Supplementary Information

Supplementary Methods

Construction of plasmids harboring *lacZ* fusions to *mgtM*

PCR fragments corresponding to nucleotides 1 to 59 of the *mgtCBR* leader were amplified with primer 9801, which includes the sequence corresponding to the p$lac_{1\text{-}6}$ promoter, and either primer 9802 or 9803 (creating a stop codon) using 14028s genomic DNA as a template. The resulting PCR products were digested with *Sma*I and *Xba*I and cloned into plasmid pACYC-'*lacZ* digested with the same enzymes. The sequences of the resulting constructs were verified by DNA sequencing.

Construction of plasmids harboring fusions to a promoterless *gfp* gene

pGFP303, a plasmid with the PhoP-dependent *mgtCBR* promoter and the wild-type *mgtC* leader fused to a promoterless *gfp* gene, was constructed as follows: a PCR fragment generated with primers 1746 and 8117 using 14028s genomic DNA as a template and digested with *Eco*RI and *Xba*I was cloned into plasmid pfpv25 digested with the same enzymes.

Derivatives of pGFP303 with nucleotides substitution in the *mgtCBR* leader region were constructed by cloning PCR fragments generated by two rounds of PCR reactions. For the $A_{44\text{-}46}\rightarrow$T substitution in the *mgtC* leader, a first PCR fragment was generated with primers 1746 and 11727, and a second fragment was generated with primers 11726 and 8117 using 14028s genomic DNA as a template. A third PCR was performed with primers 1746 and 8117 using the two PCR-generated DNA fragments as templates. The

resulting PCR product was cloned into pfpv25 using the same restriction enzymes used for construction of pGFP303. All other substitutions were generated in a similar way using the following primer pairs: $A_{44\text{-}46}$→G (1746/11725 and 11724/8117), $A_{44\text{-}46}$→C (1746/11399 and 11398/8117), $A_{56\text{-}57}$→G (1746/11723 and 11722/8117), and $mgtM_{\text{scrambled}}$ (1746/11950 and 11949/8117).

pfpv25 *mgtA*, a plasmid with the PhoP-dependent *mgtA* promoter and wild-type *mgtA* leader fused to a promoterless *gfp* gene, was constructed as follows: a PCR fragment generated with primers 6208 and 11737 using 14028s genomic DNA as a template and digested with *Eco*RI and *Xba*I and then cloned into pfpv25 digested with the same enzymes. The sequences of the resulting constructs were verified by DNA sequencing.

Construction of plasmids harboring the *mgtA* and *mgtM* ORFs

Plasmid p*mgtA* was constructed as follows: a PCR fragment corresponding to the *mgtA* gene generated by PCR with primers 1561 and 7285 using 14028s genomic DNA as a template, was digested with *Hin*dIII and *Bam*HI and cloned into pUHE 21-2$lacI^q$ digested with the same enzymes.

Plasmid p*mgtM* was constructed as follows: a PCR fragment corresponding to the *mgtM* gene generated by PCR with primers 10074 and 10075 using 14028s genomic DNA as a template, was digested with *Hin*dIII and *Bam*HI and cloned into pUHE 21-2$lacI^q$ digested with the same enzymes.

Construction of a strain with chromosomal deletions of the *mgtC*, *mgtB*, or *mgtCB* genes

Salmonella strains deleted for the *mgtC*, *mgtB*, or *mgtCB* genes were generated by the one-step gene inactivation method [36]. A Km^R cassette was PCR amplified from plasmid pKD4 using primers 1908 and 1909 for the *mgtC* gene and Cm^R cassettes was PCR amplified from plasmid pKD3 using primers 1910 and 1911 for the *mgtB* gene, 1908 and 1911 for the *mgtCB* genes deletion and the resulting PCR products were integrated into 14028s chromosome to generate strains EG13241 (*mgtC*::Km^R), EG13245 (*mgtB*::Cm^R), EG16736 (*mgtCB*::Cm^R), respectively. The *mgtC* (EL4), *mgtB* (EL5) and *mgtCB* deletion (EL6) strains were made by removing the antibiotic resistance cassette from EG13241, EG13245 and EG16736, respectively, using plasmid pCP20 as described [36].

Construction of strains with chromosomal mutations in the *mgtCBR* leader region

Two different methods were used to generate strains with chromosomal mutations in the *mgtCBR* leader. To create stop codon mutants in *mgtM* and with nucleotides substitution in the *mgtCBR* leader region, we used the fusaric acid method as described [16]. We introduced a Tet^R cassette in the *mgtCBR* leader region as follows: we generated a 1990 bp PCR product harboring the *tetRA* genes using as template chromosomal DNA from strain MS7953s and primers 7310/7370. The product was purified and used to electroporate 14028s or EG9527 *Salmonella* containing plasmid pKD46. The resulting *mgtCBR leader*::*tetRA* strains containing plasmid pKD46 (EG18715 and EG18798) were kept at 30°C. Then, we replaced the *tetRA* cassette in the *mgtCBR* leader region using DNA fragments carrying a mutation to create nucleotide substitutions. This was accomplished by preparing a DNA fragment harboring nucleotide substitutions in the *mgtCBR* leader region by a two-step PCR process.

To create strains with stop codon mutations in *mgtM*, DNA fragments carrying stop codons in *mgtM* were prepared as follows: we used two primer pairs 8118/8769 and

8770/7308 (for UAG), 8118/8878 and 8880/7308 (for UAA1), 8118/8881 and 8879/7308 (for UAA2), 8118/8986 and 8987/7308 (for UGA3), 8118/8882 and 8883/7308 (for UAA4) or 8118/9851 and 9850/7308 (for UAG2) and 14028s genomic DNA as a template in the first PCR reaction. For the second PCR reaction, we mixed the two PCR products from the first PCR reaction as templates and amplified the DNA fragment with expected substitution using primers 8118 and 7308. The resulting PCR products were purified and integrated into the EG18798 or EG18715 (for EG19307 and EG19322) chromosome and selected against Tet^R with media containing fusaric acid to generate strains EG19269, EG19285, EG19289, EG19293, EG19298, EL90 (or EL91), EG19307 and EG19322, Tet^S Amp^S chromosomal mutants, respectively.

DNA fragments carrying the A44-46 to T substitution in the *mgtC* leader were prepared by a two-step PCR reaction. For the first PCR reaction, we used two primer pairs 8118/11727 and 11726/7308, and 14028s genomic DNA as a template. For the second PCR reaction, we mixed the two PCR products from the first PCR reaction as templates and amplified the DNA fragment with expected substitution using primers 8118 and 7308. The resulting PCR products were purified and integrated into the EG18715 chromosome and selected against Tet^R with media containing fusaric acid to generate EL341, a Tet^S Amp^S chromosomal mutant. The presence of the expected substitution was verified by sequencing.

All other chromosomal mutants with substitutions in the *mgtCBR* leader were constructed by a multiple step PCR process. Strain EL92 was constructed by inserting a Cm^R cassette in the *yicL* gene, which is 278 nt upstream from *mgtC* transcription start site. The Cm^R cassette was amplified from plasmid pKD3 using primers 4801 and 4802 and the resulting PCR products were integrated into 14028s chromosome to generate EL92 (*yicL*::Cm^R). Then, we prepared DNA fragments containing a Cm^R cassette and

the proper nucleotide substitutions in the *mgtCBR* leader using two primer pairs and EL92 genomic DNA as a template: primer pairs 10077/8265 and 8264/7308 for the C87→G G95→C substitution and primer 10077/8769 and 8770/7308 for UAG mutation in the *mgtM*. The two resulting DNA fragments from the first PCR reactions were mixed and used as PCR templates to amplify DNA fragments containing Cm^R cassette and the proper nucleotide substitution using 10077 and 7308. For EL98, DNA fragments were generated using primer pairs 10077/8265 and 8264/7308 and EL97 genomic DNA as a template. The resulting DNA fragments were purified and integrated into 14028s chromosome by the one-step inactivation method [36] and mutants were selected for resistance to chloramphenicol. The presence of the expected substitution was verified by DNA sequencing.

Quantitative RT-PCR

Total RNA was isolated using RNeasy Kit (Qiagen) according to the manufacturer's instructions. The purified RNA was quantified using a Nanodrop machine (NanoDrop Technologies). cDNA was synthesized using High Capacity RNA-to cDNA Master Mix (Applied Biosystems). The mRNA levels of the *mgtC, mgtB, mgtA* and *rrs* genes were measured by quantification of cDNA using Fast SYBR Green PCR Master Mix (Applied Biosystems, Foster City) and appropriate primers (*mgtC* leader: 6962/6963, *mgtC* coding: 7530/7531, *mgtB* coding: 7763/7764, *mgtA* leader: 7225/7226 and *mgtA* coding: 4308/4309) and monitored using a Fast ABI7500 machine (Applied Biosystems, Foster City). Data were normalized to the levels of 16S ribosomal RNA amplified with primers 6970 and 6971.

β-galactosidase assays

Cells were grown overnight in N-minimal media and washed twice in N-minimal media before resuspending them in N-minimal media with different $MgCl_2$ concentrations for 4 h at 37°C with shaking. The activity was determined as described [37]. Data correspond to two independent experiments conducted in duplicate.

Determining intracellular pH in *Salmonella*

We measured intracellular pH using green fluorescence protein as described with modification [38]. Bacteria harboring a plasmid containing the *gfp* gene expressed from heterologous promoter (pfpv25.1) were grown as shown in Figures 1 and 2b. Cells were normalized by OD_{600} and resuspended in 150 μl of phosphate-buffered saline (PBS) in a 96-well black microplate. Excitation spectra were measured at 30°C from 480 to 510 nm (slit width, 2 nm), using an emission wavelength of 545 nm (slit width, 20 nm) by a Synergy H1 plate reader (BioTek). Spectra were measured for three biological replicates at each pH. Standard curve was determined for green fluorescence protein by measuring fluorescence of samples resuspended in the same buffer at pH 5.5, 6.0, 6.5, 7.0 or 7.5 with addition of 20 mM sodium benzoate, a permeant acid equilibrating cytoplasmic pH with external pH.

In-line probing

Experiments were carried out as described [39] with the following modifications: the *mgtC* leader RNA was synthesized *in vitro* with T7 RiboMAX Large Scale RNA production system (Promega) from the DNA template amplified from wild-type 14028s and primers (8675/11562 for the *mgtC* leader 1-195. DNA templates with $G_{95}\rightarrow C$, $A_{44\text{-}46}\rightarrow T$

or $A_{56\text{-}57}$→G subtitutions were prepared by a two-step PCR reaction. For the first PCR reaction, we used two primer pairs 8675/8175 and 8174/11562 for G_{95}→C substitution, 8675/11727 and 11726/11562 for $A_{44\text{-}46}$→T substitution, 8675/11723 and 11722/11562 for $A_{56\text{-}57}$→G substitution and 14028s genomic DNA as a template. For the second PCR reaction, we mixed the two PCR products from the first PCR reaction as templates and amplified the DNA fragment with expected substitution using primers 8675 and 11562. To probe the structures at different Mg^{2+} concentrations, 1 pmol of 5′ end-labeled *mgtC* leader RNA was incubated in buffer (100 mM KCl, 50 mM Tris (pH 8.3)) with 1, 5, or 20 mM Mg^{2+} for 40 h at room temperature. Reactions were quenched with urea gel loading buffer II (Ambion) and analyzed on a 10 % denaturing polyacrylamide gel.

Examining effect of pH on RNA structure

To determine pH effect on structure of the *mgtC* leader, we used lead (II) acetate as it cleaves single-stranded RNAs nonspecifically. 1 pmol of 5′-end labeled wild-type 1-195 RNA or mutant RNA with $A_{44\text{-}46}$→T substitution was incubated in 10 μl of either pH 8.3 (100 mM KCl, 10 mM Tris (pH 8.3), 10 mM $MgCl_2$) or pH 7.0 (100 mM KCl, 10 mM Tris (pH 7.0), 10 mM $MgCl_2$) buffer for 10 min at 37°C to allow RNA folding. Then, 1 μl of fresh solution of lead (II) acetate (50 mM) was added and incubated for 2 min at 37°C. Reaction was stopped by adding 10 μl of gel loading buffer II (Ambion) on ice and analyzed on a 10 % denaturing polyacrylamide gel.

Supplementary Table 1. Bacterial strains and plasmids used in this study

Strain or Plasmid	Description	Reference or Source
***S. enterica* serovar Typhimurium**		
14028s	wild-type	[40]
EG9521	*mgtA9226*::Mu*d*J	[15]
EG9527	*mgtCB9232*::Mu*d*J	[9]
EG9652	*purB877*::Tn*10*	[9]
EG10232	*phoP* phoQ5996*::Tn*10*	[19]
EG13241	*mgtC*::Km^R	This work
EG13245	*mgtB*::Cm^R	This work
EG16736	*mgtCB*::Cm^R	This work
EG18715	*mgtCBR leader*::*tetRA*/pKD46	This work
EG18798	*mgtCBR leader*::*tetRA mgtC-lac*/pKD46	This work
EG19269	*mgtM* (UAG) *mgtC-lac*	This work
EG19285	*mgtM* (UAA1) *mgtC-lac*	This work
EG19289	*mgtM* (UAA2) *mgtC-lac*	This work
EG19293	*mgtM* (UAA4) *mgtC-lac*	This work
EG19298	*mgtM* (UGA3) *mgtC-lac*	This work
EG19307	*mgtM* (UAG)	This work
EG19322	*mgtM* (UAA4)	This work
EL4	*mgtC*	This work

EL5	*mgtB*	This work
EL6	*mgtCB*	This work
EL86	*mgtC-lac*/pUHE21	This work
EL87	*mgtC-lac*/p*supF*	This work
EL88	*mgtM* (UAA2) *mgtC-lac*/ pUHE21	This work
EL89	*mgtM* (UAA2) *mgtC-lac*/ p*supF*	This work
EL90	*mgtM* (UAG2) *mgtC-lac*/ pUHE21	This work
EL91	*mgtM* (UAG2) *mgtC-lac*/ p*supF*	This work
EL92	*yicL*::Cm^R, *mgtC-lac*	This work
EL96	*yicL*::Cm^R, *mgtC-lac* C_{87}→G G_{95}→C	This work
EL97	*yicL*::Cm^R, *mgtC-lac mgtM* (UAG)	This work
EL98	*yicL*::Cm^R, *mgtC-lac mgtM* (UAG) C_{87}→G G_{95}→C	This work
EL341	*mgtM* (A_{44-46}→T)	This work
EL486	*phoP* phoQ5996*::Tn*10*, *mgtM* (A_{44-46}→T)	This work
EL487	*phoP* phoQ5996*::Tn*10*, *mgtC*	This work

MS7953s	*phoP*::Tn*10*	[40]
Plasmid		
pACYC-*'lacZ*	rep_{p15A} Cm^R *'lacZ*	[30]
pCP20	$rep_{pSC101}{}^{ts}$ Ap^R Cm^R FLP^+ λ *c*I857$^+$	[36]
pKD3	$repR_{R6K}$ Ap^R FRT Cm^R FRT	[36]
pKD46	$rep_{pSC101}{}^{ts}$ Ap^R p_{araBAD} γ β exo	[36]
p*mgtM-'lacZ*	rep_{p15A} Cm^R p_{lac1-6} *mgtM-'lacZ*	This work
p*mgtM* stop-*'lacZ*	rep_{p15A} Cm^R p_{lac1-6} *mgtM* stop-*'lacZ*	This work
pfpv25	ColE1 ori, Ap^R, promoterless *gfp*	[41]
pGFP303	pfpv25 p_{mgtC}-*mgtC* leader 303-*gfp*	This work
pGFP303 A_{44-46}→T	pfpv25 p_{mgtC}-*mgtC* leader 303 (A_{44-46}→T)-*gfp*	This work
pGFP303 A_{44-46}→G	pfpv25 p_{mgtC}-*mgtC* leader 303 (A_{44-46}→G)-*gfp*	This work
pGFP303 A_{44-46}→C	pfpv25 p_{mgtC}-*mgtC* leader 303 (A_{44-46}→C)-*gfp*	This work
pGFP303 A_{56-57}→G	pfpv25 p_{mgtC}-*mgtC* leader 303 (A_{56-57}→G)-*gfp*	This work

pGFP303 $mgtM_{\text{scrambled}}$	pfpv25 p_{mgtC}-*mgtC* leader 303 ($mgtM_{\text{scrambled}}$)-*gfp*	This work
pfpv25 *mgtA*	pfpv25 p_{mgtA}-*mgtA* leader-*gfp*	This work
pfpv25.1	pfpv25 p_{rpsM}-*gfpmut3*	[41]
pUHE21-2*lacI*^{q}	rep_{pMBI} Ap^{R} *lacI*^{q}	[42]
p*mgtC*	pUHE21-*mgtC*	[43]
p*mgtB*	pUHE21-*mgtB*	[43]
p*mgtA*	pUHE21-*mgtA*	This work
p*mgtM*	pUHE21-*mgtM*	This work
p*supF*	pUHE21-s*upF*	[30]

Supplementary Table 2. Primers used in this study

No.	**Sequence (from 5′ to 3′)**
1561	CGCGGATCCATGCTAAAAATCATTACCCGC
1746	GGAATTCCTTTGCTCCATGATGTAC
1908	ATATAGCACGTACTTATTCTTCCAGAAAAAGTGTAGGCTGGAG CTGCTTC
1909	TGGCGTAATGTTGCAATTGAATAAAAAACTACATATGAATATC CTCCTTA
1910	AACACTACACCTTAATTTTGGGGATTCATCGTGTAGGCTGGAG CTGCTTC

1911	AGCGATTCATCTGGGCGATCCTCAAACATTACATATGAATATC CTCCTTA
4308	ACCGCGGTAAATGCGACTAT
4309	TGCCGCGACTTTCAGACA
4801	AAAAGGGATGCTAAACGTCCTGATTGCCGCCGTATTGTGGATA TGAATATCCTCCTTAGT
4802	TTTGCTCCATGATGTACTGCGCGCAAACGCCTGAACTCCCGTG TAGGCTGGAGCTGCTTC
6208	CGGAATTCCGCTCGCGGGTACGTTCGCTTACG
6962	GCAGGAGTAATATGTTGGACAGTCAC
6963	GGGAGATTGCTGCCCACC
6970	CCAGCAGCCGCGGTAAT
6971	TTTACGCCCAGTAATTCCGATT
7225	TTCAGGGTCCATGTCGCC
7226	CCACAAAACTTATGGATTTATGCGT
7285	CCCAAGCTTCTTACTGCCAGCCATAACGTCTGC
7308	ATTGGCGCAAAGAATAATGATCG
7310	AACACCATAGCGTTGCCGCAGTGTTCAGCCCGCGCACATTCTA AGCACTTGTCTCCTGTTTAC
7370	ATCGACATAATATTGTCATTTTTTGTCATAAGAAAAAATTTAA GACCCACTTTCACATTTAAG
7530	CAGCCCGCGCACATTC
7531	TTGTCTCTGGGATTGGCTTTCT
7763	TCAGAAAATGATAAGCAGCATAAAAAA

7764	CCCTGACGATGGCTGTTCA
8117	AAGTCTAGATTAACATACGTTCCTCCATT
8118	TACGTGCAGGCATCATAACAGAGC
8174	GCTTGCCGAACCGCTGGGCAGCAATCTC
8175	GAGATTGCTGCCCAGCGGTTCGGCAAGC
8264	CTGCGCTGCTTGGCGAACCGCTGGGCAGCAATC
8265	GATTGCTGCCCAGCGGTTCGCCAAGCAGCGCAG
8675	GCTCTAGATAATACGACTCACTATAGCAAATTCATGCAGGAGT AATATGTTGG
8769	TGATTTACGTAAAAGTGACTGTCCTACATATTACTCCTGCATG AATTTG
8770	CAAATTCATGCAGGAGTAATATGTAGGACAGTCACTTTTACGT AAATCA
8878	CTTGCCAGATGATTTACGTAAAATTAACTGTCCAACATATTAC TCCTG
8879	GTAATATGTTGGACAGTCACTTTTAAGTAAATCATCTGGCAAG TTAACG
8880	CAGGAGTAATATGTTGGACAGTTAATTTTACGTAAATCATCTG GCAAG
8881	CGTTAACTTGCCAGATGATTTACTTAAAAGTGACTGTCCAACA TATTAC
8882	GAATAGCGTGCGTTAACTTGCCAGTTAATTTACGTAAAAGTGA CTGTCC
8883	GGACAGTCACTTTTACGTAAATTAACTGGCAAGTTAACGCACG

	CTATTC
8986	GCGTTAACTTGCCAGATGATTTCAGTAAAAGTGACTGTCCAAC AT
8987	ATGTTGGACAGTCACTTTTACTGAAATCATCTGGCAAGTTAAC GC
9801	GCTCTAGACTTTACACTTTAAGCTTTTTATGTTTATGTTGTGTGG ACAAATTCATGCAGGAGTAATATG
9802	GTCCCGGGACTTGCCAGATGATTTACGTAAAAGTGACT
9803	GTCCCGGGACTTGCCAGATGATTTACCTAAAAGTGACT
9850	GGACAGTCACTTTTAGGTAAATCATCTGGCA
9851	TGCCAGATGATTTACCTAAAAGTGACTGTCC
10074	CGCGGATCCTTGGACAGTCACTTTTAC
10075	CCCAAGCTTTTAACTTGCCAGATGATT
10077	ACGTATCATC GTCAGGAACTG
11398	GGACAGTCACTTTTACGTCCCTCATCTGGCAAGTT
11399	AACTTGCCAGATGAGGGACGTAAAAGTGACTGTCC
11562	TAAAGCGTGTTTAAACATGA
11722	AAATCATCTGGCGGGTTAACGCACG
11723	CGTGCGTTAACCCGCCAGATGATTT
11724	GGACAGTCACTTTTACGTGGGTCATCTGGCAAGTT
11725	AACTTGCCAGATGACCCACGTAAAAGTGACTGTCC
11726	GGACAGTCACTTTTACGTTTTTCATCTGGCAAGTT
11727	AACTTGCCAGATGAAAAACGTAAAAGTGACTGTCC
11737	GCTCTAGACGGTGAACCAGACGATAAGGC

11949	AGGAGTAATATGTTGGACACGTACCCTAGTTTAAACATCATGG CAAGTTAACGCACGC
11950	GCGTGCGTTAACTTGCCATGATGTTTAAACTAGGGTACGTGTC CAACATATTACTCCT

Supplementary References

36. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A **97**, 6640-6645 (2000).

37. Miller, J. H. Experiments in Molecular Genetics. (Cold Spring Harbor Lab, 1972).

38. Wilks, J. C. & Slonczewski, J. L. pH of the cytoplasm and periplasm of *Escherichia coli*: rapid measurement by green fluorescent protein fluorimetry. J Bacteriol **189**, 5601-5607 (2007).

39. Regulski, E. E. & Breaker, R. R. In-line probing analysis of riboswitches. Methods Mol Biol **419**, 53-67 (2008).

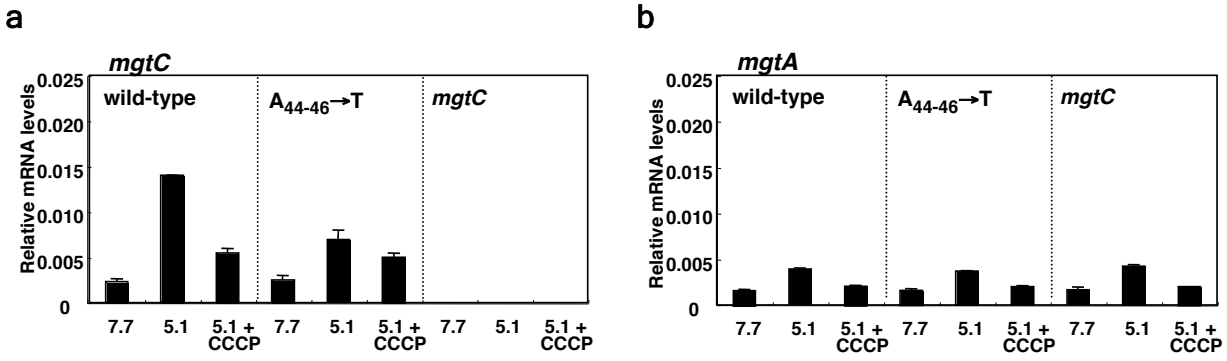
40. Fields, P. I., Groisman, E. A. & Heffron, F. A *Salmonella* locus that controls resistance to microbicidal proteins from phagocytic cells. Science **243**, 1059-1062 (1989).

41. Valdivia, R. H. & Falkow, S. Bacterial genetics by flow cytometry: rapid isolation of *Salmonella typhimurium* acid-inducible promoters by differential fluorescence induction. Mol Microbiol **22**, 367-378 (1996).

42. Soncini, F. C., Vescovi, E. G. & Groisman, E. A. Transcriptional autoregulation of the *Salmonella typhimurium phoPQ* operon. J Bacteriol **177**, 4364-4371 (1995).

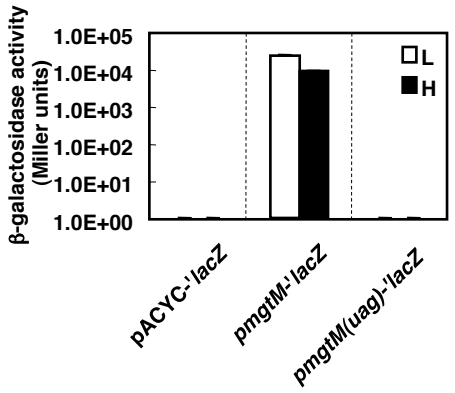
43. Chamnongpol, S. & Groisman, E. A. Mg^{2+} homeostasis and avoidance of metal toxicity. Mol Microbiol **44**, 561-571 (2002).

Supplementary Figure 1

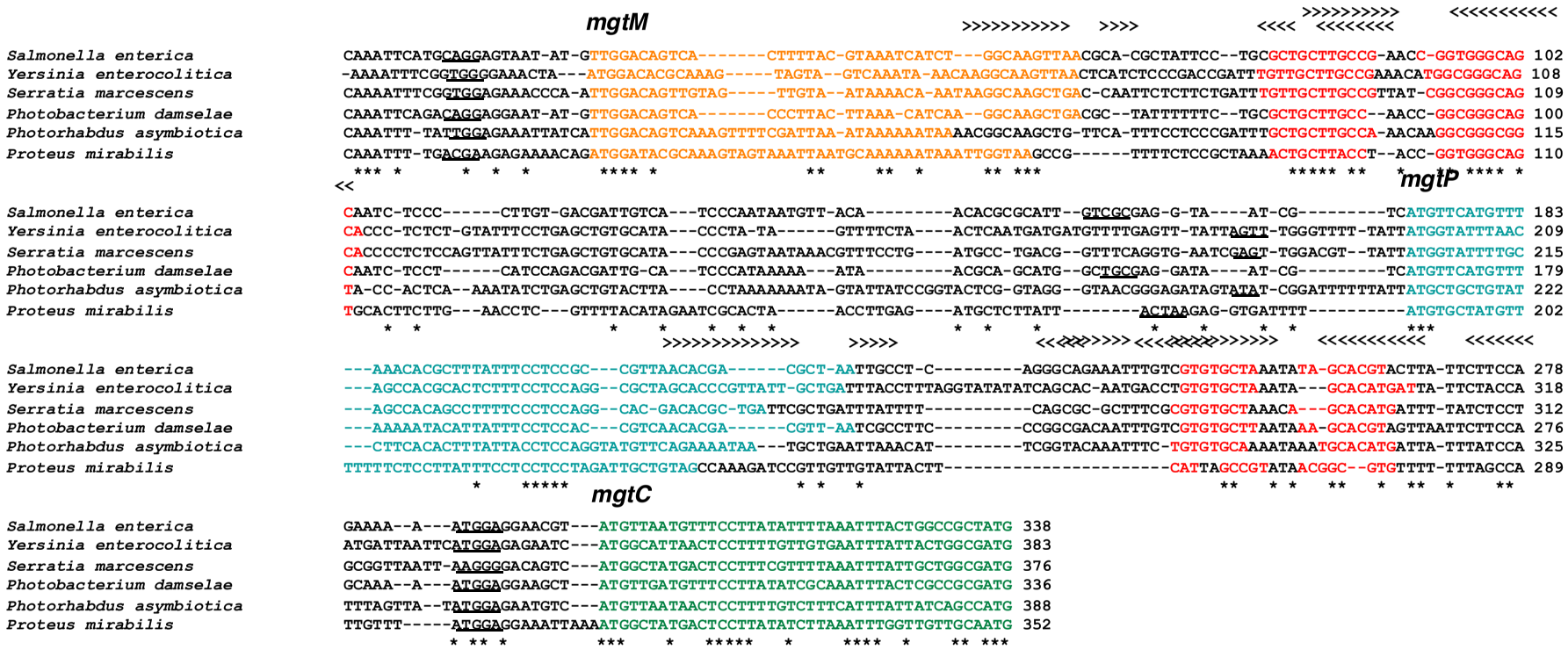


Supplementary Figure 1. Mild acid pH promotes transcription of the *mgtC* coding region in wild-type *Salmonella*. Relative mRNA levels of the coding regions of the *mgtC* **(a)** and *mgtA* **(b)** genes produced by wild-type (14028s) *Salmonella* or derivatives with substitutions of conserved A nucleotides at position 44-46 by Ts in the *mgtCBR* leader (EL341) or deletion of the *mgtC* gene (EL4). Bacteria were grown in N-minimal media with 500 μM Mg^{2+} at pH 7.7 for 1 h and then for an additional 1 h in the same media at pH 5.1 in the absence or presence of 0.5 μM of the protonophore carbonyl cyanide 3-chlorophenylhydrazone (CCCP). Expression levels of target genes were normalized to that of 16S ribosomal RNA *rrs* gene. Shown are the mean and SD from two independent experiments.

Supplementary Figure 2

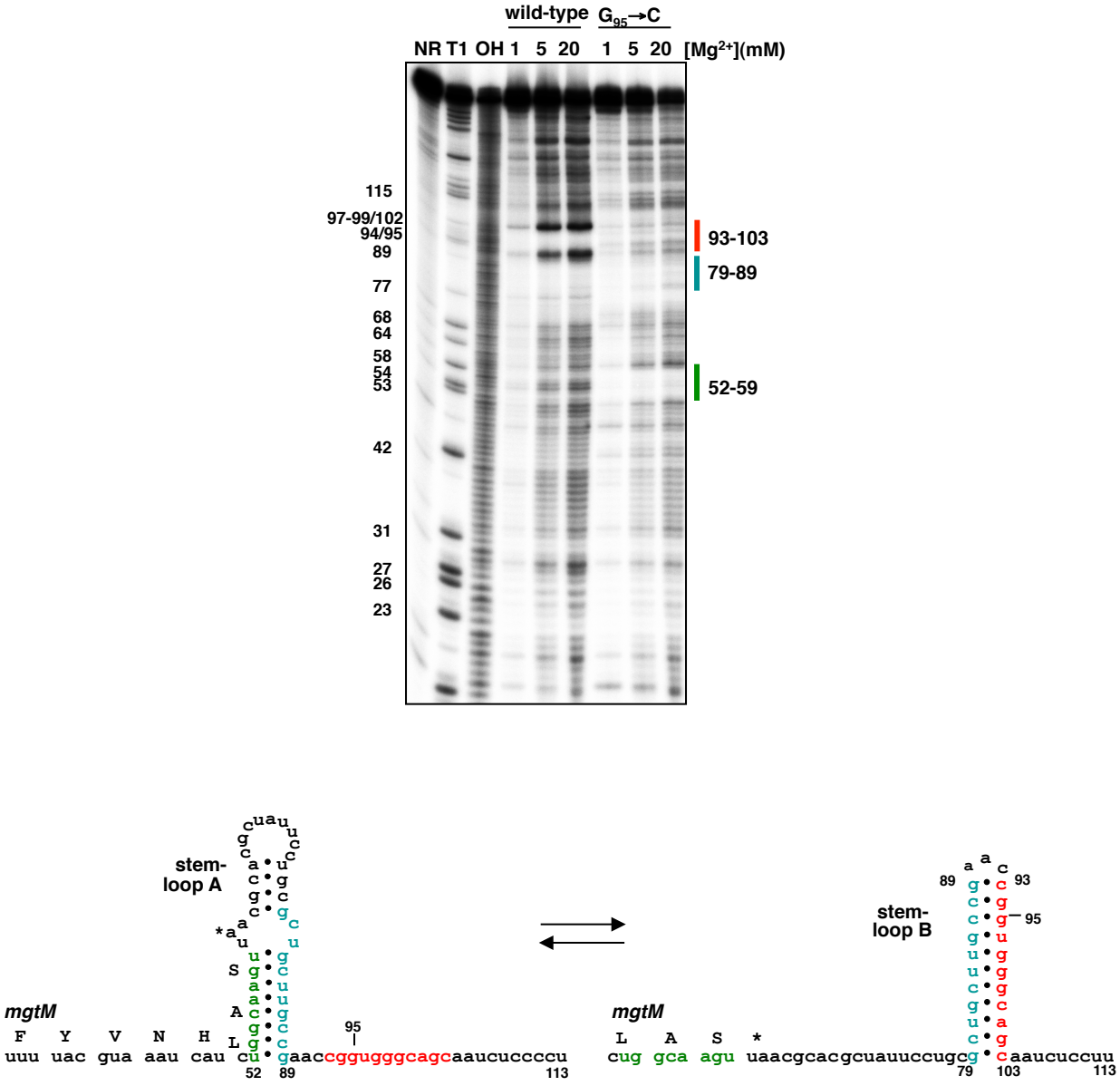


Supplementary Figure 2. The *mgtM* ORF is translated *in vivo*. β-galactosidase activity (Miller units) produced by wild-type *Salmonella* (14028s) harboring either the plasmid vector (pACYC-'*lacZ*), or derivatives with a *lac* translational fusion to the last *mgtM* sense codon (p*mgtM*-'*lacZ*) or following the last (i.e., stop) *mgtM* codon (p*mgtM*(uag)-'*lacZ*). Bacteria were grown in N-minimal media with 10 μM (L) or 10 mM (H) Mg^{2+} for 4 h as described in Methods.



Supplementary Figure 3. Conservation of the *mgtCBR* leader sequence in enteric species. Alignment of the nucleotide sequences corresponding to the *mgtCBR* leader region from *Salmonella enterica*, *Yersinia enterocolitica*, *Serratia marcescens*, *Photobacterium damselae*, *Photorhabdus asymbiotica* and *Proteus mirabilis*. Sequences in orange, blue and green correspond to *mgtM, mgtP* and part of *mgtC,* respectively. Sequences in red represent regions involved in stem-loop structures shown in the following figures. Bracket arrows correspond to sequences with the potential to adopt alternative stem-loop structures. The predicted ribosome binding site for each of the ORFs is underlined. Asterisks correspond to nucleotides conserved in all listed species.

Supplementary Figure 4

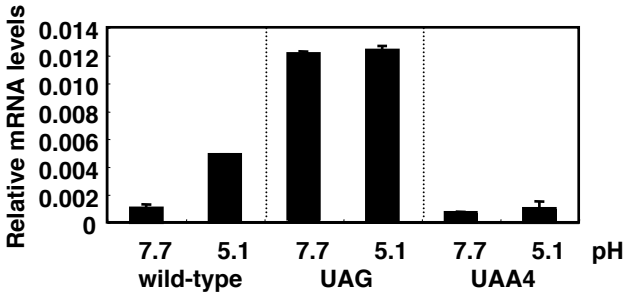


Supplementary Figure 4. Stem-loops A and B can form *in vitro* in a wild-type and a mutant *mgtCBR* leader with G_{95}→C substitution, respectively. In-line probing analysis of 5' ^{32}P-labeled RNA corresponding to nucleotides 1-195 from the wild-type or a mutant with the G_{95}→C substitution in the *mgtCBR* leader. NR, T1, and OH identify rows containing untreated RNA, or RNA subjected to partial digestion with the ribonuclease T1 (which cleaves at unpaired G-residues) or alkali (OH), respectively. Remaining lanes correspond to RNA incubated in the presence of 1, 5 and 20 mM Mg^{2+} as described in Methods. Lines in color correspond to the left and right arms of stem-loop A and B shown at the bottom of the figure.

Supplementary Figure 5

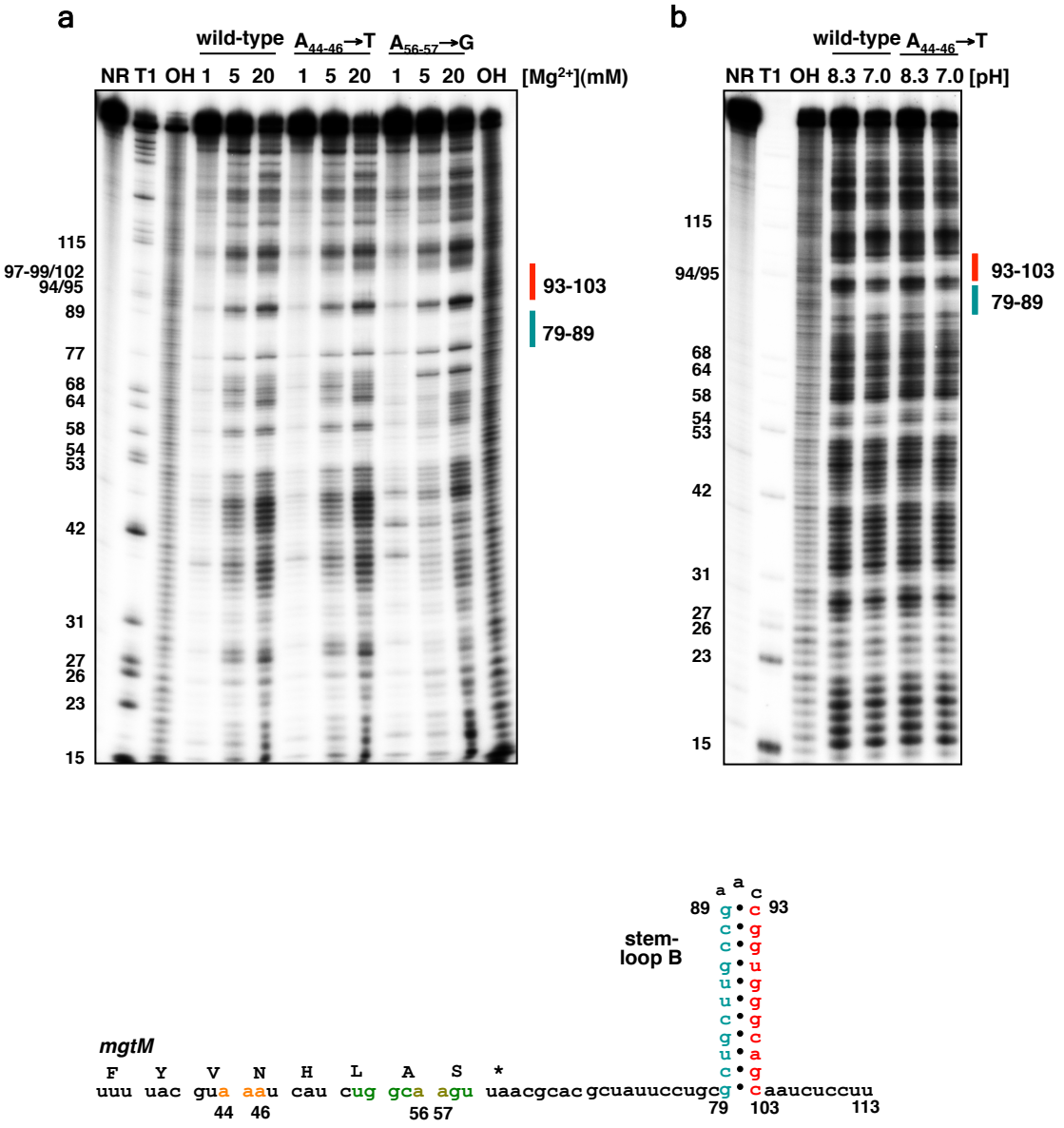
Supplementary Figure 5. Alignment of the deduced amino acid sequences corresponding to *mgtM* from the indicated species. Asterisks correspond to positions conserved in all listed species.

Supplementary Figure 6



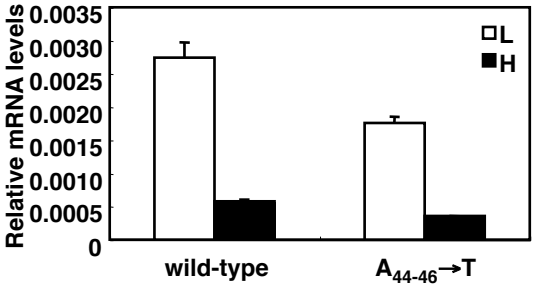
Supplementary Figure 6. Mutation at start codon in *mgtM* derepresses expression of the *mgtC* coding region following mild acid pH induction. Relative mRNA levels of the coding regions of the *mgtC* gene produced by wild-type (14028s) *Salmonella* or derivatives with mutation of the *mgtM* start codon (EG19307) or with a stop codon at the 9th position of *mgtM* (EG19322). Bacteria were grown in N-minimal media with 500 μM Mg^{2+} at pH 7.7 for 1 h and then for an additional 1 h in the same media at pH 5.1. Expression levels of target genes were normalized to that of 16S ribosomal RNA *rrs* gene. Shown are the mean and SD from two independent experiments.

Supplementary Figure 7



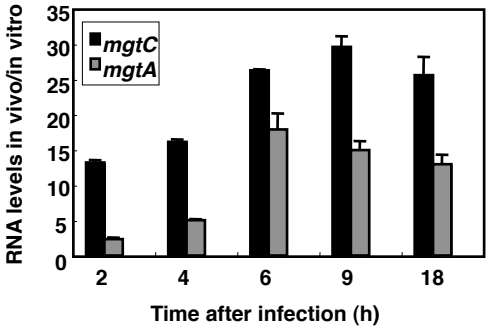
Supplementary Figure 7. Nucleotide substitutions at $A_{44\text{-}46}$ or $A_{56\text{-}57}$, or a change in pH do not affect the structure of the *mgtCBR* leader RNA. **a,** In-line probing analysis of 5' ^{32}P-labeled RNA corresponding to nucleotides 1-195 from the wild-type or mutant RNA with nucleotide substitutions at $A_{44\text{-}46}$ or $A_{56\text{-}57}$ in the *mgtCBR* leader. NR, T1, and OH identify rows containing untreated RNA, or RNA subjected to partial digestion with the ribonuclease T1 (which cleaves at unpaired G-residues) or alkali (OH), respectively. Remaining lanes correspond to RNA incubated in the presence of 1, 5 and 20 mM Mg^{2+} as described in Methods. Lines in color correspond to the left and right arms of stem-loop B. **b,** lead acetate cleavage analysis of 5' ^{32}P-labeled RNA corresponding to nucleotides 1-195 from the wild-type or a mutant RNA with nucleotides substitution at $A_{44\text{-}46}$ in the *mgtCBR* leader. Reactions were performed as described in Supplementary Methods. Lines with numbers correspond to the regions shown at the bottom of the figure.

Supplementary Figure 8



Supplementary Figure 8. A *mgtCBR* leader with conserved A nucleotides at position 44-46 substituted by Ts retains a wild-type ability to respond to Mg^{2+}. Relative mRNA levels of the coding region of the *mgtC* gene produced by *Salmonella* strains lacking the Mg^{2+} sensor PhoQ and harboring a *phoQ*-independent allele of *phoP* (termed *phoP**) (EG10232) or a derivative with conserved A 44-46 nucleotides in the chromosomal region corresponding to the *mgtCBR* leader region substituted by Ts (EL486) following growth for 4 h in N-minimal media with 10 μM Mg^{2+} (L) or 10 mM (H) Mg^{2+} as described in Methods.

Supplementary Figure 9



Supplementary Figure 9. Expression of the *mgtC* and *mgtA* leader regions increases inside macrophage. Relative mRNA levels of the *mgtC* and *mgtA* leader regions of wild-type *Salmonella* (14028s) inside J774 A.1 macrophages at the indicated times after infection. Analysis was carried out as described in the legend to Figure 3 and in Methods.