**Title: A set of diverse genes influence the frequency of white-opaque switching in *Candida albicans***

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**File S3:** Supplemental Information, consisting of Supplemental Materials and Methods and Supplemental Results. Contains details on comparisons between white-to-opaque and opaque-to-white switching frequencies, GO terms, and genes whose transcripts are differentially regulated between cell types.

**SI Materials and Methods**

**GO Term and Existing Dataset Enrichment Testing**

Subsets of genes associated with a given function, component, or process were created using the GO-SLIM mapping tool at the *Candida* Genome Database by taking all Function, Component, and Process entries with at least ten genes from our library assigned to them (as well as the “peptidase activity” functional group which had only nine genes assigned to it). There were a total of 42 GO-SLIM gene sets (ten Function, 11 Component, 21 Process) resulting from this process. We also developed groups of genes previously reported to exhibit either two- or four-fold cell type enrichment and/or that have a She3-associated transcript. Finally, we also considered genes with five-fold switching effects in the opposite direction (e.g. genes with a white-to-opaque effect that also had an opaque-to-white effect) for a total of 48 comparisons for both the white-to-opaque and opaque-to-white directions. A five-fold change in switching rate threshold was used for all comparisons. We then evaluated whether the ability of each set of genes to predict a change in switching rates was significantly different from that expected by chance using the Hypergeometric Distribution. All of the comparisons for a given type of assay (e.g. all gene sets and white-to-opaque switching assay results) were pooled for the multiple comparisons correction step, giving a number of hypotheses, m, of 48 for both the white-to-opaque switching and the opaque-to-white switching assays (Bonferroni Correction, α = 0.05, for a final threshold of 1.04 x 10-3). Due to the relatively small number of genes in the various subsets (white cell average 25, median 20; opaque cell average 24, median 21) and the resulting low number of genes that would be expected to have an effect based on the overall switching rates for the library, our statistical testing approach does not have an ability to detect an underrepresentation of genes affecting switching rates in any given category. Lists of genes in each category as well as the statistical testing results for each category can be found in File S4.

**SI Results**

**Enrichment and Switching Frequencies**

As with the frequency of genes affecting switching rates, many other switching-related trends for this deletion library are similar to those previously reported for the transcriptional regulator deletion library (Lohse *et al.* 2016). We found that 23% of the genes affecting switching rates (7 of 31) were differentially regulated at least two-fold between cell types (File S2), slightly less than the 38% reported for transcriptional regulators (16 of 42). In other words, roughly three out of four genes that affected white-opaque switching rates were not differentially expressed between cell types. Likewise, only eleven percent (7 of 61) of the differentially expressed genes tested in this screen had five-fold effects on switching rates (File S2).

**GO term and Switching Frequencies**

Looking at the genes whose deletion affected white-opaque switching, we observed a wide range of gene functions, including kinases (*cek1*, *hsl1*, *pkh2*, *prk1*, *ssn3*), phosphatases (*pho15*, *ptc1*, *sit4*, *C3\_00570C\_A*), and cyclins (*ccn1*, *pcl5*). Likewise, we observed a wide range of cellular components commonly associated with the genes (e.g. nucleus, cytoplasm, golgi, cell wall). Given this diversity, we examined whether any criteria (e.g. gene function, cellular component, She3 associated transcripts, two- or four-fold cell type enrichment) were over-represented in the set of genes that affected white-to-opaque or opaque-to-white switching (our screen lacked sufficient statistical power to evaluate under-representation). To do this, we compared the subsets of genes associated with a given function, component, or process (using the GO-SLIM mapping tool at the *Candida* Genome Database) as well as existing datasets for She3 associated transcripts (Elson *et al.* 2009) and transcriptional enrichment between white and opaque cells (Tuch *et al.* 2010). Only two categories out of 42 GO-SLIM sets tested were enriched for genes whose deletion affected white-to-opaque switching rates (regulation of biological process, p= 2.59 x 10-7; signal transduction, 5.95 x 10-4;, hypergeometric distribution with Bonferroni Correction) (File S4). None of the 42 GO-SLIM sets tested showed a statistically significant enrichment for genes affecting opaque-to-white switching rates (File S4). Furthermore, there was not any statistically significant enrichment of genes whose transcripts were She3-associated or that exhibit two- or four-fold cell type enrichment among the genes affecting switching in either direction (File S4). As such, consistent with our observations from the transcriptional regulator deletion library screen (Lohse *et al.* 2016), no simple criteria, be they genome-wide data sets or GO terms, reliably predicts genes whose deletion would affect white-to-opaque and/or opaque-to-white switching rates. Although our screen lacks the power to detect underrepresentation of types of genes, the wide range of genes that affected switching rates underscores previous observations that regulation of white-opaque switching is tightly integrated with many aspects of *C. albicans*’ biology (Ene *et al.* 2016; Lohse *et al.* 2016).

**Literature Cited**

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