



**Figure S7. Spike-in libraries provide a method for accurate normalization between samples.** **A, B.** Browser shot of the Spt4 locus in *S. cerevisiae* (A) and *S. pombe* (B). Displayed are the PRO-seq (top four tracks) and mRNA-seq data (lower four tracks) for two biological replicates of WT cells (green) and *spt4Δ* (purple). The gene upstream of the Spt4 locus in *S. cerevisiae* (YGR0465C) was found to be significantly changed at the mRNA level in *spt4Δ* cells. **C-F.** Scatter plots of PRO-seq gene body read density (reads per mappable base) between biological replicates. Counts are normalized based on the relative amount of reads that align uniquely to the spiked-in genome for each library. **G, H.** Stacked bar plots displaying the fraction of uniquely mapped reads aligning to the genome of interest or the spike-in (reference) genome for each PRO-seq sample in *S. cerevisiae* (G) and *S. pombe* (H).