

Figure 1S

**Figure 1S.** Antibodies raised against peptide MGQTNDGAYRDPTDNN recognized CotH1, CotH2 and CotH3 proteins heterologously expressed by *S. cerevisiae*. *S. cerevisiae* heterologously expressing the indicated CotH proteins were stained with the anti-CotH antibodies, followed by a FITC labeled antirabbit goat antibody prior to imaging the cells with confocal microscopy. Scale bar = 15 mM.



## Figure 2S

Figure 2S. Anti-CotH antibodies has no effect on respiration, germlings formation or growth rate of *R. oryzae*. *R. oryzae* 99-880 spores were incubated without IgG, with rabbit 10 mg/ml of pre-immune IgG, or Anti-CotH IgG for varying time periods at 37°C. *R. oryzae* respiration was assessed after 6 h incubation with pre-immune IgG or anti-CotH IgG and quantified spectrophotometrically (A, n=12) using XTT-menadione assay. The effect of anti-CotH IgG on germ tube formation (n=50 per arm) was measured microscopically after 6 h incubation with the antibodies (B). To determine the effect of the antibody on growth rate, *R. oryzae* spores ( $10^{7}$ / ml) were incubated with 10 mg/ml IgG for 1,3, or 6 h prior to plating  $10^5$  cells on YPD plates. The diameter of the colony (C, n=6 per arm) was measured after 24 h incubation at 37°C. Data are expressed as median  $\pm$  interquartile range.



Figure 3S. RNA-i construct targeting *CotH2* and *CotH3* has no effect on the growth, the pattern of germination, cell size of or respiration of *R. oryzae*. The two transformants with reduced *CotH2* and *CotH3* expression had similar growth rate on YPD or minimal medium (YNB) without uracil plates (A), germination pattern in YPD medium at 37°C for different time periods (B), cell size as determined by flow cytometry (C), or cell metabolism (D) when compared to the wild-type cells or cells transformed with the empty plasmid. Data in A (n=9 per time point from three experiments), B (n=100 per time point from three experiments) represent median  $\pm$  interquartile ranges.



Congo red

Wild-type

Empty plasmid RNA-i

5.0 10.0 0.0 Diameter of hyphae (mm)

15.0 20.0 25.0

20.0

25.0

SDS





**Calcofluor White** 

Wild-type Empty plasmid RNA-i



Triton x100





0.0 10.0 15.0 20.0 25.0 5.0 Diameter of hyphae (mm)

## Figure 4S

Figure 4S. RNA-i construct targeting *CotH2* and *CotH3* did not alter the cell wall integrity of *R. oryzae*. *R. oryzae* 99-880 (wild-type), *R. oryzae* transformed with empty plasmid or RNA-i construct were grown at 10 fold dilution  $(10^5-10^2)$  on YNB medium without uracil containing the cell wall stressors Congo red (2 mg/ml), calcofluor white (0.1 mg/ml), hydrogen peroxide, SDS (0.004%) or Triton x100 (0.1%). Plates were incubated for 16 h at 37°C before measuring the diameter of growth of the. Data (n=6) are presented as the average colony diameter (mm)  $\pm$  SD of the 10<sup>5</sup> inoculum.



Figure 5S. Attenuation of *CotH2* and *CotH3* expression did not alter susceptibility of *R. oryzae* to mouse macrophage cells lines or mouse neutrophils (PMNs). *R. oryzae* spores (10<sup>4</sup>) transformed with empty plasmid or RNA-i construct were grown with mouse macrophage cell RAW or J774 ( $2 \times 10^5$ ), or primary neutrophils ( $1 \times 10^5$ ) to give an target:effector ratio of 1:20 or 1:10, respectively. The cells were co-incubated for 6 h at 37<sup>o</sup>C prior to determining macrophage-mediated cell damage by using the XTT assay. Data is presented as median optical density of 8 replica from two independent experiments  $\pm$  interquartile range.

## Figure 5S