

Description of Additional Supplementary Files

Supplementary Data 1: Metadata QC.

Supplementary Data 2: QC report for STRT2 sequencing with MultiQC report.

Supplementary Data 3: Summary of the results of the PCR validation experiments of the selected alternative promoters. The transcripts of five robustly expressed candidate genes, *ATP5ME*, *MTIF3*, *TNNI3*, *TBC1D22B* and *GPT* (Table 1), with putative new first exons or TSSs based on gene models annotated in CanFam3.1, were investigated by RT-PCR and Sanger sequencing with several different primers (Figs. 1-4). The sequences were merged to create the consensus sequence for each cDNA. According to the UCSC's BLAT search on CanFam4.1, all except one (*TNNI3*) of the new CanFam3.1 TSSs were present in CanFam4.1, indicating the improved reference and validating our experiments. The new *TNNI3* transcript TSS is located upstream of exon 3 (Fig. 3C).

Supplementary Data 4: List of tissue-enriched genes.

Supplementary Data 5: List of tissue enriched promoters.

Supplementary Data 6: PCR primers for promoter validation.