



1 Article

# 2 The Organization of Mitochondrial Supercomplexes 3 is Modulated by Oxidative Stress in vivo in Mouse 4 Models of Mitochondrial Encephalopathy

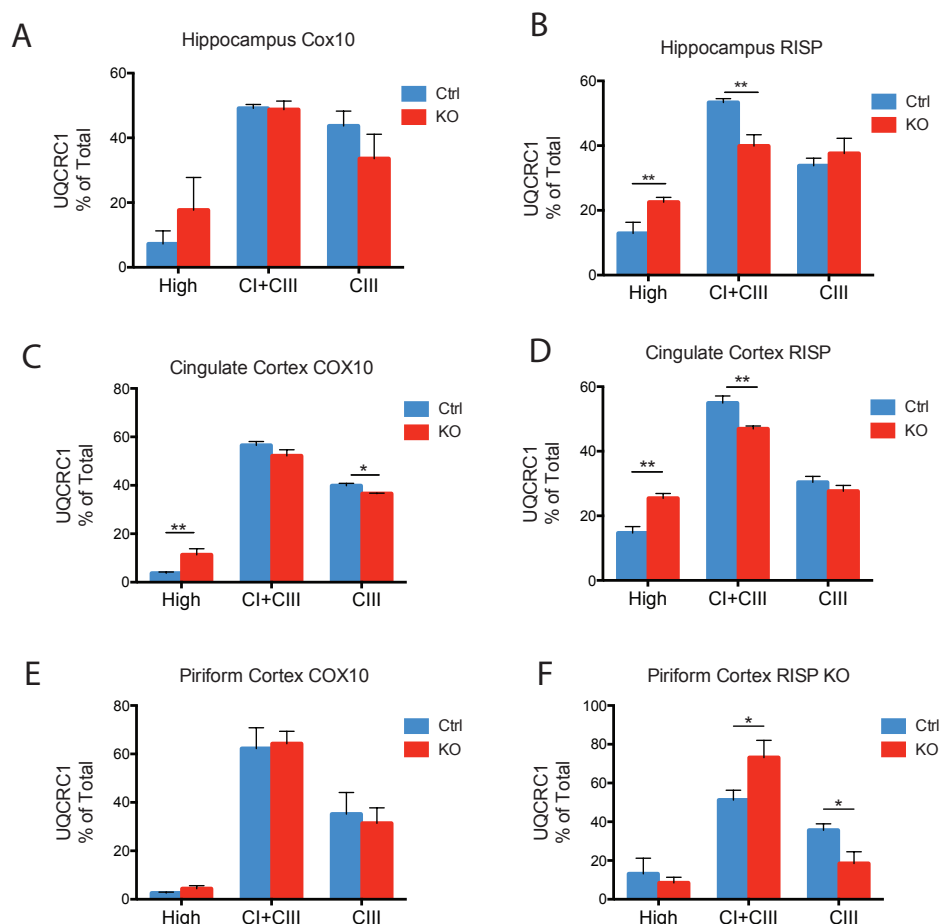
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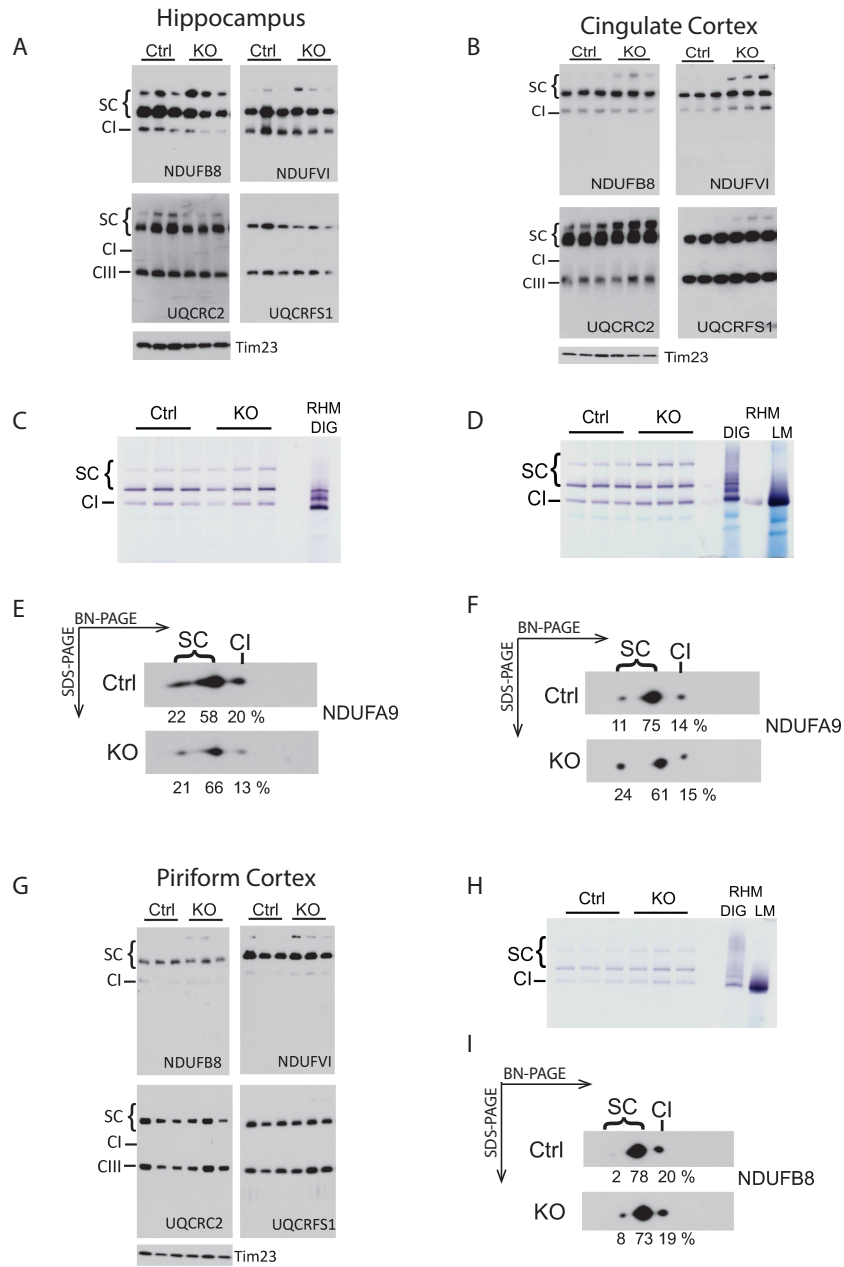
9 Supplementary Materials



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11 **Fig S1.** Quantification of UQCRC1 signal in blue native gels of mitochondria from hippocampus,  
12 cingulate cortex and piriform cortex of COX10 and RISP KO mice. Mitochondria were extracted for  
13 BN-PAGE from Ctrl and KO mice from: A) and B) Hippocampus from COX10 and RISP KO  
14 respectively; C) and D) Cingulate cortex from COX10 and RISP KO respectively; E) and F) Piriform  
15 cortex from COX10 and RISP KO. Signals obtained using the UQCRC1 antibody shown in Fig1 were  
16 quantified by densitometry using Image J and expressed as % of total signal. Bars represent mean and  
17 standard deviation. (\*) p<0.05 and (\*\*) p<0.01 indicate statistical significance, n=3.

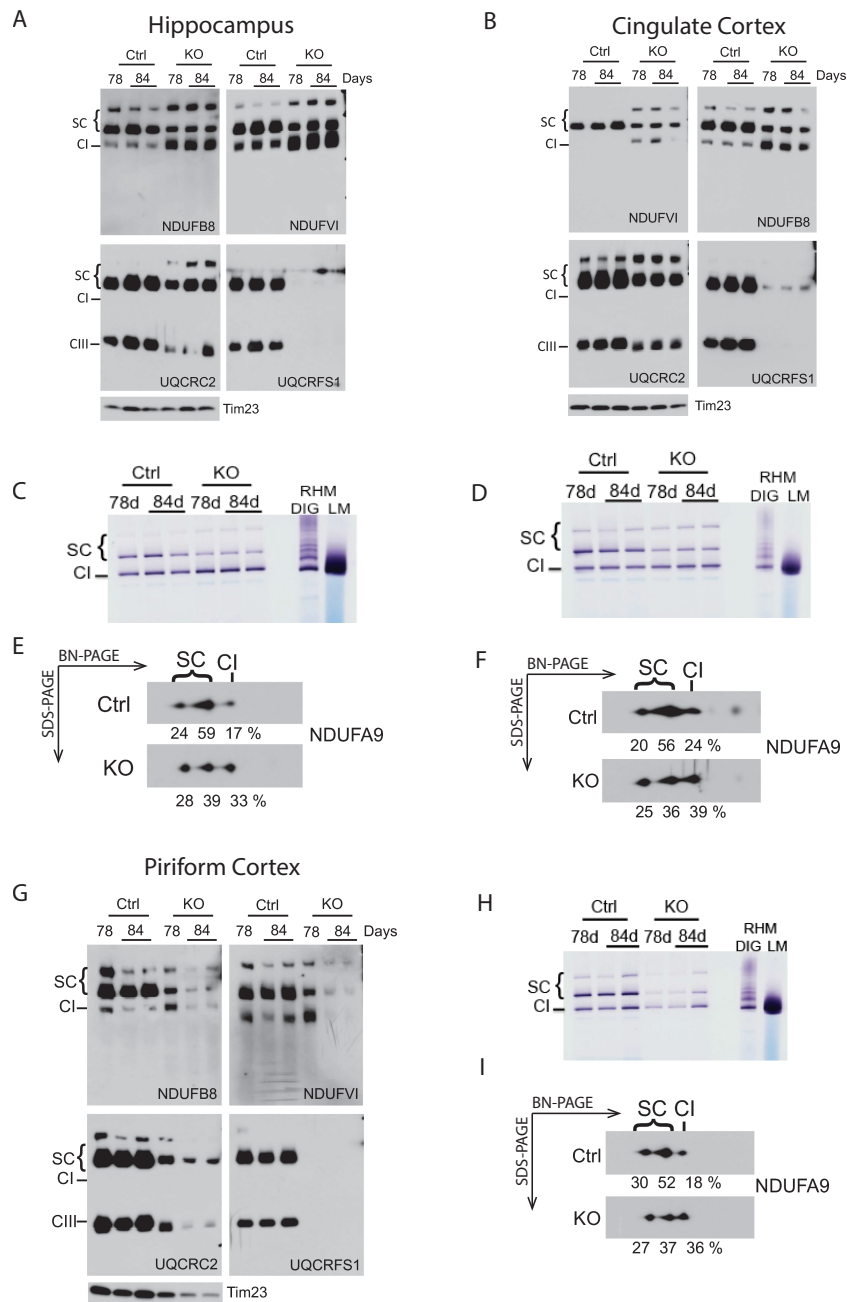
COX10 KO



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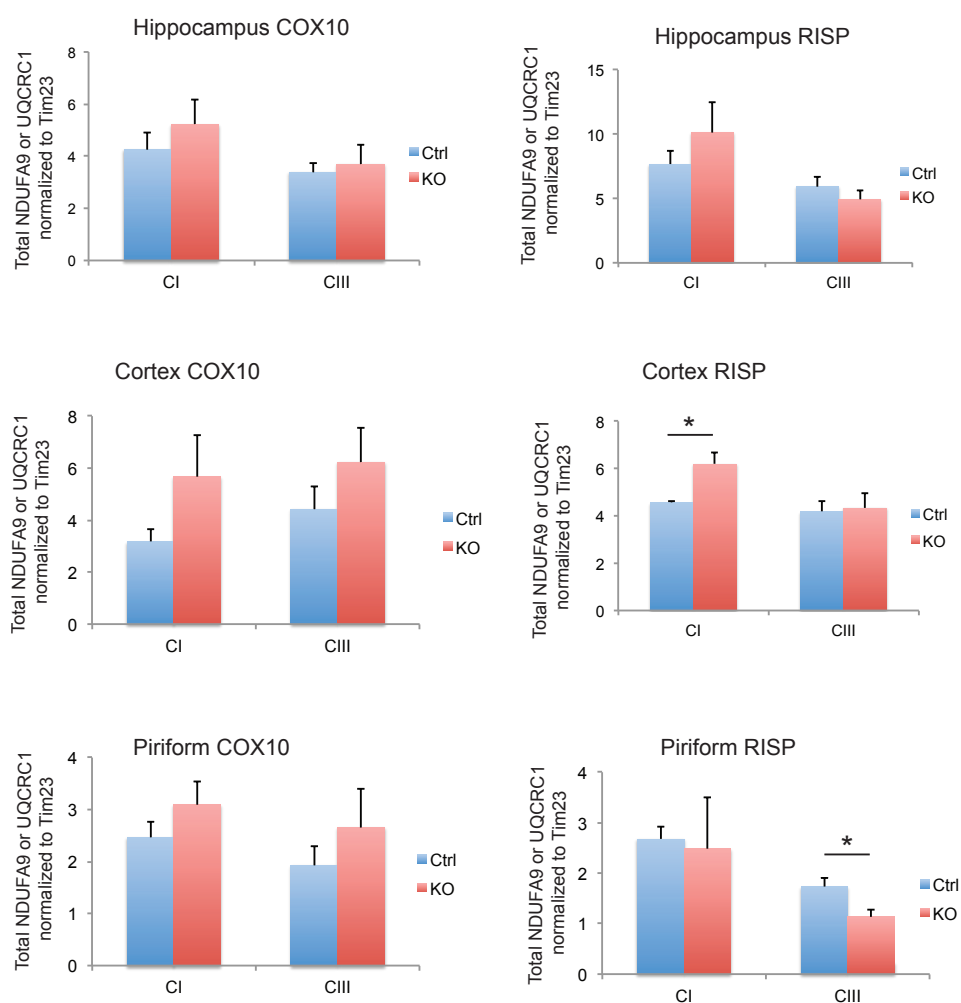
**Fig S2. Analysis of mitochondria from hippocampus, cingulate cortex and piriform cortex from COX10 KO mice.** A) B) G) BN-PAGE of mitochondria from control and COX10 KO mice from hippocampus, cingulate cortex and piriform cortex respectively using antibodies against CI (NDUFA9, NDUFB8 and NDUFV1) and CIII (UQCRC1, UQCRC2 and UQCRF51/RISP) subunits. C) D) H) Complex I *in gel* activity. E) F) I) two dimensional-BN-PAGE showing % of total signal for each spot.

RISP KO



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**Fig S3. Analysis of mitochondria from hippocampus, cingulate cortex and piriform cortex from RISP KO mice.** A) B) G) BN-PAGE of mitochondria from control and RISP KO mice from hippocampus, cingulate cortex and piriform cortex respectively using antibodies against CI (NDUFA9, NDUFB8 and NDUFV1) and CIII (UQCRC1, UQCRC2 and UQCRES1 or RISP) subunits. C) D) H) Complex I in gel activity. E) F) I) two dimension-BN-PAGE showing % of total signal for each spot.



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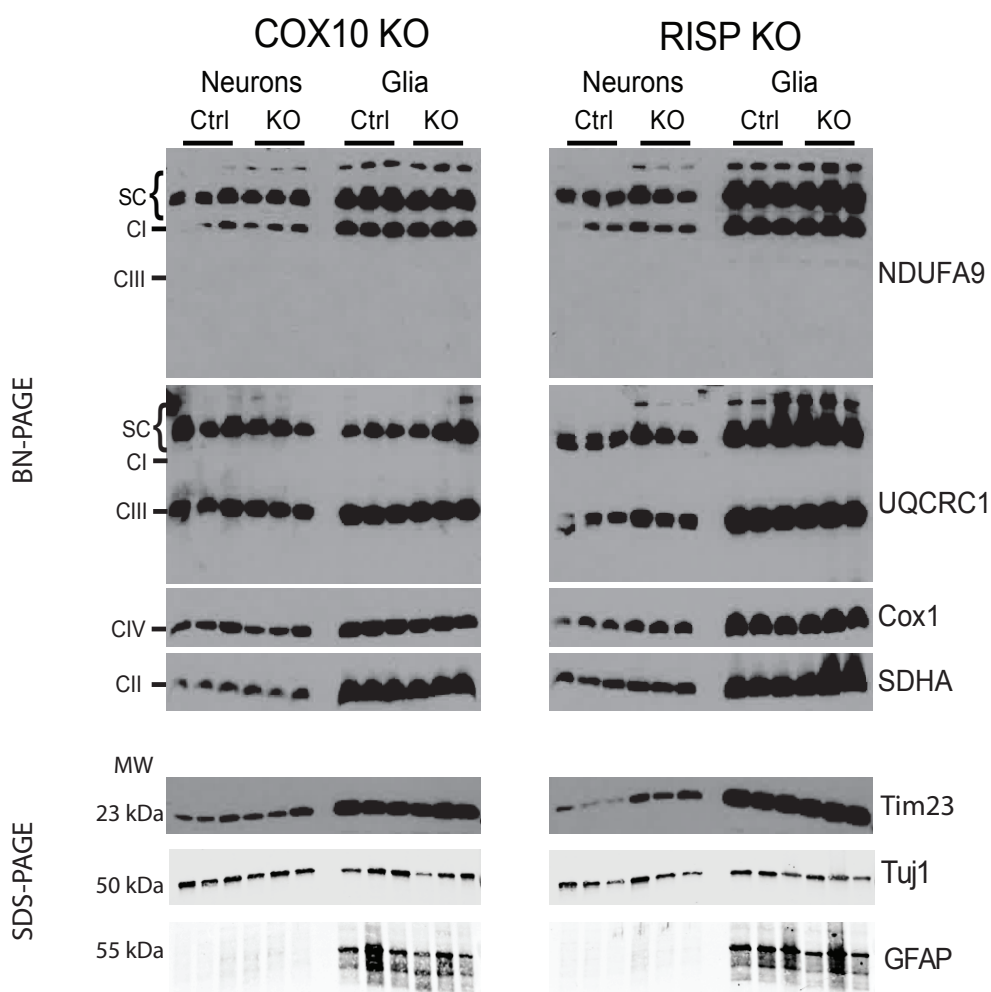
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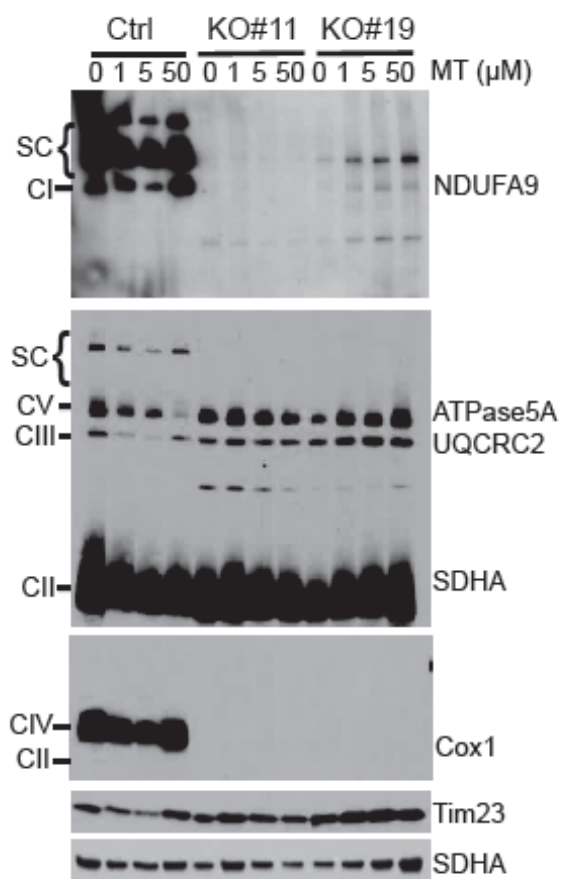
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**Fig S4. Quantification of total NADUFA9 and UQCRC1 signal in hippocampus, cortex and piriform cortex of COX10 and RISP KO.** Total signals of NDUFA9 (CI) and UQCRC1 (CIII) blots from different brain regions in Fig 1 were quantified using Image J software and normalized to Tim23 signal. (\*)  $p < 0.05$  indicates statistical significance,  $n=3$ .



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**Fig S5. Mitochondrial supercomplexes in neuron and glial fraction isolated from COX10 and RISP KO.** Neurons and glial cells were isolated from whole brains from control and KO mice using MACS technology and analyzed by BN-PAGE. Purity of cell fractions was assessed by blotting with antibodies against Tuj1 a neuronal marker and GFAP and astrocyte marker. Tim23 was used as loading control. Molecular weight (MW) of proteins are indicated.



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45 **Fig S6. Mitochondrial supercomplexes in COX10 KO fibroblasts treated with mitoTEMPO.** Control

46 and COX10 KO mouse fibroblast (#11 and #19) were incubated with different concentrations of

47 MitoTEMPO (MT) for 24hr. Cell homogenates were prepared for the SCs analyzed by BN-PAGE and

48 western blot to detect SCs, CI, CIII, CIV and CII using antibodies against NDUFA9, ATPase5a,

49 UQCRC1, Cox1 and SDHA subunits respectively. Tim23 was used as mitochondria loading control

for BN-PAGE.