SUPPORTING INFORMATION

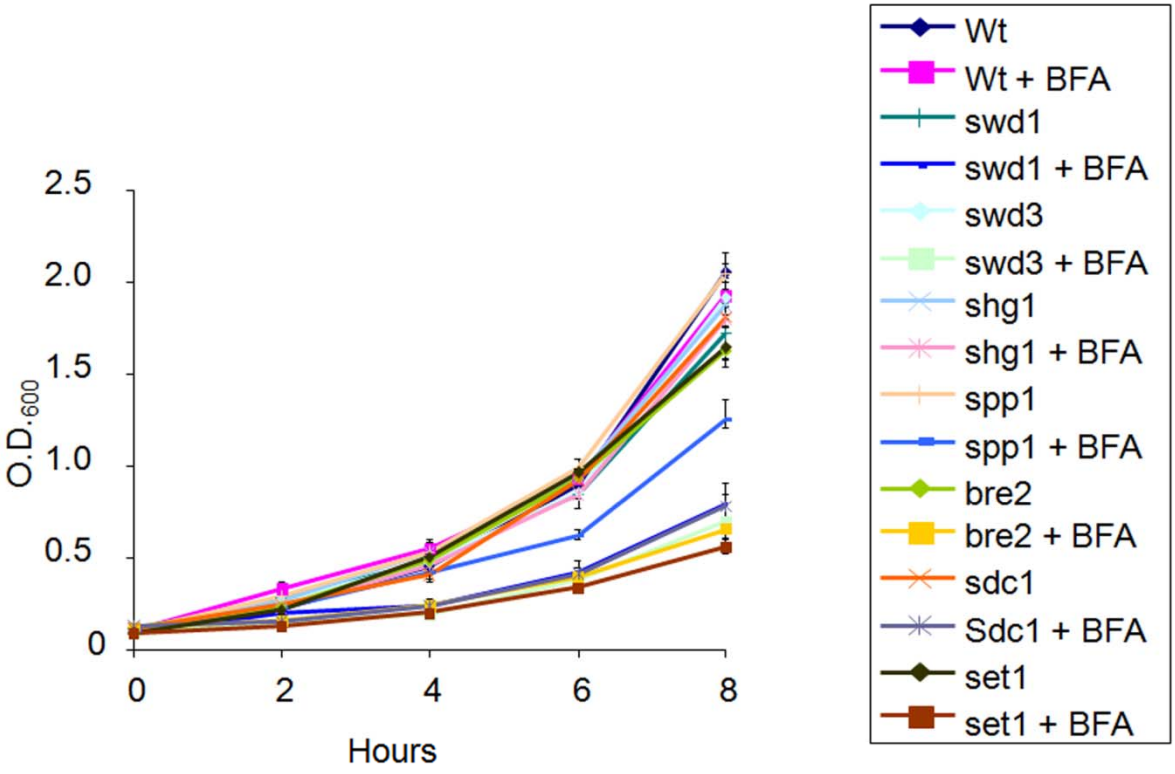

Figure S1. Set1 complex subunits are necessary for resistance to Brefeldin A. Growth curve analysis over 8 hours of strains containing deletion cassettes at the Set1 complex subunit loci. Strains were either treated with100 µg/ml Brefeldin A dissolved in ethanol or in an equal volume of ethanol.

SUPPORTING INFORMATION

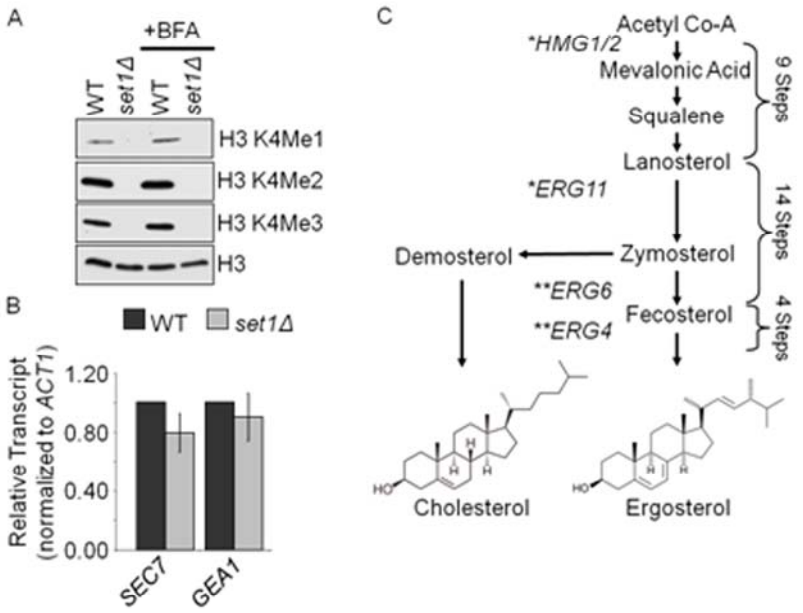

Figure S2. Brefeldin A does not affect H3K4 methylation. A. Western blot analysis of WT and *set1Δ* strains in the presence of Brefeldin A. H3K4 methylation was determined using antibodies specific to mono-, di-, and trimethylation of H3K4. H3 antibody was used as a loading control. C. Schematic of the ergosterol and cholesterol biosynthetic pathway with indicated names of conserved and yeast specific genes tested for gene expression analysis. Single asterisks indicate genes conserved between the ergosterol and cholesterol pathways. Double asterisks indicate ergosterol specific genes. B. Expression of *SEC7* and *GEA1* was determined in the *set1Δ* strain by qRT-PCR analysis. All expression analysis was relative to wild-type (WT) strain using Actin (*ACT1*) as an internal control to normalize expression levels. Data were analyzed from a minimum of three biological repeats with three technical repeats each.

SUPPORTING INFORMATION

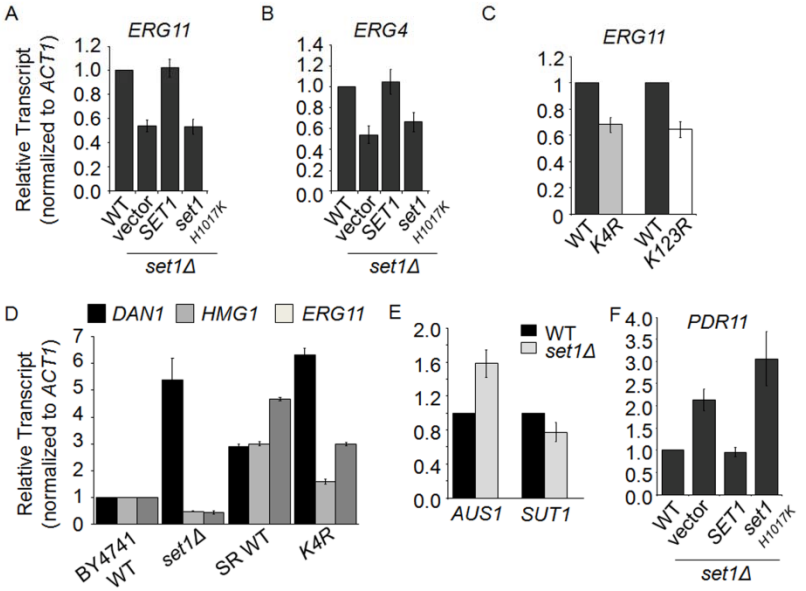

Figure S3. H3K4 methylation and Set1 methyltransferase activity are necessary for proper gene expression. A-F. Relative transcript levels of *DAN1, ERG11, ERG4, AUS1, SUT1* and *HMG1* were determined in the indicated strains by qRT-PCR analysis. All expression analysis was relative to wild-type (WT) strain using Actin (*ACT1*) as an internal control to normalize expression levels. Data were analyzed from three biological repeats with three technical repeats. Each error bar represents the standard error of the mean. Statistical significance of gene expression analysis is indicated in Tables S6-S11.

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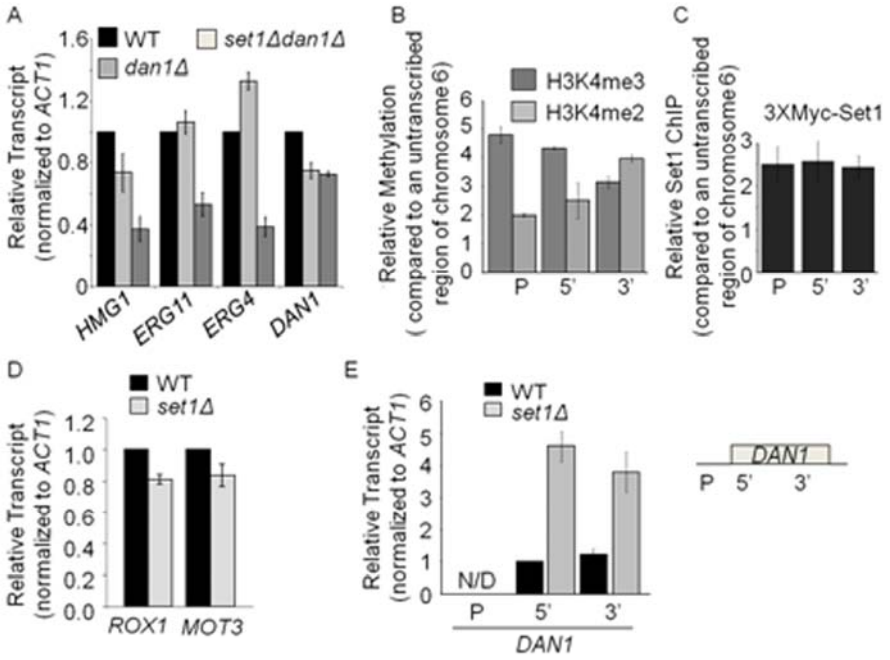

Figure S4. Set1 mediated repression of *DAN1* is not through reduced expression of *DAN1* repressors. A. Relative transcript levels of *DAN1, ERG11, ERG4,* and *HMG1* were determined in the indicated strains by qRT-PCR analysis. All expression analysis was relative to a wild-type (WT) strain using Actin (*ACT1*) as an internal control to normalize expression levels. Data were analyzed from three biological repeats with three technical repeats. Each error bar represents the standard error of the mean. Statistical significance of gene expression analysis is indicated in Tables S6-S11. B-C. ChIP analysis from the indicated strains were performed using antibodies specific to a 3XMYC-tag for detection of Set1, histone H3K4 di- and trimethylation, and histone H3. ChIP analysis for H3K4 di- and trimethylation was normalized to input and histone H3 and relative to a ChIP from an untranscribed region of chromosome 6. ChIP analysis for 3XMYC-Set1 was normalized to input and relative to WT untagged strain and relative to a ChIP from an untranscribed region of chromosome 6. All ChIP analysis was performed using three biological repeats with three technical replicates; error bars represent the standard error of the mean. D. Relative transcript levels of *ROX1* and *MOT3* were determined in the indicated strains by qRT-PCR analysis. E. Gene expression analysis using probes specific to the promoter, 5' and 3' regions of *DAN1* in WT and *set1Δ* strains. All expression analysis was relative to WT strain using Actin (*ACT1*) as an internal control to normalize expression levels. Data were analyzed from a minimum of three biological repeats with three technical repeats each.

SUPPORTING INFORMATION

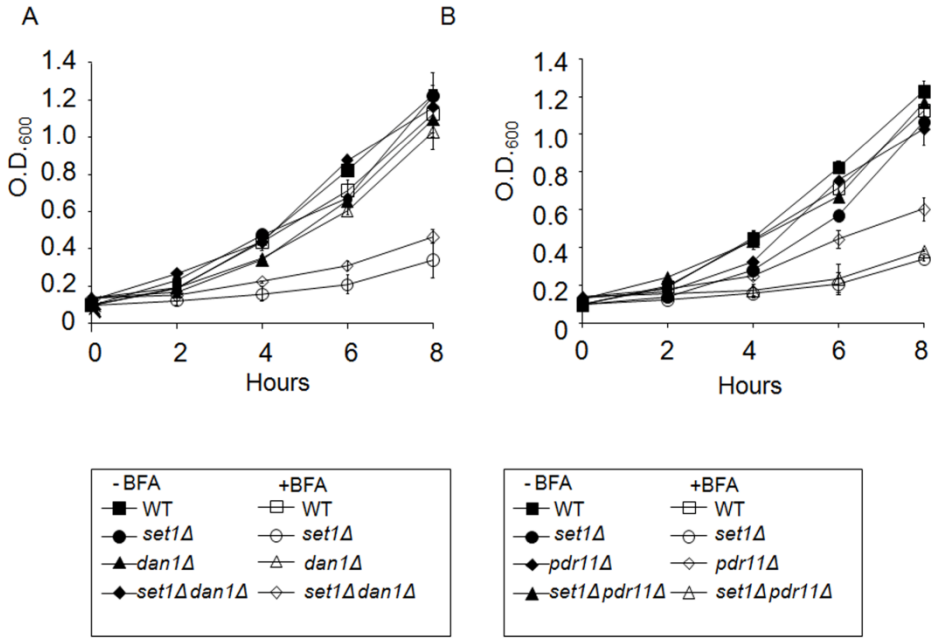

Figure S5. Set1 dependent growth in the presence of BFA is not dependent on ergosterol uptake genes in the absence of exogenous ergosterol. A-B, Growth curve analysis over 8 hours of the indicated strains. Strains were either treated with 100 µg/ml Brefeldin A dissolved in ethanol or in an equal volume of ethanol in the absence of exogenous ergosterol. Data were combined from three biological repeats. Error bars are calculated standard error of the mean.

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Table S1. Strains

Yeast strain	Genotype	Reference
BY4741	*MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0*	Open Biosystems
bre2Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 bre2Δ::KanMX*	Open Biosystems
sdc1Δ	BY4741*: MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 sdc1Δ::KanMX*	Open Biosystems
spp1Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 spp1Δ::KanMX*	Open Biosystems
swd1Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 swd1Δ::KanMX*	Open Biosystems
swd3Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 swd3Δ::KanMX*	Open Biosystems
shg1Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 shg1Δ::KanMX*	Open Biosystems
erg4Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 erg4Δ::KanMX*	Open Biosystems
bre1Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 bre1Δ::KanMX*	Open Biosystems
set2Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 set2Δ::KanMX*	Open Biosystems
dot1Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 dot1Δ::KanMX*	Open Biosystems
dan1Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 dan1Δ::KanMX*	Open Biosystems
pdr11Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 prd11Δ::KanMX*	Open Biosystems
set3Δ	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 set3Δ::KanMX*	Open Biosystems
SDBY1210	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 set1Δ::HygMX*	This study
SDBY1215	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 dan1Δ::KanMX set1Δ::HygMX*	This study
SDBY1216	BY4741: *MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 pdr11Δ::KanMX set1Δ::HygMX*	This study
SDBY1217	*MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 HMG1 3xFLAG::KanMX*	This study
SDBY1218	*MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 HMG1 3xFLAG::KanMX set1Δ::HygMX*	This study
SDBY1001	*MAT***a**, *ura3−52*, *lys2−801*, *ade2−101*, *trp1Δ63*, *his3Δ200*, *leu2Δ1*, *hht1-hhf1*::pWZ405-F2F9-LEU2,*hht2-hhf2*::pWZ403-F4F10-HIS3::pJH18	(1)
SDBY1004	*MAT***a**, *ura3−52*, *lys2−801*, *ade2−101*, *trp1Δ63*, *his3Δ200*, *leu2Δ1*, *hht1-hhf1*::pWZ405-F2F9-LEU2,*hht2-hhf2*::pWZ403-F4F10-HIS3::pHCL54	(1)
SDBY1005	*MAT***a**, *ura3−52*, *lys2−801*, *ade2−101*, *trp1Δ63*, *his3Δ200*, *leu2Δ1*, *hht1-hhf1*::pWZ405-F2F9-LEU2,*hht2-hhf2*::pWZ403-F4F10-HIS3::pHCL55	(1)

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SDBY1006	*MAT***a**, *ura3−52*, *lys2−801*, *ade2−101*, *trp1Δ63*, *his3Δ200*, *leu2Δ1*, *hht1-hhf1*::pWZ405-F2F9-LEU2,*hht2-hhf2*::pWZ403-F4F10-HIS3::pHCL56	(1)
SDBY1211	as SDBY1210 with pRS415	This study
SDBY1212	as SDBY1210 with pDPM32	This study
SDBY1213	as SDBY1210 with pPFS56	This study
SDBY1214	as BY4741 with pRS415	This study
FY406	MATa (*hta1-htb1*)*Δ::LEU2* (*hta2-htb2*)*Δ::TRP1 leu2Δ1 ura3-52 lys2Δ1 lys2Δ128 his3Δ200 trp1Δ63 <psab6>*	(2)
YKG002	MATa (*hta1-htb1*)*Δ::LEU2* (*hta2-htb2*)*Δ::TRP1 leu2Δ1 ura3-52 lys2Δ1 lys2Δ128 his3Δ200 trp1Δ63 <pZS146 (K123R)>*	(2)

Table S2. Plasmids

Plasmid	Inserted Gene	Promoter	Vector	Source
pRS415	None		pRS415	
pDPM32	*3xMYC-SET1*	*SET1*	pRS415	This Study
pPFS56	*3xMYC-set1 H1017K*	*SET1*	pRS415	This Study

Table S3. Primers for qRT-PCR

Primer name	Sequence
ACT1-001F	TGGATTCCGGTGATGGTGTT
ACT1-002R	TCAAAATGGCGTGAGGTAGAGA
HMG1-001F	CACCATATTCGGCCTCTTCAA
HMG1-002R	GGCGCTCAACCAAAAATTTG
ERG4-001F	CAACTCGGTGTTCCCATGGT
ERG4-002R	AAGGCTCTGTGAATCAGGACAAC
ERG6-001F	AAGAACAACTCTGCCCAAAAGG
ERG6-002R	CGGTTCTACCATCCCAATTTCTC
ERG11-001F	CACGAATTTGTCTTCAACGCTAA
ERG11-002R	AGTCAAATGAGCGTAAGCAGCTT
HMG2-001F	ACATGAGGAAAATCGGATCAAAG
HMG2-002R	GCGCATGCAGAGTTTGAA
DAN1-001F	GGCGATTTCACCACCATGTT
DAN1-002R	CCGGTGATCATTCTGGTCACT
AUS1-001F	TGAGGACAGCATGCCGAAT
AUS1-002R	GCCTCTGCTTGGAAGGTTATGT
PDR11-001F	CGGTGGAGCAAACCATTGAT
PDR11-002R	ACGCTCTTGCTTGGGAATGT
SUT1-001F	GCACGCTGTTGCGGAATAT
SUT1-002R	GCGTGAGCCTGTACCCTCAA
SEC7-001F	GGCTATCAGAACAATCTACAACGTTT

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SEC7-002R	AGGTCGCCTGTGCAATACCT
GEA1-001F	CAGTCGAGGATGGGTGTGAAG
GEA1-002R	TGGAACGACTAGAAAGATCAATAATAGAA
ROX1-001F	CGACCCTTCAACGAGACATCA
ROX1-002R	TCGAGTTAGCCCTTGGTTGAG
MOT3-001F	TGGCGTCACAGCCTTTCAA
MOT3-002R	TTCACCATAACGTCGTCCTTGT

Table S4. Probe sets for ChIP analysis

Primer name	Sequence 5'-3'
HMG1 promoter	/56-FAM/AGTGCGAAG/ZEN/AAAACGTAGTGCGAGT/3IABkFQ/
	GAAGAGTTGGAAGACCTCAGC
	AGATCCTATAGCTAGTACGGAC
HMG1 5'	/56-FAM/TGAATGACG/ZEN/GATAGATAAGCGAATGCGG/3IABkFQ/
	CGGCGAAACGACCAATTC
	GTGTTGGAGTCTTTATTTGGAGC
HMG1 3'	/56-FAM/AGGTTTTGT/ZEN/TGGTTCAGCAGGTTTCC/3IABkFQ/
	CGGCCATTTGGTTCAAAGTC
	ACGATTTATATCAGTGGCGTCC
DAN1 promoter	/56-FAM/AGATCTTGG/ZEN/CATTTTCAAAGGCACGC/3IABkFQ/
	GTTTCATGTTTCCTGCGCG
	GGACCCTTTTGCATTTCTAGC
DAN1 5'	/56-FAM/AAATCGCCA/ZEN/CCGGCAAAAACAGC/3IABkFQ/
	CACCTGAAATACCAGTCAACATG
	AAGACTGAACATACCCACCTG
DAN1 3'	/56-FAM/AGCCAGTCT/ZEN/CCTCCAAAGCTCAATC/3IABkFQ/
	AGACTCCAAGCTTCCACTGTTG
	TGACGATGTGACAGAAGTAGC
ERG6 promoter	/56-FAM/ACCTCCAAT/ZEN/ACTTGCTGTTGCCGA/3IABkFQ/
	TGCTCCACTTCGTCTCAATG
	GGATAGGAGAAAACACCCGAAC
ERG6 5'	/56-FAM/TCTGAACGG/ZEN/CTTCCTTTTGGGCA/3IABkFQ/
	GGTTTGAGTGCATTGATGTCG
	TCGGCATCTTTATCGGTTCTAC
ERG6 3'	/56-FAM/AGGAAGCCA/ZEN/GAAAACGCCGAAAC/3IABkFQ/
	TCGGCATCTTTATCGGTTCTAC
	TTCTTGGGAAGTTTGGGAGG
ERG11 promoter	/56-FAM/TCGTTAACT/ZEN/CGTGGAGATGCACAATAGG/3IABkFQ/
	GCAGGAGACATCGATTTTATGC
	GAGGCTTTTCGAATACATGCG
ERG11 5'	/56-FAM/TGGGCCAAT/ZEN/GGTAAAGCCAAGAAATG/3IABkFQ/

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	TTGGAGAGGCATTGGAATACG
	TCCTTTCTCAAAGAATATAGTAATTGCC
ERG11 3'	/56-FAM/ACCGTTCCA/ZEN/CCTCCTGACTTTACATCT/3IABkFQ/
	AAATGGCATTACCCAGAGGG
	TTTGTTCTGGATTTCTCTTTTCCC
Untranscribed	/56-FAM/TGCCATGTA/ZEN/TGCAACTGCTCTACCA/3IABkFQ/
region of	ACACCCGTCTTTGATGCTAG
chromosome 6	ATGTTCTCACGCTCTGTTCTAG

Table S5. Doubling time over 8 hours at 30° C

Strain	-BFA	+BFA
BY4741 WT	1.93	1.93
bre2Δ	2.12	3.24
bre1Δ	2.00	4.55
erg6Δ	1.86	5.83
set1Δ	2.07	3.54
spp1Δ	1.91	2.22
swd1Δ	1.94	2.67
swd3Δ	1.93	2.91
shg1Δ	2.00	2.02
erg4Δ	1.94	5.56
sdc1Δ	2.02	3.08
set2Δ	2.16	2.25
dot1Δ	1.99	2.03
dan1Δ	2.45	2.93
pdr11Δ	2.56	3.61
set3Δ	3.03	5.16
set1Δdan1Δ	2.50	3.93
set1Δpdr11Δ	2.39	4.23
WT + ergosterol	1.91	1.83
set1Δ + ergosterol	1.82	1.74
dan1Δ + ergosterol	2.66	2.92
pdr11Δ + ergosterol	2.72	3.78
set1Δdan1Δ + ergosterol	2.80	3.50
set1Δpdr11Δ + ergosterol	2.51	5.41
WZY42 WT	2.49	2.63
K4R	2.74	4.88
K36R	2.43	2.51
K79R	2.46	2.60
FY406 WT	2.20	2.28
YKG002 K123R	2.32	3.16

Table S6. qRT-PCR analysis of *set1Δ* strain relative to WT

Gene name	RQ	Fold change relative to WT	P<.001
HMG1	0.46	-2.17 +/- 0.06	P<.001
HMG2	0.33	-3.03 +/- 0.06	P<.001
ERG6	0.84	-1.19 +/- 0.12	0.042
ERG11	0.43	-2.32 +/- 0.05	P<.001
ERG5	0.42	-2.38 +/- 0.03	P<.001
ERG4	0.39	-2.56 +/- 0.07	P<.001
DAN1	5.59	5.59 +/- 0.81	P<.001
AUS1	1.58	1.58 +/- 0.16	0.003
PDR11	2.45	2.45 +/- 0.34	P<.001
SUT1	0.78	-1.28 +/- 0.11	0.036
SEC7	0.79	-1.26 +/- 0.13	.059
GEA1	0.90	-1.11 +/- 0.16	.282
ROX1	0.81	-1.23 +/- 0.04	.002
MOT3	0.83	-1.20 +/- 0.09	.077

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Table S7. qRT-PCR analysis of Wt, *set1Δ, set1Δ* +Set1, *set1Δ*+ Set1 H1017K

Strain	Gene name	RQ	Fold change relative to WT	P<.001
WT +pRS415	*HMG1*	1	0	
set1Δ +pRS415	*HMG1*	0.55	-1.81 +/- 0.08	P<.001
set1Δ +Set1	*HMG1*	0.88	-1.13 +/- 0.18	0.804
set1Δ +Set1 H1017K	*HMG1*	0.55	-1.81 +/- 0.13	P<.01
WT +pRS415	*ERG11*	1	0	
set1Δ +pRS415	*ERG11*	0.54	-1.85 +/- 0.04	P<.001
set1Δ +Set1	*ERG11*	1.02	1.02 +/- 0.07	0.373
set1Δ +*Set1* H1017K	*ERG11*	0.53	-1.89 +/- 0.06	P<.001
WT +pRS415	*ERG4*	1	0	
set1Δ +pRS415	*ERG4*	0.54	-1.85 +/- 0.08	P<.001
set1Δ +Set1	*ERG4*	1.04	1.04 +/- 0.18	0.707
set1Δ +Set1 H1017K	*ERG4*	0.66	-1.51 +/- 0.13	P<.001
WT +pRS415	*DAN1*	1	0	
set1Δ +pRS415	*DAN1*	4.44	4.44 +/- 0.89	P<.001
set1Δ +Set1	*DAN1*	1.17	1.17 +/- 0.05	0.019
set1Δ +Set1 H1017K	*DAN1*	5.33	5.33 +/- 1.01	P<.001
WT +pRS415	*PDR11*	1	0	
set1Δ +pRS415	*PDR11*	2.14	2.14 +/- 0.24	P<.001
set1Δ +Set1	*PDR11*	0.95	-1.05 +/- 0.11	0.678
set1Δ +Set1 H1017K	*PDR11*	3.05	3.05 +/- 0.60	P<.001

Table S8. qRT-PCR analysis of *set3Δ* strain relative to WT

Gene name	RQ	Fold change relative to WT	P<.001
HMG1	0.48	-2.08 +/- 0.16	P<.001
ERG11	0.41	-2.43 +/- 0.08	P<.001
DAN1	1.34	1.34 +/- 0.46	0.52

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Table S9. qRT-PCR analysis of *bre1Δ* strain relative to WT

Gene name	RQ	Fold change relative to WT	P<.001
HMG1	0.35	-2.86 +/- 0.10	P<.001
ERG11	0.26	-3.85 +/- 0.07	P<.001
DAN1	4.03	4.03 +/- 1.25	P<.001

Table S1.0 qRT-PCR analysis of *dan1Δ* and *set1Δdan1Δ* strain relative to WT

strain	Gene name	RQ	Fold change relative to WT	P<.001
dan1Δ	*HMG1*	0.73	-1.36 +/- 0.02	P<.001
	ERG11	1.06	-1.06 +/- 0.25	0.67
	ERG4	1.33	1.33+/- 0.13	0.03
set1Δdan1Δ	*HMG1*	0.37	-2.70 +/- 0.02	P<.001
	ERG11	0.52	-1.92 +/- 0.25	P<.001
	ER G4	0.38	-2.63 +/- 0.13	P<.001

Table S11.
qRT-PCR analysis of *K4R* and *K123R* strains relative to their respective WT strain

Strain	Gene name	RQ	Fold change relative to WT	P<.001
K4R	*HMG1*	0.52	-1.91 +/- 0.06	P<.001
	ERG11	0.64	-1.56 +/- 0.06	P<.001
	DAN1	2.19	2.19+/- 0.10	P<.001
K123R	*HMG1*	0.40	-2.5 +/- 0.09	P<.001
	ERG11	0.68	-1.47 +/- 0.05	P<.001
	DAN1	2.51	2.51 +/- 0.11	P<.001

Supporting References

1. Fingerman IM, Li HC, & Briggs SD (2007) A charge-based interaction between histone H4 and Dot1 is required for H3K79 methylation and telomere silencing: identification of a new trans-histone pathway. *Genes & development* 21(16):2018-2029.

2. Gardner KE, Zhou L, Parra MA, Chen X, & Strahl BD (2011) Identification of lysine 37 of histone H2B as a novel site of methylation. *PLoS.One.* 6(1):e16244.