SUPPLEMENTARY DATA

Figure S1. DksA is required for *S*. Typhimurium growth in minimal medium, but not in rich LB broth. (**A**) The growth of the clinical isolates 85982 (containing N88D amino acid substitution in DksA) and 87541 (harboring a wild-type DksA) and isolate 85982 harboring pWSK29 or pWSK29::$dksA_{87541}$ was compared in LB minimal medium at 37°C under aerobic growth conditions. (**B**). *S*. Typhimurium SL1344 (WT), its derivative *dksA* null mutant strain, and the *dksA* mutant harboring the plasmid pWSK29 or the *dksA* gene (from SL1344) cloned into pWSK29 were grown in LB for 16 h, washed, diluted 1:100 into M9 and grown at 37°C with aeration. Optical density (OD_{600}) was recorded at the indicated time points. Each time point shows the mean OD_{600} of three independent cultures and the standard error of the mean (SEM) is indicated by the error bars.

Figure S2. DksA is required for *S*. Typhimurium host cells invasion, but not adhesion. *S*. Typhimurium SL1344 (WT) and its derived *invA* and *dksA* null mutant strains were grown in LB at 37°C and used to infect Caco-2 epithelial cell-line (**A**) and Raw 264.7 macrophage-like cells (**B**). Adhesion was determined in the presence of cytochalasin D and is shown as the percentage of cell-associated bacteria from the total number of CFU used to infect the cells. Invasion into HeLa cells (**C**) was determined following mild centrifugation (500 RPM for 5 min) using the gentamycin protection assay and is calculated as the percentage of intracellular bacteria (CFU) recovered at 2 h p.i from the total number of CFU used to infect the cells. Graph bars represent the mean and SEM of four independent infections. ANOVA with Dunnett's Multiple Comparison Test was used to determine differences between data sets. ***, $p<0.0001$; ns, not significant.

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

Strain or plasmid	Genotype and description	Reference or source

S. Typhimurium SL1344	wild type Sm^r *xyl hisG rpsL*	SGSC
S. Typhimurium *invA*	SL1344 Δ*invA*	(1)
S. Typhimurium *dksA*	SL1344 Δ*dksA*	This study
S. Typhimurium *fliC fljB*	SL1344 *ΔfliC ΔfljB*	(2)
S. Typhimurium 85982	clinical isolate *dksA* N88D	This study
S. Typhimurium 87541	clinical isolate	This study
Plasmids		
pKD3		(3)
pKD46		(3)
pCP20		(3)
pWSK29	Amp^r low copy number cloning vector	(4)
pWSK129	Km^r low copy number cloning vector	(4)
pCS26	Kan^r cloning vector for *luxCDABE* fusion	(5)
pWSK29::*dksA*	*S.* Typhimurium SL1344 *dksA* cloned into pWSK29	This study
pWSK29::$dksA_{87541}$	*S.* Typhimurium 87541 *dksA* cloned into pWSK29	This study
p**CS26::**P*dksA*	*S.* Typhimurium SL1344 *dksA* regulatory region cloned into pCS26	This study
pCS26::P*rpoD*	*S.* Typhimurium SL1344 *rpoD* regulatory region cloned into pCS26	(5)
pDE-fliC::2HA $_{STM}$	*S.* Typhimurium SL1344 *fliC* fused to 2HA tag cloned into pWSK29	(6)
pDE-sopB::2HA $_{STM}$	*S.* Typhimurium SL1344 *sipB* fused to 2HA tag cloned into pWSK29	(6)
pDE-sopE2::2HA $_{STM}$	*S.* Typhimurium SL1344 *sopE2*	(6)

	fused to 2HA tag cloned into pACYC184

SGSC – *Salmonella* genetic Stock Center the University of Calgary.

Table S2. Primers used in the study.

Primer	**Sequence 5'-3'***
P1 dksA KO	CTGCACTCCGCCATTACAC
P2 dksA KO cm5'	GAAGCAGCTCCAGCCTACACATTGCCCTTCTTGCATGTTG CTTCTCC
P3 dksA KO	CGCTGTGATTGCCATAAGAC
P4 dksA KO cm3'	CTAAGGAGGATATTCATATGCAGATGGCGGGTTAATCCCG TTCGTTTCTACC
Clone dskA Fw	TTTTGAGCTCAACGAACCAGTACCCATAACG
Clone dskA Rv	TTTTTCTAGAGGTAGAAACGAACGGGATTAACC
dksA promoter Fw	AAAACTCGAGATACGCGCAGCTATTGAGCGAG
dksA promoter Rev	AAAAGGATCCCATGTTGCTTCTCCTTAACACGC
rpoD RT-PCR F	GGTCTGACCATCGAACAGGTG
rpoD RT-PCR R	ATCAGACCGATGTTGCCTTC
invF RT-PCR F	TGTCGCACCAGTATCAGGAG
invF RT-PCR R	ACTCGCAGCGTTTACGATC
ssaR RT-PCR F	ATGTCTTCAGGCCAGGTTCG
ssaR RT-PCR R	TTCTGGACGTCTGAGTGGG
ssrB RT-PCR F	TGGCCTGGATATCATTCCTC
ssrB RT-PCR R	TTGCAATGCCGCTAACAGAAC
sifA RT-PCR F	CAACTCCCCAAGGAATACG
sifA RT-PCR R	ATCTCTGTAAGCCGCTCTCG
invA RT-PCR F	TCCACGAATATGCTCCACAAG
invA RT-PCR R	CAGACATGCCACGGTACAAC
sopB RT-PCR F	GAAAATCGGCGCAAAAGATATC
sopB RT-PCR R	TCATGATAGGGGGAAAGCAC
sipB RT-PCR F	GTGGGCAAAAATACGGAAG
sipB RT-PCR R	CCCGATACATCCCATAATGC
sopE2 RT-PCR R	CCGACTACCCATTTTCATCG
sopE2 RT-PCR R	GCTTCGCATGTCTGACGAGC
hilA RT-PCR F	ATATGCCGTTCTGGTCATCC
hilA RT-PCR R	GCCCTGTCCGTACAGTGTTTC
hilD RT-PCR F	GAGATACCGACGCAACGAC
hilD RT-PCR R	CTGCGCTTTCTCTGTGGG
hilE RT-PCR F	CTGTACGGACAGGGCTATCG
hilE RT-PCR R	CACGCTCAGGCCAAAGG
fliA RT-PCR F	GATTGAATCGCTGCCGGAAC

fliA RT-PCR R	ACTATGCAACTGGCTGACCC
fliC RT-PCR F	AAGAGAGGACGTTTTGCGG
fliC RT-PCR R	AGAATCCGCCTTTGTTGG
flhD RT-PCR F	TGTTCCGCCTCGGTATCAAC
flhD RT-PCR R	CGCGAATCCTGAGTCAAACG
csgD Fw RT	CTGCATAATATTCAACGTTCTCTGG
csgD Rev RT	GCCAGTTTTCAATTTCACGGTAG
csgA RT Fw	CGGCGGCAATAGTTCC
csgA RT Rev	CCGTTACCATAACCGCTCTG
bcsA RT Fw	GCTGGTGATGATCTCGTCG
bcsA RT Rev	GTTGCTAAGATGTCCCAGCTC
bapA RT Fw	CCATGAAGCAGGCGGC
bapA RT Rev	GGGATTACCGTCAGGATTGGTG

* The sequence of restriction sites added to the primers is underlined.

References

1. **Galan JE, Curtiss R, 3rd.** 1991. Distribution of the invA, -B, -C, and -D genes of Salmonella typhimurium among other Salmonella serovars: invA mutants of Salmonella typhi are deficient for entry into mammalian cells. Infect Immun **59:**2901-2908.
2. **Elhadad D, Desai P, Rahav G, McClelland M, Gal-Mor O.** 2015. Flagellin Is Required for Host Cell Invasion and Normal Salmonella Pathogenicity Island 1 Expression by Salmonella enterica Serovar Paratyphi A. Infect Immun **83:**3355-3368.
3. **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.
4. **Wang RF, Kushner SR.** 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. Gene **100:**195-199.
5. **Bjarnason J, Southward CM, Surette MG.** 2003. Genomic profiling of iron-responsive genes in Salmonella enterica serovar typhimurium by high-throughput screening of a random promoter library. J Bacteriol **185:**4973-4982.
6. **Elhadad D, McClelland M, Rahav G, Gal-Mor O.** 2015. Feverlike Temperature is a Virulence Regulatory Cue Controlling the Motility and Host Cell Entry of Typhoidal Salmonella. J Infect Dis **212:**147-156.