Table S1 Primers used in this study

Primer	Sequence (5'-3')[a]
for cloning into pZE21MCS	
TTC0113F-Kpn	AAAGGTACC**ATG**GGAAGGCGCTACCTCCTAGAC
TTC0113R-Sal	AAAGTCGAC**TCA**CCACAGGGTCTCCACGC
TTC0114F-Kpn	AAAGGTACC**ATG**AAGGCCTTCACCGTGCAC
TTC0125F-Kpn	AAAGGTACC**ATG**GTGCTGGACGCTTCCGC
TTC0125R-Sal	AAAGTCGAC**TCA**GGAGGGCTTCCACGCCA
TTC0126F-Kpn	AAAGGTACC**ATG**GCCCTCACCATCCGCAAC
TTC1207F-Kpn	AAAGGTACC**ATG**AGGGTCGTCCTGGACACC
TTC1207R-Sal	AAAGTCGAC**TCA**GCTCACCTCCCGCAAAAAG
TTC1208F-Kpn	AAAGGTACC**ATG**CGGCGCATTGCCCTGC
TTC1395F-Kpn	AAAGGTACC**GTG**AGCCCCCGCCTCATCC
TTC1395R-Sal	AAAGTCGAC**TCA**ACGGGATCCAAGGCGCTTTTG
TTC1394F-Kpn	AAAGGTACC**AGA**TGAGGCGGCGTTACCG
TTC1549F-Kpn	AAAGGTACC**ATG**GCCAAGGGGGGGCAC
TTC1549R-Sal	AAAGTCGAC **CTA**GAGGTTATGGAACTC
TTC1548F-Kpn	AAAGGTACC**ATG**CTCAAGTACACCGCCCTCC
TTC1705F-Kpn	AAAGGTACC**ATG**GCGAGGCGGCTTAGGC
TTC1705R-Sal	AAAGTCGAC**TCA**CTCCAGCCATTCCTCCTCCTC
TTC1704F-Kpn	AAAGGTACC**ATG**GACGGGATGGGCACCCTG
TTC1805F-Kpn	AAAGGTACC**ATG**ATGCATCGCAAGCGCGTC
TTC1805R-Sal	AAAGTCGAC**CTA**GCGTCCAGCACCACCCAG
TTC1804F-Kpn	AAAGGTACC**ATG**AGCTGGGTGGTGCTGGACGC
for cloning into pET28a	
TTC0126F-Nde	AAACAT**ATG**GCCCTCACCATCCGCAAC
TTC0126R-Xho	AAACTCGAG**TCA**GTACCCCTCCTTGCCGTAC
TTC0125R-Xho	AAACTCGAG**TCA**GGAGGGCTTCCACGCCA
tetRF-Nde	CCGGCAT**ATG**GCACGGCTGAACAGAGA
tetRR-Xho	CCGGCTCGAG**TCA**GCAAAAGGGGATGATAAG
TTP0042F-Nde	GAGAGCAT**ATG**ACCGAGAACGCCGAAAAATTC
TTP0042R-Xho	AATTCTCGAG**TTA**GGTCTGGGCCCGCGCG
for Northern blotting	
tRNAMet	*biotin*-CCATCATTATGAGTGATGTG
for site-directed mutagenesis	
D4AF	GCCATATGGTGCTGG*C*CGCTTCCGC
D4AR	GCGGAAGCG*G*CCAGCACCATATGGC
E23AF	GAGGAGCTTTTGG*C*GGAACTCCGGCG
E23AR	CGCCGGAGTTCC*G*CCAAAAGCTCCTC
E40AF	CCACCCTGGCCG*C*AGCGGGGATCGTC
E40AR	GACGATCCCCGCT*G*CGGCCAGGGTGG
F71AF	CCGAGATCGTGCCC*GC*TACCGAAAGGCATG
F71AR	CATGCCTTTCGGTA*GC*GGGCACGATCTCGG
T72AF	GATCGTGCCCTTT*G*CCGAAAGGCATG
T72AR	CATGCCTTTCGG*C*AAAGGGCACGATC
A76DF	CCGAAAGGCATG*A*CCGGGAGGCCATC

A76DR	GATGGCCTCCCGG*T*CATGCCTTTCGG
G94DF	GGCACCCCGCCG*AT*CTCAACTTCGGG
G94DR	CCCGAAGTTGAG*AT*CGGCGGGGTGCC
G98DF	GGGCTCAACTTCGACG*AC*TGCCTGAGC
G98DR	GCTCAGGCAGTC*GT*CGAAGTTGAGCCC
D99AF	CTCAACTTCGGGG*C*CTGCCTGAGCTAC
D99AR	GTAGCTCAGGCAG*G*CCCCGAAGTTGAG
S102DF	GGGACTGCCTG*GAT*TACGCCCTGGCC
S102DR	GGCCAGGGCGTA*ATC*CAGGCAGTCCC
L105DF	CTGAGCTACGCC*GAC*GCCCGGGTGGAG
L105DR	CTCCACCCGGGC*GTC*GGCGTAGCTCAG
V108DF	CCCTGGCCCGGG*AC*GAGGGGGAACC
V108DR	GGTTCCCCCTC*GT*CCCGGGCCAGGG
D119AF	CTACAAGGGCCAGG*C*CTTTGACCGGACG
D119AR	CGTCCGGTCAAAG*G*CCTGGCCCTTGTAG
D124AF	CTTTGACCGGACGG*C*CCTGGCGTGGAAG
D124AR	CTTCCACGCCAGG*G*CCGTCCGGTCAAAG

[a]The restriction sites introduced are underlined, and the mutations are shown in italics. The initiation and termination codons are shown in bold.

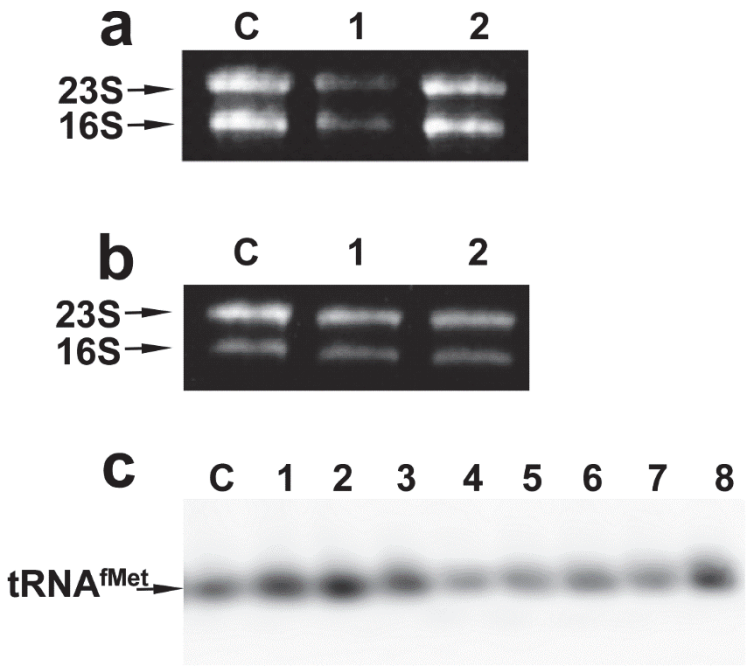

Fig. S1 RNA degradation assay with intact ribosomes from *E. coli* (**a**)*, T. thermophilus* (**b**), and with initiator tRNA (**c**)

(**a, b**) Intact ribosomes were incubated with *Tt*VapC (lane 1) or the *Tt*VapBC complex (lane 2) at 50 °C for 60 min. Then, RNA was prepared and loaded onto a denaturing agarose gel. Lane C indicates a control experiment in which ribosomes were incubated in the reaction buffer for 60 min. (**c**) Total RNA from *E. coli* was incubated with *Tt*VapC for 0 (lane 1), 15 (lane 2), 30 (lane 3), or 60 min. (lane 4), or with the *Tt*VapBC complex for 0 (lane 5), 15 (lane 6), 30 (lane 7), or 60 min. (lane 8), and resolved by denaturing PAGE. The initiator tRNA was detected by Northern blotting. Lane C indicates a control experiment without incubation with *Tt*VapC. The positions of 23S, 16S rRNA, and $tRNA^{fMet}$ are indicated by arrows.

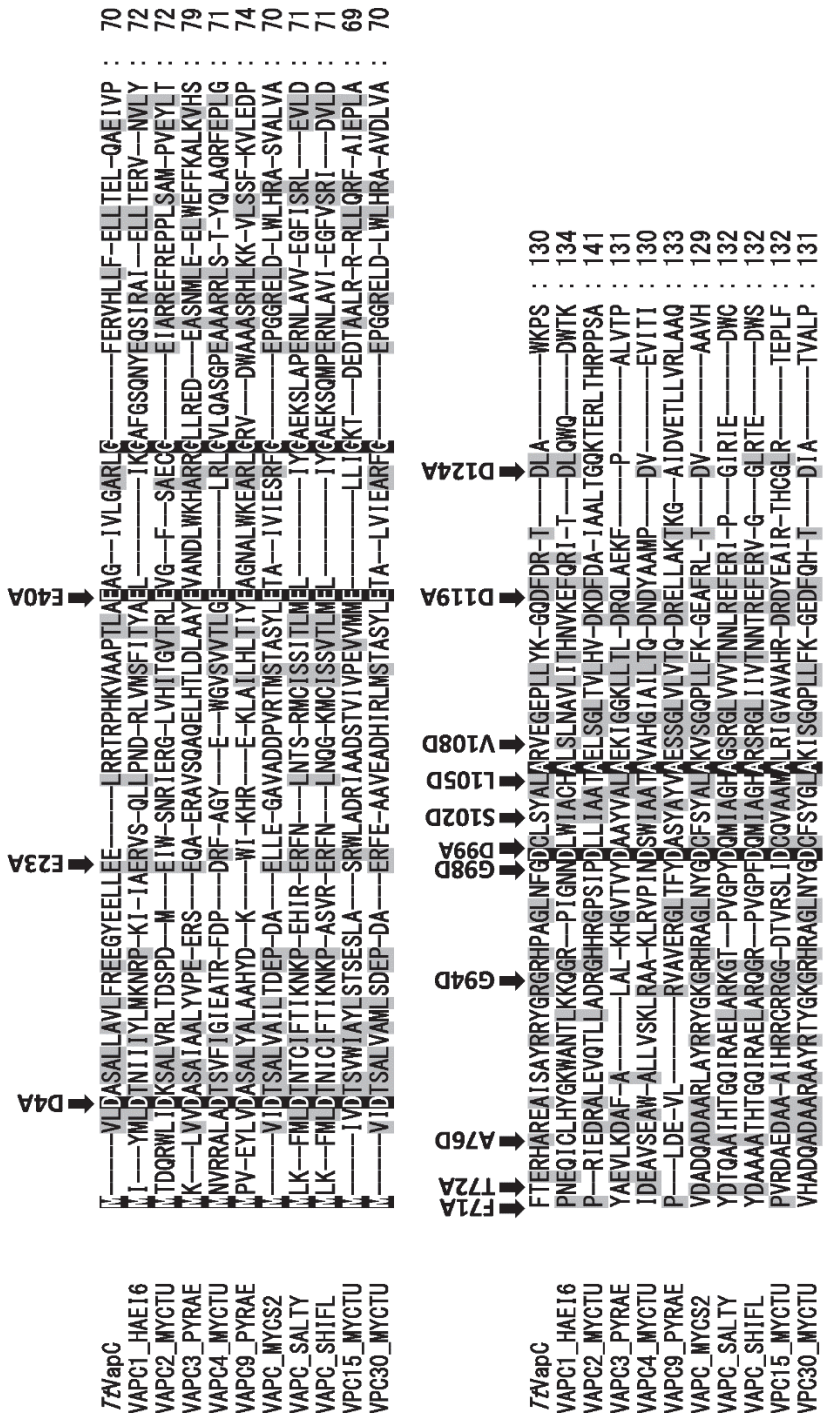

Fig. S2 Amino acid sequence alignment of *Tt*VapC and the "reviewed" VapCs in the Uniprot database

The alignment was generated by the T-Coffee program. Conserved residues are shaded, of which those conserved among all of the sequences are in black. Amino acid substitutions introduced in *Tt*VapC are indicated above the alignment.

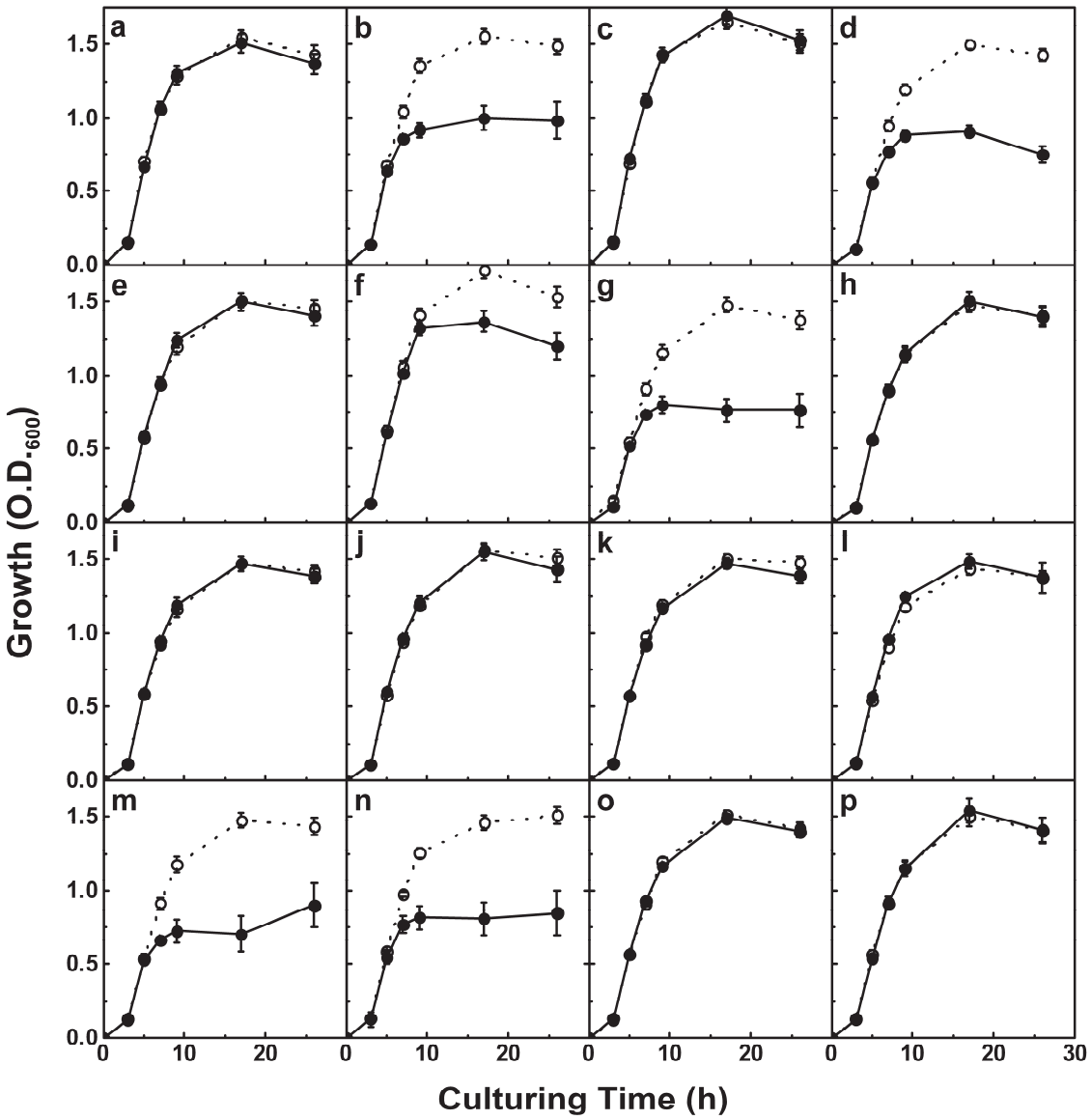

Fig. S3 Growth inhibition assay of *Tt*VapC mutants

E. coli DH5αZ1 harboring pZE21MCS (**a**), or the plasmid containing the wild type *TtvapC* (**b**), D4A (**c**), E23A (**d**), E40A (**e**), F71A (**f**), T72A (**g**), A76D (**h**), G94D (**i**), G98D (**j**), D99A (**k**), S102D (**l**), L105D (**m**), V108D (**n**), D119A (**o**), or D124A (**p**) were cultured in LB medium containing kanamycin at 43 ºC, and cell growth was monitored by measuring $O.D._{600}$. At $O.D._{600} \approx 0.2$, Dox was added to induce gene expression. Open circles with dotted lines indicate the culture without induction, and filled circles with solid lines indicate the culture with induction. Culturing was conducted three times, and the mean values ± standard deviations are shown.