGCTGGCAAGAACACAACATGCAAAACAAATCTGGAACTGGATATTGTCTGGAAAGTGATACG
 ATGGGTAACCGCAATTCTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTGCTGAAACGT
 CTTATTCTGACATTTCACATGCCATGGTACTTATGCCAATGATGCTACCATGGTCATTGTT
 ATTAACAACCAAGGCTCGGTACCTTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
 GCTAAATTATATAATGGCAAACCAACCTGCTACCTTGGCTCTCTATGATGGTGCTCTGGTCA
 GACTTCTTATATCATCCTGGTAACCTCGATGGATACTCTCTGGTTGCCAACACAGCCAATCCT
 AATGGTGAAGCTTATGAAGCTTGTACCTCTCTGTAAGGCCTGGAACGAGACGACCTAACCGAT
 AATACAGCCATTGCAAACCTTGGAAAAATGTTGATCTCGACCGTTCATGCGTTATGGTTATT
 GAATACTTGACTGCCGATTGGGATGGTTACTGGATGGACAGACCAATGATGGTGCTATCGTGAT
 CCAACTGATAATAACAAGTGGACTTTAGATCAAGACTTGTGATGGTACTTTGGTGTCAACTTG
 GCTGCACCCGAAGGCAATGCTTTCTTGATGTTCTTACAAGGATTCCCTCTCGTTACCGTGG
 GCTGTCATGATCAAACACCTTACAGAATGCTGATAAAAAGGCCACCTTGGAAAAATTTGACT
 GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTC
 CTCTGCCTGATCTGAATGGGATCGTTCGATCGTCAACAATCTCTGGTATTAACCTTGGTTGG
 ACATTGATCAAGTCAACTAAAACCTGTGGCAAGGTGTCACTGCACCCAAATAACAATGGAGGTGG
 GCTGCTTTGGTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTAAATATT
 TCTATCGTTCCAACCTGTTGGCCCTCCTCTGCTAACGGTACTACTGCTGCTGCTCCTGCTCCT
 GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTCTAGCAGTGCTTCATCCAACAAA
 ACCTCGGCTCAAAGCACATCAGGTGCTCTCGTTCAAGACTGCGCCATGTTAGCCATTCC
 GCTTAGCTCCTGTATTCTAA

FIGURE 11

>RO3G_16295 (mw=40.59)

MIATPFEMFQCQMYILCLVLIAFSFTCVNTQQLCNGYAEYCNKPYNSTYLLTHNSYGYVSNPAAN
 QLCPIITQLADGVRGIKLSAVKATNATTDTGTTADSIYLCHTSCIILNAGPAVNTLRTIKEWVEQN
 PNEVVTIMWNNVDAFDGNAFEAAYNASGIEEYSYQQPKKNYTWP TLGELIASGKRVINF GDTYYQQ
 DLPWLLTEYDYVFETPYENHNESSFSCTIDRPQDPASPTEFLYVMNHFLYGSLQLGSLPIEIPQKG
 IANTTNSDNSLMKQAKTCTEKFGRQPNFLEIDFYNLGDALKITAELNNVTYKGSGSLQCDTYAAQQ
 ASSSSTDSEAIQTISISSVSLLTLIAATFFIFF*

Coding sequence (exons only): 1098nt

ATGATTGCTACCCCTTTGAAATGTTCAATGTCAAATGTATATTATGTTAGTACTCATTGCA
 TTTCAATTACTGTGTCAATACTCAACAGCTGTAAATGGGTATGCAGAGTATTGTAATAAGCCT
 TACAACTCGCTCACCTACCTCTGACACATAATTATGGTTATGTATCTAACCGCTGCTAAT
 CAACTCTGCTCTACACTACTCAACTTGCAGATGGTGTTCGAGGCATCAAGCTGTCAGCCGTTAAA
 GCAACAAATGCAACTACAGATGGTACCATTACAGCAGCAGCATTATCTTGTACACATCGTGT
 ATTATATTAAATGCTGGTCCAGCAGTCAATAACCTTCGTACGATTAAGAAATGGGTGAGCAAAAC
 CCTAATGAAGTAGTGACAATCATGTGGAATAATGTGGATGCCATTGATGGGAATGCATTGAGGCT
 GCTTATAATGCAAGTGGTATCATTGAATATAGTTATCAGCAACCTAAAAAAACTATACTTGGCCA
 ACGTTAGGTGAATTAATTGCTAGTGGAAAAAGAGTAATTAAATTGGTGTACTTATTATCACAG
 GATCTCCCTGGTTATTAACAGAAATATGATTATGTGTTGAGACACCTATGAAAATCATAACGAA
 AGTCATTAGTTGCACTATTGATCGACCTCAAGATCCTGCCAGCCAACCGAATTCTTATACGTC
 ATGAACCATTGTTGTTGACTTCATTGATGGTCACTTCATTGGGTTCACTACCTATCGAGATACCTCAAAAGGC
 ATAGCCAACACAACCAACTCTGATAATTGCTCATGAAACAAAGCTAAACATGTACCGAGAAATT
 GGTCGTCAACCCAACTTTAGAAATAGATTTTATAACCTGGGTGATGCACTCAAGATTACTGCA
 GAATTAAACAATGTACCTACAAAGGTTAGGAAGTTGCAGTGTGATACATATGCTGCTAACAA
 GCAAGTAGTTCAAGTACAGACTCATCCGAAGCAATTCAAACAATTAGTATTAGTTAGTTAGTTA
 CTATTAACCTTAATTGCTGCAACATTCTTATTTCCTTTAA

FIGURE 12

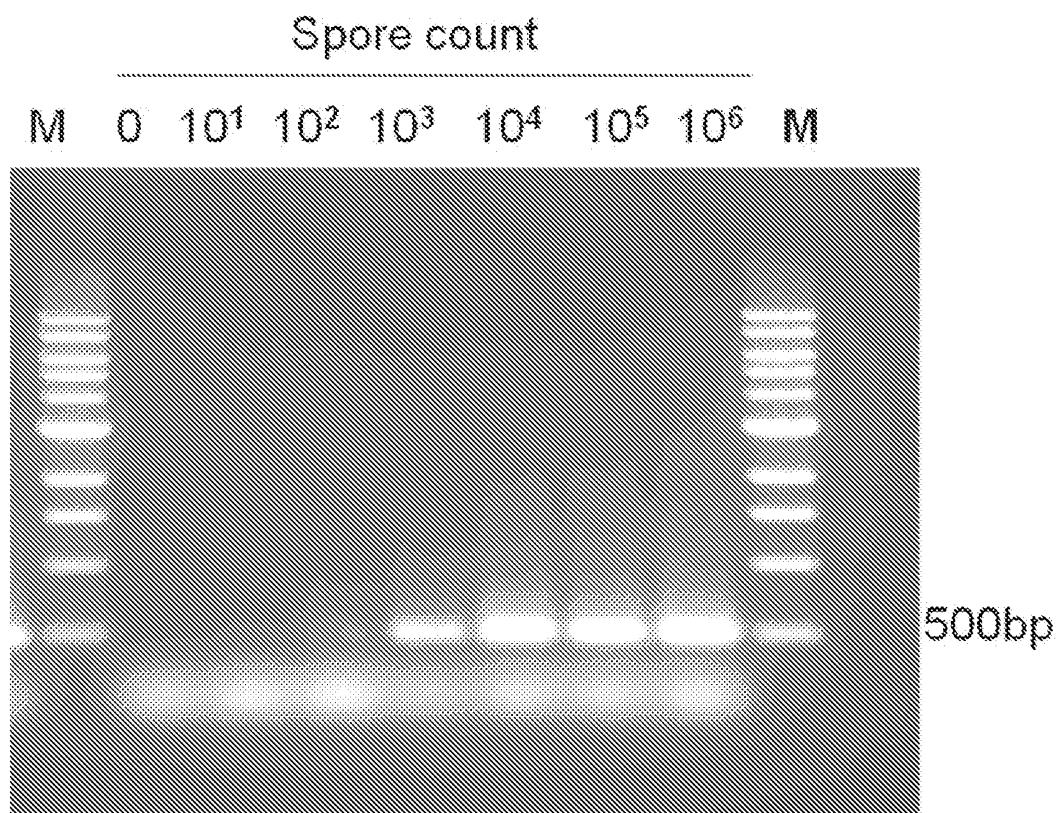


FIGURE 13

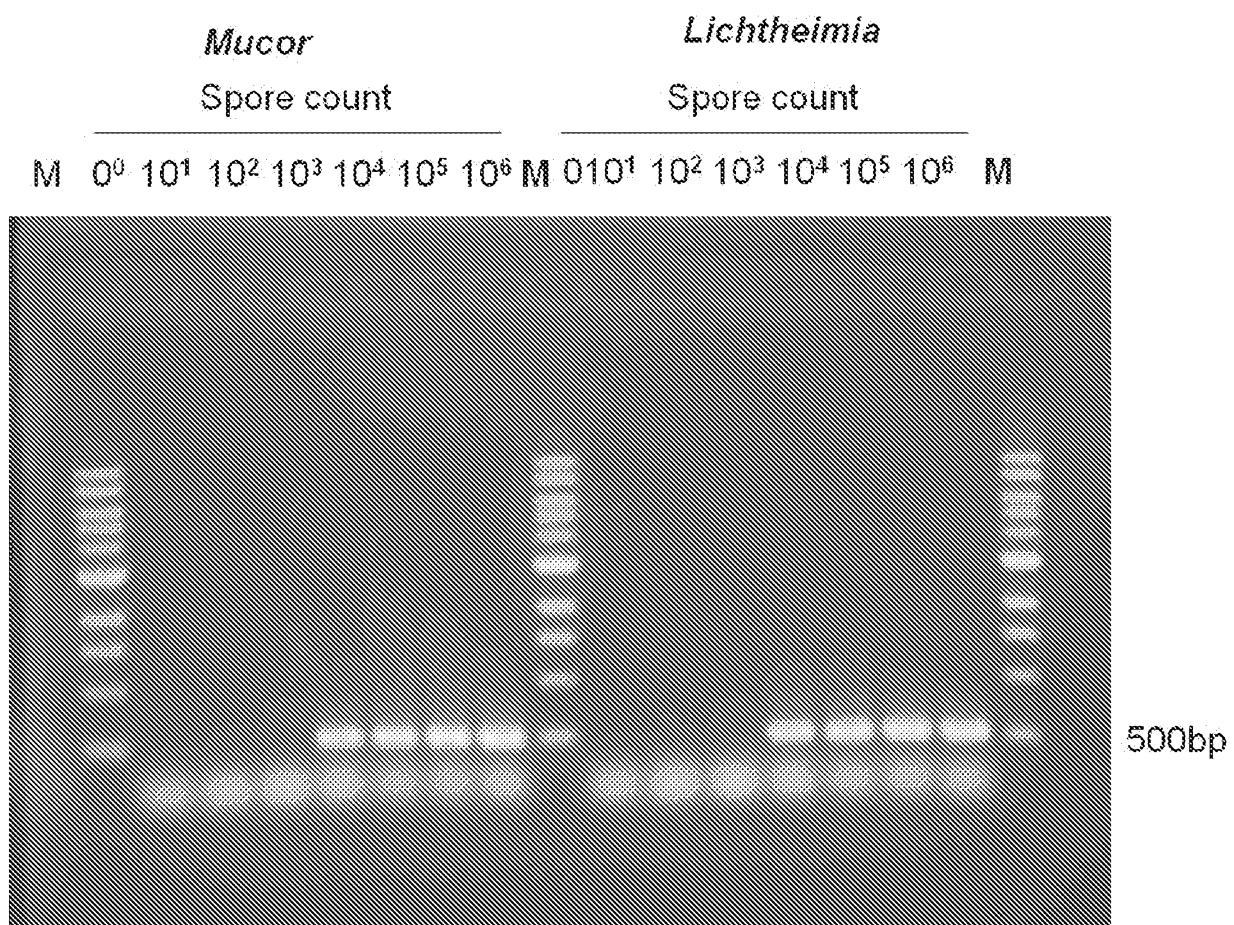


FIGURE 14

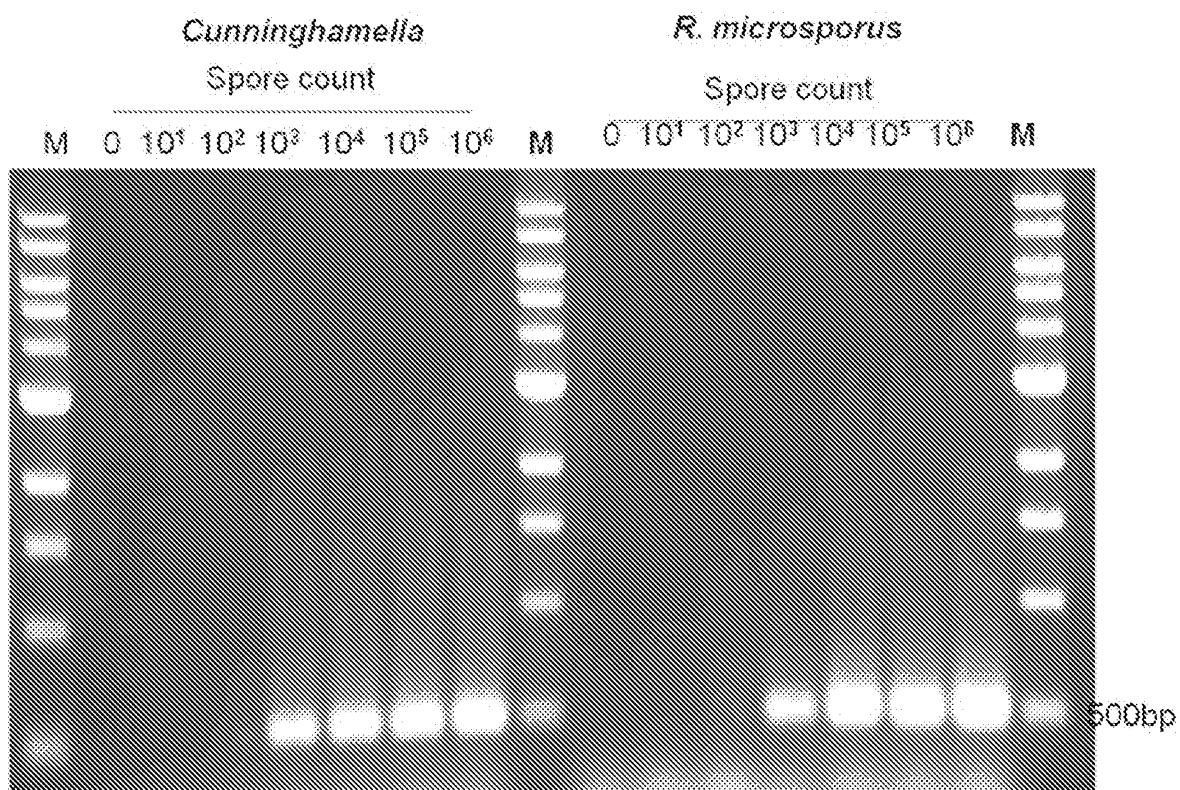


FIGURE 15

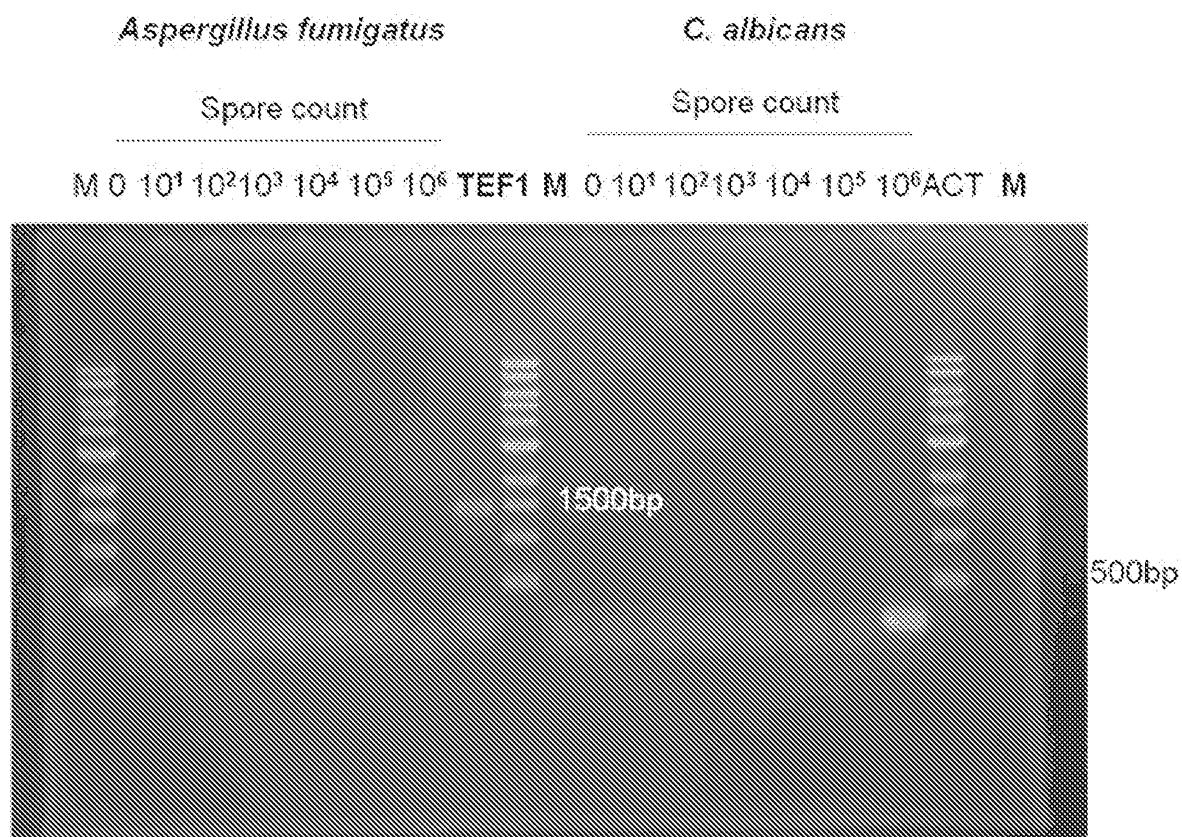


FIGURE 16

Total alignment length : 626
Overall identity : 18.05 % (113/626)

FIGURE 17

Total alignment length: 478
Overall identity : 25.94 % (124/478)
Inner consensus gaps : 39
Sequence lengths:
RO3G_16295 365
Talaromyces 453

FIGURE 18

Total alignment length: 477
Overall identity : 24.11 % (115/477)
Inner consensus gaps : 41
Sequence lengths:
R03G_16295.3 365
Penicillium 449

FIGURE 19

Total alignment length: 485
Overall identity : 25.15 % (122/485)
Inner consensus gaps : 38
Sequence lengths:
R03G_16295.3 365
Aspergillus 460

FIGURE 20

Total alignment length: 495
Overall identity : 23.43 % (116/495)
Inner consensus gaps : 38
Sequence lengths:
RO3G_16295.3 365
Aspergillus 470

FIGURE 21

Total alignment length: 385
Overall identity : 29.35 % (113/385)
Inner consensus gaps : 17
Sequence lengths:
R03G_16295.3 365
Ustilago 378

FIGURE 22

```

RO3G_16295.3
Coccidioides
MEESFELAVASLRLRSTYDQETIPLGQINPYPISLVADPTDPLTQGLKRYDSDPFTVYR
RO3G_16295.3
Coccidioides
NIAATPPFEMFQCQSQSEELCIVLLAFASTUV      NT
DQGQWQAPRTPEPTAFTSSEPTAISQGQWQWQWQWQWQWQWQWQWQWQWQWQWQWQWQWQ
RO3G_16295.3
Coccidioides
QQLCNGSYAEPYCNKPFYSSLEIYALRNS  YGYVNPANQLCPTTQLADGVRVTEKLSASVKA
TYPNCNSYFEPYCPKSYRNIITIAAKNSFVYVFRONTAAGQHLPVTTQLNDEGTRELAQFET
RO3G_16295.3
Coccidioides
***** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
TNAATDGTITADSYLQHBTMILNATPAVNLGRTIKEWVQEQPNFVVVITLNNVYLAPOG
KTYNPTIR  LAKSSSCDNLAVGPLHDFYLKVLADLWLPNFTVVSILMNNPFPYLP
RO3G_16295.3
Coccidioides
NAFRAAQNASCYIIBSYQQPERNYI  NPTFLGRITASGVRVTPGDTYYCQ  DLPSELT
TRYTYPFENSGLILYVYTYPKPFDALINPFLAENFILQYKAIVYFLDFTYRANQUTVPPYIAD
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
RO3G_16295.3
Coccidioides
SYDVSFEEPYHNHNSSEFSGCTIDRFQDQFASPT  BFLYVMMHFLYQSLQLGSLDPTIPLQK
EPFQGQWETPPNQFPTED  FPTVYKRPPFELGANDAKRFLYKNSRFLNTEVSL&GABFLNPN
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
RO3G_16295.3
Coccidioides
GIAINTNSDN  SLMKQANTCTCFKSFGRQPNPLKHDYFLNLDG  ALKUTAFLRNNTYK
VLLNFTNAVSGYGBAAGAMAGNCTEQWTREPNFTIUVDDYNTQGYNQNSUVFVAANUNVNTYK
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
RO3G_16295.3
Coccidioides
GGGSILQDNTYAAQQAASSSSSTDSSEA IQLTISISSVLLFLIAAUPPNFPP
RKNDQG  QTSAAAGKQISSLSSAKQSFVFLQIATITTSIPLTIP
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
Total alignment length: 469
Overall identity :23.98 % (112/469)
Inner consensus gaps : 36
Sequence lengths:
RO3G_16295.3 365
Coccidioides 449

```

FIGURE 23

Total alignment length: 410
Overall identity : 25.12 % (103/410)
Inner consensus gaps : 24
Sequence lengths:
R03G_16295.3 365
Neurospora 396

FIGURE 24

RO3G_16295.3 Cryptococcus	MIATPFPEMFQGQKELCIVLIAASPTIIVVNTQQLCNGFABTCNPFYNSLTYLILHNSYGVV MAPFLILSLAKTIFALPAVFRATTCGNSSELCSRLYLNVTFRKNSDLYAVG
RO3G_16295.3 Cryptococcus	SNPAANQICPITIQLADGVRITKLSAVKAATNATTDFITIADSIYCHTSCILINAGPANN GIVVADQGQKQVPTQKNGGUTPLQIQLQANRAS
RO3G_16295.3 Cryptococcus	TIRTFKRNVEQNPNEVVTIINNNVDAFDGUNAFEGAYNSGIIIPYFVY YLCFTVSEVNVNENPNEVNTIETIVNBDNLPTTFPUPVPRAGLEGLSKVYTFVPAQSPFQLEDWPS
RO3G_16295.3 Cryptococcus	LGQIATASGURVINSFHDYDITQ QILFLPLIETDYYVPTTFYRNNRSESPCTTILRQFLQAFASP LSDWMLQAGTIVVAFMDYKHTTSVVPYIILDRFAAMEDAY GYTTQRFKCAVNRSESGDTSB
RO3G_16295.3 Cryptococcus	TEFLTYVNNRFLYGSLIQLGSLSIPRIPQKTFIANITNSDN QPFIL TNNFLDSTYSPSSLQVYFVPPNTDQLNARTNARTYETGTSIGVYVNNCQQLWGRNPNSL
RO3G_16295.3 Cryptococcus	BIDFY NLGHDALKITARLNKVVYK TSSGELQCDTIAAQQASSESTDSSEBAIQTISIGSVS LIDSYDNEENSPFVVAAGLNSNVEAP TTTTFTGTAAGTBSGCTAAYVVSQDLSLG GYTSBIE
RO3G_16295.3 Cryptococcus	LLAFLILIAKTFPSIIP GIAKXGTAAGTGGVNSVLSQVQVNGVQVRYVSL

Total alignment length: 385
Overall identity : 25.97 % (100/385)
Inner consensus gaps : 34
Sequence lengths:
R03G_16285.3 365
Cryptococcus 360

FIGURE 25

Total alignment length: 773
Overall identity : 11.51 % (89/773)
Inner consensus gaps : 59
Sequence lengths:
R03G_16295 365
S.lividans 740 V

FIGURE 26

>CotH3 from Rhizopus oryzae 99-880 (including introns from Data base)

ATGAAATTATCTATTATCCGCTGCCTTTAGTGGCTATTACACACGCTGCTCAATAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTATTCACTGGTCAGCTGAAGTGGTGCCTCAAAGACATACAAA
GTAATCATAAATTAGTTGAATTCAATGAGATTAAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAAGTTGATCGTTCTTGATGGAATCACAAACTAACACTTAATGATTTTATAA
CCGCCCCGTCACTTATGCTAACCTCCTCAATTACCTTGGCAATTGAAAAAGACGTAAGTTATTT
TATTTTATCTTCTCAGCTAAAATAAACATTGTCTTCTCAGCCTCAGTGGACTCGTTCTGGAA
GCAAAGCCGACATTTCGATGACAATTATTCAGCGTTTCCACGGAGATGACAGTCAG
TCCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTATTGGATCTA
ATTACGTCTACTCTTCCAGAATGTCTCATTGGTATTACGGTGCTGGCAAGAAACACAACAATG
CAAACAAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGTAACCGCAATTCTTAAGC
TTCGACATATGGAAGAAGATCCTACACAGATTGTAACGTCTTATTCTGACATTACATGCCA
TGGTACTTATGCCAATGATGCTACCATGGTCGATTGTTATTAAACAACCAAGGCTCGGTACCT
TCAACATGTTGGATGATATCACTCAATTCTCTATATCAATGCTAAATTATAATGGCAAACCAC
CTGCTACCTGGTCCTCTATGATGGTGCGCTCTGGTGCAAGACTTCTTATATCATCCTGGTAACC
TCGATGGATACTCTTCTTGGGTGCCAACACAGCCAATCTAATGGTAAGCTTATGAAGCTCTG
ATCCTCTCTGTAAGGCCTGGAACGAGAAGACCTATACCGATAATACAGCCATTGCAAACCTTGAAA
AAATGTTGATCTGACCGTTCATGCGTTCATGGTTATTGAATACTTGACTGCCATTGGGATG
GTTACTGGATGGGACAGACCAATGATGGTGCGCTATCGTGATCCAACGTGATAATAACAAGTGGTACT
TTTAGATCAAGACTTGATGGTACTTTGGTGTCAACTGGCTGCACCGAAGGCAATGCTTTC
TTGATGTTCTTACAAGGATTCCCTCTCGTTACCGCTGGCGCTGTCATGATCAACAAACCTTTAC
AGAATGCTGATAAAAAGGCCACCTTGAAAAATATTGACTGAGACTGTGCGTGTGCTGTTCAATA
ATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTCCTCTGCGCTGATCTGAAATGGGATC
GTTCGATCGTCAACAATCTCCTGGTATTAACCTTGTTGGACATTGATCAAGTCACCAAACACT
TGTGGCAAGGTGTCACTGCACCAATAACAATGGAGGTGGTGTGCTGCTTTGGTTAGTTGAATATA
TTGCTGCAAAGGCACAAGCTGAGCTAAGGAATTAAATATTCTATCGTTCCCAACCTGTTGGCC
CTCCTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAG
GAGGAAATCAATCTATTCTAGCAGTGCTCATCCAACAAACCTCGGCTCAAAGCACATCAGGTG
CTTCTCGTCCAAGACTGCGCCATGTTAGCCATTCCGCTTAGCTCCTTGTATTCTAA

FIGURE 27

CotH3 from Rhizopus oryzae 99-880 (exon only from Data base)

ATGAAATTATCTATTATATCCGCTGCCTTTAGTGGCTATTACACACGGCTGCTCAATAAAGTTT
 AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
 GCTCAGATGCAAATGTCCTTATTCACTGGTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
 TATGTTGCAGGTGGAACAGAAAGTTGATCGTTCTTGATGGAATCACAAACTCAACACTT
 AATGATTTTATAACCGCCCCGTCACTTATGCTAACCTCCTCAATTACCTGGCCAATTGAAAAA
 GACCCCTCAGTGGACTCGTTCTGGAAGCAAAGCCGACATTTCGATGACAATTATATTCCCAGCGTT
 TTTTCCACGGAGATGACAGTCAAGTCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGT
 GGTACACTGACCTTATTGGATCTAATTACGTACTCTTCCAGAATGTCTCATTGGTATTAC
 GGTGCTGGCAAGAACACAATGCAAAACAATCTGGAACTGGATATTGTCTGGAAGTGATACG
 ATGGGTAACCGCAATTCTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTGTAACGT
 CTTTATTCTGACATTTCATGCCATGGTACTTATGCCAATGATGCTACCATGGTCATTGTT
 ATTAACAACCAAGGCTCGGTACCTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
 GCTAAATTATATAATGGCAAACACACCTGCTACCTGGTCTCTATGATGGTGCCTCTGGTCA
 GACTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTGGTGCACACAGCCAATCCT
 AATGGTGAAGCTTATGAAGCTTGTGATCCTCTGTAAGGCTGGAACGAGACGACCTATACCGAT
 AATACAGCCATTGCAAACCTTGAAAAAATGTTGATCTGACCGTTCATGCGTTCATGGTTATT
 GAATACTTGACTGCCGATTGGATGGTTACTGGATGGACAGACCAATGATGGTGCCTATCGTGAT
 CCAACTGATAATAACAAGTGGTACTTTAGATCAAGACTTGTGATGGTACTTTGGTGTCAACTTG
 GCTGCACCGAAGGCAATGCTTCTTGATGTTCTACAAGGATTCCCTCTCGTTACCGTGGC
 GCTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTGAAAAAATATTGACT
 GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTC
 CTCTGCGTGTGATCTGAAATGGATCGTCAACAATCTCCTGGTATTAACCTGGTTGG
 ACATTGATCAAGTCAACTCAAACCTGTGGCAAGGTGTCAGTCACCCAAATAACAATGGAGGTGGT
 GCTGCTTTGGTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTAAATATT
 TCTATCGTTCCCAACCTGTTGGCCCTCCTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCT
 GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTCTAGCAGTGCTCATCCAACAAA
 ACCTCGGCTCAAAGCACATCAGGTGCTCTCGTTCCAAGACTGCGCCCATGTTAGCCATTCC
 GCTTAGCTCCTGTATTCTAA

FIGURE 28

>CotH3 from *Rhizopus oryzae* 99-880 (predicted amino acids)

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
YVAGGTEESFDRSLDGITNSTLNDFYNRPVTYANLPQLPWPIEKDPQWTRSGSKADIFDDNYIPSV
FFHGDDSQVQNVVKNVPADRIISGTLTFIGSNYVYSFQNVSFGIHGAGKKHNNAKQSWNWILSGSDT
MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGYANDATMVRLFINNQGFGTFNMLDDITQFSYIN
AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNGEAYEALDPLCKAWNNETTYTD
NTAIANFEKMFDFDRFMFMVIEYLTADWDGYWMGQTNDGAYRDPTDNNKWFYFLDQDFDGTFGVNL
AAPEGNAFLDVSYKDFPSRYPGAVMINNLLQNAKKATFEKYLTETVRVLFNNVLTNRVLALHNF
LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGVTAPNNNGGAAFGLVEYIAAKAQAVAKEFNI
SIVSQPVGPPSANGTTAAAPAPAAGNSTGKGGNQSISSSASSNKTSAQSTSGASRSKTAPIVLAIS
ALALLVF*

FIGURE 29

>CotH3 from Rhizopus oryzae 99-880 (including introns from sequenced data)

ATGAAATTATCTATTATCCGCTGCCTTTAGTGGCTATTACACACGCTGCTCAATAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTTATTCACTGGTCAGCTGAAGTGGTGCCTCAAAGACATACAAA
GTAATCATAAATTAGTTGAATTCAATGAGATTAAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAAGTTGATCGTCTCTGATGGAATCACAAACTCAACACTTAATGATTTTATAA
CCGCCCGTCACTTATGCTAACCTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATT
TATTTTATCTTCTCAGCTAAAATAAACATTGTCTTCTCAGCCTCAGTGGACTCGTTCTGGAA
GCAAAGCCGACATTTCGATGACAATTATTCAGCGTTTCCACGGAGATGACAGTCAAG
TCCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTATTGGATCTA
ATTACGTCTACTCTTCCAGAATGTCTCATTGGTATTACGGTGCTGGCAAGAAACACAACAATG
CAAACAAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGTAACCGCAATTCTTAAGC
TTCGACATATGGAAGAAGATCCTACACAGATTGTAACGTCTTATTCTGACATTACATGCCA
TGGTACTTATGCCAATGATGCTACCATGGTCGATTGTTATTAAACAACCAAGGCTCGGTACCT
TCAACATGTTGGATGATATCACTCAATTCTCTATATCAATGCTAAATTATAATGGCAAACCAC
CTGCTACCTGGTCCTCTATGATGGTGCTCTGGTGCAAGCTTCTTATATCATCCTGGTAACC
TCGATGGATACTCTTCTGGTGCACACAGCCAATCTTAATGGTGAAGCTTATGAAGCTCTG
ATCCTCTGTAAGGCCTGGAACGAGACGACCTACCGATAATACAGCCATTGCAAACCTTGAAA
AAATGTTGATCTGACCGTTCATGCGTTCATGGTTATTGAATACTTGACTGCCATTGGGATG
GTTACTGGATGGGACAGACCAATGATGGTGCTATCGTGAACGATAATAACAAGTGGTACT
TTTAGATCAAGACTTGATGGTACTTTGGTGTCAACTGGCTGCACCGAAGGCAATGCTTTC
TTGATGTTCTTACAAGGATTCCCTCTCGTTACCGCTGGCGCTGTCATGATCAACAAACCTTAC
AGAATGCTGATAAAAAGGCCACCTTGAAAAATATTGACTGAGACTGTGCGTGTGCTTCAATA
ATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTCCTCTGCCTGATCTTGAATGGGATC
GTTCGATCGTTCAACAATCTCCTGGTATTAACCTTGTTGGACATTGATCAAGTCACTCAAAC
TGTGGCAAGGTGTCACTGCACCAATAACAATGGAGGTGGTGCCTTGGTTAGTTGAATATA
TTGCTGCAAAGGCACAAGCTGTAAGGAATTAAATATTCTATCGTTCCCAACCTGTTGGCC
CTCCTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAG
GAGGAAATCAATCTATTCTAGCAGTGCTCATCCAACAAACCTCGGCTCAAAGCACATCAGGTG
CTTCTCGTTCCAAGACTGCGCCATGTTAGCCATTCCGCTTAGCTCTCCTGTATTCTAAA

FIGURE 30

CotH3 from Rhizopus oryzae 99-892 (exons only from Sequenced data)

ATGAAATTATCTATTATCCGCTGCCTTTAGTGGCTATAACACACGCTGCTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTACTTCACTGGTCAGCTGAAGTTGGTCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGGTTTGATCGTTCTTGATGGAATCACAAACTAACATTT
AATGATTTTATAATCGCCCCATCACTTATGCTAACCTCCTCAATTACCTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAACAAAGCCGACATTTCGATGACAATTATATTCCCAGCATT
TTTTCCACGGAGATGACAGTCAAGTCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTATCGGATCTAATTACGTACTCTTCCAGAATGTCATTTGGTATTAC
GGTGGCTGGCAAGAACACAACAAATGCAAAGCAATCTGGAACTGGATCTTGTCTGGAAAGTGATACG
ATGGGTAAACGTAATTCTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGT
CTTTATTCTGACATTTCACATGCCATGGTACTTATGCCAATGATGCTACCATGGTCATTGTT
ATTAACAATCAAGGCTTCGGTACCTCAACATGTTGGATGACATCACTCAATTCTTATATCAAT
GCTAAATTCTATAATGGCAAACACCACCTGCTACCTTGGTCCTCTATGATGGTGCCTCTGGTGC
GATTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCTAAC
TAATGGTGAAGCTTATGAAGCTTGTGATCCTCTGTAAAGGCTGGAACAGAGACGACCTATACCGA
TAATACAGGCATTGGGAACCTTGAaaaaATGTTGATCTGACCGTTCATGGCTTATGGTT
TGAATACTTGGCTGCCGATTGGGATGGTTACTGGATGGACAGACCAATGATGGTGCCTATCGTGA
TCCAACGTATAAAACAAGTGGTACTTTAGATCAAGACTTGTGATGGTACCTTGGTGTCAACTT
GGCTGCACCCGAAGGCAATGCTTTCTGATATTCTTACAAGATTCCCTCTCGTTACCCCTGG
CGCTGTATGATCAACAAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTGAaaaACTTGAC
TGAGACTGTGCGTGTGCTGTTCAATAATGTCACCTGACTAACCGTGTCTGGCCCTTCACA
CCTCTGCTGACCTTGAATGGGATCGTGCATCGTTCAACAAACTCCTGGTATTAACCTTGGTT
GACATTGATCAAGTCAACTAAACTTGTGGCAAGGTGTCAATGCACCCAAATAACAACGGAGGTGG
TGCTGCTATTGGTTAGTTGAATATATTGCTACAAAGGCACAAGTTGTAGCTAAGAATTAAATATTA
CTATCGTTCCAACCTGTTGGCCCTCCTCTGCTAATGGTACTACTGCTGCTAACTGCTCCTA
CTGCTGGTATCTACTGGAAAAGGAAGAAATCATCCCATTCTAGCAGTGCTCATCAACAAACTCG
CTCAAGTAACATCAAGTGCTTCGTCAGACTGCGCCAATCATTAGGCAATTCCGCTTAGCC
CTCCCCCGTTGTGATTCTCAAAA

FIGURE 31

>CotH3 from *Rhizopus oryzae* 99-892 (including intron from Sequenced data)

ATGAAATTATCTATTATCCGCTGCCTTTAGTGGCTATAACACACAGCTGCTTCAATAAAGTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTTACTTCAGCTGAAGTTGGTCCTCAAAGACATACAAA
GTAATCATAAATTATTGAATTCAATCATATTAAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAGGTTTGATCGTTCTTGTGAAATCACAAACTCAACATTAAATGATTAA
TCGCCCATCACTTATGCTAACCTCCTCAATTACCTTGGCAATTGAAAAAGACGTAAGTTATT
TATTTTATCTTCTCAGCTAAACATTGCTTCTCAGCCTCAGTGGACTCGTTCTGGAA
ACAAAGCCGACATTTGATGACAATTATATTCCCAGCATTTCACGGAGATGACAGTCAG
TCCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTATCGGATCTA
ATTACGTCTACTCTTCCAGAATGTCTCATTGGTATTACGGTGCTGGCAAGAACACAACATG
CAAAGCAATCTTGGAACTGGATCTGTGGAAAGTGATACGATGGTAACCGTAATTCTTAAGC
TTCGACATATGGAAGAAGATCCTACACAGATCCGTGAAACGTCTTATTGACATTACATGCCA
TGGGTACTTATGCCAATGATGCTTACCATGGTTCGATTGTTATTAAACATCAAGGCTCGGTACCT
TCAACATGTTGGATGACATCAATTCTCTTATATCAATGCTAAATTCTATAATGGCAAACAC
CTGCTACCTGGTCCCTCTATGATGGTGCCTCTGGTGCAGATTCTTATATCATCCTGGTAACC
TCGATGGATACTCTTCTTGGTGCACACAGCTAATCCTAATGGTGAAGCTTATGAAGCTCTT
GATCCTCTGTAAAGGCTGGAACAGACGACCTATACCGATAATACGCCATTGCGAACTTGAA
AAAATGTTGATCTTGGACCGTTCATGGTGCATTGTTGAATACTGGCTGCCGATTGGGAT
GGTTACTGGATGGACAGACCAATGATGGTGCCTATCGTATCCAACGGTACGATAATAACAGTGGTAC
TTTTAGATCAAGACTTGGTACCTTGGTGTCAACTTGGCTGCACCCGAAGGCAATGCTTT
CTTGATATTCTTACAAAGATTCCCTCTCGTTACCGTGGCTGTGATCAACAAACCTCTTA
CAGAATGCTGATAAAAAGGCCACCTTGGAAAAAATACCTGACTGAGACTGTGCGTGTGTTCAAT
AATGTCACCTGACTAACCGTGTCTTGGCCCTTCACAACCTCCTCTGCCTGACCTGAAATGGGAT
CGTCGATCGTTCAACAATCTCTGGTATTAAACCTTGGTGGACATTGATCAAGTCACCAAC
TTGGCAAGGGTGTCAATGCACCCAATAACAAACGGAGGGTGGTGCCTATTGGTTAGTTGAATAT
ATTGCTACAAAGGCACAAGTTGTAGCTAAGAATTAAATTACTATCGTCCCAACCTGTTGGCCCT
CCTCTGCTAATGGTACTACTGCTGCTAACCTGCTCCTACTGCTGGTATCTACTGGAAAAGGAA
GAAATCATCCCATTCTAGCAGTGCTCATCAACAAACTCGCTCAAGTAACATCAAGTGCTTCTCG
TCAAGACTGCGCCAATCATTAGGCAATTCCGCTTAGCCCTCCTGTGATTCTCAAAA

FIGURE 32

>Predicted amino acid sequence of CotH3 from *R. oryzae* 99-892
(excluding introns)

MKLSTIISAAFLVAITHAASIKFNVIAPNATDVKSVNGQQVTLTASDANVPYFTGSAEVGSSKTYK
VIINLF*IQSY*SSYSIVCCRWNRRRF*SFS*WNHKLNI**FL*SPHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFLSLSGLVILETKPTFSMTIIFPAFFSTEMTVKSKMWLKTYLLTESVVH*PLSDL
ITSTLSRMSHLVFTVLARNTTMQSNLGTGSCLEVIRWVTVISLSFDIWKKILHRSVNVFILTFYMP
WVLMPMMLPWFDCLLTIKASVPSTCWMTSLNSLISMLNSIMANHLLPWVLSMMVPLVQISYIILV
SMDTLSWVANTANPNGEAYEALDPLCKAWNNETTYTDNTAIANFEKMFDFLDRFMKFMVVEYLAADWD
GYWMGQTNDGAYRDPTDNNKWFQFLDQDFGTFGVNLAAPEGNAFLDISYKDFPSRYPGAVMINNLL
QNADKKATFEKYLTEVRLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQN
LWQGVNAPNNNGGAAIGLVEYIATKAQVVAKN*YYYRSQPGPPSANGTTAACNSCYCWYLLEKE
ETIPFLAVLHQQTRESSNIKCFSSRLRQSF*AISA*PSL*FSK

FIGURE 33

>Predicted amino acid sequence of CotH3 from *R. oryzae* 99-892 (including introns)

MKLSTIISAAFLVAITHAASIKENVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGSSKTYK
VIINLF*IQSY*SSYSIVCCRWNRRRF*SFS*WNHKLNI**FL*SPHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFLSLSGLVLETKPTFSMTIIIFPAFFSTEMTVKSKMWLKYLLTESVvh*PLSDL
ITSTLSRMSHLVFTVLARNTTMQSNLGTGSCLEVIRWTVVISLSFDIWKKILHRSVNVFILTFYMP
WVLMPMMLPWFDCLLTIKASVPSTCWMTSLNSLISMLNSIMANHLLPWVLSMMPVPLVQISYIILVT
SMDTLSWVANTANPNEAYEALDPLCKAWNEDTYTDNTAIANFEKMFDLDRFMRFMVVEYLAADWD
GYWMGQTNDGAYRDPTDNNKwyFLDQDFDGTFGVNLAAPEGNAFLDISYKDFPSRYPGAVMINNLL
QNADKKATFEKYLTETVRVLFNNVTLTNRVLALHNFLPDLEWDRSIVQQSPGINFGWTFDQVTQN
LWQGVNAPNNNGGAAIGLVEYIATKAQVVAKN*YYRSQPVGPPSANGTTAACNSCYCWYLLEKE
EIPFLAVLHQQT RSSNIKCFSSRLRQSF*AISA*PSPVVILK

FIGURE 34

>CotH3 sequence from Mucor sp. 99-932 (exons only from sequenced data)

ATGAAATTATCTATTATCCGCTGCCTTTAGGGCTATAACACACAGCTGCTTCATAAAGTTA
ATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGAAATGGACAGCAAGTGACACTTACTG
CTTCAGATGCAAATGTCCTTACTTCAGCTGGTCAAGTTGGTCTCAAAGACATACAAAT
ATGTTGCAGGTGGAACAGAAGGTTTGATCGTCTCTGATGGAATCACAACTAACATTAA
ATGATTTTATAATCGCCCCATCACTTATGCTAACCTCCTCAATTACCTGGCAATTGAAAAAG
ACCCTCAGTGGACTCGTCTGGAAACAAAGCCGACATTTCGATGACAATTATATTCCCAGCATT
TTTCCACGGAGATGACAGTCAAGTCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGTG
GTACACTGACCTTATCGGATCTAATTACGTCTACTCTTCCAGAATGTCTCATTGGTATTACG
GTGCTGGCAAGAAACACAATGCAAAGCAATCTGGAACTGGATCTGTCTGGAAGTGATACGA
TGGGTAAACCGTAATTCTTAAGCTCGACATATGGAAGAAGATCCTACACAGATCCGTGAAACGTC
TTTATTCTGACATTTACATGCCATGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTA
TTAACAAATCAAGGCTCGGTACCTCAACATGTTGGATGACATCACTCAATTCTCTTATATCAATG
CTAAATTCTATAATGGCAAACACCACCTGCTACCTGGTCTCTCTATGATGGTGCCTCTGGTGCAG
ATTCTTATATCATCCTGGAACCTCGATGGAATACTCTTCTGGTGCACACAGCTAATCCTA
ATGGTGAAGCTTATGAAGCTCTGATCCTCTGTAAAGGCCTGGAAACGAGACGACCTATTACCG
ATAATACAGCCAATTGCGAACCTTGAAAGGAAATGTTGATCTGACGTTCATGCGTCCATGCTGGT
GATACTGGGCTGCCGAATGAATGCTACTGCAATGGAAGACATGAATCGTGTCTATTGATGCCAA
CTGAATAATACCAGTCGGTACTTTAGATCAAGACTTGTGTTGACTTTGGTGTCAACTGGC
TGCACCCGAAGGCAATGCTTCTGATGTTCTTACAAGGATTCCCTCTCGTTACCGTGGC
TGTCAATGATCAACACCTCTTACAGAACGACTGATGATAAAAGGCCACCTTGAAAATATTGACTGA
GACTGTGCGTGTGCTGTTCAATAATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTCCT
CTTGCCTGATCTGAATGGGATCGTTGATCGTCAACAACTCCTGGTATTAACCTGGTGGAC
ATTGATCAAGTCACTCAAACCTGTTGCAAGGTGTCAGTCACCCAAATAACAATGGAGGTGGTGC
TGCTTTGGTTAGTGAATATATTGCTGCAAAGGACAAGACTGTTGACTAACGAAATTAAATATTTC
TATCGTTCCAAACCTGTTGGCCCTCCTCTGCTAAATGGTACTACTGCTGCTGCTCCTGCTCCTGC
TGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTCTAGCAGTGCTTCATCCAACAAAC
CTCGGCTCAAAGCACATCAGGTGCTCTCGTTCCAAGACTGCGCCATGTTAGCCATTCCGC
TTAGCTCTCCTGGTATTCTAAA

FIGURE 35

>Predicted amino acid sequence of CotH3 from Mucor 99-932 (AA exons only)

MKLSIISAAFLVAITHAAS*SLM*LLLMQLMSKYL*MDSK*HLLLQMQMQLTSLVQLKLVQRHTN
MLQVEQKKVLIIVLLMESQTQHLMIFI IAPSLMLTFLNYLGQLKKTLSGLVLETKPTFSMTIIIFPAF
FSTEMTVKSkmWLKTYLITE SVVH*PLSDLITSTLSRMSHLVFTVLARNTTMQSNLGTGSCLEVIR
WVTVISLSFDIWKKILHRSVNVFILTIFYMPWVLMPMMLPWFDCLLTIKASVPSTCWMTSLNSLISM
LNSIMANHLLPWVLSMMVPLVQISYIILVTSMDTLLGLPTQLTLMVKLMKLLILLCKAWKRDDLLP
IIQPIANFEKMFDTFHFAGDTGLPNECYCNGRHESCLFVIQLNNNTSRVLFRSRL*WYFWCQLG
CTRRCQFS*CFLQGFPFSLPWRCHDQQPLTEC**KGHL*KIFD*DCACAVQ*CHLD*PCLGPSQLP
LA*S*MGSFDRSTISWY*LWLDI*SSHSKLVARCHCTQ*QWRWCCFWFS*IYCCKGTSCS*GI*YF
YRFPTCWPSCFC*WYYCCCSGCCWQFYWKRRKSIYF*QCFIQQNLGSKHIRCFSFQDCAHRSF
FSSPWYSK

FIGURE 36

>DNA sequence of CotH3 from Mucor 99-932 (including introns)

ATGAAATTATCTATTATCCGCTGCCTTTAGGGCTATAACACACAGCTGCTTCATAAAGTTA
ATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACTG
CTTCAGATGCAAATGTCCTACTTCAGCTGGTCAAGTTGGTCTCAAAGACATACAAAG
TAATCATAAATTATTTGAATTCAATCATATTAAATCATCTTATTCTATAGTATGTTGCAGGTGGA
ACAGAAGAAGGTTTGATCGTTCTTGATGGAATCACAAACTAACATTAAATGATTTTATAAT
CGCCCCATCACTTATGCTAACCTTCCTCAATTACCTGGCCAATTGAAAAAGACGTAAGTTATT
ATTTTATCTTCTCAGCTAAACATTGTCTTCAGCCTCAGTGGACTCGTTCTGGAAA
CAAAGCCGACATTTGATGACAATTATTCAGCATTTCACGGAGATGACAGTCAGT
CCAAATGTGGTAAAAACGTACCTGCTGACCGAACAGTGGTACACTGACCTTATCGGATCTAA
TTACGTCTACTCTTCCAGAACATGTCTCATTTGGTATTCAACGGTCTGGCAAGAACACAACATGC
AAAGCAATCTTGGAACTGGATCTGTCTGGAAAGTGATGGTAACCGTAATTCTTAAGCT
TCGACATATGGAAAGAAGATCCTACACAGATCCGTGACCGTCTTATTCTGACATTTCATGCCAT
GGGTACTTATGCCAATGATGCTTACCATGGTCGATTGTTATTAAACAATCAAGGCTCGGTACCTT
CAACATGTTGGATGACATCACTCAATTCTCTTATATCAATGCTAAATTCTATAATGGCAAACACC
TGCTACCTTGGTCTCTCATGATGGTGCCTCTGGTGCAGATTCTTATATCATCCTGGTAACCT
CGATGGATACTCTTCTTGGGTTGCCAACACAGCTAACCTTAATGGTGAAGCTTATGAAGCTTGA
TCCTCTCTGTAAGGCCTGGAAACGAGACGACCTATTACCGATAATACAGCCAATTGCGAACCTTG
AAAAATGTTGATCTGACGTTCATGCGTTCCATGCTGGTGAATCTGGGCTGCCGAATGAATGCT
ACTGCAATGGAAGACATGAATCGTGTCTATTGATCCAATGCTGAATAATACAGTCGGTACTTT
TTAGATCAAGACTTTGATGGTACTTTGGTGTCAACTTGGCTGCACCGAAGGCAATGCTTTCTT
GATGTTCTTACAAGGATTCCTCTCGTTACCCCTGGCGCTGTGATCAACAAACCTCTTACAG
AATGCTGATAAAAAGGCCACCTTGAAAAATATTGACTGAGACTGTGCGTGTGCTGTTCAATAAT
GTCACCTTGACTAACCGTGTCTGGCCCTTCACAACTTCTCTGCTGATCTGAATGGATCGT
TCGATCGTTCAACAATCTCTGGTATTAACCTTGGTGGACATTGATCAAGTCACTCAAAACTTG
TGGCAAGGGTGTCACTGCACCCAATAACAATGGAGGTGGTGTGCTTTGGTTAGTTGAATATATT
GCTGCAAAGGCACAAGCTGTAGCTAAGGAATTAAATATTCTATCGTTCCCAACCTGTTGGCCCT
CCTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAGGA
GGAAATCAATCTATTCTAGCAGTGCTCATCCAACAAACCTCGGCTCAAAGCACATCAGGTGCT
TCTCGTTCCAAGACTGCGCCATCGTTAGCCATTCCGCTTAGCTCTCCTGGTATTCTAAA

FIGURE 37

>Predicted amino acid sequence of CotH3 from Mucor 99-932 (including introns)

MKLSTIISAAFLVAITHAAS*SLM*LLLMQLMSKYL*MDSK*HLLLQMOMSLTSLVQLKLVPQRHTK
*S*IYFEFNHINHLIL*YVAGGTEEGFDRSLDGITNSTFNDFYNRPITYANLPQLPWPIEKDVSYF
IFIFSQLK*TLSFSASVDSFWKQSRHFR*QLYSQHFFPRR*QSSPKCG*KRTC*PNQWYTDLYRI*
LRLLFPECLIWYSRCWQETQQCKAILELDLVWK*YDG*P*FL*ASTYGRRSYTDP*TSLF*HFTCH
GYLCQ*CYHGGSIVY*QSRLRYLQHVG*HHSILLYQC*IL*WQTTCYLGSSL*WCLWCRFLISSW*P
RWILFLGCQHS*S*W*SL*SS*SFSVRPGNETTYYR*YSQLRTLKKCLI*RFMRSMLVILGCRMNA
TAMEDMNRVYS*SN*TIPVGYFLDQDFDGTFGVNLAAPEGNAFLDVSYKDFPSRYPGAVMINNLIQ
NADKKATFEKYLTELTVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFOGWTFDQVTQNL
WQGVVTAPNNNGGAAAGLVEYIAAKAQAVAKEFNISIVSQPGPPSANGTTAAAPAPAAGNSTGKG
GNQSISSSASSNKTSAQSTSGASRSKTAPIVLAISALALLGIL

FIGURE 38

>CotH3 sequence from *Lichtheimia corymbifera* (exons only from sequenced data)

ATGAAATTATCTATTATCCGCTGCCTTTAGGGCTATTACACACGCTGCTTCAATAAAGTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTTATTCAGCTGAAGTTGGTCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTGATCGTTCTTGATGGAATCACAAACTAACACTT
AATGATTTTATAATGCCCGTCACTTATGCTAACCTCCTCAATTACCTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGAAACAAAGCCGACATTTCGATGACAATTATATTCCCAGCGTT
TTTTCCACGGAGATGACAGTCAAGTCAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTATTGGATCTAATTACGTCTACTCTTCCAGAATGTCTCATTGGTATTAC
GGTGCCTGGCAAGAACACAATGCAAAACAAATCTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAACCGCAATTCTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATCCGTGAAACGT
CTTATTCTGACATTTCACATGCCATGGTACTTATGCCAATGATGCTACCATGGTCATTGTT
ATTAACAACCAAGGCTCGTACCTCAACATGTTGGATGATATCACTCAATTCTCTTATATCAAT
GCTAAATTATATAATGGCAAACACCACCTGCTACCTTGGGCTCTCTATGATGGTGCCTCTGGTGC
GATTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCT
AATGGTGAAGCTTATGAAGCTTGGGATGGTACTGGATGGACAGACCAATGATGGTGCCTATCGT
CCAACGTATAACAACGCTTGGTACTTTAGATCAAGACTTGGATGGTACTTTGGTGTCAACTTG
GCTGCACCCGAAGGCAATGCTTCTTGGGATGGTACTGGATGGACAGACCAATGATGGTGCCTACCTGG
GCTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTATGAAAAATATTGACT
GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTTC
CTCTTGCCTGATCTGAATGGGATCGTTCGATCGTCAACAAATCTCCTGGTATTAACCTTGGTGG
ACATTGATCAAGTCACTAAAACCTGTGGCAAGGTGTCAGTGCACCCAAATAACAATGGAGGTGGT
GCTGCTTTGGTTAGTGAATATATTGCTACAAAGGCACAAGCTGTAGCTAAGGAATTAAATATT
TCTATCGTTCCAAACCTGTTGGCCCTCCTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCT
GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTCTAGCAGTGCTTCAACACAAA
ACCTCGGCTCAAAGCACATCAGGTGCTCTCGTCAAGACTGCGCCCATCATTAGCCATTTC
CGCTTAGCTCTCCCTGTATTCTAAA

FIGURE 39

>Predicted amino acid sequence of CotH3 from Lichtheimia corymbifera (exons only from sequenced data)

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKS VNGQQVLTASDANVPYFTGSAEVGSSKYK YVAGGTEESFDRSLDGITNSTLNDFYNRPVTYANLPQLPWPIEKDPQWTRSGNKADIFDDNYIPS VFFHGDDSQVQNVVKNVPADRI SGTLFIGSNYVYSFQNVSGIHAGKKHNNAKQSWNWILSGSDT MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGTYANDATMVRLFINNQGFTFNMLDDITQFSYIN AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNGEAYEALDPLCKAWNNETTYTD NTAIANFEKMF DLD RFRM VIEYLTADWDGYWMGQTNDGAYRDPTDNNK WYFLDQDFDGTFGVNL AAPEGNAFLDV SYKDFPSRYPGAVMINNLLQNA DKKATYEKYL TETVRVLFNNVLTNRVLALHNF LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGV TAPNNNGGAAFGLVEYIATKAQAVAKEFNI SIVSQPVGPPSANGTTAAAPAPAAGNSTGKGGNQSISSSASSNKTSAQSTSGASRSKTAPIIFSHF RFSSPLYSK

FIGURE 40

>CotH3 sequence from *Lichtheimia corymbifera* (including introns)

ATGAAATTATCTATTATCCGCTGCCCTTTAGTGGCTATTACACACGCTGCTCAATAAAGTTT
 AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
 GCTTCAGATGCAAATGTCCTTATTCACTGGTCAGCTGAAGTTGGTCCTCAAAGACATACAAA
 GTAATCATAAATTAGTTGAATTCAATGAGATTAATCATCTTATTCTATAGTATGTTGCAGGTGG
 AACAGAAGAAAGTTGATCGTTCTTGATGGAATCACAACACTCAACACTTAATGATTTTATAA
 TCGCCCCGTCACTTATGCTAACCTCCTCAATTACCTTGGCAATTGAAAAAGACGTAAGTTATT
 TATTTTATCTTCTCAGCTAAAATAAACATTGTCTTCAGCCTCAGTGGACTCGTTCTGGAA
 ACAAAGCCGACATTTCGATGACAATTATATTCCAGCGTTTTCCACGGAGATGACAGTCAAG
 TCCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTATTGGATCTA
 ATTACGTCTACTCTTCCAGAATGTCTCATTGGTATTACGGTGCTGGCAAGAAACACAACAATG
 CAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGTAACCGCAATTCTTAAGC
 TTCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGTCTTATTCTGACATTACATGCCA
 TGGTACTTATGCCAATGATGCTACCATGGTCGATTGTTATTAAACAACCAAGGCTCGGTACCT
 TCAACATGTTGGATGATATCACTCAATTCTCTTATATCAATGCTAAATTTATAATGGCAAACCAC
 CTGCTACCTGGTCCTCTTATGATGGTGCGCTCTGGTGCGATTCTTATATCATCCTGGTAACC
 TCGATGGATACTCTTCTGGGTTGCCAACACAGCCAATCTTAATGGTGAAGCTTATGAAGCTCTG
 ATCCTCTGTAAGGCCTGGAACGAGACGACCTATACCGATAATACAGCCATTGCAAACCTTGAAA
 AAATGTTGATCTGACCGTTCATGCGTTCATGGTTATTGAATACTTGACTGCCATTGGGATG
 GTTACTGGATGGGACAGACCAATGATGGTGCGCTATCGTGATCCAACGTGATAATAACAAGTGGTACT
 TTTAGATCAAGACTTGATGGTACTTTGGTGTCAACTGGCTGCACCGAAGGCAATGCTTTC
 TTGATGTTCTTACAAGGATTCCCTCTCGTTACCGCTGGCGCTGTCATGATCAACAAACCTTAC
 AGAATGCTGATAAAAAGGCCACCTATGAAAAATATTGACTGAGACTGTGCGTGTGCTGTTCAATA
 ATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTCCTCTGCCTGATCTTGAATGGGATC
 GTTCGATCGTTCAACAATCTCCTGGTATTAACCTTGTTGGACATTGATCAAGTCACTCAAACACT
 TGTGGCAAGGTGTCACTGCACCAATAACAATGGAGGTGGTGTGCTGCTTTGGTTAGTTGAATATA
 TTGCTACAAAGGCACAAGCTGTAAGGAATTAAATATTCTATCGTTCCCAACCTGTTGGCC
 CTCCTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAG
 GAGGAAATCAATCTATTCTAGCAGTGCTCATCCAACAAACCTCGGCTCAAAGCACATCAGGTG
 CTTCTCGTTCCAAGACTGCGCCCATTAGCCATTCCGCTTAGCTCTCCCTGTATTCTA
 AA

FIGURE 41

>Predicted amino acid sequence of CotH3 from Lichtheimia corymbifera (including introns)

MKLSTIISAAFLVAITHAASIKFNVIAPNATDVKSVNGQQVTLTASDANVPYFTGSAEVGSSKYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*SPRHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFLSGLVLETKPTFSMTIIFPAFFSTEMTVSKMWLKTYLLTESVVH*PILLDL
ITSTLSRMSHLVFTVLARNTTMQNNLGTGYCLEVIRWTAISLSFDIWKKILHRSVNVFILTFYMP
WVLMPMMLPWFDCLLTTKASVPSTCWMISLNSLISMLNFI^{MANHLL}PWVL^{SMMV}PLVQISYIILV
SMDTLLGLPTQPILMVKLMKLLILSVRPGTRRPIPIIQPLQTLKKCLISTVSCVSWLLNT*LPIGM
VTGWDRPMMPVIVIQLIITS^{GTF}*IKTLMVLLVSTWLHPKAMLFILMFLTRISLLVTI^{ALS}*STTSY
RMLIKRPPMKNI*LRLCVCCSIMSP*LT^VSWPFTTSSCLILNGIVRSFNNLLVLTLVGHLIKSLKT
CGKVSLHPI^TMEV^VLLL^V*LNILLQRHKL*LRNLIFLSFPNLLALLLMV^VLLLLL^VLLLLL^VAILLEK
EEINLFLAVLHPTKPR^LKAHQVLLV^PRLRPSFLAISALALPCIL

FIGURE 42

>CotH3 sequence from Cunninghamella bertholletiae(exons only)

ATGAAATTATCTATTATACTCGCTGCCCTTTAGTGGCTATTACACACGCTGCTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTTATTCACTGGTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTGATCGTTCTTGATGGAATCACAAACTAACACTT
AATGATTTTATAACCGCCCCGTCACTTATGCTAACCTCCTCAATTACCTGGCCAATTGAAAAAA
GACCCTCAGTGGACTCGTTCTGGAAGCAAAGCCGACATTTCGATGACAATTATATTCCCAGCGTT
TTTTCCACGGAGATGACAGTCAAGTCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTATTGGATCTAATTACGTACTCTTCAGAATGTCATTTGGTATTAC
GGTGGCTGGCAAGAACACAACAATGCAAAACAATCTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAACCGCAATTCTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTGTCGACGT
CTTTATTCTGACATTTCACATGCCATGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTT
ATTAACAACCAAGGCTTCGGTACCTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
GCTAAATTATAATGGCAAACCAACCTGCTACCTGGTCTCTATGATGGTGCCTCTGGTGC
GACTTCTTATATCATCCTGGTAAACCTCGATGGATACTCTCTGGTGCACACAGCCAATCCT
AATGTGAAGCTTATGAAGCCTCTTGATCCTCTGTAGCCTGGAACAGAGACGACCTAATACCGATA
ATACAGCCATTGCAAACCTTGGAAAAATGTTGATCTCGACCGTTCATGGTTCATGGTTATTG
AATACTGACTGCCATTGGGATGGTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGCATC
CAACTGATAATAACAAGTGGTACTTTAGATCAAGACTTGTAGGGTACTTTGGTGTCAACTTGG
CTGCACCCGAAGGCAATGCTTTCTTGATGTTCTTACAAGGATTCCCTCTCGTTACCCGGCG
CTGTCATGATCAACAAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTGAAAAATATTGACTG
AGACTGTGCGTGTGCTGTCATAATGTCACCTGACTAACCGTGTCTGGCCCTTCACAACCTCC
TCTTGCCTGATCTTGAATGGGATCGTCAACATGTCGTTCAACAATCTCCTGGTATTAACCTGGTGG
CATTGATCAAGTCACTCAAACCTGTTGGCAAGGTGTCAGTGCACCCAAATAACAATGGAGGTGGT
CTGCTTTGGTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGAGCTAAGGAATTAAATATT
CTATCGTTCCCAACCTGTTGGCCCTCTGCTAATGGTACTACTGCTGCTGCTGCT
GCTGCAATTCTACTGGAAAAGAGAAATCAATCTATTTCAGCAGTGCTCATCAACAAAGCTCGG
CTCAAGGCACATCAGTGCCTCTCGATCAAGACTGCGCCATCGATTAAGCAGTTGCTTAGCTT
CCCTGGGGTAATCTCCAAAAAA

FIGURE 43

>Predicted amino acid sequence of CotH3 from Cunninghamella bertholletiae (exons only from sequenced data)

MKLSTIISAAFLVAITHAASIKFNVIAPNATDVKSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*PPRHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFSASVDSFWKQSRHFR*QLYSQRFFPRR*QSSPKCG*KRTC*PNQWYTDLYWI*
LRLLFPECLIWYSRCWQETQQCKTILELDIVWK*YDG*PQFL*ASTYGRRSYTDS*TSLF*HFTCH
GYLCQ*CYHGSIVY*QPRRLRYLQHVG*YHSILLYQC*IL*WQTTCYLGSSL*WCLWCRLLISSW*P
RWILFLGCQHSQS*CEAYEAS*SSL*PGTRRPNTDNTAIANFEKMF DLD RFM R M VIE YLTADWDG
YWMGQTNDGAYRDPTDNNKWYFLDQDFDGTGVNLAAPEGNAFLDVSYKDFPSRYPGAVMINNLIQ
NADKKATFEKYL TETVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQNL
WQGV TAPNNNGGAAAGLVEYIAAKAQAVAKEFNISIVSQPGPPSANGTTAAALLCCNSTGKEK
SIYFLAVLHQSSAQGTSVPSRSRLRPSIKQFALASLG*SPK

FIGURE 44

>CotH3 sequence from Cunninghamella bertholletiae (including introns)

ATGAAATTATCTATTATCCGCTGCCTTTAGGGCTATTACACACGCTGCTTCAATAAAGTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTTATTCACTGGTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
GTAATCATAAATTAGTTGAATTCAATGAGATTAACTCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAAGTTGATCGTTCTGATGGAATCACAAACTCAACACTTAATGATTTTATAA
CCGCCCGTCACTTATGCTAACCTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATT
TATTTTATCTTCTCAGCTAAACATTGCTTCAGCCTCAGTGGACTCGTTCTGGAAG
CAAAGCCGACATTTGATGACAATTATATTCCAGCCTTTTCCACGGAGATGACAGTCAGT
CCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTATTGGATCTAA
TTACGTCTACTCTTCCAGAACATGTCTCATTGGTATTCAACGGTCTGGCAAGAAACACAACATGC
AAAACAATCTTGGAACTGGATATTGCTGGAAGTGATGGTAACCGCAATTCTTAAGCT
TCGACATATGGAAGAAGATCCTACACAGATTGCTAACGTCTTATTCTGACATTACATGCCAT
GGGTACTTATGCCAATGATGCTTACCATGGTCATTGTTATTACAACCAAGGCTCGGTACCTT
CAACATGTTGGATGATATCACTCAATTCTCCTATATCAATGCTAAATTATGAAACACC
TGCTACCTTGGGTCTCTATGATGGTGCCTCTGGTGCAGACTTCTTATATCATCCTGGTAACCT
CGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCTAATGTAAGCTTATGAAAGCCTCTTGA
TCCTCTCTGTAGCCTGGAACGAGACGACCTAATACCGATAATACAGCCATTGCAAACCTTGAAAAAA
ATGTTGATCTGACCGTTCATGCGTTCATGGTATTGAAATACCTGACTGCCATTGGGATGGT
TACTGGATGGACAGACCAATGATGGTGCCTATCGTATGCAACTGATAATAACAAGTGGTACTTT
TTAGATCAAGACTTGTGATGGTACTTTGGTGTCAACTTGGCTGCACCGAAGGCAATGCTTTCTT
GATGTTCTTACAAGGATTCCCTCTCGTTACCCCTGGCGCTGTATGATCAACAAACCTCTTACAG
AATGCTGATAAAAAGGCCACCTTGAAAAATATTGACTGAGACTGTGCGTGTGCTGTTCAATAAT
GTCACCTTGACTAACCGTGTCTGGCCCTTCACAACCTCCTTGCCTGATCTGAATGGATCGT
TCGATCGTTCAACAATCTCCTGGTATTAACCTTGGTGGACATTGATCAAGTCACTCAAAACTTG
TGGCAAGGGTGTCACTGCACCCAATAACAATGGAGGTGGTGCCTTGGTTAGTGAATATATT
GCTGCAAAGGCACAAGCTGTAGCTAAGGAATTAAATATTCTATCGTTCCCAACCTGTTGGCCCT
CCTCTGCTAATGGTACTACTGCTGCTGCTGCTGCTGCAATTCTACTGGAAAAGAGAAA
TCAATCTATTCTAGCAGTGCTTCATCAACAAAGCTCGGCTCAAGGCACATCAGTGCCTCTCGA
TCAAGACTGCGCCATCGATTAAGCAGTCGCTTAGCTCCCTGGGTAATCTCCAAAAAA

FIGURE 45

>Predicted amino acid sequence of CotH3 from Cunninghamella bertholetiae (including introns from sequenced data)

MKLSTIISAAFLVAITHAASIKFNVIAPNATDVKSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*PPRHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFSASVDSFWKQSRHFR*QLYSQRFFPRR*QSSPKCG*KRTC*PNQWYTDLYWI*
LRLLFPECLIWYSRCWQETQQCKTILELDIVWK*YDG*PQFL*ASTYGRRSYTDS*TSLF*HFTCH
GYLCQ*CYHGSIVY*QPRRLRYLQHVG*YHSILLYQC*IL*WQTTCYLGSSL*WCLWCRLLISSW*P
RWILFLGCQHSQS*CEAYEAS*SSL*PGTRRPNTDNTAIANFEKMF DLDRFMRFMVIEYLTADWDG
YWMGQTNDGAYRDPTDNNKWYFLDQDFDGTGVNLAAPEGNAFLDVSYKDFPSRYPGAVMINNLLQ
NADKKATFEKYL TETVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQNL
WQGV TAPNNNGGAAAGLVEYIAAKAQAVAKEFNISIVSQPGPPSANGTTAAALLCCNSTGKEK
SIYFLAVLHQSSAQGTSVPSRSRLRPSIKQFALASLG*SPK

FIGURE 46

>CotH3 sequence from *R. mirosporus* (exons only from sequenced data)

ATGAAATTATCTATTATCCGCTGCCTTTAGTGGCTATTACACACGCTGCTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTTATTCACTGGTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTGATCGTTCTTGATGGAATCACAAACTAACACTT
AATGATTTTATAACCGCCCCGTCACTTATGCTAACCTCCTCAATTACCTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAGCAAAGCCGACATTTCGATGACAATTATATTCCCAGCGTT
TTTTCCACGGAGATGACAGTCAAGTCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTATTGGATCTAATTACGTACTCTTCCAGAATGTCTCATTTGGTATTAC
GGTGGCTGGCAAGAACACAACAATGCAAAACAACTTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAAACCGCAATTCTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTGTAACGT
CTTTATTCTGACATTTCACATGCCATGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTT
ATTAACAACCAAGGCTTCGGTACCTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
GCTAAATTATAATGGCAAACACACCTGCTACCTTGGTCCTCTCATGATGGTGCCTCTGGTGC
GACTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTGGGTTGCCAACACAGCCAATCCT
AATGGTGAAGCTTATGAAGCTTGTGATCCTCTGTAAAGGCTGGAACGAGACGACCTATACCGAT
AATACAGCCATTGCAAACATTGAAAAATGTTGATCTCGACCGTTGATGCGTTCATGGTATT
GAATACTTGACTGCCGATTGGGATGGTTACTGGATGGACAGACCAATGATGGTGCCTATCGT
CCAACGTGATAATAACAAGTGGTACTTTAGATCAAGACTTGTGATGGTACTTTGGTGTCAACTG
GCTGCACCCGAAGGCAATGCTTTCTGATGTTCTACAAGGATTTCCCTCTCGTTACCGTGG
GCTGTCATGATCAACAAACCTTACAGAATGCTGATAAAAAGGCCACCTTGAAAAATATTGACT
GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTGACTAACCGTGTCTGGCCCTCACAACCTC
CTCTGCCTGATCTGAATGGGATGTTGATCGTCAACAATCTCCTGGTATTAACCTTGGTGG
ACATTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCATAACAATGGAGGTGG
GCTGCTTTGGTTAGTTGAATATATTGCTGCAAAGGACAAGCTGTAGCTAAGGAATTAAATATT
TCTATCGTTCCCAACCTGTTGGCCCTCCTCTGCTAATGGTACTACTGCTGCTGCTCCT
GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTCTAGCAGTGCTTCATCCAACAAA
ACCTCGGCTCAAAGCACATCAGGTGCTCTCGTTCAAGACTGCGCCATGTTAGCCATTCC
GCTTAGCTCCTGTATTCTAAA

FIGURE 47

>Predicted amino acid sequence of CotH3 from *R. mirosporus* (exons only from sequenced data)

MKLSIISAAFLVAITHAASIKENVIAFPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
YVAGGTEESFDRSLDGITNSTLNDFYNRPVTYANLPQLPWPIEKDPQWTRSGSKADIFDDNYIPSV
FFHGDDSQVQNVVKNVPADRIGSTLTFIGSNYVYSFQNVSFGIHAGGKHHNNAKQSWNWILSGSDT
MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGYANDATMVRLFINNQGFGTFNMLDDITQFSYIN
AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNGEAYEALDPLCKAWNNETTYTD
NTAIANFEKMFDDLDRFMRFMVIEYLTADWDGYWMGQTNDGAYRDPDTNNKWWYFLDQDFDGTFGVNL
AAPEGNAFLDVSYKDFPSRYPGAVMINNLLQNAKKATFEKYLTEVRVLFNNVTLNRVLALHNF
LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGVTAPNNNGGAAFGLVEYIAAKAQAVAKEFNI
SIVSQPVGPPSANGTTAAAPAPAAGNSTGKGGNQSISSSASSNKTSAQSTSGASRSKTAPIVLAIS
ALALLVF*

FIGURE 48

>CotH3 sequence from *R. mirosporus* (including introns, has only one intron)

ATGAAATTATCTATTATCCGCTGCCTTTAGGGCTATTACACACGCTGCTCAATAAGTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCTTATTCAGCTGAAAGTTGGATCGTTCTCTGATGGAATCACAAACTCAACACTT
ATGTTGCAGGTGGAACAGAAAGTTGGATCGTTCTCTGATGGAATCACAAACTCAACACTT
AATGATTTTATAACCGCCCCGTCACTTATGCTAACCTCCTCAATTACCTGGCCAATTGAAAAA
GACGTAAGTTATTTATTTATCTTCTCAGCTAAAATAAACATTGTCTTCTCAGCCTCAGTG
GACTCGTTCTGGAAGCAAAGCCGACATTTGATGACAATTATATTCCAGCGTTTTCCACGG
AGATGACAGTCAGTCCAAAATGTGGTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGAC
CTTATTGGATCTAATTACGTCTACTCTTCCAGAATGTCTCATTGGTATTACGGTCTGGCAA
GAAACACAACAATGCAAACAAATCTTGGAACTGGATATTGTCTGGAAGTGTACGATGGTAACCG
CAATTCTTAAGCTCGACATATGGAAGAAGATCCTACACAGATTGTAACGTCTTATTCTGA
CATTTACATGCCATGGTACTTATGCCAATGATGCTACCATGGTCGATTGTTATTAACAACCA
AGGCTTCGGTACCTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAATGCTAAATTAA
TAATGGCAAACCAACCTGCTACCTTGGGCCTCTATGATGGTGCCTCTGGTCAGACTCTTATA
TCATCCTGGTAACCTCGATGGATACTCTTGGTGCCTACAGCCAACTCCTAACGGTAAAGC
TTATGAAGCTCTGATCCTCTGTAAAGGCCTGGAACGAGACGACCTATACCGATAATACAGCCAT
TGCAAACCTTGGAAATGTTGATCTGACCGTTCATGCGTTCATGGTATTGAATACTGAC
TGCGATTGGGATGGTACTGGATGGACAGACCAATGATGGTGCCTATCGTGTACCAACTGATAA
TAACAAGTGGTACTTTAGATCAAGACTTGTGATGGTACTTTGGTGTCAACTGGCTGCACCGA
AGGCAATGCTTCTGATGTTCTACAAGGATTTCCCTCTCGTTACCCCTGGCGCTGTGATGAT
CAACAACCTTACAGAATGCTGATAAAAAGGCCACCTTGGAAAATATTGACTGAGACTGTGCG
TGTGCTGTTCAATAATGTCACCTGACTAACCGTGTCTGGCCCTCACAACCTCCTTGCCTGA
TCTTGAATGGGATCGTCGATCGTTCAACAATCTCCTGGTATTAACTTGGTGGACATTGATCA
AGTCACTAAAATTGTCGCAAGGTGTCAGTCACCCAAATAACAATGGAGGTGGTGTGCTTTGG
TTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTAAATATTCTATCGTTTC
CCAACCTGTTGGCCCTCTGCTAAATGGTACTACTGCTGCTGCTCCTGCTGCTGGCAA
TTCTACTGGAAAAGGAGGAAATCAATCTATTCTAGCAGTGCTTCATCCAACAAACCTCGGCTCA
AAGCACATCAGGTGCTTCTCGTTCCAAGACTGCGCCCATCGTTAGCCATTCCGCTTAGCTCT
CCTGTATTCTAAA

FIGURE 49

>Predicted amino acid sequence of CotH3 from *R. mirosporus* (including introns)

MKLSTIISAAFLVAITHAASIKFNVIAPNATDVKSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*PPRHLC*PSSITLAN*KRRKLF
YFYILFSAKINIVFLSLSGLVLEAKPTFSMTIIFPAFFSTEMTVKSKMWLKTYLLTESVVH*PILLDL
ITSTLSRMSHLVFTVLARNTTMQNNLGTGYCLEVIRWVTAISLSFDIWKKILHRFVNVFILTFYMP
WVLMPMMLPWFDCLLTTKASVPSTCWMSLNSPISMLNFIMANHLLPWVLSMMVPLVQTSYIILV
SMDTLLGLPTQPILMVKLMKLLILSVRPGTRRPIPIIQPLQTLKKCLISTVSCVSWLLNT*LPIGM
VTGWDRPMMPVIVIQLIITSQTF*IKTLMVLLVSTWLHPKAMLFLIMFLTRISLLVTIALS*STTSY
RMLIKRPPLKNI*LRLCVCCSIMSP*LTWSWPFTTSSCLILNGIVRSFNNLLVLTLVGHLIKSLKT
CGKVSLHPIITMEVVLLV*LNILLQRHKL*LRNLIFLSFPNLLALLLMVLLLLLLLAILLEK
EEINLFLAVLHPTKPRLKAHQVLLVPRLRPSF*PFPL*LSLYSK

FIGURE 50

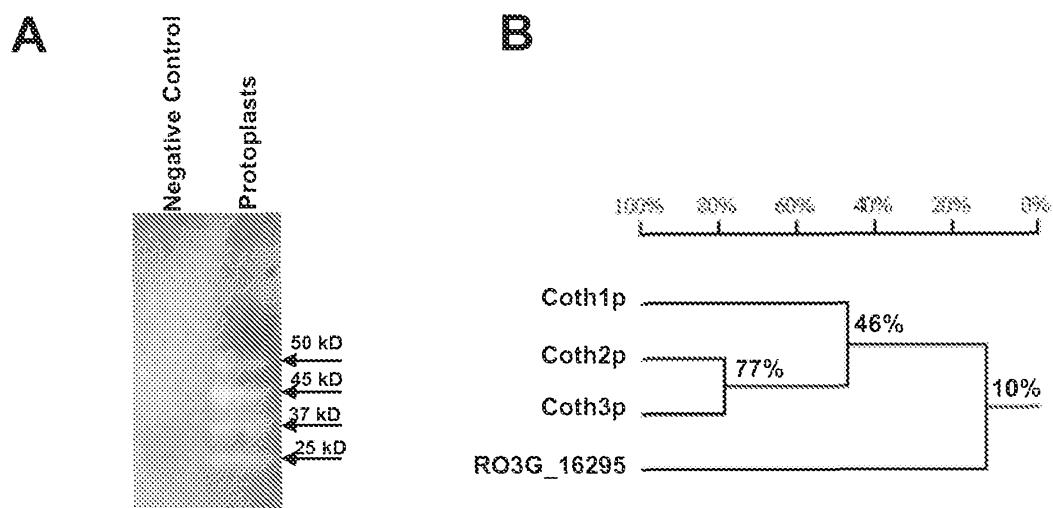


FIGURE 51

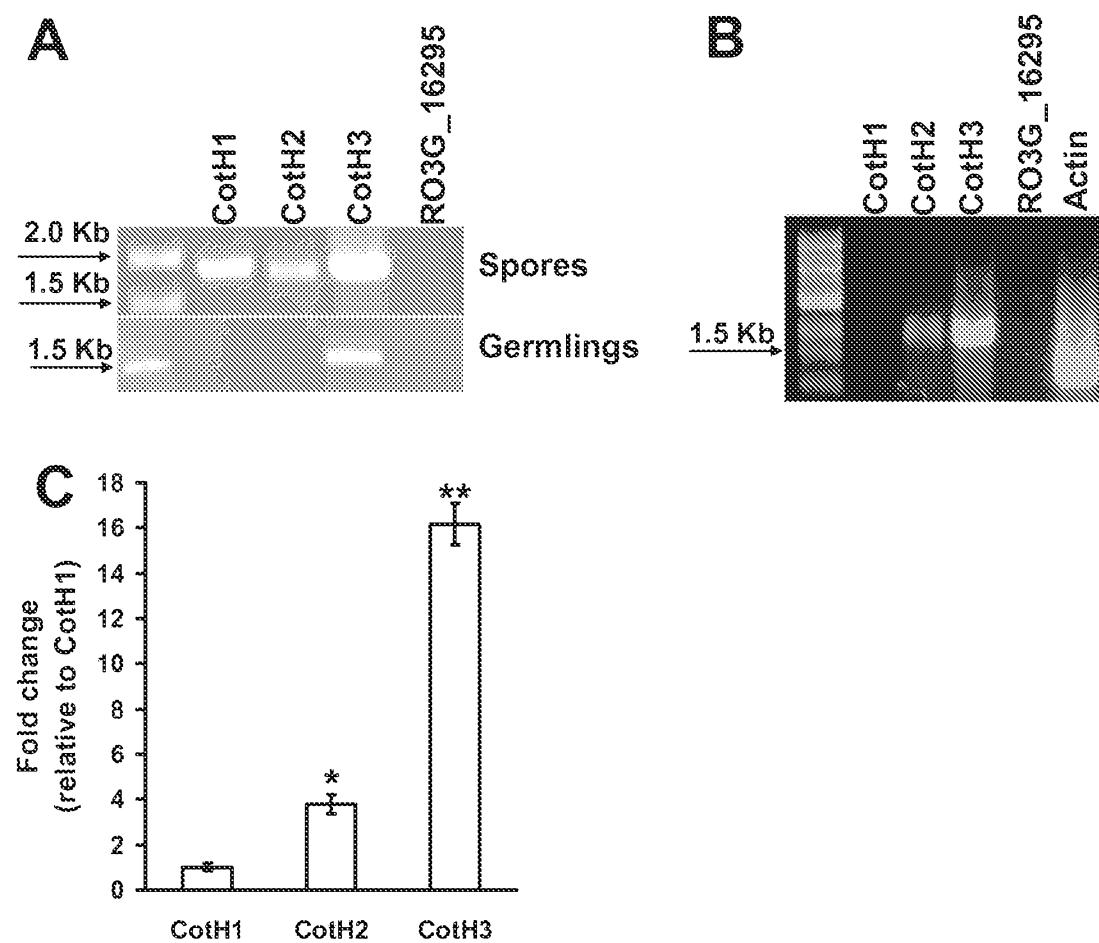


FIGURE 52

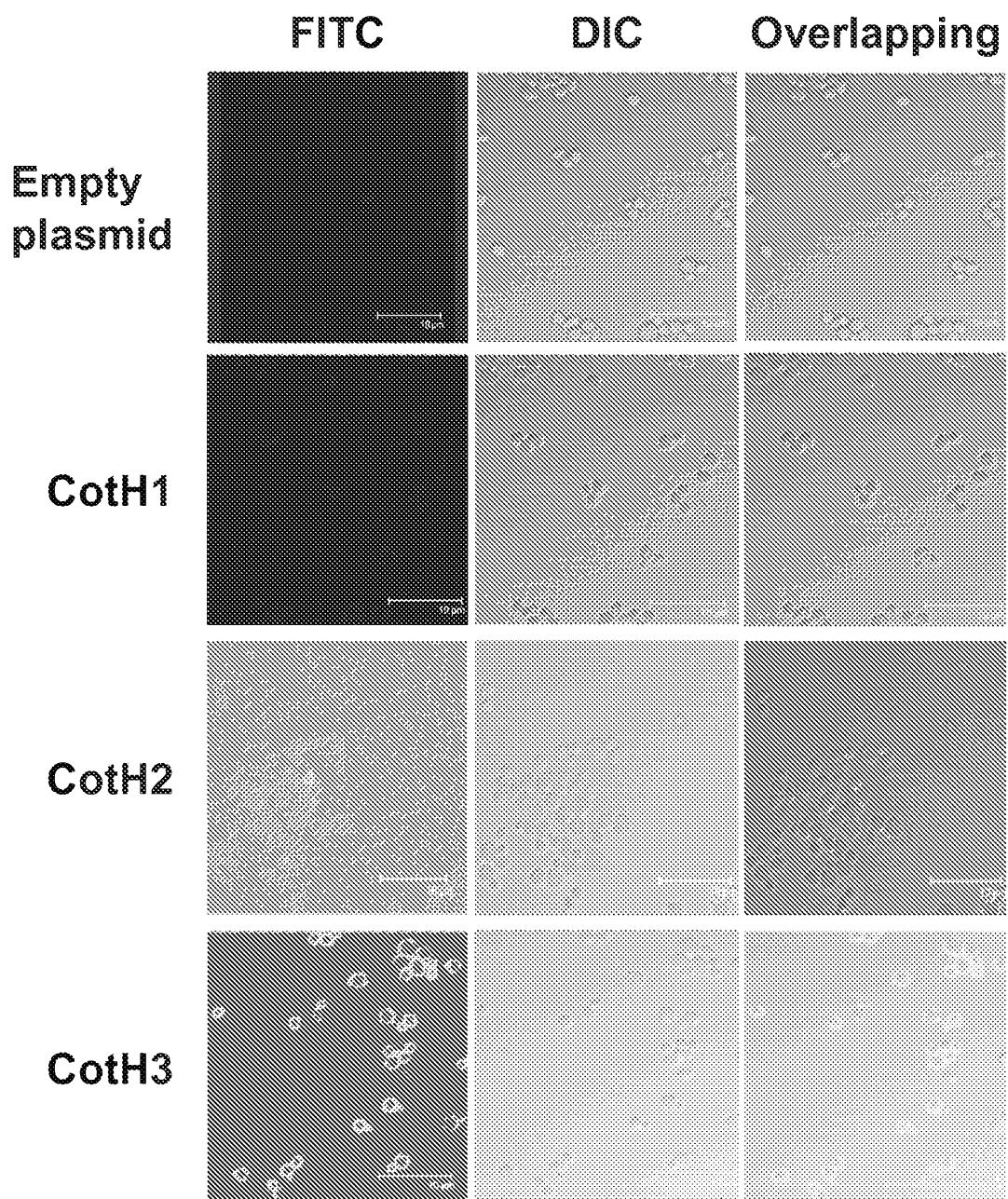


FIGURE 53

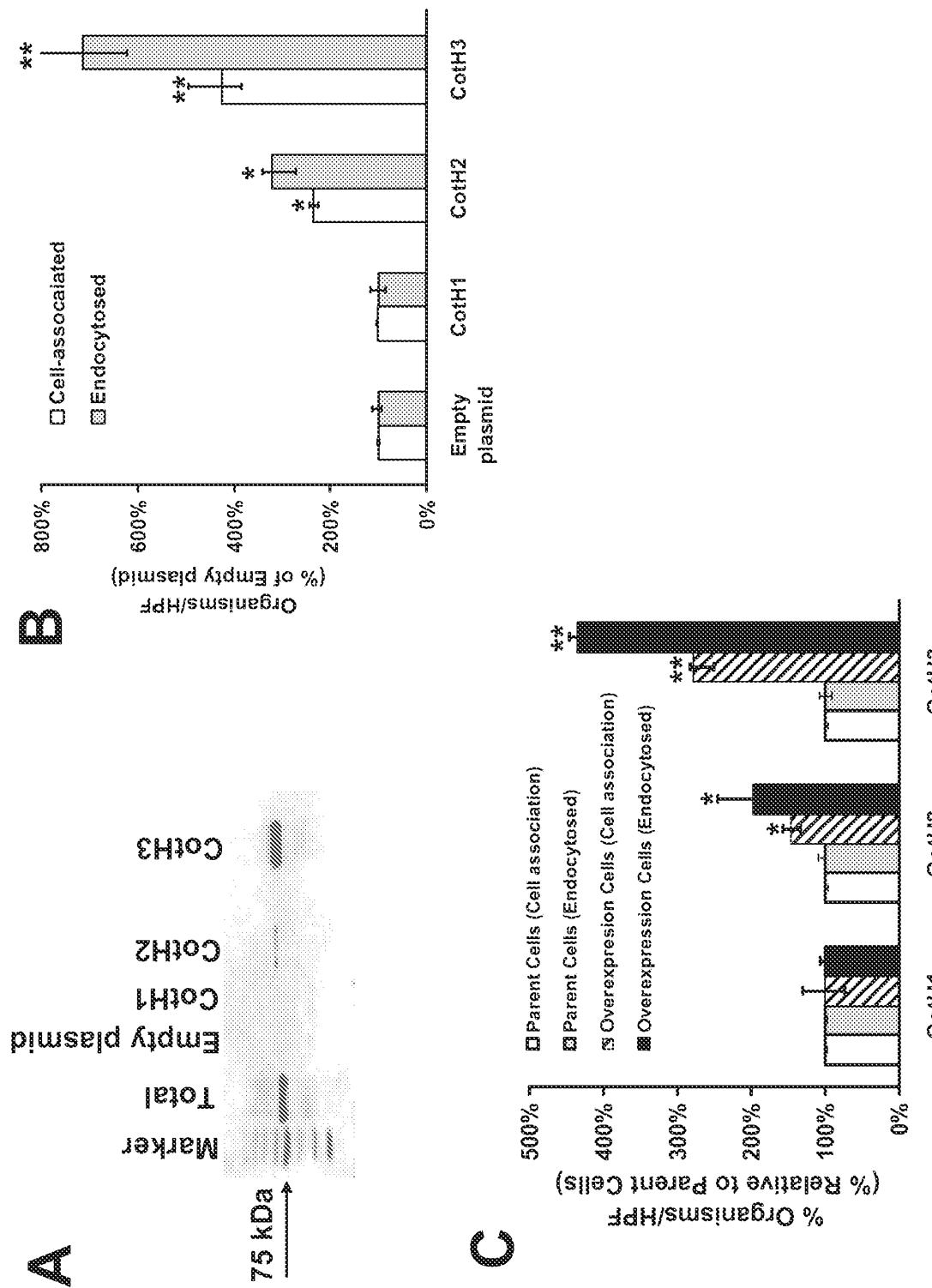


FIGURE 54

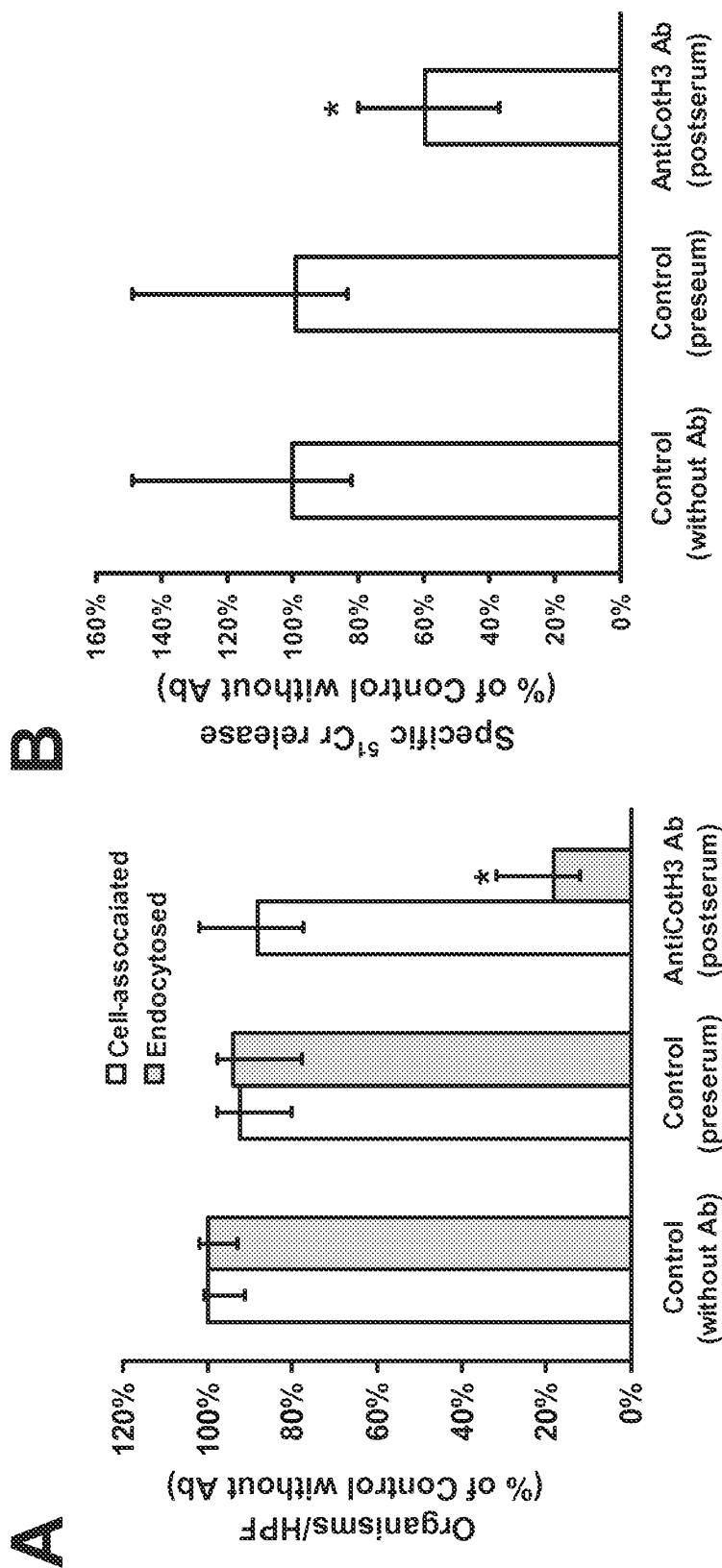


FIGURE 55

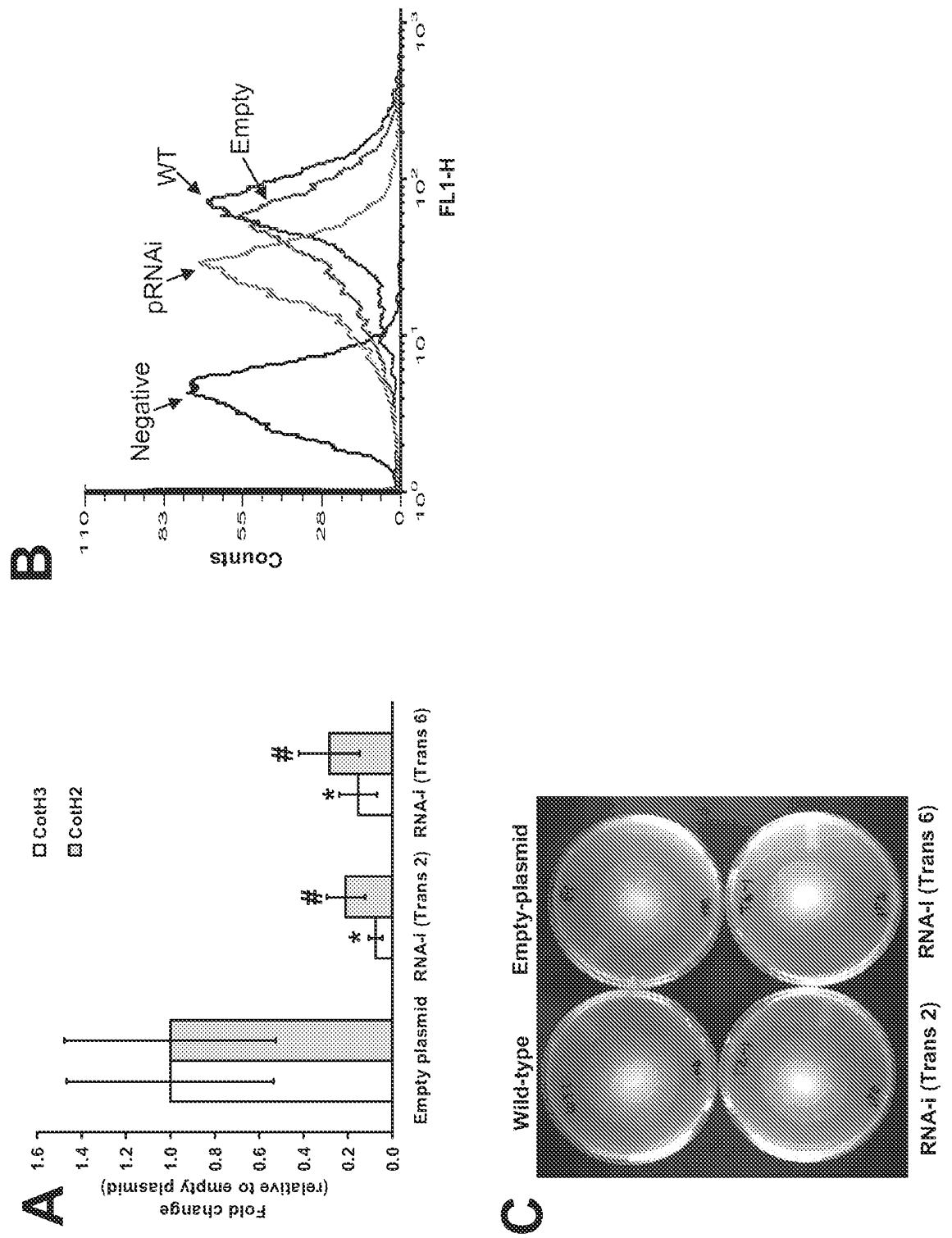


FIGURE 56

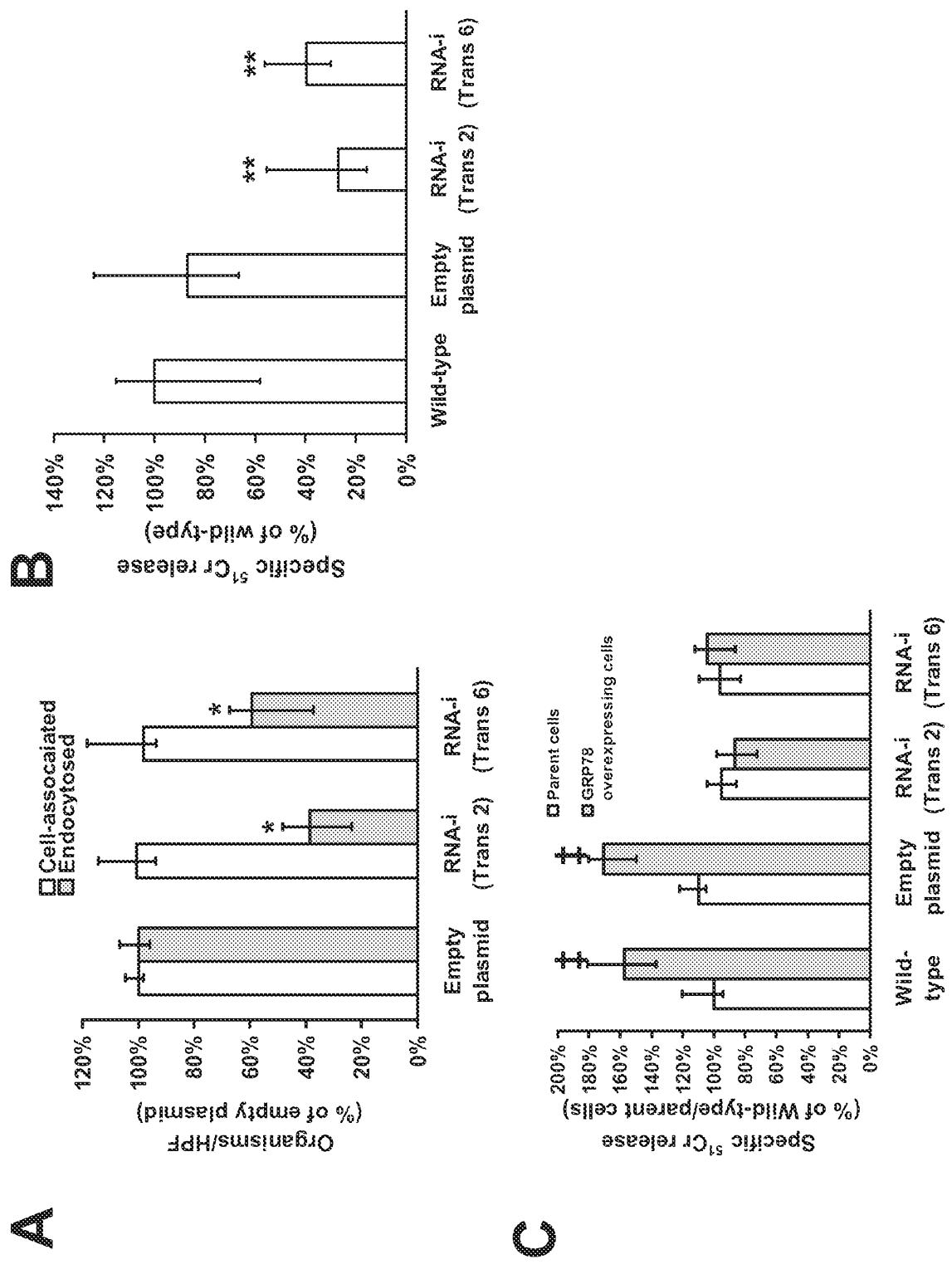


FIGURE 57

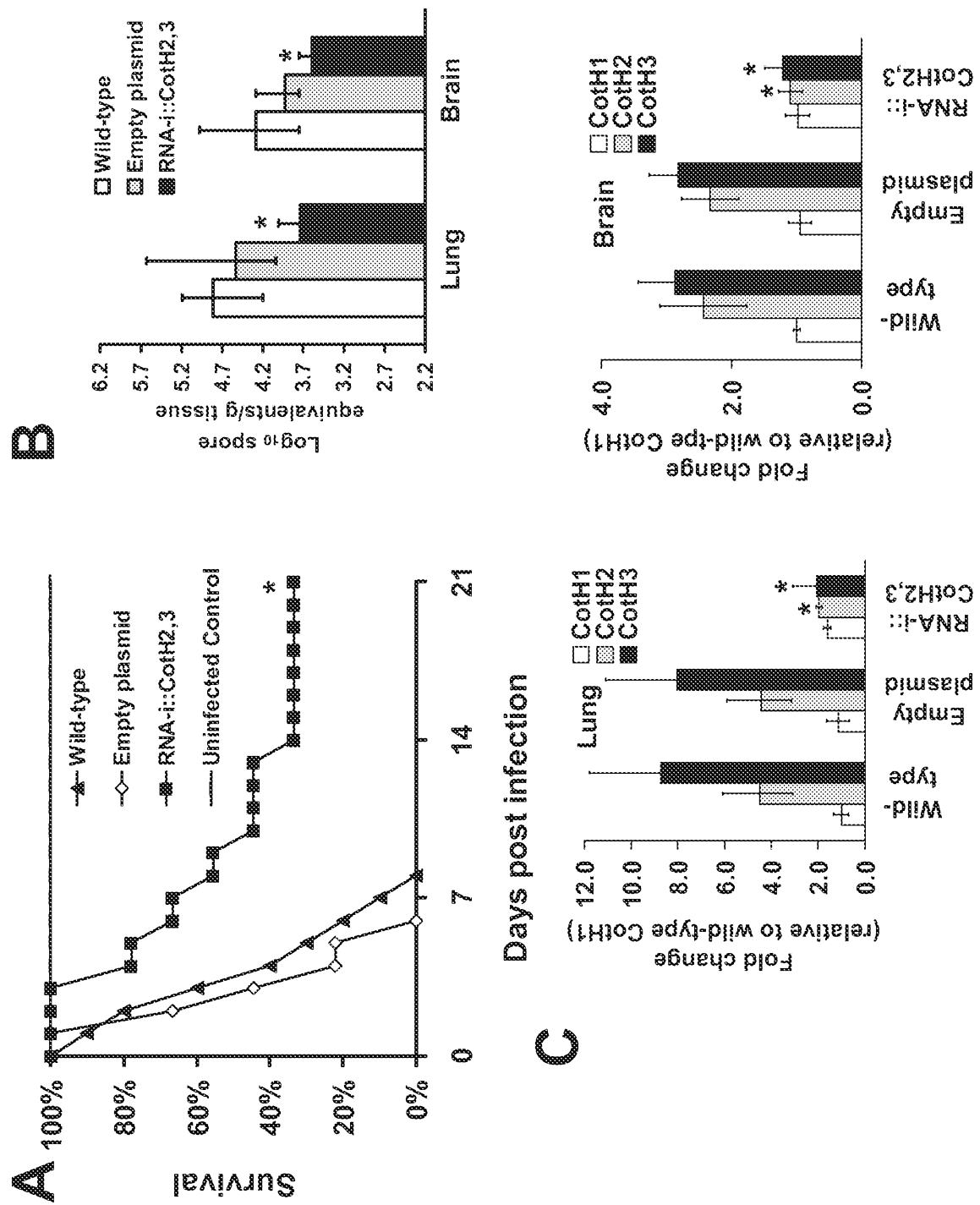


FIGURE 58

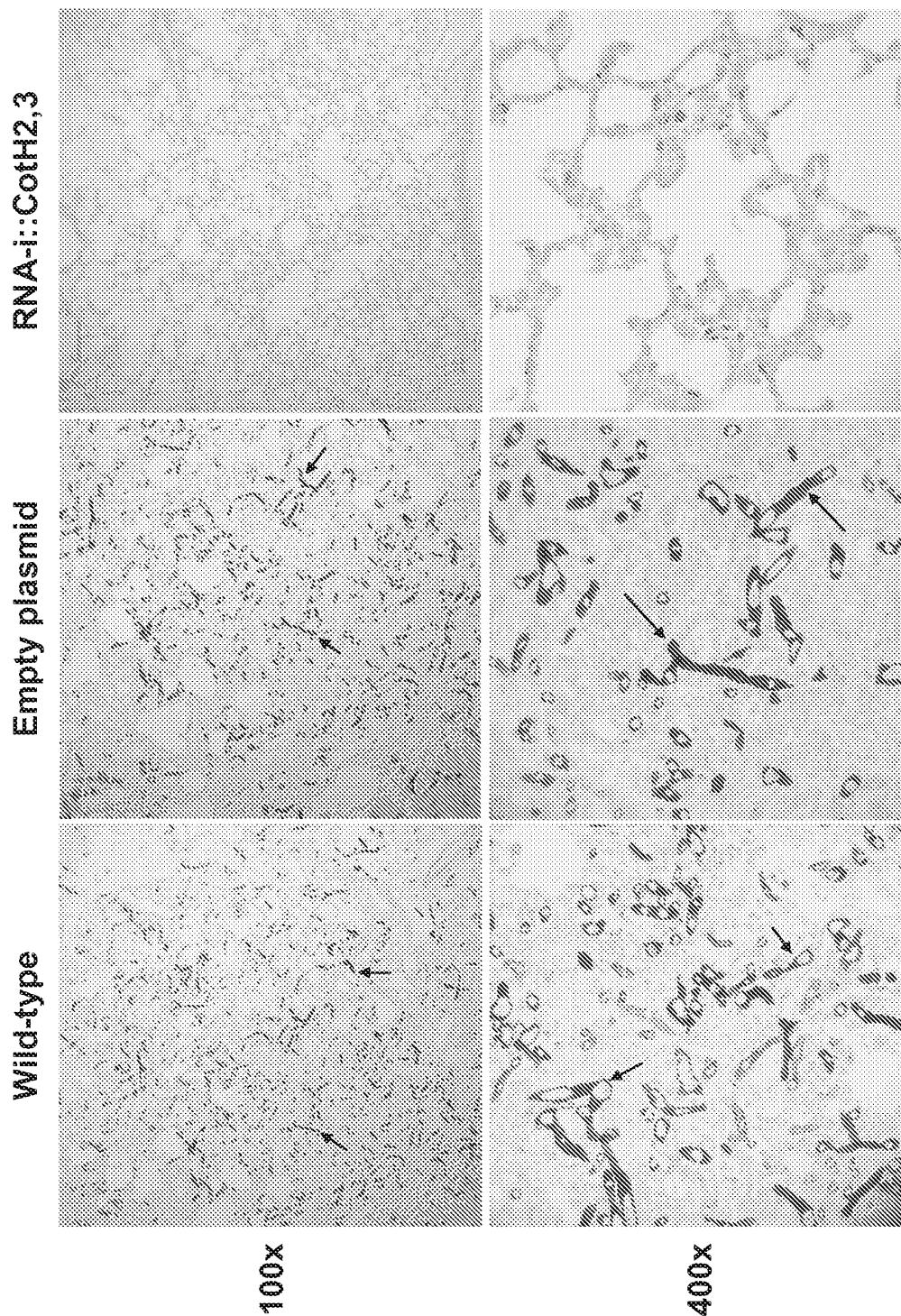


FIGURE 59

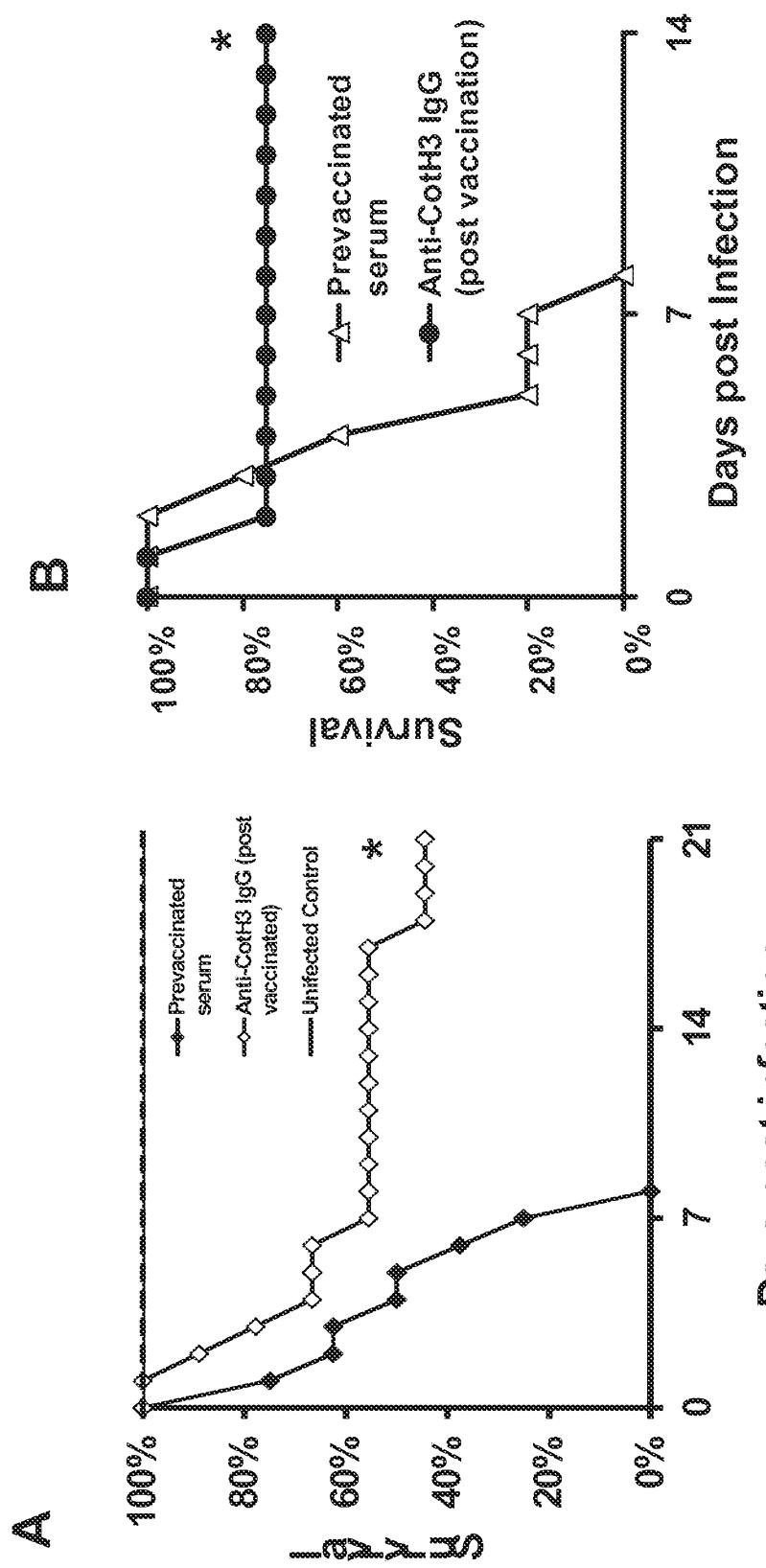


FIGURE 60

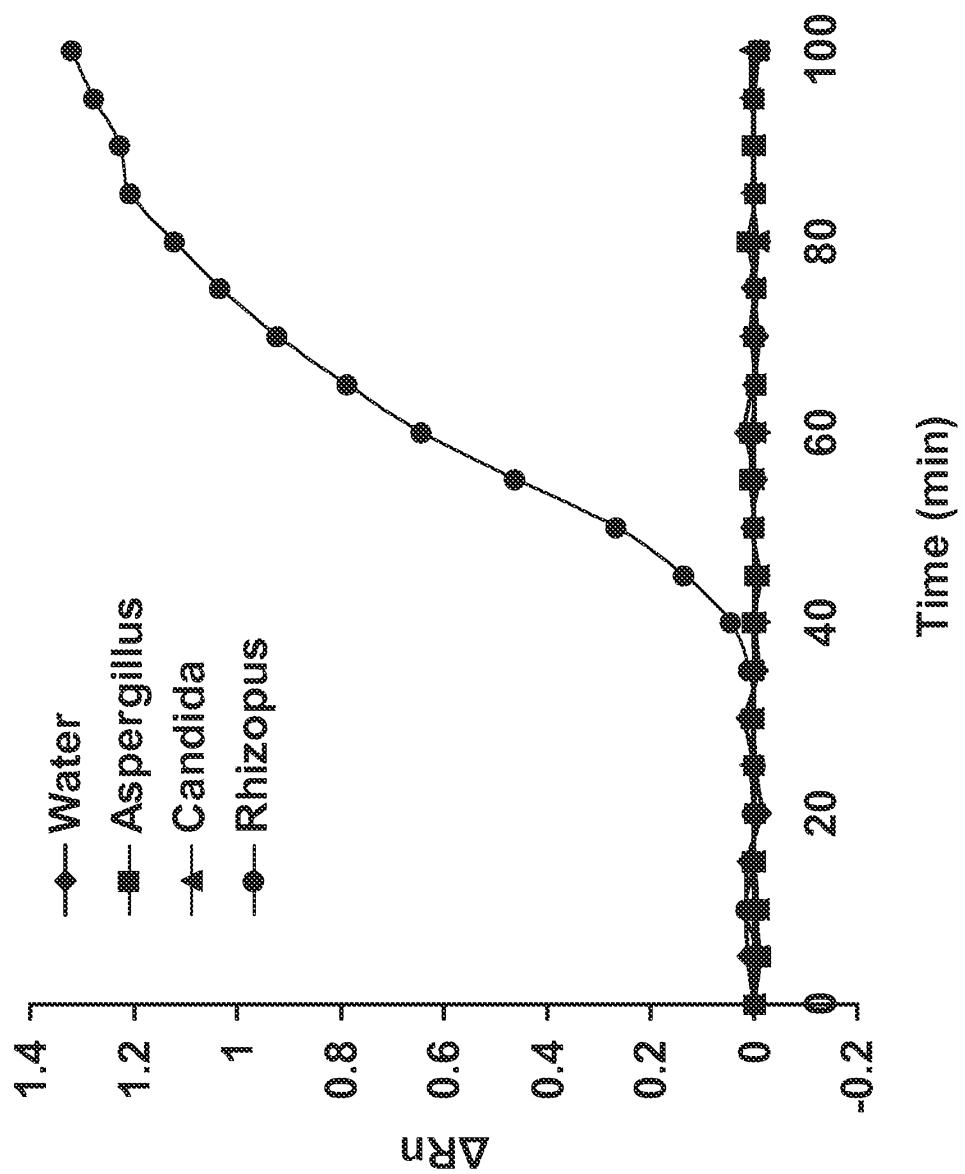


FIGURE 61

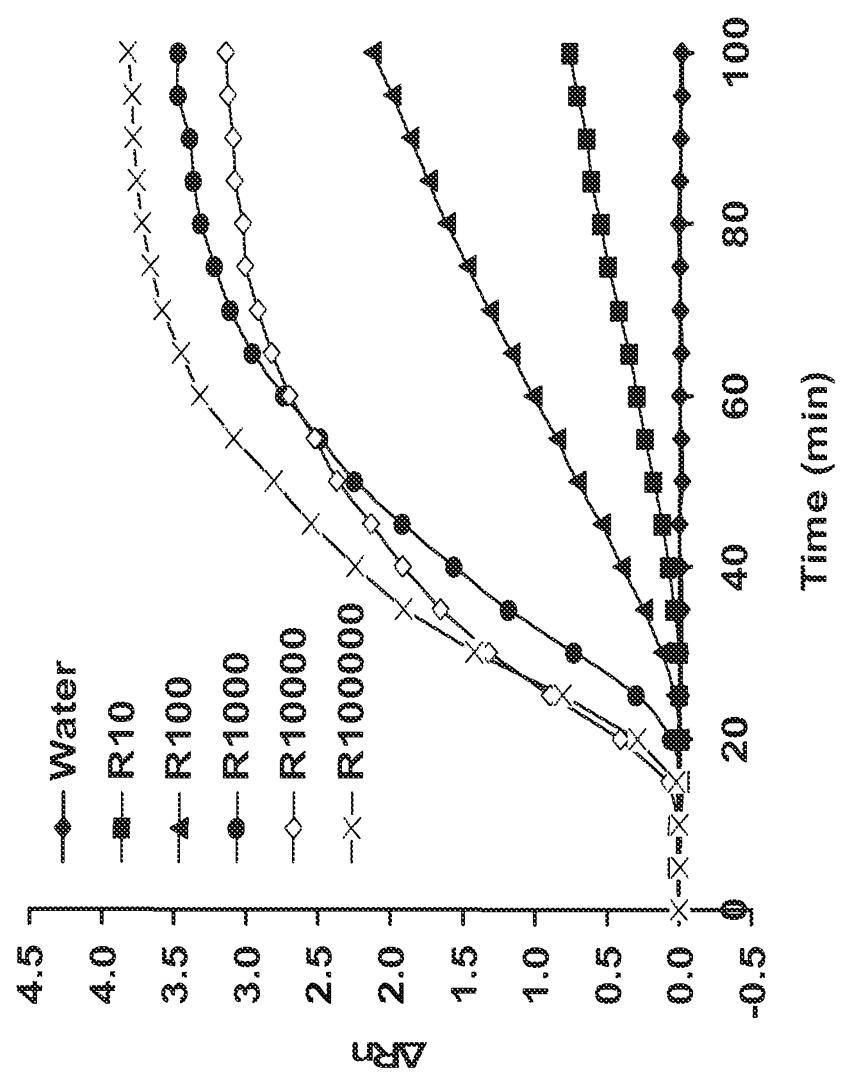


FIGURE 62

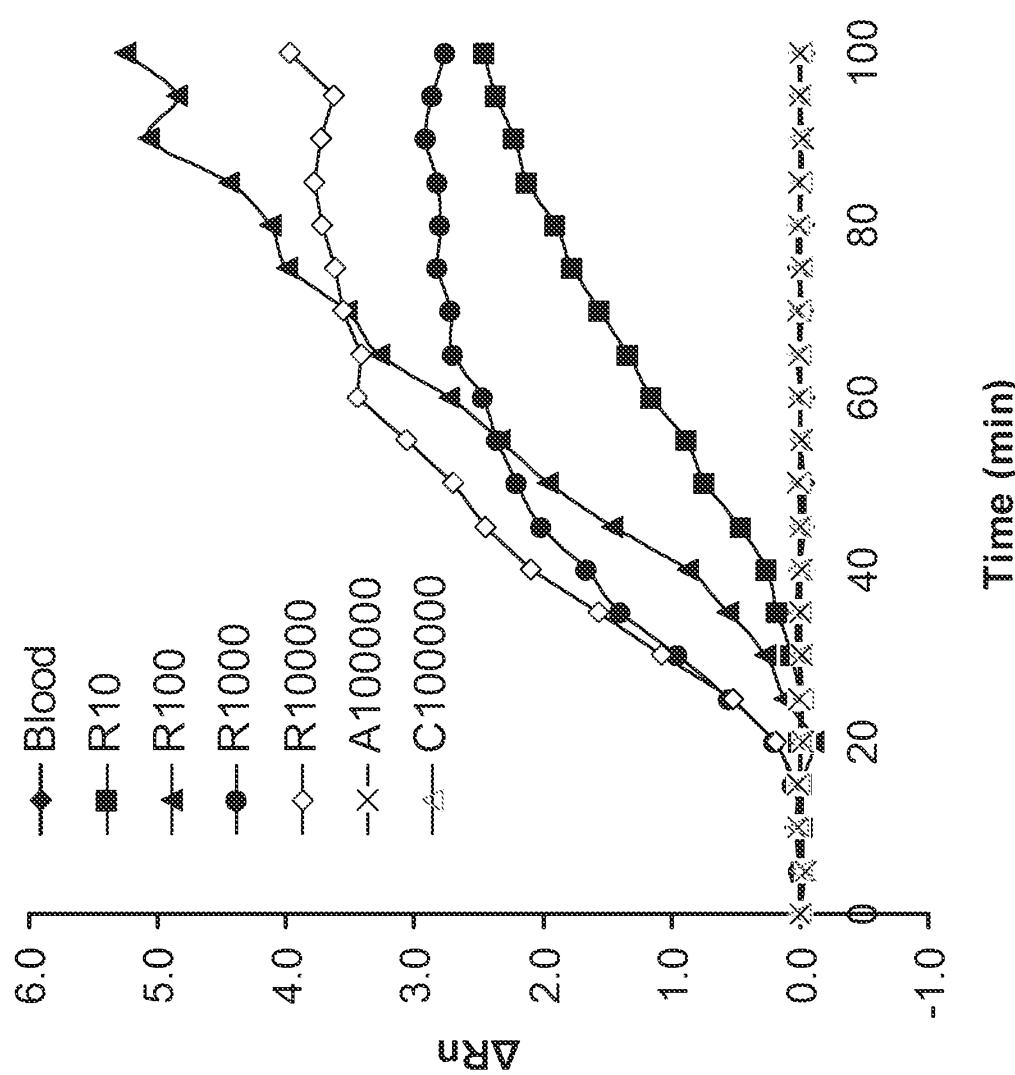


FIGURE 63

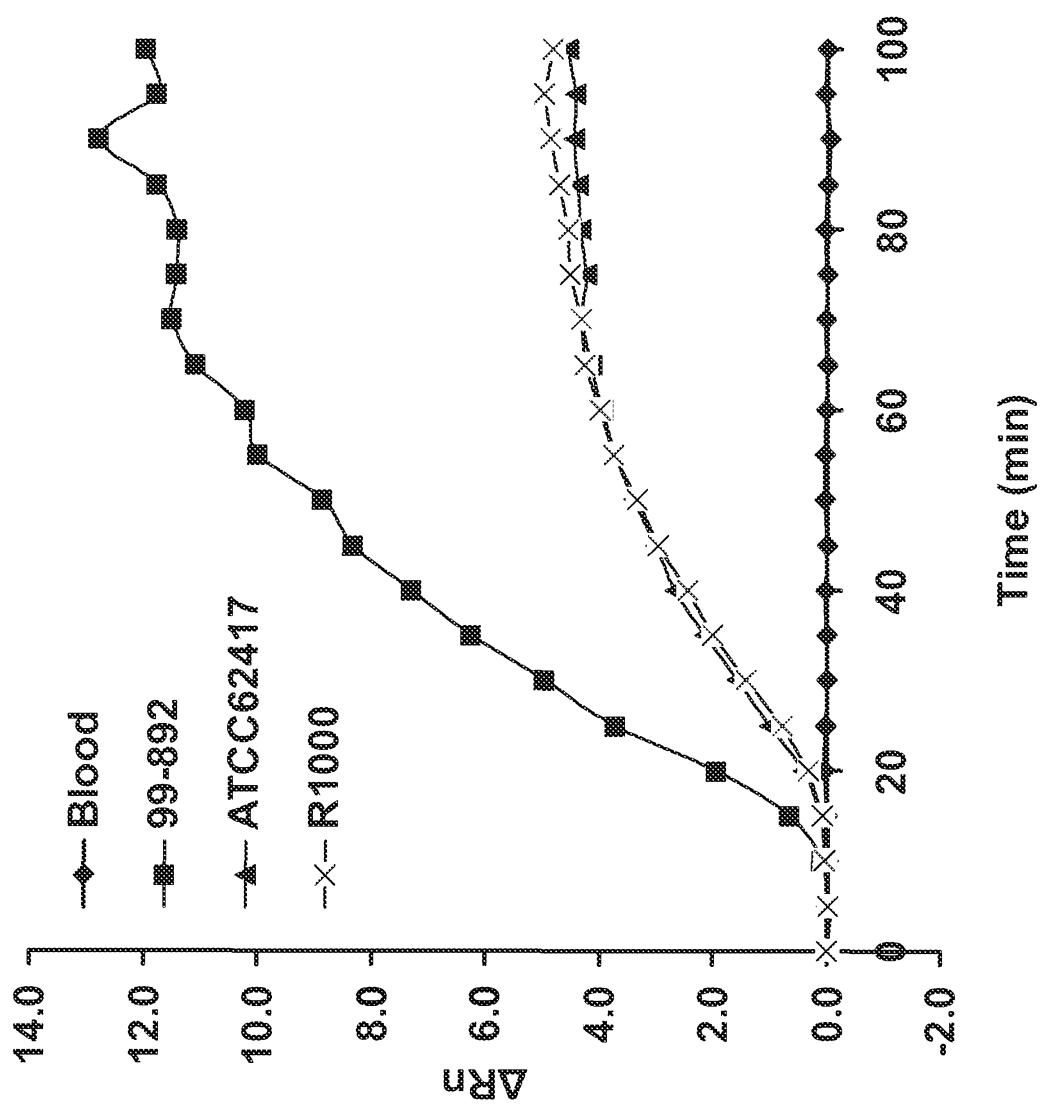


FIGURE 64