Supplemental material

Essential histone chaperones collaborate to regulate transcription and chromatin integrity

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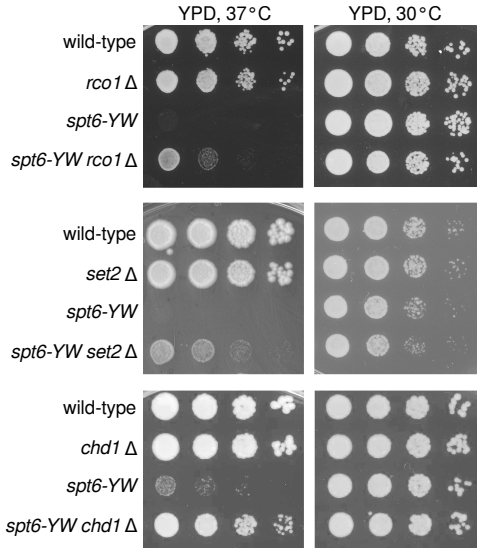
Supplemental Figures S1-S6

Supplemental Tables S1, S2, S4, S5

Supplemental Materials and Methods

Figure S1 (related to Figure 1)

A



B

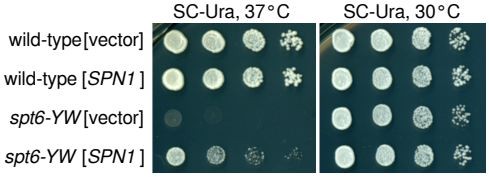


Figure S1 (related to Figure 1). The *spt6-YW* mutant phenotype is suppressed by *SPN1* overexpression.

(A) Analysis of genetic interactions between *spt6*, *rco1*, *set2*, and *chd1* mutations. Strains were grown to saturation in YPD, serially diluted 10-fold, spotted on YPD, and grown at the indicated temperature.

(B) Analysis of effects of *SPN1* overexpression on *spt6-YW* temperature sensitivity. Strains FY3276 and FY3277, transformed with either plasmid FB2701 [*SPN1-Myc*, *CEN*, *URA3*, *HIS3*] or an empty vector, were grown to saturation in liquid media without uracil, serially diluted 10-fold, spotted on the indicated media, and grown at the indicated temperature.

Figure S2 (related to Figure 2)

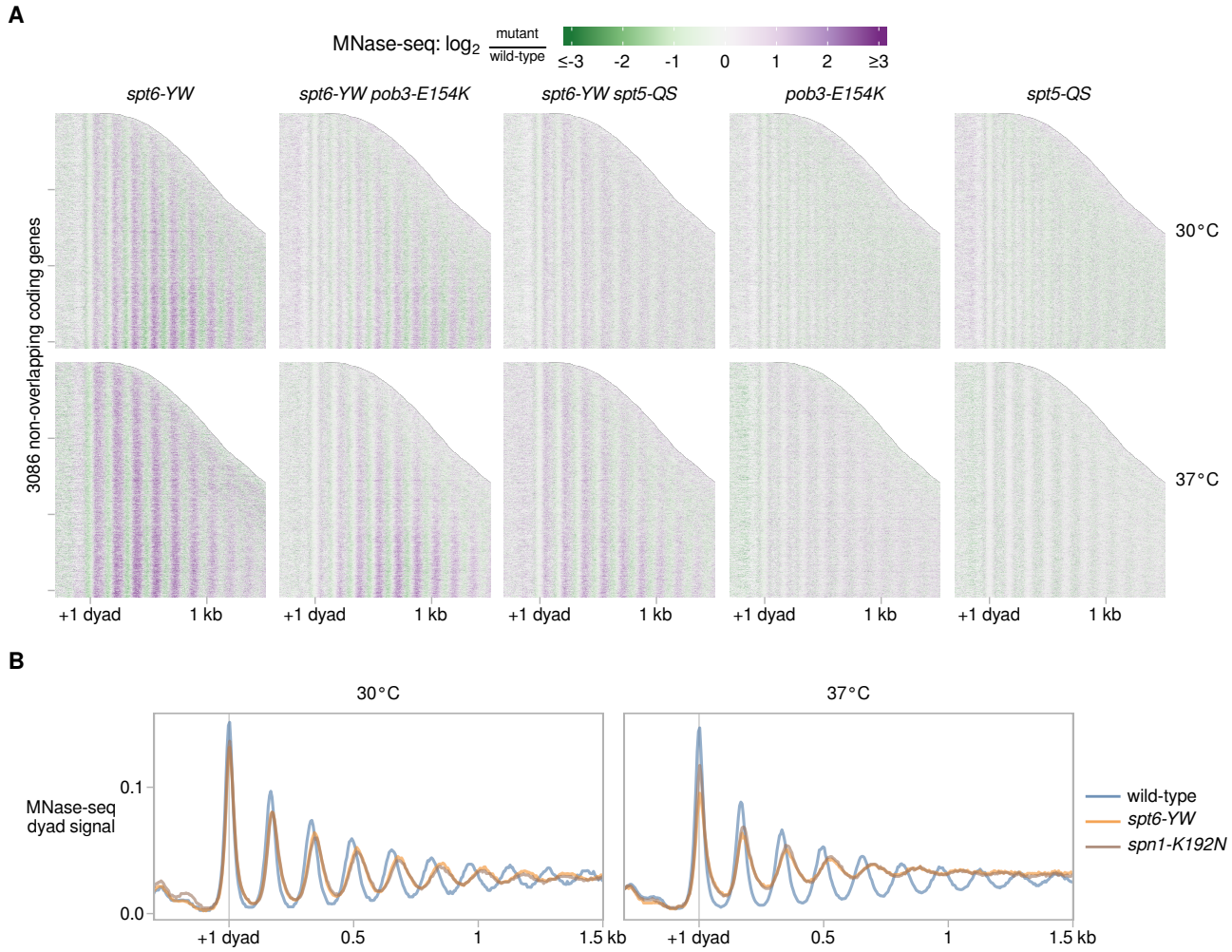


Figure S2 (related to Figure 2). Spt6, FACT, Spt5, and Spn1 functionally interact to modulate nucleosome organization *in vivo*.

(A) Heatmaps of changes to MNase-seq dyad signal in mutants over wild-type for 3086 non-overlapping coding genes aligned by wild-type +1 nucleosome dyad and sorted by gene length, for strains grown either at 30°C or with an 80-minute shift to 37°C.

(B) Average MNase-seq dyad signal over 3,086 non-overlapping verified coding genes aligned by 30°C wild-type +1 nucleosome dyad, for wild-type (FY87), *spt6-YW* (FY3223), *spn1-K192N* (FY3272) strains grown at 30°C or with an 80-minute shift to 37°C. Values are the mean of the mean library-size normalized coverage over the genes considered, over at least two replicates.

Figure S3 (related to Figure 3)

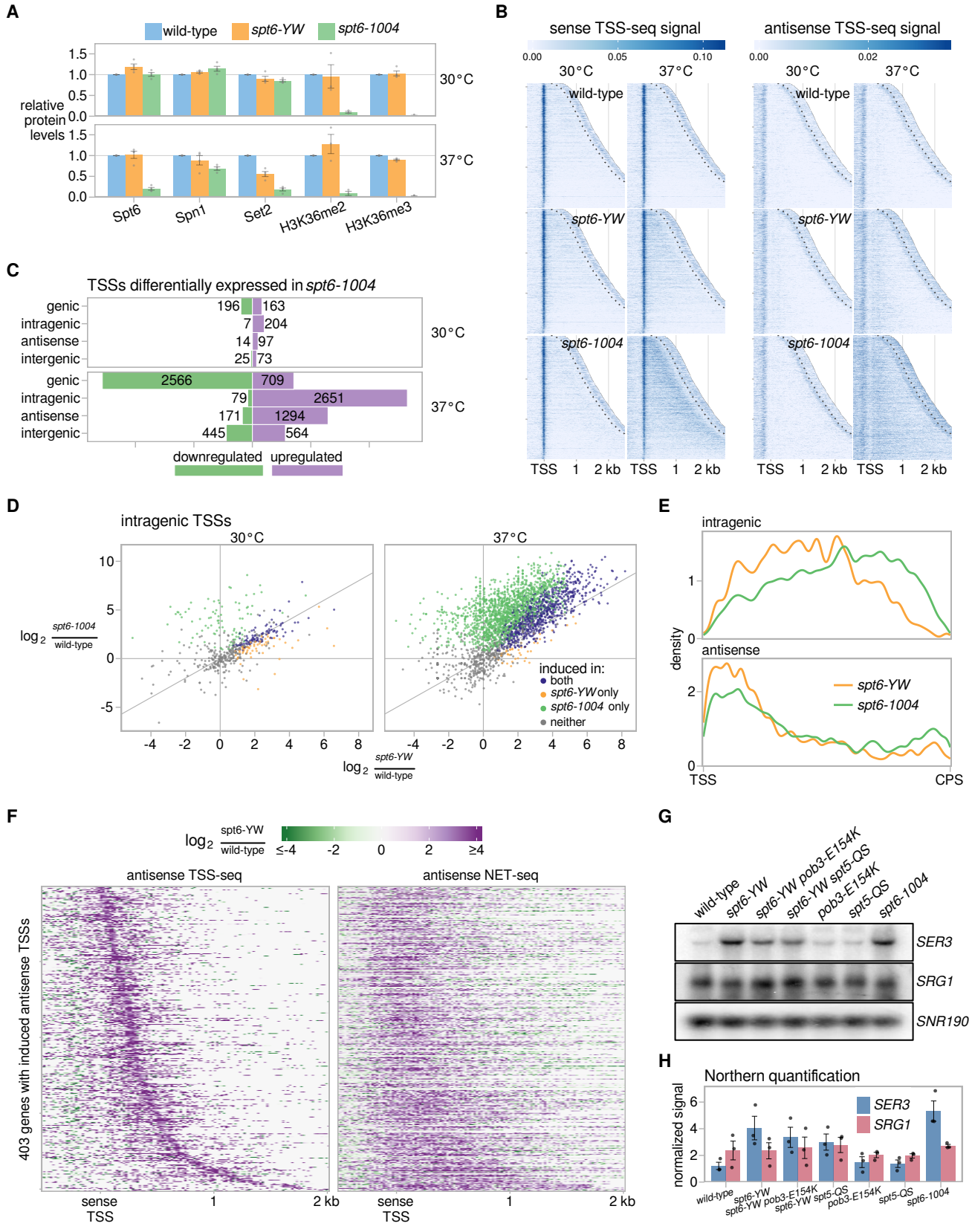


Figure S3 (related to Figure 3). The *spt6-YW* mutation causes altered sense and antisense transcription.

(A) Quantification of western blots for protein abundance in whole cell lysates of wild-type, *spt6-YW*, and *spt6-1004* strains grown at 30°C or shifted to 37°C for 80 minutes. Spt6, Spn1, and Set2 signal were normalized to a Pgk1 loading control, and histone modification signal was normalized to histone H3. Error bars indicate the mean ± standard error of the replicates shown. Samples from a *set2Δ* strain were included as a negative control for the histone modifications (data not shown).

(B) Heatmaps of sense and antisense TSS-seq signal in wild-type, *spt6-YW*, and *spt6-1004* strains, grown either at 30°C or shifted to 37°C for 80 minutes. Data are shown for 3,087 non-overlapping verified coding genes aligned by wild-type genic TSS and sorted by length, up to 300 nt 3′ of the cleavage and polyadenylation site indicated by the dotted line.

(C) Bar plots showing the number of TSS-seq peaks differentially expressed in *spt6-1004* versus wild-type, separated by genomic class.

(D) Scatterplots showing changes in sense strand intragenic transcript abundance versus wild-type strains, comparing *spt6-1004* to *spt6-YW*. TSS peaks are colored based on significant upregulation in one, both, or neither mutant.

(E) Distributions of the positions of upregulated intragenic TSSs on the sense (top) and antisense (bottom) strands along the scaled length of transcripts for *spt6-YW* (orange) and *spt6-1004* (green) at 37°C.

(F) Heatmaps of change in antisense TSS-seq and NET-seq signal in *spt6-YW* versus wild-type at 37°C. Data are shown over 403 genes with a significantly induced antisense TSS peak in *spt6-YW* at 37°C, aligned by wild-type sense TSS and arranged by the distance from the sense TSS to the *spt6-YW*-induced antisense TSS.

(G) Northern blots for the *SER3* and *SRG1* transcripts in yeast cultures grown at 30°C. *SNR190* is shown as a loading control.

(H) Quantification of Northern blots for *SER3* and *SRG1*. *SER3* and *SRG1* signal were normalized to the *SNR190* loading control. Error bars indicate the mean ± standard error of the replicates shown.

Figure S4 (related to Figure 4)

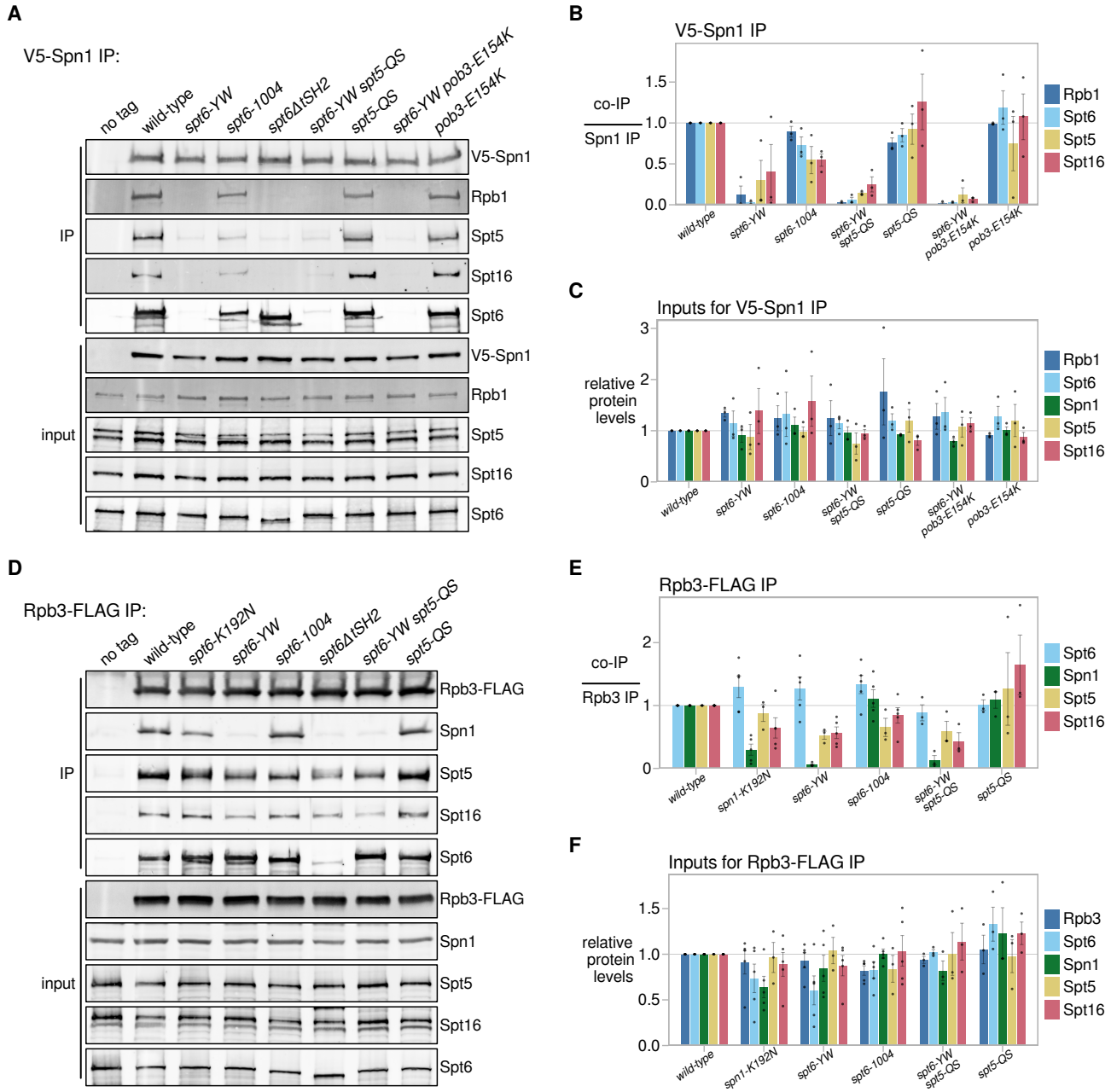


Figure S4 (related to Figure 4). The *pob3-E154K* and *spt5-QS* suppressors do not restore the Spt6-Spn1 interaction.

(A) Western blots showing the levels of V5-Spn1, Rpb1, Spt5, Spt16 and Spt6 in V5-Spn1 immunoprecipitation (IP) samples and their corresponding inputs, detected with respective antibodies (Table S5).

(B) Quantification of V5-Spn1 co-IP experiments. Error bars indicate the mean ± standard error of the relative co-IP signal normalized to V5-Spn1 pull-down signal in the replicates shown.

(C) Quantification of protein abundance from western blots of the inputs for V5-Spn1 co-IP experiments. Error bars indicate the mean ± standard error of the relative western blot signal from the replicates shown.

(D) Western blots showing the levels of Rpb3-FLAG, Spn1, Spt5, Spt16 and Spt6 in Rpb3-FLAG IP samples and their corresponding inputs, detected with respective antibodies (Table S5).

(E) As in (B), but for Rpb3-FLAG.

(F) As in (C), but for Rpb3-FLAG.

Figure S5 (related to Figure 5)

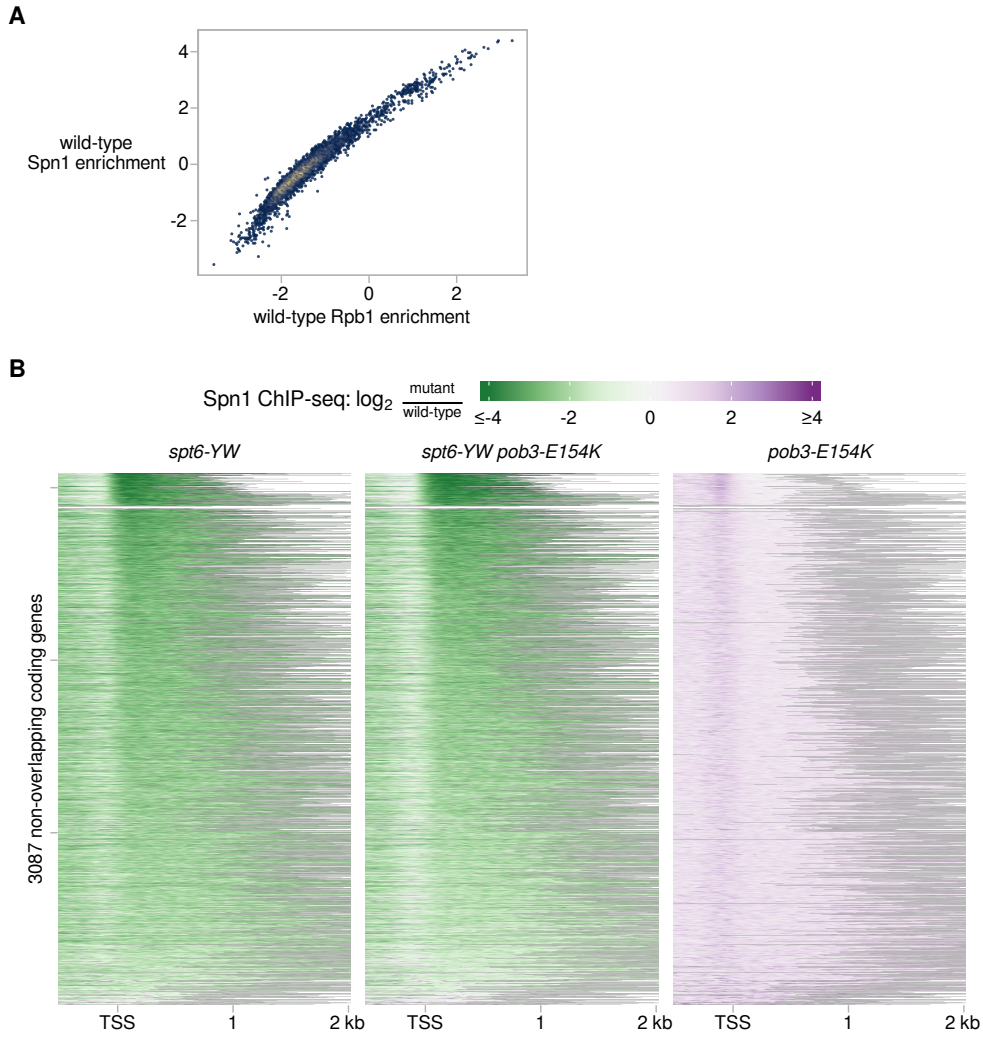


Figure S5 (related to Figure 5). The Spt6-dependent recruitment of Spn1 to chromatin is not altered by *pob3-E154K*.

(A) Scatterplot of Spn1 ChIP enrichment versus Rpb1 enrichment for 5,091 verified coding genes in wild-type cells. Enrichment values are the relative $\log_2$ enrichment of IP over input.

(B) Heatmaps of the change in Spn1 enrichment in mutants over wild-type for 3,087 non-overlapping verified coding genes aligned by TSS and arranged top to bottom by decreasing wild-type Rpb1 enrichment.

Figure S6 (related to Figure 6)

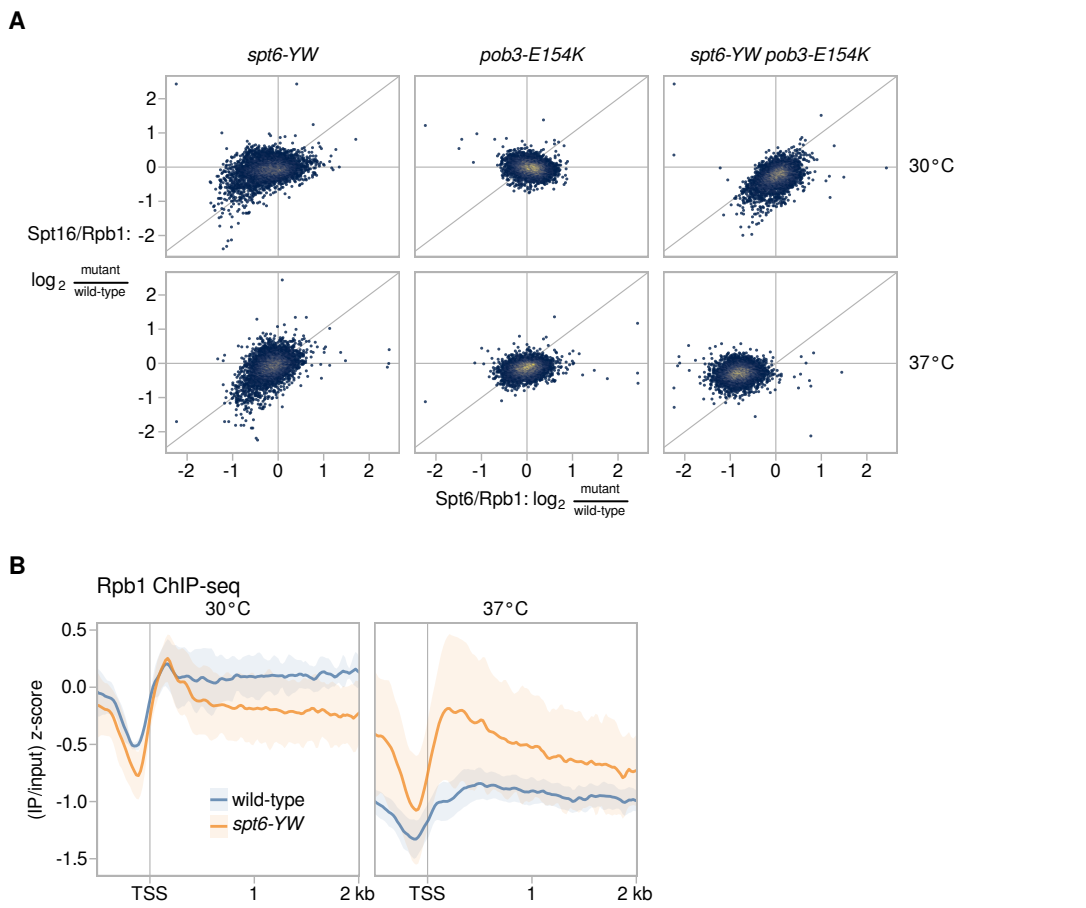


Figure S6 (related to Figure 6). Mutant FACT suppresses the Spt6-Spn1 defect by restoring the balance between FACT and Spt6 on chromatin.

(A) Scatterplots showing changes in Rpb1-normalized Spt16 ChIP enrichment in mutants over wild-type versus changes in Rpb1-normalized Spt6 ChIP enrichment, for 5,091 verified coding genes.

(B) Average Rpb1 ChIP enrichment over 3,087 non-overlapping verified coding genes aligned by TSS in wild-type (FY3276) and *spt6-YW* (FY3277) strains, grown either at 30°C or with an 80-minute shift to 37°C. For each gene, the spike-in normalized ratio of IP over input signal is standardized to the mean and standard deviation of the 30°C wild-type signal over the region. The solid line and shading are the mean and 95% confidence interval of the mean standard score over the genes considered from two to five replicates.

Table S1 (related to Figure 1): Identification and genetic verification of the suppressors for *spt6-YW* temperature-sensitive phenotype.

Gene	Mutation or amino acid change	Times isolated	Genetic verification		
			Phenotype linkage[1]	Complementation by respective WT[2]	Allele reconstitution[3]
Intragenic to *SPT6*					
SPT6	**P231L**, Y255A, W257A	3	Linked to *SPT6*	ND	ND
Extragenic					
HTA1	I79S	1	Linked to *HTA1*	phenotype rescue by *HTA1*	ND
RCO1	S558X	1	Linked to *RCO1*	phenotype rescue by *RCO1*	*rco1Δ* suppresses *spt6-YW*
	C440X	1			
SET2	K213E G219K	1	Linked to *SET2*	phenotype rescue by *SET2*	*set2Δ* suppresses *spt6-YW*
CHD1	frameshift after codon 812	1	Linked to *CHD1*	ND	*chd1Δ* suppresses *spt6-YW*
SPT5	Q342H S343del (3 bp deletion)	1	Linked to *SPT5*	phenotype rescue by *SPT5*	*spt5-QS* suppresses *spt6-YW*
POB3	E154K	7	Linked to *POB3*	phenotype rescue by *POB3*	*pob3-E154K* suppresses *spt6-YW*
	P253L	1			
SPT16	T627K	1	Linked to *SPT16*	phenotype rescue by *SPT16*	ND
	E656K	1			
	K579E	1			
Disomic					
chr XVI disomy[4]		8	NA	NA	*SPN1* on CEN plasmid suppresses *spt6-YW*

[1] Phenotype linkage was assessed by the analysis of the Ts^+ phenotypes in the progeny segregating the indicated suppressor allele in the *spt6-YW* backgrounds.

[2] Complementation was examined by transforming the indicated *spt6-YW* suppressor strains with a plasmid expressing a respective wild-type allele for the suppressor gene (see Table S5) followed by the phenotype analysis of the transformants. Phenotype rescue was scored when transformants had a Ts^- phenotype (complete rescue), similar to the *spt6-YW* parent strains, or had an intermediate $Ts^\pm$ phenotype (partial rescue). "ND" stands for "not determined".

[3] In case of the non-essential genes (*RCO1*, *SET2*, and *CHD1*), the null alleles were shown to suppress the Ts- phenotype of *spt6-YW* (Table S2, Figure S1A). In case of FACT and Spt5, the identified mutations were introduced to *SPT5* and *POB3* genes, reconstituting the *spt5-QS* and *pob3-E154K* alleles, and genetic suppression of *spt6-YW* by either allele was confirmed (Figure 1C). "ND" stands for "not determined".

[4] In several cases the chromosome XVI disomy was accompanied by other disomies, including chr.I, III or XI. None of the disomies other than the chr.XVI disomy were specifically associated with the Ts^+ suppressor phenotype.

Table S2 (related to Figure 1). The phenotypes for different mutant combinations used to determine genetic interactions.

Strain	30°C YPD	37°C YPD	25°C YPD	Spt[1] (SC -Lys)	Phleomycin[2]	Hydroxyurea[3]
wild type	++++	++++	++++	-	+++	++++
pob3-E154K	++++	++++	++++	-	+++	++++
spt5-QS	++++	++++	++++	-	++	++++
set2Δ	++++	++++	++++	-	+++	++++
rco1Δ	++++	++++	++++	-	ND	ND
chd1Δ	++++	++++	++++	ND	ND	++++
pob3-272 (I282K)	+++	+++	+++	++++	ND	ND
spt16-197 (G132D)	++++	+	++++	++	ND	ND
spt5-4 (E338K)	+++	++	++	++	ND	ND
spt5-194 (S324F)	+++	+++	ND	+++	ND	ND
spt5-242 (A268V)	++++	++++	-	+	ND	ND
spt4Δ	++++	++	ND	ND	ND	ND
spt6-YW	++++	-	++++	++++	+	+
spt6-YW pob3-E154K	++++	++++	++++	++	++	+++
spt6-YW spt5-QS	++++	++++	++++	++++	++	+++
spt6-YW set2Δ	++++	++	++++	+++	+	++
spt6-YW rco1Δ	++++	++	++++	++++	ND	ND
spt6-YW chd1Δ	++++	++	++++	ND	ND	+++
spt6-YW pob3-272	+++	-	+++	+++	ND	ND
spt6-YW spt16-197	++++	-	++++	+++	ND	ND
spt6-YW spt5-4	inviable					
spt6-YW spt5-194	inviable					
spt6-YW spt5-242	++++	-	+++	++++	ND	ND
spt6-YW spt4Δ	inviable					
spt6-F249K	++++	-	++++	++	ND	ND
spt6-F249K pob3-E154K	++++	++++	++++	-	ND	ND
spt6-F249K spt5-QS	++++	++++	++++	++++	ND	ND
spt6-F249K rco1Δ	++++	+++	++++	++++	ND	ND
spt6-1004	++++	+	++++	++++	ND	ND
spt6-1004 pob3-E154K	++++	-	++++	+++	ND	ND
spt6-1004 spt5-QS	++++	-	++++	++++	ND	ND
spt6-1004 rco1Δ	+++	-	+++	+++	ND	ND
spn1-K192N	++++	-	++++	++++	+	+
spn1-K192N pob3-E154K	++++	++++	++++	++++	+++	++++
spn1-K192N spt5-QS	++++	++++	++++	++++	+	++++
spn1-K192N chd1Δ	++++	+[4]	++++	ND	ND	+++
spt6-YW spn1-K192N	inviable					
spt6-YW spn1-K192N pob3-E154K	++++	++	++++	+++	ND	ND
spt6-YW spn1-K192N spt5-QS	inviable					
spn1Δ	inviable					
spn1Δ pob3-E154K	+++	++	ND	+++	ND	ND
spn1Δ spt5-QS	inviable					

The phenotypes were analyzed based on the yeast growth under indicated conditions in comparison to the wild-type strain using the spot test assay and scored after two days of incubation, unless indicated otherwise. The number of "+" indicates growth of each of the 10-fold serially diluted cultures. "-" indicates a very weak growth, if any. "ND" stands for "not determined".

[1] Spt phenotype was scored as growth on synthetic media without lysine due to suppression of the *lys2-128δ* allele.

[2] Phleomycin was added to the YPD media at concentration of 13 μg/ml; plates were incubated at 30°C for three days before scoring.

[3] Hydroxyurea was supplemented to YPD at 150 mM final concentration; plates were incubated at 30°C.

[4] *spn1-K192N chd1Δ* mutant was scored for growth at 37°C after 3 days of incubation, indicating weak suppression of Ts- phenotype.

Table S4. The list of strains used in the study.

Strain	Genotype	Used for
S. cerevisiae		
FY87	*MATα lys2-128δ ura3-52 leu2Δ1*	Genetics, TSS-seq, MNase-seq,
FY3223	*MATα spt6-YW lys2-128δ ura3-52 leu2Δ1 [pRS316]*	
FY3207	*MATα spt6-YW lys2-128δ ura3-52 leu2Δ1*	
FY3205	*MATα spt6-YW pob3-E154K lys2-128δ ura3-52 leu2Δ1 [pRS316]*	
FY3206	*MATα pob3-E154K ura3-52 lys2-128δ [pRS316]*	
FY3125	*MATα spt6-1004 lys2-128δ ura3-52 leu2Δ1*	
FY3272	*MATα spn1-K192N::URA3 leu2D0 lys2-128δ ura3D0*	
FY3273	*MATα spt5-QS lys2-128δ ura3-52 leu2Δ1*	
FY3274	*MATα spt5-QS spt6-YW lys2-128δ ura3-52 leu2Δ1*	
FY3220	*MATα spt6-YW pob3-E154K lys2-128δ ura3-52 leu2Δ1*	
FY3221	*MATα pob3-E154K ura3-52 leu2Δ1 lys2-128δ*	
FY3276	*MATα SPT6-(FLAG)$_{X3}$ lys2-128δ ura3-52 leu2Δ1*	Spt6 ChIP-seq Spt6 pulldown
FY3277	*MATα spt6-YW-(FLAG)x3 lys2-128δ ura3-52 leu2Δ1*	
FY3278	*MATα spt6-YW-(FLAG)x3 spt5-QS ura3-52 leu2Δ1 lys2-128δ*	
FY3279	*MATα SPT6-(FLAG)x3 spt5-QS ura3-52 leu2Δ1 lys2-128δ*	
FY3280	*MATα SPT6-(FLAG)x3 spn1-K192N::URA3 spt5-QS ura3-52 leu2Δ1 lys2-128δ*	
FY3281	*MATα spt6-YW-(FLAG)x3 pob3-E153K ura3-52 leu2Δ1 lys2-128δ*	
FY3282	*MATα SPT6-(FLAG)x3 pob3-E153K ura3-52 leu2Δ1 lys2-128δ*	
FY3283	*MATα spt6-1004-(FLAG)x3 lys2-128δ ura3-52 leu2Δ1*	
FY2912	*MATα RPB3-(FLAG)x3::NatMx ura3-52 his4-912δ lys2-128δ*	NET-seq Rpb3 pulldown and/or IP-MS
FY3019	*MATα RPB3-(FLAG)x3::NatMx spt6-YW his4-912δ lys2-128δ ura3-52*	
FY3021	*MATα RPB3-(FLAG)x3::NatMx spt6-1004 his4-912δ lys2-128δ ura3-52*	
FY3284	*MATα RPB3-(FLAG)x3::NatMx spt6-YW spt5-QS his4-912δ lys2-128δ ura3-52*	
FY3285	*MATα RPB3-(FLAG)x3::NatMx spt5-QS his4-912δ lys2-128δ ura3-52*	
FY3286	*MATα RPB3-(FLAG)x3::NatMx spn1-K192N::URA3 his4-912δ lys2-128δ ura3-52*	
FY3287	*MATα RPB3-(FLAG)x3::NatMx spt6-YW pob3-E154K his4-912δ lys2-128δ ura3-52*	
FY3288	*MATα RPB3-(FLAG)x3::NatMx pob3-E154K his4-912δ lys2-128δ ura3-52*	
FY3289	*MATα (V5)x3-SPN1 spt6-YW ura3-52 lys2-128δ leu2Δ1 his3Δ200*	Spn1 ChIP-seq Spn1 pulldown
FY3290	*MATα (V5)x3-SPN1 spt6-YW spt5-QS ura3-52 lys2-128δ leu2Δ1 his3Δ200*	
FY3291	*MATα (V5)x3-SPN1 spt5-QS ura3-52 lys2-128δ leu2Δ1 his3Δ200*	
FY3292	*MATα (V5)x3-SPN1 ura3-52 lys2-128δ leu2Δ1 his3Δ200*	
FY3293	*MATα (V5)x3-SPN1 pob3-E154K spt6-YW ura3-52 lys2-128δ leu2Δ1 his3Δ200*	
FY3294	*MATα (V5)x3-SPN1 pob3-E154K ura3-52 lys2-128δ leu2Δ1 his3Δ200*	
FY3296	*MATα (V5)x3-SPN1 spt6-1004 ura3-52 lys2-128δ leu2Δ1 his3Δ200*	
FY3297	*MAT**a** spt6-YW his4-912δ lys2-128δ ura3-52 leu2Δ1*	suppressor isolation
FY3298	*MATα spt6-YW his4-912δ lys2-128δ ura3-52*	
FY3299	*MATα SPT16-Myc leu2Δ1 ura3-52 lys2-128δ*	Spt16 ChIP-seq
FY3300	*MATα SPT16-Myc spt6-YW pob3-E154K leu2Δ1 ura3-52 lys2-128δ*	
FY3301	*MATα SPT16-Myc spt6-YW leu2Δ1 ura3-52 lys2-128δ*	
FY3302	*MATα SPT16-Myc pob3-E154K leu2Δ1 ura3-52 lys2-128δ*	
FY3303	*MAT**a** pob3-E154K-(V5)x3 spt6-YW lys2-128δ ura3-52 leu2Δ1*	Pob3 ChIP Pob3 pulldown
FY3304	*MAT**a** POB3-(V5)x3 lys2-128δ ura3-52 leu2Δ1*	
FY3305	*MAT**a** POB3-(V5)x3 spt6-YW lys2-128δ ura3-52 leu2Δ1*	
FY3306	*MAT**a** pob3-E154K-(V5)x3 lys2-128δ ura3-52 leu2Δ1*	

FY3307	*MAT**a** set2Δ::KanMX his3D200 lys2-128δ ura3-52 leu2Δ1 FLO8-URA3*	Genetic Interactions
FY3308	*MATα rco1Δ::KanMX ura3-52 his4-912δ lys2-128δ*	
FY3309	*MAT**a** chd1D::hphMX ura3-52 his4-912δ leu2D0 lys2-128δ*	
O877	*MATα pob3-272 his4-912δ lys2-128δ leu2Δ1 ura3-52 suc2dUAS*	
FY346	*MAT**a** spt16-197 ura3-52 leu2Δ1 lys2-128δ*	
FY1668	*MAT**a** spt5-4 his4-912δ lys2-128δ*	
FY300	*MAT**a** spt5-194 his4-912δ lys2-128δ ura3-52 leu2Δ1*	
FY1672	*MATα spt5-242 lys2-128δ leu2Δ1 ura3-52*	
FY247	*MAT**a** spt4Δ::URA3 ura3-52 leu2Δ1*	
FY3310	*MATα set2Δ::kanMX6 spt6-YW ura3-52 lys2-128δ*	
FY3311	*MAT**a** spt6-YW rco1Δ::KanMX ura3-52 lys2-128δ his4-912δ*	
FY3312	*MATα spt6-YW chd1Δ::hphMX lys2-128δ ura3-52 his4-912δ leu2Δ1*	
FY3313	*MATα spt6-YW pob3-272 ura3-52 lys2-128δ his4-912δ leu2Δ1 suc2dUAS*	
FY3314	*MAT**a** spt6-YW spt16-197 lys2-128δ his4-912δ*	
FY3315	*MATα spt6-YW spt5-242 lys2-128δ his4-912δ leu2Δ1*	
FY3316	*MATα spt6-F249K(-424, URA3) lys2-128δ ura3-52 his4-921δ*	
FY3317	*MATα spt6-F249K(-424, URA3) pob3-E154K lys2-128δ ura3-52 leu2Δ1 his4-921δ*	
FY3318	*MATα spt6-F249K(-424, URA3) spt5-QS lys2-128δ ura3-52*	
FY3319	*MATα spt6-F249K(-424, URA3) rco1Δ::KanMx his4-912δ lys2-128δ ura3-52*	
FY3320	*MATα spt6-1004 pob3-E154K ura3-52 lys2-128δ leu2Δ1*	
FY3321	*MAT**a** spt6-1004 spt5-QS lys2-128δ ura3-52 leu2Δ1*	
FY3322	*MATα spt6-1004 rco1Δ::KanMx his4-912δ lys2-128δ ura3-52*	
FY3323	*MATα spn1-K192N::URA3 pob3-E154K lys2-128δ ura3 leu2Δ1*	
FY3324	*MATα spn1-K192N::URA3 spt5-QS lys2-128δ ura3 leu2D0*	
FY3325	*MATα spn1-K192N::URA3 chd1Δ::hphMX ura3-52 leu2D0 lys2-128δ*	
FY3326	*MATα spt6-YW spn1-K192N::URA3 pob3-E154K ura3 lys2-128δ*	
FY3327	*MATα spn1Δ::KanMX his3d200 leu2Δ1 ura3-52 lys2-128δ [SPN1, URA3]*	
FY3328	*MATα spn1Δ::KanMX pob3-E154K his3d200 lys2-128δ ura3-52 [SPN1, URA3]*	
FY3329	*MATα spn1Δ::KanMX spt5-QS his3d200 lys2-128δ ura3-52 [SPN1, URA3]*	
S. pombe		
FWP570	*h+ spt5::spt5-V5-IAA::KanMx rpb3-3XFLAG::ura4+ ctr9-Myc::KanMx ura4-D18 ade6-210*	spike-in for ChIP-seq
972	*h- wild-type*	spike-in for TSS-seq and MNase-seq

Table S5. Reagents.

Reagent or resource	Source	Identifier
Antibodies		
Mouse monoclonal anti-Rpb1 (8WG16)	Millipore Sigma	Cat# 05-952, RRID:AB_492629
Rabbit polyclonal anti-Spt6	Winston lab	NA
Rabbit polyclonal anti-Spn1	Laurie Stargell lab	NA
Rabbit polyclonal anti-Spt16	Tim Formosa lab	NA
Rabbit polyclonal anti-Spt5	Grant Hartzog lab	NA
Mouse monoclonal anti-FLAG (M2)	Millipore Sigma	Cat# F1804, RRID:AB_262044
Mouse monoclonal anti-Myc (9E10)	Santa Cruz Biotechnology	Cat# 05-419, RRID:AB_309725
Mouse monoclonal anti-V5	Thermo Fisher Scientific	Cat# R960-25, RRID:AB_2556564
Rabbit polyclonal anti-H3	Karen Arndt lab	NA
Bacterial and Virus Strains		
E. coli strain DH5 alpha	Winston lab	NA
Chemicals, Peptides, and Recombinant Proteins		
Hydroxyurea	SIGMA	Cat# H8627
Phleomycin	SIGMA	Cat# P9564
Anti-FLAG M2 Affinity Gel	SIGMA	Cat# A2220
Protein G Sepharose beads, Fast Flow	GE Healthcare	Cat# 17-0618-01
SIGMAFAST, Protease Inhibitor Cocktail Tablets	SIGMA	Cat# S8830
PhosSTOP EASYpack, Phosphatase Inhibitor Cocktail	SIGMA	Cat# 04 906837001
Anti-V5-Conjugated Magnetic Beads, clone 1H6	MBL International Corporation	Cat# M167-11 006 10
FLAG Peptide	Winston lab	NA
Critical Commercial Assays		
GeneRead DNA Library I Core Kit	Qiagen	Cat# 180434
Phusion High-Fidelity DNA Polymerase	New England Bio Labs	Cat# M0530
Bio-Rad Protein Assay	BioRad	Cat# 500-0006
BioAnalyzer High Sensitivity DNA Kit	Agilent Technologies	Cat# 5067-4626
Qubit dsDNA High Sensitivity Assay Kit	Thermo Fisher Scientific	Cat# Q32851
Brilliant III Ultra-Fast SYBR Green QPCR Master Mix	Agilent Technologies	Cat# 600882
AMPure XP beads	Beckman Coulter	Cat# A63881
Deposited Data		
High-throughput sequencing data	This study	GEO: GSE16081
Raw data and analyses	This study	https://doi.org/10.5281/zenodo.4174464
Experimental Models: Organisms/Strains		
S. cerevisiae	This study and other sources	Table S4

S. pombe	This study and other sources	Table S4
Oligonucleotides		
5' GCTTCTAAAATCTAACAGTAGTAAGAATAG AATGAACAACTACCGTAGGGAACAAAAGCTGG 3' (to tag Spt6)	This study	NA
5' GGTCAAAGTAATAATAAAATTAATAATAACAATG GACACTACATACGCATCTATAGGGCGAATTGG 3' (to tag Spt6)	This study	NA
5' GGGGTAGCGACGAAGAAAGGCCTTCGAA GAAGCCTAAGGTAGAAAGGGAACAAAAGCTGG 3' (to tag Pob3)	This study	NA
5' TACACAATATCAATAAATTTAACTTATACAGTCT AAAATTATTACTATAGGGCGAATTGG 3' (to tag Pob3)	This study	NA
5'CTTTTAGTAATAAAAGGCAAAAACATATCAATTAGC AATGAGGGAACAAAAGCTGG 3' (to tag Spn1)	This study	NA
5' TCGCTTCCACCACTTTGGGTTGTTCTTGATCGGCT GTACTCTGTAGGGCGAATTGG 3' (to tag Spn1)	This study	NA
5' TGTCCTATCATTATCGTCTAACATCT 3' (*PMA1* qPCR primer)	This study	NA
5' TCACTATTGGTGTTATAGGAAAGAAAG 3' (*PMA1* qPCR primer)	This study	NA
5' CAGCATCTTCTGTTTCAGCTCA 3' (*PMA1* qPCR primer)	This study	NA
5' TTCAGATGCAGCGTCATCGT 3' (*PMA1* qPCR primer)	This study	NA
5' TCGTTATGTTTTTCGTCGGTCC 3' (*PMA1* qPCR primer)	This study	NA
5' GACAAACCGGCAGCCAAAAT 3' (*PMA1* qPCR primer)	This study	NA
5' TCGATCAATCTGCTATTACTGGTGA 3' (*PMA1* qPCR primer)	This study	NA
5' AGAAGAGAAAGTTTGGTCACCGT 3' (*PMA1* qPCR primer)	This study	NA
5' TTAGGTCTAGGTGGCGGTGG 3' (*PMA1* qPCR primer)	This study	NA
5' GAAACCATCGGCATTTTCAACA 3' (*PMA1* qPCR primer)	This study	NA
5' TCGAAGACTTCATGGCTGCT 3' (*PMA1* qPCR primer)	This study	NA
5' GCTACTTCAACAGGATTAGGTTTCC 3' (*PMA1* qPCR primer)	This study	NA
5' GCTCCATTTGGAAGTCTCGC 3' (*PMA1* qPCR primer)	This study	NA
5' ACGTTCATGTAAGTGTGTATCTTGA 3' (*PMA1* qPCR primer)	This study	NA
5' GGCCCTGATGATAATG 3' (*SNR190* Northern probe PCR)	Martens et al., 2004	NA
5' GGCTCAGATCTGCATG 3' (*SNR190* Northern probe PCR)	Martens et al., 2004	NA
5' ACAGATGCTCACCAATCCTG 3' (*DSK2* Northern probe PCR)	This study	NA
5' TTAAACATCGCCGTTCAGTAG 3' (*DSK2* Northern probe PCR)	This study	NA

5’ CGTTCCACAGCGCTTGAATG 3’ (*SER3* Northern probe PCR)	Martens et al., 2004	NA
5’ CGCTTTGGTCAACAGAAGAG 3’ (*SER3* Northern probe PCR)	Martens et al., 2004	NA
5’ CTATGTGCAAATATCACAAA 3’ (*SRG1* Northern probe PCR)	Martens et al., 2004	NA
5’ TTTCCTTATCCTCTGCTCCC 3' (*SRG1* Northern probe PCR)	Martens et al., 2004	NA
Recombinant DNA		
pJC102 [*HTA1*, CEN, *URA3*] plasmid	Michael Grunstein lab	NA
pCYY23 [*SET2*, CEN, *URA3*] plasmid	Greg Prelich lab	NA
pJW4 [*POB3*, CEN, *URA3*] plasmid	Tim Formosa lab	NA
pJW11 [*POB3*, CEN, *LEU2*] plasmid	Tim Formosa lab	NA
pTF128 [*SPT16*, CEN, *LEU2*] plasmid	Tim Formosa lab	NA
FB2701 [*SPN1*-Myc, CEN, *URA3*, *HIS3*] plasmid	Winston lab	NA
[pGAL-*RCO1*, CEN, *URA3*] plasmid	from pGAL collection	NA
[pGAL-*SPT5*, CEN, *URA3*] plasmid	from pGAL collection	NA
pRS316 [CEN, *URA3*] plasmid	Winston lab	NA
ZM467 (to introduce FLAG epitope)	Kevin Struhl lab	NA
ZM474 (to introduce V5 epitope)	Kevin Struhl lab	NA
Software and Algorithms		
Snakemake	Köster and Rahmann, 2012	https://snakemake.readthedocs.io/en/stable/
cutadapt	Martin, 2011	https://cutadapt.readthedocs.io/en/stable/
TopHat2	Kim et al., 2013	https://ccb.jhu.edu/software/tophat/index.shtml
Samtools	Li et al., 2009	http://www.htslib.org/
BEDTools	Quinlan and Hall, 2010	https://bedtools.readthedocs.io/en/latest/
IDR	Li et al., 2011	https://github.com/nboley/idr
DESeq2	Love et al., 2014	http://bioconductor.org/packages/release/bioc/html/DESeq2.html
fastq-multx	Aronesty, 2013	https://expressionanalysis.github.io/ea-utils/
Bowtie 2	Langmead and Salzberg, 2012	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
MACS2	Zhang et al., 2008	https://github.com/macs3-project/MACS
Bowtie	Langmead et al., 2009	http://bowtie-bio.sourceforge.net/index.shtml
DANPOS2	Chen et al., 2013	https://sites.google.com/site/danposdoc/

Supplemental Materials and Methods.

Data Analysis

Genome builds and annotations

The genome builds used were *S. cerevisiae* R64-2-1 {Engel, 2014 #374} and *S. pombe* ASM294v2 {Wood, 2002 #375}. *S. cerevisiae* transcript coordinates were generated from TIF-seq {Pelechano, 2013 #376} and TSS-seq data, as previously described {Doris, 2018 #300}.

TSS-seq library processing

Removal of adapter sequences and random hexamer sequences from the 3′ end of the read and 3′ quality trimming were performed using cutadapt {Martin, 2011 #377}. The random hexamer molecular barcode on the 5′ end of the read was then removed and processed using a Python script. Reads were aligned to the combined *S. cerevisiae* and *S. pombe* reference genomes using Tophat2 {Kim, 2013 #378} without a reference transcriptome, and uniquely mapping alignments were selected using SAMtools {Li, 2009 #379}. Alignments mapping to the same location as another alignment with the same molecular barcode were identified as PCR duplicates and removed using a Python script. Coverage of the 5′-most base, corresponding to the TSS, was extracted using bedtools genomecov {Quinlan, 2010 #380}. Due to high variability in the proportion of *S. pombe* spike-in alignments among the libraries for certain conditions, coverage was normalized to the total number of alignments uniquely mapping to the *S. cerevisiae* genome. The quality of raw, cleaned, non-aligning, and uniquely aligning non-duplicate reads was assessed using FastQC {Andrews, 2014 #381}.

TSS-seq peak calling

TSS peak calling was performed using 1D watershed segmentation followed by filtering for reproducibility by the Irreproducible Discovery Rate method (IDR=0.1) {Li, 2011 #382}, as previously described in {Doris, 2018 #300}, except using the maximum signal within a putative peak to estimate the probability of the peak being generated by noise rather than the sum of the signal within the peak. To allow for direct comparison between *spt6-YW* and *spt6-1004,* unified TSS peak sets were generated by using bedtools multiinter to combine peaks called in wild-type, *spt6-YW,* and *spt6-1004* at 30°C or 37°C.

TSS-seq differential expression analysis

For TSS-seq differential expression analyses, counts of alignments overlapping the unified TSS peak sets described above were used as the input to differential expression analysis by DESeq2 {Love, 2014 #383}, with a null hypothesis of no change between conditions and a false discovery rate of 0.1.

Classification of TSS peaks into genomic categories

TSS peak classification was performed as described {Doris, 2018 #300}, except the summit of intragenic and antisense peaks were used to determine overlap with open/closed reading frames or transcripts. In brief, a genic region was defined for each gene using its transcript and open/closed reading frame annotations. TSS peaks were classified as genic if they overlapped a genic region on the same strand. TSS peaks were classified as intragenic if they were not classified as genic and their summit overlapped an open or closed reading frame on the same strand. TSS peaks were classified as antisense if they were not classified as genic and their

summit overlapped a transcript on the opposite strand. TSS peaks not overlapping genic regions, transcripts, or reading frames were classified as intragenic.

NET-seq library processing

Removal of adapter sequences from the 3′ end of the read and 3′ quality trimming were performed using cutadapt {Martin, 2011 #377}. Reads were aligned to the *S. cerevisiae* genome using Tophat2 without a reference transcriptome (kim2013), and uniquely mapping alignments were selected using SAMtools {Li, 2009 #379}. Coverage of the 5′-most base of the read, corresponding to the 3′-most base of the nascent RNA and the active site of elongating RNA polymerase, was extracted using bedtools genomecov {Quinlan, 2010 #380}, and normalized to the total number of uniquely mapping alignments. The quality of raw, cleaned, non-aligning, and uniquely aligning reads was assessed using FastQC {Andrews, 2014 #381}.

ChIP-seq library processing

Reads were demultiplexed using fastq-multx {Aronesty, 2103 #384}*,* allowing one mismatch to the index sequence and A-tail. Cutadapt {Martin, 2011 #377} was then used to remove index sequences and low-quality base calls from the 3′ end of the read. Reads were aligned to the combined *S. cerevisiae* and *S. pombe* genome using Bowtie 2 {Langmead, 2012 #385}, and alignments with a mapping quality of at least 5 were selected using SAMtools {Li, 2009 #379}. The median fragment size estimated by MACS2 {Zhang, 2008 #386} cross-correlation over all samples of a factor was used to generate coverage of fragments and fragment midpoints by extending alignments to the median fragment size or by shifting the 5′ end of alignments by half the median fragment size, respectively. The quality of raw, cleaned, non-aligning, and uniquely aligning reads was assessed using FastQC {Andrews, 2014 #381}.

ChIP-seq normalization

For ChIP-seq coverage from IP samples, spike-in normalization was accomplished by scaling coverage proportionally to the normalization factor $N_{input,\ spike\text{-}in}$ / $N_{IP,\ spike\text{-}in}$ * $N_{input,\ experimental}$, where $N_{IP,\ spike\text{-}in}$ is the number of *S. pombe* alignments in the IP sample, $N_{input,\ spike\text{-}in}$ is the number of *S. pombe* alignments in the corresponding input sample, and $N_{input,\ experimental}$ is the number of *S. cerevisiae* alignments in the input sample. Coverage from input samples was normalized to $N_{input,\ experimental}$. Relative estimates of the total abundance of each ChIP target on chromatin were also obtained by multiplying the normalization factor with the number of *S. cerevisiae* alignments in an IP sample. Coverage of the relative ratio of IP over input was obtained by first smoothing normalized IP and input fragment midpoint coverage using a Gaussian with 20 bp bandwidth, and then taking the ratio. Coverage of the relative ratio of one factor to another (e.g. Spn1 over Rpb1) was obtained as follows: For each factor, coverage of IP over input in each sample was standardized using the genome-wide mean and standard deviation over all samples, weighted such that each condition had equal contribution. Standardized coverage of the normalizing factor was then subtracted from the matched coverage of the factor to be normalized.

ChIP-seq differential occupancy analysis

For differential occupancy analyses of single factors over verified coding genes, IP and input fragment midpoints overlapping the transcript annotation of these genes were counted using bedtools {Quinlan, 2010 #380}. These counts were used to perform a differential occupancy analysis using DEseq2 {Love, 2014 #383}, at a false discovery rate of 0.1. The design formula used was a generalized linear model with variables for sample type (IP or input), condition

(strain and temperature), and the interaction of sample type with condition. Fold changes were extracted from the coefficients of the interaction of sample type with condition, and represent the change in IP signal between conditions, corrected for change in input signal. To normalize to the spike-in control, size factors obtained from *S. pombe* counts over peaks called with MACS2 {Zhang, 2008 #386} and IDR {Li, 2011 #382} were used for each sample.

MNase-seq library processing and quantification

Paired-end reads were demultiplexed using fastq-multx {Aronesty, 2103 #384} allowing one mismatch to the barcode. Filtering for the barcode on read 2 and 3′ quality trimming were performed with cutadapt {Martin, 2011 #377}. Reads were aligned to the combined *S. cerevisiae* and *S. pombe* genome using Bowtie 1 {Langmead, 2009 #402}, and correctly paired alignments were selected using SAMtools {Li, 2009 #379}. Coverage of nucleosome protection and nucleosome dyads were extracted using bedtools {Quinlan, 2010 #380} and shell scripts to get the entire fragment or the midpoint of the fragment, respectively. Smoothed nucleosome dyad coverage was generated by smoothing dyad coverage was generated by smoothing dyad coverage with a Gaussian kernel of 20 bp bandwidth. Due to differences in the proportion of *S. pombe* DNA added between sequencing runs, coverage was normalized to the total number of correctly paired *S. cerevisiae* fragments. The quality of raw, cleaned, non-aligning, and correctly paired reads was assessed using FastQC {Andrews, 2014 #381}. Nucleosome regions for each condition were called using DANPOS2 {Chen, 2013 #365}. Nucleosome 'fuzziness' was calculated for each nucleosome region in each sample by taking the standard deviation of nucleosome dyad positions in the region.