Supplemental Figure 1, related to Figure 1

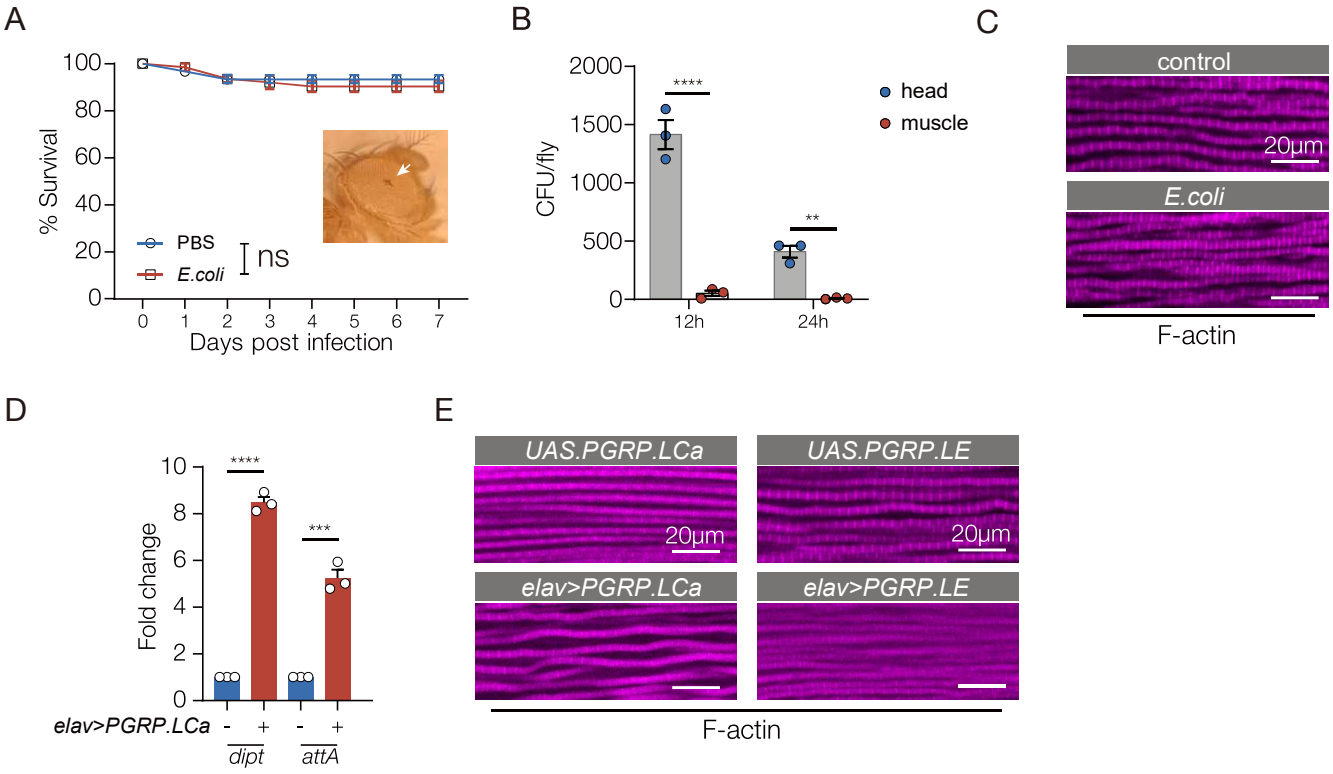


Figure S1. Related to Figure 1. A. Survival curve. Sterile-injected (PBS, control) and *E. coli* injected flies showed comparable longevity. White arrow shows the injection site. n=3 cohorts per genotype, with n≥20 flies per cohort. **B.** Colony forming unit (CFU) assay. Bacterial load in the brain dramatically decreased between 12- and 24- hours after *E. coli* injection. Muscle showed minimal bacterial infection. Each data point represents one biological replicate, with n=5 flies per replicate. **C.** Confocal micrographs of indirect flight muscle stained with phalloidin to visualize F-actin (violet). Muscle morphology was comparable between sterile-injected (PBS, control) and *E. coli* injected flies. **D.** qRT-PCR. Transcripts encoding the antimicrobial peptides *Dipt* and *attA* in the brain was enriched in *elav>PGRPLCa* flies compared to controls (*UAS.PGRPLCa*). Data points represent independent biological replicates, with n≥10 flies per cohort. **E.** Confocal micrographs of indirect flight muscle stained with phalloidin to visualize F-actin (violet). Muscle morphology was comparable among *elav>PGRPLCa* flies, *elav>PGRPLE* flies, and controls (*UAS.PGRPLCa, UAS.PGRPLE*). Significance was determined by Kaplan–Meier tests (A), or two-way ANOVA (B, D). Error bars represent SEM. (*) $p< 0.05$, (**) $p< 0.01$, (***) $p< 0.001$, (****) $p < 0.0001$, (ns) non-significant.

Supplemental Figure 2, related to Figure 2

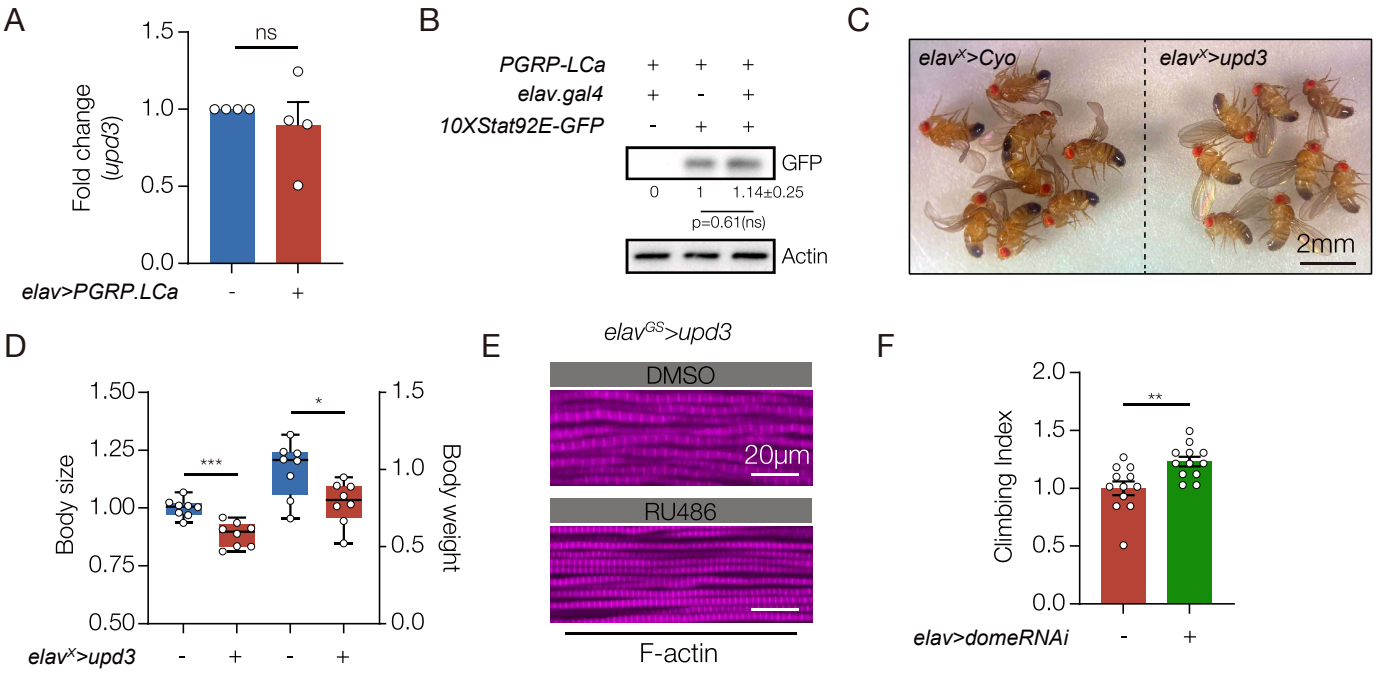


Figure S2. Related to Figure 2. A. qRT-PCR. Flies that expressed PGRP in the CNS (*elav>PGRP.LCa)* showed similar levels of *upd3* mRNA in the brain as control flies. **B.** Western blot. GFP expression from the JAK/Stat activity reporter *10XStat92E.GFP* in muscle was similar between control and *elav>PGRP.LCa* flies. Relative expression was determined for three biological replicates. **C.** Micrographs of adult flies 3-5 days after eclosion. $elav^{X}$>*upd3* flies were smaller than controls. **D.** Normalized body size (left Y axis) and body weight (right Y axis) of control and $elav^{X}$>*upd3* flies. **E.** Confocal micrographs of indirect flight muscles stained with phalloidin to visualize F-actin (violet). $elav^{GS}$>*upd3* flies treated with RU486 or DMSO showed similar myofiber morphology **F.** Climbing index. $dome^{RNAi}$ was used to knock down Dome expression in skeletal muscle of *E. coli* infected flies. Infected flies with reduced Dome expression (*Mef2>dome* ^{RNAi}) showed improved climbing capacity compared to controls at 2 dpi. Significance was determined by two-sided unpaired student's t-test. For qRT-PCR, data points represent biological replicates, with n≥10 flies per cohort. See Fig. 1 legend for Climbing Index data points. Error bars represent SEM. (*) p< 0.05, (**) p< 0.01, (***) p< 0.001, (****) p < 0.0001, (ns) non-significant.

Supplemental Figure 3, related to Figure 4

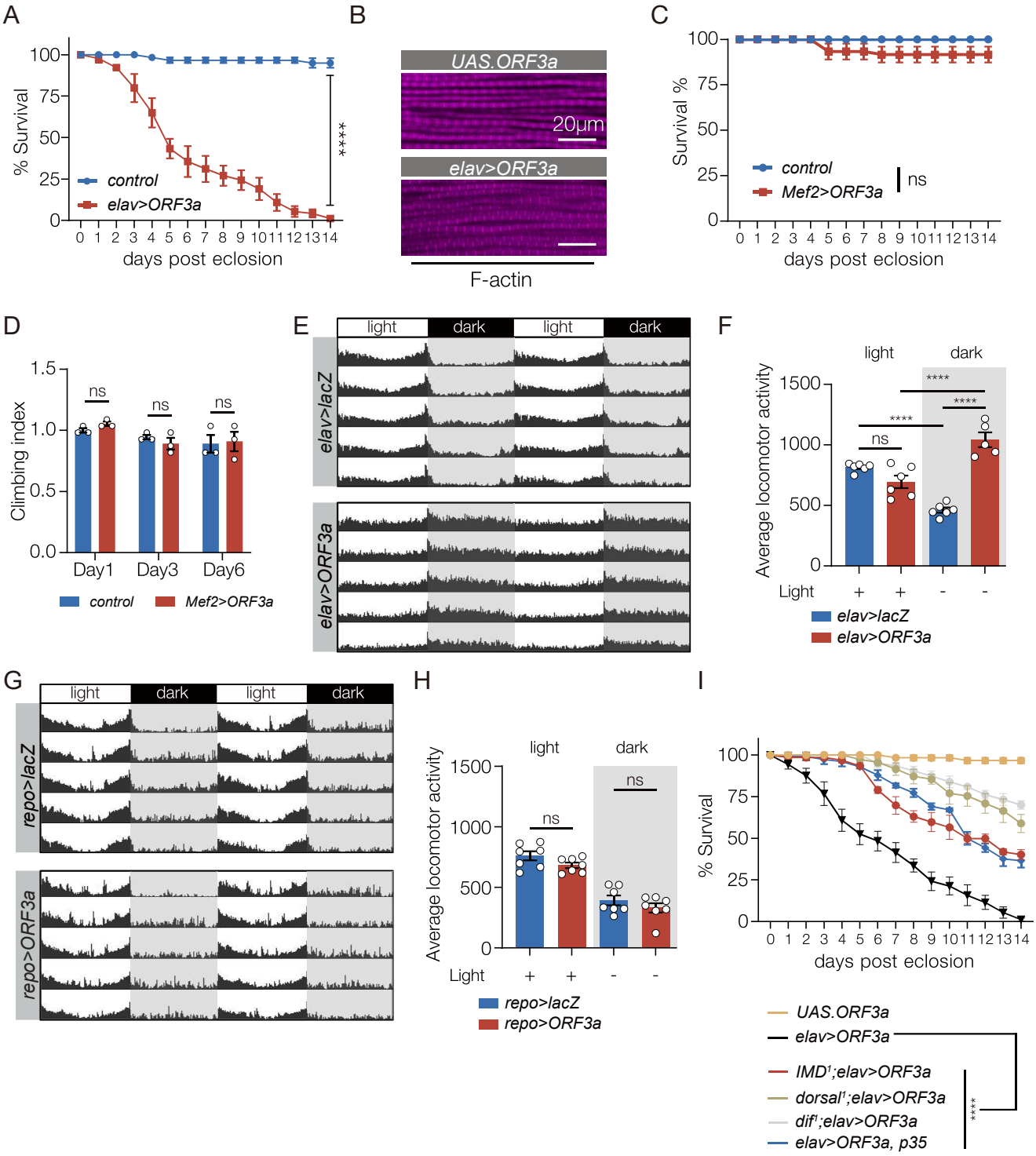


Figure S3 Related to Figure 4.

A. Survival curves. *elav>ORF3a* flies showed a significant reduction in longevity compared to control flies (*UAS.ORF3a*). n=5 cohorts per genotype, with n≥20 flies per cohort. **B.** Confocal micrographs of indirect flight muscles stained with phalloidin to visualize F-actin (violet). *elav>ORF3a* flies and control flies (*UAS.ORF3a*) showed similar myofiber morphology. **C.** Survival curve. *Mef2>ORF3a* flies and control flies (*UAS.ORF3a*) showed comparable longevity. n=3 cohorts per genotype, with n≥20 flies per cohort. **D.** Climbing index. *Mef2>ORF3a* flies showed and control flies (*UAS.ORF3a*) showed similar climbing capacity at 1-, 3-, and 6-days after eclosion. **E,G.** Actograms. Average activity of flies over 2-days is shown. **E.** Flies that expressed ORF3a broadly in the CNS (*elav>ORF3a*) flies were more active in dark cycles than control flies (*elav>lacZ*). **F.** Quantification of data shown in E. n = 28 flies per each genotype. **G.** Flies that expressed ORF3a only in glial cells (*repo>ORF3a*) flies showed similar activity in light and dark cycles as control flies (*repo>lacZ*). **H.** Quantification of data shown in G. n = 16 flies per each genotype. **I.** Survival curves. *elav>ORF3a* flies with homozygous mutations affecting the IMD pathway (IMD^1) or the Toll pathway ($dorsal^1$, dif^1) showed improved longevity compared to *elav>ORF3a* flies. *elav>ORF3a* flies that expressed the inhibitor of apoptosis p35 in the CNS also showed improved longevity compared to *elav>ORF3a* flies. n=3 cohorts per genotype, with n≥20 flies per cohort. Significance was determined by Kaplan–Meier test (A, C, I), and two-way ANOVA (D, F, H). Data represent the average of at least three independent tests. Error bars represent SEM. (****) $p < 0.0001$, (ns) not significant.

Supplemental Figure 4, related to Figure 5

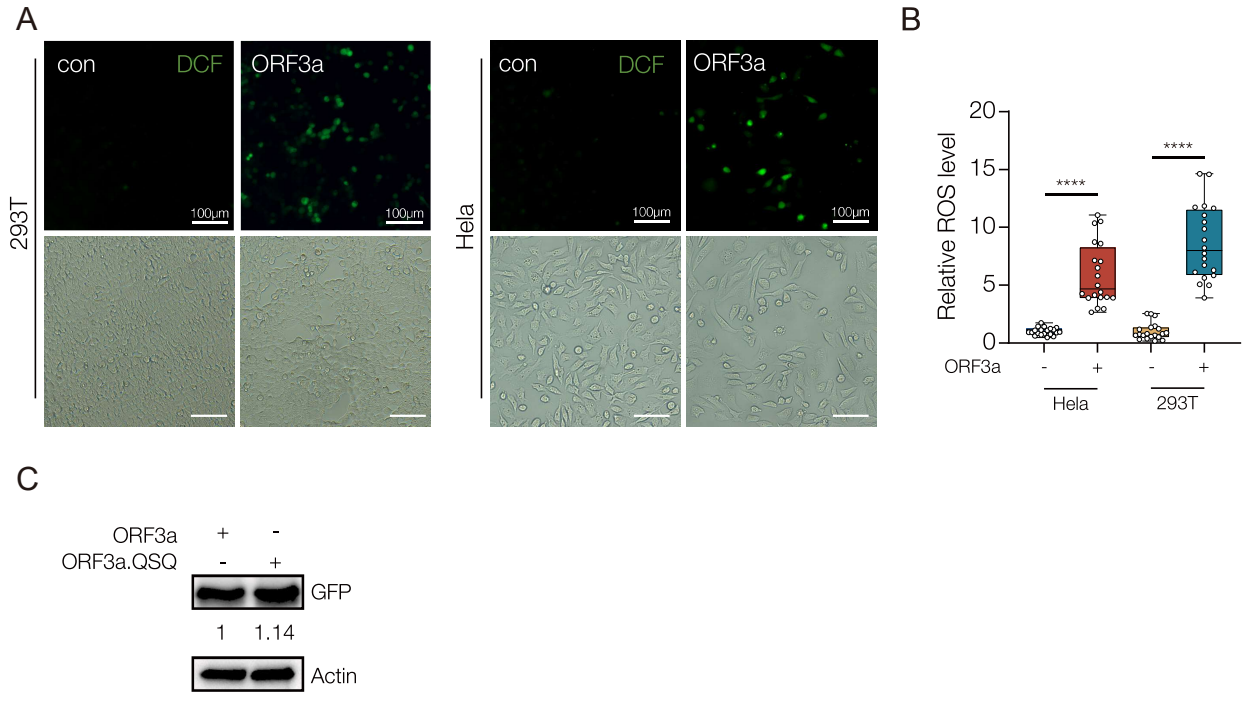
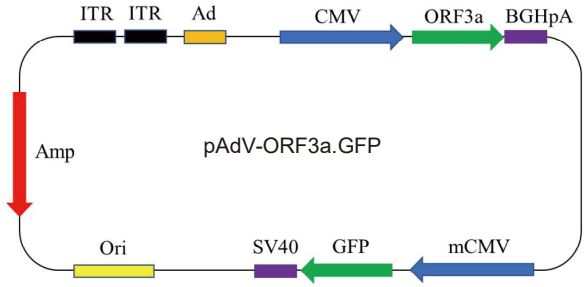


Figure S4 Related to Figure 5.

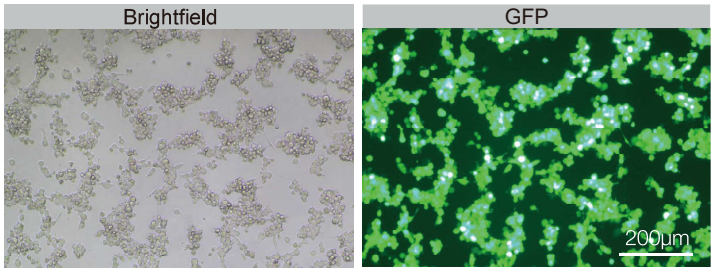
A. ROS assay. H2DCFDA was used to measure ROS in cultured cells. Micrographs of DCF fluorescence (green) in HEK293T cells (left) and HeLa cells (right) transfected with wild-type ORF3a. ORF3a transfected cells produced more ROS than untransfected controls. **B.** Quantification of ROS levels shown in A. Data points represent fluorescence in a single cell normalized to control cells. n=5 fields **C.** Western blot. HEK293T cells transfected with wild-type ORF3a and ORF3a.QSQ showed similar levels of ORF3a protein expression. Significance was determined by two-sided unpaired student's t-test (B). Error bars represent SEM. (****) $p < 0.0001$.

Supplemental Figure 5, related to Figure 6

A



B



C

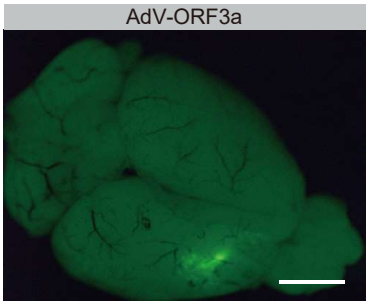
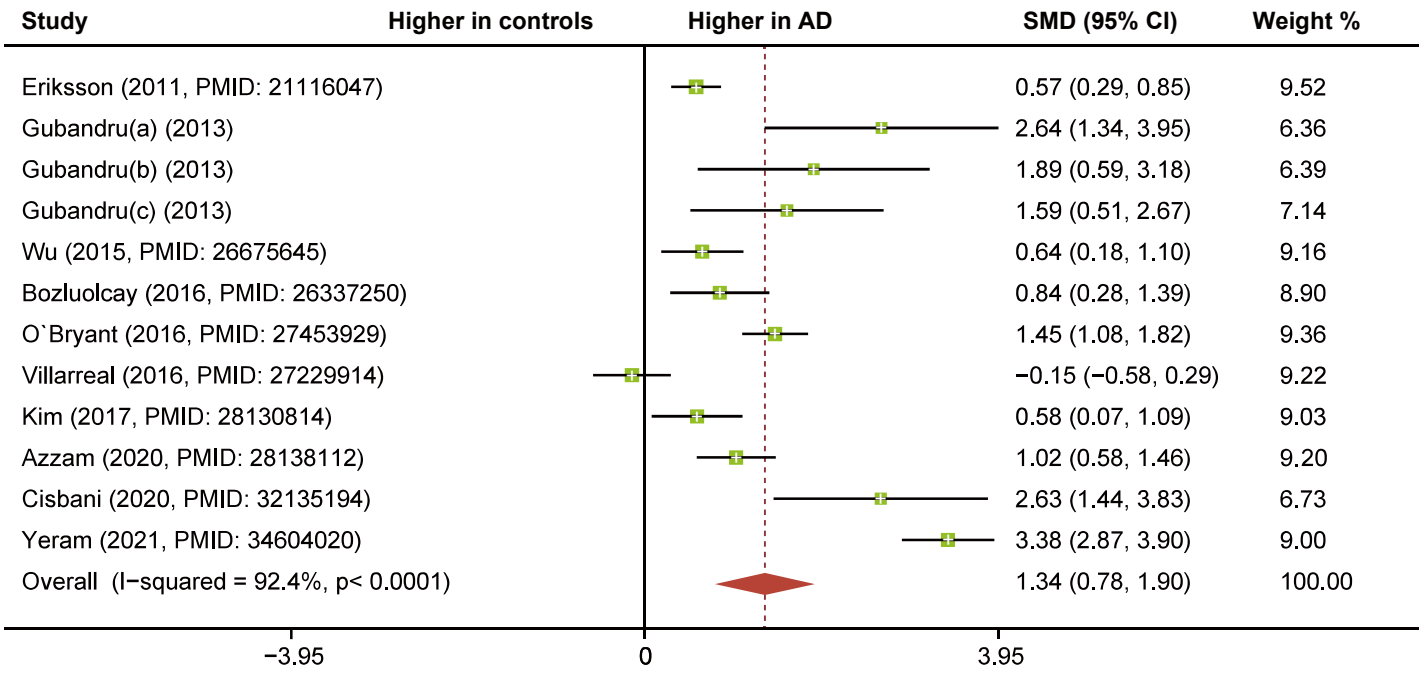


Figure S5 Related to Figure 6.

A. Vector map of AdV-ORF3a.GFP. **B.** Micrograph of HEK293 cells transduced with AdV-ORF3a.GFP. Transduced cells expressed ORF3a.GFP. **C.** Micrograph of a whole mount brain after retro-orbital injection of AdV-ORF3a.GFP. Transduced neural tissue expressed ORF3a.GFP.

Supplemental Figure 6, related to Figure 7

A



NOTE: Weights are from random effects analysis

B

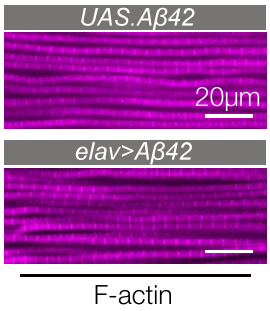


Figure S6 Related to Figure 7.

A. Forest plot depicting IL-6 levels in AD patients. Squares represent the odds ratio and horizontal lines show the 95% confidence intervals. The solid vertical line corresponds to no effect. The red diamond shows the summary measure, indicating the serum levels of IL-6 were increased in AD patients (n=585) compared to health controls (n=439). **B.** Confocal micrographs of indirect flight muscle stained with phalloidin to visualize F-actin (violet). Muscle morphology was comparable between control (*elav>Gal4*) and *elav>Aβ42* flies.