Supplementary Table 1. Virion proteins of phage JBD30 detect by mass spectrometry, Related to Figure 7A

JBD30 ORF	Accession ID	Protein Length (amino acids)	Function
32	YP_007392339.1	526	portal protein
33	YP_007392340.1	428	homolog of phage Mu gpF
37	YP_007392344.1	365	protease/scaffold
38	YP_007392345.1	304	major head
41	YP_007392348.1	138	head-tail joining
42	YP_007392349.1	157	tail terminator
44	YP_007392351.1	256	tail tube
46	YP_007392353.1	1158	tape measure
47	YP_007392354.1	318	putative tail protein
48	YP_007392355.1	307	putative tail protein
49	YP_007392356.1	567	tail protein; phage lambda gpM-like
50	YP_007392357.1	273	tail protein; phage lambda gpL-like
53	YP_007392360.1	735	central fiber
54	YP_007392361.1	382	putative pilus binding protein

Supplementary Table 2. List of genomes and anti-CRISPR protein identifiers used in Figure 1

Source	Genome ID	Anti-CRISPR ID
Pseudomonas phage JBD30	NC_020198.1	YP_007392342.1
Alcanivorax sp. KX64203	NZ_LVIC01000002.1	WP_063139756.1
Pectobacterium phage ZF40	NC_019522.1	YP_007006940.1
Escherichia coli SE11	NC_011413	WP_000765122.1
Neisseria meningitidis 23231	NZ_OALB01000002.1	WP_042743676.1; WP_042743678.1
Listeria monocytogenes J0161	NC_017545.1	WP_003722517.1; WP_003722518.1
Streptococcus phage Sfi21	NC_000872.1	NP_049988.1
Streptococcus phage D1811	MH000604.1	AVO22749.1
Sulfolobus islandicus rudivirus 3	NC_030884.1	YP_009272954.1

Supplementary Table 3. List of genomes and Aca protein identifiers used in Supplementary Figures 6 and 7

Source	**Genome ID**	**Aca ID**
Pectobacterium phage ZF40	NC_019522.1	YP_007006939.1
Vibrio parahaemolyticus	NZ_JPKT01000003.1	WP_080285139.1
Vibrio cyclitrophicus	KP795522.1	AKN37111.1
Phaseolibacter flectens	NZ_JAEE01000001.1	WP_036985669.1
Serratia marcescens	NZ_CP013046.2	WP_060559942.1
Proteus penneri	GG661994.1	EEG86165.1
Shewanella xiamenensis	JGVI01000034.1	KEK29120.1
Oceanimonas smirnovii	NZ_KB908455.1	WP_019933869.1
Brackiella oedipodis	NZ_KK211205.1	WP_028357637.1
Nme NmSL13x2	NZ_NGAT01000003.1	WP_002212356.1
Nme 22472	NZ_OAFV01000002.1	WP_002255676.1
Nme M40030	NZ_QQEW01000023.1	WP_118803841.1
Nme 2842STDY5881035	NZ_FERW01000005.1	WP_042743680.1
Nme NM80179	NZ_ALXV01000004.1	WP_002231710.1
Nme 2842STDY5881013	NZ_FERN01000021.1	WP_061695140.1
Nme WUE2121	NZ_CP012394.1	WP_061384811.1

Supplementary Table 4. Oligonucleotides used in this study, Related to STAR Methos

Purpose	**Sequence (5’-3’)**
Cloning of JBD30 anti-CRISPR locus into pBTK30 transposon	F: GGGCCCGTCGACTGGCCACTTTCGGACAAG
	R: CCCGGGGTCGACTCACGCAGATGGCGGGTCGT
Generation of RT-qPCR standard for gene *A*	F: TGGTTCAGCCCTCAACAACT
	R: TCTTGAGCATGGCGAGCA
RT-qPCR of gene *A*	F: GCCTCGGTTCAACAGTACGA
	R: AACGTGGTACTCCATCGCTTT
Generation of RT-qPCR standard for gene *G*	F: AGTTCGCCTTTATGGACGAG
	R: ATTTCGGCTCAAGGCTGTTA
RT-qPCR of gene *G*	F: CGGGTCCAACTTGGTCTATG
	R: TTTCGTCGAACGGCAGATA
Generation of RT-qPCR standard for *acrIF1*	F: ATGAAGTTCATCAAATACCTC
	R: TCAGGGGTTTTCACGCCGGG
RT-qPCR of *acrIF1*	F: AATACCTCAGCACCGCTCAC
	R: TTGCCGTTTACGACGTTCTC
Generation of RT-qPCR standard for *aca1*	F: ATGAGATTTCCCGGCGTGAA
	R: TCACGCAGATGGCGGGTCGT
RT-qPCR of *aca1*	F: TCAAGAAAGCCGGCATCA
	R: TCCTTGATGTCCTCGCTCAG
Generation of RT-qPCR standard for gene *37* (protease/scaffold)	F: GAAAAGAACCGCCTACTCGTT
	R: TGGCTTTCAGGAGTTCATCC
RT-qPCR of gene *37*	F: ATGAGCACCAGACCCTCAAG
	R: GGGCTGTGTATTCGACACG
Generation of RT-qPCR standard for *clpX*	F: CTGCAAGAGTTTCTGGATGATG
	R: CTTTATCTGCGACGAGTGTGTC
RT-qPCR of *clpX*	F: CGCTTGTAGTGGTTGTATACCG
	R: AAAGTAGTGGGCACAAACTTCC
Generation of RT-qPCR standard for *rpoD*	F: GAGATGCGGTTGAGCTTGTT
	R: GTCGACAGCGTCCTGAAGAG
RT-qPCR of *rpoD*	F: GGGCGAAGAAGGAAATGGTC
	R: CAGGTGGCGTAGGTAGAGAA
Cloning of anti-CRISPR promoter from JBD30	F: CCCGGGCCCCATGGTGGCCACTTTCGGACAAG
	R: CCCGGGAAGCTTGGTTTGAATCCTTGTTGGCGCC
Generation anti-CRISPR promoter deletion recombination cassette	F: AGCCGAAATCGGTAGAACGGCGAGGCGCCAACAAG
	R: CTACCGATTTCGGCTCAAG
Cloning Aca1	F: CCCGGGCCATGGCCAGATTTCCCGGCGTGAA
	R: CCCGGGAAGCTTTCACGCAGATGGCGGGTCGT

Purpose	Sequence (5’-3’)
Wild-type anti-CRISPR promoter substrate for EMSA	sense: ACAAGCGGCACACTGTGCCTATTGCGAATTAGGCACAATGTGCCTAATCTAACG
	anti-sense: GGTTAGATTAGGCACATTGTGCCTAATTCGCAATAGGCACAGTGTGCCGCTTGT
IR1 mutant anti-CRISPR promoter substrate for EMSA	sense: ACAAGCGTCGTACTGTGCCTATTGCGAATTAGGCACAATGTGCCTAATCTAACG
	anti-sense: CGTTAGATTAGGCACATTGTGCCTAATTCGCAATAGGCACAGTACGACGCTTGT
IR2 mutant anti-CRISPR promoter substrate for EMSA	sense: ACAAGCGGCACACTGTGCCTATTGCGAGCTAGTCCCAATGTGCCTAATCTAACG
	anti-sense: CGTTAGATTAGGCACATTGGGACTAGCTCGCAATAGGCACAGTGTGCCGCTTGT
IR1+IR2 mutant anti-CRISPR promoter substrate for EMSA	sense: ACAAGCGTCGTACTGTGCCTATTGCGAGCTAGTCCCAATGTGCCTAATCTAACG
	anti-sense: CGTTAGATTAGGCACATTGGGACTAGCTCGCAATAGGCACAGTACGACGCTTGT
Generation of IR1 mutations in anti-CRISPR promoter	sense: GGCCACTTTCGGACAAGCGTCGTACTGTGCCTATTGCGAATT
	anti-sense: AATTCGCAATAGGCACAGTACGACGCTTGTCCGAAAGTGGCC
Generation of IR2 mutations in anti-CRISPR promoter	sense: TGACGTTAGATTAGGCACATTGGGACTGCTTCGCAATAGGCACAGTGTGCC
	anti-sense: GGCACACTGTGCCTATTGCGAAGCAGTCCCAATGTGCCTAATCTAACGTCA
Generation of R33A Aca1 mutant	sense: TCGGCTGCGCGCGCCTGGCTGATGCC
	anti-sense: GGCATCAGCCAGGCGCGCGCAGCCGA
Generation of R34A Aca1 mutant	sense: CAGCTCGGCTGCGGCCCGCTGGCTGATG
	anti-sense: CATCAGCCAGCGGGCCGCAGCCGAGCTG
Generation of R33A/R34A Aca1 mutant	sense: CAGCTCGGCTGCGGCCGCCTGGCTGATGCCG
	anti-sense: CGGCATCAGCCAGGCGGCCGCAGCCGAGCTG
Generation of R44A Aca1 mutant	sense: GTAATAGCGCATCACCGCGTCACTGAGGCCGAGC
	anti-sense: GCTCGGCCTCAGTGACGCGGTGATGCGCTATTAC
Amplification of *rrnB* T1 terminator	F: ACGACCCGCCATCTGCGTGAAGGGAACTGCCAGGCATC
	R: TTTGCGGGCGGATCAGGTGACGTTCACCGACAAACAACAG
Generation of terminator insertion recombination cassette	F: TCACCTGATCCGCCCGCA
	R: TCACGCAGATGGCGGGTC
Cloning of Aca2	F: TAGTTGCGGCCGCAAAATGGATGAATGGTCAAGAATTAAAAAAAG
	R: GCGGCCGCAGGCAAAGGATATTAGATTAAATCCGCGTGAC

Purpose	**Sequence (5’-3’)**
Cloning of Aca3	F: TAGTTGCGGCCGCAAAATGGATGAAGAAATTTGAAGCCC
	R: GCGGCCGCAGGCAAAGGATATTATTTTAATGAATCCAAAAGTTTTTG
Amplification of pCM-Str for Aca cloning	F: TATCCTTTGCCTGCGGCC
	R: CCATTTTGCGGCCGCAAC
Cloning of *aca2* associated upstream region	F: CCCGGGCCATGGAGCCTCACCTCCGGCG
	R: CCCGGGAAGCTTCTCGAACCGATGAATAAATTATATGT
Cloning of *aca3* associated upstream region	F: CCCGGGCCATGGAATTGAATCCGCAATGGTGAAA
	R: CCCGGGAAGCTTTTTGAAATCCTTTCGTTTATCCTTG

Supplementary Table 5. Plasmids used in this study, Related to STAR Methods

Plasmid ID	**Purpose**	**Backbone**
pES102	Overexpression of JBD44-targeting crRNA in *P. aeruginosa*	pHERD30T
pSY100	Overexpression of JBD30 Aca1 in *P. aeruginosa*	pHERD30T
pSY115	Overexpression of R33A Aca1 mutant in *P. aeruginosa*	pHERD30T
pSY116	Overexpression of R34A Aca1 mutant in *P. aeruginosa*	pHERD30T
pSY117	Overexpression of R33A/34A Aca1 mutant in *P. aeruginosa*	pHERD30T
pSY118	Overexpression of R44A Aca1 mutant in *P. aeruginosa*	pHERD30T
pSY099	Overexpression of AcrIF1 in *P. aeruginosa*	pHERD30T
pSY107	Generation of JBD30ΔP*acr*	pHERD20T
pSY108	Generation of JBD30 IR1 mut	pHERD20T
pSY109	Generation of JBD30 IR2 mut	pHERD20T
pSY110	Generation of JBD30 IR1+IR2 mut	pHERD20T
pSY119	Generation of JBD30*aca*^{R33A}	pHERD20T
pSY120	Generation of JBD30*aca*^{R34A}	pHERD20T
pSY121	Generation of JBD30*aca*$^{R33A/R34A}$	pHERD20T
pSY122	Generation of JBD30*aca*^{R44A}	pHERD20T
pSY182	Generation of JBD30*aca*^{R44A} IR1+IR2 mut	pHERD20T
pSY183	Generation of JBD30+*rrnB* T1	pHERD20T
pSY184	Generation of JBD30*aca*^{R44A} +*rrnB* T1	pHERD20T
pSY105	Encodes anti-CRISPR locus carrying transposon	pBTK30
pSY101	Determining anti-CRISPR promoter region activity	pQF50
pSY102	Determining IR1 mutant promoter activity	pQF50
pSY103	Determining IR2 mutant promoter activity	pQF50
pSY104	Determining IR1+IR2 mutant promoter activity	pQF50
pSY138	Determining *aca2*-associated promoter activity	pQF50
pSY139	Determining *aca3*-associated promoter activity	pQF50
pSY123	Expression and purification of JBD30 Aca1	p15TV-L
pSY124	Expression and purification of R33A Aca1 mutant	p15TV-L
pSY125	Expression and purification of R34A Aca1 mutant	p15TV-L
pSY126	Expression and purification R33A/34A Aca1 mutant	p15TV-L
pSY127	Expression and purification of R44A Aca1 mutant	p15TV-L
pSY146	Constitutive expression of *aca1* in *E. coli*	pCM-Str
pSY144	Constitutive expression of *aca2* in *E. coli*	pCM-Str

pSY145	Constitutive expression of *aca3* in *E. coli*	pCM-Str

Supplementary Table 6. Sequences for cloning of *aca2*, *aca3*, and associated promoter regions, Related to STAR Methods

Description	Reference Sequence	Sequence
Anti-CRISPR locus phage ZF40	NC_019522.1	AGCCTCACCTCCGGCGTTGCCGTGGCGCTGTGTGATTTACAGGAAATAAAAAGGCCACGAATGCGGCCTTAGCGATTAAAAAATATGAAATGCCTTGCTTGTTCGCGATTGCGAACATATAATTTATTCATCGGTTCGAGATGGCTCGAATCGCTCCTAACGAGGATTCCACAATGTCTACTGCTTACATCATCTTTAACTCATCCGTCGCGGCCGTAGTTGATACTGAGATCGCTAATGGCGCTAATGTCACATTCTCAACAGTGACCGTTAAAGAAGAAATTAACGCGAACCGTGATTTCAATCTGGTTAACGCTCAGAACGGGAAAATCTCACGCGCAAAACGCTGGGGAAACGAGGCGTCAAAATGTGAGTATTTTGGCCGAGAAATAAACCCAACCGAGTTTTTCATCAAATAATGTGGTCAAAATGACAAACAAAGAACTTCAGGCAATCAGAAAACTGTTAATGCTGGATGTATCAGAAGCGGCTGAACACATTGGCCGCGTTTCCGCCCGGAGTTGGCAATATTGGGAGTCTGGACGCTCTGCTGTTCCTGATGATGTTGAGCAGGAAATGTTGGATTTAGCGTCAGTCAGGATAGAAATGATGTCCGCTATAGACAAGCGTCTCGCCGATGGCGAACGTCCTAAATTACGTTTTTATAACAAGTTGGATGAATACCTGGCTGACAACCCCGATCACAATGTGATCGGGTGGCGTCTGAGCCAGTCTGTTGCCGCACTCTATTACACTGAGGGTCACGCGGATTTAATCTAA
Anti-CRISPR promoter region and *aca3* gene from *Neisseria meningitidis*	NZ_FERW01000005.1	TCCCAATTACCTGTTTGAAGCAGTATTTGTTTCTCAAATGACCAATTTTTAACCAAAGGCCGCTAATGTGGCCGTTTTTTTTGTTCTCATACTCTTCTAATTTAGGGTCTCTGCCTCCAAGCTCCCGGTCTCGCCGCCGACGGCTCGGGAGCAGGGCATAGCCATAAAAGCTTACATTGTGTGCTAGACTATATCAAACTACAACTACGAAAGGAAATCCGAACACTATGAATAAAACTTATAAAATTGGAAAAAATGCCGGGTATGATGGCTGCGGTCTTTGTCTTGCGGCCATTTCTGAAAATGAAGCTATCAAAGTTAAGTATTTGCGCGACATTTGTCCTGATTACGATGGCGATGATAAAGCTGAGGATTGGCTGAGATGGGGAACGGACAGCCGCGTCAAAGCAGCCGCTCTTGAAATGGAGCAGTACGCATATACGTCGGTTGGTATGGCCTCATGTTGGGAGTTTGTTGAA

	CTATGAAGAAATTTGAAGCCCCTGAAATTGGCTATACACCTGCCAATCTTAAAGCACTGAGAAAACAATTTGGGCTTACACAAGCTCAGGTAGCAGAAATTACTGGTACAAAAACCGGATACAGCGTCCGCAGGTGGGAAGCAGCAATTGATGCCAAAAATCGCGCGGATATGCCGCTCGTAAAATGGCAAAAACTTTTGGATTCATTAAAATAATGA