Supplementary Data

1. Supplementary Methods

1.1 Table S1: Bacterial strains and cell lines

Bacterial Strains	**Description**	**Source and Reference**
EHEC O157:H7 EDL933	*stx1a*; *stx2a*	[1]
EHEC/EAEC O104:H4 11-02027	*stx2a,* resistances: tetracyclines, streptomycin, trimethoprim/sulphonamides, ampicillin, nalidixic acid, cephalosporins	[2]
C. rodentium DBS770	stx_{2dact}-expressing *C. rodentium* DBS100 lysogenized with phage ϕ1720a-02	[3]
C600W34	*E. coli* C600 transduced with phage 933W from EHEC strain EDL933	[4]
C. rodentium stx2d::*Gluc* (MBK22)	DBS770 stx_{2dact}*::gluc, aphT*	This study
C600W34 *stx2a*::*Gluc* (JLG5)	C600 W34 stx_{2a}*::gluc, aphT*	This study
Cell lines	**Medium**	**Source**
VeroB4	OptiPRO SFM (Gibco) supplemented with 1x glutamine	DSMZ-AC33
LLC-PK1	Medium 199 (Biochrom) supplemented with 5% FBS (Sigma)	ATCC CL-101

1.2 Table S2: Plasmids and Oligonucleotides used in this study.

Plasmids	**Description/genotype**	**Reference**
pKD46	repA101(ts), oriR101, *bla*, P_{araB} (*gam*, *bet*, *exo*)	[5]
pMBK4	pSB377 *stx2aA::gluc, aphT*	This study
pSB377	*tetAB,* oriR6K	[6]
pWRG701	High-copy vector, colE1-replicon; carries Gaussia luciferase (glow kinetics) *GlucM43LM110L aphT* flanked by FRT sequences; ampicillin resistance	kind gift from Roman Gerlach
Oligonucleotides		
Designation	**Sequence (5‘-3‘)**	**Reference**
Stx2-Gluc fusion Fw	TTTTATATCTGCGCCGGGTCTGGTGCTGA TTACTTCAGCCAAAAGGAACACCTGTATA TGAAACCGACCGAAAACAACGA	This study
Stx2_Com fusion Rev	ATTAACAGAAGCTAATGCAAATAAAACCG CCATAAACATCTTCTTCATGCTTAACTCCT CGTGTAGGCTGGAGCTGCTTC	This study
Stx2A outside Fw	AGACGGTCAGGGAAGTTCAG	This study
Stx2B primer Rev	AATCCGGAGCCTGATTCACA	This study
DBS770 outside Rev	GCGCGGATCCTTCGAATAAA	This study
DBS Stx2B dn Rev	TAACAAATTCTCAGTCGGGCA	This study
Seq Com Rev	TCGGAATAGGAACTAAGGAGGA	This study
GA DBS Stx2 up Fw	GGCTGACATGGGAATTCCTGCAGCCCGG GGGATCCTCAGTCAGAACGGATGATATTG CAG	This study
GA DBS Stx2 up Rev	TTCGGTCGGTTTCATATACAGGTGTTCCT TTTGGCTGAAG	This study
GA DBS GlucKan Fw	AAGGAACACCTGTATATGAAACCGACCGA AAACAACGAAG	This study
GA DBS GlucKan Rev	AGCTAAGGAAGCTAACGTGTAGGCTGGA GCTGC	This study
GANewDBS Stx2B dn Fw	GCTCCAGCCTACACGCAGGAGTTAAATAT GAAGAAGATATTTGTAGCGG	This study
GA DBS Stx2B dn Rev	AAGCTCAATAAAAAGCCCCACCGCGGTG GCGGCCGCACCATGCAGGATTTTTTTTTT AACAAATTCTCAG	This study

1.3 Table S3: Antibiotics used in this study.

Stock solutions including solvent and supplier are listed for all antibiotics used in this study.

Antibiotic	stock solution	Supplier
Azithromycin	100 mg/ml in EtOH	Sigma Aldrich / Merck
Carbenicillin	3 mg/ml in H_2O	Carl Roth GmbH + Co. KG
Cefalexin	1 mg/ml in H_2O	Sigma Aldrich / Merck
Chloramphenicol	30 mg/ml in DMSO	Carl Roth GmbH + Co. KG
Ciprofloxacin hydrochloride	5 mg/ml in H_2O	Sigma Aldrich / Merck
Enrofloxacin	50 µg/ml in DMSO	Sigma Aldrich / Merck
Kanamycin	5 mg/ml in H_2O	Carl Roth GmbH + Co. KG
Meropenem	10 mg/ml in H_2O	Sigma Aldrich / Merck
Rifampicin	20 mg/ml in DMSO	SERVA Electrophoresis GmbH
Tetracycline	15 mg/ml in H_2O	SERVA Electrophoresis GmbH
Trimethoprim	5 mg/ml in DMSO	Sigma Aldrich / Merck
Tigecycline	100 mg/ml in H_2O	LKT Laboratories, Inc.
Vancomycin	100 mg/ml in H_2O	SERVA Electrophoresis GmbH

1.4 Generation of *Gaussia* luciferase reporter strains

All reporter strains were generated by λ-Red recombination as described (5). The strains C600W34 and *C. rodentium* DBS770 were transformed with pKD46 (5). The template plasmid pWRG701 was used to amplify reporter gene *Gaussia princeps luc* with M43L/M110L mutations (here: *Gluc*) and a kanamycin-cassette flanked by Flp/FRT sites (FRT *aphT* FRT). To create C600W34 ϕ*stx2a::Gluc*, a PCR product was amplified using primers Stx2-Gluc fusion Fw and Stx2_Com fusion *Rev* containing the reporter gene and the resistance cassette flanked by regions homologous to ~50-60 bp adjacent to the insertion site immediately upstream of *stx2aA* and *stx2aB*. All primer sequences are listed in supplementary **Table S2**. Correct insertion of the reporter was verified by PCR using the primers Stx2A outside Fw and Stx2B primer Rev and sequencing (additional oligonucleotide: Seq Com Rev). To construct the *C. rodentium* DBS770 reporter strain mutant, Gibson assembly

was used to create a template plasmid containing the reporter gene followed by the kanamycin resistance cassette and flanked by 400 bp regions homologous to the target region in DBS770. The oligonucleotides GA DBS Stx2 up Fw and GA DBS Stx2 up Rev were used to amplify the 400 bp region upstream of *stx2A* and the primers GANewDBS Stx2B dn Fw and GA DBS Stx2B dn Rev were used to amplify the region downstream of *stx2d*A. For both PCR reactions genomic DNA of DBS770 was used as a template. The *Gluc* gene and the Kanamycin cassette were amplified by PCR with the primers GA DBS GlucKan Fw and GANewDBS Stx2 Rev with a linearized pWRG701 as the template. The pSB377 backbone was cut via *BamH*I and *Not*I. Gibson assembly was performed with the three purified PCR products and the cut vector using the Gibson assembly master mix (NEB) following the manufacturer's instructions. The resulting plasmid (pMBK4) was linearized and used for λ-Red recombination. Correct insertion of the reporter was verified by PCR using the primers Stx2A outside Fw and DBS770 outside Rev and sequenced (additional primer: Seq Com Rev).

1.5 Serology

Upon necropsy, blood was taken by cardiac puncture and serum was prepared by centrifugation and separation in Microvette® 500 Z-Gel tubes (Sarstedt) and frozen for serology. Blood urea nitrogen (BUN) was measured using a Fuji DRI-CHEM NX500 automatic dry-chemistry analyzer (Fujifilm Corporation).

1.6 Histology and pathological evaluation

Colon and kidneys were removed and fixed in Roti-Histofix (Roth) for 24 hours

and stored in 70% ethanol until further use. Samples were embedded in paraffin and 3 µm thick sections were stained with hematoxylin-eosin according to standard laboratory procedures. Sections were analyzed randomized and blinded to the experimental groups. Tubulus necrosis was graded as follows: 1 = sporadic tubulus necrosis or dilation, 2 = 30-50% of the tubuli show necrosis, 3 = more than 50% of the tubuli show necrosis. The colon was scored by 5 markers as follows: inflammation: 1 = few inflammatory cells in lamina propria, 2 = clearly visible inflammatory cells reaching submucosa, 3 = transmural invasion of inflammatory cells. Epithelial erosion: 1 = sporadic erosion of epithelial cells, 2 = clearly visible erosion of epithelial cells, with moderate amounts of sloughed cells in lumen, 3 = thinning of crypt walls and large amounts of sloughed cells in lumen. Goblet cell loss: 1 = slightly reduced number of goblet cells, 2 = moderate loss of goblet cells with sporadic increase in size, 3 = severe loss of goblet cells with marked increase in size of goblet cells. Epithelial hyperplasia: 1 = up to 100% increase in thickness, 2 = more than 100% increase in thickness, 3= more than 100% increase in thickness and altered morphology. Area involved: 1= up to 30%, 2= 40-70%, 3= more than 70%.

1.7 References:

1. Perna NT, Plunkett G , Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Pósfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA, Blattner FR. Genome sequence of

enterohaemorrhagic *Escherichia coli* O157:H7. Nature. 2001; 409: 529-533.

2. Tietze E, Dabrowski PW, Prager R, et al. Comparative genomic analysis of two novel sporadic Shiga toxin-producing *Escherichia coli* O104:H4 strains isolated 2011 in Germany. PLoS ONE. 2015; 10(4): e0122074.

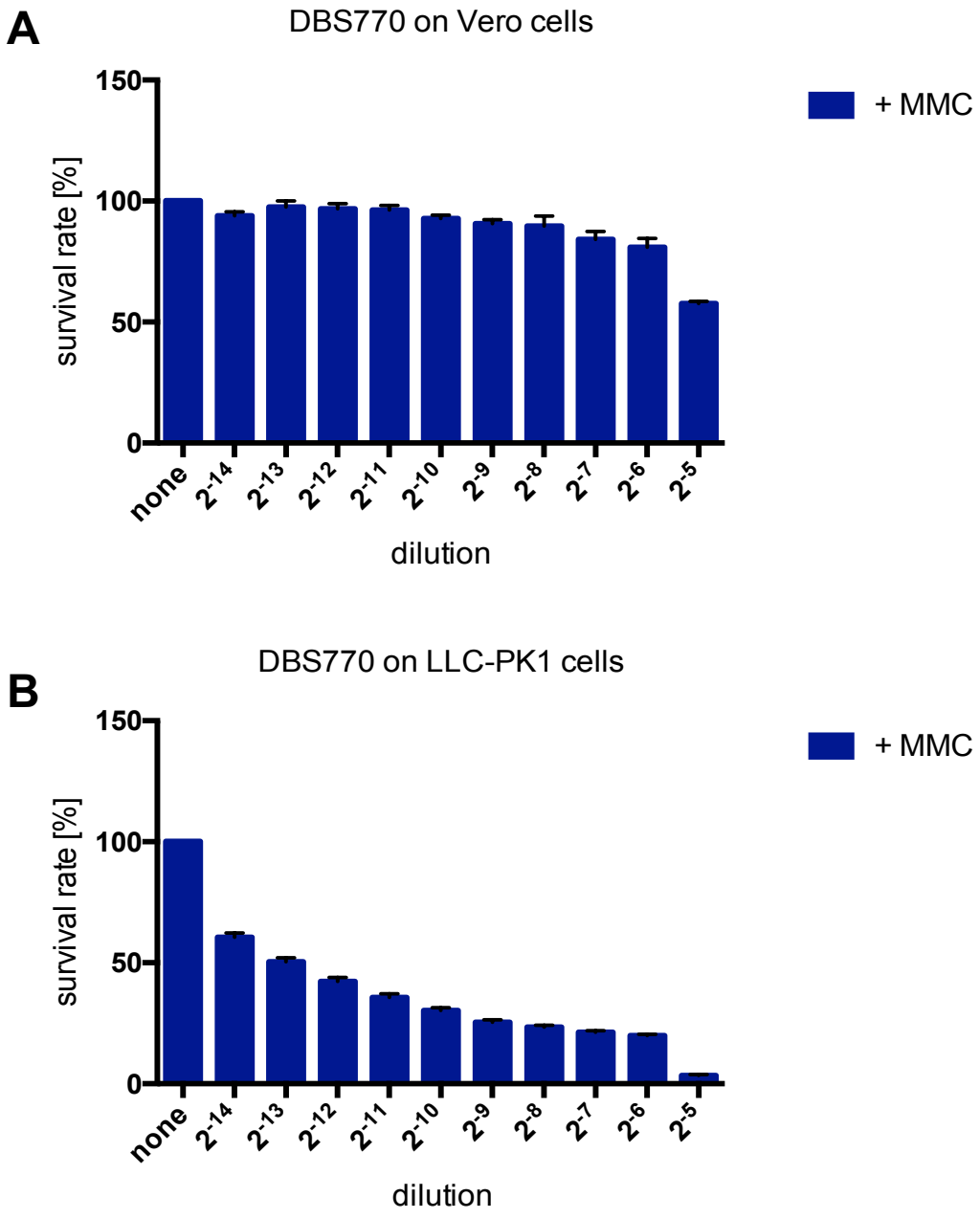
3. Mallick EM, McBee ME, Vanguri VK, et al. A novel murine infection model for Shiga toxin-producing *Escherichia coli*. J Clin Invest. 2012; 122(11):4012–24.

4. O'Brien, Alison D., et al. "Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea." Science. 1984; 226.4675: 694-696.

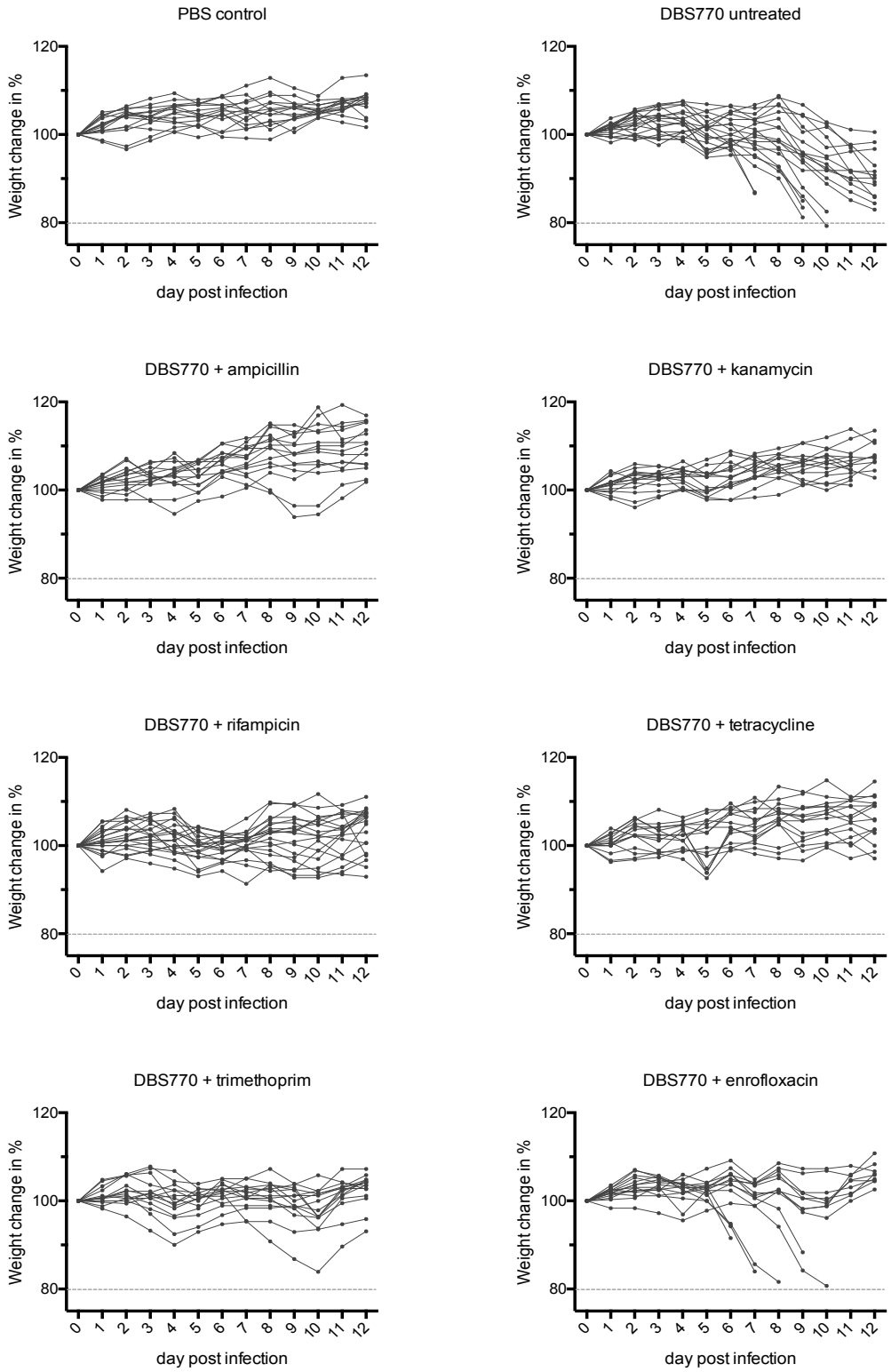
5. Datsenko KA, Wanner B. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA 2000; 97:6640-6645.

6. Mirold, S., W. Rabsch, M. Rohde, S. Stender, H. Tschape, H. Russmann, E. Igwe, and W. D. Hardt. Isolation of a temperate bacteriophage encoding the type III effector protein SopE from an epidemic *Salmonella typhimurium* strain. Proc. Natl. Acad. Sci. USA 1999; 96:9840-9850.

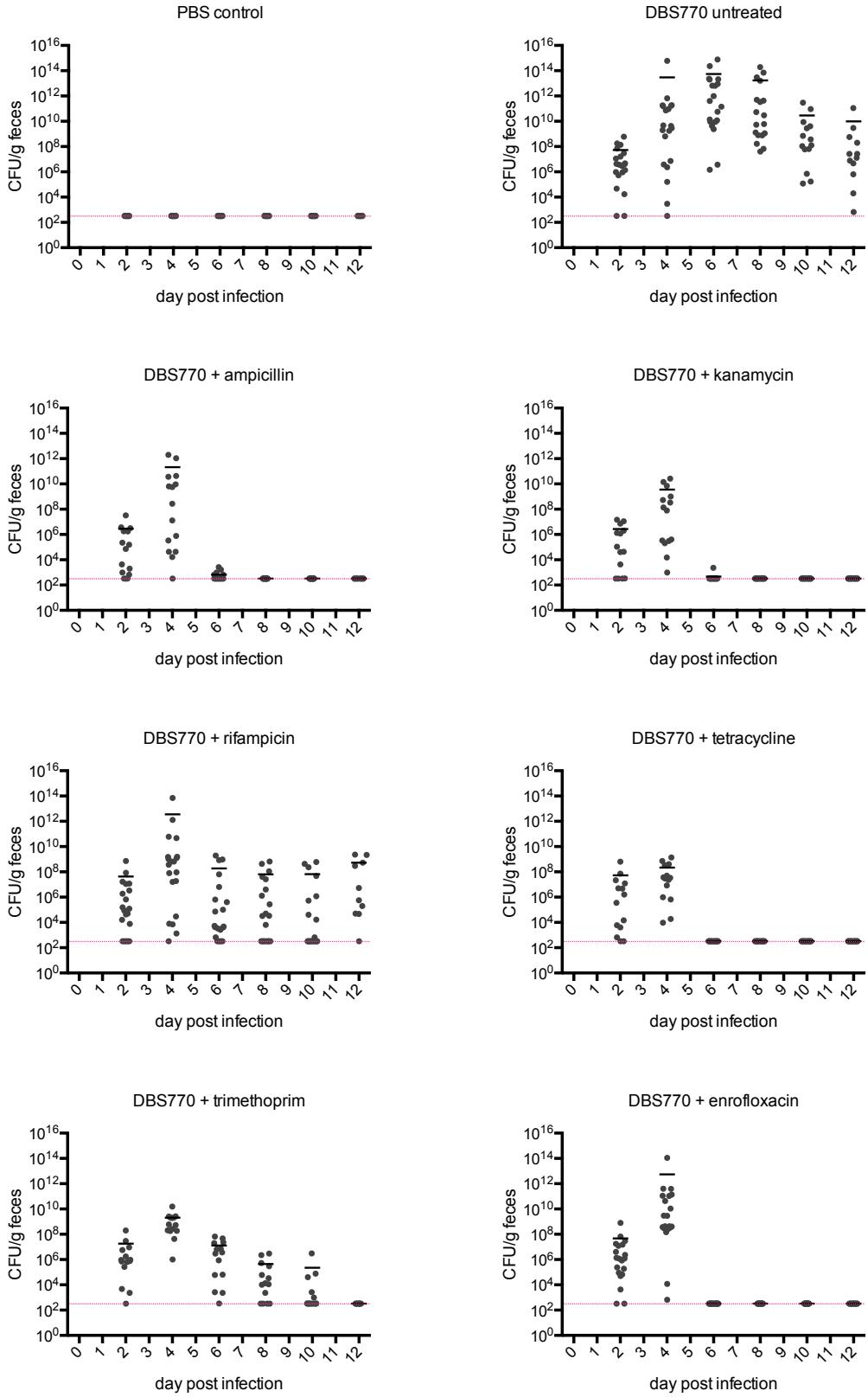
2. Supplementary Figures



Supplementary Figure 1. Differences in cytotoxic activity of *C. rodentium* ϕ*stx*$_{2dact}$ supernatants on VeroB4 and LLC-PK1 cells.
Dilutions of bacteria-free *C. rodentium* ϕ*stx*$_{2dact}$ supernatants were added to VeroB4 cells (**A**) or LLC-PK1 cells (**B**). After incubation for 72 hours, XTT solution was added to PBS-washed cells for 2 hours. 0.01% Triton-X100 treated cells were used as positive (dead) control, while untreated cells were used as negative (live) control. After incubation, the absorbance of the samples was determined at 475 nm and the cell viability of untreated cells set to 100%. Shown are the mean values (±SEM) of three independent experiments performed in quadruplicates compared to untreated control. Statistical analysis was performed via unpaired Mann-Whitney tests (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



Supplementary Figure 2. Influence of antibiotic treatment of *C. rodentium* ϕ*stx*$_{2dact}$-infected mice on the weight of individual mice.
Weight of each mouse is given for every study group (uninfected, untreated and antibiotics-treated mice). The weight of each mice at day 0 was set to 100%. The dashed line at 80% indicates the official termination point.



Supplementary Figure 3. Influence of antibiotic treatment of *C. rodentium* ϕ*stx*$_{2dact}$-infected mice on the bacterial colonization in individual mice.
The colonization with *C. rodentium* ϕ*stx*$_{2dact}$ of each mouse on alternate days is given for every study group (uninfected, untreated and antibiotics-treated mice). Colonization was determined by feces count. Each dot represents one mouse, the means are indicated. The dotted line denotes the detection limit of the analysis.