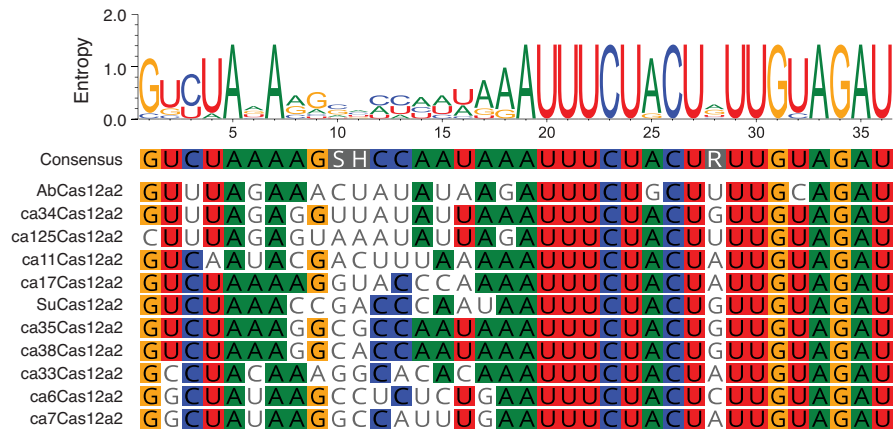


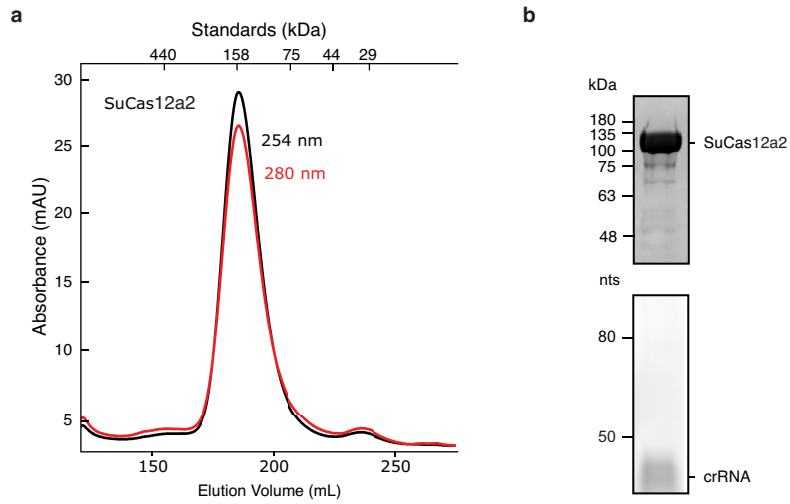
SUPPLEMENTARY FIGURES

Supplementary Figure 1 | Gel source data. See the separate PDF file. Raw gel images of Fig. 2a (**a**), Fig. 2b (**b**), Fig. 2d (**c**), Fig. 2e (**d**), Fig. 4c and ED Fig. 7a (**e**), ED Fig. 3c (**f**), ED Fig. 3d (**g**), ED Fig. 4a (**h-s**), ED Fig. 4b (**t-v**), ED Fig. 4c (**w**), ED Fig. 4d (**x**), ED Fig. 4e (**y** and **z**), ED Fig. 10e (**za**), SI Fig. 4b (**zb** and **zc**).

Supplementary Figure 2 | Portion of an amino-acid sequence alignment containing the fragmented RuvC domain within Cas12a2 and Cas12a orthologs. The domains in FnCas12a⁶⁷, LbCas12a⁶⁸, and AsCas12a⁶⁹ are based on crystal structures. The domains in SuCas12a2 were predicted using MOTIF search. The highlighted residues represent SuCas12a2 amino acids conserved in other Cas12a2 and Cas12a sequences. Numbering is shown in relation to SuCas12a2.



Supplementary Figure 3 | Nucleotide alignment of the direct repeats from CRISPR arrays associated with *cas12a2* genes. Flanking arrays could not be identified for some *cas12a2* genes.



Supplementary Figure 4 | Purification of SuCas12a2. **a**, Size exclusion chromatogram of purified SuCas12a2 bound to a co-expressed 3X crRNA guide over a Superdex 200 pg 26/600 column. Absorbance was measured at 254 nm and 280 nm. Molecular weight standards are indicated (top). **b**, SDS-PAGE of purified SuCas12a2 + crRNA (top). Urea-PAGE gel of RNA acid-phenol-chloroform extracted from purified SuCas12a2 + crRNA (bottom). For gel source data, see Supplementary Figure 1.

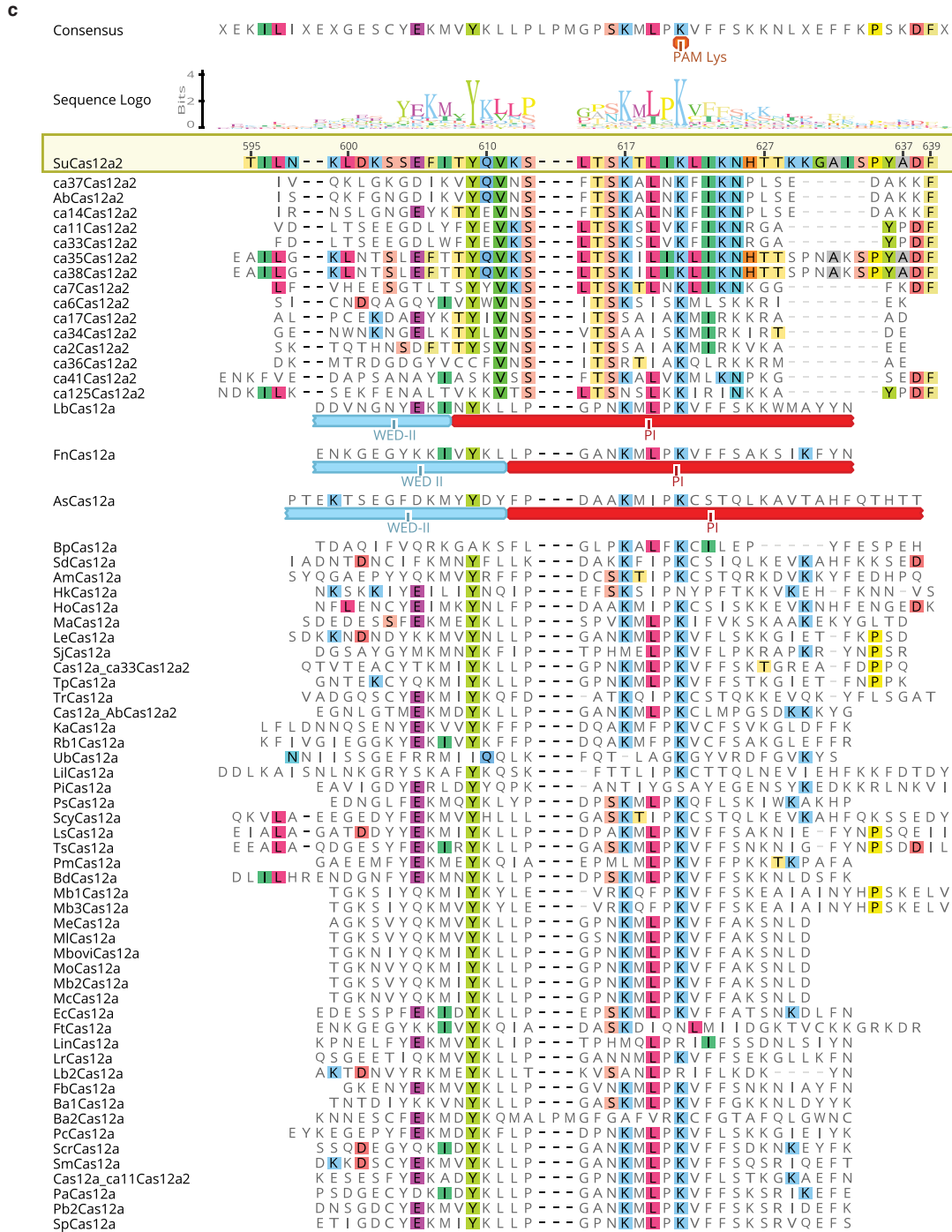
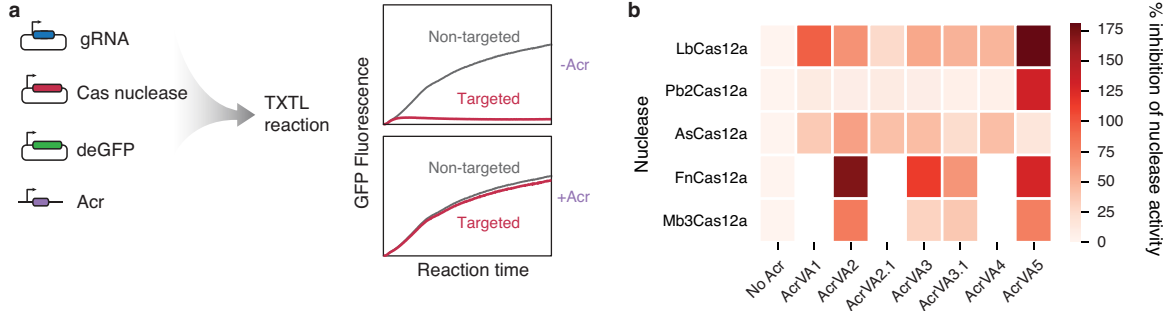
crRNA Processing Loop

SuCas12a2WP_041148111.1	-----KRYGRLQFVCAFNAHIVPQN-17-DVQKRNVTDFNKKVNHAL----SDKEYVVIGIDRGL	851
LnCas12aWP_044910712.1	KVKVLESESRVWWSKRYDEQFAVTFVSKKNAD-----CLDTTKDLNAEVMQYSE-----SNRLILIRNT	886
BrCas12aWP_013282991.1	AIKKANEDIIRNRRYTEDKFFLSLSTYTKNAD-----ISARTLDYINDKVEEDTQD-----SRMAVIVTRNL	892
ParcbacCas12aKKR91555.1	-DGNKEKEVIQHRRFAKDALTLHLKIRLNFN-----KHV-NLFDNFNKLVNTELFQ---KVPVKILGMDRGE	985
EuCAGCas12aWP_022501477.1	GHRKSKIDIVKDKRYTEDKYFLYLPITINYG-----IED---ENVNSKIEIYIAK---QDNMNVIGIDRGE	893
EuCas12aWP_012739647.1	EVRTAQKDIVKDYRYTVDKYFIHTPITINYK-----VTA--RNNVNDMVMVKYIAQ---NDDIHVIGIDRGE	883
MetCas12aWP_015504779.1	KTKKADHDIVKDRRFTVDKMMFHVPIAMNFK-----AIS--KPNLNKKVIDGIID---DQDLKIIGIDRGE	851
CanCas12aAI256868.1	RYFKAHYDITKDRRYLNDKIYFHVPLTLNFK-----ANG--KKNLNKMVIEKFLS---DEKAHIIGIDRGE	862
HcCas12aWP_005398606.1	KYKEARFDI IKDRRYSESQFFFHVPITFNWD-----IKT--NKNVNVQIVQGMIKD---GEIKHIIGIDRGE	920
AcCas12aWP_021736722.1	ITKEVSHEI IKDRRFTSDKFFFHVPITLNYQ-----AAN-SPSKFNQRVNAYLKE---HPETPIIGIDRGE	911
SuccCas12aWP_031492824.1	IKKEATHDITKDKRFTSDKFFFHCPITINYK-----EGD--TKQFNNEVLSFLRG---NPDINIIGIDRGE	937
MiCas12aKKQ38174.1	-----GNKVIDHKRYSENKIFFHVPLTLNRT-----KNDSY--RFNAQINNF---LANNKDINIIGVDRGE	894
PcCas12aKKT48220.1	-----GDRAYKRYRYTEKIMFMSLVLNTG-----KGEIKQVQFNKIINQRISSDNEMRVNVIGIDRGE	926
AnCas12aWP_027407524.1	KESVFNVDLIKDKRYTERKFFHCPITLNFN-----ADK--PIKYNEKINRFVEN---NPDVCIIGIDRGE	843
ProteoCas12aWP_028830240.1	LKDKFDYPI IKDKRYSQDKFFFHVPIMVINYK-----SEKLSKSLNRRTNENLQ---FTHIIGIDRGE	784
PdCas12aWP_004356401.1	KVSLFTYDIYKNRRYEMENKFLFHLISIVQNYK-----AAN-DSAQLNSSATEYIRK---ADDLHIIGIDRGE	946
LnNCCas12aWP_027109509.1	KESIFSVDIVKDKRYTVDKFFIHLPIITLNYK-----EQN--VSRFNDYIREILK---SKNIRVIGIDRGE	815
BuFbCas12aWP_027216152.1	PTRRLDYDIVKDRRYSDKFMHLHSIIMNFG-----AEE--NVSFNDIVNGVLRN---EDKVNVIIGIDRGE	837
OriCas12aWP_049895985.1	KTSTFDYDIVKDRRYSDKFMHLHPIITVNFN-----TNE--SGKFNELVNNAIRA---DKDVNVIGIDRGE	834
PseudoCas12aWP_028248456.1	PTSKFGYDI IKDKRYSQDKFMHLHPIITVNFN-----VDE--TRRFNDVNDALRN---DEKVRVIGIDRGE	817
LnmaCas12aWP_044919442.1	ETSTFSYDIVKDKRYSKDKFTLHPIITMNFN-----VDE--VSRFNDVAVNSAIRI---DENVNVIGIDRGE	818
FnCas12a_3WP_004339290.1	KESVFEYDLIKDKRFTEDKFFFHCPITINFK-----SSG--ANKFNDEINLLK---KANDVHILSIDRGE	927
PmCas12aWP_018359861.1	ETSLFNVDLVKDKRFTEDKFFFHVPISINYK-----NKK--ITNVNQVMRDYIAQ---NDDLQIIGIDRGE	863
MxCas12aKDN25524.1	-KRQFVYDI IKDKRYTQDKFMHLHVPITMNFN-----VQGMTIKEFNKKVNSIQ---YDEVNVIGIDRGE	989
LpCas12aWP_020988726.1	LFEKLYPILKDKRYSQDKFMHLHPIITLNFN-----SKE--RLNFNLKVNVEFLK---NKDINIIGIDRGE	876
SmCas12aWP_037385181.1	ATSTFNVDIVKDKRYTIDKQFHVPIITMNFN-----AEG--IFNMNQVRVQFLKA---NPDINIIGIDRGE	866
FbCas12aCCB70584.1	NEKNKTIDI IKDKRFTVDKQFHVPIITMNFN-----ATG--GSYINQTVLEYLQN---NPEVKIIGLDRGE	932
FlavbCas12aWP_045971446.1	AKNTFAYDLIKDKRYTVDKQFHVPIITMNFN-----ATG--NSYINQDVLAYLKD---NPEVNIIGLDRGE	888
LnMCCas12aWP_044910713.1	EVSVPYDI IKNRRYTVDKQFHVPLKMNFK-----ADE--KKRINDDYIEAIRS---NKGIHVIGIDRGE	842
LnNDCas12aWP_035635841.1	KTTTSLSYDVYKDKRFSQDYELHPIAINK-----PKN--IFKINTEVRVLLKH---DDNPYVIGIDRGE	835
LnCoeCas12aWP_016301126.1	KESMFDYDI IKDKRFTCDKQFHVPIITMNFN-----ALG--ENHFNRKVNRLIHD---AENMHIIGIDRGE	845
PorpCas12aWP_023936172.1	EESLFEYDLVKDRRYTMDKQFHVPIITMNFN-----CSA--GSKVNDMVAHIRE---AKDMHVIGIDRGE	883
PrevbCas12aWP_044110123.1	QQSNFEYDLIKDKRYTVDKFMFHVPIITLNFN-----GMG--NGDINMQVREYIKT---TDDLHFVIGIDRGE	877
BoCas12aWP_009217842.1	KESKFEYDLIKDRRYTVDKFHLFHVPIITMNFN-----SVG--GSNINQLVRRHIRS---ATDLHIIGIDRGE	868
PvCas12aWP_024988992.1	HTSTFKYDI IKDKRRYTVDKQFHVPIITINFK-----ATG--QNNINPIVQEVIRQ---NGITHIIGIDRGE	865
PbB_Cas12aEFI70750.1	KESIFDYDLVKDKRYTVDKQFHVPIITMNFN-----STG--NTNINQVVIDYLRT---EDDTHIIGIDRGE	869

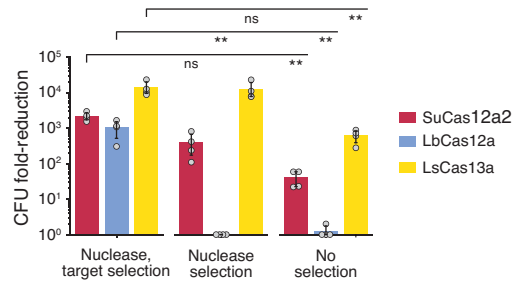
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Positively Charged Active Site Residues

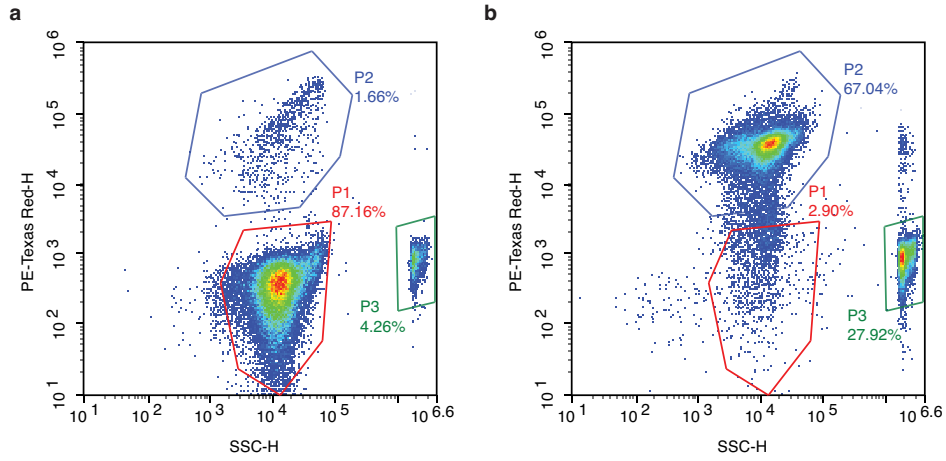
Supplementary Figure 5 | Clustal Omega amino acid sequence alignment of SuCas12a2 with Cas12a sequences. Cas12a amino acids located within the loop involved in crRNA-processing are indicated in red. Conserved positively charged residues involved in processing²⁰ are indicated. Although the putative crRNA-processing loop of Cas12a2 is not as long, the positively charged residues appear to be conserved.



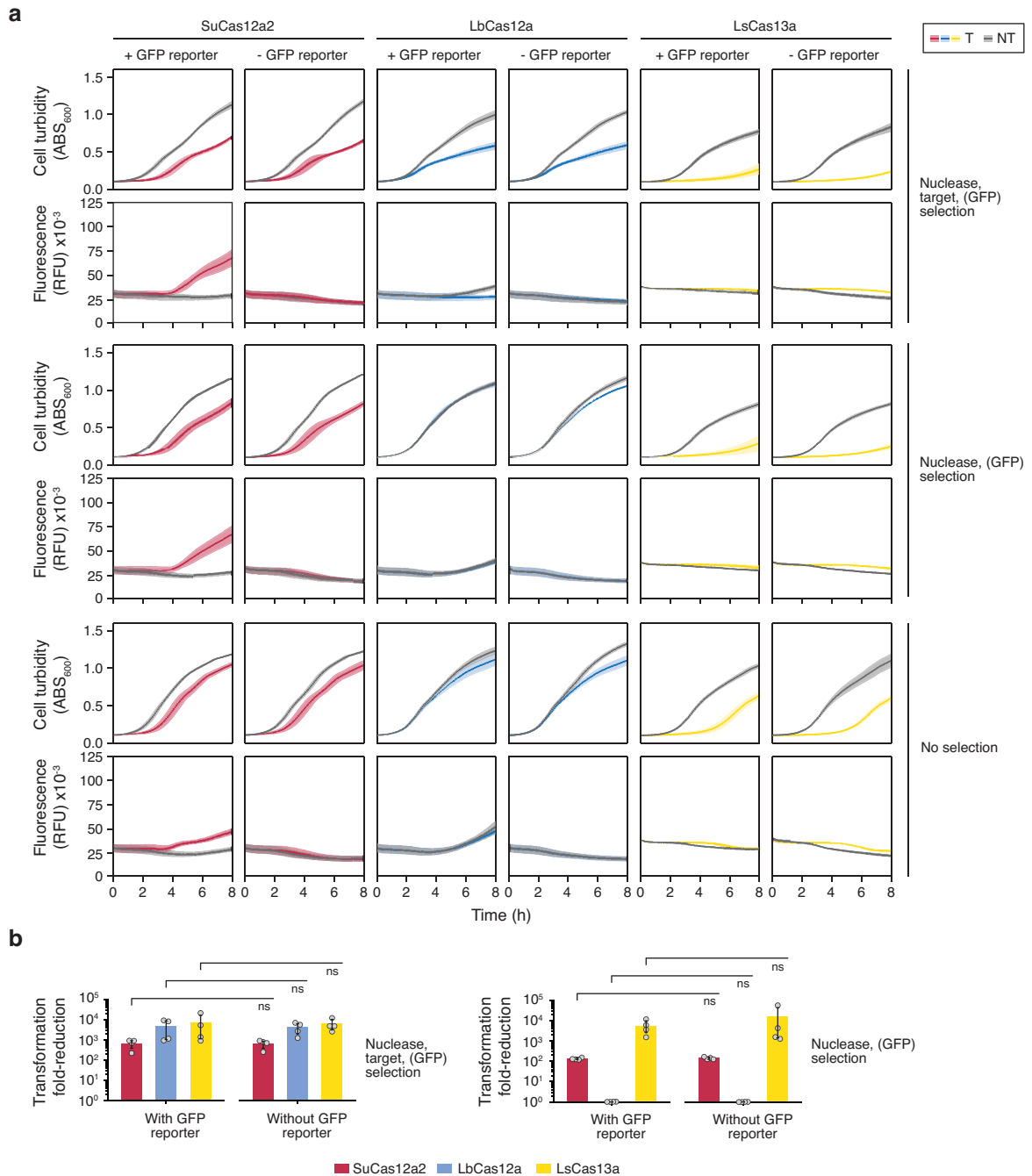
Supplementary Figure 6 | Verification of DNA targeting inhibition by AcrVA proteins in TXTL. **a**, Schematic of the Acr targeting inhibition assay in TXTL. **b**, Percent inhibition of Cas12a activity by the known type V-A anti-CRISPR proteins in TXTL. Inhibition above 100% reflects higher GFP levels for the target versus the non-target reaction in the presence of a given Acr. The mean of at least three biological replicates are shown. **c**, Amino-acid sequence alignment of the locus containing the lysine residue acetylated by AcrVA5 in Cas12a³⁹. The alignment shows that this residue is also present in Cas12a2 orthologs. The highlighted residues represent conserved amino acids relative to SuCas12a2. Numbering is shown in relation to SuCas12a2. The domains in FnCas12a⁶⁷, LbCas12a⁶⁸, and AsCas12a⁶⁹ are based on crystal structures.



Supplementary Figure 7 | Reduction in colony-forming units (CFU) following nuclease and crRNA induction under different antibiotic selection conditions in *E. coli* BL21(AI). See Fig. 4a for the corresponding time course data. Values are means \pm s.t.d. of 4 independent experiments started from separate colonies. ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.005$ calculated with one-tailed Welch's t-test.



Supplementary Figure 8 | Examples of the gating strategy used in Figure 4b. a, A sample used to gate live cells (P1). **b,** A sample used to gate dead cells treated with isopropanol (P2). Gate P3 encompasses beads supplied with the LIVE/DEAD BacLight™ Bacterial Viability and Counting Kit (Molecular Probes, L34856).



Supplementary Figure 9 | Inclusion of the SOS reporter construct does not perturb plasmid interference by the different Cas nucleases. a, Turbidity and fluorescence time course measurements when assessing the SOS-responsive GFP reporter under different antibiotic selection conditions in *E. coli* BL21(AI). Darker bands represent the mean values of four independent experiments. The shaded areas depict one standard deviation from the mean. **b**, Impact of the SOS-responsive GFP reporter on plasmid interference in *E. coli* BL21(AI) under different selection conditions. Bar heights represent mean values of four independent experiments. Values are means \pm s.t.d. of 4 independent experiments started from separate colonies. ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.005$ calculated with one-tailed Welch's t-test.