

Supplemental Material

Disease associated mutations in mitochondrial precursor tRNAs affect binding, m1R9 methylation and tRNA processing by mtRNase P

Agnes Karasik¹, Catherine A. Wilhelm², Carol A. Fierke³, Markos Koutmos^{4*}

¹Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, Bethesda, MD, 20814, USA

²Department of Chemistry, University of Michigan, Ann Arbor, MI, 48109, USA

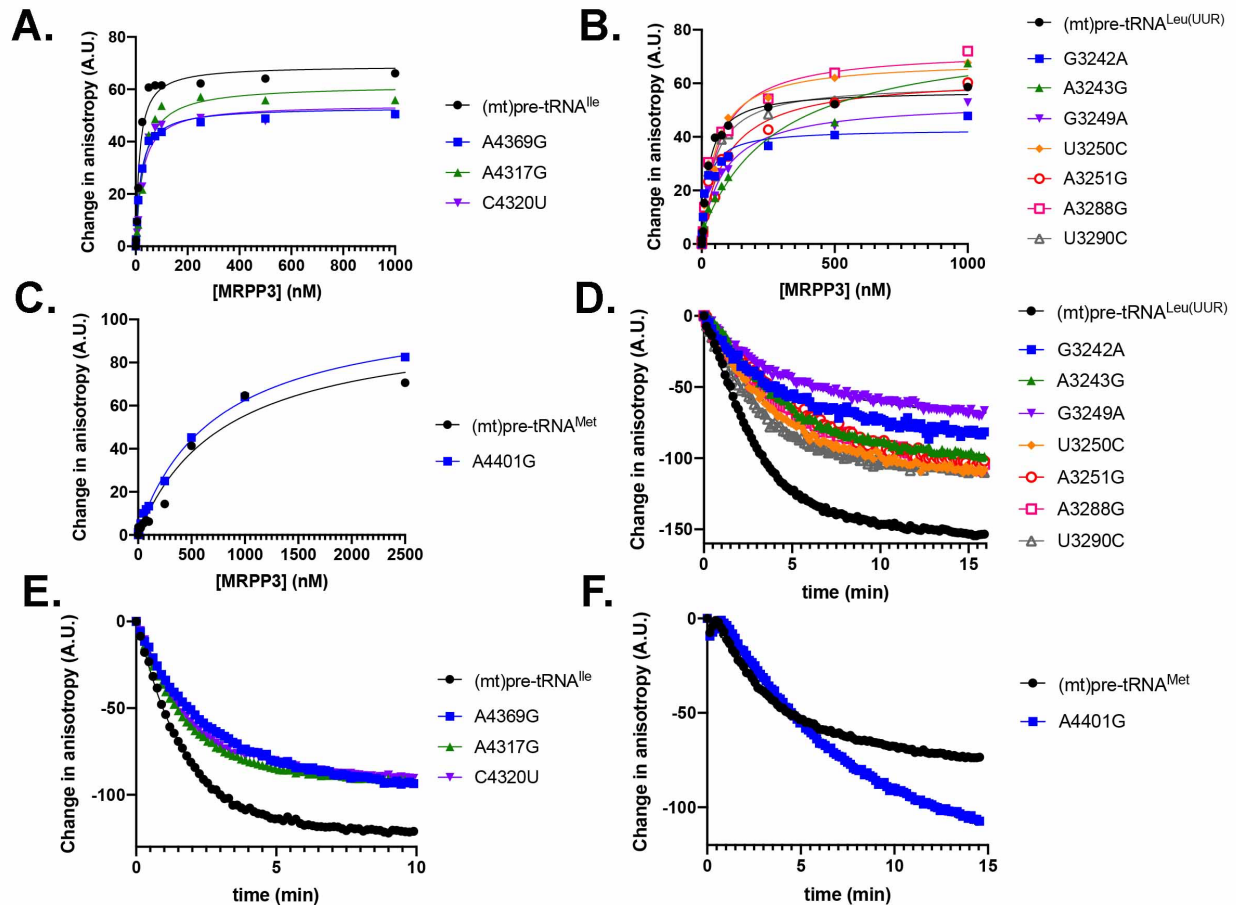
³Department of Chemistry, Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109, USA

⁴Department of Chemistry, Program in Biophysics, University of Michigan, Ann Arbor, MI, 48109, USA

Supplemental Table 1. Melting temperature values for first transitions for wild type (mt)pre-tRNAs and (mt)pre-tRNA^{Ile} mutants.

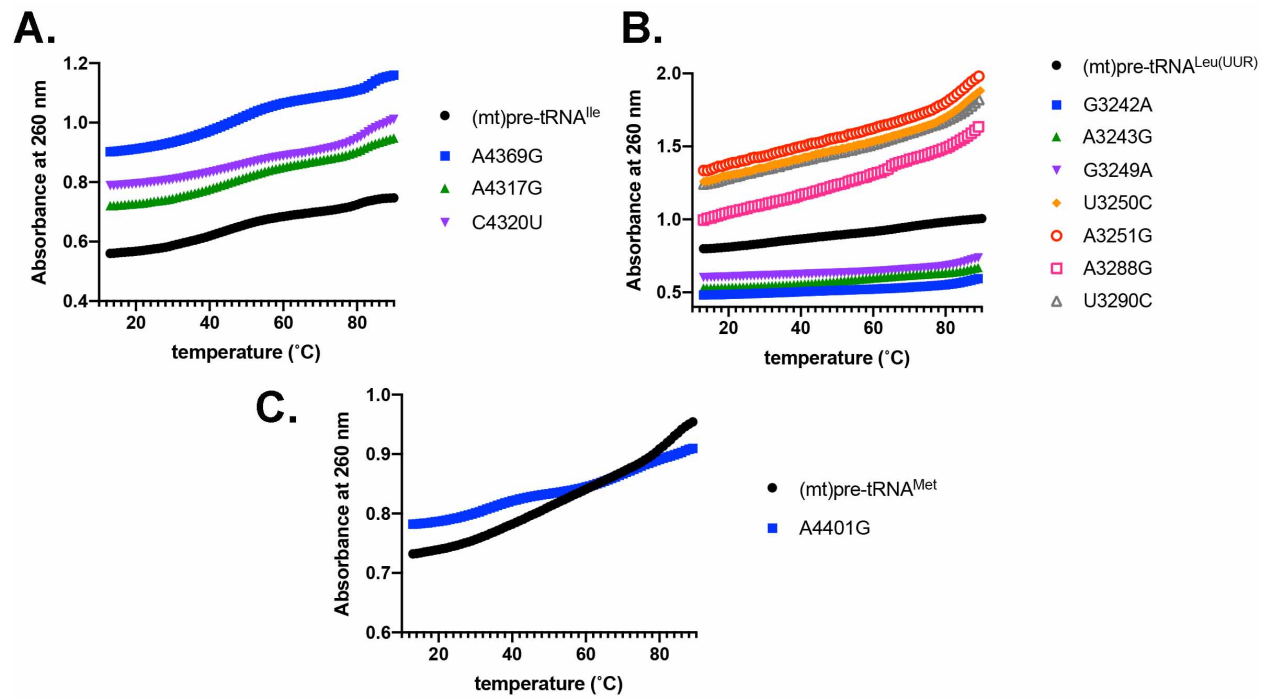
human mitochondrial pre-tRNA	T _{m1} (°C)	T _{m2} (°C)
(mt)pre-tRNA ^{Leu(UUR)}	28.7 ± 0.1	-
(mt)pre-tRNA ^{Met}	32.6 ± 1.1	
(mt)pre-tRNA ^{Ile}	47.7 ± 1.6	
A4269G	40.4 ± 5.4	49.3 ± 1.0
A4317G	42.5 ± 0.9	56.2 ± 0.5
C4320U	27.7 ± 3.7	47.5 ± 0.4

Supplemental Figure 1.



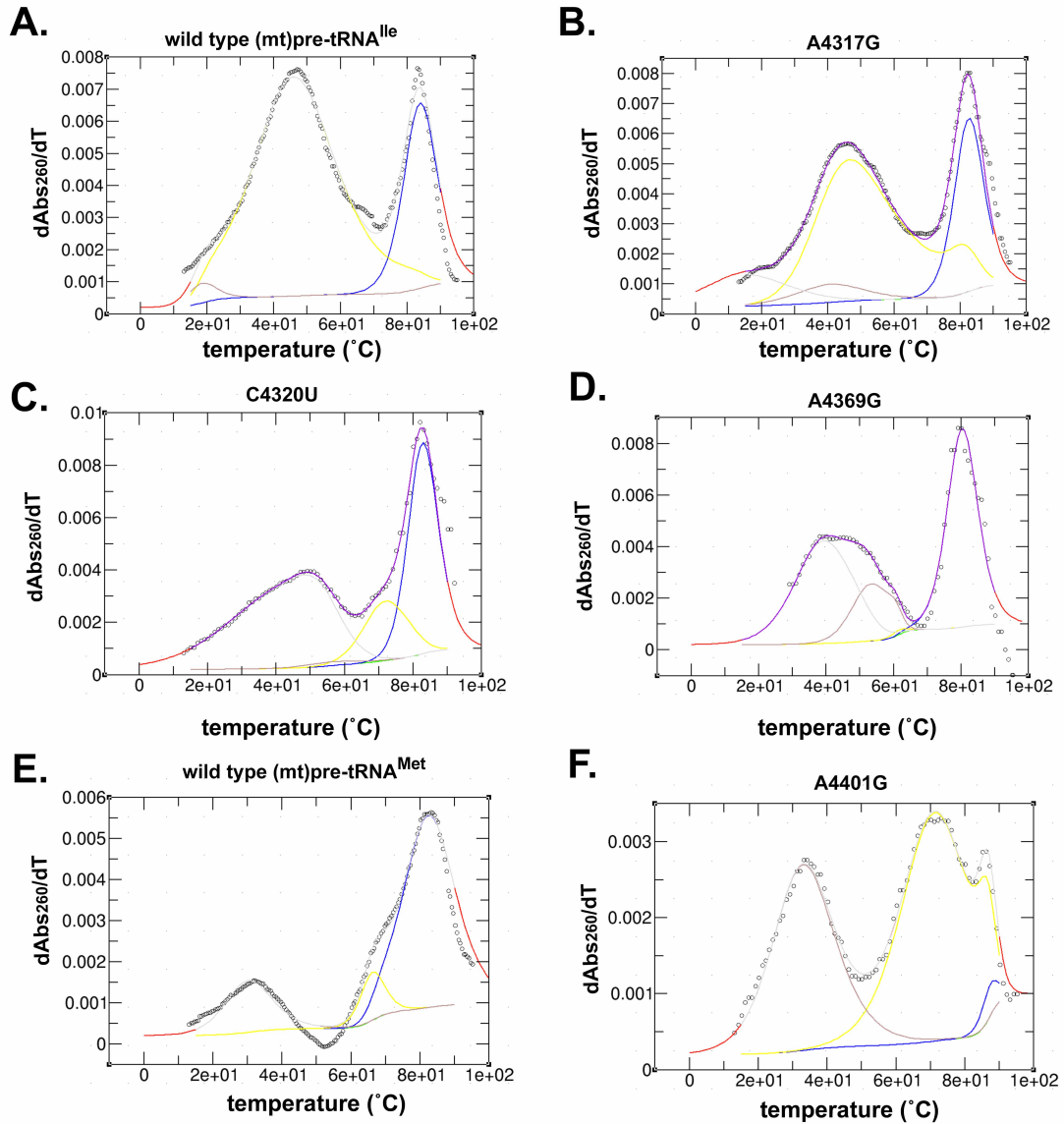
Supplemental Figure 1. A. Representative curves with fitting from fluorescent polarization binding (**A-C**) and single turnover assays (**D-F**). Wild type and a mutant (mt)pre-tRNA binding in the function of MRPP3 concentrations were measured in the presence of 150 nM MRPP1/2 and using standard binding assay conditions (see Methods). Single turnover cleavage of a wild type and a mutant (mt)pre-tRNA were obtained using standard cleavage assay conditions. Plots were generated by Prism 8.1.0.

Supplemental Figure 2.

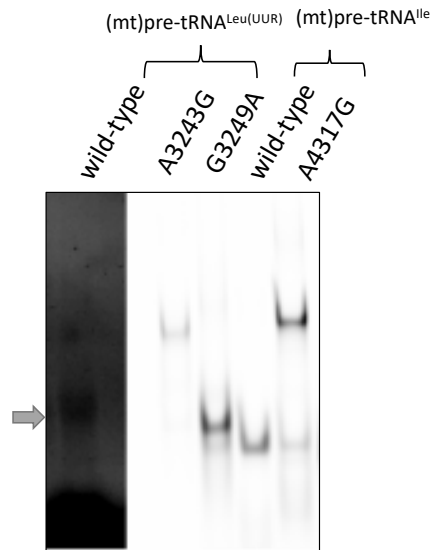


Supplemental Figure 2. Representative UV melting curves for the investigated pre-tRNAs used in this study. Plots were generated by Prism 8.1.0.

Supplemental Figure 3.



Supplemental Figure 3. Examples of fitting data from UV melting experiments by fitUVData.py and Global Melt Fit for different (mt)pre-tRNAs.



Supplemental Figure 4. Native polyacrylamide gel electrophoresis (PAGE) of selected mutant (mt)pre-tRNA investigated in this study. Note that left panel is the image of the same overexposed gel as in the right panel. This overexposure was necessary to visualize wild type (mt)tRNA^{Leu(UUR)} (grey arrow).