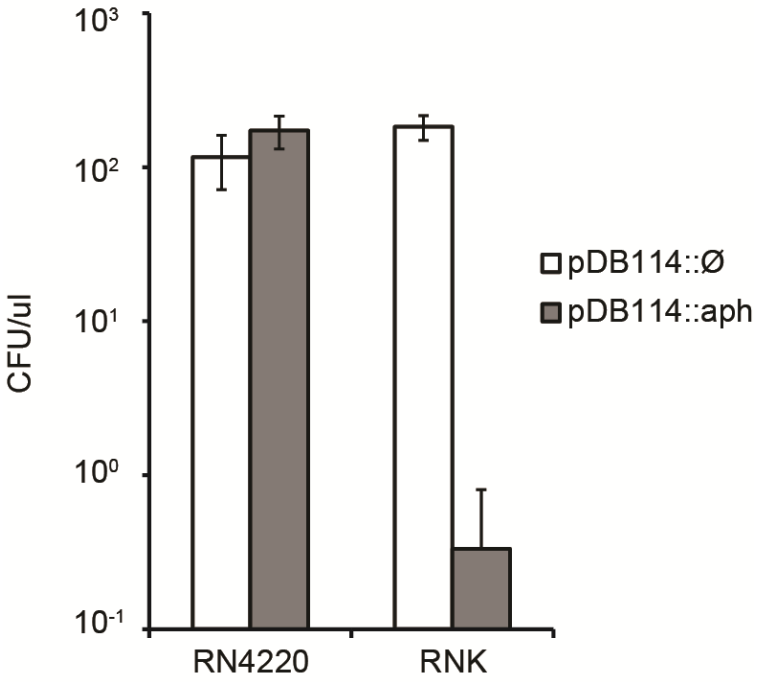
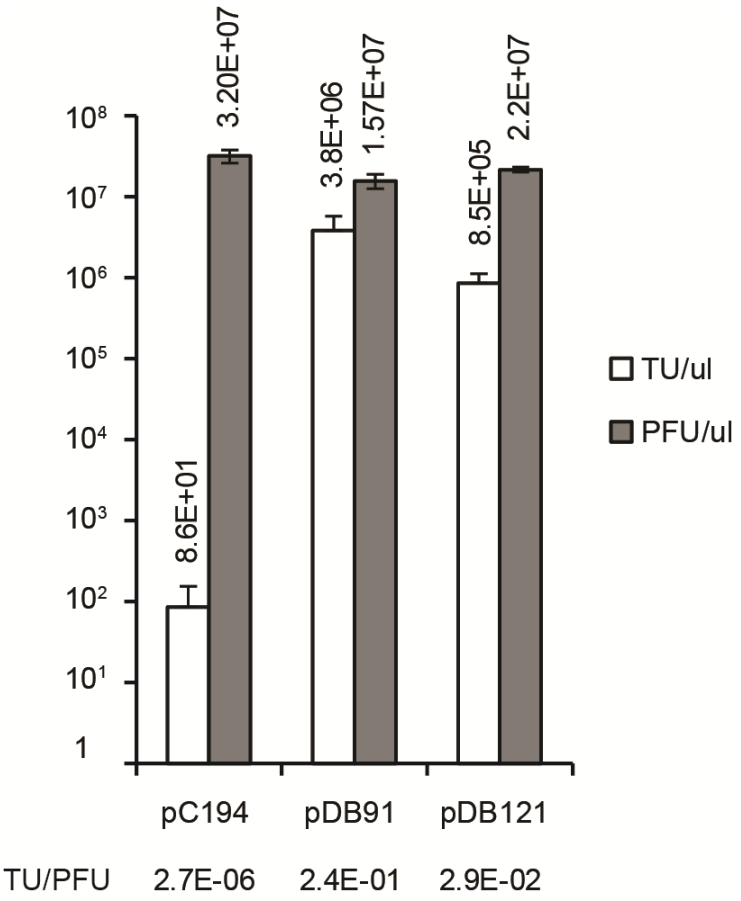
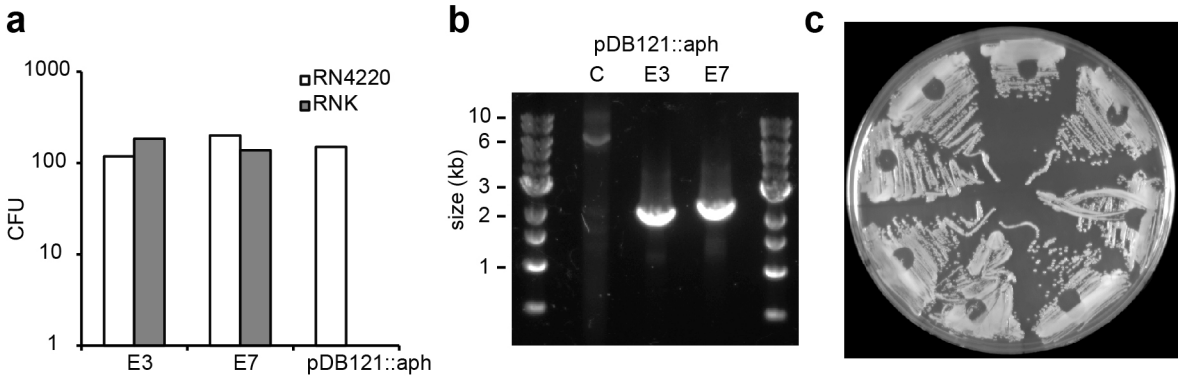
SUPPLEMENTARY MATERIALS
Supplementary Figures



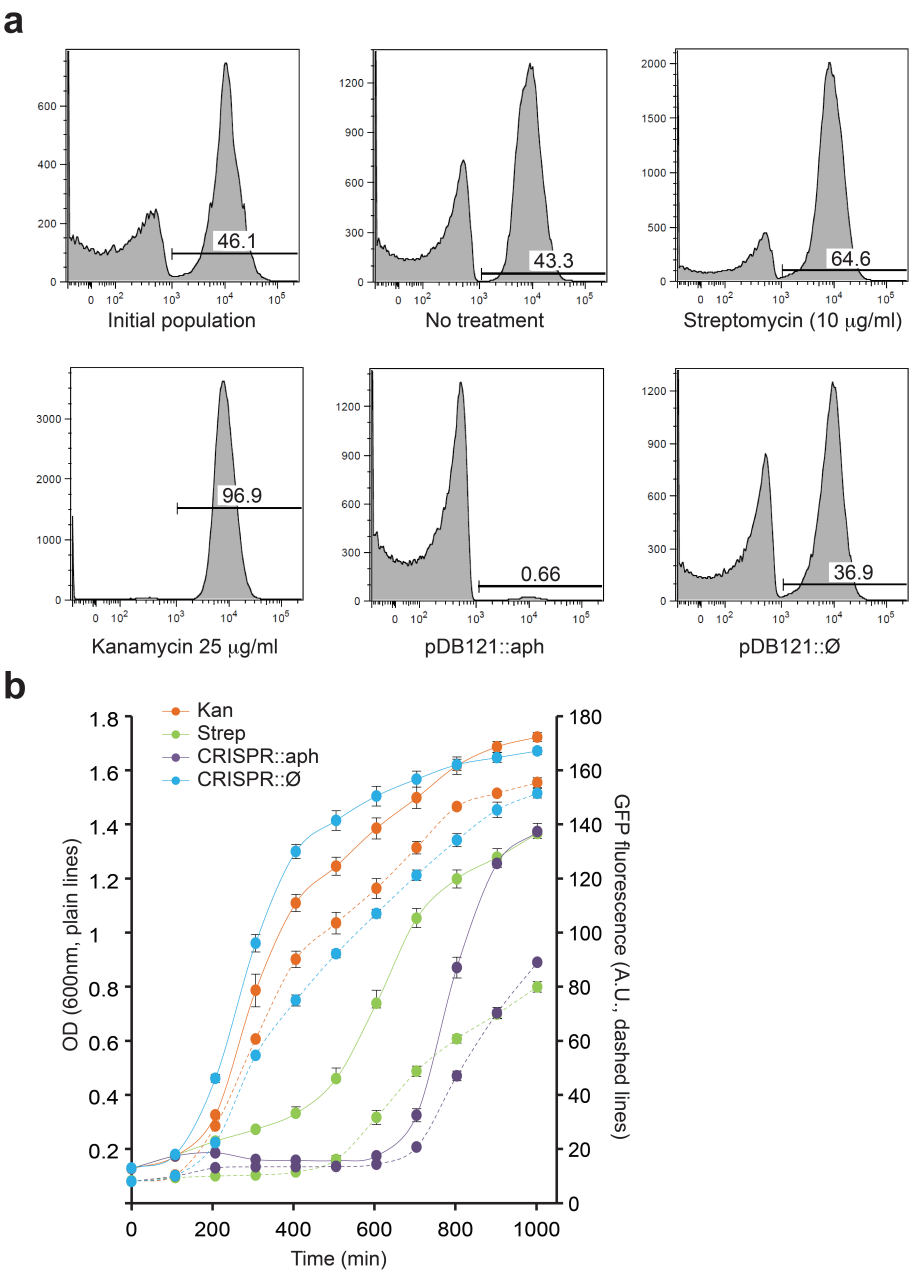
Supplementary Figure 1. The *S. pyogenes* CRISPR-Cas system can be programmed to kill *S. aureus*. Plasmid pDB114 carries the *S. pyogenes* tracrRNA, *cas9* and a minimal array containing two repeats separated by a sequence containing BsaI restriction sites used to clone crRNA guide sequences using annealed oligonucleotides. pDB114 was programmed to target the *aph-3* kanamycin resistance gene and transformed either in electrocompetent RN4220 cells or RNK cells carrying *aph-3* in the chromosome. Chloramphenicol resistant CFU obtained in three independent assays are reported (mean ± s.d.).



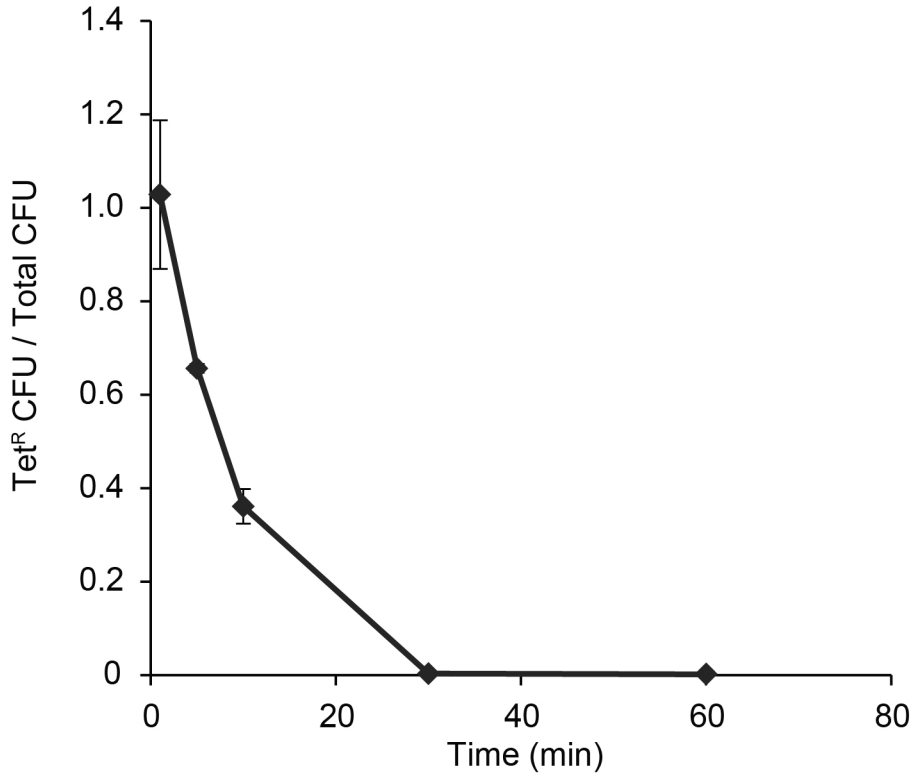
Supplementary Figure 2. Phagemid transduction efficiency. ΦNM1 lysates were prepared on RN4220 cells containing either pC194, pDB91 or pDB121. PFU/µl and TU/µl were measured as described in materials and methods (mean ± s.d.).



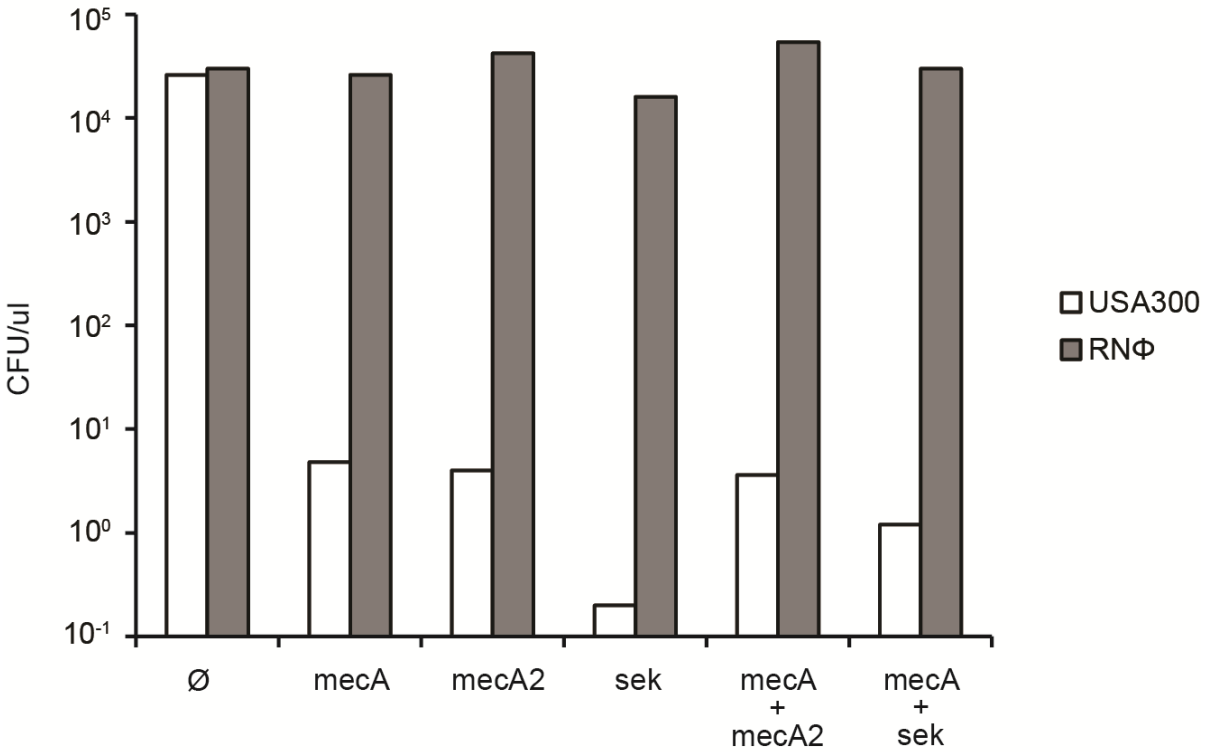
Supplementary Figure 3. Analysis of survivor colonies. Eight colonies of RNK$^{\Phi}$ that survived a treatment with pDB121::aph were re-streaked on TSA-chloramphenicol plates. Two chloramphenicol-resistant colonies were isolated, indicating that they carried the pDB121 plasmid (E3 and E7). (**a**) The plasmids from E3 and E7 were purified and retransformed in RN4220 or RNK electrocompetent cells and plated on TSA-chloramphenicol. While a control pDB121::aph plasmid did not yield any colonies when transformed in RNK, plasmids prepared from E3 and E7 could be efficiently transformed, indicating that the CRISPR system was no longer functional. (**b**) PCR amplification of the Cas9 region with primers B113 and W270 on pDB121::aph (control, C) and the E3 and E7 escapers, showing a deletion of the whole region. The expected size of the control is 7,137bp. (**c**) In order to assess the integrity of the target, the survivor colonies were streaked on a HIA containing 5 mM $CaCl_2$ plate and a drop of pDB121::aph phagemid was added on top. Clearance indicates that the isolated colonies are still sensitive; hence the target is still intact.



Supplementary Figure 4. Determination of GFP fluorescence as a measure of cell growth after phagemid or antibiotic treatment. (**a**) Flow cytometry of cells shown in Fig. 1d either before, without or with different treatments. Y-axis, GFP fluorescence in absolute units); X-axis, number of cells. (**b**) Same as Fig. 1d but using a monoculture of RNK cells.



Supplementary Figure 5. Measurement of pUSA02-mediated tetracycline resistance after the addition of the CRISPR-Cas9 phagemid. S. aureus Newman cells carrying the pUSA02 plasmid were treated with phagemid pDB251, targeting the tetracycline-resistance (Tet^R) gene carried by the plasmid. At different times after phagemid addition to the culture, aliquots were plated in selective and non-selective media to calculate the percentage of cells harboring the plasmid. Results indicate the average and standard deviation of three independent experiments.



Supplementary Figure 6. Killing with multiple crRNA guides. Lysates of the pDB121 phagemid targeting carrying mecA, mecA2 or sek either alone or in combination were used to infect USA300 or RNK$^{\Phi}$ cells with a MOI of ~10. Cells were plated on non-selective TSA plates.

Supplementary Tables

Supplementary Table 1. Cfu counts of data represented in Fig. 3b (three independent experiments were performed for each treatment: 1-3).

PBS	Mouse #	GFP CFU	TOTAL CFU	GFP%
1	17	4.50E+05	1.05E+06	42.86
	18	6.50E+06	1.50E+07	43.33
	19	4.50E+07	1.05E+08	42.86
	20	1.00E+05	3.50E+05	28.57
2	24	8.00E+03	2.15E+04	37.21
	25	1.50E+04	5.00E+04	30.00
	26	5.00E+06	2.40E+07	20.83
	27	2.00E+05	9.00E+05	22.22
	28	6.50E+06	1.50E+07	43.33
3	20	1.50E+05	4.00E+05	37.50
	21	5.00E+04	3.00E+05	16.67
	22	2.00E+05	6.50E+05	30.77
	23	2.00E+04	9.50E+04	21.05
	24	5.00E+05	2.50E+06	20.00
	25	1.00E+04	2.50E+04	40.00
	Mean	**4.31E+06**	**1.10E+07**	**31.81**
	SD	**1.15E+07**	**2.70E+07**	**9.82**

ΦNM1	Mouse #	GFP CFU	TOTAL CFU	GFP%
1	11	2.50E+06	9.00E+06	27.78
	12	9.00E+06	2.00E+07	45.00
	13	1.20E+07	2.95E+07	40.68
	14	2.50E+06	6.00E+06	41.67
	15	1.50E+06	6.00E+06	25.00
	16	5.00E+05	2.50E+06	20.00
2	19	4.00E+06	9.50E+06	42.11
	20	5.00E+05	3.00E+06	16.67
	21	1.00E+05	3.00E+05	33.33
	22	1.40E+07	3.00E+07	46.67
	23	3.50E+06	1.90E+07	18.42
3	14	2.00E+05	4.50E+05	44.44
	16	3.50E+04	7.50E+04	46.67
	17	4.00E+04	1.90E+05	21.05
	18	1.50E+03	5.00E+03	30.00
	19	1.50E+04	5.00E+04	30.00
	Mean	**3.15E+06**	**8.47E+06**	**33.09**
	SD	**4.52E+06**	**1.05E+07**	**10.85**

DB121::aph	Mouse #	GFP CFU	TOTAL CFU	GFP%
1	1	5.00E+05	7.00E+06	7.14
	2	2.00E+05	1.50E+06	13.33
	3	2.00E+05	1.90E+06	10.53
	4	5.00E+04	1.00E+06	5.00
	5	2.50E+06	2.00E+07	12.50
2	1	5.00E+04	1.40E+06	3.57

	2	2.00E+05	1.05E+06	19.05
	3	2.00E+04	1.05E+05	19.05
	4	5.00E+06	2.70E+07	18.52
	5	5.00E+04	9.00E+05	5.56
	6	1.50E+04	1.05E+05	14.29
3	**1**	5.00E+05	6.50E+06	7.69
	3	5.00E+05	9.00E+06	5.56
	4	1.00E+06	5.50E+06	18.18
	Mean	**7.70E+05**	**5.93E+06**	**11.43**
	SD	**1.38E+06**	**8.09E+06**	**5.75**

Mupirocin	**Mouse #**	GFP CFU	TOTAL CFU	GFP%
1	**6**	2.00E+05	4.50E+05	44.44
	7	2.00E+04	8.00E+04	25.00
	8	3.00E+04	9.50E+04	31.58
	9	1.00E+03	4.00E+03	25.00
	10	<500	<500	
2	**7**	<500	<500	
	8	5.00E+03	2.00E+04	25.00
	9	2.00E+06	1.15E+07	17.39
	10	<500	<500	
	11	<500	<500	
3	**8**	<500	<500	
	9	2.00E+03	6.50E+03	30.77
	10	1.50E+03	5.00E+03	30.00
	11	1.50E+04	3.00E+04	50.00
	12	4.50E+04	8.00E+04	56.25
	13	<500	<500	
	Mean*	**2.32E+05**	**1.23E+06**	**33.54**
	SD*	**6.24E+05**	**3.61E+06**	**12.51**

* Mean and StDev calculated using only values above the limit of detection of the assay (500 cfus)

Supplementary Table 2. Spacers used in this study.

Target	Spacer sequence (5'-3')
aph	TCATGAGTGAGGCCGATGGCGTCCTTTGCT
mecA	TTTTGAGTTGAACCTGGTGAAGTTGTAATC
mecA2	CATTTTCTTTGCTAGAGTAGCACTCGAATT
sek	GATTATCAATTCCTATATCACCTTGAGCGC
pUSA01	CTTATGTAACTTCAAATAGCCTTCATCAGT
pUSA02	AGGAGTAGTATTAAAATGATTTGCAATATC

Supplementary Table 3. Oligonucleotides used in this study.

Number	Sequence (5'-3')
B113	ATTATAAAAGCCAGTCATTAGGCCTA
B127	AAAAGCATGCAAATATGAGCCAAATAAATATATTC
B233	TACTGGTACCTTTAAAAGCTTCTGTAGGTTTTTAG
B234	TTTAGGTACCAAGAGCGAGAGATAGAGATATTAAG
B235	TTTAGCATGCCTATAATCCTAGAGATTTTATTGTGT
B333	CTTTATCCAATTTTCGTTTGAACTCAACAAGTCTCAGTGTGCTG
B334	ACACTGAGACTTGTTGAGTTCAAACGAAAATTGGATAAAGTGGG
B351	ATCGTTTATCGTCGTCATCACAATAACTTTTAAAGATCTTGCATAATTCACGCTGAC
B628	AGTGGGAAACAACGCCCATGGAG
B629	GTTGAACGCATAAATCCAACAAG
B632	AGTCACCTCAAGTAAAGAGGTAA
B633	TGAAGGACCTAACCCTTCACCTA
L316	TTAAGGGTTCTTCTCAACGCAC
L318	TTAAAAGTTATTGTGATGACGACG
L362	aaactcgtgGATTCTGTGATTTGGATCCTTCC
L409	CGTGGTAAATCGGATAACGTTCCAAGTGAAG
L410	CTTCACTTGGAACGTTATCCGATTTACCACG
L482	aaaCTCGAGCTGAGAGTGCACCATATGCGG
L483	aaaCTCGAGCTTAATAGCTCACGCTATGCCG
L484	aaaCTCGAGCGCGCAAGCTGGGGATCCG
L485	aaaCTCGAGTAGGTACTAAAACAATTCATCCAG
W270	aaaaagatctTGCATAATTCACGCTGACCTC
W278	aaaaagatctTATGACTGTTATGTGGTTATCG
W282	aaaacacgagCGTTTGTTGAACTAATGGGTGC