## **1** Supplemental Figure

- 2 Figure S1: Screening of the MGD::kan library in C2BBe1 epithelial cells. Caco-2 C2BBe1 3 cells were seeded in 48-well plates and infected with S. Typhimurium 14028s wild type (WT) or 4 MGD::kan strain (genetic regions deleted in each MGD mutant are indicated). The MOI was 5 100, except where indicated by #, in which case the MOI was 1000. \* indicates strains that were 6 non-invasive or very poorly invasive. These bacteria were added to monolayers at an MOI of 7 100, followed by centrifugation at 600 xg for 5 min. The proportion of cytosolic:vacuolar bacteria 8 at 7 h p.i. was determined by the CHQ resistance assay (CHQ-resistant CFU/(Total CFU-CHQ-9 resistant CFU)). Each data point is the average of duplicate wells from 1-5 independent 10 experiments. The shaded area indicates 14028s wild type mean ± 2SD. Arrows indicate MGD
- 11 mutants that were the focus of this study.



Table S1: Oligonucleotides used in this study

Oligonucleotide name	Sequence (5'-3')	For construction of
asmA F2	GGCTCTGCCCTTTGGCCAAAAA <b>CTACAT</b> GCTACCCTCAATGGACCGGCAG	∆asmA
asmA R1	CTGCCGGTCCATTGAGGGTAGC <b>ATGTAG</b> TTTTTGGCCAAAGGGCAGAGCC	∆asmA
asmA-XbaF1	GC <u>TCTAGA</u> ACTGACCGACGCGCGTTTG	∆asmA
asmA-XmaR2	TCCC <u>CCCGGGG</u> CCGTTTTCTTTCCGCTAAC	∆asmA
corA OL-F	CCGGTC <b>ATG</b> CTGAGCTGGCTG <b>TAA</b> GACATTAAGGCCAGGGTG	ΔcorA
corA OL-R	AATGTC <b>TTA</b> CAGCCAGCTCAG <b>CAT</b> GACCGGGACTCCCAATG	ΔcorA
Xma corA	CCC <u>CCCGGG</u> CCATGCAGAAACTGACGCTAAC	ΔcorA
Kpn corA new	GG <u>GGTACC</u> ATATTGTCATGCTAACGACG	ΔcorA
Hha OL-F	GAATTT <b>ATG</b> TCTGATATTCGT <b>TAA</b> TCACGCTACATTACTTTTAG	Δhha
Hha OL-R	GCGTG <b>ATT</b> AACGAATATCAGA <b>CAT</b> AAATTCTACCTATGATTG	Δhha
Xmal hha	CCC <u>CCCGGG</u> CTGTTATCGCGTTTTTCACGG	Δhha
Kpnl hha	GG <u>GGTACC</u> ATCCTTACTGCGTTAAAGGC	Δhha
recA F2	CCGCCCCACCATCACCTGATGA <b>TTACAT</b> TATTACTCCTGTCATGCAACTT	∆recA
recA R1	AAGTTGCATGACAGGAGTAATAATGTAATCATCAGGTGATGGTGGGGCGG	∆recA
recA-XmaF1	TCCCCCGGGATTGGTAAACAACAGAGTG	∆recA
recA-XbaR2	GCTCTAGACTATATCCCAGACATCCC	∆recA
recA-BamHIF	CCACCG <u>GGATCC</u> ACAGCCGTAGTTGACAGG	pWSK29- <i>recA</i>
recA-KpnIR	CTTGGG <u>GGTACC</u> TTAACGTTTTGCTGAATGG	pWSK29- <i>recA</i>
srlR F2	ATAAGTCAGGGTAATCACGCCT <b>ATGTGA</b> TGCACTACTAAACGCGGGCCGT	ΔsrlR
srlR R1	ACGGCCCGCGTTTAGTAGTGCA <b>TCACAT</b> AGGCGTGATTACCCTGACTTAT	ΔsrlR
srlR-Kpn2R2	GG <u>GGTACC</u> GACGCCTTTCACAACATG	∆srlR
srlR-Xba2F1	GC <u>TCTAGA</u> CTGGGCATTAAGCCC	ΔsrlR
ydgT F2	TTGATGTTAAACGCTACTTTCT <b>TTACAT</b> ATTAAATATAATGCCAACGGAG	∆ydgT
ydgT R1	CTCCGTTGGCATTATATTTAAT <b>ATGTAA</b> AGAAAGTAGCGTTTAACATCAA	∆ydgT
ydgT-KpnF1	GG <u>GGTACC</u> CCCAGAACCGCACAGTTAG	∆ydgT
ydgT-XbaR2	GC <u>TCTAGA</u> CGCGCCTGTGACAGTGTCAC	ΔydgT
ydgT-KpnIF	CGG <u>GGTACC</u> TTGTGGAAGACGGAAAGTACC	pWSK29- <i>ydgT</i> (1)
ydgT-SacIR	CC <u>GAGCTC</u> AACGCTACTTTCTTTATTGCACA	pWSK29- <i>ydgT</i> (1)

Start and stop codons are in bold. Engineered restriction sites are underlined.

1. Coombes BK, Wickham ME, Lowden MJ, Brown NF, Finlay BB. 2005. Negative regulation of *Salmonella* pathogenicity island 2 is required for contextual control of virulence during typhoid. Proc Natl Acad Sci USA **102**:17460-17465.