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 ^{is} SET1::NatMx (isogenic to W303-1A)						
YGM99	rsc9 ^{ts} set2	rsc9 ^{is} 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 ^{is} SWD1::KanMx4 (isogenic to W303-1A)						
YEN372	rsc9 ^{ts} bre2	<i>rsc9^{ts} BRE2::KanMx4</i> (isogenic to W303-1A)						
YEN363	rsc9 ^{ts} sdc1	<i>rsc9^{ts}</i> SDC1::KanMx4 (isogenic to W303-1A)						
YGM267	rsc9 ^{ts} spp1	rsc9 ^{is} SPP1::KanMx4 (isogenic to W303-1A)						
YEN436	rsc9 ^{ts} set1 isw1	rsc9 ^{is} SET1::NatMx ISW1::KanMx4 (isogenic to W303-1A)						
YEN455	rsc9 ^{ts} set1 eaf7	rsc9 ^{is} 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	<i>rsc9</i> ^{ts} Bdf1-6HA	rsc9 ^{ts} BDF1-6HA-HIS3 (isogenic to W303-1A)						
YMN228	<i>rsc9^{ts} set1</i> 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	<i>rsc9^{ts} set1</i> pGAL-Swr1 ADGEV	<i>rsc9^{is} SET1::NatMx KAN::pGAL1-SWR1 Gal4DBD-hER- VP16::URA3</i> (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 GIn489 of RSC9.

(**) Gift from T. Kouzarides.

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		l							
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	ТСТАБСАТАСААБТТАБААТАААТААААА	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						

Table 2: Oligonucleotides used in this study

(*) 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 °C, shifted to 37 °C 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 °C up to OD₆₆₀ 0.5, were shifted to 37 °C for 2 hours and were then subjected to heat shock at 42 °C 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 $rpd3\Delta$ and $spt20\Delta$ 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 ($hog1\Delta$, $set1\Delta$ 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 °C and shifted to non-permissive temperature (37°C) 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.

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			wt			rsc9 degron		rsc9 degron on set1∆					ı			
0.4 M NaCl	0	5	10	15	30	0	5	10	15	30	0	5	10	15	30	min
STL1					-								+	=		
CTT1			1						1	ŧ.			1	7		
ACT1	1	-	1	t	=		*	-		1					÷	

В

Α

	control	5 mM caffeine				
wt		🔵 🍇 🧞				
hog1∆	🕒 🏶 😗	🌰 🎕 📩				
rsc9 deg	۰ کې کې					
rsc9 deg set1∆	🌲 🌒 🔵					

С

		wt			rs	c9 ^{ts}		r					
42 °C	0	5	10	15	0	5	10	15	0	5	10	15	min
CTT1			**	**									
ACT1	-	-		***	••			••					



	wt	rpd3∆	rpd3∆ set1∆ spt20.	spt20∆ ∆ set1∆
0.4 M NaCl	0 5 15 30	0 5 15 30	0 5 15 30 0 5 15	30 0 5 15 30 min
CTT1		-		
ACT1				

В



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