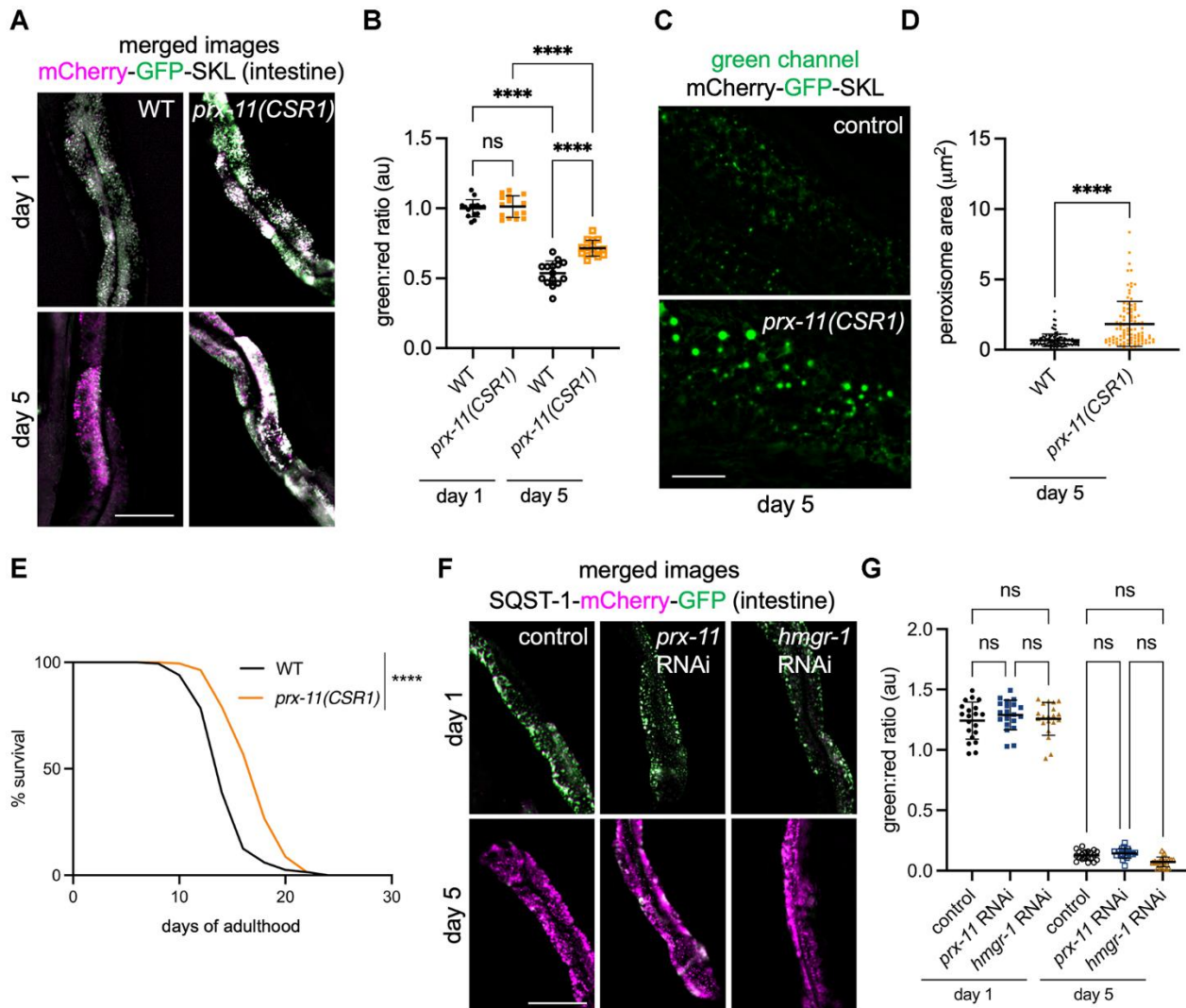
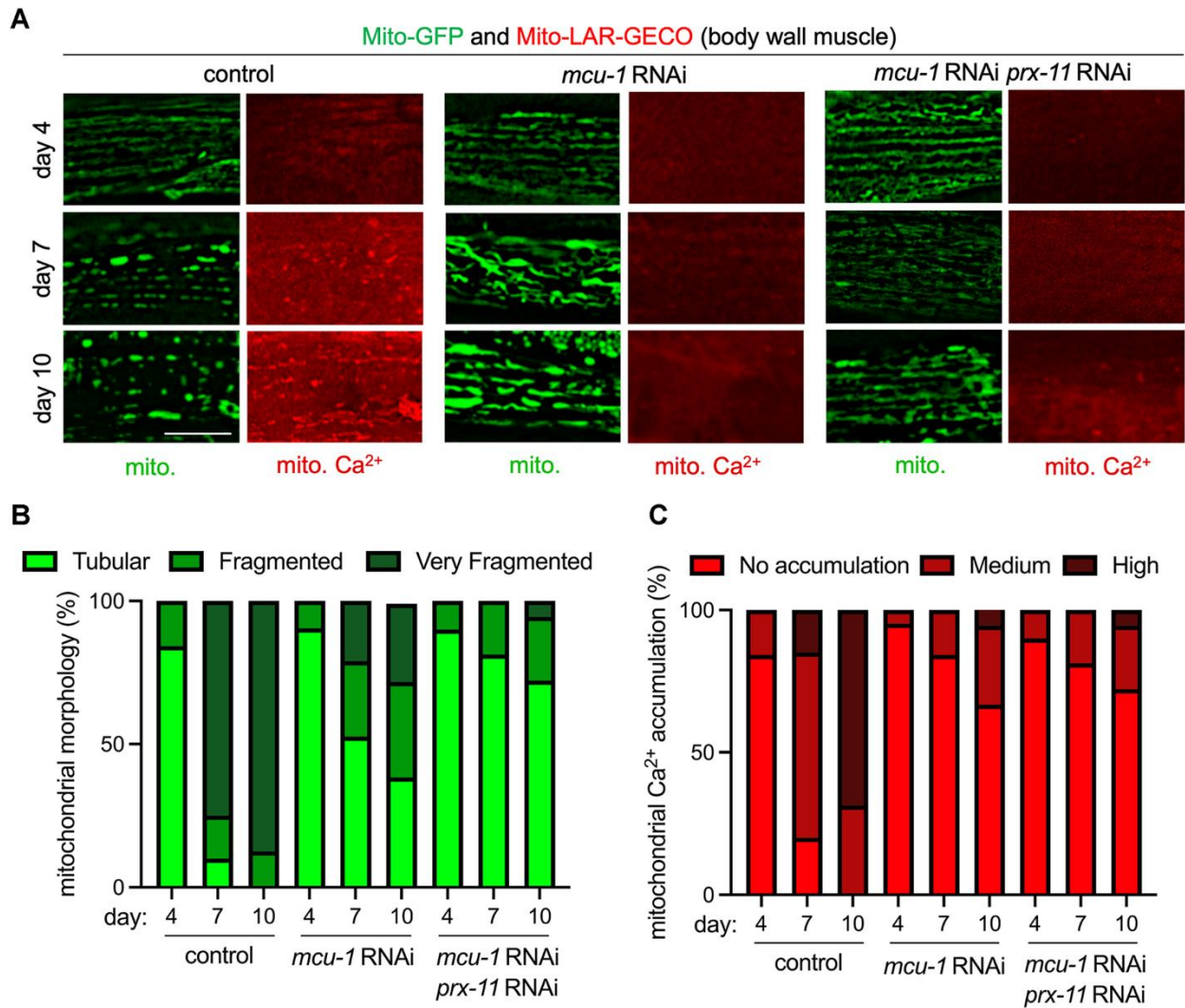


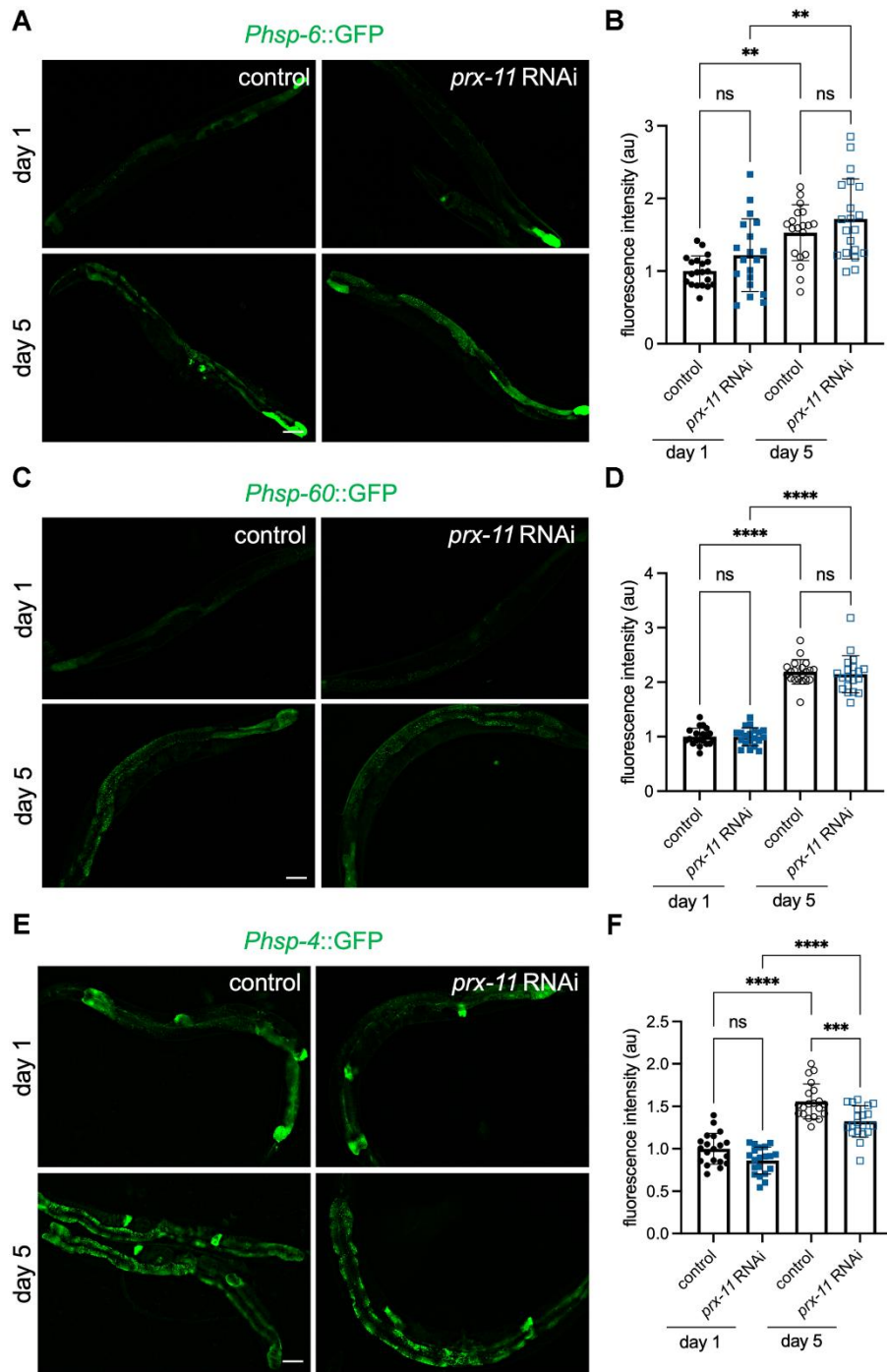
SUPPLEMENTARY FIGURES



**Supplementary Figure 1. Loss of *prx-11* function, such as in *prx-11(CSR1)* mutants, is associated with peroxisome enlargement, lifespan extension, and a reduction in pexophagy but not autophagy in general.** (A) Representative merged green and red fluorescence images of the mCherry-GFP-SKL pexophagy reporter at day 1 and day 5 of adulthood in wild-type and *prx-11(CSR1)* animals. Pexophagy is indicated by a decrease in green fluorescence, which is sensitive to acidic pH, relative to red fluorescence, which is insensitive to acidic pH. (B) Quantification of green:red fluorescence ratios for mCherry-GFP-SKL at day 1 and day 5 of adulthood in wild-type and *prx-11(CSR1)* animals ( $n = 20$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*\*\*,  $p \leq 0.0001$ ; ns, not significant. One-way ANOVA with Tukey's multiple comparisons. (C) Representative green channel images of mCherry-GFP-SKL at day 5 of adulthood in wild-type and *prx-11(CSR1)* animals. (D) Quantification of individual peroxisome areas at day 5 of adulthood in wild-type and *prx-11(CSR1)* animals. Data are presented as mean  $\pm$  SD. \*\*\*\*,  $p \leq 0.0001$ . Unpaired  $t$ -test. (E) Lifespan analysis of wild-type and *prx-11(CSR1)* animals. Wild-type: Mean lifespan = 14.66 days,  $n = 199$  worms. *prx-11(CSR1)*: Mean lifespan = 17.37 days,  $n = 195$  worms. Significance was determined using a log-rank test. Wild-type vs. *prx-11(CSR1)*:  $\chi^2 = 79.33$ ,  $p \leq 0.0001$ . (F) Representative merged green and red fluorescence images of the SQST-1-mCherry-GFP autophagy reporter at day 1 and day 5 of adulthood in animals fed either control, *prx-11*, or *hmgr-1* RNAi. Autophagic activity is indicated by a decrease in green fluorescence, which is sensitive to acidic pH, relative to red fluorescence, which is insensitive to acidic pH. (G) Quantification of green:red fluorescence ratios for SQST-1-mCherry-GFP at day 1 and day 5 of adulthood in animals fed either control, *prx-11*, or *hmgr-1* RNAi ( $n = 20$  worms per condition). Data are presented as mean  $\pm$  SD. ns, not significant. One-way ANOVA with Tukey's multiple comparisons. Bars: 10  $\mu\text{m}$  (C) or 100  $\mu\text{m}$  (A, F).

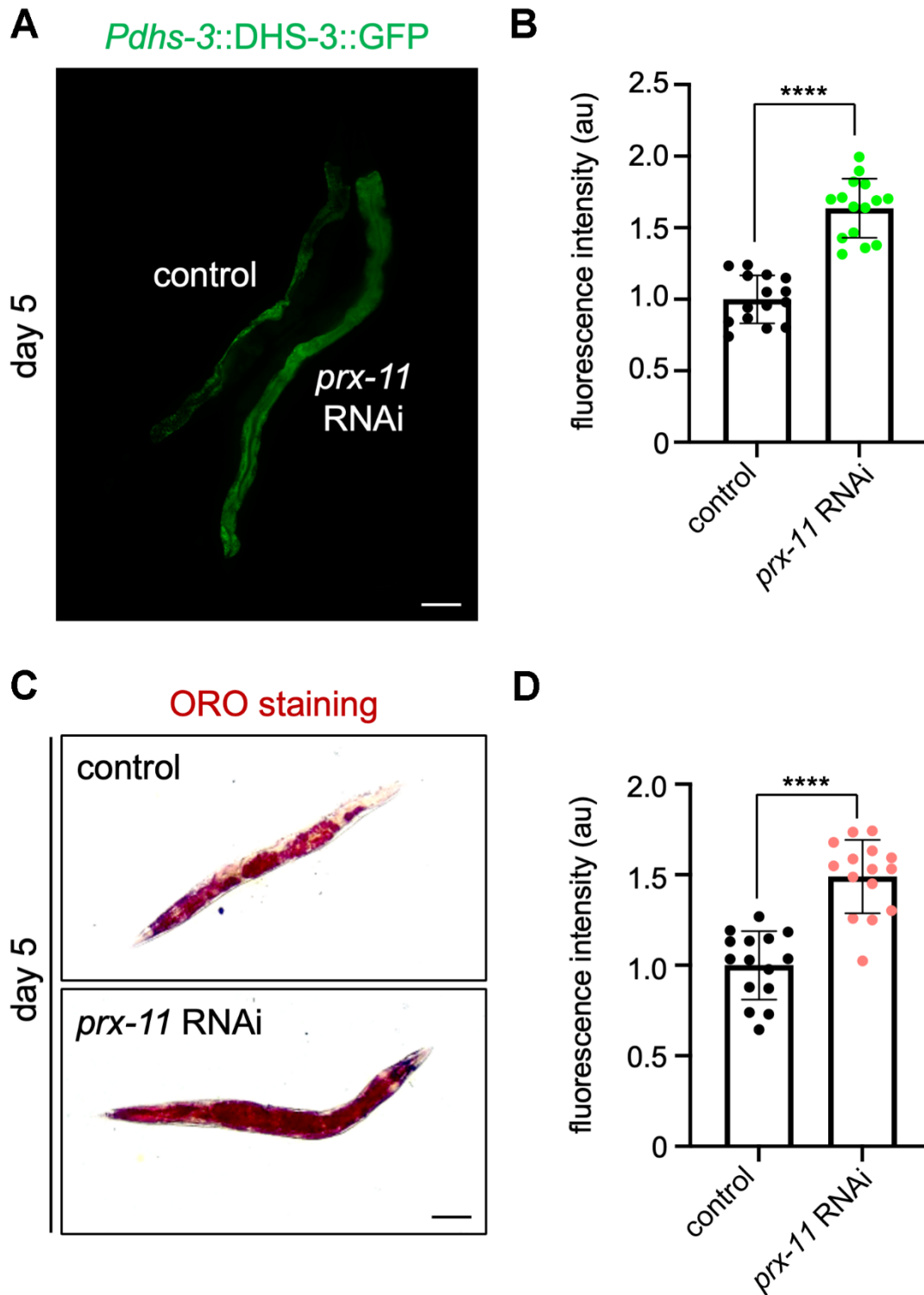


**Supplementary Figure 2. *mcu-1* knockdown suppresses age-dependent mitochondrial Ca<sup>2+</sup> accumulation and, partly, mitochondrial fragmentation.** (A) Representative images of Mito-GFP and the Mito-LAR-GECO Ca<sup>2+</sup> reporter in body wall muscle of day 4, day 7, and day 10 adult hermaphrodite animals fed either control RNAi alone, *mcu-1* RNAi alone, or *mcu-1* RNAi in combination with *prx-11* RNAi. (B, C) Classification of mitochondrial morphology and mitochondrial Ca<sup>2+</sup> accumulation in day 4, day 7, and day 10 adult hermaphrodite animals fed either control RNAi alone, *mcu-1* RNAi alone, or *mcu-1* RNAi in combination with *prx-11* RNAi. Bar: 10  $\mu$ m.

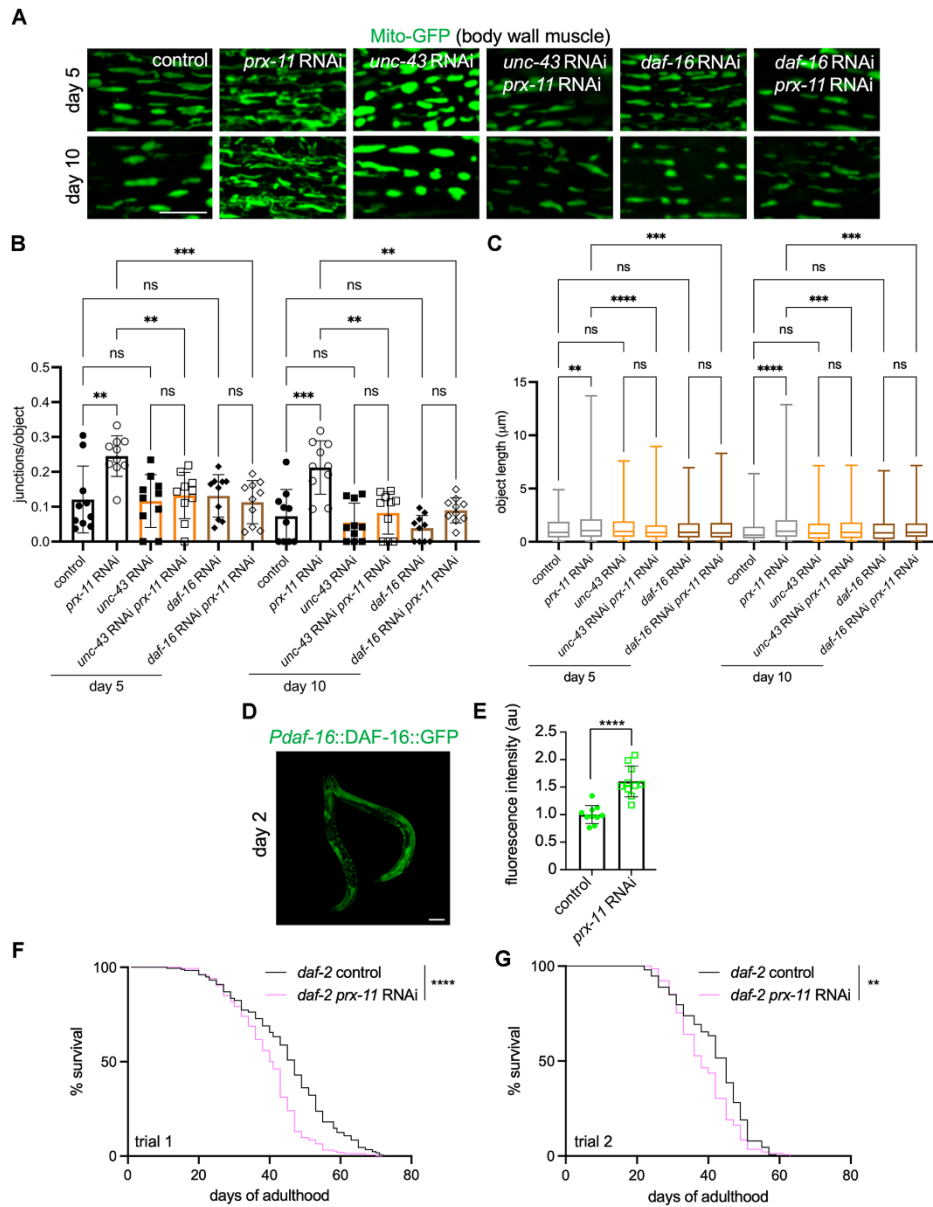


**Supplementary Figure 3. *prx-11* RNAi does not activate mitochondrial or endoplasmic reticulum unfolded protein responses.**

(A) Representative images of *Phsp-6::GFP* in day 1 and day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi. (B) Quantification of *Phsp-6::GFP* fluorescence intensities in day 1 and day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi ( $n = 20$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*,  $p \leq 0.01$ ; ns, not significant. One-way ANOVA with Tukey's multiple comparisons. (C) Representative images of *Phsp-60::GFP* in day 1 and day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi. (D) Quantification of *Phsp-60::GFP* fluorescence intensities in day 1 and day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi ( $n = 20$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*\*\*,  $p \leq 0.0001$ ; ns, not significant. One-way ANOVA with Tukey's multiple comparisons. (E) Representative images of *Phsp-4::GFP* in day 1 and day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi. (F) Quantification of *Phsp-4::GFP* fluorescence intensities in day 1 and day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi ( $n = 20$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*\*\*,  $p \leq 0.0001$ ; \*\*\*,  $p \leq 0.001$ ; ns, not significant. One-way ANOVA with Tukey's multiple comparisons. Bars: 100  $\mu$ m.



**Supplementary Figure 4. *prx-11*-RNAi animals have increased lipid content.** (A) Representative image of *Pdhs-3::DHS-3::GFP* fluorescence in day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi. (B) Quantification of *Pdhs-3::DHS-3::GFP* fluorescence intensities in day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi ( $n = 15$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*\*\*,  $p \leq 0.0001$ . Unpaired  $t$ -test. (C) Representative images of ORO-stained day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi. (D) Quantification of ORO staining fluorescence intensities in day 5 adult hermaphrodite animals fed either control or *prx-11* RNAi ( $n = 15$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*\*\*,  $p \leq 0.0001$ . Unpaired  $t$ -test. Bars: 100  $\mu$ m.



**Supplementary Figure 5. Mitochondrial tubulation during aging in *prx-11*-RNAi animals requires *daf-16* and *unc-43*.** (A) Representative images of Mito-GFP in body wall muscles of day 5 and day 10 adult hermaphrodite animals fed either control RNAi alone, *prx-11* RNAi alone, *unc-43* RNAi alone, *unc-43* and *prx-11* RNAi in combination, *daf-16* RNAi alone, or *daf-16* and *prx-11* RNAi in combination. (B) Quantification of mitochondrial junctions per object in day 5 and day 10 adult hermaphrodite animals fed either control RNAi alone, *prx-11* RNAi alone, *unc-43* RNAi alone, *unc-43* and *prx-11* RNAi in combination, *daf-16* RNAi alone, or *daf-16* and *prx-11* RNAi in combination ( $n = 10$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*\*,  $p \leq 0.001$ ; \*\*,  $p \leq 0.01$ ; ns, not significant. One-way ANOVA with Tukey's multiple comparisons. (C) Mito-GFP object lengths in day 5 and day 10 adult hermaphrodite animals fed either control RNAi alone, *prx-11* RNAi alone, *unc-43* RNAi alone, *unc-43* and *prx-11* RNAi in combination, *daf-16* RNAi alone, or *daf-16* and *prx-11* RNAi in combination ( $n = 10$  worms per condition). Data are presented as box-and-whisker plots (minimum, 25<sup>th</sup> percentile, median, 75<sup>th</sup> percentile, maximum). \*\*\*\*,  $p \leq 0.0001$ ; \*\*\*,  $p \leq 0.001$ ; \*\*,  $p \leq 0.01$ ; ns, not significant. One-way ANOVA with Tukey's multiple comparisons. (D) Representative image of *Pdaf-16::DAF-16::GFP* fluorescence in day 2 adult hermaphrodite animals fed either control or *prx-11* RNAi. (E) Quantification of *Pdaf-16::DAF-16::GFP* fluorescence intensities in day 2 adult hermaphrodite animals fed either control or *prx-11* RNAi ( $n = 10$  worms per condition). Data are presented as mean  $\pm$  SD. \*\*\*\*,  $p \leq 0.0001$ . Unpaired *t*-test. (F) Trial 1 lifespan analysis of *daf-2* hermaphrodite animals fed either control or *prx-11* RNAi. *daf-2* control: Mean lifespan = 44.93 days,  $n = 177$  worms. *daf-2 prx-11* RNAi: Mean lifespan = 39.38 days,  $n = 154$  worms. Significance was determined using a log-rank test. *daf-2* control vs. *daf-2 prx-11* RNAi:  $\chi^2 = 30.41$ ,  $p \leq 0.0001$ . (G) Trial 2 lifespan analysis of *daf-2* hermaphrodite animals fed either control or *prx-11* RNAi. *daf-2* control: Mean lifespan = 41.67 days,  $n = 153$  worms. *daf-2 prx-11* RNAi: Mean lifespan = 38.77 days,  $n = 142$  worms. Significance was determined using a log-rank test. *daf-2* control vs. *daf-2 prx-11* RNAi:  $\chi^2 = 9.69$ ,  $p \leq 0.01$ . Bars: 10  $\mu\text{m}$  (A) or 100  $\mu\text{m}$  (D).