Cas13-induced cellular dormancy prevents the rise of CRISPR-resistant bacteriophage

Alexander J. Meeske1*, Sandra Nakandakari-Higa1 & Luciano A. Marraffini1,2*

Clustered, regularly interspaced, short palindromic repeat (CRISPR) loci in prokaryotes are composed of 30–40-base-pair repeats separated by equally short sequences of plasmid and bacteriophage origin known as spacers4–6. These loci are transcribed and processed into short CRISPR RNAs (crRNAs) that are used as guides by CRISPR-associated (Cas) nucleases to recognize and destroy complementary sequences (known as protospacers) in foreign nucleic acids4–6. In contrast to most Cas nucleases, which destroy invader DNA4–7, the type VI effector nuclease Cas13 uses RNA guides to locate complementary transcripts and catalyse both sequence-specific cis- and non-specific trans-RNA cleavage8. Although it has been hypothesized that Cas13 naturally defends against RNA phages8–10, type VI spacer sequences have exclusively been found to match the genomes of double-stranded DNA phages8–10, suggesting that Cas13 can provide immunity against these invaders. However, whether and how Cas13 uses its cis- and/or trans-RNA cleavage activities to defend against double-stranded DNA phages is not understood. Here we show that trans-cleavage of transcripts halts the growth of the host cell and is sufficient to abort the infectious cycle. This depletes the phage population and provides herd immunity to uninfected bacteria. Phages that harbour target mutations, which easily evade DNA-targeting CRISPR systems11–13, are also neutralized when Cas13 is activated by wild-type phages. Thus, by acting on the host rather than directly targeting the virus, type VI CRISPR systems not only provide robust defence against DNA phages but also prevent outbreaks of CRISPR-resistant phage.

We investigated the ability of Cas13a to provide phage resistance to Listeria, a natural host for the type VI-A CRISPR–Cas system, which we previously established as a model to study immunity against plasmid transfer14. We constructed Listeria ivanovii ΔCRISPRVI, a strain that is susceptible to infection by the phage φRR4 and carries the type VI-A CRISPR locus of Listeria seeligeri ATCC35967 (Extended Data Fig. 1a–c). To study Cas13a-mediated phage immunity, we generated a spacer library containing 41,276 unique φRR4 sequences, tiled every two nucleotides across both strands of the phage genome (Supplementary Information 1). We introduced the spacer library into L. ivanovii ΔCRISPRVI and infected the cells with φRR4. We collected intact cells before and five hours after infection, and used next-generation sequencing to determine spacer abundance. Wild-type L. ivanovii RR3 cells decreased in optical density at 600 nm (OD600) after infection, but the diverse set of spacers in the library provided protection from lysis (Extended Data Fig. 1d). We calculated enrichment ratios for each spacer as the read count after infection divided by the number of initial reads. The observed enrichment ratios were modest, consistent with previously reported growth defects associated with Cas13a activation5–7. As expected, given the fact that Cas13a targets RNA, we found a strong strand bias in spacer efficacy: spacers that generate a crRNA complementary to predicted transcripts (based on open reading frame direction) showed substantially higher enrichment ratios than those for which there should not be a target transcript (Fig. 1a, b, Extended Data Fig. 1e, Supplementary Information 1). To confirm this, we performed RNA sequencing (RNA-seq) analysis of phage-infected cells (Extended Data Fig. 2a) and found a strong correlation between protospacer transcription and the protection conferred by the corresponding spacer (Fig. 1c, Extended Data Fig. 1b, Extended Data Fig. 2b, Supplementary Information 1). We selected three individual spacers from the library for further characterization: spcA, which targets the φRR4 anti-CRISPR region; spcE, which targets the early lytic genes; and spcL, which targets the late lytic genes (Extended Data Fig. 1a). Each spacer strongly reduced both the efficiency of infective centre formation (Fig. 1d) and the phage burst size (Fig. 1e). Together, these results show that Cas13a uses crRNAs complementary to phage transcripts to prevent the establishment and maintenance of infection.

The experiments described above demonstrated that, although different individual spacer sequences supported different levels of defence, spacers that matched either essential or non-essential transcripts (such as those produced by phage anti-CRISPR genes, see below), and early- or late-expressed RNAs, could mediate a robust defence. This indicates

![Image](https://doi.org/10.1038/s41586-019-1257-5)

**Fig. 1 | Cas13a halts DNA phage infection upon protospacer transcription.** a, b, Enrichment of spacers targeting the top (a) or bottom (b) strands of the φRR4 genome after phage exposure, plotted by position. ac, anti-CRISPR; lg, lyosgeny. c, Correlation of target transcript expression (RPM, reads per million mapped reads) and enrichment of the corresponding spacer. d, Mean (± s.e.m.) efficiency of φRR4 infective centre formation (ECOI, efficiency of centre of infection; n = 3 biological replicates) on a strain lacking CRISPR (RR3) or ΔCRISPRVI strains programmed with spcE, spcL or spcA. e, Mean (± s.e.m.) φRR4 burst size for the strains tested in d (n = 3 biological replicates). ND, not detectable.

---

1Laboratory of Bacteriology, The Rockefeller University, New York, NY, USA. 2Howard Hughes Medical Institute, The Rockefeller University, New York, NY, USA. *e-mail: ameeske@rockefeller.edu; marraffini@rockefeller.edu
that the function of the target transcript cleaved by the cis-RNase activity of Cas13a does not affect the potency of the type VI-A immune response. To determine the extent of Cas13a-mediated trans-RNA degradation in vivo, we used conventional RNA-seq (Extended Data Fig. 3a) as well as global mapping of RNA 5′ ends, a method that detects both the transcription start site (TSS) of primary transcripts and the products of Cas13a cleavage. We analysed both host- and phage-derived transcripts in the RR3, ΩCRISPR VI (spcE) and ΩCRISPR VI (spcL) strains of L. ivanovii after 105 min of infection with oRR4. In the absence of type VI-A immunity, we detected abundant peaks for each TSS over a background signal that is presumably derived from spurious RNA cleavage or alternative transcription initiation (Fig. 2a–d). By contrast, in ΩCRISPR VI (spcE) cells we detected extensive cleavage within the oRR4 early lytic transcript (Fig. 2a) as well as within host transcripts (Fig. 2c, Extended Data Fig. 3b). Moreover, the average cleavage ratio (defined as the ratio of cleavage products to primary transcript reads) in ΩCRISPR VI (spcE) cells was significantly higher than in RR3 control cells across all host transcripts (Fig. 2e, Supplementary Information 2). Transcripts of the late lytic operon were undetectable in ΩCRISPR VI (spcE) cells (Fig. 2a, Extended Data Fig. 3a) probably because the products of phage early lytic genes are required for late gene transcription. Similar results were obtained for ΩCRISPR VI (spcL) cells (Fig. 2b, d, f, Extended Data Fig. 3a, Supplementary Information 3). We also detected cleavage of the early lytic transcripts in this strain, presumably as a consequence of trans-RNA degradation by Cas13a. Finally, similar degradation of host RNA was observed in the absence of phage infection, 15 min after activation of Cas13a by a chromosomally encoded target in L. sakei ATCC35967 (Fig. 3g, h, Extended Data Fig. 3c, Supplementary Information 4). Together, these results indicate that trans-RNA cleavage by Cas13a leads to massive degradation of both host and invader RNA in vivo.

Whereas DNA-cleaving CRISPR–Cas systems provide immunity through direct destruction of the phage genome, we reasoned that the collateral RNA degradation deployed by type VI-A systems might lead to the depletion of host and phage factors that are required for phage DNA replication and thereby indirectly support the clearance of invader genomes. We used quantitative PCR (qPCR) to measure the abundance of phage DNA in the RR3 and ΩCRISPR VI strains over the course of oRR4 infection (Fig. 3a). Phage DNA did not accumulate when early-expressed transcripts were targeted by spcA or spcE, probably because destruction of host and phage transcripts by Cas13a early in the lytic cycle prevented phage replication. By contrast, when Cas13a was programmed with spcL, phage DNA accumulated to similar levels to the RR3 control. This result indicates that Cas13a can infer with phage replication at any time during the viral lytic cycle, through a mechanism that does not necessarily prevent the accumulation of phage DNA. We hypothesized that such a mechanism could be based on the growth defect observed during targeting of plasmids by Cas13 in both heterologous and native hosts. We looked for similar growth defects during phage infection by measuring the OD₆₀₀ of L. ivanovii RR3 and ΩCRISPR VI cultures after infection. Although we detected only mild growth defects at a multiplicity of infection (MOI) of 1 (Extended Data Fig. 4a), at an MOI of 5, CRISPR targeting resulted in a marked growth delay of the cultures (Fig. 3b). It has been hypothesized that, similarly to abortive infection systems that elicit phage defence in other bacteria, massive host transcript degradation could result in dormancy of infected cells and thus prevent viral propagation. To test this idea, we assessed cell viability and phage titres before and immediately after infection at an MOI of 2, as well as after the first phage burst 4 h later (Fig. 3c, Extended Data Fig. 4b). Only 10–20% of RR3 control cells lacking CRISPR remained viable immediately after infection, and these suffered a more pronounced loss after 4 h. By contrast, all three ΩCRISPR VI strains exhibited the same initial loss of viability but then recovered at 4 h, although they showed reduced numbers compared to uninfected control cultures (Fig. 3c, Extended Data Fig. 4c). Phage titres mirrored these results, with substantial phage proliferation in RR3 cells during the first 4 h of infection, but a substantial decrease in phage propagation, even immediately after infection, in cells equipped with type VI-A immunity (Fig. 3d).

These results support a model for Cas13a-mediated immunity in which infected cells stop proliferating and inactivate the phages that invaded them, thus providing passive protection to uninfected cells, which survive and proliferate. Indeed, a viability of 10–20% corresponds to the expected number of uninfected cells at an MOI of 2, which calculated using the Poisson distribution is 13%. To test this model, we first tested whether Cas13a activation elicits cell dormancy. We monitored growth (OD₆₀₀) after induction of a Pₐᵣ₄-controlled target transcript by anhydrotetracycline (aTc) in the native type VI-A host, L. sakei ATCC35967 (Extended Data Fig. 5a). Wild-type (but not ACRISPR) cells exhibited growth arrest, with no evidence of lysis. These cultures recovered upon removal of aTc and remained sensitive to re-exposure to the inducer (Extended Data Fig. 5b), suggesting that the Cas13a-induced dormancy state is reversible. To test this more rigorously, we counted viable cells after induction of Cas13a targeting, plating culture samples on solid medium without aTc. Over the course of 9 h of target transcription and Cas13a activation, we observed a stable population of viable cells that could form colonies when aTc was removed (Fig. 3e), demonstrating that cells in which Cas13a was
activated cease to grow but do not die. Notably, these cells were not escape mutants that had lost CRISPR function; these accounted for only about 1% of the viable population after 9 h (Fig. 3e). Furthermore, induction of Cas13a in wild-type cells, but not in ΔCRISPRR3 cells, provided tolerance to transient exposure to the bactericidal antibiotics ampicillin, ciprofloxacin and streptomycin, which are otherwise lethal to growing cells (Fig. 3f). Finally, to test whether cells enter a dormant state during phage targeting, we took samples of L. ivanovii RR3 and ΔCRISPRR3 cultures at different times after φRR4 infection to count viable cells and determine the vitality of the culture using the reagent resorufin, which is quantitatively converted to fluorescent resorufin by the reducing cytosol of living cells29. The colony-forming unit (CFU) values mimicked the results shown in Fig. 3c (Extended Data Fig. 5c). By contrast, resorufin fluorescence remained constant over the course of the experiment in ΔCRISPRVI cultures (Extended Data Fig. 5d), showing that most cells in the culture, including those not able to form a colony, stayed alive during infection. Together, these data show that, when activated by a target transcript, the RNase activity of Cas13a promotes a state of cell dormancy, rather than cell death. Although dormancy can be reversed by the inhibition of target transcription, it is maintained in infected cells where active phages continue to produce protospeck RNA.

Next, we investigated whether infected cells could provide herd immunity to uninfected cells in the population, as has been observed for other CRISPR types1,2,11,12. We found that phase-sensitive, chloramphenicol-resistant RR3 cells were protected from φRR4 infection when co-cultured with ΔCRISPRVI (spcE) cells (Fig. 3g). Finally, we directly tested whether the cell dormancy triggered by activation of Cas13a is sufficient to protect against phage infection. We introduced a plasmid harbouring a target under an αTc-inducible promoter into L. ivanovii ΔCRISPRVI (spcP), which carries a spacer that matches the plasmid target, but not any of the phase transcripts. Pre-activation of Cas13a by an αTc-induced target transcription resulted in a 6.7-fold reduction in φRR4 infection efficiency (Fig. 3h). Furthermore, the plasmid-targeting strain showed a significant (P = 5 × 10⁻⁴) survival advantage after 7 h of φRR4 infection (Extended Data Fig. 5e). Together, these results demonstrate that the trans- RNA degradation activity of Cas13a is sufficient to provide immunity against double-stranded DNA (dsDNA) phages by inducing dormancy in infected cells, which prevents viral proliferation and protects uninfected cells in the population.

Our observation that plasmid-activated type VI-A CRISPR immunity also protected against infection by a phage not recognized by the crRNA guide of Cas13a demonstrates that these systems can provide general, non-specific immunity. This suggests that type VI-A systems could neutralize ‘escaper’ phages in the population, which contain target mutations that prevent their recognition by the crRNA guide. Such escaper phages easily overcome DNA-targeting CRISPR systems11–13 but should not be able to successfully infect a host in which a previous infection by a wild-type phage activated Cas13a (a very likely scenario when escapers are rare in the phage population). Indeed, we were not able to detect escaper plaques on lawns of L. ivanovii ΔCRISPRVI (spcE) or ΔCRISPRVI (spcA) cells (Extended Data Fig. 6a, b). To investigate the ability of type VI-A systems to protect against escaper phages, we engineered two φRR4 mutants that could escape spcA (φRR4acr) and spcE (φRR4ΩCRISPRVI) targeting (Extended Data Figs. 6a–d). We infected L. ivanovii ΔCRISPRVI cells with these mutants and found that escaper phage propagation was reduced by 1–2 orders of magnitude when cells were co-infected (1:10⁵ ratio) with wild-type φRR4 (Fig. 4a, c). Moreover, we obtained similar results when we used the virulent mycovirus AS1123, a Listeria phage unrelated to φRR4, instead of the mutants (Fig. 4b, c).

Finally, we tested the prediction that the extent of escaper neutralization should increase with the MOI, as the probability of infection with a wild-type phage also increases. Indeed, cells pre-infected at higher MOIs with wild-type φRR4 showed reduced propagation of escaper phages (added at MOI 0.1 to pre-infected cells) (Fig. 4d). Adsorption efficiency was unchanged after the first infection, excluding the possibility that competition for phage binding sites prevented the second

![Fig. 3](https://example.com/fig3.jpg) Cas13a-induced cell dormancy is sufficient to abort lytic infection and limit phage propagation. a, Mean (± s.e.m., n = 3 biological replicates) phage DNA content after infection of L. ivanovii RR3 and ΔCRISPRVI (spcA, spcE or spcL) cultures after addition of φRR4 at an MOI of 1, normalized to value for RR3 cells 10 min after infection. b, Mean (± s.e.m., n = 3 biological replicates) OD₆₀₀ values of L. ivanovii RR3 and ΔCRISPRVI (spcA, spcE or spcL) cultures before (P) and immediately after (e), and 4 h after infection with φRR4 at an MOI of 5. RR3-UN, uninfected RR3. c, Mean (± s.e.m., n = 6 biological replicates) CFU present in L. ivanovii RR3 and ΔCRISPRVI (spcA, spcE or spcL) cultures before (P), immediately after (0), and 4 h after infection with φRR4 at an MOI of 2. d, As in c but measuring PFU titre. e, Mean (± s.e.m., n = 3 biological replicates) CFU from L. seeligeri wild-type and ΔCRISPRR3 cultures after transcription of a chromosomal target and plating on medium lacking αTc. Escaper mutants in the wild-type culture were counted on plates with αTc. f, Mean (± s.e.m., n = 3 biological replicates) survival of L. seeligeri wild-type and ΔCRISPR cultures in the presence of ampicillin (Amp), streptomycin (Str) or ciprofloxacin (Cip) after activation of Cas13. g, Mean (± s.e.m., n = 3 biological replicates) chloramphenicol-resistant CFU per ml before (P), 7 h after (e) and without (UN) infection with φRR4 at an MOI of 1 of a 1:1 mix of phage-susceptible, chloramphenicol-resistant (cmR) L. ivanovii RR3 and chloramphenicol-sensitive L. ivanovii RR3 or ΔCRISPRVI (spcE) strains. h, Mean (± s.e.m., n = 3 biological replicates) ECOI after addition of φRR4 to phage-susceptible L. ivanovii ΔCRISPRVI (spcP) cells harbouring a plasmid with an αTc-inducible spcP target or an empty vector control.
infection by escaper phages (Extended Data Fig. 6e). As a control, we infected a ΔCRISPRV (Δs) strain that carries the type II-A CRISPR system from Streptococcus pyogenes programmed with sCwE with ΔR44, which lacks the anti-CRISPR genes that inhibit Cas9 cleavage (Extended Data Fig. 6c, f). We isolated one Cas9-resistant escaper (a Cas9 escape mutant derived from ΔR44+) phage on CRISPR VI (Δs) in the presence and absence of excess ΔR44.

We have shown that during the type VI-A CRISPR–Cas response against ssDNA phages, the RNAse Cas13a performs crRNA-guided recognition of phage RNA, resulting in massive degradation of host and phage transcripts. Although it is possible that this degradation interferes with the phage lytic cycle to contribute to overall defense, we believe that the fundamental feature of type VI-A immunity is the dormancy of the host cell produced by the destruction of host transcripts. This is similar to other phage defense strategies known as abortive infection26. We postulate that, in the absence of a mechanism to specifically destroy the phage DNA, target transcription continues, Cas13a remains active and the host cells neither resume growth nor lyse. Therefore, the infected cell can continue to adsorb phages, abort their infectious cycle and eliminate them from the culture.

To our knowledge, all other CRISPR types studied possess DNA cleavage activities that can destroy the genome of an infecting phage,6,7,15,25,26. Therefore immunity is achieved by a direct attack on the invader, followed by survival of the infected cells. A consequence of direct interference with the phage lytic cycle is that mutant escapers can overcome targeting, kill the infected cell and propagate. By contrast, during type VI-A CRISPR–Cas immunity, the small fraction of escape phages present in the viral population are likely to end up infecting a cell in which Cas13a was previously activated by a wild-type, target-bearing phage, and are therefore also neutralized. Phage density, escape frequency, and spacer targeting efficiency are all likely to affect the extent of this cross-protection mechanism.

Instead of using abortive infection to limit the propagation of escape phages, the DNA-degrading CRISPR systems usually acquire new spacer sequences that can target other regions of the phage25. Given the negative selection suffered by cells in which type VI-A immunity is triggered, and the strong protection against escapers provided by Cas13a, it is unclear how spacer acquisition might operate in these systems. One possibility is that spacers are acquired from the injected genomes of defective phage particles that fail to activate their transcriptional program27. Alternatively, phage genomes could occasionally be cleared from the cell after Cas13a activation, permitting the cell to recover from dormancy. If such a mechanism exists, the infected cell itself would benefit from the presence of the type VI CRISPR system and immunity would not be an exclusively altruistic event.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1257-5.

Received: 8 January 2019; Accepted: 30 April 2019; Published online 29 May 2019.

1. Mojica, F. J., Déz-Villaseñor, C., García-Martínez, J. & Soria, E. Interfering sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J. Mol. Biol. 303, 185–199 (2000).
2. Bolotin, A., Quinquis, B., Sorokin, A. & Ehrlich, S. D. Clustered regularly interspaced short palindromic repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 151, 2551–2551 (2005).
3. Praz, C., Boven, C., Salvignol, G. & Vergnaud, G. CRISPR elements in meningococcus and gonococcus are spread by transposons and integrated into other mobile elements. J. Bacteriol. 187, 1169–1180 (2005).
4. Marraffini, L. A. & Sontheimer, E. J. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Cell 127, 499–511 (2006).
5. Marraffini, L. A. & Sontheimer, E. J. A Cas9–CRISPR array offers high-fidelity sequence-specific nucleases. Science 339, 823–825 (2013).
6. Garneau, J. E. et al. The CRISPR/Cas bacterial immune system cleaves DNA. Nature 468, 67–71 (2010).
7. Abudayyeh, O. O. et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science 353, eaaf5573 (2016).
8. Saedler, H. & Ehrlich, S. D. A comparison of type I and type II CRISPR-Cas systems. Microbiol. Mol. Biol. Rev. 70, 282–308 (2006).
9. Katahira, K. et al. DNA cleavage specificity of the CRISPR endonuclease Cas13a. Nat. Biotechnol. 34, 158–162 (2016).
10. Yan, W. X. et al. Cas13a is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYD-domain-containing accessory protein. Mol. Cell 70, 327–339 (2017).
11. Deveau, H. et al. Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. J. Bacteriol. 190, 1390–1400 (2008).
12. van Houte, S. et al. The diversity-generating benefits of a prokaryotic adaptive immune system. Nature 532, 385–388 (2016).
13. Lyubartsev, A. V., Tang, W. & Marraffini, L. A. Broad targeting specificity during bacterial type III CRISPR-Cas immunity constrains viral escape. Cell Host Microbe 22, 343–353 (2017).
14. Meese, A. J. & Marraffini, L. A. RNA guide complementarity prevents self-targeting in type VI CRISPR systems. Mol. Cell 71, 791–801 (2018).
15. Wimmer, E. R. & Darzynkiewicz, Z. Flow cytometry: a biochemistry of life-end death events. Nat. Protoc. 3, 1–9 (2008).
16. Parreira, R., Ehrlich, S. D. & Choppin, M. C. Dramatic decay of phage transcripts in lactococcal cells carrying the abortive infection determinant AbB. Mol. Microbiol. 19, 221–230 (1996).
17. Fineran, P. C. et al. The phage abortive infection system, ToxiN, functions as a protein-RNA toxin-antitoxin pair. Proc. Natl Acad. Sci. USA 106, 894–899 (2009).
19. Short, F. L. et al. Selectivity and self-assembly in the control of a bacterial toxin by an antitoxic noncoding RNA pseudoknot. Proc. Natl Acad. Sci. USA 110, E241–E249 (2013).
20. Shiloh, M. U., Ruan, J. & Nathan, C. Evaluation of bacterial survival and phagocyte function with a fluorescence-based microplate assay. Infect. Immun. 65, 3193–3198 (1997).
21. Watson, B. N. J., Staals, R. H. J. & Fineran, P. C. CRISPR–Cas-mediated phage resistance enhances horizontal gene transfer by transduction. MBio 9, e02406-17 (2018).
22. Payne, P., Geyrhofer, L., Barton, N. H. & Bollback, J. P. CRISPR-based herd immunity can limit phage epidemics in bacterial populations. eLife 7, e32035 (2018).
23. Klumpp, J. et al. The terminally redundant, nonpermuted genome of Listeria bacteriophage AS11: a model for the SPO1-like myoviruses of gram-positive bacteria. J. Bacteriol. 190, 5753–5765 (2008).
24. Rostøl, J. T. & Marraffini, L. (Ph)ighting phages: how bacteria resist their parasites. Cell Host Microbe 25, 184–194 (2019).
25. Yan, W. X. et al. Functionally diverse type V CRISPR–Cas systems. Science 363, 88–91 (2019).
26. Zetsche, B. et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR–Cas system. Cell 163, 759–771 (2015).
27. Hynes, A. P., Villon, M. & Moineau, S. Adaptation in bacterial CRISPR–Cas immunity can be driven by defective phages. Nat. Commun. 5, 4399 (2014).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2019
Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Bacterial strains and growth conditions. The type V CRISPR system was derived from L. seeligeri ATCC strain 35967 (Rocourt and Grimont). L. seeligeri RR4 and L. ivanovii RR3 were obtained from J.-P. Lemaître (INRA, Dijon, France). All Listeria strains were propagated in Brain Heart Infusion (BHI) broth or agar at 30°C. Unless otherwise stated, plasmids were cloned in Escherichia coli DH5α, mini-prepped, and transformed into the conjugative donor strain E. coli SM10 for conjugative mating. All E. coli strains were cultured in lysogeny broth (LB) at 37°C.

Phage isolation and propagation. Isolation of φRR4 was carried out by overnight induction of L. seeligeri RR4 at OD 0.1 using micotycin C at 2 μg/ml. The induced culture supernatant was diluted to single plaques on a lawn of L. ivanovii, and a single plaque was purified twice to ensure homogeneity. To generate high-titre phage lysates, 50 ml of L. ivanovii culture was infected with φRR4 at MOI 0.1, OD 0.1 and the infection proceeded overnight. The lysate was filtered and concentrated to 1 ml using Amicon Ultra 100 MWCO columns.

Infective centre and burst size quantification. L. ivanovii strains were grown to mid-exponential phase, and 0.6 ml of culture at OD₆₀₀ 0.5 was infected with φRR4 or derivatives at an MOI of 0.1. Adsorption was carried out for 5 min, followed by three washes with 1 ml BHI to remove unbound phage, and the samples were resuspended to a final volume of 1 ml. Infective centres were enumerated by measuring PU Ftu titres on a lawn of naïve L. ivanovii RR3 at the initial time point. Burst size was calculated by dividing the Pfu after the first burst by the starting Pfu. E. coli–Listeria shuttle vectors were constructed by engineering a BsaI site into the PSA vector (pAM521). L. ivanovii strains carrying E. coli–Listeria shuttle vectors were cultured in LB containing 100 μg/ml ampicillin (for pAM8-derived vectors) or 25 μg/ml chloramphenicol (for pPL2e-derived vectors). Recipient L. seeligeri or L. ivanovii strains were cultured in BHI. Saturated donor and recipient cultures (100 μl each) were combined in 10 ml BHI, and concentrated onto a 0.45-μm membrane filter, which was then overlaid onto a BHI agar plate containing 8 μg/ml oxacillin, which weakens the cell wall, enhancing conjugation. Mating plates were incubated for 4 h at 37°C, then cells were resuspended in 2 ml BHI and plated at 30°C on selective BHI medium containing 50 μg/ml nalidixic acid (which kills donor E. coli but not recipient L. seeligeri). Selection for plasmid recipients was performed with either 15 μg/ml chloramphenicol (for pAM8-derived vectors) or 1 μg/ml erythromycin (for pPL2e-derived vectors).

Cloning of CRISPR locus and spacer library construction. The L. seeligeri type V CRISPR locus was introduced into L. ivanovii RR3 by cloning into the PSA site-specific integrating vector pPL2e via three-piece Gibson assembly using (1) a synthetic gene fragment containing the native promoter and CRISPR array promoter modified to contain only two repeats and Bsal sites for spacer cloning (amplified with pAM462/127), (2) the cas13a gene (amplified with pAM463/94), and (3) BstXI-digested Bsal-DNA. Donor E. coli but not recipient L. seeligeri. Selection for plasmid recipients was performed by plating a 1:1 mixture of donor E. coli and recipient L. seeligeri strains carrying either 15 μg/ml chloramphenicol (for pAM8-derived vectors) or 1 μg/ml erythromycin (for pPL2e-derived vectors).

RNA sequencing. To isolate RNA from L. seeligeri, the equivalent of 3 ml culture at OD₆₀₀ 0.3 was pelleted and lysed by 3 min treatment with 2 mg/ml lysozyme followed by the addition of sarkosyl at 1%. A fixed amount of Staphylococcus aureus RNA was added to each sample immediately after lysis for global RNA normalization. RNA was purified from these lysates using the Zymo Direct-Zol RNA miniprep plus kit according to the manufacturer’s instructions. Genomic DNA was eliminated using the Ambion Turbo DNase-free kit.Ribosomal RNA was depleted using the Illumina Ribo-Zero RNA removal (Bacteria) kit. After rRNA removal, libraries were prepared for deep sequencing using the TruSeq Stranded mRNA Library Prep kit, skipping mRNA purification and beginning at the fragmentation step. Paired-end (2 × 75-bp) sequencing was performed on the Illumina HiSeq 3000 using PE libraries. 1:1 (4) mixture of pPL2e-derived L. ivanovii RR3, L. ivanovii RR3 ΔspcE, L. seeligeri RR3, and L. seeligeri RR3 Δspc4, OCRISPRV(spce), and OCRISPRV(spcl) strains, as well as aTc-treated L. seeligeri wild-type (Ptet-spct) and OCRISPR (Ptet-spct) strains, have been deposited under BioProject accession number PRJNA512236.

Results

For 5′ end mapping, RNA was first purified, DNase-treated and RNA-depleted as above, then concentrated using the Zymo RNA Clean & Concentrator 5 kit and 5′ phosphorylated with T4 polynucleotide kinase (NEB). 5′ triphosphates were removed using E. coli polynucleotase, and a 5′ RNA adaptor was added using T4 RNA ligase. The library was fragmented using Agilent RNA fragmentation reagents, and reverse transcribed using SuperScript IV with an adaptor primer ending in nine randomized nucleotides. The resulting cDNA libraries were amplified by PCR and purified using AMPure beads, then single-end 150-bp sequencing was carried out on the MiSeq platform. 5′ end sequencing data for phage-infected L. ivanovii RR3, OCRISPRV(spce), and OCRISPRV(spcl) strains, as well as aTc-treated L. seeligeri wild-type (Ptet-spct) and OCRISPR (Ptet-spct) strains, have been deposited under BioProject accession number PRJNA512236.

RNA-seq analysis. RNA-seq reads were mapped to the corresponding genome using bowtie2. Coverage was calculated at each genome position and normalized to the total number of S. aureus reads (representing the spike-in), and the ratio of CRISPR ‘+CRISPR’ reads was calculated. If zero reads were detected, a pseudocount was added. For RNA 5′ end mapping analysis, reads were mapped as above, with only the 5′ end position tabulated. Operonic transcripts were manually annotated from the paired-end transcriptomic data, and these boundaries were used to calculate the fraction of reads that mapped within or outside the TSS. The site within 500 nucleotides upstream of the start codon containing the most 5′ reads in wild-type cells was designated as the TSS. Reads mapping within five nucleotides of this site were counted as TSS reads. For transcripts in which at least 20 reads were detected, the cleavage ratio was calculated as the number of reads downstream of the TSS divided by that within the TSS. Normalization to the TSS accounts for differences in gene expression generated by pleiotropic effects such as the degradation of transcription factor mRNAs. This cleavage

Letter
ratio also allowed us to capture events in which Cas13a-mediated degradation did not produce stable cleavage products. Tables are provided showing cleavage ratios for each transcript in phage-infected L. ivanovii RR4 and ΩCRISPRVI(spE) (Supplementary Information 2), ΩCRISPRIII(spC) (Supplementary Information 3), and aTc-treated L. seeligeri wild-type (Ptet-spE) and ΔCRISPR (Ptet-spC4) (Supplementary Information 4) strains.

**δRR4 escaper mutant construction.** δRR4 mutants were generated by allelic exchange using the integration of the suicide vector pAM215 into the L. seeligeri RR4 lysogen as described above. Mutant prophages were confirmed by PCR and sequencing, and induced with mitomycin C as described above.

**Herd immunity experiment.** L. ivanovii RR3 cells carrying a cat gene encoding resistance to chloramphenicol were co-cultured at a 1:1 ratio with either wild-type RR3 or ΔCRISPR(spE) cells, then the population was infected with δRR4 at OD600 = 0.1, MOI 1, for 7 h at 30 °C. Viable chloramphenicol-resistant cells were counted before and after infection.

**Immunity by plasmid targeting.** L. ivanovii ΔCRISPRIII(spE)-p cells were transformed with pAM211 (empty vector) or pAM212 (aTc-inducible spP target) via conjugative mating. Cells carrying each plasmid and the CRISPR locus were grown to OD600 = 0.1, and exposed to 100 ng/ml aTc for 1 h. Then, cells were infected with δRR4 at MOI 1 for 7 h at 30 °C. Viable CFUs were counted before and after infection. Alternatively, the efficiency of δRR4 infective centre formation was measured on cells 1 h after aTc treatment.

**Cell vitality assay.** Cell vitality was measured with the resazurin-based cell vitality reagent alamarBlue HS (ThermoFisher) according to the manufacturer’s instructions. In brief, 100 µl of cell culture was mixed with 10 µl of alamarBlue HS and incubated for 20 min while shaking at 37 °C, and fluorescence (excitation: 560 nm, emission: 590 nm) was measured on a Tecan Infinite M200 Pro plate reader with a monochromator module with a fixed gain setting of 79. Uninfected cells in the same medium as all experimental samples were used as live cell standards, and the same samples heated for 5 min at 95 °C were used as dead cell standards. We made 10% and 50% live cell mixture standards to assess the accuracy of vitality measurements. Dead cell signal was subtracted as background from all resorufin signal values. Resorufin signal values for all samples were normalized to values before infection.

**Phage co-infection plaque assays.** For phage co-infection assays to assess the efficiency of escaper infection in the presence and absence of co-infecting wild-type phage, escaper phage stocks were diluted to approximately 5 × 10⁸ PFU/ml in BHI medium, then mixed with BHI containing either no phage or 5 × 10⁸ PFU/ml wild-type phage. Serial tenfold dilutions of phage mixtures were made and 2 µl of each dilution was spotted onto BHI top agar lawns seeded with 100 µl of saturated cultures of ΔCRISPRIII strains. δRR4acr escaper mixtures were spotted onto ΔCRISPRIII(spA) lawns, and δRR4acr escaper mixtures were spotted on ΔCRISPRIII(spE) lawns. A511 mixtures were spotted onto both target lawns. Co-infections comparing Cas9 and Cas13 were conducted in the same way, except that δRR4acr was used as the activating phage, and was mixed in 10-fold excess with δRR4acr or A511. Mixtures were spotted onto BHI top agar lawns seeded with 100 µl of saturated ΔCRISPRIII(spE) or ΔCRISPRIII(spE) culture.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability** The L. seeligeri RR4 and L. ivanovii RR3 genome sequences, along with raw reads from the spacer library deep sequencing, paired-end RNA-seq, and S5′ end mapping have been deposited in the Sequence Read Archive under BioProject accession number PRJNA512236. Lists of strains, plasmids, and oligonucleotides used in this study are available in Supplementary Information 5.

**Code availability** Custom scripts used in analysis of spacer library data as well as RNA S5′ end mapping data are available upon request.

28. Lauer, P., Chow, M. Y., Loessner, M. J., Portnoy, D. A. & Calendar, R. Construction, characterization, and use of two Listeria monocytogenes site-specific phage integration vectors. J. Bacteriol. 184, 4177–4186 (2002).

**Acknowledgements** We thank all members of the Marraffini laboratory for advice and encouragement. A. Varble for discussions, and J. T. Rostal for critical reading of the manuscript. L. seeligeri RR4 and L. ivanovii RR3 were gifts from J.-P. Lemaître. Support for this work comes from the National Institute of Health Director’s Pioneer Award 1DP1GM128184-01 (to L.A.M.). L.A.M. is an investigator of the Howard Hughes Medical Institute. A.J.M. is a Helen Hay Whitney postdoctoral fellow.

29. Roccourt, J., Schrettenbrunner, A., Hof, H. & Espaze, E. P. (New species of the genus Listeria: Listeria seeligeri), Pathol. Biol. (Paris) 35, 1075–1080 (1987).

30. Lemaître, J. P., Duroux, A., Pimpie, R., Duez, J. M. & Milat, M. L. Listeria phage and phage tail induction triggered by components of bacterial growth media (phosphate, LiCl, nalidixic acid, and acriflavine). Appl. Environ. Microbiol. 81, 2117–2124 (2015).

31. Loessner, M. J., Inman, R. B., Lauer, P. & Calendar, R. Complete nucleotide sequence, molecular analysis and genome structure of bacteriophage A118 of Listeria monocytogenes: implications for phage evolution. Mol. Microbiol. 35, 324–340 (2000).

**Author contributions** Experiments were designed by A.J.M. and L.A.M. A.J.M. conducted spacer library construction and testing, all RNA sequencing, cell dormancy experiments, phage mutant construction, and escaper cross-protection assays, as well analysis of all next-generation sequencing data. S.N.-H. assisted with sequencing the L. seeligeri RR4 and L. ivanovii RR3 genomes and initial testing of spacers in δRR4 immunity. The paper was written by A.J.M. and L.A.M.

**Competing interests** L.A.M. is a cofounder and Scientific Advisory Board member of Intellia Therapeutics, and a co-founder of Eligo Biosciences. The other authors declare no competing interests.

**Additional information**

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41586-019-1257-5.

**Correspondence and requests for materials** should be addressed to A.J.M. or L.A.M.

**Peer review information** Nature thanks Peter Fineran, Edzve Westra and the other anonymous reviewer(s) for their contribution to the peer review of this work.

**Reprints and permissions information** is available at http://www.nature.com/reprints.
Extended Data Fig. 1 | *Listeria* phage infection model for studying type VI-A CRISPR immunity. **a**, Diagram of the φRR4 genome, with individual genes depicted within the anti-CRISPR, early lytic, late lytic, and lysogenic regions. *L. seeligeri* ATCC35967 harbours a five-spacer type VI-A CRISPR locus, but phages that infect this strain have not yet been identified. We sequenced the genome of *L. seeligeri* RR4 and found that it contains a 42-kb prophage, φRR4, that is similar to the A118 listeriophage. Although φRR4 particles induced from the lysogen did not infect *L. seeligeri* ATCC35967, φRR4 propagated in the closely related *L. ivanovii* RR3 strain (99.2% 16S rRNA identity). **b**, The type VI-A CRISPR locus of *L. seeligeri* ATCC35967 was inserted into the tRNAArg gene of *L. ivanovii* RR3 using the vector pAM125, generating *L. ivanovii* ΩCRISPRVI. Different strains with either the five spacers naturally present in this system (spc1–5), individual spacers matching the genome of φRR4 (spcA, spcE, spcL), or a φRR4 spacer library (spc lib), were generated. **c**, Test of type VI-A anti-plasmid immunity in *L. ivanovii* ΩCRISPRVI(spcl-5). Plasmids with spc2 or spc4 targets in the chloramphenicol resistance cassette were conjugated into *L. ivanovii* RR3 or ΩCRISPRVI(spcl-5) and transconjugants selected on nalidixic acid and chloramphenicol. Transconjugants that received an empty vector lacking a target sequence are shown as a negative control (none). Representative of two biological replicates. **d**, Prevention of φRR4 lytic infection by the type VI spacer library. Spacer library cells (yellow–orange gradient) or cells lacking CRISPR (grey) were infected with φRR4 at OD600 = 0.1, MOI = 1, and OD600 was monitored over time. Representative of two biological replicates. **e**, One-hundred-base-pair sliding window average spacer enrichment ratio (post-infection/pre-infection spacer abundance) for spacers targeting the top (orange) and bottom (brown) strands.

For the latter, 41,276 φRR4-matching spacers were selected, tiled every two nucleotides across the phage genome, with both strands equally represented. **c**, Test of type VI-A anti-plasmid immunity in *L. ivanovii* ΩCRISPRVI(spcl-5). Plasmids with spc2 or spc4 targets in the chloramphenicol resistance cassette were conjugated into *L. ivanovii* RR3 or ΩCRISPRVI(spcl-5) and transconjugants selected on nalidixic acid and chloramphenicol. Transconjugants that received an empty vector lacking a target sequence are shown as a negative control (none). Representative of two biological replicates. **d**, Prevention of φRR4 lytic infection by the type VI spacer library. Spacer library cells (yellow–orange gradient) or cells lacking CRISPR (grey) were infected with φRR4 at OD600 = 0.1, MOI = 1, and OD600 was monitored over time. Representative of two biological replicates. **e**, One-hundred-base-pair sliding window average spacer enrichment ratio (post-infection/pre-infection spacer abundance) for spacers targeting the top (orange) and bottom (brown) strands.
Extended Data Fig. 2 | φRR4 transcriptome and enrichment of corresponding targeting spacers. a, RNA-seq over the course of φRR4 infection. Wild-type *L. ivanovii* RR3 was infected with φRR4 at an MOI of 1 and samples were collected for transcriptomic analysis by paired-end RNA-seq at the indicated time points. Reads were mapped to the φRR4 genome and normalized to the total reads per sample. Orange, top strand; brown, bottom strand. Representative of two biological replicates. b, Spacer enrichment correlates with target transcription, with no additional protection conferred above a critical expression threshold. Spacer abundance in the library was assessed before infection as well as 5 h after infection with φRR4 at an MOI of 1. Spacer enrichment distributions are shown, with individual histograms representing different tiers of target transcript abundance for the corresponding protospacer.
Extended Data Fig. 3 | Cas13a-mediated cleavage of phage and host transcripts detected by RNA-seq. a, Abundance of phage transcripts assessed by conventional paired-end RNA-seq 1.75 h after infection with \( \phi RR4 \) in \( L. \) annovii RR3 wild-type, \( \Omega CRISPR VI(spce) \) or \( \Omega CRISPR VI(spcl) \) strains. Reads were mapped to the \( \phi RR4 \) genome and normalized to the abundance of a spike-in RNA. Both \( \Omega CRISPR VI(spce) \) and \( \Omega CRISPR VI(spcl) \) targeting result in elevated early transcript cleavage products (Fig. 2a, b) and a reduction in late transcript abundance. Orange, top strand; brown, bottom strand. Representative of two biological replicates. b, \( L. \) annovii host mRNA cleavage detected by 5' end mapping in \( L. \) annovii RR3 wild-type (grey) and \( \Omega CRISPR VI(spce) \) (green) strains 1.75 h after infection with \( \phi RR4 \). The height of each peak represents the detected abundance of the corresponding mRNA 5' end. Grey arrowheads, TSSs. Four regions of the genome are depicted: murA1, ftsEX/isp, isdCD, division and cell wall (dcw) cluster. Abundant intragenic cleavage products are generated in the \( \Omega CRISPR VI(spce) \) strain. Representative of two biological replicates. c, The four genomic regions in b shown for the native type VI CRISPR host \( L. \) seeligeri, wild-type (red) and \( \Delta CRISPR \) (grey), 15 min after aTc-mediated induction of a target transcript. The dcw cluster is broken into two operons in \( L. \) seeligeri. Representative of two biological replicates.
Extended Data Fig. 4 | Trans-RNase activity is sufficient to limit growth of both φRR4 phage and ΔCRISPR13 host. a, *L. ivanovii* RR3 and ΔCRISPR13(spca, spce or spcl) strains at OD<sub>600</sub> = 0.05 were infected with φRR4 at an MOI of 1 and growth was monitored over 24 h. Each curve represents the mean ± s.e.m. of three biological replicates.

b, Quantification of φRR4 infective centres over time on wild-type *L. ivanovii* RR3. Cells were infected with φRR4 at an MOI of 0.1 and allowed to adsorb for 5 min, and then cells were washed three times to remove free phage. Infective centres were counted every 30 min by counting plaque-forming units on a lawn of phage-susceptible RR3 cells. Each data point represents the mean ± s.e.m. of three biological replicates.

c, Survival of the indicated strains during φRR4 infection at an MOI of 2. CFU titres were measured before infection (P) and 4 h after infection (IN) or mock infection (UN). Each bar represents the mean ± s.e.m. of three biological replicates.
Extended Data Fig. 5 | Activation of Cas13a induces reversible dormancy of host cells. a, Growth arrest (measured as culture OD$_{600}$) induced by target transcription in wild-type L. seeligeri (but not the ΔCRISPR mutant) harbouring an aTc-inducible protospacer RNA. Arrowhead indicates addition of 100 ng ml$^{-1}$ aTc. Each data point represents the mean $\pm$ s.e.m. of three biological replicates. b, Wild-type and ΔCRISPR L. seeligeri cultures carrying an aTc-inducible target transcript were exposed to 100 ng ml$^{-1}$ aTc for 3 h as in a, then diluted (at time 0 h) to OD$_{600}$ = 0.05 in fresh medium in the presence or absence of aTc, and growth was monitored over 24 h. Each curve represents the mean $\pm$ s.e.m. of three biological replicates. c, Immediate reduction in CFU upon phage infection of L. ivanovii RR3 or ΔCRISPRV$^7$ strains. The indicated strains were infected with φRR4 at an MOI of 2, and CFU titres in the infected cultures were monitored over time. Pre-infection (P) and mock-infection titres were also measured. Each bar represents the mean $\pm$ s.e.m. of three biological replicates. d, Cell vitality within ΔCRISPRV$^7$ cultures during phage infection. Cell vitality was measured in samples of cultures from c by monitoring conversion of nonfluorescent resazurin to fluorescent resorufin at each time point. The resorufin signal from heat-killed cells was subtracted from all samples as background, and each signal was normalized to the pre-infection value. Live cell standards (10% and 50%, mixed with heat-killed cells) are shown to demonstrate the quantitative capability of the vitality assay. Each bar represents the mean $\pm$ s.e.m. of three biological replicates. e, Phage-susceptible L. ivanovii ΔCRISPRV$^7$(spcP) cells harbouring a spacer against an aTc-inducible plasmid target RNA (or empty vector control) were treated with aTc for 1 h to pre-activate Cas13a, then infected with φRR4 at an MOI of 1. Viable CFUs were counted before infection (PRE), 7 h after infection (T7) or after mock infection (UN). Two-sided Student’s t-test, ***$P = 0.0005$. Each bar represents the mean $\pm$ s.e.m. of three biological replicates.
**Extended Data Fig. 6** | Absence of CRISPR-resistant escape mutants and validation of engineered escaper phage. **a**, Efficiency of plaque-forming assays with wild-type φRR4 and engineered spcA-escaper phage φRR4acr infecting *L. ivanovii* RR3 and ΩCRISPRVI(spcA) strains. Phages were diluted and spotted onto top agar lawns containing the indicated strain. Escaper plaques were not observed in the presence of type VI CRISPR targeting. The φRR4acr mutant, which lacks the acr region targeted by spcA, is viable and evades CRISPR targeting. Representative of two biological replicates.

**b**, As in a, but testing the spcE-escaper phage φRR4acr and the ΩCRISPRVI(spcE) strain. Representative of two biological replicates.

**c**, Design of the φRR4acr mutant, harbouring a deletion of the putative anti-CRISPR genes of φRR4.

**d**, Design of the φRR4acr mutant, depicting the wild-type and mutant spcE target sequence.

**e**, Cells pre-infected with wild-type φRR4 continue to adsorb escaper phage 2 h after infection. ΩCRISPRVI(spcE) cells were infected with wild-type φRR4 at an MOI of 5 for 2 h (an uninfected control is shown for comparison), then washed three times with fresh medium to remove free phages. φRR4acr was added to cells (or to a cell-free control) at an MOI of 0.1 for 5 min, cells and bound phage were pelleted, and free phage in the supernatant were quantified as PFUs on a lawn of ΩCRISPRVI(spcE) cells. Mean PFU values are shown from two biological replicates.

**f**, Efficiency of plaque-forming assay using RR3 and ΩCRISPRII(spcE) strains. We generated a ΩCRISPRII(spcE) strain carrying the type II-A CRISPR system from *S. pyogenes* programmed with spcE against φRR4. This strain has very limited immunity to wild-type φRR4, but is highly immune to the φRR4acr mutant, which lacks anti-CRISPR–Cas9 genes. Cas9-resistant φRR4acr escaper plaques are evident in the plaque assay (yellow arrowheads). One escaper, φRR4acr-esc, was isolated and confirmed to be resistant to Cas9 targeting. Representative of two biological replicates.
### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- **n/a** Confirmed
- [ ] The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement
- [ ] A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [ ] The statistical test(s) used AND whether they are one- or two-sided
  - *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- [ ] A description of all covariates tested
- [ ] A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- [ ] A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- [ ] For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted
  - *Give $P$ values as exact values whenever suitable.*
- [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- [ ] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- [ ] Estimates of effect sizes (e.g. Cohen's $d$, Pearson's $r$), indicating how they were calculated

*Our web collection on [statistics for biologists](https://www.ncbi.nlm.nih.gov/books/NBK541098/) contains articles on many of the points above.*

### Software and code

**Policy information about [availability of computer code](https://www.nature.com/nr/misc/how-to-include-software-data-availability-policy.pdf)**

| Data collection | Genome sequencing, spacer library sequencing, and transcript 5' end mapping data were collected using Illumina MiSeq Control Software v2.6.2.1
|                 | Conventional RNA-seq data was collected using Illumina NextSeq 500 Control Software v4.0
|                 | qPCR data in Figure 3a was collected using Applied Biosystems QuantStudio 12k flex
|                 | Growth curve data in Figs 3b, Extended Data 4b and 5b were collected with Tecan i-control v2.0
|                 | Resorufin fluorescence data in Extended Data 5d were collected with Tecan i-control v2.0 |

**Data analysis**

For genome sequencing:

- Reads were quality-trimmed using Sickle ([https://github.com/najoshi/sickle](https://github.com/najoshi/sickle))
- Reads were assembled into contigs using Abyss ([https://github.com/bcgsc/abyss](https://github.com/bcgsc/abyss))
- Contigs were mapped to reference L. seeligeri and L. ivanovii genomes using Medusa ([http://combo.dbe.unifi.it/medusa](http://combo.dbe.unifi.it/medusa))

Spacer library analysis:

- Spacer reads were mapped to the phage genome using bowtie2 ([http://bowtie-bio.sourceforge.net/bowtie2/index.shtml](http://bowtie-bio.sourceforge.net/bowtie2/index.shtml))
- Custom Perl scripts (custom scripts) were used to normalize spacer read counts, filter spacers containing fewer than 10 reads (pre-infection), and calculate enrichment ratios. These scripts are available upon request, as mentioned in the Code Availability statement below (and in the Methods section).

RNA-seq analysis:

- RNA-seq reads (both conventional and 5’ end mapping) were mapped to the host and phage genomes using bowtie2 ([http://bowtiebio.sourceforge.net/bowtie2/index.shtml](http://bowtiebio.sourceforge.net/bowtie2/index.shtml))
- Custom Perl scripts (custom scripts) were used to designate transcriptional start sites, count transcript 5’ ends within and downstream of the transcriptional start sites, filter transcripts containing fewer than 20 reads, and calculate corresponding cleavage ratios for each transcript. These scripts are available upon request, as mentioned in the Code Availability statement below (and in the Methods section).
Code Availability statement: Custom scripts used in analysis of spacer library data as well as RNA 5’ end mapping data are available upon request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The L. seeligeri RR4 and L. ivanovii RR3 genome sequences, along with raw reads from the spacer library deep sequencing, paired end RNA-seq, and 5’ end mapping have been deposited in the Sequence Read Archive under BioProject accession number PRJNA512236. Lists of strains, plasmids, and oligonucleotides used in this study are available in Supplementary Information 5.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No sample size calculation was performed. All experiments were performed with sample sizes based on standard protocols in the field. |
|-------------|-------------------------------------------------------------------------------------------------------------------|
| Data exclusions | In spacer library analysis, spacers with fewer than 10 reads (pre-infection) were discarded to reduce noise and report accurate enrichment values. For transcript 5’ end analysis, the transcriptional start sites could not be accurately determined for transcripts with fewer than 20 reads, therefore these data were excluded from analysis. |
| Replication | All measurements of phage infection efficiency, phage DNA content, and cell survival were performed in at least biological triplicate. NGS experiments were performed in biological duplicates. |
| Randomization | n/a. Animal or human research subjects were not involved in this study. None of the experiments were randomized. |
| Blinding | n/a. Animal or human research subjects were not involved in this study. None of the investigators were blinded. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ✗   | Antibodies            |
| ✗   | Eukaryotic cell lines |
| ✗   | Palaeontology         |
| ✗   | Animals and other organisms |
| ✗   | Human research participants |
| ✗   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ✗   | ChIP-seq              |
| ✗   | Flow cytometry        |
| ✗   | MRI-based neuroimaging |