

## Supplementary Information

### **Type I-F CRISPR-Cas resistance against virulent phages results in abortive infection and provides population-level immunity**

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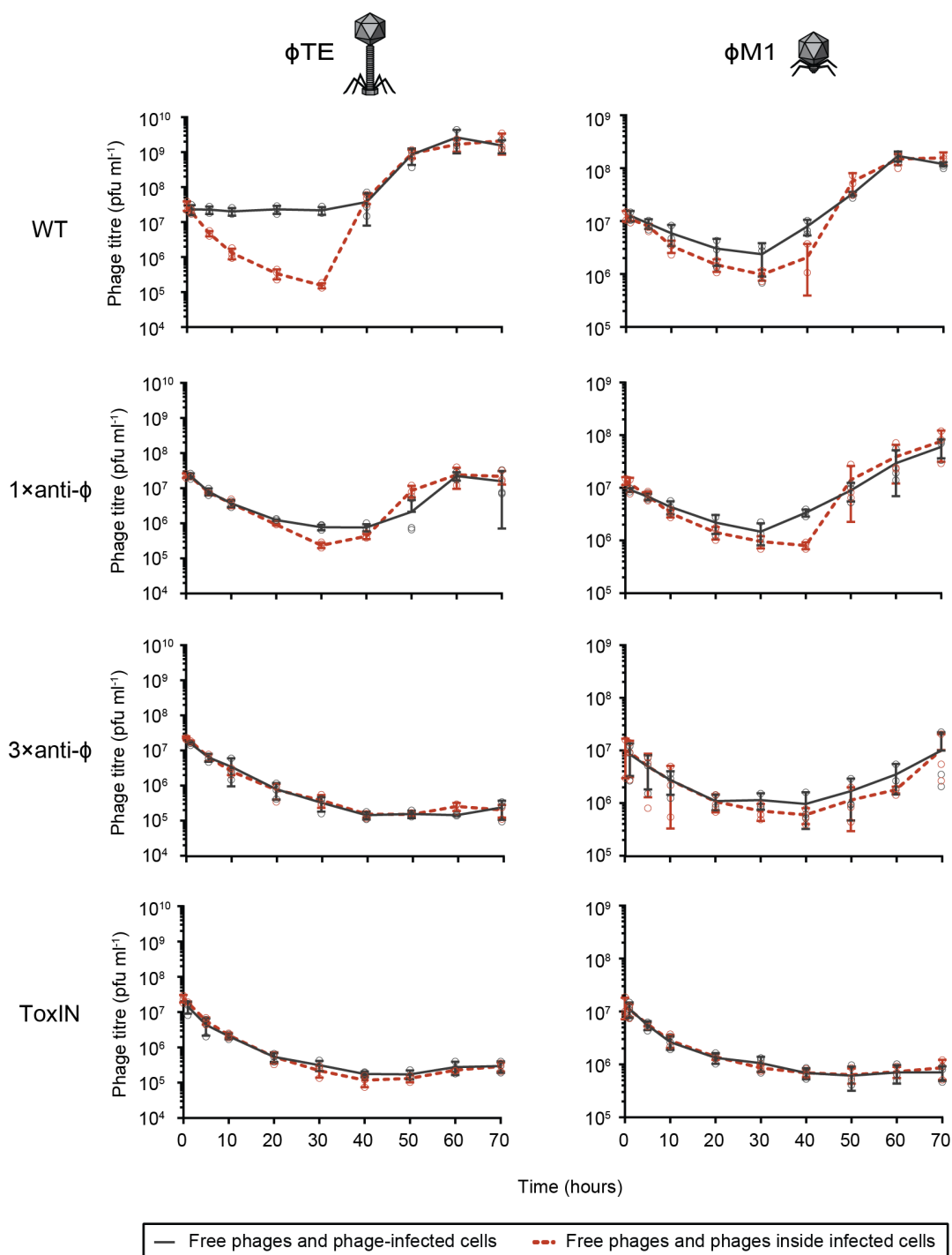
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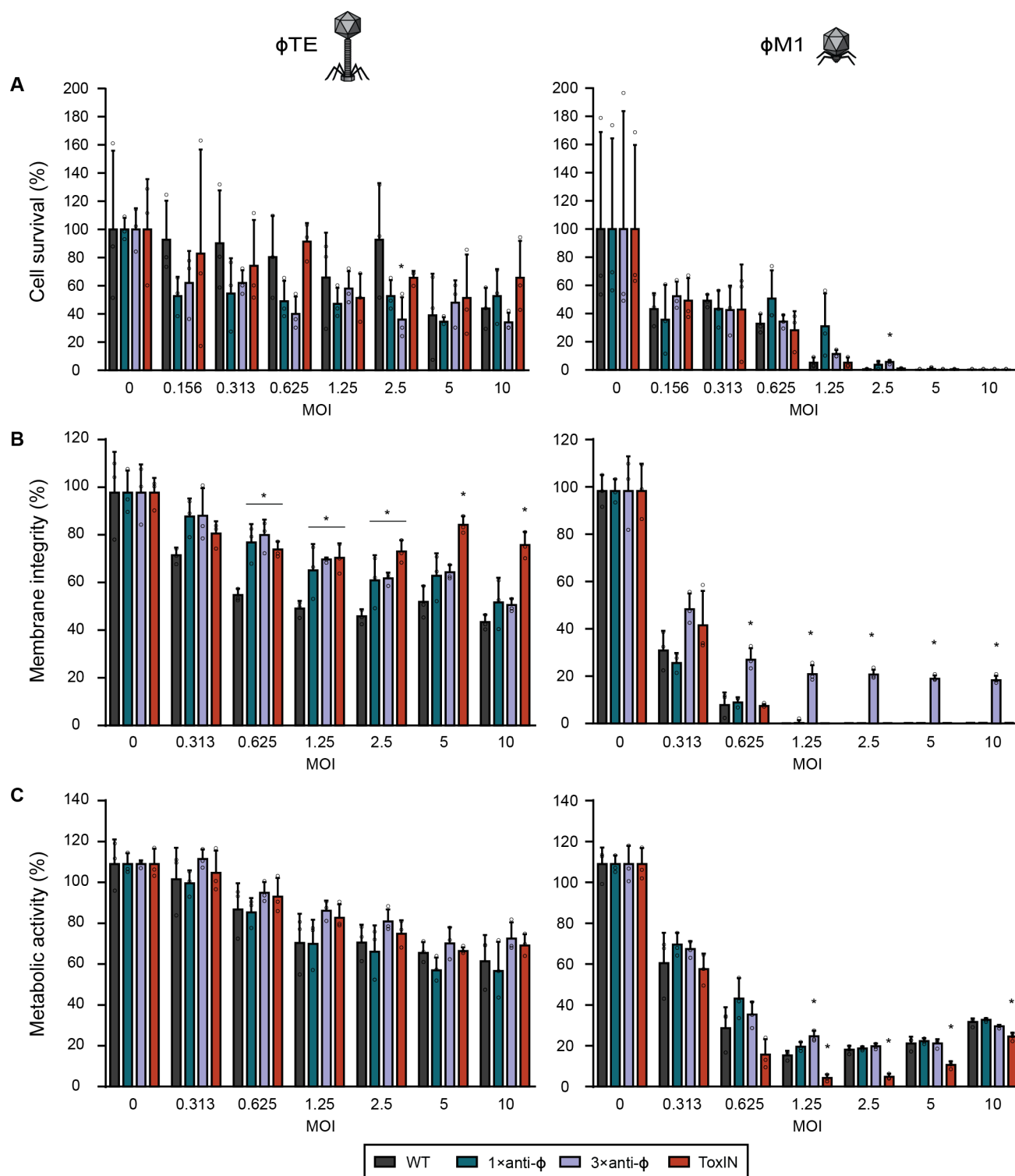
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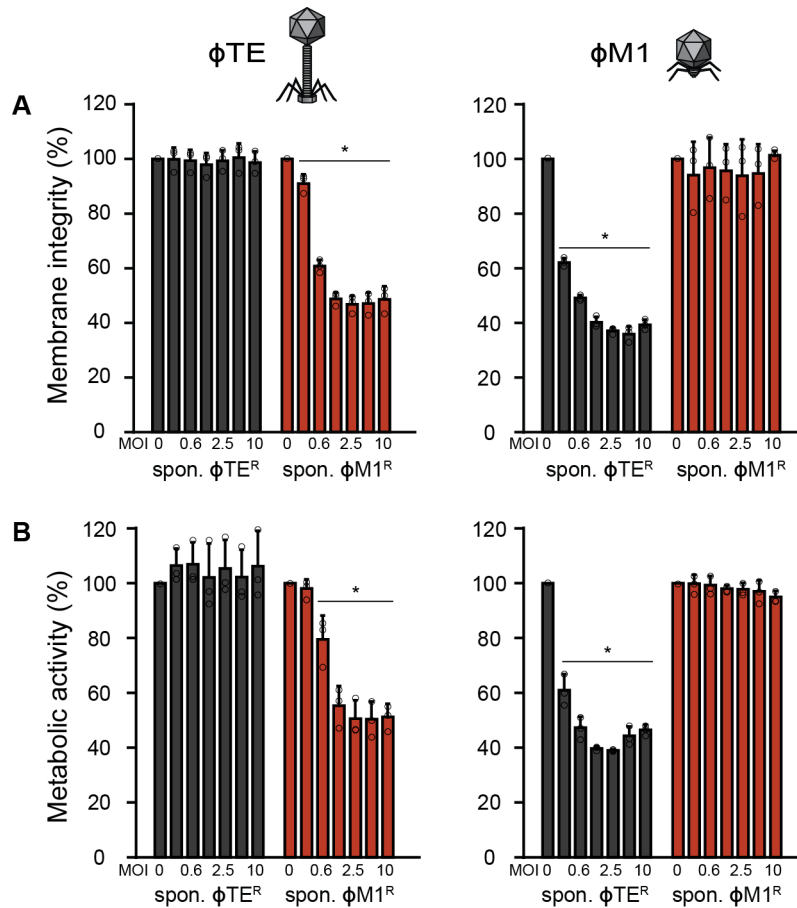
## Supplementary Figures



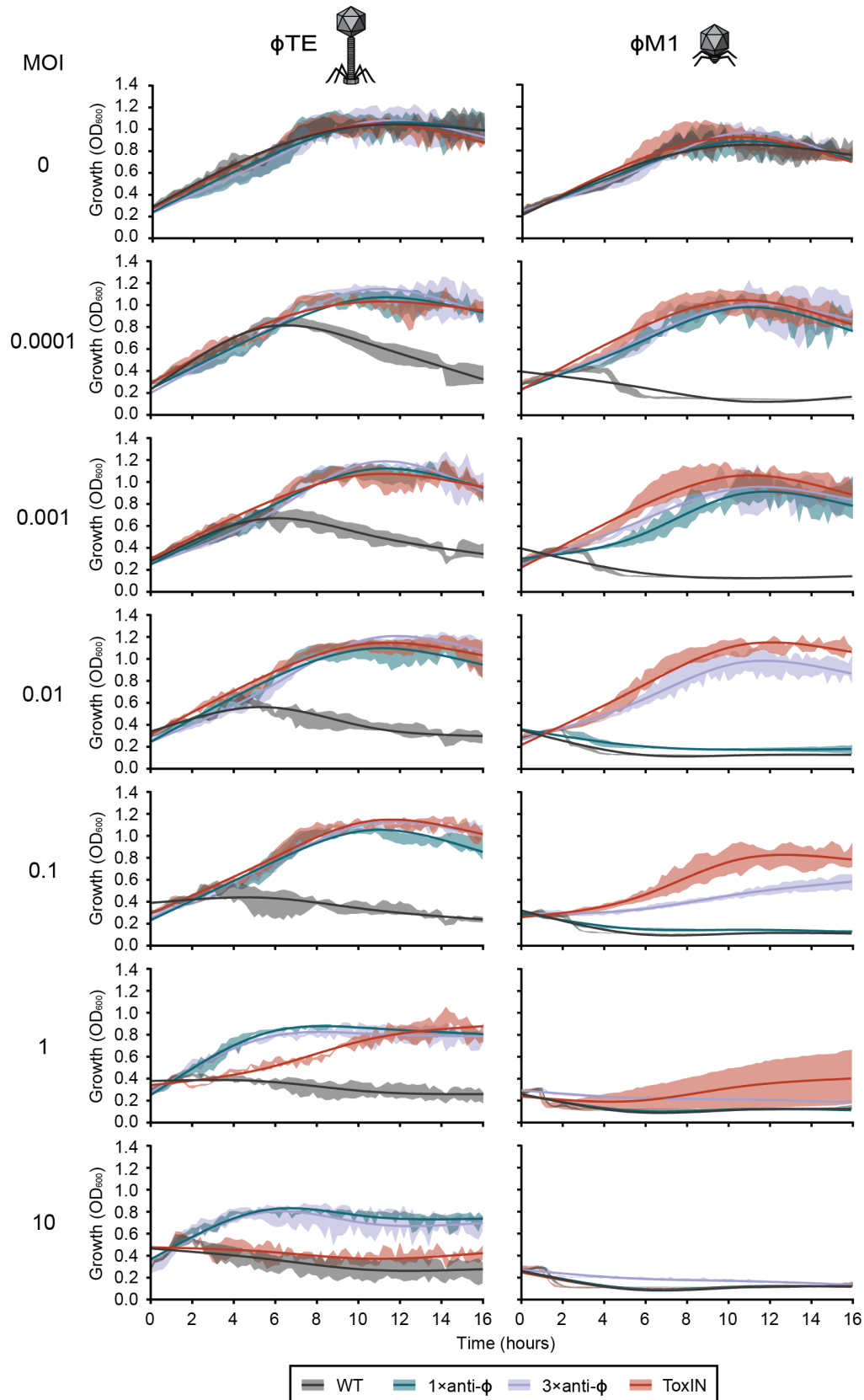
**Supplementary Figure 1. One-step growth curves provide insight into adsorption and phage burst size.** Assays were performed on strains infected at an MOI of ~0.1. Samples were non-treated (free phages and phage-infected cells, black line) or treated with chloroform (free phages and phages accumulated inside infected cells, red dashed line). In the  $\phi$ TE infected WT cells, the red line decreases since phages have adsorbed to the cell and have injected their DNA. As these samples are treated with chloroform to lyse the cells these ‘infecting’ phages are not seen as plaques. The black line does not go down, since the ‘infecting’ phages can continue replicating once the intact cells are plated. Strains with immunity mediated by CRISPR-Cas or ToxIN do not show this trend as there is reduced phage replication. Phage burst size and adsorption data was calculated for Fig. 1 and Table S1. Source data are provided as a Source Data file.



**Supplementary Figure 2. CRISPR-Cas immunity does not promote cell survival at a range of MOIs.** **A** Cell survival, **B** membrane integrity and **C** metabolic activity was assessed at a range of MOIs for WT, 1× and 3×anti-φ strains and ToxIN, using both φTE and φM1. Statistical significance was calculated using one-way ANOVA using Dunnett's multiple comparison test, comparing strains with targeting spacers to the control with no-targeting spacers. No significance was detected, unless indicated (\*  $p \leq 0.05$ ). Source data are provided as a Source Data file.



**Supplementary Figure 3. Spontaneous phage-resistant mutants are active in the presence of phages.** Spontaneous  $\phi TE^R$  and  $\phi M1^R$  mutants were infected with  $\phi TE$  and  $\phi M1$  at different MOIs (0, 0.3, 0.6, 1.25, 2.5, 5 and 10) and **A** membrane integrity **B** cell activity levels was assessed following one round of infection. Statistical significance was calculated using one-way ANOVA using Dunnett's multiple comparison test, comparing the phage-infected samples to the uninfected sample for each strain. No significance was detected, unless indicated (\*  $p \leq 0.05$ ). Source data are provided as a Source Data file.



**Supplementary Figure 4. Anti- $\phi$  strains grow in the presence of phages up to a MOI of 1.** Strains were grown in the presence of phages at different MOIs and OD<sub>600</sub> measurements were taken every 12 min for 16 hours. Solid lines: restricted cubic spline curve of the OD<sub>600</sub> values, shaded colour: one SD of the mean OD<sub>600</sub>. These are the full data from what is presented in Fig. 4. Source data are provided as a Source Data file.

## Supplementary Tables

**Supplementary Table 1.** Characteristics of phages  $\phi$ TE and  $\phi$ M1.

Phage/ host	EOP	ECOI (%)	Latent period (min)	Adsorption (%)	Burst size (phages)
<b><math>\phi</math>TE</b>					
WT	$1.0 \times 10^0 \pm 6.0 \times 10^{-2}$	$100 \pm 18.7$	$30 \pm 0$	$99 \pm 0.00$	$75 \pm 44$
1 $\times$ anti- $\phi$ TE	$2.0 \times 10^{-3} \pm 1.2 \times 10^{-3}$	$4.1 \pm 1.6$	$33 \pm 6$	$99 \pm 0.00$	$1 \pm 0$
3 $\times$ anti- $\phi$ TE	$1.1 \times 10^{-5} \pm 5.6 \times 10^{-6}$	$0.9 \pm 0.3$	n/d	$98 \pm 0.01$	<1
ToxIN	$1.9 \times 10^{-6} \pm 4.1 \times 10^{-7}$	$1.1 \pm 0.4$	n/d	$99 \pm 0.00$	<1
<b><math>\phi</math>M1</b>					
WT	$1.0 \times 10^0 \pm 4.2 \times 10^{-1}$	$100 \pm 50.7$	$37 \pm 6$	$92 \pm 0.04$	$13 \pm 3$
1 $\times$ anti- $\phi$ M1	$1.5 \times 10^{-1} \pm 6.5 \times 10^{-2}$	$22.5 \pm 17.4$	$37 \pm 6$	$93 \pm 0.03$	$6 \pm 3$
3 $\times$ anti- $\phi$ M1	$4.7 \times 10^{-3} \pm 2.1 \times 10^{-4}$	$6.3 \pm 3.5$	$40 \pm 0$	$90 \pm 0.08$	$1 \pm 0$
ToxIN	$2.3 \times 10^{-5} \pm 7.4 \times 10^{-6}$	$1.5 \pm 0.6$	n/d	$93 \pm 0.02$	<1

Data shown is the mean  $\pm$ SD. n/a not applicable. n/d no data the pfu values continue to decrease and there was no detectable phage burst.

**Supplementary Table 2.** Bacterial strains and plasmids used in this study.

Strain/Plasmid	Relevant Genotype/Phenotype	Reference
<b>Strains</b>		
<b><i>Escherichia coli</i></b>		
DH5α	F <sup>-</sup> , φ80Δ <i>lac</i> ZM15, Δ( <i>lac</i> ZYA– <i>arg</i> F)U169, <i>endA</i> 1, <i>recA</i> 1, <i>hsdR</i> 17 (r <sup>-</sup> m <sup>+</sup> ), <i>deoR</i> , <i>thi</i> -1, <i>supE</i> 44, λ <sup>-</sup> , <i>gyrA</i> 96, <i>relA</i> 1	Gibco/BRL
ST18	<i>recA</i> , <i>pro</i> , <i>hsdR</i> , <i>recA</i> ::RP4-2-Tc::Mu, λ <i>pir</i> , Tmp <sup>R</sup> , Sp <sup>R</sup> , Sm <sup>R</sup> , Δ <i>hemA</i>	1
<b><i>Pectobacterium atrosepticum</i></b>		
SCRI1043	Wild type (WT)	2
PCF81	SCRI1043 Δ <i>expl</i> :: <i>cat</i> , Cm <sup>R</sup>	3
PCF188	SCRI1043 with 3x anti-φTE spacers (in CRISPR1+2)	4
PCF190	SCRI1043 with 1x anti-φTE spacer (in CRISPR1)	5
PCF254	SCRI1043 with 1x anti-φM1 spacer (in CRISPR1)	5
PCF256	SCRI1043 with 3x anti-φM1 spacers (in CRISPR1+2)	5
PCF333	SCRI1043 with spontaneous φTE <sup>R</sup>	This study
PCF334	SCRI1043 with spontaneous φM1 <sup>R</sup>	This study
PCF610	SCRI1043 with integrated pPF1814 for <i>cas</i> operon overexpression	This study
<b>Plasmids</b>		
pBR322	Cloning vector, ColE1 ori, Tc <sup>R</sup> , Ap <sup>R</sup>	6
pPF260	pQE-80L derivative with RP4 oriT, Km <sup>R</sup>	7
pPF445	mini-CRISPR with 1 repeat, pBAD30-derivative (aka pC1-16), p15a ori, Ap <sup>R</sup>	3
pPF452	mini-CRISPR with single spacer targeting <i>expl</i> , pPF445 -	3
(“pCRISPR”)	derivative (aka pE1-16)), Ap <sup>R</sup>	
pPF459	pPF260-derivative with <i>P. atrosepticum expl</i> gene, Km <sup>R</sup>	This study
(“pTargeted”)		
pPF975	pPF260-derivative, IPTG-inducible CRISPR locus for expressing crRNAs, Km <sup>R</sup>	8
pPF1421	pPF975-derivative with the spacer from PCF254	This study
pPF1423	pPF975-derivative with the spacer from PCF190	This study
pPF1814	pSEVA511-derivative with T5/ <i>lac</i> promoter, MCS and <i>lacI</i> from pQE-80L-stuffer, and 500 bp of <i>cas1</i>	This study
pQE-80L-stuffer	pQE-80L (Qiagen) with the 6His removed by digestion with EcoRI and BamHI and these sites restored, Ap <sup>R</sup>	Josh Ramsay; unpublished
pSEVA511	R6K ori, Tc <sup>R</sup>	9
pTA46	pBR322-derivative containing <i>toxIN</i> , Ap <sup>R</sup>	10

**Supplementary Table 3.** Oligonucleotide sequences used in this study

Name	Sequence (5'-3')	Description
PF210	GTCATTACTGGATCTATCAACAGG	R 100 bp downstream of CRISPR locus in pPF975
PF314	TTTGGTACCGGATCCGTGGCAATGATTA CTCCATC	F for amplifying <i>expI</i> from <i>P. atropeticum</i> (BamHI)
PF317	TTTTCTAGACTGATGAATGGGTGAATCT C	R for amplifying <i>expI</i> from <i>P. atropeticum</i> (XbaI)
PF357	GACGAATTCTTACGGAAGAAAATACATT ATGG	F for amplifying <i>cas1</i> N-terminal (EcoRI)
PF669	TTTCCCGGGAAAGGTAAAGCGCGATT AC	R for amplifying 500 bp into <i>cas1</i> (XmaI)
PF2511	TCTCCCGGGAGGCATCAAATAAACGA	F for amplifying <i>lacI</i> from pQE-80L (XmaI)
PF2512	TCTGTCGACACACCATCGAATGGTGCA	R for amplifying <i>lacI</i> from pQE-80L (SalI)
PF2565	GAAAACTAGCGTCTGTAGTGGGTCGTT GTGCAAGTAG	F for cloning PCF254 spacer into pPF975
PF2566	TGAACTACTTGCACAACGACCCACTACA GACGCTAGT	R for cloning PCF254 spacer into pPF975
PF2569	GAAATGACACAGCCAACGCCCTGAAAA TCGGCACAGG	F for cloning PCF190 spacer into pPF975
PF2570	TGAACCTGTGCCGATTTTCAGGGCGTT GGCTGTGTCA	R for cloning PCF190 spacer into pPF975
PF3494	TTTGCGGCCGCTCGTCTTCACCTCGAG AAATC	F for amplifying pQE-80L MCS (NotI)
PF3495	TTTGCGGCCGCGTCATTACTGGATCTAT CAACAGG	R for amplifying pQE-80L MCS (NotI)



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