SUPPLEMENTAL MATERIAL: Lee and Gottesman

SUPPLEMENTARY FIGURES

SUPPLEMENTARY TABLES:

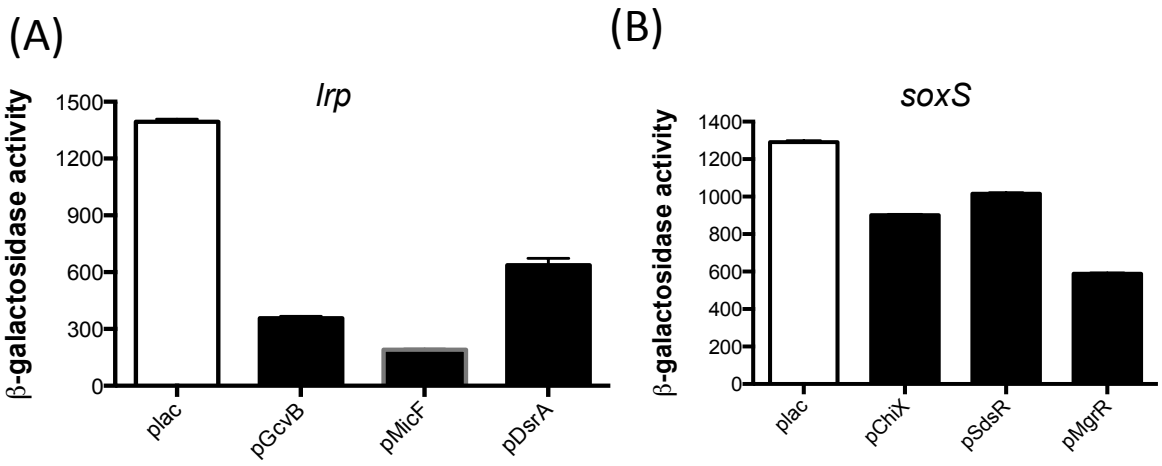


Fig. S1: Regulation of *lrp* and *soxS* by sRNAs.

(A) HL1100, a strain carrying the P_{BAD}-*lrp-lacZ* translational fusion and deletions in *dsrA*, *gcvB* and *micF*, was transformed with plasmids overexpressing GcvB, MicF, or DsrA, or with the pBRplac empty vector. Transformed cells were grown on LB medium containing 100 μg/ml ampicillin, 100 μM IPTG, and 0.001% arabinose. Samples were collected at stationary phase (OD_{600} of between 2.5 and 3) and assayed for β-galactosidase.

(B) HL1064, a strain carrying the P_{BAD}-*soxS-lacZ* translational fusion, was transformed with plasmids overexpression ChiX, SdsR, MgrR, or with the pBRplac empty vector. Transformed cells were grown in LB medium containing 100 μg/ml ampicillin, 100 μM IPTG, and 0.0002% arabinose. Samples were collected at stationary phase (OD_{600} of 2.0) and assayed for β-galactosidase. Error bars indicate standard deviation throughout.

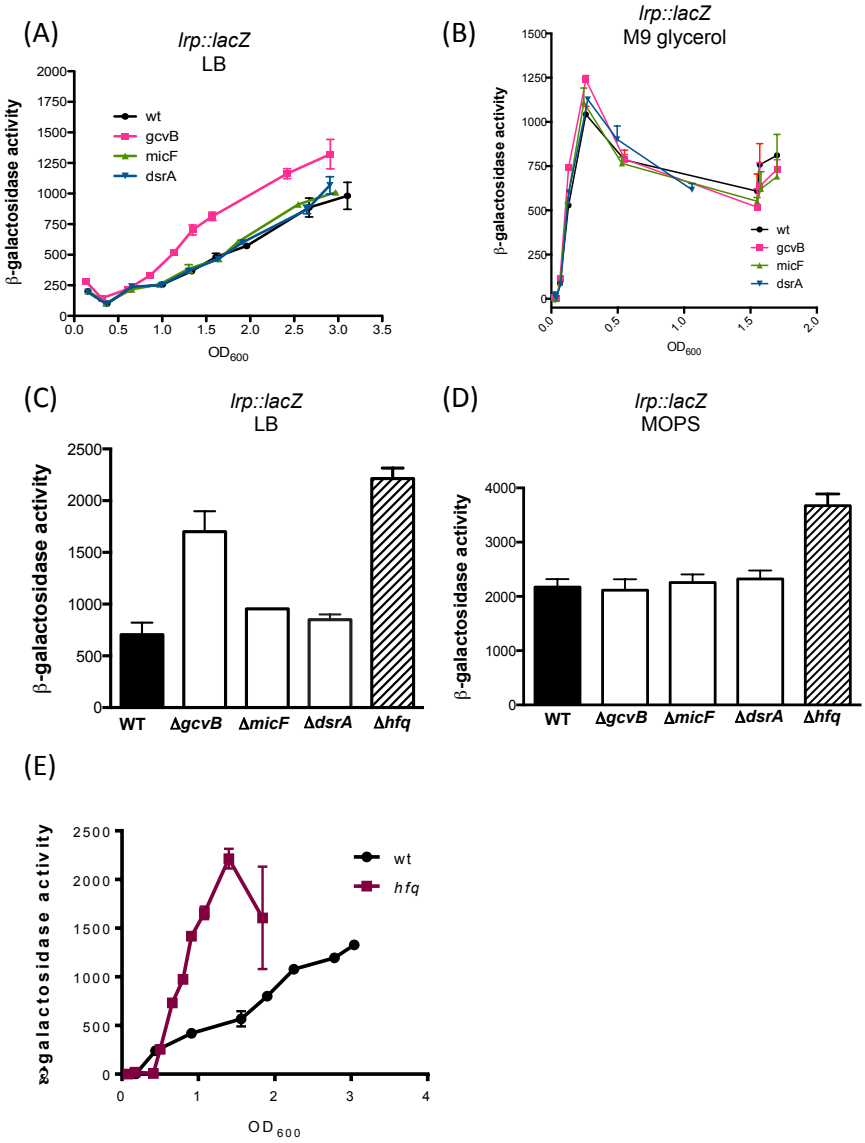


Fig. S2: Effect of sRNA deletions and Hfq on expression of an *lrp*::*lacZ* fusion during growth.

(A, C, E) HL1044 (WT), HL1071 (Δ*gcvB*), HL1078 (Δ*micF*), HL1108 (Δ*dsrA*) and HL1189 (Δ*hfq*) cells were grown in LB medium containing 0.001% arabinose and samples collected across the growth curve (A, E) or at OD_{600} of 1.5 (C) and assayed for β-galactosidase. Error bars indicate standard deviation.

(B, D) Activity in minimal medium. Strains used in Fig. S2A, C, E were grown on M9 glycerol medium (B) and samples collected across the growth curve or (D) in MOPS (Glycerol) minimal medium containing 0.001% arabinose and samples collected at mid-exponential phase (OD_{600} of 0.5) and assayed for β-galactosidase.

Fig. S3. *lrp* leader and mutations:

Alignment of *lrp* leader showing deletions and mutants discussed in this work above the sequence lines. Deletions are shown with XXX in front of the

remaining sequence. The region within the *Salmonella* leader predicted by Sharma et al (1) to base pair with GcvB sequence is highlighted in dark grey with white lettering; the region predicted by Modi et al (2) is highlighted in light grey. Two potential Hfq binding sites (ARN repeats) are shown with blue letters; one overlaps the initiating ATG of the Lrp open reading frame as well as the site predicted by Modi et al, while the other is close to one site of GcvB binding. The ATG is highlighted in green. The GACAG repeats protected by GcvB in vitro (BS1 and BS2) are highlighted in yellow. Double mutants at the BS1 and BS2 sites are shown with an A or B superscript to indicate existence of a second region of the mutation elsewhere. A possible ORF within the leader, pointed out by a reviewer, is underlined.

Fig. S4.

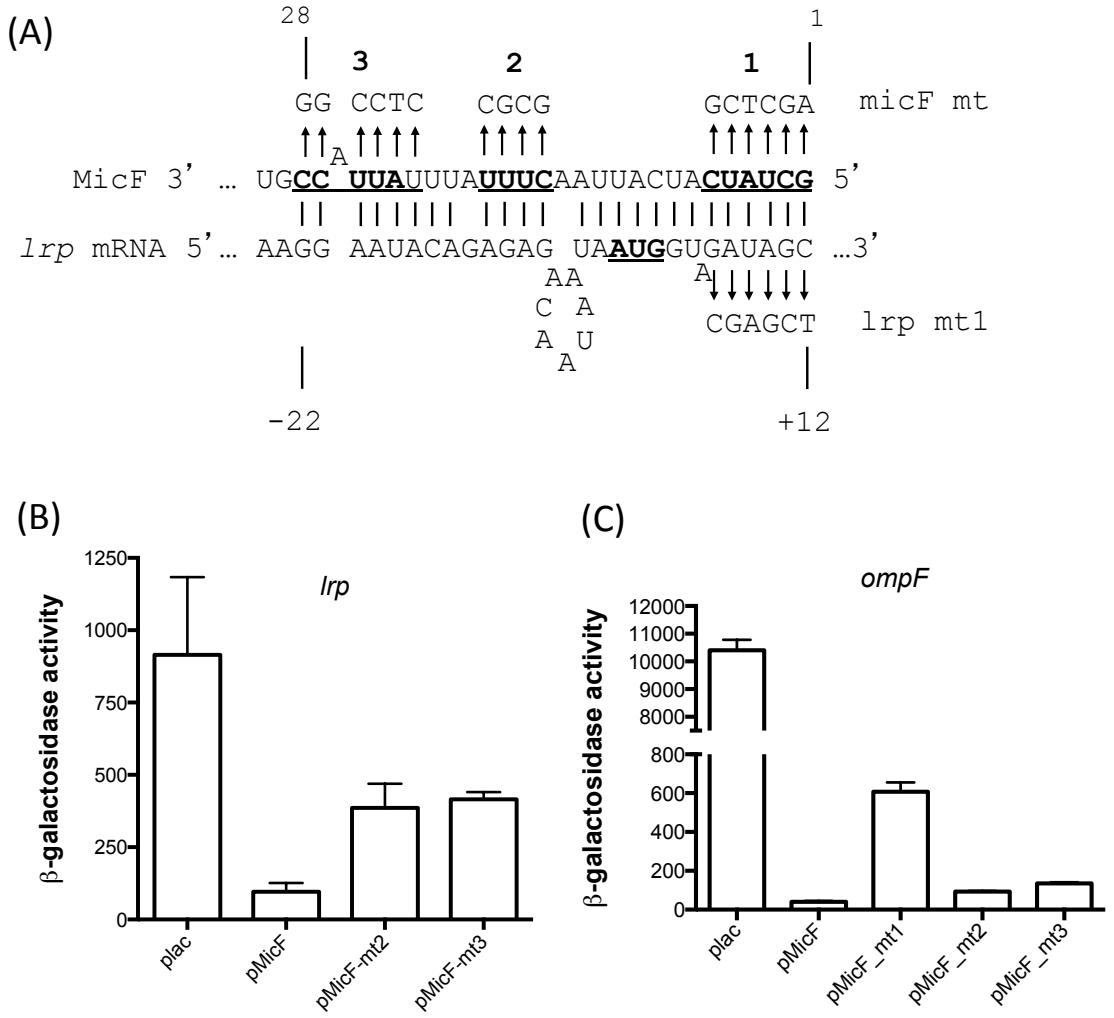


Fig. S4. Regulation by mutant derivatives of MicF.

(A) Pairing predicted between MicF and *lrp*, with nucleotides mutated in MicF underlined and changes to MicF shown above the sequence.

(B) β-galactosidase activity from cells with *lrp-lacZ* (HL1044) translational fusions, in the presence of plasmids that either overexpress wild-type pMicF or mutant MicF as indicated. Cells were grown in LB Ampicillin with IPTG, at 37°C, to OD_{600} 2.5. Error bars indicate standard deviation.

(C) β-galactosidase activity of strain HL1214 (pBAD-*ompF-lacZ*) transformed with plasmids overexpressing MicF or MicF_mt1, MicF_mt2. MicF_mt3, or with the pBRplac empty vector. Cells were grown in LB Ampicillin with IPTG, at 37°C, to OD_{600} 2.5-3. Error bars indicate standard deviation.

Fig. S5.

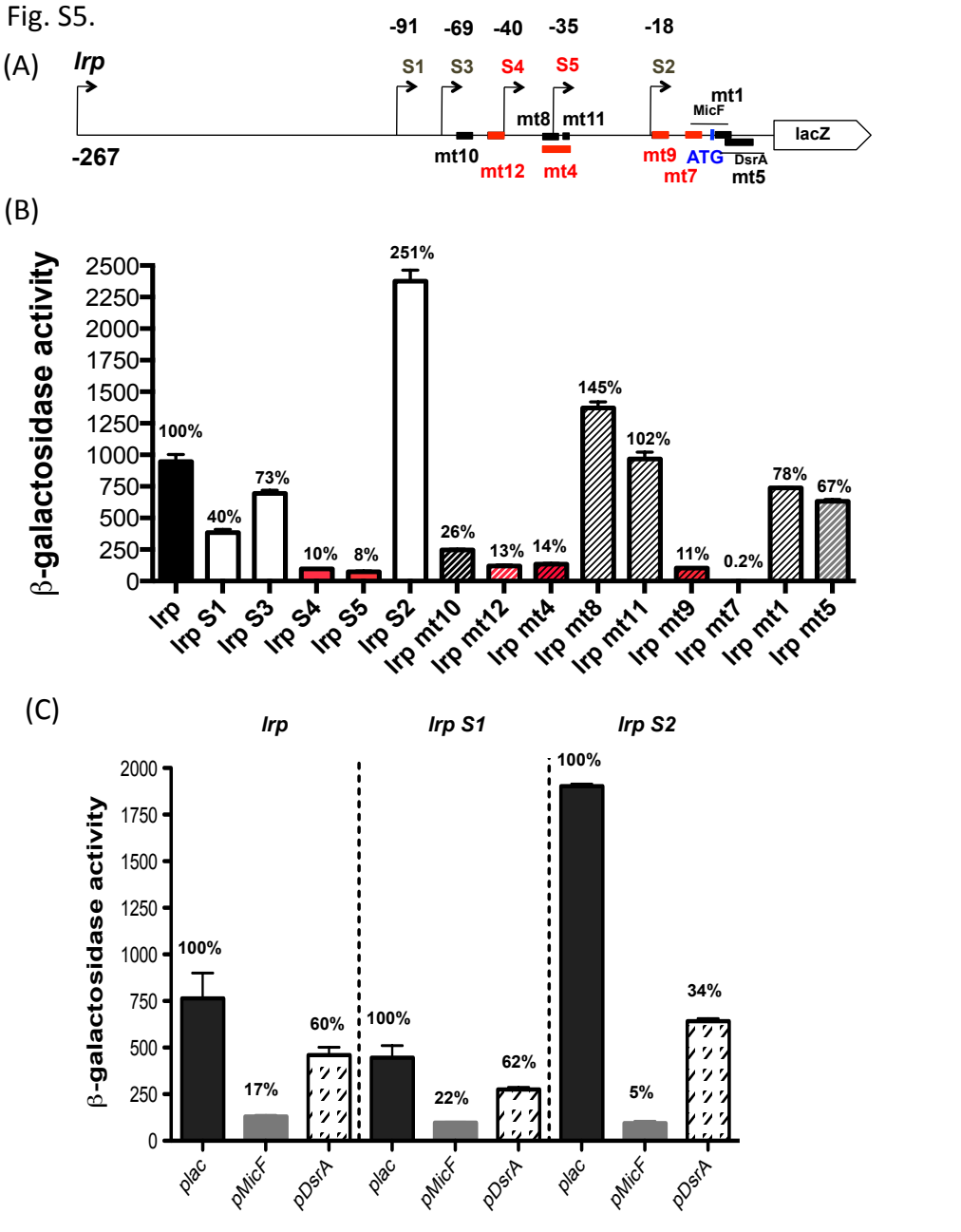


Fig. S5. Translation of *lrp* deletions and point mutations and regulation by MicF and DsrA.

(A) Schematic of end-points of deletions (arrows) and point mutations (heavy bars) on the lrp 5' UTR and initial region of the ORF. Bars and labels in red are those with <15% wild-type activity (data in (B)). Initiating ATG is in blue, with a blue bar on the schematic.

(B) A set of strains carrying deletions or point mutations in the *lrp* leader, wild type for all sRNAs, were grown in LB medium containing 0.001% arabinose at 37°C to OD_{600} of 2.5 and β-galactosidase activity from the *lrp-lacZ* fusion was measured. Percentage expression compared to WT is shown on top of each bar. Deletions have clear bars; point mutations are hatched bars. Deletions or mutants with less than 15% of wild-type have red fill. Sequence changes for mutants (*lrp*_mt) and end points for deletions (*lrp* S1-S5) are shown in Fig. S3. Strains: *lrp* (HL1044), *lrp S1 (HL1503), lrp S2* (HL1505), *lrp S3* (HL1695), *lrp S4* (HL1696), *lrp S5* (HL1697), *lrp mt1* (HL1079), *lrp mt4* (HL1149), *lrp mt5* (HL1150), *lrp mt7* (HL1551), *lrp mt8* (HL1693), *lrp mt9* (HL1694), *lrp mt10* (HL1717), *lrp mt11* (HL1731), *and lrp mt12* (HL1732)-*lacZ* translational fusions. Error bars indicate standard deviation.

(C) β-galactosidase activity from cells carrying full-length and truncated (*lrp*_S1 and *lrp*_S2; see Fig. 3 and Fig. S3) *lrp-lacZ* translational fusions. All strains are deleted for *gcvB* and were transformed with either the empty vector (plac), the plac-MicF plasmid (pMicF) or the plac-DsrA plasmid (pDsrA). Cells were grown in LB medium containing ampicillin, 100 μM IPTG, and 0.001% arabinose at 37°C. Samples are collected at OD_{600} of between 1.5 and 2) and assayed for β-galactosidase. Strains used: *lrp* (HL1071), *lrp S1* (HL1517), *and lrp S2* (HL1518)-*lacZ* translational fusions.

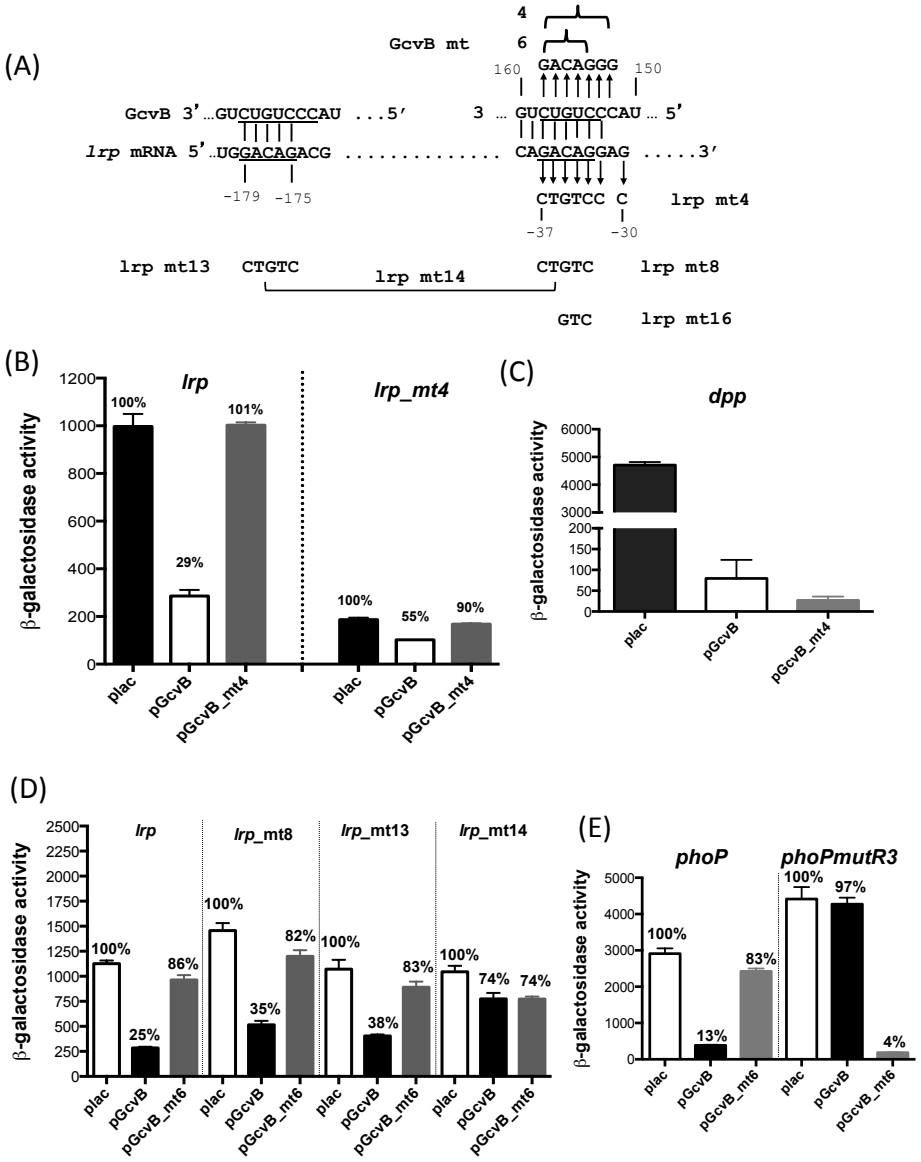


Fig. S6. Effect of GcvB mutations on *lrp* regulation.

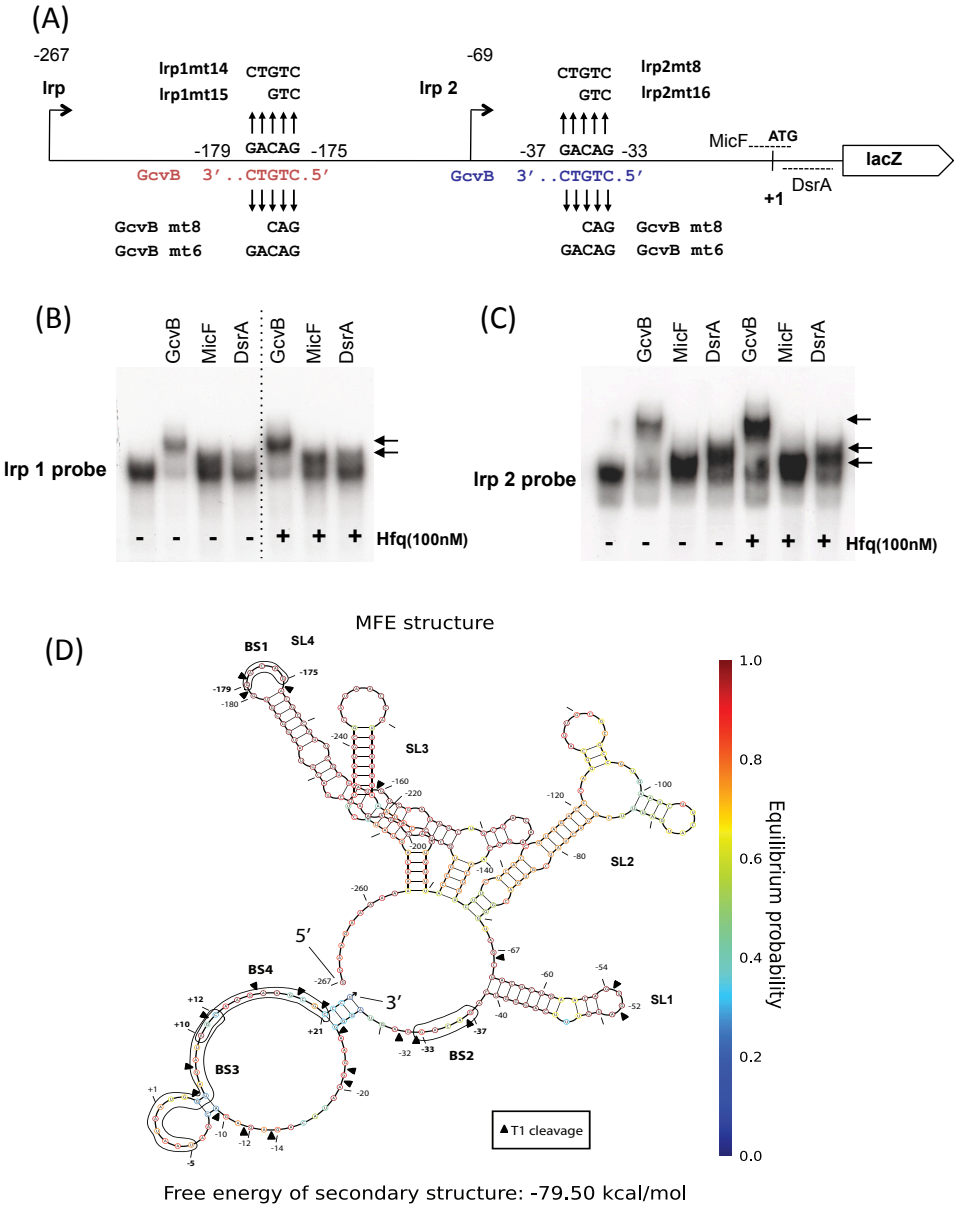
(A) Predicted pairing between GcvB and two regions of the *lrp* 5' UTR. Numbering for the *lrp* sequences are with respect to the translation start codon. Mutants used in panels are shown.

(B) β-galactosidase activity from cells with *lrp-lacZ* (HL1071) and *lrp_mt4-lacZ* (HL1525) translational fusion plasmids, in the presence of plasmids that either overexpress wild-type pGcvB or pGcvB_mt4. Cells were grown at 37°C to stationary phase (OD_{600} of 2.5) and assayed. Error bars indicate standard deviation throughout.

(C) β-galactosidase activity from cells with *dppA-lacZ* (KM339) translational fusion plasmids, in the presence of plasmids that either overexpress wild-type GcvB or GcvB_mt4.

(D) β-galactosidase activity from cells with *lrp* (HL1071), *lrpmt8* (HL1699), *lrpmt13* (HL1803), and *lrpmt14* (HL1804)-*lacZ* translational fusions, in the presence of plasmid overexpressing GcvB or GcvB_mt6 or with the pBRplac empty vector. Normalization of each *lrp* fusion to the vector control is shown.

(E) β-galactosidase activity from cells with *phoP*::*lacZ* (MG1521) or *phoP*mutR3::*lacZ* (MG1586) translational fusions, assayed in the presence of plasmids carrying either wild-type GcvB, GcvB_mt6 (identical to mutant described by Coornaert et al (3), or a vector. All strains were grown and assayed as in Fig. 2; strains are described in Coornaert et al (3).


(A)
-267
lrp
lrp1mt14
lrp1mt15
CTGTC
GTC
-179 GACAG -175
GcvB 3′..CTGTC.5′
GcvB mt8
GcvB mt6
CAG
GACAG
-69
lrp 2
CTGTC
GTC
lrp2mt8
lrp2mt16
-37 GACAG -33
GcvB 3′..CTGTC.5′
CAG
GACAG
GcvB mt8
GcvB mt6
MicF
ATG
lacZ
DsrA
+1
(B)
GcvB
MicF
DsrA
GcvB
MicF
DsrA
lrp 1 probe
- - - - + + +
Hfq(100nM)
(C)
GcvB
MicF
DsrA
GcvB
MicF
DsrA
lrp 2 probe
- - - - + + +
Hfq(100nM)
(D)
MFE structure
BS1
SL4
SL3
SL2
SL1
BS2
BS3
BS4
5′
3′
▲T1 cleavage
Equilibrium probability
1.0
0.8
0.6
0.4
0.2
0.0
Free energy of secondary structure: -79.50 kcal/mol

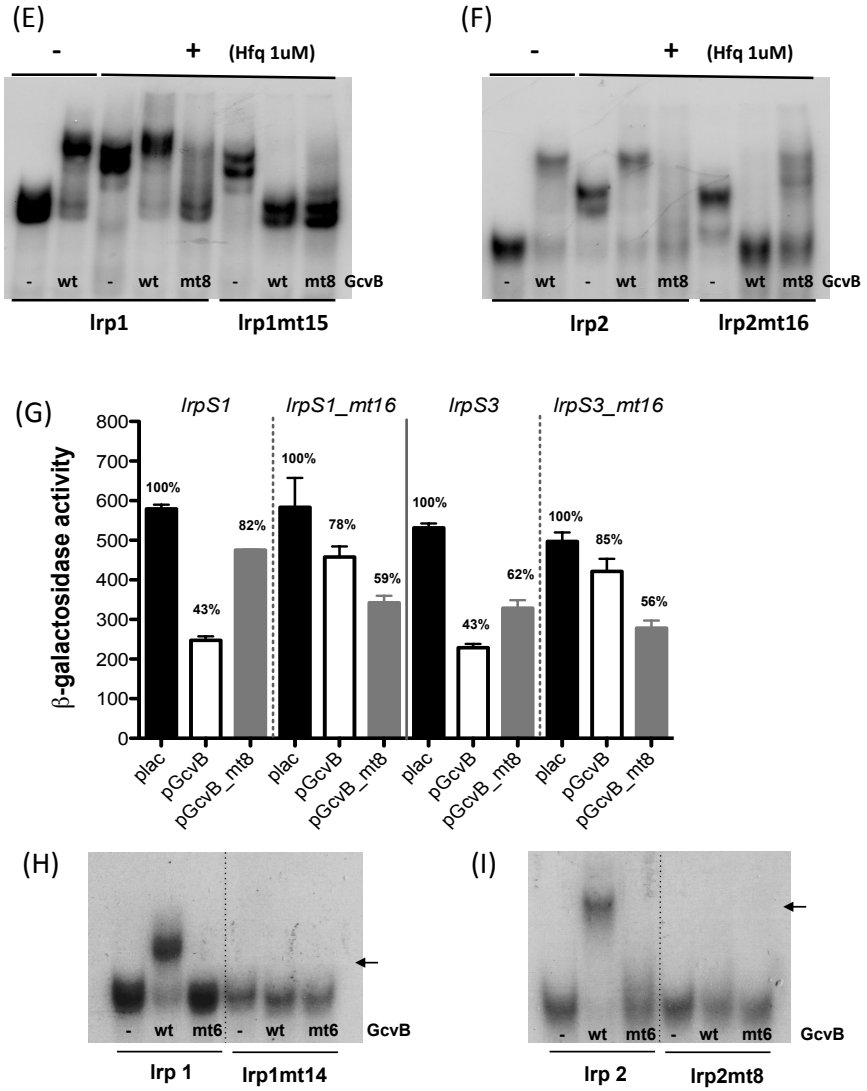


Fig. S7. *in vitro* interaction of *lrp* and sRNAs.

(A) Schematic of *lrp* 5' UTR and regions mutated for *in vitro* analysis (lrp1mt14, lrp1mt15, lrp2mt8, lrp2mt16), and pairing to wild-type and mutant GcvB_mt8 and GcvB_mt6.

(B, C) Gel-mobility shift assays were carried out with each small RNA and either the full 5' UTR, lrp1 (from -267 to +60 relative to the ATG) (A) or a portion of the 5' UTR, lrp2 (from -69 to + 60 relative to the ATG) (B) in vitro. ^{32}P labeled lrp RNAs were incubated with each unlabeled small RNA with or without 100 nM of Hfq protein.

(D) Summary of *in vitro* structure probing experiments (see Fig. 5) on predicted secondary structure for RNA of wild-type *lrp* 5' UTR and initial translated region. Small RNA binding regions on *lrp* strand are indicated as

binding site (BS1, 2, 3, and 4). Positions cleaved after G nucleotides indicated with filled triangles (RNase T1).

(E, F) Gel shift, as for B, C, using both wild-type lrp, lrpmt15 and lrpmt16 derivatives (see panel A), with wild-type GcvB or GcvB_mt8.

(G) β-galactosidase activity from cells with *lrpS1* (HL1517), *lrpS1mt*16 (HL1815), *lrpS3* (HL1701), and *lrpS3mt*16 (HL1816)-*lacZ* translational fusions, in the presence of plasmid overexpressing GcvB or GcvB_mt8 or with the pBRplac empty vector. Error bars indicate standard deviation.

(H) Gel-mobility shift assays as for B, C, but with wild-type lrp, lrpmt14, and lrpmt8 derivatives (see panel A), with wild-type small RNA GcvB or GcvB_mt6.

Fig. S8.

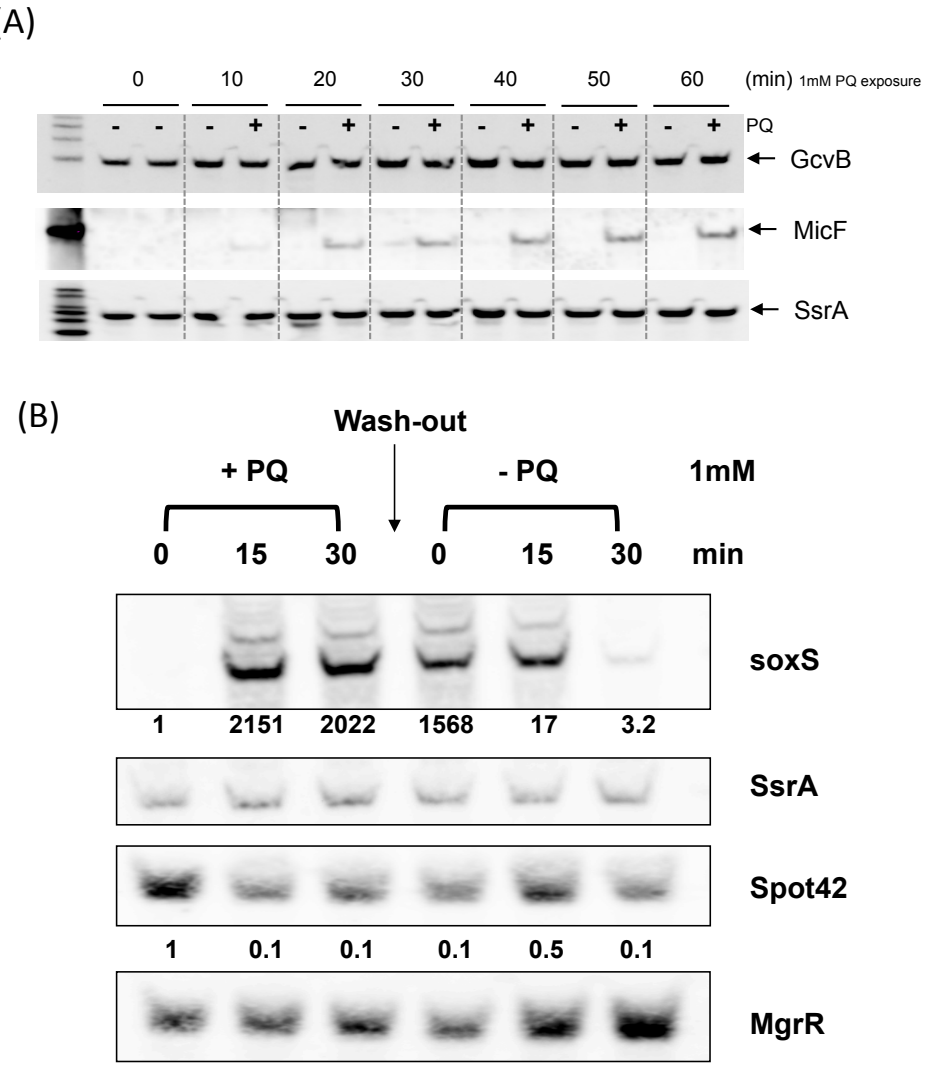


Fig. S8: sRNA levels after paraquat treatment.

(A) Paraquat effects on GcvB and MicF sRNAs. Cells (MG1655) were grown in LB (to OD_{600} of 0.5) and treated with or without 1.0 mM paraquat and allowed to grow for the times indicated. RNA was extracted and probed for GcvB, MicF and SsrA (as a loading control) as described in Materials and Methods.

(B) Paraquat effect on MgrR and *soxS*. MG1655 cells were grown in LB, treated with paraquat for 30 minutes, filtered to remove the paraquat and resuspended and grown. Samples were taken as indicated and analyzed by Northern blot.

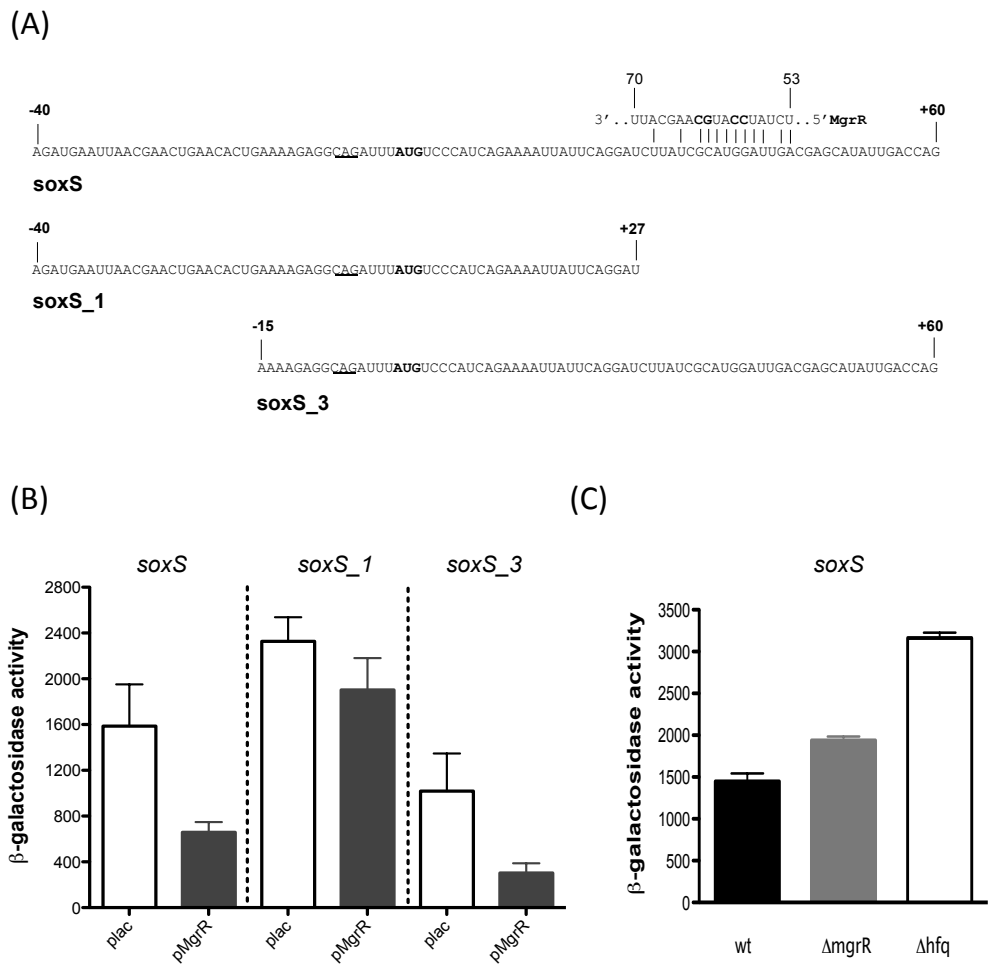


Fig. S9. Deletion analysis of *soxS* fusions.

(A) Sequence of *soxS* 5' UTR and initial translated region to +60 is included in translational fusion and truncated derivatives. Predicted pairing with MgrR is shown. Translation initiation AUG is underlined.

(B) The translational fusions shown in A, *soxS* (HL1064), *soxS*_1 (HL1117), and *soxS*_3 (HL1698) were grown to an OD_{600} of 3 in LB medium containing ampicillin, 100 μM IPTG, and 0.0002% arabinose and assayed for β-galactosidase in the presence of the vector or plasmid expressing MgrR. Error bars indicate standard deviation throughout.

(C) Derivatives of the *soxS* translational fusion containing deletion of *mgrR* (HL1099) or *hfq* (HL1190) were grown to an OD_{600} of 3 in LB medium containing ampicillin, 100 μM IPTG, and 0.0002% arabinose and assayed for β-galactosidase.

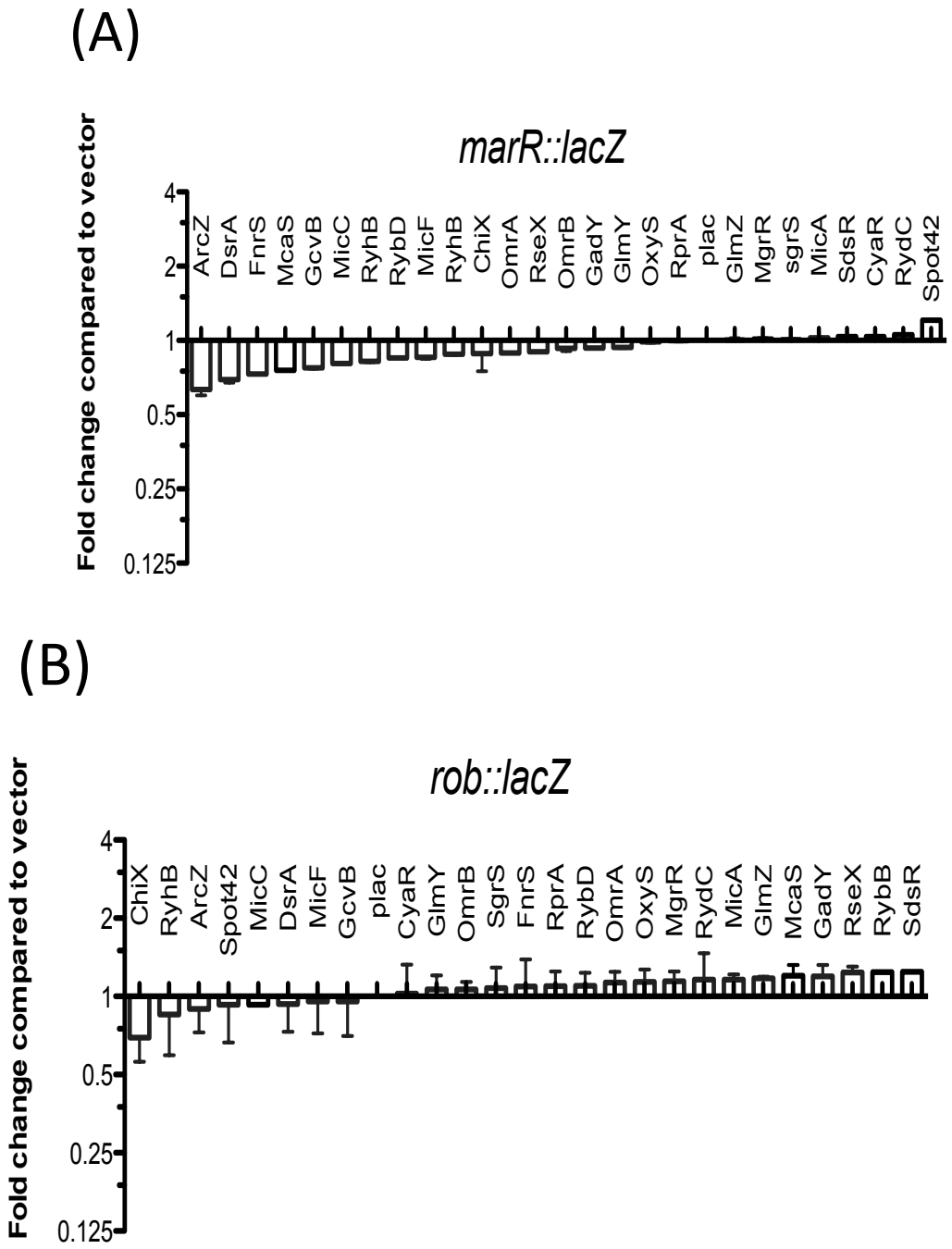


Fig. S10: Mar, Rob sRNA landscapes.

Strains carrying either the (A) *marR*::*lacZ* (HL1667) or (B) *rob*::*lacZ* translational fusion (HL1065) were grown and assayed in the presence of the sRNA plasmid library as for Fig. 1.

Supplementary Tables
Table S1. Strains and plasmids used in this study.

Strain or plasmid	Description	Reference or Source
Strains		
MG1655	Wild type *E. coli*	Laboratory strain
PM1205	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$* *lacI'*::P_{BAD}-*cat-sacB-lacZ* , mini-λtet^R , ϕ80 lysogen	(4)
PM1805	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$* *lacI'*::P_{BAD}-*cat-sacB-lacZ* , mini-λtet^R *trpE+*	PM1205 without ϕ80
KM339	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$ lacI'*::P_{BAD}-*dppA-lacZ*	(5)
HL1044	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$ lacI'*::P_{BAD}-*lrp-lacZ*	PM1205 + PCR (PBAD-LRP-F + lacZ-LRP-R)
HL1061	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$ lacI'*::P_{BAD}-*hns-lacZ*	PM1205 + PCR (PBAD-HNS-F + lacZ-HNS-R)
HL1064	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$ lacI'*::P_{BAD}-*soxS-lacZ*	PM1205 + PCR (PBAD-SOXS-F + lacZ-SOXS-R)
HL1065	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$ lacI'*::P_{BAD}-*rob-lacZ*	PM1205 + PCR (PBAD-ROB-F + lacZ-ROB-R)
HL1069	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$ lacI'*::P_{BAD}-*fnr-lacZ*	PM1205 + PCR (PBAD-FNR-F + lacZ-FNR-R)
HL1070	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$ lacI'*::P_{BAD}-*crp-lacZ*	PM1205 + PCR (PBAD-CRP-F + lacZ-CRP-R)
HL1071	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$* *lacI'*::P_{BAD}-*lrp-lacZ* Δ*gcvB::kan*	HL1044 + P1(Δ*gcvB::kan*)
HL1078	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$* *lacI'*::P_{BAD}-*lrp-lacZ* Δ*micF::cat*	HL1044 + P1(Δ*micF::cat*)
HL1079	MG1655 *mal::lacI^q* Δ*araBAD araC$^+$*	PM1205 + PCR (lrp mt1)

	lacI'::P_{BAD}-*lrp mt1-lacZ*	
HL1083	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp-lacZ* Δ*gcvB::kan* Δ*micF::cat*	HL1071 + P1(Δ*micF::cat*)
HL1098	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS-lacZ,* Δ*spf::cat*	HL1064 + P1(Δ*spf::cat*)
HL1099	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS-lacZ* Δ*mgrR::kan*	HL1064 + P1(Δ*mgrR::kan*)
HL1100	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp-lacZ* Δ*gcvB::kan* Δ*micF::cat* Δ*dsrA::tet*	HL1083 + P1(Δ*dsrA::tet*)
HL1108	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp-lacZ* Δ*dsrA::tet*	HL1044 + P1(Δ*dsrA::tet*)
HL1117	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS 1-lacZ*	PM1205 + PCR (PBAD-SOXS-F + lacZ-SOXS_1-R)
HL1123	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*cat-sacB-lacZ* , mini-λtet^{R,} araE'	PM1205 + araE' (6)
HL1131	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS-lacZ,* Δ*spf::cat, araE'*	HL1098 + araE' (6)
HL1149	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt4-lacZ*	PM1205 + PCR (lrp mt4)
HL1150	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt5-lacZ*	PM1205 + PCR (lrp mt5)
HL1151	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt5-lacZ* Δ*micF::cat*	HL1150 + P1(Δ*micF::cat*)
HL1152	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$	HL1151 + P1(Δ*gcvB::kan*)

	lacI'::P_{BAD}-*lrp mt5-lacZ* Δ*micF::cat* Δ*gcvB::kan*	
HL1154	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt5-lacZ* Δ*micF::cat* Δ*gcvB::kan* Δ*dsrA::tet*	HL1152 + P1(Δ*dsrA::tet*)
HL1189	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp-lacZ* Δ*hfq::cat*	HL1044 + P1(Δ*hfq::cat*)
HL1190	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS-lacZ* Δ*hfq::cat*	HL1064 + P1(Δ*hfq::cat*)
HL1213	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ*	PM1205 + PCR(ilvIH -327F + ilvIH +33R)
HL1214	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ompF-lacZ*	PM1205 + PCR(PBAD-ompF-F + lacZ-ompF-R)
HL1231	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ* Δ*lrp::kan*	HL1213 + P1(Δ*lrp::kan*)
HL1232	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ* Δ*micF::cat*	HL1213 + P1(Δ*micF::cat*)
NM1100	MG1655 mini-λ-Red-tet	(7)
HL1235	MG1655 *lrp*::SPA::kan	NM1100 + PCR(Lrp_SPA_F + Lrp_SPA_R)
HL1434	MG1655 *lrp*::SPA	HL1235 pCP20
HL1437	MG1655 *lrp*::SPA Δ*micF::cat*	HL1434 + P1(Δ*micF::cat*)
HL1438	MG1655 *lrp*::SPA Δ*gcvB::kan*	HL1434 + P1(Δ*gcvB::kan*)
HL1440	MG1655 *lrp*::SPA Δ*gcvB::kan* Δ*micF::cat*	HL1438 + P1(Δ*micF::cat*)
HL1503	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S1-lacZ trpE*+	PM1805 + PCR (PBADlrp_S1_F + lacZ-LRP-R)
HL1505	MG1655 *mal::lacI*^{q}	PM1805 + PCR

	Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S2-lacZ trpE*+	(PBADlrp_S2_F + lacZ-LRP-R)
HL1517	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S1-lacZ trpE*+ Δ*gcvB::kan*	HL1503 + P1(Δ*gcvB::kan*)
HL1518	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S2-lacZ trpE*+ Δ*gcvB::kan*	HL1505 + P1(Δ*gcvB::kan*)
HL1525	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt4-lacZ* Δ*gcvB::kan*	HL1149 + P1(Δ*gcvB::kan*)
HL1551	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt7-lacZ trpE*+	PM1805 + PCR (lrp mt7)
HL1562	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ* Δ*dsrA::zeo*	HL1213 + P1(Δ*dsrA::zeo*)
HL1567	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ* Δ*gcvB::kan*	HL1213 + P1(Δ*gcvB::kan*)
HL1579	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ* Δ*hfq::cat*	HL1213 + P1(Δ*hfq::cat*)
HL1576	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ* Δ*dsrA::zeo* Δ*gcvB::kan*	HL1562 + P1(Δ*gcvB::kan*)
HL1586	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*ilvIH-lacZ* Δ*dsrA::zeo* Δ*gcvB::kan* Δ*micF::cat*	HL1576 + P1(Δ*micF::cat*)
HL1667	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*marR-lacZ trpE*+	PM1805 + PCR (PBAD-ROB-F + lacZ-ROB-R)
HL1693	MG1655 *mal::lacI*^{q}	PM1805 + PCR (lrp

	Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt8-lacZ trpE*+	mt8)
HL1694	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt9-lacZ trpE*+	PM1805 + PCR (lrp mt9)
HL1695	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S3-lacZ trpE*+	PM1805 + PCR (PBADlrp_S3_F + lacZ-LRP-R)
HL1696	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S4-lacZ trpE*+	PM1805 + PCR (PBADlrp_S4_F + lacZ-LRP-R)
HL1697	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S5-lacZ trpE*+	PM1805 + PCR (PBADlrp_S5_F + lacZ-LRP-R)
HL1698	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS_3-lacZ trpE*+	PM1805 + PCR (PBAD-SOXS_3F + lacZ-SOXS-R)
HL1699	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt8-lacZ trpE*+ Δ*gcvB::kan*	HL1693 + P1 (Δ*gcvB::kan*)
HL1700	MG1655 *mal::lacI*^{q} D*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt9-lacZ trpE*+ Δ*gcvB::kan*	HL1694 + P1 (Δ*gcvB::kan*)
HL1701	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S3-lacZ trpE*+ Δ*gcvB::kan*	HL1695 + P1 (Δ*gcvB::kan*)
HL1702	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S4-lacZ trpE*+ Δ*gcvB::kan*	HL1696 + P1 (Δ*gcvB::kan*)
HL1703	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp S5-lacZ trpE*+ Δ*gcvB::kan*	HL1697 + P1 (Δ*gcvB::kan*)
HL1717	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$	PM1805 + PCR (lrp mt10)

	lacI'::P_{BAD}-*lrp mt10-lacZ trpE+*	
HL1719	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt10-lacZ trpE+* Δ*gcvB::kan*	HL1717 + P1 (Δ*gcvB::kan*)
HL1731	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt11-lacZ trpE+*	PM1805 + PCR (lrp mt11)
HL1732	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt12-lacZ trpE+*	PM1805 + PCR (lrp mt12)
HL1734	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt11-lacZ trpE+* Δ*gcvB::kan*	HL1731 + P1 (Δ*gcvB::kan*)
HL1735	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*lrp mt12-lacZ trpE+* Δ*gcvB::kan*	HL1732 + P1 (Δ*gcvB::kan*)
HL1772	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS mt7-lacZ, araE'*	HL1123 + PCR (PBAD-SOX-F + lacZ-SOXS-mt7-R)
HL1755	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS-lacZ,* Δ*spf::cat, araE'*, Δ*mgrR::kan*	HL1131 + P1 (Δ*mgrR::kan*)
HL1775	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS mt7-lacZ, araE'*, Δ*mgrR::kan*	HL1772 + P1 (Δ*mgrR::kan*)
HL1788	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS mt6-lacZ, araE'*	HL1123 + PCR (PBAD-SOX-F + lacZ-SOXS-mt6-R)
HL1790	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$ *lacI'*::P_{BAD}-*soxS mt6-lacZ, araE',* Δ*spf::cat*	HL1788 + P1 (Δ*spf::cat*)
HL1791	MG1655 *mal::lacI*^{q} Δ*araBAD araC*$^+$	HL1790 + P1 (Δ*mgrR::kan*)

	lacI'::P_{BAD}-*soxS mt6-lacZ, araE', Δspf::cat, ΔmgrR::kan*	
HL1799	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp mt13-lacZ trpE+*	PM1805 + gblock (lrp mt13)
HL1801	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp mt14-lacZ trpE+*	PM1805 + gblock (lrp mt14)
HL1803	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp mt13-lacZ trpE+ ΔgcvB::kan*	HL1799 + P1 (Δ*gcvB::kan*)
HL1804	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp mt14-lacZ trpE+ ΔgcvB::kan*	HL1801 + P1 (Δ*gcvB::kan*)
HL1808	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp mt15-lacZ trpE+*	PM1805 + gblock (lrp mt15)
HL1810	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp mt15-lacZ trpE+ ΔgcvB::kan*	HL1808 + P1 (Δ*gcvB::kan*)
HL1813	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp S1mt16-lacZ trpE+*	PM1805 + PCR(lrp2mt*; PBADlrp_S1_F + lacZ-LRP-R)
HL1814	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp S3mt16-lacZ trpE+*	PM1805 + PCR(lrp2mt*; PBADlrp_S3_F + lacZ-LRP-R)
HL1815	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp S1mt16-lacZ trpE+ ΔgcvB::kan*	HL1813 + P1(Δ*gcvB::kan*)
HL1816	MG1655 *mal::lacI^q ΔaraBAD araC$^+$ lacI'*::P_{BAD}-*lrp S3mt16-lacZ trpE+ ΔgcvB::kan*	HL1814 + P1(Δ*gcvB::kan*)

Table S2. Primers and probes used in this study

Primer or probe	Sequence (5' to 3')
Construction of *lacZ* translational fusions	
PBAD-CRP-F1	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GATGCTACAGTAATACATTG
lacZ-CRP-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCGTGGCAATGAGACAAGAACCA
PBAD-FNR-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT ATATCAATTACGGCTTGAGC
lacZ-FNR-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCGCAATGGATAGCACAACCGCC
PBAD-HNS-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT AACAAACCACCCCAATATAA
lacZ-HNS-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCTTCTCTTGCCTGCGCACGAAG
PBAD-LRP-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAAACAGTATT
lacZ-LRP-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCAAGAATGTTACGATCGATACG
PBAD-SOXS-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT AGATGAATTAACGAACTGAA
lacZ-SOXS-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCCTGGTCAATATGCTCGTCAAT
PBAD-ROB-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT ACCTGATGTCAGGTGCTCGT
lacZ-ROB-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCCTGATCCAGATGACCTTCCAG
PBAD-MARRA-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT AACTAATTACTTGCCAGGGCAAC
lacZ-MARRA-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCTTTCTTCTGATTAACCATATGG
PBAD-OMPF-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT AGACACATAAAGACACCAAA
lacZ-OMPF-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCTAACAGAGCAGGGACGATCAC
PBADlrp_Sl_F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAATTTCGGTCTATCGTGACGGG
PBAD-MARR-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT AACTAATTACTTGCCAGGGCAAC
lacZ-MARR-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCTTTCTTCTGATTAACCATATGG
PBAD-ROB-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT ACCTGATGTCAGGTGCTCGT
PBAD-ROB-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCCTGATCCAGATGACCTTCCAG

PBADlrp_S2_F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAATACAGAGAGACAATAATAATG
PBADlrp_S3_F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAATAGCGACTCTGAACAGTGAT
PBADlrp_S4_F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAATCAGACAGGAGTAGGGAAGG
PBADlrp_S5_F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAACAGGAGTAGGGAAGGAATAC
ilvIH -327F	CGAAGCGGCATGCATTTACGTTGACACCATCGAATGGC GCTCAGTGGATG GAAGAGCAAT TAGTCTCAAT
PBAD-SOXS_3-F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT AGATGAATTAAAAAGAGGCAGATTTATGTCCCATCAG
lacZ-SOXS_1-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCATCCTGAATAATTTTCTGAT
lacZ-SOXS-mt6-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCCTGGTCAATATGCTCGTCAATCCATCGGATAAG
lacZ-SOXS-mt7-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCCTGGTCAATATGCTCGTCAATGGATGCGATAAG
ilvIH +33R	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACG ACCATAGCTGTTTCCTGTGTGAGGCCTGCCTCACTGTTT GACGGAAAAAATG
Chromosomal mutation	
Lrp-mt1-F	GGAATACAGAGAGACAATAATAATGGTACGAGCTAAG AAGCGCCCTGGCAAAGATCTCG
Lrp-mt1-R	AAGAATGTTACGATCGATACGGTCGAGATCTTTGCCAG GGCGCTTCTTAGCTCGTACCATTATTATTGTCTCTCTGTA TTCC
Lrp-mt4-F	CTGAACAGTGATGTTTCAGGGTCACTGTCCACTAGGGA AGGAATACAGAGAGAC
Lrp-mt4-R	GTCTCTCTGTATTCCTTCCCTAGTGGACAGTGACCCTGA AACATCACTGTTCAG
Lrp-mt5-R	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG GCAAGAATGTTACGATCGATACGGTCGAGATCTTTGCC AGGGCCGTTGTTCGTATCTAC
Lrp-mt13 (gblock)	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAAACAGTATTCTTATATGCGCATAACCA TGCATGTAAATACCATGTTTACCGTGCTAGTGAAATCTA CGTATGGCGTGCTGTCACGCCATTCGTGATGTCGATAGC TGCCACAAGGCAACGGTCTTCTCACCGTAGACCCAGGC ATTGCGCGCCGTGAATCTTCATGATTTCGGTCTATCGTG ACGGGTAGCGACTCTGAACAGTGATGTTTCAGGGTCAG ACAGGAGTAGGGAAGGAATACAGAGAGACAATAATAA TGGTAGATAGCAAGAAGCGCCCTGGCAAAGATCTCGAC CGTATCGATCGTAACATTCTT
Lrp-mt14 (gblock)	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAAACAGTATTCTTATATGCGCATAACCA

	TGCATGTAAATACCATGTTTACCGTGCTAGTGAAATCTA CGTATGGCGTGCTGTCACGCCATTCGTGATGTCGATAGC TGCCACAAGGCAACGGTCTTCTCACCGTAGACCCAGGC ATTGCGCGCCGTGAATCTTCATGATTTCGGTCTATCGTG ACGGGTAGCGACTCTGAACAGTGATGTTTCAGGGTCAC TGTCGAGTAGGGAAGGAATACAGAGAGACAATAATAAT GGTAGATAGCAAGAAGCGCCCTGGCAAAGATCTCGACC GTATCGATCGTAACATTCTT
Lrp-mt15 (gblock)	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT GGAAGAAAAAAAACAGTATTCTTATATGCGCATAACCA TGCATGTAAATACCATGTTTACCGTGCTAGTGAAATCTA CGTATGGCGTGGAGTCACGCCATTCGTGATGTCGATAG CTGCCACAAGGCAACGGTCTTCTCACCGTAGACCCAGG CATTGCGCGCCGTGAATCTTCATGATTTCGGTCTATCGT GACGGGTAGCGACTCTGAACAGTGATGTTTCAGGGTCA GAGTCGAGTAGGGAAGGAATACAGAGAGACAATAATA ATGGTAGATAGCAAGAAGCGCCCTGGCAAAGATCTCGA CCGTATCGATCGTAACATTCTT
Construction of plasmids	
micF_mt1_F	CAAGATACTGACGTCAGCTCGATCATTAACTTTATTTAT TACC
micF_mt1_R	GGTAATAAATAAAGTTAATGATCGAGCTGACGTCAGTA TCTTG
micF_mt2_F	GACGTCGCTATCATCATTAAGCGCATTTATTACCGTCAT TC
micF_mt2_R	GAATGACGGTAATAAATGCGCTTAATGATGATAGCGAC GTC
micF_mt3_F	CGTCGCTATCATCATTAACTTTATTCTCCAGGGTCATTC ATTTCTGAATGTC
micF_mt3_R	GACATTCAGAAATGAATGACCCTGGAGAATAAAGTTAA TGATGATAGCGACG
dsrA_mt1_F	GGTGTAACGAATTTTTTAAGTCGTTGTTCGTTAAGCAAG TTTCATCCCGACCC
dsrA_mt1_R	GGGTCGGGATGAAACTTGCTTAACGAACAACGACTTAA AAAATTCGTTACACC
gcvB_mt1_F	CGTGTTCTGGTGAACTTTTGGCTTAGAATTGTGATGTTG TGTTGTTGTG
gcvB_mt1_R	CACAACAACACAACATCACAATTCTAAGCCAAAAGTTC ACCAGAACACG
gcvB_mt2_F	TCTGGTGAACTTTTGGCTTACGGTTATAATGTTGTGTTG TTGTGTTTGC
gcvB_mt2_R	GCAAACACAACAACACAACATTATAACCGTAAGCCAAA AGTTCACCAGA
gcvB_mt3_F	CTTTTGGCTTACGGTTGTGACGCTGTGTTGTTGTGTTTGC ACCC
gcvB_mt3_R	GGGTGCAAACACAACAACACAGCGTCACAACCGTAAGC

	CAAAAG
gcvB_mt4_F	CTTTTTTCACTTCCTGTACATTTAGGGACAGTGTCCATA GTGATTAATGTAGCAC
gcvB_mt4_R	GTGCTACATTAATCACTATGGACACTGTCCCTAAATGTA CAGGAAGTGAAAAAAG
gcvB_mt6_F	CTTTTTTCACTTCCTGTACATTTACCGACAGTGTCCATAG TGATTAATGTAGCAC
gcvB_mt6_R	GTGCTACATTAATCACTATGGACACTGTCGGTAAATGTA CAGGAAGTGAAAAAAG
gcvB_mt7_F	CTTTTTTCACTTCCTGTACATTTTGGGACTCTGTCCATAG TGATTAATGTAGCAC
gcvB_mt7_R	GTGCTACATTAATCACTATGGACAGAGTCCCAAAATGT ACAGGAAGTGAAAAAAG
gcvB_mt8_F	CTTTTTTCACTTCCTGTACATTTACCGACTCTGTCCATAG TGATTAATGTAGCAC
gcvB_mt8_R	GTGCTACATTAATCACTATGGACAGAGTCGGTAAATGT ACAGGAAGTGAAAAAAG
In vitro transcription	
Lrp iv -267F	ACG CAC GCT GTA ATA CGA CTC ACT ATA GG GGAAGAAAAA AAACAGTATT
lrp iv -69F	ACG CAC GCT GTA ATA CGA CTC ACT ATA GG TA GCGACTCTGA ACAGTGAT
lrp iv +60R	AAGAATGTTACGATCGATACG
GcvB-VTF	ACG CAC GCT GTA ATA CGA CTC ACT ATA GG ACTTCCTGAG CCGGAACGAA
GcvB-VTR	AAA AAA AGC ACC GCA ATT AGG CGG TGC TAC ATT AAT CAC TAT GGA CAG ACA GG
GcvBmt6-VTR	AAAAAAAGCACCGCAATTAGGCGGTGCTACATTAATCA CTATGGACACTGTCG
GcvBmt8-VTR	AAAAAAAGCACCGCAATTAGGCGGTGCTACATTAATCA CTATGGACAGAGTCGG
VT_T7DsrAF	ACGCACGCTGTAATACGACTCACTATAGGAACACATCA GATTTCCTGGTGTAACGAATTTTTTAAGTGCTTCTTGCTT AAGCAAGTTTCATCCCGACCCCCTCAGGGTCGGGATTT
GC-VT_T7DsrAR	AAATCCCGACCCTGAGGGGGTCGGGATGAAACTTGCTT AAGCAAGAAGCACTTAAAAAATTCGTTACACCAGGAAA TCTGATGTGTTCCTATAGTGAGTCGTATTACAGCGTGCG T
VT_MicFF	ACGCACGCTGTAATACGACTCACTATAGGGCTATCATC ATTAACTTTATTTATTACCGTCATTCATTTCTGAATGTCT GTTTACCCCTATTTCAACCGGATGCCTCGCATTCGGTTT TTTTT
GC-VT_MicFR	AAAAAAAACCGAATGCGAGGCATCCGGTTGAAATAGG GGTAAACAGACATTCAGAAATGAATGACGGTAATAAAT AAAGTTAATGATGATAGCCCTATAGTGAGTCGTATTAC AGCGTGCGT

Construction of SPA tag	
Lrp_SPA_F	AGAAGTCAAGCAGAGTAATCGTCTGGTTATTAAGACGC GCTCCATGGAAAAGAGAAG
Lrp_SPA_R	GAGTGTAATCAAAATACGCCGATTTTGCACCTGTTCCGT G CATATGAATATCCTCCTTAG
Biotinylated probes for Northern blot	
Lrp probe	ATTCAGAGTAATCTCAACGAATACCAGAAGTGATGC
MicF probe	TCCGGTTGAAATAGGGGTAAACAGACATTCAGAAA
Spot 42 probe	GAAGTAAAAGGTCTGAAAGATAGAACATCTTACCTC
GcvB probe	CCAGAACACGCATTCCGATAAAACTTTTCGTTCCGGCTCA
MgrR probe	CAGTAAACCGGCGGTGAATGCTTGCATGGATAG
SoxS probe	GGAACATTCGTTGCAAGTACCACTTTGAATAGCCTG
SsrA probe	CGCCACTAACAAACTAGCCTGATTAAGTTTTAACGCTTCA

Table S3. Regulation of mutant and wild-type *lrp* fusions with sRNAs.

	Strain name	plac	pGcvB	pGcvB_ mt4	pMicF	pMicF_ mt1	pDsrA	pDsrA_ mt1
Lrp::lacZ	HL1044	1,394 (14) 100%	357 (10) 26%	1,002.6 (12.2) 72%	190 (3.6) 14%	865.3 (74.6) 62%	637 (36) 46%	905.5 (95) 65%
Lrp (MicF) mt1::lacZ	HL1079	1,006 (30.6) 100%	350 (86) 35%	ND	859 (180) 85%	198.3 (32.7) 20%	**744.3 (18.4) 74%**	ND
Lrp (GcvB) mt4::lacZ	HL1149	187 (8.3) 100%	103 (0) 55%	168.4 (3.3) 90%	ND	ND	ND	ND
Lrp (DsrA) mt5::lacZ	HL1150	964 (53) 100%	284 (2.6) 29%	ND	**993.5 (6.1) 100%**	ND	495 (18) 51%	355 (30) 37%

Results are the average value of three independent experiments. Errors are the numbers in parentheses. ND: Not done. Level of expression of plac vector control was set to 100%, and expression levels in the presence of an sRNA are compared to this value for each row. Numbers in bold demonstrate effect of *lrp* mutations that lose regulation by MicF on the ability of DsrA to repress and vice versa.

Table S4. **Mutational tests of GcvB regulation of *lrp***

	Strain name & condition	plac	pGcvB	pGcvB_mt4	pGcvB_mt6	pGcvB_mt7	pGcvB_mt8
lrp wt	HL1071 (Δ*gcvB*)	997 (100%)	286 (29%)	1003 (101%)			
lrp wt	HL1071 (Δ*gcvB*)	1049 (100%)	310 (30%)	981 (94%)	994 (95%)	915 (87%)	568 (48%)*
lrp mt1	HL1079	1006 (100%)	350 (35%)				
lrp mt4	HL1149	187 (100%)	102 (55%)	168 (90%)			
lrp mt4	10 uM IPTG	190 (100%)	90 (48%)	160 (84%)			
lrp mt5	HL1150	964 (100%)	284 (29%)				
lrp mt8	HL1699 (Δ*gcvB*)	1633 (100%)	692 (42%)		1517 (93%)		
lrp mt8	10 uM IPTG	1602 (100%)	757 (47%)		1785 (111%)		
lrp mt9	HL1700 (Δ*gcvB*)	142 (100%)	74 (52%)		120 (84%)		
lrp mt10	HL1719 (Δ*gcvB*)	247 (100%)	127 (51%)	267 (108%)			
lrp mt11	HL1734 (Δ*gcvB*)	1046 (100%)	185 (18%)				
lrp mt12	HL1735 (Δ*gcvB*)	128 (100%)	61 (48%)			116 (90%)	
lrp mt15*	HL1810 (Δ*gcvB*)	906 (100%)	711 (78%)				517 (57%)

β-galactosidase activity assay with wild-type, mutant, and truncated *lrp* fusions with wild-type and mutant forms of GcvB multicopy sRNAs. Percentages for each fusion with the plac vector control were set to 100%. The chromosomal gcvB allele is deleted in many of these strains (shown under strain name). IPTG was used at 100 uM unless otherwise noted. Mutant locations in the *lrp* leader are shown in Fig. S3; strains are listed in Table S1. Many of these assays were carried out to try to identify direct pairing by using mutations in possible pairing regions and compensating mutations. However, in general, compensating mutations did not work well, suggesting that secondary or tertiary structure in the lrp leader may be an important factor for proper regulation. * These assays were done separately from others, and the value for pGcvB/*lrp*$^+$ is normalized to the control for that group of experiments (see Fig. 4).

Table S5. Activity of *lrp* translational fusions and *ilvIH* transcriptional fusions in various genetic backgrounds with MOPS glycerol minimal media.

	wt	Δ*dsrA*	Δ*micF*	Δ*gcvB*	Δ*dsrA,micF,gcvB*	Δ*hfq*	Δ*lrp*
Strain name	HL1044	HL1108	HL1078	HL1071	HL1100	HL1189	
lrp::lacZ	2216 (160)	2296 (87)	2326 (147)	1951 (101)	2222 (35)	3417 (167)	
Strain name	HL1213	HL1562	HL1232	HL1567	HL1586	HL1579	HL1231
ilvIH::lacZ	211 (5.5)	218 (2.4)	227 (2.4)	207 (7.5)	214 (12.4)	176 (2.9)	26 (4.0)

Cells were grown in MOPS minimal glycerol media; samples were collected at OD_{600} 0.5. Errors are the numbers in parentheses.

References

1. Sharma, C.M., Papenfort, K., Pernitzsch, S.R., Mollenkopf, H.-J., Hinton, J.C.D. and Vogel, J. (2011) Pervasive post-transcriptional control of genes involved in amino acid metabolism by the Hfq-dependent GcvB small RNA. *Molec. Microbiol.*, **81**, 1144-1165.
2. Modi, S.R., Camacho, D.M., Kohanski, M.A., Walker, G.C. and Collins, J.J. (2011) Functional characterization of bacterial sRNAs using a network biology approach. *Proc Natl Acad Sci U S A*, **108**, 15522-15527.
3. Coornaert, A., Chiaruttini, C., Springer, M. and Guillier, M. (2013) Post-transcriptional control of the Escherichia coli PhoQ-PhoP two-component system by multiple sRNAs involves a novel pairing region of GcvB. *PLoS genetics*, **9**, e1003156.
4. Mandin, P. and Gottesman, S. (2009) A genetic approach for finding small RNAs regulators of genes of interest identifies RybC as regulating the DpiA/DpiB two-component system. *Mol Microbiol*, **72**, 551-565.
5. Moon, K. and Gottesman, S. (2011) Competition among Hfq-binding small RNAs in *Escherichia coli*. *Molecular microbiology*, **82**, 1545-1562.
6. Khlebnikov, A., Datsenko, K.A., Skaug, T., Wanner, B.L. and Keasling, J.D. (2001) Homogeneous expression of the P(BAD) promoter in *Escherichia coli* by constitutive expression of the low-affinity high-capacity AraE transporter. *Microbiology*, **147**, 3241-3247.
7. Bougdour, A., Cunning, C., Baptiste, P.J., Elliott, T. and Gottesman, S. (2008) Multiple pathways for regulation of sigmaS (RpoS) stability in *Escherichia coli* via the action of multiple anti-adaptors. *Molecular microbiology*, **68**, 298-313.