SUPPLEMENTAL MATERIAL

Bacterial strains, plasmids constructions, and reagents

S. Typhimurium strain 14028s and *S.* Enteritidis 31194 are the parental strains used for this study. Bacterial strains used in this study are listed in **Table S1**. For the generation of mutants (i.e., *fnr':ha* NC1040, *fur::cat* BTNC0001, *lon::cat* BTNC0021, and *clpPX::cat* BTNC0022), the λ Red protocol was used as described previously (1). Primers used in this study are listed in **Table S2**. P22 transduction was used to transfer mutations into 14028s or 14028s-derived backgrounds and colony PCR followed by sequencing was used to confirm the genotype(s). Transductants were purified on Evans-Blue-Uranine (EBU) agar plates.

To construct the Kan^R 'wild-type' *fnr':ha* strain (NC1040), the 3' end of the *fnr* gene was tagged with the hemagluttinin epitope (HA) using the approach described in (2). This was accomplished by generating the template plasmid p*ha:aph* as follows. Primers BamHApKD4 and HindpKD4 were used to amplify a 1.5 kb product from pKD4. The DNA encoding the HA epitope is in italics. The PCR product was gel extracted, cut with BamHI-HindIII, and ligated overnight at 16°C with a BamHI-HindIII cut pACYC184, which was gel extracted following digestion. The *fnr':ha* PCR product was generated using primers FNRHA fwd and FNRHA rev, where the nucleotides in italics have complementarity to p*ha:aph*. The *fnr':ha* PCR product was used to generate strain NC1040 using the λ Red approach above. The sequence of NC1040 was confirmed by sequencing the PCR product from amplification with primers Fnr middlefwd and Fnr to ydaA rev. The construction of a spontaneous rifampicin resistant isolate of *S.* serovar Enteritidis 31194 (BTNC0025) was accomplished by plating 100 µL of an

overnight culture on LB agar containing 100 µg/mL of rifampicin. An isolate was purified by three passages on LB rifampicin and a glycerol stock was stored at -80°C until used.

The generation of strain BTNC0001 was accomplished as above using primers furKD3 fwd and furKD3 rev and sequenced with fur fwd and fur rev. P22 transduction was used to move the *fur::cat* mutation for generating strains BTNC0006-BTNC0009 and BTNC0017 **(Table S1)**. The generation of strains BTNC0020 and BTNC0021 was accomplished using primers LonpKD3 fwd, LonpKD3 rev, and ClpPXKD3 fwd, ClpPXKD3 rev, respectively. P22 transduction was used as above to transfer the *lon::cat* or *clpPX::cat* mutation into the appropriate genetic backgrounds to generate strains BTNC0011, BTNC0013, BTNC0014, BTNC0016, and BTNC0012, respectively **(Table S1)**. Sequences were confirmed using primers Lon fwd, Lon rev, and ClpPX fwd, ClpPX rev. To generate strain BTNC0015, a P22 lysate from strain JS953 (17) was generated and transduced into 14028s with selection for apramycin and lac^+ phenotypes.

Construction of P_{hilC}-*lacZ*, P_{rtsA}-*lacZ*, and P_{hilD}-*lacZ* was accomplished as follows. DNA from 14028s was used as template for PCR amplification of the P_{hilC}, P_{rtsA}, and P_{hilD} promoters with the primers EcoRIhilC, NotIhilC, EcoRI rtsA, NotI rtsA rev, EcoRIhilD, and NotIhilD, respectively. PCR products were gel extracted, digested with EcoRI-NotI, ligated into the EcoRI-Not digested pSP417, and cloned into chemically competent *Escherichia coli* strain DH5α. Colony PCR was used to screen successful inserts in clones that were Amp^R and lac^+ using primers pSP417 Fwd and pSP417 Rev. Strain NC1040 was electroporated with plasmid preparations from correct DH5α clones and the inserts were sequenced using pSP417 fwd and rev.

To generate p*fur-flag*, p*rtsA-flag*, and p*hilC-flag*, DNA from 14028s was used as template for PCR with the primers furBamHIIPTG , furFLAGHindIII rev , rtsABamHIIPTG fwd, rtsAFLAGEcoRV rev , hilCBamHIIPTG fwd , and hilCFLAGHindIII rev , respectively. The DNA sequence for the *flag* epitope is shown in italics and for all primer sequences shown above the restriction enzyme site is underlined. PCR products were gel purified, digested with BamHI-HindIII or BamHI, ligated to pUHE21-2*lacI*^{q}, and cloned into DH5α. Clones were screened for inserts using the primers pUHE fwd and pUHE rev. Plasmid prepared from correct clones was transformed into BTNC0015 and sequenced using pUHE fwd and rev.

For molecular biology techniques, all DNA amplified by PCR used Phusion® high-fidelity PCR master mix with HF buffer (2X) from New England Biolabs (NEB; Ipswitc, MA). Dimethyl sulfoxide (DMSO) was added to reactions to a final concentration between 1-3%, depending on the target DNA. Plasmid DNA was purified using a GenElute maxiprep kit (Sigma-Aldrich; St. Louis, MO). PCR products were gel extracted using the QIAquick Gel extraction kit (Qiagen; Valencia, CA) and sequencing reactions were performed by Genewiz, Inc. (South Plainfield, NJ). Kanamycin sulfate (65 μg/mL), chloramphenicol (20 μg/mL), tetracycline HCl (12.5 μg/mL for resistance and 2.5 μg/mL for induction), and ortho-nitrophenyl-β-galactoside (ONPG) were purchased from Sigma-Aldrich. Ampicillin (125 μg/ml), 3-morpholinopropane-1-sulfonic acid (MOPS), acrylamide, methanol, rifampicin, and isopropyl β-D-1 thiogalactopyranoside (IPTG) were purchased from Fisher Scientific (Pittsburgh, PA). Apramycin sulfate (100 μg/mL) was purchased from Indofine Chemical company (Hillsborough, NJ).

TABLE S1 – Strains and plasmids used in this study

Strain	Genotype[a]	Source
Salmonella enterica serovar Typhimurium 14028s		ATCC[b]
Salmonella enterica serovar Enteritidis 31194		ATCC
Escherichia coli strain DH5α		Lab stock
BTNC0025	*S.* Enteritidis ATCC 31194 (Rif^R)	This study
NC1040	*fnr':ha* (Kan^R)	This study
BTNC0001	*fur::cat* (Cm^R)	This study
RM5938	*hilA-lacZY* (Tet^R)	(1)
AV0305	*hmpA-lacZY* (Kan^R)	(2)
CA701	*invF-lacZY* (Tet^R)	(1)
RM5385	*sipC-lacZY* (Tet^R)	(3)
BTNC0002	*fnr':ha* pSP417 (Kan^R, Amp^R)	This study
BTNC0003	*fnr':ha* pP_{hilC}-*lacZ* (Kan^R, Amp^R)	This study
BTNC0004	*fnr':ha* pP_{rtsA}-*lacZ* (Kan^R, Amp^R)	This study
BTNC0005	*fnr':ha* pP_{hilD}-*lacZ* (Kan^R, Amp^R)	This study
BTNC0006	*fnr':ha fur::cat* pSP417 (Kan^R, Amp^R, Cm^R)	This study
BTNC0007	*fnr':ha fur::cat* pP_{hilC}-*lacZ* (Kan^R, Amp^R, Cm^R)	This study
BTNC0008	*fnr':ha fur::cat* pP_{rtsA}-*lacZ* (Kan^R, Amp^R, Cm^R)	This study
BTNC0009	*fnr':ha fur::cat* pP_{hilD}-*lacZ* (Kan^R, Amp^R, Cm^R)	This study
BTNC0010	*fnr':ha sipC-lacZY* (Kan^R, Tet^R)	This study
BTNC0011	*fnr':ha lon::cat sipC-lacZY* (Kan^R, Cm^R, Tet^R)	This study
BTNC0012	*fnr':ha clpPX::cat sipC-lacZY* (Kan^R, Cm^R, Tet^R)	This study
BTNC0013	*fnr':ha lon::cat* pP_{hilC}-*lacZ* (Kan^R, Cm^R, Amp^R)	This study
BTNC0014	*fnr':ha lon::cat* pP_{rtsA}-*lacZ* (Kan^R, Cm^R, Amp^R)	This study
BTNC0015	*attλ*::pDX1::*hilA-lacZ* (Ap^R)	This study
BTNC0016	*lon::cat attλ::pDX1::hilA-lacZ* (Ap^R, Cm^R)	This study
BTNC0017	*fur::cat* pUHE21-2*lacI*^{q} *fur-flag attλ*::pDX1::*hilA-lacZ* (Cm^R, Ap^R, Amp^R)	This study
JS1180	*attλ*::pDX1::*hilA-lacZ tetRA-hilD3Xflag* (Ap^R)	(4)
BTNC0018	p*fliZ-flag attλ*::pDX1::*hilA-lacZ* (Ap^R, Amp^R)	This study
BTNC0019	p*rtsA-flag attλ*::pDX1::*hilA-lacZ* (Ap^R, Amp^R)	This study
BTNC0020	p*hilC-flag attλ*::pDX1::*hilA-lacZ* (Ap^R, Amp^R)	This study
BTNC0021	*lon::cat*	This study
BTNC0022	*clpPX::cat*	This study
BTNC0023	RM5385 with p*rtsA-flag*	This study
BTNC0024	RM5385 with p*hilC-flag*	This study

BTNC0026	*S.* Enteritidis ATCC 31194 with pSP417 (Rif^R, Amp^R)	This study
BTNC0027	*S.* Enteritidis ATCC 31194 with pP_{hilC}-*lacZ* (Rif^R, Amp^R)	This study
BTNC0028	*S.* Enteritidis ATCC 31194 with pP_{rtsA}-*lacZ* (Rif^R, Amp^R)	This study
BTNC0029	*S.* Enteritidis ATCC 31194 with pP_{hilD}-*lacZ* (Rif^R, Amp^R)	This study

Plasmids

p*ha-aph*	*The ha* epitope with the aph gene from pKD4 cloned into the BamHI-HindIII site of pACYC184	This study
pSP417	Promoterless *lacZ* shuttle vector derived from pRS415 (Amp^R)	(5)
pP_{hilC}-*lacZ*	-344 to -10 relative to the ATG start codon of *hilC* (*STM14_3465*) from 14028s cloned into the EcoRI-NotI site of pSP417 (Amp^R)	This study
pP_{rtsA}-*lacZ*	-330 to -11 relative to the the ATG start codon of *rtsA* (*STM14_5188*) from 14028s cloned into the EcoRI-NotI site of pSP417 (Amp^R)	This study
pP_{hilD}-*lacZ*	-315 to -9 relative to the ATG start codon of *hilD* (*STM14_3474*) from 14028s cloned into the EcoRI-NotI site of pSP417 (Amp^R)	This study
pUHE21-2*lacI*^{q}	P_{lac} rep_{pMB1} *lacI*^{q} (Amp^R)	(6)
p*fur-flag*	The coding sequence of the *fur (STM14_0808*) gene with in-frame 3' addition of the *flag* epitope gene cloned into pUHE-21-2*lacI*^{q} (Amp^R)	This study
p*fliZ-flag*	The coding sequence of the *fliZ* (*STM14_2373*) gene with the in-fram 3' addition of the *flag* epitope gene cloned into pUHE-21-2*lacI*^{q} (Amp^R)	This study
p*hilC-flag*	The coding sequence of the *hilC (STM14_3465*) gene with in-frame 3' addition of the *flag* epitope gene cloned into pUHE-21-2*lacI*^{q} (Amp^R)	This study
p*rtsA-flag*	The coding sequence of the *rtsA (STM14_5188*) gene with in-frame 3' addition of the *flag* epitope gene cloned into pUHE-21-2*lacI*^{q} (Amp^R)	This study
pKD46	Phage *λ gam-bet-exo* under P_{araB} (Amp^R)	(7)
pKD3	*bla* FRT *cat* FRT PS1 PS2 oriR6K (Amp^R, Cm^R)	(7)
pKD4	*bla* FRT *aph* FRT PS1 PS2 oriR6K (Amp^R, Kan^R)	(7)

[a] Kan^R (kanamycin resistance), Cm^R (chloramphenicol resistance), Tet^R (tetracycline resistance, Amp^R (ampicillin resistance), Ap^R (apramycin resistance).
[b] American Type Culture Collection.

Table S2 Primer Sequences

Primer	Sequence[a]
BamHApKD4	ATA GGA TCC GAC TAC CCT TAT GAC GTA CCA GAC TAC GCA TAA GTG TAG GCT GGA GCT GCT
HindpKD4	ATA AAG CTT CAT GGG AAT TAG CCA TGG TCC
FNRHA fwd	TGC GCT GGC GGC CCT CGC CGG TCA TAC CCG CAA CGT CGC TGA CTA CCC TTA TGA CGT ACC
FNRHA rev	GCC AGA TCA ATA AAT GAG AAA AAT TTA ACG ATA TGG CAG ACA TGG GAA TTA GCC ATG GTC
Fnr middlefwd	ACC TGC GTC AGC AAA TGA TG
Fnr to ydaA rev	AGG GTG GTC ATC TCA TAC G
furKD3 fwd	TCT AAT GAA GTG AAT CGT TTA GCA ACA GGA CAG ATT CCG CCA TGG GAA TTA GCC ATG GTC
furKD3 rev	GCC AAC CGG GCG GTT GGC TCT TCG AAA GAT TTA CAC AAA AGT GTA GGC TGG AGC TGC TTC
fur fwd	TAT TAA CAT CTG CGA GAG ACT TGC
fur rev	TAA CTG GCG AAG CTC CAT TCC
LonpKD3 fwd	ATC TGA TTA CCT GGC GGA CAC TAA ACT AAG AGA GAG CTC TCA TGG GAA TTA GCC ATG GTC
LonpKD3 rev	TGC CAG CCC TGT TTT TAT TAG CGC TAT TTG CGC GAG GTC AGT GTA GGC TGG AGC TGC TTC
ClpPXKD3 fwd	AGT ACA GCA GAT TTT TTC AAT TTT TAT CCA GGA GAC GGA ACA TGG GAA TTA GCC ATG GTC
ClpPXKD3 rev	ATC CCC CCT TTT TTG GCT AAC TGA TTG TAT GAA TGT TTA AGT GTA GGC TGG AGC TGC TTC
Lon fwd	TGA CGT ACA TGT TAT AGG TGG TAT GG
Lon rev	TTA GCT ATA CAA AAA AAG GCT GGC
ClpPX fwd	GCG TTA AAA GCA CGA AAT TTG C
ClpPX rev	AAT CAG ATA GTA TAG CTG TGC TCC
EcoRIhilC	ATA GAA TTC ACC CCC ATT GCT GGC GAT TTG
NotIhilC	ATA TGC GGC CGC GTG TGC TAT AAG GAA CTC AAA ATC G
EcoRIrtsA	ATA GAA TTC CAT GCT AAC TAC CTC CAT GAA ATT
NotIrtsA	ATA TGC GGC CGC TTT ATT AAA TGT GCG TGT AA
EcoRIhilD	ATA GAA TTC ATA TAC TGT TAG CGA TGT
NotIhilD	ATA TGC GGC CGC TTG TTG ATG TTA TTT TAA TGT TCC
pSP417 Fwd	AGT AGG ACA AAT CCG CCG GG
pSP417 Rev	GTT GTA AAA CGA CGG CCA GT
furBamHIIPTG	ATA TAT GGA TCC ATG ACT GAC AAC AAT ACC GC

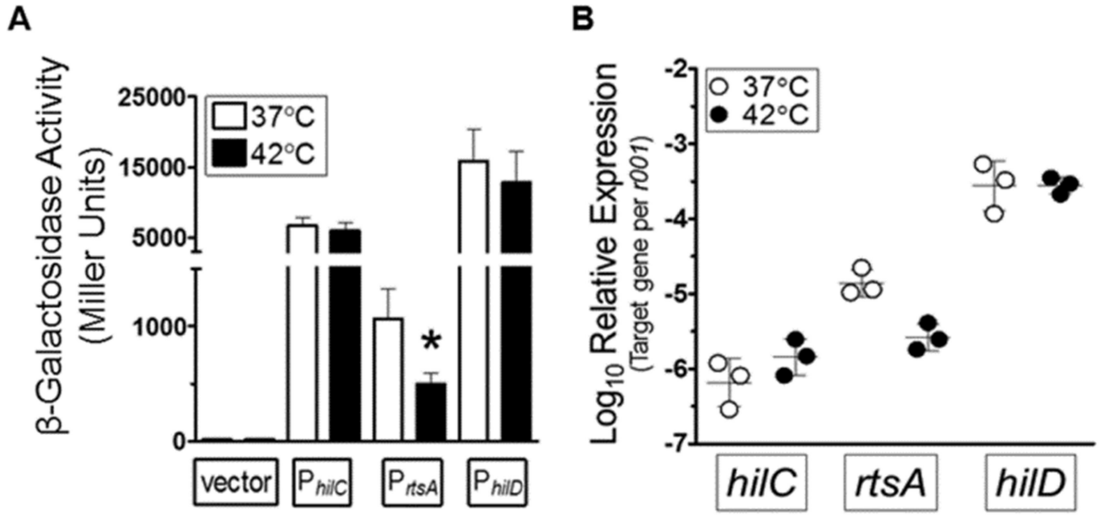
furFLAGHindIII	ATA TAA AGC TTT TAC TTG TCG TCA TCG TCT TTG TAG TCT TTA GTC GCG TCA TCG TG
rtsABamHIIPTG	ATA TAT GGA TCC ATG CTA AAA GTA TTT AAT CC
rtsAFLAGEcoRV	ATA TAT GAT ATC TTA CTT GTC GTC ATC GTC TTT GTA GTC ATT AAC ATA TTG ATG ACG AGA GG
hilCBamHIIPTG	ATA TAT GGA TCC ATG GTA TTG CCT TCA ATG AAT AAA TC
hilCFLAGHindIII	ATA TAA AGC TTT TAC TTG TCG TCA TCG TCT TTG TAG TCA TGG TTC ATT GTA CGC
pUHE fwd	TTG ACT TGT GAG CGG ATA ACA ATG
pUHE rev	GAT GGA GTT CTG AGG TCA TTA CTG G
qRThilC fwd	GAA ACT ATA ATG ATC GAG AGC
qRThilC rev	CTC ACA TTA CTA CAA ACC CG
qRTrtsA fwd	TAA AAT TTC AAT TAC GAC ATC GTC
qRTrtsA rev	AAG TCT GCA TGT TCA TAC AG
qRThilD fwd	TCA CGA TAC GAT TTA CTG TG
qRThilD rev	ATT TTC TGC GCT TTC TCT G
qRT16S rRNA fwd	AAA GTA CAA TGG CGC ATA C
qRT16S rRNA rev	GCT ACC TAC TTC TTT TGC

[a] Underlined sequences correspond to restriction enzyme sites.

SUPPLMENTAL FIGURE LEGENDS

Figure S1 – The SPI-1 activator *rtsA* is differentially regulated by growth at 42°C within *S.* Enteritidis. (A) The promoters of *hilC*, *rtsA*, and *hilD* were cloned into the multi copy shuttle vector pSP417 (empty vector) upstream of a promoterless lacZ gene. Bacteria were grown as in **Fig. 1B** and diluted 1:1,000 into LB-MOPS medium with 1 mM glucose at pH 7.4 at 37°C or 42°C. β-galactosidase activity was measured after overnight growth. Data are from 4 separate experiments and a statistical significant compared to activity at 37°C was determined. The strains used were BTNC0026 BTNC0029. **(B)** Bacteria were grown in LB-MOPS medium with 1 mM glucose at pH 7.4 at 37°C or 42°C. Total RNA was extracted at OD_{600} ~ 1. cDNA was generated using gene specific primers and expressions were normalized to the 16S rRNA gene *r001*.

Fig S1



SUPPLEMENTAL REFERENCES

1. **Bajaj V, Lucas RL, Hwang C, Lee CA.** 1996. Co-ordinate regulation of *Salmonella typhimurium* invasion genes by environmental and regulatory factors is mediated by control of *hilA* expression. Mol Microbiol **22:**703-714.
2. **McCollister BD, Bourret TJ, Gill R, Jones-Carson J, Vazquez-Torres A.** 2005. Repression of SPI2 transcription by nitric oxide-producing, IFNgamma-activated macrophages promotes maturation of *Salmonella* phagosomes. J Exp Med **202:**625-635.
3. **Altier C, Suyemoto M, Lawhon SD.** 2000. Regulation of *Salmonella enterica* serovar *typhimurium* invasion genes by *csrA*. Infect Immun **68:**6790-6797.
4. **Hung CC, Garner CD, Slauch JM, Dwyer ZW, Lawhon SD, Frye JG, McClelland M, Ahmer BM, Altier C.** 2013. The intestinal fatty acid propionate inhibits *Salmonella* invasion through the post-translational control of HilD. Mol Microbiol **87:**1045-1060.
5. **Podkovyrov SM, Larson TJ.** 1995. A new vector-host system for construction of *lacZ* transcriptional fusions where only low-level gene expression is desirable. Gene **156:**151-152.
6. **Soncini FC, Vescovi EG, Groisman EA.** 1995. Transcriptional autoregulation of the *Salmonella typhimurium phoPQ* operon. J Bacteriol **177:**4364-4371.
7. **Datsenko KA, Wanner BL.** 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.