SUPPLEMENTARY INFORMATION

Non-specific degradation of transcripts promotes plasmid clearance during type III-A CRISPR-Cas immunity.

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Supplementary figures and legends

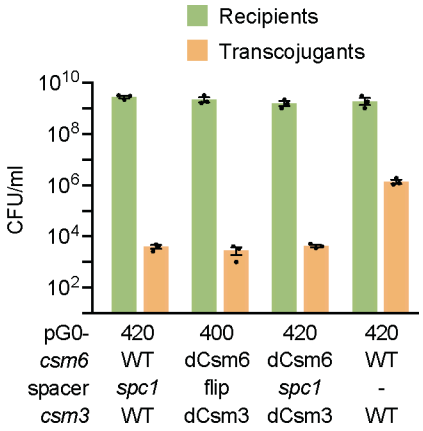


Figure S1 – Csm3 RNase activity is not required for interference against a well-transcribed target in pG0400/pG0420. Like Fig. 1c, but with catalytic Csm3 mutants (dCsm3) where indicated. With well-transcribed targets, neither the RNase activity of Csm3 nor Csm6 is required for interference. Each bar represents the mean of three biological replicates ±s.e.m.

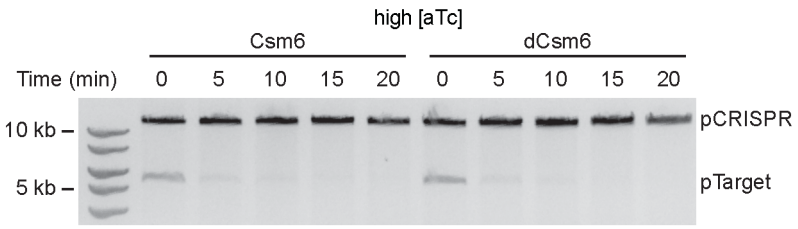


Figure S2 – Plasmid degradation is rapid for both WT and dCsm6 under high transcription conditions. Same as Figure 2c, but assaying earlier timepoints after induction of transcription. Gel image is representative of two independent experiments.

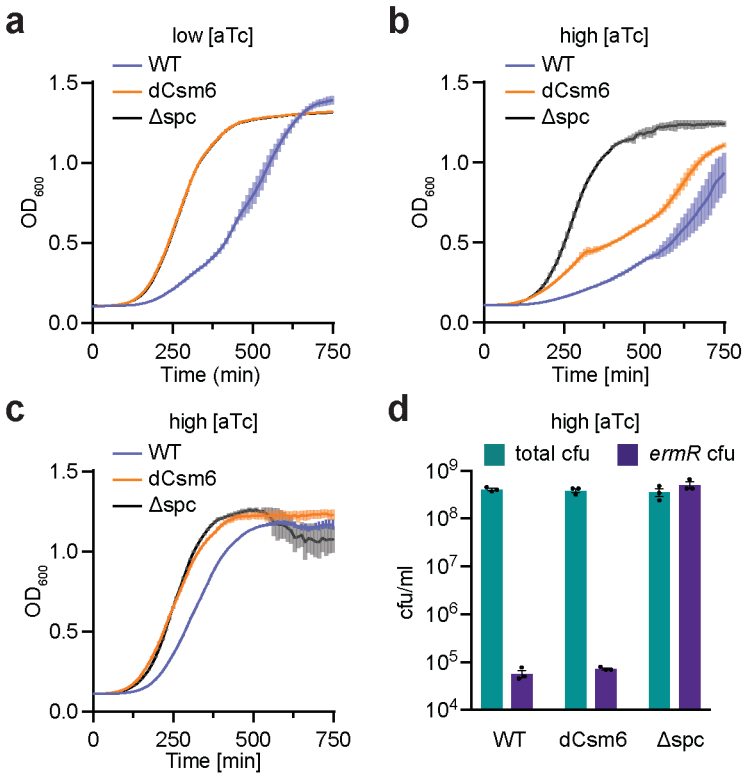


Figure S3 – Csm6 is required for pTarget clearance. a, The OD_{600} of staphylococci harbouring pTarget and the specified pCRISPR is measured every 10 minutes after induction of protospacer transcription by the addition of low levels of aTc, in the presence of erythromycin. Each data point represents the mean of three biological replicates ±s.e.m. **b**, Same as panel **a**, but with high levels of aTc. **c**, Same as panel **b**, but without erythromycin. For the Δ*spc* control curve, the same data as from Fig. 5a was used. **d**, Cells at the end of the experiment in panel **c** are spotted onto TSB plates in the presence or absence of erythromycin, selecting for the presence of pTarget, and enumerated. Each bar represents the mean of three replicates ±s.e.m.

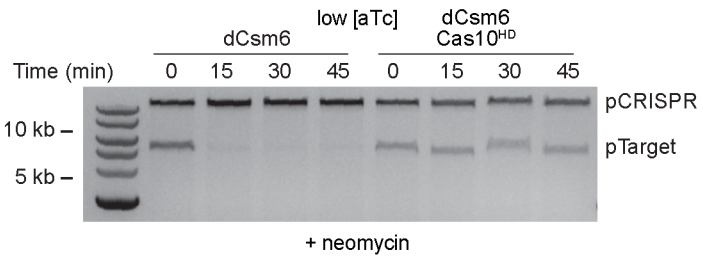


Figure S4 – The Cas10 HD domain is required for plasmid depletion in the presence of neomycin. Same as Fig. 4c, but testing cells that express both Cas10^{HD} and dCsm6, where pTarget is not depleted. The experiment was performed once.

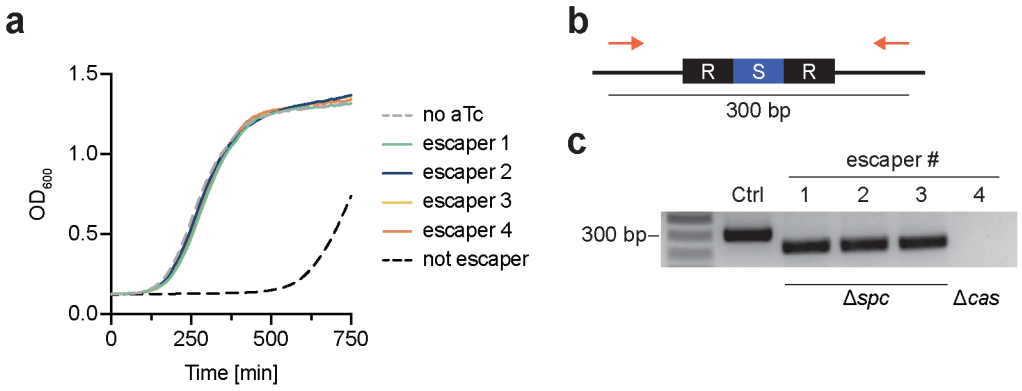
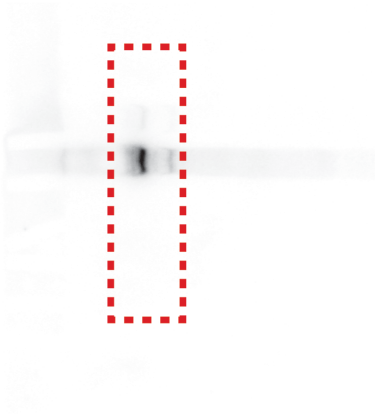


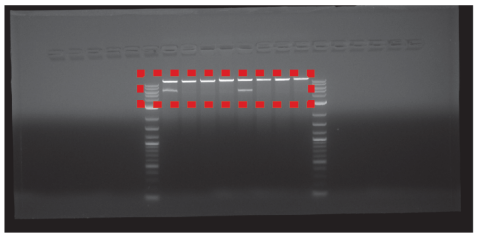
Figure S5 – Analysis of staphylococci expressing Cas10^{HD} in the presence of a target transcript (high aTc). a, Cells that recovered at the end of the experiment in shown in Figure 5a (expressing the Cas10^{HD} mutant) were streaked out to obtain single colonies. Four colonies were picked and seeded into a new microwell plate where growth was tracked by measuring the OD_{600} every 10 minutes in the presence of high levels of aTc. The four isolates displayed unrestricted growth, similar to a control culture lacking aTc induction of CRISPR immunity. A culture that had previously not grown in the presence of aTc (non-escaper) is shown as a targeting control. **b,** All four escapers were analysed by PCR using primers that include the CRISPR locus. and Sanger sequencing to determine the presence of escaper mutations. A 300 bp PCR amplicon spanning the pTarget-specific spacer (S) and its two flanking repeats (R) is expected from wild-type loci. **c,** Agarose gel electrophoresis of the PCR products from the amplification illustrated in panel **b.** Gel image is representative of two independent experiments. A wild-type locus was used a control (ctrl). Escapers 1 through 3 contained a reduced CRISPR array. Sanger sequencing revealed that they carry a deletion of the spacer and a one of the flanking repeats (Δ*spc*). Escaper 4 did not produce a PCR product. Sanger sequencing of plasmid DNA isolated from this escaper showed that it harboured a complete deletion (~9kb) of the *cas* operon (Δ*cas*). All these deletions render pCRISPR unable to target pTarget, and therefore allow to escape the Csm6-mediated growth arrest.

a



b

c



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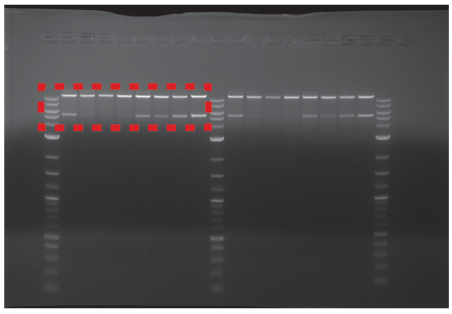

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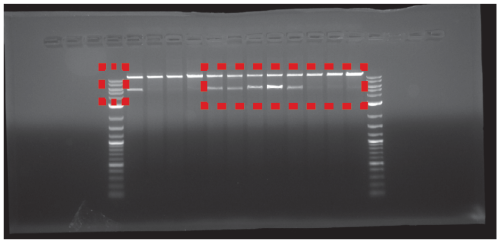

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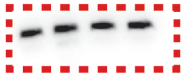

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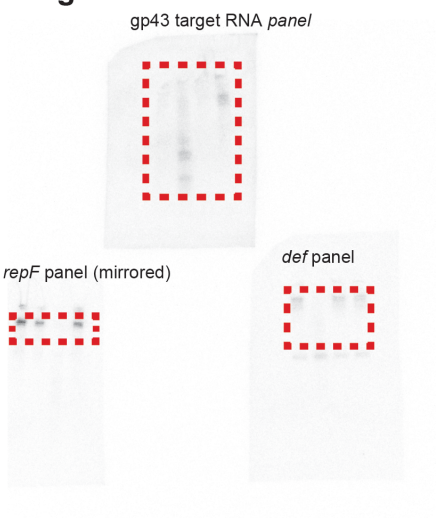

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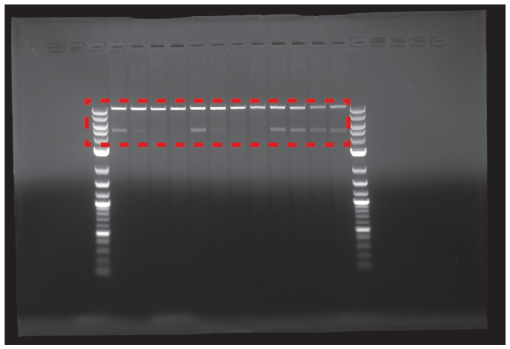

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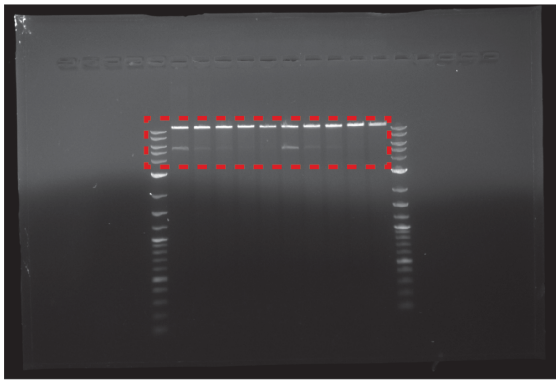
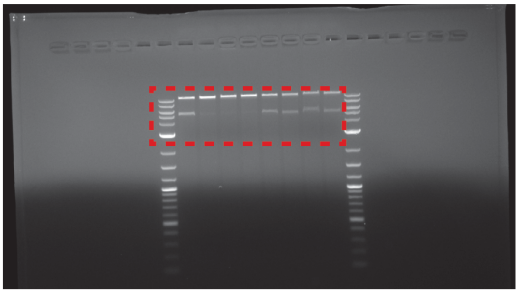


j



k

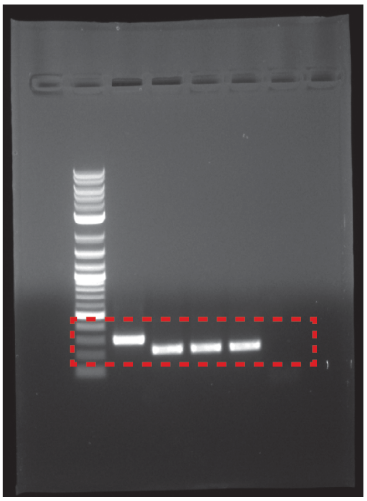


Fig S6 – Original images for data used in this study. **a** Image used for gp43 target RNA panel of Fig. 2b. The image was rotated 90 degrees clockwise and mirrored. **b** Image used for 5S rRNA panel of Fig. 2b. The image was rotated 90 degrees and mirrored. **c**. Image used for Fig. 2c. **d** Image used for Fig. 2d. The samples in the right part of the image represent a different aTc concentration. **e** Image used for Fig. 2e. **f** Image used for 5S rRNA panel of Fig. 3c. **g** Image used for gp43 target RNA, *repF*, and *def* panels of 3c. The top of *repF* cropped out represents RNA that did not enter the gel **h** Image used for Fig. 4c. **i** Image used for Fig. S2. **j** image used for Fig. S4. **k** image used for Fig S5c.

Supplementary tables

Supplementary table S1. Oligonucleotides used in this study.

Name	Sequence
AV552	tcgagtcagaaaaatatacctgtatct
AV553	cctagaccatgggtatggacagatc
GG12	gttctcgtccccttttcttcggggtgggtatcgatcctttgtactgatgatttatatacttc
GG58	gaagaaaaggggacgagaactaaatctaacaacactctaaaaaattg
GG424	catattgcctgatgaagtgaatag
GG425	ctattcacttcatcaggcaatatg
JTR390	atgcggccgcgtgacatatcatataatcttgtactagtgattgtc
JTR405	gaacctttgtactgatgatttatatacttcggcatacgt
JTR406	gatcacgtatgccgaagtatataaatcatcagtacaaag
JTR592	ttttgtgtgttgcggctcctattctcccgactttggtacc
JTR595	gcgggaaccaatcatcaaatttaaacttcattgcataatc
JTR601	caacattatcatcgacactaaggcaaccttcaccagttgg
JTR606	gtgttcggcatgggaacaggtgtgacctcc
PS465	gaatctagtatgattggagcaattgcttctcctgtagttagagatttgcaaacc
PS466	ggtttgcaaatctctaactacaggagaagcaattgctccaatcatactagattc
W614	ggttatactaaaagtcgtttgttgg
W852	ccaacaaacgacttttagtataacc
W1022	aataacatctttcatttttccatcc

Supplementary table S2. Plasmids used in this study

Plasmid name	Plasmid contents	Parental plasmid	Oligos used	Made in this study?
pE194	pTarget backbone			No[1]
pG0420	pG0400 with promoter	pG0400		Yes
pGG25	pCRISPR with *spc1* spacer only	pWJ30β	GG12+GG58	Yes
pGG-BsaI-R	pCRISPR with no spacer			No[2]
pJTR109	pCRISPR with gp43 spacer, dHD	pWJ191, pJTR119	(W852+GG425)+(W614+GG424)	Yes
pJTR111	pCRISPR with *spc1* spacer, dCsm6	pGG25, pWJ241	(W852+GG425)+(W614+GG424)	Yes
pJTR121	pCRISPR with gp43 spacer, dHD, dPalm	pWJ191, pJTR120	(W852+GG425)+(W614+GG424)	Yes
pJTR125	pCRISPR with gp43 spacer, dHD, dCsm6	pWJ191, pJTR124	(W852+GG425)+(W614+GG424)	Yes
pJTR135	pCRISPR with *spc1*-flip spacer	pGG-BsaI-R	JTR405+JTR406 (BsaI cloning)	Yes
pJTR138	pCRISPR with *spc1*-flip spacer, dCsm6	pJTR135, pWJ241	(W852+GG425)+(W614+GG424)	Yes
pJTR162	pTarget	pJTR77		Yes
pJTR175	pCRISPR with *spc1* spacer, dHD	pGG25, pJTR109	(W852+GG425)+(W614+GG424)	Yes
pJTR177	pCRISPR with *spc1* spacer, dHD, dCsm6	pGG25, pJTR125	(W852+GG425)+(W614+GG424)	Yes
pJTR193	Genome editing plasmid, with spacer targeting homology arm of pG0400, and homology arms with a promoter inside	pJTR173, pJTR190		Yes
pJTR240	pCRISPR with *spc1* spacer, dCsm3, dCsm6	pJTR111	(PS465+W614)+(PS466+W852)	Yes
pJTR241	pCRISPR with *spc1*-flip spacer, dCsm3, dCsm6	pJTR138	(PS465+W614)+(PS466+W852)	Yes
pWJ191	pCRISPR with gp43 spacer			No [3]
pWJ241	pCRISPR with gp43 spacer, dCsm6			No [3]

Supplementary table S3. Cloning strategies for plasmids used in this study

Name	Sequence
pG0420	Homology-directed allelic exchange with Cas9 elimination was performed as described in reference [4]. Briefly, a plasmid (pJTR193) with the promoter to be inserted between 1 kb homology arms was transformed into pG0400-containing *S. aureus* RN4220 cells. This plasmid also contained Cas9 with a spacer targeting the unedited pG0400 template. Subsequently, pJTR193 was cured by growing the cells at the non-permissive temperature (37°C)
pGG25	PCR amplification of pWJ30β with GG12/GG58, followed by a Gibson assembly of the PCR product
pJTR109	PCR amplification of pWJ191 with W852/GG425, and pJTR119 with W614/GG424, followed by a Gibson assembly of the two products
pJTR111	PCR amplification of pGG25 with W852/GG425, and pWJ241 with W614/GG424, followed by a Gibson assembly of the two products
pJTR121	PCR amplification of pWJ191 with W852/GG425, and pJTR120 with W614/GG424, followed by a Gibson assembly of the two products
pJTR125	PCR amplification of pWJ191 with W852/GG425, and pJTR124 with W614/GG424, followed by a Gibson assembly of the two products
pJTR135	BsaI-HF (NEB) digestion of plasmid pGG-BsaI-R, followed by ligation with annealed oligos JTR405 and JTR406 with compatible overhangs
pJTR138	PCR amplification of pJTR135 with W852/GG425, and pWJ241 with W614/GG424, followed by a Gibson assembly of the two products
pJTR162	Digestion of pJTR77 with with KpnI-HF and MfeI-HF, and ligation with chromosomal fragment from *S. aureus*
pJTR175	PCR amplification of pGG25 with W852/GG425, and pJTR109 with W614/GG424, followed by a Gibson assembly of the two products
pJTR177	PCR amplification of pGG25 with W852/GG425, and pJTR25 with W614/GG424, followed by a Gibson assembly of the two products
pJTR193	PCR amplification of pJTR173 with AV552/W614 and AV553/W852, and pJTR190 with JTR530/JTR531, followed by a Gibson assembly of the three products
pJTR240	PCR amplification of pJTR111 with PS465/W614, and with PS466/W852, followed by a Gibson assembly of the two products
pJTR241	PCR amplification of pJTR138 with PS465/W614, and with PS466/W852, followed by a Gibson assembly of the two products

Supplementary references.

1 Horinouchi, S. & Weisblum, B. Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibodies. *J. Bacteriol.* **150**, 804-814 (1982).

2 Goldberg, G. W., Jiang, W., Bikard, D. & Marraffini, L. A. Conditional tolerance of temperate phages via transcription-dependent CRISPR-Cas targeting. *Nature* **514**, 633-637 (2014).

3 Jiang, W., Samai, P. & Marraffini, L. A. Degradation of phage transcripts by CRISPR-associated RNases enables type III CRISPR-Cas immunity. *Cell* **164**, 710-721 (2016).

4 Chen, W., Zhang, Y., Yeo, W. S., Bae, T. & Ji, Q. Rapid and Efficient Genome Editing in Staphylococcus aureus by Using an Engineered CRISPR/Cas9 System. *J. Am. Chem. Soc.* **139**, 3790-3795 (2017).