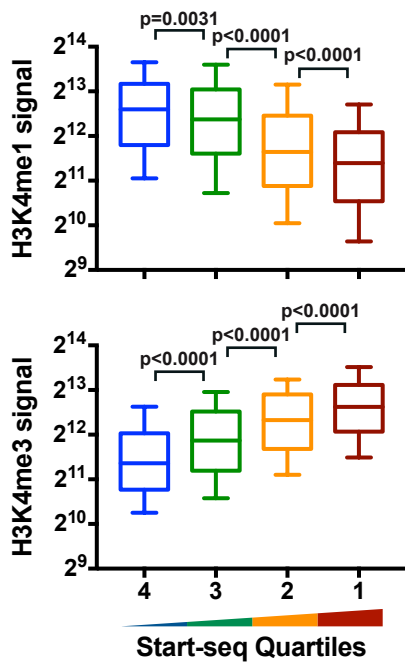
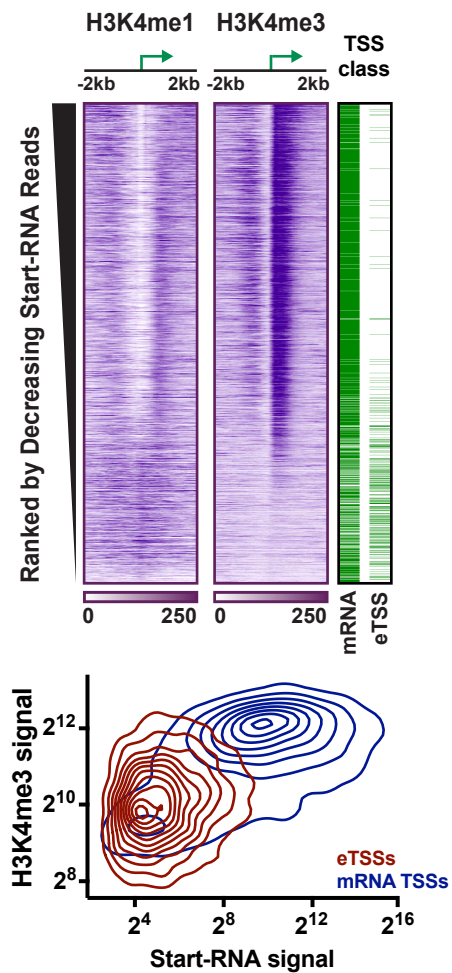


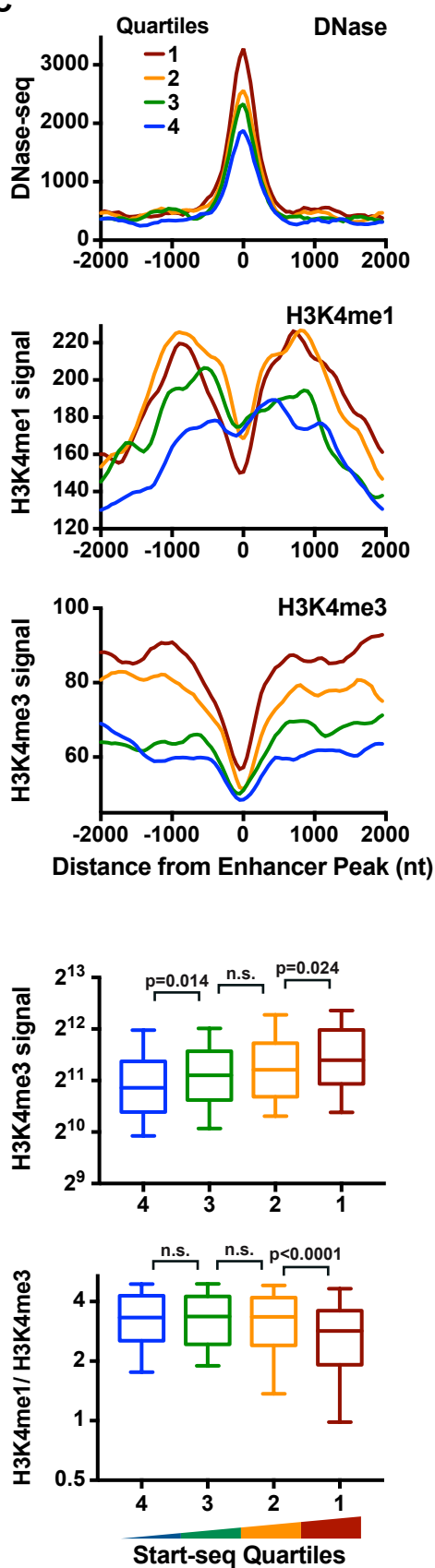
A



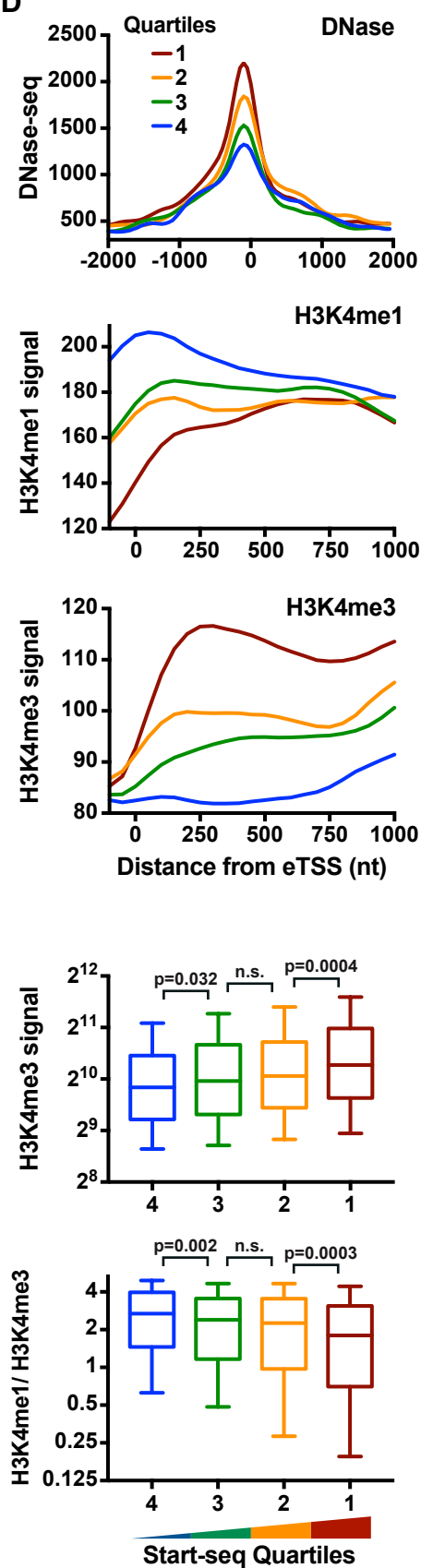
B



C



D



Supplemental Figure S2. Relationship of Start-RNAs and chromatin marks with enhancer activity. **A**, Relationships of chromatin marks with activity at functionally-defined enhancers that are both accessible and contain a defined eTSS (N=3,692). Quartiles are defined by levels of Start-RNAs (± 200 bp from enhancer peak). H3K4me1 and H3K4me3 ChIP-seq reads were summed ± 1 kb from enhancer peak. **B**, (Top) Heatmap representations of H3K4me1 and H3K4me3 ChIP-seq signal around mRNA TSSs (N=10,162) and eTSSs lacking a protein-coding TSS within 1.25kb downstream (N=3,372). Data is aligned around TSSs and rank ordered by Start-RNAs levels (± 50 bp from TSS). TSS class is noted at right. (Bottom) Density contour plots of Start-RNA levels (± 50 bp from TSS) versus H3K4me3 levels (+50 to +750bp from TSS) around TSSs shown at top. **C**, Average distributions of DNase-seq, H3K4me1 and H3K4me3 histone modifications at functionally-defined enhancers that are transcriptionally active (≥ 5 reads ± 200 bp from enhancer peak center) and lack an mRNA TSS within ± 2 kb from enhancer peak center (N=1,254). Data is shown across quartiles of Start-RNAs reads. Despite markedly different spatial distributions, there are no statistically significant differences across quartiles for H3K4me1 levels (not shown). Box plots depict levels of H3K4me3 ChIP-seq signals and ratio of H3K4me1 to H3K4me3 (± 1 kb from enhancer peak center) at these loci. **D**, Average distributions of DNase-seq, H3K4me1 and H3K4me3 histone modifications at eTSSs lacking a protein-coding TSS within 1.25kb downstream of eTSS (N=3,372), across quartiles defined by Start-RNAs levels. Box plots below are ChIP-seq signals for H3K4me3 and ratios of H3K4me1 to H3K4me3 (+50 to +750bp from eTSS) at these eTSSs. Throughout this figure, read counts were summed in 50 nt bins, centered on the TSS, and the moving average across 5 bins was plotted. Box plots show 25th-75th percentiles and error bars depict 10-90th percentiles. P-values are from Kruskal-Wallis test. Related to Fig. 2.