Supplementary Tables

Supplemental Table 1. Strains used in this study.

Strain	Description and relevant phenotype	Reference
DBS100	*Citrobacter rodentium* wild type strain (prototype TMCH isolate, ATTC 51459, original biotype 4280)	(1, 2)
DBS132	DBS100/pTROY09,Nal^R	(3, 4)
EC1720a	EC1720a containing Φ1720a-01 and Φ1720a-02.	(5)
JB-13-06	EC1720a containing Φ1720a-02 Δ*Rz*::*cat*, Cm^R	This study
JB-13-28	Φ1720a Δ*Rz*::*cat* Δstx_{2dact}*AB*::*kan*, Cm^R, Km^R	This study
DBS770	DBS100 lysogenized with Φ1720a-02 Δ*Rz*::*cat*, Cm^R	This study
DBS771	DBS100 lysogenized with Φ 1720a-02 Δ*Rz*::*cat* Δstx_{2dact}*AB*::*kan*, Cm^R, Km^R	This study
EDL933	EHEC O157:H7 strain encoding stx_1 and stx_2	(6)
TUV93-0	EDL933 cured of Stx1 and 2-producing phages	(7)
B2F1	O91:H21 serotype containing 2 copies of Stx2d	(8)
EH250	EHEC strain containing non-activatable Stx2d toxin	(9)

Nal^R, nalidixic acid resistant
Cm^R, chloramphenicol resistant
Km^R, kanamycin resistant

Supplemental Table 2. Plasmids used in this study.

Plasmids	Description	Reference
pTROY9	expresses LamB, Nal^R, Tet^R	(3, 4)
pKD46	Expresses lambda Red recombinase; Amp^R	(10)
pK184	Cloning vector, Km^R	(11)
pMB46	pK184 containing EPEC *tir* promoter - EHEC *tir* - EHEC *cesT*, Km^R in the KpnI and HindIII sites.	This study
pEM123	pMB46ΔEHEC *tir - cesT* with new restriction sites (MluI and SacII), Km^R	This study
pEM129 (pTir)	pEM123 containing *C. rodentium tir – cesT*, Km^R	This study
pDONRzeo	Contains zeocin gene	Invitrogen
pBJK5	pUC-based plasmid containing *cat* cassette flanked by 707 bp upstream and 692 bp downstream of *Rz* in EC1720a in the SphI and SacI site; Cm^R	This study
pBR328	Cloning vector; Amp^R	(12)
pUC18K	Cloning vector; Km^R	(13)

Nal^R, nalidixic acid resistant
Tet^R, tetracycline resistant
Amp^R, ampicillin resistant
Km^R, kanamycin resistant
Cm^R, chloramphenicol resistant

Supplemental Table 3. Oligonucleotide sequences used in this study.

Primer	Nucleotide sequence (from 5' to 3')[a]
PanHandle PCR	
F-Adaptor1	CTA ATA CGA CTC ACT ATA GGG CTC GAG CGG CCG CCC GGG CAG GT
R-Adaptor2	/5Phos/ACCTGCCCGG/3AmMC7/
F-AP1 primer	GGA TCC TAA TAC GAC TCA CTA TAG GG
R-AP2 primer	AAT AGG GCT CGA GCG GC
Sequencing of Φ1720a-02 (λ*stx*$_{2dact}$)	
R-Oli210	TGT ACC GTT CAT GCA TGG TG
F-Oli218	ACT GTC TGA AAC TGC TCC TGT G
R-Oli219	CAA ATC CTG AAC CTG ACG CAC AGG
R-Oli220	TTC GGA TGG TTA AGG CGG CT
F-Oli221	CGC TTC AGG CAG ATA CAG AG
F-Oli222	ACG GAC AGA GAT ATC GAC CC
R-Oli223	CTG TAA CTA CAT TGC TGC AC
F-Oli224	ATG AAG TGT ATA TTA TTT AAA TGG GTA CTG TGC CTG
F-Oli226	GGT GCT GAT TAC TTC AGC CAA
F-Oli228	GAA TCC AGT ACA ACG CGC CAC A
Antibiotic marking of Φ1720a-02	
F-425	AAAAAAGCATGCCTGCTGACATATCTGACGAAC
R-426	AAAAAAGGATCCTTTGTAGGTGATGGCGTTATC
R-427	AAAAAAGAGCTCATATCAATAACTTACACTGGT
F-259	AAAAAAGGATCCGCGCAGCGCGATAAAAAAGCC
F-265	AAAAAGGATCCTTCGAATAAATACCTGTGACGGAAGATCAC
R-266	AAAAAGGATCCTTCGAATTTCTGCCATTCATCCGCTTATTA
Stx-deletion within Φ1720a-02Δ*Rz*::*cat*	
F-2dH1	CGT CAC TCA CTG GTT TCA TCA TAT CTG GCG TCC CGG GTG ACT AAC TAG GAG GAA TAA ATG
F-498	GGC GCG TTT TGA CCA TTT CGT TTG ATT
R-2dH2	TGA TTT GAT TGT TAC CGT CAT TCC TGT TAA TCC CCG GGT CAT TAT TCC CTC CAG GTA CTA
R-2dH1AP	CAT TTA TTC CTC CTA GTT AGT CAC CCG GGA CGC CAG ATA TGA TGA AAC CAG TGA GTG ACG
F-2dH2AP	TAG TAC CTG GAG GGA ATA ATG ACC CGG GGA TTA ACA GGA ATG ACG GTA ACA ATC AAA TCA
R-499	CTG TGA CGC TGA TAT GCC CCG CCG CTC
F-316	CTC AGG GGA CCA CAT CGG
R-398	GCC GCC CTG ACC ACA TCG
Generation of pTir	
F-MluI-Cr-tirU	CAT CAT ACG CGT ATG CCT ATT GGT AAT CTT GGT AAT AAT AAT ATA AGT AAC
R-SacII-Cr-tirL	CAT CAT CCG CGG GTT ATA GGC TCC ACC ACA ATG AGT TAG AAT GAG TAG TAA

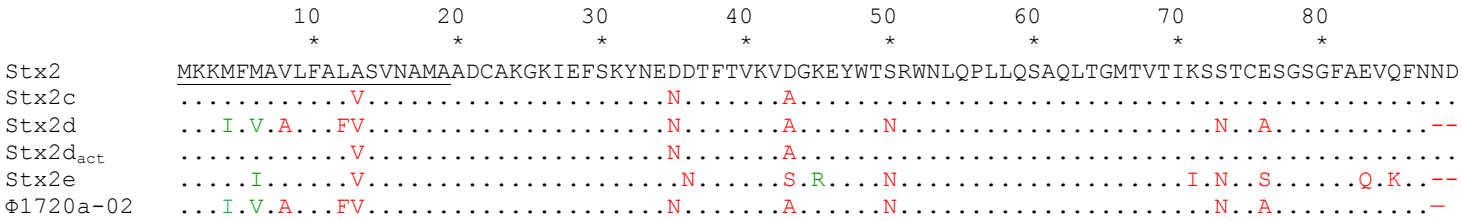
[a]F, forward (top strand) primer; R, reverse (bottom strand) primer. Restriction sites are underlined.
[b]Primers used to construct *C. rodentium*Δ*tir*. Italicized regions indicate homology to zeocin cassette. Numbers in primer name correspond to nucleotide positions primer is located within *tir* gene. Int represents screening primers internal to the gene (*tir* or *zeocin*) and Ext represents screening primers that are external to the gene.

Supplementary Figures and Figure Legends

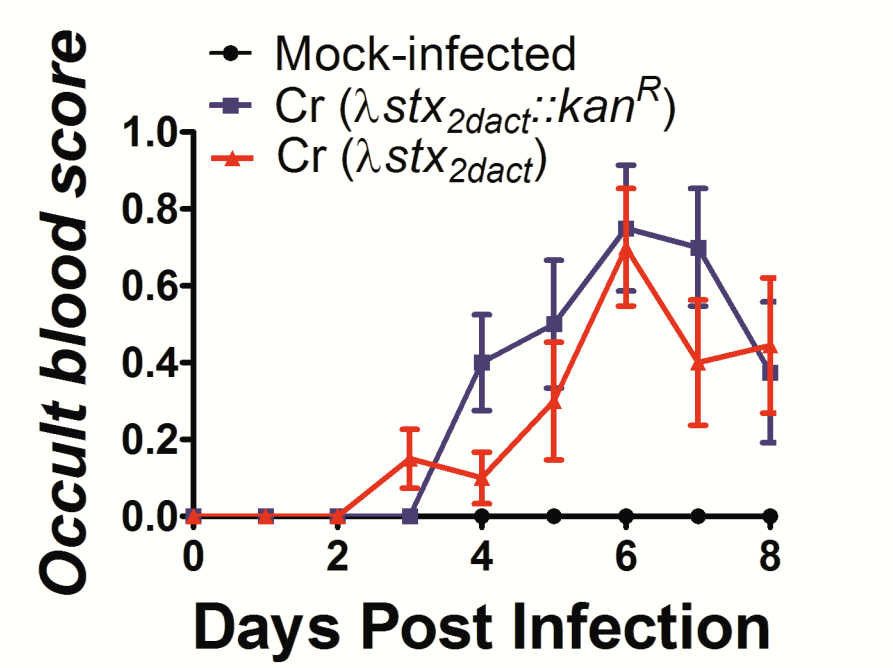
A

```
                10        20        30        40        50        60        70        80        90        100
                 *         *         *         *         *         *         *         *         *         *
Stx2            MKCILFKWVLCLLLGFSSVSYSREFTIDFSTQQSYVSSLNSIRTEISTPLEHISQGTTSVSVINHTPPGSYFAVDIRGLDVYQARFDHLRLIIEQNNLYV
Stx2c           ..........................................................................................
Stx2d           .....L.....................................C..............................................
Stx2d_act       ..........................................................................................
Stx2e           .....L..I.............Q.....................A...........A..............IS.G........E..........R.....
Φ1729a-02       ........................M.................................................................

                110       120       130       140       150       160       170       180       190       200
                 *         *         *         *         *         *         *         *         *         *
Stx2            AGFVNTATNTFYRFSDFTHISVPGVTTVSMTTDSSYTTLQRVAALERSGMQISRHSLVSSYLALMEFSGNTMTRDASRAVLRFVTVTAEALRFRQIQREF
Stx2c           ..........................................................................................
Stx2d           ................A.........................................................A.............
Stx2d_act       ..........................................................................................
Stx2e           ......T.........A...L.....I................................................................
Φ1729a-02       ..........................................................................................

                210       220       230       240       250       260       270       280       290       300
                 *         *         *         *         *         *         *         *         *         *
Stx2            RQALSETAPVYTMTPGDVDLTLNWGRISNVLPEYRGEDGVRVGRISFNNISAILGTVAVILNCHHQGARSVRAVNEESQPECQITGDRPVIKINNTLWES
Stx2c           ..........................................................................................
Stx2d           .L............EE..............F...G.................................S....I............RL.......
Stx2d_act       ..........................................................................................
Stx2e           .L............E.....................A.....................................................
Φ1729a-02       ..............EE..............F.....................................................

                310
                 *
Stx2            NTAAAFLNRKSQFLYTTGK
Stx2c           ...................
Stx2d           .........RAHS.N.S.E
Stx2d_act       ............S.....E
Stx2e           ............S.....E
Φ1729a-02       .........RAHS.N.S.E
```

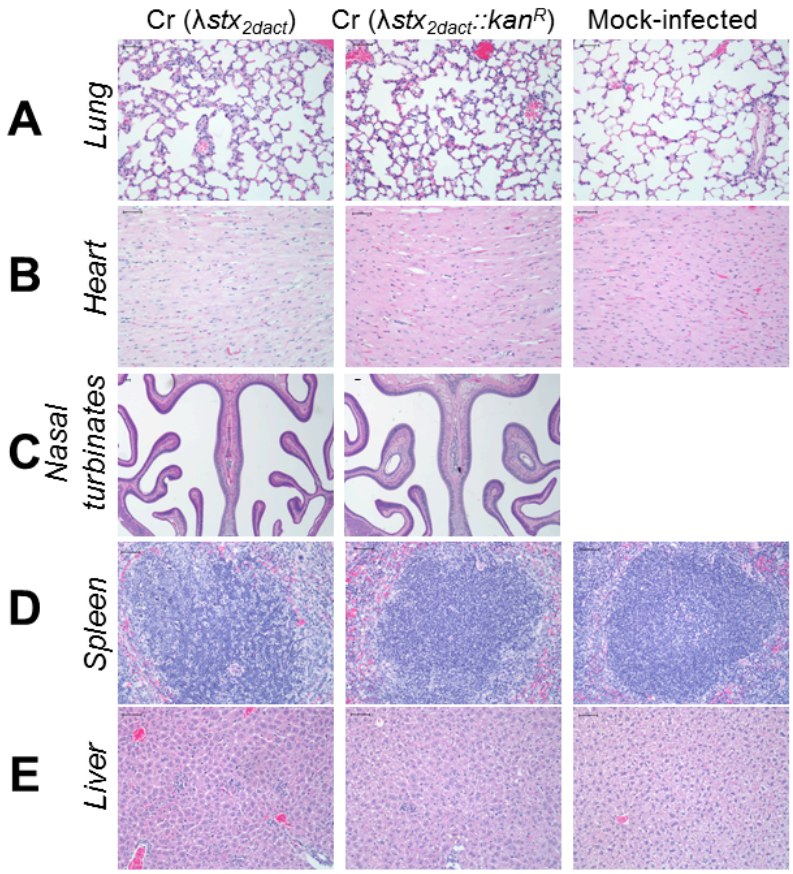
B



Supplementary Figure 1. Amino acid alignment of Stx A and B subunits of ϕ1720a-02 from *C. rodentium* (λ*stx*$_{2dact}$) with other Stx A and B subunits from various STEC strains. The A **(Panel A)** or B **(Panel B)** subunits of Φ1720a-02 (for simplicity termed "λ*stx*$_{2dact}$" in this study) was aligned with the corresponding subunits of Stx2 from EDL933 (Genbank accession number X078655), Stx2c from E32511 (Genbank accession number M59432), Stx2d from EH250 (Genbank accession number AF043627), Stx2dact (formerly called Stx2vhb) from B2F1 (Genbank accession number AF479829), and Stx2e from S1191 (Genbank accession number M21534). The sequences were aligned using the Align Plus 5 program, version 5.03 (Scientific & Educational Software) following the global-ref alignment procedure (14) and the scoring matrix BLOSUM 62 (15). The underlined regions indicate the processed signal sequence, dots depict identical residues, green or red letters highlight conserved or non-conserved residues respectively, and a red dash indicates a gap in the alignment.



Supplementary Figure 2. Infection by *C. rodentium* (λ*stx*$_{2dact}$) and *C. rodentium* (λ*stx*$_{2dact}$*::kan*^{R}) results in the presence of fecal occult blood. Fecal samples were collected from mice daily throughout infection and assessed for the presence of occult blood. Occult blood scores were assigned as follows: 0=no occult blood, 0.5=trace amounts of occult blood, and 1=presence of occult blood. Data are representative of the average fecal occult blood scores (±SEM) of groups of ten mice infected with either *C. rodentium* (λ*stx*$_{2dact}$) or *C. rodentium* (λ*stx*$_{2dact}$*::kan*^{R}) or mock-infected.



Supplementary Figure 3. Infection by *C. rodentium* (λ*stx*$_{2dact}$) does not result in significant histological damage to the lung, heart, nasal turbinates, spleen or liver. Tissue sections of the lung **(A)**, heart **(B)**, nasal turbinates **(C)**, spleen **(D)**, and liver **(E)** were taken from mock-infected mice or mice infected with *C. rodentium* (λ*stx*$_{2dact}$) or *C. rodentium* (λ*stx*$_{2dact}$*::kan*^{R}) at seven days post-infection, stained with H&E, and analyzed histologically by a board certified pathologist. Magnification is 200X for all samples except for nasal turbinates for which the image was taken at 40X magnification. For spleen, heart, lung, and liver samples, data are representative of one mock-infected mouse, four mice infected with *C. rodentium* (λ*stx*$_{2dact}$) and two mice infected with *C. rodentium* (λ*stx*$_{2dact}$*::kan*^{R}). For nasal turbinate samples, data are representative of five mice infected with *C. rodentium* (λ*stx*$_{2dact}$) and five mice infected with *C. rodentium* (λ*stx*$_{2dact}$*::kan*^{R}). Scale bars measure 50 μm.

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