SUPPLEMENTARY MATERIAL

Supplementary Tables

Table 1: Yeast strains used in this study

Strain Name		Genotype
W303-1A	W303-1A	*MATa ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100*
	rsc9^{ts} (*)	*fae1-2*::*TRP1* (isogenic to W303-1A)
YEN405	*set1*	*SET1*::*KanMx4* (isogenic to W303-1A)
YGM108	*rsc9^{ts} set1*	*rsc9^{ts} SET1*::*NatMx* (isogenic to W303-1A)
YGM99	*rsc9^{ts} set2*	*rsc9^{ts} SET2*::*KanMx4* (isogenic to W303-1A)
YSL151	*H3K4A* (**)	*MATa his3Δ200 leu2Δ1 ura3-5 trp1Δ63 lys2-128δ (hht1-hhf1Δ)::LEU2 (hht2-hhf2Δ)::HIS3 TRP1-plasm (HHT2-HHF2)*
YGM208	*H3K4A rsc9ts*	*MATa his3Δ200 leu2Δ1 ura3-5 trp1Δ63 lys2-128δ (hht1-hhf1Δ)::LEU2 (hht2-hhf2Δ)::HIS3 TRP1-plasm (HHT2-HHF2) rsc9-G489STOP*::*KanMx4*
YEN354	*rsc9^{ts} swd1*	*rsc9^{ts} SWD1*::*KanMx4* (isogenic to W303-1A)
YEN372	*rsc9^{ts} bre2*	*rsc9^{ts} BRE2*::*KanMx4* (isogenic to W303-1A)
YEN363	*rsc9^{ts} sdc1*	*rsc9^{ts} SDC1*::*KanMx4* (isogenic to W303-1A)
YGM267	*rsc9^{ts} spp1*	*rsc9^{ts} SPP1*::*KanMx4* (isogenic to W303-1A)
YEN436	*rsc9^{ts} set1 isw1*	*rsc9^{ts} SET1*::*NatMx ISW1*::*KanMx4* (isogenic to W303-1A)
YEN455	*rsc9^{ts} set1 eaf7*	*rsc9^{ts} SET1*::*NatMx EAF7*::*KanMx4* (isogenic to W303-1A)
YMN164	*rsc9^{ts} set1 snf2*	*rsc9^{ts} SET1*::*NatMx SNF2*::*HPH* (isogenic to W303-1A)
YMN206	*rsc9^{ts} set1 bdf1*	*rsc9^{ts} SET1*::*NatMx BDF1*::*KanMx4* (isogenic to W303-1A)
YMN225	Bdf1-6HA	*BDF1-6HA-HIS3* (isogenic to W303-1A)
YMN226	*set1* Bdf1-6HA	*SET1*::*NatMx BDF1-6HA-HIS3* (isogenic to W303-1A)
YMN227	*rsc9^{ts}* Bdf1-6HA	*rsc9^{ts} BDF1-6HA-HIS3* (isogenic to W303-1A)
YMN228	*rsc9^{ts} set1* Bdf1-6HA	*rsc9^{ts} SET1*::*NatMx BDF1-6HA-HIS3* (isogenic to W303-1A)
YMN208	*rsc9^{ts} set1 htz1*	*rsc9^{ts} SET1*::*NatMx HTZ1*::*KanMx4* (isogenic to W303-1A)
YMN219	*rsc9^{ts} set1* pGAL-Swr1 ADGEV	*rsc9^{ts} SET1*::*NatMx KAN*::*pGAL1-SWR1 Gal4DBD-hER- VP16*::*URA3* (isogenic to W303-1A)
Euroscarf	*rsc9degron*	*MATa his3-11,15 leu2-3,112 trp1-11 ura3-1 Δlys2*::*rKWD50N, pGAL1-10-myc*::*UBR1*::*HIS3 pCUP1-degron*::*RSC9*::*URA3*
YGM185	*rsc9degron set1*	*MATa his3-11,15 leu2-3,112 trp1-11 ura3-1 Δlys2*::*rKWD50N, pGAL1-10-myc*::*UBR1*::*HIS3 pCUP1-degron*::*RSC9*::*URA3 SET1*::*kanMx4*
YAL74	*rpd3*	*RPD3*::*KanMx4* (isogenic to W303-1A)
YGM277	*rpd3 set1*	*RPD3*::*KanMx4 SET1*::*NatMx* (isogenic to W303-1A)
BY4741	wild type	*MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0*
Euroscarf	*spt20*	*SPT20*::*KanMx4* (isogenic to BY4741)
YGM61	*hog1*	*HOG1*::*KanMx4* (isogenic to BY4741)
YGM283	*spt20 set1*	*SET1*::*NatMx SPT20*::*kanMx4* (isogenic to BY4741)
YGM222	Set1-HA	*SET1-6HA-HIS3* (isogenic to BY4741)

(*) *fae1-2*::*TRP1* is a nonsense mutation at Gln489 of *RSC9*.

(**) Gift from T. Kouzarides.

Table 2: Oligonucleotides used in this study

Amplicon	Position (*)	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
Northern blot probes			
STL1	+402/+1280	GGCCAGTTTATCATCGGAAG	GCGTTTGTTGATGCACGAAC
CTT1	+1/+1722	CGGGATCCATGCCAATAAGATCAATC	GGGGATCCTTAATTGGCACTTGCAATGGAC
ACT1	-389 / +30	TGTCACCCGGCCTCTATTTT	GTGCAAGCGCTAGAACATAC
RDN18	+1/+1000	TATCTGGTTGATCCTGCCAG	GATCATCTTCGATCCCCTAA
ENO1	+1/+1310	ATGGCTGTCTCTAAAGTTTA	AATTTGTCACCGTGGTGGAA
ChIP assays			
stl1 1/2 prom	-372 / -112	TTGGTTAATCCTCGCCAGGT	TATGAGTGTGACTACTCCTG
stl1 III a/b ORF	+402 / +630	GGCCAGTTTATCATCGGAAG	GATTTGCATTGACACGGGGA
ctt1 3/4 prom	-452 / -160	AGGCACATGGGGATAGAACC	GGGAGTCGGGGGCATTATCG
ctt1 II a/b ORF	+422 / +669	GAACCATGACTGGGTCTTCA	GAAGGAATGACCAGAGTACG
tel rt a/b	269437-637	ACCACTCAAAGAGAAATTTACTGGAAGA	CTCGTTAGGATCACGTTCGAATC
tel2 a/b	269493-982	AGTGCAAGCGTAACAAAGCC	GCCTCACTGGTTTTTACCCT
GAL1-10 prom	-537 / -215	AGAGCCCCATTATCTTAGCC	CGCATTATCATCCTATGGTTG
MNase assay			
-583,5_stl1	-635/-534	ACTTTGACTCAACTTGCGTCATTT	AAATGCAGGTAGCGGAGGC
-498,5_stl1	-552/-447	GCCTCCGCTACCTGCATTT	TCCGCTTATTTTAGTTTCATGCAAC
-415_stl1	-468/-364	GCATGAAACTAAAATAAGCGGAAAG	ATTAACCAACCAAAAACGGTATATAGC
-326_stl1	-384/-271	TACCGTTTTTGGTTGGTTAATCCTCGC	GTGAACCAAAAGTGTTTCCCTTACCGG
-255_stl1	-309/-203	AAAGTGCAGATCCCGGTAAGG	TAAGCTTTCTCAATTTCTCTATCTACTTCCT
-170_stl1	-221/-121	GAGAAATTGAGAAAGCTTAAGTGAGATG	TGACTACTCCTGAACTGCAATTCTG
-96_stl1	-157/-44	GCCTTAGAGGGTCAGAATTGCAGTT	TCTAACTTGTATGCTAGAACACGTATACA
-5,5_stl1	-61/+49	TCTAGCATACAAGTTAGAATAAATAAAAAA	TTCTGCTTATAAATTTGCCTTTGAA
74_stl1	+24/+124	TTTCAAAGGCAAATTTATAAGCAGAA	ACAGGGAGAAGCCCGTCATA
158,5_stl1	+96/+219	TATCGCATCTATGACGGGCTTCTCC	TCTGTCATGATCGCCATTTTCTTTG
247_stl1	+197/+297	AAGAAAATGGCGATCATGACAGA	GAACATAACGAATAGAGAACCTGCG
330,5_stl1	+280/+381	TCTCTATTCGTTATGTTCTGCGGT	TGCGCATGTAGAAATAACGGC
405_stl1	+354/+456	CATTGGTGCCGTTATTTCTACA	TGTATTCAACCCTGTTCCAACA
487_stl1	+436/+537	GTTGGAACAGGGTTGAATACATCTAC	AATTGTGGAACCTTCTAAATTGACC
562,5_stl1	+512/+614	TGGTCAATTTAGAAGGTTCCACAA	GGGAATCTCCACTGAACAGAACTG
647,5_stl1	+594/+703	TTCTGTTCAGTGGAGATTCCCC	GACTTTGAGAAATCAGCCAACGT
739_stl1	+683/+796	GTTGGCTGATTTCTCAAAGT	CATCGTGAAGCATAGCAACT
830,5_stl1	+780/+883	TGCTATGCTTCACGATGCTGT	CAATCAAAGCCCTCTGAAGATTTT
902,5_stl1	+851/+956	GCAGGTCCCAAAATCTTCAGAG	TTGAATAATACAGTAGAGTAGTATATGGCAGC

(*) Position indicates the distance of the 5' end from the respective ATG initiation codon.

Supplementary Figure Legends

Figure S1. Specificity of *SET1* deletion effects for osmostress-responsive gene expression.

(A) Deletion of *SET1* restores gene expression in response to osmostress in an *rsc9* degron strain. Wild-type and *rsc9* degron and *rsc9* degron *set1* mutant strains were grown in YPGal at 25 ºC, shifted to 37 ºC for 2 h and then subjected to osmostress (0.4 M NaCl) for the indicated times. RNA was probed for analysis of *STL1*, *CTT1* and *ACT1* (as a loading control) expression.

(B) Sensitivity to caffeine that is associated with RSC mutants is not suppressed by deletion of *SET1*. Wild type and the indicated mutant strains were spotted on plates without and with 5 mM caffeine. Growth was scored after 4 days.

(C) The transcriptional defect of the *rsc9^{ts}* strain after heat stress is not restored by deletion of *SET1*. The indicated strains were grown in YPD at 25 ºC up to OD_{660} 0.5, were shifted to 37 ºC for 2 hours and were then subjected to heat shock at 42 ºC for the indicated times. RNA was probed for analysis of *CTT1* and *ACT1* (as a loading control) expression.

Figure S2. Role of H3K4 methylation in osmostress gene expression.

(A) Deletion of *SET1* does not suppress the transcriptional defect of *rpd3Δ* and *spt20*Δ mutant strains. The indicated strains were grown in YPD until mid-log phase and were then subjected to osmostress (0.4 M NaCl) for the indicated times. Total RNA was assayed by northern blot for *CTT1* and *ACT1* (as a loading control) expression.

(B) Cells deficient in H3K4 trimethylation express stress-genes in a similar manner to wild type. mRNA was obtained from wild type and mutant strains (*hog1*Δ*, set1*Δ and H3K4A strains) that were untreated or treated with a mild osmotic shock (0.4 M NaCl) for the indicated times. Total RNA was assayed by Northern blot for *STL1*, *CTT1* and *ACT1* (as a loading control) expression.

Figure S3. The levels of H3K4 trimethylation at the *STL1* promoter under basal conditions are similar to those observed at the *GAL10* promoter after galactose induction.

Wild type and *set1*Δ strains were grown in YP with either raffinose 2% or glucose 2%, and galactose 2% was then added to the cultures growing in raffinose. At mid-log phase samples were taken for ChIP analysis of the levels of trimethyl H3K4 (using the Abcam antibody) at the promoters of *GAL10* and *STL1*. As a control, chromatin from the wild type and *set1*Δ cells was immunoprecipitated without antibody (no Ab). PCR samples were amplified with *TEL* as a loading control (upper band). Quantification of the bands is shown at the bottom.

Figure S4. Histones are not evicted from the stress-responsive locus upon stress in an *rsc9^{ts}* mutant.

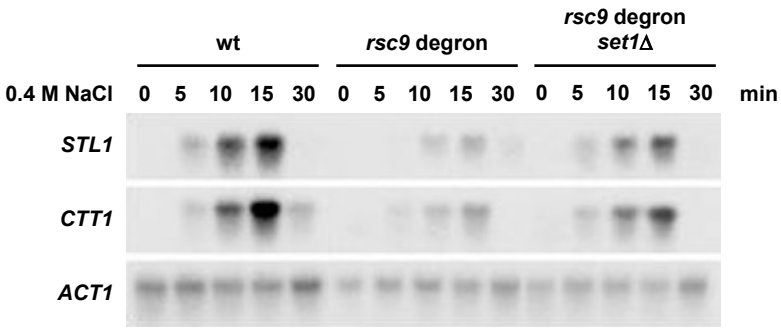
Cells were grown at 25 ºC and shifted to non-permissive temperature (37ºC) for 2 hours. Histone H3 occupancy of the *STL1* ORF in wild type and *rsc9^{ts}* mutant cells untreated or treated

with a mild osmotic shock (0.4 M NaCl) at the indicated times was analyzed using ChIP assay followed by real time PCR. Histone H3 occupancy was normalized to that of a telomere control and H3 levels of untreated samples were set to one as a reference of each strain. Data represent the mean and standard deviation of three independent experiments.

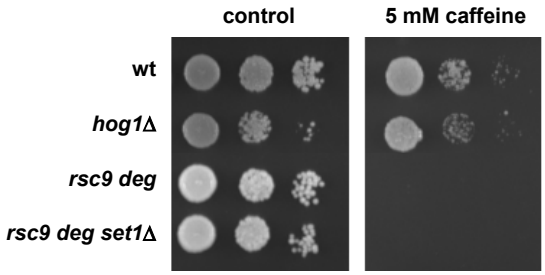
Figure S5. The deletion of *SET1* does not change Bdf1 recruitment at *STL1* in the absence of stress.

Association of Bdf1-6HA with the promoter and ORF of *STL1* was analyzed in the indicated strains in the absence of stress using ChIP assays. The real-time PCR results are shown as fold induction of untreated wild type cells relative to *set1*, *rsc9*^{ts} and *set1 rsc9*^{ts} mutant cells. Data are means and standard deviation of three independent experiments.

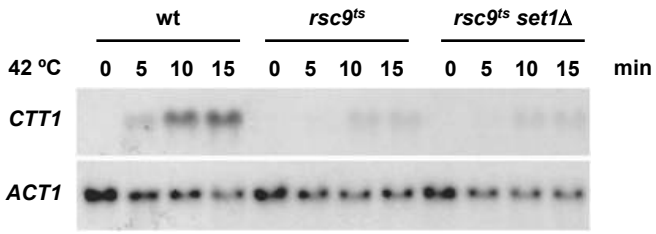
A


wt
rsc9 degron
rsc9 degron set1Δ
0.4 M NaCl
0 5 10 15 30 0 5 10 15 30 0 5 10 15 30 min
STL1
CTT1
ACT1

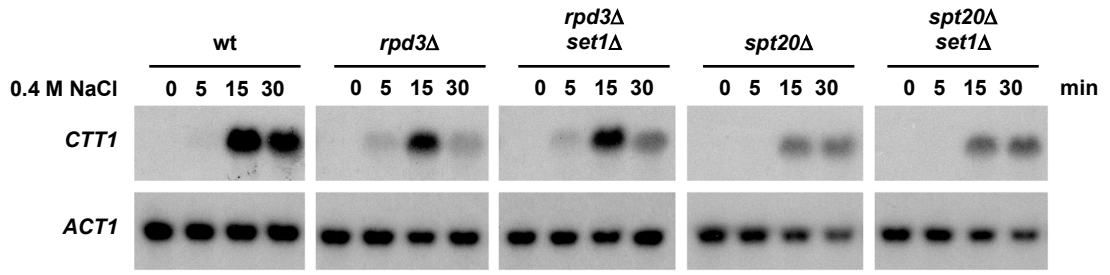
B


control
5 mM caffeine
wt
hog1Δ
rsc9 deg
rsc9 deg set1Δ

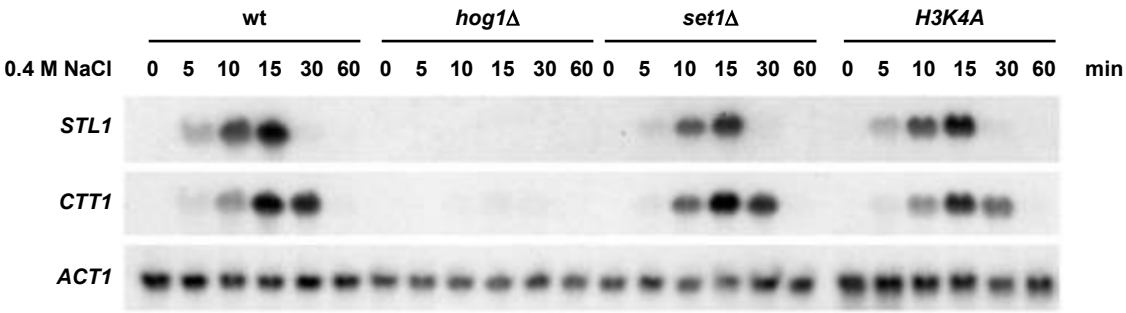
C

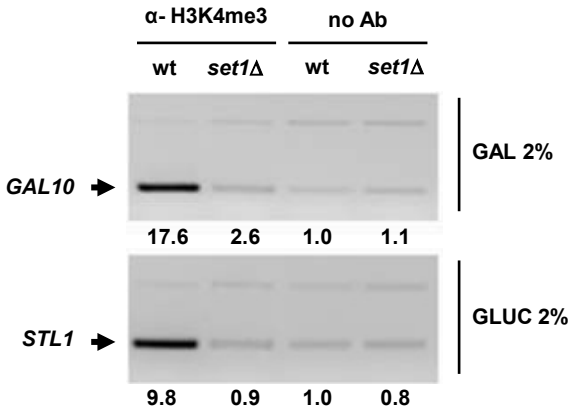

wt
rsc9ts
rsc9ts set1Δ
42 ºC
0 5 10 15 0 5 10 15 0 5 10 15 min
CTT1
ACT1

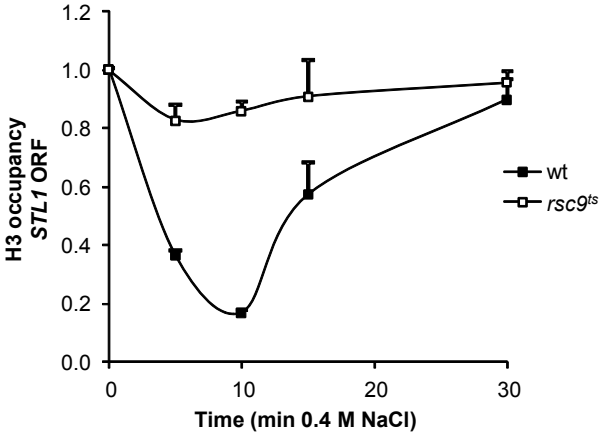
A

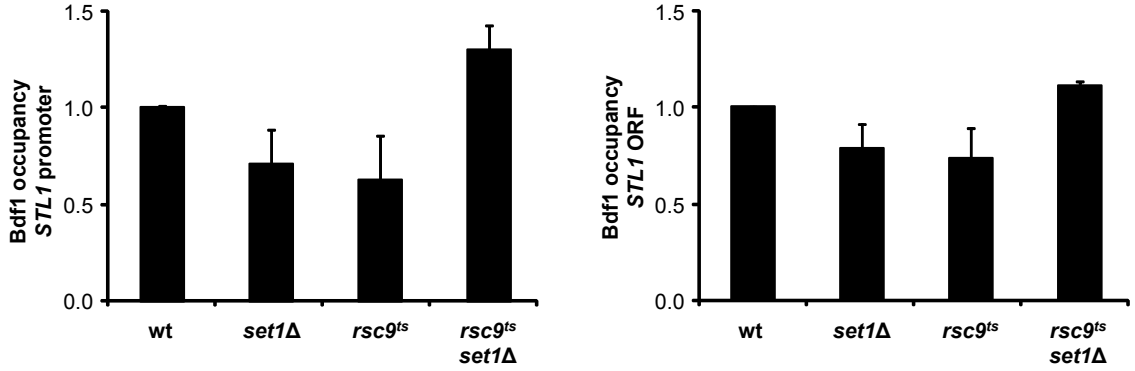

wt
rpd3Δ
rpd3Δ set1Δ
spt20Δ
spt20Δ set1Δ
0.4 M NaCl
0 5 15 30
min
CTT1
ACT1

B


wt
hog1Δ
set1Δ
H3K4A
0.4 M NaCl
0 5 10 15 30 60
min
STL1
CTT1
ACT1


α- H3K4me3
no Ab
wt
set1Δ
wt
set1Δ
GAL10
GAL 2%
17.6
2.6
1.0
1.1
STL1
GLUC 2%
9.8
0.9
1.0
0.8


1.2
1.0
0.8
0.6
0.4
0.2
0.0
H3 occupancy
STL1 ORF
0
10
20
30
Time (min 0.4 M NaCl)
wt
rsc9ts


Bdf1 occupancy
STL1 promoter
1.5
1.0
0.5
0.0
wt
set1Δ
rsc9ts
rsc9ts
set1Δ
Bdf1 occupancy
STL1 ORF
1.5
1.0
0.5
0.0
wt
set1Δ
rsc9ts
rsc9ts
set1Δ