



Supplemental Figure S4. All RNAPII-dependent transcripts are affected by Spt5 loss. **A**, Western blot analysis shows that >90% of Spt5 protein is lost within 2 days of treating S2 cells with double-stranded RNA targeting Spt5. TFIIIS is a loading control. **B**, Extended depletion of Spt5 causes defects in S2 cell growth, in agreement with Spt5 being an essential gene. **C**, Broad reduction in 4sU RNA-seq signals upon depletion of Spt5, across RNAPII-dependent transcript classes. For this analysis, Flybase gene models were separated into annotated gene groups and 4sU RNA-seq reads were counted using normalized (n=5) data sets from Control versus Spt5-depleted S2 cells. **D**, Levels of RNAPII ChIP-seq (± 750 bp) from Control or Spt5-dep. cells centered at functionally-defined, transcriptionally active enhancers lacking a mRNA TSS within ± 2 kb from peak center (N=1,254). P-values are from Mann-Whitney test. **E**, Levels of Start-RNAs 5'-ends (± 50 bp) from Control or Spt5-dep. cells centered on eTSSs. P-values are from Mann-Whitney test. Box plots show 25th-75th percentiles and error bars depict 10-90th percentiles. Related to Fig. 4.