Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure Legends

Supplemental Figure 1. The electron transport chain complexes activities in *mt:CoI*^{T300I} flies. (A) Cytochrome c oxidase activity in 2-day-old wt and *mt:CoI*^{T300I} flies cultured at 25°C. COX activity was measured and normalized to the average of wt values (means ± s.d., n=3). (B) Blue Native PAGE analysis of mitochondrial extracts from wt and *mt:CoI*^{T300I} flies cultured at 25°C or 29°C. The Complexes I, III, IV and V are indicated. (C) Activities of mitochondrial NADH dehydrogenase (Complex I), NADH-cytochrome c oxidoreductase (Complexes I/III) and succinate-cytochrome c oxidoreductase (Complexes II/III) in wt and *mt:CoI*^{T300I} flies (means ± s.d., n=3).

Supplemental Figure 2. AOX improves the respiration of *mt:CoI*^{T300I} flies. (A) AOX can transfer electrons (e^-) directly from ubiquinone (Q) to molecular oxygen (O_2), bypassing the cytochrome oxidase Complexes III and IV. H^+: protons. C: cytochrome c. Complex I-V and the number of nuclear- and mtDNA-encoded protein subunits of each complex are shown. For instance, Complex I contains 39 nuclear-encoded and 7 mtDNA-encoded protein subunits. AOX is encoded in the nucleus. Complex IV, which is disrupted in *mt:CoI*^{T300I} flies, is highlighted in red. (B) Representative graph showing CO_2 production by each male fly in an open flow system. Note that the respiration of *mt:CoI*^{T300I} flies is decreased compared to wt control, and AOX expression under control of *ac-Gal4* (*ac>AOX* (*mt:CoI*^{T300I})) partially rescues respiration. (C) Rates of CO_2 production (amounts of CO_2 produced per unit time) by each fly with indicated genotypes (means ± s.d., n=3) as described in (B). (D) Mitochondrial membrane potential as shown by TMRM fluorescence (means ± s.d.) in primary motor neurons labeled by MitoGFP

under control of *D42-Gal4* in wt or *mt:CoI*^{T300I} background. Note that co-expression of AOX and MitoGFP under control of *D42-Gal4* (*AOX+mitoGFP*) does not restore membrane potential in *mt:CoI*^{T300I} mitochondria. *mitoGFP* (wt), n=13; *mitoGFP* (*mt:CoI*^{T300I}), n=13; *AOX+mitoGFP*, n=10; *AOX+mitoGFP* (*mt:CoI*^{T300I}), n=10. (E) Average amplitude of responses of Mitycam, a mitochondrial Ca^{2+} reporter, in wt or *mt:CoI*^{T300I} motor neurons upon acetylcholine stimulation (means ± s.d., n=5). Note that co-expression of AOX and Mitycam under control of *D42-Gal4* (*AOX+mitycam*) does not restore Ca^{2+} uptake in *mt:CoI*^{T300I} mitochondria. **p*<0.05, **p<0.005, n.s., not significant in (C) and (D). Nuclear genotypes are: *ac-Gal4/UAS-AOX* (*ac>AOX*), *D42-Gal4/UAS-mitoGFP* (*mitoGFP*), *UAS-AOX/+; D42-Gal4/UAS-mitoGFP* (*AOX+mitoGFP*), *D42-Gal4/UAS-mitycam* (*mitycam*), *UAS-AOX/+; D42-Gal4/UAS-mitycam* (*AOX+mitycam*).

Supplemental Figure 3. Lifespan, but not ROS level is affected in *mt:CoI*^{T300I} flies. (A) AOX can extend the lifespan of the mutant flies, while SOD2/catalase does not. Males carrying *UAS-AOX (ac>AOX)*, *UAS-mitoGFP (ac>mitoGFP)*, or *UAS-SOD2/catalase* (*ac>SOD2+cat*) transgenes were crossed with female *actin-Gal4/CyO* flies carrying homoplasmic *mt:CoI*^{T300I} mtDNA, and *non-Cy* progeny were used for the lifespan assay performed at 25°C. w^{1118} (*mt:CoI*^{T300I}), *ac-Gal4* (*mt:CoI*^{T300I}) and *ac>mitoGFP* (*mt:CoI*^{T300I}) flies were used as control. AOX expression (green line) extended the median lifespan of *mt:CoI*^{T300I} flies from 14.1 days to 16.8 days. However, expression of SOD2 and catalase (black line) had no effect on the lifespan of *mt:CoI*^{T300I} flies (median lifespan was 10.9 days). (B) Reactive oxygen species levels are comparable in mutant

and wt flies. Data were normalized to the mean value of young wt flies (means ± s.d., n=3). (C) Immunoblots showing the protein carbonylation of mitochondrial proteins (mito.) or of whole-cell extracts (whole cell) isolated from wild type (W) or *mt:CoI*^{T300I} (T). ATP synthase α-subunit and β-tubulin were used as loading controls. Y: young, 2-day-old flies. O: old, 2-week-old flies. Nuclear genotypes are: *ac-Gal4/UAS-SOD2*, *UAS-catalase* (*ac>SOD2+cat*), *ac-Gal4/UAS-mitoGFP* (*ac>mitoGFP*), *ac-Gal4/UAS-AOX* (*ac>AOX*).

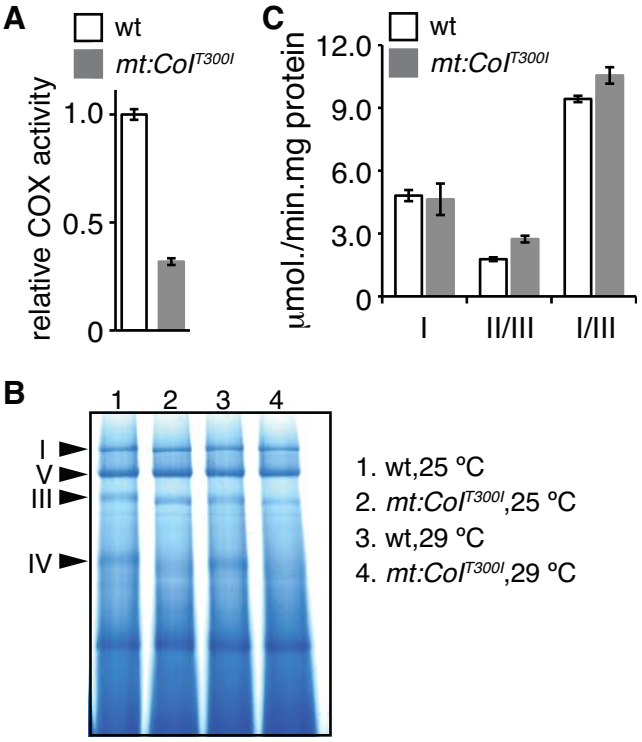
Supplemental Figure 4. Generating tissue-specific *mt:CoI*^{T300I} homoplasmic mosaics in heteroplasmic flies. (A) The principle of shifting from *mt:CoI*^{T300I} heteroplasmy to homoplasmy by expressing MitoXhoI, which eliminates wt genome. Heteroplasmic *mt:CoI*^{T300I} flies contain both wt and mutant mtDNA. wt mtDNA has an XhoI site, whereas *mt:CoI*^{T300I} mtDNA does not. Expression of MitoXhoI in heteroplasmic background destroys wt mtDNA and results in 100% of *mt:CoI*^{T300I} genome. Expression of MitoXhoI driven by different tissue specific Gal4 drivers can lead to tissue-specific homoplasmy. (B) Expression of MitoXhoI generates homoplasmy in heteroplasmic *mt:CoI*^{T300I} flies. A 4.0 kb mtDNA fragment (PCR) flanking the XhoI site was amplified from flies with indicated genotypes (1-4), and digested by XhoI enzyme (PCR/XhoI). wt mtDNA carrying the XhoI recognition site could be digested into two fragments (1.6 kb and 2.4 kb), while mutant mtDNA was resistant to XhoI digestion. Note that ubiquitous expression of MitoXhoI under control of *tub-Gal4* (*tub>mitoXhoI*) completely eliminates wt mtDNA and results in 100% of *mt:CoI*^{T300I} in heteroplasmic flies, while *UAS-mitoXhoI* transgene alone has no effect. (C) mtDNA amounts in heteroplasmic flies. Total

DNA was prepared from different lines with indicated genotypes described in (B). mtDNA and nuclear DNA copy numbers were measured by real-time qPCR using primers specific for *mt:CoI* and *histone4* (*his4)*, respectively. Ratio of mtDNA to nuclear DNA in various mutants (2-4) was normalized to mean ratio in wild type (1) and averages were plotted (means ± s.d., n=3). Abbreviation, *tub>mitoXhoI* stands for *UAS-mitoXhoI/+; tub-Gal4/+*.

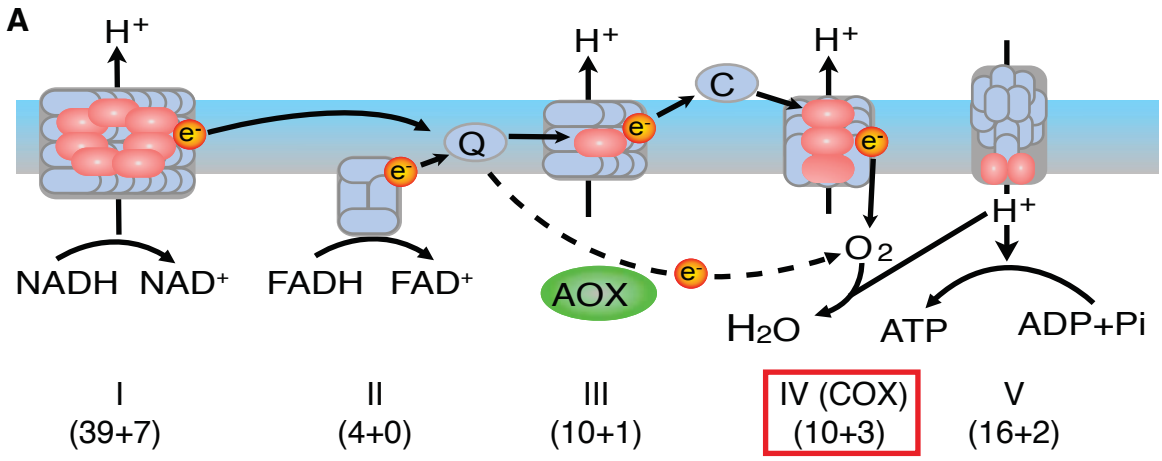
Supplemental Movie 1. Mitochondrial Ca^{2+} dynamics in wt motor neuron expressing Mitycam (*D42-Gal4/UAS-mitycam*) stimulated with acetylcholine. The movie was recorded over a 1-minute span at speed of 2 frames/second, and played back at 10 frames/second. The time point of application of acetylcholine is indicated.

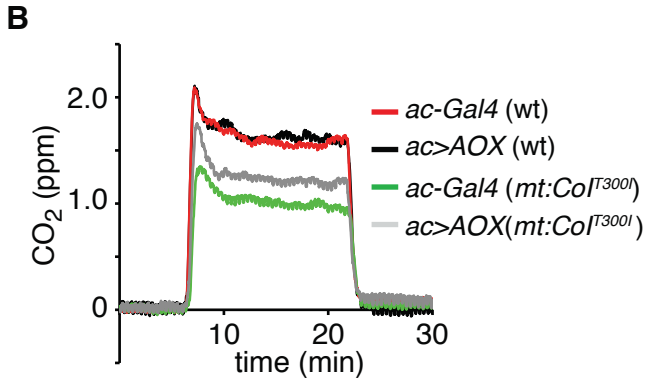
Supplemental Movie 2. Mitochondrial Ca^{2+} dynamics in *mt:CoI*^{T300I} motor neuron expressing Mitycam (*D42-Gal4/UAS-mitycam*) stimulated with acetylcholine. The movie was recorded over a 1-minute span at speed of 2 frames/second, and played back at 10 frames/second. The time point of application of acetylcholine is indicated.

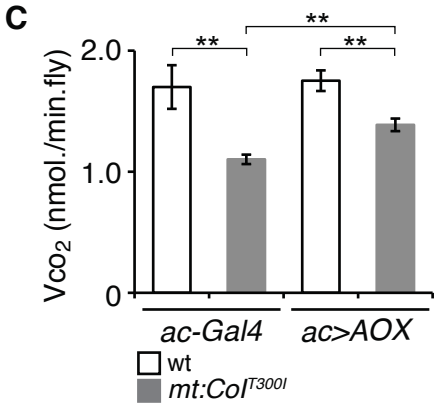
Supplemental Figure 1

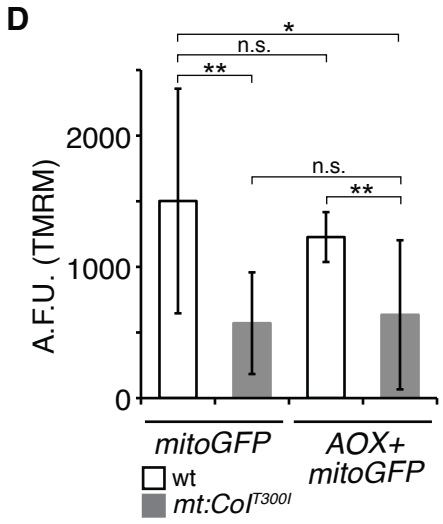


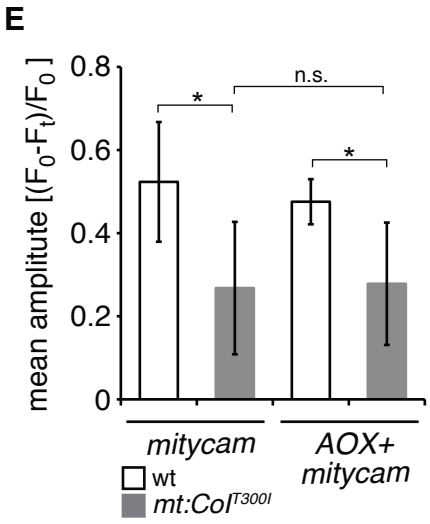
Supplemental Figure 2



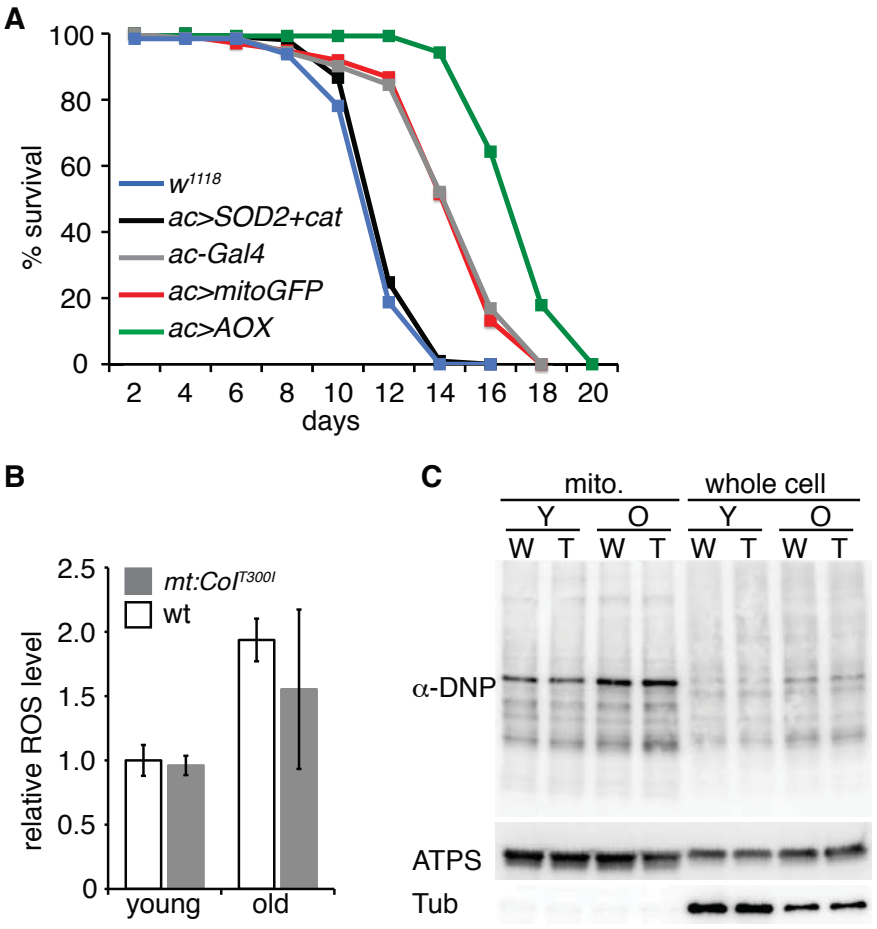








Supplemental Figure 3



Supplemental Figure 4