Supplementary Data

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

Strains	Genotype/Phenotype	Source
E. coli	BW25141/pkD4 amp^R kan^R	(1)
E. coli	BL21 (DE3) pLysE/*hfq::aph* + pCSB12 (*csrA* cloned into the *Nde*I and *Bam*HI sites of pET21a+)	This study
S. Typhimurium	SL1344	(2)
S. Typhimurium	SL1344 Δ *acrB*	(3)
S. Typhimurium	SL1344 *acrA::aph*	(3)
S. Typhimurium	SL1344 *ramA*::*aph*	This study
S. Typhimurium	SL1344(pACYC-*ramA*::3xFLAG::*aph*)	(4)
S. Typhimurium	SL1344 *csrA*::aph + (pACYC-*ramA*::3xFLAG::*aph*)	This study
S. Typhimurium	SL1344 pKD46 amp^R	(5)
S. Typhimurium	ATCC 14028S	(6)
S. Typhimurium	SL1344 *ramR*::aph	(7)
S. Typhimurium	SL1344 pMW82p*ramA*	(8)
S. Typhimurium	SL1344 pMW82p*acrAB*	This study
S. Typhimurium	SL1344 *csrA*::aph (Kan^R)	This study
S. Typhimurium	14028S *csrA*::aph (Kan^R)	This study
S. Typhimurium	SL1344 *csrA*::aph + pMW82-p*acrAB*	This study
S. Typhimurium	SL1344 *csrA*::aph + pMW82-p*ramA*	This study
S. Typhimurium	SL1344 *csrA*::aph + pWSK30*csrA*	This study
S. Typhimurium	SL1344 pMW82-p*acrAB* (CsrA BS-SDM)	This study
S. Typhimurium	Ciprofloxacin selected MDR mutant	(5)
S. Typhimurium	Ciprofloxacin selected MDR mutant/*csrA::aph*	This study
S. Typhimurium	*ΔramR/csrA::aph* (Kan^R)	This study
S. Typhimurium	pAcrA-TF-WT	This study
S. Typhimurium	pAcrA-TF-SDM	This study
Plasmids		

pMW82	Promoter trap vector containing a promoterless *gfp* gene	(9)
pMW82-p*ramA*	DNA fragment carrying the *ramA* promoter region, flanked by *BamH*I and *Xba*I restriction sites cloned into pMW82	(8)
pMW82-p*acrAB*	DNA fragment carrying the *acrAB* promoter region, flanked by *BamH*I and *Xba*I restriction sites cloned into pMW82	This study
pMW82-p*acrAB* (CsrA BS-SDM)	DNA fragment carrying the acr*AB* promoter region with mutated CsrA binding sites, flanked by *BamH*I and *Xba*I restriction sites cloned into pMW82	This study
pMW82-p*acrAB* (RNase E- DEL)	DNA fragment carrying the acr*AB* promoter region with putative RNase E cleavage site region deleted, flanked by *BamH*I and *Xba*I restriction sites cloned into pMW82	This study
pWSK30	Multi-purpose low-copy-number vector	(10)
pWSK30*csrA*	*csrA* gene flanked by *BamH*I and *Hind*III restriction sites cloned into pWSK30	This study
pACYC-*ramA*::3xFLAG::*aph*	DNA fragment carrying the *ramA*::3XFLAG::aph flanked by *Hind*III and *Nru*I restriction sites cloned into the multicopy plasmid pACYC	(4)
pCR®II-TOPO®	Cloning vector with LacZ reporter, T7 and SP6 promoter sites, amp & kan resistance	Thermo Fisher
pCR®II-p*ramA*	DNA fragment carrying the *ramA* promoter region cloned into pCR®II-TOPO®	This study
pCR®II-p*acrAB*	DNA fragment carrying the *acrAB* promoter region cloned into pCR®II-TOPO®	This study
pCR®II-p*hfq*	DNA fragment carrying the *hfq* promoter region cloned into pCR®II-TOPO®	This study
pCR®II- p*acrAB* (CsrA BS-SDM)	DNA fragment carrying the acr*AB* promoter region with mutated CsrA binding sites cloned into pCR®II-TOPO®	This study

pAcrA-TF-WT	DNA fragment carrying the WT *acrAB* promoter region plus the *acrA* CDS minus stop codon flanked by *BamH*I and *Kpn*I restriction sites cloned into pDOC-G	This study
pAcrA-TF-SDM	DNA fragment carrying the *acrAB* promoter region with nucleotide substitutions plus the *acrA* CDS minus stop codon flanked by *BamH*I and *Kpn*I restriction sites cloned into pDOC-G	This study
pT7-*acrA*-GFP	DNA fragment carrying the *acrA*-GFP translational fusion with wildtype leader sequence cloned into the pET-20b(+) vector (Novagen)	This study
pT7-*acrA*-GFP Mut CsrA BS	DNA fragment carrying the *acrA*-GFP translational fusion with mutated CsrA binding sites in the leader sequence cloned into the pET-20b(+) vector (Novagen)	This study

Table S2.

Phenotype of *Salmonella* Typhimurium *csrA::aph* and MDR mutants

Strain	Antimicrobial susceptibilities					Growth in LB and MOPs minimal medium		Biofilm formation
	MIC (mg/L)					Generation time (mins)		OD (600nm)
	CIP	NAL	CHL	TET	EtBr	LB	MOPs	
SL1344	0.015	4	2	1	2048	47.5 +/- 5.4	100.2 +/- 8.9	0.29 +/- 0.06
SL1344 *csrA::aph*	0.015	4	2	1	2048	182.5 +/- 12.5*	374.3 +/- 16.2*	0.05 +/- 0.02*
SL1344 *csrA::aph* (pWSK30*csrA*)	0.015	4	2	1	2048	49.3 +/- 6.2	105.4 +/- 6.7	0.31 +/- 0.04
SL1344 *ramR::aph*	0.06	16	16	8	2048	ND	ND	ND
SL1344 Δ*ramR/csrA::aph*	0.03	8	8	4	256	ND	ND	ND
SL1344 CIP selected MDR mutant	0.12	32	16	8	2048	ND	ND	ND
SL1344 CIP selected MDR mutant/*csrA::aph*	0.03	8	4	2	512	ND	ND	ND

CIP, ciprofloxacin; NAL, nalidixic acid; CHL, chloramphenicol; TET, tetracycline; EtBr, ethidium bromide; LB, Luria-Bertani broth; MOPs, MOPs minimal medium. ND, not done. *$P < 0.05$ by Student's t-test.

Table S3. Analysis of the second elution fraction analysed by Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry.

Accession	Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
A7ZQC4	Carbon storage regulator OS=Escherichia coli O139:H28 (strain E24377A / ETEC) GN=csrA PE=3 SV=1 - [CSRA_ECO24]	25.83	63.93	5	2	5	7	61	6.9	8.62
A0A080J9U6	Carbon storage regulator OS=Escherichia coli 2-460-02_S1_C2 GN=AB34_3203 PE=4 SV=1 - [A0A080J9U6_ECOLX]	14.93	67.35	4	1	4	5	49	5.5	7.25
A0A086VAZ1	Molybdopterin synthase small subunit OS=Escherichia coli GN=moaD PE=4 SV=1 - [A0A086VAZ1_ECOLX]	9.48	29.17	38	1	1	2	72	7.7	4.42
D7XFN1	Murein-lipoprotein (Fragment) OS=Escherichia coli MS 198-1 GN=lpp PE=4 SV=1 - [D7XFN1_ECOLX]	5.87	35.62	16	2	2	2	73	7.7	6.60
E9YIC2	Entericidin EcnA/B family protein OS=Escherichia coli TA007 GN=ERHG_03462 PE=4 SV=1 - [E9YIC2_ECOLX]	3.90	40.43	6	1	1	1	47	4.7	8.21
P49064	Serum albumin OS=Felis catus GN=ALB PE=1 SV=1 - [ALBU_FELCA]	2.50	2.47	1	1	1	1	608	68.6	5.66
K3GH09	Phage portal family protein (Fragment) OS=Escherichia coli 5412 GN=EC5412_1619 PE=4 SV=1 - [K3GH09_ECOLX]	0.00	6.59	1	1	1	1	91	10.2	6.51
T8NXA3	Inner membrane protein ytfF OS=Escherichia coli UMEA 3097-1 GN=G907_04361 PE=4 SV=1 - [T8NXA3_ECOLX]	0.00	13.19	142	1	1	3	235	26.1	9.07

Figure S1

Expression of GFP from pMW82p*ramA* from eight random transposon mutants, chosen from the four FACS gated populations (P4, P5, P6 and P7).

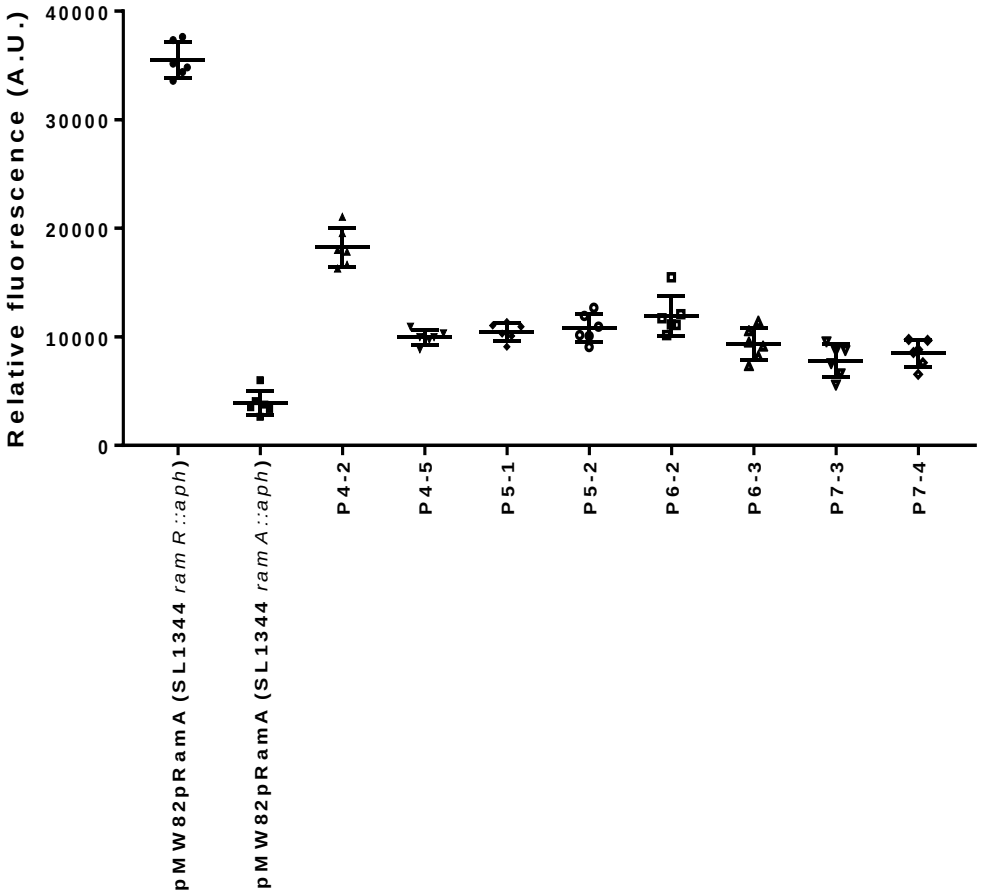


Figure S2 Infection assays with SL1344 and SL1344 *csrA::aph* mutant in INT-407 human embryonic intestine cells. Association and invasion of the SL1344 *csrA::aph* mutant into INT-407 cells was compared to that of the parental wild-type strain, SL1344.

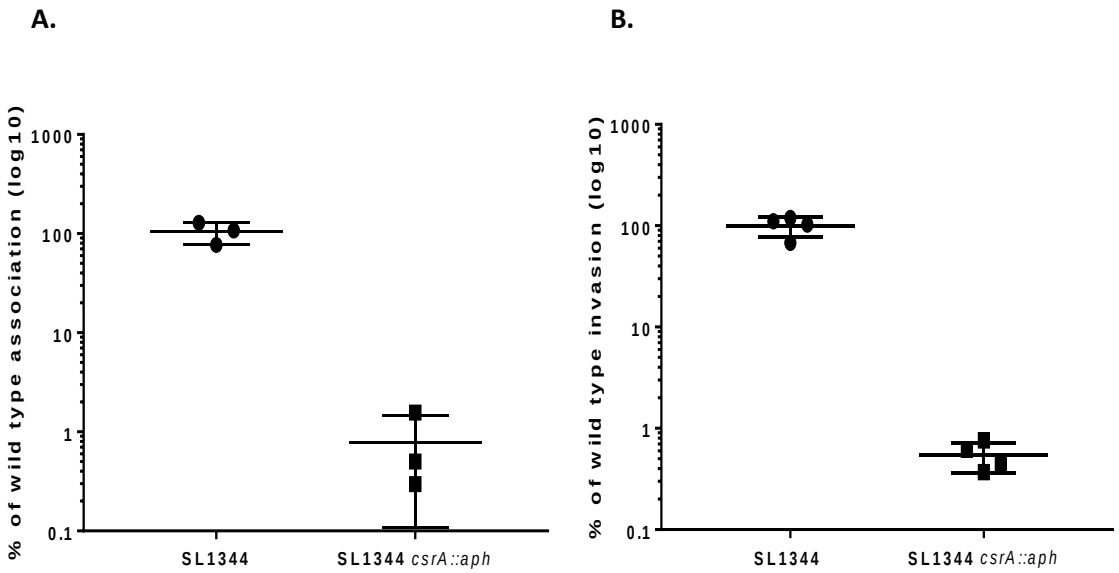


Figure S3.

Rate of efflux of ethidium bromide. **A.** (▬), SL1344; (▬), SL1344 *csrA::aph.* **B.** (▬), SL1344 Δ*ramR*; (▬), SL1344 Δ*ramR/csrA::aph.* **C.** (▬), SL1344 MDR mutant; (▬), SL1344 MDR/*csrA::aph.*

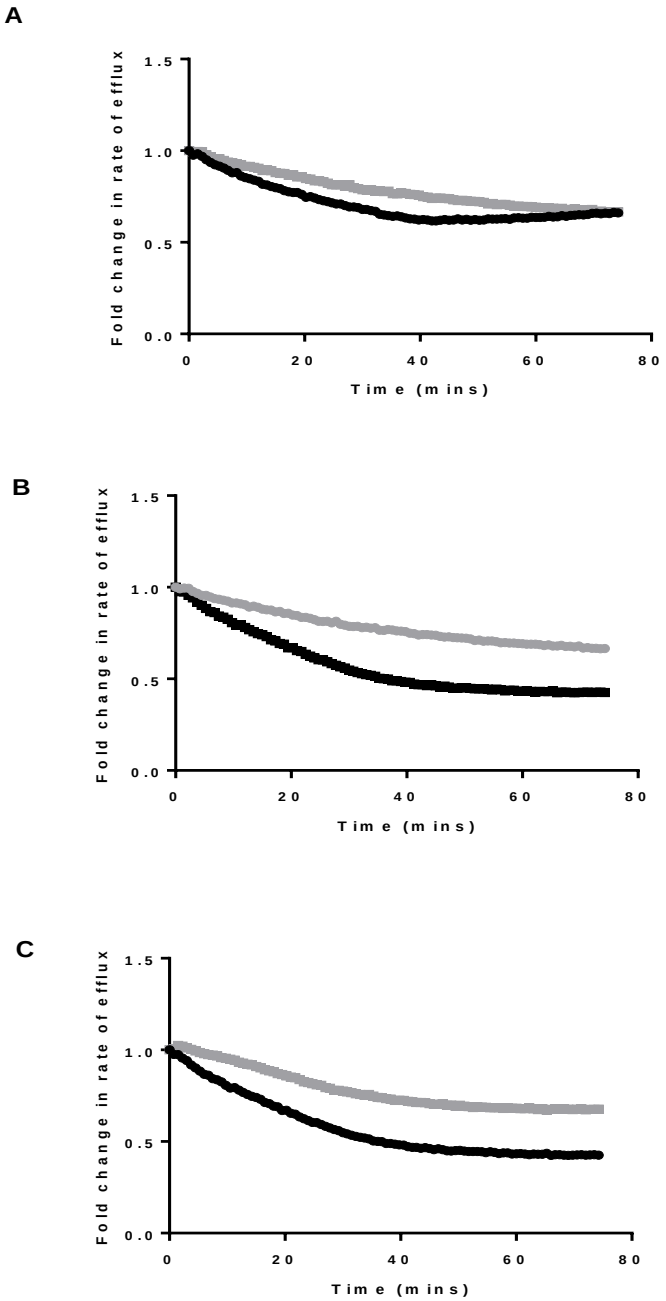


Figure S4: Uncropped gel images. The images correspond to EMSA gels presented in Figures 4 A-D.

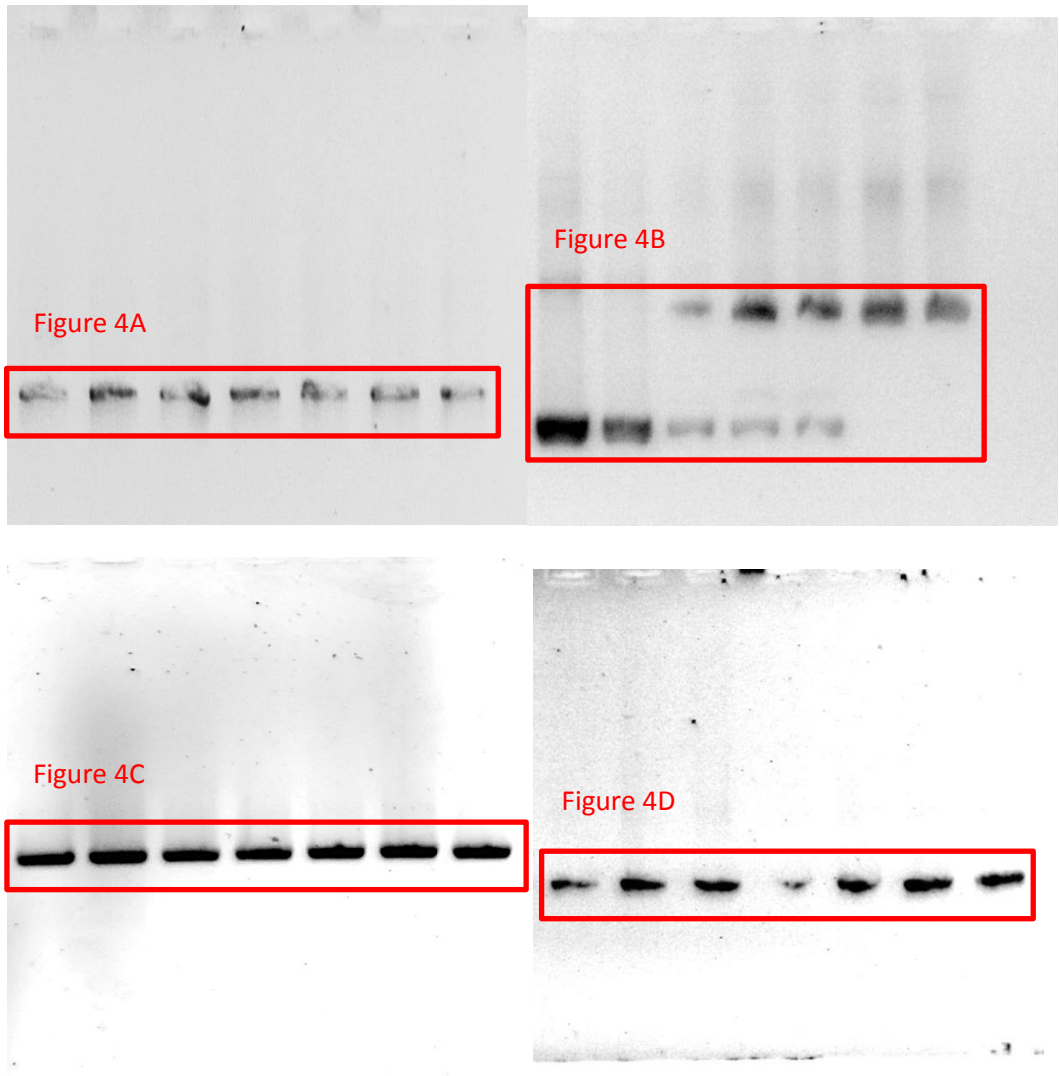


Figure S5. Coomassie blue stained SDS-PAGE gel (A) and anti-His tag Western blot (B) of fractions derived from purification of CsrA-His protein. M = Protein ladder, 1 = Flow through, 2 = Wash 1, 3 = Wash 2, 4 = Elute 1, 5 = Elute 2.

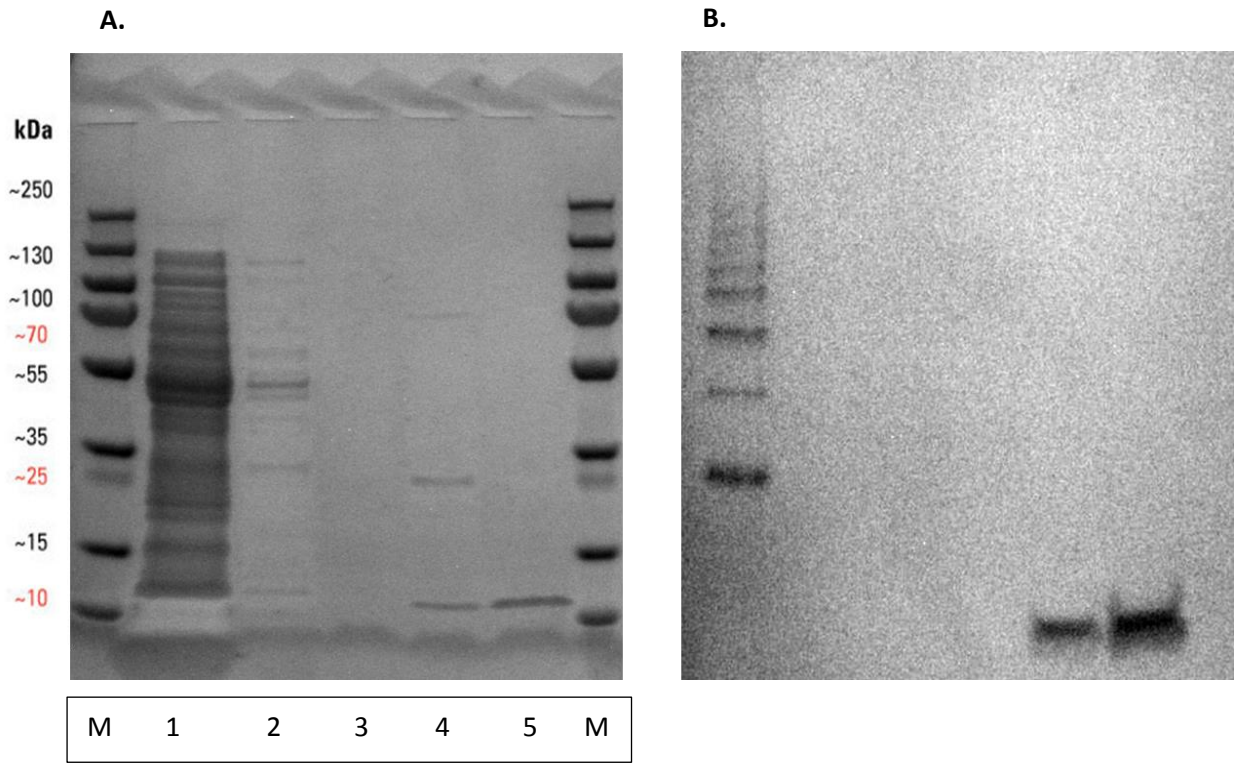
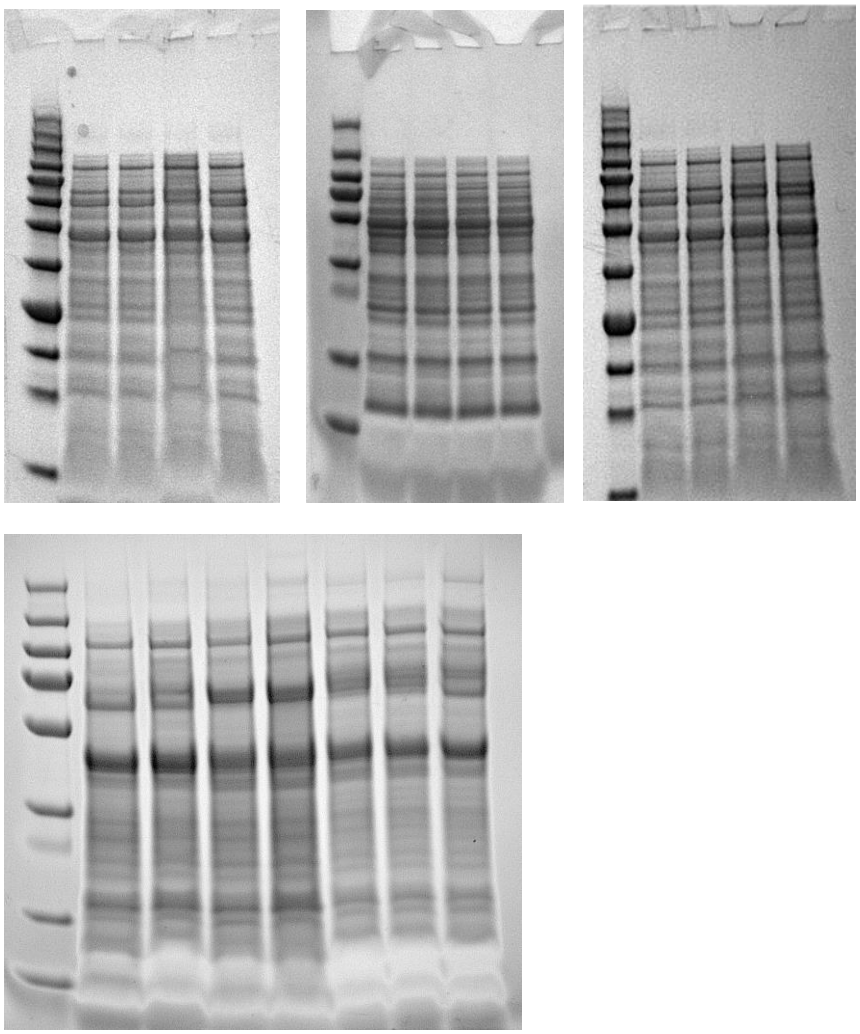


Figure S6. Coomassie blue stained protein gels corresponding to the Western blots described in Figures 3 and 11.



Supplementary References

1. Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A*, **97**, 6640-6645.
2. Wray, C. and Sojka, W.J. (1978) Experimental *Salmonella typhimurium* infection in calves. *Res Vet Sci*, **25**, 139-143.
3. Buckley, A.M., Webber, M.A., Cooles, S., Randall, L.P., La Ragione, R.M., Woodward, M.J. and Piddock, L.J. (2006) The AcrAB-TolC efflux system of *Salmonella enterica* serovar Typhimurium plays a role in pathogenesis. *Cell Microbiol*, **8**, 847-856.
4. Ricci, V., Blair, J.M. and Piddock, L.J. (2014) RamA, which controls expression of the MDR efflux pump AcrAB-TolC, is regulated by the Lon protease. *J Antimicrob Chemother*, **69**, 643-650.
5. Ricci, V., Tzakas, P., Buckley, A. and Piddock, L.J. (2006) Ciprofloxacin-resistant *Salmonella enterica* serovar Typhimurium strains are difficult to select in the absence of AcrB and TolC. *Antimicrob Agents Chemother*, **50**, 38-42.
6. Fields, P.I., Swanson, R.V., Haidaris, C.G. and Heffron, F. (1986) Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. *Proc Natl Acad Sci U S A*, **83**, 5189-5193.
7. Ricci, V., Busby, S.J. and Piddock, L.J. (2012) Regulation of RamA by RamR in *Salmonella enterica* serovar Typhimurium: isolation of a RamR superrepressor. *Antimicrob Agents Chemother*, **56**, 6037-6040.
8. Lawler, A.J., Ricci, V., Busby, S.J. and Piddock, L.J. (2013) Genetic inactivation of *acrAB* or inhibition of efflux induces expression of *ramA*. *J Antimicrob Chemother*, **68**, 1551-1557.
9. Bumann, D. and Valdivia, R.H. (2007) Identification of host-induced pathogen genes by differential fluorescence induction reporter systems. *Nat Protoc*, **2**, 770-777.
10. Wang, R.F. and Kushner, S.R. (1991) Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene*, **100**, 195-199.