Supporting Information

Hämäläinen et al. 10.1073/pnas.1311660110

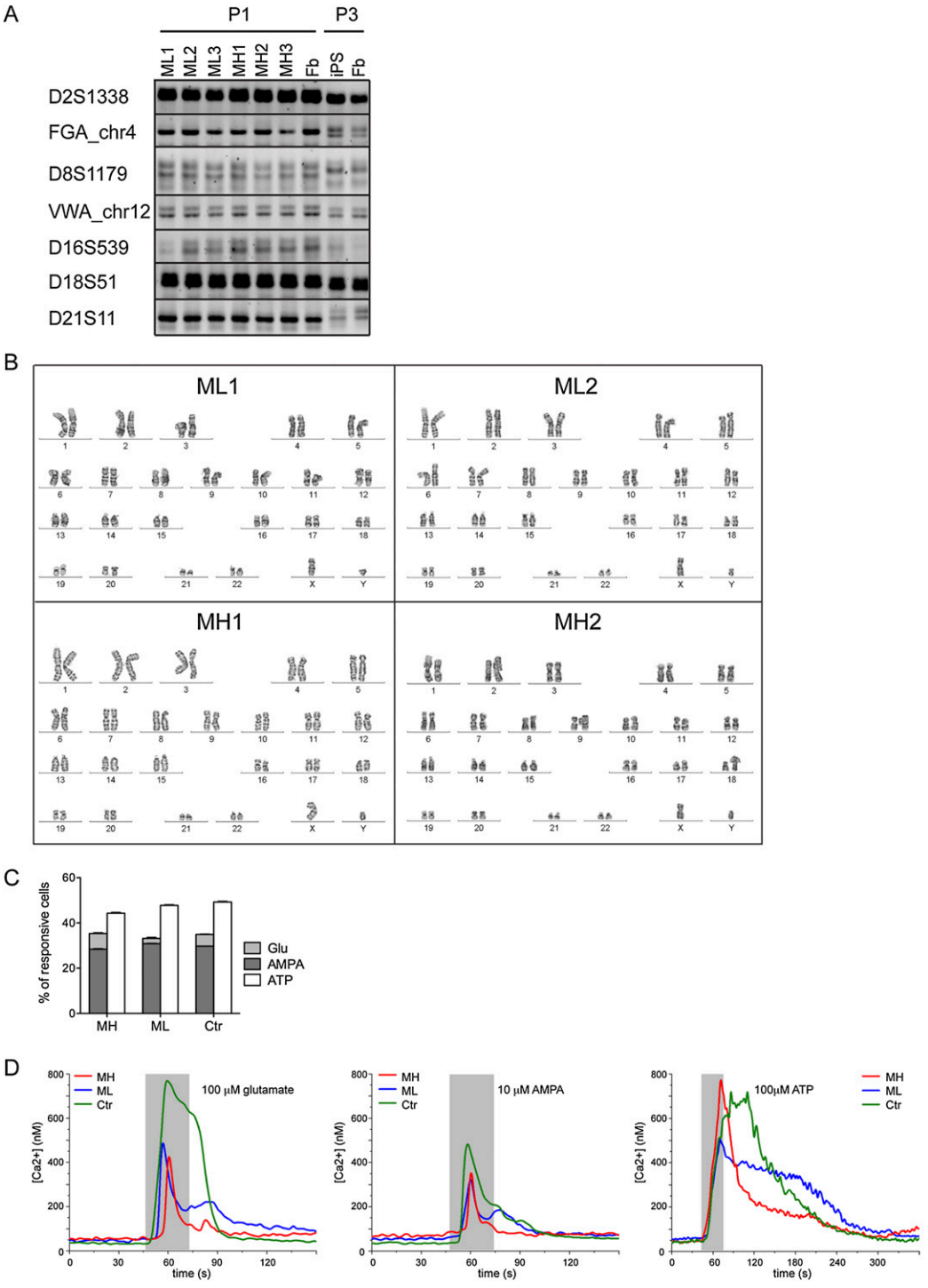

Fig. S1. Characterization of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) patient induced pluripotent stem cell (iPSC) lines. (*A*) Verification of parental identity of iPSC clones. DNA fingerprints of polymorphic DNA-repeat regions of iPSC lines chosen for further analyses. Identical results compared with the parental fibroblasts verify the origin of the studied iPSC lines. P1 and P3, MELAS patients that the original fibroblasts (Fb) were from; MH, MELAS lines with high mtDNA mutant load; ML, MELAS lines with low mtDNA mutant load. (*B*) Karyotypes of MELAS patient iPSC lines. All four lines analyzed show normal diploid karyotypes. (*C*) Calcium responses of iPSC-derived neuronal cells. Cytosolic-free Ca^{2+} concentration measurements

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from neuronal cells stimulated with glutamate or AMPA (for glutamate receptor activation in neurons) and ATP (for purinoreceptor activation in astrocytes). Cells were stimulated with high potassium (KCl) to probe their membrane potential; the percentages of KCl-sensitive cells responding to different agonists are shown. Functional neurons and astrocytes existed in all cultures. (*D*) Traces from representative single cells from the cytosolic-free Ca^{2+} concentration measurements. Ctr, control; gray bars, timing of the exposure to the agonist.

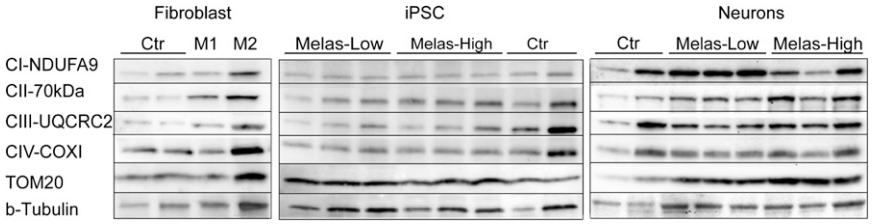

Fig. S2. Western blot analysis of mitochondrial respiratory chain complexes in different cultured cell types. CI, complex I; CII, complex II; CIII, complex III; CIV, complex IV; COX1, cytochrome *c* oxidase subunit 1; M1, M2, MELAS patients; NDUFA9, NADH dehydrogenase 39-kDa subunit; TOM20, mitochondrial outer membrane protein; UQRC2, ubiquinol cytochrome *c* oxidoreductase subunit core 2.

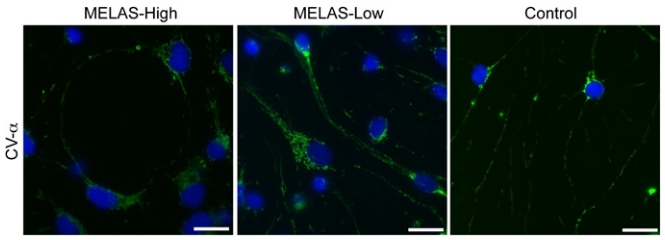

Fig. S3. Immunofluorescence analysis of neuronal respiratory chain complex V (CV). MELAS-high neurons stained normally for CV-α (ATP synthase subunit α). DAPI counterstaining in blue. (Scale bar, 50 μm.)

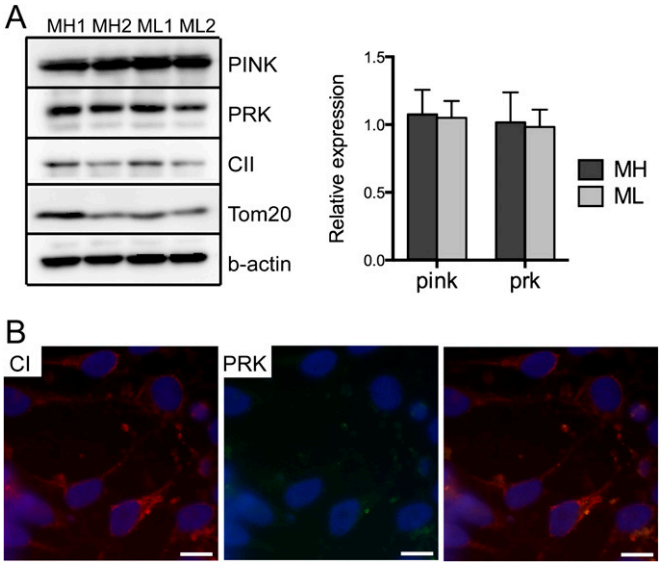

Fig. S4. PINK1 and Parkin (PRK) expression are not significantly up-regulated in MELAS neurons. (*A*) Western blot analysis of the mixed neuronal culture shows no significant difference in PINK1 or PRK expression in the MELAS neurons with high mutation load. (CII, 70-kD subunit.) Relative levels compared with MELAS-low cells are presented as mean ± SEM. (*B*) Immunofluorescence analysis of CI and PRK in control neurons with low mtDNA mutation amount shows only random colocalization of PRK with the CI-positive mitochondrial network.

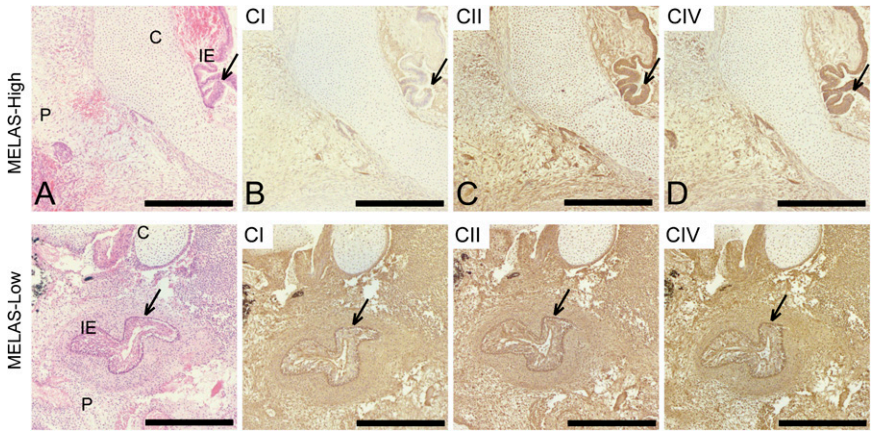

Fig. S5. Low respiratory chain CI amount in teratomas with high MELAS mutant amount. (*A*) Hematoxylin-eosin staining of serial sections of paraffin-embedded, formalin-fixed teratoma tissue with high or low MELAS mutant mtDNA load. C, cartilage; IE, intestinal-like epithelia; P, parenchyma. (*B*) CI (NDUFS3 antibody) immunohistochemical reactivity in teratomas. Arrow points to an example of a region showing decreased reactivity in teratoma with high MELAS mutant load. (*C*) CII (70-kDa subunit antibody) immunohistochemical reactivity. (*D*) CIV (subunit COX1 antibody) immunohistochemical reactivity. (Scale bar, 400 μm.)

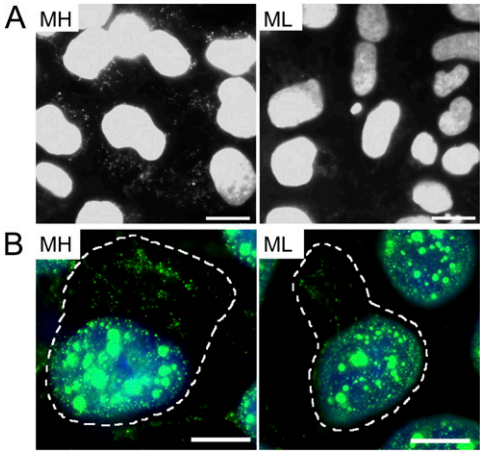

Fig. S6. Visualization of mitochondrial nucleoids. (*A*) Picogreen staining of nucleoids and (*B*) immunostaining of DNA visualizing nucleus and mitochondrial nucleoids in MELAS iPSCs with high (MH) or low (ML) mutation load. The number of nucleoids is increased in MH cells, with the most prominent increase seen in the largest nucleoids. (Scale bar, 10 μm.)