The American Journal of Human Genetics

Supplemental Data

TRMT5 Mutations Cause a Defect in Post-transcriptional Modification of Mitochondrial tRNA Associated with Multiple Respiratory-Chain Deficiencies

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Control Subject #73901

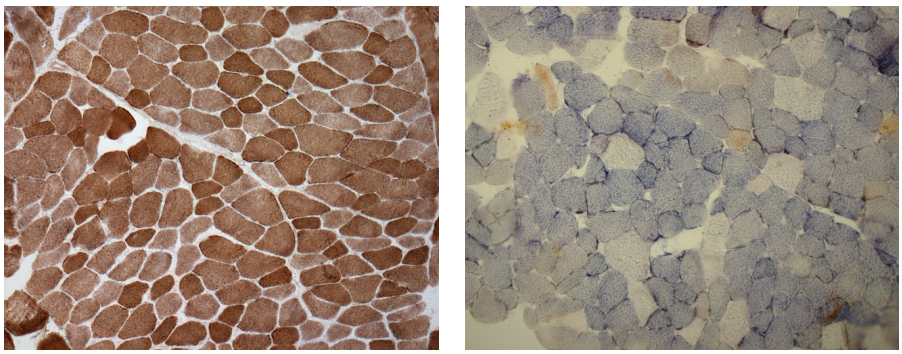

Figure S1 - COX histochemistry of skeletal muscle biopsy of subject #73901

Skeletal muscle biopsy of #73901 and healthy control reacted for sequential succinate dehydrogenase (SDH) and cytochrome-*c*-oxidase (COX) activities, highlighting a deficiency in COX activity in the majority of fibers in the patient.

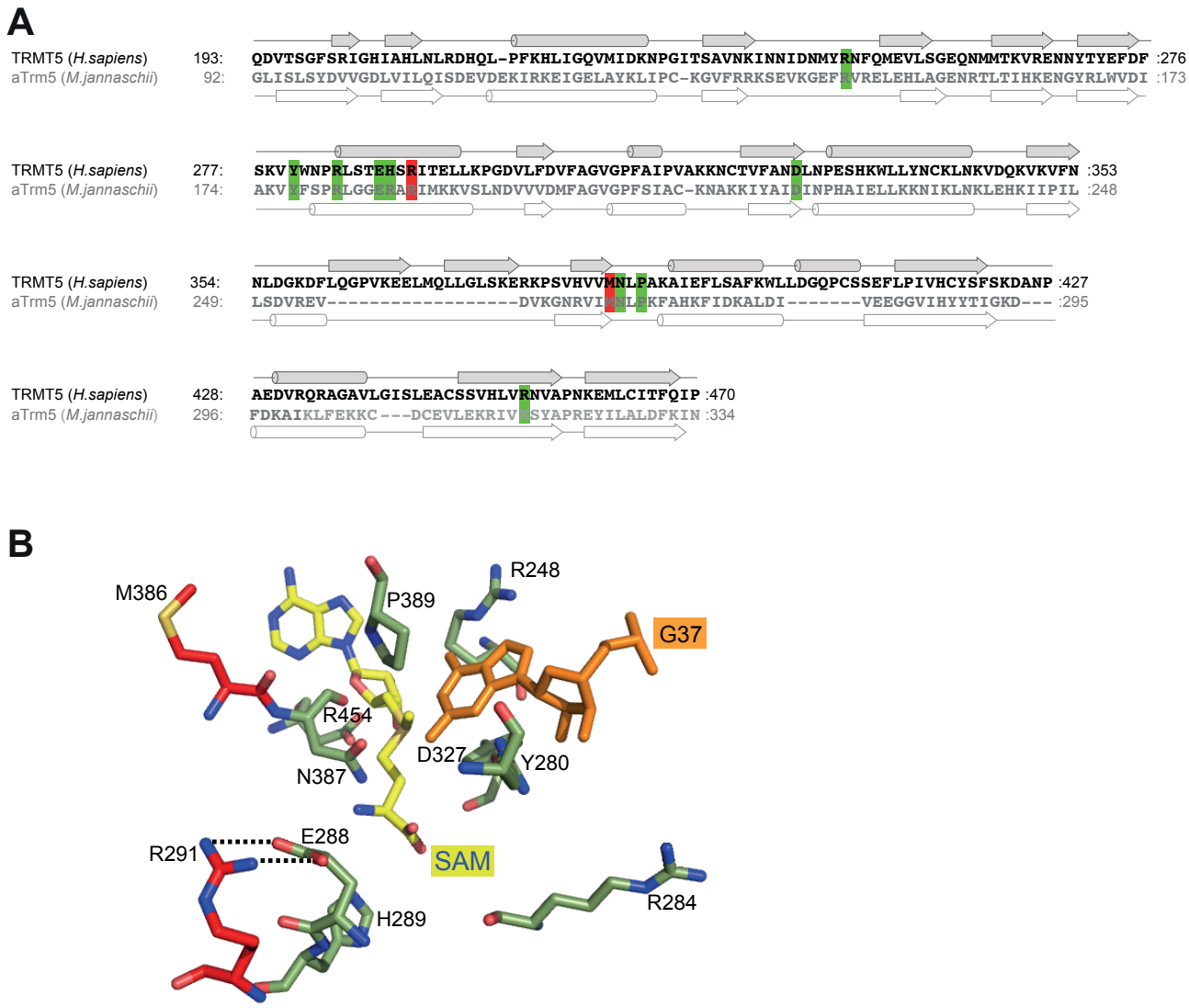

Figure S2 - Three-dimensional model of human TRMT5

(A) Sequence alignment of human TRMT5 and the homologous protein from *M. jannaschii* (aTrm5). Predicted (TRMT5) and observed (aTrm5, Protein Data Bank [PDB], 2ZZN) secondary structure elements are indicated (Arrows for β-sheets and cylinders for α-helices). Important catalytic residues of aTrm5 and corresponding residues in human TRMT5 are highlighted in green. Mutation sites detected in the human protein are in red.

(B) Detailed view of the 3D model of the predicted catalytic site of human TRMT5. TRMT5 residues corresponding to the catalytically important amino acids in aTrm5 are indicated in dark green. The residues mutated in the #65205 and #73901 individuals, Met389 and Arg291, respectively, are indicated in red. Arg291 is predicted to form a hydrogen bond interaction with catalytically critical Glu288 (dashed line). *S*-Adenosyl methionine in (SAM) is indicated as stick models in yellow. The guanosine residue at tRNA position 37 (G37) is indicated in orange.

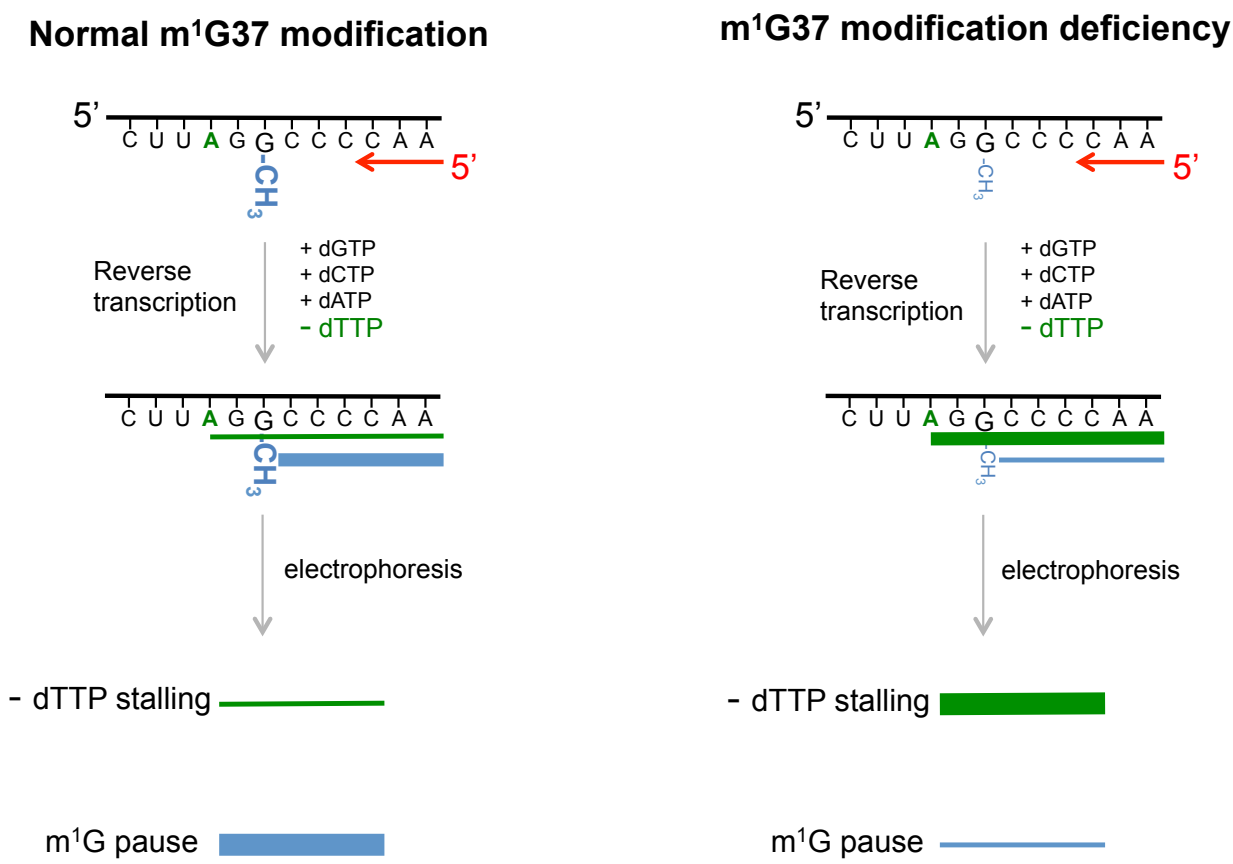

Figure S3 - Detection of N^1-Methylguanosine (m^1G) by Reverse Transcription Primer Extension (RT-PEx).

A DNA primer (red arrow) is extended on an RNA template towards the modification site (blue "–CH3"). N^1-methylguanosine does not efficiently form the standard Watson-Crick base G:C pair, thereby causing the reverse transcriptase to pause when incorporating cytosine and blocking extension past the modification site (thick blue line). In addition, the reverse transcription reaction is performed in the absence of a dNTP, in this example dTTP (green). The lack of dTTP causes stalling of the reaction shortly downstream from the modification site when an adenine nucleotide is encountered (thin green line). Deficient modification (right) causes less prominent pausing at the m^1G site (thin blue line) and allows for a more prolonged extension reaction until the reverse transcriptase stalls owing to the lack of dTTP (thick green line). The ratio between m^1G-specific extension product (blue) and –dTTP-specific readthrough product (green) is proportional to the abundance of the modification.

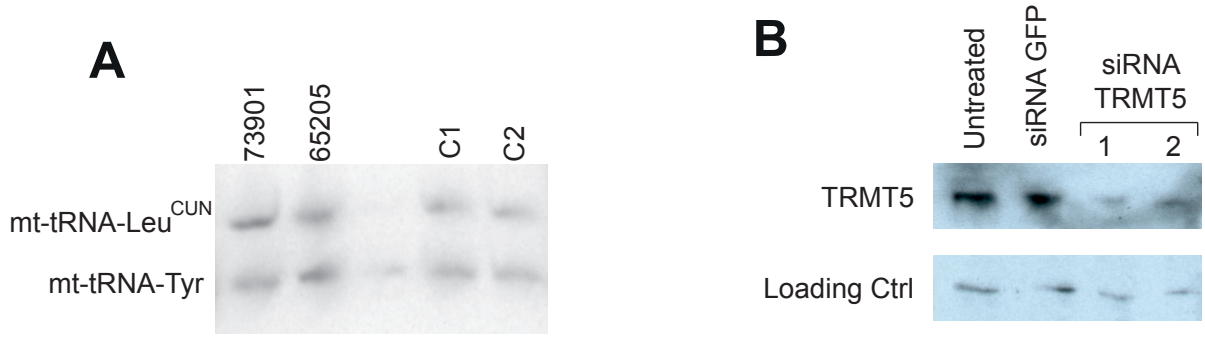

Figure S4 - The steady-state levels of mt-tRNA in TRMT5 fibroblasts and downregulation of TRMT5 expression by siRNA

(A) High-resolution northern blot analysis of total RNA isolated from the *TRMT5*-mutated (73901 and 65205) or control (C1 and C2) primary fibroblasts. The blots were probed with the mt-tRNA-specific probes as indicated.

(B) Western blot of total HeLa cell lysate transfected with two different siRNAs to TRMT5 for 6 days. siRNA to GFP was used as transfection control. A control band is shown to evidence equal protein sample loading.

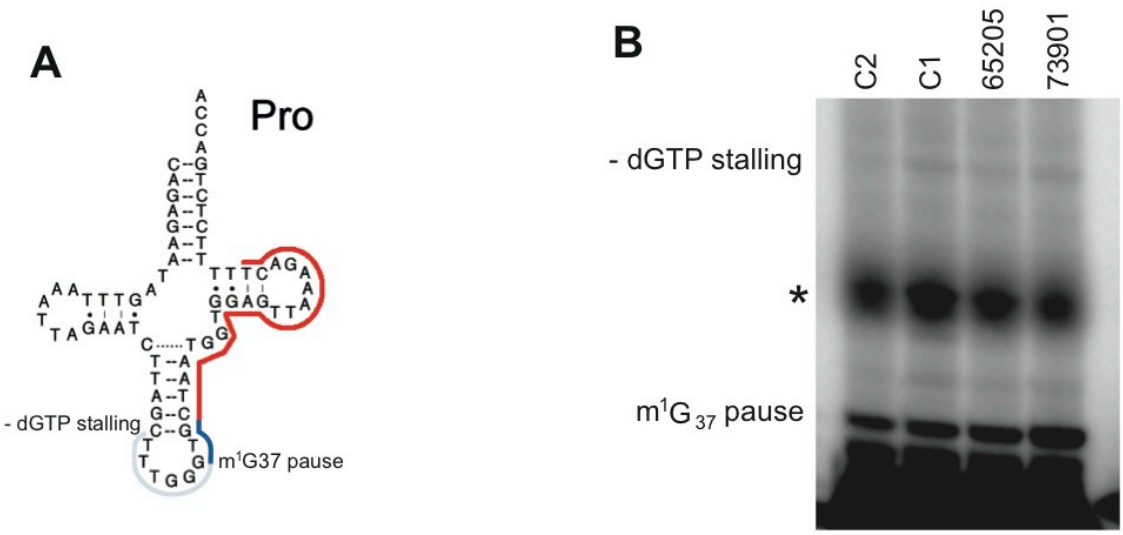

Figure S5. Modification of G37 in mitochondrial tRNA^{Pro} in affected individuals

(A) A radioactively-labeled, complementary primer (red) is annealed to mt-tRNA^{Pro} and subjected to a reverse transcriptase primer extension (RT-PEx) reaction. The presence of m^{1}G37 results in RT-Ex pausing producing a shorter product (dark blue). The extension reaction stalls due to the absence of a dGTP (light blue), producing a longer product.

(B) Separation and detection of products of RT-PEx reactions as per (A) on control (C1 and C2) and patient-derived (65205 and 73901) RNA extracted from skin fibroblasts. The asterisk denotes unspecific RT-PEx band.

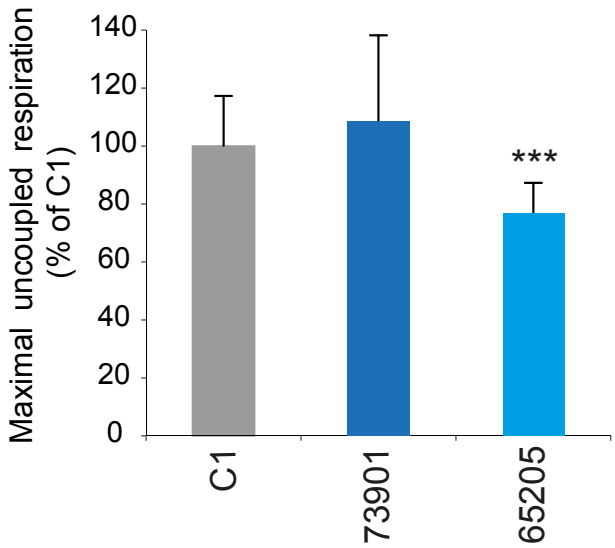

Figure S6. Cellular respiration of fibroblasts from affected individuals

Maximal uncoupled respiration of fibroblast cell lines from affected individuals and a control (C1) cultured in high-glucose medium. Each analysis was performed in more than 20 replicates. Data has been normalized to the number of cells after the respiration measurement and is expressed as percentage of control (C1). Error bar = 1SD, *** = $p<0.001$. (Students unpaired T-Test).