

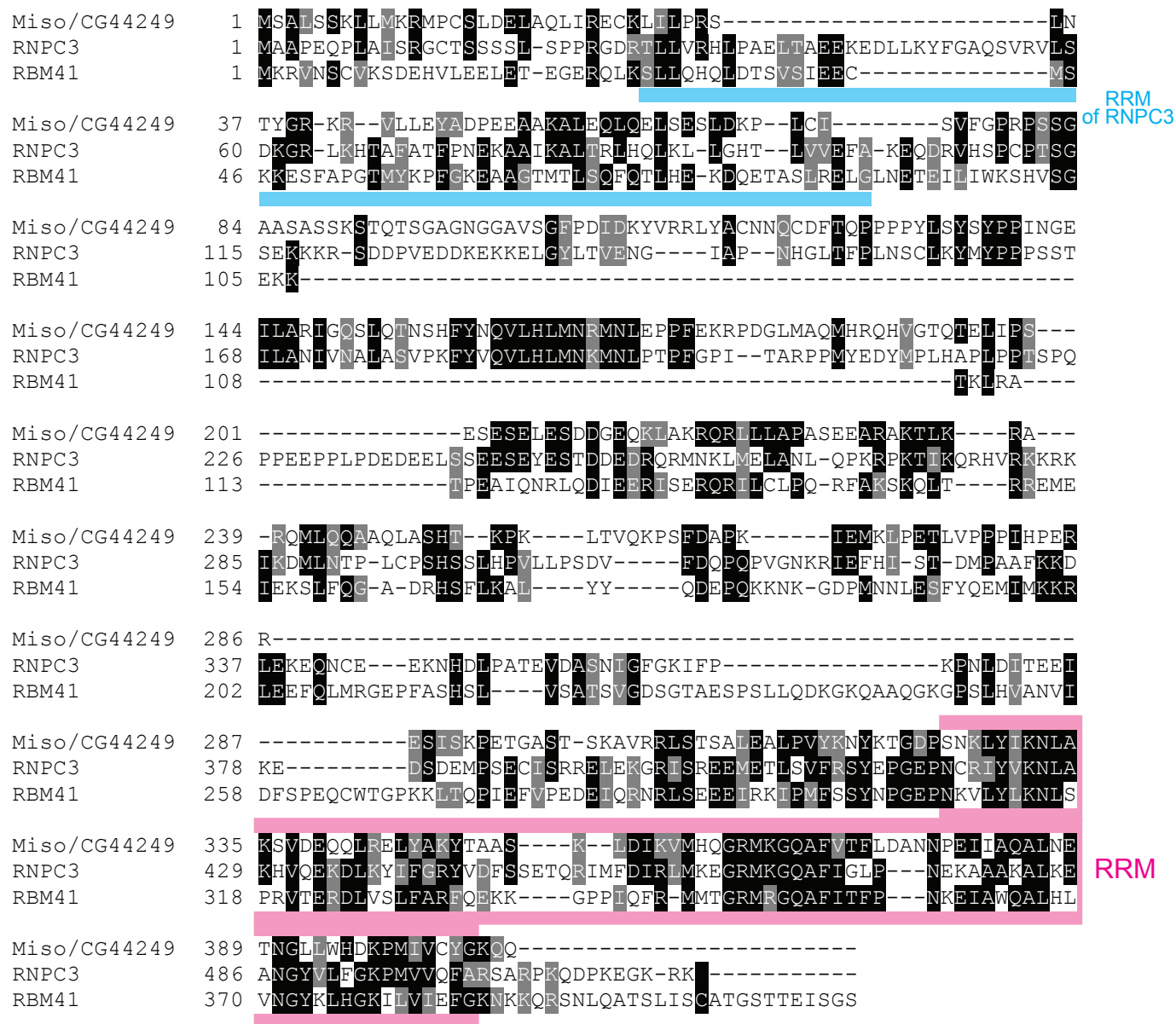
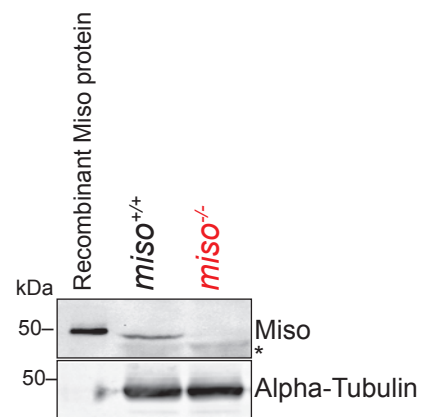


Fig S1. Multiple sequence alignment of Drosophila Miso/CG44249, human RNPC3, and human RBM41. Drosophila Miso/CG44249 and human RNPC3 share 31% sequence identity and 45% similarity. Drosophila Miso/CG44249 and human RBM41 share 27% sequence identity and 38% similarity. The N-terminal RNA-recognition motif (RRMs) in RNPC3 is indicated in cyan and the C-terminal RRM in Miso/CG44249, RNPC3, and RBM41 are indicated in pink.

**Fig S2. Miso Western blot**

Western blot analysis of Miso protein expression in ovaries using purified recombinant Miso protein as a control. The purified recombinant Miso protein (MW 45.6 kDa) has additional three amino acid residues (Gly-Pro-Val) at the N-terminus compared with the annotated Miso protein (MW 45.4 kDa).

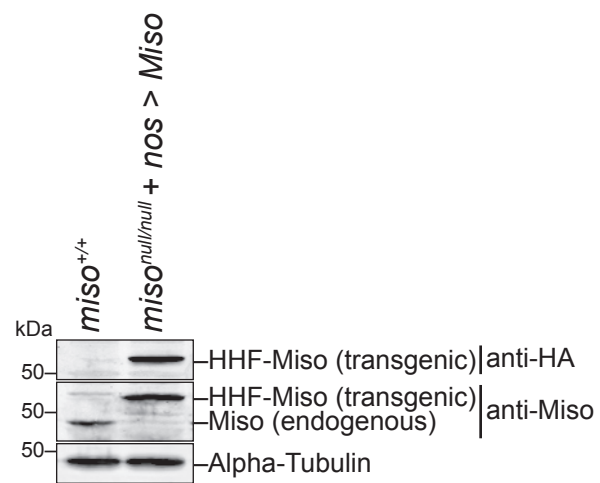
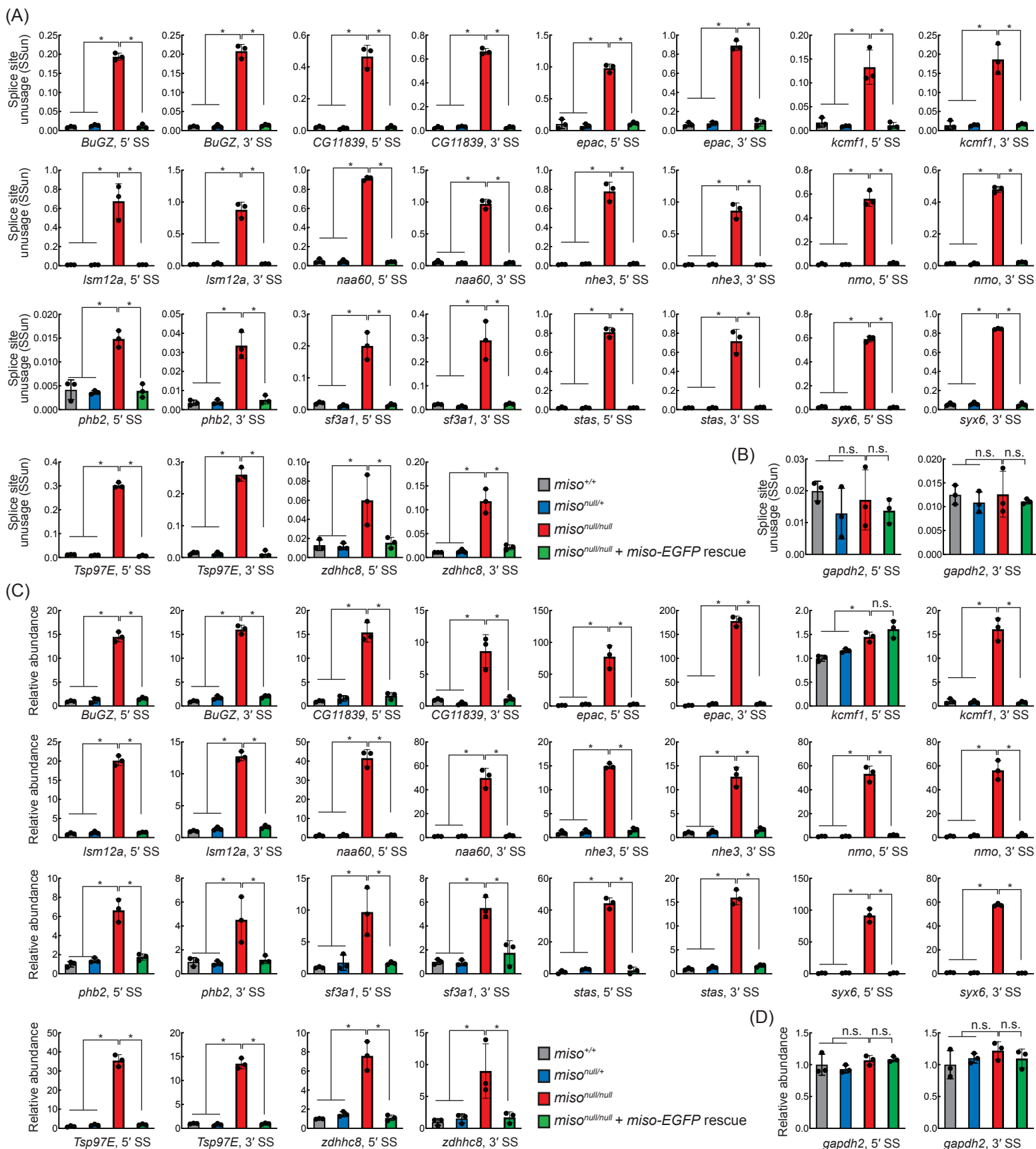


Fig S3. Miso Western blot of *nos* > *Miso* rescue ovaries

Western blot analysis of Miso protein expression in ovaries from control and *miso*^{null/null} + *nos* > *Miso* flies.

**Fig S4. Minor intron retention in *miso* mutant ovaries**

(A) Splice site usage (SSun) of 5' and 3' splice sites of minor introns, as determined by RNA-seq of ovary RNAs.

Mean $\pm$ SD (n=3). p-value < 0.05 (Student's t-test, unpaired, two-tailed) is indicated by *.(B) SSun of a major intron in *gapdh2* determined by RNA-seq of ovary RNAs. Mean $\pm$ SD (n=3).(C) RT-qPCR quantification of RNAs with retained minor introns at 5' and 3' splice sites, normalized to *act5c* mRNA.Mean $\pm$ SD (n=3). p-value < 0.05 (Student's t-test, unpaired, two-tailed) is indicated by *.(D) RT-qPCR quantification of *gapdh2* RNAs with retained major introns at 5' and 3' splice sites, normalized to *act5c* mRNA.Mean $\pm$ SD (n=3).

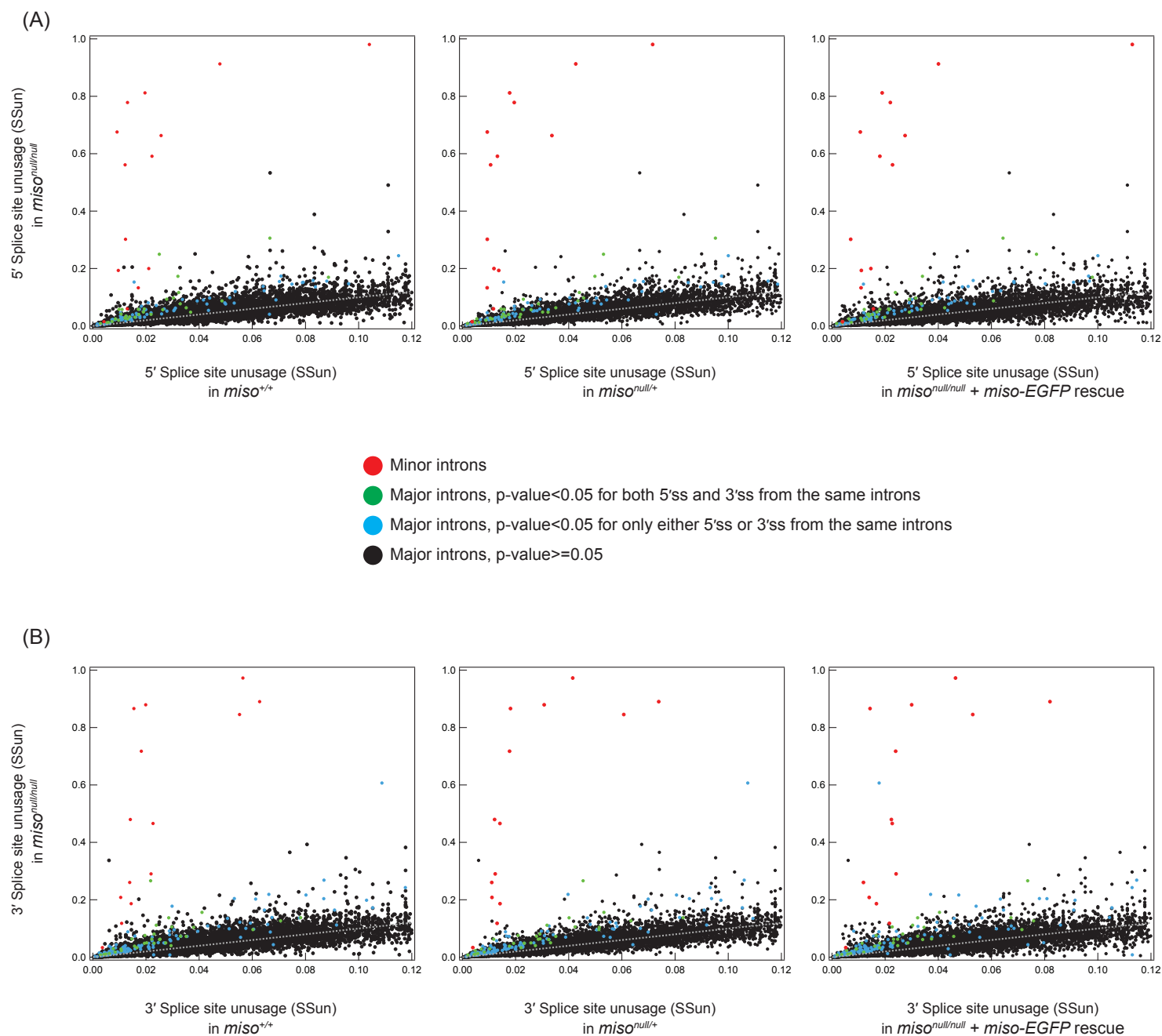


Fig S5. Global intron retention analysis in miso mutant ovaries

Scatter plots showing splice site usage (SSun) of (A) 5' and (B) 3' splice sites of major and minor introns, as determined RNA-seq of ovary RNAs. Mean (n=3). Each dot represents an intron. Minor introns are shown in red. Major introns whose 5' and 3' splice sites are both significantly (p-value <0.05) more retained in *miso*^{null/null} than in all the other three genotypes are shown in green. Major introns with either 5' or 3' splice site (but not both) is significantly (p-value <0.05) more retained in *miso*^{null/null} than in all the other three genotypes are shown in blue.

SSun changes are much bigger for minor introns than major introns. Higher retention of some major introns might be secondary effects of reduction of Sf3a1, one of the MIGs, which is a component of the SF3A protein complex in U2 snRNP for major spliceosome.

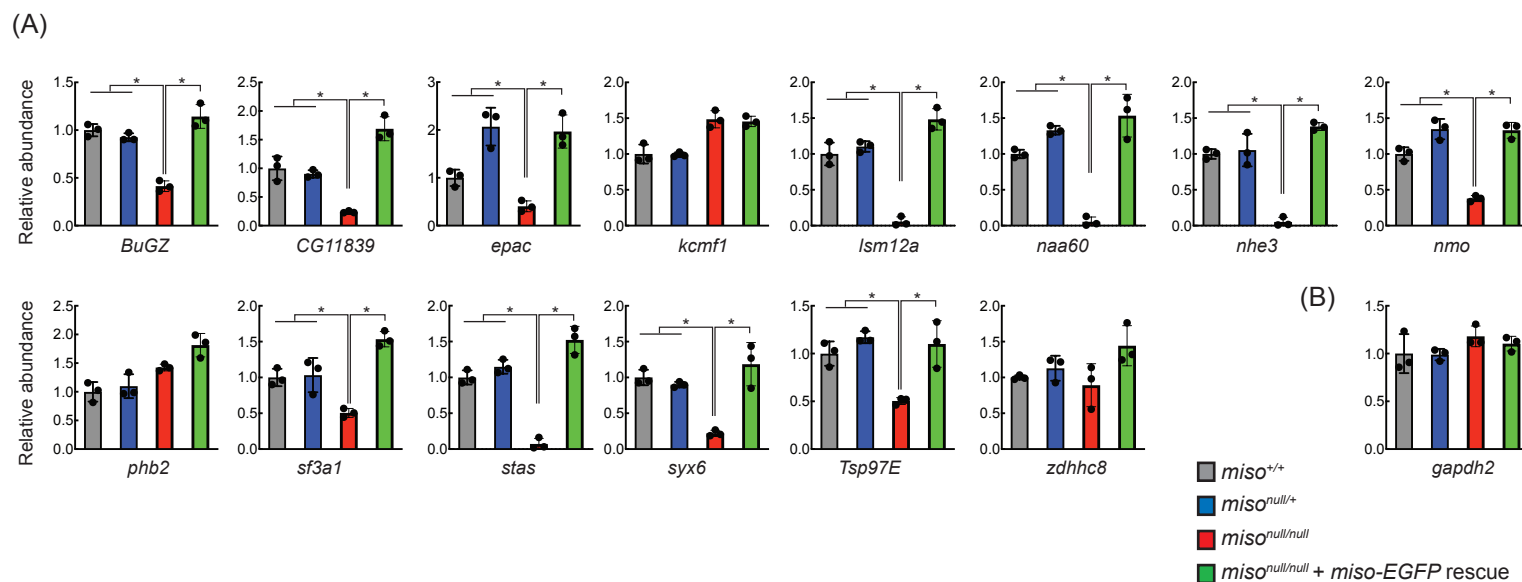


Fig S6. Reduced spliced MIG mRNAs in *miso* mutant ovaries

RT-qPCR quantification of spliced mRNAs from (A) MIGs and (B) *gapdh2* in ovaries, normalized to *act5c* mRNA. Mean $\pm$ SD (n=3). p-value < 0.05 (Student's t-test, unpaired, two-tailed) is indicated by *.

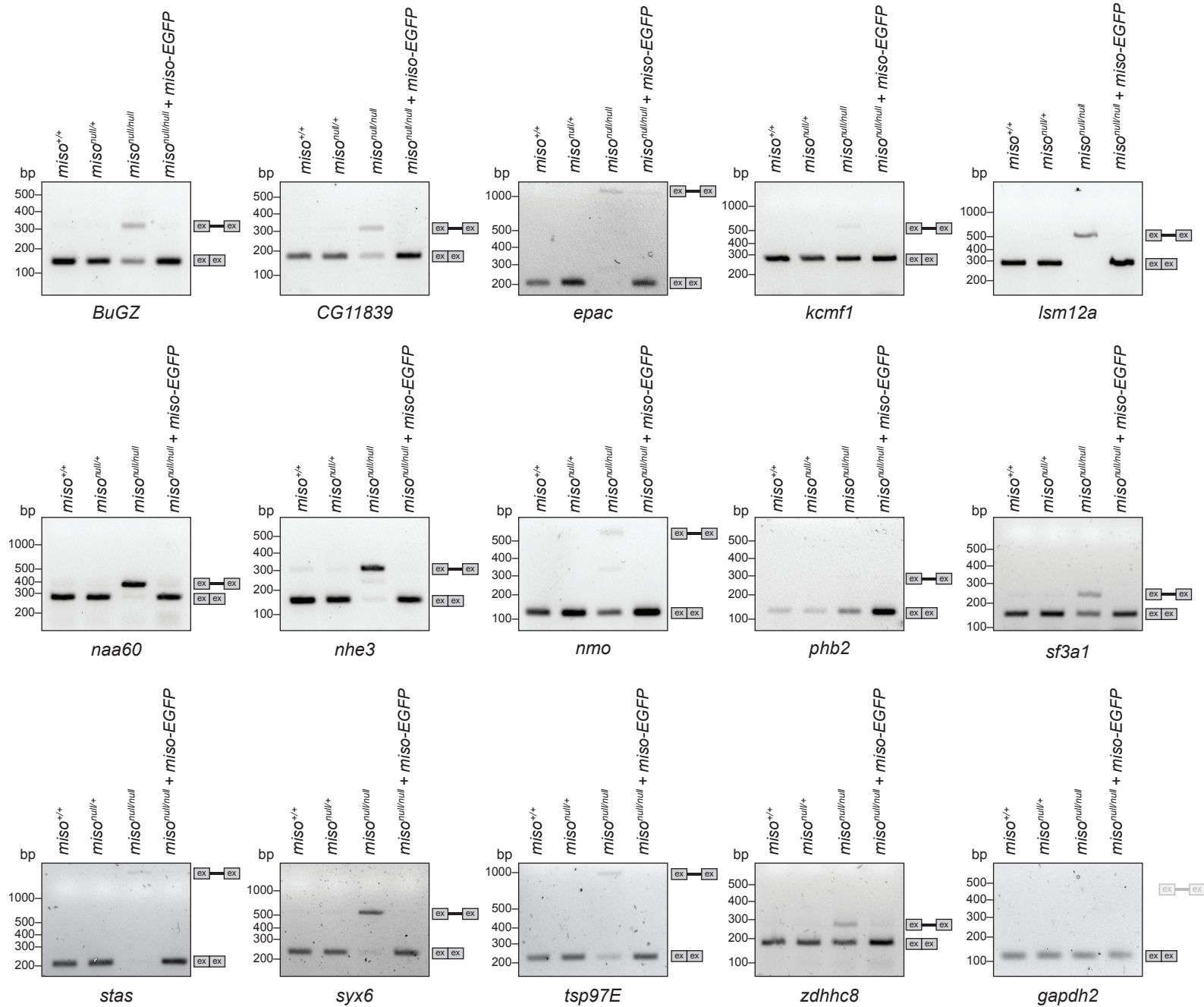
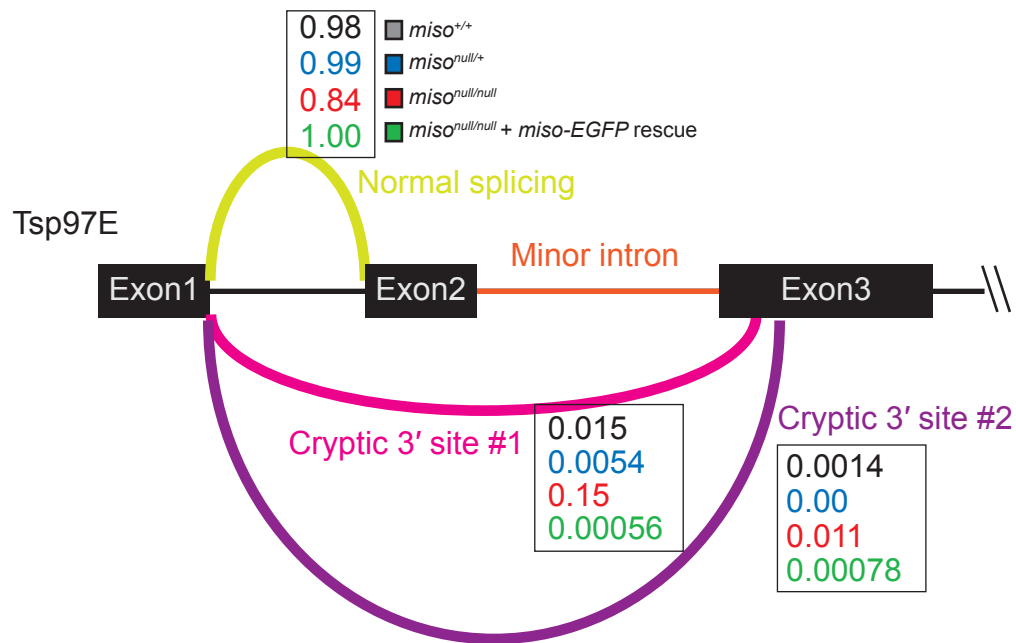


Fig S7. Minor introns retention and reduced spliced mRNAs from MIGs in miso mutant ovaries

Agarose gel electrophoresis of RT-PCR products from ovary RNAs, stained with SYBR Safe. Bands representing spliced and unspliced RNAs are indicated.

**Fig S8. Cryptic splicing in *Tsp97E***

Leafcutter analysis results of the relative ratio of splicing events in *Tsp97E* exons 1, 2, and 3 in *miso*^{+/+}, *miso*^{null/+}, *miso*^{null/null}, and *miso*^{null/null} + *miso*-EGFP using ovary RNA-seq dataset. The ratio of alternative splicing events using the normal 5' site of intron 1 (between exons 1 and 2) and the cryptic 3' sites #1 and #2, which are located within exon3, are significantly increased in *miso*^{null/null}.

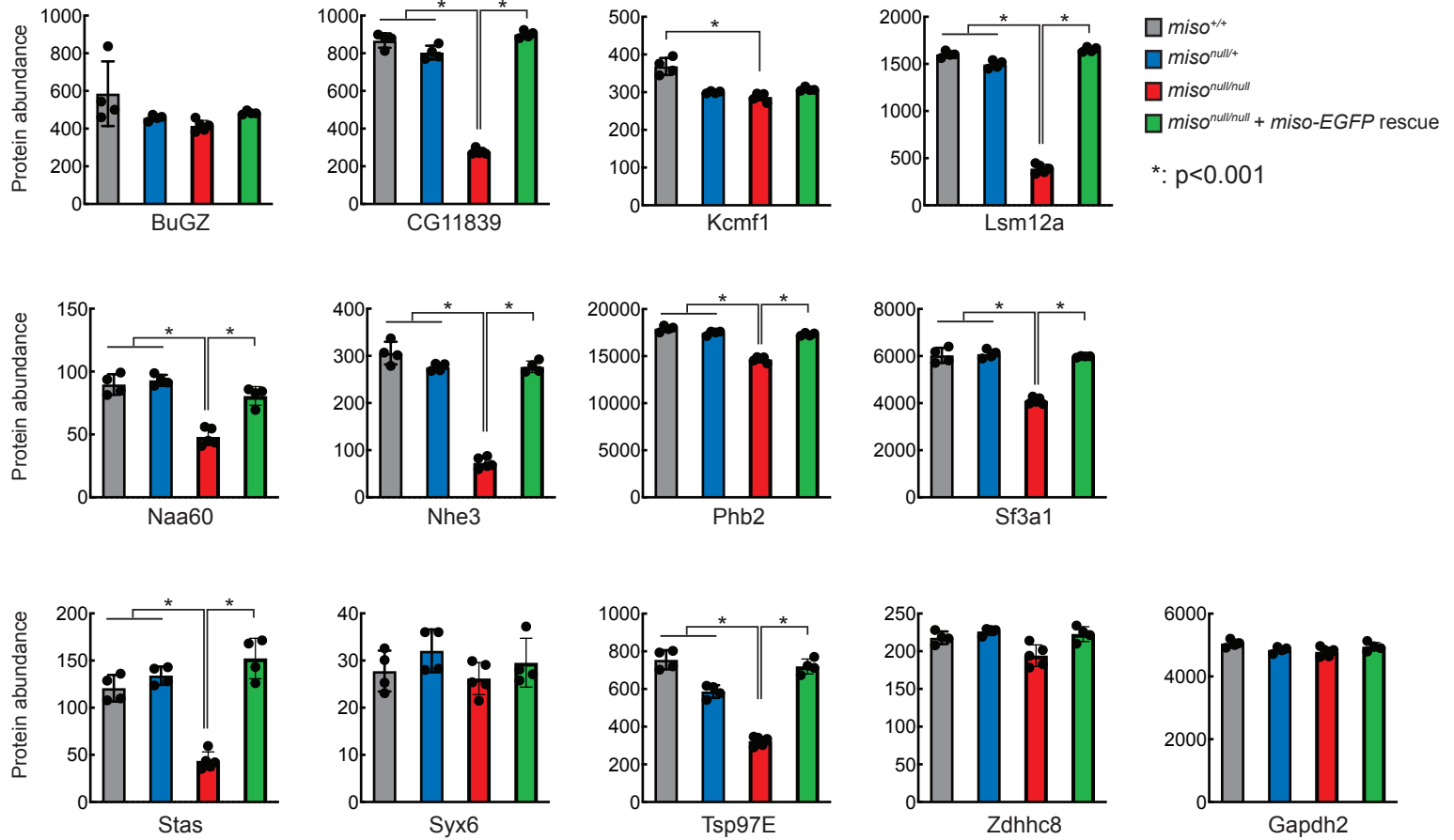


Fig S9. Reduced protein levels of MIGs in miso mutant ovaries

Protein levels of MIGs and Gapdh2 in ovaries, quantified by tandem mass tag (TMT) mass spectrometry. Mean $\pm$ SD (n=4 for *miso*^{+/+}, *miso*^{null/+}, and *miso*^{null/null} + *miso*-EGFP; 5 for *miso*^{null/null}). p-value < 0.001 (Student's t-test, unpaired, two-tailed) is indicated by *.