SUPPLEMENTARY INFORMATION

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

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Supplementary Figures and Legends

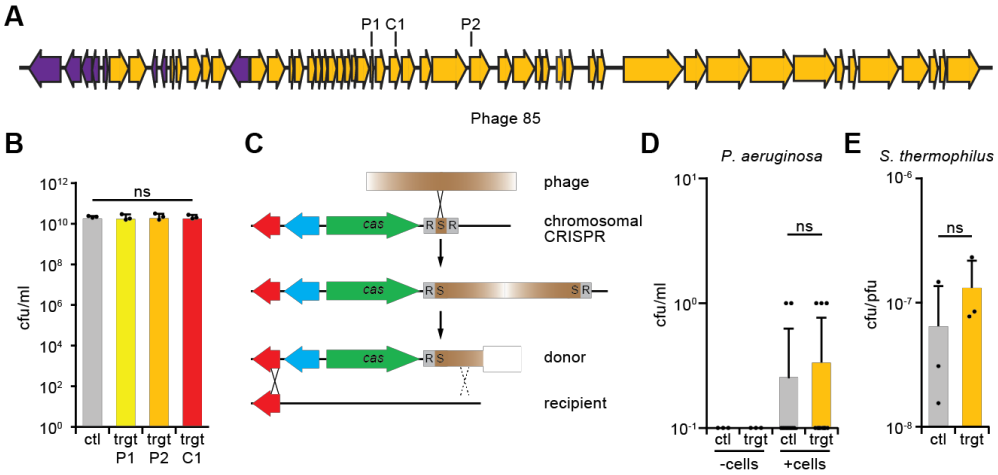

Fig. S1. Spacer-mediated transduction of chromosomal loci. (**A**) Schematic of *S. aureus* phage 85. P1 and P2 indicate the insertion sites of the spacer 1 target from the chromosomally encoded type III-A system of 08BA02176. C1 indicates target location of a spacer engineered into the type III-A system locus. (**B**) Recipient cells from the experiment in 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. One-way ANOVA used to calculate *P* value; ns, not significant ($p = 0.92$). (**C**) Recombination between the spacer of a chromosomal CRISPR-Cas locus and the protospacer in the phage genome. After transfer assisted by the phage packaging machinery, the recombinant molecule only has homology for a single crossover event (solid lines), while lacking homology for the second crossover (dotted lines), and therefore the transfer of a complete CRISPR-Cas locus is highly unlikely. (**D**) Spacer-mediated transduction of the *P. aeruginosa* type I-F CRISPR-Cas system. Overnight cultures (+cells) with (trgt) or without (ctl) a phage-targeting spacer were infected on soft agar plates with a DMS3*vir* phage containing a target for recombination with the spacer but with a PAM mutation to allow its propagation. Plates without cells were used as a control (-cells). 24 hours post-infection phage was harvested from the soft agar and transducing phage particles were determined by infecting recipients lacking CRISPR and plating them with the appropriate antibiotic selection. CRISPR-Cas transduction was confirmed by PCR (not shown). Mean + STD of 3 (-cell) or 8 biological replicates (+cells) are reported. Two-tailed unpaired *t*-test was used to calculate *P* value; ns, not significant ($p = 0.63$). (**E**) Spacer-mediated transduction of the *S. thermophilus* type II-A CRISPR-Cas system. Liquid cultures with (trgt) or without (ctl) a phage-targeting spacer were infected with phage 2972 carrying a target for recombination with the spacer but with a PAM mutation to allow its propagation. Three hours post-infection lysates were harvested and transducing phage particles were determined by infecting a culture lacking CRISPR-Cas at an MOI of 1 and plating for antibiotic resistance. CRISPR-Cas transduction was confirmed by PCR (not shown). Mean + STD of 3 biological replicates are reported. Two-tailed unpaired *t*-test was used to calculate *P* value; ns, not significant ($p = 0.36$).

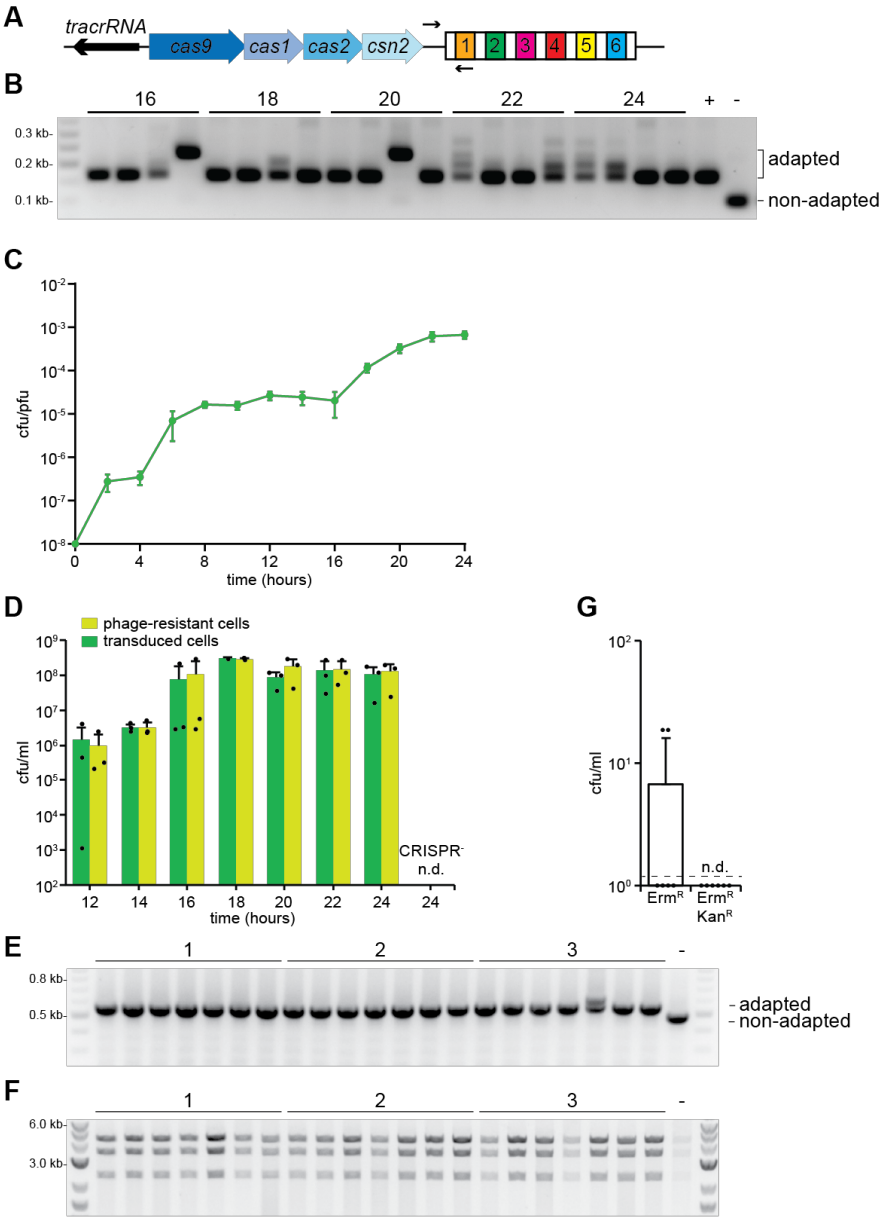

Fig. S2. Analysis of pCRISPR transductants. (**A**) Organization of *S. pyogenes* CRISPR-*cas* locus. Arrows indicated annealing positions of primers used to detect spacer acquisition (**B**) PCR amplification and analysis of the CRISPR array of pCRISPR transductants obtained in Fig. 1D. Numbers indicate the time point (in hours) of the collection of the transducing lysates. Four transductant colonies were check at each time point. (+) single spacer acquisition positive control; (-) no spacer acquisition negative control. These results were representative of three independent experiments. (**C**) Transduction rate data from Fig. 1D reported as cfu/pfu, to enable comparison with other experiments in the manuscript. Mean ± STD of 3 biological replicates are reported. (**D**) Comparison of pCRISPR transductants (chloramphenicol-resistant cfu) and total phage-resistant bacteria (total cfu) after infection of staphylococci lacking CRISPR with lysates from the different time points of Fig. 1D. The data shows that

almost all recipients gain phage resistance through pCRISPR transduction. Limit of detection is 100 cfu/ml. Mean + STD of 3 biological replicates are reported. (**E**) Phage-resistant transductants from Fig. 1D contained expanded CRISPR arrays. PCR-based analysis to check for spacer acquisition in transduced colonies collected in Fig. 1D at 18 hours post infection. Numbers indicate different replicates. A non-adapted control is also shown (-). These results were representative of three independent experiments. (**F**) Phage-resistant transductants from Fig. 1D contain intact pCRISPR plasmids. Restriction enzyme digest (HindIII) of pCRISPR plasmid DNA from transduced colonies collected in Fig. 1D at 18 hours post infection. Numbers indicate different replicates. Non-adapted control is also shown. These results were representative of three independent experiments. (**G**) The recipient cells from Fig. 1E contained two chromosomal antibiotic tags to determine the possibility of reverse transduction from the recipient cells into the pCRISPR staphylococci that could confound results, but these double-marker transduction events were undetectable. 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 6 biological replicates are reported.

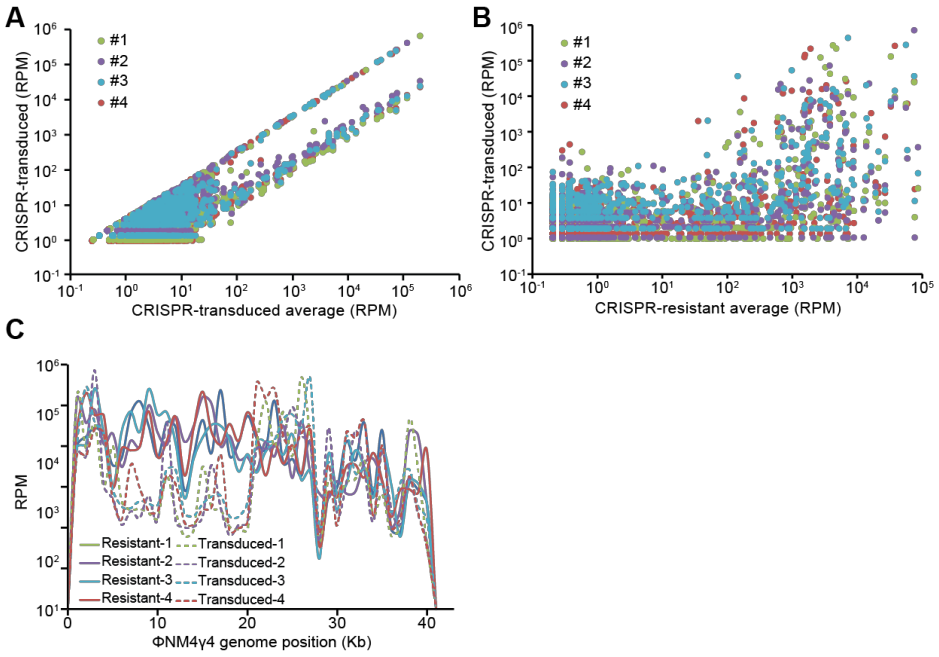

Fig. S3. Distribution pattern of acquired and transduced spacers. (**A**) The average frequency of CRISPR-transduced reads plotted against the 4 individual replicates of CRISPR-transduced reads (#1-4). Each point represents a single spacer and its representation in sequenced populations. (**B**) The 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.

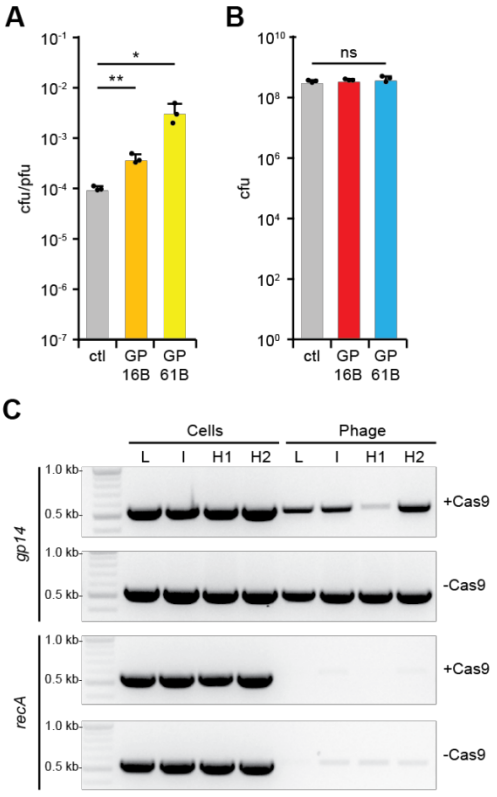

Fig. S4. Phage recombination with CRISPR spacers enhance transduction. (**A**) Enhanced levels of transduction when the type III-A system from *S. epidermidis* RP62A contains spacers matching the infecting phage genome. Transduction of plasmids containing the type III-A locus from *S. epidermidis* RP62A, with either a control spacer (ctl) or ΦNM1γ6 spacers, complimentary to bottom-strand (GP16B, GP61B). Cultures were infected with ΦNM1γ6 at a MOI of 50 and phage was collected 1.5 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. Two-tailed unpaired *t*-test was used to calculate *P* value,**p* = 0.022, ***p* = 0.0043 (**B**) Enhanced levels of transduction observed in Fig. S4A are not due to differences in viability of the recipient cells.Transduction recipient cells from Fig. S4A 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. One-way ANOVA used to calculate *P* value; ns, not significant (*p* = 0.29). (**C**) PCR check of cell and phage DNA in the samples of Fig. 2D. Amplification of phage locus *gp14* and of chromosomal locus *recA* were used to check for the presence of phage DNA inside cells (effective infection) and for the lack of cell DNA in filtered lysates, respectively. These results were representative of three independent experiments.

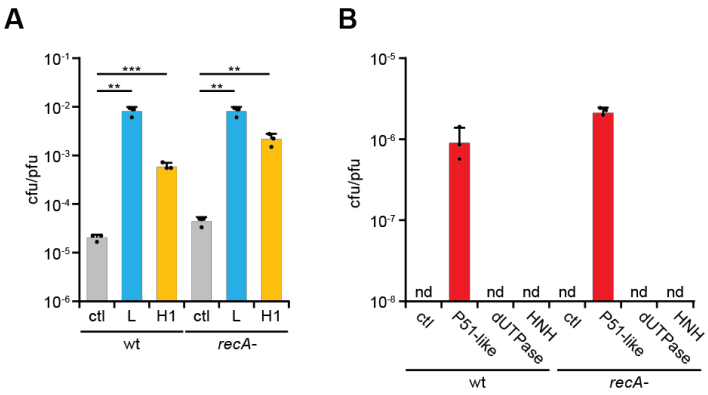

Fig. S5. RecA is not required for spacer-enhanced transduction. (**A**) Spacer-mediated enhancement of transduction was observed in the absence of *recA*, suggesting that the phage, rather than the host recombinases, are required for this phenomenon. Transduction of pSpacer plasmids carrying either a control spacer (ctl) or ΦNM4γ4-targeting (L, H1) spacers in wild-type (wt) or *recA*- donor cells. 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. Two-tailed unpaired *t*-test was used to calculate *P* value; wt: ***p* = 0.0014, ****p* = 0.0004; *recA*-: ***p*(L) = 0.0014, ***p*(H1) = 0.0046 (**B**) Generalized transduction is typically detected with *pac* phages such as ΦNM4γ4, but not with *cos* phages. However, transduction of DNA molecules that result from recombination events between the CRISPR spacer and the phage protospacer could be viewed as an alternative form of specialized transduction, which can be achieved by both *pac* and *cos* phages. Therefore, we investigated if the *cos* phage Φ12γ3 could support spacer-mediated transduction. As expected for *cos* phages, no transduction is observed when using a control spacer (a generalized transduction event), and one out of the three spacers targeting Φ12γ3 was able to support CRISPR-Cas transfer independently of *recA*, suggesting that, as in the case of ΦNM4γ4-mediated transduction, there could be high- and low-transducing spacers. 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 pCRISPR plasmid. Mean + STD of 3 biological replicates are reported.

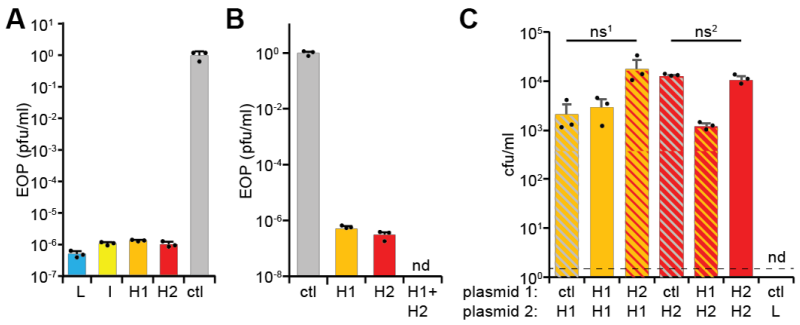

Fig. S6. CRISPR escaper levels do not affect pCRISPR transduction. (**A**) The rate of ΦNM4γ4 escape from the immunity provided by H1, H2, I or L spacers was measured and found that escapers of all four spacers at occurred at equal rates. ΦNM4γ4 plaquing efficiency on soft agar lawns of high, intermediate, 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**) Cells harboring both H1 and H2 spacers drastically diminish the production of escapers. ΦNM4γ4 plaquing efficiency on soft agar lawns of individual spacers or combined spacers (H1+H2) expressed from different plasmids. Limit of detection is 10^{-9} pfu/ml. Mean + STD of 3 biological replicates are reported. (**C**) Despite a drastic reduction in the number of escapers, the combination of H1 and H2 spacers did not significantly alter the efficiency of transduction of the CRISPR-Cas system. 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-Cas plasmid. Data condensed from (Fig. 4C). Limit of detection is 1.5 cfu/ml (dotted line). Mean + STD of 3 biological replicates are reported. Two-tailed unpaired *t*-test was used to calculate *P* value; ns, not significant ($p^1 = 0.075$, $p^2 = 0.20$).

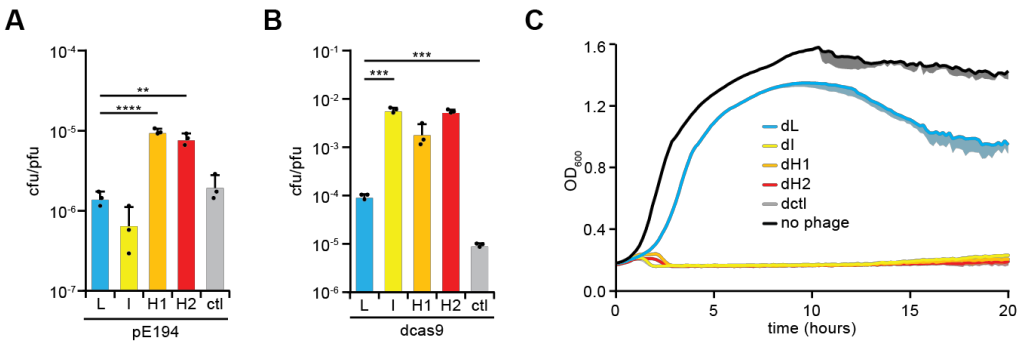

Fig. S7. Levels of CRISPR immunity dictate transduction of pCRISPR. (**A**) Transduction of a second plasmid (pE194, erythromycin-resistant) from cells also containing the different pCRISPR plasmids (with spacers L, I, H1, H2 or a nontargeting spacer, ctl). Two-plasmid cultures were infected with ΦNM4γ4 at a MOI of 50 and lysates were collected 90 minutes post infection. pE194 transduction was determined by infecting a susceptible culture at a MOI of 1 with the lysates and plating transductants in the presence of erythromycin. Mean + STD of 3 biological replicates are reported. Two-tailed unpaired *t*-test was used to calculate *P* value, ***p* = 0.0014,*****p* = 0.000059. (**B**) Enhanced transduction for all pCRISPR plasmids was observed in a dCas9 background, but at lower level for spacer L when compared to experiments were Cas9 was entirely eliminated. Transducing particles produced by cells harboring dCas9 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 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 transduced plasmid. Mean + STD of 3 biological replicates are reported. Two-tailed unpaired *t*-test was used to calculate *P* value, ****p*(ctl) = 0.0002, ****p*(I) =0.0005. **(C)** The reduction in transduction efficiency of spacer L can be attributed to the unexpectedly high protection provided by dCas9 programmed with this spacer, as opposed to the complete susceptibly to phage infection in the presence of I, H1, and H2 crRNAs. Growth of cells harboring dCas9 and control (ctl), or targeting spacers (L, I H1, H2). Indicated strains were infected with ΦNM4γ4 at an MOI of 1. Growth was determined by optical density measured at 600 nm (OD_{600}) and compared to an uninfected control (no phage). Mean -STD of 3 biological replicates are reported.

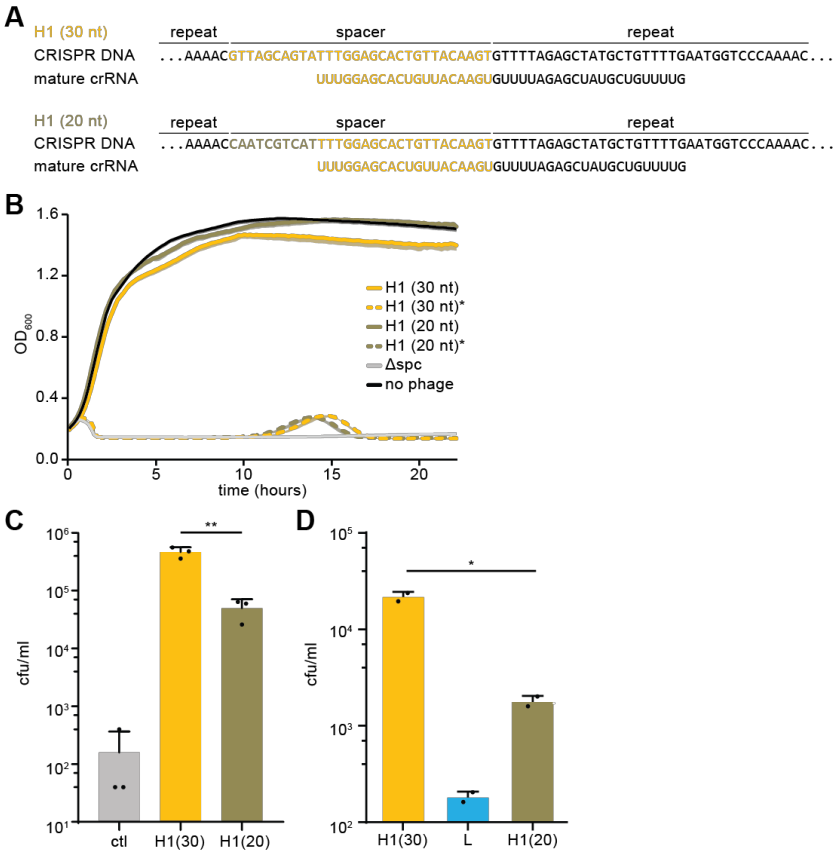

Fig. S8. Spacer sequences dispensable for targeting enhance transduction. (**A**) To test whether the 10 additional nucleotides in the spacer DNA of type II-A facilitates transduction, 10 mismatches were introduced at the 5' end of the highly transduced H1 spacer. Schematic of CRISPR DNA locus and mature crRNA for full-length H1 (30 nt) or truncated H1 (20 nt), containing 10 mismatches in the 5' end of the spacer (**B**) The truncated spacer provided equal levels of protection to the host as the full length H1 spacer. 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, we added an excess of non-CRISPR cells to allow high levels of phage propagation. Growth was determined by optical density measured at 600 nm (OD_{600}) and compared to an uninfected control (no phage). Mean -STD of 3 biological replicates are reported. (**C**) The truncated spacer reduced the rate of transduction of pSpacer by an order of magnitude. Cells harboring plasmids with either a control spacer (ctl) or the targeting spacers from (A) were infected with ΦNM4γ4 at a MOI of 1 and phage filtrates were 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. Two-tailed unpaired *t*-test was used to calculate *P* value, $^{**}p = .0022$. (**D**) The truncated spacer reduced the rate of transduction of the CRISPR-*cas* locus by an order of magnitude in the presence of Cas9. Cells harboring pCRISPR plasmids with H1(30), H1(20) or L spacers were infected with ΦNM4γ4 at a MOI of 50 and phage filtrates 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 2 biological replicates are reported. Two-tailed unpaired *t*-test was used to calculate *P* value, **p* = 0.010.

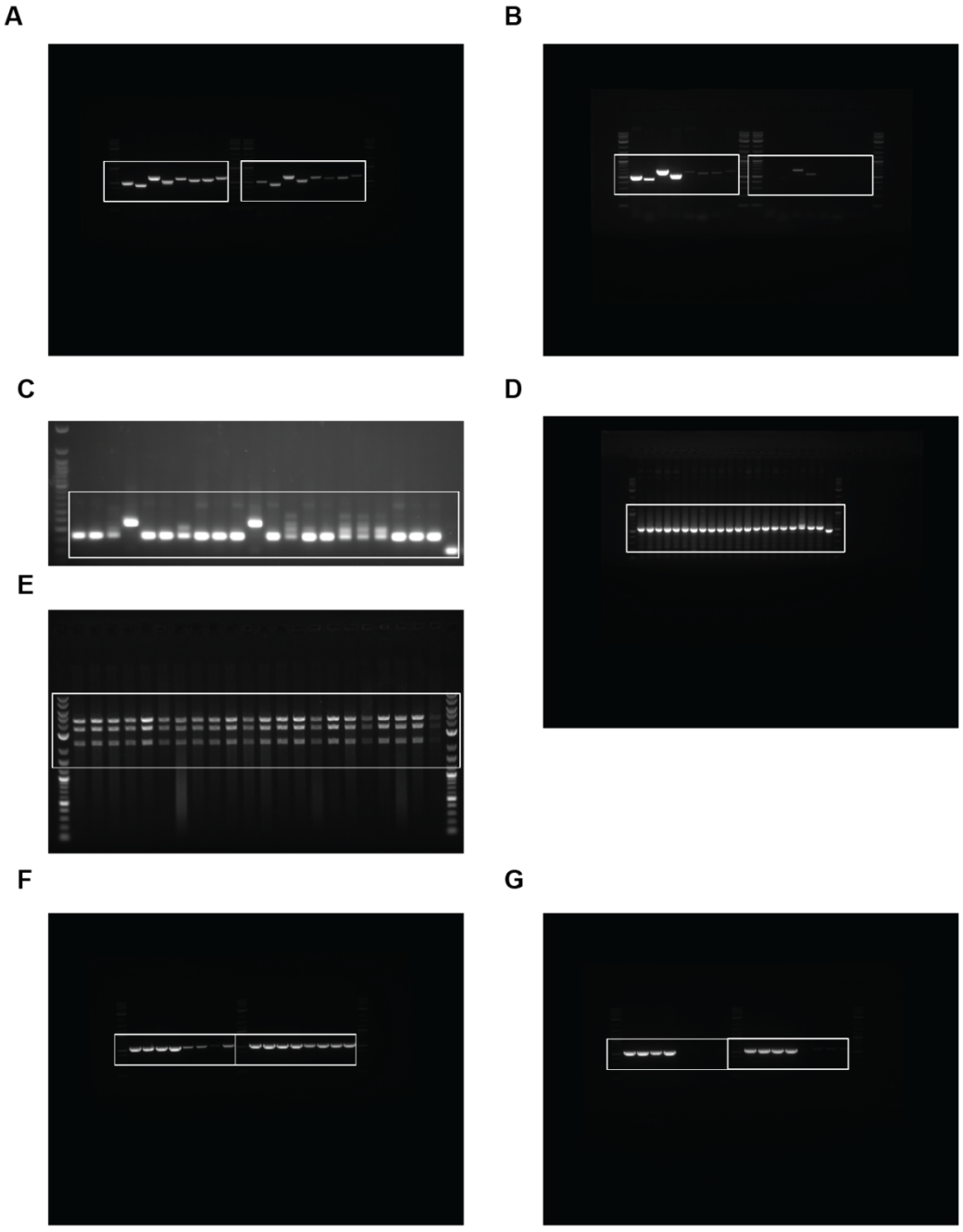

Fig. S9. Original images used in his study. (**A**) Fig. 2D –Cas9. **(B)** Fig. 2D. +Cas9. **(C)** Fig. S2B. **(D)** Fig. S2E. **(E)** Fig. S2F. **(F)** Fig. S4D. *gp14* **(G)** Fig. S4D *recA*. Boxes represent the area cropped and included in the corresponding figure.

Supplementary Tables.

Supplementary table S1. Strains used in this study.

Strains	Description	Reference
RN4220	*S. aureus* strain	[1]
OS2	Chromosomal erythromycin resistance RN4220	[2]
08BA02176	*S. aureus* MRSA with CRISPR-Cas type III-A	[3]
DGCC7710	*S. thermophilus* strain	[4]
PA14	*P. aeruginosa* strain	[5]
PA14--SM	*P. aeruginosa* strain streptomycin tagged type I-F	This study
JW263	Chromosomal kanamycin resistance RN4220	[6]
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-Cas system on pC194	[7]
pWJ244	*E. coli* ColE1 vector for genome engineering	[6]
pWJ326	*S. aureus* temperature-sensitive phagemid	[6]
pC194	Chloramphenicol-resistant *S. aureus* plasmid	[8]
pE194	Erythromycin-resistant *S. aureus* plasmid	[9]
pT181	Tetracycline-resistant *S. aureus* plasmid	[10]
pLZ12spec	Spectinomycin resistant cloning vector	[11]
TM17	Chromosomal expression of pT181 *repC*	[12]
pTM402	pT181 *ds repC- cop 623* replication origin	[12]
pBAM1-Sm	Streptomycin resistance cassette	[13]
pHERD30T	*E. coli/P. aeruginosa* shuttle vector	[14]
pGG4	Type III-A CRISPR-Cas GP16B targeting plasmid on pC194	[15]
pGG12	Type III-A CRISPR-Cas DUF1318 targeting plasmid on pC194	[15]
pGG15	Type III-A CRISPR-Cas GP61B targeting plasmid on pC194	[15]
pGG-BsaIR	Type III-A CRISPR-Cas control spacer on pC194	[6]
pDB114	Control spacer with *cas9*	[16]
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

pAV305	H1 with *dcas9* on pC194	This study
pAV306	L with *dcas9* on pC194	This study
pAV307	I with *dcas9* on pC194	This study
pAV308	H2 with *dcas9* on pC194	This study
pAV309	Control spacer with *dcas9* on pC194	This study
ΦNM1γ6	Obligately lytic *S. aureus pac* phage	[15]
ΦNM4γ4	Obligately lytic *S. aureus pac* phage	[7]
Φ12γ3	Obligately lytic *S. aureus cos* phage	[6]
85	*S. aureus* phage	[17]
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	[18]
2972α1	2972 JAV33 PAM escaper phage	This study
DMS3vir	Virulent *P. aeruginosa* mu-like phage	[19]

Supplementary table S2. DNA oligonucleotides used in this study.

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
AV439	TAATACGACTCACTATAGGGCTAAATTCAGTGATGTATTCGTTTTAGAGCTATGCTGTT
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
AV622	CTCGGAAGGTATACAGTCCACTTTTACCACTTTTTTAGAGTGAC
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
AV868	CATAGAAAAATACGGTTCTCAAGGAAG
AV869	CTACGGATTGAAGAACGGTTTAGC
AV876	CCAAGACCCTGAATTGGAAGTC
AV877	GCATTGATTTCTTTTTCAATGCGC
AV878	GCTGTCCTTGTGAGTGATCGATAACTACCCCGAATAACAGGGGACGAGAATTCTATA
AV879	CACTCACAAGGACAGCGTATGTTGAAGAATGTTGAAAATTCTCGTCCCCTGTTATTC
AV880	GCTCGGAAGGTATACAGTCGAATAATGGCTCTATTACAACGGTAC
AV881	CACTCTCGGACAATACTCCATCCCCTAAAAATTAATCAATGCG
B337	GACGCTATTTGTGCCGATAGCTAAGCCTATTGAGTATTTC
B338	GAAATACTCAATAGGCTTAGCTATCGGCACAAATAGCGTC
B339	GGAAACTTTGTGGAACAATGGCATCGACATCATAATCACT
B340	AGTGATTATGATGTCGATGCCATTGTTCCACAAAGTTTCC
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
JW696[a]	aaaacAACGATGTGAAAGTGTTTAGATTAGCGCTC
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 information Data File 1 (separate file)

Next-generation sequencing data used to generate figures 2A, S2A, S2B, and S2C.

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