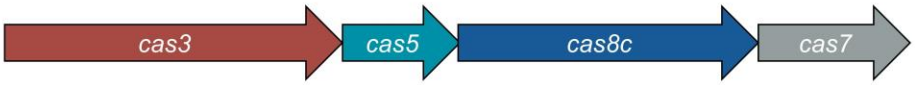
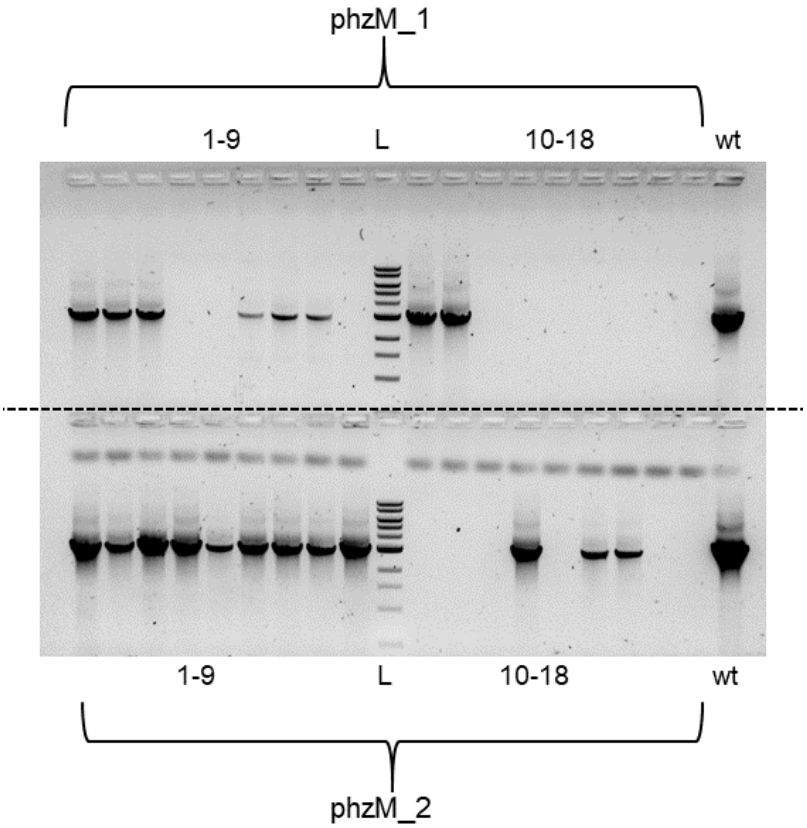
A



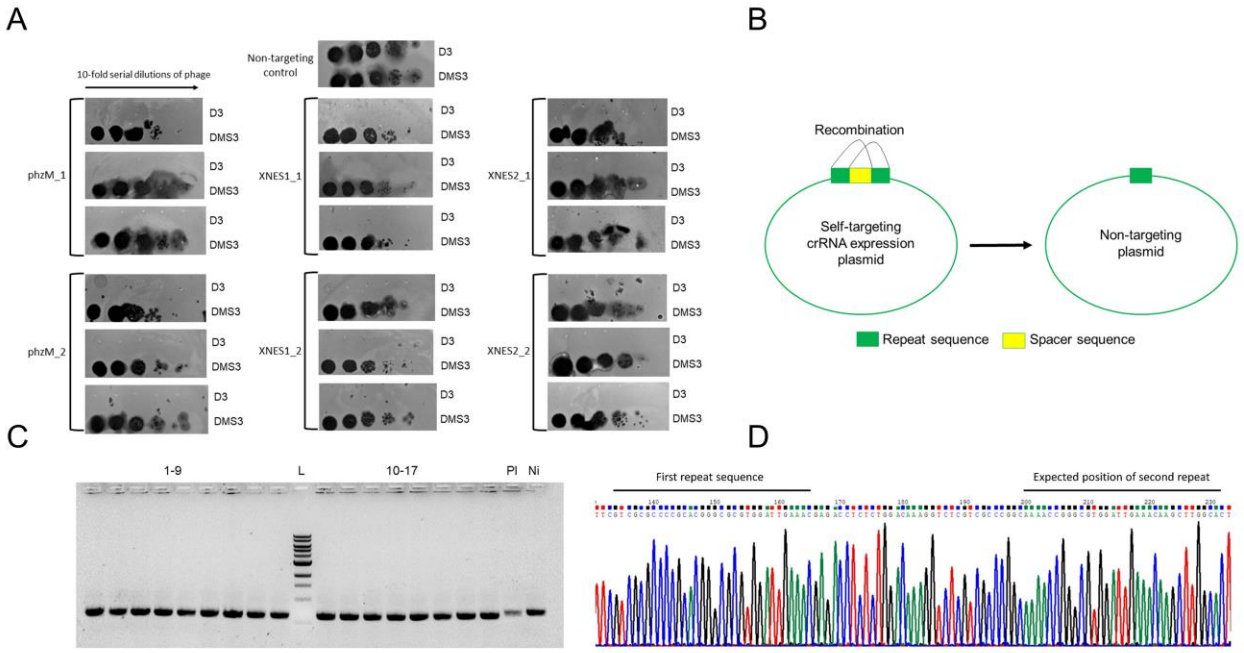
	Cas3 (%)	Cas5 (%)	Cas8 (%)	Cas7 (%)
*Bacillus halodurans**	Query cover.: 98 ID: 32	Query cover.: 93 ID: 31	Query cover.: 96 ID: 24	Query cover.: 94 ID: 35
Bacillus cereus	Query cover.: 91 ID: 31	Query cover.: 93 ID: 29	Query cover.: 19 ID: 29	Query cover.: 94 ID: 33
Lactobacillus fermentum	Query cover.: 97 ID: 27	Query cover.: 93 ID: 28	Query cover.: 78 ID: 22	Query cover.: 87 ID: 31
Mycobacterium canettii	Query cover.: 93 ID: 36	Query cover.: 93 ID: 41	Query cover.: 99 ID: 28	Query cover.: 95 ID: 45
Streptococcus pyogenes	Query cover.: 92 ID: 28	Query cover.: 91 ID: 29	Query cover.: 23 ID: 31	Query cover.: 84 ID: 34

B

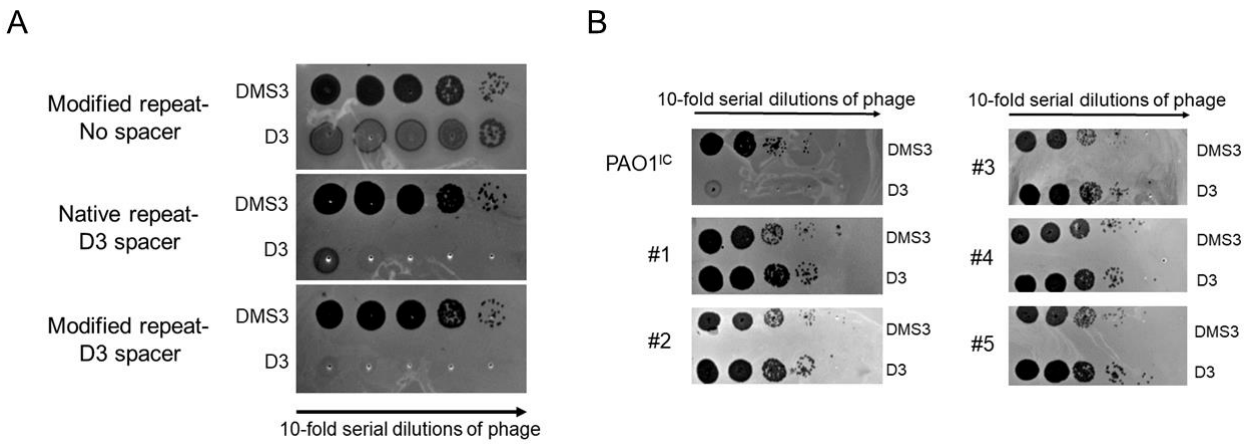


Supplementary Figure 1. A) Comparison of Type I-C CRISPR system from *P. aeruginosa* used in the study, to various other previously identified I-C systems from a range of different bacteria**.** Values show query coverage and percent identity (ID) percentages comparing the four

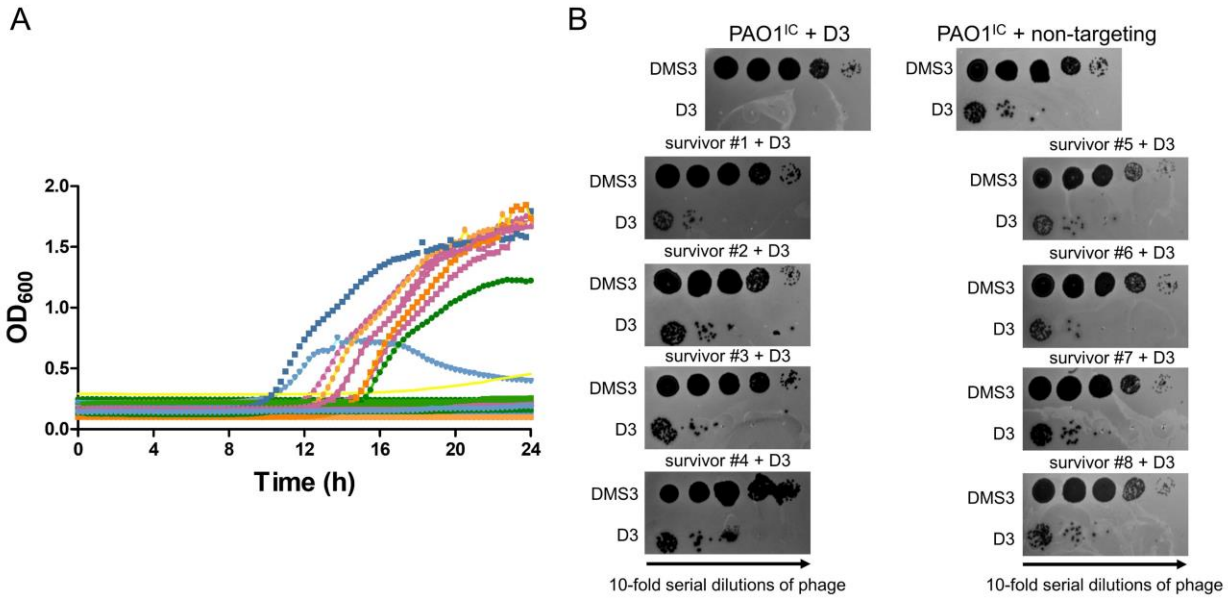
genes of the *P. aeruginosa* system to each of the other four. * Denotes the reference Type I-C CRISPR system referred to in Ref. 1. **B)** PCR amplification of a 3 kb genomic fragment flanking the *phzM* gene targeted using two different crRNAs, phzM_1 and phzM_2. Colony PCRs were performed on 18 biological replicates of self-targeted strains for each crRNA. The PAO1^{IC} parental strain is used as a positive control (wt). L indicates a 1 kb DNA ladder.



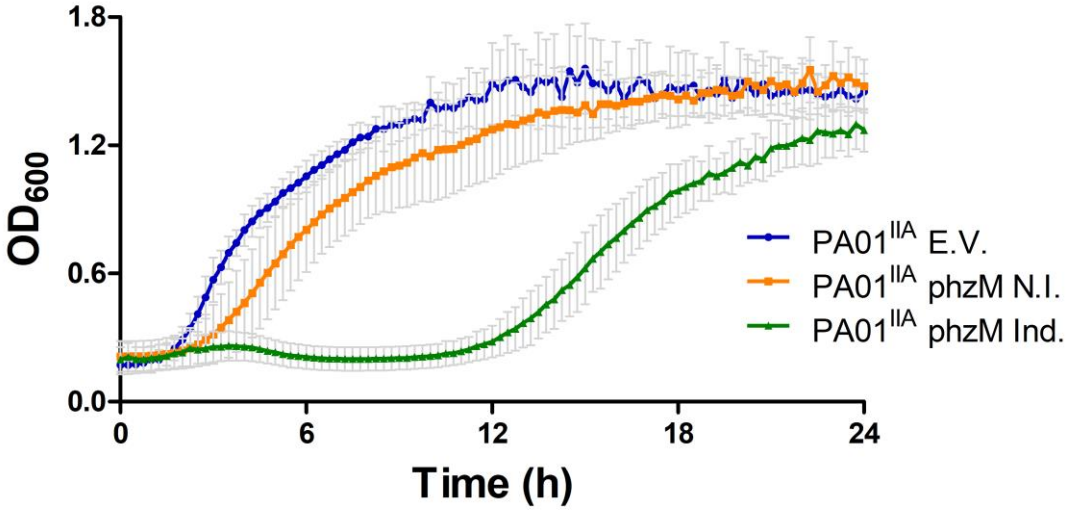
Supplementary Figure 2. A) Phage targeting assays with survivors that had no discernable deletion of the crRNA-targeted genomic site. Strains were transformed with a D3 phage-targeting crRNA to assay for IC CRISPR-Cas3 activity. Three unique survivors were isolated from six self-targeting assays for a total of 18 survivors. Control is a non-targeting crRNA. **B)** Schematic of spacer excision events where the two direct repeats recombine, resulting the loss of the targeting spacer. **C)** PCR amplification of the crRNA sequence from plasmids isolated from 17 non-deletion self-targeted survivors. Pl indicates the original plasmid as the PCR template, Ni indicates a sample where the crRNA was not induced, L indicates a 1 kb DNA ladder. **D)** Sample chromatogram of a sequenced plasmid with the spacer flipped out. Only one 32 bp repeat sequence remains in the plasmid, the 34 bp spacer sequence and other 32 bp repeat are missing.



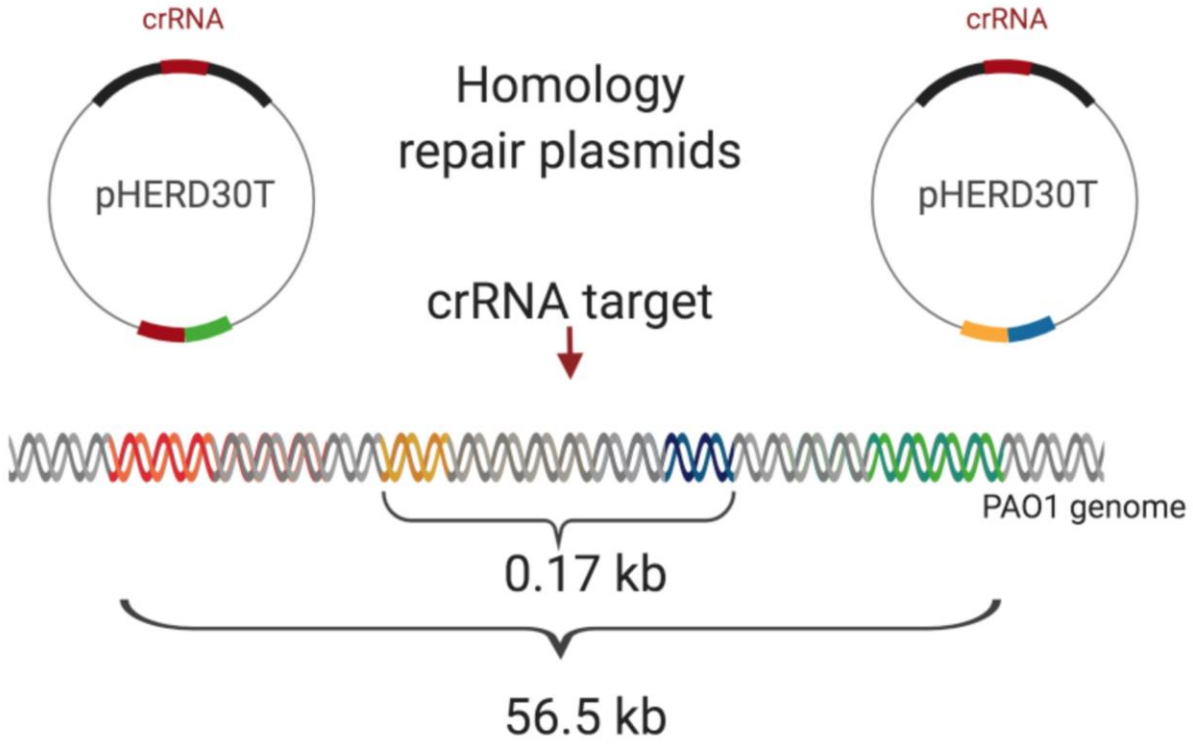
Supplementary Figure 3. A) Phage-targeting assay showing the activity of the modified repeat crRNA constructs. Ten-fold serial dilutions of DMS3 phage and D3 phage were spotted on lawns of PAO1^{IC} expressing either empty vector (top), a crRNA targeting D3 with WT direct repeats (middle), or a crRNA targeting D3 with modified repeats (bottom). **B)** Phage targeting assay of five non-deletion self-targeting survivors expressing a D3 phage targeting crRNA. Unsuccessful targeting of phage indicates a non-functional CRISPR-Cas system in these strains. The parental PAO1^{IC} strain with a functional CRISPR-Cas system was used as a control.



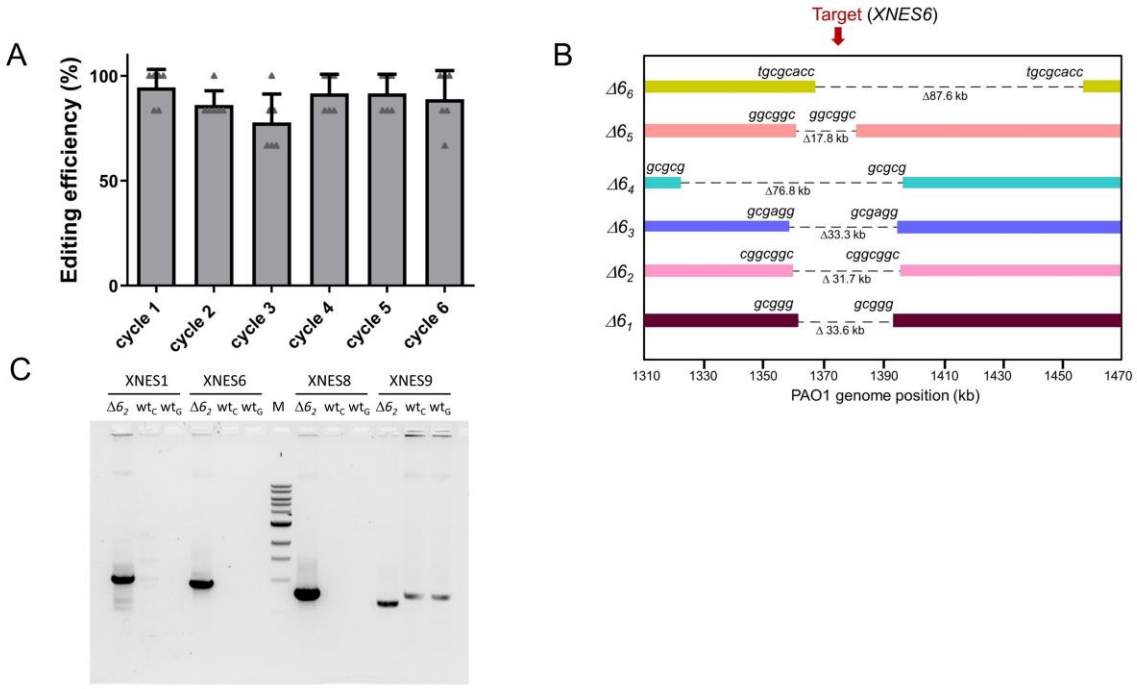
Supplementary Figure 4. A) Growth curves of 36 PAO1^{IC} biological replicates targeting the essential gene, *rplQ*, using the MR crRNA plasmid. **B)** Phage targeting assays with eight isolated *rplQ*-targeted survivors to assay for I-C CRISPR-Cas activity. Serial dilutions of DMS3 phage and D3 phage were spotted on lawns of PAO1^{IC} expressing a crRNA targeting phage D3. The parent PAO1^{IC} strain expressing a D3 targeting crRNA (top left) was used as a positive control, while PAO1^{IC} expressing a non-targeting crRNA was used as a negative control.



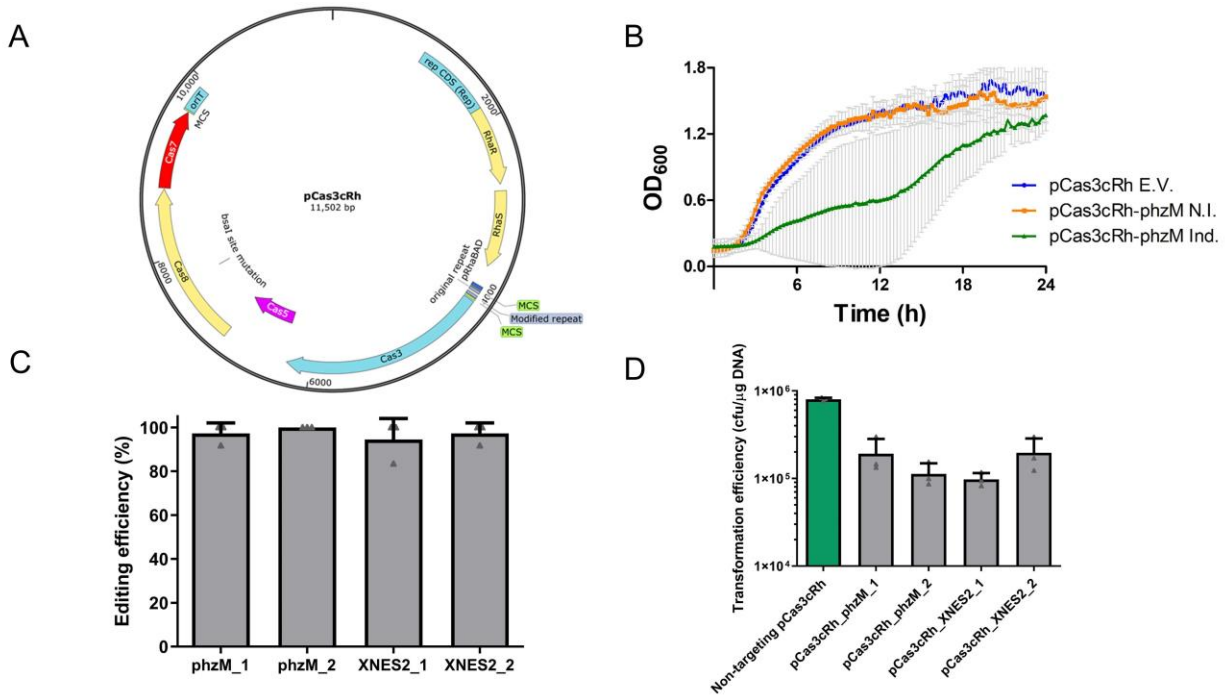
Supplementary Figure 5. Growth of self-targeting strains of PAO1^{IIA} expressing a self-targeting gRNA targeting the genome at *phzM* (Ind.). An empty vector (E.V.) and a non-induced *phzM* targeting strain (N.I.) were used as controls. Mean OD values measured at 600 nm are shown for 8 biological replicates each, error bars indicate SD values.



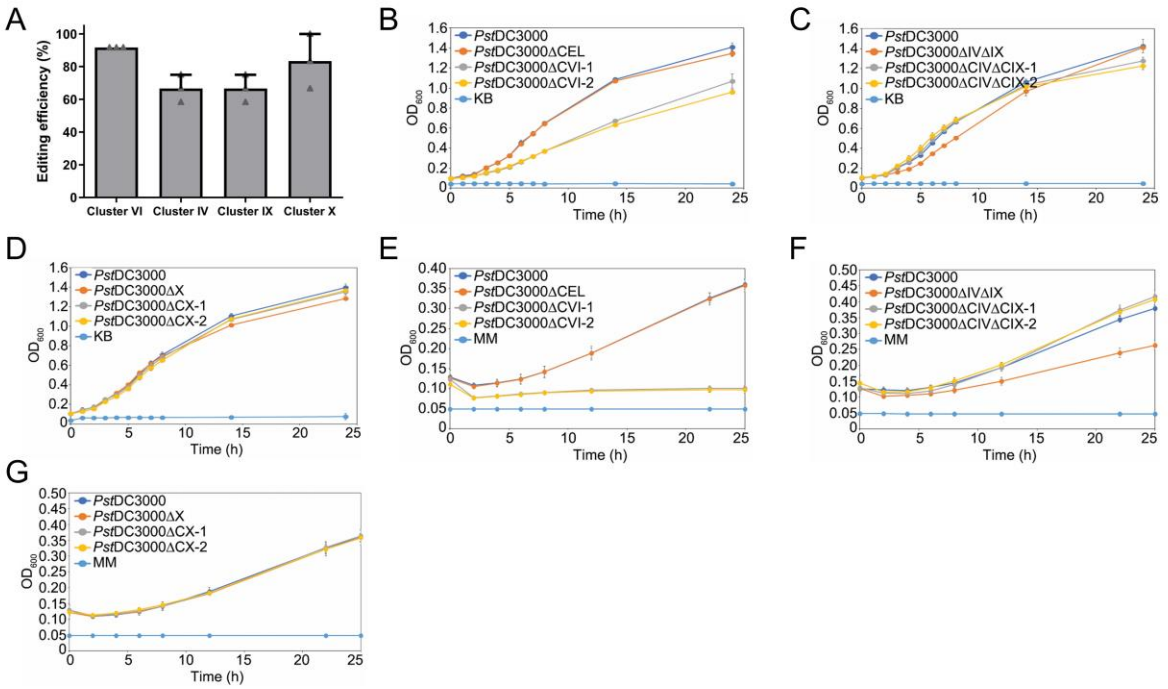
Supplementary Figure 6. Schematic overview of the generation of deletions with predetermined coordinates of various sizes. Sequences with ~400 bp homology to genomic sites (purple and yellow boxes for the short deletion, red and orange boxes for the long deletion) were cloned into the vector crRNA vector.



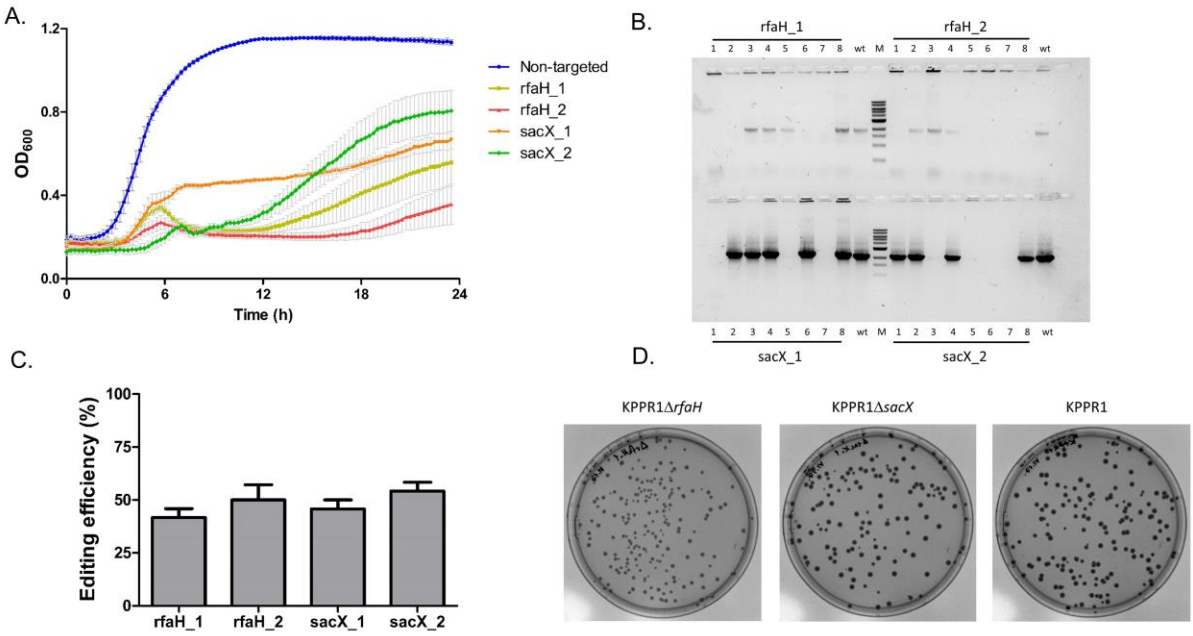
Supplementary Figure 7. A) Deletion efficiencies observed over six cycles of iterative self-targeting. Six genomic targets were targeted in six different orders. Six survivors were analyzed using site-specific PCR after each cycle, for a total of 36 analyzed colonies (6*6) after each cycle. **B)** Deletion junctions at *XNES6* target site of the 6 PAO1^{IC} strains with 6 iterative targeting events each. Sequences of each specific microhomology for the junctions are shown for each strain above the bars representing the given genomes at both ends, deletion sizes are shown below dashed lines for each strain. **C)** PCR analysis using a representative set of primers amplifying various large deletion junctions (at XNES1, 6, 8, and 9 regions) of the whole-genome sequenced $\Delta 6_2$ strain. $\Delta 6_2$ served as a positive control template, while wt_C represents untargeted PAO1$_{IC}$ cells scraped from a lawn of colonies grown on plates serving as templates, and wt_G represents isolated genomic DNA from 1.5 ml overnight culture of untargeted PAO1$_{IC}$ used as templates. Bands appearing for the XNES9 deletion junction for the PAO1$_{IC}$ samples were aspecific and when sequenced, did not match any genomic region of the PAO1$_{IC}$ genome.



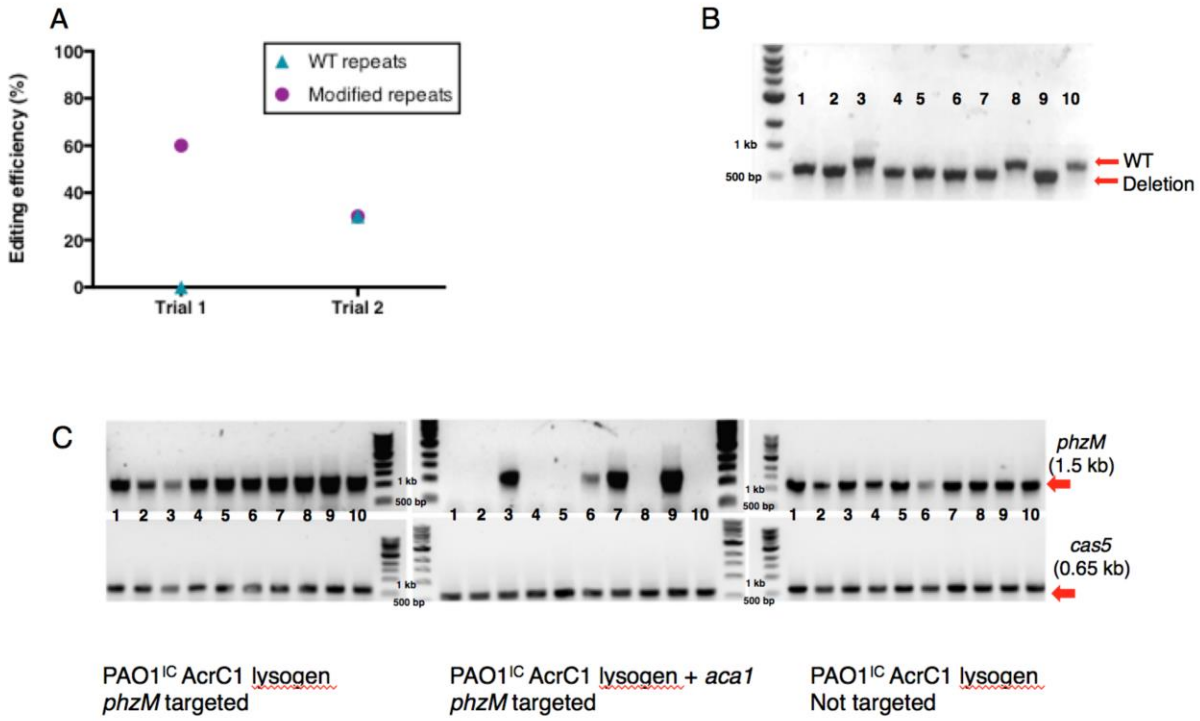
Supplementary Figure 8. A) Map of the I-C CRISPR-Cas all-in-one plasmid pCas3cRh carrying I-C crRNA and genes *cas3*, *cas5*, *cas8*, and *cas7* under the control of the rhamnose-inducible *rhaSR*-Prha$_{BAD}$ system. **B)** Growth curve of PAO1 transformed with the pCas3cRh vector expressing a self-targeting crRNA targeting *phzM* (Ind.). An empty vector (E.V.) and a non-induced *phzM* targeting strain (N.I.) were used as controls. Mean OD values measured at 600 nm are shown for six biological replicates each. **C)** Deletion efficiencies for WT PAO1 using the all-in-one vector pCas3cRh carrying all necessary components of the I-C CRISPR-Cas system. Values are averages of three replicates where 12 individual colonies were analyzed using site-specific PCR. Error bars show standard deviations. **D)** Transformation efficiencies with self-targeting pCas3cRh vectors expressing crRNAs for *phzM* or XNES 2 compared to a non-targeting control (green bar) in PAO1. Values are means of 3 replicates each, error bars represent SD values.



Supplementary Figure 9. A) Percentage of survivors with targeted deletions in clusters of non-essential virulence effector genes in *P. syringae* pv. *tomato* DC3000. Values are averages of three biological replicates where 12 individual colonies were analyzed using site-specific PCR for each, error bars show standard deviations. **B)** *In vitro* growth of cluster VI deletion strains in King's medium B (KB). ΔCEL is the previously published polymutant, while ΔCVI-1 and ΔCVI-2 are Cas3-generated mutants. Error bars represent standard deviation, n =4. **C)** *In vitro* growth of cluster IV, cluster IX deletion strains in KB. ΔCEL is the previously published polymutant, while ΔCIVΔCIX-1 and ΔCIVΔCIX-2 are Cas3-generated mutants. Error bars represent standard deviation, n = 4. **D)** *In vitro* growth of cluster X deletion strains in KB. ΔCEL is the previously published polymutant, while ΔCX-1 and ΔCX-2 are Cas3-generated mutants. Error bars represent standard deviation, n = 4. **E)** *In vitro* growth of cluster VI deletion strains in apoplast mimicking minimal media (MM). ΔCEL is the previously published polymutant, while ΔCVI-1 and ΔCVI-2 are Cas3-generated mutants. Error bars represent standard deviation, n = 4. **F)** *In vitro* growth of cluster IV, cluster IX deletion strains in MM. ΔCEL is the previously published polymutant, while ΔCIVΔCIX-1 and ΔCIVΔCIX-2 are Cas3-generated mutants. Error bars represent standard deviation, n = 4. **G)** *In vitro* growth of cluster X deletion strains in MM. ΔCEL is the previously published polymutant, while ΔCX-1 and ΔCX-2 are Cas3-generated mutants. Error bars represent standard deviation, n = 4.



Supplementary Figure 10. CRISPR-Cas3 editing in *Klebsiella pneumoniae*. **A)** Growth curves of *K. pneumoniae* strains expressing distinct crRNAs targeting *rfaH* and *sacX* (2 each). Non-targeting crRNA expressing control is marked in blue. Values depicted are averages of 8 biological replicates each. **B)** Representative gel electrophoresis of PCR fragments amplified from 8 total surviving colonies each from the 4 crRNA targeting constructs. Primer pairs amplified regions flanking the targeted position at *rfaH* and *sacX*. Wild-type KPPR1 (wt) colonies were used as controls, M represents 1 kb DNA marker ladder. **C)** Percentage of survivors with targeted deletions at the targeted genomic positions. Values are averages of three biological replicates where 8 individual colonies were analyzed using site-specific PCR for each, error bars show standard deviations. **D)** Colony morphologies of deletion candidate strains of *rfaH* and *sacX* compared to wild-type *K. pneumoniae* KPPR1.



Supplementary Figure 11. A) Editing efficiencies for the *Pseudomonas aeruginosa* environmental isolate naturally expressing the Type I-C *cas* genes, transformed with a plasmid targeting *phzM* with WT repeats or modified repeats. Each data point represents the fraction of isolates with the deletion out of ten isolates assayed. **B)** Genotyping results for the *Pseudomonas aeruginosa* environmental isolate using the 0.17 kb HDR template. Larger band corresponds to the WT sequence, smaller band corresponds to a genome reduced by 0.17 kb. **C)** Genotyping results of PAO1^{IC} AcrC1 lysogens after self-targeting induction in the presence or absence of *aca1* and a non-targeted control. Ten biological replicates per strain were assayed. gDNA was extracted from each replicate and PCR analysis for the *phzM* gene (targeted gene, top row of gels) or *cas5* gene (non-targeted gene, bottom row) was conducted. Only cells that co-expressed *aca1* with the crRNA showed loss of the *phzM* band, indicating genome editing. All replicates had a *cas5* band, indicating successful gDNA extraction and target specificity for the *phzM* locus.

Region	Coordinates	Size
XNES 1	27535 – 142359	114 kb
XNES 2	143267 – 371151	228 kb
XNES 3	491900 – 606160	114 kb
XNES 4	841825 – 986817	145 kb
XNES 5	1147815 – 1249907	102 kb
XNES 6	1260442 – 1491913	232 kb
XNES 7	1974210 – 2150828	176 kb
XNES 8	2216121 – 2375804	160 kb
XNES 9	2376541 – 2923367	546 kb
XNES 10	2972700 – 3079197	106 kb
XNES 11	3155072 – 3309411	154 kb
XNES 12	3587303 – 3802567	216 kb
XNES 13	3897357 – 4062426	165 kb
XNES 14	4294208 – 4457362	163 kb
XNES 15	4576324 – 4753990	178 kb
XNES 16	6025305 – 6180942	156 kb

Supplementary Table 1. Extended, non-essential regions (XNES) of *P. aeruginosa* PAO1 genome with contiguous, individually non-essential genes in a complex laboratory medium exceeding 100 kb. Data based on a transposon sequencing dataset from Turner *et al*.[39].

884 **Supplementary Table 2.** Genomic coordinates and extent of homologous sequences at
885 genomic deletion junctions of whole-genome sequenced self-targeting strains of *P. aeruginosa,*
886 *P. syringae*, and *E. coli*. See separate Excel File.

887

Strain	Edited gene	HR edits (%)	Nontemplated edits (%)	No edits (%)	n	Designed deletion (bp)	HR template length (left + right, bp)
PA14	*psiF*	100	0	0	12	0.5	600 + 600
PA14	*rebB*	50	50	0	16	4.1	600 + 600
z8	*ghlO*	100	0	0	5	0.2	600 + 600
z8	*mexZ*	75	25	0	12	0.6	722 + 600
z8	*psiF*	100	0	0	12	0.5	600 + 600
z8	*qsrO*	29(80)*	71	0	14	0.4	751 + 596
z8	*teg*	75	25	0	4	6.3	800 + 809

Supplementary Table 3. Summary of HR-mediated genome editing experiments using the Type I-F CRISPR-Cas3 system. Genes were targeted for deletion in the strains PA14 and z8. Experiments targeted 4 single genes and 2 gene blocks, *teg* and *rebB*. Transformants were classified as 1) 'HR edits' that have the HR designed deletion; 2) 'non-templated edits' that have a non-designed deletion encompassing the targeted gene, 3) 'no edits' where the targeted gene is intact. (*) two colony morphologies with different editing frequencies were obtained in this experiment. The HR arms were designed to be of 600 bp on average. The arm length was increased to ~800 bp for the deletion >5 kb. The variability around the average arm length values comes from technical constraints, such as selection of adequate primer sequences or avoidance of regions not optimal for Gibson assembly.

Supplementary Table 4. List of oligonucleotides (including crRNA sequences) used in the study. See separate Excel File.