Table S1. Fungal Strains, Plasmids, and Gene Fragments

Fungal strains

Strain	Description	Genotype	Phenotype	Reference
CY4353	*Saccharomyces cerevisiae* expressing wild-type *TRA1*	*MATα his3Δ0 leu2Δ0 ura3Δ0 TRA1-HIS3 +DED1pr-YHR100-LEU2*	wild-type *TRA1*	Hoke *et al. Curr Genet* (2010)
CY6582	*Saccharomyces cerevisiae tra1Q3* mutant strain with 3 point mutations in the gene *TRA1* (R3389Q, R3390Q, R3456Q)	*MATα his3Δ0 leu2Δ0 ura3Δ0 tra1Q3-HIS3+DED1pr-YHR 100-LEU2*	*tra1Q3*	Berg *et al. G3* 2018
fRS302	Wild-type *Candida albicans* strain SC5314 containing empty CRISPR-Cas9 backbone (pRS252), selected for based on NAT resistance background; the control strain for the mutant *tra1Q3*	*NAT-Cas9*	*NATr*	
fRS318	*Candida albicans tra1Q3* mutant strain with 3 point mutations in the gene *TRA1* (K3471Q, R3472Q, R3538Q). Plasmid pRS290 (CRISPR-Cas9 plasmid targeting *TRA1*) was co-transformed with gene block gRS11 (repair template containing 3 *TRA1* mutations) into the WT *C. albicans* strain SC5314. Mutations were verified using Sanger sequencing. One of two mutant strains used.	*NAT-Cas9 tra1Q3/tra1Q3 (K3471Q, R3472Q, R3538Q)*	*NATr tra1Q3*	
fRS319	*Candida albicans tra1Q3* mutant strain with 3 point mutations in the gene *TRA1* (K3471Q, R3472Q, R3538Q). Plasmid pRS290 (CRISPR-Cas9 plasmid targeting *TRA1*) was	*NAT-Cas9 tra1Q3/tra1Q3 (K3471Q, R3472Q, R3538Q)*	*NATr tra1Q3*	

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	co-transformed with gene block gRS11 (repair template containing 3 *TRA1* mutations) into the WT *C. albicans* strain SC5314. Mutations were verified using Sanger sequencing. One of two mutant strains used.			
fRS572	*Candida albicans tra1* mutant strain, with a deletion of the *TRA1* open reading frame. Plasmid pRS477 (CRISPR-Cas9 plasmid targeting *TRA1*) was co-transformed with gene block gRS83 (repair template) into the WT *C. albicans* strain SC5314. Mutations were verified by PCR. One of two mutant strains used.	*NAT-Cas9 tra1/tra1*	*NATr tra1*	
fRS573	*Candida albicans tra1* mutant strain, with a deletion of the *TRA1* open reading frame. Plasmid pRS477 (CRISPR-Cas9 plasmid targeting *TRA1*) was co-transformed with gene block gRS83 (repair template) into the WT *C. albicans* strain SC5314. Mutations were verified by PCR. One of two mutant strains used.	*NAT-Cas9 tra1/tra1*	*NATr tra1*	

Plasmids

Plasmid	**Description**	**aka**	**Citation**

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pRS252	CRISPR-Cas9 optimized for *Candida* plasmid backbone; contains homology regions for NEUT5L integration, C. albicans-optimized Cas9, NAT resistance marker, and AMP selection. Linearized with PacI for integration. Linearized with NgoMIV for Gibson assembly.	Addgene ID: 89576, pJK-caCas9-NatMX-Neut5L	Shapiro et al. Nature Microbiology, 2018
pRS290	CRISPR-Cas9 *C. albicans* plasmid assembled by cloning the plasmid pRS252 and the geneblock fragment gRS10; CRISPR-Cas9 targets *TRA1* with two sgRNAs flanking the Q3 loci. Contains homology regions for *NEUT5L* integration, *C. albicans*-optimized Cas9, NAT resistance marker, and AMP selection. Linearized with PacI for integration.		
pRS477	CRISPR-Cas9 *C. albicans* plasmid assembled by cloning the plasmid pRS252 and the geneblock fragment gRS83; CRISPR-Cas9 targets *TRA1* with two sgRNAs flanking the *TRA1* open reading frame. Contains homology regions for *NEUT5L* integration, *C. albicans*-optimized Cas9, NAT resistance marker, and AMP selection. Linearized with PacI for integration.		

Gene fragments

Gene block	Description	Sequence
gRS10	Gene block to be cloned into pRS252 to target *TRA1* for cleavage with 2 sgRNAs. Block contains regions of homology to plasmid for Gibson assembly, sgRNA promoter, two sgRNAs (containing sgRNA tail)	GAGATCCAGAAAACTGAATTGTGCTTGAATACCACTTG TTTAGCCTAAAAGTCATTGGTCAATAACTATACTCGAGT ATTGCCTCATCAAAGAAACAATCAAATATTATAGATACT CACTCCATCACGTGATAATTTCACTGGTATGGAAAAGT GGAAAATTTTATAAAAAAAAATTTGATGCCTTTGGCATA GCTGAAACTTCGGCCCAATAGGATTGGAGAATATGTTT TCGCAGCGTTCTTACAATTAAATTGTGGTGGAAGTTCG AGACTTGCGTAAACTATTTTTAATTTGACCACGAGCTAA ATCAATGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGA

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		GTCGGTGGTGCGTATCGGGTTTGGGTAATCTTGTTTTA GAGCTATGCTGAAAAGCATAGCAAGTTAAAATAAGGCA GTGATTTTTAATCCAGTCCGTACACAACTTGAAAAAGT GCGCACCGATTCGGTGCTTTTTTTTGGCGAACGTGGC GAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCG CTAG
gRS11	Gene block acts as a repair template to be used with CRISPR plasmid pRS290. Repair template contains homology to *TRA1* and contains 5 mutations: 3 Q3 mutations (R3471Q, R3472Q, R3538Q), and 2 synonymous mutations in the sgRNA PAM sites to block further CRISPR activity after integration of repair template	CGAAGCTAATATAACCAGATTTGCTGAAACTGTACTTC CAAAACAGATTAGAGCTGAATTTGAAAAAGATTTGGTA ATTTCGAAACCAAACTTGGAAACTTATATTTCTAAATTA AGAAATTGGAGAGATAGGTTAGAGGACAAATTAGATAG ACGGTTTTCGCAAGTCAATTTGGAGAATTTATGTCCTC ATTTGAGTGAATTTCATCATCAGAAATTTGAAGAAATTG AAGTTCCAGGGCAATATTTGTTGAACAAaGATAGCAAC GCCCATTTTGTTAAAATTGAAAGATTCCTTTCgACCATT GATTTAGCTCGTGGTTCTAGTGCCTGTTATcAGcaATTG AGAATTCGTGGTCATGATGGTAGTTTACACACATTTGC GGTCCAATTTCCAGCGGCTCGTAATTGTCGTCGTGAG GAATCGGTATTTCAACTTTTCAGAATATTTAACGACAGC ATTTCGAGAAAAGTTGAGACTCGTCGTCGTAATATCCA ATTTACTTTACCAATTGCCGTTCCATTATCTCCACATATT CaaATTGTCAATGATGATACTAGAGATGTGACATTACAA AGAGTTTATGAAGATTTCTGTAAAAAGAATGGTAAGAG TCGTGATGAACCATTTATTTATACAGTcGAGAAATTAAG AGCCGCTTTTGAtCAAAGATTACCCAAACCCGATATTG CTAGTGTTAAAGTTGAAATCTTGAGTGCAATTCAATCAT TATTGGTTCCCTCCACGGTGTTGAAAAACCATTTTATC AATTTGTATCCAAATTTTGAAGATTTCTGGTTATTCCGT AAGCAATTCACTTCACAATATGCGTCATTTATTTTCACC ACATACATGATGTGTATTAATGCTAGACAACCACAGAAA ATTCATGTTAATAGAGGTTCAGGAGCTGTTTGGACTTC TGATATGTTACCATACAGATTATCAACCAGAAATAATGG TGAAATTACCCATAATAAACAATCTCCTATATTTGTT
gRS83	Gene block to be cloned into pRS252 to target the *TRA1* open reading frame for cleavage with 2 sgRNAs. Block contains regions of homology to plasmid for Gibson assembly, sgRNA promoter, two sgRNAs (containing sgRNA tail)	GAGATCCAGAAAACTGAATTGTGCTTGAATACCACTTG TTTAGCCGTATTATGCCTTTGTCAATGTGTGTAGAATTT GGTTATTACATATCCATGTGTAATATATATGTTGATCAAA AAACGCGATCTTCTCTTTGGTGTAGTGTGTTACACAAA AAATTCACTAGTCTAGGTCACATGATAATCACGTGAAA ATCAAAAATTTGTTGAAATTGAATTTCCTCAATTTTGAA ATTTTGTTTGAAATAAAAGTCATTGGTCAATAACTATACT CGAGTATTGCCTCATCAAAGAAACAATCAAATATTATAG ATACTCACTCCATCACGTGATAATTTCACTGGTATGGAA AAGTGGAAAATTTTATAAAAAAAAATTTGATGCCTTTGG CATAGCTGAAACTTCGGCCCAATAGGATTGGAGAATAT

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		GTTTTCGCAGCGTTCTTACAATTAAATTGTGGTGGAAG TTCGAGACTTGCGTAAACTATTTTTAATTTGGTTACGCG ACTTAATGATAGGTTTTAGAGCTAGAAATAGCAAGTTAA AATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC CGAGTCGGTGGTGCGTAGCTTCAGCTGATACCTTAGT TTTAGAGCTATGCTGAAAAGCATAGCAAGTTAAAATAA GGCAGTGATTTTTAATCCAGTCCGTACACAACTTGAAA AAGTGCGCACCGATTCGGTGCTTTTTTTTATTGTATTG TATTTATATATGTTTATTAAACTATTAGTATTATTGTTGTAT TTATTAAATTTTCCCCATTTTATTGTTATCATTGACTCAA ACTGAGTAATACCTATCTAATCTATATCGATAATCATCTA TTGATTCAGAACCGATACGAATCTTTCTATTTCGTTTAA GTACTCTTCTATCAACTTTATTACCAATATATTCTAAAGC CTTTTCACAAATTATGGCGAACGTGGCGAGAAAGGAA GGGAAGAAAGCGAAAGGAGCGGGCGCTAG