

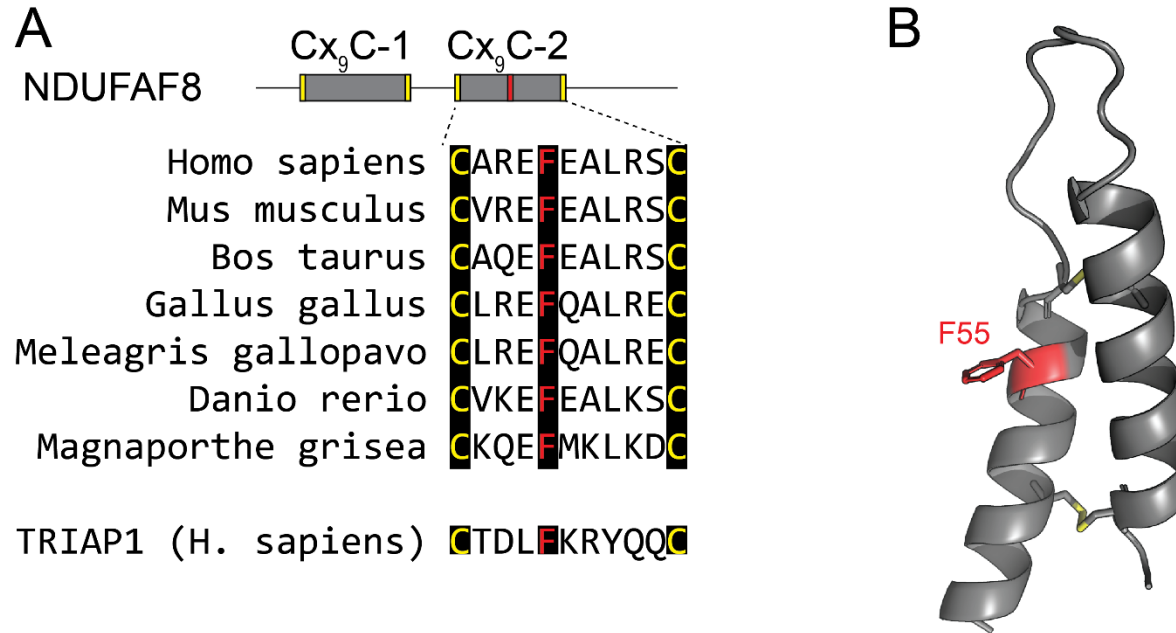
Supplemental Data

Pathogenic Bi-allelic Mutations in *NDUFAF8* Cause

Leigh Syndrome with an Isolated Complex I Deficiency

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Supplemental Figures



Supplemental Figure S1. Conservation of the Phe55 residue and predicted tertiary structure of NDUFAF8.

A Alignment of the second Cx₉C motif across several species with NDUFAF8 according to CLIME [1]. Human TRIAP1 is also included in the alignment as its crystal structure was used to model NDUFAF8 (**B**). **B** SWISS MODEL prediction of the tertiary structure of NDUFAF8 based on the crystal structure of TRIAP1 [2] demonstrating the disulfide bonds arising from the twin Cx₉C motifs. The p.Phe55 variant that is mutated in Subject 3 is labelled (F55) and is situated within the Cx₉C-derived hairpin.

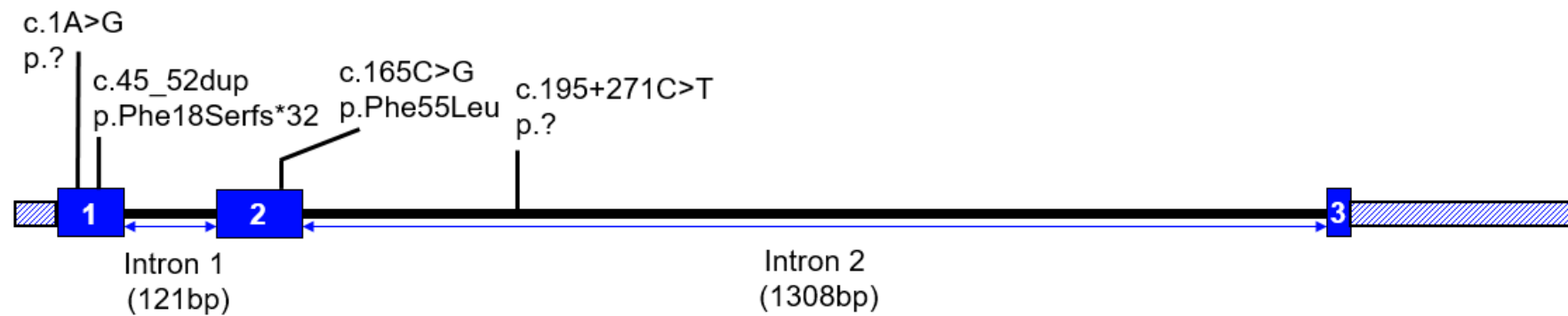


Figure S2: Pathogenic *NDUFAF8* variants and gene structure.

NDUFAF8 is a small gene (<3kb genomic DNA) with just three exons. Identified gene variants are mapped onto the gene schematic; the small size of the introns facilitated identification of the intronic c.195+271C>T variant in the whole exome sequencing data for Subject 2.

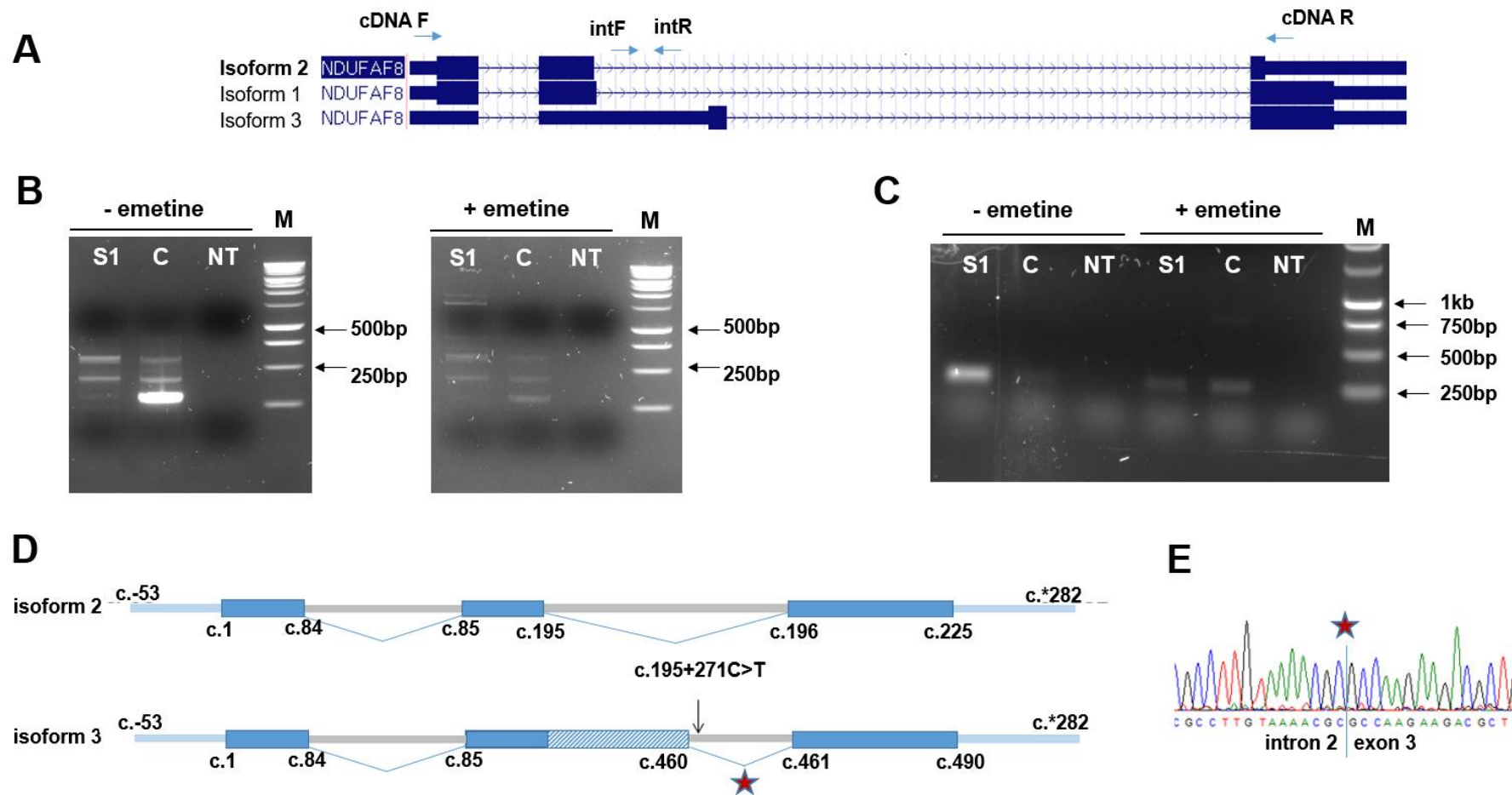
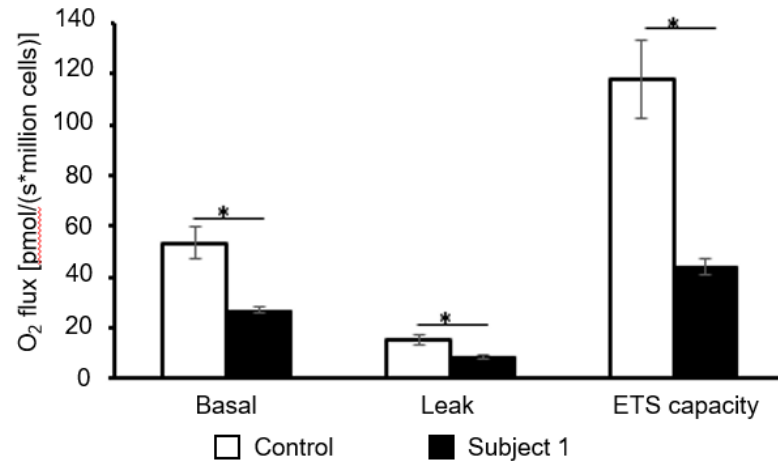
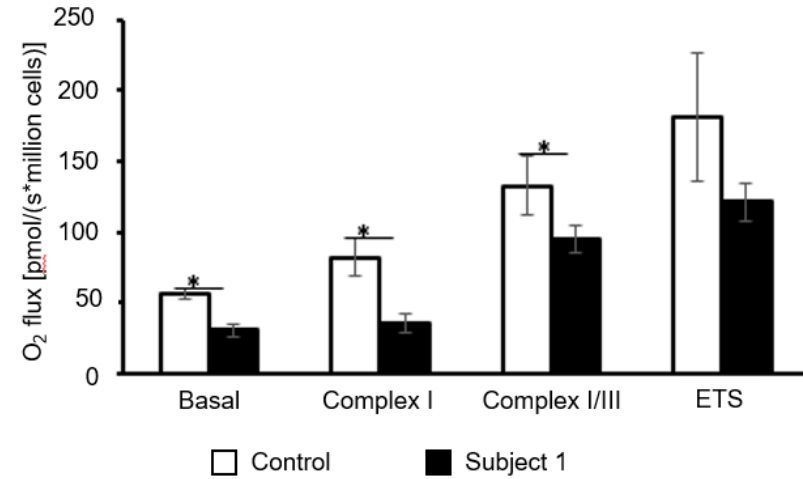


Figure S3: cDNA analysis of Subject 1 fibroblasts provides evidence of aberrant splicing.

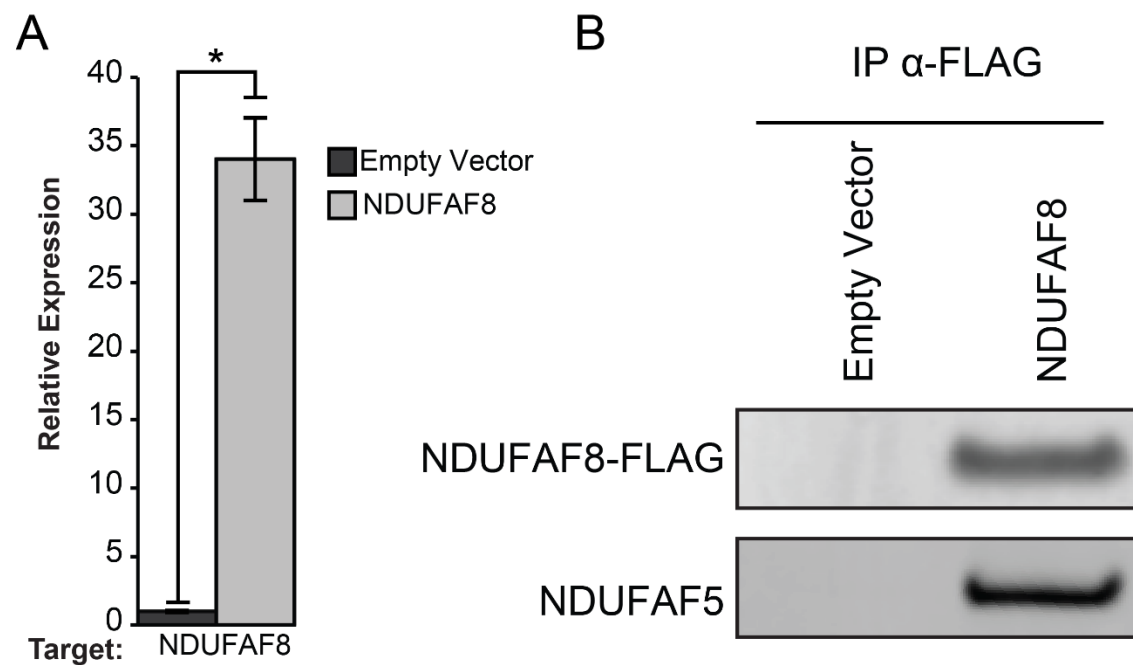
A. Three isoforms of NDUFAF8 exist; the functional isoform being isoform 2. Primers used for the NDUFAF8 cDNA amplification are denoted cDNA F/R (full length NDUFAF8 cDNA molecule, with primers common to all three transcripts) and a sequencing intF/R primer, specific to

isoform 3. **B.** Amplification products of Subject 1 (S1) and Control (C) fibroblast-derived cDNA samples using NDUF8 cDNA/R primers, with or without overnight exposure to emetine; nt=no template; M=Promega 1kb ladder. A clear reduction in the smallest (~150bp) amplicon is observed in Subject 1 untreated fibroblasts compared to the control. Additionally, high molecular weight amplicons are present in Subject 1's emetine-treated fibroblasts, compared to the control, suggestive of aberrantly spliced transcripts that are anticipated to be subject to nonsense mediated decay *in vivo*. **C.** Amplification of Subject 1 and control fibroblast-derived cDNA using a forward primer specific to NDUF8 isoform 3 is supportive of considerably higher levels compared to control; the presence of NDUF8 isoform 3 at apparently similar levels in emetine treated cells support the predominant turnover of this isoform *in vivo*. **D.** Retention of intronic sequence distinguishes isoforms 2 and 3, a star denotes the junction, visualised in the sequencing chromatogram (**E**).

A**B**

Supplemental Figure S4: Mutation of NDUFAF8 causes dramatic defects on cell respiration and complex I.

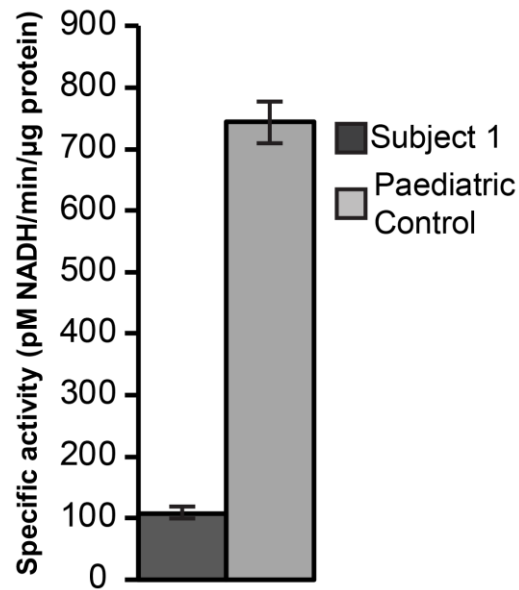
A. High-resolution respirometry of patient-derived fibroblasts (black bars) compared to control fibroblasts (white bars). Basal- oxygen consumption rate of intact cells in growth medium, Leak- oxygen consumption rate upon ATP synthase inhibition with Oligomycin, ETS capacity- maximal uncoupled respiration. Data are mean $\pm$ SD from 3 experiments. **p<0.01; values are mean $\pm$ SD. **B.** High-resolution respirometry of patient-derived fibroblasts (black bars) compared to control fibroblasts (white bars). After measurement of basal respiration cells were permeabilized with digitonin and mitochondrial respiratory rates are measured for complex I (CI) and complex I+II (CI+II) OXPHOS capacity followed by the measurement of maximal uncoupled respiration after stepwise titration of FCCP (ETS). Data are mean $\pm$ SD from 3 experiments.*p<0.05, **p<0.01; values are mean $\pm$ SD



Supplemental Figure S5. NDUF8-FLAG is being expressed in the EF1 α based rescue cell line.

A, qPCR of NDUF8 levels using primers targeting both endogenous NDUF8 as well as the NDUF8-FLAG overexpression construct. Error bars are at ± 1 standard deviation, data shown are from three technical replicates.

B, α -FLAG IP from transformed cell lines Western blotted for FLAG or NDUF8.



Supplemental Figure S6. Complex I activity in primary fibroblasts from Subject 1 is impaired. Spectrophotometric analysis using a colorimetric complex I activity assay (ab109721, Life Technologies) demonstrates a marked complex I deficiency in the primary fibroblast cell line from Subject 1 compared to a paediatric control. Error bars are at ± 1 standard deviation, data shown are from three technical replicates.

Supplemental references

1. Li Y, Calvo SE, Gutman R, Liu JS, Mootha VK. Expansion of biological pathways based on evolutionary inference. *Cell*. 2014;158(1):213-25.
2. Miliara X, Garnett JA, Tatsuta T, Abid Ali F, Baldie H, Pérez-Dorado I, Simpson P, Yague E, Langer T, Matthews S. Structural insight into the TRIAP1/PRELI-like domain family of mitochondrial phospholipid transfer complexes. *EMBO Rep*. 2015;16(7):824-35.