Supplementary Materials for

A riboswitch-containing sRNA controls gene expression by sequestration of a response regulator

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Materials and Methods

Bacterial strains and media

All bacterial strains used in this study are listed in Table S2. Media was purchased from DIFCO and chemicals from Sigma, unless otherwise mentioned. *E. coli* strains were routinely cultured in Luria Bertani broth at 37°C. Antibiotics were used at the following concentrations (μg/ml): ampicillin, 100; spectinomycin, 100; erythromycin 300. *E. faecalis* strains were cultured in Brain Heart Infusion (BHI) medium at 37°C, unless otherwise noted. The media conditions used to induce *eut* gene expression and microcompartment formation were previously described (*5*). Antibiotics were added at the following concentrations (μg/ml): erythromycin, 50; and rifampicin, 100.

Plasmid Construction

All translational *lacZ* constructs were generated using the previously described plasmid, pSD2 (*5*). The 5' UTR of the different variants of *eutG* were amplified using the primers listed in Table S2. Primers KAF103 and SD133 were used to amplify the riboswitch to generate the *riboswitch-lacZ* fusion. The SD158 (*eutGΔriboswitch*) strain was used as template to amplify the *eutGΔriboswitch* leader using primers KAF103 and KAF235. The *eutGΔP3P4* and *eutGΔriboswitch ΔP3P4* reporters were constructed by joining two PCR fragments using overlap PCR. The primers KAF103 and SD170 were used to amplify the upper fragment from OG1RF and SD158 genomic DNA for *eutGΔP3P4* and *eutGΔriboswitchΔP3P4,* respectively. The lower fragment for both was amplified from OG1RF genomic DNA with primers SD171 and SD161. PCR products were joined by overlap PCR using KAF103 and KAF235. Amplified products were then digested with BamHI and SalI and inserted into pSD2 digested with BglII and SalI, to create the respective plasmids. Point mutations in the *eutG* leader were introduced by site-directed mutagenesis. A *eutG* 5'UTR clone in pTOPO PCR/GW8 was altered using the primers SD186/187 (mut1), SD188/189 (mut2) and SD228/229 (mut3) listed in Table S2. The different mutated versions were then amplified using KAF103 and KAF235, and cloned into pSD2 as described above. The constructs were introduced into *E. faecalis* by electroporation.

Strain Construction

To generate the *E. faecalis* genomic background lacking both the riboswitch and the P3P4 loops used in this study, overlap PCR was employed as described in the previous section. Primer sets SD198/199 and SD171/200 were used to amplify the two fragments, and joined using SD198/SD200. Fragments were then cloned into pCJK47 and introduced into the *E. faecalis* genome using allele exchange method (*15*). Different reporter plasmids were then introduced into these strains by electroporation.

β-galactosidase Assays

All β-galactosidase assays were performed as described previously (*5*). Each experiment was replicated independently at least three times. The data shown reflects the average, and the error bars are the standard deviation.

RNA Preparation and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

E. faecalis strains were grown anaerobically as described previously (*5*). RNA was isolated using the RiboPure Kit (Ambion) as per manufacturer's instruction. RNA was treated with DNaseI to remove contaminating DNA using the TurboDNA free kit (Applied Biosystems). RNA was quantitated and qRT-PCR was performed on an ABI7500 instrument using Power SYBR Green RNA-to-C_T 1 step kit (Applied Biosystems). Comparitive C_T method was used to determine fold changes relative to *gyrA*. Primers used for sets A (SD296/297), B (SD320/321), C (SD303/304), D (SD260/261), *eutG*(214/215) and *gyrA* (SD220/221) are listed in Table S2.

Transmission Electron Microscopy

E. faecalis strains OG1RF, AR2 and SD289 were grown in a previously described minimal medium with and without EA and AdoCbl to induce *eut* gene expression (*5*). At 3.5 hours, the specimens were fixed in 3% glutaraldehyde overnight. Then they were rinsed in Millonig's buffer for 5 minutes. The Millonig's buffer was drawn off and the sample was layered with 2% OsO_4 for 60 minutes at 4°C. The samples were then rinsed for 5 minutes with deionized H_2O and dehydrated at room temperature (50% EtOH 5 minutes, 70% EtOH 10 minutes, 95% EtOH 10 minutes, 100% EtOH 10 minutes x 3, 100% propylene oxide 10 minutes x 3). The samples were then permeated with 50% LX-112 resin and 50% propylene oxide for 4 hours followed by 100% LX-112 overnight. The next day the specimens were transferred to fresh 100% LX-112 for 6 hours at room temperature then embedded in 100% LX-112 in BEEM capsules and allowed to polymerize overnight at 70°C. 500 nm thick sections were cut using a glass knife and a Leica Ultracut-R microtome. They were heat-fixed to glass slides and stained for 60 seconds with Toludine Blue to select the most appropriate areas for imaging. The blocks were then trimmed and 120 nm thin sections were cut using the same microtome and a diamond knife (Daitome-U.S.), floated on 100 and 150 mesh copper grids (EMS), heat fixed and stained for 15 minutes with 2% uranyl acetate, rinsed with deionized H_2O and further stained for 5 minutes with Renold's lead citrate. The grids were imaged using a JEOL 1200 transmission electron microscope at 60 kv and images were captured with a 2k x 2k Gatan CCD camera.

RNA Extraction

Cells were harvested by centrifugation at 5,700 rpm for 10 minutes at which point pellets were frozen at -80°C until ready for RNA extraction. To extract total RNA, the cell pellet was briefly thawed and resuspended in 750 µL LETS buffer (0.1 M LiCl, 10 mM EDTA pH 8.0, 10 mM Tris-HCl pH 7.4, and 1% SDS) and disrupted by continuous vortexing with 400 µL glass beads for four minutes followed by incubation at 55°C for five minutes. The suspension was centrifuged for ten minutes at 15,000 rpm and the supernatant collected and mixed with 1 mL TRI reagent (Ambion AM9738) with incubation at room temperature for five minutes. 200 µL chloroform was then added and the suspension was mixed vigorously for 15 seconds followed by a two minute incubation at room temperature. The samples were centrifuged at 15,000 rpm for 15 minutes and the top 60% (approximately 600 µL) of the phase-separated mixture was collected and precipitated with 1 mL isopropanol for 10 minutes at room temperature.

Precipitated RNA was washed with 200 µL of 70% ethanol and resuspended in 20 µL purified water.

RACE

With 5' adenylated linkers: E. faecalis OG1RF was grown in minimal medium containing supplemental AdoCbl and EA, or EA alone. After 3.5 hours of anaerobic growth, cells were harvested and total RNA was extracted as described above. Total RNA was then DNase I-treated (Promega) and subjected to the RiboZero ^{TM} Magnetic Kit (Gram-Positive Bacteria) to remove ribosomal RNA according to manufacturers' protocol. A pre-adenylated linker (NEB Universal miRNA Cloning Linker) was incubated with T4 RNA ligase KQ (NEB) for 1 hour at 25°C. The reaction was then purified using the Zymo-RNA Clean & Concentrator kit. This RNA was incubated with a DNA oligonucleotide primer complementary to the pre-adenylated linker (ATTGATGGTGCCTACAG), and incubated for 10 minutes at 65°C. Then iScript reverse transcriptase, buffer, and 5 mM dNTPs were added and the reactions were incubated at 42°C, 50°C, 55°C, 60°C for 15 minutes each, followed by 90°C for 5 minutes. The reaction was then treated with 0.5 µg/µL RNase A for 20 minutes at 37°C and again purified by the Zymo-DNA Clean & Concentrator kit. This cDNA was used as template for PCR amplification, using oligos MG283 and MG285 (Table S2). The resulting PCR products were subcloned by TOPO TA reactions according to manufacturers' protocol (Invitrogen) and transformed into Top10 cells (Invitrogen) on LB containing 50 µg/mL ampicillin and 40 µg/mL X-Gal. White colonies were chosen and the appropriate insert sequences were analyzed by Sanger sequencing.

From circularized RNA templates: Total RNA was treated with DNase I and then incubated with Terminator™ 5'-phosphate dependent exonuclease (Epicentre), according to manufacturers' protocol. Terminator exonuclease-treated RNA was then incubated with Tobacco Acid Pyrophosphatase (Epicentre) for full conversion of 5'-triphosphates to 5'-monophosphates. The RNA was then incubated with T4 RNA ligase (NED) under relatively dilute conditions to promote intramolecular ligations. cDNA synthesis and PCR amplification were performed as above using oligos MG293 and MG294 (Table S2). The subsequent sub-cloning, PCR amplification and sequencing analyses were performed as above.

Northern Blot

Total RNA was extracted as described above. Total RNA samples (10-20 µg) were heated at 65°C for ten minutes in gel loading buffer (45 mM Tris-borate, 4 M urea, 10% sucrose [w/v], 5 mM EDTA, 0.05% SDS, 0.025% xylene cyanol FF, 0.025% bromophenol blue) and resolved on a 6% denaturing (8 M urea) polyacrylamide gel. Aliquots of an Ambion® Century™ RNA Marker were also resolved within neighboring lanes in order to generate an RNA size standard curve. RNA within the polyacrylamide gels was transferred to BrightStar-Plus nylon membranes (Ambion) using a semi-dry electroblotting apparatus (Owl Scientific) in 1X NAQ buffer (4 mM MOPS, 1 mM sodium acetate, 0.1 mM EDTA) at 2 mA/cm^2 for 1 hour. The blots were UV-crosslinked at 125 mJ and hybridized overnight at 42°C in UltraHyb Oligo Buffer (Ambion) in the presence of different radiolabeled (^{32}P) antisense RNA probes. The blots were the washed twice for 15 minutes using low stringency buffer (1X SSC, 0.1% SDS, 1 mM EDTA) and

then for 30 minutes using high stringency buffer (0.2X SSC, 0.01% SDS, 1 mM EDTA) Radioactive bands were visualized using ImageJ software and a FLA5000 Phosphorimager.

In Vitro Transcription Assays

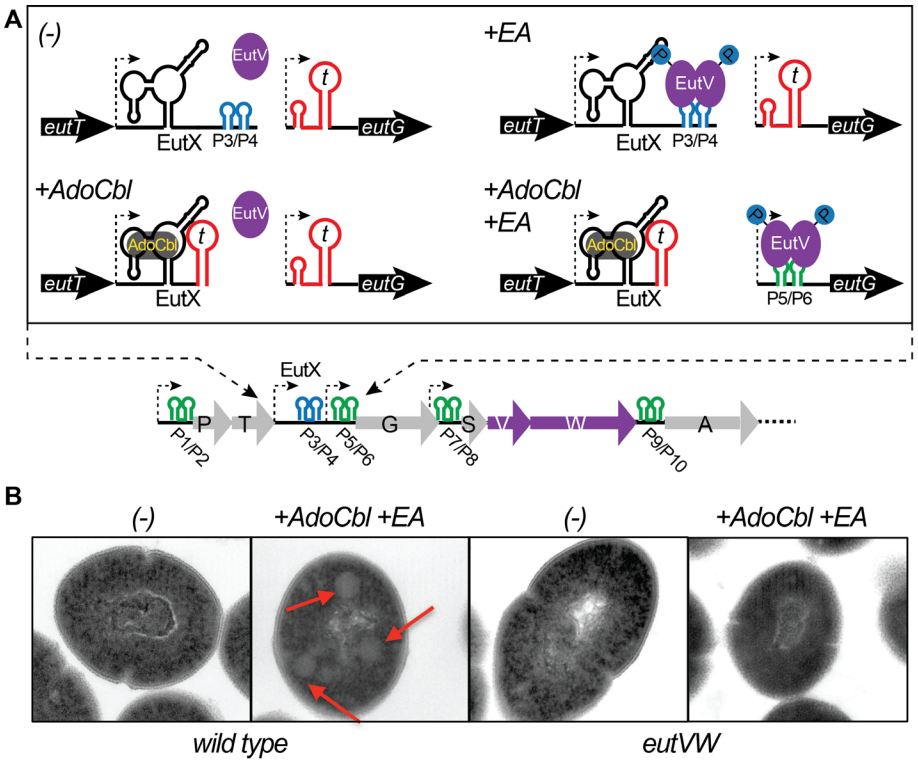
The double-stranded DNA template for transcription of the EutX-associated riboswitch consisted of a 308 base-pair (bp) PCR fragment. This sequence included from -135 to +1 of the *B. subtilis glyQS* promoter, followed by the EutX-associated AdoCbl riboswitch. The DNA template ended at 20 nts after the putative riboswitch intrinsic terminator, which was predicted by 3' RACE. A separate control template included a mutation (M3) of three cytosines changed to guanines, located within the L5 loop, which is known to perturb AdoCbl binding. Synchronized transcription assays were carried out as described (*16*). Template DNA (10 nM) was incubated in 1X transcription buffer (10 mM Tris pH 8.0, 20 mM NaCl, 14 mM $MgCl_2$, 14 mM β-ME, and 0.1 mM EDTA pH 8.0) with *E. coli* RNAP (Epicentre). The dinucleotide ApU (150 μM, Tri Link Biotechnologies) was also included to assist transcription initiation. ATP and GTP were added at 2.5 μM, UTP was added to 0.75 μM and [α-^{32}P] UTP (3000 Ci/mmol) was added to 0.33 μM. Transcription was initiated in the absence of CTP. The first C in the DNA template sequence was located at +30; therefore, the transcription elongation complex halted after synthesis of 29 nt under these conditions. The initiation reaction mixtures were incubated at 37°C for 10 min and were then placed on ice. Heparin (20 μg/mL, Sigma) was added to block reinitiation, and elongation was triggered by the addition of NTPs to 1 mM final. Transcription reactions were terminated with 2x urea loading buffer (8 M urea, 20% sucrose [w/v], 0.1% SDS, 0.05% bromophenol blue [w/v], 0.05% xylene cyanol FF [w/v], 0.09 M Tris-HCl pH 7.5, 0.09 M borate, and 1 mM EDTA pH 8.0) and resolved by 6% denaturing polyacrylamide gel electrophoresis and visualized and quantified using ImageJ software and a FLA5000 phosphorimager.

RNA–binding studies using electrophoretic mobility shift assays (EMSA) or differential radial capillary action of ligand assay (DRaCALA)

These studies were generally carried out as previously described (*5*) with the following modifications. An RNA transcript corresponding to a 117 nt portion of *eutX*, which includes P3/P4, was transcribed and radiolabeled with γ-^{32}P ATP. A sequence that includes P1/P2 was also generated for control reactions. Similar reactions, minus γ-^{32}P ATP, were run to generate cold competitor RNA, which was added at 30 μM to certain controls. RNA (~2 fmol) was incubated with increasing concentrations of protein in a 10 μl reaction containing 50 mM Hepes pH 7.5, 150 mM sodium chloride, 10 mM $MgCl_2$ and 2.5 ng/μl yeast tRNA for 30 minutes at 25°C. A mutant form of EutV, containing alteration of D54E was used for this assay, as it exhibits high affinity RNA-binding activity in the absence of ethanolamine and phosphoryl transfer from EutW (*5*). The samples were then subjected to either EMSA or DRaCALA (*12*). For EMSA, 10 μl of each sample was run on non-denaturing 5% TBE polyacrylamide gels (5% acrylamide: bis (80:1). Gels were pre-run for 30 minutes at 40 volts, electrophoresed at 40 volts for 2.5 hours, with 0.56 TBE running buffer and dried for 45 mins. For DRaCALA, 3 μl of each sample was spotted on a nitrocellulose membrane and allowed to air dry for 30

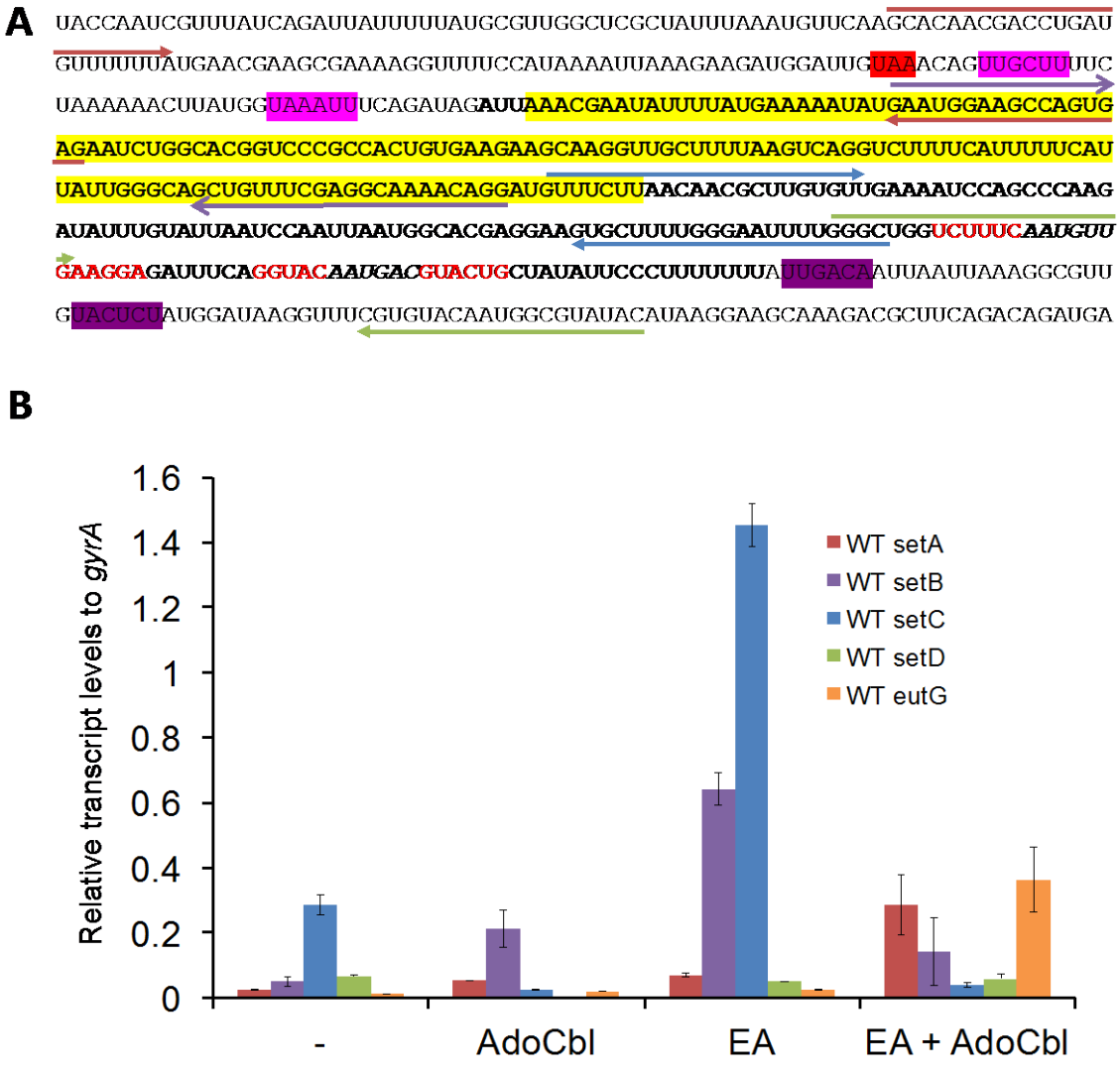
mins. Gels for EMSA and nitrocellulose membranes for DRaCALA were exposed overnight and visualized using a PhosphorImager (GE Health Sciences).

Fig. S1.


A
(-)
EutV
eutT
EutX
P3/P4
t
eutG
+EA
+AdoCbl
AdoCbl
+AdoCbl
+EA
P5/P6
P1/P2
P
T
G
S
V
W
A
P7/P8
P9/P10
B
(-)
+AdoCbl +EA
wild type
eutVW

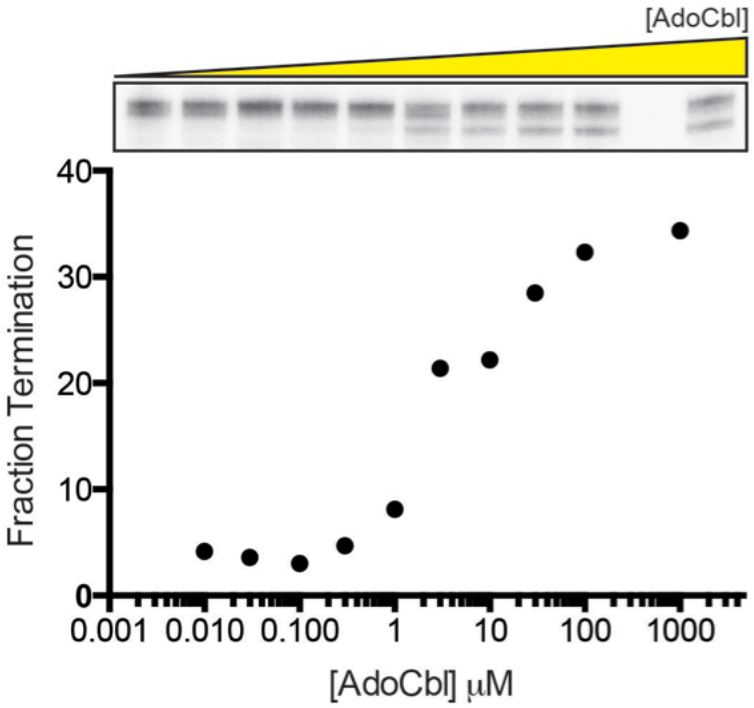
(A). Model for how EutX uses both a riboswitch and an antiterminator binding site to regulate *eut* gene expression. See main text for details **(B). Microcompartment formation occurs in wild type cells grown in EA and AdoCbl**. Shown are typical cells observed by TEM. The wild type cells (OG1RF) grown in EA and AdoCbl contained microcompartments, while the *eutVW* cells (AR2), containing a deletion of the two-component system, and/or without both inducing compounds, had none. The light grey, icosahedral-like structures within these cells are indicative of these structures, and are marked with red arrows.

Fig. S2


A
UACCAAUCGUUUAUCAGAUUAUUUUUUAUGCGUUGGCUCGCUAUUUAAAUGUUCAAGCACAACGACCUGAU
GUUUUUUUAUGAACGAAGCGAAAAGGUUUUCCAUAAAAUUAAAGAAGAUGGAUUGUAAACAGUUGCUUUUC
UAAAAAACUUAUGGUAAAUUUCAGAUAGAUUAAACGAAUAUUUUAUGAAAAAUAUGAAUGGAAGCCAGUG
AGAAUCUGGCACGGUCCCGCCACUGUGAAGAAGCAAGGUUGCUUUUAAGUCAGGUCUUUUCAUUUUUCAU
UAUUGGGCAGCUGUUUCGAGGCAAAACAGGAUGUUUCUUAACAACGCUUGUGUUGAAAAUCCAGCCCAAG
AUAUUUGUAUUAAUCCAAUUAAUGGCACGAGGAAGUGCUUUUGGGAAUUUUGGGCUGGUCUUUCAAUGUU
GAAGGAGAUUUCAGGUACAAUGACGUACUGCUAUAUUCCCUUUUUUUAUUGACAAUUAAUUAAAGGCGUU
GUACUCUAUGGAUAAGGUUUCGUGUACAAUGGCGUAUACAUAAGGAAGCAAAGACGCUUCAGACAGAUGA
B
Relative transcript levels to gyrA
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT setA
WT setB
WT setC
WT setD
WT eutG
-
AdoCbl
EA
EA + AdoCbl

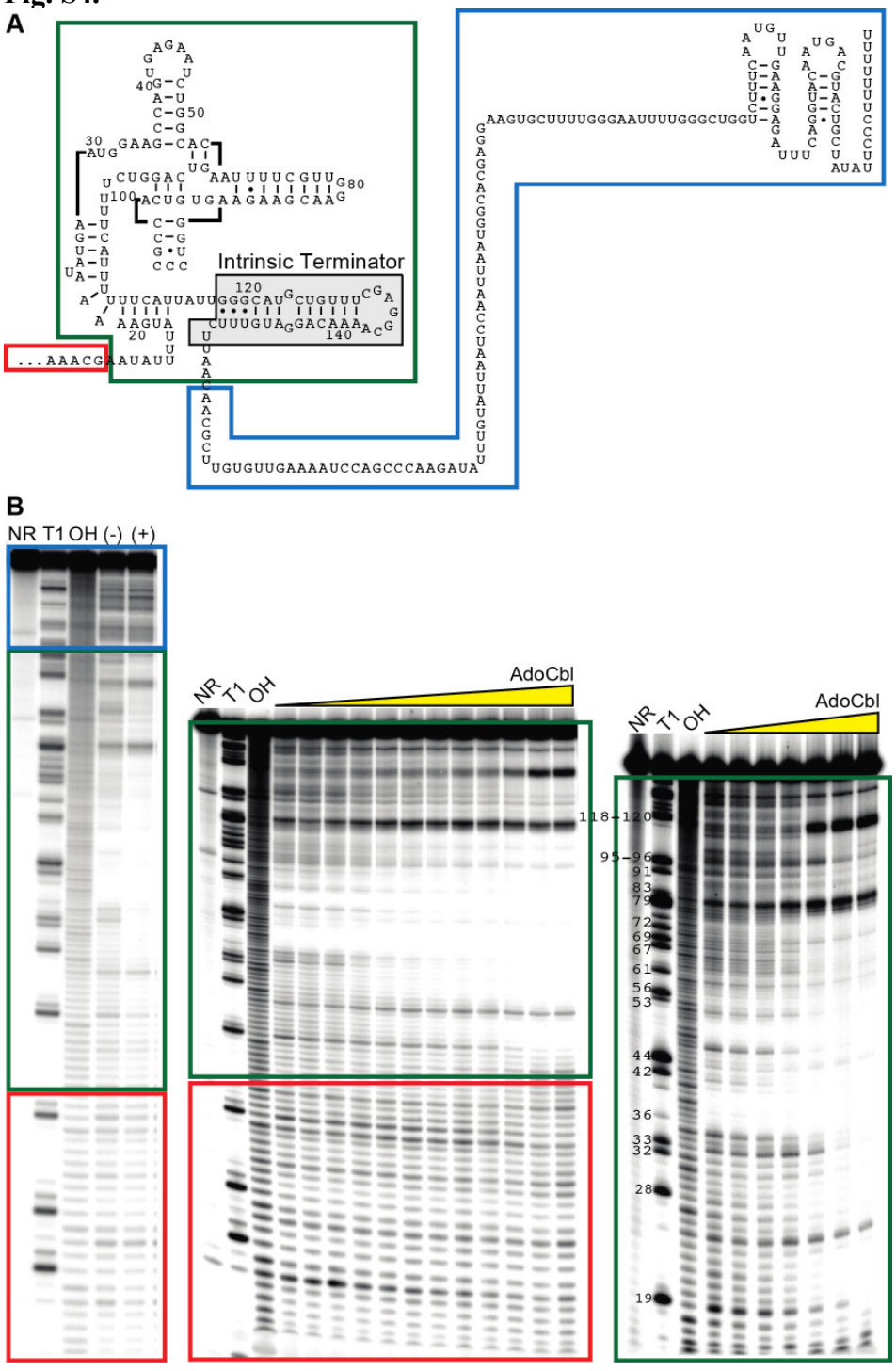
Detection of the EutX sRNA by qRT-PCR. (**A**). The primers used for qRT-PCR are shown as arrows along the sequence encompassing the end of *eutT* to shortly after the promoter for *eutG.* The stop codon for *eutT* is highlighted in red. The pink highlight indicates the -35 and -10 for the promoter in front of the riboswitch aptamer, whereas purple highlight indicates the promoter associated with *eutG.* The bolded sequence is EutX. The yellow highlight is the riboswitch aptamer, and the red sequences indicate the paired stems of the P3/P4 dual hairpin. The italicized sequence is the loops of these hairpins. (**B**). The setC primers, designed to detect full-length EutX, detect high amounts in EA, a moderate amount in plain modified minimal medium, and hardly any when AdoCbl is present, consistent with the Northern data in Fig. 2. The setB primers, designed to detect just the riboswitch aptamer, detect a moderate amount in AdoCbl. A high amount is also detected with EA, as the riboswitch aptamer is part of full-length EutX. Readthrough between *eutT* and *eutX* only occur in medium containing EA and AdoCbl (setA primers). No readthrough occurs between the end of *eutX* and the downstream sequence (setD primers). The expression of *eutG* (primer set not shown) is induced by EA and AdoCbl, consistent with the *lacZ* fusion (Fig. S6). The data presented in (**B**) is the average of three or more independent experiments and the error bars represent the standard deviation.

Fig. S3


[AdoCbl]
Fraction Termination
40
30
20
10
0
0.001
0.010
0.100
1
10
100
1000
[AdoCbl] μM

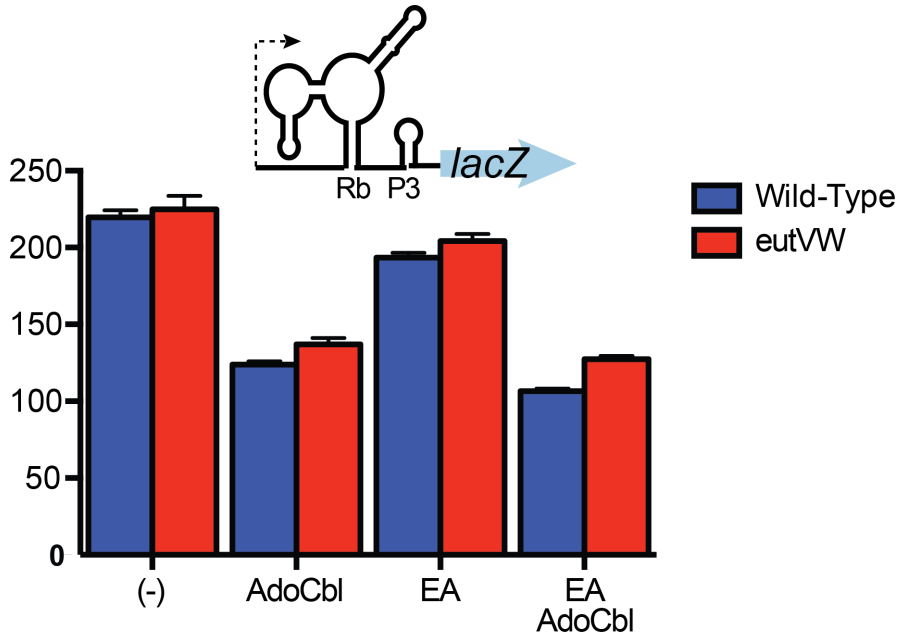
AdoCbl-induced termination by the AdoCbl riboswitch. A PCR-generated DNA template encompassing the AdoCbl riboswitch plus an additional ~20 nucleotides was incubated with *E. coli* RNA polymerase (Epicentre), nucleotides, magnesium and varying amounts of AdoCbl. Premature termination was observed as AdoCbl was added to the single-round transcription reactions. Note that the second to last lane was left intentionally blank to make sure there was not carryover between lanes.

Fig. S4.


A
Intrinsic Terminator
B
NR T1 OH (-) (+)
NR T1 OH
AdoCbl
NR T1 OH
AdoCbl

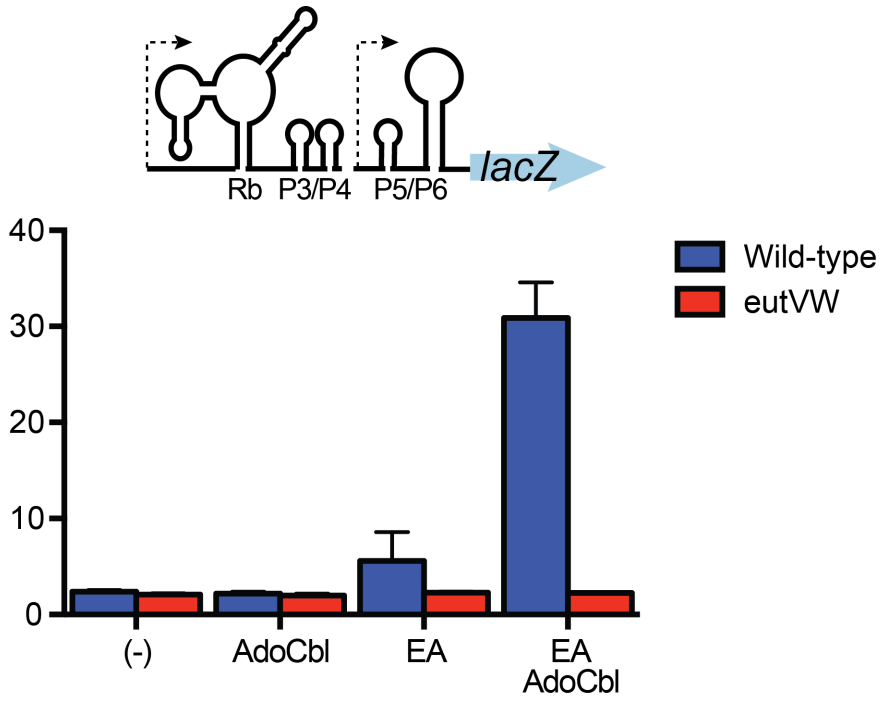
Structural probing of EutX. (A) The proposed secondary structure of EutX is shown. The red box indicates sequences upstream of the AdoCbl riboswitch aptamer. The green box denotes the AdoCbl riboswitch, including the intrinsic terminator hairpin (marked by a gray-shaded box). The blue-boxed region indicates the downstream portion of the EutX sRNA which includes the P3/P4 motif. (**B**) In-line probing of EutX RNAs. RNAs were transcribed in vitro that corresponded to full-length EutX, the AdoCbl riboswitch plus additional upstream sequence, or only the AdoCbl riboswitch. These RNAs were 5' radiolabeled and incubated for 40 hours at room temperature in 20 mM $MgCl_2$, 50 mM Tris-HCl pH 8.0, and 100 mM KCl. The products of these reactions were resolved by denaturing 6% PAGE. An unreacted control lane ('NR') is included alongside these reactions. Also, a control lane is shown for cleavages at G residues (denoted by partial digests by RNase T1 ('T1')). Finally, a hydroxyl cleavage ladder, denoting cleavages at all positions, is included in each analysis. As increasing concentrations of AdoCbl are included in these reactions, structural changes are observed only within the AdoCbl riboswitch portion of EutX (green box).

Fig. S5.


Rb
P3
lacZ
250
200
150
100
50
0
(-)
AdoCbl
EA
EA
AdoCbl
Wild-Type
eutVW

Addition of AdoCbl reduces expression of a *riboswitch-lacZ* construct. Displayed are β-galactosidase assays of wild type and *eutVW* mutant cells containing the *riboswitch-lacZ* fusion, strains SD159 and SD160 respectively. The cells were grown in modified minimal medium with or without EA and/or AdoCbl. The data presented is the average of three or more independent experiments and the error bars represent the standard deviation.

Fig. S6.


Rb
P3/P4
P5/P6
lacZ
40
30
20
10
0
(-)
AdoCbl
EA
EA
AdoCbl
Wild-type
eutVW

Expression of *eutG-lacZ* requires the EutVW two-component system, EA and AdoCbl. Displayed are β-galactosidase assays of wild type and *eutVW* mutant cells containing the *eutG-lacZ* fusion, strains SD72 and SD73 respectively. The cells were grown in modified minimal medium with or without EA and/or AdoCbl. The data presented is the average of three or more independent experiments and the error bars represent the standard deviation.

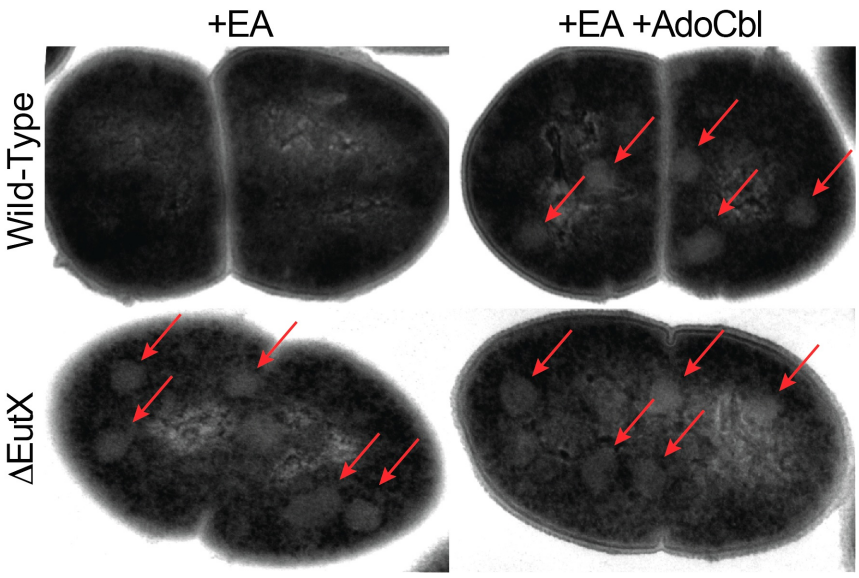

+EA
+EA +AdoCbl
Wild-Type
ΔEutX

Fig. S7. EA alone induces microcompartment formation in a strain lacking EutX. Wild type and *eutX* strains were grown in minimal medium containing EA or EA and AdoCbl and visualized by TEM. Microcompartments, indicated by the red arrows, were visible in the strain lacking EutX when grown only in EA, unlike the wild type strain.

Table S1.

RNA-seq analysis of *eut* pathway gene cluster

		Gene RPKM		
Locus Tag	Gene Name	MM	MM+AdoCbl+EA	Ratio BE/MM
OG1RF_11331	*parE*	166.8287903	464.843067	2.786348005
OG1RF_11333	*eutQ*	41.02036694	536.7842244	13.08579773
OG1RF_11334	*eutH*	58.64112503	339.9438723	5.797021666
OG1RF_11335	*eutN*	19.51546326	81.67609073	4.185198662
OG1RF_11337	*eutY*	58.83059555	624.3986245	10.61350168
OG1RF_11338	*eutZ*	43.38874841	338.5809816	7.80342817
OG1RF_11339	*eutK*	17.99969912	986.0513981	54.78154893
OG1RF_11340	*eutE*	68.87253295	1560.120417	22.6522875
OG1RF_11341	*eutM*	48.40971711	1972.102796	40.73774675
OG1RF_11342	*eutL*	21.59963894	268.9762082	12.45281039
OG1RF_11343	*eutC*	50.58862806	964.2451474	19.06051191
OG1RF_11344	*eutB*	66.78835726	624.5933232	9.351829403
OG1RF_11345	*eutA*	108.09293	585.0694938	5.412652741
OG1RF_11346	*eutW*	121.3558662	676.0911205	5.571144945
OG1RF_11347	*eutV*	80.90391079	794.2732113	9.817488469
OG1RF_11348	*eutS*	48.40971711	432.6204377	8.936644614
OG1RF_11349	*eutG*	121.3558662	623.2304325	5.135560826
OG1RF_11350	*eutT*	49.64127547	404.7785283	8.154071878
OG1RF_11351	*eutP*	45.18871832	447.4175364	9.901089321

Table S2.

Strains, plasmids and primers used in this study

Strains	**Description**	**Source**
E. faecalis		
OG1RF	Wild type strain, Fa^R, Rf^R	(*17*)
AR2	Δ*eutVW*, Fa^R, Rf^R	(*4*)
SD72	OG1RF pSD20, Fa^R, Rf^R, Em^R	This work
SD73	AR2 pSD20, Fa^R, Rf^R, Em^R	This work
SD158	OG1RF*Δriboswitch*, Fa^R, Rf^R	This work
SD419	OG1RF pSD22, Fa^R, Rf^R, Em^R	This work
SD420	OG1RF pSD21, Fa^R, Rf^R, Em^R	This work
SD391	OG1RF pSD23, Fa^R, Rf^R, Em^R	This work
SD289	OG1RF*ΔeutX*, Fa^R, Rf^R	This work
SD293	SD289 pSD20, Fa^R, Rf^R, Em^R	This work
SD296	SD289 pSD21, Fa^R, Rf^R, Em^R	This work
SD297	SD289 pSD23, Fa^R, Rf^R, Em^R	This work
SD295	SD289 pSD22, Fa^R, Rf^R, Em^R	This work
SD307	SD289 pSD24, Fa^R, Rf^R, Em^R	This work
SD308	SD289 pSD25, Fa^R, Rf^R, Em^R	This work
SD351	SD289 pSD26, Fa^R, Rf^R, Em^R	This work
SD159	OG1RF pSD27, Fa^R, Rf^R, Em^R	This work
SD160	AR2 pSD27, Fa^R, Rf^R, Em^R	This work
E. coli		
TOP10	Strain used for construction of pSD2-based plasmids	Lab Stock
XL-1 Blue	Strain used for maintenance of pTOPO GW8 based site-directed mutagenesis clones	Lab Stock
Plasmids		
pCJK47	Vector for allelic exchange	(*15*)
pSD2	*lacZ* reporter plasmid backbone	(*5*)
pSD20	pSD2*eutG-lacZ*	This work
pSD21	pSD2*eutGΔP3/P4*	This work
pSD22	pSD2*eutGΔriboswitch*	This work
pSD23	pSD2*eutGΔriboswitchΔP3/P4*	This work
pSD24	pSD2*eutGmut1*	This work
pSD25	pSD2*eutGmut2*	This work
pSD26	pSD2*eutGmut3*	This work
pSD27	pSD2 *riboswitch-lacZ*	This work
Oligos		
KAF103	5’ AAAGTCGACGCAGAACGCATCATTGTTCG 3’	This work
KAF235	5’ AAAGGATCCCCCATAGTTCGGTAGGGAAATGAATTGT 3’	This work
SD133	5’ AAAGGATCCGAATTGTTTTCATCTCAATTCCTCCTTAATAA	This work

	AAAAAGGG 3’	
SD161	5’ GGTGCCAGTTTCAGCAACCGTAATCACTG 3’	This work
SD170	5' GTACAACGCCTTTAATTAATTGTCAATAAAAAAAGGGAAT ATAGCCCA GCCCAAAATTCCCAAAAGCACTTCC 3'	This work
SD171	5’ GCTATATTCCCTTTTTTTATTGACAATTAATTAAAGGCG 3’	This work
SD186	5’GGTCTTTCTATATTGAAGGAGATTTCAGGTACAATGACGTA CTGC 3’	This work
SD187	5' GCAGTACGTCATTGTACCTGAAATCTCCTTCAATATAGAAA GACC 3'	This work
SD188	5’ GAAGGAGATTTCAGGTACTATAACGTACTGCTATATTCCC 3’	This work
SD189	5' GGGAATATAGCAGTACGTTATAGTACCTGAAATCTCCTTC 3'	This work
SD198	5’GCTCTAGAGCTATATTATCAGCCAACTTCCAC 3’	This work
SD199	5' CAACGCCTTTAATTAATTGTCAATAAAAAAAGGGAATATAG CAATCTA TCTGAAATTTACCATAAG 3'	This work
SD200	5' AACCCGGGGCAATAAACTCAGCAATCTGAGTTTG 3'	This work
SD214	5’ ATGGCGTTTAATGTTACTTCGTTAGG 3’	This work
SD215	5’ TAAGAGCATTGCATTCATTCGTC 3’	This work
SD220	5’ AGATGTGAGTGCCTCTGTTGTGTTA 3’	This work
SD221	5’ TCGGTACGCCTTTTTCGATG 3’	This work
SD228	5’ GAGAATCTGGCACGGTGGGGCCACTGTGAAGAAGCAAGGT TGC 3’	This work
SD229	5' GCAACCTTGCTTCTTCACAGTGGCCCCACCGTGCCAGATTC TC 3'	This work
SD260	5’GGGCTGGTCTTTCAATGTTG	This work
SD261	5’GTATACGCCATTGTACACG 3’	This work
SD296	5’ GCACA ACGACCTGATGTTTTTTATG 3’	This work
SD297	5’ CTCACTGGCTTCCATTCATATTTTTC 3’	This work
SD303	5’ CAGGATGTTTCTTAACAACGC 3’	This work
SD304	5’ GCCCAAAATTCCCAAAAGCAC 3’	This work
SD320	5’GAATGGAAGCCAGTGAG 3’	This work
SD321	5’CCTGTTTTGCCTCGAAACAGC 3’	This work
MG283	5’ CAAGGTTGCTTTTAAGTCAGGTC 3’	This work
MG285	GCATGCATTGATGGTGCCTACAG	This work
MG290/291	5’ TAATACGACTCACTATAGGGAATATAGCAGTACGTCATTGT ACCTGAAATCTCCTTCAACATTGAAAGACCAGCCCAAAATTC CCAAAAGCACTTCCTCGTGCCATTAATTGGATTAATACAAAT ATCTTGGGCTGGATTTTCAACACAAGCGTTGTTAAGAAACAT CCTGTTTTGCCTCGAAACAGCATGCCCAATAATGAAAAAATG AAAAGACCTGACTTAAAAGCAACCTTGCTTCTTCACAGTGGC GGGACCGTGCCAGATTCTCACTGGCTTCCATTCATATTTTTCA TAAAATATTCGTTTAAT 3’	This work
MG279	5’ CCTGACTTAAAAGCAACCTTGCTTC 3’	This work
JRG175	5’ GTTAAGAAACATCCTGTTTTGCCTCG 3’	This work
MG280	5’ CAATAAAAAAAGGGAATATAGCAGTACGTC 3’	This work

MG293	5’ CAACCTTGCTTCTTCACAGTGG 3’	This work
MG294	5’ CTTTCAATGTTGAAGGAGATTTCAGG 3’	This work
Strains	**Description**	**Source**
E. faecalis		
OG1RF	Wild type strain, Fa^R, Rf^R	(*16*)
AR2	Δ*eutVW*, Fa^R, Rf^R	(*4*)
SD72	OG1RF pSD20, Fa^R, Rf^R, Em^R	This work
SD73	AR2 pSD20, Fa^R, Rf^R, Em^R	This work
SD158	OG1RF*Δriboswitch*, Fa^R, Rf^R	This work
SD419	OG1RF pSD22, Fa^R, Rf^R, Em^R	This work
SD420	OG1RF pSD21, Fa^R, Rf^R, Em^R	This work
SD391	OG1RF pSD23, Fa^R, Rf^R, Em^R	This work
SD289	OG1RF*ΔeutX*, Fa^R, Rf^R	This work
SD293	SD289 pSD20, Fa^R, Rf^R, Em^R	This work
SD296	SD289 pSD21, Fa^R, Rf^R, Em^R	This work
SD297	SD289 pSD23, Fa^R, Rf^R, Em^R	This work
SD295	SD289 pSD22, Fa^R, Rf^R, Em^R	This work
SD307	SD289 pSD24, Fa^R, Rf^R, Em^R	This work
SD308	SD289 pSD25, Fa^R, Rf^R, Em^R	This work
SD351	SD289 pSD26, Fa^R, Rf^R, Em^R	This work
SD159	OG1RF pSD27, Fa^R, Rf^R, Em^R	This work
SD160	AR2 pSD27, Fa^R, Rf^R, Em^R	This work
E. coli		
TOP10	Strain used for construction of pSD2-based plasmids	Lab Stock
XL-1 Blue	Strain used for maintenance of pTOPO GW8 based site-directed mutagenesis clones	Lab Stock
Plasmids		
pCJK47	Vector for allelic exchange	(*14*)
pSD2	*lacZ* reporter plasmid backbone	(*5*)
pSD20	pSD2*eutG-lacZ*	This work
pSD21	pSD2*eutGΔP3/P4*	This work
pSD22	pSD2*eutGΔriboswitch*	This work
pSD23	pSD2*eutGΔriboswitchΔP3/P4*	This work
pSD24	pSD2*eutGmut1*	This work
pSD25	pSD2*eutGmut2*	This work
pSD26	pSD2*eutGmut3*	This work
pSD27	pSD2 *riboswitch-lacZ*	This work
Oligos		
KAF103	5’ AAAGTCGACGCAGAACGCATCATTGTTCG 3’	This work
KAF235	5’ AAAGGATCCCCCATAGTTCGGTAGGGAAATGAATTGT 3’	This work
SD133	5’ AAAGGATCCGAATTGTTTTCATCTCAATTCCTCCTTAATAA	This work

	AAAAAGGG 3’	
SD161	5’ GGTGCCAGTTTCAGCAACCGTAATCACTG 3’	This work
SD170	5' GTACAACGCCTTTAATTAATTGTCAATAAAAAAAGGGAAT ATAGCCCA GCCCAAAATTCCCAAAAGCACTTCC 3'	This work
SD171	5’ GCTATATTCCCTTTTTTTATTGACAATTAATTAAAGGCG 3’	This work
SD186	5’GGTCTTTCTATATTGAAGGAGATTTCAGGTACAATGACGTA CTGC 3’	This work
SD187	5' GCAGTACGTCATTGTACCTGAAATCTCCTTCAATATAGAAA GACC 3'	This work
SD188	5’ GAAGGAGATTTCAGGTACTATAACGTACTGCTATATTCCC 3’	This work
SD189	5' GGGAATATAGCAGTACGTTATAGTACCTGAAATCTCCTTC 3'	This work
SD198	5’GCTCTAGAGCTATATTATCAGCCAACTTCCAC 3’	This work
SD199	5' CAACGCCTTTAATTAATTGTCAATAAAAAAAGGGAATATAG CAATCTA TCTGAAATTTACCATAAG 3'	This work
SD200	5' AACCCGGGGCAATAAACTCAGCAATCTGAGTTTG 3'	This work
SD214	5’ ATGGCGTTTAATGTTACTTCGTTAGG 3’	This work
SD215	5’ TAAGAGCATTGCATTCATTCGTC 3’	This work
SD220	5’ AGATGTGAGTGCCTCTGTTGTGTTA 3’	This work
SD221	5’ TCGGTACGCCTTTTTCGATG 3’	This work
SD228	5’ GAGAATCTGGCACGGTGGGGCCACTGTGAAGAAGCAAGG TTGC 3’	This work
SD229	5' GCAACCTTGCTTCTTCACAGTGGCCCCACCGTGCCAGATTC TC 3'	This work
SD260	5’ GGGCTGGTCTTTCAATGTTG	This work
SD261	5’GTATACGCCATTGTACACG 3’	This work
SD296	5’ GCACA ACGACCTGATGTTTTTTATG 3’	This work
SD297	5’ CTCACTGGCTTCCATTCATATTTTTC 3’	This work
SD303	5’ CAGGATGTTTCTTAACAACGC 3’	This work
SD304	5’ GCCCAAAATTCCCAAAAGCAC 3’	This work
SD320	5’ GAATGGAAGCCAGTGAG 3’	This work
SD321	5’ CCTGTTTTGCCTCGAAACAGC 3’	This work
MG283	5’ CAAGGTTGCTTTTAAGTCAGGTC 3’	This work
MG285	GCATGCATTGATGGTGCCTACAG	This work
MG290/291	5’ TAATACGACTCACTATAGGGAATATAGCAGTACGTCATTGT ACCTGAAATCTCCTTCAACATTGAAAGACCAGCCCAAAATTC CCAAAAGCACTTCCTCGTGCCATTAATTGGATTAATACAAAT ATCTTGGGCTGGATTTTCAACACAAGCGTTGTTAAGAAACAT CCTGTTTTGCCTCGAAACAGCATGCCCAATAATGAAAAAATG AAAAGACCTGACTTAAAAGCAACCTTGCTTCTTCACAGTGGC GGGACCGTGCCAGATTCTCACTGGCTTCCATTCATATTTTTCA TAAAATATTCGTTTAAT 3’	This work
MG279	5’ CCTGACTTAAAAGCAACCTTGCTTC 3’	This work
JRG175	5’ GTTAAGAAACATCCTGTTTTGCCTCG 3’	This work
MG280	5’ CAATAAAAAAAGGGAATATAGCAGTACGTC 3’	This work

MG293	5’ CAACCTTGCTTCTTCACAGTGG 3’	This work
MG294	5’ CTTTCAATGTTGAAGGAGATTTCAGG 3’	This work

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