Supplementary information

Complete chemical structures of human mitochondrial tRNAs

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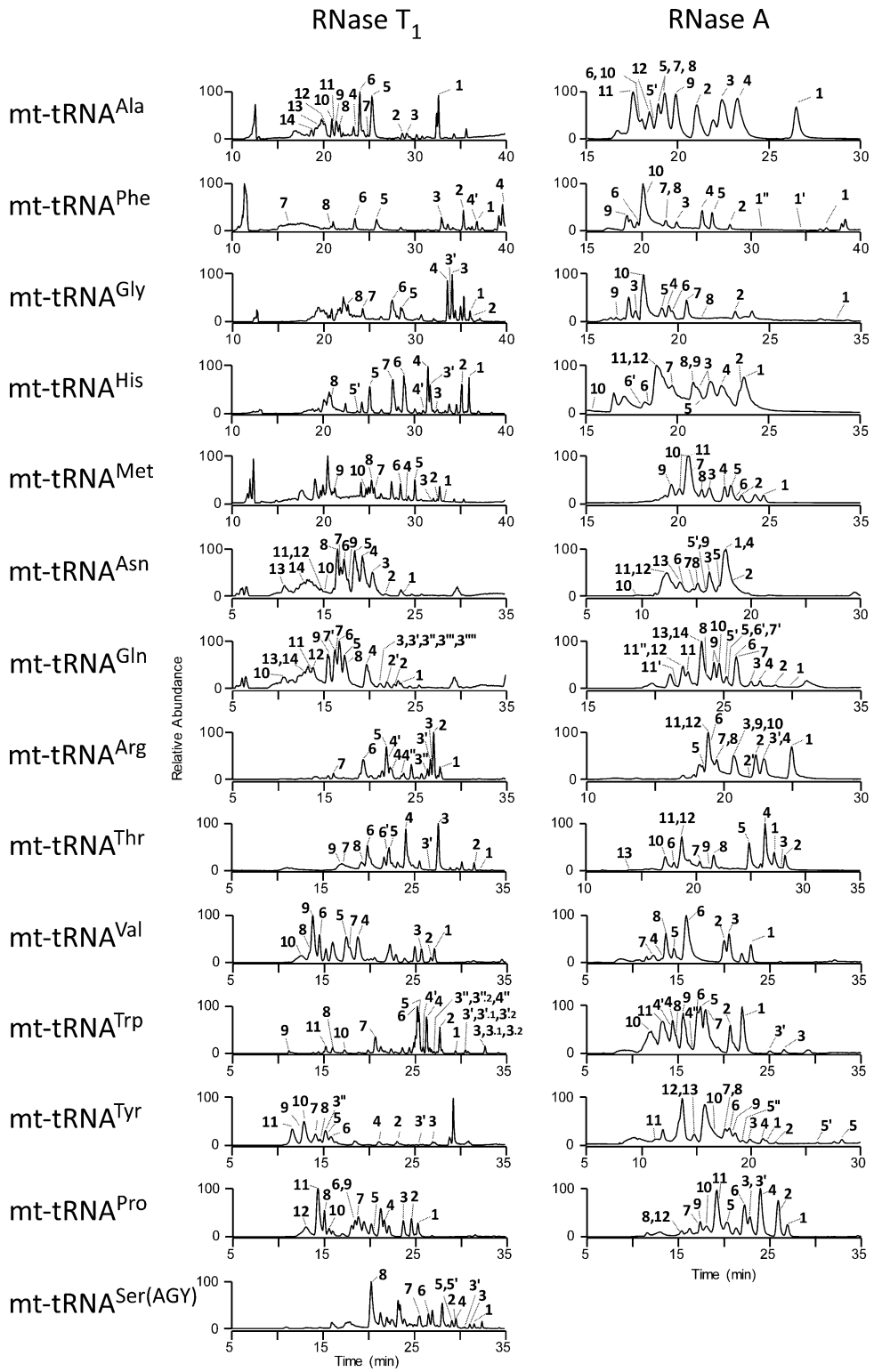
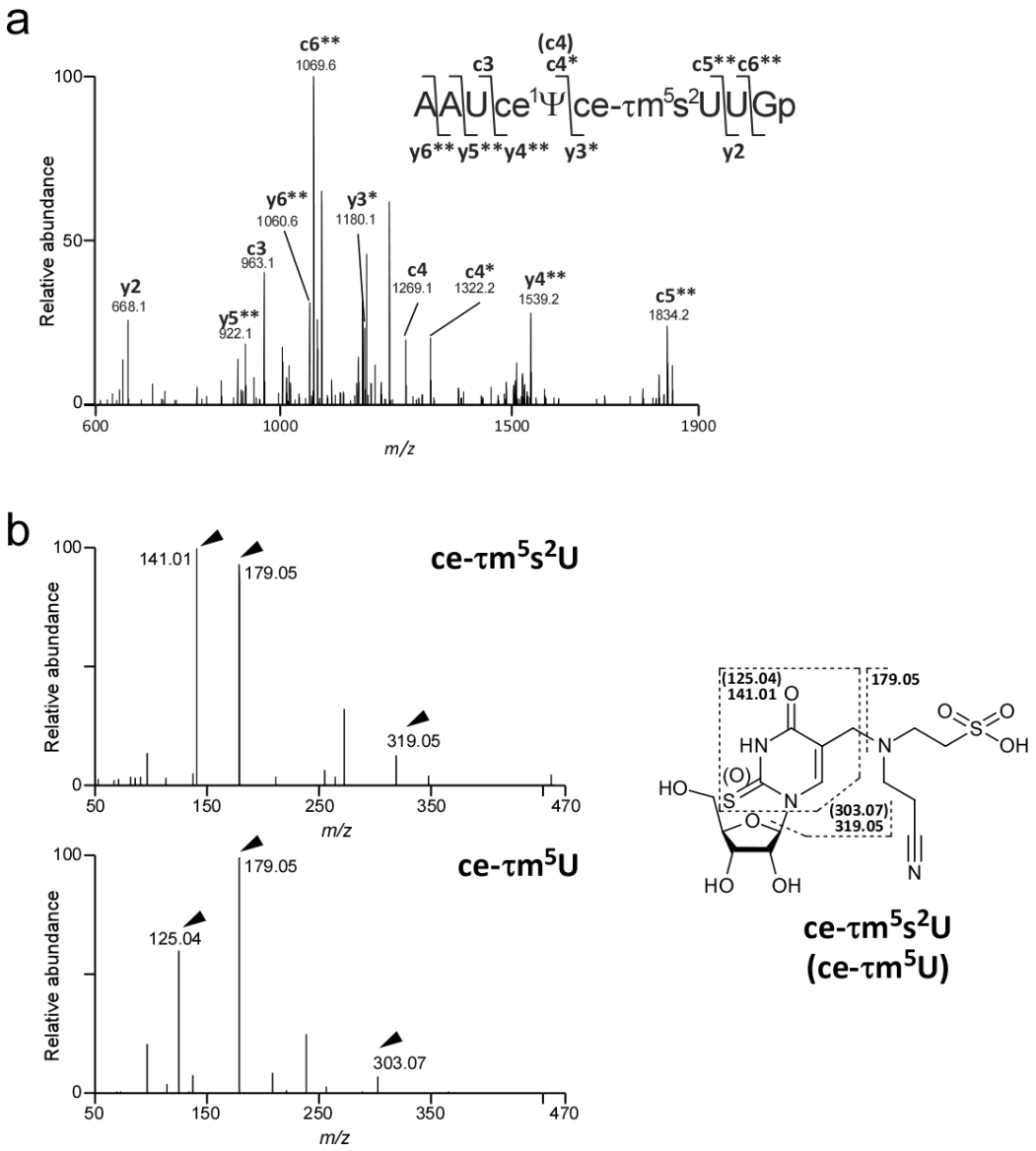


Supplementary Figure 1. RNA fragment analysis of human mt-tRNAs.

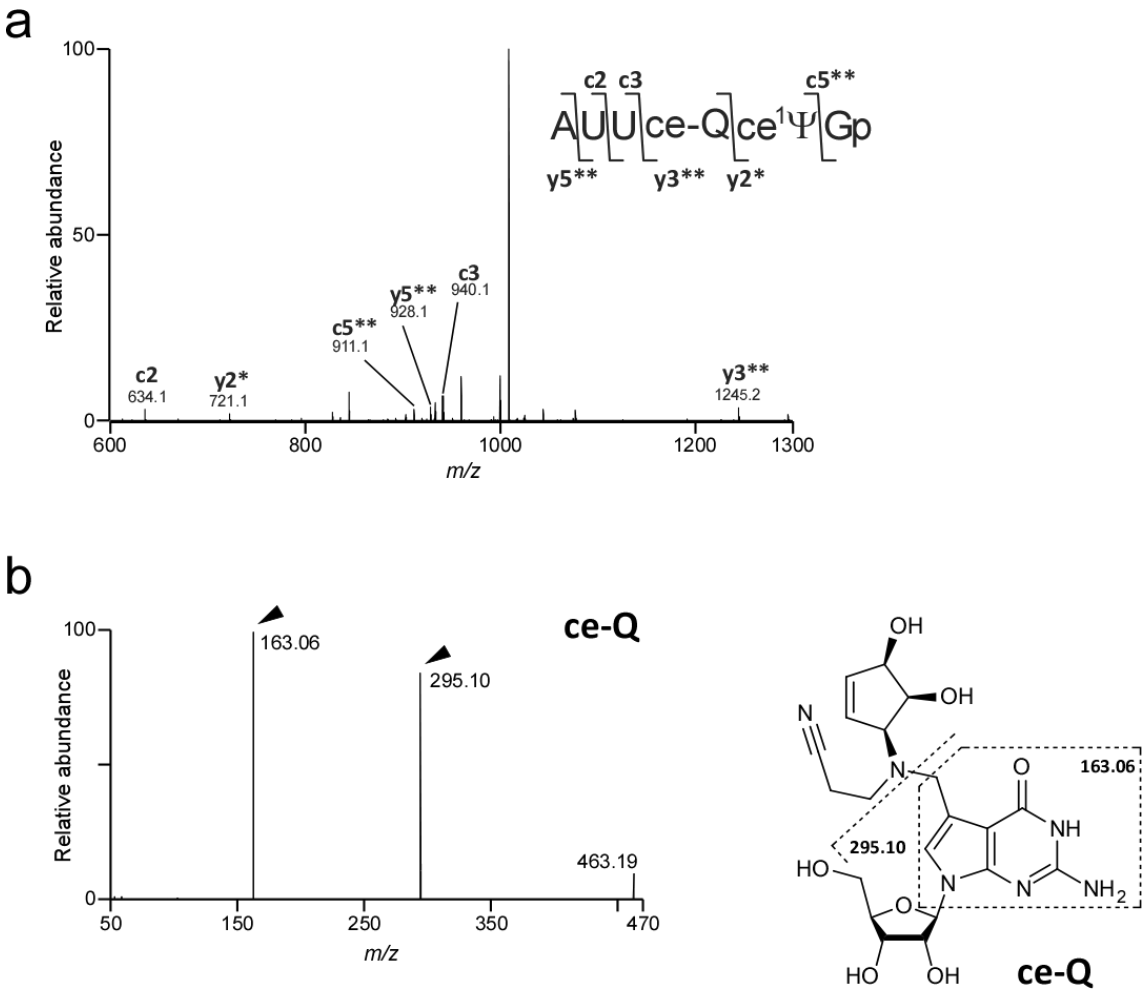
Base peak chromatogram (BPC) for each tRNA digested by RNase T_1 (left panels) and RNase A (right panels). All assigned fragments (numbered) listed in Supplementary Data 1.



Supplementary Figure 2. Cyanoethylation of τm^{5}s^{2}U34

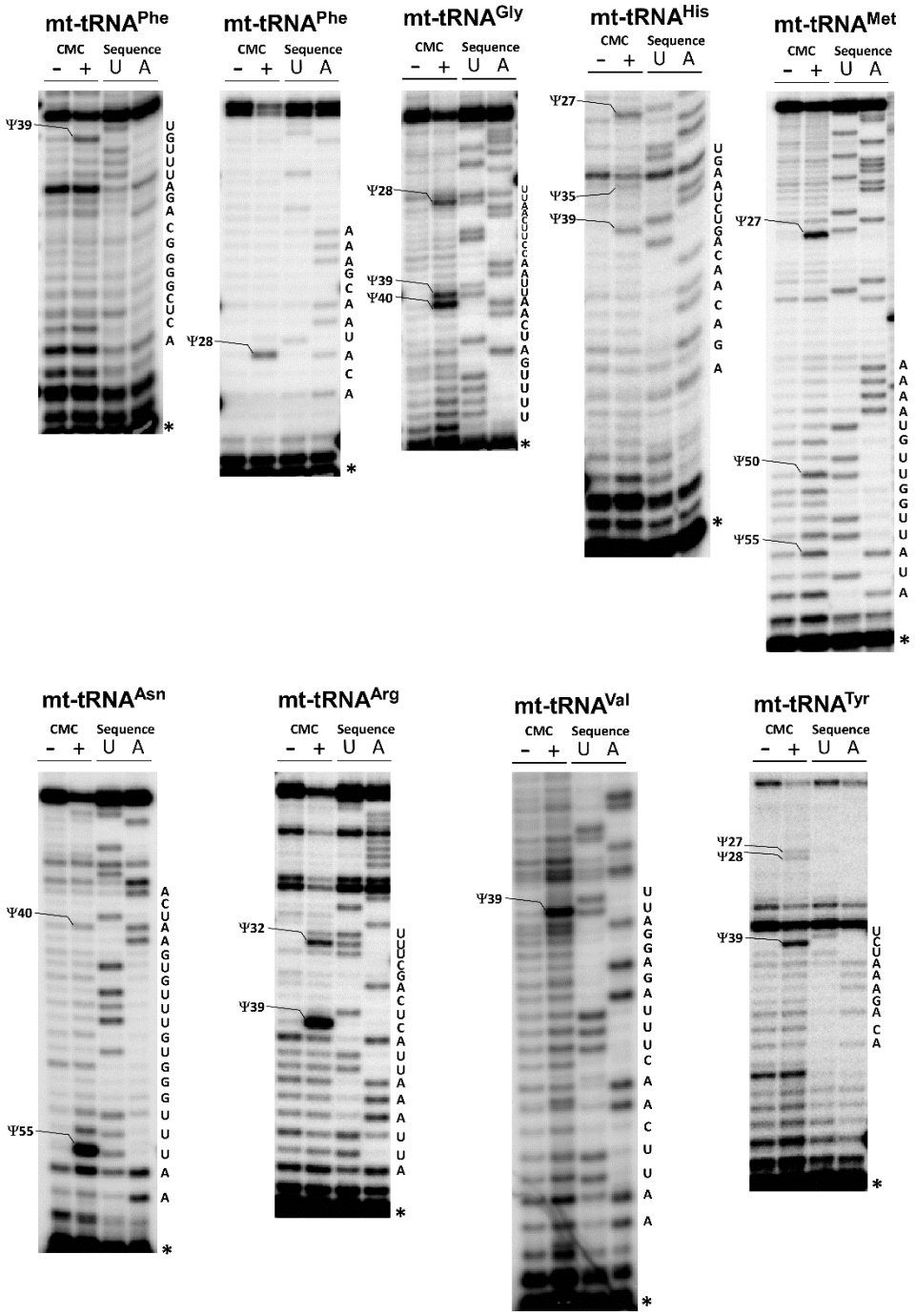
(a) CID spectrum of a di-cyanoethylated fragment from human mt-tRNA^{Gln}. The doubly charged negative ion of the RNA fragment (*m/z* 1251.1) was used as a precursor for CID. Assignment of the product ions revealed two cyanoethylation sites at Ψ33 and τm^{5}s^{2}U34. Asterisks marked on the product ions indicate the number of cyanoethylations.

(b) CID spectra (left panels) of the cyanoethylated τm^{5}s^{2}U and τm^{5}U nucleosides [ce-τm^5(s^2)U] indicate that cyanoethylation occurs at the N atom in the taurine moiety. The predicted chemical structure of the derivative and its dissociation patterns, with *m/z* values of the product ions, are shown on the right. Assigned product ions are indicated by arrowheads in the CID spectra.



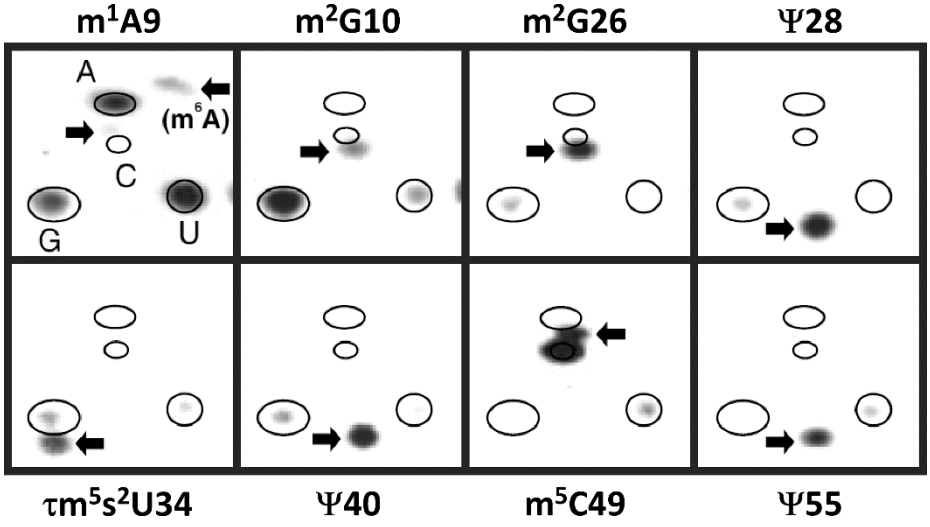
Supplementary Figure 3. Cyanoethylation of Q34

(a) CID spectrum of a di-cyanoethylated fragment from human mt-tRNA^{His}. The doubly charged negative ion of the RNA fragment (*m/z* 1092.7) was used as a precursor for CID. Assignment of the product ions revealed two cyanoethylation sites at Q34 and Ψ35. Asterisks marked on the product ions indicate the number of cyanoethylations. (b) CID spectrum (left panel) of the cyanoethylated Q34 nucleosides (ce-Q) indicates that cyanoethylation occurs at the N atom in the side chain. The predicted chemical structure of the derivative and its dissociation patterns, with *m/z* values of the product ions, are shown on the right. Assigned product ions are indicated by arrowheads in the CID spectrum.



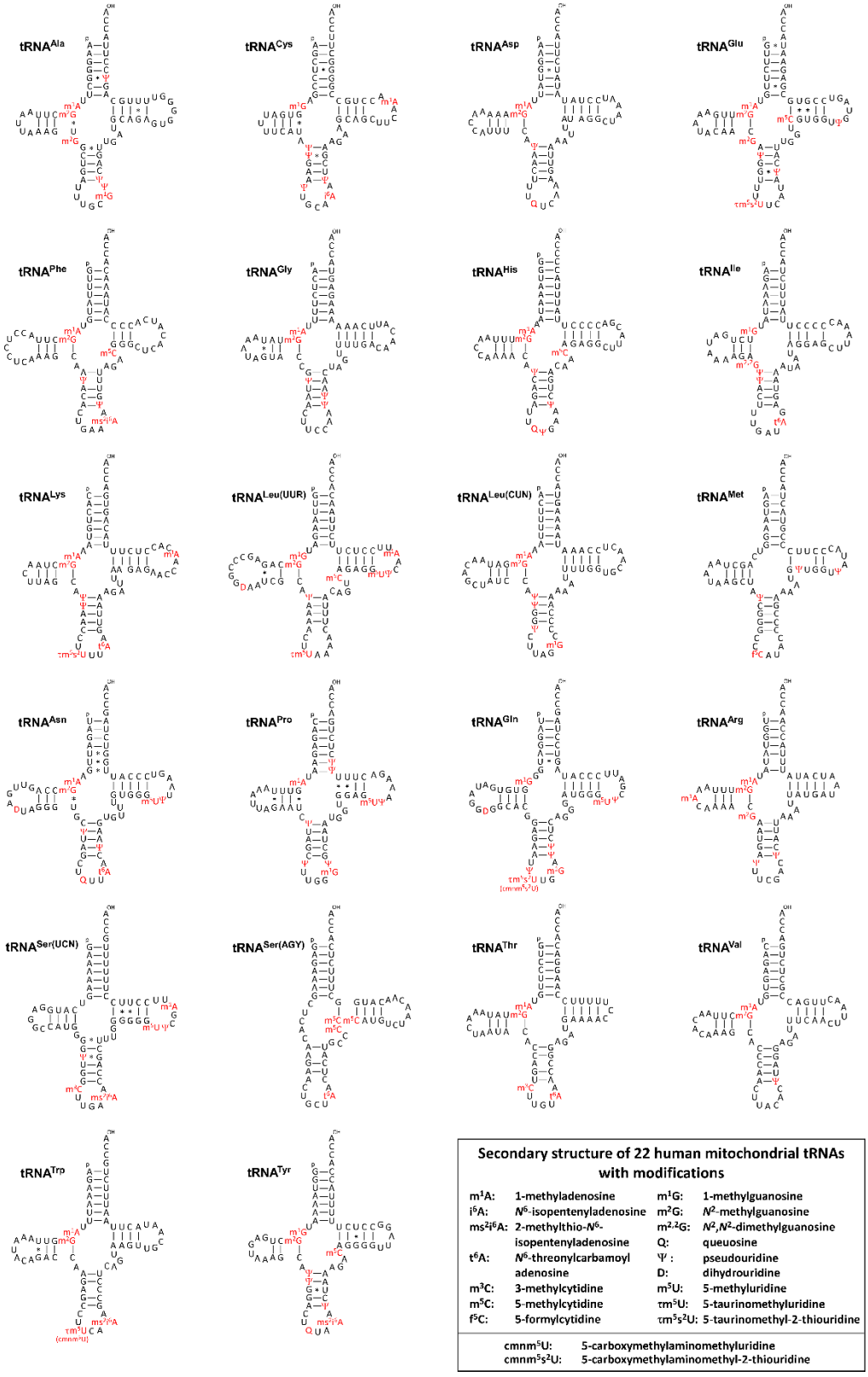
Supplementary Figure 4. CMC-PE analysis for detection of Ψ sites in human mt-tRNAs

HeLa total RNA treated with (+) or without (-) CMC were reverse-transcribed with a primer specific for each human mt-tRNA. The sequence ladders for U and A were generated under the same conditions in the presence of ddATP and ddTTP, respectively. Positions of Ψs and sequence are shown with each gel image. Source data are provided as a Source Data file.



Supplementary Figure 5. Post-transcriptional modifications in human mt-tRNA^{Glu}, determined by the post-labeling method.

Eight modifications in human mt-tRNA^{Glu} were determined by 2D-TLC. The spot corresponding to each modification is indicated by an arrow. Separation patterns for unmodified nucleotide 5'-monophosphates (A, C, G and U) are indicated by ellipses.



Supplementary Figure 6. Post-transcriptional modifications in all species of human mt-tRNAs.

Supplementary Table 1. List of confirmed and predicted genes responsible for post-transcriptional modifications in human mitochondrial tRNAs

Position [a]	tRNA species	Modification [b]	Confirmed gene(s) in human or mammals	Predicted gene(s) in human	Human diseases
9	Ala, Asp, Glu, Phe, Gly, His, Lys, Leu(CUN), Asn, Pro, Arg, Thr, Val, Trp	m^1A	*MRPP1* and *MRPP2* (formerly known as *TRMT10C* and *SDR5C1*, respectively) [1]		HSD10 disease [2]
9	Cys, Ile, Leu(UUR), Gln, Tyr	m^1G	*MRPP1* and *MRPP2* [1]		HSD10 disease [2]
10	Ala, Asp, Glu, Phe, Gly, His, Lys, Leu(UUR), Leu(CUN), Asn, Arg, Thr, Val, Trp, Tyr	m^2G		*TRMT11* and *TRMT112* [3]	
16	Arg	m^1A		*TRMT61B* [4,5]	
20	Leu(UUR), Asn, Gln	D		*DUS2* [6]	
26	Ala, Glu, Arg	m^2G	*TRMT1* [7]		Intellectual disability [8,9]
26	Ile	$m^{2,2}G$	*TRMT1* [7,10,11]		Intellectual disability [8,9]
27	Cys, Asp, His, Ile, Lys, Leu(UUR), Leu(CUN), Met, Pro, Tyr	Ψ	*PUS1* [12]		MLASA [13]
28	Cys, Glu, Phe, Gly, Ile, Lys, Leu(CUN), Asn, Ser(UCN), Tyr	Ψ	*PUS1* [12]		MLASA [13]
31	Leu(CUN)	Ψ		*RPUSD1, 2, 3 or 4*	
32	Cys, Pro, Arg	Ψ		*RPUSD1, 2, 3 or 4*	

32	Ser(UCN), Thr	m^3C		*METTL2A*, *2B*, *6* or *8* [14-16]	
33	Gln	Ψ		*RPUSD1, 2, 3 or 4*	
34	Leu(UUR), Trp, (Glu, Lys, Gln)	τm^5U	*GTPBP3* and *MTO1* [17,18]		MELAS (lack of τm^5U in mutant $tRNA^{Leu(UUR)}$) [19,20] Hypertrophic cardiomyopathy and lactic acidosis and encephalopathy, Leigh syndrome [21,22] Hypertrophic cardiomyopathy and lactic acidosis [23-25]
34	Glu, Lys, Gln	τm^5s^2U [c]	*MTU1* [c] [26,27] and	*NFS1* [c] [28]	MERRF (lack of τm^5s^2U in mutant $tRNA^{Lys}$) [29] RILF [30-33]
34	Met	f^5C	*NSUN3* [34-36] and *ALKBH1* [36,37]		Combined mitochondrial respiratory chain complex deficiency [35]
34	Asp, His, Asn, Tyr	Q	*QTRT1* and *QTRT2* (This work)		
35	His	Ψ		*PUS7* [38]	
37	Ile, Lys, Asn, Ser(AGY), Thr	t^6A	*YRDC* and *OSGEPL1* [39]		
37	Cys, (Phe, Ser(UCN), Trp, Tyr)	i^6A	*TRIT1* [40]		Encephalopathy and myoclonic epilepsy with multiple OXPHOS deficiencies [41].
37	Phe, Ser(UCN), Trp, Tyr	ms^2i^6A [d]	*CDK5RAP1* [d] [42,43]		
37	Ala, Leu(CUN), Pro, Gln	m^1G	*TRMT5* [44,45]		Multiple mitochondrial respiratory chain complex deficiencies [45]
38	Ala, Pro	Ψ		*PUS3* [46]	
39	Ala, Cys, Phe, Gly,	Ψ	*RPUSD4* [e] [47]		

	His, Gln, Arg, Val, Tyr				
40	Glu, Gly, Asn, Gln	Ψ		*PUS3*	
48	Phe, His, Leu(UUR), Ser(AGY), Tyr	m^5C	*NSUN2* [48,49]		Intellectual disability [50-52]
49	Glu, Ser(AGY)	m^5C	*NSUN2* [48,49]		Intellectual disability [50-52]
50	Ser(AGY)	m^5C	*NSUN2* [48,49]		Intellectual disability [50-52]
50	Met	Ψ		*RPUSD4* [47]	
54	Leu(UUR), Asn, Pro, Gln, Ser(UCN)	m^5U	*TRMT2B* [53,54]		
55	Glu, Leu(UUR), Met, Asn, Pro, Gln, Ser(UCN)	Ψ		*TRUB2* [55]	
58	Cys, Lys, Leu(UUR), Ser(UCN)	m^1A	*TRMT61B* [4]		
66	Pro	Ψ		*PUS1*	
67	Pro	Ψ		*PUS1* [56]	
68	Ala	Ψ		*PUS1*	

a: The numbering system for tRNA refers to the tRNAdb compilation [57].

b: Symbols for modifications originate from MODOMICS (http://modomics.genesilico.pl/) [58].

c: MTU1 and NFS1 are involved in 2-thiolation of τm^5s^2U34. *MTU1* (Mitochondrial tRNA-specific 2-thiouridylase 1) is also known as *TRMU*; the name originated from bacterial *trmU* (tRNA methyltransferase U). However, *trmU* (renamed *mnmA*) was mis-annotated, as it is not a tRNA methyltransferase. **d**: CDK5RAP1 is required for 2-methylthiolation of ms^2i^6A37 following 6-isopentenylation of A37. **e**: The literature [47] described that Ψ39 in $tRNA^{Gly}$ was not affected by knockdown of the gene.

MLASA, mitochondrial myopathy and dideroblastic anemia. MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes. MERRF, myoclonus epilepsy associated with ragged-red fibers. RILF, reversible infantile liver failure.

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