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Supplemental Data

Mutations in *GTPBP3* Cause a Mitochondrial Translation Defect Associated with Hypertrophic Cardiomyopathy, Lactic Acidosis, and Encephalopathy

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Figure S1

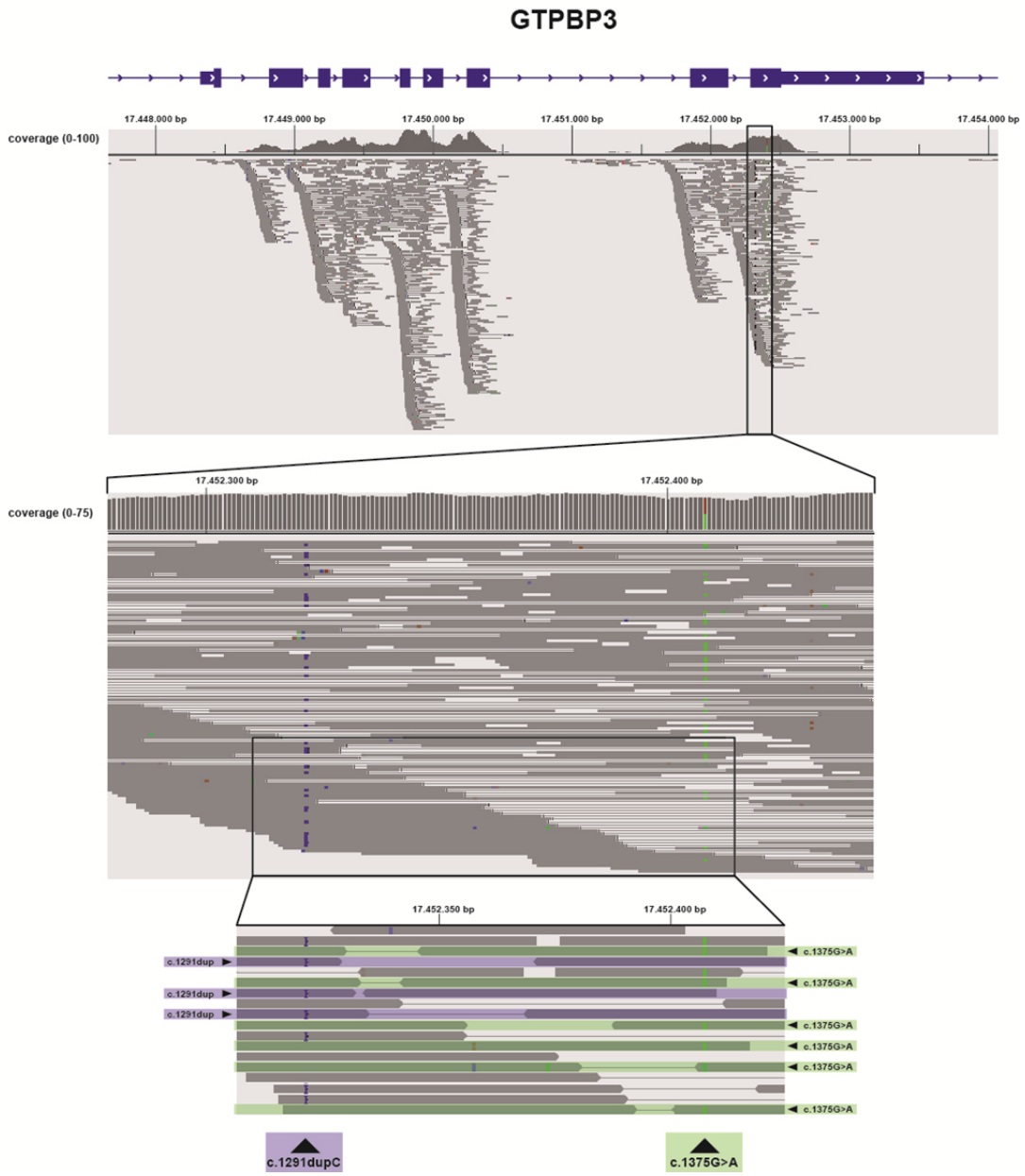

Figure S1) Segregation analysis in family F1 in WES data

The two mutations identified in family F1 (c.1291dupC and c.1375G>A) are only separated by 97 bp which allowed analysis of both alleles despite the lack of parental material. 13 paired sequence reads were identified which covered the region of both variants. All reads contained either of the two mutations demonstrating a compound heterozygous status of the two variants. Figure S1 shows three sequence reads containing the c.1291dupC variant and five reads containing the c.1375G>A variant.

Figure S2

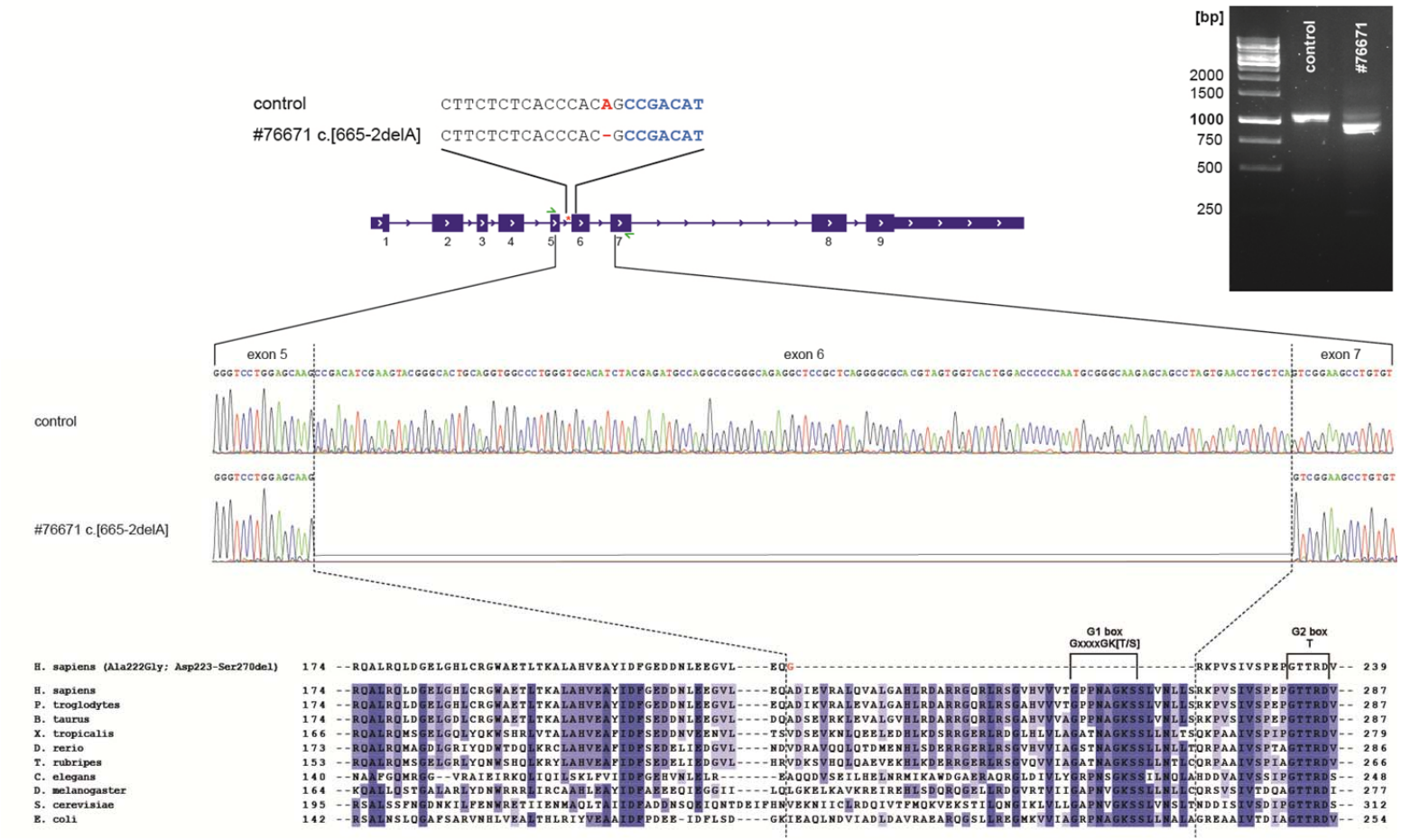

Figure S2) Splice site mutation in individual #76671 causes skipping of exon 6

Analysis of cDNA derived from fibroblasts of individual #76671 yielded a smaller than expected PCR product, indicating alternative splicing. Sanger sequencing revealed that the c.665-2delA mutation affects the conserved splice acceptor site. The splice acceptor upstream of exon 7 is alternatively used, yielding a mature mRNA that lacks exon 6. The resulting protein product is predicted to contain a 1 amino acid exchange followed by a 48 amino acid deletion (p.Ala322Gly; Asp223-270del).

Figure S3

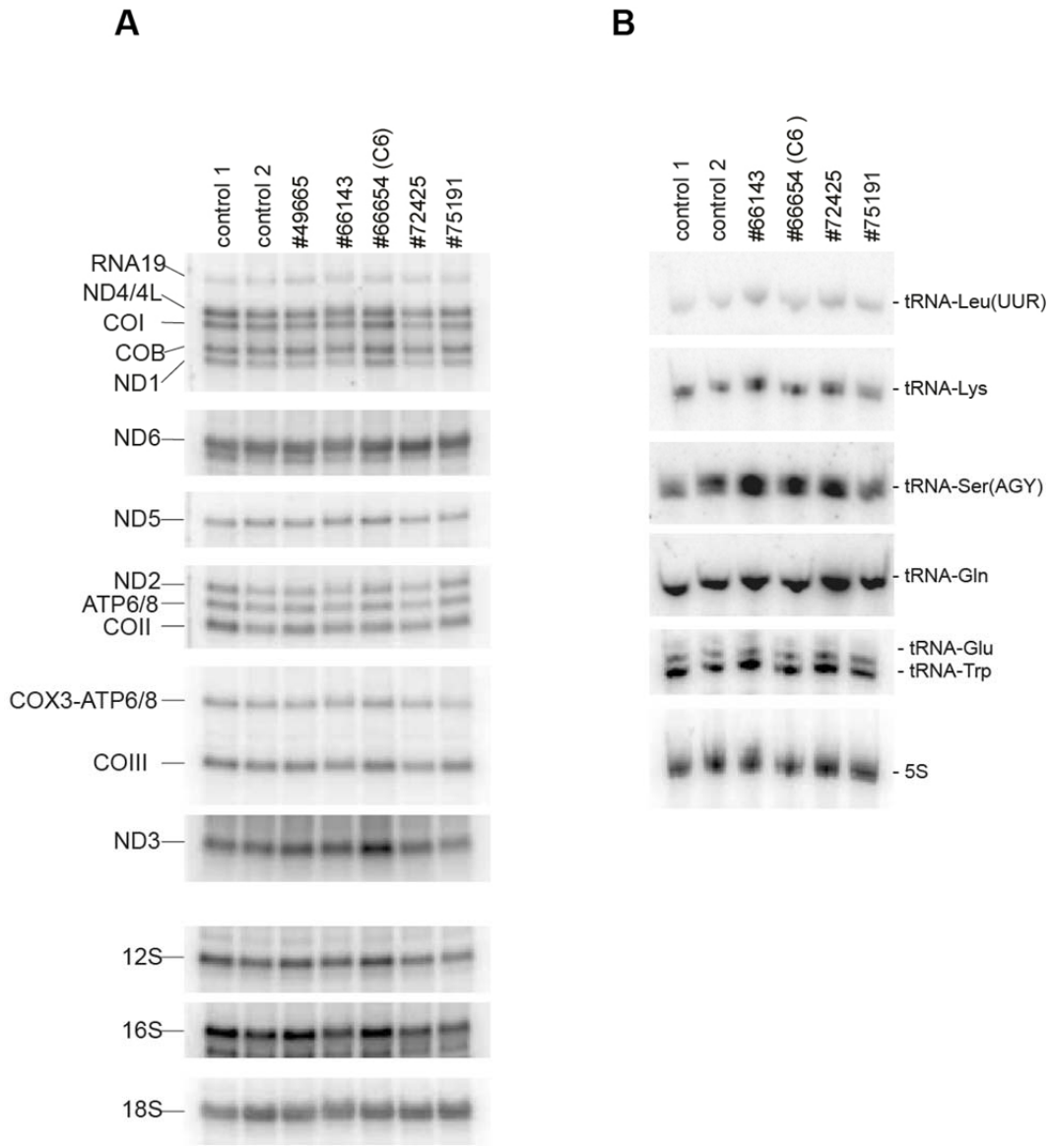

Figure S3) Northern blot analysis of the steady-state levels of mitochondrial transcripts in GTPBP3 patient fibroblasts.

A) Northern blot analysis of total RNA isolated from the *GTPBP3* patient or control primary fibroblasts. The blots were probed with the mt-mRNA- and mt-rRNA-specific probes as indicated. The cytosolic 18S rRNA was used as a loading control.

B)) High-resolution Northern blot analysis of total RNA isolated from the *GTPBP3* patient or control primary fibroblasts. The blots were probed with the mt-tRNA -specific probes as indicated. The cytosolic 5S rRNA was used as a loading control.

Figure S4

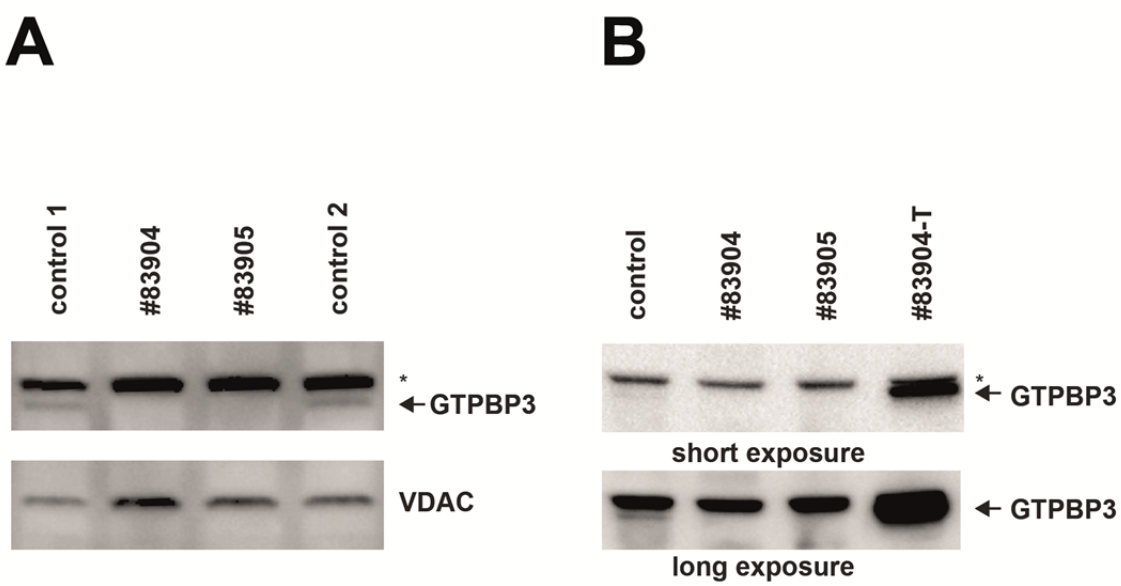

Figure S4) Analysis of GTPBP3 protein levels in patient fibroblasts

A) Immunoblot analysis of GTPBP3 protein levels in fibroblasts from affected individuals #83904 and #83905 from family F9. VDAC served as a mitochondrial loading control. (Asterics indicates a non-specific band.)

B) Comparison of the electrophoretic migration of GTPBP3 in un-transfected cells (lane control) and cells derived from one of the affected individuals transfected with a plasmid (pIRES2-EGFP) for *GTPBP3* cDNA expression (lane #83904-T). (Asterics indicates a non-specific band.)