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Supplemental Information

Opa1 Overexpression Ameliorates the Phenotype

of Two Mitochondrial Disease Mouse Models

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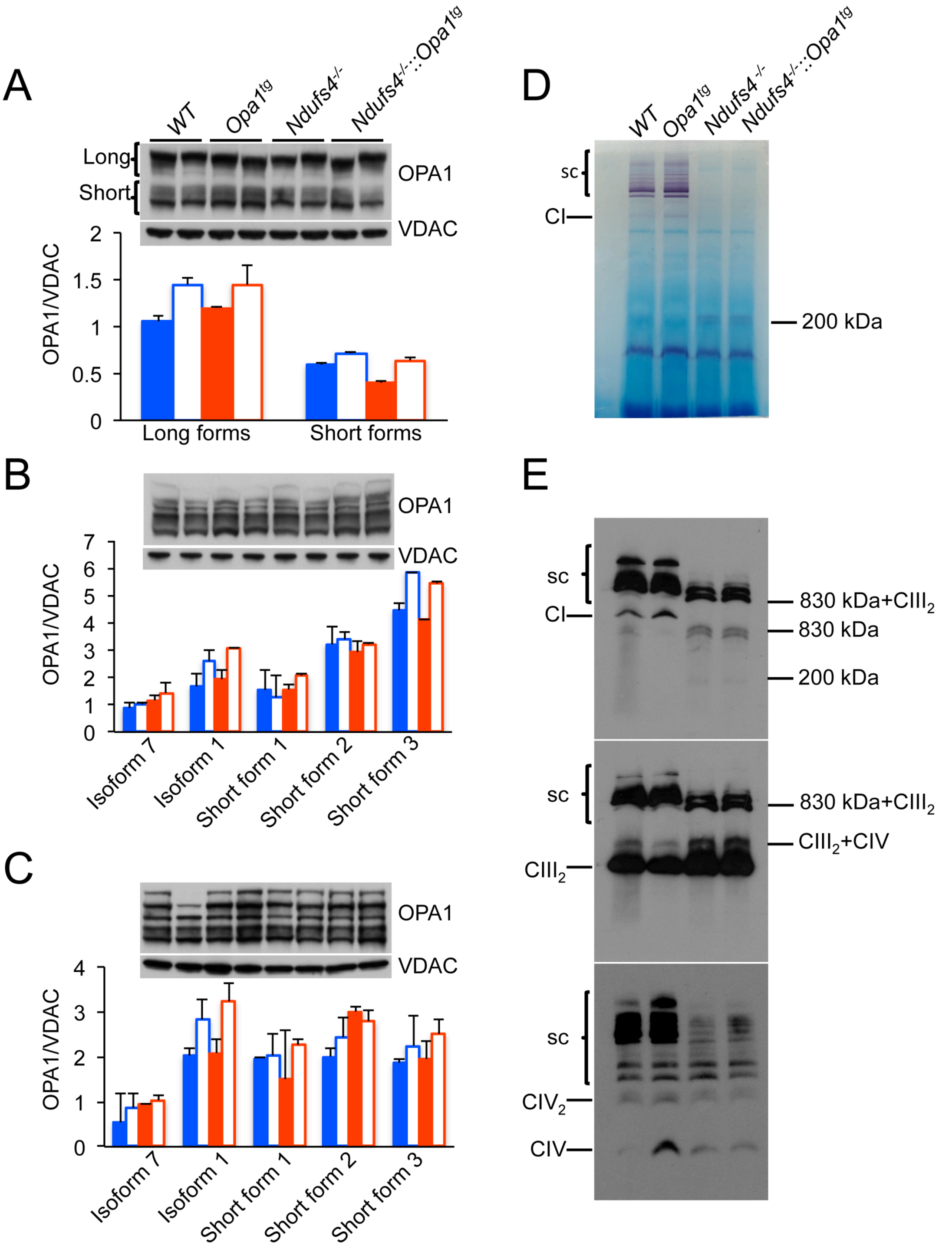
Supplemental Material Online

Figure S1, related to figure 1.

Analysis of the *Ndufs4*$^{-/-}$ and *Ndufs4*$^{-/-}$*::Opa*^{Tg} mouse models.



A) Western blot immunovisualization (upper panel) and densitometric analysis (lower panel) of OPA1 long (L) and Short (S) isoforms in brain mitochondria. Solid blue: *WT*; blue outline: *Opa1*^{tg}; solid red: *Cox15*$^{sm/sm}$; red outline: *Cox15*$^{sm/sm}$*::Opa1*^{Tg}. Error bars represent SEM. VDAC was used as a loading control.

B) Western blot immunovisualization (upper panel) and densitometric analysis (lower panel) of OPA1 forms in skeletal muscle mitochondria of *WT, Opa*^{Tg}*, Ndufs4*$^{-/-}$*, Ndufs4*$^{-/-}$*::Opa*^{Tg} mice. Solid blue: *WT*; blue outline: *Opa1*^{tg}; solid red: *Cox15*$^{sm/sm}$; red outline: *Cox15*$^{sm/sm}$*::Opa1*^{Tg}. Error bars represent SEM. VDAC was used as a loading control.

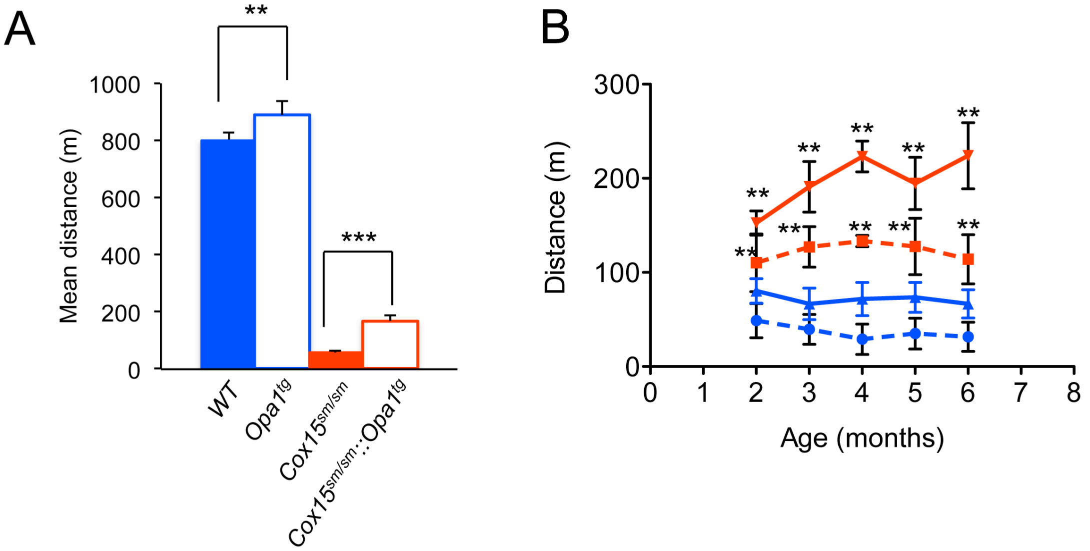
C) Western blot immunovisualization (upper panel) and densitometric analysis (lower panel) of OPA1 forms in heart mitochondria of *WT, Opa*^{Tg}*, Ndufs4*$^{-/-}$*, Ndufs4*$^{-/-}$*::Opa*^{Tg} mice. Solid blue: *WT*; blue outline: *Opa1*^{tg}; solid red: *Cox15*$^{sm/sm}$; red outline: *Cox15*$^{sm/sm}$*::Opa1*^{Tg}. Error bars represent SEM. VDAC was used as a loading control.

D) BNGE-in-gel activities of MRC CI from BNGE of digitonin-treated isolated mitochondria of *WT, Opa*^{Tg}*, Ndufs4*$^{-/-}$*, Ndufs4*$^{-/-}$*::Opa*^{Tg} samples. See main text for details.

E) Western-blot immunovisualization of MRC CI, CIII, and CIV from BNGE of digitonin-treated isolated mitochondria of *WT, Opa*^{Tg}*, Ndufs4*$^{-/-}$*, Ndufs4*$^{-/-}$*::Opa*^{Tg} samples. See main text for details. Anti-COX1, anti-NDUFB8 and anti-UQCRC1 antibodies were used for CIV, CI, and CIII.

Figure S2, related to figure 2.

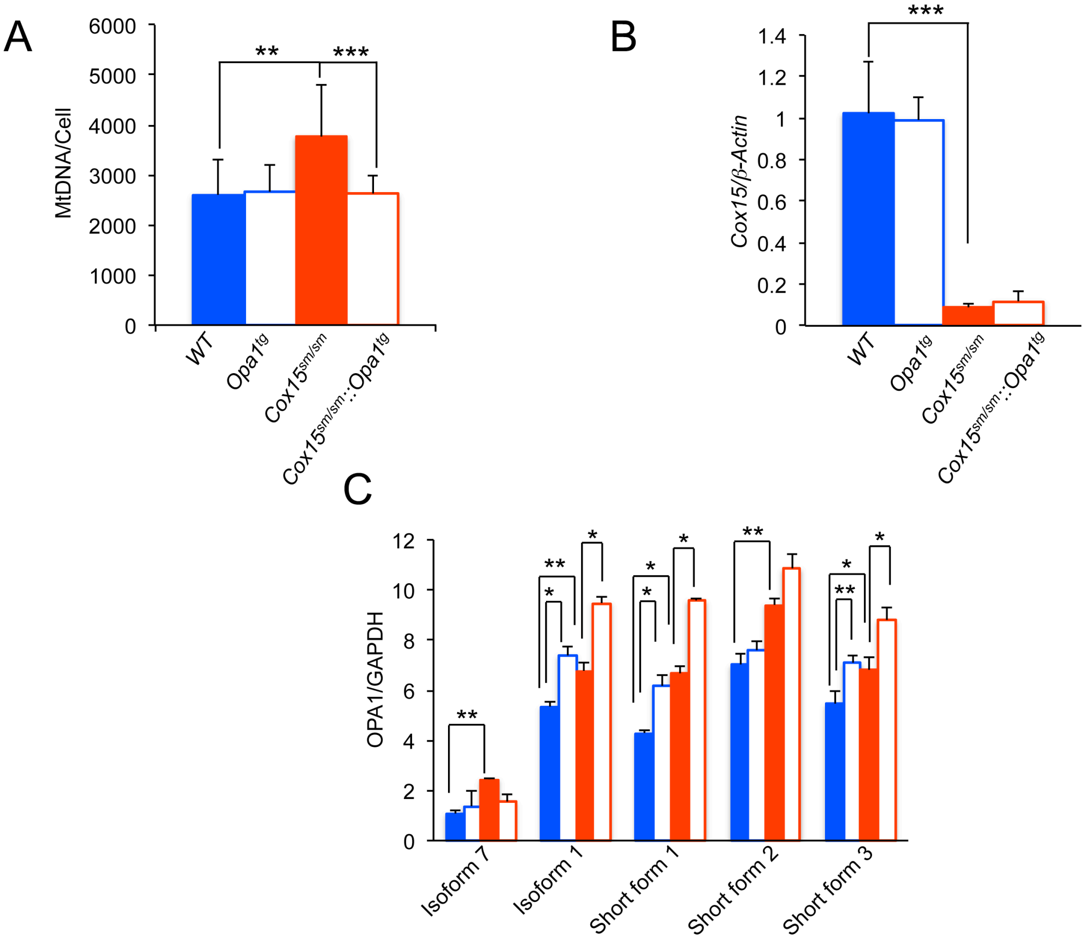
***In vivo* phenotypic characterization of *Cox15$^{sm/sm}$* and *Cox15$^{sm/sm}$::Opa1^{Tg}* mouse models**



A) Means of weekly-performed treadmill tests over 5 weeks. Solid blue: *WT* (n=8); blue outline: *Opa1^{tg}* (n=9); solid red: *Cox15$^{sm/sm}$* (n=9); red outline: *Cox15$^{sm/sm}$::Opa1^{Tg}* (n=10). Error bars represent SEM. Statistical significance was calculated by unpaired, 2-tail Student's t test: $^{**}p<0.01$: $^{***}p<0.005$.

B) Motor performance by treadmill tests in males (continuous lines) and females (dashed lines). Red lines: *Cox15$^{sm/sm}$::Opa1^{Tg}*; blue lines: *Cox15$^{sm/sm}$* individuals. Asterisks refer to significant differences between *Cox15$^{sm/sm}$::Opa1^{Tg}* vs *Cox15$^{sm/sm}$* calculated by unpaired, 2-tail Student's *t* test: $^{**}p<0.01$. Values between genders were not significantly different for either genotype. Error bars represent SEM.

Figure S3, related to figure 6.
Quantitative analyses in skeletal muscle



A) Quantification of Cox15 transcript. Solid blue: *WT* (n=4); blue outline: $Opa1^{tg}$ (n=4); solid red: $Cox15^{sm/sm}$ (n=4); red outline: $Cox15^{sm/sm}::Opa1^{tg}$ (n=4). Error bars represent SEM. Statistical significance was calculated by unpaired, 2-tail Student's t test: ***p<0.005.

B) Quantification of mtDNA copy number. Solid blue: *WT* (n=4); blue outline: $Opa1^{tg}$ (n=4); solid red: $Cox15^{sm/sm}$ (n=4); red outline: $Cox15^{sm/sm}::Opa1^{tg}$ (n=4). Error bars represent SEM. Statistical significance was calculated by unpaired, 2-tail Student's t test: **p<0.01; ***p<0.005.

C) Densitometric analysis of Opa1 isoforms in skeletal muscle on n=4 samples for each genotype. Error bars represent SEM. Statistical significance was calculated by unpaired, 2-tail Student's t test: *p<0.05; **p<0.01; ***p<0.005.