SUPPLEMENTARY MATERIALS

Supplementary Table S1. Cas12a orthologs and their ability to edit the genomes of human cells

Cas12a orthologs	Short title	Scaffold nucleotide sequence, 5'-3'	Paper where was showed human cell gene editing, doi
Lachnospira eligens ATCC 27750	EeCas12a	UAAUUUCUACU UUGUAGAU	10.1038/ s41598-019-50423-6
Moraxella bovoculi 237	MbCas12a	UAAUUUCUACU **UUU**GUAGAU	10.1093/nar/gky815
Eubacterium rectale	ErCas12a	UAAUUUCUACU **CUU**GUAGAU	10.1089/crispr.2019.0026
Acidaminococcus sp	AsCas12a	UAAUUUCUACU **CUU**GUAGAU	10.1093/nar/gky815
Ruminococcus bromii sp.	**RbCas12a**	UAAUUUCUACU **AUU**GUAGAU	**current study**
Butyrivibrio sp. NC3005	BsCas12a	UAAUUUCUACU **AUU**GUAGAU	10.1186/ s13059-019-1620-8
Lachnospiraceae bacterium MA2020	Lb2Cas12a	UAAUUUCUACU **AUU**GUAGAU	10.1096/fj.202001013RR
Helcococcus kunzii ATCC 51366	HkCas12a	UAAUUUCUACU **AUU**GUAGAU	10.1186/ s13059-019-1620-8
Pseudobutyrivibrio xylanivorans strain DSM 10317	PxCas12a	UAAUUUCUACU **AUU**GUAGAU	10.1186/ s13059-019-1620-8
Francisella novicida	FnCas12a	UAAUUUCUACU **GUU**GUAGAU	10.1093/nar/gky815
Thiomicrospira sp. XS5	TsCas12a	UAAUUUCUACU **GUU**GUAGAU	10.2302/kjm.2019-0009-OA
Agathobacter rectalis strain 2789STDY5834884	ArCas12a	UAAUUUCUACU **GUU**GUAGAU	10.1186/ s13059-019-1620-8
Butyrivibrio fibrisolvens MD2001	BfCas12a	UAAUUUCUACU **GUU**GUAGAU	10.1186/ s13059-020-01989-2
Lachnospiraceae bacterium ND2006	LbCas12a	UAAUUUCUACU**AAGU**GUAGAU	10.1093/nar/gky815
Moraxella bovoculi AAX08_00205	Mb2Cas12a	UAAUUUCUACU**GUUU**GUAGAU	10.2302/kjm.2019-0009-OA
Moraxella bovoculi sp.	Mb3Cas12a	UAAUUUCUACU**GUUU**GUAGAU	10.1126/science.aau5174
Coprococcus eutactus sp.	CeCas12a	UAAUUUCUACU **UCG**GUAGAU	10.1186/ s13059-020-01989-2
Lachnospira pectinoschiza strain 2789STDY5834886	LpCas12a	UAAUUUCUACU**GUGU**GUAGAU	10.1186/ s13059-019-1620-8
Pseudobutyrivibrio ruminis CF1b	PrCas12a	UAAUUUCUACU**GUGU**GUAGAU	10.1186/ s13059-019-1620-8

Supplementary Table S 2. Sequence of oligonucleotides used in the study (T7 promoter complementary region of oligonucleotides is highlighted green, protospacer complementary region is highlighted red, scaffold is black)

DNA oligonucleotides 5'->3'	
Fwd_test	GGATGCAACTGAATCCTGTAG
Rev_test	TACCGCATAAGCCGGAATAAG
Fwd_RbCpf_Nde	TGAACATATGATGCAAGAGCGTAAAAAAATATCGCATC
Rev_RbCpf_Xho	ATCTACTCGAGATTATTCGCCATATCATTCTCCTGAACA
Fwd_NLS_Nhe_Hind	CTAGATGCCGAAGAAAAAGCGCAAGGTCA
Rev_NLS_Nhe_Hind	AGCTTGACCTTGCGCTTTTTCTTCGGCAT
Fwd_NLS_Hind	AGCTCCCGAAGAAAAAGCGCAAGGTCA
Rev_NLS_Hind	AGCTTGACCTTGCGCTTTTTCTTCGGG
Fwd_dnmt1_spacer1	TAGAAAGGAAGTCTTGGCTGGCCTTCC
Rev_dnmt1_spacer1	AAAAGGAAGGCCAGCCAAGACTTCCTT
Fwd_dnmt1_spacer2	TAGATCACGGGACTTCTGGCTGAGGTCA
Rev_dnmt1_spacer2	AAAATGACCTCAGCCAGAAGTCCCGTGA
Fwd_dnmt1_spacer3	TAGATCTGATGGTCCATGTCTGTTACTC
Rev_dnmt1_spacer3	AAAAGAGTAACAGACATGGACCATCAGA
Fwd_vegfa_spacer	TAGATCTAGGAATATTGAAGGGGGCAGG
Rev_vegfa_spacer	AAAACCTGCCCCCTTCAATATTCCTAGA
Fwd_emx1_spacer	TAGATTCCTCCGGTTCTGGAACCACACC
Rev_emx1_spacer	AAAAGGTGTGGTTCCAGAACCGGAGGAA

Fwd_dnmt1_del_ngs	GACAGACACACGGAGTGTCTAGCT
Rev_dnmt1_del_ngs	AATTTGGCTCAGCAGGCACC
Fwd_dnmt1_sp1_ngs	CAAGGGCAGCTCAGTGGTGACTT
Rev_dnmt1_sp1_ngs	CTGAGTCACGTGAGTTGATCCCCAT
Fwd_dnmt1_sp2_ngs	GACAGACACACGGAGTGTCTAGCT
Rev_dnmt1_sp2_ngs	GAAGCTGTTGTGTGAGGTTCGCTTATC
Fwd_dnmt1_sp3_ngs	CAGAACTAGTCCTTAGCAGCT
Rev_dnmt1_sp3_ngs	CTCTGGGGACCGTTTGAG
Fwd_vegfa_ngs	GGGTCACTCCAGGATTCC
Rev_vegfa_ngs	CCAAGGTTCACAGCCTGAAA
Fwd_emx1_ngs	GCCTCCTGAGTTTCTCATCTG
Rev_emx1_ngs	CTAGTCATTGGAGGTGACATCG
Fwd_SDM_T	TTTGGAATTTTGTGCCACTTCTG
Fwd_SDM_A	TTTGGAATTTAGTGCCACTTCTG
Fwd_SDM_C	TTTGGAATTTCGTGCCACTTCTG
Rev_SDM	GGTTTACCTTGACCCCTATAG
Fwd_PAM_library	GTAAAACGACGGCCAGTGCCGCAGTACTGATCATNNNNNNNNCCCCTCTATTGATCCCCACCTCCAAATATCTCATCAACAACGTCATAGCTGTTTCCTG

Rev_PAM_library	CAGGAAACAGCTATGACGTTGTTGATGAGATATTTGGAGGTGGGGA TCAATAGAGGGGNNNNNNNNATGATCAGTACTGCGGCACTGGCCG TCGTTTTAC
Fwd_RbCpf_RT	AGTGACGACTTGAAGGCTGA
Rev_RbCpf_RT	CGTCACATGGCAGCTTAGAC
Fwd_T7_guide	TAATACGACTCACTATAGG
Rev_TCA_guide	CTAAGAAACCATTATTATCAATCTACAAGAGTAGAAATTACCCTATA GTGAGTCGTATTA
Rev_TCG_guide	GGGTTCCGCGCACATTTCCCATCTACAAGAGTAGAAATTACCCTATA GTGAGTCGTATTA
Rev_TCC_guide	CGTCAGGTGGCACTTTTCGGATCTACAAGAGTAGAAATTACCCTAT AGTGAGTCGTATTA
Rev_TCT_guide	AAAGTGCCACCTGACGTCTAATCTACAAGAGTAGAAATTACCCTAT AGTGAGTCGTATTA
Rev_CTA_guide	TATTTAGAAAAATAAACAAAATCTACAAGAGTAGAAATTACCCTATA GTGAGTCGTATTA
Rev_CTG_guide	AATAATGGTTTCTTAGACGTATCTACAAGAGTAGAAATTACCCTATA GTGAGTCGTATTA
Rev_CTC_guide	CCGTCATCACCGAAACGCGCATCTACAAGAGTAGAAATTACCCTAT AGTGAGTCGTATTA
Rev_CTT_guide	AAAAGTGCCACCTGACGTCTATCTACAAGAGTAGAAATTACCCTAT AGTGAGTCGTATTA
Rev_CCA_guide	ACATTTCCCCGAAAAGTGCCATCTACAAGAGTAGAAATTACCCTAT AGTGAGTCGTATTA
Rev_CCG_guide	AGACGTCAGGTGGCACTTTTATCTACAAGAGTAGAAATTACCCTATA GTGAGTCGTATTA
Rev_CCC_guide	GACGTCAGGTGGCACTTTTCATCTACAAGAGTAGAAATTACCCTAT AGTGAGTCGTATTA
Rev_CCT_guide	ATAATGGTTTCTTAGACGTCATCTACAAGAGTAGAAATTACCCTATA GTGAGTCGTATTA
Rev_TTC_guide	GTCAGGTGGCACTTTTCGGGATCTACAAGAGTAGAAATTACCCTAT AGTGAGTCGTATTA
crRNA_site1	rUrArArUrUrUrCrUrArCrUrArUrUrGrUrArGrArUrCrCrCrCrUrCrUrArUr UrGrArUrCrCrCrCrArCrC
Fwd_gDNA_site1	TCTTGCACTCATGAGCTGTC
Rev_gDNA_site1	GTTGAGGGTTATGAGAGTAGC

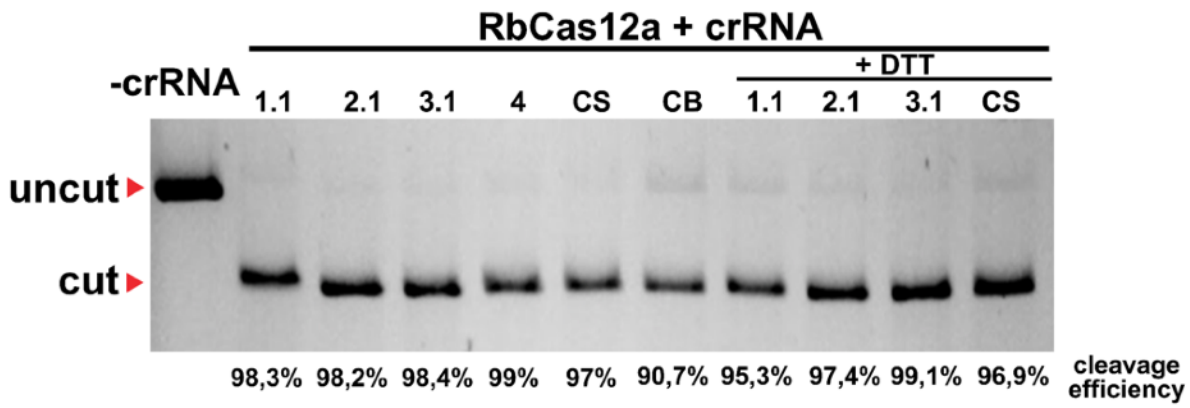

Figure S1. Effect of buffers on RbCas12a crRNA cleavage activity. Target DNA cleavage by RbCas12a programmed with 500 nM crRNA at a 1:3:30 target:RbCas12a:RNA ratio in various conditions. The effect of NEBuffer 1.1 (1.1), NEBuffer 2.1 (2.1), NEBuffer 3.1 (3.1), NEBuffer 4 (4), CutSmart (CS) and cleavage buffer (CB) either supplemented or not with DTT were tested. Mean cleavage efficiencies from three independent experiments are shown below the gel.

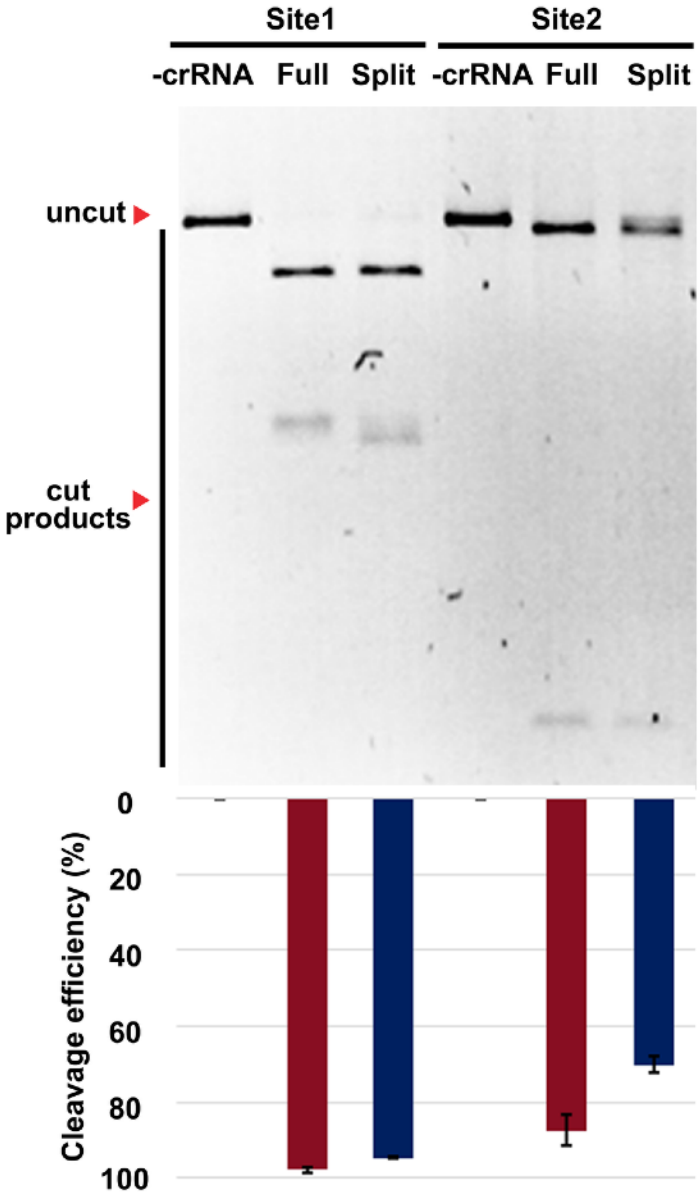

Figure S2. Split crRNA activity is not dependent on the sequence of the crRNA spacer moiety. Target DNA *in vitro* cleavage by RbCas12a loaded with full-sized or split crRNAs (500 nM) bearing different guide sequences ('Site1' and 'Site2' as indicated above the panel). The cleavage efficiencies calculated from one experiment are shown on the bottom. Mean cleavage efficiencies and standard deviations calculated from three independent experiments are shown.

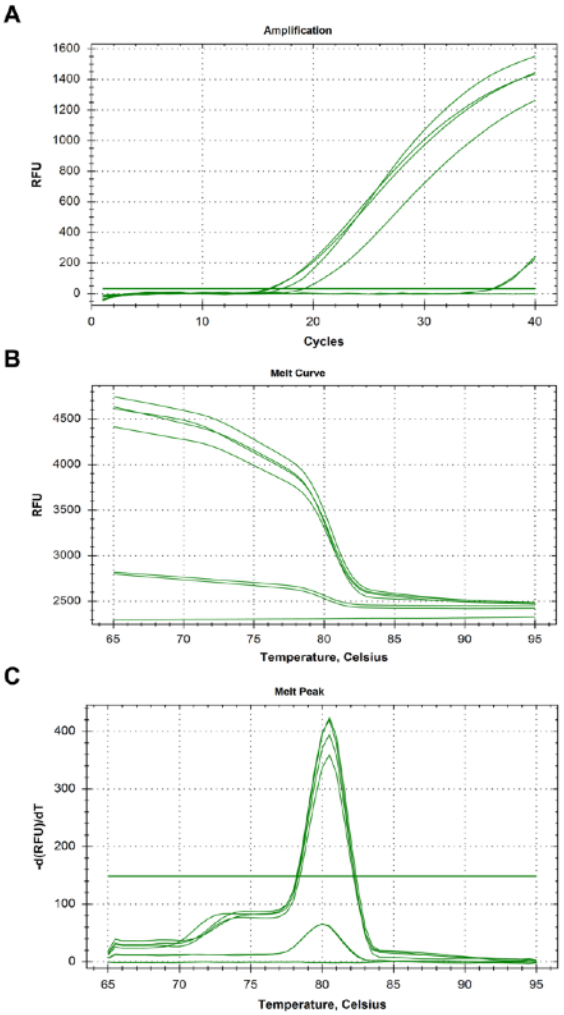

Figure S3. Real-time RT-PCR of RbCas12a mRNA extracted from HEK293T in 24 and 48 hours after transfection (500 ng and 2500 ng of hRbCas12a plasmid vector). **(A)** The signal grows exponentially from cycle 15 in four samples with hRbCas12a plasmid vector. In two mock samples where the plasmid vector was not added the signal is observed from cycle 35. **(B), (C)** Melt curves and peaks indicate specificity of the primer pair.

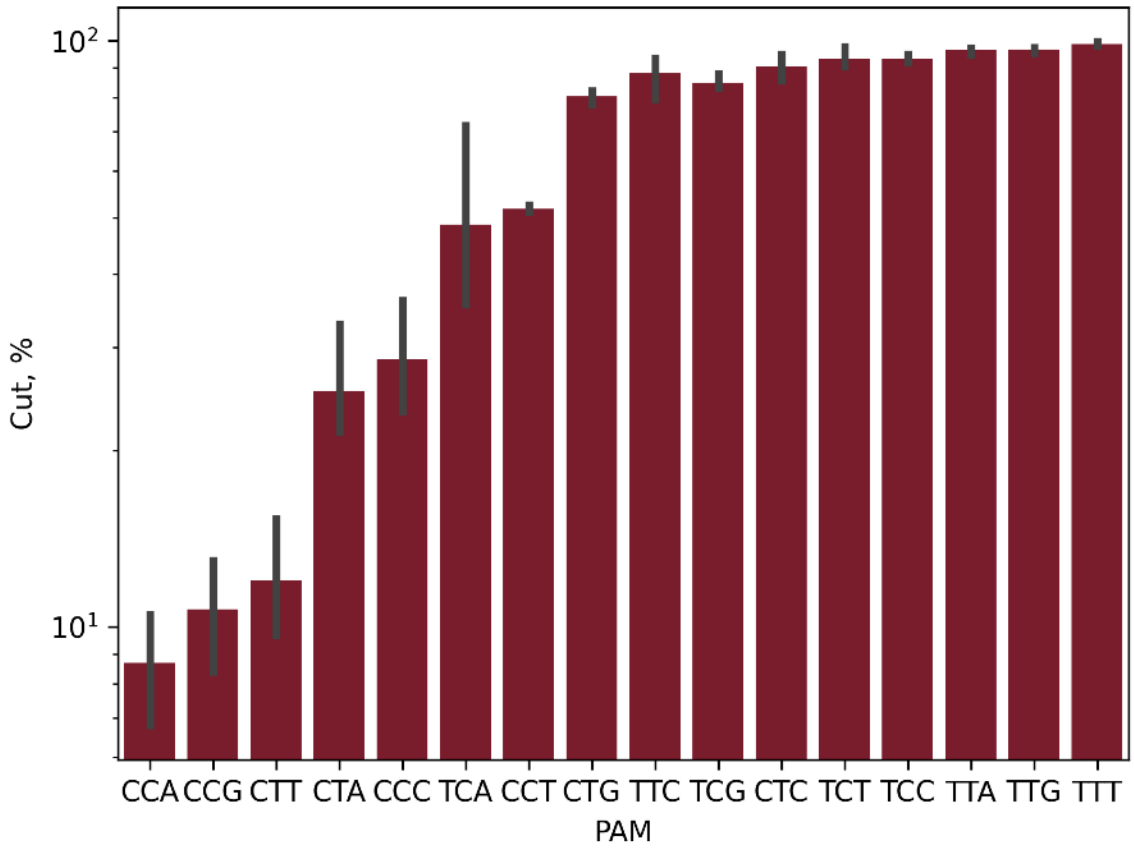

Figure S4. Investigation of RbCas12a 5'-YYN PAM sequence by *in vitro* cleavage assay. Bar plots display the LFC depletion of PAM containing DNA sequences following treatment with RbCas12a.

Nucleotide sequence of RbCas12a

ATGCAAGAGCGTAAAAAAATATCGCATCTTACACACAGAAATTCAGTTAAAAAAACAATTAGGATGCAACTGAATCCTGTAGGT
AAAACAATGGATTATTTTCAAGCAAAGCAAATTCTTGAAAATGATGAAAAGCTTAAAGAGAACTATCAGAAAATCAAGGAAAT
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CTATCACTGTAATACGGATGCAGACCGAAAAAGACTTGATGAATGTGCATCGGAATTAAGGAAAGAAATCGTCAAAAATTTTA
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Amino-acid sequence of RbCas12a

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HCNTDADRKRLDECASELRKEIVKNFKNRDEYNKLFDKRMIEIVLPQHLKNEDEKEVVASFKNFTTYFTGFFTNRKNMYSDGEEST
AIAYRCINENLPKHLDNVKAFEKAISKLSKNAIDDLDATYSGLCGTNLYDVFTVDYFNFLLPQSGITEYNKIIGGYTTSDGTKVKGIN
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DNVSTVEPVIHNDKWLKFVQENDMANNLE

Human optimized nucleotide sequence of RbCas12a with nuclear localization signals

(SV40 NLS is highlighted red, nucleoplasmin NLS is blue)

ATGCCGAAGAAAAAGCGCAAGGTCAAGCTCCCGAAGAAAAAGCGCAAGGTCAAGCTCCCGAAGAAAAAGCGCAAGGTCAA
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