SUPPLEMENTARY INFORMATION

Supplementary Figures

S1.

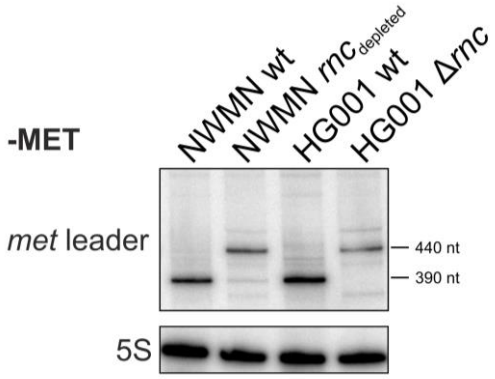

Figure S1. *met* leader cleavage does not occur in an RNase III deletion mutant. Total RNA isolated from *S. aureus* Newman (NWMN wt), isogenic RNase III mutant (NWMN $rnc_{depleted}$), *S. aureus* HG001 (HG001 wt) and isogenic RNase III deletion mutant (HG001 Δ*rnc*) grown in CDM without methionine ('-MET') was run on a PAA gel. Northern blot was hybridized with a *met* leader-specific probe and subsequently re-hybridized with a 5S rRNA-specific probe as loading control. Approximate transcript lengths are indicated on the right.

S2.

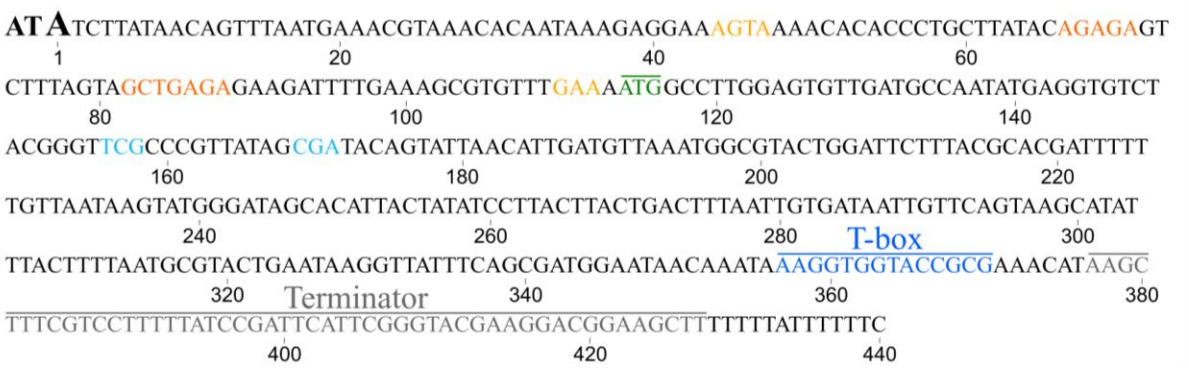

Figure S2. Transcriptional start sites (TSS) and sequence of *met* leader. Transcriptional start sites (TSS) determined by cRACE are shown in bold, major TSS enlarged and numbered with '1', interacting nucleotides of specifier loop are shown in yellow, base pairing nucleotides of AG-bulge and apical loop of stem I in orange, specifier codon in green, potentially interacting nucleotides of Stem IIA/B pseudoknot in light blue, T-box sequence is highlighted in dark blue, terminator sequence is shown in gray.

S3.

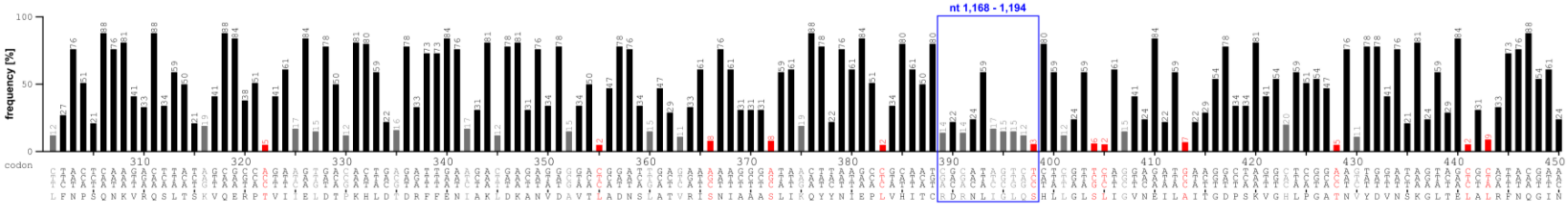

Figure S3. Stretch of rare codons present within last 800 nt of *metF*. Frequency of each codon from 300-450 (= nt 900-1,350) of *metF* is given in percent (‘%’) for *S. aureus*. ‘AUG’ has a frequency of 100 % as it is the only base triplet coding for methionine. A frequency of 80 % means that the respective codon is used by *S. aureus* in 80 % of cases, when the particular amino acid is encoded. Codons with frequencies below 20 % (grey) and 10 % (red) are regarded as rare codons. Region of nt 1,168-1,194 (= codons 389-398) within *metF*, where 29 % of 5'-ends were detected with 5' RACE (see Figure 7) is highlighted by a blue box. Base triplet and amino acid in single letter code is given for each position.

Supplementary Material

Supplementary Material 1

Composition of chemically defined medium (CDM).

CDM consisted of a basic salt medium (12.5 mM Na_2HPO_4, 10 mM KH_2PO_4, 1.65 mM $MgSO_4$, 9.25 mM NH_4Cl, 8.5 mM NaCl), 0.142 mM sodium citrate tribasic dihydrate, 75 mM α-D(+) glucose and 1 mM of all 19 L-amino acids, except for cysteine (1.2 mM) and tyrosine (0.1 mM). L-methionine (MET) was only added when indicated. Vitamins were supplemented as follows: 4-aminobenzoic acid (870 nM), Ca-D-panthothenic acid (630 nM), cyanocobalamin (108 nM), D-biotin (120 nM), nicotinamide (2430 nM), pyridoxine hydrochloride (1860 nM), thiamine hydrochloride (870 nM), riboflavin (600 nM). Trace elements were supplemented as follows: $NiCl_2$ (100 nM) and $FeCl_3$ (750 nM). $ZnSO_4$, $MnCl_2$, H_3BO_3, $Co(NO_3)_2$, $CuSO_4$ and Na_2MoO_4 were supplemented by adding the 1,000x 'Trace Metal Mix A5 with Co' (Sigma-Aldrich, # 92949) to a final concentration of 1x.

Supplementary Methods

Supplementary Method 1

Cloning and mutagenesis to obtain *met* leader mutant constructs.

Two different cloning techniques were used for the generation of plasmids. Either the conventional 'cut-and-paste' method using restrictions enzymes and ligases or the '*in vivo E. coli* cloning' (iVEC) method were employed (1). PCR site-directed mutagenesis methods in combination with iVEC were used to introduce point mutations, insertions or deletions into a plasmid sequence.

All plasmids and oligonucleotides used in this study are listed in Tables 1, 2 and Tables S1 and S2, respectively. Plasmids were constructed as described in the following paragraphs.

pBASE *met* leader mutant vectors

Vector pBASE_metleader+1kb flanking (pFW001) was generated by amplifying the *met* leader sequence with its 1kb flanking regions with PCR using genomic DNA of *S. aureus* Newman as template. PCR product was digested with BglII (Thermo Scientific, FastDigest) and cloned into pBASE6. The resulting plasmid, pFW001, was used as template for outward PCRs to generate plasmid pBASE_ΔAntiTer&Ter. PCR products were DpnI-digested, cleaned up using the innuPREP PCRpure kit (Analytikjena (Endress+Hauser Company), #845-KS-5010) and used for a ligation reaction. (Note that an actual ligation cannot take place due to the blunt end and tri-phosphate nature of the PCR products. However, iVEC turned out to be more efficient in presence of the ligation buffer. In later cloning approaches the ligase was omitted from the 'ligation' reaction.)

Vectors pBASE_Ter_destab, pBASE_Ter_mutated_ 2, 3 and 4 were generated by PCR site-directed mutagenesis amplifying the pFW001 vector in two fragments that overlapped in the ampicillin resistance cassette and in the region containing the point mutations introduced by the primers. The 1.7 kb and the 7.1

kb fragments were DpnI-digested, cleaned up using the innuPREP PCRpure kit and used for a 'ligation' reaction (see above).

Vector pBASE6_Ter_mutated_1 was generated in several steps because cloning as described above was unsuccessful. First, two fragments overlapping in the region containing the point mutations introduced by the primers were amplified by PCR using the pBASE_Ter_destab plasmid as template. The 800 bp-fragment contained a BglII restriction site, the 3' flanking region of the *met* leader and the 3' region of the *met* leader, the 1.4 kb-fragment contained a BglII restriction site, the 5'-flanking region of the *met* leader and most of the *met* leader sequence (both fragments overlapped in the Terminator region). PCR reactions were treated with DpnI and cleaned up using the innuPREP PCRpure kit. 50-100 ng of each fragment were used as template for an overlap PCR using the primers FW099 and FW100. The resulting 2.2 kb fragment was size-separated from unwanted PCR products via gel electrophoresis, cut out from the agarose gel and purified using the NucleoSpin® Gel and PCR Clean-up kit (Machery-Nagel, #740588.250). Purified fragment was subjected to A-tailing using Dream-Taq polymerase (Thermo Scientific, #EP0703), following manufacturer's instructions, for cloning into the pGEM-T-easy vector system I (Promega, #A1360). The resulting plasmid, pGEM-T-easy+Ter_mut_1, was treated with BglII (Thermo Scientific, FastDigest) to cut out the Ter_mut_1+1kb_flanking region fragment. pBASE6 vector was linearized using BglII and dephosphorylated using CIP (alkaline phosphatase calf intestinal, NEB, #M0290) according to manufacturer's instructions. The fragment and the linearized vector were size-separated on an agarose gel, cut out from gel and purified using the NucleoSpin® Gel and PCR Clean-up kit. The fragment was cloned into pBASE6.

pEB01-met leader-metI

Vector pEB01-met leader-metI was generated by amplifying the *met* leader sequence (from the -35 signal on) until nt 215 of *metI* with PCR using genomic DNA of *S. aureus* Newman as template. PCR product was digested with BamHI (Thermo Scientific, FastDigest) and cloned into pEB01.

pJC1_tRNAi_deletion

Vector pJC1_tRNAi_deletion was generated by amplifying the pJC1-MetTBox-metleader-cl-pR-eYFP vector in two fragments that overlapped in the kanamycin resistance cassette. The 5.2 kb fragment included the 3'-end of the tRNA sequence on the non-overlapping end that was subsequently removed by Pfl23II (Thermo Scientific, FastDigest) digestion. The 4.5 kb fragment had a flanking Pfl23II restriction site on the non-overlapping end. Both fragments were DpnI- and Pfl23II-digested, cleaned up using the innuPREP PCRpure kit and used for a ligation reaction.

Accuracy of all plasmids was verified by Sanger sequencing.

Supplementary Method 2

Rifampicin assay Northern blot quantification.

Fiji software was used for quantification of Northern blot bands obtained from rifampicin assays. The procedure is described in detail online (https://di.uq.edu.au/community-and-alumni/sparq-ed/sparq-ed-services/using-imagej-quantify-blots).

Probe signals were displayed in *.tiff files. These files were opened in Fiji and the region of interest was selected by using the rectangle tool covering the band of interest. After measuring the last band, the signals were plotted and the background noise was removed by closing the area under the curve. The enclosed areas under the curves were measured by the programme. The data were exported from the results table. Percentage of transcript remaining was calculated by setting the t0 value (derived from the t0 band signal of the rifampicin assay) as 100 %. 5S rRNA signals were quantified. Only, if values were equal for all time points, signals of *met* leader transcripts were quantified. The 'percentage of transcript remaining' was plotted against time to obtain the graph shown in Figure 4B.

Supplementary Method 3

In-line probing assays.

In-line probing was used to determine the secondary structure of the *met* leader RNA. The experimental set up was adapted from (2). *In vitro* transcribed RNA of either the full-length (440 nt) or a shortened (237 nt) *met* leader version were used for in-line probing. Column-purified PCR products containing a T7 promoter sequence immediately upstream of the *met* leader sequence were used as template for *in vitro* transcription. To increase transcription efficacy, the full-length *met* leader template sequence contains two additional 5'-guanosines and the shortened *met* leader one additional 5'-guanosine. Sequences of the resulting T7 transcripts are listed in Table S3.

In vitro transcription was carried out using the MEGAscript T7 Kit (Ambion, #AM1333) according to the manufacturer's instructions for short transcripts. Following, template DNA was digested by incubation with 1 µl Turbo DNase for 15 min at 37 °C, reaction volume was increased to 100 µl with RNase-free ddH_2O and RNA was purified with 100 µl phenol/chloroform/isoamylalcohol solution (P/C/I, 25:24:1) in a Phase Lock Gel (PLG) 'heavy' tube (5 Prime, #2302830). After vigorous shaking reaction was centrifuged for 12 min at 13,000 rpm and 15 °C and upper aqueous phase cleaned up via a G-25 column (illustra MicroSpin G-25 column, GE Healthcare, #27-5325-01) according to manufacturer's instructions. RNA was precipitated with 300 µl 30:1 ethanol/sodium acetate mix for 2 to 3 h at -80 °C or overnight at -20 °C. When precipitated at -80 °C solution was thawed on ice for 20 to 30 min prior to centrifugation for 30 min at 14,000 rpm and 4 °C. RNA pellet was washed with 100 µl 70-75 % ethanol, centrifuged for 10 min at 14,000 rpm and 4 °C and then dried at room temperature. To redissolve the RNA 33 µl RNase-free ddH_2O were added and solution was incubated for 5 min at 65 °C and 1,000 rpm in a heating block with shaking function and

vortexed one to two times in between. RNA concentration and quality was determined by measuring a 1:10 diluted and an undiluted aliquot of the sample with a spectrophotometer (NanoDrop). 10 µM stock solutions of the *in vitro* transcribed RNA were set up with RNase-free ddH_2O for downstream application and stored at -80 °C until use.

End labelling of the *in vitro* transcribed RNA with radioactive [γ-^{32}P]-ATP required preceding dephosphorylation to obtain a 5'-monophosphate. 20 pmol *in vitro* transcribed RNA were mixed with 15 µl RNase-free ddH_2O, incubated at 95 °C for 1 min and chilled on ice. Then 2 µl 10x CIP (alkaline phosphatase calf intestinal, NEB) reaction buffer and 2 µl CIP (NEB, #M0290) were added and reaction was incubated for 1 h at 37 °C. Ensuing reaction volume was increased to 110 µl with RNase-free ddH_2O and RNA purified with 110 µl phenol/chloroform/isoamylalcohol solution (25:24:1) in a PLG heavy tube. After vigorous shaking reaction was centrifuged for 12 min at 13,000 rpm and 15 °C and upper aqueous phase was transferred into a fresh tube. 1.1 µl GlycoBlue™ coprecipitant (15 mg/ml, Ambion) were added and RNA was precipitated with 300 µl 30:1 ethanol/sodium acetate mix for 3 h at -80 °C or overnight at -20 °C. RNA was cleaned up and redissolved in 16 µl RNase-free ddH_2O as described above. RNA concentration was determined by measuring an undiluted aliquot of the sample with a spectrophotometer (NanoDrop).

20 pmol of dephosphorylated *in vitro* transcribed RNA mixed with RNase-free ddH_2O to a final volume of 15 µl were incubated at 95 °C for 1 min and chilled on ice. Then 2 µl 10x PNK (T4 polynucleotid kinase, Thermo Scientific) buffer A, 1 µl PNK (Thermo Scientific, #EK0031) and 2 µl [γ-^{32}P]-ATP (6000 Ci/mmol, 10 µCi/µl, Hartmann Analytic GmbH, Germany, #SRP-501) were added and incubated for 1 h at 37 °C. Labelling reaction was cleaned up via a G-25 column (illustra MicroSpin G-25 column, GE Healthcare) according to manufacturer's instructions and mixed with 20 µl 2x RNA gel loading dye. Sample was incubated at 95 °C for 1 min, chilled on ice and loaded in two lanes of a denaturing 5 or 6 % PAA gel with 7 M urea and deep wells. Electrophoresis proceeded at 300 V for 1h and 45 min in 1x TBE buffer at room temperature. Subsequently, bands of wanted RNA (entire transcript) were excised from gel, transferred into a reaction tube and 750 µl RNA elution buffer (0.1 M sodium acetate, 0.1 % (w/v) SDS, 10 mM EDTA) were added. RNA was eluted from gel by incubation at 8 °C and 850 rpm overnight in a cooling block with shaking function. Then reaction was briefly centrifuged, about 700 µl supernatant was transferred into a PLG heavy tube prefilled with 750 µl phenol/chloroform/isoamylalcohol solution (25:24:1) and vigorously shaken. After centrifugation for 12 min at 13,000 rpm and 15 °C upper aqueous phase was transferred equally into 2 fresh tubes and RNA was precipitated with 1 ml absolute ethanol for at least 3 h or overnight at -20 °C. RNA was spun down for 30 min at 13,000 rpm and 4 °C, pellet was air-dried and then redissolved in 25-50 µl RNase-free ddH_2O. When the signal detected with the Geiger counter was less than 1,000 counts per second, the RNA was regarded as weakly labelled and double the quantity was used for the in-line probing reaction. RNA concentration was determined by measuring an undiluted aliquot of the sample with a spectrophotometer (NanoDrop) and a 0.2 pmol/µl stock was set up with RNase-free ddH_2O.

For the in-line probing reaction 0.2 pmol [γ-^{32}P]-ATP labelled RNA was mixed with RNase-free ddH_2O to a final volume of 5 µl and incubated at 95 °C for 1 min, then chilled on ice for 5 min and subsequently

incubated at 37 °C for 15 min. After addition of 5 µl 2x in-line reaction buffer (100 mM Tris-HCl (pH 8.3), 40 mM $MgCl_2$, 200 mM KCl) reaction was incubated for 40 h at room temperature. Then 10 µl 2x colourless gel loading solution (10 M urea, 1.5 mM EDTA (pH 8.0)) were added to in-line probing reaction and kept on ice until loading. For the ladder and control reactions 0.2 pmol [γ-^{32}P]-ATP labelled RNA was used. To prepare the T1 ladder 0.2 pmol [γ-^{32}P]-ATP labelled RNA was mixed with 8 µl RNA sequencing buffer (Ambion), incubated for 1 min at 95 °C and immediately chilled on ice for 2 min. Then 1 µl RNase T1 (0.1 U/µl, 1:10 diultion of stock was set up with RNase-free ddH_2O, Ambion, #AM2283) was added, reaction incubated for 5 min at 37 °C and stopped by adding 12 µl 2x RNA gel loading dye. Ladder was kept on ice until loading.

To prepare the OH ladder 0.2 pmol [γ-^{32}P]-ATP labelled RNA was mixed with 9 µl alkaline hydrolysis buffer (Ambion), incubated for 5 min at 95 °C and reaction stopped by adding 12 µl 2x RNA gel loading dye. Ladder was kept on ice until loading. To prepare the control (RNA integrity) reaction 0.2 pmol [γ-^{32}P]-ATP labelled RNA was mixed with 9 µl RNase-free ddH_2O, 12 µl 2x RNA gel loading dye was added and control reaction was kept on ice until loading. 10 µl of the in-line, ladder and control reactions were loaded per lane of a denaturing PAA sequencing gel with 7 M urea, respectively. 4, 5, 6 and 10 % PAA gels were used to obtain satisfying size separation of the complete RNA molecule. One gel consisted of 50 ml PAA premix (4, 5, 6 or 10 % of a 40 % PAA solution (19:1 acrylamide/bis-acrylamide), 7 M urea, 10 % 10x TBE buffer and purified ('VE') water), 500 µl 10 % APS (ammonium persulfate) and 50 µl TEMED (tetramethylethylendiamin). Gel was pre-warmed to 40-45 °C by running in 1x TBE buffer for 1 h at 40 W. Then samples were loaded and electrophoresis proceeded at 40 W for 1 to 4 h (depending on PAA percentage and region of the RNA molecule supposed to be well separated) in 1x TBE buffer at room temperature. Ensuing gel was transferred onto blotting paper (Whatman), dried for 45 min at 80 °C with a vacuum applied and exposed to a storage phosphor screen for 1 to 2 days. Screen was read out using the Typhoon™ FLA 7000 laser scanner (GE Healthcare).

Supplementary Tables

Table S1. List of additional plasmids used in this work. In case of shuttle vectors selection is detailed for Gram-negative and Gram-positive bacteria.

Plasmid name	Properties	Size (bp)	Selection	Reference
pGEM®-T Easy Vector System I	ori pBR322, 3´-T overhang, system for the cloning of PCR products, blue/white screening	3,015	Gram (-): Amp (100)	Promega (#A1360)
pCR-XL-2-TOPO™	ori pUC, *lac* promoter, (linearized and topoisomerase 1-activated)	3,960	Gram (-): Amp (100), Kana (50)	ThermoFisher Scientific Inc. (K8050-10, Invitrogen™)
pJC1-MetTBox-metleader-cl-pR-eYFP	initiator tRNA NWMN_tRNA23, *met* leader (T-Box), *metI* 30 bp, cl repressor with LVA-fast degradation tag, *eyfp* under pR promoter, kana, *orfX*, *repA*, *per*	9,552	Gram (-): Kana (50) Gram (+): Kana (25)	R. Mahr (unpublished)

Table S2. List of additional oligonucleotides used in this work.

Purpose	Template	Name	Sequence
General primers			
PCR and sequencing pGEM-T-Easy, pCR-XL-2-TOPO		M13-Fow	GTAAAACGACGGCCAG
		M13-Rev	CAGGAAACAGCTATGAC
PCR and sequencing pBASE constructs		pBASE_MCS_F	GATGCCTCAAGCTAGAGAGTCATTACC
		pBASE_MCS_R	CCATGTATTCACTACTTCTTTCAAACTCTCTC
pEB01 constructs		pCN33_seq_for	CTGATTCTGTGGATAACCGTATTACC
		pCN47_seq_rv	CTCGAAAATAATAGAGGGAAAATCAGT

Purpose	Template	Name	Sequence
Cloning *met* leader mutants			
Universal			
Binds within ampicillin resistance cassette	Various, see below	FW107	GGCGAGTTACATGATCCCCCATG TTGT
Binds within ampicillin resistance cassette	"	FW108	GGGGGATCATGTAACTCGCCTTG AT
Binds within kanamycin resistance cassette	"	FW26	GCGATCGCGTATTTCGTCTCGCT CAGGCGCAAT
Binds within kanamycin resistance cassette	"	FW27	GCCTGAGCGAGACGAAATACGCG ATCGCTGTTA
Construct-specific (universal primers listed again for clarity)			
pBASE_metleader+ 1kb flanking (pFW001)	Newman	FW099	AGTATAAGATCTCGCTCAATGCGT AAATGCAAAGTT
		FW100	GTGATAAGATCTCCTACGATTTAA TTGTTCAA TTA
pBASE_ΔAntiTer&Ter	pFW001	DSt003	CTTATAGGAGGGTCTTAATATGAA GGATACA
		DSt004	GTTATTCCATCGCTGAAATAACCT TATTCAGTA
1.7 kb fragment pBASE_Ter_destab	pFW001	Sa_Ter4 01_out1	CATTCCTTGACGATTCAACGACTC TTTTTTTATTTTTTC
		FW107	GGCGAGTTACATGATCCCCCATG TTGT
7.1 kb fragment pBASE_Ter_destab	pFW001	Sa_Ter4 27_out2	AAAGAGTCGTTGAATCGTCAAGG AATGAATCGGATAAAAAG
		FW108	GGGGGATCATGTAACTCGCCTTG AT
800 bp 3' fragment *met* leader Ter_mutated_1	pBASE_Ter_destab	FW099	AGTATAAGATCTCGCTCAATGCGT AAATGCAAAGTT
		FW166	GGGATTCATTCCCTGACGAGGCA ACGATCCTTTTTTT
1.4 kb 5' fragment *met* leader Ter_mutated_1	pBASE_Ter_destab	FW100	GTGATAAGATCTCCTACGATTTAA TTGTTCAATTA
		FW165	CGTCAGGGAATGAATCCCAGAAA AAGCAACAATCCTTATGTT

Purpose	**Template**	**Name**	**Sequence**
2.2 kb overlap PCR *met* leader Ter_mutated_1 fragment	800 bp 3' fragment *met* leader Ter_mutated_1 and 1.4 kb 5' fragment *met* leader Ter_mutated_1	FW099	AGTATAAGATCTCGCTCAATGCGTAAATGCAAAGTT
		FW100	GTGATAAGATCTCCTACGATTTAATTGTTCAA TTA
1.7 kb fragment pBASE_Ter_mutated_2	pFW001	FW204	GCTTTTTATCCGATTCATTCGGGTACGAAGCACGGAA
		FW107	GGCGAGTTACATGATCCCCCATGTTGT
7.1 kb fragment pBASE_Ter_mutated_2	pFW001	FW203	GCTTCGTACCCGAATGAATCGGATAAAAAGCACGAAA
		FW108	GGGGGATCATGTAACTCGCCTTGAT
1.7 kb fragment pBASE_Ter_mutated_3	pFW001	FW206	GCTTTTTATCCGATTCATTCGGGTACGAGGCACGGAA
		FW107	GGCGAGTTACATGATCCCCCATGTTGT
7.1 kb fragment pBASE_Ter_mutated_3	pFW001	FW205	GCCTCGTACCCGAATGAATCGGATAAAAAGCACGAAA
		FW108	GGGGGATCATGTAACTCGCCTTGAT
1.7 kb fragment pBASE_Ter_mutated_4	pFW001	FW208	TGGCTTTTTATCCGATTCATTCGGGTACGAAGCCAGGAA
		FW107	GGCGAGTTACATGATCCCCCATGTTGT
7.1 kb fragment pBASE_Ter_mutated_4	pFW001	FW207	TGGCTTCGTACCCGAATGAATCGGATAAAAAGCCAGAAA
		FW108	GGGGGATCATGTAACTCGCCTTGAT

(Continued on next page.)

Purpose	Template	Name	Sequence
Cloning pEB01-met leader-metI			
met leader-*metI* sequence (from -35 until nt 215 of *metI*)	Newman	FW198	AATTATGGATCCCCACTTGATGTAGCGAATGATGCAATA
		FW199	AATTATGGATCCGTTGACAAATCTTTTTTTACTCTGTAA
Cloning pJC1_tRNAi_deletion			
Cloning 4.5 kb fragment pJC1_tRNAi_deletion	pJC1-MetTBox-metleader-cl-pR-eYFP	FW19	GGGGCGTACGAAAACGCTACGTTCCAAAATGTGG
		FW26	GCGATCGCGTATTTCGTCTCGCTCAGGCGCAAT
Cloning 5.2 kb fragment pJC1_tRNAi_deletion	pJC1-MetTBox-metleader-cl-pR-eYFP	FW27	GCCTGAGCGAGACGAAATACGCGATCGCTGTTA
		FW28	GGTGGTTCAAATCCGCCTCCCGCAA CGTACGGTTTTT

Table S3. Sequences of T7 transcripts. Point mutations introduced are highlighted in red.

Name	Sequence (5'→3')
met leader	GGGUCUUAUAACAGUUUAAUGAAACGUAAACACAAUAAAGAGGAAAGUAAAACACACCCUGCUUAUACAGAGAGUCUUUAGUAGCUGAGAGAAGAUUUUGAAAGCGUGUUUGAAAAUGGCCUUGGAGUGUUGAUGCCAAUAUGAGGUGUCUACGGGUUCGCCCGUUAUAGCGAUACAGUAUUAACAUUGAUGUUAAAUGGCGUACUGGAUUCUUUACGCACGAUUUUUUGUUAAUAAGUAUGGGAUAGCACAUUACUAUAUCCUUACUUACUGACUUUAAUUGUGAUAAUUGUUCAGUAAGCAUAUUUACUUUUAAUGCGUACUGAAUAAGGUUAUUUCAGCGAUGGAAUAACAAAUAAAGGUGGUACCGCGAAACAUAAGCUUUCGUCCUUUUUAUCCGAUUCAUUCGGGUACGAAGGACGGAAGCUUUUUUUAUUUUUUC
short *met* leader	GGGAUUCUUUACGCACGAUUUUUUGUUAAUAAGUAUGGGAUAGCACAUUACUAUAUCCUUACUUACUGACUUUAAUUGUGAUAAUUGUUCAGUAAGCAUAUUUACUUUUAAUGCGUACUGAAUAAGGUUAUUUCAGCGAUGGAAUAACAAAUAAAGGUGGUACCGCGAAACAUAAGCUUUCGUCCUUUUUAUCCGAUUCAUUCGGGUACGAAGGACGGAAGCUUUUUUUAUUUUUUC

Name	Sequence (5'→3')
short *met* leader Ter_destab	GGGAUUCUUUACGCACGAUUUUUUGUUAAUAAGUAUGGGAUAGCACAUUA CUAUAUCCUUACUUACUGACUUUAAUUGUGAUAAUUGUUCAGUAAGCAUAU UUACUUUUAAUGCGUACUGAAUAAGGUUAUUUCAGCGAUGGAAUAACAAAU AAAGGUGGUACCGCGAAACAUAAGCUUUCGUCCUUUUUAUCCGAUUCAUU CCUUGACGAUUCAACGACUCUUUUUUUAUUUUUUC
short *met* leader Ter_mutated_1	GGGAUUCUUUACGCACGAUUUUUUGUUAAUAAGUAUGGGAUAGCACAUUA CUAUAUCCUUACUUACUGACUUUAAUUGUGAUAAUUGUUCAGUAAGCAUAU UUACUUUUAAUGCGUACUGAAUAAGGUUAUUUCAGCGAUGGAAUAACAAAU AAAGGUGGUACCGCGAAACAUAAGGAUUGUUGCUUUUUCUGGGAUUCAUU CCCUGACGAGGCAACGAUCCUUUUUUUAUUUUUUC
short *met* leader Ter_mutated_2	GGGAUUCUUUACGCACGAUUUUUUGUUAAUAAGUAUGGGAUAGCACAUUA CUAUAUCCUUACUUACUGACUUUAAUUGUGAUAAUUGUUCAGUAAGCAUAU UUACUUUUAAUGCGUACUGAAUAAGGUUAUUUCAGCGAUGGAAUAACAAAU AAAGGUGGUACCGCGAAACAUAAGCUUUCGUGCUUUUUAUCCGAUUCAUU CGGGUACGAAGCACGGAAGCUUUUUUUAUUUUUUC
short *met* leader Ter_mutated_3	GGGAUUCUUUACGCACGAUUUUUUGUUAAUAAGUAUGGGAUAGCACAUUA CUAUAUCCUUACUUACUGACUUUAAUUGUGAUAAUUGUUCAGUAAGCAUAU UUACUUUUAAUGCGUACUGAAUAAGGUUAUUUCAGCGAUGGAAUAACAAAU AAAGGUGGUACCGCGAAACAUAAGCUUUCGUGCUUUUUAUCCGAUUCAUU CGGGUACGAGGCACGGAAGCUUUUUUUAUUUUUUC
short *met* leader Ter_mutated_4	GGGAUUCUUUACGCACGAUUUUUUGUUAAUAAGUAUGGGAUAGCACAUUA CUAUAUCCUUACUUACUGACUUUAAUUGUGAUAAUUGUUCAGUAAGCAUAU UUACUUUUAAUGCGUACUGAAUAAGGUUAUUUCAGCGAUGGAAUAACAAAU AAAGGUGGUACCGCGAAACAUAAGCUUUCUGGCUUUUUAUCCGAUUCAUU CGGGUACGAAGCCAGGAAGCUUUUUUUAUUUUUUC

Table S4. 5' RACE data obtained with *metI*-specific primer (FW200) for cDNA synthesis.

Gene/region	Position (nt)	% 5' ends
met leader	418	50
	-11 (*metI* 5' UTR)	4.2
metI	201-300	8.3
metI	301-400	29.2
metI	401-500	8.3
∑		**100 (n = 24)**

SI References

1. Nozaki,S. and Niki,H. (2019) Exonuclease III (XthA) Enforces *In Vivo* DNA Cloning of *Escherichia coli* To Create Cohesive Ends. *Journal of Bacteriology*, **201**, e00660-18.
2. Regulski,E.E. and Breaker,R.R. (2008) In-line probing analysis of riboswitches. *Methods Mol. Biol.*, **419**, 53–67.