Supplementary Information

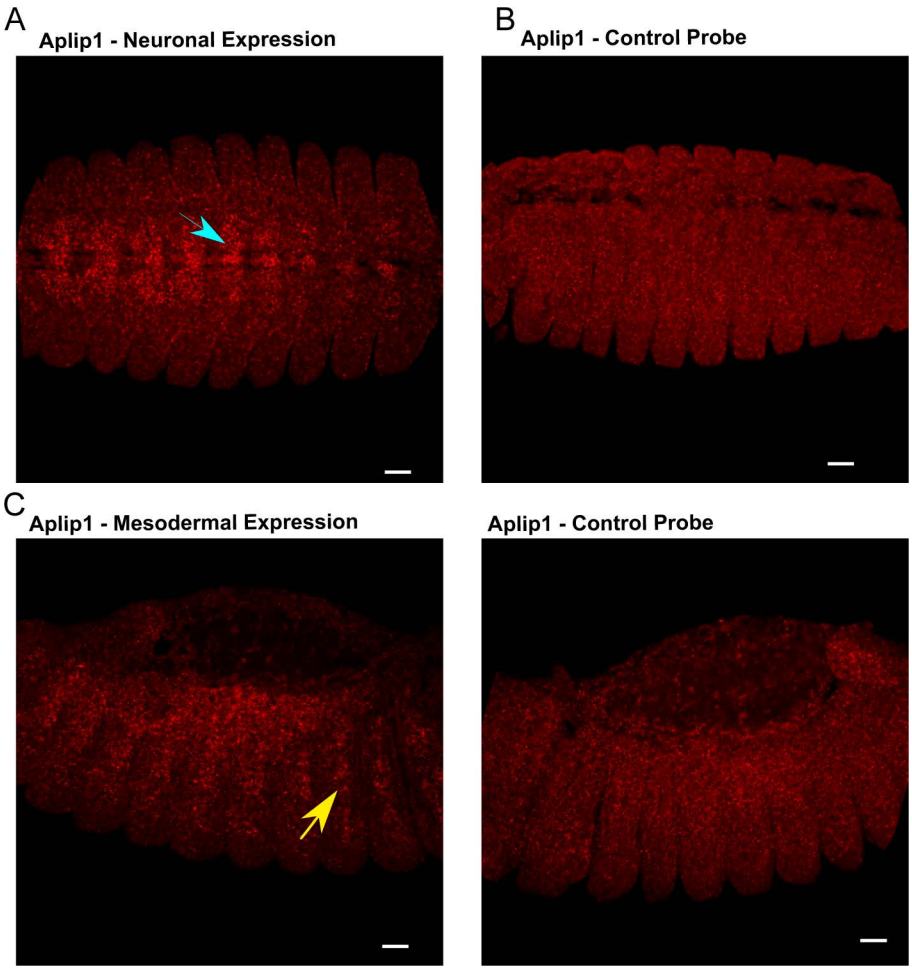


Figure S1. Fluorescent in situ hybridization (FISH) of *Aplip1* in Drosophila embyos.
A-B) Stage 13 embryo showing neuronal expression (cyan arrow) of Aplip1 using an antisense probe **(A)** or a control sense probe to indicate background signal **(B) C-D)** Stage 13 embryo showing mesodermal expression (yellow arrow) of Aplip1 using an antisense probe (Red) **(C)** or a control sense probe showing background signal **(D)**

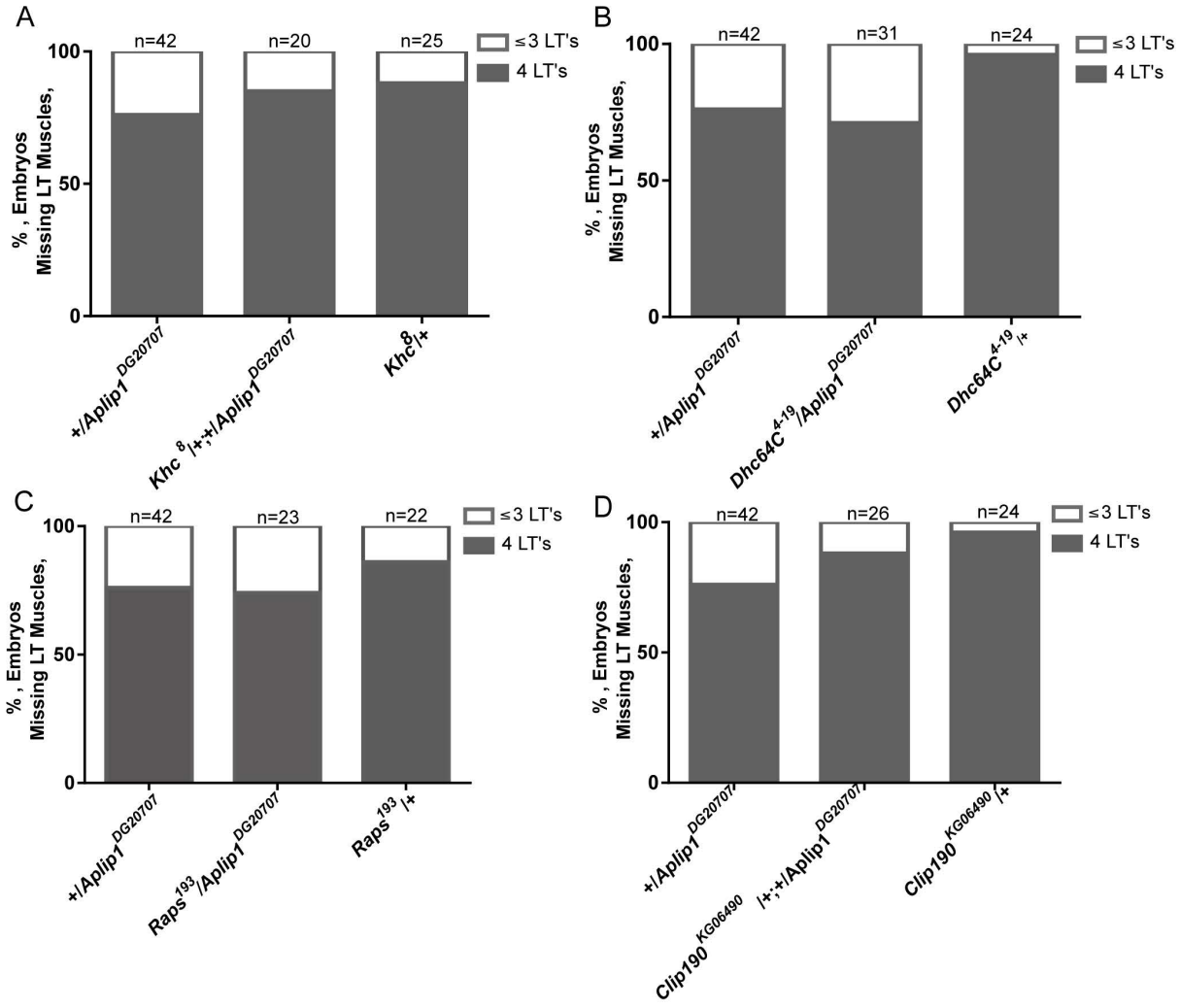


Figure S2. *Aplip1* does not interact with cytoskeletal proteins to regulate muscle stability. A-D) Graphs indicating that the percentage of embryos with < 4 LTs is not significantly different when *Aplip1* heterozygous mutants (+/*Aplip1*^{DG20707}) and *Kinesin* heterozygous mutants (*Khc*^{8}/+) are compared to *Kinesin* and *Aplip1* doubly heterozygous mutant embryos (*Khc*^{8}/+; +/*Aplip1*^{DG20707}) **(A),** *Aplip1* heterozygous mutants (+/*Aplip1*^{DG20707}) and *Dynein* heterozygous mutants (*Dhc64C*$^{4-19}$/+) compared to *Dynein* and *Aplip1* double heterozygous mutant embryo (*Dhc64C*$^{4-19}$/*Aplip1*^{DG20707}) **(B),** *Aplip1* heterozygous mutants (+/*Aplip1*^{DG20707}) and *Raps* heterozygous mutants (*Raps*^{193}/+) compared to *Raps* and *Aplip1* double heterozygous mutant embryos (*Raps*^{193}/*Aplip1*^{DG20707}) **(C)** and *Aplip1* heterozygous mutants (+/*Aplip1*^{DG20707}) and *Clip-190* heterozygous mutants (*Clip-190*^{KG06490}/+) compared to *Clip-190* and *Aplip1* double heterozygous mutant embryo (*Clip-190*^{KG06490}/+; +/*Aplip1*^{DG20707}) **(D).**

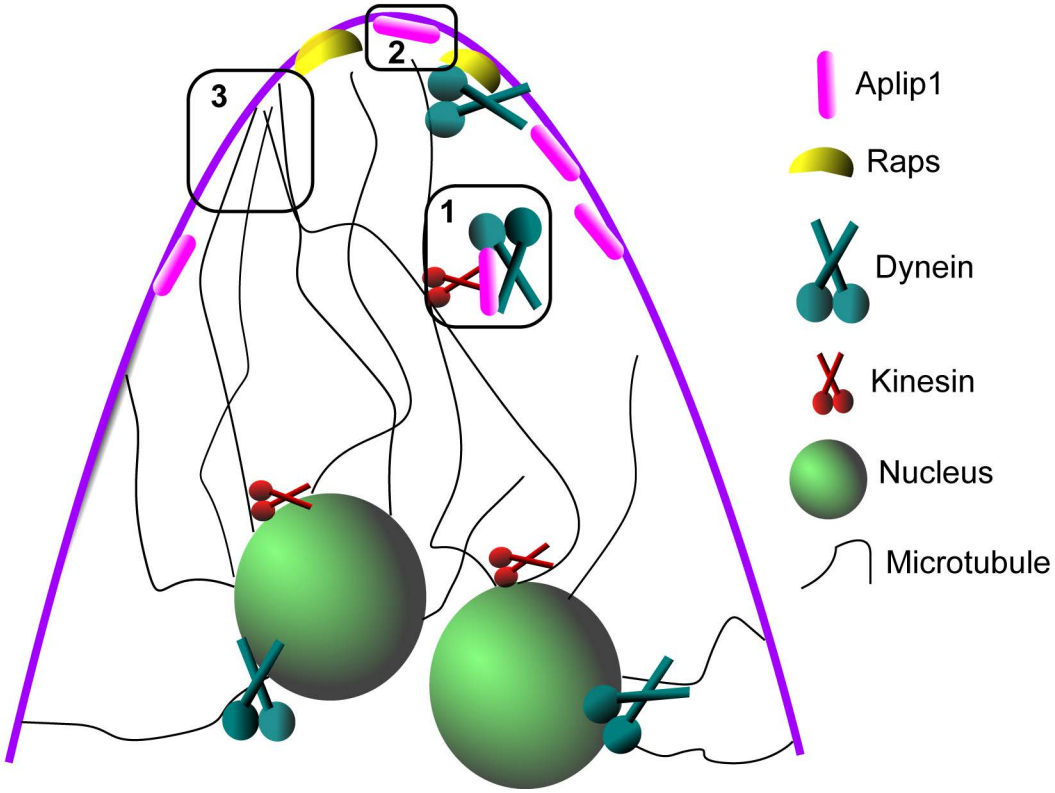
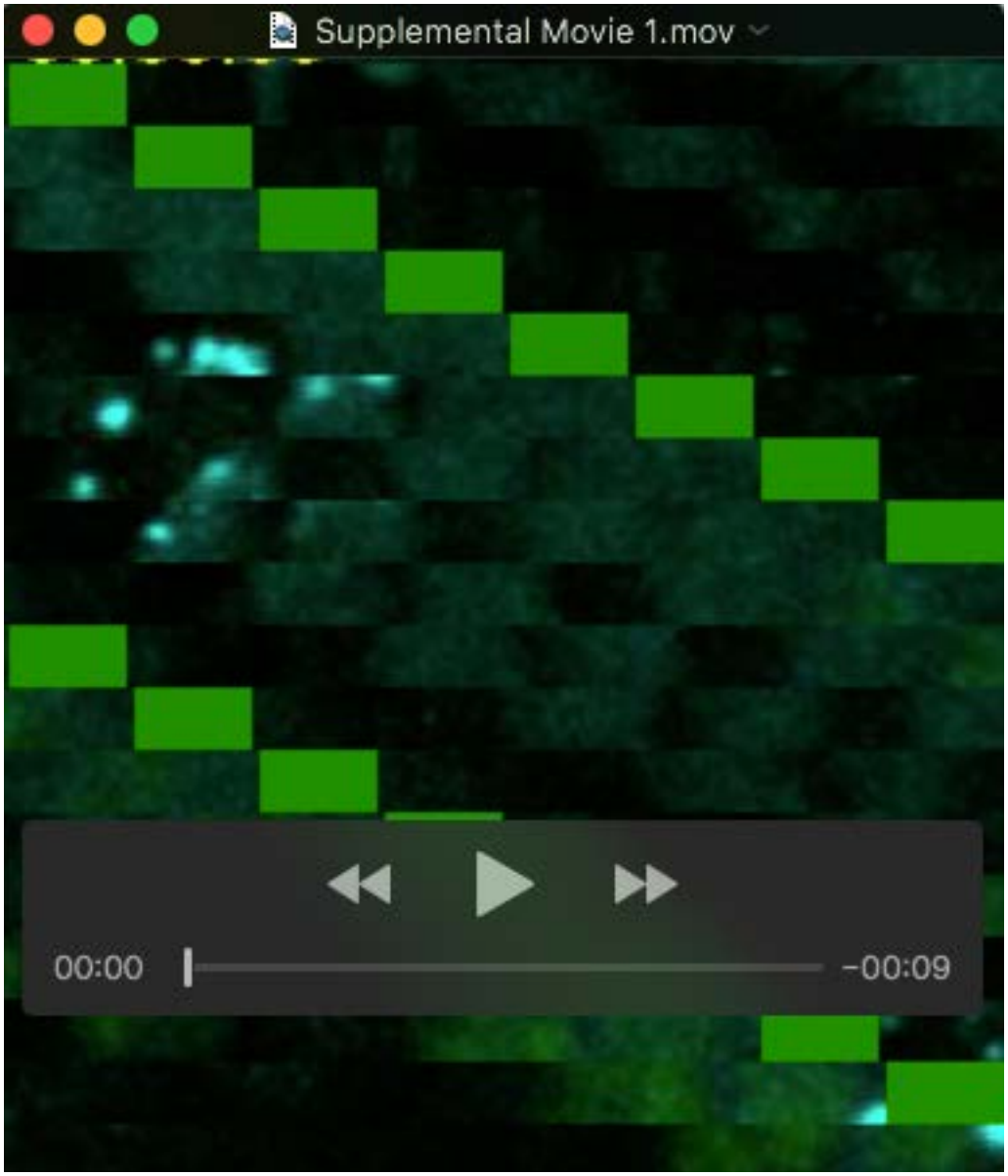


Figure S3. Model of the role of Aplip1 in myonuclear positioning and muscle stability. In Aplip1 mutant embryos there are reduced levels of both Dynein and Kinesin near the muscle ends. This suggests that one function of Aplip1 may be to regulate Kinesin-dependent transport of Dynein toward the muscle end (1). Additionally, there is a strong genetic interaction between Aplip1 and Raps, a gene previously demonstrated to be important for Dynein localization. This suggests that Aplip1 may additionally function in the anchoring and/or activation of anchored Dynein for the purpose of myonuclear movement (2). Finally, there is an increase in the abundance of MTs at the ends of muscles in Aplip1 mutants. This suggests either that there is a compensation mechanism to stabilize microtubules directed toward the muscle end or that Aplip1 directly contributes to the dynamics of microtubules near the muscle end (3).



Movie S1. Aplip1eGFP dynamics at the MTJ

LT muscles of the *Drosophila* embryo expressing Aplip1-eGFP (Cyan) with nuclei (green). Aplip1-eGFP accumulates at MTJs where it forms dynamic puncta extending from the muscle pole. Time-lapse movies shown as maximum projections. Z-stacks were acquired at a rate of 1 frame/10 secs. Movie plays at 10 frames/s. Scale bar, 5 µm.

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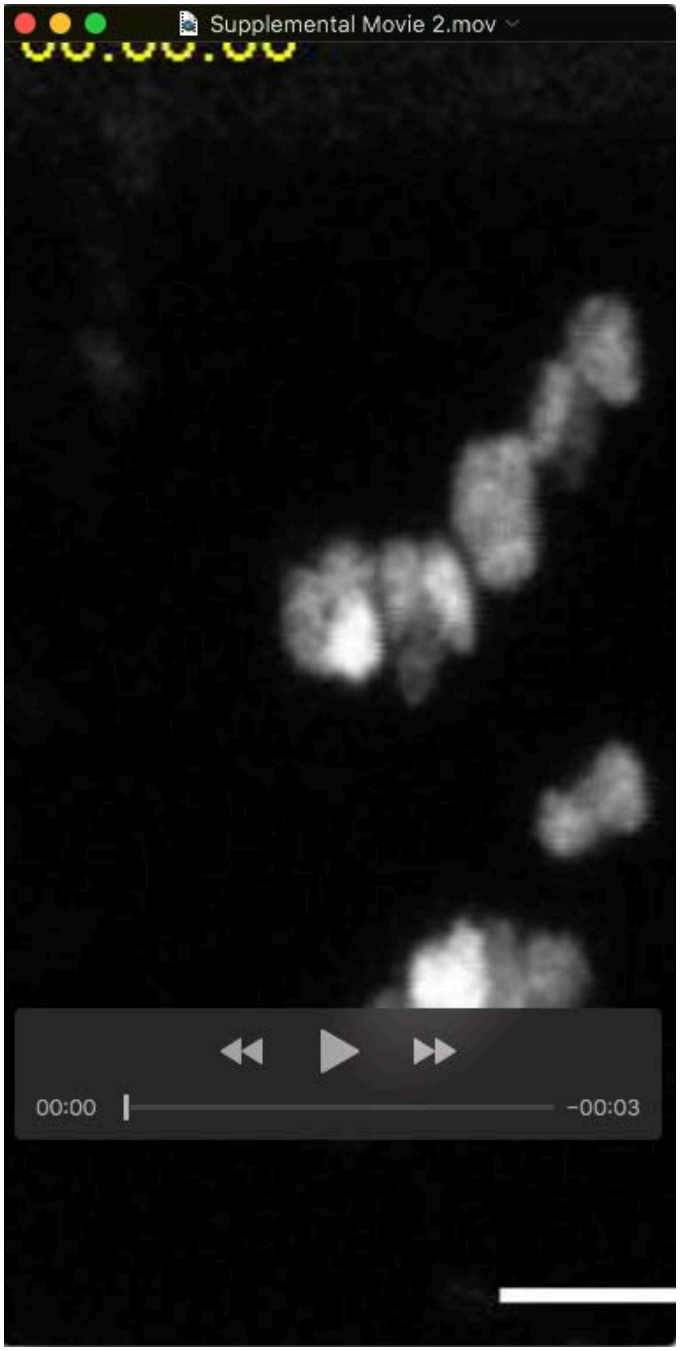

Movie S2. Myonuclear movement in control embryos

LT muscles from a *Drosophila* embryo that expressed apRed (green). Movie shows clusters of myonuclei moving toward the muscle pole in late stage 15 embryos. Time-lapse movie shown as maximum projections. Z-stacks acquired at a rate of 1 frame/2 mins. Movie plays at 10 frames/s. Scale bar, 10 µm.

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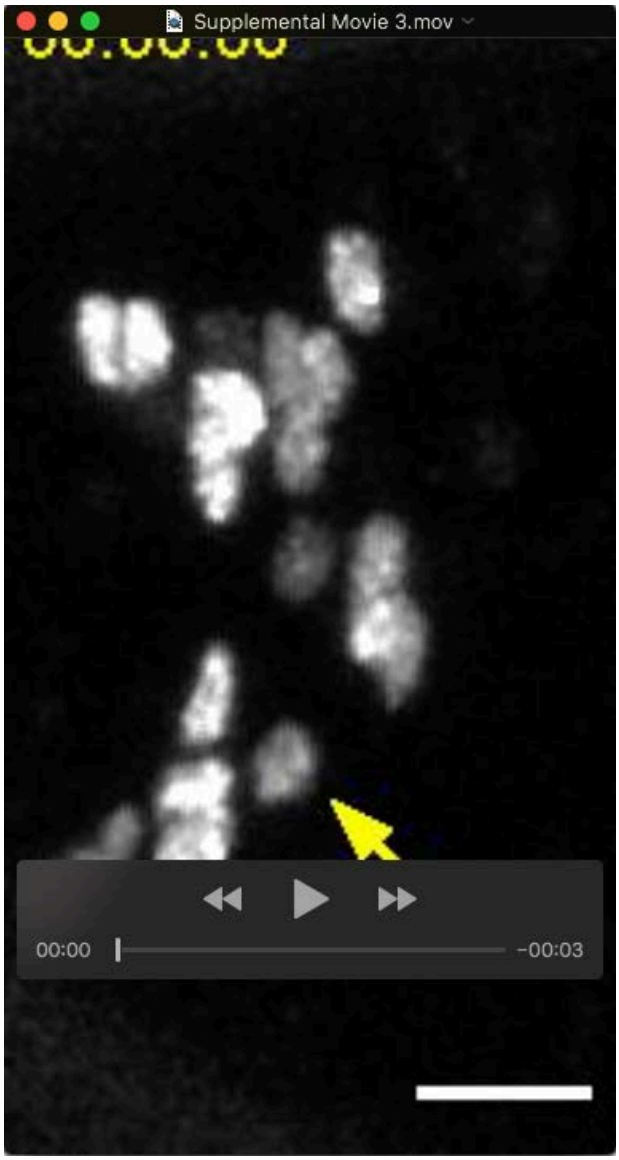

Movie S3. Myonuclei collapsing in *Aplip1*^{DG20707} embryos

LT muscles of the *Drosophila* embryo expressing apRed (green). Movie shows myonuclear collapse in ventral to dorsal direction in an LT muscle (indicated with yellow arrow). Time-lapse movie shown as maximum projections. Z-stacks acquired at a rate of 1 frame/2 mins. Movie plays at 10 frames/s. Scale bar, 10 µm.

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