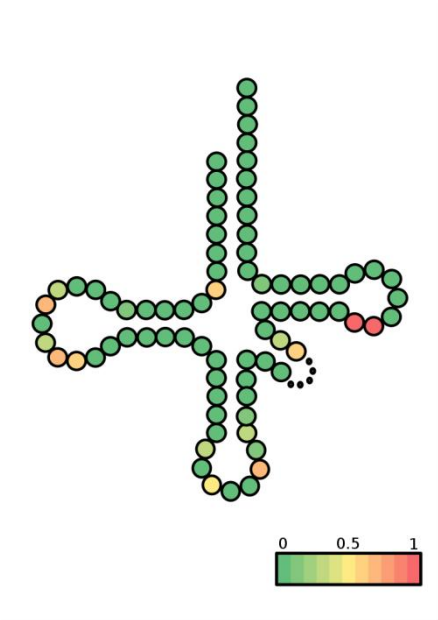
A



B

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fMet-CAT-1-1      1 CGCGGGGTGGAGCAGCCtGGTAGCTCGTCGGGCTCATAACCCGAAGGtCGTCGGTTCAAATCCGGCCCCCGCAA  74
                    ||||||||||||||||||||||||||||||||||||||||||||||||.||||||||||||||||||||||||||
fMet-CAT-2-1      1 CGCGGGGTGGAGCAGCCtGGTAGCTCGTCGGGCTCATAACCCGAAGAtCGTCGGTTCAAATCCGGCCCCCGCAA  74

Leu-CAG-1-1       1 GCGAAGGTGGCGGAATTGGTaGACGCGCTAGCTTCAGGTGTTAGTGTCCTTACGGACGTGGGGGTTCAAGTCCCCCCCCTCGCA  84
                    ||||||||||||||||||||||||||||||||||||||||||||||||||.||||||||||||||||||||||||||||||||||||
Leu-CAG-2-1       1 GCGAAGGTGGCGGAATTGGTaGACGCGCTAGCTTCAGGTGTTAGTGTTCTTACGGACGTGGGGGTTCAAGTCCCCCCCCTCGCA  84

Thr-CGT-1-1       1 GCCGATATAGCT-CAGTTGGTAGAGCAGCGCATTCGTAATGCGAAGGtCGTAGGTTCGACTCCTATTATCGGCA  73
                    ||..|..|||.| .|..||....|.||.|||||||||||||||||||||||||||||||||||||||||||||||
Thr-CGT-2-1       1 GCTCAAGTAGTTAAAAATGCaTTAACATCGCATTCGTAATGCGAAGGtCGTAGGTTCGACTCCTATTATCGGCA  74

Tyr-GTA-1-1       1 GGTGGGGTTCCCGAGCGGCcaAAGGGAGCAGACTGTAAATCTGCCGTCACAGACTtCGAAGGTTCGAATCCTTCCCCCCACCA  82
                    ||||||||||||||||||||||||||||||||||||||||||||||||||..||||||||||||||||||||||||||||||
Tyr-GTA-2-2       1 GGTGGGGTTCCCGAGCGGCcaAAGGGAGCAGACTGTAAATCTGCCGTCATCGACTtCGAAGGTTCGAATCCTTCCCCCACCA  82
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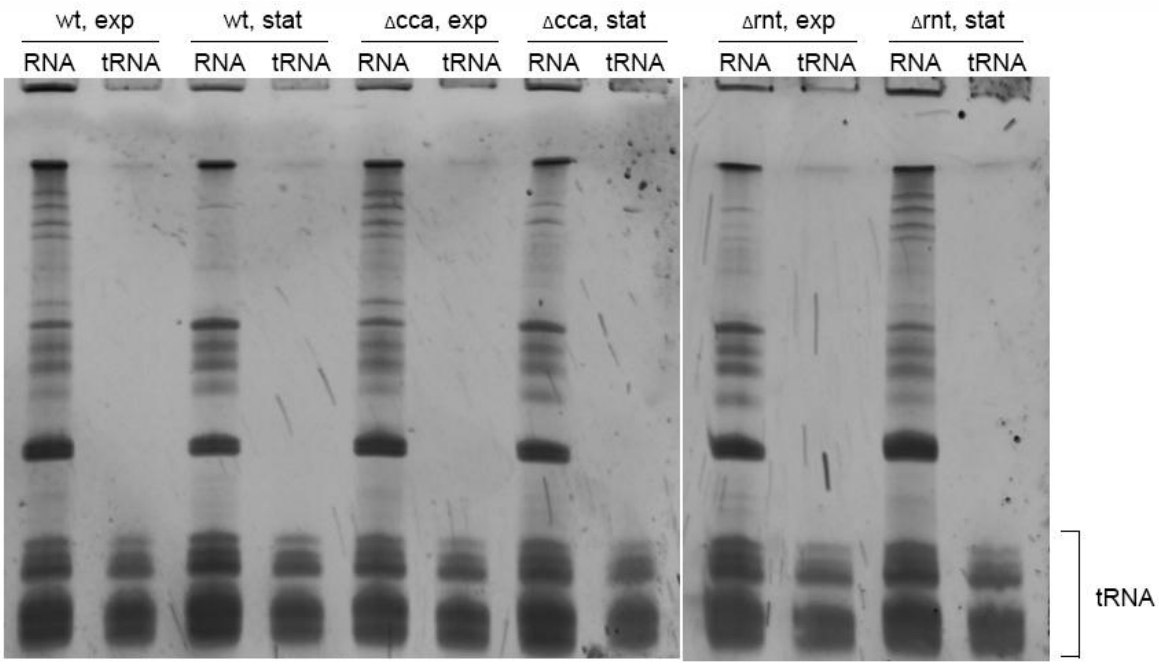
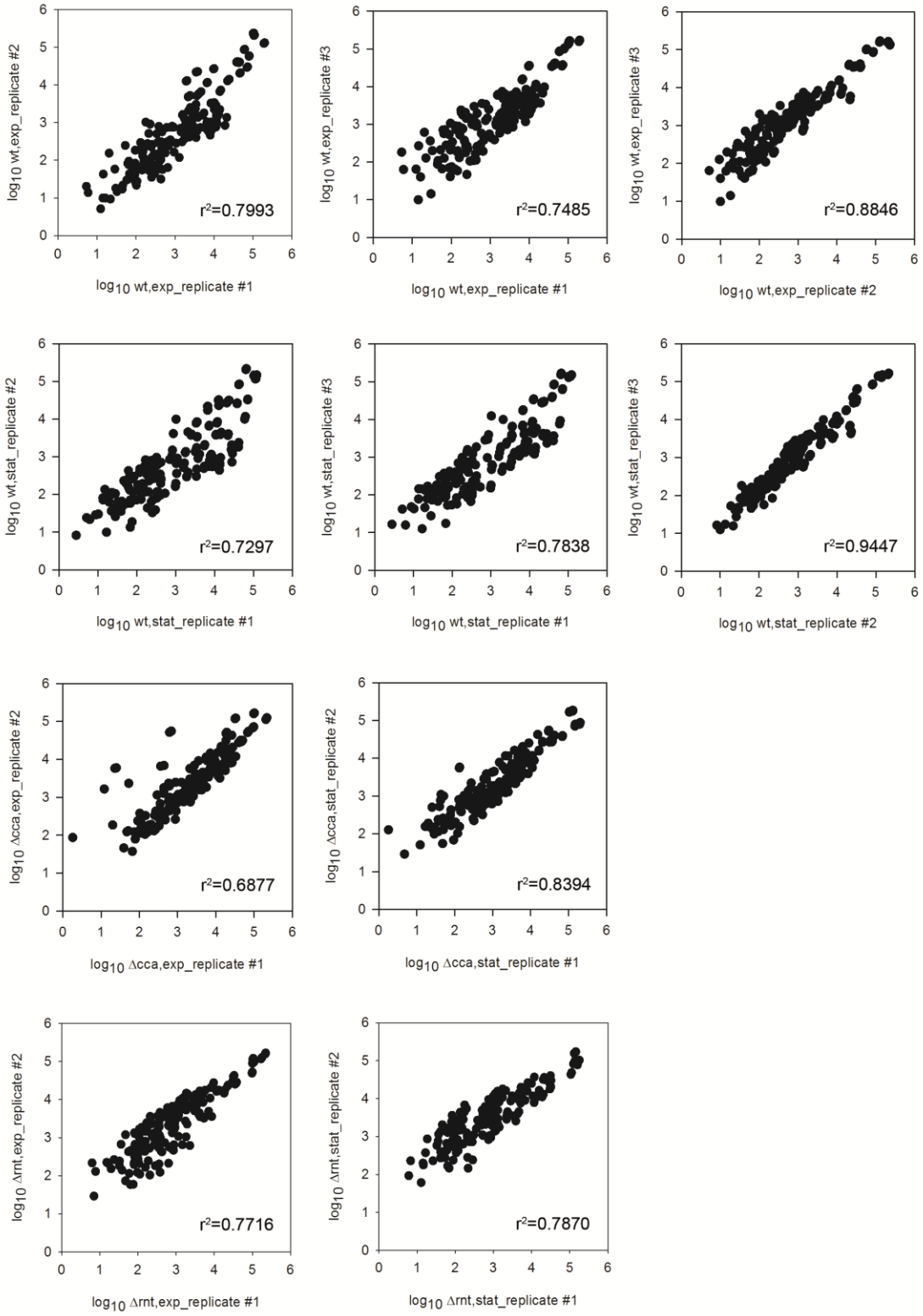
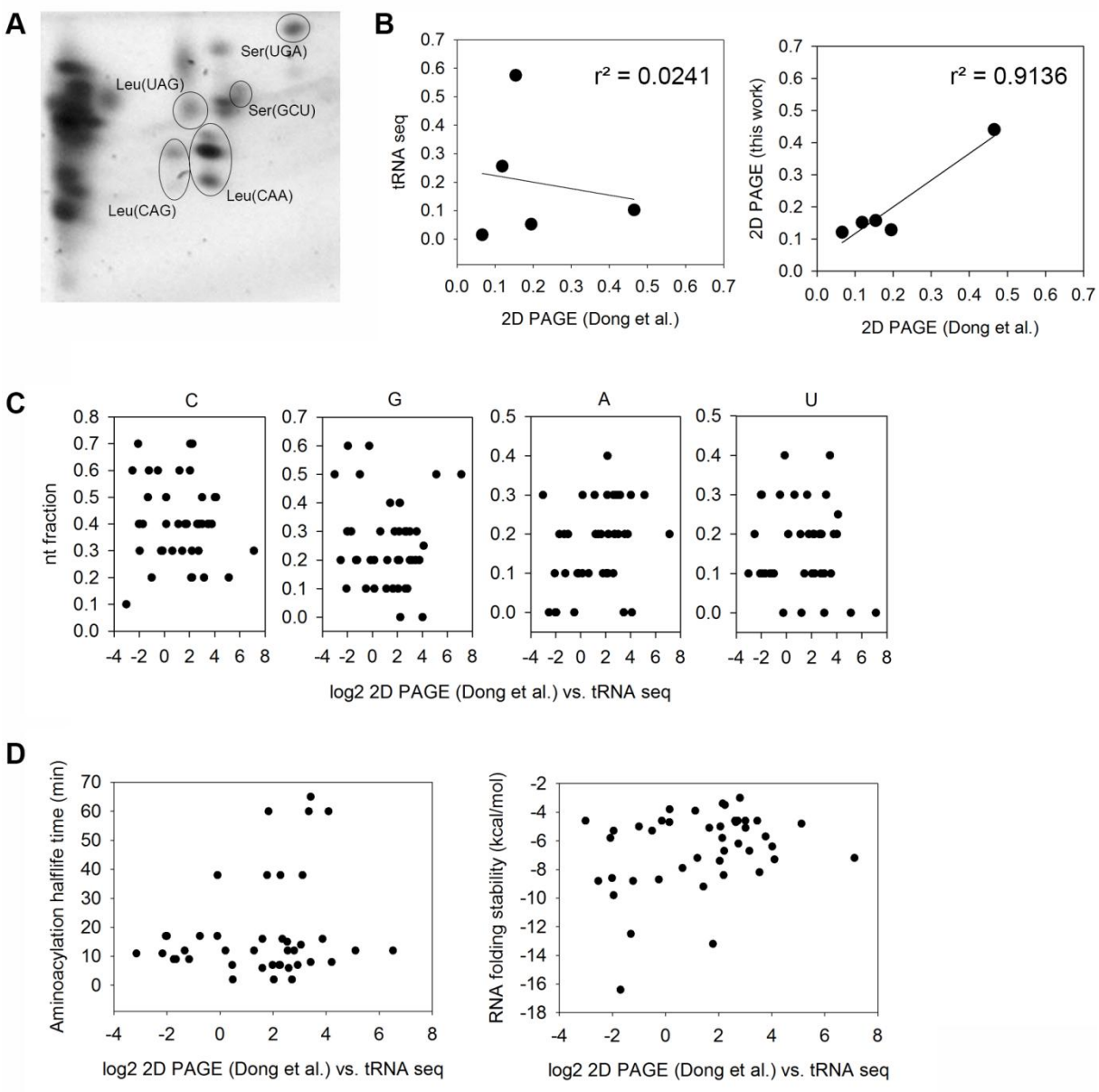
Supplementary Figure 1: A Modification of *E. coli* tRNA. For each position the fraction of modified tRNA was calculated based on the tRNA database tRNAdb 2009 (trnadb.bioinf.uni-leipzig.de/). **B** Alignment of four isodecoder pairs which cannot be discriminated by sequencing tRNA 3'-portion. The last 10 nt (yellow) were used for read mapping.



Supplementary Figure 2: PAGE-purification of tRNA from total RNA.



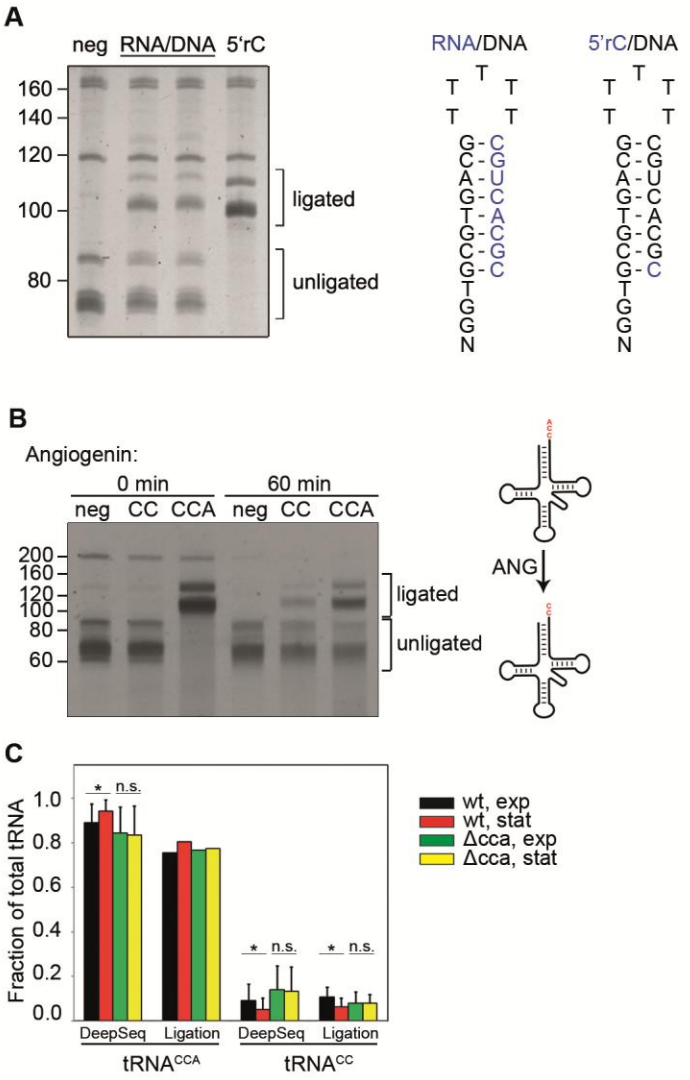
Supplementary Figure 3: Correlation of deep sequencing replicates. Points represent rpM of total fraction of one tRNA species and sub-fractions bearing CC, CCA and 3'-trailer.



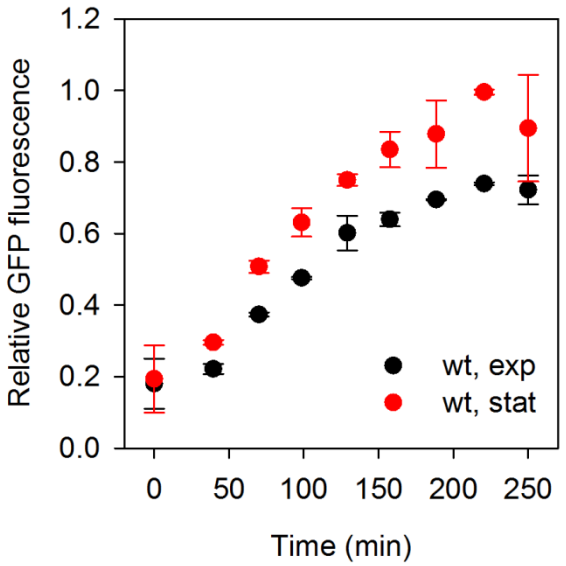
Supplementary Figure 4: Comparing results from tRNA 3'-terminus sequencing with 2D gel electrophoresis (PAGE). **A** Representative tRNA 2D gel. Five labeled spots were identified by Northern Blotting and quantified densitometrically. **B** tRNA sequencing reads (means of three replicates) or spot intensities from 2D gel electrophoresis (means of three replicates) were correlated to 2D gel electrophoresis results from Dong et al.(Dong et al. 1996). Data are represented as fractions of these five tRNA species. **C** For each tRNA the difference between results from Dong et. al. (Dong et al. 1996) were plotted against the frequency of each of the four nucleotides within the sequenced tRNA part. **D** As in **C**, the difference between 2D gel electrophoresis and tRNA sequencing was plotted against aminoacylation half-life times in 0.1 M Tris-HCl pH 8.6 at 37 °C (Hentzen et al. 1972) and against RNA folding stability of the sequenced tRNA part in conjunction with the 3'-adapter. Folding stabilities were calculated by the Vienna Fold Package (rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi).

Dong H, Nilsson L, Kurland CG. 1996. Co-variation of tRNA Abundance and Codon Usage in Escherichia coli at Different Growth Rates. *J Mol Biol* **260**: 649–663.

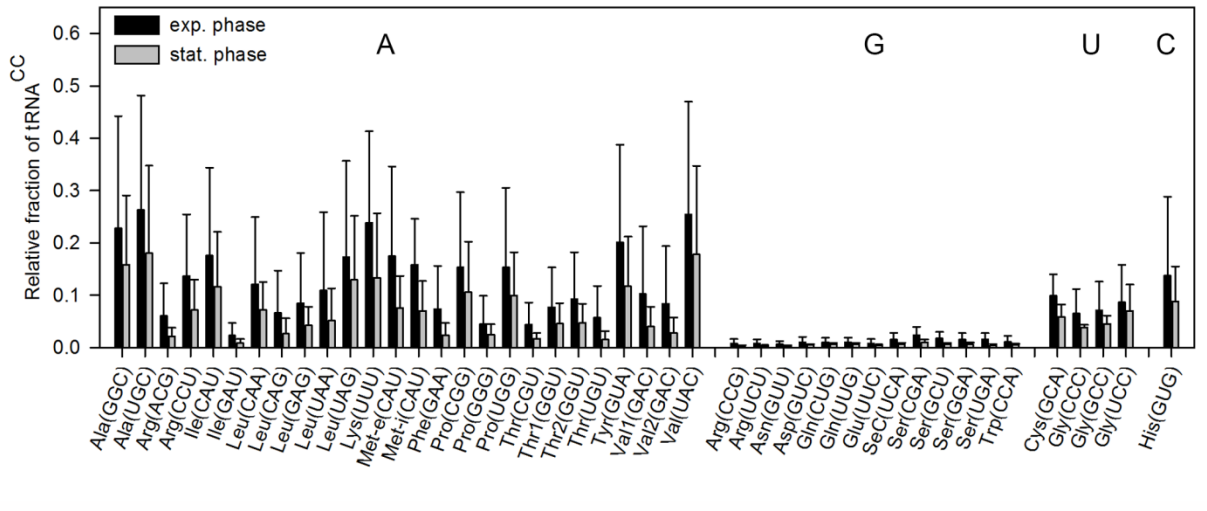
Hentzen D, Mandel P, Garel J-P. 1972. Relation between aminoacyl-tRNA stability and the fixed amino acid. *Biochim Biophys Acta - Nucleic Acids Protein Synth* **281**: 228–232.



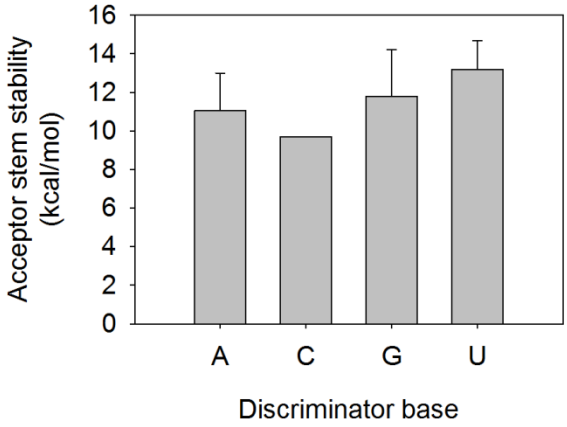
Supplementary Figure 5: A Improvement of tRNA ligation by reducing the RNA part of the oligonucleotide. Ligation of total RNA was performed without (neg), DNA/RNA or 5'rC oligonucleotide. **B** Proof of principal for CC-specific tRNA ligation assay. 3'-terminal adenosine cleaving angiogenin reduces CCA-specific and improves CC-specific ligation. Ligation of total RNA was performed without (neg) oligonucleotide, with CC- (CC) and CCA-specific (CCA) oligonucleotide and assessed by PAGE and SYBR Gold staining. **C** Comparison of tRNA fractions derived from deep sequencing and quantification of ligation assay. Data is represented as means±SD, Student's t-test.



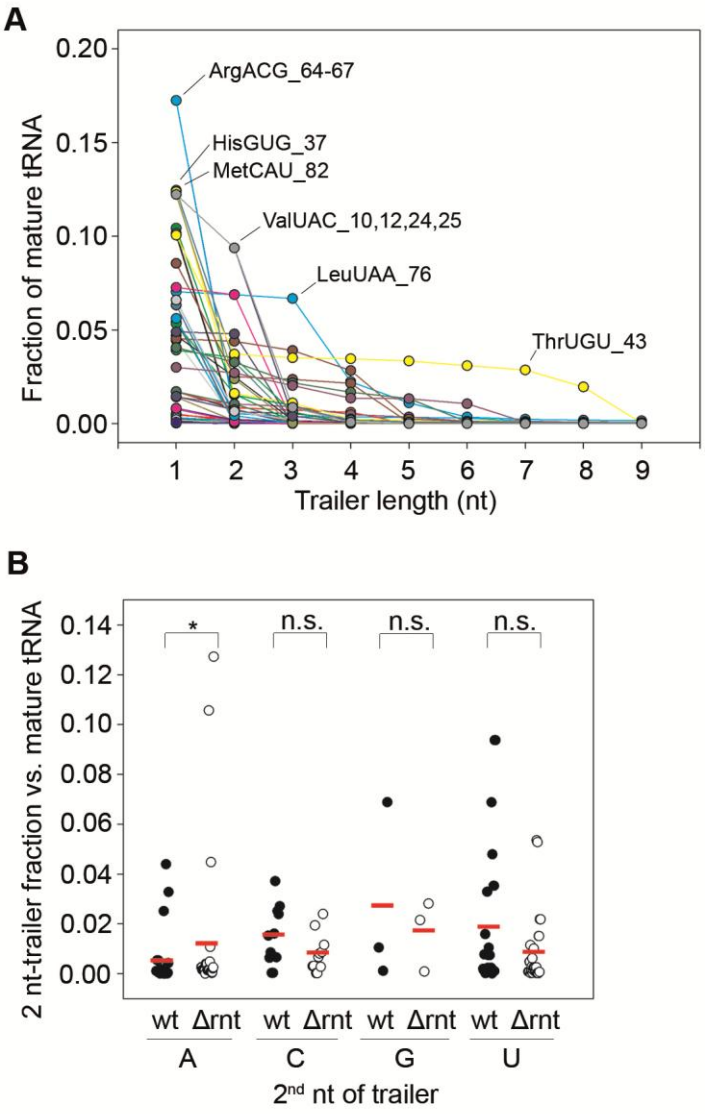
Supplementary Figure 6: *In vitro* translation assay with tRNA isolated from wt cells from exponential (exp) and stationary (stat) growth phase. Data is represented as means±SD (*n* = 2).



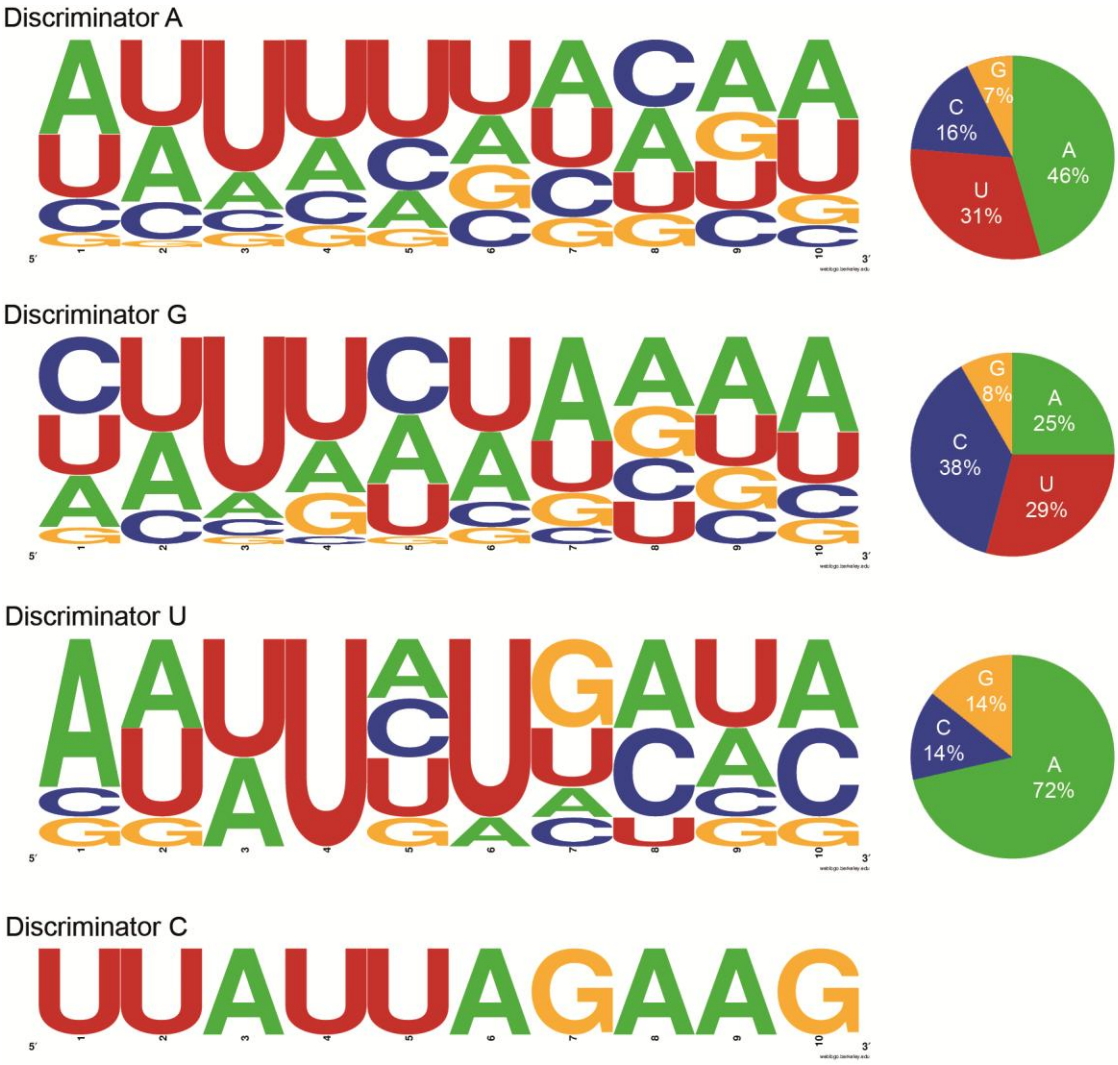
Supplementary Figure 7: Fractions of non-intact tRNAs bearing CC-tail among different species measured by deep sequencing. Data is represented as means±SD (*n* = 3).



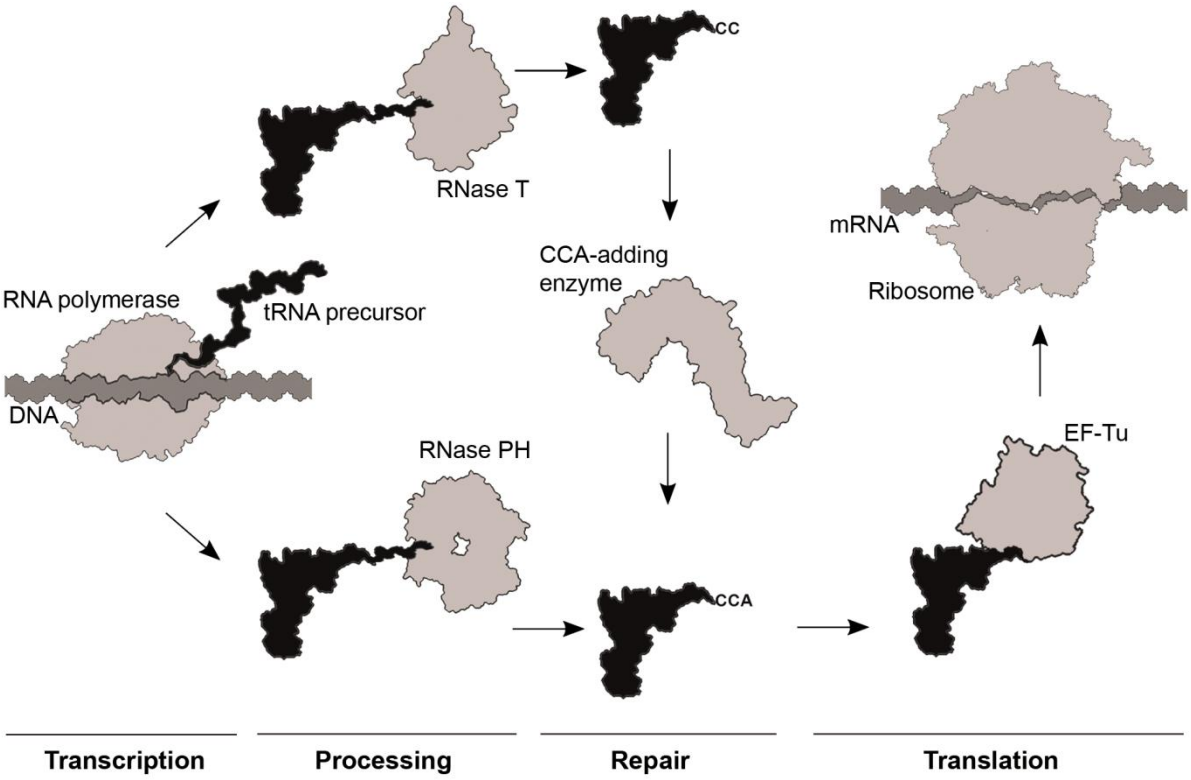
Supplementary Figure 8: Comparison of acceptor stem stability among tRNA groups bearing different discriminator bases. Folding stabilities were calculated for each tRNA by the Vienna Fold Package (rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) and means±SD were calculated for each group of discriminator base.



Supplementary Figure 9: Maturation of tRNA precursor varies among tRNA species. **A** Fractions of 3'-trailers of different lengths normalized by the amount of respective mature tRNA. Data is represented as means (*n* = 2). **B** Fractions of 2 nt-trailer precursors grouped by nucleotide identity (*n* = 2), Wilcoxon rank-sum test.



Supplementary Figure 10: Consensus sequences of 3'-trailers grouped by discriminator base (left) and quantification of first nucleotide in each group (right). Weblogos were obtained from weblogo.berkeley.edu.



Supplementary Figure 11: Model for different maturation pathways for tRNA precursors processed by RNase T or RNase PH. Processing by RNase T can lead to CCA-tail damage which needs to be repaired before entering the pool of functional tRNAs for translation.