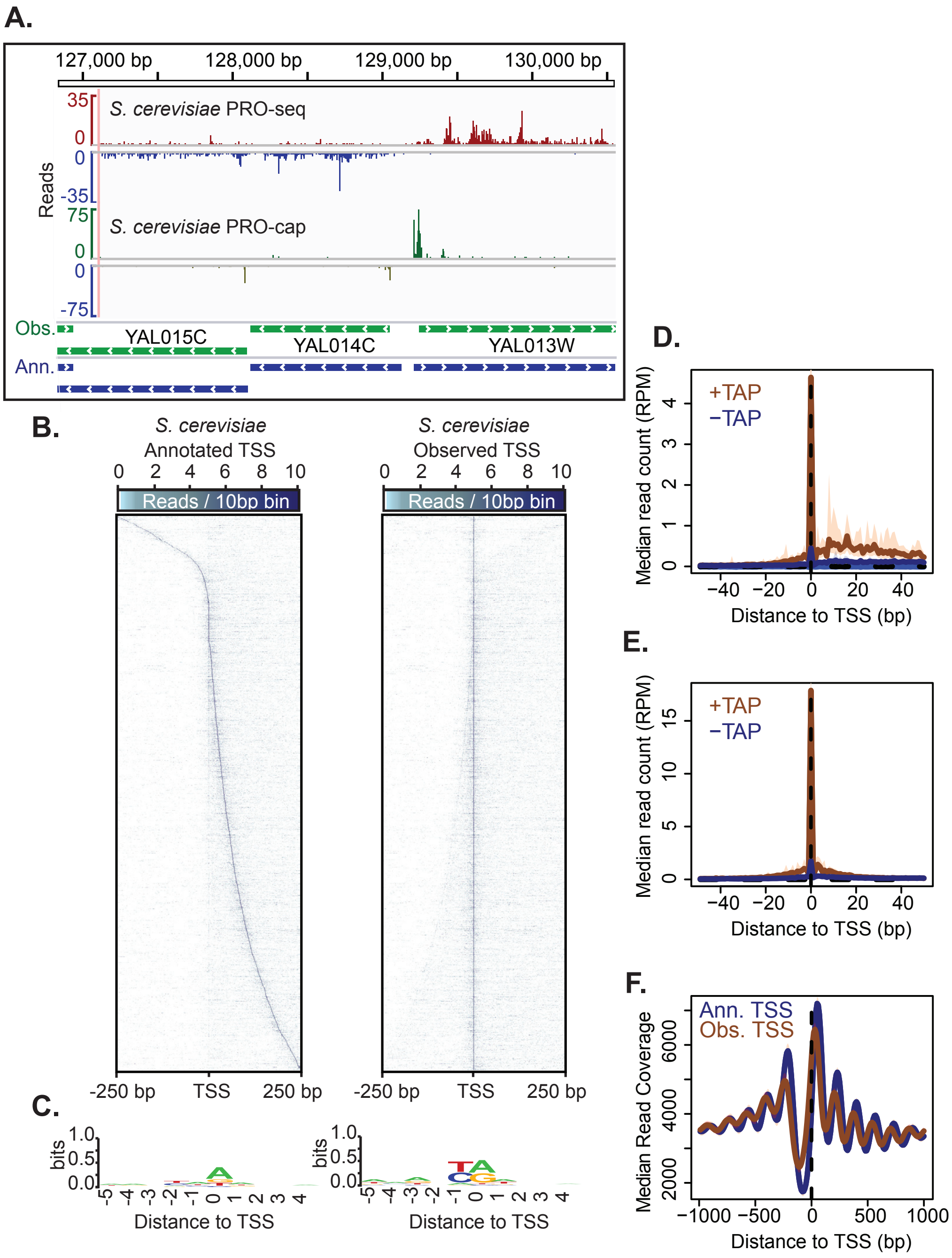

Figure S1. PRO-seq and PRO-cap capture transcription elongation and initiation genome-wide in *S. cerevisiae.* **A.** Browser tracks of PRO-seq (plus strand: red; minus strand: blue) and PRO-cap data (plus strand: green; minus strand: yellow) derived from *S. cerevisiae*. Green gene models show the re-annotated, 'observed' transcription start-sites based on PRO-cap data. Blue gene models correspond to the longest major transcript isoform identified by (Pelechano et al. 2013). **B.** Heatmaps of *S. cerevisiae* PRO-cap signal for each base within +/- 250 bp around either the annotated TSS (left) or PRO-cap-observed TSS (right) for all active and filtered genes (N = 3403). Genes within heatmaps are sorted by increasing downstream distance of observed TSS relative to annotated TSS. **C.** Sequence logos of 10 bp sequence centered on annotated TSS (left) and observed TSS (right) were generated using WebLogo (Crooks et al. 2004). **D.** Median PRO-cap signal from samples prepared either with or without Tobacco Acid Pyrophosphatase (TAP) treatment centered on annotated TSS. The TAP-minus samples represent empirical levels of background for each genomic position. **E.** Median PRO-cap signal from samples prepared either with or without TAP treatment centered on observed TSS. **F.** Median MNase-seq coverage centered on annotated TSSs (blue) or observed TSSs (brown). For the meta-gene plots, the y-axis shows the median read counts for each base-pair (D & E), or median read coverage within 10 bp bins (F). In figures D, E and F, the 12.5% and 87.5% quantiles are shown in lightly shaded regions.