



**Figure S8: Analysis of upstream, divergent and intragenic, antisense transcription.**  
**A-D.** Correspond to analysis of *S. pombe* PRO-seq/PRO-cap data. **A.** Composite profile of PRO-cap signal (background subtracted) for plus (+) and minus (-) strands around *S. pombe* TSSs of genes filtered for analysis of upstream, antisense transcription (described

below). **B.** Composite profile of PRO-seq data on the plus and minus strand for wild type and *spt4Δ* strains around the TSS of genes used in A. **C.** Histogram of upstream, antisense to downstream, sense ratios calculated as reads per mappable base-pair on the antisense strand from -500 to TSS over reads per mappable base-pair on sense strand from TSS to +500. **D.** Scatter plot comparing the ratio of reads per mappable base-pair on the antisense strand over that of the sense strand across the entire transcription unit between wild type and *spt4Δ* strains. Genes used in scatter plot were filtered for analysis of intragenic, antisense transcription (described below). **E-H.** Correspond to the same set of analyses as performed in A-D, but for *S. cerevisiae* PRO-seq and PRO-cap data. Genes used for intragenic, antisense analysis were selected because they did not contain functional transcription units on the antisense strand within 500 bp of either boundary (*S. cerevisiae*, n = 2432; *S. pombe*, n = 2407). Genes used for analysis of divergent (upstream, antisense) promoter transcription were additionally required to be tandemly located downstream of a gene on the same strand (*S. cerevisiae*, n = 1329; *S. pombe*, n = 1250).