Expanded View Figures

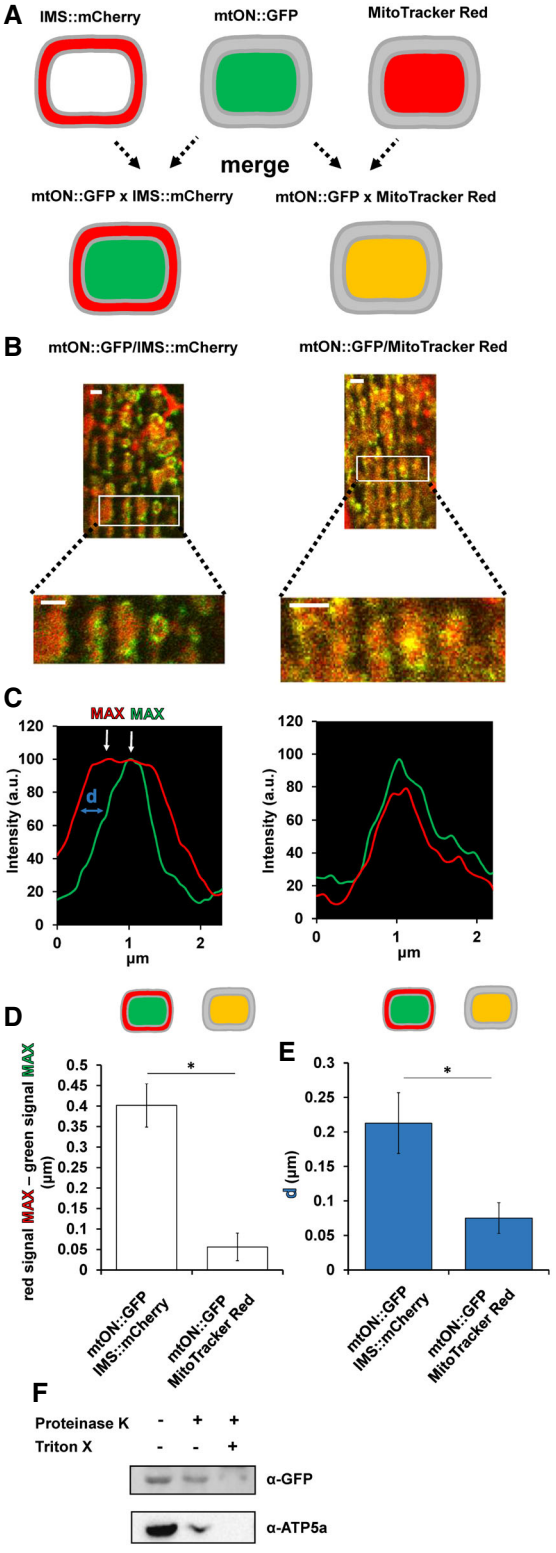

Figure EV1. mtON is targeted to mitochondria.

A Schematic showing expected fluorescence localization of mtON::GFP in the matrix, intermembrane space (IMS)::mCherry, and MitoTracker Red CMXRos™ in the matrix. The combinations of mtON::GFP and the mCherry and MitoTracker controls are shown below as predicted merged images.

B Images of muscle mitochondria from mtON::GFP × IMS::mCherry (left) and mtON::GFP mitochondria stained with MitoTracker (right). White scale bars are each 2 μm.

C Representative profile intensity plots of single mitochondria from the images in B. Maximum intensities for each color are indicated (MAX), as well as the distance between inflection points (d). Larger values will correspond to mtON::GFP being in a separate compartment from the control. mtON::GFP was predicted to have larger values when coexpressed with IMS::mCherry, as the GFP would be targeted to the matrix-side C-terminus of mtON. Smaller values were predicted when mtON::GFP was quantified with MitoTracker stain, as MitoTracker accumulates in the matrix, overlapping with the predicted location of GFP.

D Quantification of the distance between maximum fluorescence (red signal MAX—green signal MAX, μm) for both mtON::GFP × IMS::mCherry (left bar) and mtON::GFP mitochondria stained with MitoTracker (right bar). Two-sample 2-tailed unpaired *t*-test, $^*P = 0.0164$, $n = 10$ mitochondria. Data show mean ± SEM.

E Quantification of the distance between inflection points (d, μm), for both mtON::GFP x IMS::mCherry (left bar) and mtON::GFP mitochondria stained with MitoTracker (right bar). Two-sample 2-tailed unpaired *t*-test, $^*P = 0.0009$, left bar $n = 5$ mitochondria, right bar $n = 7$ mitochondria. Data show mean ± SEM.

F Immunoblot of the same membrane showing proteinase K treatment of isolated mitochondria. GFP signal at the predicted molecular weight of mtON was protected along with the mitochondrial inner membrane protein, ATP5a, demonstrating mtON expression in the inner mitochondrial membrane. Triton X treatment permeabilizes mitochondria and both GFP and ATP5a were susceptible to proteinase K digestion, as expected.

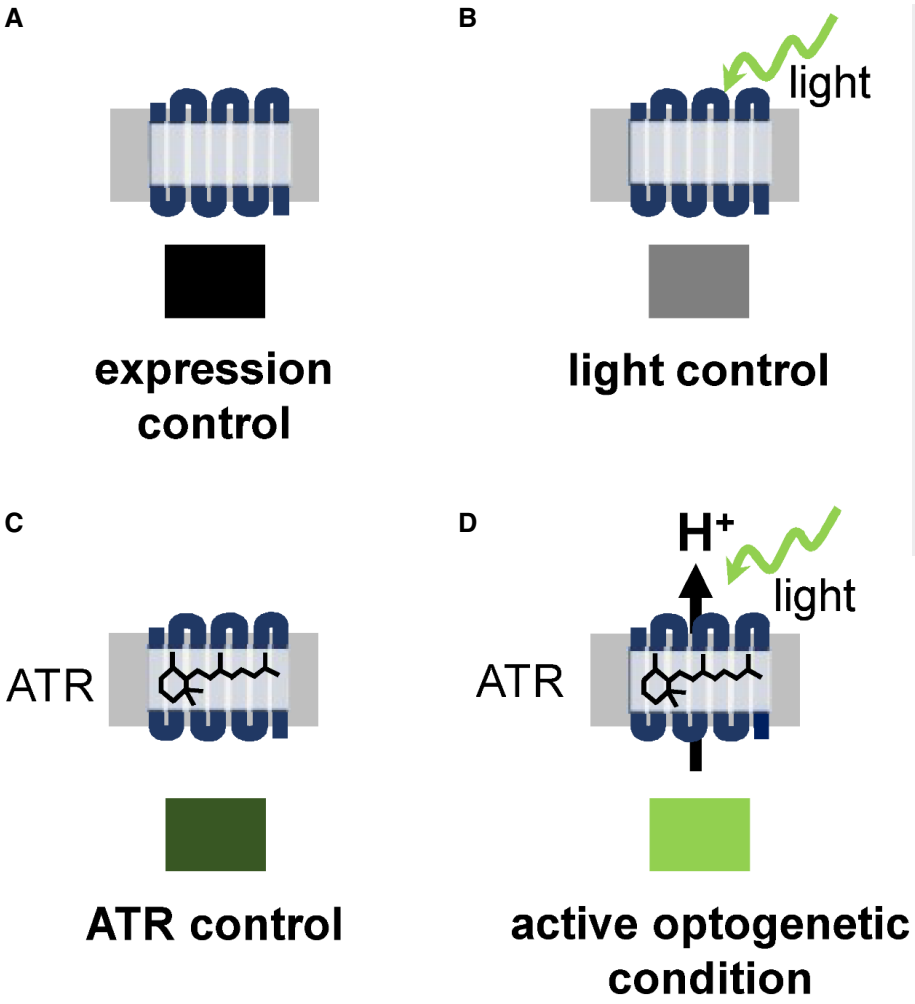

Figure EV2. Schematic of experimental conditions.

A Expression control: baseline condition where animals express mtON, but have not been supplemented with ATR or exposed to light. This condition is represented by the color black throughout.

B Light control: condition exposed to light where mtON is illuminated but not supplemented with ATR, resulting in an inactive proton pump. This condition is represented by the color gray throughout.

C ATR control: condition supplemented with ATR but not exposed to light, where proton pumping is possible, but no light activation has occurred. This condition is represented by dark green throughout.

D Active optogenetic condition: supplemented with ATR and exposed to light, where proton pumping activity is expected. This condition is represented by bright green throughout.

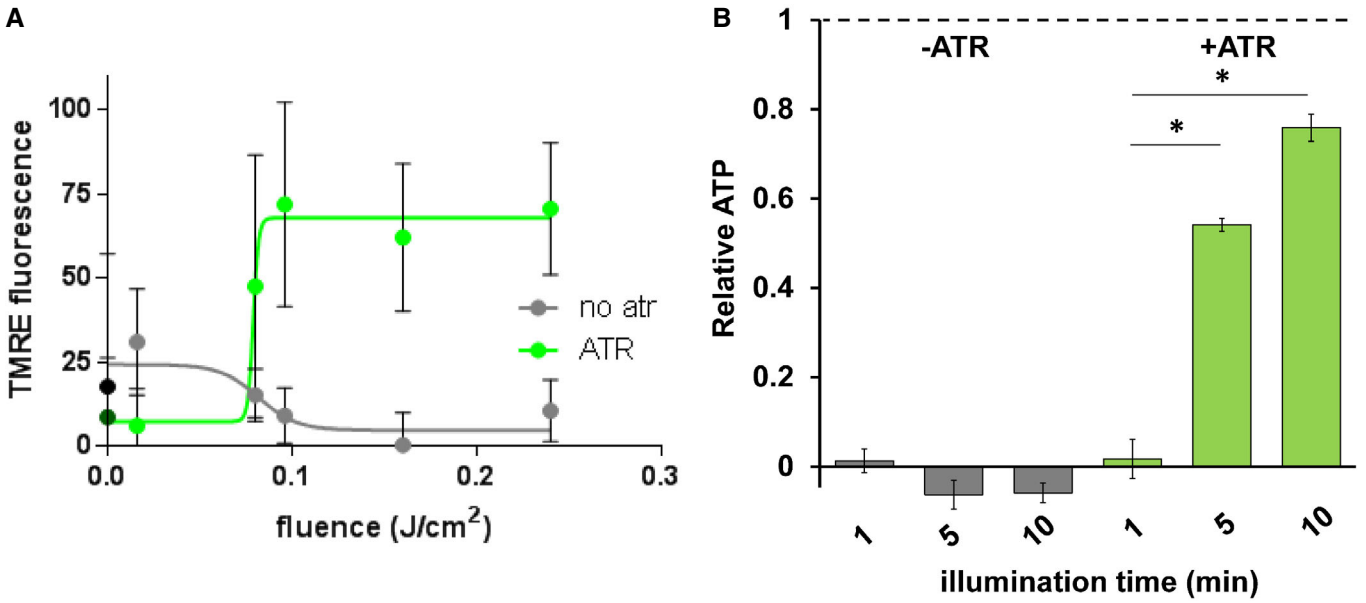

Figure EV3. mtON increases the PMF and ATP synthesis light dose-dependently.

A Change in TMRE fluorescence with increasing light dose (fluence 0, 0.016, 0.08, 0.096, 0.16, 0.24 J/cm^2). Two-way ANOVA with Sidak's multiple comparisons test, +ATR: 0 versus 0.096 J/cm^2 $P = 0.0232$, 0 versus 0.24 J/cm^2 $P = 0.0149$, 0.016 versus 0.096 J/cm^2 $P = 0.0162$, 0.016 versus 0.16 J/cm^2 $P = 0.0376$, 0.016 versus 0.24 J/cm^2 $P = 0.0101$. $n = 3$–6 mitochondrial preparations. Non-linear dose–response fit carried out using GraphPad Prism. Data show mean ± standard deviation.

B ATP levels in response to increasing illumination normalized to maximum ATP synthesis given by succinate respiration (dotted line). Ten-minute illumination time from Fig 2E. Two-way ANOVA with Sidak's multiple comparison test, −ATR: succinate versus 1-, 5-, and 10-min light each $P < 0.0001$. +ATR: succinate versus 1-min light, $P < 0.0001$, 1-min light versus 5-min light *$P = 0.0098$, 1-min light versus 10-min light *$P = 0.0001$. $n = 7$ independent mitochondrial preparations. Data show mean ± SEM.

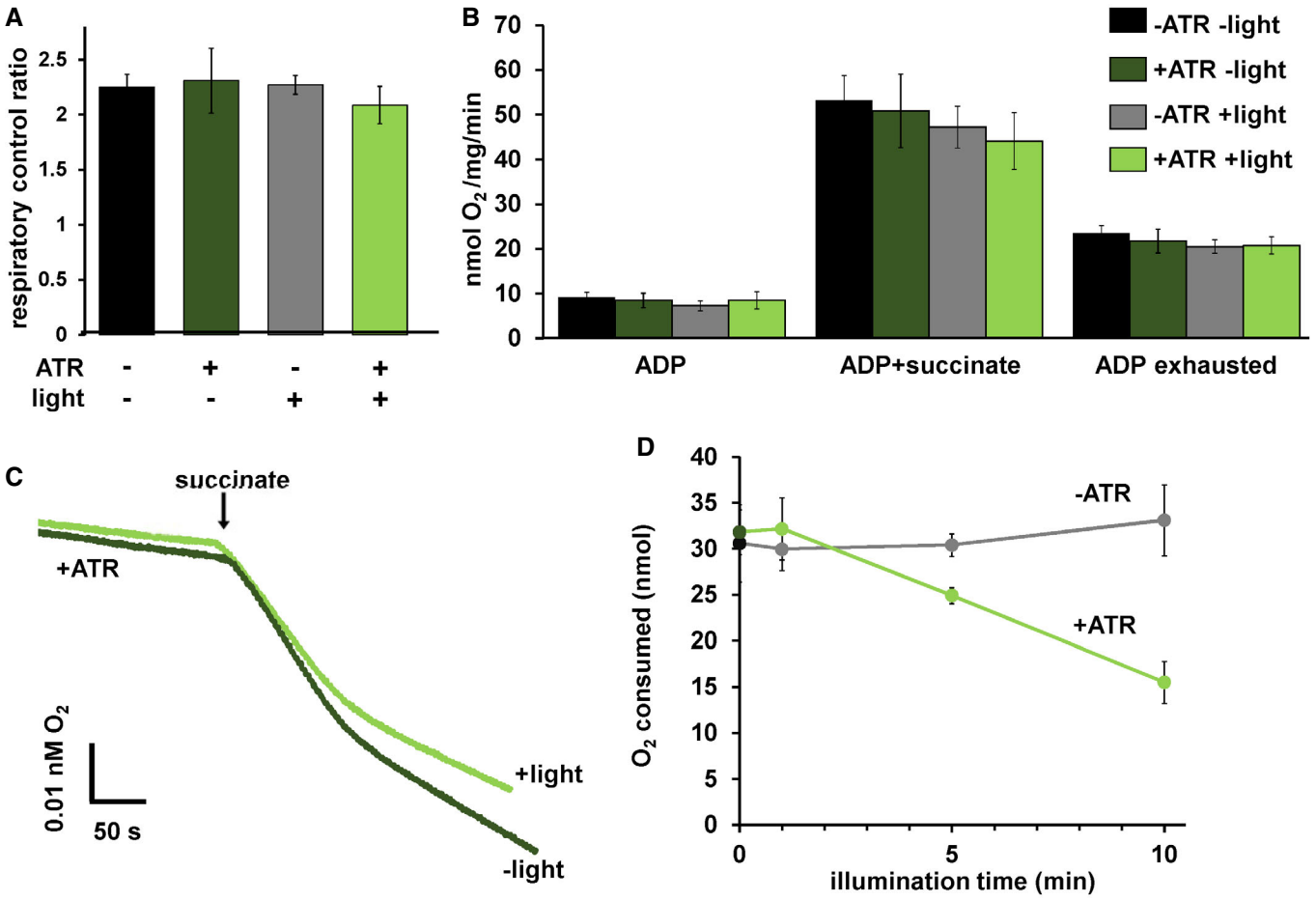

Figure EV4. mtON effects on respiration.

A Respiratory control ratios (indicative of ability of isolated mitochondria to respond to energy demand) were calculated by dividing the rate of O_2 consumption with ADP and succinate by the rate after ADP was depleted. One-way ANOVA with Tukey's post hoc test, $P = 0.72$, $n = 6$ mitochondrial preparations. Data show mean ± SEM.

B O_2 consumption rates under different states of respiration comparing the control conditions. One-way ANOVA with Tukey's post hoc tests performed for each respiration state. ADP rate $P = 0.66$, succinate rate $P = 0.61$. ADP exhausted rate $P = 0.83$. $n = 6$ mitochondrial preparations. Data show mean ± SEM.

C Representative traces depicting O_2 consumption rate. +/− light for mitochondria with ATR present. Light exposure was 10 min before the addition of succinate. Traces show an initial rapid depletion of ADP before transitioning to a lower rate of O_2 consumption.

D Activation of mtON decreases the amount of O_2 required to consume 50 nmoles ADP light dose-dependently. Isolated mitochondria were exposed to light for the indicated time and then succinate was added (example traces in panel C). Dark and 10-min illumination data were used for analysis in Fig 2F. Linear regression showed a negative relationship between O_2 required to consume ADP and illumination time in mitochondria from animals with mtON that were supplemented with ATR, $R^2 = 0.98$ $P = 0.007$, $n = 6$ mitochondrial preparations. Linear regression shows no relationship between O_2 required to consume ADP and illumination in mitochondria from animals with mtON not supplemented with ATR, $R^2 = 0.756$ $P = 0.130$, dark $n = 5$, 1-min light $n = 5$, 5-min light $n = 6$, 10-min light $n = 6$. Each n is an independent mitochondrial preparation. Data show mean ± SEM.

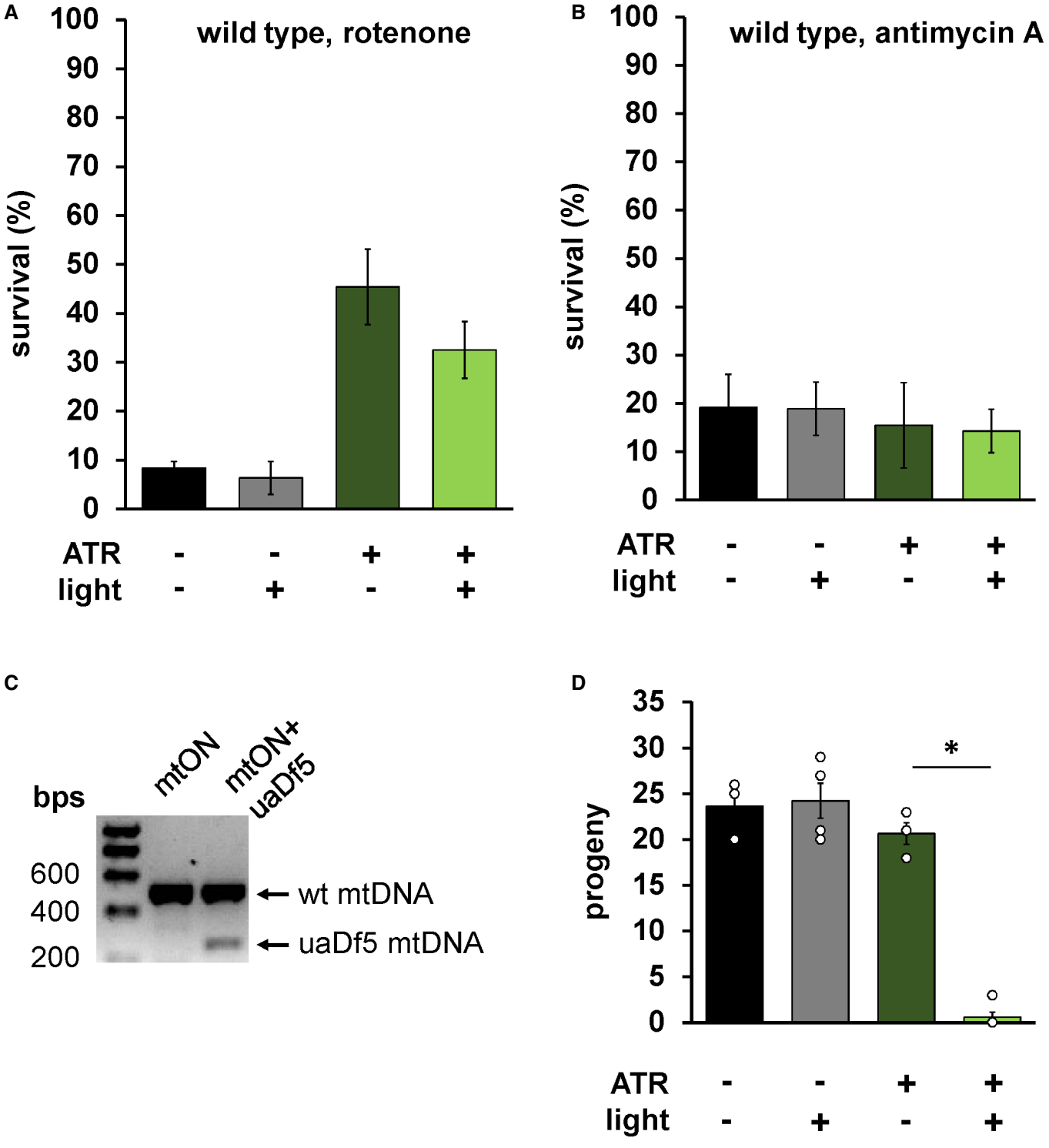

Figure EV5.

Figure EV5. ATR and light have no effect on wild-type survival, and mtON abolishes viability of animals harboring the uaDf5 mitochondrial genome deletion.

A Same experimental conditions from Fig 3A, here testing wild-type animals. No difference in survival within ATR conditions, suggesting the interaction between ATR and light has no effect on its own. One-way ANOVA with Tukey's multiple comparison test. Again, ATR alone was protective (−ATR, −light versus +ATR, −light $P = 0.0111$. $n = 3, 3, 7, 6$ plates each bar from left to right). Data show mean ± SEM.

B Same experimental conditions from Fig 3B, here testing wild-type animals, showing no difference in survival. One-way ANOVA with Tukey's multiple comparison test. $n = 3$ plates each condition. Data show mean ± SEM.

C PCR amplification of mitochondrial DNA (mtDNA) confirming uaDf5 mtDNA at ~299 base pairs (bps) in mtON-expressing animals. Wild-type mtDNA is amplified at ~500 bp. The presence of both bands indicated animals harbor the uaDf5 mtDNA deletion (see Materials and Methods).

D Number of progeny that reached L4 stage after 4 days of illumination of one parent animal. One-way ANOVA with Tukey's post hoc test, $^*P < 0.0001$ (−ATR, −light versus +ATR, +light $P < 0.0001$, −ATR, +light versus +ATR, +light $P < 0.0001$. $n = 3, 4, 3, 5$ plates each bar from left to right). Some individual points overlap. Data show mean ± SEM.