Supporting Information

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SI Materials and Methods

Construction of a Plasmid Harboring the *proC* ORF. Plasmid p*proC* was constructed as follows: a PCR fragment corresponding to the *proC* gene, generated by PCR with primers W481 and W482 using 14028s genomic DNA as a template, was digested with HindIII and BamHI and cloned into pBR322 digested with the same enzymes. The sequence of the resulting construct was verified by DNA sequencing.

Construction of a Strain with Chromosomal Deletions of the *proC* or *thrB* Genes. *Salmonella* strains deleted for the *proC* or *thrB* genes were generated by the one-step gene inactivation method (1). A chloramphenicol resistance Cm^R cassette was PCR amplified from plasmid pKD3 using primers W469/W470 (for *proC*) and W463/W464 (for *thrB*), and the resulting PCR product was integrated into the 14028s chromosome to generate EL605 (*proC*::Cm^R) and EL601 (*thrB*::Cm^R), respectively.

Construction of Strains with Chromosomal Mutations in the *mgtCBR* Leader Region. To generate strains with chromosomal mutations in the *mgtCBR* leader, we used the fusaric acid method as described (2). DNA fragments carrying proline to either leucine or threonine codons substitutions in *mgtP* were prepared by a two-step PCR. For the first PCR, we used two sets of primer pairs 8118/W472 and W471/7308 (for leucine substitution) and 8118/W466 and W465/7308 (for threonine substitution), and 14028s genomic DNA as template. For the second PCR, we mixed the two PCR products from the first PCR as templates and amplified a DNA fragment using primers 8118 and 7308. The resulting PCR products were purified and integrated into the EG18715 chromosome and selected against tetracycline resistance Tet^R with media containing fusaric acid to generate EL606 ($mgtP_{Pro\rightarrow Leu}$) and EL611 ($mgtP_{Pro\rightarrow Thr}$), tetracycline-sensitive, ampicillin-sensitive Tet^S Amp^S chromosomal mutants, respectively. The presence of the expected nucleotide substitutions was verified by DNA sequencing.

A P22 phage lysate grown in strain EG19886 was used to transduce strains EL606, EL611, and EL605 *Salmonella* selecting for tetracycline resistance to generate EL373 (*proB1657*::Tn*10*, $mgtP_{Pro\rightarrow Leu}$), EL417 (*proB1657*::Tn*10,* $mgtP_{Pro\rightarrow Thr}$), and EL625 (*proB1657*::Tn*10, proC*::Cm^R), respectively.

1. Prost LR, et al. (2007) Activation of the bacterial sensor kinase PhoQ by acidic pH. *Mol Cell* 26(2):165–174.

2. Bader MW, et al. (2005) Recognition of antimicrobial peptides by a bacterial sensor kinase. *Cell* 122(3):461–472.

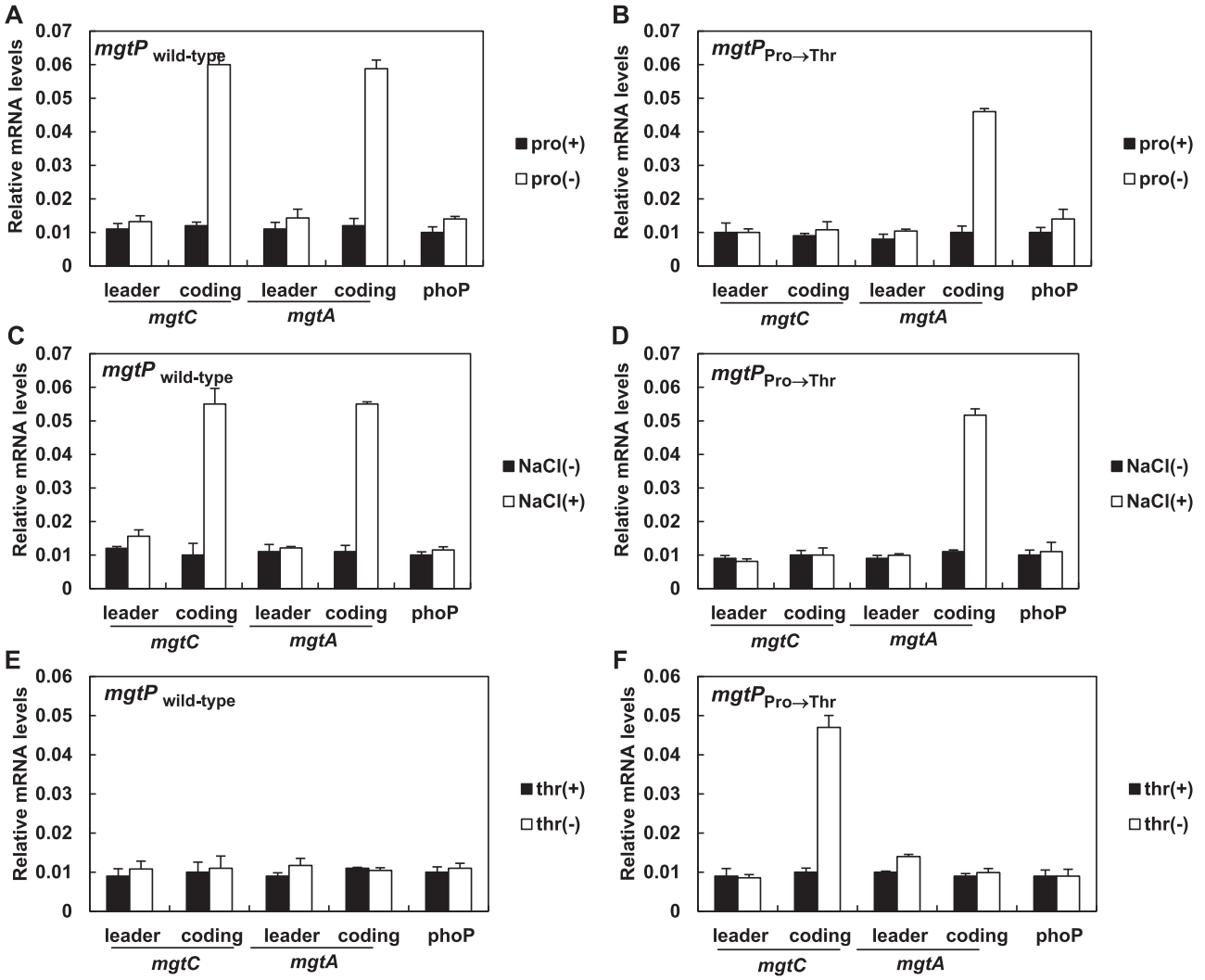

Fig. S1. mRNA levels of the *mgtA* and *mgtC* leader regions and the *mgtA*, *mgtC*, and *phoP* coding regions (relative to those of 16S rRNA *rrs* gene). The ratios of these data are presented in Fig. 2. The legend to Fig. 2 describes the strains and experimental details.

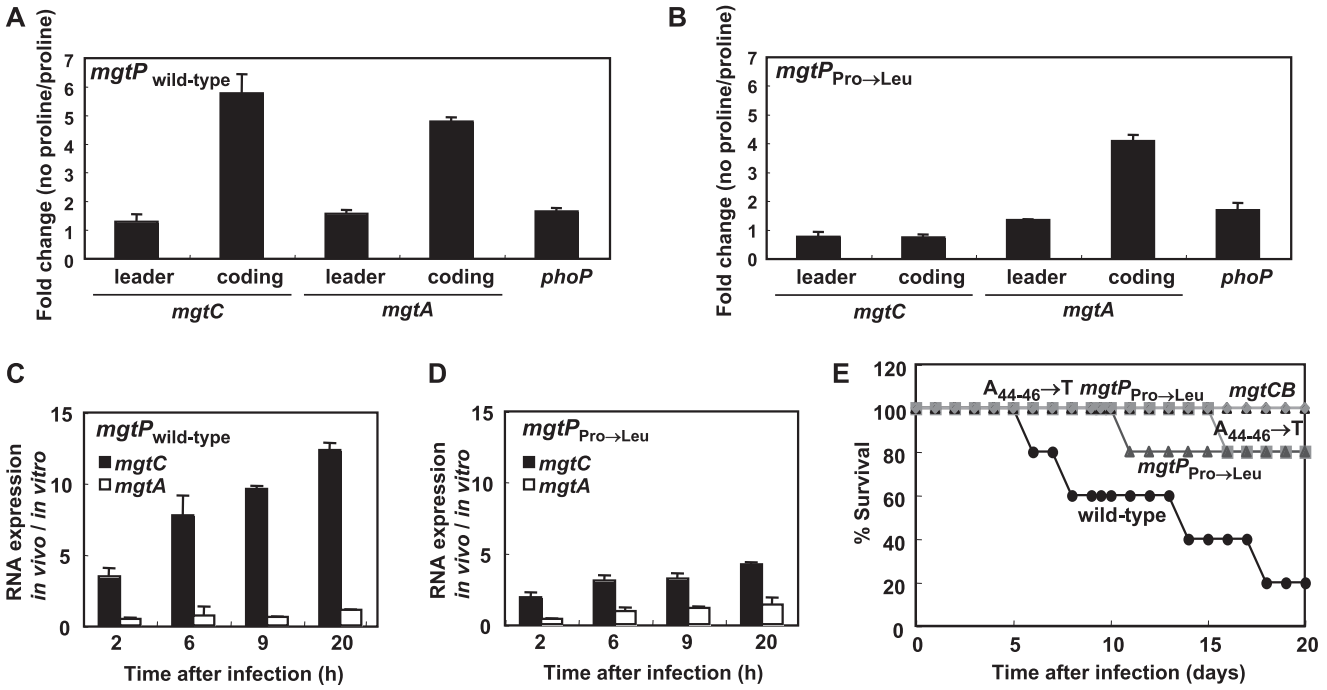

Fig. S2. Substitution of proline codons in *mgtP* by leucine codons decreases *mgtC* expression inside macrophages and attenuates virulence in mice. (*A* and *B*) Fold change in the mRNA levels of the leader regions of the *mgtC* and *mgtA* transcripts and the coding regions of the *mgtC*, *mgtA*, and *phoP* genes produced by a proline auxotroph harboring either the wild-type *mgtCBR* leader (*A*) (EG19886) or a derivative where the three *mgtP* Pro codons were substituted by Leu codons (*B*) ($mgtP_{Pro\rightarrow Leu}$; EL373). Bacteria were grown in *N*-minimal media with 500 μM Mg^{2+} in the presence of 1 mM proline for 1 h, and then grown for 45 min in media containing or lacking proline. Expression levels of target genes were normalized to that of the 16S ribosomal RNA *rrs* gene. Fold change was calculated by dividing the mRNA levels of cells grown in the absence of proline by that of cells grown in the presence of proline. Shown are the means and SDs from two independent experiments. (*C*) Relative mRNA levels of the *mgtC* and *mgtA* coding regions of wild-type *Salmonella* harboring either the wild-type *mgtCBR* leader (14028s) (*C*) or a mutant *Salmonella* with the Pro codons in the *mgtP* substituted by Leu codons (EL606) (*D*) inside J774 A.1 macrophages at the indicated times after infection. (*E*) Survival of C3H/HeN mice inoculated intraperitoneally with ~10^3 colony-forming units of wild-type *Salmonella* (14028s) or an *mgtP* mutant with the Pro codons replaced by Leu codons (EL606) or ATP-sensing defective leader mutant (EL341) or both (EL610) or deleted for both the *mgtC* and *mgtB* coding regions (EL6).

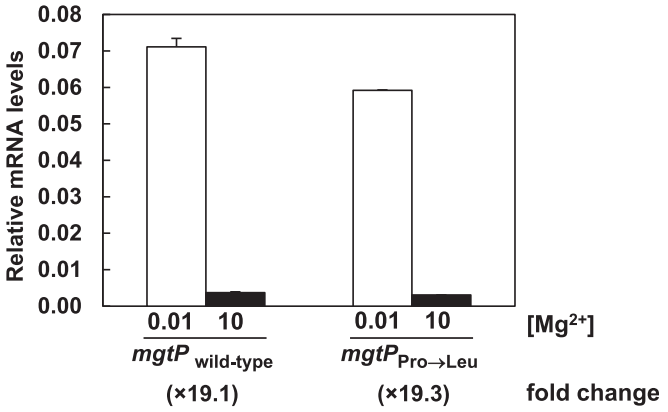

Fig. S3. A *Salmonella* mutant where the *mgtP* proline codons were substituted by leucine codons retains a wild-type ability to promote expression of the *mgtC* coding region in response to low Mg^{2+}. Relative mRNA levels of the *mgtC* coding region of wild-type *Salmonella* harboring either the wild-type *mgtCBR* leader (14028s) or a mutant *Salmonella* with the Pro codons in the *mgtP* substituted by Leu codons (EL606) grown in *N*-minimal media containing either 0.01 or 10 mM Mg^{2+}. Bacteria were grown in *N*-minimal media with 0.01 or 10 Mg^{2+} for 4 h. Expression levels of target genes were normalized to that of 16S ribosomal RNA *rrs* gene. Shown are the means and SDs from two independent experiments.

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
Salmonella enterica serovar Typhimurium strains		
14028s	Wild type	(1)
EG18715	*mgtCB leader*::*tetRA*/pKD46	(2)
EG19886	*proB1657*::Tn*10*	(3)
EL6	*mgtCB*	(2)
EL341	*mgtM* ($A_{44–46}$→T)	(2)
EL373	*proB1657*::Tn*10*, $mgtP_{Pro→Leu}$	This work
EL417	*proB1657*::Tn*10*, $mgtP_{Pro→Thr}$	This work
EL601	*thrB*::Cm^R	This work
EL602	*mgtM* ($A_{44–46}$→T) $mgtP_{Pro→Thr}$	This work
EL605	*proC*::Cm^R	This work
EL606	$mgtP_{Pro→Leu}$	This work
EL610	*mgtM* ($A_{44–46}$→T) $mgtP_{Pro→Leu}$	This work
EL611	$mgtP_{Pro→Thr}$	This work
EL621	*thrB*::Cm^R $mgtP_{Pro→Thr}$	This work
EL625	*proB1657*::Tn*10, proC*::Cm^R	This work
Plasmids		
pCP20	$rep_{pSC101}{}^{ts}$ Ap^R Cm^R FLP^+ λ cI857$^+$	(4)
pKD3	$repR_{R6K}$ Ap^R FRT Cm^R FRT	(4)
pKD46	$rep_{pSC101}{}^{ts}$ Ap^R p_{araBAD} γ β exo	(4)
pBR322	pMB1 ori, Ap^R Tc^R	(5)
p*proC*	pBR322-*proC*	This work

1. Heithoff DM, et al. (1999) Coordinate intracellular expression of Salmonella genes induced during infection. *J Bacteriol* 181(3):799–807.
2. Lee EJ, Groisman EA (2012) Control of a *Salmonella* virulence locus by an ATP-sensing leader messenger RNA. *Nature* 486(7402):271–275.
3. Park SY, Cromie MJ, Lee EJ, Groisman EA (2010) A bacterial mRNA leader that employs different mechanisms to sense disparate intracellular signals. *Cell* 142(5):737–748.
4. Prost LR, et al. (2007) Activation of the bacterial sensor kinase PhoQ by acidic pH. *Mol Cell* 26(2):165–174.
5. Cromie MJ, Shi Y, Latifi T, Groisman EA (2006) An RNA sensor for intracellular Mg(2+). *Cell* 125(1):71–84.

Table S2. Primers used in this study

No.	Sequence, from 5′ to 3′
4308	ACCGCGGTAAATGCGACTAT
4309	TGCCGCGACTTTCAGACA
4489	GATGAAGACGGCCTTTCCTTAA
4490	GAACCGGCAGTGAAACATCA
6962	GCAGGAGTAATATGTTGGACAGTCAC
6963	GGGAGATTGCTGCCCACC
6970	CCAGCAGCCGCGGTAAT
6971	TTTACGCCCAGTAATTCCGATT
7225	TTCAGGGTCCATGTCGCC
7226	CCACAAAACTTATGGATTTATGCGT
7308	ATTGGCGCAAAGAATAATGATCG
7530	CAGCCCGCGCACATTC
7531	TTGTCTCTGGGATTGGCTTTCT
8118	TACGTGCAGGCATCATAACAGAGC
W463	GTTTGCCGATCTGTTACGGACCCTCTCATGGAAGTTAGGAGTTTAACATGGTGTAGGCTGGAGCTGCTTC
W464	AAGCTGACCTGCTCATTATGGTCTTTCAGATTATAGAGTTTCATTGATTAATATGAATATCCTCCTTAG
W465	GTTTAAACACGCTTTATTTACCACCACCTTAACACGACGCTAATTGC
W466	GCAATTAGCGTCGTGTTAAGGTGGTGGTAAATAAAGCGTGTTTAAAC
W469	GTTAAGCTAACCATTCCCCATAACACACAAACATAGGGAGTGACGAGATGGTGTAGGCTGGAGCTGCTTC
W470	GCCGGACGTAACCGCACCGAAGTGGCGGCATGACGTCCAGCCGGGCTTCAATATGAATATCCTCCTTAG
W471	TCATGTTTAAACACGCTTTATTTCTCCTCCTCTTAACACGACGCTAATTGC
W472	GCAATTAGCGTCGTGTTAAGAGGAGGAGAAATAAAGCGTGTTTAAACATGA
W481	CGCGGATCCCGCGGTACAAAATTTCTTTA
W482	CCCAAGCTTCGAGTGCATGAACGGCTAAA
W688	GGATTATCCGGCTCGGGTAA
W689	GAGCGCAAATGACTGGAAGAC
W696	GCGCCGACATCAACGTATTT
W697	GCCAACAAAGCGCAGAACTT
W718	CGACCCCCGACACCCCATGACGGT
W722	ACGACCTTCGCATTACGAATGCGC