Supplemental material

Table S1. List of primers and plasmids used in the study

Primer purpose	Gene	Primer name and sequence
Amplification of *CaCas9* cassette	*CaCas9*	CaCas9 F, ATCTCATTAGATTTGGAACTTGTGGGGTT CaCas9 R, TTCGAGCGTCCCAAAACCTTCT
Amplification of *SNR52* promoter and sgRNA scaffold		SNR52 F, AAGAAAGAAAGAAAACCAGGAGTGAA sgRNA R, ACAAATATTTAAACTCGGGACCTGG
Amplification of final sgRNA expression cassette		SNR52/N F, GCGGCCGCAAGTGATTAGACT sgRNA/N R, GCAGCTCAGTGATTAAGAGTAAAGATGG
Amplification of sgRNA promoter and *SNR52* scaffold with overlapping ORF/gene guide sequence	*ECS1* (orf.19.1766)	sgRNA/F_19.1766 F, ATTCAGCTATTAATGCCACAGTTTTAGAGCTAGAAATAGCAAGTTAAA SNR52/R_19.1766 R, TGTGGCATTAATAGCTGAATCAAATTAAAAATAGTTTACGCAAGTC
Same as above	*ECS2* (orf.19.6867)	sgRNA/F_19.6867 F, GGGAACGAGTGAAAATAGGGGTTTTAGAGCTAGAAATAGCAAGTTAAA SNR52/R_19.6867 R, CCCTATTTTCACTCGTTCCCCAAATTAAAAATAGTTTACGCAAGTC
	LEU42	sgRNA/F_19.1375 F, GCTTGGATAAACCACCAAGAGTTTTAGAGCTAGAAATAGCAAGTTAAA SNR52/R_19.1375 R, TCTTGGTGGTTTATCCAAGC

Same as above	(orf.19.1375)	CAAATTAAAAATAGTTTACGCAAGTC
Same as above	*PR26* (orf.19.5793)	sgRNA/F_19.5793 F, GGGCACAAGAAGAAGTCAA GGTTTTAGAGCTAGAAATAGCAAGTTAAA SNR52/R_19.5793 R, CTTGACTTCTTCTTGTGCCC CAAATTAAAAATAGTTTACGCAAGTC
Same as above	*ECS3* (orf.19.5833)	sgRNA/F_19.5833 F, AACTTGTTTAAAGCTGATGG GTTTTAGAGCTAGAAATAGCAAGTTAAA SNR52/R_19.5833 R, CCATCAGCTTTAAACAAGTT CAAATTAAAAATAGTTTACGCAAGTC
Amplification of ORF/gene deletion cassette with *NAT1*	*ECS1* (orf.19.1766)	orf19.1766 F, TATTTCTGAAAATCCTTGTTTGTTTG TTTTTTTTTTTTTTTCAAATATCCTATAAATCCTAG TTATATTTAATAAATGATGATGATTCATCCCATTCA TTCCATC orf19.1766 R, TTATAATCATAAATAAATAAATAATT CCATACTTCAAAGAAGAATATAAATTTTATCAAAT GTTAAAATACTGTTATTTTGCCGCTCTAGAACTAG TGGATCT
Same as above	*ECS2* (orf.19.6867)	orf19.6867 F, GAAAGTATCAATTCCTTAACTTTTTCTA TATCAACCTAGAGTTAACAATCTTTTGTTTGCTTT ATAATTGCTGAGCTACAGATGATTCATCCCATTCA TTCCATC orf19.6867 R, AACTATCATATAACGCCTATTATGAA AAAAAAAAAACAAAATATTAAACTCAAACGCTAAT CAGTGAAACATACTCTTGAGCCGCTCTAGAACTA GTGGATCT

Same as above	*LEU42* (orf.19.1375)	orf19.1375 F, TTCTTACTATTTTTCTTTTTTGAAGTTTT GTTCAGTTTATTATATCTAATTAACTTACCATTAAC TAATCCACCATGCCTGATGATTCATCCCATTCATT CCATC orf19.1375 R, ATTGAAGAACTACCATACTTCTTGTT GTTGGTTAACCTTCGTGCCTTATCACTTGCCCATT ATACCTATTCTTTGTCTTCGCCGCTCTAGAACTAG TGGATCT
Same as above	*PR26* (orf.19.5793)	orf19.5793 F, TGATTGAAAACTCTGTCGCCACTTG AATTTCTAGTCAGCAATACTAAACCTTATTATTCA CCACAAAGAAGTTTTAGGAGGATGATTCATCCCA TTCATTCCATC orf19.5793 R, TAAATCAGAGAAGCAACGTTTTGTT TTATTTAATTTTATTTCATTCTTTACTTTACAAAATA ATAAAAAAGCTAGTAAATGCCGCTCTAGAACTAG TGGATCT
Same as above	*ECS3* (orf.19.5833)	orf19.5833 F, AACCTACACACAAAAGAAATTATAC GCAATTGCTACTTTTGAATATAACTATTTTCTTTCT CAATCATCATATATTAACAGATGATTCATCCCATT CATTCCATC orf19.5833 R, TTTTTGTACCTATAAAAATATAGAGT TTATTCCTATAATATTCCTGTTTCTTGTATATTTCA CATTATTACTACGAGAATGCCGCTCTAGAACTAG TGGATCT
		orf19.1766-fwd F, AGTAGTCAATCAATCACCTTAAT

Confirmation of deletion of ORF/gene	*ECS1* (orf.19.1766)	ACC orf19.1766-rev R, CATAGCAACACCAATGGTATAAGT Flk19.1766-rev R, CAATTCATCTATTATTTCCATTCTAAGTTTGTTT
Same as above	*ECS2* (orf.19.6867)	orf19.6867-fwd F, TTGTTTGAAGTATTGAGAGGGAA orf19.6867-rev R, CAATTATACCTTGCTGCTTTTCATC Flk19.6867-rev R, CCTACATGAAATTATCAATACTCCTACATCTA
Same as above	*LEU42* (orf.19.1375)	orf19.1375-fwd F, CAAAAAACTCCTGACTCATCTTCAAATA orf19.1375-rev R, ACGGTCAGTAATATGAGTGGC Flk19.1375-rev R, AAATTTACACGATGAGAAGTTCAAAAGA
Same as above	*PR26* (orf.19.5793)	orf19.5793-fwd F, CAGTAAAGTTTAATGGAACAGTAGC orf19.5793-rev R, CCCATCCATTTGATTCAATAACTC Flk19.5793-rev R, CCCAGTTTGGAACTACACTTTTC
	ECS3	orf19.5833-fwd F, GAAACTAAGTTGAATGGCTGCA orf19.5833-rev R, CAGTGGTTCAACTGTCTTTGG Flk19.5833-rev R, CAAATGAAAGCTGAAGAACAAG

Same as above	(orf.19.5833)	GAA
Confirmation of deletion of ORF/gene	*NAT1*	NAT1 RP4, CACAGACGCGTTGAATTGT
Plasmids	**Description and Purpose**	
pV1093	*CaCas9/gRNA* cassette carrying ampicillin resistance gene, amplification of *CaCas9/gRNA* cassette	
pJK863	*CaNAT1-FLP* cassette carrying nourseothricin resistance gene, amplification of *NAT* cassette	

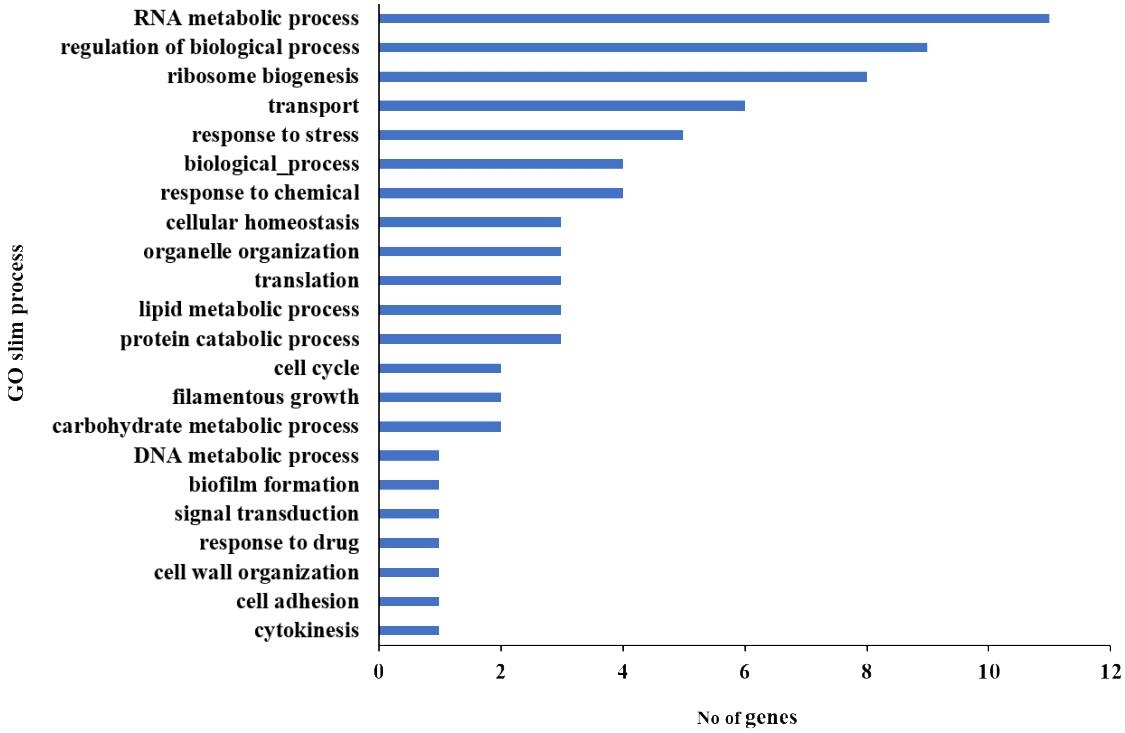

Fig. S1. GO Slim Mapper analysis of 35 differentially expressed genes that are similarly changed across all caspofungin-adapted mutants: three aneuploids lacking one Ch5 (SMC60-2-5, SMC60-3-4 and JMC200-3-4) and two normal diploids (JMC160-2-5 and JMC200-2-5). X-axis represents number of genes, whereas Y-axis represents categories.

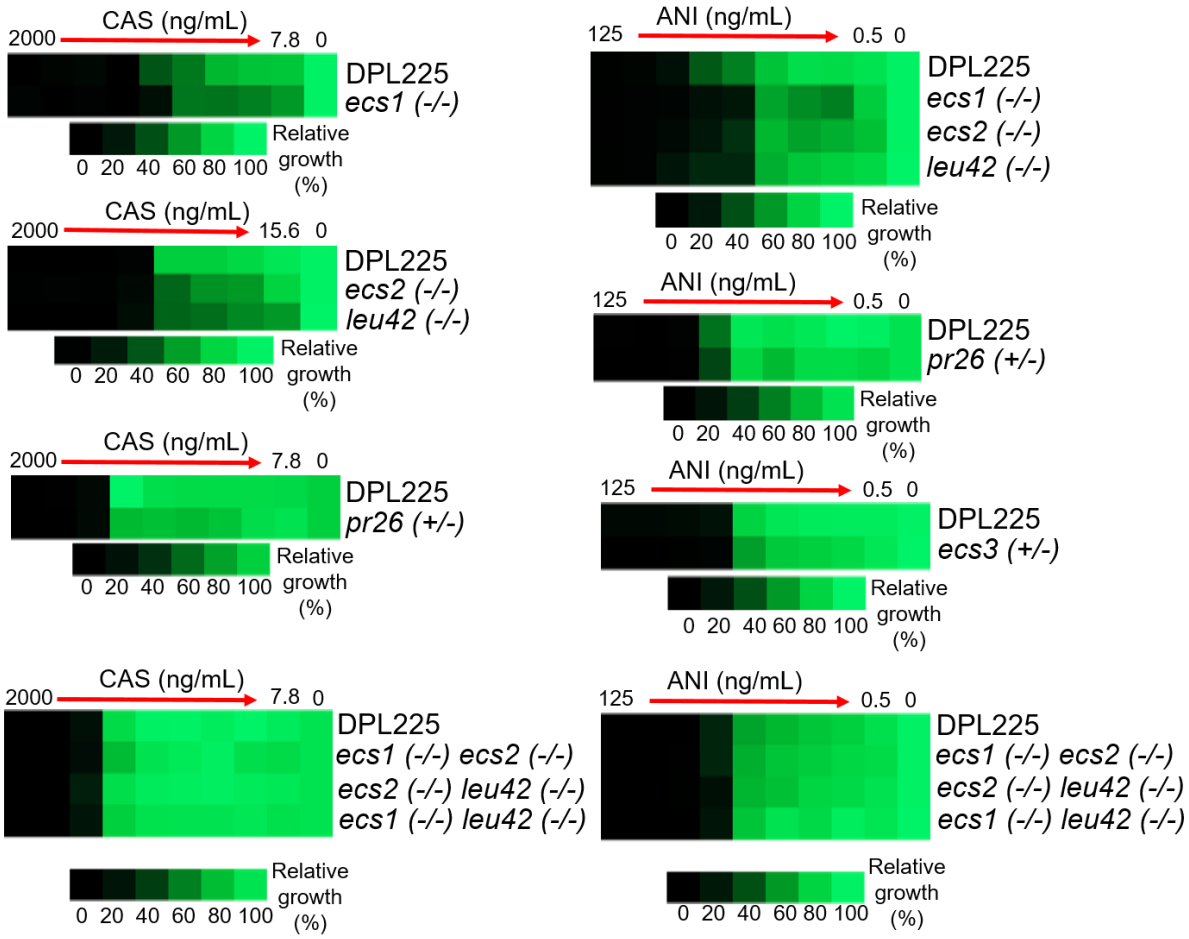

FIG S2. Heat map presenting growth of various *C. albicans* strains by broth microdilution assay. CAS and ANI refer to caspofungin and anidulafungin, correspondingly. Shown are parental strain DPL225, additional biological repeats of null mutants lacking *ECS1*, *ECS2*, or *LEU42*, as well as additional biological repeats of mutants lacking one copy of *PR26* or *ECS3*. Additional biological repeats of double-deletion mutants lacking *ECS1 ECS2*, *ECS2 LEU42* and *ECS1 LEU42*, as well as parental strain DPL225 are shown at the bottom. Names and genotypes of strains are indicated on the right. Assay was conducted according to CLSI method in medium RPMI 1640 with 2% glucose. Assay included maximum caspofungin concentration of 2 µg/ml or anidulafungin concentration of 0.125 µg/ml and two-fold serial dilutions. 10^3 cells were inoculated in each well in either duplicates or triplicates (technical replicates) and tray was

incubated at 35°C for 24 h. Control wells without drug and cells were included. No cells control was used to subtract background. No drug control was used for normalization. Color bar for % growth is presented for each experiment underneath. Note diminished growth of deletion mutants vs parental strain. Also note elevated CAS MIC of DPL225.

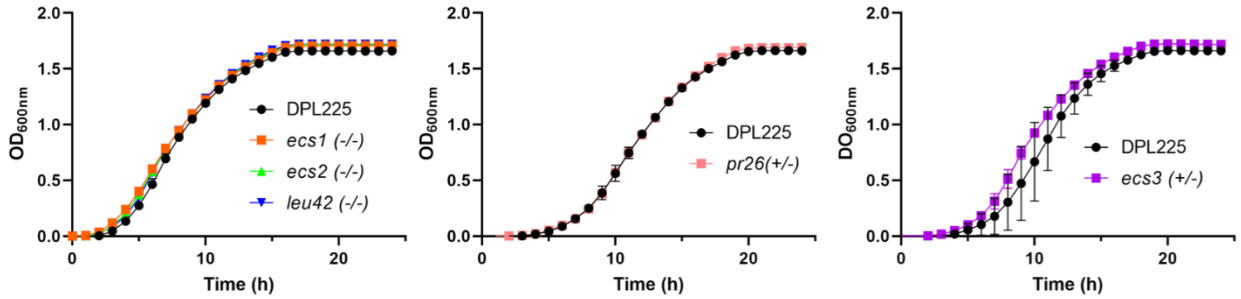

FIG S3. Growth curves of *C. albicans* deletion mutants vs parental DPL225. Shown are DPL225, *ecs1* $^{-/-}$, *ecs2* $^{-/-}$ and *leu42* $^{-/-}$ (left panel); DPL225 and *pr26* $^{+/-}$ (central panel); DPL225 and *ecs3* $^{+/-}$ (right panel). Cell growth was conducted in YPD medium at 35°C. Optical density was measured at 600 nm and plotted against time. Mean and standard deviation of optical density were calculated from three experiments.

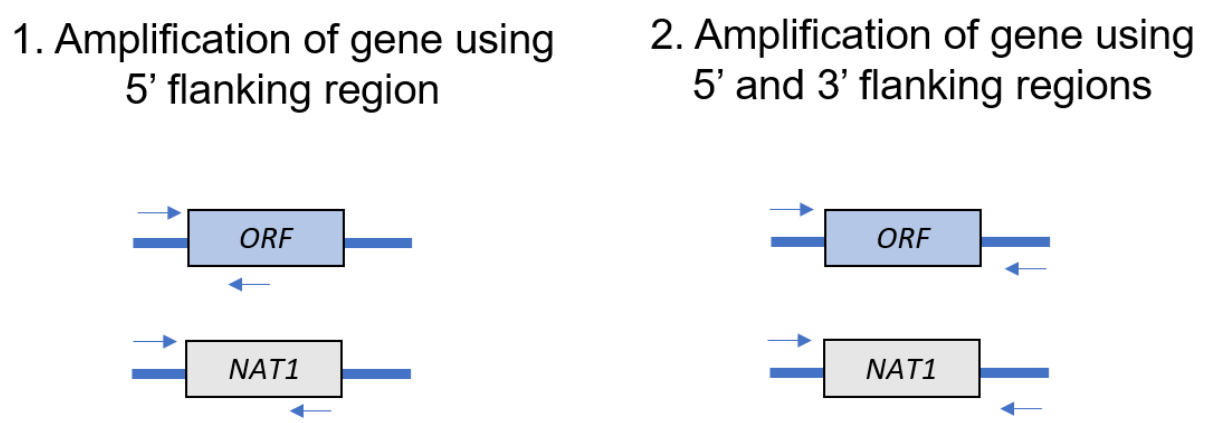

FIG S4. Cartoon showing strategy used to confirm deletion of a candidate gene. Presented are amplification of 5’ junction of ORF of either a candidate gene or *NAT1*, as well as amplification of a candidate gene using flanking regions.