Supplementary Information

Different genetic and morphological outcomes for phages targeted by single or multiple CRISPR-Cas spacers

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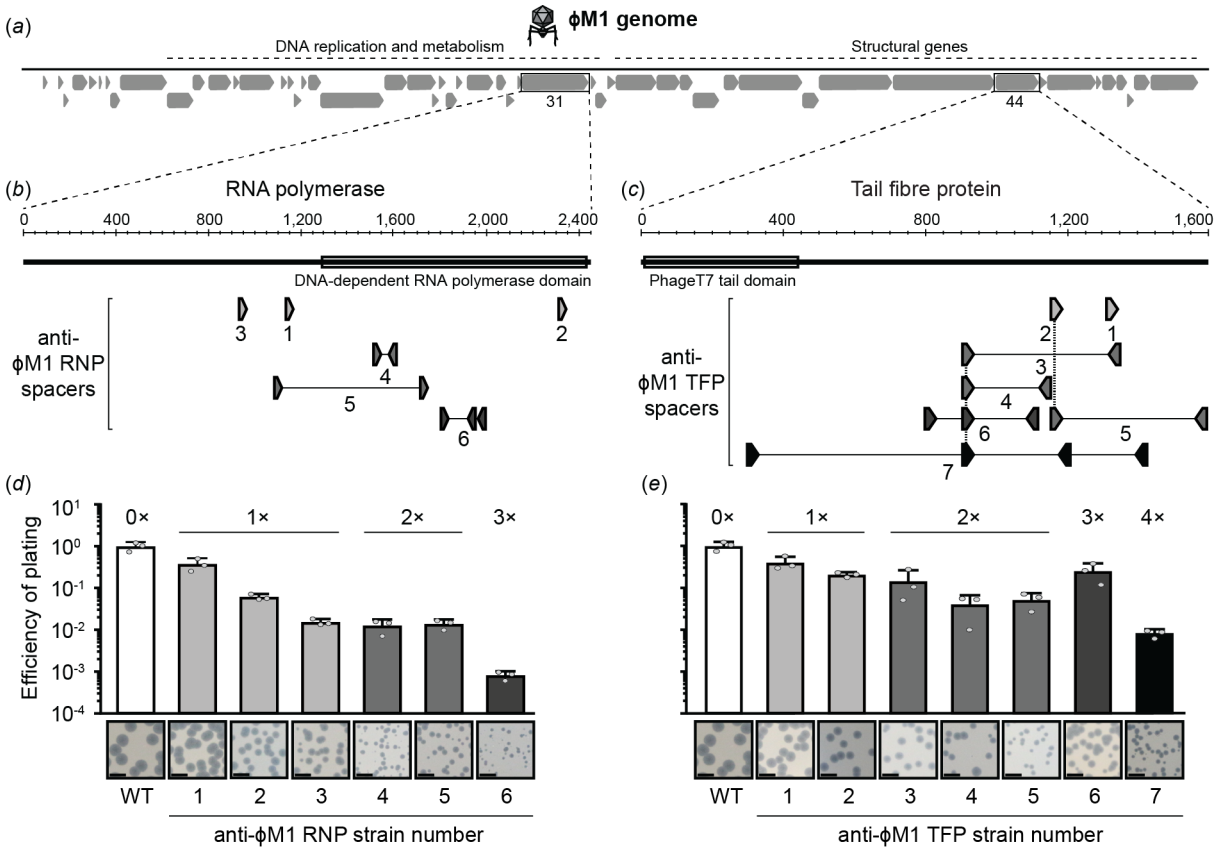

Figure S1. Phage-targeting spacers reduce phage infectivity. (*a*) Schematic of the ϕM1 genome showing the locations of (*b*) the RNA polymerase gene (RNP, phiM1_31) with the targeting spacers, and (*c*) a tail fibre protein gene (TFP, phiM1_44) with the targeting spacers. EOPs and representative plaque morphology images of the (*d*) anti-ϕM1 RNP and (*e*) anti-ϕM1 TFP strains, with white bars: WT (0 spacers), light grey: 1×, mid grey: 2×, dark grey: 3× and black: 4×anti-ϕ spacers. Data shown in the mean +SD (*n*=3). Spacers shared by different strains have been identified with vertical dashed lines (*c*) and the limit of detection in (*d*) and (*e*) is 1×10^{-10}. The plaque image scale bars represent 5 mm. Full details of EOP and plaque size measurements are in table S3.

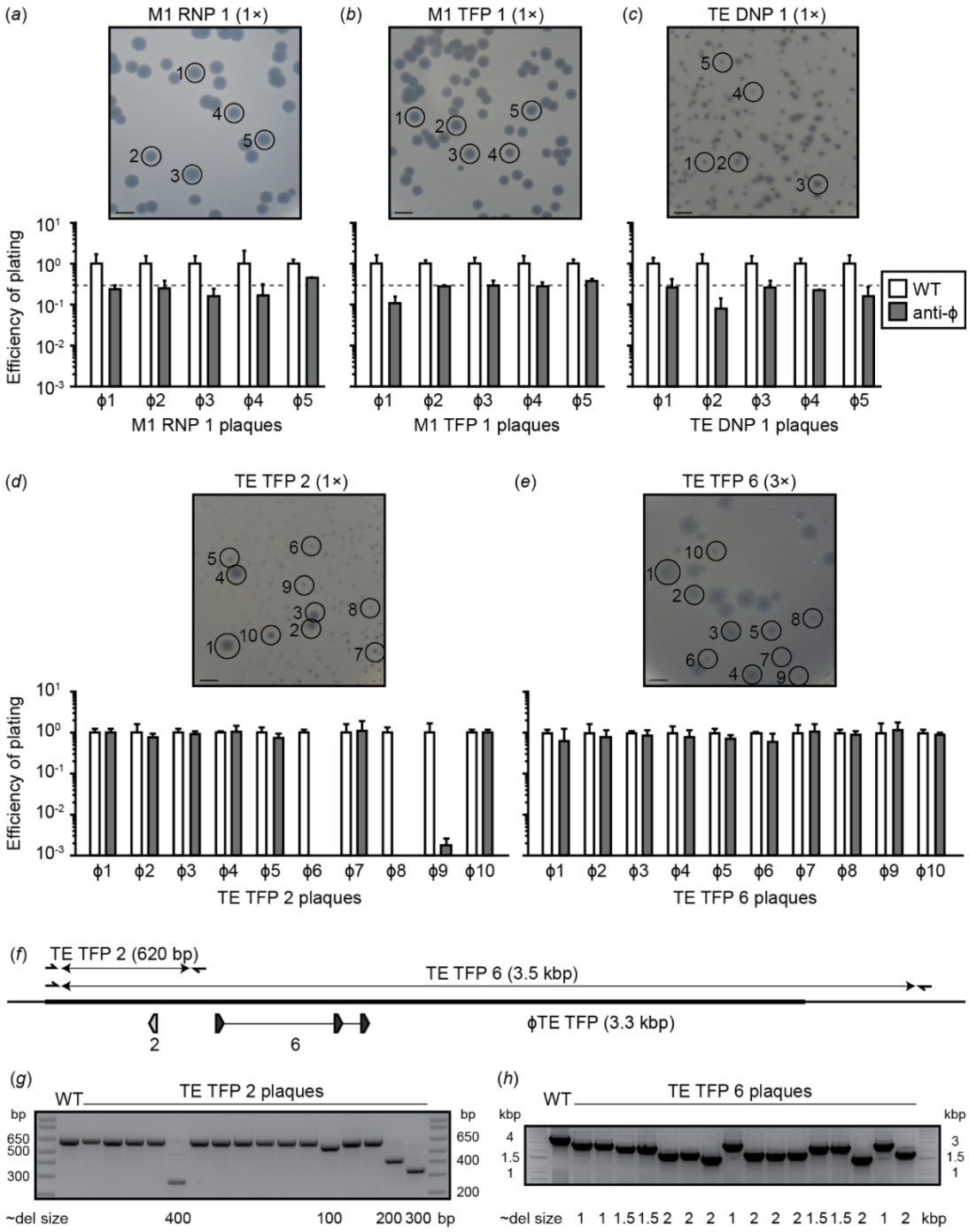

Figure S2. Phages that replicate on phage-resistant strains are phenotypic or genotypic escapes. (*a*), (*b*) & (*c*) Five plaques were retitred on WT (white) and the anti-ϕ strain (grey) from (*a*) M1 RNP 1, (*b*) M1 TFP 1 and (*c*) TE DNP 1. The dashed line represents the EOPs for the strains from figure 1. (*d*) & (*e*) 10 plaques were picked and retitred on WT (white) and the anti-ϕ strain (grey) from (*d*) TE TFP 2 and (*e*) TE TFP 6. The EOPs for the strains were below the limit of detection. (*f*), (*g*) & (*h*) 16 genotypic escape phages, that infected the anti-ϕ strains with an EOP of 1, from (*g*) TE TFP 2 and (*h*) TE TFP 6 were screened for deletions using PCR. (*f*) Schematic showing the ϕTE TFP gene and the primers used to amplify the region targeted by the (*g*) TE TFP 2 spacer (PF1635 and PF2146) and the entire gene, plus 200 bp downstream for (*h*) TE TFP 6 (PF1635 and PF2144, table S2). Product sizes were compared to the Invitrogen 1 kb Plus DNA ladder.

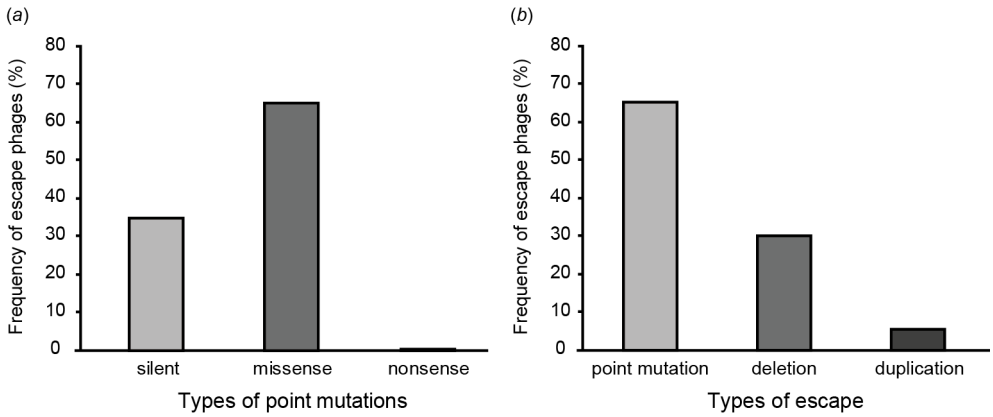

Figure S3. More phages evade CRISPR-Cas targeting of a single spacer through point mutation than deletion or duplication. (a) The consequences of the point mutations on the coding sequence and (b) the frequency type of escape.

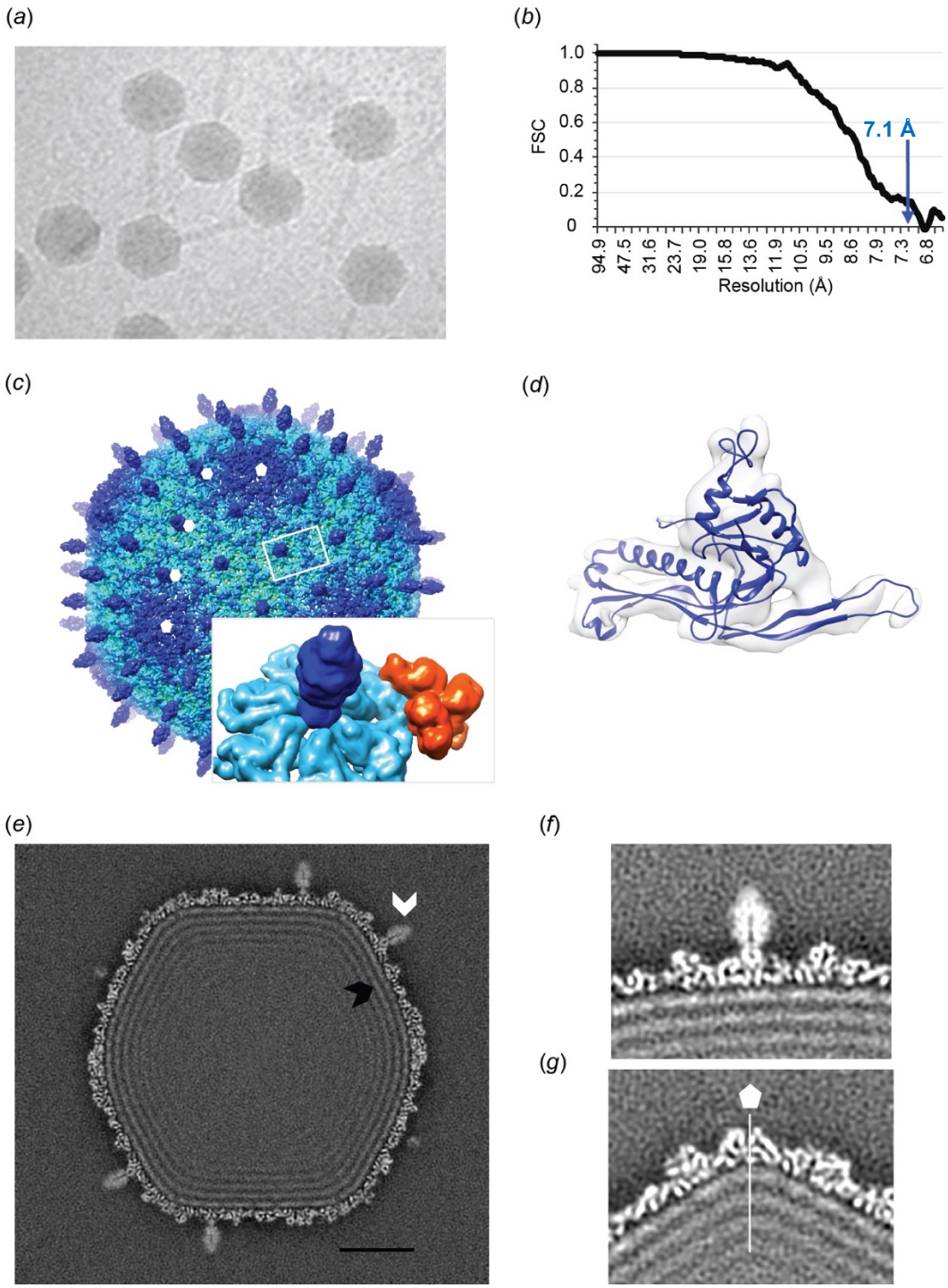

Figure S4. Cryo-EM analysis of wild type ϕTE. (*a*) Area of a cryo electron micrograph showing ϕTE phages. (*b*) Fourier shell correlation (FSC) curve showing a 7.1Å resolution for the full capsid. (*c*) Radially coloured surface representation of the capsid showing the T=13 triangulation number. A total of 780 copies of the major capsid protein form 12 pentamers and 120 hexamers that assemble into an icosahedral capsid, which has a maximum diameter of 980 Å. The five-fold axes are indicated by a pentagon, while the centre of the hexamers are indicated by hexagons. At the bottom, the insert shows the segmented densities corresponding to the decoration proteins (blue), hexamers (cyan), and trifolium-like density (red). The decoration protein appears to have a thin, elongated cavity at its interior. (*d*) Individual density of the major capsid protein (transparent) shows the specific HK97 fold (blue ribbon – protein data bank accession no: 2FT1). (*e*) Central slice through the map; concentric rings of DNA with a 25Å spacing are visible inside the capsid (black arrow) and pagoda-shaped decoration proteins (white arrow) are present at the exterior of the capsid and (*f*) are shown as a slice through the map. (*g*) Slice through the cryo-EM along the five-fold axis showing the induced curvature on the capsid coat and the interior genome shells.

Table S1. Strains with phage-targeting spacers

Strain	PCF#	#*	Pos†	CRISPR1 sequence‡	Pos	CRISPR2 sequence	Ref
TE DNP							
1	PCF452	1	-1	AACTTATTGAAAAGTCTAAGATCGTATCCGTA			This study
2	PCF461	1	-2	CCTGCTTGCCGTTGAGACTTCCCATGCACTTG			This study
			-1	*GAGCGCTTTTCCGCTGCATAACCCTGCTTCGG*			
3	PCF454	1	-1	TCACGCAGTTTAAGCGCCGCCTCTTCGTCAAA			This study
4	PCF455	1	*-1*	*GCGACGATAGTCATGCCCCGCGCCCACCGGAA*	-1	GTCCTCGATTACACGGTCGCAATACTTCCACA	This study
5	PCF456	2	-2	ATGATCAGGCCGTTAAGTTTATTGCTTCAAAA			This study
			-1	TTGTCAGGACGCTGTTTCTTAAACTGGTCCTT			
6	PCF453	2	-2	GTATCCGTAGGCATTGTGACATGCCAAAGCCC	-1	AGATAATTGGCATATGTTTCTACCCAAAGAAA	This study
			-1	*CTTGATCCGGCAAACAAACCACCGCTGGTAGC*			
7	PCF457	3	-2	TCTCCGGGCAACCACGGGGAAGGGGTCGATCG	-2	CCCAAGTGCATGGGAAGTCTCAACGGCAAGCA	This study
			-1	ATGATCAGGCCGTTAAGTTTATTGCTTCAAAA	*-1*	*TAGGTTGAGGCCGTTGAGCACCGCCGCCGCAA*	
8	PCF460	3			-4	CTGTTTCGCCATGTGTCCTTCTACCCTTTCGA	This study
					-3	GATCGTATCGACGTGATGATGAGGGACATCGA	
					-2	*TTCTGCATTACGGGGCCGTCGGAGGGGAAGTT*	
					-1	GATTTCAAAGGACCTCCGGCGAAGTCGTTCAA	
9	PCF458	4	-2	GTTCATCCTTGAACATAAACCTTTTATCGTCT	-2	ATGATCAGGCCGTTAAGTTTATTGCTTCAAAA	This study
			-1	AAAATGAAGCCCGTTGGTCCGCACGGACTGGA	-1	CCCAAGTGCATGGGAAGTCTCAACGGCAAGCA	
TE TFP							
1	PCF194	1	-1	GGCCAGTCCCTTGCGGACCTTCCGGTCCGATT			This study
2	PCF190	1	-1	CTGTGCCGATTTTCAGGGCGTTGGCTGTGTCA			This study
3	PCF189	1	-2	TCAGAACTGGCAACATAAAGACCCTGCTCACT			This study
			-1	*TGTTCCTTCTTCACTGTCCCTTATTCGCACCT*			
4	PCF192	1	-2	TCACATCAACCTGGGTCTTGGCCTTTCCAGTT			This study
			-1	*AGCTGTCCCTGATGGTCGTCATCTACCTGCCT*			
5	PCF191	2	-2	TGGGTCGGCTTTGATTTCCAGAGTCTTCTTCG			This study
			-1	CAGATCCTTCTACGAGCACCTCTGGCGGGTCT			
6	PCF188	3	-2	AGAAGGGTGACACCGGAGACACCGGTGCAGTG	-1	AAGAATGCCAACGCCGTAAACATCCTCGGTAA	(1)
			-1	GTTTGGCTGGATAACGGTCACACTGGTTCCCA			
M1 DNP							
1	PCF247	1	-2	CTCCTTAATGCTCAAGTACTTAACGGAGTTCA			This study
			-1	*CCGTACATGAACTGAGGGGACAGGATGTCCCA*			
2	PCF254	1	-1	TACTTGCACAACGACCCACTACAGACGCTAGT			This study
3	PCF253	1	-1	TGCGTAGTGTGTATCAAACCGGTATCTATAAT			This study
4	PCF249	2	-2	ACCGCCGATAGGGTCACGCATTACTGCTGAGA	-1	TCGCCAGAGACTTGGGAGACGGGTATCGCAGT	This study
			-1	*GGGATGTCGGCGGGGTGCTTCACGTAGGCCTT*			
5	PCF252	2	*-3*	*CTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCT*			This study
			-2	ACTTGCGGGTACAGCCGCCTCAATCCCTTTGC			
			-1	ATCCAGAGCAGGCACTGTACTGGGGCACGCAC			
6	PCF256	2	-2	ACATTGAATAGTTGCACGGAGTACGTGTACTT	-1	ATCCTTGGGCATCTGTCCTGCCAAACCACGCA	This study
			-1	ACTTGCGGGTACAGCCGCCTCAATCCCTTTGC			
M1 TFP							
1	PCF289	1			-1	AGCCTTAGTTTACCTGCGGGCGATTGGGAACT	This study
2	PCF290	1	-2	ACAACGATATCTCAAACCTAGCTGCCCTGACA			This study
			-1	*CTGACGCTCAGTGGAACGAAAACTCACGTTAA*			

(Table 3.1 continued)

Strain	PCF#	#*	Pos†	CRISPR1 sequence‡	Pos	CRISPR2 sequence	Ref
3	PCF294	2	-2	GTTTAACCACGCCACTTAGTGCTGCACAGGGT	-1	TTCCAGTTCCCAATCGCCCGCAGGTAAACTAA	This study
			-1	*GGTGTGGTCGCCATGATCGCGTAGTCGATAGT*			
4	PCF293	2	-1	GTTTAACCACGCCACTTAGTGCTGCACAGGGT	-1	TAGCGCACGTGCTAACCTCGGCGCAGCTATGA	This study
5	PCF292	2	-2	ACAACGATATCTCAAACCTAGCTGCCCTGACA			This study
			-1	CTAACCCGCTCGCGCGTACGTACCCATTAGC			
6	PCF295	3	-3	GTTTAACCACGCCACTTAGTGCTGCACAGGGT	*-2*	*TCCGCACCAACGCGCAGCCCGGACTCGGTAAT*	This study
			-2	*AAATCAAAACTGGTGAAACTCACCCAGGGATT*	-1	GCACAGCTTAGTGCGGTACAGGCAAACCTACA	
			-1	CGGAGCCAGCCCCGGTACCCCCATTAGTAACT			
7	PCF296	4	-4	GTTTAACCACGCCACTTAGTGCTGCACAGGGT	*-2*	*ATGTCATGATAATAATGGTTTCTTAGACGTCA*	This study
			-3	TTAGCGAGGTGCCGCCGGCTTCCATTCAGGTC	-1	CAGATGATTTACCTTGCGCAGGAGTTCACTGA	
			-2	TAACCCAGTAATACCCCCTGTCAGGGCAGCTA			
			-1	GGTAACACCCCTGTGGTGGTGCTGACACCAAA			
TE TMP							
1	PCF258	1			-1	GGATGCCTTCTCCCACTCTTTGCCAACCTGAC	This study
2	PCF267	1	-1	TCTTGATGGTGATGTCACCTTCGACTTTCTGC			This study
3	PCF269	1	-1	TACCCCATAGGCTGCTCCCGCATTCAGCAGTT			This study
4	PCF273	1	-1	CTGAATGCCTTGTCCTGCACCGTCTCGTCCAA			This study
5	PCF266	1	*-2*	*TGACGATGAGCGCATTGTTAGATTTCATACAC*			This study
			-1	TCAGTCTCAAAAGTGACTCGGTTCGTGGTTCT			
6	PCF275	1	-1	GCCCAGCAGATGCGCCTGGTGAATAAGCGTTT			This study
7	PCF271	2	-1	CATTGCAACACGGTTACTTTCCAATGCTTTCG	-1	GCATTCAGGACTTGAACCGCCAGTTCCGTGCT	This study
8	PCF264	2	*-2*	*GTCGATAGTGGCTCCAAGTAGCGAAGCGAGCA*	-2	GAGGAGCAGAGTTGATCCGGGTAACATTCATC	This study
			-1	*CCGATGCCGCCGGAAGCGAGAAGAATCATAAT*	-1	ACGAATTTGATTCGCTGGCCCGCCTCCTCAGA	
9	PCF263	2	-1	AGCAGAGTTGATCCGGGTAACATTCATCGGGC	-2	GCACCTGCACCACCGCCAAGACCTGCGCCGTT	This study
					-1	*CGATAGCGGAGTGTATACTGGCTTAACTATGC*	
10	PCF261	2	-1	GAGGAGCAGAGTTGATCCGGGTAACATTCATC	-1	ATACGCTGCTGGGCCATTACATCATCAATGCG	This study
11	PCF268	2	-2	GGAGGCCGCATTCATACCAATCGTGGCTTTAC	*-1*	*TCATGATAATAATGGTTTCTTAGACGTCAGGT*	This study
			-1	TTTGAGACTGACAAGGCGTCCTATGCCCGGGC			
12	PCF274	3	-3	CCAGTCGAATACTTCTTTCAACAGGTCTCCTT			This study
			-2	TCAGGGACTTGGCAACGATCCTCGACAGTAAT			
			-1	TCGACAAGAAGCTTGCAGTCCTTGAAGCGCCA			
13	PCF257	3	-3	TTCACCGAAACCACCTTGGAAGATTTGGTTCT			This study
			-2	CATGAAGCCGTATTCTTTCTGGAATGTCTCTT			
			-1	GAATTGAAAGGTCAGTTGGCGGAAGGTATCCC			
14	PCF259	2	-3	AGCAGGTACAGCAGATGACATCTCCTCGTGCA			This study
			-2	TTTGAGACTGACAAGGCGTCCTATGCCCGGGC			
			-1	ACTACGGATACGGCCTGCCATCGCACGGTTTT			
15	PCF272	3	-1	GTCGCAGCTACGAAGGCCGCCTTGGACGAGAC	-2	CCCGGATCAACTCTGCTCCTCTCTCCCCTCGT	This study
					-1	AATACCGACGCGCCGGAGGCGAATACCCCATA	
16	PCF262	3	-2	GCCTACGACAAATCAGCCCAGCAGATGCGCCT	-1	GAGGAGCAGAGTTGATCCGGGTAACATTCATC	This study
			-1	TACCCCATAGGCTGCTCCCGCATTCAGCAGTT			

*number of phage-targeting spacers †spacer position in array ‡Anti-ϕ spacers with a GG PAM, anti-ϕ spacer with a GA PAM, *anti-plasmid spacers with a GG PAM*.

Table S2. Plasmids and oligonucleotides used in this study

Name	Description/ sequence (5'-3')	Reference/ description
Plasmids		
pPF712	Priming protospacer, Tc^R and mCherry	(1)
pPF718	pPF712 with ϕTE tail fibre protein gene (phiTE_215)	(1)
pPF971	pPF712 with ϕM1 RNA polymerase gene (phiM1_31)	This study
pPF981	pPF712 with ϕTE tape measure protein gene (phiTE_228)	This study
pPF1251	pPF712 with ϕTE DNA polymerase gene (phiTE_10)	This study
pPF1252	pPF712 with ϕM1 tail fibre protein gene (phiTE_44)	This study
Oligonucleotides		
PF174	CGTTAGAGTGATCGGGCTAC	F for CRISPR1 (binds in leader)
PF175	CAATGGCTCAGGGGATTC	R for CRISPR1 (binds spacer 2)
PF176	GGTAACTACCGTAAAATAGGAACG	F for CRISPR2 (binds in leader)
PF177	GCCTTTAAGCGCATGTCG	R for CRISPR2 (binds spacer 2)
PF178	CTTTAATAATCTGGTTGTTAGTGTG	F for CRISPR3 (binds in leader)
PF179	CCTCAGAAAGCCGACTTC	R for CRISPR3 (binds spacer 2)
PF1635	TTTGGTACCATAAGGTCGTTGAAGTTCTGG	F for amplifying ϕTE TFP (KpnI)
PF1636	TTTCTCGAGCTACAGTAGCAACAGTACCTTTC	R for amplifying ϕTE TFP (XhoI)
PF1637	TTTGGTACCACAAGAGTTTATATCAGCAACTGG	F for amplifying ϕM1 RNP (KpnI)
PF1638	TTTCCCGGGATGAAGAATTCAGACTCTCTCACC	R for amplifying ϕM1 RNP (SmaI)
PF2021	TTTGGTACCACTAATGGCTGCATCTGG	F for amplifying ϕTE TMP (KpnI)
PF2022	TTTCTCGAGATTGATTAGTTAGGCCCACC	R for amplifying ϕTE TMP (XhoI)
PF2144	CATGAACCATACAATAGCCC	R 200 bp ds of ϕTE TFP gene
PF2146	TCACGGTTCCAACAAAAATGC	R 150 bp ds of ϕTE TFP 2 spacer
PF2371	TTTCTCGAGATGGATGCACCTTGTAAGTTACC	F for amplifying ϕTE DNP (XhoI)
PF2372	TTTGGTACCGTATATGATAGCGAGGCCAGC	R for amplifying ϕTE DNP (KpnI)
PF2373	TTTCTCGAGTACCATTCCTGAGTATCCTACCG	F for amplifying ϕM1 TFP (XhoI)
PF2374	TTTCCCGGGAGTCATCAAGCATAACCTCTGC	R for amplifying ϕM1 TFP (SmaI)

Table S3. Efficiency of plating, plaque sizes and escape type

Strain	No. spacers	EOP ± sd	Plaque size range (mm)*	Esc type †
WT	0	$1.0\times10^{0} \pm 2.3\times10^{-1}$	2.5-5	
TE DNP				
1	1	$3.6\times10^{-1} \pm 3.9\times10^{-2}$	0.5-2.5	P/G
2	1	$9.2\times10^{-2} \pm 8.7\times10^{-3}$	0.5-2.5	P/G
3	1	$9.3\times10^{-2} \pm 3.3\times10^{-2}$	<0.5-1	P/G
4	1	$1.6\times10^{-2} \pm 3.8\times10^{-4}$	0.5-1.5	P/G
5	2	$2.2\times10^{-2} \pm 4.7\times10^{-3}$	<0.5-1.5	P
6	2	$4.0\times10^{-3} \pm 9.0\times10^{-4}$	<0.5-1	P
7	3	$7.9\times10^{-3} \pm 2.0\times10^{-3}$	<0.5-1	P
8	3	$1.5\times10^{-4} \pm 6.9\times10^{-5}$	All <0.5	P
9	4	$3.0\times10^{-5} \pm 1.5\times10^{-5}$	All <0.5	P
TE TFP				
1	1	$1.9\times10^{-1} \pm 3.4\times10^{-2}$	0.5-3	P/G
2	1	$9.2\times10^{-3} \pm 2.4\times10^{-3}$	<0.5-3.5	P/G
3	1	$1.4\times10^{-2} \pm 3.0\times10^{-3}$	All <0.5	P/G
4	1	$9.1\times10^{-3} \pm 1.7\times10^{-3}$	0.5-4	P/G
5	2	$2.1\times10^{-5} \pm 0.0\times10^{-0}$	All <0.5	P
6	3	$1.1\times10^{-5} \pm 3.6\times10^{-6}$	0.5-3.5	G
WT	0	$1.0\times10^{0} \pm 2.4\times10^{-1}$	2.5-4	
M1 DNP				
1	1	$3.8\times10^{-1} \pm 1.3\times10^{-1}$	2-4	P
2	1	$6.1\times10^{-2} \pm 9.8\times10^{-3}$	0.5-2.5	P
3	1	$1.5\times10^{-2} \pm 2.7\times10^{-3}$	0.5-2	P
4	2	$1.3\times10^{-2} \pm 4.8\times10^{-3}$	<0.5-2	P
5	2	$1.4\times10^{-2} \pm 3.5\times10^{-3}$	<0.5-2	P
6	3	$8.3\times10^{-4} \pm 1.9\times10^{-4}$	<0.5-2	P
M1 TFP				
1	1	$4.0\times10^{-1} \pm 1.9\times10^{-4}$	2-3	P
2	1	$2.1\times10^{-1} \pm 2.7\times10^{-2}$	1-2.5	P
3	2	$1.4\times10^{-1} \pm 1.2\times10^{-1}$	1-2.5	P
4	2	$4.0\times10^{-2} \pm 2.6\times10^{-2}$	0.5-1.5	P
5	2	$3.1\times10^{-2} \pm 2.9\times10^{-2}$	0.5-1.5	P
6	3	$2.5\times10^{-1} \pm 1.3\times10^{-1}$	1-2.5	P
7	3	$8.4\times10^{-3} \pm 1.8\times10^{-3}$	0.5-1.5	P
WT	0	$1.0\times10^{0} \pm 2.3\times10^{-1}$	2.5-5	
TE TMP				
1	1	$5.1\times10^{-2} \pm 1.2\times10^{-2}$	0.5-2.5	P/G
2	1	$6.9\times10^{-2} \pm 9.2\times10^{-3}$	0.5-1.5	P/G
3	1	$7.5\times10^{-2} \pm 9.6\times10^{-3}$	0.5-1.5	P/G
4	1	$1.5\times10^{-2} \pm 5.0\times10^{-3}$	0.5-1	P/G
5	1	$3.5\times10^{-3} \pm 2.3\times10^{-3}$	All <0.5	P/G
6	1	$5.9\times10^{-4} \pm 1.4\times10^{-4}$	<0.5-3	P/G
7	2	$1.1\times10^{-3} \pm 4.8\times10^{-4}$	All <0.5	P
8	2	$2.2\times10^{-3} \pm 3.4\times10^{-4}$	All <0.5	P
9	2	$2.3\times10^{-3} \pm 5.0\times10^{-4}$	All <0.5	P
10	2	$8.7\times10^{-5} \pm 6.2\times10^{-5}$	All <0.5	P
11	2	$1.1\times10^{-4} \pm 2.7\times10^{-5}$	All <0.5	P
12	3	$1.5\times10^{-3} \pm 1.2\times10^{-4}$	All <0.5	P
13	3	$1.8\times10^{-4} \pm 5.2\times10^{-5}$	All <0.5	P
14	2	$6.1\times10^{-5} \pm 1.7\times10^{-5}$	All <0.5	P
15	3	$2.1\times10^{-5} \pm 0.0\times10^{-0}$	All <0.5	P
16	3	$2.1\times10^{-5} \pm 0.0\times10^{-0}$	All <0.5	P

*across three replicates; † P=phages that did not heritably escape CRISPR-Cas, G=genotypic escape phages

Table S4. Genotypic escape phages

Esc	Host (TE..)	#*	Mech†	Pos.§	Base changeǁ	Codon change	Del/insert size (bp)	Homology/ dup. seq	EOP ±SD
1	TFP 2		del				147	CACG	1.0 ±0.4
2	TFP 2		del				292	AGCCAACG	0.9 ±0.2
3	TFP 2	4	pm	3	A-G	D-G			0.8 ±0.2
4	TFP 2	4	pm	7	A-G				1.0 ±0.2
5	TMP 6	2	del				120	GGCTAAAGCC	0.8 ±0.5
6	TMP 1		pm	2	T-G				1.1 ±0.1
7	TMP 2	2	pm	-1	C-G (CG)	P-R			1.1 ±0.3
8	TMP 1		pm	-1	C-T (AG)	R-C			1.2 ±0.1
9	TMP 1	2	pm	2	T-C				0.8 ±0.1
10	TMP 3		pm	1	A-G	R-S			0.9 ±0.6
11	TMP 6	2	pm	5	C-A	Q-R			0.8 ±0.1
12	TFP 2		pm	5	A-G	T-A			0.9 ±0.2
13	TMP 2		pm	2	C-G	Q-E			0.8 ±0.1
14	TFP 6		del				1,089	TGAAGGGCGA	1.3 ±0.2
15	TFP 6		del				990	GGTGACACCGG	0.8 ±0.6
16	TMP 6		pm	1	T-C	L-S			0.7 ±0.3
17	TMP 4	2	dup‡				15	AGACGTGCAGG ACA	1.0 ±0.2
18	TMP 4	2	dup				15	AGACGTGCAGG ACA	0.9 ±0.1
19	TMP 1	2	pm	25,26, 32	A-T, G-T, C-G	K-I			1.1 ±0.7
20	TFP 3		pm	-2	C-T (GA)	S-F			1.0 ±0.2
21	TMP 3		pm	2	A-G				0.8 ±0.4
22	TMP 4	3	pm	-2	C-A (GT)				1.1 ±0.1
23	TFP 6	3	del				1,968	GACA	1.3 ±0.6
24	TFP 6		del				1,986	GGTCC	1.0 ±0.1
25	TFP 4		pm	-2	C-G (GC)	P-A			0.9 ±0.2
26	TFP 4	2	pm	4	T-C				0.9 ±0.1
27	TFP 4		pm	3	C-T	T-I			0.8 ±0.5
28	TFP 2		del				57	AGCC	1.0 ±0.2
29	TFP 2		pm	-2	C-A (GT)	P-T			1.1 ±0.2
30	TFP 2		del				144	CCTGTG	0.9 ±0.8
31	TFP 2		pm	-1	C-G (CG)	P-R			1.0 ±0.2
32	TFP 3		del				246	GTGTC/ GTATC	1.2 ±0.2
33	TFP 3		pm	1	A-C	S-R			1.1 ±0.2
34	TFP 3		pm	1	A-G	S-G			1.3 ±0.2
35	TFP 1& TFP 6	2	del				1,719	AAGGGTGACAC	0.7 ±0.5 0.8 ±0.2
36	TFP 1		del				462	CCAATCGG/CC GCAGGG	1.4 ±0.4
37	TFP 1		del				729	GGTGACAC	1.2 ±0.3
38	TFP 1		del				783	AAGGGTGACAC TGGCCCAACTG GT	0.7 ±0.4
39	TMP 4		pm‡	-2	C-T (GA)				1.0 ±0.3
40	TMP 4		del‡				45	CCGCC	0.8 ±0.2
41	TMP 5		pm	-1	C-T (AG)				1.0 ±0.0
42	TFP 6		del				2,004	TACTGGTCCAA CAGG	1.1 ±0.0
43	TFP 6		del				1.968	CCGCAGGGTG	1.1 ±0.0
44	TFP 6		del				1,968	AGGTCCAG	1.0 ±0.1

*****Number of times isolated; **†** Mechanism of escape: pm=point mutation, del=deletion, dup=duplication; **‡**Esc 5 (120 bp del) background; **§** position of point mutation: -1, -2=PAM, 1,2,3…=protospacer; ǁ top/coding strand, () new PAM sequence.

Supplementary Reference

1. Pawluk A, Staals RHJ, Taylor C, Watson BNJ, Saha S, Fineran PC, Maxwell KL, Davidson AR. 2016 Inactivation of CRISPR-Cas systems by anti-CRISPR proteins in diverse bacterial species. *Nature Microbiology*; 1(8):16085.