Supplementary Information for "Altered tRNA processing is linked to a distinct and unusual La protein in *Tetrahymena thermophila*"

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Table S3. Raw counts for 3'-U ending and 3'-CCA ending tRNAs from raw .fastq files from wild type (WT) and partial Mlp1 knockout (KO) strains.

Table S4. Number of tRNA genes encoded in the genome of different eukaryotic species for each isotype (top) and isoacceptor (bottom).

Table S5. List of oligonucleotides used in this study.

Table S6. NCBI accession numbers of primary amino acid sequences used for conservation analysis.

S1

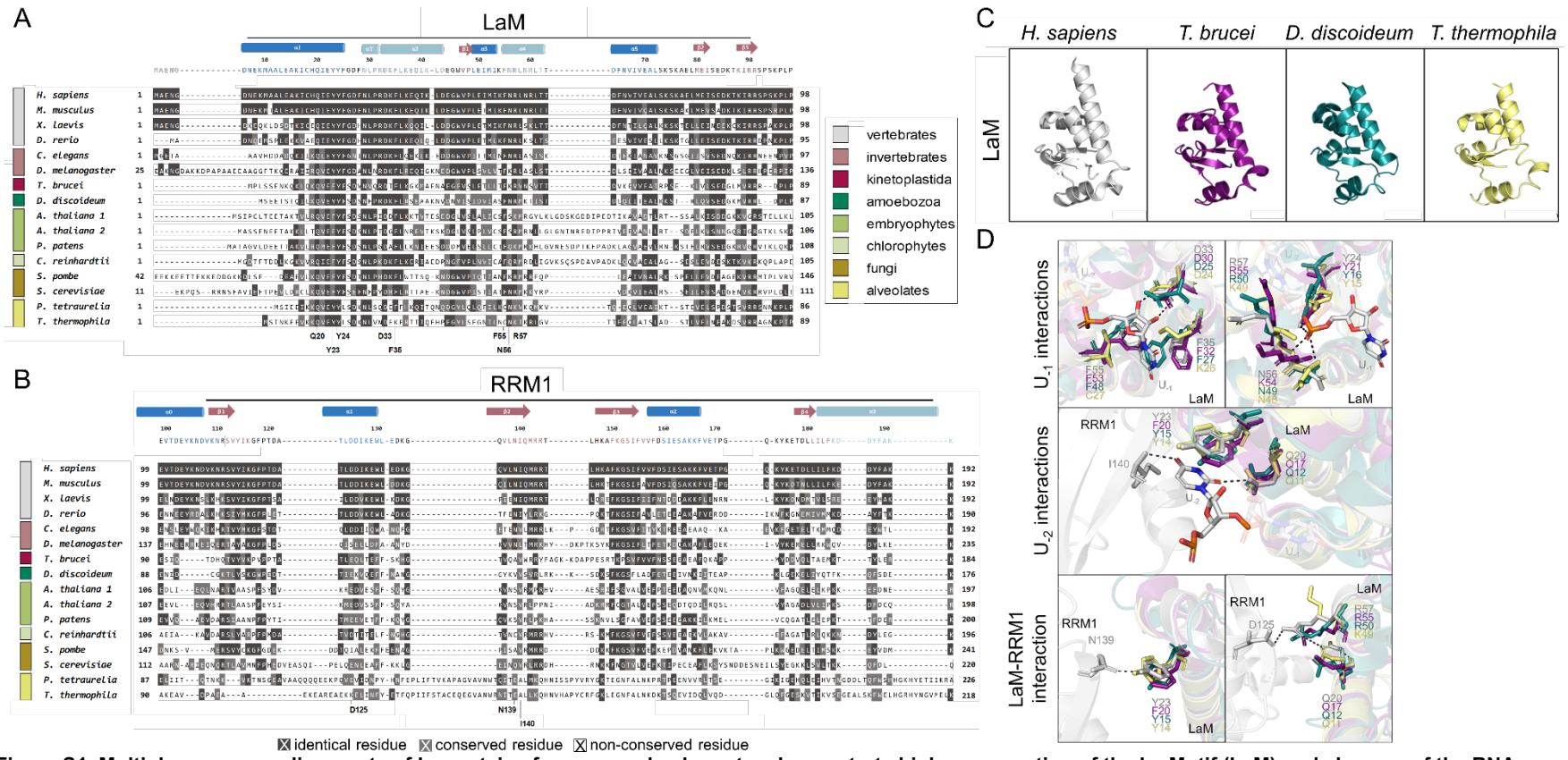


Figure S1. Multiple sequence alignments of La proteins from several eukaryotes demonstrate high conservation of the La Motif (LaM) and absence of the RNA recognition motif-1 (RRM1).

(A,B) Primary amino acid alignments of full RNA-binding domains LaM and RRM1 from different eukaryotic lineages. A dark grey background indicates identical residues, light grey conserved residues and white indicates no conservation. Most uridylate binding residues are conserved in the LaM of alveolates, whereas residues in the RRM1 are more variable. Important uridylate binding residues are shown at the bottom. The secondary structure motif of the high-resolution hLa protein structure is shown at the top with β-sheets shown in red and α-helices in blue (dark blue: typical α-helices found in the winged-helix fold and classic RRM, light blue: inserted α-helices found in La proteins specifically) (PDB: 2VOD).

(C) High-resolution structures of the LaM in different species: *Homo sapiens* in white (PDB: 2VOD)[14], *Trypanosoma brucei* in purple (PDB: 1S29)[10], *Dictyostelium discoideum* in teal (PDB: 2M5W)[56] and the predicted tertiary structure *for Tetrahymena thermophila* in yellow[42].

(D) Magnified views of La-U$_{-1}$ RNA, La-U$_{-2}$ and LaM-RRM interactions. U$_{-1}$ is the most 3'-terminal uridylate possessing 2'-OH and 3'-OH ends. Most uridylate binding residues located in the LaM are conserved in *Tetrahymena thermophila,* except for F35 and F55 (hLa numbering) which bind the most 3'-terminal U$_{-1}$. Carbon atoms within the RNA (U$_{-1}$ and U$_{-2}$) from the *Homo sapiens* La structure are colored in white with other atoms colored by type: oxygen in red; nitrogen in blue and phosphorous in orange. Hydrogen bonds are shown as black dashed lines.

S2

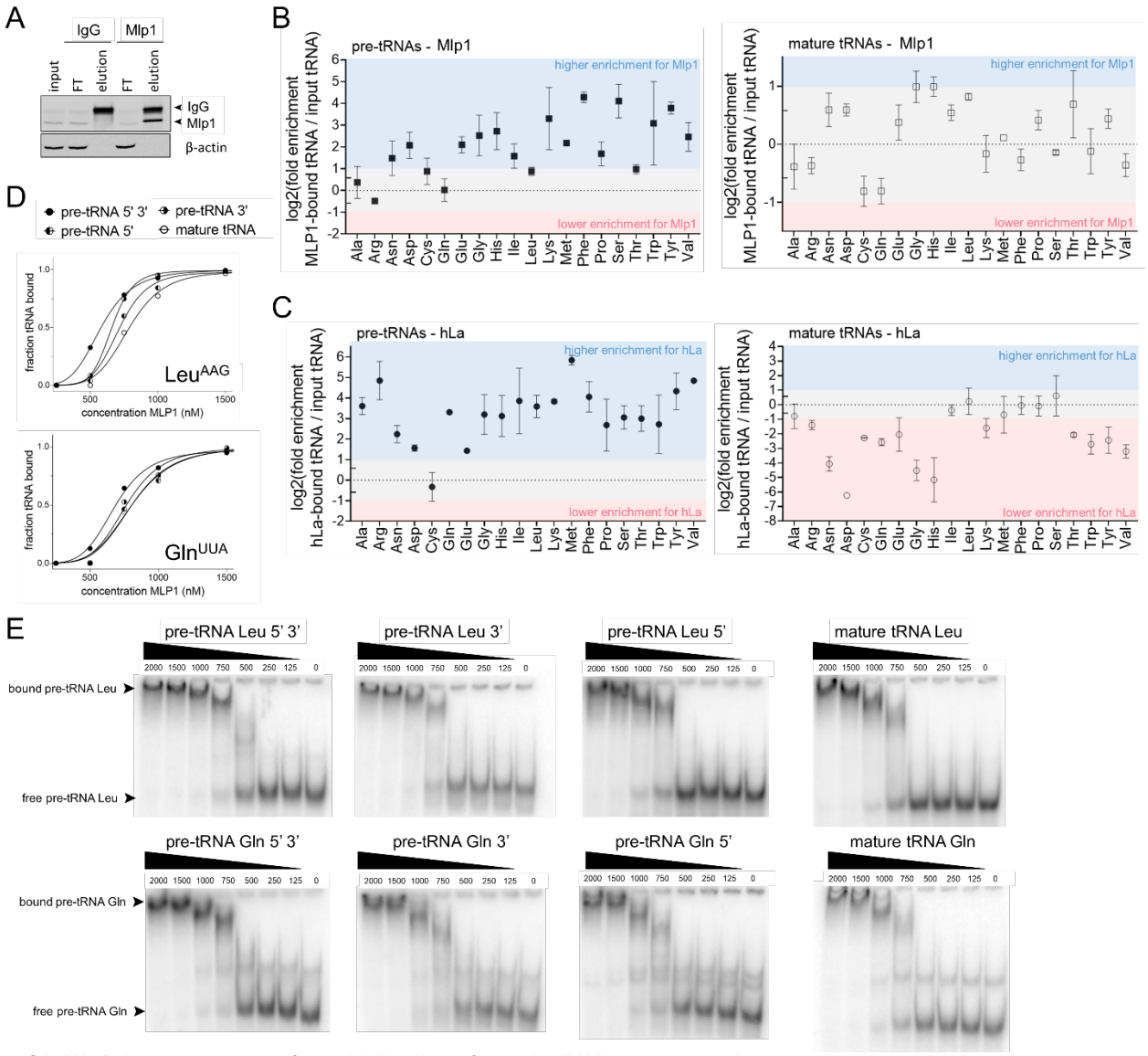


Figure S2. Mlp1 demonstrates preferential binding of certain tRNA isotypes and unprocessed pre-tRNAs.
(A) Western blot confirming Mlp1-specific immunoprecipitation from *Tetrahymena thermophila* using an affinity purified rabbit anti-Mlp1 antibody and a rabbit isotype immunoglobin G (IgG) control. The Mlp1-specific antibody was used for both ribonucleoprotein-immunoprecipitation (RNP-IP) and western blotting. The heavy chain (50 kDa) of the antibodies was detected with the secondary anti-rabbit antibody and shown as IgG. Loading control: β-actin.
(B-C) Next generation sequencing data of tRNAs split by tRNA isotypes for Mlp1 (n=3) (B) and hLa from Gogakos *et al*. (n=2) (C). Fold enrichment is shown as the log2 transformed ratio between Mlp1- or hLa-immunoprecipitated tRNAs and input tRNAs. Both Mlp1 and hLa have a higher binding affinity for pre-tRNAs compared to mature tRNAs.
(D-E) Binding curves from EMSAs (D) and corresponding native gels (E) comparing binding affinity of Mlp1 between ^{32}P-labeled pre-tRNA containing 5'- and 3'-extensions or 5'- or 3'-extensions and mature tRNA for Leu^{AAG} and Gln^{UUA}. The highest binding affinity is found for 5'- and 3'-end containing pre-tRNAs. See Table S1 for Kd quantifications.

S3

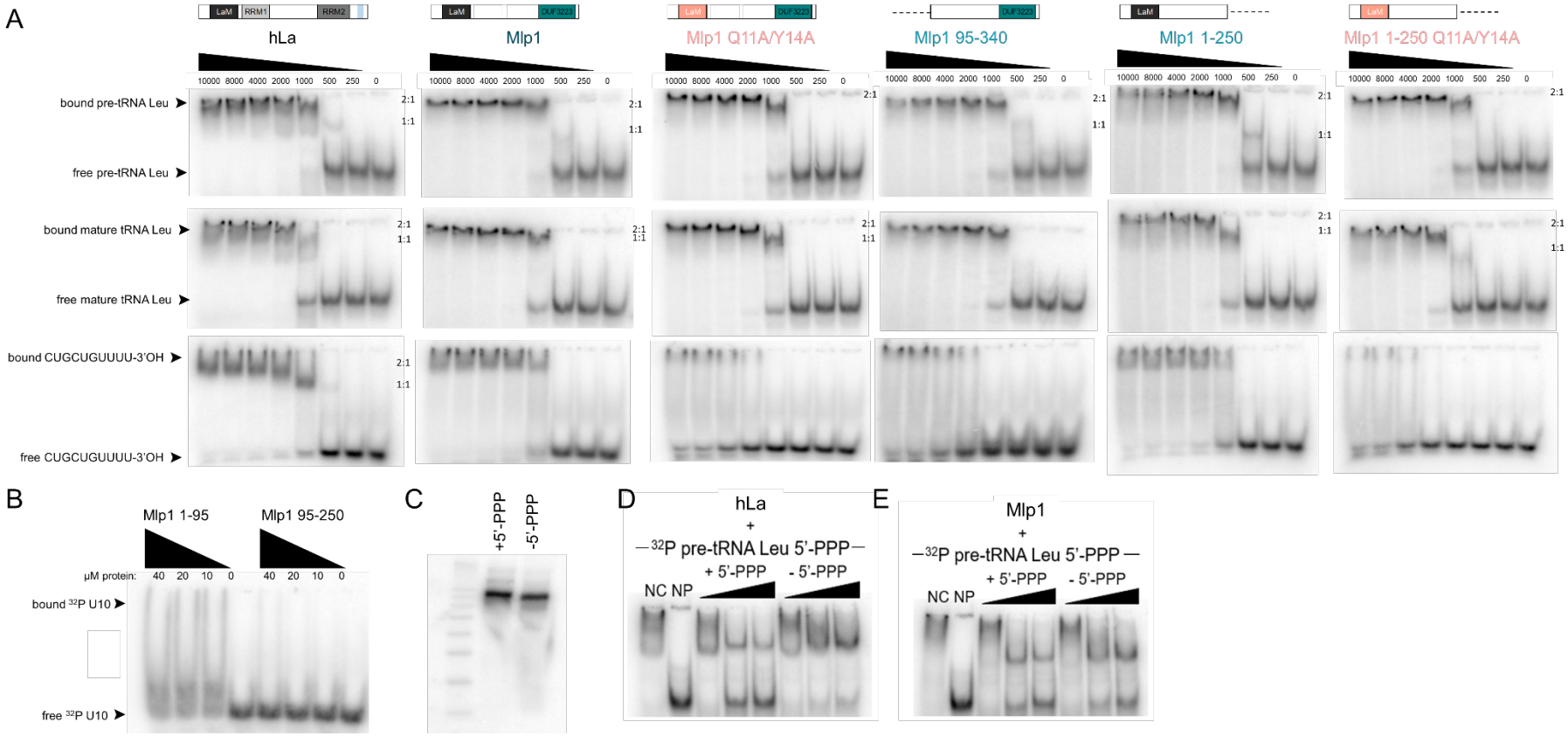


Figure S3. Mlp1 binding to typical La protein target RNAs.

(A) Native gels comparing binding to ^{32}P-labeled pre-tRNAs, mature tRNAs and a 3'-trailer sequence CUGCUGUUUU-3'OH. Unbound RNA is found at the bottom of the gel, while protein-RNA complexes shift upwards. The numbering on the side of the gels indicate binding events. A single protein bound to the RNA is indicated at 1:1 and two proteins bound to the same RNA is denoted at 2:1.

(B) Native gels comparing binding to ^{32}P-labeled U10 RNA for mutants Mlp1 1-95 (LaM only) and Mlp1 95-250 (middle domain only).

(C) Denaturing gel of *in vitro* transcribed ^{32}P-labeled 5'-leader containing, 3'-trailer processed pre-tRNAs produced side-by-side as unlabelled-competitor pre-tRNAs (lacking ^{32}P-labeled labeling) used in (E,F) to confirm successful removal of the 5'-triphosphate group.

(D,E) Native gels comparing unlabelled-competitors 5'-triphosphate containing pre-tRNA (+5'PPP) and dephosphorylated pre-tRNA (-5'PPP) for ^{32}P-labeled +5'PPP binding on hLa (D) or Mlp1 (E). Competition is seen as a decrease in protein-RNA complex and increase in unbound RNA. NP: no protein lane, NC: no unlabeled competitor lane

S4

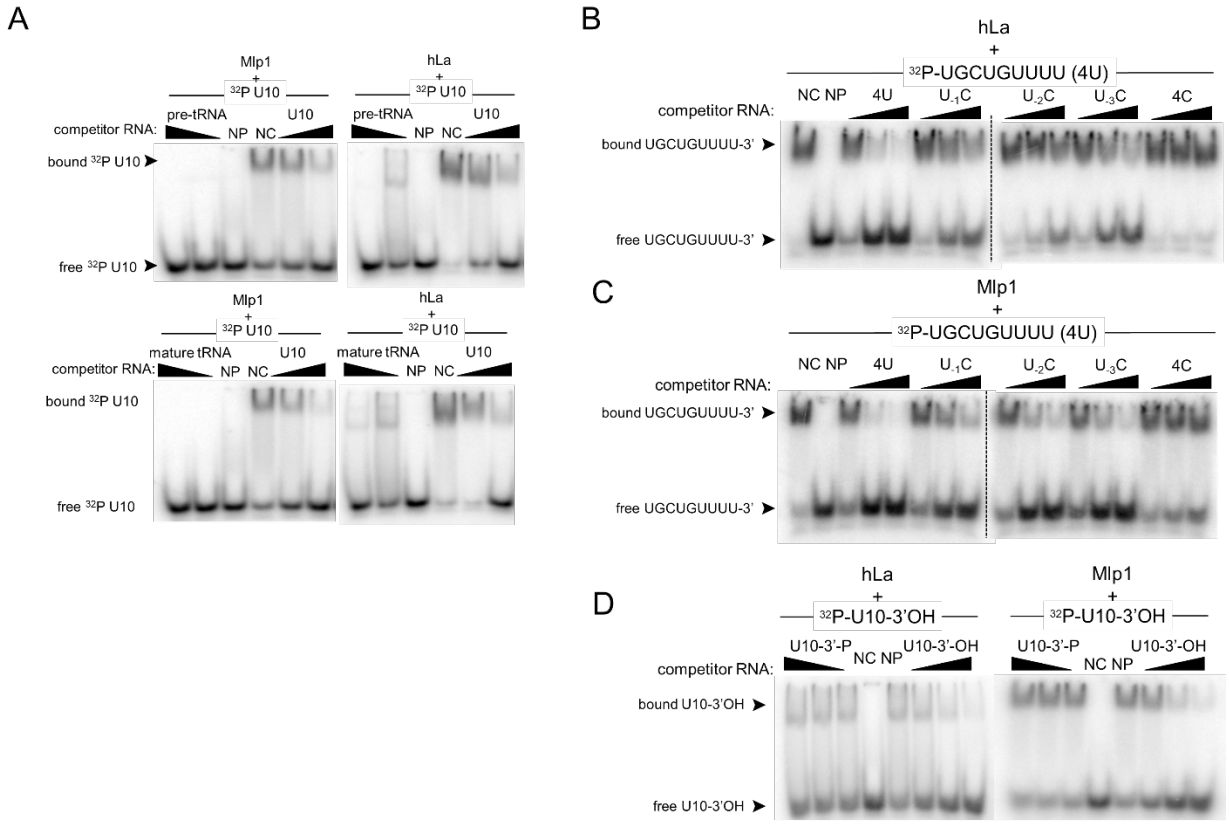


Figure S4. Mlp1 binding to uridylate RNA does not discriminate between the position of the uridylate.
(A) Native gels comparing unlabelled-competitors U10 (positive control), pre-tRNA and mature tRNA for ^{32}P-labeled U10 binding. Competition is seen as a decrease in protein-RNA complex and increase in unbound RNA. NP: no protein lane, NC: no competitor lane.
(B-C) Native gels comparing unlabelled-competitors CUGCUGUUUU (4U), CUGCUGUUUC (U$_{-1}$C), CUGCUGUUCU (U$_{-2}$C), CUGCUGUCUU (U$_{-3}$C) and CUGCUGCCCC (4C) for ^{32}P-labeled 4U binding on hLa (B) or Mlp1 (C). Competition is seen as a decrease in protein-RNA complex and increase in unbound RNA. NP: no protein lane, NC: no competitor lane.
(D) Native gels comparing a 3'-phosphorylated substrate (U10-3'-P) and normal 3'-OH containing substrate (U10-3'OH) for binding of ^{32}P-labeled U10-3'-OH. Competition is seen as a decrease in protein-RNA complex and increase in unbound RNA. No competition was observed with the phosphorylated target for either Mlp1 or hLa. NP: no protein lane, NC: no competitor lane.

S5

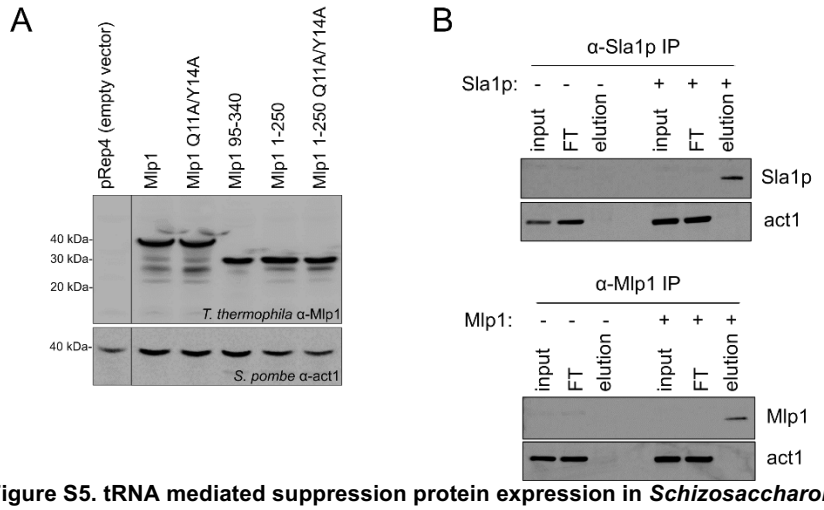


Figure S5. tRNA mediated suppression protein expression in *Schizosaccharomyces pombe*.
(A) Western blot confirming Mlp1 protein expression levels in *Schizosaccharomyces pombe* following pRep4 plasmid transformation in the tRNA-mediated suppression assay Figure 4A. Loading control: act1.
(B) Western blot confirming immunoprecipitation (IP) of Sla1p and Mlp1 from Sla1p- and Mlp1-transformed *Schizosaccharomyces pombe* ySH9 tRNA suppressor strains. pRep4 empty vector transformed strains were used as a control for background immunoprecipitation using Sla1p and Mlp1 antibodies. Loading control: act1. FT: flow through.

S6

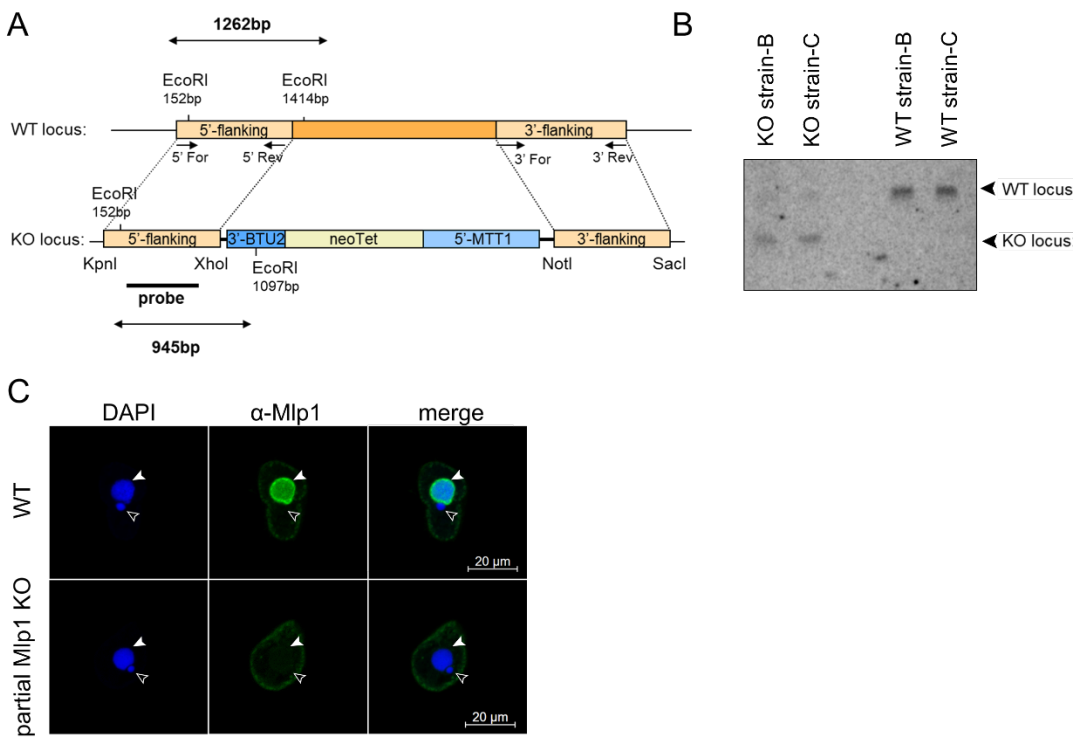


Figure S6. Confirmation of the partial Mlp1 *Tetrahymena thermophila* knockout strain.
(A) Schematic overview of the *Tetrahymena thermophila* wild type (WT) locus encoding endogenous Mlp1 and knockout (KO) locus following homologous recombination between 5'- and 3'-flanking regions of the genome with transformation plasmid encoding the neomycin (neoTet) selection marker. Restriction enzymes used for cloning 5'- and 3'-flanking regions for homologous recombination are shown below the KO locus. Genomic DNA digest before southern blotting were performed with *EcoRI* restriction enzyme generating a 1262 bp fragment for the WT locus and a 945 bp fragment for the KO locus.
(B) Southern blot of genomic DNA digested with restriction enzyme *EcoRI* probed with ^{32}P-labeled PCR-generated probe reveals almost complete KO of Mlp1 (see schematic in A).
(C) Indirect immunofluorescent staining of *Tetrahymena thermophila* WT and partial Mlp1 KO strains using the polyclonal rabbit anti-Mlp1 antibody. Nuclei were stained using DAPI. Full white arrows are denoting the transcriptionally active macronucleus and empty white arrows show the transcriptionally inactive micronucleus.

S7

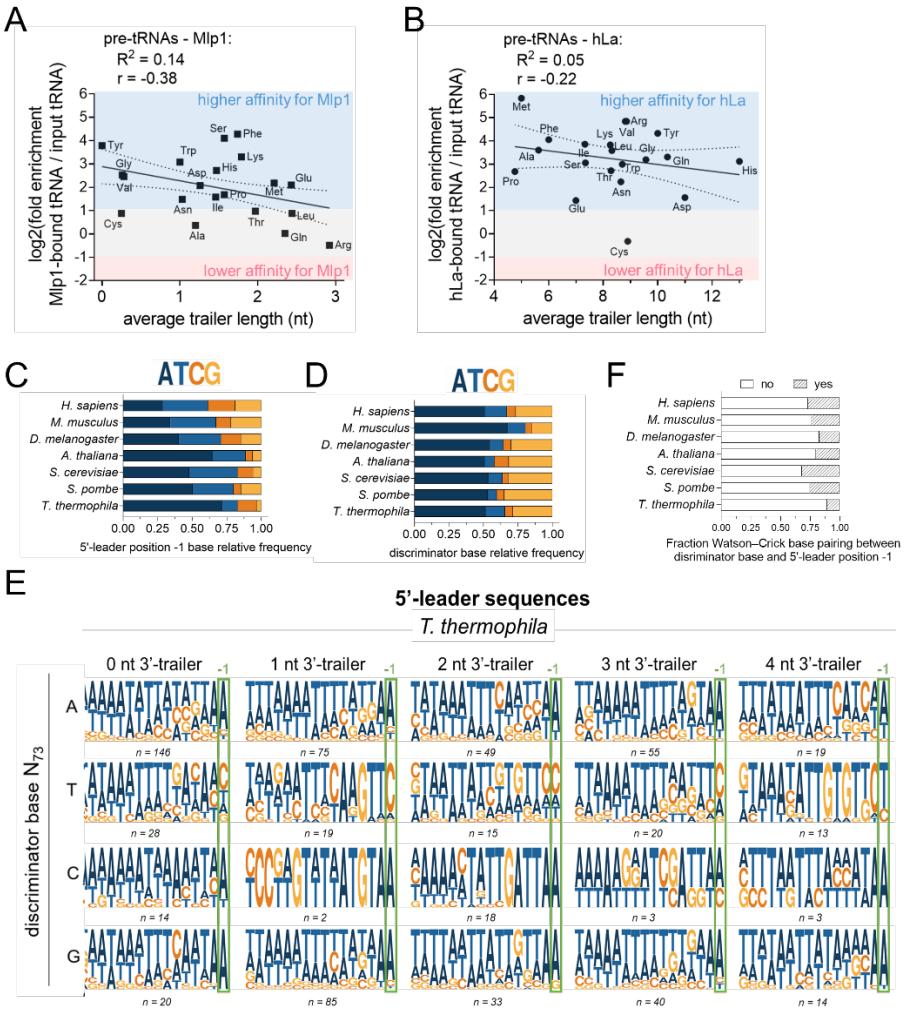
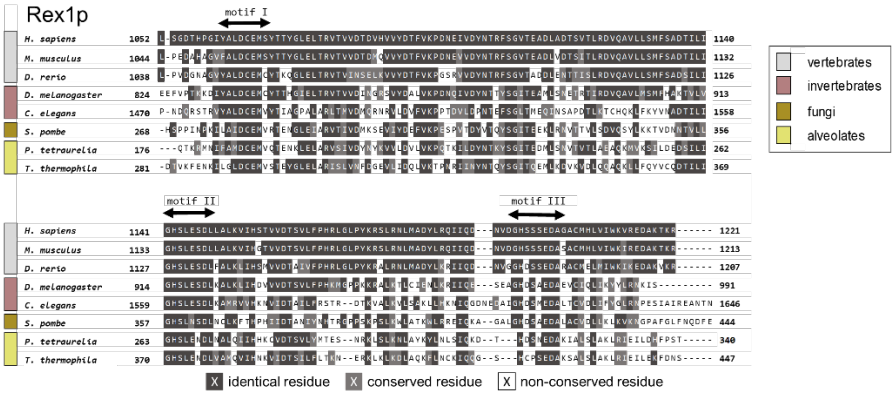


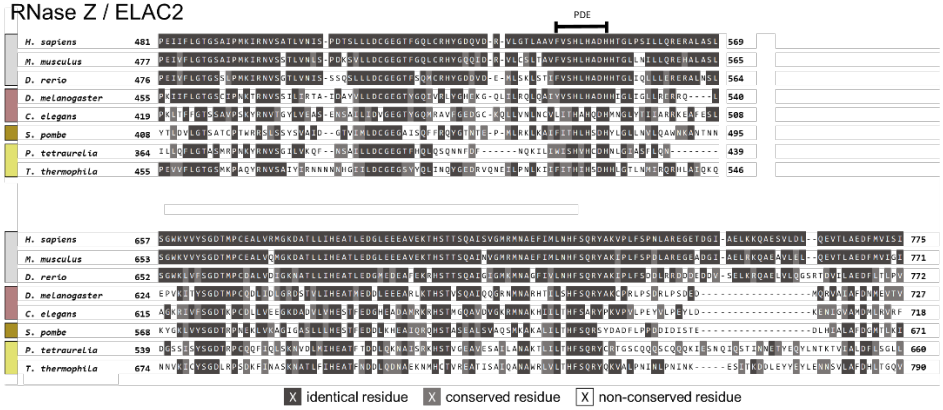
Figure S7. The effect of short 3'-trailer sequences on 5'-leader composition and Mlp1 binding affinity.
(A,B) Next generation sequencing data of Mlp1-bound pre-tRNAs (n=3) (A) and hLa-bound pre-tRNAs (n=2) (B) split by tRNA isotypes compared against 3'-trailer lengths.
(C) Distribution of the nucleotide at the most 3'-terminal position in the 5'-leader of pre-tRNAs from different eukaryotes. *Tetrahymena thermophila* has a high frequency of adenosines in this position.
(D) Distribution of the nucleotide found as the discriminator base (N_{73}) in different eukaryotes. Distribution is equal between different species.
(E) Logo analysis of *Tetrahymena thermophila* 5'-leader sequences split by the discriminator base identity and 3'-trailer length. The number of analyzed pre-tRNAs is shown underneath each logo.
(F) Distribution of pre-tRNAs containing a perfect Watson-Crick base pairing between the discriminator base (N_{73}) preceding the 3'-trailer and most 3'-terminal 5'-leader nucleotide (N_{-1}).

S8

A


Rex1p
motif I
motif II
motif III
H. sapiens
M. musculus
D. rerio
D. melanogaster
C. elegans
S. pombe
P. tetraurelia
T. thermophila
vertebrates
invertebrates
fungi
alveolates
X identical residue
X conserved residue
X non-conserved residue

B


RNase Z / ELAC2
PDE
H. sapiens
M. musculus
D. rerio
D. melanogaster
C. elegans
S. pombe
P. tetraurelia
T. thermophila
X identical residue
X conserved residue
X non-conserved residue

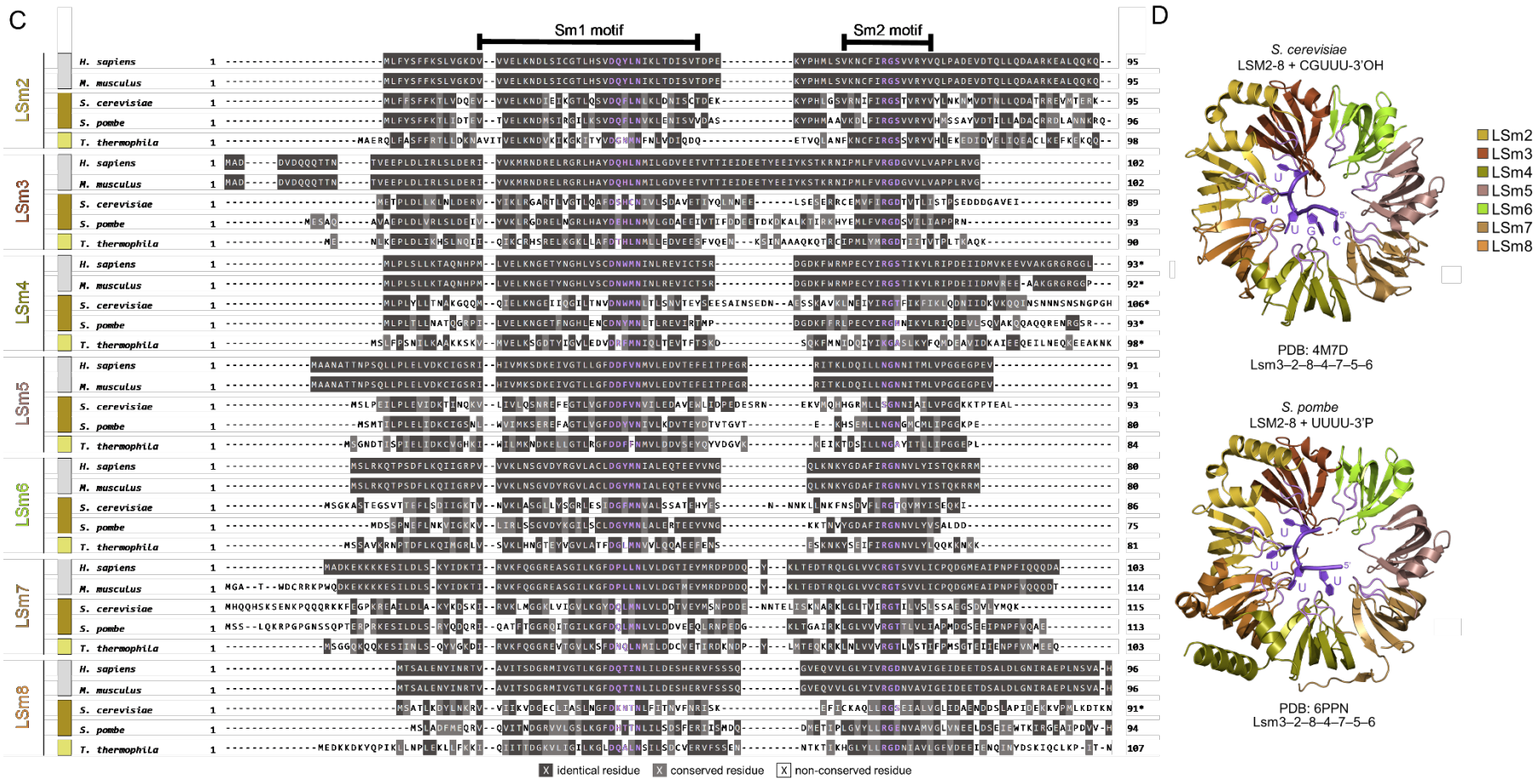


Figure S8. Primary sequence alignments of the 3'-exonuclease Rex1p, 3'-endonuclease RNase Z / ELAC2 and LSm2-8 complex from different eukaryotic species.

(A-C) Primary amino acid alignments from different eukaryotic lineages revealing conservation of the 3'-exonuclease Rex1p (A), 3'-endonuclease RNase Z/ELAC2 (B) and LSm2-8 complex (C). Regions important for Rex1p function are highlighted at motif I, motif II and motif III. The RNase Z catalytic domain is shown as PDE. LSm2-8 complex conserved RNA interacting residues are highlighted in purple and conserved Sm1 and Sm2 motifs are shown. A dark grey background indicates identical residues, light grey conserved residues and white indicates no conservation.

(D) High-resolution structures of the Lsm2-8 complex in *Saccharomyces cerevisiae* (PDB: 4M7D) and *Schizosaccharomyces pombe* (PDB: 6PPN) in complex with 3'-uridylate RNA shown in dark purple. The conserved RNA interacting residues, highlighted in purple C, are also shown in light purple and are found in the loops.

Table S1. Kd values from EMSAs determining binding of Mlp1 to different processed pre-tRNA intermediates and mature tRNA.

	Leu AAG K_d **(nM)**	**Gln UUA** K_d **(nM)**
pre-tRNA 5' 3'	586 ± 15	687 ± 44
pre-tRNA 3'	658 ± 36	740 ± 40
pre-tRNA 5'	714 ± 21	804 ± 16
mature tRNA	831 ± 33	801 ± 62

Table S2. Raw counts for 3'-U ending and 3'-CCA ending tRNAs from raw .fastq files from wild type (WT) input tRNA and Mlp1-immunoprecipitated tRNA (IP). Data was obtained using a custom python script specifically "fishing" sequence specific reads (see Additional Table 2 for fishing sequences) from the raw .fastq file based on the 3'-ends of the tRNA. Data normalization was done by dividing the raw counts by the scaling factor (sum of transcript counts for each replicate divided by 1,000,000), giving counts per million (CPM) values. Fold enrichment of Mlp1-bound mature tRNAs (3'-CCA) and pre-tRNAs (summation of 3'-U) was calculated by taking the ratio of Mlp1-bound tRNA counts and input WT tRNA counts.

		pre-tRNA (U-ending) and mature tRNA (CCA-ending) raw counts for WT total tRNAs																																
tRNA isotype	**tRNA anticodon**	**CCA_1**	**CCA_2**	**CCA_3**	**U1_1**	**U1_2**	**U1_3**	**U2_1**	**U2_2**	**U2_3**	**U3_1**	**U3_2**	**U3_3**	**U4_1**	**U4_2**	**U4_3**	**U5_1**	**U5_2**	**U5_3**	**U6_1**	**U6_2**	**U6_3**	**U7_1**	**U7_2**	**U7_3**	**U8_1**	**U8_2**	**U8_3**	**U9_1**	**U9_2**	**U9_3**	**U10_1**	**U10_2**	**U10_3**
Ala	AGC	101773	49045	61265	41	30	52	18	5	10	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	CGC	17674	9944	13321	9	1	9	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	TGC	58804	25680	28108	32	20	48	3	1	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	ACG	69845	56357	77456	60	56	120	19	6	10	2	7	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCG	1906	1188	1692	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCT	6877	5023	6250	3	4	10	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCG	4727	3567	5295	5	8	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCT	655451	515155	774760	718	487	948	51	47	106	16	20	30	7	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asn	GTT	93198	47317	61766	63	27	41	3	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asp	GTC	108197	64370	83561	87	67	81	8	23	7	2	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cys	GCA	123990	72413	107691	119	76	179	4	8	18	0	0	0	0	8	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	CTA	274808	148637	185931	183	109	190	29	23	22	1	0	10	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTA	83090	51452	64155	312	180	364	9	1	18	2	0	4	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTG	589597	422248	526962	490	320	569	66	20	95	24	4	15	7	8	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	CTC	21233	12157	13254	18	18	23	4	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	TTC	91903	60273	70481	110	117	123	11	14	18	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	CCC	2	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	GCC	109151	49790	64024	57	11	44	2	0	2	14	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	TCC	38765	20931	32257	68	22	53	2	0	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
His	GTG	46189	28199	28886	23	18	19	1	1	9	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	AAT	194897	139549	167646	123	53	113	23	12	5	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	TAT	25298	16946	19701	27	10	46	0	0	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	AAG	736823	371241	387961	372	230	274	38	36	10	0	0	8	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Leu	CAA	534721	385328	330488	673	587	587	259	130	206	38	5	15	4	3	8	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	CAG	694731	350058	366549	287	174	207	33	35	8	0	0	8	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Leu	TAA	81109	57291	76435	151	84	151	17	5	18	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	TAG	54400	31709	35419	24	13	20	5	2	0	7	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lys	TTT	81449	40102	47166	27	47	32	3	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Met	CAT	122134	83729	115064	176	119	213	9	11	19	2	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	AAA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	GAA	73674	50329	56535	52	42	31	4	5	3	0	0	10	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	AGG	64362	35241	51175	131	49	118	24	7	17	2	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	CGG	4039	1924	2166	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	TGG	19765	10741	15525	42	33	27	0	2	2	0	0	3	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	AGA	228626	145814	205261	239	110	306	47	25	81	6	0	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	CGA	19689	17749	21887	25	14	39	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	GCT	83449	46174	61004	60	24	54	6	2	0	9	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	TGA	74794	52775	69731	48	16	36	9	0	13	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	AGT	76120	43784	53703	76	29	121	9	14	13	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	CGT	3019	2628	2513	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	TGT	226719	90263	66532	99	45	38	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trp	CCA	50436	29034	36424	49	62	35	2	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyr	GTA	11817	9834	11524	27	37	82	20	26	79	31	7	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	AAC	43617	21142	23822	32	21	47	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	CAC	6095	2944	4309	35	12	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	TAC	47244	23873	28946	35	15	29	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		premature (U-ending) and mature (CCA-ending) tRNA counts for MLP1-immunoprecipitated tRNAs																																
tRNA isotype	**tRNA anticodon**	**CCA_1**	**CCA_2**	**CCA_3**	**U1_1**	**U1_2**	**U1_3**	**U2_1**	**U2_2**	**U2_3**	**U3_1**	**U3_2**	**U3_3**	**U4_1**	**U4_2**	**U4_3**	**U5_1**	**U5_2**	**U5_3**	**U6_1**	**U6_2**	**U6_3**	**U7_1**	**U7_2**	**U7_3**	**U8_1**	**U8_2**	**U8_3**	**U9_1**	**U9_2**	**U9_3**	**U10_1**	**U10_2**	**U10_3**
Ala	AGC	12790	18607	13616	27	19	7	3	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	CGC	1807	2306	2050	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	TGC	7717	10867	10919	4	2	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	ACG	12057	14757	16553	27	27	39	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCG	485	734	684	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCT	3471	4118	2867	4	0	2	0	2	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCG	1282	1895	1583	2	4	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCT	121075	172409	153047	82	125	117	4	31	2	27	29	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asn	GTT	24429	36169	28634	12	34	27	0	17	2	12	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asp	GTC	33001	45745	35673	21	52	43	64	45	71	16	16	23	0	0	7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cys	GCA	12559	20982	18480	30	15	8	16	18	31	26	16	52	0	3	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	CTA	20308	31636	27251	12	33	43	15	3	10	33	76	101	7	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTA	4181	6675	5297	15	100	115	6	17	44	17	17	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTG	83793	115812	107411	40	104	60	6	6	3	15	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	CTC	2810	4166	4151	9	3	6	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	TTC	26920	33372	32085	99	122	104	25	68	81	30	30	10	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	CCC	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	GCC	47487	63069	52703	9	18	30	0	0	0	0	56	0	21	63	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	TCC	4397	6995	5856	42	13	42	25	21	24	16	18	0	5	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
His	GTG	18117	25645	17210	2	6	19	28	26	29	0	19	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	AAT	58883	105760	71718	24	71	36	2	2	0	0	24	1	13	2	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	TAT	3852	6245	5081	26	2	34	0	31	7	0	1	0	27	32	0	14	0	4	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	AAG	303102	366843	206948	148	186	127	38	74	35	23	1	55	80	39	23	0	22	0	17	1	0	0	0	1	0	5	31	0	0	0	0	0	0
Leu	CAA	162010	209902	143378	56	88	94	74	15	38	0	3	5	0	0	12	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	CAG	284604	345178	194258	112	142	90	38	71	3	22	1	52	80	39	21	0	20	0	14	1	0	0	0	1	0	4	31	0	0	0	0	0	0
Leu	TAA	13122	16157	15068	65	47	26	24	25	5	0	0	8	0	14	31	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	TAG	26395	30450	22376	7	37	47	2	1	1	26	24	27	0	84	30	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lys	TTT	19857	13620	10960	35	54	32	14	0	0	28	0	18	72	0	30	12	2	1	0	31	0	0	0	0	18	0	7	0	0	0	0	0	0
Met	CAT	27999	43139	33348	131	236	262	23	38	15	8	13	5	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	AAA	12421	18200	14340	172	376	262	50	2	34	0	0	28	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	GAA	16540	23446	15822	68	65	33	23	45	9	1	0	7	16	0	17	0	0	0	0	0	0	0	18	0	0	0	0	0	10	0	0	0	0
Pro	AGG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	CGG	1214	1558	1427	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	TGG	5158	8927	6768	23	31	15	0	0	1	9	16	0	13	8	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	AGA	45732	57271	42418	130	47	79	37	16	1	140	135	19	668	225	129	156	16	25	7	10	0	11	0	0	0	0	0	0	0	0	0	11	0
Ser	CGA	6195	7190	8046	224	111	219	711	472	274	0	0	20	0	26	6	9	7	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	GCT	18535	28469	19044	148	70	146	137	119	188	44	13	7	12	0	54	0	69	3	0	63	0	0	15	11	0	0	0	0	0	0	0	0	0
Ser	TGA	12304	17710	14669	47	5	9	74	39	9	222	226	162	1	1	1	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
Thr	AGT	12275	18076	12647	2	22	26	15	0	9	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	CGT	1484	2567	1843	3	23	0	38	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	6	17	0	0	0	0	0	0	0
Thr	TGT	55865	91243	60231	16	36	28	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trp	CCA	8134	11600	11939	184	46	129	12	0	19	0	0	25	0	12	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyr	GTA	3551	5553	4597	12	5	23	100	205	251	136	175	239	9	1	7	0	0	0	25	0	0	0	0	13	9	33	12	0	0	0	0	0	0
Val	AAC	7018	8275	6321	93	125	55	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	CAC	1399	1881	1464	5	0	52	0	0	1	9	34	18	0	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	TAC	5806	7978	5496	1	54	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3. Raw counts for 3'-U ending and 3'-CCA ending tRNAs from raw .fastq files. Data was obtained using a custom python script specifically "fishing" sequence specific reads (see Additional Table 2 for fishing sequences) from the raw .fastq file based on the 3'-ends of the tRNA. Data normalization was done by dividing the raw counts by the scaling factor (sum of transcript counts for each replicate divided by 1,000,000), giving counts per million (CPM) values. The average log10 value of the cumulative TPM is shown in the heatmap in Figure 5C.

		premature (U-ending) and mature (CCA-ending) tRNA counts for wild type (WT) total tRNAs																																	
tRNA isotype	tRNA anticodon	CCA_1	CCA_2	CCA_3	U1_1	U1_2	U1_3	U2_1	U2_2	U2_3	U3_1	U3_2	U3_3	U4_1	U4_2	U4_3	U5_1	U5_2	U5_3	U6_1	U6_2	U6_3	U7_1	U7_2	U7_3	U8_1	U8_2	U8_3	U9_1	U9_2	U9_3	U10_1	U10_2	U10_3
Ala	AGC	101773	49045	61265	41	30	52	18	5	10	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	CGC	17674	9944	13321	9	1	9	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	TGC	58804	25680	28108	32	20	48	3	1	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	ACG	69845	56357	77456	60	56	120	19	6	10	2	7	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCG	1906	1188	1692	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCT	6877	5023	6250	3	4	10	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCG	4727	3567	5295	5	8	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCT	655451	515155	774760	718	487	948	51	47	106	16	20	30	7	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asn	GTT	93198	47317	61766	63	27	41	3	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asp	GTC	108197	64370	83561	87	67	81	8	23	7	2	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cys	GCA	123990	72413	107691	119	76	179	4	8	18	0	0	0	0	8	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	CTA	274808	148637	185931	183	109	190	29	23	22	1	0	10	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTA	83090	51452	64155	312	180	364	9	1	18	2	0	4	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTG	589597	422248	526962	490	320	569	66	20	95	24	4	15	7	8	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	CTC	21233	12157	13254	18	18	23	4	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	TTC	91903	60273	70481	110	117	123	11	14	18	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	CCC	2	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	GCC	109151	49790	64024	57	11	44	2	0	2	14	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	TCC	38765	20931	32257	68	22	53	2	0	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
His	GTG	46189	28199	28886	23	18	19	1	1	9	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	AAT	194897	139549	167646	123	53	113	23	12	5	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	TAT	25298	16946	19701	27	10	46	0	0	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	AAG	736823	371241	387961	372	230	274	38	36	10	0	0	8	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Leu	CAA	534721	385328	330488	673	587	587	259	130	206	38	5	15	4	3	8	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	CAG	694731	350058	366549	287	174	207	33	35	8	0	0	8	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Leu	TAA	81109	57291	76435	151	84	151	17	5	18	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	TAG	54400	31709	35419	24	13	20	5	2	0	7	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lys	TTT	81449	40102	47166	27	47	32	3	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Met	CAT	122134	83729	115064	176	119	213	9	11	19	2	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	AAA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	GAA	73674	50329	56535	52	42	31	4	5	3	0	0	10	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	AGG	64362	35241	51175	131	49	118	24	7	17	2	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	CGG	4039	1924	2166	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	TGG	19765	10741	15525	42	33	27	0	2	2	0	0	3	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	AGA	228626	145814	205261	239	110	306	47	25	81	6	0	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	CGA	19689	17749	21887	25	14	39	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	GCT	83449	46174	61004	60	24	54	6	2	0	9	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	TGA	74794	52775	69731	48	16	36	9	0	13	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	AGT	76120	43784	53703	76	29	121	9	14	13	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	CGT	3019	2628	2513	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	TGT	226719	90263	66532	99	45	38	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trp	CCA	50436	29034	36424	49	62	35	2	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyr	GTA	11817	9834	11524	27	37	82	20	26	79	31	7	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	AAC	43617	21142	23822	32	21	47	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	CAC	6095	2944	4309	35	12	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	TAC	47244	23873	28946	35	15	29	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		premature (U-ending) and mature (CCA-ending) tRNA counts for partial MLP1 knockout (KO) total tRNAs																																
tRNA isotype	**tRNA anticodon**	**CCA_1**	**CCA_2**	**CCA_3**	**U1_1**	**U1_2**	**U1_3**	**U2_1**	**U2_2**	**U2_3**	**U3_1**	**U3_2**	**U3_3**	**U4_1**	**U4_2**	**U4_3**	**U5_1**	**U5_2**	**U5_3**	**U6_1**	**U6_2**	**U6_3**	**U7_1**	**U7_2**	**U7_3**	**U8_1**	**U8_2**	**U8_3**	**U9_1**	**U9_2**	**U9_3**	**U10_1**	**U10_2**	**U10_3**
Ala	AGC	71772	37393	39213	83	32	17	23	17	12	11	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	CGC	21716	14584	15613	11	5	4	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	TGC	69207	31515	30878	30	28	23	5	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	ACG	82876	39548	53154	85	45	58	3	0	8	0	0	0	8	16	3	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCG	1188	712	692	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	CCT	10167	4736	5402	8	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCG	4147	2343	1809	4	5	11	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	TCT	815051	508887	566999	717	546	491	61	56	30	7	18	51	0	13	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asn	GTT	116604	44529	53692	89	34	31	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asp	GTC	120380	70875	65211	93	66	63	23	14	12	38	40	20	6	9	3	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0
Cys	GCA	110936	75986	74105	103	62	84	8	11	7	18	9	6	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	CTA	259803	139250	158230	254	129	171	5	1	1	0	40	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTA	99472	56703	67782	425	273	285	14	4	0	32	15	12	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	TTG	991452	578704	656541	651	462	447	121	40	57	48	11	49	0	10	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	CTC	22690	9612	8511	37	30	9	24	11	9	8	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	TTC	144526	63140	57303	202	122	97	18	51	16	0	8	6	0	1	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0
Gly	CCC	167	54	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	GCC	239527	111217	108213	182	47	32	7	9	2	12	6	34	34	15	6	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	TCC	31857	19319	17784	35	33	12	11	3	7	0	20	2	10	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
His	GTG	45057	19064	21395	22	26	32	21	5	21	0	23	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	AAT	328129	136498	118534	224	80	74	23	5	8	0	1	3	0	1	5	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	TAT	26956	12451	13939	36	38	21	0	11	8	0	0	1	17	7	5	20	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Leu	AAG	318831	137406	155545	157	85	74	3	11	4	26	5	7	0	4	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	CAA	1553032	485986	295666	1635	732	260	406	220	78	16	26	8	14	6	5	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	CAG	300318	129195	147071	124	67	65	3	10	0	12	5	7	0	4	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	TAA	110701	56916	71315	237	97	123	20	9	7	0	4	6	8	1	4	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	TAG	47973	22171	24927	27	12	13	0	3	0	10	10	4	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lys	TTT	107921	63792	65459	27	71	27	17	23	0	13	0	0	14	1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Met	CAT	124565	64897	72966	110	74	86	10	11	6	0	5	10	2	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	AAA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	GAA	54969	27885	31151	132	97	105	40	28	70	38	22	42	0	4	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	AGG	27663	12788	16490	102	99	83	18	53	24	4	5	8	8	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	CGG	6115	2984	3118	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	TGG	17471	7496	8721	61	17	11	0	13	8	15	71	77	23	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	AGA	224694	101158	133828	157	61	83	22	11	11	22	4	4	0	2	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	CGA	29419	14279	19091	9	22	10	12	3	1	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	GCT	50080	28857	25310	63	28	24	43	9	28	0	0	2	0	1	9	12	1	7	26	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Ser	TGA	111315	51610	59788	52	24	34	21	5	10	33	7	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	AGT	91344	56141	57326	105	66	81	15	25	11	0	0	0	0	0	7	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	CGT	6923	3715	3077	16	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	TGT	369704	137505	107236	168	50	45	4	4	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trp	CCA	60240	33614	36027	58	94	64	58	16	18	39	8	9	0	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyr	GTA	16837	8815	8195	112	35	19	96	37	58	172	86	130	20	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	AAC	42983	22066	22492	263	252	186	83	31	19	7	5	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	CAC	3451	1898	1811	105	103	62	0	2	0	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	TAC	24016	12533	12597	71	80	100	3	3	15	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S4. Number of tRNA genes encoded in the genome of different eukaryotic species for each isotype (top) and isoacceptor (bottom). The number of tRNA genes was obtained by counting the number of entries for each tRNA isotype and anticodon from the Genomic tRNA Database (GtRNAdb) and from the UCSC Genome Browser for *Tetrahymena thermophila* in Additional Table 3. NNN anticodons were excluded from the analysis.

tRNA isotype	*T. thermophila*	*S. pombe*	*S. cerevisiae*	*A. thaliana*	*D. melanogaster*	*M. musculus*	*H. sapiens*
Ala	44	12	16	33	17	46	45
Arg	36	13	19	37	26	25	31
Asn	31	6	10	16	10	7	36
Asp	31	8	16	27	14	22	20
Cys	16	3	4	16	7	62	32
Gln	52	6	10	17	12	19	31
Glu	44	10	16	27	21	22	28
Gly	42	12	21	41	20	32	36
His	17	4	7	10	6	11	10
Ile	41	9	15	22	12	18	24
iMet	0	4	5	2	6	10	10
Leu	55	13	21	42	22	29	38
Lys	29	12	21	32	19	41	40
Met	33	3	5	23	6	9	10
Phe	38	5	10	16	8	7	17
Pro	28	9	12	67	17	20	25
Ser	46	13	17	64	20	22	28
Sup	0	0	0	1	0	0	1
Thr	29	10	16	23	18	1	22
Trp	21	3	6	14	8	0	7
Tyr	19	4	8	76	10	0	16
Val	32	12	18	30	15	0	34
Sum	**684**	**171**	**273**	**636**	**294**	**403**	**541**

tRNA isotype	anticodon	*T. thermophila*	*S. pombe*	*S. cerevisiae*	*A. thaliana*	*D. melanogaster*	*M. musculus*	*H. sapiens*
Ala		**44**	**12**	**16**	**33**	**17**	**46**	**45**
	AGC	31	9	11	16	12	22	30
	CGC	2	1		7	3	10	5
	GGC						3	
	TGC	11	2	5	10	2	11	10
Arg		**36**	**13**	**19**	**37**	**26**	**25**	**31**
	ACG	8	8	6	9	10	6	7
	CCG	1	1	1	4		3	4
	CCT	2	1	1	8	3	5	8
	TCG	1	1		6	10	5	6
	TCT	24	2	11	10	3	6	6
Asn		**31**	**6**	**10**	**16**	**10**	**7**	**36**
	ATT							2
	GTT	31	6	10	16	10	13	35
Asp		**31**	**8**	**16**	**27**	**14**	**22**	**20**
	GTC	31	8	16	27	14	16	19
Cys		**16**	**3**	**4**	**16**	**7**	**62**	**32**
	ACA						1	1
	GCA	16	3	4	16	7	61	31
Gln		**52**	**6**	**10**	**17**	**12**	**19**	**31**
	CTA *	9						
	CTG		2	1	9	8	12	22
	TTA *	30						
	TTG	13	4	9	8	4	7	9
Glu		**44**	**10**	**16**	**27**	**21**	**22**	**28**
	CTC	7	6	2	13	15	14	12
	TTC	37	4	14	14	6	8	16
Gly		**42**	**12**	**21**	**41**	**20**	**32**	**36**
	ACC						2	
	CCC	1	1	2	5		7	10
	GCC	29	8	16	23	14	15	15
	TCC	12	3	3	13	6	8	11
His		**17**	**4**	**7**	**10**	**6**	**11**	**10**
	ATG						1	
	GTG	17	4	7	10	6	10	10
Ile		**41**	**9**	**15**	**22**	**12**	**18**	**24**
	AAT	29	8	13	17	10	12	16
	GAT						1	3
	TAT	12	1	2	5	2	5	5
iMet			**4**	**5**	**2**	**6**	**10**	**10**
	CAT		4	5	2	6	10	10
Leu		**55**	**13**	**21**	**42**	**22**	**29**	**38**
	AAG	19	5		12	4	5	13
	CAA	11	4	10	10	4	4	7
	CAG	2	1		3	8	10	9
	GAG			1	1			
	TAA	19	2	7	6	4	7	5
	TAG	4	1	3	10	2	3	4
Lys		**29**	**12**	**21**	**32**	**19**	**41**	**40**
	CTT		9	14	18	13	26	21
	TTT	29	3	7	14	6	15	19
Met		**33**	**3**	**5**	**23**	**6**	**9**	**10**
	CAT	33	3	5	23	6	9	10
Phe		**38**	**5**	**10**	**16**	**8**	**7**	**17**
	AAA	1						
	GAA	37	5	10	16	8	7	17
Pro		**28**	**9**	**12**	**67**	**17**	**20**	**25**
	AGG	20	6	2	16	7	8	11
	CGG	1	1		5	5	3	4
	GGG						1	1
	TGG	7	2	10	45	5	8	9
Ser		**46**	**13**	**17**	**64**	**20**	**22**	**28**
	ACT							1
	AGA	20	7	11	37	8	9	11
	CGA	2	1	1	4	4	3	4
	GCT	15	3	2	13	6	9	8
	GGA				1			
	TGA	9	2	3	9	2	1	4
Sup					**1**			**1**
	TTA				1			1
Thr		**29**	**10**	**16**	**23**	**18**	**1**	**22**
	AGT	22	7	11	10	9	1	10
	CGT	1	1	1	5	3		6
	TGT	6	2	4	8	6		6
Trp		**21**	**3**	**6**	**14**	**8**		**7**
	CCA	21	3	6	14	8		7
Tyr		**19**	**4**	**8**	**76**	**10**		**16**
	ATA							1
	GTA	19	4	8	76	10		15
Val		**32**	**12**	**18**	**30**	**15**		**34**
	AAC	21	9	14	15	6		11
	CAC	5	1	2	8	7		18
	TAC	6	2	2	7	2		5
Sum		**684**	**171**	**273**	**636**	**294**	**403**	**541**

* Anticodons encoding stop codons in eukaryotes except in *T. thermophila*

Table S5. List of oligonucleotides used in this study.

gBlock Gene Fragments used as DNA template for cloning MLP1	
gBlock MLP1 CDS codon optimized	ATGTCAATCAATAAAGAAGAGGTAAAAAAGCAAGTCGAATACTATCTGTCGGACAAGAACTTGGTAAATGATGAGAAATTTCGCACTATCATTCAAGAGCACCCAGAG GGGTATTTATCCTTCGGCAATATCTTAAACTGTAATAAGATTAAGCGTCTGGGTGTGACGACATTCGAACAACTGGCAACGTCCCTTGCTGATAGTACCTTAGTTGAA TTAAATGAGGCAAAGGACTCTGTGCGTCGTGCCGGAAATAAACCCATTCCAGCGAAGGAAGCAGTTGACCCCGCTGAAGCTGCAGAAAAAGAAGCGCGTGAGGC CGAAAAAAAGGAACTGATCAACTTTTATGAAACTTTTCAACCGATTATCTTCTCAACCGCCTGCGAACAAGAGGGGGTAGCGAACTGGCGCAACATCACAGAGGCCT TGCTGAAGCAACATAACGTTCATGCTCCGTATTGCCGCTTTGGCAAGTTGGAGGGTAACTTTGCATTGAATAAAGACAAAACTTCGCAAGAGGTTATCGACCAGCTT GTGCAAGACGGGCTTCAATTTGGAGAGTCTAAGGTGACTATCAAAGTGAGTGAGGGCGAAGCCCTGTCAAAGTTTTGGGAGCTGCATGGACGCCACTACAACGGT GTGATGGAATTGAAAAAGAAGGAGGTCAATCAGACAGTTAAAGCTAAGAAAGATAAAAAGGAGAAAAAACAAAAACGTGAATTCGAATTCGGGGGAGAAAAATATAC AGATGTATTGACTATTAAAAATTTGTTTAAGGGAATCTTAGGGCGTACGGCGAATGGACAAAAGATCATTAGTCCTTATCACGAGATGTTGAAGAGCCTTCTTGAGTA TCACAATAACAAAGAAGCCAAATTAAAAGATTTAGATCACTTCACGGTAGACGTCCATCCTGAGCATAAAGATACACGTTGTTTCTTTGTCGTCAAATCGGACGGAAC GAAGGAAGATTTCTCAGCGGTTAAGTGCATCTCAAACTTTGAGGAAAAACTTAAACTTTGA

Oligonucleotides used for cloning MLP1 and MLP1-mutants in pET28a (bacterial expression - protein purification) and pRep4 (yeast expression - tRNA mediated suppression assay). Restriction enzyme cleavage sites are underlined. 6X His tag is shown in bold and blue. Point mutations are shown in bold and pink.		
MLP1 SalI 6X His For	pRep4	5' GCGCGGTCGACATG**CACCATCACCATCACCAT**TCAATCAATAAAGAAGAGGTAAAAAAGC
MLP1 Q11A/Y14A SalI 6X His For	pRep4	5' GCGCGGTCGACATG**CACCATCACCATCACCAT**TCAATCAATAAAGAAGAGGTAAAAAAG**GCA**GTCGAA**GCC**TATCTGTCGGACAAGAACTTGGTAAATG
MLP1 95 SalI 6X His For	pRep4	5' GCGCGGTCGACATG**CACCATCACCATCACCAT**GACCCCGCTGAAGCTGCAGAAAAAGAAGCGCGTG
MLP1 NheI For	pET28a	5' GCGCGGCTAGCATGTCAATCAATAAAGAAGAGGTAAAAAAGCAAGTC
MLP1 Q11A/Y14A NheI For	pET28a	5' GCGCGGCTAGCATGTCAATCAATAAAGAAGAGGTAAAAAAG**GCA**GTCGAA**GCC**TATCTGTCGGACAAGAACTTGG
MLP1 95 NheI For	pET28a	5' GCGCGGCTAGCATGGACCCCGCTGAAGCTGCAGAAAAAGAAGCGCGTG
MLP1 BamHI Rev	pET28a/pRep4	5' GCGCGGGATCCTCAAAGTTTAAGTTTTTCCTCAAAGTTTGAG
MLP1 95 BamHI Rev	pET28a/pRep4	5' GCGCGGGATCCTCAGTCAACTGCTTCCTTCGCTGGAATGGGTTTATTTCCG
MLP1 250 BamHI Rev	pET28a/pRep4	5' GCGCGGGATCCTCAATCTGTATATTTTTCTCCCCCGAATTCGAATTC

Oligonucleotides used to generate T7 DNA template for *in vitro* transcription of tRNAs for electromobility shift assays (EMSAs). T7 promotor sequence is shown in bold and blue.	
Leu AAG DNA template	5' GATGAAGTGGCCGAGCGGTCTAAGGCGGTAGATTAAGGCTCTATTCCGAAAGGGCGCGAGTTCGAATCTCGCCTTCATCA
Leu AAG **T7 promoter** + 5'-leader For	5' **GCTAATACGACTCACTATA**gagcgaaGATGAAGTGGCCGAGCGGTC
Leu AAG **T7 promoter** + mature For	5' **GCTAATACGACTCACTATA**GATGAAGTGGCCGAGCGGTC
Leu AAG 3'-trailer + $(U)_5$ Rev	5' aaaaatgTGATGAAGGCGAGATTCGAAC
Leu AAG mature Rev	5' TGATGAAGGCGAGATTCGAAC
Gln TTA DNA template	5' GGTTCCATAGTATAGTGGTTAGTACTGGGGACTTTAAATCCCTTGACCTGGGTTCGAATCCCAGTGGGACCT
Gln TTA **T7 promoter** + 5'-leader For	5' **GCTAATACGACTCACTATA**gcatgtccGGTTCCATAGTATAGTGGTTAGTAC
Gln TTA **T7 promoter** + mature For	5' **GCTAATACGACTCACTATA**GGTTCCATAGTATAGTGGTTAGTAC
Gln TTA 3'-trailer + $(U)_5$ Rev	5' aaaaagcAGGTCCCACTGGGATTCGAACCC
Gln TTA mature Rev	5' AGGTCCCACTGGGATTCGAACCC

Oligonucleotides used for cloning MLP1 partial knockout strain		
MLP1 5'-flanking KpnI For	pNeo4	5' CGCGGGTACCGGTATGTTGTTTGAGATCTTATTAGAATCAC
MLP1 5'-flanking XhoI Rev	pNeo4	5' CGCGCTCGAGTTATTCAAAAGTAGTAACTCCGAGTCTCTT
MLP1 3'-flanking NotI For	pNeo4	5' CGCGGCGGCCGCTCACGAAATGGTAAATAAATAAATACGATT
MLP1 3'-flanking SacI Rev	pNeo4	5' ATTAGAGCTCAGTATATTAAAAGCAATCAATAAATATAAA

DNA probes used for northern blotting. Anticodon sequence shown in bold and 5'-leader, 3'-trailer and intron sequences in lowercase if applicable.	
T. thermophila U5 snRNA	5' CACATTGAAAAACCCAGCTCACGG
T. thermophila 5.8S rRNA	5' CTGCAATTCGCATTGCGT
T. thermophila Tyr GTA pre-tRNA 3'-trailer	5' aaaaaaaaTCCGAACTCCCGGG
T. thermophila Tyr GTA mature tRNA	5' GACCACCGGATTTACAGTCCG
T. thermophila Ile TAT pre-tRNA intron	5' GACacacttttataaggttggtcagcaaaT**ATA**AATC
T. thermophila Ile TAT/Leu TAA pre-tRNA 3'-trailer	5' aaaaaaaaagTAGCTCAGAGAGGGTTC
T. thermophila Ile TAT/Leu TAA pre-tRNA 5'-leader	5' ACCACTGAGCCtttcaaag
T. thermophila Leu TAA pre-tRNA intron	5' GACactcttttctaaatgtaggaggatgaacG
T. thermophila Val CAC pre-tRNA intron	5' TTAAGGTTtattagaccttC**GTG**ATA
T. thermophila Val CAC pre-tRNA 3'-trailer	5' aaaaaaGTCGACACTAGGGTTTGAA
T. thermophila Val CAC pre-tRNA 5'-leader	5' ACTACTTCACCATGCCGACtatagcag
T. thermophila Arg TCT mature tRNA	5' CAACCAAGGTGGGACTCGAACCCAC
S. pombe U5 snRNA	5' CTGGTAAAAGGCAAGAAACAGATACG
S. pombe suppressor Ser TCA pre-tRNA intron	5' gaatacaggaT**TGA**AGTCT
S. pombe suppressor Ser TCA pre-tRNA intron (unlabelled probe)	5' gaatacaggaT**TCA**AGTCT
S. pombe suppressor Ser TCA mature tRNA	5' **TGA**AGTCTAACTCCTT
S. pombe suppressor Ser TCA mature tRNA (unlabelled probe)	5' **TCA**AGTCTAACTCCTT
S. pombe Lys CTT pre-tRNA intron	5' CTTCTGATaccattcgT**AAG**AGTC
S. pombe Lys CTT pre-tRNA 3'-trailer	5' aaattaaccTCCCAAG
S. pombe Lys CTT pre-tRNA 5'-leader	5' ATTGAGCCACTCGGGAcgcgtt
S. pombe Lys CTT mature tRNA	5' CTCCCAAGGCGAGACTCGAACTCGCAA

DNA probes used for pre-tRNA intron-specific PCR amplification for Sanger sequencing RIP-3'-RACE	
Lys CTT pre-tRNA intron	5' TCTGACTCTTATGATGGTAATCAGA
Tyr GTA pre-tRNA intron	5' CCGGCTGTAATTATAAAGATACC

RNA for electromobility shift assays (EMSAs)	
U10	5' rUrUrUrUrUrUrUrUrU
U10-3'-P	5' rUrUrUrUrUrUrUrUrU/3Phos/
4U (wild type)	5' rCrUrGrCrUrGrUrUrU
U_1C	5' rCrUrGrCrUrGrUrUrC
U_2C	5' rCrUrGrCrUrGrUrCrU
U_3C	5' rCrUrGrCrUrGrUrCrU
4C	5' rCrUrGrCrIrGrCrCrC

Table S6. NCBI accession numbers of primary amino acid sequences used for conservation analysis

		La	Rex1p	RNase Z / ELAC2	LSm2	LSm3	LSm4	LSm5	LSm6	LSm7	LSm8
Vertebrates	*Homo sapiens*	NP_003133.1	NP_065746.3	NP_060597.4	NP_067000.1	NP_055278.1	NP_036453.1	NP_036454.1	NP_009011.1	NP_057283.1	NP_057284.1
	Mus musculus	NP_033304.1	NP_080128.2	NP_001349912.1	NP_001103571.1	NP_080585.1	NP_056631.2	NP_079796.1	NP_001177933.1	NP_001346078.1	NP_598700.1
	Xenopus laevis	NP_001081021.1									
	Danio rerio	NP_955841	NP_001119888.1	NP_001243133.1							
Invertebrates	*Caenorhabditis elegans*	NP_491411.1	NP_498135.2	NP_001023110.1							
	Drosophila melanogaster	NP_477014.1	NP_001034073.1	NP_724916.1							
Amoebozoa	*Dictyostelium discoideum*	XP_640625.1									
Kinetoplastide	*Trypanosoma brucei*	XP_822491.1									
Embryophytes	*Arabidopsis thaliana*	NP_567904.1 NP_178106.2									
	Physcomitrella patens	XP_024359573.1									
Chlorophytes	*Chlamydomonas reinhardtii*	XP_042914771.1									
Fungi	*Schizosaccharomyces pombe*	NP_593315.1	NP_594627.2	NP_595514.1	NP_588459.1	NP_595747.1	NP_001342832.1	NP_596373.1	NP_594380.1	NP_588340.1	NP_588509.1
	Saccharomyces cerevisiae	NP_010232.1			NP_009527.1	NP_013543.3	NP_011037.3	NP_011073.1	NP_010666.2	NP_014252.2	NP_012556.2
Alveolates	*Paramecium tetraurelia*	XP_001439258.1	XP_001435945.1	XP_001346883.1							
	Tetrahymena thermophila	XP_001019287.2	XP_001033374.2	XP_001031902.2	XP_012654399	XP_012656392.1	XP_001008519.2	XP_012655775.1	XP_012653392.1	XP_001031297.1	XP_001470817.1

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