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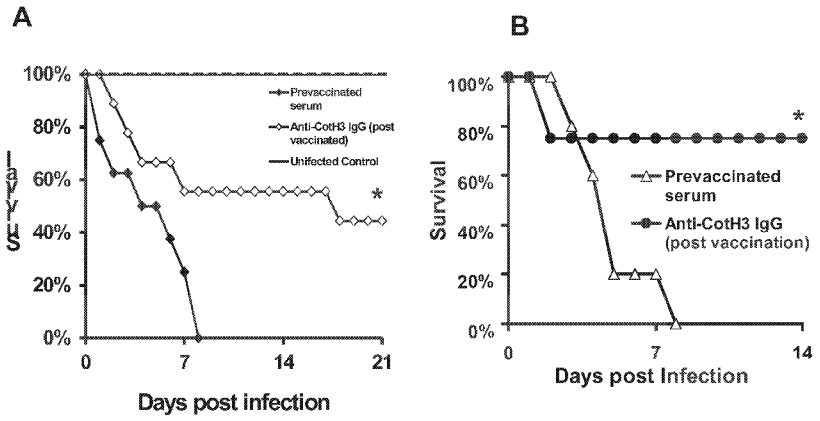

FIGURE 60

(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.

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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.

[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

[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.

[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*, *Asergillus* 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 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 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 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*)).

[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 and burns, malignant hematological disorders, and deferoxamine chelation-therapy in subjects receiving hemodialysis.

[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 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 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.

[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

[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. 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 glycosylphosphatidylinisotol (GPI) anchored predicted to act as ligands to GRP78.

[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.

[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 bertholetiae and R. microsporus.

[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*.

[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*.

[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.

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

[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).

[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 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).

[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) 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 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* 99-880 (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).

[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).

[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).

[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).

[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).

[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 bertholetiae* (exons only) (SEQ ID NO: 25).

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

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

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

[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).

[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 ($2x10^8$), 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 ± 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 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 μg/ml anti-CotH3 or with serum from the same rabbit prior to vaccination (control) for 1 hour prior to addition of *R. oryzae* germlings. 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-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 ± interquartile range.

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

[0067] Figure 58, panels A-C, show inhibition of CotH2 and CotH3 expression 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 x 10^3, 2.8 x 10^3, and 2.5 x 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 and brain fungal burden of diabetic ketoacidotic mice (n=9 per group) infected intratracheally with wild type (1.7 x 10^3), empty plasmid (3.0 x 10^3) or RNA-i (3.1 x 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 ± interquartile range. * $P<0.001$ compared to wild type or empty plasmid infected mice by 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 ± SD. * $P<0.001$ vs. wild type or empty plasmid.

[0068] Figure 59 shows histopathological examination of lungs harvested from 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 (control) 2 hr prior to infecting intratracheally with 2.4 x 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 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 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 (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 (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 µl of blood) , A: *A. fumigants*, C: *C. albicans* each used to spike 350 µl of blood at 10^5 cells.

[0073] Figure 64 shows the specificity of the CotH3 molecular beacon probe in 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 10^3 spores.

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. cerevisae*, the *S. cerevisae* 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 Mucorales 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 Mucorales 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 Mucorales CotH but does not include proximal genes that do not encode Mucorales 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-seudaticae*, *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 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 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 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 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 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 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% polyvinylpyrolidone, 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 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 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 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 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 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

= 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. 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 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).

[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, 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 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 oligonucleotide disclosed 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.

[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 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 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.

[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 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 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 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.

[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 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.

[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% 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 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 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 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 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 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, 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 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 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 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 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 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 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 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 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 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 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 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 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; 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.

[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 transducted, transfected or otherwise genetically modified. Exemplary host cells that can be used to express recombinant Mucorales CotH molecules 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*.

[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 (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 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. 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 unrearranged human immunoglobulin gene, see, for example, U.S. Patent No. 5,877,397. 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 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 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.

[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, Duncan E.S. Stewart-Tull (ed.), John Wiley & Sons Ltd, ISBN 0-471-95170-6, and also in "Vaccines: New Generationn 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.

[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 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.

[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, 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.

[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 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.

[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 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 found described in, for example, Merck Manual, Sixteenth Edition, 1992, and Spellberg et al., *Clin. Microbio. Rev.* 18:556-69 (2005).

[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, 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 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 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.

[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 sporotichosis), and systemic mycoses (i.e., deep-seated 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 (hyalohyphomycoses), blastomycosis, penicilliosis or sporotrichosis.

[00142] As used herein, the term “zygomycosis” is intended to mean a fungal 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, rinocerebral 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 μg 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. 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 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 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, 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 polypetide. 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.

[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 be particularly useful when targeted to a muscel 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 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 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); 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.*, 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 exparssion 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); metalothionein promoter induced by heavy metals; insect 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 mammory tumor virus (MMTV) induced by steroids such as glucocortocoid and estrogen (Lee et al., *Nature*, 294:228-232 (1981); and heat shock promoters inducible by temperature changes.

[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 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, 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 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 monoclongal antibodies having specific reactivity with a Mucorales CotH polypeptide, an immunogenic fragment thereof, or functional fragment thereof.

[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 x 10^5 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 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 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 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, 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 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 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, 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 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 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 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 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, 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 constrict 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.

[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 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.

[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 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.

[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.

[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 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. 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), 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.

[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 seqeunce 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 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 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 μm ID C18 pre-Trap column and washed for 10 min with 100% Buffer A (2% acetonitrile containing 0.1% formic acid) at a flow rate of 5 μl/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 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 (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 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) 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:

- 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 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);
- Penicillium marneffei ATCC 18224 (XP_002144175);
- Aspergillus niger (XP_001392236);
- Aspergillus nidulans (XP_658934);
- Ustilago maydis (XP_760027);
- Coccidioides immitis (XP_001243211);
- 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 facilitated by CotH3, or CotH2 binding to GRP78.

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

CotH 3	R. oryzae 99-880	R. oryzae 99-892	Mucor 99-932	Absidia Corymbifera	Cunninghamela bertholetiae	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 %
Cunninghamela bertholetiae	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 Ca^{2+} and Mg^{2+}).

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 SalI*. 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	AAAAAACCCCGGATCCTATGAAATCCCTACTTTTTGTTGTATTC	RT-PCR and to clone CotH1 in to expression vector pESC-Leu
CotH1-R	TCTGTTCCATGTCGACCTAGAAGAAAGAGGCAAATAAAGTGC	RT-PCR and to clone CotH2 in to expression vector
CotH2-F	AAAAACCCCGGATCCTATGAAATTATCACTCACTATAGTATCCTCT	RT-PCR to clone CotH3 in to expression vector
CotH2-R	TCTGTTCCATGTCGACTTAAAAGATAGCAGTGGCAACTAAAG	RT-PCR to clone CotH3 in to expression vector
CotH3-F	AAAAAACCCCGGATCCTATGAAATTATCTATTATATCCGCTGCC	RT-PCR to clone CotH3 in to expression vector
CotH3-R	TCTGTTCCATGTCGACTTAGAATACAAGGAGAGCTAAAGCG	Detection in mucorales
Ligand#4-F RO3G_16295	AAAAAACCCCGGATCCTATGATTGCTACCCCTTTTGAAA	RT-PCR
Ligand#4-R RO3G_16295	TCTGTTCCATGTCGACTTAAAAGAAAATAAAGAATGTTGCAGC	RT-PCR
CotH3-F-ORF	ATGAAATTATCTATTATATCCGCTGCC	Detection IN OTHER MUCORALS1.9Kb
CotH3-R-ORF	TTAGAATACAAGGAGAGCTAAAGCG	Detection IN OTHER MUCORALS1.9Kb
RNAi-cotHF-F	GCATGCTAGAACAGAAGAAAGTTTTGATCGTTC	RNAi-forward
RNAi-ccoHF-R	GTACGACGTTCACGAATCTGTGTAGG	RNAi-forward
RNAi-I-F	CCGCGGGACGTTCACGAATCTGTGTAGG	RNAi-Reverse
RNAi-I-R	GCTAGCAGAACAGAAGAAAGTTTTGATCGTTC	RNAi-Reverse
CotH1-F	CAAACAAATGATGGGGCCTA	qRT-PCR for Expression
CotH1-R	CGTTTTTGTTCAAGATTTACACCA	qRTPCR for Expression
CotH2-F	CCTAATAAGGACAACGCAAACG	qRT-PCR for Expression
CotH2-R	TTGGCAATGGCTGTGTTATC	qRT-PCR for Expression
CotH3-F	GCCAATCCTAATGGTGAAGC	qRT-PCR for Expression
CotH3-R	CATGAAACGGTCGAGATCAA	qRT-PCR for Expression

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RO Actin-F	AGCTCCTTTGAACCCCAAGT	qRT-PCR for Expression
RO Actin-R	ACGACCAGAGGCATACAAGG	qRT-PCR for Expression
RO 18sRNA-F	GCGGATCGCATGGCC	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 contract (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 10^4 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 confluency in 96-well tissue culture plates (Costar, Van Nuys, CA) on fibronectin (BD Biosciences). All incubations were in 5% CO_2 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 x *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-ß-D-glucopyranoside (w/v) (Cal BioChem) and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 µg/ml pepstatin A, 1 µg/ml leupeptin, and 1 µg/ml aprotinin) (Sigma). The cell debris was removed by centrifugation at 5000 x *g* for 5 min at 4°C. The supernatant was collected and centrifuged at 100,000 x *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 x 10^8) expressing CotH1, CotH2, CotH3, or empty plasmid were incubated for 1 h on ice with 250 μg of biotin-labeled endothelial cell surface proteins in PBS-CM plus 1.5% n-octyl-ß-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 confluency. 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 $Na_2{}^{51}CrO_4$ (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: [(experimental release x 2)-(spontaneous release x 2)]/[total incorporation -(spontaneous release x 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 (≥20g) (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* (*delemar*) 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 none-adherent none 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 reduce 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 µl (per reaction) in MicroAmp® 96-Well Reaction Plate (Applied Biosystems) and contained 5.4 µl of sterile water, 0.4 µl of each primer, 0.2 µl probe, 4 µl of 5 x NASBA buffer. Then 5 µl 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 µl 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. fumigants* 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. 10µl of samples containing 10 to 10^5 of germlings were aliquoted into 250 µl of sheep blood. Total RNA was isolated from the spiked blood samples with RNeasy Plant Mini Kit (Qiagen) and eluted in 30 µl elution buffer. Five microliter of the total RNA was added to each NASBA reaction. Applification 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 µl of YPD broth each. 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. No amplification products were detected in samples inoculated with 10^6 spores of *A. fumigatus* or *C. albicans*, whereas each sample inoculated with 10 to 10^4 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 AAACGTACCTGCTGACCGAATC (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 AAACGTACCTGCTGACCGAATC (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 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.

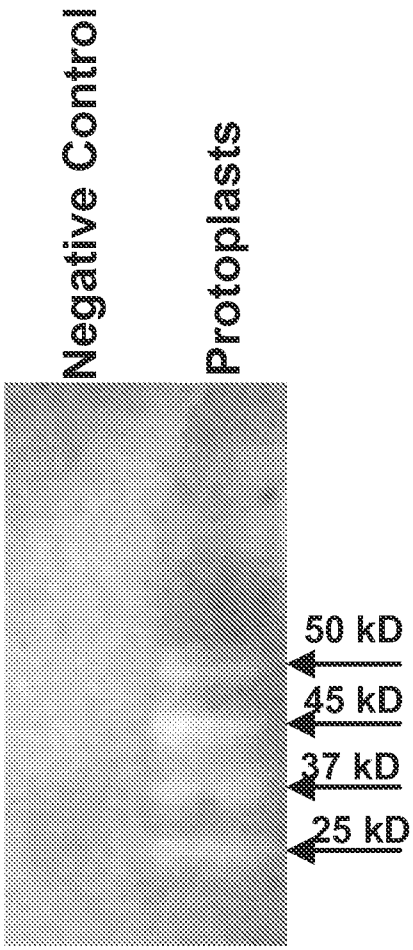


FIGURE 1

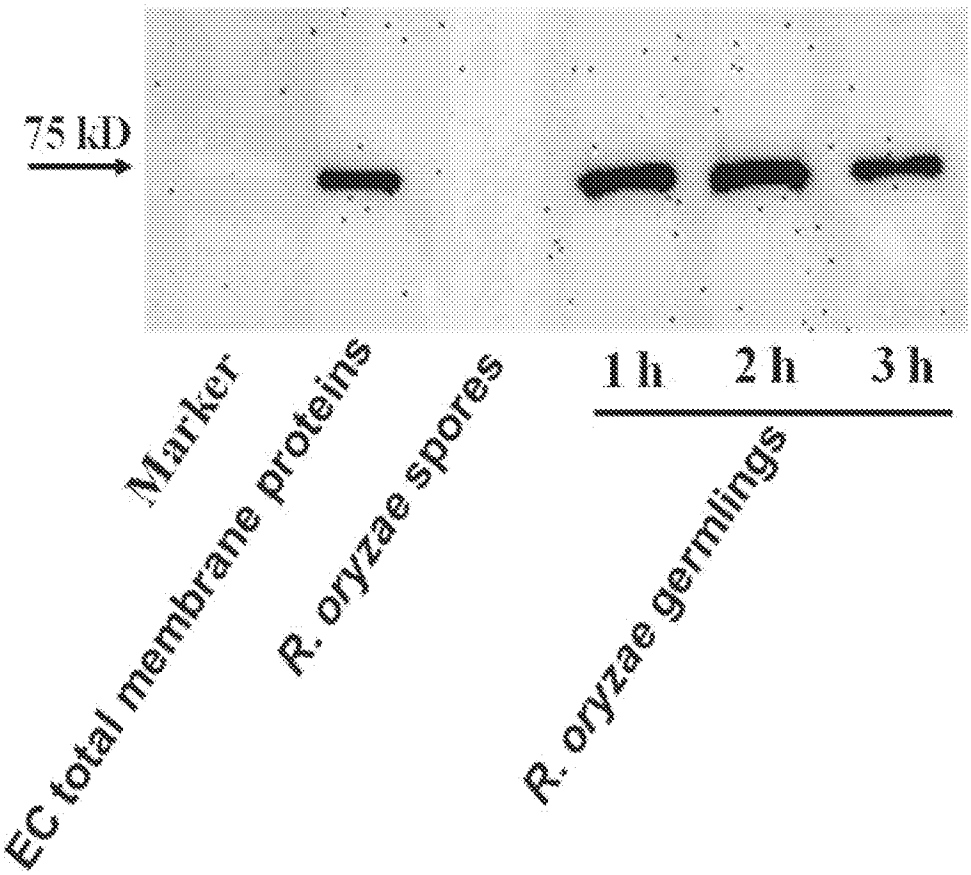

75 kD
Marker
EC total membrane proteins
R. oryzae spores
1 h
2 h
3 h
R. oryzae germlings

FIGURE 2

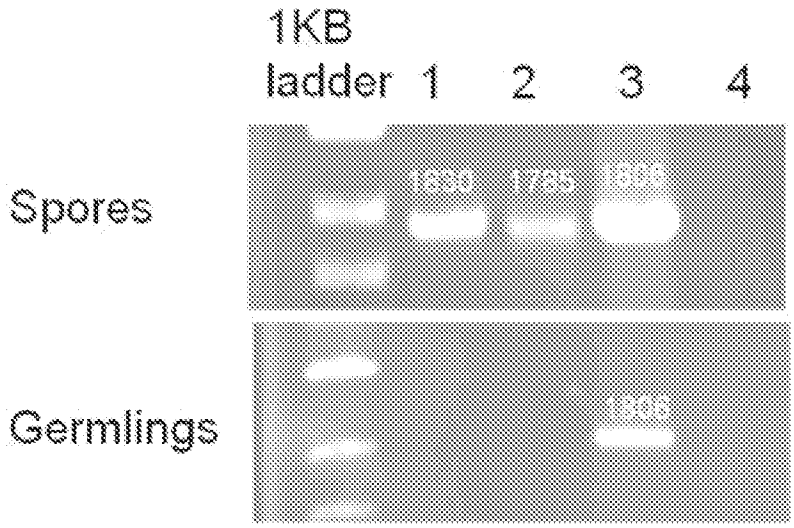
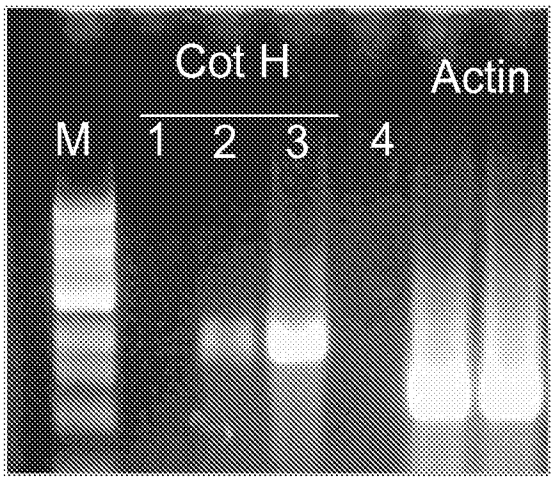


FIGURE 3

A.


Cot H
Actin
M
1
2
3
4

B.

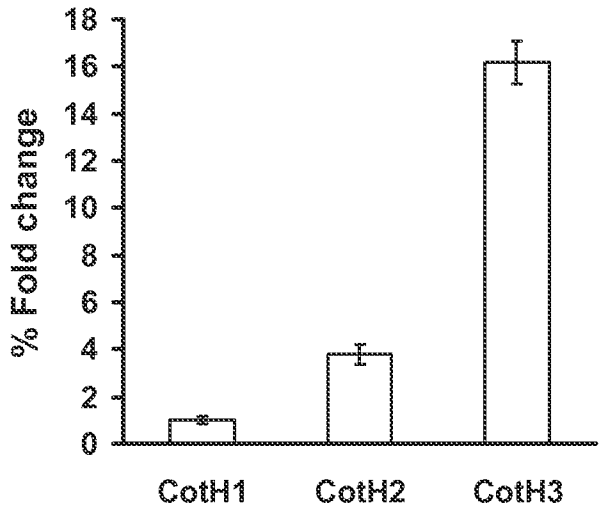

% Fold change
18
16
14
12
10
8
6
4
2
0
CotH1
CotH2
CotH3

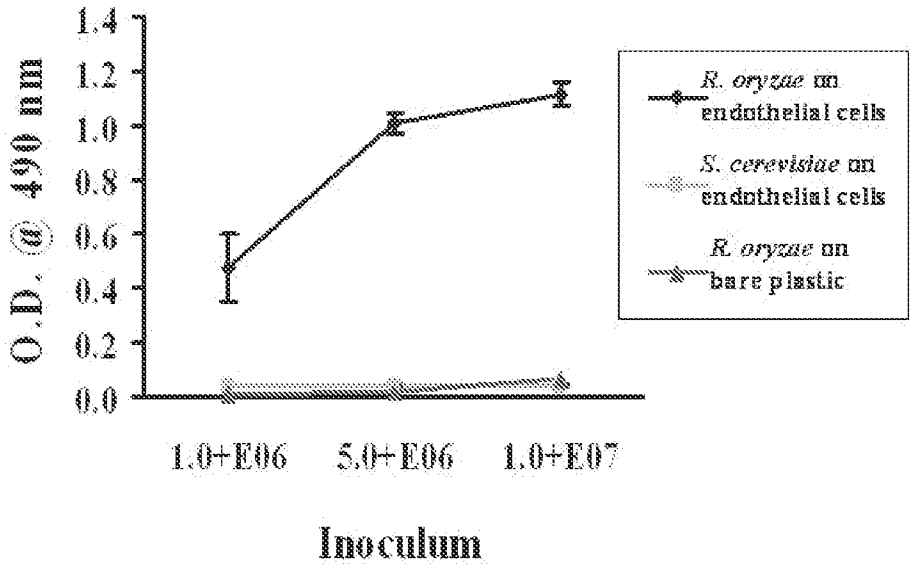
FIGURE 4

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

A.


O.D. @ 490 nm
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0+E06
5.0+E06
1.0+E07
Inoculum
R. oryzae on endothelial cells
S. cerevisiae on endothelial cells
R. oryzae on bare plastic

B.

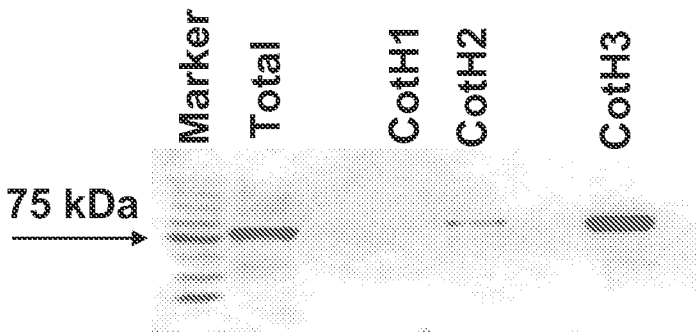
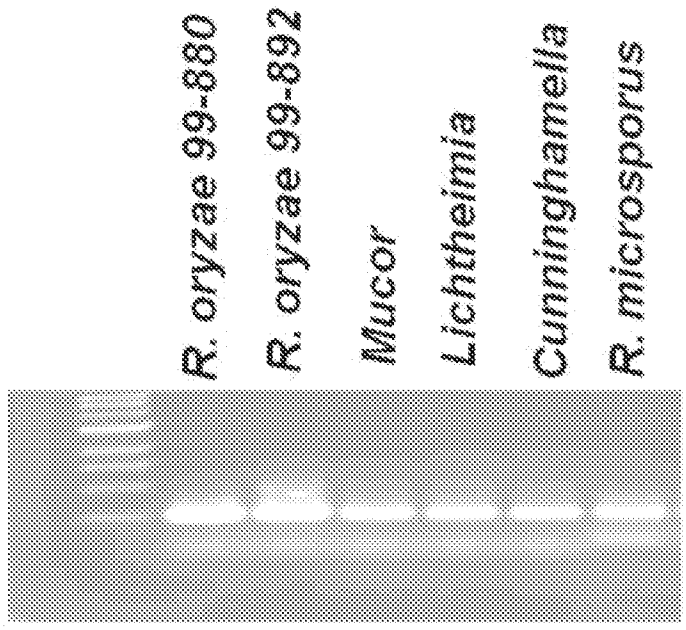

Marker
Total
CotH1
CotH2
CotH3
75 kDa

FIGURE 6



> 90% homology at the nucleotide and amino acid levels

FIGURE 7

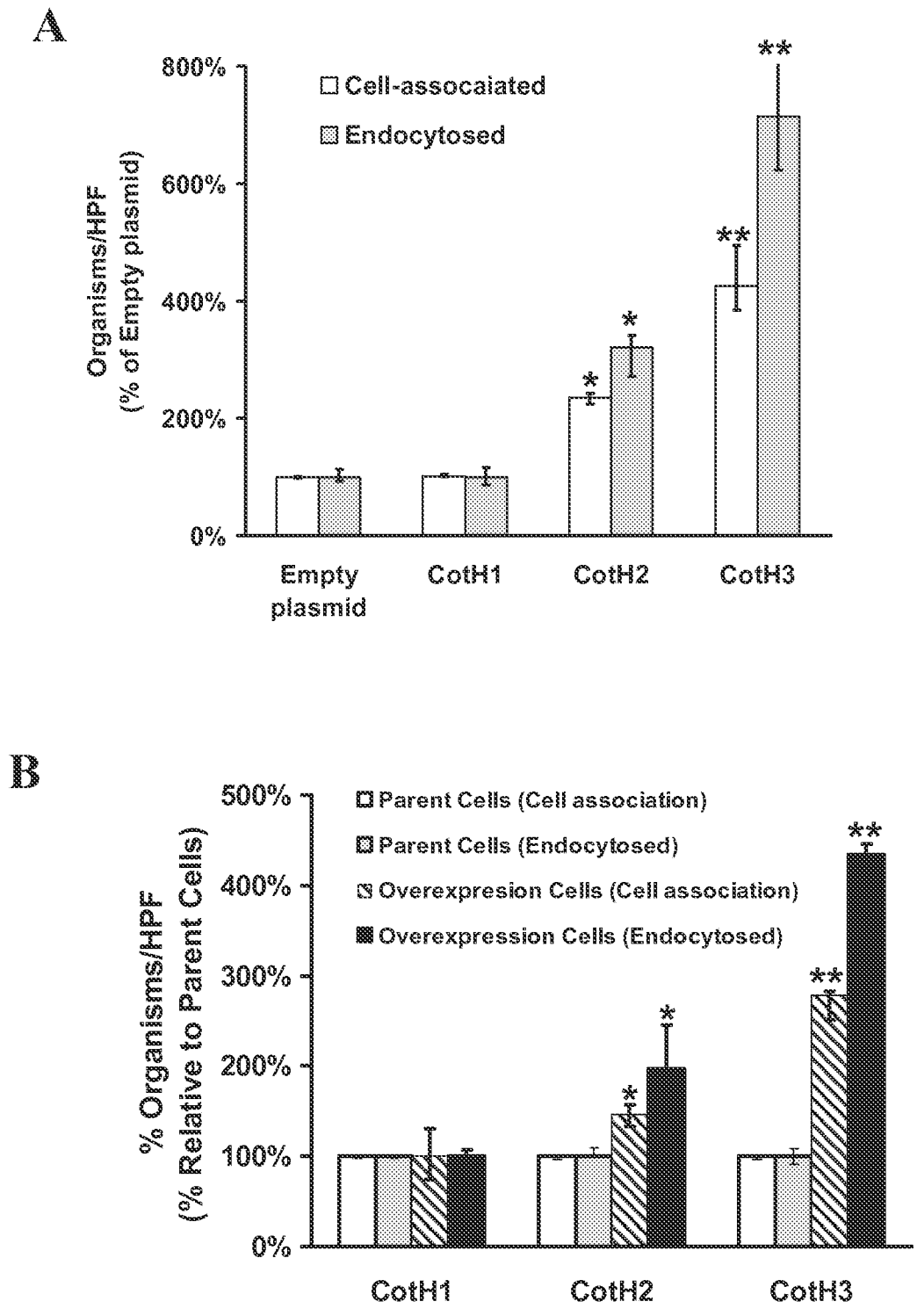

A
800%
600%
400%
200%
0%
Organisms/HPF
(% of Empty plasmid)
Cell-assocaiated
Endocytosed
*
*
**
**
Empty plasmid
CotH1
CotH2
CotH3
B
500%
400%
300%
200%
100%
0%
% Organisms/HPF
(% Relative to Parent Cells)
Parent Cells (Cell association)
Parent Cells (Endocytosed)
Overexpresion Cells (Cell association)
Overexpression Cells (Endocytosed)
**
*
**
**
CotH1
CotH2
CotH3

FIGURE 8

>CotH1 (RO3G_05018) (mw=68.77)

MKSLLFVVFIFLTTTYAAKVSFKVIAPDAKNRVHVNINGVLVELKASDPDVPYYTGFAELKHGQSY
NYVVDGNAEPFKRLLNGSSTKNEFFNRPVTYATNIPELPSILTEGSWTRGDTSNPIWDSNYVPSIF
VTGNPREMNELIENVKKNTYKTKITFIGPETINTFEGCTLGLHKPGRKHNDAKQSWIWALPEGQFM
ANRNWFKIRHMEEDPTQLREKLYADILRKMGTYANEANMVRFFINKEGMGIFNMLDDVIMYSYINA
MFYHGDTPEQLGGLYDGASGASFNFPGDFDSFIPNVESPLDQDAIEPFSKAFTSIDFLEDEQVKTI
GKYFDYDQFLRFMVMEFLTGDWDGYWQEQTNDGAYIDINDHNKIYYLGQDFDATFGVNLEQKREFV
NVSYTEYPKLFPGGVLINRLLQNPGVKKTFENYLKITVQEIFNNATLGPYVTARHEFLAPDLQWDR
SIKQRSPGNIFGWTFEQTYENLFEGVTAPGKNSGGADWGLLEWVAAKEKAVKSYLSSSEAADAATV
TQVPEAPGTDGTPSESTAWPHANTRFRQAEASNTHKIGTSSPSNFIVKIKQGTVSSSSSIKRTPCI
LPLVILASTLFASFF*

Coding sequence (exons only):1830nt

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AAGGCCAGTGATCCAGATGTTCCTTACTACACCGGTTTTGCTGAACTAAAGCATGGACAAAGTTAT
AATTACGTTGTCGATGGAAATGCAGAGCCATTCAAACGTCTATTGAATGGCTCTTCTACTAAAAAC
GAGTTCTTCAATCGACCTGTAACCTACGCTACCAACATTCCCGAGCTACCCAGCATTCTTACTGAA
GGTAGCTGGACTCGTGGAGATACGAGTAATCCTATTTGGGATAGTAACTATGTCCCTTCTATCTTT
GTCACTGGAAATCCAAGGGAAATGAATGAATTAATTGAGAATGTGAAAAAGAACACATATAAAACA
AAAATCACTTTTATTGGACCTGAGACTATCAACACATTTGAGGGTTGTACCCTTGGACTTCATAAA
CCTGGGCGTAAACATAATGATGCTAAACAATCTTGGATATGGGCTCTCCCTGAAGGTCAATTTATG
GCGAATCGAAATTGGTTTAAGATTCGACATATGGAAGAAGATCCTACACAACTTCGTGAAAAGCTT
TATGCAGATATCCTTCGAAAGATGGGAACCTATGCGAATGAGGCCAATATGGTTCGATTCTTTATA
AACAAGGAAGGCATGGGTATCTTTAATATGTTGGACGATGTTATTATGTATTCTTATATTAATGCC
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TTCAATTTTCCTGGTGACTTTGATAGCTTCATCCCGAATGTCGAATCCCCGCTTGACCAAGATGCT
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GGAAAGTATTTTGATTATGACCAATTCCTTCGTTTCATGGTCATGGAGTTCCTTACCGGTGACTGG
GATGGCTACTGGCAAGAGCAAACAAATGATGGGGCCTATATTGATATCAACGACCACAACAAAATT
TATTACTTGGGGCAGGATTTTGACGCCACCTTTGGTGTAAATCTTGAACAAAAACGAGAGTTTGTC
AATGTGTCTTATACTGAATACCCCAAACTGTTTCCTGGAGGTGTCTTGATCAACAGACTTCTTCAA
AACCCAGGTGTCAAGAAAACGTTTGAAAACTATCTGAAAATCACAGTACAAGAGATCTTCAACAAC
GCCACGCTCGGCCCCTATGTCACTGCTCGCCACGAATTCCTTGCTCCAGATCTTCAGTGGGATCGT
TCGATAAAACAACGGTCTCCTGGAAATATCTTTGGTTGGACGTTCGAGCAGACATATGAAAATCTA
TTTGAAGGTGTCACTGCTCCTGGAAAAAACTCGGGTGGTGCTGATTGGGGTCTTCTTGAATGGGTG
GCTGCAAAGGAAAAAGCAGTCAAAAGTTACCTCAGTTCGTCTGAAGCCGCTGATGCTGCCACTGTT
ACGCAAGTACCAGAAGCTCCTGGTACAGATGGCACTCCTTCCGAATCAACTGCCTGGCCTCATGCC
AATACAAGGTTCAGACAAGCCGAAGCTTCTAATACTCATAAAATAGGCACCTCATCGCCTTCTAAT
TTTATTGTTAAAATCAAGCAAGGTACTGTGTCATCGTCTTCATCTATCAAAAGAACCCCATGTATT
CTACCTCTTGTTATCTTG GCTAGCACTTTATTTGCCTCTTTCTTCTAG

FIGURE 9

>CotH2 (RO3G_08029) (mw=65.26)

MKLSLTIVSSSFLVAIAHAASVQFNLIAPSATDVKVSVNGQQVALTASDPNVPYFTGSAEVGGTEE
SFERSLAGITNSTFNDFYNRPVTYANLPQLPWPIENDPQWTRKGKKAEIFDDNYIPSVFFHGDDSQ
VQDLVKNVPKDKVTGTLTFIGSNYVHSFANVSFGIHGAGKKHNNAKQSWKWTLSGTDTMGNRNHFK
LRHMEEDPTQIRERLYADILHAMGTYANETTMVRLFINGQGFGTFNMLDDITEFSYINAMFYGGNP
PATLGPLFDGASGADFIYHPGNLDGYSSWKPNKDNANGEGYEAFDPLCKAWNETDYTDNTAIANFE
KMFDTEHFLRFMVIEYLTAHWDGYWMGQTNDGAYRDPSDNNKWYFLDQDFDATFGVNLDVPENKDF
ISVSYKDFPSRYPAGVMANGLLQNADKKAKFEQYLTETVRVLFNNVTLTNRVLAIHNFLSPDLEWD
RSIVQQSPGTNFGWTFEQTSQNLWQGVSAPNNNGGGAEWGLVEYIAAKSQAMAKEFNITIVSEPVG
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*

Coding sequence (exons only):1785nt

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CTTACAGCTTCAGACCCTAATGTGCCTTATTTCACCGGATCTGCTGAAGTTGGTGGAACTGAAGAA
AGCTTTGAGCGTTCTCTCGCTGGTATTACAAACTCCACATTCAATGATTTTTATAACCGTCCTGTT
ACTTACGCTAACCTTCCTCAATTACCATGGCCAATTGAAAATGATCCACAATGGACTCGCAAAGGA
AAGAAAGCTGAAATATTTGACGACAACTACATCCCCTCCGTATTCTTTCACGGTGATGATAGCCAA
GTCCAAGATTTGGTTAAAAATGTGCCCAAAGACAAAGTTACTGGCACCTTGACTTTCATTGGGTCC
AATTACGTTCATTCTTTCGCAAATGTCTCCTTTGGAATTCATGGTGCAGGAAAGAAGCACAACAAT
GCAAAGCAATCCTGGAAGTGGACCTTGTCTGGTACTGATACTATGGGCAACCGTAACCATTTTAAG
CTTCGTCATATGGAAGAAGATCCTACACAGATCCGTGAACGTCTTTATGCTGATATATTGCATGCT
ATGGGAACCTATGCCAATGAGACCACTATGGTCCGATTGTTTATTAACGGTCAAGGTTTTGGTACC
TTCAACATGCTAGACGATATTACTGAATTTTCTTACATCAATGCCATGTTCTATGGTGGTAATCCT
CCTGCTACTTTAGGACCTCTATTTGATGGTGCAAGCGGTGCAGACTTTATTTACCATCCTGGTAAT
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GATCCTCTATGTAAGGCTTGGAACGAAACCGACTATACCGATAACACAGCCATTGCCAACTTTGAA
AAAATGTTTGATACCGAGCACTTTTTACGATTCATGGTGATTGAATATCTAACTGCTCACTGGGAT
GGTTATTGGATGGGACAGACCAACGATGGTGCTTATCGTGACCCAAGTGACAATAACAAGTGGTAC
TTTTTGGATCAAGATTTTGATGCCACATTTGGTGTCAATTTGGACGTTCCTGAGAATAAAGACTTT
ATCAGTGTCTCCTACAAGGATTTCCCATCTCGTTACCCTGCTGGTGTCATGGCCAATGGTCTCTTA
CAGAATGCTGATAAAAAAGCCAAGTTTGAACAGTACTTGACTGAAACTGTTCGCGTCTTGTTCAAT
AATGTCACTTTGACTAATCGTGTCTTGGCTATCCACAACTTCCTCTCTCCTGATCTTGAATGGGAT
CGATCCATCGTTCAACAGTCGCCTGGTACTAATTTTGGATGGACCTTTGAGCAAACTTCTCAAAAC
TTATGGCAAGGTGTCTCAGCCCCAAATAACAACGGAGGTGGTGCTGAGTGGGGCTTGGTTGAATAT
ATCGCAGCAAAATCCCAAGCCATGGCTAAGGAATTTAATATCACTATTGTCTCTGAACCTGTAGGT
CCTCCTGCTGCTAATGGAACTGCAACTTCTACTAATGATGGTGGTAACACTCATACCGCTGCCGGA
GAAAGTAAGCCTGCCTCAAGTTCTGAATCTTCCGGTTCGAAAATTGCTTCTCAAAGCGTATCAGGT
GCTTCCCGTTCTGCTGTATCTACCGTCTTATTAGGTGTTACAGCTTTAGTTGCCACTGCTATCTTT
TAA

FIGURE 10

>CotH3 (RO3G_11882) (mw=65.77)

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
YVAGGTEESFDRSLDGITNSTLNDFYNRPVTYANLPQLPWPIEKDPQWTRSGSKADIFDDNYIPSV
FFHGDDSQVQNVVKNVPADRISGTLTFIGSNYVYSFQNVSFGIHGAGKKHNNAKQSWNWILSGSDT
MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGTYANDATMVRLFINNQGFGTFNMLDDITQFSYIN
AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNGEAYEALDPLCKAWNETTYTD
NTAIANFEKMFDLDRFMRFMVIEYLTADWDGYWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNL
AAPEGNAFLDVSYKDFPSRYPGAVMINNLLQNADKKATFEKYLTETVRVLFNNVTLTNRVLALHNF
LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGVTAPNNNGGGAAFGLVEYIAAKAQAVAKEFNI
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ALALLVF*

Coding sequence (exons only):1806nt

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GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
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AATGATTTTTATAACCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAGCAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTT
TTTTTCCACGGAGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTTATTGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCAC
GGTGCTGGCAAGAAACACAACAATGCAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAACCGCAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGT
CTTTATTCTGACATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTT
ATTAACAACCAAGGCTTCGGTACCTTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
GCTAAATTTTATAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCA
GACTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCT
AATGGTGAAGCTTATGAAGCTCTTGATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGAT
AATACAGCCATTGCAAACTTTGAAAAAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATT
GAATACTTGACTGCCGATTGGGATGGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGAT
CCAACTGATAATAACAAGTGGTACTTTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTG
GCTGCACCCGAAGGCAATGCTTTTCTTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGC
GCTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACT
GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTC
CTCTTGCCTGATCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGG
ACATTTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGT
GCTGCTTTTGGTTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATT
TCTATCGTTTCCCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCT
GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAA
ACCTCGGCTCAAAGCACATCAGGTGCTTCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCC
GCTTTAGCTCTCCTTGTATTCTAA

FIGURE 11

>RO3G_16295 (mw=40.59)

MIATPFEMFQCQMYILCLVLIAFSFTCVNTQQLCNGYAEYCNKPYNSLTYLLTHNSYGYVSNPAAN
QLCPITTQLADGVRGIKLSAVKATNATTDGTITADSIYLCHTSCIILNAGPAVNTLRTIKEWVEQN
PNEVVTIMWNNVDAFDGNAFEAAYNASGIIEYSYQQPKKNYTWPTLGELIASGKRVINFGDTYYQQ
DLPWLLTEYDYVFETPYENHNESSFSCTIDRPQDPASPTEFLYVMNHFLYGSLQLGSLPIEIPQKG
IANTTNSDNSLMKQAKTCTEKFGRQPNFLEIDFYNLGDALKITAELNNVTYKGSGSLQCDTYAAQQ
ASSSSTDSSEAIQTISISSVSLLLTLIAATFFIFF*

Coding sequence (exons only):1098nt

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TACAACTCGCTCACCTACCTCTTGACACATAATTCATATGGTTATGTATCTAACCCTGCTGCTAAT
CAACTCTGTCCTATCACTACTCAACTTGCAGATGGTGTTCGAGGCATCAAGCTGTCAGCCGTTAAA
GCAACAAATGCAACTACAGATGGTACCATTACAGCGGACAGCATTTATCTTTGTCACACATCGTGT
ATTATATTAAATGCTGGTCCAGCAGTCAATACCCTTCGTACGATTAAAGAATGGGTCGAGCAAAAC
CCTAATGAAGTAGTGACAATCATGTGGAATAATGTGGATGCCTTTGATGGGAATGCATTTGAGGCT
GCTTATAATGCAAGTGGTATCATTGAATATAGTTATCAGCAACCTAAAAAAAAACTATACTTGGCCA
ACGTTAGGTGAATTAATTGCTAGTGGAAAAAGAGTAATTAATTTTGGTGATACTTATTATCAACAG
GATCTTCCCTGGTTATTAACAGAATATGATTATGTGTTCGAGACACCTTATGAAAATCATAACGAA
AGTTCATTTAGTTGCACTATTGATCGACCTCAAGATCCTGCCAGCCCAACCGAATTCTTATACGTC
ATGAACCATTTTTTGTATGGTTCACTTCAATTGGGTTCACTACCTATCGAGATACCTCAAAAAGGC
ATAGCCAACACAACCAACTCTGATAATTCGCTCATGAAACAAGCTAAAACATGTACCGAGAAATTT
GGTCGTCAACCCAACTTTTTAGAAATAGATTTTTATAACCTGGGTGATGCACTCAAGATTACTGCA
GAATTAAACAATGTCACCTACAAAGGTTCAGGAAGTTTGCAGTGTGATACATATGCTGCTCAACAA
GCAAGTAGTTCAAGTACAGACTCATCCGAAGCAATTCAAACAATTAGTATTAGTTCAGTTAGTTTA
CTATTAACTTTAATTGCTGCAACATTCTTTATTTTCTTTTAA

FIGURE 12

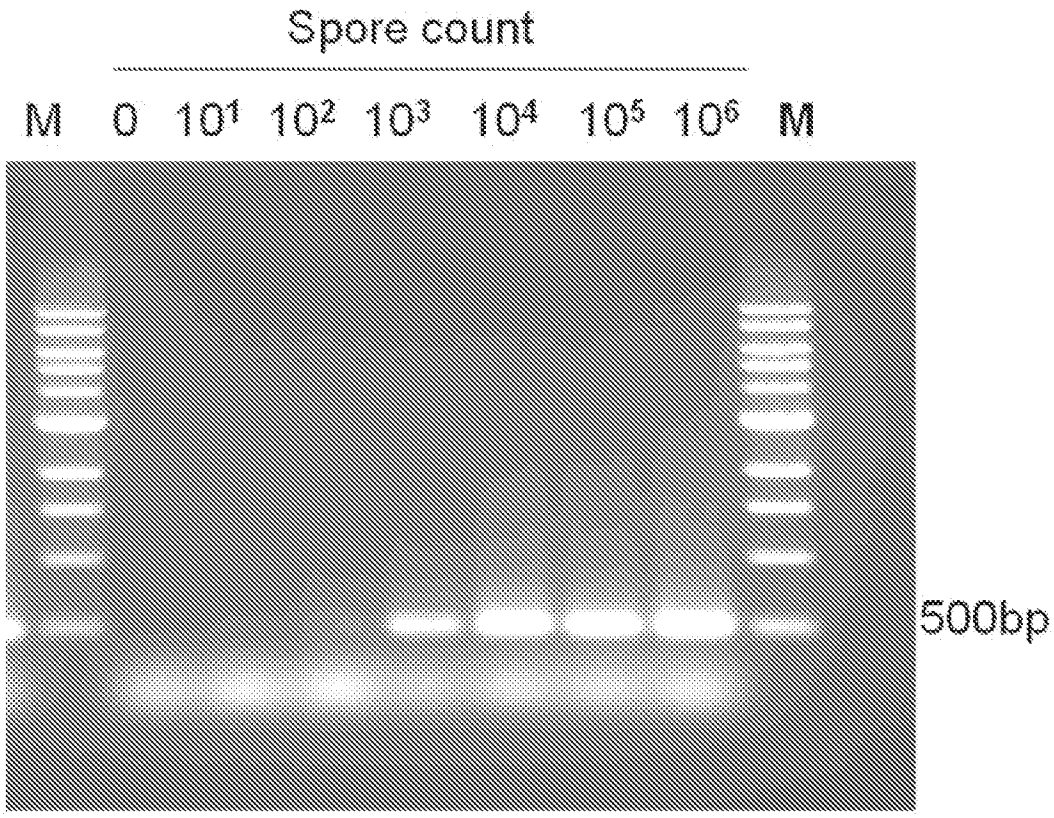


FIGURE 13

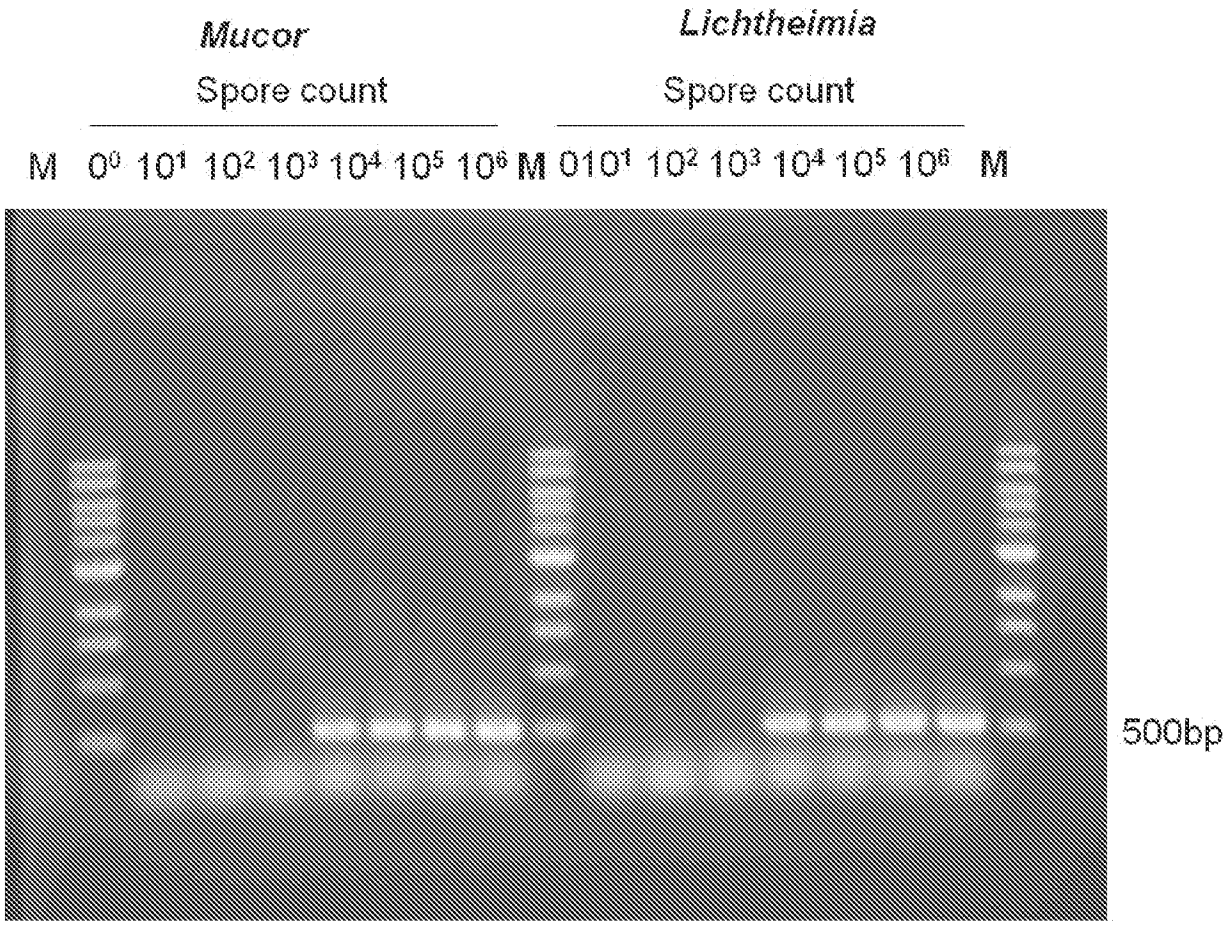

Mucor
Spore count
Lichtheimia
Spore count
M 0⁰ 10¹ 10² 10³ 10⁴ 10⁵ 10⁶ M 010¹ 10² 10³ 10⁴ 10⁵ 10⁶ M
500bp

FIGURE 14

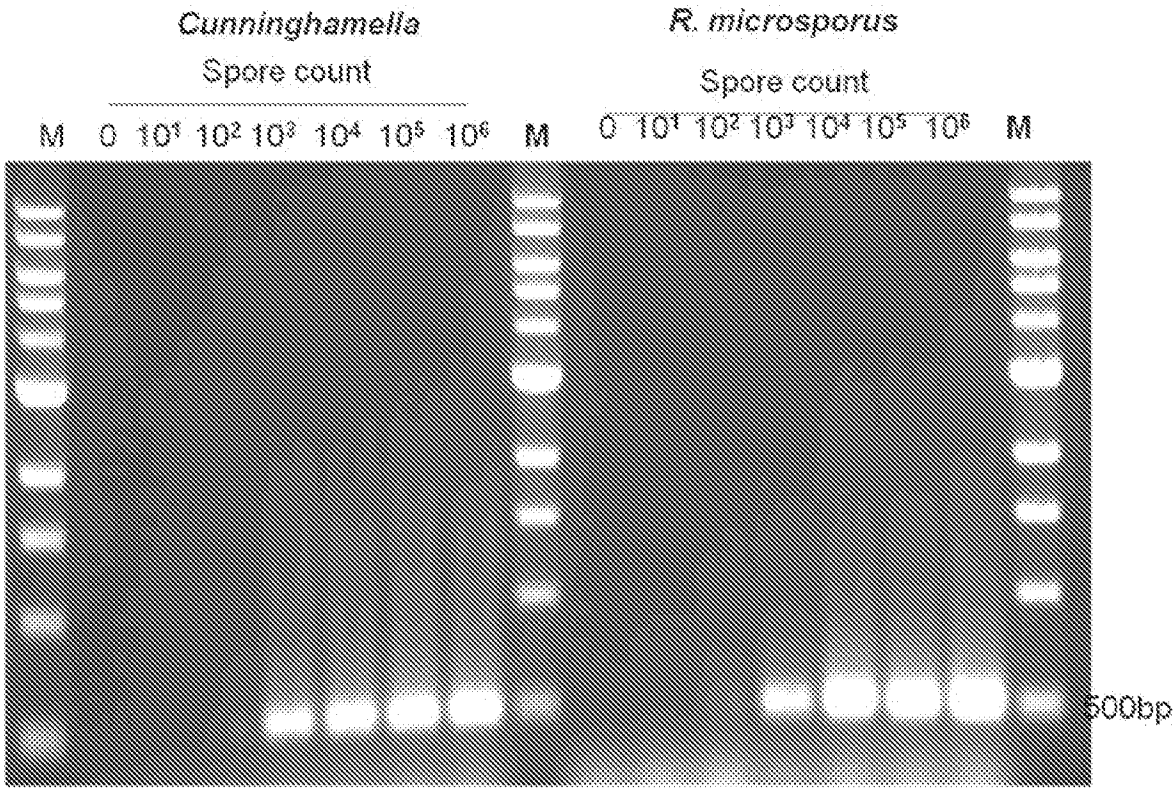

Cunninghamella
Spore count
M 0 10¹ 10² 10³ 10⁴ 10⁵ 10⁶ M
R. microsporus
Spore count
0 10¹ 10² 10³ 10⁴ 10⁵ 10⁶ M
500bp

FIGURE 15

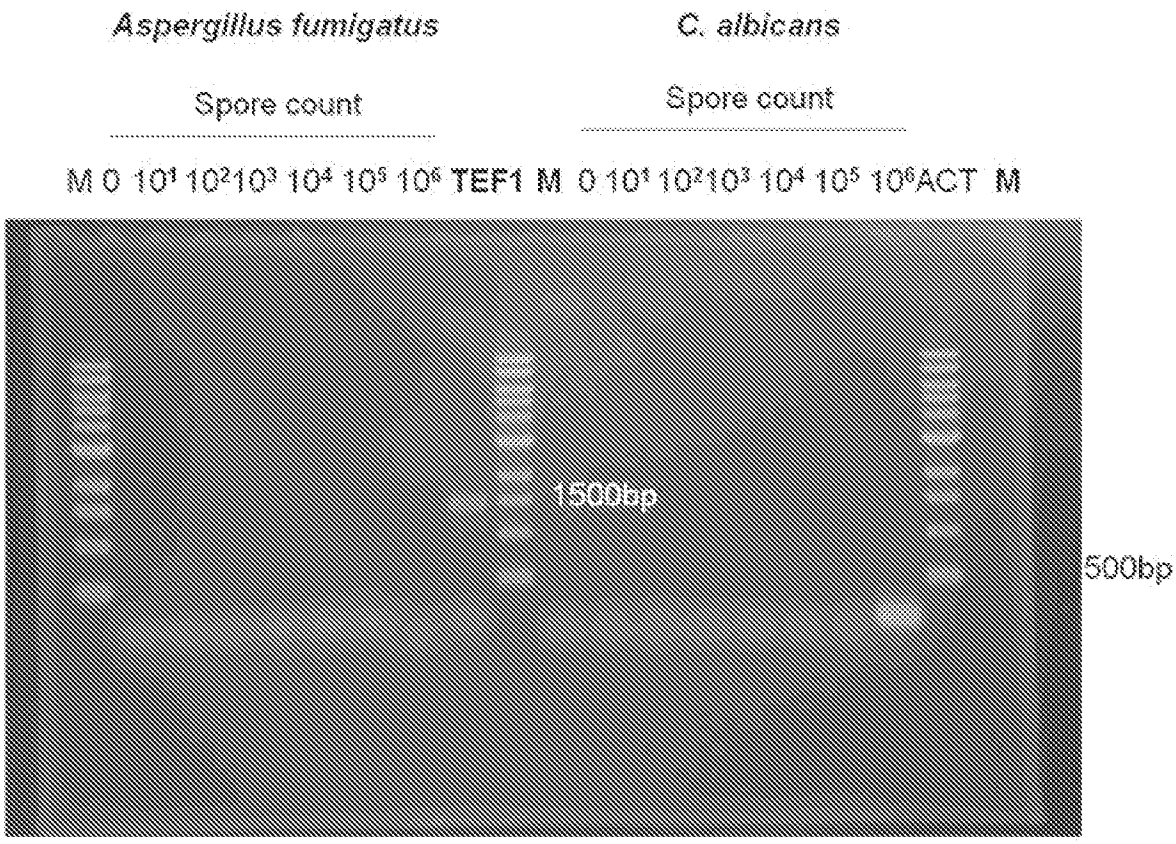
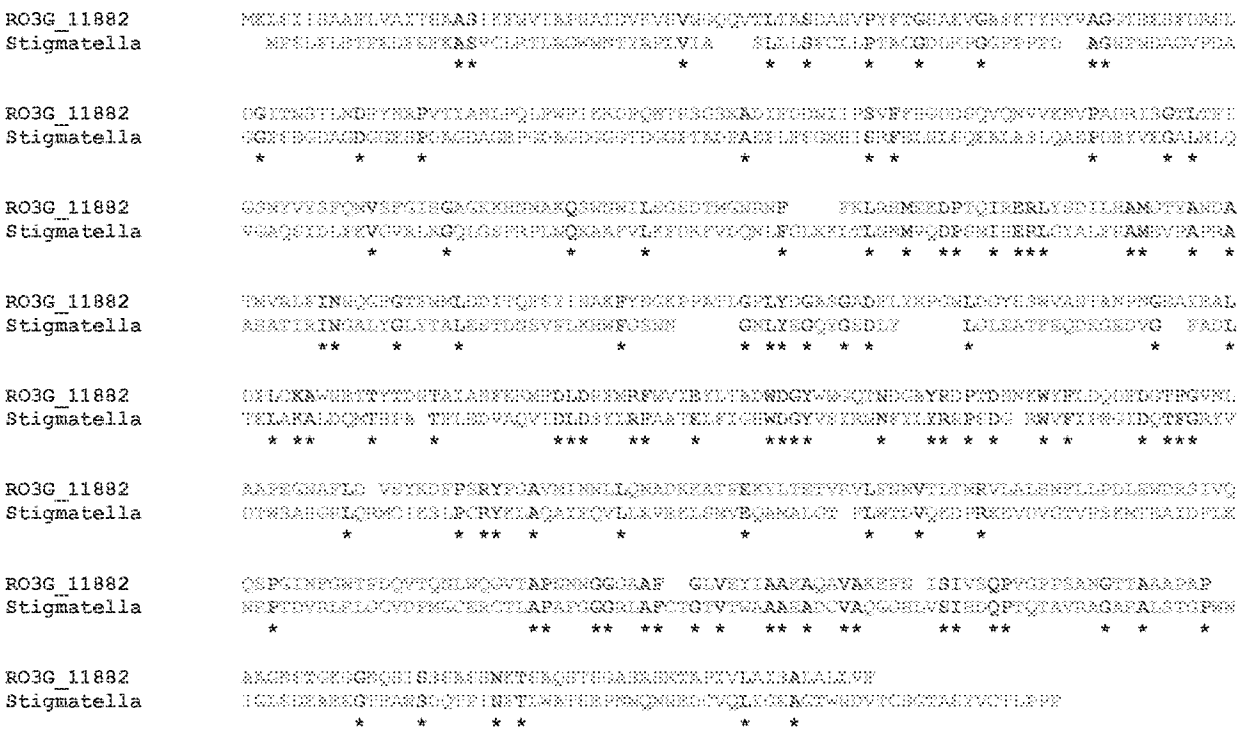

Aspergillus fumigatus
C. albicans
Spore count
Spore count
M 0 10¹ 10²10³ 10⁴ 10⁵ 10⁶ TEF1 M 0 10¹ 10²10³ 10⁴ 10⁵ 10⁶ACT M
1500bp
500bp

FIGURE 16



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

FIGURE 17

```
RO3G_16295
Talaromyces       MRGLWIVATLLTGALASTTTTASTTTSGATDTAAIVTLGGTVDFLGLRGATGSVTYPGVT

RO3G_16295                                                  MIATFFEMFQCQMYILCLVLIA
Talaromyces       TTITLSTPKDGETSTGTGTRSGNVTDAYTTTSGTVTMLVGSQGTSTLAPNATALRNSTAT
                                                                            *
RO3G_16295        FSFTCVNTQ QLCNGYAEYCNKFYNSLTYLLTHNS YGYVGNPAANQLCFITTQLADGVR
Talaromyces       TSTTFLPTNTQPCNGYVEFCARNYSNITYVAAHNSFFDRKGNIAGNQQYSVTTQLNDGIR
                   * *   *  * **** * *   *   **   ***      * * **    **** ** *
RO3G_16295        GIKLSAVKATNATTDGTIHADGIYLCHTSCIILNAGPAVNILETIESWVSQNFNEVVTIM
Talaromyces       MLQFQA     HLQNGTIR     LCHTSCDLLNVGPLEEYLTTVTPWLNNNPYEVITIL
                        *       ***       ******  ** **    * *   *   ** ** **
RO3G_16295        WNNVDAFDGNAFEAAYNASGIIEYSYQQPEKNYT   WPTLGELIASGKRVINFGD TYY
Talaromyces       MGNYDLVGVGNFTAPIINSGLSRYVYTPPKIPMCLNDWPVLSELILTQKRVTIFMDYNAN
                    * *       * *    **   * *  **       ** * ***   **** * *
RO3G_16295        QQDLPWLLTEYDYVFETPYENNNESSFSCTTDRPQD  PASFTEPLYVMNNFLYGSLQLG
Talaromyces       QTEVPYTLDEPTQMWETFF SPTDPAFPCTVQRPPNLGPESAKQILYMANNNLNVRIGPS
                  *   *  * *     ***        * **  **    * *    **  ** *
RO3G_16295        SLPIEIPQKGIANTTNSDN   SLMKQAKTCTEKFGRQPNFLEIDFYNLGDA    LKIT
Talaromyces       GLDLLIPNTAVLNETNGVSGYRSLGLMANSCTTTWGRFPNFLLVDYYNEGSSPGSVPEVA
                   *   **     * **       **   *  **   ** ****  * ** *
RO3G_16295        ARLMNVTYEGSGSLQCDTYAAQQASSSSTDSSEAIQTTSTSSVSLLLLTLIAATFFIPP
Talaromyces       ANMNNVTYNGN      CCGGNTSGALRLQTPDAVNMFVVAALSVLLCMN
                  *  ***** *                      *         * **
```

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

FIGURE 18

```
RO3G_16295.3
Penicillium         MPSLWMAVALLTGALVQSTTAASSSSGTLSSASDTLSAGTVTLSGTVDPLSIDGATGSVT

RO3G_16295.3                                                  MIATPFFSMFQCQMYLLCLVLIA
Penicillium         YFSVTTTTTLSTDSSTISGTVTPTTTDVTTTTVLVGSQAATILAPNATASINSTTATGTA
                                                                                  *
RO3G_16295.3        PSFTCVNTQQLCNGYAEYCNKFYNSLTYLLTRNS YSYVSNPAANQLCPITTQLADGVRS
Penicillium         TTAFLPTNTQPCNGYVSFCAPNYSNITYVAAHNSPFNKQGNIASNQQYFVTTQLNDGIRM
                          * **** * *   *   **   ***      * * **  * **** ** *
RO3G_16295.3        IKLSAVKATNATTDGTITADSIYLCHTSCTILNAGPAVSTLSTIKSWVEQNPNSVVTIMN
Penicillium         LQFQV          SLQNGSLYLCHTSCDLLNVGTLQLYLTTVTKWLNNNPIEVITLM
                                 * *******  ** *     * *   *   ** ** **
RO3G_16295.3        NNVDAFDGNAPRAAYNASGITRYSYQQPPKNY   TWPTLGRLIASGKRVTNPGD TYYQ
Penicillium         GNYDLIGVGNFTDFTVRSGLSKYAYQPPKITPWGLDDWPMLSELILIQKRAIIEMDYNANQ
                     * *      *      **   * ** **       ** * ***   ** * * *    *
RO3G_16295.3        QDLPWLLTRYDIVFETPYRDNNSSFSCTIDRPQDPASPT  RFLIVMNHFLYGSLQLGS
Penicillium         TEVFYILDEFTQWWETPF SPTDPWFSCTVQPPFNLSTRRAKSIMYWANHNLNVRLIFSG
                       *  * *     ***       * **  **          *  ** *
RO3G_16295.3        LPIEIPQPGIANTTNSD   NSLMKQARTCTRKFGRQPNFLEIDFYNLGD    ALFITA
Penicillium         IDILIPNTAVLNETNGVFGVKSLGLMANNCTATWGRPPNFLIVDYTNNGNFPGSVFQVAA
                    * * **     * **      **   *  **   ** ****  * ** *          *
RO3G_16295.3        ELNNVTYKGSGSLQCDTYAAQQASSSSTDSSEAIQTISISSVSLLLTLIAATFFIFF
Penicillium         EMNNVTYSGRCCRSMASGALRLSLFGHWMFAMAVSAFLFI
                    * ***** *          *             *
```

Total alignment length: 477
Overall identity :24.11 % (115/477)
Inner consensus gaps : 41
Sequence lengths:
RO3G_16295.3 365
Penicillium 449

FIGURE 19

```
RO3G_16295.3
Aspergillus       MRLIAMLLPLLAVGVWPGLAKDDSTTTTTTTGNWGGLTLEGTVTSSTSRATLPTGHTLSY

RO3G_16295.3                                                      MLATPFPMFQCQMY
Aspergillus       TTTMTLDDGHTVTSTGARSATTTSNSTTTSGNFTTTVTSSSQSLTLIVGGQTGGVNGTNA

RO3G_16295.3      LLGLVLIAFSFTCVNTQQLCNGYAEYCRKPYNSLTYLLTHNS YSIVSNPAANQLCPITT
Aspergillus       TTTATGTASSTSVVNTQP CNGMAEFCARKYGNITWVAAHNSPFVKPGNAAANQALKVTA
                    * *   **** *** ** *   *   *    ***       * ****    *
RO3G_16295.3      QLADGVRGLKLSAVKATNATTLGTITADSIYLCHTSCTILNAGPAVNTLPTIKEWVEQNP
Aspergillus       QLDDGIRMLQFQTHLVNN           TLYLCHTSCDLLNWGPLEEYLTTVTKWVKTHP
                  ** ** *          *            *******  ** **    * *   **   *
RO3G_16295.3      NEVVTIWWNNVDAFDGNAFRAAVNASGITEYSIQQPKKNYT   WPTLGELIASGKRVIN
Aspergillus       YDVVTILIGNYDTVDPGNFTGFMQNSGLMDIVFTPSKIFMALDLWPTMSSMILSGKPAVV
                    ****   * *  *   *      **   *     *       ***    * ****
RO3G_16295.3      PGDTYYQQDL PWLLTEYDIVFETPYTMRNNRSFSCTIDRPQDFASPT  EFLIVMWHFL
Aspergillus       FWDYQANQTAYFWLMDEFSQWMETPF SPTDAAFPCTEQRPFDLSAQDAFDSMYWANHNL
                  * *    *   ***  *     ***       * **  ** *          *  ** *
RO3G_16295.3      YGSLQLGSLPIEIPQEGIANTTNGDN   SLWKQARTCTEKFGRQPNFLEIDFYNLGD
Aspergillus       NLDINIASISLLIPNTASLNQTNAVSGYGSLGEMARNCTAWWLRFPNFLIVDIYNYGNIN
                    *    **      * **      ** * *  **    * ****  * ** *
RO3G_16295.3        ALKITAELMNVTYKGSGSLQCDTYAAQQASSSSTDSSSAIQTISTSSVSLLLFLIAAT
Aspergillus       GSVFSVAAEMNNVTWDG    KC CSAASAASSVMFSVSVMGTLLLIAGVQYWASIF
                    ** ****  *     *   **  ***     *       *  *
RO3G_16295.3      FFIFF
Aspergillus
```

```
Total alignment length: 485
Overall identity  :25.15 % (122/485)
Inner consensus gaps : 38
Sequence lengths:
RO3G_16295.3 365
Aspergillus 460
```

FIGURE 20

```
RO3G_16295.3
Aspergillus         MRLTWLLTLLAASRVLSQNTLSDSDSDSDSSTTTDSNREAISQSLAETASAITTTVDLAT

RO3G_16295.3                                                              MIAT
Aspergillus         VFSGDYIYYSTTVVLRGTHGTVIGSTAVQVTGTPNANATTSANATITSTSDTVTVLIGGQ

RO3G_16295.3        PFRMPQCQMYILCLVLIAFSFTCVNTQQLCNGYAEYCNKFYNSLTYLLTHNS YGYVSNP
Aspergillus         TTISGNSTGNSTHSATPSFSQTFVVNTQPCNGNFEFCLPKYENITQVAAHNSFFVAQGNV
                                    * * *   * ***  * *   *   *    ***      *
RO3G_16295.3        AANQLCPITTQLADGVRGTFLSAVKATNATTDGTITADSIYLCHTSCITLNAGPAVNTLA
Aspergillus         AANQALDVEYQLLDGVRMLQFQT            HIMNGTNYLCHTSCDLLNVGPLEDYLS
                    ****      ** ****                  *     *******  ** **    *
RO3G_16295.3        TTEEWVEQNPNENVVTINNNNNVDAFDGNAFEAAYNASGLIEYSYQQPYENY   TWPTLGE
Aspergillus         NITENLNQNFYDVVTILIGNTDYVDPGNFTTPMENSGLMDFVFTPPMIPNGLDDWPTLGS
                     * **  * *  **** * *  *  *      **       *       *****
RO3G_16295.3        LIASGKRVINFGDTYYQQDL PWLLTEYLYVFETPYENNNESGFSCTIDRPQDPASPT
Aspergillus         IILSGKRAIVFMDYQANQTAIPWLMEEFSQNWETPF EPTERDFPCTVQRPPLLAAEDAR
                     * **** * * *    *   ***  *    ***       * **  ** * *
RO3G_16295.3        EPLYVMNHFLVSGLQLGSLPIEIPQKGIANTTNSDN   SLMEQAETCTERFGRQPNFLE
Aspergillus         KRNTMANHNLNIDFSIASLNLLIPNTALLNETNADHGTGSVGRMAENCTTLWNRPPNFLL
                      *  ** *       **   **      * ** *    *    *  **    * ****
RO3G_16295.3        IDFYNLGD    ALKITARLNETVTYRGSGSLQCDTYAAQQASSSSTDSSRAIQTISISSV
Aspergillus         VDYYNEGNFNGSVFQVAADMNGVSYDPDSC  CGTLSAASSLGFGAMMSAVLFFVGLQVL
                     * ** *          *  * * *       * *  *          *
RO3G_16295.3        SLLLTLIAATFFIFF
Aspergillus         AWL
                     *
Total alignment length: 495
Overall identity  :23.43 % (116/495)
Inner consensus gaps : 38
Sequence lengths:
RO3G_16295.3 365
Aspergillus 470
```

FIGURE 21

```
RO3G_16295.3                    MIATPFRMPQCQMYILCLVLIAPGFTCVNTQQLCNGYADYCNKPYNSLTYL
Ustilago               MFQFIQLLSLVGALVIVSGLVGAVPHPVLDAAVETLVKRASVCNGDASLCSPLVSNVTYI
                                                ** *   * *     *** *  *   *   **
RO3G_16295.3           LTHNSYGVVSNPAA    NQLCPITTQLADGVRGIYLSAVKATNATTDGTTTADSIVLCH
Ustilago               GARNSYAVGTLAGASVGKNQEQSVTQQLTDGIRLLQVQARKGSNSTS     GSGINLCH
                         ****       *    **    * ** ** *     * *  * *          * ***
RO3G_16295.3           TSCIILNAGPAVNTLRTIKSWVSQNPNNVVTIMWNNVDAFDGNAFRAAYNASGLIEYSYQ
Ustilago               SSCQIDNGGTLENYLSEVKTWVDSNPNDVITILIVNSDNQPVSGFSTAFQSTGLASKAIS
                        ** * * *   * *   * **  *** * **   * *      *  *   *    *
RO3G_16295.3           QPKSNT   TWPTLGELIASGKSVINFGDTYYQ QDLPWLLTSYLYVFETPYENNNSGSF
Ustilago               PGTAALAKLSWPTLGSLIDSGRNLVVFIDNSADVSSVPYILPHSQNTWSNPY NQISVPF
                         ***** ** ***    * *       *  *       * ** *      *
RO3G_16295.3           SCTIDPPQSPASPTSFLYWNNFLYQSLQLGSLPIELPQYGIANTTNSDNSLWKQAKTCT
Ustilago               NCSVDRINSGSSPSNLWYLINHYLDSSPNLPGTTVFVPRTAQLNTTNSLSSIMTDAGNCA
                        *  **     *    *  ** *  *  *      *     *****  * *  *  *
RO3G_16295.3           SNFGR QPNFLSIDFYNLGDA  LKITAELNNVTYKSGSLQCLTYAAQQASGSSTDSSS
Ustilago               SLAGTGYPTTVLTDFYDVGDGSVFQAAAQMNGVQYTAKPIGNATKSGSSSGSGSSSGAA
                         *   *     ***  **       *  * * *                 * **
RO3G_16295.3           AIQTISISSVSLLLTLIAATFFIPF
Ustilago               STSVNAIAAVATFWTMFALASTLA
                         *  *    *  *
Total alignment length: 385
Overall identity  :29.35 % (113/385)
Inner consensus gaps : 17
Sequence lengths:
RO3G_16295.3 365
Ustilago 378
```

FIGURE 22

```
RO3G_16295.3
Coccidioides        MLLSFRLLAVASLLRSTYADEIITLTGTNIPPSLSVSDPTPSLTSGLYRSYSSVVTVSAT

RO3G_16295.3                                    MIATPFPMFQCQMYILCLVLLAFSFTCV   NT
Coccidioides        DKQLESAPTGTETATGSERTATSDGGELIIGSKRVSTTNGTTLSGNATATSTESAAVFTN
                                                                 *     * *
RO3G_16295.3        QQLCNGYARYCNKFYNSLTYLLTRNS YGYVSNPAANQLCPITTQLADGVRGIKLSAVKA
Coccidioides        TPPCNGYSEFCEPKYSNITHIAAHNSFVKPGNIAGNQELAVTIQLNDGIPMLQFQT
                      **** * *   *   *    ***      * * **    * ** ** *
RO3G_16295.3        TNATTDGTITADSIYLCHTSCITLNAGPAVNTLKTIKEWVEQNPNEVVTIMWNNFYAPDG
Coccidioides          NTINGTIR     LCHSSCDLLDVGPLEDYLRKVADWLRANPYDVVSILMGNSNFILP
                        ***       *** **  *  **    **    *   **  ** *   *
RO3G_16295.3        NAFRAAYNASGITEYSYQQPKNYT   WPTLGELIASGKRVINPGDTYYQQ DLPWLLT
Coccidioides        TNYTEFIENSGLILDYVTTPPKIPMALDDWFLLSNFLITGQRAIVYLDYRANQTEVPYLLD
                      ** * * *  **       ** *   *  * * *   *    *   * **
RO3G_16295.3        EYDYVFETPYENNNESSFSCTIDRPQDPASPT  EFLYVMNHFLYGSLQLGSLPIEIPQF
Coccidioides        EFSQNWETPFSETNKD FFCVVKPPPGLGARDAKNFLYMANENLNTEVELAGASLAVPNT
                    *     ***    *   * *   **          **  ** *    *       *
RO3G_16295.3        GIANTTNSDN   SLMKQAETCTEKFGRQPNFLEIDFYNLGD    ALKITAELMNVTYK
Coccidioides        VLLNETNAVSGTGSAGAMAGNCTEQWTRPPNFTLVDYTNTGNFNGSVFEVAAWCNNVTYN
                      * **      *    *  ***   * ***   * ** *          *  *****
RO3G_16295.3        GSGSLQCDTYAAQQASSSSTDSSEAIQTISISSVSLLLTLIAATFSTFF
Coccidioides        EKCCGK QTSAASKGLSSGAKQSFFVGLIATITTSLLFTLF
                      * **    **    *           *** **
Total alignment length: 469
Overall identity  :23.88 % (112/469)
Inner consensus gaps : 36
Sequence lengths:
RO3G_16295.3 365
Coccidioides 449
```

FIGURE 23

```
RO3G_16295.3                                                    MIATPPEMFQCQWTILCLVLIAFSPT
Neurospora          MFSLISSLATALLLVSGICATPQGPSGAESGIVSAVSAAAVSTAGVAVSQATTASPSTSN

RO3G_16295.3        CVNTQQLCNGYAEYCNYPYNSLTYLLTENSYGYYSNPAANQLCPITTQLADGVAGILKLSA
Neurospora          AASGISACNNSPLICDRAYNNVTRWGANDSSFLRDASTSDSLAGNQYFNATVALDAGLRL
                      **      *   **  *    * *          *       *
RO3G_16295.3        VKATNATTDGTITADSIYLCHTSCIILMASPAVNTLNTIKENVEQNPNEVVTIMWNNVDA
Neurospora          LQAQVHEVNGTLQ     LCHTSCSLLDAGPLQLWLAKIKFWNDNNPNEVVTLLLVNSDN
                      *      **       ******  * ***    *  ** *   ********   * *
RO3G_16295.3        FDGNAFEAATNASGTIEYSYQQPR   KNYTWPTLGSLIASGKRVINF SDTYYQQDLPW
Neurospora          KLVSDYAAVSEGSGISTYGVQLSNGSSASNTWPTLGDMITSNKRLVTFIASIDYSPTYPY
                      *    ***  * **          ******  * * **   *     *    *
RO3G_16295.3        LLTEYDYVFETPYENNNSSFSCTIDREQDFASPTE     FLYYNNHFLYGSLQLGSLP
Neurospora          LLSEFDHVFETAYNVLSLSGFNCTIDPPKGQGSAGDAISAGLMFLNNHFADSLLIQGVQI
                    ** * * **** *     * * ** ***    *              ****    *  *
RO3G_16295.3        IEIPQKGIANT  TNSLNSLMYQANTCTEYPGKQPNFLEIDFYNLGDALEITAELNNVTY
Neurospora          PDETDIIITNSEDTETTGNLGLMAQTCVEQWGVKPTFILVDFFQHGPAIDTALPLNGITA
                      * *   *     *   * **    *  * *   **   * *       **  *
RO3G_16295.3        EGSGSLQCDTYAAQQASSSTLSGRAIQTTSISSVSLLLTLIAATFFIFF
Neurospora          TGPRSVSGES KGNTSGAGSNHSFMGMNVALIAFVVFALAMV
                     *  *                *        *  *   *
```

Total alignment length: 410
Overall identity :25.12 % (103/410)
Inner consensus gaps : 24
Sequence lengths:
RO3G_16295.3 365
Neurospora 396

FIGURE 24

```
RO3G_16295.3        MIATPFEMFQCQMIILCLVLIAFSPTCVNTQQLCNGYAETCNFPYNGLTYLLTHNSYGIV
Cryptococcus                MLPHLILSLASIFALPAVFAATTCNGHSELCSKLYSNVTFTSAHDSYAVG
                                          *    *     ***  * *   *   *    * **
RO3G_16295.3        SNPAANQLCPITTQLADGVRGIKLSAVKATNATTDGTITADSIYLCHTSCTILNAGPAVN
Cryptococcus        SSVADDQDKDVTSQLNDGIRTLQIQAHNAS           DGIHLCHSSCSILDGGLNSD
                    *  *  *    * ** ** *     *  *           * * *** **  *  *
RO3G_16295.3        TLNTIKSNVEQNPNSVVTIMWNNVDAFDGNAFSAAVNAGGIIEYSY   QQPYRNYTWPT
Cryptococcus        YLGTVASWVSENPSEVITIVINSDNLPPTSFSVFSSAGLSSKVYTPASQPTQLSDWPS
                     * *   **  *** * **   * *     *       *     *    **     **
RO3G_16295.3        LGSLIASGERVINFGDTYQ QDLPWLLTEYDYVFRTPYSNNNRSSPSCTIDRPQLPASP
Cryptococcus        LSDNIIAGTTVVAFWDYSADTSSVPYLLDSFAAWSEDAY GVTTQSFSCAVNRSSGCTSS
                    *   *  *  *  * *         * ** *     *  *       * *   *     *
RO3G_16295.3        TSFLVVWNNFLVGSLQLGSLPIRIPQKGIANTTNSDN   SLMYQAYTCTSSFGPQPNFL
Cryptococcus        QFFL  INNFLDSTYSFSSIQVFVFNSDKLNSTNARTGTGSIGVSVNNCSQLWGRNPNSI
                      **   ****      *    * *   * **     *       *    ** **
RO3G_16295.3        SIDFY  NLGDALKITARLNNVTYKGSGSLQCDTYAAQQASGSSTDSSSAIQTISISSVS
Cryptococcus        LLDFYDSNGNSSFNVAASLNGVSAP TNTVTSGTASATSSGTSAAVVSTQSLSG SVTSIE
                      ***  *       * ** *           *  *         *       *  *
RO3G_16295.3        LLLTLIAATFFTFF
Cryptococcus        GTARGTTLGFGVMLGVGMGVGEVFL
                          *   *
Total alignment length: 385
Overall identity  :25.97 % (100/385)
Inner consensus gaps : 34
Sequence lengths:
RO3G_16295.3 365
Cryptococcus 360
```

FIGURE 25


RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
RO3G_16295
S.lividans
Total alignment length: 773
Overall identity :11.51 % (89/773)
Inner consensus gaps : 59
Sequence lengths:
RO3G_16295 365
S.lividans 740 V

FIGURE 26

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
GTAATCATAAATTTAGTTTGAATTCAATGAGATTAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTTAATGATTTTTATAA
CCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATTT
TATTTTTATCTTTTCTCAGCTAAAATAAACATTGTCTTTCTCAGCCTCAGTGGACTCGTTCTGGAA
GCAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTTTTTTTCCACGGAGATGACAGTCAAG
TCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTTATTGGATCTA
ATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACGGTGCTGGCAAGAAACACAACAATG
CAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGGTAACCGCAATTTCTTTAAGC
TTCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGTCTTTATTCTGACATTTTACATGCCA
TGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTATTAACAACCAAGGCTTCGGTACCT
TCAACATGTTGGATGATATCACTCAATTCTCCTATATCAATGCTAAATTTTATAATGGCAAACCAC
CTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAGACTTCTTATATCATCCTGGTAACC
TCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCTAATGGTGAAGCTTATGAAGCTCTTG
ATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGATAATACAGCCATTGCAAACTTTGAAA
AAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATTGAATACTTGACTGCCGATTGGGATG
GTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGATCCAACTGATAATAACAAGTGGTACT
TTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGGCTGCACCCGAAGGCAATGCTTTTC
TTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCGCTGTCATGATCAACAACCTCTTAC
AGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACTGAGACTGTGCGTGTGCTGTTCAATA
ATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCTCTTGCCTGATCTTGAATGGGATC
GTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGACATTTGATCAAGTCACTCAAAACT
TGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTGCTGCTTTTGGTTTAGTTGAATATA
TTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTTCTATCGTTTCCCAACCTGTTGGCC
CTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAG
GAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAAACCTCGGCTCAAAGCACATCAGGTG
CTTCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCCGCTTTAGCTCTCCTTGTATTCTAA

FIGURE 27

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTT
AATGATTTTTATAACCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAGCAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTT
TTTTTCCACGGAGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTTATTGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCAC
GGTGCTGGCAAGAAACACAACAATGCAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAACCGCAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGT
CTTTATTCTGACATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTT
ATTAACAACCAAGGCTTCGGTACCTTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
GCTAAATTTTATAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCA
GACTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCT
AATGGTGAAGCTTATGAAGCTCTTGATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGAT
AATACAGCCATTGCAAACTTTGAAAAAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATT
GAATACTTGACTGCCGATTGGGATGGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGAT
CCAACTGATAATAACAAGTGGTACTTTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTG
GCTGCACCCGAAGGCAATGCTTTTCTTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGC
GCTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACT
GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTC
CTCTTGCCTGATCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGG
ACATTTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGT
GCTGCTTTTGGTTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATT
TCTATCGTTTCCCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCT
GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAA
ACCTCGGCTCAAAGCACATCAGGTGCTTCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCC
GCTTTAGCTCTCCTTGTATTCTAA

FIGURE 28

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
YVAGGTEESFDRSLDGITNSTLNDFYNRPVTYANLPQLPWPIEKDPQWTRSGSKADIFDDNYIPSV
FFHGDDSQVQNVVKNVPADRISGTLTFIGSNYVYSFQNVSFGIHGAGKKHNNAKQSWNWILSGSDT
MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGTYANDATMVRLFINNQGFGTFNMLDDITQFSYIN
AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNGEAYEALDPLCKAWNETTYTD
NTAIANFEKMFDLDRFMRFMVIEYLTADWDGYWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNL
AAPEGNAFLDVSYKDFPSRYPGAVMINNLLQNADKKATFEKYLTETVRVLFNNVTLTNRVLALHNF
LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGVTAPNNNGGGAAFGLVEYIAAKAQAVAKEFNI
SIVSQPVGPPSANGTTAAAPAPAAGNSTGKGGNQSISSSASSNKTSAQSTSGASRSKTAPIVLAIS
ALALLVF*

FIGURE 29

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
GTAATCATAAATTTAGTTTGAATTCAATGAGATTAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTTAATGATTTTTATAA
CCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATTT
TATTTTTATCTTTTCTCAGCTAAAATAAACATTGTCTTTCTCAGCCTCAGTGGACTCGTTCTGGAA
GCAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTTTTTTTCCACGGAGATGACAGTCAAG
TCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTTATTGGATCTA
ATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACGGTGCTGGCAAGAAACACAACAATG
CAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGGTAACCGCAATTTCTTTAAGC
TTCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGTCTTTATTCTGACATTTTACATGCCA
TGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTATTAACAACCAAGGCTTCGGTACCT
TCAACATGTTGGATGATATCACTCAATTCTCCTATATCAATGCTAAATTTTATAATGGCAAACCAC
CTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAGACTTCTTATATCATCCTGGTAACC
TCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCTAATGGTGAAGCTTATGAAGCTCTTG
ATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGATAATACAGCCATTGCAAACTTTGAAA
AAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATTGAATACTTGACTGCCGATTGGGATG
GTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGATCCAACTGATAATAACAAGTGGTACT
TTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGGCTGCACCCGAAGGCAATGCTTTTC
TTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCGCTGTCATGATCAACAACCTCTTAC
AGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACTGAGACTGTGCGTGTGCTGTTCAATA
ATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCTCTTGCCTGATCTTGAATGGGATC
GTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGACATTTGATCAAGTCACTCAAAACT
TGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTGCTGCTTTTGGTTTAGTTGAATATA
TTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTTCTATCGTTTCCCAACCTGTTGGCC
CTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAG
GAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAAACCTCGGCTCAAAGCACATCAGGTG
CTTCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCCGCTTTAGCTCTCCTTGTATTCTAAA

FIGURE 30

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATAACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTACTTCACTGGTTCAGCTGAAGTTGGTTCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAGGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACATTT
AATGATTTTTATAATCGCCCCATCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAACAAAGCCGACATTTTCGATGACAATTATATTCCCAGCATT
TTTTTCCACGGAGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTTATCGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCAC
GGTGCTGGCAAGAAACACAACAATGCAAAGCAATCTTGGAACTGGATCTTGTCTGGAAGTGATACG
ATGGGTAACCGTAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGT
CTTTATTCTGACATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTT
ATTAACAATCAAGGCTTCGGTACCTTCAACATGTTGGATGACATCACTCAATTCTCTTATATCAAT
GCTAAATTCTATAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCA
GATTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTTCTTGGGTTGCCAACACAGCTAATCC
TAATGGTGAAGCTTATGAAGCTCTTGATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGA
TAATACAGCCATTGCGAACTTTGAAAAAATGTTTGATCTTGACCGTTTCATGCGTTTCATGGTTGT
TGAATACTTGGCTGCCGATTGGGATGGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGA
TCCAACTGATAATAACAAGTGGTACTTTTTAGATCAAGACTTTGATGGTACCTTTGGTGTCAACTT
GGCTGCACCCGAAGGCAATGCTTTTCTTGATATTTCTTACAAAGATTTCCCTTCTCGTTACCCTGG
CGCTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTTGAAAAATACTTGAC
TGAGACTGTGCGTGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTT
CCTCTTGCCTGACCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTG
GACATTTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCAATGCACCCAATAACAACGGAGGTGG
TGCTGCTATTGGTTTAGTTGAATATATTGCTACAAAGGCACAAGTTGTAGCTAAGAATTAATATTA
CTATCGTTCCCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTACTGCTGCTAACTGCTCCTA
CTGCTGGTATCTACTGGAAAAGGAAGAAATCATCCCATTTCTAGCAGTGCTTCATCAACAAACTCG
CTCAAGTAACATCAAGTGCTTCTCGTCAAGACTGCGCCAATCATTTTAGGCAATTTCCGCTTAGCC
CTCCCCCGTTGTGATTCTCAAAA

FIGURE 31

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATAACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTACTTCACTGGTTCAGCTGAAGTTGGTTCCTCAAAGACATACAAA
GTAATCATAAATTTATTTTGAATTCAATCATATTAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAGGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACATTTAATGATTTTTATAA
TCGCCCCATCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATTT
TATTTTTATCTTTTCTCAGCTAAAATAAACATTGTCTTTCTCAGCCTCAGTGGACTCGTTCTGGAA
ACAAAGCCGACATTTTCGATGACAATTATATTCCCAGCATTTTTTTCCACGGAGATGACAGTCAAG
TCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTTATCGGATCTA
ATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACGGTGCTGGCAAGAAACACAACAATG
CAAAGCAATCTTGGAACTGGATCTTGTCTGGAAGTGATACGATGGGTAACCGTAATTTCTTTAAGC
TTCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGTCTTTATTCTGACATTTTACATGCCA
TGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTATTAACAATCAAGGCTTCGGTACCT
TCAACATGTTGGATGACATCACTCAATTCTCTTATATCAATGCTAAATTCTATAATGGCAAACCAC
CTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAGATTTCTTATATCATCCTGGTAACC
TCGATGGATACTCTTTCTTGGGTTGCCAACACAGCTAATCCTAATGGTGAAGCTTATGAAGCTCTT
GATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGATAATACAGCCATTGCGAACTTTGAA
AAAATGTTTGATCTTGACCGTTTCATGCGTTTCATGGTTGTTGAATACTTGGCTGCCGATTGGGAT
GGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGATCCAACTGATAATAACAAGTGGTAC
TTTTTAGATCAAGACTTTGATGGTACCTTTGGTGTCAACTTGGCTGCACCCGAAGGCAATGCTTTT
CTTGATATTTCTTACAAAGATTTCCCTTCTCGTTACCCTGGCGCTGTCATGATCAACAACCTCTTA
CAGAATGCTGATAAAAAGGCCACCTTTGAAAAATACTTGACTGAGACTGTGCGTGTGCTGTTCAAT
AATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCTCTTGCCTGACCTTGAATGGGAT
CGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGACATTTGATCAAGTCACTCAAAAC
TTGTGGCAAGGTGTCAATGCACCCAATAACAACGGAGGTGGTGCTGCTATTGGTTTAGTTGAATAT
ATTGCTACAAAGGCACAAGTTGTAGCTAAGAATTAATATTACTATCGTTCCCAACCTGTTGGCCCT
CCTTCTGCTAATGGTACTACTACTGCTGCTAACTGCTCCTACTGCTGGTATCTACTGGAAAAGGAA
GAAATCATCCCATTTCTAGCAGTGCTTCATCAACAAACTCGCTCAAGTAACATCAAGTGCTTCTCG
TCAAGACTGCGCCAATCATTTTAGGCAATTTCCGCTTAGCCCTCCTTGTGATTCTCAAAA

FIGURE 32

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGSSKTYK
VIINLF*IQSY*SSYSIVCCRWNRRRF*SFS*WNHKLNI**FL*SPHHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFLSLSGLVLETKPTFSMTIIFPAFFSTEMTVKSKMWLKTYLLTESVVH*PLSDL
ITSTLSRMSHLVFTVLARNTTMQSNLGTGSCLEVIRWVTVISLSFDIWKKILHRSVNVFILTFYMP
WVLMPMMLPWFDCLLTIKASVPSTCWMTSLNSLISMLNSIMANHLLPWVLSMMVPLVQISYIILVT
SMDTLSWVANTANPNGEAYEALDPLCKAWNETTYTDNTAIANFEKMFDLDRFMRFMVVEYLAADWD
GYWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNLAAPEGNAFLDISYKDFPSRYPGAVMINNLL
QNADKKATFEKYLTETVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQN
LWQGVNAPNNNGGGAAIGLVEYIATKAQVVAKN*YYYRSQPVGPPSANGTTTAANCSYCWYLLEKE
EIIPFLAVLHQQTRSSNIKCFSSRLRQSF*AISA*PSL*FSK

FIGURE 33

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGSSKTYK
VIINLF*IQSY*SSYSIVCCRWNRRRF*SFS*WNHKLNI**FL*SPHHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFLSLSGLVLETKPTFSMTIIFPAFFSTEMTVKSKMWLKTYLLTESVVH*PLSDL
ITSTLSRMSHLVFTVLARNTTMQSNLGTGSCLEVIRWVTVISLSFDIWKKILHRSVNVFILTFYMP
WVLMPMMLPWFDCLLTIKASVPSTCWMTSLNSLISMLNSIMANHLLPWVLSMMVPLVQISYIILVT
SMDTLSWVANTANPNGEAYEALDPLCKAWNETTYTDNTAIANFEKMFDLDRFMRFMVVEYLAADWD
GYWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNLAAPEGNAFLDISYKDFPSRYPGAVMINNLL
QNADKKATFEKYLTETVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQN
LWQGVNAPNNNGGGAAIGLVEYIATKAQVVAKN*YYYRSQPVGPPSANGTTTAANCSYCWYLLEKE
EIIPFLAVLHQQTRSSNIKCFSSRLRQSF*AISA*PSPVVILK

FIGURE 34

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATAACACACGCTGCTTCATAAAGTTTA
ATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACTG
CTTCAGATGCAAATGTCCCTTACTTCACTGGTTCAGCTGAAGTTGGTTCCTCAAAGACATACAAAT
ATGTTGCAGGTGGAACAGAAGAAGGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACATTTA
ATGATTTTTATAATCGCCCCATCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAAG
ACCCTCAGTGGACTCGTTCTGGAAACAAAGCCGACATTTTCGATGACAATTATATTCCCAGCATTT
TTTTCCACGGAGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTG
GTACACTGACCTTTATCGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACG
GTGCTGGCAAGAAACACAACAATGCAAAGCAATCTTGGAACTGGATCTTGTCTGGAAGTGATACGA
TGGGTAACCGTAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGTC
TTTATTCTGACATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTA
TTAACAATCAAGGCTTCGGTACCTTCAACATGTTGGATGACATCACTCAATTCTCTTATATCAATG
CTAAATTCTATAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAG
ATTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCTAATCCTA
ATGGTGAAGCTTATGAAGCTCTTGATCCTTCTCTGTAAGGCCTGGAAACGAGACGACCTATTACCG
ATAATACAGCCAATTGCGAACTTTGAAAAAATGTTTGATCTGACGTTTCATGCGTTCCATGCTGGT
GATACTGGGCTGCCGAATGAATGCTACTGCAATGGAAGACATGAATCGTGTCTATTCGTGATCCAA
CTGAATAATACCAGTCGGGTACTTTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGGC
TGCACCCGAAGGCAATGCTTTTCTTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCGC
TGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACTGA
GACTGTGCGTGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCT
CTTGCCTGATCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGAC
ATTTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTGC
TGCTTTTGGTTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTTC
TATCGTTTCCCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCTGC
TGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAAAC
CTCGGCTCAAAGCACATCAGGTGCTTCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCCGC
TTTAGCTCTCCTTGGTATTCTAAA

FIGURE 35

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

MKLSIISAAFLVAITHAAS*SLM*LLLMQLMSKYL*MDSK*HLLLQMQMSLTSLVQLKLVPQRHTN
MLQVEQKKVLIVLLMESQTQHLMIFIIAPSLMLTFLNYLGQLKKTLSGLVLETKPTFSMTIIFPAF
FSTEMTVKSKMWLKTYLLTESVVH*PLSDLITSTLSRMSHLVFTVLARNTTMQSNLGTGSCLEVIR
WVTVISLSFDIWKKILHRSVNVFILTFYMPWVLMPMMLPWFDCLLTIKASVPSTCWMTSLNSLISM
LNSIMANHLLPWVLSMMVPLVQISYIILVTSMDTLLGLPTQLILMVKLMKLLILLCKAWKRDDLLP
IIQPIANFEKMFDLTFHAFHAGDTGLPNECYCNGRHESCLFVIQLNNTSRVLFRSRL*WYFWCQLG
CTRRQCFS*CFLQGFPFSLPWRCHDQQPLTEC**KGHL*KIFD*DCACAVQ*CHLD*PCLGPSQLP
LA*S*MGSFDRSTISWY*LWLDI*SSHSKLVARCHCTQ*QWRWCCFWFS*IYCCKGTSCS*GI*YF
YRFPTCWPSFC*WYYCCCSCSCCWQFYWKRRKSIYF*QCFIQQNLGSKHIRCFSFQDCAHRFSHFR
FSSPWYSK

FIGURE 36

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATAACACACGCTGCTTCATAAAGTTTA
ATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACTG
CTTCAGATGCAAATGTCCCTTACTTCACTGGTTCAGCTGAAGTTGGTTCCTCAAAGACATACAAAG
TAATCATAAATTTATTTTGAATTCAATCATATTAATCATCTTATTCTATAGTATGTTGCAGGTGGA
ACAGAAGAAGGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACATTTAATGATTTTTATAAT
CGCCCCATCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATTTT
ATTTTTATCTTTTCTCAGCTAAAATAAACATTGTCTTTCTCAGCCTCAGTGGACTCGTTCTGGAAA
CAAAGCCGACATTTTCGATGACAATTATATTCCCAGCATTTTTTTCCACGGAGATGACAGTCAAGT
CCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTTATCGGATCTAA
TTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACGGTGCTGGCAAGAAACACAACAATGC
AAAGCAATCTTGGAACTGGATCTTGTCTGGAAGTGATACGATGGGTAACCGTAATTTCTTTAAGCT
TCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGTCTTTATTCTGACATTTTACATGCCAT
GGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTATTAACAATCAAGGCTTCGGTACCTT
CAACATGTTGGATGACATCACTCAATTCTCTTATATCAATGCTAAATTCTATAATGGCAAACCACC
TGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAGATTTCTTATATCATCCTGGTAACCT
CGATGGATACTCTTCTTGGGTTGCCAACACAGCTAATCCTAATGGTGAAGCTTATGAAGCTCTTGA
TCCTTCTCTGTAAGGCCTGGAAACGAGACGACCTATTACCGATAATACAGCCAATTGCGAACTTTG
AAAAAATGTTTGATCTGACGTTTCATGCGTTCCATGCTGGTGATACTGGGCTGCCGAATGAATGCT
ACTGCAATGGAAGACATGAATCGTGTCTATTCGTGATCCAACTGAATAATACCAGTCGGGTACTTT
TTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGGCTGCACCCGAAGGCAATGCTTTTCTT
GATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCGCTGTCATGATCAACAACCTCTTACAG
AATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACTGAGACTGTGCGTGTGCTGTTCAATAAT
GTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCTCTTGCCTGATCTTGAATGGGATCGT
TCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGACATTTGATCAAGTCACTCAAAACTTG
TGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTGCTGCTTTTGGTTTAGTTGAATATATT
GCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTTCTATCGTTTCCCAACCTGTTGGCCCT
CCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAGGA
GGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAAACCTCGGCTCAAAGCACATCAGGTGCT
TCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCCGCTTTAGCTCTCCTTGGTATTCTAAA

FIGURE 37

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

MKLSIISAAFLVAITHAAS*SLM*LLLMQLMSKYL*MDSK*HLLLQMQMSLTSLVQLKLVPQRHTK
*S*IYFEFNHINHLIL*YVAGGTEEGFDRSLDGITNSTFNDFYNRPITYANLPQLPWPIEKDVSYF
IFIFSQLK*TLSFSASVDSFWKQSRHFR*QLYSQHFFPRR*QSSPKCG*KRTC*PNQWYTDLYRI*
LRLLFPECLIWYSRCWQETQQCKAILELDLVWK*YDG*P*FL*ASTYGRRSYTDP*TSLF*HFTCH
GYLCQ*CYHGSIVY*QSRLRYLQHVG*HHSILLYQC*IL*WQTTCYLGSSL*WCLWCRFLISSW*P
RWILFLGCQHS*S*W*SL*SS*SFSVRPGNETTYYR*YSQLRTLKKCLI*RFMRSMLVILGCRMNA
TAMEDMNRVYS*SN*IIPVGYFLDQDFDGTFGVNLAAPEGNAFLDVSYKDFPSRYPGAVMINNLLQ
NADKKATFEKYLTETVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQNL
WQGVTAPNNNGGGAAFGLVEYIAAKAQAVAKEFNISIVSQPVGPPSANGTTAAAPAPAAGNSTGKG
GNQSISSSASSNKTSAQSTSGASRSKTAPIVLAISALALLGIL

FIGURE 38

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTTCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTT
AATGATTTTTATAATCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAACAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTT
TTTTTCCACGGAGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTTATTGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCAC
GGTGCTGGCAAGAAACACAACAATGCAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAACCGCAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGT
CTTTATTCTGACATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTT
ATTAACAACCAAGGCTTCGGTACCTTCAACATGTTGGATGATATCACTCAATTCTCTTATATCAAT
GCTAAATTTTATAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCA
GATTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCT
AATGGTGAAGCTTATGAAGCTCTTGATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGAT
AATACAGCCATTGCAAACTTTGAAAAAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATT
GAATACTTGACTGCCGATTGGGATGGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGAT
CCAACTGATAATAACAAGTGGTACTTTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTG
GCTGCACCCGAAGGCAATGCTTTTCTTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGC
GCTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTATGAAAAATATTTGACT
GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTC
CTCTTGCCTGATCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGG
ACATTTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGT
GCTGCTTTTGGTTTAGTTGAATATATTGCTACAAAGGCACAAGCTGTAGCTAAGGAATTTAATATT
TCTATCGTTTCCCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCT
GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAA
ACCTCGGCTCAAAGCACATCAGGTGCTTCTCGTTCCAAGACTGCGCCCATCATTTTTAGCCATTTC
CGCTTTAGCTCTCCCTTGTATTCTAAA

FIGURE 39

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGSSKTYK
YVAGGTEESFDRSLDGITNSTLNDFYNRPVTYANLPQLPWPIEKDPQWTRSGNKADIFDDNYIPSV
FFHGDDSQVQNVVKNVPADRISGTLTFIGSNYVYSFQNVSFGIHGAGKKHNNAKQSWNWILSGSDT
MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGTYANDATMVRLFINNQGFGTFNMLDDITQFSYIN
AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNGEAYEALDPLCKAWNETTYTD
NTAIANFEKMFDLDRFMRFMVIEYLTADWDGYWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNL
AAPEGNAFLDVSYKDFPSRYPGAVMINNLLQNADKKATYEKYLTETVRVLFNNVTLTNRVLALHNF
LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGVTAPNNNGGGAAFGLVEYIATKAQAVAKEFNI
SIVSQPVGPPSANGTTAAAPAPAAGNSTGKGGNQSISSSASSNKTSAQSTSGASRSKTAPIIFSHF
RFSSPLYSK

FIGURE 40

>CotH3 sequence from Lichtheimia corymbifera(including introns)

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTTCCTCAAAGACATACAAA
GTAATCATAAATTTAGTTTGAATTCAATGAGATTAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTTAATGATTTTTATAA
TCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATTT
TATTTTTATCTTTTCTCAGCTAAAATAAACATTGTCTTTCTCAGCCTCAGTGGACTCGTTCTGGAA
ACAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTTTTTTTCCACGGAGATGACAGTCAAG
TCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTTATTGGATCTA
ATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACGGTGCTGGCAAGAAACACAACAATG
CAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGGTAACCGCAATTTCTTTAAGC
TTCGACATATGGAAGAAGATCCTACACAGATCCGTGAACGTCTTTATTCTGACATTTTACATGCCA
TGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTATTAACAACCAAGGCTTCGGTACCT
TCAACATGTTGGATGATATCACTCAATTCTCTTATATCAATGCTAAATTTTATAATGGCAAACCAC
CTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAGATTTCTTATATCATCCTGGTAACC
TCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCTAATGGTGAAGCTTATGAAGCTCTTG
ATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGATAATACAGCCATTGCAAACTTTGAAA
AAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATTGAATACTTGACTGCCGATTGGGATG
GTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGATCCAACTGATAATAACAAGTGGTACT
TTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGGCTGCACCCGAAGGCAATGCTTTTC
TTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCGCTGTCATGATCAACAACCTCTTAC
AGAATGCTGATAAAAAGGCCACCTATGAAAAATATTTGACTGAGACTGTGCGTGTGCTGTTCAATA
ATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCTCTTGCCTGATCTTGAATGGGATC
GTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGACATTTGATCAAGTCACTCAAAACT
TGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTGCTGCTTTTGGTTTAGTTGAATATA
TTGCTACAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTTCTATCGTTTCCCAACCTGTTGGCC
CTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCTGCTGCTGGCAATTCTACTGGAAAAG
GAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAAACCTCGGCTCAAAGCACATCAGGTG
CTTCTCGTTCCAAGACTGCGCCCATCATTTTTAGCCATTTCCGCTTTAGCTCTCCCTTGTATTCTA
AA

FIGURE 41

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGSSKTYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*SPRHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFLSLSGLVLETKPTFSMTIIFPAFFSTEMTVKSKMWLKTYLLTESVVH*PLLDL
ITSTLSRMSHLVFTVLARNTTMQNNLGTGYCLEVIRWVTAISLSFDIWKKILHRSVNVFILTFYMP
WVLMPMMLPWFDCLLTTKASVPSTCWMISLNSLISMLNFIMANHLLPWVLSMMVPLVQISYIILVT
SMDTLLGLPTQPILMVKLMKLLILSVRPGTRRPIPIIQPLQTLKKCLISTVSCVSWLLNT*LPIGM
VTGWDRPMMVPIVIQLIITSGTF*IKTLMVLLVSTWLHPKAMLFLMFLTRISLLVTLALS*STTSY
RMLIKRPPMKNI*LRLCVCCSIMSP*LTVSWPFTTSSCLILNGIVRSFNNLLVLTLVGHLIKSLKT
CGKVSLHPITMEVVLLLV*LNILLQRHKL*LRNLIFLSFPNLLALLLLMVLLLLLLLLLLAILLEK
EEINLFLAVLHPTKPRLKAHQVLLVPRLRPSFLAISALALPCIL

FIGURE 42

>CotH3 sequence from Cunninghamella bertholetiae(exons only)

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTT
AATGATTTTTATAACCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAGCAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTT
TTTTTCCACGGAGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTTATTGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCAC
GGTGCTGGCAAGAAACACAACAATGCAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAACCGCAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGT
CTTTATTCTGACATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTT
ATTAACAACCAAGGCTTCGGTACCTTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
GCTAAATTTTATAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCA
GACTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCT
AATGTGAAGCTTATGAAGCCTCTTGATCCTCTCTGTAGCCTGGAACGAGACGACCTAATACCGATA
ATACAGCCATTGCAAACTTTGAAAAAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATTG
AATACTTGACTGCCGATTGGGATGGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGATC
CAACTGATAATAACAAGTGGTACTTTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGG
CTGCACCCGAAGGCAATGCTTTTCTTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCG
CTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACTG
AGACTGTGCGTGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCC
TCTTGCCTGATCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGA
CATTTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTG
CTGCTTTTGGTTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTT
CTATCGTTTCCCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTGCTGCTGCTCTGCTCTGCT
GCTGCAATTCTACTGGAAAAGAGAAATCAATCTATTTTCTAGCAGTGCTTCATCAACAAAGCTCGG
CTCAAGGCACATCAGTGCCTTCTCGATCAAGACTGCGCCCATCGATTAAGCAGTTCGCTTTAGCTT
CCCTGGGGTAATCTCCAAAAA

FIGURE 43

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*PPRHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFSASVDSFWKQSRHFR*QLYSQRFFPRR*QSSPKCG*KRTC*PNQWYTDLYWI*
LRLLFPECLIWYSRCWQETQQCKTILELDIVWK*YDG*PQFL*ASTYGRRSYTDS*TSLF*HFTCH
GYLCQ*CYHGSIVY*QPRLRYLQHVG*YHSILLYQC*IL*WQTTCYLGSSL*WCLWCRLLISSW*P
RWILFLGCQHSQS*CEAYEAS*SSL*PGTRRPNTDNTAIANFEKMFDLDRFMRFMVIEYLTADWDG
YWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNLAAPEGNAFLDVSYKDFPSRYPGAVMINNLLQ
NADKKATFEKYLTETVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQNL
WQGVTAPNNNGGGAAFGLVEYIAAKAQAVAKEFNISIVSQPVGPPSANGTTAAALLCCCNSTGKEK
SIYFLAVLHQQSSAQGTSVPSRSRLRPSIKQFALASLG*SPK

FIGURE 44

>CotH3 sequence from Cunninghamella bertholetiae(including introns)

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
GTAATCATAAATTTAGTTTGAATTCAATGAGATTAATCATCTTATTCTATAGTATGTTGCAGGTGG
AACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTTAATGATTTTTATAA
CCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAAGACGTAAGTTATTT
TATTTTTATCTTTTCTCAGCTAAAATAAACATTGTCTTCTCAGCCTCAGTGGACTCGTTCTGGAAG
CAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTTTTTTTCCACGGAGATGACAGTCAAGT
CCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGACCTTTATTGGATCTAA
TTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACGGTGCTGGCAAGAAACACAACAATGC
AAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGGTAACCGCAATTTCTTTAAGCT
TCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGTCTTTATTCTGACATTTTACATGCCAT
GGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTATTAACAACCAAGGCTTCGGTACCTT
CAACATGTTGGATGATATCACTCAATTCTCCTATATCAATGCTAAATTTTATAATGGCAAACCACC
TGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAGACTTCTTATATCATCCTGGTAACCT
CGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCTAATGTGAAGCTTATGAAGCCTCTTGA
TCCTCTCTGTAGCCTGGAACGAGACGACCTAATACCGATAATACAGCCATTGCAAACTTTGAAAAA
ATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATTGAATACTTGACTGCCGATTGGGATGGT
TACTGGATGGGACAGACCAATGATGGTGCCTATCGTGATCCAACTGATAATAACAAGTGGTACTTT
TTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGGCTGCACCCGAAGGCAATGCTTTTCTT
GATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCGCTGTCATGATCAACAACCTCTTACAG
AATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACTGAGACTGTGCGTGTGCTGTTCAATAAT
GTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCTCTTGCCTGATCTTGAATGGGATCGT
TCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGACATTTGATCAAGTCACTCAAAACTTG
TGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTGCTGCTTTTGGTTTAGTTGAATATATT
GCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTTCTATCGTTTCCCAACCTGTTGGCCCT
CCTTCTGCTAATGGTACTACTGCTGCTGCTCTGCTCTGCTGCTGCAATTCTACTGGAAAAGAGAAA
TCAATCTATTTTCTAGCAGTGCTTCATCAACAAAGCTCGGCTCAAGGCACATCAGTGCCTTCTCGA
TCAAGACTGCGCCCATCGATTAAGCAGTTCGCTTTAGCTTCCCTGGGGTAATCTCCAAAAA

FIGURE 45

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*PPRHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFSASVDSFWKQSRHFR*QLYSQRFFPRR*QSSPKCG*KRTC*PNQWYTDLYWI*
LRLLFPECLIWYSRCWQETQQCKTILELDIVWK*YDG*PQFL*ASTYGRRSYTDS*TSLF*HFTCH
GYLCQ*CYHGSIVY*QPRLRYLQHVG*YHSILLYQC*IL*WQTTCYLGSSL*WCLWCRLLISSW*P
RWILFLGCQHSQS*CEAYEAS*SSL*PGTRRPNTDNTAIANFEKMFDLDRFMRFMVIEYLTADWDG
YWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNLAAPEGNAFLDVSYKDFPSRYPGAVMINNLLQ
NADKKATFEKYLTETVRVLFNNVTLTNRVLALHNFLLPDLEWDRSIVQQSPGINFGWTFDQVTQNL
WQGVTAPNNNGGGAAFGLVEYIAAKAQAVAKEFNISIVSQPVGPPSANGTTAAALLCCCNSTGKEK
SIYFLAVLHQQSSAQGTSVPSRSRLRPSIKQFALASLG*SPK

FIGURE 46

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTT
AATGATTTTTATAACCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAA
GACCCTCAGTGGACTCGTTCTGGAAGCAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTT
TTTTTCCACGGAGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGT
GGTACACTGACCTTTATTGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCAC
GGTGCTGGCAAGAAACACAACAATGCAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACG
ATGGGTAACCGCAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGT
CTTTATTCTGACATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTT
ATTAACAACCAAGGCTTCGGTACCTTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAAT
GCTAAATTTTATAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCA
GACTTCTTATATCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCT
AATGGTGAAGCTTATGAAGCTCTTGATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGAT
AATACAGCCATTGCAAACTTTGAAAAAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATT
GAATACTTGACTGCCGATTGGGATGGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGAT
CCAACTGATAATAACAAGTGGTACTTTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTG
GCTGCACCCGAAGGCAATGCTTTTCTTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGC
GCTGTCATGATCAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACT
GAGACTGTGCGTGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTC
CTCTTGCCTGATCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGG
ACATTTGATCAAGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGT
GCTGCTTTTGGTTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATT
TCTATCGTTTCCCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCT
GCTGCTGGCAATTCTACTGGAAAAGGAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAA
ACCTCGGCTCAAAGCACATCAGGTGCTTCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCC
GCTTTAGCTCTCCTTGTATTCTAAA

FIGURE 47

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
YVAGGTEESFDRSLDGITNSTLNDFYNRPVTYANLPQLPWPIEKDPQWTRSGSKADIFDDNYIPSV
FFHGDDSQVQNVVKNVPADRISGTLTFIGSNYVYSFQNVSFGIHGAGKKHNNAKQSWNWILSGSDT
MGNRNFFKLRHMEEDPTQIRERLYSDILHAMGTYANDATMVRLFINNQGFGTFNMLDDITQFSYIN
AKFYNGKPPATLGPLYDGASGADFLYHPGNLDGYSSWVANTANPNGEAYEALDPLCKAWNETTYTD
NTAIANFEKMFDLDRFMRFMVIEYLTADWDGYWMGQTNDGAYRDPTDNNKWYFLDQDFDGTFGVNL
AAPEGNAFLDVSYKDFPSRYPGAVMINNLLQNADKKATFEKYLTETVRVLFNNVTLTNRVLALHNF
LLPDLEWDRSIVQQSPGINFGWTFDQVTQNLWQGVTAPNNNGGGAAFGLVEYIAAKAQAVAKEFNI
SIVSQPVGPPSANGTTAAAPAPAAGNSTGKGGNQSISSSASSNKTSAQSTSGASRSKTAPIVLAIS
ALALLVF*

FIGURE 48

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

ATGAAATTATCTATTATATCCGCTGCCTTTTTAGTGGCTATTACACACGCTGCTTCAATAAAGTTT
AATGTAATTGCTCCTAATGCAACTGATGTCAAAGTATCTGTAAATGGACAGCAAGTGACACTTACT
GCTTCAGATGCAAATGTCCCTTATTTCACTGGTTCAGCTGAAGTTGGTGCCTCAAAGACATACAAA
TATGTTGCAGGTGGAACAGAAGAAAGTTTTGATCGTTCTCTTGATGGAATCACAAACTCAACACTT
AATGATTTTTATAACCGCCCCGTCACTTATGCTAACCTTCCTCAATTACCTTGGCCAATTGAAAAA
GACGTAAGTTATTTTATTTTTATCTTTTCTCAGCTAAAATAAACATTGTCTTTCTCAGCCTCAGTG
GACTCGTTCTGGAAGCAAAGCCGACATTTTCGATGACAATTATATTCCCAGCGTTTTTTTCCACGG
AGATGACAGTCAAGTCCAAAATGTGGTTAAAAACGTACCTGCTGACCGAATCAGTGGTACACTGAC
CTTTATTGGATCTAATTACGTCTACTCTTTCCAGAATGTCTCATTTGGTATTCACGGTGCTGGCAA
GAAACACAACAATGCAAAACAATCTTGGAACTGGATATTGTCTGGAAGTGATACGATGGGTAACCG
CAATTTCTTTAAGCTTCGACATATGGAAGAAGATCCTACACAGATTCGTGAACGTCTTTATTCTGA
CATTTTACATGCCATGGGTACTTATGCCAATGATGCTACCATGGTTCGATTGTTTATTAACAACCA
AGGCTTCGGTACCTTCAACATGTTGGATGATATCACTCAATTCTCCTATATCAATGCTAAATTTTA
TAATGGCAAACCACCTGCTACCTTGGGTCCTCTCTATGATGGTGCCTCTGGTGCAGACTTCTTATA
TCATCCTGGTAACCTCGATGGATACTCTTCTTGGGTTGCCAACACAGCCAATCCTAATGGTGAAGC
TTATGAAGCTCTTGATCCTCTCTGTAAGGCCTGGAACGAGACGACCTATACCGATAATACAGCCAT
TGCAAACTTTGAAAAAATGTTTGATCTCGACCGTTTCATGCGTTTCATGGTTATTGAATACTTGAC
TGCCGATTGGGATGGTTACTGGATGGGACAGACCAATGATGGTGCCTATCGTGATCCAACTGATAA
TAACAAGTGGTACTTTTTAGATCAAGACTTTGATGGTACTTTTGGTGTCAACTTGGCTGCACCCGA
AGGCAATGCTTTTCTTGATGTTTCTTACAAGGATTTCCCTTCTCGTTACCCTGGCGCTGTCATGAT
CAACAACCTCTTACAGAATGCTGATAAAAAGGCCACCTTTGAAAAATATTTGACTGAGACTGTGCG
TGTGCTGTTCAATAATGTCACCTTGACTAACCGTGTCTTGGCCCTTCACAACTTCCTCTTGCCTGA
TCTTGAATGGGATCGTTCGATCGTTCAACAATCTCCTGGTATTAACTTTGGTTGGACATTTGATCA
AGTCACTCAAAACTTGTGGCAAGGTGTCACTGCACCCAATAACAATGGAGGTGGTGCTGCTTTTGG
TTTAGTTGAATATATTGCTGCAAAGGCACAAGCTGTAGCTAAGGAATTTAATATTTCTATCGTTTC
CCAACCTGTTGGCCCTCCTTCTGCTAATGGTACTACTGCTGCTGCTCCTGCTCCTGCTGCTGGCAA
TTCTACTGGAAAAGGAGGAAATCAATCTATTTCTAGCAGTGCTTCATCCAACAAAACCTCGGCTCA
AAGCACATCAGGTGCTTCTCGTTCCAAGACTGCGCCCATCGTTTTAGCCATTTCCGCTTTAGCTCT
CCTTGTATTCTAAA

FIGURE 49

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

MKLSIISAAFLVAITHAASIKFNVIAPNATDVKVSVNGQQVTLTASDANVPYFTGSAEVGASKTYK
VIINLV*IQ*D*SSYSIVCCRWNRRKF*SFS*WNHKLNT**FL*PPRHLC*PSSITLAN*KRRKLF
YFYLFSAKINIVFLSLSGLVLEAKPTFSMTIIFPAFFSTEMTVKSKMWLKTYLLTESVVH*PLLDL
ITSTLSRMSHLVFTVLARNTTMQNNLGTGYCLEVIRWVTAISLSFDIWKKILHRFVNVFILTFYMP
WVLMPMMLPWFDCLLTTKASVPSTCWMISLNSPISMLNFIMANHLLPWVLSMMVPLVQTSYIILVT
SMDTLLGLPTQPILMVKLMKLLILSVRPGTRRPIPIIQPLQTLKKCLISTVSCVSWLLNT*LPIGM
VTGWDRPMMVPIVIQLIITSGTF*IKTLMVLLVSTWLHPKAMLFLMFLTRISLLVTLALS*STTSY
RMLIKRPPLKNI*LRLCVCCSIMSP*LTVSWPFTTSSCLILNGIVRSFNNLLVLTLVGHLIKSLKT
CGKVSLHPITMEVVLLLV*LNILLQRHKL*LRNLIFLSFPNLLALLLLMVLLLLLLLLLLAILLEK
EEINLFLAVLHPTKPRLKAHQVLLVPRLRPSF*PFPL*LSLYSK

FIGURE 50

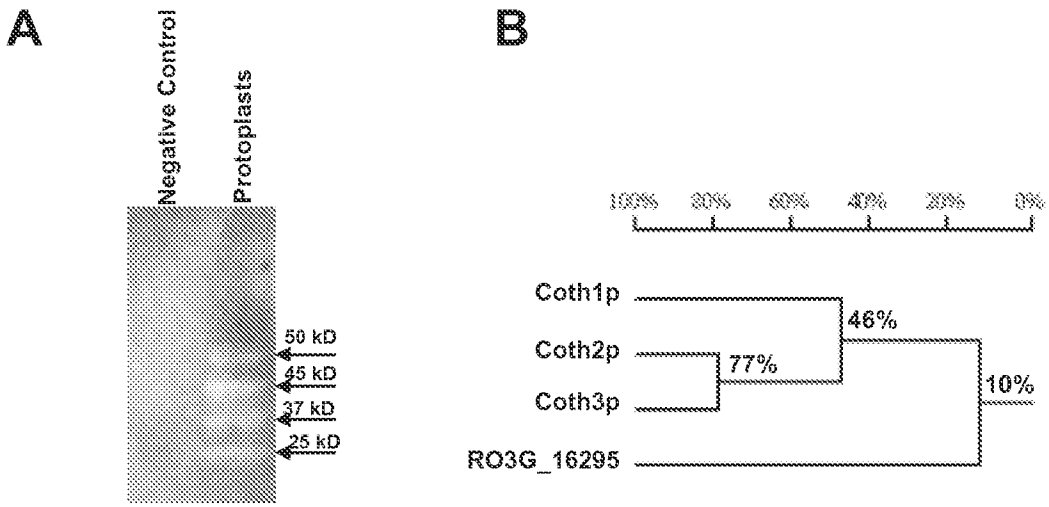

A
Negative Control
Protoplasts
50 kD
45 kD
37 kD
25 kD
B
100%
80%
60%
40%
20%
0%
Coth1p
Coth2p
Coth3p
RO3G_16295
46%
77%
10%

FIGURE 51

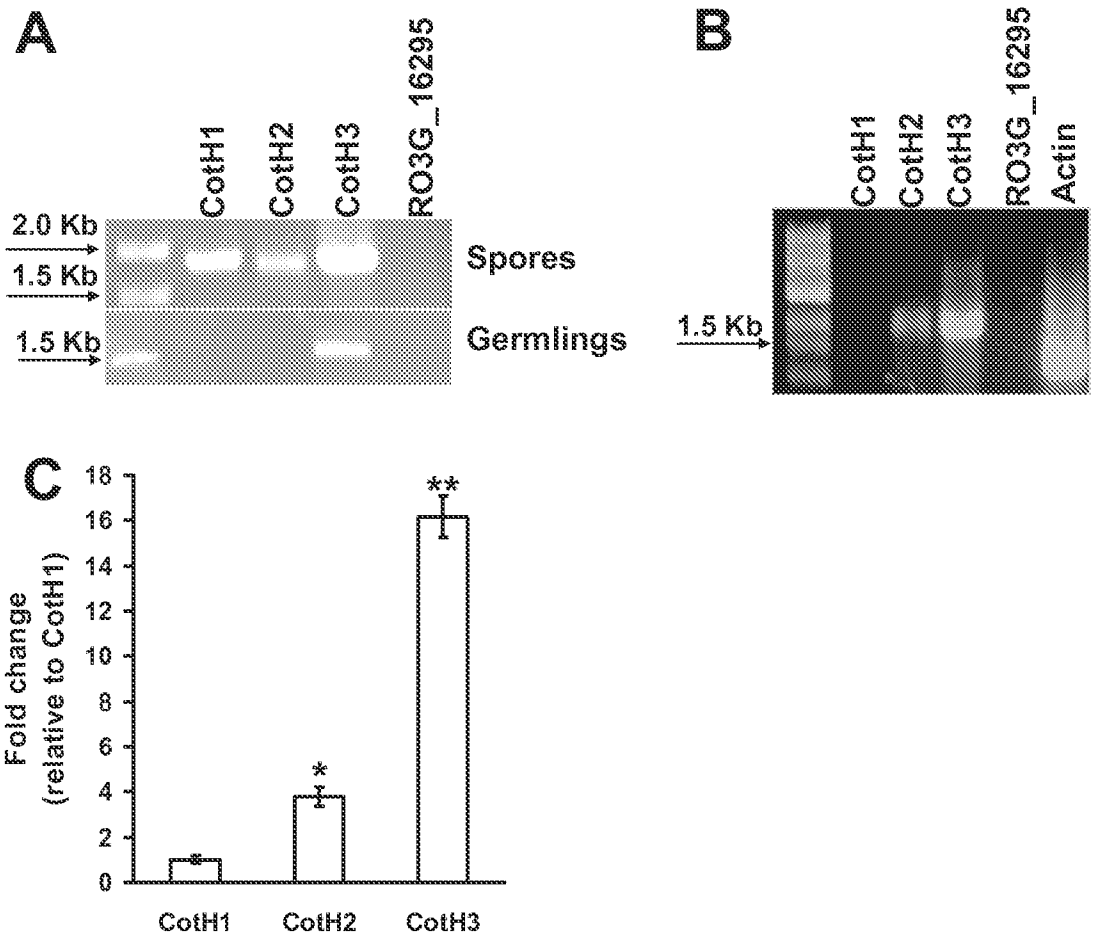

A
CotH1
CotH2
CotH3
RO3G_16295
2.0 Kb
1.5 Kb
Spores
1.5 Kb
Germlings
B
CotH1
CotH2
CotH3
RO3G_16295
Actin
1.5 Kb
C
Fold change
(relative to CotH1)
18
16
14
12
10
8
6
4
2
0
**
*
CotH1
CotH2
CotH3

FIGURE 52

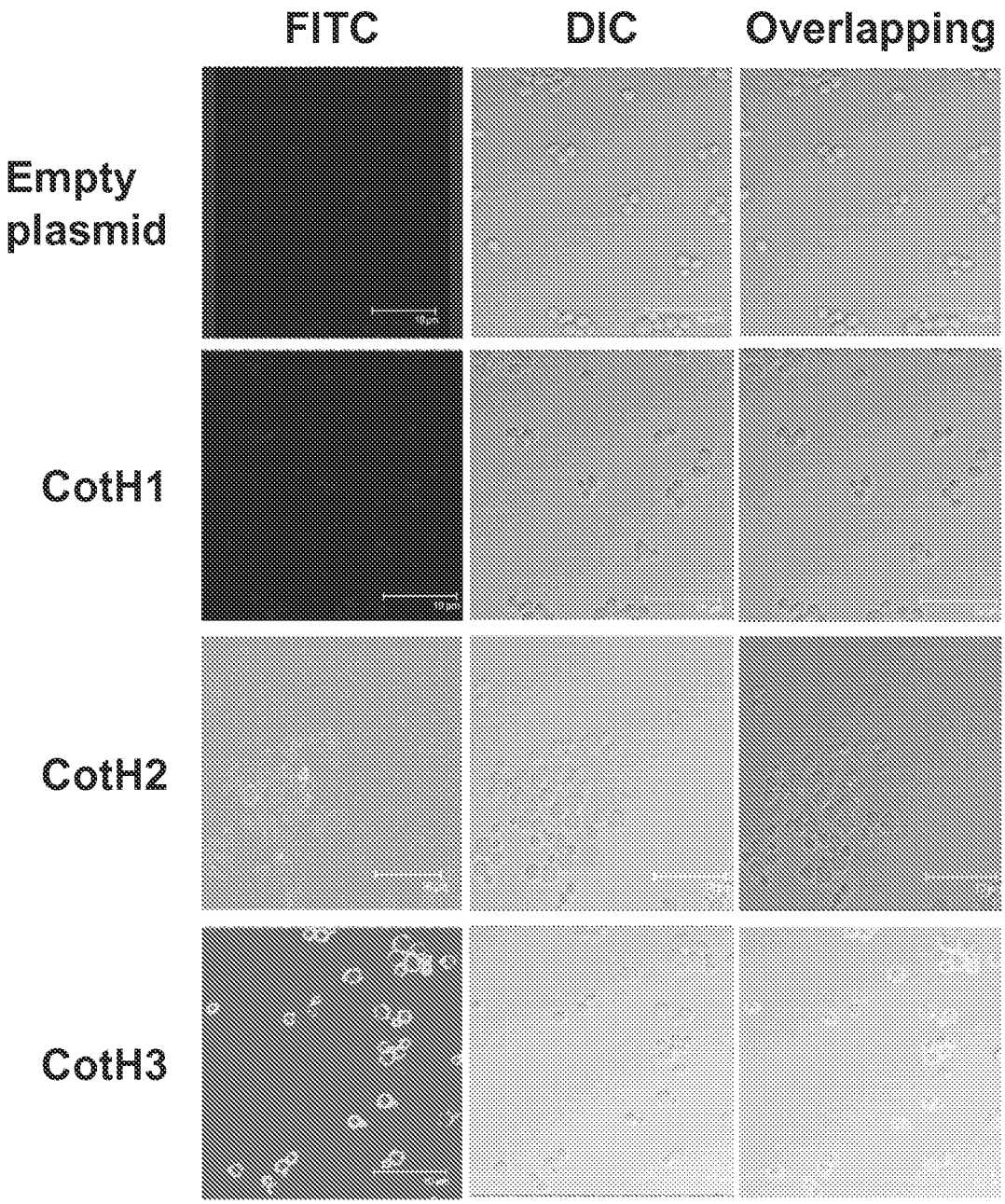


FIGURE 53

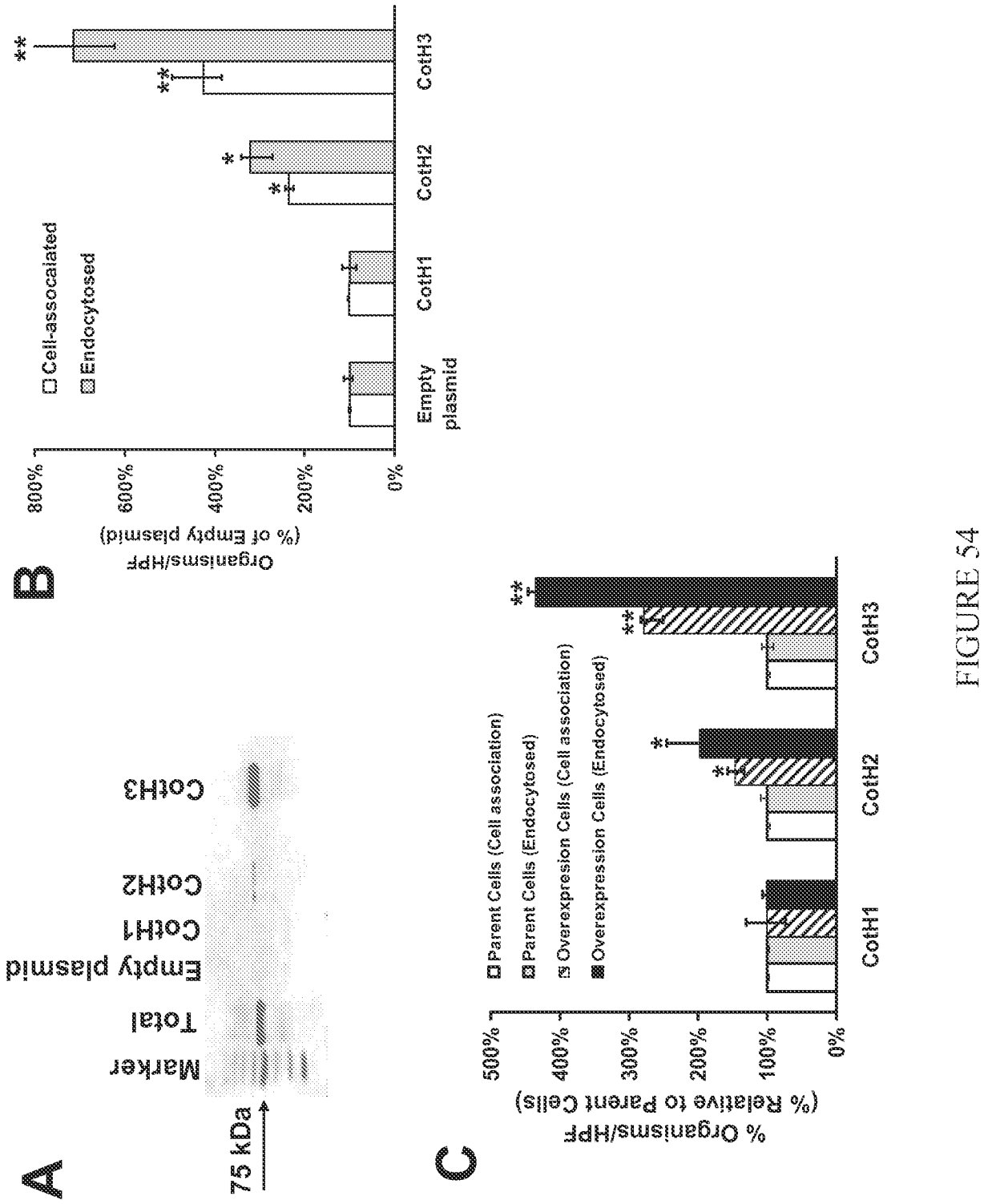

A
75 kDa
Marker
Total
Empty plasmid
CotH1
CotH2
CotH3
B
Organisms/HPF
(% of Empty plasmid)
800%
600%
400%
200%
0%
Cell-associated
Endocytosed
Empty plasmid
CotH1
CotH2
CotH3
C
% Organisms/HPF
(% Relative to Parent Cells)
500%
400%
300%
200%
100%
0%
Parent Cells (Cell association)
Parent Cells (Endocytosed)
Overexpression Cells (Cell association)
Overexpression Cells (Endocytosed)
CotH1
CotH2
CotH3

FIGURE 54

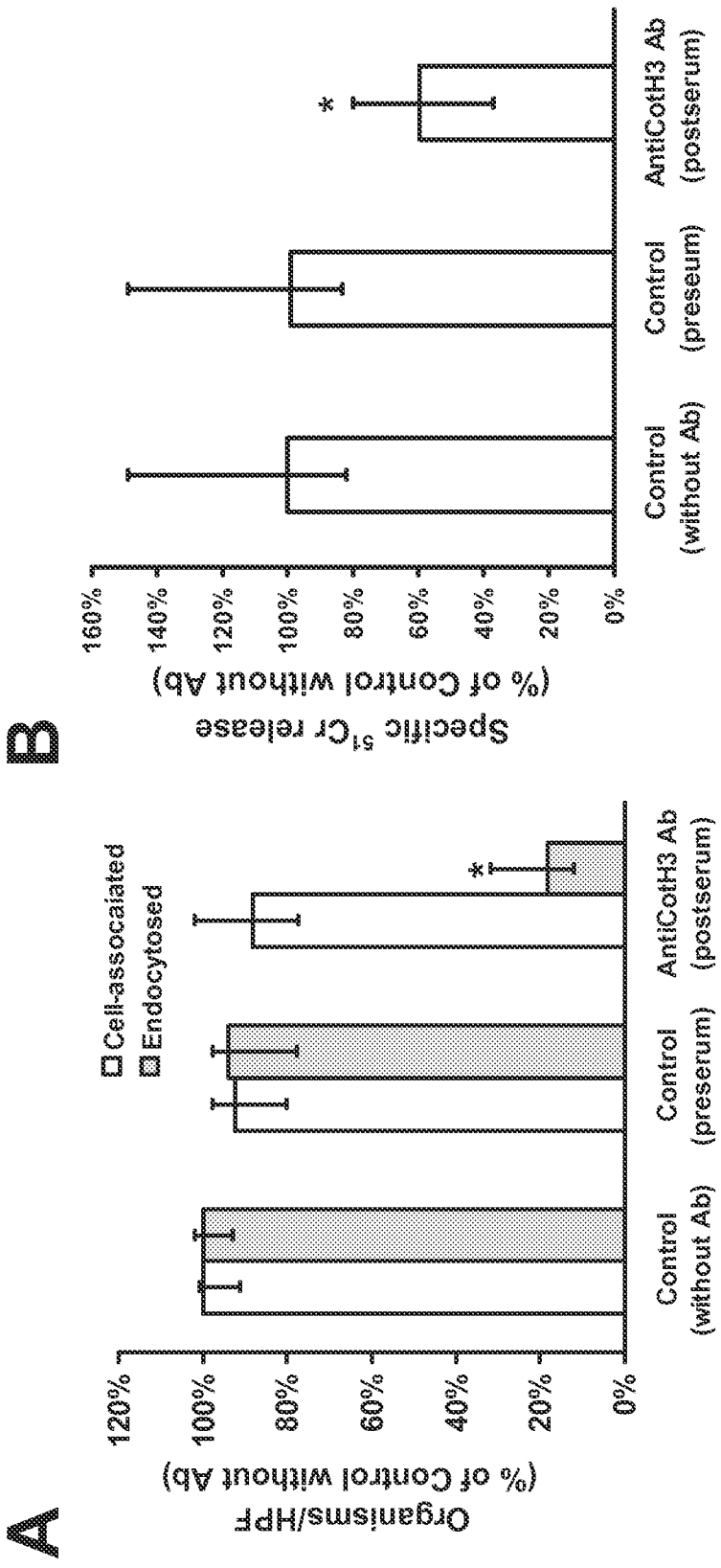

A
Organisms/HPF
(% of Control without Ab)
120%
100%
80%
60%
40%
20%
0%
Cell-associaiated
Endocytosed
*
Control (without Ab)
Control (preserum)
AntiCotH3 Ab (postserum)
B
Specific 51Cr release
(% of Control without Ab)
160%
140%
120%
100%
80%
60%
40%
20%
0%
*
Control (without Ab)
Control (preseum)
AntiCotH3 Ab (postserum)

FIGURE 55

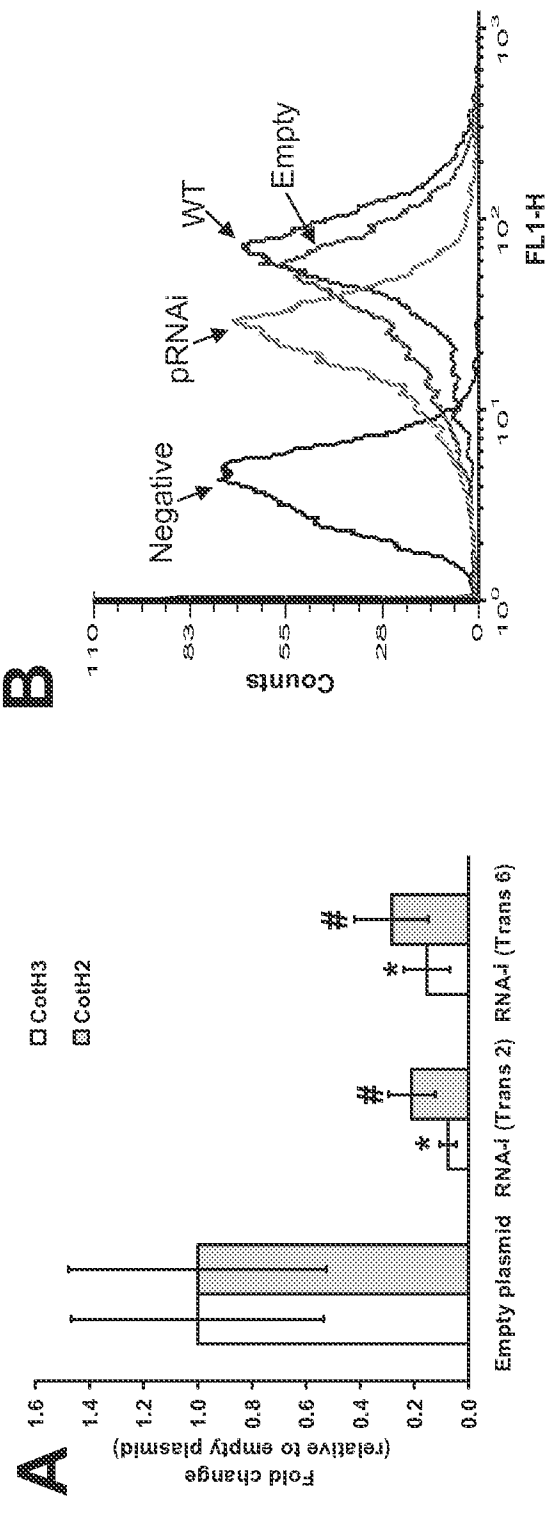

A
Fold change (relative to empty plasmid)
CotH3
CotH2
Empty plasmid
RNA-i (Trans 2)
RNA-i (Trans 6)
B
Counts
Negative
pRNAi
WT
Empty
FL1-H
C
Wild-type
Empty-plasmid
RNA-i (Trans 2)
RNA-i (Trans 6)

FIGURE 56

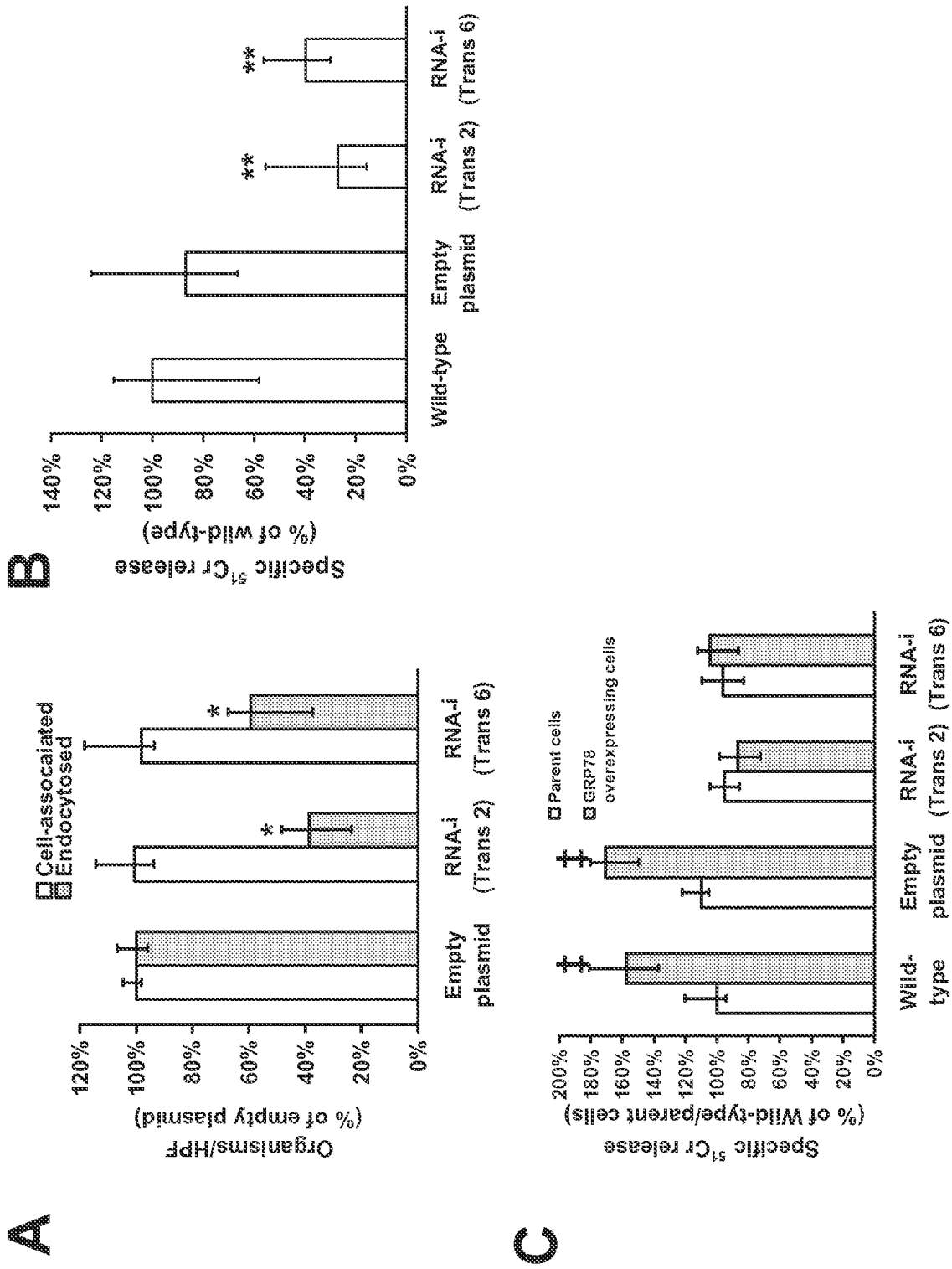

A
Cell-associated
Endocytosed
Organisms/HPF
(% of empty plasmid)
120%
100%
80%
60%
40%
20%
0%
*
*
Empty plasmid
RNA-i (Trans 2)
RNA-i (Trans 6)
B
Specific 51Cr release
(% of wild-type)
140%
120%
100%
80%
60%
40%
20%
0%
**
**
Wild-type
Empty plasmid
RNA-i (Trans 2)
RNA-i (Trans 6)
C
Parent cells
GRP78 overexpressing cells
Specific 51Cr release
(% of Wild-type/parent cells)
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
Wild-type
Empty plasmid
RNA-i (Trans 2)
RNA-i (Trans 6)

FIGURE 57

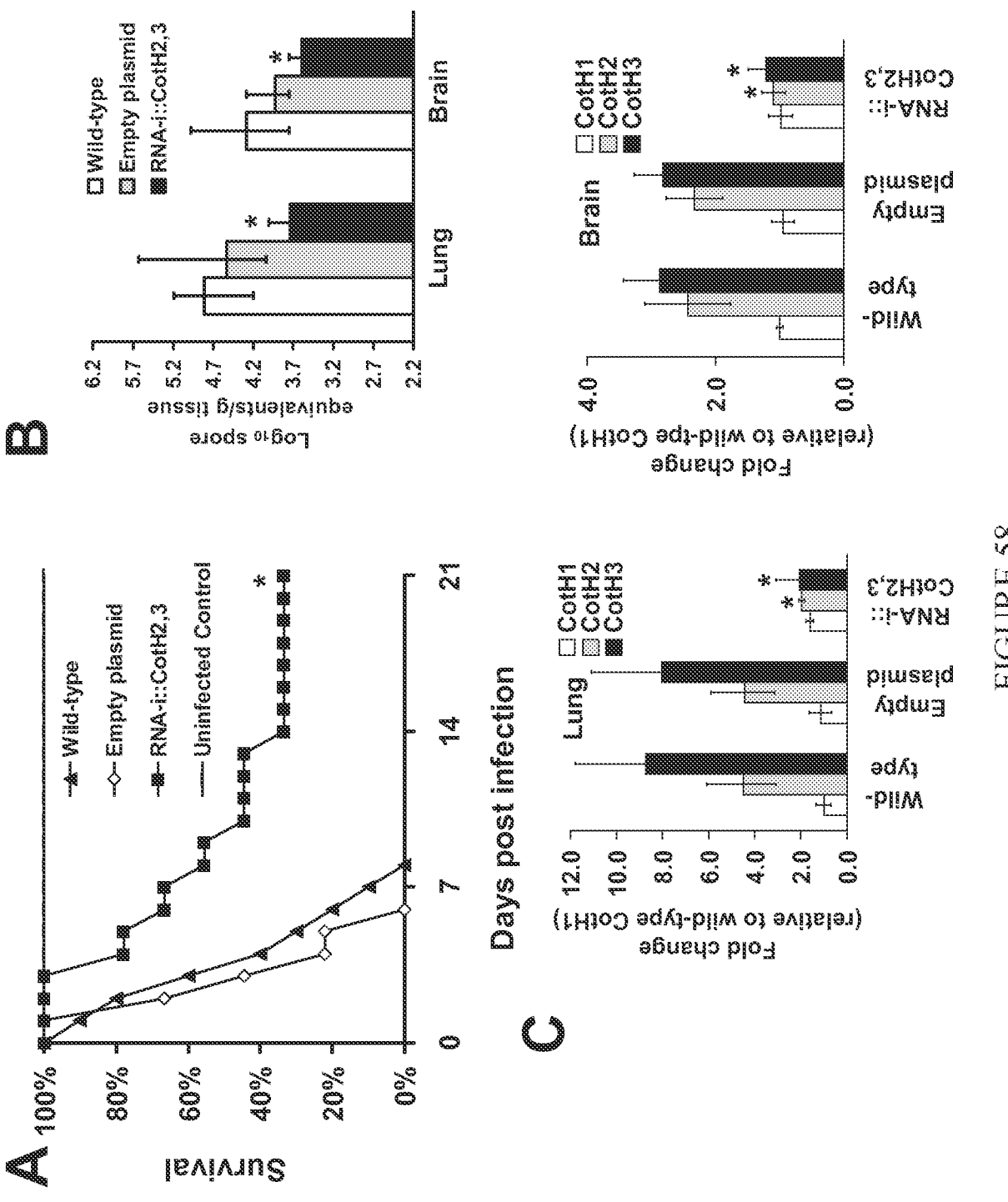

A
Survival
100%
80%
60%
40%
20%
0%
0
7
14
21
Days post infection
Wild-type
Empty plasmid
RNA-i::CotH2,3
Uninfected Control
*
B
Log10 spore equivalents/g tissue
6.2
5.7
5.2
4.7
4.2
3.7
3.2
2.7
2.2
Lung
Brain
Wild-type
Empty plasmid
RNA-i::CotH2,3
*
*
C
Fold change (relative to wild-type CotH1)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Lung
Brain
CotH1
CotH2
CotH3
Wild-type
Empty plasmid
RNA-i::CotH2,3
*
*
*
Fold change (relative to wild-type CotH1)
4.0
2.0
0.0
Wild-type
Empty plasmid
RNA-i::CotH2,3
*
*

FIGURE 58

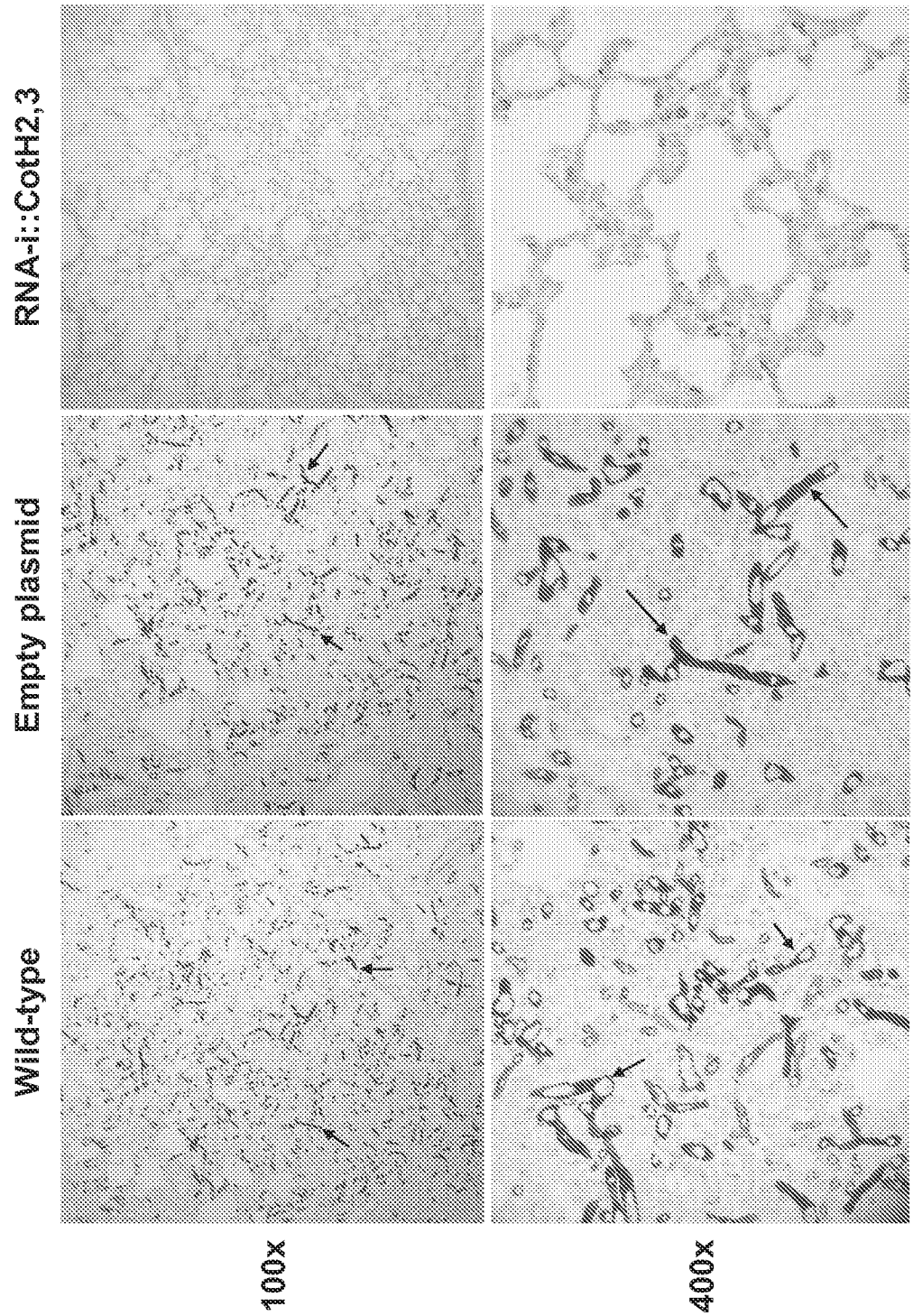


FIGURE 59

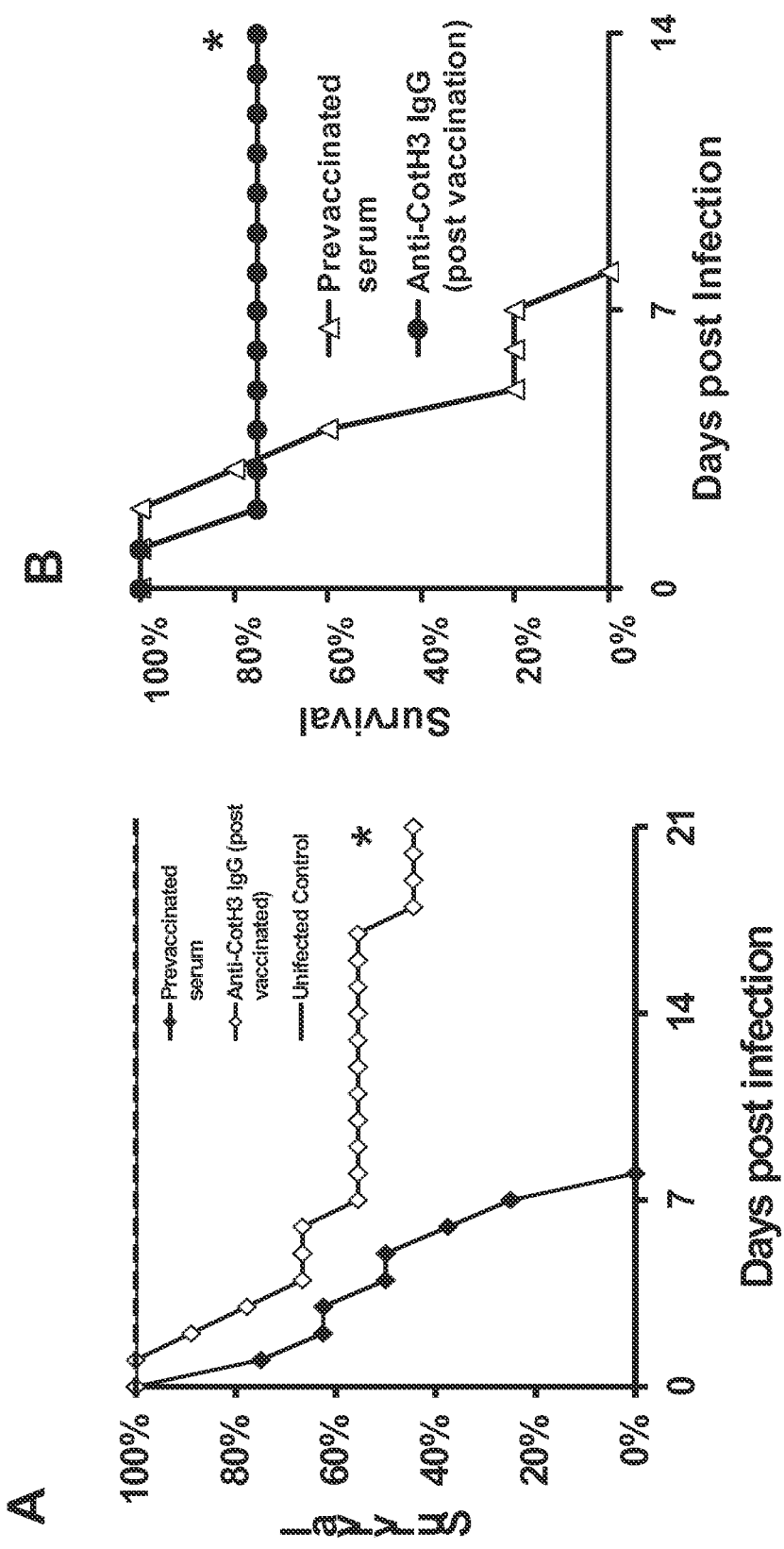

A
100%
80%
60%
40%
20%
0%
Survival
0
7
14
21
Days post infection
Prevaccinated serum
Anti-CotH3 IgG (post vaccinated)
Uninfected Control
*
B
100%
80%
60%
40%
20%
0%
Survival
0
7
14
Days post Infection
Prevaccinated serum
Anti-CotH3 IgG (post vaccination)
*

FIGURE 60

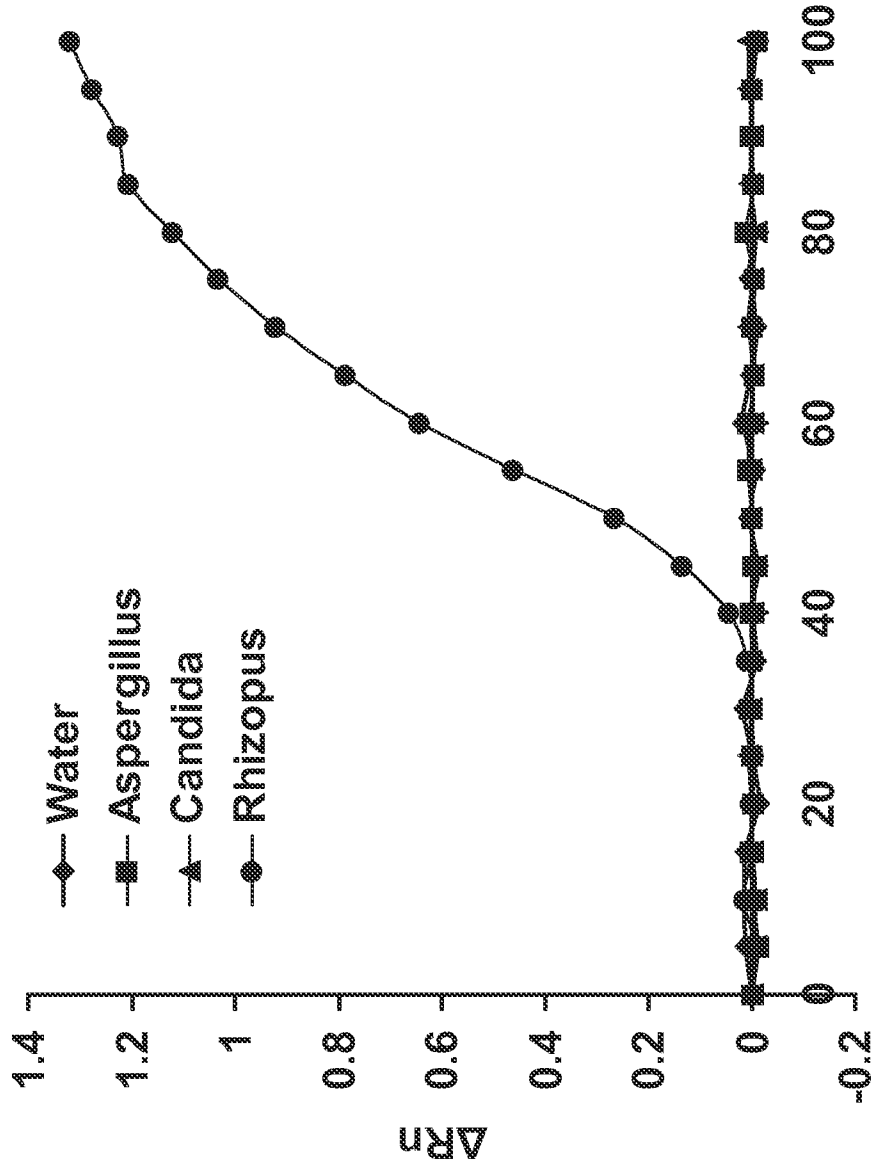

Water
Aspergillus
Candida
Rhizopus
ΔRn
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
20
40
60
80
100
Time (min)

FIGURE 61

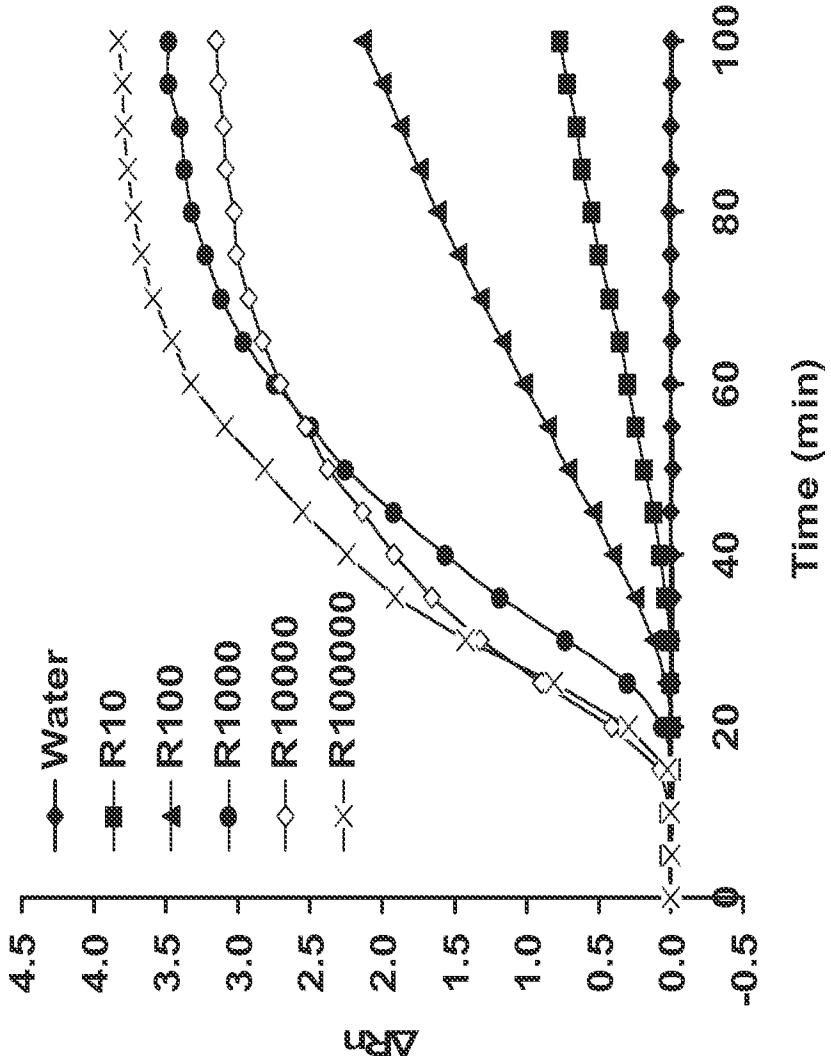

Water
R10
R100
R1000
R10000
R100000
ΔP
Time (min)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
20
40
60
80
100

FIGURE 62

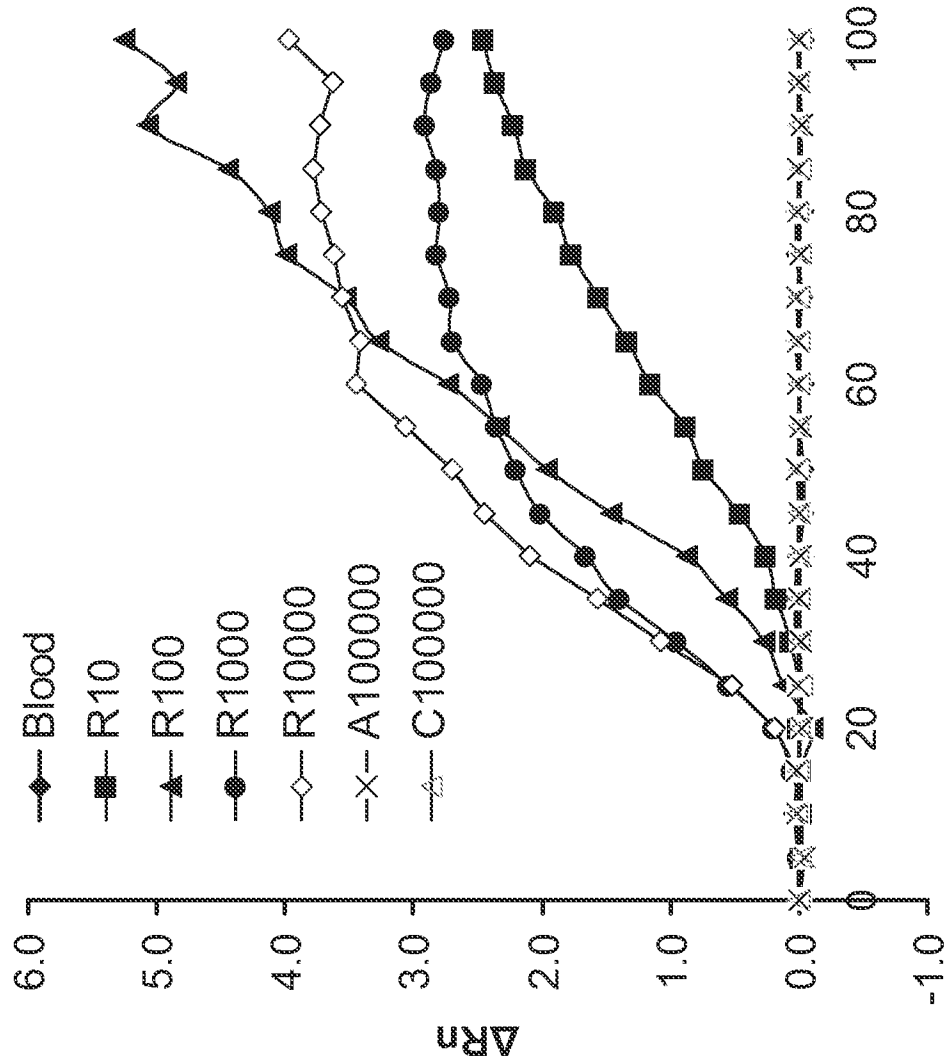

Blood
R10
R100
R1000
R10000
A100000
C100000
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
ΔRn
0
20
40
60
80
100
Time (min)

FIGURE 63

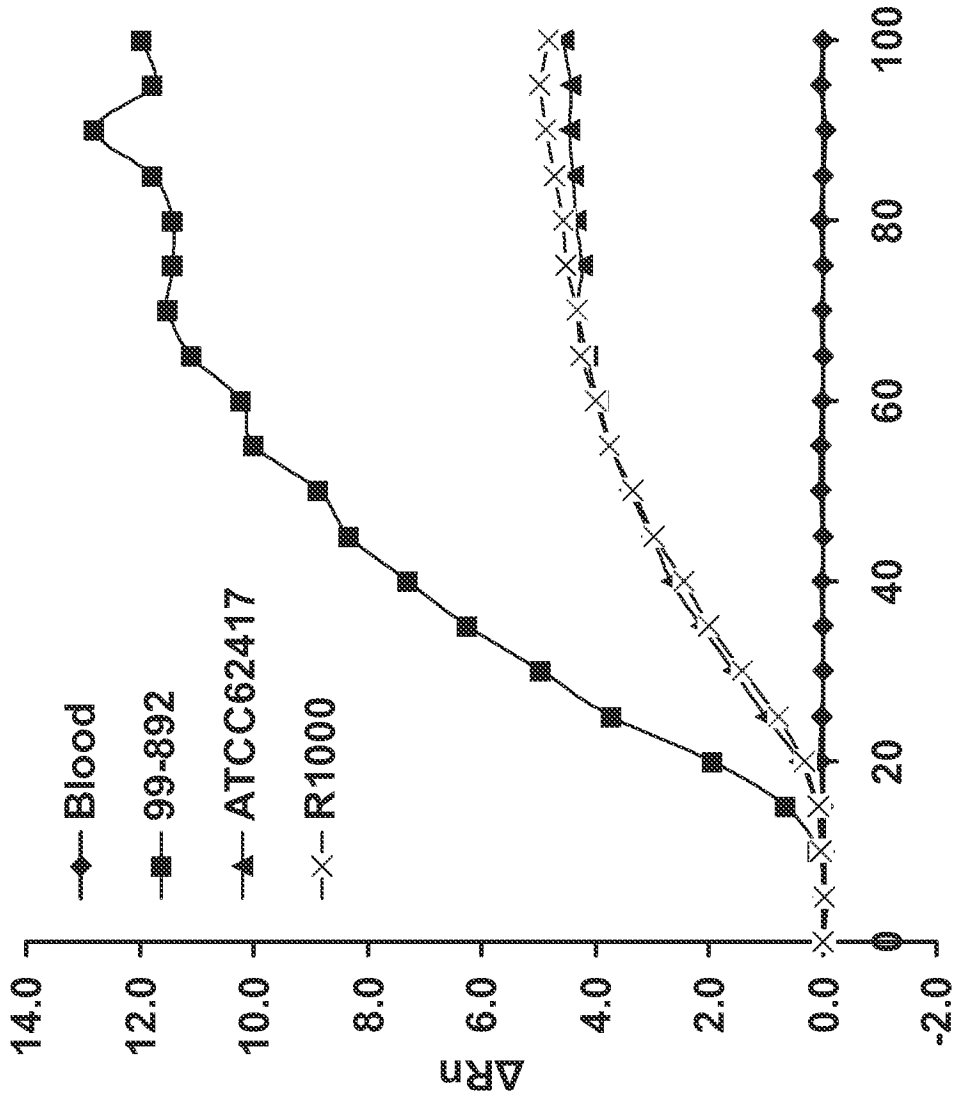

Blood
99-892
ATCC62417
R1000
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
ΔRn
0
20
40
60
80
100
Time (min)

FIGURE 64