Supporting Information

Oxygen-Dependent Regulation of SPI1 Type Three Secretion System by Small RNAs in *Salmonella enterica* serovar Typhimurium

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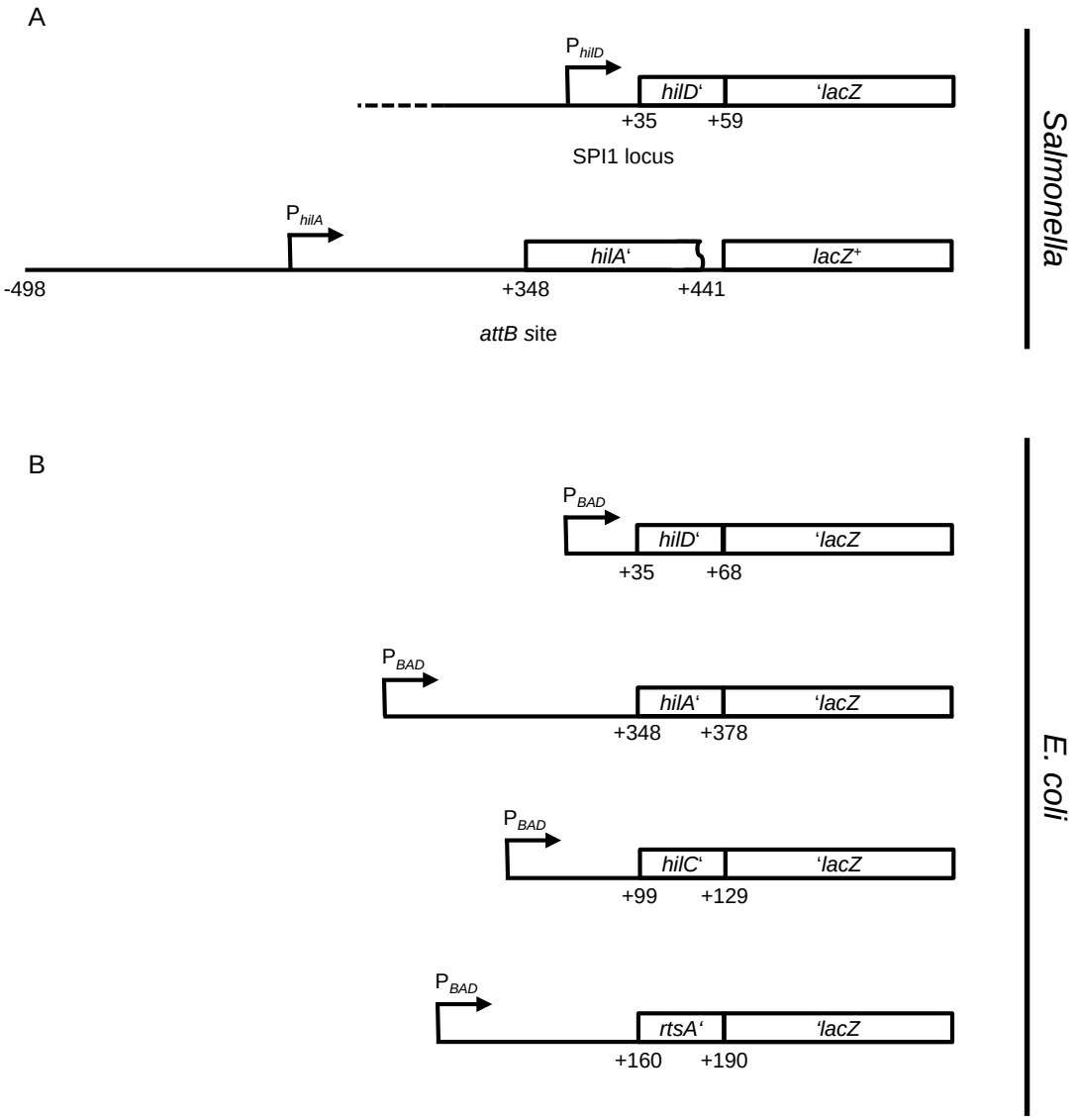

Figure S1. Schematic representation of the *lacZ* reporter systems used in this study. The following *lacZ* reporter fusions were used to identify sRNAs affecting SPI1 gene expression and to characterize the mechanism of regulation. (A) In *Salmonella*, an in locus *hilD'-'lacZ* translational fusion (and, therefore, *hilD* null), or a *hilA'-lacZ*$^{+}$ transcriptional fusion integrated at the *attB* site in the chromosome both controlled by the native promoters. (B) In *E.coli*, *hilD'-'lacZ, hilC'-'lacZ, rtsA'-'lacZ,* or *hilA'-'lacZ* translational fusions were constructed in the PM1205 background. Each translational fusion contains the full 5' UTR and the partial coding region of the corresponding gene under the control of the P_{BAD} promoter.

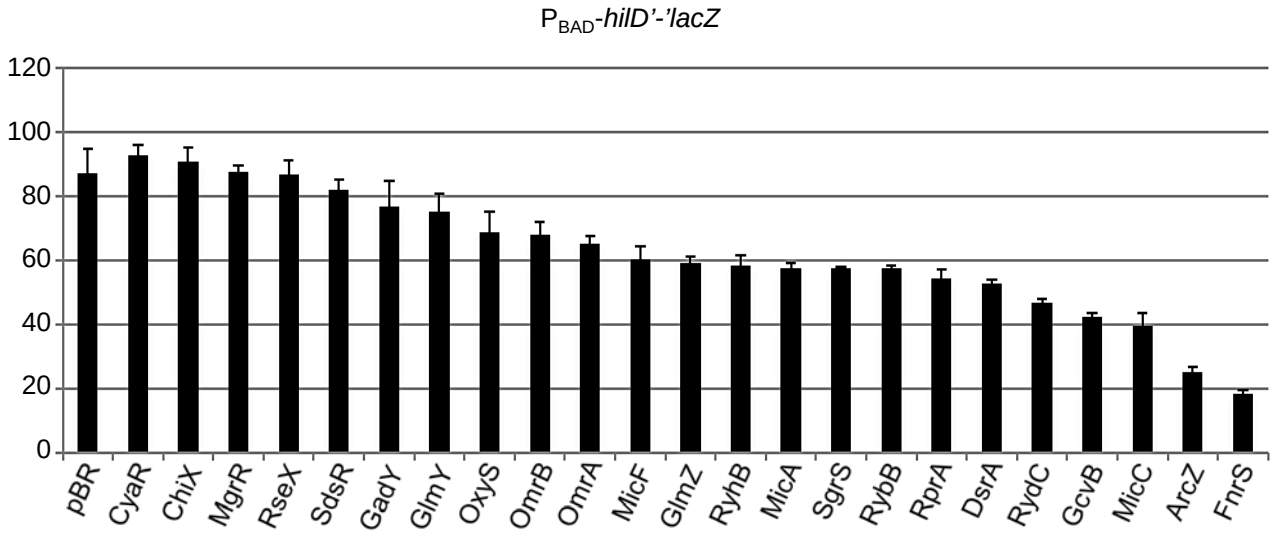

Figure S2. Screen for sRNAs that repress *hilD* translation.
β-galactosidase activity in *E. coli* strains containing the *hilD'-'lacZ* translational fusion and plasmids overexpressing the indicated *E. coli* sRNA. Strains were grown in the presence of 100 μM IPTG and 0.001% arabinose to induce the sRNA expression and the fusion *lacZ* protein expression, respectively. β-galactosidase activity units are defined as (μmol of ONP formed min^{-1}) x 10^6/(OD_{600} x ml of cell suspension) and are reported as mean ± standard deviation where n=3. Strains used: JMS6500 with plasmid pBRplac or this vector expressing the indicated sRNA.

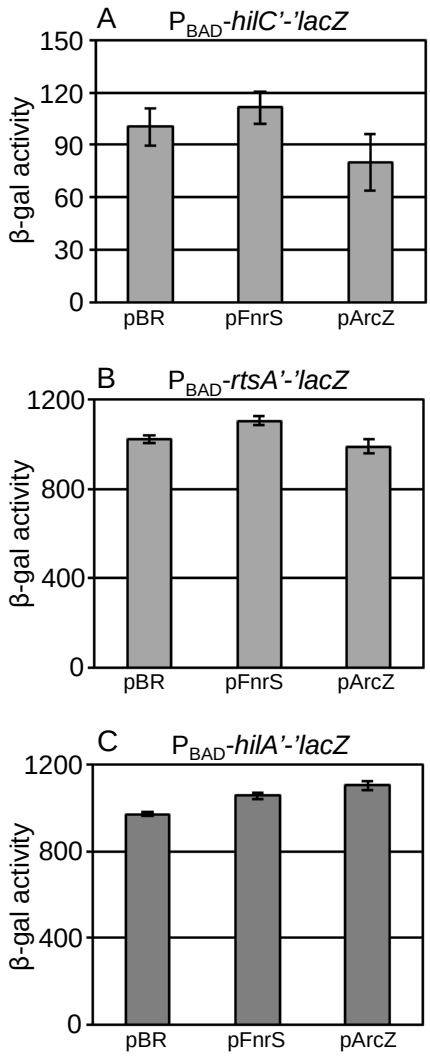

Figure S3. FnrS and ArcZ do not regulate *hilC, rtsA, or hilA* translation in *E. coli.* β-galactosidase activity in *E.coli* strains containing the (A) *hilC'-'lacZ* , (B) *rtsA'-'lacZ* , or (C) *hilA'-'lacZ* translational fusions and plasmids overexpressing FnrS or ArcZ grown in the presence of 100 μM IPTG and 0.001% arabinose to induce the sRNA expression and the fusion *lacZ* protein expression, respectively. β-galactosidase activity units are defined as (μmol of ONP formed min^{-1}) x 10^6/(OD_{600} x ml of cell suspension) and are reported as mean ± standard deviation where n=3. Strains used: JMS6503, JMS6504, and JMS6505, each with plasmid pBRplac, pFnrS-SM, or pArcZ-SM.

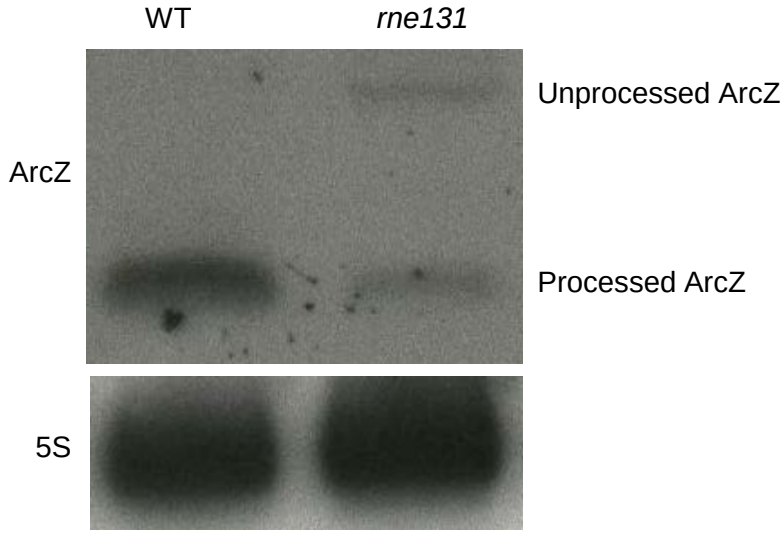

Figure S4. Processing of ArcZ in *rne131* background.
Total RNA was isolated from strains grown under high aeration conditions and processed as described in Experimental procedures. The RNA was probed for ArcZ sRNA and 5S RNA (loading control). This gel is representative of two independent experiments. Strains used: 14028 and JS2117.

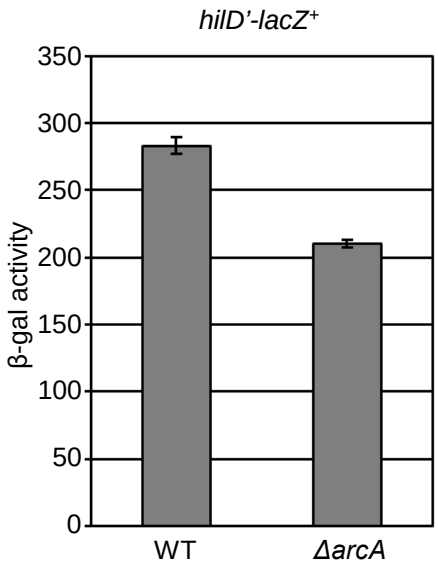

Figure S5. ArcA activates *hilD* transcription during aerobic growth. β-galactosidase activity in *Salmonella* strains containing the *hilD'-lacZ*$^{+}$ transcriptional fusion in the wild type or Δ*arcA* background grown in high aeration conditions. Note that this strain is *hilD* null. β-galactosidase activity units are defined as (μmol of ONP formed min^{-1}) x 10^{6}/(OD_{600} x ml of cell suspension) and are reported as mean ± standard deviation where n=3. Strains used: JS883 and JS2161.

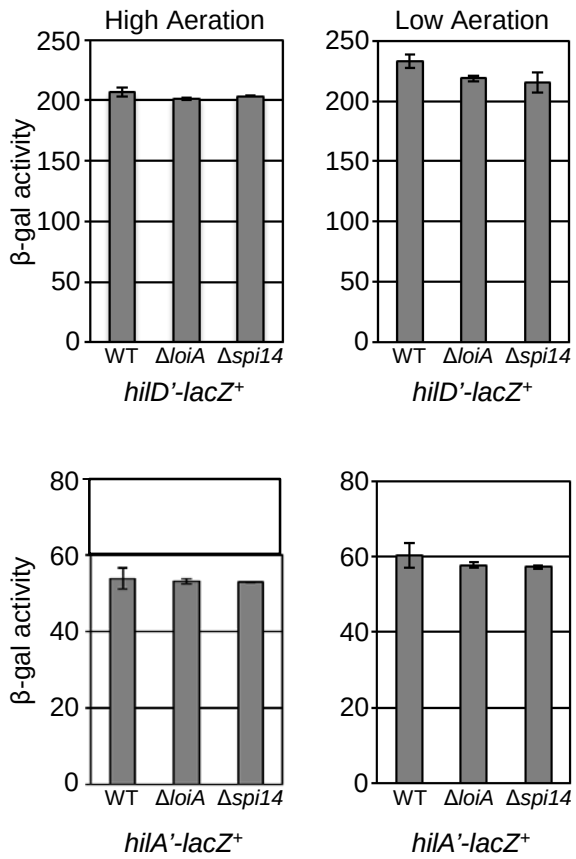

Figure S6. Loss of LoiA or SPI-14 does not affect *hilD* or *hilA* transcription. β-galactosidase activity in *Salmonella* strains containing the *hilD'-lacZ*$^+$ or the *hilA'-lacZ*$^+$ transcriptional fusion in the wild type, Δ*loiA*, or Δ*spi14* background grown in either high oxygen or low oxygen conditions. β-galactosidase activity units are defined as (μmol of ONP formed min^{-1}) x 10^6/(OD_{600} x ml of cell suspension) and are reported as mean ± standard deviation where n=3. Strains used: JS883, JS2156, JS2157, JS749, JS2158, and JS2159.

Table S1. Bacterial strains and plasmids

Strain	Genotype	Deletion endpoints[a]	Source or reference[b]
Salmonella			
14028	Wild type		ATCC[c]
JS481	Δ(*invH-avrA*)*2916*::*Cm* (called Δ*spi1-2916*::*Cm*)		(Ellermeier *et al.*, 2005)
JS570	Δ*hfq11*::*Cm*		(Ellermeier & Slauch, 2008)
JS892	Φ(*hilD'-'lacZ*)*hyb139*		(Chubiz *et al.*, 2010)
JS749	*attλ*::*pDX1*::*hilA'-lacZ*$^+$		(Lin *et al.*, 2008)
JS2117	*rne131*::*Cm*	1226517 - 1227962	
JS2118	Φ(*hilD'-'lacZ*)*hyb139* Δ*hfq11*::*Cm*		
JS2119	Φ(*hilD'-'lacZ*)*hyb139 rne131*::*Cm*		
JS2120	Δ*fnrS3*::*Cm*	176026 - 1759907	
JS2121	Δ*arcZ252*::*Kn*	3504279 - 3504387	
JS2122	Δ*arcZ252*::*Cm*	3504279 - 3504387	
JS2123	Φ(*hilD'-'lacZ*)*hyb139* Δ*fnrS3*::*Cm*		
JS2124	Φ(*hilD'-'lacZ*)*hyb139* Δ*arcZ252*::*Cm*		
JS2125	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*fnrS3*::*Cm*		
JS2126	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*arcZ252*::*Cm*		
JS2127	Δ*fnr4*::*Kn*	1764312 - 1765103	
JS1046	Δ*fnr1*::*Cm*		(Golubeva *et al.*, 2012)
JS1063	Δ*fnr2*::*Tet*		(Golubeva *et al.*, 2012)
JS2128	*fnrS5::cm*	1759979 - 1759907	
JS2129	*fnrS5'-lacZ*		
JS2130	*fnrS5'-lacZ*+ Δ*fnr2*::*Tet*		
JS2131	*fnrS5'-lacZ*+ Δ*fur42*::*Tet*		
JS583	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*fur42*::*Tet*		(Ellermeier and Slauch, 2008)
JS2132	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*fur42*::*Tet* Δ*fnrS3*::*Cm*		

JS619	Φ(*sodB111'-'lac*)*hyb*		(Ellermeier and Slauch, 2008)
JS2133	Φ(*sodB111'-'lac*)*hyb* Δ*fnrS3*::*Cm*		
JS620	Φ(*sodB111'-'lac*)*hyb* Δ*fur42*::*Tet*		(Ellermeier and Slauch, 2008)
JS2134	Φ(*sodB111'-'lac*)*hyb* Δ*fur42*::*Tet* Δ*fnrS3*::*Cm*		
JS2135	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*fnr2*::*Tet*		
JS2136	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*fnr2*::*Tet* Δ*fnrS3*::*Cm*		
JS2137	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT purA*::*tetRA-rtsA*		
JS2138	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT purA*::*tetRA-rtsA* Δ*fnrS3*::*Cm*		
JS2139	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT purA*::*tetRA-rtsA* Δ*fnr4*::*Kn*		
JS2140	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT purA*::*tetRA-rtsA* Δ*fnr4*::*Kn* Δ*fnrS3*::*Cm*		
JS2141	Δ*arcA253*::*cm*	4868202 - 4868920	
JS2142	Δ*arcA254*::*Tet*	4868242 - 4868863	
JS2077	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ ∆*spi1-2916*::*FRT* ∆*rtsA5*::*FRT purA*::*tetRA-rtsA*		(Golubeva *et al.*, 2016)
JS2143	Φ(*hilD'-'lacZ*)*hyb139* Δ*arcA253*::*Cm*		
JS2144	Φ(*hilD'-'lacZ*)*hyb139* Δ*arcA253*::*Cm* Δ*arcZ252*::*Kn*		
JS2145	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*arcA253*::*Cm*		
JS2146	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*arcA253*::*Cm* Δ*arcZ252*::*Kn*		
JS2147	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT*		
JS2148	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT* Δ*arcZ252*::*Kn*		
JS2149	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT* Δ*arcA253*::*Cm*		
JS2150	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ Δ*hilD138*::*FRT* Δ*arcZ252*::*Kn* Δ*arcA253*::*Cm*		
JS2151	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ ∆*spi1-2916*::*FRT* ∆*rtsA5*::*FRT purA*::*tetRA-rtsA* Δ*arcA253*::*Cm*		
JS2152	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ ∆*spi1-2916*::*FRT* ∆*rtsA5*::*FRT purA*::*tetRA-rtsA* Δ*arcZ252*::*Kn*		
JS2153	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ ∆*spi1-2916*::*FRT* ∆*rtsA5*::*FRT purA*::*tetRA-rtsA* Δ*arcA253*::*Cm* Δ*arcZ252*::*Kn*		
JS883	Φ(*hilD'-lac*$^+$)*139*		(Chubiz *et al.*, 2010)
JS2154	Δ*loiA*::*Cm*	933078 - 934003	
JS2155	Δ*spi14*::*Cm*	927271 - 934003	

JS2156	Φ(*hilD'-lac*$^+$)*139 ΔloiA*::*Cm*		
JS2157	Φ(*hilD'-lac*$^+$)*139 Δspi14*::*Cm*		
JS2158	*att*λ::pDX1::*hilA'-lacZ*$^+$ *ΔloiA*::*Cm*		
JS2159	*att*λ::pDX1::*hilA'-lacZ*$^+$ *Δspi14*::*Cm*		
JS2160	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ *ΔfnrS3*::*Cm ΔarcZ252*::*Kn*		
JS2161	Φ(*hilD'-lac*$^+$)*139 ΔarcA254*::*Tet*		
JS2075	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ *Δspi1-2916*::*FRT ΔrtsA*::*FRT*		(Golubeva *et al.*, 2016)
JS2162	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ *Δspi1-2916*::*FRT ΔrtsA*::*FRT ΔfnrS3*::*Cm ΔarcZ252*::*Kn*		
JS107	*zjg8101*::*Kn*		(Mann and Slauch, 1997)
JS2163	*att*λ::pDX1::*hilA'-lacZ*$^+$ *zjg8101*::*Kn*		
JS2164	*attλ*::*pDX1*::*hilA'-lacZ*$^+$ *ΔarcZ252*::*Kn*		
JS2165	*ΔfnrS3::Kn*	176026 - 1759907	
JS2166	*rne131*::*Cm ΔfnrS3::Kn*		
JS2167	*rne131*::*Cm ΔarcZ252*::*Kn*		
E. coli			
PM1205	MG1655 *mal*::*lacI*^{q}, *ΔaraBAD araC*$^+$, *lacI'*::P_{BAD}*-cat-sacB*:*lacZ, miniλtet*^{R}		(Mandin and Gottesman, 2009)
JMS6500	PM1205 *lacI'*::P_{BAD}*-hilD'-'lacZ*		
JMS6501	PM1205 *lacI'*::P_{BAD}*-hilD*mt1*'-'lacZ*		
JMS6502	PM1205 *lacI'*::P_{BAD}*-hilD*mt2*'-'lacZ*		
JMS6503	PM1205 *lacI'*::P_{BAD}*-hilC'-'lacZ*		
JMS6504	PM1205 *lacI'*::P_{BAD}*-rtsA'-'lacZ*		
JMS6505	PM1205 *lacI'*::P_{BAD}*-hilA'-'lacZ*		
Plasmids			
pBRplac	Amp^R, p*lac* promoter based expression vector		(Guillier and Gottesman, 2006)

pFnrS-EC	AatII-EcoRI *fnrS*(*E.coli*) containing fragment cloned into pBRplac		(Mandin and Gottesman, 2009)
pFnrS-SM	AatII-EcoRI *fnrS*(*Salmonella*) containing fragment cloned into pBRplac		
pFnrS-mt	C47G site directed mutation in pFnrS-SM		
pArcZ-EC	AatII-EcoRI *arcZ*(*E.coli*) containing fragment cloned into pBRplac		(Mandin and Gottesman, 2009)
pArcZ-SM	AatII-EcoRI *arcZ*(*Salmonella*) containing fragment cloned into pBRplac		
pArcZ-mt	C66G, C67G, G70C, G71T, T72A, G73C, T74A site directed mutations in pArcZ-SM		

a Numbers indicate the base pairs that are deleted (inclusive) as defined in the *S. enterica* serovar Typhimurium 14028 genome sequence (National Center for Biotechnology Information; CP001363.1).
b This study unless specified otherwise.
c ATCC, American Type Culture Collection.

Table S2. Primers used

Brief description	Primer Sequence
F-AatII-ArcZ	GACTGACGTCGTGCGGCCTGAAAACAGGACTGCGC
R-EcoRI-ArcZ	GACTGAATTCCCTGGGTGGCAAACGCGGAAAA
F-AatII-FnrS	GACTGACGTCGCAGGTGAATGCAACGTCAAGCGAT
R-EcoRI-FnrS	GACTGAATTCGACGCAGATAGTCTACAGGCAAAA
F-mt-ArcZ	TAAGCACGGCGCAGCCACGATTTGGCTCTACATGGCGCAGT ATTCGCGCACCCCGG
R-mt-ArcZ	CCGGGGTGCGCGAATACTGCGCCATGTAGAGCCAAATCGTG GCTGCGCCGTGCTTA
F-mt-FnrS	CGATGGGCGTTGCGCTCCATATTGACTTACTTCCTTTTTTGAA TTACT
R-mt-FnrS	AGTAATTCAAAAAAGGAAGTAAGTCAATATGGAGCGCAACGC CCATCG
F-C-rne131-KO	CTGGCGGTCGGCTTTGTATCAGCATTTACATGTAGGCTGGAG CTG
R-C-rne131-KO	ACCGTCGAAACAGCCGCGCCGAAAGCGGAATAACATATGAA TATCCTC
F-hilD'-'lacZ E. coli	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATAAG GAACATTAAAATAACAT
R-hilD'-'lacZ E. coli	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACAT GACTATTACTTACAAAGG
F-hilD'-'lacZ mt1	TTTATCGCAACTCTCTACTGTTTCTCCATAAGGAACATTAAAA TAACATCAACAAAGGGT
F-hilD'-'lacZ mt2	TTTTATCGCAACTCTCTACTGTTTCTCCATAAGGAACATTAAA ATATGTGGAACAAAGCC
F-hilA'-'lacZ E. coli	ACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATAAAACTAA TCTCTATTGCAATGAGG
R-hilA'-'lacZ E. coli	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCG ATACAGGAACAGGATTAA
F-rtsA'-'lacZ E. coli	TTTTATCGCAACTCTCTACTGTTTCTCCATAGAAATGCAATAT AAAATAGCATTTTCCAT
R-rtsA'-'lacZ E. coli	GTTTTCCCAGTCACGACGTTGTAAAACGACCTGGACAGGTGA GGGATTAAATACTTTTAG
F-hilC'-'lacZ E. coli	CTTTTTATCGCAACTCTCTACTGTTTCTCCATGAGTTCCTTAT AGCACACAGGATAAAAT
F-hilC'-'lacZ E. coli	AGGGTTTTCCCAGTCACGACGTTGTAAAACGACCTCAACTGA TTTATTCATTGAAGGCAA
F-KO-Fnr	TCCTTCTCCGGGATAGCTCAGACTTACGCGCTCACCAAAAAG ATGTGTAGGCTGGAGCTG
R-KO-Fnr	ATTTAACGATATGGCAGAAGATAACATCAATGGTTTAGCTGA CGTCATATGAATATCCTC
F-KO-FnrS	AGTCAATAAACCATCTACCTATTCGGGGCAATATCTCTCTCG CAGTGTAGGCTGGAGCTG
R-KO-FnrS	ATGTCATTCAGACTCTTAAAGGGTAGACGCAGATAGTCTACA GGCCATATGAATATCCTC
F-KO-FnrS20	TGAATGCAACGTCAAGCGATGGGCGTTGCGCTCCATATTGTC TTATGTAGGCTGGAGCTG

F-KO-ArcZ109	ATTCATGTAACAAATCATTTAGGATTTGCTATCTTAACTGCGT GCTGTAGGCTGGAGCTG
R-KO-ArcZ109	GTCGCGGTGCTGAAAGCCTGGGTGGCAAACGCGGAAAAAAA ATGACATATGAATATCCTC
F-ArcA-KO	ACCGGCTGTTTTTACAGTTTGGCGCCTGGGCCGAATGTAGG CTGGAGCTG
R-ArcA-KO	CTGTTTCGATTTAGTTGGCAATTTAGGTAGCAAACCATATGAA TATCCTC
F-KO-LoiA	ATGCAAACGTTAGAACGGTTTTTTCTTTTCGTTACGGGGTGTA GGCTGGAGCTG
F-KO-spi14	AACGGACCAAATTATACAGGGATGTAACGCTATCACTCAGTC TGTGTAGGCTGGAGCTG
R-KO-LoiA	AAAGCAGCACACTGTATTATACGTTAATTATGAGCCACAACG ATGCATATGAATATCCTC
AO-ArcZ	GAATACTGCGCCAACACCAG
AO-RrfA	CTACGGCGTTTCACTTCTGAGTTC
hilD-CDS-F	ATGGAAAATGTAACCTTTGTAAGTAATAGTCATCAG
hilD-CDS-R	TTAATGGTTCGCCATTTTTATGAATGTCGATGGCGT

Table S3. In vivo competitions between *hilA-lac* pDX1 and wild type *S*. Typhimurium.

Route of infection	Tissue	Geometric Mean CI	Number of Mice	P
IP	Spleen	0.91	6	NS
Oral	Small Intestine	0.86	5	NS
	Spleen	0.60	5	NS

Strains used: JS107, JS2163

Table S4. In vitro competition assays

Strain A	Strain B	No. of replicates	CI	P
13 mm tubes: 2 mL HSLB, roller drum, 16 h, 37°C				
hilA-lac pDX1	wt	6	0.93	NS
fnrS arcZ hilA-lac pDX1	*hilA-lac* pDX1	6	1.35	0.0024
fnrS arcZ spi1 hilA-lac pDX1	*spi1 hilA-lac* pDX1	6	1.27	0.009
125 mL flasks: 4 mL HSLB, shaker 200 rpm, 16 h, 37°C				
hilA-lac pDX1	wt	6	0.95	NS
fnrS arcZ hilA-lac pDX1	*hilA-lac* pDX1	6	1.17	0.005
fnrS arcZ spi1 hilA-lac pDX1	*spi1 hilA-lac* pDX1	6	1.03	NS

Strains were grown the same as described in Experimental procedures for a mice competition assays, then mixed 1:1, diluted and inoculated into indicated medium/condition at 10^3 bacteria per tube/flask, then grown for 16h in indicated conditions. Strains used: JS107, JS2163, JS749, JS2075, JS2162, and JS2160.

References

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