Dual-acting riboswitch control of translation initiation and mRNA decay

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Supporting Information

SI Materials and Methods

Strains and plasmids

Strains used in all experiments were derivatives of *E. coli* MG1655 (see Table S1). The *E. coli* strain DH5α was used for routine cloning procedures. The deletion/insertion mutations in *lysC* were performed by a method described previously (1). Chloramphenicol resistance cassette was amplified from strain EM1047 by PCR with sequences homologous of *lysC* (EM1742-EM1743). The resulting PCR product was electroporated into EM1237 after induction of λ*red* system (1) and selecting for chloramphenicol resistance (30 μg/ml). The recombinant product was verified by PCR. Then, the resulting plasmid pBAD-*lysC* (EM1339-EM1729) (MPC70) and pBAD-*lysC*-ΔSite1 (MPC66) (PCR1: EM1729-EM1684, PCR2: EM1683-EM1339; PCR1 and PCR2 serve as DNA template for PCR3 which was amplified using primers EM1729-EM1339) were digested with EcoR1 and MSC1, cloned in PNM12 (pBAD24 derivative) and selected by ampicillin at 50 μg/ml. Strains constructed by phage P1 transduction were selected with appropriate antibiotic-resistant markers.

The *lysC* translational and transcriptional *lacZ* fusions, containing the riboswitch domain with the first 57 nucleotides of *lysC* ORF, were constructed with the PM1205 strain (2). Briefly, cells were heat-shocked to activate the λ*red* recombination system as described previously (2). Then, cells were electroporated with a PCR product, which produced a chromosomal insertion by homologous recombination. The PCR product for the *lysC-lacZ* translational fusion (MPC7) was prepared with oligonucleotides EM845 and EM908 from the genomic DNA. The *lysC-lacZ* transcriptional fusion (MPC16) harboring a translation stop in the *lysC* ORF was constructed with a four-step PCR amplification. The PCR1 (EM908-EM844) was performed from genomic DNA to serve as a template for the PCR2 (EM991-EM992) and PCR3 (EM993-EM994). Then the PCR4 was prepared with the products of PCR2 and PCR3 with the oligonucleotides EM991 and EM994 (see Table S3). The The transcriptional *lacZ* fusions of the ON and OFF mutants of the *lysC* riboswitch were also prepared by four-step PCR amplifications (see Table S3). The *lysC* promoter fusions to *lacZ* $P_{1lysC}P_{2lysC}$-*lacZ* (MPC29) and P_{2lysC}-*lacZ* (MPC30) were constructed with a three-step PCR amplification. Briefly, PCR1 and PCR2 were performed from the genomic DNA and the PCR3 was performed with the products of PCR1 and PCR2 (see Table S3). To construct other mutants, all PCR were performed with three-step PCR amplifications from the wild type *lysC-lacZ* fusions. Then, the PCR1 and PCR2 served as DNA template for the PCR3. The *thiM lacZ* fusions were constructed as described for the *lysC* fusion (see Table S3). All obtained *lacZ* fusions were sequenced to ensure the integrity of the constructs.

3' rapid amplification of cDNA ends (RACE)

Total RNA was extracted with the hot phenol method (3) and 20 μg of RNA was treated with 8 U of Turbo DNase (Ambion) for 30 min at 37°C. RNA was extracted by phenol-chloroform-isoamyl and precipitated with ethanol. Then, the RNA was polyadenylated with 4 U of Poly-(A) Polymerase following the manufacturer's protocol (Ambion). The RNA was extracted again with phenol-chloroform-isoamyl and precipitated with ethanol. A reverse transcription was performed with SuperScript II (Invitrogen) and the poly-dT oligonucleotide EM1363. The resulting cDNA was used as template in a PCR reaction using oligonucleotides EM656-EM1364. The PCR

product was cloned using the Zero Blunt TOPO kit (Invitrogen) and sequenced. At least three independent experiments were carried out.

***In vitro* RNA synthesis**

RNA was transcribed *in vitro* with purified T7 RNA polymerase from PCR product (see Table S4 for oligonucleotides used to generate DNA templates). The reaction was performed in transcription buffer (40 mM Tris-HCl pH 8.0, 0.01% triton, 20 mM $MgCl_2$, 2 mM spermidine), 2.5 mM NTPs (ATP, CTP, GTP and UTP), 1 mM DTT, 10 pmol DNA template and 10 μg of T7 polymerase. The mixture was incubated for 3h at 37°C. The synthesized RNA was extracted by phenol-chlorom-isoamyl alcohol, precipitated with ethanol and purified using a 4% polyacrylamide 8M urea gel.

Radiolabeled probes used for Northern blot analysis were transcribed using a transcription buffer (400 mM Hepes-KOH pH 7.5, 120 mM $MgCl_2$, 200 mM DTT, 10 mM spermidine), 400 μM NTPs (ATP, CTP and GTP), 10 μM UTP, 3 μl of α-^{32}P-UTP (3000 Ci/mmol), 20 U RNase OUT (Invitrogen) and 1 μg of DNA template. After 4h of incubation at 37°C, 2 U of Turbo DNase (Ambion) was added for 15 min. The labeled RNA was extracted by phenol-chloroform-isoamyl and purified with a G-50 Sephadex column to remove free nucleotides. See Table S3 for oligonucleotides used to generate DNA templates.

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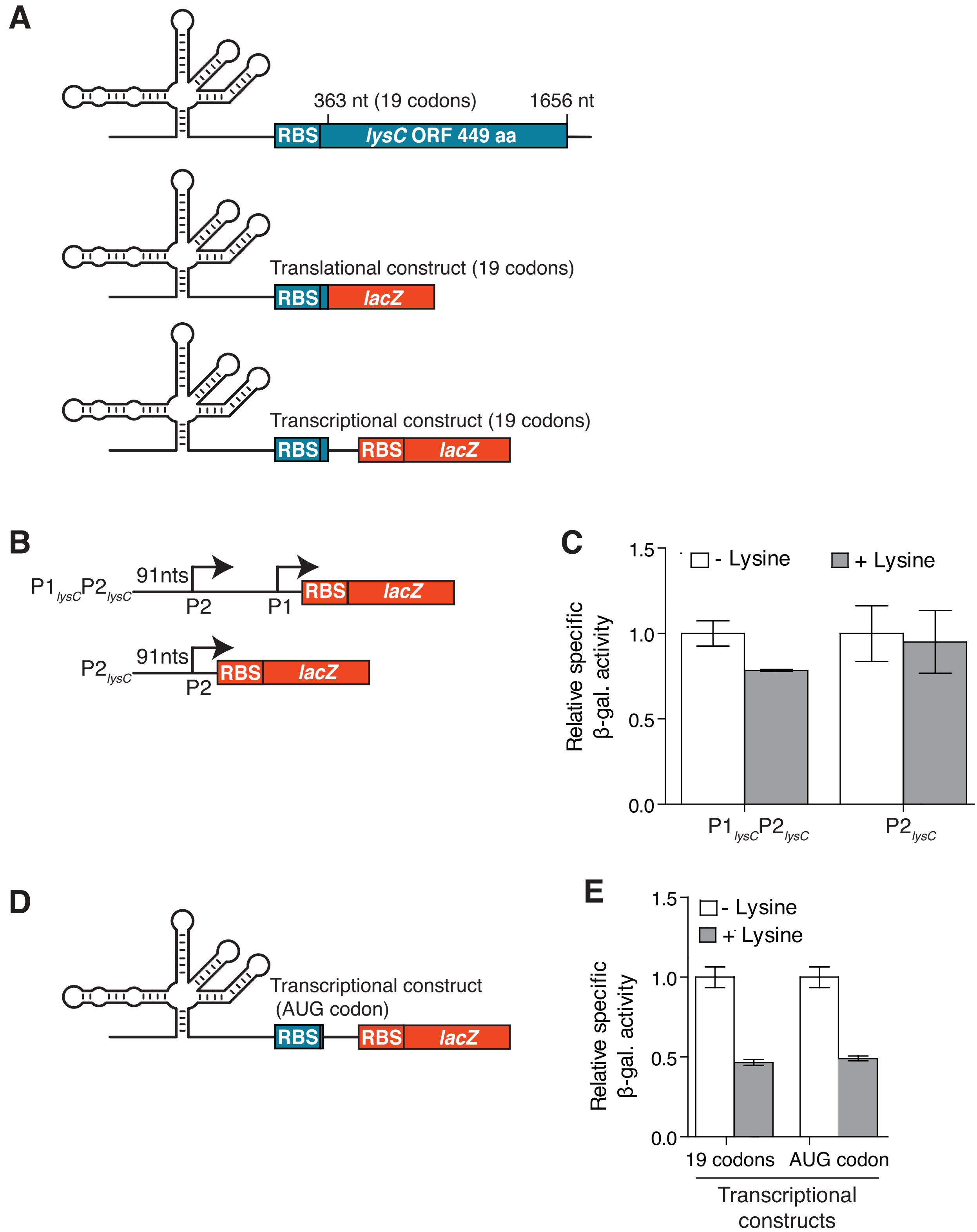


Fig. S1. Influence of lysine on the regulatory activity of the *lysC* riboswitch.

(*A*) Schematic of the wild-type *lysC* mRNA (top) compared with translational (middle) and transcriptional (bottom) fusions. Both fusions are expressed from an arabinose inducible promoter (P_{BAD}) that is fused to the *lysC* riboswitch domain encompassing positions +1 to +363, relatively to the P1 transcription start site (4), which therefore contains 57 nucleotides (19 codons) of the *lysC* ORF. In the case of the transcriptional fusion, because translation initiation of *lacZ* is performed from its own RBS which is not modulated by the riboswitch upon lysine binding, the expression of *lacZ* is thus strictly dependent on the level of *lysC* mRNA.

(*B*) Schematic representation of constructs used in β-galactosidase assays to monitor the expression of the *lysC* promoter region. Constructs $P1_{lysC}P2_{lysC}$ and $P2_{lysC}$ contained regions encompassing positions -80 to +105 and -80 to -12, respectively, relatively to the P2 transcription start site (4, 5). While the fused reporter *lacZ* gene in $P1_{lysC}P2_{lysC}$ construct starts at position +105 (13 nucleotides after the promoter P1), the *lacZ* gene starts at position +12 after the promoter P2 in the $P2_{lysC}$ construct. The identification of both promoters is based on previous studies (4, 5).

(*C*) β-galactosidase assays of $P1_{lysC}P2_{lysC}$ and $P2_{lysC}$ *lacZ* fusions. Enzymatic activities were measured in absence and presence of 10 μg/ml lysine. Values were normalized to enzymatic activity obtained in absence of lysine. The average values of three independent experiments with standard deviations are shown. While the expression of the $P2_{lysC}$ promoter is not affected by the presence of lysine, the expression of the $P1_{lysC}P2_{lysC}$ promoter is decreased by ~22% with lysine suggesting that the P1 promoter is modulated to some degree by lysine. This extent of promoter regulation is significantly less than what was observed using the riboswitch domain (~70% decrease, Fig. 1*B* and *C*).

(*D*) Schematic representation of the transcriptional *lysC*-*lacZ* fusion construct containing only the AUG start codon of *lysC* ORF.

(*E*) β-galactosidase assays of transcriptional *lysC*-*lacZ* fusions containing 19 codons or only the AUG start codon of *lysC* ORF. Enzymatic activities were measured in absence and presence of 10 μg/ml lysine. Values were normalized to the activity obtained in absence of lysine.

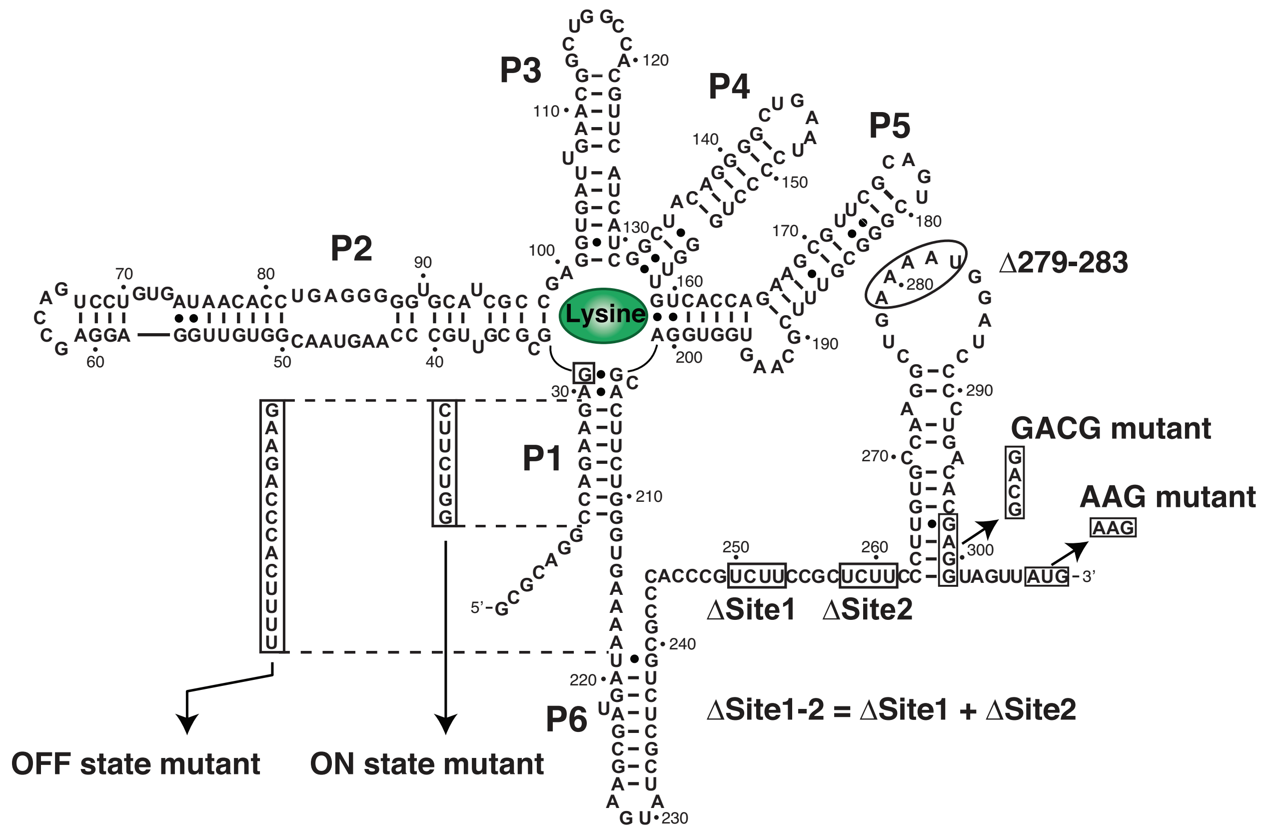
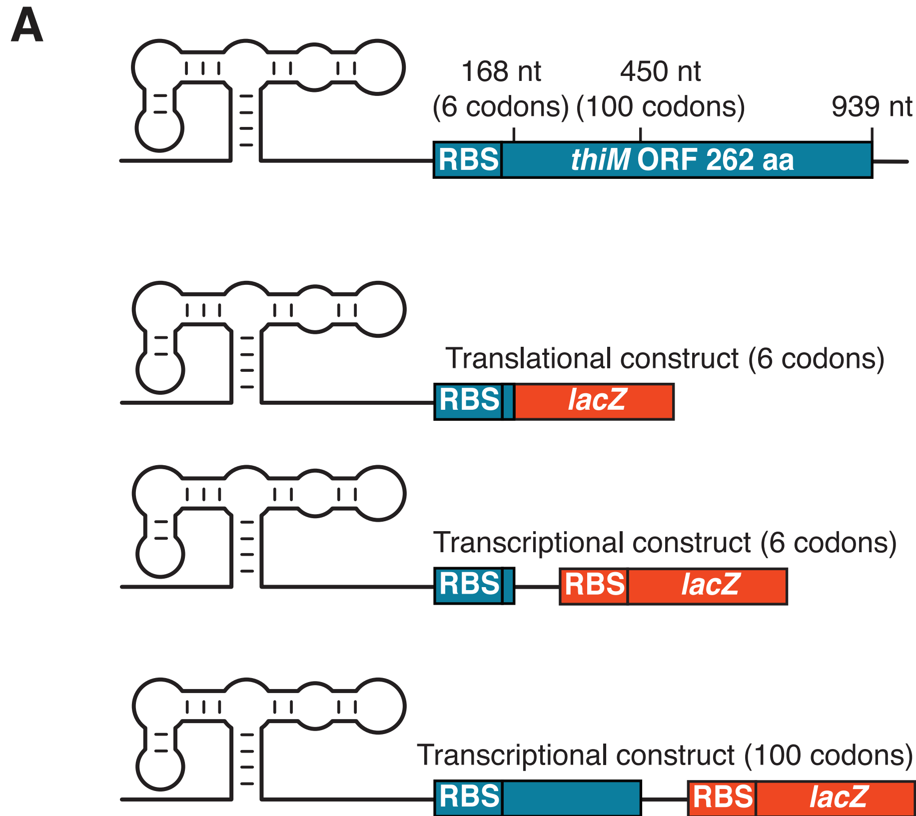


Fig. S2. Secondary structure of the wild-type lysine riboswitch and mutants used in this study.

The secondary structure of the *lysC* riboswitch is shown in the predicted ligand-bound conformation (OFF state). Based on the riboswitch regulation model (Fig. 1*A*), ligand binding to the riboswitch induces the formation of the P1 stem, which is important for the adoption of the OFF state (6). Mutations introduced in the P1 stem to favor the ON or OFF state are shown. The G31C mutation previously shown to inhibit ligand binding is shown in a box at position 31 (6-8). The ribosome binding site (RBS; positions 298-301) and the AUG start codon (positions 307-309) are indicated. Deleted sequences to generate mutants ΔSite1, ΔSite2 and Δ279-283 are shown. The ΔSite1-2 mutant was generated by incorporating both ΔSite1 and ΔSite2 mutations. The numbering and nomenclature of the *lysC* riboswitch are based from previous studies (7).



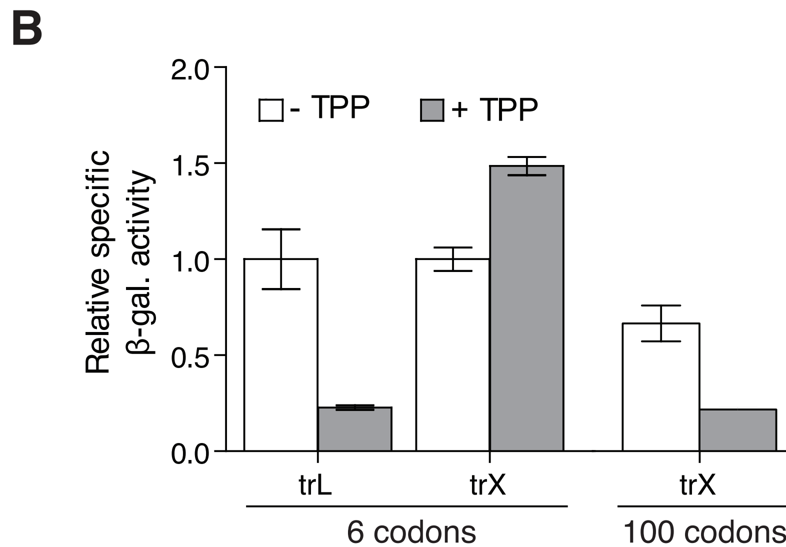


Fig. S3. Genetic control of the *E. coli thiM* riboswitch.

(*A*) Schematic description of translational and transcriptional β-galactosidase constructs used to monitor *thiM* expression. These constructs are expressed from an arabinose-inducible promoter that is fused to the *thiM* riboswitch domain encompassing various lengths of *thiM* ORF (6 or 100 codons). In the case of the transcriptional fusion, the expression of *lacZ* strictly depends on the level of *thiM* mRNA.

(*B*) β-galactosidase assays of translational (trL: ThiM-LacZ) and transcriptional (trX: *thiM-lacZ)* fusions. Cells were grown in M63 medium with 0.2% glycerol to mid-log phase and induced with arabinose (0.1% final). Enzymatic activities were measured in absence and presence of 500 μg/ml TPP. Values were normalized to enzymatic activity obtained in absence of TPP (6 codons). The average values of three independent experiments with standard deviations are shown.

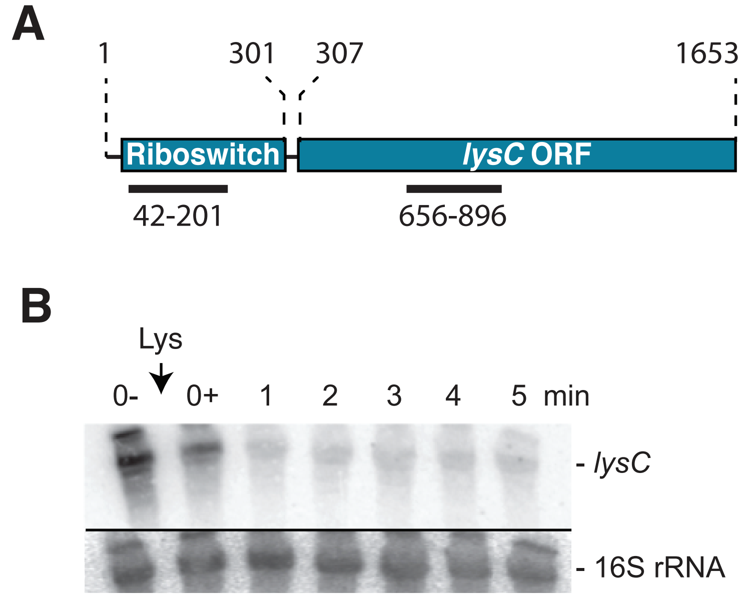


Fig. S4. Northern blot analysis of the *lysC* mRNA.

(*A*) Localization of RNA probes used for Northern blots. The lysine riboswitch (positions 1-301) and the *lysC* ORF (positions 307-1653) are delimited by dotted lines. RNA probes designed to target the riboswitch (positions 42-201) and ORF domains (positions 656-896) are indicated by black horizontal lines.

(*B*) Northern blot analysis of the *lysC* mRNA using a probe targeting the ORF domain. Wild-type *E. coli* strain MG1655 was grown to mid-log phase in M63 minimal medium with 0.2% glucose at 37°C and total RNA was extracted at the indicated times immediately before (0-) and after (0+) addition of lysine (10 μg/ml). The probe was designed to detect the ORF region (positions 656-896) of the *lysC* mRNA. The 16S rRNA was used as a loading control.

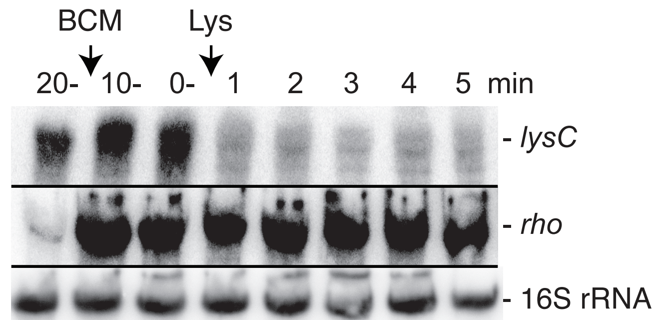


Fig. S5. Investigation of the Rho transcription factor in the lysine-dependent *lysC* mRNA regulation.

Northern blot analysis of the *lysC* mRNA in presence of bicyclomycin. Wild-type *E. coli* strain MG1655 was grown to mid-log phase in M63 minimal medium with 0.2% glucose at 37°C and total RNA was extracted at the indicated times. Bicyclomycin (BCM, 20 μg/ml) was added 20 min before the addition of lysine (10 μg/ml). Negative time points are relativized to the addition of lysine. The addition of BCM does not prevent the lysine-induced mRNA decrease of *lysC*, consistent with Rho not being involved in the regulation of *lysC*. As a control, a strong BCM effect on the level *rho* mRNA is observed, in agreement with Rho autoregulation mechanism. Probes were designed to detect the *lysC* riboswitch region (positions 42 to 201; Fig. S4*A*) and positions 256 to 455 of *rho* mRNA (relatively to the transcription start site). The 16S rRNA was used as a loading control.

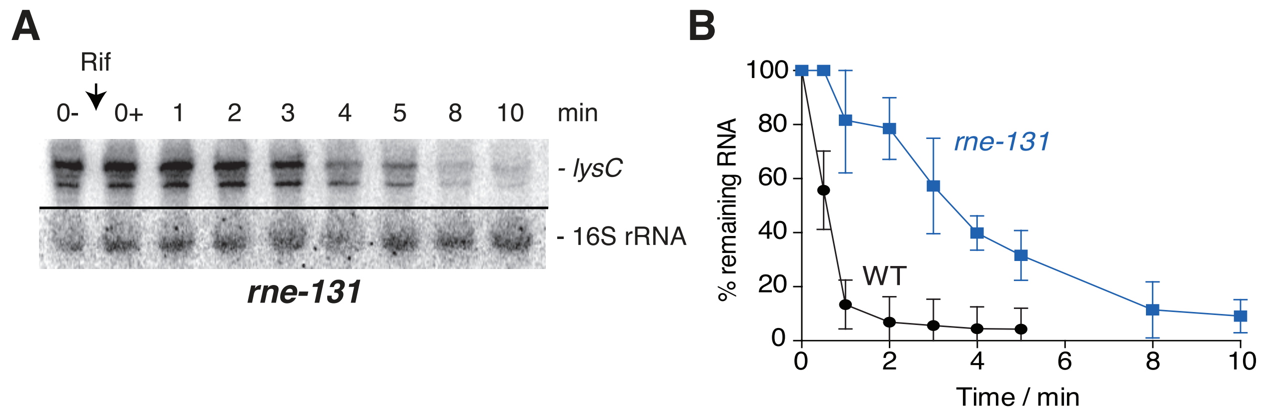


Fig. S6. Determination of the *lysC* mRNA stability in the context of the *rne-131* strain.

(*A*) Northern blot analysis of the *lysC* mRNA level in the context of the *rne-131* strain. The *E. coli* strain *rne-131* was grown to mid-log phase in M63 minimal medium with 0.2% glucose at 37°C and total RNA was isolated at the indicated times immediately before (0-) and after (0+) addition of rifampicin (250 μg/ml). The 16S rRNA was used as a loading control.

(*B*) Quantification analysis of Northern blots shown in panel (*A*) and Fig. 1*F*. The average values of three independent experiments with standard deviations are shown.

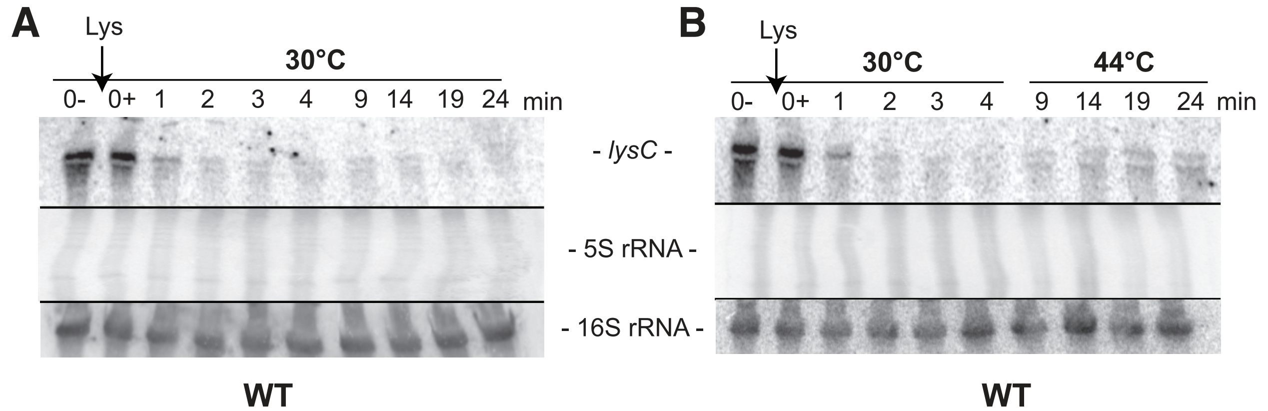


Fig. S7. Northern blot analysis of the *lysC* mRNA at 30°C and 44°C.

(*A*) Northern blot analysis of the *lysC* mRNA in the context of wild-type (WT) *E. coli* strain grown at 30°C. The strain was grown to mid-log phase in M63 glucose minimal medium with 0.2% glucose at 30°C. Total RNA was isolated at the indicated times before (0-) and after (0+) addition of lysine (10 μg/ml). The 16S rRNA was used as a loading control.

(*B*) Northern blots analysis of the *lysC* mRNA in the context of the wild-type (WT) *E. coli* strain grown at 30°C followed by a temperature shift at 44˚C. Bacterial growth culture and RNA extractions were performed as described in (*A*). Cells were incubated at 30˚C from 0 to 4 min and at 44˚C from 4 to 24 min. The 16S rRNA was used as a loading control. The absence of 5S rRNA intermediates suggests that RNase E is still active in these conditions at 44°C (9), which is in contrast to what was obtained in the *rne-3071* (RNase E(TS)) strain (Fig. 2*F*).

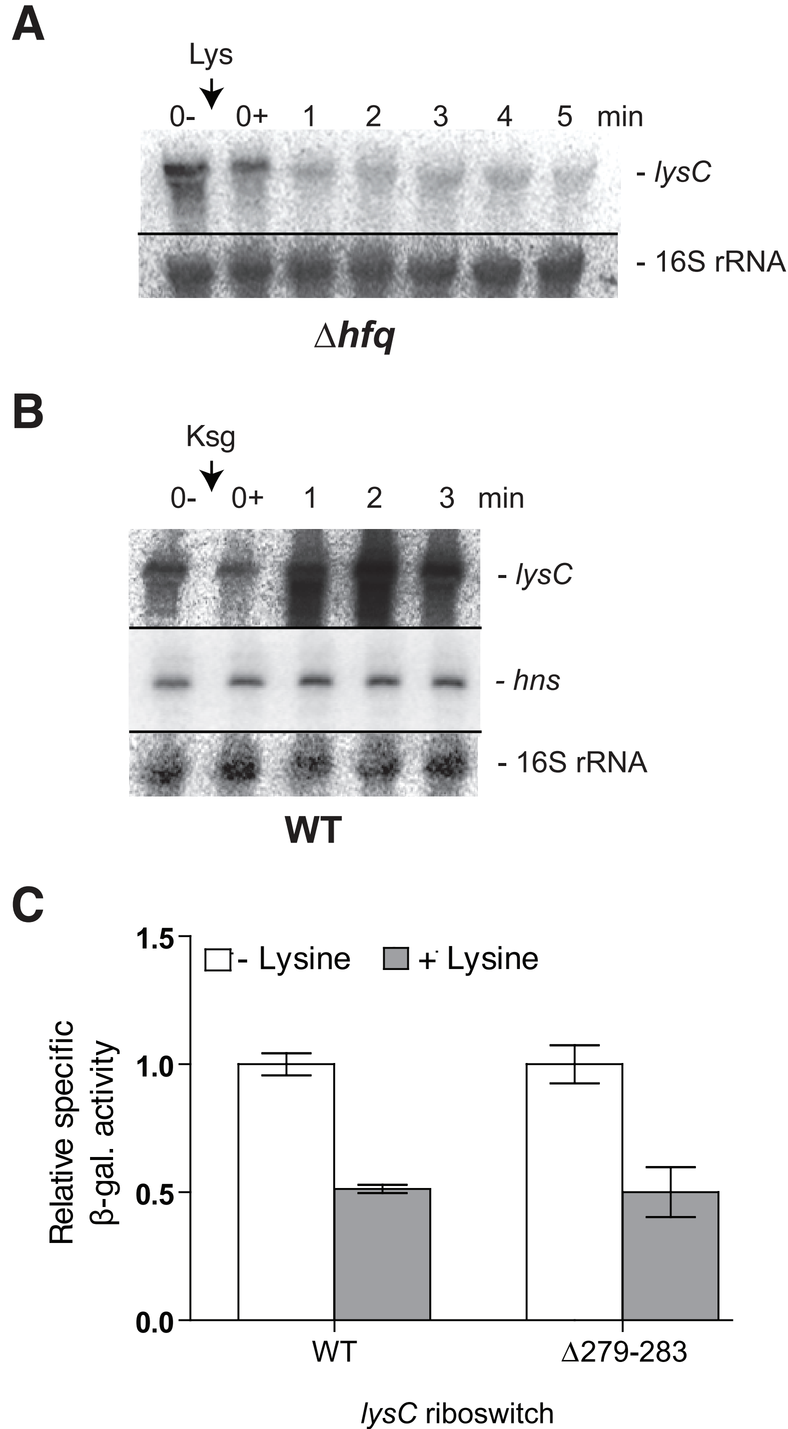


Fig. S8. Investigation of various *cis*- and *trans*-acting factors potentially involved in *lysC* mechanism.

(*A*) Northern blot analysis of the *lysC* mRNA in *hfq* mutant strain (10) grown at 37°C to mid-log phase in M63 0.2% glucose minimal media. Total RNA was isolated at the indicated times before (0-) and after (0+) addition of lysine (10 μg/ml). The 16S rRNA was used as a loading control.

(*B*) Northern blot analysis of the *lysC* mRNA grown in M63 minimal medium with 0.2% glucose at 37°C to mid-log phase as a function of kasugamycin (500 μg/ml). Total RNA was isolated at the indicated times before (0-) and after (0+) addition of kasugamycin (Ksg), which specifically blocks ribosomes at the RBS (11). These results are consistent with the idea that ribosome stalling at the RBS of the *lysC* mRNA prevents RNase E cleavages in the riboswitch domain, resulting the in accumulation of the *lysC* mRNA at 1-3 min. The *hns* mRNA was used as a negative control. The 16S rRNA was used as a loading control.

(*C*) β-galactosidase assays of transcriptional *lysC-lacZ* fusions for the mutant Δ279-283 (see Fig. S2 for construct). Enzymatic activities were measured in absence and presence of 10 μg/ml lysine. Values were normalized to the enzymatic activity obtained for the WT in absence of lysine. The average values of three independent experiments with standard deviations are shown.

Table S1. Summary of strains or plasmids used in this study.

Strains	Relevant marker	References
EM1055	MG1655 Δ*lacZ* X174	(14)
EM1277	EM1055 *rne-3071*(Ts) *zce-726*::Tn*10*	(13)
EM1377	EM1055 *rne131 zce-726*::Tn*10*	(13)
EM1047	DH5α + pACYC184	Laboratory collection
PM1205	*lacI′*::P_{BAD}-*cat-sacB-lacZ*, mini λ tet^R	(2)
EM1237	DY330 [W3110 delta-lacU169 gal490 lambda-cI857 delta-(*cro-bioA*)]	(1)
HFS10	*supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21 recD1009* Δ*hfq::cat*	(10)
EM1264	EM1055 Δ*hfq::cat*	This study EM1055+P1 HFS10
MPC58	EM1237 Δ*lysC*::*cat*	This study
MPC59	EM1055 Δ*lysC*::*cat*	This study EM1055+P1 Δ*lysC*::*cat* (MPC58)
MPC66	EM1055Δ*lysC*::*cat* pBAD *lysC*-ΔSite1	This study
MPC70	EM1055Δ*lysC*::*cat* pBAD *lysC*-WT	This study
MPC7	PM1205 *lacI′*::P_{BAD}-LysC-LacZ	This study
MPC16	PM1205 *lacI′*::P_{BAD}-*lysC-lacZ*	This study
MPC19	PM1205 *lacI′*::P_{BAD}-LysC-mutON-LacZ	This study
MPC20	PM1205 *lacI′*::P_{BAD}-LysC-mutOFF-LacZ	This study
MPC24	PM1205 *lacI′*::P_{BAD}-*lysC*$_{AUG}$-*lacZ*	This study
MPC25	PM1205 *lacI′*::P_{BAD}-*lysC-mutON-lacZ*	This study
MPC26	PM1205 *lacI′*::P_{BAD}-*lysC-mutOFF-lacZ*	This study
MPC29	PM1205 *lacI′*::$P_{2lysC}P_{1lysC}$-*lacZ*	This study
MPC30	PM1205 *lacI′*::P_{2lysC}-*lacZ*	This study
MPC40	PM1205 *lacI′*::P_{BAD}-LysC-G31C-LacZ	This study
MPC41	PM1205 *lacI′*::P_{BAD}-*lysC-ΔSite1-2-lacZ*	This study
MPC42	PM1205 *lacI′*::P_{BAD}-LysC-ΔSite1-2-LacZ	This study
MPC43	PM1205 *lacI′*::P_{BAD}-*lysC-G31C-lacZ*	This study
MPC44	PM1205 *lacI′*::P_{BAD}-*lysC-mutAAG-lacZ*	This study
MPC45	PM1205 *lacI′*::P_{BAD}-*lysC-mutGACG-lacZ*	This study
MPC46	PM1205 *lacI′*::P_{BAD}-LysC-mutAAG-LacZ	This study
MPC47	PM1205 *lacI′*::P_{BAD}-LysC-mutGACG-LacZ	This study
MPC50	PM1205 *lacI′*::P_{BAD}-*lysC-ΔSite1-lacZ*	This study
MPC51	PM1205 *lacI′*::P_{BAD}-LysC-ΔSite2-LacZ	This study
MPC52	PM1205 *lacI′*::P_{BAD}-*lysC-ΔSite2-lacZ*	This study
MPC53	PM1205 *lacI′*::P_{BAD}-LysC-ΔSite1-LacZ	This study
TPP3	PM1205 *lacI′*::P_{BAD}-*thiM-lacZ*	This study
TPP4	PM1205 *lacI′*::P_{BAD}-ThiM-LacZ	This study
TPP5	PM1205 *lacI′*::P_{BAD}-*thiM*$_{300}$-*lacZ*	This study

Plasmids	Relevant marker	References
pNM12	pBAD24 derivative	(12)
pBAD *lysC*-ΔSite1	pBAD24 + *lysC*-ΔSite1 (arabinose inducible promoter)	This study
pBAD *lysC*-WT	pBAD24 + *lysC* (arabinose inducible promoter)	This study

Table S2. Summary of oligonucleotides used in this study.

Oligonucleotides	Sequence 5'-3'
EM168	TCACACTTTGCTATGCCATAGC
EM169	CTGCAGGTCGACTCTAGAGG
EM192	TAATACGACTCACTATAGGGAGATGCCTGGCAGTTCCCTACTC
EM193	TGCCTGGCGGCAGTAGCG
EM293	TAATACGACTCACTATAGGGAGACGCTTTACGCCCAGTAATTCC
EM294	CTCCTACGGGAGGCAGCAGT
EM411	ACATCCGTACTCTTCGTGCG
EM412	TGTAATACGACTCACTATAGGAGTCCAGGTTTTAGTTTCGCC
EM649	TGTAATACGACTCACTATAGTCCACCACTTGCGAAACGCC
EM656	CAAGTAACGGTGTTGGAGGA
EM674	CATTCAATGCCCCATTTGCG
EM675	TGTAATACGACTCACTATAGCCAAAAAGTTAAGGACGTGG
EM703	GATTTATCATCGCAACCAAAC
EM704	TGTAATACGACTCACTATAGCTGCCATAACGTGAAGAAGC
EM840	GAATCTGGTGTATATGGCGAGC
EM841	GGGGGATGTGCTGCAAGGC
EM844	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATAGCTGTTTCCTGT GTGAGGCGTCAAAATCAGCTACGCT
EM845	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCGTCAAAATCAGCT ACGCT
EM908	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGTACTACCTGCGCTAGC
EM991	ACCTGACGCTTTTTATCGCAAC
EM993	GATCCGGCATTTTAACTTTCTTTATCACACAGGAAACAGCTATGG
EM994	TAACGCCAGGGTTTTCCCAG
EM1023	TGTAATACGACTCACTATAGGTGAAAATAGTAGCGAAGTATCGC
EM1024	CATAACTACCTCGTGTCAGGG
EM1065	GGCGTCAAAATCAGCTACGC
EM1067	CGGGTAGCAAAACAGATCGAA
EM1069	TAAAGAAAGTTAAAATGCCGGATCGGCGTCAAAATCAGCTACGCT
EM1094	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATAGCTGTTTCCTGT GTGACATGGCTACCTCGTGTCAGGGGATC
EM1098	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATTTTTCACCCAGAAGAGG CGCGTTGC
EM1099	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGGTCTTCAGGCGCGTTG CCCAAGTAAC
EM1101	TGTAATACGACTCACTATAGGGTCTTCAGGCGCGTTGCCCAAGTAAC
EM1102	TAAAGAAAGTTAAAATGCCGGATCCATGGCTACCTCGTGTCAGGGGATC
EM1166	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATAGCTGTTTCCTGT GTGAGCGCAGGTAGTACATTTAT
EM1167	TAAAGAAAGTTAAAATGCCGGATCGCGCAGGTAGTACATTTAT

EM1168	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATAGCTGTTTCCTGT GTGAGGCGGTCAGTAGTTCAGC
EM1169	TAAAGAAAGTTAAAATGCCGGATCGGCGGTCAGTAGTTCAGC
EM1337	GCGCAGGCCAGAAGACGC
EM1338	GCGTCTTCTGGCCTGCGC
EM1339	ACAGTAGAGAGTTGCGATAAAAAGCGTCGATGGTGCCCTCAGTGAGCC
EM1363	GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTTT
EM1364	GGCCACGCGTCGACTAGTAC
EM1372	TGTAATACGACTCACTATAGGGGTTTTCACCCAGAAGAGGCGCGTTGC
EM1444	CGTACGCTGGTACCGCC
EM1467	GTATCGCTCTGCGCCCACCCGCCGCCCCTTGTGCCAAGGCTGAAAATG
EM1468	CATTTTCAGCCTTGGCACAAGGGGCGGCGGGTGGGCGCAGAGCGATAC
EM1492	CACGAGGTAGTTAAGTCTGAAATTG
EM1493	CAATTTCAGACTTAACTACCTCGTG
EM1494	TCCCCTGACACGACGTAGTTATGTCTG
EM1495	CAGACATAACTACGTCGTGTCAGGGGA
EM1552	TGTAATACGACTCACTATAGGCCAGAAGAGGCGCGTTGC
EM1652	CGTAACGTCTTTCATTTCATCG
EM1683	CGCTCTGCGCCCACCCGCCGCTCTTCCCTTGTGCCAAGG
EM1684	CCTTGGCACAAGGGAAGAGCGGCGGGTGGGCGCAGAGCG
EM1685	CTGCGCCCACCCGTCTTCCGCCCCTTGTGCCAAGGCTG
EM1686	CAGCCTTGGCACAAGGGGCGGAAGACGGGTGGGCGCAG
EM1687	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATAGCTGTTTCCTGT GTGACAGCAGGTCGACTTGCATAGTTTG
EM1688	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCAGCAGGTCGACTTGC ATAGTTTG
EM1701	TAAAGAAAGTTAAAATGCCGGATCCAGCAGGTCGACTTGCATAGTTTG
EM1729	CCATGTACTACCTGCGCTAGC
EM1742	CGCCAGTCACAGAAAAATGTGATGGTTTTAGTGCCGTTAGACCAGCAATAGACATA AGCG
EM1743	CGTTTATTGATGAGCATAGTGACAAGAAAATCAATACGGTGTGACGGAAGATCACT TCG
EM1786	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGATTTATCATCGCAACCA AAC
EM1787	TGTAATACGACTCACTATAGCCAAGCGTCGTTGTACGAC
EM1788	CGGCGAGCTGATGTCGAC
LB1	GTGTGATAAAGAAAGTTAAAATGCCGGATCTGGATCAAGCGTCCAGGGTGT
MSR1	TAATACGACTCACTATAGGGGAACGGAGGAAACCAAATCCATC
MSR2	ATGAATCTTACCGAATTAAAG

Table S3. Summary of *lacZ* fusions used in this study.

Strains	Relevant marker	Oligonucleotides
MPC7	PM1205 *lacI'*::P_{BAD}-LysC-LacZ	EM845-EM908 (genomic DNA)
MPC16	PM1205 *lacI'*::P_{BAD}-*lysC-lacZ*	PCR1: EM908-EM844 (genomic DNA) PCR2: EM991-EM1069 (PCR1) PCR3: EM993-EM994 (PCR1) PCR4: EM991-EM994 (PCR2-3)
MPC19	PM1205 *lacI'*::P_{BAD}-LysC-mutON-LacZ	EM845-EM1099 (genomic DNA)
MPC20	PM1205 *lacI'*::P_{BAD}-LysC-mutOFF-LacZ	EM845-EM1098 (genomic DNA)
MPC24	PM1205 *lacI'*::P_{BAD}-*lysC$_{AUG}$-lacZ*	PCR1: EM1908-EM1094 (genomic DNA) PCR2: EM991-EM1102 (PCR1) PCR3: EM993-EM994 (PCR1) PCR4: EM991-EM994 (PCR2-3)
MPC25	PM1205 *lacI'*::P_{BAD}-*lysC-mutON-lacZ*	PCR1: EM1099-EM844 (genomic DNA) PCR2: EM991-EM1069 (PCR1) PCR3: EM993-EM994 (PCR1) PCR4: EM991-EM994 (PCR2-3)
MPC26	PM1205 *lacI'*::P_{BAD}-*lysC-mutOFF-lacZ*	PCR1: EM1098-EM844 (genomic DNA) PCR2: EM991-EM1069 (PCR1) PCR3: EM993-EM994 (PCR1) PCR4: EM991-EM994 (PCR2-3)
MPC29	PM1205 *lacI'*::$P_{2lysC}P_{1lysC}$-*lacZ*	PCR1: EM846-EM1166 (genomic DNA) PCR2: EM1067-EM1167 (PCR1) PCR3: EM993-EM994 (PCR1) PCR4: EM1067-EM994 (PCR2-3)
MPC30	PM1205 *lacI'*::P_{2lysC}-*lacZ*	PCR1: EM846-EM1168 (genomic DNA) PCR2: EM1067-EM1169 (PCR1) PCR3: EM993-EM994 (PCR1) PCR4: EM1067-EM994 PCR2-3)
MPC40	PM1205 *lacI'*::P_{BAD}-LysC-G31C-LacZ	PCR1: EM991-EM1338 (From MPC7) PCR2: EM994-EM1337 (From MPC7) PCR3: EM991-EM994 (PCR1-2)
MPC41	PM1205 *lacI'*::P_{BAD}-*lysC-ΔSite1-2-lacZ*	PCR1: EM991-EM1468 (From MPC16) PCR2: EM994-EM1467 (From MPC16) PCR3: EM991-EM994 (PCR1-2)
MPC42	PM1205 *lacI'*::P_{BAD}-LysC-ΔSite1-2-LacZ	PCR1: EM991-EM1468 (From MPC7) PCR2: EM994-EM1467 (From MPC7) PCR3: EM991-EM994 (PCR1-2)
MPC43	PM1205 *lacI'*::P_{BAD}-*lysC-G31C-lacZ*	PCR1: EM991-EM1338 (From MPC16) PCR2: EM994-EM1337 (From MPC16) PCR3: EM991-EM994 (PCR1-2)
MPC44	PM1205 *lacI'*::P_{BAD}-*lysC-mutAAG-lacZ*	PCR1: EM991-EM1493 (From MPC16) PCR2: EM994-EM1492 (From MPC16) PCR3: EM991-EM994 (PCR1-2)

MPC45	PM1205 *lacI′*::P_{BAD}-*lysC-mutGACG-lacZ*	PCR1: EM991-EM1495 (From MPC16) PCR2: EM994-EM1494 (From MPC16) PCR3: EM991-EM994 (PCR1-2)
MPC46	PM1205 *lacI′*::P_{BAD}-LysC-mutAAG-LacZ	PCR1: EM991-EM1493 (From MPC7) PCR2: EM994-EM1492 (From MPC7) PCR3: EM991-EM994 (PCR1-2)
MPC47	PM1205 *lacI′*::P_{BAD}-LysC-mutGACG-LacZ	PCR1: EM991-EM1495 (From MPC7) PCR2: EM994-EM1494 (From MPC7) PCR3: EM991-EM994 (PCR1-2)
MPC50	PM1205 *lacI′*::P_{BAD}-*lysC*-Δ*Site1-lacZ*	PCR1:EM991-EM1684 (From MPC16) PCR2:EM994-EM1683 (From MPC16) PCR3:EM991-EM994 (PCR1-2)
MPC51	PM1205 *lacI′*::P_{BAD}-LysC-ΔSite2-LacZ	PCR1:EM991-EM1684 (From MPC7) PCR2:EM994-EM1683 (From MPC7) PCR3:EM991-EM994 (PCR1-2)
MPC52	PM1205 *lacI′*::P_{BAD}-*lysC*-Δ*Site2-lacZ*	PCR1:EM991-EM1686 (From MPC16) PCR2:EM994-EM1685 (From MPC16) PCR3:EM991-EM994 (PCR1-2)
MPC53	PM1205 *lacI′*::P_{BAD}-LysC-ΔSite1-LacZ	PCR1:EM991-EM1686 (From MPC7) PCR2:EM994-EM1685 (From MPC7) PCR3:EM991-EM994 (PCR1-2)
TPP3	PM1205 *lacI′*::P_{BAD}-*thiM-lacZ*	PCR1: EM1786-EM1687 (genomic DNA) PCR2:EM991-EM1701 (PCR1) PCR3:EM993-EM994 (PCR1) PCR4:E991-EM994 (PCR2-3)
TPP4	PM1205 *lacI′*::P_{BAD}-ThiM-LacZ	EM1688-EM1786 (genomic DNA)
TPP5	PM1205 *lacI′*::P_{BAD}-*thiM*$_{300}$*-lacZ*	PCR1: EM1786-LB1 (genomic DNA) PCR2:EM991-EM1701 (PCR1) PCR3:EM993-EM994 (PCR1) PCR4:E991-EM994 (PCR2-3)

Table S4. PCR constructs used for *in vitro* RNA synthesis.

Constructions	**Oligonucleotides**
lysC- expression platform	EM1023-EM1024
lysC-ON mutant structure	EM1065-EM1101
lysC-OFF mutant structure	EM1065-EM1372
lysC-WT structure	EM1065-EM1552
lysC-ΔSite1-2	PCR1: EM1065-EM1467 PCR2: EM1552-EM1468 PCR3: EM1065-EM1552
lysC-ΔSite1-2/OFF	PCR1: EM1065-EM1372 PCR2: EM1372-EM1468 PCR3: EM1065-EM1467 PCR4: EM1065-EM1372
lysC-G31C	PCR1: EM1065-EM1337 PCR2: EM1552-EM1338 PCR3: EM1065-EM1552
lysC riboswitch RNA probe	EM649-EM656
lysC ORF RNA probe	EM1787-EM1788
thiM riboswitch RNA probe	EM703-EM704
hns ORF probe	EM411-EM412
rho ORF probe	MSR1-MSR2
16S rRNA probe	EM293-EM294
5S rRNA probe	EM192-EM193