Supporting Information

Andrews et al. 10.1073/pnas.1319247110

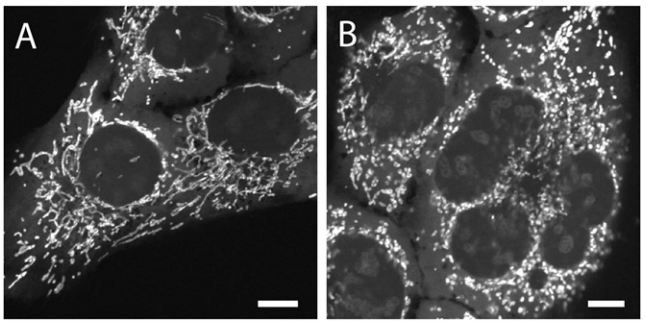

Fig. S1. Comparison of morphology of mitochondria in control cells and in cells in which expression of subunit NDUFA11 has been suppressed transiently. (*A*) Human 143B cells transfected with (*A*) control siRNA and (*B*) siRNA targeted against subunit NDUFA11. The cells were stained with MitoTracker 72 h after transfection, fixed, and visualized by confocal microscopy. (Scale bars, 10 μm.)

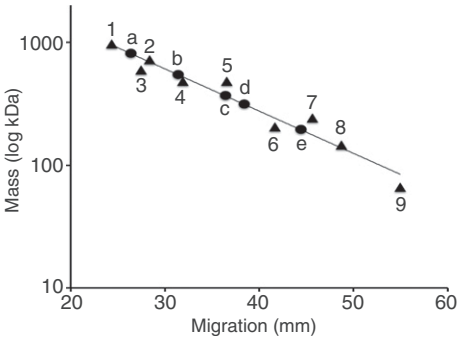

Fig. S2. Reestimation of molecular masses of subcomplexes of human complex I by blue native (BN)-PAGE. The gels were calibrated with the following proteins and protein complexes with their calculated molecular masses given in kilodaltons in parentheses: 1, bovine complex I (971); 2, apoferritin 1 (720); 3, bovine ATP synthase (597); 4, bovine complex III (482); 5, apoferritin 2 (480); 6, bovine complex IV (205); 7, B-phycoerythrin (242); 8, lactate dehydrogenase (146); and 9, BSA (66). ▲, standard proteins and complexes; ●, subcomplexes of complex I; their molecular masses as measured here are as follows, with previous estimates for a–d (1) in parentheses: a, 815 (830) kDa; b, 550 (650) kDa; c, 370 (460) kDa; d, 315 (400) kDa; and e, 200 kDa.

1. Mimaki M, Wang X, McKenzie M, Thorburn DR, Ryan MT (2012) Understanding mitochondrial complex I assembly in health and disease. *Biochim Biophys Acta* 1817(6):851–862.

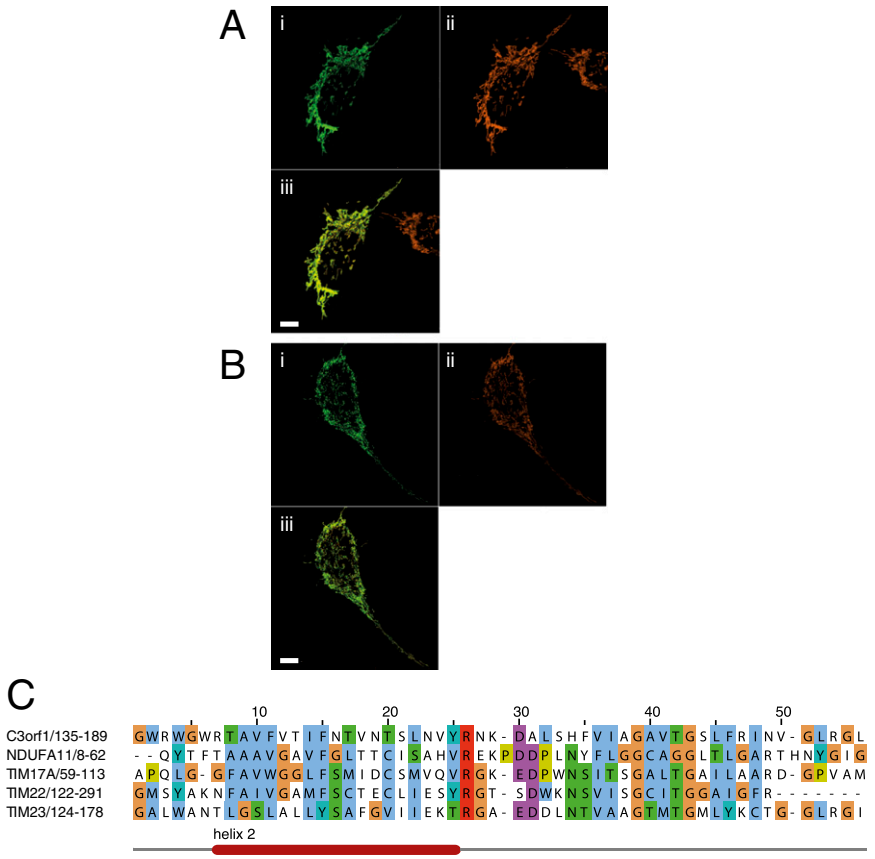

Fig. S3. Colocalization of C3orf1 and TMEM126B with mitochondria, and members of the C3orf1 protein family. The C-terminal FLAG and StrepII-tagged C3orf1 (*A*, *i–iii*) and C-terminal FLAG-tagged TMEM126B (*B*, *i–iii*) were visualized in HeLa cells. Plasmid pcDNA5/FRT/TO containing the C-terminal-tagged sequence of interest was transfected into HeLa cells, which were treated with MitoTracker 24 h later, fixed, permeabilized with Triton X-100, and immunostained. (*A*, *i* and *B*, *i*) Alexa Fluor 488 anti-mouse secondary antibody conjugated to mouse anti-FLAG antibody; (*A*, *ii* and *B*, *ii*) MitoTracker Orange staining; (*A*, *iii*) *A*, *i* and *A*, *ii* merged; (*B*, *iii*) *B*, *i* and *B*, *ii* merged. (Scale bar in each merged image, 10 μm.) (*C*) Alignment of sequence sections of C3orf1 and other protein family members NDUFA11, TIM17, TIM22, and TIM23 (PFAM: PF02466). The positions of transmembrane α-helices predicted with HMMTOP (1) are indicated by red bars beneath the sequences. A Clustal X color scheme is used for aligned residues: hydrophobic (A, C, I, L, M, F, W, and V), blue; basic (R and K), red; acidic (D and E), magenta; amide (N and Q) and nucleophilic (S and T), green; H and Y, cyan; G, orange; and P, yellow.

1. Tusnády GE, Simon I (2001) The HMMTOP transmembrane topology prediction server. *Bioinformatics* 17(9):849–850.

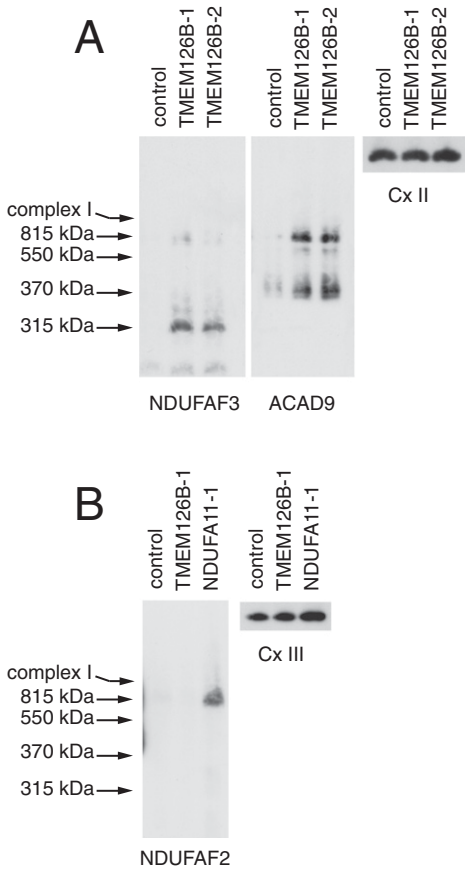

Fig. S4. Presence of NDUFAF3, ACAD9, and NDUFAF2 in subassemblies of complex I. Human 143B cells were treated for 96 h with siRNAs against TMEM126B (100 nM) and NDUFA11 (30 nM). Mitoplast proteins from those cells were separated by BN-PAGE and Western blotted with antibodies against: (*A*) NDUFAF3, ACAD9, and complex II and (*B*) NDUFAF2 and complex III.

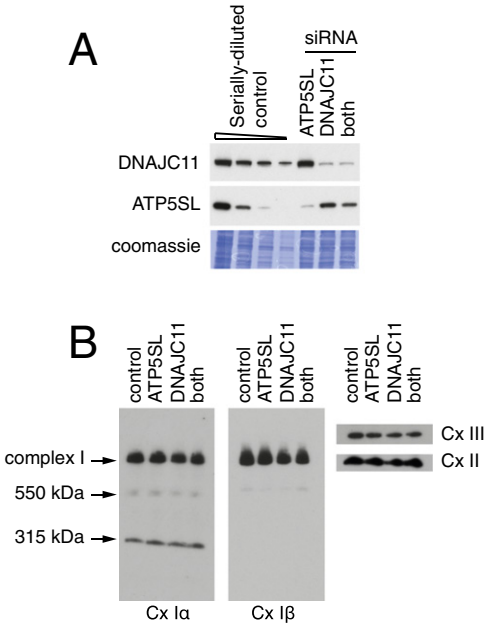

Fig. S5. Suppression of expression of ATP5SL and DNAJC11 in human 143B cells. Western blot analyses of mitoplasts from human 143B cells prepared 96 h after addition of siRNAs (50 nM) targeted against ATP5SL and DNAJC11, both singly and together. The proteins were fractionated by SDS/PAGE (*A*) and BN-PAGE (*B*). Detection with antibodies against, in *A*, DNAJC11 and ATP5SL, with the membrane stained with Coomassie blue as a loading control, and in *B*, against the NDUFS2 subunit, a component of subcomplex Iα and subunit NDUFB8, a component of subcomplex Iβ. The levels of complexes II and III are shown on the *Right*.

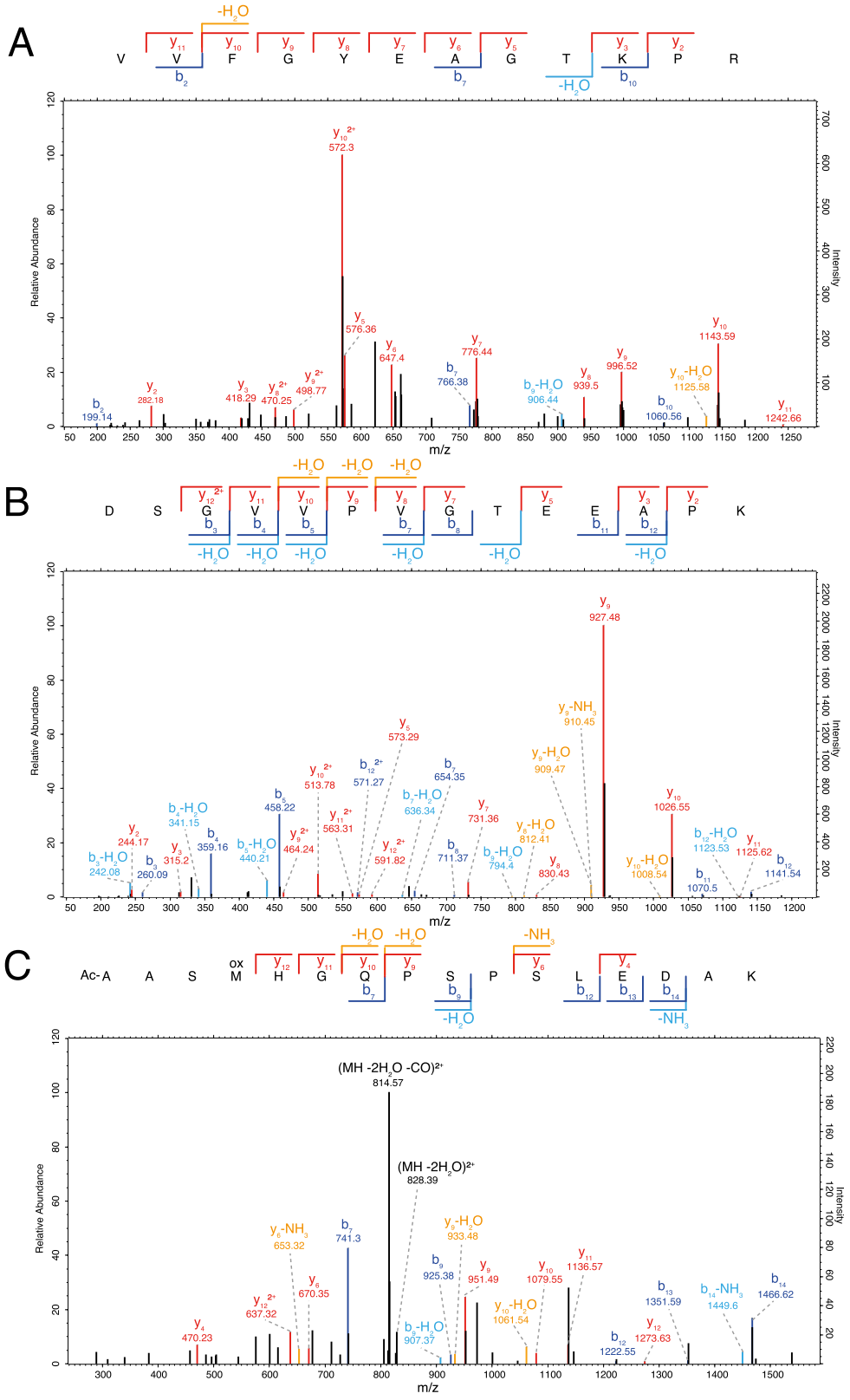

Fig. S6. Fragment ion mass spectra of three peptides from TMEM126B that are diagnostic of isoforms 1 and 5. The N-terminal region of isoform 5 is truncated by 30 residues relative to isoform 1. (*A*) N-terminal peptide (*m/z* 671.32^{2+}) of TMEM126B isoform 1, lacking the initiator methionine residue; (*B*) peptide (residues 14–27; *m/z* 692.85^{2+}) from isoform 1-specific region of TMEM126B; (*C*) N-terminal peptide (*m/z* 846.39^{2+}) lacking initiator methionine from TMEM126B isoform 5. The N-terminal alanine is acetylated.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)