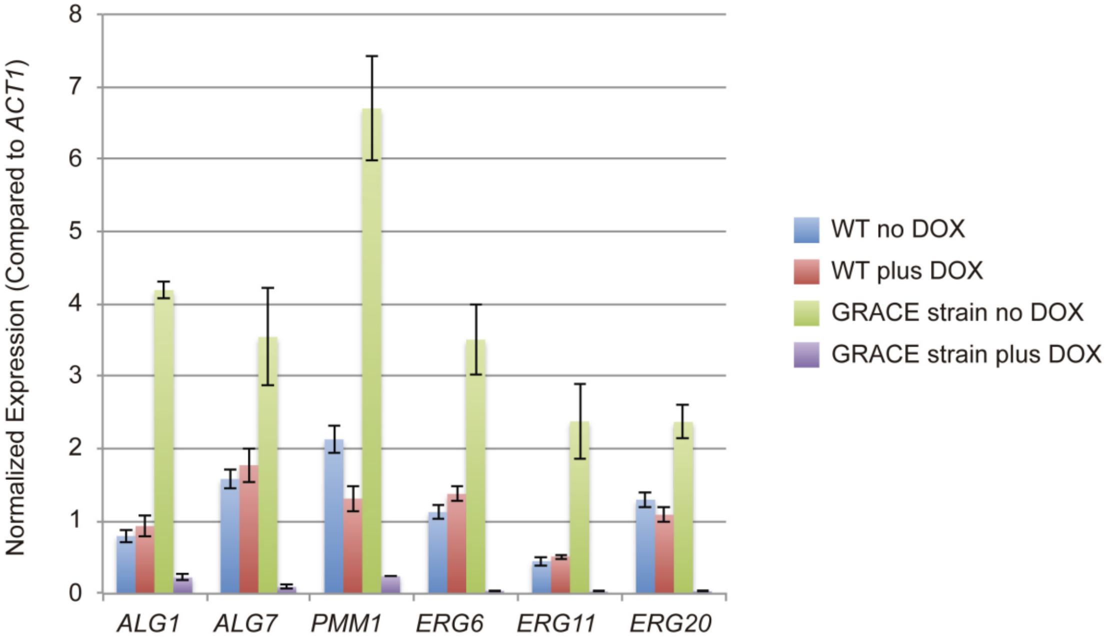
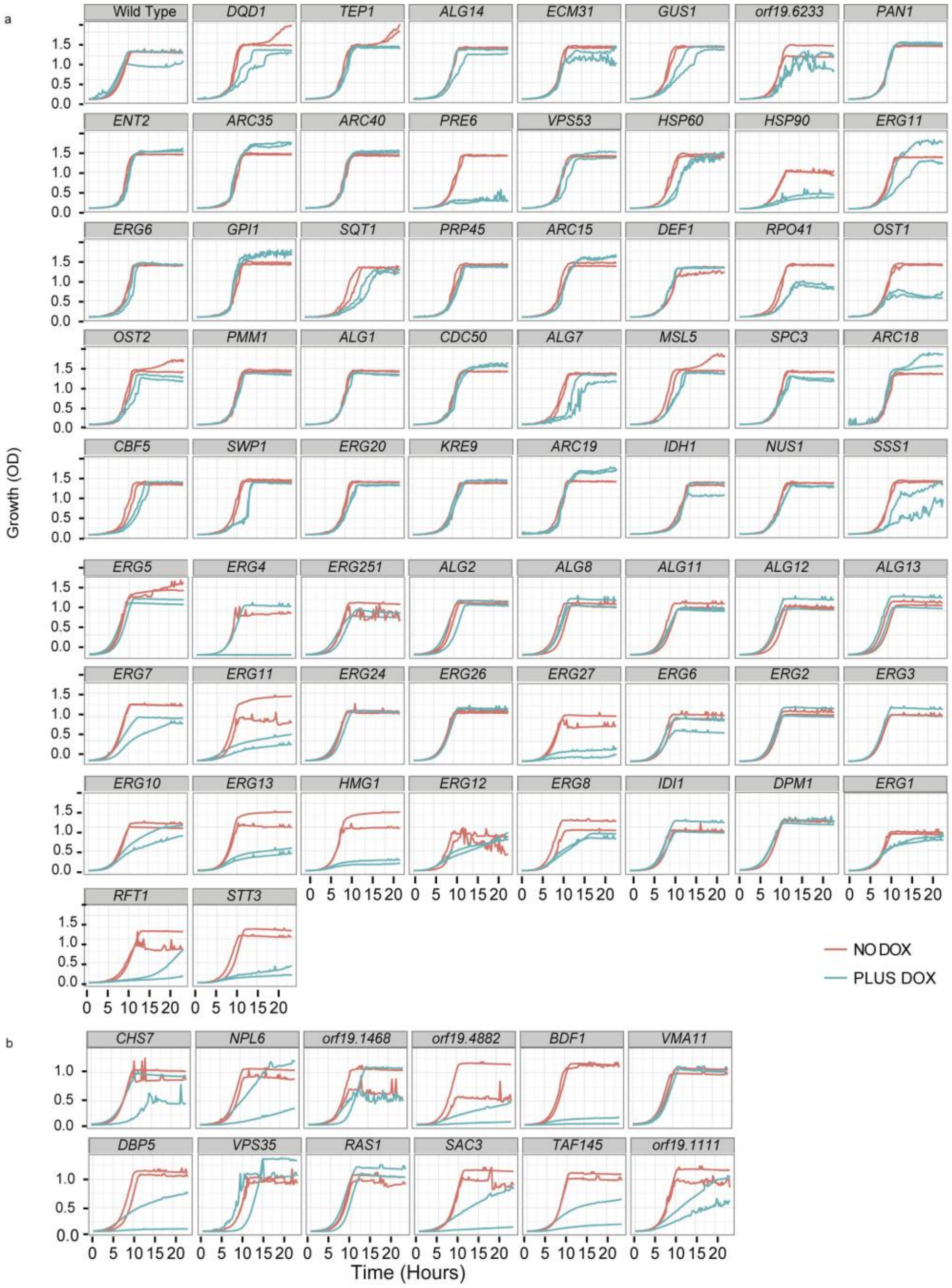
# Supplementary Information

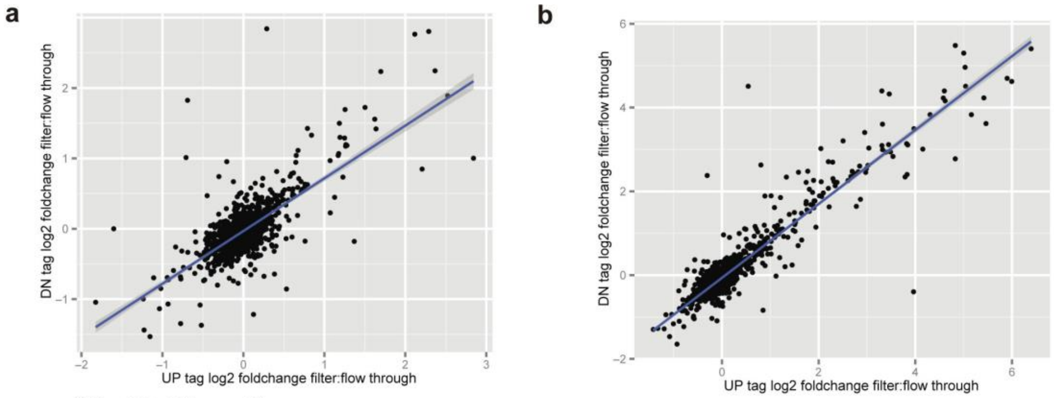


**Supplementary Figure 1:** Target gene transcript levels are depleted upon treatment with doxycycline (DOX). Strains were grown in the absence or presence of 0.05 µg mL$^{-1}$ DOX overnight, subcultured into fresh medium of the same condition and grown to mid-log phase. Target gene transcript levels were normalized to *ACT1*. Data are means +/- S.D. for triplicate samples. Figure represents one of two biological replicates.



**Supplementary Figure 2:** Growth kinetics of GRACE strains. Strains were inoculated from overnight cultures into 96-well culture plates in the absence (in blue) and presence of DOX (in red) and incubated with shaking in a TECAN plate reader at 37°C. The OD600 values were measured every 15 minutes for 24 hours. Each graph displays two biological replicates performed in triplicate. **(A)** Strains grown in 0.05 μg mL$^{-1}$ DOX. **(B)** Strains grown in 0.5 μg mL$^{-1}$ DOX.

**Supplementary Figure 3:** Correlation between virulence in mice and morphology score in response to serum. Color bar indicates growth in rich medium. Morphology scores indicate the degree of filamentation, where 0 indicates yeast form and 3 indicates filaments. Virulence data from Becker *et al.* [1]

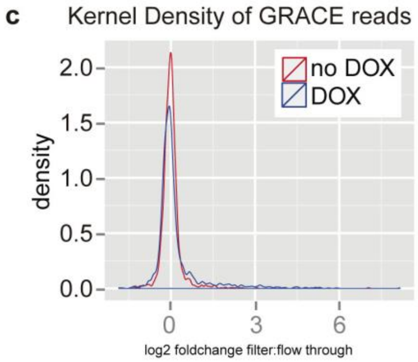


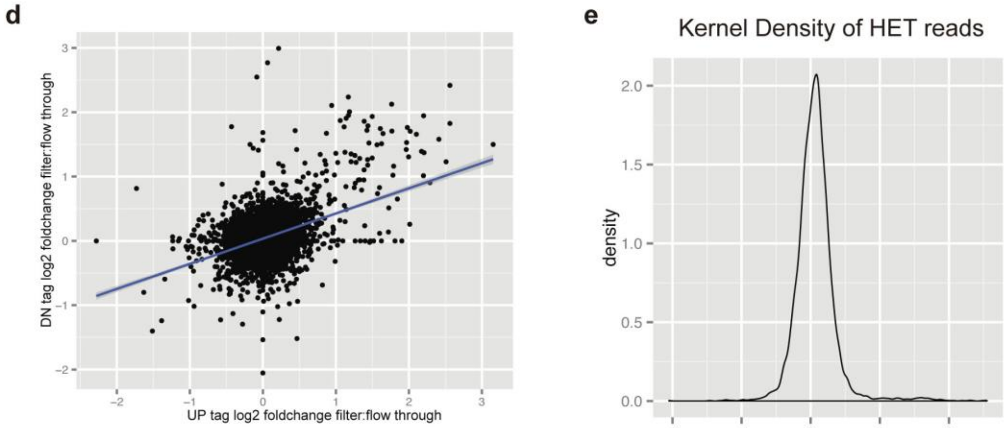
0 Dox UP v DN correlation

Residual standard error: 0.2405 on 1229 degrees of freedom
Multiple R-squared: 0.5136,
Adjusted R-squared: 0.5132
F-statistic: 1298 on 1 and 1229 DF, p-value: < 2.2e-16

0.5 Dox UP v DN correlation

Residual standard error: 0.3334 on 1216 degrees of freedom
Multiple R-squared: 0.8575,
Adjusted R-squared: 0.8574
F-statistic: 7320 on 1 and 1216 DF, p-value: < 2.2e-16

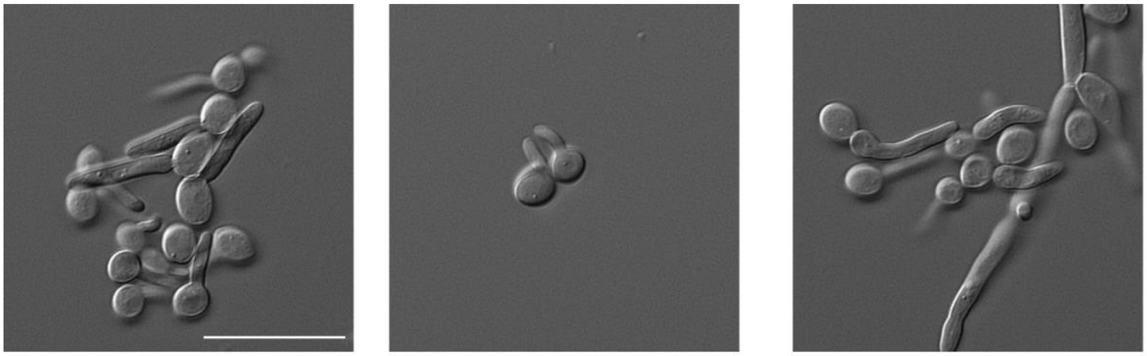




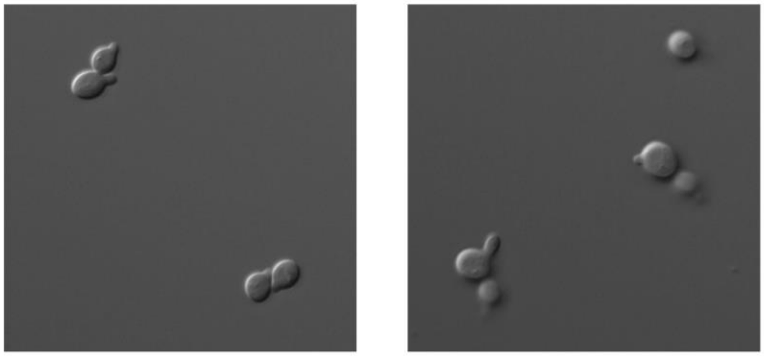
HET UP vs DN correlation
Residual standard error: 0.2946 on 5283 degrees of freedom
Multiple R-squared: 0.1728,
Adjusted R-squared: 0.1727
F-statistic: 1104 on 1 and 5283 DF, p-value: < 2.2e-16

**Supplementary Figure 4:** Correlation between the $\log_2$ fold-change of normalized UPTAG and DNTAG barcode reads in the GRACE strains for the 0 μg $mL^{-1}$ DOX **(A)** and 0.5 μg $mL^{-1}$ DOX **(B)** treated pooled samples. **(C)** Kernal density graph of averaged

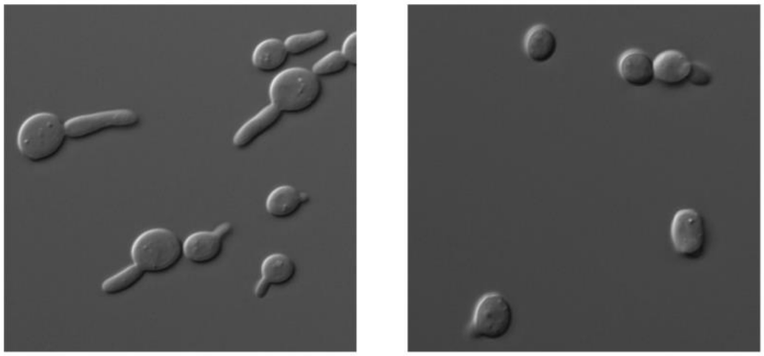
UPTAG and DNTAG $\log_2$ reads in the GRACE strains for the 0 μg $mL^{-1}$ DOX and 0.5 μg $mL^{-1}$ DOX samples. **(D)** Correlation between the $\log_2$ fold-change of normalized UPTAG and DNTAG barcode reads between filter retailed and flow-through HET strains. **(E)** Kernal density graph of averaged UPTAG and DNTAG $\log_2$ reads in the HET strains.



Wild Type *PCL1/pcl1* *NRG1/nrg1*

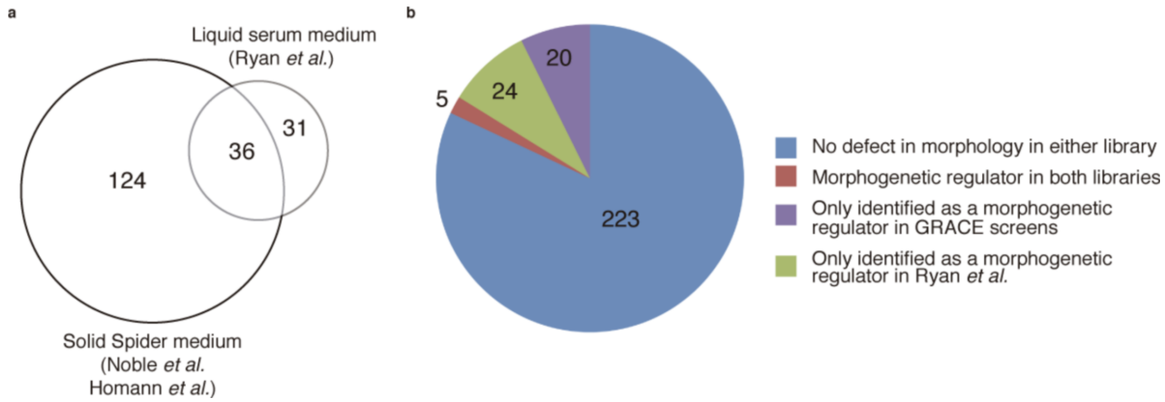


*RPL20B/rpl20b* *ORF19.4612/orf19.4612*

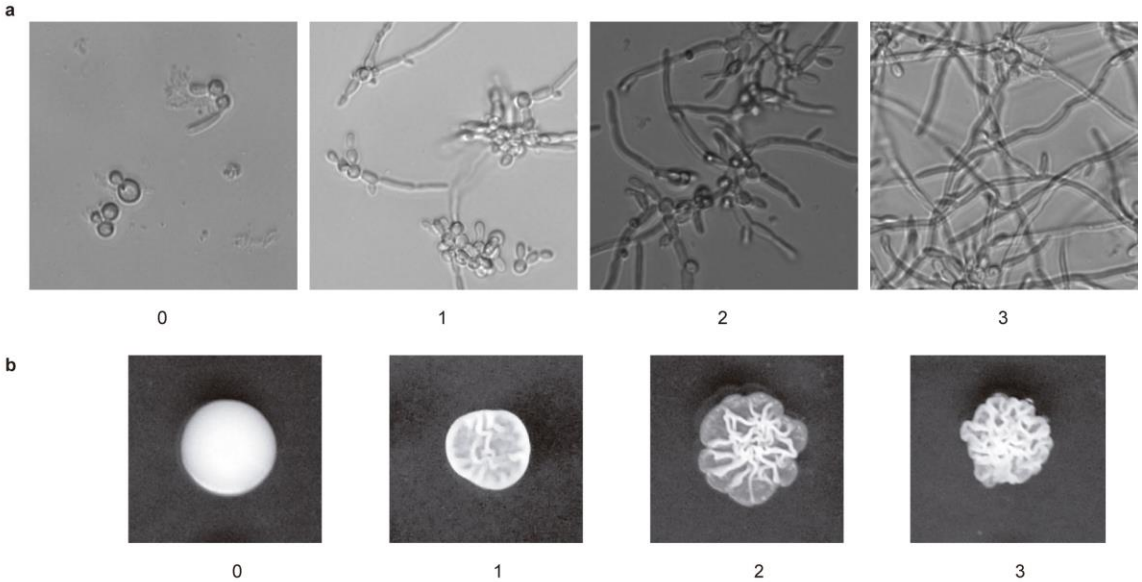


*ORF19.5730/orf19.5730* *ORF19.199/orf19.199*

**Supplementary Figure 5:** Pooled analysis of the HET collection revealed mutants with altered morphogenesis. Phenotypes were verified after incubation for 30 minutes at 37°C in medium containing 1% v/v serum. All images taken at 40X magnification. Scale bar represents 20 μm.



**Supplementary Figure 6:** Comparison of morphogenetic regulators identified in different studies. **(A)** Comparison of morphogenetic regulators identified in screens on Spider solid performed by Noble *et al.*[2] and Homann *et al.*[3] and those identified with the same libraries by Ryan *et al.*[6] in serum liquid conditions. (**B)** Comparison of morphogenetic regulators identified for mutants covered in the GRACE library and in the screens performed by Ryan *et al.*[4].



**Supplementary Figure 7: (A)** Microscopy images were scored from 0 to 3, based on the degree of filamentation. Representative images of each score are displayed here. (**B)** Solid plate filamentation was scored from 0 to 3, based on the colony morphology. Representative images of each score are displayed here.

# Supplementary Table 1: Macrophage lysis and morphology scores

| GRACE strain | No DOX | | Plus DOX | |
|---|---|---|---|---|
| | Lysis Rate (%) | Morphology | Lysis Rate (%) | Morphology |
| *TAF145* | 18.36 | filament | 0.61 | pseudohyphae |
| *BDF1* | 21.52 | filament | 0.69 | yeast |
| *orf19.1111* | 17.73 | filament | 0.74 | short filaments |
| *DBP5* | 19.14 | filament | 0.9 | yeast |
| *orf19.4882* | 17.65 | filament | 1.03 | yeast |
| *NPL6* | 14.6 | filament | 1.15 | short filaments |
| *VMA11* | 15.05 | filament | 2.17 | yeast |
| *VPS35* | 15.92 | filament | 2.59 | filament |
| *RFT1* | 18.1 | filament | 2.84 | hyperfilament |
| *SPC3* | 21.15 | filament | 3.03 | yeast |
| *ERG11* | 20.54 | filament | 3.39 | pseudohyphae |
| *CBF5* | 20.85 | filament | 3.48 | yeast |
| *PRE6* | 18.54 | filament | 3.61 | pseudohyphae |
| *RPO41* | 8.33 | filament | 4 | yeast |
| *ERG7* | 16.94 | filament | 4.36 | yeast |
| *SWP1* | 15.17 | filament | 4.38 | yeast |
| *ERG8* | 15.67 | filament | 4.41 | yeast |
| *STT3* | 17.73 | filament | 4.73 | yeast |
| *DQD1* | 23.51 | filament | 5.36 | yeast |
| *GUS1* | 23.32 | filament | 5.84 | yeast |
| *HSP60* | 22.77 | filament | 6.04 | yeast |
| *ERG6* | 25.27 | filament | 6.28 | yeast |
| *KRE9* | 18.1 | filament | 6.28 | yeast |
| *OST1* | 18.58 | filament | 6.38 | yeast |
| *SQT1* | 28.92 | filament | 6.75 | short filaments |
| *RAS1* | 21.09 | filament | 6.95 | short filaments |
| *ARC40* | 22.7 | filament | 7.65 | pseudohyphae |
| *PRP45* | 13.5 | filament | 8.14 | yeast |
| *WBP1* | 16.61 | filament | 8.2 | yeast |
| *NUS1* | 16.14 | filament | 8.28 | yeast |
| *GPI16* | 22.99 | filament | 8.33 | yeast |
| *IDH1* | 14.29 | filament | 8.41 | filament |
| *ARC15* | 23.14 | filament | 8.67 | pseudohyphae |
| *ALG14* | 15.26 | filament | 8.72 | pseudohyphae |
| *DEF1* | 21.58 | filament | 9.29 | short filaments |
| *ARC18* | 18.92 | filament | 9.68 | pseudohyphae |
| *CDC50* | 21.74 | filament | 9.7 | yeast |
| *ERG20* | 19.93 | filament | 9.7 | yeast |

| | | | | |
|---|---|---|---|---|
| *SSS1* | 17.83 | filament | 9.95 | yeast |
| *HSP90* | 22.29 | filament | 10.32 | short filaments |
| *ENT2* | 23.4 | filament | 10.45 | pseudohyphae |
| *VPS53* | 22.64 | filament | 10.51 | short filaments |
| *MSL5* | 15.87 | filament | 10.55 | yeast |
| *OST2* | 20.15 | filament | 10.84 | yeast |
| *ALG7* | 18.9 | filament | 12.38 | yeast |
| *PAN1* | 18.69 | filament | 12.47 | pseudohyphae |
| *ALG11* | 12.9 | filament | 12.6 | yeast |
| *ECM31* | 21.64 | filament | 12.62 | yeast |
| *ARC35* | 22.16 | filament | 12.68 | pseudohyphae |
| *ARC19* | 12.88 | filament | 12.79 | pseudohyphae |
| *PMM1* | 19.87 | filament | 13.33 | yeast |
| *SAC3* | 17.02 | filament | 13.61 | short filaments |
| *orf19.6233* | 25.98 | filament | 15.98 | yeast |
| *CDC55* | 20.6 | filament | 16.67 | pseudohyphae |
| *TEP1* | 30.95 | filament | 21.67 | filament |
| WT | 24.63 | filament | 24.02 | filament |
| *ALG1* | 24.25 | filament | 24.51 | yeast |
| *BMT3* | 21.7 | filament | 25.19 | filament |
| *GPI10* | 23.65 | filament | 21.86 | filament |
| *GWT1* | 21.39 | filament | 20 | short filaments |
| *MCD4* | 19.33 | filament | 11.73 | short filaments |
| *MNN1* | 23.93 | filament | 24.6 | short filaments |
| *MNN24* | 21.94 | filament | 23.74 | filament |
| *MNT1* | 21.96 | filament | 23.51 | filament |
| *MNN2* | 26.29 | filament | 20.2 | filament |
| *OCH1* | 22.18 | filament | 23.53 | short filaments |
| *PMT1* | 16.52 | filament | 17.07 | filament |
| *VAN1* | 25.88 | filament | 24.67 | filament |

## Supplementary Table 2: Macrophage lysis rates in response to pre-treatments and species

| Strain | Pre-incubation | Treatment | Average Lysis Rate (%) | Standard Deviation | Comments |
|---|---|---|---|---|---|
| WT | YEPD | live | 19.02 | 1.73 | |
| WT | YEPD | heat killed | 3.2 | 1.85 | |
| WT | conditioned media | heat killed | 3.11 | 0.49 | |
| WT | 30min phagocytosis | heat killed | 6.34 | 1.67 | |
| WT | 60min phagocytosis | heat killed | 17.51 | 4.84 | |
| WT | 60min phagocytosis | formalin killed | 13.95 | 5.13 | |
| WT | 90min phagocytosis | heat killed | 18.37 | 2.16 | |
| WT | unphagocytized | heat killed | 5.66 | 1.14 | |
| WT | EndoH | heat killed | 4.49 | 2.69 | |
| *ALG1* | unphagocytized | heat killed | 5.66 | 1.61 | |
| *ALG1* | 60min phagocytosis | heat killed | 19.82 | 1.97 | |
| *ALG1* | EndoH | heat killed | 5.33 | 4.65 | |
| *orf19.6233* | 60min phagocytosis | heat killed | 16.38 | 2.16 | |
| *orf19.6233* | 60min phagocytosis, fomalin killed | formalin killed | 14.18 | 3.08 | |
| *orf19.6233* | EndoH | heat killed | 4.72 | 3.43 | |
| *OST1* | unphagocytized | heat killed | 1.38 | 1.96 | |
| *OST1* | 60min phagocytosis | heat killed | 5.1 | 3.79 | |
| *ERG6* | 60min phagocytosis | heat killed | 5.91 | 0.56 | |
| *S. cerevisiae* | unphagocytized | heat killed | 3 | 4.24 | |
| *S. cerevisiae* | 60min phagocytosis | heat killed | 5.34 | 1.4 | |
| *C. neoformans* WT | unphagocytized | heat killed | 0 | 0 | very few phagocytosis events |
| *C. neoformans* WT | 60min phagocytosis | heat killed | 2.56 | 3.63 | very few phagocytosis events |
| *C. neoformans cap59* | YEPD | live | 7.97 | 1.16 | |
| *C. neoformans cap59* | unphagocytized | heat killed | 1.6 | 0.8 | |
| *C. neoformans cap59* | 60min phagocytosis | heat killed | 13.92 | 3.97 | |
| *C. neoformans cap59* | EndoH | heat killed | 1.56 | 2.21 | |

## Supplementary Table 3: Oligonucleotides used in this study

| Primer | Sequence (5’ to 3’) |
|---|---|
| oLC1131- *ERG11*- Forward | GATGTTTCTGCTGAAGATGC |
| oLC1132- *ERG11*- Reverse | ATAGTTGAGCAAATGAACGG |
| oLC2285- *ACT1*-Forward | GACCTTGAGATACCCAATTG |
| oLC2286- *ACT1*- Reverse | CAGCTTGAATGGAAACGTAG |
| oLC3546- *ALG1*-Forward | CTCAACTTCATTTACTCCTG |
| oLC3547- *ALG1*-Reverse | GTACTTTAGGATAGTCCTCTG |
| oLC3548- *PMM1*- Forward | CACCAATTGGTAGAAATGCT |
| oLC3549- *PMM1*- Reverse | GTTCATCTTCAACGTGTTGT |
| oLC3550- *ALG7*- Forward | TTTGTCGGAGTGTCATTGGC |
| oLC3551- *ALG7*- Reverse | CTTGGACAGGGCAAGATGTG |
| oLC3552- *ERG6*- Forward | TTATGCCATTGAAGCTACCG |
| oLC3553- *ERG6*- Reverse | GCTTGTTCAGCAACTTTACG |
| oLC3554- *ERG20*- Forward | TAGAAGGGGCCATTTATATC |
| oLC3555- *ERG20*- Reverse | CCAATTTGTTCTGGAGTACC |
| UPTAG universal amplification primer | AATGATACGGCGACCACCGAGATCTACACCGAGGTCGAGAATGATGTCCACGAGGTCTCT |
| UPTAG index amplification primer | CAAGCAGAAGACGGCATACGAGATNNNNNNGCCATTTGTCTGTCGACCTGCAGCGTACG |
| DNTAG universal amplification primer | AATGATACGGCGACCACCGAGATCTACACCACATGATATGTTGAGCGGTGTCGGTCTCGTAG |
| DNTAG index amplification primer | CAAGCAGAAGACGGCATACGAGATNNNNNNGAGTATCTGTATCTGGCC GAGCTCGAATTCATCGAT |
| UPTAG sequencing primer | CGAGGTCGAGAATGATGTCCACGAGGTCTCT |
| DNTAG sequencing primer | CACATGATATGTTGAGCGGTGTCGGTCTCGTAG |
| UPTAG index sequencing primer | CGTACGCTGCAGGTCGACAGACAAATGGC |
| DNTAG index sequencing primer | ATCGATGAATTCGAGCTCGGCCAGATACAGATACTC |

## Supplementary References:

1. Becker, J. M. *et al.* Pathway analysis of *Candida albicans* survival and virulence determinants in a murine infection model. *Proc Natl Acad Sci* **107,** 22044–22049 (2010).
2. Noble, S. M., French, S., Kohn, L. A., Chen, V. & Johnson, A. D. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nat Genet* **42,** 590–598 (2010).
3. Homann, O. R., Dea, J., Noble, S. M. & Johnson, A. D. A phenotypic profile of the *Candida albicans* regulatory network. *PLoS Genet* **5,** e1000783 (2009).
4. Ryan, O. *et al.* Global gene deletion analysis exploring yeast filamentous growth. *Science* **337,** 1353–1356 (2012).