

Supplementary Information

Targeted dual base editing with *Campylobacter jejuni* Cas9 by single AAV-mediated delivery

Jiyeon Kweon^{1, 2}, An-Hee Jang^{1, 2}, Eunji Kwon^{3,4}, Ha Rim Shin^{1, 2}, Jieun See^{1, 2}, Gayoung Jang^{1, 2}, Chaeyeon Lee^{1, 2}, Taeyoung Koo^{3,4}, Yongsub Kim^{1, 2, *}

¹ Department of Biomedical Sciences, Asan Medical Institute of Convergence Science and Technology, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, Republic of Korea

² Stem Cell Immunomodulation Research Center, University of Ulsan College of Medicine, Seoul 05505, Republic of Korea

³ Department of Fundamental Pharmaceutical Sciences, Kyung Hee University, Seoul, 02447, Republic of Korea

⁴ Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University, Seoul, 02447, Republic of Korea

* Corresponding author: Yongsub Kim, PhD (email: yongsub1.kim@gmail.com)

Table of Contents

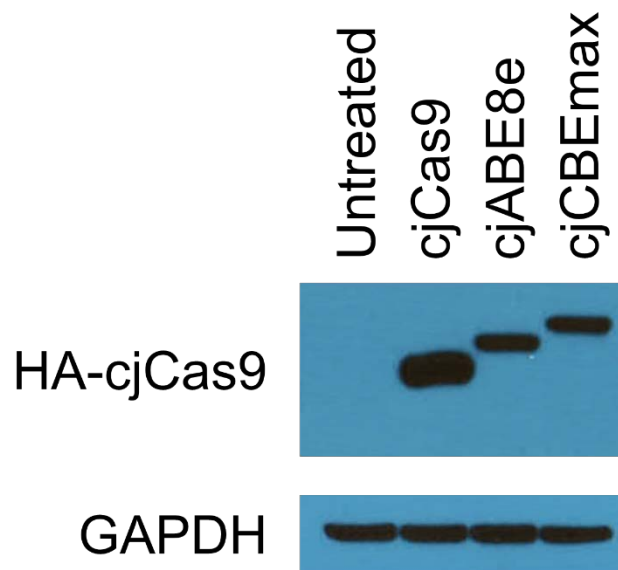
Supplementary Figure 1. Protein expression levels of cjCBEmax and cjABE8e.

Supplementary Figure 2. Base editing frequency of individual adenine and cytosine within a 50-nt window around target sites.

Supplementary Figure 3. Base editing frequency of individual adenine by cjCas9 (L58Y/D900K) and e-scaffold gRNAs.

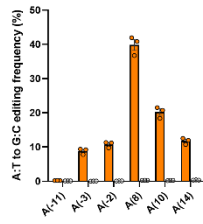
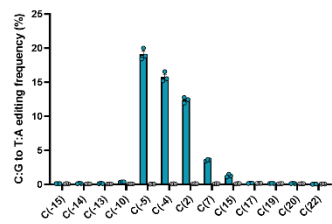
Supplementary Table 1. List of target sequences and PCR primers used in this study.

Supplementary Table 2. List of potential off-target sites of cjABE target sites in the human genome.

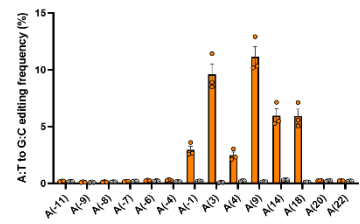
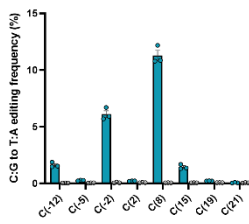


Supplementary Fig. 1. Protein expression levels of cjCBEmax and cjABE8e. Western blotting of HEK293T cells transfected with cjCas9, cjCBEmax, and cjABE8e. GAPDH served as the loading control.

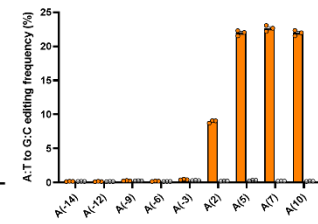
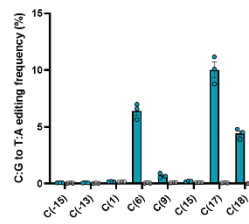
ANGPT2
 C₋₁₅C₋₁₄C₋₁₃TA₋₁₁C₋₁₀GTGTC₋₅C₋₄A₋₃A₋₂TGC₂TGTGC₇A₈GA₁₀GGGA₁₄C₁₅GC₁₇GC₁₈C₂₀GC₂₂TCGAATAC



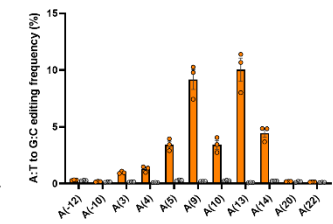
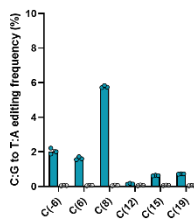
EPAS1-1
 TGTC₋₁₂A₋₁₁GA₋₉A₋₈A₋₇A₋₆C₋₅A₋₄TC₋₂A₋₁GC₃A₄GTTCA₈ATGGGA₁₄C₁₅TTA₁₈C₁₉A₂₀C₂₁A₂₂GGTGACAC



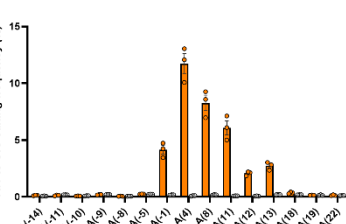
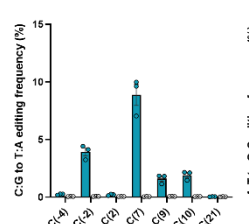
EPAS1-2
 C₋₁₅A₋₁₄C₋₁₃A₋₁₂TGA₋₉TGA₋₈TGA₋₃GGC₋₁A₂GA₅C₆A₇GC₉AGGGGC₁₅TC₁₇TTGTAGCCACAC



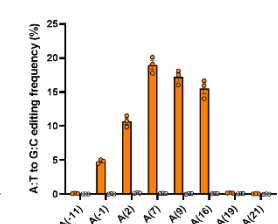
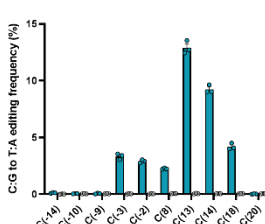
HIF1A-1
 TGGA₋₁₂TA₋₁₀TGTC₋₈TGGGTTGA₃A₄A₅C₆TC₈A₉A₁₀GC₁₂A₁₃A₁₄C₁₅TGTC₁₉A₂₀TA₂₂TATAACAC



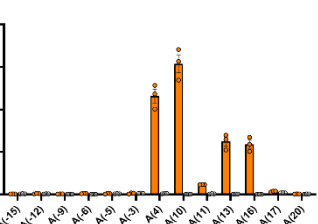
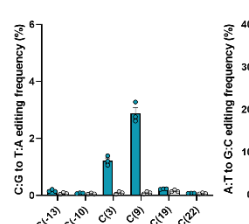
HIF1A-2
 TA₋₁₄TGA₋₁₃A₋₁₀A₋₉TGA₋₅C₋₄TC₋₂A₋₁GC₂TA₄TTC₇A₈C₉C₁₀A₁₁A₁₂A₁₃GTTGA₁₆A₁₉TC₂₁A₂₂GAAGATAC



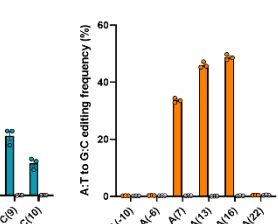
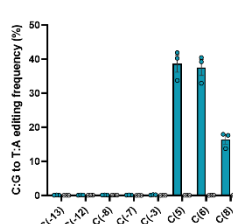
SERPINC1
 TC₋₁₄TTA₋₁₁C₋₁₀C₋₉TTTTTC₋₅C₋₂A₋₁GA₃GGTTA₇C₈A₉GTTCA₁₃TA₁₆TC₁₈A₁₉C₂₀A₂₁TTGGAATAC



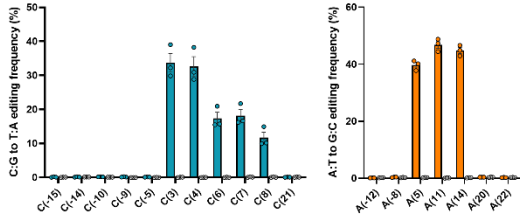
TFPI
 A₋₁₅TC₋₁₃A₋₁₂GC₋₁₀A₋₉TTA₋₅GA₋₃GGGGC₃AGGGGC₉A₁₀A₁₁GA₁₃TTA₁₆A₁₇GC₁₉A₂₀GC₂₂AGGCATAC



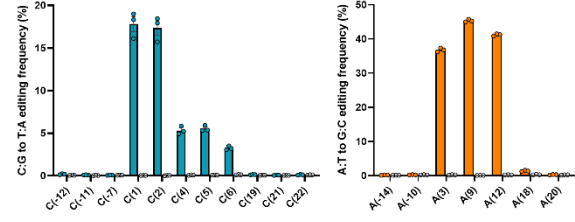
HPD-1
 GGC₋₁₃C₋₁₂GA₋₁₀GC₋₈C₋₇A₋₆GGC₋₃TGTTTTC₅C₆A₇C₈C₉GTAT₁₃GTAT₁₆TGGGGA₂₂CACCACAC



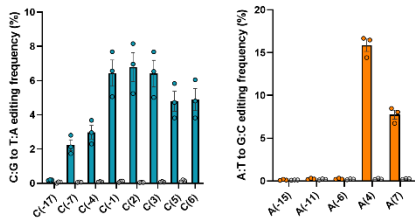
HPD-2
 C₋₁₅C₋₁₄GA₋₁₂GC₋₁₀C₋₉A₋₈GGC₋₅TGTTTTC₃C₄A₅C₆C₇C₈GT₁₁GT₁₄TGGGGA₂₈C₂₁A₂₂CCACACAC



HPD-3
 GA₋₁₄GC₋₁₂C₋₁₁A₋₁₀GGC₋₇TGTTTTC₁C₂A₃C₄C₅C₆GT₉GT₁₂TGGGGA₁₈C₁₉A₂₀C₂₁C₂₂ACACACAC

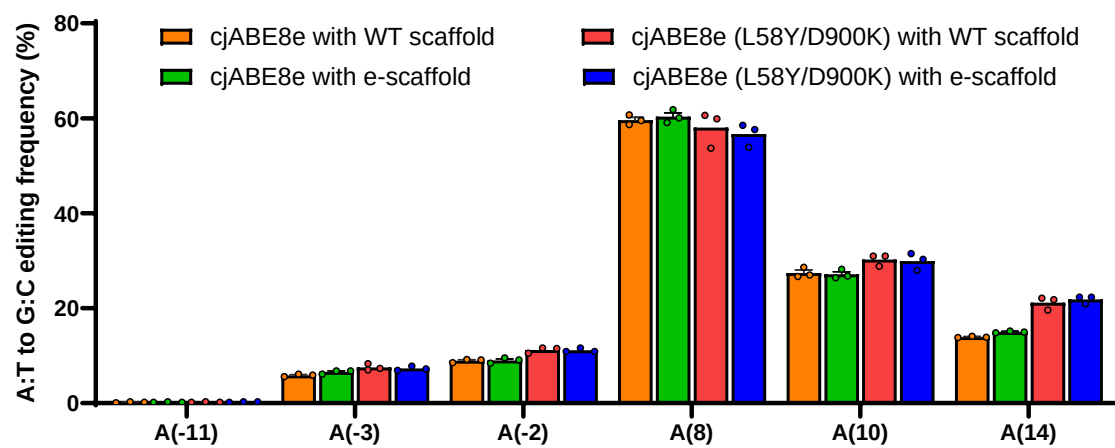


HPD-4
 A₋₁₅TGTA₋₁₁GTTC₋₇A₋₆TC₋₄TTC₋₁TC₂C₃A₄C₅C₆A₉GGGTGTGTGGTGTCCCCATAC

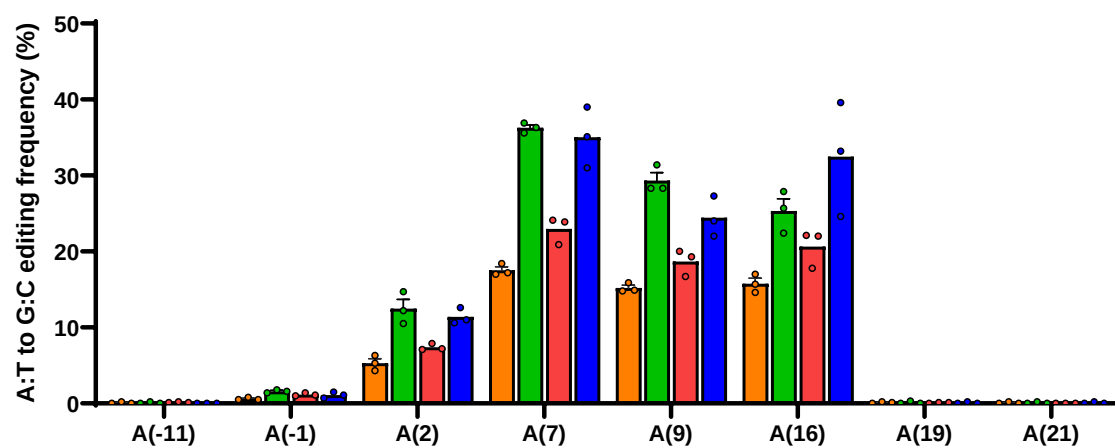


Supplementary Fig. 2. Base editing frequency of individual adenine and cytosine within a 50-nt window around target sites. Within the 50-nt window, adenine and cytosine are highlighted in red and green, respectively. The PAM sequences are highlighted in blue. The position of each nucleotide was numbered relative to the starting position of the target spacer sequences. All experiments were conducted in biologically independent triplicates. Error bars indicate standard error of the mean.

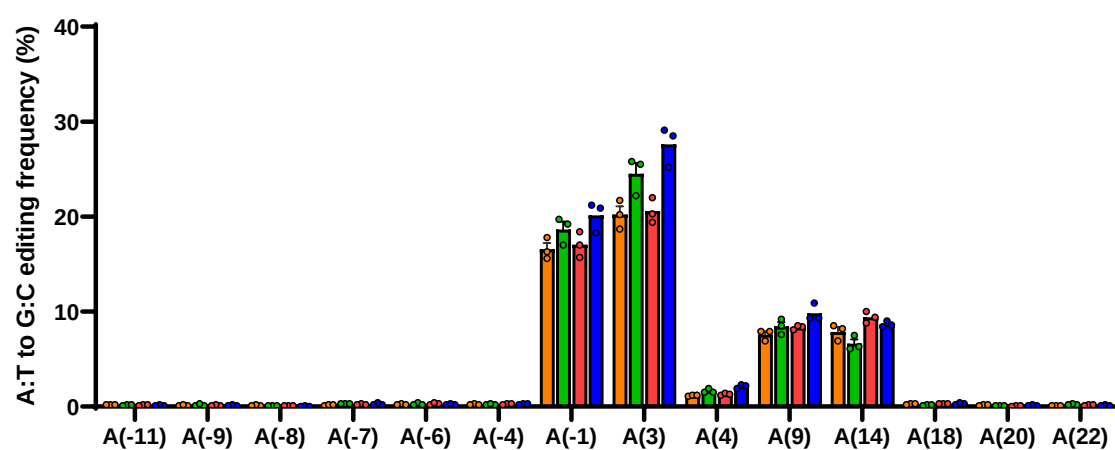
(a)



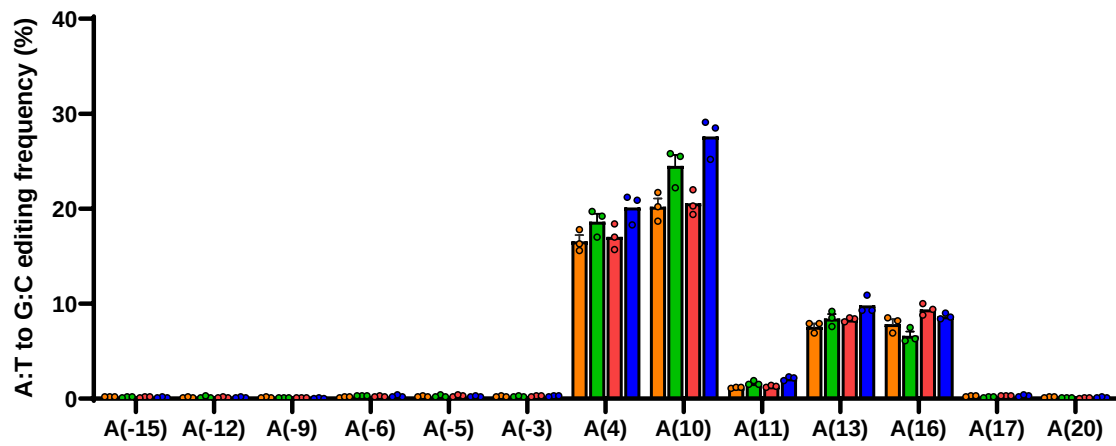
(b)



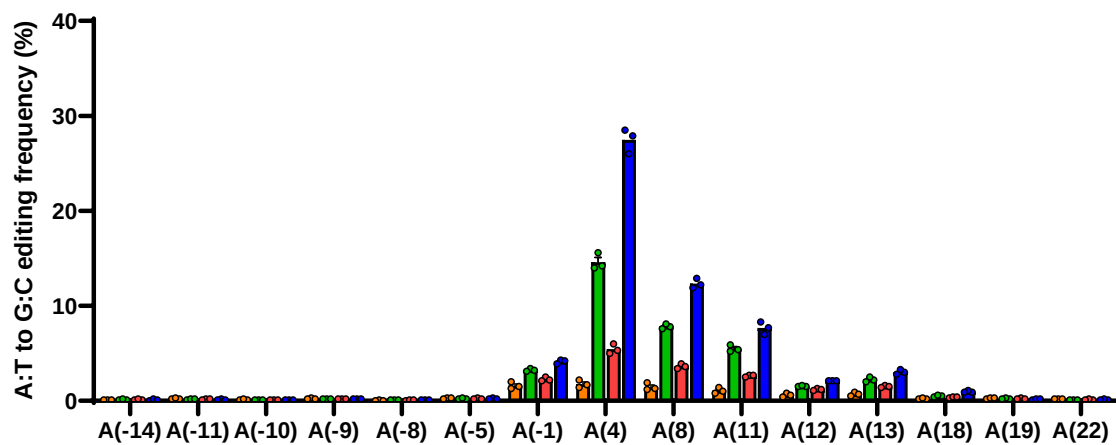
(c)



(d)



(e)



Supplementary Fig. 3. Base editing frequency of individual adenine by cjCas9 (L58Y/D900K) and e-scaffold gRNAs. The encjCas9 showed improved base editing frequencies and had synergistic effects with e-scaffold gRNAs without altering base editing windows across five target sites: (a) *ANGPT*, (b) *SERPINC1*, (c) *EPAS1-1*, (d) *TFPI*, and (e) *HIF1A-2*. The position of each nucleotide was numbered relative to the starting position of the target spacer sequences. All experiments were conducted in biologically independent triplicates. Error bars indicate standard error of the mean.

Supplementary Table 1. List of target sequences and PCR primers used in this study.

gRNA name	Target sequences (5' to 3')	PAM	PCR-F	PCR-R
SERPINC1	GAGGTTACAGTTCCTATCACAT	TGGAATAC	GGTGTGGAGGTCATTCTG	GTCCTCTGGAACCTCTGC
TFPI	GGCAGGGGCAAGATTAAGCAGC	AGGCATAC	TGGGTTCTGTATTTTCAGAGATGA	TTCCCTCCACAATGGAAAAC
EPAS1-1	GCAAGTTCATGGGACTTACACA	GGTGACAC	AAGCCTTGGAGGGTTTCATT	GTGGCTAGCACCTTCCACTC
ANGPT2	GCTGTGCAGAGGGACGCGCCGC	TCGAATAC	ATGGGTCTGCAGCTACACT	TTCCATGATGTTCTCCAGCA
EPAS1-2	CAGGACAGCAGGGGCTCCTTGT	AGCCACAC	GGGCCAGGTGAAAGTCTACA	TCTCTGCCACAGCATACAC
HIF1A-1	TGAAACTCAAGCAACTGTCATA	TATAACAC	GGACAAGTCACCACAGGACA	TCCCCAAAAATGTTTATTTCTCA
HIF1A-2	GCTATTCACCAAAGTTGAATCA	GAAGATAC	CCCTTCCCCTCACTGTATCA	GGCCAGCAAAGTTAAAGCAT
HPD-1	TTTTCCACCCGTAGTATGGGGA	CACCACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2	TTCCACCCGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-3	CCACCCGTAGTATGGGGACACC	ACACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-4	TCCACCAGGGTGTGTGTGGTGT	CCCCATAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
AAVS1-2	GAGTAGAGGCGGCCACGACCTG	GTGAACAC	AGAGCAGGGCCTTAGGGAAG	CGTGCGTCAGTTTACCTGT
AAVS1-8	GGAGTGTGACAGCCTGGGGCCC	AGGCACAC	GCAGATACTCTGCAGGAACG	CCCAAGTTTTGGACCCCTAA
HPD-2-M1-1	TTCCACCCGTAGTATGGGGACg	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-2	TTCCACCCGTAGTATGGGGgCA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-3	TTCCACCCGTAGTATGGgACACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-4	TTCCACCCGTAGTATaGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-5	TTCCACCCGTAGTgTGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-6	TTCCACCCGTAAaTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-7	TTCCACCCGcAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-8	TTCCACcTgTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-9	TTCCAtCCGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-10	TTCTACCCGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M1-11	TcCCACCCGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-1	TTCCACCCGTAGTATGGGGAtg	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-2	TTCCACCCGTAGTATGGGAgCA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-3	TTCCACCCGTAGTATGaaGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-4	TTCCACCCGTAGTAcagGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-5	TTCCACCCGTAGcgTGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-6	TTCCACCCGTgaTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-7	TTCCACCCacAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-8	TTCCActtGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-9	TTCCgtCCGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-10	TTttACCCGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG
HPD-2-M2-11	ccCCACCCGTAGTATGGGGACA	CCACACAC	GGAAGTAGGGGTCCATGA	ACGCATCTGGTTAGGGTCAG

Supplementary Table 2. List of potential off-target sites of cjABE target sites in the human genome.

gRNA name		Target sequences (5' to 3')	PAM	Mismatches (bp)
ANGPT2	ON	GCTGTGCAGAGGGACGCGCCGC	TCGAATAC	N/A
	OT1	tCTGTGaAGAGGtACaCGCCGC	CCGAGTAC	4
EPAS1-1	ON	CAGGACAGCAGGGGCTCCTTGT	AGCCACAC	N/A
	OT1	aAGGAgAGCtGGGGCaCCTTGT	AATCATAc	4
HIF1A-1	ON	TGAAACTCAAGCAACTGTCATA	TATAACAC	N/A
	OT1	TaAAACaCAAGCAaATGtGATA	AGAGATAC	4
HPD-1	ON	TTTTCCACCCGTAGTATGGGGA	CACCACAC	N/A
	OT1	TaTgtCgCCCGTAGTATGGGGA	AACAGCAC	4
AAVS1-8	ON	GGAGTGTGACAGCCTGGGGCCC	AGGCACAC	N/A
	OT1	tGAGTGTGActGCCTGGGGCCC	AGTCACAC	2