Supplementary Information for:

A Recently Evolved Transcriptional Network Controls Biofilm Development in *Candida albicans*

Clarissa J. Nobile, Emily P. Fox, Jeniel E. Nett, Trevor R. Sorrells, Quinn M. Mitrovich, Aaron D. Hernday, Brian B. Tuch, David R. Andes, and Alexander D. Johnson

Supplemental Figure Legends

Figure S1 (related to Figure 1). Characterization of *in vitro* biofilm-defective mutants over time. The wild-type reference strain SN250 and the six transcription regulator mutant strains were grown under biofilm conditions at the following time points: 0 h, 8 h, 24 h, and 48 h post adherence, and visualized by CSLM. The upper panel for each image shows the top view and the lower panel shows the side view. Scale bars represent 20 μm.

Figure S2 (related to Figure 1 and Dataset S1). Screening and characterization of *in vitro* biofilm-defective transcription regulator mutants. Nine regulator mutant strains: TF022, TF091, TF095, TF103, TF110, TF115, TF117, TF137, and TF156 were initially found to have biofilm defects. Visual phenotypic characterization of the mutants is shown in panel A. Of these combined nine mutants, we did not follow up on TF091 (*orf19.1685*Δ/Δ) because its biofilm defect is due to a growth defect specific to Spider medium, TF103 (*orf19.3063*Δ/Δ) because its biofilm defect is the result of an overall growth defect, and TF117 (*tup1*Δ/Δ) because this mutant is highly pleiotropic and its phenotype interfered with the biofilm assay. Panel B shows CSLM side view images of the six complemented strains where a wild-type allele was added back into the six core regulator mutant strains. Reintroduction of an ectopic copy of the wild-type allele back into each mutant reversed the biofilm-formation defect of each mutant to near wild-type levels by CSLM (panel B, upper images), and to complete wild-type levels by the visual plate assay (panel B, bottom images). Scale bars represent 20 μm.

Figure S3 (related to Figure S1). Assessment of hyphal-formation abilities for the six core biofilm-defective regulator mutants (related to Figure 1). Images of true hyphae found suspended in the medium (surrounding the biofilm) under biofilm-forming conditions are shown in panel A. Panels on the left show evidence of hyphal formation; panels on the right are more representative of the entire suspended cell population. The percentage of true hyphae produced by each strain under the three indicated planktonic conditions is shown quantitatively in panel B. Hyphal defects have been previously reported for *efg1*Δ/Δ, *tec1*Δ/Δ, and *ndt80*Δ/Δ *in vitro* (Lo et al., 1997; Ramage et al., 2002; Schweizer et al., 2000; Sellam et al., 2010; Stoldt et al., 1997).

Figure S4 (related to Figure 2). Biofilm formation in the *in vivo* rat catheter model (panel A). The wild-type reference strain SN250, and the six transcription regulator mutant strains were inoculated into rat intravenous catheters, and the resulting biofilms were visualized after 24 h of growth by SEM. These SEM Images show catheter luminal

surfaces at magnifications of 50X. Extensive bacterial biofilm formation in the *in vivo* rat denture model on the dentures of the six transcription regulator mutant strains (panel B). The regulator mutant strains were inoculated into rat dentures, and extensive bacterial biofilms were visualized on the denture surfaces after 24 h of growth by SEM. SEM images show the denture surfaces at magnifications of 2000X.

Figure S5 (related to Figure 5 and Dataset S4). Hierarchical cascade of target gene regulation. Deletion of any one of the six biofilm regulators results in downregulation of eight target genes; however the promoters of these genes are not bound by every regulator. Indirect regulation by each biofilm regulator can be explained by direct regulation by at least one of the other regulators. The diagram shows potential regulation cascades from each regulator to each target gene. The arrows represent direct regulation (binding and differential regulation as determined by ChIP-chip and gene expression microarray).

Figure S6 (related to Figure 6). The target gene ectopic expression strains that rescued the defects of their corresponding transcription regulator deletion mutant. CSLM side view images of the wild-type and the eighteen target gene ectopic expression strains that exhibited a rescuing phenotype are shown. Scale bars represent 20 μm.

Figure S7 (related to Figure 7). Validation of transcriptional regulation of the biofilm regulatory network by quantitative real time PCR (qPCR) (panel A). Expression levels of the six biofilm regulators (*BCR1, TEC1, EFG1, NDT80, ROB1,* and *BRG1*) in the background of each regulator mutant under biofilm conditions are shown in panel A. Normalized gene expression values were calculated using the ΔΔCt method using *TAF145* as a reference gene. Results are the means of three determinations. For ease of interpretation, the reference strain expression level values were set to 1.0 for each gene set, and the normalized expression level of each gene relative to *TAF145* expression is shown. Validation of transcriptional regulation of the biofilm regulators on their own promoters (panel B). Transcriptional reporters of the biofilm transcriptional regulators (TRs) were constructed where one copy of each regulator's promoter was fused to an mCherry transcriptional reporter in both a homozygous regulator mutant as well as a heterozygous regulator mutant. Normalized expression levels of these reporter strains under biofilm conditions were calculated using the ΔΔCt method using *TAF145* as a reference gene. Means of three determinations was determined. The relative fold increase of expression in the TRp-mCherry in the heterozygote deletion strain to its respective TRp-mCherry in the homozygote deletion strain is shown in panel B. Enrichment of regulator motifs across yeast species (panel C). Motifs for several biofilm regulators and two control regulators governing ribosomal biogenesis were scored across thirteen yeast species. Known targets of each regulator were mapped to orthologs in other species, and enrichment of the motif was determined relative to the rest of the genome for each species. Ndt80 and Efg1 were the only biofilm regulators with motifs containing sufficient predictive power in *C. albicans* to be scored across the other yeast species. As a positive control, we analyzed conservation of motifs upstream of two ribosomal protein genes (Tbf1 and Cbf1) from Lavoie et. al. 2010. A cutoff for the motif score from MAST was selected to maximize the log-ratio of each regulator in *C.*

albicans and was then used for the other species. Colored squares indicate significant enrichment of the regulator motif. Question marks indicate borderline significance.

Supplemental Experimental Procedures

C. albicans strains used in this study:

Strain	Genotype	Reference
CJN1700	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::CdHIS1 BRG1-13XMyc-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 BRG1*	This study
CJN1707	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::CdHIS1 NDT80-13XMyc-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 NDT80*	This study
CJN1734	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::CdHIS1 BRG1-13XMyc-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 BRG1*	This study
CJN1748	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::CdHIS1 NDT80-13XMyc-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 NDT80*	This study
CJN1775	*ura3Δ::λimm434 ARG4:URA3::arg4::hisG his1::hisG::pHIS1 EFG1-13XMyc-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG EFG1*	This study
CJN1781	*ura3Δ::λimm434 ARG4:URA3::arg4::hisG his1::hisG::pHIS1 EFG1-13XMyc-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG EFG1*	This study
CJN1785	*ura3Δ::λimm434 ARG4:URA3::arg4::hisG his1::hisG::pHIS1 BCR1-13XMyc-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG BCR1*	This study
CJN1787	*ura3Δ::λimm434 ARG4:URA3::arg4::hisG his1::hisG::pHIS1 BCR1-13XMyc-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG BCR1*	This study
CJN2144	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::CdHIS1 7XMyc-FRT-FLP-SAT1-FRT-ROB1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 ROB1*	This study
CJN2208	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::CdHIS1 7XMyc-FRT-ROB1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 ROB1*	This study
CJN2302	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::CdARG4 efg1Δ::CmLEU2* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1*	This study
CJN2318	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::EFG1-CdARG4 efg1Δ::CmLEU2* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1*	This study
CJN2320	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG::TEC1-CdARG4 tec1Δ::CmLEU2* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG tec1Δ::CdHIS1*	This study

CJN2322	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::BCR1-CdARG4* *bcr1Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *bcr1Δ::CdHIS1*	This study
CJN2324	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::BRG1-CdARG4* *brg1Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *brg1Δ::CdHIS1*	This study
CJN2326	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::ROB1-CdARG4* *rob1Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *rob1Δ::CdHIS1*	This study
CJN2328	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::NDT80-CdARG4* *ndt80Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *ndt80Δ::CdHIS1*	This study
CJN2330	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::CdARG4* *tec1Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *tec1Δ::CdHIS1*	This study
CJN2334	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::CdARG4* *bcr1Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *bcr1Δ::CdHIS1*	This study
CJN2338	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::CdARG4* *brg1Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *brg1Δ::CdHIS1*	This study
CJN2351	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *brg1Δ::CmLEU2* *ORF19.4000::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.4000* *ura3Δ::λimm434* *arg4::hisG his1::hisG leu2::hisG* *brg1Δ::CdHIS1* *ORF19.4000*	This Study
CJN2354	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *rob1Δ::CmLEU2* *ORF19.4000::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.4000* *ura3Δ::λimm434* *arg4::hisG his1::hisG leu2::hisG* *rob1Δ::CdHIS1* *ORF19.4000*	This Study
CJN2395	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *ndt80Δ::CmLEU2* *ORF19.3337::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.3337* *ura3Δ::λimm434* *arg4::hisG his1::hisG leu2::hisG* *ndt80Δ::CdHIS1* *ORF19.3337*	This Study
CJN2397	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *efg1Δ::CmLEU2* *ORF19.3337::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.3337* *ura3Δ::λimm434* *arg4::hisG his1::hisG leu2::hisG* *efg1Δ::CdHIS1* *ORF19.3337*	This Study
CJN2408	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::CdARG4* *rob1Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *rob1Δ::CdHIS1*	This study
CJN2412	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::CdARG4* *ndt80Δ::CmLEU2* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *ndt80Δ::CdHIS1*	This study
CJN2473	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *ndt80Δ::CmLEU2* *ALS1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ALS1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *ndt80Δ::CdHIS1* *ALS1*	This Study
CJN2476	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *brg1Δ::CmLEU2* *ALS1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ALS1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *brg1Δ::CdHIS1* *ALS1*	This Study
CJN2479	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *efg1Δ::CmLEU2* *ALS1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ALS1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *efg1Δ::CdHIS1* *ALS1*	This Study
CJN2480	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *bcr1Δ::CmLEU2* *ALS1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ALS1*	This Study

	ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CdHIS1 ALS1	
CJN2483	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG tec1Δ::CmLEU2 ALS1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ALS1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG tec1Δ::CdHIS1 ALS1*	This Study
CJN2486	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG rob1Δ::CmLEU2 ALS1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ALS1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG rob1Δ::CdHIS1 ALS1*	This Study
CJN2499	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG rob1Δ::CmLEU2 ORF19.3337::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.3337* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG rob1Δ::CdHIS1 ORF19.3337*	This Study
CJN2527	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG brg1Δ::CmLEU2 HWP1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HWP1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG brg1Δ::CdHIS1 HWP1*	This Study
CJN2530	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CmLEU2 HWP1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HWP1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CdHIS1 HWP1*	This Study
CJN2531	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG tec1Δ::CmLEU2 HWP1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HWP1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG tec1Δ::CdHIS1 HWP1*	This Study
CJN2533	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG rob1Δ::CmLEU2 HWP1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HWP1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG rob1Δ::CdHIS1 HWP1*	This Study
CJN2536	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CmLEU2 ORF19.3337::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.3337* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CdHIS1 ORF19.3337*	This Study
CJN2537	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CmLEU2 TPO4::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO4* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CdHIS1 TPO4*	This Study
CJN2539	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG brg1Δ::CmLEU2 TPO4::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO4* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG brg1Δ::CdHIS1 TPO4*	This Study
CJN2541	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CmLEU2 ORF19.4000::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.4000* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CdHIS1 ORF19.4000*	This Study
CJN2544	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CmLEU2 ORF19.4000::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.4000* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CdHIS1 ORF19.4000*	This Study
CJN2546	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG tec1Δ::CmLEU2 ORF19.4000::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.4000* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG tec1Δ::CdHIS1 ORF19.4000*	This Study
CJN2549	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG brg1Δ::CmLEU2 HYR1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HYR1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG brg1Δ::CdHIS1 HYR1*	This Study
CJN2552	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CmLEU2 HYR1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HYR1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CdHIS1 HYR1*	This Study
CJN2555	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CmLEU2 HWP1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HWP1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CdHIS1 HWP1*	This study
CJN2557	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG efg1Δ::CmLEU2 TPO4::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO4* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1 TPO4*	This Study

CJN2558	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*bcr1Δ::CmLEU2* *bcr1Δ::CdHIS1*	*TPO4::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO4* *TPO4*	This Study
CJN2561	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*tec1Δ::CmLEU2* *tec1Δ::CdHIS1*	*TPO4::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO4* *TPO4*	This Study
CJN2564	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*rob1Δ::CmLEU2* *rob1Δ::CdHIS1*	*TPO4::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO4* *TPO4*	This Study
CJN2567	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*ndt80Δ::CmLEU2* *ndt80Δ::CdHIS1*	*HYR1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HYR1* *HYR1*	This Study
CJN2569	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*tec1Δ::CmLEU2* *tec1Δ::CdHIS1*	*HYR1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HYR1* *HYR1*	This Study
CJN2570	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*rob1Δ::CmLEU2* *rob1Δ::CdHIS1*	*HYR1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HYR1* *HYR1*	This Study
CJN2573	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*ndt80Δ::CmLEU2* *ndt80Δ::CdHIS1*	*CAN2::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-CAN2* *CAN2*	This Study
CJN2576	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*brg1Δ::CmLEU2* *brg1Δ::CdHIS1*	*CAN2::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-CAN2* *CAN2*	This Study
CJN2578	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*bcr1Δ::CmLEU2* *bcr1Δ::CdHIS1*	*CAN2::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-CAN2* *CAN2*	This Study
CJN2580	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*tec1Δ::CmLEU2* *tec1Δ::CdHIS1*	*CAN2::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-CAN2* *CAN2*	This Study
CJN2583	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*rob1Δ::CmLEU2* *rob1Δ::CdHIS1*	*CAN2::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-CAN2* *CAN2*	This Study
CJN2585	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*ndt80Δ::CmLEU2* *ndt80Δ::CdHIS1*	*EHT1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-EHT1* *EHT1*	This Study
CJN2587	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*brg1Δ::CmLEU2* *brg1Δ::CdHIS1*	*EHT1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-EHT1* *EHT1*	This Study
CJN2590	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*efg1Δ::CmLEU2* *efg1Δ::CdHIS1*	*EHT1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-EHT1* *EHT1*	This Study
CJN2591	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*bcr1Δ::CmLEU2* *bcr1Δ::CdHIS1*	*EHT1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-EHT1* *EHT1*	This Study
CJN2595	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*tec1Δ::CmLEU2* *tec1Δ::CdHIS1*	*EHT1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-EHT1* *EHT1*	This Study
CJN2597	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*rob1Δ::CmLEU2* *rob1Δ::CdHIS1*	*EHT1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-EHT1* *EHT1*	This Study

CJN2600	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG efg1Δ::CmLEU2 HWP1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HWP1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1 HWP1*	This Study
CJN2601	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG efg1Δ::CmLEU2 ORF19.4000::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.4000* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1 ORF19.4000*	This Study
CJN2604	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG efg1Δ::CmLEU2 HYR1::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-HYR1* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1 HYR1*	This Study
CJN2607	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG efg1Δ::CmLEU2 CAN2::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-CAN2* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1 CAN2*	This Study
CJN2609	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG brg1Δ::CmLEU2 ORF19.3337::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.3337* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG brg1Δ::CdHIS1 ORF19.3337*	This Study
CJN2611	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG tec1Δ::CmLEU2 ORF19.3337::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-ORF19.3337* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG tec1Δ::CdHIS1 ORF19.3337*	This Study
CJN2614	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG::CdARG4 his1::hisG leu2::hisG::CdHIS1 BCR1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 BCR1*	This Study
CJN2616	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG::CdARG4 his1::hisG leu2::hisG::CdHIS1 TEC1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 TEC1*	This Study
CJN2619	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG::CdARG4 his1::hisG leu2::hisG::CdHIS1 EFG1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 EFG1*	This Study
CJN2621	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG::CdARG4 his1::hisG leu2::hisG::CdHIS1 BRG1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 BRG1*	This Study
CJN2629	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG::CdARG4 his1::hisG leu2::hisG::CdHIS1 ROB1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 ROB1*	This Study
CJN2672	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG::CdARG4 his1::hisG leu2::hisG::CdHIS1 NDT801p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG::CmLEU2 NDT80*	This Study
CJN2684	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG brg1Δ::CmLEU2 TPO5::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO5* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG brg1Δ::CdHIS1 TPO5*	This Study
CJN2687	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG rob1Δ::CmLEU2 TPO5::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO5* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG rob1Δ::CdHIS1 TPO5*	This Study
CJN2690	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG tec1Δ::CmLEU2 TPO5::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO5* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG tec1Δ::CdHIS1 TPO5*	This Study
CJN2691	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CmLEU2 TPO5::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO5* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG bcr1Δ::CdHIS1 TPO5*	This Study
CJN2700	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG efg1Δ::CmLEU2 TPO5::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO5* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG efg1Δ::CdHIS1 TPO5*	This Study
CJN2704	*ura3Δ::λimm434::URA3-IRO1 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CmLEU2 TPO5::AgTEF1p-NAT1-AgTEF1UTR-TDH3p-TPO5* *ura3Δ::λimm434 arg4::hisG his1::hisG leu2::hisG ndt80Δ::CdHIS1 TPO5*	This Study

CJN2708	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *BCR1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *bcr1Δ::CdHIS1*	This Study
CJN2710	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *TEC1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *tec1Δ::CdHIS1*	This Study
CJN2712	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *EFG1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *efg1Δ::CdHIS1*	This Study
CJN2715	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *ROB1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *rob1Δ::CdHIS1*	This Study
CJN2718	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *BRG1p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *brg1Δ::CdHIS1*	This Study
CJN2724	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *BCR1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *bcr1Δ::CdHIS1*	This Study
CJN2725	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *TEC1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *tec1Δ::CdHIS1*	This Study
CJN2726	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *EFG1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *efg1Δ::CdHIS1*	This Study
CJN2727	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *NDT80* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *ndt80Δ::CdHIS1*	This Study
CJN2728	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *ROB1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *rob1Δ::CdHIS1*	This Study
CJN2729	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *BRG1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *brg1Δ::CdHIS1*	This Study
CJN2736	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *NDT80p-mCherry-FRT-FLP-SAT1-FRT* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG* *ndt80Δ::CdHIS1*	This Study
QMY23	*ura3Δ::λimm434::URA3-IRO1* *his1::hisG* *leu2::hisG::CdHIS1* *ura3Δ::λimm434* *his1::hisG* *leu2::hisG::CmLEU2*	(Mitrovich et al., 2007)
SN87	*ura3Δ::λimm434::URA3-IRO1* *his1::hisG* *leu2::hisG* *ura3Δ::λimm434* *his1::hisG* *leu2::hisG*	(Noble and Johnson, 2005)
SN152	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG*	(Noble and Johnson, 2005)
SN250	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG* *his1::hisG* *leu2::hisG::CdHIS1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG::CmLEU2*	(Noble et al., 2010)
SN425	*ura3Δ::λimm434::URA3-IRO1* *arg4::hisG::CdARG4* *his1::hisG* *leu2::hisG::CdHIS1* *ura3Δ::λimm434* *arg4::hisG* *his1::hisG* *leu2::hisG::CmLEU2*	(Noble et al., 2010)

TF021	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*orf19.4000Δ::CmLEU2* *orf19.4000Δ::CdHIS1*	(Homann et al., 2009)
TF022	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*brg1Δ::CmLEU2* *brg1Δ::CdHIS1*	(Homann et al., 2009)
TF095	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*ndt80Δ::CmLEU2* *ndt80Δ::CdHIS1*	(Homann et al., 2009)
TF110	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*rob1Δ::CmLEU2* *rob1Δ::CdHIS1*	(Homann et al., 2009)
TF115	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*tec1Δ::CmLEU2* *tec1Δ::CdHIS1*	(Homann et al., 2009)
TF137	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*bcr1Δ::CmLEU2* *bcr1Δ::CdHIS1*	(Homann et al., 2009)
TF156	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*efg1Δ::CmLEU2* *efg1Δ::CdHIS1*	(Homann et al., 2009)
TFT54a	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*hyr1Δ::CmLEU2* *hyr1Δ::CdHIS1*	This Study
TFT60d	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*hwp1Δ::CmLEU2* *hwp1Δ::CdHIS1*	This Study
TFT64b	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*als1Δ::CmLEU2* *als1Δ::CdHIS1*	This Study
TFT66a	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*orf19.3337Δ::CmLEU2* *orf19.3337Δ::CdHIS1*	This Study
TFT68b	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*tpo4Δ::CmLEU2* *tpo4Δ::CdHIS1*	This Study
TFT70a	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*eht1Δ::CmLEU2* *eht1Δ::CdHIS1*	This Study
TFT72b	*ura3Δ::λimm434::URA3-IRO1* *ura3Δ::λimm434*	*arg4::hisG* *arg4::hisG*	*his1::hisG* *his1::hisG*	*leu2::hisG* *leu2::hisG*	*can2Δ::CmLEU2* *can2Δ::CdHIS1*	This Study

Primers used in this study:

Primer Name	Description	Sequence (5'-3')
AHO276	F-Myc-detect	AACTCGAGCGGATCCCCGGGTTAATTAA
AHO279	F-detect-Efg1-13XMyc	TGTCTGGTGCTTCTGGTGCAG
AHO281	R-detect-Efg1-13XMyc	CAGCCATCGAGTAAAATATACTTGTTC
AHO283	R-detect-flp	GGCGGCCGCTCTAGAACTAGTGGATC
AHO300	R-detect-13XMyc-flank	CCGTTAATTAACCCGGGGATC
AHO301	F-detect-Myc-flank	GGAACTTCAGATCCACTAGTTCTAGAGC
AHO302	F-detect-Myc-flp	TCACTAGTGAATTCGCGCTCGAG
AHO385	F-Efg1-13XMyc	GTTCAGTCATTGACTCAAGGTTCAGTTCACCCTTCACCCCAACAACATCAAGCTAATCAATCAGCTAGCACTG TTGCCAAAGAAGAAAAGCGGATCCCCGGGTTAATTAACGG
AHO386	R-Efg1-13XMyc	AACTTTCCAATCATTTCTTAATGAAATATATGCTATAATCTAATTTGGAATTTATGGCAGAAAGCAGAAGGTGAT GTACACGAATGATATGGCGGCCGCTCTAGAACTAGTGGATC
AHO613	F-detect-Nterm-tag	GTGGGCACTAAGCAGACAGC
AHO624	F-detect-Nterm-flank	CAATGAAATCCAGACAGTCGAG
AHO628	F-detect-Nterm-flp	CCTCCACCGAAGTTCCTATTC
AHO702	R-detect-mCherry	CGTTAACAGAACCTTCCATGTG
CJNO782	F-OE-prom-ALS1	ATCTTCAACCCAAGTTGTCAATTGCAAAAATTTCCAACTGGTATTGCCAATATTAACAGCTATTTATGAAAATG ATATGGGTCTGATTGACTTTGATTACATCAAGCTTGCCTCGTCCCC
CJNO792	F-OE-prom-HWP1	TACATCAACTGGATGTTATTTGCATCTACTACTATAAGCTCAAACAAATTATCTTTCAAAAATGTTATAATTAAC AAGTCATCTATAATTCTTTGGATCCATCAAGCTTGCCTCGTCCCC
CJNO804	F-OE-prom-HYR1	GAGTAATTGTATCATGCTGGGCACTGATTGAAATTATTGAATATTTGAAAAGGGCAAATGCTTAGTATGACAG CCATTAATTTATGTTTACTCATCCTATATCAAGCTTGCCTCGTCCCC
CJNO806	F-OE-detect-HYR1	CTGCTCAAAATGATAGTATT
CJNO839	F-OE-detect-ALS1	CGATAACCCGCCTCGAATCT
CJNO875	R-OE-detect-NAT	GAAACAACAACGAAACCAGC
CJNO949	R-OE-TDH3p-ALS1	TGGACCAAGTTAATGAATTAAAACTATCAAAAACACCAGTGATTGTCTTTGCACTTGCAATTGACAAATATAGG AATAACAATGTAAATTGTTGAAGCATATTTGAATTCAATTGTGATG
CJNO1012	F-ORF19.4056-Myc-nostop	CAATCTTGTCATTCATCAACCACCACCACAACAACAACAACAACAACAGCAACAAACAACCATATGTCGGATC CCCGGGTTAATTAACGG
CJNO1013	R-ORF19.4056-Myc-UTR	TTAAAACTATTTTAATGACGAATTAAAGGAATTTGGGTTGGGTAAGCAACAGGAATACCGCCAGGGCGGCCG CTCTAGAACTAGTGGATC
CJNO1014	F-ORF19.4056-detect-UpMyc	GCACAAAATCAACCATCACACCC
CJNO1015	R-ORF19.4056-detect-DownMyc	CATTAACAAGTGTTGATTACCCAACAAATACTTGAA
CJNO1024	F-ORF19.2119-Myc-nostop	AAGCTACCACAAAGATAGAACTTCTGGGTATAGGGCTACAAACACCCCAACCCCTACTCCTCCACAGCGGAT CCCCGGGTTAATTAACGG
CJNO1025	R-ORF19.2119-Myc-UTR	TTTAAACTTTAAAATCAACCTTTCTTCGTCATCATCAAAAAAAAAAAAAAAATCTATAGTTTTGCGGCGGCCGCT CTAGAACTAGTGGATC
CJNO1026	F-ORF19.2119-detect-UpMyc	GCAATTAGGTTAGTGGCCAAGTGTTC
CJNO1027	R-ORF19.2119-detect-DownMyc	CTTACGTTGGCTCCTGTAGTGGGGAGA
CJNO1046	F-BCR1-Myc-nostop	AACAACAAATACAAATACAAATACAAATAGTACGGCTACTACTAATGCTACTGGGTATGTTTCGAAAAAACCTA CGATAAATAATTTAATATCACAACGGATCCCCGGGTTAATTAACGG
CJNO1047	R-BCR1-Myc-UTR	AGAAACAACATCAAAAACATCACAAGACATAAGCAAATAAATAAATAAATAAATAAAAATACATTTTTCACATAC TTTCTTCTTATTATTATTGGGCGGCCGCTCTAGAACTAGTGGATC
CJNO1048	F-BCR1-detect-UpMyc	CTACAAGATCCAAAATTAGCATCAC
CJNO1049	R-BCR1-detect-DownMyc	CAGGCAAATGGTATATATTGAATGG
CJNO1137	F-ORF19.4998-7xMyc	AGAAGTCTAAAACTAAAAAAAAAATATAAAGAACGATTCTGAACTATTAGAACATTCAGGAACACCATGCGGAT CCCCGGGTTAATTAAC
CJNO1140	R-ORF19.4998-Myc&Flag	AAAGTAACAATCATATAAATACATACGAAGTACTTCTTCTTTGCTTAATTTTTTTAGTTGAACTTGGTGTACCTC CACCGAAGTTCCTAT
CJNO1141	F-ORF19.4998-detect-Myc&Flag	GAGGCGGTTGCATTATTTGTGT
CJNO1142	R-ORF19.4998-detect-Myc&Flag	GTCTAAAAACAGACTCGAGGATTCG
CJNO1172	R-OE-TDH3p-HWP1	CCGTCTACCTGTGGGACAGTGGCCCCAATTGATAACATGTAATAAGCGATAGCAATAAGTTGAGCAGTTGAT AATCTCATATTTGAATTCAATTGTGATG
CJNO1173	F-OE-detect-HWP1	ACACATAACACTTTGAGTATGATAATATCAACC
CJNO1186	F-OE-prom-ORF19.3337	GGGCACCTTGCATACAGAAAAATAATATAATTGTTAACTCCTTTATACACAAAAGCACGACCATCATCACTACT ATTATCATCAAGCTTGCCTCGTCCCC
CJNO1187	R-OE-TDH3p-ORF19.3337	AAGTCTTGTTGTTCTTCAAATTCATGAATGGAAAATTGAGTATTGTTAAACAGTTGGTTATGATCAGTAGGATC TAACATATTTGAATTCAATTGTGATG

CJNO1188	F-OE-detect-ORF19.3337	CAAACTACTAGTTGCGTGTACTCTGTAC
CJNO1251	F-OE-prom-ORF19.4000	TCAGTGTTGCTAGTGAAAAAGGAAGTAGTTGAGTCTACGTAAACAGGTCAATTTTACAGAGTTACAAAAAATC AAGCTTGCCTCGTCCCC
CJNO1252	R-OE-TDH3p-ORF19.4000	ATAATGATGTTGGCGGAGTAGGTGTAGATAGATCCTGGTTCACCAGTGATGAAATAGAGTCAGGAGACATAT TTGAATTCAATTGTGATG
CJNO1253	F-OE-detect-ORF19.4000	GTGTGTGTAAATACCAATTTGAGAATCATTC
		CTTAAATTTTCAAAAACAACAACTACAACAACTATAATTAATAATACTATCTCGATATATATCATGCGGATCCCC
CJNO1265	F-mCherry-BCR1	GGGTTAATTAACGGT
		AAGCAAATAAATAAATAAATAAATAAAAATACATTTTTCACATACTTTCTTCTTATTATTATTGGGCGGCCGCTC
CJNO1266	R-mCherry-BCR1	TAGAACTAGTGGATC
CJNO1267	F-mCherry-det-BCR1	CCTAGATTGTTGTGTGTCATTCAC
		TTTTTTTCCCTTTTATAAATTATCCGTTAAGTTTACCGCTTTTTTTTTTGACTATTTTAAATTATGCGGATCCCCG
CJNO1268	F-mCherry-TEC1	GGTTAATTAACGGT
		CTAAACTAATGTATCCAACAGTGAGTAAGTGATAATATTTTCTTCTCTTTTCTCATTTTGTTCAGGCGGCCGCT
CJNO1269	R-mCherry-TEC1	CTAGAACTAGTGGATC
CJNO1270	F-mCherry-det-TEC1	GCACCTCAAATACAAACAACATCAAATAC
		CCCTTAACCCATTAACGAATTAAGATTTGTTCTATTTGACTACCAAGAATATAACCCATATTAATGCGGATCCC
CJNO1271	F-mCherry-EFG1	CGGGTTAATTAACGGT
		GCAACAGTGCTAGCTGATTGATTAGCTTGATGTTGTTGGGGTGAAGGGTGAACTGAACCTTGAGGGCGGCC
CJNO1272	R-mCherry-EFG1	GCTCTAGAACTAGTGGATC
CJNO1273	F-mCherry-det-EFG1	CCCACTTAACTTACAATTGAAGAGACAAGC
		CACCATCCTATTCACTATCATGGTATTGCTCAGCAACAACAGTCCCAGCTTCCTCATTTCGCTATGCGGATCC
CJNO1274	F-mCherry-NDT80	CCGGGTTAATTAACGGT
		TTTAAACTTTAAAATCAACCTTTCTTCGTCATCATCAAAAAAAAAAAAAAAATCTATAGTTTTGCGGCGGCCGCT
CJNO1275	R-mCherry-NDT80	CTAGAACTAGTGGATC
CJNO1276	F-mCherry-det-NDT80	CCTTTCCCATCTCCATATTACCATCTTCC
		AGTCTAAAACTAAAAAAAAAATATAAAGAACGATTCTGAACTATTAGAACATTCAGGAACACCATGCGGATCCC
CJNO1277	F-mCherry-ROB1	CGGGTTAATTAACGGT
		TTGAAAAAACTATTTAGAATTTAAAATAAAAAACAAAAAATAAAAACTAGTAATAATAATAACTGGCGGCCGCTC
CJNO1278	R-mCherry-ROB1	TAGAACTAGTGGATC
CJNO1279	F-mCherry-det-ROB1	GAGCGATTTAGAATGGATAGAATGGAATATTAAACG
		GTTATTTCTCCATCCATACTTGTTACATTATAAATATTCACTACTATTCCAGAAATTCAAATTATGCGGATCCCC
CJNO1280	F-mCherry-BRG1	GGGTTAATTAACGGT
		TTAAAACTATTTTAATGACGAATTAAAGGAATTTGGGTTGGGTAAGCAACAGGAATACCGCCAGGGCGGCCG
CJNO1281	R-mCherry-BRG1	CTCTAGAACTAGTGGATC
CJNO1282	F-mCherry-det-BRG1	GTGGTTAATTTTAGGTTTCTGCATTTTTAAATTTTTTGC
CJNO1298	R-OE-TDH3p-HYR1	CTGCTTGTGACAACTTCTAATGCAGCACTGAGGTTGAGAGTAAGCAAAATTGTGAATATAAAGTTTGATACCA
		CTTTCATATTTGAATTCAATTGTGATG
CJNO1299	F-OE-prom-TPO4	GGTTAGAATACTCTATTTATAACTAGAATTGAGGAGAAGAAGGGTTATGATAATTGAGTTTTGTTATTATATCA AGCTTGCCTCGTCCCC
CJNO1300	R-OE-TDH3p-TPO4	TTTCGGATGTTGTTAAAACCGCTTGTCTAAGTTGAGATTCTTCCTCTTTTGAAGTCCCAAAAAAACCCATATTT GAATTCAATTGTGATG
CJNO1301	F-OE-detect-TPO4	GAAGCTGATCTGGATTTTGGTTTGTTTGAC
CJNO1302	F-OE-prom-CAN2	GATATTTAGTTACCAGCAAGCAATGATGACAGTATTCCCAAGAAGAAGAGACAATGGCTACAGCAACTGGATC AAGCTTGCCTCGTCCCC
CJNO1303	R-OE-TDH3p-CAN2	CACCACGAGATTCTATGTCATTTGAACTACTGCTGCTTTCAACAGGATATTTTTCACCTACTTCAAACATATTT GAATTCAATTGTGATG
CJNO1304	F-OE-detect-CAN2	CACAGAAGTAAAGATATTGTTCATGCTTATTGTGC
CJNO1305	F-OE-prom-EHT1	CTTAGAGAACTAAGTTTTAGGTTTTGGTTGGTTGGTTGGTATGGTTATGTTTTGCTGCACCTAATCTCCAATCA AGCTTGCCTCGTCCCC
CJNO1306	R-OE-TDH3p-EHT1	TAGCAGAACCTTCATCGCCTGACTGATGAATCTTAATGTTTGATCTGAAACCCCAGCCTAATATCCCCATATTT GAATTCAATTGTGATG
CJNO1307	F-OE-detect-EHT1	TGCTAGATCCGTAGTTTTGGTATATCAATTAGAAG
		TGATGGTGAAGAAGAAGAAGATAGTGATTTTGATTTTTAATCTAAACACAATTGATATTATATAATAGATATTAC
CJNO1330	F-OE-prom-TPO5	ATAATATCAAGCTTGCCTCGTCCCC
		GGAGGTGGTGGAGAAGACGATGAAGAATTGTCATTGTGTGGTTGCTGTAGCATTGGTTCGTAAGAAGAATTT
CJNO1331	R-OE--TDH3p-TPO5	ATTGTCATATTTGAATTCAATTGTGATG
CJNO1332	F-OE-detect-TPO5	GTGGTGGTGGTGAAGGAATTATAAAAGA
EFO237	F-HWP1- fusion	GAGAGTTTTGGTAGGCTCATAATCG
EFO238	F-HWP1-flank	CACGGCGCGCCTAGCAGCGGGAGTGACTATAGGAGATTCCTGTTGTG
EFO239	R-HWP1-flank	GTCAGCGGCCGCATCCCTGCTTGGTAGAAAATGTGAGCTCTGT
EFO240	R-HWP1-fusion	TCTTTTATTGCGGCTGATCCTC
EFO241	F-detect-HWP1-flank	CAAGGAATTCGGAAATTCTGACG
EFO242	R-detect-HWP1-flank	CCCTGTTGAAGTACGTAATAAC
EFO243	F-HWP1-intraORFcheck	GACAATCCTCCTCAACCTGATGTT
EFO244	R-HWP1-intraORFcheck	ATTTGGAGTAGTAGCTGGAGCAGA
EFO277	F-HYR1- fusion	ACACGAATACAATGGGAACACGA
EFO278	F-HYR1-flank	CACGGCGCGCCTAGCAGCGGCTGGAGCAATTTCAAGAAGGA
EFO279	R-HYR1-flank	GTCAGCGGCCGCATCCCTGCGCTTACTGTTATCATTATCACCCA

EFO280	R-HYR1-fusion	CGCCAATTCTTGAATACTCCTC
EFO281	F-detect-HYR1-flank	CCAAGCGAGGTGAAATCAAATC
EFO282	R-detect-HYR1-flank	GGAAACTCTTTACACGTGGTG
EFO283a	F-HYR1-intraORFcheck	TGGTTGTGTTACTGCTGATGAAGAC
EFO284a	R-HYR1-intraORFcheck	AAGGGCAGCAGCTCTTAATTGT
EFO317	F-ALS1- fusion	TGCTAATCATCTTTGGAGATATTCG
EFO318	F-ALS1-flank	CACGGCGCGCCTAGCAGCGGAATTGAGAGGAGGAAAGAGCCT
EFO319	R-ALS1-flank	GTCAGCGGCCGCATCCCTGCGGCTTGATCTAGTTCACATTTATCTTT
EFO320	R-ALS1-fusion	GCGAATGCTAGAAAATAAACTGA
EFO321	F-detect-ALS1-flank	TCCTATCCGATAACCCGCCT
EFO322	R-detect-ALS1-flank	GTCAGCTGTGATCGAGGATATAC
EFO323	F-ALS1-intraORFcheck	AGAGAACCACCAAATCACACTGT
EFO324	R-ALS1-intraORFcheck	GATTGAGGATTCATTGCTATCTGG
EFO325	F-ORF19.3337- fusion	GCACGACCATCATCACTACTATTATC
EFO326	F-ORF19.3337-flank	CACGGCGCGCCTAGCAGCGGGCGATTGGAAGGTATTATATTTGG
EFO327	R-ORF19.3337-flank	GTCAGCGGCCGCATCCCTGCCTTCGATGAATTCTTTCTTGGAC
EFO328	R-ORF19.3337-fusion	GCCTTACCCTCCATTGATTCTT
EFO329	F-detect-ORF19.3337-flank	TAGGGCACCTTGCATACAGA
EFO330	R-detect-ORF19.3337-flank	GCTCCATTCAAATTTAGATTCATCGAG
EFO331	F-ORF19.3337-intraORFcheck	CAATTTCAGGAACAAGAATCCAT
EFO332	R-ORF19.3337-intraORFcheck	TGTACTTGCTGTTGAGGAGCACTA
EFO333	F-TPO4- fusion	GGAGAAGAAGGGTTATGATAATTGAGTT
EFO334	F-TPO4-flank	CACGGCGCGCCTAGCAGCGGGCACTTTTACCTCTTGATCTGTTG
EFO335	R-TPO4-flank	GTCAGCGGCCGCATCCCTGCCGTAGTTCTAAGCATTTTTATTGCATCT
EFO336	R-TPO4-fusion	CTGAGATCCATTATTCATTCTTGCAC
EFO337	F-detect-TPO4-flank	CATTGCTAACAAAGCTCATGTC
EFO338	R-detect-TPO4-flank	GAGGTTGTTGGCCATTATTG
EFO339	F-TPO4-intraORFcheck	CTCCTTACCAATAGCCATGTTGTT
EFO340	R-TPO4-intraORFcheck	CAATGAGCAACTTCAAAGGCA
EFO341	F-EHT1- fusion	GCTTAGAGAACTAAGTTTTAGGTTTTGG
EFO342	F-EHT1-flank	CACGGCGCGCCTAGCAGCGGTCTATAGTCATCTTATAACCTTGATGCT
EFO343	R-EHT1-flank	GTCAGCGGCCGCATCCCTGCATTCATTTGCATGGGCTCTTC
EFO344	R-EHT1-fusion	ATTCAACCGTGGTGACCCATAA
EFO345	F-detect-EHT1-flank	TTGTTAGAGATGCTAGATCCG
EFO346	R-detect-EHT1-flank	GGGTCATAATTCACTATTCAGTG
EFO347	F-EHT1-intraORFcheck	GATTACAACCCCGTTGCAGATACT
EFO348	R-EHT1-intraORFcheck	AAGGACAACCAACAGTACAAGCAG
EFO349	F-CAN2- fusion	AACACGGCACCAATGAATGA
EFO350	F-CAN2-flank	CACGGCGCGCCTAGCAGCGGGCAAGGTGTAAGATTGAAGACTTT
EFO351	R-CAN2-flank	GTCAGCGGCCGCATCCCTGCCGCCGGTTATACAAACTCAA
EFO352	R-CAN2-fusion	GCCCTTGAATGTAATTGAAGGTG
EFO353	F-detect-CAN2-flank	CATGCTTATTGTGCTAACCTGATGTA
EFO354	R-detect-CAN2-flank	TAAATCCGCTGGAAATGCAGTA
EFO355	F-CAN2-intraORFcheck	AAGTAATTGCTATTGTTGGGTGGC
EFO356	R-CAN2-intraORFcheck	CGTGATCCCGAATAAACATTGG
RZO37	F-universal-KO	CCGCTGCTAGGCGCGCCGTGACCAGTGTGATGGATATCTGC
RZO38	R-universal-KO	GCAGGGATGCGGCCGCTGACAGCTCGGATCCACTAGTAACG
RZO39	F-LEU2-check	TCTTTCTAGACATGGGTAG
RZO40	R-LEU2-check	CTCAAACCTCTTTCTTGACC
RZO41	F-HIS1-check	AAACAGTTCACCTGGTACGG
RZO42	R-HIS1-check	CACATTTCACACCCAGCTCG

Media

C.albicans strains were grown at 30°C in YPD medium (2% Bacto Peptone, 2% dextrose,1% yeast extract) unless otherwise indicated. Transformants were selected for on SC medium (2% dextrose, 6.7% YNB with ammonium sulfate, and auxotrophic supplements) or on YPD+clonNAT400 (2% Bacto Peptone, 2% dextrose,1% yeast extract, and 400 µg/mL nourseothricin (clonNAT, WERNER BioAgents)) for nourseothricin-resistant isolates. To obtain nourseothricin-sensitive isolates having flipped out the *SAT1* marker (Reuss et al., 2004), nourseothricin-resistant transformants were grown for 10 h in YPM liquid medium, plated at a cell density of 200 cells/plate on

YPD+clonNat25 (2% Bacto Peptone, 2% dextrose,1% yeast extract, and 25 µg/mL nourseothricin (clonNAT, WERNER BioAgents)), and allowed to grow for 24 h at 30°C as previously described (Reuss et al., 2004). Biofilms were grown in Spider medium (Liu et al., 1994) at 37°C. Planktonic cells were grown in Spider medium at 37°C or in SC+Uri medium (SC supplemented with 100 mg/L uridine) at 23°C.

Plasmid and Strain Construction

All *C. albicans* strains used in this study are listed in the table above with the exception of the transcription regulator (TR) mutant deletion library. This TF library, consisting of 165 strains, has been previously described (Homann et al., 2009), and is publicly available at the Fungal Genetics Stock Center (http://www.fgsc.net/). Strains were constructed in isogenic strain backgrounds, and were derived from strain SN87 (His-Leu-) or SN152 (His-Leu-Arg-) (Noble and Johnson, 2005). The transcription regulator mutant deletion strain library (His+Leu+Arg- background), and reference strains SN250 (His+Leu+Arg-), SN425 (His+Leu+Arg+), and QMY23 (His+Leu+Arg+) have been previously described (Homann et al., 2009; Mitrovich et al., 2007; Noble et al., 2010). All primer sequences used in this study are listed in the primer table above.

All genotypes were verified for correct integration by colony PCR using corresponding flanking detection primers. Plasmids for complementation (pCJN598 (*EFG1*), pCJN600 (*TEC1*), pCJN602 (*BCR1*), pCJN604 (*BRG1*), pCJN606 (*ROB1*), pCJN608 (*NDT80*)) were constructed using PCR and homologous recombination in *S. cerevisiae*, and were designed to contain a wild-type copy for each gene of interest with upstream promoter and downstream UTR sequences, followed by the *C. dubliniensis ARG4* gene between upstream and downstream flanking *C. albicans LEU2* gene sequences. The complemented strains CJN2318 (*EFG1*), CJN2320 (*TEC1*), CJN2322 (*BCR1*), CJN2324 (*BRG1*), CJN2326 (*ROB1*), and CJN2328 (*NDT80*) were made by transforming the transcription regulator mutant strains TF156 (*efg1Δ/Δ*), TF115 (*tec1Δ/Δ*), TF137 (*bcr1Δ/Δ*), TF022 (*brg1Δ/Δ*), TF110 (*rob1Δ/Δ*), and TF095 (*ndt80Δ/Δ)*, respectively, with their corresponding PmeI-digested complementation plasmid described above. The transcription regulator mutant strains were made Arg+ by transforming with PmeI-digested pSN105 (Noble et al., 2010) to yield strains CJN2302 (*efg1Δ/Δ*), CJN2330 (*tec1Δ/Δ*), CJN2334 (*bcr1Δ/Δ*), CJN2338 (*brg1Δ/Δ*), CJN2408 (*rob1Δ/Δ*), and CJN2412 (*ndt80Δ/Δ*). SN425 (His+Leu+Arg+) (Noble et al., 2010) was used as the marker-matched prototrophic reference strain for all Arg+ add-back strains. Wild-type reference strain QMY23 (Mitrovich et al., 2007) was used for the RNA sequencing of biofilm and planktonic cells (See below).

The N-terminal Myc-tagged nourseothricin-resistant Myc-Rob1 (CJN2144) strain was constructed by transforming the reference strain SN250 using PCR products from template plasmid pADH70 (containing a 7XMyc epitope tag immediately preceding the *SAT1*-flipper cassette (see description below)) and primers CJNO1137 and CJNO1140. These primers amplify the entire 7XMyc epitope tag and complete *SAT1* flipper cassette with 66 bp of hanging homology to *ROB1* up to the start codon for the forward primer

and 70 bp of hanging homology to *ROB1* precisely after the start codon for the reverse primer. The homology in these primers allows recombination of the entire 7XMyc epitope tag and complete *SAT* flipper cassette directly upstream of *ROB1,* so that *ROB1* contains an in frame N-terminal 7XMyc epitope tag translational fusion after the marker has been flipped out. Correct integration of the N-terminal 7XMyc epitope tag and *SAT1* flipper for Rob1 was verified by colony PCR using detection primers CJNO1141 and AHO624 to check the upstream integration, and CJNO1142 and AHO613 to check the downstream integration. The N-terminal tagged nourseothricin-sensitive Myc-Rob1 (CJN2208) was constructed by flipping out the *SAT1* cassette from strain CJN2144, as described previously (Reuss et al., 2004). The following primer pairs were used in colony PCR to confirm the clean "flipping out" of the *SAT1*-flipper cassette for *ROB1*: CJNO1141 with AHO628 and CJNO1142 with AHO276. The 7XMyc epitope tag and the region of homology to the 5' end of the ORF of interest used for integration of the *SAT1*-flipper cassette was confirmed by sequencing the colony PCR product generated using primers CJNO1141 and AHO628.

The C-terminal Myc-tagged nourseothricin-resistant Efg1-Myc (CJN1775), Bcr1-Myc (CJN1785), Brg1-Myc (CJN1700), and Ndt80-Myc (CJN1707) strains were constructed by transforming the reference strain SN250 using PCR products from template plasmid pADH34 (containing a 13XMyc epitope tag immediately preceding the *SAT1*-flipper cassette, described in (Nobile et al., 2009)) and primers AHO385 and AHO386 (for Efg1), CJNO1046 and CJNO1047 (for Bcr1), CJNO1012 and CJNO1013 (for Brg1), and CJNO1024 and CJNO1025 (for Ndt80), respectively. These primers amplify the entire 13XMyc epitope tag and complete *SAT1* flipper cassette with 65 bp of hanging homology to the ORF of interest (minus stop codon) for the forward primer and 65 bp of hanging homology to the 3' UTR of the ORF of interest (precisely downstream of the stop codon) for the reverse primer. The homology in these primers allows recombination of the entire 13XMyc epitope tag and complete *SAT1* flipper cassette directly downstream of the ORF of interest, lacking its natural stop codon, so that the ORF contains a C-terminal 13XMyc epitope tag translational fusion. Correct integration of the C-terminal 13XMyc epitope tag and *SAT1* flipper was verified by colony PCR using detection primers AHO279 and AHO300 (for Efg1), CJNO1048 and AHO300 (for Bcr1), CJNO1014 and AHO300 (for Brg1), and CJNO1026 and AHO300 (for Ndt80) to check the upstream integration, and AHO281 and AHO301 (for Efg1), CJNO1049 and AHO301 (for Bcr1), CJNO1015 and AHO301 (for Brg1), and CJNO1027 and AHO301 (for Ndt80) to check the downstream integration. The C-terminal tagged nourseothricin-sensitive Efg1-Myc (CJN1781), Bcr1-Myc (CJN1787), Brg1-Myc (CJN1734), and Ndt80-Myc (CJN1748) strains were constructed by flipping out the *SAT1* cassette from strains CJN1775, CJN1785, CJN1700, and CJN1707, respectively, as described previously (Reuss et al., 2004). The following primer pairs were used in colony PCR to confirm the clean "flipping out" of the *SAT1*-flipper cassette for the ORF of interest: AHO279 with AHO300 and AHO281 with AHO302 (for Efg1), CJNO1048 with AHO300 and CJNO1049 with AHO302 (for Bcr1), CJNO1014 with AHO300 and CJNO1015 with AHO302 (for Brg1), and CJNO1026 with AHO300 and CJNO1027 with AHO302 (for Ndt80). The 13XMyc epitope tag and the region of homology to the 3' end of the ORF of interest used for integration of the *SAT1*-flipper cassette was confirmed by sequencing the colony PCR product generated using primers AHO279 with AHO283 (for Efg1),

CJNO1048 with AHO283 (for Bcr1), CJNO1014 with AHO283 (for Brg1), and CJNO1026 with AHO283 (for Ndt80).

The *NAT1-TDH3* promoter plasmid pCJN542 (Nobile et al., 2008) was used for gene overexpression. All overexpression strains (CJN2555, CJN2527, CJN2600, CJN2530, CJN2531, CJN2533, CJN2567, CJN2549, CJN2604, CJN2552, CJN2569, CJN2570, CJN2473, CJN2476, CJN2479, CJN2480, CJN2483, CJN2486, CJN2395, CJN2609, CJN2397, CJN2536, CJN2611, CJN2499, CJN2537, CJN2539, CJN2557, CJN2558, CJN2561, CJN2564, CJN2573, CJN2576, CJN2607, CJN2578, CJN2580, CJN2583, CJN2541, CJN2351, CJN2601, CJN2544, CJN2546, CJN2354, CJN2585, CJN2587, CJN2590, CJN2591, CJN2595, CJN2597, CJN2704, CJN2684, CJN2700, CJN2691, CJN2690, CJN2687) were constructed by transforming the various transcription regulator mutant strains, using PCR products from template plasmid pCJN542 and specific primers (listed in the primer table) that amplify the entire *Ashbya gossypii TEF1* promoter, the *C. albicans NAT1* open reading frame, the *A. gossypii TEF1* terminator, and the *C. albicans TDH3* promoter with 70-100 bp of hanging homology to 500 bp upstream into the promoter of the gene being overexpressed for the forward primer and 70-100 bp of hanging homology from exactly the start codon of the gene being overexpressed. The homology in these primers allows for homologous recombination of the entire cassette directly upstream of the natural locus of the gene being overexpressed so that its expression is driven by the *TDH3* promoter instead of its natural promoter. Transformation into *C. albicans* strains and selection on YPD+clonNAT400 plates has been described for this construct (Nobile et al., 2006). Integration of the constructs was verified by colony PCR with a gene-specific forward detection primer annealing to a sequence within the promoter of each gene and the reverse primer CJNO875 annealing to a sequence found in the *NAT* gene (listed in the primer table). The overexpression strains were assayed by quantitative real time PCR (qPCR) to ensure that the chosen genes of interest were overexpressed in their corresponding strain backgrounds (data not shown).

The target gene deletion mutant strains TFT66a (*orf19.3337Δ/Δ*), TFT64b (*als1Δ/Δ*), TFT68b (*tpo4Δ/Δ*), TFT70a (*eht1Δ/Δ*), TFT54a (*hyr1Δ/Δ*), TFT60d (*hwp1Δ/Δ*), and TFT72b (*can2Δ/Δ*) were constructed with the method previously described (Homann et al., 2009; Noble and Johnson, 2005) by fusion PCR using the primers listed in Supplemental Experimental Procedures. Target gene deletion mutant strain TF021 (*orf19.4000Δ/Δ*) was previously constructed (Homann et al., 2009). All deletions were verified by diagnostic PCR of the flanks surrounding the introduced markers and by attempting to amplify a small internal fragment of the ORF (for a successful deletion, this intra-ORF PCR yielded no product while a wild-type control yielded a clear product).

Transcriptional reporter strains of the biofilm transcriptional regulators (where one copy of each regulator's promoter was fused to an mCherry transcriptional reporter in both a homozygous regulator mutant as well as a heterozygous regulator mutant) were constructed as follows. The mCherry heterozygous reporter strains CJN2614, CJN2616, CJN1619, CJN2672, CJN2629, and CJN2621 were constructed by transforming SN425, using PCR products from template plasmid pADH77 (see description below) and gene-

specific primers (listed in the primer table); transformants were selected on YPD+clonNAT400 plates, as described above. Integration of the constructs was verified by colony PCR with a gene-specific forward detection primer and the reverse primer AHO702 (see primer table). The mCherry homozygous reporter strains CJN2708, CJN2710, CJN2712, CJN2736, CJN2715, and CJN2718 were constructed by transforming CJN2724, CJN2725, CJN2726, CJN2727, CJN2728, and CJN2729, respectively, using PCR products from template plasmid pADH77 and gene-specific primers (listed in the primer table); transformants were selected on SC-His+clonNAT200 plates, as described above. Integration of the constructs was verified by colony PCR with a gene-specific forward detection primer and the reverse primer AHO702 (see primer table).

Description of N-terminal Myc-tagging Plasmid pADH70

The sequence of plasmid pADH70 (containing a 7XMyc epitope tag immediately preceding the *SAT1*-flipper cassette) used for N-terminal myc-tagging is available at GenBank (www.ncbi.nlm.nih.gov/genbank), accession # JN795133).

Description of mCherry tagging Plasmid pADH77

The sequence of plasmid pADH77 (containing an mCherry fluorescent tag immediately preceding the *SAT1*-flipper cassette) used for creating transcriptional reporters of the biofilm regulators is available at GenBank (www.ncbi.nlm.nih.gov/genbank), accession # JN795134).

In vitro Biofilm Growth, Confocal Microscopy, and Biomass Determination

In vitro biofilm growth assays were carried out in Spider medium by growing the biofilm on either the silicone square substrate (Cardiovascular Instruments Corp, PR72034-060N) as described in (Nobile and Mitchell, 2005), or directly on the bottom of 12-well polystyrene plates (BD Falcon), as follows. Strains were grown overnight in YPD at 30°C, diluted to an optical density at 600 nm (OD_{600}) of 0.5 in 2 mL Spider medium. The 12-well plate alone or 12-well plate with silicone squares had been pretreated overnight with bovine serum (Sigma, B-9433) and washed with 2 mL phosphate-buffered saline (PBS) to prepare it for the biofilm assay. The inoculated plate was incubated at 37°C for 90 min at 200 rpm agitation for initial adhesion of cells in an ELMI digital thermostatic shaker. The plates were washed with 2 mL PBS, and 2 mL of fresh Spider medium was added. The plate was incubated at 37°C for an additional 48 h at 200 rpm agitation to allow biofilm formation. For visualization of strains over time, biofilms were allowed to form for 0 h (imaging was done directly after the 90 min incubation step), 8 h, 24 h, or 48 h. Biofilms grown on the silicone squares were used for confocal scanning laser microscopy (CSLM) visualization. For CSLM, biofilms were stained with 50 µg/mL of concanavalin A Alexa Fluor 594 conjugate (conA-594) (Molecular Probes, C-11253) in the dark for 1 h with 200 rpm agitation at 37°C. CSLM was performed in the Nikon Imaging Center at UCSF with a Nikon Eclipse C1si upright spectral imaging confocal

microscope using a 40x/0.80W Nikon objective. For conA-594 visualization, a 561 nm laser line was used. Images were acquired using Nikon EZ-C1 Version 3.80 software, and assembled into maximum intensity Z-stack projections using Nikon NIS Elements Version 3.00 software. Biofilms grown on the bottom of the 12-well plates were used for biomass determination. For dry mass measurements, five replicate wells containing biofilms were used. The medium was removed, 2 mL of PBS was added to each well, the biofilms were disrupted and resuspended by pipetting, and the contents of each well were vacuum filtered over a pre-weighed 0.8 µm nitrocellulose filter (Millipore, AAWG02500). A control well with no cells added was also vacuum filtered. The biofilm-containing filters were dried overnight, and weighed the following day. The average total biomass for each strain was calculated from five independent samples after subtracting the mass of the filter with no cells added. Statistical significance (*P* values) was calculated with a Student's one-tailed paired *t* test.

In vivo Rat Catheter Biofilm Model

A well established rat central-venous catheter infection model (Andes et al., 2004) was used for *in vivo* biofilm modeling to mimic human catheter infections, as described previously (Andes et al., 2004; Nobile et al., 2006). For this model, specific-pathogen-free female Sprague-Dawley rats weighing 400 g (Harlan Sprague-Dawley) were used. A heparinized (100 U/mL) polyethylene catheter with 0.76 mm inner and 1.52 mm outer diameters was inserted into the external jugular vein and advanced to a site above the right atrium. The catheter was secured to the vein with the proximal end tunneled subcutaneously to the midscapular space and externalized through the skin. The catheters were inserted 24 h prior to infection to permit a conditioning period for deposition of host protein on the catheter surface. Infection was achieved by intraluminal instillation of 500 µL *C. albicans* cells (10^6 cells/mL). After a 4 h dwelling period, the catheter volume was withdrawn and the catheter flushed with heparinized 0.15 M NaCl. Catheters were removed after 24 h of *C. albicans* infection to assay biofilm development on the intraluminal surface by scanning electron microscopy (SEM). Catheter segments were washed with 0.1 M phosphate buffer, pH 7.2, fixed in 1% glutaraldehyde/4% formaldehyde, washed again with phosphate buffer for 5 min, and placed in 1% osmium tetroxide for 30 min. The samples were dehydrated in a series of 10 min ethanol washes (30%, 50%, 70%, 85%, 95%, and 100%), followed by critical point drying. Specimens were mounted on aluminum stubs, sputter coated with gold, and imaged using a Hitachi S-5700 or JEOL JSM-6100 scanning electron microscope in the high-vacuum mode at 10 kV. Images were assembled using Adobe Photoshop Version 7.0.1 software.

In vivo Rat Denture Biofilm Model

A recently developed rat denture stomatitis infection model (Nett et al., 2010) was used for *in vivo* biofilm modeling to mimic human denture infections, as described in Nett et al., with certain modifications described here. For this model, specific-pathogen-free male Sprague-Dawley rats weighing 350 g (Harlan Sprague-Dawley) were used. Rats were immunosuppressed with a single dose of 200 mg/kg subcutaneous cortisone at

the time of denture placement. For denture placement, a stainless steel wire was threaded between the cheek teeth, a metal spatula was placed over the hard palate to create a space for *C. albicans* inoculation, and acrylic denture material (Maxitemp HP) was applied over the cheek teeth and wire to create the denture surface. The denture material was then molded into place, allowed to solidify for 5 min, and the spatula was removed. For denture infection, the hard palate beneath the acrylic denture was inoculated with 100 uL *C. albicans* cells (10^8 cells/mL). In order to reduce enteric bacterial colonization, ampicillin sodium/sulbactam sodium at 100 mg/kg was subcutaneously administered twice daily while the dentures were in place. Dentures were removed after 24 h post *C. albicans* infection to assay biofilm development on the denture surface by SEM. Dentures were processed for SEM as described previously (Andes et al., 2004) and briefly above, for the catheters.

RNA Sample Preparation and Extraction

Biofilms for gene expression microarray and RNA-seq analysis were grown in Spider medium at 37°C directly on the bottom of 6-well polystyrene plates, as described above, but with all volumes at 4 mL. One 6-well plate containing biofilms for one strain yields sufficient RNA for gene expression microarray and RNA-seq analysis. Biofilms were harvested by gently pipetting up and down along the bottoms of the 6-well plates, and combining the biofilm slurry of the same strain from each well of one 6-well plate in a 50 mL conical tube. Biofilm slurries were then centrifuged at 3,000 x g for 5 min, and total RNA was extracted using the RiboPure-Yeast RNA kit (Ambion, AM1926) or by the hot-phenol method (Hernday, 2010). To isolate total RNA for gene expression microarrays from planktonic cells, Spider medium was inoculated with organisms from a YPD 30°C overnight culture to obtain a starting OD_{600} of 0.05, incubated at 37°C until harvested by vacuum filtration when the OD_{600} was 1.0, snap-frozen in liquid nitrogen, and total RNA was extracted using the RiboPure-Yeast RNA kit (Ambion, AM1926). To isolate RNA for RNA-seq from planktonic cells, cells were grown in SC+Uri medium to an OD_{600} of 1.0 at 30°C, harvested by centrifugation (5 min, 2,000 x g), and snap-frozen in liquid nitrogen; total RNA was extracted by the hot phenol method (Hernday, 2010). For all total RNA samples used for RNA-seq, poly(A) RNA was isolated from 50 µg of total RNA by two rounds of purification using the Poly(A)Purist MAG kit (Ambion, AM1922).

Gene Expression Microarray Design and Analysis

Synthesis of cDNA and dye coupling were performed as previously described (Nobile et al., 2009). The gene expression microarrays were custom-designed oligonucleotide microarrays, containing at least two independent probes for each ORF from the *C. albicans* Assembly 21 genome (http://www.candidagenome.org/), and printed by Agilent Technologies (AMADID #020166). The gene expression microarray experiments were performed and analyzed as previously described using LOWESS normalization (Lohse and Johnson, 2010). Microarray data was clustered using Cluster Version 3.0 (de Hoon et al., 2004), and visualized using Java TreeView Version 1.13 (Saldanha, 2004). Expression microarray data are reported in Dataset S4 as the median of three

independent experiments. We used a cutoff of twofold in both directions ($\log_2 > 1.0$, and $\log_2 < 1.0$) for the differential expression of biofilm versus planktonic cells, and 1.5-fold in both directions ($\log_2 > 0.58$, and $\log_2 < -0.58$) for the differential expression of mutant over wild-type. Raw gene expression array data are available at the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo, accession # GSE30474).

Full Genome Chromatin Immunoprecipitation Tiling Microarray (ChIP-chip)

The ChIP-chip tiling microarrays were designed by tiling 181,900 probes of 60 bp length across 14.3 Mb included in the *C. albicans* Assembly 21 genome (http://www.candidagenome.org/), as previously described (Tuch et al., 2008), and printed by Agilent Technologies (AMADID #016350). All Myc-tagged regulator strains, untagged strains, and delete strains were grown under the same biofilm-inducing conditions as the strains grown for gene expression microarray analysis. The ChIP-chip experiments were performed as previously described (Nobile et al., 2009) with two independent biological replicates for each strain. Normalized enrichment values were determined for every probe on the microarray by LOWESS normalization using Agilent Chip Analytics Version 1.2 software (see Agilent Chip Analytics manual for details). Display, analysis and identification of the binding events were determined using MochiView Version 1.45 software (http://johnsonlab.ucsf.edu/sj/mochiview-start/) (Homann and Johnson, 2010), where peaks for the Myc-tagged strain (plus Myc antibody) or the wild-type strain (plus custom antibody), are compared to peaks from an untagged reference strain (plus Myc antibody) or the deletion strain (plus custom antibody). All ChIP-chip data analysis was performed with MochiView by mapping to the *Candida albicans* Assembly 21 genome (http://www.candidagenome.org/) downloaded on 4/1/2010. Identification of binding events for both independent biological replicates was determined by smoothing the two data sets together using the "Extract Peaks from Data Set(s)" utility described in detail in the MochiView manual. Briefly, a smoothing function is first applied to the Chip Analytics $\log_2$ enrichment values, followed by the application of a peak detection algorithm, where all binding peaks are assigned a *P* value using permutation testing. Peak-finding significance thresholds were kept at the default settings, $P \leq 0.001$ for the experimental IPs (i.e. Myc-tagged regulator strains and wild-type strains with custom antibodies), and $P \leq 0.05$ for the control IPs (i.e. untagged strains and deletion strains). For greater confidence, the amount of sampling was increased tenfold from the default setting to 100,000 (number of random samples to compare against each peak), and 100 (maximum number of random samples passing for inclusion of peak). The user-defined cut-offs for the minimum value for peak inclusion post-smoothing (values ranging from 0.40-0.58) were determined using the distribution of log-ratios for each regulator, and were set at two standard deviations from the mean of $\log_2$-transformed fold enrichments. User-defined cut-offs for the minimum value for peak inclusion post-smoothing ranging from 0.27-0.36 (1.5 standard deviations from the mean of $\log_2$-transformed fold enrichments) were used for the untagged and delete IP control data sets. We note that adjustments to these peak inclusion cut-offs do not alter the primary conclusions that we make from our ChIP-chip data. We have chosen highly stringent significance thresholds ($P \leq 0.001$) for the analysis of our ChIP-chip data in order to maximize our confidence in indicating a direct binding event

through a ChIP signal, and note that, as with all ChIP-chip data, the lack of a called peak does not necessarily indicate the absence of a direct binding event in that region. Raw ChIP-chip data are available at the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo, accession # GSE29785).

Assessment of Myc-tagged Proteins for Functionality

We did not observe a biofilm phenotype for our six heterozygous transcriptional regulator mutant strains. Thus, to ensure that Myc-tagging the regulators in the wild-type background did not interfere with the function of the regulator of interest, we additionally created a Myc-tagged allele over a deletion to confirm that the Myc-tagged allele is functional in promoting biofilm production. In addition, when Myc-tagging our regulators of interest, the constructs are sequenced to ensure that there are no obvious coding errors, and a Western blot is performed before attempting a ChIP experiment.

Custom Antibody for Tec1

Since C-terminal and N-terminal Myc-tagging of Tec1 interfered with the function of the protein, we used a custom-designed polyclonal antibody to epitope ELPSSAKPQVRLQK from amino acids 612-625 of the Tec1 protein. The peptide was synthesized, conjugated, polyclonal antibodies were produced in rabbits, and the antibody was affinity purified by Promab Biotechnologies. This Tec1 antibody (Promab, 29307) was used at a concentration of 10 μg/mL instead of the Myc antibody during the Tec1 ChIP-chip experiments.

Motif Analysis

Motif finding and assessment was performed with MochiView's "motif finder" function using 250 bp centered on the midpoint of half of the extracted peaks for each regulator, and analyzed for significance of enrichment in the remaining half of extracted peaks for that regulator using MochiView's "enrichment" function (Dataset S5). This utility determines the likelihood of finding the identified motif by chance in random intergenic regions of the same length. MEME Version 3.4.7 (http://meme.nbcr.net) software using 250 bp centered on the midpoint of all of the significantly called peaks was also used to independently verify the motifs found by MochiView. Locations of motif instances for each regulator, identified within their corresponding binding peaks, are shown in Dataset S2. Overall, the fraction of bound locations containing instances of the motifs depends on the regulator, and ranges from 30-100% (30% for Bcr1, 100% for Tec1, 100% for Efg1, 93% for Ndt80, 43% for Rob1, 44% for Brg1).

Motif distribution summary plots (Dataset S5) were constructed with MochiView's "compact motif/data/location plot" function using the minimum LOD score cutoffs established from the enrichment plots for each of the regulator motifs.

Motif distributions relative to binding peaks and to start codons (Dataset S5) were determined using MochiView's "motif→distribution→relative to locations" function. *P* values are calculated for the motif distributions at 50%, 60%, 70%, 80%, 90%, and 100% of the maximum motif score using a bootstrapped chi-square goodness of fit test (see MochiView manual). Motif occurrences for Tec1, Efg1, and Ndt80 were significantly centered at the area of ChIP-chip binding peak enrichment (Dataset S5). Motifs for all regulators were uniformly distributed across promoters, and there was no significant bias towards the start codon (Dataset S5).

RNA Sequencing (RNA-seq) of Biofilm and Planktonic Cells

Strand-specific, massively-parallel SOLiD System sequencing of RNA from wild-type *C. albicans* biofilm and planktonic cells and mapping of resulting reads were performed as previously described (Tuch et al., 2010) using poly(A) RNA. Library amplification was performed using barcoded SOLiD PCR Primer Sets 5 and 10 for planktonic and biofilm samples, respectively. Sequencing was performed on a full slide with eight other samples (not presented here), and resulted in 18 million planktonic and 28 million biofilm ~50 nt strand-specific sequence reads mappable to the *C. albicans* genome. Sequence data are available at the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo, accession # GSE21291).

Identification of Novel Transcriptionally Active Regions (nTARs) in Biofilms

nTARs were identified using the biofilm RNA-seq dataset and the "Create data set by extracting enriched regions from tiled set" feature of MochiView (Homann and Johnson, 2010). A previously published transcript annotation (Tuch et al., 2010) was used as a starting scaffold, and additional transcribed regions identified using a sliding window size of 125 nt, a trim multiplier of 0.01, a minimum location size of 50 nt, a location threshold cutoff of 20 and a location merge interval of 50 nt. This approach identified 783 biofilm nTARs distinct from those in the previous annotation (Dataset S6).

Differential Expression Analysis of RNA-seq Data

For every transcribed region in our expanded biofilm genome annotation, mean per-nucleotide sequence coverage was extracted from both biofilm and planktonic WIG files (generated using Life Technologies' SOLiD Whole Transcriptome Pipeline, available at http://solidsoftwaretools.com/gf/project/transcriptome), and transformed into pseudo-RPKM values (reads per kilobase per million mapped reads). The DEGseq package (Wang et al., 2010) was then used to determine which transcripts were differentially expressed between the two datasets, using a likelihood ratio test with a false discovery rate (q-value) cutoff of 0.005 (Storey and Tibshirani, 2003); an expression change of at least twofold was also required. The union of the RNA-seq and microarray datasets was

used to determine the final set of differentially expressed genes. The union was chosen (rather than the intersection) of differentially expressed genes because we wanted to increase the sensitivity and be more inclusive of the differentially expressed targets in the biofilm network. We noticed that genes called by only one set were often just below the significance threshold in the other set, but still appeared to be differentially expressed. We found that some of these genes proved to be important for biofilm formation, and thus we wanted to be sure to include them. There are very few cases (24 out of 2235 genes) where the directions between the microarrays and RNA-seq data were conflicting, and these genes are listed as "unchanged" in Dataset S6. Statistical significance (*P* values) for the association of binding and differential transcription was calculated using a two-tailed Fisher's exact test.

Association of Transcription Regulator Binding Sites with Adjacent Transcripts

To determine the association between transcription regulator binding and differential gene expression, the binding peaks identified by ChIP-chip were mapped to immediately adjacent, divergently transcribed (i.e., downstream) genes (with no threshold on distance). In our experience, certain chromosomal loci frequently exhibit non-specific (artifactual) chromatin immunoprecipitation; such loci were removed from our sets of predicted binding sites by screening out those that overlapped centromeres (0.7% of peaks) or heavily transcribed regions (mean biofilm RNA-seq coverage of >60 within 100 bp surrounding peak on either strand; 4% of peaks). The Assembly 21 annotation of the *C. albicans* genome was used (van het Hoog et al., 2007), but with transcription start sites defined by RNA-seq (Tuch et al., 2010), and supplemented with previously annotated nTARs (Tuch et al., 2010) and with biofilm nTARs annotated herein. Likely spurious genes (identified as those with mappable sequences having a maximal pseudo-RPKM value of <1 in all of our RNA-seq datasets) were removed from the annotation prior to peak association. A transcription regulator binding site was considered to be associated with differential expression if at least one divergent flanking transcript was differentially expressed in either the microarray or the RNA-seq comparison, using the criteria described above. The expected background association between transcription regulator binding and differential expression (0.32) was calculated as the average likelihood of a chance association across all binding sites; the likelihood for individual binding events was calculated as 0 for regions flanked by convergent transcripts, 0.26 (i.e., the overall proportion of differentially expressed genes) for regions flanked by one divergent transcript, and the probability that at least one of two randomly selected genes is differentially expressed ($1 – (1 – 0.26)^2$) for regions flanked by two divergent transcripts).

Exploring Orthology Relationships and Defining Gene Age Categories

The hemiascomycete species include the model yeast, *Saccharomyces cerevisiae*, and the predominant fungal pathogen of humans, *Candida albicans* (Pappas et al., 2004). These species are estimated to have diverged from a common ancestor between 300-700 million years ago (Hedges et al., 2004); in terms of protein conservation, the two

yeasts differ from each other to about the same extent as humans and fish (Dujon et al., 2004).

Orthologs of the *C. albicans* and *S. cerevisiae* biofilm regulators were identified by orthology mappings using SYNERGY (Remm et al., 2001) from The Fungal Orthogroups Repository (http://www.broad.mit.edu/regev/orthogroups/) and InParanoid (Wapinski et al., 2007) from the *Candida* Genome Database (CGD) (Costanzo et al., 2006) (www.candidagenome.org). In the cases of ambiguous orthology relationships from these two sources, further analysis was performed by hand annotation using gene trees generated from alignments of similar genes from 32 fungal genomes as identified by PSI-BLAST. Orthology comparisons for the direct targets of the regulators were based on orthology tables from The Fungal Orthogroups Repository (http://www.broad.mit.edu/regev/orthogroups/) using our *C. albicans* ChIP-chip direct targets, and *S. cerevisiae* direct targets from the YEASTRACT database (Teixeira et al., 2006) (http://www.yeastract.com/).

Tec1, a TEA/ATTS protein family member, represents the simplest case. The *C. albicans* Tec1, which regulates hyphal growth in biofilms (Nobile and Mitchell, 2005; Schweizer et al., 2000), has a one-to-one orthologous relationship to Tec1 in *S. cerevisiae*, where it controls pseudohyphal development (Gavrias et al., 1996; Liu et al., 1993). (*S. cerevisiae* does not make true hyphae.) Thus, Tec1 promotes aspects of cell morphology in both species. Despite the fact that the Tec1 protein and its recognition motif are conserved between *S. cerevisiae* and *C. albicans*, the set of genes controlled by Tec1 in the two species (as determined by ChIP-chip) has diverged considerably; for example, only 3% of the *C. albicans* Tec1-controlled genes are also controlled by Tec1 in *S. cerevisiae* (data presented here compared with that of Borneman et al., 2007). This situation is not unusual: even though a transcription regulator might control the same general process in *S. cerevisiae* and *C. albicans* (in this case, production of an elongated cell morphology), the genes controlled can vary significantly (Bennett et al., 2003; Booth et al., 2010; Borneman et al., 2007).

The situation with Efg1, a basic-helix-loop-helix protein, is similar in principle. *C. albicans* Efg1 and the closely related *C. albicans* protein Efh1 have a two-to-two orthology relationship with *S. cerevisiae* Phd1 and Sok2, which, like Tec1, are regulators of pseudohyphal growth (Gimeno and Fink, 1994; Ward et al., 1995). (We note that deletion of Efh1 had no apparent effect on *C. albicans* biofilm formation in our screen (Figure 1)). Like Tec1, Efg1 likely orchestrated changes in cell morphology (from spherical yeast form cells to more elongated forms) in the common ancestor of *S. cerevisiae* and *C. albicans*.

Ndt80, a member of an unusual class of DNA-binding proteins, seems to have very different roles in *C. albicans* and *S. cerevisiae* despite an almost identical recognition motif. *C. albicans* has two Ndt80 paralogs (the other is Orf19.513, whose deletion has no affect on biofilm formation (Figure 1)), while *S. cerevisiae* has a single ortholog, which is produced only in meiosis and is devoted to regulating the middle meiosis genes (Hepworth et al., 1998; Unal et al., 2011). *C. albicans* does not appear to undergo

meiosis (having a parasexual cycle (Bennett and Johnson, 2005)), and, as we show here, Ndt80 in this species plays a prominent role in biofilm production. A universal feature of microbial biofilms is their resistance to drugs and other antimicrobial agents, and, although *NDT80* is required for formation of biofilms in *C. albicans*, it also plays a role in regulating drug resistance by controlling the expression of *CDR1*, which encodes a major drug efflux pump (Chen et al., 2004). Indeed, our data also indicate that Ndt80 directly and indirectly controls the expression of many drug efflux pumps in biofilms (for example, *CDR3,* and *CDR4* directly; *CDR11* indirectly). At this point, we do not know whether Ndt80 controlled meiosis or biofilm production in the ancestor of *S. cerevisiae* and *C. albicans*, but it is clear that the regulator plays very different roles in the two modern species.

Finally, *C. albicans* Bcr1, a C_2H_2 zinc finger protein, has a one-to-two orthology relationship (as a result of the whole genome duplication) with *S. cerevisiae* Usv1 and Rgm1. However, neither of these genes has been extensively studied, and it is therefore not possible to meaningfully compare their function between the two species. *C. albicans* Rob1, a zinc cluster protein, and Brg1, a GATA protein, have no identifiable orthologs outside of the *Candida* (CTG) clade (with the possible exception of a Brg1 ortholog in *Yarrowia lipolytica*, a species recently reported to form biofilms (Dusane et al., 2008).

Genes of different age groups have been shown to have different functional properties (Capra et al., 2010). Gene ages were defined using orthology assignments from The Fungal Orthogroups Repository (http://www.broad.mit.edu/regev/orthogroups/). “Old” *C. albicans* genes are members of gene families found in all Ascomycetes. “Middle-aged” *C. albicans* genes are members of gene families that arose after the divergence of *S. pombe* and *S. japonicus* but before the divergence of the CTG clade. “Young” genes are found only in CTG clade species. Overlap of age categories with biofilm-induced genes is described by the hypergeometric distribution, which was approximated by the Pearson’s chi-square test without Yates’ continuity correction to obtain *P* values. For all age categories, P<1.23E-9) Similar results to those in Figure 4H were obtained using the expression, binding, and intersection of those datasets.

Details on Identification and Phenotypic Characterization of Biofilm-Defective Transcription Regulator Mutants in vitro

Of the nine mutants that were revealed from our visual and biomass biofilm screens, we did not follow up on TF091 (*orf19.1685*Δ/Δ) because its biofilm defect is due to a growth defect specific to Spider medium (the mutant is unable to utilize mannitol as a carbon source (Homann et al., 2009)), TF103 (*orf19.3063*Δ/Δ) because its biofilm defect is the result of an overall growth defect (Homann et al., 2009), and TF117 (*tup1*Δ/Δ) because this mutant is highly pleiotropic (Braun and Johnson, 1997; Homann et al., 2009; Zhao et al., 2002) and its phenotype interfered with the biofilm assay.

A comprehensive phenotypic characterization of the homozygous transcription regulator mutant library that we describe here has been previously reported (Homann et al.,

2009). Homann et al. assessed phenotypes of this mutant library under 55 different growth conditions, including several conditions for assaying colony morphologies and drug susceptibilities (Homann et al., 2009). Biofilm formation assays, however, were not previously performed on this library. Our six biofilm defective transcription regulator mutants that we identify here, do not appear to have any one phenotype in common based on the phenotypic profiles reported in Homann et al. We note that *tec1Δ/Δ* strains exhibited reduced colony wrinkling and reduced peripheral filamentation in several media, *bcr1Δ/Δ* and *ndt80Δ/Δ* strains exhibited enhanced invasion on several media, and *efg1Δ/Δ*, *brg1Δ/Δ*, and *rob1Δ/Δ* strains exhibited reduced invasion on several media (Homann et al., 2009). Based on this information, it is possible that reductions in peripheral filamentation, or the presence of an invasive growth phenotype in either direction (hyper- or hypo- invasion), may potentially be an indicator of a biofilm defect. However, there are many mutants in the library that display reductions in peripheral filamentation (e.g. *rim101Δ/Δ*, *isw2Δ/Δ*, *orf19.7381Δ/Δ*, and *orf19.1168Δ/Δ*), enhanced invasive growth (e.g. *sfl1Δ/Δ*, *stp4Δ/Δ*, *orf19.6102Δ/Δ*, *orf19.6874Δ/Δ*, and *orf19.6798Δ/Δ*), and reduced invasive growth (e.g. *cph2Δ/Δ* and *orf19.921Δ/Δ*) (Homann et al., 2009), that we did not find to be defective in biofilm formation (Dataset S1 and Figure 1). Thus, the characteristics that contribute to defining a biofilm-defective mutant cannot be predicted based on other morphological attributes of the mutant.

Assessing Hyphal Formation Under Biofilm and Planktonic Conditions in vitro

For assessing hyphal formation under biofilm conditions, strains were grown under standard biofilm-inducing conditions in Spider medium at 37°C for 48 h. Two hundred cells free-floating in the medium were counted and analyzed for hyphal formation by light microscopy. From this, the percentage of true hyphae was determined for each transcription regulator mutant strain under biofilm conditions (Figure S3). For assessing hyphal formation under planktonic conditions, strains were grown planktonically at 37°C under three types of filament-inducing conditions: 1) RPMI medium for 90 min, 2) Spider medium for 3 h, and 3) YPD+10% Serum for 2 h. Strains were inoculated from a saturated overnight YPD culture into the corresponding filament-inducing medium at an OD_{600} = 0.2. Two hundred cells from each medium were counted and analyzed for hyphal formation by light microscopy. From this, the percentage of true hyphae was determined for each transcription regulator mutant strain under planktonic conditions (Figure S3).

We found that, with the exception of the *efg1Δ/Δ* strain, we were able to detect true hyphae suspended in the medium surrounding the biofilm (but not necessarily in the biofilm itself) for the mutants under biofilm conditions (Figure S3A). Note that cells dispersed from biofilms are predominantly in the yeast form; 21% of cells suspended in the medium from a wild-type biofilm are hyphae (Figure S3A). The *efg1Δ/Δ* strain was the only strain completely defective for hyphal formation under biofilm conditions; 0% of cells suspended in the medium from an *efg1Δ/Δ* strain are true hyphae (Figure S3A). The *efg1Δ/Δ* strain was also defective for hyphal formation under every planktonic hyphal-inducing condition that we tested (Figure S3B). Thus, consistent with previous

findings (Lo et al., 1997; Ramage et al., 2002; Stoldt et al., 1997), *efg1Δ/Δ* has a strong hyphal defect under various *in vitro* conditions, including biofilm conditions, and has the strongest hyphal defect of our six biofilm-defective regulator mutants. We also observed that the absolute number of true hyphae suspended in the medium for the *tec1Δ/Δ, ndt80Δ/Δ*, and *rob1Δ/Δ* strains was slightly reduced (6%, 7%, and 10% hyphae, respectively), compared to wild-type (21% hyphae), under our biofilm conditions (Figure S3A). In addition, the *tec1Δ/Δ*, *ndt80Δ/Δ*, and *rob1Δ/Δ* strains were defective for hyphal formation, compared to wild-type, under some planktonic hyphal-inducing conditions that we tested (Figure S3B). Hyphal defects have been previously reported for *tec1Δ/Δ* and *ndt80Δ/Δ in vitro* (Schweizer et al., 2000; Sellam et al., 2010). We did not detect any hyphal defects for the *bcr1Δ/Δ* or *brg1Δ/Δ* strains under our biofilm conditions (Figure S3A) or under the planktonic hyphal-inducing conditions that we tested (Figure S3B). This finding is consistent with the previous finding that Bcr1 is not required for hyphal formation per se, but is important for overall adherence of hyphae and yeast cells (Nobile and Mitchell, 2005). We note that our newly identified biofilm regulator mutant, *brg1Δ/Δ*, at least in terms of its hyphal formation phenotype, appears most similar to *bcr1Δ/Δ*.

Additional Information on the Characterization of Biofilm-Defective Transcription Regulator Mutants in vitro

Based solely on the *in vitro* biofilm phenotypes of the six core transcription regulator mutants that we describe, below we attempt to categorize the mutants according to their appearances in our assay. We visualized each regulator mutant over time during the development of the biofilm (Figure S1). At the 0 h time-point (90 min post-adherence), the wild-type reference strain and the *bcr1Δ/Δ* strain both had a uniform distribution of yeast-form cells, many of which began to form hyphae. In contrast, the *rob1Δ/Δ*, *ndt80Δ/Δ*, *brg1Δ/Δ*, and *tec1Δ/Δ* strains formed a dense mat of primarily yeast-form cells. The *efg1Δ/Δ* strain only sparsely colonized the substrate and occasional pseudohyphae were observed. The *bcr1Δ/Δ* strain did not appear deficient at the 0 h time-point, however it began to show a defect at the 8 h time-point. At 8 h, the wild-type strain contained many hyphal cells and was 4 times thicker than at the 0 h time-point, while the *bcr1Δ/Δ strain* had not changed substantially since the 0 h time-point. The other mutant strains also made minimal progress and were thicker and/or denser at 8 h than at 0 h, but were largely lacking hyphae within their biofilms. The *tec1Δ/Δ* strain appeared to contain some of the same long and vertical hyphae as seen in wild-type, although they were sparse compared to wild-type and disappeared by the 24 h time-point. At 24 h, wild-type formed a robust biofilm, while most of the mutants were still rudimentary mats composed largely of yeast-form and pseudohyphal cells. Interestingly, the *bcr1Δ/Δ* was capable of forming a morphologically intact biofilm at this time-point, however, it was extremely delicate and sloughed off the substrate, leaving behind a few scattered yeast and hyphal cells. It appeared that by 48 h, the *bcr1Δ/Δ* began to fill in the sloughed-off regions with yeast and hyphal cells, and somewhat resembled the other mutant regulators at this stage. From this phenotypic description over time, we

can categorize the *bcr1Δ/Δ* as being defective at a later time-point than the other mutants. The other mutants were deficient at either making or retaining hyphal cells immediately after adhesion as well as throughout biofilm development. The *efg1Δ/Δ* strain had the most severe phenotype overall as it appeared defective in adhesion, hyphal formation, and thickness at every stage of biofilm formation. The *tec1Δ/Δ* and *bcr1Δ/Δ* strains were the most successful at incorporating hyphae into the biofilm, at least transiently. While none of the mutants appeared retarded at any particular developmental stage throughout biofilm development, per se, *rob1Δ/Δ*, *brg1Δ/Δ*, *ndt80Δ/Δ*, and *efg1Δ/Δ* strains most closely resembled wild-type at the 0 h time-point, while *tec1Δ/Δ* and *bcr1Δ/Δ* strains, in terms of biofilm architecture, made some attempts at forming the components of a mature biofilm, but ultimately failed by the 48 h time-point likely due to adherence deficiencies.

Additional Information on the Characterization of Biofilm-Defective Transcription Regulator Mutants in a Rat Denture in vivo Model

In the initial characterization of this in vivo denture model, the *bcr1Δ/Δ* strain was the strain that was used to validate this model, and our findings are consistent with what was observed by Nett et al. (Nett et al., 2010). Nett et al. reported that the *bcr1Δ/Δ* biofilm formed on the rat denture contained 4-fold fewer adherent *C. albicans* cells, and a 50% higher bacterial burden compared to the reference strain (Nett et al., 2010). One simple hypothesis for this finding is that, during oral colonization of the denture, the presence of fewer colonizing *C. albicans* cells will result in an increase in bacterial colonization of the denture. This inference is consistent with what we observe for the other five mutant strains, which had little to no colonizing *C. albicans* cells on their dentures (Figure 2); we were, however, able to detect extensive bacterial biofilms consisting of both cocci and rod bacteria on their dentures (Figure S4B).

Additional Information on the Analysis of the ChIP-chip Data

To identify genes directly regulated by Bcr1, Tec1, Efg1, Ndt80, Rob1, and Brg1, we performed full genome chromatin immunoprecipitation microarray (ChIP-chip) to map the position across the genome to which each of the six transcription regulators are bound. We tagged each transcription regulator with a Myc tag at the C- or N-terminal end of the protein (choosing the scheme that did not interfere with the protein's activity) in a wild-type reference strain background. (In the case of Tec1, tagging the protein at either the C- or N- terminal end interfered with the protein's activity, and we used a custom-designed polyclonal antibody against an epitope near the C terminus of the Tec1 protein.) We then grew the tagged strains under standard biofilm conditions (because the tags do not compromise function, the strains form normal biofilms), and harvested the biofilm cells for chromatin immunoprecipitation. After precipitation using the commercially available Myc antibody or the custom Tec1 antibody, the immunoprecipitated DNA and whole-cell extract were amplified and competitively hybridized to custom whole-genome oligonucleotide tiling microarrays.

For analysis of the ChIP-chip data, peaks for the Myc-tagged strain (plus Myc antibody) or the wild-type strain (plus custom antibody), are compared to peaks from an untagged reference strain (plus Myc antibody) or the deletion strain (plus custom antibody), respectively, by mapping the data onto each chromosome. We chose stringent significant thresholds P<0.001 for the peak-finding analysis of our ChIP-chip data in order to maximize our confidence in indicating a direct binding event through a ChIP signal, with the caveat in mind that the lack of a called significant peak does not necessarily indicate the absence of a direct binding event in that region. Our analysis revealed the following number of significant binding peaks (binding events): 314 for Bcr1, 90 for Tec1, 393 for Efg1, 660 for Ndt80, 100 for Rob1, and 309 for Brg1 (see Dataset S2 for a complete list of every bound location for each regulator and Dataset S3 for MochiView image plots of every called significant peak for each regulator). The numbers of peaks that we report here include counting multiple peaks that may bind the same intergenic region. We then mapped these called significant peaks to intergenic regions of the *C. albicans* genome by taking the maximum peak enrichment value ($\log_2$ normalized enrichment) of the peak/s restricted to an intergenic region, thereby only counting a single maximum peak value per bound intergenic region. Our analysis based on intergenic regions containing a peak from the ChIP-chip signals that meets our significance thresholds indicates the following number of bound regions for each regulator: 211 for Bcr1, 76 for Tec1, 328 for Efg1, 558 for Ndt80, 95 for Rob1, and 283 for Brg1 (see Dataset S2 for a list of every intergenic region containing a significant called peak). Our analysis indicates that the biofilm regulatory network consists of 831 intergenic regions bound by one or more regulator, 350 intergenic regions bound by two or more, 186 intergenic regions bound by three or more, 111 intergenic regions bound by four or more, 55 intergenic regions bound by five or more, and 18 intergenic regions bound by all six of the biofilm regulators (Dataset S2). Indeed, 42% of the 831 bound intergenic regions are bound by at least two of the six regulators.

Finally, to determine which target genes contain a significant called peak in their upstream promoter regions, we assigned peak enrichment values to promoters (defined as in (Tuch et al., 2010)), and listed bound target genes based on the presence of a binding event in their upstream promoter region (see Dataset S4 for a list of every gene significantly bound in their upstream promoter by each regulator). In the case of the latter dataset, binding events are listed twice if the upstream promoter region is flanked by two divergent ORFs, thus listing each candidate target gene's promoter; and binding events occurring outside of promoter regions (e.g. between two convergent ORFs) are not listed. From our analysis, we found the following number of bound promoters containing a significant called peak from the ChIP-chip signals of each regulator: 252 for Bcr1, 107 for Tec1, 447 for Efg1, 836 for Ndt80, 96 for Rob1, and 311 for Brg1 (see Dataset S4 for a list of every promoter containing a significant called peak). Of these 1,061 target genes - 23 are bound by all six, 77 are bound by five or more, 165 are bound by four or more, 265 are bound by three or more, and 458 (43%) are bound by two or more of the biofilm regulators.

Additional Information on Identifying Functionally Relevant Target Genes of the Core Biofilm Network

We observed the following transcriptional responses from largest to smallest when *EFG1*, *ROB1*, *BRG1, NDT80*, *TEC1*, and *BCR1* were deleted (48%, 35%, 29%, 16%, 7%, and 7% of the genome changing, respectively, based on our significance threshold of $\log_2 > 0.58$, and $\log_2 < -0.58$) (Dataset S4). For Bcr1, we found 234 genes that were downregulated and 173 genes that were upregulated in the *bcr1Δ/Δ* mutant (Dataset S4). Of these 407 differentially regulated genes, 46 of them (11%) were also directly bound in their promoters by Bcr1 (Dataset S4). Precisely half of these 46 direct target genes were downregulated and half of them were upregulated in the *bcr1Δ/Δ* mutant, arguing that although Bcr1 is typically thought of as an activator of its target genes (Nobile and Mitchell, 2005), we show that Bcr1 can act as both an activator and repressor of its biofilm-relevant direct targets. For Tec1, we found 233 genes that were downregulated and 226 genes that were upregulated in the *tec1Δ/Δ* mutant (Dataset S4). Of these 459 differentially regulated genes, 40 of them (9%) were also directly bound in their promoters by Tec1 (Dataset S4). 33 of these directly bound target genes (90%) were downregulated in the *tec1Δ/Δ* mutant, while only 4 direct target genes were upregulated in the *tec1Δ/Δ* mutant, suggesting that Tec1 is predominantly an activator of its biofilm-relevant direct targets. For Efg1, we found 1,410 genes that were downregulated and 1,537 genes that were upregulated in the *efg1Δ/Δ* mutant (Dataset S4). Of these 2,947 differentially regulated genes, 276 (9%) were also directly bound in their promoters by Efg1 (Dataset S4). 179 of these directly bound target genes (65%) were downregulated in the *efg1Δ/Δ* mutant, and 97 (35%) were upregulated in the *efg1Δ/Δ* mutant, suggesting a dual role for Efg1 as both an activator and repressor of its biofilm-relevant direct targets. For Ndt80, we found 593 genes that were downregulated and 406 genes that were upregulated in the *ndt80Δ/Δ* mutant (Dataset S4). Of these 999 differentially regulated genes, 273 (27%) were also directly bound in their promoters by Ndt80 (Dataset S4). 143 of these directly bound target genes (52%) were downregulated in the *ndt80Δ/Δ* mutant, and 130 (48%) were upregulated in the *ndt80Δ/Δ* mutant, suggesting that Ndt80 is both an activator and repressor of its biofilm-relevant direct targets. For Rob1, we found 1,121 genes that were downregulated in and 1,029 genes that were upregulated in the *rob1Δ/Δ* mutant (Dataset S4). Of these 2,150 differentially regulated genes, 46 (2%) were also directly bound in their promoters by Rob1 (Dataset S4). 33 of these directly bound target genes (72%) were downregulated in the *rob1Δ/Δ* mutant, and 13 (28%) were upregulated in the *rob1Δ/Δ* mutant, suggesting that Rob1 is both an activator and repressor of its biofilm-relevant direct targets. For Brg1, we found 822 genes that were downregulated and 931 genes that were upregulated in the *brg1Δ/Δ* mutant (Dataset S4). Of these 1,753 differentially regulated genes, 130 (7%) were also directly bound in their promoters by Brg1 (Dataset S4). 101 of these directly bound target genes (78%) were downregulated in the *brg1Δ/Δ* mutant, and 29 (22%) were upregulated in the *brg1Δ/Δ* mutant, suggesting that Brg1 is both an activator and repressor of its biofilm-relevant direct targets.

By looking at the correlation between our ChIP-chip binding data and our gene expression array data when that regulator is deleted, we note that we do not find an improvement in this correlation by considering regions bound by only the relevant transcriptional regulator (or a smaller subset of the regulators). Thus, the lack of a complete correlation between binding and regulation is unlikely to be a result of redundancy of binding (see Worksheet “Correlation Bound and Regulated” in Dataset S4).

In Figure 5, we identified eight target genes that are positively regulated by all six transcriptional regulators; however they are not bound by all six regulators. We find that a hierarchical cascade between the biofilm regulators can explain this indirect regulation. These eight genes are not direct targets of all six regulators, however they are direct targets of at least one regulator, and indirect targets can be explained by regulators that are direct targets (Figure S5). For example, Ndt80 binds and directly regulates six out of the eight target genes. Ndt80 also directly regulates *BRG1*, which in turn directly regulates *TEC1*, which then directly regulates *EFG1*, which then directly regulates the other two target genes (Dataset S4; Figure S5). The hierarchical cascade can be followed in this way, from each transcriptional regulator to each of the eight target genes, explaining the differential regulation of these genes, even in the absence of a direct binding event by the initial regulator.

In addition to the identifying the direct targets of each regulator, the ChIP-chip and expression profiling data indicate that differential expression of indirect target genes of any one regulator may be explained via regulation of the other five transcriptional regulators (see Worksheet “Direct and Indirect Target Reg” in Dataset S4). For example, Rob1 directly regulates only 2% of its targets, however it also directly regulates Brg1, Bcr1, and Tec1; at least one of each binds an additional 12% of Rob1’s indirect targets. If the other biofilm regulators (Ndt80 and Efg1) are included, 9% more of Rob1’s indirect targets are bound, making a total of 23% of Rob1’s indirect targets bound by at least one regulator in the biofilm circuit. If this analysis is done for each biofilm regulator, 6% of Ndt80’s indirect targets (and 33% of all of its targets) are explained; 18% of Brg1’s indirect targets (and 25% of all of its targets) are explained; 11% of Efg1’s indirect targets (and 21% of all of its targets) are explained; 31% of Bcr1’s indirect targets (and 42% of all of its targets) are explained; and 27% of Tec1’s indirect targets (and 36% of all of its targets) are explained. Additionally, Ndt80, Brg1, Efg1, Bcr1, Tec1, and Rob1 bind and regulate, respectively, 29, 26, 29, 4, 4, and 2, transcriptional regulators other than the 6 regulators in the biofilm circuit. Thus, it is possible that indirect targets of the biofilm regulators may be regulated by these other regulators. Interestingly, the direct targets of the biofilm regulators are significantly enriched for transcriptional regulators (*P*=4.573E-12; Pearson’s chi-square test): 11% of Ndt80 targets, 21% of Brg1 targets, 12% of Efg1 targets, 11% of Bcr1 targets, 15% of Tec1 targets, and 11% of Rob1 targets); only 5% of genes in the genome encode transcriptional regulators (see Worksheet “TR Enrichment of Direct Targets” in Dataset S4). We note that of the 52 transcriptional regulators directly regulated by a member of the biofilm circuit, we screened deletion mutants for 34 of these (as they were in the library), and found no biofilm defects by dry weight. It is possible that deletion of one of

the remaining 15 transcriptional regulators may have a biofilm defect, but 8 of these may be essential (as homozygous deletion mutants were not obtained) and the other 7 are yet to be attempted.

Similar to the biofilm network, the regulators in the pseudohyphal growth circuit of *S. cerevisiae* bind many additional regulators outside of the main circuit (Borneman et al., 2006). In contrast, transcriptional regulators that are not major players in complex regulatory networks, but rather control their targets in more simple genetic pathways, such as Zap1 in *C. albicans* and Gal4 in *S. cerevisiae*, bind only a few, if any transcriptional regulators (Nobile et al., 2009; Ren et al., 2000). Thus, we suggest that transcriptional regulators that are main players in complex networks may be more likely to regulate other transcriptional regulators.

GO Term Functional Analysis of Biofilm Target Genes

Genes specifically regulated by only one biofilm transcriptional regulator or genes that are regulated by multiple biofilm transcriptional regulators may share common functions. However, 75% of the *C. albicans* genome remains uncharacterized, which makes it difficult to assign function to groups of genes; we attempted to do this analysis using Gene Ontology. While most of the targets of the regulators were not significantly enriched for any particular GO category, there were some exceptions. Targets that were regulated by all 6 biofilm transcriptional regulators were enriched for the following GO terms: adhesion, biofilms, and hexose biosynthesis (*P*=1E-4 to 0.05). Targets that were regulated by 5 biofilm transcriptional regulators were enriched for multi-organism processes including biofilm formation and host interaction (*P*=1E-8 to 1E-6). Targets that were regulated by 4 biofilm transcriptional regulators were enriched for organic substrate transport and membrane transport (*P*=1E-6 to 1E-4). For genes that were regulated by only one biofilm transcription regulator, only the targets of Ndt80 and Rob1 had any sort of functional enrichment. Ndt80 targets were enriched for genes involved in reproduction (*P*=1E-4 to 0.05), including *KIC1*, *HHF1*, *HHF22*, and *VPS11*. Rob1 targets were enriched for intracellular transport (*P*=1E-4 to 0.05), including *PEP7*, *VPS21*, *REI1*, *ORF19.3128*, *ORF19.479.2*, and *ORF19.7202*. While these GO term categories provide a hint to the function of various target gene sets, they explain only a small fraction of regulated genes. Note that the majority of genes in the *C. albicans* genome do not have a GO term, and those that do are based on not only hand-curated entries but also high throughput data and computational prediction.

Additional Information on Target Gene Ectopic Expression

We note that there were no genes among similar functional classes as the eight target genes that we chose for overexpression that were not differentially regulated in all of the regulator mutants compared to the reference strain. However, among target genes

within similar functional classes, there was one gene that was differentially regulated in just one of the regulator mutants. Therefore, we overexpressed this gene, *TPO5 (ORF19.151)*, which is predicted to be within the same functional class as *TPO4 (ORF19.473)*, one of the target genes we implicate in playing a role in biofilm formation. While *TPO4* is differentially regulated in all six regulator mutants compared to reference strain, *TPO5* is differentially regulated (just under 2-fold) only in the *brg1*Δ/Δ mutant strain background. When *TPO5* is overexpressed in the backgrounds of all of the regulator mutants, it is not able to rescue biofilm formation (data not shown). We believe that this is consistent with our hypothesis that some of our target genes are important in biofilm formation, while other genes (even from similar functional classes) are not.

Additional Information on the Functionally Relevant Target Genes Controlled by the Biofilm Circuit

In this study, we prioritized our focus on the eight target genes that were downregulated in all six transcription regulator mutant strains when compared to WT (as it turned out, they were also bound by at least one regulator). We made homozygous deletion mutant strains for each of these eight genes and also created strains where each target gene was overexpressed in the background of each regulator mutant. We analyzed the resulting 56 strains by dry weight assays and CSLM. From the deletion strains, three were deficient in biofilm formation: *als1*Δ/Δ and *hwp1*Δ/Δ (both of which were previously known to have biofilm defects (Nobile et al., 2006a; Nobile et al., 2006b; Nobile et al., 2008)), and also *can2*Δ/Δ, which has not been identified previously as important for biofilm formation. All three of these proteins are predicted to be cell-surface localized. Hwp1 is a hyphal-specific protein and Als1 is expressed in both yeast and hyphal cells (Coleman et al., 2010; Green et al., 2005; Staab et al., 1996). Als1 and Hwp1 both play roles in cell-cell adhesion as well as surface adhesion, and it is thought that decreased adhesion contributes to poor biofilm formation in the null mutants.

CAN2 is predicted to encode an amino acid permease, and the protein sequence has 81% identity to Can1, a confirmed lysine/arginine/histidine permease. Amino acid permeases not only provide amino acid substrates for metabolic pathways, but also transport drugs and toxic substances and may be involved in nutrient sensing and signaling pathways (Hundal and Taylor, 2009; Sophianopoulou and Diallinas, 1995). In *C. albicans*, arginine activates the filamentation pathway, and arginine biosynthesis is required for *C. albicans* to escape macrophages via filamentation (Ghosh et al., 2009). It is possible that Can2 contributes to filamentation pathways via arginine uptake. Alternatively, Can2 may uptake other small molecules or participate in nutrient signaling pathways important for biofilm formation. Interestingly, *CAN2* expression was previously found to be upregulated in wild-type biofilms formed in the rat catheter model (Nett et al., 2009), whereas the other known amino acid permease, *CAN1* was not differentially regulated. This data indicates that Can2 plays an important role in the host environment.

Three of the eight top target gene candidates were required for normal biofilm formation, however, all but two of them (*ORF19.3337* and *HYR1*) were able to partially rescue biofilm formation when overexpressed in at least one transcription regulator mutant strain background. This is not surprising, given that many proteins have functional redundancy. Overexpression of six of the eight target genes was able to partially rescue both the *bcr1Δ/Δ* and *tec1Δ/Δ* mutants. Previous work has shown that *BCR1* expression is dependent on Tec1, so this may explain why these mutant backgrounds are similarly rescued (Nobile and Mitchell, 2005).

Further study will be needed to fully understand the roles of each of these target genes in biofilm formation. However, we can form some hypotheses based on previous work on some of these proteins in *C. albicans* and also homology to *S. cerevisiae* proteins. As described above, Als1 and Hwp1 are both adhesin proteins, thus enhancing adhesion between biofilm cells; Als1 has no clear orthologs in *S. cerevisiae*. Little is known about the other four genes that resulted in partial rescue. *ORF19.4000* is a predicted homeodomain transcription regulator, potentially regulating its own set of target genes that may compensate for the gene sets no longer regulated by the lack of Bcr1 or Tec1. Like *CAN2*, *TPO4* encodes a putative transporter, but with similarity to both polyamine and MFS drug transporters. Polyamines are essential for normal cell growth and polyamine levels are carefully regulated in *E. coli*, *S. cerevisiae*, and higher eukaryotes (Igrashi and Kashiwagi, 2010a; Igrashi and Kashiwagi, 2010b). It is unclear what the roles of Tpo4 and Can2 are in *C. albicans*, but both may be transporting small molecules that affect biofilm formation. The final rescue was mediated by overexpression of *EHT1*. *EHT1* encodes a putative alcohol acyl transferase, and contains a predicted hydrolase catalytic domain. The *S. cerevisiae* othologs, Eht1 and Eeb1, play roles in lipid metabolism (Athenstaedt et al., 1999). In *C. albicans*, *EHT1* is induced in response to alpha pheromone, thus it may play a role in polarized growth, which is also important in biofilm formation (Bennett and Johnson, 2006).

Our genome wide approach provided us with an unbiased list of candidate target genes potentially involved in biofilm formation. We chose to focus first on the eight genes that are positively regulated by all six of the biofilm regulators. Of the 8 target genes, 6 had a biofilm phenotype, which validates our approach for selecting a high confidence set of target genes. Future work will examine the roles of these 6 genes in biofilm formation and will also screen additional target genes identified here for biofilm phenotypes.

Additional Information on the Evolutionary Conservation of the Biofilm Network

Our evolutionary analysis indicated that biofilm genes are enriched for young and middle-aged genes, while old genes are underrepresented in the biofilm network. In addition, we noticed that the intergenic regions bound by biofilm regulators were longer than the genome-wide average. Because young genes tend to have longer intergenic regions (Sugino and Innan, 2011), we asked whether the enrichment of young genes can explain the increased length of biofilm regulator targets. However, biofilm targets had much longer intergenic regions than most young genes (P<2.2E-16) (Figure 4I).

We note that the enrichment of young and middle-aged genes and underrepresentation of old genes is also true for regulator-bound genes. By incorporating the array data, we find that old genes are indirectly regulated, whereas young genes are directly regulated by the biofilm regulators (see Worksheet “Age of Targets and Expression” in Dataset S6).

The biofilm network has additional features that may point to its rapid evolution. For example, the mean length of intergenic regions bound by each individual biofilm regulator is longer than those that are not bound by the biofilm regulators (see Figure 4I and Worksheet “Intergenic Lengths Bound” in Dataset S6). In addition, and in contrast to other transcription regulators whose position is confined to a narrow region upstream of the transcription start site (e.g. Matα2 in *S. cerevisiae*; Johnson and Herskowitz, 1985), the regulatory sites for the biofilm regulators were found interspersed throughout intergenic regions (Dataset S5).

Information on the Enrichment of the Biofilm Regulator Motifs Across Other Yeast Species

Genes that are known targets of biofilm regulators in *C. albicans* were mapped to gene families using The Fungal Orthogroups Repository (http://www.broad.mit.edu/regev/orthogroups/). Upstream intergenic regions from thirteen species were scored using the motifs of each biofilm regulator and two control regulators (Lavoie et. al., 2010) using MAST (Bailey and Gribskov, 1998) (Figure S7C). Ndt80 and Efg1 were the only biofilm regulators with motifs containing sufficient predictive power in *C. albicans* to be scored across the other yeast species (Figure S7C). Enrichment of the motif upstream of biofilm regulator targets was quantified using a log-ratio. A cutoff for the motif score from MAST was selected to maximize the log-ratio of each regulator in *C. albicans* and was then used for the other species. Based on the control regulator, we considered a log ratio greater than 2.8 as significant, below 2.0 as insignificant, and in between 2.0 and 2.8 as borderline significant. Overall we found that the *C. albicans* biofilm targets were not well conserved to other fungal species. Nonetheless, *C. albicans* Ndt80 biofilm targets were the most conserved, where we observed significant conservation in *C. tropicalis, C. parapsilosis,* and *L. elongisporus*. Based on Ndt80 and Efg1 biofilm target gene conservation, there does not appear to be any trend in targeting between pathogenic and non-pathogenic fungal species (Figure S7C).

Evolutionary Analysis of C. albicans Genes without Orthologs in S. cerevisiae

According to the current CGD orthology mappings, there are 1801 ORFs with no orthologs in *S. cerevisiae*. We find that these 1801 *C. albicans* ORFs with no orthologs in *S. cerevisiae* map to both pathogenic and non-pathogenic species of the *Candida* clade. Overall, 1199 map to *C. tropicalis* (pathogenic), 1047 map to *D. hansenii* (non-

pathogenic), 1040 map to *C. parapsilosis* (pathogenic), 984 map to *L. elongisporus* (non-pathogenic), 977 map to *C. guillermondii* (pathogenic), and 925 map to *C. lusitaniae* (pathogenic). A total of 468 of these ORFs are specific to *C. albicans*. Of these genes that do not map to *S. cerevisiae*, 35 map to *C. glabrata*, an independently-evolved pathogen, which diverged after the whole genome duplication. We find that 649 of these ORFs map to all CTG clade species. Genes found only in pathogenic species are potentially interesting because they may identify a pathogenesis module (Butler et al., 2009). We find that 722 of these ORFs (half of which are not yet characterized) map to all pathogenic CTG clade species (this excludes *D. hansenii* and *L. elongisporus*). If we include *C. glabrata* in addition to all of the pathogenic CTG clade species, 19 of these ORFs (half of which are not yet characterized) map to these species. Some of these ORFs that may form a pathogenesis module contain some genes that are in the biofilm network (e.g. gene families encoding HYR/Iff-like, Als-like adhesins, Pga-like, and major facilitator transporters proteins), however they are not statistically enriched for biofilm genes ($P > 0.05$).

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