Supplemental Materials

Liu Z, Rossi, JM, and Myers LC. '*C. albicans* Zn Cluster Transcription Factors Tac1 and Znc1 are Activated by Farnesol to Up Regulate a Transcriptional Program Including the Multi-Drug Efflux Pump *CDR1*'

Contents:

Fig. S1

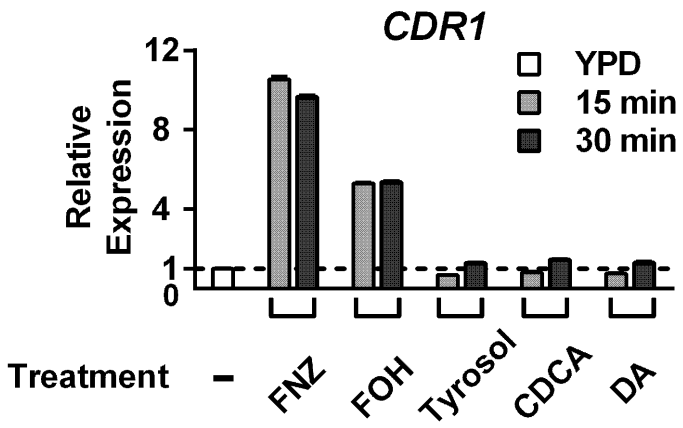

Fig. S1 *CDR1* induction by potential small molecule inducers

RT-qPCR analysis of changes in *CDR1* expression upon exposure to molecules structurally or functionally related to FOH, including chenodeoxycholic acid (CDCA) and deoxycholic acid (DA). Each compound tested (or an equal volume of methanol) was added into log phase cultures of a wild type strain (yLM167) at a final concentration of 50 μM. *CDR1* mRNA level in the methanol treated samples (15 min) was set to '1'. *ACT1* level was used as an internal reference. Results from one representative experiment were presented by the mean and standard deviation (value may not be large enough to give a visible error bar) of two qPCR measurements on the same set of cDNA samples.

Fig. S2

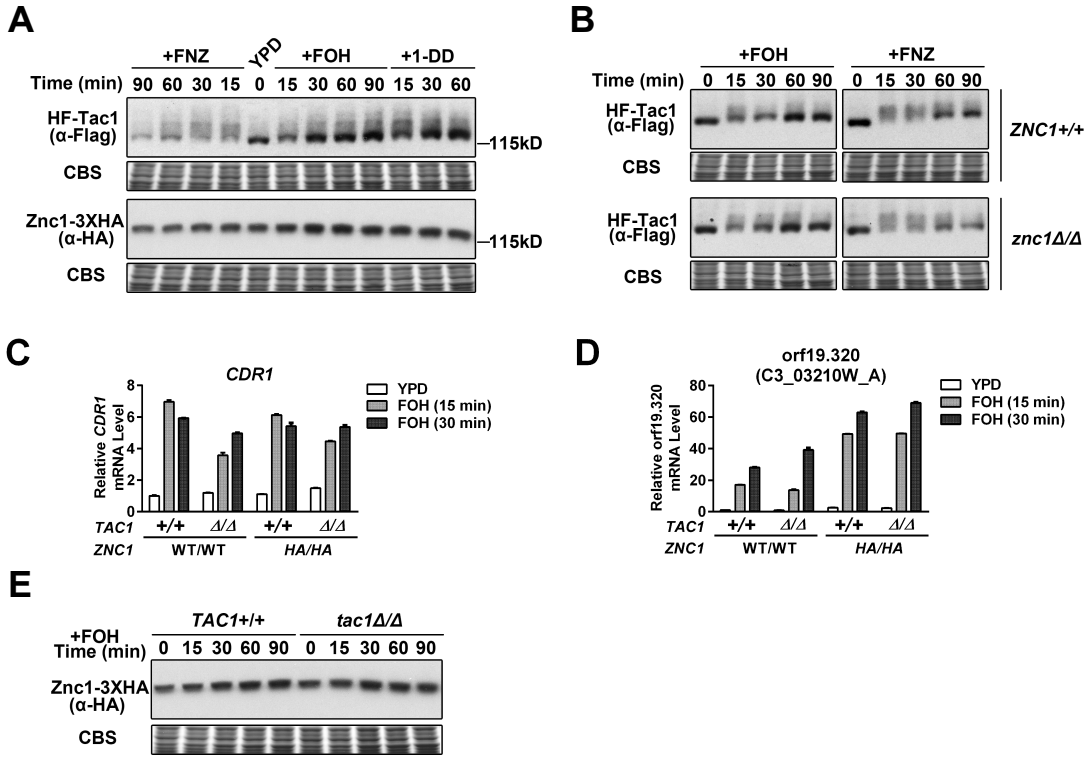

Fig. S2 Analysis of tagged Tac1 and Znc1 SDS-PAGE mobility upon hyperactivation

(A) Immunoblot analysis Tac1 and Znc1 expression and SDS-PAGE mobility in strains expressing N-terminally 6His3Flag tagged Tac1 ('HF-Tac1',yLM682) or C-terminally 3XHA tagged Znc1 ('Znc1-3XHA'; yLM684) treated with FNZ (25μM), FOH (50 μM) and 1-DD (50 μM) in log phase YPD cultures. The blots were correspondingly probed by an anti-Flag or an anti-HA antibody. **(B)** Anti-Flag immunoblot analysis comparing Tac1 SDS-PAGE mobility in extracts from wild type ('*ZNC1*+/+'; yLM682) and *znc1* deletion ('*znc1Δ/Δ*'; yLM683) strains treated with 25 μM FNZ or 50 μM FOH. **(C-D)** RT-qPCR analysis of *CDR1* **(C)** and orf19.320 **(D)** mRNA expression in *TAC1* wild type ('+/+'; yLM660 and yLM684) and deletion mutants ('*Δ/Δ*'; yLM663 and yLM685) treated with FOH (50 μM) and carrying homozygous native ('WT/WT') or C-terminally 3XHA tagged *ZNC1* ('*HA/HA*'). Expression of each gene, in the absence of FOH treatment, in the untagged wild type strain (yLM660) was set to '1'. **(E)** Anti-HA immunoblot analysis of Znc1 SDS-PAGE mobility in wild type ('*TAC1*+/+'; yLM684) and *tac1* deletion ('*tac1Δ/Δ*'; yLM685) strains treated with FOH (50 μM). Coomassie Blue staining (CBS) served as loading control in **(A)**, **(B)** and **(E)**.

Fig. S3

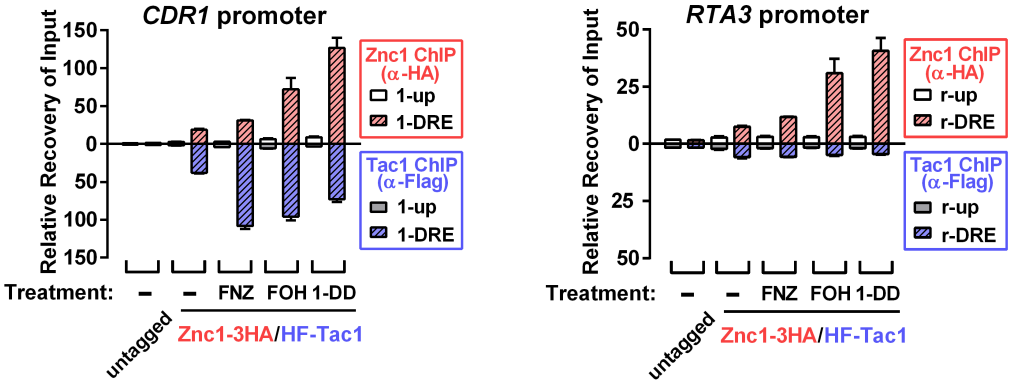

Fig. S3 Tac1 and Znc1 occupancy at *CDR1* and *RTA3* promoters with different inducers

(A-B) RT-qPCR ChIP analysis of Tac1 and Znc1 occupancy at the *CDR1* **(A)** and *RTA3* **(B)** promoters upon treatment with FNZ, FOH or 1-DD. A strain carrying two copies of N-terminally 6His3Flag-tagged *TAC1* and two copies of C-terminally 3XHA tagged *ZNC1* ('Znc1-3HA/HF-Tac1'; yLM686) and a strain with native *TAC1* and *ZNC1* ('untagged'; yLM660) were treated with FNZ (25 μM), FOH (50 μM), 1-DD (50 μM), or vehicle ('Veh.'; methanol) for 15 minutes before fixation. Each sample was immunoprecipitated by an anti-Flag antibody and an anti-HA antibody in separated reactions. Promoter regions tested for Tac1 and Znc1 binding and their relative positions to the known Tac1 *cis* elements at the *CDR1* and *RTA3* promoters (See Fig. 5). Percent recovery of input (Input%) at the *CDR1* promoter '1-up' region in the anti-Flag/anti-HA ChIP products obtained from the methanol-treated untagged strain was set to '1' to normalize Tac1/Znc1 binding across conditions and promoter regions. Hence, the strength of ChIP signals (Y axis value) can be compared across panels.

Fig. S4

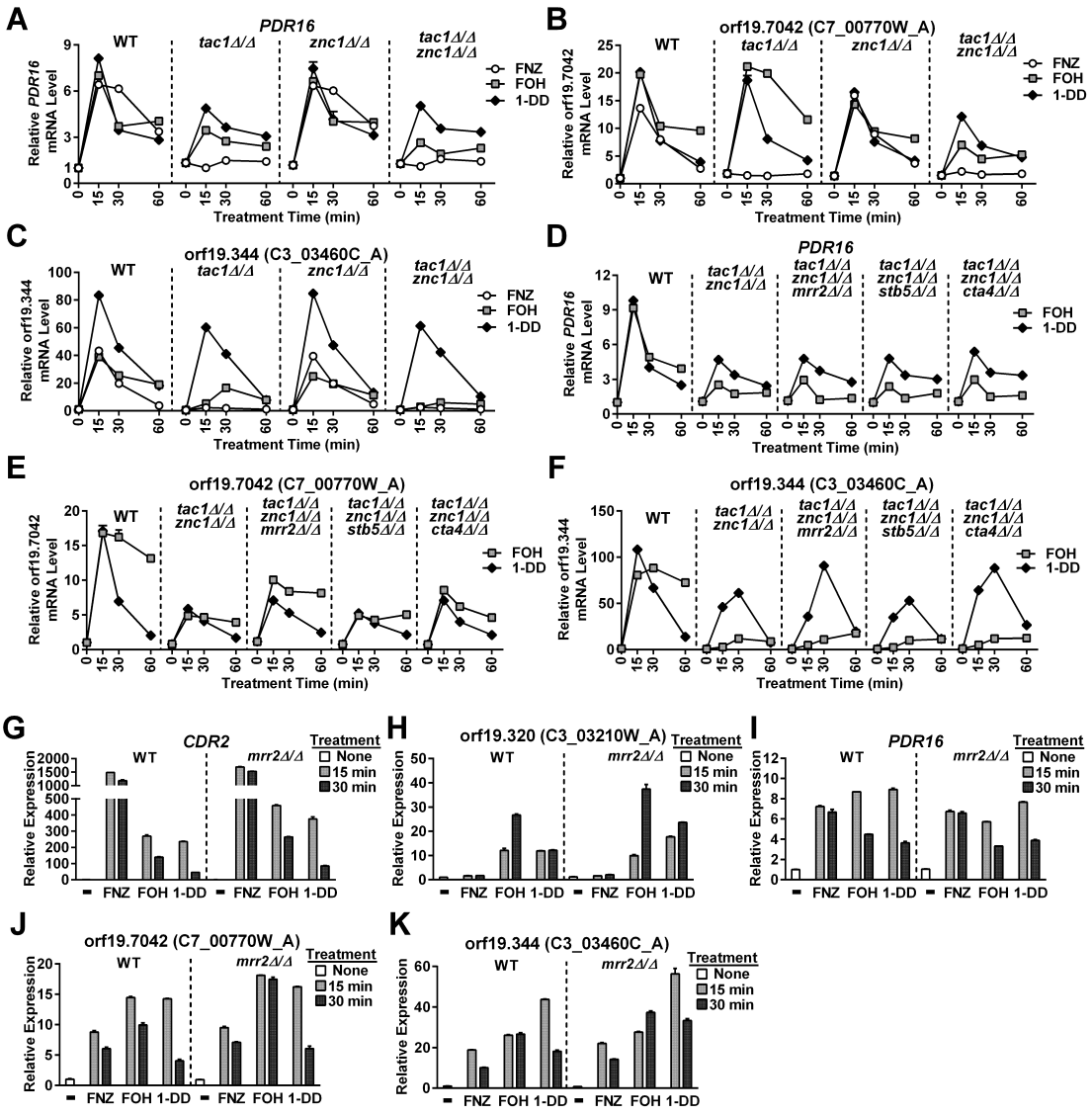

Fig. S4 FNZ, FOH and 1-DD induction of target genes in *mrr2*, *stb5* or *cta4* deletion backgrounds

(A-C) RT-qPCR analysis of *PDR16* **(A)**, orf19.7042 **(B)** and orf19.344 **(C)** mRNA expression in wild type (yLM660), *tac1Δ/Δ* (yLM663), *znc1Δ/Δ* (yLM661) and *tac1Δ/Δ znc1Δ/Δ* (yLM664) strains treated with FNZ (25 μM), FOH (50 μM) and 1-DD (50 μM). Expression of each gene, in the absence of treatment, in the wild type strain was individually set to '1'. **(D-F)** RT-qPCR analysis of *PDR16* **(D)**, orf19.7042 **(E)** and orf19.344 **(F)** mRNA expression in wild type (yLM660), *tac1Δ/Δ znc1Δ/Δ* (yLM771), and the three indicated transcription factor triple deletion (yLM772, yLM773 and yLM774) strains after treatment with 50 μM FOH or 50 μM 1-DD. Expression of each gene in the wild type strain, in the absence of treatment, was set to '1'. **(G-K)** RT-qPCR analysis of *CDR2* **(G)**, orf19.320 **(H)**, *PDR16* **(I)**, orf19.7042 **(J)** and orf19.344 **(K)** mRNA expression in wild type

(yLM660) and *mrr2* deletion (yLM662) strains treated with FNZ (25 μM), FOH (50 μM) or 1-DD (50 μM). Expression of each gene in the wild type strain, in the absence of treatment, was set to '1'.

Fig. S5

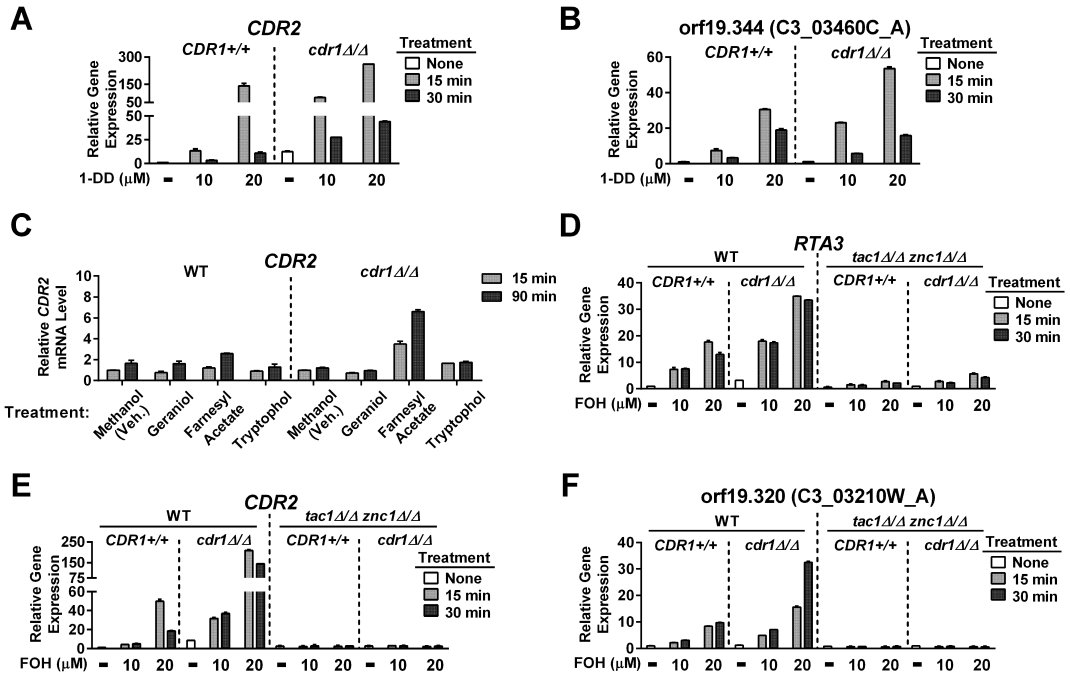

Fig. S5 Impact of *CDR1* on Tac1/Znc1 dependent gene activation by specific inducers

(A-B) RT-qPCR analysis of *CDR2* **(A)** and orf19.344 **(B)** mRNA expression in *cdr1* deletion mutant (yLM708) and wild type (yLM660) strains upon treatment with 1-DD. mRNA expression in the wild strain, in the absence of treatment, was set to '1'. **(C)** RT-qPCR analysis of *CDR2* mRNA expression in a wild type strain (yLM167) and a *cdr1Δ/Δ* strain (yLM611) after treatment with geraniol (50 μM), farnesyl acetate (50 μM) or tryptophol (50 μM). *CDR2* expression, upon 15 min. treatment with vehicle (methanol) in the wild type and *cdr1Δ/Δ* strains were individually set to '1' (not shown) to normalize expression in each strain. **(D-F)** RT-qPCR of *RTA3* **(D)**, *CDR2* **(E)** and orf19.320 **(F)** mRNA expression in *TAC1*/*ZNC1* wild type strains (yLM660 and yLM708), and *tac1Δ/Δ znc1Δ/Δ* (yLM664 and yLM711) strains, in a *CDR1* wild type or null background, treated with FOH. Expression of each gene in the wild type strain (yLM660), in the absence of treatment, was set to '1'.

Fig. S6

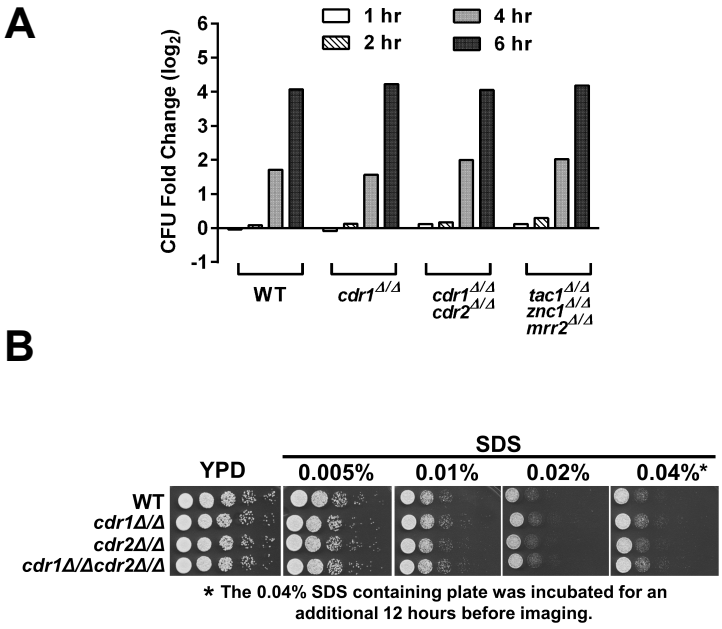

Fig. S6 Effect of methanol and SDS on cell growth of *CDR1* expression *C. albicans* mutant strains

(A) Colony formation analysis comparing cell viability between wild type and *cdr1* mutant strains after one to six hours methanol exposure. As a vehicle control experiment for Fig. 9A, stains with wild type (yLM660), *cdr1* null (yLM708), *cdr1 cdr2* double deletion (yLM710), or *tac1 znc1 mrr2* triple deletion (yLM702) genotypes were each diluted from overnight culture and treated with 0.1% (v/v) methanol in YPD media. Experiments and data presentation were performed in the same way as in Fig. 9A. **(B)** Spot growth assay showing the sensitivity of wild type (yLM167), *cdr1Δ/Δ* (yLM611), *cdr2Δ/Δ* (yLM716), and *cdr1Δ/Δ cdr2Δ/Δ* (yLM712) strains to increasing concentrations of SDS.

Table S1 Fluconazole MIC measurement showing deletion of *znc1* does not affect fluconazole resistance in *TAC1* GOF mutant strains.

	Fluconazole MIC (μg/mL)	
	***ZNC1*+/+**	***znc1Δ/Δ***
***TAC1* WT**	**0.75-1** (yLM687)	**0.75-1** (yLM691)
TAC1 ΔM677	**8** (yLM688)	**8** (yLM692)
TAC1 N972D	**24** (yLM689)	**16-24** (yLM693)
TAC1 N977D	**12-16** (yLM690)	**12-16** (yLM694)

Fluconazole MIC was measured by E-test at 30°C on YPD plates. Intermediate values, between scale marks, are presented as intervals. Exact strain used for each MIC measurement is listed in parentheses.

Table S2 Strains used in this study

Strain Name [a]	Parental Strain	Genotype	Reference
yLM167 (*TAC1*^{WT})	-	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**TAC1-1**/URA3*	DSY2937-35 (1)
yLM232	-	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**TAC1-1**/URA3 **med3Δ::FRT/ med3Δ::FRT***	(2)
yLM169 (*TAC1*^{A736V})	-	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**TAC1-1-A736V**/URA3*	yLM169 (3)
yLM496 (*TAC1*^{N977D})		*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**TAC1-1-N977D**/URA3*	(2)
yLM505	-	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**TAC1-1**/URA3 CDR1/**CDR1::CDR1-3HA***	(2)
cRC106	-	*ura3Δ::λimm*^{434}*/ura3Δ::λimm*^{434} *ade2::hisG/ade2::hisG::[pOPlacZ]*	(4)
yLM567	-	*ura3Δ::λimm*^{434}*/ura3Δ::λimm*^{434} *ade2::hisG/ade2::hisG::[pOPlacZ] RPS10/rps10Δ::[**LexA**]/URA3*	(2)
yLM611 (*TAC1*^{WT} *cdr1*$^{Δ/Δ}$)	-	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**P**$_{TAC1}$**-TAC1-1**/URA3 **cdr1Δ::FRT/cdr1Δ::FRT***	(2)
yLM612 (*TAC1*^{A736V} *cdr1*$^{Δ/Δ}$)	-	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**P**$_{TAC1}$**-TAC1-1-A736V**/URA3 **cdr1Δ::FRT/cdr1Δ::FRT***	(2)
yLM614 (*TAC1*^{N977D} *cdr1*$^{Δ/Δ}$)	-	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::**P**$_{TAC1}$**-TAC1-1-N977D**/URA3 **cdr1Δ::FRT/ cdr1Δ::FRT***	(2)
AZC2	-	**SC5314 (a/a) in white state**	
AZC11	AZC2	**SC5314 (a/a) in opaque state**	
yLM660 (wild type)	-	*arg4Δ/arg4Δ, leu2Δ/leu2Δ::LEU2, his1Δ/his1Δ::HIS1,URA3/ura3Δ, IRO1/iro1Δ*	Wild type strain (5)
yLM661 (*znc1*$^{Δ/Δ}$)	-	*arg4Δ/arg4Δ, leu2Δ/leu2Δ, his1Δ/his1Δ, URA3/ura3Δ, IRO1/iro1Δ, **znc1Δ::LEU2/znc1Δ::HIS1***	*znc1* null mutant (5)
yLM662 (*mrr2*$^{Δ/Δ}$)	-	*arg4Δ/arg4Δ, leu2Δ/leu2Δ, his1Δ/his1Δ, URA3/ura3Δ, IRO1/iro1Δ, **mrr2Δ::LEU2/mrr2Δ::HIS1***	*mrr2* null mutant (5)
yLM663 (*tac1*$^{Δ/Δ}$)	yLM660	*arg4Δ/arg4Δ, leu2Δ/leu2Δ::LEU2, his1Δ/his1Δ::HIS1, URA3/ura3Δ, IRO1/iro1, **tac1Δ::ARG4/tac1Δ::ARG4***	This study
yLM664 (*tac1*$^{Δ/Δ}$ *znc1*$^{Δ/Δ}$)	yLM661	*arg4Δ/arg4Δ, leu2Δ/leu2Δ, his1Δ/his1Δ, URA3/ura3Δ, IRO1/iro1Δ, **tac1Δ::ARG4 znc1Δ::LEU2/tac1Δ::ARG4 znc1Δ::HIS1***	This study
yLM665	yLM660	*arg4Δ/arg4Δ, leu2Δ/leu2Δ::LEU2, his1Δ/his1Δ::HIS1, URA3/ura3Δ, IRO1/iro1Δ **CDR1/CDR1::CDR1-3HA-SAT1***	This study

yLM666	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1,,* ***tac1Δ::ARG4/tac1Δ::ARG4 CDR1/CDR1::CDR1-3HA-SAT1***	This study
yLM667	yLM661	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***znc1Δ::LEU2/znc1Δ::HIS1 CDR1/CDR1::CDR1-3HA-SAT1***	This study
yLM668	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***tac1Δ::ARG4 znc1Δ::LEU2/tac1Δ::ARG4 znc1Δ::HIS1 CDR1/CDR1::CDR1-3HA-SAT1***	This study
yLM669 [b]	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,****(tac1-znc1)Δ::ZNC1_A-SAT1/tac1Δ::ARG4 znc1Δ::LEU2***	This study
yLM670	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::SAT1(mock)/tac1Δ::ARG4 znc1Δ::LEU2***	This study
yLM671	yLM669	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::ZNC1_A-SAT1/tac1Δ::TAC1-HIS1 znc1Δ::LEU2***	This study
yLM672	yLM669	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::ZNC1_A-SAT1/tac1Δ::HIS1(mock) znc1Δ::LEU2***	This study
yLM673	yLM670	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::SAT1(mock)/tac1Δ::TAC1-HIS1 znc1Δ::LEU2***	This study
yLM674	yLM670	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::SAT1(mock)/tac1Δ::HIS1(mock) znc1Δ::LEU2***	This study
yLM675	yLM671	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::ZNC1_A-SAT1/tac1Δ::TAC1-HIS1 znc1Δ::ZNC1_B-ARG4***	This study
yLM676	yLM672	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::ZNC1_A-SAT1/tac1Δ::HIS1(mock) znc1Δ::ZNC1_B-ARG4***	This study
yLM677	yLM673	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::SAT1(mock)/tac1Δ::TAC1-HIS1 znc1Δ::ARG(mock)***	This study
yLM678	yLM674	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ,* ***(tac1-znc1)Δ::SAT1(mock)/tac1Δ::HIS1(mock) znc1Δ::ARG(mock)***	This study
yLM568		*ura3Δ::λimm^{434}/ura3Δ::λimm^{434} ade2::hisG/ade2::hisG::[pOPlacZ] RPS10/rps10Δ::[**LexA-Tac1$^{130-981}$**]/URA3*	(2)

yLM679 (*znc1*$^{\Delta/\Delta}$ *cdr1*$^{\Delta/\Delta}$)	yLM661	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***znc1Δ::LEU2/znc1Δ::HIS1 cdr1Δ::FRT/cdr1Δ::FRT***	This study
yLM680	cRC106	*ura3Δ::λimm*^{434}*/ura3Δ::λimm*^{434} *ade2::hisG/ade2::hisG::[pOPlacZ] RPS10/rps10Δ::[**LexA-Znc1**$^{127-922}$]/URA3*	This study
yLM681	cRC106	*ura3Δ::λimm*^{434}*/ura3Δ::λimm*^{434} *ade2::hisG/ade2::hisG::[pOPlacZ] RPS10/rps10Δ::[**LexA-Hal9**$^{116-1010}$]/URA3*	This study
yLM682	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1*, ***tac1Δ::ARG4/tac1Δ::HF-TAC1-SAT1***	This study
yLM683	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***znc1Δ::LEU2/znc1Δ::HIS1 tac1Δ::ARG4/tac1Δ::HF-TAC1-SAT1***	This study
yLM684	yLM660	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1Δ* ***ZNC1::ZNC1-3HA-SAT1/ZNC1::ZNC1-3HA-SAT1***	This study
yLM685	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1*, ***tac1Δ::ARG4/tac1Δ::ARG4 ZNC1::ZNC1-3HA-SAT1/ZNC1::ZNC1-3HA-SAT1***	This study
yLM686	yLM660	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1Δ* ***(TAC1 ZNC1)::ZNC1-3HA-SAT1-HF-TAC1/ (TAC1 ZNC1)::ZNC1-3HA-SAT1-HF-TAC1***	This study
yLM687	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1*, ***tac1Δ::ARG4/tac1Δ::TAC1***^{WT}***-SAT1***	This study
yLM688	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1*, ***tac1Δ::ARG4/tac1Δ::TAC1***$^{\Delta M677}$***-SAT1***	This study
yLM689	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1*, ***tac1Δ::ARG4/tac1Δ::TAC1***^{N972D}***-SAT1***	This study
yLM690	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1*, ***tac1Δ::ARG4/tac1Δ::TAC1***^{N977D}***-SAT1***	This study
yLM691	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***znc1Δ::LEU2/znc1Δ::HIS1 tac1Δ::ARG4/tac1Δ::TAC1***^{WT}***-SAT1***	This study
yLM692	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***znc1Δ::LEU2/znc1Δ::HIS1 tac1Δ::ARG4/tac1Δ::TAC1***$^{\Delta M677}$***-SAT1***	This study

yLM693	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***znc1Δ::LEU2/znc1Δ::HIS1 tac1Δ::ARG4/tac1Δ::TAC1^{N972D}-SAT1***	This study
yLM694	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***znc1Δ::LEU2/znc1Δ::HIS1 tac1Δ::ARG4/tac1Δ::TAC1^{N977D}-SAT1***	This study
yLM695	yLM660	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1Δ* ***MED17/MED17::MED17-3HA-SAT1***	This study
yLM696	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1*, ***tac1Δ::ARG4/tac1Δ::ARG4 MED17/MED17::MED17-3HA-SAT1***	This study
yLM697	yLM661	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***znc1Δ::LEU2/znc1Δ::HIS1 MED17/MED17::MED17-3HA-SAT1***	This study
yLM698	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***tac1Δ::ARG4 znc1Δ::LEU2/ tac1Δ::ARG4 znc1Δ::HIS1 MED17/MED17::MED17-3HA-SAT1***	This study
yLM699	-	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***stb5Δ::LEU2/stb5Δ::HIS1***	*stb5* null mutant (5)
yLM700	-	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***cta4Δ::LEU2/cta4Δ::HIS1***	*cta4* null mutant (5)
yLM701	yLM660	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1Δ* ***(tac1 znc1)::SAT1/(tac1 znc1)::SAT1***	This study
yLM702 (*tac1$^{Δ/Δ}$ znc1$^{Δ/Δ}$ mrr2$^{Δ/Δ}$*)	yLM662	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***mrr2Δ::LEU2/mrr2Δ::HIS1 (tac1 znc1)::SAT1/(tac1 znc1)::SAT1***	This study
yLM703	yLM699	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***stb5Δ::LEU2/stb5Δ::HIS1 (tac1 znc1)::SAT1/(tac1 znc1)::SAT1***	This study
yLM704	yLM700	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***cta4Δ::LEU2/cta4Δ::HIS1 (tac1 znc1)::SAT1/(tac1 znc1)::SAT1***	This study
yLM705	yLM701	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *URA3/ura3Δ*, *IRO1/iro1Δ* ***(tac1 znc1)::SAT1/(tac1 znc1)::SAT1 CDR1/CDR1::CDR1-3HA-ARG4***	This study
yLM706	yLM702	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO1/iro1Δ*, ***mrr2Δ::LEU2/mrr2Δ::HIS1 (tac1 znc1)::SAT1/(tac1 znc1)::SAT1 CDR1/CDR1::CDR1-3HA-ARG4***	This study

yLM707	yLM662	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO 1/iro1Δ,* ***mrr2Δ::LEU2/mrr2Δ::HIS1 CDR1/CDR1::CDR1-3HA-ARG4***	This study
yLM708 (*cdr1*$^{\Delta/\Delta}$)	yLM660	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *UR A3/ura3Δ*, *IRO1/iro1Δ* ***cdr1Δ::FRT/cdr1Δ::FRT***	This study
yLM709 (*cdr2*$^{\Delta/\Delta}$)	yLM660	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *UR A3/ura3Δ*, *IRO1/iro1Δ* ***cdr2Δ::FRT/cdr2Δ::FRT***	This study
yLM710 (*cdr1*$^{\Delta/\Delta}$ *cdr2*$^{\Delta/\Delta}$)	yLM708	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *UR A3/ura3Δ*, *IRO1/iro1Δ* ***cdr1Δ::FRT/cdr1Δ::FRT cdr2Δ::FRT/cdr2Δ::FRT***	This study
yLM711 (*tac1*$^{\Delta/\Delta}$ *znc1*$^{\Delta/\Delta}$ *cdr1*$^{\Delta/\Delta}$)	yLM664	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *URA3/ura3Δ*, *IRO 1/iro1Δ,* ***tac1Δ::ARG4 znc1Δ::LEU2/ tac1Δ::ARG4 znc1Δ::HIS1 cdr1Δ::FRT/cdr1Δ::FRT***	This study
yLM712 (*TAC1*^{WT} *cdr1*$^{\Delta/\Delta}$ *cdr2*$^{\Delta/\Delta}$)	yLM611	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::****TAC1-1****/URA3* ***cdr1Δ::FRT/cdr1Δ::FRT cdr2Δ::FRT/cdr2Δ::FRT***	This study
yLM713 (*TAC1*^{A736V} *cdr1*$^{\Delta/\Delta}$ *cdr2*$^{\Delta/\Delta}$)	yLM612	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::****P$_{TAC1}$-TAC1-1-A736V****/URA3* ***cdr1Δ::FRT/cdr1Δ::FRT cdr2Δ::FRT/cdr2Δ::FRT***	This study
yLM714 (*tac1*$^{\Delta/\Delta}$ *cdr1*$^{\Delta/\Delta}$)	yLM663	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::LEU2*, *his1Δ/his1Δ::HIS1*, *UR A3/ura3Δ*, *IRO1/iro1,* ***tac1Δ::ARG4/tac1Δ::ARG4 cdr1Δ::FRT/cdr1Δ::FRT***	This study
yLM715 (*TAC1*^{N977D} *cdr1*$^{\Delta/\Delta}$ *cdr2*$^{\Delta/\Delta}$)	yLM614	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::****P$_{TAC1}$-TAC1-1-N977D****/URA3* ***cdr1Δ::FRT/cdr1Δ::FRT cdr2Δ::FRT/cdr2Δ::FRT***	This study
yLM716	yLM167	*tac1-1Δ::hisG/ tac1-2Δ::hisG LEU2::****TAC1-1****/URA3* ***cdr2Δ::FRT/cdr2Δ::FRT***	This study
yLM122		Wü284	(6) collection
yLM751		CD38	(6) collection
yLM718		CD36	(6) collection
yLM753		CD57	(6) collection
yLM754		CD506	(6) collection
yLM756		CM1	(6) collection

yLM757		CAN6	(6) collection
yLM758		p7718	(6) collection
yLM759		CBS8500	(6) collection
yLM763 (CD57 *cdr1*$^{\Delta/\Delta}$)	CD57	*cdr1Δ::SAT1/cdr1Δ::SAT1*	This study
yLM764 (CD57 *tac1*$^{\Delta/\Delta}$)	CD57	*tac1Δ::SAT1/tac1Δ::SAT1*	This study
yLM765 (CD57 *tac1*$^{\Delta/\Delta}$ *znc1*$^{\Delta/\Delta}$)	CD57	*(tac1-znc1)Δ::SAT1/(tac1-znc1)Δ::SAT1*	This study
yLM766	cRC106	*ura3Δ::λimm*^{434}*/ura3Δ::λimm*^{434} *ade2::hisG/ade2::hisG::[pOPlacZ] RPS10/rps10Δ::[**LexA-CdTac1**$^{131-989}$]/URA3*	This study
yLM767	Wü284	*cdr1*^{756stop}*Δ::SAT1/cdr1*^{756stop}*Δ::SAT1*	This study

[a] The genotypic features in the parentheses identify the strains tested in Table 1 and Table 2.

[b] '(*tac1-znc1*)' or '(*TAC1-ZNC1*)' in the presentation of strain genotype refers to a genetic modification event (deletion, tagging or complementation) which affects both *TAC1* and *ZNC1* loci. Details are described in the Supplemental Method/Strain Construction session.

Table S3 Plasmids used in this study

Plasmid	Description	Reference
pV1093	A tool plasmid for *C. albicans* CRISPR-Cas9 system	(7)
pFA-3HA-SAT1	Cloning vector/Template for amplifying C-terminal 3xHA tagging cassettes with a ***SAT1*** marker	(8)
pFA-3HA-ARG4	Template for amplifying C-terminal 3xHA tagging cassettes with an ***ARG4*** marker	(8)
pNAT	Plasmid template for amplifying gene deletion cassettes with a ***SAT1*** marker	(9)
pSFS2-CDR1KO	*SAT1* flipper construct for deleting *C. albicans* ***CDR1*** ORF	(2)
pSFS2-CDR2KO	*SAT1* flipper construct for deleting *C. albicans* ***CDR2*** ORF	(2)
pRS-ARG4ΔSpe1	Plasmid template for amplifying gene deletion cassettes with an ***ARG4*** marker	(10)
CIp-LexA	Parental vector for cloning and expressing LexA fusion proteins in a *C. albicans* LacZ reporter strain	(4)
pFA-3HA-SAT1-Z3	Intermediate plasmid	This study
pFA-3HA-SAT1-T3	Intermediate plasmid	This study
pFA-3HA-HIS-T3	Intermediate plasmid	This study
pFA-ZNC1_A-SAT1-Z3	Intermediate plasmid	This study
pFA-ZNC1_B-SAT1-Z3	Intermediate plasmid	This study
pFA-ZNC1_A-SAT1-T3	Plasmid for introducing ***ZNC1*** **allele A (C5_01850C_A) with a** ***SAT1*** **marker** to bridge the *ZNC1* upstream region and the *TAC1* downstream region in a *tac1 znc1* double deletion locus	This study
pFA-ZNC1mock-SAT1-Z3	Intermediate plasmid	This study
pFA-TAC1mock-HIS1-T3	Plasmid for introducing **a** ***HIS1*** **marker as a mock complement** into a *tac1* deletion locus	This study
pFA-TAC1-HIS1-T3	Plasmid for re-introducing **wild type** ***TAC1*** **with a** ***HIS1*** **marker** into a *tac1* deletion locus	This study
pFA-TAC1-SAT1-T3	Plasmid for introducing **wild type** ***TAC1*** **with a** ***SAT1*** **marker** into a *tac1* deletion locus	This study
pFA-HF-TAC1-HISI-T3	Intermediate plasmid	This study
pFA-HF-TAC1-SAT1-T3	Plasmid for introducing **N-terminally 6HisFlag tagged** ***TAC1*** **with a** ***SAT1*** **marker** into a *tac1* deletion locus	This study
pFA-TAC1-ΔM677 -HISI-T3	Intermediate plasmid	This study
pFA-TAC1-ΔM677 -SAT1-T3	Plasmid for introducing ***TAC1 ΔM677*** **GOF mutant with a** ***SAT1*** **marker** into a *tac1* deletion locus	This study
pFA-TAC1-N972D -HIS1-T3	Intermediate plasmid	This study
pFA-TAC1-N972D -SAT1-T3	Plasmid for introducing ***TAC1 N972D*** **GOF mutant with a** ***SAT1*** **marker** into *tac1* deletion locus	This study

pFA-TAC1-N977D -HIS1-T3	Intermediate plasmid	This study
pFA-TAC1-N977D -SAT1-T3	Plasmid for introducing ***TAC1 N977D* GOF mutant with a *SAT1* marker** into a *tac1* deletion locus	This study
pFA-ZNC1mock-SAT1-T3	Plasmid for introducing **a *SAT1* marker as a mock complement** to bridge the *ZNC1* upstream region and the *TAC1* downstream region in a *tac1 znc1* double deletion locus	This study
pFA-ZNC1mock-ARG4-Z3	Plasmid for introducing **an *ARG4* marker as a mock complement** into a *znc1* deletion locus	This study
pFA-ZNC1_B-ARG4-Z3	Plasmid for re-introducing ***ZNC1* allele B (C5_01850C_B) with an *ARG4* marker** into a *znc1* deletion locus	This study
CIp-LexA-Znc1$^{127\text{-}922}$	Plasmid for expressing **LexA-Znc1$^{127\text{-}922}$** fusion protein in a *LacZ* reporter strain; targeting a *RPS10* locus with **a URA3 marker**	This study
CIp-LexA-Hal9$^{116\text{-}1010}$	Plasmid for expressing **LexA-Hal9$^{116\text{-}1010}$** fusion protein in a *LacZ* reporter strain; targeting a *RPS10* locus with **a URA3 marker**	This study
CIp-LexA-CdTac1$^{131\text{-}989}$	Plasmid for expressing **LexA-CdTac1$^{131\text{-}989}$** fusion protein in a *LacZ* reporter strain; targeting a *RPS10* locus with **a URA3 marker**	This study

Table S4 Primers used in this study

Primer Name	Primer Sequence	Note *
ZL514	ATTGACGACGATGATGAC	1-up_F (2)
ZL515	ATAAGAAGTTGAGGCGAAG	1-up_R (2)
ZL851	TGCGTGACCCAAACATAATCT	1-2_F (2)
ZL852	TGTGACGAGGTGGCTGAT	1-2_R (2)
ZL518	tttcaacatattagaatcgaatcattacg	1-DRE_F (11)
ZL519	gcggctgtgtgtttgtgtg	1-DRE_R (11)
ZL528	CATCTTGCGGTTCTAATAG	2-up_F (2)
ZL529	CACTCTAATCTGATATGGTTC	2-up_R (2)
ZL861	TGTGACCAGGTAGTGATAGT	2-4_F (2)
ZL862	GCATTGCTGAGAGTGGAA	2-4_R (2)
ZL532	aattcaaacacaaacaataaggctgt	2-DRE_F (11)
ZL533	gcaatcattgtggtatacatcgga	2-DRE_R (11)
ZL833	ACACAACAATTTAACTGCGAATAG	i-1_F
ZL834	CCAGAGATGCCAGTGACT	i-1_R
ZL1082	AGTCACTGGCATCTCTGG	i-2_F
ZL1083	AACGGCACGGTTAGAATT	i-2_R
ZL835	GGTAGTTTGTATGCGGGAAAT	i-3_F
ZL836	TCAACGAAGGGTGGAAGT	i-3_R
ZL1052	AACCTTTTCCGTATAGATG	z-0_F
ZL1053	CGAACTTTCATTCTTGTATT	z-0_R
ZL1056	CATTCCTAGACCTGGTAACA	z-2_F
ZL1057	AGTAAGCACCATTCTCTGT	z-2_R
ZL1060	TGCCATCAAAGTAAACTAGG	z-PZM_F
ZL1061	AGGCTATTACTGTGGTATCTA	z-PZM_R
ZL1064	ATGTGTATGTCGTGGTTCA	r-up_F
ZL1065	GTGGTCAGCCTCCTAATC	r-up_R
ZL1068	AATACACTTATCCTACAAGATCC	r-2_F
ZL1069	GCTTATCTCCGCATTCAA	r-2_R
ZL1072	CACACGGAACTCGGAAAT	r-DRE_F
ZL1073	GGACACGCCAATAATAATCATAA	r-DRE_R
ZL1010	ATTTAATAATATGATATTCAGTGAGCTTAATGAATTG CCAGACTTTTTCAATTCACCGTCTTTAGGATTTAATG AGCAAAATATAggtcgacggatcccc	
ZL1011	TTCGTGTGATTTTGCATCATCTCCGAAACGGAAGTGC GGAGCACGGAAGAAGCAACGGAAAATAAAAAGTAA AAATATCCGGtcgatgaattcgagctcg	
ZL1012	**CTATTTCCATTGTATTCTTC**CAAATTAAAAATAGTT TACGCAAGTC	
ZL1013	**GAAGAATACAATGGAAATAG**GTTTTAGAGCTAGAA ATAGCAAGTTAAA	

ZL1014	GGGAACATGATGTTAATGAATGGTAAC	
ZL1015	GATCGAGAATCAAAGTCTAAGTTTAAACC	
ZL1033	tcgatgaattcgagctcg	
ZL1034	cgagctcgaattcatcgaAAGAAGAAGTGGATAATTTTGATTAAC	
ZL1035	TGAGGCACTTTCTCTATGCCAACC	
LM077	gcaaggatccaagaagaagtggataattttgattaac	BamHI (12)
ZL487	CTATTAGTATCGTTAGGGTCATTCC	
ZL941	ATTCAGATTCCCTTTCAGCCAAGAAAAAACTCCAAGAAAAGAAATAGAGCCTTTCTCCTTCTCTCATAAATAATGGACAC**TAATGAttTTTCCCAGTCACGACGTT**	
ZL942	GGAAAAAATATATGAAACAATAAATATTTACAAAGATATACATTATACATCGCTTTCACCAATTACAACTCTTTTTTAACCC**GTGGAATTGTGAGCGGATA**	
ZL943	**CCCGTAGTGGATAAATTGCA**CAAATTAAAAATAGTTTACGCAAGTC	
ZL944	**TGCAATTTATCCACTACGGG**GTTTTAGAGCTAGAAATAGCAAGTTAAA	
ZL948	TGCCGACGAATATCAATA	
ZL955	AACAGTGGTGCTATTAGG	
ZL540	ATTCTAAGATGTCGTCGCAAGATG	(13)
ZL541	AGTTCTGGCTAAATTCTGAATGTTTTC	(13)
ZL542	TAGTCCATTCAACGGCAACATT	(13)
ZL543	CACCCAGTATTTGGCATTGAAA	(13)
ZL712	TGGTGATGGTGTTACTCACG	(14)
ZL713	GACAATTTCTCTTTCAGCAC	(14)
ZL544	AACTTCAACAACTCTATCC	
ZL545	GAGGCACTAATGTAATCC	
M2PT-1	GTTGCTACTACTGGTTCA	
M2PT-2	GGATATGTGATTCGGATGA	
M2PT-23	TGATTCTCCTTGTGAAGTGAT	
M2PT-24	TGTAGATGTAGATGTAGATGTAGC	
M2PT-15	TGATGACAATACTTCTAACAAC	
M2PT-16	TGGAGATGATGATGATGAG	
ZL823	ATGGGTGAGGTTGATGAA	
ZL824	CCAAACGCTTGACAGATG	
ZL951	TAATACTGGACTTGGTTATG	
ZL958	CGAATGTCACTGTTACTAA	
AZcp007	CACGAACAACAGGAGTAGG	(15)
AZcp008	GCCATTACCACCACTAAC	(15)
ZL578	CAGTGTTATCAGTGAAGG	(2)
ZL579	TGCTCTATGAAGACCAAT	(2)
LM21	CTAATTAACGTGTGTGTATGGATC	

ZL959	AGGT**GTTTAAAC**GAGTCAATTCACGTTGAGACGG	PmeI
ZL960	CAATT**CCGCGG**TTCGTGTGATTTTGCATCATCTCCG	SacII
ZL962	TAACCA**ggatcc**AA**CCCGGG**TAGATACAAGTTGGTTTG CAACAGC	BamHI XmaI
ZL963	AATTC**CTGCAG**TC**GGCGCGCC**TTATATATTTTGCTC ATTAAATCCTAAAG	AscI PstI
ZL961	TTCA**GGCGCGCC**AACTCTTTTTTAACCCTTAAATCCC	AscI
ZL994	AGGG**GTTTAAAC**GAGTTGTAATTGGTGAAAGCG	PmeI
ZL995	GATTTT**CCGCGG**agtatattctgttgggaaaggggtgag	SacII
ZL964	AGCTGGTACCCGTGGTGGTGGTGGTGGTGCATTTTTA ATTAAAGCAACTATTTGTCAGTGTGAAGCTTGG	KpnI
ZL633	GTATGGGTACCcCATTATTTATGAGAGAAGGAGA AAGGC	KpnI
ZL968	**ggtccacgcgttGGTGGAGGTCCAGGTGGA**AGTTCG TTCACAAATGGCAAT	MluI
ZL988	**CTGAAacgcgttGGTGGAGGTCCAGGTGGA**AGCA AGCTGAACAGGGACCGGC	MluI
ZL989	AATGGCTATTCAGaCCTGTGCTTAGCA	
ZL990	TGCTAAGCACAGGtCTGAATAGCCATT	
ZL991	cCCg**CTGCAG**cC**GGCGCGCCt**TTTATTAGTTATA AAATATATCAGGAAAGTTCAAGTC	AscI PstI
ZL1008	ACATATTAGGATGGTCCA	
ZL1009	GTCCACATTCAAATTCAC	
ZL1045	**CTGAAacgcgttGGTGGAGGTCCAGGTGGA**GATA TAGAATCAAGATTGAGTAGAATTG	MluI
ZL1046	CAAATGGCGCGCCtctaCTTAACTATTTTTAGATTC CCAAATTATTGTCAAAGAAAAAATTGGG	AscI
ZL1084	**CCAAATGGTCAAACTTGTTC**CAAATTAAAAAT AGTTTACGCAAGTC	
ZL1085	**GAACAAGTTTGACCATTTGG**GTTTTAGAGCTA GAAATAGCAAGTTAAA	
ZL1086	CCCTAATATAAGTTAAGATTATGTTAGATTCTAA GATGTCGTCGCAAGATGAATCTAAACTAGAAAG GGCAACA**TAATGAttTTTCCCAGTCACGACGTT**	
ZL1087	ATTTTCAACGGAATAGTCGGCAACCCACGTTCA ACTAACAATTACTACAATCCCAAAAACCTGGAC GACAACAAGAATCTC**GTGGAATTGTGAGCGGA TA**	
ZL1090	**CCACTGAAAGGTGAAGCACC**CAAATTAAAAAT AGTTTACGCAAGTC	
ZL1091	**GGTGCTTCACCTTTCAGTGG**GTTTTAGAGCTA GAAATAGCAAGTTAAA	

ZL1092	GAACCACCCTGGAAACCTCCCATAAATAATGGA TAATTTACCATCACTGGAGACTCACCATTCATCT TTAGATA**TAATGAttTTTCCCAGTCACGACGTT**	
ZL1093	ATCTCCGGACTGGAATATCAAGTCTCGGATATA GTTTATCTGATTTCCAGGAATCTGACTAGGTAAT CGGGTATCTCCTAC**GTGGAATTGTGAGCGGAT A**	
ZL1096	GAAAGAAAACTCCATGAATACCCATTAGC	
ZL1097	TAAGTGTGTCATTAGTCTCCAGCAT	
ZL1098	CTTGATATTCCAGTCCGGAGAT	
ZL1099	CTTTCGTATGTGCTGAAGGAGATG	
ZL1100	ATGCTGGAGACTAATGACACACTTA**TAATGAttT TTCCCAGTCACGACGTT**	
ZL1102	TGTTGATTATGAATATTGCCATTGTGC	
ZL1103	CAGTTAACGTAGTGACATCTTCC	
ZL1104	GATTTTAATGAAGAACGAATCGCCAC	
ZL1105	GTGTTGATCACTGGAGGTTTACCTG	

***** The 'Note' column denotes the restriction enzyme cutting site(s) added for cloning, the region probed by a ChIP assay primer (referring to **Fig. 5**), or the reference for a published primer.

Supplemental Methods

Plasmid construction

A DNA fragment with *ZNC1* downstream sequence ('*Z3*') was amplified by ZL959/ZL960 from *C. albicans* genomic DNA, digested by Pme1/SacII, and inserted into a *pFA-3HA-SAT1* backbone cut by the same enzymes. The resulting plasmid, *pFA-3HA-SAT1-Z3*, was digested by XmaI/AscI for introduction of *ZNC1* promoter driven *ZNC1* coding sequence amplified by ZL962/ZL963 from *C. albicans* genomic DNA to generate *pFA-ZNC1_A-SAT1-Z3* and *pFA-ZNC1_B-SAT1-Z3* which respectively contain *ZNC1* A allele (C5_01850C_A) and *ZNC1* B allele (C5_01850C_B) sequence. The former plasmid was digested by Pme1/SacII for insertion of *TAC1* downstream sequence ('*T3*') amplified by ZL994/ZL995 to generate *pFA-ZNC1_A-SAT1-T3*, and the latter by AscI/PmeI for replacement of the *SAT1* selective marker with an *ARG4* selective marker digested by the same enzymes from a *pFA-myc-ARG4* vector to generate *pFA-ZNC1_B-ARG4-Z3.* DNA with *ZNC1* promoter sequence was amplified by ZL962/ZL964 from a genomic DNA sample, digested by BamHI/KpnI and ligated into a *pFA-6His3Flag-SAT1* backbone, and then sub-cloned into *pFA-3HA-SAT1-Z3* to generate *pFA-ZNC1mock-SAT1-Z3.* The *ZNC1* downstream sequence between the PmeI/SacII sites on this plasmid was replaced by the *TAC1* downstream sequence amplified by ZL994/ZL995 to generate *pFA-ZNC1mock-SAT1-T3. pFA-ZNC1mock-ARG-Z3* was generated by substituting the *SAT1* selective marker on *pFA-ZNC1mock-SAT1-Z3* with the *ARG4* selective marker digested by AscI/PmeI from *pFA-myc-ARG4*.

The *TAC1* downstream sequence ('*T3*') amplified by ZL994/ZL995 was also inserted into *pFA-3HA-SAT1* between the PmeI/SacII sites. The resulting plasmid, *pFA-3HA-SAT1-T3*, was cut by AscI/PmeI for replacing the *SAT1* marker with a *HIS1* marker cut from a *pFA-myc-HIS1* plasmid by the same enzymes to generate *pFA-3HA-HIS1-T3. pFA-3HA-HIS1-T3* was digested by BamHI/AscI for individual insertion of *TAC1* promoter driven *6Hi3Flag-TAC1*, wild type *TAC1*, $TAC1^{\Delta M677}$, $TAC1^{N972D}$ and $TAC1^{N977D}$ amplified by LM077/ZL961 respectively from the *TAC1InteHF-WT*, pDS1097, *TAC1InteΔM677*, *TAC1InteN972D* and *TAC1InteN977D* (2, 3, 12) to generate *pFA-HF-TAC1-HIS-T3*, *pFA-TAC1-HIS-T3*, *pFA-TAC1-ΔM677-HIS-T3*, *pFA-TAC1-N972D-HIS-T3 and pFA-TAC1-N977D-HIS-T3.* The *HIS1* marker between the AscI/PmeI sites on

these constructs were replaced by a *SAT1* marker to generate *pFA-HF-TAC1-SAT1-T3*, *pFA-TAC1-SAT1-T3*, *pFA-TAC1-ΔM677-SAT1-T3*, *pFA-TAC1-N972D-SAT1-T3* and *pFA-TAC1-N977D-SAT1-T3*. *pFA-TAC1mock-HIS-T3* contains only *TAC1* promoter sequence between the BamHI/AscI sites, which was amplified by LM077/ZL633 and introduced by sub-cloning.

CIp-LexA-Znc1$^{127\text{-}922}$ was generated by insertion of a *ZNC1* fragment amplified by ZL968/ZL963 from *C. albicans* genomic DNA into a *CIp-LexA* vector between the MluI/PstI sites. The *ZNC1* sequence cloned in *CIp-LexA-Znc1*$^{127\text{-}922}$ matches the C5_01850C_A sequence. A StuI-free DNA fragment which encodes Hal9 aa116-1010 was generated by sealing ZL988/ZL990 and ZL989/ZL991 amplicons from a genomic DNA sample by fusion PCR. This fragment was cloned into a *CIp-LexA* vector between the MluI/PstI sites to generate *CIp-LexA-Hal9*$^{116\text{-}1010}$. A DNA fragment which encodes *CdTac1* aa131-989 was amplified by ZL1045/ZL1046 from *C. dubliniensis* genomic DNA, digested with MluI/AscI and inserted into MluI cut *CIp-LexA* in an appropriate orientation to generate *CIp-LexA-CdTac1*$^{131\text{-}989}$.

Further details on the construction of particular plasmids and their sequences can be obtained by contacting the authors.

Strain construction:

Both conventional homologous recombination and CRISPR-Cas9 (7, 9) systems were used to genetically engineer *C. albicans* strains in this study. The latter method requires co-transforming cells with a transient Cas9 expressing cassette amplified from pV1093 (7) and target specific sgRNA expressing cassette and repairing cassette. sgRNA expressing cassettes were amplified from pV1093 (7) by a nested fusion PCR strategy (9) and referred to as 'ZL(number 1)/ZL(number 2) (name of the primers which specify the gene target) sgRNA' herein.

C-terminal 3xHA tagging of Cdr1 (yLM665-yLM668) or Srb4 (yLM695-yLM698) was performed as described previously (2). *pFA-3HA-ARG4* was used as the template to generate a *CDR1*-3xHA tagging cassette which contains an *ARG4* selective marker to construct yLM705-yLM707. ZL1012/ZL1013 sgRNA was used to guide the integration of the ZL1010/ZL1011 *pFA-3HA-SAT1* amplicon to tag both copies of Znc1 with C-terminal 3xHA in wild type and *tac1Δ/Δ* background (yLM684 and yLM685). Successful tagging at both alleles was confirmed by loss of

wild type size ZL1014/ZL1015 amplicon in genotyping PCR tests. The ZL1012/ZL1013 sgRNA was also used to guide the integration of a '*ZNC1-3HA-SAT1-HF-TAC1*' cassette to generate yLM686 which carries two copies of N-terminally 6His3Flag tagged *TAC1* and two copies of C-terminally 3xHA tagged *ZNC1*. The '*ZNC1-3HA-SAT1-HF-TAC1*' was a fusion PCR product generated by sealing ZL1010/ZL1033 *pFA-3HA-SAT1* amplicon (the '*ZNC1-3HA-SAT1*' half) and ZL1034/ZL1035 *pFA-HF-TAC1-SAT1-T3* amplicon (the '*HF-TAC1*' half). Transformants with the wanted genotype were screened by positive amplification by ZL1014/LM21 and loss of wild type size amplicon by LM077/ZL487 in genotyping tests.

Deletion of *cdr1* and *cdr2* was performed in the same way as described previously (2). Deletion of *tac1* was performed by using ZL943/ZL944 sgRNA and an *ARG4* repairing cassettes amplified by ZL941/ZL942 from *pRS-ARG4ΔSpe1* (10). The same sgRNA was used to transform *C. albicans* with XmaI/SacII digested *pFA-ZNC1mock-SAT1-T3* to disrupt the *TAC1-ZNC1* locus as an entirety. Successful deletion of *znc1* and *tac1* was respectively confirmed by loss of the ZL948/ZL955 and ZL578/ZL579 amplicons in genotyping PCR tests. Generally, for each new strain made in this study, 4-6 PCR verified transformants were picked from the primary transformation plates, streaked on fresh plates for single colonies and double-checked by PCR. Colony size and appearance was confirmed to be comparable by eye. Since many mutants used in this work show growth changes on farnesol and/or fluconazole, spot growth assays on agar plates containing these compounds were performed for all PCR verified isolates. Typically, the isolates showed highly similar growth/sensitivity. Occasionally, however, one out of the 4-6 isolates would exhibit different extent of phenotype compared to the rest, and not be included in further analysis. The occurrence of oddly behaving colonies had no clear correlation with the gene editing methods used (conventional homologs recombination versus CRISPR). Further support for comparable performance of the CRISPR and conventional methodology came from our finding that generation of *tac1 znc1* double deletion strains by sequential deletion of the two genes or by a single step CRISPR disruption behaved identically.

A series of complementation strains were generated in *ARG+ LEU+ HIS+ sat1-* yLM664 which carries deletions in both *tac1* and *znc1*. The genotype at the *TAC1-ZNC1* loci in yLM664 was referred to as '*tac1Δ*::*ARG4 znc1Δ*::*HIS1*'/'*tac1Δ*::*ARG4 znc1Δ*::*LEU2*' in this session. First,

yLM664 was transformed with XmaI/SacII digested *pFA-ZNC1mock-SAT1-T3* or *pFA-ZNC1_A-SAT1-T3*. To search for integration at the '*tac1Δ*::*ARG4 znc1Δ*::*HIS1*' locus, clonat resistant transformants were further selected for histidine auxotrophy. The resulting *ARG+ LEU+ his- SAT1* strains were named yLM670 or yLM669. yLM669 was next transformed with BamHI/SacII digested *pFA-TAC1mock-HIS-T3* or *pFA-TAC1-HIS-T3* to replace the *ARG4* marker at the '*tac1Δ*::*ARG4 znc1Δ*::*LEU2*' locus with either a vector control or a *TAC1* ORF to generate *arg-LEU+ HIS+ SAT1+* yLM672 and yLM671. yLM673 and yLM674 were generated from yLM670 by using the same strategy. yLM671 and yLM672 were further transformed with *pFA-ZNC1_B-ARG4-Z3* to replace the *LEU2* at the remaining *znc1* deletion locus to generate *ARG+ leu- HIS+ SAT1+* yLM675 and yLM676 respectively. yLM677 and yLM678 were similarly generated by introduction of *pFA-ZNC1mock-ARG-Z3* into yLM673 and yLM674 respectively.

A *tac1Δ/Δ* strain (yLM663) was transformed with BamHI/SacII digested *pFA-HF-TAC1-SAT1-T3*, *pFA-TAC1-SAT1-T3* and its GOF mutation variants to generate yLM682 and yLM687-yLM690. A same set of transformation was performed in a *tac1Δ/Δ znc1Δ/Δ* strain (yLM664) to generate yLM683 and yLM691-yLM694.

LacZ reporter strains yLM680, yLM681 and yLM766 were generated by transforming cRC106 (4) with the corresponding *CIp-LexA* plasmids. The *CIp-LexA* plasmids were linearized by Stu1 digestion before used for transformation.

The transient Cas9 system (9) was also used for gene deletion in *C. dubliniensis* strains. ZL1084/ZL1085 and ZL1090/ZL1091 were respectively used to generate sgRNA cassettes targeting Cd*CDR1* and Cd*TAC1* ORF sequence, and the repairing cassettes with a *SAT1* selective marker was respectively amplified by ZL1086/ZL1087 and ZL1092/ZL1093 marker from *pNAT* (9). One step disruption of both the *TAC1* and *ZNC1* ORFs was performed by using ZL1090/ZL1091 sgRNA and a fusion PCR product as the repairing template, which seals *ZNC1* upstream region amplified by ZL1096/ZL1097 from genomic DNA, a *SAT1* marker amplified by ZL1100/ZL1093 from *pNAT* and *TAC1* downstream region amplified by ZL1098/ZL1099 from genomic DNA. Successful deletion of *cdr1*, *tac1* or *znc1* in yLM763, yLM764, yLM765 and yLM767 was respectively tested by loss of specific amplicons by ZL541/ZL542, ZL1104/ZL1105 and ZL1102/ZL1103 in genotyping PCR tests.

Further details on the construction of particular strains can be obtained by contacting the authors.

Fluconazole MIC measurement by E-test

Overnight YPD culture of each strain to be tested was diluted in 0.85% NaCl solution to OD 0.05 ($5x10^5$ cells mL^{-1}) and spread on a YPD plate (supplemented with 0.1 mM uridine) by a cotton swab. After placement of fluconazole E-test strips (bioMérieux, MIC range: 0.016-256 µg/mL), plates were incubated for 36 hours at 30°C before reading the MIC.

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