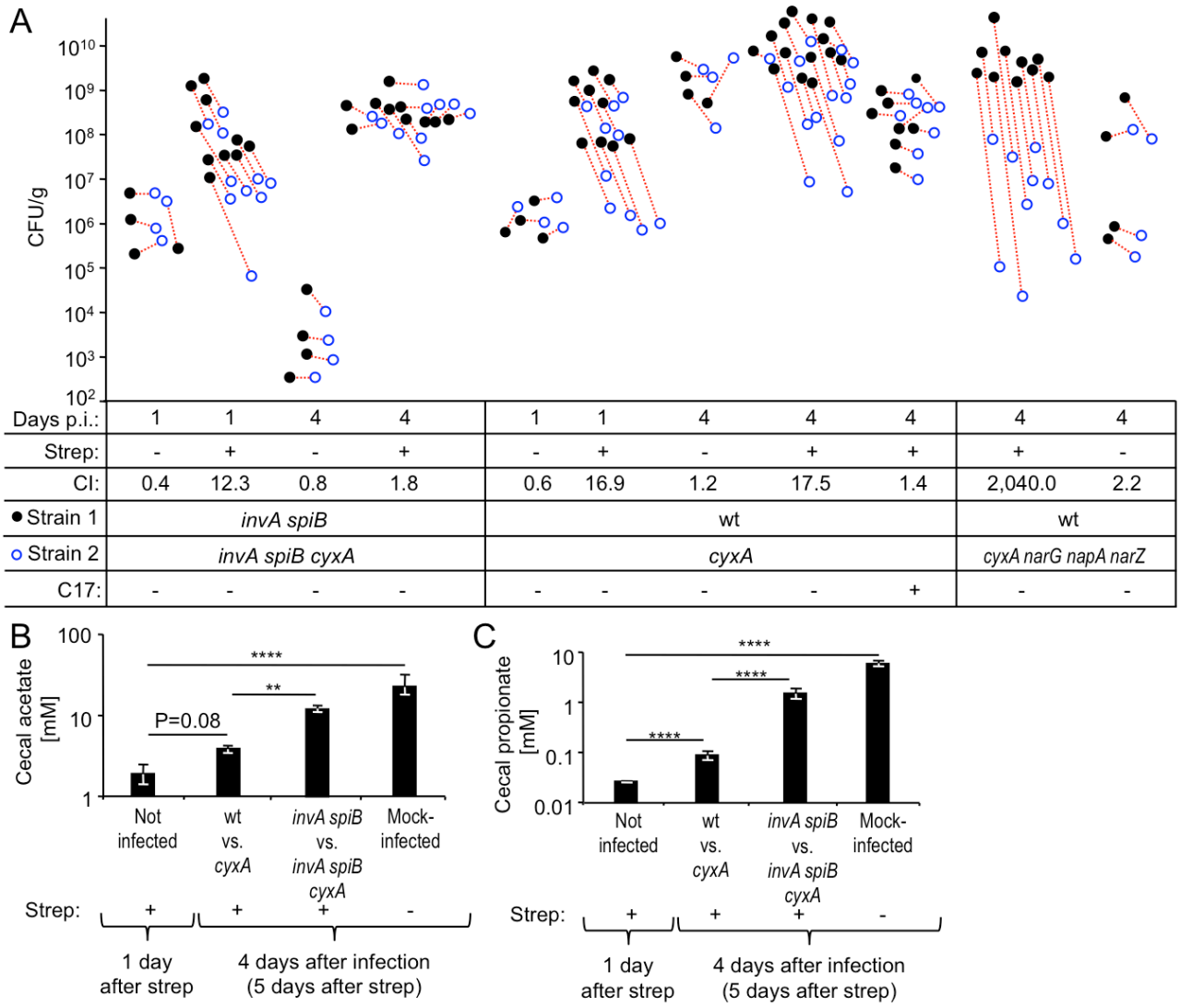

Days p.i.:	1	1	4	4	1	1	4	4	4	4	4
Strep:	-	+	-	+	-	+	-	+	+	+	-
CI:	0.4	12.3	0.8	1.8	0.6	16.9	1.2	17.5	1.4	2,040.0	2.2
● Strain 1	*invA spiB*				wt					wt	
○ Strain 2	*invA spiB cyxA*				*cyxA*					*cyxA narG napA narZ*	
C17:	-	-	-	-	-	-	-	-	+	-	-

Figure S1: Streptomycin treatment depletes short-chain fatty acids and drives an aerobic luminal expansion of *Salmonella* (related to Figures 1 - 3).

(A) Colony-forming units (CFU) recovered from individual mice in experiments shown in figure panels 1D, 1E, and 3A. Groups of C57BL/6 mice were mock-treated or treated with streptomycin (Strep) and one day later infected intragastrically with a 1:1 mixture of the indicated *S.* Typhimurium strains. One day after infection, some mice were inoculated intragastrically with a culture of 17 human *Clostridia* isolates (C17). Samples were collected at the indicated days after infection (days p.i.). Dotted red lines connect strains recovered from the same animal. (B and C) Groups of C57BL/6 mice were treated with streptomycin (Strep) or mock-treated (-) and one day later were mock-infected or infected intragastrically with the indicated *S.* Typhimurium strain mixtures (*N* is indicated in Fig. 1H). The concentration of acetate (B) and propionate (C) was measured in cecal contents at the indicated time points after streptomycin treatment. Bars represent geometric means ± standard error. **, $P < 0.01$; ****, $P < 0.001$; wt, *S.* Typhimurium wild type.

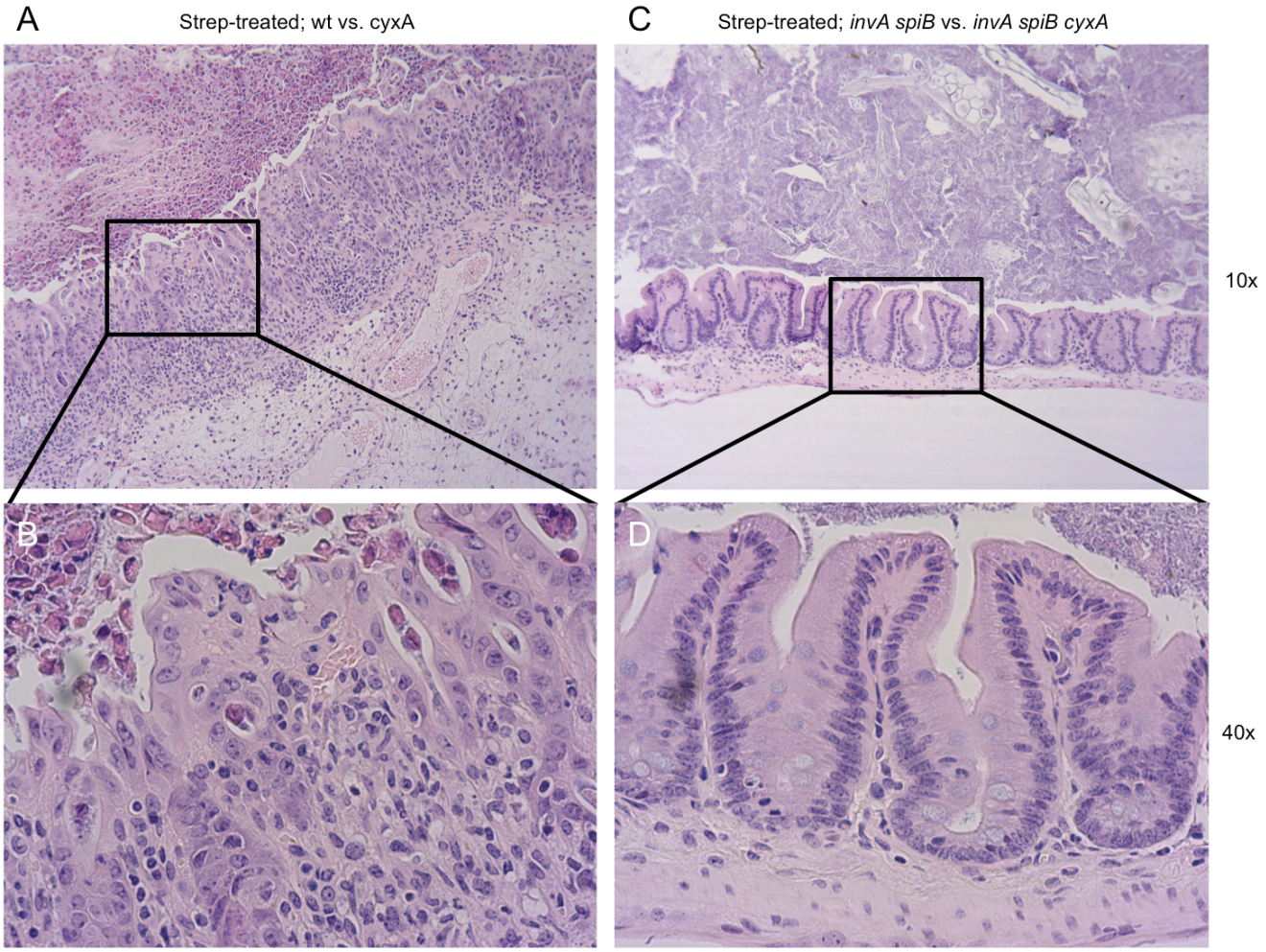

Figure S2: Virulence factors drive pathological changes during *S.* Typhimurium infection (related to Figure 3B).

Groups of C57BL/6 mice (*N* is shown in Fig. S1A) were treated with streptomycin (Strep), infected one day later with the indicated strain mixtures and tissue collected four days after infection. (A-D) Representative images of H/E-stained cecal sections scored in Fig. 3B were taken with a 10x (A and C) or 40x (B and D) objective.

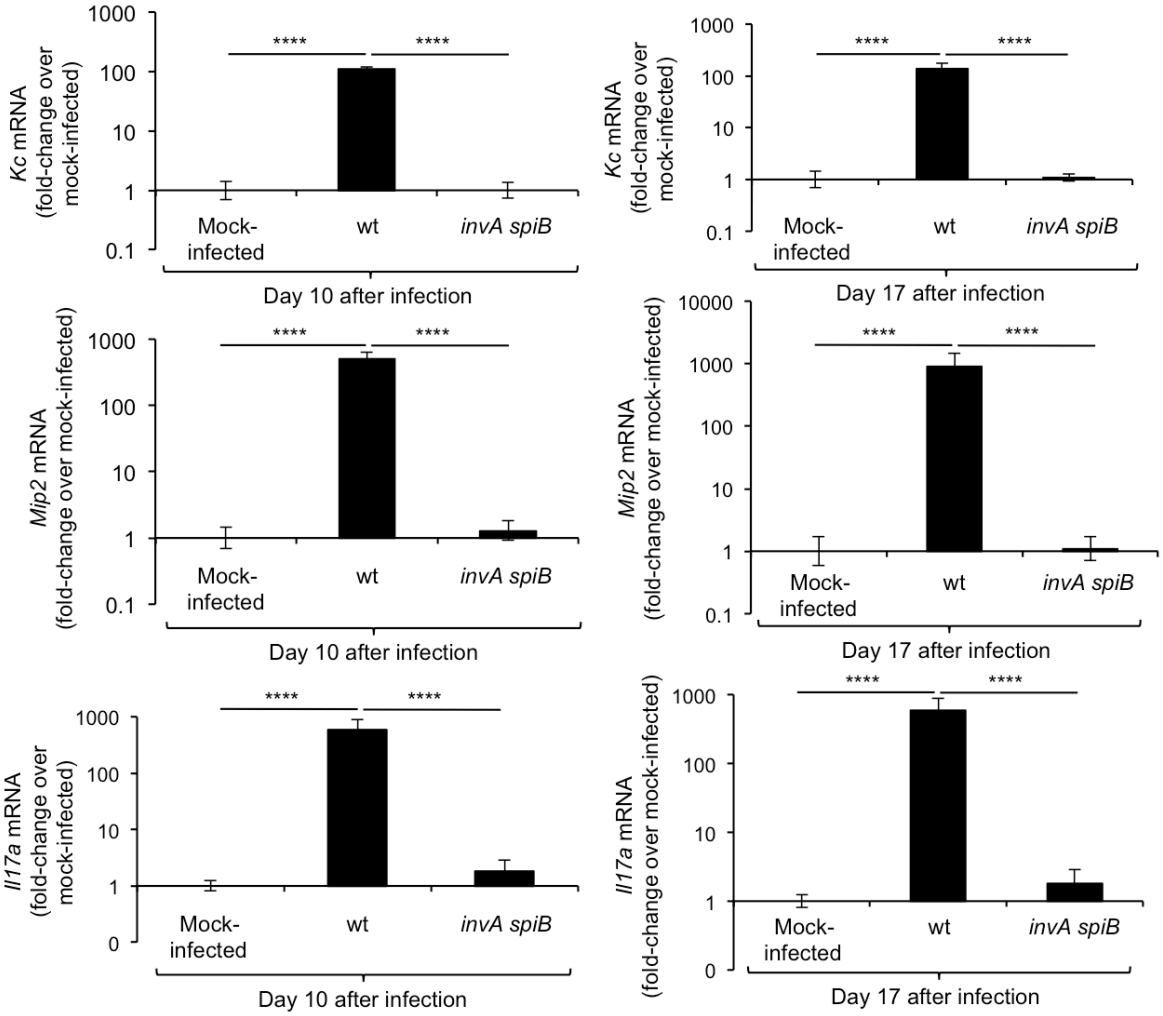

Figure S3: *S.* Typhimurium virulence factors induce elevated transcript levels of pro-inflammatory genes in the cecal mucosa (related to Figure 4A).

Groups of CBA mice (N = 6) were mock-infected or infected intragastrically with 1 x 10^9 CFU/animal of the virulent *S.* Typhimurium wild type (wt) or the avirulent *invA spiB* mutant (*invA spiB*) and the cecum was collected at the indicated time points to isolate RNA. Transcript levels of the indicated genes were determined by quantitative real-time PCR. Data represent geometric means ± standard error of fold-changes over transcript levels detected in mock-infected animals. ****, P < 0.001.

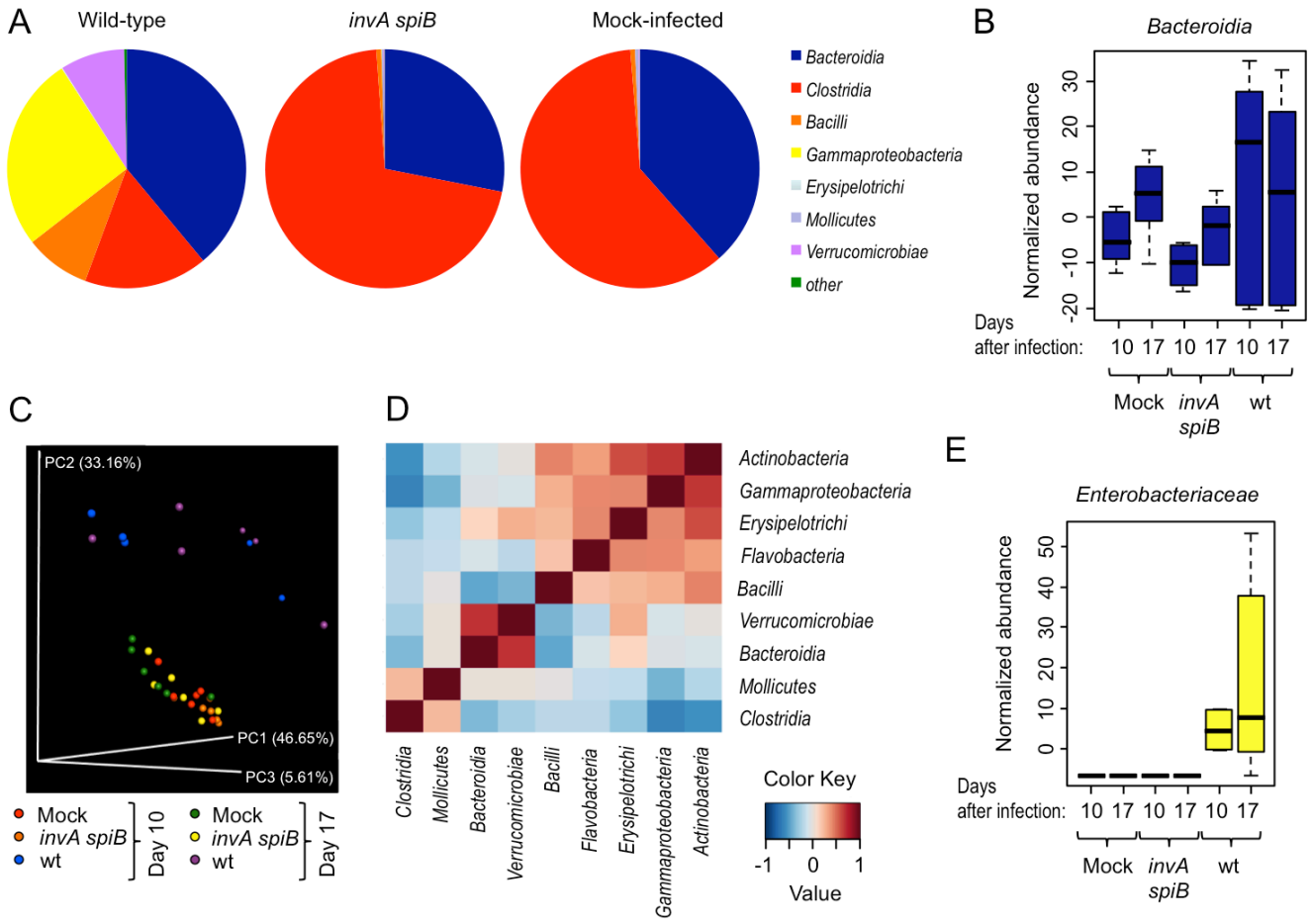

Figure S4: *S.* Typhimurium virulence factors are required for *Clostridia* depletion in the absence of antibiotic treatment (related to Figure 4).

Groups of CBA mice (*N* = 6) were mock-infected or infected intragastrically with 1 x 10^9 CFU/animal of the virulent *S.* Typhimurium wild type (wt) or the avirulent *invA spiB* mutant (*invA spiB*) and samples were collected at the indicated time points for 16S profiling of the microbial community present in colon contents. (A) The pie graphs show the average relative abundances of phylogenetic groupings at the class level on day 17 after infection. (B and E) Normalized abundances of members of the class *Bacteroidia* (B) and members of the family *Enterobacteriaceae* (E) in colon contents at the indicated days after infection. Boxes in whisker plots represent the second and third quartiles, while lines indicate the first and fourth quartiles. (C) Weighted principal coordinate analysis of 16S profiling data. Each dot represents data from one animal. (D) The heat map indicates positive (red) and negative (blue) correlations between the average abundances of phylogenetic groupings at the class level detected at 10 and 17 days after infection.

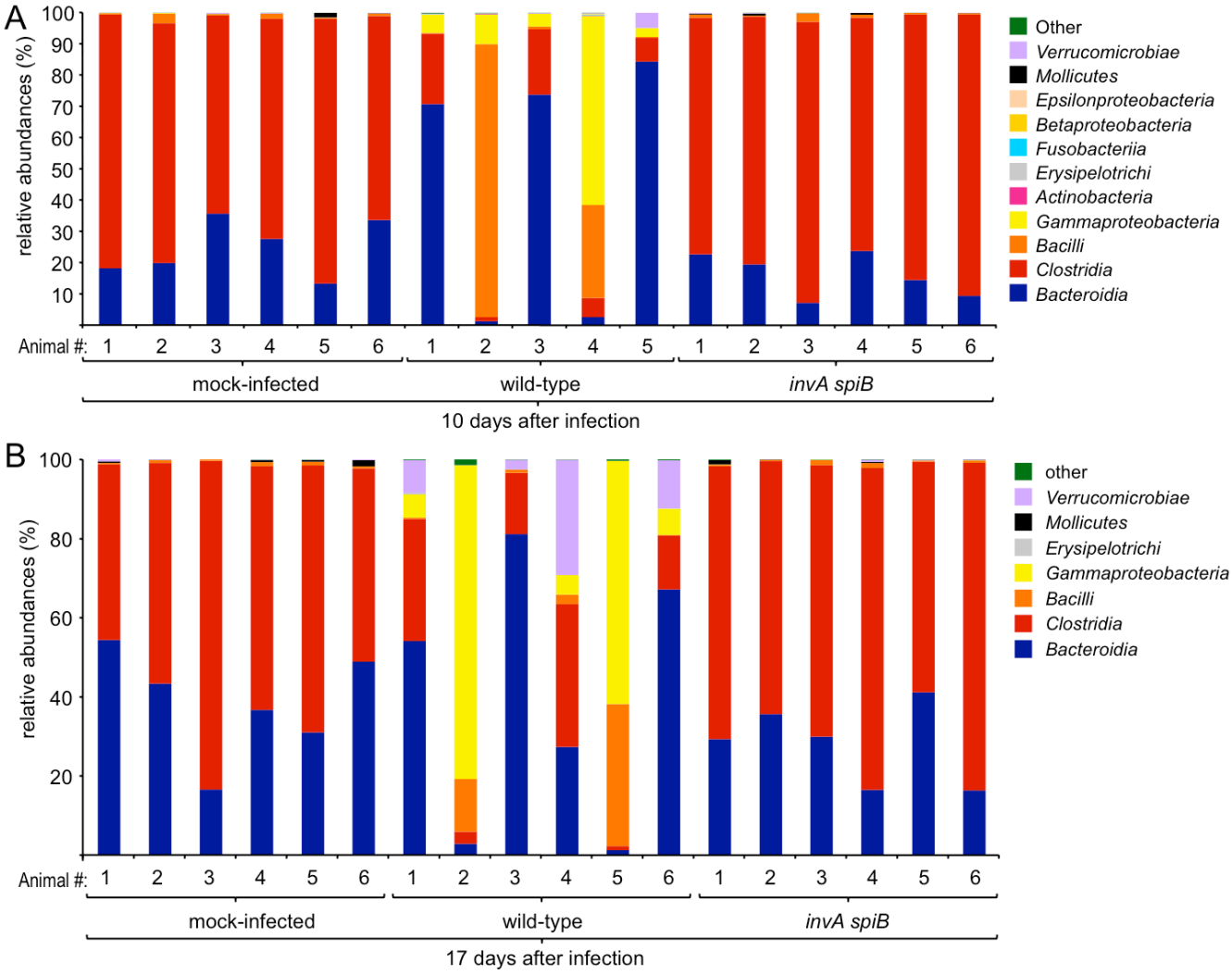

Figure S5: Relative abundances of phylogenetic groupings at the class level during *S.* Typhimurium infection (related to Figure 4).

Groups of CBA mice (*N* is indicated in each graph) were mock-infected or infected intragastrically with 1 x 10^9 CFU/animal of the virulent *S.* Typhimurium wild type (wt) or the avirulent *invA spiB* mutant (*invA spiB*) and samples were collected at the indicated time points for 16S profiling of the microbial community present in colon contents. The bar graphs show the average relative abundances of phylogenetic groupings at the class level on days 10 (A) and 17 (B) after infection for individual animals.

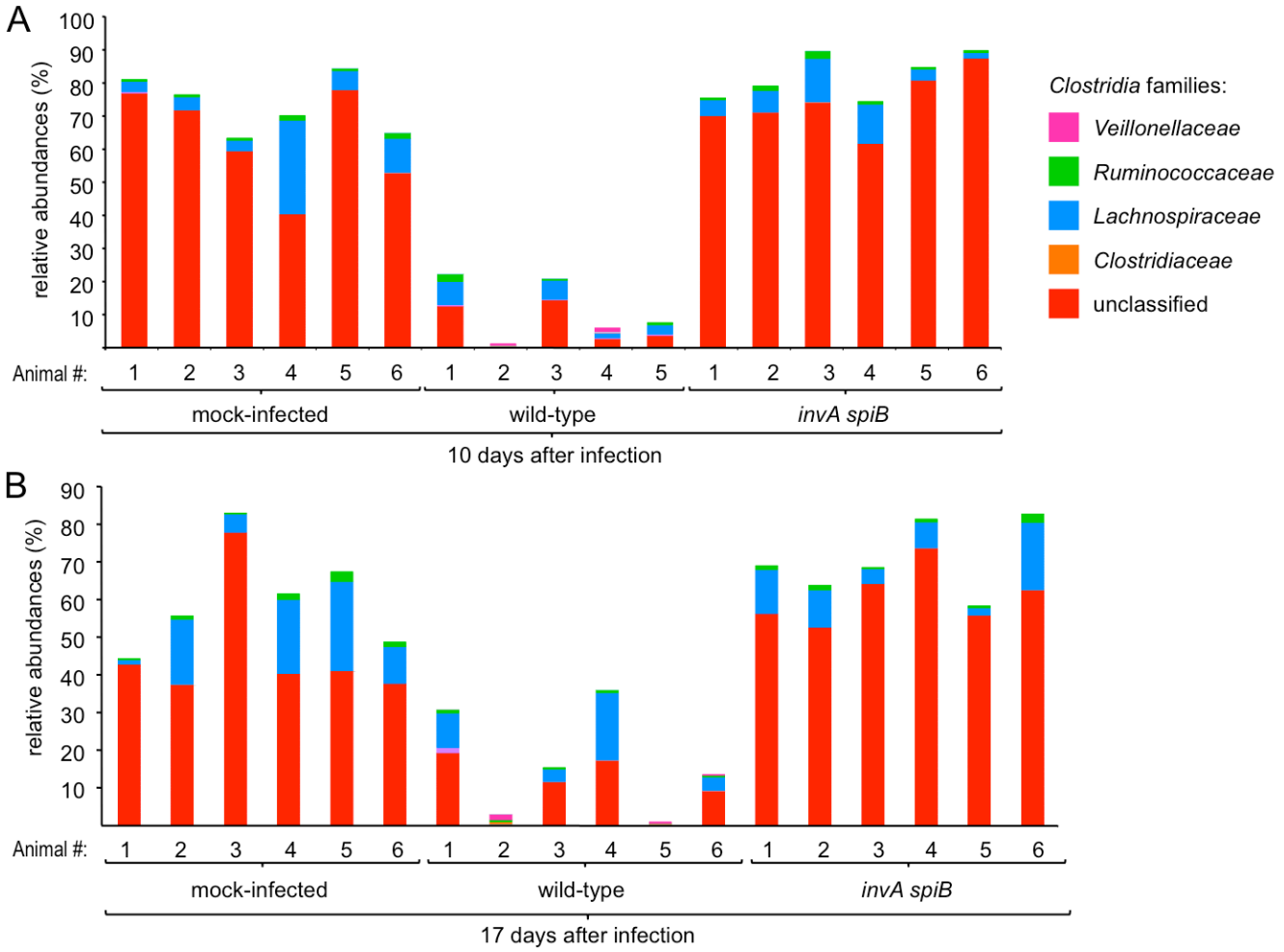

Fig. S6: Relative abundances of *Clostridia* families during *S.* Typhimurium infection (related to Figure 4).

Groups of CBA mice (*N* is indicated in each graph) were mock-infected or infected intragastrically with 1 x 10^9 CFU/animal of the virulent *S.* Typhimurium wild type (wild-type) or the avirulent *invA spiB* mutant (*invA spiB*) and samples were collected at the indicated time points for 16S profiling of the microbial community present in colon contents. The bar graphs show the average relative abundances of *Clostridia* families on days 10 (A) and 17 (B) after infection for individual animals.

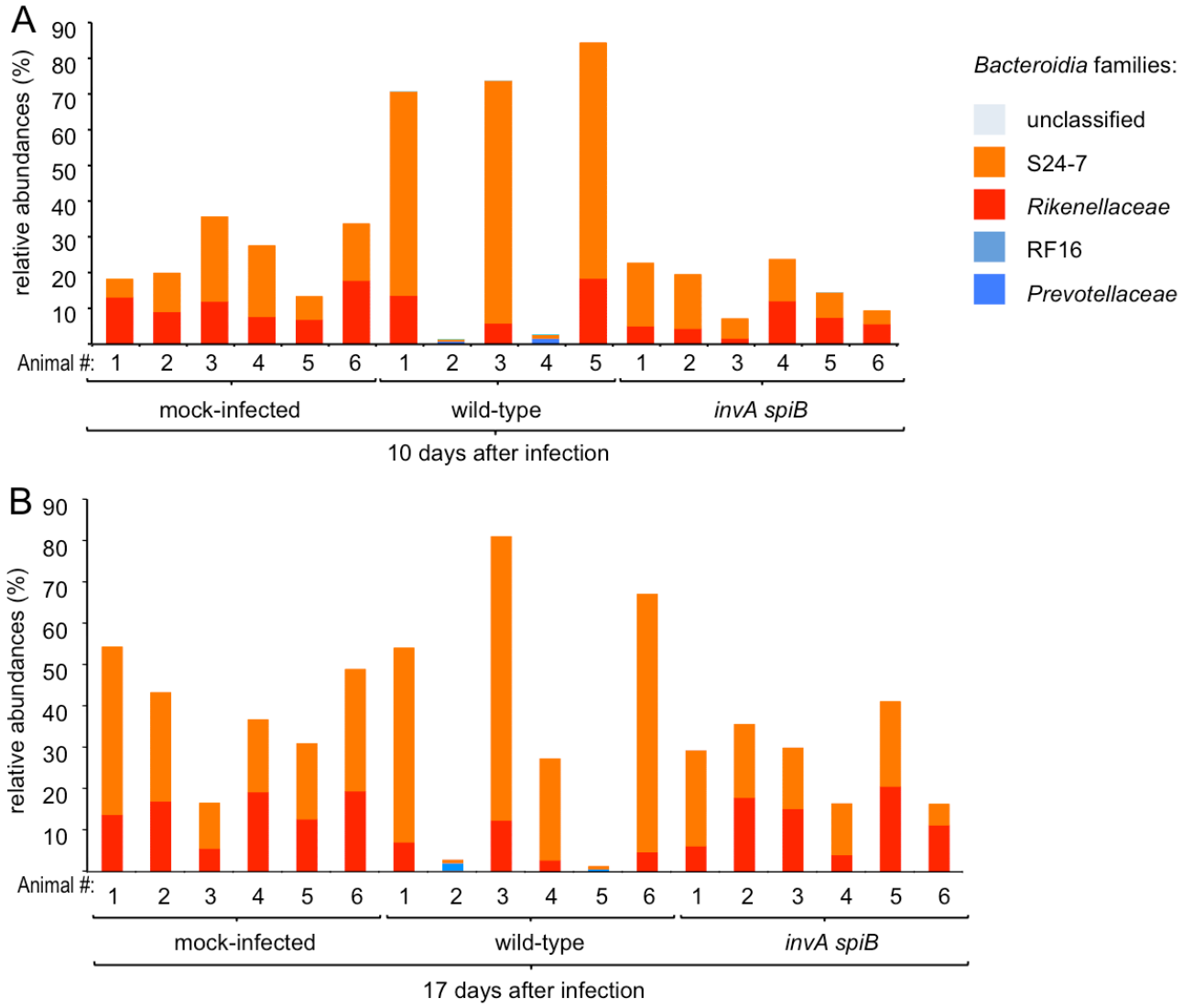

Fig. S7: Relative abundances of *Bacteroidia* families during *S.* Typhimurium infection (related to Figure 4).

Groups of CBA mice (*N* is indicated in each graph) were mock-infected or infected intragastrically with 1 x 10^9 CFU/animal of the virulent *S.* Typhimurium wild type (wild-type) or the avirulent *invA spiB* mutant (*invA spiB*) and samples were collected at the indicated time points for 16S profiling of the microbial community present in colon contents. The bar graphs show the average relative abundances of *Bacteroidia* families on days 10 (A) and 17 (B) after infection for individual animals.

SUPPLEMANTARY TABLES

Table S1: *S.* Typhimurium and *E. coli* strains used in this study (related to *Bacterial Strains and Plasmids* section in *Experimental Procedures*)

S. Typhimurium		
Strain	Genotype	Reference
IR715	Nalidixic acid-resistant derivative of ATCC 14028	(Stojiljkovic et al., 1995)
FR102	IR715 *cyxA*::pPT48 ($Carb^r$)	This Study
FR103	IR715 *cydA*::pFR9 (Cm^r)	This Study
FR104	IR715 *cyxA*::pPT48 Δ*napA* Δ*narZ narG*:: pCAL18 ($Carb^r$,Cm^r)	This Study
FR105	IR715 Δ*invA*::*tetRA* Δ*spiB*::KSAC *cyxA*:: pPT48 (Tet^r; Kan^r, $Carb^r$)*	This Study
CAL82	IR715 Δ*napA* Δ*narZ narG*::pCAL18 (Cm^r)	This Study
CAL49	IR715 Δ*napA* Δ*narZ*	This Study
CAL45	IR715 *narZ*::pCAL10 (Cm^r)	(Lopez et al., 2012)
CAL46	IR715 Δ*napA narZ*::pCAL10	(Lopez et al., 2012)
SPN452	IR715 Δ*invA*::*tetRA* Δ*spiB*::KSAC (Tet^r; Kan^r)	(Raffatellu et al., 2009)
E. coli		
Strain	Genotype	Reference
DH5α λ*pir*	F^- *endA1 hsdR17* ($r^- m^+$) *supE44 thi-1 recA1 gyrA96 relA1* Δ(*lacZYA-argF*)U169 *deoR nupG* ϕ80*lacZ*ΔM15 λ*pir*	(Pal et al., 2005)
S17-1 λ*pir*	*zxx*::RP4-2-Tet^r::Mu-Kan^r::Tn*7 recA1 thi*	(Simon et al., 1983)

	pro hsdR ($r^- m^+$) λ*pir*	
TOP10	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 *lacX74 recA1 araD139* Δ(*ara - leu*)*7697 galE15 galK rpsL endA1 nupG*	Invitrogen

*Cm^r: chloramphenicol resistance; $Carb^r$: carbenicillin resistance; Kan^r: kanamycin resistance

Table S2: Plasmids used in this study (related to *Bacterial Strains and Plasmids* section in *Experimental Procedures*)

Designation	Relevant Characteristics	Reference/source
pCR2.1	Cloning vector	Invitrogen
pGP704	*ori*(R6K) *mobRP4* ($Carb^r$)	(Miller and Mekalanos, 1988)
pEP185.2	*ori*(R6K) *mobRP4* (Cm^r)	(Kinder et al., 1993)
pRDH10	sacRB *ori*(R6K) *mobRP4* (Tet^r Cm^r)	(Kingsley et al., 1999)
pPT48	*'cyxA'* gene fragment cloned into pGP704; insertion of pPT48 into the IR715 chromosome *cyxA* gene.	This Study
pFR9	*'cydA'* gene fragment cloned into pEP185.2; insertion of pFR9 into the IR715 chromosome *cydA* gene.	This Study
pCAL18	*'narG'* gene fragment from pCAL5 cloned into pEP185.2; insertion of pCAL18 into the IR715 chromosome disrupts *narG*.	This Study
pCAL5	*'narG'* gene fragment cloned into pGP704	(Lopez et al., 2012)
pCAL10	Upstream and downstream regions of *'narZ'* cloned into pRDH10	(Lopez et al., 2012)

Table S3: Primers used in this study (related to *Bacterial Strains and Plasmids* section in *Experimental Procedures*)

Target gene / organism	Sequence	Reference
cyxA / *S.* Typhimurium	5'-GGTACCCTTACCAGTTCGGAAC-3' 5'-GATATCGCCTACCAGCGTAAG-3'	This study
cydA / *S.* Typhimurium	5'- CTGCAGCCCGGGGGATCCACT AGTTGCGATGTTCATCCTCGGT-3' 5'- GAGCTCCACCGCGGTGGCGGC CGCTGATCTGAATCGCCAGATG-3'	This study
Gapdh / *Mus musculus*	5'-TGTAGACCATGTAGTTGAGGTCA-3' 5'-AGGTCGGTGTGAACGGATTTG-3'	(Godinez et al., 2008)
Kc / *Mus musculus*	5'-TGCACCCAAACCGAAGTCAT-3' 5'-TTGTCAGAAGCCAGCGTTCAC-3'	(Godinez et al., 2008)
Mip2 / *Mus musculus*	5'-AGTGAACTGCGCTGTCAATGC-3' 5'-AGGCAAACTTTTTGACCGCC-3'	(Godinez et al., 2008)
16S rRNA gene from members of the class *Clostridia*	5'-ACTCCTACGGGAGGCAGC-3' 5'-GCTTCTTTAGTCAGGTACCGTCAT-3'	(Croswell et al., 2009)

Table S4: Criteria for blinded scoring of histopathological changes (related to Figure 3B)

Score	Submucosal edema	Epithelial damage	Exudate	Infiltration by PMNs[a,b]	Mononuclear cell infiltration[a]
0	No changes	No changes	No changes	No changes (0-5)	No changes (0)
1	Detectable (<10%)	Desquamation	Slight accumulation	6-20	5-10
2	Mild (10%-20%)	Mild erosion	Mild accumulation	21-60	10-20
3	Moderate (20%-40%)	Marked erosion	Moderate accumulation	60-100	20-40
4	Marked (>40%)	Ulceration	Marked accumulation	>100	>40

[a] Number of cells per high power field (400x)
[b] PMN, polymorphonuclear cells

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Bacterial culture conditions

Unless indicated otherwise, *S.* Typhimurium and *E. coli* strains were routinely grown aerobically at 37°C in LB broth (BD Biosciences) or on LB plates. Antibiotics were added to the media at the following concentrations: 0.03 mg/ml chloramphenicol, 0.1 mg/ml carbenicillin, 0.05 mg/ml kanamycin, and 0.05 mg/ml nalidixic acid. For growth under different microaerobic or anaerobic conditions, minimal medium containing glycerol as the sole carbon source was inoculated with the indicated strain mixtures and incubated at 37°C either in a hypoxia chamber (set at 8% or 0.8% oxygen) or in an anaerobe chamber (0% oxygen).

Spore preparations

Spores were isolated from cecal contents of mice as described previously (Momose et al., 2009), with some modifications. Briefly, cecal contents from C57BL/6 mice were diluted 1:10 in PBS and chloroform was added to a final concentration of 3%. The mixture was incubated in a 37°C shaker for at least 30 minutes. The chloroform was allowed to settle to the bottom of the tube and the top layer containing spores was collected into a new tube.

Construction of plasmids

PCR products were cloned into vectors using Gibson Assembly (New England Biolabs, Ipswich, MA) or cloned into pCR2.1 using the TOPO TA cloning kit (Invitrogen, Carlsbad) and sequenced (SeqWright Fisher Scientific, Houston) prior to subcloning into the appropriate vectors.

An internal fragment of the *cyxA* gene was amplified by PCR using the primers listed in Table S3. The *cyxA* PCR product was then cloned into pGP704 using the KpnI and EcorV restriction enzyme sites to construct pPT48. A fragment of the *cydA* gene was amplified by PCR and cloned into pEP185.2 using Gibson Assembly to create pFR9. An internal fragment of the *narG* gene from pCAL5 was cloned into pEP185.2 using the SacI and XbaI restriction enzyme sites creating pCAL18.

To generate plasmids that served as standards for real-time PCR, primer sets (Table S3) were used in a standard PCR reaction to amplify the respective target genes, which were subsequently inserted into pCR2.1 using the TOPO cloning kit (Life Technologies). The resulting plasmids were sequenced to ensure accuracy.

Generalized Phage Transduction

Phage P22 HT *int-105* was used for generalized transduction. Transductants were routinely purified from phage contamination on Evans blue-Uranine agar and then cross-struck against P22 H5 to confirm phage sensitivity.

Using phage transduction, the *cyxA*::pPT48 mutation from FR102 was introduced into CAL82 and SPN452 to yield strains FR104 and FR105, respectively.

Construction of *S.* Typhimurium mutants

Suicide plasmids were propagated in *E. coli* DH5α λ*pir* and introduced into the *S.* Typhimurium strain IR715 by conjugation using *E. coli* S17-1 λ*pir* as a donor strain. Exconjugants were selected on LB agar plates containing nalidixic acid and antibiotics selecting for integration of the suicide plasmid. Integration of the plasmids in the correct location on the chromosome was verified by PCR. Using this methodology, pPT48 and pFR9 were integrated into the *cyxA* and *cydA* genes of IR715 to yield strains FR102 and FR103, respectively.

The *narZ*::pCAL10 mutation from CAL45 was introduced into CAL46 and sucrose counter-selection was performed as described previously (Kingsley et al., 1999) to remove the Cm^r marker. A strain that was sucrose-tolerant and Cm^s was verified by PCR and designated CAL49.

pCAL18 was integrated into the *narG* gene of strain CAL49 to yield CAL82.

Animal Experiments

C57BL/6 mice were treated with 20 mg/animal streptomycin or mock-treated and orally inoculated 24 hours later with 0.1 ml sterile LB broth (mock infection), with an equal mixture of 5 x 10^8

CFU of each *S*. Typhimurium strain in 0.1 ml LB broth or with 5 x 10^8 CFU of individual *S*. Typhimurium strains. After euthanasia, organs were collected for RNA purification and bacteriological analysis. For competitive infections, the ratio of recovered bacterial strains (output ratio) was divided by the ratio present in the inoculum (input ratio) to determine the competitive index (CI). For treatment of infected C57BL/6 mice with a community of 17 *Clostridia* strains or with spore preparations, animals were treated with 20 mg/animal streptomycin and orally inoculated 24 hours later with *S*. Typhimurium. One-day after infection, mice were inoculated with 0.2 ml of spores or with 17 human Clostridia isolates by oral gavage.

CBA mice were inoculated with 0.1 ml of a suspension (LB broth) containing an equal mixture of 5×10^7 CFU of each bacterial strain. For single strain infections, animals were inoculated with approximately 1 x 10^8 CFU per animal or 1 x 10^9 CFU per animal. For treatment of CBA mice with a mixture of 17 *Clostridia* strains, animals were infected with *S.* Typhimurium strains and 5, 7 and 10 days after infection mice were inoculated with 0.2 ml of a culture of 17 human Clostridia isolates by oral gavage.

For transmission studies, mice from the same cage were split into two new cages (two mice per cage). Two mice were mock-treated and the other two mice (donors) were infected with 1 x 10^8 CFU of the indicated *S*. Typhimurium strain. Ten days after infection, two mock-treated mice (recipients) were co-housed with two infected mice. Feces were collected after 7 days of co-housing for bacterial enumeration. Colon contents were collected after 18 days of co-housing. Successful "transmission" was defined as recipient mice harboring detectable levels of *S*. Typhimurium in their feces at one of the indicated time points.

Microbiota analysis

Bacterial DNA was amplified via a PCR enrichment of the 16S rRNA gene (V4 region) using primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) modified by addition of barcodes for multiplexing. Libraries were sequenced with an Illumina MiSeq system. Sample sequences were demultiplexed and trimmed, followed by filtering for quality. QIIME

open-source software (http://qiime.org) (Caporaso et al., 2010) was used for initial identification of operational taxonomic units (OTU), clustering, and phylogenetic analysis. Principal Coordinate (PC) analysis taxa summaries using weighted UniFrac were created through QIIME. Samples containing less than 1000 quality reads were removed from dataset. Subsequent data transformation was performed using MEGAN 4 software (http://ab.inf.uni-tuebingen.de/software/megan4/) (Huson and Weber, 2013) and then further analyzed using Explicit (http://www.explicet.org/) (Robertson et al., 2013) and METAGENassist (http://www.metagenassist.ca/) (Arndt et al., 2012). Data are available at the NCBI BioSample database under Submission ID: SUB1356628 accession numbers SAMN04543794 through SAMN04543828.

Hypoxia Staining

Colon and cecal samples were fixed in 10% buffered formalin phosphate and paraffin-embedded tissue was probed with mouse anti-pimonidazole monoclonal IgG1 (MAb1 4.3.11.3) and then stained with Cy-3 conjugated goat anti-mouse antibody (Jackson Immuno Research Laboratories). Samples were counterstained with DAPI using SlowFade Gold mountant.

Histopathology

Formalin fixed cecal tissue sections were stained with hematoxylin and eosin, and a veterinary pathologist performed a blinded evaluation using criteria shown in Table S4 as described previously (Spees et al., 2013). Representative images were obtained using an Olympus BX41 microscope and the brightness adjusted (Adobe Photoshop CS2).

RNA isolation

For murine RNA isolation, colon tissue sections were homogenized in a Mini-Beadbeater (BioSpec Products, Bartlesville, OK) and RNA was isolated by the TRI-Reagent method (Molecular Research Center, Inc.) following the manufacturer's protocol. Contaminating DNA was removed using the DNA-free kit (Applied Biosystems) and RNA was stored at -80°C.

Measurements of short-chain fatty acid concentrations

Samples of cecal and colon contents were diluted with 80% ethanol (10 µl/mg) and gently agitated overnight at 4°C. The homogenized samples were centrifuged at 21,000 x g for 5 min. 200 µl of the supernatants were transferred centrifuged at 21,000 x g again for 20 min. For each sample, 20 µl of the supernatant was mixed with 20 µl of 200 mM N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (1-EDC HCl) (SIGMA) in 5% pyridine (SIGMA) and 40 uL of 100 mM 2-Nitrophenylhydrazine (2-NPH) (SIGMA) in 80% acetonitrile (ACN) (SIGMA) with 50 mM HCl. The mixture was incubated at 40°C for 30 min. After reacting, 400 µl of 10% ACN was added to the solution. Then 1 µl the solution was injected into an Agilent 6490 triple quadruple mass spectrometer for analysis.

REFERENCES FOR SUPPLEMENTAL MATERIALS

Arndt, D., Xia, J., Liu, Y., Zhou, Y., Guo, A.C., Cruz, J.A., Sinelnikov, I., Budwill, K., Nesbo, C.L., and Wishart, D.S. (2012). METAGENassist: a comprehensive web server for comparative metagenomics. Nucleic Acids Res *40*, W88-95.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I.*, et al.* (2010). QIIME allows analysis of high-throughput community sequencing data. Nat Methods *7*, 335-336.

Croswell, A., Amir, E., Teggatz, P., Barman, M., and Salzman, N.H. (2009). Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric Salmonella infection. Infection and immunity *77*, 2741-2753.

Godinez, I., Haneda, T., Raffatellu, M., George, M.D., Paixao, T.A., Rolan, H.G., Santos, R.L., Dandekar, S., Tsolis, R.M., and Baumler, A.J. (2008). T cells help to amplify inflammatory responses induced by Salmonella enterica serotype Typhimurium in the intestinal mucosa. Infection and immunity *76*, 2008-2017.

Huson, D.H., and Weber, N. (2013). Microbial community analysis using MEGAN. Methods Enzymol *531*, 465-485.

Kinder, S.A., Badger, J.L., Bryant, G.O., Pepe, J.C., and Miller, V.L. (1993). Cloning of the YenI restriction endonuclease and methyltransferase from Yersinia enterocolitica serotype O8 and construction of a transformable R-M+ mutant. Gene *136*, 271-275.

Kingsley, R.A., Reissbrodt, R., Rabsch, W., Ketley, J.M., Tsolis, R.M., Everest, P., Dougan, G., Baumler, A.J., Roberts, M., and Williams, P.H. (1999). Ferrioxamine-mediated Iron(III) utilization by Salmonella enterica. Appl Environ Microbiol *65*, 1610-1618.

Lopez, C.A., Winter, S.E., Rivera-Chavez, F., Xavier, M.N., Poon, V., Nuccio, S.P., Tsolis, R.M., and Baumler, A.J. (2012). Phage-mediated acquisition of a type III secreted effector protein boosts growth of salmonella by nitrate respiration. mBio *3*.

Miller, V.L., and Mekalanos, J.J. (1988). A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in Vibrio cholerae requires toxR. Journal of bacteriology *170*, 2575-2583.

Momose, Y., Maruyama, A., Iwasaki, T., Miyamoto, Y., and Itoh, K. (2009). 16S rRNA gene sequence-based analysis of clostridia related to conversion of germfree mice to the normal state. J Appl Microbiol *107*, 2088-2097.

Pal, D., Venkova-Canova, T., Srivastava, P., and Chattoraj, D.K. (2005). Multipartite regulation of rctB, the replication initiator gene of Vibrio cholerae chromosome II. Journal of bacteriology *187*, 7167-7175.

Raffatellu, M., George, M.D., Akiyama, Y., Hornsby, M.J., Nuccio, S.P., Paixao, T.A., Butler, B.P., Chu, H., Santos, R.L., Berger, T.*, et al.* (2009). Lipocalin-2 resistance confers an advantage to Salmonella enterica serotype Typhimurium for growth and survival in the inflamed intestine. Cell Host Microbe *5*, 476-486.

Robertson, C.E., Harris, J.K., Wagner, B.D., Granger, D., Browne, K., Tatem, B., Feazel, L.M., Park, K., Pace, N.R., and Frank, D.N. (2013). Explicet: graphical user interface software for metadata-driven management, analysis and visualization of microbiome data. Bioinformatics *29*, 3100-3101.

Simon, R., Priefer, U., and Puhler, A. (1983). A Broad Host Range Mobilization System for In Vivo Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria. Nat Biotech *1*, 784-791.

Spees, A.M., Wangdi, T., Lopez, C.A., Kingsbury, D.D., Xavier, M.N., Winter, S.E., Tsolis, R.M., and Baumler, A.J. (2013). Streptomycin-Induced Inflammation Enhances Escherichia coli Gut Colonization Through Nitrate Respiration. mBio *4*, e00430-00413.

Stojiljkovic, I., Baumler, A.J., and Heffron, F. (1995). Ethanolamine utilization in Salmonella typhimurium: nucleotide sequence, protein expression, and mutational analysis of the cchA cchB eutE eutJ eutG eutH gene cluster. Journal of bacteriology *177*, 1357-1366.