Supplemental Information

Shutoff of host transcription triggers a toxin-antitoxin system to cleave phage RNA and abort infection

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A

ToxN sequences

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E. coli        MKFYTISSSYIKYLKDFDDKVPNSEDPTYNNPKAFIGIVLEIEGHKYLAPLTSP    54
P. atrosepticum MKFYTISSKYIEYLKEFDDKVPNSEDPTYQNPKAFIGIVLEIQGHKYLAPLTSP    54

E. coli        KAWHANVKESSPAFFKLHENGVPDNQLGLINLKFMIPIIEAEVSLLDLDSMPDT   108
P. atrosepticum KKWHNNVKESSLSCFKLHENGVPENQLGLINLKFMIPIIEAEVSLLDLGNMPNT   108

E. coli        PYKRMLYKQLQFIRVNEDKISEKSKLLRNLALQGRMQGTCDFAVLEEKYQHFGK   162
P. atrosepticum PYKRMLYKQLQFIRANSDKIASKSDTLRNLVLQGKMQGTCNFSLLEEKYRDFGK   162

E. coli        KPEDMEIDD                                                171
P. atrosepticum EAEDTEEGE                                                171
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B

toxI sequences

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E. coli        AAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTTAGGTGATTTGCTACCTT    54
P. atrosepticum AAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTTAGGTGATTTGCTACCTT    54

E. coli        TAAGTGCAGCTAGAAATTTAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATT   108
P. atrosepticum TAAGTGCAGCTAGAAATTCAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATT   108

E. coli        CAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTCAGGTGATTTGCTACCTT   162
P. atrosepticum CAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTCAGGTGATTTGCTACCTT   162

E. coli        TAAGTGCAGCTAGAAATTTAGGTCATTTACTACCTTAAAGTAATAAAGT       211
P. atrosepticum TAAGTGCAGCTAGAAATTCAGGTGATTTACTACCTTTAAGTAATAAAGT       211
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C

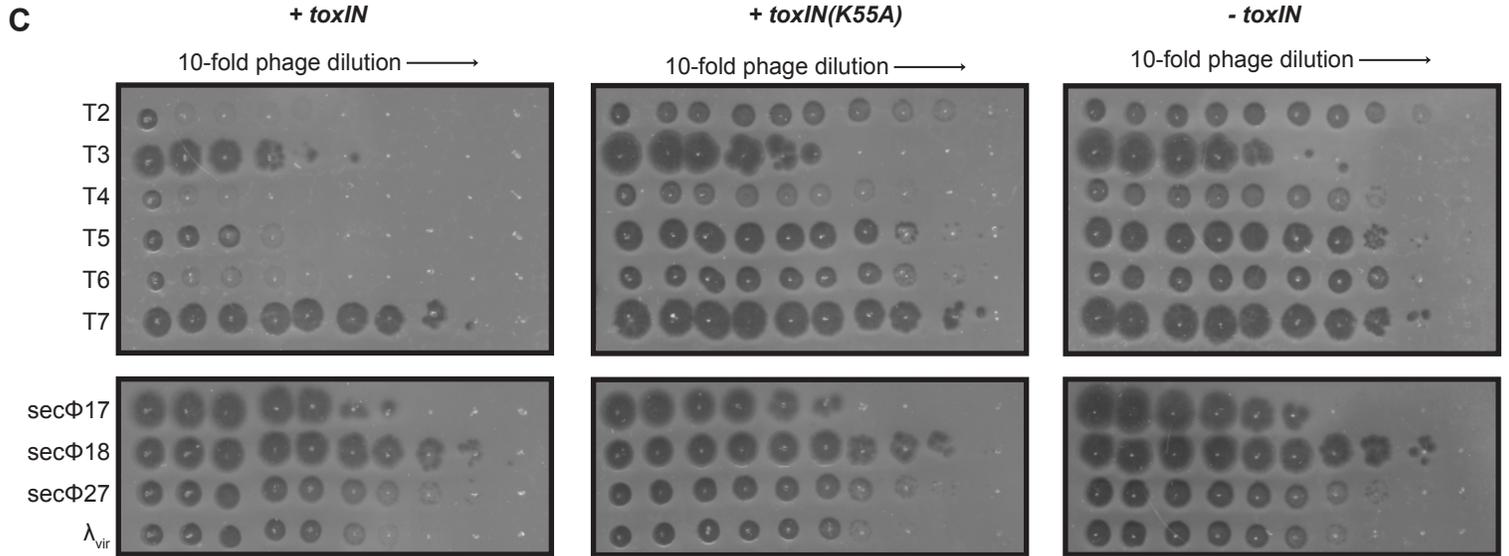| + *toxIN*	+ *toxIN(K55A)*	- *toxIN*
10-fold phage dilution ⟶	10-fold phage dilution ⟶	10-fold phage dilution ⟶

T2
T3
T4
T5
T6
T7

secΦ17
secΦ18
secΦ27
λvir

Fig. S1. *E. coli toxIN* is a type III toxin-antitoxin system that protects against multiple coliphage, Related to Fig. 1.

(A) Sequence alignment of *E. coli* and *P. atrosepticum* ToxN.

(B) Sequence alignment of *E. coli* and *P. atrosepticum toxI* arrays, with ToxN cleavage motifs highlighted.

(C) Representative serial dilution plaque assays a panel of coliphage spotted on *+toxIN, +toxI toxN(K55A)*, and *–toxIN* cells.
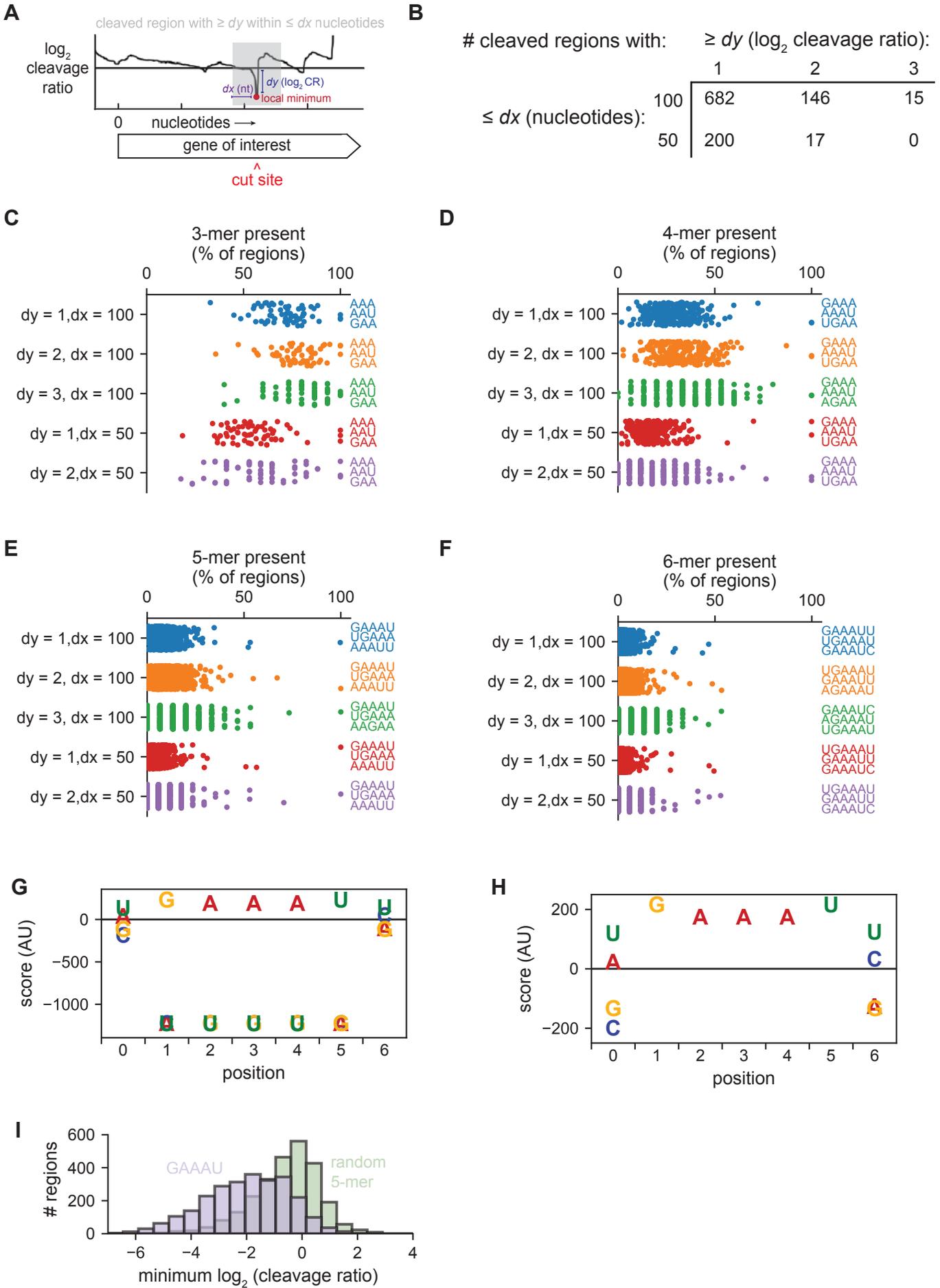
A

log$_2$ cleavage ratio

cleaved region with ≥ dy within ≤ dx nucleotides

dx (nt) dy (log$_2$ CR)
local minimum

0   nucleotides →

gene of interest

cut site

B

# cleaved regions with:	≥ dy (log$_2$ cleavage ratio):		
	1	2	3
≤ dx (nucleotides): 100	682	146	15
50	200	17	0

C

3-mer present (% of regions)

D

4-mer present (% of regions)

E

5-mer present (% of regions)

F

6-mer present (% of regions)

G

score (AU)   position

H

score (AU)   position

I

regions

GAAAU   random 5-mer

minimum log$_2$ (cleavage ratio)

Fig. S2. ToxN recognizes the 5-mer GAAAU, Related to Fig. 2.

(A) Schematic overview of computational approach for identifying ToxN cleavage sites. Briefly, we identified local minima in the cleavage ratio array and looked for 'cleavage valleys' in which the cleavage ratio increased by a given dy within a nucleotide distance dx from the local minimum. We then extracted the sequences of these so-called cleaved regions for further analysis.

(B) Number of cleavage valleys identified using the dx and dy thresholds indicated.

(C-F) Scatterplots showing the percent of cleaved regions identified with the thresholds indicated that contain each possible 3-mer (C), 4-mer (D), 5-mer (E), or 6-mer (F). Top three most frequent k-mers are indicated on the right.

(G) Position weight matrix for sites associated with ToxN-cleaved regions of a maximum width of 100 nt. Scores were calculated using MEME.

(H) Zoomed-in position weight matrix shown in (G).

(I) Histograms showing the distribution of the minimum cleavage ratios within well-expressed GAAAU sequences in *E. coli* transcripts (purple, $n = 2579$) compared to the minimum cleavage ratios within the same number of random 5-mers in the transcriptome (green).
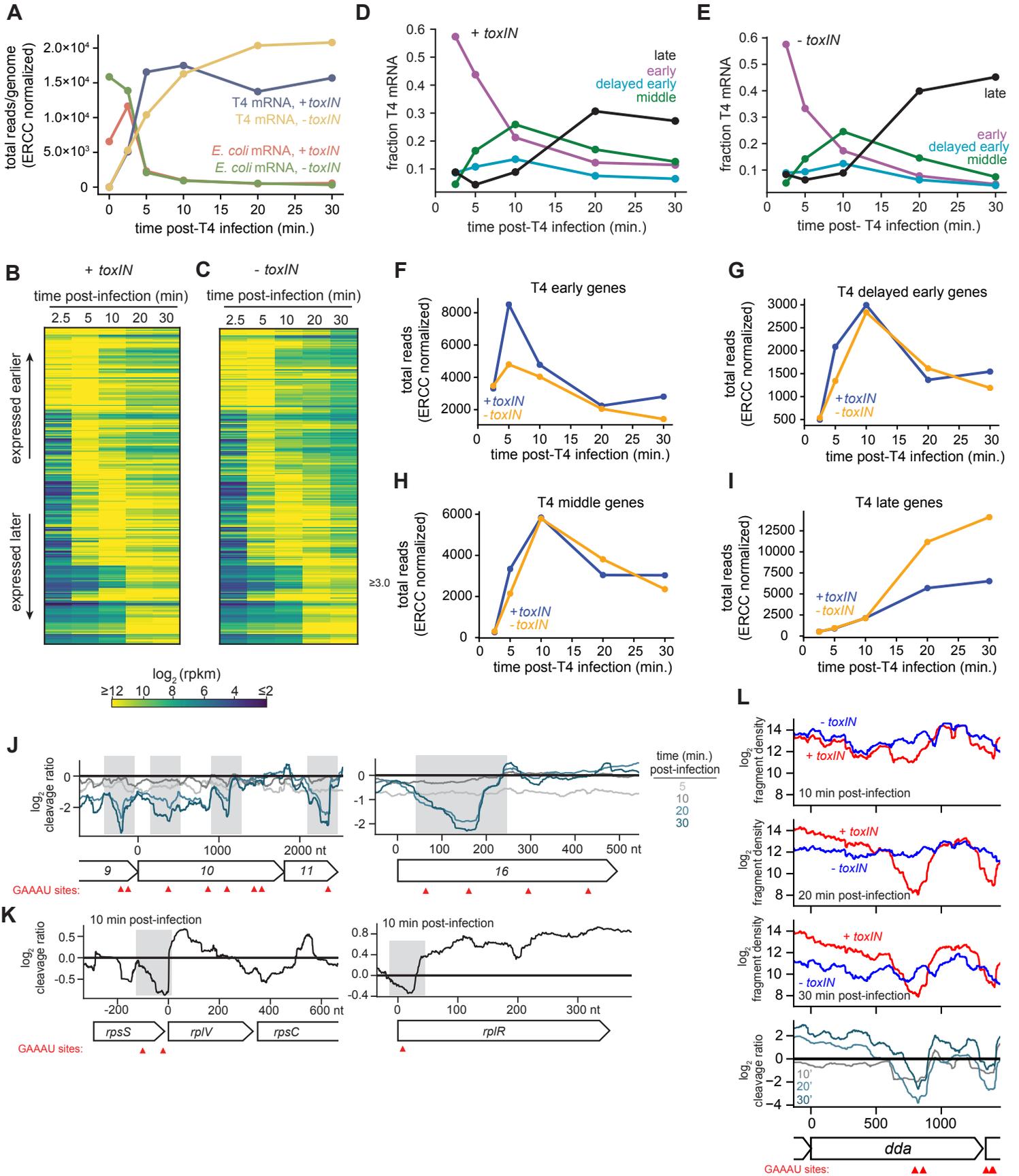
Fig. S3. ToxN disrupts progression of the T4 life cycle and cleaves phage and host transcripts, Related to Fig. 3.

(A) Plot showing the total RNA-seq signal coming from host (*E. coli*) and phage (T4) mRNAs relative to the total counts coming from a spike-in control (ERCC standard) at the indicated times post-infection. Note that we included the spike-in control in only one of the two RNA-seq replicates during T4 infection.

(B-C) Heatmaps showing the $\log_2$(rpkm) (reads per kilobase per million) for each T4 transcript at each time post-infection in *+toxIN* (B) or *–toxIN* (C) cells. Each row corresponds to an individual gene, and genes are ordered based on their time of peak expression in the *–toxIN* dataset. Time of peak expression was determined by comparing the rpkm for a particular transcript relative to the entire T4 transcriptome and determining the timepoint at which a given gene made up the largest fraction of the T4 transcriptome.

(D-E) Plots showing the fraction of total T4 mRNA signal in RNA-seq coming from different classes of T4 transcripts (early, delayed early, middle, and late) in *+toxIN* (C) or *–toxIN* (D) cells at the indicated times post-infection.

(F-I) Plots showing the total counts coming from T4 early (A), delayed early (B), middle (C), or late (D) mRNAs relative to the total counts coming from a spike-in control (ERCC standard) at the indicated times post-infection in *+toxIN* and *–toxIN* cells. Note that we included the spike-in control in only one of our RNA-seq replicates during T4 infection.

(J) Cleavage profiles for the regions surrounding T4 genes *10* and *16* at 5, 10, 20, and 30 min post-infection. Sites of GAAAU motifs are indicated with red triangles.

(K) Cleavage profiles for the regions surrounding the *E. coli* ribosomal protein genes *rplV* and *rplR* at 10 min post-infection. Sites of GAAAU motifs are indicated with red triangles. See Fig. 2D for the cleavage profile of these regions following ToxN overexpression.

(L) RNA fragment density profiles for *+toxIN* and *–toxIN* cells (top three panels) and cleavage profiles (bottom panel) for the T4 gene *dda* at 10, 20, and 30 min post-infection. As infection proceeds, GAAAU sites are cleaved in the *+toxIN* sample, but the surrounding regions remain stable relative to the *–toxIN* sample, likely due to defects in progression of the T4 life cycle. Thus,

at 20 and 30 min post-infection, the cleavage ratio becomes positive (>0) in regions of *dda* surrounding the cut site. Also see Fig. 3C.
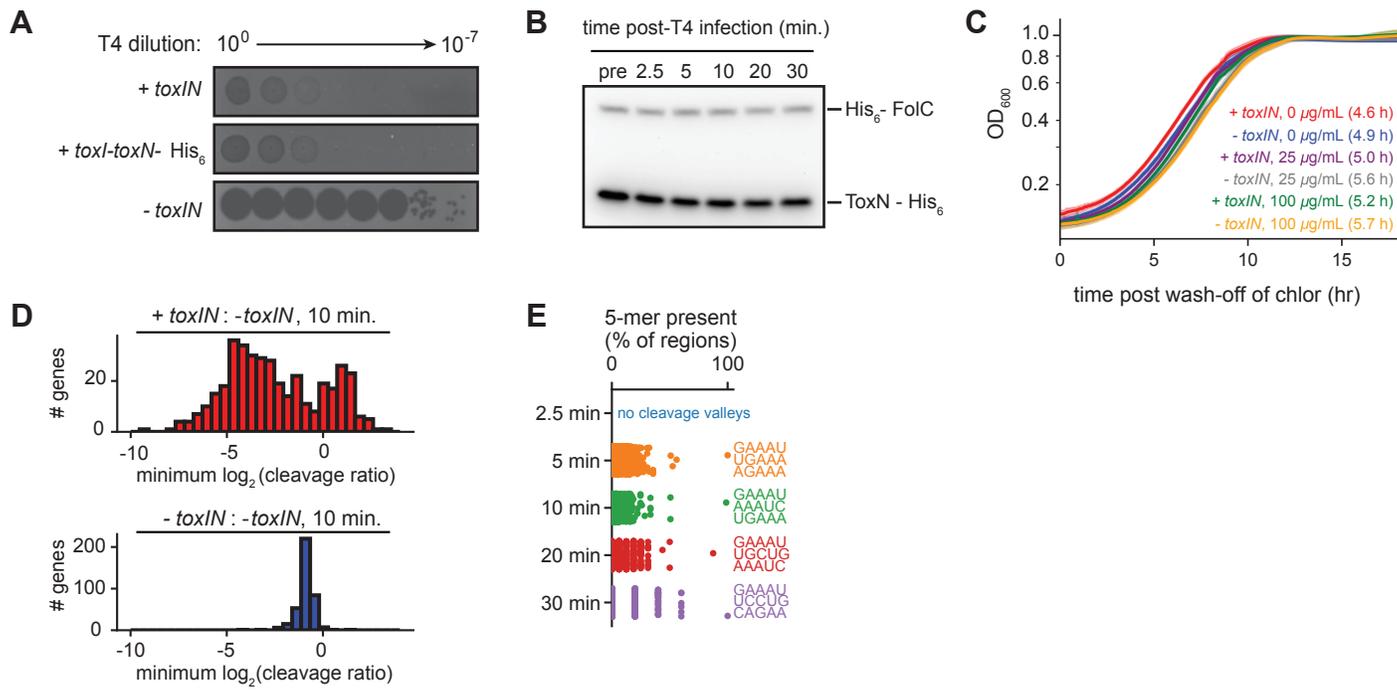
A

T4 dilution: 10^0 ⟶ 10^{-7}

+ *toxIN*

+ *toxI-toxN*- His$_6$

- *toxIN*

B

time post-T4 infection (min.)

pre 2.5 5 10 20 30

— His$_6$- FolC

— ToxN - His$_6$

C

OD$_{600}$

time post wash-off of chlor (hr)

+ *toxIN*, 0 µg/mL (4.6 h)
- *toxIN*, 0 µg/mL (4.9 h)
+ *toxIN*, 25 µg/mL (5.0 h)
- *toxIN*, 25 µg/mL (5.6 h)
+ *toxIN*, 100 µg/mL (5.2 h)
- *toxIN*, 100 µg/mL (5.7 h)

D

+ *toxIN* : -*toxIN*, 10 min.

genes

minimum log$_2$(cleavage ratio)

- *toxIN* : -*toxIN*, 10 min.

genes

minimum log$_2$(cleavage ratio)

E

5-mer present
(% of regions)

0   100

2.5 min — no cleavage valleys

5 min — GAAAU
UGAAA
AGAAA

10 min — GAAAU
AAAUC
UGAAA

20 min — GAAAU
UGCUG
AAAUC

30 min — GAAAU
UCCUG
CAGAA

Fig. S4. ToxN is activated by transcription shutoff of *toxIN*, Related to Fig. 4.

(A) Serial dilution plaque assays for T4 spotted on +*toxIN,* +*toxI toxN*-His$_6$, and –*toxIN* cells.

(B) Western blot of ToxN-His$_6$ during T4 infection with purified His$_6$-FolC included as a loading control during cell harvesting. Also see Fig. 4B.

(C) Growth curves for +*toxIN* and –*toxIN* cells following chloramphenicol treatment at the indicated concentrations for 30 min. Data are the mean of two technical replicates each of three biological replicates, and shaded areas report the S.D. Estimated lag time is reported in parentheses.

(D) Histograms showing the distribution of the minimum cleavage ratios within well-expressed coding regions ($n = 389$) in *E. coli* when comparing +*toxIN* to –*toxIN* cells (red, top) or comparing two independent replicates of –*toxIN* cells (blue, bottom) following rif treatment for 10 min.

(E) Percent of well-cleaved regions in the *E. coli* transcriptome that contain each possible 5-mer at the timepoints indicated following rif treatment. Top three most frequent 5-mers are indicated on the right.
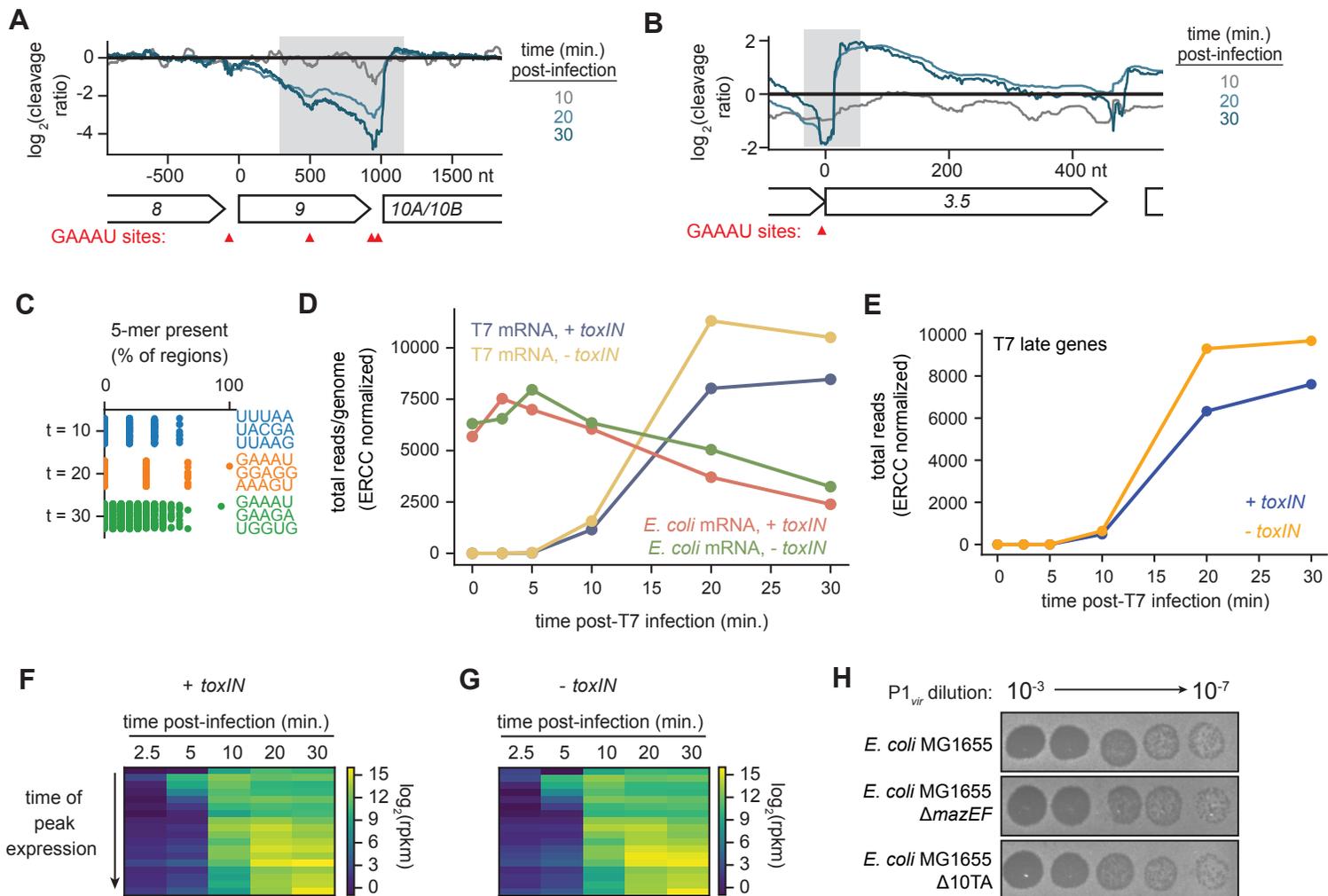
A

log$_2$(cleavage ratio)

time (min.) post-infection: 10, 20, 30

-500, 0, 500, 1000, 1500 nt

8, 9, 10A/10B

GAAAU sites:

B

log$_2$(cleavage ratio)

time (min.) post-infection: 10, 20, 30

0, 200, 400 nt

3.5

GAAAU sites:

C

5-mer present (% of regions)

0, 100

t = 10: UUUAA, UACGA, UUAAG

t = 20: GAAAU, GGAGG, AAAGU

t = 30: GAAAU, GAAGA, UGGUG

D

total reads/genome (ERCC normalized)

T7 mRNA, + toxIN
T7 mRNA, - toxIN
E. coli mRNA, + toxIN
E. coli mRNA, - toxIN

0, 5, 10, 15, 20, 25, 30
time post-T7 infection (min.)

E

total reads (ERCC normalized)

T7 late genes

+ toxIN
- toxIN

0, 5, 10, 15, 20, 25, 30
time post-T7 infection (min)

F

+ toxIN

time post-infection (min.): 2.5, 5, 10, 20, 30

time of peak expression

log$_2$(rpkm): 0, 3, 6, 9, 12, 15

G

- toxIN

time post-infection (min.): 2.5, 5, 10, 20, 30

log$_2$(rpkm): 0, 3, 6, 9, 12, 15

H

P1$_{vir}$ dilution: 10^{-3} ⟶ 10^{-7}

E. coli MG1655

E. coli MG1655 ΔmazEF

E. coli MG1655 Δ10TA

Fig. S5. ToxN is activated during T7 infection, Related to Fig. 5.

(A-B) Cleavage profiles for the region surrounding the T7 genes *9* (A) and *3.5* (B) at 10, 20 and 30 min post-infection. Sites of GAAAU motifs are indicated with red triangles.

(C) Scatterplots showing the percent of well-cleaved regions in the T7 transcriptome that contain each possible 5-mer at the times indicated post-infection. Top three most frequent 5-mers are indicated on the right.

(D) Plot showing the total RNA-seq signal coming from host (*E. coli*) and phage (T7) mRNAs relative to the total counts coming from a spike-in control (ERCC standard) at the indicated times post-infection.

(E) Plot showing the total counts coming from T7 late mRNAs relative to the total counts coming from a spike-in control (ERCC standard) at the indicated times post-infection in *+toxIN* and *–toxIN* cells. Late transcripts were defined as those whose time of peak expression (panel G) was 20 or 30 min post-infection.

(F-G) Heatmaps showing the $\log_2$(rpkm) (reads per kilobase per million) for each T7 mRNA at each timepoint post-infection in *–toxIN* (F) and *+toxIN* (G) cells. Each row corresponds to an individual mRNA, and mRNAs are ordered based on their time of peak expression in the *–toxIN* dataset. Time of peak expression was determined by comparing the rpkm for a particular transcript relative to the entire T7 transcriptome and determining the timepoint at which a given mRNA made up the largest fraction of the T7 transcriptome.

(H) Serial dilution plaque assays for P1 spotted on *E. coli* MG1655, *E. coli* MG1655 Δ*mazEF*, and *E. coli* MG1655 Δ*mazEF* Δ*relBE* Δ*chpB* Δ*dinJ-yafQ* Δ*yefM-yoeB* Δ*yafNO* Δ*prlF-yhaV* Δ*hicAB* Δ*ygiMN* Δ*mqsRA* (Δ10TA).

- toxIN

+ toxIN

T4 infects, replicates, releases progeny

T4 infects, but ToxIN blocks replication

progeny infect neighbor cells

infected cell still dies, but no progeny released
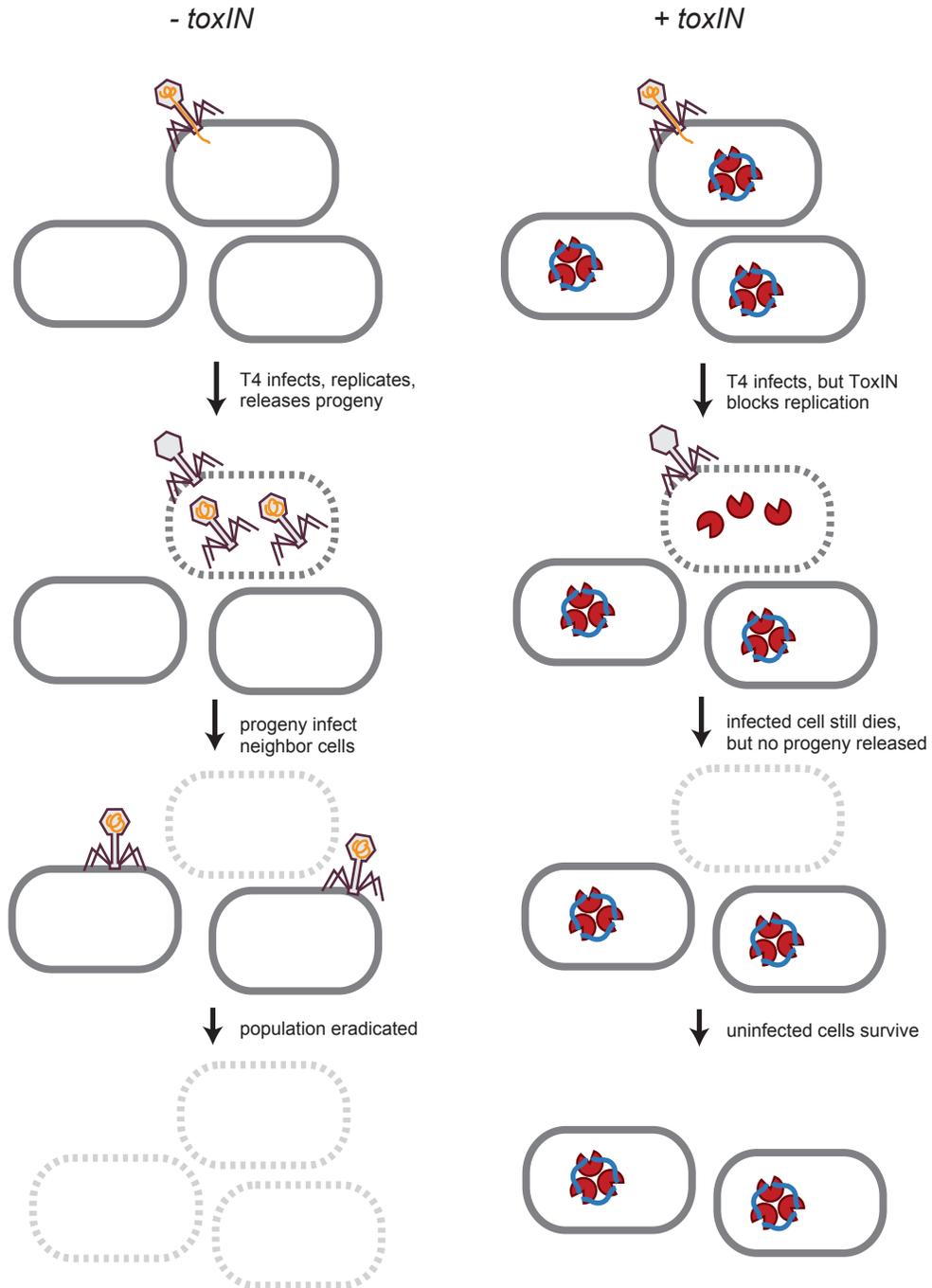
population eradicated

uninfected cells survive

Fig. S6. Population-level model for phage defense by type III TA systems, Related to Fig. 6.

(Left) Following infection of a *–toxIN* cell, T4 replicates and releases progeny, which go on to infect neighboring cells and eradicate the cell population. (Right) Following infection of a *+toxIN* cell, ToxN is activated and blocks production of new phage particles. The initially infected cell dies, but because no new progeny are released, uninfected cells in the clonal population survive.

Table S1. Strains, Related to STAR Methods

Name	Genotype	Source
ML6	MG1655	
DH5α	Cloning strain	Invitrogen
ML3358	MG1655 Δ*mazEF*	This study
ML3221	MG1655	Goeders et al., 2013
ML3222	MG1655 Δ*mazEF* Δ*relBE* Δ*chpB* Δ*dinJ-yafQ* Δ*yefM-yoeB* Δ*yafNO* Δ*prlF-yhaV* Δ*hicAB* Δ*ygjMN* Δ*mqsRA*	Goeders et al., 2013
ML3326	MG1655 pBAD33-*toxN* pEXT20-*toxI*	This study
ML3327	MG1655 pBAD33 pEXT20	This study
ML3328	MG1655 pBR322-*toxIN*	This study
ML3329	MG1655 pBR322-*toxI-toxN*(K55A)	This study
ML3330	MG1655 pBR322	This study
ML3331	MG1655 pBR322-*toxI-toxN*-His$_6$	This study
ML3332	MG1655 pBR322-*toxIN* pdCas9deg-*toxIN* gRNA	This study
ML3333	MG1655 pBR322-*toxI-toxN*(K55A) pdCas9deg-*toxIN* gRNA	This study
ML3334	MG1655 pBR322 pdCas9deg-*toxIN* gRNA	This study
ML3335	MG1655 pACYC-*toxIN* pBluescript-P$_{35.3 \ (T4 \ early)}$-*toxI*	This study
ML3336	MG1655 pACYC-*toxIN* pBluescript-P$_{rIIB2 \ (T4 \ middle)}$ -*toxI*	This study
ML3337	MG1655 pACYC-*toxIN* pBluescript-P$_{soc \ (T4 \ late)}$-*toxI*	This study
ML3338	MG1655 pACYC-*toxIN* pBluescript II SK(+)	This study
ML3339	MG1655 pACYC pBluescript-P$_{35.3 \ (T4 \ early)}$-*toxI*	This study
ML3340	MG1655 pACYC pBluescript-P$_{rIIB2 \ (T4 \ middle)}$ -*toxI*	This study
ML3341	MG1655 pACYC pBluescript-P$_{soc \ (T4 \ late)}$-*toxI*	This study
ML3342	MG1655 pACYC pBluescript II SK(+)	This study
ML3343	DH5α pBAD33-*toxN*	This study
ML3344	DH5α pBAD33	This study
ML3345	DH5α pEXT20-*toxI*	This study
ML1978	DH5α pEXT20	E. coli Genetic Stock Center, #12325
ML3346	DH5α pBR322-*toxIN*	This study
ML3347	DH5α pBR322-*toxI-toxN*(K55A)	This study
ML3348	DH5α pBR322	This study
ML3349	DH5α pBR322-*toxI-toxN*-His$_6$	This study
ML3350	DH5α pdCas9deg-*toxIN* gRNA	This study
ML3351	DH5α pACYC-*toxIN*	This study
ML3352	DH5α pACYC	This study
ML3353	DH5α pBluescript-P$_{35.3 \ (T4 \ early)}$-*toxI*	This study
ML3354	DH5α pBluescript-P$_{rIIB2 \ (T4 \ middle)}$ -*toxI*	This study
ML3355	DH5α pBluescript-P$_{soc \ (T4 \ late)}$-*toxI*	This study
ML3356	DH5α pBluescript II SK(+)	This study

Table S2. Plasmids, Related to STAR Methods

Plasmid	Description	Source
pBAD33	Arabinose inducible vector	Guzman et al., 1995
pBAD33-*toxN*	Arabinose inducible ToxN expression	This study
pEXT20	IPTG inducible vector	E. coli Genetic Stock Center, #12325
pEXT20-*toxI*	IPTG inducible *toxI* expression	This study
pBR322-*toxIN*	Full *toxIN* locus	This study
pBR322-*toxI-toxN(*K55A*)*	*toxIN* locus with active-site ToxN mutant	This study
pBR322-*toxI-toxN*-His$_6$	*toxIN* locus with C-terminal His$_6$-tagged ToxN	This study
pBR322 empty vector	Derivative of pBR322 with pTet removed	This study
pBluescript II SK(+)	Control for P$_{T4}$-*toxI* experiments	D. Alley
pBluescript-P$_{35.3\ (T4\ early)}$-*toxI*	Expression of *toxI* during T4 infection	This study
pBluescript-P$_{rIIB2\ (T4\ middle)}$-*toxI*	Expression of *toxI* during T4 infection	This study
pBluescript-P$_{soc\ (T4\ late)}$-*toxI*	Expression of *toxI* during T4 infection	This study
pACYC empty vector	Derivative of pACYC-Duet with *lacI* and two multiple cloning sites removed	This study
pACYC-*toxIN*	Full *toxIN* locus	This study
pdCas9deg-*toxIN* gRNA	aTc-inducible expression of catalytically-inactive Cas9 for repression of *toxIN* promoter	This study

Table S3. Primers, Related to STAR Methods.

Number	Purpose	Sequence (5′→3′)
CG-1	*toxN* into pBAD33 (forward)	ATGCAGAGCTCATGAAATTTTACACAATATCAAGCAGTTACATAAAATACCTG
CG-2	*toxN* into pBAD33 (reverse)	GCCGTGTCGACTTAATCGTCTATTTCCATGTCTTCAGGTTTTTTACCG
CG-3	Ultramer to construct *toxI* locus with 3.5 tandem repeats (sense strand)	AGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTCAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTCAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTTAGGTCATTTACTACCTTAAAGTAATAAAGTAAAAAAGGCGACTATACACAGTCGCCTTTTTTACGAGAAAA
CG-4	Ultramer to construct *toxI* locus with 3.5 tandem repeats (antisense strand)	TTTTTCTCGTAAAAAAGGCGACTGTGTATAGTCGCCTTTTTTACTTTATTACTTTAAGGTAGTAAATGACCTAAATTTCTAGCTGCACTTAAAGGTAGCAAATCACCTGAATTTCTAGCTGCACTTAAAGGTAGCAAATCACCTGAATTTCTAGCTGCACTTAAAGGTAGCAAATCACCT
CG-5	*toxI* into pEXT20 (forward)	AGGAAACAGAATTCGAGCTCAGGTGATTTGCTACCTTTAAG
CG-6	*toxI* into pEXT20 (reverse)	GCTTGCATGCCTGCAGGTCGACCATTTTTTCTCGTAAAAAAGGCGACTGTG
CG-7	Ultramer to construct full *toxI* locus using overlap PCR (sense strand)	GCTACCTTTAAGTGCAGCTAGAAATTTAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTTAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTCAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTCAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTTAGGTCATTTACTACCTTAAAGTAATAAAG
CG-8	Construct full *toxI* locus (forward, PCR1)	CATGTATACTTAGTTCAAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTT
CG-9	Construct full *toxI* locus (reverse forward, PCR1,2&3)	TGTAACTGCTTGATATTGTGTAAAATTTCATTTTTTCTCGTAAAAAAGGCGACTGTG
CG-10	Construct full *toxI* locus (forward, PCR2)	GTAAGCCCAAGCGAAGATCATGTATACTTAGTTCAAGGTGATTTGC
CG-11	Construct full *toxI* locus (forward, PCR3)	CTGAACATCCCATGCTAAGCACCTAGTTGTAAGCCCAAGCGAAGATCATG
CG-12	Amplify full *toxIN* locus (forward)	TTTATCACAGTTAAATTGCTAACGCAGTCAG
CG-13	Amplify full *toxIN* locus (reverse)	GGAGTGGTGAATCCGTTAGCG
CG-14	Construct pBR322 empty vector and assemble pBR322-*toxIN* (forward)	ATGGAAGCCGGCGGCAC
CG-15	Construct pBR322 empty vector and assemble pBR322-*toxIN* (reverse)	ACATGAGAATTCTTGAAGACGAAAGGGC
CG-16	Construct pBR322-*toxI-toxN*(K55A) (forward)	GCGGCATGGCATGCTAATGTAAAAGAGTCATC
CG-17	Construct pBR322-*toxI-toxN*(K55A) (reverse)	TGGCGATGTTAAAGGTGCTAAATATTTATGTCC
CG-18	Construct pBR322-*toxI-toxN*-His₆ (forward)	CATCATCACCATCACCATTAACCATTCTTTTTTATGGAATTGAAGGTCCAAAATC
CG-19	Construct pBR322-*toxI-toxN*-His₆ (reverse)	GCTTCCGCTTCCATCGTCTATTTC
CG-20	Construct pACYC-*toxIN* (forward)	GTGAGCTAACTTACATTAATTGCGTTGCGCCTGAACATCCCATGCTAAGCACC
CG-21	Construct pACYC-*toxIN* (reverse)	TCTCAAATGCCTGAGGTTTCAGCTTATATTGGATGAGAGCAAAAAAAATAGGTCCAAAAC
CG-22	Construct pACYC empty vector and assemble pACYC-*toxIN* (forward)	CTGAAACCTCAGGCATTTGAGAAGCAC

CG-23	Construct pACYC empty vector and assemble pACYC-*toxIN* (reverse)	GCGCAACGCAATTAATGTAAGTTAGCTCAC
CG-22	Amplify T4 *35.3* promoter (forward)	TAGAGGATCTGCTCATGTTTGACAGCTTATCCCTGGCATCGTAGAATTCATTATCC
CG-23	Amplify T4 *35.3* promoter (reverse)	ATGATCTCCGACTAGTACATATGAATCAAGTTTCAGTTACTATAACACGTACTGATAGATTTGTAAAATC
CG-24	Amplify T4 *rIIB2* promoter (forward)	TAGAGGATCTGCTCATGTTTGACAGCTTATCTCATGACATTTGGACAGTAACTAATCTTTTTG
CG-25	Amplify T4 *rIIB2* promoter (reverse)	ATGATCTCCGACTAGTACATATGAATCAAGTTAGCAACCATTTTATCACTGTTTTTTGAAGC
CG-26	Amplify T4 *soc* promoter (forward)	TAGAGGATCTGCTCATGTTTGACAGCTTATCCTTAAGAATTTAATTTACTTATTCAGAAAAGAAGATG
CG-27	Amplify T4 *soc* promoter (reverse)	ATGATCTCCGACTAGTACATATGAATCAAGTTATGATTATTTATACATCTTCTTTTCTGAATAAG
CG-28	Amplify pKVS45 backbone to assemble pKVS45-P$_{T4}$ (forward)	ATGCCCGTCGACAGGGAACTGCCAGGCATCAAATA
CG-29	Amplify pKVS45 backbone to assemble pKVS45-P$_{T4}$ (reverse)	GATAAGCTGTCAAACATGAGCAGATCCTCTACG
CG-30	Amplify *toxIN* for Gibson assembly into pKVS45-P$_{T4}$ (forward)	TTTATTTGATGCCTGGCAGTTCCCTCTTATATTGGATGAGAGCAAAAAAAATAGGTCC
CG-31	Amplify *toxIN* for Gibson assembly into pKVS45-P$_{T4}$ (reverse)	ATTTGATGCCTGGCAGTTCCCTCATTTTTTCTCGTAAAAAAGGCGACTGTGTATAG
CG-32	Amplify pKVS45-P$_{T4}$ for Gibson assembly with *toxIN* (forward)	ATACATGATGTCGGACAACTAGGTGCTTAGATGATCTCCGACTAGTACATATGAATCAAG
CG-33	Amplify pKVS45-P$_{T4}$ for Gibson assembly with *toxIN* (reverse)	AGGGAACTGCCAGGCATCAAATA
CG-34	Remove region upstream of *toxIN* in pKVS45-P$_{35.3}$-*toxIN* (forward)	TCAGTTACTATAACACGTACTGATAGATTTGTAAAATCTTTATAG
CG-35	Remove region upstream of *toxIN* in pKVS45-P$_{rIIB2}$-*toxIN* (forward)	AGCAACCATTTTATCACTGTTTTTTGAAGC
CG-36	Remove region upstream of *toxIN* in pKVS45-P$_{soc}$-*toxIN* (forward)	ATGATTATTTATACATCTTCTTTTCTGAATAAGTAAATTAAATTC
CG-37	Remove region upstream of *toxIN* in pKVS45-P$_{T4}$-*toxIN* (reverse)	AAGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTT
CG-38	Amplify P$_{35.3}$-*toxI* for construction of pBluescript- P$_{35.3}$-*toxI* (forward)	TAGAACTAGTGGATCCCCCGGGCTGCAGGACCTGGCATCGTAGAATTCATTATCC
CG-39	Amplify P$_{T4}$-*toxI* for construction of pBluescript- P$_{T4}$-*toxI* (reverse)	CCTCGAGGTCGACGGTATCGATTTACATTTTTTCTCGTAAAAAAGGCGACTGTG
CG-40	Amplify P$_{rIIB2}$-*toxI* for construction of pBluescript- P$_{rIIB2}$-*toxI* (forward)	TAGAACTAGTGGATCCCCCGGGCTGCAGGATCATGACATTTGGACAGTAACTAATCTTTTTG
CG-41	Amplify P$_{soc}$-*toxI* for construction of pBluescript- P$_{soc}$-*toxI* (forward)	CTAGAACTAGTGGATCCCCCGGGCTGCAGGACTTAAGAATTTAATTTACTTATTCAGAAAAGAAGATG
CG-42	Linearize pBluescript II SK(+) for construction of pBluescript- P$_{T4}$-*toxI* (forward)	TCCTGCAGCCCGGGGGATC
CG-43	Linearize pBluescript II SK(+) for construction of pBluescript- P$_{T4}$-*toxI* (reverse)	TAAATCGATACCGTCGACCTCGAGG
CG-44	Construct pdCas9deg-*toxIN* gRNA (forward)	CCACTAGTTTACAACTAGGTGCTTAGCAGTTTTAGAGCTAGAAATAGCAAG

CG-45	Construct pdCas9deg-*toxIN* gRNA (reverse)	GGACTAGTATTATACCTAGGACTGAG
CG-46	Northern blot probe for *toxI*	CTAGCTGCACTTAAAGGTAGCAAATCACCT
CG-47	Northern blot probe for *groL*	GTCGTTAGCGGCTTTAGTAATCTGGATACC