SUPPLEMENTARY FIGURES

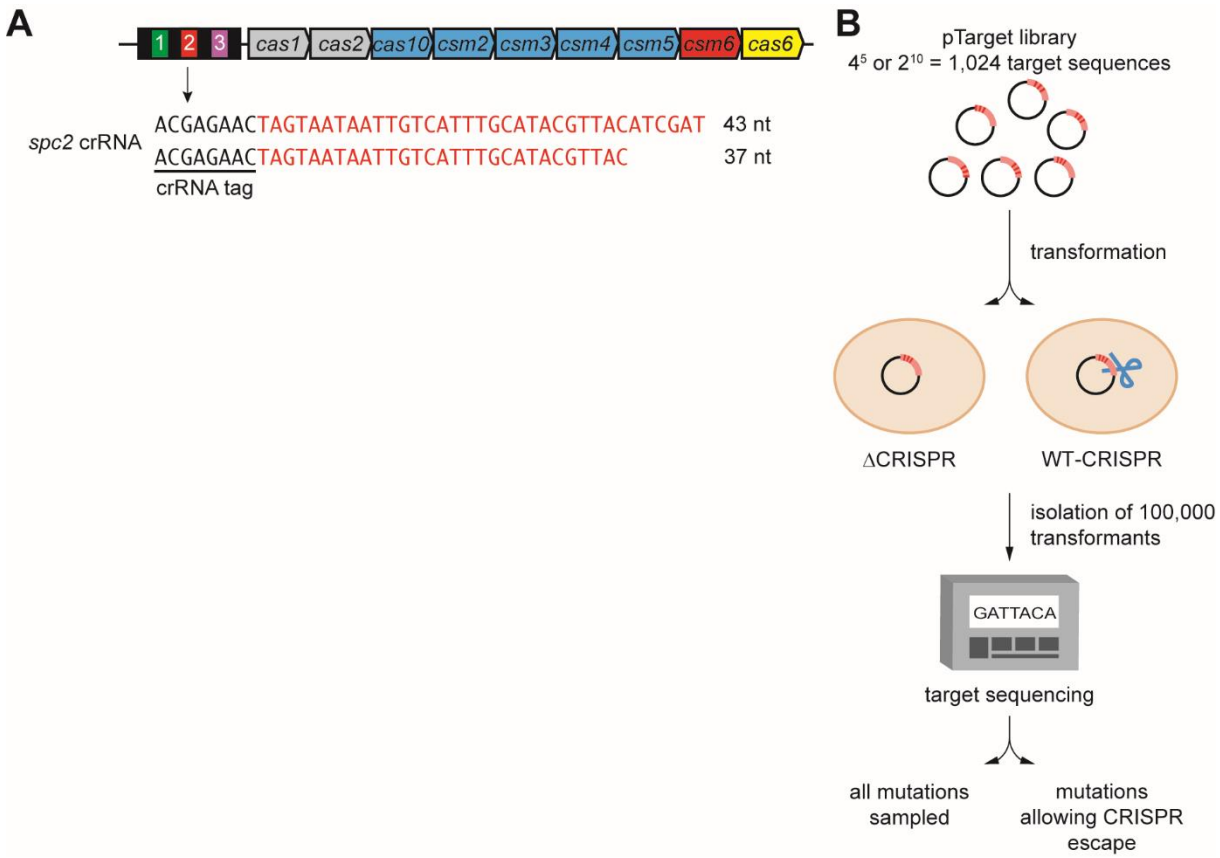

Fig. S1. Target library construction and next-generation sequencing. Related to Fig. 1. (**A**) Genetic organization of the S. epidermidis RP62a type III-A CRISPR-*cas* locus. This locus harbors three spacers (numbered, colored boxes) and nine *cas* genes. *cas1-2* (grey) are involved in the integration of new spacers into the CRISPR array. *cas10* and *csm2-5* (blue) encode a protein complex that contains the crRNA. *csm6* (red) encodes a non-sequence specific RNase that degrades target transcripts. *cas6* (yellow) encodes for the endoribonuclease that cleaves the crRNA precursor at every repeat sequence. After this cleavage, mature crRNAs are generated that contain an 8-nucleotide repeat seqeunce known as the “tag” and a variable spacer sequence length. Previous studies have identified the most abuntant mature crRNA species being 43- and 37-nucleotides long, differing by 6-nucleotides at the 3’ end. (**B**) Target plasmid libraries generated in this study contained either completely randomized 5-nucleotide sequences (4^5=1024 different sequences) or 10-nucleotide seqeunces with only two different bases in each position (2^{10}=1024 different sequences). Target library plasmids were introduced via electroporation into S. aureus OS2 cells harboring plasmids with either a wild-type type III-A CRISPR-Cas system (WT-CRISPR) or a mutant system in which the repeats and spacers are deleted (ΔCRISPR). Approximately 100,000 transformats of each strain were collected, DNA extracted, the target amplified by PCR and subjected to next generation sequencing to determine the number of reads and seqeunce of targets that survive type III-A CRISPR-Cas immunity and compare it to the number of reads obtained for the non-targeting ΔCRISPR control.

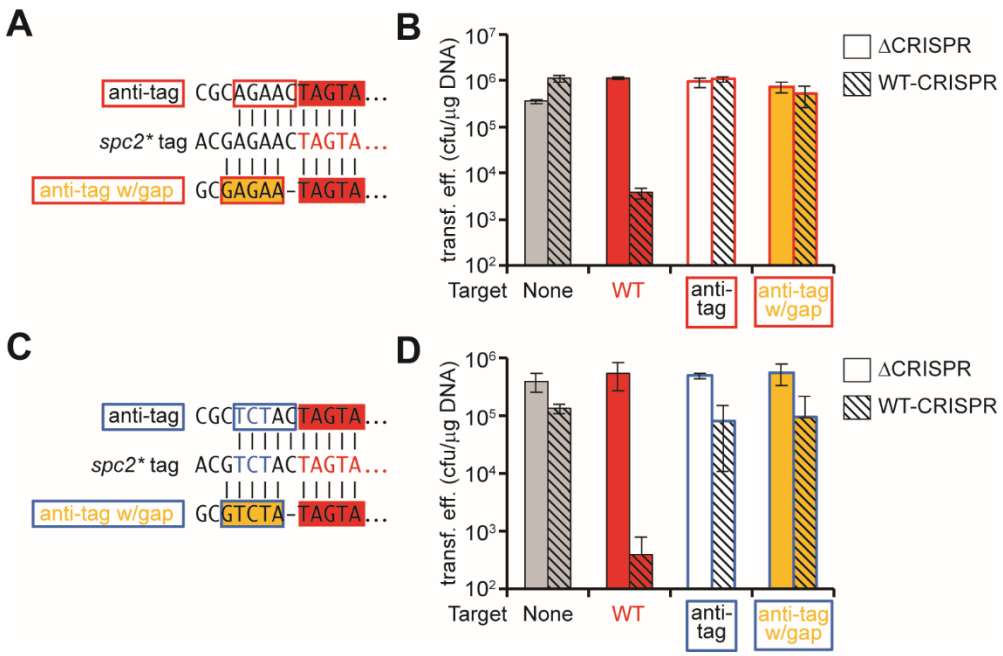

Fig. S2. A gap in the alignement of the tag and anti-tag sequences allows escape from type III-A targeting. Related to Fig. 2. (**A**) Constuction of a perfect and gapped anti-tag targets. The aligment of the protospacer flanking sequences to the CRISPR repeat upstream of *spc2* is shown. (**B**) Transformation efficiencies of pTargets shown in (**A**) into WT-CRISPR and ΔCRISPR cells. Transformation efficiencies of plasmids harboring either no target or a target with a wild-type 5' end flanking sequence are shown as controls. The mean ± SD of 3 independent experiments are reported. (**C**) Constuction of a perfect and gapped anti-tag targets for the *spc2** mutant repeat. The aligment of the protospacer flanking sequences to the CRISPR repeat upstream of *spc2** is shown. (**D**) Transformation efficiencies of pTargets shown in (**C**) into WT-CRISPR and ΔCRISPR cells. Transformation efficiencies of plasmids harboring either no target or a target with a wild-type 5' end flanking sequence are shown as controls. The mean ± SD of 3 independent experiments are reported.

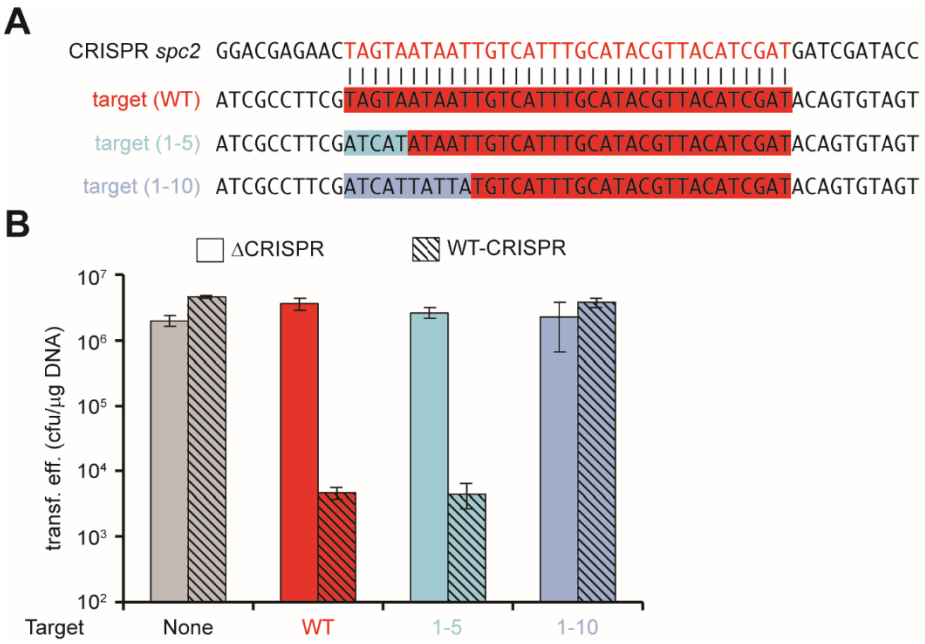

Fig. S3. Ten, but not five, mismatches in the first 10 nucleotides of the protospacer abrogate type III-A CRISPR-Cas immunity. Related to Fig. 3. (**A**) Either the first five or ten bases of the pTarget protospacer were mutated by changing the wild-type nucleotide to its Watson-Crick complementary base. (**B**) Transformation efficiencies for different pTargets plasmids shown in (**A**) into cells containing the pWT-CRISPR or pΔCRISPR plasmids. Transformation efficiencies of plasmids harboring either no target or a wild-type target are shown as controls. The mean ± SD of 3 independent experiments are reported.

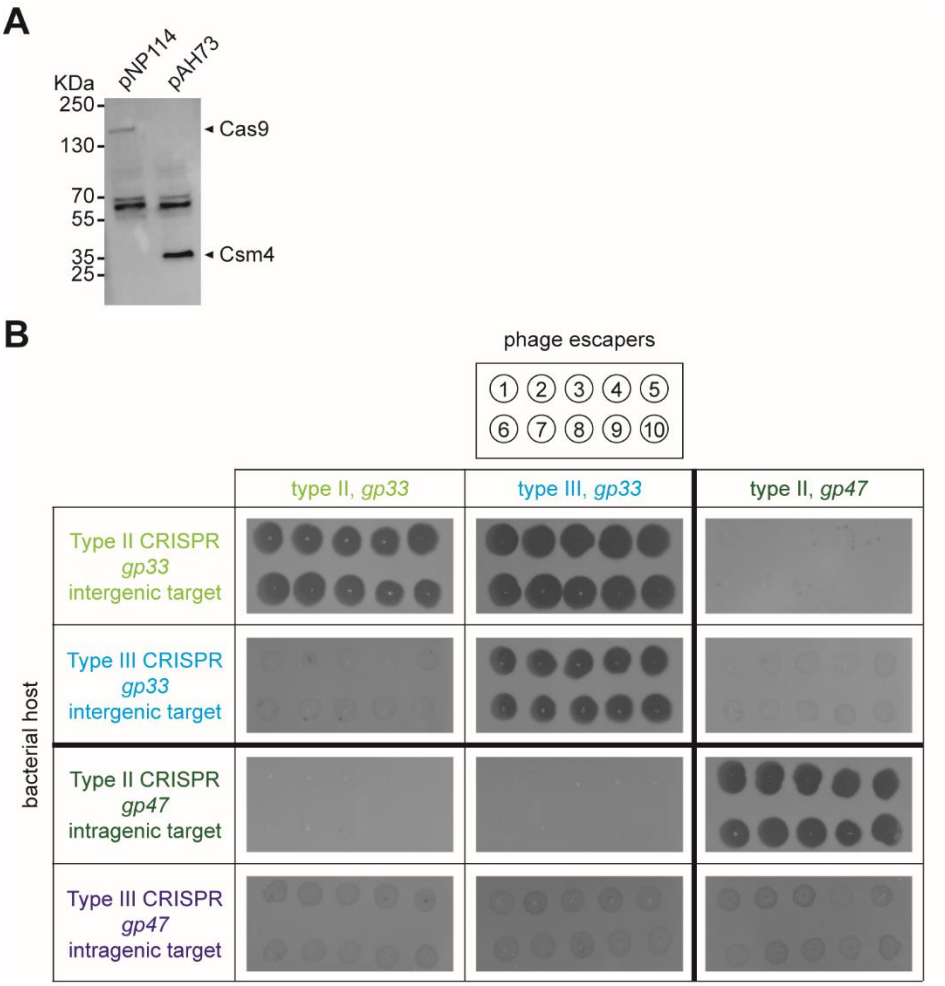

Fig. S4. Phages that escape type III-A CRISPR-Cas immunity are not suceptible to type II-A targeting. Related to Fig. 5. (**A**) Western blot analysis of type II and III expression. In order to compare the experssion levels of Cas9 and the Cas10-Csm complex we added a hexa-histidyl tag to Cas9 and Csm4, respectively, and performed western blot analysis of total protein extracts using an anti-His-tag antibody. pNP114 and pAH73, protein extracts from staphylococci expressing tagged versions of Cas9 and Csm4, respectively. (**B**) Ten different phages that escape the different type II-A and III-A targeting were spotted onto bacterial lawns harboring these different CRISPR-Cas systems. Bacteriophages that are able to lyse the bacteria in the lawn create a clear zone of inhibition of growth.

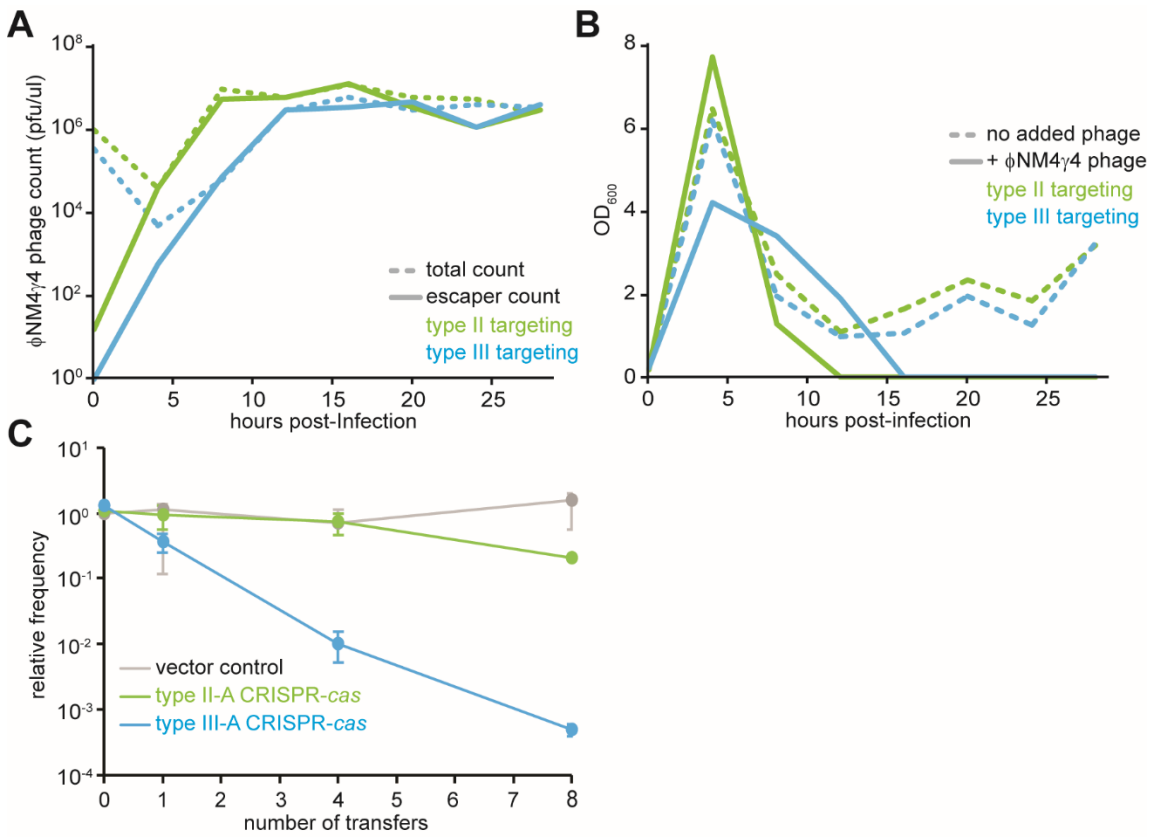

Fig. S5. Deletions of non-essential phage target regions allows for escape from type III-A CRISPR-Cas immunity. Related to Fig. 6. (**A**) ϕNM4γ4 phage titers measured as pfu/ml every 4 hours after infection of cells carrying type II-A (light green) or type III-A (light blue) systems targeting the *gp33* non-essential gene. Total phage titers (dotted line) were calculated after plating samples on non-CRISPR strain RN4220. Escaper phage titers (full line) were calculated by plating samples on CRISPR-immune bacterial strains. (**B**) Bacterial growth measured as OD_{600} every 4 hours after ϕNM4γ4 infection of cells carrying type II-A (light green) or type III-A (light blue) systems targeting the *gp33* non-essential gene. Growth measurements of non-infected cells (dotted lines) are shown as controls. (**C**) Pairwise competition analysis of staphylococci harboring the erythromycin-resistant pE194 plasmid or a chloramphenicol-resistant plasmid either without any insert or harboring the *S. pyogenes* type II-A or the *S. epidermidis* III-A CRISPR-*cas* locus. Cultures were mixed in a 1:1 ratio and grown for 24 hs before plating serial dilutions into media containing either erythromycin or choramphenicol to count the number of bacteria containing each plasmid and calculate the realtive frequency of chloramphenicol-resistant to erythromycin-resistant staphlococci. The experiment was performed in triplicate, with counting on days 0, 1, 4 and 8. The rapid decrease of the number of chloramphenicol-resistant cells relative to the erythromycin-resistant cells when they harbor a type III-A CRISPR-Cas system reveals a fitness cost for this locus, not present in staphylococci harboring the type II-A locus. The toxicity associated with the type III-A system may be due to higher rate of off-target chromosomal cleavage compared with the type II-A system, however it is important to note that the spacer content of these systems (which should determine the number of off-targets) is different.

SUPPLEMENTARY TABLES

Supplementary table S1. Related to STAR Methods. Oligonucleotides used in this study.

Primer name	Sequence
GG211	CATCATCTGCCTCCTCAACATTG
GG212	TTAGAGATGCCAGACGATGAATC
GG213	CTTTGCTATACATCTACTCTGAC
GG215	TTTAAATTTTATCCCGACCAACAAGCAATTGTTATAGTGA
H29[(a)]	aaacAAAAATGTTTTAACACCTATTAACGTAGTATg
H30[(a)]	aaaacATACTACGTTAATAGGTGTTAAAACATTTTT
H117[(a)]	aaacCTTTAAATGTTTTAAAAGAATAGCATCATTg
H118[(a)]	aaaacAATGATGCTATTCTTTTAAAACATTTAAAG
H346[(a)]	aaacATACGTGTAAAGACATATTAGATCGAGTCAg
H347[(a)]	aaaacTGACTCGATCTAATATGTCTTTACACGTAT
H433[(a)]	aaacAAACAGTGACAGAAACTATTGAGTACGAGGg
H434[(a)]	aaaacCCTCGTACTCAATAGTTTCTGTCACTGTTT
H478[(a)]	aaacTTATTTTGCGTTAGAATTGACACCTCAAGAg
H479[(a)]	aaaacTCTTGAGGTGTCAATTCTAACGCAAAATAA
H480[(a)]	aaacTTATCGTGAGTGGGAGAAATATAAGCGAAAg
H481[(a)]	aaaacTTTCGCTTATATTTCTCCCACTCACGATAA
H482[(a)]	aaacGACAAATGCTATTCAACATTCAGTTAAAGAg
H483[(a)]	aaaacTCTTTAACTGAATGTTGAATAGCATTTGTC
H488[(a)]	aaacTTGTTTATCGATTGGAGCATGCAAATAACTg
H489[(a)]	aaaacAGTTATTTGCATGCTCCAATCGATAAACAA
H494[(a)]	aaacGGATATTGTCGTTTTCCCGTCAAAGTATGGg
H495[(a)]	aaaacCCATACTTTGACGGGAAAACGACAATATCC
L55	TAAATCTAACAACACTCTAA
L56	TTAGAGTGTTGTTAGATTTA
L65	GTGATGGCATTTGTTAAAGTATC
L66	GATACTTTAACAAATGCCATCAC
L409	CGTGGTAAATCGGATAACGTTCCAAGTGAAG
L410	CTTCACTTGGAACGTTATCCGATTTACCACG
NP24	CACATCAGAAAATGGAATATCAGGTAGTAATTCC
NP25	GGAATTACTACCTGATATTCCATTTTCTGATGTG
NP32	CCCTGACGAAAGTCCAAGGGTTTAT
NP61	CCAGACCAGACATTACGAACTGG
NP62	AGACCAGACATTACGAACTGGCA
NP63	ACCAGACATTACGAACTGGCACA
NP64	CAGACATTACGAACTGGCACAGA
NP65	TGAATAAGAACGGTGCTCTCCAAAT
NP66	GCTGAATAAGAACGGTGCTCTCC
NP67	TAGCTGAATAAGAACGGTGCTCTCC
NP68	AATAGCTGAATAAGAACGGTGCTCT
NP99	ATCGCCTTCGTAGTAATAATTGTCATTTGCATACGTTACATCGATACAGTGTAGTTGGTCATAACCTGAAGGAAGATCTGG
NP100	ACTACACTGTATCGATGTAACGTATGCAAATGACAATTATTACTACGAAGGCGATTCTGTGCCAGTTCGTAATGTCTGG
NP102[(b)]	ATTACGAACTGGCACAGAATCGCNNNNNTAGTAATAATTGTCATTTGCATACGT
NP103	CGAAGGCGATTCTGTGCCAG
NP104[(b)]	ACGAACTGGCACAGAATCGCCTTCGNNNNNATAATTGTCATTTGCATACGTTACA
NP105[(b)]	CTGGCACAGAATCGCCTTCGWWSWWWWWWWTGTCATTTGCATACGTTACATCG
NP106	ATTATTACTACGAAGGCGATTCTG

NP107[b]	GAATCGCCTTCGTAGTAATAATWSWSWWWWSSATACGTTACATCGATACAGTGTAG
NP108	CGTATGCAAATGACAATTATTACTACG
NP109[b]	TAATAATTGTCATTTGCATACGWWWSWWSSWWACAGTGTAGTTGGTCATAACCTGAAG
NP120	GACATTACGAACTGGCACAGAAT
NP121	ATTACGAACTGGCACAGAATCG
NP122	TACGAACTGGCACAGAATCGC
NP123	GAACTGGCACAGAATCGCC
NP124	TGGCACAGAATCGCCTTCG
NP125	GCACAGAATCGCCTTCGTAG
NP135[a]	gaacACCACTAGTTGAAGTGTTGTAAGGTGATTTAGTACg
NP136[a]	cgatcGTACTAAATCACCTTACAACACTTCAACTAGTGGT
NP137[a]	gaacCTCCTTGACTCGATCTAATATGTCTTTACACGTATg
NP138[a]	cgatcATACGTGTAAAGACATATTAGATCGAGTCAAGGAG
NP183	CGAGCGAGTGGCACATATCC
NP184	CCCTACATCTTGTGCAGGAGC
NP189	CTGGCACAGAATCGCAGAACTAGTAATAATTGTCATTTGCATACGTTACATCG
NP190	TGACAATTATTACTAGTTCTGCGATTCTGTGCCAGTTCGTAATG
NP191	CTGGCACAGAATCGCGAGAATAGTAATAATTGTCATTTGCATACGTTACATCG
NP192	TGACAATTATTACTATTCTCGCGATTCTGTGCCAGTTCGTAATG
NP197	CTGGCACAGAATCGCTCTACTAGTAATAATTGTCATTTGCATACGTTACATCG
NP198	TGACAATTATTACTAGTAGAGCGATTCTGTGCCAGTTCGTAATG
NP199	CTGGCACAGAATCGCGTCTATAGTAATAATTGTCATTTGCATACGTTACATCG
NP200	TGACAATTATTACTATAGACGCGATTCTGTGCCAGTTCGTAATG
NP218	CCTTCGATCATATAATTGTCATTTGCATACGTTACATCGAT
NP219	ACAATTATATGATCGAAGGCGATTCTGTGCCAG
NP220	GCCTTCGATCATTATTATGTCATTTGCATACGTTACATCG
NP221	CAAATGACATAATAATGATCGAAGGCGATTCTGTGCC
NP342	ctttttattttaggaggcaaaaATGGGCCATCATCATCATCATCATC
NP343	ATGGCCCATttttgcctcctaaaataaaaagtttaaattaaatcc
PN71[a]	aaacATTCACAACTTGCCAAGGAAATCCTATGCAg
PN72[a]	aaaacTGCATAGGATTTCCTTGGCAAGTTGTGAAT
PN87[a]	aaacTACTAAATCACCTTACAACACTTCAACTAGg
PN88[a]	aaaacCTAGTTGAAGTGTTGTAAGGTGATTTAGTA
PN95	AAGAAATGGCGAAAGAAATCAATATTAG
PN96	TCTTCTGCAGGATTAGCCATC

Supplementary Sequence. Related to STAR Methods. pNP61 CRISPR array.

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CATCGATGATCGATACCCACCCCGAAGAAAAGGGGACGAGAACTAGTACGGTCGT
GAACATTTTTTCTTGATTCTCTGATCGATAGCCACCCCGAAGAAAAGGGGGCAGAG
TGTAAATCTAACAACACTCTAAAAAATTGTAG

SUPPLEMENTARY DATA FILE S1. Related to STAR Methods. Analysis of next generation sequencing data.