

Table S1. *Candida albicans* strains used in this study.

Strain Number	Strain Name	Genotype	Source
JBV3532	SC5314	Prototrophic	[1]
JBV3880	Chr3 x 3 ABB	SC5314 Chr3 x3 ABB	[2]
YDJ4095	SN95	<i>arg4Δ/arg4Δ his1Δ/his1Δ</i> <i>URA3/ura3Δ::imm434 IRO1/iro1::imm434</i>	[3]
YDJ6059	CaCiS-1	Prototrophic	This study
YDJ6060	CaCiS-2	Prototrophic	This study
YDJ6065	CaCiS-7	Prototrophic	This study
YDJ6122	CaCi-1	Prototrophic	[4]
YDJ6277	N1	SN95 naïve lineage 1	This study
YDJ6278	N2	SN95 naïve lineage 2	This study
YDJ6279	N3	SN95 naïve lineage 3	This study
YDJ6280	ParaR L1	SN95 pararesistant lineage 1	This study
YDJ6281	ParaR L2	SN95 pararesistant lineage 2	This study
YDJ6282	ParaR L3	SN95 pararesistant lineage 3	This study
YDJ6283	S1	SN95 sensitive lineage 1	This study
YDJ6284	S2	SN95 sensitive lineage 2	This study
YDJ6285	S3	SN95 sensitive lineage 3	This study
YDJ6309	SCUPC2R32A	SC5314 <i>UPC2A643T-FRT/UPC2</i>	[5]
YDJ9231	<i>SNT1</i> deletion	SN95 <i>snt1Δ/snt1Δ</i>	This study
YDJ9229	<i>SKO1</i> deletion	SN95 <i>sko1Δ/sko1Δ</i>	This study
YDJ8230	<i>SKO1-GFP</i>	SN95 <i>SKO1-GFP-ARG/SKO1</i>	This study
YDJ8522	<i>HST1</i> deletion	SN95 <i>hst1Δ/hst1Δ</i>	This study
	<i>MAC1</i> deletion	SN152 <i>mac1Δ/mac1Δ</i>	[6]
	<i>SKO1</i> deletion	SN152 <i>sko1Δ/sko1Δ</i>	[6]
	<i>EFG1</i> deletion	SN152 <i>efg1Δ/efg1Δ</i>	[6]
	<i>ACE2</i> deletion	SN152 <i>ace2Δ/ace2Δ</i>	[6]
YDJ9280	<i>HOG1</i> deletion	SN95 <i>hog1Δ/hog1Δ</i>	This study
YDJ13917	<i>CDR1</i> overexpression	SN95 <i>TAr-FRT-tetO-CDR1/CDR1</i>	This study
YDJ13959	<i>SNT1-GFP</i>	SN95 <i>SNT1-GFP-ARG/SNT1</i>	This study
YDJ13981	<i>RAP1-GFP</i>	SN95 <i>RAP1-GFP-ARG/RAP1</i>	This study
YDJ14006	ParaR <i>RAP1-GFP-1</i>	SN95 <i>RAP1-GFP-ARG/RAP1</i> ParaR 4-D1	This study

Strain Constructions

YDJ6277/YDJ6278/YDJ6279: These three isolates were fluconazole-sensitive and had no prior drug exposure. To obtain the single-colony derivatives of the naïve wild-type strain, SN95 was subcultured in YPD at 30°C for 48 hours. Cultures were then plated on YPD agar and incubated at 30°C for an additional 48 hours. Single colonies were subsequently grown in YPD and archived in 25% glycerol. MIC assays confirmed that the archived strains remained fluconazole-sensitive.

YDJ6280/YDJ6281/YDJ6282: These three isolates developed resistance following pre-exposure to fluconazole. To obtain the single-colony derivatives, SN95 was subcultured in YPD supplemented with 0.5 µg/ml fluconazole at 30°C for 48 hours. Cultures were then plated on YPD agar and incubated at 30°C for an additional 48 hours. Single colonies were subsequently grown in YPD and archived in 25% glycerol. Growth curves from MIC assays confirmed that the archived strains were adapted to 0.5 µg/ml fluconazole.

YDJ6283/YDJ6284/YDJ6285: These three isolates had prior exposure to fluconazole but remained fluconazole-sensitive. To obtain single-colony derivatives following drug pre-exposure, SN95 was subcultured in YPD with 0.5 µg/ml fluconazole at 30°C for 48 hours. Cultures were then plated on YPD agar and incubated at 30°C for an additional 48 hours. Single colonies were subsequently grown in YPD and archived in 25% glycerol. MIC assays confirmed that the archived strains retained fluconazole sensitivity.

YDJ9231: PDJ2042 was digested with MssI to release His1-FRT-pENO1-Cas9-pMAL1-FLP-NAT (1 of 2). Fragment A (1066 bp) was amplified from PDJ2045 with oLX191 and oLX192. Fragment B (704 bp) was amplified from YDJ2055 with oLX446 and oLX193. Fragment C (1714 bp) was amplified by stitching Fragment A and Fragment B with oLX199 and oLX200. Donor DNA was generated by annealing oLX447 and oLX448. SNT1 deletion was verified by colony PCR with oLX459 and oLX442 (770 bp for SNT1 deletion and 3774 bp for WT SNT1) and oLX459 and oLX516 (464 bp for WT SNT1, no amplification for deleted allele). The MAL1 promoter was induced to drive expression of FLP recombinase to excise the NAT flipper cassette.

YDJ9229: PDJ2042 was digested with MssI to release His1-FRT-pENO1-Cas9-pMAL1-FLP-NAT (1 of 2). Fragment A (1066 bp) was amplified from PDJ2045 with oLX191 and oLX192. Fragment B (704 bp) was amplified from PDJ2055 with oLX510 and oLX193. Fragment C (1714 bp) was amplified by stitching Fragment A and Fragment B with oLX199 and oLX200. Donor DNA was generated by annealing oLX291 and oLX292. SKO1 deletion was verified by colony PCR with oLX300 and oLX277 (430 bp for SKO1 deletion and 2276 bp for WT SKO1) and oLX300 and oLX316 (344 bp for WT SKO1, no amplification for deleted allele). The MAL1 promoter was induced to drive expression of FLP recombinase to excise the NAT flipper cassette.

YDJ8230: SKO1-GFP-ARG (3244 bp) was amplified from PDJ1973 with oLX278 and oLX279 and transformed into YDJ4095. Upstream integration (520 bp) was verified with oLX275 and oLX021CaGFP-R. Downstream integration (685 bp) was verified with oLX136 and oLX277.

YDJ8522: PDJ2042 was digested with MssI to release His1-FRT-pENO1-Cas9-pMAL1-FLP-NAT (1 of 2). Fragment A (1066 bp) was amplified from YDJ2045 with oLX191 and oLX192. Fragment B (704 bp) was amplified from YDJ2055 with oLX494 and oLX193. Fragment C (1714 bp) was amplified by stitching Fragment A and Fragment B with oLX199 and oLX200. Donor DNA was generated by annealing oLX291 and oLX292. HST1 deletion was verified by colony PCR with oLX497 and oLX498 (390 bp for HST1 deletion and 2358 for WT HST1) and oLX499 and oLX498 (421 bp for WT HST1, no amplification for deleted allele). The MAL1 promoter was induced to drive expression of FLP recombinase to excise the NAT flipper cassette.

YDJ9280: PDJ2042 was digested with MssI to release His1-FRT-pENO1-Cas9-pMAL1-FLP-NAT (1 of 2). Fragment A (1066 bp) was amplified from YDJ2045 with oLX191 and oLX192. Fragment B (704 bp) was amplified from YDJ2055 with oLX511 and oLX193. Fragment C (1714 bp) was amplified by stitching Fragment A and Fragment B with oLX199 and oLX200. Donor DNA was generated by annealing oLX298 and oLX299. HOG1 deletion was verified by colony PCR with oLX301 and oLX302 (452 bp for HOG1 deletion and 2373 for WT HOG1) and oLX301 and oLX515 (287 bp for WT HOG1, no amplification for deleted allele). The MAL1 promoter was induced to drive expression of FLP recombinase to excise the NAT flipper cassette.

YDJ13917: TAr-NAT-tetO-CDR1 (7175 bp) was amplified from PDJ1763 with oLX312 and oLX313 and transformed into YDJ4095. Upstream integration (433 bp) was verified with oLX317 and oLX314. Downstream integration (397 bp) was verified with oLX315 and oLX318. The NAT cassette and the FLP recombinase were excised by growing the positive colony in YNB-BSA for 2 days at 30°C. The SAP2 promoter was induced to drive expression of FLP recombinase to excise the NAT flipper cassette.

YDJ13959: SNT1-GFP-ARG (3244 bp) was amplified from PDJ1973 with oLX439 and oLX440 and transformed into YDJ4095. Upstream Integration (486 bp) was verified with oLX441 and oLX128. Downstream Integration (823 bp) was verified with oLX136 and oLX442.

YDJ13981: RAP1-GFP-ARG (3244 bp) was amplified from PDJ1973 with oLX433 and oLX434 and transformed into YDJ4095. Upstream integration (490 bp) was verified with oLX435 and oLX128. Downstream integration (390 bp) was verified with oLX136 and oLX436.

YDJ14006/YDJ14007: These isolates developed resistance following pre-exposure to fluconazole. To obtain these single-colony derivatives, YDJ13981 was subcultured in 200 µl of YPD supplemented with 0.5 µg/ml fluconazole at 30°C for 48 hours. Cultures were then plated on YPD agar and incubated at 30°C for an additional 48 hours. Single colonies were used to 100 µl of YPD and archived directly in 25% glycerol. A significant increase in colony size on YPD plates supplemented with 0.6 µg/ml fluconazole confirmed that the archived strains were adapted to fluconazole. The strains were subsequently grown in YPD and plated for single colonies on PDR plates. They gave rise to mostly large light colonies and few small dark colonies, confirming that the resistance state is both heritable and reversible.

Table S2. Bacterial plasmids used in this study.

Strain Name	Description	Source
PDJ2042	<i>C. albicans</i> HIS/FLP CAS9 expression plasmid	[7]
PDJ2045	Universal plasmid template for Fragment A for cloning-free stitching of gRNA expression cassette	[7]
PDJ1973	For tagging genes with GFP at the C-terminus	[8]

Table S3 Oligonucleotides used in this study.

Strain Name	Description	Source
oLX128	GFP+368_R	gtatcaccttcaaacttgac
oLX136	ARG+1161_F	ggagttccatttagagaaac
oLX191	gRNA Universal Fragment A_F	gacggcacggccacgcgttaaaccgcc
oLX192	gRNA Universal Fragment A_R	caaattaaaaatagtttacgcaag
oLX193	gRNA Fragment B_R	cccgccaggcgctggggtttaacaccg
oLX199	Fragment C_F	aggtgatgctgaagctattgaag
oLX200	Fragment C for HIS-FLP_R	TAAAGCTGCCACAAGAGGTATTTC
oLX277	SKO1+1994_R	CTTGAAGAAGGTTTCAGTAAC
oLX291	CaSKO1 deletion dDNA_F	TCAGATCTTTATTCATTCTTTATTTTTTT TAGTTCCAAAAAGACGGATTGTTCTTGTCT TGTTATTATTATCATTAATGTATGATTTTTG TTT
oLX292	CaSKO1 deletion dDNA_R	AAACAAAAATACATACATTTAATGATAATAAT AACAAGAACAAGAACAATCCGTCTTTTTGGA ACTAAAAAAAATAAAGAATGAATGAATAAAG ATCTGA
oLX300	CaSKO1-172_F	CCACAACATATTCTTCTTGC
oLX312	CaCDR1-M13R_F	TATATCATATATATATTCTATTTATTTTTTGTA CTTAATAATTTCTTTAAAAGGTCAAAAACGAA AAAATTGGAAACAGCTATGACCATG
oLX313	tetO-CaCDR1_R	AAGAAGAGTCTTGACTAATTGCCTTTTCTAAT TTAGATTCATCTTGCGACGACATCTTAGAAT CTGACATcgactatttatattgtatg
oLX314	CaTAR+72_R	gtacgatggagatagttac
oLX315	tetO+473_F	atgtttgtcgtttctgatgg
oLX316	CaSKO1+172_R	GCAAATGATCTTTCGAATGG
oLX317	CaCDR1-293_F	TTCTTTTCCACTGGTACTAC
oLX318	CaCDR1+180_R	TGACGAGTCATCTTTGAAAG
oLX433	CaRAP1-GFP_F	TTATAAAGAATATTATGAAAATTGTGAAAAAG TAGGTATGGCTCCTAGATGTTTAACAAGAAT TGCTCCAggtgctggcgcaggtgcttc
oLX434	CaRAP1-ARG_R	TTCAATTCTCCCTGATTAACCCCTTTAATAATAA AGTTACTTCTTTCTCTGTAAGGTCCCTTTCTTT TTTTTAgattaccctgttatcccta
oLX435	CaRAP1+1169_F	CTTGGAGAGATAGATTTAGG

oLX436	CaRAP1+1610_R	GGGTAAAACTAATGAACGTG
oLX439	CaSNT1-GFP_F	AAAAAATGGTTGGGATGCTATTGTTGCTAAT CAGAAAAGTTTCGAAAATGTCAATCATGAGTT TGTTAAATggtgctggcgcaggtgcttc
oLX440	CaSNT1-ARG_R	TTGATCTGTTTAAACATTATTAACCTATTATTG TTGTTATTTTACTACTGATATATATATCTACAT ATGTAagattaccctgttatcccta
oLX441	CaSNT1+2886_F	CACAAAATCAACAACCATGG
oLX442	CaSNT1+3410_R	CCCTTGTTTTATTAAGTGGC
oLX446	CaSNT1 gRNA+2310_R	CGTAAACTATTTTTAATTTGGGTTTCACCAGA CTCAACAGGTTTTAGAGCTAGAAATAGC
oLX447	CaSNT1 deletion dDNA_F	AACACCATCATTGTCAAATTACTGTCAAATA ATTAATTTTATCATAATGGTACATATGTAGAT ATATATATCAGTAGTAAATAACAACAATAAT AGGT
oLX448	CaSNT1 deletion dDNA_R	ACCTATTATTGTTGTTATTTTACTACTGATAT ATATATCTACATATGTACCATTATGATAAAAT TAATTATTTTGACAGTAATTTGACAATGATGG TGTT
oLX459	CaSNT1-364_F	TCACTCCTTTGTTGTTGTTG
oLX510	CaSKO1 gRNA+720_R	CGTAAACTATTTTTAATTTGGGGCACTAATAA ACTCAGAGGTTTTAGAGCTAGAAATAGC
oLX516	CaSNT1+101_R	GAATAAAAATTTTTCCTTCG

Citations

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