Supporting Information

Global secretome characterization of the pathogenic yeast *Candida glabrata*

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Supporting Figures S1 to S4

Supporting Tables S1 to S28

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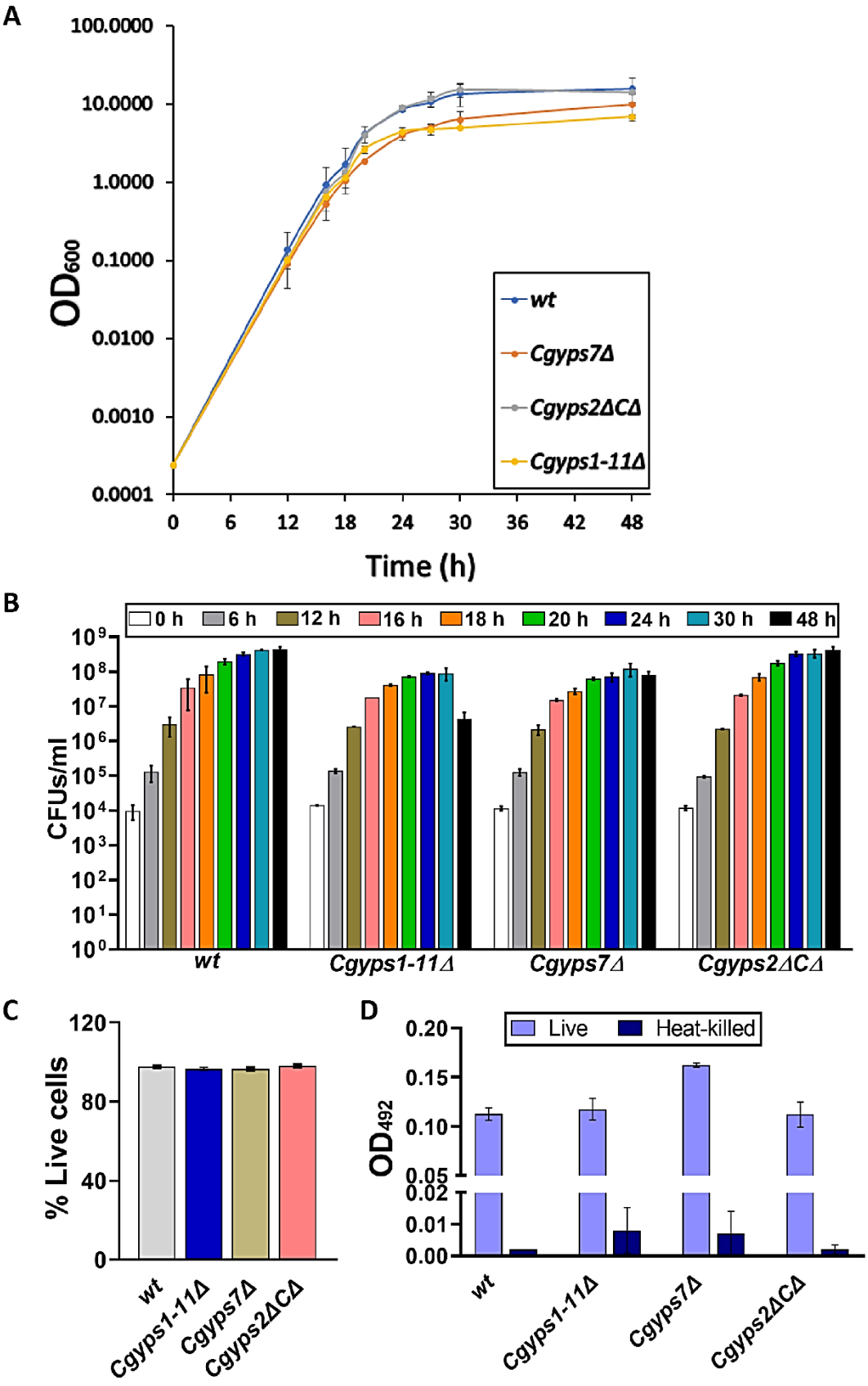
Table S28: Enriched GO terms for biological process (BP), cellular component (CC) and molecular function (MF) categories for the *Cgyps2ΔCΔ* secretome, as determined by the FungiFun tool.

Figure S1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18
24
30
36
42
48
Time (h)
wt
Cgyps7Δ
Cgyps2ΔCΔ
Cgyps1-11Δ
B
0 h
6 h
12 h
16 h
18 h
20 h
24 h
30 h
48 h
CFUs/ml
wt
Cgyps1-11Δ
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% Live cells
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80
40
0
wt
Cgyps1-11Δ
Cgyps7Δ
Cgyps2ΔCΔ
D
Live
Heat-killed
OD492
0.20
0.15
0.10
0.05
0.02
0.01
0.00
wt
Cgyps1-11Δ
Cgyps7Δ
Cgyps2ΔCΔ

Figure S1: Similar growth and viability profiles of *C. glabrata wild-type* (*wt*) and *Cgyps1-11*Δ strains at the time of secretome collection.

A. Growth curve analysis. Indicated *C. glabrata* strains were grown overnight in YPD medium and inoculated at an initial OD_{600} of 0.0002 in YNB medium. The OD_{600}, once reached the measurable range of 0.1, was monitored at regular intervals up to 48 h. Absorbance data are plotted against time and represent mean ± SD of two independent experiments. Secretome collection was done from cultures, once they showed an OD_{600} of 1.2 to 1.5.

B. CFU-based cell viability analysis: At indicated time intervals, an aliquot of *C. glabrata* cultures, from the time-course analysis shown in panel A, was taken, diluted appropriately in PBS and plated on YPD medium. After multiplying the number of colonies appeared by dilution factor, data were plotted, and represent mean ± SD of two independent experiments. No statistically significant differences were observed between *wt* and *Cgyps* mutant CFUs at the time of inoculation (0 h) or secretome collection (16-20 h). Consistent with an earlier report (Bairwa and Kaur, *Mol Microbiol* **79:** 900-913, 2011), viability loss for *Cgyps1-11*Δ mutant was observed under post-diauxic shift conditions between 30 and 48 h.

C. Methylene blue staining-based cell viability analysis: Indicated *C. glabrata* strains were grown overnight in YPD medium and inoculated at an initial OD_{600} of 0.0002 in YNB medium. At 18 h post inoculation, 2 ml culture, corresponding to ~ 2 OD_{600}, was taken, and cells were pelleted down and stained with 30 µl of 1 X methylene blue solution (Qualigens # 38883). After 15 min, cells were mounted on a slide and visualized using 100 X objective (Nikon Eclipse 80i). A minimum of 500 cells were counted in five arbitrary chosen fields, and the number of live (unstained) and dead cells (darkly stained) recorded. Data (mean ± SD; n=2) represent the of % live cells in *C. glabrata* cultures.

D. XTT assay-based cell viability analysis: Indicated *C. glabrata* strains were grown overnight in YPD medium and inoculated at an initial OD_{600} of 0.0002 in YNB medium. At 18 h post inoculation, 0.5 OD_{600} cells were taken and treated with 50 µl of XTT sodium salt solution (1 mg/ml) and 4 µL of menadione solution (250 mM; prepared in acetone). After incubation at 37°C for 5 h with constant shaking, cells were spun down and absorbance at 492 nm, as a read-out of amount of formazan produced, was measured, Head-killed cells, prepared by incubation at 95°C for 20 min, were used as a negative control. Data represent mean ± SD of two independent experiments.

Figure S2

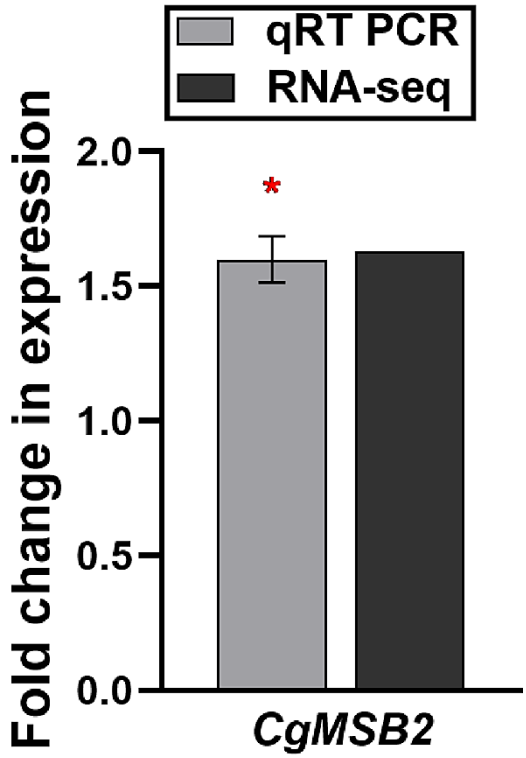

Figure S2: The *CgMSB2* gene is modestly upregulated in the *Cgyps1-11*Δ mutant. Using the acid phenol extraction method, total RNA was extracted from log-phase *wt* and *Cgyps1-11*Δ cultures. *CgMSB2* (*CAGL0F08833g*) transcript levels were measured by qPCR. Data (mean ± S.E., n = 3) were normalized against the *CgACT1* mRNA control, and represent fold increase in expression in the *Cgyps1-11*Δ mutant compared to the *wt* strain. The second bar represents *CgMSB2* gene expression, as reported previously in RNA-sequencing-based transcriptional profiling of log-phase *Cgyps1-11*Δ cells [Rasheed *et al., J Biol Chem* **293:**6410-6433, 2018]. *, $p<0.05$, paired two tailed student's t-test.

Figure S3

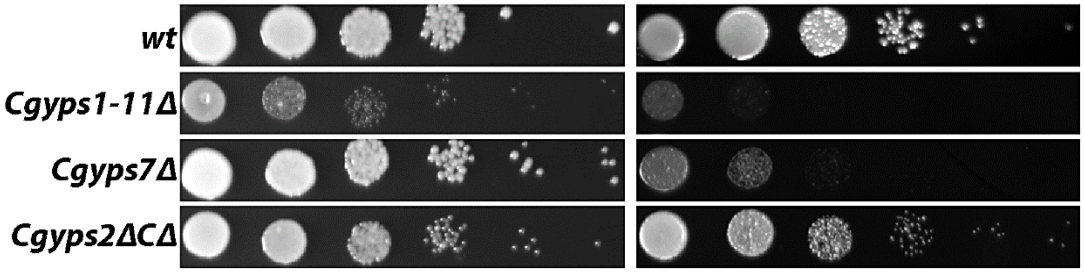

Figure S3: The *Cgyps1-11*Δ and *Cgyps7*Δ mutants show enhanced sensitivity to the cell wall stressor congo red. Indicated *C. glabrata* strains were grown overnight in YPD medium and OD_{600} was normalized to 1.0. Cultures were 10-fold serially diluted, and 3 µl of each dilution were spotted on the YPD medium lacking or containing congo red (2 mg/ml). Plates were incubated at 30˚C for 1-2 days, and images were captured.

Figure S4

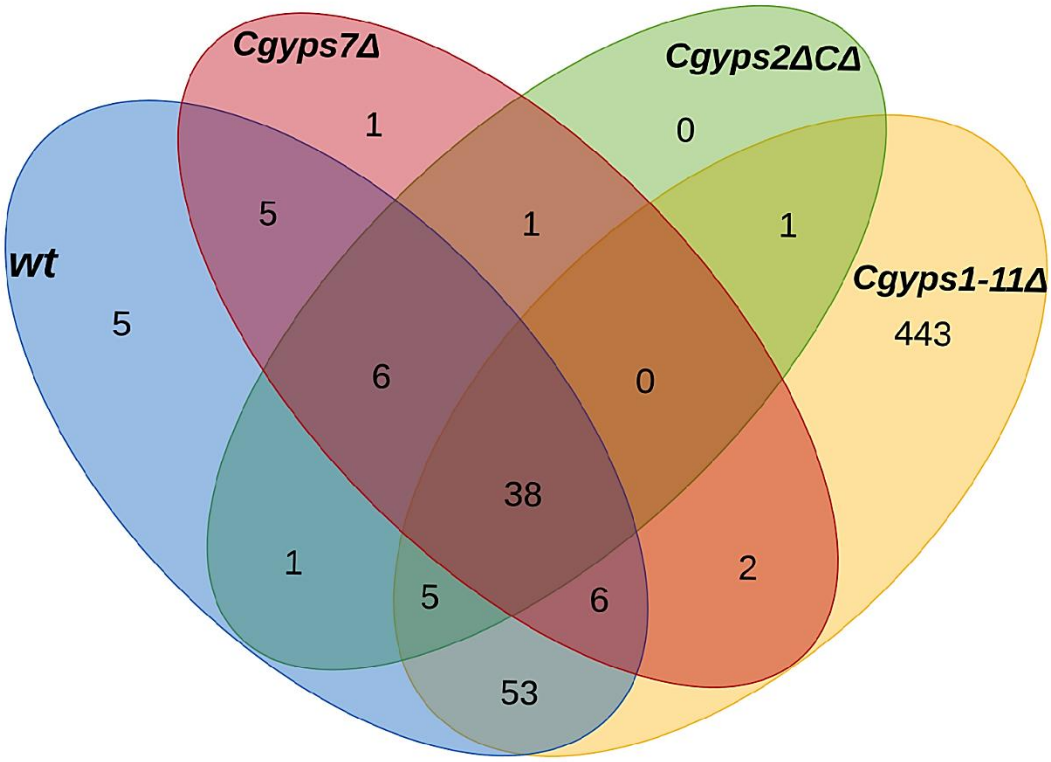

Figure S4: Comparative analysis of global secretomes of *wild-type, Cgyps1-11*Δ, *Cgyps7*Δ and *Cgyps2*Δ*C*Δ strains. Venn diagram illustrating overlap in proteins identified in global secretomes of *wild-type* (*wt*), *Cgyps1-11*Δ, *Cgyps7*Δ and *Cgyps2*Δ*C*Δ strains.