

Supplemental Figures

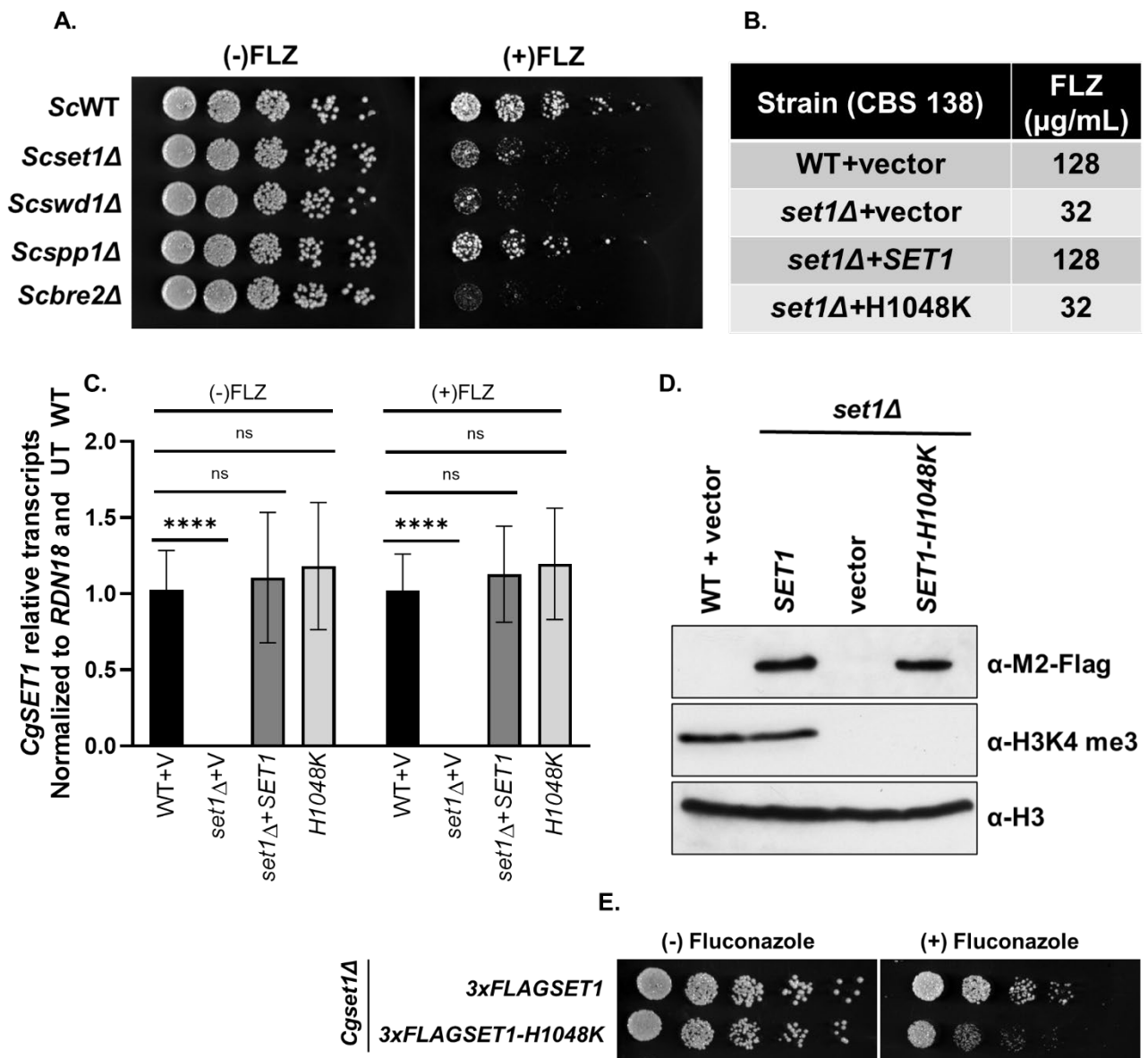


FIG S1. Loss of Set1 complex members results in altered azole efficacy. (A) Five-fold serial dilution spot assays of the indicated *S. cerevisiae* strains were grown on SC plates with or without 8 μg/mL fluconazole. (B) MIC assay of the indicated strains performed in SC media at 35°C and results recorded after 24 h of incubation. (C) Expression of SET1 was determined in the indicated mutants treated with and without 64 μg/mL fluconazole for 3 h by qRT-PCR analysis. Gene expression analysis was set relative to the untreated wild-type and expression was normalized to *RDN18* mRNA levels. Data were analyzed from 4 biological replicates with three technical replicates each. (D) Whole cell extracts isolated from the indicated strains were immunoblotted using trimethyl-specific and anti-M2-Flag mouse antibodies. Histone H3 was used as a loading control. (E) Five-fold serial dilution spot assays of the indicated 3xFLAG-*SET1* strains were grown on SC plates with or without 32 μg/mL fluconazole. Statistics were performed using Graphpad Prism student t-test version 9.2.0. **** $p < 0.0001$. Error bars represent SD.

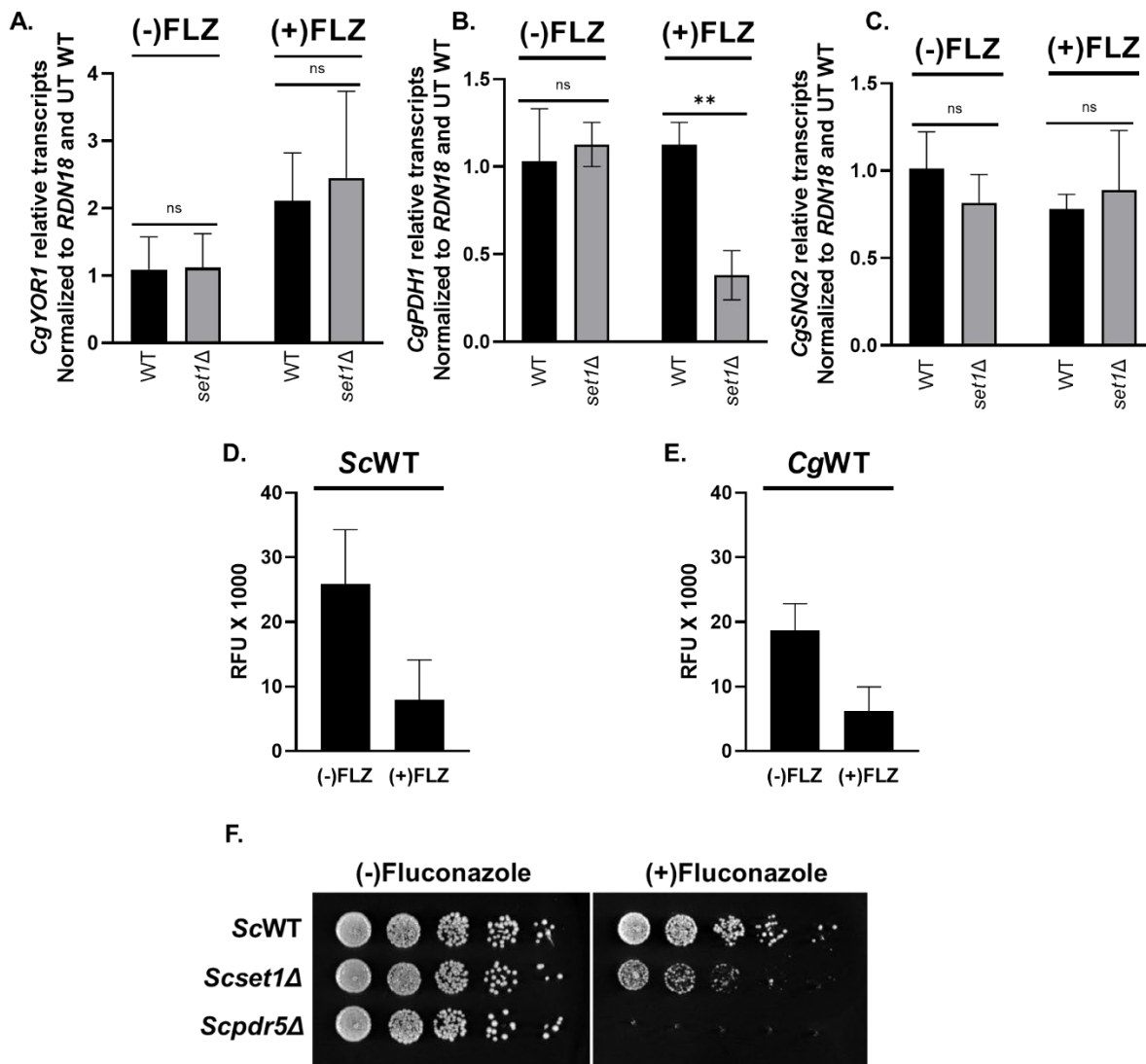


Fig S2. Transcript levels of drug transporters in a *set1Δ* strain compared to wild type. (A-C) Expression of the indicated genes were determined in the indicated mutants treated with and without 64 μ M fluconazole for 3 h by qRT-PCR analysis. Gene expression analysis was set relative to the untreated wild-type and expression was normalized to *RDN18* mRNA levels. Data were analyzed from ≥ 3 biological replicates with three technical replicates each. Statistics were performed using Graphpad Prism student t-test version 9.2.0. $**p < 0.01$. Error bars represent SD. (D-E) Relative fluorescent units of Nile red in wild-type *C. glabrata* and *S. cerevisiae* with and without 64 μ M fluconazole. (F) Five-fold dilution spot assay of indicated *S. cerevisiae* strains with and without 8 μ M fluconazole on minimal media.

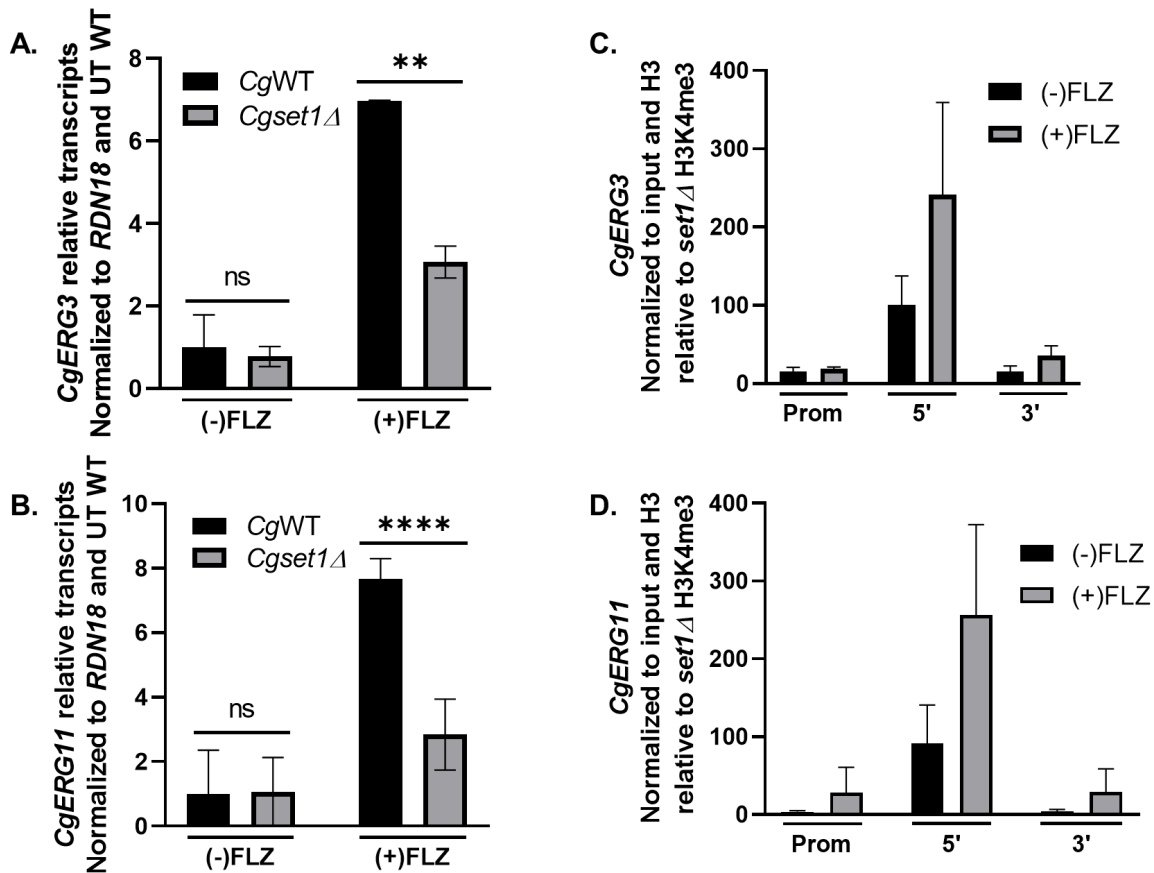
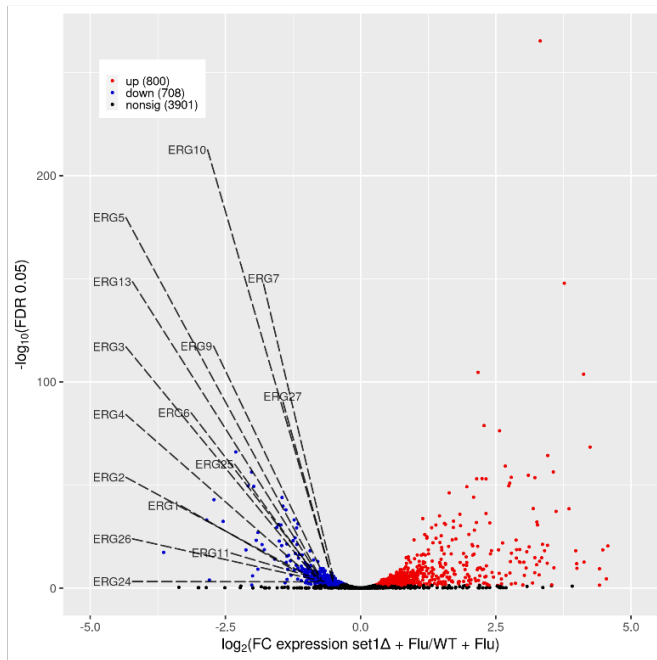


Fig S3. Histone H3K4 trimethylation is enriched on ERG gene chromatin and Set1-mediated histone H3K4 methyltransferase activity is required for azole induction of ERG genes in saturated cells. (A, B) Expression of genes was determined in the indicated strains treated with and without 64 $\mu\text{g}/\text{mL}$ fluconazole in a saturated culture for 3 h by qRT-PCR analysis. Gene expression analysis was set relative to the untreated wild-type expression was normalized to *RDN18* mRNA levels. Data were analyzed from ≥ 3 biological replicates with three technical replicates each. (C, D) ChIP analysis of histone H3K4 tri-methylation levels at the promoter, 5', and 3' regions of *ERG11* and *ERG3* in a wild-type *C. glabrata* strain with and without 64 $\mu\text{g}/\text{mL}$ fluconazole treatment in saturated cell cultures. ChIP analysis was set relative to a *set1* Δ strain and normalized to histone H3 and DNA input levels. Data were analyzed from 3 biological replicates with three technical replicates each. Statistics were performed using Graphpad Prism student t-test version 9.2.0. **** $p < 0.0001$ and ** $p < 0.01$. Error bars represent SD.

A.



B.

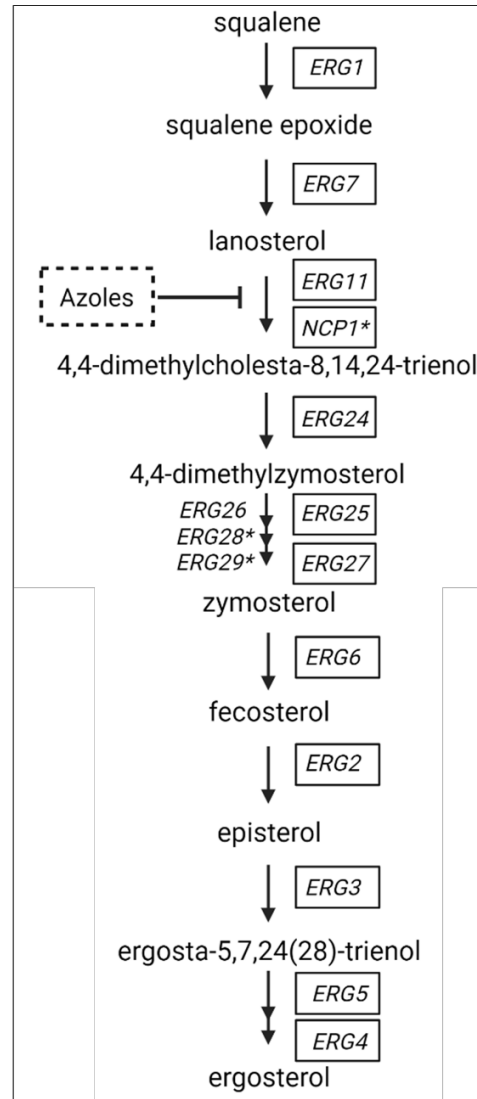


Fig S4. Genes encoding enzymes of the late ergosterol pathway are down in a *set1Δ* strain upon azole treatment in *C. glabrata*. (A) Volcano plot showing the significance [$-\log_2(\text{FDR})$, y-axis] vs. the fold change (x-axis) of the DEGs identified in the *set1Δ* azole treated samples relative to WT azole treated samples. Genes with significant differential expression ($\text{FDR} < 0.05$) are highlighted in red or blue for up- and downregulated genes, respectively. Down-regulated *ERG* genes are labelled in the plot which include 12 of the *ERG* genes in the late pathway and two *ERG* genes in the early pathway. Black highlighted genes are considered nonsignificant. (B) Depiction of the late ergosterol pathway in *C. glabrata*. Azoles inhibit lanosterol 14- α -demethylase, the enzyme encoded by *ERG11*. *NCP1** and *ERG28** interact with ergosterol synthesizing enzymes. *ERG29** is a protein of unknown function involved in ergosterol biosynthesis. Loss of *SET1* results in lower transcript levels of 12 of the 12 genes in the late ergosterol biosynthesis pathway compared to a wild-type strain upon azole treatment. Genes with decreased transcript levels due the loss of *SET1* are surrounded by a solid square.

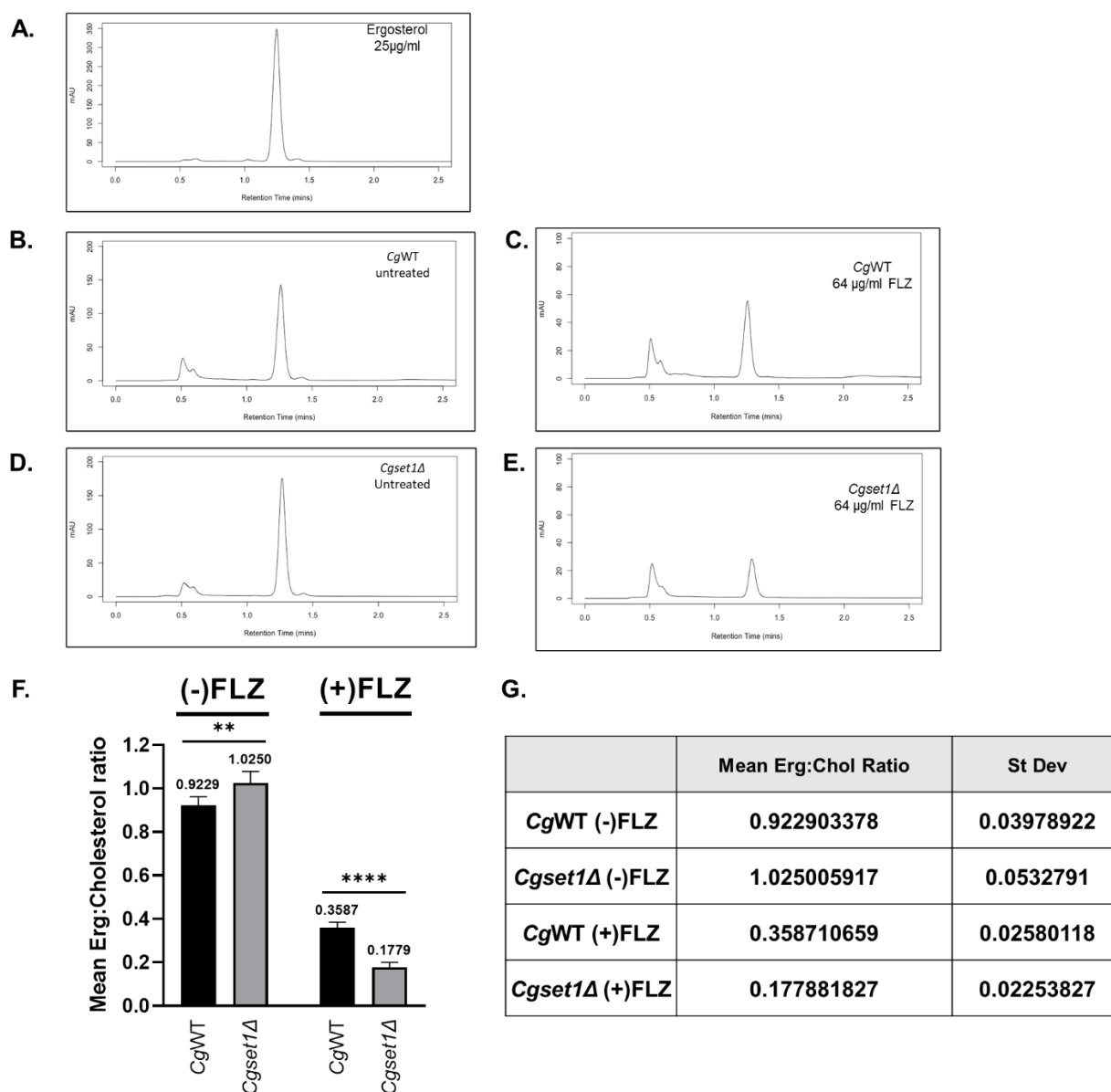


Fig. S5 HPLC analysis of total cellular ergosterol. (A-E) Representative chromatograms of ergosterol extracted from CgWT and Cgset1Δ cells with and without 64 µg/mL fluconazole treatment compared with purified ergosterol (MilliporeSigma). Purified and extracted ergosterol had a retention time of ~1.2 min. Purified cholesterol (MilliporeSigma) was added to the extract yeast as an internal control (data not shown). Standard curves were generated for ergosterol and cholesterol to determine the ergosterol:cholesterol ratio (F) Bar graph and mean ergosterol:cholesterol ratio of the indicated *C. glabrata* strains treated with and without 64 µg/mL fluconazole. (G) Table of values used to generate Figure S5F and Figure 7. Data was obtained from 6 biological replicates.

Supplemental Tables

Table S1.	Yeast Strains		
Strains	Genotype	Reference	Strain Name
<i>S. cerevisiae</i> BY4741	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0</i>	Open Biosystems	ScWT
<i>Scswd1Δ</i>	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 swd1Δ::KanMX</i>	Open Biosystems	<i>Scswd1Δ</i>
<i>Scspp1Δ</i>	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 spp1Δ::KanMX</i>	Open Biosystems	<i>Scspp1Δ</i>
<i>Scbre2Δ</i>	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 bre2Δ::KanMX</i>	Open Biosystems	<i>Scbre2Δ</i>
SDBY1420	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 set1Δ::HphMX</i>	Zhang et al. Yeast 2017	<i>Scset1Δ</i>
SDBY1600	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 HHT1::K4R</i>	This study	<i>ScH3K4R1</i>
SDBY1601	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0ura3Δ0 HHT1::K4R HHT2::K4R</i>	This study	<i>ScH3K4R2</i>
<i>Scpdr5Δ</i>	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 pdr5Δ::KanMX</i>	Open Biosystems	<i>Scpdr5Δ</i>
ATCC 2001 CgWT	<i>C. glabrata</i> wild type strain	www.atcc.org	CgWT
SDBY1602	<i>set1Δ::HphMX</i>	This study	<i>Cgset1Δ</i>
SDBY1603	<i>swd1Δ::HphMX</i>	This study	<i>Cgswd1Δ</i>
SDBY1604	<i>spp1Δ::HphMX</i>	This study	<i>Cgspp1Δ</i>
SDBY1605	<i>bre2Δ::HphMX</i>	This study	<i>Cgbre2Δ</i>
SDBY1606	<i>pdr1Δ::NatMX</i>	This study	<i>Cgpdr1Δ</i>
ATCC 200989 CgWT	<i>his3Δ trp1Δ ura3Δ</i>	www.atcc.org	CgWT
SDBY1607	<i>his3Δ trp1Δ ura3Δ set1Δ::HphMX</i> <i>C. glabrata</i> isolate fluconazole susceptible	This study	<i>Cgset1Δ</i>
SM1	<i>C. glabrata</i> isolate fluconazole resistant	Magill et al. JCM	SM1
SM3	<i>C. glabrata</i> isolate fluconazole resistant	Magill et al. JCM Caudle et al. Euk. Cell	SM3
<i>SM1pdr1Δ</i>	<i>pdr1::FRT</i>		<i>SM1pdr1Δ</i>
SDBY1608	<i>set1Δ::HphMX</i>	This study	<i>SM1set1Δ</i>
SDBY1609	<i>set1Δ::HphMX</i>	This study	<i>SM3set1Δ</i>
SDBY1610	<i>set1Δ::HphMX pdr1::FRT</i>	This study	<i>SM1set1Δpdr1Δ</i>

Table S2. Plasmids				
Plasmid	Inserted Gene	Promoter	Vector	Source
pGRB2.0	None		pGRB2.0	Zordan et al.
pGRB2.0	<i>SET1</i>	<i>SET1</i>	pGRB2.0	Zordan et al.
pGRB2.0	<i>SET1/H1048K</i>	<i>SET1</i>	pGRB2.0	Zordan et al.
pGRB2.0	<i>3XFLAGSET1</i>	<i>SET1</i>	pGRB2.0	Zordan et al.
pGRB2.0	<i>3XFLAGSET1/H1048K</i>	<i>SET1</i>	pGRB2.0	Zordan et al.

Table S3. Primers for qRT-PCR	
Primer Name	Sequence
<i>CgRDN18-001F</i>	ACGGAGCCAGCGAGTCTAAC
<i>CgRDN18-002R</i>	CGACGGAGTTTCACAAGATTACC
<i>CgERG3-001F</i>	TGGGAGCACCACGGTCTAAG
<i>CgERG3-002R</i>	CAGTCGGTGAAGAAGATGAAAGTG
<i>CgERG11-001F</i>	GGGTCCAAAGGGTCACGAA
<i>CgERG11-002R</i>	GCAGCTTCAGCGGAAACATC
<i>CgCDR1-001F</i>	GTCTATGGAAGGTGCCGTC
<i>CgCDR1-002R</i>	TGAACCAGGTCTACCTAGCACAAC
<i>CgPDR1-001F</i>	TCGGCGAGGGTAAATTCAAC
<i>CgPDR1-002R</i>	CAACTGCGTTTGATTCTTAAGC
<i>CgSET1-001F</i>	CCAACCAAAGCCGATACTCATC
<i>CgSET1-002R</i>	GCGTTGACTACCGCGAGATT
<i>CgYOR1-001F</i>	GTACAGAGTGTGCCGGAACAAG
<i>CgYOR1-002R</i>	TGCGTCACCTTCTCTATGTCGTT
<i>CgPDH1-001F</i>	GAGTCCGCCGATGTGTCAT
<i>CgPDH1-002R</i>	CAGCTTCACGGGCAGGTT
<i>CgSNQ2-001F</i>	CCCCGGCGGATAAACTAAC
<i>CgSNQ2-002R</i>	CTGGCTCGCAGTGTCTCTTG
<i>ScRDN18-001F</i>	TGGTGCATGGCCGTTCTTA
<i>ScRDN18-002R</i>	GGTCTCGTTTCGTTATCGCAATT
<i>ScERG11-001F</i>	CTACCTACAAGGATGGTGTGAAG
<i>ScERG11-002R</i>	CAGAAGTGGCAGCAGAAGTAT
<i>ScPDR5-001F</i>	ATTCACCAACCCTCTGCTATTT
<i>ScPDR5-002R</i>	TTACAACCTTCGCCCAAGTC
<i>ScPDR1-001F</i>	CGGGCACTTCATGCTTACTA
<i>ScPDR1-002R</i>	CTCCGGTAGTGAGTTGTTTCTC

Table S4. Probe sets for ChIP Analysis	
Probe Name	Sequence 5'-3'
<i>ERG11 Promoter</i>	/56-FAM/CCTTGTTCC/ZEN/AACTACAATCGAGTGAGCT/3IABkFQ/ CGAATACGAGGCCATTTGTAAAC CTGTGCTCCCATCTCACTATAAC
<i>ERG11 5'</i>	/56-FAM/TCGTACTTC/ZEN/CAAGCTCTGCCATTGG/3IABkFQ/ GAGTACGTGAAGCTTGGTCTT

TGGCAAGGCGACCATAATAG
 ERG11 3' /56-FAM/CGGCATGAC/ZEN/TTAAGCTGGTTGTTTCG/3IABkFQ/
 ACGGGATATACGCTGATTCATT
 AGCAGCAAAGCCCTCTAAA
 ERG3 Promoter /56-FAM/AGCGAGAGC/ZEN/TGCTAGAGCTGAGAA/3IABkFQ/
 GAGACTATACGAGTGTGCTCTTTG
 TCTTCTTCCAGGCCTCATCT
 ERG3 5' /56-FAM/TCGACGACT/ZEN/CGTTGGTCAATGCTT/3IABkFQ/
 CGACGATGTGTATGCCAAGA
 ATGCAGCAGCGTAGAGTTAG
 ERG3 3' /56-FAM/CCAAGAGGT/ZEN/GGAAGGTGACGACAC/3IABkFQ/
 AGAAACCGCCGCTTACAT
 CCGGTGTTTCTGTCTAGTT

Table S5.

qRT-PCR Values

Figure 4A: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev	N	P-Value
<i>ScPDR5</i>	ScWT	Untreated	1.027	0.28	4	n.s.
<i>ScPDR5</i>	ScWT	(+) fluconazole	1.16	0.30	4	n.s.
<i>ScPDR5</i>	Scset1Δ	Untreated	0.45	0.08	4	<0.01
<i>ScPDR5</i>	Scset1Δ	(+) fluconazole	0.515	0.17	4	<0.01

Figure 4B: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev	N	P-Value
<i>ScPDR1</i>	ScWT	Untreated	1.13	0.58	3	n.s.
<i>ScPDR1</i>	ScWT	(+) fluconazole	1.39	0.1	3	n.s.
<i>ScPDR1</i>	Scset1Δ	Untreated	1.17	0.57	3	n.s.
<i>ScPDR1</i>	Scset1Δ	(+) fluconazole	1.73	0.37	3	n.s.

Figure 4D: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev	N	P-Value
<i>CgCDR1</i>	CgWT	Untreated	1	0.75	7	n.s.
<i>CgCDR1</i>	CgWT	(+) fluconazole	4.51	1.07	7	n.s.
<i>CgCDR1</i>	Cgset1Δ	Untreated	1.1	0.97	7	n.s.
<i>CgCDR1</i>	Cgset1Δ	(+) fluconazole	4.04	0.61	7	n.s.

Figure 4E: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev	N	P-Value
<i>CgPDR1</i>	CgWT	Untreated	1	0.26	3	n.s.
<i>CgPDR1</i>	CgWT	(+) fluconazole	2.29	0.20	3	n.s.
<i>CgPDR1</i>	Cgset1Δ	Untreated	1.02	0.35	3	n.s.
<i>CgPDR1</i>	Cgset1Δ	(+) fluconazole	2.29	0.25	3	n.s.

Figure 5E: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev	N	P-Value
<i>CgERG11</i>	CgWT	Untreated	1	0.32	3	n.s.
<i>CgERG11</i>	CgWT	(+) fluconazole	10.29	0.23	3	n.s.
<i>CgERG11</i>	Cgset1Δ	Untreated	1.32	0.65	3	n.s.
<i>CgERG11</i>	Cgset1Δ	(+) fluconazole	3.83	0.26	3	<0.0001

Figure 5F: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev		P-Value
<i>CgERG3</i>	CgWT	Untreated	1	0.1	3	n.s.
<i>CgERG3</i>	CgWT	(+) fluconazole	3.63	0.69	3	n.s.
<i>CgERG3</i>	<i>Cgset1Δ</i>	Untreated	1.14	0.05	3	n.s.
<i>CgERG3</i>	<i>Cgset1Δ</i>	(+) fluconazole	0.9	0.18	3	<0.0001

Figure 5G: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev		P-Value
<i>ScERG11</i>	ScWT	Untreated	1.022	0.12	4	n.s.
<i>ScERG11</i>	ScWT	(+) fluconazole	3.649	0.85	4	n.s.
<i>ScERG11</i>	<i>Scset1Δ</i>	Untreated	0.696	0.11	4	<0.001
<i>ScERG11</i>	<i>Scset1Δ</i>	(+) fluconazole	3.151	1.17	4	n.s.

Figure 6A: ChIP qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev		P-Value
<i>CgERG11</i>	CgWT promoter	Untreated	11.24	5.57	3	n.s.
<i>CgERG11</i>	CgWT promoter	(+) fluconazole	22.138	12.01	3	n.s.
<i>CgERG11</i>	CgWT 5'	Untreated	62.531	1.45	3	n.s.
<i>CgERG11</i>	CgWT 5'	(+) fluconazole	133.274	20.39	3	<0.001
<i>CgERG11</i>	CgWT 3'	Untreated	11.412	3.19	3	n.s.
<i>CgERG11</i>	CgWT 3'	(+) fluconazole	26.019	7.85	3	<0.05

Figure 6B: ChIP qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev		P-Value
<i>CgERG3</i>	CgWT	Untreated	35.768	18.27	3	n.s.
<i>CgERG3</i>	CgWT	(+) fluconazole	57.996	35.32	3	n.s.
<i>CgERG3</i>	CgWT 5'	Untreated	145.877	42.57	3	n.s.
<i>CgERG3</i>	CgWT 5'	(+) fluconazole	281.392	69.12	3	<0.05
<i>CgERG3</i>	CgWT 3'	Untreated	50.262	13.74	3	n.s.
<i>CgERG3</i>	CgWT 3'	(+) fluconazole	133.95	59.06	3	n.s.

Figure 6C: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev		P-Value
<i>CgERG11</i>	CgWT+V	Untreated	0.951	0.38	4	n.s.
<i>CgERG11</i>	CgWT+V	(+) fluconazole	6.791	1.99	4	n.s.
<i>CgERG11</i>	<i>Cgset1Δ</i> +V	Untreated	1.342	0.55	4	n.s.
<i>CgERG11</i>	<i>Cgset1Δ</i> +V	(+) fluconazole	2.603	1.04	4	<0.01
<i>CgERG11</i>	<i>Cgset1Δ/SET1</i>	Untreated	1.227	0.63	4	n.s.
<i>CgERG11</i>	<i>Cgset1Δ/SET1</i>	(+) fluconazole	6.438	2.78	4	n.s.
<i>CgERG11</i>	<i>Cgset1Δ/H1048K</i>	Untreated	1.346	0.43	4	n.s.
<i>CgERG11</i>	<i>Cgset1Δ/H1048K</i>	(+) fluconazole	3.370	0.87	4	<0.05

Figure 6D: qRT-PCR

Gene	Strain	Condition	Mean RQ	Standard Deviation		P-Value
<i>CgERG3</i>	CgWT+V	Untreated	1.266	1.14	4	n.s.
<i>CgERG3</i>	CgWT+V	(+) fluconazole	5.686	2.14	4	n.s.
<i>CgERG3</i>	<i>Cgset1Δ</i> +V	Untreated	0.814	0.35	4	n.s.
<i>CgERG3</i>	<i>Cgset1Δ</i> +V	(+) fluconazole	1.512	0.43	4	n.s.
<i>CgERG3</i>	<i>Cgset1Δ/SET1</i>	Untreated	1.034	0.64	4	n.s.
<i>CgERG3</i>	<i>Cgset1Δ/SET1</i>	(+) fluconazole	7.518	4.23	4	<0.001
<i>CgERG3</i>	<i>Cgset1Δ/H1048K</i>	Untreated	0.888	0.21	4	n.s.

<i>CgERG3</i>	<i>Cgset1Δ/H1048K</i>	(+) fluconazole	2.357	0.54	4	<0.05
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Figure 8E: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev		P-Value
<i>CgERG11</i>	SM1 <i>CgWT</i>	Untreated	1.15	0.72	3	n.s.
<i>CgERG11</i>	SM1 <i>CgWT</i>	(+) fluconazole	19.69	6.49	3	n.s.
<i>CgERG11</i>	SM1 <i>Cgset1Δ</i>	Untreated	1.04	0.89	3	n.s.
<i>CgERG11</i>	SM1 <i>Cgset1Δ</i>	(+) fluconazole	6.3	3.80	3	<0.05

Figure 8F: qRT-PCR

Gene	Strain	Condition	Mean RQ	St Dev		P-Value
<i>CgERG11</i>	SM3 <i>CgWT</i>	Untreated	1.51	1.66	5	n.s.
<i>CgERG11</i>	SM3 <i>CgWT</i>	(+) fluconazole	6.15	1.63	5	n.s.
<i>CgERG11</i>	SM3 <i>Cgset1Δ</i>	Untreated	1.54	0.76	5	n.s.
<i>CgERG11</i>	SM3 <i>Cgset1Δ</i>	(+) fluconazole	2.87	0.42	5	<0.01