Figure S1

A

	Enzyme	Gene	Fold change in array (log_2)
	Positively regulated by Cra		
Gluconeogenic	PEP synthase	*ppsA*	2 (Increase)
	PEP carboxykinase	*pckA*	2.1 (Increase)
	Malate synthase; isocitrate lyase	*aceBA*	4.1 (Increase)
	Isocitrate dehyrogenase	*icd*	1.4 (Increase)
	Fructose-1,6-bisphosphatase	*fbp*	1.3 (Increase)
	Negatively regulated by Cra		
Glycolytic	Fructose catabolic enzymes	*fruBKA*	0.4 (Decrease)
	HPr, Enzyme I	*ptsHI*	0.1 (Decrease)
	Enterner-Doudoroff enzymes	*edd-eda*	-0.1 (Decrease)
	Phosphofructokinase	*pfk*	0 (Decrease)
	Erythrose-4-phosphate dehydrogenase	*gapB/epd*	0 (Decrease)
	Pyruvate kinase	*pykF*	-0.2 (Decrease)

B

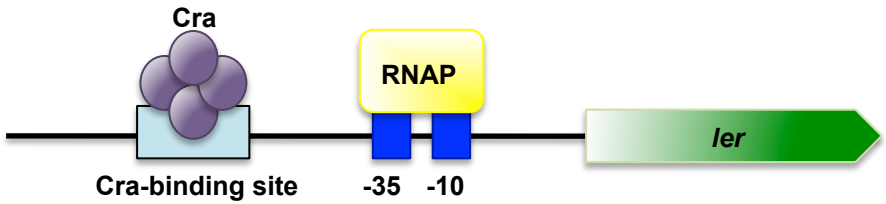

Figure S1, related to Fig. 2: Microarray results correspond with *Bt* regulation of EHEC gene expression through Cra. A, Cra positively regulates genes associated with gluconeogenesis and negatively regulates genes associated with glycolysis. Microarray results comparing EHEC cultured in the presence of *Bt* to EHEC grown alone correspond with a role of *Bt* positively and negatively regulating known Cra targets. Cra targets were based on the findings by Chin et al. (1) and by Feldheim et al. (2) reviewed in (Saier and Ramseier, 1996). **B,** Cra positively regulates expression of *ler*, the master transcriptional regulator of the LEE pathogenicity island, under gluconeogenic conditions.

[1] Chin, A.M., Feldheim, D.A., and Saier, Jr., M.H. (1989). Altered transcriptional patterns affecting several metabolic pathways in strains of *Salmonella typhimurium* which overexpress the fructose regulon. J. Bacteriol. *171,* 2424-2434.

[2] Feldheim, D.A., Chin, A.M., Nierva, C.T., Feucht, B.U., Cao, Y.W., Xu, Y.F., Sutrina, S.L., and Saier, Jr., M.H. (1990). Physiological consequences of the complete loss of the phosphoryl transfer proteins HPr and FPr of the phosphoenolpyruvate:sugar phosphotransferase system, and analysis of fructose (*fru)* operon expression in *Salmonella tymphimurium*. J. Bacteriol. *172,* 5459-5469.

[3] Saier, Jr., M.H. and Ramseier, T.M. (1996). Catabolite Repressor/Activator (Cra) Protein of Enteric Bacteria. J. Bacteriol. *178,* 3411-3417.

Figure S2

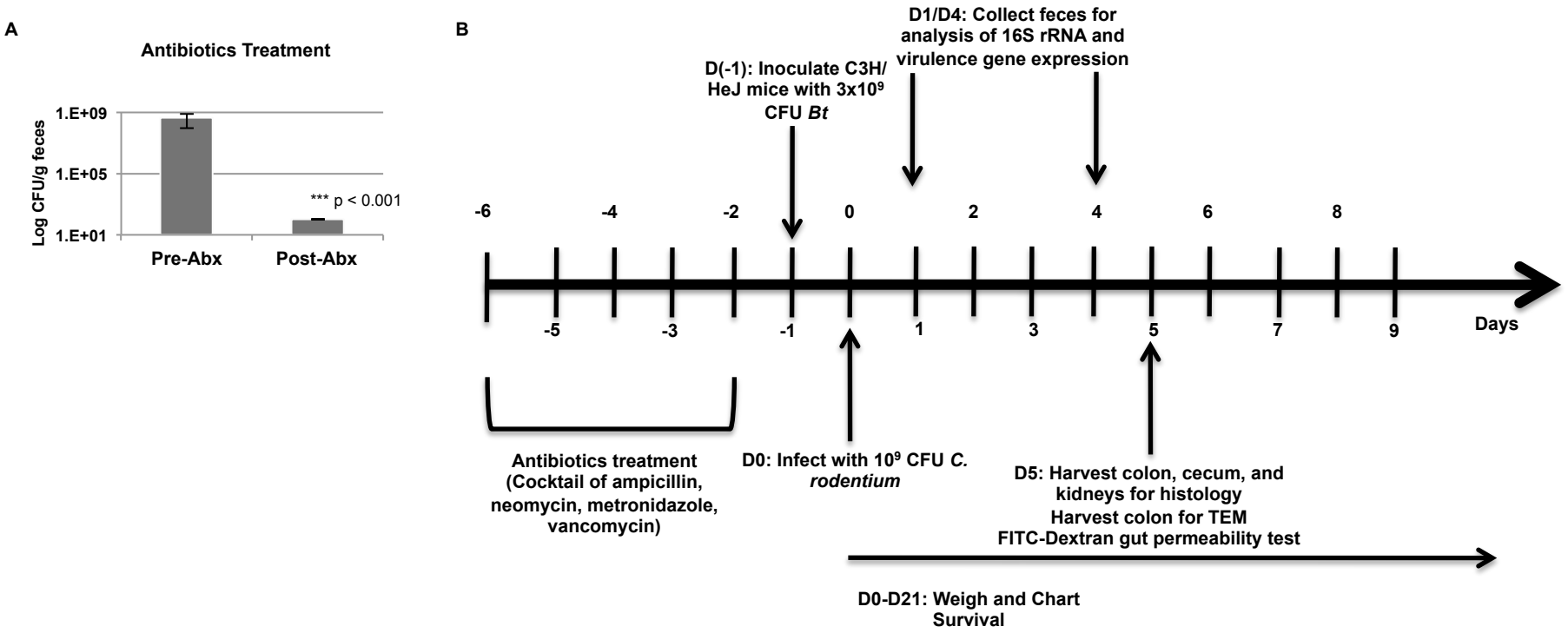

Figure S2, related to Fig. 5: Outline of *C. rodentium in vivo* infection model. A, Mice were administered a cocktail of antibiotics (ampicillin, vancomycin, neomycin, and metronidazole) for 5 days via oral gavage to deplete the indigenous microflora. Fecal pellets were collected before and after antibiotics treatment and plated on blood agar plates to determine colony forming units (CFU). **B,** Schematic of *B. thetaiotaomicron* colonization and *C. rodentium* infection model.

Figure S3

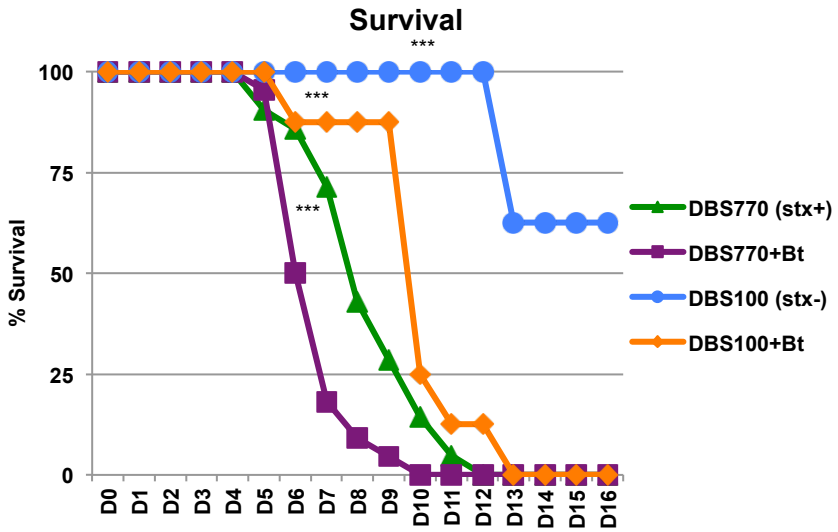

Figure S3, related to Fig. 5. Wild-type DBS100 strain behaves similarly to the DBS770 Stx+ strain. Survival curves of C3H/HeJ mice were treated for 5 days with an antibiotics regimen to deplete gut microbiota and then infected with *C. rodentium* WT DBS100 (blue and orange) or *C. rode*nitum DBS770 (Stx+) (green and purple) in the absence or presence of *Bt*.

Figure S4

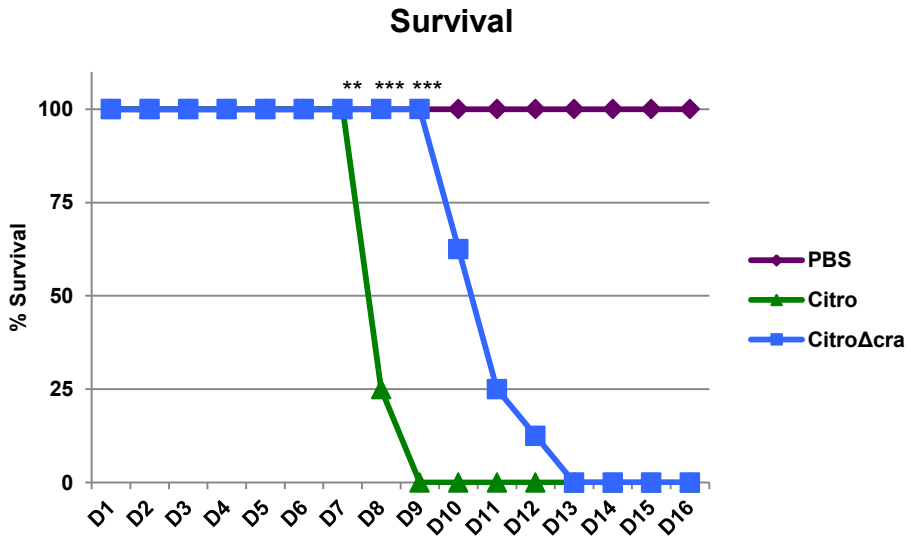

Figure S4, related to Fig. 5. The Δ*cra* mutant is attenuated for infection, highlighting the importance of fluctuations of carbon metabolites during infection. Survival curves of C3H/HeJ mice infected with *C. rodentium* WT or Δ*cra* infection. (n = 8 mice/group; error bars, s.d.; *** $P < 0.001$, ** $P < 0.01$

Figure S5

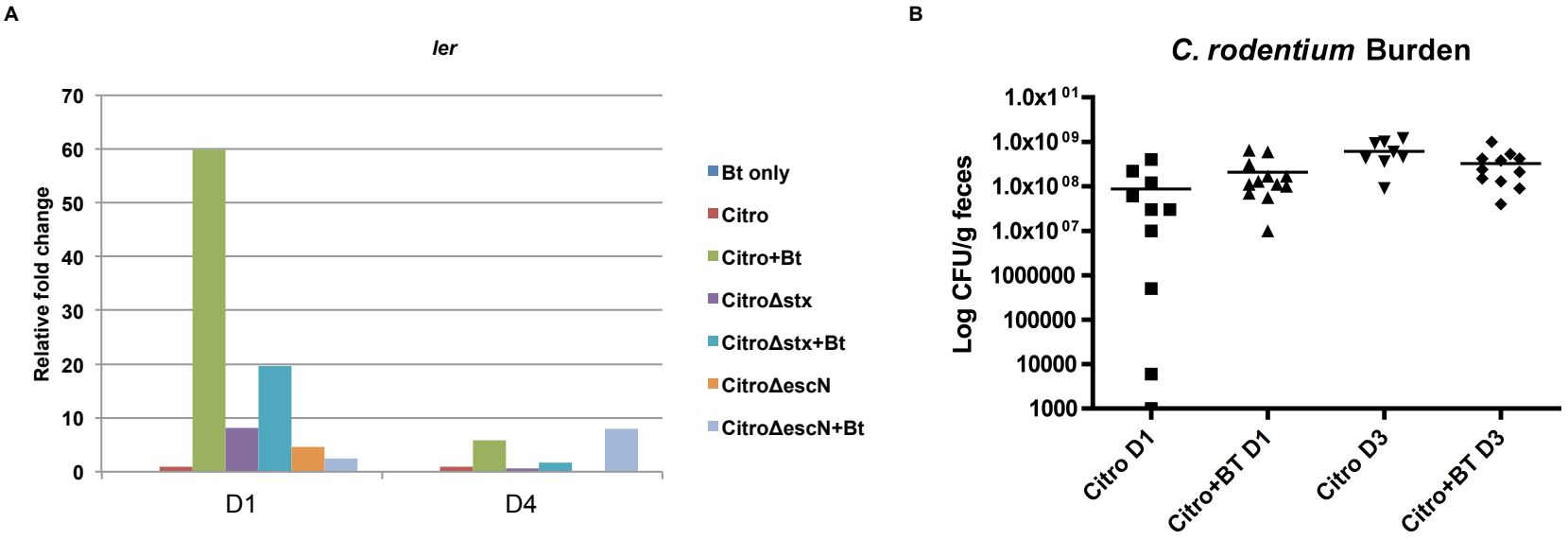

Figure S5, related to Fig. 5: *C. rodentium* virulence gene expression increases in the presence of *Bt* during *in vivo* infection, but the bacterial burden did not differ significantly. A, qRT-PCR of the *C. rodentium* virulence gene *ler*. Bacterial RNA was isolated from fecal pellets collected on day 1 and day 4 post-infection from mice either deplete of gut microbiota or reconstituted with *B. thetaiotaomicron* ((+) *Bt*) and then infected with *C. rodentium* (*Citro*, Stx+), *C. rodentiumΔstx (CitroΔstx,* Stx-), or *C. rodentiumΔescN (CitroΔescN).* Fecal pellets were collected from each mouse and pooled as a group to increase RNA yield. Experiments were performed at least twice with at least 3 mice/experiment. **B,** Bacterial burden of *C. rodentium* in the feces of infected mice in the absence (Citro) and presence of *Bt* (Citro +Bt) one day (D1) or three days post-infection (D3).

Figure S6

	Edema	Crypt Integrity	Neutrophil	Apoptosis	Bact. Attachment	Goblet Cell	Vasculitis
PBS	0.50±0.58	1.0±0	1.0±1.2	1.4±0.25	0±0	3.8±0.29	0±0
Bt	0.50±0.58	1.0±0	0.50±0.58	2.0±0.82	0±0	3.6±0.48	0±0
Citro	2.5±1.0	2.5±0	2.8±1.3	6.5±2.7	2.3±.0.96	3.4±0.95	2.8±0.96
Citro+Bt	4.8±0.50	3.8±0.96	4.0±1.2	9.5±1.0	2.3±0.50	2.0±0	3.5±1.0
CitroΔstx	1.8±0.96	2.0±0.82	2.4±0.75	4.0±2.8	1.8±0.96	4.0±0.41	1.5±0.58
CitroΔstx+Bt	3.0±0	2.8±0.50	4.3±1.3	9.6±2.3	2.8±0.5	3.6±0.25	2.5±0.58
CitroΔescN	1.0±0	1.0±0	1.5±2.4	2.5±0.58	0±0	3.9±0.48	0±0
CitroΔescN+Bt	1.9±0.25	1.0±0	1.8±1.5	2.5±0.58	0±0	3.9±0.48	0±0

Figure S6, related to Fig. 6: Host pathology increased in *C. rodentium*-infected animals reconstituted with *Bt* compared to infected animals deplete of gut microbiota. Double-blind pathology scoring presented in table form. Antibiotics-treated C3H/HeJ mice either deplete of gut microbiota or reconstituted with *Bt* (*+Bt)* were mock-infected (PBS, *Bt* only) or infected with *C. rodentium* (*Citro*, Stx+), *C. rodentiumΔstx (CitroΔstx,* Stx-), or *C. rodentiumΔescN (CitroΔescN)*. Scoring was performed blindly, and the scores for each parameter are an average of the cecum and distal colon, taken from two independent experiments with 3 mice/experiment.

Figure S7

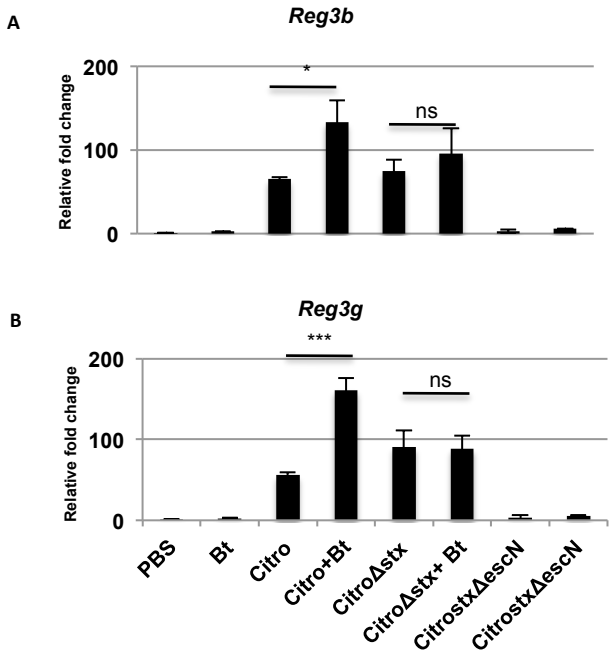

Figure S7, related to Fig. 6: Expression of the host antibacterial genes *Reg3b* and *Reg3g* increases during *C. rodentium* infection. A,B, Colonic tissue was harvested from mice on day 5 post-infection and qRT-PCR was performed for the host antibacterial genes *Reg3b* and *Reg3g*. (n = 6; error bars, s.d.; ***$P < 0.001$; * $P < 0.05$; ns not significant.

Supplemental Material

Table S1, related to Experimental Procedures (Strains and culture conditions). Bacterial strains used in this study

Strain	Relevant properties	Source or reference
EHEC		
wild-type (WT)	wt O157:H7 (86-24 clinical isolate)	(Griffin et al., 1988)
VS138	Δ*qseC*	(Sperandio et al., 2002)
NR01	Δ*qseE*	(Reading et al., 2007)
JN01	Δ*cra*	(Njoroge et al., 2012)
DH11	Δ*kdpE*	(Hughes et al., 2009)
JN02	Δ*cra*Δ*kdpE*	(Njoroge et al., 2012)
ARP01	Δ*fusK*	(Pacheco et al., 2012)
Bacteroides thetaiotaomicron (Bt)		
VPI 5482	wt, Gm^r	ATCC 29148
Citrobacter rodentium		
DBS100	WT *C. rodentium*	
DBS770	Stx+ *C. rodentium*	(Mallick et al., 2012)
DBS771	Stx- *C. rodentium*	(Mallick et al., 2012)
MMC01	Δ*escN,* isogenic mutant of DBS770	This study
MMC02	Δ*cra*, isogenic mutant of DBS770	This study
Enterococcus faecalis		
V583	*E. faecalis* (human blood isolate)	(Paulsen et al., 2003)

Table S2, related to Experimental Procedures (Real time qPCR). Oligonucleotides used in this study

Primer name	Sequence	Description
qPCR		
rpoA F	GCGCTCATCTTCTTCCGAAT	EHEC (endogenous ctrl)
rpoA R	CGCGGTCGTGGTTATGTG	EHEC
ler F	CGACCAGGTCTGCCC	EHEC (*LEE1)*
ler R	GCGCGGAACTCATC	EHEC
escC F	GCGTAAACTGGTCCGGTACGT	EHEC *(LEE2)*
escC R	TGCGGTAGAGCTATTAAAGGCAAT	EHEC
escV F	TCGCCCCGTCCATTGA	EHEC (*LEE3)*
escV R	CGCTCCCGAGTGCAAAA	EHEC
tir F	CCATGGAGAGCAGACGTAGCT	EHEC (*LEE5)*
tir R	CGGTGATCCTGGATTTAACCTT	EHEC
espA F	TCAGAATCGCAGCCTGAAAA	EHEC (*LEE4)*
espA R	CGAAGGATGAGGTGGTTAAGCT	EHEC
CR_*rpoA*_F	ACGTCAGCCGGAAGTGAAAGAAGA	*C. rodentium* (endo. ctrl)
CR_*rpoA*_R	AGCGGACAGTCAATTCCAGATCGT	
CR_*ler*_F	ACAGTTTGAATCTCCTGCTCACGC	*C. rodentium (LEE1)*
CR_*ler*_R	AATTCGCCCACAACAAGCCCATAC	
CR_*eae*_F	AATTCTCCGGGATTCTGTCCGGTT	*C. rodentium (LEE5)*
CR_*eae*_R	ACATCCATCCCGGAAAGGTCATCA	
CR_*espA*_F	ACGAGGTAACAACCATGCGAGTGT	*C. rodentium (LEE4)*
CR_*espA*_R	CTGCCTGGCATTGCTTTCCAGAAT	
CR_*p1411*_F	CGACCAGTTTACCGTCCTTATC	*C. rodentium* (mucinase)
CR_*1411*_R	GCCGATATCCCGTATCAGTTATT	
GAPDH F	ATTGTCAGCAATGCATCCTG	Murine (endo. ctrl)
GAPDH R	ATGGACTGTGGTCATGAGCC	
Tff3 F	CCTGGTTGCTGGGTCCTCTG	Murine (*Trefoil factor 3*)
Tff3 R	GCCACGGTTGTTACACTGCTC	
Muc2 F	GCTGACGAGTGGTTGGTGAATG	Murine (*Mucin2)*
Muc2 R	GATGAGGTGGCAGACAGGAGAC	
Reg3g F	AAGCTTCCTTCCTGTCCTCC	Murine (*Reg3 gamma)*
Reg3g R	TCCACCTCTGTTGGGTTCAT	
Reg3b F	GGCTTCATTCTTGTCCTCCA	Murine (*Reg3 beta)*
Reg3b R	TCCACCTCCATTGGGTTCT	

Lambda Red primers

CR_*escN*_F (MMC01):
CCGTGCTCTCTTTTCAGGAAGTTGGTAATAATATCGAACTGAAAGTATTAGGAACGGTAAGTGTAGGCTGGAGCTGCTTCG

CR_*escN*_R (MMC01):
TCGACTCCCTCAAACGATTCGCTCTATTTCTACGAATAGATAAAATTCTGTCCAACATATCATATGAATATCCTCCTTAG

CR_*cra*_F (MMC02): CAAGCTACGT GATAAACGGA AAAGCAAAGC AATACCGCGT GAGCGACAAG
ACTGTCGAAAGTGTAGGCTGGAGCTGCTTCG

CR_*cra*_R (MMC02):
CCGGCTTCGGCTTGCGCGGCTCATCCAGGCTCGCCAGCACGATCTCCAGCACGCGCTCCGCATATGAATATCCTCCTTAG

Supplemental Methods

Transmission Electron Microscopy

A portion of distal colon harvested from mock-infected or *C. rodentium*-infected mice was fixed in 2.5% glutaraldehyde, 0.1 M cacodylate buffer for a minimum of 2 h. Samples were then submitted to the UT Southwestern Electron Microscopy Core and prepared as follows: The tissue was rinsed in cacodylate buffer and placed into 1% osmium with 0.8% ferricyanide for 1.5 h, then rinsed with deionized water and en bloc stained with uranyl acetate for 2 h. The tissue then underwent a series of dehydration steps from 50% to 100% ethanol, was infiltrated with propylene oxide and resin, embedded in pure resin, and cured in a 70°C oven overnight. After sectioning, the grids were then post-stained with 2% uranyl acetate in deionized water and lead citrate. The grids were imaged on a Technai G2 Spirit Transmission Electron Microscope (Tecnai) using iTEM software (Olympus).

Muc2 Staining

Portions of the distal colon and cecum were harvested five days post-infection with *C. rodentium*. The tissues were washed in PBS and then fixed in Bouin's fixative for 48h. The tissues were embedded in paraffin and cut into 5-µm sections. For deparaffinization, the slides were washed with 100% xylene → 50% xylene : 50% ethanol → 100% ethanol → 95% ethanol → 70% ethanol → 50% ethanol and then rinsed with cold water. The slides were then heated in a 90°C oven in 0.01 M citrate buffer, pH 5.0 for 15 minutes for antigen retrieval. The slides were rinsed with Tris buffer, stained with Muc2 antibody (clone H-300, SantaCruz, rabbit polyclonal IgG) at 4°C, and counterstained with DAPI (Molecular Probes) and anti-IgG FITC (Sigma) at 4°C. ProLong Gold Antifade (Life Technologies) was added, and the slides were visualized with a Zeiss Axiovert microscope.

Metabolomics and Determination of abundances of succinate, fumarate and pyruvate using LC/MS/MS

Cecal contents were harvested on day 2 post-infection and resuspended in water to 0.5 g/ml. Two ml of chloroform were added to the cecal contents and vigorously vortexed, followed by 2 ml of methanol with vigorous vortexing, and 1 ml of water with vigorous vortexing. The samples were centrifuged at 1000 rpm for 5 min, and the aqueous layer was submitted for analysis by liquid chromatography-mass spectrometry. Following this, 300 µL of the samples added with 1.2 mL of cold methanol/water (80/20, v/v). After rigorous vortexing, the debris was pelleted by centrifugation at 16,000 × *g* and 4°C for 15 min. The supernatant was transferred to a new tube and evaporated to dryness using a SpeedVac concentrator (Thermo Savant, Holbrook, NY). Metabolites were reconstituted in 100 µL of 0.03% formic acid in analytical-grade water, vortex-mixed and centrifuged to remove debris. Thereafter, the supernatant was transferred to a HPLC vial for the analysis of succinate, fumarate and pyruvate.

The analysis was performed using a liquid chromatography-mass spectrometry/mass spectrometry (LC/MS/MS) approach. Separation was achieved on a Phenomenex Synergi Polar-RP HPLC column (150 × 2 mm, 4 µm, 80 Å) using a Nexera Ultra High Performance Liquid Chromatograph (UHPLC) system (Shimadzu Corporation, Kyoto, Japan). The mobile phases employed were 0.03% formic acid in water (A) and 0.03% formic acid in acetonitrile (B). The gradient program was as follows: 0-3 min, 100% A; 3-5 min, 100% - 0% A; 5-6 min, 0% A; 6-6.1 min, 0% - 100% A; 6.1-8 min, 100% A. The column was maintained at 35°C and the samples kept in the autosampler at 4°C. The flow rate was 0.5 mL/min, and injection volume 10 µL. The mass spectrometer was an AB QTRAP 5500 (Applied Biosystems SCIEX, Foster City, CA) with electrospray ionization (ESI) source in multiple reaction monitoring (MRM) mode. Sample analysis was performed in negative mode. Declustering potential (DP) and collision energy (CE) were optimized by direct infusion of reference standards using syringe pump prior to sample analysis. The MRM MS/MS detector conditions were set as follows: curtain gas 30 psi; ion spray voltages 5000 V; temperature 650°C; ion source gas 1 50 psi; ion source gas 2 50 psi; interface heater on; entrance potential 10 V. The MRM transitions (*m/z*), DPs (V) and CEs (V) of the detected metabolites are 117 > 73, -60 V, -18 V (succinate); 115 > 71, -60 V, -10 V (fumarate); and 87 > 43, -60 V, -12 V (pyruvate), respectively. Dwell time for each transition was set at 50 msec. Samples were analyzed in a randomized order, and MRM data was acquired using Analyst 1.6.1 software (Applied Biosystems SCIEX, Foster City, CA). Chromatogram review and peak area integration were performed using MultiQuant software version 2.1 (Applied Biosystems SCIEX, Foster City, CA). The peak area values were used as variables for the following statistical data analysis. Statistical differences of the metabolites between two groups were analyzed using Student's *t*-test. The results with $p < 0.05$ were considered as statistically significant. The relative abundance from the total metabolomics analysis can be found in Supplementary Table 3.

To confirm a role for succinate, EHEC was grown with increasing concentrations of succinate (0.5 g/ml stock, pH 7.5) added to DMEM containing 0.1% glucose. Total cell lysate or concentrated culture supernatant was run on an SDS-PAGE, transferred to nitrocellulose membrane, and incubated with either anti-RpoA monoclonal antibody as a loading control or anti-EspA polyclonal antibody.

For total metabolomics analysis, sample preparation from cecal contents is as described above. The samples were subjected to LC/MS/MS, and chromatogram review and peak area integration were performed using MultiQuant software version 2.1 (Applied Biosystems SCIEX, Foster City, CA). Although the numbers of cells were very similar and each sample was processed identically and randomly, the peak area for each detected metabolite was normalized against the protein content of that sample to correct any variations introduced from sample handling through instrument analysis. The normalized area values were used as variables for the multivariate and univariate statistical data analysis. The chromatographically co-eluted metabolites with shared MRM transitions were shown in a grouped format, i.e., leucine/isoleucine. All multivariate analyses and modeling on the normalized data were carried out using SIMCA-P (version 13.0.1, Umetrics, Umeå, Sweden). The pre-processed datasets were mean-centered and unit-variance scaled, and then evaluated by principal component analysis (PCA) to visualize the clustering trend, as well as to detect and exclude outlier datasets. After that, the data were further subjected to Partial Least Squares Discriminant Analysis (PLS-DA) for identification of discriminant metabolites that characterized the different groups. Univariate statistical differences of the metabolites between two groups were analyzed using Student's t-test.

Principal component analysis (PCA) is proven to be suitable for differentiating sample groups on the basis of metabolite composition. It is an unsupervised technique that assumes no *a priori* knowledge of class structure and transforms the variables in a data set into a smaller number of new latent variables called principal components (PCs), which are uncorrelated with each other and account for decreasing proportions of the total variance of the original variables. Each new PC is a linear combination of the original variables such that a compact description of the variation within a data set is generated. Observations are assigned scores according to the variation measured by the PCs with those having similar scores clustering together.

Table S3, related to Fig. 7. Total metabolomics analysis

Metabolite	Avg	Std dev	Avg (Bt)	Std dev (Bt)	Avg (Citro)	Std dev (Citro)	Avg (C+Bt)	Std dev (C+Bt)
Class	PBS		Bt only		Citro		Citro+Bt	
2-hydroxyglutaric acid	1.22E-06	1.32E-07	4.72E-05	4.52E-05	1.09E-06	5.15E-08	2.80E-04	1.40E-04
acetyl-carnitine	1.94E-03	1.24E-03	9.85E-04	4.61E-04	1.53E-03	5.95E-04	1.19E-03	3.02E-04
choline	9.12E-03	1.99E-03	6.97E-03	1.05E-03	9.00E-03	2.15E-03	8.91E-03	2.03E-03
CMP	5.91E-05	6.70E-05	6.34E-05	3.84E-05	6.36E-05	4.22E-05	2.16E-04	1.82E-04
creatine	1.12E-02	1.85E-03	7.95E-03	2.69E-03	8.79E-03	1.86E-03	6.25E-03	1.01E-03
cytidine	1.00E-02	3.12E-03	6.50E-03	2.08E-03	6.46E-04	2.76E-04	3.98E-03	8.78E-04
gluconic acid	1.33E-03	4.16E-04	8.79E-04	3.74E-04	2.94E-04	7.93E-05	1.21E-04	2.95E-05
guanidoacetic acid	3.58E-03	2.03E-03	1.77E-03	5.34E-04	3.63E-03	9.95E-04	1.43E-03	3.14E-04
guanosine	1.08E-02	3.67E-03	4.49E-03	2.38E-03	9.04E-04	3.71E-04	2.80E-03	4.58E-04
inosine	1.12E-02	2.10E-03	6.61E-03	2.10E-03	4.00E-03	1.81E-03	7.03E-03	7.50E-04
leucine/isoleucine	2.38E-02	6.98E-03	1.56E-02	2.57E-03	2.50E-02	4.70E-03	1.27E-02	2.18E-03
methionine	5.04E-03	2.14E-03	3.55E-03	1.09E-03	5.68E-03	1.79E-03	3.54E-03	2.59E-04
phenyl-alanine	8.68E-03	3.74E-03	5.07E-03	2.15E-03	8.44E-03	2.81E-03	5.36E-03	1.64E-03
proline	2.79E-02	6.83E-03	2.04E-02	7.44E-03	2.68E-02	4.82E-03	2.06E-02	3.92E-03
shikimic acid	1.79E-02	4.48E-03	1.06E-02	2.89E-03	1.55E-02	6.15E-03	1.14E-02	2.97E-03
tryptophan	1.07E-02	2.58E-03	5.80E-03	8.75E-04	1.22E-02	2.89E-03	6.34E-03	9.05E-04
tyrosine	6.23E-03	1.49E-03	4.03E-03	6.73E-04	6.31E-03	1.15E-03	4.20E-03	1.28E-03
urea	6.05E-03	4.70E-04	4.37E-03	1.83E-03	4.72E-03	4.84E-04	2.02E-03	5.39E-04
yamma-aminobutyric acid	4.19E-03	1.18E-03	2.78E-03	1.05E-03	3.59E-03	1.28E-03	4.05E-03	2.08E-04
1-methyl-adenosine	1.78E-02	2.21E-03	1.25E-02	7.10E-03	5.11E-03	1.62E-03	4.29E-03	1.52E-03
1-methyl-histidine	3.32E-04	8.82E-05	3.10E-04	5.57E-05	5.50E-04	2.00E-04	5.42E-04	1.14E-04
1-methyl-nicotinamide	1.15E-02	6.52E-03	1.44E-02	2.87E-03	1.40E-02	6.81E-03	7.92E-03	4.54E-03
2-deoxycytidine	1.29E-02	3.49E-03	9.44E-03	4.70E-03	5.87E-04	1.62E-04	3.50E-03	7.87E-04
2-isopropylmalic acid	8.47E-04	4.23E-04	1.20E-03	6.25E-04	2.07E-03	1.62E-03	5.58E-04	3.89E-04
2-ketohaxonic acid	4.19E-04	2.36E-04	3.31E-03	1.36E-03	6.15E-04	8.62E-05	1.97E-03	5.69E-04
3-hydroxybutyrate	4.12E-04	1.32E-04	8.73E-04	2.70E-04	4.65E-04	1.39E-04	9.73E-04	1.60E-04
4-pyridoxic acid	7.88E-03	2.15E-03	9.82E-03	3.74E-03	1.06E-02	4.12E-03	5.24E-03	1.20E-03
5-aminolevulinic acid	7.12E-03	2.21E-03	7.58E-03	2.25E-03	6.36E-03	1.56E-03	6.55E-03	5.66E-04
7-methylguanosine	1.03E-03	3.75E-04	5.29E-03	2.93E-03	8.99E-04	2.08E-04	3.38E-03	1.17E-03
8-hydroxy-2-deoxyguanosine	4.78E-04	1.23E-04	3.83E-04	1.44E-04	3.78E-04	9.93E-05	2.51E-04	3.67E-05
acetyl-choline	3.06E-02	6.36E-03	2.62E-02	8.93E-03	3.55E-02	8.12E-03	2.95E-02	8.56E-03
acetyllysine	5.99E-03	1.82E-03	5.54E-03	6.92E-04	8.82E-03	2.33E-03	8.72E-03	2.28E-03
adenine	1.85E-02	4.76E-03	1.37E-02	1.33E-03	2.02E-02	2.30E-03	1.24E-02	2.31E-03
adenosine	1.50E-02	6.58E-03	7.77E-02	3.49E-02	2.79E-02	6.89E-03	8.44E-02	8.86E-03
AICAR	2.78E-04	4.15E-05	3.71E-04	1.23E-04	3.20E-04	6.75E-05	2.65E-04	5.34E-05
alanine/sarcosine	8.67E-03	2.75E-03	9.62E-03	2.18E-03	1.34E-02	2.04E-03	1.66E-02	4.46E-03
allantoin	2.00E-03	2.71E-04	2.93E-03	3.48E-04	2.31E-03	6.46E-04	2.13E-03	5.13E-04
aminoadipic acid	4.31E-03	1.14E-03	4.36E-03	7.31E-04	3.73E-03	3.97E-04	3.03E-03	3.66E-04
arginine	3.21E-02	2.22E-03	2.26E-02	5.07E-03	3.08E-02	4.01E-03	2.00E-02	3.76E-03
ascorbic acid	6.00E-04	2.97E-04	8.10E-04	4.19E-04	1.43E-03	1.13E-03	3.78E-04	2.47E-04
asparagine	9.50E-02	1.21E-02	7.36E-02	1.00E-02	9.67E-02	6.71E-03	6.14E-02	5.92E-03
aspartate	3.74E-03	6.64E-04	2.99E-03	3.01E-04	4.17E-03	3.43E-04	2.58E-03	2.49E-04
betaine	2.00E-02	3.92E-03	1.82E-02	3.06E-03	2.43E-02	4.00E-03	3.79E-02	3.19E-03
cadaverine	7.59E-05	9.60E-05	1.30E-04	1.12E-04	4.35E-04	2.37E-04	7.13E-04	2.72E-04
carnitine	1.03E-02	3.01E-03	6.16E-03	1.76E-03	9.40E-03	1.47E-03	6.38E-03	1.40E-03
carnitine-C3	3.99E-03	3.76E-04	1.27E-02	5.79E-03	3.84E-03	1.02E-03	3.53E-02	3.66E-03
carnitine-C4	9.04E-03	1.51E-03	5.64E-03	3.27E-03	4.50E-03	1.01E-03	5.87E-03	1.32E-03
citrate/isocitrate	1.40E-03	7.08E-04	2.46E-03	9.84E-04	3.32E-03	1.49E-03	3.93E-03	2.00E-03
citrulline	5.72E-03	2.81E-03	1.13E-02	5.50E-03	1.39E-02	4.57E-03	1.77E-02	6.04E-03
creatinine	2.71E-02	4.96E-03	3.37E-02	6.83E-03	3.04E-02	9.26E-03	1.74E-02	7.33E-03
cysteine	1.22E-06	1.32E-07	3.44E-04	4.21E-04	1.89E-04	2.95E-04	6.90E-04	1.93E-04
cytosine	1.43E-03	4.46E-04	1.07E-03	4.95E-04	7.16E-04	1.06E-04	8.91E-04	1.30E-04
dAMP	3.50E-04	8.93E-05	2.81E-04	1.40E-04	2.79E-04	6.53E-05	1.85E-03	2.91E-03
deoxyinosine	3.17E-02	9.05E-03	1.61E-02	1.32E-02	9.74E-04	2.89E-04	4.80E-03	9.21E-04
deoxyuridine	6.17E-04	1.79E-04	2.22E-04	2.93E-04	1.09E-06	5.15E-08	6.39E-05	2.29E-05
dihydroorotate	1.09E-04	1.09E-05	2.29E-04	6.26E-05	1.75E-04	5.65E-05	2.79E-04	5.34E-05
dimethylglycine	2.67E-02	2.63E-03	2.41E-02	3.98E-03	2.61E-02	3.15E-03	2.68E-02	2.80E-03
flavin adenine dinucleotide	7.44E-05	4.99E-05	2.33E-04	9.98E-05	9.70E-05	9.27E-05	2.64E-04	2.44E-04

Table S3, related to Fig. 7. Total metabolomics analysis

Metabolite	Avg	Std dev	Avg (Bt)	Std dev (Bt)	Avg (Citro)	Std dev (Citro)	Avg (C+Bt)	Std dev (C+Bt)
Class	PBS		Bt only		Citro		Citro+Bt	
fructose	2.24E-04	1.07E-04	2.23E-04	9.15E-05	1.78E-04	8.61E-05	1.20E-04	3.05E-05
glucarate	9.26E-05	8.68E-05	7.32E-05	4.19E-05	2.36E-05	3.16E-05	5.67E-05	2.78E-05
glucosamine	4.30E-04	9.00E-05	3.10E-04	6.98E-05	3.47E-04	3.39E-05	2.82E-04	1.03E-04
glucose/fructose	1.73E-04	1.45E-04	1.55E-04	1.24E-04	1.03E-04	2.74E-05	1.10E-04	3.70E-05
glutamate/NMDA	3.98E-04	8.14E-05	8.15E-04	3.58E-04	9.10E-04	3.26E-04	2.05E-03	4.69E-04
glutamine	1.13E-02	8.88E-04	7.26E-03	1.50E-03	1.63E-02	2.11E-03	1.07E-02	2.98E-03
glycerate	1.22E-06	1.32E-07	5.92E-05	5.40E-05	1.09E-06	5.15E-08	8.83E-05	1.91E-05
glycerophosphorylcholine	8.97E-03	2.79E-03	1.16E-02	5.19E-03	1.97E-03	3.19E-04	1.21E-03	2.28E-04
glycine	1.01E-04	3.74E-05	7.21E-05	1.50E-05	2.59E-04	9.01E-05	2.47E-04	7.31E-05
GSH	1.22E-06	1.32E-07	1.00E-04	9.19E-05	1.09E-06	5.15E-08	4.23E-03	2.30E-03
guanine	3.21E-02	9.22E-03	1.93E-02	1.07E-02	4.02E-03	9.62E-04	1.20E-02	4.22E-04
histidine	7.33E-03	1.46E-03	4.84E-03	7.72E-04	1.05E-02	2.43E-03	7.32E-03	1.16E-03
homocysteic acid	1.82E-03	3.86E-04	1.53E-03	2.61E-04	2.17E-03	3.25E-04	1.35E-03	1.79E-04
homoserine/threonine	8.94E-03	4.81E-03	4.72E-03	1.35E-03	4.73E-03	6.69E-04	6.88E-03	2.62E-03
hydroxyisocaproic acid	1.22E-06	1.32E-07	8.82E-03	7.44E-03	2.33E-03	1.15E-03	2.43E-03	1.10E-03
hydroxyphenylpyruvate	6.46E-02	8.32E-03	5.19E-02	5.76E-03	6.63E-02	8.16E-03	4.36E-02	4.20E-03
hydroxy-proline	5.65E-03	2.19E-03	8.74E-03	2.91E-03	5.99E-03	1.45E-03	1.41E-02	2.98E-03
hypoxanthine	8.40E-03	2.62E-03	1.26E-02	4.37E-03	2.85E-02	6.07E-03	1.78E-02	5.36E-03
indole	3.69E-02	6.92E-03	3.41E-02	5.22E-03	4.38E-02	4.31E-03	3.51E-02	5.40E-03
kynurenine	3.24E-04	8.29E-05	2.71E-04	7.93E-05	3.92E-04	7.96E-05	1.08E-03	5.04E-04
lactate	1.08E-04	4.26E-05	1.65E-04	1.13E-04	1.62E-04	2.07E-05	2.29E-04	6.34E-05
lysine	2.97E-02	6.27E-03	2.06E-02	4.39E-03	3.51E-02	4.03E-03	1.91E-02	5.06E-03
malic acid	5.16E-04	3.39E-04	3.32E-03	2.12E-03	1.98E-03	3.35E-04	6.16E-03	1.41E-03
malonate	3.90E-04	9.67E-05	8.01E-04	2.40E-04	4.50E-04	1.35E-04	9.22E-04	1.57E-04
methionine sulfoxide	1.38E-03	5.44E-04	1.16E-03	2.33E-04	8.34E-04	1.38E-04	5.85E-04	7.63E-05
myo-inositol	4.40E-04	1.53E-04	7.74E-04	2.85E-04	4.85E-04	1.59E-04	4.50E-04	1.47E-04
N-acetyl-alanine	2.64E-04	1.29E-04	2.03E-04	9.22E-05	3.75E-04	9.27E-05	2.55E-04	1.34E-04
NAD	7.19E-05	1.98E-05	6.12E-04	4.56E-04	1.99E-04	3.44E-05	1.58E-03	4.55E-04
Ng,Ng-dimethyl-arginine	6.05E-03	8.39E-04	7.16E-03	8.65E-04	4.99E-03	2.41E-04	6.31E-03	9.03E-04
nicotinamide	1.16E-02	3.86E-03	5.80E-03	4.56E-03	6.24E-03	3.45E-03	1.66E-02	2.05E-03
nicotinate	1.76E-02	1.50E-03	4.16E-02	1.72E-02	5.24E-02	5.22E-03	3.40E-02	2.35E-03
O-acetyl-serine	9.20E-04	2.46E-04	1.24E-03	2.36E-04	1.50E-03	2.88E-04	7.29E-04	6.22E-05
ornithine	5.23E-04	1.29E-04	4.25E-03	3.18E-03	3.79E-03	7.39E-03	3.10E-03	2.03E-03
pantothenic acid	1.58E-03	2.47E-04	3.40E-03	1.59E-03	2.84E-03	1.48E-03	6.05E-03	6.40E-04
phenyl-lactic acid	1.22E-06	1.32E-07	2.88E-04	2.04E-04	1.70E-04	2.83E-05	4.89E-04	1.17E-04
phosphorylcholine	3.83E-04	5.32E-04	1.77E-04	1.68E-04	3.60E-04	1.19E-04	2.42E-03	3.15E-03
pipecolic acid	1.39E-02	2.05E-03	1.69E-02	1.79E-03	1.65E-02	3.15E-03	2.52E-02	3.60E-03
propionylcholine	1.40E-02	8.67E-04	1.08E-02	6.82E-03	1.04E-02	1.22E-03	3.25E-03	6.44E-04
putrescine	9.34E-03	7.00E-03	1.32E-02	7.97E-03	1.93E-02	7.40E-03	2.62E-02	5.23E-03
pyridoxamine	1.18E-03	3.88E-04	1.57E-03	5.83E-04	2.52E-03	3.97E-04	3.04E-03	4.39E-04
pyridoxine	1.31E-03	4.53E-04	3.61E-03	9.38E-04	1.17E-03	6.40E-04	2.25E-03	3.58E-04
S-adenosyl-homocysteine	1.22E-06	1.32E-07	1.61E-04	2.03E-04	1.09E-06	5.15E-08	1.11E-04	4.11E-05
S-adenosyl-methionine	7.34E-03	1.79E-03	6.62E-03	1.74E-03	8.57E-03	2.79E-03	1.01E-02	1.59E-03
succinate/methyl-malonic acid	3.19E-04	1.58E-04	6.40E-02	4.40E-02	1.18E-02	2.24E-03	6.42E-02	2.02E-02
taurine	7.01E-04	4.29E-04	6.26E-04	1.93E-04	1.04E-03	1.56E-04	1.68E-03	4.23E-04
tetrahydrobiopterin	1.23E-03	3.22E-04	9.44E-04	3.91E-04	7.79E-04	7.38E-05	5.19E-04	6.11E-05
thiamine	1.52E-02	3.93E-03	5.58E-03	7.34E-03	1.64E-02	4.81E-03	1.10E-03	5.71E-04
thymidine	3.33E-02	1.07E-02	1.90E-02	1.20E-02	1.14E-03	2.78E-04	7.48E-03	1.73E-03
trimethyllysine	8.10E-03	1.56E-03	7.71E-03	1.74E-03	1.11E-02	2.17E-03	1.15E-02	2.34E-03
UDP	1.59E-05	3.29E-05	1.66E-05	3.46E-05	8.79E-05	1.10E-04	2.53E-04	1.78E-04
uracil	7.77E-04	9.40E-05	7.24E-04	3.75E-04	7.00E-04	1.07E-04	8.39E-04	8.38E-05
uridine	4.20E-04	6.00E-05	2.42E-04	1.08E-04	6.13E-05	2.03E-05	1.90E-04	3.42E-05
valine	3.86E-02	8.63E-03	3.47E-02	4.31E-03	4.87E-02	5.23E-03	3.51E-02	4.39E-03
xanthine	8.42E-04	1.92E-04	6.20E-04	2.07E-04	1.89E-03	4.07E-04	1.34E-03	6.26E-04
xanthosine	8.24E-03	3.37E-03	6.86E-03	1.56E-03	4.52E-03	1.17E-03	6.15E-03	3.83E-04

References

Griffin, P.M., Ostroff, S.M., Tauxe, R.V., Greene, K.D., Wells, J.G., Lewis, J.H., and Blake, P.A. (1988). Illnesses associated with Escherichia coli O157:H7 infections. A broad clinical spectrum. Ann Intern Med *109*, 705-712.

Hughes, D.T., Clarke, M.B., Yamamoto, K., Rasko, D.A., and Sperandio, V. (2009). The QseC adrenergic signaling cascade in Enterohemorrhagic E. coli (EHEC). PLoS Pathog *5*, e1000553.

Mallick, E.M., McBee, M.E., Vanguri, V.K., Melton-Celsa, A.R., Schlieper, K., Karalius, B.J., O'Brien, A.D., Butterton, J.R., Leong, J.M., and Schauer, D.B. (2012). A novel murine infection model for Shiga toxin-producing Escherichia coli. J Clin Invest *122*, 4012-4024.

Njoroge, J.W., Nguyen, Y., Curtis, M.M., Moreira, C.G., and Sperandio, V. (2012). Virulence meets metabolism: Cra and KdpE gene regulation in enterohemorrhagic Escherichia coli. MBio *3*, e00280-00212.

Pacheco, A.R., Curtis, M.M., Ritchie, J.M., Munera, D., Waldor, M.K., Moreira, C.G., and Sperandio, V. (2012). Fucose sensing regulates bacterial intestinal colonization. Nature *492*, 113-117.

Paulsen, I.T., Banerjei, L., Myers, G.S., Nelson, K.E., Seshadri, R., Read, T.D., Fouts, D.E., Eisen, J.A., Gill, S.R., Heidelberg, J.F*., et al.* (2003). Role of mobile DNA in the evolution of vancomycin-resistant Enterococcus faecalis. Science *299*, 2071-2074.

Reading, N.C., Torres, A.G., Kendall, M.M., Hughes, D.T., Yamamoto, K., and Sperandio, V. (2007). A novel two-component signaling system that activates transcription of an enterohemorrhagic Escherichia coli effector involved in remodeling of host actin. J Bacteriol *189*, 2468-2476.

Sperandio, V., Torres, A.G., and Kaper, J.B. (2002). Quorum sensing Escherichia coli regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in E. coli. Mol Microbiol *43*, 809-821.