

Figure S1. Intestinal-specific rescue of *fzo-1* rescues fragmented mitochondrial morphology in intestine but not muscle cells of *fzo-1* mutant worms. Related to Figure 3.

Mitochondrial networks in intestinal cells (**A**) and muscle cells (**B**) from wild-type, *fzo-1* mutant and *fzo-1* mutant with intestinal-specific *fzo-1* rescue (*ges1p::fzo-1*).

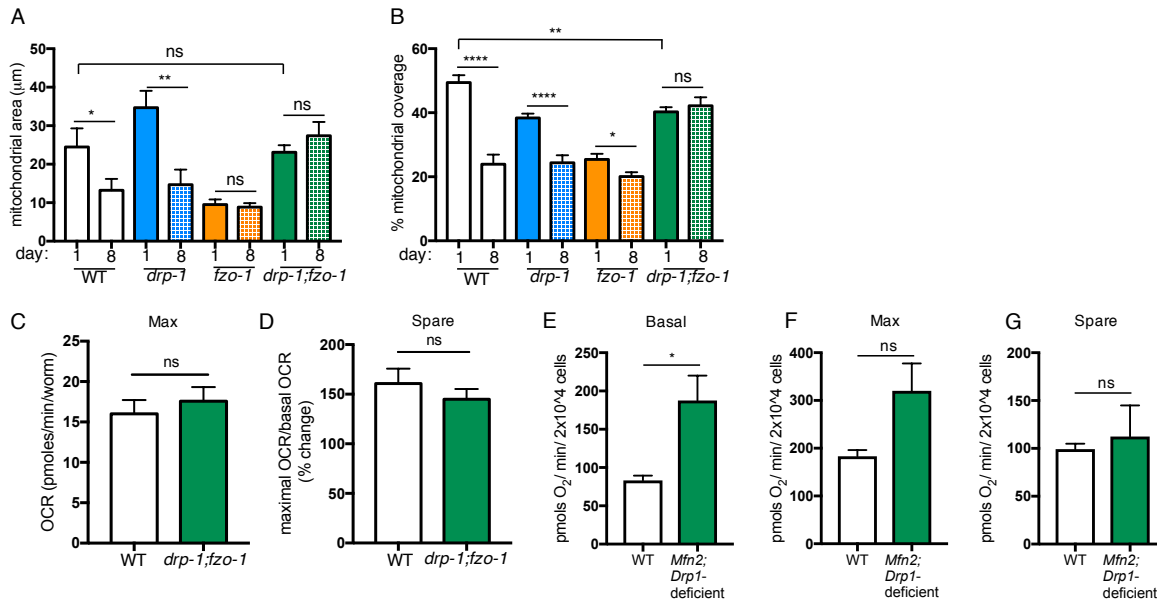


Figure S2. Mitochondrial content and size are maintained with age in *drp-1;fzo-1* double mutant animals. Related to Figure 5.

(A) and (B) Quantification of muscle cell mitochondrial networks from wild-type, *drp-1* mutant, *fzo-1* mutant, and *drp-1;fzo-1* double mutant worms on day 1 and day 8 of adulthood. Mitochondrial size (A) and mitochondrial content (B). Mean \pm SEM of n=11-18 muscle cells from different worms. Maximal oxygen consumption rate (OCR, (C)) and spare respiratory capacity (D) of day 11 wild-type and *drp-1;fzo-1* double mutant worms are not significantly different. (E) - (G) Basal respiration (E), maximal respiration (F) and spare respiratory capacity in WT and *Mfn2;Drp1*-deficient MEFs in the presence of 20mM galactose, 4mM pyruvate and 2mM glutamine. Mean \pm SEM of n=3-5 independent experiments. *p<0.05, **p<0.01, ****p<0.0001 by t test.

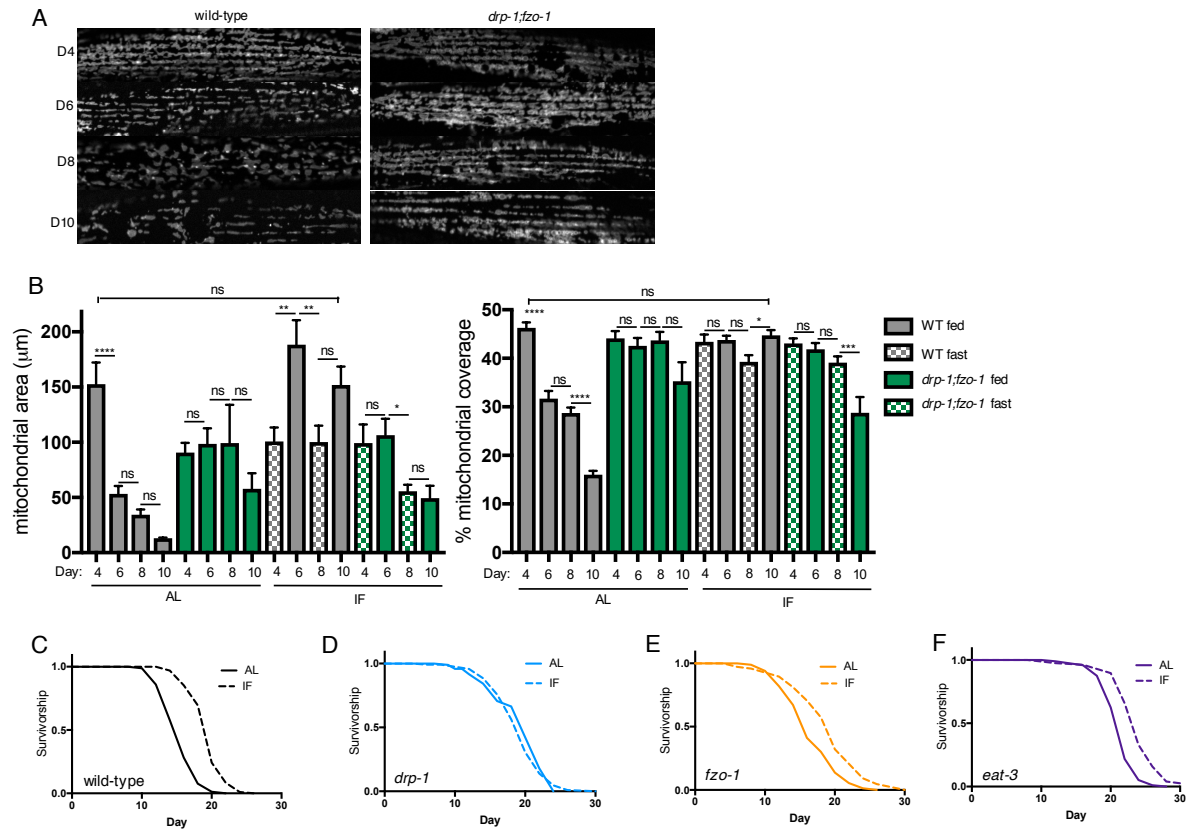


Figure S3. Mitochondrial plasticity and network remodeling is required for IF-mediated longevity. Related to Figure 5.

(A) Representative images of mitochondrial networks in wild-type and *drp-1;fzo-1* mutant worms fed *ad libitum* (AL) on day 4, 6, 8, and 10 (B) Mitochondrial area and content on day 4, 6, 8, and 10, from wild-type and *drp-1;fzo-1* mutant worms fed AL or with IF. Worms in the IF group were fasted from day 2-4 and day 6-8. Mean \pm SEM of $n=12-26$ muscle cells from 2 independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ by one-way ANOVA with Tukey's multiple comparisons test. (C)-(F) Survival curves for wild-type (C), *drp-1* (D), *fzo-1* (E) and *eat-3* (F) worms fed AL or with IF. See Table S1 for lifespan statistics.

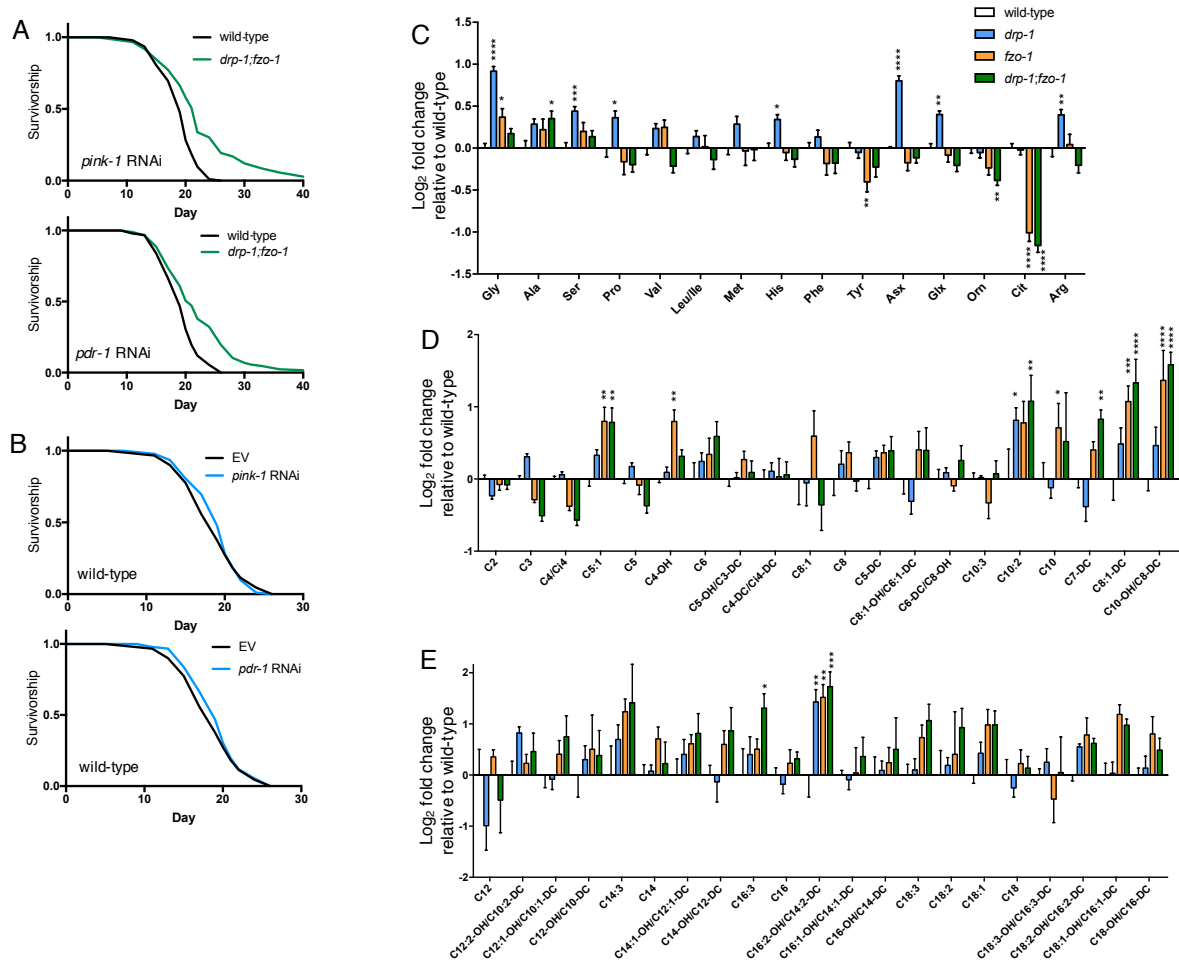


Figure S4. *drp-1;fzo-1* double mutants do not require mitophagy to increase lifespan but reprogram mitochondrial metabolism. Related to Figure 6.

(A) *drp-1;fzo-1* double mutant animals are long-lived with *pink-1* and *pdr-1* RNAi. (B) *pink-1* and *pdr-1* RNAi do not alter wild-type lifespan. See Table S1 for lifespan statistics. (C)-(E) Metabolomic analyses of wild-type, *drp-1* mutant, *fzo-1* mutant, and *drp-1;fzo-1* double mutant worms on day 1 of adulthood. Levels of amino acids (C), acylcarnitines C2-C10 (D) and C12-C18 (E) are expressed as log₂ fold change relative to wild type levels. Mean ± SEM of 5-6 biological replicates. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 by two-way ANOVA with Tukey's multiple comparisons test. Statistics shown are relative to wild type levels.

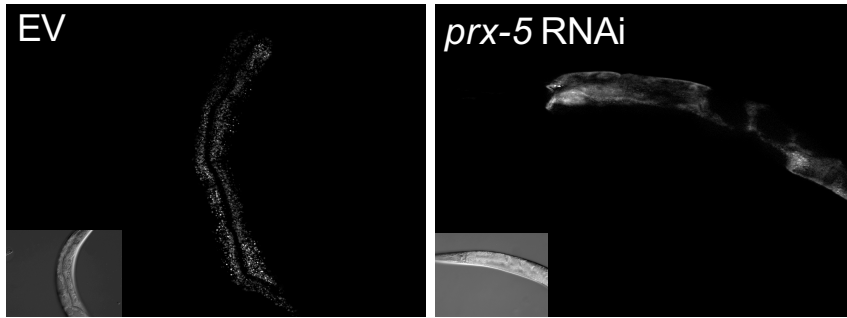


Figure S5. *prx-5* RNAi inhibits peroxisomal protein import. Related to Figure 6.

Fluorescence and DIC (inset) images of L4 wild-type worms expressing *vha-6p::mRFP-PTS1*. Fluorescence signal is punctate on empty vector control (EV, **(A)**), but diffuse on *prx-5* RNAi (**(B)**), indicating that mRFP fails to import into peroxisomes.

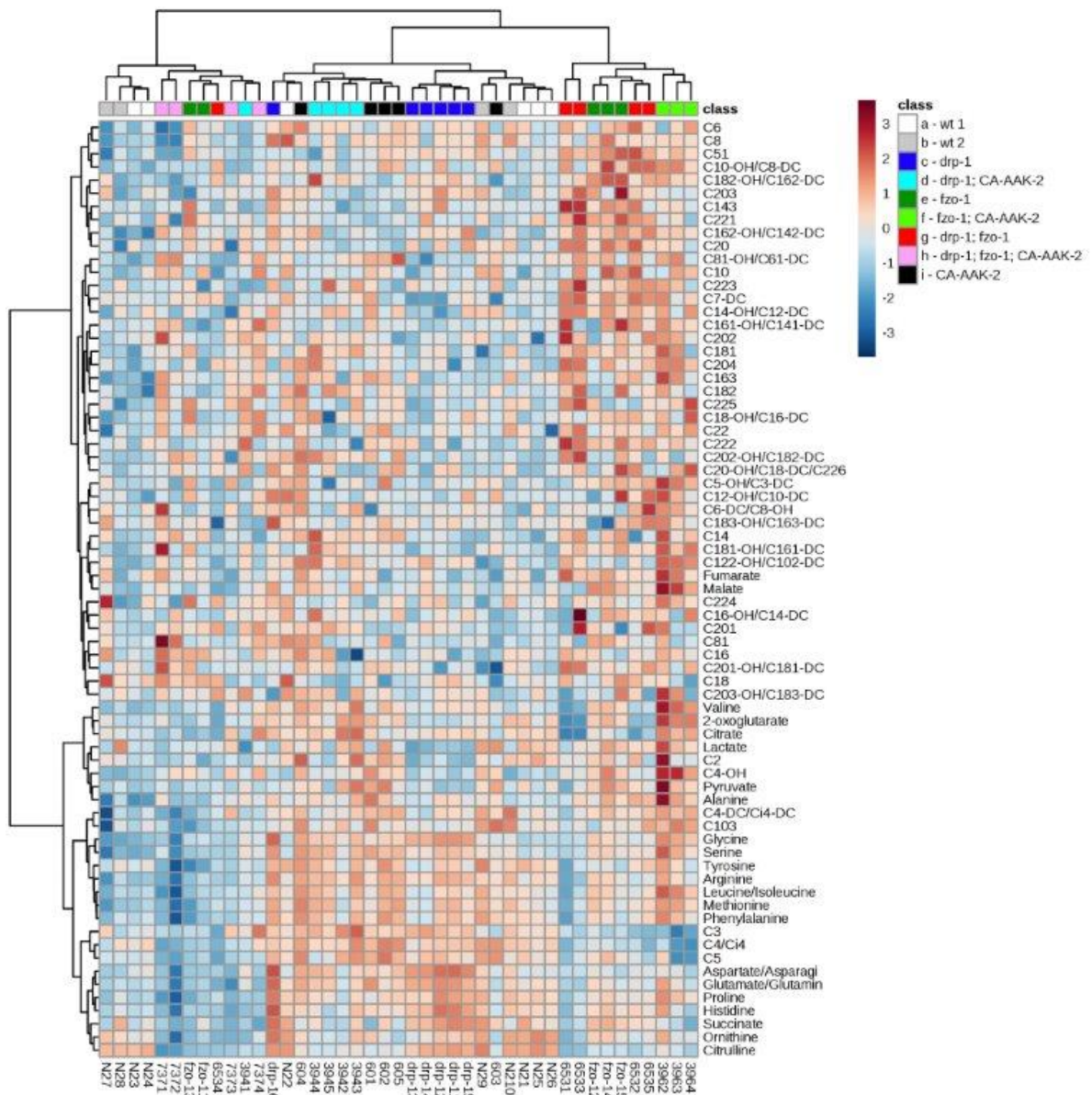


Figure S6. Metabolite profiles of *drp-1*, *fzo-1*, and *drp-1;fzo-1* mutants, with and without AMPK activation. Related to Figure 7.

Heatmap with hierarchical clustering of TCA intermediates, amino acids and acylcarnitines from wild-type, *drp-1*, *fzo-1*, and *drp-1;fzo-1* mutant worms, with and without CA-AAK-2.

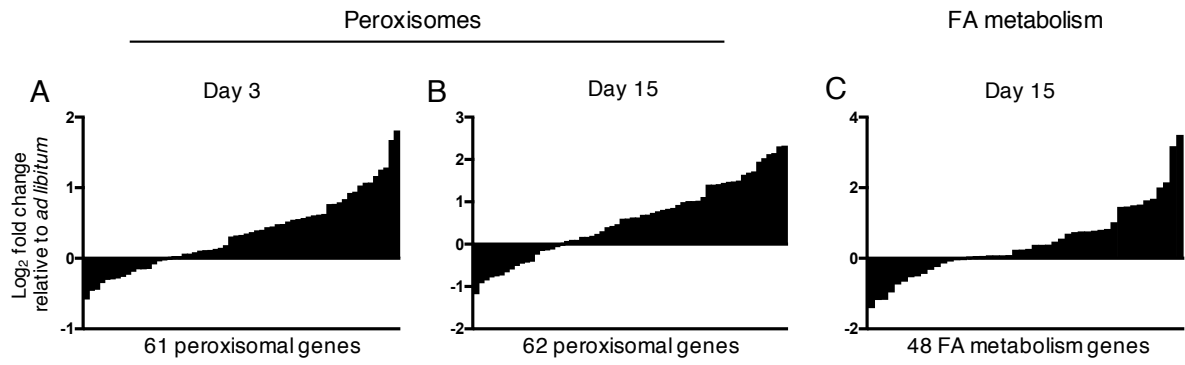


Figure S7. Peroxisome and FA metabolism genes are significantly enriched by DR.

Related to Figure 7.

(A) and (B) Genes in the KEGG pathway 'Peroxisome' are upregulated in *eat-2(ad1116)* mutants (DR) vs wild-type animals (*ad libitum*) on day 3 (A) and day 15 (B). (C) Genes in the KEGG pathway 'Fatty acid metabolism' are upregulated in *eat-2(ad1116)* mutants vs wild-type animals on day 15. See also Tables S2-S4.