Methods:

Samples were obtained and quality controlled using methods from Swamy et al.\* Each sample was aligned to the hg38 gencode V[] assembly using STAR\* and sorted using SAMTOOLS sort\*. A transcriptome was built for each sample using Stringtie\*, and the resulting gtfs for each sample were merged using StringTie merge at the subtissue level to create a tissue specific transcriptome, containing both reference and novel transcripts. Next, we extracted transcript sequences using gffread\* for each tissue specific transcriptome, built a transcriptomic alignment index, and quantified transcript expression using the alignment free quantification tool Salmon\*. Transcripts were then removed from the transcriptomes using 2 metrics. Transcripts were removed first based on their quantification, removing transcripts that lacked an average count of 1 per sample. Using 100 bootstrap quantifications for each sample, we calculated the quantification variance for both reference and novel transcripts, generating a distribution of quantification variance for each set of transcripts. We removed novel transcripts that had a variance greater than the 99th percentile of the reference transcript variance distribution. The transcriptomic index was rebuilt using the filtered transcriptome and samples were requantified. The resulting filtered tissue specific transcriptomes were merged into a single annotation file using the tools gffcompare\*. For novel transcripts in this transcriptome, we used TransDecoder to find open reading frames for each transcript. Transcripts with a detectable open reading frame are classified as protein-coding, and transcripts lacking an ORF are considered non-coding.

For each filtered tissue specific transcriptome, we took the resulting gtf and used it as the reference annotation for the splicing analysis tools rMATS\*, comparing each tissue against a synthetic set of samples constructed from random sampling from each tissue type. We then found the global percent spliced in(PSI) for each alternatively spliced exon by summing all inclusion reads and all exclusion reads, (scaled for length) for each AS event associated with a given exon.