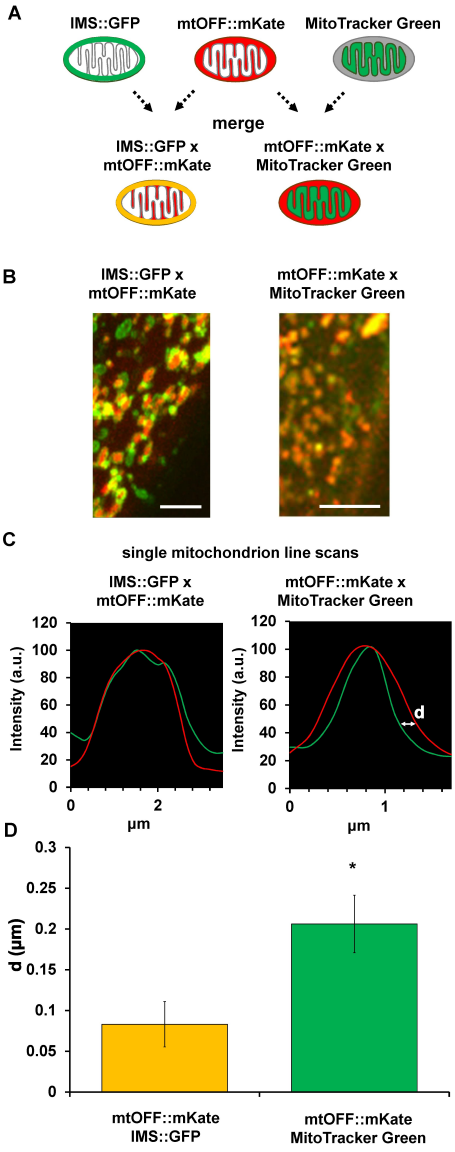


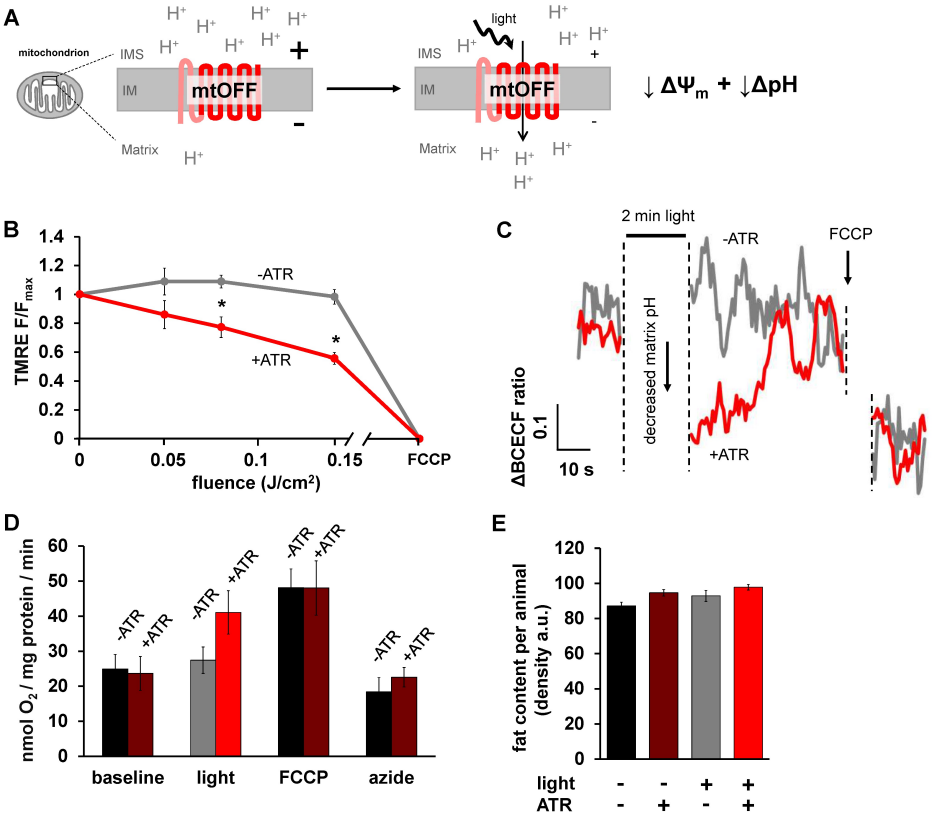
Supplementary Figure 1. Mitochondrial localization of mtOFF.

A) Schematic showing expected fluorescence localization in single mitochondria for mtOFF::mKate, intermembrane space (IMS)::GFP, and MitoTracker Green. Bottom row shows the expected fluorescence pattern of merged images.

B) Fluorescent images of muscle mitochondria in live *C. elegans* coexpressing IMS::GFP and mtOFF::mKate (left) or expressing mtOFF::mKate and stained with MitoTracker Green. Scale bars are 5 µm.

C) Representative profile fluorescence intensity plots for single mitochondria from the images in panel B. The white letter d shows the distance between inflection points of the red and green fluorescent signals.

D) The distance between inflection points was quantified (examples shown in panel d). mtOFF::mKate localized close to IMS::GFP signal, and distant from the matrix MitoTracker Green signal as expected, with the C terminal mKate predicted to be in the IMS. Two-tailed unpaired t test was performed, *p = 0.0137. Data are means $\pm$ SEM, n = 14 mitochondria from distinct animals for each condition.



Supplementary Figure 2. mtOFF decreases the PMF

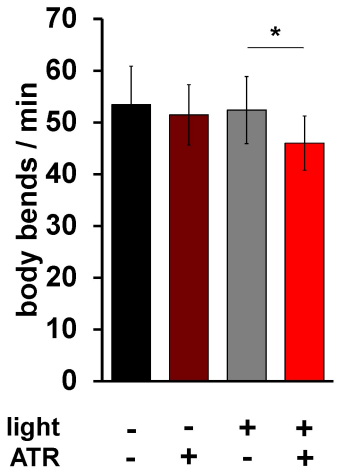
A) Schematic showing mtOFF decreasing both components of the protonmotive force (PMF), the $\Delta\Psi_m$ and the ΔpH, upon light exposure. mtOFF pumps protons (H^+) from the intermembrane space (IMS) across the inner membrane (IM) into the matrix.

B) TMRE fluorescence was measured in response to increasing light doses. Increasing fluence (light dose, Joules / cm^2) results in progressively decreased PMF in isolated mitochondria supplied with succinate. Data from 0 and 0.15 J/cm^2 are presented in Figure 1C. Two-way ANOVA with Holm-Sidak test for multiple comparisons was performed, 0.08 J/cm^2 *p = 0.0068, 0.15 J/cm^2 *p = 0.018, n = 4 independent mitochondrial isolations. Data are means ± SEM.

C) Representative BCECF-AM fluorescence ratio trace. Baseline level of mitochondria supplied with succinate from animals with and without ATR is shown followed by light treatment (no BCECF-AM fluorescence measured), and signal immediately after illumination. Mitochondria with ATR have a decreased matrix pH, indicating proton entry through mtOFF during light exposure. Rapid reestablishment of baseline pH shows the reversibility of mtOFF when light is removed. FCCP was then added to establish minimum signal.

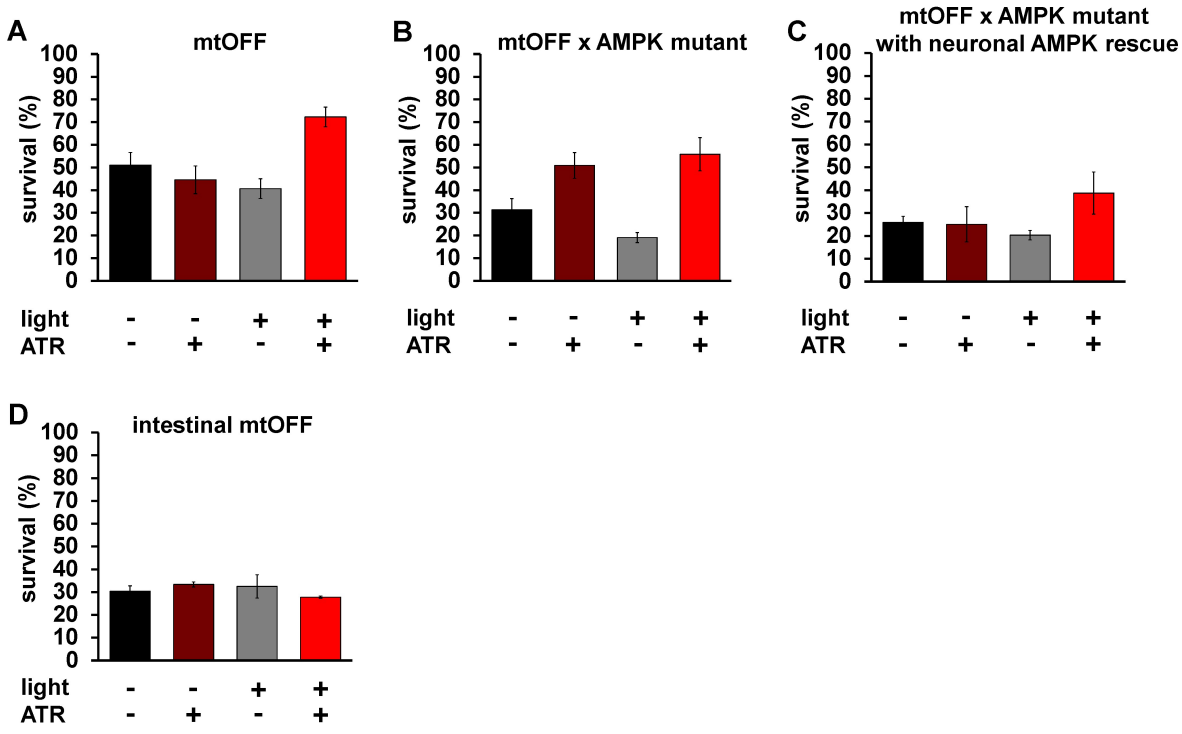
D) Raw O_2 consumption values under baseline, light treatment, maximal respiration, and minimum respiration states in whole animals. Maximum respiration was induced by FCCP treatment, and minimum respiration was induced with azide treatment. Data are presented for animals with and without ATR and are means ± SEM, n = 5, where one n is one O_2 consumption rate from ~1500 animals in a Clark type O_2 electrode. Normalized baseline data are presented in Figure 1E.

E) Oil Red O density was quantified (a.u. is arbitrary units) in whole animals stained immediately after 10 minute activation of mtOFF, as performed in Figure 1E and Supplementary Figure 2D. mtOFF had no effect on fat stores. One way ANOVA was performed, p > 0.05. Data are means ± SEM.



Supplementary Figure 3. mtOFF starved locomotion.

mtOFF activation in animals off of food resulted in a small but significant decrease in locomotion. Data was collected on at least two different experimental days for each condition. One way ANOVA with Tukey's test for multiple comparisons was performed, -ATR –light vs +ATR +light $p < 0.0001$, +ATR –light vs +ATR +light $p = 0.0056$, -ATR +light vs +ATR +light *$p = 0.0008$, n = 30 – 60 animals each condition. Data are means $\pm$ standard deviation.



Supplementary Figure 4. Raw survival data following hypoxia for Figure 4.

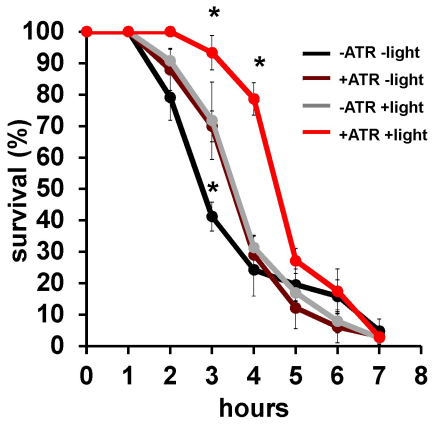
These raw data were used to calculate protection % in Figure 4 to account for ATR and light exposure effects. See Figure 4 legend and methods section for details on statistics. For each set of experiments, n = 3, where one n is an average of three technical replicates of plates containing 15-50 animals. Data are means ± SEM.

A) Survival % for animals expressing mtOFF after hypoxia exposure. Protection % presented in Figure 4B.

B) Survival % for animals expressing mtOFF in the AMPK mutant background after hypoxia exposure. Protection % presented in Figure 4C.

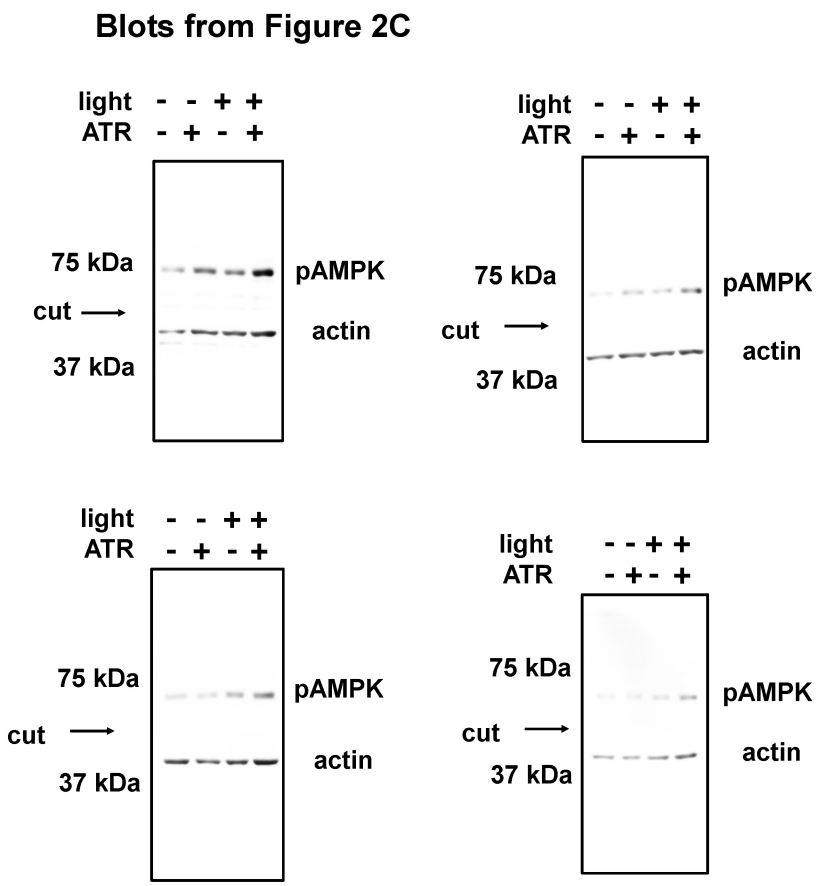
C) Survival % for animals expressing mtOFF in the AMPK mutant background with AMPK expression rescued in neurons after hypoxia exposure. Protection % presented in Figure 4D.

D) Survival % for animals expressing mtOFF in intestine after hypoxia exposure. Protection % presented in Figure 4E.



Supplementary Figure 5. mtOFF activation results in oxidative stress resistance.

Day1 adults were exposed to 200 mM paraquat. Continuous mtOFF activation resulted in increased survival over time. Two-way ANOVA with Tukey's test for multiple comparisons was performed. For hour 3, -ATR -light vs. +ATR +light *$p < 0.0001$, +ATR -light vs. +ATR +light *$p = 0.0297$, -ATR -light vs. -ATR +light *$p = .0024$. For hour 4, -ATR -light vs. +ATR +light *$p < 0.0001$, +ATR -light vs. +ATR +light *$p < 0.0001$, -ATR +light vs. +ATR +light *$p < 0.0001$. Data are means $\pm$ SEM, n = 3 biological replicates with at least 30 animals for each condition.



Supplementary Figure 6. Full length blots for Figure 2 immunoblot.

These raw data are full length blots, the first of which has been cropped for presentation in Figure 2C. Each blot shows separate biological replicates.