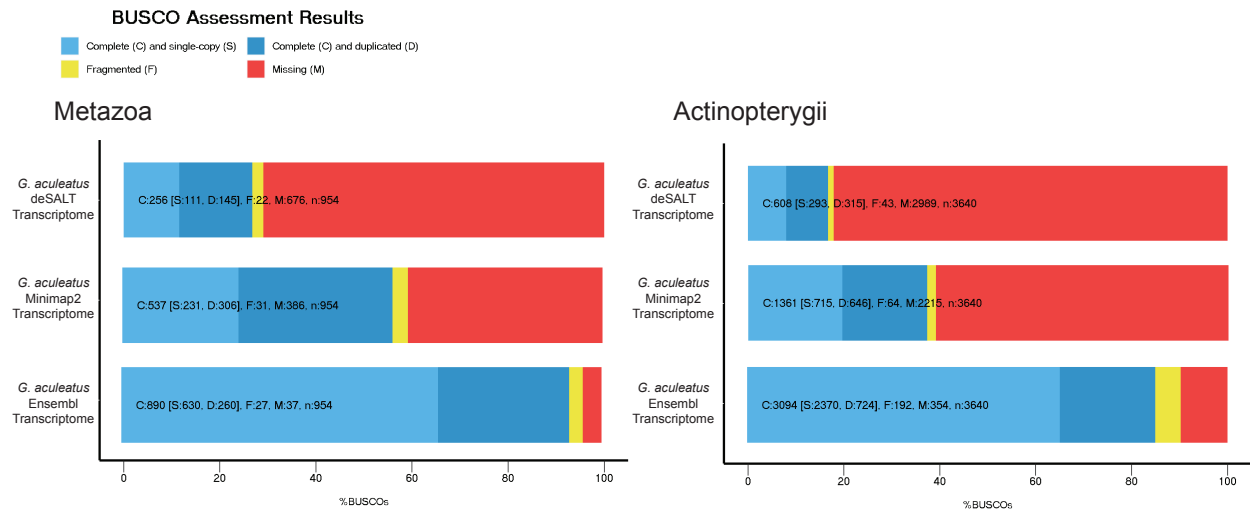
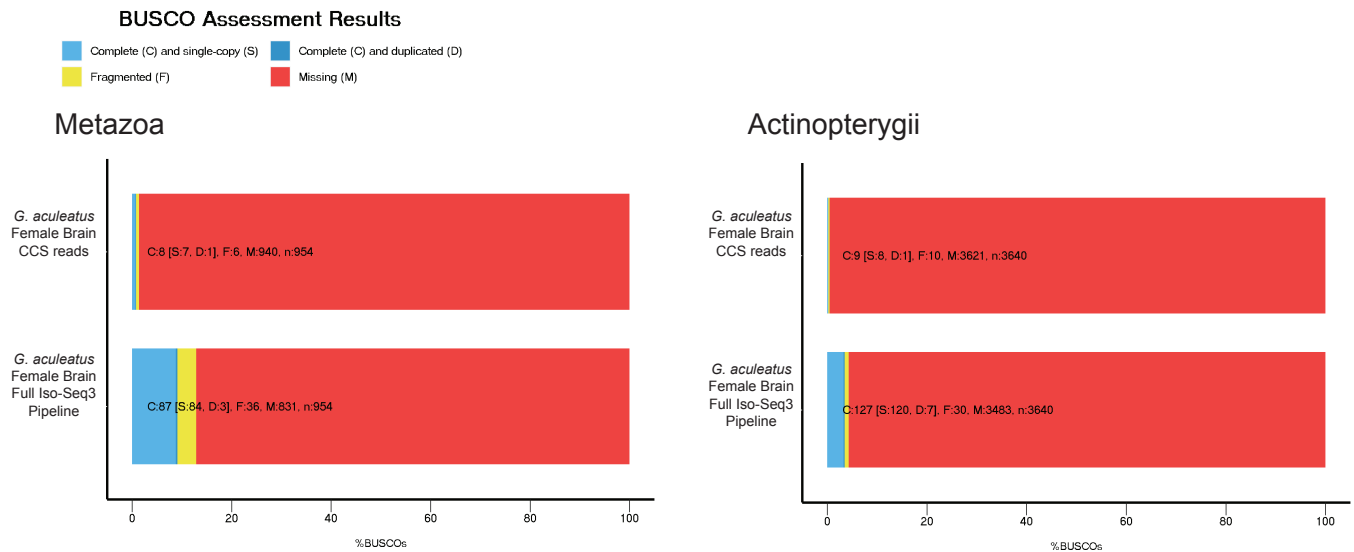


Supplemental_fig_S1.pdf



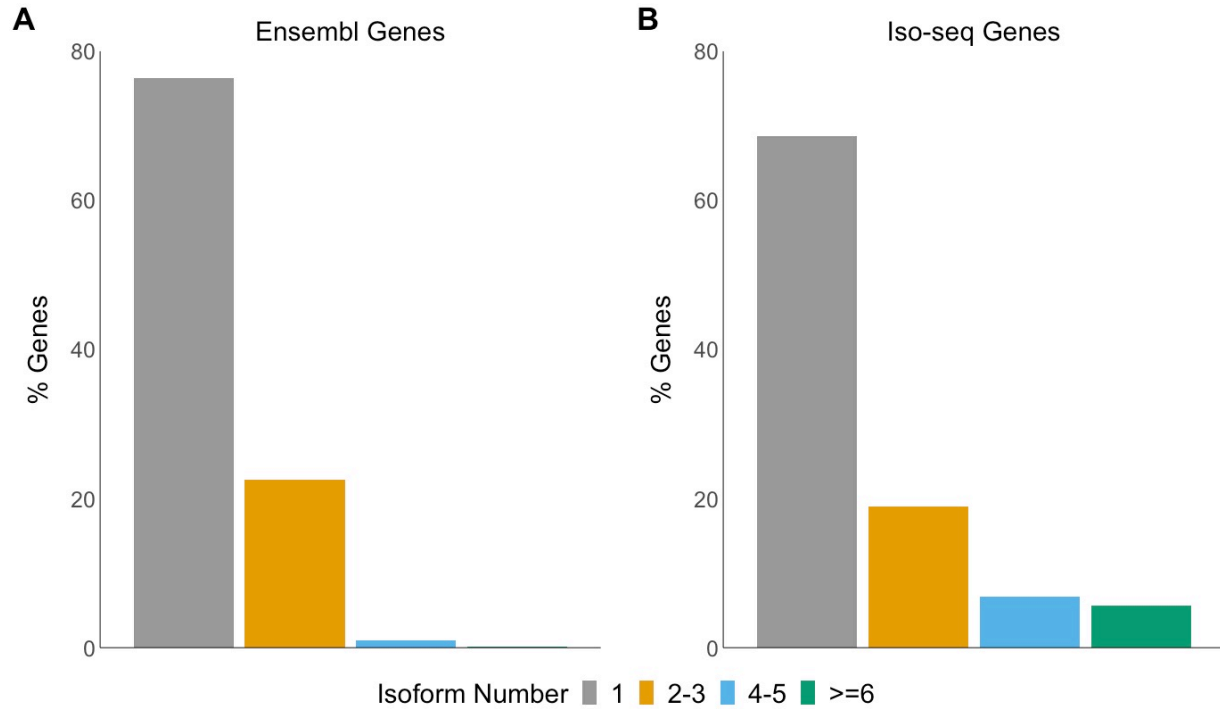
Supplemental Fig S1. The Minimap2 transcriptome contains fewer complete orthologs than the Ensembl transcriptome but is more complete than the deSALT transcriptome. The Ensembl transcriptome is almost complete in Metazoan (93.3% complete orthologs) and Actinopterygian (85.0% complete orthologs) lineages. The Minimap2 transcriptome, while not complete, represents 56.3% of complete Metazoan orthologs and 37.4% of complete Actinopterygian orthologs. The deSALT transcriptome was the least complete, representing only 27.0% of complete Metazoan orthologs and 16.9% of complete Actinopterygian orthologs.

Supplemental_Fig_S2.pdf



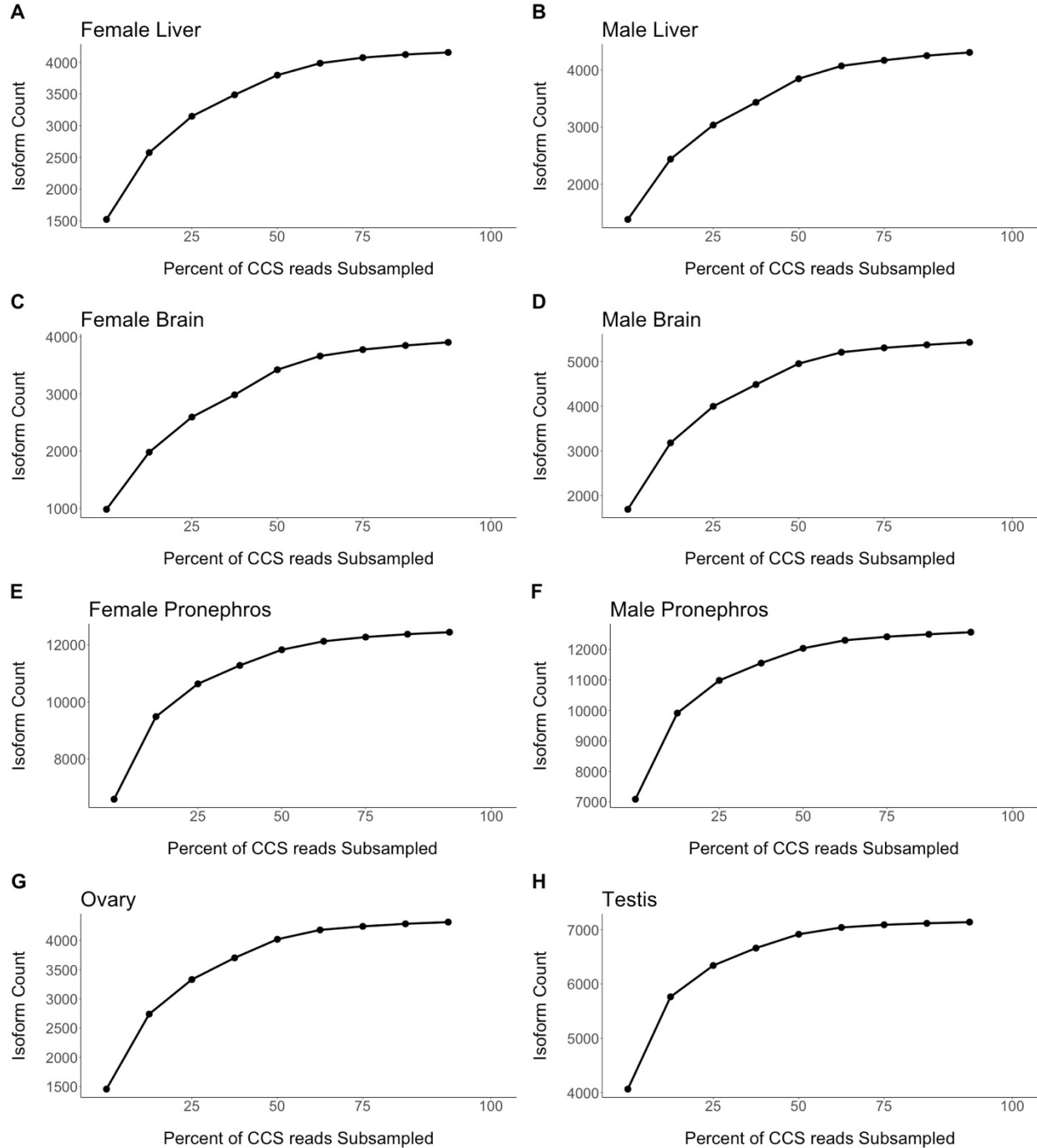
Supplemental Fig S2. The CCS reads produced a less complete transcriptome compared to the full Iso-Seq3 pipeline. Here we examined the female brain sample as a representative sample. The CCS reads transcriptome only had 0.8% of the complete Metazoan orthologs and 0.2% of complete Actinopterygian orthologs. The Iso-Seq3 transcriptome was more complete, representing 9.1% of complete Metazoan orthologs and 3.5% of complete Actinopterygian orthologs.

Supplemental_Fig_S3.jpeg



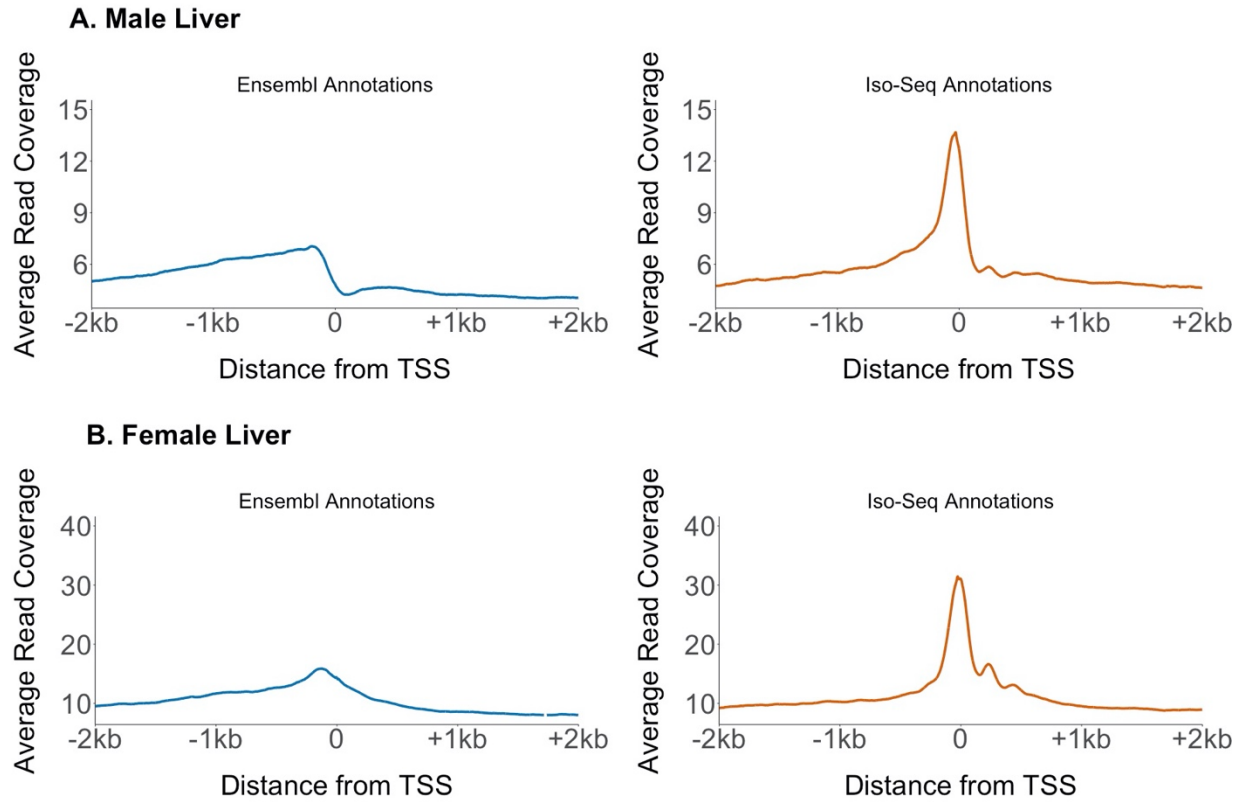
Supplemental Fig S3. There are more isoforms per gene in the Iso-Seq transcriptome compared to the Ensembl transcriptome. (A) In the Ensembl transcriptome, only 24% of genes have more than one isoform. (B) In the Iso-Seq transcriptome, 31% of all genes have at least two isoforms.

Supplemental_Fig_S4.jpeg



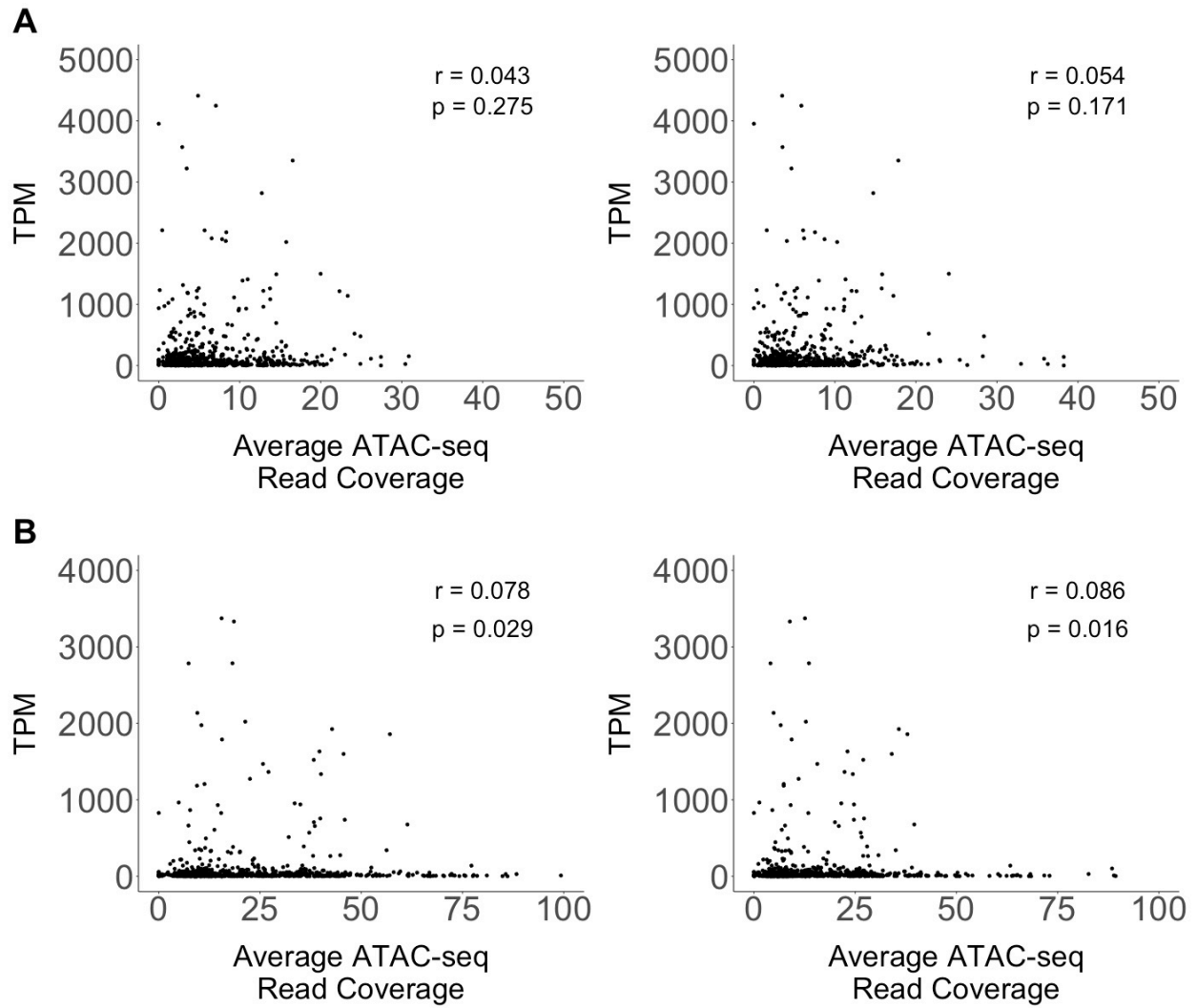
Supplemental Fig S4. Over 90% of total isoforms are recovered when subsampling CCS reads. 90% of total isoforms are recovered at the following percent of subsampled CCS reads: (A) 65% for the female liver, (B) 85% for the male liver, (C) 85% for the female brain, (D) 65% for the male brain, (E) 65% for the female pronephros, (F) 50% for the male pronephros, (G) 65% for the ovary, and (H) 35% for the testis.

Supplemental_Fig_S5.jpeg



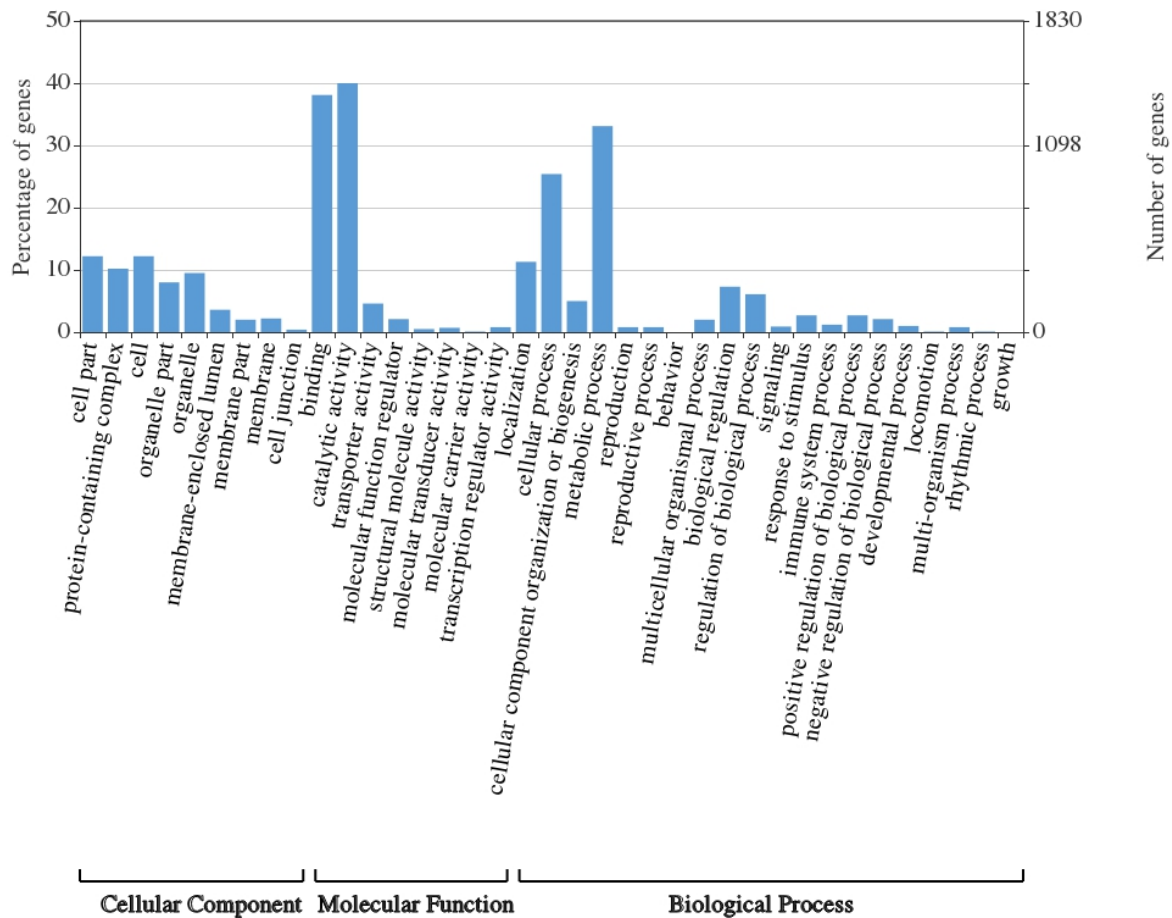
Supplemental Fig S5. Accessible chromatin is localized in narrow peaks around the Iso-Seq transcription start sites. We compared ATAC-seq read coverage at all Ensembl TSSs and Iso-Seq TSSs across the autosomes in a male liver (A) and female liver (B). ATAC-seq reads show an enrichment at the Iso-Seq TSS compared to the Ensembl TSS. This indicates a more accurate positioning of the TSS using Iso-Seq. A second male and female replicate is shown in Figure 3.

Supplemental_Fig_S6.jpeg

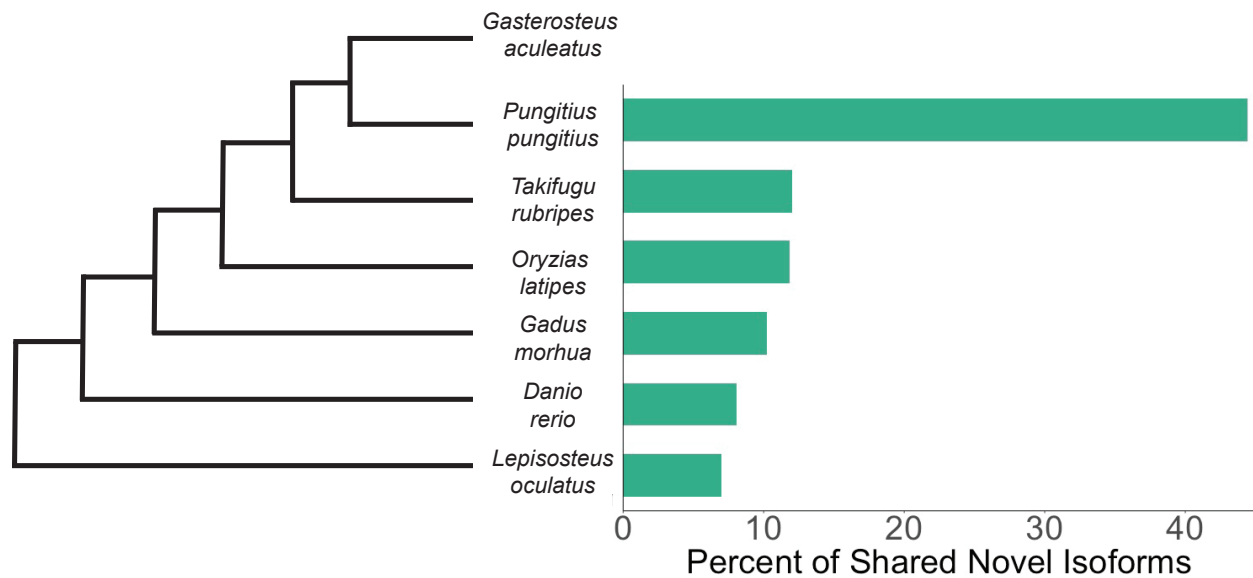


Supplemental Fig S6. Isoform expression and ATAC-seq read coverage were weakly correlated. (A) Male liver ATAC samples (individual 1: left panel; individual 2: right panel) both had a weak positive correlation with isoform expression levels, but were not significantly correlation (Spearman's rank correlation; $p > 0.05$). (B) Female liver ATAC samples (individual 1: left panel; individual 2: right panel) also both had weak positive correlations with isoform expression, but were significantly correlation (Spearman's rank correlation; $p < 0.05$).

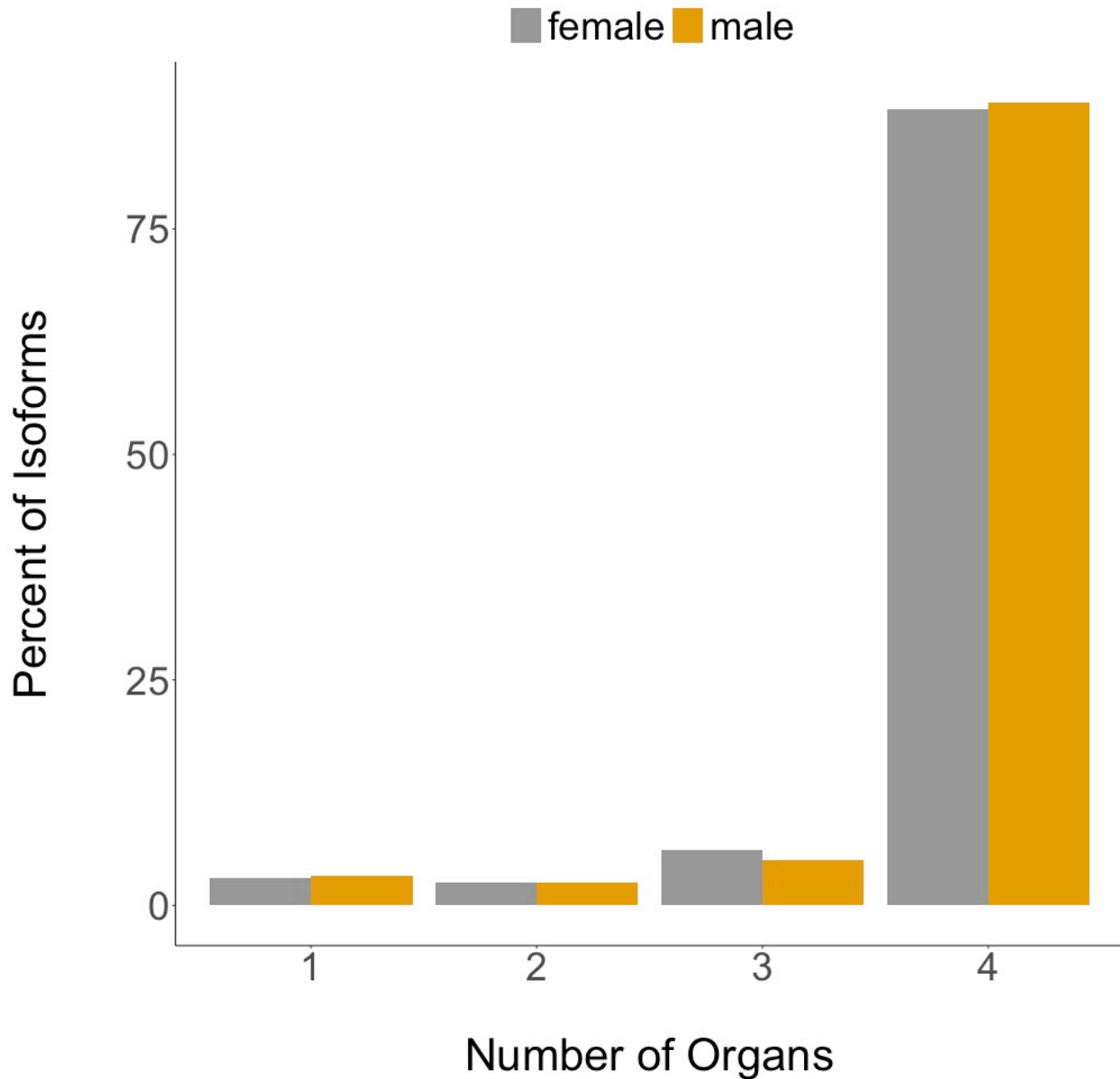
Supplemental Fig S7.jpeg



Supplemental Fig S7. Novel isoforms have many general cellular functions. 595 GO terms were significantly enriched ($P < 0.001$) after a Bonferroni correction. GO terms were collapsed into more general categories using WEGO (web gene ontology annotation plot).

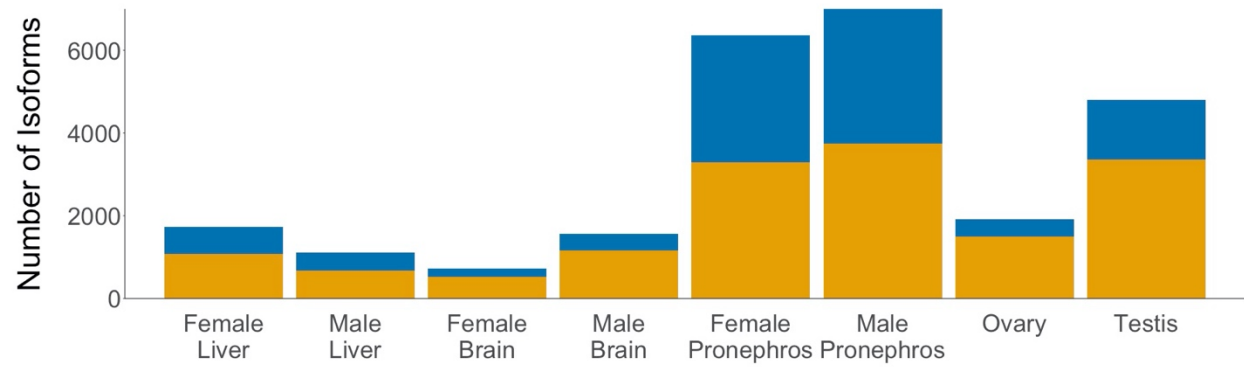


Supplemental Fig S8. Novel isoforms are largely stickleback specific. Using BLAST, we examined if novel isoforms were present across six different fish species. We looked for novel isoforms in ninespine stickleback (*Pungitius pungitius*), fugu (*Takifugu rubripes*), medaka (*Oryzias latipes*), Atlantic cod (*Gadus morhua*), zebrafish (*Danio rerio*), and spotted gar (*Lepisosteus oculatus*). We found that 44.5% of the novel isoforms were shared between threespine and ninespine stickleback fish. The percentage of shared novel isoforms decreased as divergence time increased between threespine stickleback and the remaining species (fugu: 12.0%; medaka: 11.8%; Atlantic cod: 10.2%; zebrafish: 8.0%; spotted gar: 7.0%).

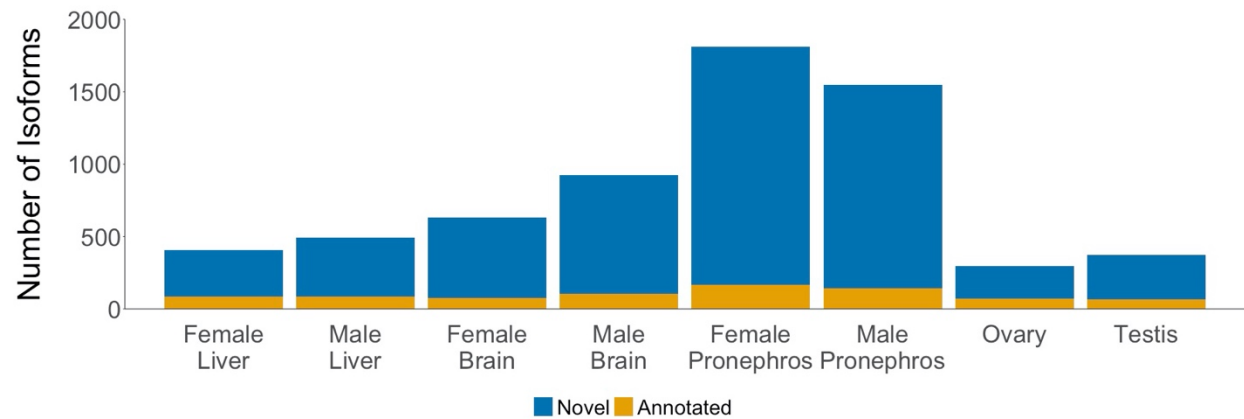


Supplemental Fig S9. Most sex-specific alternatively spliced isoforms are expressed across all four organs examined. From 4,842 genes that were expressed in both sexes, 1,590 isoforms were female-specific and 2,103 were male-specific. We then utilized short-read RNA-seq to measure the expression of these sex-specific isoforms across all four organs from each sex. For the female-specific isoforms, 88.3% were expressed in all female organs. For male-specific isoforms, 89.1% were expressed across all male organs.

A. Protein Coding Isoforms



B. Non-protein Coding Isoforms



Supplemental Fig S10. Most of the novel isoforms are non-protein coding. (A) Most protein-coding isoforms were previously annotated in the Ensembl transcriptome. The pronephros and the testis have the largest number of novel isoforms. (B) A majority of non-protein coding isoforms across all samples are novel isoforms.