

**Table S1, related to Star Methods (Strain and culture conditions) Bacterial Strains. Details of construction of strains can be found in full methods for strains constructed in this work, or in the respective cited reference.**

Strain	Genotype
86-24	Stx2A+ EHEC strain serotype O157:H7
EDL933	Stx2A+-Stx1 EHEC strain serotype O157:H7
Sakai	Stx2A+-Stx1 EHEC strain serotype O157:H7
JHD01	86-24 phage <i>N</i> antiterminator isogenic mutant
JHD02	86-24 phage <i>Q</i> antiterminator isogenic mutant
JHD03	86-24 phage <i>SR</i> holins isogenic double mutant
JHD04	86-24 phage <i>cro</i> regulatory protein isogenic mutant
JHD05	86-24 <i>espA</i> translocon isogenic mutant
JHD06	86-24 $\Delta N$ - complemented pJHD13
JHD07	86-24 $\Delta Q$ - complemented pJHD14
JHD08	86-24 $\Delta\Delta SR$ - complemented pJHD15
JHD09	86-24 $\Delta cro$ -complemented pJHD16
JHD10	86-24 <i>cro</i> -pet21a in BL21
JHD11	Sakaic <i>cro</i> -pet21a in BL21
JHD12	EDL933 <i>cro</i> -pet21a in BL21
JHD13	86-24 $\Delta cro$ complemented pJHD020
JHD14	86-24 $\Delta cro$ complemented pJHD021
JHD15	EDL933 phage <i>cro</i> -stx2 isogenic mutant
JHD16	EDL933 phage <i>cro</i> -stx1 isogenic mutant
JHD17	EDL933 phage <i>cro</i> -cryptic isogenic mutant
JHD18	EDL933 phage- <i>cro</i> -stx2-stx1 double mutant
JHD19	EDL933 phage- <i>cro</i> -stx2-cryptic double mutant
JHD20	EDL933 phage- <i>cro</i> -stx2-sxt1-cryptic triple mutant
JHD21	Sakai phage <i>cro</i> -stx2 isogenic mutant
JHD22	Sakai phage <i>cro</i> -stx1 isogenic mutant
JHD23	Sakai phage <i>cro</i> -cryptic isogenic mutant
JHD24	Sakai phage- <i>cro</i> -stx2-stx1 double mutant
JHD25	Sakai phage- <i>cro</i> -stx2-cryptic double mutant

JHD26	Sakai phage- <i>cro</i> - <i>stx2</i> - <i>sxt1</i> -cryptic triple mutant
BL21	F- ompT hsdSB(rB-, mB-) gal dcm (DE3)
TOPO10	Host <i>E. coli</i> strain for protein expression
<i>E. coli</i> W3350	<i>galk2(Oc) galT22 IN(rrnD-rrnE)1</i>
<i>Citrobacter rodentium</i>	WT murine pathogen
DBS100	
<i>Citrobacter rodentium</i> C.r-j11	<i>C. rodentium</i> with EHEC <i>ler</i> promoter region
<i>Citrobacter rodentium</i> -C.r-j11- <i>pcro</i>	<i>C.rodentium</i> C.r-j11 complemented pJHD016

**Table S2, related to Star Methods. Oligonucleotides used in this study.**

	Primers used for mutagenesis and cloning
N-86-24red-F	CACCACCAAAGTTCATCAGGAGGTCTATATGACACGCAGAACTCAGTT CAGTGTAGGCTGGAGCTGCTTC
N-86-24red-R	TCACCTCAAATAAGTGGTTTGTCTGCCTAATTTCAATTTCTGGCGACCAA CCCATATGAATATCCTCCTT
Ncomp-F	GTTTCTGCGGCCGCATCAGGAGGTCTATATGACA clone <i>N</i> antiterminator in PWSK129
Ncomp-R	GTTTCTGGATCCTCATTCTGGCGACCAAC clone <i>N</i> antiterminator in PWSK129
Q-86-24red-F	AAGGGAAAAGACACGCTATCGTCAACGGTGTCTTATGGTTCACCGCG GATGTGTAGGCTGGAGCTGCTTC
Q-86-24red-R	TCGACTGCGTGGCAATGTAACCACTTTATCATGATATGCAGATTTTTTA CGCCATATGAATATCCTCCTT
Qcomp-F	GTTTCTGCGGCCGCACGCTATCGTCAACG clone <i>Q</i> antiterminator in PWSK129
Qcomp-R	GTTTCTGGATCCCATGATATGCAGATTTTTACGATC clone <i>Q</i> antiterminator in PWSK129
SR-86-24red-F	ACGGGGAGTCAGGGCCATCAGTAAACAGCTGCTGGCCTTTTTCATGTT GTGTGTAGGCTGGAGCTGCTTC
SR-86-24red-R	TTATCTGTCGATTCCCAGCACGCCAGCGCGCTCTCCTGGTCACGACG GGATCCATATGAATATCCTCCTT
SR-lysis-comp-F	GTTTCTGCGGCCGCATCAGTAAACAGCTGCT clone <i>SR</i> lysis in PWSK129
SR-lysis-comp-R	GTTTCTGGATCCTTATCTGTCGATTCCCAGC clone <i>SR</i> lysis in PWSK129
Cro-86-24red-F	TGCGGATGATTTATCAAATGGTAAAGTTGTTCTGTGTGATAAACGGAG GCAGTGTAGGCTGGAGCTGCTTC
Cro-86-24red-R	TCCATTGTGAAAATAGCGGTGTTACTTATGCAGCCGATGCTCTACGCG ATACCATATGAATATCCTCCTT
Cro-comp-F	GTTTCTGCGGCCGCCTGTGTGATAAACGGAGGCA clone regulatory <i>cro</i> in PWSK129
Cro-comp-R	GTTTCTGGATCCTCCATTGTGAAAATAGCGGTG clone regulatory <i>cro</i> in PWSK129
Cro-pet21a-F	GTTTCTGGATCCATGAGTAATGAACTACTACGC clone 86-24- <i>cro</i> in PET21a
Cro-pet21a-R	GTTTCTGCGGCCGCTGCAGCCGATGCTCTACG clone 86-24- <i>cro</i> in PET21a
CroSak-pet21F	GTTTCTGGATCCATGAGCAACCTACGAAAATATC clone Sakai- <i>cro</i> in PET21a
CroSak-pet21R	GTTTCTGCGGCCGCAGCGGCTTTGTGCTCCG clone Sakai- <i>cro</i> in PET21a
CroEdl-pet21F	GTTTCTGGATCCATGCAAATCTTGATGAGCCG clone EDL933- <i>cro</i> in PET21a
CroEdl-pet21R	GTTTCTGCGGCCGCTGCAGCCAGAAGTTCTTTT clone EDL933- <i>cro</i> in PET21a
CroSak-comF	GTTTCTGCGGCCGCGTGGTTACTATGGAGGGCAT clone VT-Stx2- <i>cro</i> in pWSK129
CroSak-comR	GTTTCTGGATCCATTAAGCGGCTTTGTGCTCC clone VT-Stx2- <i>cro</i> in pWSK129
CroEdl-comF	GTTTCTGCGGCCGCTACTGAAAGTACGAAAAGGATA clone BP933W- <i>cro</i> in pWSK129
CroEdl-comR	GTTTCTGGATCCATAGCGGTGTTACTTATGCAG clone BP933W- <i>cro</i> in

pWSK129

EspA-86-24red-F	TTATTTACCAAGGGATATTGCTGAAATAGTTCTATATTGTAGAGATTGC GTGTAGGCTGGAGCTGCTTC
EspA-86-24red-R	ATGGATACATCAAATGCAACATCCGTTGTTAATGTGAGTGCGAGTTCTT CCCATATGAATATCCTCCTT
Cro-stx2edl-F	GTAAGTAAAGTACGAAAAGGATATTCCTATGCAAAATCTTGATGAGCC GTGTAGGCTGGAGCTGCTTC
Cro-stx2edl-R	CCATTGTGAAAATAGCGGTGTTACTTATGCAGCCAGAAGGTTCTTTTTG CCCATATGAATATCCTCCTT
Cro-stx1edl-F	TTAGAGGTGAAAGGCCATGATGAAAGAGCGGGTACTGCTTAGGCGGC TTGTGTAGGCTGGAGCTGCTTC
Cro-stx1edl-R	GGGCAATTTTGTTCATTGCGGTCTCCTTAGCATTAAATCACACACCCATT ACCATATGAATATCCTCCTT
Cro-crypticedl-F	GGTTGCATGTACTAAGGAGTTGTATGGAACAACGCATAACCCTGAAA GGTGTAGGCTGGAGCTGCTTC
Cro-crypticedl-R	TAAGAGCGGGGTTATTTATGCTGTTGTTTTTTTGTACTCGGGAAGGGC TCCATATGAATATCCTCCTT
Cro-stx2Sak-F	TTCGTGGTTACTATGGAGGGCATATGAGCAACCTACGAAAATATCGAG AGGTGTAGGCTGGAGCTGCTTC
Cro-stx2Sak-R	CGCTTATTAAGCGGCTTTGTGCTCCGGCGGGAACACGTCATCAAGACT TACCATATGAATATCCTCCTT
Cro-stx1Sak-F	CCCAATGGATTTGCCGCTGATGTTTGTCTACCCGGTTAGAGGTGAAAG GCGTGTAGGCTGGAGCTGCTTC
Cro-stx1Sak-R	GAGACCGCAATGAACAAAATTGCCAGCAGCGAAAAAAAATCGGAGTT TCGCCATATGAATATCCTCCTT
Cro-crypticSak-F	GGTGATAATGGTTGCATGTACTAAGGAGTTGTATGGAACAACGCATA ACGTGTAGGCTGGAGCTGCTTC
Cro-crypticSak-R	TGTAAGAGCGGGGTTATTTATGCTGTTGTTTTTTTGTACTCGGGAAGG GCCATATGAATATCCTCCTT
CroStx1edlF	TTAGAGGTGAAAGGCCATGA
CroStx1edlR	TAATGGGTGTGTGATTAATGCT
CrocrypdlF	GTTGCATGTACTAAGGAGGT
CrocrypdlR	TAAGAGCGGGGTTATTTATGC
CroStx1SakF	CCCAATGGATTTGCCGCT
CroStx1SakR	GAGACCGCAATGAACAAAATTG
CrocrypSakF	GGTGATAATGGTTGCATGTACT
CrocrypSakR	TGTAAGAGCGGGGTTATTTATG
CRlerF	GAGCTCGGTACCCGGGGATCTTAAATGTTATTCAGAGATGTTAC
CRlerR	GATTAATTGTTGGTCCTTCCT
EHlerPF	CCTCATGCTTTAATATTTTAAGCTA
EHlerPRev	GTATAGGAACCTTCGAAGCAGCTCCAGCCTACACAGTATCATATAGCAT CATATAGTGT
CMf	TGTGTAGGCTGGAGCTGCTTC
CMr	ATCGGGTACGCGATCTGTTGCCCTGGAAAGTTGATCTGAGCTCTCAGT AACCATATGAATATCCTCCTTAGT
Tris66F	TACTGAGAGCTCAGATCAAC
Tris66R	CGACCTGCAGGCATGCAAGCATGGACACCTCACTTGCTC
FlaRzF	TGCAAAAACAACCTGGAAGGAACCCAGAAGTATATTAATGAGCAGTGCA GAGACTACAAAGACCATGACGG
FlagRzR	CGCTGGAAGCGCGGGTGTATTGCTCACAAATAATTGCATGAGTTGCCCA TCCATATGAATATCCTCCTTAG
RzBorF	ATGAACCGTGTTCTGTGTGTG
RzBorR	GCTGGCCCTGCTTATTACAG

LamCroF	ATGTAATAAGGAGGTTGTATG
LamCroR	TGCATACACCATAGGTGTG
Stx2AF	ATGAAGTGTATATTATTTAAATGGG
Stx2AR	TTTACCCGTTGTATATAAAAACTG

Oligonucleotides used for Real Time PCR

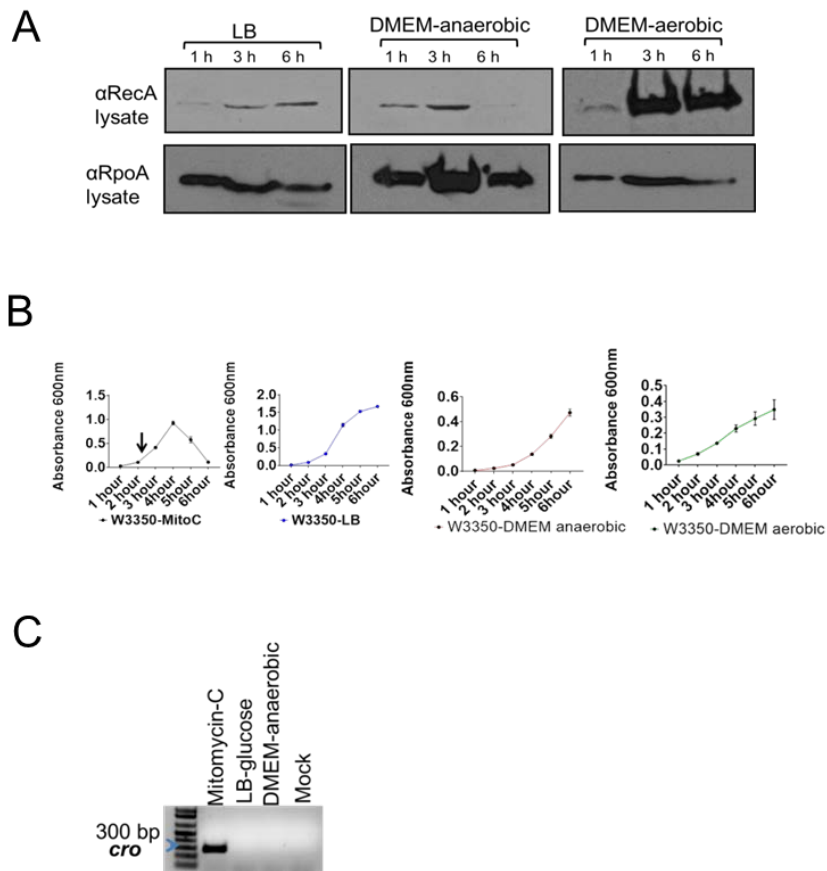
Ler-F	CGACCAGGTCTGCCCTTCT
Ler-R	GCGCGGAACCTCATCGAAA
Tir-F	CCATGGAGAGCAGACGTAGCT
Tir-R	CGGTGATCCTGGATTTAACCTT
sepL-F	GCCTGGGATTTCGAAAGGT
sepL-R	CTCTTGCATATCATTGAGCAGCTT
espA-F	TCAGAATCGCAGCCTGAAAA
espA-R	CGAAGGATGAGGTGGTTAAGCT
rpoA-F	GCGCTCATCTTCTTCCGAAT
rpoA-R	GCGGGTGGTGGTTATGTG
cro-86-24F	GTGAGGAATGGAAGCGACTC
cro-86-24R	TGGTCAAGATAGCCAAGTGAAG
cl-86-24F	CGTGAGTTGAGAAGGGCTAAC
cl-86-24R	ATCGCGGGTAACACTAATGC
dinIF	GTAATGATACGTGGCCTTCA
dinIR	GAACTTTCCCGCCGATTCA
fliC-F	GTCATCCTTCGCGCTGTTAA
fliC-R	TCTGCGCTGTCGAGTTCTAT
NantiF	GTAGGGCGGTGTAATACTTC
NantiR	ATTAGCTAACGGCGTACTGG
phnB-F	CTGTCCCTGCGCTGATTTTG
phnB-R	GGCGCTGAACTGCTCTATAA
proPF	ACCGCTTGGCGGACTCTT
proPR	TCTTCTGGCGACCATATTTATC
rncF	GCTCAGAATAGAGTCGCCTA
rncR	CAGCAGGCATTAACCTCATCG
trpDF	GCGCGGTAAGCTGCCTAT
trpDR	GTAAGCTTCGACAATCGCCT
wcaMF	GTGATATTCGCCACGACAAAAG
wcaMR	GGAAGCACTTACGATAATAATTAC
Cr-rpoA-F	ACGTCAGCCGGAAGTAAAGAAGA
Cr-rpoA-R	AGCGGACAGTCAATTCCAGATCGT
Cr-espA-F	ACGAGGTAACAACCATGCGAGTGT
Cr-espA-R	CTGCCTGGCATTGCTTTCCAGAAT

Oligonucleotides used for EMSAS

Ler-173 F	CGGGATCCCGATGATTTTCTTCTATATCATTG
Ler-42 R	CGGAATTCGCGACCTTATCAGGAAGGACC
KanF	CCGGAATTGCCAGCTGGGGCG
KanR	TCTTGTTCAATCATGCGAAACGATCC
OrprmFv	CGCCAGATTCGATTTGCGAAT
OrprmRv	ACTAGAAGCCTCTTTTCGCC

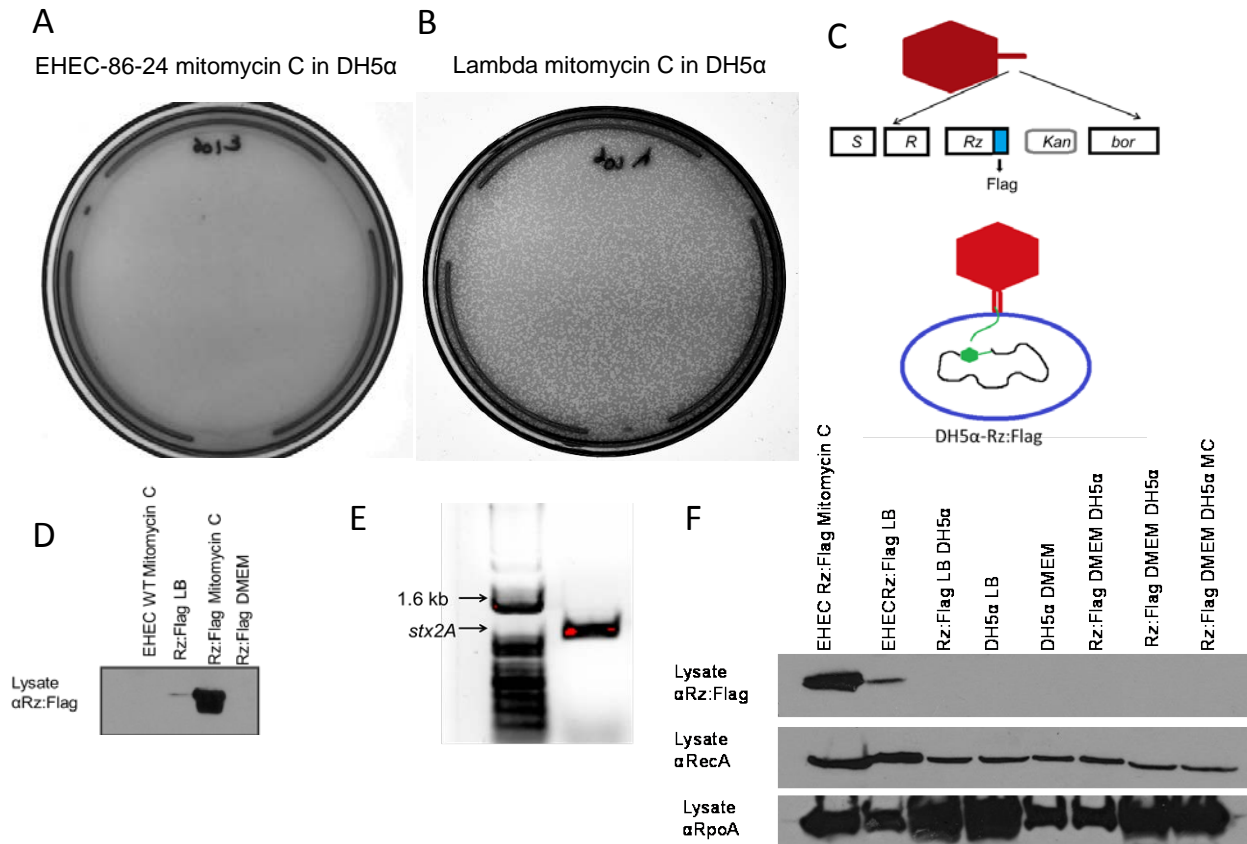
**Table S3, related to Star Methods. Plasmids.**

	Genotype
pet21a	N-terminal His-Tag vector
pWSK129	pLG339 <i>ori, kan, lacZ<math>\alpha</math></i>
pkd4	$\lambda$ red template plasmid
pkd46	$\lambda$ red recombinase expression plasmid
pcp20	TS replication and thermal induction of FLP synthesis
pJHD013	86-24 phage <i>N</i> antiterminator in pwsk129
pJHD014	86-24 phage <i>Q</i> - antiterminator in pwsk129
pJHD015	86-24 phage <i>SR</i> - holins in pwsk129
pJHD016	86-24 phage <i>cro</i> -transcription factor in pWSK129
pJHD017	86-24 phage <i>cro</i> -transcription factor in pet21a
pJHD018	EDL933-933w- <i>cro</i> -transcription factor in pet21a
pJHD019	Sakai-Vt-Stx2- <i>cro</i> -transcription factor in pet21a
pJHD020	EDL933-933w- <i>cro</i> -transcription factor in pWSK129
pJHD021	Sakai-Vt-Stx2- <i>cro</i> -transcription factor in pWSK129
pRS551	lacZ reporter gene fusion vector



**Figure S1. Related to Figure 1. Bacteriophage activity in *E. coli* W3350.**

(A) Protein levels of the DNA repair protein, RecA, from whole-cell lysate of *E. coli* W3350 in LB aerobic, DMEM anaerobic and DMEM aerobic conditions. RpoA levels serves as a loading control (B) Growth curves of *E. coli* W3350 using LB-mitomycin C, LB-glucose, DMEM anaerobic and DMEM aerobic. The experiments were conducted with 9 biological replicates. Data are represented as mean  $\pm$  SEM. (C) Bacteriophage particles isolated from *E. coli* W3350 using PEG8000 under LB mitomycin C, LB-glucose, DMEM anaerobic and DMEM aerobic conditions, and PCR reaction was performed targeting the *cro* gene as readout.



**Figure S2. Related to Figure 1. Differences between EHEC 8624 and *E. coli* W3350 bacteriophages.**

EHEC and *E. coli* W3350 were grown in LB medium and treated with Mitomycin C (10 µg/ml), centrifuged, filter sterilized and serially diluted in SM buffer and an aliquot (100 µl) was mixed with host strain *E. coli* (DH5α), incubated and poured onto LB plates to be incubated overnight. Plaques were visualized with a digital camera.

(A) EHEC Stx2 bacteriophage did not form plaques on *E. coli* DH5α.

(B) Lambda phage from strain W3350 was able to produce thousands of plaques on *E.*



*coli* DH5 $\alpha$ . (C) Cartoon depicting the strategy to Flag-tag *Rz* gene from the 8624 bacteriophage, which is a non-essential gene for bacteriophage. *Rz* is a gene located downstream of the lysis genes *SR*, and between *Rz* flag and *bor* a kanamycin resistant gene was introduced as a selection marker. (D) Western Blot experiment of EHEC 8624 *Rz*-flag strain where the bacterium was grown on different conditions, Mitomycin C, LB, and DMEM anaerobic for 6 hours to obtain whole cell lysate to be probed against anti-Flag antibody (Sigma) . WT EHEC was added to test the specificity of the Flag antibody. The Flag tag was highly expressed by the *Rz*-Flag 8624 strain in Mitomycin C. In the absence of mitomycin C it was expressed at lower levels in LB and not in DMEM. (E) PCR of DH5 $\alpha$  clone transduced with 8624 *Rz*:Flag bacteriophage depicting the presence of *stx2A* gene in the chromosome. (F) Western blot of DH5 $\alpha$  transduced with *Rz*:Flag bacteriophage under different growth conditions. The first to lines are EHEC 8624 *Rz*:flag under mitomycin C and LB conditions used as positive controls. (D) Western Blot experiment of EHEC 8624 *Rz*-flag strain where the bacterium was grown on different conditions, Mitomycin C, LB, and DMEM anaerobic for 6 hours to obtain whole cell lysate to be probed against anti-Flag antibody (Sigma) . WT EHEC was added to test the specificity of the Flag antibody. The Flag tag was highly expressed by the *Rz*-Flag 8624 strain in Mitomycin C. In the absence of mitomycin C it was expressed at lower levels in LB and not in DMEM. (E) PCR of DH5 $\alpha$  clone transduced with 8624 *Rz*:Flag bacteriophage depicting the presence of *stx2A* gene in the chromosome.

(F) Western blot of DH5 $\alpha$  transduced with Rz:Flag bacteriophage under different growth conditions. The first two lines are EHEC 8624 Rz:flag under mitomycin C and LB conditions used as positive controls.

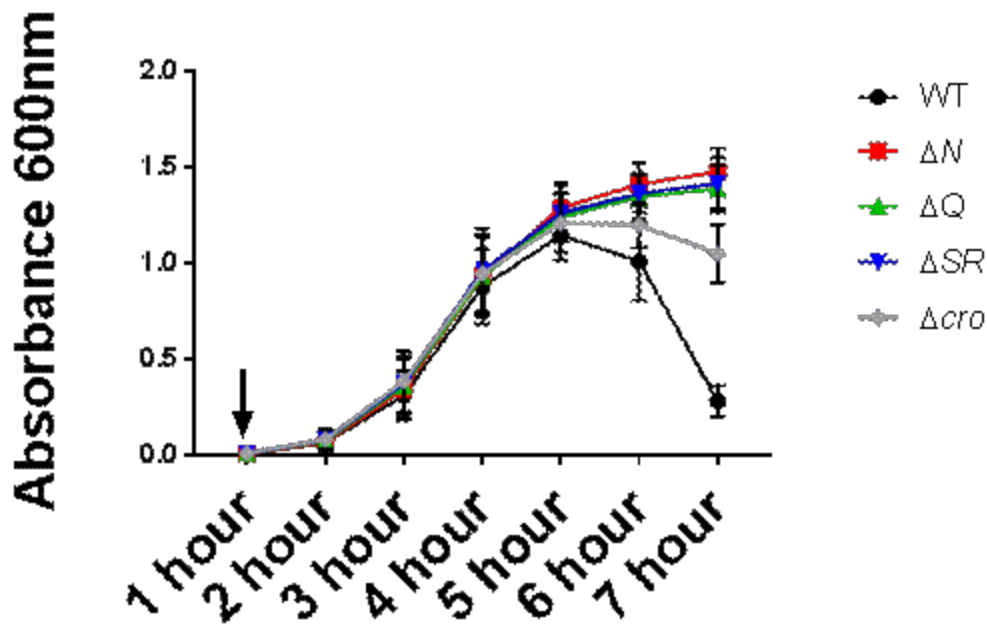
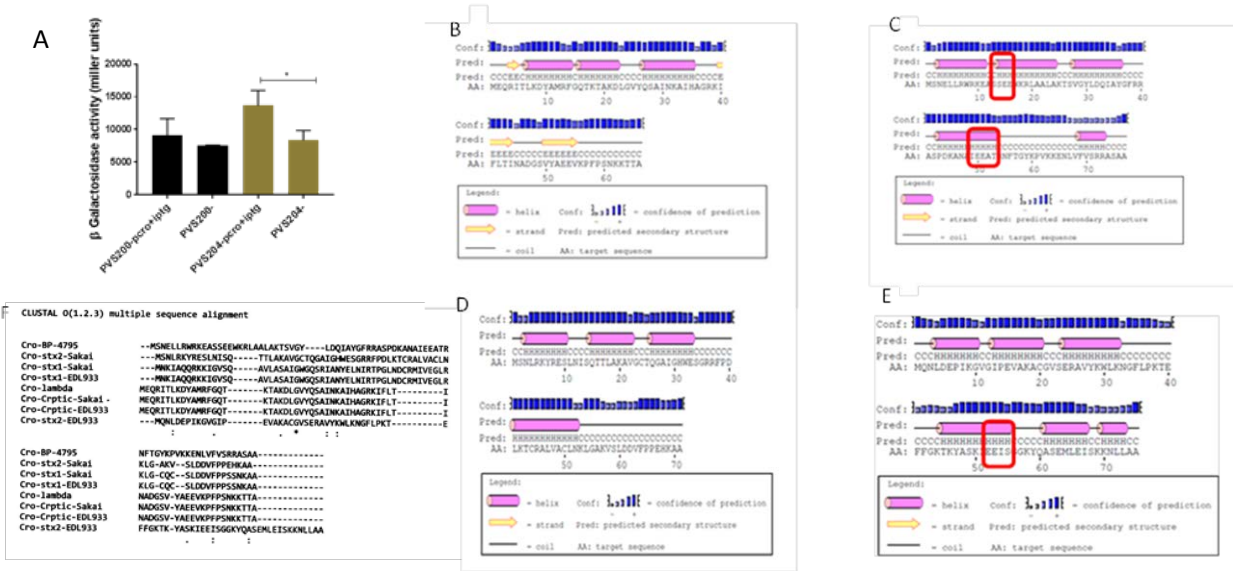


Figure S3. Related to Figures 2 and 3. EHEC 8624 bacteriophage mutants are lysogenic

Growth curves of EHEC 8624 WT (Black line) and isogenic bacteriophage mutations  $\Delta N$  (Red line),  $\Delta Q$  (Green line)  $\Delta SR$  (Blue line) and  $\Delta cro$  (Grey line). The strains were grown in LB medium supplemented with a low concentration of Mitomycin C (200 ng/ml) under aerobic conditions, and induction was done at the beginning of the experiment (0 hour) (n=3, error bars, standard deviation).



**Figure S4. Related to Figure 4. Cro regulation of ler and Different Cro proteins.**

(A) Beta galactosidase assay of *ler-lacZ* fusions in *E. coli* K-12 MC4100 in the absence (vector control) or presence of pCro. IPTG is used in all cultures because it induces *cro* expression. Cultures were grown in DMEM. pVS204 contains the -343 to +86 regulatory region of *ler*. pVS200 does not contain *ler* P2 promoter (Sharp and Sperandio, 2007) . P<0.05 as significant. (B) The presence of acidic patches in the DNA domain of a transcription factor are required for transcriptional activation(Bushman and Ptashne, 1988; Ko et al., 2008). Cro proteins from the different phages were evaluated using the software Protein Sequence Analysis Workbench (PSPIRED)(Buchan et al., 2013). a, Lambda ( $\lambda$ ) Cro protein, accession number NP\_040629.1 was used for comparison purposes showing the software's accuracy to predict the Cro DNA binding domain at amino acid residual 30 , QSANK showed as pink bars. Noticeably, this domain does not contain acidic patches which is typical for this type of Cro. (C) EHEC 86-24 Cro

accession number YP\_001449261.1 contains two domains , at residuals 20 and 50 which have acidic patches SEE and EE-T respectively (red boxes). (D) EHEC Sakai VT-Stx2 Cro accession number WP\_000067727, does not have any residual patches in the predicted helix domains. (E) EHEC EDL933 933W Cro accession number NP\_286964, contain one domain at residual 50 with acid patch EE-S (red box). (F) Clustal Omega alignments of all the Cro used in the study. Phage BP-4795 Cro is 100 percent identical to EHEC strain 86-24 phage Cro. EHEC strain EDL933 contain three different phages: Shiga toxin 2 (Stx2) phage ( $\Phi$ BP933W ), Shiga toxin 1 (Stx1) phage ( $\Phi$ CP933V) and Cryptic phage. EHEC strain Sakai contain also three different phages: Shiga toxin 2 (Stx2) phage ( $\Phi$ VTX2), Shiga toxin 1 (Stx1) phage ( $\Phi$ ECs2989), and Cryptic phage ( $\Phi$  ECs0275). Lambda phage Cro was added for comparison purposes. Cro accession numbers used in the alignment were : YP\_001449261.1, NP\_040629.1, WP\_000067727, NP\_286964.1 , WP\_000437875, WP\_000437875.1, WP\_001033078.1. (E) EHEC EDL933 933W Cro accession number NP\_286964, contain one domain at residual 50 with acid patch EE-S (red box). (F) Clustal Omega alignments of all the Cro used in the study. Phage BP-4795 Cro is 100 percent identical to EHEC strain 86-24 phage Cro. EHEC strain EDL933 contain three different phages: Shiga toxin 2 (Stx2) phage ( $\Phi$ BP933W ), Shiga toxin 1 (Stx1) phage ( $\Phi$ CP933V) and Cryptic phage. EHEC strain Sakai contain also three different phages: Shiga toxin 2 (Stx2) phage ( $\Phi$ VTX2), Shiga toxin 1 (Stx1) phage ( $\Phi$ ECs2989), and Cryptic phage ( $\Phi$  ECs0275). Lambda phage Cro was added for comparison purposes. Cro accession numbers used in the alignment were : YP\_001449261.1, NP\_040629.1,

WP\_000067727, NP\_286964.1 , WP\_000437875, WP\_000437875.1,  
WP\_001033078.1.

**A** Down regulated genes affected by  $\Delta cro$

Virulence	Dna-repair	Peptidoglycan-cell-wall	Metabolism	Hypothetical-protein	tRNA-rRNA	Phage-related	Transport	Total
49	52	30	181	87	81	42	62	584

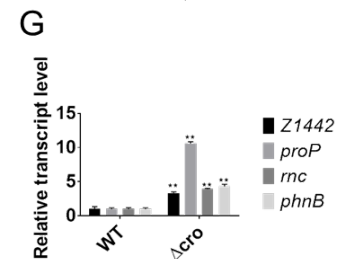
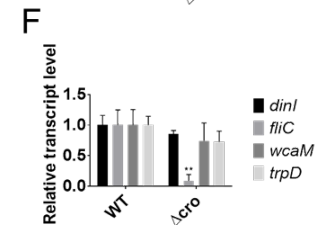
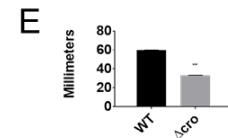
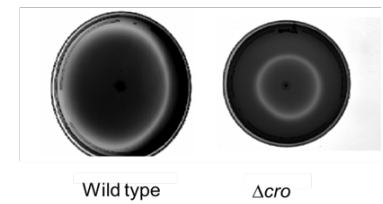
**B** Up-regulated genes affected by  $\Delta cro$

Virulence	Dna-repair	Peptidoglycan-cell-wall	Metabolism	Hypothetical-protein	tRNA-rRNA	Phage-related	Transport	Total
9	40	23	84	64	44	15	28	307

**C** TypeIII-Secretion system Down regulated genes in  $\Delta cro$ .

Gene name	Fold Change	Biological function
<i>espA</i>	0.061	protein EspA
<i>espD</i>	0.081	protein EspD
<i>Z5111</i>	0.105	Tir chaperone protein (CesT) family
<i>Z3071</i>	0.119	secretion protein EspJ
<i>Z5104</i>	0.122	pathogenicity island 2 effector protein SseE
<i>espF</i>	0.179	secretion protein EspF
<i>espB</i>	0.247	Enterobacterial EspB protein
<i>Z3921</i>	0.249	T3SS effector protein NleG8
<i>Z5102</i>	0.297	EscG/YscG/SsaH family type III secretion system
<i>Z5125</i>	0.329	type III secretion system protein SepD
<i>sepZ</i>	0.338	type III secretion system protein SepZ
<i>Z5121</i>	0.359	Hypothetical protein (type III secretion)
<i>Z5116</i>	0.365	type III secretion system protein SepQ
<i>Z5114</i>	0.408	regulation of protein secretion, Tir chaperone protein (CesT) family (hypothetical protein)
<i>Z1829</i>	0.478	type III secretion protein GogB

**D**



**Figure S5. Related to Figures 1-4. Microarray analysis of EHEC 8624  $\Delta cro$  grown**

**anaerobically in DMEM.** (A-B). Transcriptomic profile of a mutation in the bacteriophage transcription factor, *cro*, where approximately 800 genes were affected and grouped in categories. Majority of the affected genes were down regulated in  $\Delta cro$  (approximately 500) and the remaining genes were up regulated in  $\Delta cro$  (300).

(C) Summary of genes affected (down regulated) in the T3SS comprising genes related to the needle apparatus such as *espA*, and secreted encoded effectors like *espF*.

(D) Motility plates were conducted using a low concentration of agar (0.25%) in DMEM anaerobically at 37°C overnight. Wild type EHEC 86-24 and isogenic  $\Delta cro$  were grown

in LB medium overnight, plates were stabbed in the middle with the respective strain and incubated anaerobically overnight.

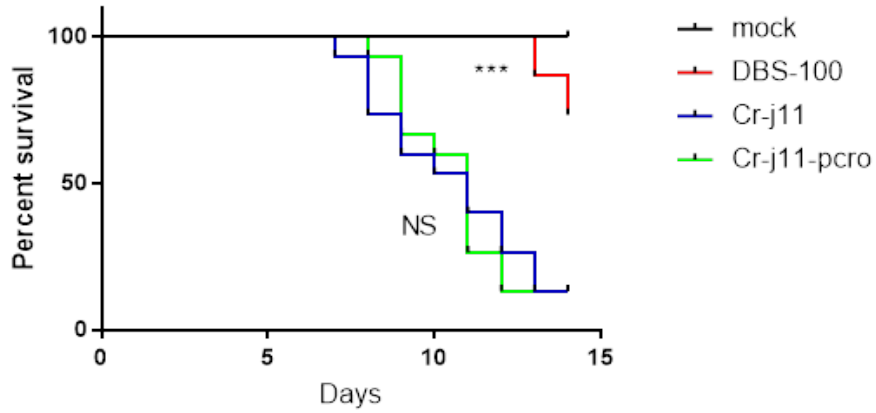
(E) Motility experiments were conducted in triplicates at three independent times having a total of 9 replicates, the diameter was measured and a Student's *t*-test was conducted showing statistical significance at  $p < 0.01$  (\*\*). qRT-PCR of selected genes from the microarray experiments. RNA was extracted from EHEC 86-24 strains grown anaerobically in DMEM to an OD<sub>600</sub> of 0.6 (n=6 replicates per strain; asterisks,  $p < 0.01$ ; Student's *t*-test).

(F) Up-regulated genes and

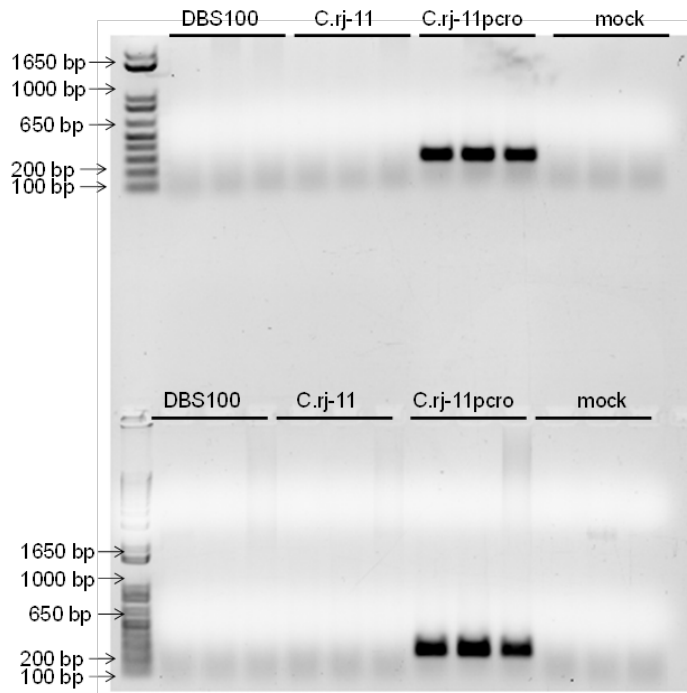
(G) Down regulated genes affected by  $\Delta$ *cro* compared to wild type (WT) EHEC.



A



B



**Figure S6. Related to Figures 5 and 6. The disease prognosis in mice can be influenced utilizing the EHEC 8624 bacteriophage Cro expressed in Trans**

(A) Survival curves of mouse after infection with mock (PBS) and different *C. rodentium*

strains. The curves show a drastically difference between *Cr-j11*, *Cr-j11-pcro* and *C. rodentium* DBS100. There was a highly statistical significance comparing DBS100 either *Cr-j11* or *Cr-j11-pcro* (\*\* $p < 0.01$ ). Nonetheless, there was not statistical significance within *Cr-j11* and *Cr-j11-pcro* (n=15, analysis was done using Mantel-cox, and Grehan-Breslow-Wilcoxon tests in GraphPad prism). (B) 8624 *cro* phage gene isolated from mouse distal colon. The RNA from distal colon was extracted and cDNA converted . PCR reaction was conducted using primers to target the *cro* gene complemented in trans into pwsk129 plasmid. Each lane denotes a single mouse from different groups.