OMTN, Volume *17*

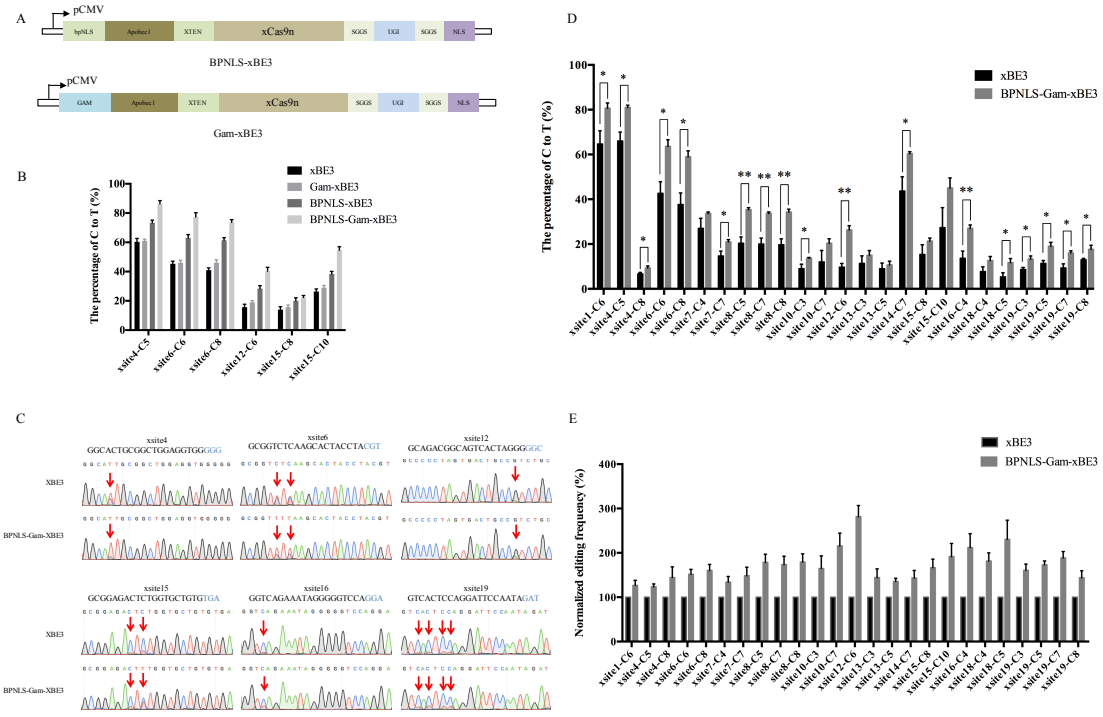
Supplemental Information

Improving Editing Efficiency for the Sequences with NGH PAM Using xCas9-Derived Base Editors

Xinyi Liu, Guanglei Li, Xueliang Zhou, Yunbo Qiao, Ruixuan Wang, Shaohui Tang, Jianqiao Liu, Lisheng Wang, and Xingxu Huang

Supplemental Information

Supplementary figure 1



Supplementary figure 1. Editing frequency of xBE3 and BPNLS-Gam-xBE3 on xsites in human cells.

A. Schematic diagram illustrating the design of expression vectors of BPNLS-xBE3 and Gam-xBE3.

B. Histogram of editing efficiency between xBE3, BPNLS-xBE3, Gam-xBE3, and BPNLS-Gam-xBE3. The editing efficiency was indicated as the percentage of targeted C-to-T base substitution at indicated sites. Data from three independent experiments are shown as means ± s.e.m.

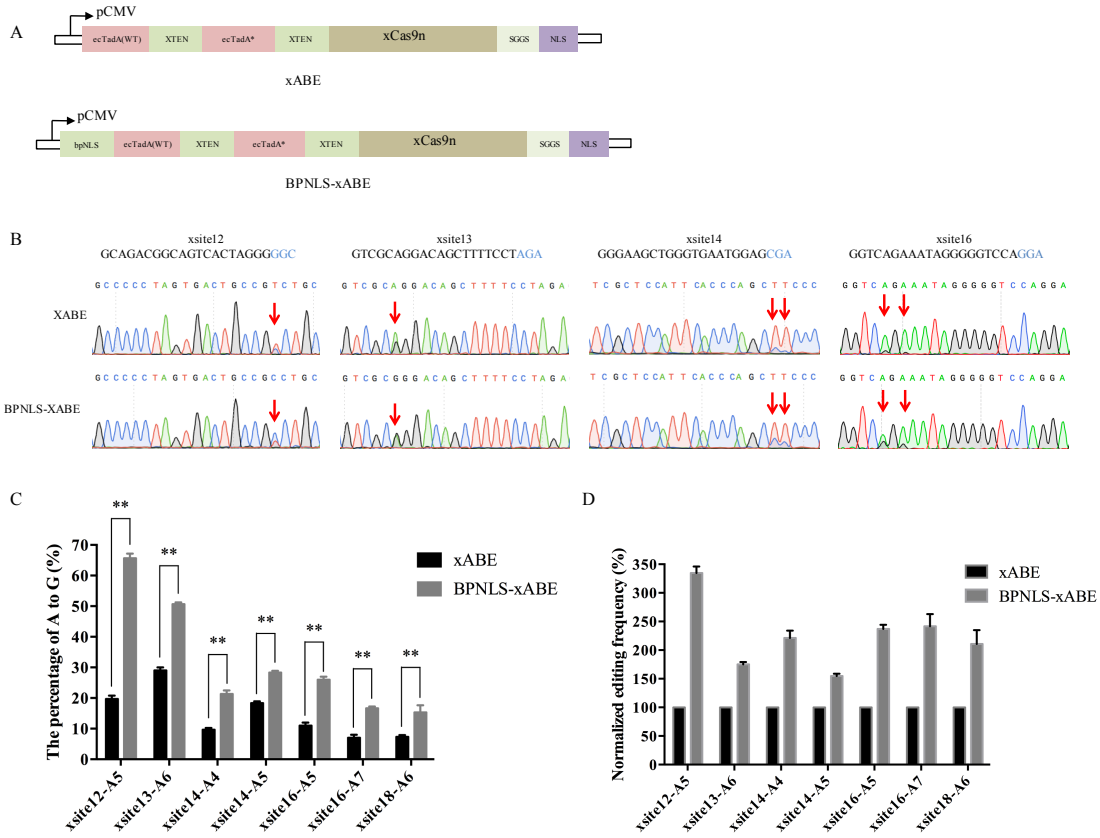
C. Sequence chromatogram of representative sites with different PAM (NGG, NGT, NGC, NGA and GAT) were presented, the PAM sequences are highlighted in blue. Red arrows indicate the edited bases.

D. The editing frequencies of indicated loci edited by xBE3 or BPNLS-Gam-

xBE3 were presented. PCR products were subjected for Sanger sequencing and the results were quantified by online software EditR. Data from three independent experiments are shown as means ± s.e.m, the asterisks denote statistical significance, “*”, $P<0.05$, “*”, $P<0.01$.

E. Normalized editing frequency of xBE3and BPNLS-Gam-xBE3. Set the editing frequency of xBE3 as 100 percent.

Supplementary figure 2



Supplementary figure 2. Comparison of editing efficiency of xABE and BPNLS-xABE in human cells.

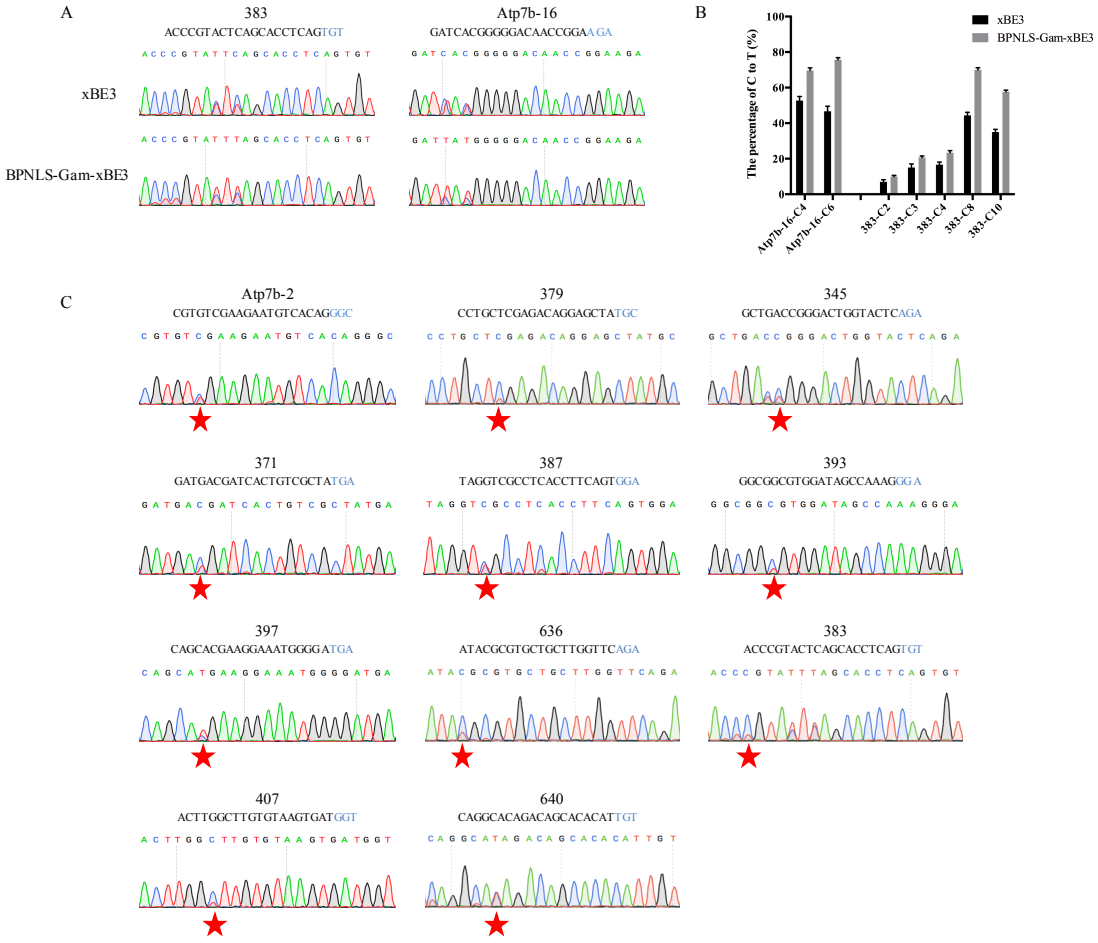
A. Schematic diagram illustrating the design of expression vectors of xABE and BPNLS-xABE.

B. Sequence chromatogram of representative sites edited by xABE and BPNLS-xABE. Red arrows indicate bases edited by xABE or BPNLS-xABE, the PAM sequences are highlighted in blue.

C. The editing frequencies of xABE and BPNLS-xABE. The editing efficiency was indicated as the percentage of A-to-G conversion at indicated loci. Data from three independent experiments are shown as means ± s.e.m. , the asterisks denote statistical significance, "*", $P<0.05$, "*", $P<0.01$.

D. Normalized editing frequency of BPNLS-xABE to xABE, set the editing frequency of xABE as 100 percent.

Supplementary figure 3



Supplementary figure 3. Pathogenic mutations created by xBE3 and BPNLS-Gam-xBE3 in human cells.

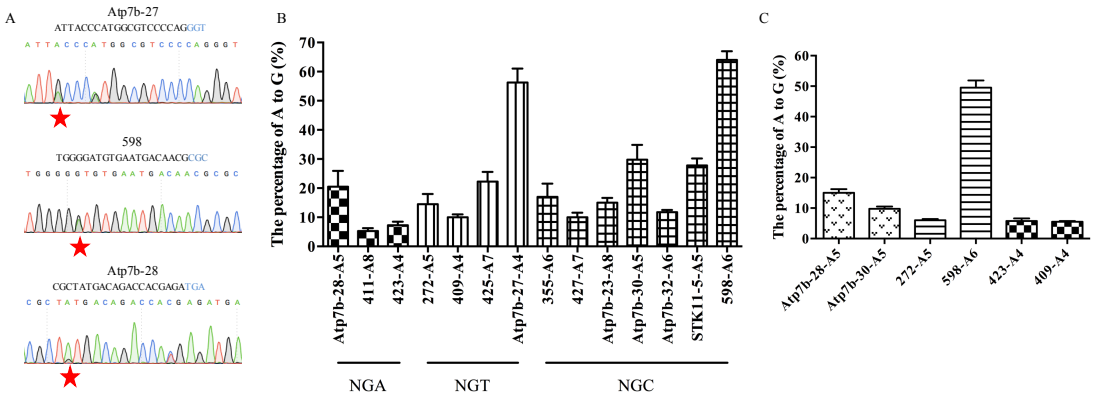
A. Sequence chromatogram of editing pattern of xBE3 and BPNLS-Gam-xBE3 at pathogenic sites 383 and Atp7b-16.

B. Histogram of editing frequency of xBE3 and BPNLS-Gam-xBE3 at each edited position.

C. Chromatogram of representative pathogenic sites with different PAM edited by BPNLS-Gam-xBE3. The PAM sequences are highlighted in blue. Red

stars indicate the desired pathogenic mutations introduced by BPNLS-Gam-xBE3.

Supplementary figure 4



Supplementary figure 4. BPNLS-xABE can efficiently mimic pathogenic mutations in human cells.

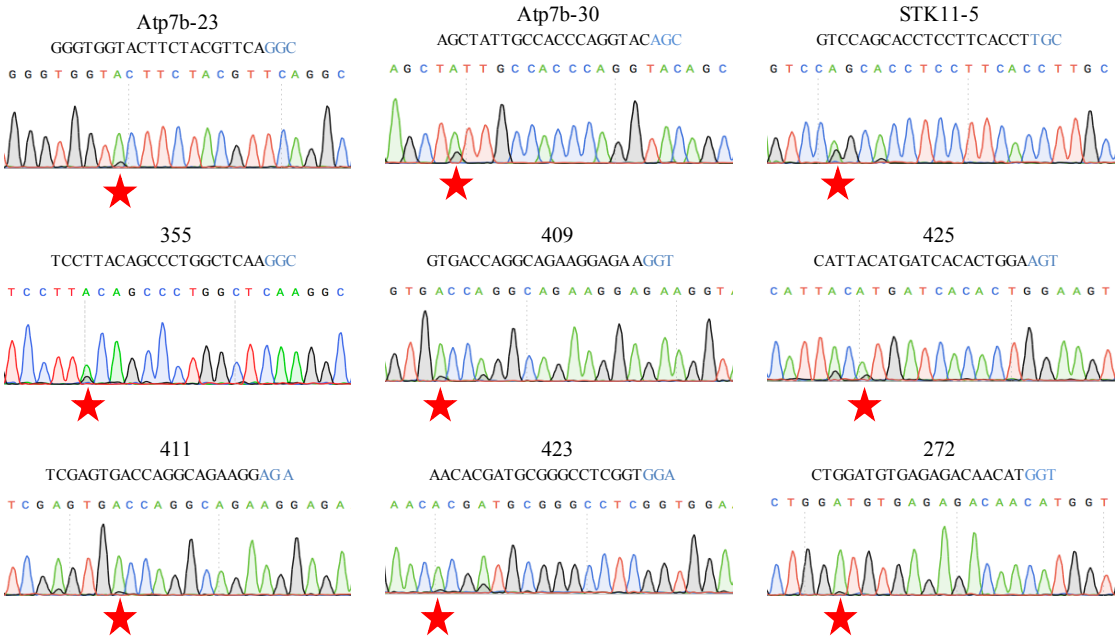
A. Sequence chromatogram of 3 representative pathogenic sites with NGA, NGT or NGC PAM were presented. The PAM sequences are highlighted in blue, red stars indicate the desired pathogenic mutations introduced by BPNLS-xABE.

B. The histogram of editing efficiency of BPNLS-xABE on different pathogenic sites with NGH PAM. The editing frequencies were presented as the percentage of A-to-G conversion, all data were from at least three independent experiments, the target loci with same PAM were underlined and indicated. Data from three independent experiments are shown as means ± s.e.m.

C. The histogram of editing efficiency of BPNLS-xABE with two targeting loci simultaneously in human cells, the sgRNAs transfected simultaneously were filled in same pattern. Data from three independent

experiments are shown as means ± s.e.m.

Supplementary figure 5



Supplementary figure 5. Sequence chromatogram of pathogenic mutations introduced by BPNLS-xABE in human cells. Cells were transfected with BPNLS-xABE and sgRNAs, genomic DNA was extracted and amplified for Sanger sequencing. The sequence chromatogram of representative sites with different PAM were presented, the PAM sequences are highlighted in blue. Red stars indicate the desired pathogenic mutation introduced by BPNLS-xABE.

Supplementary Table 1. sgRNAs from previous study used in this study

sgRNA name	Spacer	PAM
xsite1	GATGACAGGCAGGGGCACCG	CGG
xsite4	GGCACTGCGGCTGGAGGTGG	GGG
xsite6	GCGGTCTCAAGCACTACCTA	CGT
xsite7	GAGCTGCACATACTAGCCCC	TGT
xsite8	GCTTCTCCAGCCCTGGCCTG	GGT
xsite10	GACAGGCAGGGGCACCGCGG	CGC
xsite12	GCAGACGGCAGTCACTAGGG	GGC
xsite13	GTCGCAGGACAGCTTTTCCT	AGA

xsite14	GGGAAGCTGGGTGAATGGAG	CGA
xsite15	GCGGAGACTCTGGTGCTGTG	TGA
xsite16	GGTCAGAAATAGGGGGTCCA	GGA
xsite18	GATCCAGGTGCTGCAGAAGG	GAT
xsite19	GTCACTCCAGGATTCCAATA	GAT

Supplementary Table 2. Pathogenic sites used in this study

Pathogenic sites	Sequence	PAM
337	GGGCACGTTGTGGGTGACGC	CGT
345	GCTGACCGGGACTGGTACTC	AGA
371	GATGACGATCACTGTCGCTA	TGA
379	CCTGCTCGAGACAGGAGCTA	TGC
383	ACCCGTACTCAGCACCTCAG	TGT
387	TAGGTCGCCTCACCTTCAGT	GGA
393	GGCGGCGTGGATAGCCAAAG	GGA
397	CAGCACGAAGGAAATGGGGA	TGA
407	ACTTGGCTTGTGTAAGTGAT	GGT
Atp7b-2	CGTGTCGAAGAATGTCACAG	GGC
Atp7b-16	GATCACGGGGGACAACCGGA	AGA
STK11-2	CAGGTCGGAGATTTTGAGGG	TGC
636	ATACGCGTGCTGCTTGGTTC	AGA
640	CAGGCACAGACAGCACACAT	TGT
272	CTGGATGTGAGAGACAACAT	GGT
355	TCCTTACAGCCCTGGCTCAA	GGC
409	GTGACCAGGCAGAAGGAGAA	GGT
411	TCGAGTGACCAGGCAGAAGG	AGA
423	AACACGATGCGGGCCTCGGT	GGA
425	CATTACATGATCACACTGGA	AGT
427	ATGATCACACTGGAAGTCCC	TGC
ATP7b-23	GGGTGGTACTTCTACGTTCA	GGC
ATP7b-27	ATTACCCATGGCGTCCCCAG	GGT
ATP7b-28	CGCTATGACAGACCACGAGA	TGA
ATP7b-30	AGCTATTGCCACCCAGGTAC	AGC
ATP7b-32	CATCCAGGGCTGCAGCACAA	TGC
STK11-5	GTCCAGCACCTCCTTCACCT	TGC
598	TGGGGATGTGAATGACAACG	CGC
A16-mut-Correction	GTCCCCCATGATCAGAACCA	CGT
A27-mut-Correction	ATGGGCAATGGTGCCAGTCT	TGT

Supplementary Table 3. Primers used for PCR amplification of sgRNAs

Target sgRNAs	Forward	Reverse
xsite1	GAACCCAGGTAGCCAGAGAC	TCCTTTCAACCCGAACGGAG
xsite4	GAACCCAGGTAGCCAGAGAC	TCCTTTCAACCCGAACGGAG

xsite6	GGGGTCCCAGGTGCTGAC	CATTGCAGAGAGGCGTATCA
xsite7	ATGTGGGCTGCCTAGAAAGG	CCCAGCCAAACTTGTCAACC
xsite8	ATGTGGGCTGCCTAGAAAGG	CCCAGCCAAACTTGTCAACC
xsite10	GAACCCAGGTAGCCAGAGAC	TCCTTTCAACCCGAACGGAG
xsite12	GCCCATTCCCTCTTTAGCCA	CTCAACCCCACACGCACA
xsite13	ATGTGGGCTGCCTAGAAAGG	CCCAGCCAAACTTGTCAACC
xsite14	GCCCATTCCCTCTTTAGCCA	CTCAACCCCACACGCACA
xsite15	GAACCCAGGTAGCCAGAGAC	TCCTTTCAACCCGAACGGAG
xsite16	GTCAGAGGGACACACTGTGG	AATGGGCTTTGGAAAGGGGG
xsite18	CATTGCAGAGAGGCGTATCA	GGGGTCCCAGGTGCTGAC
xsite19	GAGGACGTGTGTGTCTGTGT	ATATTGAAGGGGGCAGGGGA
337	CGCACAGGCATCGTGCTG	GAATGCCCGCCGACTCCA
345	TTCTGGCTGCTCGGCTAC	GTCAATGTCCCTGATGTTATGC
371	ACCTCATCCAAATGTCCC	GAATGAAAACCAAAACAAGA
379	TCCCATCTCCACTGTTCC	CAGCTCCCATCATTCTTTT
383	TCCCATCTCCACTGTTCC	CAGCTCCCATCATTCTTTT
387	TTTTATGCTGGGCTATGAC	TCTGGAACATCTGGGTCA
393	GGCACAGCTCCACCTAAT	AAACAGCACAGCCAAGAC
397	GCAGTCCCACTCCTACCCA	GTCCGCACCTTTCCCTAC
407	CTTTCTTGCGTTTCTACTTCC	TGGTACACTGGCCCCTGA
Atp7b-2	ATTTCATAAACGCCCATCA	ATGGAGGTTTCCTATTTC
Atp7b-16	ATGATCGCAATCGCAGAC	CAGCAAGGGAGAAAGAGC
STK11-2	CCTGCTGGACCTAGCCTTTC	ATCTCGGGCGGCTGGAAA
636	TGAACTCCCTGAGTGGTA	ACTGCAATTTGCCCTTAA
640	CATCTGCCAGTTACAAGA	AAAGCATTACTCACCCTC
272	AGGCTGGTCTCAAACTCG	GAAGGCTGGACTCTAAAA
355	TTCTGGCTGCTCGGCTAC	GTCAATGTCCCTGATGTTATGC
409	GACAGGTTGCAGGTGGATT	TGGGACAGAGGTGGGTCT
411	GACAGGTTGCAGGTGGATT	TGGGACAGAGGTGGGTCT
423	TTTTATGCTGGGCTATGAC	TCTGGAACATCTGGGTCA
425	TCCCATCTCCACTGTTCC	CAGCTCCCATCATTCTTTT
427	TCCCATCTCCACTGTTCC	CAGCTCCCATCATTCTTTT
ATP7b-23	ATTTCATAAACGCCCATCA	ATGGAGGTTTCCTATTTC
ATP7b-27	GAACCCTGAGATTGAACGA	CCTTTGTGATAACCTGGAAC
ATP7b-28	CAAGAGGTGCTTACAAGG	GAGCCAGTGGAAAGAATGAA
ATP7b-30	ATGATCGCAATCGCAGAC	CAGCAAGGGAGAAAGAGC
ATP7b-32	TGTGAGTGCGAGTTCTTTCTT	CCTGCTCATGGTGCTGATA
STK11-5	TCGACTCCACCGAGGTCA	CAAGAGCCCATGCAAACG
598	CCTCAGCACCGCCTAACT	CTGGGCAGAAGAGCCACA
A16-mut-Correct	ATGATCGCAATCGCAGAC	CAGCAAGGGAGAAAGAGC
A27-mut-Correct	GAACCCTGAGATTGAACGA	CCTTTGTGATAACCTGGAAC

Supplementary Table 4. Primers used for on-target deep sequencing

On-targets	Forward	Reverse
xsite4	TGACCAAGAACCCAGGTAGCCAGAGAC	TGACCAATCCTTTCAACCCGAACGGAG
xsite6	AGTTCCGGGGTCCCAGGTGCTGAC	AGTTCCCATTGCAGAGAGGCGTATCA
xsite7	CCGTCCATGTGGGCTGCCTAGAAAGG	CCGTCCCCCAGCCAAACTTGTCAACC
xsite12	GAGTGGAGCCCATTCCCTCTTTAGCCA	GAGTGGACTCAACCCCACACGCACA
xsite13	GTGAAATGATGTGGGCTGCCTAGAAAGG	GTGAAATGCCCAGCCAAACTTGTCAACC
xsite14	CACCGGTGGCCCATTCCCTCTTTAGCCA	CACCGGTGCTCAACCCCACACGCACA
xsite15	CTTGTAGAACCCAGGTAGCCAGAGAC	CTTGTATCCTTTCAACCCGAACGGAG
xsite16	TCCCGAGTCAGAGGGACACACTGTGG	TCCCGAAATGGGCTTTGGAAAGGGGG
xsite18	TCCCGATGGGGGTCCCAGGTGCTGAC	TCCCGATGCATTGCAGAGAGGCGTATCA
xsite19	TACAGCGAGGACGTGTGTGTCTGTGT	TACAGCATATTGAAGGGGGCAGGGGA
383	CACTGGAAGTCCCTGCTC	CTCCCATCATTCTTTTCTACTC
387	TTTTATGCTGGGCTATGA	TGCCTGGCTCCTTCCTAC

Supplementary Table 5. Primers used for off-target deep sequencing

Off-targets	Forward	Reverse
387-OT1	GAATAAAGGCAAGGCAAAC	AATGAGAAAGGAGGAGGG
387-OT2	TAACCTGCCCAAGTTTGC	CCCTTCCCGACTTTCTTC
387-OT3	TCAAGAAGATAAAGCGAAGA	TCACATTGATGAGGCAGT
387-OT4	AGTGATCGGGCTAAGTCA	TCTCATCGGTGAAACTGG
387-OT5	CCAGATTTTGTCCCACTC	GAAATAAAGGCAAAGTTCTA
387-OT6	ACCTGTCTGTCCCTTCCA	AGGGTGCTTTGACCTTTT
387-OT7	AGCCAGAAATACCAAATCC	CTGCCATGAAGCACAGAG
387-OT8	AGAATGGGATGAAATGGA	AAGGATGGACTCTTGGGT
387-OT9	GCCGTGTCCTGAATGGTG	AAAGGCTGCTGGGTTTGG
387-OT10	CTTTGCCATCGGTCTTTG	GCTTAGCGACTACAGGGA
387-OT11	TTTTCAGAGTGTTCACCCAT	CCAGCAACAACATTCTTACCT
387-OT12	AGGCCCTCAGTAAGTTGG	AGAGTGGCGATTTCTATC
387-OT13	GAGACAAGCTGCTTCCTG	TTTTCTCCAAAGTACAAC
387-OT14	GCTATACTGCCATCACTT	GTAACATTCCTGGGGTAA
387-OT15	ACCTGCGTCCCTCCTACA	CTCCGAAGCTCCAGTGCTAT
387-OT16	GATGAAGCCACTGTTAGG	AAGGCATGTGGTAAACTC
383-OT1	CACCACGGGCACATAGAC	GGGAAGGGTACTGGGTTC
383-OT2	ACGCAGCCTTCATGTTAA	ACACTGCTCCAGGGACAC
383-OT3	TTCCTACCTTGGTGCTAT	GACTTCTGGGATGTCTGG
383-OT4	GGCAGAATGGCTTTGTTT	GCATCATTGCATTGGATA
383-OT5	GCAGCCAAAGATGAAGAG	ATGGGTGAAATTATGACTGAAC
383-OT6	TGTTCCCTTCCCTTTCTT	AGAGCCACTGGACTTTGAG
383-OT7	TGGCTGGAGCGAAATAAA	CGTAGTGGGCACATCATAG
383-OT8	GGGCATTGGCACCTGTCT	ACCCGCTCACTGGATGGA
383-OT9	AACAACCCTATCACCACT	AACAACCCTATCACCACT
383-OT10	TGAGGTAAACGGATGGTA	ATCAATGGGAAGAGTGGG

383-OT11	ATGGCTGCAACAACCTTC	ACCTATCTCCCTCCTCCC
383-OT12	GTGCGCTGCCGTAAATCT	TTCCCCTGGTAAACATCC
383-OT13	TCACTATGTCATGGAAGAGC	AAGAAAAGGAAAGGGAGA
383-OT14	CTCACTTTGACAGGGCTTGG	GGCAGAACAGGGATTGGA
383-OT15	GCTGGGTGAATGAAAGAG	GTGTTGCGGATAACCTTT
383-OT16	CTCCACGCCCACCTAACA	AGCCTGAATCCCACCTCC