Supplementary Information

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

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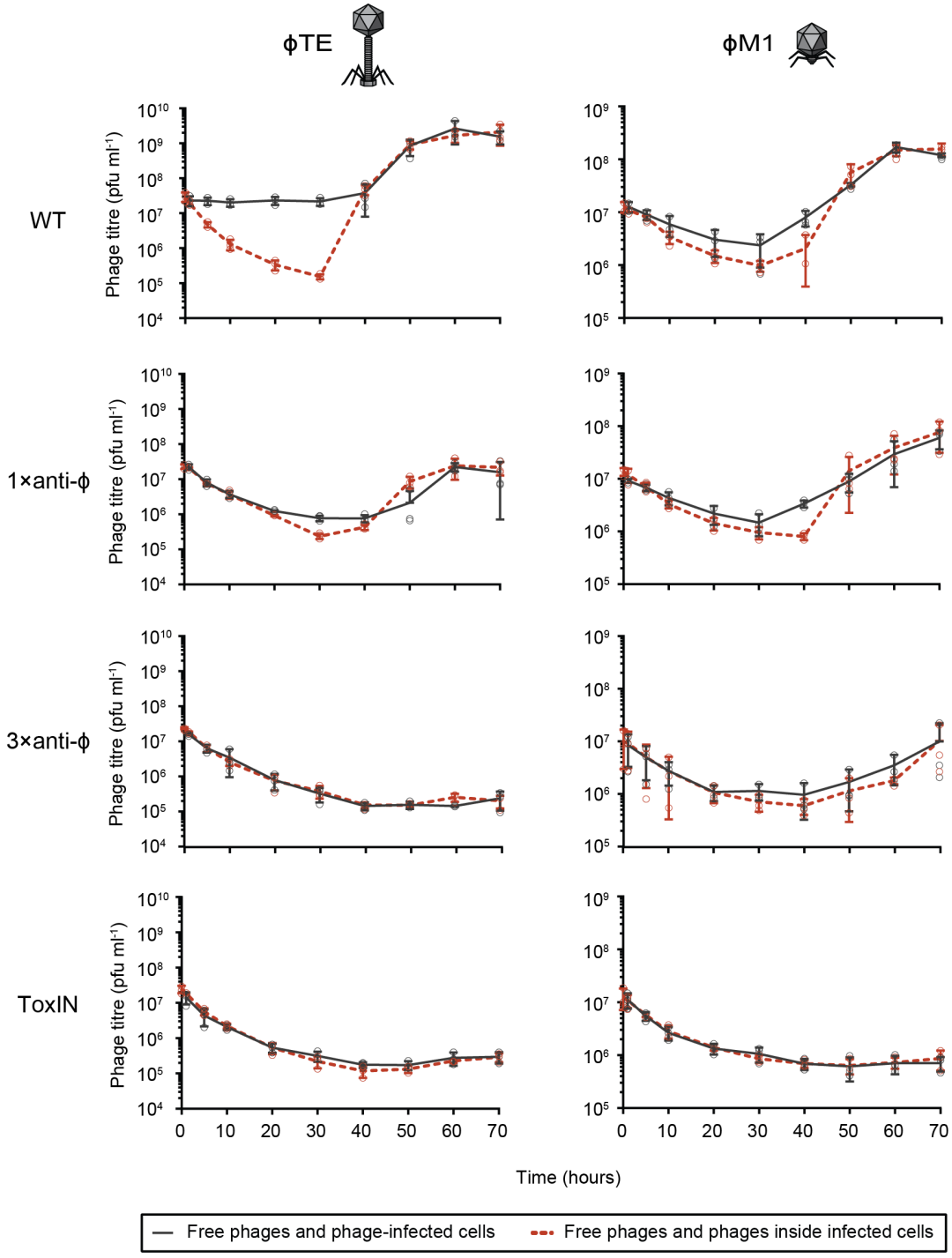
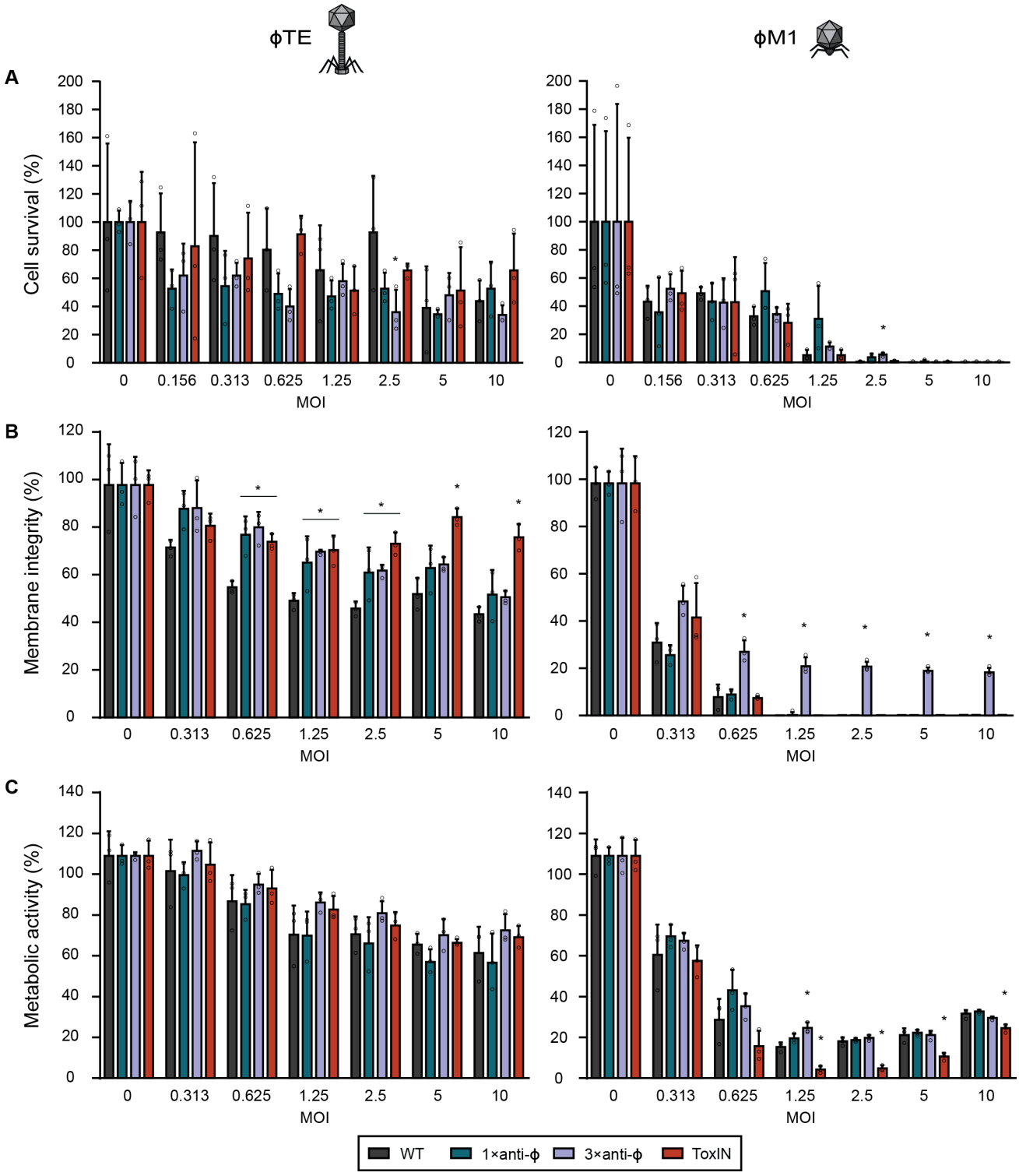
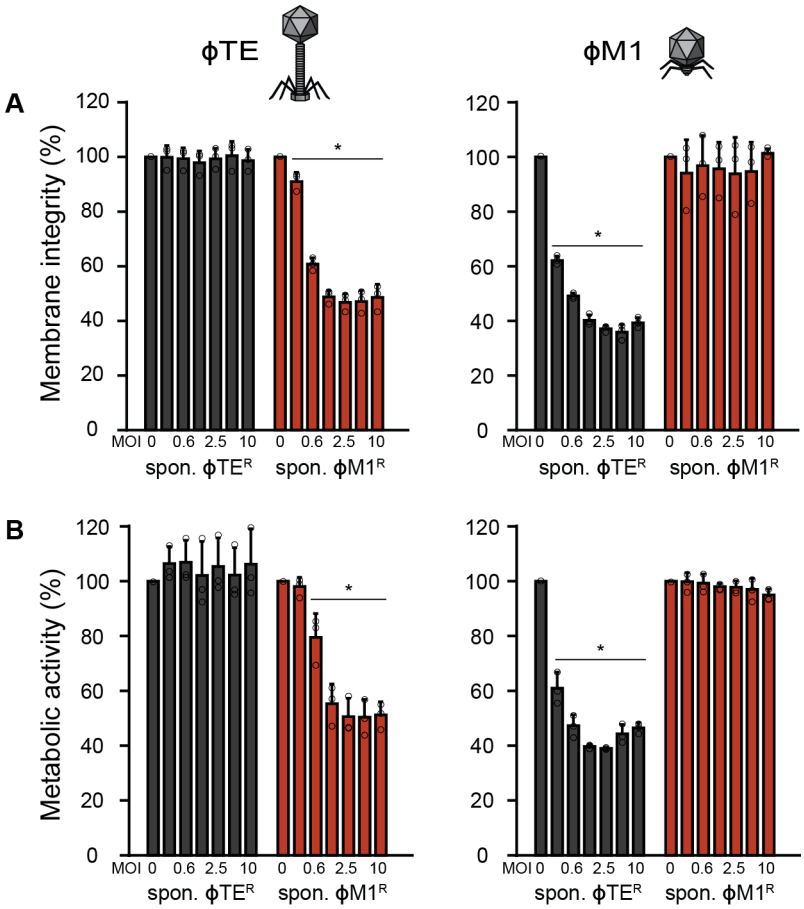
Supplementary Figures



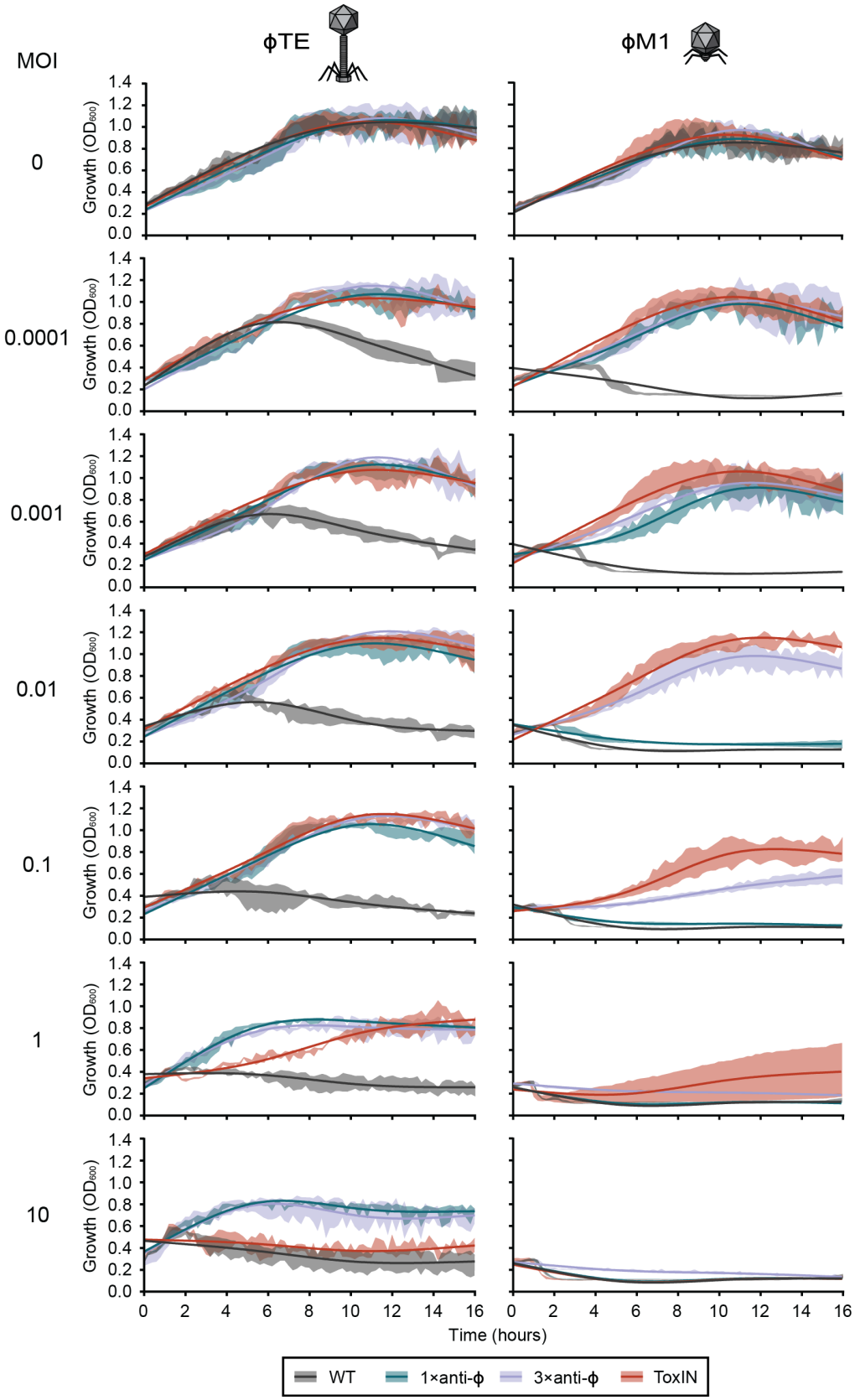
Supplementary Figure 1. One-step growth curves provide insight into adsorption and phage burst size. Assays were performed on strains infected at an MOI of ~0.1. Samples were non-treated (free phages and phage-infected cells, black line) or treated with chloroform (free phages and phages accumulated inside infected cells, red dashed line). In the ϕTE 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 survivial 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 ϕTE^R and ϕM1^R mutants were infected with ϕTE and ϕM1 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-ϕ 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_{600} measurements were taken every 12 min for 16 hours. Solid lines: restricted cubic spline curve of the OD_{600} values, shaded colour: one SD of the mean OD_{600}. 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 ϕTE and ϕM1.

Phage/ host	EOP	ECOI (%)	Latent period (min)	Adsorption (%)	Burst size (phages)
ϕTE					
WT	$1.0\times10^{0} \pm 6.0\times10^{-2}$	100 ±18.7	30 ±0	99 ±0.00	75 ±44
1×anti-ϕTE	$2.0\times10^{-3} \pm 1.2\times10^{-3}$	4.1 ±1.6	33 ±6	99 ±0.00	1 ±0
3×anti-ϕTE	$1.1\times10^{-5} \pm 5.6\times10^{-6}$	0.9 ±0.3	n/d	98 ±0.01	<1
ToxIN	$1.9\times10^{-6} \pm 4.1\times10^{-7}$	1.1 ±0.4	n/d	99 ±0.00	<1
ϕM1					
WT	$1.0\times10^{0} \pm 4.2\times10^{-1}$	100 ±50.7	37 ±6	92 ±0.04	13 ±3
1×anti-ϕM1	$1.5\times10^{-1} \pm 6.5\times10^{-2}$	22.5 ±17.4	37 ±6	93 ±0.03	6 ±3
3×anti-ϕM1	$4.7\times10^{-3} \pm 2.1\times10^{-4}$	6.3 ±3.5	40 ±0	90 ±0.08	1 ±0
ToxIN	$2.3\times10^{-5} \pm 7.4\times10^{-6}$	1.5 ±0.6	n/d	93 ±0.02	<1

Data shown is the mean ±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
Strains		
Escherichia coli		
DH5α	F-, φ80Δ*dlacZ*M15, Δ(*lacZYA–argF*)U169, *endA1*, *recA1*, *hsd*R17 ($r_K^- m_K^+$), *deoR*, *thi-1*, *supE*44, λ-, *gyr*A96, *rel*A1	Gibco/BRL
ST18	*recA, pro, hsdR, recA*::RP4-2-Tc::Mu, λ*pir*, Tmp^R, Sp^R, Sm^R, Δ*hemA*	[1]
Pectobacterium atrosepticum		
SCRI1043	Wild type (WT)	[2]
PCF81	SCRI1043 Δ*expI*::*cat*, Cm^R	[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^R	This study
PCF334	SCRI1043 with spontaneous $\phi M1^R$	This study
PCF610	SCRI1043 with integrated pPF1814 for *cas* operon overexpression	This study
Plasmids		
pBR322	Cloning vector, ColE1 ori, Tc^R, Ap^R	[6]
pPF260	pQE-80L derivative with RP4 oriT, Km^R	[7]
pPF445 ("pControl")	mini-CRISPR with 1 repeat, pBAD30-derivative (aka pC1-16), p15a ori, Ap^R	[3]
pPF452 ("pCRISPR")	mini-CRISPR with single spacer targeting *expI*, pPF445 -derivative (aka pE1-16)), Ap^R	[3]
pPF459 ("pTargeted")	pPF260-derivative with *P. atrosepticum expI* gene, Km^R	This study
pPF975	pPF260-derivative, IPTG-inducible CRISPR locus for expressing crRNAs, Km^R	[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/*lac* promoter, MCS and *lacI* from pQE-80L-stuffer, and 500 bp of *cas1*	This study
pQE-80L-stuffer	pQE-80L (Qiagen) with the 6His removed by digestion with EcoRI and BamHI and these sites restored, Ap^R	Josh Ramsay; unpublished
pSEVA511	R6K ori, Tc^R	[9]
pTA46	pBR322-derivative containing *toxIN*, Ap^R	[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	TTTGGTACCGGATCCGTGGCAATGATTACTCCATC	F for amplifying *expI* from *P. atrospeticum* (BamHI)
PF317	TTTTCTAGACTGATGAATGGGTGAATCTC	R for amplifying *expI* from *P. atrospeticum* (XbaI)
PF357	GACGAATTCTTACGGAAGAAAATACATTATGG	F for amplifying *cas1* N-terminal (EcoRI)
PF669	TTTCCCGGGAAAGGTAAAGCGCGATTCAC	R for amplifying 500 bp into *cas1* (XmaI)
PF2511	TCTCCCGGGAGGCATCAAATAAAACGA	F for amplifying *lacI* from pQE-80L (XmaI)
PF2512	TCTGTCGACACACCATCGAATGGTGCA	R for amplifying *lacI* from pQE-80L (SalI)
PF2565	GAAAACTAGCGTCTGTAGTGGGTCGTTGTGCAAGTAG	F for cloning PCF254 spacer into pPF975
PF2566	TGAACTACTTGCACAACGACCCACTACAGACGCTAGT	R for cloning PCF254 spacer into pPF975
PF2569	GAAATGACACAGCCAACGCCCTGAAAATCGGCACAGG	F for cloning PCF190 spacer into pPF975
PF2570	TGAACCTGTGCCGATTTTCAGGGCGTTGGCTGTGTCA	R for cloning PCF190 spacer into pPF975
PF3494	TTTGCGGCCGCTCGTCTTCACCTCGAGAAATC	F for amplifying pQE-80L MCS (NotI)
PF3495	TTTGCGGCCGCGTCATTACTGGATCTATCAACAGG	R for amplifying pQE-80L MCS (NotI)

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