Supplementary Material

Different modification pathways for m^1A58 incorporation in yeast elongator and initiator tRNAs

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SUPPLEMENTARY TABLES

Supplementary Table S1 – Yeast and *E. coli* strains used in this study

Strain	Genotype	Source
BY4741	*MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0*	Euroscarf
BY4741-*dus1Δ*	BY4741, *YML080w::kanMX*	Euroscarf
BY4741-*dus3Δ*	BY4741, YLR401c*::kanMX*	Euroscarf
BY4741-*pus4Δ*	BY4741, *YNL292w::kanMX*	Euroscarf
BY4741-*trm1Δ*	BY4741, *YDR120c::kanMX*	Euroscarf
BY4741-*trm2Δ*	BY4741, *YKR056w::kanMX*	Euroscarf
BY4741-*trm4Δ*	BY4741, *YBL024w::kanMX*	Euroscarf
BY4741-*trm8Δ*	BY4741, *YDL201w::kanMX*	Euroscarf
BY4741-*trm10Δ*	BY4741, *YOL093w::kanMX*	Euroscarf
BY4741-*trm11Δ*	BY4741, *YOL124c::kanMX*	Euroscarf
BY4741-*rit1Δ*	BY4741, *YMR283c::kanMX*	Euroscarf
c13-ABYS-86	*MATα ura3Δ5 leu2-3,112 his3 pra1-1 prb1-1 prc1-1 cps1-3*	(1)
c13-ABYS-86-*trm4Δ*	c13-ABYS-86, *YBL024w::kanMX*	(2)
BW25113-*ygghΔ*	*E. coli* K-12 *lacI*$^{+}$*rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR*514 Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$ *rph-1* Δ*(araB–D)567* Δ*(rhaD–B)568* Δ*lacZ4787*(::*rrnB-3*) *hsdR514 rph-1 yggH::kan*	(3)
BL21-CodonPlus (DE3)-RIL	*E. coli* B F^{-} ompT hsdS($r_B^{-}m_B^{-}$) dcm^{+} Tet^{r} gal λ(DE3) endA Hte [argU ileY leuW Cam^{r}]	Agilent
BL21-CodonPlus (DE3)-RIL-*yggHΔ*	*E. coli* BL21-CodonPlus(DE3)-RIL *yggH::kan*	This study

Supplementary Table S2 – List of primers used in this study

Pus4-M1_EcoRI_Fwd: CAG-GCG-AAT-TCA-TGA-ATG-GAA-TAT-TTG-CTA-TT
Pus4-V403_NotI_Rev: TAC-AAG-GCG-GCC-GCT-TAC-ACC-TGT-TCG-ATT-TT

Trm2-M1_EcoRI_Fwd: GAC-AAA-GAA-TTC-ATG-TAC-GAA-CAG-TTT-GAA-TTT
Trm2-I639_NotI_Rev: TAT-TTT-GCG-GCC-GCA-ATT-AGA-TTC-TCT-TCA-TTA
Trm2_BamHI-del_Fwd: GTT-ATC-TTG-GAC-CCA-CCA-CGC-AAG-GGC-TGT-GAC-GAA-TTA-TTC
Trm2_BamHI-del_Rev: TGG-TGG-GTC-CAA-GAT-AAC-GGA-AGT-GTT-TTC-ACT-TGG-AGT-ATC-AAT-AG
Trm2-V116_BamHI_Fwd: CGA-TGG-ATC-CGT-TGA-AAC-AAC-TTC-TCC-GAT-GG
Trm2-I639_XhoI_Rev: GCG-CCT-CGA-GTT-AGA-TTC-TCT-TCA-TTA-TAC-ACA-C

Trm6-M1_BamHI_Fwd: AAT-TTA-GGA-TCC-GAT-GAA-TGC-TTT-GAC-AAC
Trm6-I478_NotI_Rev: CTC-TGC-GGC-CGC-TTA-TAT-CTT-TTG-TTT-CTT-AG
Trm61-M1_NdeI_Fwd: TTT-ACA-TAT-GTC-AAC-AAA-TTG-TTT-TTC-CGG-TTA
Trm61-K383_XhoI_Rev: TAA-TCC-TCG-AGT-TAT-TTT-TCC-GTG-GAT-CGA-AGA

yggH_A: GGT-GCG-TAC-CTC-ATC-CAG-TT
yggH_B: GTA-TCG-TTC-AGG-TGC-CGT-TT
yggH_C: CTG-CTC-AGG-GCG-ATC-TTT-AG
yggH_D: CGC-CAT-AAA-GCG-TTC-AAA-AT

k1: CAG-TCA-TAG-CCG-AAT-AGC-CT
k2: CGG-TGC-CCT-GAA-TGA-ACT-GC

rit1_C: TCT-AGG-AAA-AGT-GAG-TTC-AGG-CTT-A
rit1_D: TCG-TGT-AAA-ACC-TCA-GAA-CAC-TGT-A

dus3_C: GGT-CTA-GGC-AAC-AAA-GGT-ACA-CTA-A
dus3_D: TAT-TTT-GAT-TTT-CTT-GGA-ACC-CAT-A

trm10_C: TAC-CAA-TTA-TGA-AAA-CTG-GAA-CCA-T
trm10_D: TAC-AAC-ATC-AAA-GCA-AAA-TAA-GCA-A

kanC: TGA-TTT-TGA-TGA-CGA-GCG-TAA-T

T7 promoter: 5'TAA-TAC-GAC-TCA-CTA-TAG 3'

yeast $tRNA^{Phe}$-WT DNA template :
3' ATTATGCTGAGTGATATCGCCTAAATCGAGTCAACCCTCTCGCGGTCTGACTTCTAGACCTCCAG
GACACAAGCTAGGTGTCTTAAGCGTGGT 5'
yeast $tRNA^{Phe}$-ΔU17 DNA template:
3' ATTATGCTGAGTGATATCGCCTAAATCGAGTCACCCTCTCGCGGTCTGACTTCTAGACCTCCAGG
ACACAAGCTAGGTGTCTTAAGCGTGGT 5'
yeast $tRNA^{Phe}$-A20A60 DNA template:
3' ATTATGCTGAGTGATATCGCCTAAATCGAGTCAACCTTCTCGCGGTCTGACTTCTAGACCTCCAG
GACACAAGCTATGTGTCTTAAGCGTGGT 5'
yeast $tRNA^{Phe}$-A54 DNA template:
3' ATTATGCTGAGTGATATCGCCTAAATCGAGTCAACCCTCTCGCGGTCTGACTTCTAGACCTCCAG
GACACTAGCTAGGTGTCTTAAGCGTGGT 5'
yeast $tRNA^{Phe}$-UAAA DNA template:
3' ATTATGCTGAGTGATATCGCCTAAATCGAGTCACCTTCTCGCGGTCTGACTTCTAGACCTCCAGG
ACACTAGCTATGTGTCTTAAGCGTGGT 5'

yeast $tRNA_i^{Met}$-WT DNA template:
3′ ATTATGCTGAGTGATATCCGCGGCACCGCGTCACCTTCGCGCGTCCCGAGTATTGGGACTACAGG
AGCCTAGCTTTGGCTCGCCGCGGTGGT 5′
yeast $tRNA_i^{Met}$-U17 DNA template:
3′ ATTATGCTGAGTGATATCCGCGGCACCGCGTCAACCTTCGCGCGTCCCGAGTATTGGGACTACAG
GAGCCTAGCTTTGGCTCGCCGCGGTGGT 5′
yeast $tRNA_i^{Met}$-G20C60 DNA template:
3′ ATTATGCTGAGTGATATCCGCGGCACCGCGTCACCCTCGCGCGTCCCGAGTATTGGGACTACAGG
AGCCTAGCTTGGGCTCGCCGCGGTGGT 5′
yeast $tRNA_i^{Met}$-U54 DNA template:
3′ ATTATGCTGAGTGATATCCGCGGCACCGCGTCACCTTCGCGCGTCCCGAGTATTGGGACTACAGG
AGCCAAGCTTTGGCTCGCCGCGGTGGT 5′
yeast $tRNA_i^{Met}$-UGCU DNA template:
3′ ATTATGCTGAGTGATATCCGCGGCACCGCGTCAACCCTCGCGCGTCCCGAGTATTGGGACTACAG
GAGCCAAGCTTGGGCTCGCCGCGGTGGT 5′
Remark: The A1-U72 base pair was replaced with a G1-C72 base pair to improve in vitro transcription efficiency for all $tRNA_i^{Met}$ variants.

reverse complementary oligo for yeast $tRNA_i^{Met}$ purification:
5′ [Biotin]-AAA-TCG-GTT-TCG-ATC-CGA-GGA-CAT-CAG-GGT-TAT-GA 3′

Supplementary Table S3 – Purification conditions of the modification enzymes used in this study

	Pus4	Trm2	Trm6/Trm61	Trm4
Cooled down at	30°C	25°C	18°C	30°C
IPTG induction concentration	0.5 mM	0.5 mM	0.4 mM	0.5 mM
Growing time after induction	6 h	6 h	22 h	17 h
Lysis Buffer	50 mM Tris-HCl pH 8.0, 300 mM NaCl, 10 mM BME, 1 mM PMSF, 10% (v/v) glycerol, 1 mM EDTA	50 mM Tris-HCl pH 8.0, 1 M NaCl, 5 mM $MgCl_2$, 1 mM DTT, 5% (v/v) glycerol, 1 mM EDTA	50 mM Tris-HCl pH 8.0, 300 mM NaCl, 1 mM PMSF, 1 mM DTT, 5% (v/v) glycerol, 1 mM EDTA	50 mM Tris-HCl pH 8.0, 0.5 M NaCl, 1 mM DTT, 0.1% (v/v) Triton X-100, 1 mM EDTA
Purification steps	-HisTrap HP -Superdex 75	-HisTrap HP -HiPrep Phenyl HP -Superdex 75	-HisTrap HP -Superdex 200	-HisTrap HP -Superdex 200
Storage Buffer	50 mM Tris-HCl pH 8.0, 150 mM NaCl, 2 mM BME	50 mM Tris-HCl pH 8.0, 400 mM NaCl, 1 mM DTT	50 mM Tris-HCl pH 8.0, 300 mM NaCl, 5 mM DTT	50 mM Tris-HCl pH 8.0, 300 mM NaCl, 1 mM DTT, 5% (v/v) glycerol
Concentration	to ~10 mg/mL with Amicon 10,000 MWCO	to ~5 mg/mL with Amicon 50,000 MWCO	to ~5 mg/mL with Amicon 50,000 MWCO	to ~8 mg/mL with Amicon 50,000 MWCO
mM extinction coefficient	30.4 $mM^{-1}.cm^{-1}$	30.8 $mM^{-1}.cm^{-1}$	89 $mM^{-1}.cm^{-1}$	80.9 $mM^{-1}.cm^{-1}$
Vector	pET28-Pus4	pSUMO-Trm2	pETDuet-Trm6/Trm61	pET28-Trm4
Expression strain	*E. coli* BL21(DE3) CodonPlus-RIL	*E. coli* BL21(DE3) CodonPlus-RIL	*E. coli* BL21(DE3) CodonPlus-RIL *yggH::kan*	*E. coli* BL21(DE3) CodonPlus-RIL
Purification adapted from reference	(4)	-	-	(5)

IPTG: isopropyl-β-D-thiogalactopyranoside; BME : 2-mercaptoethanol; PMSF: phenylmethanesulfonylfluoride; EDTA: ethylenediaminetetraacetic acid; DTT: dithiothreitol

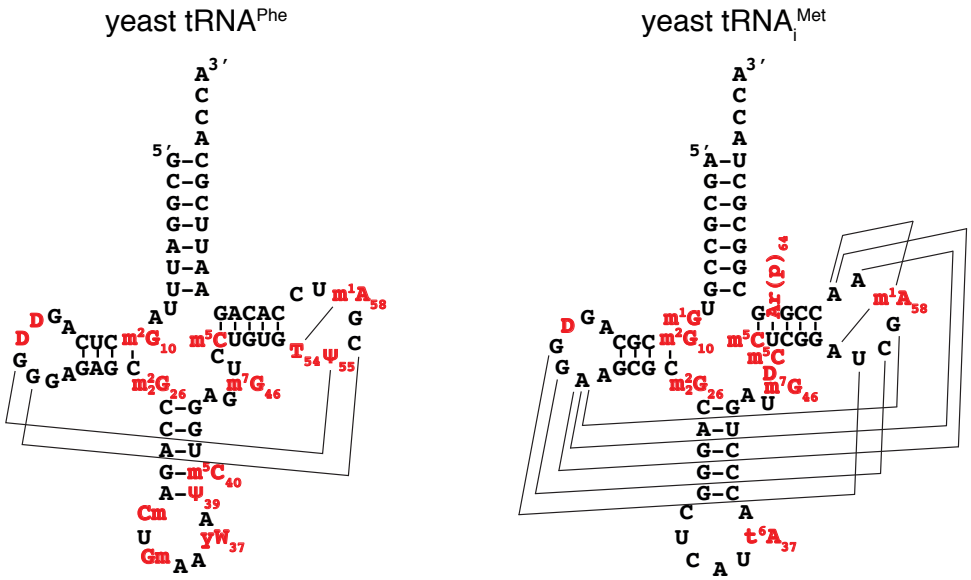
Supplementary Table S4

Enzyme	Trm2		Trm6/Trm61					
tRNA quantity	500 pmol		500 pmol					
Yeast tRNAs	unmodified - $tRNA^{Phe}$	Ψ55- $tRNA^{Phe}$	unmodified - $tRNA^{Phe}$	T54- $tRNA^{Phe}$	Ψ55- $tRNA^{Phe}$	Ψ55-T54- $tRNA^{Phe}$	unmodified- $tRNA_i^{Met}$	$m^5C48,49$- $tRNA_i^{Met}$
Enzyme (pmol)	20	2.5	15	15	5	2.5	7.5	7.5
SAM (pmol)	880	880	880	880	880	880	880	880
[^{3}H]-SAM (pmol)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Reaction conditions	MB1X 30°C 30 min	MB1X 30°C 30 min	MB1X 30°C 96 min	MB1X 30°C 30 min	MB1X 30°C 30 min	MB1X 30°C 30 min	MB1X 30°C 30 min	MB1X 30°C 30 min

The different kinetic reaction mixes and conditions to determine the initial velocities of Trm2 introducing the T54 modification and Trm6/Trm61 introducing the m^1A58 modification to a set of specifically modified yeast tRNAs. MB corresponds the maturation buffer: 100 mM NaH_2PO_4/K_2HPO_4 pH 7.0, 5 mM NH_4Cl, 2 mM DTT and 0.1 mM EDTA. These quantities correspond to a volume of 50 µL of reaction.

SUPPLEMENTARY FIGURES

a



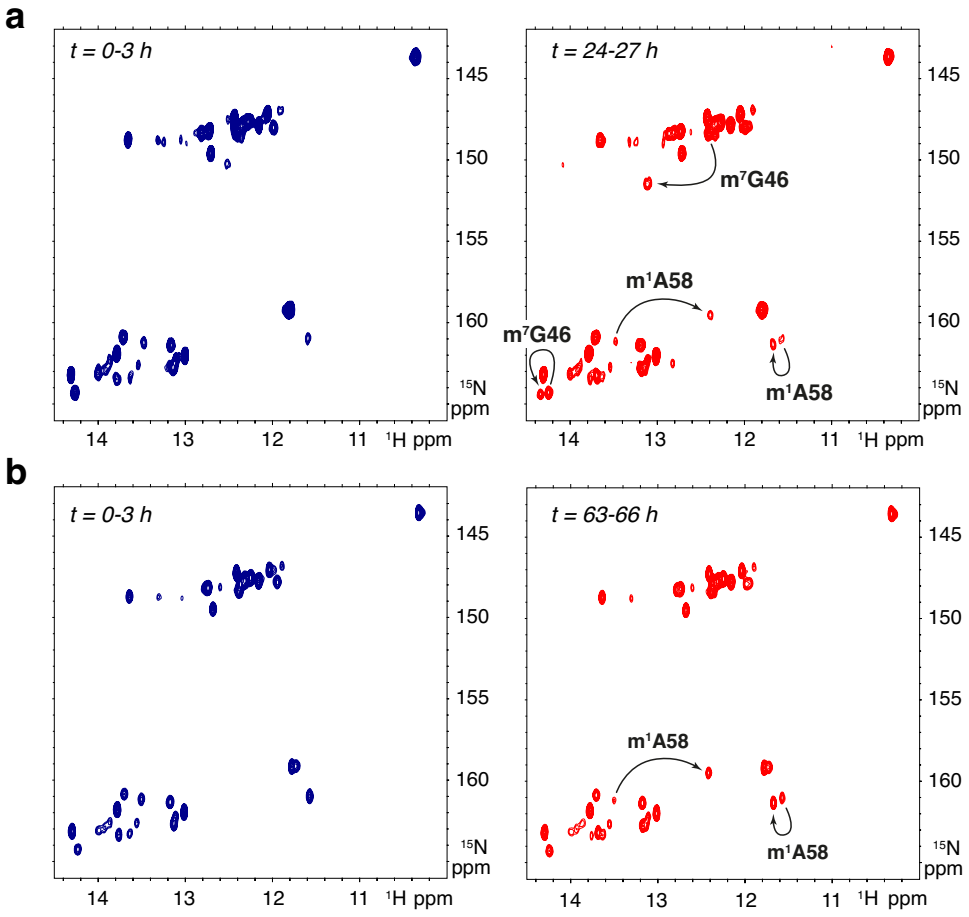
b

Modification	Enzyme	Partner protein
m²G10	Trm11	Trm112
D16	Dus1	-
D17	Dus1	-
m²₂G26	Trm1	-
Cm32	Trm7	Trm732
Gm34	Trm7	Trm734
yW37	Trm5, TYW1-4	-
Ψ39	Pus3	-
m⁵C40	Trm4	-
m⁷G46	Trm8	Trm82
m⁵C49	Trm4	-
m⁵U54 (T54)	Trm2	-
Ψ55	Pus4	-
m¹A58	Trm61	Trm6

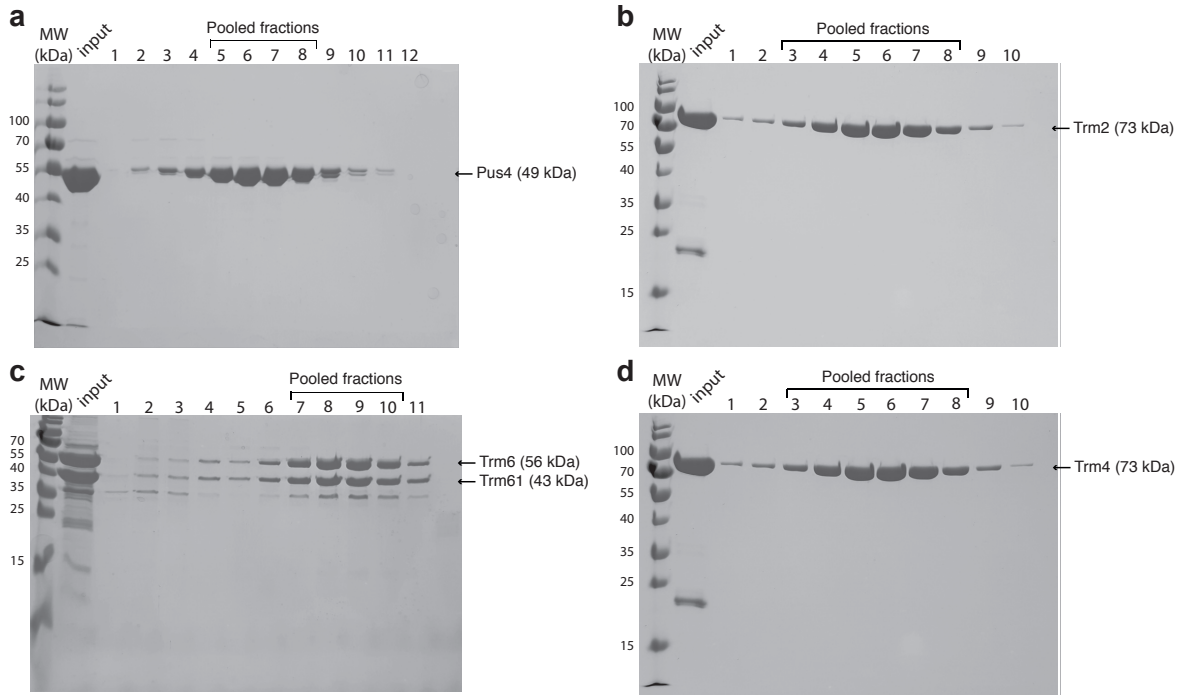
Modification	Enzyme	Partner protein
m¹G9	Trm10	-
m²G10	Trm11	Trm112
D16	Dus1	-
m²₂G26	Trm1	-
t⁶A37	Sua5	EKC/KEOPS
m⁷G46	Trm8	Trm82
D47	Dus3	-
m⁵C48	Trm4	-
m⁵C49	Trm4	-
m¹A58	Trm61	Trm6
Ar(p)64	Rit1	-

Supplementary Figure S1: Sequence and modifications of yeast elongator tRNA^{Phe} and yeast initiator tRNA$_i^{Met}$

(**a**) Sequence and cloverleaf representation of yeast tRNA^{Phe} (*left*) and yeast tRNA$_i^{Met}$ (*right*). Main tertiary interaction between the D- and T-loops are represented with thin lines. Modifications are highlighted in red. (**b**) Table of correspondence between the modifications and the enzyme responsible for their introduction in yeast tRNA^{Phe} (*left*) and in yeast tRNA$_i^{Met}$ (*right*).

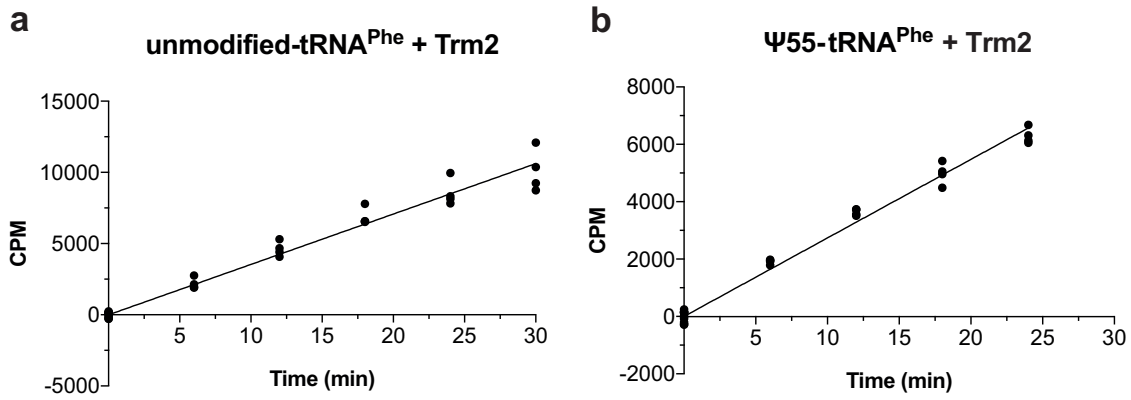


Supplementary Figure S2: Contamination of Trm6/Trm61 with m^{7}G46 activity
(**a**) (^{1}H,^{15}N)-BEST-TROSY experiments on ^{15}N-labelled tRNA^{Phe} reporting on Trm6/Trm61 enzymatic activity purified from *E. coli* BL21(DE3)CodonPlus-RIL cells. Concentrations: tRNA^{Phe} at 100 µM and Trm6/Trm61 at 2 µM. Although the m^{1}A58 activity is clearly observable, the Trm6/Trm61 sample is contaminated with m^{7}G46 activity. (**b**) (^{1}H,^{15}N)-BEST-TROSY experiments on ^{15}N-labelled tRNA^{Phe} reporting on Trm6/Trm61 enzymatic activity purified from *E. coli* BL21(DE3)CodonPlus-RIL *yggh::kan* cells. The sample is no longer contaminated with m^{7}G46 activity.



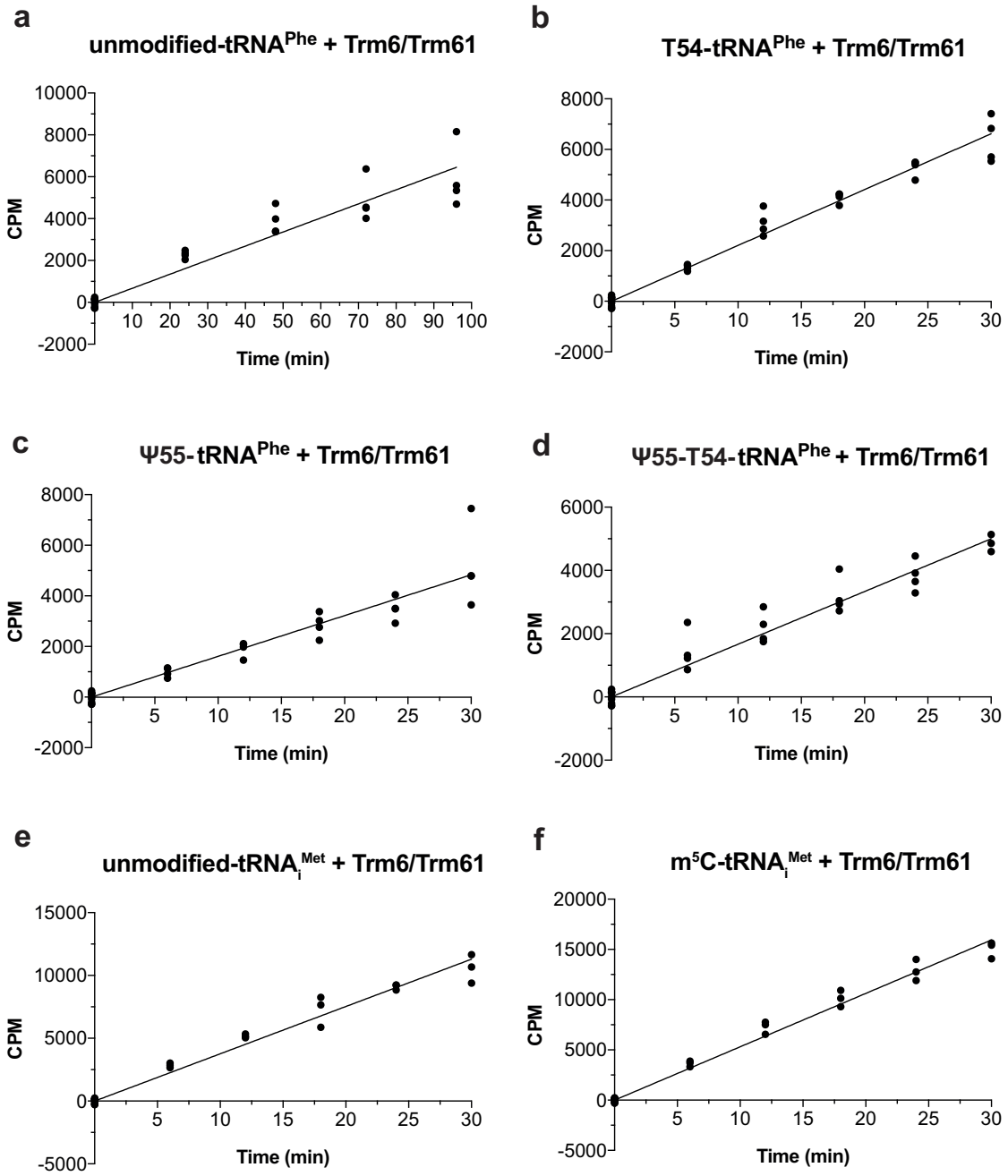
Supplementary Figure S3: Quality control in the last step of purification of the modification enzymes

(**a**) SDS-PAGE of the fractions of the Superdex 75 size exclusion column of Pus4 purification. (**b**) SDS-PAGE of the fractions of the Superdex 200 size exclusion column of Trm2 purification. (**c**) SDS-PAGE of the fractions of the Superdex 200 size exclusion column of Trm6/Trm61 purification. (**d**) SDS-PAGE of the fractions of the Superdex 200 size exclusion column of Trm4 purification. (MW): protein ladder, size in kDa. (input): fraction injected onto the column. For each purification, the pooled fractions are indicated above the gel.



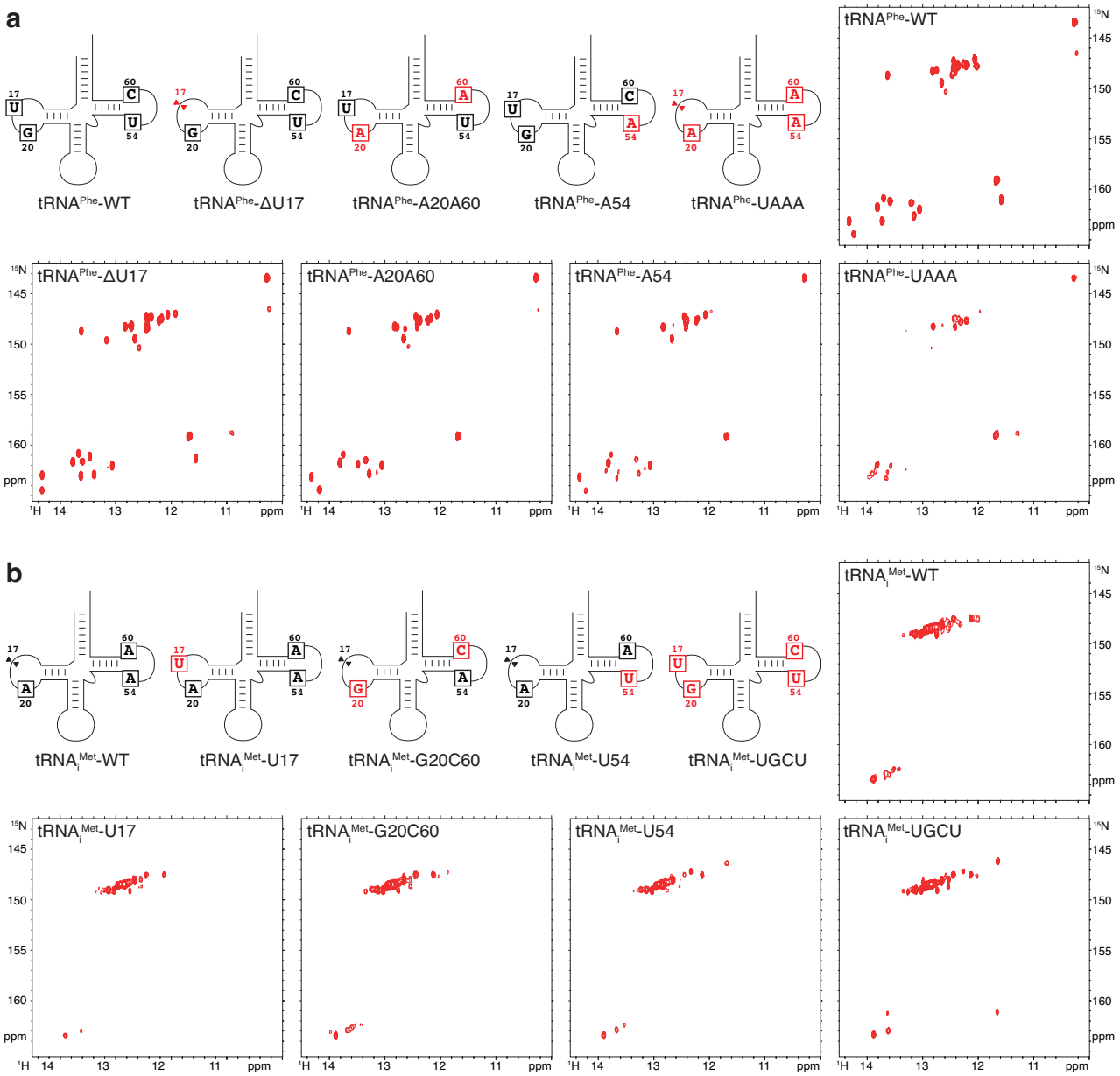
Supplementary Figure S4: Kinetic efficiency of Trm2 depending on the modification profiles of yeast tRNA^{Phe}

(**a**) Raw counts per minute (CPM) data corresponding to T54 introduction by Trm2 on the yeast unmodified-tRNA^{Phe}. (**b**) Raw CPM data corresponding to T54 introduction by Trm2 on the yeast tRNA^{Phe} containing the Ψ55 modification (Ψ55-tRNA^{Phe}). CPM are measured for 4 or 5 time points in four independent experiments (N=4). Black dots represent individual measurements.



Supplementary Figure S5: Kinetic efficiency of Trm6/Trm61 depending on the modification profiles of yeast tRNA^{Phe} and tRNA$_i^{Met}$.

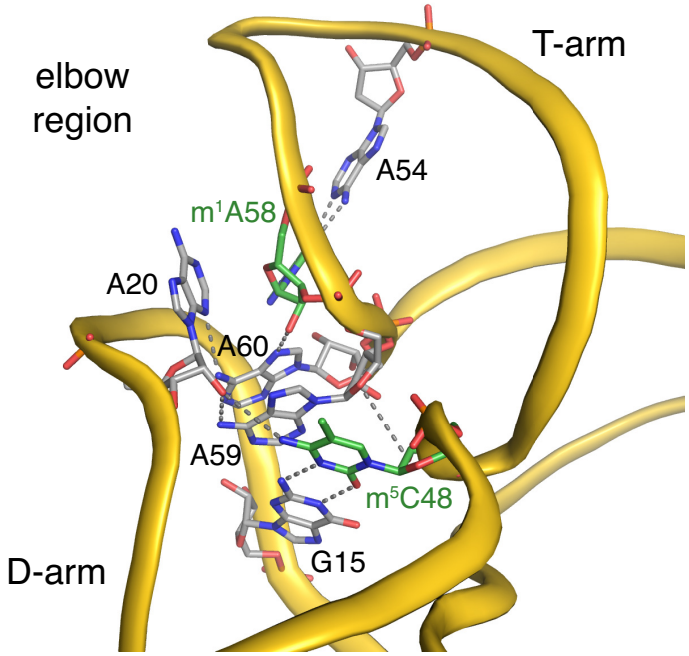
(**a**) Raw counts per minute (CPM) data corresponding to the m^{1}A58 introduction by Trm6/Trm61 on the yeast unmodified-tRNA^{Phe}. (**b**) Raw CPM data corresponding to the m^{1}A58 introduction by Trm6/Trm61 on the tRNA^{Phe} containing the T54 modification (T54-tRNA^{Phe}). (**c**) Raw CPM data corresponding to the m^{1}A58 introduction by Trm6/Trm61 on the tRNA^{Phe} containing the Ψ55 modification (Ψ55-tRNA^{Phe}). (**d**) Raw CPM data corresponding to the m^{1}A58 introduction by Trm6/Trm61 on the tRNA^{Phe} containing both the Ψ55 and T54 modifications (Ψ55-T54-tRNA^{Phe}). (**e**) Raw CPM data corresponding to the m^{1}A58 introduction by Trm6/Trm61 on the unmodified-tRNA$_i^{Met}$. (**f**) Raw CPM data corresponding to the m^{1}A58 introduction by Trm6/Trm61 on the tRNA$_i^{Met}$ containing the m^{5}C48,49 modifications (m^{5}C-tRNA$_i^{Met}$). CPM are measured for 4 or 5 time points in at least three independent experiments (N=3 or 4). Black dots represent individual measurements.



Supplementary Figure S6: Effect of nucleotide mutations on the structural properties of yeast tRNA^{Phe} and yeast tRNA$_i^{Met}$.

(**a**) (*top left*) Schematic cloverleaf representation of yeast elongator tRNA^{Phe} showing residues (boxed) characteristic of elongator tRNAs. Residues in black represent nucleotides that correspond to the WT sequence. Residues in red represent mutated nucleotides that correspond to the initiator tRNA$_i^{Met}$ sequence. The triangles in the D-loop denote that the U17 is absent. (*bottom right*) Imino (^{1}H,^{15}N) correlation spectra of ^{15}N-labelled tRNA^{Phe} WT and variants measured at 38°C in the NMR buffer.

(**b**) (*top left*) Schematic cloverleaf representation of yeast initiator tRNA$_i^{Met}$ showing residues (boxed) characteristic of initiator tRNA. Residues in black represent nucleotides that correspond to the WT sequence. Residues in red represent mutated nucleotides that correspond to the elongator tRNA^{Phe} sequence. The triangles in the D-loop denote that the U17 is absent. (*bottom right*) Imino (^{1}H,^{15}N) correlation spectra of ^{15}N-labelled tRNA$_i^{Met}$ WT and variants measured at 38°C in the NMR buffer.



Supplementary Figure S7: Close-up view of the intricate network of interaction at the level of the elbow region of initiator $tRNA_i^{Met}$

Close-up view of yeast $tRNA_i^{Met}$ illustrating the proximity between nucleotides m^5C48 and m^1A58. The intricate network of interactions between nucleotides G15, m^5C48, A20, A59, A60, and m^1A58 is shown with potential hydrogen bonds as grey dashes. tRNA backbone is shown as a cartoon in yellow. Modified nucleotides m^5C48 and m^1A58 are shown in green.

SUPPLEMENTARY REFERENCES

(1) Heinemeyer W, Kleinschmidt JA, Saidowsky J, Escher C, Wolf DH. Proteinase yscE, the yeast proteasome/multicatalytic-multifunctional proteinase: mutants unravel its function in stress induced proteolysis and uncover its necessity for cell survival. *EMBO J* (1991) 10:555-562.

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(3) Baba T, Ara T, Hasegawa M, et al. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* (2006) 2:2006.0008.

(4) Becker HF, Motorin Y, Planta RJ, Grosjean H. The yeast gene YNL292w encodes a pseudouridine synthase (Pus4) catalyzing the formation of psi55 in both mitochondrial and cytoplasmic tRNAs. *Nucleic Acids Res* (1997) 25:4493-99.

(5) Motorin Y, Grosjean H. Multisite-specific tRNA:m5C-methyltransferase (Trm4) in yeast Saccharomyces cerevisiae: identification of the gene and substrate specificity of the enzyme. *RNA* (1999) 5:1105-18.