Supplementary Information

Mechanistic insights into tRNA cleavage by a contact-dependent growth inhibitor protein and translation factors

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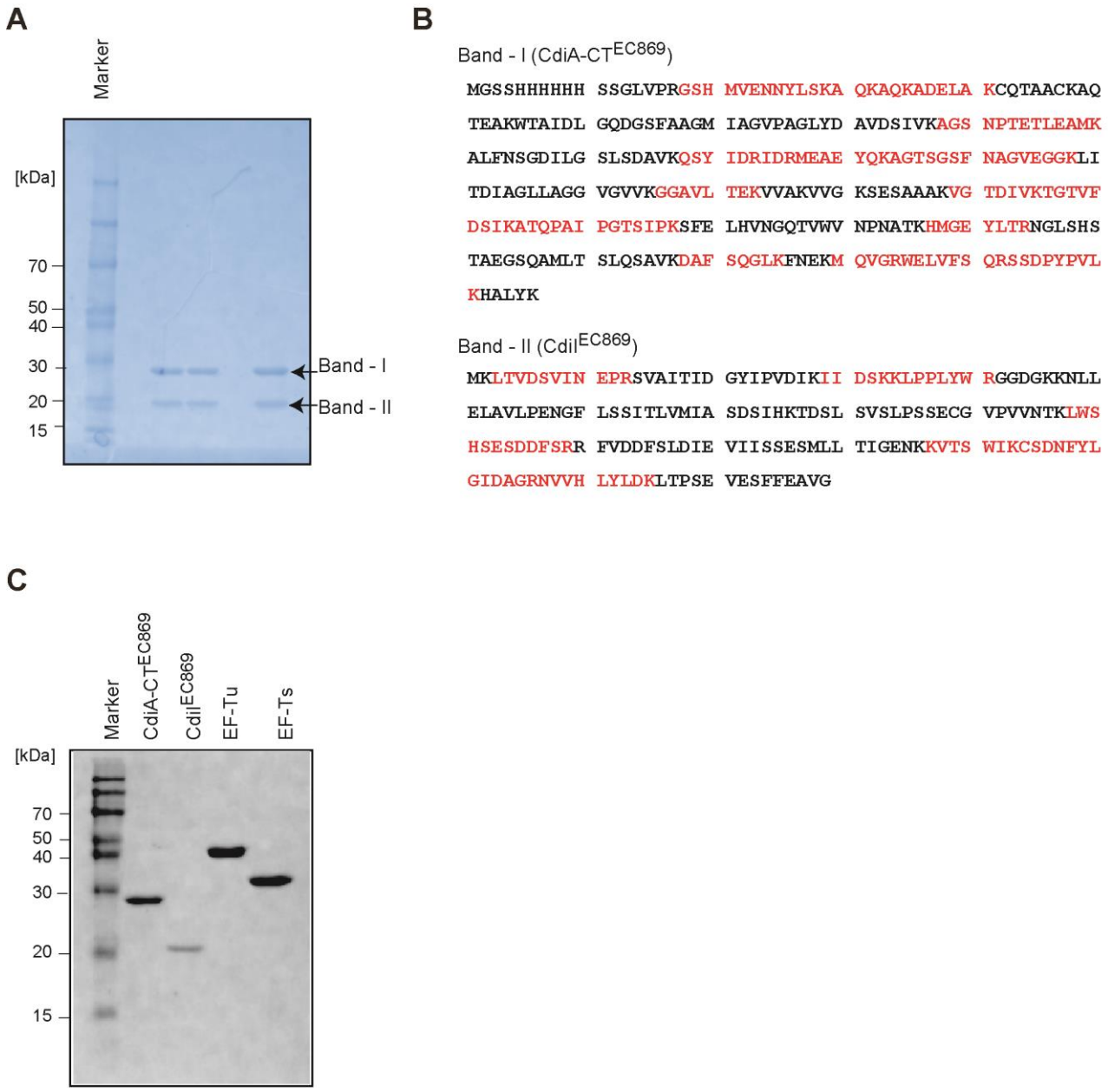
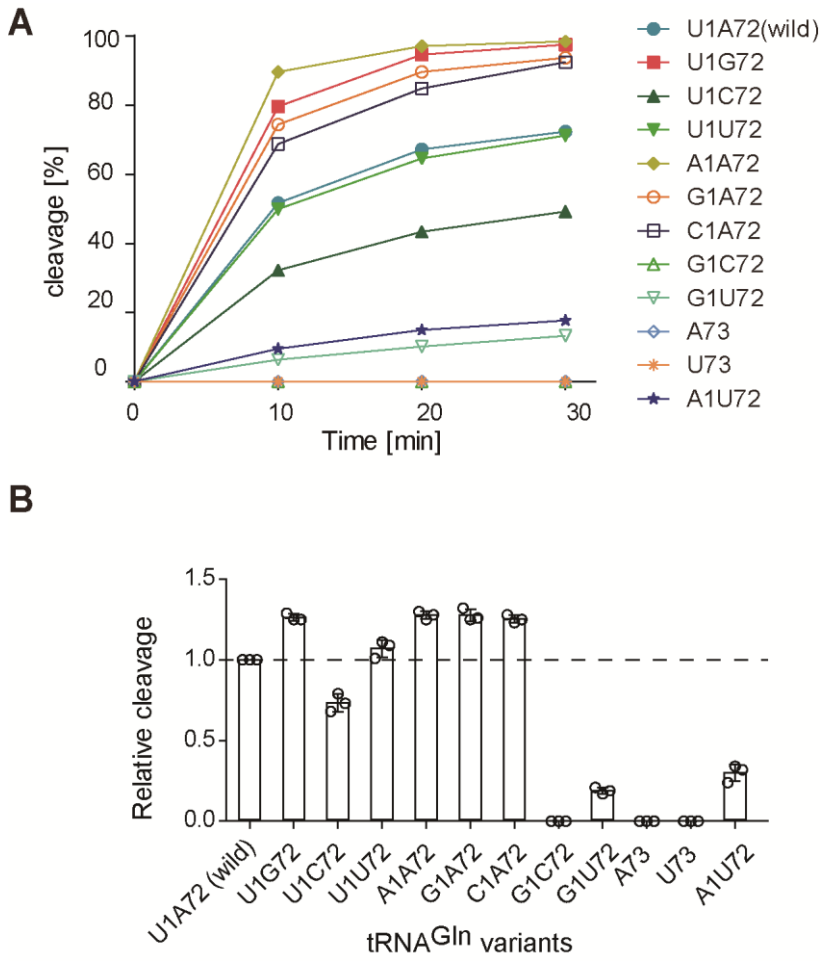
Figure S1



Supplementary Figure S1: Purification of recombinant proteins.

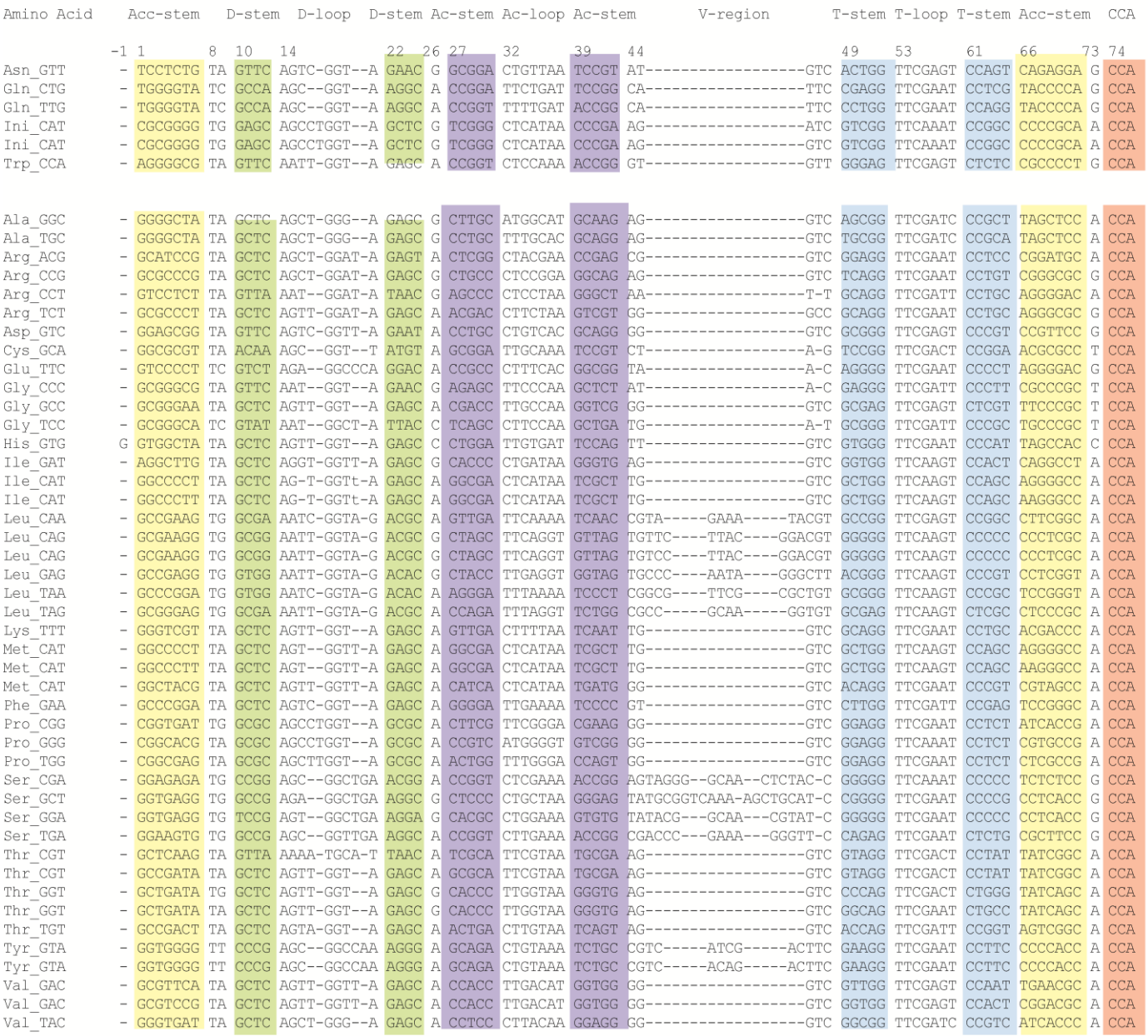
(**A**) Purification of the CdiA-CT^{EC869}:CdiI^{EC869} complex. After size-exclusion column chromatography, the fraction containing the CdiA-CT^{EC869}:CdiI^{EC869} complex was analyzed by electrophoresis on a 5–20% (w/v) SuperSep Ace gel (FujiFilm, Japan), which was stained with Quick-CBB Plus (Wako, Japan). Band-I and Band-II were digested with trypsin, and the peptides were analyzed by LC/MS, as described (1). (**B**) Peptides identified by LC/MS from the trypsin-digested Band-I and Band-II in (A) were mapped onto the amino acid sequences of recombinant CdiA-CT^{EC869} and CdiI^{EC869}, respectively. The amino acid sequences of recombinant CdiA-CT^{EC869} and CdiI^{EC869} are shown, and the identified peptide sequences are colored red. CdiI^{EC869} was co-purified with histidine-tagged CdiA-CT^{EC869}. (**C**) The purified Tu, Ts, CdiA-CT^{EC869}, and CdiI^{EC869} used for this study were separated by 15% (w/v) SDS PAGE, and the gel was stained with Coomassie Brilliant Blue.

Figure S2



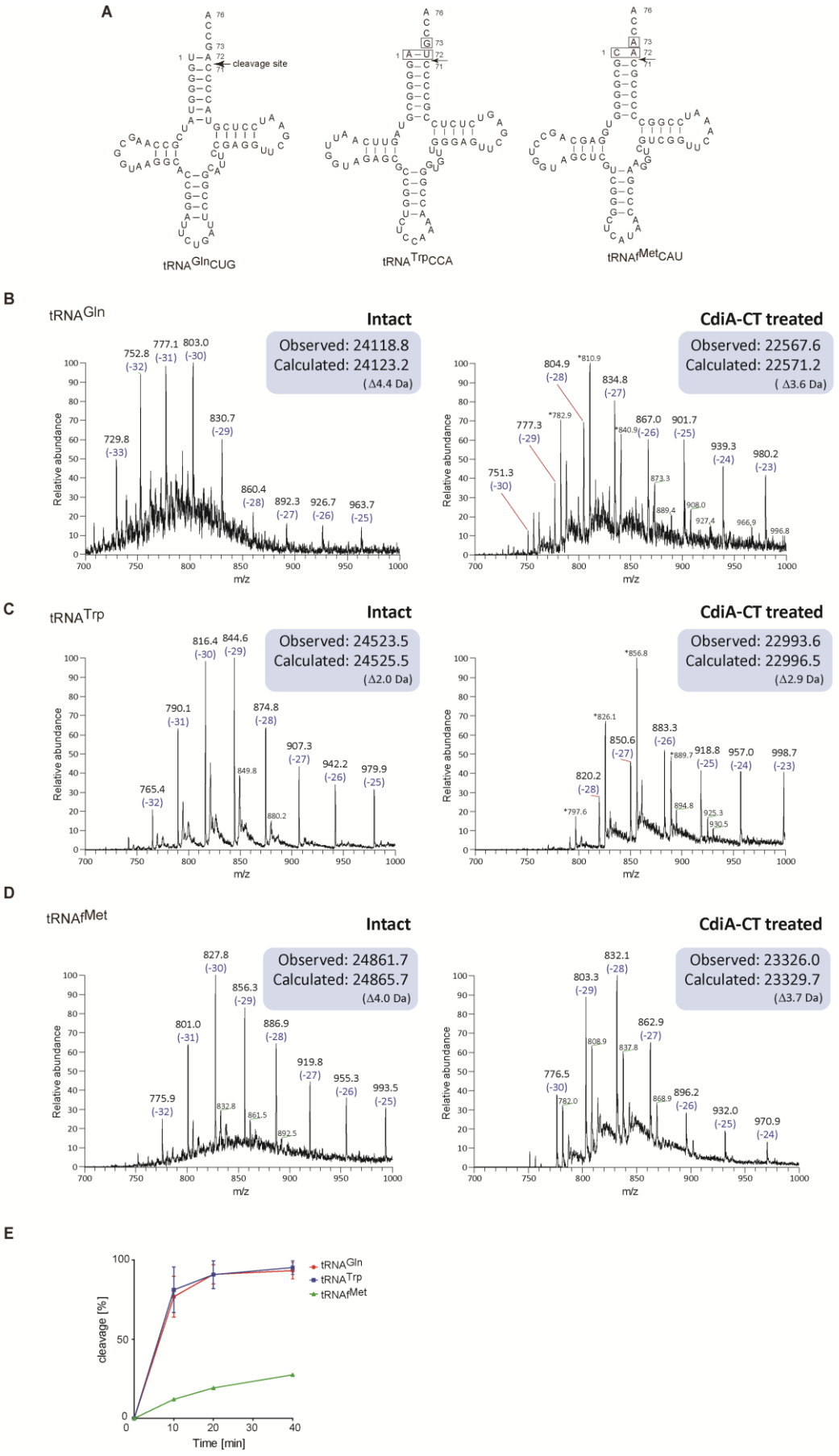
Supplementary Figure S2: Cleavage of tRNA^{Gln} variants by CdiA-CT^{EC869} under acidic conditions. (**A**) Time course of cleavage of tRNA^{Gln} variants by CdiA-CT^{EC869} under lower-pH conditions (pH 5.7) in the absence of Tu, Ts, and GTP. The tRNA^{Gln} variants (1.0 μM) were incubated with 0.1 μM of CdiA-CT^{EC869} at 37°C. The fraction of cleaved tRNA was quantified at the indicated time points. (**B**) The cleavage of tRNA^{Gln} variants by CdiA-CT^{EC869} in the absence of Tu, Ts, and GTP. The tRNA^{Gln} transcript variants (1.0 μM) were incubated with 0.1 μM of CdiA-CT^{EC869} at 37°C for 10 min. The cleavage level of wild-type tRNA^{Gln} (U1A72) was defined as 1.0. The bars in the graphs are SDs of more than three independent experiments, and the data are presented as mean values ± SD.

Figure S3



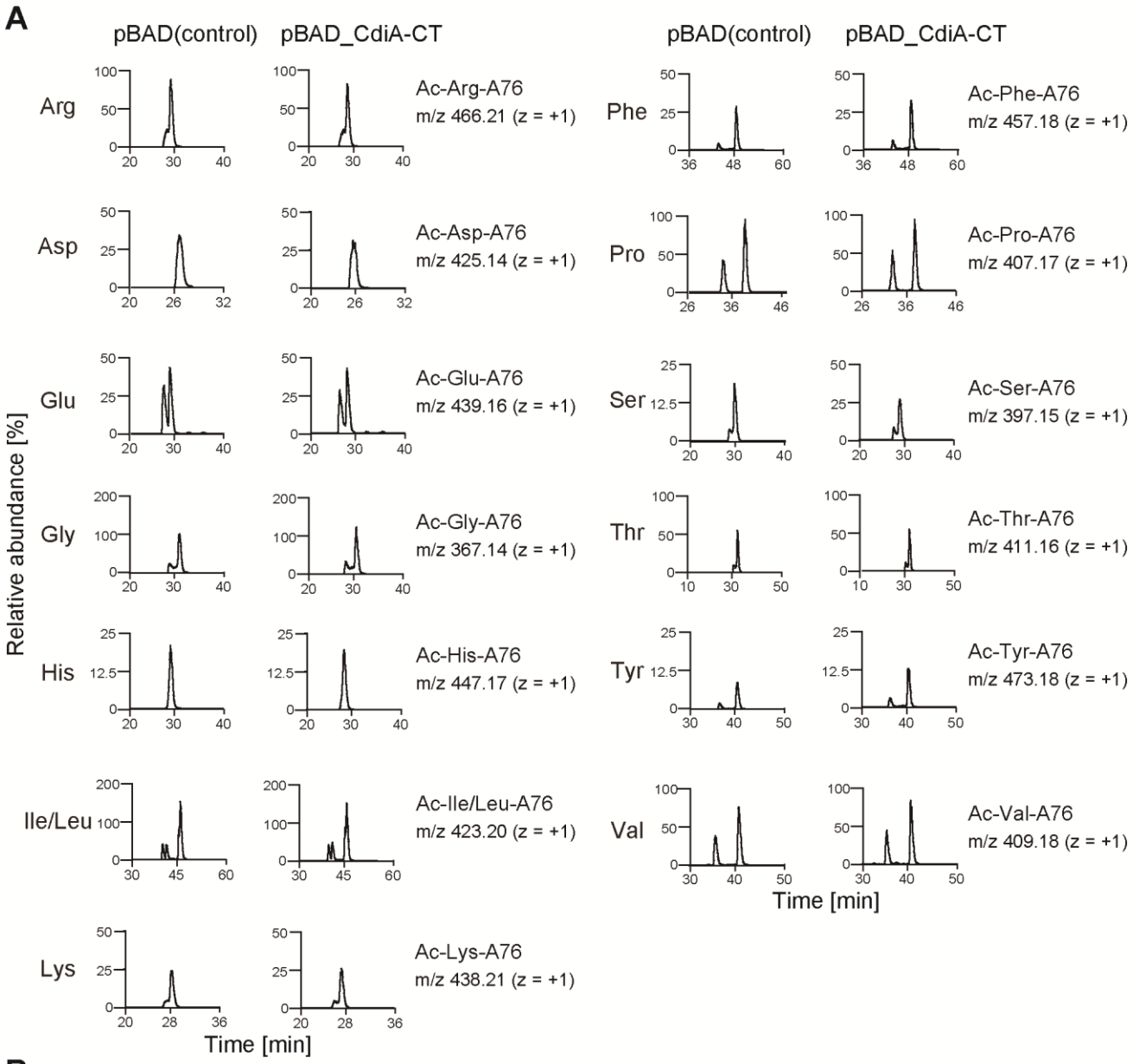
Supplementary Figure S3: Sequence alignments of *E. coli* tRNA genes (2).

Figure S4


A
cleavage site
tRNAGlnCUG
tRNATrpCCA
tRNAfMetCAU
B
tRNAGln
Intact
Observed: 24118.8
Calculated: 24123.2
(Δ4.4 Da)
CdiA-CT treated
Observed: 22567.6
Calculated: 22571.2
(Δ3.6 Da)
C
tRNATrp
Intact
Observed: 24523.5
Calculated: 24525.5
(Δ2.0 Da)
CdiA-CT treated
Observed: 22993.6
Calculated: 22996.5
(Δ2.9 Da)
D
tRNAfMet
Intact
Observed: 24861.7
Calculated: 24865.7
(Δ4.0 Da)
CdiA-CT treated
Observed: 23326.0
Calculated: 23329.7
(Δ3.7 Da)
Relative abundance
m/z
E
cleavage [%]
Time [min]
tRNAGln
tRNATrp
tRNAfMet

Supplementary Figure S4: Cleavage sites in $tRNA_f^{Met}$ and $tRNA^{Trp}$ by $CdiA\text{-}CT^{EC869}$ *in vitro*.
(**A**) Clover-leaf structures of *E. coli* $tRNA^{Gln}CUG$, $tRNA^{Trp}CCA$, and $tRNA_f^{Met}CAU$. Cleavage sites in tRNAs are indicated by arrows. (**B**)-(**D**) Whole-mass analysis of CdiA-CT-treated tRNAs for Gln (B), Trp (C), and fMet (D). After tRNA cleavage by CdiA-CT in the presence of Tu, Ts and GTP, the cleaved products were separated by PAGE, purified, and analyzed. Left and right panels show ESI-MS of intact and CdiA-CT-treated tRNAs, respectively. A series of multiply charged negative ions (black) with charge values (blue) are shown in each mass spectrum. The deconvoluted molecular mass (Observed) and calculated value (Calculated) are shown in the inset. MS peaks of HFIP adducts are asterisked. The molecular mass reductions of tRNAs upon CdiA treatment indicated that these tRNAs are cleaved between positions 71 and 72, yielding terminal 2'-3' cyclic phosphate ends. (**E**) Time courses of the cleavage of $tRNA^{Trp}$ and $tRNA_f^{Met}$ by $CdiA\text{-}CT^{EC869}$ in the presence of Tu, Ts, and GTP at pH 7.4. The tRNAs (1.0 μM) were incubated with 0.6 μM of $CdiA\text{-}CT^{EC869}$ at 37°C in the presence of 0.6 μM Tu, 0.6 μM Ts, and 1 mM GTP. The fraction of each cleaved tRNA was quantified at the indicated time points. The bars in the graphs are SDs of more than three independent experiments.

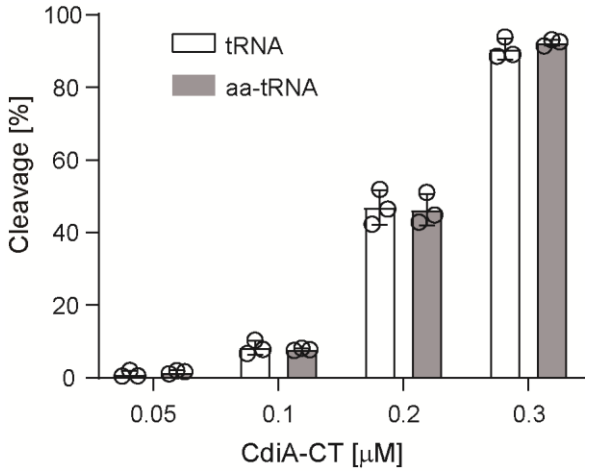
Figure S5


A
pBAD(control)
pBAD_CdiA-CT
Arg
Ac-Arg-A76
m/z 466.21 (z = +1)
Asp
Ac-Asp-A76
m/z 425.14 (z = +1)
Glu
Ac-Glu-A76
m/z 439.16 (z = +1)
Gly
Ac-Gly-A76
m/z 367.14 (z = +1)
His
Ac-His-A76
m/z 447.17 (z = +1)
Ile/Leu
Ac-Ile/Leu-A76
m/z 423.20 (z = +1)
Lys
Ac-Lys-A76
m/z 438.21 (z = +1)
Phe
Ac-Phe-A76
m/z 457.18 (z = +1)
Pro
Ac-Pro-A76
m/z 407.17 (z = +1)
Ser
Ac-Ser-A76
m/z 397.15 (z = +1)
Thr
Ac-Thr-A76
m/z 411.16 (z = +1)
Tyr
Ac-Tyr-A76
m/z 473.18 (z = +1)
Val
Ac-Val-A76
m/z 409.18 (z = +1)
Relative abundance [%]
Time [min]
B
Relative amount of aa-tRNA
pBAD33_empty
pBAD33_CdiA-CT
Ala
Arg
Asn
Asp
Cys
Gln
Glu
Gly
His
Ile/Leu
Lys
Lys-2
Met
fMet
Phe
Pro
Ser
Thr
Trp
Tyr
Tyr-2
Val

Supplementary Figure S5: Changes of aa-tRNA levels after CdiA-CT^{EC869} expression.

(**A**) LC/MS analyses of RNase I–digested RNAs prepared from *E. coli* with or without CdiA-CT^{EC869} induction. The amount of each Ac-aa-A76 derived from the aminoacyl-tRNA prepared from cells, with or without induction of CdiA-CT^{EC869}, is expressed relative to the amount of Ac-Ala-A76 in each cell, with or without induction of CdiA-CT^{EC869}, respectively. (**B**) Quantification of relative amounts of each aa-tRNA (or formyl-Met-tRNA$_f^{Met}$: fMet) in *E. coli* with (pBAD33_CdiA-CT) or without (pBAD33_empty) induction of CdiA-CT^{EC869}. The amount of Ala-tRNA^{Ala} in each cell with or without CdiA-CT^{EC869} induction was set to 1.0. Lys-2 and Tyr-2 correspond to the products acetylated at the amino group and hydroxy group of the side chains, respectively, in addition to the α-amino groups. The bars in the graphs are SDs of more than three independent experiments.

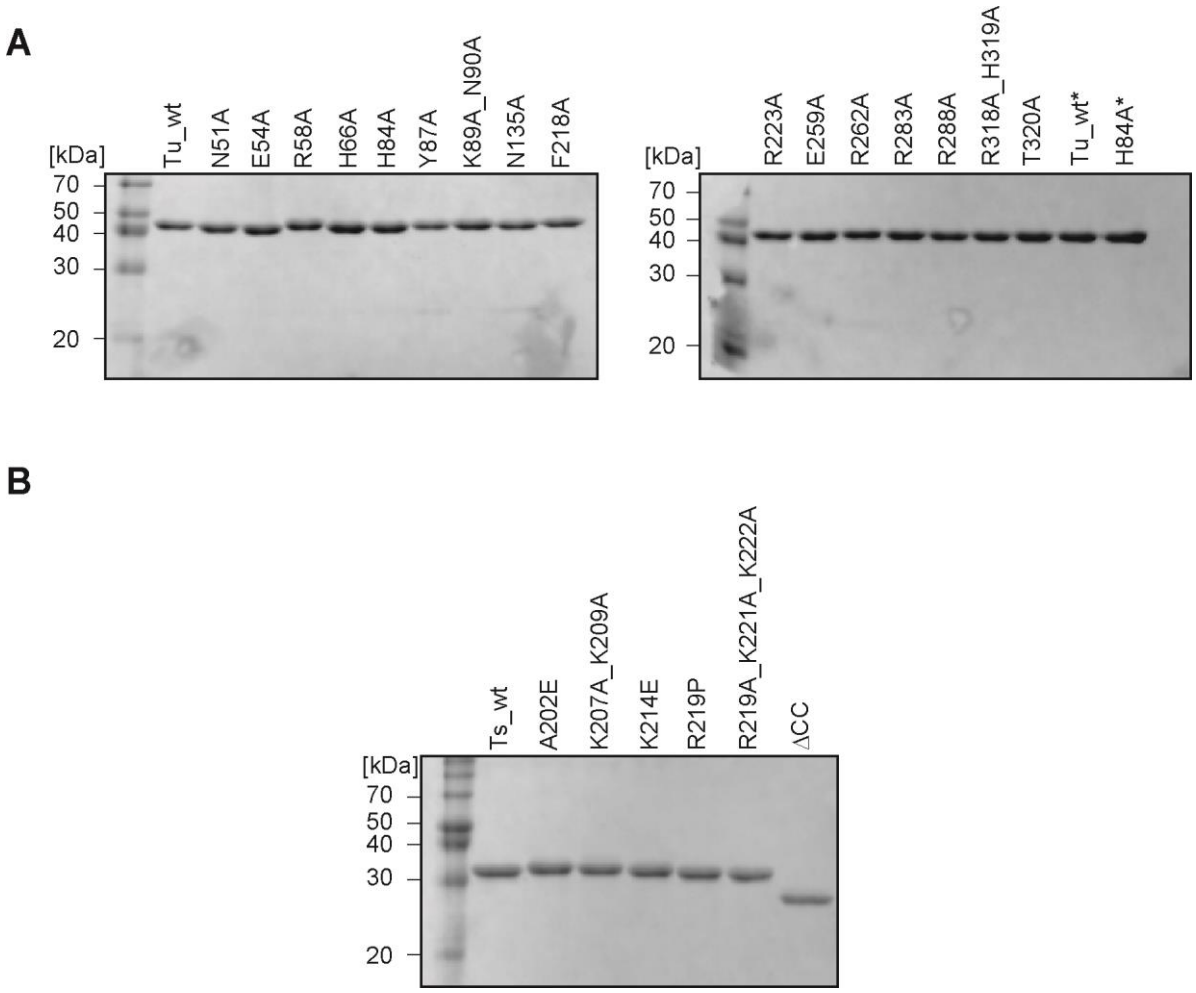
Figure S6



Supplementary Figure S6: Uncharged- and aminoacylated-tRNAs are cleaved by CdiA-CT^{EC869} in the presence of translation factors.

tRNA^{Gln} (tRNA, open box) and Gln-tRNA^{Gln} (aa-tRNA, closed box) cleavage by CdiA-CT^{EC869} in the presence of Tu, Ts, and GTP at 37°C. For cleavage of Gln-tRNA^{Gln}, 1.0 μM tRNA^{Gln} was first aminoacylated by GlnRS (1.0 μM) for 60 min in buffer containing 20 mM Tris-Cl, pH 7.4, 150 mM KCl, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol, 4 mM ATP, and 200 μM glutamine, and was then cleaved by increasing amounts of CdiA-CT^{EC869} (0.05, 0.1, 0.2, and 0.3 μM), in the presence of translation factors (0.05, 0.1, 0.2, and 0.3 μM each) and GTP (1 mM) for 10 min. The tRNA cleavage levels were quantified. The bars in the graphs are SDs of more than three independent experiments.

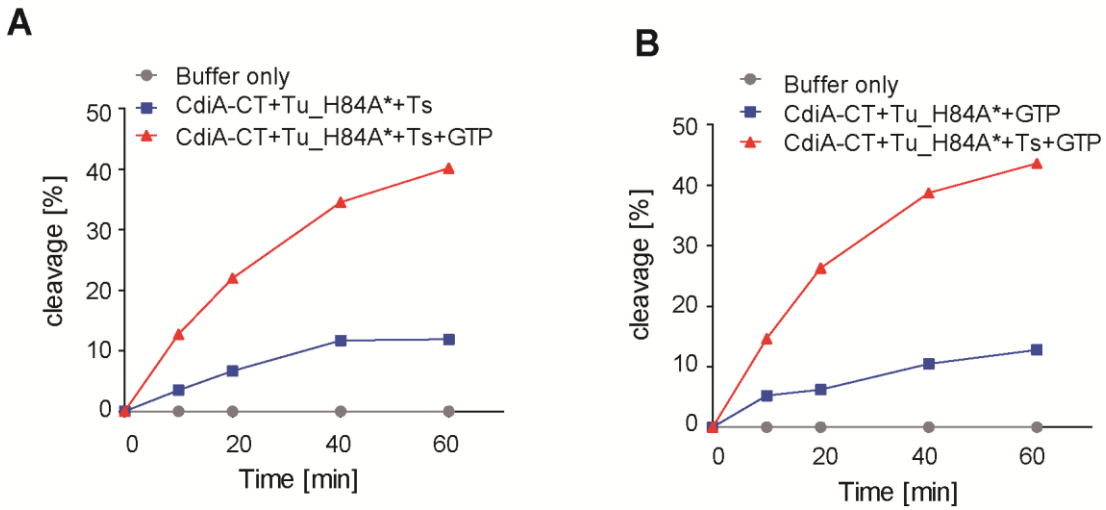
Figure S7



Supplementary Figure S7: Purification of Tu and Ts variants used in this study.

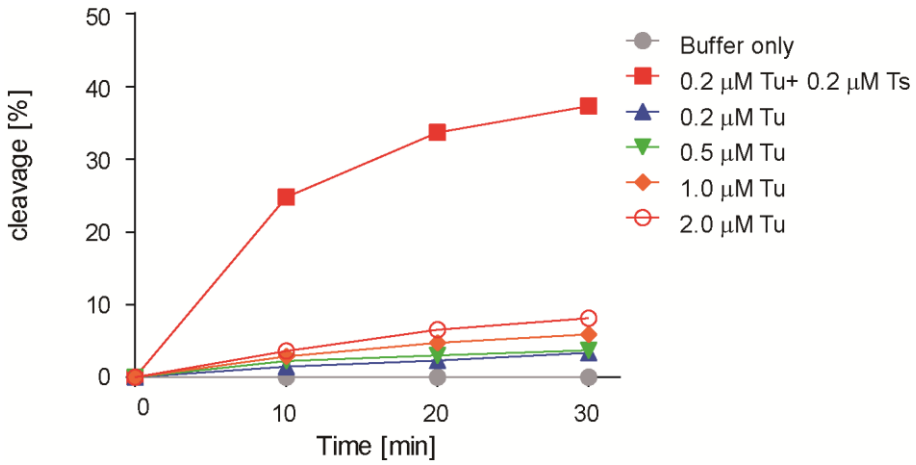
(**A**) Tu variants and (**B**) Ts variants were separated by 15% (w/v) SDS PAGE, and the gels were stained with Coomassie Brilliant Blue. Tu_wt* and H84A* in (A) were purified as previously described (3).

Figure S8



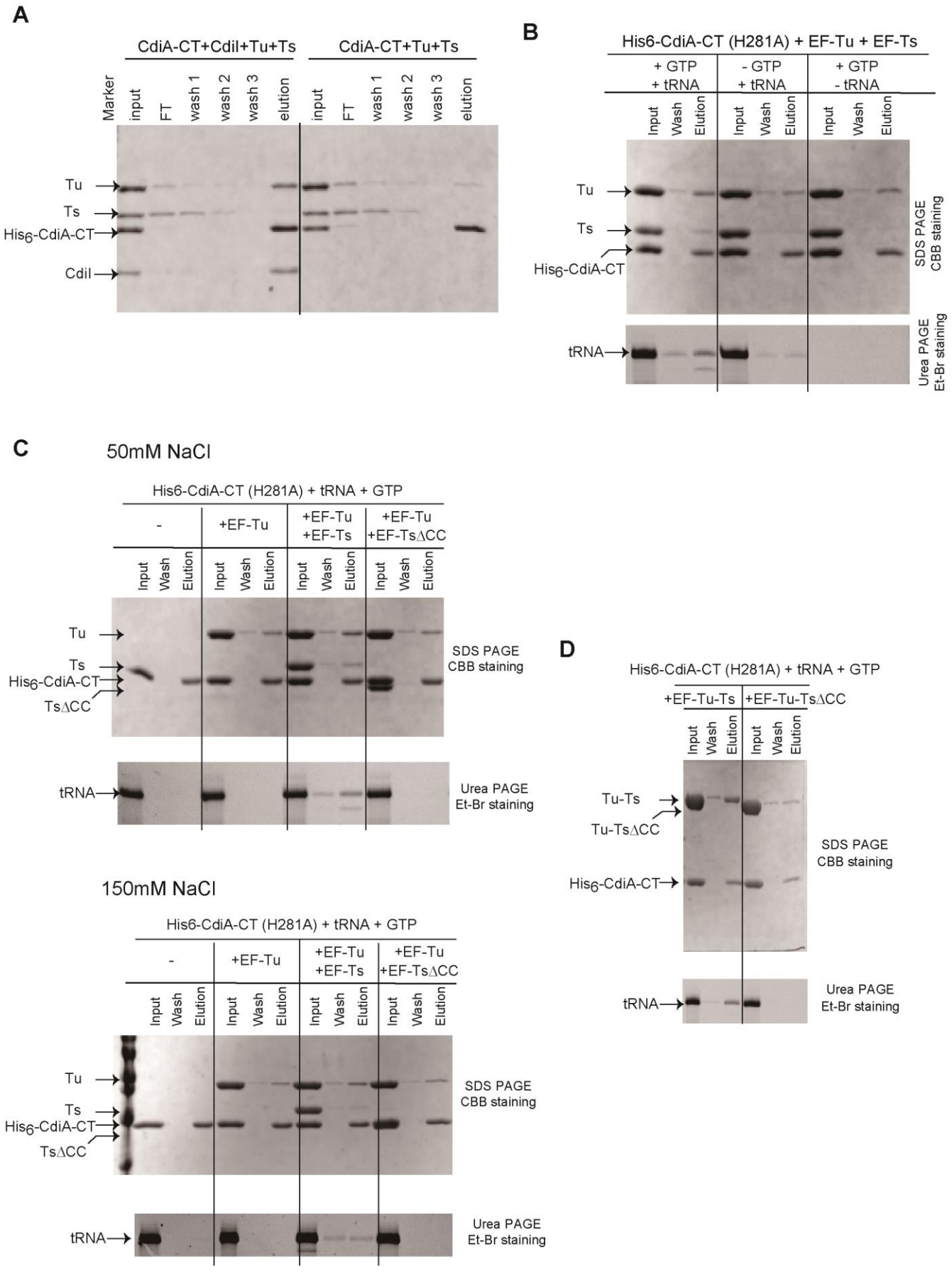
Supplementary Figure S8: The role of Tu_H84A in the enhancement of tRNA cleavage by CdiA-CT^{EC869}. (**A**) GTP requirement for the enhancement of tRNA cleavage by CdiA-CT^{EC869} in the presence of Tu_H84A and Ts. The tRNA^{Gln} transcript (1.0 μM) was incubated at 37°C with 0.1 μM of CdiA-CT^{EC869} in the presence of Tu_H84A (0.1 μM) and Ts (0.1 μM) with or without GTP (1 mM). (**B**) Ts requirement for the enhancement of tRNA cleavage by CdiA-CT^{EC869} in the presence of Tu_H84A and GTP. The tRNA^{Gln} transcript (1.0 μM) was incubated at 37°C with 0.1 μM of CdiA-CT^{EC869} in the presence of Tu_H84A (0.1 μM) and GTP (1 mM), with or without Ts (0.1 μM).

Figure S9



Supplementary Figure S9: Excess Tu does not compensate for the absence of Ts.
Cleavage of $tRNA^{Gln}$ by $CdiA\text{-}CT^{EC869}$ in the presence of increasing amounts of Tu and GTP, in the absence of Ts. The $tRNA^{Gln}$ transcript (1.0 μM) was incubated at 37°C with 0.2 μM of $CdiA\text{-}CT^{EC869}$ in the presence of Tu (0.2–2.0 μM) and GTP (1 mM). Addition of 0.2 μM Ts promoted cleavage of $tRNA^{Gln}$ at pH 7.4.

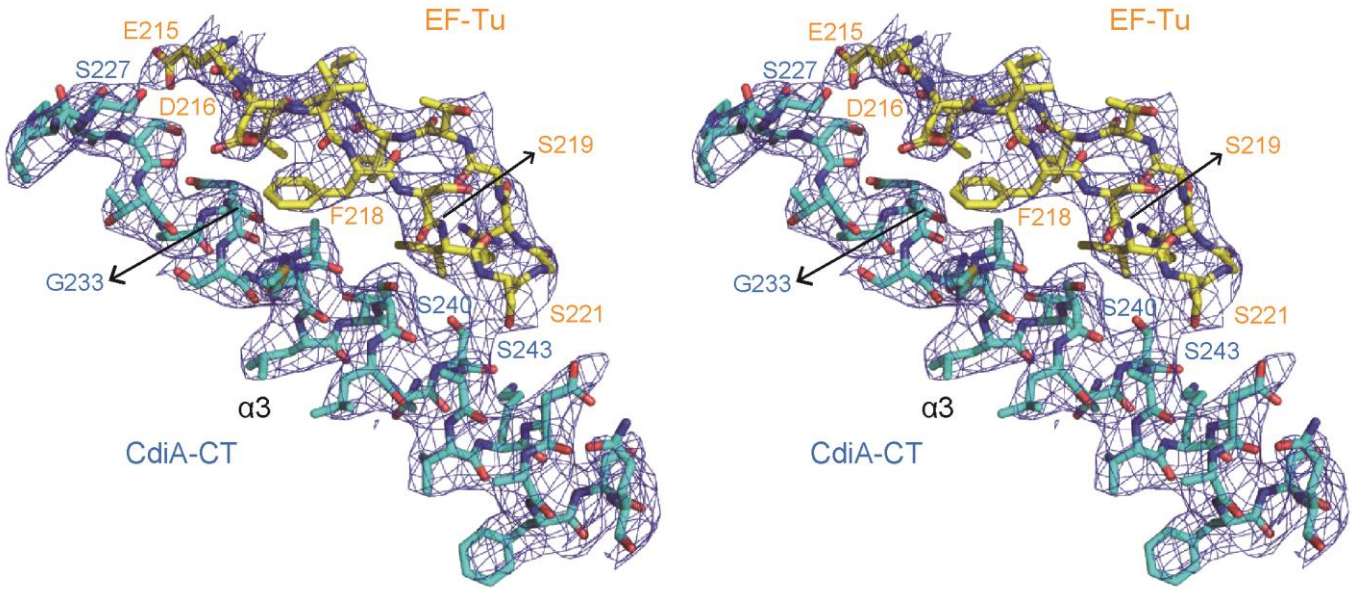
Figure S10


A
CdiA-CT+CdiI+Tu+Ts
CdiA-CT+Tu+Ts
Marker
input
FT
wash 1
wash 2
wash 3
elution
Tu
Ts
His6-CdiA-CT
CdiI
B
His6-CdiA-CT (H281A) + EF-Tu + EF-Ts
+ GTP + tRNA
- GTP + tRNA
+ GTP - tRNA
Input
Wash
Elution
Tu
Ts
His6-CdiA-CT
tRNA
SDS PAGE CBB staining
Urea PAGE Et-Br staining
C
50mM NaCl
His6-CdiA-CT (H281A) + tRNA + GTP
-
+EF-Tu
+EF-Tu +EF-Ts
+EF-Tu +EF-TsΔCC
Tu
Ts
His6-CdiA-CT
TsΔCC
tRNA
SDS PAGE CBB staining
Urea PAGE Et-Br staining
150mM NaCl
D
His6-CdiA-CT (H281A) + tRNA + GTP
+EF-Tu-Ts
+EF-Tu-TsΔCC
Tu-Ts
Tu-TsΔCC
His6-CdiA-CT
tRNA
SDS PAGE CBB staining
Urea PAGE Et-Br staining

Supplementary Figure S10: Interaction between CdiA-CT^{EC869} and Tu, Ts, and tRNA.

(**A**) Histidine-tagged CdiA-CT^{EC869} (5 μM, His_6-CdiA-CT) was mixed with equal amounts of Tu and Ts (and CdiI^{EC869}) in 200 μl of A-buffer, containing 20 mM Tris-Cl, pH 7.0, 150 mM NaCl, 5 mM β-mercaptoethanol, and 20 mM imidazole (input), and the mixture was loaded onto Ni-NTA resin (150 μl) in a Poly-Prep chromatography column (Bio-Rad, Japan). The flow-through (FT) was collected, and the Ni-NTA was washed three times with 500 μl of A-buffer (washes 1, 2, and 3). His_6-CdiA-CT was eluted from the resin with 200 μl of buffer, containing 20 mM Tris-Cl, pH 7.0, 150 mM NaCl, 5 mM β-mercaptoethanol, and 500 mM imidazole (elution). The samples were separated by 15% (w/v) SDS-PAGE and the gel was stained with Coomassie Brilliant Blue. (**B**) His-tagged CdiA-CT(H281A) was mixed with Tu and Ts in the presence or absence of tRNA or GTP, and the mixture was loaded onto a Ni-NTA column. The column was washed, and finally, CdiA-CT was eluted from the column. Proteins were visualized by SDS-PAGE and staining with CBB, and tRNA was visualized by PAGE and staining with ethidium bromide as in Figure 5G. (**C**) His-tagged CdiA-CT(H281A) was mixed with Tu or Tu and Ts (or Ts_ΔCC) in the presence of tRNA and GTP under two different salt conditions (upper panel: 50 mM NaCl and lower panel: 150 mM NaCl), and the mixture was loaded onto a Ni-NTA column. The column was washed, and finally, CdiA-CT was eluted from the column as in (B). (**D**) His-tagged CdiA-CT(H281A) was mixed with sg-Tu-Ts (or sgEF-Tu-TsΔCC) in the presence of tRNA and GTP. The column was washed, and finally, CdiA-CT was eluted from the column as in (B). For the experiments in (B)-(D), we used catalytically inactive CdiA-CT with H281A mutation, CdiA-CT (H281A), instead of using wild-type CdiA-CT, because tRNA could be cleaved during the pre-incubation of tRNA with wild-type CdiA-CT.

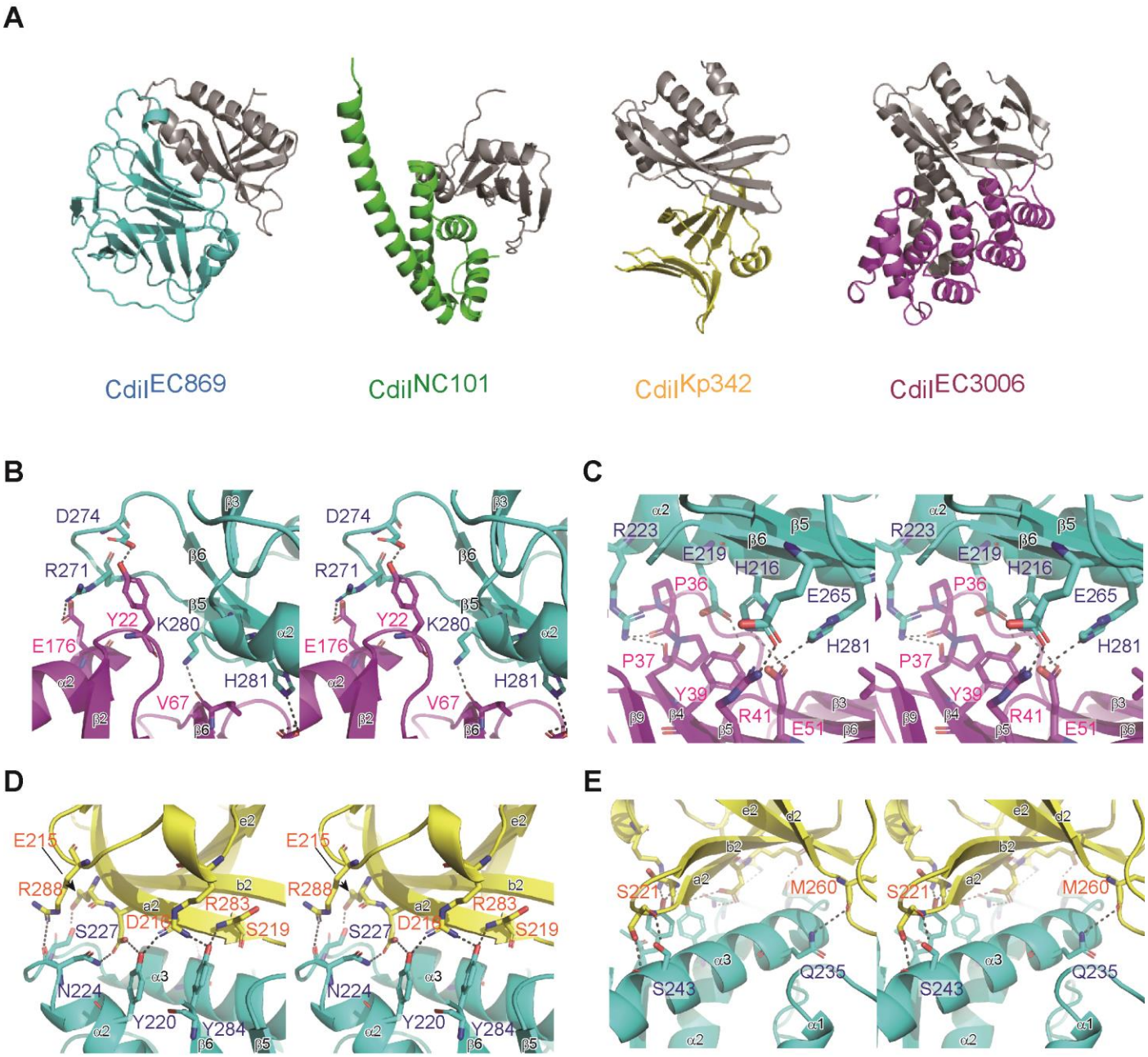
Figure S11



Supplementary Figure S11: Electron density map.

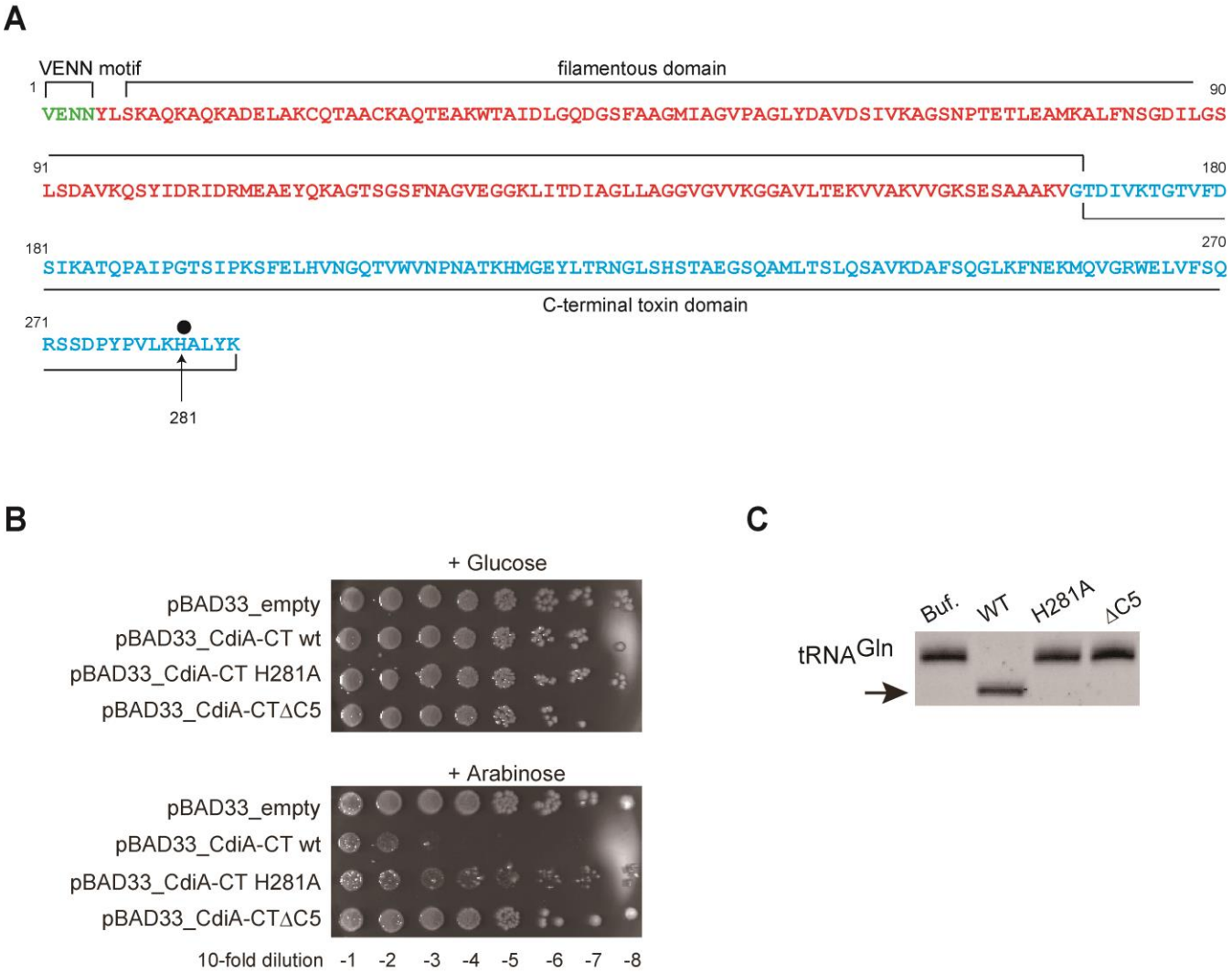
Stereoviews of the 2m*F*o-D*F*c electron densities of the residues around the CdiA-CT and Tu interface are shown in blue contoured at 1.0 σ.

Figure S12



Supplementary Figure S12: (A) Structural comparison of CdiA-CT:CdiI complexes from *E. coli* EC869 (cyan), NC101 (green) (4), *Klebsiella pneumoniae* 342 (yellow) (5), and *E. coli* EC3006 (magenta) (5). For clarity, CdiA-CTs are colored in gray. (**B, C**) Interactions between CdiA-CT^{EC869} (cyan) and CdiI^{EC869} (magenta). (**D**, **E**) Interactions between CdiA-CT^{EC869} (cyan) and domain II of Tu (yellow). Tu secondary structure elements are labeled according to the literature (6).

Figure S13



Supplementary Figure S13: CdiA-CT^{EC869} variants with reduced toxicity and tRNase activity.
(A) Amino acid sequence of CdiA-CT^{EC869}. The VENN motif (green), filamentous domain (red) and toxin domain (cyan) of CdiA-CT^{EC869} are depicted. **(B)** The cytotoxic activity of CdiA-CT^{EC869} is reduced by the His281Ala mutation (H218A) or deletion of five C-terminal amino acid residues (ΔC5). Overnight cultures of *E. coli* MG1655 transformed with pBAD33_CdiA-CT^{EC869}, pBAD33_CdiA-CT^{EC869}_H281A, and pBAD33_CdiA-CT^{EC869}_ ΔC5 were serially diluted, and the dilutions were spotted on LB agar plates containing 50 μg/ml chloramphenicol supplemented with 1% (w/v) arabinose (lower panel) or 1% (w/v) glucose (upper panel). **(C)** The His281Ala mutation (H281A) or deletion of the C-terminal five amino acids (ΔC5) decreased tRNA cleavage activity *in vitro.* Reactions were performed in the presence of Tu, Ts, and GTP, and the tRNA^{Gln} transcript was used as the substrate. The tRNA^{Gln} transcript (1.0 μM) was incubated at 37°C for 10 min with 0.5 μM of CdiA-CT^{EC869} or its variants in the presence of Tu (0.5 μM), Ts (0.5 μM), and GTP (1 mM). The arrow indicates tRNA^{Gln} cleaved by CdiA-CT^{EC869}.

Supplementary Table S1

Nucleotide sequences of the synthetic *E. coli* tRNAs and CdiA-CT/CdiI^{EC869} genes

Gene	Nucleotide sequence
$tRNA^{Gln}$ U1A72 (wt)	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGATGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCAGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$U1G72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGATGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCGGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$U1C72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGATGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCCGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$U1U72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGATGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCTGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$A1A72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCAGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$G1A72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAGGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCAGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$C1A72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGACGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCAGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$A1U72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCTGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$G1C72	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAGGG

	GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCCGCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$A73	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGATGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCAACCAggctagctaCATCCAAGCTT-3'
$tRNA^{Gln}$U73	5'- gaattcTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGATGG GGTATCGCCAAGCGGTAAGGCACCGGATTCTGATTCCGGCATTCCGAGGTT CGAATCCTCGTACCCCATCCAggctagctaCATCCAAGCTT-3'
$tRNA^{Trp}$	5'- GAATTCTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAA GGGGCGTAGTTCAATTGGTAGAGCACCGGTCTCCAAAACCGGGTGTTGGGA GTTCGAGTCTCTCCGCCCCTGCCAGGCTAGCTACATCCAAGCTT-3'
CdiA-CT^{EC869} (amino acid residues 1–286) and $CdiI^{EC869}$ (blue letters; amino acid residues 1–179) coding sequences	5'- GTTGAGAATAACTATCTGAGTAAAGCCCAGAAAGCTCAAAAAGCCGATGA GCTGGCTAAATGTCAGACTGCTGCCTGTAAGGCTCAGACAGAAGCAAAAT GGACGGCAATTGACCTGGGGCAGGATGGTAGCTTTGCTGCAGGAATGATTG CTGGGGTTCCTGCCGGGCTGTATGATGCTGTGGATAGTATTGTAAAAGCAG GTTCTAACCCGACGGAAACCCTGGAGGCAATGAAAGCGCTGTTTAACAGC GGCGATATCTTGGGTTCGTTATCGGATGCGGTTAAGCAATCTTATATAGAC CGTATTGACCGAATGGAAGCCGAATATCAGAAAGCTGGTACCAGCGGTTC GTTTAATGCAGGCGTTGAAGGCGGTAAGCTGATTACTGATATTGCCGGGCT GCTGGCTGGCGGCGTTGGCGTTGTGAAAGGTGGTGCGGTACTGACGGAGA AGGTTGTTGCTAAGGTTGTGGGTAAGTCTGAATCAGCAGCGGCAAAAGTTG GTACAGATATTGTTAAAACAGGAACCGTTTTCGACTCAATTAAAGCGACTC AACCTGCGATACCAGGAACATCCATTCCAAAATCTTTTGAATTACATGTTA ACGGACAAACCGTTTGGGTAAACCCAAATGCAACTAAACATATGGGCGAA TATTTAACACGGAATGGATTGTCTCACAGTACAGCAGAAGGAAGCCAAGC CATGCTGACCAGTCTTCAAAGTGCGGTTAAAGATGCATTTTCGCAGGGATT AAAATTTAACGAAAAAATGCAAGTTGGACGCTGGGAACTGGTATTTAGCC AACGGTCTTCAGATCCTTATCCAGTATTAAAGCATGCGTTGTATAAATAAT GAAATTAACTGTAGATAGCGTTATTAATGAACCTAGAAGCGTAGCCATTAC TATTGATGGTTATATTCCCGTTGATATAAAGATTATTGATTCTAAAAAGCTT CCGCCTTTGTATTGGCGGGGCGGGGATGGCAAAAAAAACCTACTTGAACTG GCTGTACTACCAGAAAATGGTTTTTTATCATCCATCACATTGGTAATGATAG CATCAGACTCAATTCATAAAACAGACTCCTTGTCAGTATCTTTACCAAGCA GTGAGTGTGGAGTTCCTGTAGTGAATACAAAACTGTGGAGCCACTCAGAAA GTGATGATTTTAGTCGTCGTTTTGTTGACGATTTTAGCCTTGATATTGAGGT GATTATATCATCAGAGTCTATGTTATTAACGATTGGAGAGAATAAAAAGGT

	AACTAGCTGGATAAAATGTAGCGATAATTTTTATCTCGGGATAGATGCAGG AAGAAATGTCGTTCATTTGTATTTGGATAAGTTAACACCAAGTGAAGTGGA AAGTTTTTTTGAGGCAGTAGGTTAG-3'

Nucleotide sequences of Tu and Ts

Tu (amino acid residues 1–394) coding sequence	5'- TCTAAAGAAAAGTTTGAACGTACAAAACCGCACGTTAACGTCGGTACTAT CGGCCACGTTGACCATGGTAAAACAACGCTGACCGCTGCAATCACTACCG TACTGGCTAAAACCTACGGCGGTGCTGCTCGCGCATTCGACCAGATCGAT AACGCGCCGGAAGAAAAAGCTCGTGGTATCACCATCAACACTTCTCACGT TGAATACGACACCCCGACCCGTCACTACGCACACGTAGACTGCCCGGGGC ACGCCGACTATGTTAAAAACATGATCACCGGTGCTGCGCAGATGGACGG CGCGATCCTGGTAGTTGCTGCGACTGACGGCCCGATGCCGCAGACTCGTG AGCACATCCTGCTGGGTCGTCAGGTAGGCGTTCCGTACATCATCGTGTTC CTGAACAAATGCGACATGGTTGATGACGAAGAGCTGCTGGAACTGGTTG AAATGGAAGTTCGTGAACTTCTGTCTCAGTACGACTTCCCGGGCGACGAC ACTCCGATCGTTCGTGGTTCTGCTCTGAAAGCGCTGGAAGGCGACGCAGA GTGGGAAGCGAAAATCCTGGAACTGGCTGGCTTCCTGGATTCTTACATTC CGGAACCAGAGCGTGCGATTGACAAGCCGTTCCTGCTGCCGATCGAAGA CGTATTCTCCATCTCCGGTCGTGGTACCGTTGTTACCGGTCGTGTAGAACG CGGTATCATCAAAGTTGGTGAAGAAGTTGAAATCGTTGGTATCAAAGAG ACTCAGAAGTCTACCTGTACTGGCGTTGAAATGTTCCGCAAACTGCTGGA CGAAGGCCGTGCTGGTGAGAACGTAGGTGTTCTGCTGCGTGGTATCAAAC GTGAAGAAATCGAACGTGGTCAGGTACTGGCTAAGCCGGGCACCATCAA GCCGCACACCAAGTTCGAATCTGAAGTGTACATTCTGTCCAAAGATGAAG GCGGCCGTCATACTCCGTTCTTCAAAGGCTACCGTCCGCAGTTCTACTTCC GTACTACTGACGTGACTGGTACCATCGAACTGCCGGAAGGCGTAGAGAT GGTAATGCCGGGCGACAACATCAAAATGGTTGTTACCCTGATCCACCCGA TCGCGATGGACGACGGTCTGCGTTTCGCAATCCGTGAAGGCGGCCGTACC GTTGGCGCGGGCGTTGTAGCAAAAGTTCTGAGCTAA-3'
Ts (amino acid residues 1–283) coding sequence	5'- GCTGAAATTACCGCATCCCTGGTAAAAGAGCTGCGTGAGCGTACTGGCG CAGGCATGATGGATTGCAAAAAAGCACTGACTGAAGCTAACGGCGACAT CGAGCTGGCAATCGAAAACATGCGTAAGTCCGGTGCTATTAAAGCAGCG AAAAAAGCAGGCAACGTTGCTGCTGACGGCGTGATCAAAACCAAAATCG ACGGCAACTACGGCATCATTCTGGAAGTTAACTGCCAGACTGACTTCGTT GCAAAAGACGCTGGTTTCCAGGCGTTCGCAGACAAAGTTCTGGACGCAG CTGTTGCTGGCAAAATCACTGACGTTGAAGTTCTGAAAGCACAGTTCGAA GAAGAACGTGTTGCGCTGGTAGCGAAAATTGGTGAAAACATCAACATTC

	GCCGCGTTGCTGCGCTGGAAGGCGACGTTCTGGGTTCTTATCAGCACGGT GCGCGTATCGGCGTTCTGGTTGCTGCTAAAGGCGCTGACGAAGAGCTGGT TAAACACATCGCTATGCACGTTGCTGCAAGCAAGCCAGAATTCATCAAA CCGGAAGACGTATCCGCTGAAGTGGTAGAAAAAGAATACCAGGTACAGC TGGATATCGCGATGCAGTCTGGTAAGCCGAAAGAAATCGCAGAGAAAAT GGTTGAAGGCCGCATGAAGAAATTCACCGGCGAAGTTTCTCTGACCGGT CAGCCGTTCGTTATGGAACCAAGCAAAACTGTTGGTCAGCTGCTGAAAG AGCATAACGCTGAAGTGACTGGCTTCATCCGCTTCGAAGTGGGTGAAGG CATCGAGAAAGTTGAGACTGACTTTACAGCAGAAGTTGCTGCGATGTCC AAGCAGTCTTGA-3'

Supplementary Table S2: Oligonucleotide sequences

Oligonucleotide	Sequence (5′ to 3′ direction)
GlnRS-for_NdeI	AGCTAGCTCATATGAGTGAGGCAGAAGCCCG
GlnRS-rev_XhoI	AGCTCTCGAGCTCGCCTACTTTCGCCCAGGTA
Tu_N51A-for	gcgGCGCCGGAAGAAAAAGCTCGTGGTATCACC
Tu_N51A-rev	ATCGATCTGGTCGAATGCGCGAGCAGCACC
Tu_E54A-for	gcgGAAAAAGCTCGTGGTATCACCATCAACACTTC
Tu_E54A-rev	CGGCGCGTTATCGATCTGGTCGAATGCGCGAGC
Tu_R58A-for	gcgGGTATCACCATCAACACTTCTCACGTTG
Tu_R58A-rev	AGCTTTTTCTTCCGGCGCGTTATCGATCTGGTCG
Tu_H66A-for	gcgGTTGAATACGACACCCCGACCCGTCACTACG
Tu_H66A-rev	AGAAGTGTTGATGGTGATACCACGAGCTTTTTC
Tu_H84A-for	gcgGCCGACTATGTTAAAAACATGATCACCGG
Tu_H84A-rev	CCCCGGGCAGTCTACGTGTGCGTAGTGACGGGTC
Tu_Y87A-for	gcgGTTAAAAACATGATCACCGGTGCTGCGCAGA
Tu_Y87A-rev	GTCGGCGTGCCCCGGGCAGTCTACGTGTGC
Tu_K89A N90A-for	gcggcgATGATCACCGGTGCTGCGCAGATGGAC
Tu_K89A N90A-rev	AACATAGTCGGCGTGCCCCGGGCAGTCTACGTG
Tu_N135A-for	gcgAAATGCGACATGGTTGATGACGAAGAGCTGC
Tu_N135A-rev	CAGGAACACGATGATGTACGGAACGCCTACCTG
Tu_F218A-for	gcgTCCATCTCCGGTCGTGGTACCGTTGTTACCGG
Tu_F218A-rev	TACGTCTTCGATCGGCAGCAGGAACGGCTTG
Tu_R223A-for	gcgGGTACCGTTGTTACCGGTCGTGTAGAACGCGG
Tu_R223A-rev	ACCGGAGATGGAGAATACGTCTTCGATCGGCAGC
Tu_E259A-for	gcgATGTTCCGCAAACTGCTGGACGAAGGCCG
Tu_E259A-rev	AACGCCAGTACAGGTAGACTTCTGAGTCTCTTTG
Tu_R262A-for	gcgAAACTGCTGGACGAAGGCCGTGCTGGTGAG
Tu_R262A-rev	GAACATTTCAACGCCAGTACAGGTAGACTTCTGAG
Tu_R283A-for	gcgGAAGAAATCGAACGTGGTCAGGTACTGGC
Tu_R283A-rev	TTTGATACCACGCAGCAGAACACCTACGTTCTC
Tu_R288A-for	gcgGGTCAGGTACTGGCTAAGCCGGGCACCATC
Tu_R288A-rev	TTCGATTTCTTCACGTTTGATACCACGCAGCAG
Tu_R318A H319A-for	gcggcgACTCCGTTCTTCAAAGGCTACCGTCCGC
Tu_R319A H319A-rev	GCCGCCTTCATCTTTGGACAGAATGTACACTTCAG
Tu_T320A-for	gcgCCGTTCTTCAAAGGCTACCGTCCGCAG
Tu_T320A-rev	ATGACGGCCGCCTTCATCTTTGGACAGAATGTAC
Ts_A202E-for	gaaATGCAGTCTGGTAAGCCGAAAGAAATC
Ts_A202E-rev	GATATCCAGCTGTACCTGGTATTCTTTTTCTACC

Ts_K207A K209A-for	gcgCCGgcgGAAATCGCAGAGAAAATGGTTGAAGGC
Ts_K207A K209A-rev	ACCAGACTGCATCGCGATATCCAGCTGTACC
Ts_K214E-for	gaaATGGTTGAAGGCCGCATGAAGAAATTCACC
Ts_K214E-rev	CTCTGCGATTTCTTTCGGCTTACCAGACTGCATC
Ts_R219P-for	ccgATGAAGAAATTCACCGGCGAAGTTTCTCTG
Ts_R219P-rev	GCCTTCAACCATTTTCTCTGCGATTTCTTTCGG
Ts_R219A K221A K222A-for	gcgATGgcggcgTTCACCGGCGAAGTTTCTCTG
Ts_R219A K221A K222A-rev	GCCTTCAACCATTTTCTCTGCGATTTCTTTCGG
CdiA-for_NdeI	AGCTAGCTCATATGGTTGAGAATAACTATCTGAGTAAAGCC
CdiA-rev_XhoI_HindIII	AGCTAAGCTTCTCGAGTTATTTATACAACGCATGCTTTAATACTGG
CdiI-for_NdeI	AGCTAGCTCATATGAAATTAACTGTAGATAGCGTT
CdiI-rev_XhoI	AGCTCTCGAGCTAACCTACTGCCTCAAA
CdiA_H281A-for	gctGCGTTGTATAAATAATGAAATTAACTG
CdiA_H281A-rev	CTTTAATACTGGATAAGGATCTG
CdiAΔC5-rev_XhoI_HindIII	AGCTAAGCTTCTCGAGTTACTTTAATACTGGATAAGGATCTGAAGACCG
CdiA_H216A-for	GCGATGGGCGAATATTTAACACGGAATGG
CdiA_H216A-rev	TTTAGTTGCATTTGGGTTTACCCAAACGG
CdiA_Y220F-for	TTTTTAACACGGAATGGATTGTCTCACAG
CdiA_Y220F-rev	TTCGCCCATGTGTTTAGTTGCATTTGGG
CdiA_R263A-for	gccTGGGAACTGGTATTTAGCCAACGG
CdiA_R263A-rev	TCCAACTTGCATTTTTTCGTTAAATTTTAATCC
CdiA_R271A-for	gcgTCTTCAGATCCTTATCCAGTATTAAAG
CdiA_R271A-rev	TTGGCTAAATACCAGTTCCCAGCGTCC
Ala_probe	TGGAGCTAAGCGGGATCGAACCGCTGaCCTCTTGC
Asn_probe	CTCCTCTGACTGGACTCGAACCAGTGaCATACGGA
Gln_probe	CTGGGGTACGAGGATTCGAACCTCGGaATGCCGGA
Met_probe	TGGCTACGACGGGATTCGAACCTGTGACCCCATCA
fMet_probe	TTGCGGGGGCCGGATTTGAACCGACGaCCTTCGGG
Trp_probe	CAGGGGCGGAGAGACTCGAACTCCCAaCACCCGGT
Infusion_sumo_TsTu_For	aacagattggtggtCATATGGCTGAAATTACCGCATCCCTG
Infusion_sumo_TsTu_Rev	ccctcgagcccgggCATATGTTAGCTCAGAACTTTTGCTACAACG

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