Ecdysone signaling at metamorphosis triggers apoptosis of *Drosophil*a abdominal muscles

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Supporting Figures

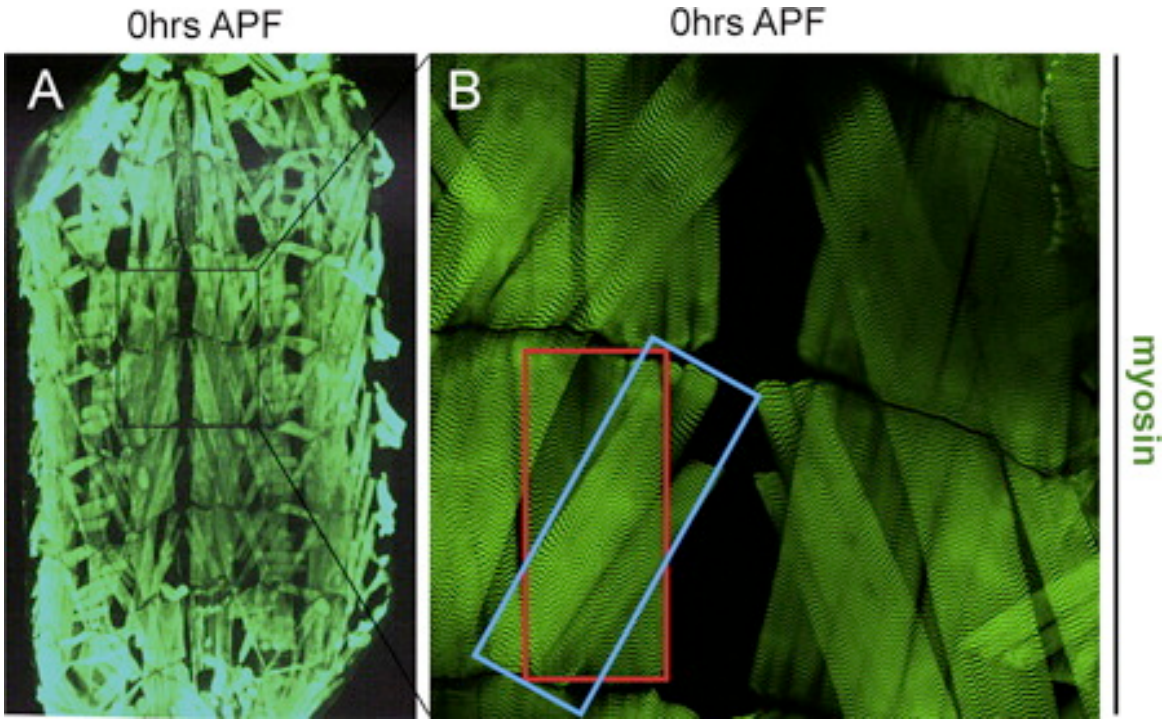

Fig. S1.

Abdominal muscles at the onset of pupariation. (A) *Drosophila* body wall muscles from the *weeP*26 transgenic line at 0 h APF, expressing a GFP-tagged Myosin heavy chain protein (green). (B) Magnification of the two segments of dorsal abdominal muscles. The dorsal external oblique muscle (DEOM) is marked with a red outline. The dorsal internal oblique muscle (DIOM) is marked with blue outline.

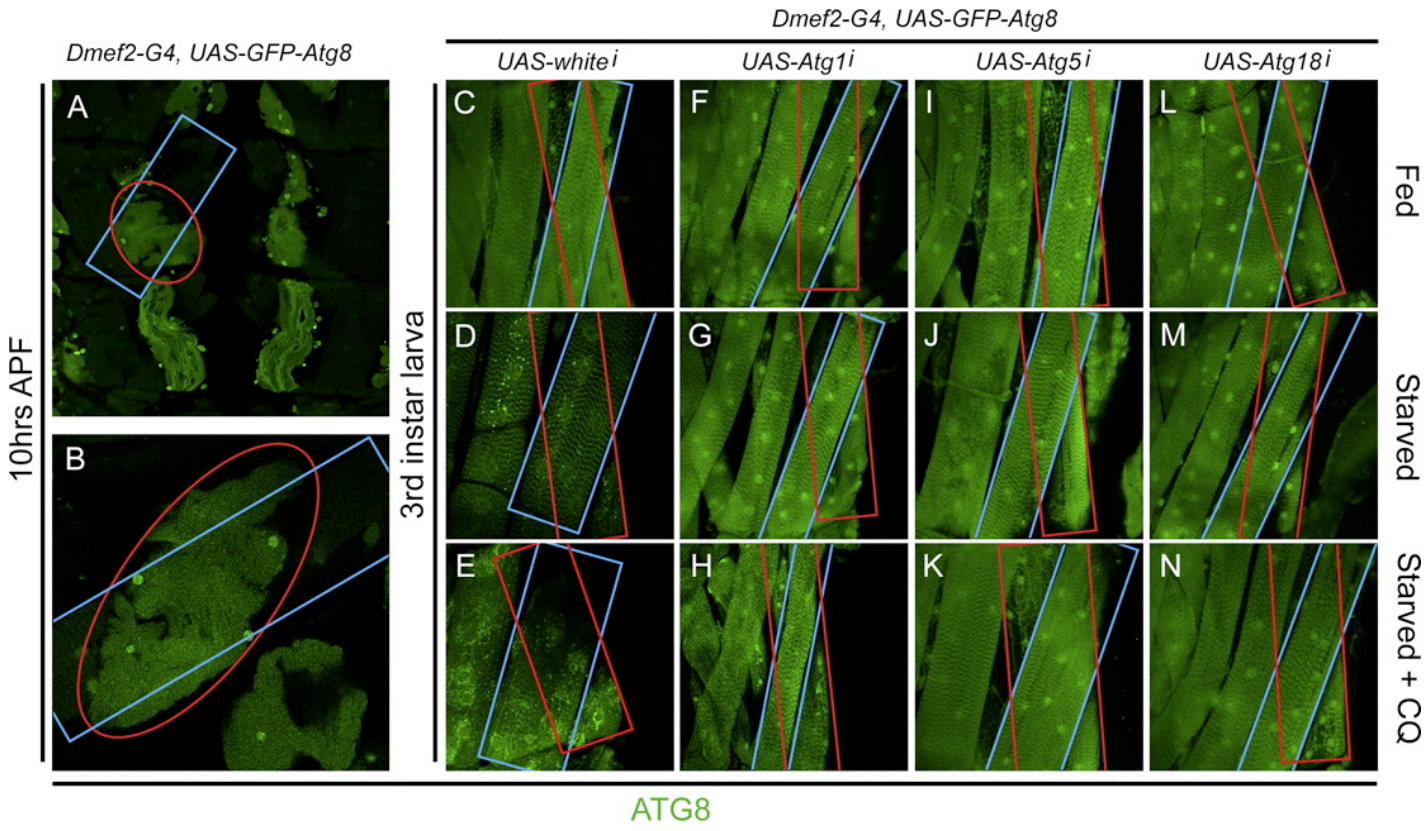

Fig. S2.

Autophagosome formation in larval and pupal abdominal muscles. (A-B) Muscles from *Dmef2-Gal*4, *UAS-GFP-Atg*8 animals at 10 h APF. Expression of the autophagosome marker GFP-Atg8 (green) is heightened in degenerating DEOMs (red outline) relative to DIOMs (blue outline). (B) Higher magnification of DEOM showing no obvious increase in autophagosomes. (C-N) Muscles from late third instar *Dmef2-Gal*4, *UAS-GFP-Atg*8 larvae expressing RNAi targeting the core autophagy pathway genes, *Atg*1, *Atg*5, and *Atg*18, or *white* RNAi control. The DEOM and DIOM are marked with red and blue lines, respectively. Larvae were given a diet of rich food (fed), low nutrient food (starved), or low nutrient food+chloroquine (starved +CQ). (C–D) *white* RNAi control larvae. (C) In fed animals, GFP-Atg8 is localized throughout the cytoplasm and nuclei. (D) Starvation induces the formation of GFP-Atg8 vesicles primarily around the muscle nuclei of both DEOMs and DIOMs. (E) CQ treatment causes the accumulation of greater numbers of GFP-Atg8 vesicles in the muscles from starved animals. Knockdown of Atg1 (F–H), Atg5 (I–J), and Atg18 (L–N) has no effect on GFP-Atg8 in the fed animals, but completely blocks the formation of GFP-Atg8 vesicles in both starved and starved+CQ animals.

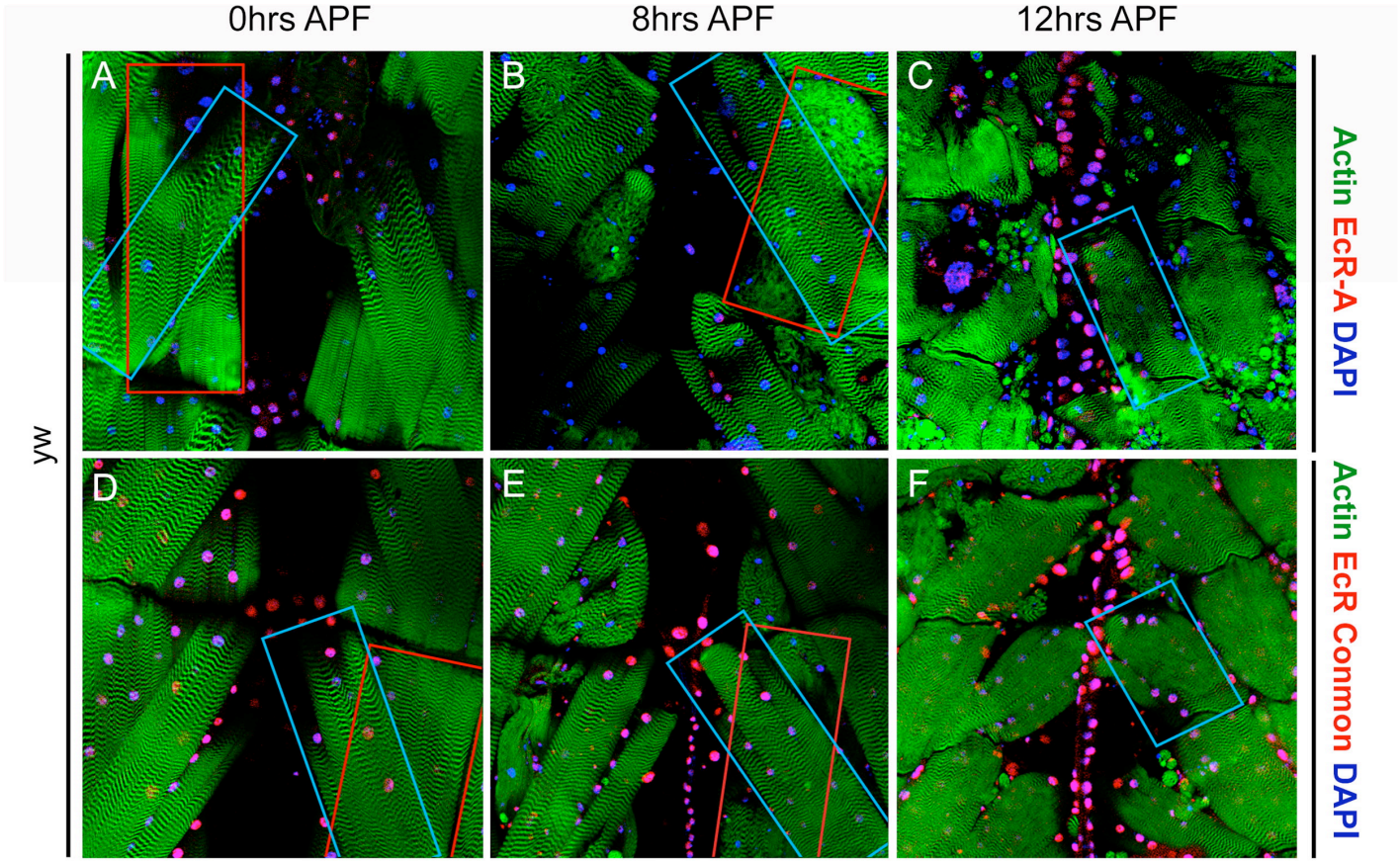

Fig. S3.

Expression of EcR isoforms in abdominal muscles. In all panels the red outline indicates the DEOM and the blue outline indicates the DIOM. (A-C) Time course of abdominal muscles from *yw* larvae stained with α-EcR-A (red), Phalloidin/actin (green), and DAPI (blue). Neither the DEOMs nor DIOMs express EcR-A throughout the period of muscle histolysis. (D-F) Time course of abdominal muscles from *yw* larvae stained with Phalloidin/actin (green), DAPI (blue), and α-EcR-C (red), which recognizes all EcR isoforms. Both the DEOMs and DIOMs express one of the EcR isoforms throughout the period of muscle histolysis.

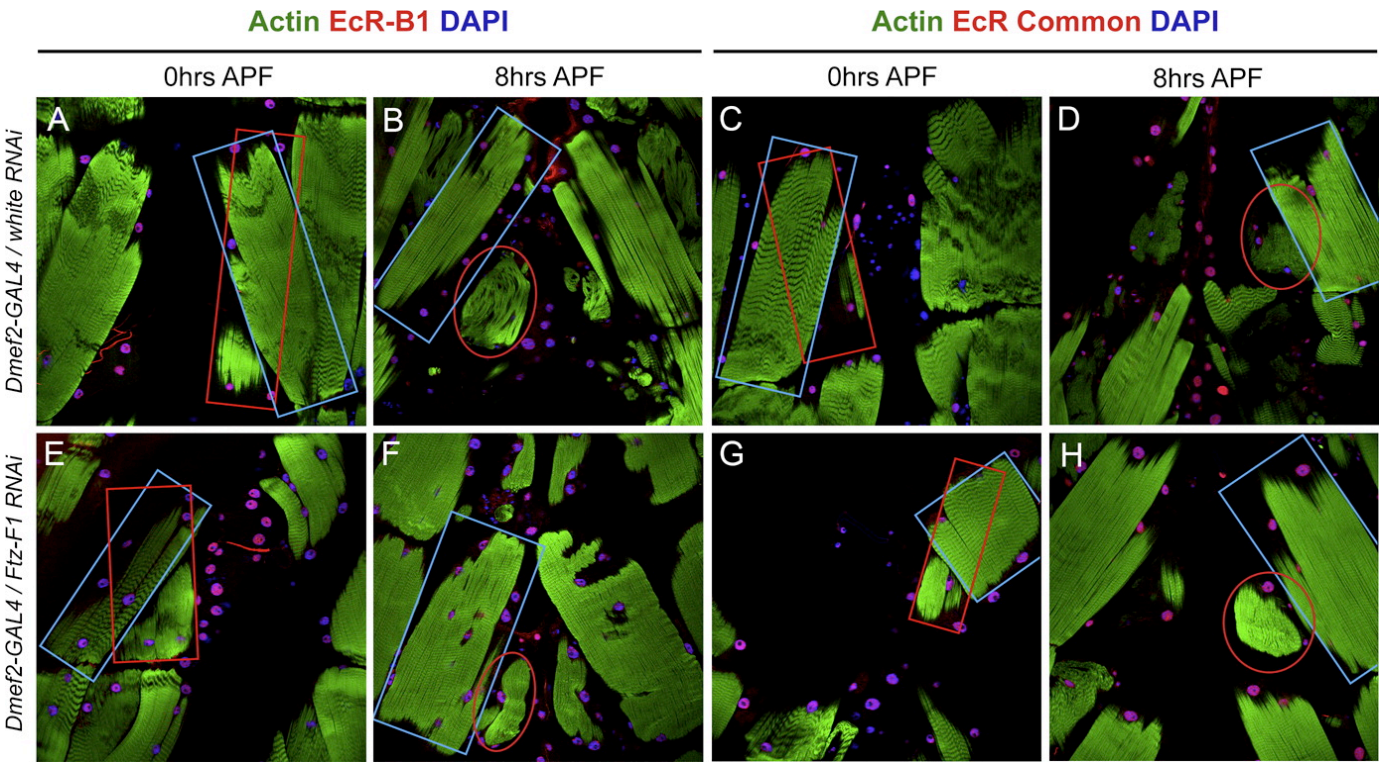

Fig. S4.

Effect of FTZ-F1 on EcR expression. In all panels the red outline indicates the DEOM and the blue outline indicates the DIOM. (A–D) Abdominal muscles from *Dmef2-Gal4/UAS-white RNAi* larvae stained with Phalloidin/actin (green), DAPI (blue) and either α-EcR-B1 or α-EcR-C (red). EcR-B1 (A-B) and EcR-C (C–D) are expressed in both DIOMs and DEOMs at 0 and 8 h APF. (E-H) Abdominal muscles from *Dmef2-Gal4/UAS-ftz-f1 RNAi* larvae stained with Phalloidin/actin (green), DAPI (blue) and either α-EcR-B1 or α-EcR-C (red). EcR-B1 (E-F) and EcR-C (G-H) are expressed in both DIOMs and DEOMs at 0 and 8 h APF.

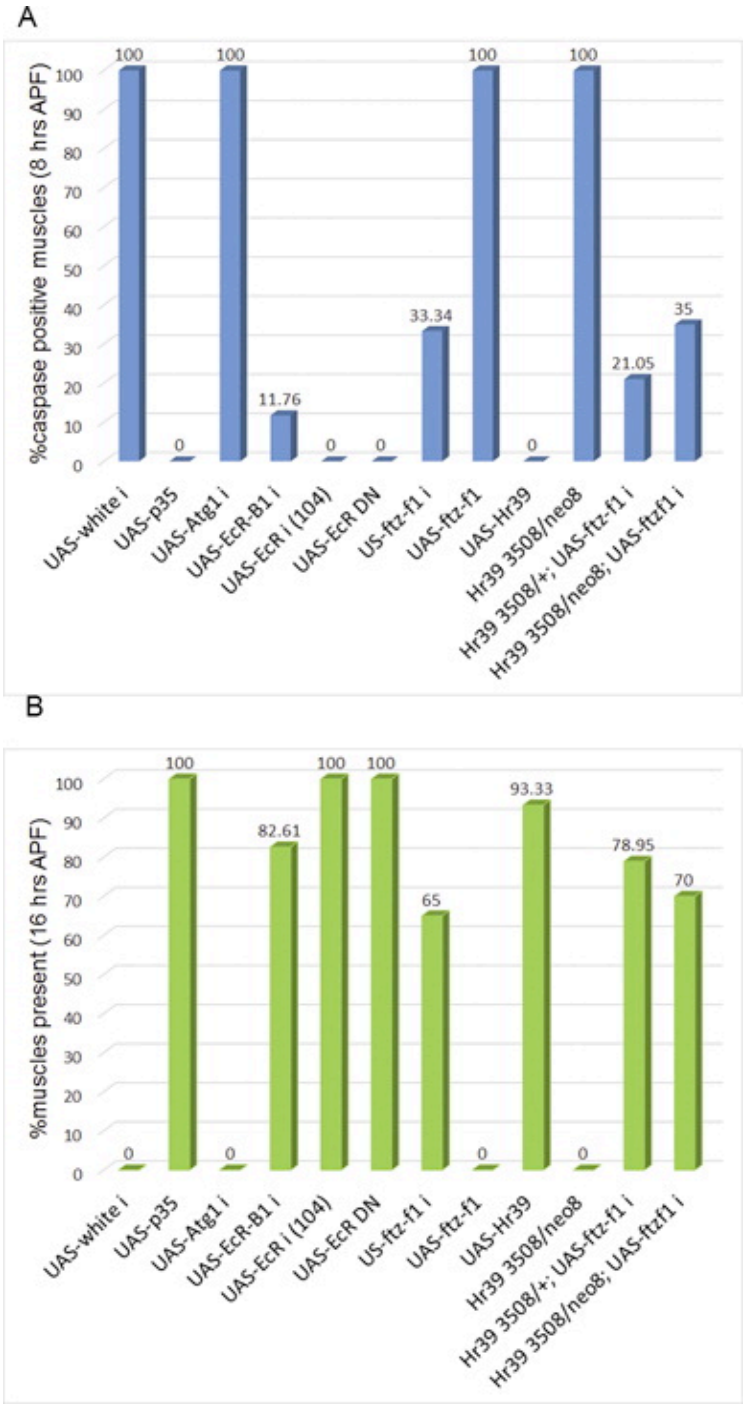

Fig. S5.

Quantification of muscle phenotypes. (A) The percentage of caspase positive muscles at 8 h APF and (B) the percentage of muscles remaining at 16 h APF. Measurements were obtained from the DEOMs of the fourth abdominal segment (*n*>10 larvae). All genotypes included *Dmef*2-*Gal*4 to drive expression of the UAS transgenes.

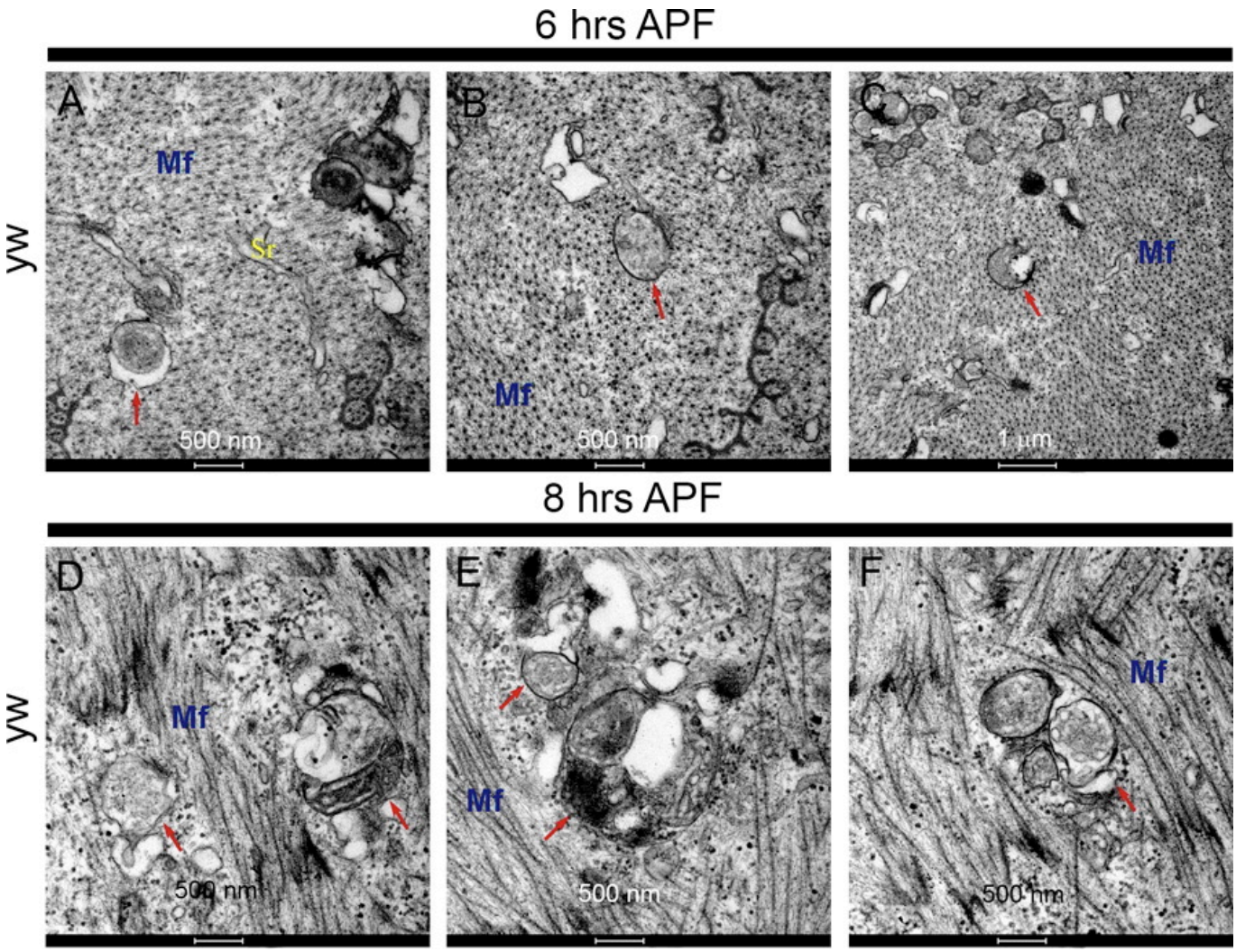

Fig. S6.

EM of vesicles in degenerating muscle. (A–C) Cross section of DEOM at 6 h APF. The muscles retain their larval sarcomere pattern, and vesicles occasionally appear between the myofibrils (red arrows). (D–F) Longitudinal section of DEOM at 8 h APF. Myofibrils are present, but are disorganized, while vesicles and multi-vesicular bodies (red arrows) are more prevalent. Abbreviations: Mf (myofibrils), Sr (sarcoplasmic reticulum).