Supplementary materials to

Mutations in the accessory subunit *NDUFB10* result in isolated complex I deficiency and illustrate the critical role of intermembrane space import for complex I holoenzyme assembly

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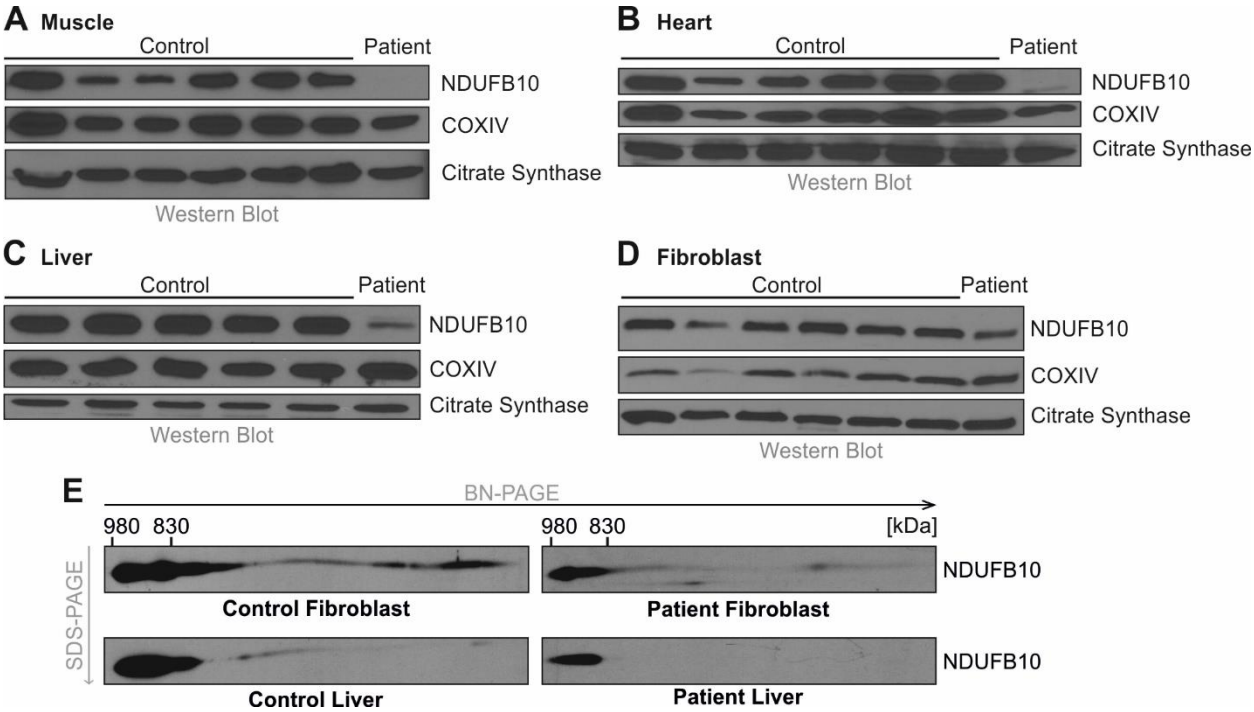
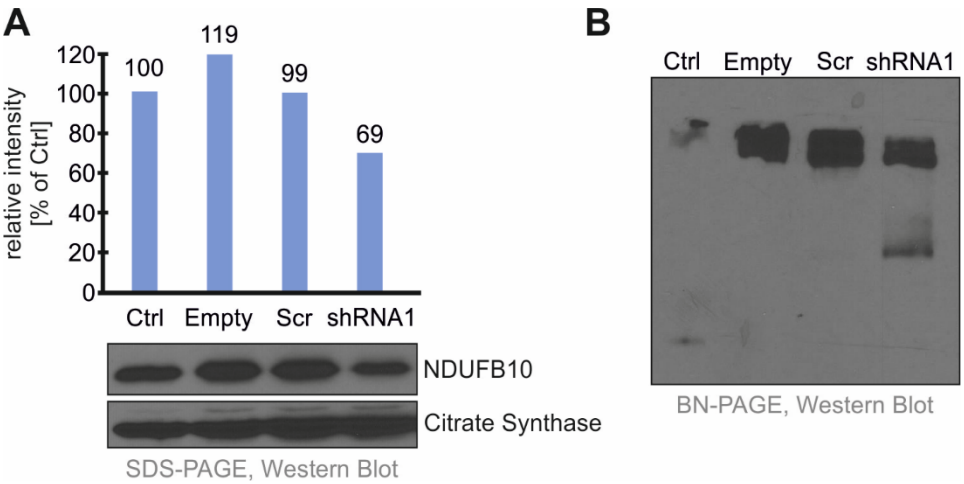
Supplemental figures



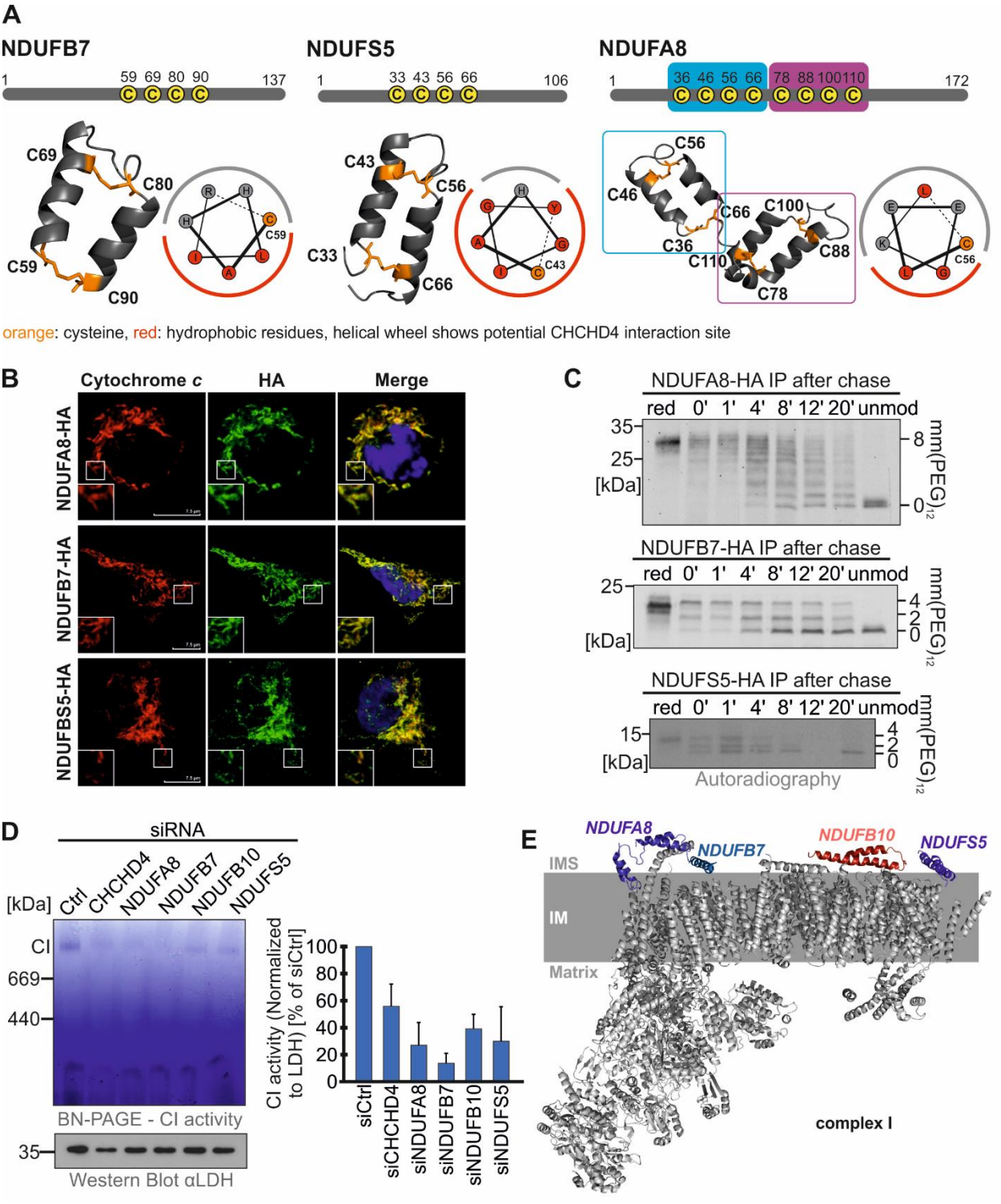
Supplemental figure 1: Western blot of NDUFB10

Western blotting in muscle, heart, liver and fibroblasts from 6 controls and the patient is shown (A-D). The mitochondrial proteins COXIV and citrate synthase are provided as loading controls. (E) Two-dimensional gel analysis with blue native PAGE in the first dimension followed by SDS-PAGE in the second dimension of liver and fibroblast of the patient. After Western blotting, the membrane was probed with antibody against NDUFB10. NDUFB10 mainly localizes to the size representing fully assembled complex I indicating introduction at a late assembly stage.



Supplemental figure 2: Knock-down of NDUFB10 by shRNA

Lentiviral transfection of HepG2 cells with shRNA against NDUFB10 resulted in partial decrease of NDUFB10 protein levels not present in empty vector or scrambled control (A). This resulted in the present of some subassembly intermediates of complex I, not present in multiple control samples.



Supplemental figure 3: Complex I contains three additional CHCHD4 substrates.

(A) NDUFA8, NDUFB7, and NDUFS5 contain potential CHCHD4 recognition motifs. The surroundings of the conserved cysteine residues in the proteins were analysed for their respective secondary structure and the presence of an amphipathic helix. (B) NDUFA8, NDUFB7, and NDUFS5 localize to

mitochondria as detected by immunofluorescence. (C) NDUFA8, NDUFB7, and NDUFS5 form disulfide bonds during their maturation. Proteins were radioactively labeled, and followed for different chase times. After the chase proteins were modified with mmPEG12 to distinguish reduced (i.e. modified) from oxidized proteins. Then proteins were isolated by immunoprecipiation. (D) Complex I activity is reduced upon depletion of CHCHD4 substrates. Indicated proteins were depleted by siRNA-mediated knockdown in HeLa cells. 72 h after transfection complex I activity was assessed by in gel activity assay on BN-PAGE. n=3 (E) Localization of CHCHD4 substrates in mature complex I suggests a role for these proteins in clamping together and protecting complex I on its IMS face. Structure modified from (46) (pdb 4UQ8)

Supplemental tables

Supplemental Table 1: Primers for Sanger sequencing of NDUFB10:

Exon 1 forward	TGT AAA ACG ACG GCC AGT ACTACAAGTCCCATGGTGCATC
Exon 2 forward	TGT AAA ACG ACG GCC AGT TCTAAACGCTGGAACACTCAGG
Exon 3 forward	TGT AAA ACG ACG GCC AGT ATTTTAGGGGTGACATGGGAGG
Exon 4 forward	TGT AAA ACG ACG GCC AGTAAATCTTCCCTCCCCTCCCTTG
Exon 1 reverse	TCA CAC AGG AAA CAG CTA TGA C CCAAAGGAGGCGACGGAG
Exon 2 reverse	TCA CAC AGG AAA CAG CTA TGA C CTAAAATCTGGGCATGCCACC
Exon 3 reverse	TCA CAC AGG AAA CAG CTA TGA C GCATCCTCTGCCTCTGTTTGG
Exon 4 reverse	TCA CAC AGG AAA CAG CTA TGA C GGGCCAATAGATCACACCAGAC

Supplemental Table 2: High-resolution respirometry in permeabilized fibroblasts

Parameter	**Patient (n=5)**	**Control (n=20)**	**Control range P5-P95**
Routine	15.79 ±3.61	19.56 ±2.86	15.96 – 24.33
Malate*	14.67 ±3.34	19.29 ±3.04	16.41 – 24.39
Pyruvate	18.08 ± 3.61	20.63 ±3.13	16.82 – 25.06
Digitonin	8.48 ±1.93	9.00 ±1.85	6.18 – 11.30
ADP**	18.01 ± 4.18	28.46 ±5.79	21.31 – 38.48
Glutamate**	20.50 ± 4.32	31.39 ±6.17	24.21 – 42.37
Succinate	51.15 ± 18.73	40.46 ±7.58	31.09 – 54.12
CCCP	56.87 ± 16.28	60.56 ±9.82	48.58 – 78.89
Rotenone	26.82 ± 12.54	18.91 ±3.71	14.73 – 27.24
TMP+ascorbate*	163.36 ±45.4	107.58 ±11.12	88.97 – 118.39
Azide*	96.47 ± 33.02	61.03 ±6.91	52.23 – 72.14
Derived function			
Acceptor control (ADP/digitonin)**	2.13 ± 0.17	3.26 ±0.80	2.18 – 4.46
[Glutamate–(ADP,pyruvate)]/Glutamate	12.4% ± 4.7%	9.4% ± 2.8%	5.5% - 12.8%
Q control (Succinate/glutamate)**	2.45 ± 0.51	1.29 ±0.07	1.19 – 1.37
% coupling (Succinate/CCCP)**	89% ± 8%	67% ± 10%	55% - 83%
Complex I (CCCP - rotenone)	30.05 ± 6.90	41.65 ±7.23	32.80-54.05
Complex IV (TMPD+ascorbate – azide)	66.89 ± 23.34	46.54 ±9.86	32.99-60.62

Legend: $1x10^6$ fibroblasts cells / 2 mL are incubated in respiration medium in a Oroboros Oxygraph 2k cell and oxygen rate as pmol O_2 /sec/10^6 cells recorded with sequential addition of substrates, uncoupler, and inhibitors according to a SUIT protocol. The patient's fibroblasts were analyzed five times and compared to 20 different control fibroblasts. Results are shown as mean and standard deviation, and 5th and 95th percentile of controls, with * $p < 0.05$, ** $p < 0.01$ by Student t-testing.