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Supplemental Information

The Respiratory Chain Supercomplex Organization Is Independent of *COX7a2l* Isoforms

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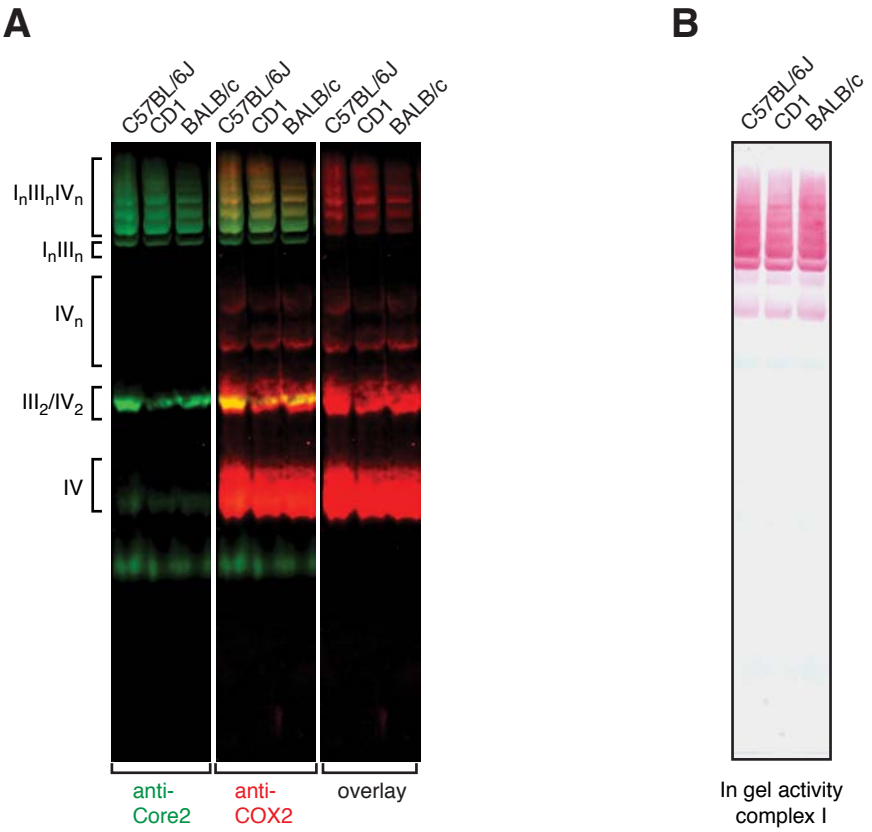
A
C57BL/6J
CD1
BALB/c
C57BL/6J
CD1
BALB/c
C57BL/6J
CD1
BALB/c
$I_nIII_nIV_n$
I_nIII_n
IV_n
III_2/IV_2
IV
anti-
Core2
anti-
COX2
overlay
B
C57BL/6J
CD1
BALB/c
In gel activity
complex I

Figure S1

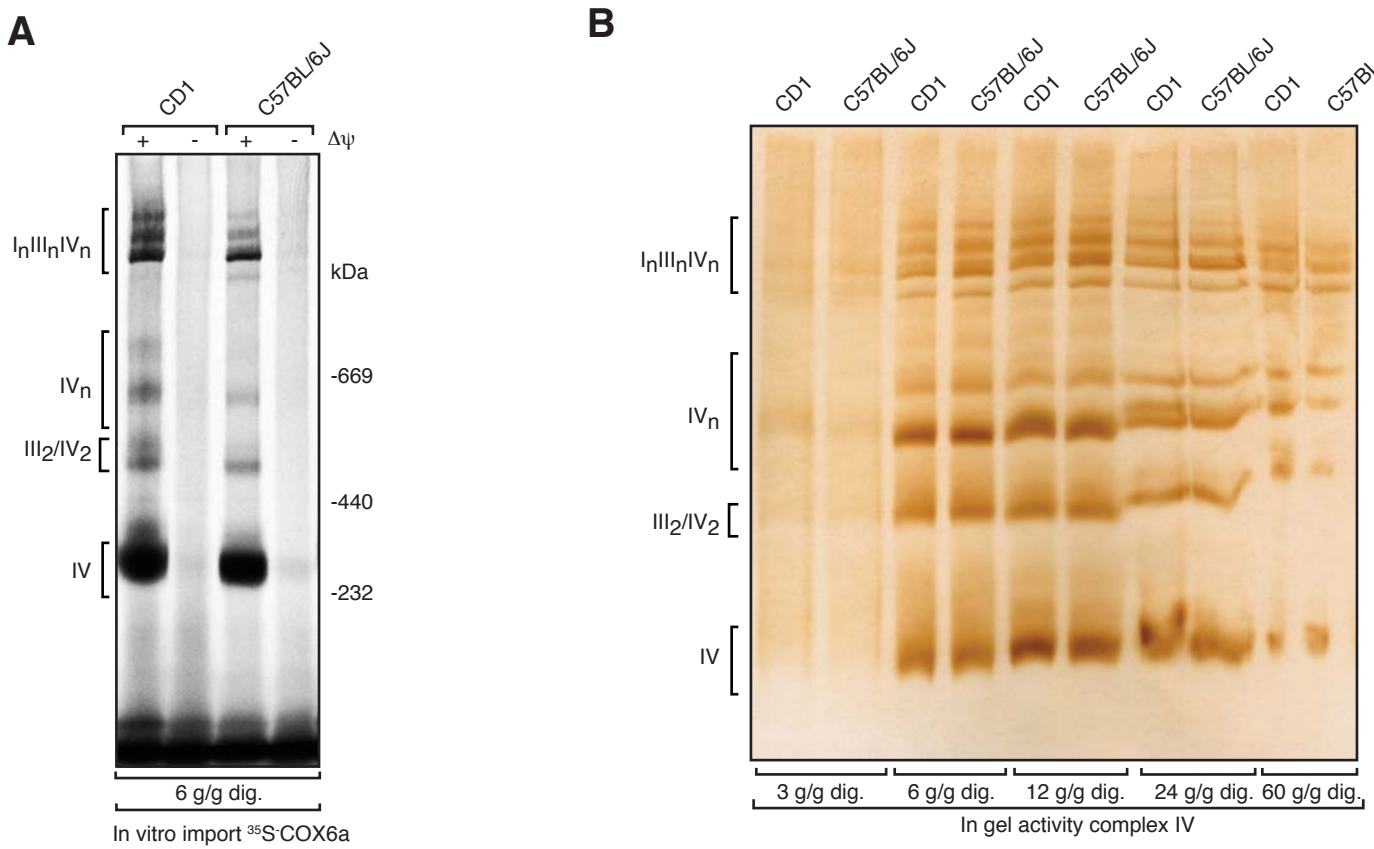
A
CD1
C57BL/6J
+
-
+
-
Δψ
$I_nIII_nIV_n$
IV_n
III_2/IV_2
IV
kDa
-669
-440
-232
6 g/g dig.
In vitro import ^{35}S-COX6a
B
CD1
C57BL/6J
$I_nIII_nIV_n$
IV_n
III_2/IV_2
IV
3 g/g dig.
6 g/g dig.
12 g/g dig.
24 g/g dig.
60 g/g dig.
In gel activity complex IV

Figure S2

SUPPLEMENTAL FIGURE LEGEND

Figure S1 (related to Figure 2) The supramolecular organization of the respiratory chain is not altered in BALB/c mouse strain

(A) Supramolecular organization of the respiratory chain in different wild type mouse strains. Western blot analyses with double fluorescent detection of complex IV (anti-COX2, red color) and complex III (anti-Core2, green color).

(B) In gel enzyme activities of complexes I in different wild type mouse strains.

Figure S2 (related to Figure 4). The short *Cox7a2l* isoform does not impair the formation of complex IV containing respiratory supercomplexes

(A) In vitro import of the radiolabelled precursor of Cox6a into isolated liver mitochondria from the CD1 and C57BL/6J mouse strains. After 60 minutes incubation in the presence or absence of mitochondrial membrane potential ($\Delta\psi$), the mitochondria were solubilized with digitonin (6g/g mitochondrial protein) and further analyzed by BN-PAGE.

(B) Analysis of complex IV in gel enzyme activity after BN-PAGE of heart mitochondria isolated from CD1 and C57BL/6J mice. The mitochondria were solubilized in buffers containing different digitonin concentrations (6-60 g/g mitochondrial protein).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mouse breeding

About 20 mice per genotype (CD1, C57BL/6) and 3 BALB/c mice were obtained directly from Charles River and Jackson laboratory and sacrificed for analyses a few weeks after maintenance in our mouse breeding facility.

Mitochondria isolation

Isolation of mitochondria was performed by differential centrifugation as previously described (1). Briefly, mice were sacrificed by cervical dislocation, and tissues were quickly collected in

ice-cold DPBS (Gibco), minced and homogenized with few strokes of a Potter S homogenizer (Sartorius) in 5 ml of ice-cold mitochondria isolation buffer (MIB; 310 mM sucrose, 20 mM Tris-HCl, 1 mM EGTA, pH 7.2). Mitochondria were purified by differential centrifugation (1200g for 10 minutes) and supernatants were subsequently centrifuged at 12000g for 10 minutes. The crude mitochondrial pellet was resuspended in an appropriate volume of MIB. The mitochondrial protein concentration was determined using the Protein DC Lawry based assay (Bio-Rad).

Oxygen consumption measurements:

Mitochondrial oxygen consumption flux was measured as previously described (2) at 37°C using 65–125 µg of crude mitochondria diluted in 2.1 ml of mitochondrial respiration buffer (120 mM sucrose, 50 mM KCl, 20 mM Tris-HCl, 4 mM KH_2PO_4, 2 mM $MgCl_2$, 1 mM EGTA, pH 7.2) in an Oxygraph-2k (OROBOROS INSTRUMENTS, Innsbruck, Austria). The oxygen consumption rate was measured by using either 10 mM pyruvate, 5 mM glutamate and 5 mM malate or 10 mM succinate and 10 nM rotenone. Oxygen consumption was assessed in the phosphorylating state with 1 mM ADP (state 3) or in the non-phosphorylating state by adding 2.5 µg/ml oligomycin (pseudo state 4). In the control mitochondria, the respiratory control ratio (RCR) values were >10 with pyruvate/glutamate/malate as substrates. Respiration was uncoupled by successive addition of carbonyl cyanide m-chlorophenyl hydrazone (CCCP) up to 3 µM to reach maximal respiration. ETC maximal activities were obtained by incubating permeabilized mitochondria with saturating concentration of cytochrome c (2.5 mg/ml) and NADH (10 mM) and/or succinate (30 mM). The complete permeabilization was obtained after several freeze and thaw cycles and controlled by the absence of additional effects after addition of CCCP or alamethicin (10 μg/ml).

Western blot analysis

Proteins were separated by SDS–PAGE or BN–PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes (Milipore). Immunodetection was performed according to the standard techniques using enhanced chemiluminescence (Immun-Star HRP

Luminol/Enhancer Bio Rad) or fluorescence. To perform the fluorescent detection of complex III and IV, PVDF membranes were blocked using the Rockland blocking buffer (MB-070). After incubation with the primary antibody, an Alexa Fluor 680 goat anti-rabbit or an IRdye800 antimouse secondary antibody was used. The detection was performed using the Li-COR Odyssey system. Monoclonal antibodies specific for NDUFA9 (complex I) were obtained from Invitrogen. F1α (complex V) monoclonal antibodies and mitoprofile total OXPHOS cocktail antibodies were obtained from Abcam. COX1, Core1 and Core2 monoclonal antibodies were obtained from Mitoscience. Rabbit polyclonal antisera were used for detection of COX2 (3).

Polymerase chain reaction:

For microdeletion analysis, we used PCR on genomic DNA isolated from heart and liver tissue of CD1, C57BL/6J, C57BL/6N mice with the following primers Cox7a2l-fw (CTTTCTTGCTTTGCAGAAGGC and Cox7a2l-rev (GAAGGCCTCGTTTCAGGTGG). The products were analyzed on 6% TBE urea gels (Invitrogen).

SUPPLEMENTAL REFERENCES

1. Freyer, C., Cree, L.M., Mourier, A., Stewart, J.B., Koolmeister, C., Milenkovic, D., Wai, T., Floros, V.I., Hagström, E., Chatzidaki, E.E., et al. (2012) Variation in germline mtDNA heteroplasmy is determined prenatally but modified during subsequent transmission. *Nat. Genet.*, **44**, 1282–1285.