

Supplementary Figure S1

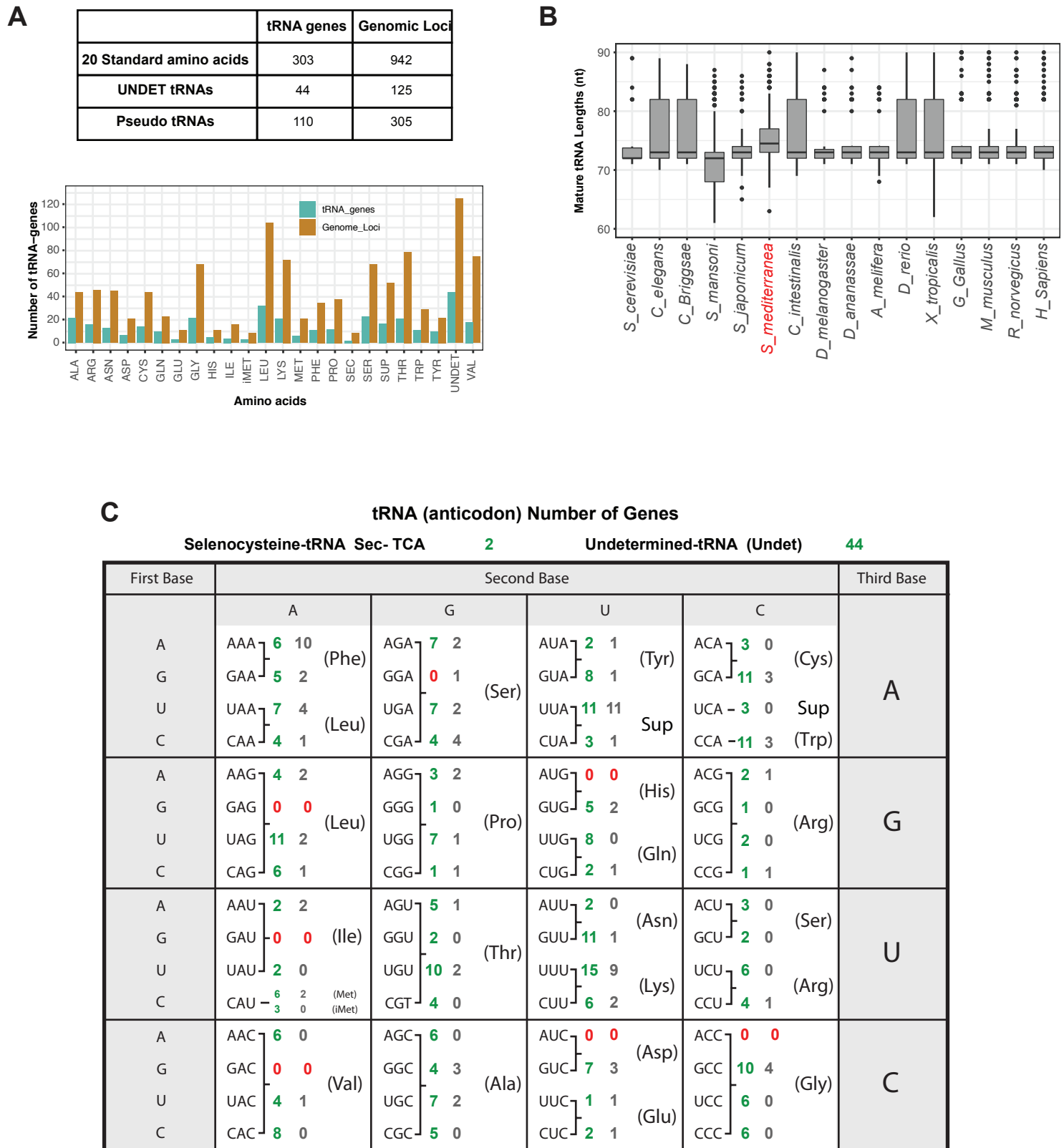


Figure S1: A) Table showing total number of tRNA genes predicted in planaria and the number of genomic loci for these tRNAs. **B)** Mature tRNA lengths across different species shows a median length of 72 nucleotides with planarian tRNAs showing a median of 75 nts. **C)** tRNA anticodon table depicting all the anticodons and the number of tRNA genes identified for each anticodon (in green) and pseudogene (in grey).

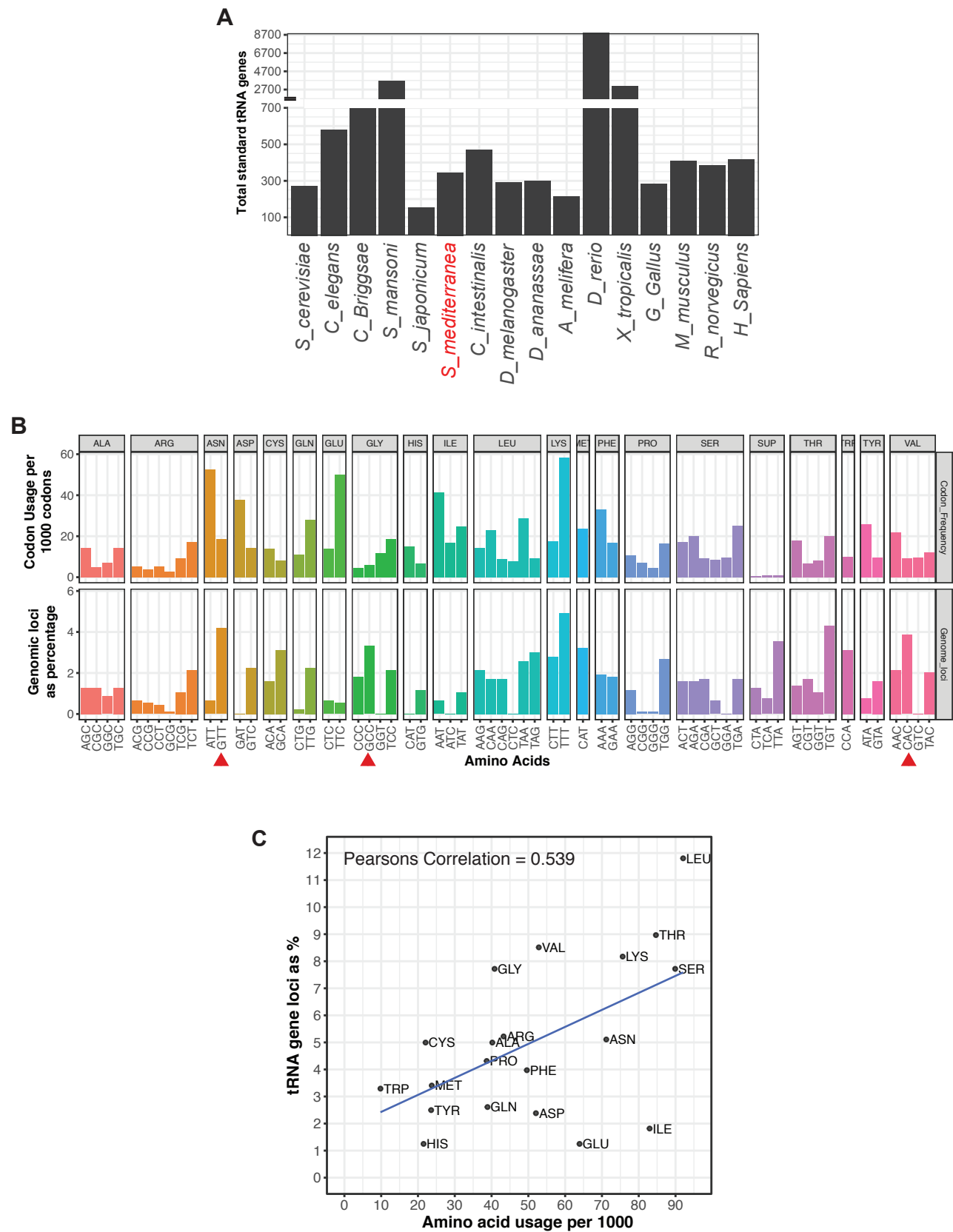


Figure S2: A) Number of tRNA gene for the standard 20 amino acids across the animal kingdom. Predicted planarian tRNA gene numbers are comparable across the species. **B)** Codon frequency across 1000 codons in the transcriptome and the abundance of its cognate anticodon tRNA in *S.mediterranea* genome. The red arrows examples of codon:anticon pairs that show opposite correlation. **C)** Correlation between tRNA gene copy number and amino acid usage in planarians.

Supplementary Figure S3

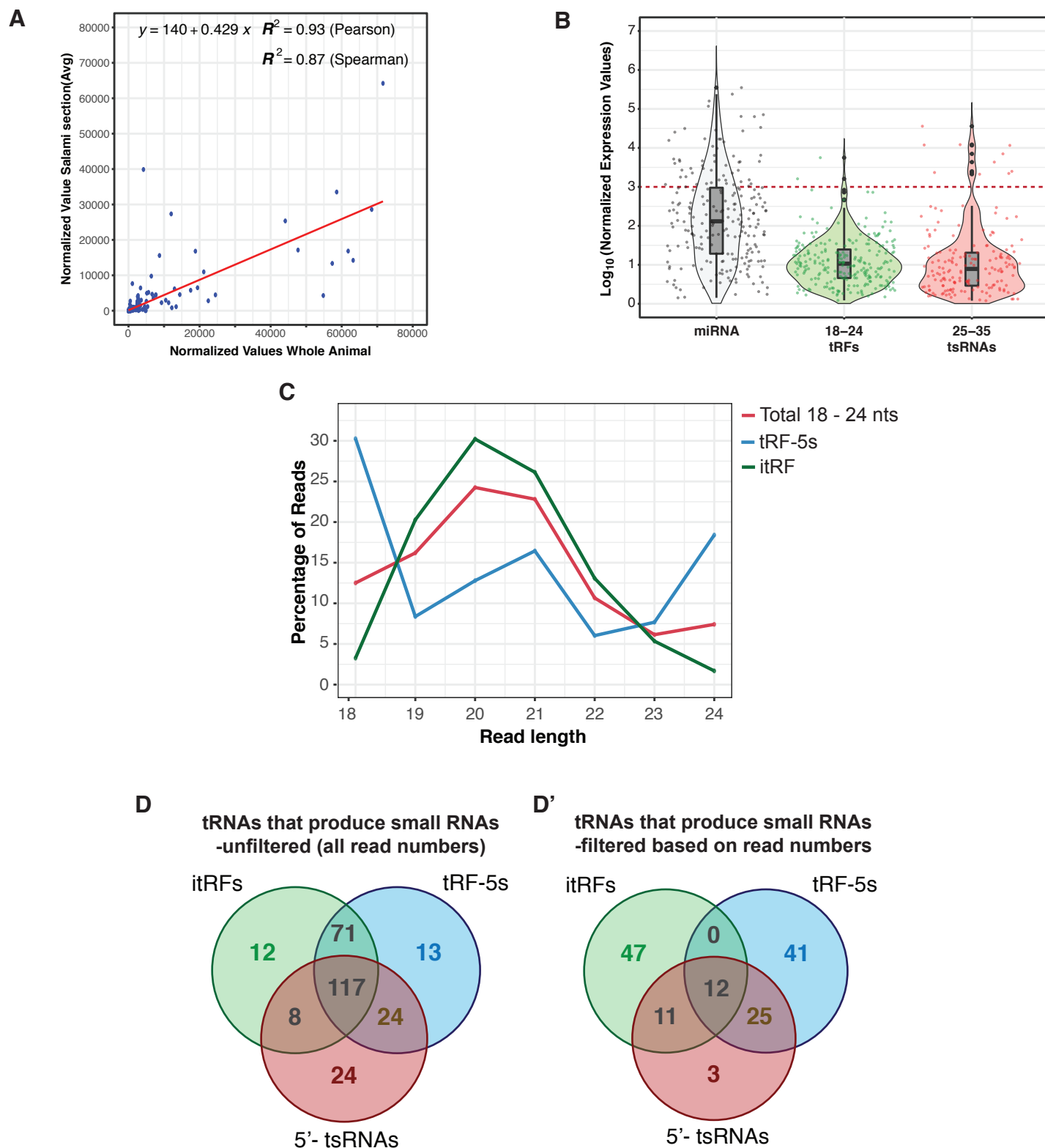


Figure S3: A) Correlation between small RNA reads mapping to tRNA in 'Uncut' and the cumulative of small RNA reads mapping to tRNA from Salami Sections. **B)** Normalised expression of all miRNAs compared to the reads mapping to tRNAs. The red dotted line indicates that the top 20 25-35 nt tsRNA species are expressed at higher levels than most miRNAs. **C)** Size distribution of itRFs and tRF-5s and the over all 18-24 reads mapping to tRNA. **D)** Venn diagram of tRNA that produce itRFs, tRF-5s and 5'-tsRNAs. **D')** Venn diagram of tRNA that produce itRFs, tRF-5s and 5'-tsRNAs using stringent criteria.

Supplementary Figure S4

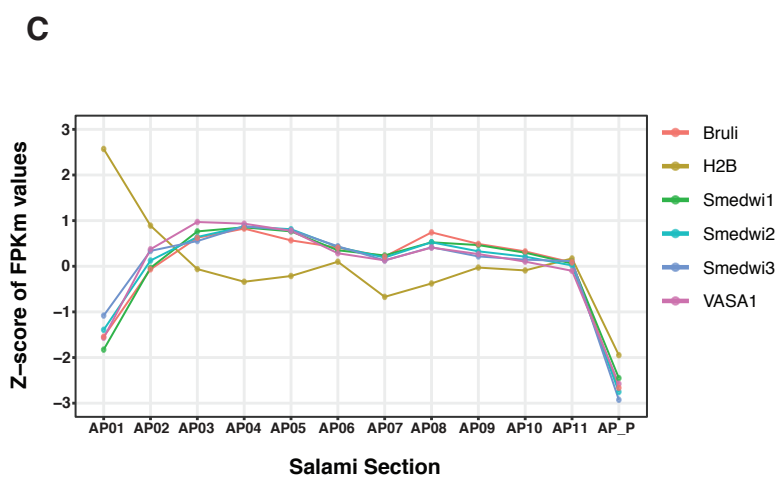
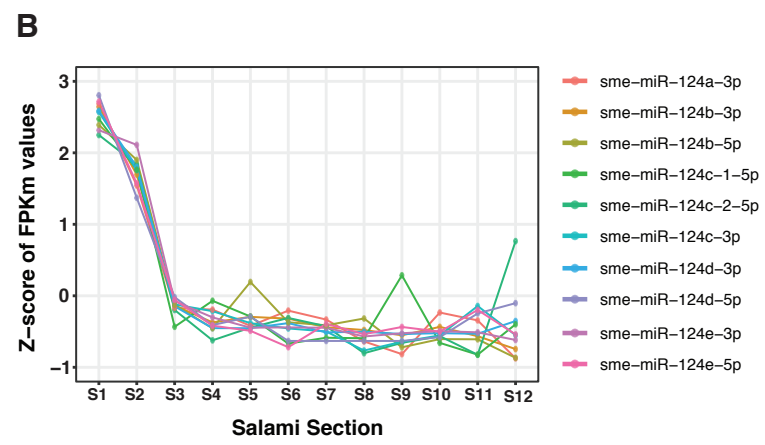
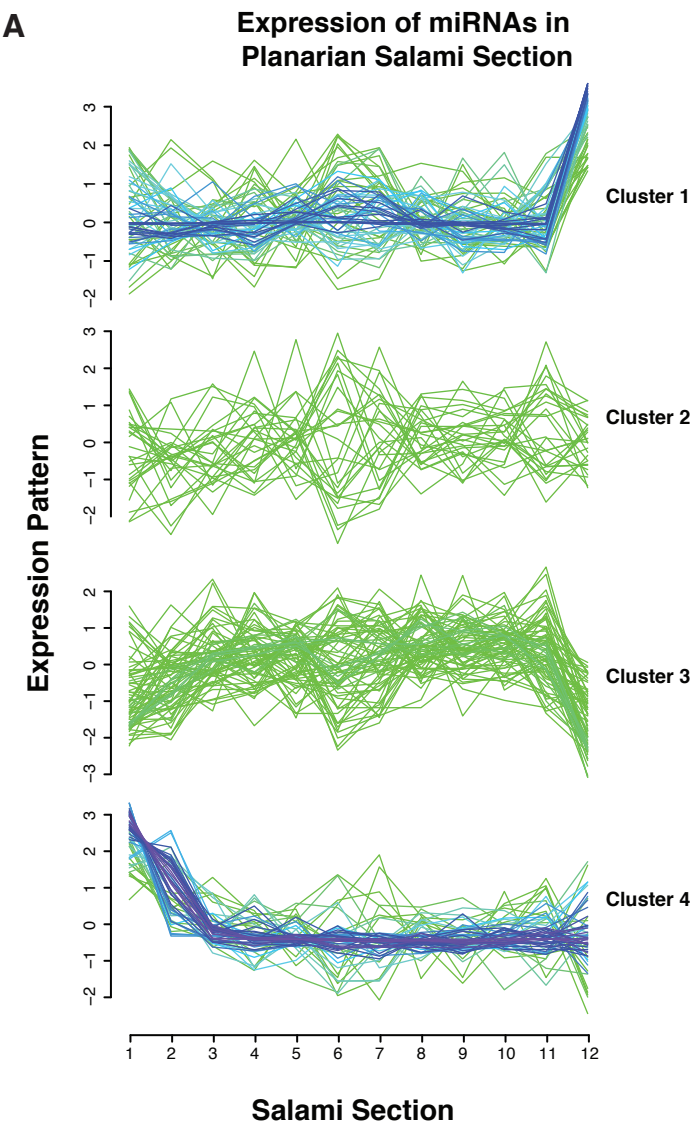


Figure S4: A) Expression clusters of miRNAs across planarian body axis identifies 4 clusters of expression. **B)** Expression of miR-124 family of miRNAs that are expressed in the brain shows enrichments in the anterior sections of planaria. **C)** Expression of transcripts expressed in neoblasts across the different salami sections.

Supplementary Figure S5

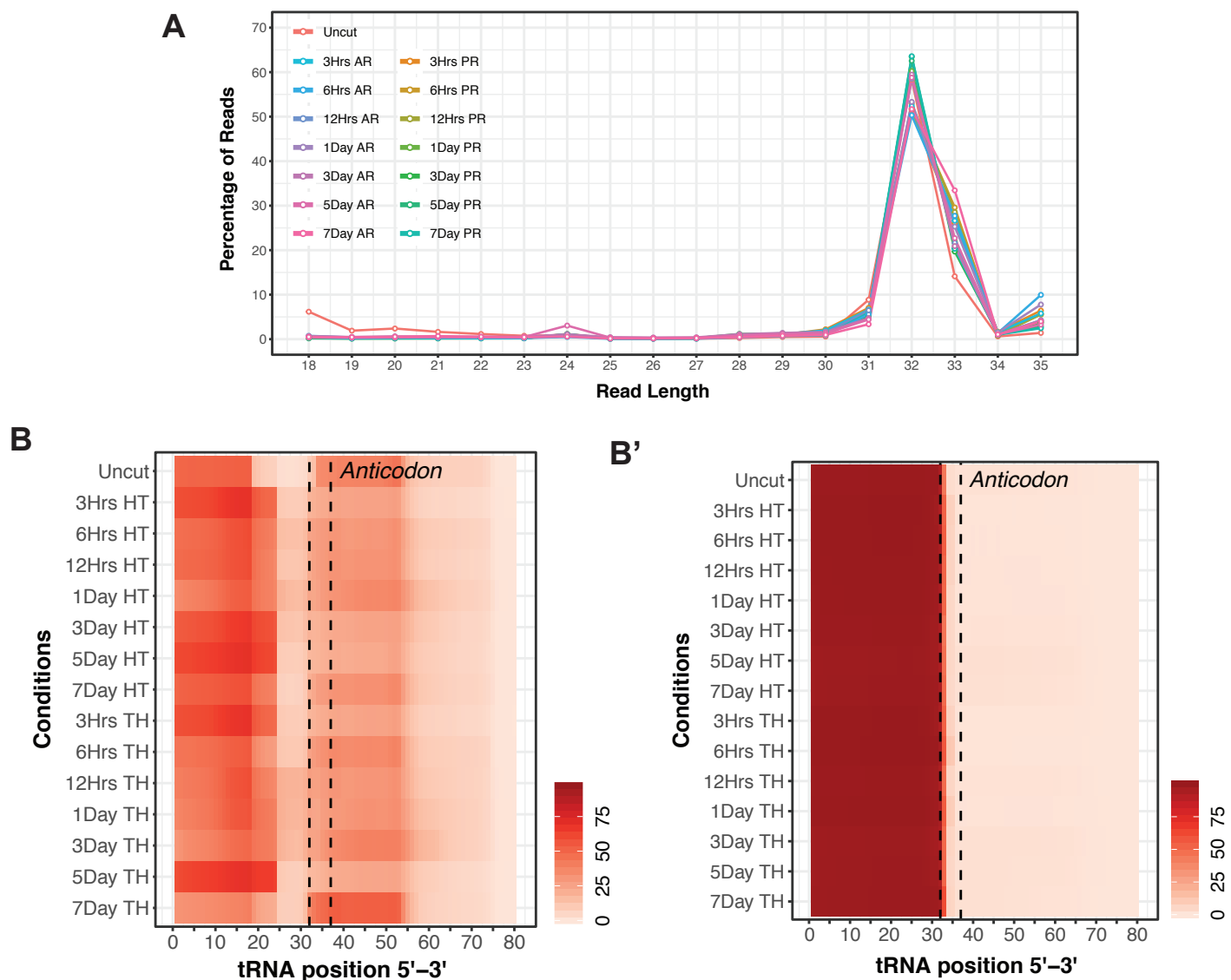
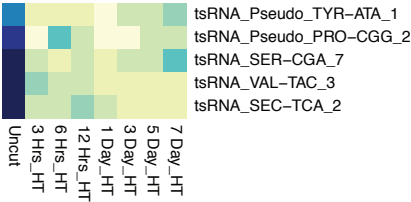


Figure S5: A) Size distribution of 18-35 nt small RNA reads that map to planarian tRNAs across regeneration timepoints. **B)** Perbase coverage of 18-24 nts of small RNA reads mapped to the tRNA length identifies two species of tRNA fragments - itRFs and tRF-5s. **B')** Perbase coverage of 25-35 nts of small RNA reads mapped to the tRNA length shows reads arise only from the 5' arm of tRNA.

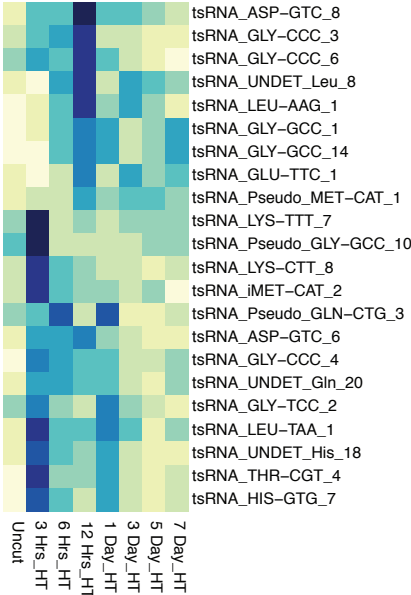
Supplementary Figure S6

A

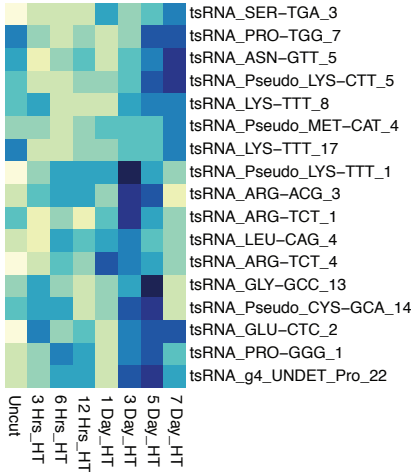
tsRNAs down regulated over the course of PR



tsRNAs upregulated early in PR

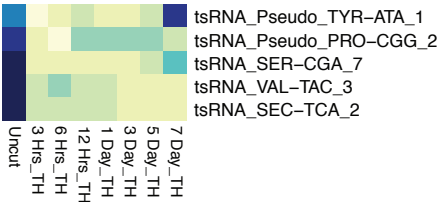


tsRNAs upregulated late in PR

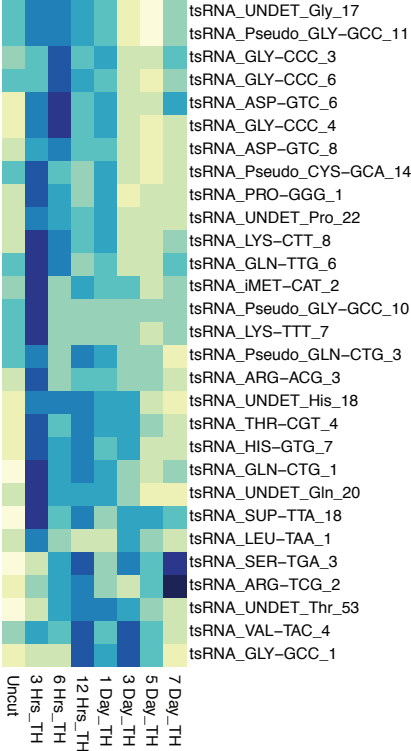


A'

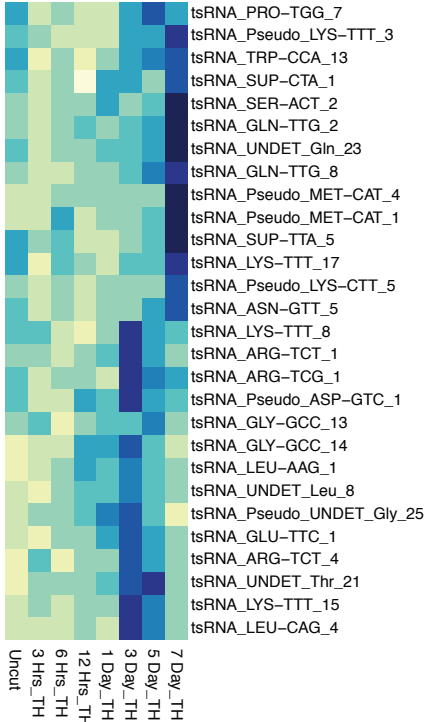
tsRNAs down regulated over the course of AR



tsRNAs upregulated early in AR



tsRNAs upregulated late in AR



B

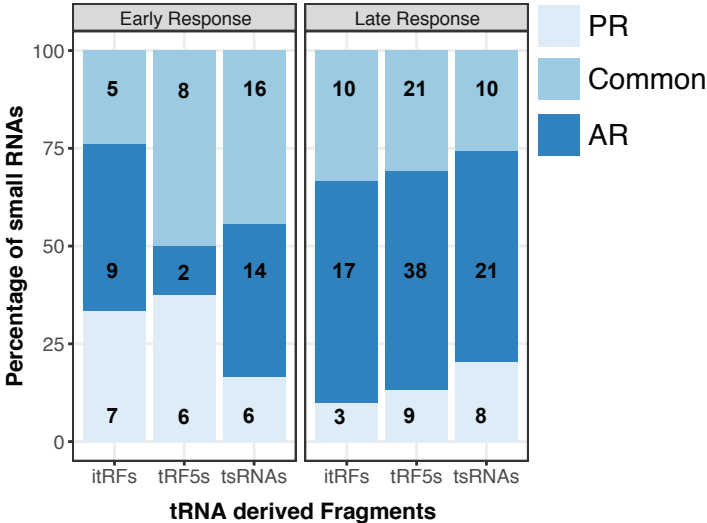


Figure S6: A) Heatmaps of 5' tsRNAs during posterior regenerating (PR). **A')** Heatmaps of 5' tsRNAs in anterior regenerating worms (AR). **B)** Percentage of tRNA-derived fragments that are common and unique in early (3hrs -1day) and late (3days - 7days) regeneration of head (HT)and tail fragments (TH).

Supplementary Figure S7

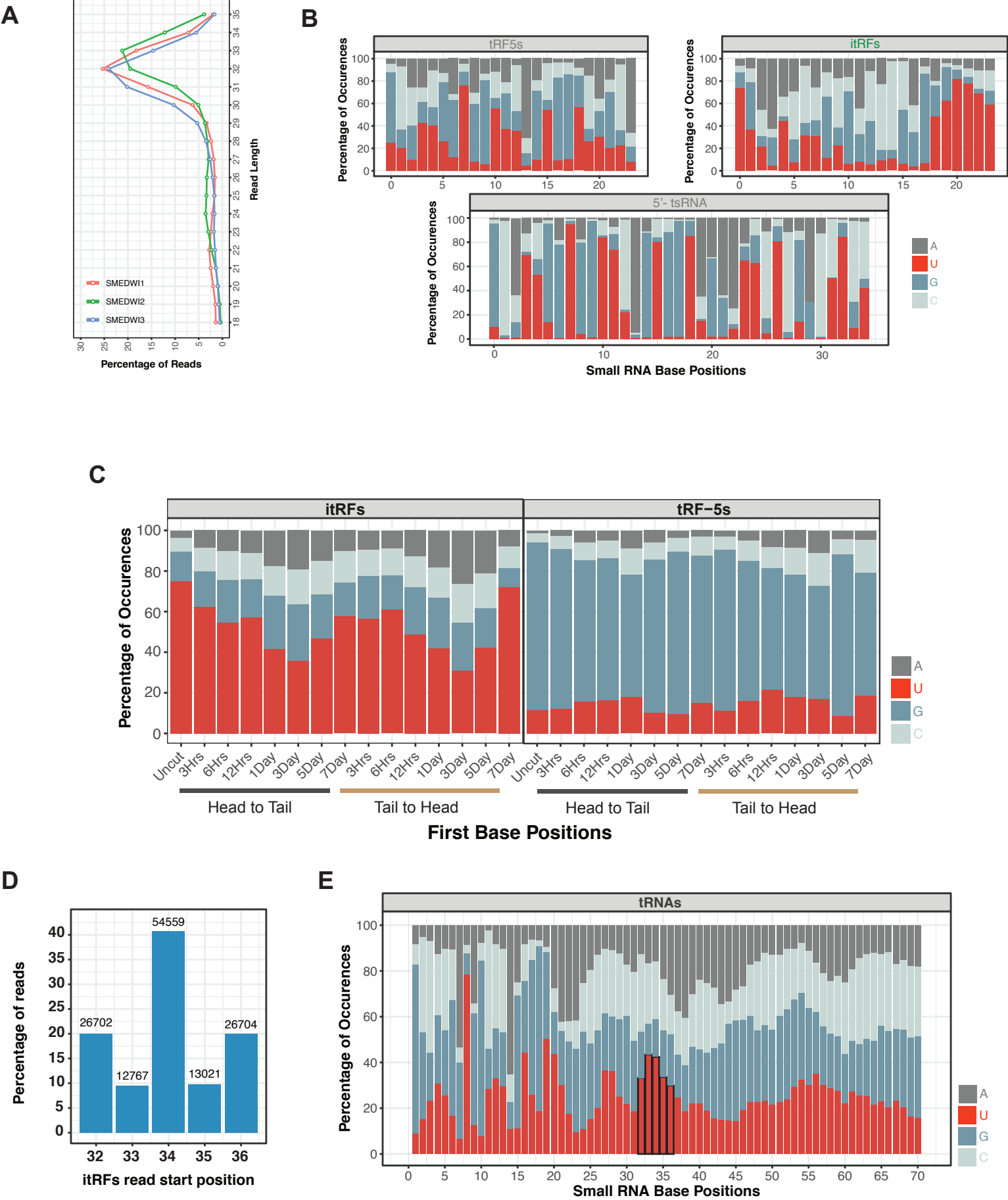


Figure S7: A) Size distribution of reads associating with SMEDWI-1, -2 and -3 that map to tRNAs. **B)** Base preference for the first base of all itRFs and tRF-5s during regeneration timepoints. **C)** Base preference across the length of tRNA-derived fragments identified in the study. **D)** Start positions of itRF fragments on the respective tRNAs from which they are processed. **E)** Base preference for the positions (from which itRFs are processed) across all tRNAs.

Supplementary Figure S8

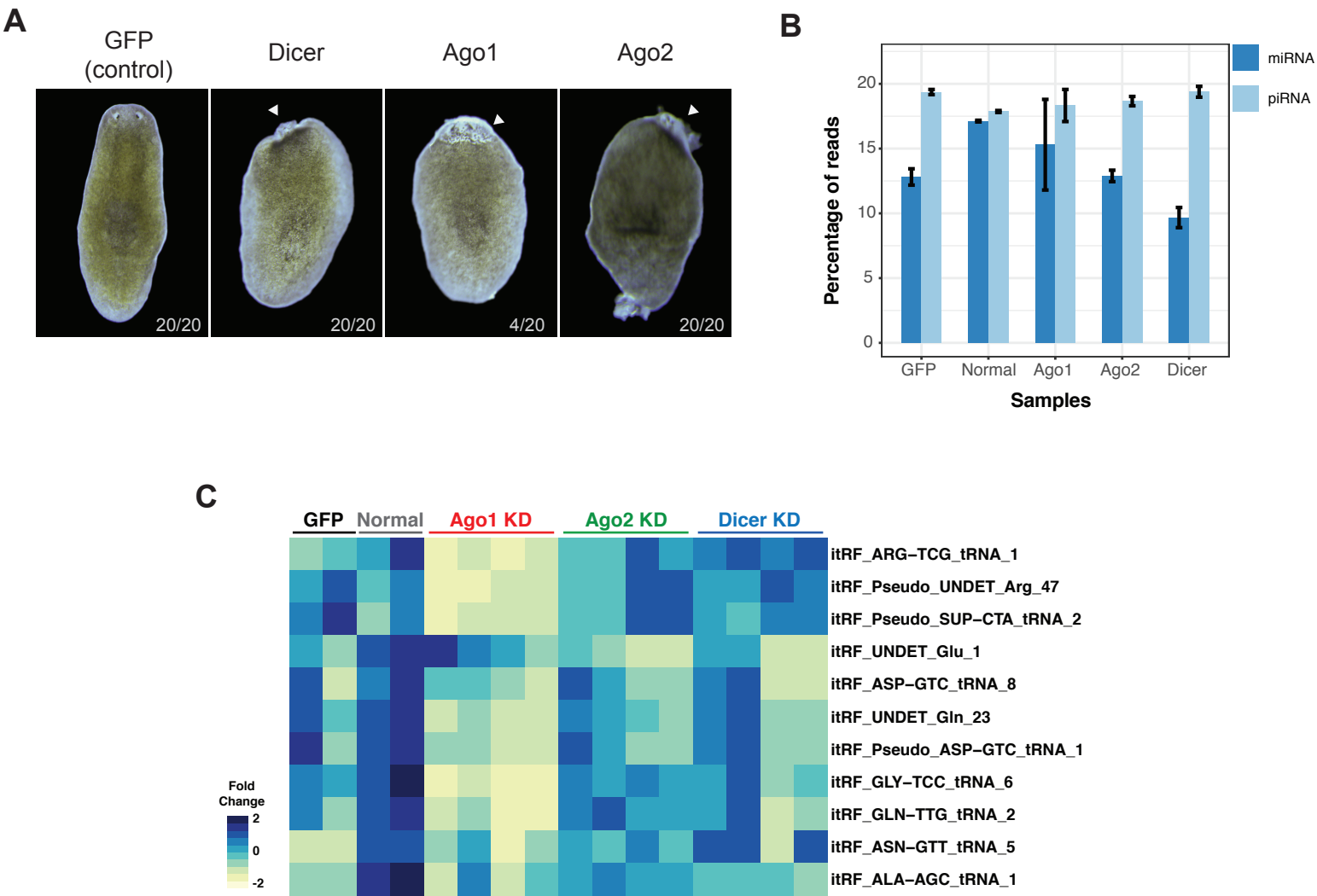


Figure S8: A) Phenotypes observed in planarians upon Ago1, Ago2 and Dicer Knockdown experiments. Inset shows then number of animals that showed phenotype out of the total animals tested (20). The arrow-heads depict head regression, lesions or lysis. **B)** miRNA and piRNA levels observed in different knockdown conditions. **C)** Expression of top 10 expressed itRFs in various knockdown conditions tested. Ago1 KD showed a decrease in the itRFs.