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Supplemental Information

TFIIH Phosphorylation of the Pol II CTD Stimulates Mediator Dissociation from the Preinitiation Complex and Promoter Escape

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SUPPLEMENTAL FIGURES

Figure S1

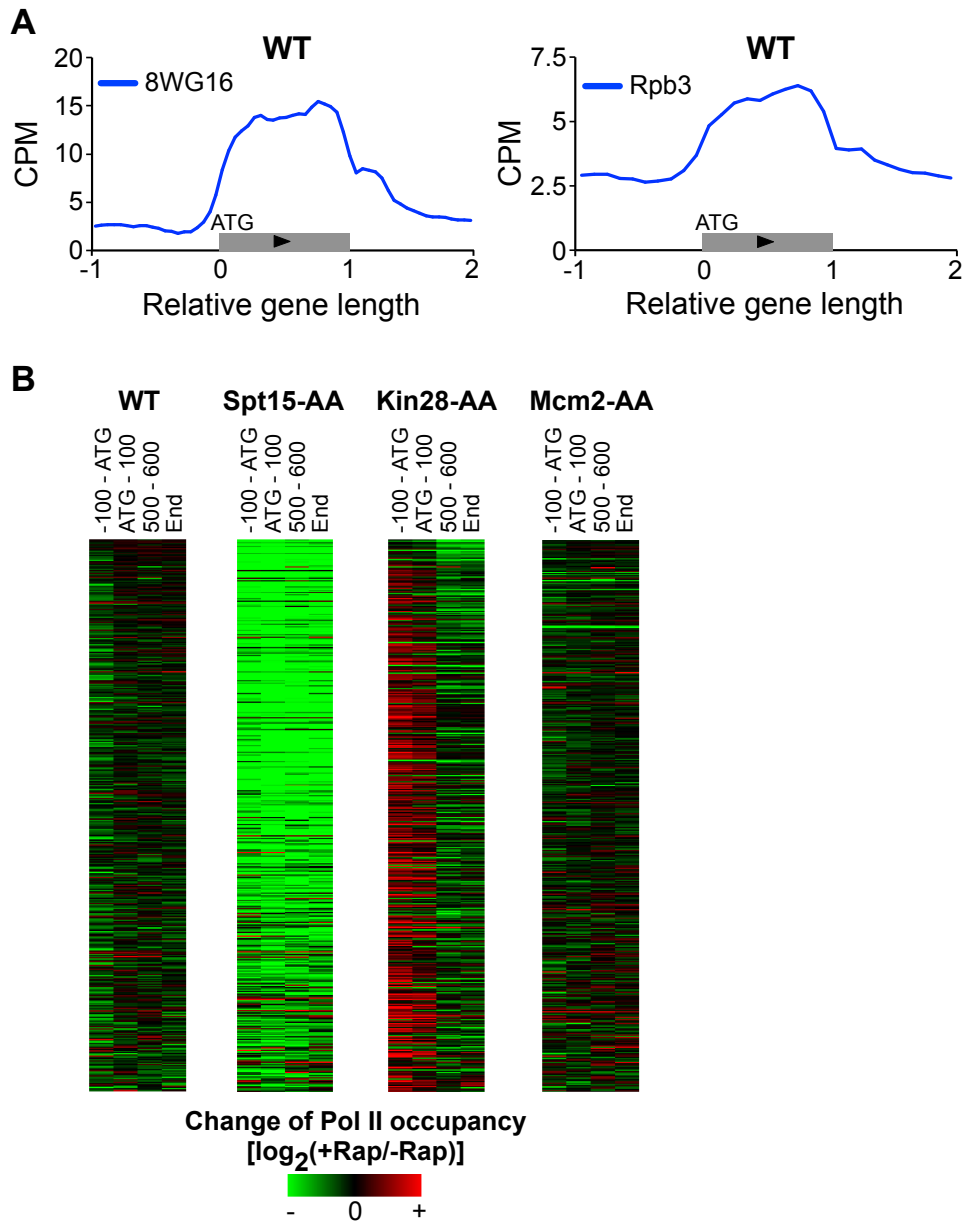


Figure S2

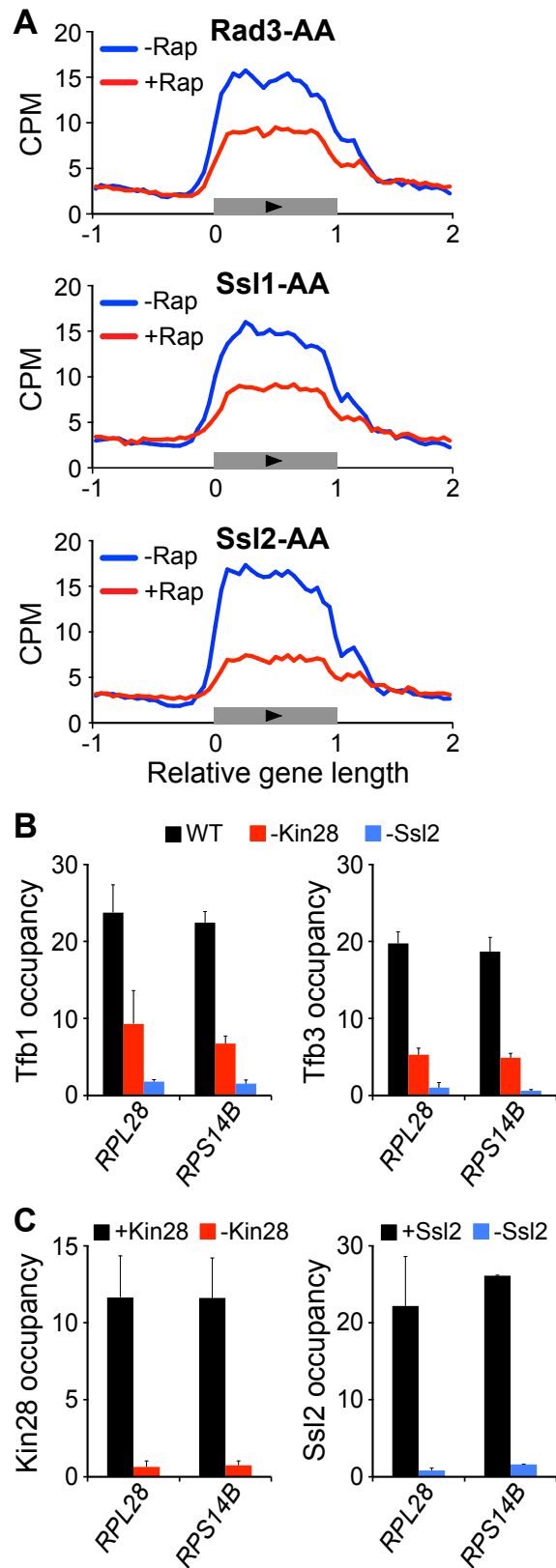
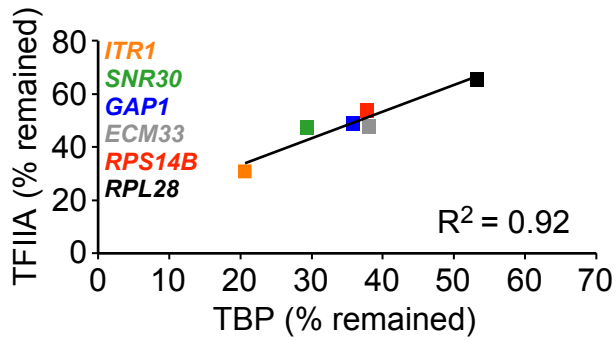
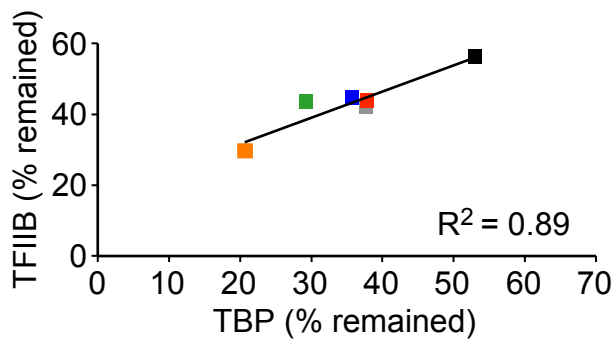


Figure S3

A



B



C

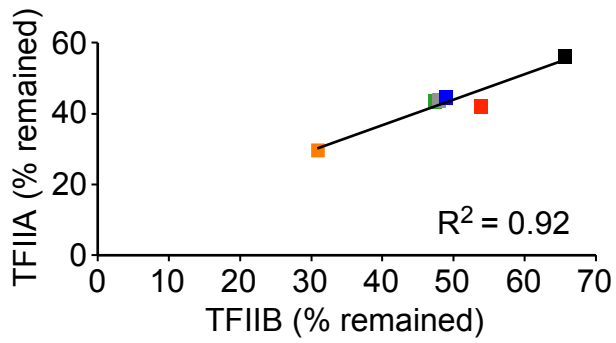


Figure S4

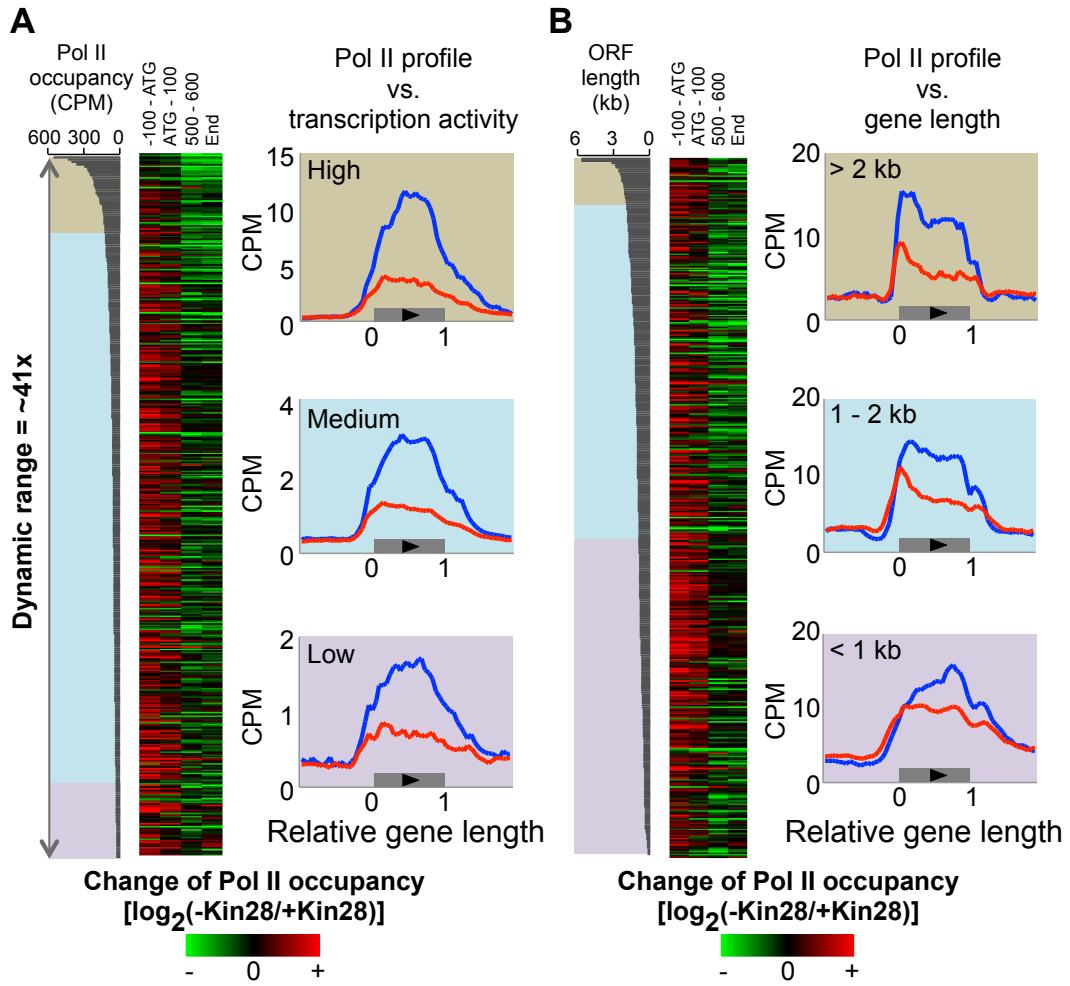
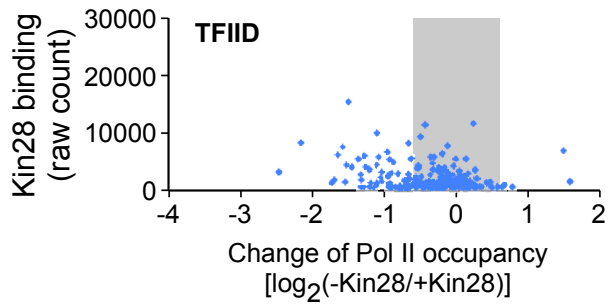
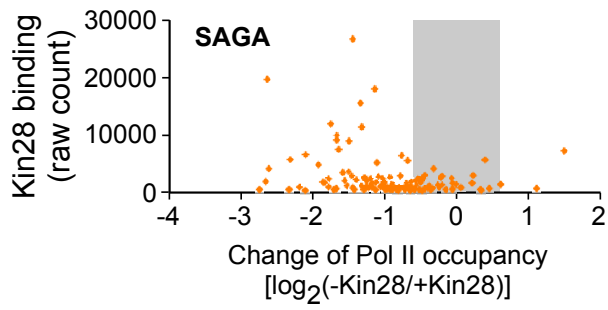


Figure S5

A



B

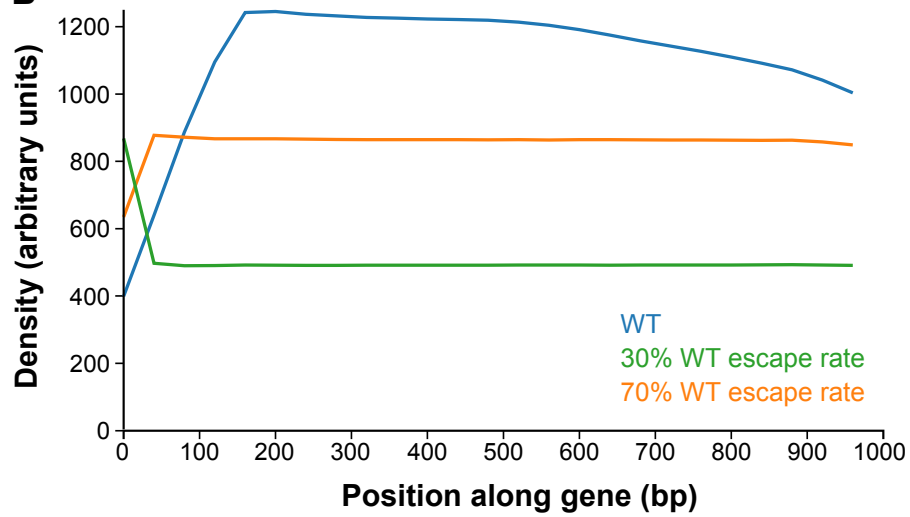


Figure S6

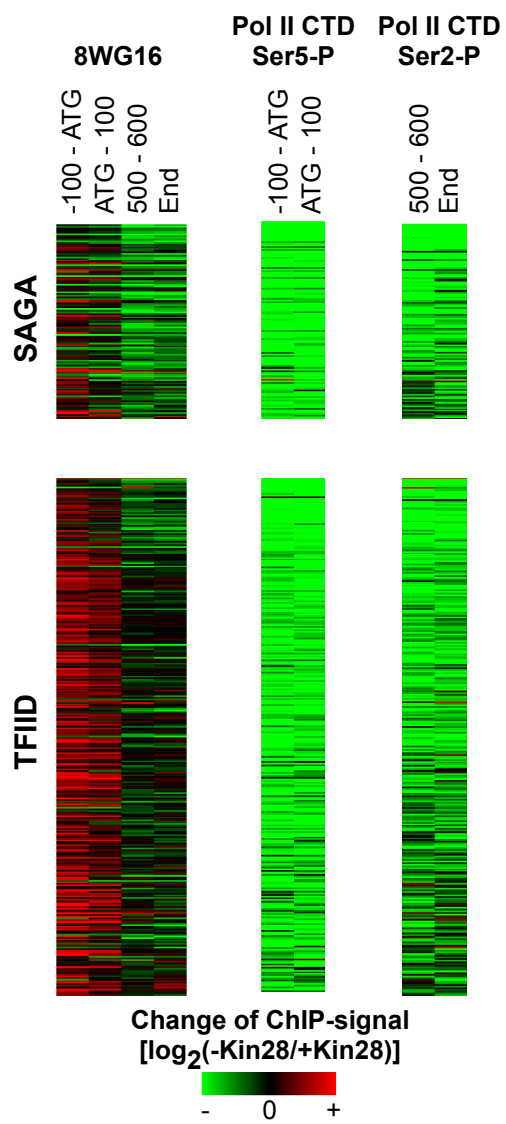
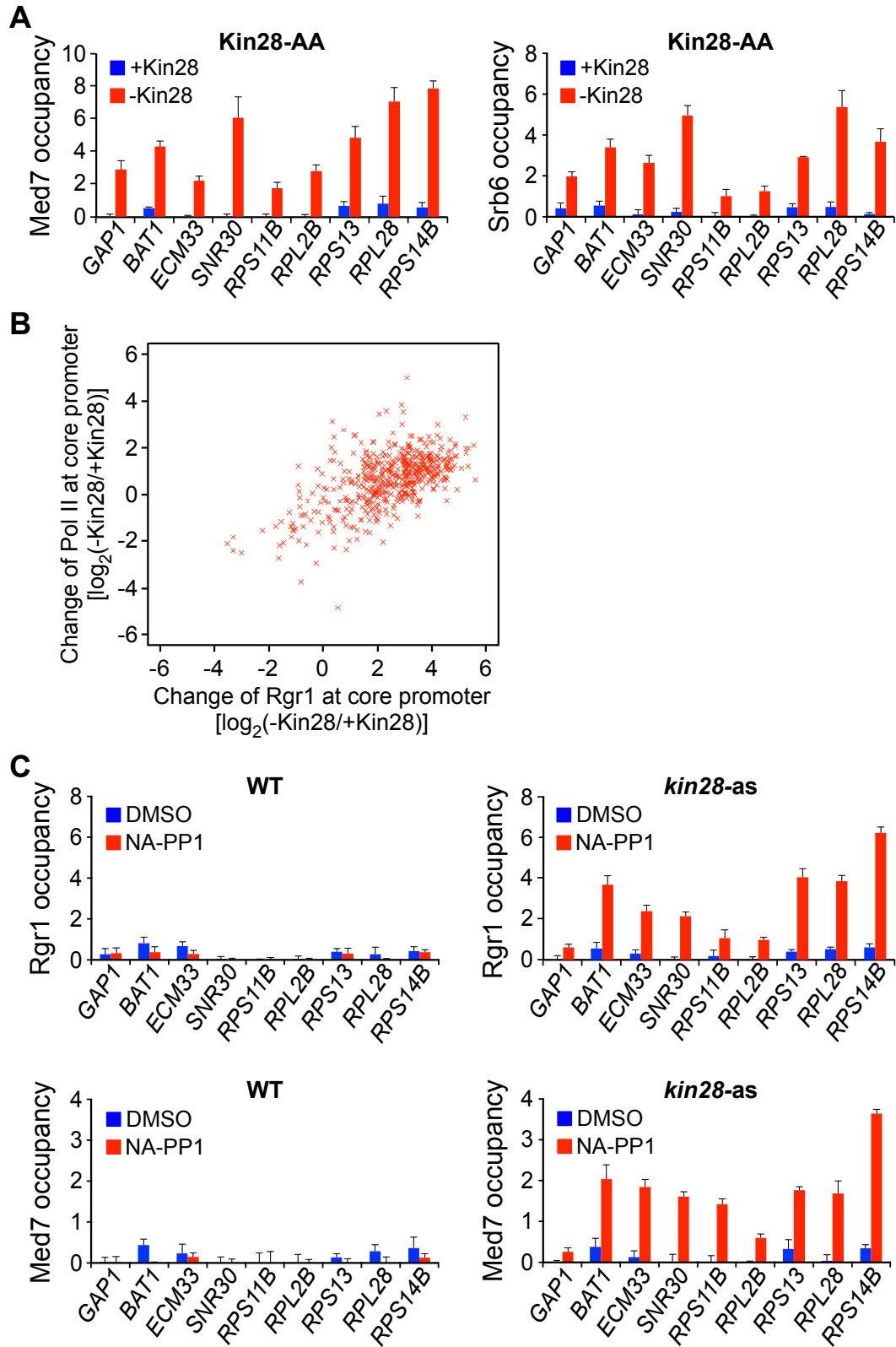


Figure S7



SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1

Transcription effect in the Kin28 anchor-away strain upon rapamycin treatment

(A) Pol II ChIP-seq profiles of Rpb3 and un-phosphorylated CTD of Rpb1 are highly comparable. Overall Pol II ChIP-seq binding profiles of active genes were measured by antibodies against Rpb3 or un-phosphorylated CTD of Rpb1 (8WG16). Coding region of each gene was divided into 20 bins and reads were averaged for each bin within the range. For each bin, the median read count over all active genes was computed, normalized to the overall number of mapped reads, and plotted as counts per million mapped reads (CPM).

(B) Heat maps showing the Pol II occupancy changes at the indicated regions of active genes in WT, Spt15-AA, Kin28-AA and Mcm2-AA strains after 1-hour rapamycin treatment.

Figure S2, related to Figure 2

Effects of depletion of the TFIIH subunit Rad3, Ssl1, Ssl2, or Kin28

(A) Depletion of the TFIIH subunit Rad3, Ssl1, or Ssl2 causes general reduction in Pol II occupancy throughout coding regions without causing Pol II re-distribution to 5' ends of genes. Overall Pol II ChIP-seq binding profiles of active genes in the Rad3-AA, Ssl1-AA and Ssl2-AA strains before and after 1 hour of rapamycin treatment were plotted as in Figure S1A.

(B) TFIIH subunits Tfb1 and Tfb3 occupancies at the core promoters of indicated genes in the Kin28-AA and Ssl2-AA strains upon 1-hour rapamycin treatment.

(C) Kin28-FRB occupancy at the core promoters of indicated genes in the Kin28-AA strain before and after Kin28 depletion. Ssl2-FRB occupancy at the core promoters of indicated genes in the Ssl2-AA strain before and after Ssl2 depletion.

Averages and standard errors of three individual experiments are presented.

Figure S3, related to Figure 3

Kin28 depletion affects PIC formation

(A) Comparison between TFIIA and TBP occupancies at the core promoters of analyzed genes in the Kin28-AA strain upon Kin28 depletion. Percentage of occupancies remained after the rapamycin treatment was calculated and plotted.

(B) Comparison between TFIIB and TBP occupancies at the core promoters of analyzed genes in the Kin28-AA strain upon Kin28 depletion.

(C) Comparison between TFIIA and TFIIB occupancies at the core promoters of analyzed genes in the Kin28-AA strain upon Kin28 depletion.

Figure S4, related to Figure 4

Kin28 effects are not correlated with transcription activities and gene lengths

(A) Genes are sorted by transcription activities (i.e. Pol II levels) on coding regions and are classified as having high (top 10%, gray shade), medium (intermediate between top and bottom 10%, blue shade) or low (bottom 10%, purple shade) transcription activity.

(B) Genes are sorted by their lengths (ORF length) and grouped into three classes; > 2 kb (gray shade), 1-2 kb (blue shade), and < 1 kb (purple shade).

Changes of Pol II occupancy at the indicated regions of active genes in the Kin28-AA strain upon Kin28 depletion are presented as \log_2 ratios of Pol II levels before and after rapamycin treatment in the heat maps. Overall Pol II ChIP-seq binding profile is also presented for each class of genes.

Figure S5, related to Figure 5

Kin28 depletion differentially affects SAGA- and TFIID-dependent genes

(A) The differential transcription effect between SAGA- and TFIID-dependent genes upon Kin28 depletion is not directly associated with the level of Kin28 binding at promoters. Transcription (as measured by Pol II occupancy at the 3' end of a gene) changes for SAGA-

and TFIID-dependent genes upon Kin28 depletion are plotted against promoter Kin28-FRB occupancies prior to its depletion. Promoter Kin28 occupancy was determined by the total count of Kin28 ChIP-seq reads within the 200 bp window immediate before the start codon (ATG) of coding region. Gray area indicates that the transcription effect is less than 2 folds. (B) Pol II ChIP-seq profiles suggest differential promoter escape rates for SAGA- and TFIID-dependent genes in the absence of Kin28. Computational simulation of Pol II density profiles based on different promoter escape rates were generated using a previously published algorithm (Ehrensberger et al., 2013).

Figure S6, related to Figure 5

Phosphorylations of Ser5 and Ser2 residues of Rpb1 CTD are similarly affected by Kin28 depletion for SAGA- and TFIID-dependent genes

Heat maps present the changes of ChIP signals of unphosphorylated Pol II and phosphorylated Ser5 or Ser2 residue of Pol II CTD at the indicated regions of active SAGA- and TFIID-dependent genes after Kin28 depletion for 1 hour.

Figure S7, related to Figure 6

Kin28 depletion causes accumulation of Mediator at core promoters of active genes

(A) Mediator subunits Med7 and Srb6 occupancies at the core promoters of indicated genes in the Kin28-AA strain before and after Kin28 depletion. Averages and standard errors of three individual experiments are presented.

(B) Pol II and Mediator accumulations at core promoters are correlated. Pol II and Mediator (Rgr1) levels of each gene were measured by the total number of ChIP-seq reads within the 100 bp window immediately upstream of the translation start codon (ATG). The read counts were then normalized to the total number of mapped reads. Pol II and Mediator (Rgr1) occupancy changes were calculated and plotted as \log_2 ratios.

(C) Inactivation of Kin28 kinase activity causes accumulation of Mediator at core promoters of active genes. Mediator subunits Rgr1 and Med7 occupancies at the core promoters of indicated genes in the WT and *kin28-as* strains upon 1-hour treatment of the inhibitor NA-PP1 or DMSO. Result of Rgr1 occupancy in the *kin28-as* strain is identical to Figure 6E, reproduced here for comparison between WT and *kin28-as* strains. Averages and standard errors of three individual experiments are presented.

Table S1, related to Experimental Procedures

Yeast strains and primer sets used in this study are listed.

SUPPLEMENTAL REFERENCES

Ehrensberger, A.H., Kelly, G.P., and Svejstrup, J.Q. (2013). Mechanistic interpretation of promoter-proximal peaks and RNAPII density maps. *Cell* 154, 713-715.