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Supplemental Data

Biallelic Mutations in *TMEM126B* Cause Severe Complex I Deficiency with a Variable Clinical Phenotype

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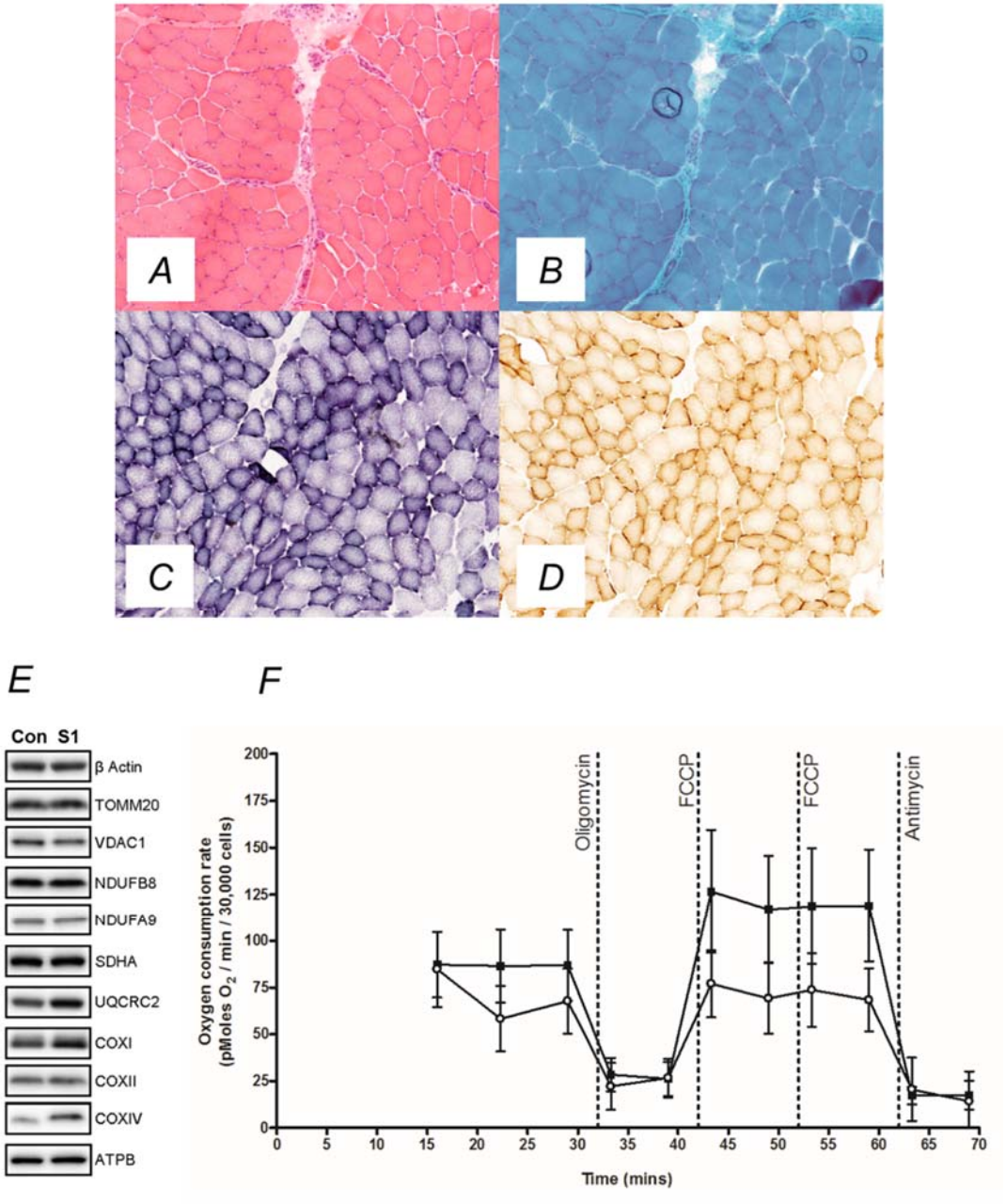

Figure S1: Functional characterization of muscle and cells from Subject 1
Histopathological analysis of a serially-sectioned skeletal muscle biopsy from Subject 1 (homozygous p.(Gly212Val) *TMEM126B* variant) showing (***A***) H&E staining, (***B***) modified Gomori Trichrome staining, (***C***) succinate dehydrogenase (SDH) and (***D***) cytochrome *c* oxidase (COX) reactions highlighting evidence of subsarcolemmal mitochondrial accumulation. Interestingly, subject fibroblasts did not show a significant OXPHOS defect, either based on immunoblotting of fibroblast mitochondrial proteins for OXPHOS components (***E***) or micro-scale oxygraphy analysis (Subject 1, n=10, white circles) compared to the combined data of control cell lines (n=5, black squares; Experimental details are described in detail previously[1]) although overall rates of oxygen consumption did appear to be generally decreased (***F***). Error bars indicate the standard deviation.

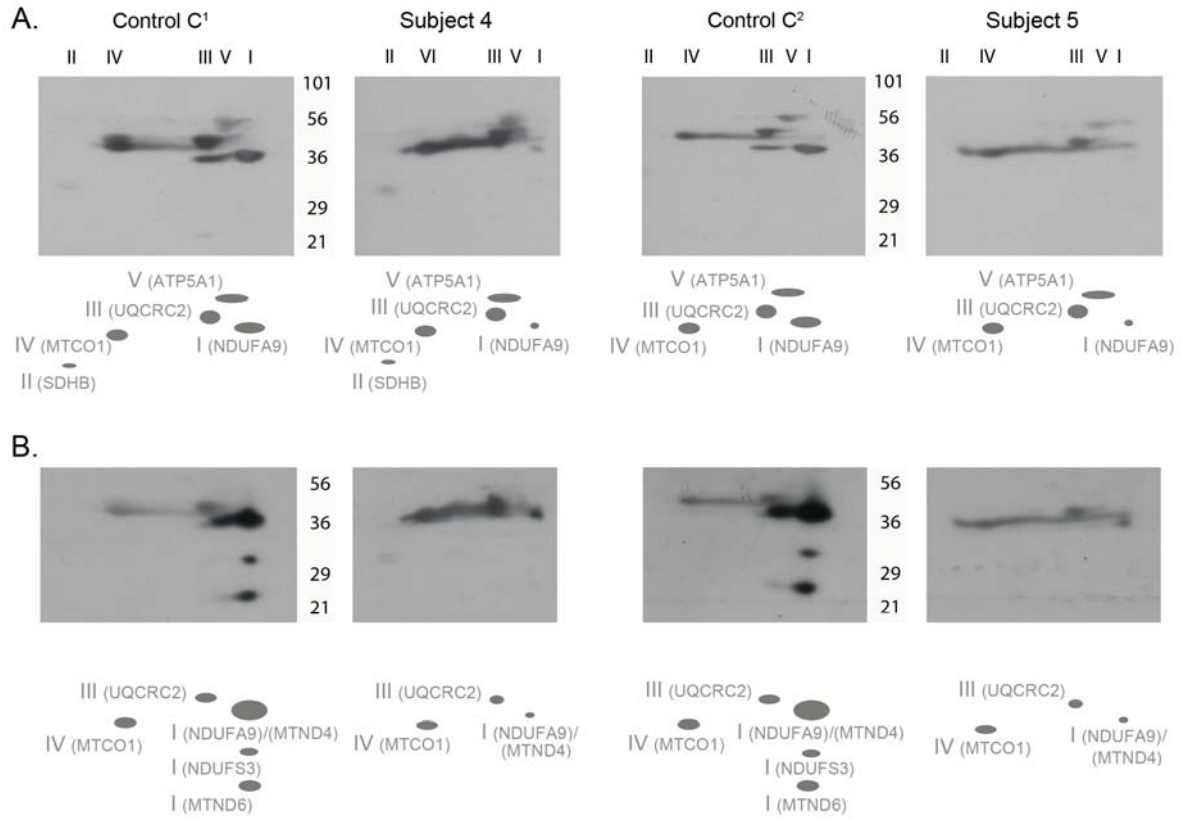

Figure S2: Western blotting following 2D BN-PAGE/tricine SDS-PAGE separation of isolated skeletal muscle mitochondria from Subjects 4 and 5
Experiments were performed according to the procedures previously described in detail[2]. (***A***) A mixture of the following antibodies was used to evaluate the abundance of the five OXPHOS protein complexes: complex I (NDUFA9), complex II (SDHB), complex III (UQCRC2), complex IV (MTCO1) and complex V (ATP5A1). (**B**) Following stripping of the antibodies, the nitrocellulose blot was reprobed using antibodies for complex I (MTND4, NDUFA9, NDUFS3 and MTND6), for complex III (UQCRC2) and for complex IV (MTCO1). An almost complete absence of signal with antibodies directed to the different complex I subunits is observed in skeletal muscle mitochondrial isolates from Subjects 4 and 5, highlighting a severe disturbance in the assembly of this OXPHOS complex.

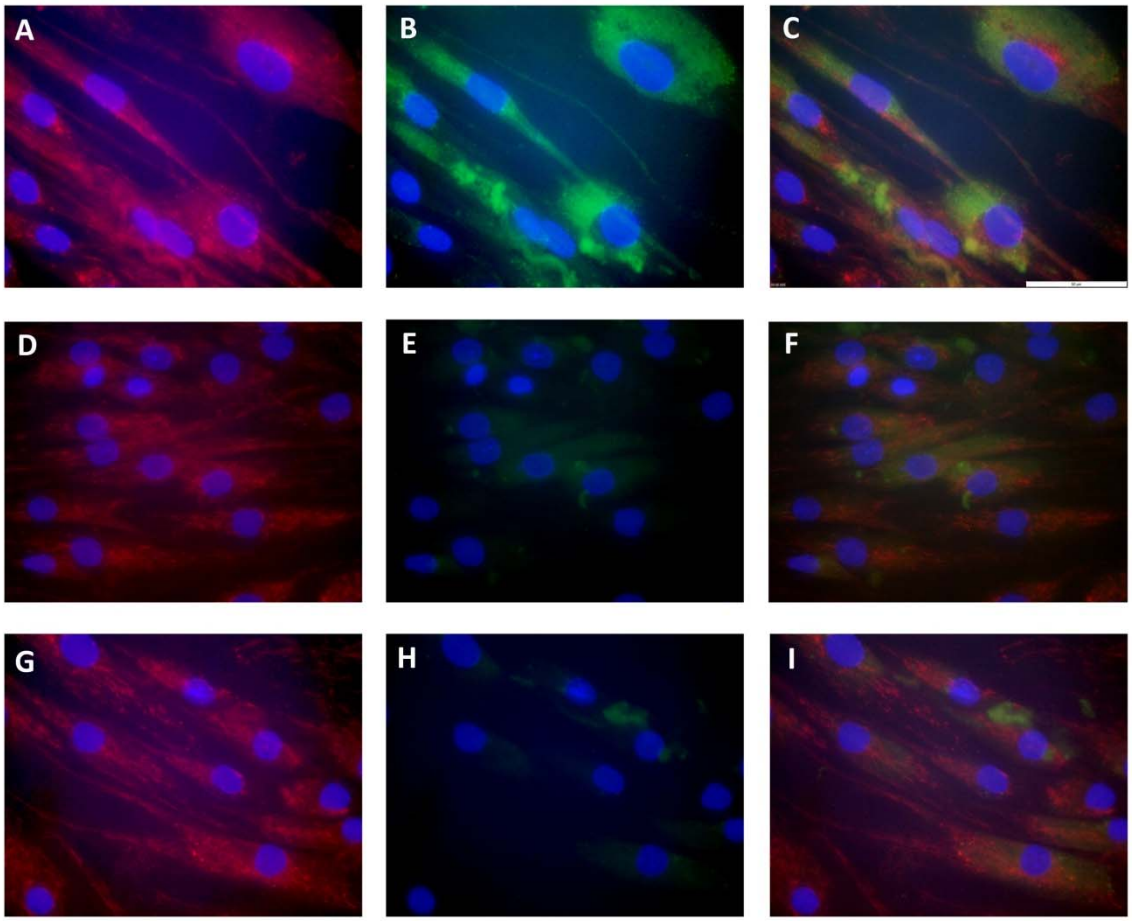

Figure S3: Cultured skin fibroblasts from Subjects 4 and 5 show severely decreased TMEM126B immunofluorescence

Double immunofluorescent staining of fibroblasts[3] from a control (***A-C***) and from Subject 4 (***D-F***) and Subject 5 (***G-I***) was performed, using MitoTracker Red CMXRos (Invitrogen) shown in red (panels ***A***, ***D*** and ***G***) and rabbit polyclonal anti-TMEM126B (AV49321, Sigma; 30µg/ml 2h room temperature) visualized with donkey anti-rabbit AlexaFluor488 (Invitrogen) shown in green (panels ***B, E*** and ***H***). Cell nuclei were counterstained with dapi shown in blue. The overlays (panels ***C, F*** and ***I***) demonstrate a reduction of TMEM126B staining in cells from both subjects (Scale bar = 50um).

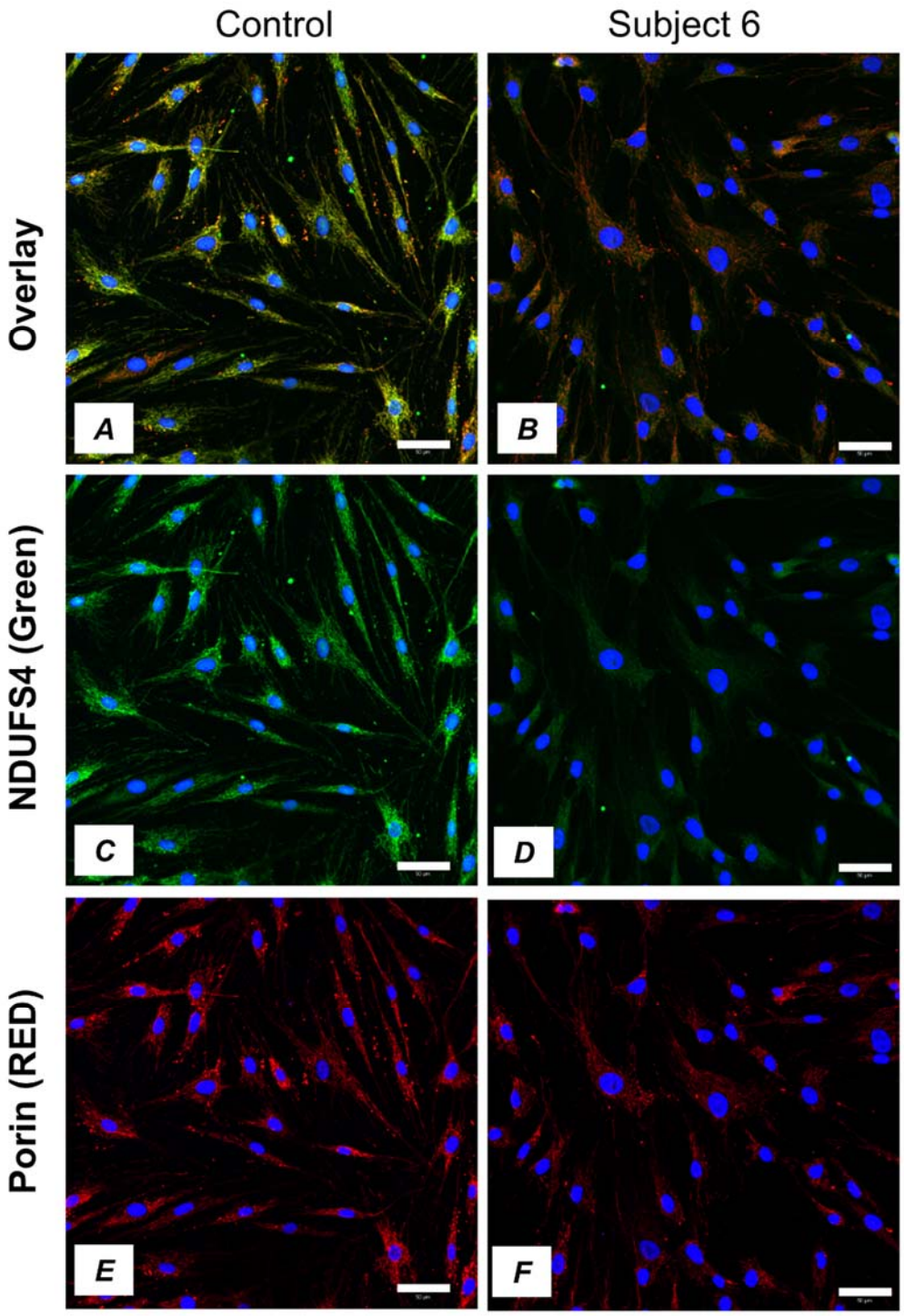

Figure S4: Cultured skin fibroblasts from Subject 6 express a complex I defect
Immunofluorescence staining of fibroblasts obtained from Subject 6 (homozygous p.(Gly212Val) *TMEM126B* variant) and a control was performed as reported [4] using the following primary antibodies: mouse monoclonal anti-NDUFS4 antibody (1:100; Abcam, Cambridge, UK) shown in green (panels ***C*** and ***D***) and rabbit polyclonal anti-VDAC1 (1:500; Abcam, Cambridge, UK) shown in red (panels ***E*** and ***F***), with the overlay (panels ***A*** and ***B***) clearly demonstrating strong staining of the NDUFS4 (complex I) protein in the control and absence in the patient cell line (Scale bar = 50um).

References

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