Materials and Methods

Bacterial strains and growth conditions

Pseudomonas aeruginosa strains UCBPP-PA14 (PA14), SMC4386, and PAO1 were used in this study. The strains were grown at 37 °C in lysogeny broth (LB) agar or liquid medium, which was supplemented with 50 μg ml^{-1} gentamicin, 30 μg ml^{-1} tetracycline, or 250 μg ml^{-1} carbenicillin as needed to retain plasmids or other selectable markers.

Phage isolation

Phage lysates were generated by mixing 10 μl phage lysate with 150 μl overnight culture of *P. aeruginosa* and pre-adsorbing for 15 min at 37 °C. The resulting mixture was then added to molten 0.7% top agar and plated on 1% LB agar overnight at 30 °C or 37 °C. The phage plaques were harvested in SM buffer, centrifuged to pellet bacteria, treated with chloroform, and stored at 4 °C.

Bacterial transformations

Transformations of *P. aeruginosa* strains were performed using standard electroporation protocols. Briefly, one mL of overnight culture was washed twice in 300 mM sucrose and concentrated tenfold. The resulting competent cells were transformed with 20 – 200 ng plasmid, incubated in antibiotic-free LB for 1 hr at 37 °C, plated on LB agar with selective media, and grown overnight at 37 °C. Bacterial transformations for cloning were performed using *E. coli* DH5α (NEB) and *E. coli* Stellar competent cells (Takara) according to the manufacturer's instructions.

Discovery of *acr* genes using *aca1*

All bacterial genome sequences used in this study were downloaded from NCBI. BLASTp was used to search the nonredundant protein database for Aca1 homologs (seed Aca1 accession: YP_007392343, e value < 0.005) in *Pseudomonas sp.* (taxid: 286) Individual genomes encoding an Aca1 homolog were then manually surveyed for *aca1* associated genes. This approach was extended to discover the Aca4 (WP_034011523.1) associated anti-CRISPR AcrIF12. tBLASTn searches to identify orthologs of VA2 in self-targeting *Moraxella bovoculi* strains were performed using the protein sequence in *Moraxella catarrhalis* BC8 strain (EGE18855.1) as the query and *Moraxella bovoculi* genome accessions as the subject (accessions: 58069 genome, CP011374.1; 58069 plasmid, CP011375.1; 22581, CP011376.1; 33362, CP011379.1; 28389, CP011378.1). Other searches for orthologs in *Moraxella sp.* were performed using BLASTp.

Discovery of anti-CRISPR associated (*aca*) gene families

Genomes with homologs of AcrIF11 were manually examined for novel anti-CRISPR associated (*aca*) genes. A gene was designated as an *aca* if it fit the following criteria: I) directly downstream of an AcrIF11 homolog in the same orientation, II) a non-identical homolog of this gene exists in the same orientation relative to a non-identical homolog of AcrIF11, and III) predicted in high confidence to contain a DNA-binding domain based on structural prediction using HHPred (probability >90%, E < 0.0005) (*19*). Genes that fit these three criteria were then grouped into sequence families, requiring that a given gene have >40% sequence identity to at least one member of the family for family membership.

Type I-C CRISPR-Cas expression in *Pseudomonas aeruginosa*

Reconstitution of the Type I-C system from a *P. aeruginosa* isolate in the Bondy-Denomy lab into PAO1 was achieved by amplifying the four effector cas genes (*cas3-5-8-7*) from genomic DNA by PCR and cloning the resulting fragment into the integrative, IPTG-inducible pUC18T-mini-Tn7T-LAC plasmid to generate the pJW31 vector. This plasmid was then electroporated into PAO1 and chromosomal integration was selected for using 50 μg ml^{-1} gentamicin. After chromosomal integration of the insert was confirmed, the gentamicin selectable marker was removed using flippase-mediated excision at the flippase recognition target (FRT) sites of the construct; the resulting strain was named LL76. CRISPR RNAs (crRNAs) consisting of a spacer that targets JBD30 phage (see Table S7 for the sequence) and two flanking repeats were cloned into the mini-CTX2 (AF140577) vector, and the resulting vector was electroporated into LL76. Stable integration of the vector at the *attB* site was selected for using 30 μg ml^{-1} tetracycline. Targeting was confirmed in the resulting strain (LL77) using phage challenge assays, as described in the "bacteriophage plaque assays" section.

Type V-A CRISPR-Cas expression in *Pseudomonas aeruginosa*

Human codon-optimized MbCas12a (*Moraxella bovoculi* 237) was amplified from the pTE4495 plasmid (Addgene #80338) by PCR and cloned into pTN7C130, a mini-Tn7 vector that integrates into the attTn7 site of *P. aeruginosa*. The pTN7C130 vector expresses MbCas12a off the araBAD promoter upon arabinose induction and contains a gentamicin selectable marker. The resulting construct, pTN7C130-MbCas12a, was used to transform the PAO1 strain of *P. aeruginosa*, and stable integration of the vector was selected for using 50 μg ml^{-1} gentamicin and confirmed by PCR. After integration, flippase was used to excise the gentamicin selectable marker from the flippase recognition target (FRT) sites of the construct.

CRISPR RNAs (crRNAs) for MbCas12a were generated by designing oligonucleotides with spacers that target gp23 and gp24 (see Table S7 for sequences) in JBD30 phage flanked by two direct repeats of the MbCas12a crRNA (*11*). The flanking repeats consist only of the sequence retained after crRNA maturation. The oligos were annealed and phosphorylated using T4 polynucleotide kinase (PNK) and ligated into NcoI and HindIII sites of pHERD30T. A fragment of the resulting plasmid that includes the araC gene, pBAD promoter, and crRNA sequence was then amplified by PCR and cloned into the mini-CTX2 plasmid. The resulting constructs were then used to transform the PAO1 tn7::MbCas12a strain, and stable integration was selected for using 30 μg ml^{-1} tetracycline. The parental strain encoding MbCas12a but no crRNA was used as the "no crRNA" control.

Cloning of candidate anti-CRISPR genes

All candidate genes were cloned into the pHERD30T shuttle vector, which replicates in both *E. coli* and *P. aeruginosa*. Novel genes found upstream of *aca1 in Pseudomonas sp.* were synthesized as gBlocks (IDT) and cloned into the SacI/PstI site of pHERD30T, which has an arabinose-inducible promoter and gentamicin selectable marker. Candidate genes derived from *Moraxella bovoculi* strains were amplified from the genomic DNA of 58069 and 22581 by PCR, whereas genes derived from *Moraxella catarrhalis* were synthesized as gBlocks (IDT). These inserts were cloned using Gibson assembly into the NcoI and HindIII sites of pHERD30T. All plasmids were sequenced using primers outside of the multiple cloning site.

Bacteriophage plaque assays

Plaque assays were performed using 1.5% LB agar plates and 0.7% LB top agar, both of which were supplemented with 10 mM MgSO4. 150 ul overnight culture was resuspended in 3-4 ml molten top agar and plated on LB agar to create a bacterial lawn. Ten-fold serial dilutions of phage were then spotted onto the plate and incubated overnight at 30 °C. Agar plates and/or top agar were supplemented with 0.5 – 1mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 0.1-0.3% arabinose for assays performed with the LL77 (I-C) strain and with 0.1-0.3% arabinose for assays performed with the SMC4386 (I-E), PA14 (I-F), and PAO1 tn7::MbCas12a (V-A) strains. The PA14 ΔCRISPR1ΔCRISPR2 (SMC5454) strain, which lacks its endogenous CRISPR arrays 1 and 2, (*20*) was used as the "no crRNA" control for type I-F assays. PAO1 strains encoding MbCas12a but no crRNA were used as the "no crRNA" control for type V-A assays. For type I-C assays, the "uninduced" control was plated on agar lacking IPTG. Agar plates were supplemented with 50 μg ml^{-1} gentamicin for pHERD30T retention, as specified in the text. Anti-CRISPR activity was assessed by measuring replication of the CRISPR-sensitive phages JBD30 (V-A, I-C), JBD8 (I-E) and DMS3m (I-F) on bacterial lawns relative to the vector control. JBD30, JBD8, and DMS3m are closely related phages, differing slightly at protospacer sequences. Plate images were obtained using Gel Doc EZ Gel Documentation System (BioRad) and Image Lab (BioRad) software.

Phylogenetic reconstructions

Homologs of AcrIF11 (accession: WP_038819808.1) were acquired through 3 iterations of psiBLASTp search the non-redundant protein database. Only hits with > 70% coverage and an E value < 0.0005 were included in the generation of the position specific scoring matrix (PSSM). A non-redundant set of high confidence homologs (> 70% coverage, E value < 0.0005) represented in unique species of bacteria were then aligned using NCBI COBALT (*21*) using default settings and a phylogeny was generated in Cobalt using the fastest minimum evolution method (*22*) employing a maximum sequence difference of 0.85 and Grishin distance to calculate the tree. The resulting phylogeny was then displayed as a phylogenetic tree using iTOL: Interactive Tree of Life (*23*). Similar analysis was performed using 3 iterations of psi-BLASTp to generate the phylogenetic reconstruction for AcrVA3, while BLASTp was used to generate the reconstructions for AcrVA1 and AcrVA2. Only hits with > 70% coverage and an E value < 0.0005 were included.

Cloning of constructs for human cell expression

Human cell Cas12a expression plasmids were generated by sub-cloning the open-reading frames of plasmids pY014, pY117, pY010, pY016, and pY004 (Addgene plasmids 69986, 92293, 69982, 69988, and 69976, respectively; gifts from Feng Zhang) into pCAG-CFP (Addgene plasmid 11179; a gift from Connie Cepko) for wild-type MbCas12a, Mb3Cas12a, AsCas12a, LbCas12a, and FnCas12a (AAS2134, RTW2500, SQT1659, SQT1665, and AAS1472, respectively). Human cell U6 promoter expression plasmids for SpyCas9 sgRNAs and Cas12a crRNAs were generated by annealing and ligating oligonucleotide duplexes into BsmBI-digested BPK1520 (*24*), BPK3079, BPK3082 (*13*). BPK4446, and BPK4449 for SpyCas9, AsCas12a, LbCas12a, FnCas12a, and MbCas12a/Mb3Cas12a, respectively. Human codon optimized AcrVA sequences were cloned with a c-terminal SV40 nuclear localization signal into a pCMV-T7 backbone via isothermal assembly. All plasmids used are listed in Table S10, all crRNA/sgRNA sequences are listed in Table S11.

Human cell culture and transfection

U2-OS cells (from Toni Cathomen, Freiburg) and U2-OS-*EGFP* cells (*18*) (containing a single integrated copy of a pCMV-*EGFP*-PEST reporter gene) were cultured in Advanced Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin-streptomycin, and 2mM GlutaMAX; a final concentration of 400 μg ml^{-1} Geneticin was added to U2-OS-*EGFP* cell culture media. All cell culture reagents purchased from Thermo Fisher Scientific. Human cells were cultured at 37°C with 5% CO_2 and were assayed bi-weekly for mycoplasma contamination. Cell line identities were confirmed by STR profiling (ATCC). All human cell electroporations were carried out using a 4-D Nucleofector (Lonza) with the SE Cell Line Kit and the DN-100 program. Unless otherwise noted, 290ng of nuclease plasmid was co-delivered with 125ng sgRNA/crRNA plasmid and 750ng of anti-CRISPR protein plasmid. Conditions listed as 'filler DNA' include 750 ng of an incompatible nuclease expression plasmid (SpyCas9 for Cas12a experiments, or AsCas12a for SpyCas9 experiments) to ensure electroporation of consistent DNA quantities. Control conditions for both *EGFP* disruption and endogenous targeting included nuclease expression plasmids co-delivered with a U6-null plasmid (in place of sgRNA/crRNA plasmids). For AcrIIA4 titration experiments with SpyCas9, a pCAG-SpyCas9 plasmid was used (SQT817) (*25*) for a comparable vector architecture relative to Cas12a expression plasmids.

Human cell nuclease assays

EGFP disruption experiments were performed as essentially previously described (*18*). Briefly, cells were electroporated as described above and were analyzed ~52h post-nucleofection for EGFP levels using a Fortessa flow cytometer (BD Biosciences). Background *EGFP* loss in negative control conditions was approximately 3% (represented as a red dashed line in figures). For T7 endonuclease I (T7E1) assays, human U2-OS cells were electroporated as described above and genomic DNA (gDNA) was extracted approximately 72 hours post-nucleofection using a custom lysis and paramagnetic bead extraction. Paramagnetic beads were prepared similar to as previously described (*26*): GE Healthcare Sera-Mag SpeedBeads (Thermo Fisher Scientific) were washed in 0.1x TE and suspended in 20% PEG-8000 (w/v), 1.5 M NaCl, 10 mM Tris-HCl pH 8, 1 mM EDTA pH 8, and 0.05% Tween20. To lyse cells, cells were washed with PBS and then subsequently incubated at 55°C for 12-20 hours in 200 μL lysis buffer (100 mM Tris HCl pH 8.0, 200 mM NaCl, 5 mM EDTA, 0.05% SDS, 1.4 mg/mL Proteinase K (New England Biolabs, NEB), and 12.5 mM DTT). The cell lysate was mixed with 165 μL paramagnetic beads and then separated on a magnetic plate. Beads were washed with 70% three times and were permitted to dry on a magnetic plate for 5 minutes before elution with 65 μL elution buffer (1.2 mM Tris-HCl pH 8.0). To perform T7E1 assays, genomic loci were amplified by PCR using ~100 ng of genomic DNA (gDNA) and Hot Start Phusion Flex DNA Polymerase (NEB). PCR products were visualized on a QIAxcel capillary electrophoresis instrument (Qiagen) to confirm amplicon size and purity, and were subsequently purified using paramagnetic beads. T7E1 assays were performed as previously described (*18*) to approximate nuclease modification of targeted genomic loci. Briefly, 200ng purified PCR product was denatured, annealed, and digested with 10U T7E1 (NEB) at 37°C for 25 minutes. Digested amplicons were purified with paramagnetic beads and quantified using a QIAxcel capillary electrophoresis machine (Qiagen) to estimate target site modification.

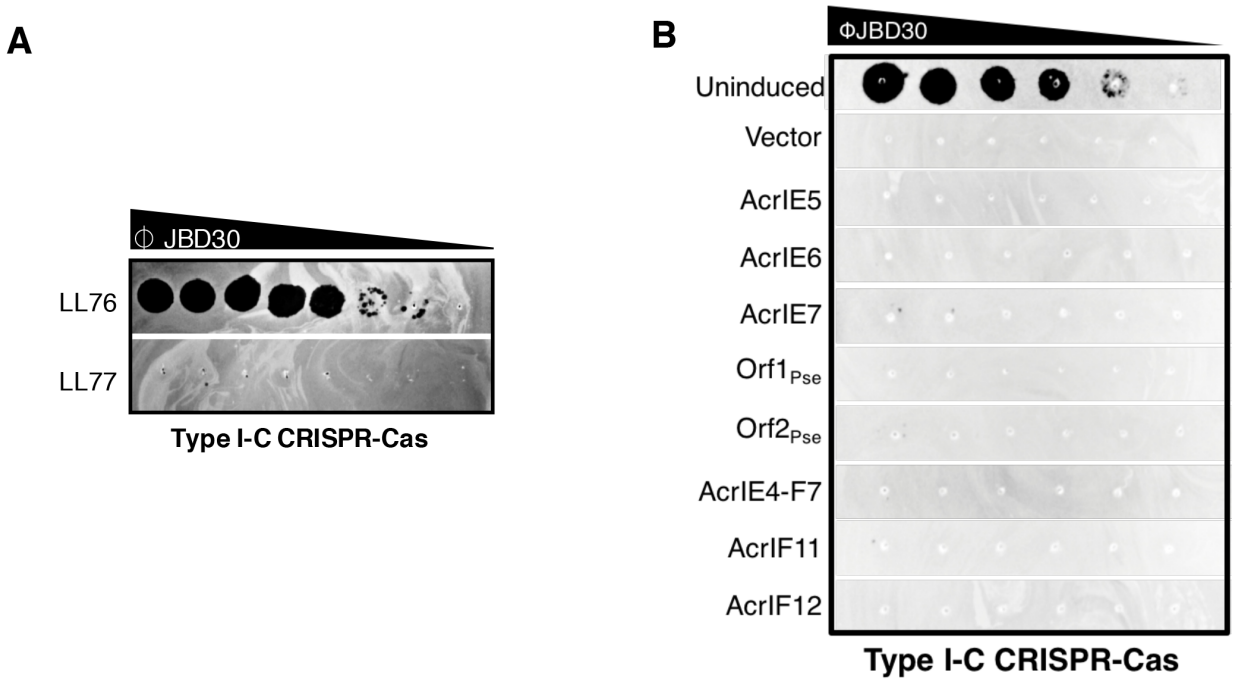


Fig. S1. All *Pseudomonas sp.* ORFs from Figure 1 are negative for anti-IC activity

(A) Ten-fold serial dilutions of JBD30 phage were applied to bacterial lawns of *P. aeruginosa* LL77 and LL76 strains. LL77 is engineered to target JBD30 with a Type I-C CRISPR-Cas immune system, whereas LL76 lacks phage-targeting crRNA. (B) Phage plaque assays to test potential Type I-C inhibition by candidate genes.

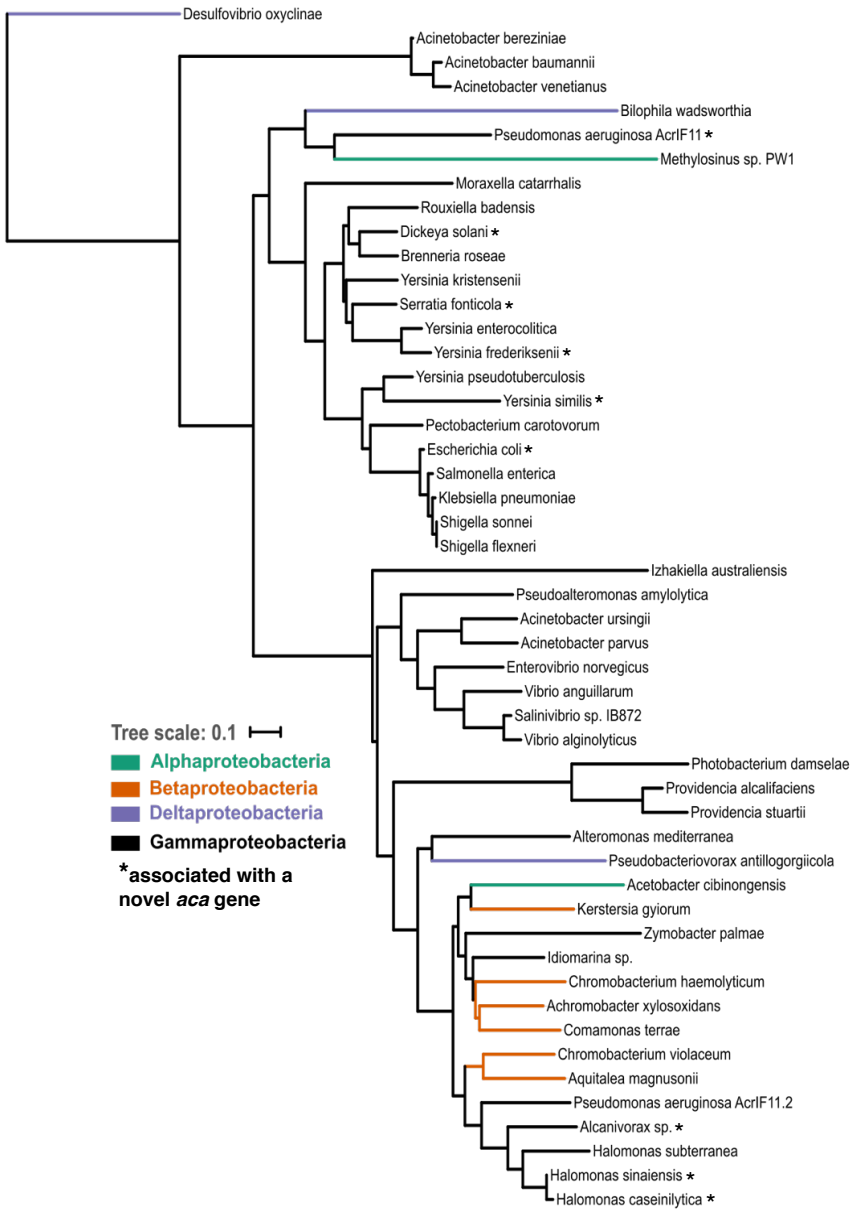


Fig. S2. AcrIF11 phylogenetic tree with *aca4-aca7* associations indicated

Midpoint rooted minimum-evolution phylogenetic tree of full-length AcrIF11 orthologues. Branches are labeled with species names and colored according to species class (see legend). Species in which AcrIF11 is associated with a novel *aca* gene (*aca4*-7) are marked with asterisks.

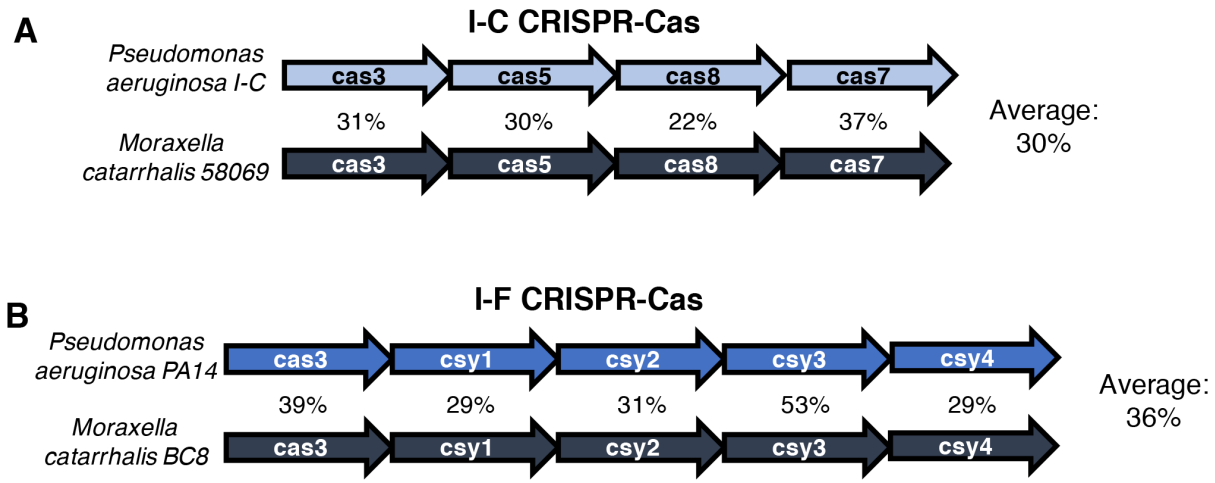


Fig. S3. Percent identity between *Pseudomonas* and *Moraxella* Cas proteins

BLASTp was used to align the indicated protein orthologues between the Type I-C (A) and Type I-F (B) systems of *Pseudomonas* and *Moraxella*. The percent sequence identity between the proteins is shown, as well as an average value for the whole system.

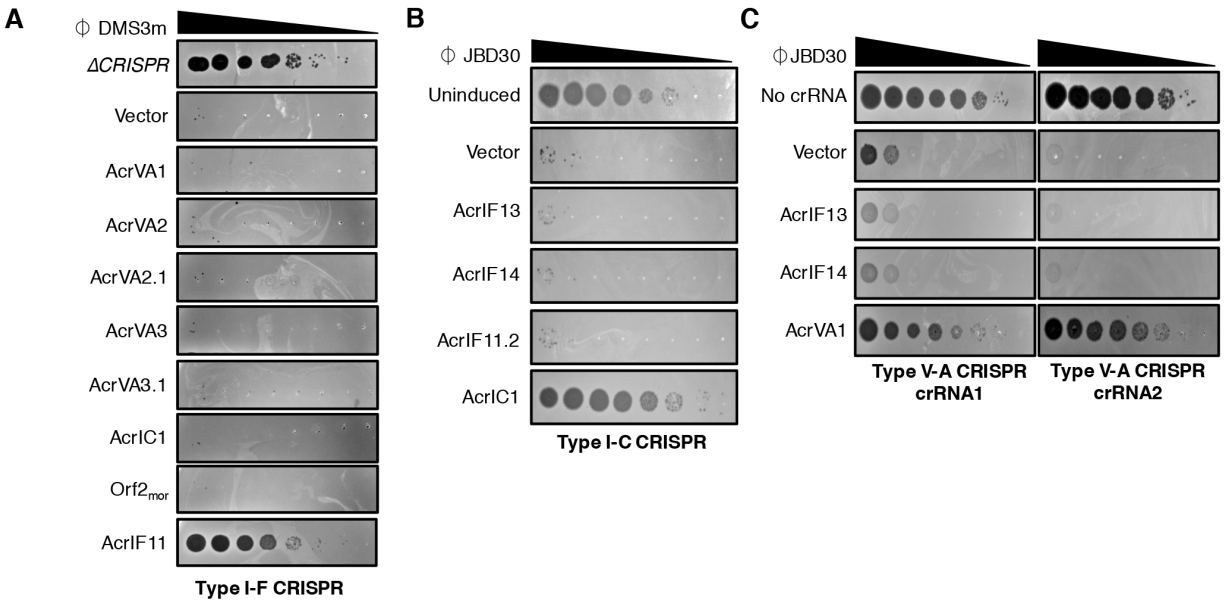


Fig. S4. Functionality of novel Acr proteins against CRISPR-Cas systems they do not inhibit.
Phage plaque assay to assess CRISPR-Cas inhibition. Ten-fold serial dilutions of (A) DMS3m or (B, C) JBD30 phage were applied to bacterial lawns of *P. aeruginosa* strain (A) UCBPP-PA14 expressing the Type I-F system, (B) PAO1 expressing the Type I-C system, or (C) PAO1 expressing the Type V-A system, transformed with candidate gene or vector control.

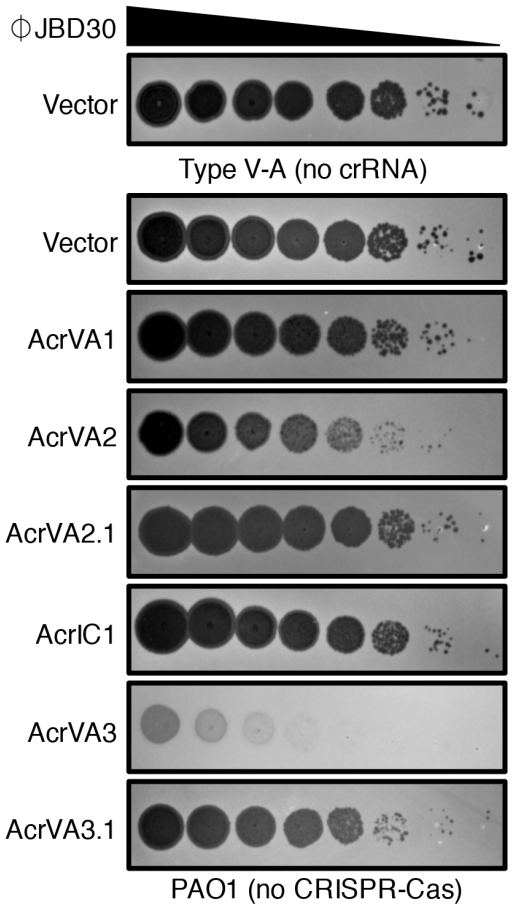


Fig S5. Effect of *acr* genes on phage growth in the absence of CRISPR-Cas.
Phage plaque assay to assess phage growth in the presence of *acr* genes and absence of CRISPR-Cas. Ten-fold serial dilutions of JBD30 phage were applied to bacterial lawns of *P. aeruginosa* strain PAO1. The strain used in the top panel expresses Cas12a but no targeting crRNA. Strains used in bottom seven panels express *acr* genes but no CRISPR-Cas system.

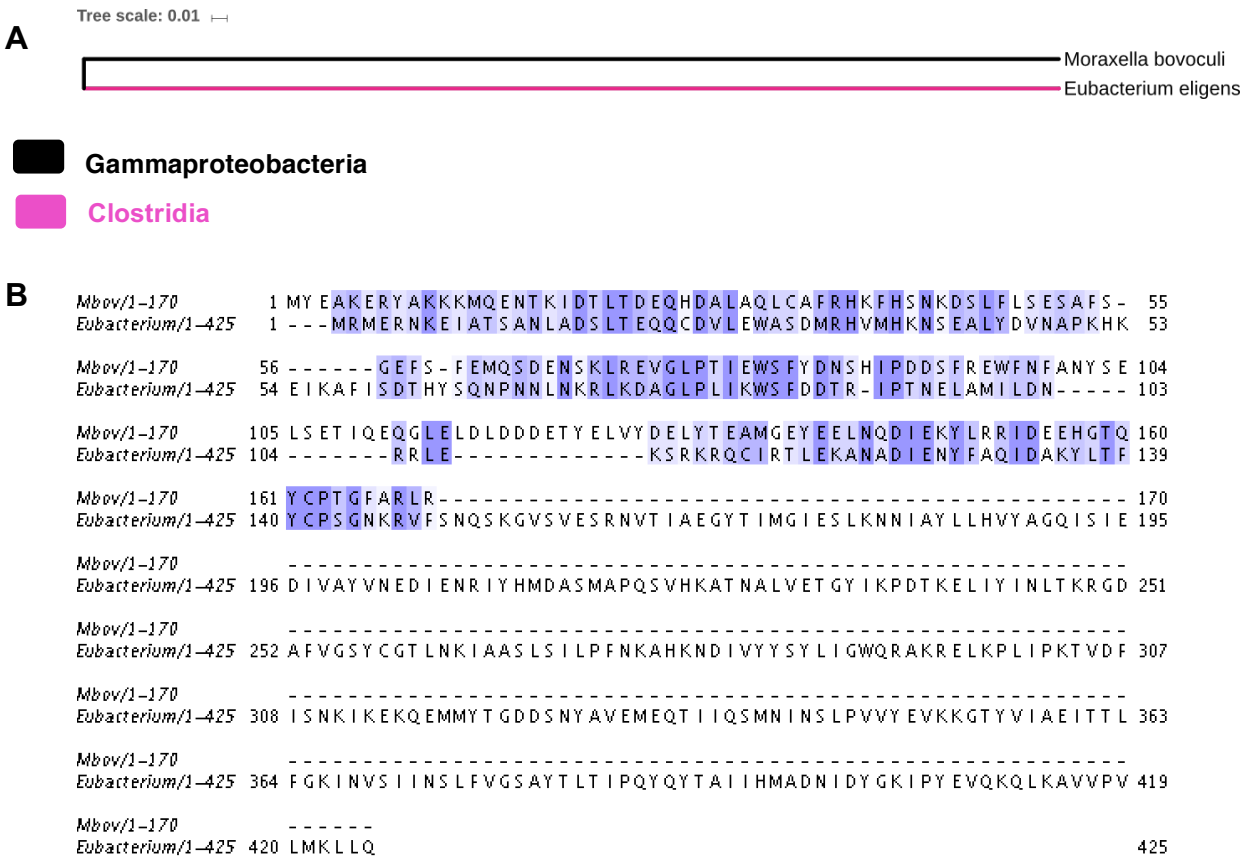
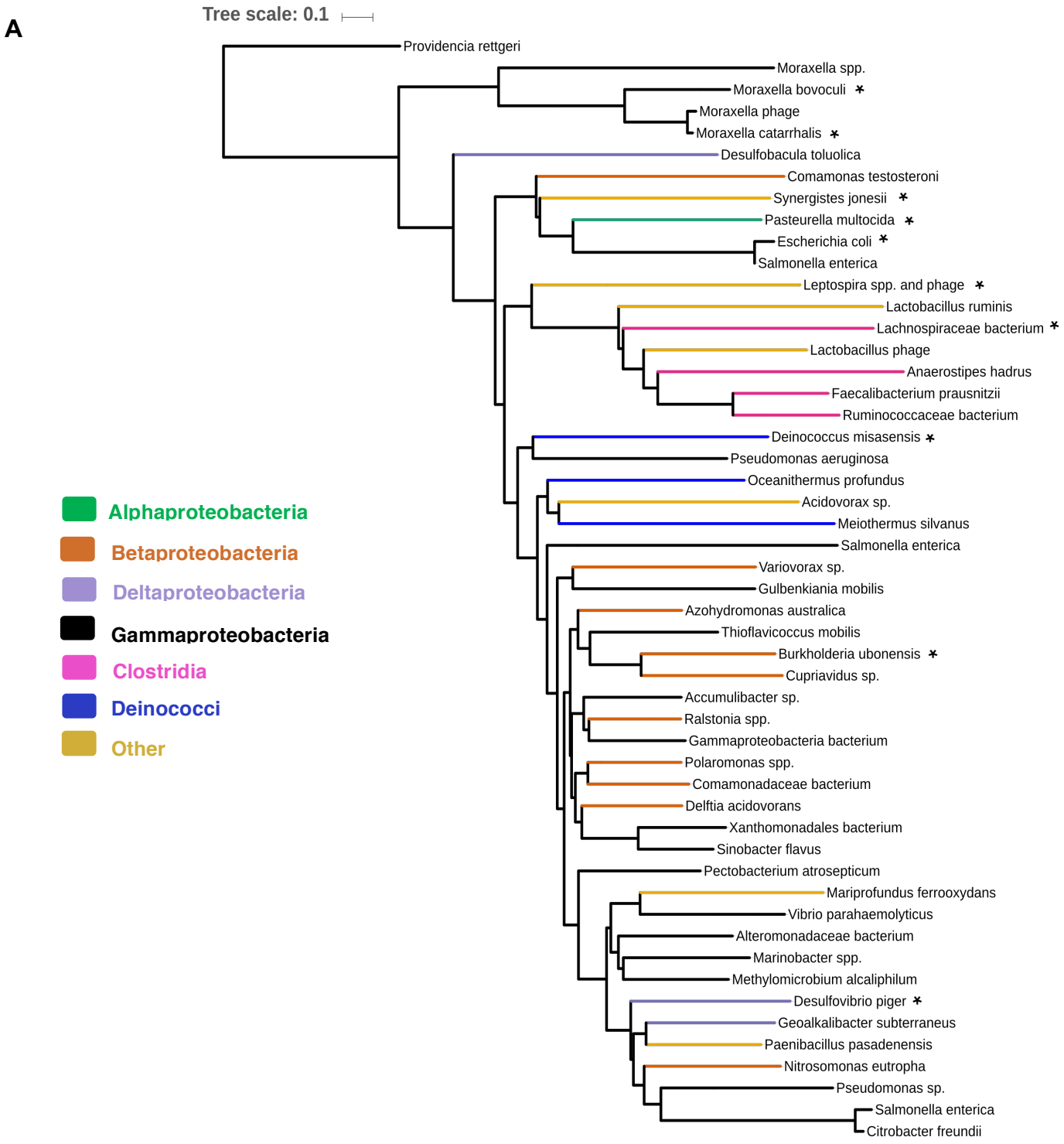


Fig S6. Phylogenetic distribution and protein sequence alignments of AcrVA1.
(A) Midpoint rooted minimum-evolution phylogenetic tree of full-length AcrVA1 orthologues. Scale bar indicates 0.01 substitutions per site. Branch colors correspond to the class of bacteria in which each homolog was found (see legend). (B) The protein sequences of AcrVA1 orthologues were aligned with Jalview and colored using BLOSUM62 scheme. Dark blue indicates that the residue matches the consensus sequence at that position, while light blue indicates the two residues have a positive Blosum62 score. Alignment includes orthologues from *Moraxella bovoculi* and *Eubacterium eligens*.


A
Tree scale: 0.1
Providencia rettgeri
Moraxella spp.
Moraxella bovoculi *
Moraxella phage
Moraxella catarrhalis *
Desulfobacula toluolica
Comamonas testosteroni
Synergistes jonesii *
Pasteurella multocida *
Escherichia coli *
Salmonella enterica
Leptospira spp. and phage *
Lactobacillus ruminis
Lachnospiraceae bacterium *
Lactobacillus phage
Anaerostipes hadrus
Faecalibacterium prausnitzii
Ruminococcaceae bacterium
Deinococcus misasensis *
Pseudomonas aeruginosa
Oceanithermus profundus
Acidovorax sp.
Meiothermus silvanus
Salmonella enterica
Variovorax sp.
Gulbenkiania mobilis
Azohydromonas australica
Thioflavicoccus mobilis
Burkholderia ubonensis *
Cupriavidus sp.
Accumulibacter sp.
Ralstonia spp.
Gammaproteobacteria bacterium
Polaromonas spp.
Comamonadaceae bacterium
Delftia acidovorans
Xanthomonadales bacterium
Sinobacter flavus
Pectobacterium atrosepticum
Mariprofundus ferrooxydans
Vibrio parahaemolyticus
Alteromonadaceae bacterium
Marinobacter spp.
Methylomicrobium alcaliphilum
Desulfovibrio piger *
Geoalkalibacter subterraneus
Paenibacillus pasadenensis
Nitrosomonas eutropha
Pseudomonas sp.
Salmonella enterica
Citrobacter freundii
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Clostridia
Deinococci
Other

B

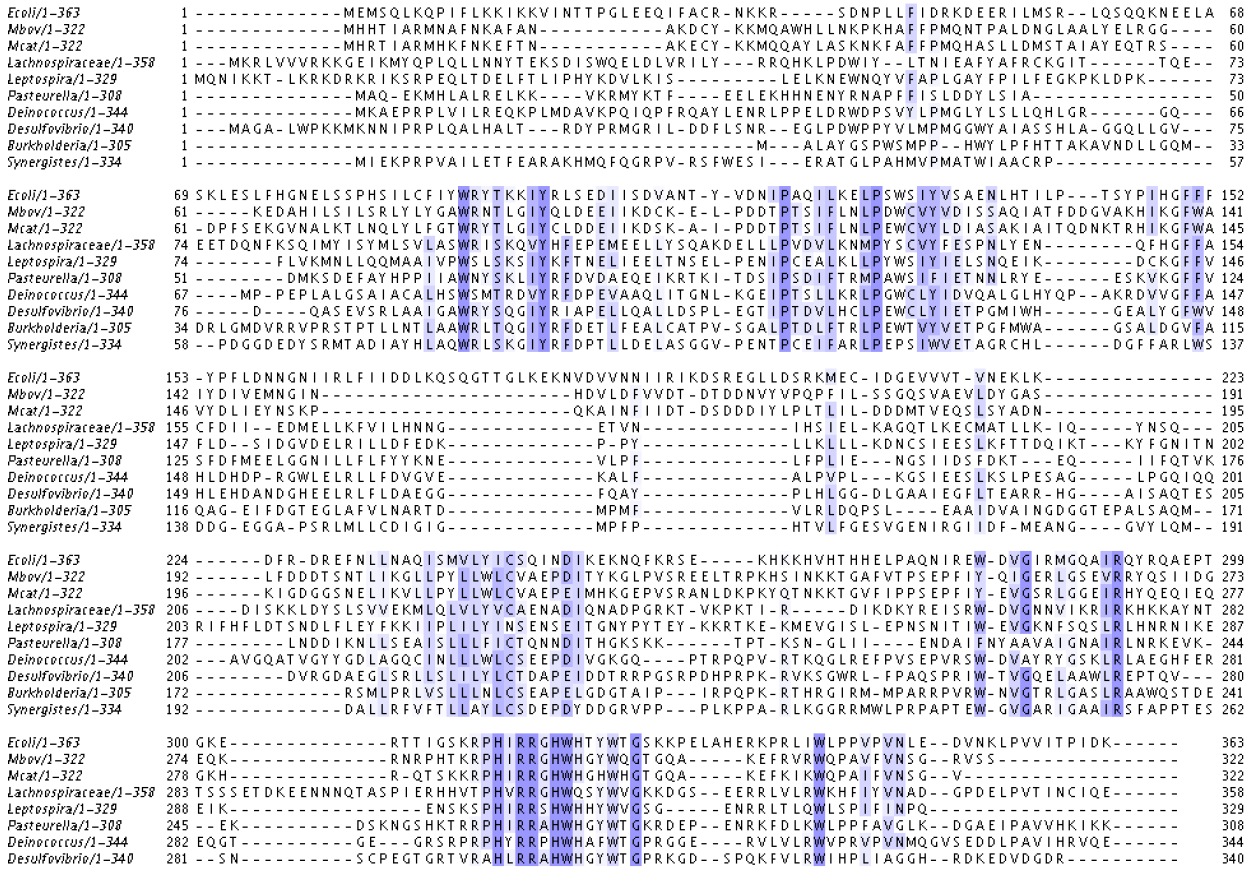
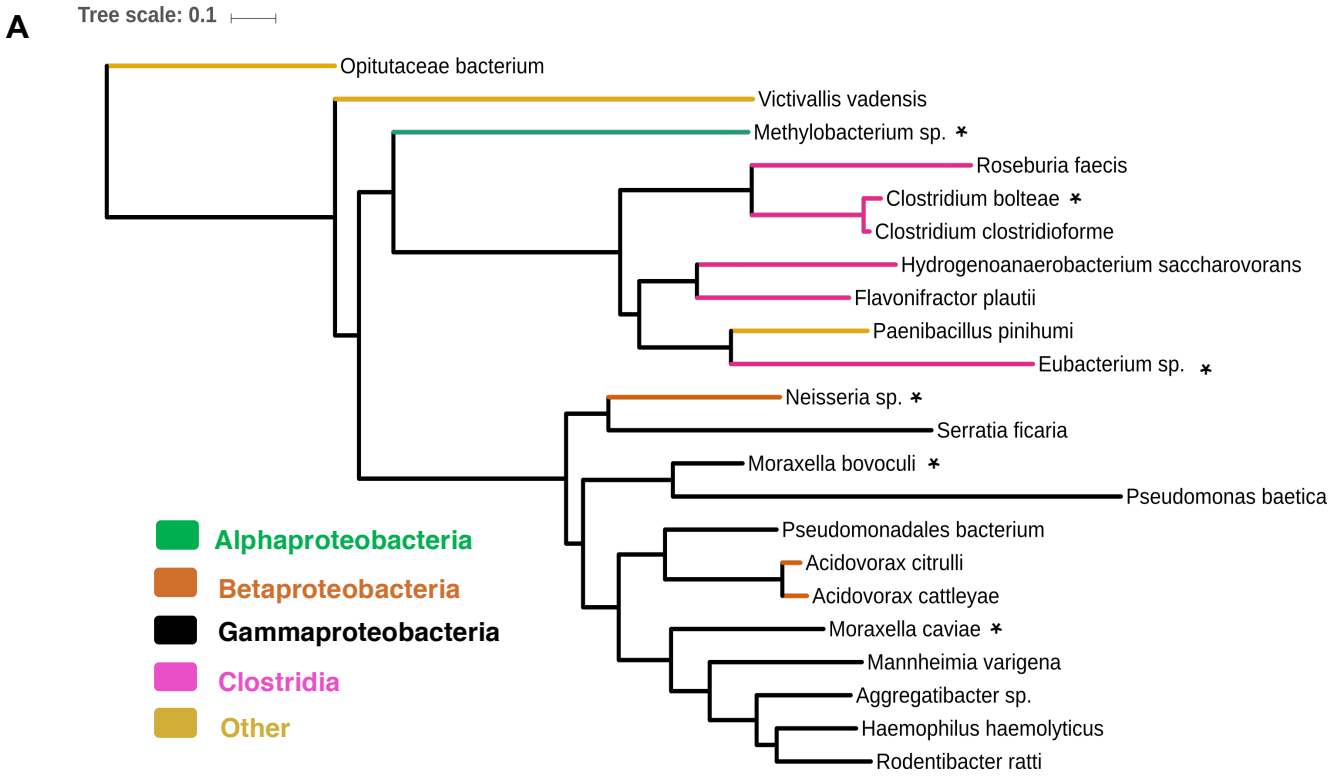


Fig. S7. Phylogenetic distribution and protein sequence alignments of AcrVA2.
(A) Midpoint rooted minimum-evolution phylogenetic tree of full-length AcrVA2 orthologues. Scale bar indicates 0.1 substitutions per site. Branch colors correspond to the class of bacteria in which each homolog was found (see legend). Asterisk indicates orthologue used in protein alignment in (B). (B) The protein sequence of different orthologues of AcrVA2 were aligned with Clustal Omega and colored in Jalview using BLOSUM62 scheme. Dark blue indicates that the residue matches the consensus sequence at that position, while light blue indicates the two residues have a positive Blosum62 score. AcrVA2 alignment includes orthologues from *Moraxella bovoculi* 58069, *Moraxella catarrhalis* BC8, *E. coli* (FinQ), *Leptospira noguchii*, *Burkholderia ubonesis*, *Synergistes jonesii*, *Lachnospiraceae bacterium* A4, *Pasteurella multocida*, *Deinococcus misasensis*, and *Desulfovibrio piger*.



B

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Eubacterium/1-188          1 MKENRFCVVCGTKLHERQIKYCSKKCMGIGKQNYKICPVCGKQFKDAATNDTVCCSHECSKTH  63
Clostridium/1-166          1 ----------------MSK----------RPPKSTKICVVCGKTFPCFPSDKTVTCGKECSKIH  38
Methylobacterium/1-156     1 ----------------------------MDNGERGK---RIIARGSV---------------TLT  19
Mcav/1-167                 1 ------------MIAHQKNRRADWESVDWTKHNDEIAQLLSRHPD---------------SVA  36
Mbov/1-168                 1 ---------------MVGKSKIDWQSIDWTKTNAQIAQECGRAYN---------------TVC  33
Neisseria/1-162            1 ------------------MPKYDWDKIDWRLSNHEIAAILQCSYD---------------TVA  30

Eubacterium/1-188         64 REQLHKSGIYDGS----------IEKMR---E----------------GFSEKIVEIGP-----E  94
Clostridium/1-166         39 RSRT-----HMGLSNAWSEESRTKKAA-----------QGKTANLALGTPAAQKSPKSGKFL  84
Methylobacterium/1-156    20 TFRAMMADDETGKTVGWTCTAAVRKPVPVDVFAARLAPHI------EAREKAKHDSPLAGPWE  76
Mcav/1-167                37 KMRTKFGAQGMAK----------RKPR-RKYKVTRK--AVPPPHTQELATAAAKISPKSGRYE  86
Mbov/1-168                34 KMRGKLGKSHQGA----------KSPR-KDKGISRPQPHLNRLEYQALATAKAKASPKAGRFE  85
Neisseria/1-162           31 SKRYRLKVGKATK----------PKTR-SDKGISRTTY-LPPKEQQRRAVEAAKASPKAGRGE  81

Eubacterium/1-188         95 KHWFSKHWVIESPSGQVYECDNLMHFIRTHPDLFDGTPTQ----------------AFDGFQK 141
Clostridium/1-166         85 TNINAKDWHLISPDGKEYKFHCLNYWLRENCDKLFGCM----------PDSKEFKNVSTGLAG 137
Methylobacterium/1-156    77 TNASAREWSLLAPDGTAHRFRDLALWLRERADLFDPADVAERPQSRT----GVSTNAQAQLGK 135
Mcav/1-167                87 TNVNAKRWLIISPSGQRFEFSNLQHFVRNHPELFAKADTVWKRQGGKRGTGGEYCNASNGLAQ 149
Mbov/1-168                86 TNTKAKTWTLKSPDNKTYTFTNLMHFVRTNPHLFDPDDVVWRTK--S--NGVEWCRASSGLAL 144
Neisseria/1-162           82 TNCHAKRWRLTDPYGKQYEFSNLHHFIRCNNNLFTRKDVVWKRTGSN--GGGEYCNASAGLQN 142

Eubacterium/1-188        142 IKATREGKRPKAPSKSWKGWHLISYSENRNKYRKGETDGRKKSKDSI                  188
Clostridium/1-166        138 AKRAM--LGKNYRCCTYKGWKVIPTEHDIKK----------------                166
Methylobacterium/1-156   136 LRP-----ERRAGRKSWKGWTWLTRQ--------------------                156
Mcav/1-167               150 A-A-----R---LNIGWKGWQAKIIKG--------------------                167
Mbov/1-168               145 L-A-----KRKKAPLSWKGWRLISLTKDNK-----------------                168
Neisseria/1-162          143 V-V-----AG--KSPAWKGWEIEEITND--------------------                162
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Fig. S8. Phylogenetic distribution and protein sequence alignments of AcrVA3. (A) Midpoint rooted minimum-evolution phylogenetic tree of full-length AcrVA3 orthologues. Scale bar indicates 0.1 substitutions per site. Branch colors correspond to the class of bacteria in which each homolog was found (see legend). Asterisk indicates orthologue used in protein alignment in (B). (B) The protein sequence of different orthologues of AcrVA3 were aligned with Clustal

Omega and colored in Jalview using BLOSUM62 scheme. Dark blue indicates that the residue matches the consensus sequence at that position, while light blue indicates the two residues have a positive Blosum62 score. AcrVA3 alignment includes orthologs from *Moraxella bovoculi* 58069, *Moraxella caviae*, *Neisseria sp*. HMSC056A03, *Clostridium bolteae* 90B7, *Methylobacterium sp*. Leaf399 and *Eubacterium sp*. An3.

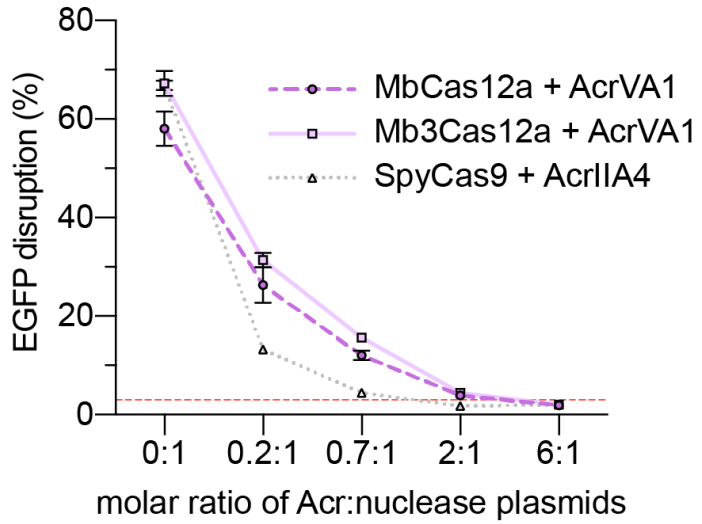

Fig. S9. Dose response curves of CRISPR nuclease inhibition by Acr proteins in human cells.

Comparison between the inhibitory activities of AcrVA1 against MbCas12a and Mb3Cas12a, and AcrIIA4 against SpyCas9, across various levels of Acr expression. EGFP disruption activities assessed by flow cytometry 52 hours post-transfection; background EGFP disruption is indicated by the red dashed line; error bars indicate s.e.m. for n = 3.

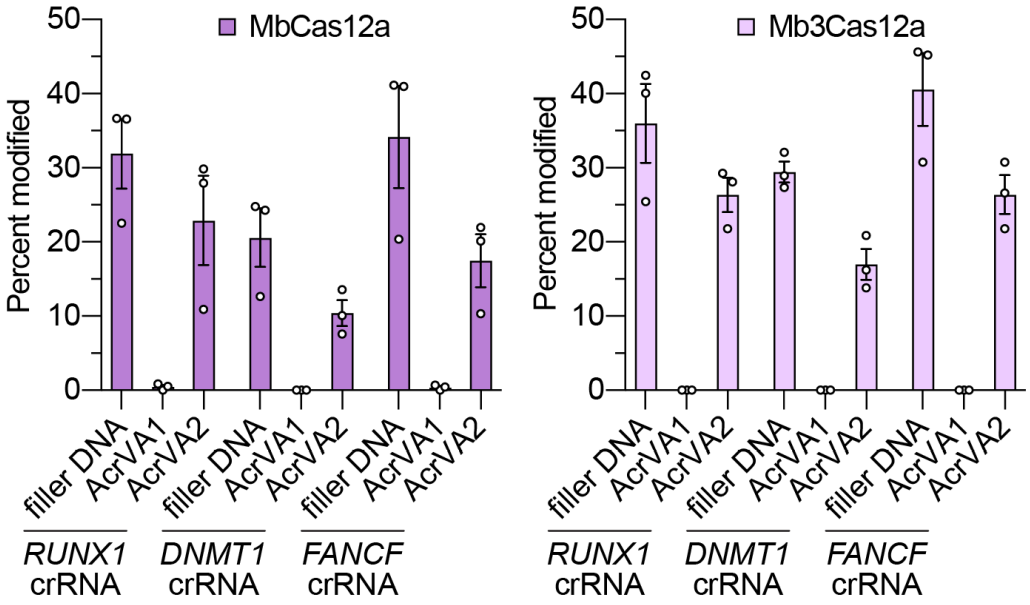

Fig. S10. Effect of AcrVA1 and AcrVA2 on endogenous gene modification by Cas12a. Inhibition of Cas12a activity against endogenous sites in human cells was assessed by co-transfecting U2-OS cells with nuclease, Acr, and crRNA or sgRNA expressions plasmids (targeted to the *RUNX1, DNMT1, or FANCF* genes). Gene modification assessed by T7 endonuclease I (T7E1) assay 72 hours post-transfection; error bars indicate s.e.m. for n = 3. There is no significant difference between "filler DNA" and "AcrVA2" for any endogenous site; Student's t-test, $p < 0.05$.

Table S1. Protein sequences and accession numbers of Acr and Aca proteins found in this study.

Name	Accession	Protein Sequences	pI
AcrIE4-F7	WP_064584002.1	MSTQYTYQQIAEDFRLWSEYVDTAGEMSKDEFNS LSTEDKVRLQVEAFGEEKSPKFSTKVTTKPDFDGF QFYIEAGRDFDGDAYTEAYGVAVPTNIAARIQAQA AELNAGEWLLVEHEA	4.2
AcrIE5	WP_074973300.1	MSNDRNGIINQIIDYTGTDRDHAERIYEELRADDRI YFDDSVGLDRQGLLIREDVDLMAVAAEIE	4.16
AcrIE6	WP_087937214.1	MNNDTEVLEQQIKAFELLADELKDRLPTLEILSPM YTAVMVTYDLIGKQLASRRAELIEILEEQYPGHAA DLSIKNLCP	4.45
AcrIE7	WP_087937215.1	MIGSEKQVNWAKSIIEKEVEAWEAIGVDVREVAAF LRSISDARVIIDNRNLIHFQSSGISYSLESSPLNSPIFL RRFSACSVGFEEIPTALQRIRSVYTAKLLEDE	5.15
AcrIF11	WP_038819808.1	MSMELFHGSYEEISEIRDSGVFGGLFGAHEKETALS HGETLHRIISPLPLTDYALNYEIESAWEVALDVAGG DENVAEAIMAKACESDSNDGWELQRLRGVLAVRL GYTSVEMEDEHGTTWLCLPGCTVEKI	4.38
AcrIF11.1	WP_033936089.1	MEIFHTSPVEITTINTQGRFGEFLCFAADEYVM TAGDHVTYRIKVDESDIIMAGSIFYHERAADLS GLVE RVMQLTGCDEDTAEELISQRIDVFNLDDIDAS DAAELSWEIQAITAKAAKTLGFRGVSMQDEQ GTCYMID MLGHDAELVRVK	4.28
AcrIF11.2	EGE18857.1	MTTLYHGSHENTAPVIKIGFAAFLPADNVFDGIFA NGDKNVARSHGDFIYAYEVDSIATNDDLDCDEAIQ IIAKELYIDEETAAPIAEAVAYEESLAEFEEHIMPRS CGDCADFGWEMQRLRGVIARKLGFDAVECVDEH GVSHLIVNANIRGSIA	4.35
AcrIF12	ABR13388.1	MAYEKTWHRDYAAESLKRAETSRWTQDANLEWT QLALECAQVVHLARQVGEELGNEKIIGIADTVLSTI EAHSQATYRRPCYKRITTAQTHLLAVTLLERFGSA RRVANAVWQLTDDEIDQAKA	6.07

AcrIF13	EGE18854.1	MKLLNIKINEFAVTANTEAGDELYLQLPHTPDSQH SINHEPLDDDDFVKEVQEICDEYFGKGDRTLARLS YAGGQAYDSYTEEDGVYTTNTGDQFVEHSYADY YNVEVYCKADLV	4.17
AcrIF14	AKI27193.1	MKKIEMIEISQNRQNLTAFLHISEIKAINAKLADGV DVDKKSFDEICSIVLEQYQAKQISNKQASEIFET LAKANKSFKIEKFRCSHGYNEIYKYSPDHEAYLFY CKGGQGQLNKLIAENGRFM	8.37
Orf1(Pse)	SDJ61947.1	MGVVVVLIIRLKARWSLHLERKLGEAGKAGIWEF HRSESSYTTDGRTTFRNAALRPAEPKEGQTVEVFIC SDSREPEEQWRAVGEGVARYE	6.74
Orf2(Pse)	WP_084336955.1	MLSVLFFWLYFYALFFIRFASSNKRARGRGMQRPA LVSIALEWGMRRELMSRSFTTRIDHLQEVSRLGRG VARLRLGHSGRNLMPLILERRDGTGLTLKLDPKAD PDEALRQLARGGIHVRVYSKYGERMRVVVDAPQA ISILRDELVDRE	11.10
Aca1	YP_007392343	MRFPGVKTPDASNHDPDPRYLRGLLKKAGISQ RRAAELLGLSDRVMRYYLSEDIKEGYRPAPYT VQFALE CLANDPPSA	8.85
Aca2	WP_019933869.1	MTHYELQALRKLLMLEVSEAAREIGDVSPRS WQYWESGRSPVPDDVANQIRNLTDMRYQLLE LRTEQIEK AGKPIQLNFYRTLDDYEAVTGKRDVVSWRLT QAVAATLFAEGDVTLVEQGGLTLE	4.79
Aca3	WP_049360086.1	MKKFEAPEIGYTPANLKALRKQFGLTQAQVA EITGTKTGYSVRRWEAAIDAKNRADMPLVKW QKLLDSLK	9.92
Aca4	ABR13385.1	MTEEQFSALAELMRLRGGPGEDAARLVLVNGLKP TDAARKTGITPQAVNKTLSSCRRGIELAKRVFT	10.01
Aca5	WP_039494319.1	MSLTEYIDKNFAGNKAAFARHMGVDAQAVN KWIKSEWFVSTTDDNKIYLSSVRREIPPVA	8.09
Aca6	WP_035450933.1	MTAMKEWRARMGWSQRRAAQELGVTLPTY QSWEKGIRLSDGSPIDPPLTALLAAAAREKGLP PIS	9.98
Aca7	WP_064702654.1	MIDARKHYDPNLAPELVRRALAVTGTQKELA ERLDVSRTYLQLLGKGQKSMSYAVQVMLEQV IQDGET	6.55

AcrIC1	AKG19229.1	MNNLKKTAITHDGVFAYKNTETVIGSVGRNDIVM AIDATHGEFNDKNFIIYADTNGNPIYLGYAYLDDN NDAHIDLAVGACNEDDDFDEKEIHEMIAEQMELA KRYQELGDTVHGTTRLAFDDDGYMTVRLDQQAY PDYRPENDDKHIMWRALALTATGKELEVFWLVED YEDEEVNSWDFDIADDWREL	4.17
Orf1(Mor)	EGE18856.1	MSKNKTPDYVLRANANYRKKHTTNKSLQLHNEK DADIIQALQNETKSFNALMKDILRNHYNLNQNQ	9.75
Orf2(Mor)	AKG19231.1	MNNPKTPEYTRKAIRAYEKNLVRKSVTFDVRKDD DMELLKMIEQDGRTFAQIARTALLEHLQK	9.45
AcrVA1	AKG19227.1	MYEAKERYAKKKMQENTKIDTLTDEQHDALAQL CAFRHKFHSNKDSLFLSESAFSGEFSFEMQSDENSK LREVGLPTIEWSFYDNSHIPDDSFREWFNFANYSEL SETIQEQGLELDLDDDETYELVYDELYTEAMGEYE ELNQDIEKYLRRIDEE HGTQYCPTGFARLR	4.39
AcrVA2	AKG19228.1	MHHTIARMNAFNKAFANAKDCYKKMQAWHLLN KPKHAFFPMQNTPALDNGLAALYELRGGKEDAHI LSILSRLYLYGAWRNTLGIYQLDEEIIKDCKELPDD TPTSIFLNLPDWCVYVDISSAQIATFDDGVAKHIKG FWAIYDIVEMNGINHDVLDFVVDTDTDDNVYVPQ PFILSSGQSVAEVLDYGASLFDDDTSNTLIKGLLPY LLWLCVAEPDITYKGLPVSREELTRPKHSINKKTG AFVTPSEPFIYQIGERLGSEVRRYQSIIDGEQKRNRP HTKRPHIRRGHWHGYWQGTGQAKEFRVRW QPAVFVNSGRVSS	6.46
AcrVA2.1	AKG12143.1	MHHTIARMNAFNKAFGNAKDCYKKMQAWHLNN KPKHIFSPLQNTLSLNEGLAALYELHGGKEDEHILS ILCCLYLYGTWRNTLGIYQLDEEIIKDCKELPDDTP TSIFLNLPDWCVYVDISSAKIATIDGGVAKHIKGFW AIYDNIEMHGVNHDVLNFIIDTDTDNNIYVPQSLIL SSEMSVAESLDYGLTLFGYDESNELVKGMLPYLL WLCVAEPDITHKGLPVSREELTKPKHGINKKTGAF VTPSEPFIYQIGERLGGEVRRYQSLIDDEKNQNRH HTKRPHIRRGHWHGYWQGTGQAKEFKVRWQPAV FVNSGV	6.24
AcrVA3	AKG19230.1	MVGKSKIDWQSIDWTKTNAQIAQECGRAYNTVCK MRGKLGKSHQGAKSPRKDKGISRPQPHLNRLEYQ ALATAKAKASPKAGRFETNTKAKTWTLKSPDNKT YTFTNLMHFVRTNPHLFDPDDVVWRTKSNGVEW CRASSGLALLAKRKKAPLSWKGWRLISLTKDNK	10.34
AcrVA3.1	OOR90252.1	MIAHQKNRRADWESVDWTKHNDEIAQLLSRHPDS VAKMRTKFGAQGMAKRKPRRKYKVTRKAVPPPH TQELATAAAKISPKSGRYETNVNAKRWLIISPSGQR FEFSNLQHFVRNHPELFAKADTVWKRQGGKRGTG GEYCNASNGLAQAARLNIGWKGWQAKIIKG	10.80

Table S2: List of all accessions of AcrIF11 homologs used to build Figure S2.

List of the accession numbers for AcrF11 homologs represented in the AcrIF11 phylogenetic tree in fig. S2. The species in which each listed accession is found is listed on the right.

AcrIF11 Accession	**Species**
WP_038819808.1	*Pseudomonas aeruginosa* (AcrIF11)
WP_102394900.1	*Enterovibrio norvegicus*
WP_033936089.1	*Pseudomonas aeruginosa* (AcrIF11.1)
WP_087698854.1	*Chromobacterium violaceum*
WP_049175110.1	*Acinetobacter ursingii*
WP_004681960.1	*Acinetobacter parvus*
WP_062681378.1	*Achromobacter xylosoxidans*
KTG25401.1	*Idiomarina sp.*
WP_059284897.1	*Aquitalea magnusonii*
WP_107732478.1	*Chromobacterium haemolyticum*
WP_071971444.1	*Alteromonas mediterranea*
WP_086652143.1	*Acetobacter cibinongensis*
OHU91773.1	*Pseudoalteromonas amylolytica*
WP_064700809.1	*Halomonas sinaiensis*
WP_064702655.1	*Halomonas caseinilytica*
WP_066478200.1	*Comamonas terrae*
WP_068370878.1	*Kerstersia gyiorum*
WP_057083778.1	*Dickeya solani*
WP_074032235.1	*Serratia fonticola*
WP_039494318.1	*Pectobacterium carotovorum*
WP_077457760.1	*Salinivibrio sp. IB872*
WP_064369479.1	*Vibrio alginolyticus*
WP_041946990.1	*Vibrio anguillarum*
WP_036292019.1	*Methylosinus sp. PW1*
WP_017725053.1	*Acinetobacter baumannii*
WP_061524032.1	*Acinetobacter venetianus*
WP_004824702.1	*Acinetobacter bereziniae*
WP_049556453.1	*Yersinia kristensenii*
WP_109055423.1	*Brenneria roseae*
WP_097468739.1	*Escherichia coli*
OZT63688.1	*Salmonella enterica*
PKT06451.1	*Klebsiella pneumoniae*
WP_084913096.1	*Rouxiella badensis*
WP_050090803.1	*Yersinia pseudotuberculosis*
WP_050879812.1	*Yersinia enterocolitica*

WP_050296286.1	*Yersinia frederiksenii*
WP_079326564.1	*Moraxella equi*
WP_003671754.1	*Moraxella catarrhalis* (AcrIF11.2)
WP_026949101.1	*Alcanivorax sp.*
WP_092828131.1	*Halomonas subterranea*
WP_027705017.1	*Zymobacter palmae*
SMF80656.1	*Pseudobacteriovorax antillogorgiicola*
WP_016360505.1	*Bilophila wadsworthia*
SMC32303.1	*Fulvimarina manganoxydans*
WP_051420249.1	*Providencia alcalifaciens*
WP_060561196.1	*Providencia stuartii*
WP_004247747.1	*Proteus mirabilis*
WP_086368795.1	*Photobacterium damselae*
WP_078005047.1	*Izhakiella australiensis*
WP_018125160.1	*Desulfovibrio oxyclinae*
OYL21963.1	*Shigella sonnei*
PAY74230.1	*Shigella flexneri*
CFQ72446.1	*Yersinia similis*

Table S3. A table of previously discovered *aca* genes (*aca1-3*) and novel *aca* genes found in this study (*aca4-7*).

All Aca proteins are predicted with high confidence to contain helix-turn-helix motifs as predicted by HHPred *(20)*.

Name	HHPred : Protein motifs	HHPred Probability, e value	Discovery	Citation
Aca1	Helix-turn-helix, DNA binding	Probability = 98%, e value = 1.6E-6	Associated with Type I-F and Type I-E inhibitors	Bondy-Denomy et al, Nature 2013, Pawluk et al. mBio 2014, Pawluk et al. Nature Micro 2016
Aca2	Helix-turn-helix, DNA binding	Probability = 98%, e value = 5E-8	Associated with Type I-F and Type II-C inhibitors	Pawluk et al. Nature Micro 2016, Pawluk et al. Cell 2016
Aca3	Helix-turn-helix, DNA binding	Probability = 98%, e value = 4.2E-8	Associated with Type II-C inhibitors	Pawluk et al. Cell 2016
Aca4	Helix-turn-helix, DNA binding	Probability = 99%, e value = 3.1E-9	Associated with AcrIF11 and AcrIF12 in *Pseudomonas sp.*	This study
Aca5	Helix-turn-helix, DNA binding	Probability = 97%, e value = 5.6E-5	Associated with AcrIF11 in *Pectobacterium carotovorum*, *Yerisnia frederiksenii*, *Escherichia coli*, *Serratia fonticola*, *Dickeya solani*, and *Enterobacter cloacae* complex members	This study
Aca6	Helix-turn-helix, DNA binding	Probability = 98%, e value = 7.8E-7	Associated with AcrIF11 in *Alcanivorax sp.*	This study
Aca7	Helix-turn-helix, DNA binding	Probability = 99%, e value = 7.2E-9	Associated with AcrIF11 in *Halomonas sp.*	This study

Table S4: List of Aca accessions.

Representative homologs of each Aca protein (Aca1-7) and its associated AcrIF11 homolog listed by accession number as well as the species of origin.

Species	Aca	AcrIF11 accession	Aca accession
Pseudomonas aeruginosa	Aca1	WP_038819808.1	WP_033971918.1
Pseudomonas aeruginosa	Aca4	WP_034011523.1	WP_079381596.1
Pectobacterium carotovorum	Aca5	WP_039494318.1	WP_039494319.1
Yersinia frederiksenii	Aca5	WP_050101208.1	WP_050101207.1
Escherichia coli	Aca5	WP_000765122.1	WP_012565004.1
Serratia fonticola	Aca5	WP_074032235.1	WP_074032234.1
Dickeya solani	Aca5	WP_057083778.1	WP_057083779.1
Pectobacterium carotovorum	Aca5	WP_039558031.1	WP_039558032.1
Enterobacter cloacae complex	Aca5	WP_045331704.1	WP_072050017.1
Alcanivorax sp.	Aca6	WP_026949101.1	WP_035450933.1
Alcanivorax sp.	Aca6	WP_063139756.1	WP_063139755.1
Halomonas caseinilytica	Aca7	WP_064702655.1	WP_064702654.1
Halomonas sinaiensis	Aca7	WP_064700809.1	WP_064700810.1

Table S5. Type V-A self-targeting spacers in *Moraxella bovoculi* strains.

List of spacers encoded in the Type V-A CRISPR array in *Moraxella bovoculi* that have matching protospacers (with PAM motif) in the same genome. 58069, 22581, 28389, and 33362 are all strains.

58069
GCTTCAATCTTGGCAAGTGTTTCATCA
AGATAGGCATTTGAAAAAGAATTTATCT
TTCGTCCTTTATACGCACCCCTTGCTT
22581
ATGGTTAATGATGATAACCCAGATTTAAT
TTTAGAAATCACGGATCATTATATATGT
ATATCCATCTACTAACCATCGCAAAAA
ATTGATGTAAACATCGATGGTGTGGTT
ATTGGTTTGTGTAACGGGGAAATTAAG
TCAAAAATGGTAGCATTTGTTAAGAAT
TGCAGGTGGTGAATCAGCGACACATTC
28389
CTAAATGCCGTGTCGTTTTGGTTCTTAT
ATGAAATAGAGCAACAGCAGAACGGTA
ATTGATGTAAACATCGATGGTGTGGTT
33362
CTAAATGCCGTGTCGTTTTGGTTCTTAT

Table S6. Type I-C self-targeting spacers in *Moraxella bovoculi* 58069.

List of spacers encoded in the Type I-C CRISPR array that have matching protospacers (with PAM motif) in the same genome of *Moraxella bovoculi* 58069.

ACCCCGTTATCTGCCACGGTGGCGTTGGCTTTGT
ACTTCGCAACATTGGCTATCCAAGTAACGCAAAC
AGCCAAGCTGGTTCGGTTGCCCTTGCCTTTGGAT
ATCGGTTTTGCATTCGGCTAAGGATTTGGGTGTA
ATTTTTAAGCACCACGCCATAATCGCCAAACACC
CAAAGACTGCTTTTTAAGCCAATCATAGTAGCTA
CCAACACGCCTAAGACACGATGACTTGTTTTTAG
TATCTCTTCAGCTTGCTCACGCCAACCCGCCTGC
TGGTGAATTTTCTTTTGAGATGCAGTCTGATGAA
TTTTTCTTGATCGATAGACGACTGATTAAACAAG

Table S7. Type V-A and I-C crRNAs used for experiments in *Pseudomonas aeruginosa.*

Type	crRNA ID	target gene	crRNA sequence (repeats in bold)	Spacer with PAM
V-A	crRNA 1-gp23	JBD30_gp23	**aaatttctactgtttgtagat**cgtttacccactgagcgaacgc**aaatttctactgtttgtagat**	**TTTG**CGTTTTACCCACTGAGCGAACGC
V-A	crRNA 2-gp24	JBD30_gp24	**aaatttctactgtttgtagat**gcccgtttcgataccgcacataa**aaatttctactgtttgtagat**	**TTTC**GCCCGTTTCGATACCGCACATAA
I-C	LL77 crRNA	JBD30_gp24	**gtcgcgccccgcacgggcgcgtggattgaaac**aacctcgcggcatccgcaacaacaaccccggcaa**gtcgcgccccgcacgggcgcgtggattgaaac**	**TTC**AACCTCGCGGCATCCGCAACAACAACCCCGGCAA

Table S8. Plasmids used for expression in *Pseudomonas aeruginosa*.

Plasmid ID	Plasmid use	Backbone	Description
NM100	Express acrVA1	pHERD30T	p30T-acrVA1
NM101	Express acrVA2	pHERD30T	p30T-acrVA2
NM102	Express acrIC1	pHERD30T	p30T-acrIC1
NM103	Express acrVA3	pHERD30T	p30T-acrVA3
NM104	Express ORF2 (Mor)	pHERD30T	p30T-ORF2 (Mor)
NM105	Express acrIF13	pHERD30T	p30T-acrIF13
NM106	Express acrIF14	pHERD30T	p30T-acrIF14
NM107	Express VA2.1	pHERD30T	p30T-acrVA2.1
NM109	Express VA3.1	pHERD30T	p30T-acrVA3.1
NM110	Express type V-A crRNA against JBD30 gp23	mini-CTX2	mini-CTX2-crRNA g23
NM111	Express type V-A crRNA against JBD30 gp24	mini-CTX2	mini-CTX2-crRNA g24 (V-A)
JDB500	Express MbCas12a (237)	pTN7C130	pTN7-MbCas12a
JDB501	Express type V-A crRNA against JBD30 gp23	pHERD30T	p30T-crRNA g23
JDB502	Express type V-A crRNA against JBD30 gp24	pHERD30T	p30T-crRNA g24
JZ_83	Express acrIE5	pHERD30T	p30T-acrIE5
JZ_99	Express acrIE6	pHERD30T	p30T-acrIE6
JZ_100	Express acrIE7	pHERD30T	p30T-acrIE7
JZ_127	Express ORF1 (Pse)	pHERD30T	p30T- ORF1 (Pse)
JZ_297	Express ORF2 (Pse)	pHERD30T	p30T- ORF2 (Pse)
JZ_298	Express acrIE4-IF7	pHERD30T	p30T-acrIE4-IF7
JZ_299	Express acrIF11	pHERD30T	p30T-acrIF11
JZ_300	Express acrIF12	pHERD30T	p30T- acrIF12
JZ_303	Express F11.1	pHERD30T	p30T-acrIF11.1
JZ_309	Express F11.2	pHERD30T	p30T-acrIF11.2
pJW31	Express type I-C cas3-5-8-7 genes	pUC18T-mini-Tn7T-LAC	pUC18T-cas3-5-8-7
LL7724	Express type I-C crRNA against JBD30 gp24	mini-CTX2	mini-CTX2-crRNA g24 (I-C)

Table S9. Strains of *P. aeruginosa* used in this study.

Strain Name	Strain Use	Parent
PAO1	Control for phage plaque formation	PAO1
PAO1 tn7::MbCas12a	"No crRNA" control for type V-A phage targeting assay	PAO1
PAO1 tn7::MbCas12a crRNA-1	Type V-A phage targeting assay	PAO1
PAO1 tn7::MbCas12a crRNA-2	Type V-A phage targeting assay	PAO1
SMC4386	Type I-E phage targeting assay	SMC4386
UCBPP-PA14 (PA14)	Type I-F phage targeting assay	UCBPP-PA14
PA14 ΔCRISPR1ΔCRISPR2 (SMC5454)	ΔCRISPR control for type I-F phage targeting assay	UCBPP-PA14
LL77	Type I-C phage targeting assay	PAO1
LL76	"No crRNA" control for type I-C phage targeting assay	PAO1

Table S10. Plasmids used for experiments in human cells.

Plasmid ID	Plasmid Use	Plasmid Description	Addgene ID
BPK3079	U6 promoter crRNA entry vector used for all AsCas12a crRNAs (clone spacer oligos into BsmBI cassette)	pUC19-U6-AsCas12a_crRNA-BsmBI_cassette	78741
BPK3082	U6 promoter crRNA entry vector used for all LbCas12a crRNAs (clone spacer oligos into BsmBI cassette)	pUC19-U6-LbCas12a_crRNA-BsmBI_cassette	78742
BPK4446	U6 promoter crRNA entry vector used for all FnCas12a crRNAs (clone spacer oligos into BsmBI cassette)	pUC19-U6-FnCas12a_crRNA-BsmBI_cassette	114087
BPK4449	U6 promoter crRNA entry vector used for all MbCas12a crRNAs (clone spacer oligos into BsmBI cassette)	pUC19-U6-MbCas12a_crRNA-BsmBI_cassette	114088
SQT1659	CAG promoter expression plasmid for human codon optimized AsCas12a nuclease with C-terminal NLS and HA tag	pCAG-hAsCas12a-NLS(nucleoplasmin)-3xHA	78743
SQT1665	CAG promoter expression plasmid for human codon optimized LbCas12a nuclease with C-terminal NLS and HA tag	pCAG-hLbCas12a-NLS(nucleoplasmin)-3xHA	78744
AAS1472	CAG promoter expression plasmid for human codon optimized FnCas12a nuclease with C-terminal NLS and HA tag	pCAG-hFnCas12a-NLS(nucleoplasmin)-3xHA	114089
AAS2134	CAG promoter expression plasmid for human codon optimized MbCas12a nuclease with C-terminal NLS and HA tag	pCAG-hMbCas12a-NLS(nucleoplasmin)-3xHA	114090
RTW2500	CAG promoter expression plasmid for human codon optimized Mb3Cas12a nuclease with C-terminal NLS and HA tag	pCAG-hMb3Cas12a-NLS(nucleoplasmin)-3xHA	115142
JDS246	CMV-T7 promoter expression plasmid for human codon optimized SpyCas9 nuclease with C-terminal NLS and HA tag	pCMV-T7-hSpCas9-NLS(sv40)-3xFLAG	43861
SQT817	CAG promoter expression plasmid for human codon optimized SpyCas9 nuclease with C-terminal NLS and HA tag	pCAG-hSpCas9-NLS(sv40)-3xFLAG	53373
BPK5050	CMV-T7 promoter expression plasmid for human codon optimized AcrVA1 anti-CRISPR protein with C-terminal NLS	pCMV-T7-hAcrVA1-NLS(sv40)	115136
AAS2283	CMV-T7 promoter expression plasmid for human codon optimized AcrVA2 anti-CRISPR protein with C-terminal NLS	pCMV-T7-hAcrVA2-NLS(sv40)	115138

BPK5059	CMV-T7 promoter expression plasmid for human codon optimized AcrVA2.1 anti-CRISPR protein with C-terminal NLS	pCMV-T7-hAcrVA2.1-NLS(sv40)	115137
BPK5077	CMV-T7 promoter expression plasmid for human codon optimized AcrVA3 anti-CRISPR protein with C-terminal NLS	pCMV-T7-hAcrVA3-NLS(sv40)	115140
RTW2624	CMV-T7 promoter expression plasmid for human codon optimized AcrVA3.1 anti-CRISPR protein with C-terminal NLS	pCMV-T7-hAcrVA3.1-NLS(sv40)	115139
BPK5095	CMV-T7 promoter expression plasmid for human codon optimized Orf2*mor* anti-CRISPR protein with C-terminal NLS	pCMV-T7-hOrf2*mor*-NLS(sv40)	115141
pJH373	CMV promoter expression plasmid for human codon optimized AcrIIA2 anti-CRISPR protein	pCMV-hAcrIIA2	86840
pJH376	CMV promoter expression plasmid for human codon optimized AcrIIA4 anti-CRISPR protein	pCMV-hAcrIIA4	86842

Table S11. crRNAs/sgRNAs used for experiments in human cells.

Type	crRNA ID	target gene	protospacer with PAM
V-A	*EGFP* crRNA 1	*EGFP*	**TTTA**CGTCGCCGTCCAGCTCGACC
V-A	*EGFP* crRNA 2	*EGFP*	**TTTG**CTCAGGGCGGACTGGGTGCT
V-A	*RUNX1* crRNA	*RUNX1*	**TTTA**CCTTCGGAGCGAAAACCAAG
V-A	*DNMT1* crRNA	*DNMT1*	**TTTG**GCTCAGCAGGCACCTGCCTC
V-A	*FANCF* crRNA	*FANCF*	**TTTG**GGCGGGGTCCAGTTCCGGGA
II-A	*EGFP* sgRNA 1	*EGFP*	GTCGCCCTCGAACTTCACCT**CGG**
II-A	*EGFP* sgRNA 2	*EGFP*	GTAGGTCAGGGTGGTCACGA**GGG**

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