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Supplemental Information

Spt5 Plays Vital Roles in the Control of Sense and Antisense Transcription Elongation

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Figure S1. Related to Figure 1

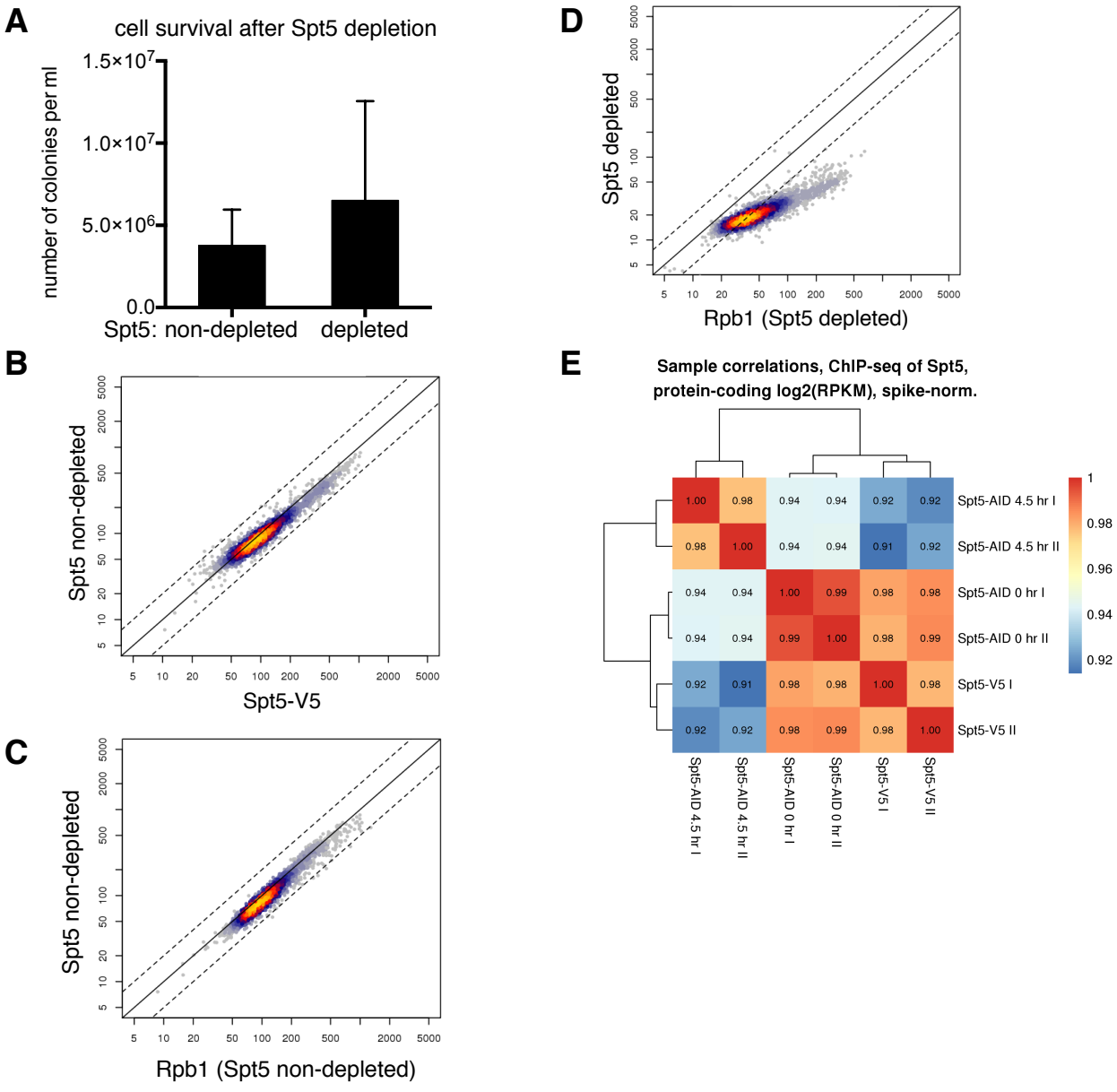


Figure S1. Spt5 depletion. (A) The number of colonies/ml were counted before and after treatment of the Spt5 depletion strain with auxin and thiamine. The graph shows the mean and standard deviation from eight biological replicates. The increased number of colonies after depletion reflects growth during the incubation after treatment. As an independent assay of viability, single cells were micro-manipulated on an EMM plate and incubated for 4-5 days at 32°C. From these results, 100% of cells from an untreated culture formed colonies (49/49 cells) and 75% of the cells from an Spt5-depleted strain formed colonies (47/63 cells) after the 4.5-hour treatment. **(B)** A scatterplot showing the spike-in normalized log2 levels of Spt5 as measured by ChIP-seq under control of its own promoter (Spt5-V5) and under control of the *nmt81* promoter (Spt5 non-depleted). **(C)** A scatterplot showing the spike-in normalized log2 levels of Rpb1 and Spt5 under non-depleted conditions. **(D)** A scatterplot showing the spike-in normalized log2 levels of Rpb1 and Spt5 after Spt5 depletion. **(E)** A correlation heatmap for the comparison of datasets. Pearson correlations were calculated from log2-scaled normalized average signal per gene for spike-in normalized ChIP-seq of Spt5. Spt5-AID 0 hr and 4.5 hr represent non-depleted and depleted; Spt5-V5 is *spt5-V5* expressed from its own promoter.

Figure S2. Related to Figure 2

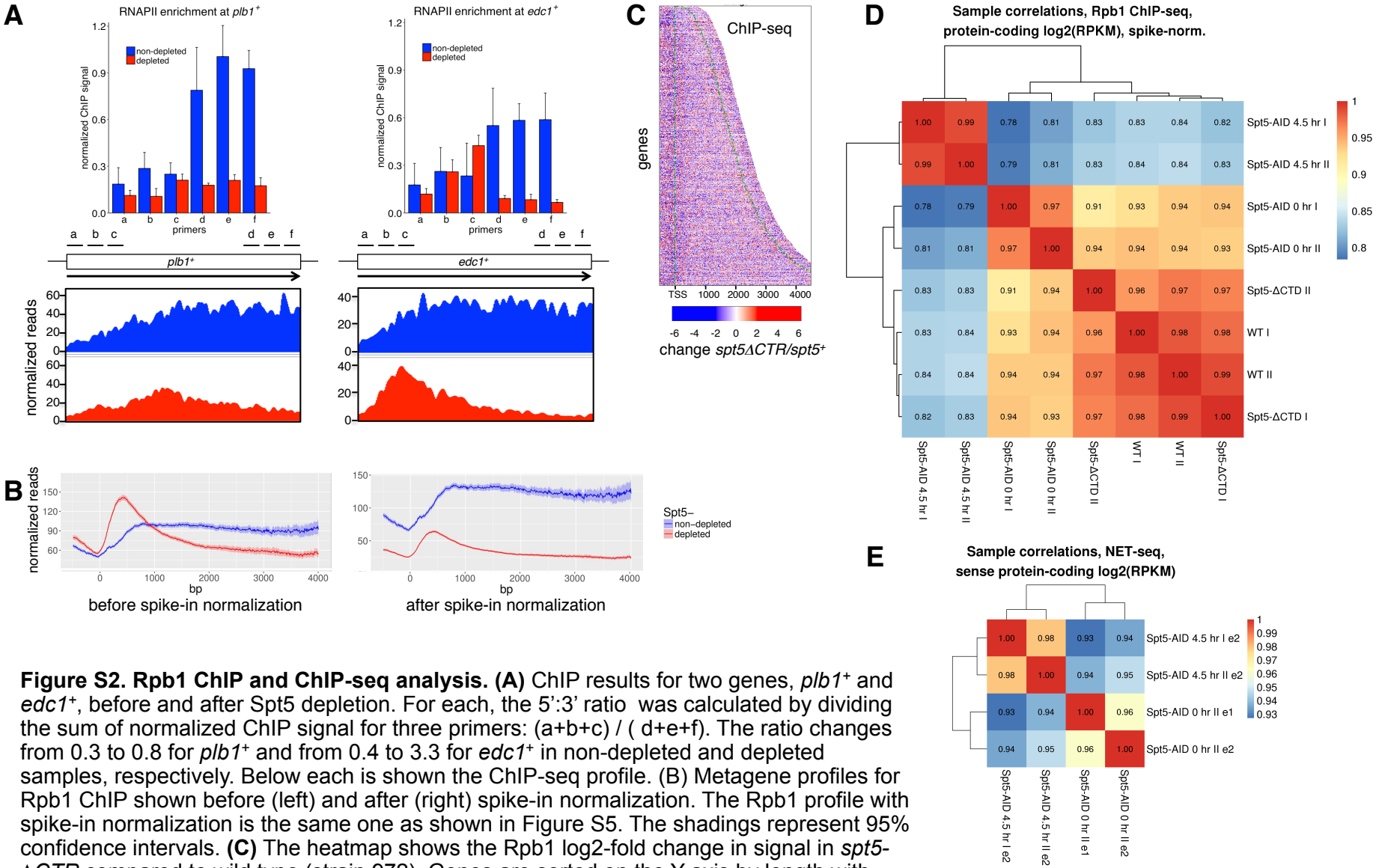
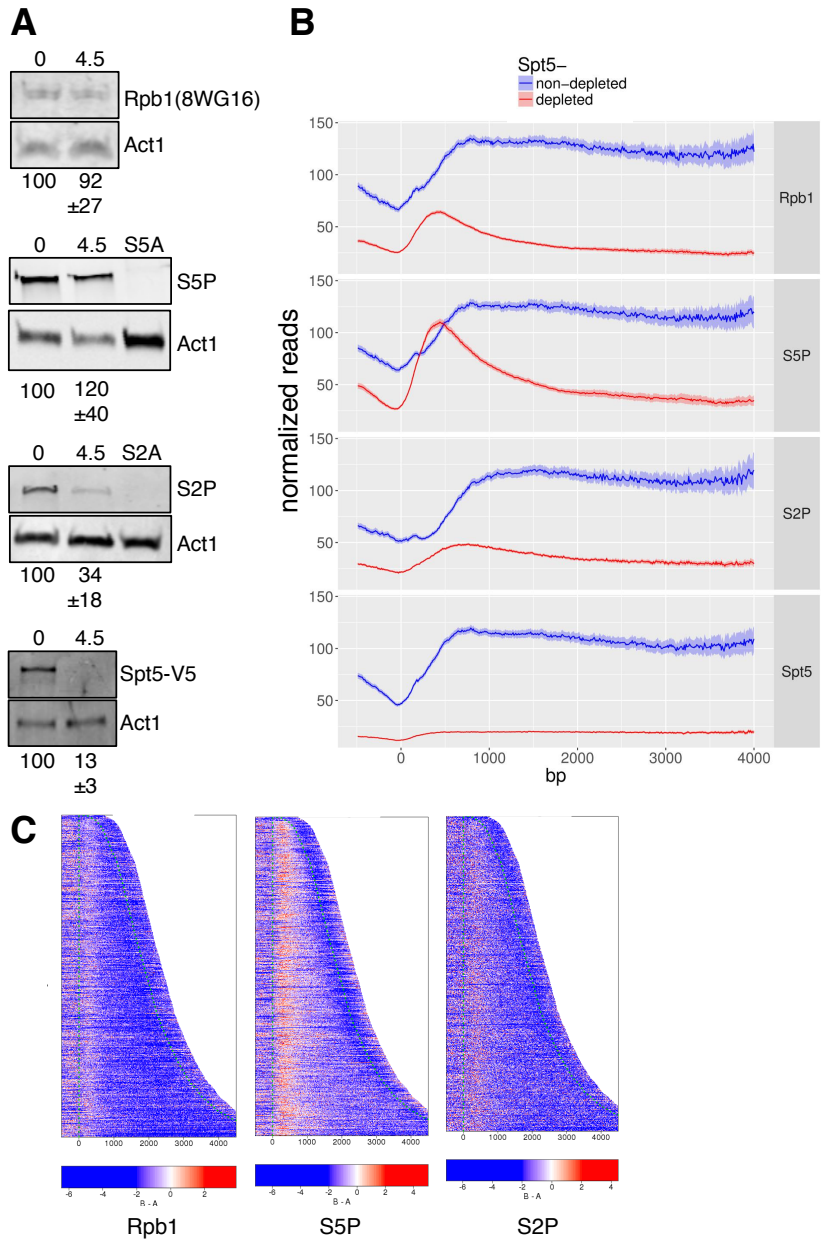
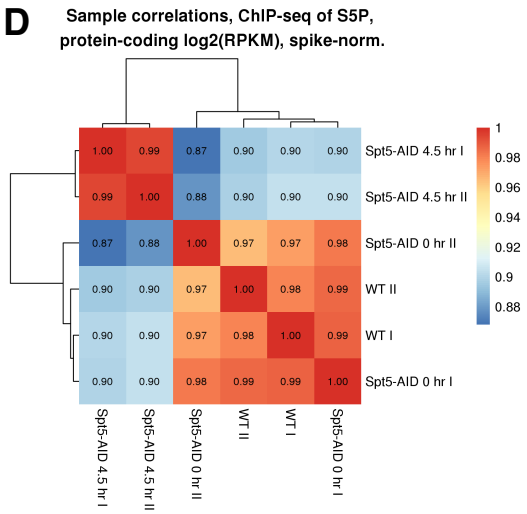
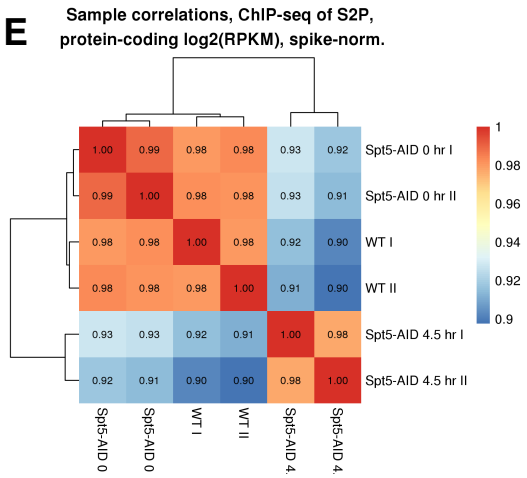


Figure S2. Rpb1 ChIP and ChIP-seq analysis. (A) ChIP results for two genes, *plb1*$^+$ and *edc1*$^+$, before and after Spt5 depletion. For each, the 5':3' ratio was calculated by dividing the sum of normalized ChIP signal for three primers: (a+b+c) / (d+e+f). The ratio changes from 0.3 to 0.8 for *plb1*$^+$ and from 0.4 to 3.3 for *edc1*$^+$ in non-depleted and depleted samples, respectively. Below each is shown the ChIP-seq profile. (B) Metagene profiles for Rpb1 ChIP shown before (left) and after (right) spike-in normalization. The Rpb1 profile with spike-in normalization is the same one as shown in Figure S5. The shadings represent 95% confidence intervals. **(C)** The heatmap shows the Rpb1 log2-fold change in signal in *spt5-ΔCTR* compared to wild type (strain 972). Genes are sorted on the Y-axis by length with their TSS and CPS indicated by the dotted green lines. **(D)** A correlation heatmap for the comparison of Rpb1 ChIP-seq datasets, showing the log2-scaled, spike-in normalized average signal per gene. **(E)** A correlation heatmap for the comparison of NET-seq datasets, showing the log2-scaled normalized average signal per gene.

Figure S3. Related to Figure 2







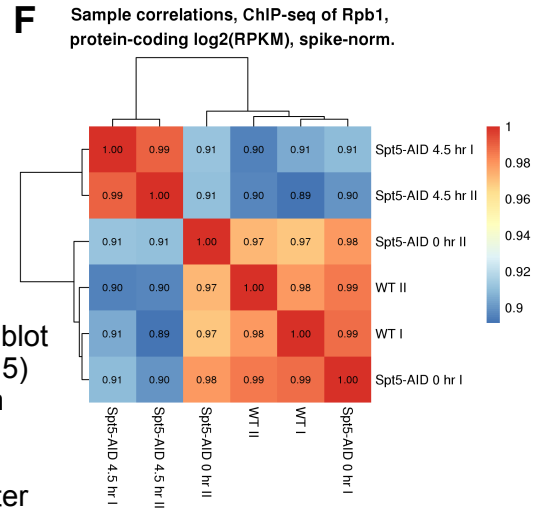


Figure S3. Analysis of Rpb1 phosphorylation. (A) A Western blot of Rpb1, Rpb1-S5P, Rpb1-S2P, and Spt5 before (0) and after (4.5) Spt5 depletion. (The Spt5 Western is the same data as shown in Figure 1.) Shown below each set of panels is the protein level, normalized to Act1 levels. **(B)** Metagene analysis of ChIP-seq results for Rpb1, Rpb1-S5P, Rpb1-S2P, and Spt5, before and after Spt5 depletion. The shading represents 95% confidence intervals. **(C)** Heatmaps that show the log2-fold change in signal before and after Spt5 depletion for Rpb1, Rpb1-S5P, and Rpb1-S2P. Genes are sorted on the Y-axis by length with their TSS and CPS indicated by the dotted green lines. **(D-F)** Correlation heatmaps for comparisons of datasets. Pearson correlations were calculated from log2-scaled normalized average signal per gene. **(D)** Spike-in normalized ChIP-seq of Rpb1-S5P. **(E)** Spike-in normalized ChIP-seq of Rpb1-S2P. **(F)** Spike-in normalized ChIP-seq of Rpb1.

Figure S4. Related to Figures 4 and 5

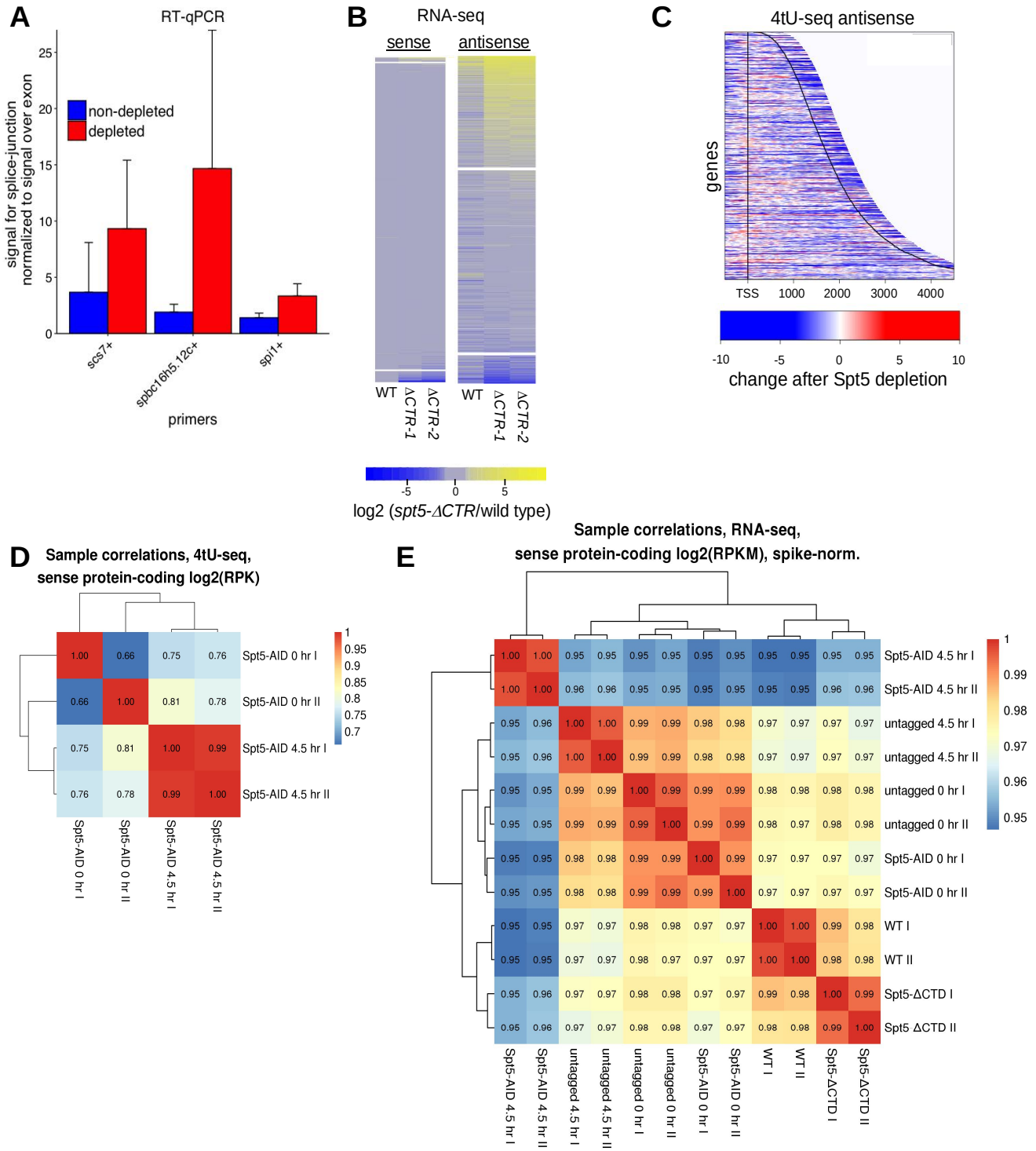


Figure S4. RNA-seq, splicing and 4tU-seq (A) RT-qPCR analysis of splicing for three genes. Bars show signal obtained for a primer set spanning the intron-exon junction normalized to signal obtained from a primer set placed within the exon downstream from the splice junction, in Spt5 depleted and non-depleted cells. The graph shows the mean and standard deviation for three biological replicates. **(B)** Comparison of RNA-seq results in wild-type and *spt5-ΔCTR* strains. Genes are arranged in rows and placed in three different bins, demarcated by the breaks, based on whether their expression is increased greater than two-fold, changed less than two-fold, or decreased greater than two-fold in the average of *spt5-ΔCTR* replicates. **(C)** The heatmap depicts the 4tU-seq log2-fold change in signal obtained for the synthesis of antisense transcripts across transcribed regions in Spt5-depleted compared to non-depleted cells. Genes are sorted on the Y-axis by length with their TSS and CPS indicated by the solid black lines. **(D)** Correlation heatmaps for comparisons of datasets. Pearson correlations were calculated from log2-scaled normalized average signal per gene. Spike-in normalized 4tU-seq. **(E)** Spike-in normalized RNA-seq.

Figure S5. Related to Figure 5

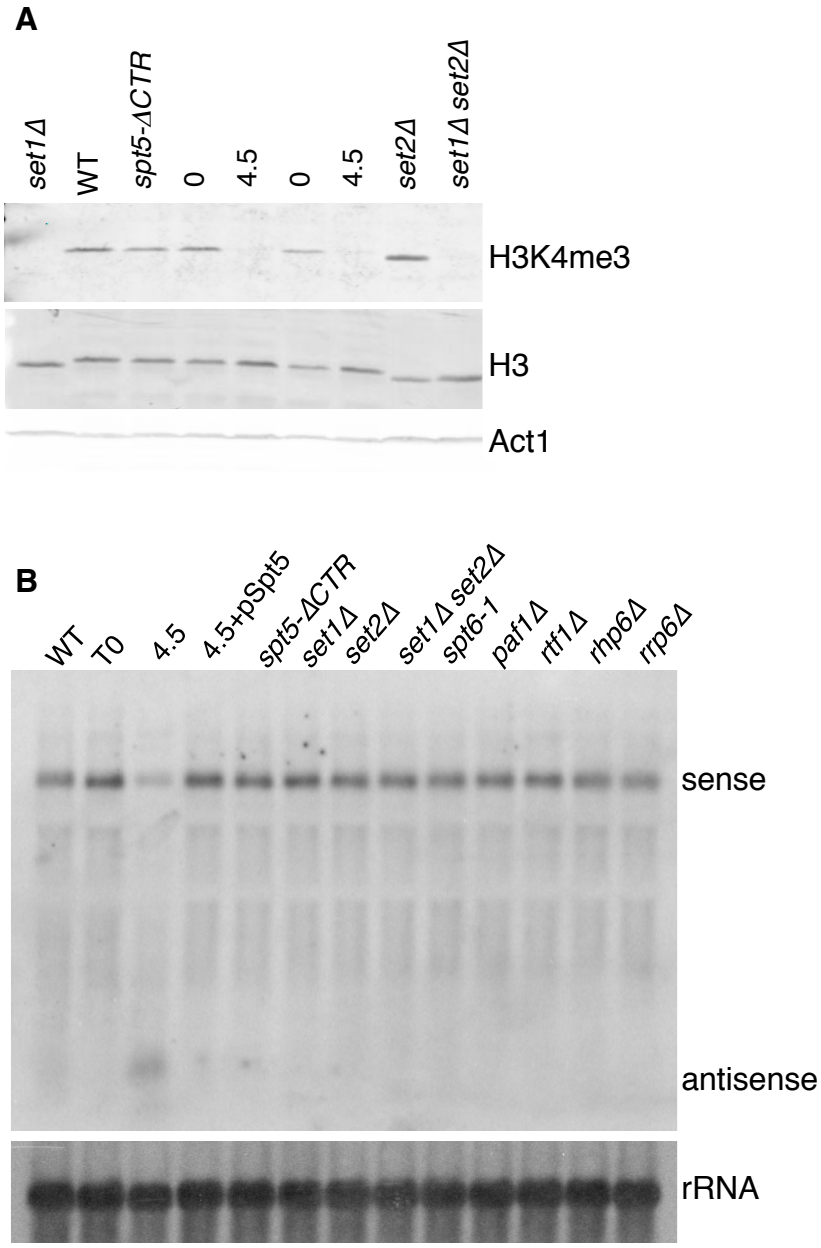


Figure S5. Effect of Spt5 on histone H3K4 modifications and transcription. (A) The levels of H3K4me3 were analyzed in WT(FWP10), *spt5-ΔCTR*, Spt5-non-depleted (T0) and depleted (T4.5) cells. Western blotting was performed by using antibodies against H3K4me3, followed by H3, and Act1 as a loading control. **(B)** Northern analysis of *rif1*$^{+}$ sense and antisense transcripts. A single probe was used that anneals to both transcripts.

Figure S6. Related to Figure 6

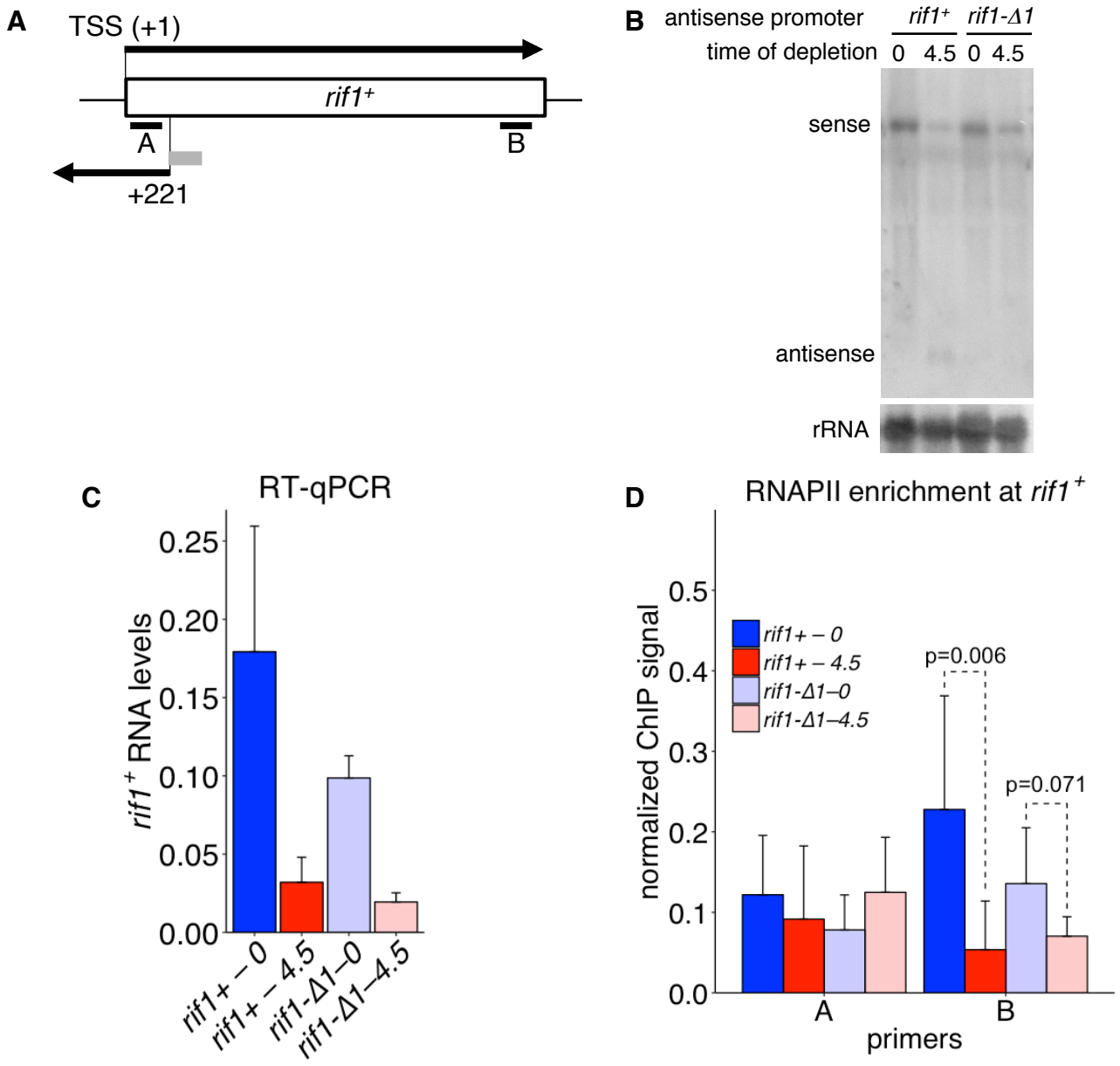


Figure S6. Regulation of RNAPII distribution by convergent antisense transcription. (A) Shown is a diagram of the *rif1*$^+$ gene with sense and antisense transcripts. The small gray box indicates the location of the 51 bp region deleted that includes the TSS and upstream sequences for the antisense transcript. The black bars labeled A and B indicated the regions tested for the level of RNAPII by the ChIP analysis shown in panel D. **(B)** A Northern blot was probed with a probe that detects both the *rif1*$^+$ sense and 5' convergent antisense transcripts. **(C)** RT-qPCR analysis was performed to measure *rif1*$^+$ sense RNA levels normalized to *adg1*$^+$ levels for the strains indicated. The graph shows the mean and standard deviation for three biological replicates. **(D)** ChIP-qPCR shows the enrichment of RNAPII at the *rif1*$^+$ locus. All strains were spiked-in with *S. cerevisiae* chromatin before immunoprecipitation. The ChIP/input signal at *rif1*$^+$ was normalized to the ChIP/input signal at *S. cerevisiae ADH1* gene. The graph shows the mean and standard deviation for six to eight biological replicates. The p values were calculated using Student's *t* test.

Figure S7. Related to Figure 6

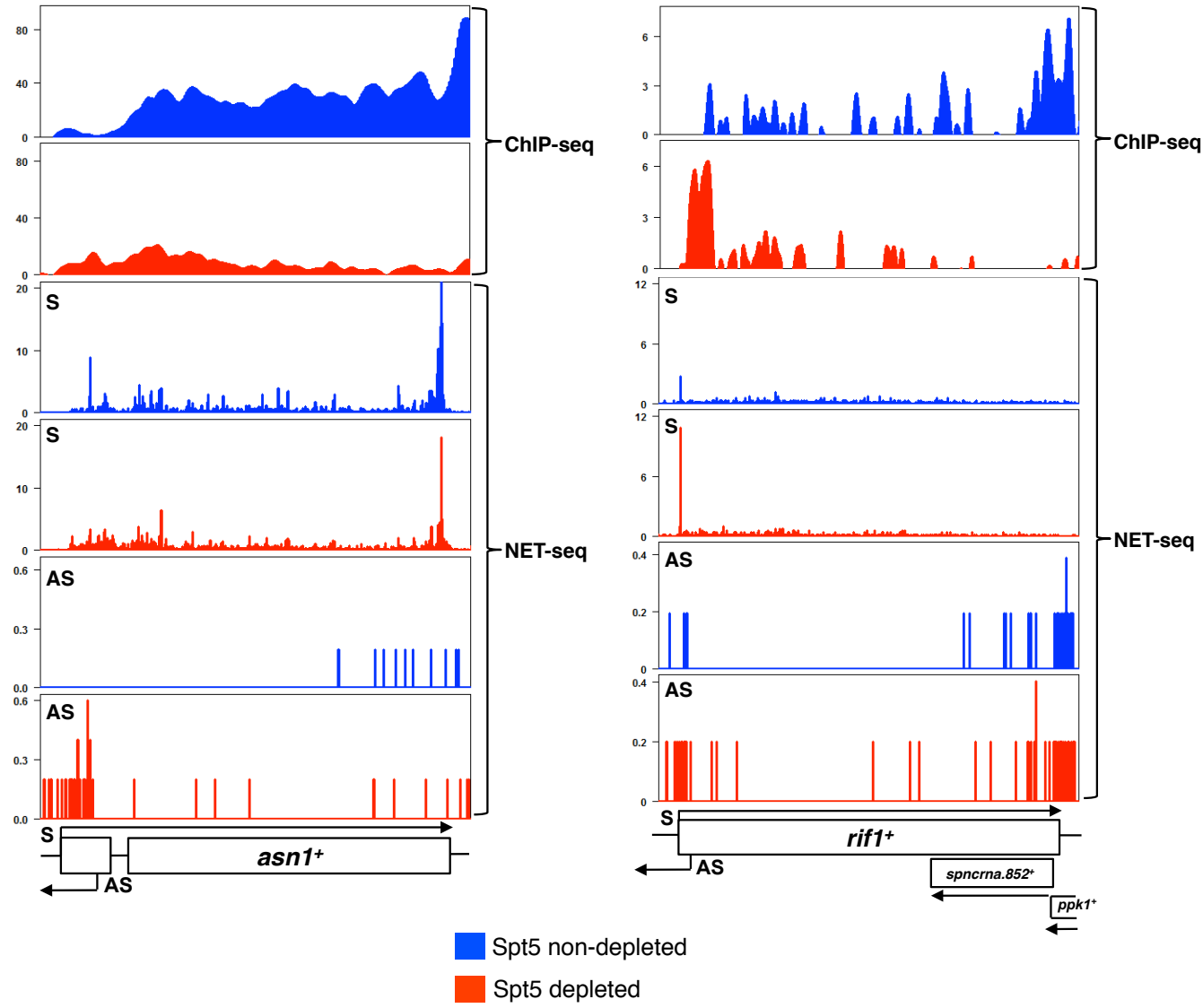


Figure S7. ChIP-seq and NET-seq profiles of the *asn1*$^{+}$ and *rif1*$^{+}$ genes. Shown are profiles for each gene for both Spt5 non-depleted and Spt5 depleted conditions. The arrows below each indicate the sense and antisense transcripts. The numbers on the Y-axis indicate normalized reads.

Table S1: Related to STAR methods. Yeast strains used in this study.

Strain name	Organism	Genotype	Purpose	Source
972	*S. pombe*	*h-*	WT in RNA-seq and ChIP-seq experiments	
FWP16	*S. pombe*	*h+ ura4-D18*	Used as WT for northern blot with antisense promoter deletion strains	Christine Grimm
FWP369	*S. pombe*	*h+ leu1-32 ade6-216 ura4-D18 set1Δ::KanR*	Used for western and northern experiments	Bioneer *S. pombe* deletion collection
FWP371	*S. pombe*	*h- ura4-D18 ade6-m210 leu1-32 spt6-1::NatMX*	Used for western and northern experiments	Winston lab
FWP484	*S. pombe*	*h- ade6::ade6$^+$-Padh15-skp1-OsTIR1-natMX6-Padh15-skp1-AtTIR1-2NLS-9myc ura4-D18*	*spt5* untagged strain used for RNA-seq normalization and ChIP-seq experiments (Strain no. 21104 from YGRC, Japan)	YGRC, Japan, Strain no. 21104
FWP485	*S. pombe*	*h- ade6::ade6+-Padh15-skp1-OsTIR1-natMX6-Padh15-skp1-AtTIR1-2NLS-9myc ura4-D18 spt5-V5-IAA17::kanMX6*	*spt5* under its native promoter fused to V5-AID sequences. Used as control to analyze Spt5 depletion by ChIP-seq	This study
FWP486	*S. pombe*	*h- ade6::ade6$^+$-Padh15-skp1-OsTIR1-natMX6-Padh15-skp1-AtTIR1-2NLS-9myc ura4-D18 hphMX6-nmt81pr::spt5-V5-IAA17::kanMX6*	Spt5-degron strain for ChIP-seq, RNA-seq, and 4tU-seq experiments	This study
FWP487	*S. pombe*	*h- ade6::ade6$^+$-Padh15-skp1-OsTIR1-natMX6-Padh15-skp1-AtTIR1-2NLS-9myc ura4-D18 Rpb3-3xFLAG::ura4$^+$ hphMX6-nmt81pr::spt5-V5-IAA17::kanMX6*	Spt5-degron strain with Rpb3-3xFLAG; used for NET-seq	This study
FWP488	*S. pombe*	*h- ura4-D18 spt5ΔCTR::ura4$^+$*	*spt5-ΔCTR* strain	This study
FWP489	*S. pombe*	*h+ leu1-32 ade6-M216 ura4DS/E otr1R(SphI)::ura4+ rrp6Δ::kanR*	Used for western and northern experiments	Moazed lab; Irvine DV. et al. 2006
FWP490	*S. pombe*	*h- set1D::KanR set2Δ::KanMX leu1-32 ade6-216 ura4-D18*	Used for western and northern experiments	This study
FWP501	*S. pombe*	*h+ ade6-216 leu1-32 ura4-D18 paf1Δ::KanMX*	Used for western and northern experiments	Winston lab; DeGennaro et al. 2013
FWP502	*S. pombe*	*h- ade6-210 leu1-32 ura4-D18 rtf1Δ::KanMX*	Used for western and northern experiments	Winston lab; Degennaro et al. 2013
FWP503	*S. pombe*	*h+ ade6-216 leu1-32 ura4-D18 rhp6Δ::KanMX*	Used for western and northern experiments	Winston lab; DeGennaro et al. 2013
FWP505	*S. pombe*	*h- ade6-210 leu1-32 ura4-D18 set2Δ::KanMX*	Used for western and northern experiments	Winston lab; DeGennaro et al. 2013
FWP541	*S. pombe*	*h- ade6::ade6+-Padh15-skp1-OsTIR1-natMX6-Padh15-skp1-AtTIR1-2NLS-9myc ura4-D18 hphMX6-nmt81pr::spt5-V5-IAA17::kanMX6 asn1-Δ1*	Spt5 -degron strain with *asn1* antisense promoter deletion	This study
FWP543	*S. pombe*	*h- ade6::ade6+-Padh15-skp1-OsTIR1-natMX6-Padh15-skp1-AtTIR1-2NLS-9myc ura4-D18 hphMX6-nmt81pr::spt5-V5-IAA17::kanMX6 rif1-Δ1*	Spt5-degron strain with *rif1* antisense promoter deletion	This study
FWP544	*S. pombe*	*h- rpb1-S5A$_{18}$-MCE1::natMX4 ade6 (216 or 210) leu1-32 ura4-D18 his3*	Rpb1-S5A strain, used as control for western experiment	Beate Schwer; Schwer et al. 2011
FWP546	*S. pombe*	*h- rpb1-S2A$_{29}$:: natMX4 ade6 (216 or 210) leu1-32 ura4-D18 his3*	Rpb1-S2A-strain, used as control for western experiment	Beate Schwer; Schwer et al. 2014
FY2912	*S. cerevisiae*	*MATα ura3-52 his4-912d lys2-128d Rpb3::3xFlag-NatMX*	Spike-in for NET-seq	Winston lab
FY3111	*S. cerevisiae*	*MATα SPT5::V5-kanMX6*	Spike-in for ChIP-seq to analyze delepletion of Spt5	This study

List of *S. pombe* and *S. cerevisiae* strains along with their experimental purpose.

Table S2: Related to STAR methods. Oligos used in this study.

Oligo Name	Oganism	Gene	Sequence	Purpose
FO9564	*S. pombe*	*18S rDNA*	GTTGTTGCAGTTAAAAAGCTCGTA	template for northern probe
FO10691	*S. pombe*	*18S rDNA*	CATTACGGCGGTCCTAGAAA	template for northern probe
FO10692	*S. pombe*	*adg1+*	TGCCAGCATTCATGTTCCTA	RT-qPCR
FO10693	*S. pombe*	*adg1+*	GGCAGAGCTAACACGGTCTC	RT-qPCR
FO9615	*S. pombe*	*asn1+*	GCCCAATTCTAGGAGATACCGTC	ChIP-qPCR
FO9723	*S. pombe*	*asn1+*	TCCCGGAATCTCTACATTTCA	ChIP-qPCR
FO10694	*S. pombe*	*asn1+*	CTAAATAGCTTCCGTTTTGGAGTTTACATTCTAAAACATCTA ACACCTAATTCTGAACTGATTGTTGGCTCGGATCCCCGGG TTAATTAA	integrate *ura4+* for deleting antisense promoter
FO10695	*S. pombe*	*asn1+*	AAAAATGGAAGATAATAAAAAATTTTTATCAACGCAGCTGG AATGATAGTTACCTTTTAAGATATGTAAAGAATTCGAGCTC GTTTAAAC	integrate *ura4+* for deleting antisense promoter
FO10696	*S. pombe*	*asn1+*	ATCTTTCGGCGGCAAACTA	delete convergent antisense promoter
FO10697	*S. pombe*	*asn1+*	CGACAAATGAAGAGCCTTGG	delete convergent antisense promoter
FO10698	*S. pombe*	*asn1+*	TACCTTTTAAGATATGTAAAAGCCAACAATCAGTTCAGAA	delete convergent antisense promoter
FO10699	*S. pombe*	*asn1+*	TTCTGAACTGATTGTTGGCTTTTACATATCTTAAAAGGTA	delete convergent antisense promoter
FO10700	*S. pombe*	*asn1+*	GGGAATGGGGTGACGATATT	ChIP-qPCR
FO10701	*S. pombe*	*asn1+*	TCAGCACACTGTCTAGGGAAGA	ChIP-qPCR
FO9721	*S. pombe*	*asn1+*	ATCTTGTAAATCATAAATTGAATACCG	template for northern probe
FO9724	*S. pombe*	*asn1+*	TGTAAACTCCAAAACGGAAGC	template for northern probe
FO10702	*S. pombe*	*asn1+*	TTTTTATCAACGCAGCTGGA	RT-qPCR
FO10703	*S. pombe*	*asn1+*	CTCTTCCAAGGAGGGAATGG	RT-qPCR
FO10704	*S. pombe*	*plb1+*	CCCTCTCTTCGGAATGTTTTT	ChIP-qPCR
FO10705	*S. pombe*	*plb1+*	TTGGAGCCGTCAACAAAACT	ChIP-qPCR
FO10706	*S. pombe*	*plb1+*	GCGAGGAATTTTGCATTTTC	ChIP-qPCR
FO10707	*S. pombe*	*plb1+*	TGCGAAAACGCTCTCTAACC	ChIP-qPCR
FO10708	*S. pombe*	*plb1+*	CACCATCGCAATCAAACAAC	ChIP-qPCR
FO10709	*S. pombe*	*plb1+*	GGAAGATGATTAGGGATTCCA	ChIP-qPCR
FO10710	*S. pombe*	*plb1+*	GATTCCCTCATTCCTTCATCA	ChIP-qPCR
FO10711	*S. pombe*	*plb1+*	GGAAATCGCACCAAAGGATA	ChIP-qPCR
FO10712	*S. pombe*	*plb1+*	GCGCATGCAAACTTTTCTCT	ChIP-qPCR
FO10713	*S. pombe*	*plb1+*	GGCATAAGCATTGCCCTCTA	ChIP-qPCR
FO10714	*S. pombe*	*plb1+*	CCAATTACGCGATATGGGTTT	ChIP-qPCR
FO10715	*S. pombe*	*plb1+*	TCTCCCTAATTAGAGCGGAGTG	ChIP-qPCR
FO10716	*S. pombe*	*edc1+*	AGTGTTGCCCCCGAAGTTAT	ChIP-qPCR
FO10717	*S. pombe*	*edc1+*	TCCCTAATGATCATGGATTCTG	ChIP-qPCR
FO10718	*S. pombe*	*edc1+*	GAGCAATGGTTGCTTCCAG	ChIP-qPCR
FO10719	*S. pombe*	*edc1+*	TACCATCTCGTTGGGGCATA	ChIP-qPCR
FO10720	*S. pombe*	*edc1+*	AGCAATGGTAAGCGAAATGG	ChIP-qPCR
FO10721	*S. pombe*	*edc1+*	ACGAGAGCCAGCATACGAAC	ChIP-qPCR
FO10722	*S. pombe*	*edc1+*	CAAATAATGTTGCATTAAGTGGTGA	ChIP-qPCR
FO10723	*S. pombe*	*edc1+*	TACACAAACGCGACACATCA	ChIP-qPCR
FO10724	*S. pombe*	*edc1+*	TCTTTCACTCGCAAATCGTT	ChIP-qPCR
FO10725	*S. pombe*	*edc1+*	TTTTAAAAAGAAATGATTCCAAAAC	ChIP-qPCR
FO10726	*S. pombe*	*edc1+*	CATGCAATGTGCTTTATGCTT	ChIP-qPCR
FO10727	*S. pombe*	*edc1+*	AGCAATGAAAGCTCAAAAACC	ChIP-qPCR
FO9677	*S. pombe*	*rif1+*	GATCGGTCCCATGAAGCTAA	delete convergent antisense promoter
FO9752	*S. pombe*	*rif1+*	CGGAACAAAACGAGGATTGTA	delete convergent antisense promoter
FO10728	*S. pombe*	*rif1+*	TGCTGTGAAGGAGGCTTCAAATATGCTTCTACAAGAACCTT CAACCCCATCATCACAAGCTGTAGGACTGCGGATCCCCGG GTTAATTAA	integrate *ura4+* for deleting antisense promoter
FO10729	*S. pombe*	*rif1+*	AAGTTTTTAAGATACCTCGTTTGGGTAAACCGAAAGATGGT CGATTTCCACCGGGGGAATTTTCTAGTTCGAATTCGAGCT CGTTTAAAC	integrate *ura4+* for deleting antisense promoter
FO10730	*S. pombe*	*rif1+*	CCGGGGGAATTTTCTAGTTCCAGTCCTACAGCTTGTGATG	delete convergent antisense promoter
FO10731	*S. pombe*	*rif1+*	CATCACAAGCTGTAGGACTGGAACTAGAAAATTCCCCCGG	delete convergent antisense promoter
FO10732	*S. pombe*	*rif1+*	GCGATCGGTCCCATGAAG	ChIP-qPCR
FO10733	*S. pombe*	*rif1+*	TTTGAAGCCTCCTTCACAGC	ChIP-qPCR
FO10734	*S. pombe*	*rif1+*	TGGTTTCCGATAATCAGCTTG	ChIP-qPCR
FO10735	*S. pombe*	*rif1+*	TTAAGGGGCTAATTGGGACA	ChIP-qPCR
FO9765	*S. pombe*	*rif1+*	TTTTTCAGTGTTGTTAAAGATCTCA	template for northern probe
FO9678	*S. pombe*	*rif1+*	CGAATCGAACTACTAGGTGAGCT	template for northern probe
FO10736	*S. pombe*	*rif1+*	TGGTTTCCGATAATCAGCTTG	RT-qPCR
FO10737	*S. pombe*	*rif1+*	TTAAGGGGCTAATTGGGACA	RT-qPCR
FO9504	*S. pombe*	*rpb3+*	CAAATTTTAAAGTGACTCTTAGGTTTTAAAACGAAAATTCTT ATTCTTGAGTAACTCTTTCCTGTAGGTCCGCCAGGGTTTTC CCAGTCA	C-terminal tagging with 3x-FLAG-*ura4+*
FO9505	*S. pombe*	*rpb3+*	GTATTTTTTATAACAACAAACATATAAAGGATGAAAATGGAT AATTTTTAGAAAAGAATTGAAGCATAGTAGCGGATAACAAT TTCACAC	C-terminal tagging with 3x-FLAG-*ura4+*
FO9145	*S. pombe*	*spt5+*	TAT ATT AGA TTA AAG GTC TAA AAA CAA AGC ATT GAG GGA ATA GGT ACT ATT TCC TCT TTT TTA AGT CAC AGA ATT CGA GCT CGT TTA AAC	place *spt5+* under control of *nmt81* promoter
FO9146	*S. pombe*	*spt5+*	CATCTTGCTCAGCTGCATCTACCTCAGTCGAATTTGCATCT TTATCAATAGATTTCGGAGAATTCGTATCCATGATTTAACAA AGCGACTATA	place *spt5+* under control of *nmt81* promoter
FO9442	*S. pombe*	*spt5+*	GATCCTAGTCGGTACCAGTAAGAAGATAGTATATGGACAC CACCTT	clone Spt5 with sequences upstream and downstream of the gene into pFY20
FO9443	*S. pombe*	*spt5+*	CTGTACGTACCCCGGGCAAAAGGCCATGGCTAAAAAGGAA AA	clone Spt5 with sequences upstream and downstream of the gene into pFY21
FO9453	*S. pombe*	*spt5+*	GGCTGCTCCCACACCAGGTGGTTGGGATGATGAAGAAGG AGATTCACCCAAATATGTACCTCCTTCTCCTGCGGCCGCT CTAGAACTAGT	C-terminal tagging with V5-AID
FO9454	*S. pombe*	*spt5+*	GTT AAT TAG AAT ATG TCT TAA CAA TTT TAA CAA AAC TAT CTG TCG ATA TTT TCA AAA ATT TGA TTT TAA ACC CCC TCG AGG TCG ACG GTA	C-terminal tagging with V5-AID
FO9478	*S. pombe*	*spt5+*	GCGTTCTAGAGGAATAAGACCTGGTAGTTAATTTAAAATCA AATTTTTGAAAATATC	delete Spt5-CTD
FO9479	*S. pombe*	*spt5+*	GATATTTTCAAAAATTTGATTTTAAATTAACTACCAGGTCTT ATTCCTCTAGAACGC	delete Spt5-CTD
F09181	*S. cerevisiae*	*ADH1*	TCCTTGTTTCTTTTTCTGCAC	for ChIP-qPCR_normalization
FO9182	*S. cerevisiae*	*ADH1*	GAGATAGTTGATTGTATGCTTGG	for ChIP-qPCR_normalization
FO9183	*S. cerevisiae*	*ADH1*	AGCCGCTCACATTCCTCAAG	for ChIP-qPCR_normalization
FO9184	*S. cerevisiae*	*ADH1*	ACGGTGATACCAGCACACAAGA	for ChIP-qPCR_normalization

List of oligos along with their experimental purpose.