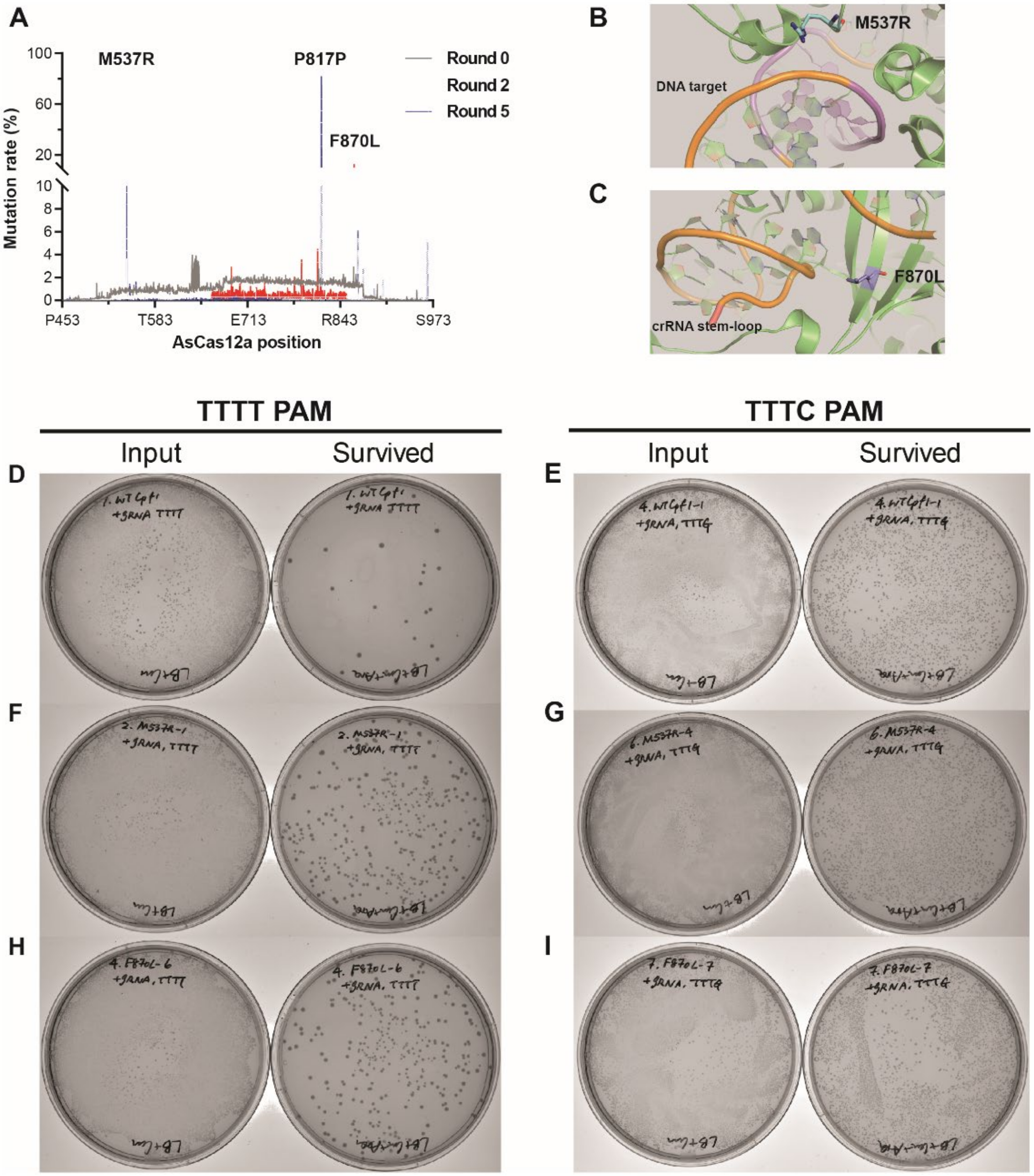
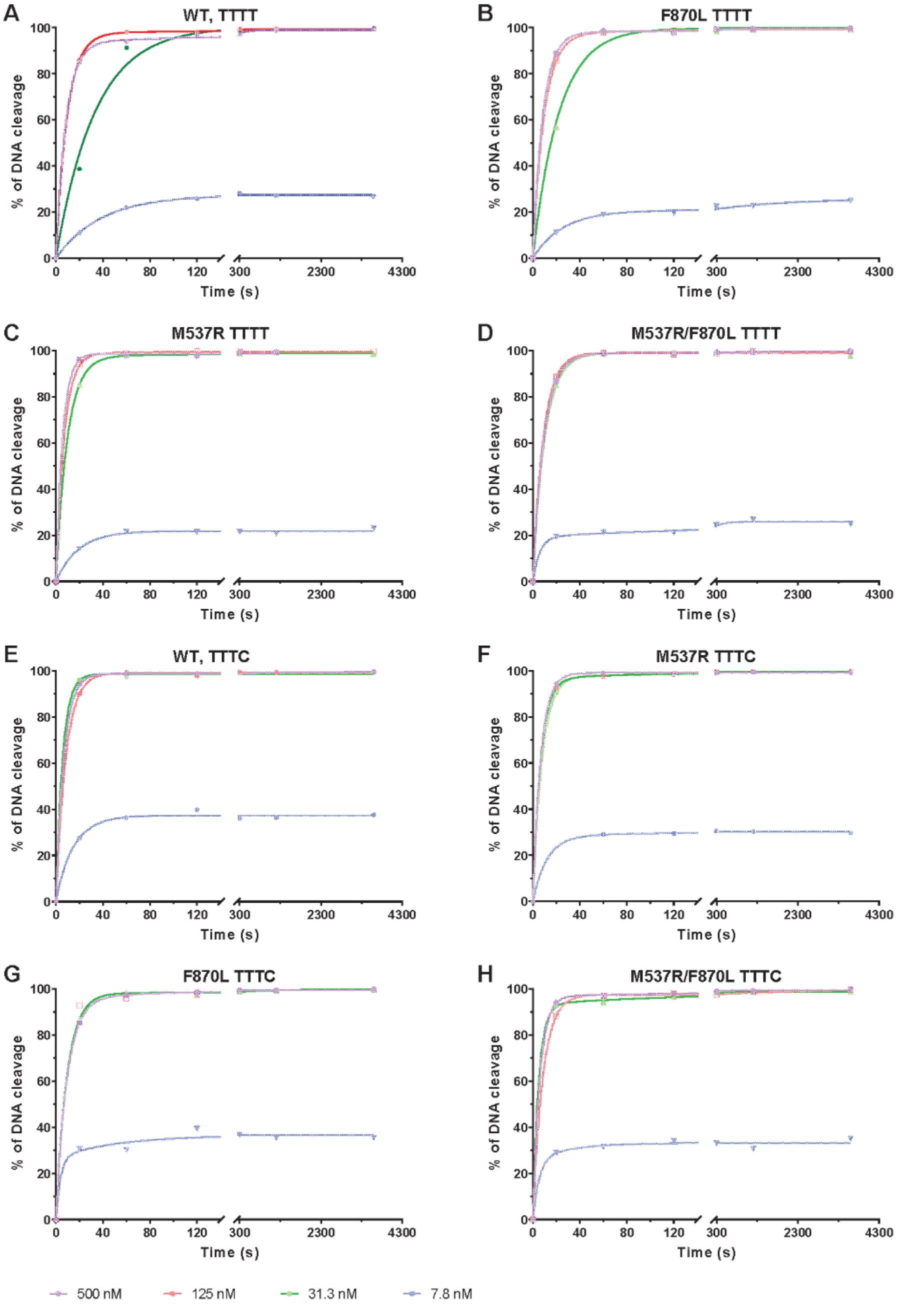
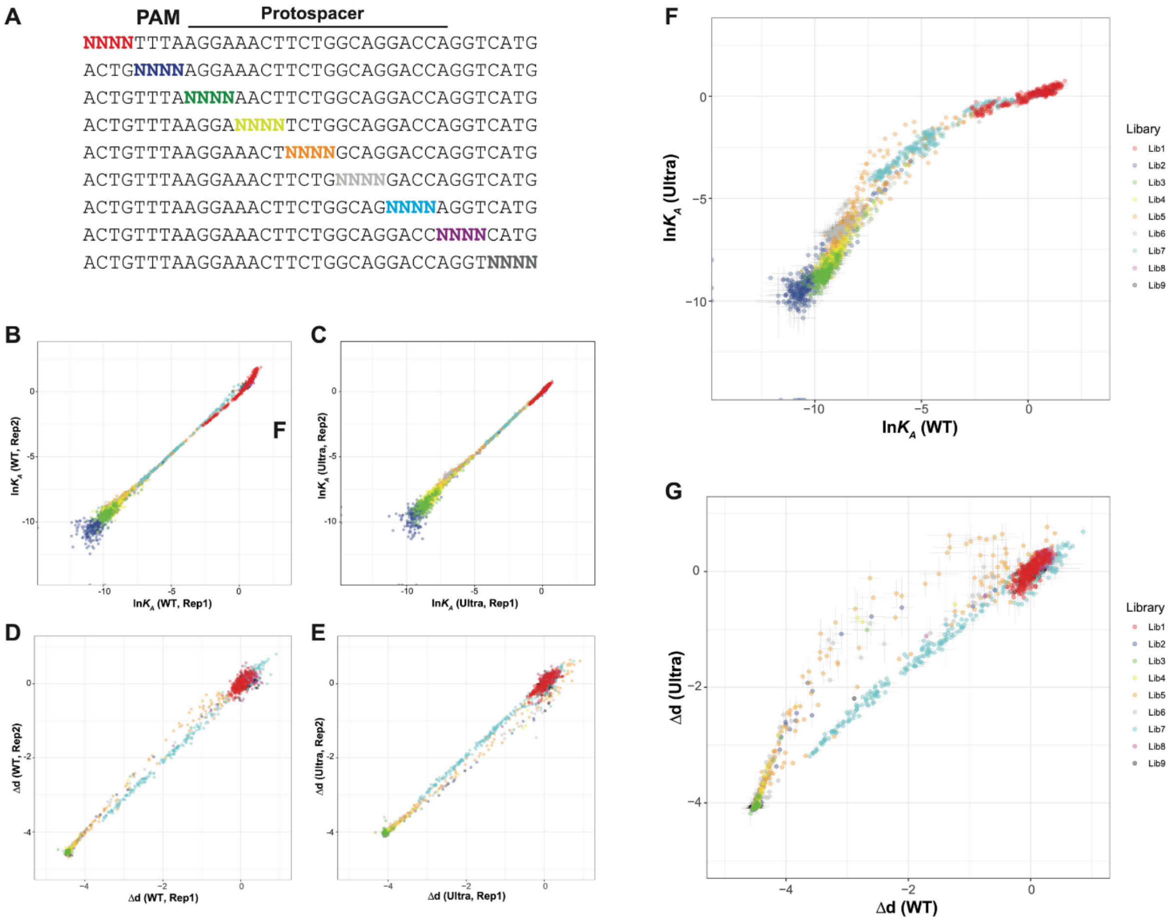


Supplementary Fig. 1 Identification and validation of AsCas12a mutants with enhanced activity in *E.coli*. a Frequency of mutations across the mutated region of AsCas12a gene over multiple rounds of selection. **b** Mapping M537R (M537R) on the crystal structure of AsCas12a (PDB: 5B43). The PAM region of DNA target is highlighted in purple. (C) Mapping the F870L on the crystal structure of AsCas12a (PDB: 5B43). **d-i** Activities of WT-AsCas12a, M537R and F870L in bacterial section assay with TTTC or TTTT PAM sequences. Higher survival rate upon arabinose selection ("Survived") relative to the transformation control (no induction, "Input") indicates a mutant with enhanced cleavage activity. Raw source data are provided in an online supplementary data file.

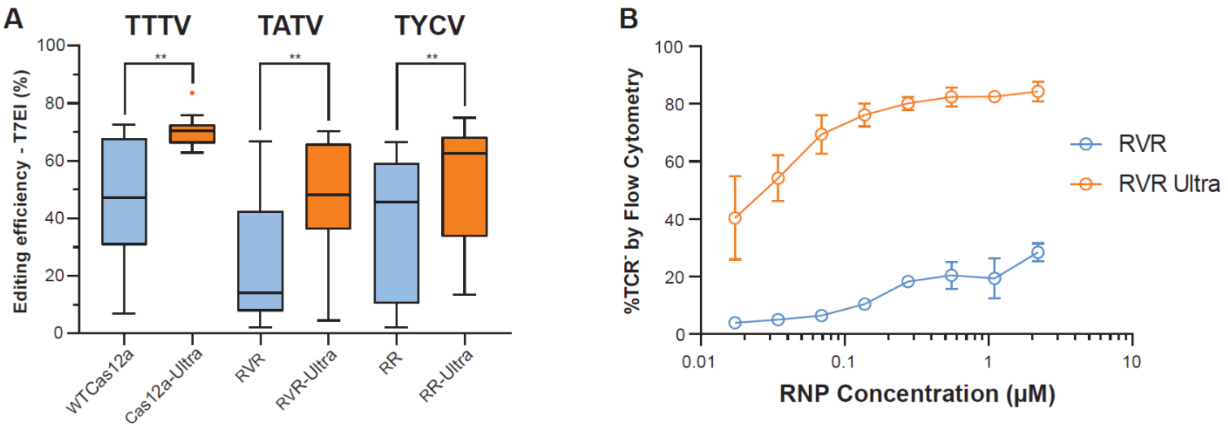
Figure S2



Supplementary Fig. 2 The *in vitro* cleavage activity of AsCas12a variants. The assembled AsCas12a RNPs were titrated into dsDNA substrates (15 nM) containing the target site used bacterial selection with TTTC or TTTT PAM sequences. Cleavage reactions were sampled at various time-points and the substrate cleavage was determined by capillary electrophoresis. protein identity and PAM sequence of DNA substrate used in each assay are listed on the top of each figure.

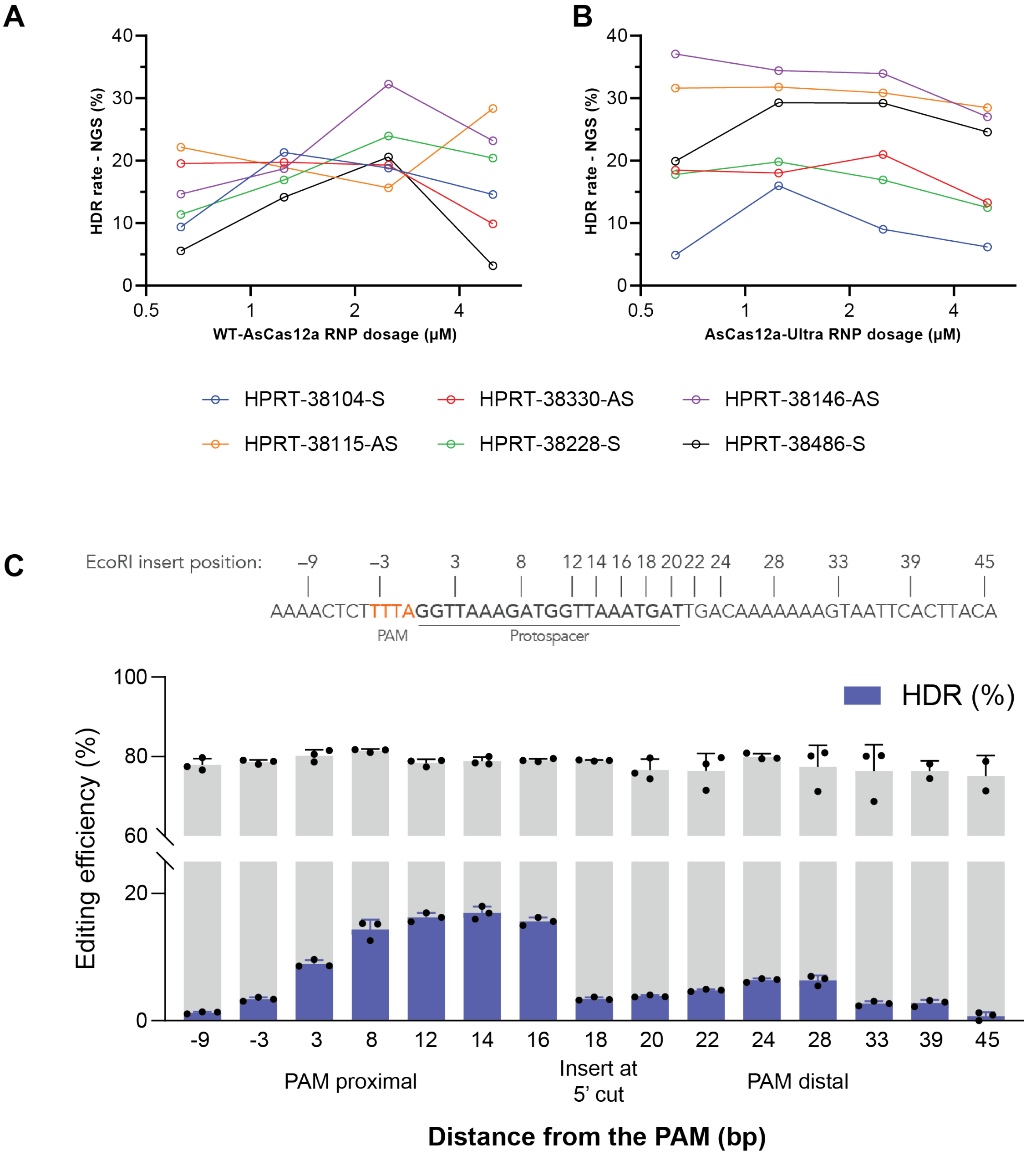


Supplementary Fig. 3 Measuring the intrinsic DNA binding and cleavage specificities of WT- and AsCas12a-Ultra by Spec-seq/SEAM-seq pair. **a** Library design with randomized region for Spec-seq/SEAM-seq. **b** Relative DNA binding affinity of nuclease-deactivated WT-AsCas12a (dAsCas12a: D908A/E993A/D1235A) over DNA sequences with mismatches in the library over two biological replicates. **c** Same as (b) but for nuclease-deactivated AsCas12a-Ultra (dAsCas12a-Ultra: D908A/E993A/D1235A/M537R/F870L). **d-e** Relative cleavage activity of WT and Ultra over DNA sequences in the library over two biological replicates. **f** Comparison of relative DNA binding affinity between WT and Ultra for each DNA sequence in the library. **g** Same as (f) but for relative DNA cleavage activity.

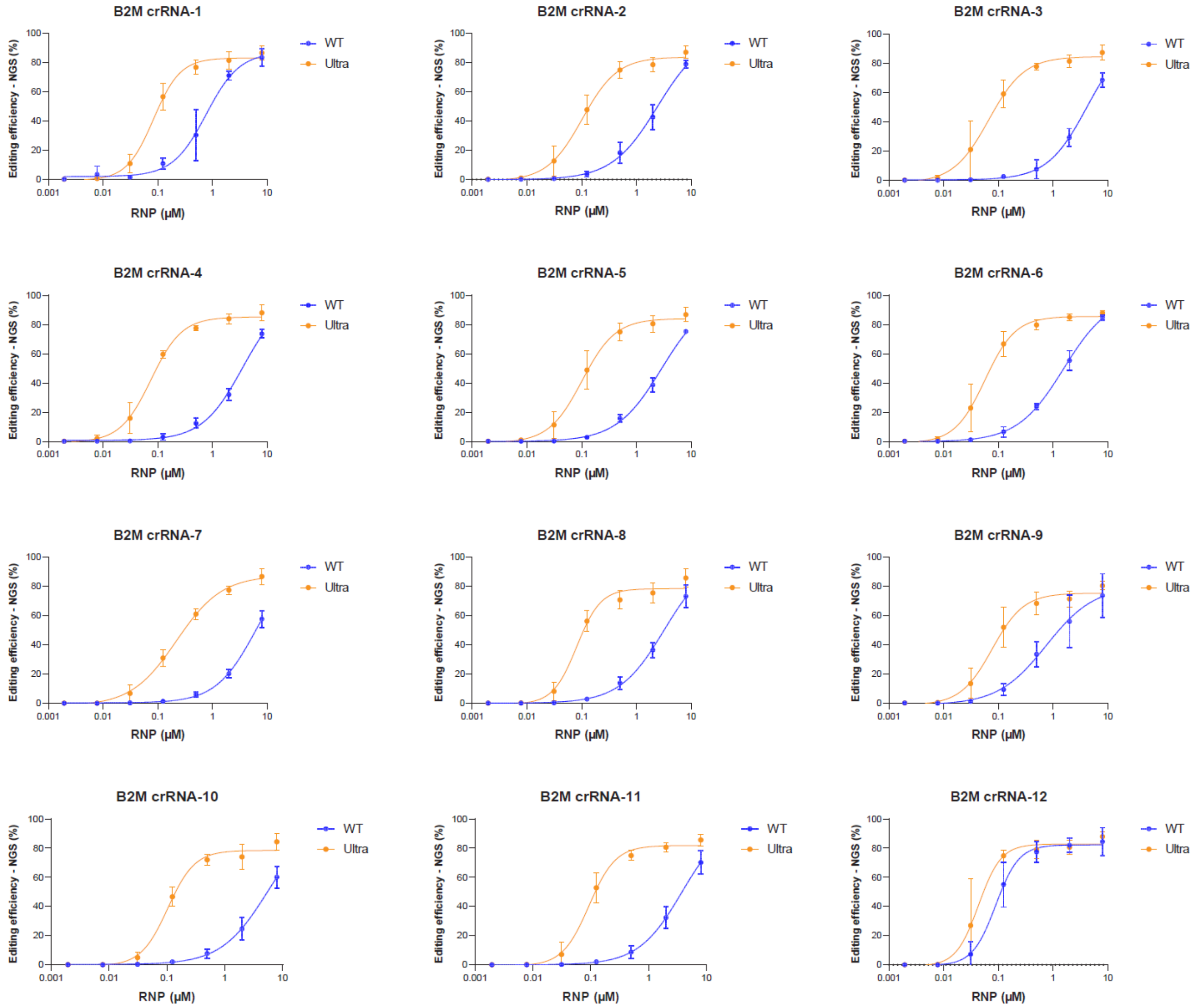


Supplementary Fig. 4 Ultra mutations substantially increased the activity of AsCas12a-RR and RVR variants. **a** Ultra mutations (M537R/F870L) were stacked on WT-, RR (S542R/K607R), and RVR (S542R/K548R/N552R) variants, and the editing efficiency of each variant was measured in HEK293 cells. Eleven crRNAs with optimal PAM sequence were tested for each variant (WT±Ultra: TTTV; RR±Ultra: TYCV; RVR±Ultra: TATV, n = 11 for each nuclease, 33 crRNAs in total). **b** Knock-out efficiency of RVR and RVR-Ultra over multiple doses in primary T cells with one crRNA (n = 1). Data are presented as mean values +/- SD. Paired, two-tailed t-test was performed to evaluate statistical significance (**: P-value = 0.0024, 0.0056, and 0.0029 respectively). Raw source data are provided in an online supplementary data file.

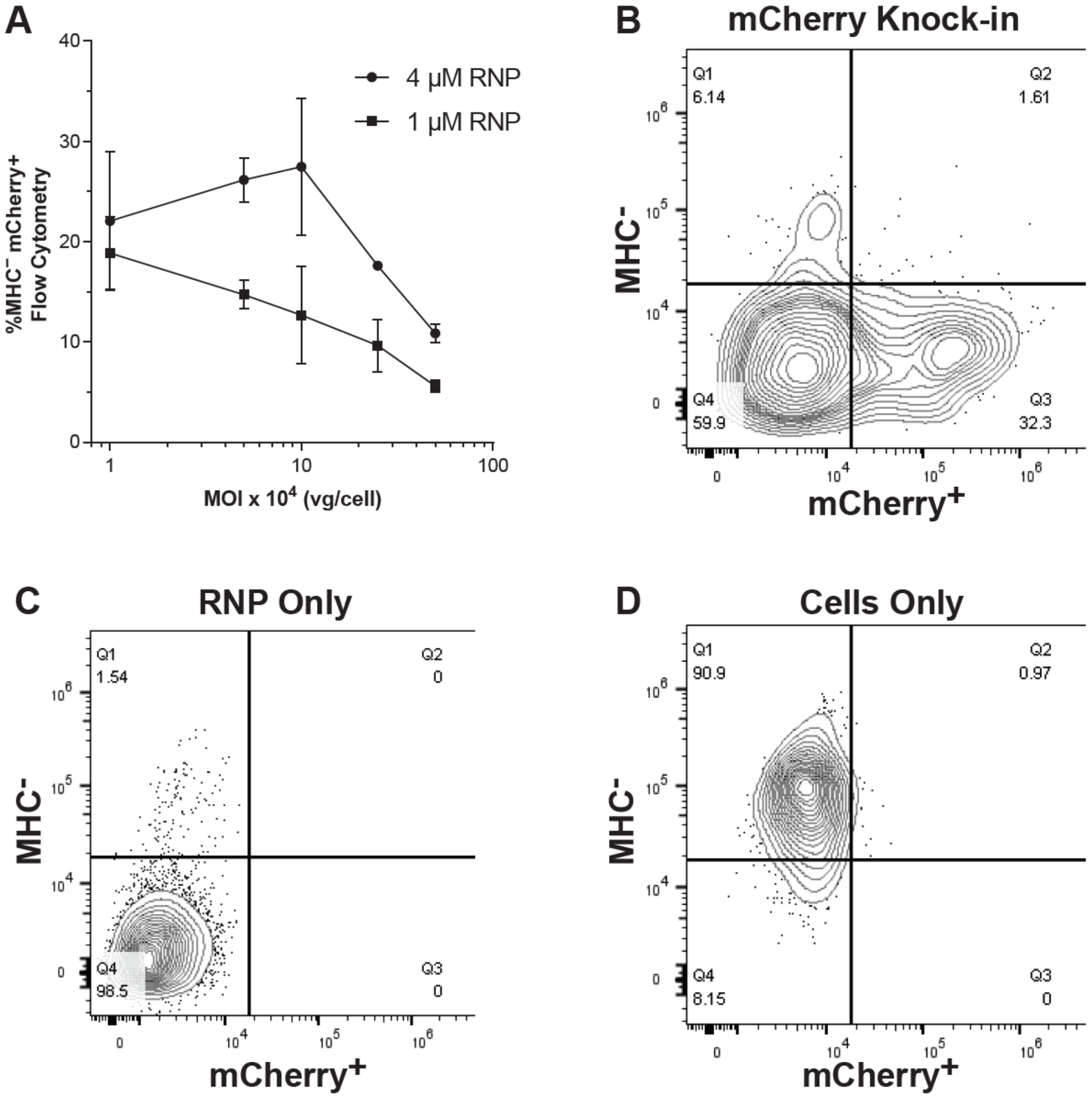
Figure S5



Supplementary Fig. 5 Optimizing experimental conditions to achieve efficient HDR rate by AsCas12a-Ultra. **a,b** Effect of RNP dosages of WT and Ultra on HDR rate. Assembled RNPs were titrated in the nucleofection reaction with fixed concentration of ssODN (3 μM) as donor template. Six pair of crRNA/ssODN were evaluated in human Jurkat cells. **c** Effect of insertion position within the target site of AsCas12a-Ultra on HDR efficiency. An EcoRI restriction sequence was inserted at various positions of the target site using ssODN (3 μM) as donor sequence. Ultra RNP was fixed at 2 μM in nucleofection reaction. Data are presented as mean values +/- SD. Raw source data are provided in an online supplementary data file.

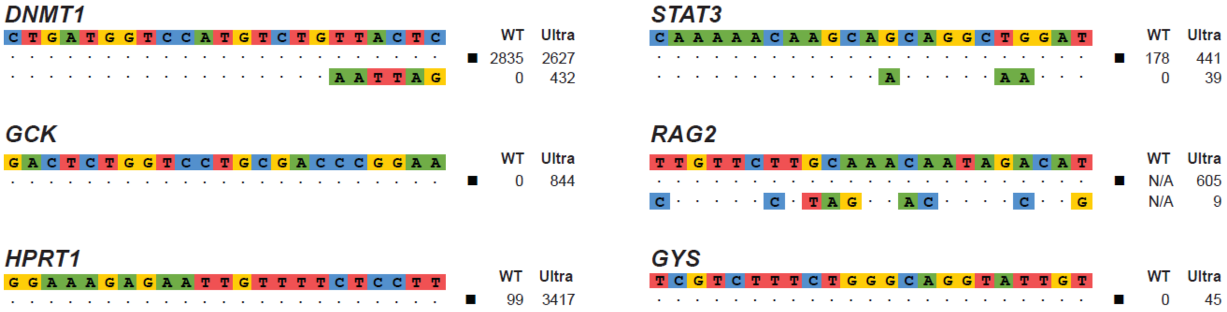


Supplementary Fig. 6 In-depth activity comparison of AsCas12a-WT and Ultra in primary T cells targeting *B2M* locus. The editing efficiency of WT and Ultra over a wide range of RNP doses at 12 crRNAs targeting *B2M* locus were measured by NGS 3-days post-electroporation. Dose-response curves were generated to estimate the EC50 of the editing, and summarized in Fig. 3c. Data are presented as mean values +/- SD. Raw source data are provided in an online supplementary data file.

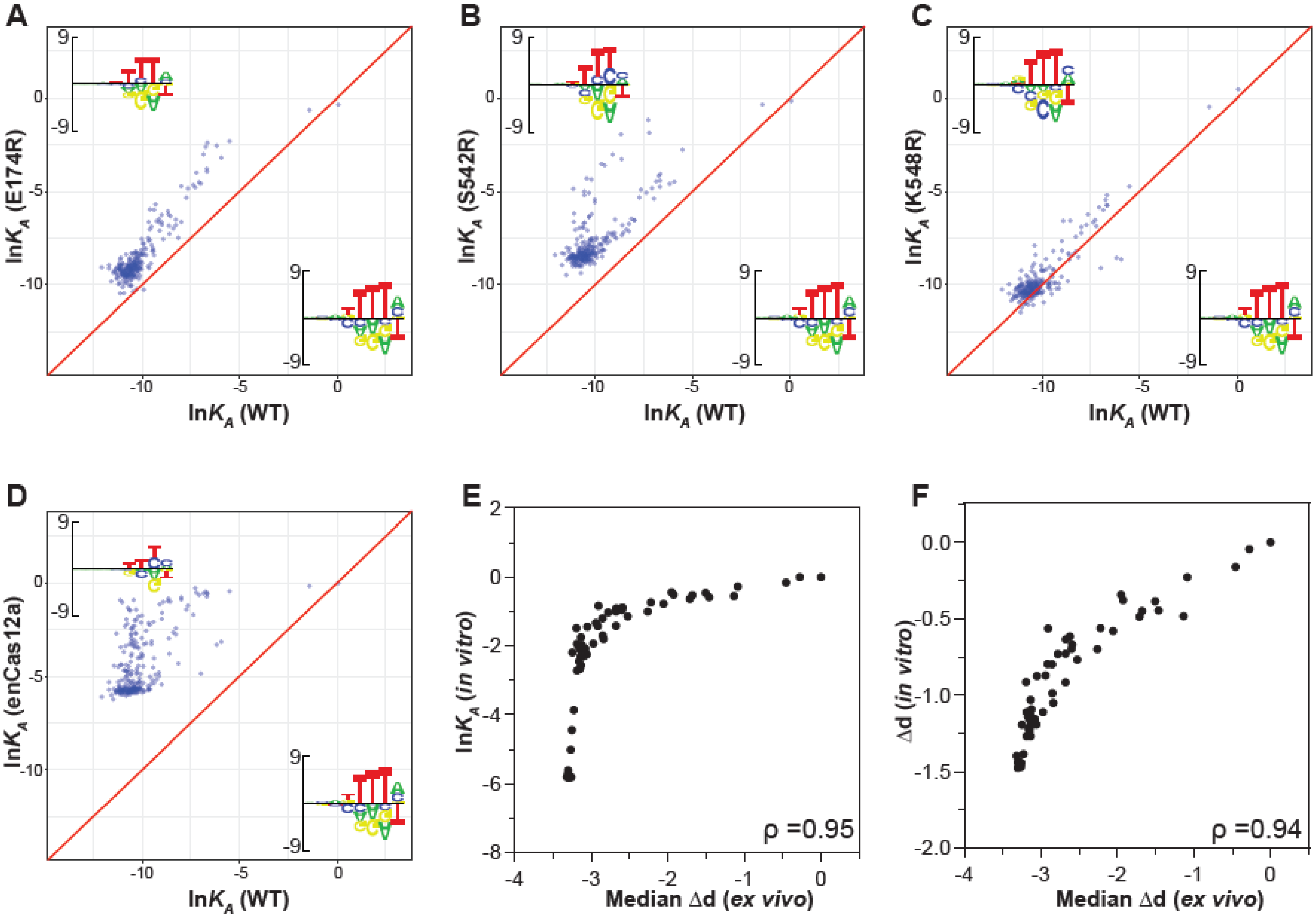


Supplementary Fig. 7 Knock-in a transgene construct at *B2M* locus in HSCs by AsCas12a-Ultra using AAV6 as donor template. a Knock-in efficiency of mCherry reporter transgene with variable MOI of AAV donor (1x10^5 vg/cell) at two RNP dosages. Data are presented as mean values +/- SD. **b-d** Representative result of flow cytometry to evaluate the knock-in efficiency of mCherry at *B2M* locus in NK cells with present of both AsCas12a RNP and AAV6 donor (b), or RNP (c) / Cell only (d). Raw source data are provided in an online supplementary data file.

Figure S8



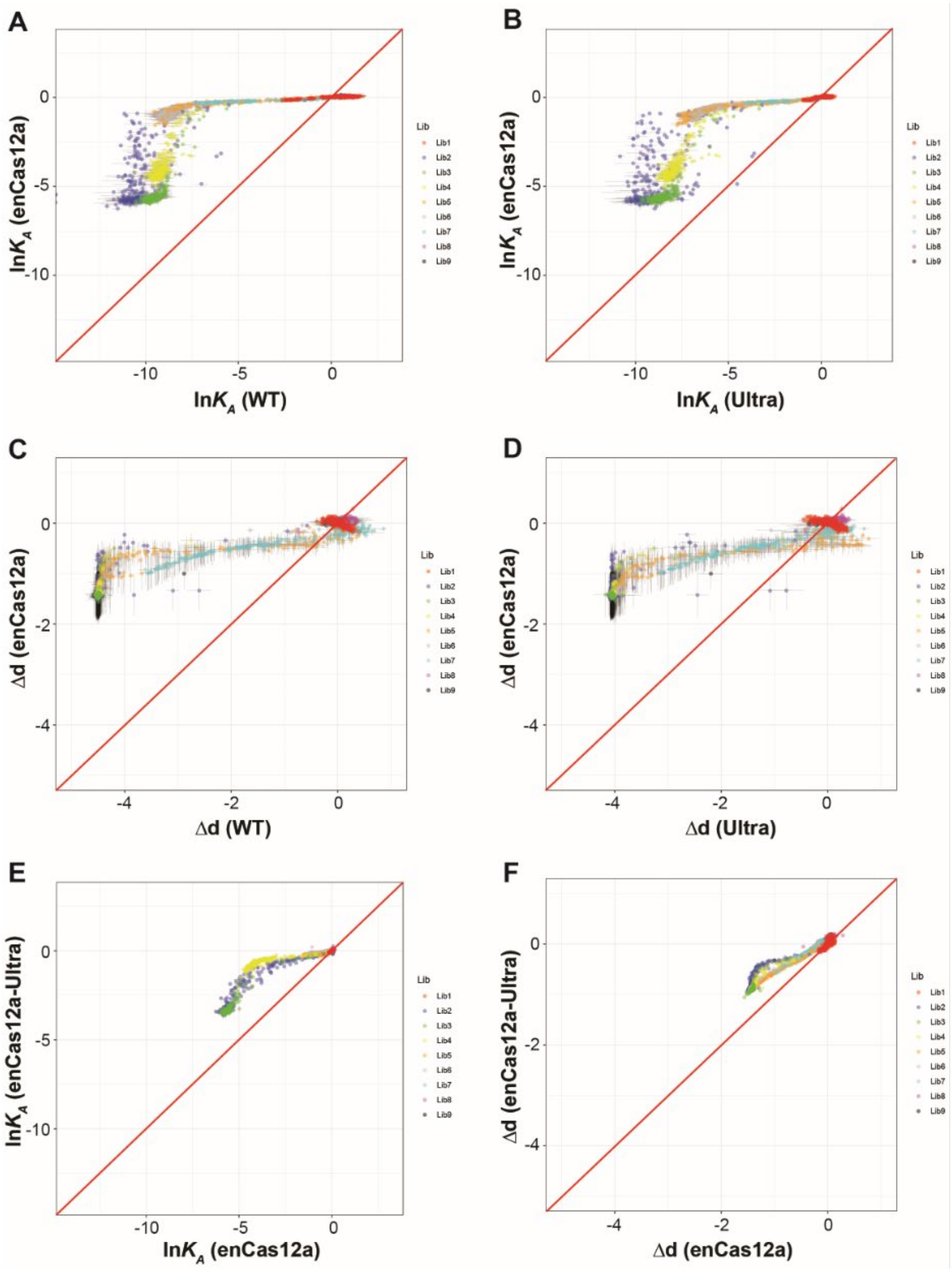
Supplementary Fig. 8 Off-target activity of AsCas12a-WT and Ultra in HEK293 cells. GUIDE-seq was performed for WT and Ultra over 6 crRNAs to detect any off-target tag integration. N/A: dataset not available.



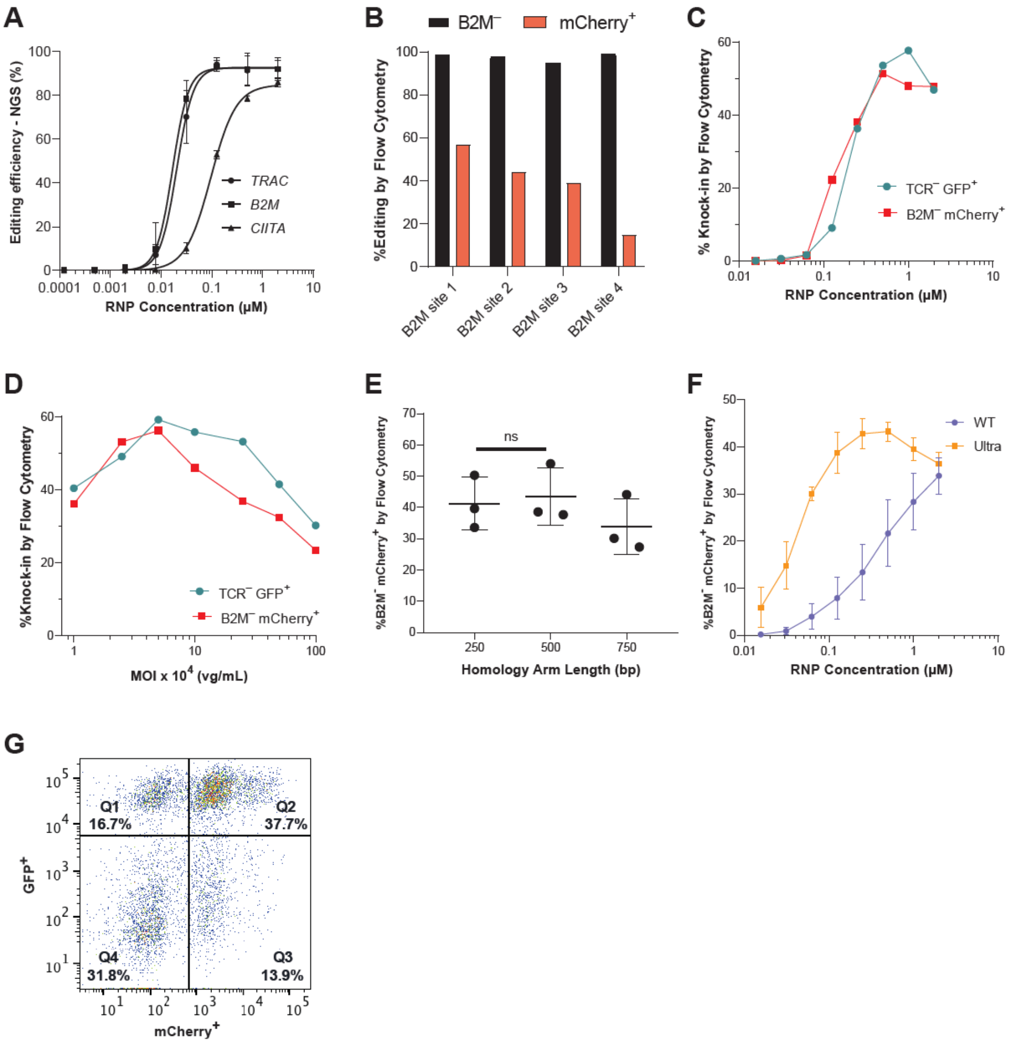
G

Rank	WT		E174R		S542R		K548R		enCas12a	
	PAM	lnK_A	PAM	lnK_A	PAM	lnK_A	PAM	lnK_A	PAM	lnK_A
1	TTTC	0.1	TTTA	0.0	TTTA	0.0	TTTC	0.5	TTTC	0.0
2	TTTA	0.0	TTTC	-0.3	TTTC	-0.1	TTTA	0.0	TTTA	0.0
3	TTTG	-1.4	TTTG	-0.6	TTTG	-0.3	TTTG	-0.5	TTTG	-0.2
4	TTTT	-5.5	TTTT	-2.3	TTCC	-1.2	TTTT	-4.7	TTCC	-0.3
5	TCTC	-5.9	CTTA	-2.4	TTCA	-1.9	ATTC	-5.2	TTTT	-0.4
6	TCTA	-6.2	TCTA	-2.5	TCCC	-1.9	ATTA	-5.4	ATTA	-0.5
7	CTTA	-6.7	ATTA	-2.6	TTTT	-2.8	CTTC	-5.7	TTCA	-0.5
8	ATTC	-6.7	GTTA	-3.0	TTCG	-3.0	CTTA	-5.8	ATTC	-0.5
9	CTTC	-6.7	TCTC	-3.2	TTCT	-3.3	GTTC	-5.8	GTTA	-0.5
10	ATTA	-6.7	CTTC	-3.8	TCCA	-3.5	GTTA	-6.0	CTTA	-0.6

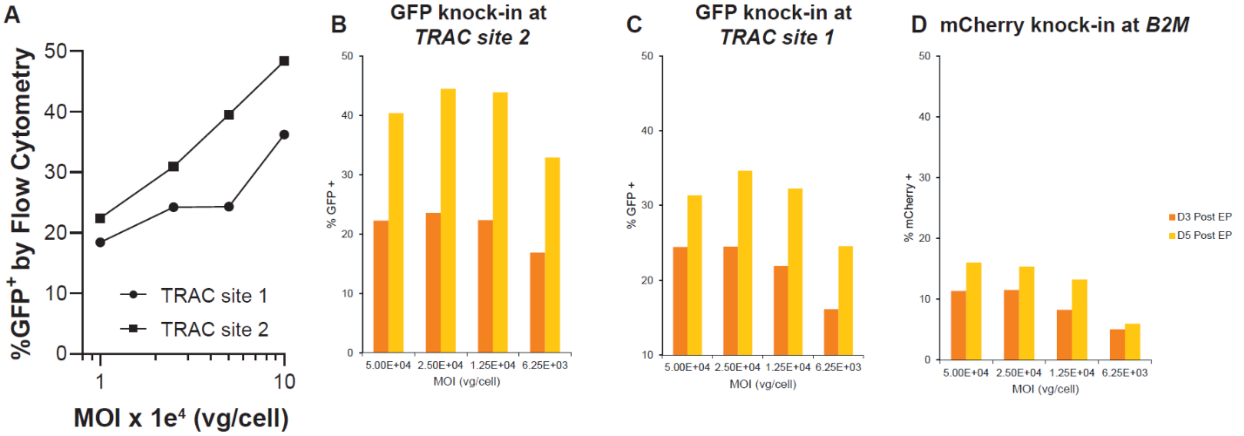
Supplementary Fig. 9 The expanded PAM flexibility of enCas12a is mediated by S542R. a-d The relative DNA binding affinities of E174R (a), S542R (b), K548R (c), and enCas12a (d) over all 256 PAM variants *in vitro*. Upper left: position-specific affinity matrix logo for each AsCas12a variant over PAM region. Bottom right: logo for WT-AsCas12a. Although all 3 point mutations globally enhanced the non-specific DNA binding affinity, only S542R significantly expanded the PAM repertoire of AsCas12a over sequences with cytosine at 3rd position of the PAM region. **e,f** PAM preference of enCas12a defined by *in vitro* approach (this study) and lentivirus-based *ex vivo* approach over 57 PAM sequences covered by both studies. Normalized depletion of each crRNA targeting essential genes in the lentivirus-based tiling assay was calculated. The natural logarithm of median depletion for each PAM sequence was plotted against the relative binding affinity (e), and cleavage specificity (f) determined *in vitro*. Highly consistent PAM preference of enCas12a was defined by two distinct approaches, as judged by the Spearman's Rho. (g) The top 10 PAM sequences targeted by each nuclease based on the *in vitro* DNA binding specificity. Raw source data are provided in an online supplementary data file.



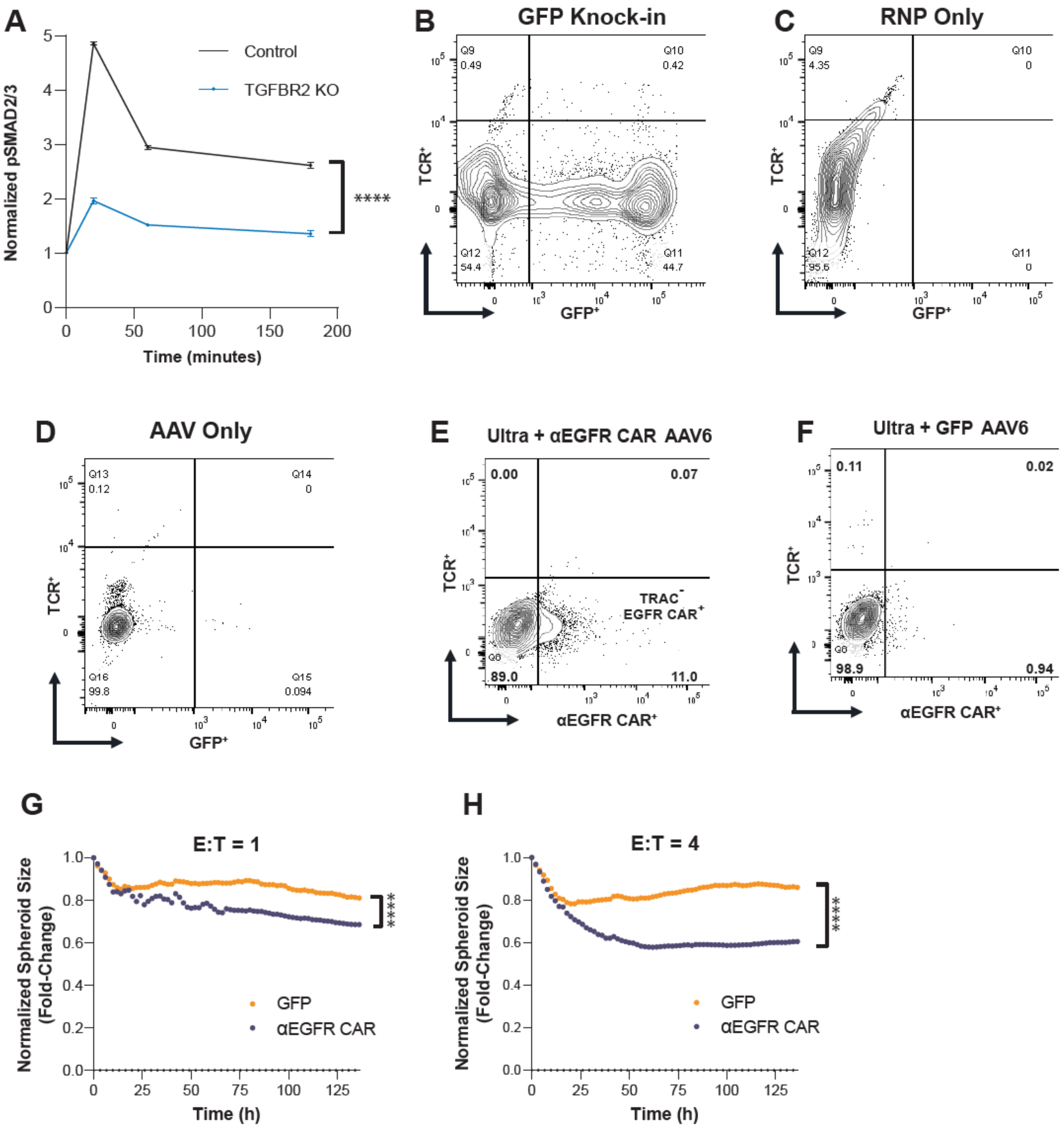
Supplementary Fig. 10 Evaluating the intrinsic DNA binding and cleavage specificities of enCas12a and enCas12a-Ultra. a,b Comparison of relative DNA binding affinity of WT (a) or Ultra (b) versus enCas12a. **c,d** Same as (a,b) but for relative DNA cleavage activity. **e,f** Comparison of relative DNA binding and cleavage between enCas12a and enCas12a-Ultra. Data are presented as mean values +/- SD.



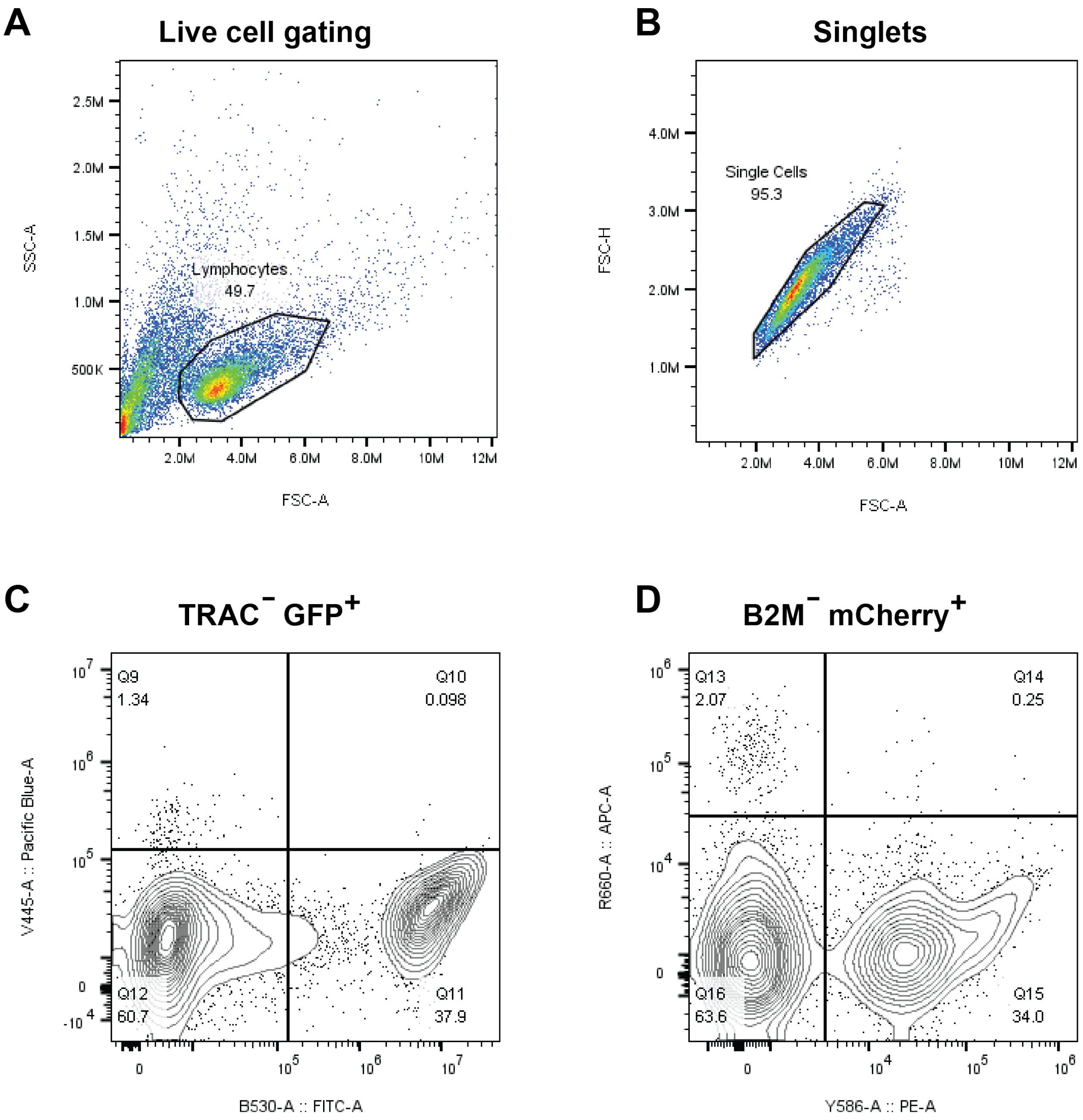
Supplementary Fig. 11 Optimizing experimental conditions to achieve efficient HDR rate by AsCas12a-Ultra using AAV6 as HDR donor template in primary T cells. **a** Editing efficiency of Ultra at *TRAC*, *B2M*, *CIITA* locus over a range of RNP dose. Note: these specific crRNAs were used for the knock-in experiments. Data are presented as mean values +/- SD. **b** Knock-in efficiency of mCherry transgene using Ultra at 4 target sites in *B2M* locus. **c** Knock-in efficiency of fluorescent reporter transgenes at each genomic locus over a range of RNP dose with fixed MOI of AAV donor (1x10^5 vg/cell). **d** Knock-in efficiency of a fluorescent reporter at either *TRAC* or *B2M* locus across a range of MOIs. RNP dose was fixed at 1 μM. **e** Knock-in efficiency of mCherry at *B2M* locus with using donor templates with different length of homology arm (RNP:1 μM; AAV MOI: 1x10^5 vg/cell). Data are presented as mean values +/- SD. One-way ANOVA was performed to evaluate statistical significance (ns: P-value > 0.05). **f** Knock-in efficiency of mCherry at *B2M* locus using WT or Ultra over a range of RNP dose. Data are presented as mean values +/- SD. **g** Representative result of flow cytometry for simultaneous double knock-in of fluorescent reporters at both *TRAC* and *B2M* loci by Ultra (RNP:1 μM; AAV MOI: 1x10^5 vg/cell). Raw source data are provided in an online supplementary data file.



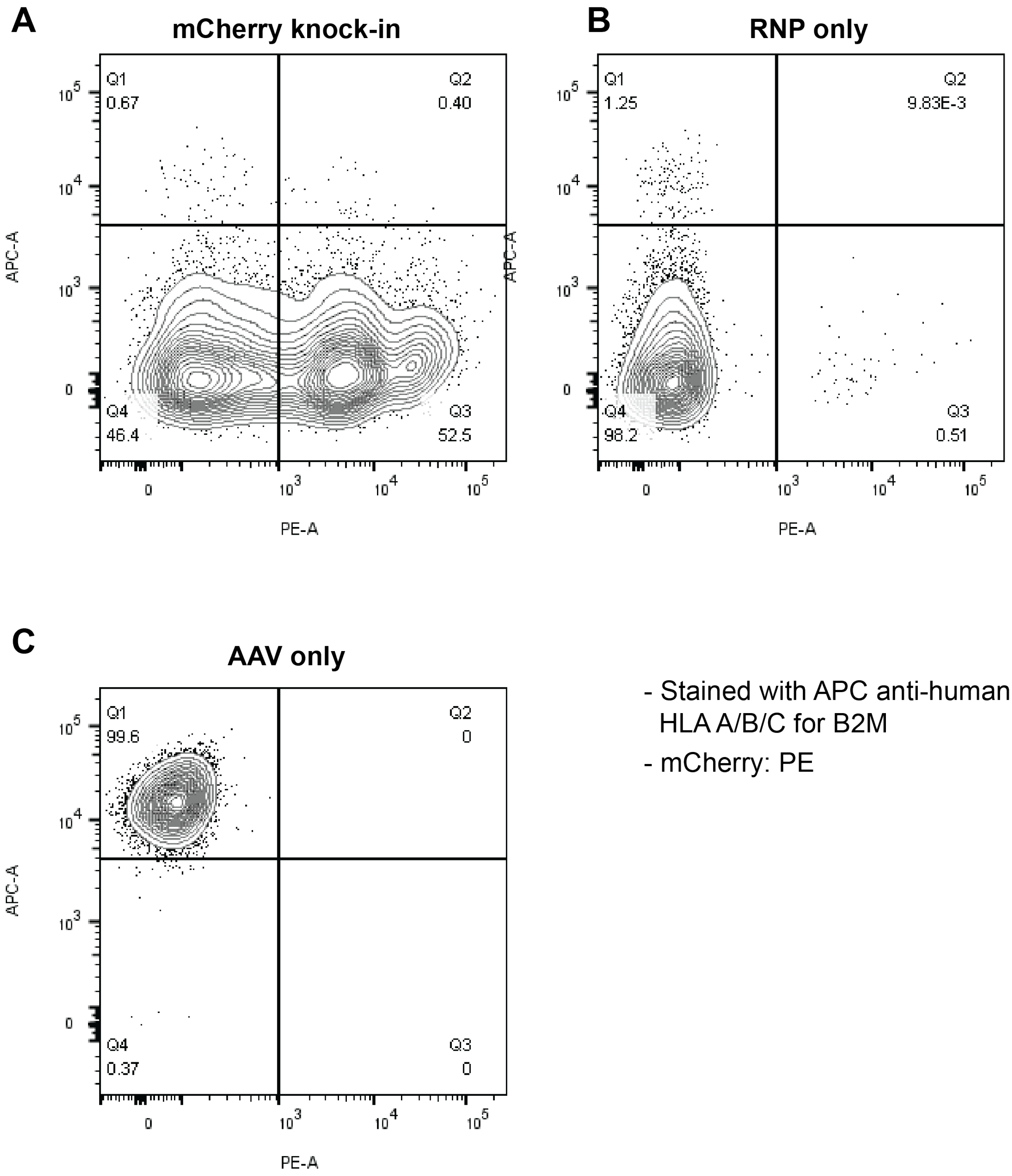
Supplementary Fig. 12 Additional optimization of knock-in condition to generate CAR-NK cells. a Transgene knock-in by AsCas12a-Ultra at two RNPs doses using variable MOI of AAV donor template. **b-d** Assessment of HDR rate by flow cytometry 3 or 5-days post-delivery over three target loci.


A
Normalized pSMAD2/3
0
1
2
3
4
5
Control
TGFBR2 KO
****
0
50
100
150
200
Time (minutes)
B
GFP Knock-in
Q9
0.49
Q10
0.42
Q12
54.4
Q11
44.7
TCR⁺
GFP⁺
C
RNP Only
Q9
4.35
Q10
0
Q12
95.6
Q11
0
TCR⁺
GFP⁺
D
AAV Only
Q13
0.12
Q14
0
Q16
99.8
Q15
0.094
TCR⁺
GFP⁺
E
Ultra + αEGFR CAR AAV6
0.00
0.07
TRAC⁻
EGFR CAR⁺
Q6
89.0
11.0
TCR⁺
αEGFR CAR⁺
F
Ultra + GFP AAV6
0.11
0.02
Q6
98.9
0.94
TCR⁺
αEGFR CAR⁺
G
E:T = 1
Normalized Spheroid Size (Fold-Change)
1.0
0.8
0.6
0.4
0.2
0.0
0
25
50
75
100
125
Time (h)
GFP
αEGFR CAR
****
H
E:T = 4
Normalized Spheroid Size (Fold-Change)
1.0
0.8
0.6
0.4
0.2
0.0
0
25
50
75
100
125
Time (h)
GFP
αEGFR CAR
****

Supplementary Fig. 13 Additional information to generate CAR-NK cells. a Phospho flow cytometry of *TGFBR2* knock-out NK cells as compared to control unedited NK cells. NK cells were cultured and edited as per SOP. 72 hours post editing, NK cells were stimulated with 10ng/mL TGF-β for indicated time, fixed and stained for pSMAD2/3 prior to interrogation by flow cytometry. Data are representative of 3 independent experiments with 4 unique NK cell donors. Error bars represent standard deviation of 3 technical replicates. Data are presented as mean values +/- SD. **b** Flow cytometry plot of GFP knock-in with AAV6 using 2.5×10^4 vg/cell MOI in NK cells. Flow cytometry plots of RNP only and AAV only controls are shown in **c** and **d**, respectively. Additional information of CAR-NK cells generated by Ultra. **e** Representative result of flow cytometry to evaluate the knock-in efficiency of *EGFR* CAR at *TRAC* locus in NK cells. **f** Same as in (a), but using an AAV6 carrying GFP transgene as negative control to evaluate the specificity of EGFR antibody used in flow analysis. **g,h** Tumor killing assay of CAR-NK at effector-to-target cells ratios (E:T) of 1 and 4, respectively. Two-way ANOVA was performed to evaluate statistical significance (****: P-value ≤ 0.0001). Raw source data are provided in an online supplementary data file.

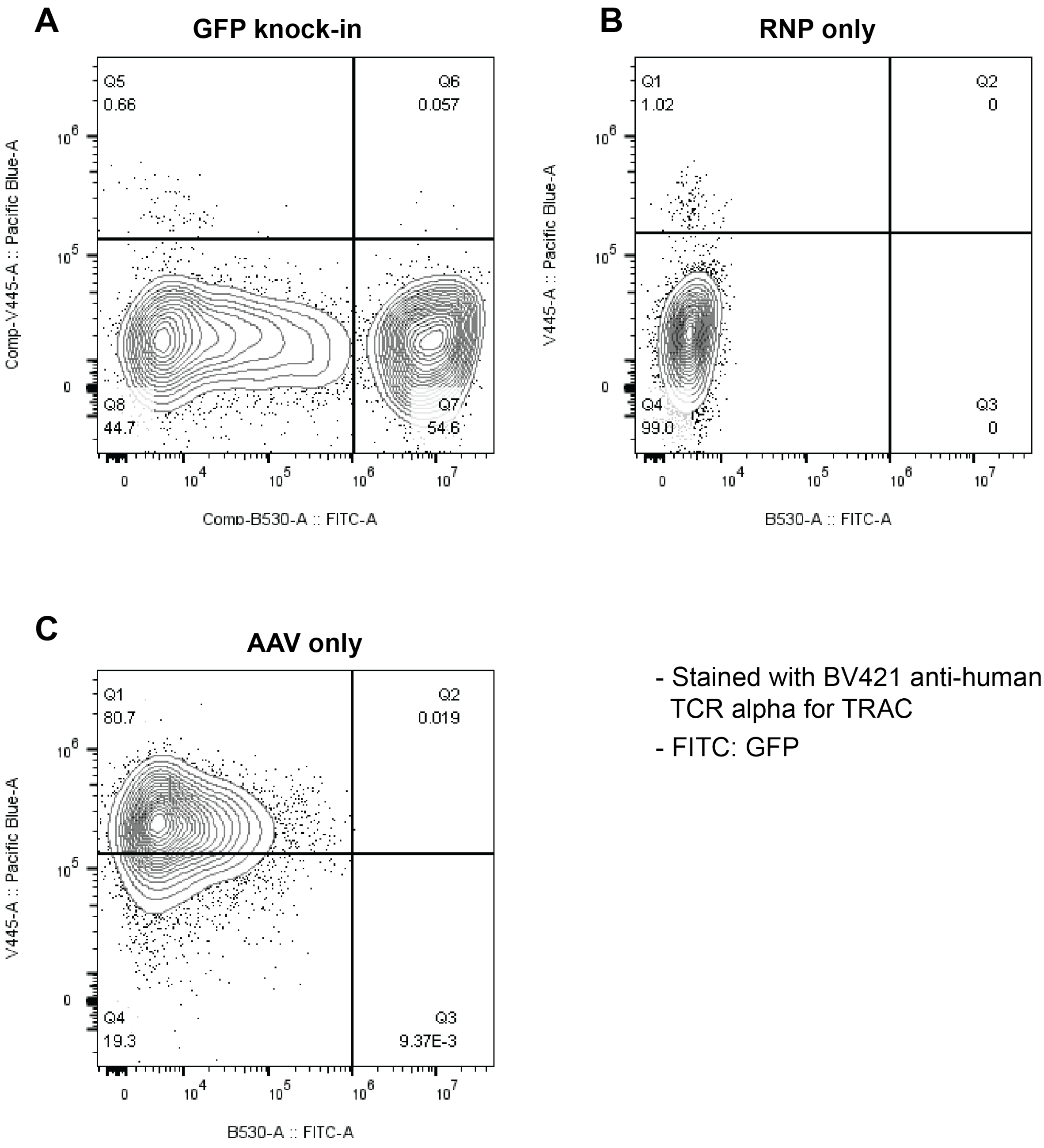


Supplementary Fig. 14 Flow cytometry gating strategy for mCherry and GFP knock-in at TRAC and B2M locus in primary T cells. **a** Gating strategy for live cells and **b** singlets. **c** Flow cytometry analysis of *TRAC*$^-$/*GFP*$^+$ or **d** *B2M*$^-$/*mCherry*$^+$ cells.



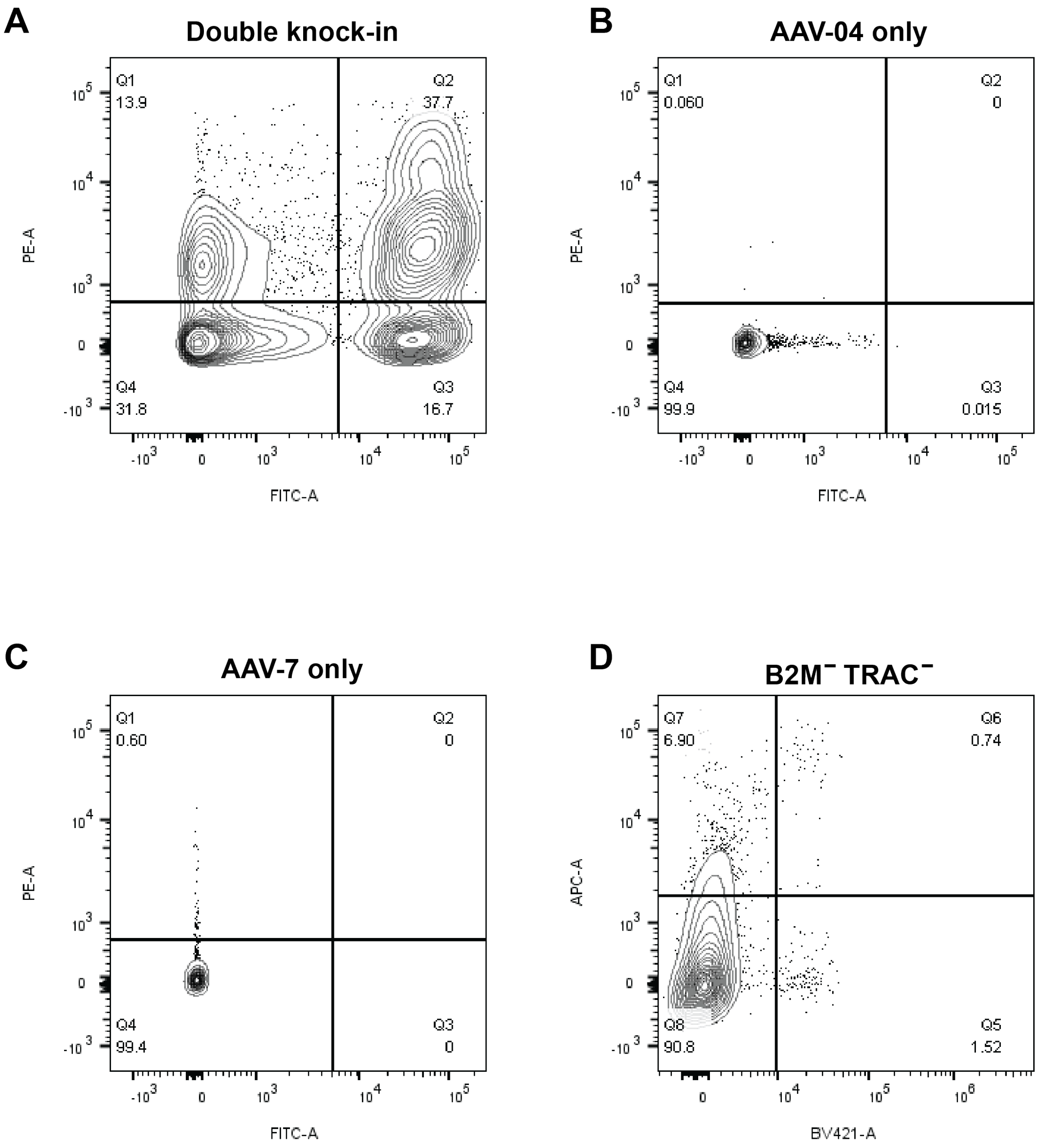
- Stained with APC anti-human HLA A/B/C for B2M
- mCherry: PE

Supplementary Fig. 15 Flow cytometry gating strategy for mCherry knock-in at B2M locus in primary T cells. a Flow cytometry results when both RNP and AAV donor are used or **b,c** when only RNP / AAV is used in the electroporation.



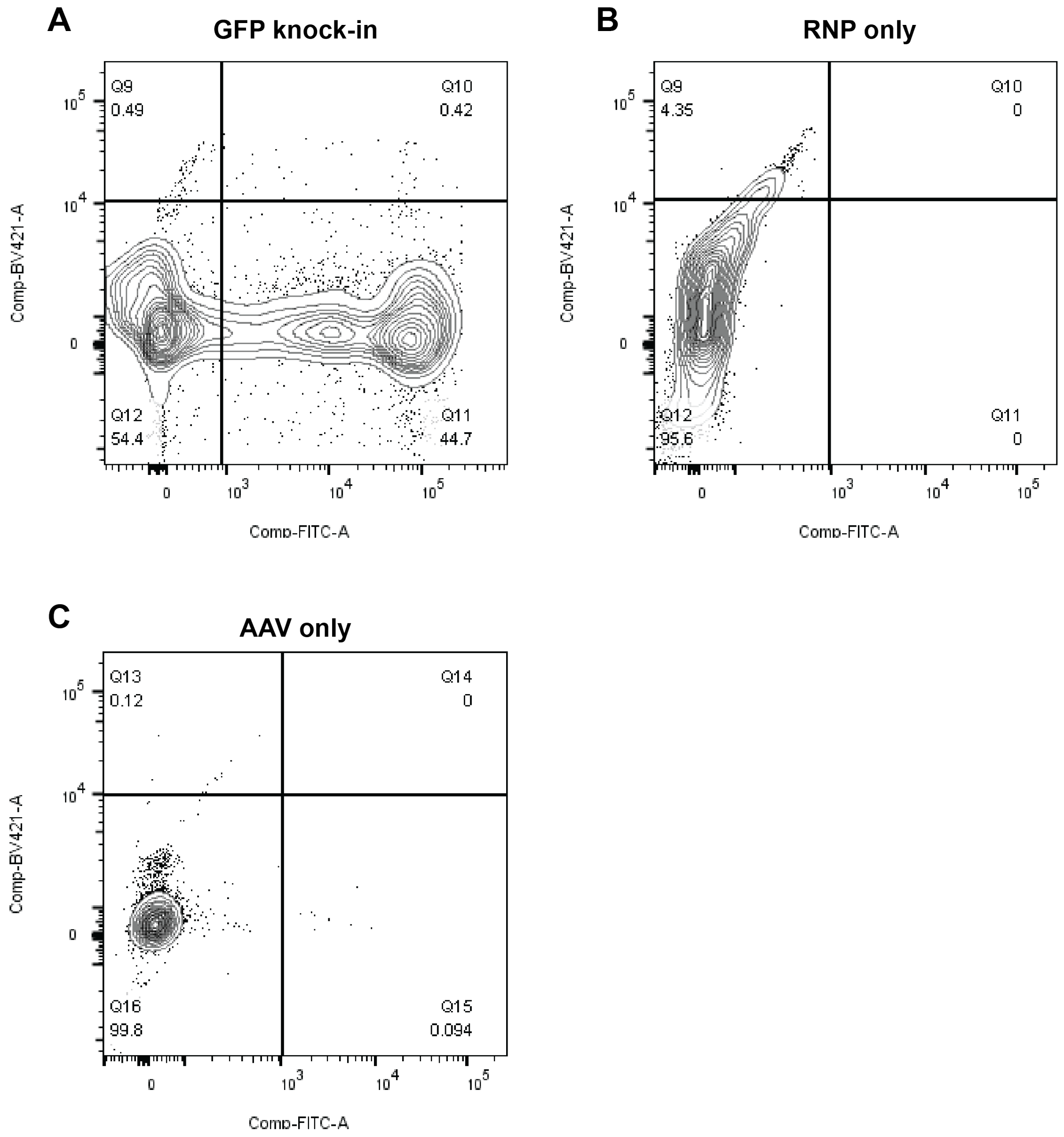
- Stained with BV421 anti-human TCR alpha for TRAC
- FITC: GFP

Supplementary Fig. 16 Flow cytometry gating strategy for GFP knock-in at B2M locus in primary T cells. a Flow cytometry results when both RNP and AAV donor are used, or **b,c** when only RNP / AAV is used in the electroporation.



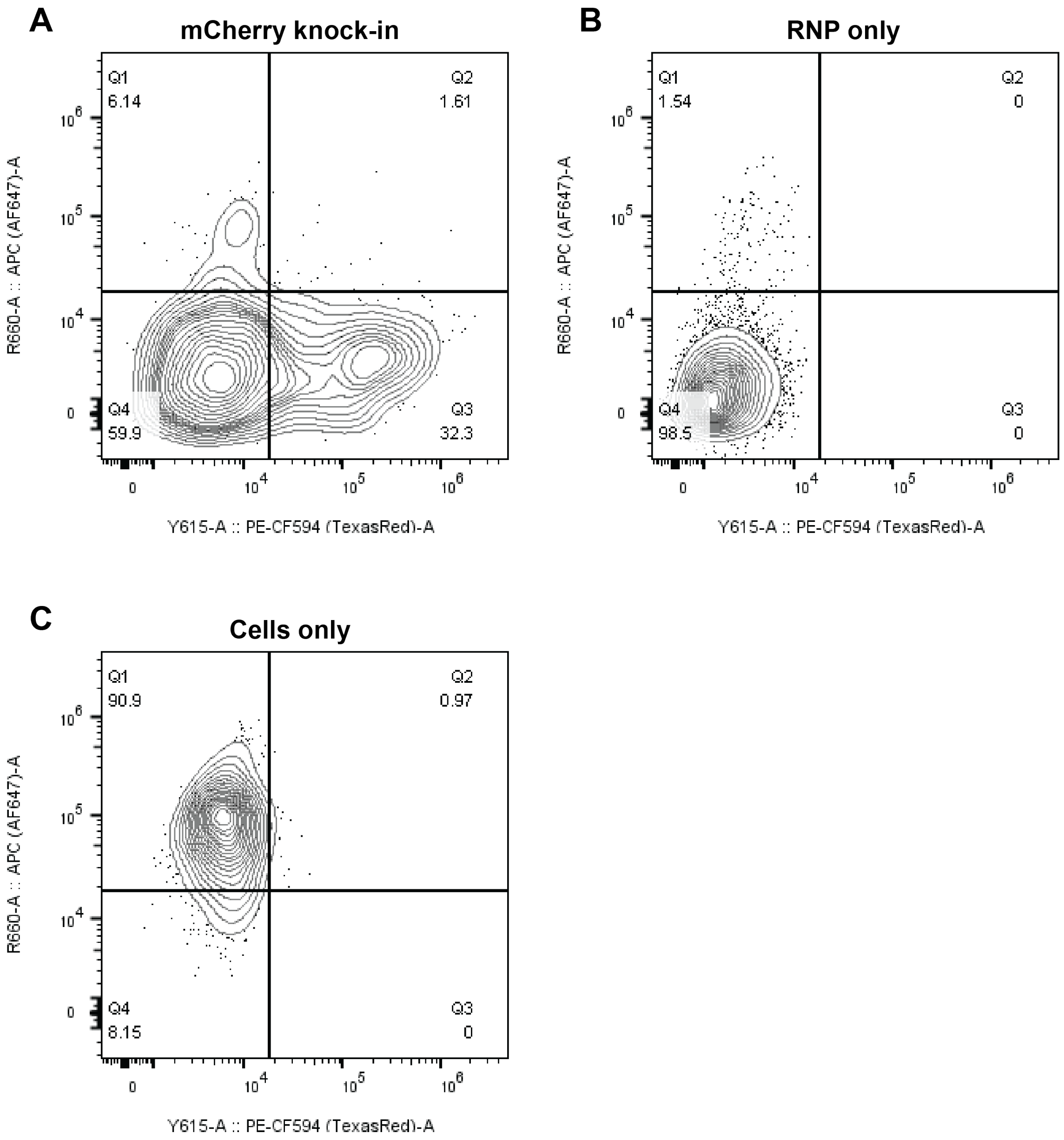
- Stained with APC anti-human HLA A/B/C for B2M
- Stained with BV421 anti-human TCR alpha for TRAC
- mCherry: PE; GFP: FITC

Supplementary Fig. 16 Flow cytometry gating strategy for GFP/mCherry double knock-in at B2M and TRAC loci in primary T cells. a Flow cytometry analysis of GFP / mCherry expression when both RNPs and AAV donors are used, or **b,c** when AAV template alone is used in the electroporation. **d** Flow cytometry analysis for B2M and TRAC expression when both RNPs and AAV donors are used.



- Stained with BV421 anti-human TCR alpha for TRAC
- GFP: FITC

Supplementary Fig. 17 Flow cytometry gating strategy for GFP knock-in at TRAC locus in primary NK cells. a Flow cytometry analysis of GFP / TRAC expression when both RNPs and AAV donors are used, or **b,c** when either AAV template alone is used in the electroporation.



- Stained with APC anti-human HLA A/B/C for B2M
- mCherry: PE

Supplementary Fig. 18 Flow cytometry gating strategy for mCherry knock-in at B2M locus in HSCs. a Flow cytometry analysis of mCherry / B2M expression when both RNPs and AAV donors, or **b** only RNP is used**. c** Expression measurement of HSC cells without RNP and AAV donor.

AsCas12a gene sequence for bacterial screen	
atgggcaagcccatccctaacccccctgttggggctggacagcaccgctcccaaaaagaaaaaggaaggtgggcattcacggcgtgcctgcggccACCCAGTTCGAGGGCTTCACCAACCTGTACCAGGTGTCCAAGACCCTGAGATTCGAGCTGATCCCCCAGGGCAAGACACTGAAGCACATCCAGGAACAGGGCTTCATCGAAGAGGACAAGGCCCGGAACGACCACTACAAAGAGCTGAAGCCCATCATCGACCGGATCTACAAGACCTACGCCGACCAGTGCCTGCAGCTGGTGCAGCTGGACTGGGAGAATCTGAGCGCCGCCATCGACAGCTACCGGAAAGAGAAAACCGAGGAAACCCGGAACGCCCTGATCGAGGAACAGGCCACCTACAGAAACGCCATCCACGACTACTTCATCGGCCGGACCGACAACCTGACCGACGCCATCAACAAGCGGCACGCCGAGATCTATAAGGGCCTGTTCAAGGCCGAGCTGTTCAACGGCAAGGTGCTGAAGCAGCTGGGCACCGTGACCACCACCGAGCACGAAAACGCCCTGCTGCGGAGCTTCGACAAGTTCACCACCTACTTCAGCGGCTTCTACGAGAACCGGAAGAACGTGTTCAGCGCCGAGGACATCAGCACCGCCATCCCCCACAGAATCGTGCAGGACAACTTCCCCAAGTTCAAAGAGAACTGCCACATCTTCACCCGGCTGATCACCGCCGTGCCCAGCCTGAGAGAACACTTCGAGAACGTGAAGAAGGCCATCGGCATCTTCGTGTCCACCAGCATCGAGGAAGTGTTCAGCTTCCCATTCTACAACCAGCTGCTGACCCAGACCCAGATCGACCTGTATAATCAGCTGCTGGGCGGCATCAGCAGAGAGGCCGGCACCGAGAAGATCAAGGGCCTGAACGAAGTGCTGAACCTGGCCATCCAGAAGAACGACGAGACAGCCCACATCATTGCCAGCCTGCCCCACCGGTTCATCCCTCTGTTCAAGCAGATCCTGAGCGACAGAAACACCCTGAGCTTCATCCTGGAAGAGTTCAAGTCCGATGAGGAAGTGATCCAGAGCTTCTGCAAGTATAAGACCCTGCTGAGGAACGAGAATGTGCTGGAAACCGCCGAGGCCCTGTTCAATGAGCTGAACAGCATCGACCTGACCCACATCTTTATCAGCCACAAGAAGCTGGAAACAATCAGCAGCGCCCTGTGCGACCACTGGGACACACTGCGGAATGCCCTGTACGAGCGGCGGATCTCTGAGCTGACCGGCAAGATCACCAAGAGCGCCAAAGAAAAGGTGCAGCGGAGCCTGAAGCACGAGGATATCAACCTGCAGGAAATCATCAGCGCCGCTGGCAAAGAACTGAGCGAGGCCTTTAAGCAGAAAACCAGCGAGATCCTGTCCCACGCCCACGCCGCACTGGATCAGCCTCTGCCTACCACCCTGAAGAAGCAGGAAGAGAAAGAGATCCTGAAGTCCCAGCTGGACAGCCTGCTGGGCCTGTACCATCTGCTGGATTGGTTCGCCGTGGACGAGAGCAACGAGGTGGACCCCGAGTTCTCCGCCAGACTGACAGGCATCAAACTGGAAATGGAACCCAGCCTGTCCTTCTACAACAAGGCCAGAAACTACGCCACCAAGAAACCCTACAGCGTGGAAAAGTTTAAGCTGAACTTCCAGATGCCCACCCTGGCCAGCGGCTGGGACGTGAACAAAGAGAAGAACAACGGCGCCATCCTGTTCGTGAAGAACGGACTGTACTACCTGGGCATCATGCCTAAGCAGAAGGGCAGATACAAGGCCCTGTCCTTTGAGCCCACCGAAAAGACCAGCGAGGGCTTTGACAAGATGTACTACGATTACTTCCCCGACGCCGCCAAGATGATCCCCAAGTGCAGCACCCAGCTGAAGGCCGTGACCGCCCACTTTCAGACCCACACCACCCCCATCCTGCTGAGCAACAACTTCATCGAGCCCCTGGAAATCACCAAAGAGATCTACGACCTGAACAACCCCGAGAAAGAGCCCAAGAAGTTCCAGACCGCCTACGCCAAGAAAACCGGCGACCAGAAGGGCTACCGCGAGGCTCTGTGCAAGTGGATCGACTTTACCCGGGACTTCCTGAGCAAGTACACCAAGACCACCTCCATCGATCTGAGCAGCCTGCGGCCCAGCTCCCAGTACAAGGATCTGGGCGAGTACTACGCCGAGCTGAACCCTCTGCTGTACCACATCAGCTTCCAGCGGATCGCCGAAAAAGAAATCATGGACGCCGTGGAAACCGGCAAGCTGTACCTGTTCCAGATCTATAACAAGGACTTCGCCAAGGGCCACCACGGCAAGCCCAATCTGCACACCCTGTACTGGACCGGCCTGTTTAGCCCCGAGAATCTGGCCAAGACCAGCATCAAGCTGAACGGCCAGGCCGAACTGTTTTACCGGCCCAAGAGCCGGATGAAGCGGATGGCCCATAGACTGGGCGAGAAGATGCTGAACAAGAAACTGAAGGACCAGAAAACCCCTATCCCCGACACACTGTATCAGGAACTGTACGACTACGTGAACCACCGGCTGAGCCACGACCTGTCCGACGAAGCTAGAGCACTGCTGCCCAACGTGATCACAAAAGAGGTGTCCCACGAGATCATCAAGGACCGGCGGTTTACCTCCGATAAGTTCTTCTTCCACGTGCCCATCACCCTGAACTACCAGGCCGCCAACAGCCCCAGCAAGTTCAACCAGAGAGTGAACGCCTACCTGAAAGAGCACCCCGAGACACCCATCATTGGCATCGACAGAGGCGAGCGGAACCTGATCTACATCACCGTGATCGACAGCACAGGCAAAATCCTGGAACAGAGAAGCCTGAACACCATCCAGCAGTTCGACTACCAGAAGAAACTGGACAACCGGGAAAAAGAACGGGTGGCCGCCAGACAGGCTTGGAGCGTCGTGGGCACCATTAAGGACCTGAAGCAGGGCTACCTGAGCCAAGTGATTCACGAGATCGTGGACCTGATGATCCACTATCAGGCTGTGGTGGTGCTGGAAAACCTGAACTTCGGCTTCAAGAGCAAGCGGACCGGAATCGCCGAGAAAGCCGTGTACCAGCAGTTTGAGAAAATGCTGATCGACAAGCTGAATTGCCTGGTGCTGAAAGACTACCCCGCTGAGAAAGTGGGAGGCGTGCTGAATCCCTACCAGCTGACCGACCAGTTCACCTCCTTTGCCAAGATGGGAACCCAGAGCGGCTTCCTGTTCTACGTGCCAGCCCCCTACACCAGCAAGATCGACCCTCTGACCGGCTTCGTGGACCCCTTCGTGTGGAAAACCATCAAGAACCACGAGTCCCGGAAGCACTTCCTGGAAGGCTTTGACTTCCTGCACTACGACGTGAAAACAGGCGATTTCATCCTGCACTTCAAGATGAATCGGAATCTGTCCTTCCAGAGGGGCCTGCCCGGCTTCATGCCTGCCTGGGATATCGTGTTCGAGAAGAATGAGACACAGTTCGACGCCAAGGGAACCCCCTTTATCGCCGGCAAGAGGATCGTGCCTGTGATCGAGAACCACAGATTCACCGGCAGATACCGGGACCTGTACCCCGCCAACGAGCTGATTGCCCTGCTGGAAGAGAAGGGCATCGTGTTCCGGGACGGCAGCAACATCCTGCCCAAGCTGCTGGAAAATGACGACAGCCACGCCATCGATACCATGGTGGCACTGATCCGCAGCGTGCTGCAGATGCGGAACAGCAATGCCGCCACCGGCGAGGACTACATCAATAGCCCAGTGCGGGACCTGAACGGCGTGTGCTTCGACAGCAGATTCCAGAACCCCGAGTGGCCCATGGATGCCGACGCCAATGGCGCCTACCACATTGCCCTGAAGGGACAGCTGCTGCTGAACCATCTGAAAGAGAGCAAAGACCTGAAACTGCAGAACGGCATCTCCAACCAGGACTGGCTGGCCTATATCCAGGAACTGCGGAACcccaaaaagaaaaaggaaggtg	
Primers for Random mutagenesis	
Cpf1_PID_mut_fwd	ATCAAACTGGAAATGGAACCCAGCCTGTCCTTCTACAACAAGGCCAGAAACTACGCCACC
Cpf1_PID_mut_REV	AATGATGGGTGTCTCGGGGTGCTCTTTCAGGTAGGCGTTCACTCTCTGGTTGAACTTGC
Primers for vector linearization	
Cpf1_VecBB_PID_rev	GGTGGCGTAGTTTCTGGCCTTGTTGTAGAAGGACAGGCTGGGTTCCATTTCCAGTTTGAT
Cpf1_VecBB_PID_fwd	AGCAAGTTCAACCAGAGAGTGAACGCCTACCTGAAAGAGCACCCCGAGACACCCATCATT
PCR primers to amplify mutagenized region for Nextera sequencing	
Cpf1_PID_nextera_F	CCTCTGCCTACCACCCTGAAG
Cpf1_PID_nextera_R	AGGTAGCCCTGCTTCAGGTC
crRNA for bacterial selection	
ACATAAAACTCTTTTAGGTTA	

Supplementary Table 1 DNA sequences used in the bacterial selection of AsCas12a Ultra.

gBlock sequence for indivudal in-vitro clevage assay	
HPRT-38346-TTTT	CTCGGCCCAGGCTGGCCATTCCGGATGATTCTCCTACTTCCTCCTCGGAGTCCTCAAGTATCACTGTCCCCTTGCACTCCCTGTCTTTGAATCTTACTTTCCATCCTTTTTACATAAAACTCTTTTAGGTTACCAAGCTGGACCCCACGAAGTGTTGGATATAAGAAAAACTCTGGAGAGCAGTGAGCCCAGCAGAGCCCCCCTAGGGATCTTCAGCCCCAGCCTGAAGTATTCATTATAGTCAAAAAAGGCTGTGGTTTCACAGGTGCAGGTCCAACCCTCCTTTCTCTGCCTTGTGGCTGCCTGTCCTGGGGCAGGTTACGTTGCCCCTGGGAGCCTCTGTTTTCTGTAA
HPRT-38346-TTTC	CTCGGCCCAGGCTGGCCATTCCGGATGATTCTCCTACTTCCTCCTCGGAGTCCTCAAGTATCACTGTCCCCTTGCACTCCCTGTCTTTGAATCTTACTTTCCATCCTTTTCACATAAAACTCTTTTAGGTTACCAAGCTGGACCCCACGAAGTGTTGGATATAAGGAAAACTCTGGAGAGCAGTGAGCCCAGCAGAGCCCCCCTAGGGATCTTCAGCCCCAGCCTGAAGTATTCATTATAGTCAAGAAAGGCTGTGGTTTCACAGGTGCAGGTCCAACCCTCCTTTCTCTGCCTTGTGGCTGCCTGTCCTGGGGCAGGTTACGTTGCCCCTGGGAGCCTCTGTTTTCTGTAA
Primers for gBlock amplification	
HPRTCF_fwd	TTACAGAAAACAGAGGCTCCCAGGG
HRPTCF_rev	CTCGGCCCAGGCTGGCCATTCCGGA
Spec-seq/SEAM-seq	
Protospacer	AGGAAACTTCTGGCAGGACC
On-target site	ACTGTTTAAGGAAACTTCTGGCAGGACCAGGTCATG
Spec-seq Library	
Lib_1	GCTATCTAGTGCTCAGTACGC(N:25252525)(N)(N)(N)TTTAAGGAAACTTCTGGCAGGACCAGGTCATGagatcggaagagcacacgtctgaactccagtca
Lib_2	GCTATCTAGTGCTCAGTACGCACTG(N:25252525)(N)(N)(N)AGGAAACTTCTGGCAGGACCAGGTCATGagatcggaagagcacacgtctgaactccagtca
Lib_3	GCTATCTAGTGCTCAGTACGCACTGTTTA(N:25252525)(N)(N)(N)AACTTCTGGCAGGACCAGGTCATGagatcggaagagcacacgtctgaactccagtca
Lib_4	GCTATCTAGTGCTCAGTACGCACTGTTTAAGGA(N:25252525)(N)(N)(N)TCTGGCAGGACCAGGTCATGagatcggaagagcacacgtctgaactccagtca
Lib_5	GCTATCTAGTGCTCAGTACGCACTGTTTAAGGAAACT(N:25252525)(N)(N)(N)GCAGGACCAGGTCATGagatcggaagagcacacgtctgaactccagtca
Lib_6	GCTATCTAGTGCTCAGTACGCACTGTTTAAGGAAACTTCTG(N:25252525)(N)(N)(N)GACCAGGTCATGagatcggaagagcacacgtctgaactccagtca
Lib_7	GCTATCTAGTGCTCAGTACGCACTGTTTAAGGAAACTTCTGGCAG(N:25252525)(N)(N)(N)AGGTCATGagatcggaagagcacacgtctgaactccagtca
Lib_8	GCTATCTAGTGCTCAGTACGCACTGTTTAAGGAAACTTCTGGCAGGACC(N:25252525)(N)(N)(N)CATGagatcggaagagcacacgtctgaactccagtca
Lib_9	GCTATCTAGTGCTCAGTACGCACTGTTTAAGGAAACTTCTGGCAGGACCAGGT(N:25252525)(N)(N)(N)agatcggaagagcacacgtctgaactccagtca
Reverse primer for Klenow extension of Spec-seq library	
Spec-Rev	TGACTGGAGTTCAGACGTGT
Primers for NGS Library amplification (P5)	
Spec-i5-1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAACTAGAGGCTATCTAGTGCTCAGTACGC
Spec-i5-2	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCATGCTGAGCTATCTAGTGCTCAGTACGC
Spec-i5-3	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGCACATCTGCTATCTAGTGCTCAGTACGC
Spec-i5-4	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATGGTTCGACGCTATCTAGTGCTCAGTACGC
Spec-i5-5	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGATCGCACTTCGCTATCTAGTGCTCAGTACGC
Spec-i5-6	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCGATAGTTGCTTGCTATCTAGTGCTCAGTACGC
Spec-i5-7	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCGATCCAGTTAGGCTATCTAGTGCTCAGTACGC
Spec-i5-8	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATCGATTTGAGCCTGCTATCTAGTGCTCAGTACGC
Spec-i5-9	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACGATCGATACACGATCGCTATCTAGTGCTCAGTACGC
Spec-i5-10	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTACGATCGATAGTCCAGAGCTATCTAGTGCTCAGTACGC
Primers for NGS Library amplification (P7)	
i7_IDT-1	CAAGCAGAAGACGGCATACGAGATACGATCAGGTGACTGGAGTTCAGACGTGT
i7_IDT-2	CAAGCAGAAGACGGCATACGAGATTCGAGAGTGTGACTGGAGTTCAGACGTGT
i7_IDT-3	CAAGCAGAAGACGGCATACGAGATCTAGCTCAGTGACTGGAGTTCAGACGTGT
i7_IDT-4	CAAGCAGAAGACGGCATACGAGATATCGTCTCGTGACTGGAGTTCAGACGTGT
i7_IDT-5	CAAGCAGAAGACGGCATACGAGATTCGACAAGGTGACTGGAGTTCAGACGTGT
i7_IDT-6	CAAGCAGAAGACGGCATACGAGATCCTTGGAAGTGACTGGAGTTCAGACGTGT
i7_IDT-7	CAAGCAGAAGACGGCATACGAGATATCATGCGGTGACTGGAGTTCAGACGTGT
i7_IDT-8	CAAGCAGAAGACGGCATACGAGATTGTTCCGTGTGACTGGAGTTCAGACGTGT
i7_IDT-9	CAAGCAGAAGACGGCATACGAGATATTAGCCGGTGACTGGAGTTCAGACGTGT
i7_IDT-10	CAAGCAGAAGACGGCATACGAGATCGATCGATGTGACTGGAGTTCAGACGTGT
i7_IDT-11	CAAGCAGAAGACGGCATACGAGATGATCTTGCGTGACTGGAGTTCAGACGTGT

i7_IDT-12	CAAGCAGAAGACGGCATACGAGATAGGATAGCGTGACTGGAGTTCAGACGTGT

Supplementary Table 2 DNA sequences used in the *in vitro* characterization of sequence specificity / activity of AsCas12a mutants

Gene	Assay	Cas12a crRNA	Cas12a PAM	Cas9 sgRNA	Forward primer	Reverse primer
A1CF	A1CF-5-S	UGGGAUGCCCCCAACAAAUAA	TTTT	UAAUCGGCAGUUGUCCACAC	acactctttccctacacgacgctcttccgatctCACCTTCAGTAACCTTTTTCATCT	gtgactggagttcagacgtgtgctcttccgatctTCAACACTATGAACCCAAACATC
	A1CF-7-S	GGAUGCCCCCAACAAAUAAUC	TTTG			
	A1CF-48-AS	UGCCAGUGUGGACAACUGCCG	TTTG			
	A1CF-125-AS	AACCUGAAUCCUACAAGGAUU	TTTC			
AADACL3	AADACL3-44-S	CGCUUUGGGACAAUCCCUGUG	TTTC	GGCAUCGUGUACUACCACGG	acactctttccctacacgacgctcttccgatctGCTCAGAATCTGTTCTATGCC	gtgactggagttcagacgtgtgctcttccgatctGGTCTGAGAAAATGGTTCTTACT
ABCG2	ABCG2-50-S	AUGAUCCCACUGUAAACACAA	TTTC	ACCUGGUCUCAACGCCAUCC	acactctttccctacacgacgctcttccgatctCACATTAAAAGTGCACAGAAAACG	gtgactggagttcagacgtgtgctcttccgatctCAGTGCCTACCCTAAATTAATAGAA
	ABCG2-69-AS	GUGUUUACAGUGGGAUCAUGA	TTTT			
ABCG5	ABCG5-127-AS	UGUCUCCUGCAGGUGGAGGCC	TTTG	CAUGUGGCAGACCGACUGAU	acactctttccctacacgacgctcttccgatctGCTAGTAACAGTTCTGGGTG	gtgactggagttcagacgtgtgctcttccgatctCCCATGAATTGAAATAGCAGC
ABHD6	ABHD6-34-S	GGUUUGAAUCCCACAGGCCUG	TTTG	GAUUGUCAGUUGAGUACUGC	acactctttccctacacgacgctcttccgatctTATTAACTCTGGGCTGCTGT	gtgactggagttcagacgtgtgctcttccgatctGACATAGGAGCAGAGCTGAAG
	ABHD6-80-S	UACAACGGCUCAAAGAACUGC	TTTG			
ABRAXAS1	ABRAXAS1-55-AS	CUGCUAUUAACACCAAGUAUA	TTTT	AAGCUGCUCUACUCAUCGAC	acactctttccctacacgacgctcttccgatctCAGTGACTGGCCAACATTTA	gtgactggagttcagacgtgtgctcttccgatctCTGCTTCACAAAAACTTGCAG
ACAT1	ACAT1-102-AS	CAAUCAAAUCUUCAAGCUUUA	TTTA	UCAAGCUUUACCCCACCAUA	acactctttccctacacgacgctcttccgatctGGGTTTGCAAAATATTTGTATTAACATT	gtgactggagttcagacgtgtgctcttccgatctCAGTATTGGACATTAGATAGCATTTAT
ACVR2A	ACVR2A-24-AS	AUUGGUUCUGUCUUUUUCCCA	TTTG	ACUGGUGUUGAACCGUGUUA	acactctttccctacacgacgctcttccgatctTAGGTGCTATACTTGGTAGATCAGAAA	gtgactggagttcagacgtgtgctcttccgatctCAGAAATATTCTTCCAGGTAGCAAAA
ADAM30	ADAM30-45-AS	ACUCGUAUGAAGUCACCAUUC	TTTG	UACGAGUCAAACUCCCCUUC	acactctttccctacacgacgctcttccgatctCTTTTAACTGCAGTAGGTAGGA	gtgactggagttcagacgtgtgctcttccgatctGACAATGCTCCTTAAGTCTCTT
ADAM7	ADAM7-44-S	UUUUUCCUUUAUGAGUUUUCC	TTTC	GCCAGUAUCAGUACGUGUAA	acactctttccctacacgacgctcttccgatctTCATTGTTTTTACCAAGGATCCAT	gtgactggagttcagacgtgtgctcttccgatctTAAATCATAGGCTACAGCTGAAA
	ADAM7-63-S	CCUAUGGAUUUUCAAAGAAGU	TTTT			
	ADAM7-105-S	AUCACUCCUGGUAUAUAGAAA	TTTT			
ADGRL3	ADGRL3-44-AS	CACCACGCCCCAGAUUGGUGG	TTTG	UUUGAGUCCGACCACCAAUC	acactctttccctacacgacgctcttccgatctCCTTCCAACAGTTTTTCTTTGTC	gtgactggagttcagacgtgtgctcttccgatctGATGAATACTCAGTCAGGGTATCA
AGAP9	AGAP9-13-S	CUUCUUUAGGUGUUUCUUUUU	TTTT	UGGUGGUGCUGGAGAUACCG	acactctttccctacacgacgctcttccgatctTTCAAAGTGCCACGTTTG	gtgactggagttcagacgtgtgctcttccgatctCTAGCCACATCAGCCT
	AGAP9-21-S	GGUGUUUCUUUUUAUUGGCAU	TTTA			
AHCTF1	AHCTF1-51-AS	UCGUAUCUGUUAAUAAAAUGG	TTTA	AAGGAUAACAUCGUUACCAC	acactctttccctacacgacgctcttccgatctGCAGAACCCACTAATACAAAGGA	gtgactggagttcagacgtgtgctcttccgatctCACAGACAAGCCCTCAGATATATT
	AHCTF1-38-S	UUAACAGAUACGAUAAAGACU	TTTA			
	AHCTF1-89-AS	AAUAGGUAAGUACAUCUUUUG	TTTT			
	AHCTF1-90-AS	UAAUAGGUAAGUACAUCUUUU	TTTT			

AHCYL1	AHCYL1-9-S	UAAGAAGUAUCCAAACGUGUU	TTTA	CGGAUCUUCUUAAACACGUU	acactctttccctacacgacgctcttccgatctGTCTTCTGGCTGTTCTATAGATC	gtgactggagttcagacgtgtgctcttccgatctTCCAGAATCTGTTACCTGTGAA
AIDA	AIDA-28-AS	UUACCAAGGUUGCCAUCGGAA	TTTA	CCAAGGUUGCCAUCGGAACC	acactctttccctacacgacgctcttccgatctCCAGCATCTTTCAAACCAATTTT	gtgactggagttcagacgtgtgctcttccgatctACTGTGAAATGATATGGAGCTTTT
AKAP10	AKAP10-70-S	CAUGGAAACUUGGUGAGUUCC	TTTC	AGAAGCGGGACUAUUUCUAC	acactctttccctacacgacgctcttccgatctCACATATGTACACACAAGAAAATCACATA	gtgactggagttcagacgtgtgctcttccgatctAATAATAGAACTAACAGCACTCAGAATCA
AKIRIN1	AKIRIN1-40-AS	CUCUAAUAAAAAAGAAACAAA	TTTG	AAGAAUAUAGUCGUUAUCAG	acactctttccctacacgacgctcttccgatctAAGATCTTTGTCTCTTCCCACTT	gtgactggagttcagacgtgtgctcttccgatctCACAAGCTTCACTCTGATTAAGAA
	AKIRIN1-31-S	UUAUUAGAGCAAAUUUUUCAG	TTTT			
	AKIRIN1-56-AS	UGUUCUGAAAAAUUUGCUCUA	TTTA			
	AKIRIN1-50-S	AGAACAUAAAACAAGAAUAUA	TTTC			
AKR1B10	AKR1B10-70-AS	GGGAAAAGGUCAUCCCCAGAC	TTTG	GAACGUUGCUUUUCCACCGA	acactctttccctacacgacgctcttccgatctAGATGTGGTATTTAGCAAGAGTCA	gtgactggagttcagacgtgtgctcttccgatctCATGGATAACTGAAGATTTCTCTCC
	AKR1B10-68-S	CCCAAAGAUGAUAAAGGUAAU	TTTC		acactctttccctacacgacgctcttccgatctAGATGTGGTATTTAGCAAGAGTCA	gtgactggagttcagacgtgtgctcttccgatctCATGGATAACTGAAGATTTCTCTCC
	AKR1B10-99-AS	CACCGAUGGCAUUACCUUUAU	TTTC		acactctttccctacacgacgctcttccgatctAGATGTGGTATTTAGCAAGAGTCA	gtgactggagttcagacgtgtgctcttccgatctCATGGATAACTGAAGATTTCTCTCC
ALS2	ALS2-22-S	GUUCCGGCACAUACUGCUGGU	TTTG	ACAAUGGUGUCGCGUACAUG	acactctttccctacacgacgctcttccgatctCGCCAACTGTAAAATCCTGA	gtgactggagttcagacgtgtgctcttccgatctTAGCCCCATTCTAGAAAATGC
AMZ2	AMZ2-36-AS	AAAAGAAAUACAUAAAUGUAU	TTTA	AUCUUUACCCAAGAGACUCG	acactctttccctacacgacgctcttccgatctCATGCAGGTGAATTACACGA	gtgactggagttcagacgtgtgctcttccgatctAATACCATCTGTCAAAGAGGC
	AMZ2-24-S	UGUAUUUCUUUUUAAAUAGGG	TTTA			
ANKRD13D	ANKRD13D-57-AS	CCAGAGACACGGCCAGCUCCA	TTTC	CUUCGACACAAUGCCAACGU	acactctttccctacacgacgctcttccgatctCTCTTCTCTCCTGCCCTTT	gtgactggagttcagacgtgtgctcttccgatctTTGTCCTCTGCAGTACCTG
ANKRD27	ANKRD27-56-AS	CAGGUCUUAGUACCCUGCAAA	TTTC	UAGACUGGAUGCUGCUCGAC	acactctttccctacacgacgctcttccgatctGGTCTGAAAATGCTCTTCCA	gtgactggagttcagacgtgtgctcttccgatctTTCTGCTGAGGTGGTAAATGG
	ANKRD27-58-AS	UCCAGGUCUUAGUACCCUGCA	TTTT			gtgactggagttcagacgtgtgctcttccgatctTTCTGCTGAGGTGGTAAATGG
ANKRD45	ANKRD45-39-S	AAAGUUUCCAAACGACCCCAG	TTTC	CAAAGUUUCCAAACGACCCC	acactctttccctacacgacgctcttccgatctCTGAGAATATCTAGCAGCAACAT	gtgactggagttcagacgtgtgctcttccgatctGTCTGTTTGATCTCACCATCTT
ANKRD50	ANKRD50-43-S	GAAAACCUAAAGAAGAGAUAA	TTTC	UCGGAAGGCAUAUAUCGUCA	acactctttccctacacgacgctcttccgatctCGCAAAGCTTCTTCTTGATCTAAAC	gtgactggagttcagacgtgtgctcttccgatctGTTGACATTCTCTTTGAAGATATGGT
	ANKRD50-61-AS	UCUCUUCUUUAGGUUUUCGAA	TTTA			
	ANKRD50-62-AS	AUCUCUUCUUUAGGUUUUCGA	TTTT			
ANTXRL	ANTXRL-63-AS	AGCCUAAGGGAAAGAAGGCAU	TTTG	GGGGGCCAACGUUUACACCC	acactctttccctacacgacgctcttccgatctCACATGCATTTCAGGACACT	gtgactggagttcagacgtgtgctcttccgatctGGTTGGCTCGAAAGTGAC
AP1S3	AP1S3-9-S	UCCAGCAGCUCCACGUAACGA	TTTG	AGAGAUUGUGCAUCGUUACG	acactctttccctacacgacgctcttccgatctGGGACTGTAGCTAATCCTAAC	gtgactggagttcagacgtgtgctcttccgatctTTCGTGGGAAAAACTGTCTC
	AP1S3-70-S	CUAUUGCACAGCAAAAAUAUA	TTTT			
	AP1S3-89-AS	UAUUUUUGCUGUGCAAUAGAA	TTTA			
	AP1S3-105-AS	AAGGUAUGCUAGUUUAUAUUU	TTTC			

	AP1S3-110-AS	AUUUCAAGGUAUGCUAGUUUA	TTTC			
AP3B1	AP3B1-57-AS	GGUGAAUAUUUUUGCACUAUU	TTTT	AAGCGCUUGCACGAAUUAGU	acactctttccctacacgacgctcttccgatctAGCATCATGATAGGTACAATAATTGG	gtgactggagttcagacgtgtgctcttccgatctTGCTATTGTCATATTACACCCTTTAAG
	AP3B1-80-AS	AAAUAUUGUAAAGUUGAAAUU	TTTA			
APAF1	APAF1-46-AS	AUCAGAAGCCCAGAUUUGUCU	TTTC	UCUUUGCACACGGUUGGAUC	acactctttccctacacgacgctcttccgatctGGCCTTAAGTTCATTATTCTTTCC	gtgactggagttcagacgtgtgctcttccgatctTAGCCTCTTCAATATTAAGTGGAA
APOPT1	APOPT1-54-AS	AGGUAUGUAAAAGUGAACAGG	TTTC	UGUAAAAGUGAACAGGUCGA	acactctttccctacacgacgctcttccgatctAAAGGTCTCAAGATTCTGCC	gtgactggagttcagacgtgtgctcttccgatctGTTTGCCCAGAACTGTTGATT
ARHGAP36	ARHGAP36-17-S	UUUUUUCUCUUUCUGUAGCUC	TTTC	AAGGUGUUCGGACGGUCAAU	acactctttccctacacgacgctcttccgatctCAGATCAGGGCATTGGGAT	gtgactggagttcagacgtgtgctcttccgatctCCTCTGCTGTCCTCTCAA
ARL1	ARL1-22-S	GAAAUGCCAAUUCGGUCUCGG	TTTG	CAUGGCAACUAACUCUGAUU	acactctttccctacacgacgctcttccgatctAATGACTGCCCCACATTTTA	gtgactggagttcagacgtgtgctcttccgatctCTCTAGGCCATACTGGAGAT
ARL16	ARL16-98-AS	UCCUAUAGGUGGGCACCAAUC	TTTT	UGCCACGAUGUCAGUAAGAU	acactctttccctacacgacgctcttccgatctGCCCATATAGGATTACAACCC	gtgactggagttcagacgtgtgctcttccgatctCTCTTCAGGAGTTCACAACG
ARL6	ARL6-26-S	AAUCACAUUAUGGGAUUGCUA	TTTG	UGCCACUAUUAUCUAGCCCA	acactctttccctacacgacgctcttccgatctCCAATATTTTCCATAACTTAAGGTGC	gtgactggagttcagacgtgtgctcttccgatctGCATCTAACAAAGATACTTACATTTGAA
ARMCX1	ARMCX1-47-S	CUUAUAAAAUUGAUGAUAUUC	TTTC	UUGAACUUGCCUCUCCGGAC	acactctttccctacacgacgctcttccgatctGGGCAAGTGGAAAATCCAAG	gtgactggagttcagacgtgtgctcttccgatctTTGTTACCCAGAGTGACCAA
	ARMCX1-86-AS	GGAGGUCGGGAGCACUCAGAA	TTTT			gtgactggagttcagacgtgtgctcttccgatctTTGTTACCCAGAGTGACCAA
ARR3	ARR3-18-S	CACCACCGGAGGCAGGCCCUG	TTTG	AGUACAAUUUGCACCACCGG	acactctttccctacacgacgctcttccgatctCTGGCTAATCTCTTGGTCTCT	gtgactggagttcagacgtgtgctcttccgatctATCAGTGAAGAAAGGGCATCA
ATP1B4	ATP1B4-79-AS	UCCUCCUCUUCUUCCUCCUCC	TTTC	GACGGUGGUGCCCAAAUCGG	acactctttccctacacgacgctcttccgatctCACTGTTCCAGGATGATCC	gtgactggagttcagacgtgtgctcttccgatctCTCTGGATCCCACAGGTATT
	ATP1B4-102-AS	CCUCCUCUUCCUCCUCUUCUU	TTTT			
AUTS2	AUTS2-53-AS	CGGAGUCCAUGGCCCCGCGUC	TTTG	AUGGACUCCGCAAAAAGCGG	acactctttccctacacgacgctcttccgatctTTTTGTGTGGCTGCGG	gtgactggagttcagacgtgtgctcttccgatctCTTGTCGGAGCCCGAC
BANF1	BANF1-16-S	UUUGGGAUUCCUAGAUUAAGC	TTTT	CCAAAAGCACCGAGACUUCG	acactctttccctacacgacgctcttccgatctTCTTACTATGAGATTGCTCGTGG	gtgactggagttcagacgtgtgctcttccgatctACACCTTGTCAAAACCCCTTTC
	BANF1-58-AS	GGAGGUUGUCAUCUUGAUCAG	TTTG			gtgactggagttcagacgtgtgctcttccgatctACACCTTGTCAAAACCCCTTTC
BBOX1	BBOX1-27-AS	UUGAGGGUGGAGAGCCACUUG	TTTC	ACCGGAGCAUCUGACAAACC	acactctttccctacacgacgctcttccgatctCAGCTACCCACTTTGGATTTT	gtgactggagttcagacgtgtgctcttccgatctGTCCTCAGTTGGGAACTATTT
	BBOX1-80-AS	AAACUUCUCCUGGUUUGUCAG	TTTG			
	BBOX1-90-AS	CCAAGUUUUGAAACUUCUCCU	TTTC			
	BBOX1-91-AS	CCCAAGUUUUGAAACUUCUCC	TTTT			
	BBOX1-122-S	AUGGGUGAGUCACCAAAUGGU	TTTT			
BLOC1S1	BLOC1S1-44-AS	UGGGGAGAGGGGGAAAAGGAA	TTTC	GAUCCACCAAAGCUUCUGUC	acactctttccctacacgacgctcttccgatctCAACTAGCAGAATAGTAATGGATGG	gtgactggagttcagacgtgtgctcttccgatctGAGGAAACTGATGTTGATAAGAGGT
	BLOC1S1-32-S	CCCCUCUCCCCAGAAAAGAGG	TTTC			gtgactggagttcagacgtgtgctcttccgatctGAGGAAACTGATGTTGATAAGAGGT
BLVRA	BLVRA-11-S	GCGUGGUGGUGGUUGGUGUUG	TTTG	ACACGAAGCCAAUCAGGUUC	acactctttccctacacgacgctcttccgatctCCTACAGTGTTTTCAGACTCCA	gtgactggagttcagacgtgtgctcttccgatctCTGGGCTTTCCATGAATTATGAA

BMT2	BMT2-59-S	CGCUCCUGCUCCCGGGGCGGC	TTTC	UGGUGAAGAGCGUCCACCGG	acactctttccctacacgacgctcttccgatctTTCCTCCCTCACTCAGC	gtgactggagttcagacgtgtgctcttccgatctGCCGAAATACTGCTCGT
BOLL	BOLL-44-AS	AAAAAGGAUUUCCAUCUAUUA	TTTA	GGUGGGACCGAAGUUACCUC	acactctttccctacacgacgctcttccgatctGGTTGATGTGTGTTATTATTTGTAATTAT	gtgactggagttcagacgtgtgctcttccgatctCTAACAACTTCAACTGGATATCCTTATA
	BOLL-44-S	UAAAUGAAAUUACUUACAGGC	TTTT			
	BOLL-46-S	AAUGAAAUUACUUACAGGCCA	TTTA			
BRAF	BRAF-28-S	GGAUUACUUACUCAAGUUGGU	TTTT	GAACUUCUGUACUACAACGC	acactctttccctacacgacgctcttccgatctCCTCACAGACTTTTAGACATCGTAG	gtgactggagttcagacgtgtgctcttccgatctTCACCTTAGCATTTTGTGACTTTT
	BRAF-29-S	GAUUACUUACUCAAGUUGGUC	TTTG			
BRD8	BRD8-66-S	CUGCUUGACGAGCUAGAACAU	TTTA	CCGGAGGUUACCCACUGUGA	acactctttccctacacgacgctcttccgatctCTCTTCCATAGTGGTTGGAGT	gtgactggagttcagacgtgtgctcttccgatctCTGCTTTAGTAATGCAACATACCT
	BRD8-87-AS	AUGUUCUAGCUCGUCAAGCAG	TTTT			
BRDT	BRDT-89-AS	AAAACUACAACAAACUUACUU	TTTA	UUCUCCCUUGAACGUGGUAC	acactctttccctacacgacgctcttccgatctCGCAACAGAAAAAGTATTTAAGCAG	gtgactggagttcagacgtgtgctcttccgatctTTGCTAGACGCTGAAGACTAATTTT
BRIX1	BRIX1-57-AS	CUCUUGGUUGCCGCCAUCUUG	TTTC	GCAACCAAGAGGAAACGGCG	acactctttccctacacgacgctcttccgatctGAGCCGCCGAACCATA	gtgactggagttcagacgtgtgctcttccgatctCGGCTCCGCATCTATTTC
BTN3A1	BTN3A1-32-AS	CAGGCCGAGGAGGGAACUUCU	TTTC	GAGGGAACUUCUGAUGGUAC	acactctttccctacacgacgctcttccgatctAGTAGCAGCATCTGTGATCAT	gtgactggagttcagacgtgtgctcttccgatctAGGTTGACTGCAGACACTAA
BUD23	BUD23-54-AS	CUUUGGCACUGUUAGGGUAGU	TTTG	GUAGUCUACCACCAUGCCAC	acactctttccctacacgacgctcttccgatctGCTGCCTTTCTTTCCTCA	gtgactggagttcagacgtgtgctcttccgatctGCTATGCCACTACCCTCC
C10orf111	C10orf111-8-S	AAAUGUUAUUCGGCCGCUUCA	TTTA	UCGGAGUCGCUGCAAAGUCG	acactctttccctacacgacgctcttccgatctGTCTTCTAAGTTCTGGCCAA	gtgactggagttcagacgtgtgctcttccgatctGAATATCCCGTCCAGTGTC
C10orf67	C10orf67-62-AS	CUCCUCCUUGAGGGGGACCUU	TTTC	GGGGGACCUUUGGCACACGC	acactctttccctacacgacgctcttccgatctTGAATTCCCGAGCTTCTCG	gtgactggagttcagacgtgtgctcttccgatctGATCGCAGGGCTCATTATGT
C16orf82	C16orf82-28-S	CUCGAGGGAGAAAAAGGGGAA	TTTT	AUCCUCUGUCCAGAAUGAGC	acactctttccctacacgacgctcttccgatctTCACAGCCAACATTCAGAG	gtgactggagttcagacgtgtgctcttccgatctGCTCTAAGCTGGGTGACT
C17orf102	C17orf102-37-AS	GCAGCCGCUCUUCCAGAGGCG	TTTT	AAGGAUGCACGCUCCCCACC	acactctttccctacacgacgctcttccgatctTGATTCCCAGCCAGTG	gtgactggagttcagacgtgtgctcttccgatctCGTTTCTCCAGGGTAGA
C1D	C1D-31-AS	UUGUUGUAUUCCUGAGGUUUG	TTTG	AACCCAAGGAGUUAAUCCUA	acactctttccctacacgacgctcttccgatctGCTCACTTTCATAATTTCAACTCGAATT	gtgactggagttcagacgtgtgctcttccgatctCATTCACCATTTTATTCCATGAAATTTTT
C1orf61	C1orf61-69-S	ACUCACCGCCCGGGAGCUGUC	TTTC	UCUAUCGUCCACACGGAGGA	acactctttccctacacgacgctcttccgatctCAAAGCTGACTGCAAACAATT	gtgactggagttcagacgtgtgctcttccgatctTCTCTTCCCTTTAGCTTCTCAC
C1orf68	C1orf68-27-AS	GAUGUAGGUCAUCUGGCAGGG	TTTT	CACCCUGGACAUAGCACGUC	acactctttccctacacgacgctcttccgatctCAACCTATGTAAAATGCCCAA	gtgactggagttcagacgtgtgctcttccgatctCTTGCAGGAGCTTGAACATA
C2orf16	C2orf16-26-AS	GGGUCAACCCCACAGAUUUCU	TTTG	GGGUUGACCCCAAAGCUAAC	acactctttccctacacgacgctcttccgatctCAGAATCTTCAGAAATGGCACAA	gtgactggagttcagacgtgtgctcttccgatctATTTTGGTACCTGAAGATCTGG
C2orf74	C2orf74-22-AS	CUGCCUUGGAAACUGAAAACA	TTTC	CCUUGUACAGAUGCAAACGG	acactctttccctacacgacgctcttccgatctCACCTTAAAATCAGGGCCATT	gtgactggagttcagacgtgtgctcttccgatctATTGCTTGTCACCACTTTGG
	C2orf74-8-S	AGUUUCCAAGGCAGGAAAGGU	TTTC			

Supplementary Table 3 Genomic targets used to compare the activity of WT and AsCas12a Ultra in HEK293 and Jurkat cells.

Assay	**AsCas12a crRNA**	**LbCas12a crRNA**
HPRT 38104-S	uaauuucuacucuuguagaucuugggugugugauuaaaagugacca	uaauuucuacuaaguguagaucuugggugugugauuaaaagugacca
HPRT 38115-AS	uaauuucuacucuuguagauacacacccaaggaaagacuau	uaauuucuacuaaguguagauacacacccaaggaaagacuauga
HPRT 38228-S	uaauuucuacucuuguagauuaauuaacagcuugcuggugaaa	uaauuucuacuaaguguagauuaauuaacagcuugcuggugaaa
HPRT 38330-AS	uaauuucuacucuuguagaugguuaaagaugguuaaaugau	uaauuucuacuaaguguagaugguuaaagaugguuaaaugauug

T7EI primer	
HPRT-FWD	AAGAATGTTGTGATAAAAGGTGATGCT
HPRT-RWD	ACACATCCATGGGACTTCTGCCTC

Supplementary Table 4 Genomic targets used to compare the activity of LbCas12a and AsCas12a Ultra in HEK293 cells at lower temperature.

Name	**PAM**	**crRNA Sequence**
Cpf1 HPRT 38094-S	TTTV	auagucuuuccuuggguguguu
Cpf1 HPRT 38104-S		cuuggguguguuaaaagugac
Cpf1 HPRT 38115-AS		acacacccaaggaaagacuau
Cpf1 HPRT 38146-AS		auccgugcugaguguaccaug
Cpf1 HPRT 38164-AS		uaaacacuguuucauuucauc
Cpf1 HPRT 38164-S		gaaacgucagucuucucuuuu
Cpf1 HPRT 38186-S		uaaugcccuguagucucucug
Cpf1 HPRT 38228-S		uaauuaacagcuugcuggug a
Cpf1 HPRT 38330-AS		gguuaaagaugguuaaaugau
Cpf1 HPRT 38343-S		ugugaaauggcuuauaauugc
Cpf1 HPRT 38455-S		guuguuggauuugaaauucca
HPRT_Cpf1_TATV_1	TATV	gggauuacuaggaaggggcag
HPRT_Cpf1_TATV_2		aaauggagagcuaaauuaugg
HPRT_Cpf1_TATV_3		uuauaugucacauuuuguaau
HPRT_Cpf1_TATV_4		ugucacauuuuguaauuaaca
HPRT_Cpf1_TATV_5		ucacauuuuguaauuaacagc
HPRT_Cpf1_TATV_6		agccagacuguaagugaauua
HPRT_Cpf1_TATV_7		caacacuucguggggucc uuu
HPRT_Cpf1_TATV_8		ugaaauggcuuauaauugcuu
HPRT_Cpf1_TATV_9		auugcuuagagaauauuugua
HPRT_Cpf1_TATV_10		uaaaugaugaauuaugauucu
HPRT_Cpf1_TATV_11		auucuuuuuaguuguuggauu
HPRT_Cpf1_TYCV_1	TYCV	uaguaauccccauaauuuagc
HPRT_Cpf1_TYCV_2		cauaauuuagcucuccauuuc
HPRT_Cpf1_TYCV_3		uuucauagucuuuccuugggu
HPRT_Cpf1_TYCV_4		uagucuuuccuuggguguguu
HPRT_Cpf1_TYCV_5		uuggguguguuaaaagugacc
HPRT_Cpf1_TYCV_6		uuucauccgugcugaguguac
HPRT_Cpf1_TYCV_7		uccgugcugaguguaccaugg
HPRT_Cpf1_TYCV_8		ugcugaguguaccauggucac
HPRT_Cpf1_TYCV_9		ccagcaagcuguuaauuacaa
HPRT_Cpf1_TYCV_10		acacuucguggggucc uuuuc
HPRT_Cpf1_TYCV_11		cuuacagucuggcuuauaucc

Supplementary Table 5 Genomic targets used to compare the editing efficiency of AsCas12a RR and RVR variants ± Ultra mutations.

Gene	Assay	crRNA	PAM	Donor sequence
ALDH2	ALDH21203-S	CUUACAAUACUGGUAGUCAGU	TTTC	T*G*CTTTTTCTTGTTTTTTTTTTTCCTTACAATACTGGTAGGAATTCTCAGTATTAGGTTGACAGCTGGTCCTGAGAACTTCTTTCCTTC*T*C
	ALDH2169-S	CUGAGUGAGUGAAGUAAUAAU	TTTG	T*C*GCAGGTGTTCATTAAGTGTTTGCTGAGTGAGTGAAGTAGAATTCATAATCTAGGGCTGGGCATGGTGACTCACACCTGCAATCCCAG*G*A
	ALDH2299-AS	UUGUAGAGAUGAGGUCCCUCU	TTTC	G*T*TCTTTTATTTACCTATTGTTTTTTGTAGAGATGAGGTCGAATTCCCTCTATATTACCCAGGCTGTCTTGAACTCCACTCAAGAGATC*G*A
	ALDH2994-S	UUACACAGCAAUGGAUAACUA	TTTG	G*C*TGCTAAGTTGTGGGCTGTTTTGTTACACAGCAATGGATGAATTCAACTAACACATGAGGCCTGGCGAGCACAGAGCAAAGCTGCCCA*G*G
APOC	APOC1198-AS	UCAAAGUAUCAGCAAACUUAU	TTTA	T*G*TTGTGCAGCACCTAACAGTTTATCAAAGTATCAGCAAAGAATTCCTTATTGTCCTGTTTGACCTTCCGCACAAAGCTGTCAAGGAAG*G*C
	APOC639-S	GGGGCUGGGUGACCGAUGGCU	TTTA	C*T*GTTGCTTCCCCTGACTGATTTAGGGGCTGGGTGACCGAGAATTCTGGCTTCAGTTCCCTGAAAGACTACTGGAGCACCGTTAAGGAC*A*A
	APOC668-AS	AGGGAACUGAAGCCAUCGGUC	TTTC	T*T*AACGGTGCTCCAGTAGTCTTTCAGGGAACTGAAGCCATGAATTCCGGTCACCCAGCCCCTAAATCAGTCAGGGGAAGCAACAGAGCA*G*G
	APOC921-AS	UUGGGAGGCCAGCAUGCCUGG	TTTA	A*T*AGCAGCTTCTTGTCCAGCTTTATTGGGAGGCCAGCATGGAATTCCCTGGAGGGGGGCCAGGCATGAGGTGGGGTAGGAGAGCACTGA*G*A
AR	AR1407-AS	AGGUCAGCGGAGCAGCUGCUU	TTTA	C*T*GGCCTCGCTCAGGATGTCTTTAAGGTCAGCGGAGCAGCGAATTCTGCTTAAGCCGGGGAAAGTGGGGCCCAGCAGGGACAACGTGGA*T*G
	AR454-AS	GCUACUGAAGACCUGACUGCC	TTTG	T*G*TTTTTGTTTGTTTTGTTTTTTGGCTACTGAAGACCTGAGAATTCCTGCCTTTTCATCTTTTGATCTCTGGCTCCGCACTCGGGGTGG*G*G
	AR490-AS	UUUCGGCUUUUUUGUUUUUGU	TTTA	C*T*GAGTTATTATCTTTTTCTTTTATTTCGGCTTTTTTGTTGAATTCTTTGTTTGTTTTGTTTTTTGGCTACTGAAGACCTGACTGCCTT*T*T
	AR710-S	CGUGGUUGCUCCCGCAAGUUU	TTTG	T*T*GAGGCTGTCAGAGCGCTTTTTGCGTGGTTGCTCCCGCAGAATTCAGTTTCCTTCTCTGGAGCTTCCCGCAGGTGGGCAGCTAGCTGC*A*G
BCAT	BCAT1172-AS	ACAGUGAAAAGAUCGUUCUCC	TTTA	G*T*TTTATATGCGATTGCAGTTTTCCAGTGTGTGAAACTAGGAATTCGAAAACAAAACACTCAACGGTGTACATCCCTACACCTAAATAG*T*C
	BCAT1396-AS	CAGUGUGUGAAACUAGGAAAA	TTTC	T*A*AAACCCGAAGAGCAACTTTTTCCAAGGAAAGTGAAGGAGAATTCTGGGCAGATATTCTTGCCATCTAACATTTTGGAATTGAGATGA*C*C
	BCAT1440-S	CAAGGAAAGUGAAGGAUGGGC	TTTC	T*C*CCTCCCCTGCTTGTGAAGTTTGGAAAGGAAGTGAGGGCGAATTCTTCTCTCAGTTTCTCCTATGCACAGGAGGTGGGGAATTTTGGA*G*A
	BCAT989-S	GAAAGGAAGUGAGGGCUUCUC	TTTG	A*A*TAAGAGGGGCCTTCTGCTTTTAACAGTGAAAAGATCGTGAATTCTCTCCCTCCCCTCTCCACCCGGGTCAACTCTTCCAGCCGCTCC*C*T
BCL2	BCL21031-AS	AUGAGGCACGUUAUUAUUAGU	TTTC	C*A*ATTCCTTTCGGATCTTTATTTCATGAGGCACGTTATTAGAATTCTTAGTAAGTATTGTTAATATCAGTCTACTTCCTCTGTGATGCT*G*A
	BCL21058-AS	UUCCAAUUCCUUUCGGAUCUU	TTTA	G*G*CATGAGATGCAGGAAATTTTTATTCCAATTCCTTTCGGGAATTCATCTTTATTTCATGAGGCACGTTATTATTAGTAAGTATTGTTA*A*T
	BCL2636-AS	AAACUUCCCAAUGAAUCAGGA	TTTG	C*A*CTCTCCAGTTATAGCTGATTTGAAACTTCCCAATGAATGAATTCCAGGAGTCGCGGGGAGAGGGAGTAAAAATTAGGAGGATTTCCA*G*A
	BCL2922-AS	CCUGUACACACUGAGUGAAAG	TTTC	T*A*AGTAAAAAATCAGGTGCGTTTCCCTGTACACACTGAGTGAATTCGAAAGCAGGGCATACACACTACAAGTAACACGGCTAAAAAGAA*T*G
C5	C5245-AS	GCACUAUGAAGAGUGGCUCAA	TTTG	A*A*GGGACCCTAAAATGGGAATTTGGCACTATGAAGAGTGGGAATTCCTCAAAGCTAGAAACCAGTAAGCCCATTGCTTTGGCCTTTCTT*C*T
	C5450-S	AAAGGAGACUAUCCUCAAAAG	TTTA	A*A*TTCTTCAAGTTCAGTTTATTTAAAAGGAGACTATCCTCGAATTCAAAAGTGTTATATCCGTGGTTTCCTGCTACCTCCAACCATGGG*C*C
	C5562-AS	CUCCUGUCCCCAGGUUUUCCC	TTTG	G*A*TAGCTTGTTTTACTTACGTTTGCTCCTGTCCCCAGGTTGAATTCTTCCCCAGGAAGATTAAAAAACAAAGTATTCCCAAAAGGCCCA*T*G
	C5729-S	AUUUGAUGAGUAGCUAACAAC	TTTG	T*G*AGAAAGTCATGGAAGCATTTTGATTTGATGAGTAGCTAGAATTCACAACATGACAGCTTTTATGGAATTGTTATTTATTAAAATTTA*C*G
CTNNB1	CTNNB1113-S	CCCUCCCUGGCUUUUAUUAUU	TTTC	A*G*CTGATTTTTGAATAGTGTTTTCCCCTCCCTGGCTTTTAGAATTCTTATTACAACTCTGTGCTTTTTCATCACCATCCTGAATATCTA*T*A
	CTNNB11210-AS	GUUCUCAAAACUGCAUUCUGA	TTTA	A*A*ACTATTATACACTAACTTTTTAGTTCTCAAAACTGCATGAATTCTCTGACTTTCAGTAAGGCAATGAAAAATAATACTCTTACCAGC*T*A
	CTNNB11293-S	GGAUAGCAAAUACUUAGGUAA	TTTA	T*A*GCAATGTCACTTTTACCATTTAGGATAGCAAATACTTAGAATTCGGTAAATGCTGAACTGTGGATAGTGAGTGTTGAATTAACCTTT*T*C
	CTNNB1803-S	UUGGCUGUCUUUCAGAUUUGA	TTTC	A*C*CCTGGCTATCATTCTGCTTTTCTTGGCTGTCTTTCAGAGAATTCTTTGACTTTATTTCTAAAAATATTTCAATGGGTCATATCACAG*A*T
CYP27A1	CYP27A1131-AS	GAAUUAUAGAAUCACCAAAAU	TTTA	G*A*AATCCCATACTCCTAGAATTTAGAATTATAGAATCACCGAATTCAAAATCTCAAGTGACAGAATCTTAGAATCCCCAAACCAGAATC*T*C

	CYP27A1600-S	CUACAUCCUGUUCGAGAAACG	TTTG	C*T*CTGTGCACTACTCAGCTATTTGCTACATCCTGTTCGAGGAATTCAAACGCATTGGCTGCCTGCAGCGATCCATCCCCGAGGACACCG*T*G
	CYP27A1791-S	GUGAGGACUCCCAGAUGGGGC	TTTG	G*T*TGGAATGCCATCTTTTCCTTTGGTGAGGACTCCCAGATGAATTCGGGGCCCAGGGAAGAGAGATGGGGGTGACTCCAGGTCTGTGCA*T*C
	CYP27A1961-S	ACAGGGAAGAAGCUGAUUGAU	TTTC	C*C*TTTCCTCTTCTCTGTTGCTTTCACAGGGAAGAAGCTGAGAATTCTTGATGAGAAGCTCGAAGATATGGAGGCCCAACTGCAGGCAGC*A*G
CYP7A1	CYP7A1139-S	UAAUAGAAUGAACAAAUGGCU	TTTC	C*A*GTTACTACCACTTTTTTTTTTCTAATAGAATGAACAAAGAATTCTGGCTAATTGTTTGCTTTGTCAACCAAGCTCAAGTTAATGGAT*C*T
	CYP7A1441-S	CCUUCAUUUUUAUACAGUAAA	TTTA	T*G*ATTCATCCATTTAATTTTTTTACCTTCATTTTTATACAGAATTCGTAAATTTGGTTTTCTATACTTACACATATTAGCATTATCTTC*C*T
	CYP7A1512-AS	AUUUAAAAAAUAAGGAAGAUA	TTTC	T*T*AAAAATTCAAATCAAATTTTTCATTTAAAAAATAAGGAGAATTCAGATAATGCTAATATGTGTAAGTATAGAAAACCAAATTTACTG*T*A
	CYP7A1591-S	GUAUUGGCAUAUACCGAUGGU	TTTA	G*A*CATATGACAGCTTCCCTTTTTAGTATTGGCATATACCGGAATTCATGGTAATATATAAATGTATATTGGTGTTAAACATAACTGACA*G*A
DICER	DICER33497-AS	CUUAGGAAAGUGUCUGUAAAC	TTTC	T*C*TTCACATAGTGCATGTATTTTCCTTAGGAAAGTGTCTGGAATTCTAAACAATAAAAATTTCCTGTGCAGCTCCTCTTGCTCATGTTT*G*A
	DICER33625-S	AAAGCGUUGAGUGGUAUAAUA	TTTG	A*A*CCATATGAGCGACAGCAGTTTGAAAGCGTTGAGTGGTAGAATTCTAATAATAGAAATCAGGATAATTATGTGTCATGGAGTGATTCT*G*A
	DICER33765-S	UGCGGAAUUAUUUUUGUGGAA	TTTG	C*C*TTCTCCTTTTACCAACATTTTGTGCGGAATTATTTTTGGAATTCTGGAAAGAAGATACACAGCAGTTGTCTTAAACAGGTAAATCTG*T*A
	DICER33781-S	UGGAAAGAAGAUACACAGCAG	TTTG	A*C*ATTTTGTGCGGAATTATTTTTGTGGAAAGAAGATACACGAATTCAGCAGTTGTCTTAAACAGGTAAATCTGTAAGTTGACTCCTTTC*A*C
EGFR	EGFR1320-AS	UCAUGGGCAGCUCCUUCAGUC	TTTC	C*C*AGCCTCTCACCCTGTAAATTTCTCATGGGCAGCTCCTTGAATTCCAGTCCGGTTTTATTTGCATCATAGTTAGATAAGACTGCTAAG*G*C
	EGFR439-S	CCAGCUAAUGUCAAGUAAUAA	TTTA	G*A*GTTTTATGAGAAAGCCATTTTACCAGCTAATGTCAAGTGAATTCAATAACTAGAAAAGGATATCAAATAGAAACAGGCTAATCTGGA*G*T
	EGFR461-AS	UAGUUAUUACUUGACAUUAGC	TTTC	C*T*GTTTCTATTTGATATCCTTTTCTAGTTATTACTTGACAGAATTCTTAGCTGGTAAAATGGCTTTCTCATAAAACTCTCGTTTGTAGC*T*C
	EGFR759-S	GAUGGGGUUUACUUAGCCUUC	TTTG	G*C*TTCAGAGTTTCACTGAATTTTGGATGGGGTTTACTTAGGAATTCCCTTCTTCTGTTTTTCTTTTACCTTTCCTTTTTAAATAAGAAA*T*A
EMX1	EMX110685-S	CAGCUAUCAGCUAUUUGUCAA	TTTC	C*C*AGGCAAGCTTACCTGTCATTTCCAGCTATCAGCTATTTGAATTCGTCAAGCATCAGTCACCCCCAACAGCCCCCCTGCCATGCATTT*C*T
	EMX110839-AS	AUGAGAGCAGACAGAAGUAUA	TTTA	A*G*CCAGTTTTGGGGAGGCAGTTTAATGAGAGCAGACAGAAGAATTCGTATATGGCTAGATGTAAAACAGCTACGGGGTGGGACAGATTA*G*A
	EMX110875-S	CGAUUUUCUAUUAGUUAUAUG	TTTC	C*C*AAAACTGGCTAATTTATATTTCCGATTTTCTATTAGTTGAATTCATATGTTCTTCCTAACAATAAGAACTATTTACCCATTTGTTCA*T*T
	EMX110955-S	CCUUCUGUGUUUCCACCAUUC	TTTA	T*C*ATTATCTTTGTCATCTATTTTACCTTCTGTGTTTCCACGAATTCCATTCATCTCAAACTTCATCTCCATAGAGCTATTTCGCAACTG*A*C
	EMX117707-AS	UGGUUGCCCACCCUAGUCAUU	TTTG	C*A*GCACTCTGCCCTCGTGGGTTTGTGGTTGCCCACCCTAGGAATTCTCATTGGAGGTGACATCGATGTCCTCCCCATTGGCCTGCTTCG*T*G
	EMX117983-S	UUUGAUGCAUUUCUGUUUUUA	TTTG	T*C*CGTGTCTCCAATCTCCCTTTTGTTTTGATGCATTTCTGGAATTCTTTTAATTTATTTTCCAGGCACCACTGTAGTTTAGTGATCCCC*A*G
	EMX118014-S	CAGGCACCACUGUAGUUUAGU	TTTC	G*C*ATTTCTGTTTTAATTTATTTTCCAGGCACCACTGTAGTGAATTCTTAGTGATCCCCAGTGTCCCCCTTCCCTATGGGAATAATAAAA*G*T
	EMX118033-S	GUGAUCCCCAGUGUCCCCCUU	TTTA	T*T*TTCCAGGCACCACTGTAGTTTAGTGATCCCCAGTGTCCGAATTCCCCTTCCCTATGGGAATAATAAAAGTCTCTCTCTTAATGACAC*G*G
ERBB2	ERBB2468-S	AAUUAAAGUUCCCGGAUUUUU	TTTA	C*T*CCGCTGAAGTCCACACAGTTTAAATTAAAGTTCCCGGAGAATTCTTTTTGTGGGCGCCTGCCCCGCCCCTCGTCCCCCTGCTGTGTC*C*A
	ERBB2579-AS	CUCAUUAACCCAUCAGGCGUC	TTTG	G*A*TCATGGAAAACACTTCAGTTTGCTCATTAACCCATCAGGAATTCGCGTCCGCCTTCCCTTAACCCTATCGCCTCGATATATGGACAC*A*G
	ERBB2612-S	AGGUAGGGCUGUUUACUGUCA	TTTG	A*A*GTGTTTTCCATGATCTTTTTTGAGGTAGGGCTGTTTACGAATTCTGTCACCACCCCTGTCGGATTTTACTTCCTAAACGTACCTGTA*A*C
	ERBB2627-S	CUGUCACCACCCCUGUCGGAU	TTTA	T*C*TTTTTTGAGGTAGGGCTGTTTACTGTCACCACCCCTGTGAATTCCGGATTTTACTTCCTAAACGTACCTGTAACTATCCACTTCTCT*C*C
G6PC	G6PC177-S	GGGGACCAGGAGGGCAGACCC	TTTC	G*T*CCTGTGTCTCTGGCCTGGTTTCGGGGACCAGGAGGGCAGAATTCGACCCTTGCACTGCCAAGAAGCATGCCAAAGTTAATCATTGGC*C*C
	G6PC753-S	AGUGGUAAGAACCAUAUAGAG	TTTA	G*A*GACTGGCTCAACCTCGTCTTTAAGTGGTAAGAACCATAGAATTCTAGAGAGGAGATCAGCAAGAAAAGAGGCTGGCATTCGCTCTCG*C*A
	G6PC923-AS	UGUCUUCCCCCUUUAUGUAGA	TTTC	T*C*AACTCACTGGTAGGATTTTTTCTGTCTTCCCCCTTTATGAATTCGTAGATGATCCAAAGTCAGAGAGAGGGGTTGGAAGCATGAGTA*G*C

	G6PC945-AS	AACUCACUGGUAGGAUUUUUU	TTTC	T*G*AAATAGGCTTTCCTGTATTTTCAACTCACTGGTAGGATGAATTCTTTTTCTGTCTTCCCCCTTTATGTAGATGATCCAAAGTCAGAG*A*G
GCK	GCK1282-S	CUGUGAAACACUGGGGGGAAU	TTTG	G*A*GAGCTGGAGACAGGGGACTTTGCTGTGAAACACTGGGGGAATTCGGAATGTGCCTGCATCACCCCAGCTGGGGGCCCAGGCAGAGTG*G*G
	GCK1284-AS	ACAGCAAAGUCCCCUGUCUCC	TTTC	C*A*GGCACATTCCCCCCAGTGTTTCACAGCAAAGTCCCCTGGAATTCTCTCCAGCTCTCTCCTTGCTCACTGCTGGGCTCAGGGCTGGCT*C*A
	GCK526-AS	AUUGUUCUCCAACGAGUCGGC	TTTC	T*T*CAGTCACAGTTTCCTCCTTTTCATTGTTCTCCAACGAGGAATTCTCGGCAAGCATTCCCTTCTGGACCCTTCCTCACTGAGCCTGGA*T*G
	GCK560-AS	GGGUUUGAGGUUCAGUCACAG	TTTG	T*G*TGGTTCTCCTCGGGCTGGTTTGGGGTTTGAGGTTCAGTGAATTCCACAGTTTCCTCCTTTTCATTGTTCTCCAACGAGTCGGCAAGC*A*T
GYS2	GYS21201-S	GGAAGAUGUAUAUGUGAUUAG	TTTA	A*G*TATAATTAAACATTTTATTTTAGGAAGATGTATATGTGGAATTCATTAGAACCAGTTGGAAACCTCAACTCTACATTCTGTTAGTAA*T*T
	GYS21337-AS	CCUUUUGAGAAGCUCCUGAAU	TTTG	T*T*CTGAAAGGAGCACAGTATTTTGCCTTTTGAGAAGCTCCGAATTCTGAATTAAAACATTAATTCCAAGCTAATTGTGAATATGAAACA*T*T
	GYS2950-AS	CUCACUCACUAGCAAUUGGUU	TTTC	T*A*TATATTCCGTTTAAATGCTTTCCTCACTCACTAGCAATGAATTCTGGTTATCACTAGAGAGTTATCTGTGAAATCTTACTCTACATT*C*C
	GYS2959-AS	AAUGCUUUCCUCACUCACUAG	TTTA	T*A*GCCTAAATATATATTCCGTTTAAATGCTTTCCTCACTCGAATTCACTAGCAATTGGTTATCACTAGAGAGTTATCTGTGAAATCTTA*C*T
HPRT	HPRT 38115-AS	ACACACCCAAGGAAAGACUAU	TTTA	C*T*GAGTGTACCATGGTCACTTTTAACACACCCAAGGAAAGGAATTCACTATGAAATGGAGAGCTAAATTATGGGGATTACTAGGAAGGG*G*C
	HPRT 38228-S	UAAUUAACAGCUUGCUGGUGA	TTTG	C*T*GTATGTTATATGTCACATTTTGTAATTAACAGCTTGCTGAATTCGGTGAAAAGGACCCCACGAAGTGTTGGATATAAGCCAGACTGT*A*A
	HPRT 38330-AS	GGUUAAAGAUGGUUAAAUGAU	TTTA	G*C*CATTTCACATAAAACTCTTTTAGGTTAAAGATGGTTAAGAATTCATGATTGACAAAAAAAGTAATTCACTTACAGTCTGGCTTATAT*C*C
	HPRT38082-S	GCUCUCCAUUUCAUAGUCUUU	TTTA	C*T*TCCTAGTAATCCCCATAATTTAGCTCTCCATTTCATAGGAATTCTCTTTCCTTGGGTGTGTTAAAAGTGACCATGGTACACTCAGCA*C*G
	HPRT38146-AS	AUCCGUGCUGAGUGUACCAUG	TTTC	C*G*TTTCTAAACACTGTTTCATTTCATCCGTGCTGAGTGTAGAATTCCCATGGTCACTTTTAACACACCCAAGGAAAGACTATGAAATGG*A*G
	HPRT38330-AS	GGUUAAAGAUGGUUAAAUGAU	TTTA	G*C*CATTTCACATAAAACTCTTTTAGGTTAAAGATGGTTAAGAATTCATGATTGACAAAAAAAGTAATTCACTTACAGTCTGGCTTATAT*C*C
	HPRT38628-S	AACAGACUGAUGGUUCCCAUU	TTTG	C*T*CCTTCCAGCACCTCATAATTTGAACAGACTGATGGTTCGAATTCCCATTAGTCACATAAAGCTGTAGTCTAGTACAGACGTCCTTAG*A*A
	HPRT411-AS	CCAGGGCUCCCCCGAGGAGGG	TTTC	C*T*TCCCACACGCAGTCCTCTTTTCCCAGGGCTCCCCCGAGGAATTCGAGGGACCCACCCCAAACCCCGCCATTCCGTCCTCCCTGCCGC*C*C
	HPRT677-S	CACACACUGUAGUUCAUCUUU	TTTG	G*A*CCAGGTTTTGCCTTTAGTTTTGCACACACTGTAGTTCAGAATTCTCTTTATGGAGATGCTCATGGCCTCATTGAAGCCCCACTACAG*C*T
	HPRT782-AS	CCUAGUUCCUUCGUGUGUCAA	TTTC	C*T*TGAAATGACCTAATGCCTTTTCCCTAGTTCCTTCGTGTGAATTCGTCAAATACGCATGGTTACCGCTACCAGAGCTGTAGTGGGGCT*T*C
	HPRT803-S	AAGCCGAAAUUCACAUGUGCU	TTTC	A*G*GGAAAAGGCATTAGGTCATTTCAAGCCGAAATTCACATGAATTCGTGCTAGAATCCAGATTCCATGCTGACCGATGCCCCAGGATAT*A*G
MET	MET1379-S	UUUACUUCUUGACGGUCCAAA	TTTA	A*T*GCCTTTGAAAGCAACAATTTTATTTACTTCTTGACGGTGAATTCCCAAAGGGAAACTCTAGATGCTCAGACTTTTCACACAAGAATA*A*T
	MET540-S	UCUUCAUUUCUGACAACUGAA	TTTC	A*A*TGGTATAGGTCTTTCAGTTTTCTCTTCATTTCTGACAAGAATTCCTGAACTGCTCTCGCCTTGAACCTGTTTTGGCAGATAAACCTC*T*C
	MET793-S	CUUGGUGCCACUAACUACAUU	TTTC	A*T*TCTACATGAGCATCACATTTTCCTTGGTGCCACTAACTGAATTCACATTTATGTTTTAAATGAGGAAGACCTTCAGAAGGTTGCTGA*G*T
	MET892-S	CCAUGUCAGGACUGCAGCAGC	TTTC	G*T*GCTGGAACACCCAGATTGTTTCCCATGTCAGGACTGCAGAATTCGCAGCAAAGCCAATTTATCAGGAGGTGTTTGGAAAGATAACAT*C*A
MYC	MYC238-AS	GUGGAUGCGGCAAGGGUUGCG	TTTC	G*C*CCGCTGCTATGGGCAAAGTTTCGTGGATGCGGCAAGGGGAATTCTTGCGGACCGCTGGCTGGGGGATCAGCGGGAGGGCTGGGCCAG*A*G
	MYC528-S	UCCCAUUCCUGCGCUAUUGAC	TTTC	A*A*TGCCTAAATAGGGTGTCTTTTCTCCCATTCCTGCGCTAGAATTCTTGACACTTTTCTCAGAGTAGTTATGGTAACTGGGGCTGGGGT*G*G
	MYC801-AS	UGCCCACCGCAAAGCAACCCC	TTTC	G*A*GCTCAGGATGCAAGGGGCTTTCTGCCCACCGCAAAGCAGAATTCACCCCCAGCCCCCCAAAACCCAGAGAGCAATTAACACAATAAA*G*C
	MYC932-S	UGACAGCCGGAGACGGACACU	TTTC	T*T*AAATTTCGGCTCACCGCATTTCTGACAGCCGGAGACGGGAATTCACACTGCGGCGCGTCCCGCCCGCCTGTCCCCGCGGCGATTCCA*A*C
RAG2	RAG2207-S	CUCAUUCAGUCCCACUGCAAG	TTTC	A*C*TGACCTAACTCCTTGGATTTTCCTCATTCAGTCCCACTGAATTCGCAAGCGTGTGGGAGGACTTAAAAAAATGCTATTCACATGTGA*A*G
	RAG2504-AS	CAUCCAGAUGGAAAACUCCAG	TTTA	T*C*AGTTTGACATGGTTATGCTTTACATCCAGATGGAAAACGAATTCTCCAGTGGGGCAGGATCTTTTGGGCCAGCCTTTTTGTCCAAAG*A*A

	RAG2544-S	UCUAAGGAUUCCUGCUACCUC	TTTC	G*T*CAAACTGAAGCCTACAATTTTCTCTAAGGATTCCTGCTGAATTCACCTCCCTCCTCTTCGCTACCCAGCCACTTGCACATTCAAAGG*C*A
	RAG2907-S	CUGGUGGAUUUUGAAUUUGGG	TTTC	G*C*TGACTGCCTGCCCTGTGTTTTCCTGGTGGATTTTGAATGAATTCTTGGGTGTGCTACATCATACATTCTTCCAGAACTTCAGGATGG*G*C
STAT3	STAT346810-AS	GUUUCCUGGGUCAUACAGUCC	TTTG	A*T*TTCTCAGGAAAAGAAACATTTGAACTGAATTTGAAGGTGAATTCGAGTGAGTTCAGGTGTGTTTGGGCTGAAGCCCAGGCCATGCTG*A*G
	STAT347118-S	AACUGAAUUUGAAGGUGAGUG	TTTG	A*G*GGACAGAGGGTAAATATTTTTGGTTTCCTGGGTCATACGAATTCAGTCCCTGCCACAAATAGCCCTGCCACTGCAGCACCAAAGCAG*C*C
	STAT347155-S	GGCUGAAGCCCAGGCCAUGCU	TTTG	G*G*TGAGTGAGTTCAGGTGTGTTTGGGCTGAAGCCCAGGCCGAATTCATGCTGAGTGGATAGCGGGTGGGAAGAGAGTGTGGAAACACAC*T*G
	STAT347205-AS	CACACUCUCUUCCCACCCGCU	TTTC	T*C*TTCCCTGCATGCAGTGTGTTTCCACACTCTCTTCCCACGAATTCCCGCTATCCACTCAGCATGGCCTGGGCTTCAGCCCAAACACAC*C*T
TTR	TTR1166-S	CUUAGCUAGGAAGUGACCAGG	TTTG	G*A*AGGATGCCCTCTTTTTGTTTTGCTTAGCTAGGAAGTGAGAATTCCCAGGAACCTGAGCATCATTTAGGGGCAGACAGTAGAGAAAAG*A*A
	TTR1297-AS	AUCACCUACAUAAAGUGUUCU	TTTC	C*T*GGGCATAGAATGTTTACTTTTCATCACCTACATAAAGTGAATTCGTTCTGTGACCCAAAAGGGTTGCAAACCACAGCTAGAGGAGAG*G*A
	TTR1439-S	GGAAAAGGUAUAAUGUGUAUU	TTTA	A*A*GTGTCCTTGTCTTAGAATTTTAGGAAAAGGTATAATGTGAATTCGTATTAACCCATTAACAAAAGGAAAGGAATTCAGAAATATTAT*T*A
	TTR959-AS	UGAACACAUGCACGGCCACAU	TTTC	C*C*CAGGTGTCATCAGCAGCCTTTCTGAACACATGCACGGCGAATTCCACATTGATGGCAGGACTGCCTCGGACAGCATCTAGAACTTTG*A*C

Supplementary Table 6 Genomic targets used to compare the HDR efficiency mediated by WT or AsCas12a Ultra in Jurkat cells.

Gene	Assay	Crispr enzyme	sgRNA	donor_sequence
A1CF	Hs.Cas9.A1CF.1	Cas9	UAAUCGGCAGUUGUCCACAC	TTTTGCAGAAATGGGCGCCTCTTAGGGGTTTGTGCCAGTGGAATTCTGGACAACTGCCGATTATTTGTTGGGGGCATCCCAAAAAC
	Cas12a_A1CF-48-AS	Cas12a	UGCCAGUGUGGACAACUGCCG	AAATGGGCGCCTCTTAGGGGTTTGTGCCAGTGTGGACAACGAATTCTGCCGATTATTTGTTGGGGGCATCCCAAAAACCAAAAAGAGAGAA
AADACL3	Hs.Cas9.AADACL3.1	Cas9	GGCAUCGUGUACUACCACGG	ATGGTTCTTACTCAAACTCCCCATGACGCCCCCGCCACCGGAATTCTGGTAGTACACGATGCCAGGCTTCAGGGTGCAGGTGGATG
	Cas12a_AADACL3-44-S	Cas12a	CGCUUUGGGACAAUCCCUGUG	CCCGATGTTGTGGTCACGGATTTCCGCTTTGGGACAATCCGAATTCCTGTGAAGCTGTACCAACCCAAGGCATCCACCTGCACCCTGAAGC
ABCG5	Hs.Cas9.ABCG5.1	Cas9	CAUGUGGCAGACCGACUGAU	CTCACCCGTGGAAATGCCCCCCAAGCTGTAGTTGCCAATCGAATTCAGTCGGTCTGCCACATGGCTCAGACTCAGCTCTGCCATGA
	Cas12a_ABCG5-127-AS	Cas12a	UGUCUCCUGCAGGUGGAGGCC	GACTCCCAGCCTTCCTGTAATTTGTGTCTCCTGCAGGTGGGAATTCAGGCCGTCATGGCAGAGCTGAGTCTGAGCCATGTGGCAGACCGAC
ABHD6	Hs.Cas9.ABHD6.1	Cas9	GAUUGUCAGUUGAGUACUGC	TATCATCAAGCCATTTGGGTTTGAATCCCACAGGCCTGCAGAATTCGTACTCAACTGACAATCAATTTGTACAACGGCTCAAAGAA
	Cas12a_ABHD6-34-S	Cas12a	GGUUUGAAUCCCACAGGCCUG	GTGGATGTATCATCAAGCCATTTGGGTTTGAATCCCACAGGAATTCGCCTGCAGTACTCAACTGACAATCAATTTGTACAACGGCTCAAAG
ABRAXAS1	Hs.Cas9.ABRAXAS1.1	Cas9	AAGCUGCUCUACUCAUCGAC	TTAACACCAAGTATAATAACAGAAAGCTGCTCTACTCATCGAATTCGACTGGAACATTCCTTATATAAACCTCAAAAAGGGTAAAT
	Cas12a_ABRAXAS1-55-AS	Cas12a	CUGCUAUUAACACCAAGUAUA	TTTTCAAACCAAGACCTTGTTTTTCTGCTATTAACACCAAGAATTCGTATAATAACAGAAAGCTGCTCTACTCATCGACTGGAACATTCCT
ACAT1	Hs.Cas9.ACAT1.1	Cas9	UCAAGCUUUACCCCACCAUA	CAATGTTCCATATGTAATGAACAGAGGATCAACACCATATGAATTCGGTGGGGTAAAGCTTGAAGATTTGATTGTAAAAGACGGGC
	Cas12a_ACAT1-102-AS	Cas12a	CAAUCAAAUCUUCAAGCUUUA	AGACATCAGTTAGCCCGTCTTTTACAATCAAATCTTCAAGGAATTCCTTTACCCCACCATATGGTGTTGATCCTCTGTTCATTACATATGG
ADAM30	Hs.Cas9.ADAM30.1	Cas9	UACGAGUCAAACUCCCCUUC	CCTTAAGTCTCTTGGCGAAGATGTAATTTTTCACCCTGAAGAATTCGGGGAGTTTGACTCGTATGAAGTCACCATTCCTGAGAAGC
	Cas12a_ADAM30-45-AS	Cas12a	ACUCGUAUGAAGUCACCAUUC	TTTTTCACCCTGAAGGGGAGTTTGACTCGTATGAAGTCACGAATTCCATTCCTGAGAAGCTGAGCTTCCGGGGAGAGGTGCAGGGTGTGGT
ADAM7	Hs.Cas9.ADAM7.1	Cas9	GCCAGUAUCAGUACGUGUAA	AAGGAAAAAGAAAGATATTCTGCATTACCTTAGACCATTAGAATTCCACGTACTGATACTGGCAGCTGAATCATATTCGTGTACTA
	Cas12a_ADAM7-63-S	Cas12a	CCUAUGGAUUUUCAAAGAAGU	CTTTCTTTTTCCTTTATGAGTTTTCCTATGGATTTTCAAAGAATTCGAAGTTAACTTTGTTGATTTTGTTTTATCACTCCTGGTATATAGA
ADGRL3	Hs.Cas9.ADGRL3.1	Cas9	UUUGAGUCCGACCACCAAUC	GCTGCAGATAAAATTTATTTCATGCCCTGGACTCCCTATCGAATTCGTACCGATACTTTAATAGAATATGCTTCTTTAGAAGATTT
	Cas12a_ADGRL3-44-AS	Cas12a	CACCACGCCCCAGAUUGGUGG	TCAGATGCCTGCAGAGGGTCTTTGCACCACGCCCCAGATTGAATTCGGTGGTCGGACTCAAACAAATGTTCACTCTGGTATACTCCTTTTA
AGAP9	Hs.Cas9.AGAP9.1	Cas9	UGGUGGUGCUGGAGAUACCG	ACATATAGCTTTGGAGTTTAACCTTTCTGCCAATCCAGAGGAATTCTCAAGCACAATATTCCAGAGGAACTCTCAAACAGAAGGTG
	Cas12a_AGAP9-21-S	Cas12a	GGUGUUUCUUUUUAUUGGCAU	AGTTGTTGGTGCTTTTCTTCTTTAGGTGTTTCTTTTTATTGAATTCGGCATGAGGAGAGGGGGGCGGGTTGAGCTTGGGGCTGGTGGTGCT
AHCTF1	Hs.Cas9.AHCTF1.1	Cas9	AAGGAUAACAUCGUUACCAC	CCGTGGGCGCTGCCGTGGCCGCCGACGCGGCTCTCCGCGCGAATTCTTTGCACTCATTGGTACTCAGAGCCTCCCGGGGCCGGCCC
	Cas12a_AHCTF1-38-S	Cas12a	UUAACAGAUACGAUAAAGACU	GTCTATCTCATCAATTCCATTTTATTAACAGATACGATAAGAATTCAGACTTTATAGTTTCAAAAGATGTACTTACCTATTAAAAAGCAAA
AKAP10	Hs.Cas9.AKAP10.1	Cas9	AGAAGCGGGACUAUUUCUAC	GGAAATGAGCCTGAAGAGGTGAAGCTGCAGAATGCCAGCAGAATTCAACAGATTGTGCAGAATGCAATCCTGCAAGCTGTGCAGCA
	Cas12a_AKAP10-70-S	Cas12a	CAUGGAAACUUGGUGAGUUCC	TGTAGAGGAAGATTCTTGGGTTTCCATGGAAACTTGGTGAGAATTCGTTCCTGCTCTGGCCATTTCAAGACGGAGAGAATGGGCACTGTCA

AKR1B10	Hs.Cas9.AKR1B10.1	Cas9	GAACGUUGCUUUUCCACCGA	CACTCTGATTAAGAACAACTTCTAAATGTCTCCACCTCTGGAATTCATAACGACTATATTCTTGTTTTATGTTCTGAAAAATTTGC
	Cas12a_AKR1B10-68-S	Cas12a	CCCAAAGAUGAUAAAGGUAAU	TCACAGTCTGGGGATGACCTTTTCCCCAAAGATGATAAAGGAATTCGTAATGCCATCGGTGGAAAAGCAACGTTCTTGGATGCCTGGGAGG
ALS2	Hs.Cas9.ALS2.1	Cas9	ACAAUGGUGUCGCGUACAUG	CCGTGACGCGCGCTCCCCTGCGCCCCCGGGGCCTCCCGTGGAATTCGGCTCCGTGCGGGGACAAAGCCAGCGCCAGCAGGAAGAGT
	Cas12a_ALS2-22-S	Cas12a	GUUCCGGCACAUACUGCUGGU	AATCAGCAATGCTGACAGGATTTGGTTCCGGCACATACTGGAATTCCTGGTTGGCTACTGCACACTGGCCAGCAGAATTCTCTCCCCACAT
AMZ2	Hs.Cas9.AMZ2.1	Cas9	AUCUUUACCCAAGAGACUCG	ATTCTGACCAGCTGGATCTCGCCCAGTTCCTCGTCCACAGGAATTCTCACGTCGTATGAATCCACCTGGGAAGGAGAACAGGCAAT
	Cas12a_AMZ2-24-S	Cas12a	UGUAUUUCUUUUUAAAUAGGG	ATATAATATACACTCATACATTTATGTATTTCTTTTTAAAGAATTCTAGGGGACATCCTGAAGTTCTTGAAAAAGAAGAAACCTGAAGATG
ANKRD13D	Hs.Cas9.ANKRD13D.1	Cas9	CUUCGACACAAUGCCAACGU	GCAGAAGTACCTGACGCGAGTGCCTGGAGTCAGACCACAGGAATTCGCGACAGGTGTAATCGTTCTTAAAATCCATCACTGAGCTA
	Cas12a_ANKRD13D-57-AS	Cas12a	CCAGAGACACGGCCAGCUCCA	GCACTCTCACAGACTCCAGGTTTCCCAGAGACACGGCCAGGAATTCCTCCAGTGGGGTCCGCCCGCGGGGTCCTCCTGTTCAATGTCGTG
ANKRD27	Hs.Cas9.ANKRD27.1	Cas9	UAGACUGGAUGCUGCUCGAC	CATCTGTCAAAGAGGCCTGTCCAAAGACAAAATTCCACGAGAATTCGTCTCTTGGGTAAAGATCAATCATTGTTATTCCCACAACA
	Cas12a_ANKRD27-56-AS	Cas12a	CAGGUCUUAGUACCCUGCAAA	AATGTTGCCCCGAATTGTTTTTTCCAGGTCTTAGTACCCTGAATTCGCAAAGGAAGCCTGTCGAGCAGCATCCAGTCTACTTGTCAGTTTG
ANKRD45	Hs.Cas9.ANKRD45.1	Cas9	CAAAGUUUCCAAACGACCCC	TGCCCGAAGAAAGAGGCTGCCCGAGATGGCCCAGCCAGTTGAATTCGACCCGGCTCACAATGTCAGCCGCCTGCACCGTGAGTGTC
	Cas12a_ANKRD45-39-S	Cas12a	AAAGUUUCCAAACGACCCCAG	ACATCCAGTTCTACCAGTGCTTTCAAAGTTTCCAAACGACGAATTCCCCAGGCTGCAGCACAATGTAAGAGTGTGTACCCTAACAAATGGA
ANTXRL	Hs.Cas9.ANTXRL.1	Cas9	GGGGGCCAACGUUUACACCC	TTGTTAGGGTACACACTCTTACATTGTGCTGCAGCCTGGGGAATTCGTCGTTTGGAAACTTTGAAAGCACTGGTAGAACTGGATGT
	Cas12a_ANTXRL-63-AS	Cas12a	AGCCUAAGGGAAAGAAGGCAU	GGCCCCCAGTTTCCGAGCCTTTTGAGCCTAAGGGAAAGAAGAATTCGGCATTGCATATTGGTCATCAAAATACATGGAGCAAAATGCTGGT
AP1S3	Hs.Cas9.AP1S3.1	Cas9	AGAGAUUGUGCAUCGUUACG	TGATCTAAACGATGAAGAATGTACTGCTGAACATCCTTGAGAATTCCGATATATGCCTTCCGAAGGTCATCTAAACTTATTTTTCG
	Cas12a_AP1S3-70-S	Cas12a	CUAUUGCACAGCAAAAAUAUA	TCAAGAGCTCATTGTCCTGATTTTCTATTGCACAGCAAAAGAATTCATATAAACTAGCATACCTTGAAATGAAAAATAACAAAAGCAAAAT
AP3B1	Hs.Cas9.AP3B1.1	Cas9	AAGCGCUUGCACGAAUUAGU	ACCGGGCCACCAAGTGTGCTGAGGCTCTGGCACCCCTGTTGAATTCGAGCAGCCTCAACGTGGCTGACAGGAGCGGGCGCAGTGCT
	Cas12a_AP3B1-57-AS	Cas12a	GGUGAAUAUUUUUGCACUAUU	AAAATATTGTAAAGTTGAAATTTTGGTGAATATTTTTGCAGAATTCCTATTTTTAGGACCCAAACCAACTAATTCGTGCAAGCGCTTTGAG
APAF1	Hs.Cas9.APAF1.1	Cas9	UCUUUGCACACGGUUGGAUC	GCTGAGATAATGCACATCAAATTGCCTCTGAAACCCAATGGAATTCATCTGAAAAACCGGTCCTCAGCCTTTGGTACACTCAACTG
	Cas12a_APAF1-46-AS	Cas12a	AUCAGAAGCCCAGAUUUGUCU	CGTGTGCAAAGATTCTGCAGTTTCATCAGAAGCCCAGATTGAATTCTGTCTTGTTTCCCAACTGAAACCCAATGCACTCCCCCTGGGAAAC
APOPT1	Hs.Cas9.APOPT1.1	Cas9	UGUAAAAGUGAACAGGUCGA	CGGATCCAGAACCTGGCACCGAGCGATGAGGAGTCCAGCTGAATTCGGACAACGTTGTCCCAAGACAGTGCCTCACCCAGCTCCCC
	Cas12a_APOPT1-54-AS	Cas12a	AGGUAUGUAAAAGUGAACAGG	TTGTTCCAATGGAGATTCATTTTCAGGTATGTAAAAGTGAGAATTCACAGGTCGAAGGTTTGAATATTTATCTGGGGGTCCTATCCAATCA
ARHGAP36	Hs.Cas9.ARHGAP36.1	Cas9	AAGGUGUUCGGACGGUCAAU	GGCGGCCACGGCGGCTCCAGGCTCTGGGACGCAACCTCTCGAATTCTCGGGGTGGCACTCCAGGGCCGACTGCGGCTGTGAAGGTT
	Cas12a_ARHGAP36-17-S	Cas12a	UUUUUUCUCUUUCUGUAGCUC	TCCTATTTGTAAGTGTATCTTTTCTTTTTTCTCTTTCTGTGAATTCAGCTCTGCCTATTGACCGTCCGAACACCTTGGATAAGTGGTTTCT
ARL1	Hs.Cas9.ARL1.1	Cas9	CAUGGCAACUAACUCUGAUU	CGAGCCGGGCATACCTTCAGGCTGCAGTGCACGGCCGACTGAATTCCGGGTGGGTACACGATGAAGTGGCGCAGAGTGAAGCCAAA

	Cas12a_ARL1-22-S	Cas12a	GAAAUGCCAAUUCGGUCUCGG	AACATGGCAACTAACTCTGATTTGGAAATGCCAATTCGGTGAATTCCTCGGTCACAACTGTCTACTACATAAATGACTGCATCTGTGTTTG
ARL16	Hs.Cas9.ARL16.1	Cas9	UGCCACGAUGUCAGUAAGAU	TCAAAATCAGAAACCACTTATCCAAGGTGTTCGGACGGTCGAATTCAATAGGCAGAGCTACAGAAAGAGAAAAAAGAAAAGATACA
	Cas12a_ARL16-98-AS	Cas12a	UCCUAUAGGUGGGCACCAAUC	TTGTTCTTTTTGCATCGTGGTTTTTCCTATAGGTGGGCACGAATTCCAATCTTACTGACATCGTGGCACAGAGAAAGATCACCATCCGGGA
ARL6	Hs.Cas9.ARL6.1	Cas9	UGCCACUAUUAUCUAGCCCA	GCAATTCTTATTTTTTAGGAAGAGCTGAGCAATGCCATGTGAATTCCGATGATCCAAGCCATCACTTCCAAACAAGAAGAAATGCA
	Cas12a_ARL6-26-S	Cas12a	AAUCACAUUAUGGGAUUGCUA	TTGCAGCTGGTTTGTAAATATTTGAATCACATTATGGGATGAATTCTGCTAGACAGACTTTCAGTCTTGCTTGGCCTGAAGAAGAAGGAGG
ARMCX1	Hs.Cas9.ARMCX1.1	Cas9	UUGAACUUGCCUCUCCGGAC	TCCCGGATGGTGATCTTTCTCTGTGCCACGATGTCAGTAAGAATTCGATTGGTGCCCACCTATAGGAAAAACCACGATGCAAAAAG
	Cas12a_ARMCX1-47-S	Cas12a	CUUAUAAAAUUGAUGAUAUUC	TCCGGAGAGGCAAGTTCAACTTTCCTTATAAAATTGATGAGAATTCTATTCTGAGTGCTCCCGACCTCCAAAAGGTCCTCAACATCCTGGA
ARR3	Hs.Cas9.ARR3.1	Cas9	AGUACAAUUUGCACCACCGG	TTGGCCTGAAGAAGAAGGAGGTTCATGTTTTGTGCCTTGGGAATTCGCTAGATAATAGTGGCAAAACGACGATCATTAACAAACTT
	Cas12a_ARR3-18-S	Cas12a	CACCACCGGAGGCAGGCCCUG	TGGTTGTCCGGAAAGTACAATTTGCACCACCGGAGGCAGGGAATTCCCCTGGCCCCTCAGCCCAGACCATCCGCCGCTTCCTTCTGTCAGC
ATP1B4	Hs.Cas9.ATP1B4.1	Cas9	GACGGUGGUGCCCAAAUCGG	TTGCTTGGTGACATTTGAAAAGGAACGCAAGGCTCCTGTGGAATTCAGACGAGGTAAAGCCATCTATTATTCAATCAGTGATCCAT
	Cas12a_ATP1B4-79-AS	Cas12a	UCCUCCUCUUCUUCCUCCUCC	TCCTCCTCTTCCTCCTCTTCTTTCTCCTCCTCTTCTTCCTGAATTCCCTCCTCCGATTTGGGCACCACCGTCACCCGAGCCTCTTCTTCTG
BANF1	Hs.Cas9.BANF1.1	Cas9	CCAAAAGCACCGAGACUUCG	CGTGACATCACTCCCCATCTCCAGGCATTGGGATCCCGTGGAATTCGCAGTTGCAGCACTGAAGTGGAGAAGGAGACCCAGGAGAA
	Cas12a_BANF1-58-AS	Cas12a	GGAGGUUGUCAUCUUGAUCAG	TGCCACGAAGTCTCGGTGCTTTTGGGAGGTTGTCATCTTGGAATTCATCAGGCTTAATCTAGGAATCCCAAAAAAAAGGCAGATTAGGGCT
BBOX1	Hs.Cas9.BBOX1.1	Cas9	ACCGGAGCAUCUGACAAACC	GCTGCGCACCGCGCACAGGCCCCGGTAGAGACCGGCCACCGAATTCTGCAGGACCGCCGGTCCCGCGCCACCCGCAGCCGCCACCA
	Cas12a_BBOX1-80-AS	Cas12a	AAACUUCUCCUGGUUUGUCAG	AACCCATCCTTTTCCCAAGTTTTGAAACTTCTCCTGGTTTGAATTCGTCAGATGCTCCGGTGAGTCTTACTATGCCTACTTTCTTGAGGGT
BLOC1S1	Hs.Cas9.BLOC1S1.1	Cas9	GAUCCACCAAAGCUUCUGUC	GAAACCCATCCTTTTCCCAAGTTTTGAAACTTCTCCTGGTGAATTCTTGTCAGATGCTCCGGTGAGTCTTACTATGCCTACTTTCT
	Cas12a_BLOC1S1-32-S	Cas12a	CCCCUCUCCCCAGAAAGAGG	AAGTCTCCCCTCCAATTCCTTTTCCCCCTCTCCCCAGAAAGAATTCAGAGGAGGCGAGAGGCTATCACTGCAGCGACCTGCCTGACAGAAG
BLVRA	Hs.Cas9.BLVRA.1	Cas9	ACACGAAGCCAAUCAGGUUC	GCAAGCCTTCCCAGAAACATGCCGGTGAATCACCAGTTCCGAATTCCCCTGGCCTCATCCATGGACCTTCTGAGCAGCAGGTCCCC
	Cas12a_BLVRA-11-S	Cas12a	GCGUGGUGGUGGUUGGUGUUG	TGTTTCAGCCCGAGAGGAAGTTTGGCGTGGTGGTGGTTGGGAATTCTGTTGGCCGAGCCGGCTCCGTGCGGATGAGGGACTTGCGGAATCC
BOLL	Hs.Cas9.BOLL.1	Cas9	GGUGGGACCGAAGUUACCUC	CCCTTTGGATGCTGGTGAATACTGTGTGCCTAGAGGAAATGAATTCCGTAGGCGGTTCCGCGTTAGGCAGCCCATCCTGCAGTATA
	Cas12a_BOLL-44-AS	Cas12a	AAAAAGGAUUUCCAUCUAUUA	TTGGCCTGTAAGTAATTTCATTTAAAAAAGGATTTCCATCGAATTCTATTACTATCAATTTTTTAATGTTTGTTATATTTCTGAAATATCT
BRAF	Hs.Cas9.BRAF.1	Cas9	GAACUUCUGUACUACAACGC	GGCTCCACCTGCCCCGGGTTTTGGAGAGGATGTGCCCAATGAATTCAGGCTTGTCGATAACATTGATATGATAGATGGAGATGGAG
	Cas12a_BRAF-28-S	Cas12a	GGAUUACUUACUCAAGUUGGU	GGTAGGTAGAAAAGAGATATTTTTGGATTACTTACTCAAGGAATTCTTGGTCATAATTAACACACATCAGTGGAACTTCTGTACTACAACG
BRD8	Hs.Cas9.BRD8.1	Cas9	CCGGAGGUUACCCACUGUGA	CTGGCGGCCGGGGAGCGGCCGTCCGACCCCGGGGAACACCGAATTCCTCAGGCCGAGCCTGGGTCTCTGGCCGAGGGGGCCGGACC
	Cas12a_BRD8-66-S	Cas12a	CUGCUUGACGAGCUAGAACAU	GTAACCTCCGGGGGGGTGTTTTTACTGCTTGACGAGCTAGGAATTCAACATAAAAGAGAACAATGTCCAAATCTTCAAGATTAAATAAATG

BRDT	Hs.Cas9.BRDT.1	Cas9	UUCUCCCUUGAACGUGGUAC	TCCGAGCTGGAGGAGGAGTCACGCCTCCAGGACGCCGACGGAATTCTGTCGGCTGCCTCGTTGCAGTCAGAACTGGCCCACAGCCT
	Cas12a_BRDT-89-AS	Cas12a	AAAACUACAACAAACUUACUU	CCAGTGTTAAAAAGCAATGATTTAAAAACTACAACAAACTGAATTCTACTTGGGCCGCAGTTTGTGAACTGGAGTTGACTGAAGCTCCCTG
BRIX1	Hs.Cas9.BRIX1.1	Cas9	GCAACCAAGAGGAAACGGCG	AGGTCCATACCCCACATTGAGGTGATCCACCAAAGCTTCTGAATTCGTCAGGCAGGTCGCTGCAGTGATAGCCTCTCGCCTCCTCT
	Cas12a_BRIX1-57-AS	Cas12a	CUCUUGGUUGCCGCCAUCUUG	ACTGCAAAGCCTCCACGCCGTTTCCTCTTGGTTGCCGCCAGAATTCTCTTGCCTCGCCGCCCGCGCTCTTGGCCTCCTTTCCGGCGCTGCC
BUD23	Hs.Cas9.BUD23.1	Cas9	GUAGUCUACCACCAUGCCAC	AGATAAGGGGTAACCCCCACCTGCTGGTCAAGTACCATAGGAATTCTGGGTTCTTCGTGGACGGGAAGTTCCTGTGTTGCCAGCAG
	Cas12a_BUD23-54-AS	Cas12a	CUUUGGCACUGUUAGGGUAGU	CGGCCCCCAGCGCTCACTTCTTTGCTTTGGCACTGTTAGGGAATTCGTAGTCTACCACCATGCCACCGGAGAAGCCTGCCTTTGTGGCCTG
C10orf111	Hs.Cas9.C10orf111.1	Cas9	UCGGAGUCGCUGCAAAGUCG	TACTTACCATAATGGTGTTGCTTATTTTCATACTCCAGAGGAATTCGTAACTTCGGTCCCACCGCCTTGGCCTGTAAGTAATTTCA
	Cas12a_C10orf111-8-S	Cas12a	AAAUGUUAUUCGGCCGCUUCA	ACAGATCCAGATTCCTGAGCTTTAAAATGTTATTCGGCCGGAATTCCTTCATCACTTGTCCCCGACTTTGCAGCGACTCCGATGGTAGCCT
C10orf67	Hs.Cas9.C10orf67.1	Cas9	GGGGGACCUUUGGCACACGC	GTGGGCAACGTGCCCTTGGAGTGGTACGATGACTTCCCCCGAATTCACGTGGGCTACGACCTGGATGGCAGGCGCATCTACAAGCC
	Cas12a_C10orf67-62-AS	Cas12a	CUCCUCCUUGAGGGGGACCUU	TATTAGATGGGTTCACTGCTTTTCCTCCTCCTTGAGGGGGGAATTCACCTTTGGCACACGCTGGGAGGCCATGAAAGCCAAGGCCACCGAG
C16orf82	Hs.Cas9.C16orf82.1	Cas9	AUCCUCUGUCCAGAAUGAGC	CTGCACTCCAGATGGATCCCTCCATCCTCATAACCCTTCAGAATTCCTTCGACGTGAAGATTAGAACCCAGTGCTGTGGAAGGATA
	Cas12a_C16orf82-28-S	Cas12a	CUCGAGGGAGAAAAAGGGGAA	CCACTTCAGCTGCCCCCCATTTTTCTCGAGGGAGAAAAAGGAATTCGGGAATCCTCTGTCCAGAATGAGCAGGAAGGAGAGCCAAGCCTAC
C1orf61	Hs.Cas9.C1orf61.1	Cas9	UCUAUCGUCCACACGGAGGA	TCTAAGCTGGGTGACTGTAGGCTTGGCTCTCCTTCCTGCTGAATTCCATTCTGGACAGAGGATTCCCCTTTTTCTCCCTCGAGAAA
	Cas12a_C1orf61-69-S	Cas12a	ACUCACCGCCCGGGAGCUGUC	AAAGGGGGTCAGCACGGCCATTTCACTCACCGCCCGGGAGGAATTCCTGTCCGTGGTGCTGGATGTGGCTCTATCGTCCACACGGAGGAAG
HPRT	Cas12a_38087-AS	Cas12a	AAUUAUGGGGAUUACUAGGA	AAAGACTATGAAATGGAGAGCTAAATTATGGGGATTACTAGAATTCGGAAGGGGCAGCAATGAGTTGACACTACAGACAAGGCACT
	Cas12a_ HPRT 38330-AS	Cas12a	GGUUAAAGAUGGUUAAAUGAUUG	CATTTCACATAAAACTCTTTTAGGTTAAAGATGGTTAAgaattcATGATTGACAAAAAAAGTAATTCACTTACAGTCTGGCTTATA

Supplementary Table 7 Genomic targets used to compare the HDR efficiency mediated by SpCas9 and AsCas12a Ultra in Jurkat cells.

gRNA Target	**Sequence Name**	**Sequence**
B2M	B2M AsCpf1-1	AGTGGGGGTGAATTCAGTGT
B2M	B2M AsCpf1-2	CCGATATTCCTCAGGTACTC
B2M	B2M AsCpf1-3	GAGTACCTGAGGAATATCGG
B2M	B2M AsCpf1-4	CTCACGTCATCCAGCAGAGA
B2M	B2M AsCpf1-5	CATTCTCTGCTGGATGACGT
B2M	B2M AsCpf1-6	ACTTTCCATTCTCTGCTGGA
B2M	B2M AsCpf1-7	CTGAATTGCTATGTGTCTGG
B2M	B2M AsCpf1-8	ATCCATCCGACATTGAAGTT
B2M	B2M AsCpf1-9	AATTCTCTCTCCATTCTTCA
B2M	B2M AsCpf1-10	AGCAAGGACTGGTCTTTCTA
B2M	B2M AsCpf1-11	TATCTCTTGTACTACACTGA
B2M	B2M AsCpf1-12	TCACAGCCCAAGATAGTTAA
BCL11A	BCL11A AsCpf1-1	CCCAGGGGGGCCTCTTTCGG
BCL11A	BCL11A AsCpf1-2	TCACAGGCTCCAGGAAGGGT
BCL11A	BCL11A AsCpf1-3	AAGCTAGTCTAGTGCAAGCT
BCL11A	BCL11A AsCpf1-4	CACTGGAATCAGCTATCTGC
BCL11A	BCL11A AsCpf1-5	AGCCATCTCACTACAGATAA
BCL11A	BCL11A AsCpf1-6	CCCATGGGGCACAGTCAGGC
TGFBR2	TGFBR2 AsCpf1-1	TGATGTGAGATTTTCCACCT
PD1	PD1 AsCpf1-1	GCACGAAGCTCTCCGATGTG
AAVS1	AAVS1 AsCpf1-1	GACCCCTAAGGGAAGAATGA
CIITA	CIITA AsCpf1-1	CCGGCCTTTTTACCTTGGGG
TRAC	TRAC AsCpf1-1	CAGATACGAACCTAAACTTT
TRAC	TRAC AsCpf1-2	GAGTCTCTCAGCTGGTACAC
CISH	CISH AsCpf1-1	ACTGACAGCGTGAACAGGTA
HBB	HBB AsCpf1-1	TATTGGTCTCCTTAAACCTGT
HBB	HBB AsCpf1-2	AGGAGACCAATAGAAACTGGG
HBB	HBB AsCpf1-3	AGGTTGCTAGTGAACACAGTT
HBB	HBB AsCpf1-4	CTTCTGACACAACTGTGTTCA

Supplementary Table 8 List of crRNA targets used in Primary cells.

Assay	**gBlock fragment**
DNMT1	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTAATTTCTACTCTTGTAGATCTGATGGTCCATGTCTGTTACTC
STAT3	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTAATTTCTACTCTTGTAGATCAAAAACAAGCAGCAGGCTGGAT
GCK	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTAATTTCTACTCTTGTAGATGACTCTGGTCCTGCGACCCGGAA
RAG2	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTAATTTCTACTCTTGTAGATTTGTTCTTGCAAACAATAGACAT
HPRT	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTAATTTCTACTCTTGTAGATGGAAAGAGAATTGTTTTCTCCTT
GYS2	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTAATTTCTACTCTTGTAGATTCGTCTTTCTGGGCAGGTATTGT

Supplementary Table 9 gBlock dsDNA fragments used to express Cas12a crRNA for GUIDE-seq in HEK293 cells.

Name	Sequence
pAAV_MND_EGFRCAR_RSQ7375_500HA	cctgcaggcagctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccggcctcagtgagcgagcgagcgcgcagagagggagtggccaactccatcactaggggttcctgcggccgcggttcctcagatctgtaatgccaacataccataaacctcccattctgctaatgcccagcctaagttggggagaccactccagattccaagatgtacagtttgctttgctgggcctttttcccatgcctgcctttactctgccagagttatattgctggggttttgaagaagatcctattaaataaaagaataagcagtattattaagtagccctgcatttcaggtttccttgagtggcaggccaggcctggccgtgaacgttcactgaaatcatggcctcttggccaagattgatagcttgtgcctgtccctgagtcccagtccatcacgagcagctggtttctaagatgctatttcccgtataaagcatgagaccgtgacttgccagccccacagagccccgcccttgtccatcactggcatctggactccagcctgggttggggcaaagagggaaatgagatcatgtcctaaccctgatcctcttgtcccacagatatccagaaccctgacccaatgaaagaccccacctgtaggtttggcaagctaggatcaaggttaggaacagagagacagcagaatatgggccaaacaggatatctgtggtaagcagttcctgccccggctcagggccaagaacagttggaacagcagaatatgggccaaacaggatatctgtggtaagcagttcctgccccggctcagggccaagaacagatggtccccagatgcggtcccgccctcagcagtttctagagaaccatcagatgtttccagggtgccccaaggacctgaaatgaccctgtgccttatttgaactaaccaatcagttcgcttctcgcttctgttcgcgcgcttctgctccccgagctcaataaaagagcccacaacccctcactcggcccggtcgccaccatggcactccccgtcaccgcccttctcttgcccctcgccctgctgctgcatgctgccaggcccatggacgaagtgcagctcgtggagtccggtggaggactcgtccaaccgggcggatcccttcgcttgtcctgcgccgcatcaggcttcagcttcaccaactatggcgtccactgggtcagacaggcccccggaaagggactggaatgggtgtccgtgatctggagcggcgggaacaccgactacaacacctccgtgaagggccggttcactattagccgcgacaactccaagaacactctgtacctccaaatgaactccctgagggccgaagatactgctgtgtactattgcgcgagagccctgacctactacgactacgagttcgcgtactggggccaggggactctcgtgaccgtgtccagcggtggtggaggttccggaggcggaggttctggtggcgggggatcagaaatcgtgctgactcagtcccctgcgaccttgtccctgagccctggagaacgggccaccctgagctgtagagccagccagagcatcgggacaaatattcactggtaccagcagaaacccggacaagcaccacggctgctgatctactacgcctccgagtcgatttccggaatcccggctcgcttttcggggtctggatcgggaacggacttcactctgaccatctcgtcgctggaacccgaggatttcgccgtgtactactgccaacagaacaacaattggccgaccacgttcggccagggcaccaagctcgagattaagggatcactggaagcggccgcaaccacaacacctgctccaaggccccccacacccgctccaactatagccagccaaccattgagcctcagacctgaagcttgcaggcccgcagcaggaggcgccgtccatacgcgaggcctggacttcgcgtgtgatatttatatttgggcccctttggccggaacatgtggggtgttgcttctctcccttgtgatcactctgtattgtaagcgcgggagaaagaagctcctgtacatcttcaagcagccttttatgcgacctgtgcaaaccactcaggaagaagatgggtgttcatgccgcttccccgaggaggaagaaggagggtgtgaactgagggtgaaattttctagaagcgccgatgctcccgcatatcagcagggtcagaatcagctctacaatgaattgaatctcggcaggcgagaagagtacgatgttctggacaagagacggggcagggatcccgagatgggggggaaagccccggagaaaaaatcctcaggagggggttgtacaatgagctgcagaaggacaagatggctgaagcctatagcgagatcggaatgaaaggcgaaagacgcagaggcaaggggcatgacggtctgtaccagggtctctctacagccaccaaggacacttatgatgcgttgcatatgcaagccttgccaccccgctaaagcggccgcgtcgagtctagagggcccgtttaaacccgctgatcagcctcgactgtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgtcattctattctggggggtggggtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgctggggatgcggtgggctctatggtgccgtgtaccagctgagagactcttaatccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaaatgtgtcacaaagtaaggattctgatgtgtatatcacagacaaaactgtgctagacatgaggtctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttgcatgtgcaaacgccttcaacaacagcattattccagaagacaccttcttccccagcccaggtaagggcagctttggtgccttcgcaggctgtttccttgcttcaggaatggccaggttctgcccagagctctggtcaatgatgtctaaaactcctctgattggtggtctcggccttatccattgccaccaaaaccctctttttactaagaaacagtgagccttgttctggcagtccagagaatgacacgggaaaaaagcagatgaagagaaggtggcaggagagggcacgtggcccagcctcagtcctggggagagatctgcggccgcaggaacccctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccgacgcccgggctttgcccgggcggcctcagtgagcgagcgagcgcgcagctgcctgcaggggcgcctgatgcggtattttctccttacgcatctgtgcggtatttcacaccgcatacgtcaaagcaaccatagtacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccggctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcgaccccaaaaaacttgatttgggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacactcaaccctatctcgggctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatttaacaaaaatttaacgcgaattttaacaaaatattaacgtttacaattttatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagccccgacacccgccaacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgagacgaaagggcctcgtgatacgcctatttttataggttaatgtcatgataataatggtttcttagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtatgagccatattcaacgggaaacgtcttgctctaggccgcgattaaattccaacatggatgctgatttatatgggtataaatgggctcgcgataatgtcgggcaatcaggtgcgacaatctatcgattgtatgggaagcccgatgcgccagagttgtttctgaaacatggcaaaggtagcgttgccaatgatgttacagatgagatggtcagactaaactggctgacggaatttatgcctcttccgaccatcaagcattttatccgtactcctgatgatgcatggttactcaccactgcgatccctgggaaaacagcattccaggtattagaagaatatcctgattcaggtgaaaatattgttgatgcgctggcagtgttcctgcgccggttgcattcgattcctgtttgtaattgtccttttaacagcgatcgcgtatttcgtctcgctcaggcgcaatcacgaatgaataacggtttggttgatgcgagtgattttgatgacgagcgtaatggctggcctgttgaacaagtctggaaagaaatgcataaacttttgccattctcaccggattcagtcgtcactcatggtgatttctcacttgataaccttatttttgacgaggggaaattaataggttgtattgatgttggacgagtcggaatcgcagaccgataccaggatcttgccatcctatggaactgcctcggtgagttttctccttcattacagaaacggctttttcaaaaatatggtattgataatcctgatatgaataaattgcagtttcatttgatgctcgatgagtttttctaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcctttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatc

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tccgatttagtgctttacggcacctcgaccccaaaaaacttgatttgggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgttc
tttaatagtggactcttgttccaaactggaacaacactcaaccctatctcgggctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgattta
acaaaaatttaacgcgaattttaacaaaatattaacgtttacaattttatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagccccgacacccgccaacacccg
ctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgagacga
aagggcctcgtgatacgcctatttttataggttaatgtcatgataataatggtttcttagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttcta
aatacattcaaatatgtatccgctcatgagacaataaccctgat

Supplementary Table 10 Plasmid sequences used to generate AAV6 as donor template.

Name	**Sequence**
WT AsCas12a	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGGSLEHHHHHH
AsCas12 Ultra	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGGSLEHHHHHH
WT LbCas12a	MSKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLVEDEKRAEDYKGVKKLLDRYYLSFINDVLHSIKLKNLNNYISLFRKKTRTEKENKELENLEINLRKEIAKAFKGNEGYKSLFKKDIIETILPEFLDDKDEIALVNSFNGFTTAFTGFFDNRENMFSEEAKSTSIAFRCINENLTRYISNMDIFEKVDAIFDKHEVQEIKEKILNSDYDVEDFFEGEFFNFVLTQEGIDVYNAIIGGFVTESGEKIKGLNEYINLYNQKTKQKLPKFKPLYKQVLSDRESLSFYGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKLEKLFKNFDEYSSAGIFVKNGPAISTISKDIFGEWNVIRDKWNAEYDDIHLKKKAVVTEKYEDDRRKSFKKIGSFSLEQLQEYADADLSVVEKLKEIIIQKVDEIYKVYGSSEKLFDADFVLEKSLKKNDAVVAIMKDLLDSVKSFENYIKAFFGEGKETNRDESFYGDFVLAYDILLKVDHIYDAIRNYVTQKPYSKDKFKLYFQNPQFMGGWDKDKETDYRATILRYGSKYYLAIMDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFSKKWMAYYNPSEDIQKIYKNGTFKKGDMFNLNDCHKLIDFFKDSISRYPKWSNAYDFNFSETEKYKDIAGFYREVEEQGYKVSFESASKKEVDKLVEEGKLYMFQIYNKDFSDKSHGTPNLHTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVVHPANSPIANKNPDNPKKTTTLSYDVYKDKRFSEDQYELHIPIAINKCPKNIFKINTEVRVLLKHDDNPYVIGIDRGERNLLYIVVVDGKGNIVEQYSLNEIINNFNGIRIKTDYHSLLDKKEKERFEARQNWTSIENIKELKAGYISQVVHKICELVEKYDAVIALEDLNSGFKNSRVKVEKQVYQKFEKMLIDKLNYMVDKKSNPCATGGALKGYQITNKFESFKSMSTQNGFIFYIPAWLTSKIDPSTGFVNLLKTKYTSIADSKKFISSFDRIMYVPEEDLFEFALDYKNFSRTDADYIKKWKLYSYGNRIRIFRNPKKNNVFDWEEVCLTSAYKELFNKYGINYQQGDIRALLCEQSDKAFYSSFMALMSLMLQMRNSITGRTDVDFLISPVKNSDGIFYDSRNYEAQENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKVKIAISNKEWLEYAQTSVKHGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGGSLEHHHHHH
LbCas12a Ultra	MSKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLVEDEKRAEDYKGVKKLLDRYYLSFINDVLHSIKLKNLNNYISLFRKKTRTEKENKELENLEINLRKEIAKAFKGNEGYKSLFKKDIIETILPEFLDDKDEIALVNSFNGFTTAFTGFFDNRENMFSEEAKSTSIAFRCINENLTRYISNMDIFEKVDAIFDKHEVQEIKEKILNSDYDVEDFFEGEFFNFVLTQEGIDVYNAIIGGFVTESGEKIKGLNEYINLYNQKTKQKLPKFKPLYKQVLSDRESLSFYGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKLEKLFKNFDEYSSAGIFVKNGPAISTISKDIFGEWNVIRDKWNAEYDDIHLKKKAVVTEKYEDDRRKSFKKIGSFSLEQLQEYADADLSVVEKLKEIIIQKVDEIYKVYGSSEKLFDADFVLEKSLKKNDAVVAIMKDLLDSVKSFENYIKAFFGEGKETNRDESFYGDFVLAYDILLKVDHIYDAIRNYVTQKPYSKDKFKLYFQNPQFMGGWDKDKETDYRATILRYGSKYYLAIMDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFSKKWMAYYNPSEDIQKIYKNGTFKKGDMFNLNDCHKLIDFFKDSISRYPKWSNAYDFNFSETEKYKDIAGFYREVEEQGYKVSFESASKKEVDKLVEEGKLYMFQIYNKDFSDKSHGTPNLHTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVVHPANSPIANKNPDNPKKTTTLSYDVYKDKRFSEDQYLLHIPIAINKCPKNIFKINTEVRVLLKHDDNPYVIGIDRGERNLLYIVVVDGKGNIVEQYSLNEIINNFNGIRIKTDYHSLLDKKEKERFEARQNWTSIENIKELKAGYISQVVHKICELVEKYDAVIALEDLNSGFKNSRVKVEKQVYQKFEKMLIDKLNYMVDKKSNPCATGGALKGYQITNKFESFKSMSTQNGFIFYIPAWLTSKIDPSTGFVNLLKTKYTSIADSKKFISSFDRIMYVPEEDLFEFALDYKNFSRTDADYIKKWKLYSYGNRIRIFRNPKKNNVFDWEEVCLTSAYKELFNKYGINYQQGDIRALLCEQSDKAFYSSFMALMSLMLQMRNSITGRTDVDFLISPVKNSDGIFYDSRNYEAQENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKVKIAISNKEWLEYAQTSVKHGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGGSLEHHHHHH
enCas12a	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYRNRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLARGWDVNREKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTP

	ILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDF AKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPI TLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFK SKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSF QRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFD SRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGGSLEHHHHHH
enCas 12a Ultra	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRH AEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYRNRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLL TQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETIS SALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARL TGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQRPTLARGWDVNREKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTP ILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDF AKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFLFHVPI TLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFK SKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSF QRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFD SRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGGSLEHHHHHH
enCas 12a*	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRH AEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYRNRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLL TQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETIS SALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARL TGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLARGWDVNREKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTP ILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDF AKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPI TLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFK SKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSF QRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFD SRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNKRPAATKKAGQAKKKKGAAALEHHHHHH
enCas 12a-Hifi*	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRH AEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYRNRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLL TQTQIDLYNQLLGGISREAGTEKIKGLNEVLALAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETIS SALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARL TGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLARGWDVNREKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTP ILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDF AKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPI TLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFK SKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSF QRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFD SRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNKRPAATKKAGQAKKKKGAAALEHHHHHH

***: enCas12a and enCas12a-Hifi with published NLS sequence (Kleinstiver BP., et al., 2019)**

Supplementary Table 11 AsCas12a protein sequences used in this study.