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Supplementary Information for

The RNA degradosome promotes tRNA quality control through clearance of hypomodified tRNA

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This PDF file includes:

Figs. S1 to S7
Tables S1 and S2
Captions for Datasets S1 to S3
References for SI reference citations

Other supplementary materials for this manuscript include the following:

Datasets S1 to S3

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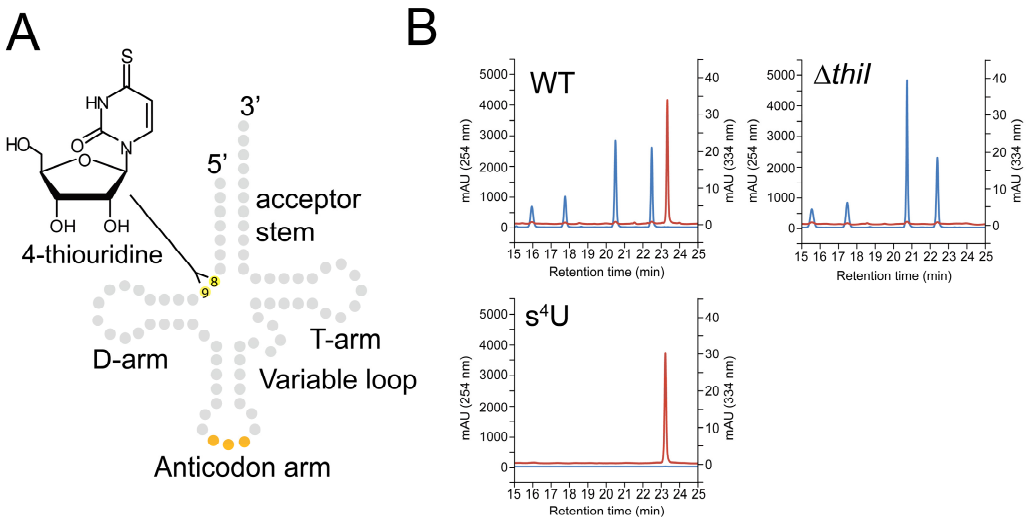

Fig. S1. ThiI accounts for s^4U synthesis in *V. cholerae*

(A) Schematic secondary structure of tRNA and chemical structure of 4-thiouridine (s^4U). Numbers indicate the positions that are modified to s^4U. The anticodon is colored in orange.

(B) Chromatograms of HPLC analysis of total nucleosides from wild-type (WT) and Δ*thiI* (Δ*thiI*) *V. cholerae* RNA and chemical standard of s^4U (s^4U). Blue and red lines represent UV traces at 254 nm and 334 nm, respectively.

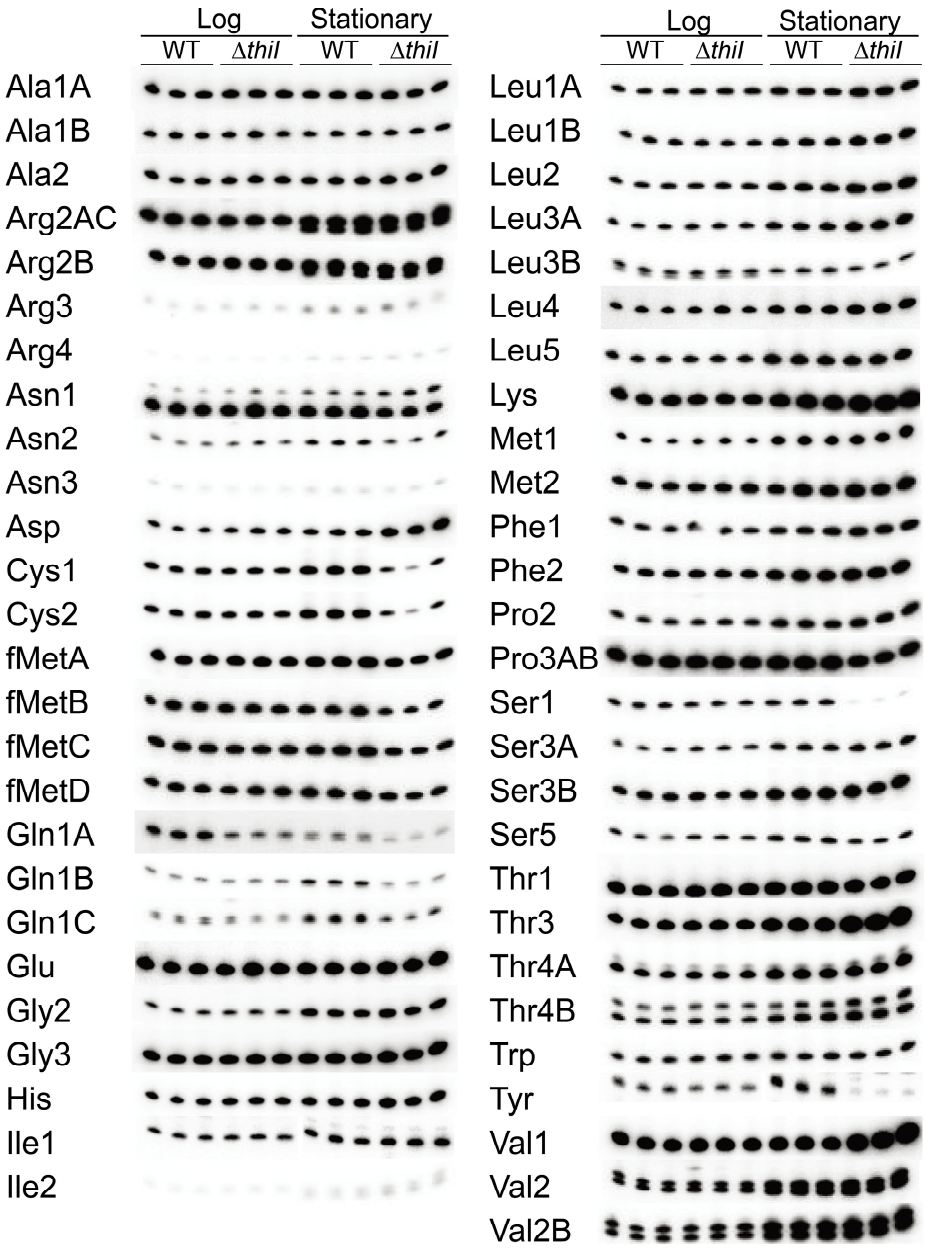

Fig. S2. Northern blots of all *V. cholerae* tRNA species derived from log and stationary phase cultures; data used for Figure 1B.

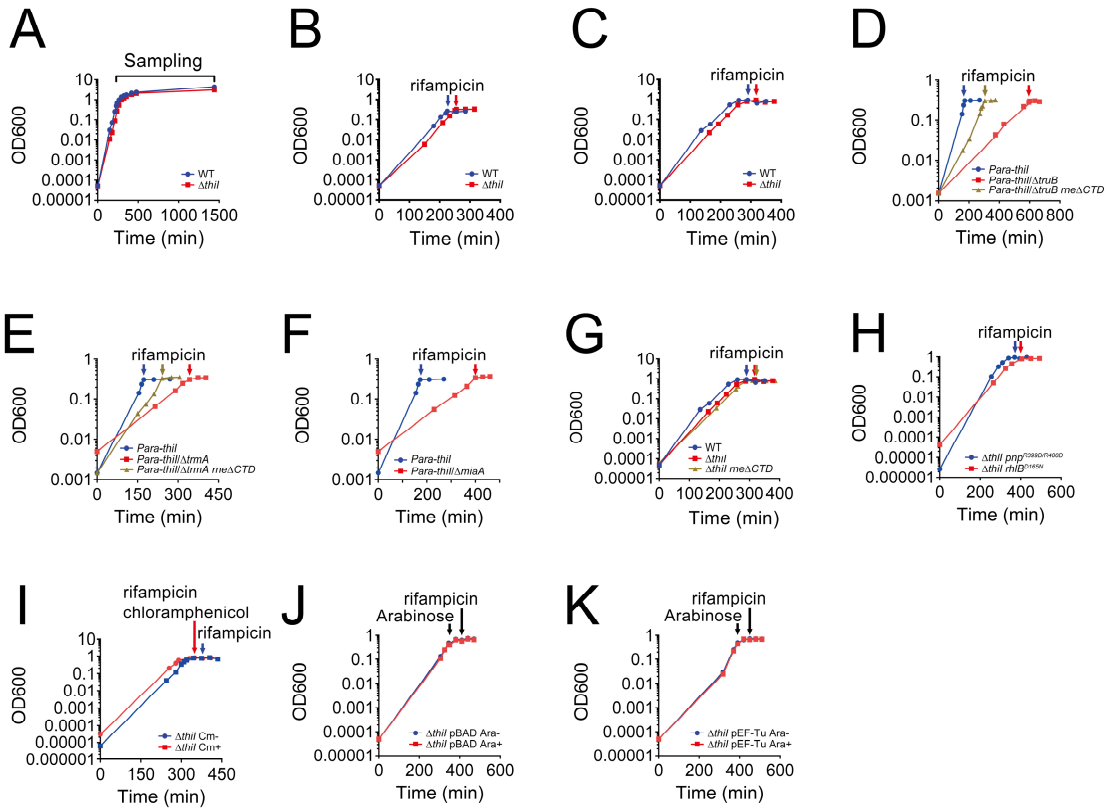

Fig. S3. Growth curves used for time course and decay measurement experiments (related to Fig. 1, 3, 4 and 6.

(A) Growth curves (culture OD_{600} over time) from time course experiment in Fig. 1D.

(B) Growth curves associated with RNA decay measurements for log phase cultures (Fig. 1E). Rifampicin was added at OD_{600} 0.3.

(C) Growth curves associated with RNA decay measurements for stationary phase cultures (Fig. 1F). Rifampicin was added one hour after OD_{600} 0.5.

(D-F) Growth curves from decay measurement in log phase (Fig. 3F). Rifampicin was added at OD_{600} 0.3.

(G and H) Growth curves from decay measurements in stationary phase (Fig. 4). Rifampicin was added one hour after OD_{600} 0.5.

(I) Growth curves from decay measurement in stationary phase (Fig. 6BC). Rifampicin and chloramphenicol (50 μg/ml) was added one hour after OD_{600} 0.5.

(J and K) Growth curves from decay measurement in stationary phase (Fig. 6D). Arabinose (0.2 %) was added at OD_{600} 0.5 and rifampicin was added one hour after OD_{600} 0.5.

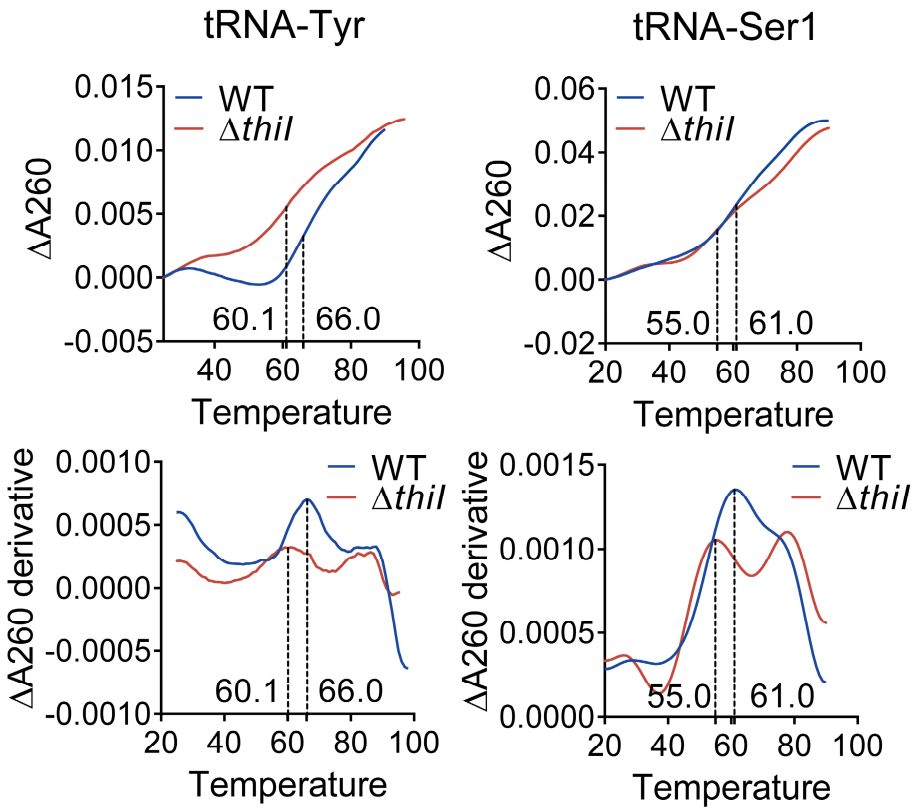

Fig. S4. Thermodynamic instability of hypomodified tRNA-Tyr and tRNA-Ser1.

Melting curves and 1st derivative curves of tRNA-Tyr and tRNA-Ser1. Upper panels are melting curves and lower panels are 1st derivative curves. Melting temperatures were determined by the position of the peak in 1st derivative curves.

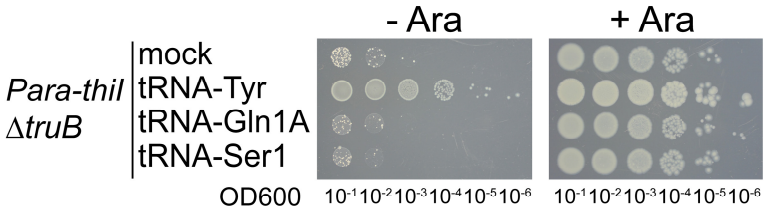

Fig. S5 Overexpression of tRNA-Gln1A and tRNA-Ser1 does not rescue the growth defect in double mutant.

Growth of *Para-thiI*/Δ*truB* double mutants with multi-copy plasmids encoding the indicated tRNA (expressed from the same promoter) with or without 0.2 % arabinose on LB plates. Plates were incubated at 37 °C for 24 hr.

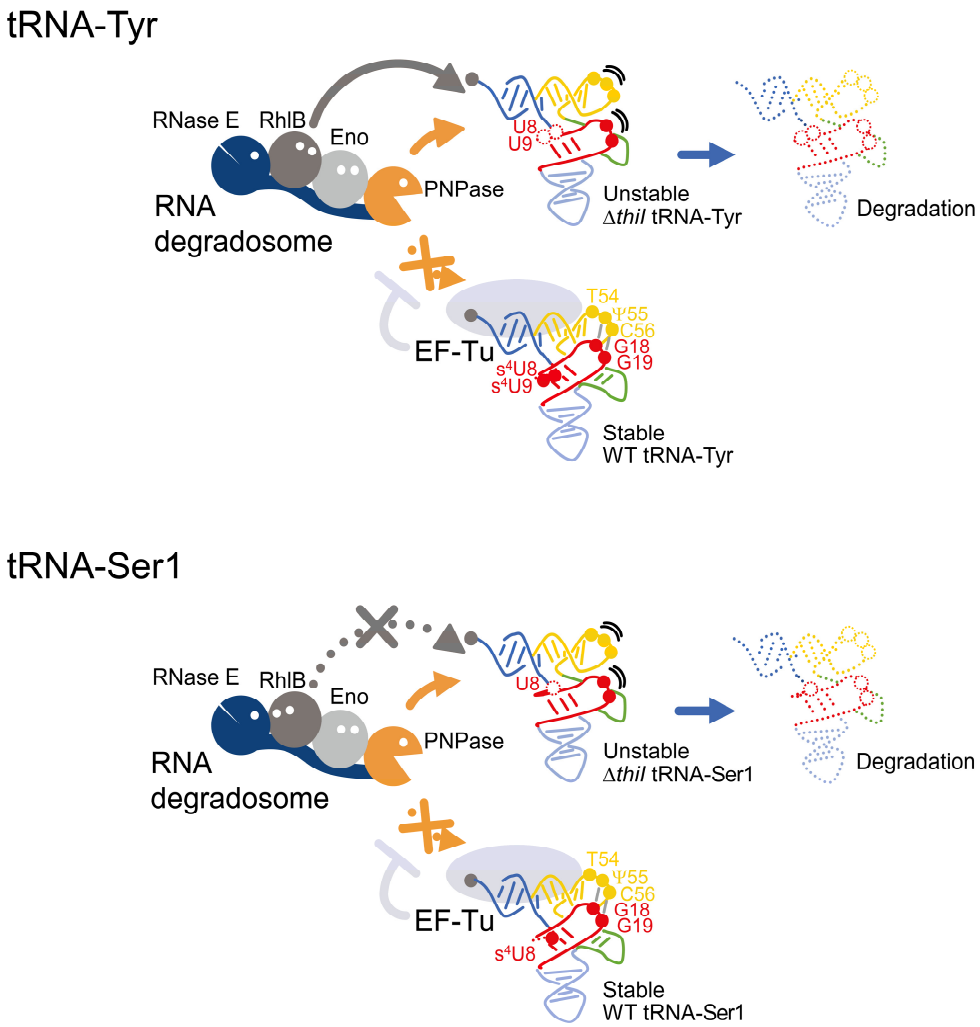

Fig. S6. Model of distinct tRNA quality control of tRNA-Tyr and tRNA-Ser1 mediated by RNA degradosomes

WT tRNA form the stable elbow with the base parings of G18-Ψ55 and G19-C56. s^4U deficient tRNAs are likely to destabilize the elbow, which could trigger targeting by RNA degradosomes. EF-Tu (gray oval) protects well folded tRNA from the degradosome. PNPases degrade tRNAs from 3' end of hypomodified tRNAs. For tRNA-Tyr degradation, RNA helicase RhlB support the degradation but not for tRNA-Ser1.

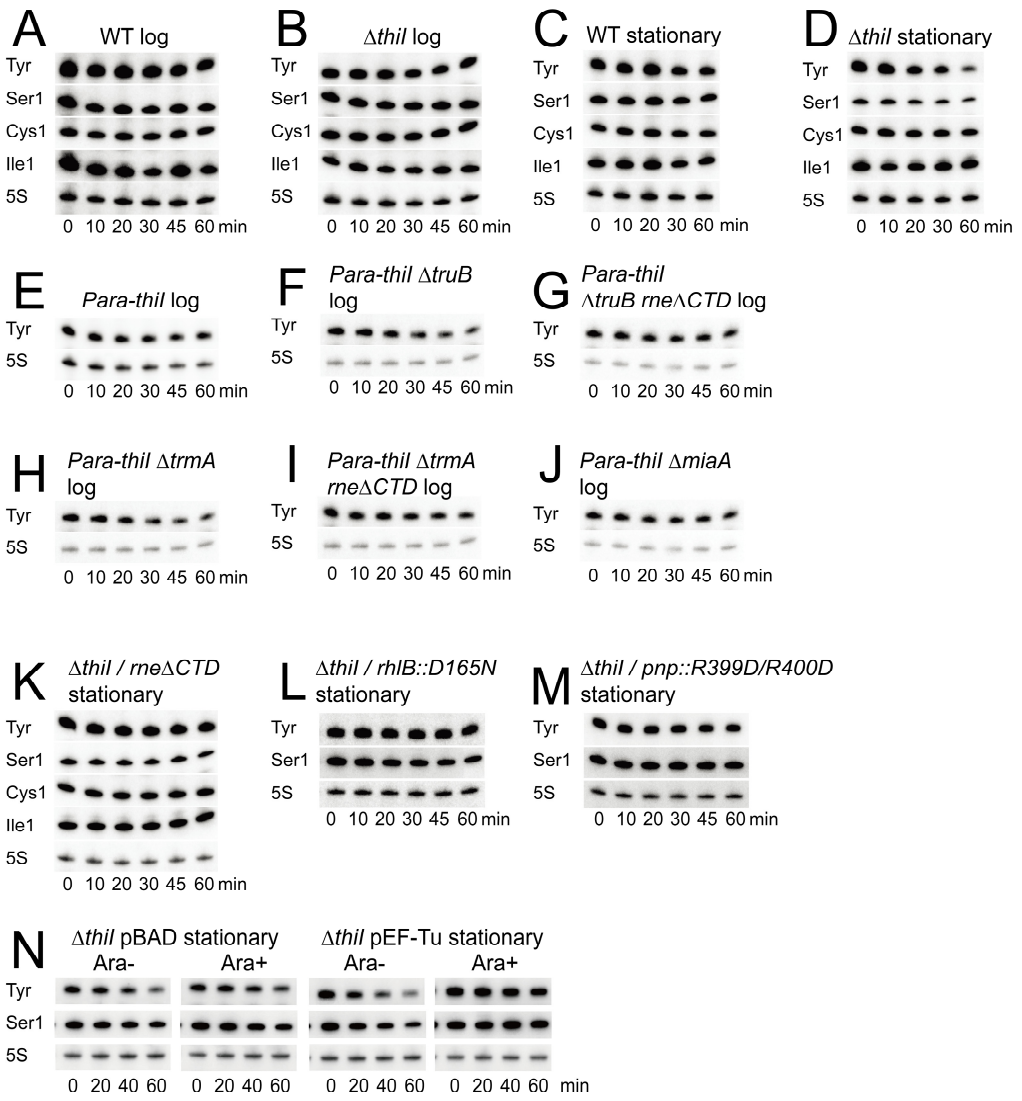

Fig. S7. Northern blot data.

(A-M) Northern blot data used to generate the decay curves in Fig. 1E (panel A and B), Fig. 1F (panel C and D), Fig. 3F (panel E-J), Fig. 4 (panel C, D, K-M), Fig 6D (panel N).

Supplementary Tables

Table S1 Strain list (related to Materials and Methods)

Table S2 Plasmid list (related to Materials and Methods)

Supplementary Datasets

Dataset S1 Results of Con-Artist (related to Fig. 2A)

Dataset S2 Primer list (related to Materials and Methods)

Dataset S3 *Vibrio cholerae* tRNA sequences with tRNA names (related to Fig. 1B)

Table S1 Strain list (related to methods)

Strains	Relevant genotype/description	Reference/source
Vibrio cholerae	C6706, wild-type El Tor clinical isolate (Sm^R)	(1)
V. cholerae Δ*thiI*	C6706 Δ*vc0894*	This work
V. cholerae Δ*miaA*	C6706 Δ*vc0346*	This work
V. cholerae Δ*trmA*	C6706 Δ*vc0154*	This work
V. cholerae Δ*truB*	C6706 Δ*vc0645*	This work
V. cholerae Para-thiI	C6706 *PthiI*::*araC-Para*	This work
V. cholerae Para-thiI Δ*trmA*	C6706 *Para-thiI* Δ*trmA*	This work
V. cholerae Para-thiI Δ*truB*	C6706 *Para-thiI* Δ*truB*	This work
V. cholerae Para-thiI Δ*miaA*	C6706 *Para-thiI* Δ*miaA*	This work
V. cholerae Para-thiI Δ*truB suppressor #1*	C6706 *Para-thiI* Δ*truB tRNA-Tyr*^{U51C}	This work
V. cholerae Para-thiI Δ*truB suppressor #2*	C6706 *Para-thiI* Δ*truB vc2030*^{E664*}	This work
V. cholerae Δ*rne*::*rne*$^{\Delta(E664\text{-}F1052)}$	C6706 Δ*vc2030*::*vc2030*$^{\Delta\ (E664\text{-}F1052)}$	This work
V. cholerae Δ*thiI* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	C6706 Δ*thiI* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	This work
V. cholerae Para-thiI Δ*trmA* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	C6706 *Para-thiI* Δ*trmA* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	This work
V. cholerae Para-thiI Δ*truB* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	C6706 *Para-thiI* Δ*truB* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	This work
V. cholerae Para-thiI Δ*miaA* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	C6706 *Para-thiI* Δ*miaA* Δ*rne*::*rne*$^{\Delta\ (E664\text{-}F1052)}$	This work
V. cholerae Δ*thiI* Δ*pnp*::*pnp*$^{R399D/R400D}$	C6706 Δ*thiI* Δ*pnp*::*pnp*$^{R399D/R400D}$	This work
V. cholerae Δ*thiI* Δ*rhlB*::*rhlB*^{D165N}	C6706 Δ*thiI* Δ*rhlB*::*rhlB*^{D165N}	This work
V. cholerae Δ*rne*::*rne-flag*	C6706 Δ*rne*::*rne-flag*	This work
V. cholerae Δ*rne*::*rne-flag* Δ*pnp*::*pnp*$^{R399D/R400D}$	C6706 Δ*rne*::*rne-flag* Δ*pnp*::*pnp*$^{R399D/R400D}$	This work
Escherichia coli DH5α λ*pir*	Cloning strain	
Escherichia coli SM10 λ*pir*	Conjugation donor	

Table S2 Plasmid list (related to methods)

Plasmid	Relevant genotype/descripton	Reference
pCVD442	Allele exchange vector	(2)
pCVD442-Δ*thiI*	Allele exchange vector for deletion of *V. cholerae vc0894*	This work
pCVD442-Δ*trmA*	Allele exchange vector for deletion of *V. cholerae vc0154*	This work
pCVD442-Δ*truB*	Allele exchange vector for deletion of *V. cholerae vc0645*	This work
pCVD442-Δ*miaA*	Allele exchange vector for deletion of *V. cholerae vc0346*	This work
pCVD442-$rne^{\Delta(E664\text{-}F1052)}$	Allele exchange vector for deletion of C-terminal domain (E664-F1052) of RNase E encoded in *vc2030*	This work
pCVD442-*ParathiI*	Allele exchange vector for replacing *V. cholerae PthiI* with *araC*::*Para* from pBAD33 (Thermo)	This work
pCVD442-$pnp^{R399D/R400D}$	Allele exchange vector for mutagenizing *pnp*	This work
pCVD442-$rhlB^{D165N}$	Allele exchange vector for mutagenizing *rhlB*	This work
pCVD442-*rne-flag*	Allele exchange vector for integrate flag tag to endogenous *rne*	This work
pSC189	Himar 1 suicide transposon vector	(3)
pACYC184	Cloning vector	
ptRNA-Tyr	*V. cholerae PtRNATyr*::*tRNATyr* on pACYC184	This work
ptRNA-Gln1A	*V. cholerae PtRNATyr*::*tRNAGln1A* on pACYC184	This work
ptRNA-Ser1	*V. cholerae PtRNATyr*::*tRNASer1* on pACYC184	This work
pEF-Tu	*V. cholerae vc0362* on pBAD33	This work

References

1. Millet YA, *et al.* (2014) Insights into Vibrio cholerae intestinal colonization from monitoring fluorescently labeled bacteria. *PLoS pathogens* 10(10):e1004405.
2. Donnenberg MS & Kaper JB (1991) Construction of an eae deletion mutant of enteropathogenic Escherichia coli by using a positive-selection suicide vector. *Infection and immunity* 59(12):4310-4317.
3. Chiang SL & Rubin EJ (2002) Construction of a mariner-based transposon for epitope-tagging and genomic targeting. *Gene* 296(1-2):179-185.