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Supplemental Data

Mutations in *DNA2* Link Progressive Myopathy to Mitochondrial DNA Instability

Dario Ronchi, Alessio Di Fonzo, Weiqiang Lin, Andreina Bordoni, Changwei Liu, Elisa Fassone, Serena Pagliarani, Mafalda Rizzuti, Li Zheng, Massimiliano Filosto, Maria Teresa Ferrò, Michela Ranieri, Francesca Magri, Lorenzo Peverelli, Hongzhi Li, Yate-Ching Yuan, Stefania Corti, Monica Sciacco, Maurizio Moggio, Nereo Bresolin, Binghui Shen, and Giacomo Pietro Comi

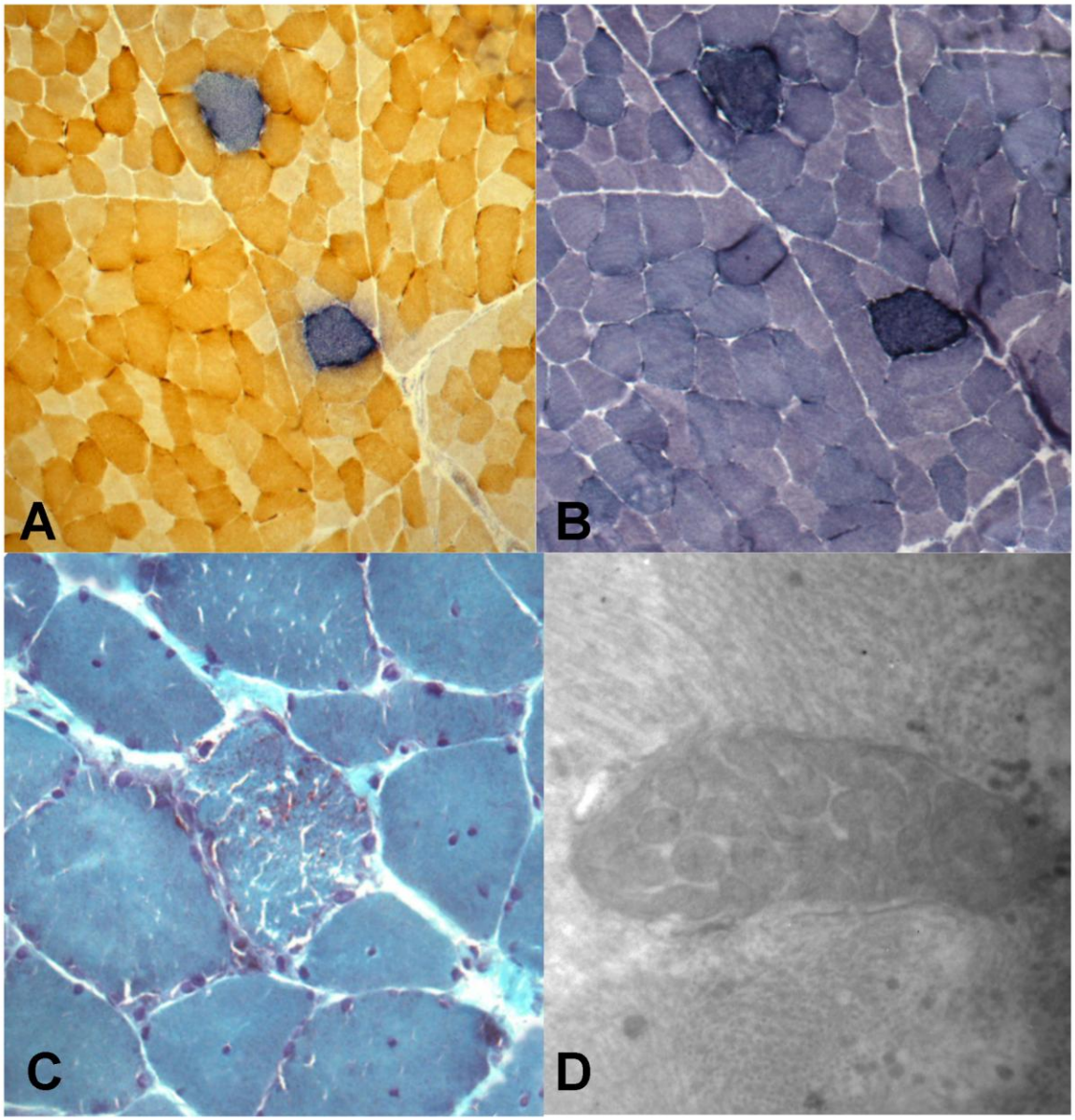

Figure S1. Histological and Ultrastructural Studies in Patients' Muscle

Muscle samples were frozen in cooled isopentane and stored in liquid nitrogen for histological and histochemical analyses, including modified Gomori trichrome staining, COX activity, SDH activity and double COX/SDH staining according to standard protocols.

(A and B) Scattered COX-negative fibers (A) with mitochondrial proliferation (B) are evident at skeletal muscle histochemistry from P4 (A: double staining for COX-SDH; C: SDH, 10X)

(C) Gomori trichrome from P1 shows mitochondrial proliferation in a skeletal muscle fiber (Ragged Red Fiber). 25X

(D) Ultrastructural examination of P2 skeletal muscle biopsy shows a mitochondrion with packed and thickened cristae. 30X

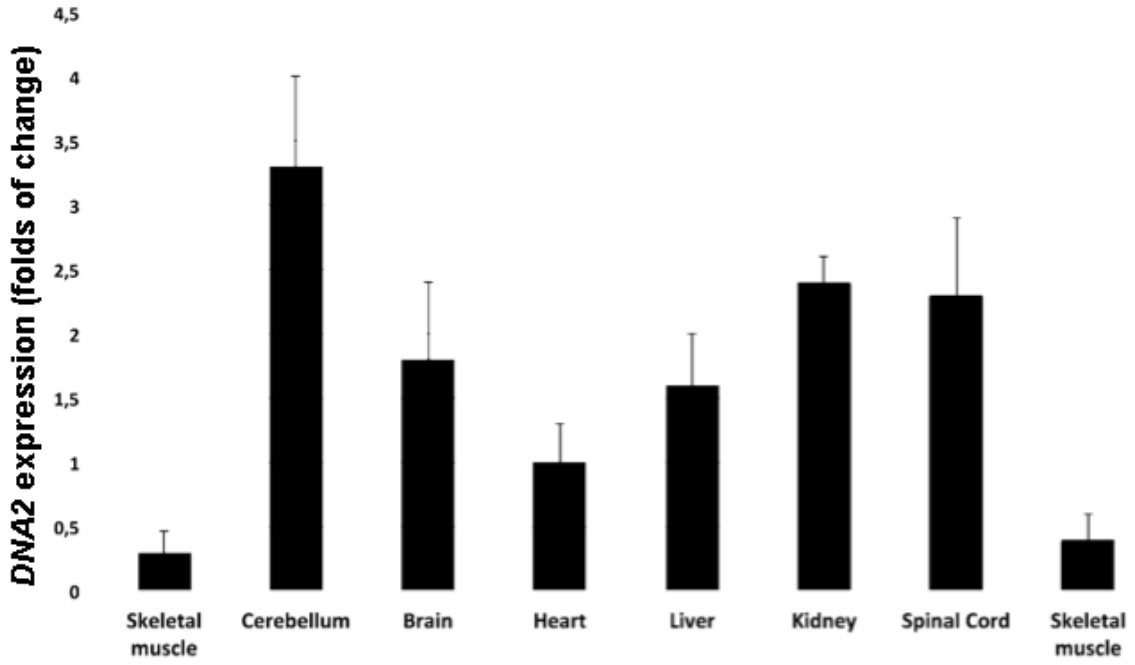

Figure S2. Quantitative RT-PCR of *DNA2* Expression in Eight Control Tissue Samples

GAPDH was used as the control housekeeping gene. The mRNA level in the heart was used for normalization of the expression data. All determinations were performed in replicates of four. Error bars indicate SEM.

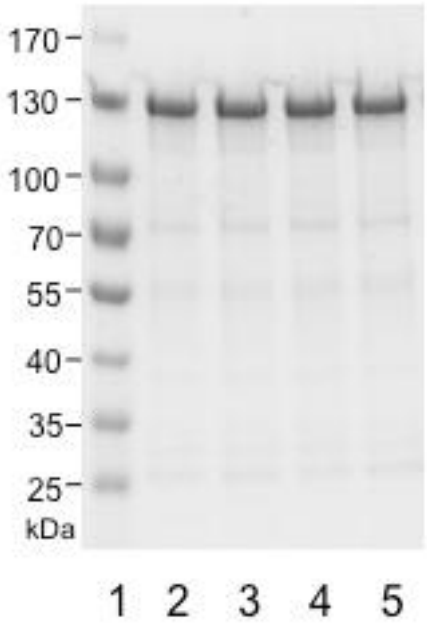

Figure S3. SDS-PAGE of Purified Recombinant DNA2

pcDNA5-*DNA2* plasmid was used as the template to amplify *DNA2* cDNA using primers DNA2-NotI_F: 5'ATAAGAATGCGGCCGC TGGAGCAGCTGAACGAACTGG3'and DNA2-BglII_R: 5'GGAAGATCTTCATTCTCT TTGAAAGTCACCC 3'. The plasmid was reported previously [reference 9]. The PCR product was double digested by NotI and BglII (New England Biolabs), and later it was subcloned into p3xflag CMV7.1 (Sigma) mammalian transient expression vector, named as pCMV7-*DNA2*, which was confirmed by sequencing. A series of *DNA2* mutants' plasmids were constructed using PCR/ mutagenesis method with pCMV7-*DNA2* as the template. The primers for making three mitochondria mutations are listed below: DNA2-Arg284His_F: 5' GTACCACTTAAATCTAAGTCAAGATGAAATAAAAC3'; DNA2-Arg284His_R: 5'TTAAGTGGTACATTTCCTTCAAATGTCTTATTTC3'; DNA2-Lys313Glu_F: 5'TGCATGAAAACACTTCGACTGACTTCCCTCAGATG3'; DNA2-Lys313Glu_R: 5'GTTTTCATGCATGAAATCTCCTGCCCATTTACAAAACG3'; DNA2-Val723Ile_ F: 5'GAAAAAGATACTTCTTTCAAAAGACTACACACTC3'; DNA2-Val723Ile_R: 5'GAAGTATCTTTTTCATCGCTTGCCTCTGAGG3'. For DNA2 helicase assay, we created the mitochondria mutation plasmids based on pCMV7-*DNA2* Asp294Ala nuclease dead plasmid to eliminate endonuclease activity. The primers for making *DNA2* Asp294Ala is listed as follows: DNA2-NM_F: 5' CAAAATAGCTGTTACAGTTGGTGTGAAAATACATCG3'; DNA2-NM_R: 5'CTGTAACAGCTATTTTGCCTTTCAATCCAAACC3'.

The expression vectors were transfected into the 293T cells using Lipofectamine 2000 (Invitrogen). Recombinant DNA2 was purified as described previously with little modification [reference 12]. In brief, cell lysate was incubated with the beads conjugated with flag antibody (SIGMA) for at least 6 hours at cold room. After wash with 500mM NaCl for 5 times, the bound proteins were eluted with Flag peptide.

Purified flag-DNA2 proteins were subjected to 4-15% SDS-PAGE. Lane1: protein marker; Lane 2-5: DNA2 WT, p.Arg284His, p.Lys313Glu and p.Val723Ile.