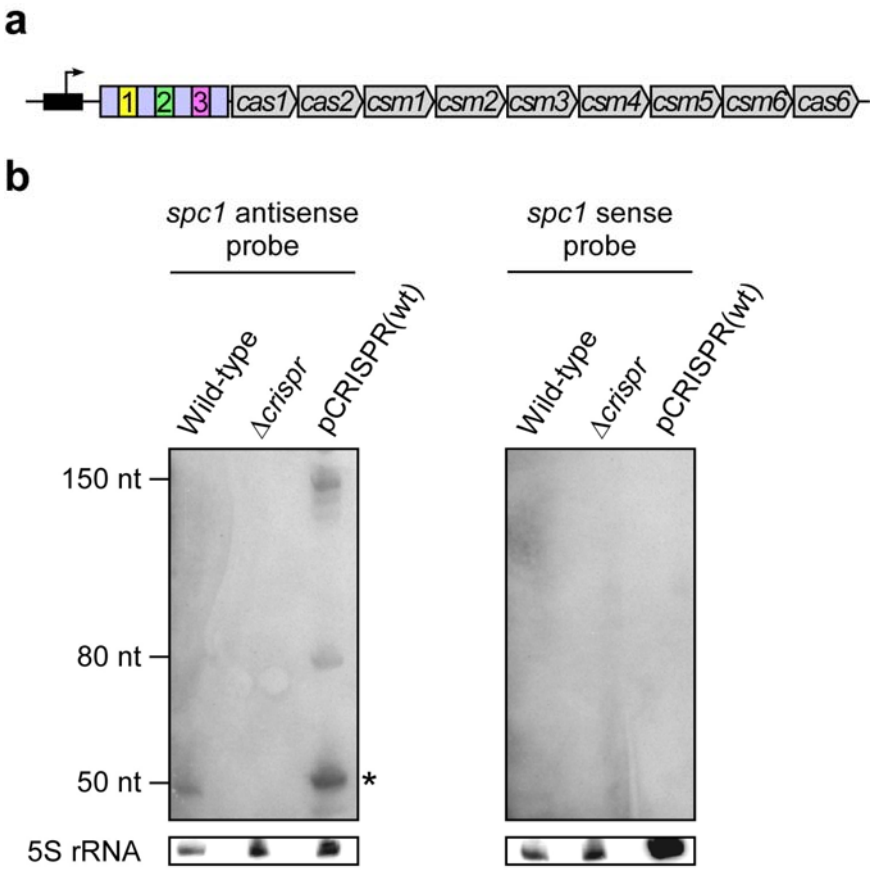
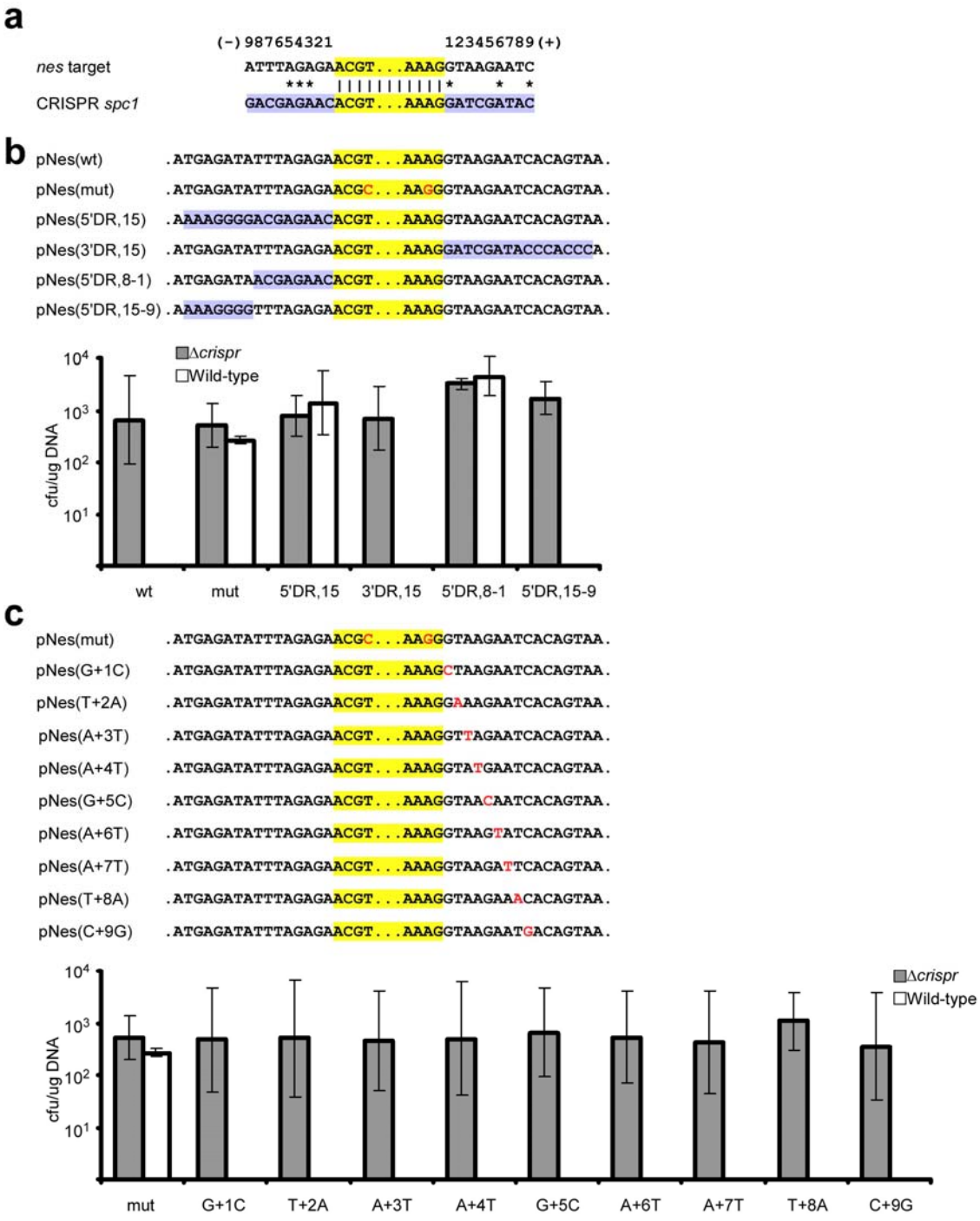


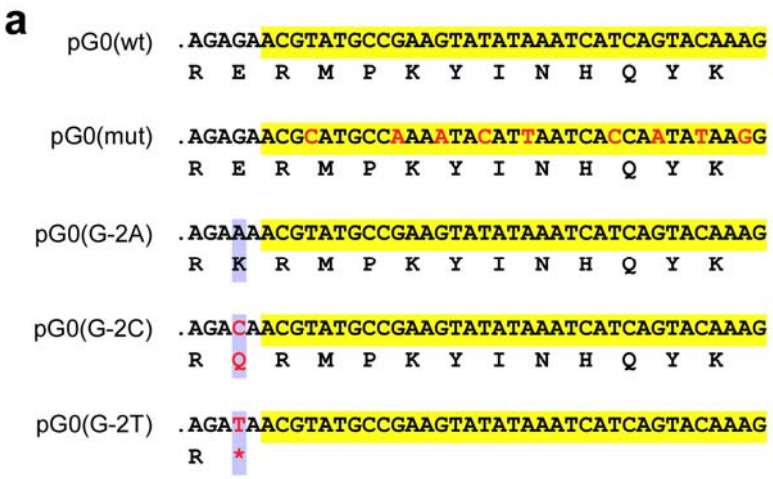
Supplementary Figure 1 | The *S. epidermidis* CRISPR locus. a, The *S. epidermidis* chromosome contains a simple CRISPR locus consisting of 4 repeats (purple boxes) and three spacer sequences (numbered boxes). *spc1* matches a sequence present in the *nickase* gene[1] of staphylococcal conjugative plasmids; the *spc2* sequence is found in *S. epidermidis* bacteriophage PH15 (ref. [2]); and *spc3* has no matches in Genbank. This cluster is preceded by a leader sequence[3] (black box) and followed by a set of nine *cas* genes. Core *cas* genes *cas1*, *cas2* and *cas6* are present, as well as *cas* subtype *Mycobacterium tuberculosis* genes[4] *csm1* to *csm6*. Transcription emanates from the leader-containing end of the locus (arrowhead). Different portions of the CRISPR/*cas* locus are not drawn to scale. **b,** Northern blot analysis of CRISPR *spc1* transcripts. Total RNA of *S. epidermidis* RP62a (Wild-type), the Δ*crispr* mutant strain (LAM104, ref. [5]) and the deletion strain complemented with the pCRISPR(wt) plasmid was separated by denaturing PAGE, transferred to a nylon membrane and hybridized with a sense or antisense *spc1* oligonucleotide probe as indicated (P61 and P62, respectively; see Supplementary Table 3). Mobilities of RNA size markers are indicated on the left. The most abundant *spc1* transcript detected in wild-type cells corresponds to a small crRNA of ~49 nt (asterisk) that is absent in Δ*crispr* extracts. No RNAs could be detected with the sense probe, consistent with the results of previous primer extension analyses[5]. The lack of a ladder-like pattern[6-12] in wild-type cells is most likely attributable to a combination of efficient processing of the crRNA precursor and

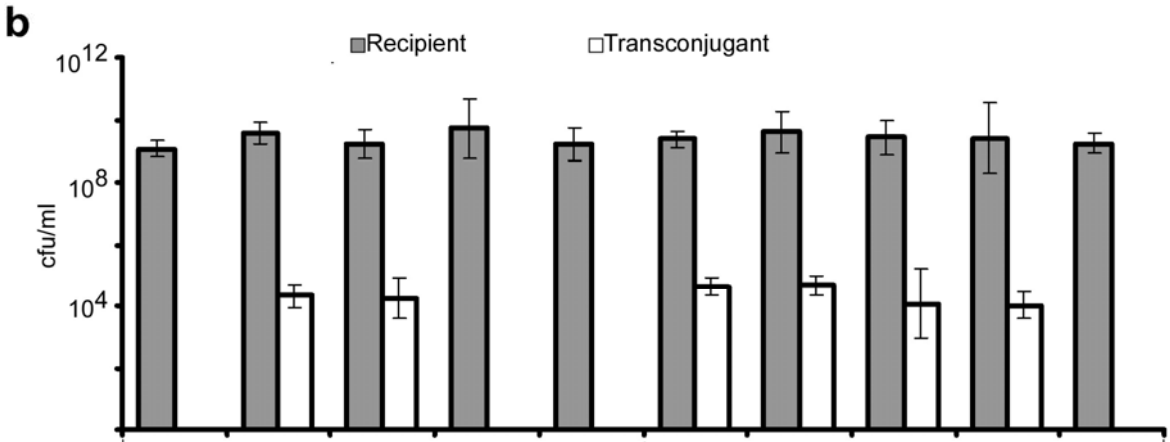
the small number of repeats and spacers in the *S. epidermidis* CRISPR cluster. The complemented mutant strain, which overexpresses crRNA from the multicopy pCRISPR(wt) plasmid, showed two additional species of ~75 and ~145 nt, which may derive from partial processing of the crRNA precursor. As a loading control (bottom), membranes were stripped of hybridized *spc1* probe and re-hybridized with a 5S rRNA antisense probe (P67).



Supplementary Figure 2 | Repeat sequences present upstream of *spc1* protect the *nes* target from CRISPR interference. a, Conservation of *nes* target and CRISPR *spc1* flanking sequences. While target and spacer sequences (highlighted in yellow) are identical, three bases are conserved at positions -5, -4 and -3 and +1, +6 and +9 between the *nes* target and *spc1* upstream and downstream flanking sequences, respectively (indicated by asterisks). **b,** Sequences flanking the 5' or 3' end of the *nes* target (highlighted in yellow) were replaced by the corresponding direct repeat (DR) sequences

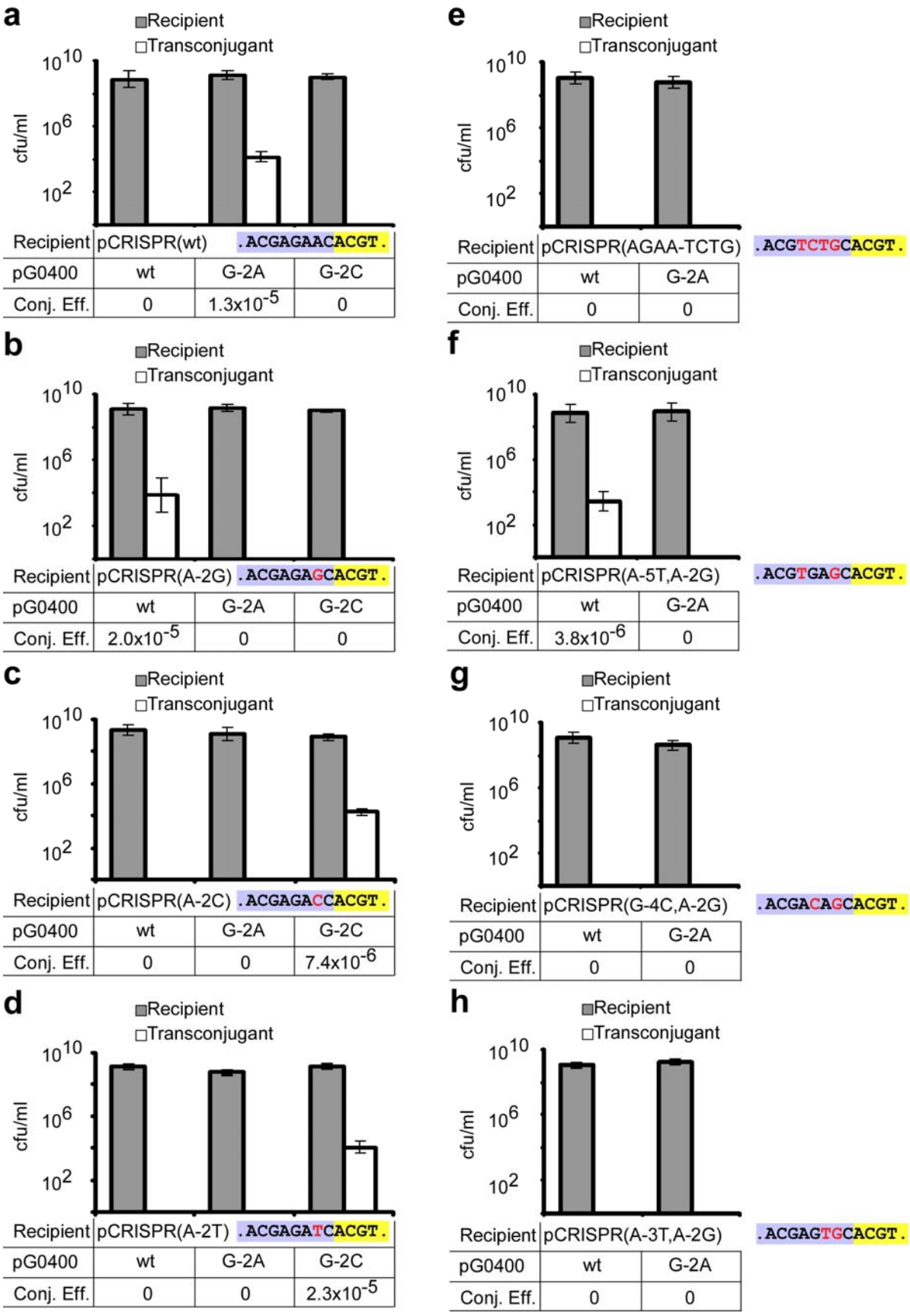
(highlighted in purple) that flank *spc1* in the CRISPR locus. 15 nt upstream or downstream of *spc1* (chosen to include approximately half of the repeat sequence) were introduced at the 5' or 3' end of the *nes* target in pNes(5'DR,15) or pNes(DR3',15), respectively. Subsets of the sequences introduced into pNes(5'DR,15) were re-introduced upstream of the *nes* target, the nearest 8 nt to *spc* (nt 8-1) and the distal 7 nt (nt 15-9) generated pNes(5'DR,8-1) and pNes(5'DR,15-9), respectively. **c,** Individual mutations were also introduced in the downstream region of the *nes* target. Each nucleotide at positions +1 to +9 following the 3' end of the *nes* target was substituted for the complementary base. Similar to pNes(DR3',15), none of these mutations altered CRISPR interference of the respective plasmid. In all cases, sequences containing the *nes* target and the described changes were cloned into pC194 (ref. [13]) and the corresponding plasmids were transformed into *S. epidermidis* wild-type and its Δ*crispr* isogenic mutant (LAM104, ref. [5]). pNes(wt) and pNes(mut) (ref. [5]) were used as positive and negative controls for CRISPR interference, respectively. The average of at least three independent measures of the transformation efficiency (determined as cfu/μg DNA) is reported and error bars indicate 1 s.d.





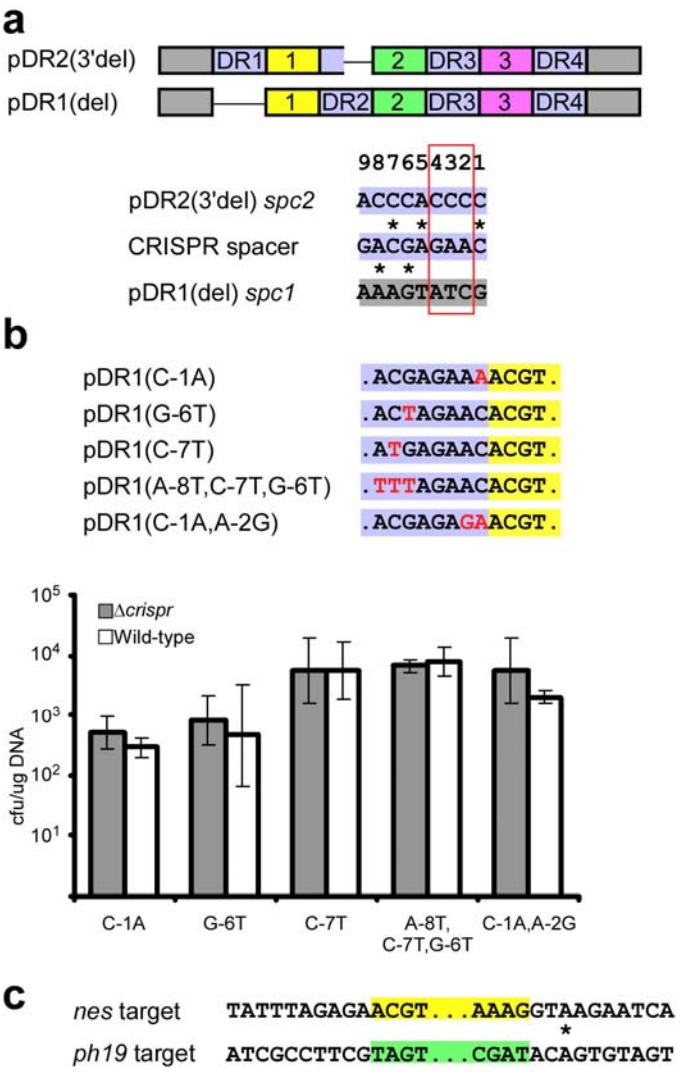
Recipient	Wild-type					Δ*crispr*				
pG0400	wt	mut	G-2A	G-2C	G-2T	wt	mut	G-2A	G-2C	G-2T
Conj. Eff.	0	$6.9x10^{-6}$	$1.3x10^{-5}$	0	0	$1.8x10^{-5}$	$7.4x10^{-6}$	$9.6x10^{-6}$	$1.7x10^{-6}$	0

Supplementary Figure 3 | Effect of pG0400 mutations at position -2 during CRISPR conjugation interference. a, Mutations introduced in pG0400 (ref. [14]). Guanosine at position -2 (G-2, two nucleotides upstream of the start of the *nes* target sequence in pG0400) was mutated to adenosine (G-2A, introducing the nucleotide present at the corresponding position upstream of *spc1*, highlighted in purple), cytosine and thymidine (G-2C and G-2T, respectively; red font and highlighted in purple). These mutations change a glutamate residue of the Nes protein (E635) to lysine (K), glutamine (Q) and to a stop codon (*), respectively. pG0400(mut) contains mutations (red) in the *nes* target that do not alter the encoded protein[5]. **b,** Conjugative plasmids were tested using *S. epidermidis* RP62a (wild-type) and Δ*crispr* as recipients. Conjugation efficiency was determined as transconjugant cfu/recipient cfu; the average of at least 3 independent experiments is reported and error bars indicate 1 s.d. Plasmids pG0400(wt) and pG0400(mut) were used as positive and negative controls of CRISPR interference[5]. pG0(G-2T) was unable to transfer into the *S. epidermidis* Δ*crispr* strain, indicating that truncation of the Nes protein prevented proper nickase function during conjugation[1]; this plasmid was not further analysed.



Supplementary Figure 4 | Complementarity between crRNA and target DNA flanking sequences is required for protection. Conjugational transfer of

pG0400 and its mutant variants into *S. epidermidis* Δ*crispr* strain. Recipient cells contained different pCRISPR complementing plasmids. Red font indicates mutations introduced in the upstream flanking sequence (highlighted in purple) of *spc1* (highlighted in yellow). Panels **a**-**h** show detailed results for each of the pCRISPR plasmids analysed in Figure 2. Conjugation efficiency was determined as transconjugant cfu/recipient cfu; the average of at least 3 independent experiments is reported. Note that all pCRISPR plasmids were able to restore CRISPR interference in Δ*crispr* cells with at least one of the conjugative plasmids, indicating that they all express functional crRNAs.



Supplementary Figure 5 | Additional mutations in upstream flanking sequences of CRISPR spacers and their effects on autoimmunity. a, Deletions upstream of *spc2* and *spc1* in plasmids pDR2(3'del) and pDR1(del), respectively, generated new 5' flanks. Asterisks indicate identity between wild-type (CRISPR spacer) and newly generated flanks. Deletions eliminated identity at positions -4, -3 or -2, resulting in lack of protection (Fig. 3a). **b**, Mutations in the upstream direct repeat (DR, highlighted in purple) of *spc1* (highlighted in yellow) are shown in red. The different CRISPR loci containing these mutations were cloned into pC194 (ref. [13]), and the resulting plasmids were transformed into *S. epidermidis* RP62a (wild-type) and its Δ*crispr* isogenic mutant (LAM104, ref. [5]). The average of at least three independent measures of the transformation efficiency (determined as cfu/μg DNA) is reported and error bars indicate 1 s.d. **c**, Only two targets are known for the CRISPR locus of *S. epidermidis* RP62a: one present in the nickase gene (*nes*) of staphylococcal conjugative plasmids[1] and another in the *ph19* gene (encoding for a putative phage pre-neck appendage protein) of the *S. epidermidis* phage PH15 (ref. [2]). Only a single nucleotide (A+3, denoted by an asterisk) is shared by *nes* and *ph19* target flanks, precluding the identification of a CRISPR motif [10, 15, 16]. Nucleotide A+3 is not essential for interference (See Supplementary Figure 2c).

Supplementary Table 1. Transformation efficiency for each plasmid used in this study.

Plasmid	Wild-type[a]	Δ*crispr*[a]	Figure[c]
pCRISPR(wt)	5.22×10^3	2.41×10^3	1a, 3a
pCRISPR(del)	3.15×10^3	4.92×10^3	1a
pNes(wt)	0.00	1.38×10^3	1b, S2b
pNes(mut)	2.71×10^2	6.42×10^2	1b, S2b, S2c
pNes(3'DR,15)	0.00	1.37×10^3	1b, S2b
pNes(5'DR,8)	5.78×10^3	3.34×10^3	1b, S2b
pNes(A-1C)	0.00	7.06×10^2	1c
pNes(G-2A)	2.92×10^2	1.04×10^3	1c
pNes(T-6G)	0.00	7.43×10^2	1c
pNes(T-7C)	0.00	1.57×10^3	1c
pNes(T-8A)	0.00	6.74×10^2	1c
pNes(G-2T)	0.00	3.80×10^2	1c
pNes(G-2C)	0.00	1.76×10^2	1c
pDR2(3'del)	0.00	3.22×10^3	3a
pDR2(5'del)	7.66×10^3	2.39×10^3	3a
pDR1(del)	0.00	2.24×10^3	3a
pDR1(wt)	3.73×10^2	1.98×10^3	3c
pDR1(AGAA-TCTG)	0.00	1.59×10^4	3c
pDR1(A-2G)	3.65×10^3	5.04×10^3	3c
pDR1(A-3T)	2.13×10^3	1.60×10^3	3c
pDR1(G-4C)	1.31×10^3	1.74×10^3	3c
pDR1(A-5T)	4.53×10^2	2.20×10^2	3c
pDR1(AGA-TCT)	0.00	4.32×10^3	3c
pDR1(A-5T/A-2G)[b]	6.27×10^2	4.11×10^3	3c
pDR1(G-4C/A-2G)[b]	1.12×10^3	2.88×10^3	3c
pDR1(A-3T/A-2G)	0.00	4.23×10^3	3c
pNes(3'DR,1-15)	0.00	1.37×10^3	S2b
pNes(5'DR,15-9)	0.00	2.01×10^3	S2b
pNes(G+1C)	0.00	1.26×10^3	S2c
pNes(T+2A)	0.00	1.64×10^3	S2c
pNes(A+3T)	0.00	1.15×10^3	S2c
pNes(A+4T)	0.00	1.50×10^3	S2c
pNes(G+5C)	0.00	1.37×10^3	S2c
pNes(A+6T)	0.00	1.19×10^3	S2c
pNes(A+7T)	0.00	1.11×10^3	S2c
pNes(T+8A)	0.00	1.56×10^3	S2c
pNes(C+9G)	0.00	9.97×10^2	S2c
pDR1(C-1A)	2.96×10^2	5.63×10^2	S5b
pDR1(G-6T)	9.87×10^2	1.00×10^3	S5b
pDR1(C-7T)	7.31×10^3	7.83×10^3	S5b
pDR1(A-8T/C-7T/G-6T)	8.62×10^3	6.80×10^3	S5b
pDR1(C-1A/A-2G)	2.07×10^3	7.77×10^3	S5b

[a] Transformation efficiency is reported in cfu/μg of DNA. The average of at least three experiments is shown.

[b] Wild-type transformant colonies were significantly smaller than Δ*crispr* transformant colonies, indicating some degree of CRISPR-dependent interference.

[c] "S" indicates supplementary figures.

Supplementary Table 2. Primers and templates used for construction of pC194-based plasmids.

Plasmid	External Primers		Internal primers		Template
	Forward	Reverse	Forward	Reverse	
pCRISPR(wt)	P92	P154			RP62a
pCRISPR(del)	P92	P154			LAM104
pNes(3'DR,15)	P70	P71	P180	P181	pG0400
pNes(5'DR,15)	P70	P71	P178	P179	pG0400
pNes(5'DR,8-1)	P70	P71	P182	P183	pG0400
pNes(5'DR,15-9)	P70	P71	P184	P185	pG0400
pNes(A-1C)	P70	P71	P188	P189	pG0400
pNes(G-2A)	P70	P71	P190	P191	pG0400
pNes(T-6G)	P70	P71	P192	P193	pG0400
pNes(T-7C)	P70	P71	P194	P195	pG0400
pNes(T-8A)	P70	P71	P196	P197	pG0400
pNes(G-2T)	P70	P71	P206	P207	pG0400
pNes(G-2C)	P70	P71	P208	P209	pG0400
pNes(G+1C)	P70	P71	P160	P161	pG0400
pNes(T+2A)	P70	P71	P162	P163	pG0400
pNes(A+3T)	P70	P71	P164	P165	pG0400
pNes(A+4T)	P70	P71	P166	P167	pG0400
pNes(G+5C)	P70	P71	P168	P169	pG0400
pNes(A+6T)	P70	P71	P170	P171	pG0400
pNes(A+7T)	P70	P71	P172	P173	pG0400
pNes(T+8A)	P70	P71	P174	P175	pG0400
pNes(C+9G)	P70	P71	P176	P177	pG0400
pDR2(3'del)	P92	P154	P143	P144	RP62a
pDR2(5'del)	P92	P154	P145	P146	RP62a
pDR1(del)	P92	P154	P147	P148	RP62a
pDR1(wt)	P155	P154			RP62a
pDR1(AGAA -TCTG)	P155	P154	P247	P248	RP62a
pDR1(A-2G)	P155	P154	P223	P224	RP62a
pDR1(A-3T)	P155	P154	P232	P233	RP62a
pDR1(G-4C)	P155	P154	P234	P235	RP62a
pDR1(A-5T)	P155	P154	P236	P237	RP62a
pDR1(AGA-TCT)	P155	P154	P260	P261	RP62a
pDR1(A-5T, A-2G)	P155	P154	P262	P263	RP62a
pDR1(G-4C, A-2G)	P155	P154	P264	P265	RP62a
pDR1(A-3T, A-2G)	P155	P154	P266	P267	RP62a
pDR1(C-1A)	P155	P154	P230	P231	RP62a
pDR1(G-6T)	P155	P154	P238	P239	RP62a
pDR1(C-7T)	P155	P154	P255	P256	RP62a
pDR1(A-8T,C-7T,G-6T)	P155	P154	P268	P269	RP62a
pDR1(C-1A,A-2G)	P155	P154	P257	P258	RP62a

Supplementary Table 3. Primers used in this study.

Primer name	Sequence[a]
P15	ggggACAAGTTTGTACAAAAAAGCAGGCTtgaagatagattaaataaaattgagg
P18	ggggACCACTTTGTACAAGAAAGCTGGGTGTTATTTAAGTGGCTGGGGGC
P61	GCCGAAGTATATAAATCATCAG
P62	CTGATGATTTATATACTTCGGC
P67	GTGACCTCCTTGCCATTGTC
P70	aaaaAAGCTTCAAGAATCCAATGAAGTAGGGG
P71	aaaaAAGCTTCTAAATTAGAACATGATACTAACG
P86	CATATAGTTTTATGCCTAAAAACC
P87	ATATATTTATTTGGCTCATATTTGC
P92	GAGCGGATAACAATTAAGCTTAACGAAATATAAAAAGAAATGAAAGG
P154	aaaAAGCTTAAATTTAATGCTATTTTCCTTCGC
P155	aaaAAGCTTGTGATGGCATTTGTTAAAGTATC
P160	CAGTACAAAGCTAAGAATCAC
P161	GTGATTCTTAGCTTTGTACTG
P162	AGTACAAAGGAAAGAATCACA
P163	TGTGATTCTTTCCTTTGTACT
P164	GTACAAAGGTTAGAATCACAG
P165	CTGTGATTCTAACCTTTGTAC
P166	TACAAAGGTATGAATCACAGT
P167	ACTGTGATTCATACCTTTGTA
P168	ACAAAGGTAACAATCACAGTA
P169	TACTGTGATTGTTACCTTTGT
P170	CAAAGGTAAGTATCACAGTAA
P171	TTACTGTGATACTTACCTTTG
P172	AAAGGTAAGATTCACAGTAAA
P173	TTTACTGTGAATCTTACCTTT
P174	AAGGTAAGAAACACAGTAAAC
P175	GTTTACTGTGTTTCTTACCTT
P176	AGGTAAGAATGACAGTAAACA
P177	TGTTTACTGTCATTCTTACCT
P178	TAAAAAGGGGACGAGAACACGTATGCCGAAGTATATAAATC
P179	CGTGTTCTCGTCCCCTTTTTAAAATTAAATATCATTGAATCTAGTC
P180	CAAAGGATCGATACCCACCCAACAGCGCCATGAGTTGAAAAATA
P181	CTGTTGGGTGGGTATCGATCCTTTGTACTGATGATTTATATAC
P182	TAATGAGATAACGAGAACACGTATGCCGAAGTATATAAATC
P183	CGTGTTCTCGTTATCTCATTAAAATTAAATATCATTGAATCTAGTC
P184	TAAAAAGGGGTTTAGAGAACGTATGCCGAAGTATATAAATC
P185	CGTTCTCTAAACCCCTTTTTAAAATTAAATATCATTGAATCTAGTC
P188	GAGATATTTAGAGCACGTATGCCGAAG
P189	CTTCGGCATACGTGCTCTAAATATCTC
P190	GAGATATTTAGAAAACGTATGCCGAAG
P191	CTTCGGCATACGTTTTCTAAATATCTC
P192	GAGATATTGAGAGAACGTATGCCGAAG
P193	CTTCGGCATACGTTCTCTCAATATCTC

P194	GAGATATCTAGAGAACGTATGCCGAAG
P195	CTTCGGCATACGTTCTCTAGATATCTC
P196	GAGATAATTAGAGAACGTATGCCGAAG
P197	CTTCGGCATACGTTCTCTAATTATCTC
P206	TGAGATATTTAGATAACGTATGCCGAAG
P207	CTTCGGCATACGTTATCTAAATATCTCA
P208	TGAGATATTTAGACAACGTATGCCGAAG
P209	CTTCGGCATACGTTGTCTAAATATCTCA
P223	GAGAGCACGTATGCCGAAG
P224	CTTCGGCATACGTGCTCTC
P230	GAGAAAACGTATGCCGAAG
P231	CTTCGGCATACGTTTTCTC
P232	CGAGTACACGTATGCCGAAG
P233	CTTCGGCATACGTGTACTCG
P234	CGACAACACGTATGCCGAAG
P235	CTTCGGCATACGTGTTGTCG
P236	GACGTGAACACGTATGCCGAAG
P237	CTTCGGCATACGTGTTCACGTC
P238	GACTAGAACACGTATGCCGAAG
P239	CTTCGGCATACGTGTTCTAGTC
P247	GACGTCTGCACGTATGCCGAAG
P248	CTTCGGCATACGTGCAGACGTC
P257	GAGAGAACGTATGCCGAAG
P258	CTTCGGCATACGTTCTCTC
P260	GACGTCTACACGTATGCCGAAG
P261	CTTCGGCATACGTGTAGACGTC
P262	GACGTGAGCACGTATGCCGAAG
P263	CTTCGGCATACGTGCTCACGTC
P264	GACGACAGCACGTATGCCGAAG
P265	CTTCGGCATACGTGCTGTCGTC
P266	GACGAGTGCACGTATGCCGAAG
P267	CTTCGGCATACGTGCACTCGTC
P268	GGTTTAGAACACGTATGCCGAAG
P269	CTTCGGCATACGTGTTCTAAACC

[b] *Hin*dIII sites used for insertion into pC194 are underlined.

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