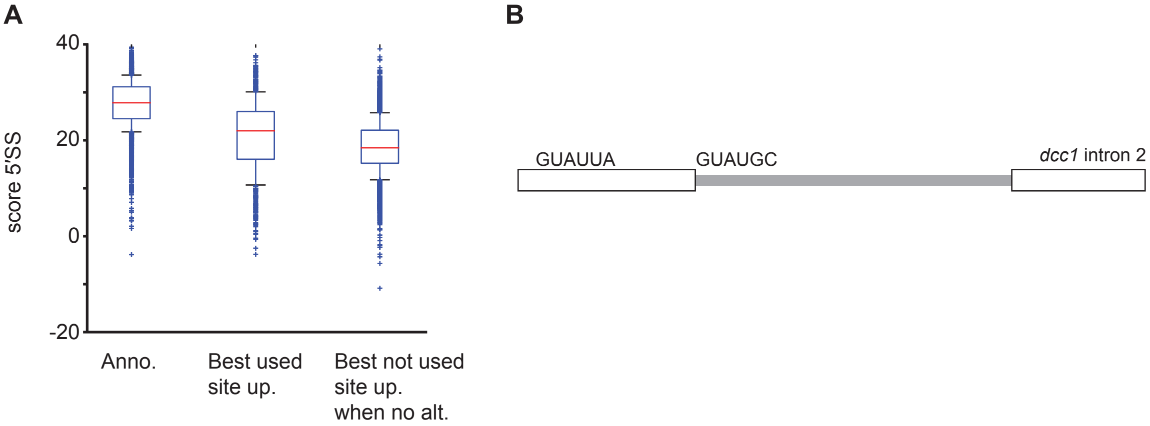

Figure S1. Unused upstream GU sites have similar splicing potential as sites that are used. (**a**) The scores of annotated splice sites vs the best scoring 5′SS upstream within 150 nt of the annotated 5′SS for introns without a found upstream alternate 5′SS. (**b**) Gene model example showing an alternate upstream 5′SS and the annotated 5′SS.

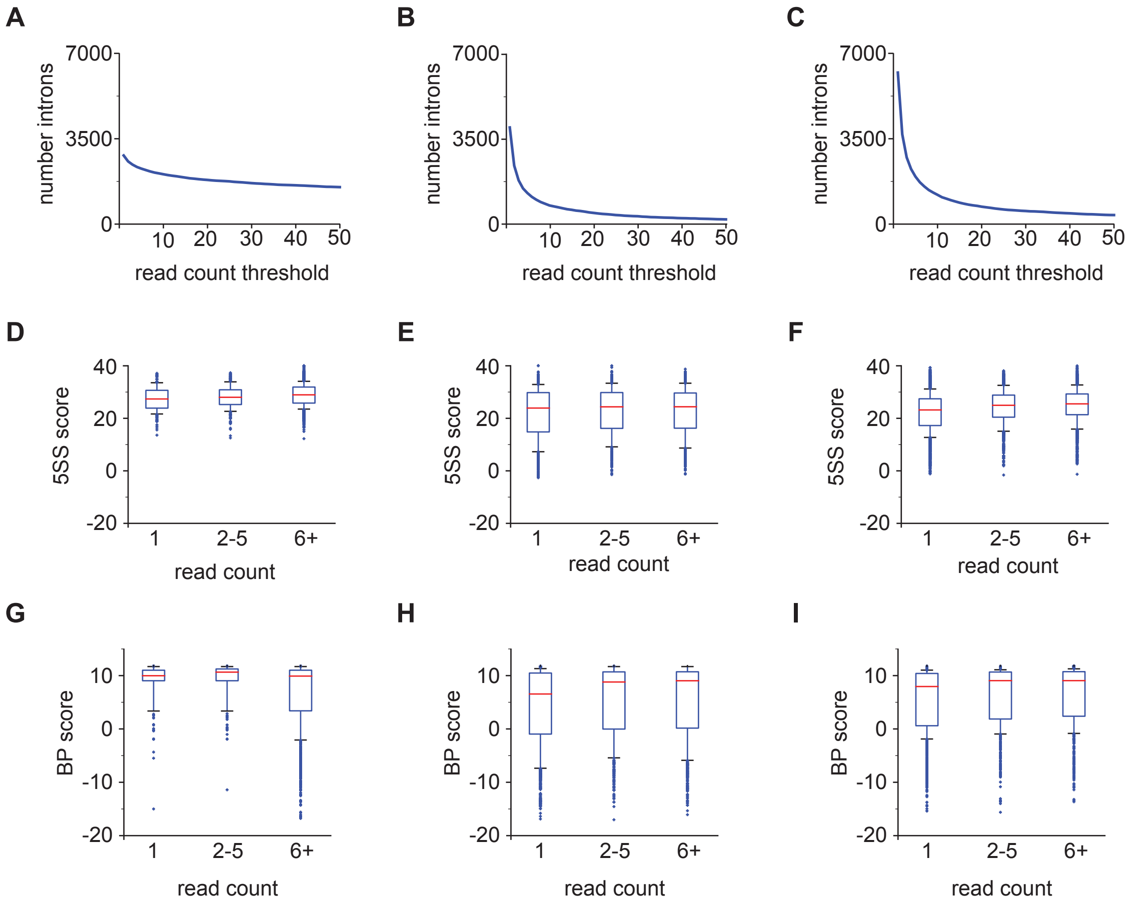

Figure S2. Relationship between read depth and introns recovered showing similarities across low and high read count events. Number of introns recovered at a read threshold for annotated, alternate, and novel introns, respectively (**a-c**). Boxplots of 5′SS splice site scores (**d-f**) and BP scores (**g-i)** for introns with the given read count ranges for annotated, alternate, and novel introns, respectively. Lower read count events are likely a composite of poorer expressed introns and introns not in our data's size sensitivity range. The marked difference between BP scores for annotated introns is the result of the method picking up low frequency events with higher read counts in the size sensitivity range.

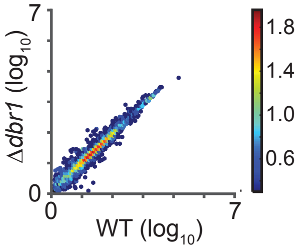

Figure S3. Scatter plot showing similarity between RNAseq transcript expression levels (shown as RPKM) between *Δdbr1* and WT *S. pombe*.

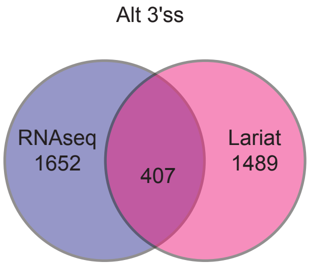

Figure S4. Venn diagram of alternate 3′SSs found by RNAseq and lariat sequencing

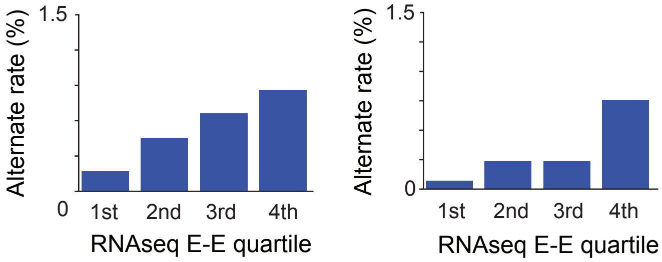

Figure S5. Alternate exon-exon junction rate for RNAseq expression quartiles calculated by log-likelihood (left) and log likelihood with unlikely events excluded using an alternate criteria (right)

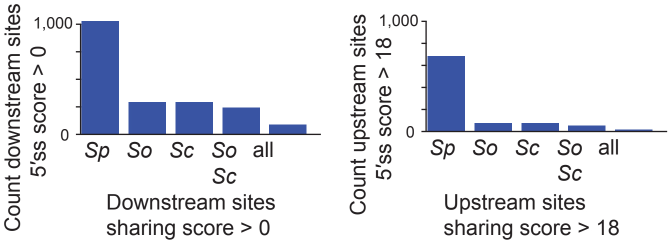

Figure S6. Alternate 5′SSs in *S. pombe* and the number of sites with scores > 0 in *S. octosporus*, *S. cryophilus*, and *S. japonicas* as indicated for downstream events (left) and upstream events (right)

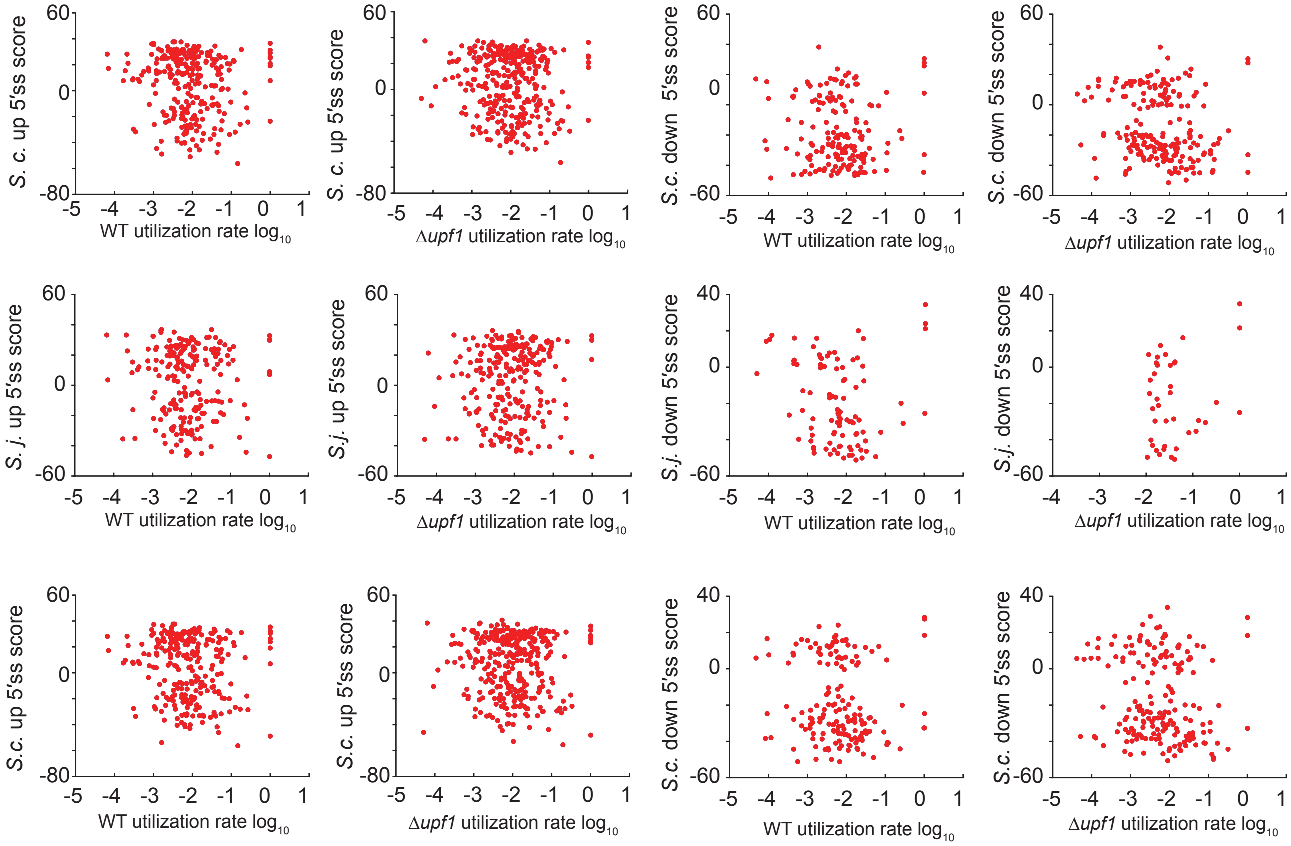

Figure S7. Orthologous 5′SS scores vs the rate of alternate splice site utilization in *S. pombe*. Scatter plot of scores of alternate 5′SS in orthologous species (*S. octosporus*, *S. cryophilus*, and *S. japonicas*) vs the alternate splice site utilization rate ($\log_{10}$) in *S. pombe*. Utilization from either upstream (up) or downstream (down) sites for either WT or *Δupf1 S. pombe*.