

**Table S1.** Bacterial strains, plasmids, and oligonucleotides used in this study.

## (A) Bacterial strains and plasmids

Name	Description	Reference
<b>S. enteric serovar Typhimurium</b>		
14028s	wild-type	Fields et al. (1986)
SM101	<i>ΔphoU</i>	Choi et al. (2022)
SM323	<i>phoU</i> Ala 147 Glu	Choi et al. (2022)
SM108	pBAD33- <i>phoU</i> -His, pTGFP	This study
SM109	pBAD33- <i>phoU</i> -His, pTGFP- <i>phoR</i>	This study
SM343	pBAD33- <i>phoU</i> Ala 147 Glu-His, pTGFP- <i>phoR</i>	This study
SM454	<i>phoR</i> -8×Myc	Choi et al. (2022)
SM443	<i>phoU</i> Ala 147 Glu, <i>phoR</i> -8×myc::Km <sup>R</sup>	This study
SM457	<i>phoU</i> Ala 147 Glu, <i>phoR</i> -8×myc	This study
<b>Escherichia coli</b>		
DH5α	<i>fhuA2 lac(del)U169 phoA glnV44 φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i> .	Taylor, Walker et al. (1993)
SM329	DH5α/pBAD33- <i>phoU</i> Ala 147 Glu-His	This study
BTH101	F-, <i>cya-854</i> , <i>recA1</i> , <i>endA1</i> , <i>gyrA96</i> ( <i>Nal</i> ), <i>thi1</i> , <i>hsdR17</i> , <i>spoT1</i> , <i>rfbD1</i> , <i>glnV44(AS)</i>	Karimova, et al. (1998)
LJ18	BTH101/pUT18-mgtC, pKT25-mgtR	Choi et al. (2019)
LJ27	BTH101/pUT18-mgtC, pKT25	Choi et al. (2019)
SM089	BTH101/pUT18- <i>phoU</i> , pKT25- <i>phoR</i>	Choi et al. (2022)
SM314	BTH101/pUT18c- <i>phoU</i> Ala 147 Glu, pKT25- <i>phoR</i>	Choi et al. (2022)
SM315	BTH101/pUT18c- <i>phoU</i> Ala 147 Lys, pKT25- <i>phoR</i>	Choi et al. (2022)
SM311	BTH101/pUT18c- <i>phoU</i> Arg 148 Ala, pKT25- <i>phoR</i>	Choi et al. (2022)
SM210	BTH101/pUT18- <i>phoU</i> , pKT25- <i>pstB</i>	This study
SM326	BTH101/pUT18c- <i>phoU</i> Ala 147 Glu, pKT25- <i>pstB</i>	This study
SM327	BTH101/pUT18c- <i>phoU</i> Ala 147 Lys, pKT25- <i>pstB</i>	This study
SM328	BTH101/pUT18c- <i>phoU</i> Arg 148 Ala, pKT25- <i>pstB</i>	This study
<b>Plasmids</b>		
pBAD33	pACYC184 ori Cm <sup>R</sup>	Guzman et al. (1995)
pBOP508	rep <sub>R6K</sub> Ap <sup>R</sup> 8×myc FRT Km <sup>R</sup> FRT	Cho et al. (2006)
pKD46	rep <sub>pSC101<sup>ts</sup></sub> Ap <sup>R</sup> P <sub>araBAD</sub> γ β exo	Datsenko and Wanner (2000)
pCP20	rep <sub>pSC101<sup>ts</sup></sub> Ap <sup>R</sup> Cm <sup>R</sup> cI857 λP <sub>Rflp</sub>	Datsenko and Wanner (2000)
pUT18	p <sub>lac</sub> ColE1 ori Ap <sup>R</sup>	Karimova, et al. (2001)
pUT18c	p <sub>lac</sub> ColE1 ori Ap <sup>R</sup>	Karimova, et al. (2001)

pKT25	<i>p<sub>lac</sub> p15Aori Km<sup>R</sup></i>	Karimova, et al. (2001)
pTGFP	<i>ColE1 ori Ap<sup>R</sup> 'gfp</i>	Lee and Groisman (2012)

(B) Primers used in this study.

Name	Sequence (from 5' to 3')*	
<b>Knockout, deletion, tagging</b>		
KU452	TGA GCT GGA TAA GCT GCT GGC GGG GAA AGA TCC GAA AGA GAT CGG ATC CAG AAT TCG TGA T	<i>phoR</i> -8×Myc Km <sup>R</sup> insertion
KU453	GGC CCG GTA AGC GCA GCG CCA CCG GGC AAA AGA ATG AGA TGA GCT CGA TCC GTC GAC C	<i>phoR</i> -8×Myc Km <sup>R</sup> insertion
<b>Cloning</b>		
KHU767	CCC AAG CTT TTA GTG GTG ATG GTG ATG ATG CTC TTT CGG ATC TTT CCC CG	<i>phoU</i> -His-HindIII-R
KHU768	GCT CTA GAT AAT TCA GGA GTG CGT AAT GGA CAG TCT GAA CCT TAA	<i>phoU</i> -XbaI-F
<b>For qRT-PCR</b>		
KHQ015	CGCTGGGAAGCTGAGTTG	<i>phoE</i> qPCR-F
KHQ016	CCAGGCCTAACATCGTACA	<i>phoE</i> qPCR-R
KHQ097	CATATTCCGCCAGTTAAC	<i>phoU</i> qPCR-F
KHQ098	CGGTGATAGCGTCAGAAAG	<i>phoU</i> qPCR-R
6970	CCAGCAGCCCGCGTAAT	<i>rrsH</i> qPCR -F
6971	TTTACGCCAGTAATTCCGATT	<i>rrsH</i> qPCR -R

## References

- Cho BK, Knight EM, Palsson BO. 2006. PCR-based tandem epitope tagging system for *Escherichia coli* genome engineering. *Biotechniques*. 40: 67–72.
- Choi S, Choi E, Cho YJ, Nam D, Lee J, et al. 2019. The *Salmonella* virulence protein MgtC promotes phosphate uptake inside macrophages. *Nat Commun*. 10: 3326.
- Choi S, Jeong G, Choi E, Lee EJ. 2022. A dual regulatory role of the PhoU protein in *Salmonella Typhimurium*. *mBio*. 13: e0081122.
- Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA*. 97: 6640–6645.
- Fields PI, Swanson RV, Haidaris CG, Heffron F. 1986. Mutants of *Salmonella Typhimurium* that cannot survive within the macrophage are avirulent. *Proc Natl Acad Sci USA*. 83: 5189–5193.
- Guzman LM, Belin D, Carson MJ, Beckwith J. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol*. 177: 4121–4130.
- Karimova G, Pidoux J, Ullmann A, Ladant D. 1998. A bacterial two-hybrid system based on a reconstituted signal transduction pathway. *Proc Natl Acad Sci USA*. 95:5752–5756.
- Karimova G, Ullmann A, Ladant D. 2001. Protein-protein interaction between *Bacillus stearothermophilus* tyrosyl-tRNA synthetase subdomains revealed by a bacterial two-hybrid system. *J Mol Microbiol Biotechnol*. 3:73–82.
- Lee EJ, Groisman EA. 2012. Tandem attenuators control expression of the *Salmonella mgtCBR* virulence operon. *Mol Microbiol*. 86:212–224.
- Taylor RG, Walker DC, McInnes RR. 1993. *E. coli* host strains significantly affect the quality of small scale plasmid DNA preparations used for sequencing. *Nucleic Acids Res*. 21:1677–1678.