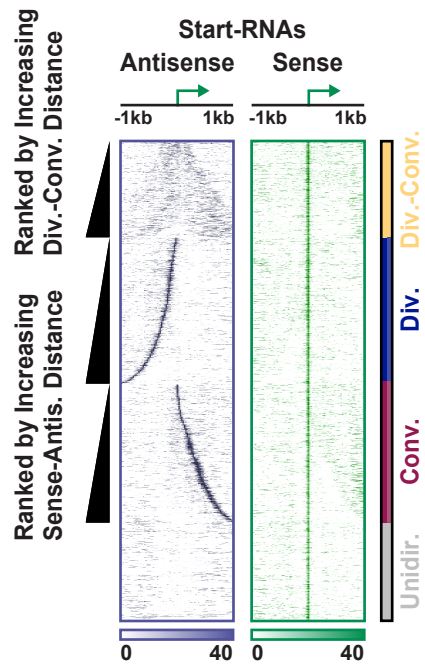
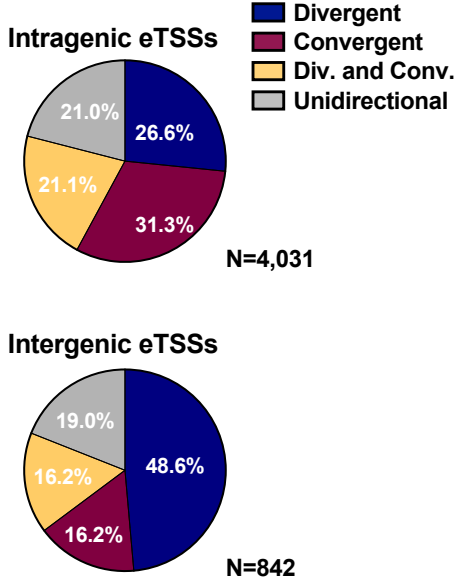
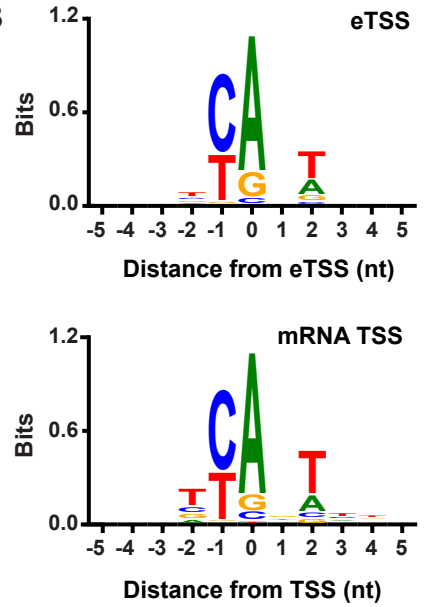


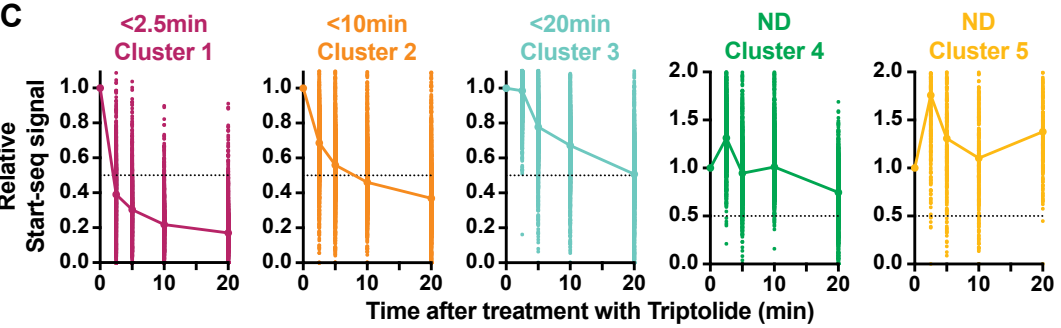
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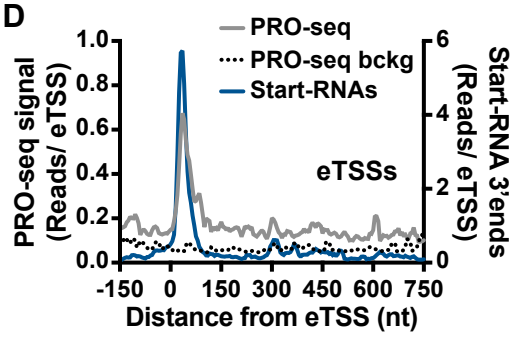
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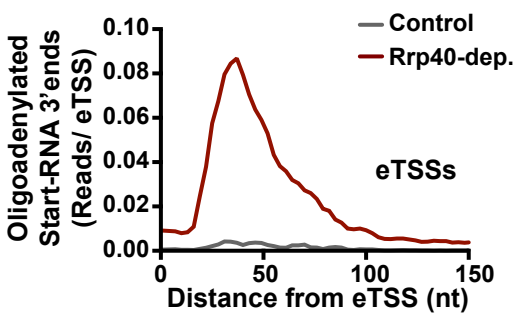
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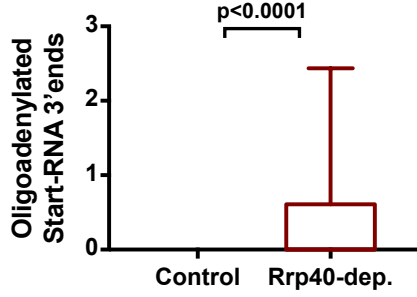
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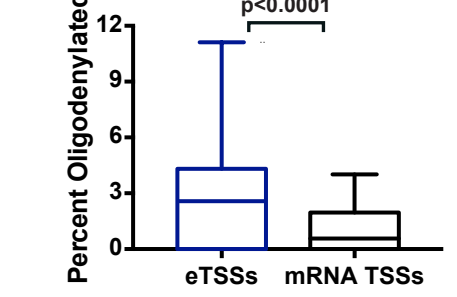
E



F



G



Supplemental Figure S3. Characteristics of enhancer TSSs include lower RNAPII stability and targeted degradation of eRNAs. **A**, Information content around TSSs from protein-coding genes (N=10,162) and eTSSs (N=4,873). **B**, (Left) Percentage of eTSSs that are located within genes (intragenic, top) or intergenic (bottom) that are classified as: divergent (blue), convergent (purple), or both divergent and convergent (yellow) with another TSS located within 1kb. eTSSs found to be unidirectional are shown in gray. We note that a considerable fraction of divergent or convergent transcription occurs between eTSSs and mRNA TSSs, as shown in Figure 3A. (Right) Sense (green) and antisense (purple) Start-RNA 5'-end reads centered around eTSSs. Data is oriented by increasing distance of divergent to convergent eTSS, or increasing distance between sense and antisense TSS. Unidirectional sites are ranked randomly. **C**, Start-RNA decay kinetics for each cluster of TSSs (includes both mRNA TSSs and eTSSs as described in Methods). Shown are the Start-RNA levels for each TSS (colored dots) and the median abundance for the cluster at each time point (colored lines). The first timepoint where the median of the relative Start-RNA amount reached 50 percent (dotted line) of its original value was used to approximate the half-life for each cluster (analysis performed as described in Krebs et al. 2017). **D**, Average distribution of PRO-seq (gray) and 3'-end Start-RNAs (blue) centered on intergenic eTSSs (N=842). For comparison, the background signal of PRO-seq is also displayed around random regions (N=842). Read counts were summed in 5 nt bins, centered on the TSS, and the moving average across 3 bins was plotted. **E**, 3'-end Oligo-adenylated Start-RNAs derived from enhancer TSSs are identified in exosome-depleted cells (Rrp40-depletion). 3'-end positions of these oligo-adenylated Start-RNAs shows that they localize just downstream of eTSSs, within the region characteristic of pausing. Read counts were summed in 3 nt bins, centered on the TSS, and the moving average across 3 bins was plotted. **F**, Levels of oligo-adenylated Start-RNAs (0-100 bp) from Control or Rrp40-dep. cells centered on eTSSs. **G**, Percentage of oligo-adenylated Start-RNA reads, as a function of total Start-RNAs from the TSS, is significantly higher at eTSSs than mRNA TSSs. Box plots show 25th-75th percentiles and error bars depict 10-90th percentiles. P-values are from Mann-Whitney test. Related to Fig. 3.