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) 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)

(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)

A

![Bar chart showing relative protein levels at 30°C and 37°C for different proteins and their mutants.]

B

![Heatmap showing sense TSS-seq signal at 30°C and 37°C for different conditions.]

C

![Table showing TSSs differentially expressed in spt6-1004.]

D

![Scatter plot showing log2 expression levels at 30°C and 37°C for different conditions.]

E

![Graph showing antisense density at TSSs and CPS for different conditions.]

F

![Heatmap showing antisense TSS-seq and NET-seq for different conditions.]

G

![Northern quantification for SER3, SRG1, and SNR190.]

H

![Bar graph showing normalized signal for SER3 and SRG1 across different conditions.]
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). 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). The Spt6-dependent recruitment of Spn1 to chromatin is not altered by \textit{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)

(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                      | Complementation by respective WT | Allele reconstitution³ |
|----------|-------------------------------|----------------|------------------------------------------|----------------------------------|-------------------------|
|          |                               |                | Phenotype linkage¹                        |                                  |                         |
| 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 disomy⁴ |                       | 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⁺ 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               | ++++             |
| spt6-YW                       | ++++     | -        | ++++     | +                    | ND               | ND               |
| spt6-YW pob3-E154K            | ++++     | ++++     | ++++     | ++                   | +++              | ++++             |
| spt6-YW spt5-QS               | ++++     | ++++     | ++++     | ++++                 | +                | ++++             |
| spt6-YW set2Δ                 | ++++     | ++       | ++++     | +                    | +                | ++               |
| spt6-YW rco1Δ                 | ++++     | ++       | ++++     | ND                   | ND               | ND               |
| spt6-YW chd1Δ                 | ++++     | ++       | ++++     | ND                   | ND               | ++++             |
| spt6-YW pob3-272              | ++++     | -        | ++++     | ND                   | ND               | ND               |
| spt6-YW spt16-197             | ++++     | -        | ++++     | ND                   | ND               | ND               |
| spt6-YW spt5-4                | inviable |          |          |                      |                  |                  |
| spt6-YW spt5-194              | inviable |          |          |                      |                  |                  |
| spt6-YW spt5-242              | ++++     | -        | ++++     | ND                   | ND               | ND               |
| spt6-YW spt4Δ                 | inviable |          |          |                      |                  |                  |
| spt6-F249K                    | ++++     | -        | ++++     | ND                   | ND               | ND               |
| spt6-F249K pob3-E154K         | ++++     | ++++     | ++++     | -                    | ND               | ND               |
| spt6-F249K spt5-QS            | ++++     | ++++     | ++++     | ND                   | ND               | ND               |
| spt6-F249K rco1Δ              | ++++     | ++++     | ++++     | ND                   | ND               | ND               |
| spt6-1004                     | ++++     | +        | ++++     | ND                   | ND               | ND               |
| spt6-1004 pob3-E154K          | ++++     | -        | ++++     | ND                   | ND               | ND               |
| spt6-1004 spt5-QS             | ++++     | -        | ++++     | ND                   | ND               | ND               |
| spt6-1004 rco1Δ               | ++++     | -        | ++++     | ND                   | ND               | ND               |
| spin1-K192N                   | ++++     | -        | ++++     | +                    | +                | ++               |
| spin1-K192N pob3-E154K        | ++++     | ++++     | ++++     | ++++                 | +                | ++++             |
| spin1-K192N spt5-QS           | ++++     | ++++     | ++++     | ++++                 | +                | ++++             |
| spin1-K192N chd1Δ             | ++++     | +        | ++++     | ND                   | ND               | ++++             |
| spin1-YW spin1-K192N          | inviable |          |          |                      |                  |                  |
| spin1-YW spin1-K192N pob3-E154K | ++++ | ++ | ++++ | ND | ND | ND |
| spin1-YW spin1-K192N spt5-QS  | inviable |          |          |                      |                  |                  |
| spin1Δ                        | inviable |          |          |                      |                  |                  |
| spin1Δ pob3-E154K             | ++++     | ++       | ND       | ++++                 | ND               | ND               |
| spin1Δ 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
2. Phleomycin
3. Hydroxyurea
Spt phenotype was scored as growth on synthetic media without lysine due to suppression of the lys2-128δ allele.

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

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

spn1-K192N chd1Δ mutant was scored for growth at 37°C after 3 days of incubation, indicating weak suppression of Ts- phenotype.
| Strain  | Genotype                                                                 | Used for                        |
|---------|---------------------------------------------------------------------------|---------------------------------|
| FY87    | MATa lys2-128δ ura3-52 leu2Δ1                                             |                                 |
| FY3223  | MATa spt6-YW lys2-128δ ura3-52 leu2Δ1 [pRS316]                             |                                 |
| FY3207  | MATa spt6-YW lys2-128δ ura3-52 leu2Δ1                                     |                                 |
| FY3205  | MATa spt6-YW pob3-E154K lys2-128δ ura3-52 leu2Δ1 [pRS316]                 |                                 |
| FY3206  | MATa pob3-E154K ura3-52 lys2-128δ [pRS316]                                 |                                 |
| FY3125  | MATa spt6-1004 lys2-128δ ura3-52 leu2Δ1                                   |                                 |
| FY3272  | MATa spn1-K192N::URA3 leu2D0 lys2-128δ ura3D0                             |                                 |
| FY3273  | MATa spt5-QS lys2-128δ ura3-52 leu2Δ1                                     |                                 |
| FY3274  | MATa spt5-QS spt6-YW lys2-128δ ura3-52 leu2Δ1                             |                                 |
| FY3220  | MATa spt6-YW pob3-E154K lys2-128δ ura3-52 leu2Δ1                           |                                 |
| FY3221  | MATa pob3-E154K ura3-52 leu2Δ1 lys2-128δ                                  |                                 |
| FY3276  | MATa SPT6-(FLAG)x3 lys2-128δ ura3-52 leu2Δ1                               |                                 |
| FY3277  | MATa spt6-YW-(FLAG)x3 lys2-128δ ura3-52 leu2Δ1                            |                                 |
| FY3278  | MATa spt6-YW-(FLAG)x3 spt5-QS ura3-52 leu2Δ1 lys2-128δ                    |                                 |
| FY3279  | MATa SPT6-(FLAG)x3 spt5-QS ura3-52 leu2Δ1 lys2-128δ                       |                                 |
| FY3280  | MATa SPT6-(FLAG)x3 spn1-K192N::URA3 spt5-QS ura3-52 leu2Δ1 lys2-128δ      |                                 |
| FY3281  | MATa spt6-YW-(FLAG)x3 pob3-E153K ura3-52 leu2Δ1 lys2-128δ                 |                                 |
| FY3282  | MATa SPT6-(FLAG)x3 pob3-E153K ura3-52 leu2Δ1 lys2-128δ                    |                                 |
| FY3283  | MATa spt6-1004-(FLAG)x3 lys2-128δ ura3-52 leu2Δ1                          |                                 |
| FY2912  | MATa RPB3-(FLAG)x3::NatMx ura3-52 his4-912Δ lys2-128δ                     |                                 |
| FY3019  | MATa RPB3-(FLAG)x3::NatMx spt6-YW his4-912Δ lys2-128δ ura3-52             |                                 |
| FY3021  | MATa RPB3-(FLAG)x3::NatMx spt6-1004 his4-912Δ lys2-128δ ura3-52           |                                 |
| FY3284  | MATa RPB3-(FLAG)x3::NatMx spt6-YW spt5-QS his4-912Δ lys2-128δ ura3-52     |                                 |
| FY3285  | MATa RPB3-(FLAG)x3::NatMx spt5-QS his4-912Δ lys2-128δ ura3-52             |                                 |
| FY3286  | MATa RPB3-(FLAG)x3::NatMx spn1-K192N::URA3 his4-912Δ lys2-128δ ura3-52    |                                 |
| FY3287  | MATa RPB3-(FLAG)x3::NatMx spt6-YW pob3-E154K his4-912Δ lys2-128δ ura3-52  |                                 |
| FY3288  | MATa RPB3-(FLAG)x3::NatMx pob3-E154K his4-912Δ lys2-128δ ura3-52           |                                 |
| FY3289  | MATa (V5)x3-SPN1 spt6-YW ura3-52 lys2-128δ leu2Δ1 his3Δ200                |                                 |
| FY3290  | MATa (V5)x3-SPN1 spt6-YW spt5-QS ura3-52 lys2-128δ leu2Δ1 his3Δ200         |                                 |
| FY3291  | MATa (V5)x3-SPN1 spt5-QS ura3-52 lys2-128δ leu2Δ1 his3Δ200                |                                 |
| FY3292  | MATa (V5)x3-SPN1 ura3-52 lys2-128δ leu2Δ1 his3Δ200                        |                                 |
| FY3293  | MATa (V5)x3-SPN1 pob3-E154K spt6-YW ura3-52 lys2-128δ leu2Δ1 his3Δ200     |                                 |
| FY3294  | MATa (V5)x3-SPN1 pob3-E154K ura3-52 lys2-128δ leu2Δ1 his3Δ200             |                                 |
| FY3296  | MATa (V5)x3-SPN1 spt6-1004 ura3-52 lys2-128δ leu2Δ1 his3Δ200              |                                 |
| FY3297  | MATa spt6-YW his4-912Δ lys2-128δ ura3-52 leu2Δ1                            |                                 |
| FY3298  | MATa spt6-YW his4-912Δ lys2-128δ ura3-52                                 |                                 |
| FY3299  | MATa SPT16-Myc leu2Δ1 ura3-52 lys2-128δ                                   |                                 |
| FY3300  | MATa SPT16-Myc spt6-YW pob3-E154K leu2Δ1 ura3-52 lys2-128δ              |                                 |
| FY3301  | MATa SPT16-Myc spt6-YW leu2Δ1 ura3-52 lys2-128δ                           |                                 |
| FY3302  | MATa SPT16-Myc pob3-E154K leu2Δ1 ura3-52 lys2-128δ                       |                                 |
| FY3303  | MATa pob3-E154K-(V5)x3 spt6-YW lys2-128δ ura3-52 leu2Δ1                  |                                 |
| FY3304  | MATa POB3-(V5)x3 lys2-128δ ura3-52 leu2Δ1                                |                                 |
| FY3305  | MATa POB3-(V5)x3 spt6-YW lys2-128δ ura3-52 leu2Δ1                        |                                 |
| FY3306  | MATa pob3-E154K-(V5)x3 lys2-128δ ura3-52 leu2Δ1                          |                                 |

**Table S4. The list of strains used in the study.**
| FY3307 | MATa set2Δ::KanMX his3D200 lys2-128δ ura3-52 leu2Δ1 FLO8-URA3 |
| FY3308 | MATa rco1Δ::KanMX ura3-52 his4-912δ lys2-128δ |
| FY3309 | MATa chd1Δ::hphMX ura3-52 his4-912δ leu2D0 lys2-128δ |
| O877   | MATa pob3-272 his4-912δ lys2-128δ leu2Δ1 ura3-52 suc2dUAS |
| FY346  | MATa spt16-197 ura3-52 leu2Δ1 lys2-128δ |
| FY1668 | MATa spt5-4 his4-912δ lys2-128δ |
| FY300  | MATa spt5-194 his4-912δ lys2-128δ ura3-52 leu2Δ1 |
| FY1672 | MATa spt5-242 lys2-128δ leu2Δ1 ura3-52 |
| FY247  | MATa spt4Δ::URA3 ura3-52 leu2Δ1 |
| FY3310 | MATa set2Δ::kanMX6 spt6-YW ura3-52 lys2-128δ |
| FY3311 | MATa spt6-YW rco1Δ::KanMX ura3-52 lys2-128δ his4-912δ |
| FY3312 | MATa spt6-YW chd1Δ::hphMX lys2-128δ ura3-52 his4-912δ leu2Δ1 |
| FY3313 | MATa spt6-YW pob3-272 ura3-52 lys2-128δ his4-912δ leu2Δ1 suc2dUAS |
| FY3314 | MATa spt6-YW spt16-197 lys2-128δ his4-912δ |
| FY3315 | MATa spt6-YW spt5-242 lys2-128δ his4-912δ leu2Δ1 |
| FY3316 | MATa spt6-F249K(-424, URA3) lys2-128δ ura3-52 his4-921δ |
| FY3317 | MATa spt6-F249K(-424, URA3) pob3-E154K lys2-128δ ura3-52 leu2Δ1 his4-921δ |
| FY3318 | MATa spt6-F249K(-424, URA3) spt5-QS lys2-128δ ura3-52 |
| FY3319 | MATa spt6-F249K(-424, URA3) rco1Δ::KanMx his4-912δ lys2-128δ ura3-52 |
| FY3320 | MATa spt6-1004 pob3-E154K ura3-52 lys2-128δ leu2Δ1 |
| FY3321 | MATa spt6-1004 spt5-QS lys2-128δ ura3-52 leu2Δ1 |
| FY3322 | MATa spt6-1004 rco1Δ::KanMx his4-912δ lys2-128δ ura3-52 |
| FY3323 | MATa spn1-K192N::URA3 pob3-E154K lys2-128δ ura3 leu2Δ1 |
| FY3324 | MATa spn1-K192N::URA3 spt5-QS lys2-128δ ura3 leu2D0 |
| FY3325 | MATa spn1-K192N::URA3 chd1Δ::hphMX ura3-52 leu2D0 lys2-128δ |
| FY3326 | MATa spt6-YW spn1-K192N::URA3 pob3-E154K ura3 lys2-128δ |
| FY3327 | MATa spn1Δ::KanMX his3d200 leu2Δ1 ura3-52 lys2-128δ [SPN1, URA3] |
| FY3328 | MATa spn1Δ::KanMX pob3-E154K his3d200 lys2-128δ ura3-52 [SPN1, URA3] |
| FY3329 | MATa spn1Δ::KanMX spt5-QS his3d200 lys2-128δ ura3-52 [SPN1, URA3] |

**Genetic Interactions**

S. pombe

| FWP570 | h+ spt5::spt5-V5-IAA::KanMx rpb3-3XFLAG::ura4+ ctr9-Myc::KanMx ura4-D18 ade6-210 |
| 972    | h- wild-type |

spike-in for ChIP-seq

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**                          | This study                 | GEO: GSE16081             |
| High-throughput sequencing data             | This study                 | https://doi.org/10.5281/zenodo.4174464 |
| Raw data and analyses                       | This study                 |                          |
| **Experimental Models: Organisms/Strains**  | This study and other sources | Table S4                |
| *S. cerevisiae*                             | This study and other sources | Table S4                |
| S. pombe        | This study and other sources | Table S4 |
|-----------------|------------------------------|----------|
| Oligonucleotides| This study                   | NA       |

5' GCTTCTAAAATCTAACAGTAGTAAGAATAG AATGAACACCTACCGTAGGGAACAAAAGCTGG 3' (to tag Spt6)

5' GGTCAAGTAATAATTAAAAATTAATAAACAATGG GACACTACATACGCATCTATAGGGGAATTTGG 3' (to tag Spt6)

5' GGGGTAGCGAAGAAAGGCCTTCGGA AAAGCCTAAGGTAAGAAAGGAACAAAAGCTGG 3' (to tag Spt6)

5' TACACAATCAAATAATTAAAAACTTATACGTCT AAAATTATATATAGGGGAATTTGG 3' (to tag Spt6)

5' GGTCAAAGTAATAATAAAATTAATAATAACAATGG GACACTACATACGCATCTATAGGGGAATTTGG 3' (to tag Spt6)

5' GGGGTAGCGACGAAGAAAGGCCTTCGGA AAAGCCTAAGGTAAGAAAGGAACAAAAGCTGG 3' (to tag Pob3)

5' TACACAATCAATAATTAAAAATTAATAACAATGG TAAATTATATAGGGGAATTTGG 3' (to tag Pob3)

5' GGGGTAGCGACGAAGAAAGGCCTTCGGA AAAGCCTAAGGTAAGAAAGGAACAAAAGCTGG 3' (to tag Pob3)

5' TACACAATCAATAATTAAAAATTAATAACAATGG TAAATTATATAGGGGAATTTGG 3' (to tag Pob3)

5' TACACTATGGTTATAGGAAAGGAAG 3' (PMA1 qPCR primer)

5' CAGCATCTTCTGTTACGCTCA 3' (PMA1 qPCR primer)

5' TTCAGATGCAGCAGTCATCGT 3' (PMA1 qPCR primer)

5' TCGTTATGTTTTCGTGGCTCC 3' (PMA1 qPCR primer)

5' GACAAACCGGCAGCCAAAAT 3' (PMA1 qPCR primer)

5' TACACTATGGTTATAGGAAAGGAAG 3' (PMA1 qPCR primer)

5' CAGCATCTTCTGTTACGCTCA 3' (PMA1 qPCR primer)

5' TTCAGATGCAGCAGTCATCGT 3' (PMA1 qPCR primer)

5' TCGTTATGTTTTCGTGGCTCC 3' (PMA1 qPCR primer)

5' GACAAACCGGCAGCCAAAAT 3' (PMA1 qPCR primer)

5' TCGATCAATCTGCTATTACTGGA 3' (PMA1 qPCR primer)

5' ACGATCGCCATTTTCAACA 3' (PMA1 qPCR primer)

5' TCGAAGCTTGCGCTGCTGCT 3' (PMA1 qPCR primer)

5' GCTACTTCAACAGGATAGTTCC 3' (PMA1 qPCR primer)

5' GCTACCTACACTCTATAGGGGAATTTGG 3' (to tag Spn1)

5' TTAAACATCGCCGTTCAGTAG 3' (PMA1 qPCR primer)

5' GGCCCTGATGATAATG 3' (SNR190 Northern probe PCR)

5' GGCTCAGATCTGCATG 3' (SNR190 Northern probe PCR)

5' ACAGATCGCCATTTTCAACA 3' (DSK2 Northern probe PCR)

5' TAAACATCGCCGTTCAGTAG 3' (DSK2 Northern probe PCR)
| DNA Probe | Sequence | Reference | Source |
|-----------|----------|-----------|--------|
| 5' CGTTCCACAGCGCTTGAATG 3' | (SER3 Northern probe PCR) | Martens et al., 2004 | NA |
| 5' CGCCTTTGGTCAACAGAAGAG 3' | (SER3 Northern probe PCR) | Martens et al., 2004 | NA |
| 5' CTATGTGCAAATATCACAAA 3' | (SRG1 Northern probe PCR) | Martens et al., 2004 | NA |
| 5' TTTCCTTATCCTGCTCCC 3' | (SRG1 Northern probe PCR) | Martens et al., 2004 | NA |

**Recombinant DNA**

| Plasmid | Description | Source |
|---------|-------------|--------|
| 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**

| Software | Reference | Website |
|----------|-----------|---------|
| Snakemake | Köster and Rahmann, 2012 | [https://snakemake.readthedocs.io/en/stable/](https://snakemake.readthedocs.io/en/stable/) |
| cutadapt | Martin, 2011 | [https://cutadapt.readthedocs.io/en/stable/](https://cutadapt.readthedocs.io/en/stable/) |
| TopHat2 | Kim et al., 2013 | [https://ccb.jhu.edu/software/tophat/index.shtml](https://ccb.jhu.edu/software/tophat/index.shtml) |
| Samtools | Li et al., 2009 | [http://www.htslib.org/](http://www.htslib.org/) |
| BEDTools | Quinlan and Hall, 2010 | [https://bedtools.readthedocs.io/en/latest/](https://bedtools.readthedocs.io/en/latest/) |
| IDR | Li et al., 2011 | [https://github.com/nboley/idr](https://github.com/nboley/idr) |
| DESeq2 | Love et al., 2014 | [http://bioconductor.org/packages/release/bioc/html/DESeq2.html](http://bioconductor.org/packages/release/bioc/html/DESeq2.html) |
| fastq-multx | Aronesty, 2013 | [https://expressionanalysis.github.io/ea-utils/](https://expressionanalysis.github.io/ea-utils/) |
| Bowtie 2 | Langmead and Salzberg, 2012 | [http://bowtie-bio.sourceforge.net/bowtie2/index.shtml](http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) |
| MACS2 | Zhang et al., 2008 | [https://github.com/macs3-project/MACS](https://github.com/macs3-project/MACS) |
| Bowtie | Langmead et al., 2009 | [http://bowtie-bio.sourceforge.net/index.shtml](http://bowtie-bio.sourceforge.net/index.shtml) |
| DANPOS2 | Chen et al., 2013 | [https://sites.google.com/site/danposdoc/](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) \cite{Li, 2011}, as previously described in \cite{Doris, 2018}, 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 \cite{Love, 2014}, 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 \cite{Doris, 2018}, 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 \( N_{\text{input, spike-in}} / N_{\text{IP, spike-in}} \cdot N_{\text{input, experimental}} \), where \( N_{\text{IP, spike-in}} \) is the number of *S. pombe* alignments in the IP sample, \( N_{\text{input, spike-in}} \) is the number of *S. pombe* alignments in the corresponding input sample, and \( N_{\text{input, experimental}} \) is the number of *S. cerevisiae* alignments in the input sample. Coverage from input samples was normalized to \( N_{\text{input, experimental}} \). 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.