Supplemental material



Figure S1. **Effects of Aret upon muscle-specific alternative splicing.** Related to Fig. 1. (A, left) Gene regions that are differentially spliced between flight and jump muscles. Broken lines indicate splice junctions; junctions of multispliced variants are color coded. (middle) Visualization of splicing products on gels identifies splicing preferences in normal (WT) flight and jump muscles and detects preference switching in *aret* KD (*aret* 1) flight muscles. (right) Schematics of spliced transcripts detected on gel. (B) Aret accumulation in WT (left) and *aret* KD (right) pharate adults. Samples were sectioned and stained for accumulation of Aret, F-actin, and nuclei. Note that the Aret protein is effectively removed from flight muscles upon the KD. Asterisks indicate flight muscles, and the jump muscle is outlined.

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Α aret atilla Transcript aret-RJ ncRNA:CR43356-RA crok–RA Ю→ aret-RB atilla-RC ←## aret-RH atilla-RB **←HH** aret-RK \rightarrow CG31759-RD aret-RI CG31759-RC ID→ aret-RG **D** aret-RF THP aret-RE DHD aret-RA TH aret-RD B



MLSSLDALAGKIATATPGTGAVTADSKNTQSLHHHHRLDYDYSAVEIEPPAEQLANSPRSERERLQQAQQAYEQQAQA ELEFGLAEVDAKLTQLKSSHSLQHHHSHHHQLQRHTALNQQQQQLHHHQPHHNHHHHPYQRPRPTSPPQQQTSS YQQQLSQLAALQDHHDLLQTQQELFHKQLATMQEYQRERERERERERERERERERERERERERERSFIDNSDYDSQTEFKICLR DSAFGMSDKSSSATNSLPNSPIHSNNNNPSLLNNNNNNSNGTSSNNSLNVGNNNSNPSLGGSNSNALVSVGSNGIMS AAGLVNNNNNPCSANRNVVAMVDDDACFRLDTDATVTYGEKEPDPDNIKMFVGQVPKSMDESQLREMFEEYGAVH SINVLRDKATGISKGCCFVTFYTRHAALKAQDALHNVKTLNGMYHPIQMKPADSENRNERKLFVGMLNKKLNENDVR KLFEVHGAIEECTVLRDQNGQSKGCAFVTFATKHAAISAIKVTLSQNKIMEGCTSPLVVKFADTQKEKEQKKIQQIQANL WNLASNINIPLGQTATSVTTPILPNPPQQPSPVLGADAITPASIQLLQQLQAVGLQHQLLQALTGLGAQQSSSATDTSA VAAAGLLTPMTVQNLAAIAAMTQPSLSNAAAAAAAATSPGSAQLTNTAALLWSDPNPMASAYMSTAAGLPQFGSAS ALSTSPLASVALSAAAAAAAGKQIEGPEGCNLFIYHLPQEFTDTDLASTFLPFGNVISAKVFIDKQTSLSKCFGFVSFDNPD SAQVAIKAMNGFQVGTKRLKVQLKKPKDSKPY

Figure S2. Identification of the aret isoform expressed in flight muscles. Related to Fig. 2. (A) Genomic structure of the aret gene (from FlyBase). Note that aret transcripts are alternatively spliced. The aret.RA isoform (boxed) is the closest annotated aret isoform to that expressed in the flight muscles. (B) Compact genomic model of aret indicating primers and transcript isoforms. Only exons are shown (boxes). Red highlight indicates translated sequences. The letters and lines above each exon indicate the isoforms harboring the exons. (C) RT-PCR analysis of WT flight muscle samples with primers listed in B. Numbers above the exons indicate primer pairs used for amplification. (D) Deduced amino acid sequence of Aret expressed in the flight muscles.

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