Supplementary Material for:

A tRNA-specific function for tRNA methyltransferase Trm10 is associated with a new tRNA quality control mechanism in *Saccharomyces cerevisiae*

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Table S1. Statistical analysis of mature tRNA levels.

***S. cerevisiae* strain**	***S. cerevisiae* strain**	**P-value[a] comparisons $tRNA^{Trp}$**	**P-value[a] comparisons $tRNA^{Gly}$**
TRM10	*trm10Δ*	3.27E-07	0.028
TRM10 + 5FU	*trm10Δ* + 5FU	0.00020	0.031
trm10Δ	*trm10Δ* + 5FU	0.00021	0.069
trm10Δ + $tRNA^{Trp}$	*trm10Δ* + $tRNA^{Trp}$ + 5FU	0.0087	0.12
TRM10	*trm10Δ* + $tRNA^{Trp}$	0.00085	0.28
TRM10 + 5FU	*trm10Δ* + $tRNA^{Trp}$ + 5FU	0.00043	0.14
trm10Δ	*trm10Δ* + $tRNA^{Trp}$ + 5FU	0.065	0.037
TRM10 + $tRNA^{Trp}$	*TRM10* + $tRNA^{Trp}$ + 5FU	0.12	0.15
trm10Δ	*trm10Δmet22Δ*	0.0048	0.13
trm10Δ + 5FU	*trm10Δmet22Δ* + 5FU	0.0019	0.069
trm10Δmet22Δ	*trm10Δmet22Δ* + *MET22*	0.061	0.11
trm10Δmet22Δ + 5FU	*trm10Δmet22Δ* + *MET22* + 5FU	0.005	0.16
trm10Δ	*trm10Δmet22Δ* + *MET22*	0.022	0.061
trm10Δ + 5FU	*trm10Δmet22Δ* + *MET22* + 5FU	0.00076	0.061

[a] Listed P-values for comparisons of quantified tRNA levels in **Figure 2** and **Figure 5**. One-tailed P-values were determined by two-sample t-tests assuming equal variances and ≤ 0.05 was defined as a significant difference.

Table S2. DNA oligonucleotide sequences used to probe northern membranes.

Northern Blot Oligo	Sequence	Source
Mature $tRNA^{Trp(CCA)}$	ATTTGGAGTCGAAAGCTCTACCATTG	Swinehart et al. 2013
Mature $tRNA^{Gly(GCC)}$	TGGCAACGTTGGATTTTACC	Swinehart et al. 2013
$tRNA^{Trp}$ intron	CGTGGAATTTCCAAGATTTAATTGGAGTCGAAAG CTCTACC	Chatterjee et al. 2022
$tRNA^{Trp}$ 5' leader (GAT)	CCATTGAGCCACCGCTTCATCTTGAAAT	This study
$tRNA^{Trp}$ 5' leader (GTT)	CCATTGAGCCACCGCTTCAACTTTTGTT	This study
5S rRNA	CTACTCGGTCAGGCTC	This study

Table S3. 5'-leader sequences for each annotated pre-$tRNA^{Trp}$ and pre-$tRNA^{Gly(GCC)}$ gene in *S. cerevisiae*.

5'-leader Sequence[a]	
$tRNA^{Gly(GCC)}$	**$tRNA^{Trp(CCA)}$**
tcaaaagtaa	atttcaagat
caattagtga	attccaagat
aataaagatc	aataaaagtt
aagaaattca	aacaaaagtt
acatgaaagc	aacaatagtt
acaatacttc	atttcaagat
taaataacaa	
caagaaataa	
aagattacta	
agttcactta	
acaattgtga	
caaatagtaa	
aagaaaacaa	
cttccaataa	
taaataacaa	
taatcagtaa	

[a] Sequences are listed 5'-3', with the last nucleotide (highlighted in red) corresponding to the -1 nucleotide that is immediately next to the RNase P cleavage site for each pre-tRNA.

Table S4. Comparison of 5FU-dependent and *trm10Δ*-dependent changes in individual pre-tRNA^{Trp} species

Pre-tRNA	*S. cerevisiae* strain	+ 5FU	- 5FU	Fold-change[b]	*trm10Δ*	*TRM10*	Fold-change
Initial transcript	*TRM10* + tRNA^{Trp}	1.55[a]	0.05	31	0.55	0.03	18
	TRM10	1.11	0.03	37			
3' processed	*TRM10* + tRNA^{Trp}	1.93	0.11	18	0.93	0.07	13
	TRM10	1.92	0.07	27			
Intron-containing	*TRM10* + tRNA^{Trp}	0.82	0.12	6.8	0.5	0.06	8.3
	TRM10	0.40	0.06	6.5			

[a] Average abundance of indicated pre-tRNA species in each strain are listed based on quantification using intron-targeting probe (green) as reported in **Figure S1**.
[b] Fold-change calculated from average abundance +5FU/-5FU for each pre-tRNA in each indicated strain.
[c] Fold-change calculated from average abundance *trm10Δ*/*TRM10* for each pre-tRNA in each indicated strain.

Table S5. OD_{600} for the indicated strains at time of harvest for RNA purification.

Strain	OD_{600}	
	- 5FU	+ 5FU
JJY 835	1.636	0.641
JJY 836	1.505	0.607
JJY 833	0.884	0.715
JJY 834	0.988	0.323
JJY 850	0.612	0.471
JJY 851	0.570	0.478
JJY 844	0.764	0.363
JJY 853	0.838	0.514

SUPPLEMENTAL FIGURE LEGENDS

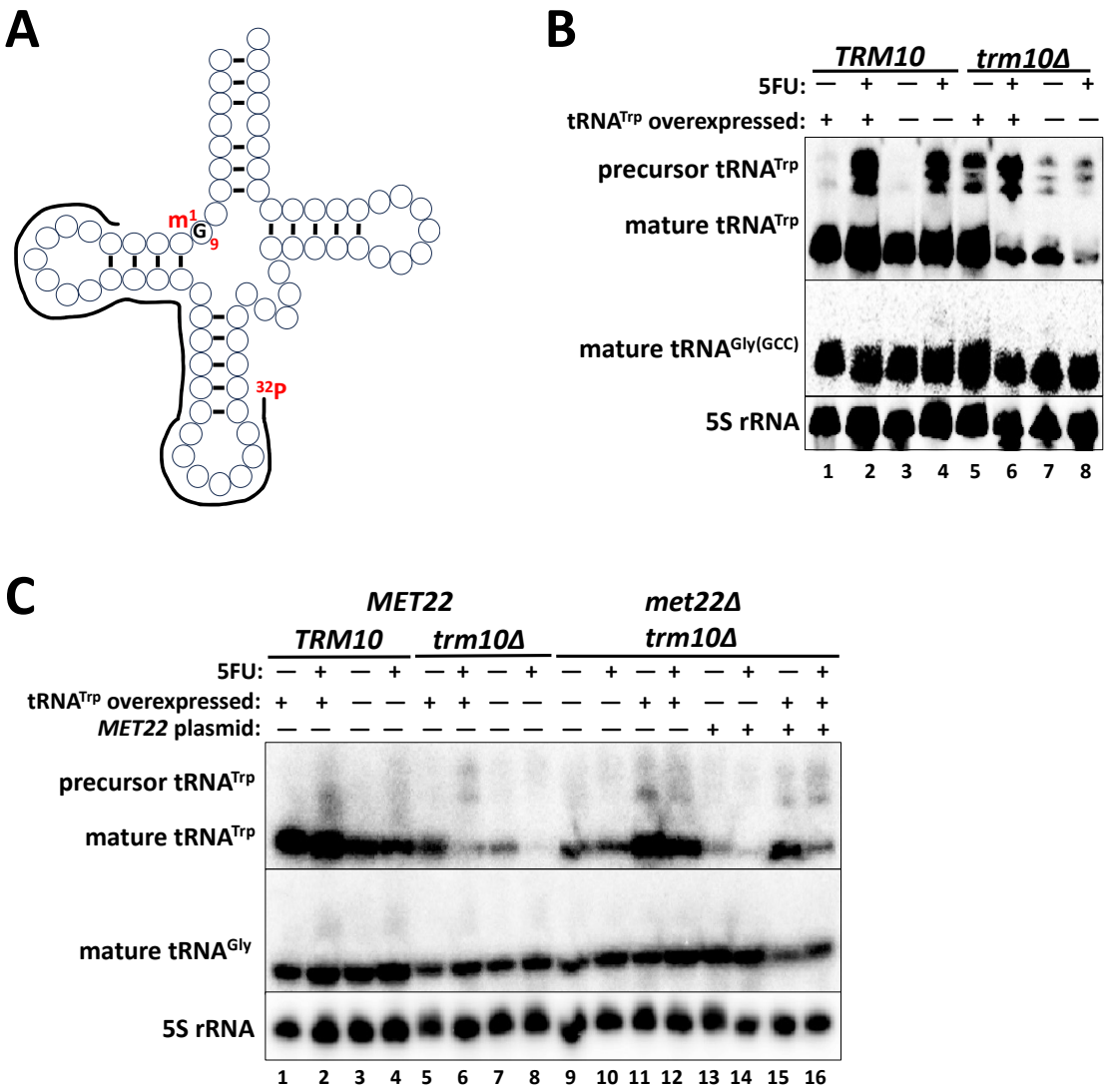
Supplementary Figure 1. Northern blot replicate experiments (A) Secondary structure depiction of mature tRNA detected by northern probes. The red line indicates hybridization of mature 5'-end radiolabeled probes (sequences listed in **Table S2**). **(B)** Northern analysis of RNA derived from the indicated strains using 5'-biotinylated probes (**Table S2**) and visualized with chemiluminescence with RNA from the same strains depicted in **Figure 2A**. The 5' biotinylated probe binds at the same location as the radiolabeled probe depicted in panel A. **(C)** Northern analysis of RNA derived from the indicated strains probed for mature $tRNA^{Trp}$, mature $tRNA^{Gly}$ and 5S rRNA, which were all detected using 5' radiolabeled probes (**Table S2)**. This northern blot is a replicate experiment probed at a different exposure level from that depicted in **Figure 5A**.

Supplementary Figure 2. *trm10Δ*- and 5FU- dependent changes to $tRNA^{Trp}$ precursor levels for individual intermediates in pre-tRNA processing. Quantification of northern analysis for each individual pre-tRNA and mature tRNA species as shown in **Figure 3**. Relative RNA levels, calculated from normalized abundance for each RNA compared to 5S rRNA are shown for **(A)** initial transcript, **(B)** 3'-processed pre-$tRNA^{Trp}$, **(C)** fully end-processed intron-containing $tRNA^{Trp}$, and **(D)** mature $tRNA^{Trp}$. Observed intensities for each species were normalized to the amount of pre-$tRNA^{Trp}$ in the *trm10Δ* +$tRNA^{Trp}$ overexpressing strain (**Figure 3B**, lane 5) to allow the full spectrum of increases and decreases in pre-tRNA abundance to be visualized. Note that the 5'-leader probes do not hybridize efficiently to mature $tRNA^{Trp}$ due to presence of the m^1G9 modification in the target region for each oligonucleotide probe, therefore leading to no signal for this species in *TRM10*-derived RNA. Colors to indicate quantification with each pre-tRNA-targeting probe correspond to the diagram in **Figure 3A**.

Supplementary Figure 3. Expression of *MET22* from a plasmid does not revert the *met22Δ* rescue of 5FU-resistant growth in *trm10Δ* strains. Strains were grown overnight in SD-Ura media if containing the [p*MET22*] *CEN URA3* plasmid or in SD-Leu if only containing the 2μ *LEU2* tRNA overexpression vector. A "–" symbol indicates no *MET22* CEN *URA3* plasmid is present, and *MET22* is genomically expressed if not indicated as *met22*Δ. Strains were serially 4-fold diluted from a starting OD_{600} of 1, plated on SD-Leu plates with indicated concentrations of 5FU, and incubated for 4 days at 30°C.

Supplementary Figure 4. $tRNA^{Trp}$ precursor levels along each step of pre-tRNA processing for the *trm10Δmet22Δ* strain. (A) Northern analysis using each of the three individual pre-tRNA targeting probes shown in **Figure 3**. **(B-D)** Quantification of individual pre-RNA^{Trp} levels from strains shown in (A) hybridized by the indicated pre-tRNA probes. Relative tRNA levels were calculated by comparing the observed intensity for each indicated RNA to the normalized abundance observed in the *trm10Δmet22Δ* strain with *met22* complementation (lane 7) set to 1.

Figure S1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32p
B
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trm10Δ
5FU:
tRNATrp overexpressed:
precursor tRNATrp
mature tRNATrp
mature tRNAGly(GCC)
5S rRNA
1 2 3 4 5 6 7 8
C
MET22
met22Δ
TRM10
trm10Δ
trm10Δ
5FU:
tRNATrp overexpressed:
MET22 plasmid:
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mature tRNATrp
mature tRNAGly
5S rRNA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure S2

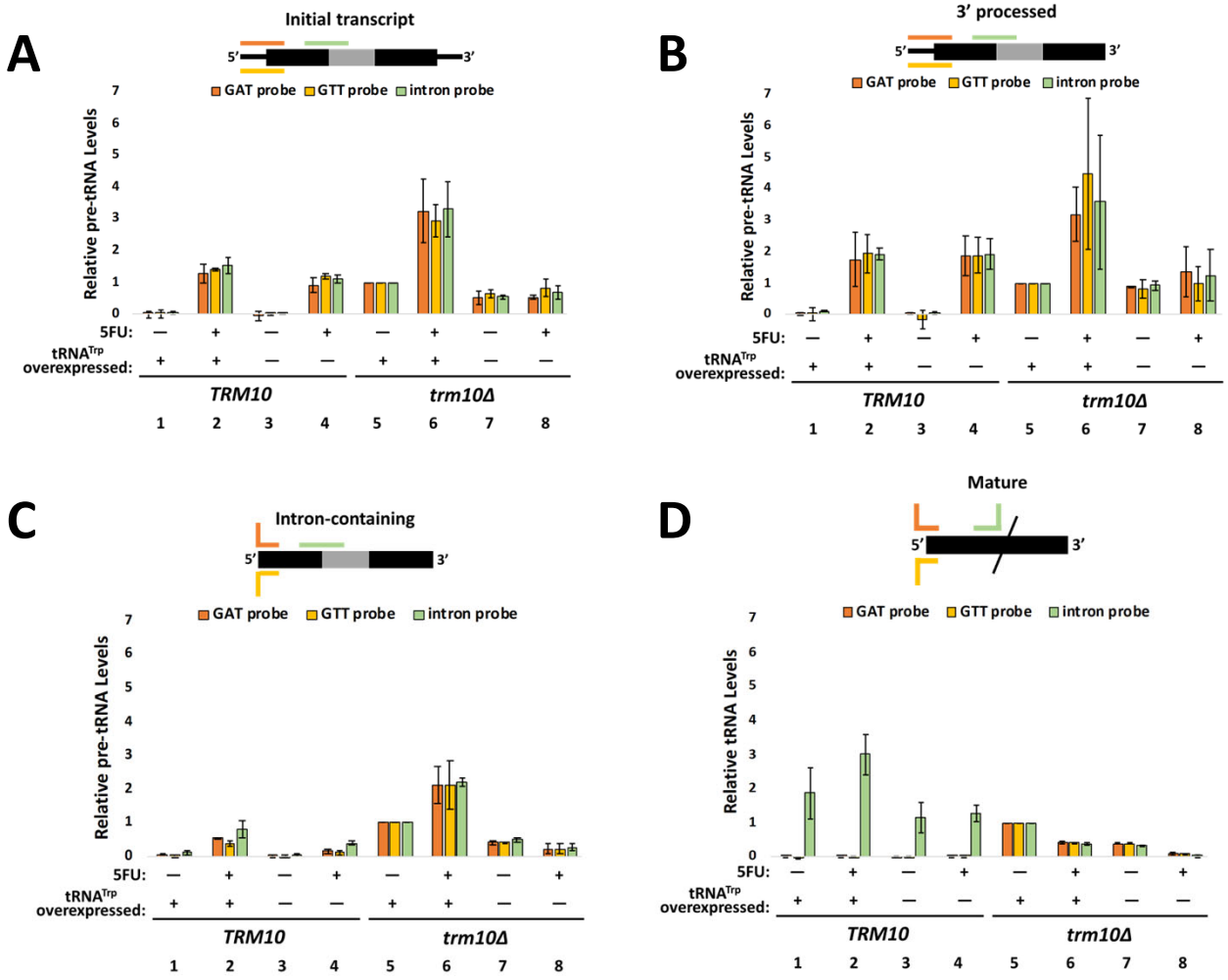

A
Initial transcript
5′
3′
GAT probe
GTT probe
intron probe
Relative pre-tRNA Levels
5FU:
tRNA^Trp overexpressed:
TRM10
trm10Δ
1 2 3 4 5 6 7 8
B
3′ processed
C
Intron-containing
D
Mature
Relative tRNA Levels

Figure S3

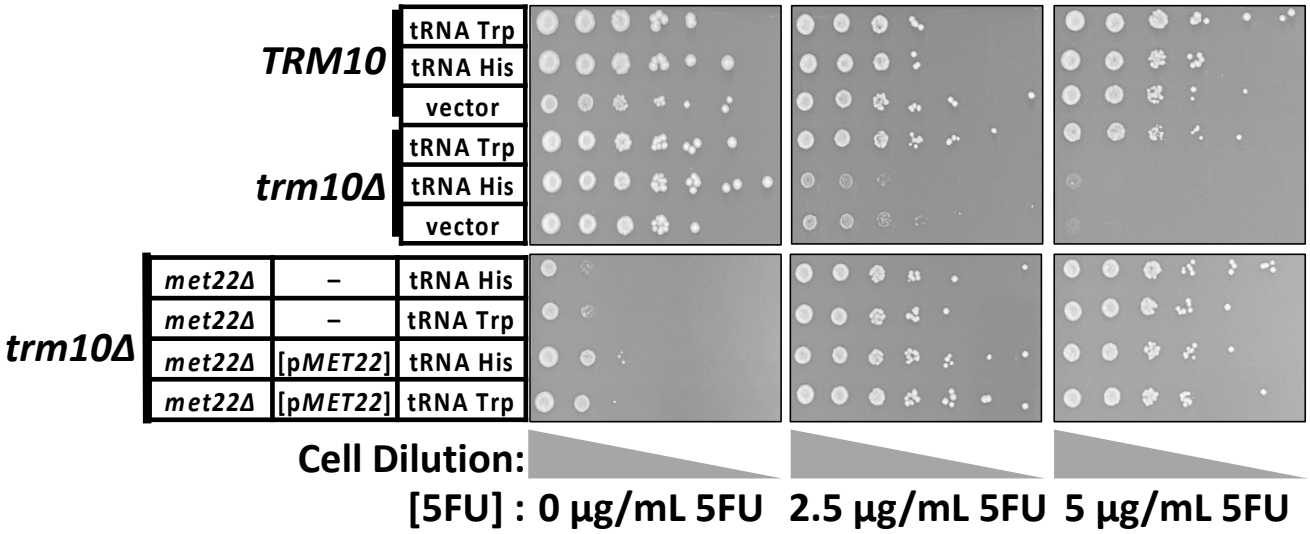
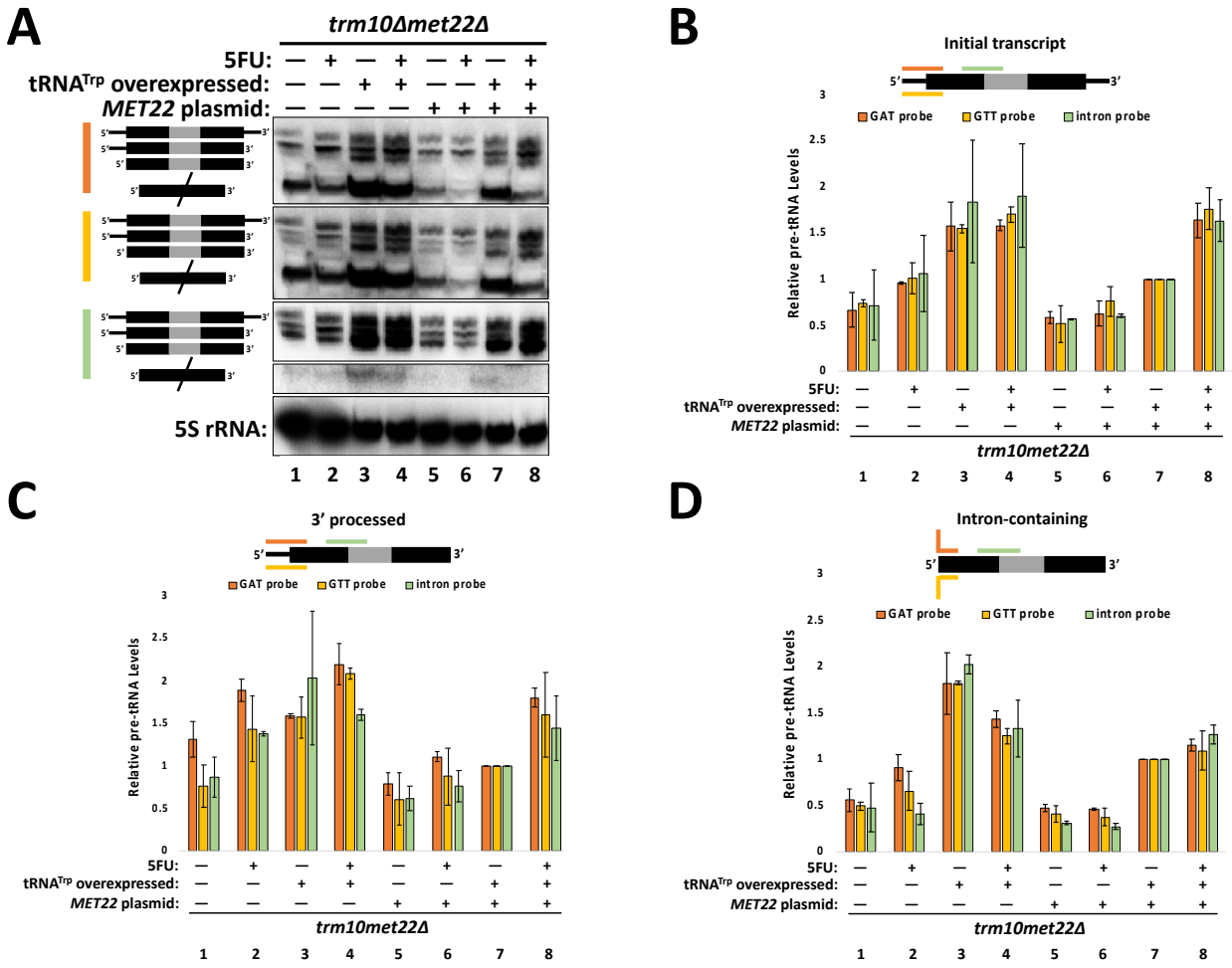

TRM10
tRNA Trp
tRNA His
vector
trm10Δ
tRNA Trp
tRNA His
vector
trm10Δ
met22Δ – tRNA His
met22Δ – tRNA Trp
met22Δ [pMET22] tRNA His
met22Δ [pMET22] tRNA Trp
Cell Dilution:
[5FU] : 0 µg/mL 5FU
2.5 µg/mL 5FU
5 µg/mL 5FU

Figure S4


A
trm10Δmet22Δ
5FU:
tRNA^Trp overexpressed:
MET22 plasmid:
5S rRNA:
1 2 3 4 5 6 7 8
B
Initial transcript
GAT probe
GTT probe
intron probe
Relative pre-tRNA Levels
trm10met22Δ
C
3' processed
D
Intron-containing