Recombination between phages and CRISPR-*cas* loci facilitates horizontal gene transfer in staphylococci

Andrew Varble[1], Sean Meaden[2], Rodolphe Barrangou[3], Edze R. Westra[2], and Luciano A. Marraffini[1]

[1]Laboratory of Bacteriology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

[2]Environment and Sustainability Institute, Centre for Ecology and Conservation, University of Exeter, Biosciences, Penryn, Cornwall, United Kingdom

[3]Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC, 27695, USA.

Correspondence to marraffini@rockefeller.edu

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) loci and their associated (*cas*) genes encode an adaptive immune system that protects prokaryotes from viral[1] and plasmid[2] invaders. Upon viral (phage) infection, a small fraction of the prokaryotic cells are able to integrate a small sequence of the invader's genome into the CRISPR array[1]. These sequences, known as spacers, are transcribed and processed into small CRISPR RNA (crRNA) guides[3-5] that associate with Cas nucleases to specify a viral target for destruction[6-9]. Although, CRISPR-*cas* loci are widely distributed throughout microbial genomes and often display hallmarks of horizontal gene transfer[10-12], the drivers of CRISPR dissemination remain unclear. Here we show that spacers can recombine with phage target sequences to mediate a form of specialized transduction of CRISPR elements. Phage targets in phage 85, ΦNM1, ΦNM4, and Φ12 can recombine with spacers in either chromosomal or plasmid-borne CRISPR loci in *Staphylococcus*, leading to either the transfer of CRISPR-adjacent genes or the propagation of acquired immunity to other bacteria in the population, respectively. Our data demonstrate that spacer sequences not only specify the targets of Cas nucleases, but also can promote horizontal gene transfer.

Bioinformatic analysis of CRISPR-*cas* loci have uncovered hallmarks of horizontal gene transfer (HGT)[10-12] and CRISPR-Cas modularity[13-15]. Phylogenies based on either CRISPR repeats or the universal Cas1 protein revealed poor correlations between bacterial species trees, suggesting evidence of HGT of CRISPR-*cas* loci between distantly related bacterial species[16-18]. Furthermore, genomic studies have suggested that CRISPR systems evolved from a common ancestor and co-opted a diverse set of effector modules, potentially via HGT[13-15]. Plasmid conjugation[16,18,19] and

bacteriophage transduction[20], fundamental routes for HGT[21], have been implicated in the dissemination of CRISPR-*cas* loci[22]. Transduction occurs during viral infection, and can be divided into generalized or specialized[23]. Generalized transduction is a rare event that occurs when the phage machinery packages any DNA from the infected (donor) cell and subsequently delivers this DNA into a recipient cell. It is mediated by *pac* but not *cos* phages. In contrast, specialized transduction is a more specific event mediated by prophages (both *pac* and *cos*) that package genes located in the vicinity of their integration site. Recently it was shown that the type I-F CRISPR-*cas* locus of *Pectobacterium atrosepticum* can be mobilized through generalized transduction at rates ~10^{-8} (ref. [22]). Here we sought to determine if specific mechanisms are in place to mediate a more efficient horizontal gene transfer of CRISPR-Cas systems, their components, or their flanking sequences.

We explored whether recombination between newly acquired spacers and their targeted phage could mediate transduction. It is well established that even short sequences with homology to a phage leads to recombination events that integrate the homologous DNA into the viral genome, leading to a drastic enhancement of the transduction rates of plasmids[24-27], for example. The acquisition of a 30-40 bp spacer sequence from the infecting virus during the CRISPR-Cas adaptive immune response would introduce homology between the phage genome and the CRISPR locus, and could facilitate recombination and elevated rates of transduction. Such mechanism would lead to the transduction of genes adjacent to the CRISPR locus (Fig. 1A). To test this, we added an erythromycin resistance gene adjacent to the chromosomal type III-A system within the methicillin resistance cassette (SCCmec) of *Staphylococcus aureus* 08BA02176[28], and inserted a target site for the first spacer of this CRISPR locus at two locations (P1 and

P2) of the staphylococcal phage 85 genome[29], or a new spacer (C1) matching *orf28* (Fig S1A). This erythromycin-resistant strain was infected with each of the three phages or a non-targeted, wild-type phage as a control, and the lysates were used to transduce the marker into the wild-type strain. We observed that spacers P1 and C1 enhanced transduction of the antibiotic resistance cassette by one order of magnitude over the non-targeting control (Fig. 1B). Since there are no differences in the viability of the recipient cells (Fig. S1B), these results suggest that recombination between spacers P1 and C1 and the phages harboring their targets can direct the transfer of genomic locations adjacent to the CRISPR locus at rates that exceed those observed for generalized transduction (mediated by the non-targeted control phage). We also investigated the possibility of spacer-mediated transduction of entire chromosomal CRISPR-*cas* loci to CRISPR-lacking strains. Using two different empirical systems, the type I-F CRISPR-Cas system of *Pseudomonas aeruginosa* and the DMS3vir phage[30] and the type II-A CRISPR-Cas system of *Streptococcus thermophilus* and the 2972 phage[1], with adjacent chromosomal markers to track transduction (Fig. S1C), we observed generalized but not spacer-mediated transduction of the entire CRISPR-Cas system (Fig. S1D,E). Most likely this is a result of the presence of only one region of homology for integration (Fig. S1C).

Although most CRISPR-Cas systems reside on chromosomes, an important fraction of CRISPR loci has been reported to be carried on plasmids[31]. Plasmid-borne CRISPR loci offer unique advantages for their lateral transfer via spacer recombination: the increased copy number elevates the probability of recombination[27,32] and the circular nature allows the insertion of the entire CRISPR-*cas* locus into the phage genome, facilitating its packaging and re-circularization into the recipient host (Fig. 1C). To

explore this, we first tested if the transduction of CRISPR-carrying plasmids can be mediated by the newly acquired spacers. We infected *S. aureus* RN4220[33] cells (which lack endogenous CRISPR-*cas* loci) carrying the type II-A locus of *Streptococcus pyogenes*[5] (Fig. S2A) into the 2.9 kb staphylococcal plasmid pC194 (pCRISPR, conferring chloramphenicol resistance), with a staphylococcal *pac* phage carrying a virulent mutation, ΦNM4γ4[33]. Staphylococci harboring pCRISPR, but not an empty vector control, recovered at 12 hours (Fig. 1D) through the acquisition of new spacers (Fig. S2B), at the same time as the phage titers began to decline (Fig. 1D). We then checked for the presence of pCRISPR-transducing particles in phage filtrates (which also contain infective phages) by infecting *S. aureus* RN4220 recipients and selecting for chloramphenicol-resistant colonies. We detected an increase to a frequency of 1 transduced colony per 10^4 plaque forming units in filtrates collected 16 hours post-infection (Figs. 1D and S2C-F). In the type II-A CRISPR-Cas immune response, only a small fraction of cells acquires new spacers, the majority are not able to adapt and succumb to phage infection. To determine if transduction could transfer expanded pCRISPR loci to non-adapted cells, we mixed pCRISPR-harboring cells (chloramphenicol-resistant) and phage-sensitive recipients (kanamycin- and erythromycin-resistant) at a 1:5 ratio and infected with ΦNM4γ4. We were able to recover transduced colonies (resistant to all three antibiotics) at a frequency of ~ 10^{-5} with respect to CRISPR-adapted colonies (chloramphenicol-resistant) (Figs. 1E and S2G). Altogether, the data presented in Figs. 1 and S2 demonstrate that CRISPR-Cas plasmids can spread to naïve cells through transduction during the course of the CRISPR-Cas immune response.

Next, we investigated whether this transduction requires the presence acquired spacers, as it seems to be the case for the transduction of chromosomal CRISPR-adjacent loci (Figs. 1AB). First, we compared the spacer repertoires of CRISPR-resistant and CRISPR-transduced cells, obtained from the experiment in Figure 1E, using next-generation sequencing. Spacer sequences from four biological replicates were mapped onto the ΦNM4γ4 genome and plotted against their average number of reads (Fig. 2A, Supplementary Data File 1). The relative frequency of transduced spacers was consistent in each experiment (Fig. S3A) and we did not detect a correlation between the frequency of spacer acquisition and transduction (Fig. S3B and S3C). Instead, we found that spacers present in CRISPR-transduced cells were enriched in the 20-30 kb region of the phage, and depleted from the immediately preceding 5-20 kb region (Fig. 2A). These results suggest that there are CRISPR-*cas* loci containing specific spacer sequences that favor or limit their transduction. To test this we selected four spacer sequences, two with high (H1, H2), one with intermediate (I), and one with low (L) transduced/resistant ratio (Fig. 2A). These spacers were cloned into pCas9, a pCRISPR derivative unable to support new spacer acquisition due to the absence of *cas1*, *cas2* and *csn2*[33]. After infection with ΦNM4γ4, the populations carrying the high transducer spacers (H1 and H2) produced approximately three orders of magnitude more transducers than populations carrying the intermediate (I) or low (L)-transducing spacers (Fig. 2B). These results indicate that the sequence of the acquired spacer determines the frequency of transduction of the CRISPR-*cas* locus which harbors it. Similar results were obtained with a plasmid-borne type III-A system, which displayed enhanced levels of transduction when the system contains a spacer matching the infecting phage genome (Figs. S4AB).

To test if the spacer sequence itself could impact transduction we generated pSpacer plasmids containing only a repeat-spacer (H1, H2, I or L)-repeat unit, without *cas9*. After infection with ΦNM4γ4, we found that all four spacers equally increased the rate of pSpacer transfer when compared to a CRISPR array containing a control spacer that does not match the phage genome (Fig. 2C), suggesting that Cas9 targeting impacts the rate of transduction. Indeed, if pCRISPR transduction occurs through the formation of recombinants between the acquired spacer and the phage protospacer sequence, these recombinants will maintain a full target in one of the recombination junctions (Fig. S4C) which could be cleaved by Cas9. To investigate this, staphylococci carrying the pSpacer or pCas9 plasmids were infected with ΦNM4γ4 and DNA was isolated from bacterial pellets (containing infected cells) or culture supernatants (containing virions) for PCR amplification of both recombination junctions (Fig. 2D) as well as chromosomal and viral genes as controls for the fractionation (Fig. S4D). pSpacer/phage recombinants were detected at both junctions, for all spacer sequences, both in infected cells and virions, a result that explains the equal transduction levels of these plasmids (Fig. 2C). In contrast, pCas9/phage recombinants were also detected but PCR products were much less abundant for the targeted junction (J2) in infected cells (Fig. 2D). In virions, we only detected the non-targeted junction (J1) for the constructs containing H1 and H2 spacers, along with a faint PCR product for the I-spacer construct; but we were unable to detect the PAM-flanked (J2) junction (Fig. 2D). These results were corroborated by next-generation sequencing of DNA extracted from the virion fraction after infection of cells containing pCas9(H1). We found abundant reads spanning the non-targeted phage-CRISPR junction (J1) were detected (Fig. 2E), while the targeted junction (J2) had relatively fewer reads (Fig. 2F). Altogether, these experiments

demonstrate two important aspects of spacer-mediated recombination. First, recombination between the spacer sequence in pCRISPR and the protospacer sequence in ΦNM4γ4 results in the formation of hybrid DNA molecules which can be encapsidated into virions during infection. This does not depend on the host recA (Fig. S5A) and can also occur via the staphylococcal *cos* phage Φ12 (Fig. S5B). Second, Cas9 targeting of the PAM-flanked spacer/protospacer junction within these molecules reduces the efficacy of their packaging into viral capsids and therefore the efficiency of transduction.

The presence of phage particles containing spacer/phage recombinants from infected CRISPR-immune cells suggests that incomplete protection of the host allows for the formation and release of the CRISPR-containing virions. Inefficient CRISPR immunity can be the result from at least two scenarios. One possibility is that the immunity provided by the acquired spacer can be bypassed by phages containing target mutations, known as "escapers"; in this case the spacers that target regions with high rate of mutation in the phage genome will be more prone to transduction. However the experiments described in Figure S6 ruled out this scenario. A second possibility is that the acquired spacer provides only partial immunity, i.e. a proportion of the adapted cells can be lysed by the phage and produce CRISPR-transducing particles. To test this, we measured immunity using an assay that reproduces the high MOI faced by cells that acquire new spacers[33]. In these conditions, CRISPR-Cas systems programmed with the L and I spacers enabled the complete recovery of the host, and cells containing H1 and H2 spacers showed only a partial recovery of the infected staphylococci (Fig. 3A). To determine the strength of the immunity mediated by all the spacers present in the CRISPR-transducing particles (not just H1 and H2), we followed the survival of naïve

168 staphylococci upon infection with phages collected during the CRISPR-Cas immune
169 response (Fig. 1D, 22 hour time-point), which contain both ΦNM4γ4 as well as
170 transducing particles that can provide immunity against the phage. We obtained similar
171 partial survival curves to those provided by the H1- and H2-containing CRISPR-Cas
172 systems (Fig. 3B). These results suggest that the complete destruction of the virus by
173 the intermediate- and low-transducing spacers limits the formation of CRISPR-
174 transducing particles. If true, these spacers should have a dominant effect on the H1
175 and H2 spacers, i.e. they will reduce their frequency of transduction. To test this, we
176 combined different pairs of pCRISPR plasmids (with different antibiotic-resistance
177 markers) in the same cell, infected them with ΦNM4γ4 and counted the number of
178 transductants (Fig. 3C). We found that the combination of CRISPR-Cas systems
179 harboring I or L spacers with either H1 or H2 spacers resulted in a low transduction
180 frequency, i.e. the effect of I and L spacer predominate over H1 and H2. Similarly, a
181 reduction in the transduction of a second plasmid (pE194, 2.9 kb) is observed in cells
182 containing I and L spacers when compared to H1 and H2 (Fig. S7A). Finally, we looked
183 at the transduction of pCRISPR plasmids harboring an inactivating mutation in Cas9
184 (dCas9[8]). Corroborating our hypothesis, the reduction in immunity caused by this
185 mutation enhanced the transduction rate for spacers I, H1, and H2 (Figs. S7BC).
186 Altogether, these results demonstrate that spacer/phage recombination is the primary
187 driver of spacer-enhanced transduction and that spacer sequences mediating highly
188 efficient CRISPR immunity prevent the transduction of the CRISPR-*cas* locus.

189 Here we show that spacers acquired by CRISPR-Cas systems can perform a form of
190 specialized transduction that requires their recombination with the phage target as well
191 as incomplete CRISPR immunity (Fig. 3D). The recombination between the spacer and

its viral target connects the locus with the packaging sequences on the phage genome, enabling foreign DNA uptake at much higher rates than observed during typical generalized transduction in our experimental system. If the CRISPR locus resides in the host chromosome, this recombination can mediate the transfer of genes adjacent to the CRISPR locus and thus facilitate the dissemination and exchange of *cas* modules. If the CRISPR-Cas system resides in a circular genetic element, spacer-mediated recombination leads to the spread of CRISPR immunity among naïve CRISPR-negative hosts. Interestingly, the host RecA is not required for protospacer-spacer recombination, a result suggesting that this phenomenon is mediated by the phage's own machinery, which can significantly elevate recombination rates[34,35]. Our analysis of four different spacers showed that transduction rates are higher for spacers that mediate poor cleavage of the pCRISPR/ΦNM4γ4 recombinants. We believe that the next-generation sequencing experiment shown in Fig. 2A, which includes data for all transduced spacers, supports this correlation: the sequences of the spacers mediating the lowest transduction rates (5 to 20 kb of the ΦNM4γ4 genome) are located around the *pac* site, which a previous study showed to be one of the regions of this phage best targeted by Cas9[36].

Our data shows that both types of spacer-enhanced transduction events we describe here occur at very low frequencies. However, as it is the case with most situations involving horizontal transfer of genetic material, the importance of these events relies not so much in their rate of occurrence, but in their capacity to increase the genetic pool of the recipients[37]; given the appropriate environmental conditions, the genes and plasmids transferred though spacer-mediated transductions could provide a crucial selective advantage to the population. For example, the exchange of CRISPR-adjacent

modules could expand the repertoire of *cas* genes of a CRISPR locus and generate the genetic diversity[13-15] required to stay ahead in the arms race with different phages and their anti-CRISPR inhibitors[38]. Plasmids and potentially excisable genomic islands harboring CRISPR-Cas loci are relatively common[31,39], and their spread though spacer-mediated transduction could provide critical spacers and/or full defense cassettes for phage defense. Even if the transduced CRISPR-Cas locus does not harbor the most efficient spacers, as our data indicates, their spread can increase the spacer diversity necessary to prevent the raise of phage escapers[40] and/or provide partial defense to enable the acquisition of more potent sequences. Finally, it is worth noting that CRISPR-Cas systems have been identified within phage genomes[41-43]. Although we do not know their origin, it is possible that these arose by the type of spacer-mediated recombination we demonstrated in this study.

It is interesting to consider that acquired spacers could have a dual role during CRISPR immunity: a major one in the generation of crRNA guides and a minor one in mediating HGT. In support of this idea, recent work has found Tn7-like transposons that harbor orphan CRISPR arrays, not flanked by effector *cas* genes, in which dissemination is likely facilitated by spacers[44]. Circumstantial evidence that this second role may be important comes from the mechanism of crRNA biogenesis. In the *S. pyogenes* type II-A CRISPR-Cas pathway, the 10 nucleotides at the 5' end of the spacer sequence on the crRNA are degraded and eliminated from the Cas9 ribonucleoprotein complex, making this region of the spacer dispensable for targeting[8]. As recombination increases with the extent of homology, it is possible that this additional 10 nucleotides in the spacer DNA could facilitate transduction (Fig. S8). Type III-A spacers also have 3' end sequences that eliminated from the spacer RNA sequence during CRISPR-RNA

maturation[45], and while not shortened, crRNA-target homology at the 3’ end of type I-E spacers is not necessary for targeting[46]. Therefore, it is tempting to speculate that the acquisition of spacers has evolved not only to incorporate foreign sequences for defense against predation, but also as a means of hijacking the mobilization machinery of these elements to spread through prokaryotic populations.

Supplementary information

Supplementary information Figures S1-S6.

Supplementary information Tables S1-S2.

Supplementary information Data File 1.

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Author Information. The authors have no conflicting financial interests. Correspondence and requests for materials should be addressed to marraffini@rockefeller.edu.

Methods

Bacterial strains and growth conditions

Culture of *Staphylococcus aureus* RN4220[47] was carried out in brain-heart infusion (BHI) medium at 37°C with agitation at 220 revolutions per minute. Liquid experiments were carried out in 3 milliliters of medium in 15 ml conical tubes unless otherwise noted. *S. aureus* media was supplemented with 10 µg/ml chloramphenicol, 10 µg/ml erythromycin, or 25 µg/ml kanamycin for plasmid maintenance and/or chromosomal marker selection.

Culture of *Streptococcus thermophilus* was carried out in M17 media supplemented with 10% lactose at 37°C without agitation, unless otherwise noted. Liquid experiments were carried out in 5 milliliters of media in 15 ml conical tubes. M17 media was supplemented with 5 µg /ml chloramphenicol, 200 µg/ml spectinomycin, or 2.5 µg/ml erythromycin for chromosomal marker selection.

Culture of *Pseudomonas aeruginosa* was carried out in LB media at 37°C with agitation at 180 revolutions per minute. LB media was supplemented with 100 µg/ml streptomycin or 30 µg/ml gentamycin for chromosomal marker selection

All strains are listed in Table S1.

Quantification of CRISPR-Cas transducing particles

In *S. aureus,* overnight cultures of pWJ40[33] or pC194[48] were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4[33] at a multiplicity of infection (MOI) 1. Phage was collected at indicated time points and filtered with 0.45-µm syringe filters (Acrodisc). Harvested phage were then used to infect lawns of *S, aureus* strain OS2[49] suspended in 50% BHI supplemented with 5 mM $CaCl_2$ at an MOI of 1 on a BHI base supplemented with erythromycin and chloramphenicol to select for recipient cells and CRISPR-Cas transduction. For quantification of transducing particles produced from strains already containing CRISPR-immunity, overnight cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 or Φ12γ3[36] at a MOI of 50. 90 minutes post infection, phage were collected and filtered with 0.45-µm syringe filters (Acrodisc). Harvested phage were then used to infect lawns of OS2 suspended in 50% BHI supplemented with 5mM $CaCl_2$ at an MOI of 1 on a BHI base supplemented with erythromycin and chloramphenicol to select for recipient cells and CRISPR-Cas transduction. Phages that were not of sufficient titers to infect at an MOI of 1 were supplemented with the appropriate phage prepared from RN4220.

Detection of spacer acquisition

To check for spacer acquisition in *S. aureus*, transduced colonies were resuspended in colony lysis buffer (250 mM KCl, 5 mM $MgCl_2$ 50 mM Tris-HCl at pH 9.0, 0.5% Triton X-100), treated with 200 ng/µl lysostaphin and incubated at 37°C for 20 minutes, then 98°C for 10 minutes. Samples were centrifuged and supernatant was used for PCR amplification with primers L400 and H50.

CRISPR adaptation and escaper phage generation

For *P. aeruginosa,* to monitor the effect of increased homology between the CRISPR system and the phage DMS3vir genome, we cultured PA14-Sm in the presence of DMS3vir and isolated a phage-resistant mutant that had acquired an additional spacer targeting the phage, following procedures previously described[30]. Next, we isolated DMS3vir 'escape' mutants by inoculating a 96 well plate with 200 µl of the CRISPR-resistant PA14-Sm strain and ~$6*10^7$ DMS3vir. After a 24-hour incubation at 37°C phages were isolated by chloroform extraction and spotted onto a lawn of the CRISPR-resistant PA14-Sm. Individual 'escape' phage clones were isolated, followed by sequencing of the amplicon containing the protospacer and PAM sequences. A single 'PAM-escape' mutant was used in the transduction assays (G>A, position 25926) along with the WT DMS3vir phage.

For *S. thermophilus,* we isolated bacterial colonies that had acquired spacers in the erythromycin-tagged CRISPR1 locus of JAV28 following infection by phage 2972 using procedures previously described[50]. Genomic DNA from strain JAV33 was amplified and sequenced with AV638-AV724 and found to have a spacer targeting the top strand beginning at position 26,553 of 2972. Phage 2972 was passaged on this strain for escapers on soft-agar. Single plaques were isolated and re-passaged to single plaques on JAV33. Phage DNA was extracted by boiling the phage and 2972α1 DNA was amplified and sequenced with AV868-AV869. 2972α1 contained a mutation in the PAM region (A>G, 26,588)

Quantification of transduction

For *S. aureus,* overnight donor cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with either ΦNM4γ4 at a MOI of 1 or 85α1-3 and ΦNM1γ6 at an MOI of 50. Following lysis of the culture at 2 hours, phages were collected and filtered with a 0.45-µm syringe filters (Acrodisc). Overnight recipient cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected at an MOI of 1 with the transducing phage. 20 minutes post-infection, 40 mM of sodium citrate was added to the cultures. For erythromycin transduction, the cells were incubated for an additional 40 minutes then pelleted and washed twice with fresh BHI supplemented with 40 mM sodium citrate, while for chloramphenicol transduction cells were washed immediately. Cells were then plated on BHI plates supplemented with the antibiotics selecting for the recipient strain and transduction marker along with 20 mM sodium citrate for type II-A plasmids and no sodium citrate for type III-A plasmids.

For *P. aeruginosa,* bacterial lawns with near-confluent lysis were generated by mixing 200 µl of PA14-Sm on overnight cultures with 20µl of ~10^4 PFU DMS3vir and 10 mL soft LB agar. Phage only controls were included by applying the same protocol, but excluding the addition of bacteria. After 24-hour incubation at 37°C, phages were harvested by soaking the lawns in 3 mL of M9 salts buffer for 1 hour at room temperature followed by chloroform extraction and titration of the resulting phage stock. As recipients, we used *P. aeruginosa* PA14 ΔCRISPR-Cas[51] transformed with pHERD30T (conferring gentamycin resistance). 10 mL LB overnight culture supplemented with 30 µg mL^{-1} gentamycin of each recipient was spun down (3000 rpm,

10 min) and re-suspended in 1 mL of LB. 100 µl of lysate was then added and statically incubated for 25 minutes. Each culture was then spun down and the whole culture was plated on LB agar supplemented with 100 µg mL^{-1} streptomycin and 30 µg mL^{-1} gentamycin (to prevent carry over of PA14-Sm cells). To estimate transduction frequency, 48 colonies were picked per replicate experiment, and screened by PCR using primers specific for the CRISPR 2 locus primers CR2_F-CR2_R.

For *S. thermophilus,* transducing phage stocks were made by infecting mid-log growth JAV33 at 42°C supplemented with 10 mM $CaCl_2$ with either 2972 or 2972α1 at an MOI of 1. Phage stocks were harvested and filtered using 0.45-µm syringe filters (Acrodisc) after the culture had cleared. JAV27 were used as recipient cells and were grown to at 42°C supplemented with 10 mM $CaCl_2$ and infected at an MOI of 0.5 when cultures reached OD_{600} = 0.4. 10 minutes following infection, 20 mM sodium citrate was added to the cultures. After 1 hour incubation at 42°C, the cultures were washed two times in M17 media supplemented with 20 mM sodium citrate and then plated on erythromycin M17 plates. Transductants were confirmed by streaking out colonies on M17 chloramphenicol plates to confirm antibiotic resistance engineered into the CRISPR3 locus.

Detection of phage-CRISPR junctions

Overnight cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 at a MOI of 50 for targeting strains or 1 for non-targeting strains. Phages were collected from indicated strains 60 minutes post-infection. Supernatants were filtered using a 0.45-µm filter and then concentrated with Ultra-4 100k centrifugal 50-ml spin columns (Amicon). Concentrates were resuspended with DNase I buffer, 20 mM Tris-HCl pH 8.0 and 2 mM $MgCl_2$ and re-concentrated two times. The suspension was then treated with 25 units of DNase I (Sigma) for one hour. Following DNAse I treatment, the enzyme was inactivated by heating at 70°C for 10 minutes and the addition of 5 mM EDTA. Phages were then incubated with 8 units of proteinase K (NEB) and 0.5% SDS at 37°C for one hour. Phage DNA was isolated using a phenol/chloroform/isoamyl alcohol extraction (Fisher). Cellular DNA was collected 15 minutes and 60 minutes post infection for non-targeting strains and targeting strains respectively. Approximately 10^9 cells were pelleted and resuspended in 100 µl of 50 mM EDTA and 1 mg/ml lysostaphin (AMBI Products) and incubated at 37°C for one hour. DNA was then extracted with the Wizard genomic purification kit (Promega) according to the manufacturer's instructions. For the non-PAM junction, primer JM117 was used with NP255, AV547, AV469, AV471 for L, I, H1, and H2, respectively. For the PAM junction, primer L400 was used with AV457, AV458, AV456, AV459 for L, I, H1, and H2, respectively. For loading controls oGG38-oGG40 were used to amplify *gp14* and JW96-W964 for *recA.*

High-throughput sequencing

Overnight cultures of pWJ40 were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 at a MOI of 1. 20 hours post-infection DNA was collected from recovered cells (CRISPR-resistant). Phages were also collected and filtered with 0.45-µm syringe filters (Acrodisc). Overnight cultures of OS2 were diluted 1:100 in fresh BHI with appropriate antibiotics

and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 collected from the pWJ40 culture at a MOI of 1. 20 hours post-infection DNA was collected from recovered cells (CRISPR-transduced). Spacers were amplified with RH50 and JW655-JW662 for sample barcoding. The sequences of the oligonucleotides used in this study are listed in Table S2. Adapted bands were gel-extracted and subjected to Illumina MiSeq sequencing. Data analysis was performed in Python. Spacer reads were extracted from the raw MiSeq FASTA files and aligned to the phage genome. Number of reads and PAM were designated for each spacer. Spacers were normalized as reads per million and plotted against the ΦNM4γ4 genome in 2000-base-pair bins.deep sequence phage-CRISPR DNA junctions, cultures containing spacer H1 were infected with ΦNM4γ4 at a MOI of 50 and phages were collected 90 minutes post infection. Phage DNA was isolated as described above. DNA was then prepped with the Illumina TruSeq Nano kit according to the manufacturer's instructions. Prepped DNA was then subject to NextSeq sequencing. BWA-MEM (arXiv:1303.3997v1) was used to align sequenced DNA to the PAM junction, which contains 200 base pairs of the upstream CRISPR sequence (leader and direct repeat) and 205 base pairs of the downstream phage sequence (Spacer, PAM, and phage genome) or the repeat junction, which contains 205 base pairs of the upstream phage sequence (phage genome and spacer, and 200 base pair downstream CRISPR sequence (direct repeat and downstream plasmid sequence). A python script was then used to sort and bin reads spanned the full 75-nucleotide read length allowing for one mismatch.

Phage titer assay

Phage titer assays were performed as previously described[52].

Efficiency of plaquing assays

Efficiency of plaquing assays were performed as previously described[52].

Simulation of CRISPR immunization

Simulation of CRISPR immunization was performed as previously described[53].

Strain construction

To make the *recA* knockout JAV9, the allelic replacement system developed by Wenyan Jiang using pWJ244 was applied as previously described[36]. Briefly, pAV44 was transformed into RN4220 and integrants were isolated. Double crossover events were selected for by a temperature sensitive *cat* targeting Cas9 phagemid, pWJ326. RecA deletion was confirmed by primers outside the homology arms, AV223 and AV224. To make JAV21, OS2 was infected with ΦNM1γ6[52] at an MOI of 1 to produce transducing particles carrying the genomic erythromycin cassette. These particles were used to infect JW263[36] as described in quantification of transduction. Colonies that were resistant to kanamycin and erythromycin were struck out 2 times on plates supplemented with 20mM sodium citrate, kanamycin, and erythromycin. JAV29 and JAV32 were constructed by transforming suicide vectors pAV253 and pAV282. Integration was confirmed using primers AV594 and AV812 for pAV253 and AV648 and AV525. JAV33 was made by infecting RN4220 at MOI of 1 in soft agar with ΦNM4γ4.

After a 24-hour incubation, a resistant colony was picked, restreaked two times, and confirmed to be insensitive to ΦNM4γ4 infection.

To create a P. aeruginosa PA14 strain carrying a streptomycin resistance cassette immediately adjacent to the Type I-F CRISPR-Cas system in the genome (PA14-Sm, with the Sm gene inserted at position 2937360), we used homologous recombination. The streptomycin (Sm) resistance gene and its promoter were PCR amplified from pBAM1-Sm[54] using primers pB_Sm_F and pB_Sm_R, and inserted into the NheI restriction site of pHERD30T, flanked by amplicons FL1 (flank1, generated using primer pairs FL1_F and FL1_R) and FL2 (flank2, generated using primer pairs FL2_F and FL2_R). To select for recombinants, a crRNA targeting the junction between the flanking sequences was expressed from the same plasmid.

To create *S. thermophilus* strains, PCR products were generated with homology arms approximately 2 kilobases long that flank antibiotic resistant cassettes and transformed into the wildtype strains. For JAV27, CRISPR1 was eliminated by amplifying homology arms with AV664-AV665 and AV666-AV667. The spectinomycin resistance cassette was amplified from pLZ12spec[55] with AV672-AV673 and a three piece Gibson assembly was used to create the final product for transformation. Also in JAV27, CRISPR3 was eliminated by amplifying homology arms with AV668-AV669 and AV682-AV683. The chloramphenicol resistance cassette was amplified from pC194[48] with W1055-W1056 and a three piece Gibson assembly was used to create the final product for transformation. JAV27 was made by first knocking out CRISPR1 and then repeating the procedure for CRISPR3. For JAV28, CRISPR1 was tagged with erythromycin resistance by amplifying homology arms with AV667-AV692 and AV693-AV694. The erythromycin cassette was amplified from pE194[56] with AV177-AV695 and a three piece Gibson assembly was used to create the final product for transformation. To transform assembled DNA fragments into cells, an overnight culture was washed once in chemically-defined medium (CDM)[57], then diluted 1:100 in one milliter of CDM. Following 1.5 hours of incubation at 37°C, 10 µl of the Gibson product along with 1 µM $ComS_{17-24}$ peptide[58] (LPYFAGCL, Genescript) were added. Following a 4-hour incubation, cells were plated with the appropriate antibiotic and incubated for 36 hours.

Phage construction

To create phages to study transduction in *S. aureus* 08BA02176[28], phage 85[29] was used to infect this strain at a high MOI on soft-agar. 85α1 was isolated for its ability to form plaques on 08BA02176. To make 85α2, the 08BA02176 type III-A target was inserted site early-genome. 85α1 was passaged on soft-agar on a strain containing pAV247, a plasmid containing ~1 kilobase phage-homology arms where a small, unessential portion of the phage genome was replaced with the type III-A spacer 1 target. This phage stock was then passaged on soft-agar on a strain containing pGG12[52], a plasmid containing a CRISPR-Cas system that targets the portion of the phage replaced with the 08BA02176 spacer 1 target. Plaques were picked from this passage and re-passaged to single plaques on soft-agar a second time. Phages were then amplified and sequenced with oGG38-oGG40 to confirm target insertion. To make 85α3, the 08BA02176 type III-A target was inserted site mid-genome. 85α1 was passaged on soft-agar on a strain containing pAV282, a plasmid containing ~1 kilobase

phage-homology arms with an insertion into the phage genome with the type III-A spacer 1 target. This phage stock was then passaged on soft-agar on a strain containing pAV284, a plasmid containing a CRISPR-Cas system that targets the portion of the phage interrupted with the 08BA02176 spacer 1 target. Plaques were picked from this passage and re-passaged to single plaques on soft-agar a second time. Phages were then amplified and sequenced with AV876-AV877 to confirm target insertion.

Plasmid construction

All plasmids were constructed using electro-competent cells as described elsewhere[52]. The sequences of the oligonucleotides used in this study are listed in Table S2. To create recA allelic exchange vector pAV44, a three-piece Gibson assembly was performed using W1005-W1055 to amplify pWJ244[36],with AV206-AV208 and AV207-AV209 to amplify the homology arms from RN4220. AV149, pAV150, pAV153, pAV155, high- and low-transducing spacers targeting ΦNM4γ4, were assembled by using BsaI cloning described in detail elsewhere[59]. Primer pairs AV404-AV405, AV406-AV407, AV412-AV413, and AV416-AV417 were annealed and ligated into pDB114[59], to construct the respective plasmids. To make Φ12γ3[36] targeting plasmids, pAV293, pAV294, pAV295, and pAV296, BsaI cloning was used to insert JW600-JW601, JW604-JW605, JW620-JW621, and JW695-JW696 into pDB114 respectively. To make pAV158, pAV159, pAV162, pAV164, and pAV165, one piece Gibson assembly was performed using H235-H236 to remove cas9 from pAV149, pAV150, pAV153, pAV155, and pDB114, respectively. To transfer high and low-transducing spacers to a pE194[56] background, a two-piece Gibson assembly was used. AV176 and AV177 were used to amplify pE194 and AV423-AV424 were used to amplify the *tracrRNA*, *cas9*, and CRISPR array cassette. pAV149, pAV150, pAV153, and pAV155 were used as templates for pAV175, pAV173, pAV174, and pAV176, respectively. To make pAV185, the last 10-basepairs of H1 were complemented. BsaI cloning was used to insert annealed oligonucleotides AV485-AV486 into pDB114. pAV195 was made with a one-piece Gibson assembly, where pAV185 was amplified with H235-H236 to remove *cas9*. To create phage 85[29] editing plasmid pAV247 a three-piece Gibson was performed where pC194[48] was amplified with AV186-AV204, and phage homology arms were amplified AV607-AV611 and AV609-AV610. To create the construct to tag the type III-A locus with erythromycin a two-piece Gibson assembly was performed, where pTM402[60] was amplified with AV590-AV591 and the homology arm was amplified with AV622-AV623 from 08BA02176 and grown in strain TM17[60]. To create phage 85 editing plasmid pAV281 a three-piece Gibson was performed where pC194 was amplified with AV186-AV204, and phage homology arms were amplified AV862-AV864 and AV863-AV865. To add a spacer that targets phage 85 (5'-TTTCAACATTCTTCAACATACGCTGTCCTTGTGAGT-3') to 08BA02176, pAV282 was made with a 3-piece Gibson assembly, where pTM402 was amplified with AV590-AV591, and homology arms were amplified with AV879-AV880 and AV878-AV881. pAV282 was grown in TM17. To make phage 85 portal-targeting plasmid pAV284, BsaI cloning was used to insert AV866-AV867 into pDB114. To make *dcas9* contstructs pAV305, pAV306, pAV307, pAV308, and pAV309 gibson assembly was performed where B338-B339 were used to amplify *cas9* from pDB114 and B337-B340 were used to amplify the plasmid backbone and spacer from pAV149, pAV150, pAV153, pAV155, and pDB114, respectively.

Statistics and Reproducibility

All experiments were independently reproduced three times unless stated otherwise in the figure legend.

Data availability statement

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Code availability statement

All code used in this study is available upon request.

Competing Interests

L.A.M. is a cofounder and Scientific Advisory Board member of Intellia Therapeutics and a cofounder of Eligo Biosciences. R.B. is a cofounder and Scientific Advisory Board member of Intellia Therapeutics, a cofounder of Locus Biosciences, an advisor to Inari Ag, and a shareholder of DuPont and Caribou Biosciences. ERW and SM declare no conflict of interest.

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Figures

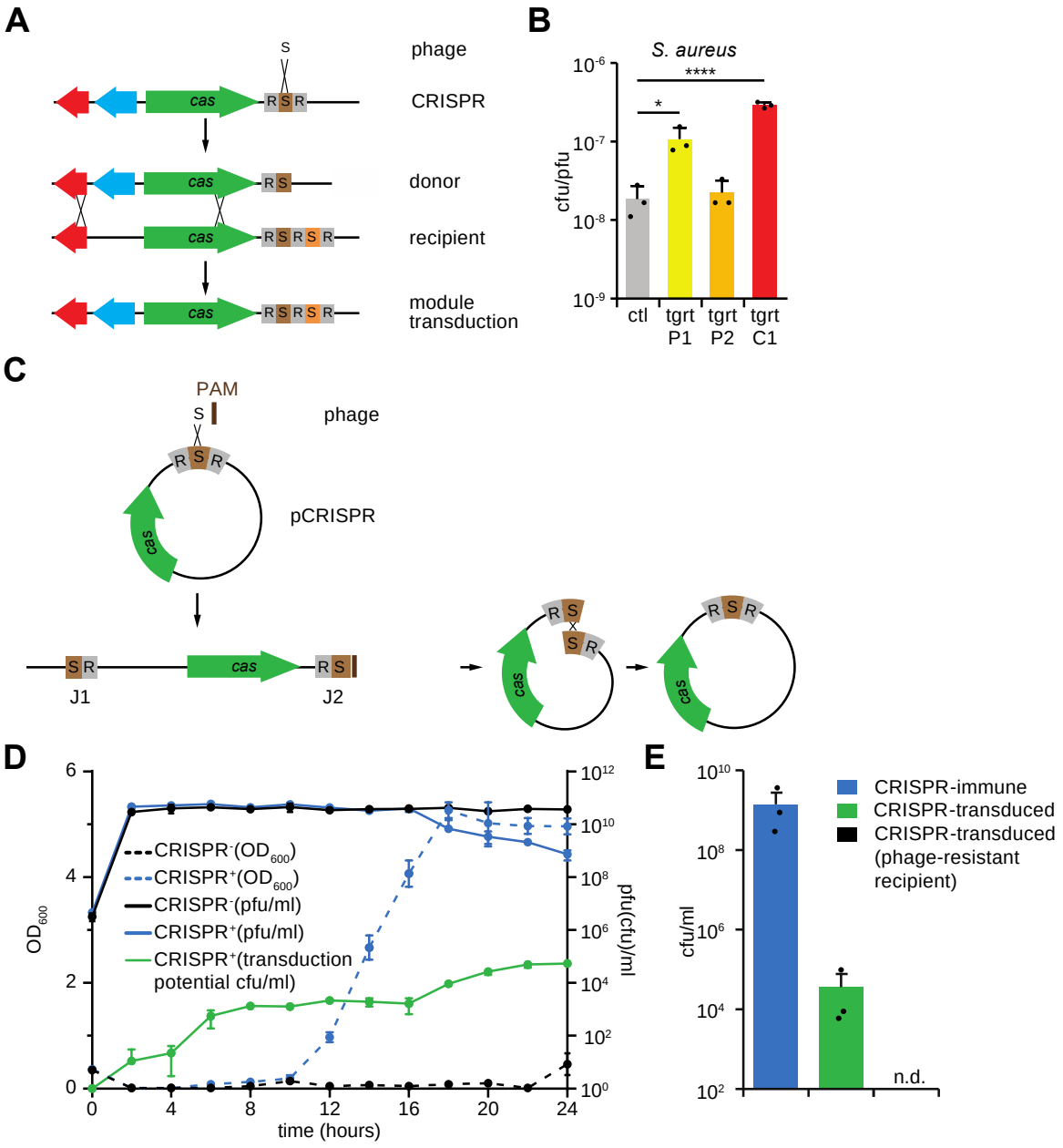
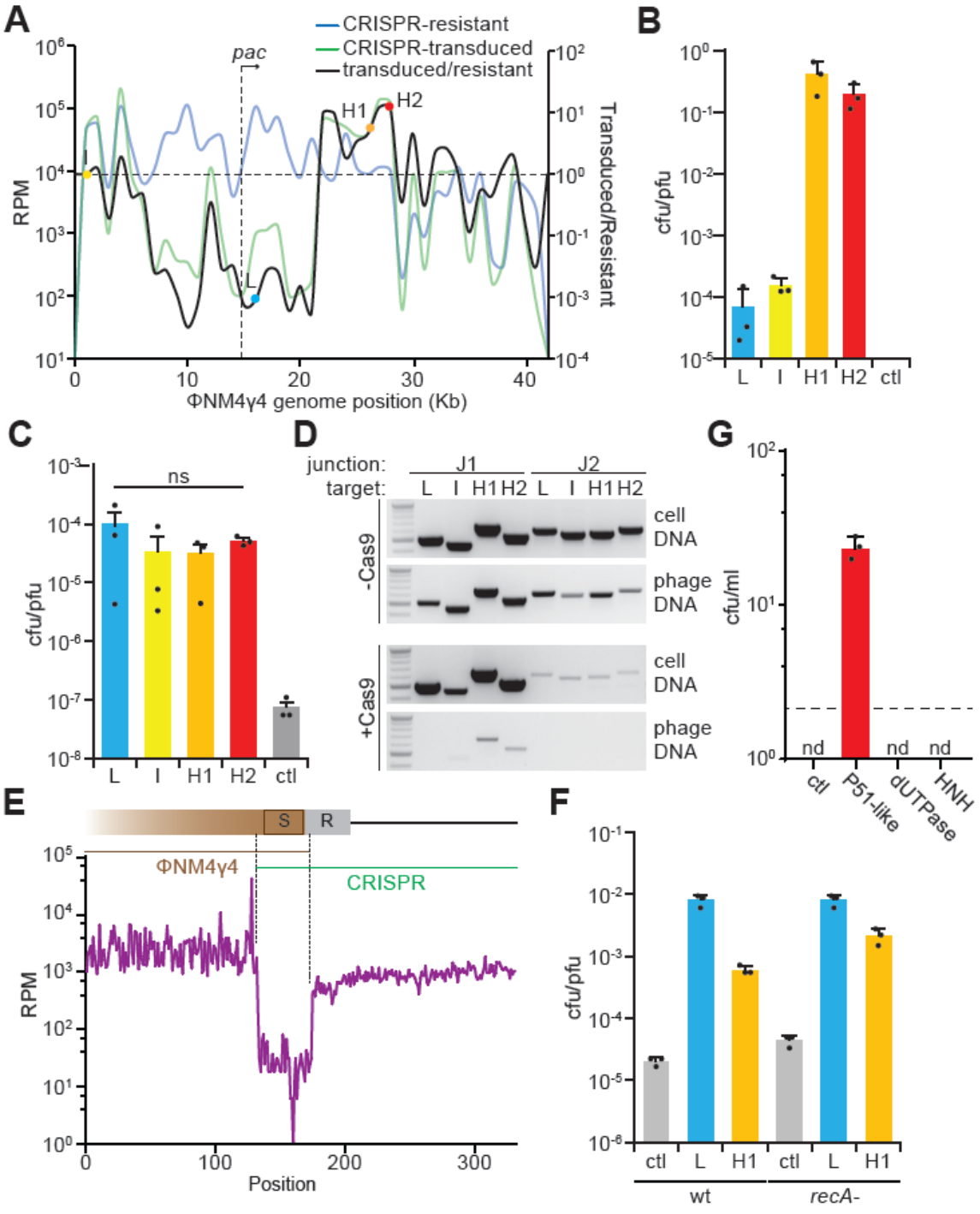


Fig. 1. Transfer of CRISPR elements through phage transduction. (**A**) Following phage recombination with a chromosomal CRISPR-Cas locus, adjacent loci can be preferentially transduced. “R” represents CRISPR repeats, “S” CRISPR spacers, brown the phage genome (**B**) Transducing particle production from *S. aureus* strain 08BA02176 tagged with an erythromycin resistance cassette. Liquid cultures were infected with 85α1 at a MOI (multiplicity of infection) of 50 for the control (ctl) or targeting strains (P1, P2, C1) and phages were collected 90 minutes post infection. Wild-type 08BA02176 was infected with transducing phages and a MOI of 1 for 20 minutes, then treating with sodium citrate. After 40 minutes cells were washed and plated on erythromycin-containing solid media. Mean + STD of 3 biological replicates are reported. (**C**) Phage recombination with a plasmid-borne CRISPR-Cas locus on

circular element. Following recombination between the spacer and the phage genome, the phage packaging machinery preferentially packages the phage-linked locus. Upon injection into the recipient cell the element re-circularizes. “R” represents CRISPR repeats, “S” CRISPR spacers, brown the phage genome. (**D**) Cell growth and titers of infected cultures containing plasmids with either the type II-A CRISPR system from *S. pyogenes* ($CRISPR^+$) or the empty vector control ($CRISPR^-$). Liquid cultures were infected at a multiplicity of infection (MOI) of 1 with ΦNM4γ4. The growth of cultures was determined by measurement of optical density at 600 nm (OD_{600}). Titers, plaque forming units/ml (pfu/ml), were determined by filtering supernatant and plaquing. Levels of transducing-immune phage particles, colony forming units/ml (cfu/ml), were determined by infecting a susceptible culture at a MOI of 1 and plating on soft agar lawns with antibiotic selection for recipient cells and the CRISPR plasmid. No transducing-immune particles were detected using a vector control. Time points were taken from 0-24 hours, every 2 hours. Mean ± STD of 3 biological replicates are reported. (**E**) Levels of transduction during adaption were determined by mixing cells at a 1:5 naïve CRISPR to $CRISPR^-$ cell ratio and infected at an MOI of 1 with ΦNM4γ4. Cultures were collected 20 hours post infection and assayed for the presence of the CRISPR-Cas locus by plating for the antibiotic resistance of the CRISPR plasmid. Levels of transduction were determined by plating for the antibiotic resistance encoded by the CRISPR-Cas plasmid and the chromosomal antibiotic resistance markers of the $CRISPR^-$ cells. As a control, cells resistant to phage infection independent of CRISPR, where mixed with naïve CRISPR cell under the conditions described above. Mean ± STD of 3 biological replicates are reported.



739

740 **Fig. 2. Spacers sequences determine frequency of CRISPR-Cas transduction.** (**A**)
741 Cultures containing the type II-A pCRISPR were infected with ΦNM4γ4 at a MOI of 1.
742 DNA was extracted 20 hours post infection and used as template for the amplification of
743 expanded CRISPR arrays. The PCR products were analyzed by next-generation
744 sequencing. Adapted spacers were normalized as reads per million and plotted against
745 the ΦNM4γ4 genome in 2000-base-pair bins (blue line, CRISPR-resistant). Phages
746 collected at 20 hours post infection were used to infect a culture lacking CRISPR-Cas
747 loci at an MOI of 1. Recovered cultures were collected at 20 hours post infection and

DNA was extracted. As described above, DNA was extracted for PCR and next-generation sequencing and plotted against the phage genome (green line, CRISPR-transduced). The ratio of transduced spacers over resistant spacers was also plotted (black line). Positions of low- (L), intermediate- (I), and highly-transduced spacers (H1, H2) used in subsequent experiments, along with phage *pac* site are indicated on graph. Mean of 4 replicates are reported. (**B**) Transducing-immune particles produced by cells expressing Cas9 and control (ctl), or targeting spacers (L, I H1, H2). Cultures were infected with ΦNM4γ4 at a MOI of 50 and phages were collected 90 minutes post infection. Levels of transducing-immune phage particles were determined by infecting a susceptible culture at a MOI of 1 and plating on soft agar lawns with antibiotic selection for recipient cells and the antibiotic resistance cassette on the pCRISPR plasmid. Mean + STD of 3 biological replicates are reported. (**C**) Transduction of plasmids containing only the CRISPR array with either a control spacer (ctl) or ΦNM4γ4-targeting (L, I, H1, H2) spacers (pSpacer). Cultures were infected with ΦNM4γ4 at a MOI of 1 and phage was collected 2 hours post infection. Levels of transduction were determined by infecting a susceptible culture at a MOI of 1 for 20 minutes, washing cells, and plating for the antibiotic resistance of the plasmid. Mean + STD of 3 biological replicates are reported. (**D**) PCR analysis of products produced from phage/CRISPR-Cas locus recombinants. Cells containing just the CRISPR array (-Cas9) or Cas9 and the CRISPR array (+Cas9) or were infected at a MOI of 1 and 50, respectively. 30 minutes post infection for –Cas9 strains or 60 minutes post infection for +Cas9 strains, genomic DNA was extracted from the cellular pellet (cell DNA). For all strains, 60 minutes post infection DNA was also extracted from filtered supernatant (phage DNA). Primers annealing to the CRISPR-Cas locus and the corresponding portion of the phage genome were used to amplify recombination products at the indicated junction (Figure 1C). (**E**) Deep sequencing of phage DNA harvested after infection of cells containing Cas9 and spacer H1. Cultures were infected with ΦNM4γ4 at a MOI of 50 and phages were collected 90 minutes post infection. Phage DNA was subject to deep sequencing and aligned to 300 base pairs of the phage CRISPR locus junction that does not contain the PAM. Each point on the graph represents the number of reads that originate at that nucleotide position and have full coverage to the right for the 75-nucleotide deep sequencing read. Dotted lines indicate portion of reads that map to the CRISPR locus and ΦNM4γ4. (**F**) Transduction of plasmids containing just the CRISPR array with either a control spacer (ctl) or ΦNM4γ4-targeting (L, H1) spacers (pSpacer) in a wildtype or RecA null (*recA*-) background. Cultures were infected with ΦNM4γ4 at a MOI of 1 and phage was collected 2 hours post infection. Levels of transduction were determined by infecting a susceptible culture at a MOI of 1 for 20 minutes, washing cells, and plating for the antibiotic resistance of the plasmid. Mean + STD of 3 biological replicates are reported. (**G**) Transducing-immune particles produced by cells expressing Cas9 and control spacers (ctl), or targeting- spacers (P51-like, duTPAse, HNH, hHydrolase H1, H2). Cultures were infected with Φ12γ3 at a MOI of 50 and phages were collected 90 minutes post infection. Levels of transducing-immune phage particles were determined by infecting a susceptible culture at a MOI of 1 and plating on soft agar lawns with antibiotic selection for recipient cells and the antibiotic resistance cassette on the CRISPR plasmid. Mean + STD of 3 biological replicates are reported. Limit of detection is 3.3 cfu/ml (dotted line).

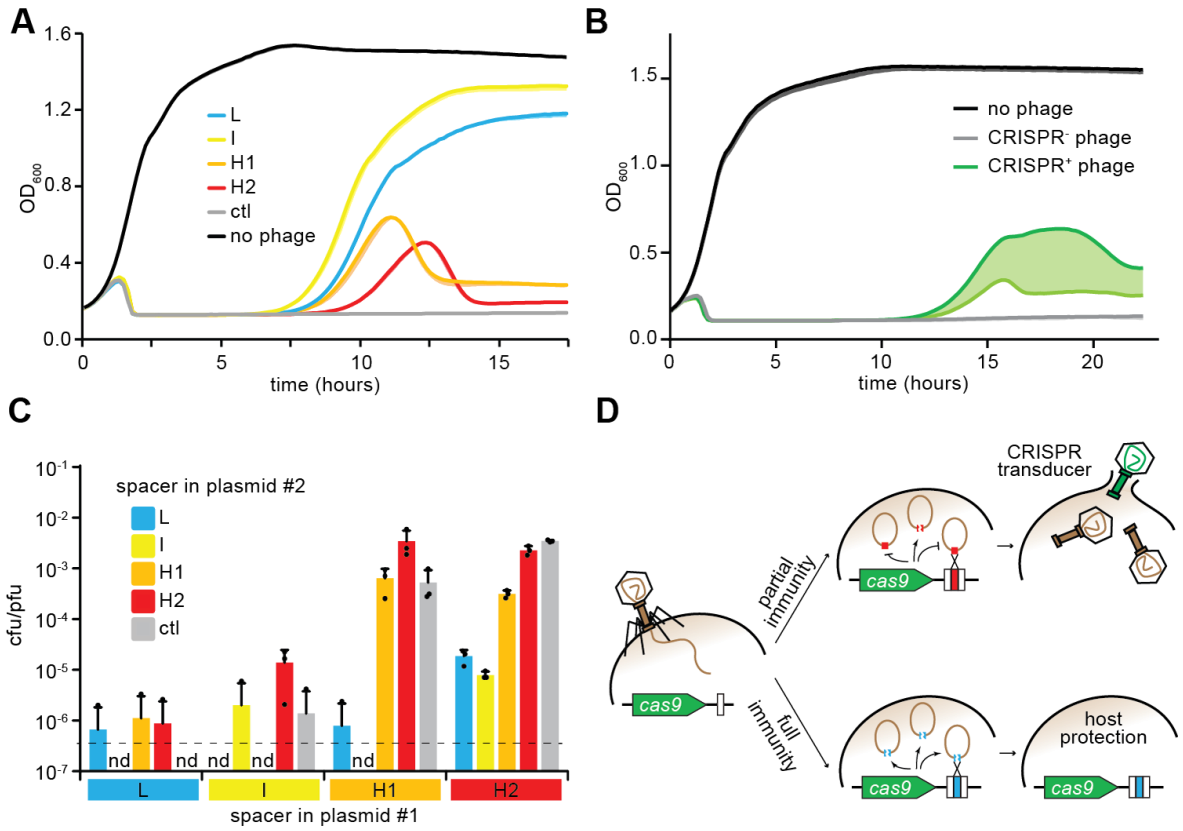


Fig. 3. Efficiency of CRISPR immunity inversely correlates with transduction rates. (**A**) Simulation of CRISPR adaptation in cells expressing spacers with high-, intermediate-, and low-transducing phenotypes (L, I, H1, H2) or a control spacer (ctl). Cells lacking CRISPR were used to dilute immune culture 1:10,000 and then infected at a final MOI of 1. These were compared to an uninfected control (no phage). Cell growth was monitored by optical density measurements at 600 nm (OD_{600}). Mean - STD of 3 biological replicates are reported. (**B**) Cells lacking CRISPR were infected at an MOI of 1 with phage collected from a culture containing an adapting CRISPR plasmid or a vector control at 22 hours post infection. Cell growth was monitored by optical density measurements at 600 nm (OD_{600}). Mean - STD of 5 biological replicates are reported. (**C**) Transducing particle production from spacers combined with a second plasmid containing the spacer indicated in figure legend/bar color. The transduction efficiency of the spacer on the x-axis was assayed for CRISPR function and antibiotic resistance. Cultures were infected with ΦNM4γ4 at a MOI of 50 and phages were collected 90 minutes post infection. Levels of transducing-immune phage particles were determined by infecting a susceptible culture at a MOI of 1 and plating on soft agar lawns with antibiotic selection for recipient cells and the antibiotic resistance cassette on the CRISPR plasmid. Limit of detection is 1.5 cfu/ml (dotted line). Mean + STD of 3 biological replicates are reported. (**D**) Spacers acquired during CRISPR adaptation can be divided in to 2 classes, spacers that provide full immunity with low levels of transduction or spacers that provide partial immunity but facilitate transduction. The acquisition of spacers provides sufficient homology to target recombination of the phage genome with the CRISPR-Cas locus. Spacers that provide partial immunity allow for occasional lysis and targeted packaging of recombination substrates.

Methods

Bacterial strains and growth conditions

Culture of *Staphylococcus aureus* RN4220[50] was carried out in brain-heart infusion (BHI) medium at 37°C with agitation at 220 revolutions per minute. Liquid experiments were carried out in 3 milliliters of medium in 15 ml conical tubes unless otherwise noted. *S. aureus* media was supplemented with 10 µg/ml chloramphenicol, 10 µg/ml erythromycin, or 25 µg/ml kanamycin for plasmid maintenance and/or chromosomal marker selection.

Culture of *Streptococcus thermophilus* was carried out in M17 media supplemented with 10% lactose at 37°C without agitation, unless otherwise noted. Liquid experiments were carried out in 5 milliliters of media in 15 ml conical tubes. M17 media was supplemented with 5 µg /ml chloramphenicol, 200 µg/ml spectinomycin, or 2.5 µg/ml erythromycin for chromosomal marker selection.

Culture of *Pseudomonas aeruginosa* was carried out in LB media at 37°C with agitation at 180 revolutions per minute. LB media was supplemented with 100 µg/ml streptomycin or 30 µg/ml gentamycin for chromosomal marker selection

All strains are listed in Table S1.

Quantification of CRISPR-Cas transducing particles

In *S. aureus,* overnight cultures of pWJ40[32] or pC194[51] were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4[32] at a multiplicity of infection (MOI) 1. Phage was collected at indicated time points and filtered with 0.45-µm syringe filters (Acrodisc). Harvested phage were then used to infect lawns of *S, aureus* strain OS2[52] suspended in 50% BHI supplemented with 5 mM $CaCl_2$ at an MOI of 1 on a BHI base supplemented with erythromycin and chloramphenicol to select for recipient cells and CRISPR transduction. For quantification of transducing particles produced from strains already containing CRISPR-immunity, overnight cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 or Φ12γ3[34] at a MOI of 50. 90 minutes post infection, phage were collected and filtered with 0.45-µm syringe filters (Acrodisc). Harvested phage were then used to infect lawns of OS2 suspended in 50% BHI supplemented with 5mM $CaCl_2$ at an MOI of 1 on a BHI base supplemented with erythromycin and chloramphenicol to select for recipient cells and CRISPR transduction. Phages that were not of sufficient titers to infect at an MOI of 1 were supplemented with ΦNM4γ4 prepared from RN4220.

Detection of spacer acquisition

To check for spacer acquisition in *S. aureus*, transduced colonies were resuspended in colony lysis buffer (250 mM KCl, 5 mM $MgCl_2$ 50 mM Tris-HCl at pH 9.0, 0.5% Triton X-100), treated with 200 ng/µl lysostaphin and incubated at 37°C for 20 minutes, then 98°C for 10 minutes. Samples were centrifuged and supernatant was used for PCR amplification with primers L400 and H50.

CRISPR adaptation and escaper phage generation

For *P. aeruginosa,* to monitor the effect of increased homology between the CRISPR system and the phage DMS3vir genome, we cultured PA14-Sm in the presence of DMS3vir and isolated a phage-resistant mutant that had acquired an additional spacer targeting the phage, following procedures previously described[28]. Next, we isolated DMS3vir 'escape' mutants by inoculating a 96 well plate with 200 µl of the CRISPR-resistant PA14-Sm strain and ~$6*10^7$ DMS3vir. After a 24-hour incubation at 37°C phages were isolated by chloroform extraction and spotted onto a lawn of the CRISPR-resistant PA14-Sm. Individual 'escape' phage clones were isolated, followed by sequencing of the amplicon containing the protospacer and PAM sequences. A single 'PAM-escape' mutant was used in the transduction assays (G>A, position 25926) along with the WT DMS3vir phage.

For *S. thermophilus,* we isolated bacterial colonies that had acquired spacers in the erythromycin-tagged CRISPR1 locus of JAV28 following infection by phage 2972 using procedures previously described[53]. Genomic DNA from strain JAV33 was amplified and sequenced with AV638-AV724 and found to have a spacer targeting the top strand beginning at position 26,553 of 2972. Phage 2972 was passaged on this strain for escapers on soft-agar. Single plaques were isolated and re-passaged to single plaques on JAV33. Phage DNA was extracted by boiling the phage and 2972α1 DNA was amplified and sequenced with AV868-AV869. 2972α1 contained a mutation in the PAM region (A>G, 26,588)

Quantification of transduction

For *S. aureus,* overnight donor cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with either ΦNM4γ4 at a MOI of 1 or 85α1-3 at an MOI of 50. Following lysis of the culture at 2 hours, phages were collected and filtered with a 0.45-µm syringe filters (Acrodisc). Overnight recipient cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected at an MOI of 1 with the transducing phage. 20 minutes post-infection, 40 mM of sodium citrate was added to the cultures. For erythromycin transduction, the cells were incubated for an additional 40 minutes then pelleted and washed twice with fresh BHI supplemented with 40 mM sodium citrate, while for chloramphenicol transduction cells were washed immediately. Cells were then plated on BHI plates supplemented with the antibiotics selecting for the recipient strain and transduction events and 20 mM sodium citrate.

For *P. aeruginosa,* bacterial lawns with near-confluent lysis were generated by mixing 200 µl of PA14-Sm on overnight cultures with 20µl of ~10^4 PFU DMS3vir and 10 mL soft LB agar. Phage only controls were included by applying the same protocol, but excluding the addition of bacteria. After 24-hour incubation at 37°C, phages were harvested by soaking the lawns in 3 mL of M9 salts buffer for 1 hour at room temperature followed by chloroform extraction and titration of the resulting phage stock. As recipients, we used *P. aeruginosa* PA14 ΔCRISPR-Cas[54] transformed with pHERD30T (conferring gentamycin resistance). 10 mL LB overnight culture supplemented with 30 µg mL^{-1} gentamycin of each recipient was spun down (3000 rpm, 10 min) and re-suspended in 1 mL of LB. 100 µl of lysate was then added and statically

incubated for 25 minutes. Each culture was then spun down and the whole culture was plated on LB agar supplemented with 100 μg mL^{-1} streptomycin and 30 μg mL^{-1} gentamycin (to prevent carry over of PA14-Sm cells). To estimate transduction frequency, 48 colonies were picked per replicate experiment, and screened by PCR using primers specific for the CRISPR 2 locus primers CR2_F-CR2_R.

For *S. thermophilus,* transducing phage stocks were made by infecting mid-log growth JAV33 at 42°C supplemented with 10 mM $CaCl_2$ with either 2972 or 2972α1 at an MOI of 1. Phage stocks were harvested and filtered using 0.45-μm syringe filters (Acrodisc) after the culture had cleared. JAV27 were used as recipient cells and were grown to at 42°C supplemented with 10 mM $CaCl_2$ and infected at an MOI of 0.5 when cultures reached OD_{600} = 0.4. 10 minutes following infection, 20 mM sodium citrate was added to the cultures. After 1 hour incubation at 42°C, the cultures were washed two times in M17 media supplemented with 20 mM sodium citrate and then plated on erythromycin M17 plates. Transductants were confirmed by streaking out colonies on M17 chloramphenicol plates to confirm antibiotic resistance engineered into the CRISPR3 locus.

Detection of phage-CRISPR junctions

Overnight cultures were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 at a MOI of 50 for targeting strains or 1 for non-targeting strains. Phages were collected from indicated strains 60 minutes post-infection. Supernatants were filtered using a 0.45-μm filter and then concentrated with Ultra-4 100k centrifugal 50-ml spin columns (Amicon). Concentrates were resuspended with DNase I buffer, 20 mM Tris-HCl pH 8.0 and 2 mM $MgCl_2$ and reconcentrated two times. The suspension was then treated with 25 units of DNase I (Sigma) for one hour. Following DNAse I treatment, the enzyme was inactivated by heating at 70°C for 10 minutes and the addition of 5 mM EDTA. Phages were then incubated with 8 units of proteinase K (NEB) and 0.5% SDS at 37°C for one hour. Phage DNA was isolated using a phenol/chloroform/isoamyl alcohol extraction (Fisher). Cellular DNA was collected 15 minutes and 60 minutes post infection for non-targeting strains and targeting strains respectively. Approximately 10^9 cells were pelleted and resuspended in 100 μl of 50 mM EDTA and 1 mg/ml lysostaphin (AMBI Products) and incubated at 37°C for one hour. DNA was then extracted with the Wizard genomic purification kit (Promega) according to the manufacturer's instructions. For the non-PAM junction, primer JM117 was used with NP255, AV547, AV469, AV471 for L, I, H1, and H2, respectively. For the PAM junction, primer L400 was used with AV457, AV458, AV456, AV459 for L, I, H1, and H2, respectively. For loading controls oGG38-oGG40 were used to amplify *gp14* and JW96-W964 for *recA.*

High-throughput sequencing

Overnight cultures of pWJ40 were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 at a MOI of 1. 20 hours post-infection DNA was collected from recovered cells (CRISPR-resistant). Phages were also collected and filtered with 0.45-μm syringe filters (Acrodisc). Overnight cultures of OS2 were diluted 1:100 in fresh BHI with appropriate antibiotics and 5 mM $CaCl_2$. At OD_{600} = 0.4, cultures were infected with ΦNM4γ4 collected from

the pWJ40 culture at a MOI of 1. 20 hours post-infection DNA was collected from recovered cells (CRISPR-transduced). Spacers were amplified with RH50 and JW655-JW662 for sample barcoding. The sequences of the oligonucleotides used in this study are listed in Table S2. Adapted bands were gel-extracted and subjected to Illumina MiSeq sequencing. Data analysis was performed in Python. Spacer reads were extracted from the raw MiSeq FASTA files and aligned to the phage genome. Number of reads and PAM were designated for each spacer.

To deep sequence phage-CRISPR DNA junctions, phage DNA was isolated as described above. DNA was then prepped with the Illumina TruSeq Nano kit according to the manufacturer's instructions. Prepped DNA was then subject to NextSeq sequencing. BWA-MEM (arXiv:1303.3997v1) was used to align sequenced DNA to the PAM junction, which contains 200 base pairs of the upstream CRISPR sequence (leader and direct repeat) and 205 base pairs of the downstream phage sequence (Spacer, PAM, and phage genome) or the repeat junction, which contains 205 base pairs of the upstream phage sequence (phage genome and spacer, and 200 base pair downstream CRISPR sequence (direct repeat and downstream plasmid sequence). A python script was then used to sort and bin reads spanned the full 75-nucleotide read length allowing for one mismatch.

Phage titer assay

Phage titer assays were performed as previously described[55].

Efficiency of plaquing assays

Efficiency of plaquing assays were performed as previously described[55].

Simulation of CRISPR immunization

Simulation of CRISPR immunization was performed as previously described[35].

Strain construction

To make the *recA* knockout JAV9, the allelic replacement system developed by Wenyan Jiang using pWJ244 was applied as previously described[34]. Briefly, pAV44 was transformed into RN4220 and integrants were isolated. Double crossover events were selected for by a temperature sensitive *cat* targeting Cas9 phagemid, pWJ326. RecA deletion was confirmed by primers outside the homology arms, AV223 and AV224. To make JAV21, OS2 was infected with ΦNM1γ6[55] at an MOI of 1 to produce transducing particles carrying the genomic erythromycin cassette. These particles were used to infect JW263[34] as described in quantification of transduction. Colonies that were resistant to kanamycin and erythromycin were struck out 2 times on plates supplemented with 20mM sodium citrate, kanamycin, and erythromycin. JAV29 and JAV32 were constructed by transforming suicide vectors pAV253 and pAV282. Integration was confirmed using primers AV594 and AV812 for pAV253 and AV648 and AV525. JAV33 was made by infecting RN4220 at MOI of 1 in soft agar with ΦNM4γ4. After a 24 hour incubation, a resistant colony was picked, restreaked two times, and confirmed to be insensitive to ΦNM4γ4 infection.

To create a P. aeruginosa PA14 strain carrying a streptomycin resistance cassette immediately adjacent to the Type I-F CRISPR-Cas system in the genome (PA14-Sm, with the Sm gene inserted at position 2937360), we used homologous recombination. The streptomycin (Sm) resistance gene and its promoter were PCR amplified from pBAM1-Sm[56] using primers pB_Sm_F and pB_Sm_R, and inserted into the NheI restriction site of pHERD30T, flanked by amplicons FL1 (flank1, generated using primer pairs FL1_F and FL1_R) and FL2 (flank2, generated using primer pairs FL2_F and FL2_R). To select for recombinants, a crRNA targeting the junction between the flanking sequences was expressed from the same plasmid.

To create *S. thermophilus* strains, PCR products were generated with homology arms approximately 2 kilobases long that flank antibiotic resistant cassettes and transformed into the wildtype strains. For JAV27, CRISPR1 was eliminated by amplifying homology arms with AV664-AV665 and AV666-AV667. The spectinomycin resistance cassette was amplified from pLZ12spec[57] with AV672-AV673 and a three piece Gibson assembly was used to create the final product for transformation. Also in JAV27, CRISPR3 was eliminated by amplifying homology arms with AV668-AV669 and AV682-AV683. The chloramphenicol resistance cassette was amplified from pC194[51] with W1055-W1056 and a three piece Gibson assembly was used to create the final product for transformation. JAV27 was made by first knocking out CRISPR1 and then repeating the procedure for CRISPR3. For JAV28, CRISPR1 was tagged with erythromycin resistance by amplifying homology arms with AV667-AV692 and AV693-AV694. The erythromycin cassette was amplified from pE194[58] with AV177-AV695 and a three piece Gibson assembly was used to create the final product for transformation. To transform assembled DNA fragments into cells, an overnight culture was washed once in chemically-defined medium (CDM)[59], then diluted 1:100 in one milliter of CDM. Following 1.5 hours of incubation at 37°C, 10 µl of the Gibson product along with 1 µM $ComS_{17-24}$ peptide[60] (LPYFAGCL, Genescript) were added. Following a 4 hour incubation, cells were plated with the appropriate antibiotic and incubated for 36 hours.

Phage construction

To create phages to study transduction in CRISPR type III-A containing strain 08BA02176[29], phage 85[30] was used to infect this strain at a high MOI on soft-agar. 85α1 was isolated for its ability to form plaques on 08BA02176. To make 85α2, the 08BA02176 type III-A target was inserted site early-genome. 85α1 was passaged on soft-agar on a strain containing pAV247, a plasmid containing ~1 kilobase phage-homology arms where a small, unessential portion of the phage genome was replaced with the type III-A spacer 1 target. This phage stock was then passaged on soft-agar on a strain containing pGG12[55], a plasmid containing a CRISPR system that targets the portion of the phage replaced with the 08BA02176 spacer 1 target. Plaques were picked from this passage and re-passaged to single plaques on soft-agar a second time. Phages were then amplified and sequenced with oGG38-oGG40 to confirm target insertion. To make 85α3, the 08BA02176 type III-A target was inserted site mid-genome. 85α1 was passaged on soft-agar on a strain containing pAV282, a plasmid containing ~1 kilobase phage-homology arms with an insertion into the phage genome with the type III-A spacer 1 target. This phage stock was then passaged on soft-agar on a strain containing pAV284, a plasmid containing a CRISPR system that targets the

portion of the phage interrupted with the 08BA02176 spacer 1 target. Plaques were picked from this passage and re-passaged to single plaques on soft-agar a second time. Phages were then amplified and sequenced with AV876-AV877 to confirm target insertion.

Plasmid construction

All plasmids were constructed using electro-competent cells as described elsewhere[55]. The sequences of the oligonucleotides used in this study are listed in Table S2. To create recA allelic exchange vector pAV44, a three-piece Gibson assembly was performed using W1005-W1055 to amplify pWJ244[34],with AV206-AV208 and AV207-AV209 to amplify the homology arms from RN4220. AV149, pAV150, pAV153, pAV155, high- and low-transducing spacers targeting ΦNM4γ4, were assembled by using BsaI cloning described in detail elsewhere[61]. Primer pairs AV404-AV405, AV406-AV407, AV412-AV413, and AV416-AV417 were annealed and ligated into pDB114[61], to construct the respective plasmids. To make Φ12γ3[34] targeting plasmids, pAV293, pAV294, pAV295, and pAV296, BsaI cloning was used to insert JW600-JW601, JW604-JW605, JW620-JW621, and JW695-JW696 into pDB114 respectively. To make pAV158, pAV159, pAV162, pAV164, and pAV165, one piece Gibson assembly was performed using H235-H236 to remove cas9 from pAV149, pAV150, pAV153, pAV155, and pDB114, respectively. To transfer high and low-transducing spacers to a pE194[58] background, a two-piece Gibson assembly was used. AV176 and AV177 were used to amplify pE194 and AV423-AV424 were used to amplify the *tracrRNA*, *cas9*, and CRISPR array cassette. pAV149, pAV150, pAV153, and pAV155 were used as templates for pAV175, pAV173, pAV174, and pAV176, respectively. To make pAV185, the last 10-basepairs of H1 were complemented. BsaI cloning was used to insert annealed oligonucleotides AV485-AV486 into pDB114. pAV195 was made with a one-piece Gibson assembly, where pAV185 was amplified with H235-H236 to remove *cas9*. To create phage 85[30] editing plasmid pAV247 a three-piece Gibson was performed where pC194[51] was amplified with AV186-AV204, and phage homology arms were amplified AV607-AV611 and AV609-AV610. To create the construct to tag the type III-A locus with erythromycin a two-piece Gibson assembly was performed, where pTM402[62] was amplified with AV590-AV591 and the homology arm was amplified with AV622-AV623 from 08BA02176 and grown in strain TM17[62]. To create phage 85 editing plasmid pAV281 a three-piece Gibson was performed where pC194 was amplified with AV186-AV204, and phage homology arms were amplified AV862-AV864 and AV863-AV865. To add a spacer that target phage 85 (5'-TTTCAACATTCTTCAACATACGCTGTCCTTGTGAGT-3') to 08BA02176, pAV282 was made with a 3-piece Gibson assembly, where pTM402 was amplified with AV590-AV591, and homology arms were amplified with AV879-AV880 and AV878-AV881. pAV282 was grown in TM17. To make phage 85 portal-targeting plasmid pAV284, BsaI cloning was used to insert AV866-AV867 into pDB114.

Table S1. Strains used in this study.

Strains	Description	Reference
RN4220	*S. aureus* strain	[50]
OS2	Chromosomal erythromycin resistance RN4220	[52]
08BA02176	*S. aureus* MRSA with CRISPR type III-A	[29]
DGCC7710	*S. thermophilus* strain	[1]
PA14	*P. aeruginosa* strain	[63]
PA14--SM	*P. aeruginosa* strain streptomycin tagged type I-F	This study
JW263	Chromosomal kanamycin resistance RN4220	[34]
JAV9	*reca*- RN4220	This study
JAV21	Chromosomal erythromycin and kanamycin RN4220	This study
JAV27	CRISPR1 and CRISPR3 knockout DGC7710	This study
JAV28	Erythromycin CRISPR1 tag DGC7710	This study
JAV33	Erythromycin CRISPR1 tag BIM DGC7710	This study
JAV29	Erythromycin type III-A tag 08BA02176	This study
JAV32	Erythromycin type III-A tag and 85 spacer 08BA02176	This study
JAV33	RN4220 ΦNM4γ4 insensitive mutant	This study
pWJ40	*S. pyogenes* type II-A CRISPR system on pC194	[32]
pWJ244	*E. coli* ColE1 vector for genome engineering	[34]
pWJ326	*S. aureus* temperature-sensitive phagemid	[34]
pC194	Chloramphenicol-resistant *S. aureus* plasmid	[51]
pE194	Erythromycin-resistant *S. aureus* plasmid	[58]
pT181	Tetracycline-resistant *S. aureus* plasmid	[64]
pLZ12spec	Spectinomycin resistant cloning vector	[57]
TM17	Chromosomal expression of pT181 *repC*	[62]
pTM402	pT181 *ds repC- cop 623* replication origin	[62]
pBAM1-Sm	Streptomycin resistance cassette	[56]
pHERD30T	*E. coli/P. aeruginosa* shuttle vector	[65]
pGG12	Type III-A DUF1318 targeting plasmid on pC194	[55]
pDB114	Control spacer with *cas9*	[61]
pAV44	recA deletion allelic exchange vector on ColE1 vector	This study
pAV149	High-transducing spacer 1 (H1) with *cas9* on pC194	This study
pAV150	Low-transducing spacer 2 (L) with *cas9* on pC194	This study
pAV153	Intermediate-transducing spacer (I) with *cas9* on pC194	This study
pAV155	High-transducing spacer 2 (H2) with *cas9* on pC194	This study
pAV158	H1, no *cas9* on pC194	This study
pAV159	L, no *cas9* on pC194	This study
pAV162	I, no *cas9* on pC194	This study
pAV164	H2, no *cas9* on pC194	This study
pAV165	Control spacer with no cas9 on pE194	This study
pAV173	L with *cas9* on pE194	This study
pAV174	I with *cas9* on pE194	This study
pAV175	H1 with *cas9* on pE194	This study
pAV176	H2 with *cas9* on pE194	This study
pAV185	H1 Truncation with *cas9* on pC194	This study
pAV195	H1 Truncation with no *cas9* on pC194	This study
pAV247	85α2 editing to add 08BA02176 spacer 1 target site on pC194	This study
pAV253	Erythromycin type III-A tag on pTM401	This study
pAV281	85α3 editing to add 08BA02176 spacer 1 target site on pC194	This study
pAV282	Erythromycin type III-A and 85 spacer on pTM401	This study
pAV284	85α3 portal protein targeting spacer on pDB114	This study
pAV293	P51-like targeting Φ12γ3 spacer on pDB114	This study
pAV294	dUTPase targeting Φ12γ3 spacer on pDB114	This study
pAV295	HNH targeting Φ12γ3 spacer on pDB114	This study
pAV296	Hydrolase targeting Φ12γ3 spacer on pDB114	This study
ΦNM4γ4	Virulent *S. aureus pac* phage	[32]
Φ12γ3	Virulent *S. aureus cos* phage	[34]
85	*S. aureus* phage	[30]
85α1	*S. aureus* phage variant infects 08BA02176	This study
85α2	85α1 targeted early-genome by spacer 1 08BA02176	This study
85α4	85α1 targeted mid-genome by spacer 1 08BA02176	This study
2972	Virulent *S. thermophilus* phage	[66]
2972α1	2972 JAV33 PAM escaper phage	This study

DMS3vir	Virulent *P. aeruginosa* mu-like phage	[54]	

Table S2. DNA oligonucleotides used in this study.

Primer Name	Sequence (5'-3')
AV122	GCTTTTTCTAAATGTTTTTTAAGTAAATCAAGTAC
AV176	GAGTGATCGTTAAATTTATACTGCAATCGG
AV177	CATGTTCATATTTATCAGAGCTCGTGC
AV186	AATCGATAACCACATAACAGTCATAAAAC
AV204	ATAGGTATGTGGTTTTGTATTGGAAT
AV206	TAATGACTTTGGTGCATCTAAAGCTTTTTGACGATCGTTATC
AV207	CAAAAAGCTTTAGATGCACCAAAGTCATTATTTGACGAA
AV208	ATATTTTAAAAATATCCCACGTGGCCCAGATTGTTGGTAAAG
AV209	CGAGGCCCTTTCGTCTTCACGATGTTTCGTCCTTCTCGTCC
AV223	CGCTAATACCAACGGACAATTTC
AV224	CCAGCTCGTTTCGCTAATGTC
AV404[a]	aaacGTTAGCAGTATTTGGAGCACTGTTACAAGTG
AV405[a]	aaaacACTTGTAACAGTGCTCCAAATACTGCTAAC
AV406[a]	aaacTCTATGTCTTCTAAATTCAGTGATGTATTCG
AV407[a]	aaaacGAATACATCACTGAATTTAGAAGACATAGA
AV412[a]	aaacATATTCATCAGATTCCAATACTACGTTAATG
AV413[a]	aaaacATTAACGTAGTATTGGAATCTGATGAATAT
AV416[a]	aaacTAAGTAAAAAGCTAAATGAAGATAGTTCTTG
AV417[a]	aaaacAAGAACTATCTTCATTTAGCTTTTTACTTA
AV423	TGATAAATATGAACATGGGATTCTGTGATTTGGATCCTTCC
AV424	AAATTTAACGATCACTCCCCACTTTATCCAATTTTCGTTTG
AV437	TAATACGACTCACTATAGGGTTTGGAGCACTGTTACAAGTGTTTTAGAGCTATGCTGTT
AV438	AACAGCATAGCTCTAAAACACTTGTAACAGTGCTCCAAACCCTATAGTGAGTCGTATTA
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AV440	AACAGCATAGCTCTAAAACGAATACATCACTGAATTTAGCCCTATAGTGAGTCGTATTA
AV456	CAACCGTACTTGTAAGTACACTTG
AV457	CATCGCTAGTCATGTGTCTGTC
AV458	GGTGTTTGACGAGATTAAGTCACG
AV459	GTCTGTTGTCCATCAAAATCACC
AV469	GCAGTAGTTGCAGTCATTGGTG
AV471	CTGATAGATTGCCTACAAACGAAGG
AV485[a]	aaacCAATCGTCATTTTGGAGCACTGTTACAAGTG
AV486[a]	aaaacACTTGTAACAGTGCTCCAAAATGACGATTG
AV523	CCCTTTAGTAACGTGTAACTTTCC
AV525	CTACATTACGCATTTGGAATACCAAC
AV547	CTCGACGACCAAGATGTTGAGG
AV590	TGTACTTTTTACAGTCGGGAATGGCATGCCGAATTGGG
AV591	GACTGTATACCTTCCGAGCCGCGGTTAGAAAGGGCTTGA
AV594	GTCTAGAGACCGGGGACTTATC
AV607	ATACAAAACCACATACCTATGGGATAGGTATTGCAAGAGCGTTG
AV609	CTGTTATGTGGTTATCGATTCTCCTCTAGCTGTTCTAGTTAGCC
AV610	GGTATCGGAATTAATGAACTTATAGACAGTTGTTGAGGCAGAGAGTAAAG
AV611	CATTAATTCCGATACCTAGATTATCTCGTCCTGTTGAATCTTTGAATGTTG
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AV623	GACTGTAAAAAGTACAGCTAAAATGCGCGTAGCTG
AV638	TGCTGAGACAACCTAGTCTCTC
AV648	TCAATCGATACATCACGAGAGGC
AV664	CTTGGGCAGAAAACCTTGTAGATG
AV665	AGTCACGTTACGTTATGAACTTGGCTTTTTAAAATACACG
AV666	ACCCTTGGACTTTCGTCACTACTAACTTGTTGGCAAGG
AV667	CAAGGGCGATGACCTTCAAGG
AV668	CTCTTAATTCATCAGGTGACCCTG
AV669	TTTTAAAAATATCCCACCTCTACTATTTTCCCACCTCATCC
AV673	GACGAAAGTCCAAGGGTTTATTG
AV672	ATAACGTAACGTGACTGGCAAGA
AV682	ACTTTTTACAGTCGGTTGTTATCACAATTTTCGGTTGACATC
AV683	CTCATAGGTGTCATCCCATTTTCC
AV692	TGATAAATATGAACATGCTAACTTGTTGGCAAGGAAATCGG
AV693	ATGCATAAACTGCATCCTAGTTTAAAATCATTTGTTCAAAAATAAAATCC
AV694	GCCTGATAAGGTGTTCGTTGTC

AV695	GGATGCAGTTTATGCATCCCTTAAC
AV724	GAATCTTGATTTGCTGTCAAACAG
AV812	GGTGGAGATTTCTACTTACGTGGC
AV862	CATTAATTCCGATACCTAGATTATCTGGCAATTACAATCATTCCTTTTTATCAAC
AV863	GGTATCGGAATTAATGAACTTATAGATAACAAAAACACTCAAGAATATTGGGAAG
AV864	ATACAAAACCACATACCTATGAAGCTCACACCACGTGAAAAC
AV865	CTGTTATGTGGTTATCGATTCTTCCGGTGAAATTTCTTTCATGC
AV866[a]	aaacTTCTTCCCAATATTCTTGAGTGTTTTTGTTg
AV867[a]	aaaacAACAAAAACACTCAAGAATATTGGGAAGAA
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AV869	CTACGGATTGAAGAACGGTTTAGC
AV876	CCAAGACCCTGAATTGGAAGTC
AV877	GCATTGATTTCTTTTTCAATGCGC
AV878	GCTGTCCTTGTGAGTGATCGATAACTACCCCGAATAACAGGGGACGAGAATTCTATA
AV879	CACTCACAAGGACAGCGTATGTTGAAGAATGTTGAAAATTCTCGTCCCCTGTTATTC
AV880	GCTCGGAAGGTATACAGTCGAATAATGGCTCTATTACAACGGTAC
AV881	CACTCTCGGACAATACTCCATCCCCTAAAAATTAATCAATGCG
H235	GATATCGGCACAAATAGCTTAGATGCCACTCTTATCCATCAATCC
H236	AAGAGTGGCATCTAAGCTATTTGTGCCGATATCTAAGCC
H50	AAAACAAAAAGCGCAAGAAGAAATCAACCAGCGCA
oGG38	AAGATAAAGAATTTGCTCAAGACG
oGG40	ACCATTAAAACTCGTCATTCTTTC
oGG50	GTTAATGTTACGAATGATGAACC
oGG96	AAGATGCAACAATGGGAACCAAG
JM117	GTTTGAACTCAACAAGTCTCAGTGTGCTG
JW96	AAAACAAAGCTGAAATTGAAGGAGAAATGGGAGAC
JW600[a]	aaacCAAAAGCAGTCCGAGACAGGTTAGTTGAAGg
JW601[a]	aaaacCTTCAACTAACCTGTCTCGGACTGCTTTTG
JW604[a]	aaacCGGAGTGTAAAGACATCTTAGATCGAGTTAg
JW605[a]	aaaactAACTCGATCTAAGATGTCTTTACACTCCG
JW620[a]	aaactGGAAGAAGTTAAGAGAGATAGCATTAGATg
JW621[a]	aaaacATCTAATGCTATCTCTCTTAACTTCTTCCA
JW655	ACACTAAGCAGTGCGATTACAAAATTTTTTAGAC
JW656	GTACGTCGCTAGTGCGATTACAAAATTTTTTAGAC
JW657	AGAAGTGCGATTACAAAATTTTTTAGAC
JW658	CTACAGTGCGATTACAAAATTTTTTAGAC
JW659	TGAAGAGTGCGATTACAAAATTTTTTAGAC
JW660	CGCATTAGTGCGATTACAAAATTTTTTAGAC
JW661	TACACGGAGTGCGATTACAAAATTTTTTAGAC
JW662	CATAAGTAAGTGCGATTACAAAATTTTTTAGAC
JW695[a]	aaacGAGCGCTAATCTAAACACTTTCACATCGTTg
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L400	CGAAATTTTTTAGACAAAAATAGTC
W964	TCGTCAAATAATGACTTTGGTGC
W1005	GTGAAGACGAAAGGGCCTCGTG
W1055	GTGGGATATTTTTAAAATATATATTTATG
W1055	GTGGGATATTTTTAAAATATATATTTATG
W1056	AACCGACTGTAAAAAGTACAGTCG
NP255	AAAACCTTTTTCTTCAATTGGTCGACGTTTGAATAT
pB_Sm_F	CCGGCCGGATCCTGGGGTACAGTCTATGCCTCGG
pB_Sm_R	CCGGCCGGATCCGACAATTGTCTTATTTGCCGAC
FL1_F	ACCAGATCCTGCCGCAGTACTGG
FL1_R	CCGCCAATTGCCCGAAGCTTCCG
FL2_F	GCCGTTCGGGCGCGAAAGGTCT
FL2_R	ACCCCGAGGTAACAGAATCGTC
crRNA _F	GTTTCGCGAGGACCGGGACGGCGATCAACCGT
crRNA_R	ACGGTTGATCGCCGTCCCGGTCCTCGCGAAAC
CR2_F	GCTCGACTACTACAACGTCCGGC
CR2_R	GGGTTTCTGGCGGGAAAAACTCG

[a]lower case sequences are compatible with the overhangs of BsaI cleavage of the CRISPR repeat, and are required for spacer cloning.

Supplementary Figures

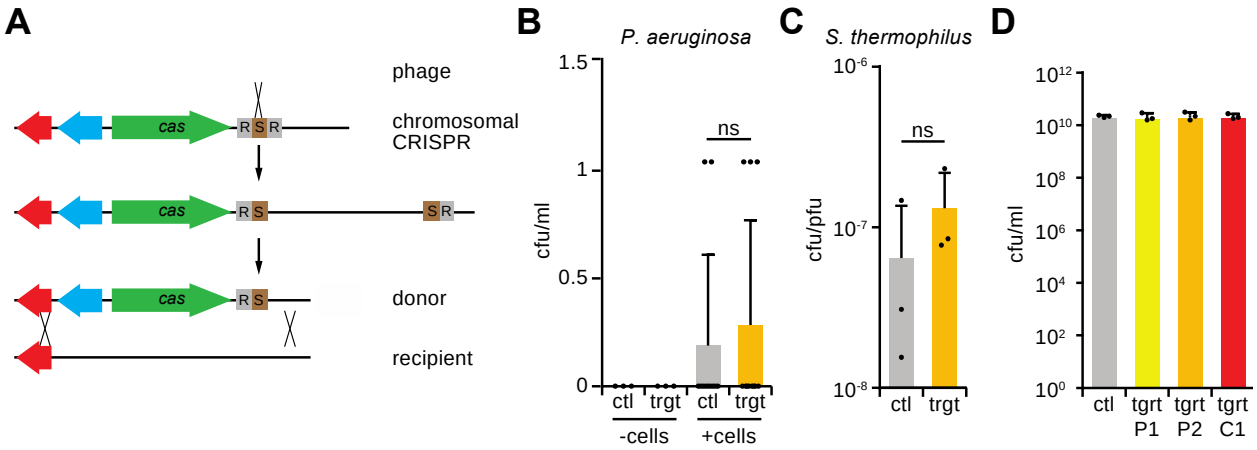

Fig. S1. Spacer-mediated transduction of chromosomal loci. (**A**) Phage recombination with a CRISPR-Cas locus on a linear element. Following recombination between the spacer and the phage genome, phage packaging machinery preferentially packages the phage-linked locus. In the case of CRISPR locus transduction, upon injection into the recipient cell the element only has homology for a single crossover event (solid lines), while lacking homology for the second crossover (dotted lines). (**B**) Transducing particle production from *P. aeruginosa*. Overnight cultures (+cells) containing the wild type array (crt) or a phage-targeting spacer (trgt) were infected on soft agar plates with a non-targeted, PAM-escaper phage. Plates without cells were used as a control (-cells). 24 hours post-infection phage was harvested and transducing phage particles were determined by infecting a CRISPR- culture and plating on soft agar lawns with antibiotic selection for recipient cells. CRISPR transduction was confirmed by PCR. Mean + STD of 3 (-cell) or 8 biological replicates (+cells) are reported. (**C**) Transducing particle production from *S. thermophilus*. Liquid cultures containing the wild type array (crt) or a phage-targeting spacer (trgt) were infected with a non-targeted phage that escaped CRISPR1 immunity through a PAM mutation. Three hours post-infection phage was harvested and transducing phage particles were determined by infecting a CRISPR- culture at an MOI of 1 and plating for antibiotic resistance. CRISPR transduction was confirmed by PCR. Mean + STD of 3 biological replicates are reported. (**D**) Transduction recipient cells from Fig. 1B were taken following phage infection and subsequent incubation and plated to count colonies to determine cell viability. Mean + STD of 3 biological replicates are reported.

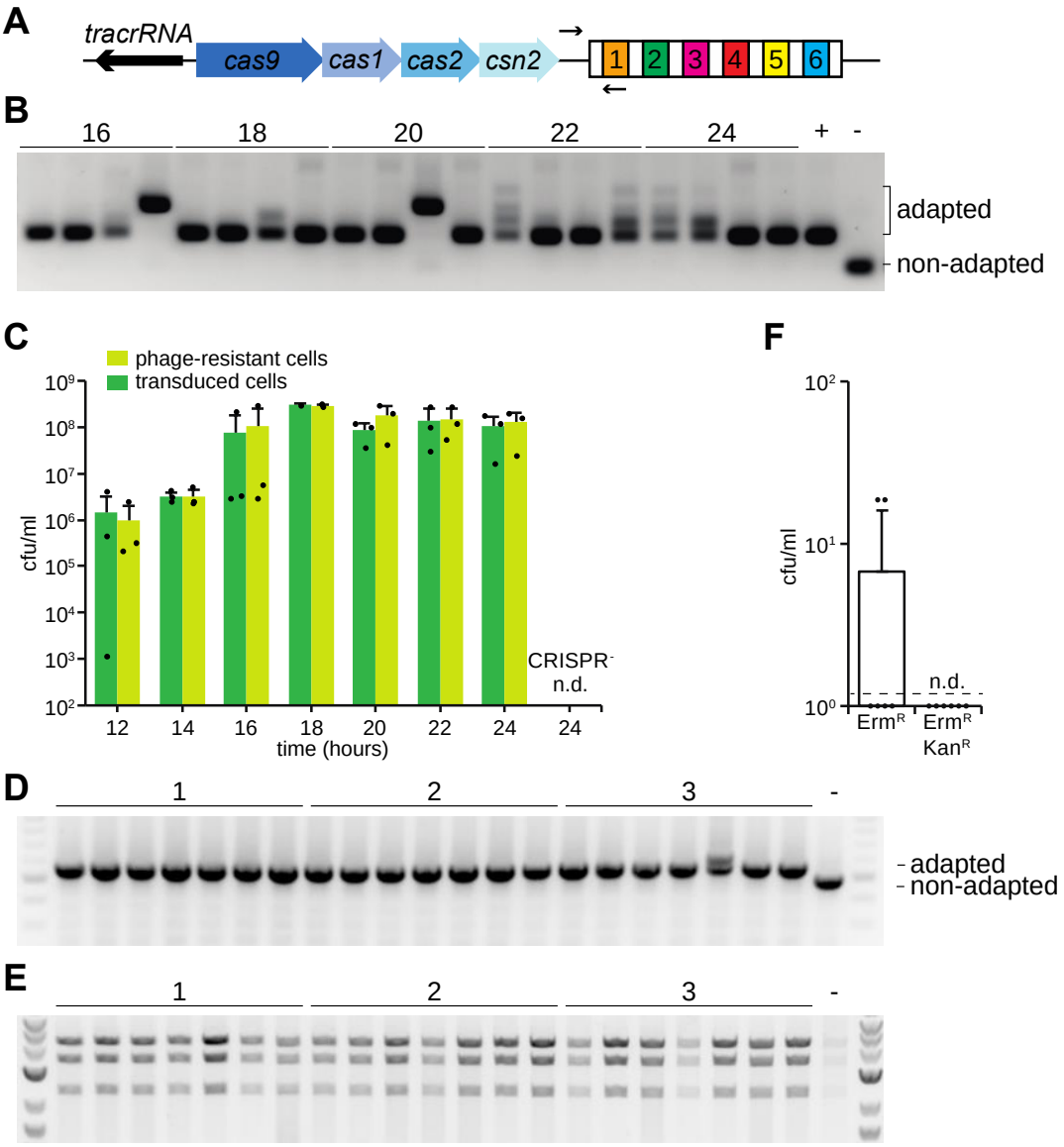

Fig. S2. Adaptation of transduced CRISPR-Cas loci and transduction of chromosomal resistance markers. (**A**) Organization of *S. pyogenes* CRISPR-Cas locus. Arrows indicated annealing positions of primers used to detect expansion of CRISPR array (**B**) PCR-based analysis to check for spacer acquisition in transduced colonies obtained in Fig. 1D. Labels indicate time point of transducing phage collection. Positive adaptation control of a single spacer acquisition indicated by +, while non-adapted control represented by (-). (**C**) Cultures without CRISPR-Cas loci infected at an MOI of 1 by ΦNM4γ4 collected from CRISPR$^+$ containing cultures at indicated time points from Fig. 1D. Cultures were plated 16 hours post infection and colonies were counted to determine overall phage resistance and also counted on plates with antibiotics selecting for the CRISPR-Cas locus to determine transduction levels. Limit of detection is 100 cfu/ml. Mean + STD of 3 biological replicates are reported. (**D**) PCR-based analysis to check for spacer acquisition in transduced colonies collected in Fig. 1D at 18 hours post infection. Labels indicate transduced colonies collected from each replicate. Non-adapted control is also shown (-). (**E**) Restriction enzyme digest of

CRISPR-Cas plasmids in transduced colonies collected in Fig. 1D at 18 hours post infection. Plasmids are digested with HindIII. Labels indicate transduced colonies collected from each replicate. Non-adapted control is also shown. (**F**) Transduction of either the chromosomal erythromycin resistance (Erm^R) or both chromosomal erythromycin and kanamycin resistance (Erm^R, Kan^R) following infection by ΦNM4γ4. Cultures harboring Erm^R and Kan^R chromosomal markers were infected with ΦNM4γ4 at a MOI of 1 and phage was collected 2 hours post infection. Levels of transduction were determined by infecting a susceptible culture at a MOI of 1 for 20 minutes, washing cells, and plating for antibiotic resistance. Limit of detection is 1.5 cfu/ml (dotted line). Mean + STD of 3 biological replicates are reported.

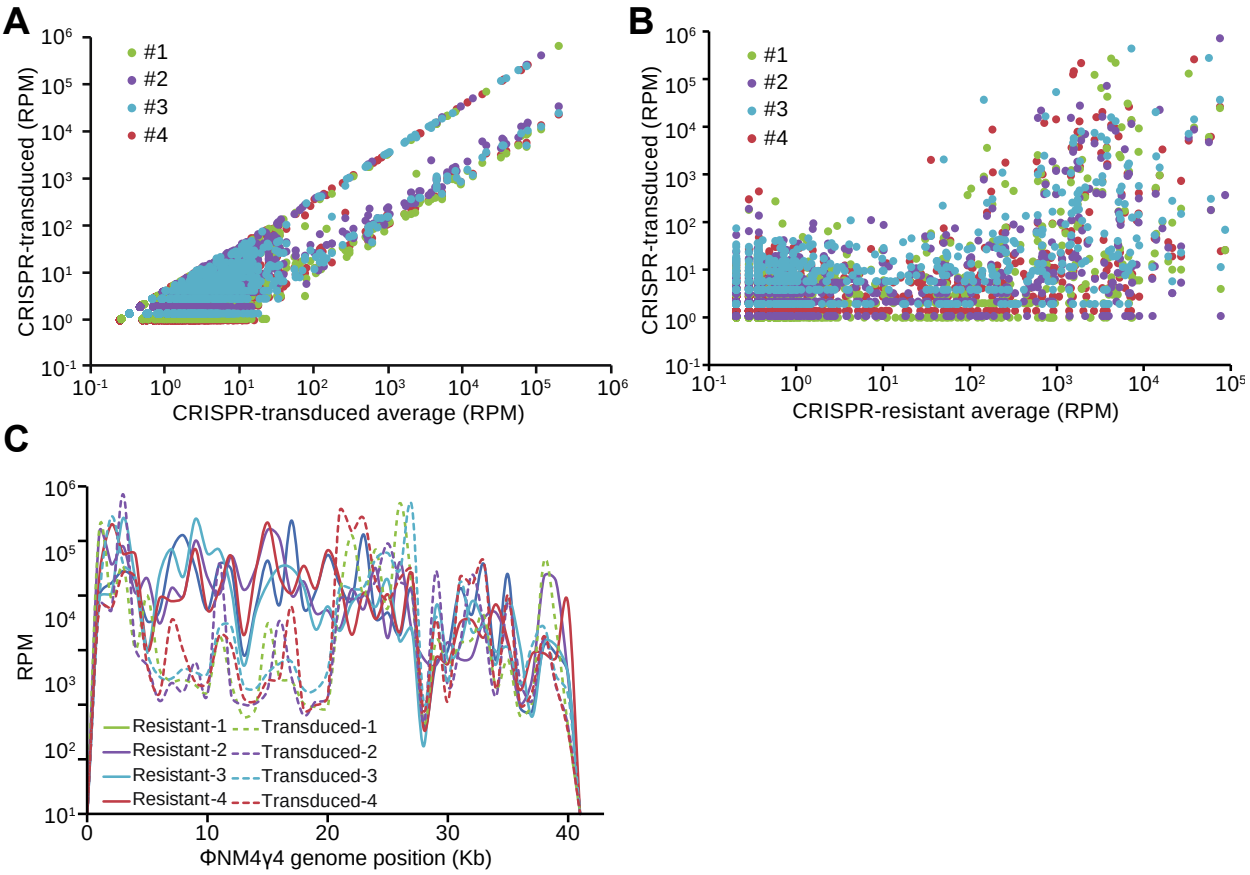

Fig. S3. Adapted and transduced spacer frequencies possess distinct profiles. (**A**) The average frequency of CRISPR-transduced reads plotted against the 4 individual replicates of CRISPR-transduced reads (#1-4). Each point represents a single spacer and its representation in sequenced populations. (**B**) The average frequency of CRISPR-resistant reads plotted against the 4 individual replicates of CRISPR-transduced reads (#1-4). Each point represents a single spacer and its representation in sequenced populations. (**C**) Data as described in Figure 2A, with each replicate mapped against the ΦNM4γ4 genome and plotted individually. Solid lines represent CRISPR-resistant replicates and dashed lines are CRISPR-transduced replicates.

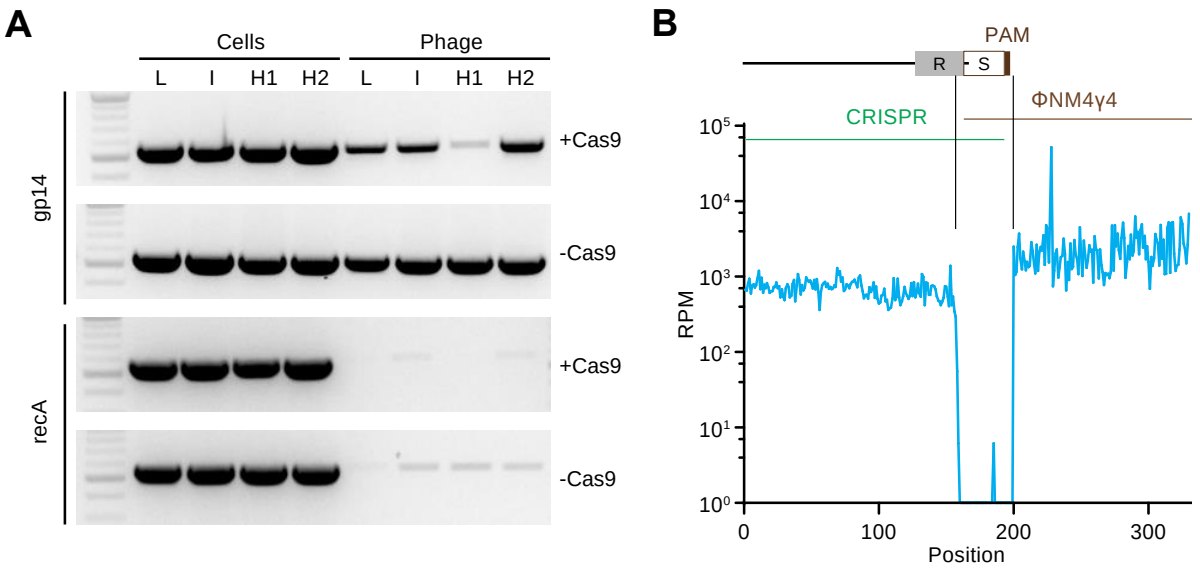

Fig. S4. Phage recombination with CRISPR spacers enhance transduction independent of RecA and in *cos*-phages. (**A**) PCR loading controls from phage/CRISPR-Cas locus recombinants. Cells containing just the CRISPR array (-Cas9) or Cas9 and the CRISPR array (+Cas9) or were infected at a MOI of 1 and 50, respectively. 30 minutes post infection for –Cas9 strains or 60 minutes post infection for +Cas9 strains, genomic DNA was extracted from the cellular pellet (cell DNA). For all strains, 60 minutes post infection DNA was also extracted from filtered supernatant (phage DNA). Primers annealing to phage locus *gp14* and chromosomal locus *recA* were used to determine DNA levels. (**B**) Deep sequencing of phage DNA harvested from cells containing Cas9 and spacer H1. Cultures were infected with ΦNM4γ4 at a MOI of 50 and phages were collected 90 minutes post infection. Phage DNA was subject to deep sequencing and aligned to 300 basepairs of the phage CRISPR locus junction that contains the PAM. Each point on the graph represents the number of reads that originate at that nucleotide position and have full coverage to the right for the 75-nucleotide deep sequencing read. Dotted lines indicate portion of reads that map to the CRISPR locus and ΦNM4γ4.

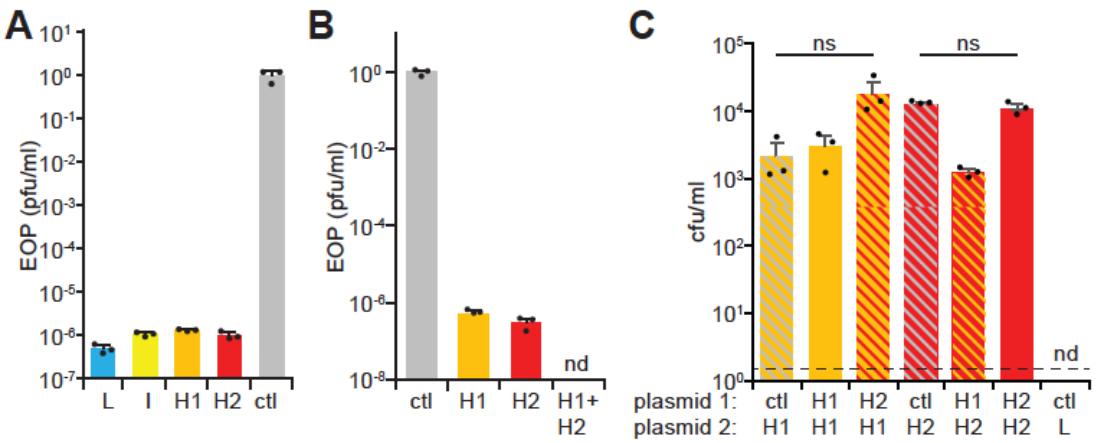

Fig. S5. CRISPR escaper levels do not affect transduction of CRISPR-Cas loci. (**A**) ΦNM4γ4 plaquing efficiency on soft agar lawns of high, intermediate, and low-transduced spacers (H1, H2, I, L) compared to a non-targeting spacer control (ctl). Mean + STD of 3 biological replicates are reported. (**B**) ΦNM4γ4 plaquing efficiency on soft agar lawns of individual spacers or combined spacers (H1+H2) expressed from different plasmids. Limit of detection is $1*10^{-9}$ pfu/ml. Mean + STD of 3 biological replicates are reported. (**C**) Transducing immune particle production from spacers combined with a second plasmid containing the spacer as indicated. The transduction efficiency of plasmid 2 is quantified. Cultures were infected with ΦNM4γ4 at a MOI of 50 and phages were collected 90 minutes post infection. Levels of transducing-immune phage particles were determined by infecting a susceptible culture at a MOI of 1 and plating on soft agar lawns with antibiotic selection for recipient cells and the antibiotic resistance cassette on the CRISPR plasmid. Data condensed from (Fig. 4C). Limit of detection is 1.5 cfu/ml (dotted line). Mean + STD of 3 biological replicates are reported.

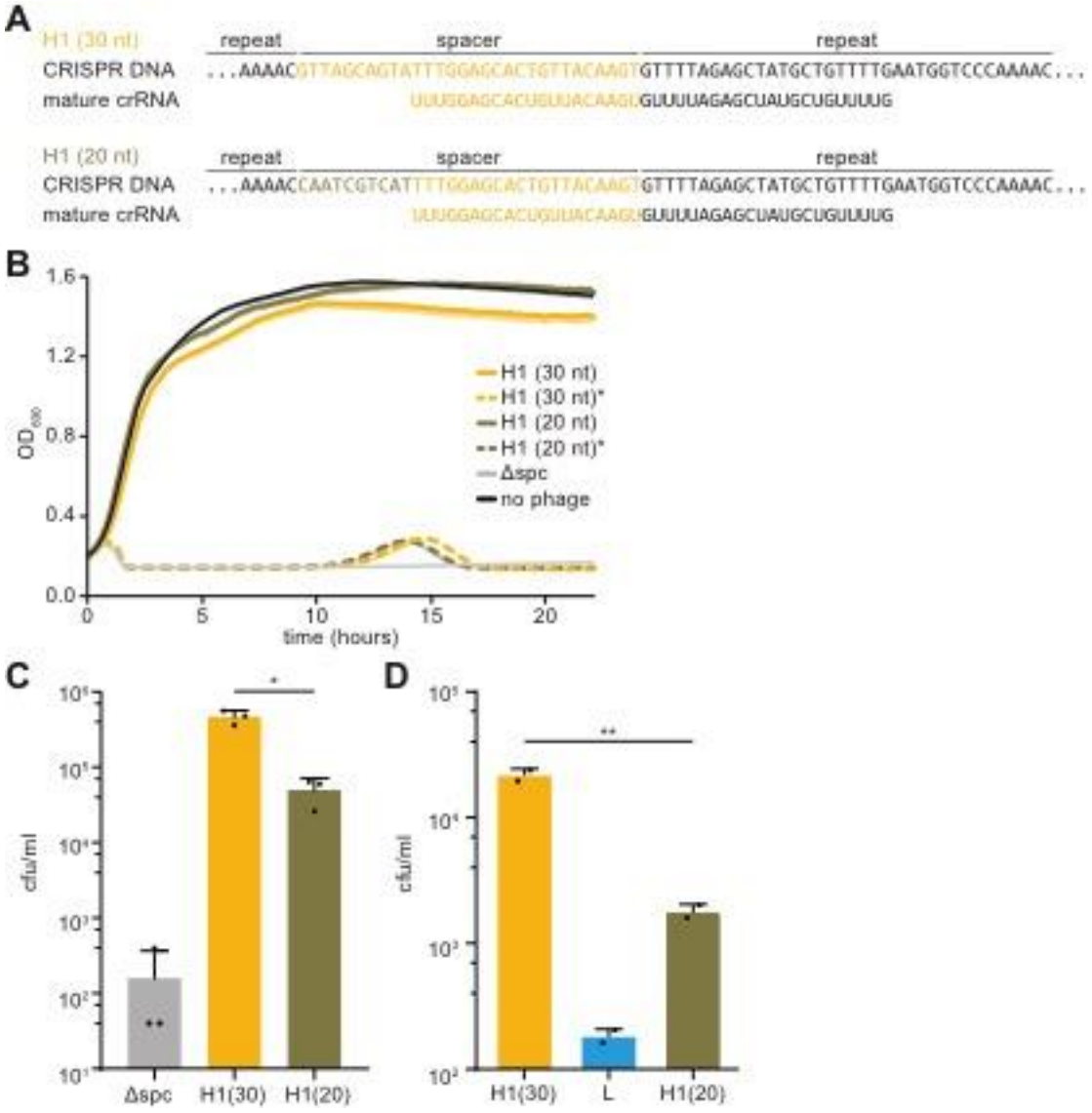

Fig. S6. Spacer sequences dispensable for targeting enhance transduction. (**A**) Schematic of CRISPR DNA locus and mature crRNA for full-length H1 (30 nt) or truncated H1 (20 nt), containing 10 mismatches in the 5' end of the spacer (**B**) Growth of H1 (30 nt) or H1 (20 nt) following infection. Strains were infected with ΦNM4γ4 at an MOI of 1. For samples denoted with an asterisk, simulation of CRISPR adaptation was performed by using cells lacking CRISPR to dilute immune culture 1:10,000. Growth was determined by optical density measured at 600nm (OD_{600}) and compared to a uninfected control (no phage). Mean -STD of 3 biological replicates are reported. (**C**) Transduction of plasmids containing just the CRISPR array with either a control spacer (ctl) or targeting spacers from (A). Cultures were infected with ΦNM4γ4 at a MOI of 1 and phage was collected 2 hours post infection. Levels of transduction were determined by infecting a susceptible culture at a MOI of 1 for 20 minutes, washing cells, and plating for the antibiotic resistance of the plasmid. Mean + STD of 3 biological replicates are reported. (**D**) Transducing-immune particles produced by strains from (A) and strain with low transducing characteristics (L). Cultures were infected with ΦNM4γ4 at a MOI of 50 and phages were collected 90 minutes post infection. Levels of transducing-

immune phage particles were determined by infecting a susceptible culture at a MOI of 1 and plating on soft agar lawns with antibiotic selection for recipient cells and the antibiotic resistance cassette on the CRISPR plasmid. Mean + STD of 3 biological replicates are reported.

Extended Data File 1 (separate file)

Deep sequencing raw data used to generate figures 2A, S2A, S2B, and S2C.