Supplementary Material

A conserved RpoS-dependent small RNA controls the synthesis of major porin OmpD

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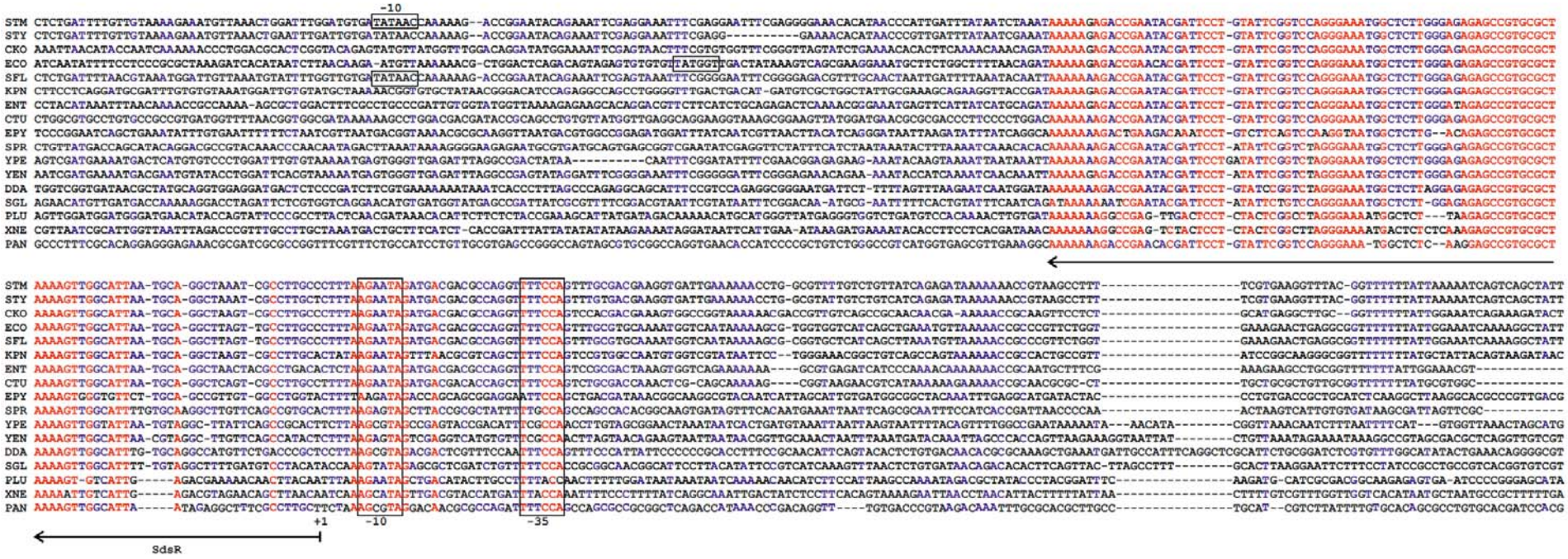
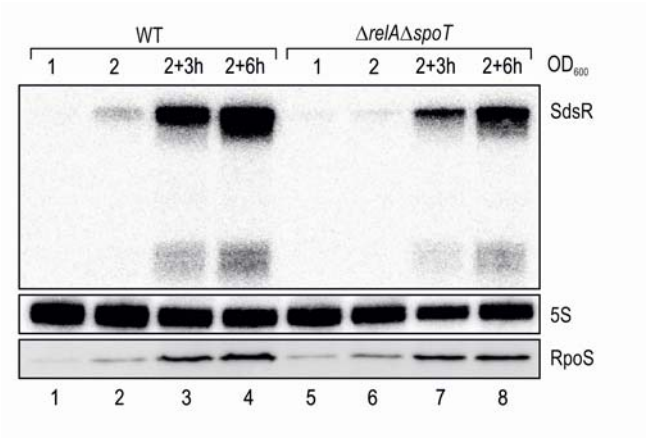
Figure S1



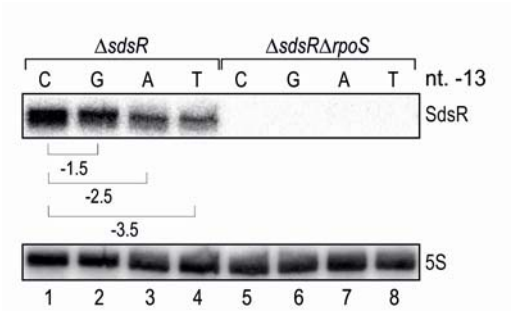
Non-redundant alignment of the *sraC* gene including the upstream promoter region from various enterobacterial species. All nucleotides are coloured regarding their degree of conservation (red: high conservation; blue: partial conservation; black: little or no conservation). STM: *Salmonella typhimurium*; STY: *Salmonella typhi*; CKO: *Citrobacter koseri*; ECO: *Escherichia coli*; SFL: *Shigella flexneri*; ENT: *Enterobacter*; CTU: *Cronobacter turicensis*; KPN: *Klebsiella pneumoniae*; SPR: *Serratia proteamaculans*; YPE: *Yersinia pestis*; YEN: *Yersinia enterolitica*; DDA: *Dickeya dadantii*; PAN: *Pantoea ananatis*; SGL: *Sodalis glossinidius*; EPY: *Erwinia pyrifoliae*; Plu: *Photorhabdus luminescens*; XNE: *Xenorhabdus nematophila*.

Figure S2



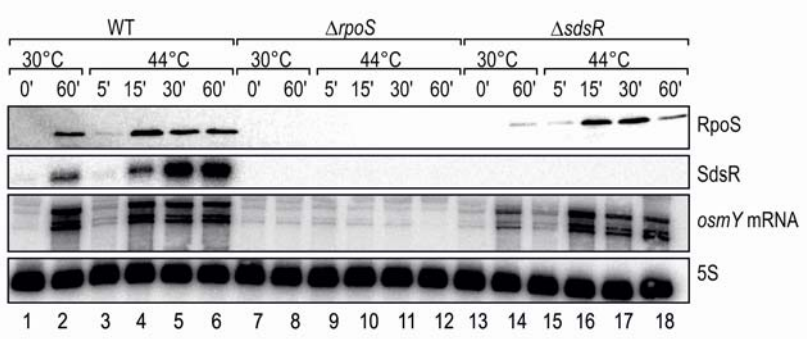
Expression of SdsR is decreased in *Salmonella* depleted of ppGpp. *Salmonella* wild-type (JVS-1574) and a Δ*relA*Δ*spoT* double mutant (JVS-1505) were grown in LB to an OD_{600} of 1.0, 2.0 and 3 or 6 hours after cells had reached an OD_{600} of 2.0. SdsR levels were determined by Northern blotting of total RNA. 5S RNA levels were analyzed to confirm equal loading. Expression of RpoS was monitored by Western blotting of protein samples taken from the same cultures.

Figure S3



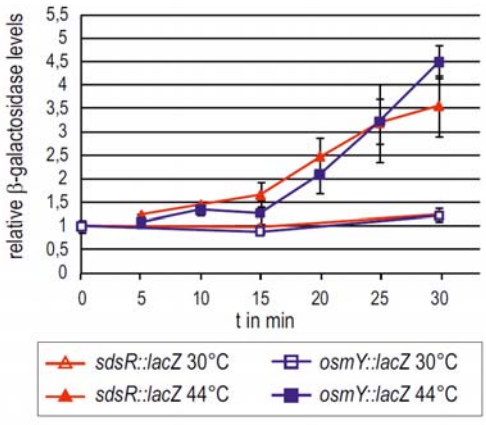
Permutation of cytosine at position -13 in the *sdsR* promoter decreases SdsR expression levels. *Salmonella* Δ*sdsR* (JVS-8827) and Δ*sdsR*Δ*rpoS* (JVS-9551) mutants carrying the complementation plasmid p*sdsR* (pVP203-1) or three derivatives differing in the nucleotide at position -13 in the *sdsR* promoter (p*sdsR* C-13G: pKF106-2; p*sdsR* C-13A: pKF107-1; p*sdsR* C-13T: pKF108-3) were grown in LB to 3 hours after the culture had reached an OD_{600} of 2.0. SdsR expression was analyzed by Northern blotting of total RNA; quantification of fold-changes in SdsR abundances is indicated below the panel. Probing for 5S RNA confirmed equal loading.

Figure S4



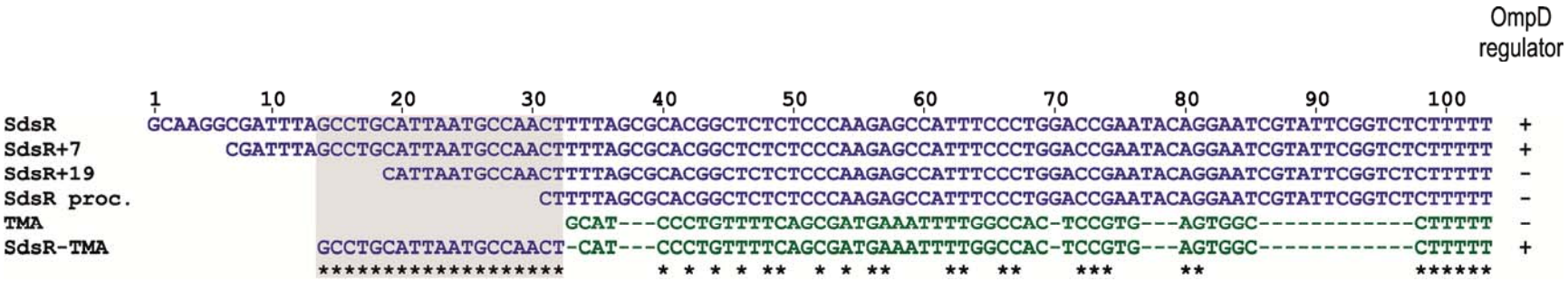
SdsR sRNA and *osmY* mRNA are rapidly induced under heat stress in wild-type but not in *rpoS* mutant bacteria. *Salmonella* wild-type (JVS-1574), Δ*rpoS* (JVS-5487) and Δ*sdsR* (JVS-8827) were grown to an OD_{600} of 0.3 at 30°C when cultures were split and either maintained at 30°C or subjected to heat-shock at 44°C. Total RNA samples withdrawn prior to and at selected time-points after temperature up-shift were analyzed by Northern blotting; SdsR and *osmY* mRNA were detected using gene-specific riboprobes, 5S levels were determined as loading control. Expression of RpoS was monitored by Western blotting.

Figure S5



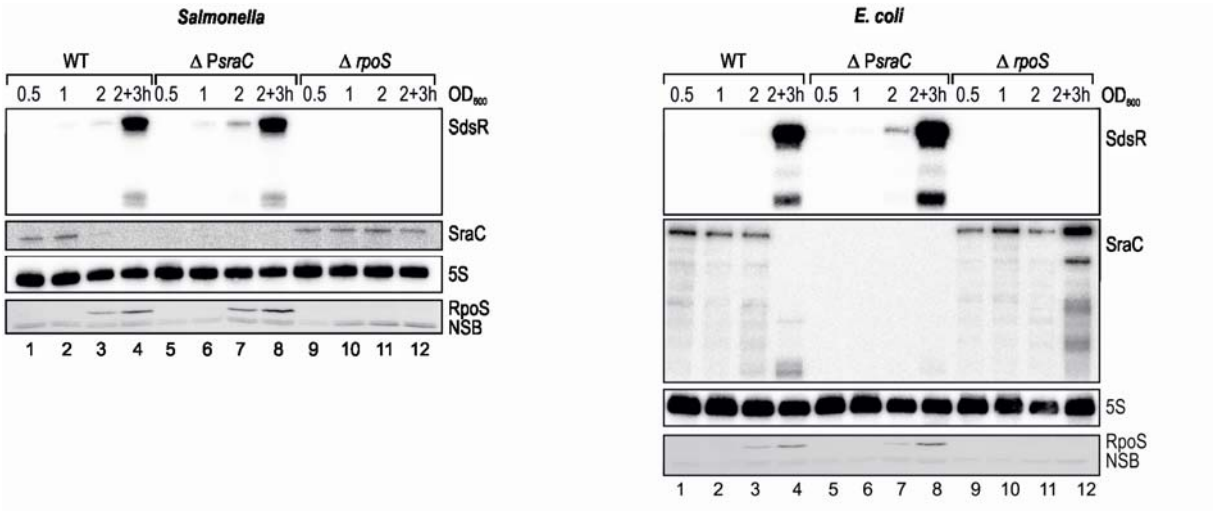
Transcriptional activity at *sdsR* and *osmY* promoters upon heat stress was compared employing *Salmonella sdsR::lacZ* (JVS-8717; red triangles) and *osmY*::*lacZ* (JVS-9145; blue squares) reporter constructs. Cells were grown to an OD_{600} of 0.3 at 30°C when cultures were split and either maintained at 30°C or subjected to heat-shock at 44°C. Relative ß-galactosidase activities were determined over the course of 30 min in culture samples grown at 30°C (filled symbols) or 44°C (open symbols). Standard deviation was calculated from three biological replicates.

Figure S6



Alignment of SdsR, truncated SdsR variants and SdsR-MicA chimera as depicted in Fig. 6A. SdsR-derived sequences are shown in blue, fragments of MicA are in green. Conserved nucleotides are marked by asterisks at the bottom of the alignment. A potential regulatory effect on OmpD is indicated (+/-), and the region of SdsR involved in *ompD* mRNA-pairing is boxed in light grey.

Figure S7



Interdependence of SdsR and SraC expression in *Salmonella* and *E. coli*. Expression of SdsR and SraC sRNAs were compared by Northern blot analysis of total RNA isolated from *Salmonella* and *E. coli* wild-type (JVS-1574 and JVS-5105, respectively), *sraC* promoter mutants (JVS-9251 and JVS-9312, respectively) and *rpoS* mutants (JVS-5487 and JVS-9322, respectively) grown in LB to an OD_{600} of 0.5, 1.0, 2.0 and 3 hours after cells had reached an OD_{600} of 2.0. Probing for 5S RNA confirmed equal loading. RpoS levels were determined by Western blotting of total protein samples; a non-specific band (NSB) served as loading control.

Table S1

name	sequence 5‘-3‘
JVO-0048	TTTCGAGGAATTTCGAGGGGAAACACATAACCCATTGATTTATAATCTAAGTGTAGGCTGGAGCTGCTTC
JVO-0049	TTTTATCTCTGATAACAGACAAAACGCCAGGTTTTTTCAATCACCTTCGTGGTCCATATGAATATCCTCCTTAG
JVO-0051	GTTTTTCTCGAGCATCCGGATGGATTGACA
JVO-0052	GTTTTTTCTAGAGTCTGGCRCCGTTTAT
JVO-0322	CTACGGCGTTTCACTTCTGAGTTC
JVO-0396	TTCATCGCTGAAAACAGG
JVO-0802	GTTTTGACGTCAAATCAATATTGAAACGG
JVO-0862	GTTTTTCTCGAGTCGACCCGCTGTACCT
JVO-0874	CCTGGCTTTCAGTACGGT
JVO-0902	5’Phosphate-GCAAGGCGATTTAGCC
JVO-0903	TTTTTCTAGAAACACATAACCCATTGATT
JVO-0997	GTTTTTTTTAATACGACTCACTATAGGCCTGTATTCGGTCCAGG
JVO-1032	GGCTCTTGGGAGAGAGC
JVO-1043	GTTTTTTCTCGAGCACATAATCTTAACAAGAATGTT
JVO-2191	GAAACTTATTATTGAACTTATGCCACTCCGTCATTTAAAAATAGTCGTATATCCAGTGATTTTTTTCTCCAT
JVO-2192	GTCACCATCACTTCCGTTATCA
JVO-2309	CTGGCGTCGTCATCTA
JVO-2390	CTGGCGTCGTCATCTA
JVO-2484	ATGAAACTTAAGTTAGTGGCAG
JVO-2678	GTTTTATGCATGCCATTGACAAACGCC
JVO-4262	5’Phosphate-TCATGTATTCTTAAAGGGCAAG
JVO-4263	5’Phosphate-TCATATATTCTTAAAGGGCAAG
JVO-4264	5’Phosphate-TCATTTATTCTTAAAGGGCAAG
JVO-4265	CGACGCCAGGTTTTCC
JVO-4314	TGCCACTAACTTAAGTTTCAT
JVO-4731	5’Phosphate-ACTTTTAGCGCACGGCTC
JVO-5738	CGGCGCTAATAGCGACGGGCGGGCCGATATTGATATTAAACTTACTGTCCCTAGTGCTTGG
JVO-5739	TAAAATGGCTCTGTCCGCAAAGACAACGACCAGTGAACGTCTGCACGGCATACTCCTTAT
JVO-6533	CTGGAAAACCTGGCGTCGTCATCTATTCTTAAAGGGCAAGGTGTAGGCTGGAGCTGCTTC
JVO-6534	TTTCGAGGGGAAACACATAACCCATTGATTTATAATCTAAGGTCCATATGAATATCCTCCTTAG
JVO-7023	GTTTTTTTTAATACGACTCACTATAGGCAAGGCGATTTAGCC
JVO-7025	AGAGACCGAATACGATTCC
JVO-7159	CGATTTAGCCTGCATTAATG
JVO-7161	CATTAATGCCAACTTTTAGCG
JVO-7163	TGGGATTAATGCAGGCTAA
JVO-7224	GCCTGCATTAATGCCAACTCATCCCTGTTTTCAGCG
JVO-7225	TTGGGAGCAGGCGTTGT
JVO-7328	5’Phosphate-CAGGGAAGTCACTGCCACTG
JVO-7692	ATGCCTGTTCAATGCGTG
JVO-7693	GTTTTTTTTTTAATACGACTCACTATAGGGAGGTTTCGGTTCCGCTTTG
JVO-7742	GCTTACGTGATGCTCTGATTTTGTTGTAAAAGAAATGTTAGGTCCATATGAATATCCTCCTTAG
JVO-7743	GTGTTTCCCCTCGAAATTCCTCGAAATTTCCTCGAATTTCGTGTAGGCTGGAGCTGCTTC
JVO-7859	AGATCACATAATCTTAACAAGAATGTTAAAAAACGCTGGAGGTCCATATGAATATCCTCCTTAG
JVO-7860	TTTATCTGTTAAAAGCCAGAAGCATTTCCTTCGCTGACTTGTGTAGGCTGGAGCTGCTTC
E1	5’Phosphate-UUCACUGUUCUUAGCGGCCGCAUGCUC-idT[1]
E3 RACE	GGCCGCTAAGAACAGTGAA
M13 fwd	GTAAAACGACGGCCAG
M13 rev	CAGGAAACAGCTATGAC
p_LLacO C	5’Phosphate-GTGCTCAGTATCTTGTTATCCG
pZE-A	GTGCCACCTGACGTCTAAGA
pZE-Cat	TGGGATATATCAACGGTGGT
pZE-T1	CGGCGGATTTGTCCTACT
pZE-Xba	TCGTTTTATTTGATGCCTCTAGA
pZE-A	GTGCCACCTGACGTCTAAGA

[1] idT: 3‘ inverted deoxythymidine

Table S2

Plasmid trivial name	Plasmid stock name	Expressed fragment	Comment	Origin, Marker	Reference
	pJV300		pP_L control plasmid, expresses a ~50 nt nonsense transcript derived from *rrnB* terminator	ColE1, Amp^R	(57)
pBAD	pKP8-35		pP_{BAD} control plasmid, expresses a ~50 nt nonsense transcript derived from *rrnB* terminator	pBR322, Amp^R	(21)
	pRH800		pP_{tac} control plasmid	pBR322, Amp^R	(62)
pP_{tac}-RpoS	pRL40.1	*rpoS*	*E. coli rpoS* expressed from constitutive P_{tac} promoter	pBR322, Amp^R	(62)
pP_L-SdsR	pKF68-3	SdsR	ColE1 plasmid based on pZE12-luc; expresses *Salmonella* SdsR from constitutive P_{Llac0} promoter	ColE1, Amp^R	This study
pP_L-SdsR proc.	pKF73-1	SdsR proc.	ColE1 plasmid based on pZE12-luc; expresses truncated *Salmonella* SdsR (starting at processing site +31) from constitutive P_{Llac0} promoter	ColE1, Amp^R	This study
pP_L-SdsR +7	pKF97-1	SdsR +7	ColE1 plasmid based on pZE12-luc; expresses truncated *Salmonella* SdsR (starting at +7) from constitutive P_{Llac0} promoter	ColE1, Amp^R	This study
pP_L-SdsR +19	pKF99-1	SdsR +19	ColE1 plasmid based on pZE12-luc; expresses truncated *Salmonella* SdsR (starting at +19) from constitutive P_{Llac0} promoter	ColE1, Amp^R	This study
pP_L-TMA	pFS135	TMA	ColE1 plasmid based on pZE12-luc; expresses truncated *Salmonella* MicA (starting at +23) from constitutive P_{Llac0} promoter	ColE1, Amp^R	(50)
pP_L-SdsR-TMA	pKF105-1	SdsR-TMA	ColE1 plasmid based on pZE12-luc; expresses truncated *Salmonella* SdsR (+14-32) fused to +23 of *Salmonella* MicA from constitutive P_{Llac0} promoter	ColE1, Amp^R	This study
pP_L-SdsR*	pKF101-26	SdsR*	ColE1 plasmid based on pZE12-luc; expresses *Salmonella* SdsR with SNE G26C from constitutive P_{Llac0} promoter	ColE1, Amp^R	This study
pBAD-SdsR	pKP19-8	SdsR	expresses *Salmonella* SdsR from arabinose-inducible P_{BAD} promoter	ColE1, Amp^R	This study
gfp	pJV859-8	*gfp*	Control plasmid, expresses *gfp* from constitutive $P_{Ltet0-1}$ promoter	pSC101*, Cam^R	(57)
D+3::*gfp*	pVP192-1	*ompD*+3::*gfp*	expresses *ompD+3::gfp* translational fusion from constitutive $P_{Ltet0-1}$ promoter	pSC101*, Cam^R	(52)
D+45::*gfp*	pVP188-1	*ompD*+45::*gfp*	expresses *ompD+45::gfp* translational fusion from constitutive $P_{Ltet0-1}$ promoter	pSC101*, Cam^R	(52)
D+78::*gfp*	pVP206-1	*ompD*+78::*gfp*	expresses *ompD+78:: gfp* translational fusion from constitutive $P_{Ltet0-1}$ promoter	pSC101*, Cam^R	(52)
D+99::*gfp*	pVP207-1	*ompD*+99::*gfp*	expresses *ompD+99::gfp* translational fusion from constitutive $P_{Ltet0-1}$ promoter	pSC101*, Cam^R	(52)
p*sdsR*	pVP203-1	SdsR; SraC	pSC101* plasmid based on pZE12-luc; expresses *Salmonella* SdsR from endogenous promoter	pSC101*, Amp^R	This study
p*sdsR* C-13G	pKF106-2	SdsR; SraC	pSC101* plasmid based on pZE12-luc; expresses *Salmonella* SdsR from endogenous promoter with SNE (C-13G)	pSC101*, Amp^R	This study
p*sdsR* C-13A	pKF107-1	SdsR; SraC	pSC101* plasmid based on pZE12-luc; expresses *Salmonella* SdsR from endogenous promoter with SNE (C-13A)	pSC101*, Amp^R	This study
p*sdsR* C-13T	pKF108-3	SdsR; SraC	pSC101* plasmid based on pZE12-luc; expresses *Salmonella* SdsR from endogenous promoter with SNE (C-13T)	pSC101*, Amp^R	This study
p*ompD*	pVP42-3	*ompD*	*ompD* complementation plasmid; expresses *ompD* from its own promoter	pSC101*, Cam^R	(52)
p*ompD**	pKF109-1	*ompD**	expresses *ompD** (C46G) from its own promoter	pSC101*, Cam^R	This study

	pZE12-*luc*	*luc*	general cloning plasmid	ColE1, Amp^R	(70)
	pVP003	*luc*	general cloning plasmid; low copy version of pZE12-luc	pSC101*, Amp^R	(57)
	pBAD Myc-His A		pBAD expression plasmid		Invitrogen
	pKD4		template plasmid KanR	oriRγ, Amp^R	(58)
	pKD46	*γ-β-exo*	Temperature-sensitive lambda red recombinase expression plasmid; expresses λRED-recombinase from arabinose-inducible P_{araB} promoter	oriR101, AmpR	(58)
	pCP20	FLP - ci857	Temperature-sensitive Flp recombinase expression plasmid	pSC101, Amp^R, Cam^R	(103)
	pKG136		For FLP-mediated *lacZ-Y* integration to construct transcriptional *lac* fusions	oriR6K, Kan^R	J.M. Slauch

Table S3

Trivial name in the manuscript	Bacterium	Stock name	Genotype; relevant markers	Source/reference
wild-type	*S. typhimurium*	JVS-0007	SL1344 *hisG rpsL xyl*	laboratory stock
wild-type	*E. coli*	JVS-5105	*relA+* derivative of MC4100 (*araD139* (*argF-lac*)*205 flb-5301 pstF25 rpsL150 deoC1 relA1*)	T. Nyström; (104)
wild-type	*Shigella flexneri*	JVS-0012	BS 176; plasmid cured derivative of *S. flexneri* M90T	Arturo Zychlinsky; (105)
Δ*rpoS*	*S. typhimurium*	JVS-5487	SL1344 Δ*rpoS::*Cm^R	K. Tedin (KT4676)
sdsR::lacZ	*S. typhimurium*	JVS-8717	SL1344 *sdsR::lacZ::*Kan^R	This study
osmY::lacZ	*S. typhimurium*	JVS-9145	SL1344 *osmY::lacZ::*Kan^R	J. Casadesus (SV6068)
	S. typhimurium	JVS-0028	SL1344 Δ*sdsR::*Kan^R	(27)
Δ*sdsR*	*S. typhimurium*	JVS-8827	SL1344 Δ*sdsR*	This study
Δ*ompD*	*S. typhimurium*	JVS-0735	SL1344 Δ*ompD*::Kan^R	(57)
Δ*sdsR ΔompD*	*S. typhimurium*	JVS-8434	SL1344 Δ*sdsR* Δ*ompD*::Kan^R	This study
	S. typhimurium	JVS-6999	SL1344 [*rluC-rne*]IG::*cat*	L. Bossi; (74)
	S. typhimurium	JVS-7000	SL1344 [*rluC-rne*]IG::*cat rne-3071* (ts)	L. Bossi; (74)
rne-ctrl.	*S. typhimurium*	JVS-9549	SL1344 [*rluC-rne*]IG::*cat ΔsdsR ΔmicC ΔrybB ΔinvR*	This study
rne-TS	*S. typhimurium*	JVS-9550	SL1344 [*rluC-rne*]IG::*cat rne-3071* (ts) Δ*sdsR* Δ*micC* Δ*rybB* Δ*invR*	This study
	S. typhimurium	JVS-8799	SL1344 Δ*sdsR ΔmicC ΔrybB ΔinvR*	This study
	S. typhimurium	JVS-8827	SL1344 *ΔmicC ΔrybB ΔinvR*	This study
	S. typhimurium	JVS-8798	SL1344 Δ*sdsR ΔmicC ΔinvR*	This study
P_{tet} *ompD*	*S. typhimurium*	JVS-9488	SL1344 Cm^R::$P_{Ltet0-1}$-*ompD*	This study
P_{tet} *ompD ΔmicC ΔrybB ΔinvR*	*S. typhimurium*	JVS-9491	SL1344 *ΔmicC ΔrybB ΔinvR* Cm^R::$P_{Ltet0-1}$-*ompD*	This study
P_{tet} *ompD* Δ*sdsR ΔmicC ΔinvR*	*S. typhimurium*	JVS-9655	SL1344 Δ*sdsR ΔmicC ΔinvR* Cm^R::$P_{Ltet0-1}$-*ompD*	This study
Δ*sdsR ompD*	*S. typhimurium*	JVS-9154	SL1344 Δ*sdsR* Cm^R::*ompD*	This study
Δ*sdsR ompD**	*S. typhimurium*	JVS-9155	SL1344 Δ*sdsR* Cm^R::*ompD**	This study
Δ*relA*Δ*spoT*	*S. typhimurium*	JVS-1505	SL1344 Δ*relA ΔspoT*211::Tn*10*	K. Tedin (KT4478)
Δ*sdsR*Δ*rpoS*	*S. typhimurium*	JVS-9551	SL1344 Δ*sdsR* Δ*rpoS::*Kan^R	This study
	S. typhimurium	JVS-0051	SL1344 Δ*micC::*Kan^R	(27)
	S. typhimurium	JVS-0127	SL1344 Δ*rybB::*Kan^R	(21)
	S. typhimurium	JVS-0175	SL1344 Δ*invR::*Kan^R	(53)
ΔP*sraC*	*S. typhimurium*	JVS-9251	SL1344 ΔP*sraC*	This study
	E. coli	JVS-1382	MC4100 Δ*rpoS::*Tn*10*	S. Altuvia
Δ*rpoS*	*E. coli*	JVS-9322	MC4100 *relA+* Δ*rpoS::*Tn*10*	This study
ΔP*sraC*	*E. coli*	JVS-9312	MC4100 *relA+* ΔP*sraC*	This study
TOP10	*E. coli*		F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lacZΔM15* Δ*lacX74 recA1 araD139* Δ(*ara-leu*)7697 *galU galK rpsL endA1 nupG* λ^-	Invitrogen

Supplementary References

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105. Zychlinsky, A., Prevost, M.C. and Sansonetti, P.J. (1992) Shigella flexneri induces apoptosis in infected macrophages. *Nature*, **358**, 167-169.