Supplementary material: Figures S1 to S7 and Tables S1 / S2.

OmpR and Prc contribute to switch the *Salmonella* morphogenetic program in response to phagosome cues

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FIG S1. Unspecific band detected with the anti-FLAG antibody in both untagged and tagged *S.* Typhimurium strains.

FIG S2. The carboxypeptidase PBP6 (DacC), which cleaves stem peptides in the PG, is not regulated by OmpR in *S.* Typhimurium.

FIG S3. The alternative sigma factors RpoS and RpoE do not regulate the expression of $PBP2_{SAL}$ and $PBP3_{SAL}$ *S.* Typhimurium

FIG S4. Effect of osmolyte concentration on the growth of *S.* Typhimurium in the nutrient-poor medium PCN at an acid pH of 4.6.

FIG S5. Micromolar amounts of magnesium and acid pH cause in *S.* Typhimurium a significant drop in PBP2/PBP3 levels.

FIG S6. The levels of MurF, enzyme required for synthesis of the PG precursor lipid II do not drop drastically in response to phagosome cues.

FIG S7. The morphology and PBP3 levels are not affected in the nutrient poor PCN medium at neutral pH irrespective of the amount of NaCl added

Table S1. Bacterial strains and plasmids used in the study.

Table S2. Oligonucleotides used in the study as primers.

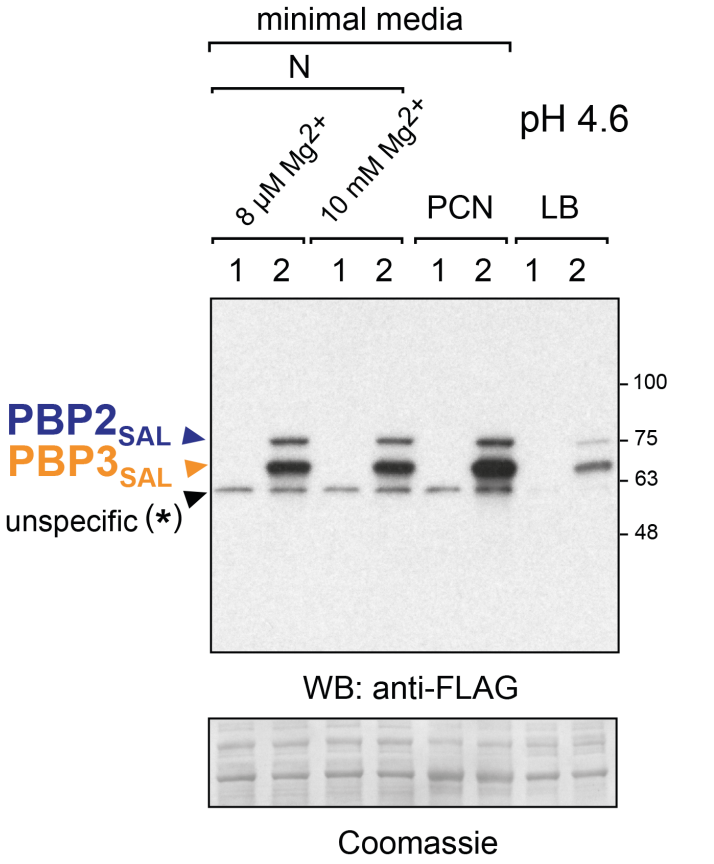

FIG. S1. Unspecific band detected with the anti-FLAG antibody in both untagged and tagged *S.* Typhimurium strains. $PBP2_{SAL}$ and $PBP3_{SAL}$ were resolved using precast 4-20% gradient gels (see Materials and Methods). Protein extracts were prepared at pH 4.6 from bacteria grown in the indicated minimal (N, PCN), and nutrient-rich (LB) media.

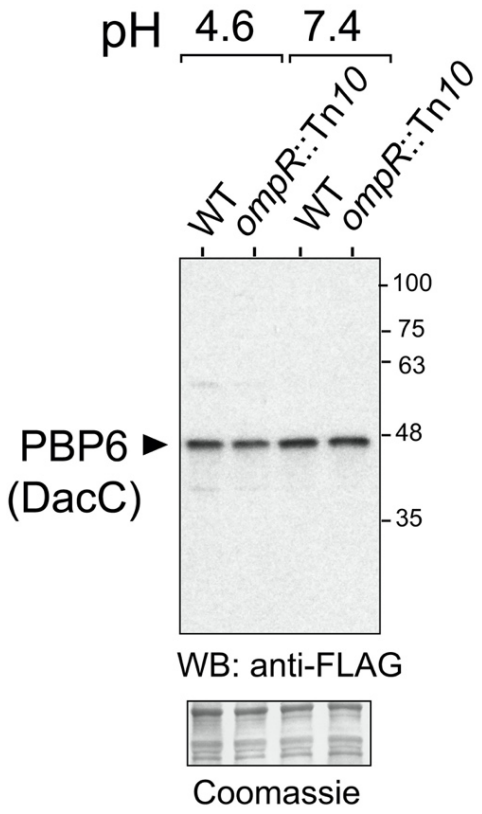

FIG S2. The carboxypeptidase PBP6 (DacC), which cleaves stem peptides in the PG, is not regulated by OmpR in *S.* Typhimurium. Shown are the levels of this enzyme detected in isogenic strains bearing a *dacC*::3xFLAG allele inserted in the native chromosomal locations and grown in nutrient poor N medium supplemented with 10 mM $MgCl_2$ at the indicated pH values. Coomassie staining is depicted as loading control. Data correspond to a representative experiment of a total of two independent biological replicates.

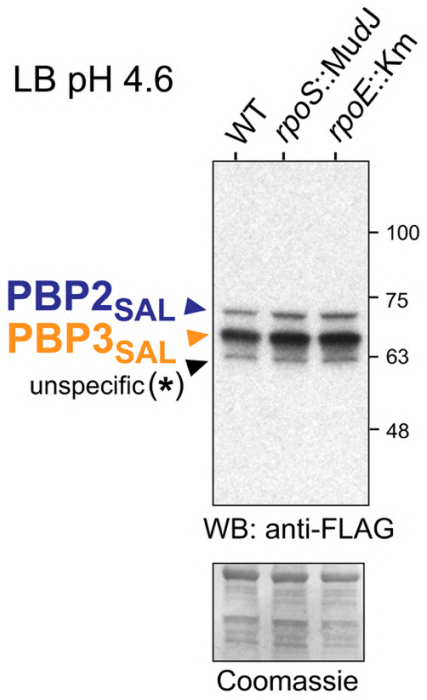

FIG. S3. The alternative sigma factors RpoS and RpoE do not regulate the expression of PBP2$_{SAL}$ and PBP3$_{SAL}$ in *S.* Typhimurium. Shown are the levels of these two enzymes in isogenic doubly tagged strains bearing *PBP2$_{SAL}$*::3xFLAG and *PBP3$_{SAL}$*::3xFLAG alleles inserted in their native chromosomal locations and grown in the nutrient rich LB medium at pH 4.6. Coomassie staining is depicted as loading control. Data correspond to a representative experiment of a total of two independent biological replicates.

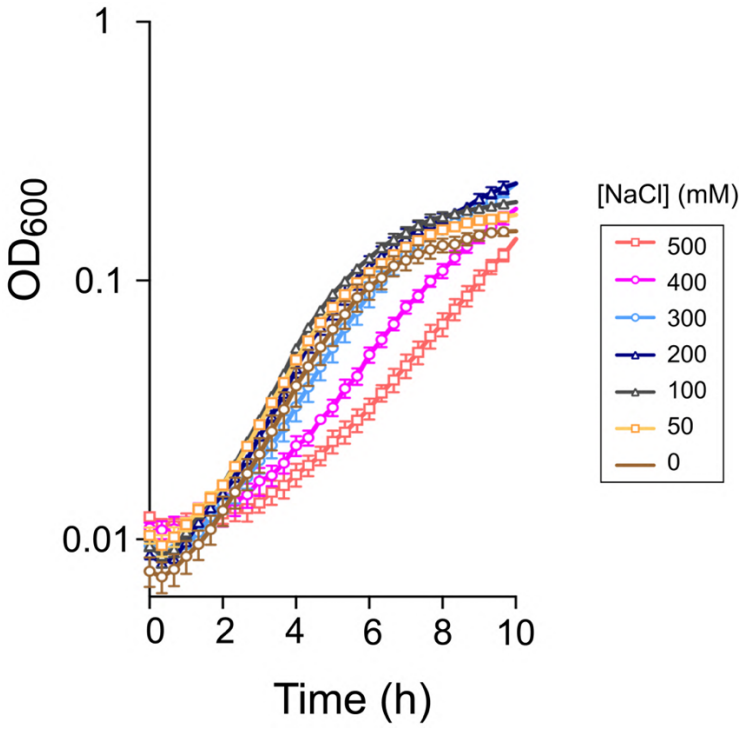

FIG S4. Effect of osmolyte concentration on the growth of *S.* Typhimurium in the nutrient-poor medium PCN at an acid pH of 4.6. Shown are the growth curves obtained with bacteria cultures supplemented with the indicated concentrations of NaCl. Data correspond to the mean and SD of a representative experiment with three technical replicates from a total of two independent biological replicates.

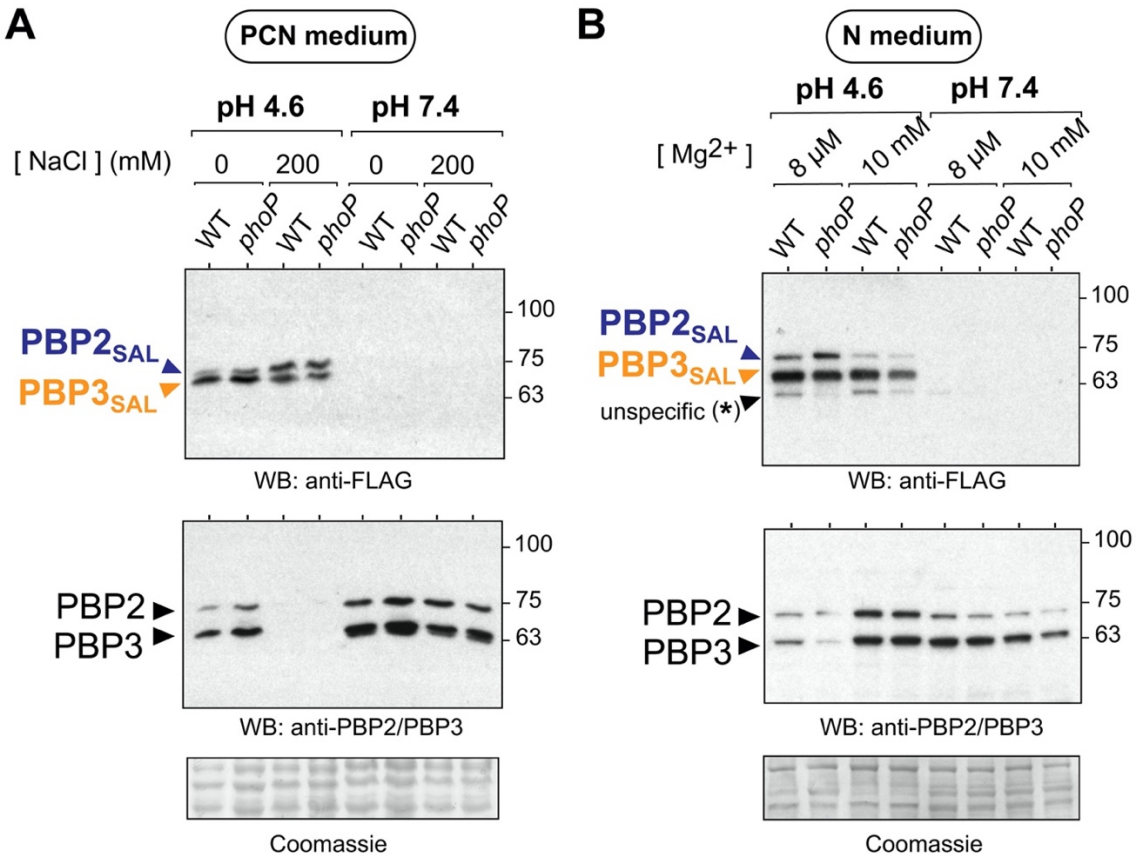

FIG. S5. Micromolar amounts of magnesium and acid pH cause in *S.* Typhimurium a significant drop in PBP2/PBP3 levels. (A) Levels of PBP2/PBP3 and $PBP2_{SAL}$/$PBP3_{SAL}$ in wild type and *phoP* isogenic strains grown in minimal PCN medium at the indicated pH and osmolarities. (B) same as in (A) but in bacteria grown in N minimal media at two different pH (4.6 and 7.4) and magnesium concentrations (8 µM and 10 mM). Low magnesium concentration (8 µM) and acid pH of 4.6 triggers the loss of PBP2 and PBP3 while the production of $PBP2_{SAL}$ and $PBP3_{SAL}$ increases notoriously. Coomassie staining is included as the loading control. Data shown are representative of a minimum of three independent biological replicates.

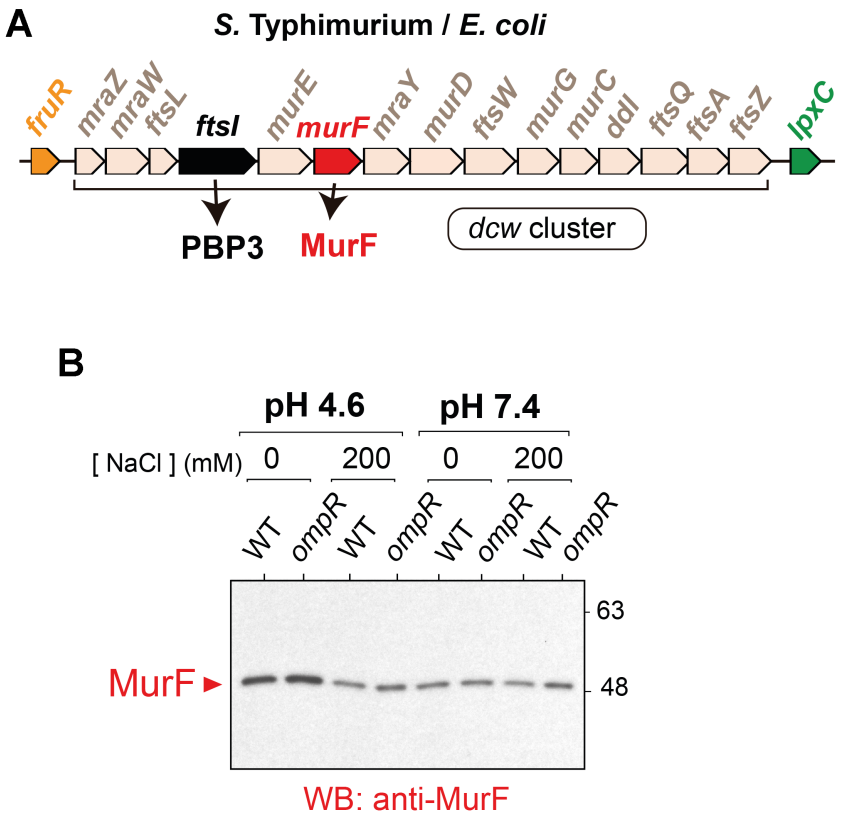

FIG S6. The levels of MurF, enzyme required for synthesis of the PG precursor lipid II do not drop drastically in response to phagosome cues. (A) Diagram of the division and cell wall (*dcw*) multicistronic gene cluster conserved in *E. coli* and *S.* Typhimurium. The positions of *ftsI,* encoding PBP3, and *murF,* encoding an essential cytosolic enzyme involved in the synthesis of the PG precursor lipid II, are depicted. (B) Levels of MurF in *S.* Typhimurium grown to mid-exponential phase in the nutrient poor PCN medium at the indicated conditions of pH and osmolarity. Shown is a representative experiment of a total of three biological replicates.

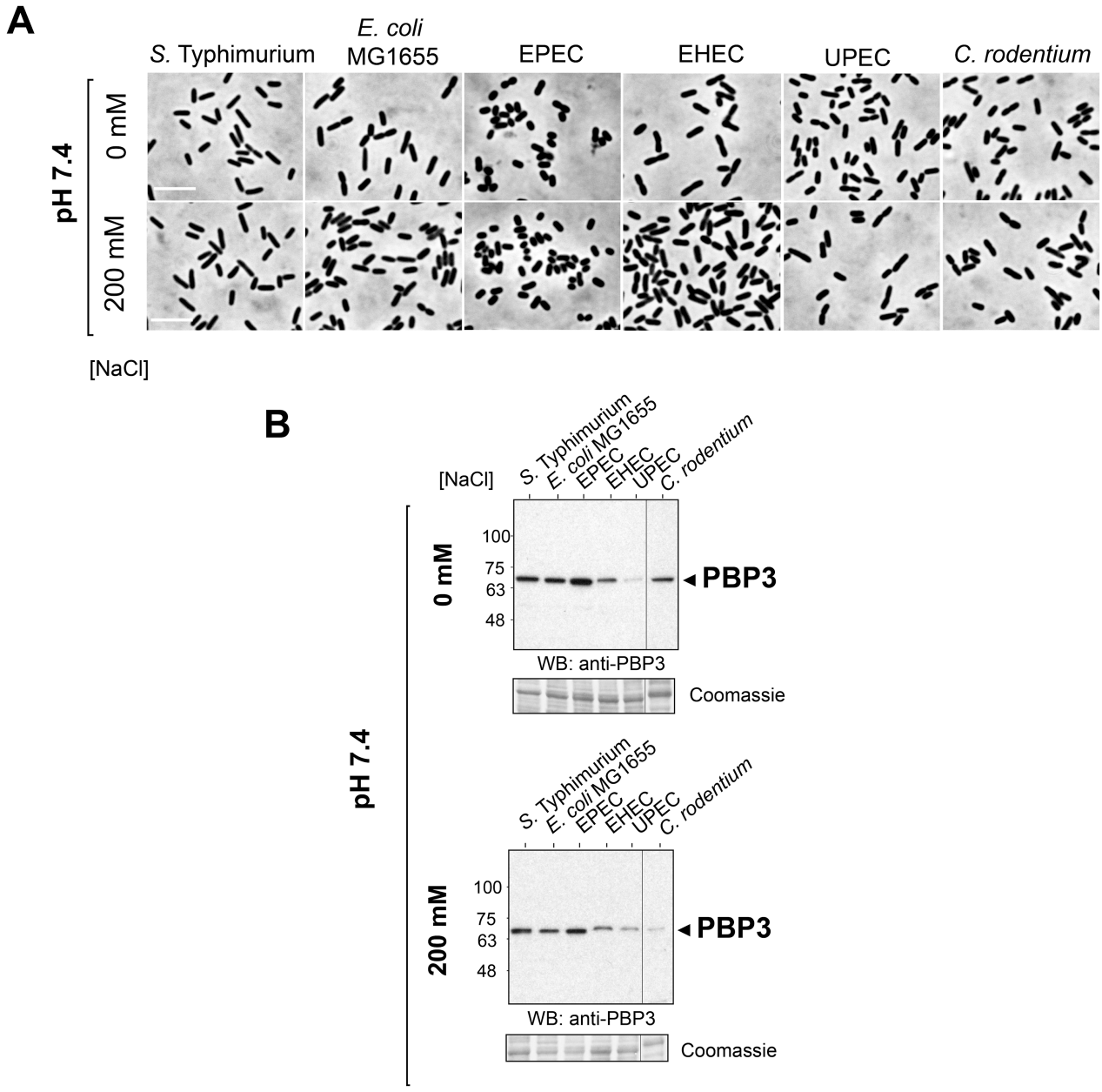

FIG S7. The morphology and PBP3 levels are not affected in the nutrient poor PCN medium at neutral pH irrespective of the amount of NaCl added. (A) Images of *S.* Typhimurium and the indicated *E. coli* isolates grown to mid-exponential phase in PCN medium at pH 7.4 at low or high osmolarity (0 mM, 200 mM NaCl). Bar, 5 μm. (B) PBP3 levels detected by immunoassay in the same samples observed at the microscope. The Coomassie staining is shown as loading control. Data are from a representative experiment of a total of two independent biological replicates.

Table S1. Bacterial strains and plasmids used in the study

Bacterial strain/ plasmid	Relevant genotype	Source/ reference
S. Typhimurium		
SV5015	SL1344 *hisG*$^{+}$	(Vivero *et al.*, 2008)
MD5064	SV5015 $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-3xFLAG	(Castanheira *et al.*, 2020)
MD5080	SV5015 *ompR1009*::Tn*10* $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-3xFLAG	This study
MD5540	SV5015 *phoP7953*::Tn*10* $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-3xFLAG	This study
MD2540	SV5015 $PBP2_{SAL}$-3xFLAG::Km^R	This study
MD3841	SV5015 *ompR1009*::Tn*10* $PBP2_{SAL}$-3xFLAG	This study
MD3896	SV5015 *phoP7953*::Tn*10* $PBP2_{SAL}$-3xFLAG	This study
MD3894	SV5015 *slyA*::pRR10Δ*trfA*Pen^R $PBP2_{SAL}$-3xFLAG::Km^R	This study
MD5140	SV5015 *ssrB*:: Km^R $PBP2_{SAL}$-3xFLAG	M. Hensel
MD2559	SV5015 $PBP3_{SAL}$-3xFLAG	(Castanheira *et al.*, 2017)
MD3842	SV5015 *ompR1009*::Tn*10* $PBP3_{SAL}$-3xFLAG	(Castanheira *et al.*, 2017)
MD3897	SV5015 *phoP7953*::Tn*10* $PBP3_{SAL}$-3xFLAG	(Castanheira *et al.*, 2017)
MD3895	SV5015 *slyA*::pRR10Δ*trfA*Pen^R $PBP3_{SAL}$-3xFLAG::Km^R	(Castanheira *et al.*, 2017)
MD5139	SV5015 *ssrB*:: Km^R $PBP3_{SAL}$-3xFLAG	M. Hensel
MD5416	SV5015 Δ*prc*::Km^R $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-3xFLAG	This study
MD5420	SV5015 *ompR1009*::Tn*10* Δ*prc*::Km^R $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-3xFLAG	This study
MD5421	SV5015 *mrcA*-3xFLAG::Km^R $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-	This study
MD5422	SV5015 *mrcB*-3xFLAG::Km^R $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-	This study
MD5425	SV5015 pAC-6xHis-*ftsI* (6xHis-PBP3)	This study
MD5426	SV5015 Δ*prc*::Km^R pAC-6xHis-*ftsI* (6xHis-PBP3)	This study
MD5427	SV5015 pAC-6xHis-$PBP3_{SAL}$	This study
MD5428	SV5015 Δ*prc*::Km^R pAC-6xHis-$PBP3_{SAL}$	This study
MD5436	SV5015 pGEN222(ΔP*ompC-ova*)-P$PBP2_{SAL}$- gfp^{TCD}	This study
MD5437	SV5015 pGEN222(ΔP*ompC-ova*)-P$PBP3_{SAL}$- gfp^{TCD}	This study
MD5438	SV5015 *ompR1009*::Tn*10* pGEN222(ΔP*ompC-ova*)-P$PBP2_{SAL}$-gfp^{TCD}	This study

MD5439	SV5015 *ompR1009*::Tn*10* pGEN222(ΔP*ompC-ova*)-P$PBP3_{SAL}$-gfp^{TCD}	This study
MD5440	SV5015 *phoP7953*::Tn*10* pGEN222(ΔP*ompC-ova*)-P$PBP2_{SAL}$-gfp^{TCD}	This study
MD5441	SV5015 *phoP7953*::Tn*10* pGEN222(ΔP*ompC-ova*)-P$PBP3_{SAL}$-gfp^{TCD}	This study
MD5446	SV5015 pGEN222(ΔP*ompC-ova*)-mut-P$PBP2_{SAL}$	This study
MD5447	SV5015 pGEN222(ΔP*ompC-ova*)-mut-P$PBP3_{SAL}$	This study
MD5449	SV5015 pGEN222(ΔP*ompC-ova*) (promoter-less vector)	This study
MD5451	SV5015 *rpoS*::Cm^R $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-3xFLAG	This study
MD5077	SV5015 *rpoE*::Km^R $PBP2_{SAL}$-3xFLAG $PBP3_{SAL}$-3xFLAG	This study
MD3238	SV5015 *dacC*-3xFLAG::Km^R	This study
MD3843	SV5015 o*mpR1009*::Tn*10 dacC*-3xFLAG::Km^R	This study
MD1168	SV5015 *ssrB*::3xFLAG:: Km^R	(Núñez-Hernández *et al.*, 2013)
MD1158	SV5015 *sseJ*::3xFLAG:: Km^R	(Núñez-Hernández *et al.*, 2013)
E. coli		
MG1655	K-12, wild type	
MD4445	MG1655 pAC	This study
MD4448	MG1655 pAC-$PBP3_{SAL}$	This study
MD5442	MG1655 pGEN222(ΔP*ompC-ova*)::P-$PBP2_{SAL}$-gfp^{TCD}	This study
MD5443	MG1655 pGEN222(ΔP*ompC-ova*)::P-$PBP3_{SAL}$- gfp^{TCD}	This study
MD5450	MG1655 pGEN222(ΔP*ompC-ova*) (promoter-less vector)	This study
E2348/69	EPEC, wild type (O127:H6)	L.A. Fernández
EDL933- Δ*stx*	EHEC, Δ*stx1* Δ*stx2* (O157:H7)	L.A. Fernández
536	UPEC, wild type (O6:K15:H31)	L.A. Fernández
C609	EIEC, wild type, ST-99 (O96:H19)	A. San Millán
C610	EIEC, wild type, ST-99 (O96:H19)	A. San Millán
Citrobacter rodentium		
ICC169	spontaneous Nal^R of wild type isolate ICC168	L.A. Fernández
Plasmids		
pAC-Plac	Cm^R	(Castanheira *et al.*, 2017)
pAC-6xHIS-Plac	Cm^R	(Castanheira *et al.*, 2017)

pCP20	FLP+, Amp^R, Cm^R	(Cherepanov and Wackernagel, 1995)
pSUB11	3xFLAG sequence, Km^R	(Uzzau *et al.*, 2001)
pKD46	γ, β, exo. Amp^R	(Datsenko and Wanner, 2000)
pGEN222	Amp^R; p15A-*ori*, *hok*/*sok* stability system, P*ompC*::*gfp*-UV	(Galen *et al.*, 1999)
pGEN222-*ova*-	pGEN222-P*ompC*-*ova*-*gfp*^{TCD}	L.A. Fernández

References - Table S1

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Núñez-Hernández, C., Tierrez, A., Ortega, A.D., Pucciarelli, M.G., Godoy, M., Eisman, B., *et al.* (2013) Genome expression analysis of nonproliferating intracellular *Salmonella enterica* serovar Typhimurium unravels an acid pH-dependent PhoP-PhoQ response essential for dormancy. *Infect Immun* **81**: 154–165.

Uzzau, S., Figueroa-Bossi, N., Rubino, S., and Bossi, L. (2001) Epitope tagging of chromosomal genes in *Salmonella*. *Proc Natl Acad Sci U S A* **98**: 15264–15269.

Vivero, A., Baños, R.C., Mariscotti, J.F., Oliveros, J.C., García-del Portillo, F., Juárez, A., and Madrid, C. (2008) Modulation of horizontally acquired genes by the Hha-YdgT proteins in *Salmonella enterica* serovar Typhimurium. *J Bacteriol* **190**: 1152–1156.

Table S2. Oligonucleotides used in the study as primers

Primer name	Sequence (5'-3') (*)
FLAG MrcA Fw	ACCATTATTGATAATGGTGAAACACACGAACTGTTCGACTACAAAGACCATGACGG
FLAG MrcA Rv	GCTAAACACAATAAAAAAGGCGCCGGAGCGCCTTTTTTGACATATGAATATCCTCCTTA
FLAG MrcB Fw	GTTGCCGGCTGGATTAAGGAGATGTTCGGCGGCAATGACTACAAAGACCATGACGG
FLAG MrcB Rv	GACCGGGTAAGCACAGCGCCACCCGGCACTATTACCGTGACATATGAATATCCTCCTTA
FLAG PBP 2* Fw	TGATCCACAGGCTGATACCACACAGCCGGATCAGGCGCCAGACTACAAAGACCATGAC
FLAG PBP 2* Rv	TCCGGCCGTATCCTTGTCTGATGGCGCTTTGCTTATTTGACATATGAATATCCTCCTTAG
FLAG PBP 3* Fw	TCTGGTGATGCATGGCAGCCACGTTGCGGTTCCGGGTTCGGACTACAAAGACCATGAC
FLAG PBP 3* Rv	GGGCGCAAGTGTAACGCGAATTGCGCCCCGGGAAAATCCTCATATGAATATCCTCCTTA
pPBP2sal_EcoRI_NotI_Fw	GC**GAATTC**GGCGCAACTGACGGCGAAG
pPBP2sal_EcoRI_NotI_Rv	ATAAGAAT**GCGGCCGC**GAACAATCCGCCGCCTAATG
pPBP3sal_EcoRI_NotI_Fw	GC**GAATTC**CCAGAAAGGTCCGGCTG
pPBP3sal_EcoRI_NotI_Rv	ATAAGAAT**GCGGCCGC**AGCCGAATCATTTTTCAGG
pPBP2sal_mut_Fw	GCCACAGCGGTATATC<u>GCTCGGAC</u>ATCACAAGGAATGAC
pPBP2sal_mut_Rv	GTCATTCCTTGTGAT<u>GTCCGAGC</u>GATATACCGCTGTGGC
pPBP3sal_mut_Fw	GCTCTCGTTAGCGATT<u>GCTCGGAC</u>AGGCGCGATGTTATTAG
pPBP3sal_mut_Rv	CTAATAACATCGCGCCT<u>GTCCGAGC</u>AATCGCTAACGAGAGC
pGEN222_Fw	GTCTCTGTTATTCAGGCAATTTC
FLAG PBP6 Fw	GCTGATGAAACTCCATCAGTGGTTTGGCAGTTGGTTCTCGGACTACAAAGACCATGACG
FLAG PBP6 Rv	CCGTAGCCGGATGCGACGCGCACCCGGCTACGGAGTTATTCATATGAATATCCTCCTTA

(*) Bold red: restriction enzyme sites; underlined: sequences of the mutated OmpR box variants; blue colour, sequence of pSUB11 vector.