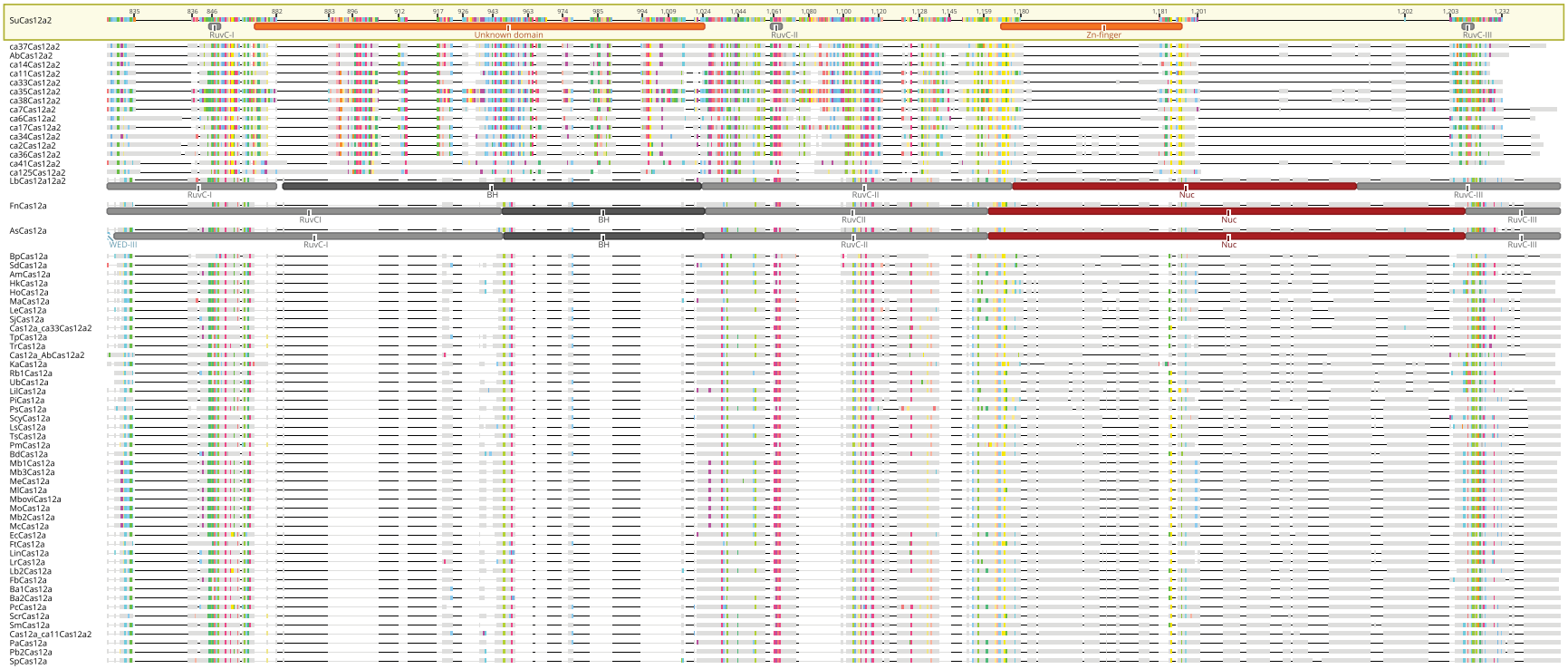
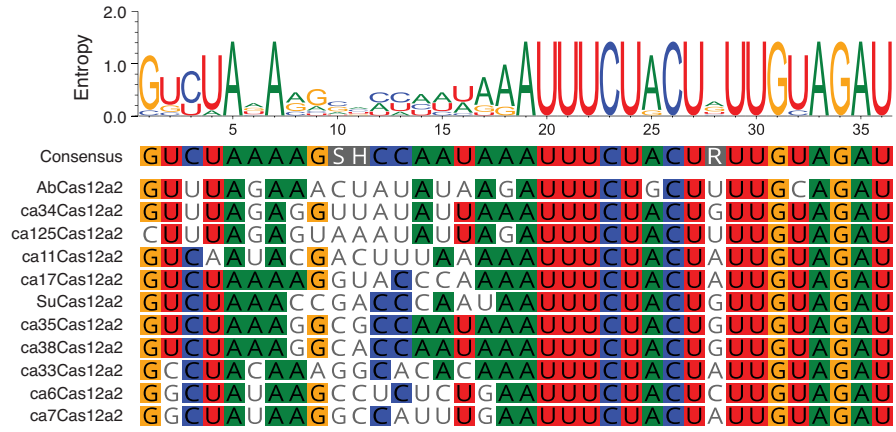
SUPPLEMENTARY FIGURES

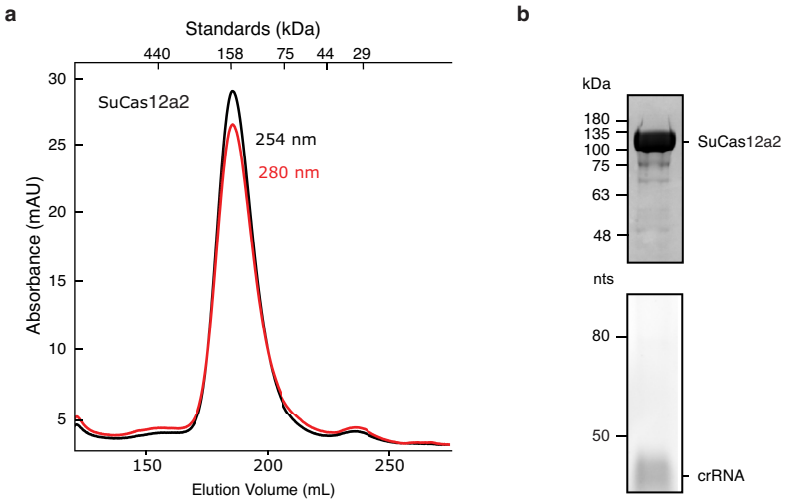
Supplementary Figure 1 | Gel source data. See the separate PDF file. Raw gel images of Fig. 2a (**a**), Fig. 2b (**b**), Fig. 2d (**c**), Fig. 2e (**d**), Fig. 4c and ED Fig. 7a (**e**), ED Fig. 3c (**f**), ED Fig. 3d (**g**), ED Fig. 4a (**h-s**), ED Fig. 4b (**t-v**), ED Fig. 4c (**w**), ED Fig. 4d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


SuCas12a2
RuvC-I
Unknown domain
RuvC-II
Zn-finger
RuvC-III
ca37Cas12a2
AbCas12a2
ca14Cas12a2
ca11Cas12a2
ca33Cas12a2
ca35Cas12a2
ca38Cas12a2
ca7Cas12a2
ca6Cas12a2
ca17Cas12a2
ca34Cas12a2
ca2Cas12a2
ca36Cas12a2
ca41Cas12a2
ca125Cas12a2
LbCas12a12a2
RuvC-I
BH
RuvC-II
Nuc
RuvC-III
FnCas12a
RuvCI
BH
RuvCII
Nuc
RuvC-III
AsCas12a
WED-III
RuvC-I
BH
RuvC-II
Nuc
RuvC-III
BpCas12a
SdCas12a
AmCas12a
HkCas12a
HoCas12a
MaCas12a
LeCas12a
SjCas12a
Cas12a_ca33Cas12a2
TpCas12a
TrCas12a
Cas12a_AbCas12a2
KaCas12a
Rb1Cas12a
UbCas12a
LiCas12a
PiCas12a
PsCas12a
ScyCas12a
LsCas12a
TsCas12a
PmCas12a
BdCas12a
Mb1Cas12a
Mb3Cas12a
MeCas12a
MlCas12a
MbovCas12a
MoCas12a
Mb2Cas12a
McCas12a
EcCas12a
FtCas12a
LinCas12a
LrCas12a
Lb2Cas12a
FbCas12a
Ba1Cas12a
Ba2Cas12a
PcCas12a
ScrCas12a
SmCas12a
Cas12a_ca11Cas12a2
PaCas12a
Pb2Cas12a
SpCas12a

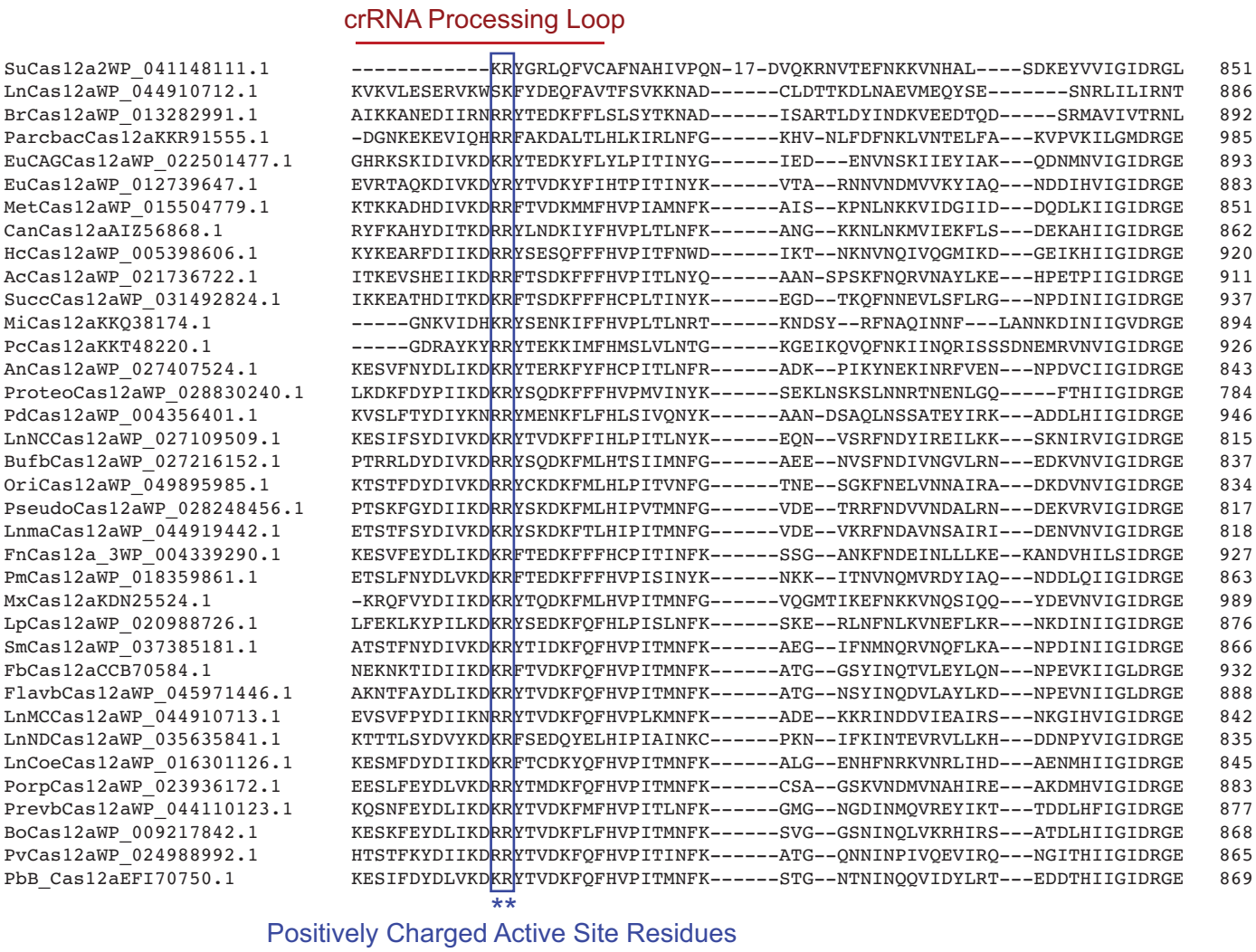
Supplementary Figure 2 | Portion of an amino-acid sequence alignment containing the fragmented RuvC domain within Cas12a2 and Cas12a orthologs. The domains in FnCas12a[67], LbCas12a[68], and AsCas12a[69] are based on crystal structures. The domains in SuCas12a2 were predicted using MOTIF search. The highlighted residues represent SuCas12a2 amino acids conserved in other Cas12a2 and Cas12a sequences. Numbering is shown in relation to SuCas12a2.



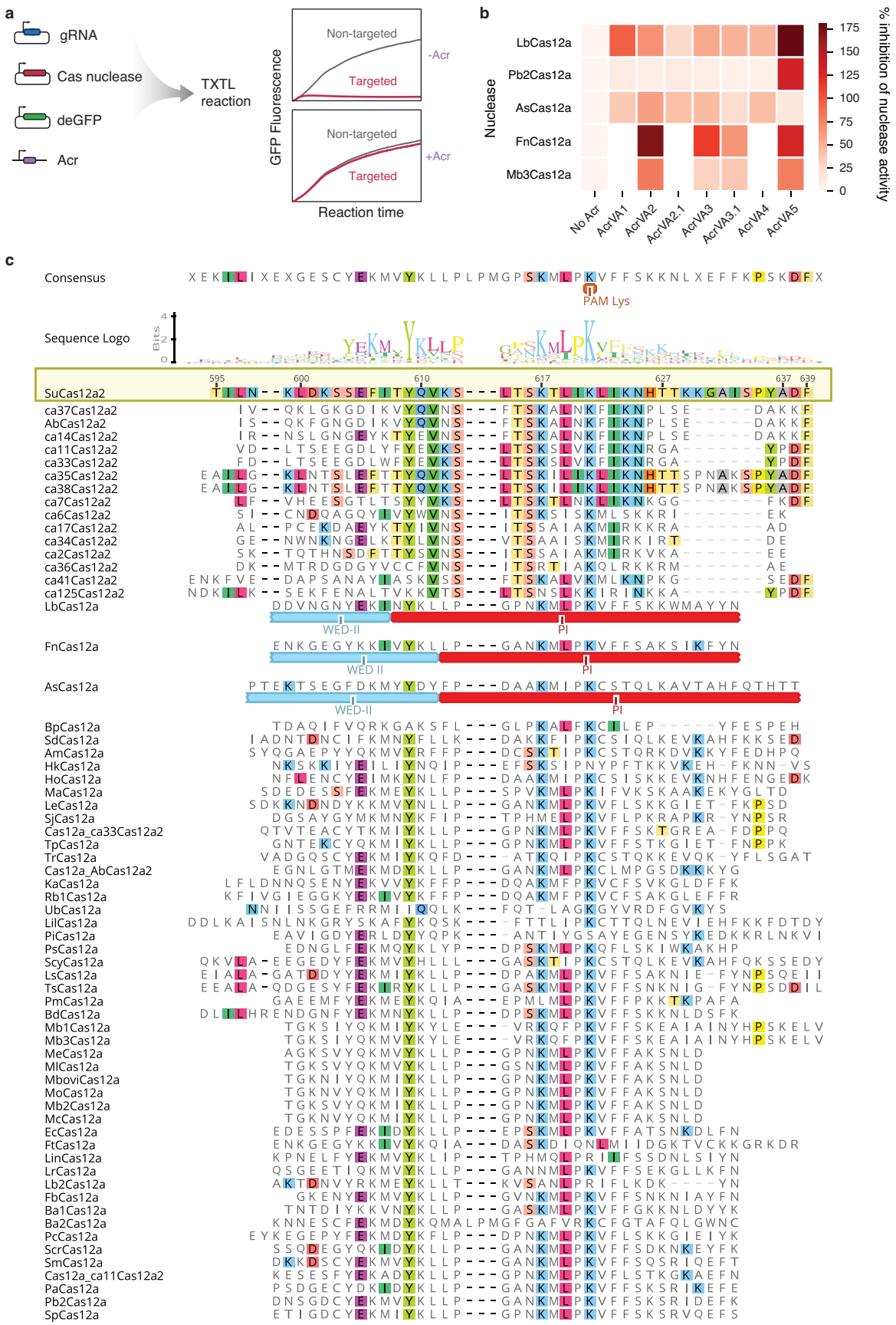
Supplementary Figure 3 | Nucleotide alignment of the direct repeats from CRISPR arrays associated with *cas12a2* genes. Flanking arrays could not be identified for some *cas12a2* genes.



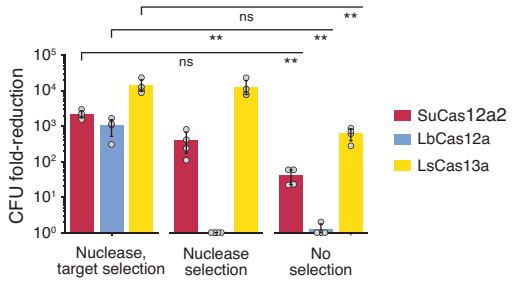
Supplementary Figure 4 | Purification of SuCas12a2. a, Size exclusion chromatogram of purified SuCas12a2 bound to a co-expressed 3X crRNA guide over a Superdex 200 pg 26/600 column. Absorbance was measured at 254 nm and 280 nm. Molecular weight standards are indicated (top). **b**, SDS-PAGE of purified 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.



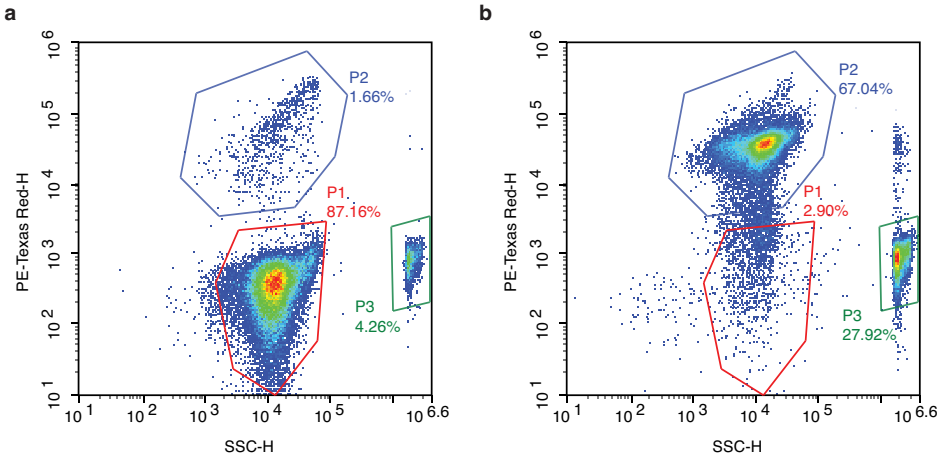
Supplementary Figure 5 | Clustal Omega amino acid sequence alignment of SuCas12a2 with Cas12a sequences. Cas12a amino acids located within the loop involved in crRNA-processing are indicated in red. Conserved positively charged residues involved in processing[20] are indicated. Although the putative crRNA-processing loop of Cas12a2 is not as long, the positively charged residues appear to be conserved.


a
gRNA
Cas nuclease
deGFP
Acr
TXTL reaction
Non-targeted
Targeted
-Acr
+Acr
GFP Fluorescence
Reaction time
b
Nuclease
LbCas12a
Pb2Cas12a
AsCas12a
FnCas12a
Mb3Cas12a
No Acr
AcrVA1
AcrVA2
AcrVA2.1
AcrVA3
AcrVA3.1
AcrVA4
AcrVA5
% inhibition of nuclease activity
175
150
125
100
75
50
25
0
c
Consensus
PAM Lys
Sequence Logo
Bits
SuCas12a2
ca37Cas12a2
AbCas12a2
ca14Cas12a2
ca11Cas12a2
ca33Cas12a2
ca35Cas12a2
ca38Cas12a2
ca7Cas12a2
ca6Cas12a2
ca17Cas12a2
ca34Cas12a2
ca2Cas12a2
ca36Cas12a2
ca41Cas12a2
ca125Cas12a2
LbCas12a
WED-II
PI
FnCas12a
WED II
PI
AsCas12a
WED-II
PI
BpCas12a
SdCas12a
AmCas12a
HkCas12a
HoCas12a
MaCas12a
LeCas12a
SjCas12a
Cas12a_ca33Cas12a2
TpCas12a
TrCas12a
Cas12a_AbCas12a2
KaCas12a
Rb1Cas12a
UbCas12a
LilCas12a
PiCas12a
PsCas12a
ScyCas12a
LsCas12a
TsCas12a
PmCas12a
BdCas12a
Mb1Cas12a
Mb3Cas12a
MeCas12a
MlCas12a
MboviCas12a
MoCas12a
Mb2Cas12a
McCas12a
EcCas12a
FtCas12a
LinCas12a
LrCas12a
Lb2Cas12a
FbCas12a
Ba1Cas12a
Ba2Cas12a
PcCas12a
ScrCas12a
SmCas12a
Cas12a_ca11Cas12a2
PaCas12a
Pb2Cas12a
SpCas12a

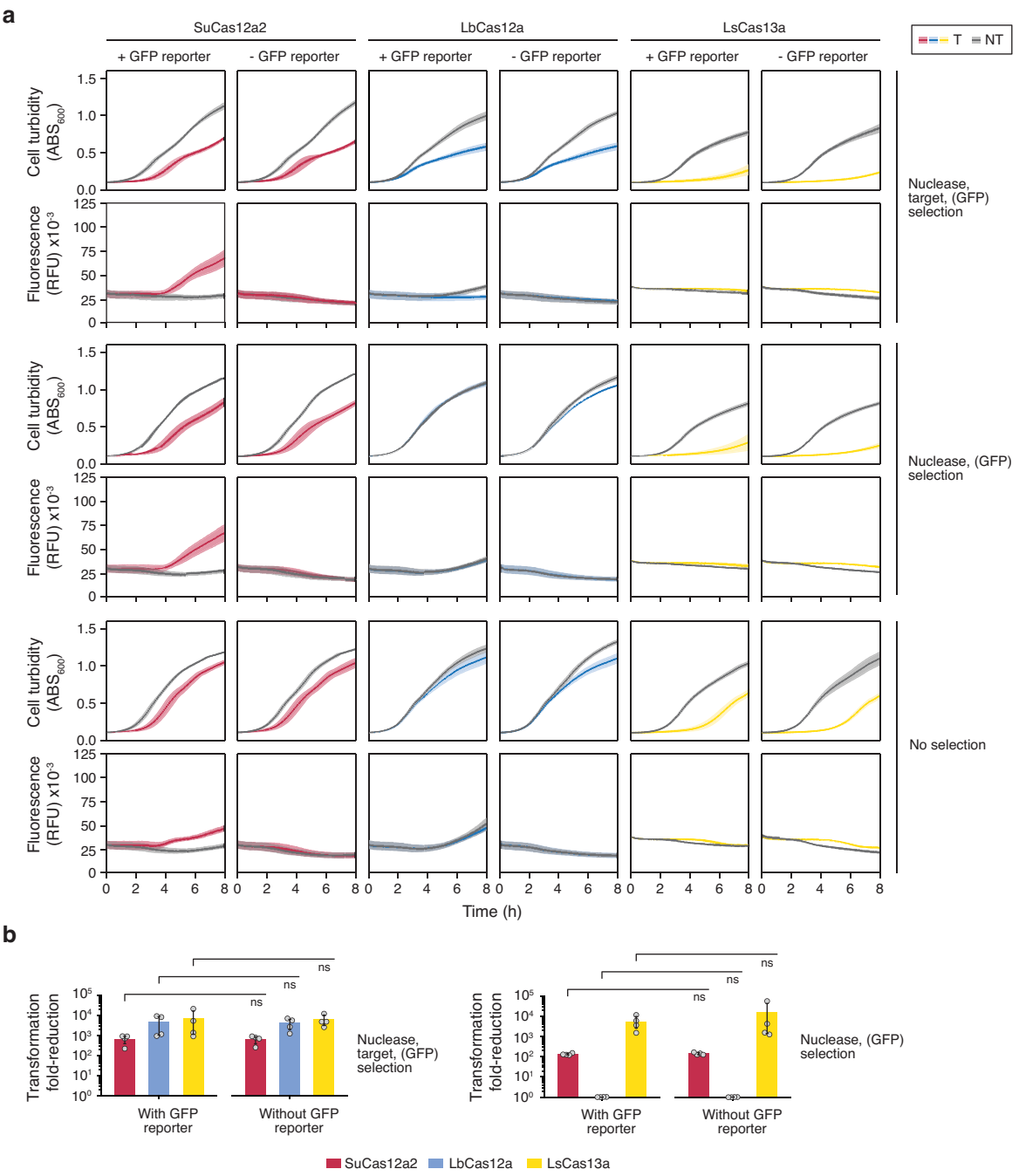
Supplementary Figure 6 | Verification of DNA targeting inhibition by AcrVA proteins in TXTL. a, Schematic of the Acr targeting inhibition assay in TXTL. **b**, Percent inhibition of Cas12a activity by the known type V-A anti-CRISPR proteins in TXTL. Inhibition above 100% reflects higher GFP levels for 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 Cas12a[39]. 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 FnCas12a[67], LbCas12a[68], and AsCas12a[69] 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 ± 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 ± 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.