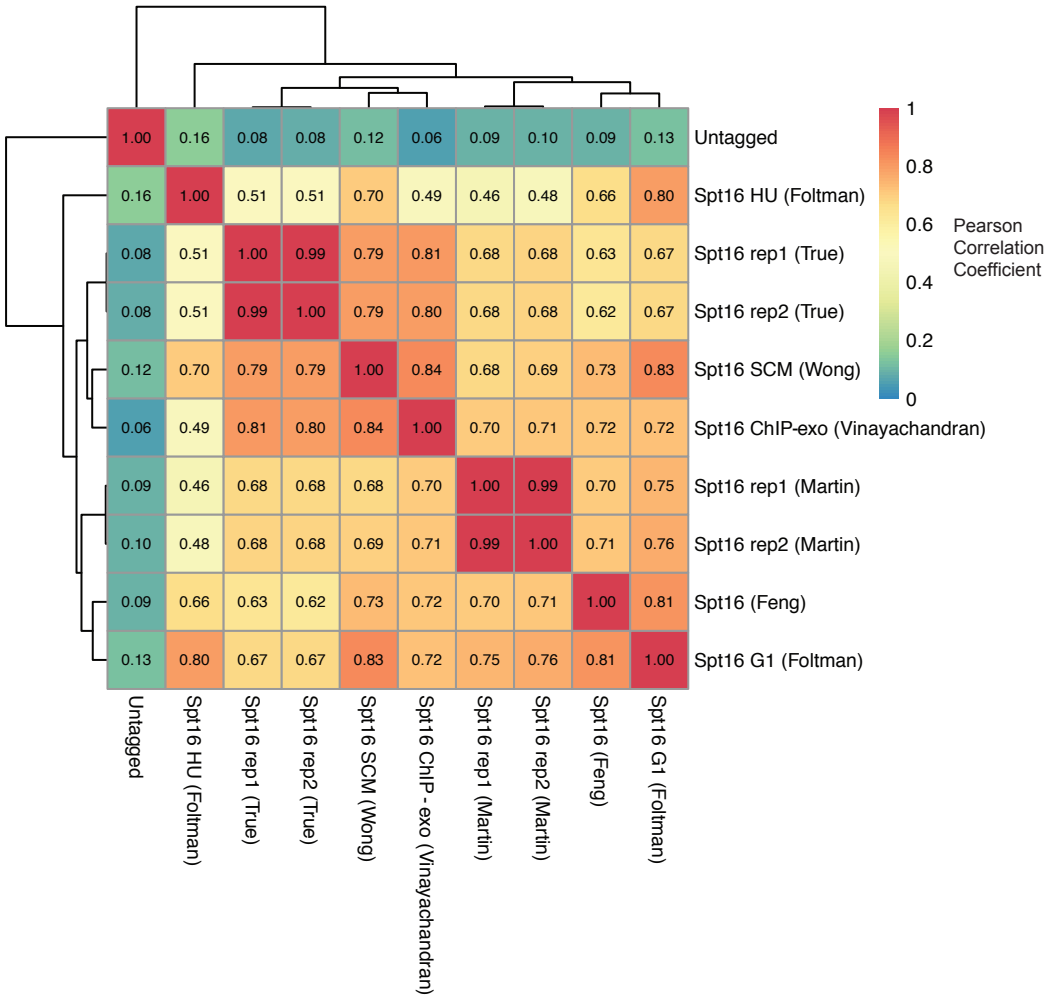

Figure S1: Comparison of Spt16 ChIPs. Pearson correlation matrix for sequence coverage of αHA ChIP from Spt16-HA_6 [two independent replicates labelled “Spt16 rep1 (Martin)” and “Spt16 rep2 (Martin)”] and from an untagged strain (”untagged”), with previously published Spt16 ChIP-seq and ChIP-exo experiments, across genome-wide 250 bp bins.

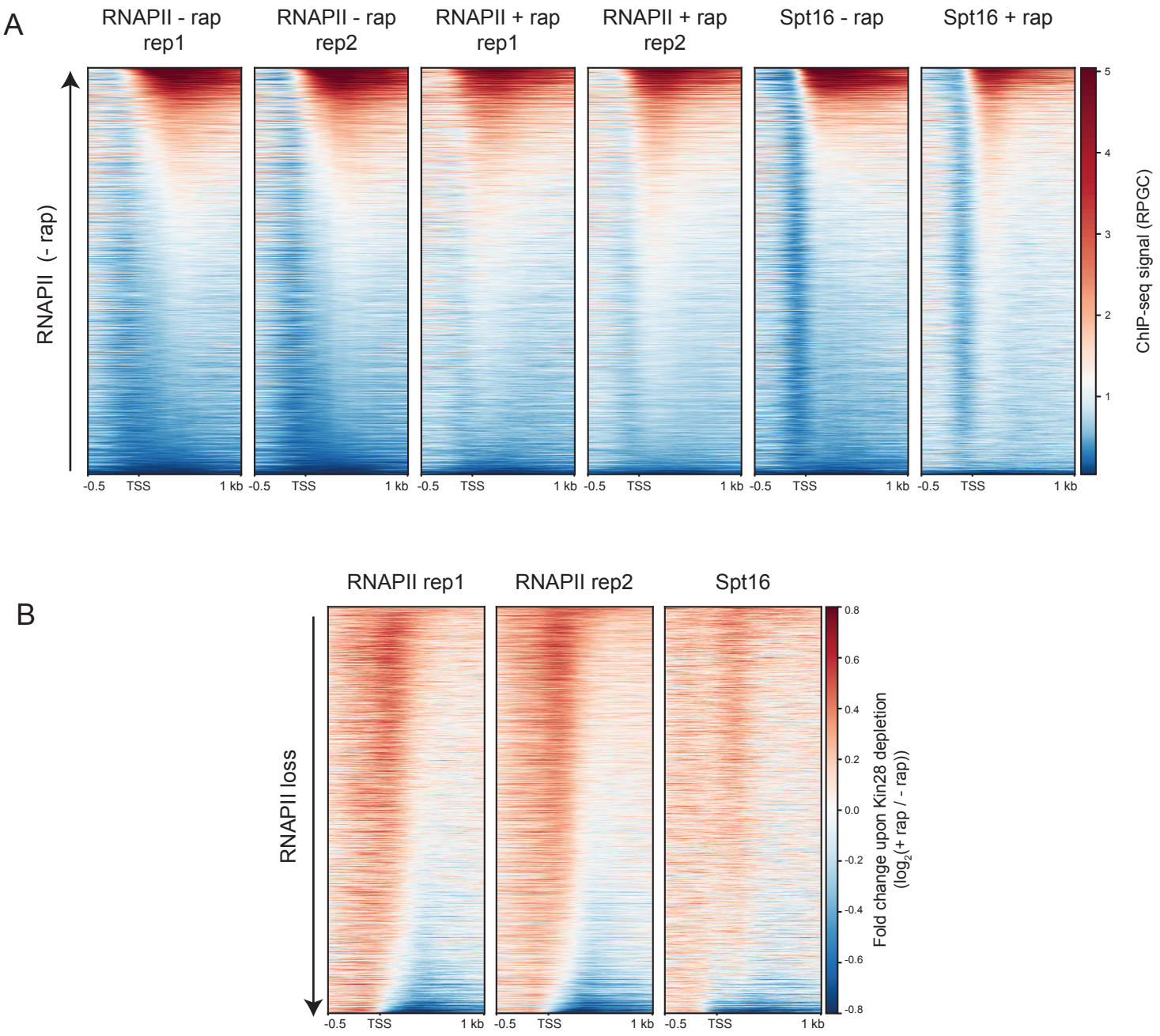

Figure S2: Changes in RNAPII and FACT following depletion of Kin28. A) RNAPII and Spt16 sequence coverage (Wong et al., 2014, downloaded from SRP036647) relative to 5502 TSSs, ordered by RNAPII signal (in first 500 bp) in untreated samples. RPGC is the reads per genomic content (reads normalized to 1x sequencing depth with sequencing depth defined as mapped reads x fragment length/effective genome size). B) The fold-change (log_2) in RNAPII upon TFIIH depletion represented by heatmap. Genes are ordered by the average fold change (in the first 500 bp) in RNAPII upon rapamycin treatment.

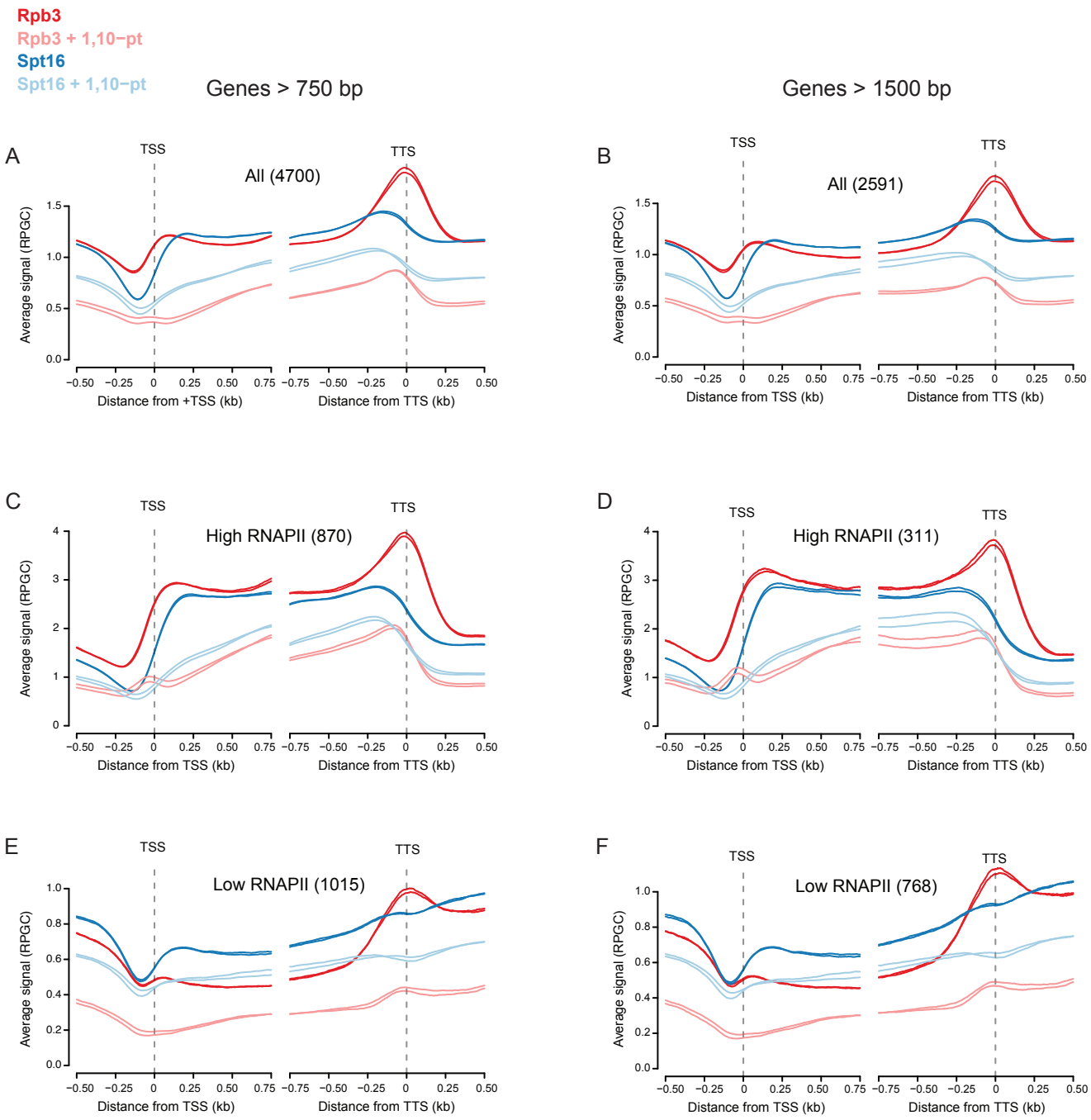

Figure S3: The interaction of FACT with chromatin is dependent on transcription. Rpb3 and Spt16-HA$_6$ sequence coverage (two independent replicates shown), from cells with and without treatment with 1,10-phenanthroline (1,10-pt), relative to the TSS and TTS for all transcription levels (A and B), for the top (C and D), and for the bottom (E and F) 20% of transcribed genes. Sequence coverage profiles are shown for transcripts longer than 750 bp (A, C, and E) or longer than 1500 bp (B, D, and F). Fragment coverage was normalized using “spike-in” control DNA. RPGC is the reads per genomic content (reads normalized to 1x sequencing depth with sequencing depth defined as mapped reads x fragment length/effective genome size).

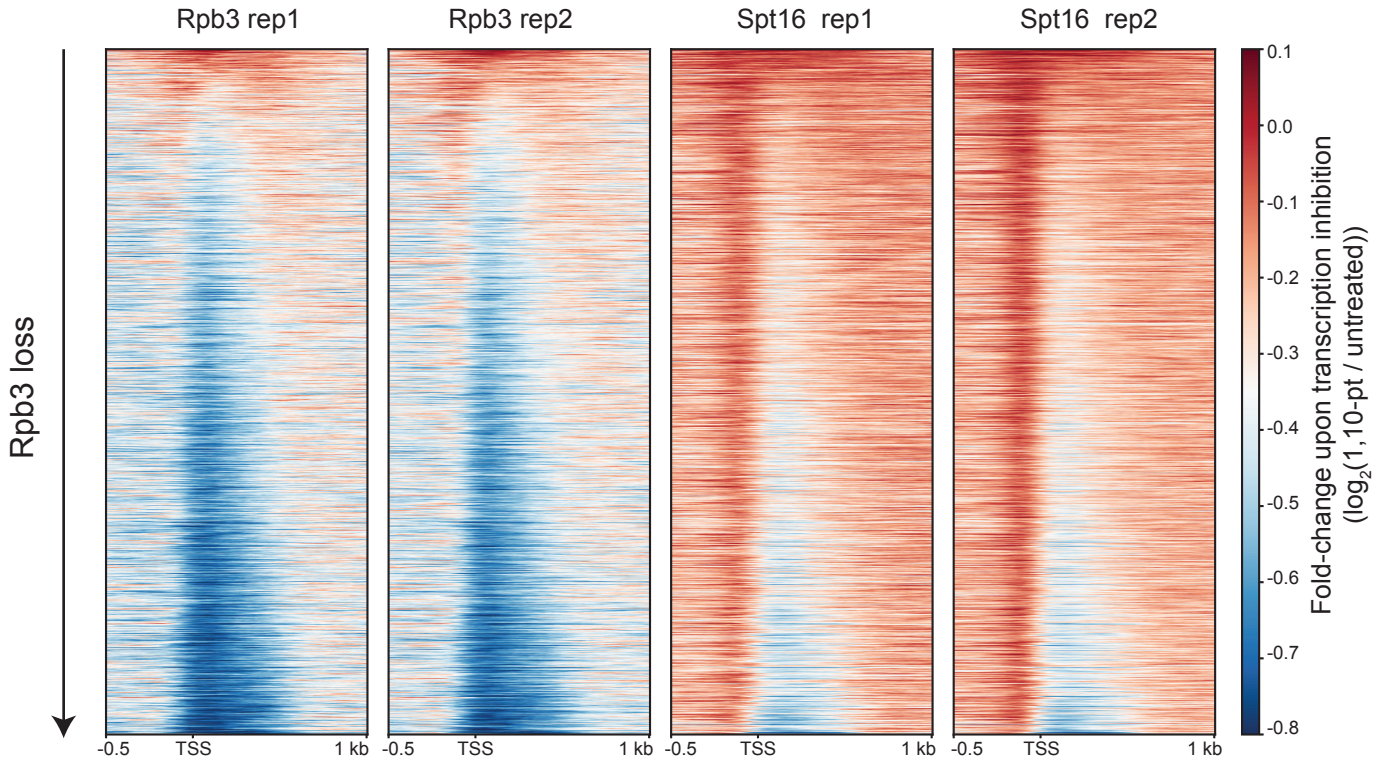

Figure S4: The interaction of FACT with chromatin is dependent on transcription. The fold-change (log_2) in Rpb3 and Spt16 (two independent replicates labelled “rep1” and “rep2”) relative to 5502 TSS upon transcription inhibition represented by heatmap . Genes were ordered by the average fold-change (in the first 500 bp) of Rpb3 upon 1,10-pt treatment.

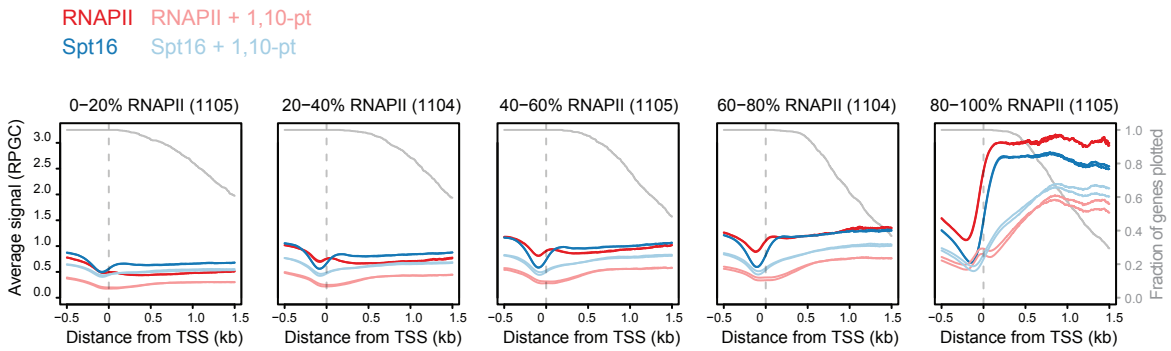

Figure S5: The interaction of FACT with chromatin is dependent on transcription. Average Rpb3 and Spt16-HA_6 sequence coverage (two independent replicates shown) relative to the TSS of genes, with the indicated quintiles of Rpb3 binding, prior to or following 15 minute treatment with 1,10-phenanthroline (1,10-pt). Fragment coverage was normalized using “spiked-in” control DNA (see methods). RPGC is the reads per genomic content (reads normalized to 1x sequencing depth with sequencing depth defined as mapped reads x fragment length/effective genome size).

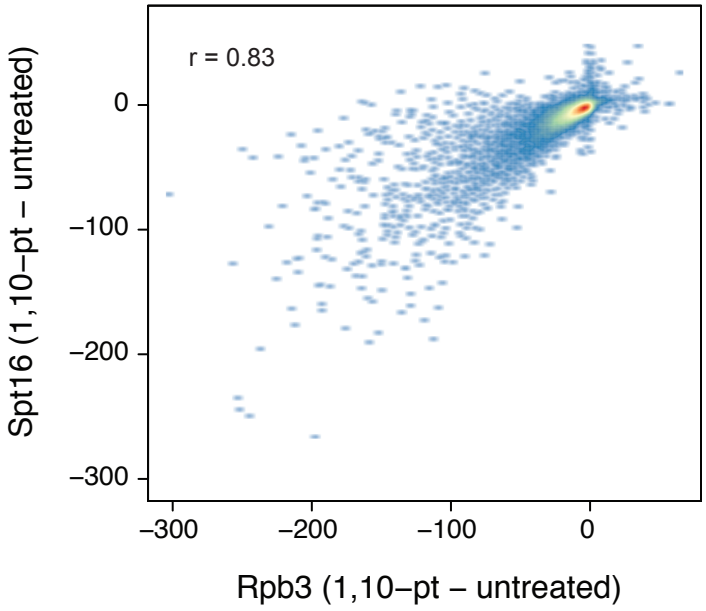

Figure S6: Genome-wide changes in FACT and RNAPII following transcription inhibition. Changes in Rpb3 and Spt16-HA_6 ChIP-seq fragment midpoint counts per million fragments in 250 bp genome-wide windows following transcription inhibition with 1,10-phenanthroline (1,10-pt) shown by smoothed scatter plot. Pearson correlation coefficient is indicated as 'r'.

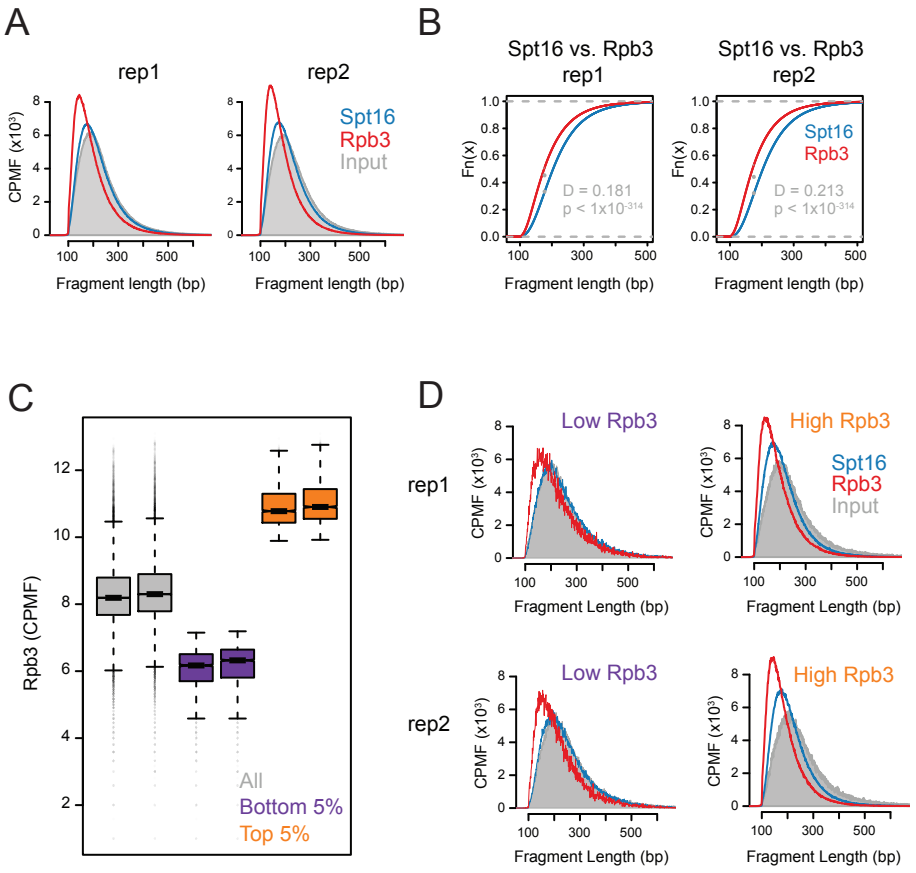

Figure S7: DNA ChIPped with Spt16 from sonicated extracts shows length differences from input and Rpb3 ChIPped DNA. A) Histograms depicting DNA fragment lengths recovered from input, Rpb3 and Spt16-HA_6 ChIPs from sonicated chromatin (two independent replicates, labelled "rep1" and "rep2"). B) Kolmogorov–Smirnov test of the distribution of fragment lengths in the Spt16-HA_6 and Rpb3 ChIPs in (A). C) Rpb3 levels in 250 bp genome-wide bins, depicted by boxplots, for all, the top 5% of Rpb3-bound bins, and the bottom 5% of Rpb3-bound bins. D) Histograms depicting DNA fragment lengths recovered from input, and Rpb3 and Spt16-HA_6 ChIPs from sonicated chromatin, for fragments overlapping the top and bottom 5% of Rpb3-bound 250 bp genome-wide bins identified in (C). CPMF is counts per million fragments.

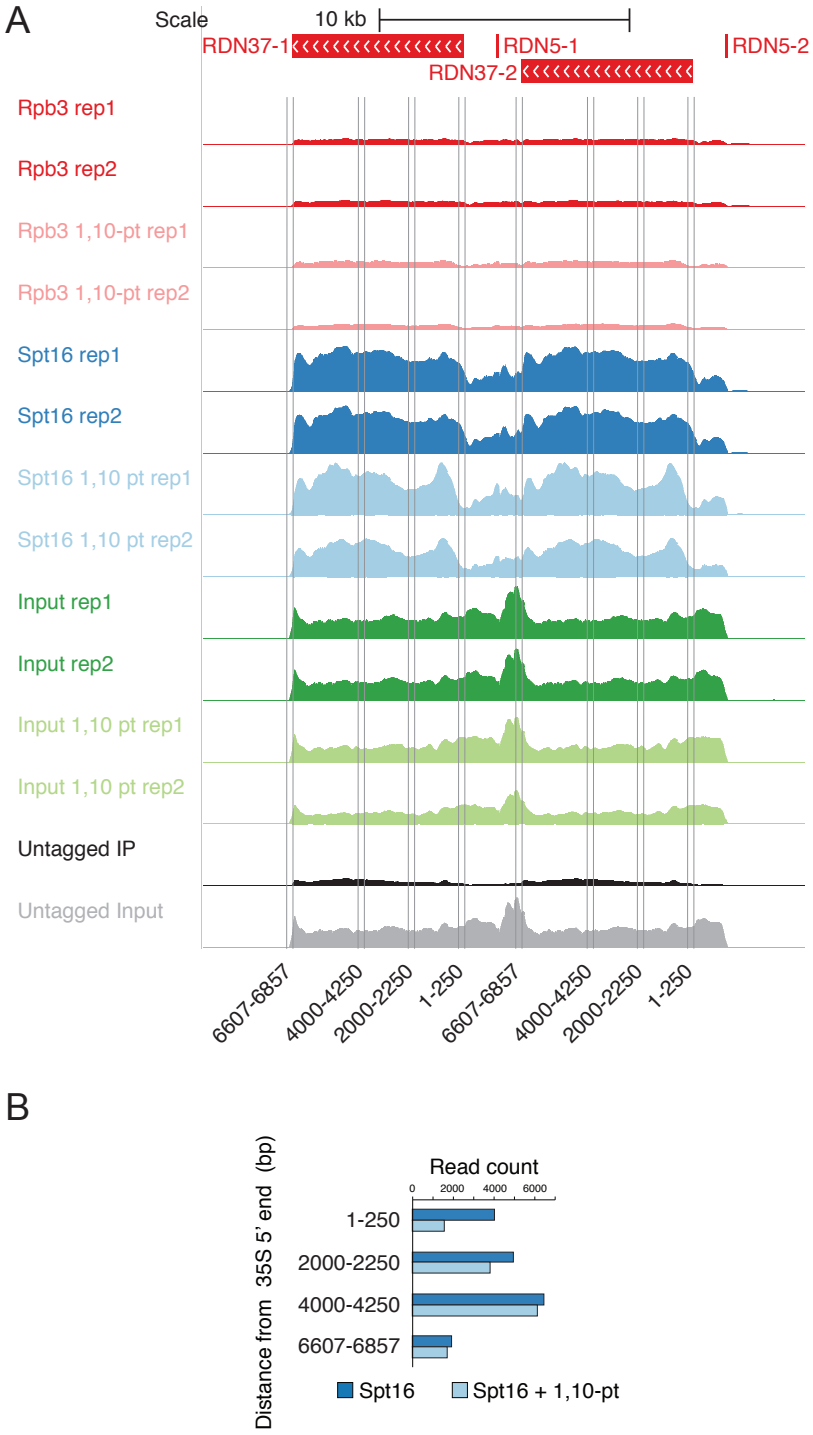

Figure S8: The interaction of FACT with RNAPI-transcribed regions is dependent on transcription. A) Genome browser screenshots of ChIP-seq data for the indicated proteins (two independent replicates, labelled "rep1" and "rep2"), over over chrXII 448000-472000. The positions of two 35S (RDN37) and 5S (RDN5) rDNA gene repeats are indicated in red. Datasets were normalized to genomic mean and samples from 1,10-phenanthroline-treated cells (1,10-pt) were further normalized to data from untreated cells based on spike-in controls. Boundaries for bins analyzed in (B) are indicated with grey lines. B) Spike-in normalized Spt16-HA_6 ChIP-seq read counts from cells with and without treatment with 1,10-phenanthroline over the bins relative to the 5' end of the 35S rRNA genes, as indicated in (A), were plotted.

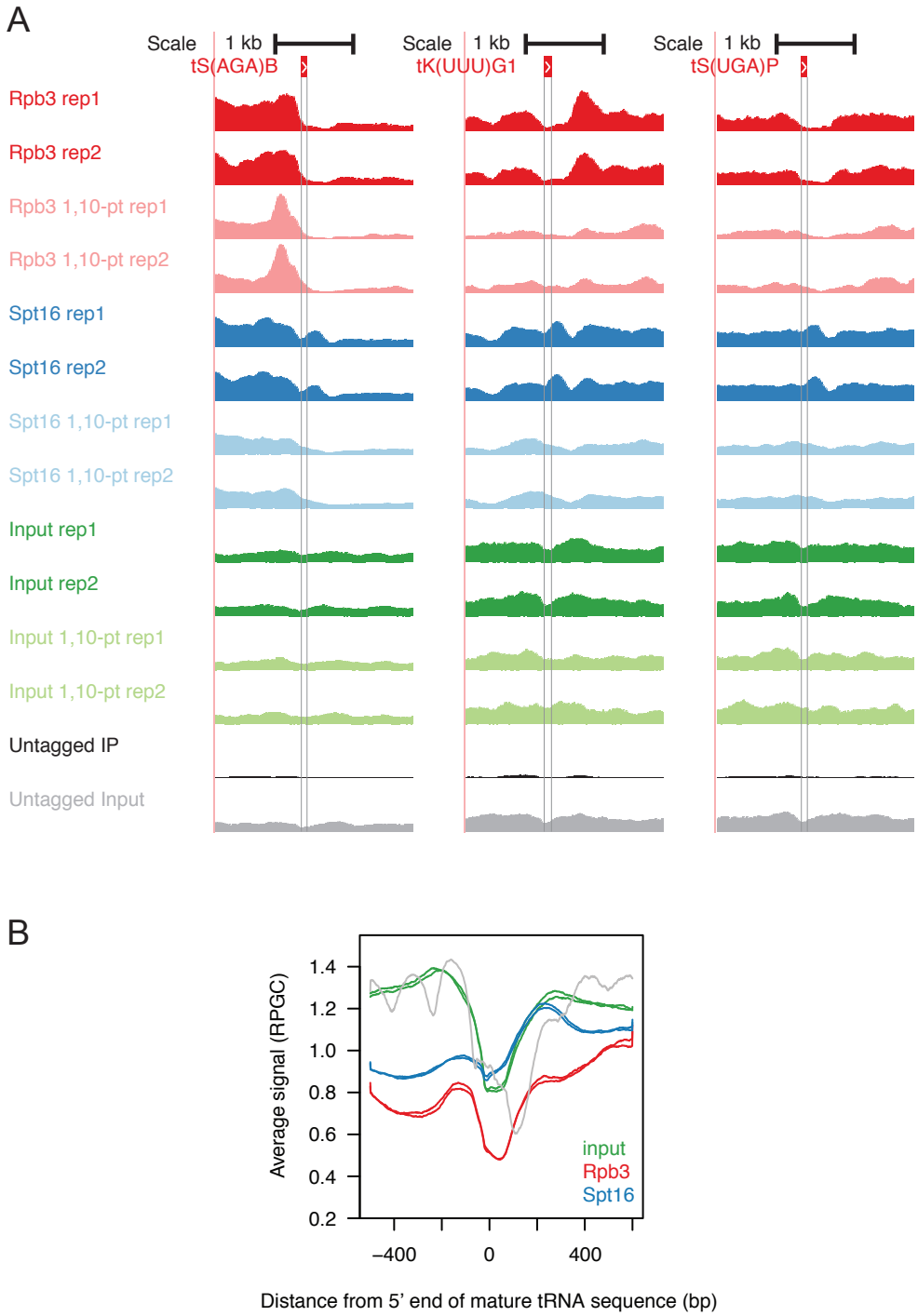

Figure S9: The interaction of FACT with RNAPIII-transcribed regions is dependent on transcription. A) Genome browser screenshots of ChIP-seq data for the indicated proteins (two independent replicates, labelled "rep1" and "rep2"), over chrII 226000-228500 (left panel), chrVII 114500-117000 (middle panel) and chrXVI 688500-691000 (right panel). tRNA genes are indicated in red, the direction of transcription with a white arrow and tRNA boundaries by verticle grey lines. Datasets were normalized to genomic mean and samples from 1,10-phenanthroline-treated (1,10-pt) cells were further normalized to data from untreated cells based on spike-in controls. B) The average Rpb3 ChIP, Spt16-HA_6 ChIP, and sonicated input sequence coverage relative to the the 5' end of mature tRNA sequence of 210 tRNA genes that lacked RNAPII [bottom three quartiles of $\log_2$(Rpb3 over input) 500 bp upstream and downstream]. RPGC is the reads per genomic content (reads normalized to 1x sequencing depth with sequencing depth defined as mapped reads x fragment length/effective genome size).

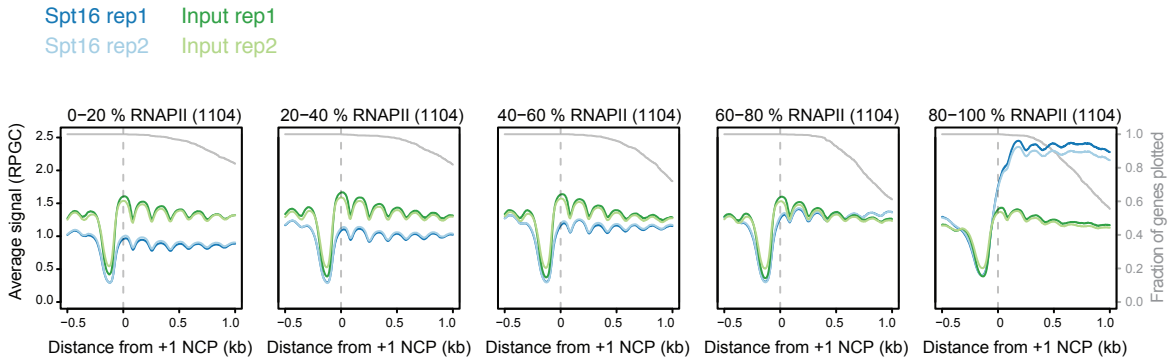

Figure S10: FACT binds nucleosomes *in vivo*. Average sequence coverage from Input and Spt16-HA_6 ChIP (two independent replicates shown) from MNase-treated extracts relative to the dyads of +1 NCPs of genes with the indicated quintiles of Rpb3 binding. RPGC is the reads per genomic content (reads normalized to 1x sequencing depth with sequencing depth defined as mapped reads x fragment length/effective genome size).

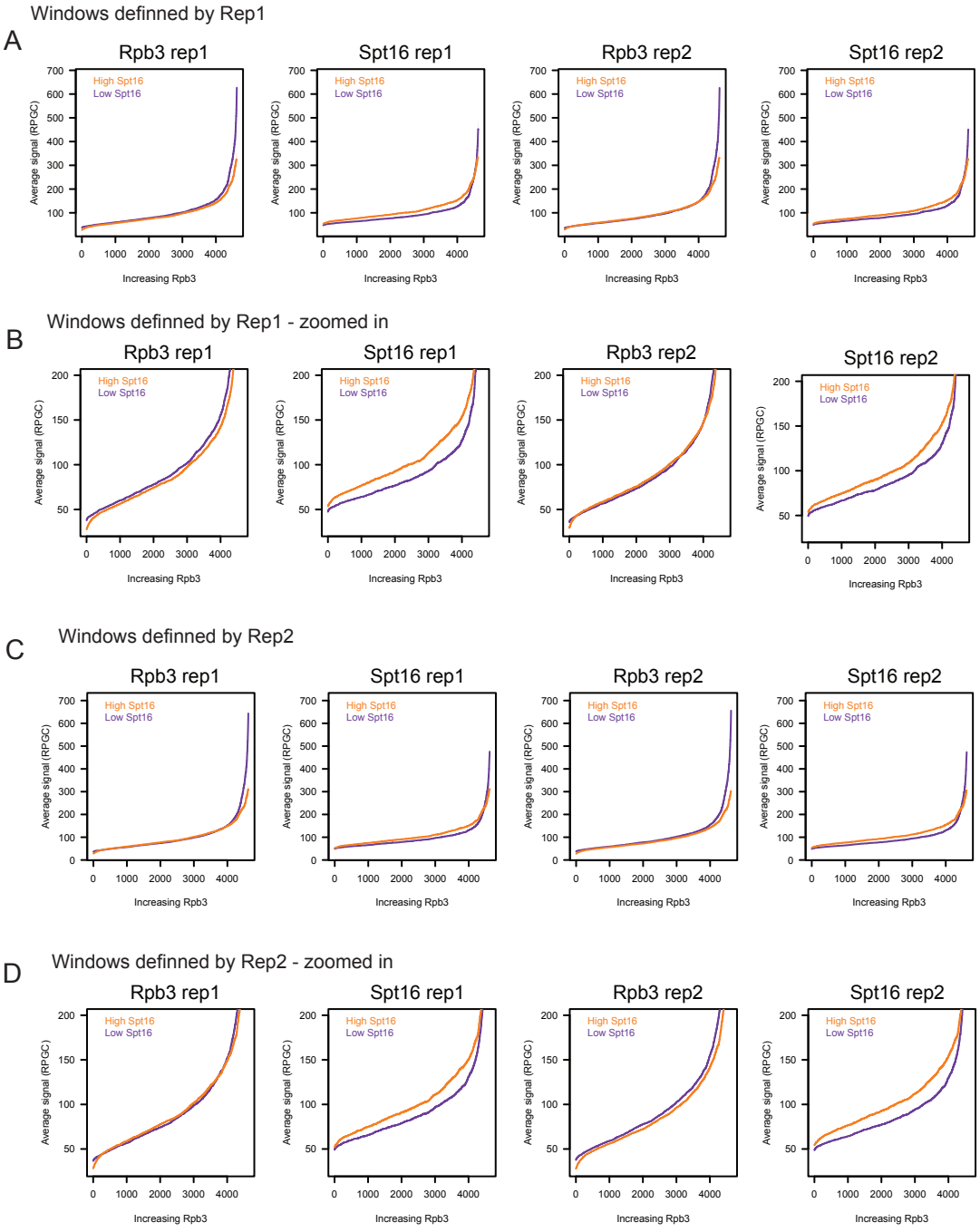

Figure S11: **Differences between Spt16 and Rpb3 levels in sliding windows is reproducible .** Genes, defined as +73 bp downstream of the +1 NCP dyad to the transcription termination site and longer than 500 bp, were ordered by Rpb3 levels and, in 500 gene sliding windows for the indicated datasets, the top and bottom 20% of Spt16-Rpb3 were designated as high and low Spt16 groups respectively. Mean enrichment of Rpb3 and Spt16, for the datasets indicated above each graph, were plotted, with the shaded regions representing the 95% confidence intervals. A and C) Plots with the full y-axes. B and D) Plots show data with y-axes truncated at 200 RPGC to better visualize changes in Spt16 at moderately transcribed genes. RPGC is the reads per genomic content (reads normalized to 1x sequencing depth with sequencing depth defined as mapped reads x fragment length/effective genome size).

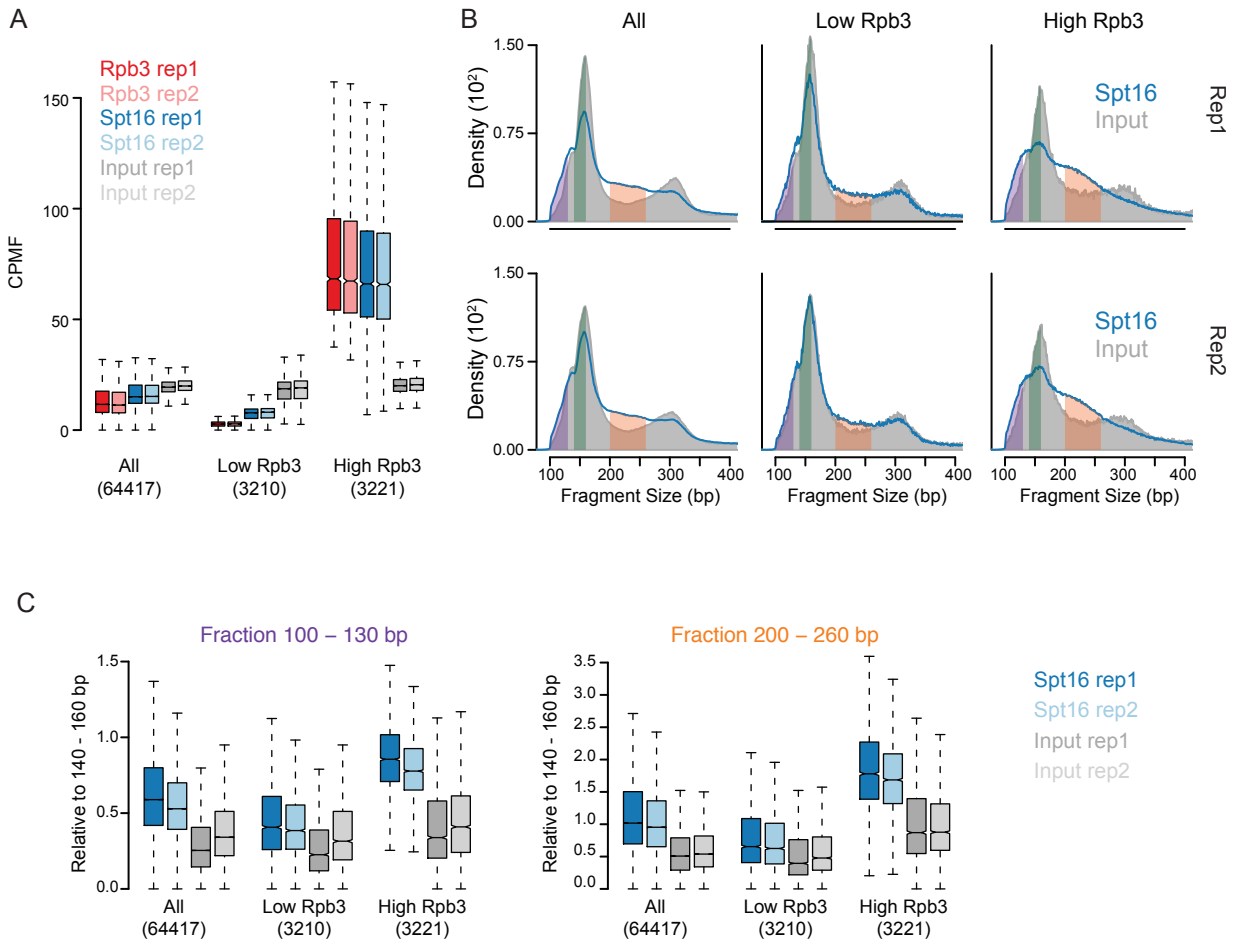

Figure S12. DNA ChIPped with Spt16 from MNase-treated extracts shows transcription-associated length differences from input. A) The number of sonicated ChIP-seq fragments overlapping annotated nucleosome dyad positions are shown as the counts per million fragments (CPMF). Nucleosome positions were filtered for coverage in the sonication input (All) and the bottom (Low Rpb3) and top (High Rpb3) 5% of Rpb3-bound nucleosome positions were selected. B) For MNase ChIP-seq fragments overlapping the nucleosome positions identified in (A), DNA fragment lengths recovered from input and Spt16 ChIP from MNase-digested chromatin are depicted by histogram. Shaded regions indicate fragment length regions of 100 - 130 bp (purple), 140 - 160 bp (green), and 200 - 260 bp (orange). C) The fraction of fragments relative to mono-nucleosome-sized fragments (140 - 160 bp), overlapping the indicated nucleosome positions identified in (A) depicted by boxplot. For boxplots, the hinges depict the first and third quartiles, while outliers are not shown. Notches extend to +/- 1.58 * IQR/sqrt(n), approximating the 95% confidence interval of the medians. IQR refers to the interquartile range.