Supplementary Material

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

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Content:

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$_{T14}$ ΔlacZ$_{WJ16}$ hsdR*514 *ΔaraBAD$_{AH33}$ ΔrhaBAD$_{LD78}$ rph-1 Δ(araB–D)567 Δ(rhaD–B)568 ΔlacZ4787(::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} DNA template:
3' ATTATGCTGAGTGATATCGGGCCTATCGAGTCAGCCATCTCGTCCCCTAACTTTTAGGGGCACAG
GAACCAAGCTAAGGCTCAGGCCCGTGGT 5'

yeast tRNA$_i^{Met}$ DNA template:
3' ATTATGCTGAGTGATATCCGCGGCACCGCGTCACCTTCGCGCGTCCCGAGTATTGGGACTACAGG
AGCCTAGCTTTGGCTCGCCGCGGTGGT 5'
Remark: The A1-U72 base pair was replaced with a G1-C72 base pair to improve in vitro transcription efficiency.

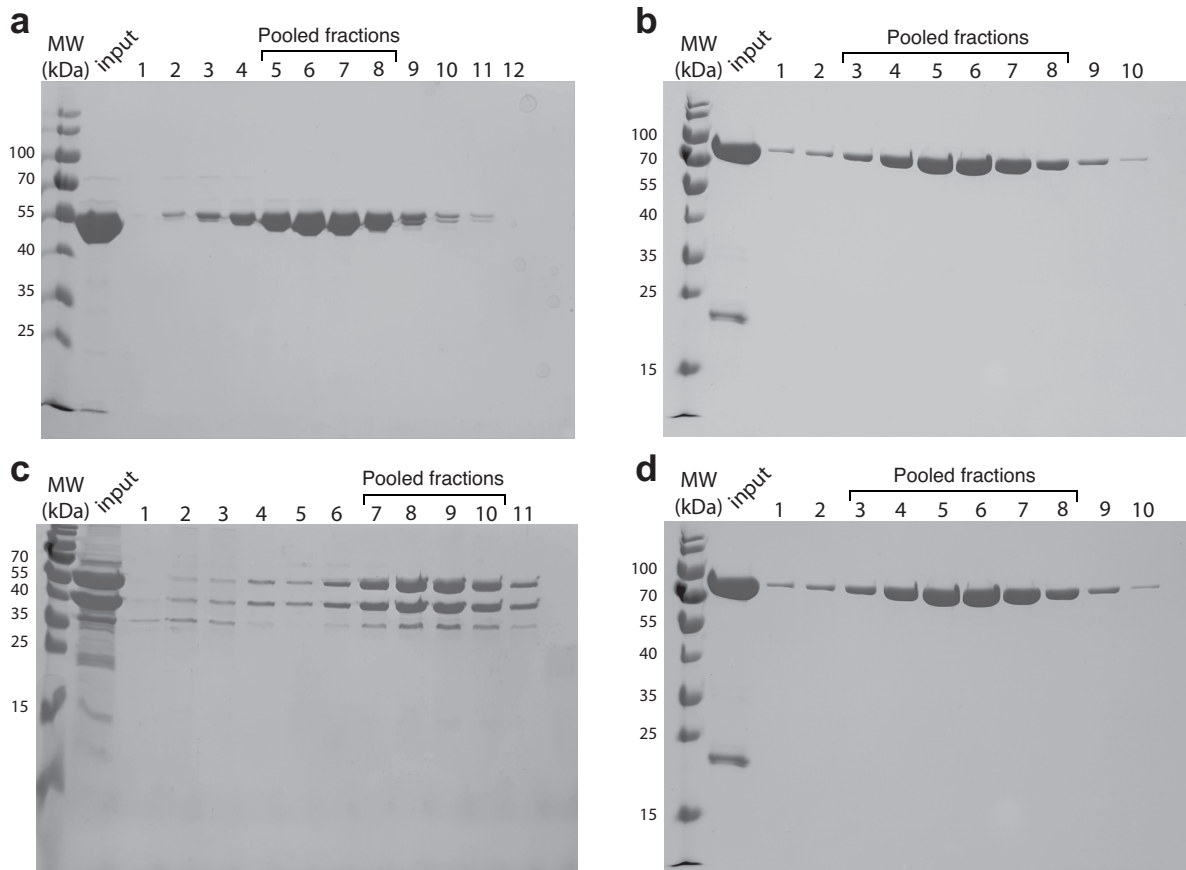reverse complementary oligo for yeast tRNA$_i^{Met}$ purification:
5' [Biotin]-AAA-TCG-GTT-TCG-ATC-CGA-GGA-CAT-CAG-GGT-TAT-GA 3'

Supplementary Table S3

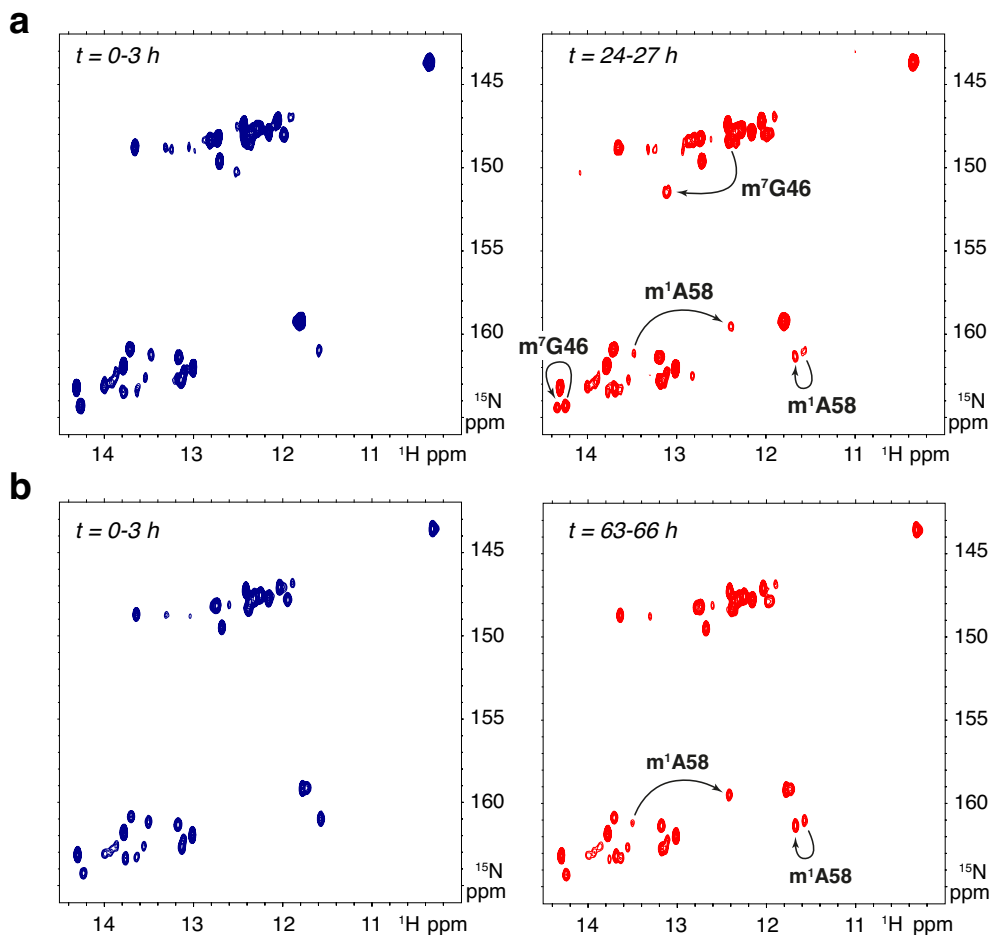| Enzyme	Trm2		Trm6/Trm61					
tRNA quantity	500 pmol		500 pmol					
Yeast tRNAs	unmodified - tRNA^{Phe}	Ψ55- tRNA^{Phe}	unmodified - tRNA^{Phe}	T54- tRNA^{Phe}	Ψ55- tRNA^{Phe}	Ψ55- T54- tRNA^{Phe}	unmodified- tRNA$_i^{Met}$	m^{5}C48,49- tRNA$_i^{Met}$
Enzyme (pmol)	20	2.5	15	15	5	2.5	7.5	7.5
SAM (pmol)	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^{1}A58 modification to a set of specifically modified yeast tRNAs. MB corresponds the maturation buffer: 100 mM NaH$_2$PO$_4$/K$_2$HPO$_4$ pH 7.0, 5 mM NH$_4$Cl, 2 mM DTT and 0.1 mM EDTA. These quantities correspond to a volume of 50 μL of reaction.
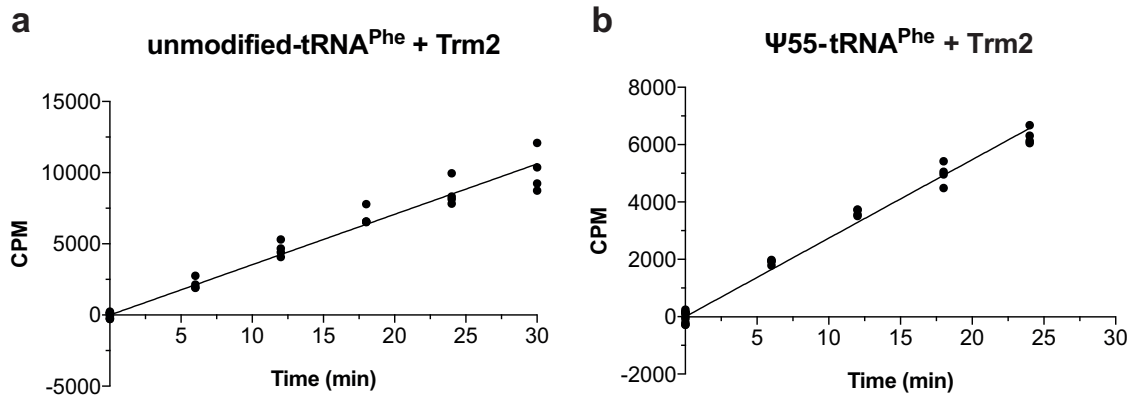
SUPPLEMENTARY FIGURES

Supplementary Figure S1: 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.
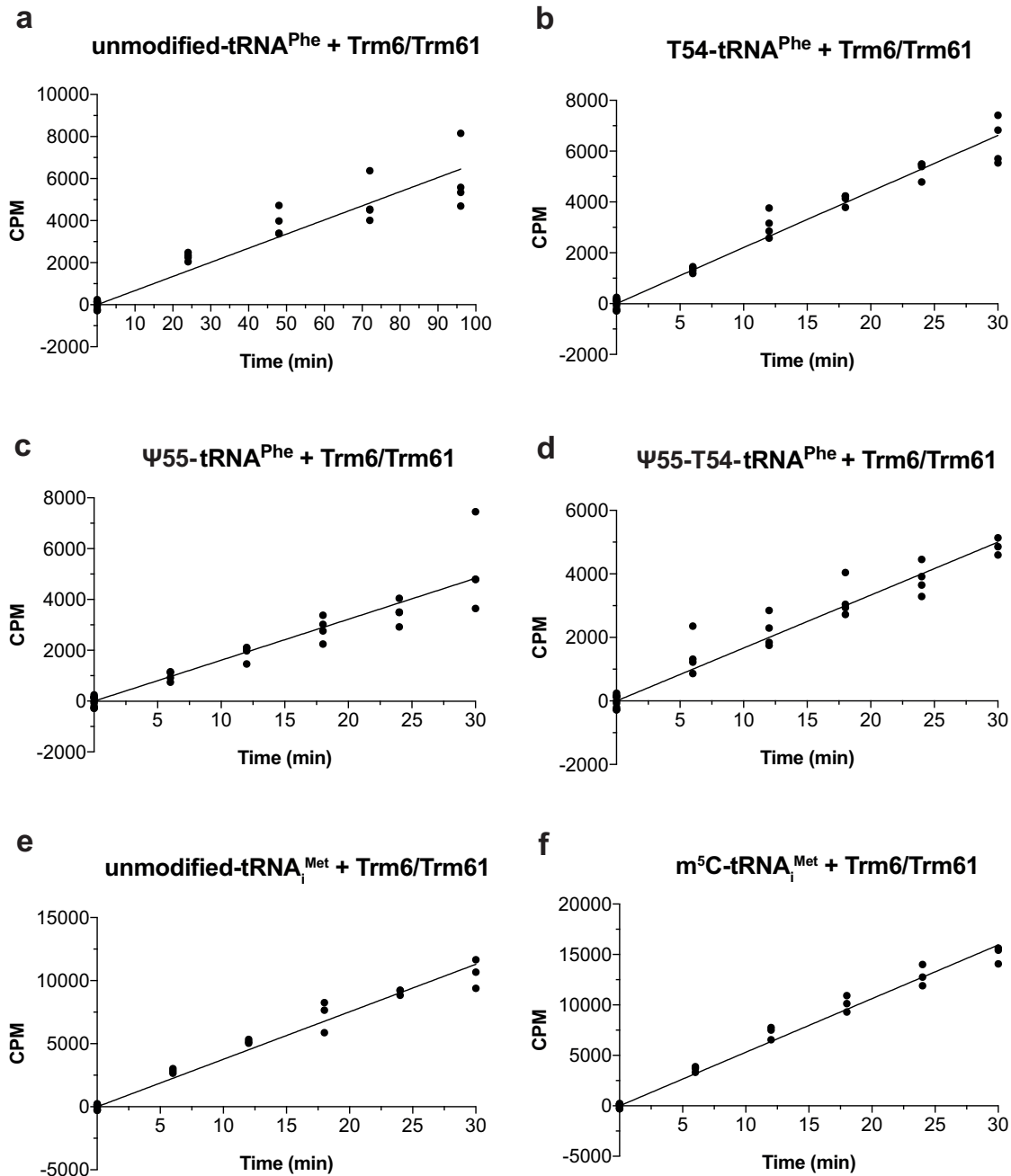
Supplementary Figure S2: Contamination of Trm6/Trm61 with m^{7}G46 activity
(**a**) (^{1}H,^{15}N)-BEST-TROSY experiments on ^{15}N-labelled tRNA^{Phe} reporting on Trm6/Trm61 enzymatic activity purified from *E. coli* BL21(DE3)CodonPlus-RIL cells. Concentrations: tRNA^{Phe} at 100 μM and Trm6/Trm61 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.

a   **unmodified-tRNA^Phe + Trm2**   **b**   **Ψ55-tRNA^Phe + Trm2**

Supplementary Figure S3: 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 S4: 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.

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SUPPLEMENTARY REFERENCES

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(2) Barraud P, Gato A, Heiss M, Catala M, Kellner S, Tisné C. Time-resolved NMR monitoring of tRNA maturation. *Nat Commun* (2019) 10:3373.

(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.