The American Journal of Human Genetics

Supplemental Data

CODAS Syndrome Is Associated with Mutations of *LONP1,* Encoding Mitochondrial AAA$^+$ Lon Protease

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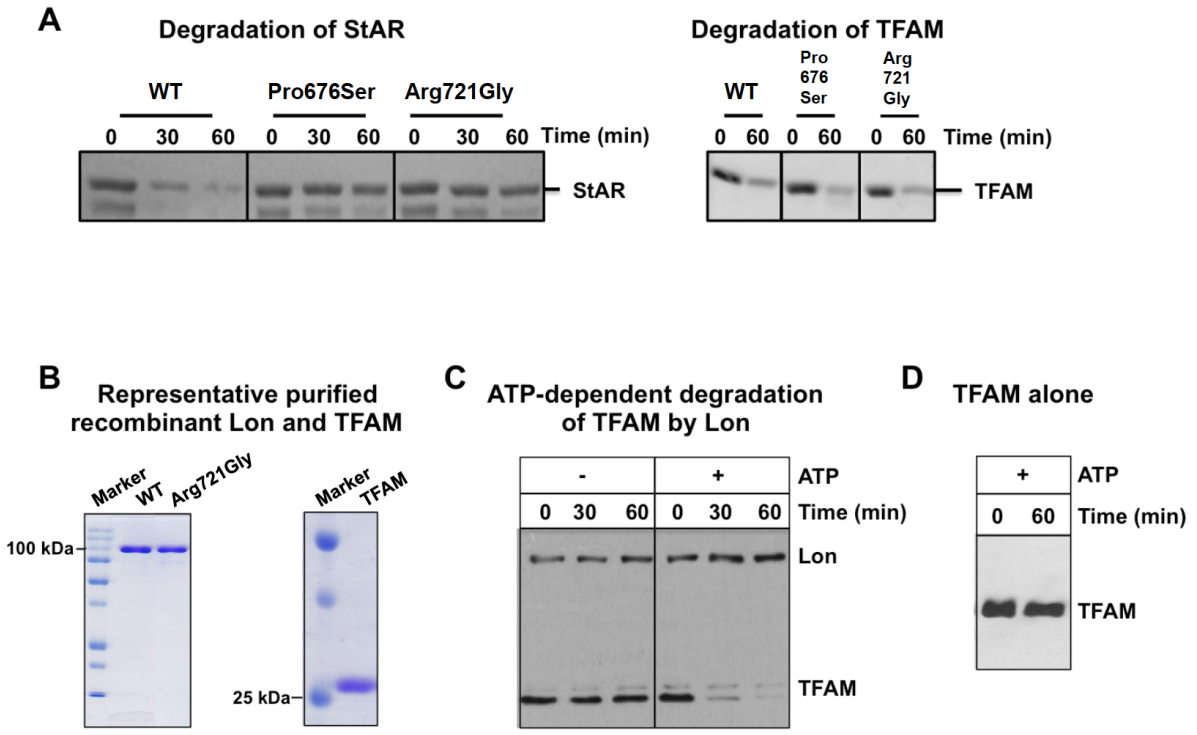

Figure S1. **Degradation of StAR and TFAM by wild type Lon and CODAS mutants Pro676Ser and Arg721Gly.** **(A)** Coomassie Brilliant Blue stained SDS-PAGE gels of StAR and TFAM degraded by wild type Lon and CODAS mutants Pro676Ser and Arg721Gly. Recombinant Lon was purified as described elsewhere[1] with the following modifications. The column was washed with Buffer A[1] (2X with 2 ml), Wash Buffer (50 mM Hepes pH 8.0, 0.15 M NaCl, 10 mM $MgCl_2$, 20% (w/v) glycerol) containing 40 mM imidazole (1X 2 ml, 4X 1 ml) and then eluted with Wash Buffer containing 0.1M imidazole (1X with 1.5 ml) and with Wash Buffer containing 0.3 M imidazole (2X with 1.5 ml). Protein concentrations were determined using the Bradford assay with BSA as a standard, and by UV absorbance at 280 nm using the extinction coefficient for Lon described in Methods. **(B)** Representative Coomassie Brilliant Blue stained SDS-PAGE gels of purified recombinant wild type Lon, CODAS mutant Arg721Gly and TFAM. **(C)** TFAM degradation by Lon is ATP-dependent. Immunoblot using anti-His tag antibody recognizing recombinant TFAM and Lon, which have a hexahistidine-tag at their respective carboxyl-termini. **(D)** Control experiment demonstrating that TFAM alone is not degraded during the 60 min incubation at 37°C. Immunoblot using anti-His tag antibody.

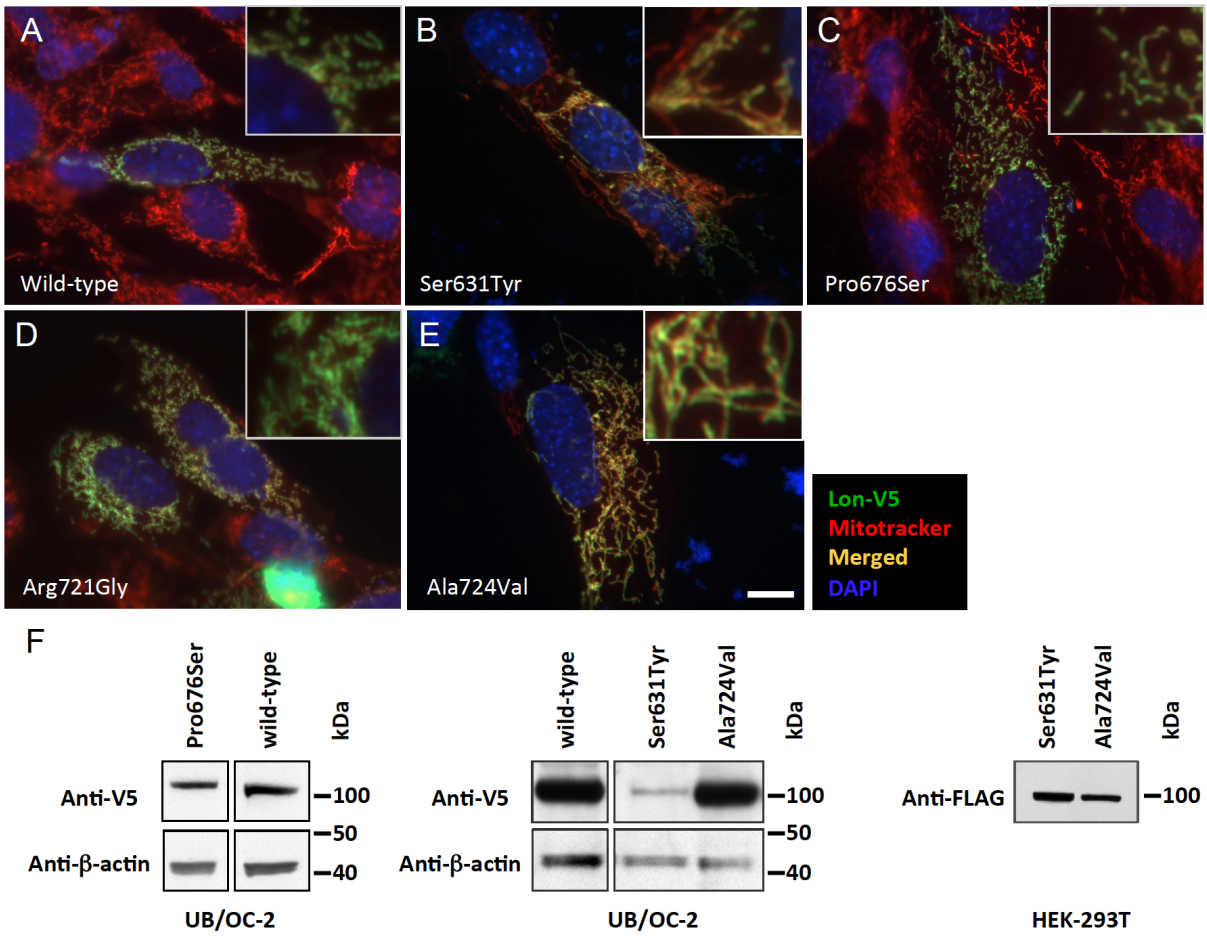

Figure S2. Recombinant Lon CODAS variant proteins localized to mitochondria in mouse UB/OC-2 auditory sensory neuron precursor cells. (A–E) Overexpression of Lon WT-V5 **(A)** and Lon-V5 CODAS variants **(B–E)** (green – V5 immunofluorescence) in UB/OC-2 cells co-labeled with Mitotracker Red (red) and DAPI (blue). **(F)** Immunoblot of lysates of UB/OC-2 and HEK-293T cells overexpressing the Lon CODAS variants indicated labeled with anti-V5, anti-FLAG, and/or β-actin (loading control) antibodies. Nontransfected cells display only red Mitotracker fluorescence. Scale bar in **E**: 10 μm **(A–E)**; 2.7 μm (insets).

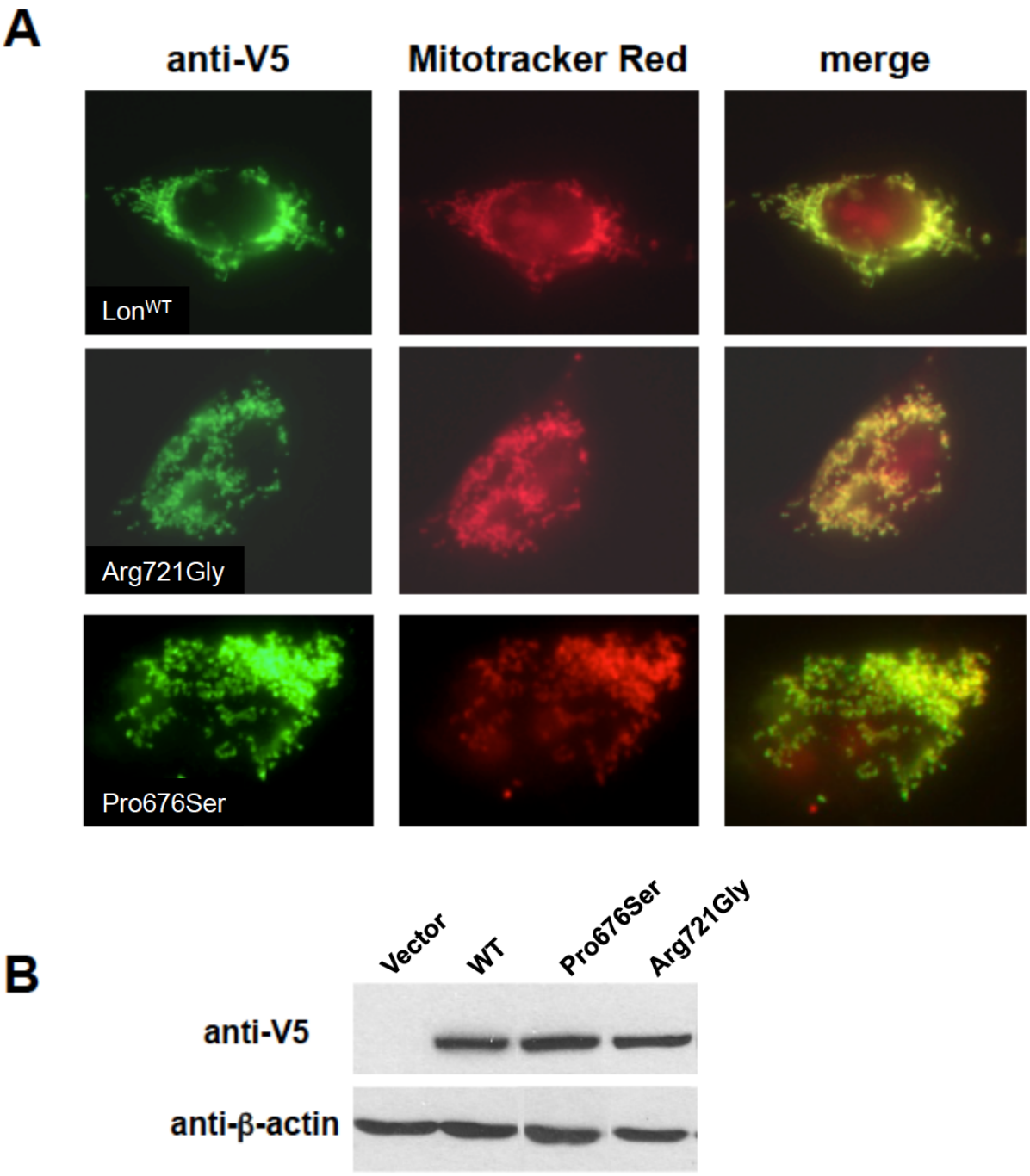

Figure S3. Recombinant wild type Lon and CODAS variants Pro676Ser and Arg721Gly are localized to mitochondria in HeLa cells. (A) Wild type and mutant Lon variants were overexpressed as carboxy-terminal V5-fusion proteins in HeLa cells, which were double labeled using anti-V5 immunofluorescence (green) and Mitotracker Red (red). **(B)** Protein extracts isolated from HeLa cells overexpressing wild type or mutant Lon CODAS variants as indicated, were immunoblotted with anti-V5 and β-actin (loading control) antibodies.

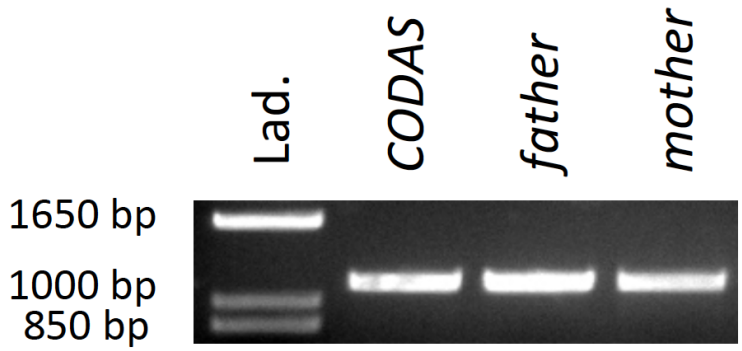

Figure S4. *LONP1* transcript abundance is comparable in CODAS lymphoblastoid cell lines (LCLs) and LCLs from unaffected parents. Reverse transcriptase PCR from total RNA and agarose gel electrophoresis were used to compare endogenous transcript abundance between homozygous CODAS proband (c.2161C>G) and heterozygous parent LCLs.

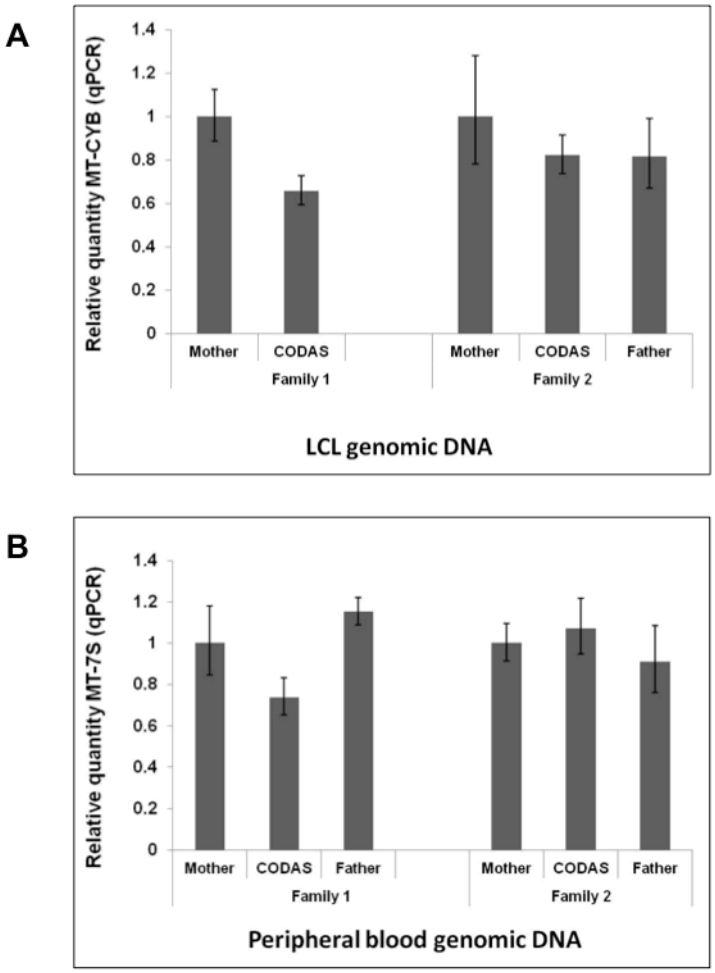

Figure S5. MtDNA copy number using total genomic DNA isolated from LCL cells generated from CODAS proband and parents. (A) Quantitative PCR (qPCR) was performed to determine the relative mtDNA copy number in CODAS probands and their respective mothers and fathers using genomic DNA isolated from LCLs generated from the Amish family members as shown. **(B)** qPCR was also performed using total genomic DNA isolated from peripheral blood lymphocytes of CODAS probands and their respective parents as shown. PCR reactions were performed using Taqman probes and primers amplifying the mtDNA-encoded *MT-CYB* gene, *MT-7S* and the nuclear DNA-encoded *APP* gene as a reference. The relative quantity of mtDNA copy number was calculated by the $\Delta\Delta C_t$-method. Error bars represent ± 1 standard deviation.

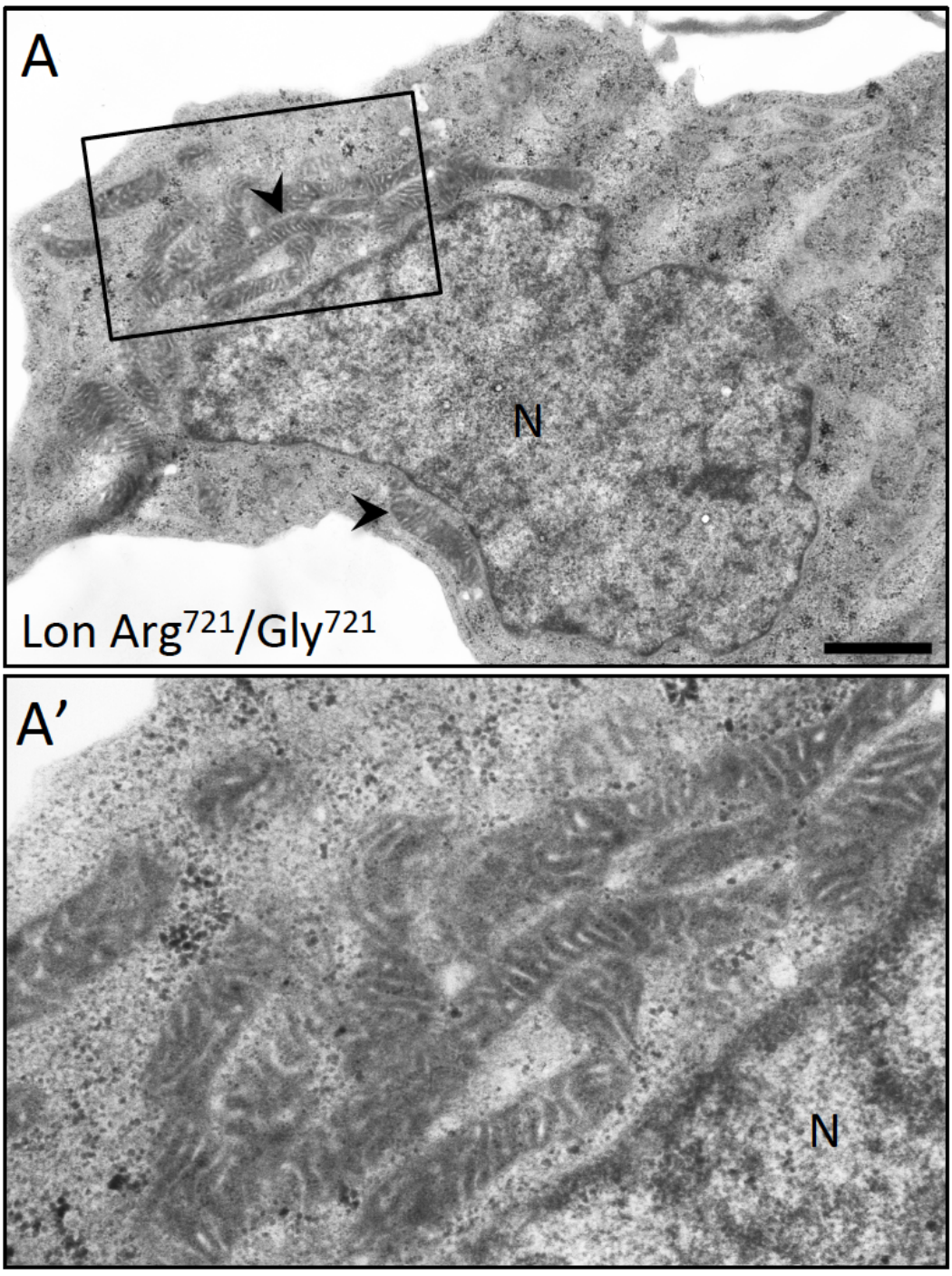

Figure S6. Lymphoblastoid cell lines (LCLs) from heterozygous Lon$^{Arg721Gly/WT}$ fathers have normal mitochondrial morphology. **(A)** Transmission electron micrographs of a B lymphocyte displaying normal mitochondrial ultrastructure (arrowhead) from the father (n = 48 cells) of the CODAS proband shown in Figure 6A. N – nucleus. Scale bar in **(A)**: 1 μm **(A)**; 0.3 μm in magnified region **(A')**.

Table S1. Next Generation sequencing of mtDNA from Amish probands ($Lon^{Arg721Gly/Arg721Gly}$), mothers and fathers ($Lon^{WT/Arg721Gly}$). Data provided in Excel spreadsheet (Table S1.xlsx). Analysis was performed by the Molecular Genetics Laboratory at Rutgers- New Jersey Medical School based on an ampliseq multiplex polymerase chain reaction. Amplicons produced served as targets for mtDNA sequencing reactions on the Ion Torrent system (Life Technologies). The amplicons were barcoded and combined with ISP beads during emulsion PCR using the Ion PGM™ Template OT2 200 Kit. This step was followed by enrichment. Upon completion, the samples were loaded onto the Ion Torrent Personal Genome Machine (PGM) for DNA sequencing with the Ion PGM™ 200 Sequencing Kit. The Ion 318™ Chip v2 provided from 600 Mb–2.0 GB of data. The sequencing results were analyzed with Nextgene software (SoftGenetics, State College PA). Variants were scored based upon NCBI Clinical Variation Database, MITOMAP, mtDB - Human Mitochondrial Genome Database, dbSNP and other relevant references.

Table S2. Primers used for genotyping lymphoblast cell lines (LCLs) and for generating CODAS *LONP1* constructs. Fwd – forward primer; Rev – reverse primer; SDM – site-directed mutagenesis (change in caps).

Primer (5'-3')	Purpose	Amplicon Size (bp)
AGAACCCCCTGATCCTCATC – Fwd	PCR genotyping – LCLs	1,140
GTGGCCGTCACCGTTCCA – Rev	PCR genotyping – LCLs	
CACCGCT**ATG**GCGGCGAGCACT – Fwd	*LONP1* CDS (start **bold**)	2,890
GTGGCCG**TCA**CCGTTCCA – Rev	*LONP1* CDS (stop **bold**)	
GCCGTGGAACGGGACGGCCACAAG	pENTR/D-TOPO/LONP1_c.2878_delT*	
gtgcccgtggacttgtAcaaggtgctgttcatc	*LONP1* c.1892C>A SDM	
cgctacctggtgTcccaggctcgcg	*LONP1* c.2026C>T SDM	
aagtggagaaggtgttaGggaaatcggcctacaag	*LONP1* c2161C>G SDM	
ggtgttacggaaatcggTctacaagattgtcagcg	*LONP1* c.2171C>T SDM	

*Used to remove *LONP1* stop codon for in-frame expression of C-terminal epitope tags (FLAG, V5) in pcDNA3.2/DEST

Supplemental References

1. Liu, T., Lu, B., Lee, I., Ondrovicova, G., Kutejova, E., and Suzuki, C.K. (2004). DNA and RNA binding by the mitochondrial lon protease is regulated by nucleotide and protein substrate. J Biol Chem 279, 13902-13910.