

Supplementary Information

Supplementary Tables

Table S1. The engineering of the CRISPR-AsCas12a/LbCas12a systems

Cas12a type	Engineering	Efficiency	Specificity	Note	Targeting Ranges
AsCas12a; <i>LbCas12a</i>	Cas-protein	↓	↑	(2017) <i>AsCas12a</i> -RR/A (S542R, K607R/K949A); <i>AsCas12a</i> -RVR/A (S542R, K548V, N552R/K949A); <i>LbCas12a</i> -RR (G532R, K595R); <i>LbCas12a</i> -RVR (G532R, K538V, Y542R)[26]	↑
<i>LbCas12a</i>	Cas-protein	--	--	(2018, 2019) <i>LbCas12a</i> -RR(G532R, K595R); -RVR (G532R, K538V, Y542R) (tested in Plant)[41-43]	↑
AsCas12a; <i>LbCas12a</i>	Cas-protein	--	↑	(2019) Synthetic chimeric Cas12a[44]	--
AsCas12a	Cas-protein	↑	↓	(2019) en <i>AsCas12a</i> (E174R, S542R, K548R); en <i>AsCas12a</i> -HF1 (N282A, E174R, S542R, K548R)[27]	↑
<i>LbCas12a</i>	Cas-protein	↑	↓	(2020) <i>LbCas12a</i> -RRVR (G532R, K538V, Y542R, K595R); imp <i>LbCas12a</i> (D156R, G532R, K538V, Y542R, K595R)[45]	↑
<i>LbCas12a</i>	Cas-protein	↑	Unknown	(2020) en <i>LbCas12a</i> (D156R, G532R, K538R); tt <i>LbCas12a</i> (D156R), (tested in Plant)[46] (2020) <i>LbCas12a</i> (D156R), (tested in Drosophila)[47]	↑
d <i>LbCas12a</i>	Cas-protein	--	--	(2020) N256A, N260A, S348A, K514A, K881A, K897A[48]	Unknown
<i>LbCas12a</i>	Cas-protein	--	Unknown	(2020) <i>LbCas12a</i> -RSR (G532R/K538S/Y548R); <i>LbCas12a</i> -RAR (G532R/K538A/Y548R)[49]	↑
AsCas12a	Cas-protein	↑	↓	(2021) <i>AsCas12a</i> ultra (M537R/F870L)[52]	↑
<i>LbCas12a</i> ; AsCas12a; <i>Lb2Cas12a</i>	Cas-protein	--	↑	(2022) Lb-K538R; As-K548R; Lb2-K548R[53]	--
AsCas12a	Cas-protein sgRNA	↑	Unknown ↓	(2020) op <i>AsCas12a</i> (E174R, S542R)[50]; (2020) open <i>AsCas12a</i> (E174R, S542R, K548R)[51]	Unknown Unknown
AsCas12a; <i>LbCas12a</i>	sgRNA	↑	--	(2017) sgRNA with chemical modifications[10]	Unknown
<i>LbCas12a</i>	sgRNA	--	--	(2017) A fusion sgRNA for both Cas9 and Cas12a[11]	Unknown
<i>LbCas12a</i>	sgRNA	↑	Unknown	(2018) tRNA-sgRNA intron[12]	Unknown
AsCas12a; <i>LbCas12a</i>	sgRNA	↑	Unknown	(2018) ribozyme-processed crRNA[9]	Unknown
<i>LbCas12a</i>	sgRNA	--	Unknown	(2018) using short and long spacer crRNA[16]	Unknown
<i>LbCas12a</i>	sgRNA	↑	Unknown	(2018) truncated pre-tRNA-like sgRNA[7]	Unknown
AsCas12a	sgRNA	↑	Unknown	(2018) Extended sgRNA[8]	Unknown

AsCas12a; <i>LbCas12a</i>	sgRNA	↑	--	(2018) A U-rich crRNA sgRNA[6]	Unknown
<i>LbCas12a</i> ; <i>FnoCas12a</i>	sgRNA	↑	--	(2019) Full-DR-sgRNA[13]	Unknown
AsCas12a	sgRNA	--	↑	(2020) Chimeric DNA-RNA sgRNA[14]	Unknown
AsCas12a; <i>LbCas12a</i>	sgRNA and Cas-protein	↑	--	(2021) Chemical modification[15]	Unknown
AsCas12a; <i>LbCas12a</i>	Cas-protein	↑	↑	This study. AsCas12a-Plus (E174R, R951K, R955A); <i>LbCas12a</i> -Plus (D156R, R883K, R887A)	↑

Note: Unless special stated, the comparison is performed with wild-type Cas-nuclease. "--", Comparable. "↑", Enhance/Improve. "↓", Decrease. "Unknow", untested in the study.

Table S2. The engineering of the CRISPR-Cas12a (excluded AsCas12a and *LbCas12a*) systems

Cas12a type	Engineering	Efficiency	Specificity	Note	Targeting Ranges
<i>FnCas12a</i>	Cas-protein	--	Unknown	(2018) <i>FnCas12a</i> -RVR(N623R, K629R and N633R) <i>FnCas12a</i> -RR(N623R, K687R) (tested in Plant)[42]	↑
<i>FnCas12a</i>	Cas-protein	↑	--	(2021) ea <i>FnCas12a</i> (Q125R)[54]	Unknown
<i>MbCas12a</i> ; <i>FnCas12a</i>	Cas-protein	--	Unknown	(2018) <i>MbCas12a</i> -RR (N576R, K637R); <i>MbCas12a</i> -RVR (N576R, K582V, N586R); <i>FnCas12a</i> -RR (N607R, K671R); <i>FnCas12a</i> -RVR (N607R, K613V, N617R)[55]	↑
<i>AaCas12a</i>	--	--	↑	(2018) Specificity improved compared to AsCas12a and <i>SpCas9</i> [56]	Unknown
<i>CeCas12a</i>	Cas-protein	↑	--	(2020) Activity improved compared to AsCas12a and <i>LbCas12a</i> [57]	↑
<i>PiCas12a</i>	Cas-protein	↑	Unknown	(2020) <i>PiCas12a</i> (F604Y)[58]	--
<i>FnCas12a</i>	Cas-protein	↑	--	(2020) T5 Exonuclease Fusion to <i>FnCas12a</i> [59]	Unknown
<i>Lb2Cas12a</i>	Cas-protein	↑	--	(2021) Lb2-KY (Q571K, C1003Y)[60]	--
<i>BsCas12a</i> ; <i>PrCas12a</i> ; <i>Mb3Cas12a</i>	Cas-protein	↑	Unknown	(2021) <i>BsCas12a</i> -3Rv (K155R/N512R/K518R); <i>PrCas12a</i> -3Rv (E162R/N519R/K525R); <i>Mb3Cas12a</i> -3Rv (D180R/N581R/K587R)[61]	↑
<i>Mb2Cas12a</i> ; <i>ErCas12a</i> ; <i>Lb5Cas12a</i> ; <i>BsCas12a</i>	Cas-protein	↑	Unknown	(2021) <i>Mb2Cas12a</i> -RVR(N563R, K569V, N573R); <i>Mb2Cas12a</i> -RVRR(N563R, K569V, N573R, K625R) <i>ErCas12a</i> -RVR(D529R, K535V, N539R); <i>Lb5Cas12a</i> -RVR(N512R, K518V, N522R); <i>BsCas12a</i> -RVR(N512R, K518V, N522R)[62]	↑

<i>PrCas12a</i> ; <i>ArCas12a</i> ; <i>BsCas12a</i> ; <i>HkCas12a</i> ; <i>LpCas12a</i> ; <i>PxCas12a</i>	sgRNA	↑	--	(2019) optimized crRNA scaffolds[63]	--
<i>TsCas12a</i> ; <i>MICas12a</i> ; <i>BoCas12a</i> ; <i>CMaCas12a</i>	others	--	--	(2021) The wild-type nuclease, tested in the plant[62]	--

Note: Unless special stated, the comparison is performed with the corresponding wild-type Cas-nuclease. "--", Comparable. "↑", Enhance/Improve/Widen. "↓", Decrease. "Unknow", untested in the study.

Table S3. The sgRNAs used in this study

Name	Sequence(5'-3')	location (hg19)
Site 6	TTTNGGGTGATCAGACCCAACAGCAGG	chr3 - 129108493 129108519
CCR5-S1	TTTNTGGGCAACATGCTGGTCATCCTC	chr3 + 46414526 46414552
CCR5-S2	TTTNAAGCAAACACAGCATGGACGACA	chr3 - 46414780 46414806
CCR5-S3	TTTNGGATTCCCAGTAGCAGATGACC	chr3 - 46415023 46415049
CD47-S1	TTTNAGAAATGAGAGAGAAACAAGATG	chr3 + 107805419 107805445
CD47-S2	TTTNATAAAACAAAATCTGTAGAATTC	chr3 - 107799148 107799174
CD47-S3	TTTNTCTCCCCTGCTTCCCACAATAA	chr3 - 107805249 107805275
CD47-S4	TTTNAAAACAGAAAGAACAATACTCATA	chr3 + 107798052 107798078
SIRPa-S1	TTTNAAAAGAGAGAGAGAGGGGGGAAA	chr20 - 1879740 1879766
SIRPa-S2	TTTNTACAGTTACAACATTCCTCAACTG	chr20 + 1902671 1902697
SIRPa-S3	TTTNTGTCCGGGACACGTTTCCATTCT	chr20 - 1903070 1903096
SIRPa-S4	TTTNACCACTAAGCAGGCATCATTAAAC	chr20 - 1905314 1905340
CXCR4-S1	TTTNAAGTCACATCTTGCTAACTCC	chr2 + 136873755 136873781
CXCR4-S2	TTTNCCACGGCATCAACTGCCAGAAG	chr2 + 136873196 136873222
PD1-S1	TTTNCCCAGAATCTCCAGTTCTGAGTC	chr2 + 242799735 242799761
PD1-S2	TTTNAGGCCAGCCAGCACTCTGGCCT	chr2 - 242792483 242792509
CTLA4-S1	TTTNTATGAAGTAGGTAACAAGAT	chr2 + 204733604 204733630
RUNX1	TTTNGCTCCGAAGGTAAGAAATCAT	chr21 - 36421242 36421268
RUNX1-S1	TTTNTGTCTCTGCCCCCTCCCCACT	chr21 + 37371826 37371852
DNMT1-S3	TTTNTGATGGTCCATGTCTGTACTC	chr19 - 10244443 10244469
DNMT1-S7	TTTNGCTCAGCAGGCACCTGCCTCAGC	chr19 - 10133918 10133944
EMX1	TTTNTCCTCCGGTCTGGAACCACACC	chr2 - 73160921 73160947
MYOD-S1	TTTNGGCTACTACGGATAAATAGCCCA	chr11 + 17741072 17741098
MYOD-S2	TTTNCCGCGGATACAGCAGTCGGGTGT	chr11 + 17740805 17740831
IL1RN-S1	TTTNCAGGAGGGTGACTCAGGCTAGCA	chr2 - 113875317 113875343
IL1RN-S3	TTTNTTAGAGCGTTGGGGACCTTGCT	chr2 + 113875106 113875132
HBG-S4	TTTNAACTACAGGCCTCACTGGAGCTA	chr11 - 5271453 5271479/ chr11 - 5276381 5276407
HBG-S5	TTTNCCTTGTCAGGCTATTGGTCAAG	chr11 + 5271181 5271207/ chr11 + 5276105 5276131
FANCF	TTTNGTCGGCATGGCCCCATTCGCACG	chr11 - 22647189 22647215

VEGFA-S1	TTTNCTGGAAAGAGCAGAAGAAAAAGG	chr6 - 43742342 43742368
BE-S3	TTTNTCCGTGTTCTTACTCTGGGCA	chr11 + 22647490 22647516
Site 16	TTTNGAAGCACATCAAGGACATTCTAA	chr21 + 38860226 38860252
Site 18	TTTNGTTTTAAACACACCGGGTTAATAA	chr9 - 110247058 110247084
NPY1R	TTTNAAGCCTCGGGAAACTGCCCTGCC	chr4 - 164254002 164254028
CHN1	TTTNAGCTAAAACGCACACCGGCCCG	chr2 - 175870275 175870301
SNX13	TTTNTCCTCTCCCAGGGCGGGCCAAC	chr7 - 17980383 17980409
Cas12a-BRAF-WT	TTTNGTCTAGCTACAGTGAAATCTCGA	chr7 - 140453126 140453152
Cas12a-BRAF-Mut	TTTNGTCTAGCTACAGAGAAATCTCGA	chr7 - 140453126 140453152
Cas9-BRAF-WT	TAGCTACAGTGAAATCTCGANGG	chr7 - 140453123 140453145
Cas9-BRAF-Mut	TAGCTACAGAGAAATCTCGANGG	chr7 - 140453123 140453145
EGFP-g1	TTTNCGTGCGCGTCCAGCTCGACCAGG	
EGFP-g2	TTTNCTCAGGGCGGACTGGGTGCTCAG	
mNeonGreen-g1	TTTNCAGGCCGCTATGGTGGATGGCAG	
mNeonGreen-g2	TTTNGCGAAGGTGTAGGTGGTCCGGGC	
Activation sgRNA		
IL1RN-23bp	TTTNCAGGAGGGTGACTCAGGCTAGCA	chr2 - 113875317 113875343
MYOD-23bp	TTTNC CGG GATACAGCAGTCGGGTGT	chr11 + 17740805 17740831
HBG-23bp	TTTNCCTTGTC AAGGCTATTGGTCAAG	chr11 + 5271181 5271207
Fgf21-23bp	TTTNTACCCAGACAGGCCCGCCACGT	chr7 + 45615513 45615539
IL1RN-15bp	TTTNCAGGAGGGTGACTCA	chr2 - 113875325 113875343
MYOD-15bp	TTTNC CGG GATACAGCAG	chr11 + 17740805 17740823
Non-canonical-PAM sgRNA		
ATTA	<u>A</u> TTAGAAGGAGATGCTCCTGTCTCCTG	chr7 + 117232231 117232257
CTTA	<u>C</u> TTATTGGGTCAGCTGTTAACATCAGT	chr19 - 10244479 10244505
GTTA	<u>G</u> TTATGAACCTGGGTGAAGTCCCAACA	chr2 - 73149328 73149354
TTTA	<u>T</u> TTAGGTCATCTGCTACTCGGGAATCC	chr3 + 46399672 46399694
TCTA	<u>T</u> CTATAGCCATTGAAGCGCAGCATGTG	chr11 + 22646901 22646927

Table S4. Primers for this study (including qPCR, T7E1/PAGE, Deep-seq)

Name	Sequence(5'-3')
qPCR-IL1RN-F	GGAATCCATGGAGGGAAGAT
qPCR-IL1RN-R	TGTTCTCGCTCAGGTCAGTG
qPCR-MYOD-F	CCGACGGCATGATGGACTAC
qPCR-MYOD-R	AGGCAGTCTAGGCTCGACAC
qPCR-HBG-F	GCTGAGTGAAGTGCAGTGTGA
qPCR-HBG-R	GAATTCCTTGCCGAAATGGA
qPCR-Fgf21-F	CAAGACACTGAAGCCACCT
qPCR-Fgf21-R	CACCCAGGATTTGAATGACC
qPCR-GAPDH-F	AGAAGGCTGGGGCTCATTG
qPCR-GAPDH-R	AGGGGCCATCCACAGTCTTC
Gapdh-qPCR-F1	GGTGAAGGTCGGTGTGAAC
Gapdh-qPCR-R1	GAGTGGAGTCATACTGGAAC
PAGE/T7E1-DMNT1-site3-F	ACGCCTGCTCTAAGCACTTG

PAGE/T7E1-DMNT1-site3-R	TAGTTCTGCCCTCCCGTCAC
DMNT1-site3-Deep-Seq-F	atcgCTCTTTCCCTACACGACGCTCTTCCGATCTIndex1GTCACCTCTGGGGAACACGC
DMNT1-site3-D Deep-Seq-R	ctctACTGGAGTTCAGACGTGTGCTCTTCCGATCTIndex2CCGTGAACGTTCCCTTAGCA
Site6-Deep-Seq-F	atcgCTCTTTCCCTACACGACGCTCTTCCGATCTIndex1CCACATCCTCACCACCTGTTT
Site6-Deep-Seq-R	ctctACTGGAGTTCAGACGTGTGCTCTTCCGATCTIndex2GGACAGGGTGGGTACCTTG
FNACF-Deep-Seq-F	atcgCTCTTTCCCTACACGACGCTCTTCCGATCTIndex1TGCCCTCCACTGGTTGTGCAG
FANCF-Deep-Seq-R	ctctACTGGAGTTCAGACGTGTGCTCTTCCGATCTIndex2CTTCTGGCGGTCTCAAGCA
MYOD-Deep-Seq-F	atcgCTCTTTCCCTACACGACGCTCTTCCGATCTIndex1CTACAGCCGCTCTACCCAT
MYOD-Deep-Seq-R	ctctACTGGAGTTCAGACGTGTGCTCTTCCGATCTIndex2TGCACTTGGCTCTCCGGCA
IL1RN-Deep-Seq-F	atcgCTCTTTCCCTACACGACGCTCTTCCGATCTIndex1TGACCTCCCATCTTACGCAG
IL1RN-Deep-Seq-R	ctctACTGGAGTTCAGACGTGTGCTCTTCCGATCTIndex2AGAGTACAAGGAGGAGCCCA

Table S5. Index information for deep-seq

Genes	sample	Index1	Index2	
DNMT1-site3	AsCas12a-WT-On	AGAGTAGA	TAAGGCGA	
	AsCas12a-WT-mm1	AGAGTAGA	CTAGTACG	
	AsCas12a-WT-mm8	AGAGTAGA	TTCTGCCT	
	AsCas12a-WT-mm9	AGAGTAGA	GCTCAGGA	
	AsCas12a-A-On	TATCCTCT	TAAGGCGA	
	AsCas12a-A-mm1	TATCCTCT	CTAGTACG	
	AsCas12a-A-mm8	TATCCTCT	TTCTGCCT	
	AsCas12a-A-mm9	TATCCTCT	GCTCAGGA	
	AsCas12a-AKK-On	TAGATCGC	TAAGGCGA	
	AsCas12a-AKK-mm1	TAGATCGC	CTAGTACG	
	AsCas12a-AKK-mm8	TAGATCGC	TTCTGCCT	
	AsCas12a-AKK-mm9	TAGATCGC	GCTCAGGA	
	AsCas12a-AKA-On	CTCTCTAT	TAAGGCGA	
	AsCas12a-AKA-mm1	CTCTCTAT	CTAGTACG	
	AsCas12a-AKA-mm8	CTCTCTAT	TTCTGCCT	
	AsCas12a-AKA-mm9	CTCTCTAT	GCTCAGGA	
	AsCas12a-KK-On	GTAAGGAG	TAAGGCGA	
	AsCas12a-KK-mm1	GTAAGGAG	CTAGTACG	
	AsCas12a-KK-mm8	GTAAGGAG	TTCTGCCT	
	AsCas12a-KK-mm9	GTAAGGAG	GCTCAGGA	
	AsCas12a-KA-On	ACTGCATA	TAAGGCGA	
	AsCas12a-KA-mm1	ACTGCATA	CTAGTACG	
	AsCas12a-KA-mm8	ACTGCATA	TTCTGCCT	
	AsCas12a-KA-mm9	ACTGCATA	GCTCAGGA	
		WT-crRNA (repeat 1)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF	GACATT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AGCGTA AGCGTA AGCGTA AGCGTA AGCGTA AGCGTA

Site6		Ctrl	AAGGAG	AGCGTA
	WT-crRNA (repeat 2)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	GACATT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	GCTCAG GCTCAG GCTCAG GCTCAG GCTCAG GCTCAG GCTCAG
	U4AU6- crRNA (repeat 1)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	GACATT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	TCGCCT TCGCCT TCGCCT TCGCCT TCGCCT TCGCCT TCGCCT
	U4AU6- crRNA (repeat 2)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	GACATT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	AGGAGT AGGAGT AGGAGT AGGAGT AGGAGT AGGAGT AGGAGT
	tRNA-crRNA (repeat 1)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	GACATT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	CTAGTA CTAGTA CTAGTA CTAGTA CTAGTA CTAGTA CTAGTA
	tRNA-crRNA (repeat 2)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	GACATT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	CATGCC CATGCC CATGCC CATGCC CATGCC CATGCC CATGCC
	WT-crRNA (repeat 1)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	TATCCT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	TTCTGC TTCTGC TTCTGC TTCTGC TTCTGC TTCTGC TTCTGC
WT-crRNA (repeat 2)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF	TATCCT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC	GCTCAG GCTCAG GCTCAG GCTCAG GCTCAG GCTCAG	

FANCF		Ctrl	AAGGAG	GCTCAG	
	U4AU6-crRNA (repeat 1)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	TATCCT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	TCGCCT TCGCCT TCGCCT TCGCCT TCGCCT TCGCCT TCGCCT	
	U4AU6-crRNA (repeat 2)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	TATCCT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	AGGAGT AGGAGT AGGAGT AGGAGT AGGAGT AGGAGT AGGAGT	
	tRNA-crRNA (repeat 1)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	TATCCT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	CTAGTA CTAGTA CTAGTA CTAGTA CTAGTA CTAGTA CTAGTA	
	tRNA-crRNA (repeat 2)	AsCas12a-WT AsCas12a-KA AsCas12a-KK AsCas12a-RKA AsCas12a-RKK AsCas12a-HF Ctrl	TATCCT GTAAGG ACTGCA CTCTCT AGAGTA TAGATC AAGGAG	CATGCC CATGCC CATGCC CATGCC CATGCC CATGCC CATGCC	
	MYOD (23bp and 15 bp)	Repeat 1	WT-VPR RKA-VPR HF-VPR Ctrl	GACATT TAGATC CTCTCT AGAGTA	AGCGTA AGCGTA AGCGTA AGCGTA
		Repeat 2	WT-VPR RKA-VPR HF-VPR Ctrl	GACATT TAGATC CTCTCT AGAGTA	TCGCCT TCGCCT TCGCCT TCGCCT
		Repeat 3	WT-VPR RKA-VPR HF-VPR Ctrl	GACATT TAGATC CTCTCT AGAGTA	CTAGTA CTAGTA CTAGTA CTAGTA
IL1RN (23bp and 15 bp)	Repeat 1	WT-VPR RKA-VPR HF-VPR Ctrl	GACATT TAGATC CTCTCT AGAGTA	AGCGTA AGCGTA AGCGTA AGCGTA	
	Repeat 2	WT-VPR RKA-VPR HF-VPR Ctrl	GACATT TAGATC CTCTCT AGAGTA	TCGCCT TCGCCT TCGCCT TCGCCT	

	Repeat 3	WT-VPR RKA-VPR HF-VPR Ctrl	GACATT TAGATC CTCTCT AGAGTA	CTAGTA CTAGTA CTAGTA CTAGTA
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Supplementary sequences: DNA and Amino acid sequences used in this study

- Amplicons for high-throughput sequencing analysis

DMNT1-site3:

GTCACTCTGGGGAACACGCCCGGTGTACGCCACTTGACAGGC **GAGTAACAGACATGGACCATCAG** GAAA
CATTAACTACTGATGTTAACAGCTGACCCAATAAGTGGCAGAGTGCTAAGGGAACGTTACGG

FANCF:

TGCCTCCACTGGTTGTGCAGCCGCGCTCCAGAGC **CGTGCGAATGGGGCCATGCCGACC** CAAAGCGCCGAT
GGATGTGGCGCAGGTAGCGCGCCCACTGCAAGGCCCGGCGCACGGTGGCGGGTCCCAGGTGCTGACG
TAGGTAGTGCTTGAGACCGCCAGAAG

Site 6:

CCACATCCTCACCACCTGTTTGTGACGCCCTCCACACTTGATAGCCCCCTGGTCCCATTTTGTCTCTCAA
CTGTCTTTCTCATTCTGGACTCCACTGGACTTCGTCGCCCCCATGA **CCTGCTGTTGGGTCTGATCACCCC**
AAAAGAAATGAGGTTTGAGTATGTTAGAGTATGCCAAGGTAACCCACCCTGTCC

MYOD:

CTACAGCCGCTCTACCCATGGGTCCCCACAAATCAGGGGACAGAGGAGTATTGAAAGTCAGCTCAGAGGT
GAGCGCGCGCAGCCAGCG TTTC **CCGCGGATACAGCAGTCGGGTG**TTGGAGAGGTTTGGAAAGGGCGTGC
CGGAGAGCCAAGTGCA

IL1RN:

TGACCTCCCATCTTACGCAGATAAGAACCAGTTTGG TTTC **TGCTAGCCTGAGTCACCCTCCTG**GAAACTGGG
CCTGCTTGGCATCAAGTCAGCCATCAGCCGGCCATCTCCTCATGCTGGCCAACCCTCTGTGAGTGTGTGG
GAGGGGAGGCTGGGCTCCTCCTTGTACTCT

- Expression of the pe-tRNA

Red: EF1a core promoter, blue: tRNA, orange: AsCpf1-crRNA scaffold, violet: SV40 polyA

GGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGATCCGGTGCCT
AGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTTCCGAGGGTGG
GGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACA
GCCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCGGCTGGAAT**AA**
TTCTACTCTTGTAGAT/23bp-sgRNA/TTTTTTGGATCCACTAGTTCTAGAAACTTGTTTATTGCAGCTTATAATGG
TTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTG
CAAACCTCATCAATGTATCTTA

- Expression of the T4AT6-crRNA

Red: U6 promoter, orange: AsCpf1-crRNA scaffold, violet: T4AT6 motif

AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGCTGTTAGAG
AGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCT
TGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGT**AA****TTCTACTCTTGTAGAT**/20bp-sgRNA/TTTTAT
TTTTT

- Expression of crRNA array

Red: U6 promoter, orange: AsCpf1-crRNA scaffold, sgRNA targeted to DNMT1-S7, EMX1, CTLA4-S1, CCR5-S1, SIRPa-S3, RUNX1-S1

AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGCTGTTAGAG
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GTAGATTGTCCGGGACACGTTTCCATTCTAATTTCTACTCTTGTAGATTGTCTCTCTGCCCCCTCCCACTTTT
TTTTG

● dWT-VPR: pCAG-dAsCpf1-WT (D908A)-NLS-3xHA-VPR

Human codon optimized Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1) is black, mutant site highlighted with red, nucleoplasmin NLS with blue, 3xHA with orange, VPR with violet

ATGACACAGTTCGAGGGCTTTACCAACCTGTATCAGGTGAGCAAGACACTGCGGTTTGAGCTGATCCCACA
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● dRKA-VPR: pCAG-dAsCpf1-RKA(E174R/R951K/R955A/D908A)-NLS-3xHA-VPR

Human codon optimized Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1) is black, mutant site highlighted with red, nucleoplasmin NLS with blue, 3xHA with orange, VPR with violet

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● dHF-VPR: pCAG-dAsCpf1-HF(E174R/N282A/S542R/K548R/D908A)-NLS-3xHA-VPR
Human codon optimized Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1) is black, mutant site highlighted with red,
nucleoplasmin NLS with blue, 3xHA with orange, VPR with violet

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● WT-VPR: pCAG-AsCpf1-WT-NLS-3xHA-VPR

Human codon optimized Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1) is black, nucleoplasm NLS with blue, 3xHA with orange, VPR with violet

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● dRKA-VPR: pCAG-dAsCpf1-RKA(E174R/R951K/R955A/D908A)-NLS-3xHA-VPR

Human codon optimized Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1) is black, mutant site highlighted with red, nucleoplamin NLS with blue, 3xHA with orange, VPR with violet

ATGACACAGTTCGAGGGCTTTACCAACCTGTATCAGGTGAGCAAGACACTGCGGTTTGAGCTGATCCCACA
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● dHF-VPR: pCAG-dAsCpf1-HF(E174R/N282A/S542R/K548R/D908A)-NLS-3xHA-VPR
Human codon optimized Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1) is black, mutant site highlighted with red,
nucleoplasmin NLS with blue, 3xHA with orange, VPR with violet
ATGACACAGTTTCGAGGGCTTTACCAACCTGTATCAGGTGAGCAAGACACTGCGGTTTGAGCTGATCCCACA
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TAAGTAA

- The amino acid sequences of the AsCpf1 variants used in this study

AsCpf1-A mutant site is K780A

AsCpf1-KA mutant sites are R951K, R955A

AsCpf1-AKA mutant sites are K780A, R951K, R955A

AsCpf1-RKA (AsCpf1-Plus) mutant sites are E174R, R951K, R955A

AsCpf1-KK mutant sites are R951K, R955K

AsCpf1-AKK mutant sites are K780A, R951K, R955K

AsCpf1-RKK mutant sites are E174R, R951K, R955K

enAsCpf1-HF mutant sites are E174R, N282A, S542R, K548R

AsCpf1-ultra mutant sites are M537R, F870L

AsCpf1-UKA mutant sites are M537R, F870L, R951K, R955A

AsCpf1-UKK mutant sites are M537R, F870L, R951K, R955K

AsCpf1-RU mutant sites are E174R, M537R, F870L

AsCpf1-RKAU mutant sites are E174R, M537R, F870L, R951K, R955A

AsCpf1-RKKU mutant sites are E174R, M537R, F870L, R951K, R955K

enAsCpf1 mutant sites are E174R, S542R, K548R

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WIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAK
GHHGKPNLHTLYWTGLFSPENLAKTSI**K(780)**LNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLY
QELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIHKDRRFTSDKF**F(870)**FHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKE**R(951)**VAAR**(955)**QAWSVVGTIKDL
KQGYLSQVIHEIVDLMIHYQAVVVLENLNFQFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGVLNPNY
QLTDQFTSFAKMGTQSGFLFVVPAPYTSKIDPLTGFVDPFVWTKIKNHESRKHFLLEGFDLHYDVKTGDFILHFK
MNRNLSFQRGLPGFMPAWDIVFEKNETQFDAQGTPFIAGKRIVPIENHRFTGRYRDLYPANELIALLEEKGIVFR

DGSNILPKLLENDSSHAIIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPPEWPMADANGAY
HIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRN

- The amino acid sequences of the *LbCpf1* variants used in this study:

LbCpf1-KA mutant sites are R883K, R887A

LbCpf1-RKA (*LbCpf1*-Plus) mutant sites are D156R, R883K, R887A

LbCpf1-KK mutant sites are R883K, R887K

LbCpf1-RKK mutant sites are D156R, R883K, R887K

LbCpf1-ultra mutant sites are N527R, E795L

LbCpf1-UKA mutant sites are N527R, E795L, R883K, R887A

LbCpf1-UKK mutant sites are N527R, E795L, R883K, R887K

LbCpf1-RU mutant sites are D156R, N527R, E795L

LbCpf1-RKAU mutant sites are D156R, N527R, E795L, R883K, R887A

LbCpf1-RKKU mutant sites are D156R, N527R, E795L, R883K, R887K

MSKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLEVEDEKRAEDYKGVKLLDRYYLSFINDVLHSIKLKNLNNY
ISLFRKKTRTEKENKELENLEINLRKEIAKAFKGNIEGYKSLFKKDIETILPEFLDDKDEIALVNSFNFGTTAFTGFFD(
156)NRENMFSEEAKSTSIAFRCINENLTRYISNMDIFEKVDIAFDKHEVQEIKEILNSDYDVEDFFEGEFFNFVLTQ
EGIDVYNAIIGGFVTESGEKIKGLNEYINLYNQKTKQKLPKFKPLYKQVLSDRESLSFYGEGYTSDEEVLEVFRNTL
NKNSEIFSSIKKLEKLFKNFDEYSSAGIFVKNNGPAISTISKDIFGEWNVIRDKWNAEYDDIHLKKAHVTEKYEDDR
RKSFKKIGSFSLEQLQEYADADLSVVEKLKEIIQKQVDEIYKVGSSSEKLFDAFVLEKSLKKNDAVVAIMKDLLDSV
KSFENYIKAFFGEGKETNRDESFYGDFVLAYDILLKVDHIYDAIRNYVTQKPYSKDKFKLYFQ(N(527)PQFMGGWD
KDKETDYRATILRYGSKYLAIMDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFSKKWMAYYNPSE
DIQKIYKNGTFKKGDMFNLNDCHKLIDFFKDSISRYPKWSNAYDFNFSETEKYKDIAGFYREVVEEQGYKVSFESA
SKKEVDKLVVEEGKLYMFQIYNKDFSDKSHGTPNLHTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVHP
ANSPIANKNPDNPKKTTTTLSYDVYKDKRFSEDQYE(795)LHIPIAINKCPKNIFKINTEVRVLLKHDDNPYVIGIDRG
ERNLLYIVVVDGKGNIVEQYSLNEIINNFNGIRIKTDYHSLLDKKEKER(883)FEAR(887)QNWTSIENIKELKAGYIS
QVVHKICELVEKYDAVIALEDLNSGFKNSRVKVEKQVYQKFEKMLIDKLNVMVDKKSNPCATGGALKGYQITNKF
ESFKSMSTQNGFIFYIPAWLTSKIDPSTGFVNLKTKYTSIADSKKFISSFDRIMYVPEEDLFEFALDYKNFSRTDAD
YIKKWKLYSYGNRIRIFRNPKNVDFWEEVCLTSAYKELFNKYGINYQQGDIRALLCEQSDKAFYSSFMALMSL
MLQMRNSITGRDVFDFLISPVKNSDGIFYDSRNYEAQENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKV
KIAISNKEWLEYAQTsvKH