Supplementary Materials for

A phage-encoded anti-CRISPR enables complete evasion of type VI-A CRISPR-Cas immunity

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Materials and Methods

Bacterial strains and growth conditions

All genetically modified *L. seeligeri* strains generated in this study are derived from *L. seeligeri* SLCC3954 (*19*). Environmental *L. seeligeri* isolates and *L. monocytogenes* strains are listed in Table S2. Unless otherwise stated, *L. seeligeri* and *L. monocytogenes* strains were cultured in Brain Heart Infusion (BHI) medium at 30°C. Where appropriate, BHI was supplemented with the following antibiotics for selection: nalidixic acid (50 µg/mL) chloramphenicol (10 µg/mL), erythromycin (1 µg/mL), or kanamycin (50 µg/mL). For cloning, plasmid preparation, and conjugative plasmid transfer, *E. coli* strains were cultured in Lysogeny Broth (LB) medium at 37°C. Where appropriate, LB was supplemented with the following antibiotics: ampicillin (100 µg/mL), chloramphenicol (25 µg/mL), kanamycin (50 µg/mL). For conjugative transfer of *E. coli – Listeria* shuttle vectors, plasmids were purified from Turbo Competent *E. coli* (New England Biolabs) and transformed into the *E. coli* conjugative donor strains SM10 λ*pir* or S17 λ*pir* (*30*).

Phage isolation and propagation

Temperate listeriaphages were isolated by prophage induction via stimulation of the SOS response with the DNA-damaging agent mitomycin C, followed by plaque isolation on the *L. seeligeri* ΔRM Δ*spc* indicator strain. Each strain of *L. seeligeri* and *L. monocytogenes* was cultured overnight and diluted to OD_{600} = 0.1, then treated with 1 µg/mL mitomycin C to activate the phage lytic cycle. Prophage induction was carried out overnight at 30°C, then culture supernatants were passed through 0.45 µm filters. Each filtrate was screened for phages by infection of ΔRM Δ*spc* using the top agar overlay method: 100ul of serially diluted induction filtrate was used to infect 100 µL of saturated ΔRM Δ*spc* culture in a 5 mL overlay of BHI containing 0.75% agar, in the presence of 5 mM $CaCl_2$. Infection plates were incubated at 30° for 24 hrs. Single plaques were resuspended in BHI, then propagated three times on ΔRM Δ*spc*, a single plaque was isolated each time to ensure phage purity. High titer phage lysates were obtained by preparing top agar infections of ΔRM Δ*spc* with plaques at near-confluent density, then soaking the agar with SM buffer (100mM NaCl, 10mM $MgSO_4$, 50mM Tris-HCl pH 7.5).

Plasmid construction and preparation

All genetic constructs for expression in *L. seeligeri* were cloned into the following three compatible shuttle vectors, each of which contains an origin of transfer sequence for mobilization by transfer genes of the IncP-type plasmid RP4. These transfer genes are integrated into the genome of the *E. coli* conjugative donor strains SM10 λ*pir* and S-17 λ*pir* (*30*).All plasmids used in this study, along with details of their construction, can be found in Table S2.

pPL2e – single-copy plasmid conferring erythromycin resistance that integrates into the $tRNA^{Arg}$ locus in the *L. seeligeri* chromosome (*31*).

pAM8 – *E. coli – Listeria* shuttle vector conferring chloramphenicol resistance (*32*).

pAM326 - *E. coli – Listeria* shuttle vector conferring kanamycin resistance (constructed in this study).

To express crRNAs containing engineered spacers, a minimal type VI CRISPR array containing the native promoter and a single repeat-spacer-repeat unit with BsaI entry sites was cloned into BamHI/SalI-digested pPL2e to generate pAM305. To clone new spacers, pAM305 was digested with BsaI, and ligated to spacer inserts consisting of annealed oligos with cohesive overhangs compatible with the sticky ends generated by BsaI-cleavage of pAM305.
All plasmid targeting assays described in this study use the pAM8-derived plasmid pAM54 (*32*), in which a protospacer matching the endogenous type VI *spc4* was cloned into the 3' untranslated region of a chloramphenicol resistance cassette. The negative control for plasmid targeting assays is pAM8, which contains the chloramphenicol cassette without a protospacer.
Putative anti-CRISPR constructs were assembled by cloning into HindIII/EagI-digested pAM326.

E. coli – *L. seeligeri* conjugation

All genetic constructs for expression in *L. seeligeri* were introduced by conjugation with the *E. coli* donor strains SM10 λ*pir*, S-17 λ*pir* (*30*), or for allelic exchange (see below), β2163 Δ*dapA* (*33*). Donor cultures were grown overnight in LB medium supplemented with the appropriate antibiotic (25 μg/mL chloramphenicol for pPL2e-derived plasmids, 100 μg/mL ampicillin for pAM8-derived plasmids, or 50 μg/mL kanamycin for pAM326-derived plasmids) at 37°C. Recipient cultures were grown overnight in BHI medium supplemented with the appropriate antibiotic (1 μg/mL erythromycin for pPL2e-derived plasmids, 10 μg/mL chloramphenicol for pAM8-derived plasmids, 50 μg/mL kanamycin for pAM326-derived plasmids) at 30°C. 100 μL each of donor and recipient culture were diluted into 10 mL of BHI medium, and concentrated onto a filter disc (Millipore-Sigma, HAWP04700) using vacuum filtration. Filter discs were laid onto BHI agar supplemented with 8 μg/mL oxacillin (which weakens the cell wall and enhances conjugation) and incubated at 37°C for 4 hr. Discs were removed, cells were resuspended in 2 mL BHI, and transconjugants were selected on medium containing 50 μg/mL nalidixic acid (which kills donor *E. coli* but not recipient *L. seeligeri*) in addition to the appropriate antibiotic for plasmid selection. Transconjugants were isolated after 2-3 days incubation at 30°C.

Gene deletions in *L. seeligeri*

Allelic exchange plasmids were generated by cloning 1kb homology arms flanking the genomic region to be deleted into the suicide vector pAM215 (*11*), which does not replicate in *Listeria*, and contains a chloramphenicol resistance cassette and *lacZ* from *Geobacillus stearothermophilus*. These plasmids were then transformed into the *E. coli* donor strain β2163 Δ*dapA*(*33*), which is auxotrophic for diaminopimelic acid (DAP), selecting on LB medium supplemented with the appropriate antibiotic and 1.2 mM DAP. Conjugation was carried out as described above, except all steps were carried out in the presence of 1.2 mM DAP. Transconjugants were selected on media lacking DAP and containing 50 μg/mL nalidixic acid, to ensure complete killing of donor *E. coli*, as well as 10 μg/mL chloramphenicol to select for integration of the pAM215-derived plasmid. Chloramphenicol-resistant colonies were patched on BHI supplemented with 100 μg/mL 5-Bromo-4-Chloro-3-Indolyl β-D-Galactopyranoside (X-gal) and confirmed *lacZ*+ by

checking for blue colony color. Plasmid integrants were passaged 3-4 times in BHI at 30° in the absence of antibiotic selection, to permit loss of the integrated plasmid. Cultures were screened for plasmid excision by dilution and plating on BHI + X-gal. White colonies were checked for chloramphenicol sensitivity, then chromosomal DNA was prepared from each, and tested for the desired deletion by PCR using primers flanking the deletion site. Deletions were finally confirmed by Sanger sequencing.

Bacterial genome sequencing, genome assembly, and ϕLS46 identification

The ϕLS46 genome was sequenced by whole-genome sequencing and assembly of its parent lysogen, *L. seeligeri* LS46. Chromosomal DNA was prepared from LS46 by lysozyme digestion of the cell wall, followed by cell lysis with 1% sarkosyl, then phenol-chloroform extraction and ethanol precipitation. 1 ng of chromosomal DNA was used to make an NGS library using the Illumina Nextera XT DNA Library Preparation Kit according to the manufacturer's instructions. Library quality was confirmed by analysis on Agilent TapeStation, then 2x150bp paired-end sequencing was carried out on the Illumina NextSeq platform. Raw reads were quality-trimmed using Sickle (https://github.com/najoshi/sickle) using a quality cutoff of 30 and length cutoff of 45. Trimmed reads were assembled using SPAdes (http://cab.spbu.ru/software/spades/) with the default parameters, which resulted in 140 assembled contigs with an N50 of 2841899. These contigs were mapped onto the completed reference genome of *L. seeligeri* SLCC3954 using Medusa (http://combo.dbe.unifi.it/medusa/) with the default parameters, which resulted in 105 scaffold assemblies. In our draft genome assembly, one scaffold (Scaffold 1) represents a 2.8 Mbp assembly, Scaffold 7 contains 46 Kbp, and each of the remaining 103 scaffolds contains between 100-1300 bp. To identify putative prophages in the assembled genome, we used Phaster (http://phaster.ca), which predicted a single prophage element, occupying the entirety of Scaffold 7. We confirmed that this prophage (ϕLS46) was the one isolated by mitomycin C induction of LS46 using PCR of the ΔRM Δ*spc*-passaged phage stock with ϕLS46-specific primers.

Construction of gene deletions in ϕLS46

Gene deletions in ϕLS46 were constructed in two ways. One group of deletions was obtained by selection of spontaneous escapers of Cas9 targeting of the anti-CRISPR locus in ϕLS46. A Cas9 spacer targeting the anti-CRISPR region (*gp4*) was cloned into the vector pAM307, which carries Cas9 from *Streptococcus pyogenes* along with a repeat-spacer-repeat construct with BsaI entry sites. This plasmid (pAM379) was introduced into ΔRM Δ*spc*, which was then infected with ten-fold serial dilutions of ϕLS46 in a plaque assay on BHI top agar. Cas9-targeting reduced the efficiency of ϕLS46 plaquing by several orders of magnitude, but spontaneous Cas9-resistant escaper plaques were isolated and checked for deletions by PCR using primers flanking the anti-CRISPR locus. The deletions were then precisely mapped by Sanger sequencing. To generate an in-frame deletion of the *acrVIA1* gene, we first assembled a homology repair template (pAM386) containing 1kb homology arms flanking an in-frame deletion of *acrVIA1*. In the deletion construct, the first six and last six codons of *acrVIA1* remain, both to avoid Rho-dependent termination of untranslated RNA, as well as to preserve the Shine-Dalgarno sequence for the *gp3* gene predicted to be present in the last six codons of *acrVIA1*. The repair template plasmid was introduced into ΔRM Δ*spc*, this strain was

infected with ϕLS46 in BHI top agar (allowing recombinants to be generated), and a phage stock was harvested. A Cas9 spacer targeting *acrVIA1* was cloned into pAM307 to generate pAM377 and introduced into ΔRM Δ*spc*. The ϕLS46 stock passaged on ΔRM Δ*spc* carrying the pAM386 repair template was used to infect ΔRM Δ*spc* carrying pAM377, and Cas9-resistant escaper mutants were isolated. Two mutant phage isolates were Sanger sequenced across the *acrVIA1* gene, and found to contain the precise deletion.

In vitro RNA cleavage assays

10 µM synthetic RNA substrates (listed in Table S1) were labeled with ATP [γ-^{32}P] for 30 min at 37° with 1ul NEB T4 Polynucleotide Kinase, then purified using GE MicroSpin G-50 columns. In a 10 µL reaction, 1 nM purified *L. seeligeri* Cas13-His6:crRNA complex was combined with 10 nM synthetic target RNA, in buffer containing 10mM HEPES pH 7.0, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM β-mercaptoethanol, and 5% glycerol, at room temperature for the indicated time. Reactions were quenched by addition of an equal volume of loading dye (95% formamide, 14 mM EDTA, 0.025% SDS, 0.04% bromophenol blue, 0.04% xylene cyanol), then denatured by boiling 5 min, then crash cooled on ice for 1 min before loading on denaturing TBE-Urea PAGE gels with 15% acrylamide. Reactions were exposed to phosphoscreen 1 hour and imaged with Beckman Coulter FLA7000IP Typhoon storage phosphorimager.

Co-immunoprecipitation

ΔCRISPR strains of *L. seeligeri* harboring pAM364 (Cas13-his6 cloned into a pPL2e backbone) and pAM395 (P_{tet}-AcrVIA1-3xFlag cloned into a pAM326 backbone) along with empty vector controls, were cultured in 50 mL BHI supplemented with 50 µg/mL kanamycin and 100 ng/mL aTc at 30°C until the OD_{600} reached 0.7. 30 mL culture samples were harvested, pelleted by centrifugation at 8,000 rpm for 2min, and frozen at -80°C. Pellets were resuspended in 0.5 mL ice-cold lysis buffer (50 mM HEPES pH 7.0, 200 mM NaCl, 5 mM $MgCl_2$, 5% glycerol, 1 mg/mL lysozyme, supplemented with Roche cOmplete EDTA-free protease inhibitor cocktail. Samples were incubated at 37°C for 5 min, then placed on ice and lysed by sonication. Insoluble material was pelleted by centrifugation at 15,000 rpm for 1 hr at 4°C. A "load" sample was harvested, then the remaining soluble fraction was applied to 30 µL of pre-equilibrated ANTI-FLAG M2 Affinity Gel (Millipore-Sigma #A2220) for 4 hr at 4°C. The resin was pelleted by centrifugation at 2,000 rpm for 1 min, then the "unbound" sample was harvested. The resin was washed three times by centrifugation and resuspension in 1 mL wash buffer (20 mM HEPES pH 7.0, 200 mM NaCl, 5 mM $MgCl_2$, 5% glycerol). All wash buffer was then removed, and the resin was resuspended in 40 µL 2x Laemmli SDS-PAGE loading buffer lacking β-mercaptoethanol, and boiled for 5 min. The resin was pelleted and supernatant was harvested as the "IP" sample. 5% β-mercaptoethanol was added to all samples before separation on 4-20% acrylamide SDS-PAGE gels. For immunoblot analysis, proteins were transferred to a methanol-activated PVDF membrane, blocked with 5% nonfat milk, and probed with anti-His6 (Genscript #A00186), anti-Flag (Sigma #F3165) and anti-$\sigma^{A}_{Bacillus\ subtilis}$ (*34*) primary antibodies, then with horseradish peroxidase-conjugated anti-mouse (Bio-Rad #170-5047) or anti-rabbit (Bio-Rad #170-

5046) secondary antibodies. Proteins were detected using Western Lightning Plus-ECL chemiluminescence reagent (Perkin-Elmer).

Electrophoretic mobility shift assay

Synthetic RNA substrates were radiolabeled as described for RNA cleavage assays. In vitro RNP assembly was performed for 30 min in a 10 µL reaction at room temperature in the presence of 5mM HEPES pH 7, 10 mM NaCl, 1 mM BME, 5 mM MgCl2, 1 µg/mL bovine serum albumin, 10 µg/mL salmon sperm DNA, and 5% glycerol. Labeled RNA substrates were added at a final concentration of 10 nM, dCas13a (R445A, H450A, R1016A, H1021A) at 500 nM, and AcrVIA1 at 1800 nM. Reactions were placed on ice 1 min, then 10 µL of non-denaturing loading dye (25% glycerol, 0.05% xylene cyanol, 0.05% bromophenol blue, 50 mM HEPES pH 7.0) was added, and samples were electrophoretically separated by 10% acrylamide native PAGE at 4°C. Gels were exposed and imaged as described for RNA cleavage assays.

RNA sequencing and analysis

L. seeligeri ΔRM Δ*spc* was infected with ϕLS46 at OD_{600} of 0.5, MOI of 0.1 in BHI medium containing 5 mM $CaCl_2$ at 30°C. At each time point, 1.5 mL of culture was harvested, pelleted by centrifugation at 8,000 rpm for 2 min, and frozen at -80°C. To harvest RNA, samples were resuspended in 90 µL of RNase-free phosphate-buffered saline containing 2 mg/mL lysozyme, and incubated at 37°C for 3 min. 10 µL of 10% sarkosyl was immediately added to lyse the cells. 300 µL of TRI Reagent (Zymo Research Direct-Zol RNA Miniprep Plus Kit) was added to each sample, then RNA was prepared according to the manufacturer's instructions, eluting in 50 µL RNase-free water. Ribosomal RNA was removed from 1 µg of purified RNA using the NEBNext rRNA Depletion Kit (Bacteria) according to the manufacturer's instructions. After rRNA removal, samples were concentrated using the Zymo Research RNA Clean and Concentrator-5 Kit according to the manufacturer's instructions, eluting RNA in 6 µL RNase-free water. Libraries were prepared for deep sequencing using the Illumina TruSeq Stranded mRNA Library Preparation Kit, skipping mRNA purification and beginning at the RNA fragmentation step. Quality control of libraries was carried out on an Agilent TapeStation. Paired-end (2x75bp) sequencing was performed on the NextSeq platform. Raw paired-end reads were mapped to the ϕLS46 genome using Bowtie2 with parameters "very-sensitive" and I = 40. Using a custom script, the coverage at each position on the ϕLS46 genome was calculated by tallying a count for each of the positions covered by each mapped read. Read counts at each genomic position were normalized to the total number of reads in each library.

Protein Expression and Purification

The *L. seeligeri* type VI CRISPR array alongside Cas13a-His6 or dCas13a-His6 (R445A, H450A, R1016A, H1021A) were cloned into pAM8 as described in Table S1, and conjugated into *L. seeligeri* Δ*spc* Δ*cas13a*. For expression, these strains were cultured at 30 °C in BHI supplemented with 10 µg/mL chloramphenicol for ~24 hr. Cells were harvested by centrifugation and resuspended in lysis buffer (20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 5% glycerol, 20 mM imidazole, 7 mM β-mercaptoethanol). The harvested cells were then lysed by an EmulsiFlex-C3 homogenizer (Avestin) and centrifuged at

20,000 rpm for 30 min in a JA-20 fixed angle rotor (Avanti J-E series centrifuge, Beckman Coulter). The supernatant was applied to 5 mL HisPur™ Cobalt Resin (Thermo Fisher Scientific). The protein was eluted with lysis buffer supplemented with 500 mM imidazole after washing the column with 10 column volumes of lysis buffer. The elution fractions were further dialyzed against buffer A (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 7mM β-mercaptoethanol), and applied on a 1 mL HiTrap SP Fast flow column (GE Healthcare). Proteins were eluted by a linear gradient from 100 mM to 1 M NaCl in 20 column volumes, and then concentrated in 50 kDa molecular mass cut-off concentrators (Amicon) before further purification over a Superdex 200 increase 10/300 GL column (GE Healthcare) pre-equilibrated in buffer B (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM DTT).

AcrVIA1 was cloned into a pRSF-Duet-1 vector (Novagen), in which the *acrVIA1* gene was attached with N-terminal His6-SUMO tag following an ubiquitin-like protease (ULP1). The vector was transformed into *Escherichia coli* BL21 (DE3) strain and expressed by induction with 0.25 mM isopropyl-β-D-1-thiogalactopyranoside (GoldBio) at 16 °C for 20 hr. Cells were harvested by centrifugation and resuspended in lysis buffer (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, 20 mM imidazole, 7 mM β-mercaptoethanol). The harvested cells were then lysed by an EmulsiFlex-C3 homogenizer (Avestin) and centrifuged at 20,000 rpm for 30 min in a JA-20 fixed angle rotor (Avanti J-E series centrifuge, Beckman Coulter). The supernatant was applied to 5 mL HisTrap Fast flow column (GE Healthcare). The protein was eluted with lysis buffer supplemented with 500 mM imidazole after washing the column with 10 column volumes of lysis buffer and 2 column volumes of lysis buffer supplemented with 40 mM imidazole. The elution fractions were further dialyzed against buffer A (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 7mM β-mercaptoethanol), and applied on a 5 mL HiTrap Q Fast flow column (GE Healthcare). Proteins were eluted by a linear gradient from 100 mM to 1 M NaCl in 20 column volumes, and then concentrated in 10 kDa molecular mass cut-off concentrators (Amicon) before further purification over a Superdex 200 increase 10/300 GL column (GE Healthcare) pre-equilibrated in buffer B (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM DTT).

Leptotrichia buccalis Cas13 purification was conducted as previously described(*32*), and the same samples were used in this study.

In Vitro Assembly of AcrVIA1-Cas13acrRNA Complex

To assemble the AcrVIA1-Cas13a^{crRNA} complex, the purified Cas13a^{crRNA} was mixed with AcrVIA1 at the molar ratio of 1:4 and incubated on ice for 30 min. The reconstructed complex was then purified by gel filtration chromatography over a Superdex 200 increase 10/300 GL column (GE Healthcare) pre-equilibrated in buffer B (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM DTT). The peak fractions containing AcrVIA1-Cas13a^{crRNA} complex were concentrated in 50 kDa molecular mass cut-off concentrators (Amicon) and added with 5 mM $MgCl_2$ before grid preparation.

Cryo-EM Sample Preparation and Data Acquisition

3.0 μl of ~0.8 mg/ml purified Cas13a^{crRNA} and AcrVIA1-Cas13a^{crRNA} complex were applied onto glow-discharged UltrAuFoil 300 mesh R1.2/1.3 grids (Quantifoil), respectively. The grids were glow-discharged for 60 s at 0.37 mBar, 15 mA in a Pelco

easiGlow air system (TED PELLA). Grids were blotted with Vitrobot™ filter paper (Electron Microscopy Sciences) for 2s at ~100% humidity and flash frozen in liquid ethane using an FEI Vitrobot Mark IV. Images were collected on FEI Titan Krios electron microscope operated at an acceleration voltage of 300 kV with a Gatan K3 Summit detector with a 1.08 Å pixel size at Memorial Sloan Kettering Cancer Center. The defocus range was set from -1.0 μm to 2.5 μm. Movies were recorded in super-resolution mode at an exposure rate of 19.9 e^-/ pixel /s with a total exposure time of 3 s, for an accumulated electron exposure of 49.3 e^-/ $Å^2$. Intermediate frames were recorded every 0.075 s for a total number of 40 frames.

Image Processing

For Cas13a^{crRNA} dataset, motion correction was performed with MotionCor2 (*35*). Contrast transfer function parameters were estimated by Ctffind4 (*36*). All other steps of image processing were performed by RELION 3 (*37*). Templates for automated particle selection were generated from 2D-averages of ~2,000 manually picked particles. Automated particle selection resulted in 4,085,566 particles from 3,249 images. After two rounds of 2D classification, a total of 1,263,761 particles were selected for 3D classification using the initial model generated by RELION as reference. Particles corresponding to the best class with the highest-resolution features were selected and subjected to the second round of 3D classification. One of 3D classes with good secondary structural features and the corresponding 443,629 particles were polished using RELION particle polishing, yielding an electron microscopy map with a resolution of 3.2 Å after 3D auto-refinement. A preferred orientation of the sample was observed during the cryo-EM reconstruction. Due to the observed directional anisotropy of the reconstruction, the corresponding 443,629 particles were further subjected to another round of 2D classification and some classes with similar orientations were discarded to reduce the impact of the preferred views on the resolution of final reconstruction. 190,543 particles were used for the final reconstruction with a resolution of 3.2 Å after 3D auto-refinement (Fig. S6). The directional resolution volumes were evaluated by the 3D FSC tool (*38*).

The dataset for AcrVIA1-Cas13a^{crRNA} complex was processed by the same procedure as above. Briefly, 4,372,366 particles were autopicked from 3,806 images, 717,725 particles were selected for the final 3D reconstruction after two rounds of 2D and 3D classification, resulting in a AcrVIA1-Cas13a^{crRNA} complex map with an overall resolution of 3.0 Å (Fig. S6).

All resolutions were estimated using RELION 'post-processing' by applying a soft mask around the protein density and the Fourier shell correlation (FSC) = 0.143 criterion. Local resolution estimates were calculated from two half data maps using cryoSPARC(*39*). Further details related to data processing and refinement are summarized in table S1.

Atomic Model Building and Refinement

For the AcrVIA1-Cas13a^{crRNA} complex, the initial models of Cas13a^{crRNA} and AcrVIA1 were manually built in COOT based on the bulky side chains to register the sequence(*40*). All models were refined against summed maps using phenix.real_space_refine by applying geometric and secondary structure restraints. For Cas13a^{crRNA} complex, the model of Cas13^{crRNA} in AcrVIA1-Cas13a^{crRNA} complex was

docked into the cryo-EM density map using UCSF Chimera (*41*) and then manually rebuilt in COOT (*40*). All models were refined against summed maps using phenix.real_space_refine (*42*) by applying geometric and secondary structure restraints. All figures were prepared by PyMol (http://www.pymol.org) or Chimera (*41*). The statistics for data collection and model refinement are shown in table S1.

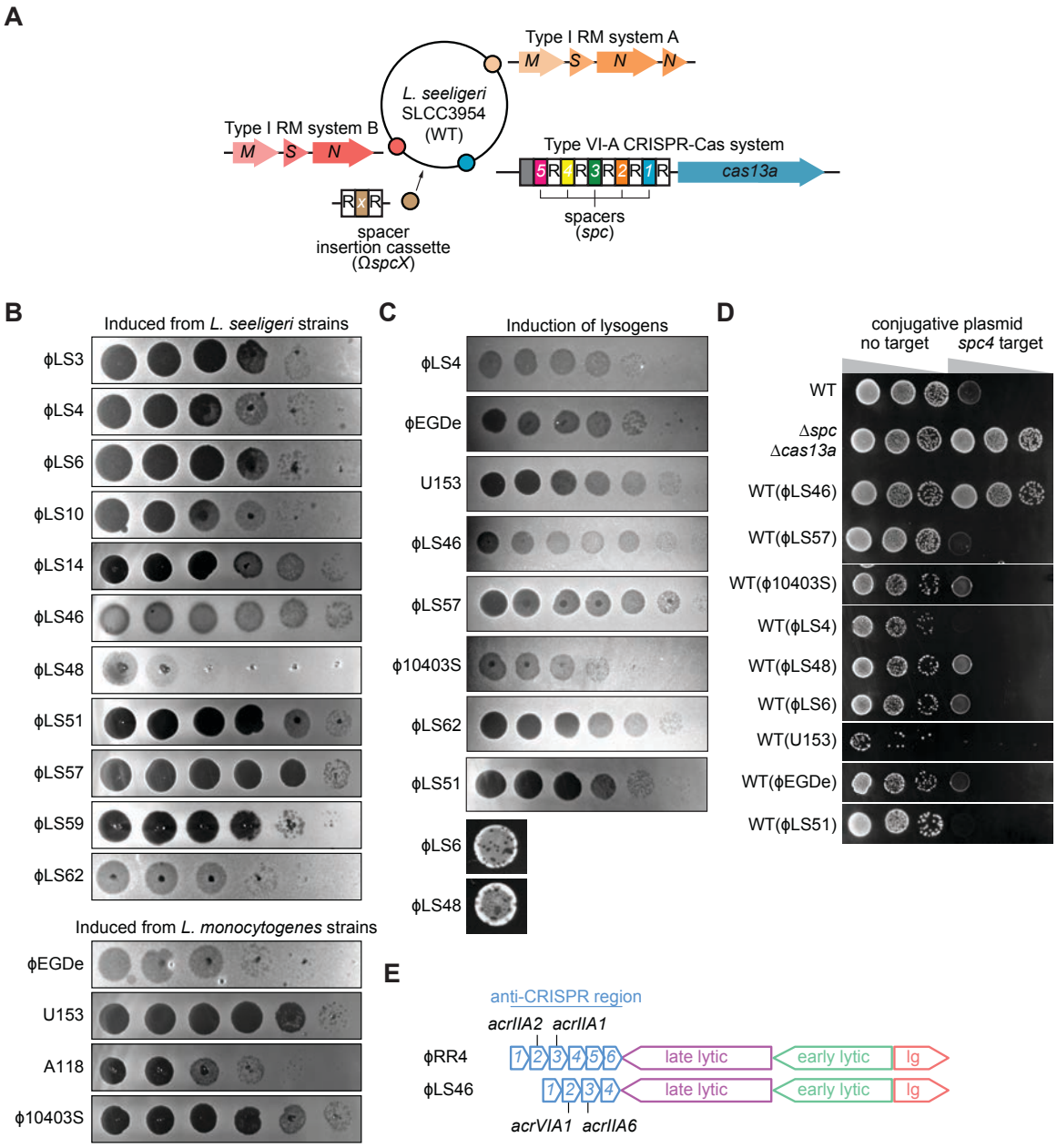

Fig. S1: Acr screen in listeriophages.

(**A**) Diagram of *L. seeligeri* SLCC3954 genetic elements modified during this study. (**B**) Detection of phages in lysates of *L. seeligeri* (11 phages isolated) or *L. monocytogenes* (4 phages isolated) strains after treatment with mitomycin C. Ten-fold dilutions of lysate were spotted on lawns of *L. seeligeri* ΔRM Δ*spc*. (**C**) Confirmation of lysogen formation by the phages isolated in (**B**). Putative lysogens were treated with mitomycin C to induce and detect integrated prophages. Ten-fold dilutions of induced culture filtrates from each lysogen were spotted on lawns of *L. seeligeri* ΔRM Δ*spc*. For ϕLS6 and ϕLS48, lysogens were less stable and spontaneous plaques were detectable during growth of the uninduced lysogen. (**D**) Transfer of a conjugative plasmid with or without the *spc4* target of the *L. seeligeri* SLCC3954 type VI-A CRISPR-Cas system into different strains: wild-

type (WT), Δ*spc* Δ*cas13a* or WT harboring the ϕLS46, ϕLS57, ϕ10403S, ϕLS4, ϕLS48, ϕLS6, U153, or ϕEGDe prophages. Ten-fold dilutions of transconjugants were plated on selective media. (**E**) Comparison of the ϕRR4 and ϕLS46 genomes, with acr regions highlighted in blue, and genes containing homology to known anti-CRISPRs indicated. The ϕRR4 and ϕLS46 genomes are organized into four regions that can be classified according to gene function: these include an early lytic region containing predicted replicases and recombinases involved in phage circularization and genome replication; a late lytic region containing phage structural genes; and a lysogeny region containing transcriptional regulators and a predicted site-specific integrase. In ϕRR4, the fourth region contains two predicted homologs of the Cas9 inhibitors AcrIIA1 and AcrIIA2, and our previous experiments showed that this region is not essential for phage viability but provides inhibitory activity against Cas9 from *Streptococcus pyogenes*. BLAST searches for each of the ORFs in the analogous operon in ϕLS46 revealed no sequence-similarity for genes of known function, but a more sensitive HHpred search revealed similarity between the gp3 protein and AcrIIA6 from Streptococcus phage DT1. We therefore hypothesized that this operon may be an anti-CRISPR region in the ϕLS46 genome.

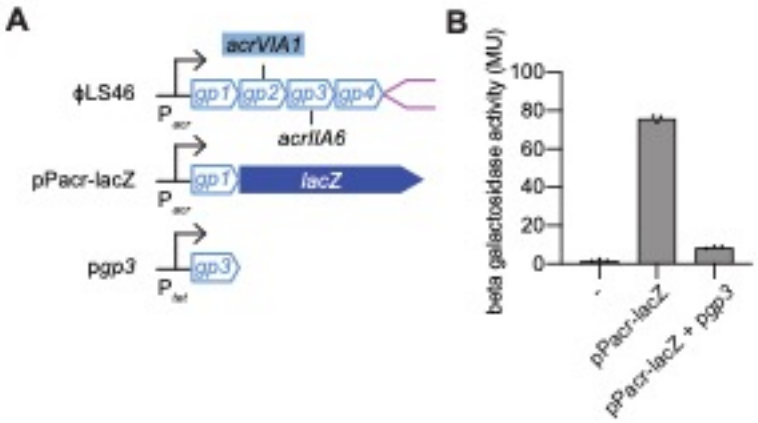

Fig. S2: ϕLS46 *gp3* encodes an AcrIIA6 homolog that represses *acr* transcription.
(**A**) Diagram of ϕLS46 *acr* operon containing four genes controlled by P_{acr} promoter. The plasmid pP_{acr}-*lacZ* contains P_{acr} fused to a *lacZ* reporter to measure transcription from the promoter. P_{acr} transcription was measured in the presence and absence of p*gp3*, which expresses the *gp3* gene. (**B**) Autorepression of P_{acr} transcription by Gp3. β-galactosidase activity was measured in the absence of lacZ reporter (-), pP_{acr}-*lacZ*, or pP_{acr}-*lacZ* in the presence of p*gp3.* Data from 3 biological replicates are shown.

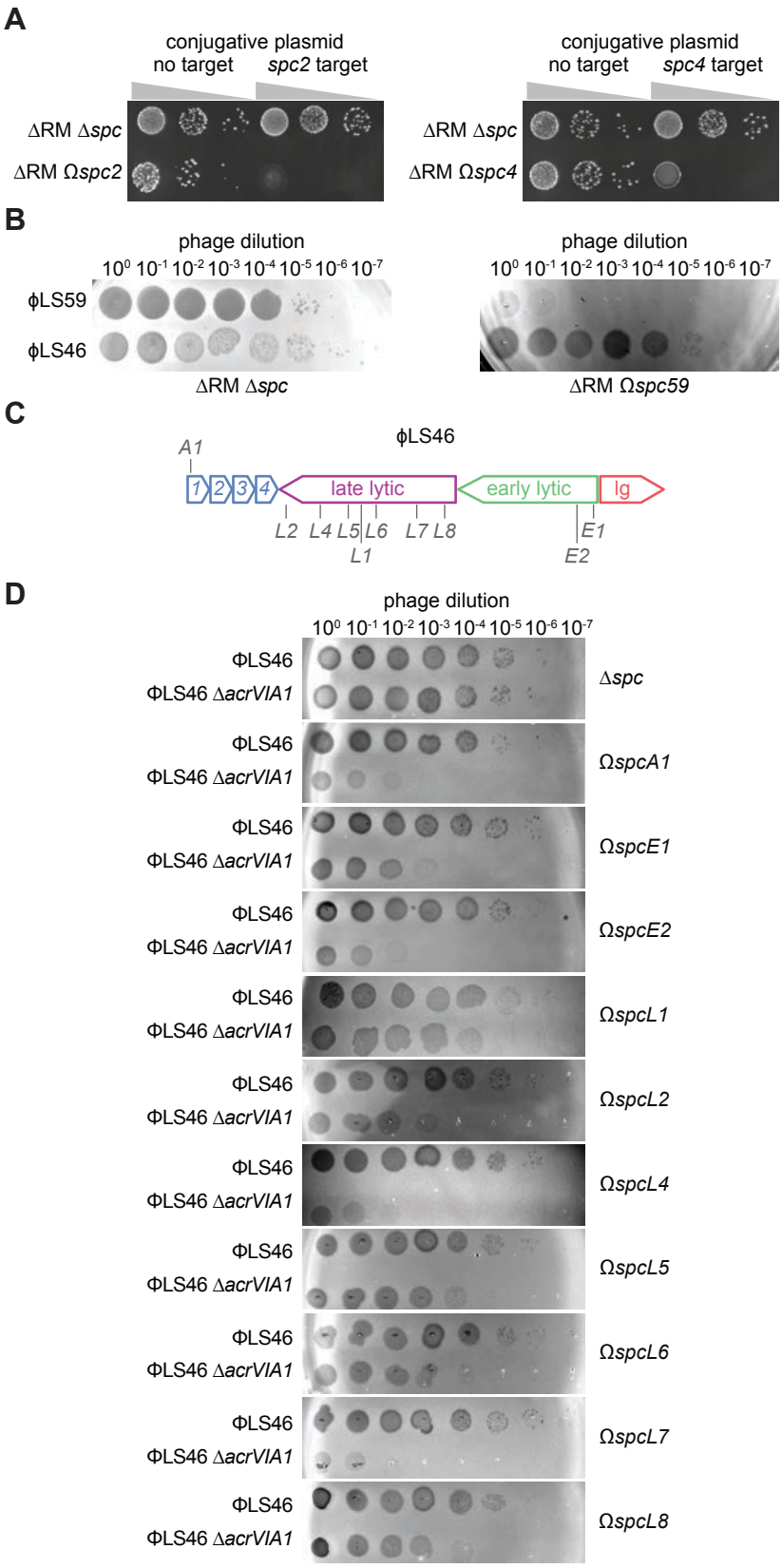

Fig. S3: AcrVIA1 inhibits type VI-A CRISPR-Cas targeting of plasmids and phages.

(**A**) Transfer of a conjugative plasmid with or without the *spc2* or *spc4* target of the *L. seeligeri* SLCC3954 type VI-A CRISPR-Cas system into strains ΔRM Δ*spc*, ΔRM Ω*spc2* or ΔRM Ω*spc4*. Ten-fold dilutions of transconjugants were plated on selective media. (**B**) Detection of phage propagation after spotting ten-fold dilutions of the phages ϕLS46 or ϕLS59, on lawns of *L. seeligeri* ΔRM Δ*spc* or ΔRM Ω*spc59*. (**C**) Schematic of the ϕLS46 genome showing the four main transcription units (*acr* in blue; lysogeny cassette in red; early- and late-expressed lytic genes in green and purple, respectively). The location of the targets of the spacers used in this study are shown in grey. Top and bottom locations refer to the DNA strand that is transcribed to produce a target transcript that is complementary to the crRNA derived from each spacer. (**C**) Same as (**B**) but spotting phages ϕLS46 or ϕLS46 Δ*acrVIA1* on lawns of bacteria expressing crRNAs from the spacers shown in (**C**).

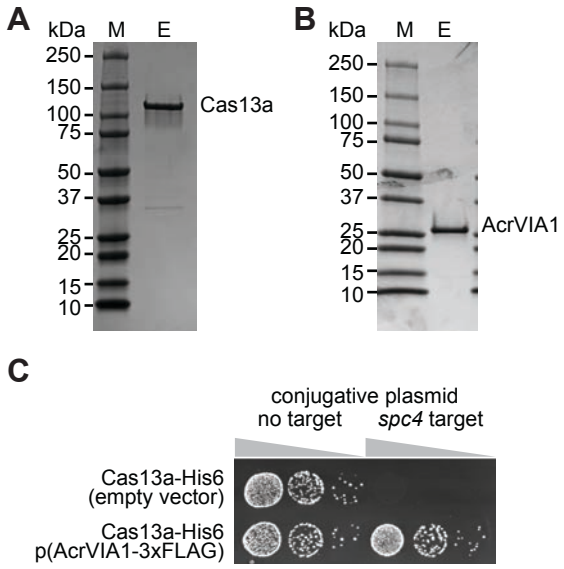

Fig. S4: Purification and functional test of Cas13a-His6 and AcrVIA1-3xFLAG

(**A**) SDS-PAGE of Cas13a-His6 after expression and purification from *L. seeligeri*. M, protein size marker; E, elution. (**B**) Same as (**A**) but for AcrVIA1 purified from *E. coli*. (**C**) Transfer of a conjugative plasmid with or without the *spc4* target of the *L. seeligeri* SLCC3954 type VI-A CRISPR-Cas system into *L. seeligeri* strains expressing Cas13a-His6 and harboring either an empty vector or p(AcrVIA1-3xFLAG). Three ten-fold dilutions of transconjugants were plated on selective media.

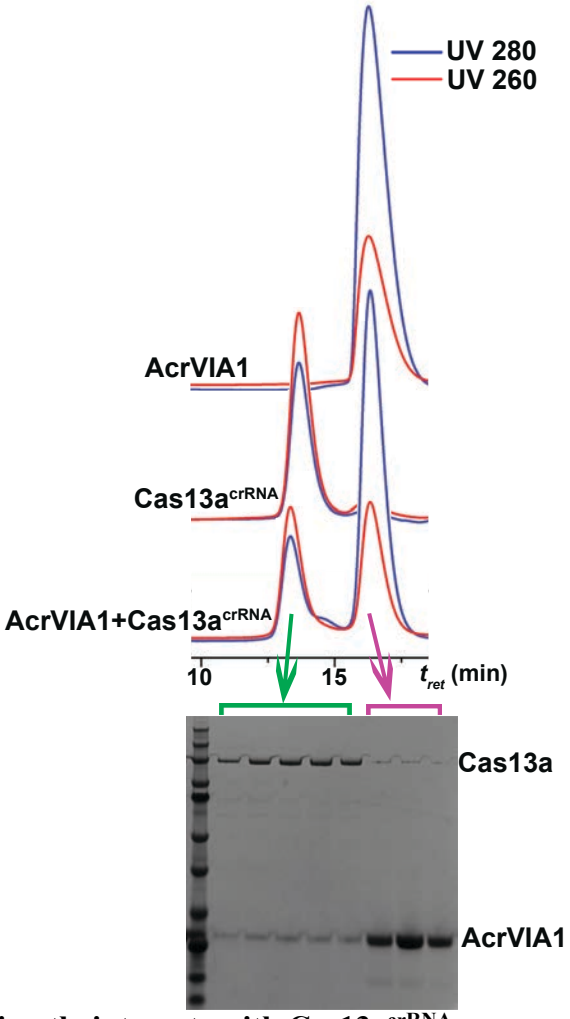

Fig. S5: AcrVIA1 directly interacts with Cas13a^{crRNA} on complex formation.
Gel filtration profiles of AcrVIA1 (upper panel), Cas13a^{crRNA} (middle panel), and AcrVIA1 bound to Cas13a^{crRNA} (bottom panel). Lower insert shows SDS-PAGE profile of AcrVIA1 with Cas13a^{crRNA} after gel filtration.

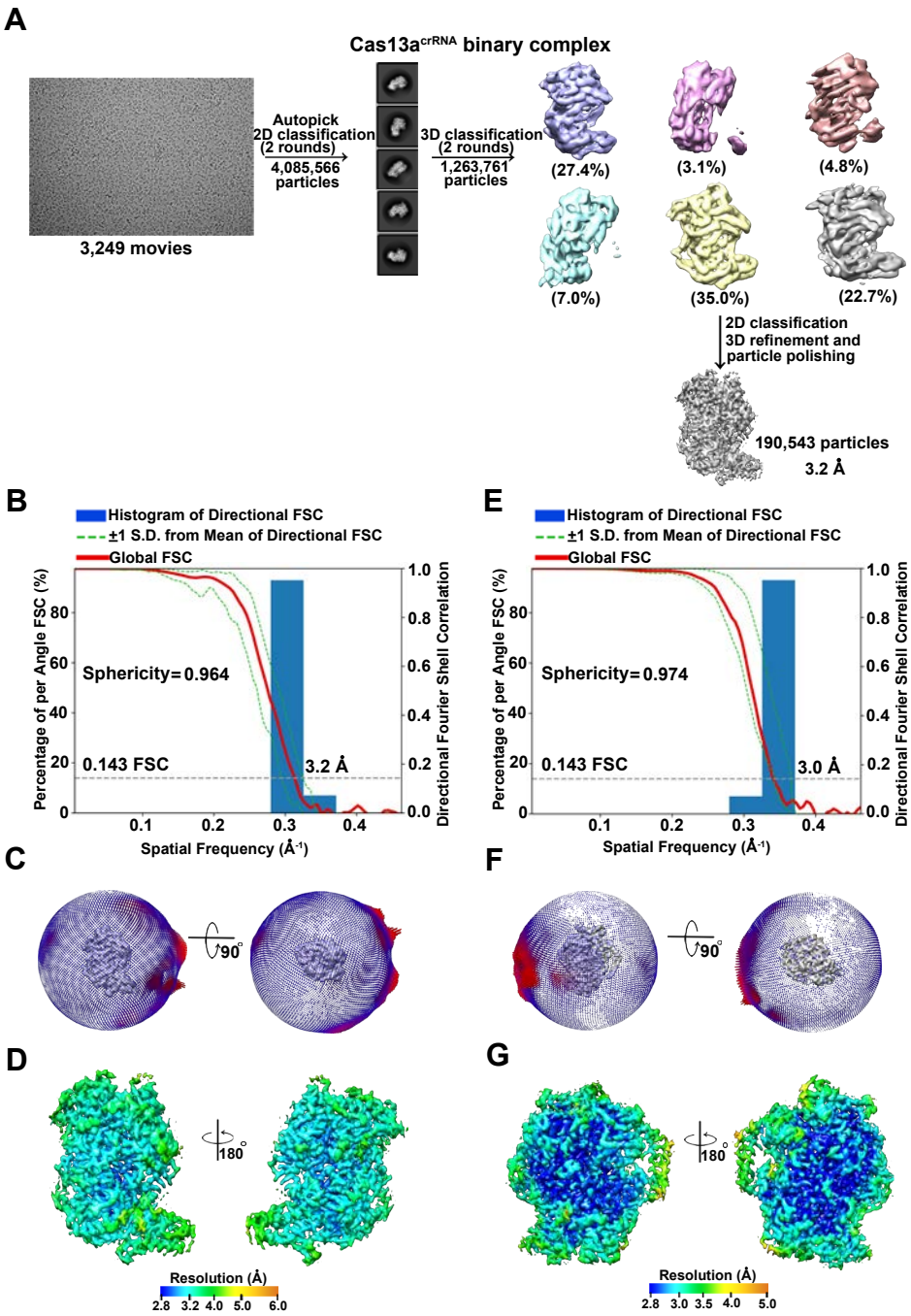

Fig. S6: Cryo-EM Reconstruction of Cas13a^{crRNA} and AcrVIA1-Cas13a^{crRNA} complexes.

(**A**) Flow chart of image processing for Cas13a^{crRNA} binary complex. (**B** and **E**) Fourier Shell Correlation (FSC) curve of Cas13a^{crRNA} (**B**) and AcrVIA1-Cas13a^{crRNA} complex (**E**) and between two half maps that were calculated from two half datasets, and between the cryo-EM map and corresponding model. (**C** and **F**) Euler angle distribution of cryo-EM particles for calculating the final EM map of Cas13a^{crRNA} (**C**) and AcrVIA1-Cas13a^{crRNA} complex (**F**). The position of each sphere relative to the density map (gray in the center) corresponds to its angular assignment, with the radius of sphere proportional to the

number of particles in that orientation. Preferred orientations of both samples were observed during the cryo-EM reconstruction. (**D** and **G**) Final 3D reconstructed map of Cas13a^{crRNA} (**D**) and AcrVIA1-Cas13a^{crRNA} complex (**G**) colored according to local resolution.

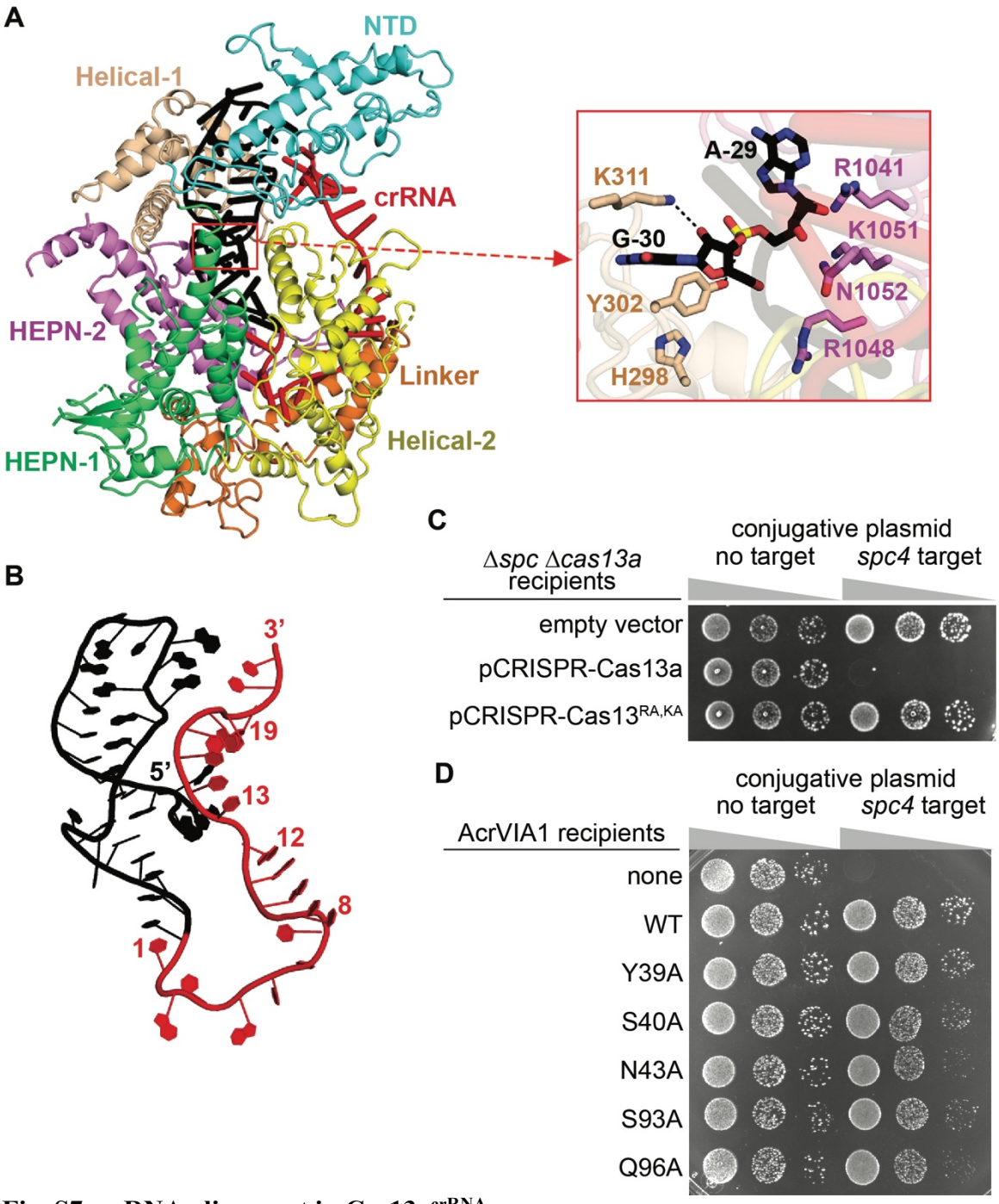

Fig. S7: crRNA alignment in Cas13a^{crRNA}

(**A**) Cryo-EM structure of Cas13a^{crRNA}. The insert shows detailed interactions between Cas13a and the cleavage site of pre-crRNA. The repeat and spacer regions within crRNA are shown in black and red, respectively. (**B**) Structure of crRNA in Cas13a^{crRNA}. A stacking alignment is observed for segments 8 to 12 and 13 to 19. (**C**) Transfer of a conjugative plasmid with or without the *spc4* target of the *L. seeligeri* type VI-A CRISPR-Cas system into Δ*spc* Δ*cas13a* harboring plasmids expressing wild-type or mutant (R1048A, K1049A) Cas13a. (**D**) Transfer of a conjugative plasmid with or

without the *spc4* target of the *L. seeligeri* type VI-A CRISPR-Cas system into wild-type *L. seeligeri* harboring p*gp2* plasmids expressing wild-type or mutant different mutant versions of AcrVIA1.

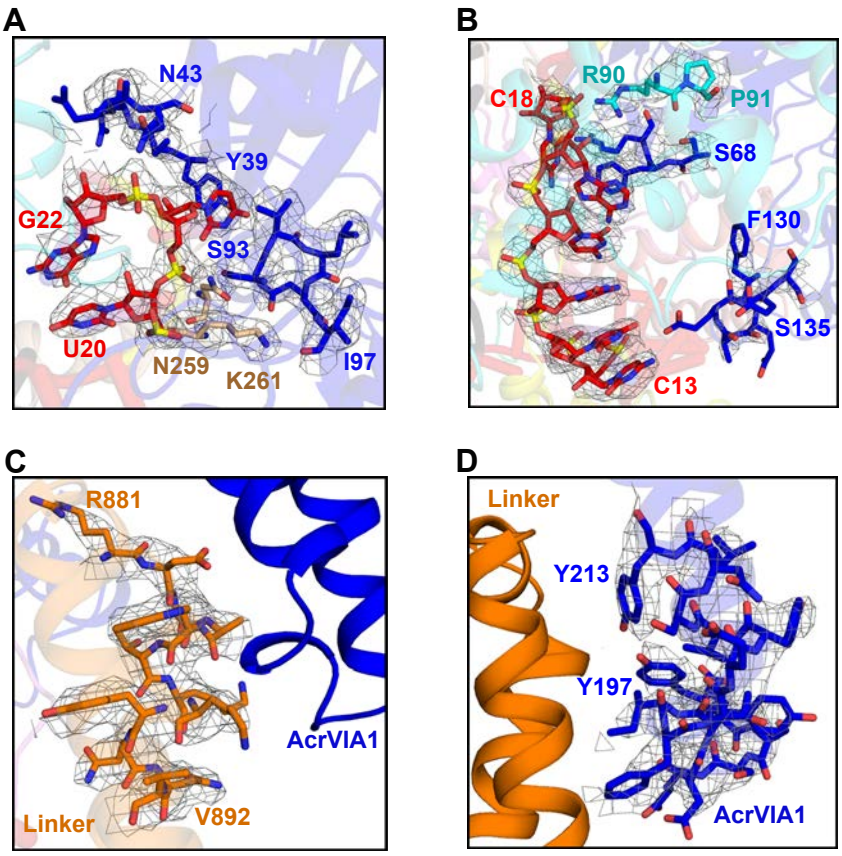

Fig. S8: EM densities for representative interfaces between AcrVIA1 and Cas13a^{crRNA}.

(**A**) EM densities for interface shown in Fig. 3F. (**B**) EM densities for interface shown in Fig. 3G. (**C** and **D**) EM densities for interface shown in Fig. 3H. The maps are contoured at 5σ.

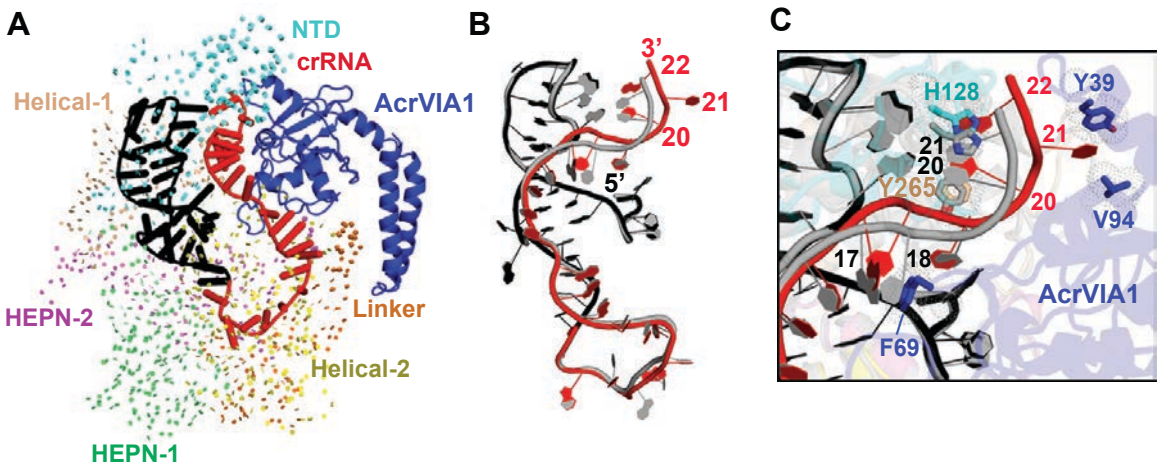

Fig. S9: Structural comparison of Cas13a^{crRNA} and AcrVIA1-Cas13a^{crRNA} complex. (**A**) Structure comparison following superposition of Cas13a^{crRNA} and AcrVIA1-Cas13a^{crRNA} complex. Vector length correlates with the domain movement scale. AcrVIA1 and crRNA originate from the AcrVIA1-Cas13a^{crRNA} complex. (**B**) Superposition of crRNA in Cas13a^{crRNA} (in grey) and AcrVIA1-Cas13a^{crRNA} complex (in red). (**C**) Detailed presentation of binding mode of 3' end of crRNA in Cas13a^{crRNA} (in grey) and AcrVIA1-Cas13a^{crRNA} complex (in red).

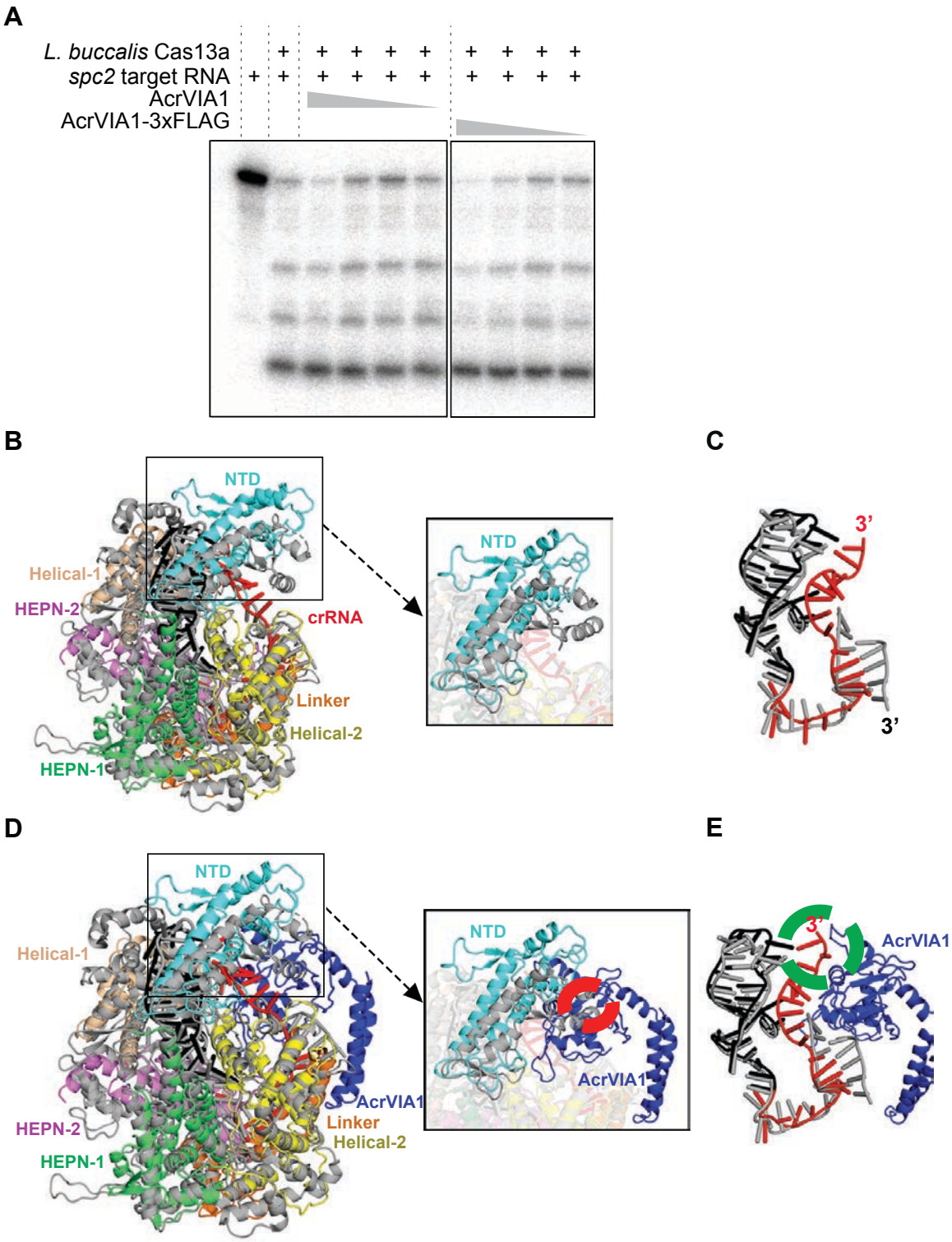

Fig. S10: Comparison of *Listeria seeligeri* Cas13a with *Leptotrichia buccalis* Cas13a. (**A**) AcrVIA1 does not inhibit *Leptotrichia buccalis* Cas13a. *cis*-RNA cleavage time course with purified *L. buccalis* Cas13a, AcrVIA1 and/or AcrVIA1-3xFLAG using radiolabeled *spc2*-target RNA substrates. Cas13a was present at 10nM, synthetic crRNA at 10nM, and AcrVIA1 or AcrVIA1-3xFLAG was added at 400, 100, 10, or 1 nM. The

products of degradation after 5, 10, and 20 minutes were analyzed by denaturing PAGE. (**B**) Structural comparison following superposition of *L. seeligeri* Cas13^{crRNA} (in color) with *L. buccalis* Cas13a^{crRNA} (in grey, PDB 5XWY). The insert presents the structural differences between NTD domains in the two species of Cas13a. (**C**) Structural comparison of crRNA in *L. seeligeri* Cas13a^{crRNA} (in color) with *L. buccalis* Cas13a^{crRNA} (in grey). (**D**) Structural comparison following superposition of *L. seeligeri* AcrVIA1-Cas13a^{crRNA} (in color) with *L. buccalis* Cas13a^{crRNA} (in grey). The insert presents the structural differences of the NTD domain of Cas13a in the two systems. The clashes between AcrVIA1 and NTD domain in *L. buccalis* Cas13a^{crRNA} (in grey) are shown in red circle. (**E**) Structure comparison of crRNA in Cas13a^{crRNA} (in color) with *L. buccalis* Cas13a^{crRNA} (in grey). The missing interactions between AcrVIA1 and crRNA in *L. buccalis* Cas13a^{crRNA} (in grey) are shown in green circle.

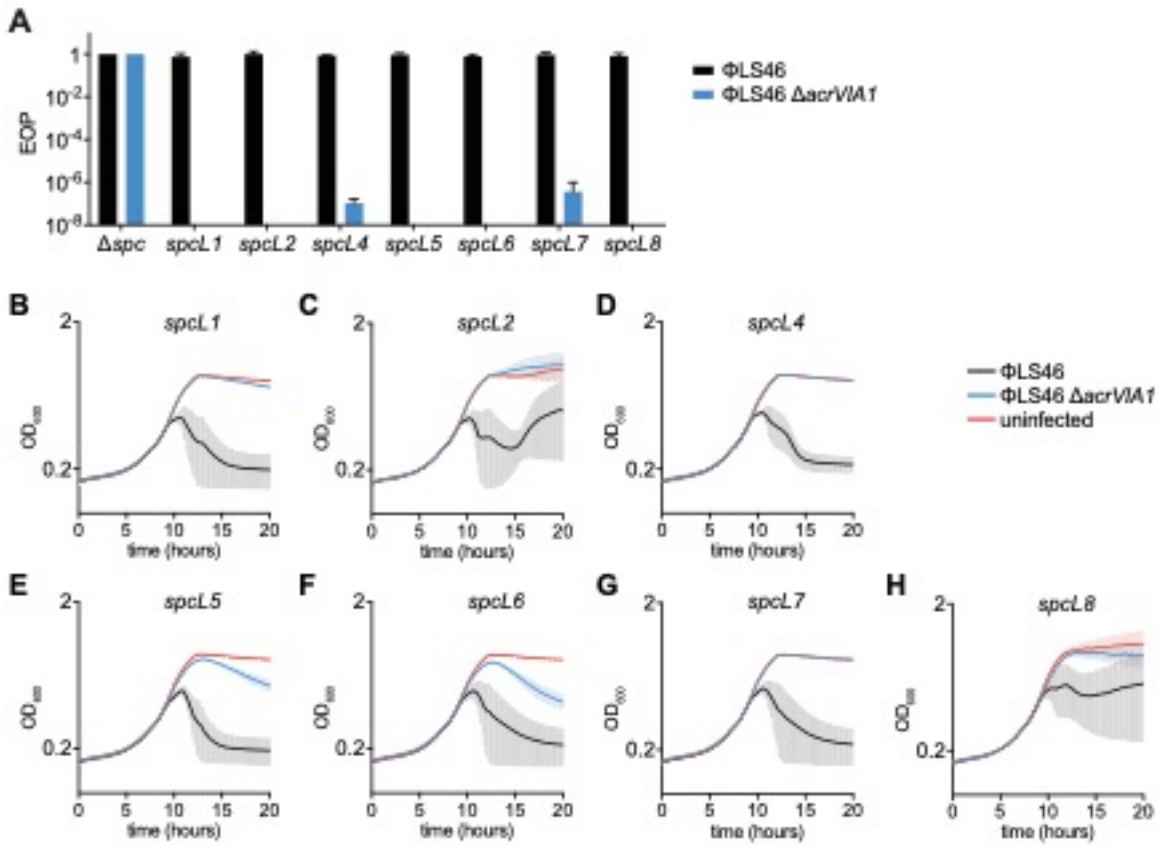

Fig. S11: AcrVIA1 enables full phage escape from type VIA CRISPR-Cas immunity.

(**A**) Efficiency of plaquing (relative to the number of plaques formed in lawns of *L. seeligeri* (ΔRM Δ*spc*)) of phages ϕLS46 or ϕLS46 Δ*acrVIA1* in lawns of bacteria expressing *spcL1*, *spcL2*, *spcL4, spcL5*, *spcL6*, *spcL7* or *spcL8*. These spacers target transcripts produced by the late-expressed region of ϕLS46; shown in Figure S3C (**B-H**) Growth of *L. seeligeri* (ΔRM Ω*spcL1*), (ΔRM Ω*spcL2*), (ΔRM Ω*spcL4*), (ΔRM Ω*spcL5*), (ΔRM Ω*spcL6*), (ΔRM Ω*spcL7*) and (ΔRM Ω*spcL8*), measured as OD_{600} over time, infected with ϕLS46 or ϕLS46 Δ*acrVIA1* phages, or uninfected. The average curves of three biological replicates are reported, with +/- SEM values shown in lighter colors.

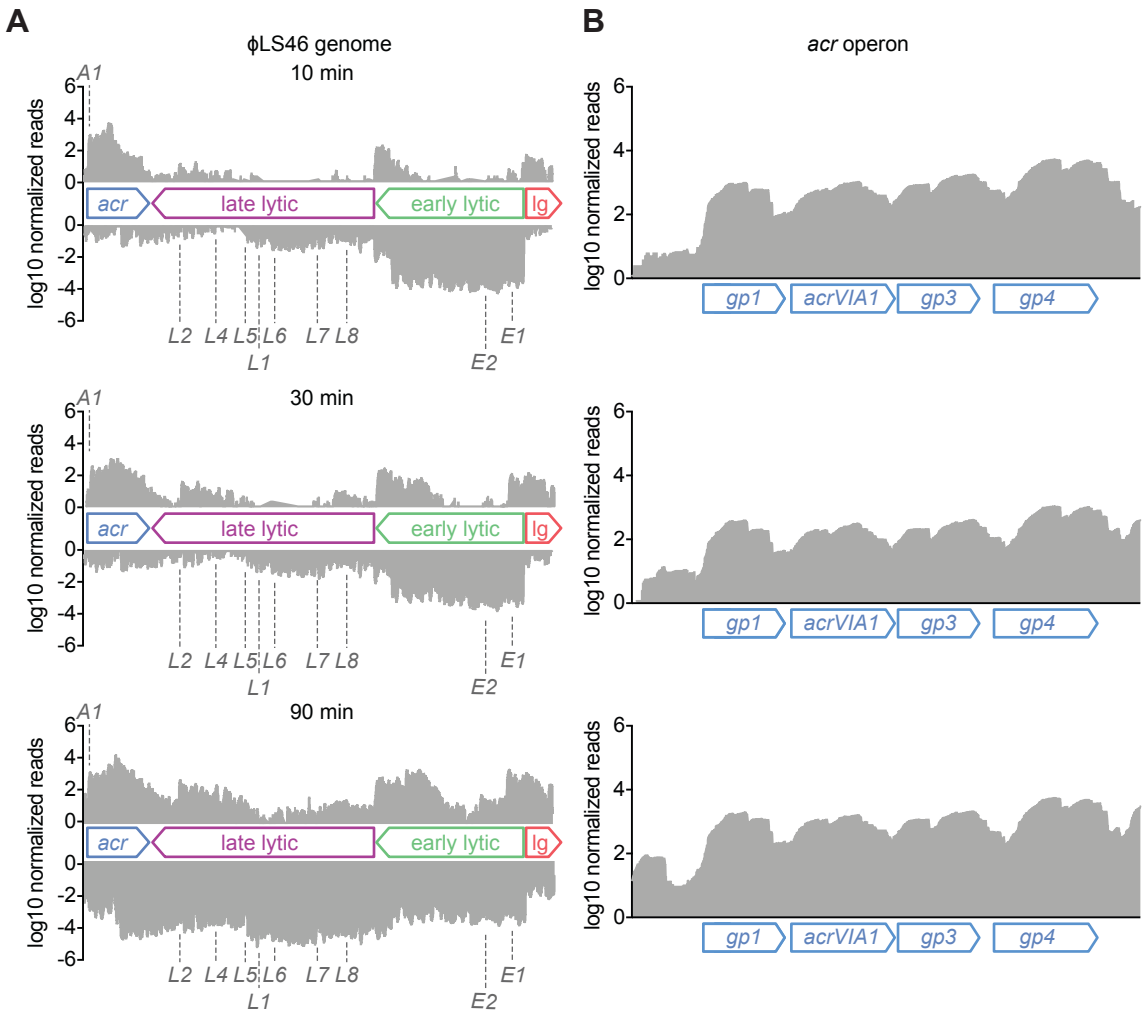

Fig. S12: RNA-seq during ϕLS46 infection.

(**A**) Strand-specific read coverage of phage-mapped reads is plotted along the ϕLS46 genome, and normalized to total reads in each sample. Targeting location of the spacers used in this study is also shown. The average of two biological replicates is shown. (**B**) Zoom-in of read coverage for the *acr* region of ϕLS46, showing co-operonic transcription of the four genes at both early and late time points during the phage life cycle. The average of two biological replicates is shown.

Table S1. Cryo-EM Statistics for Data Collection and Model Refinement.

	Cas13a^{crRNA}	AcrVIA1-Cas13a^{crRNA}
Data collection and processing		
Microscope	Titan Krios	Titan Krios
Detector	Gatan K3	Gatan K3
Automation software	SerialEM	SerialEM
Nominal magnification	22,500	22,500
Calibrated magnification	47,262	47,262
Voltage (kV)	300 kV	300 kV
Electron exposure ($e^-/Å^2$)	49.3	49.3
Exposure rate (e^-/pixel/s)	19.9	19.9
Number of frames collected	40	40
Defocus range (μm)	-1.0 to -2.5	-1.0 to -2.5
Pixel size (Å)	1.08	1.08
Micrographs (collected/used)	4,465/3,249	4,398/3,806
Total extracted particles (no.)	4,085,566	4,372,366
Refined particles (no.)	2,929,591	3,070,831
Final particles (no.)	190,543	717,725
Estimated accuracy of translations/rotations	0.749/2.479	0.513/2.160
Resolution (global)		
FSC 0.5 (unmasked/masked, Å)	4.2/3.6	3.6/3.2
FSC 0.143 (unmasked/masked, Å)	3.6/3.2	3.2/3.0
Resolution range (local, Å)	2.8~6.5	2.8~5.5
Resolution range due to anisotropy (Å)	3.2~3.5	2.8~3.0
Map sharpening B factor ($Å^2$)	-126.35	-100.0
Refinement		
Refinement package	Phenix	Phenix
Model resolution (Å)	3.22	2.98
FSC threshold	0.5	0.5
Map Correlation Coefficient	0.77	0.79
Model composition		
Protein residues	1,007	1,257
Nonhydrogen atoms	9,429	11,404
R.m.s. deviations		
Bond lengths (Å)	0.004	0.009
Bond angles (°)	0.864	1.107
Validation		
MolProbity score	1.46	1.47
Clashscore	2.55	2.21
Poor rotamers (%)	0.21	0.51
Ramachandran plot		
Favored (%)	92.2	92.1
Allowed (%)	7.8	7.9
Disallowed (%)	0	0
C-β deviations (%)	0	0
CaBLAM outliers (%)	0.88	0.48

EM-Ringer Score	3.50	3.29
Average B-factors		
protein (Å^2)	37.17	31.67
RNA (Å^2)	37.81	30.47
PDB code	6VRC	6VRB
EMDB code	EMD-21367	EMD-21366

Table S2. Bacterial strains used in this study.

Species	Strain	Alias	Genotype	Origin
L. seeligeri	LS1	wild-type	wild type	ATCC SLCC3954
L. seeligeri	LS2	RR4	wild type	(*43*)
L. seeligeri	LS3	FSL N1-067	wild type	FSL, Cornell
L. seeligeri	LS4	FSL H6-011	wild type	FSL, Cornell
L. seeligeri	LS5	FSL H6-169	wild type	FSL, Cornell
L. seeligeri	LS6	ATCC 51334	wild type	ATCC
L. seeligeri	LS7	ATCC 51335	wild type	ATCC
L. seeligeri	LS8	FSL S4-0171	wild type	FSL, Cornell
L. seeligeri	LS9	FSL B8-0099	wild type	FSL, Cornell
L. seeligeri	LS10	FSL C7-0024	wild type	FSL, Cornell
L. seeligeri	LS11	FSL S10-0305	wild type	FSL, Cornell
L. seeligeri	LS12	FSL B8-0287	wild type	FSL, Cornell
L. seeligeri	LS13	FSL C7-0049	wild type	FSL, Cornell
L. seeligeri	LS14	FSL H6-0007	wild type	FSL, Cornell
L. seeligeri	LS15	FSL C7-0030	wild type	FSL, Cornell
L. seeligeri	LS16	FSL S4-0116	wild type	FSL, Cornell
L. seeligeri	LS17	FSL S10-0823	wild type	FSL, Cornell
L. seeligeri	LS18	FSL B8-0050	wild type	FSL, Cornell
L. seeligeri	LS19	FSL C7-0481	wild type	FSL, Cornell
L. seeligeri	LS20	FSL T4-0118	wild type	FSL, Cornell
L. seeligeri	LS21	FSL S4-0037	wild type	FSL, Cornell
L. seeligeri	LS22	FSL C7-0115	wild type	FSL, Cornell
L. seeligeri	LS23	FSL S4-0039	wild type	FSL, Cornell
L. seeligeri	LS24	FSL S10-1784	wild type	FSL, Cornell
L. seeligeri	LS25	FSL B8-0253	wild type	FSL, Cornell
L. seeligeri	LS26	FSL C7-0156	wild type	FSL, Cornell
L. seeligeri	LS27	FSL S10-0300	wild type	FSL, Cornell
L. seeligeri	LS28	FSL C7-0082	wild type	FSL, Cornell
L. seeligeri	LS29	FSL L5-0045	wild type	FSL, Cornell
L. seeligeri	LS30	FSL S10-0030	wild type	FSL, Cornell
L. seeligeri	LS31	FSL F6-1136	wild type	FSL, Cornell
L. seeligeri	LS32	FSL L5-0086	wild type	FSL, Cornell
L. seeligeri	LS33	FSL S10-1611	wild type	FSL, Cornell
L. seeligeri	LS34	FSL R9-8498	wild type	FSL, Cornell
L. seeligeri	LS35	FSL C7-0251	wild type	FSL, Cornell
L. seeligeri	LS36	FSL M6-0039	wild type	FSL, Cornell
L. seeligeri	LS37	FSL S10-2970	wild type	FSL, Cornell

L. seeligeri	LS38	FSL C7-0134	wild type	FSL, Cornell
L. seeligeri	LS39	FSL C7-0462	wild type	FSL, Cornell
L. seeligeri	LS40	FSL S10-1769	wild type	FSL, Cornell
L. seeligeri	LS41	FSL H6-0027	wild type	FSL, Cornell
L. seeligeri	LS42	FSL R8-6874	wild type	FSL, Cornell
L. seeligeri	LS43	FSL S4-0544	wild type	FSL, Cornell
L. seeligeri	LS44	FSL S4-0616	wild type	FSL, Cornell
L. seeligeri	LS45	FSL S4-0939	wild type	FSL, Cornell
L. seeligeri	LS46	FSL C7-0218	wild type	FSL, Cornell
L. seeligeri	LS47	FSL C7-0499	wild type	FSL, Cornell
L. seeligeri	LS48	FSL S6-0001	wild type	FSL, Cornell
L. seeligeri	LS49	FSL L5-0058	wild type	FSL, Cornell
L. seeligeri	LS50	FSL A5-0405	wild type	FSL, Cornell
L. seeligeri	LS51	FSL C7-0081	wild type	FSL, Cornell
L. seeligeri	LS52	FSL L5-0018	wild type	FSL, Cornell
L. seeligeri	LS53	FSL R2-0626	wild type	FSL, Cornell
L. seeligeri	LS54	FSL R8-7055	wild type	FSL, Cornell
L. seeligeri	LS55	FSL C7-0498	wild type	FSL, Cornell
L. seeligeri	LS56	FSL R9-4405	wild type	FSL, Cornell
L. seeligeri	LS57	FSL S10-0788	wild type	FSL, Cornell
L. seeligeri	LS58	FSL C7-0167	wild type	FSL, Cornell
L. seeligeri	LS59	FSL M6-0259	wild type	FSL, Cornell
L. seeligeri	LS60	FSL S10-0889	wild type	FSL, Cornell
L. seeligeri	LS61	FSL B8-0378	wild type	FSL, Cornell
L. seeligeri	LS62	N/A	wild type	FSL, Cornell
L. seeligeri	LS1	N/A	Δ*spc* Δ*cas13a*	(*32*)
L. seeligeri	LS1	N/A	ΔRM, Δ*spc*	This Study
L. seeligeri	LS1		*94°::Ptet-spc4*	(*11*)
L. seeligeri	LS1		Δ*spc* Δ*cas13a, 94°::Ptet-spc4*	(*11*)
E. coli	Rosetta2	N/A	F- *ompT hsdSB*(*rB- mB-*) *gal dcm* (DE3) pRARE2 (CamR)	Millipore-Sigma
E. coli	SM10		*thi thr leu tonA lacY supE recA::RP4-2-Tc::Mu Km λpir*	(*30*)
E. coli	b2163		*dapA::erm-pir116 RP4-2-Tc::Mu Km*	(*33*)

*FSL, Food Science Lab

Table S3. Phages used in this study.

Phage	Host	Genotype	Origin
ϕLS3	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS3
ϕLS4	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS4
ϕLS6	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS6
ϕLS10	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS10
ϕLS14	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS14
ϕLS46	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS46
ϕLS48	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS48
ϕLS51	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS51
ϕLS57	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS57
ϕLS59	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS59
ϕLS62	*L. seeligeri*	wild type	This Study, induced from *L. seeligeri* LS62
ϕEGDe	*L. seeligeri*	wild type	This Study, induced from *L. monocytogenes* EGDe
ϕ10403S	*L. seeligeri*	wild type	This Study, induced from *L. monocytogenes* 10403S
U153	*L. seeligeri*	wild type	(*33*)
A118	*L. seeligeri*	wild type	(*33*)
ϕLS46	*L. seeligeri*	Δ*gp1-4*	This Study, escaper of pAM379 targeting
ϕLS46	*L. seeligeri*	Δ*acrVIA1*	This Study, pAM386-recombinant escaper of pAM377 targeting

Table S4. Plasmids used in this study.

Plasmid	Description	Source	Construction Notes
pAM8	*E. coli-Listeria* shuttle vector (cat)	Meeske, et al. 2018 Mol Cell	N/A
pAM52	*spc2* cat target in pAM8	Meeske, et al. 2018 Mol Cell	N/A
pAM54	*spc4* cat target in pAM8	Meeske, et al. 2018 Mol Cell	N/A
pAM326	*E. coli-Listeria* shuttle vector (kan)	This Study	Gibson assembly of kanR (oAM1055/1056), pSK1 ori (oAM1057/1058), oriT (oAM1059/1060)
pPL2e	*Listeria* integrating vector (erm)	Lauer, et al. 2002 J Bacteriol.	N/A
pAM215	*lacZ* suicide vector (cat)	Meeske, et al. 2019 Nature	N/A
pAM305	Type VI RSR in pPL2e with BsaI sites for spacer cloning	This Study	Gibson assembly of BamHI/SalI digested pPL2e and BsaI RSR synthetic fragment (oAM462/451)
pAM375	p*gp1-4*	This Study	Gibson assembly of HindIII/EagI digested pAM326 and gp1-4 region (oAM1176/1177)
pAM388	p*gp1*	This Study	Gibson assembly of HindIII/EagI digested pAM326, Ptet (oAM1107/572) and gp1 (oAM1178/1179)
pAM383	p*gp2*	This Study	Gibson assembly of HindIII/EagI digested pAM326, Ptet (oAM1107/572) and gp2 (oAM1180/1181)
pAM384	p*gp3*	This Study	Gibson assembly of HindIII/EagI digested pAM326, Ptet (oAM1107/572) and gp3 (oAM1182/1183)
pAM385	p*gp4*	This Study	Gibson assembly of HindIII/EagI digested pAM326, Ptet (oAM1107/572) and gp4 (oAM1184/1185)
pAM449	*spc2* in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM464/465
pAM450	*spc4* in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM770/771
pAM422	*spcA1* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1270/1271
pAM380	*spcE1* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1192/1193
pAM381	*spcE2* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1194/1195
pAM382	*spcL1* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1196/1197
pAM434	*spcL2* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1370/1371
pAM436	*spcL4* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1374/1375
pAM437	*spcL5* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1376/1377
pAM438	*spcL6* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1378/1379

pAM439	*spcL7* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1382/1383
pAM440	*spcL8* targeting ϕLS46 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM1384/1385
pAM442	Array of 3 spc (*spcA1*, *spcE1*, *spcE2*) targeting ϕLS46 in pAM305	This Study	Gibson assembly of BamHI/SalI digested pPL2e and 3spc synthetic fragment
pAM365	*spc59* targeting ϕLS59 in pAM305	This Study	Ligation of BsaI-digested pAM305 and annealed oAM559/560
pAM386	repair template for *acrVIA1* deletion	This Study	Gibson assembly of HindIII/EagI digested pAM326 and Δgp2 upstream (oAM1176/1206) and Δgp2 downstream (oAM1207/1177)
pAM307	type II CRISPR system (*S. pyogenes*) in pAM8 with SapI sites for spacer cloning	Meeske, et al. 2019 Nature	N/A
pAM377	Cas9 spc targeting *gp4* of ϕLS46 in pAM307	This Study	Ligation of SapI-digested pAM307 and annealed oAM1186/1187
pAM379	Cas9 spc targeting *gp2* of ϕLS46 in pAM307	This Study	Ligation of SapI-digested pAM307 and annealed oAM1190/1191
pAM364	CRISPR array + Cas13-His6 in pPL2e	This Study	Gibson assembly of BamHI/SalI digested pPL2e and CRISPR locus (oAM462/1111)
pAM395	AcrVIA1-3xFlag in pAM326	This Study	Gibson assembly of HindIII/EagI digested pAM326 and AcrVIA1-3xflag synthetic fragment
pAM414	p*Pacr-lacZ* in pAM8	This Study	Gibson assembly of BamHI/EagI digested pAM8, Pacr (oAM1256/1257) and lacZ (oAM304/702)
pAM417	CRISPR array + Cas13-His6 in pAM8 (for purification)	This Study	Gibson assembly of BamHI/EagI digested pAM8 and CRISPR locus (oAM631/1260)
pAM419	CRISPR array + dCas13-His6 in pAM8 (for purification)	This Study	Gibson assembly of BamHI/EagI digested pAM8 and CRISPR locus (oAM631/1260, amplified from dCas13 strain)
pAM452	AcrVIA1(Y39A, S40A, N43A, S93A, Q96A)-3xFlag in pAM326	This Study	Gibson assembly of HindIII/EagI digested pAM326 and mutant Acr allele synthetic fragment
pAM453	AcrVIA1(ΔE131-E134)-3xFlag in pAM326	This Study	Gibson assembly of HindIII/EagI digested pAM326 and mutant Acr allele synthetic fragment
pAM454	AcrVIA1(I2A, Y4A)-3xFlag in pAM326	This Study	Gibson assembly of HindIII/EagI digested pAM326 and mutant Acr allele synthetic fragment
pAM455	AcrVIA1(S68A, F69A)-3xFlag in pAM326	This Study	Gibson assembly of HindIII/EagI digested pAM326 and mutant Acr allele synthetic fragment
pAM456	AcrVIA1(ΔN173-N232)-3xFlag in pAM326	This Study	Gibson assembly of HindIII/EagI digested pAM326 mutant Acr allele synthetic fragment

Table S5. Oligonucleotide primers used in this study.

Primer	Sequence
oAM224	GGCGTGAAAATCAACGACCC
oAM225	TTTGCTTCAATGTCGCCAGC
oAM304	ATGAATGTGTTATCCTCAATTTGTT
oAM451	GTACCGGGCCCCCCCTCGAGGTTTTGTGATGCATGATTTGTTCTG
oAM462	CGGCCGCTCTAGAACTAGTGAGTGCCAAGTAACTGTGC
oAM464	AAACTTAGTCAACCCCTCGCTGCATTTTCACATT
oAM465	TTACAATGTGAAAATGCAGCGAGGGGTTGACTAA
oAM559	TTACAAAAAGAAGCTAAAGAAGTAAAAGAAGAAG
oAM560	AAACCTTCTTCTTTTACTTCTTTAGCTTCTTTTT
oAM572	GTTTAACTCACTCTATCAATGATAGAGAGCTTATTTTAATTAT
oAM631	GCAAGACGTAGCCCAGCGCGTCAGTGCCAAGTAACTGTGC
oAM770	AAACCATATTTCCAAACTCCACTTTGACTACACC
oAM702	GCGACCACACCCGTCCTGTGCTAAACCTTCCCGGCTTC
oAM771	TTACGGTGTAGTCAAAGTGGAGTTTGGAAATATG
oAM1055	CCCAGCGAACCATTTGAGG
oAM1056	TTATGCATCCCTTAACTTAAAACAATTCATCCAGTAAAATATAATATTTTATTTTCTCC
oAM1057	TACTGGATGAATTGTTTTAAGTTAAGGGATGCATAAACTGCATC
oAM1058	ATCCATGGCCTGGATCCATCAAGCTTAAAATTAGTATAATTATAGCACGAGCTCTGAT
oAM1059	GCTTGATGGATCCAGGCCATGGATGGCGGCCGCCTCTCGCCTGTCCCCTC
oAM1060	ACCTCAAATGGTTCGCTGGGTTAATCGCTTGCCCTCATCTGT
oAM1107	CTATAATTATACTAATTTTACATCACGGAAAAAGGTTATGC
oAM1111	TACCGGGCCCCCCCTCGAGGTTAATGATGATGGTGGTGATGCTTCATCGTTAATAGCGTTCTTACTAG
oAM1176	CGTGCTATAATTATACTAATTTTATATATTCGTTGACTACATTTTTCTACTATAATAGAAG
oAM1177	TGAGGGGACAGGCGAGAGGCTATTACATTACAGCTAGTGATAAGTATGTACAG
oAM1178	CATTGATAGAGTGAGTTAAACACTTTACAAGTTTAACATATTATGTTAATATATAAATATAGC
oAM1179	TGAGGGGACAGGCGAGAGGCGCCCATTTATTATTTGTTATATTTGTTGTAAAAATTTAC
oAM1180	ATTGATAGAGTGAGTTAAACCTATAGGAGGAAAAAACGATGATCTAC
oAM1181	GAGGGGACAGGCGAGAGGCTTAATTTAGCTCCTCTTTTAAAATTTGTTTGC
oAM1182	CATTGATAGAGTGAGTTAAACTAAAAGAGGAGCTAAATTAAATGACAAATTTAATC
oAM1183	GAGGGGACAGGCGAGAGGCATTTATATAAAAAGTTTAAATTTCTGCATTAAATTCTTGG
oAM1184	CATTGATAGAGTGAGTTAAACTTTAGGAGGAATTAAAATGAATAAATTTGCAT
oAM1185	TGAGGGGACAGGCGAGAGGCCTGATGTATTATATTAATCCTTGCTCTTTTTTATC
oAM1186	AAACCAAGGTAAATTTGAAGTACAGATTCAAAAA
oAM1187	AACTTTTTGAATCTGTACTTCAAATTTACCTTGG
oAM1190	AAACTCATTTCTTTTGATTTCTAAAAATATAGTA
oAM1191	AACTACTATATTTTTAGAAATCAAAAGAAATGAG

oAM1192	TTACAACAAATATGGAAAGTAATTTATTTAAATT
oAM1193	AAACAATTTAAATAAATTACTTTCCATATTTGTT
oAM1194	TTACTTTATTCGATAAAGACAGCACGAATAAAAA
oAM1195	AAACTTTTTATTCGTGCTGTCTTTATCGAATAAA
oAM1196	TTACCAACTATCGAAATTGATTGGAAAATAAATA
oAM1197	AAACTATTTATTTTCCAATCAATTTCGATAGTTG
oAM1206	CTCCTCTTTTAAAATTTGTTTGTAGATCATCGTTTTTTCCTCCTATAGTC
oAM1207	CGATGATCTACAAACAAATTTTAAAAGAGGAGCTAAATTAAATG
oAM1256	AAGACGTAGCCCAGCGCGTCTATATTCGTTGACTACATTTTTCTACTATAATAGAAG
oAM1257	CAAATTGAGGATAACACATTCATCGTTTTTTCCTCCTATAGTCGGTTATC
oAM1260	GCGACCACACCCGTCCTGTGTTAATGATGATGGTGGTGATGCTTCATCGTTAATAGCGTT CTTACTAG
oAM1270	TTACGATGATTAAAATGATGACTGAAAAACAAAA
oAM1271	AAACTTTTGTTTTTCAGTCATCATTTTAATCATC
oAM1370	TTACTACAAACAACACGTATAAAAACAAAAAATT
oAM1371	AAACAATTTTTTGTTTTTATACGTGTTGTTTGTA
oAM1374	TTACTAATAGAAGAGGTTGTAAAAGATTGTAAAG
oAM1375	AAACCTTTACAATCTTTTACAACCTCTTCTATTA
oAM1376	TTACTTTATTACAATATATTCCGCAAACATTCAA
oAM1377	AAACTTGAATGTTTGCGGAATATATTGTAATAAA
oAM1378	TTACTTGCAAAAAGAAATTACAAAGGACTTTCATT
oAM1379	AAACAATGAAAGTCCTTTGTAATTTCTTTTTGCAA
oAM1382	TTACGAATCTGGACATTTAATTGATTTTGCAAAA
oAM1383	AAACTTTTGCAAAATCAATTAAATGTCCAGATTC
oAM1384	TTACTAAAGCAATAGCGAAATACATTGAAGAAAA
oAM1385	AAACTTTTCTTCAATGTATTTCGCTATTGCTTTA
oAM1415	GTTTGCCTAAAAATGCGCTTAAATCAGC
oAM1416	CGTCGTGCAATGCTAATCAAGATTGC

Table S6. RNA oligonucleotides used in this study

RNA oligo	Sequence
non-target	GGCACACCCGCAGGGAGGAGCCAAAGCACGUCCAUCAUUCCGUUGCCACAGCAGAAGCCC
spc2 target	GGCACACCCGCAGGGAAAUGUGAAAAUGCAGCGAGGGGUUGACUAACACAGCAGAAGCCC

Table S7. Synthetic gene fragments used in this study.

Description	Sequence
Type VI RSR in with BsaI sites for spacer cloning	ccgggccccccctcgaggTTTTGTGATGCATGATTTGTTCTGTATTATCTTGCATT TCATTTTCATAAACTAACTTGCCCCCGTTTTTATCCCTAGAAATTAGTAC TTTTTTTCTATCAACCTCTACTTTAGTAATTCTCATAGTTTTCACCTCAAT GATTTTTTTCTCTCTTCTATTGTACATATAATCACAAAAAAATAAAACAC CTAAATGATGGATAAGCGTTTTTATACTTATCCAcatTAGACGTTTTAGTC CTCTTTCATATAGAGGTAGTCTCTTACTGAGACCAGTCTCGGAAGCTCA AAGGTCTCAGTTTTAGTCCTCTTTCATATAGAGGTAGTCTCTTACCCTAC TTAATAATAGTAATTAAAACAACCAATGTAAAGGATATAATCAATATAT TTAAAGTTTGCACGAGAATGCAATCATTTTATTCATAAATATCATATCAT TTATAAGCTCTATTTTCCATTTTCTAAGGCTAATAAATAAAACTGCTGTA CCTATGGATCTAAGGAAGACTTATGCACACAGTACAGCAACTTTTCAGC ATGATTTGTGTTAAAAACATTTAATTTATTGTAGCAATTTCTTGAGAATC TATAAACCATTTTCCGGTTTCAATTTTAACCAACTCCAAATCTAACAATA CTTGATTTTCGGTTTTCTTTTGATTGTCGACTTGAAAAGCAGACCAGGCT CGCACAGTTACTTGGCACTcactagttctagaGCggcc
Array of 3 spc (spcA1, spcE1, spcE2) targeting ϕLS46	TTTTGTGATGCATGATTTGTTCTGTATTATCTTGCATTTCATTTTCATAAA CTAACTTGCCCCCGTTTTTATCCCTAGAAATTAGTACTTTTTTTCTATCA ACCTCTACTTTAGTAATTCTCATAGTTTTCACCTCAATGATTTTTTTCTCT CTTCTATTGTACATATAATCACAAAAAAATAAAACACCTAAATGATGGA TAAGCGTTTTTATACTTATCCAcatTAGACGTTTTAGTCCTCTTTCATATAG AGGTAGTCTCTTACGATGATTAAAATGATGACTGAAAAACAAAAGTTTT AGTCCTCTTTCATATAGAGGTAGTCTCTTACTTTATTCGATAAAGACAGC ACGAATAAAAAGTTTTAGTCCTCTTTCATATAGAGGTAGTCTCTTACAA CAAATATGGAAAGTAATTTATTTAAATTGTTTTAGTCCTCTTTCATATAG AGGTAGTCTCTTACCCTACTTAATAATAGTAATTAAAACAACCAATGTA AAGGATATAATCAATATATTTAAAGTTTGCACGAGAATGCAATCATTTT ATTCATAAATATCATATCATTTATAAGCTCTATTTTCCATTTTCTAAGGC TAATAAATAAAACTGCTGTACCTATGGATCTAAGGAAGACTTATGCACA CAGTACAGCAACTTTTCAGCATGATTTGTGTTAAAAACATTTAATTTATT GTAGCAATTTCTTGAGAATCTATAAACCATTTTCCGGTTTCAATTTTAAC CAACTCCAAATCTAACAATACTTGATTTTCGGTTTTCTTTTGATTGTCGA CTTGAAAAGCAGACCAGGCTCGCACAGTTACTTGGCACT
AcrVIA1-3xFlag	ctcgtgctataattatactaattttACATCACGGAAAAAGGTTATGCTGCTTTTAAGACC CACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGG CCGAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAG CTTGTCGTAATAATGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCT TCTTTAGCGACTTGATGCTCTTGATCTTCCAATACGCAACCTAAAGTAA AATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGTGAAAAACC TTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTTTTT CTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGT AAAGCACATCTAAAACTTTTAGCGTTATTACGTAAAAAATCTTGCCAGC TTTCCCCTTCTAAAGGGCAAAAGTGAGTATGGTGCCTATCTAACATCTC AATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTTTTACATGCCAATAC AATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTTA AACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACT TTACTTTTATCTAATCTAGACATCATTAATTCCTCCTTTTTGTTGACATTA TATCATTGATAGAGTTATTTGTCAAACTAGTTTTTTATTTGGATCCCCTC GAGTTCATGAAAAACTAAAAAAAATATTGACACTCTATCATTGATAGAG CATAATTAAAATAAGCTCTCTATCATTGATAGAGTGAGTTAAACCTATA GGAGGAAAAAACGATGATCTACTATATAAAAGATTTAAAAGTGAAAGG AAAAATATTTGAAAATCTAATGAACAAAGAGGCTGTAGAAGGATTAAT TACTTTTTTAAAGAAAGCGGAATTTGAGATATACTCAAGAGAAAATTAT

	TCAAAATACAACAAATGGTTTGAAATGTGGAAAAGCCCAACTTCGAGC CTTGTGTTTTGGAAAAATTATAGTTTTCGCTGTCATCTTCTTTTTGTCATA GAAAAAGATGGTGAATGCCTTGGAATTCCTGCATCTGTTTTTGAATCTG TACTTCAAATTTACCTTGCGGATCCGTTCGCTCCCGATACGAAAGAACT TTTTGTTGAGGTTTGTAATTTATATGAATGTTTAGCGGATGTCACTGTCG TAGAACATTTTGAAGCGGAAGAATCAGCGTGGCATAAATTAACCCATA ATGAGACCGAAGTATCAAAAAGAGTCTATAGTAAAGATGATGACGAAC TTCTTAAATATATTCCAGAATTTCTTGACACCATAGCGACAAACAAGAA AAGTCAAAAATACAATCAAATTCAAGGAAAAATACAAGAAATTAATAA GGAAATAGCTACACTTTATGAATCGTCAGAGGATTATATATTTACTGAA TATGTTAGTAATTTATATAGAGAGTCTGCAAAGTTGGAGCAACACAGCA AACAAATTTTAAAAGAGGAGCTAAATGACTACAAGGATCATGATGGTG ATTATAAAGATCACGACATCGATTACAAAGATGATGACGATAAATAAG Cctctcgcctgtcccctcagttcagtaatttc
AcrVIA1(Y39A, S40A, N43A, S93A, Q96A)-3xFlag	ctcgtgctataattatactaattttACATCACGGAAAAAGGTTATGCTGCTTTTAAGACC CACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGG CCGAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAG CTTGTCGTAATAATGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCT TCTTTAGCGACTTGATGCTCTTGATCTTCCAATACGCAACCTAAAGTAA AATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGTGAAAAACC TTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTTTTT CTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGT AAAGCACATCTAAAACTTTTAGCGTTATTACGTAAAAAATCTTGCCAGC TTTCCCCTTCTAAAGGGCAAAAGTGAGTATGGTGCCTATCTAACATCTC AATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTTTTACATGCCAATAC AATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTTA AACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACT TTACTTTTATCTAATCTAGACATCATTAATTCCTCCTTTTTGTTGACATTA TATCATTGATAGAGTTATTTGTCAAACTAGTTTTTTATTTGGATCCCCTC GAGTTCATGAAAAACTAAAAAAAATATTGACACTCTATCATTGATAGAG CATAATTAAAATAAGCTCTCTATCATTGATAGAGTGAGTTAAACCTATA GGAGGAAAAAACGATGATCTACTATATAAAAGATTTAAAAGTGAAAGG AAAAATATTTGAAAATCTAATGAACAAAGAGGCTGTAGAAGGATTAAT TACTTTTTTAAAGAAAGCGGAATTTGAGATAGCCGCAAGAGAAGCATAT TCAAAATACAACAAATGGTTTGAAATGTGGAAAAGCCCAACTTCGAGC CTTGTGTTTTGGAAAAATTATAGTTTTCGCTGTCATCTTCTTTTTGTCATA GAAAAAGATGGTGAATGCCTTGGAATTCCTGCATCTGTTTTTGAAGCTG TACTTGCTATTTACCTTGCGGATCCGTTCGCTCCCGATACGAAAGAACTT TTTGTTGAGGTTTGTAATTTATATGAATGTTTAGCGGATGTCACTGTCGT AGAACATTTTGAAGCGGAAGAATCAGCGTGGCATAAATTAACCCATAA TGAGACCGAAGTATCAAAAAGAGTCTATAGTAAAGATGATGACGAACT TCTTAAATATATTCCAGAATTTCTTGACACCATAGCGACAAACAAGAAA AGTCAAAAATACAATCAAATTCAAGGAAAAATACAAGAAATTAATAAG GAAATAGCTACACTTTATGAATCGTCAGAGGATTATATATTTACTGAAT ATGTTAGTAATTTATATAGAGAGTCTGCAAAGTTGGAGCAACACAGCAA ACAAATTTTAAAAGAGGAGCTAAATGACTACAAGGATCATGATGGTGA TTATAAAGATCACGACATCGATTACAAAGATGATGACGATAAATAAGCc tctcgcctgtcccctcagttcagtaatttc
AcrVIA1(ΔE131-E134)-3xFlag	ctcgtgctataattatactaattttACATCACGGAAAAAGGTTATGCTGCTTTTAAGACC CACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGG CCGAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAG CTTGTCGTAATAATGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCT TCTTTAGCGACTTGATGCTCTTGATCTTCCAATACGCAACCTAAAGTAA AATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGTGAAAAACC TTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTTTTT CTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGT

	AAAGCACATCTAAAACTTTTAGCGTTATTACGTAAAAAATCTTGCCAGC TTTCCCCTTCTAAAGGGCAAAAGTGAGTATGGTGCCTATCTAACATCTC AATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTTTTACATGCCAATAC AATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTTA AACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACT TTACTTTTATCTAATCTAGACATCATTAATTCCTCCTTTTTGTTGACATTA TATCATTGATAGAGTTATTTGTCAAACTAGTTTTTTATTTGGATCCCCTC GAGTTCATGAAAAACTAAAAAAAATATTGACACTCTATCATTGATAGAG CATAATTAAAATAAGCTCTCTATCATTGATAGAGTGAGTTAAACCTATA GGAGGAAAAAACGATGATCTACTATATAAAAGATTTAAAAGTGAAAGG AAAAATATTTGAAAATCTAATGAACAAAGAGGCTGTAGAAGGATTAAT TACTTTTTTAAAGAAAGCGGAATTTGAGATATACTCAAGAGAAAATTAT TCAAAATACAACAAATGGTTTGAAATGTGGAAAAGCCCAACTTCGAGC CTTGTGTTTTGGAAAAATTATAGTTTTCGCTGTCATCTTCTTTTTGTCATA GAAAAAGATGGTGAATGCCTTGGAATTCCTGCATCTGTTTTTGAATCTG TACTTCAAATTTACCTTGCGGATCCGTTCGCTCCCGATACGAAAGAACT TTTTGTTGAGGTTTGTAATTTATATGAATGTTTAGCGGATGTCACTGTCG TAGAACATTTTTCAGCGTGGCATAAATTAACCCATAATGAGACCGAAGT ATCAAAAAGAGTCTATAGTAAAGATGATGACGAACTTCTTAAATATATT CCAGAATTTCTTGACACCATAGCGACAAACAAGAAAAGTCAAAAATAC AATCAAATTCAAGGAAAAATACAAGAAATTAATAAGGAAATAGCTACA CTTTATGAATCGTCAGAGGATTATATATTTACTGAATATGTTAGTAATTT ATATAGAGAGTCTGCAAAGTTGGAGCAACACAGCAAACAAATTTTAAA AGAGGAGCTAAATGACTACAAGGATCATGATGGTGATTATAAAGATCA CGACATCGATTACAAAGATGATGACGATAAATAAGCctctcgcctgtcccctcagtt cagtaatttc
AcrVIA1(I2A, Y4A)-3xFlag	ctcgtgctataattatactaattttACATCACGGAAAAAGGTTATGCTGCTTTTAAGACC CACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGG CCGAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAG CTTGTCGTAATAATGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCT TCTTTAGCGACTTGATGCTCTTGATCTTCCAATACGCAACCTAAAGTAA AATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGTGAAAAACC TTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTTTTT CTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGT AAAGCACATCTAAAACTTTTAGCGTTATTACGTAAAAAATCTTGCCAGC TTTCCCCTTCTAAAGGGCAAAAGTGAGTATGGTGCCTATCTAACATCTC AATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTTTTACATGCCAATAC AATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTTA AACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACT TTACTTTTATCTAATCTAGACATCATTAATTCCTCCTTTTTGTTGACATTA TATCATTGATAGAGTTATTTGTCAAACTAGTTTTTTATTTGGATCCCCTC GAGTTCATGAAAAACTAAAAAAAATATTGACACTCTATCATTGATAGAG CATAATTAAAATAAGCTCTCTATCATTGATAGAGTGAGTTAAACCTATA GGAGGAAAAAACGATGGCCTACGCTATAAAAGATTTAAAAGTGAAAGG AAAAATATTTGAAAATCTAATGAACAAAGAGGCTGTAGAAGGATTAAT TACTTTTTTAAAGAAAGCGGAATTTGAGATATACTCAAGAGAAAATTAT TCAAAATACAACAAATGGTTTGAAATGTGGAAAAGCCCAACTTCGAGC CTTGTGTTTTGGAAAAATTATAGTTTTCGCTGTCATCTTCTTTTTGTCATA GAAAAAGATGGTGAATGCCTTGGAATTCCTGCATCTGTTTTTGAATCTG TACTTCAAATTTACCTTGCGGATCCGTTCGCTCCCGATACGAAAGAACT TTTTGTTGAGGTTTGTAATTTATATGAATGTTTAGCGGATGTCACTGTCG TAGAACATTTTGAAGCGGAAGAATCAGCGTGGCATAAATTAACCCATA ATGAGACCGAAGTATCAAAAAGAGTCTATAGTAAAGATGATGACGAAC TTCTTAAATATATTCCAGAATTTCTTGACACCATAGCGACAAACAAGAA AAGTCAAAAATACAATCAAATTCAAGGAAAAATACAAGAAATTAATAA GGAAATAGCTACACTTTATGAATCGTCAGAGGATTATATATTTACTGAA

	TATGTTAGTAATTTATATAGAGAGTCTGCAAAGTTGGAGCAACACAGCA AACAAATTTTAAAAGAGGAGCTAAATGACTACAAGGATCATGATGGTG ATTATAAAGATCACGACATCGATTACAAAGATGATGACGATAAATAAG Cctctcgcctgtcccctcagttcagtaatttc
AcrVIA1(S68A, F69A)-3xFlag	ctcgtgctataattatactaattttACATCACGGAAAAAGGTTATGCTGCTTTTAAGACC CACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGG CCGAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAG CTTGTCGTAATAATGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCT TCTTTAGCGACTTGATGCTCTTGATCTTCCAATACGCAACCTAAAGTAA AATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGTGAAAAACC TTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTTTTT CTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGT AAAGCACATCTAAAACTTTTAGCGTTATTACGTAAAAAATCTTGCCAGC TTTCCCCTTCTAAAGGGCAAAAGTGAGTATGGTGCCTATCTAACATCTC AATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTTTTACATGCCAATAC AATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTTA AACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACT TTACTTTTATCTAATCTAGACATCATTAATTCCTCCTTTTTGTTGACATTA TATCATTGATAGAGTTATTTGTCAAACTAGTTTTTTATTTGGATCCCCTC GAGTTCATGAAAAACTAAAAAAAAATATTGACACTCTATCATTGATAGAG CATAATTAAAATAAGCTCTCTATCATTGATAGAGTGAGTTAAACCTATA GGAGGAAAAAACGATGATCTACTATATAAAAGATTTAAAAGTGAAAGG AAAAATATTTGAAAATCTAATGAACAAAGAGGCTGTAGAAGGATTAAT TACTTTTTTAAAGAAAGCGGAATTTGAGATATACTCAAGAGAAAATTAT TCAAAATACAACAAATGGTTTGAAATGTGGAAAAGCCCAACTTCGAGC CTTGTGTTTTGGAAAAATTACGCTGCTCGCTGTCATCTTCTTTTTGTCAT AGAAAAAGATGGTGAATGCCTTGGAATTCCTGCATCTGTTTTTGAATCT GTACTTCAAATTTACCTTGCGGATCCGTTCGCTCCCGATACGAAAGAAC TTTTTGTTGAGGTTTGTAATTTATATGAATGTTTAGCGGATGTCACTGTC GTAGAACATTTTGAAGCGGAAGAATCAGCGTGGCATAAATTAACCCAT AATGAGACCGAAGTATCAAAAAGAGTCTATAGTAAAGATGATGACGAA CTTCTTAAATATATTCCAGAATTTCTTGACACCATAGCGACAAACAAGA AAAGTCAAAAATACAATCAAATTCAAGGAAAAATACAAGAAATTAATA AGGAAATAGCTACACTTTATGAATCGTCAGAGGATTATATATTTACTGA ATATGTTAGTAATTTATATAGAGAGTCTGCAAAGTTGGAGCAACACAGC AAACAAATTTTAAAAGAGGAGCTAAATGACTACAAGGATCATGATGGT GATTATAAAGATCACGACATCGATTACAAAGATGATGACGATAAATAA GCctctcgcctgtcccctcagttcagtaatttc
AcrVIA1(ΔN173-N232)-3xFlag	ctcgtgctataattatactaattttACATCACGGAAAAAGGTTATGCTGCTTTTAAGACC CACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGG CCGAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAG CTTGTCGTAATAATGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCT TCTTTAGCGACTTGATGCTCTTGATCTTCCAATACGCAACCTAAAGTAA AATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGTGAAAAACC TTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTTTTT CTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGT AAAGCACATCTAAAACTTTTAGCGTTATTACGTAAAAAATCTTGCCAGC TTTCCCCTTCTAAAGGGCAAAAGTGAGTATGGTGCCTATCTAACATCTC AATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTTTTACATGCCAATAC AATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTTA AACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACT TTACTTTTATCTAATCTAGACATCATTAATTCCTCCTTTTTGTTGACATTA TATCATTGATAGAGTTATTTGTCAAACTAGTTTTTTATTTGGATCCCCTC GAGTTCATGAAAAACTAAAAAAAAATATTGACACTCTATCATTGATAGAG CATAATTAAAATAAGCTCTCTATCATTGATAGAGTGAGTTAAACCTATA GGAGGAAAAAACGATGATCTACTATATAAAAGATTTAAAAGTGAAAGG

	AAAAATATTTGAAAATCTAATGAACAAAGAGGCTGTAGAAGGATTAAT TACTTTTTTAAAGAAAGCGGAATTTGAGATATACTCAAGAGAAAATTAT TCAAAATACAACAAATGGTTTGAAATGTGGAAAAGCCCAACTTCGAGC CTTGTGTTTTGGAAAAATTATAGTTTTCGCTGTCATCTTCTTTTTGTCATA GAAAAAGATGGTGAATGCCTTGGAATTCCTGCATCTGTTTTTGAATCTG TACTTCAAATTTACCTTGCGGATCCGTTCGCTCCCGATACGAAAGAACT TTTTGTTGAGGTTTGTAATTTATATGAATGTTTAGCGGATGTCACTGTCG TAGAACATTTTGAAGCGGAAGAATCAGCGTGGCATAAATTAACCCATA ATGAGACCGAAGTATCAAAAAGAGTCTATAGTAAAGATGATGACGAAC TTCTTAAATATATTCCAGAATTTCTTGACACCATAGCGACAGACTACAA GGATCATGATGGTGATTATAAAGATCACGACATCGATTACAAAGATGAT GACGATAAATAAGCctctcgcctgtcccctcagttcagtaatttc