

Figure S1: Verification of anti Ma2/d specificity

A-C: Wing imaginal disc of larvae over expressing Ma2/d-GFP driven by Hh-GAL4, labeled with anti GFP (A) and anti Ma2/d antibody raised against 3 peptides (marked in D) of the extracellular domain of Ma2/d, indicating that the antibody recognizes the Ma2/d. (E) Western analysis: Left panel - Western blot of larval protein extract from wild type (WT) or larvae expressing Ma2/d-GFP in muscles (driven by *mef2*-GAL4) reacted with anti GFP. Two specific bands representing un-cleaved Ma2/d-GFP (~170kDa) and cleaved Ma2/d-GFP (~ 50kDa) are shown; The anti GFP is not expected to recognize the cleaved 120kDa polypeptide. Middle panel: Western blot of larval protein extract from WT (left lane), and larvae expressing Ma2/d-GFP in

muscles (right lane), both reacted with anti Ma2/d ab . In the lane of extract expressing Ma2/d-GFP the antibody recognizes a 170kDa band corresponding to the intact 170kDa protein and 120kDa band corresponding to the larger portion of the cleaved protein. In the wild type larvae extract this antibody recognizes a ~140kDa corresponding to endogenous non-cleaved Ma2/d, and ~120kDa band corresponding to the larger portion of the cleaved polypeptide. In both lanes, the antibody is not expected to recognize the small-cleaved polypeptide. Right panel: Western blot of protein extract from WT (left lane) and *Ma2/d* (right lane) mutant larvae both reacted with anti Ma2/d. In the WT extract lane (left lane) the 140kDa band represents non-cleaved intact Ma2/d protein, which is missing from the extract of the mutant larvae (right lane). Instead two lower molecular weight bands, absent from WT lane, appear (~100kDa and ~75kDa), presumably corresponding to the truncated Ma2/d proteins.



Figure S2: Genomic map of EPgy2^{EY2EY09750} excision

The genomic structure of *Ma2/d* is shown. The location of the original P element as well as the excised region deduced from sequence analysis is demonstrated.

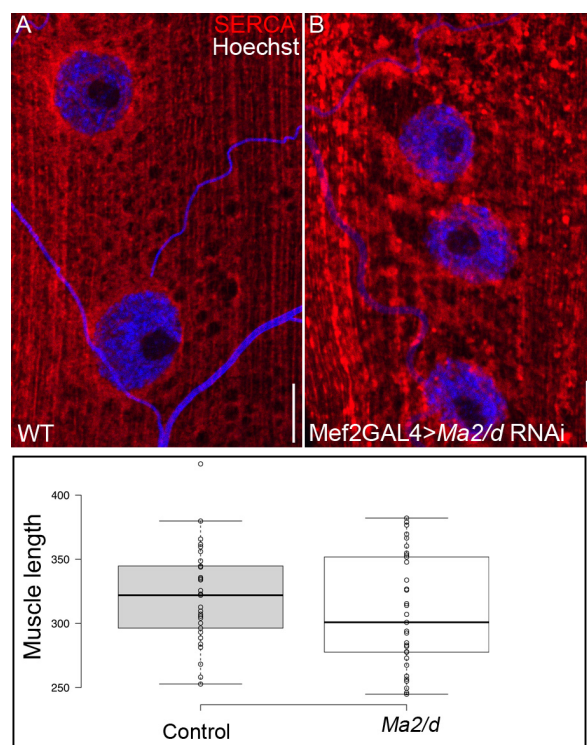


Figure S3: Aberrant nuclear position in *Ma2d* knockdown muscles

Larval muscles of wild type (A) or larvae expressing RNAi against *Ma2/d* (B) indicating a similar phenotype of aberrant nuclear position. Bar indicates 10 μ m. C – quantification of *Ma2/d* larval muscle length. No significant difference between the groups is observed (*t* test: $p=0.115$; for WT – the length of 33 muscles (muscle 6) were measured from 4 larvae, and for *Ma2d* mutant 29 muscle (muscle 6) were measured from 4 larvae.

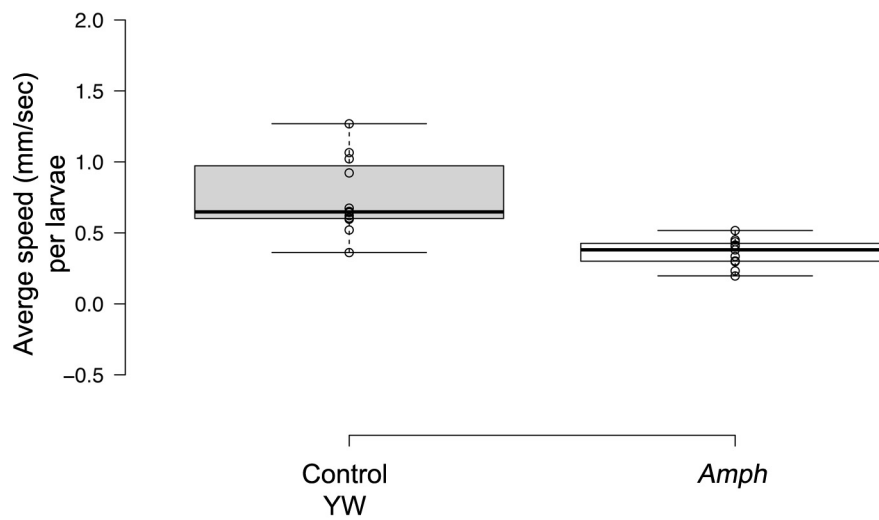
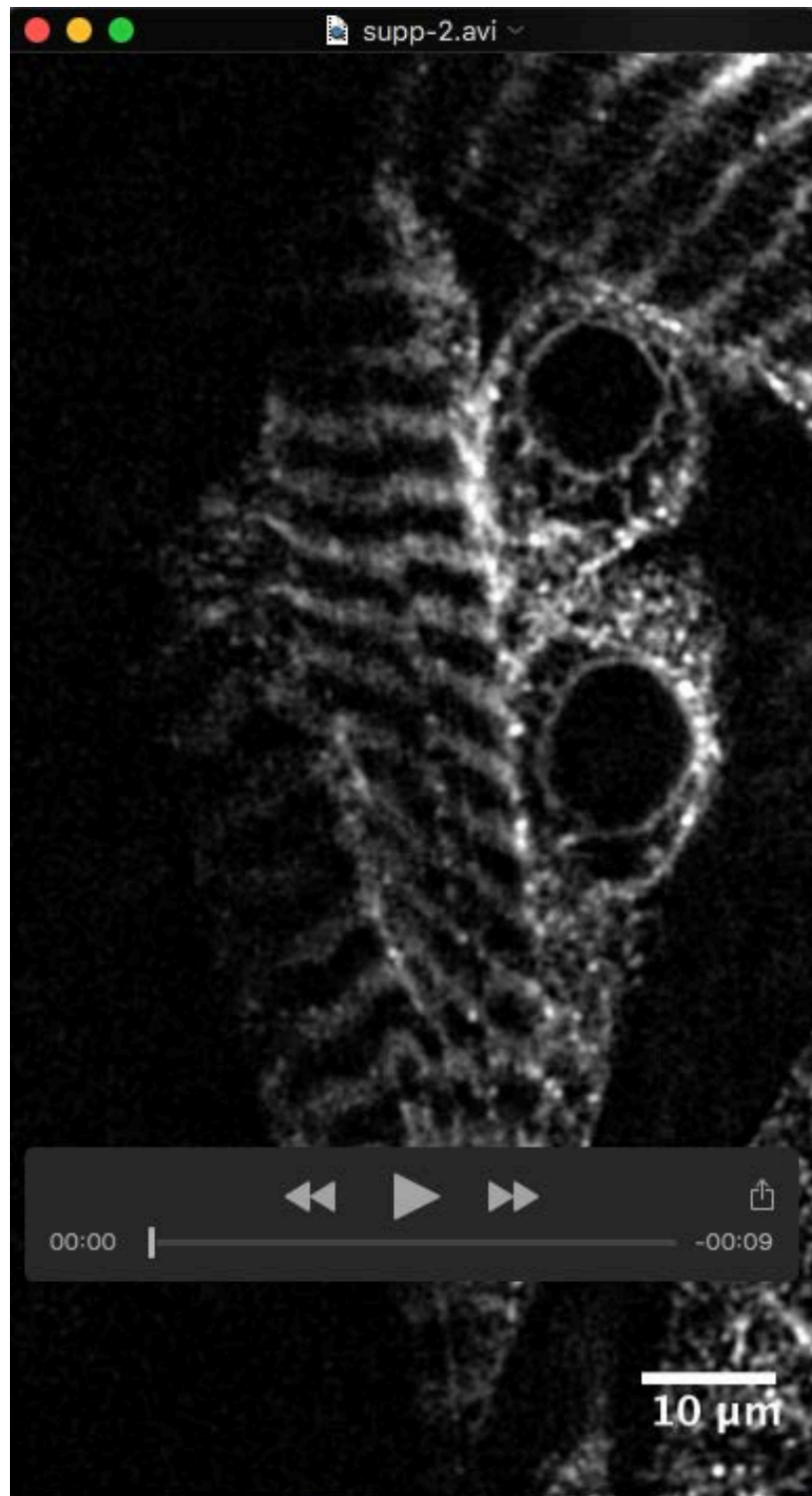


Figure S4: Locomotion assay for *Amph* mutant larvae.

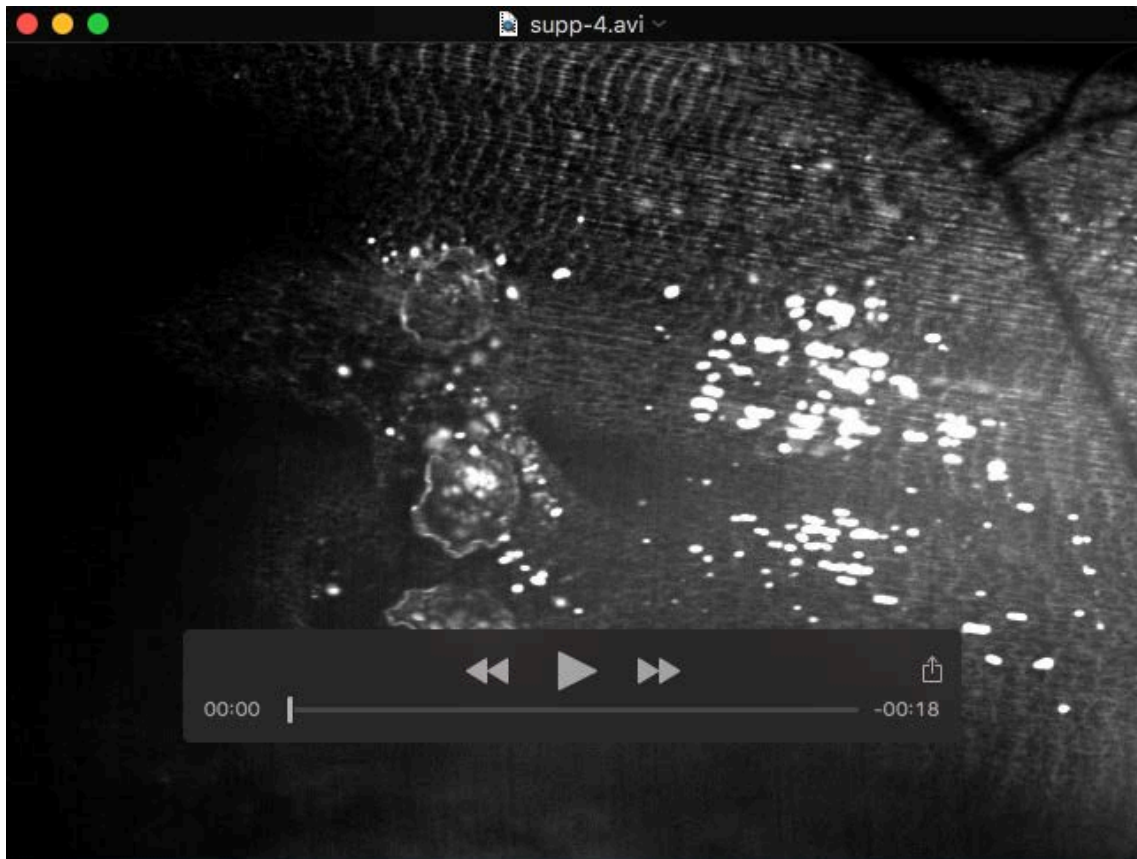
Quantification of the locomotion of control (YW) and *Amph* mutant larvae. N=10 for each group. *t*-test: $P < 0.0002$.



Movie 1: The distribution of Ma2/d-GFP in live 3rd instar larvae during muscle contraction imaged under Spinning disc microscope. Individual images of the movie are shown in Figure 5.



Movie 2: SERCA-Cherry distribution in live 3rd instar larvae during muscle contraction. Individual images of the movie are shown in Figure 5.



Movie 3: SERCA-Cherry distribution in live 3rd instar larvae mutant for *Ma2/d* during muscle contraction. Individual images of the movie are shown in Figure 5.