Supplementary Data

A “new lease of life”: FnCpf1 possesses DNA cleavage activity for genome editing in human cells

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Supplementary Data

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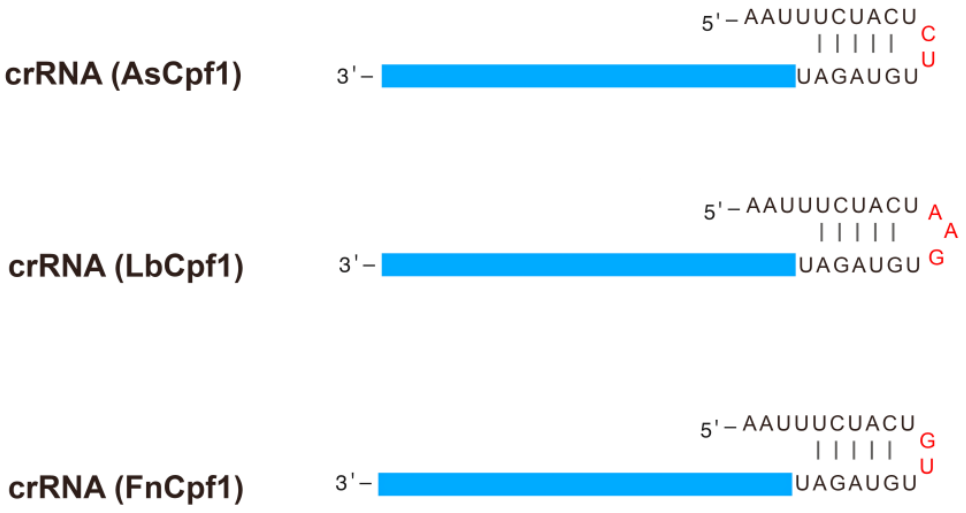

Figure S1.Schematic representation of three crRNA structures.

The difference among these three Cpf1 (AsCpf1, LbCpf1 and FnCpf1) family members is shown in red.

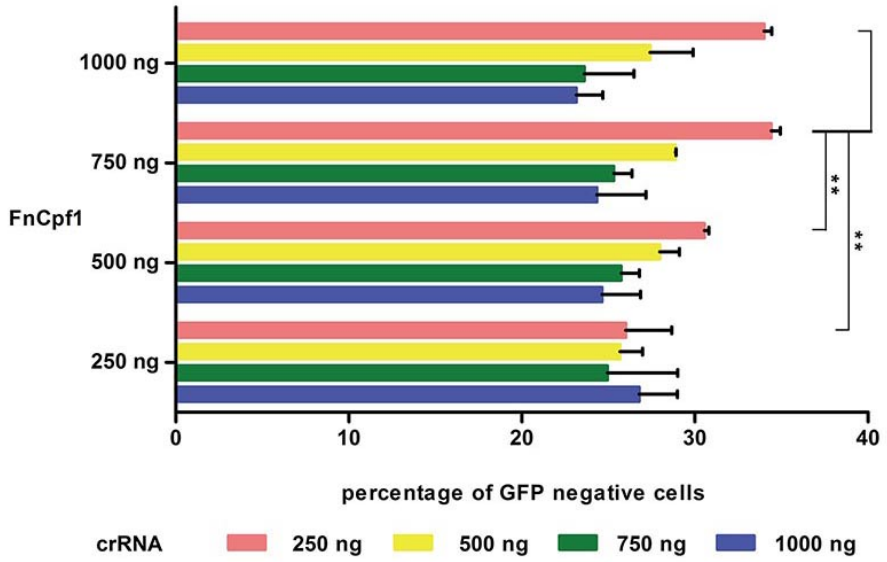

Figure S2.The effects of plasmids amount for the cleavage.

The cells were co-transfected with different amounts of expression plasmids for FnCpf1 and crRNA. The cleavage was saturated with 750 ng and 250 ng plasmids coding for FnCpf and crRNA, respectively. Error bars, s.e.m.; n=3. **P < 0.01.

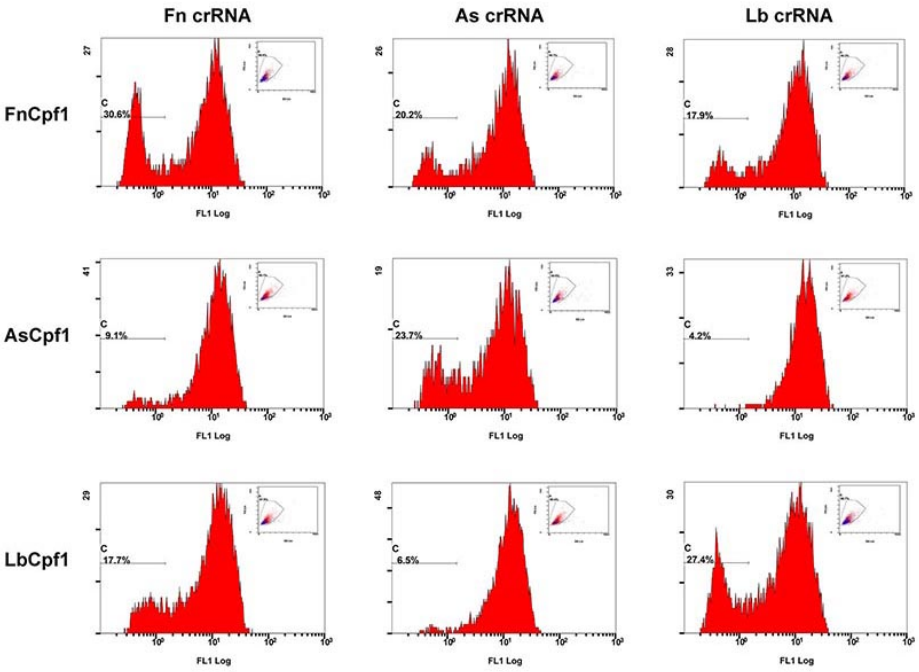

Figure S3.The cleavage efficiency of different Cpf1 with three different direct repeats.

The cleavage was the highest under the condition of each Cpf1 ortholog (FnCpf1, AsCpf1 and LbCpf1) with its own direct repeat.

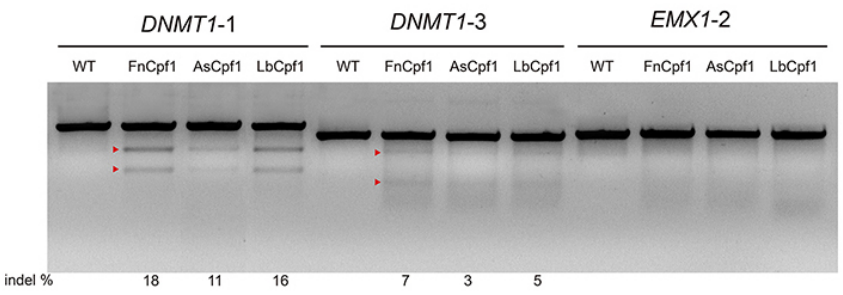

Figure S4. Gene-editing efficiency at *DNMT1* and *EMX1* loci

DNA cleavage has been observed at *DNMT1*-1 and *DNMT1*-3 with FnCpf1, respectively. No activity has been detected at *EMX1*-2 with FnCpf1, AsCpf1 and LbCpf1.Bands marker with red arrow is the cleavage bands.

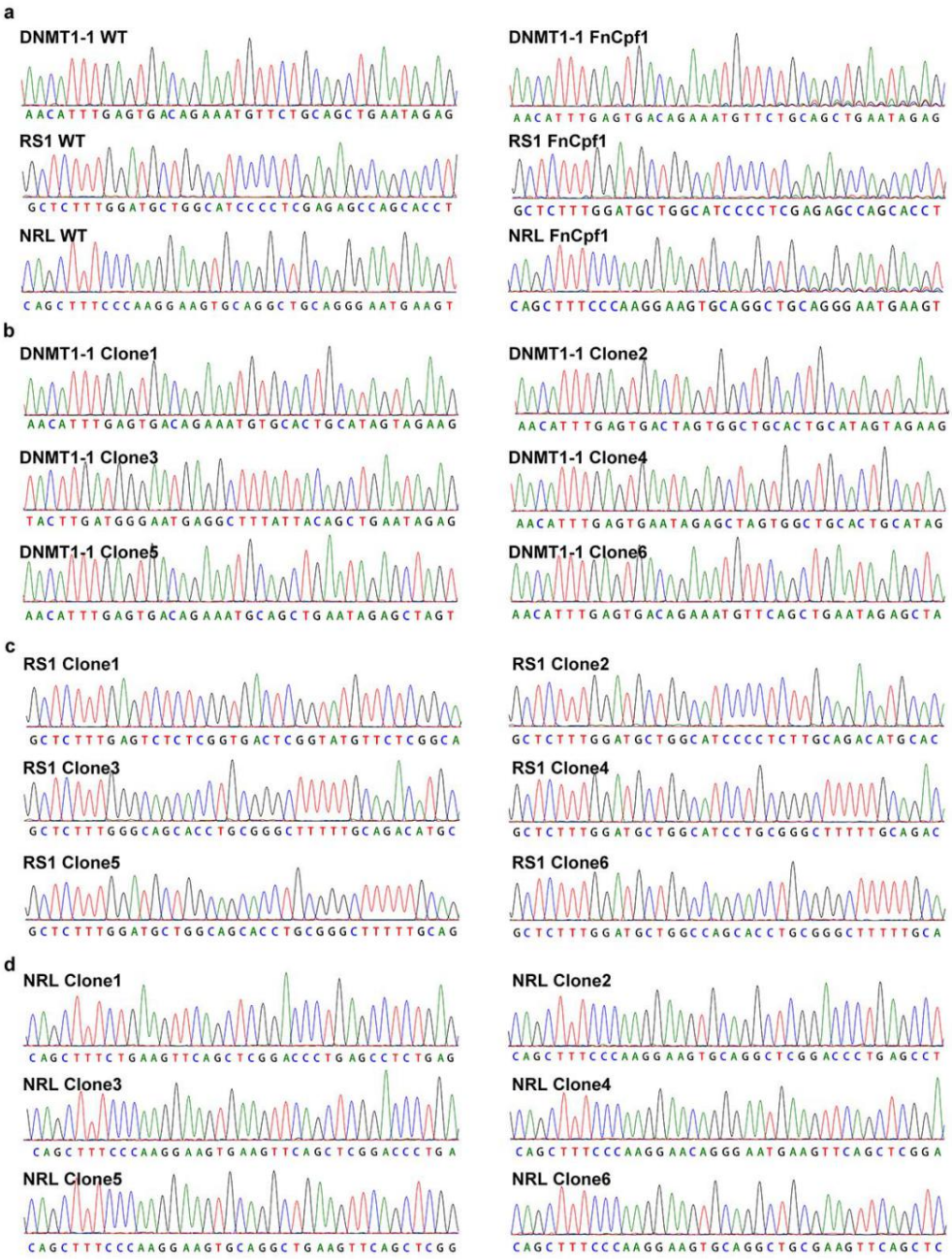

Figure S5. DNA sequencing chromatograms of *DNMT1*-1, *RS1* and *NRL* loci.

(a)DNA sequencing chromatograms of the fragment with PCR templates from the cells transfected with FnCpf1 has additional peaks, compared with that of no transfection. (b,c,d) DNA sequence analysis of single individual clone with indel.

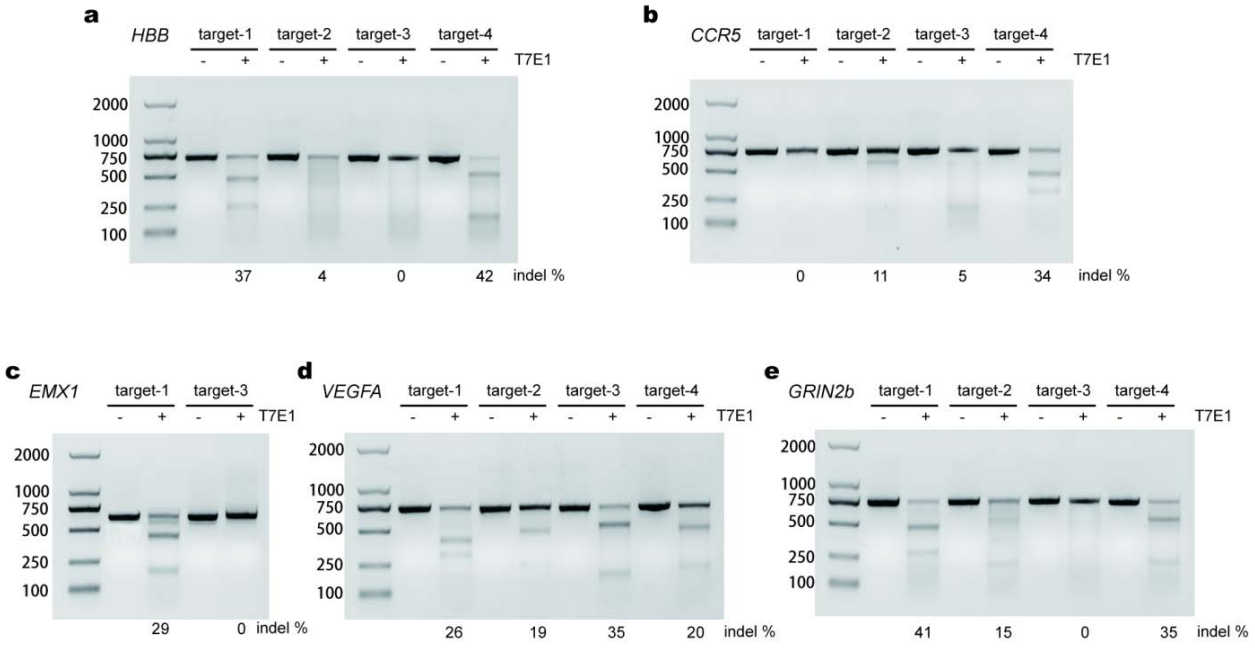

Figure S6. FnCpf1 mediated gene-editing at multiple human genomic loci.

FnCpf1 has the activity at *HBB, CCR5, EMX1, VEGFA, and GRIN2b.* We observed different activities with the different crRNA targeting the same gene.

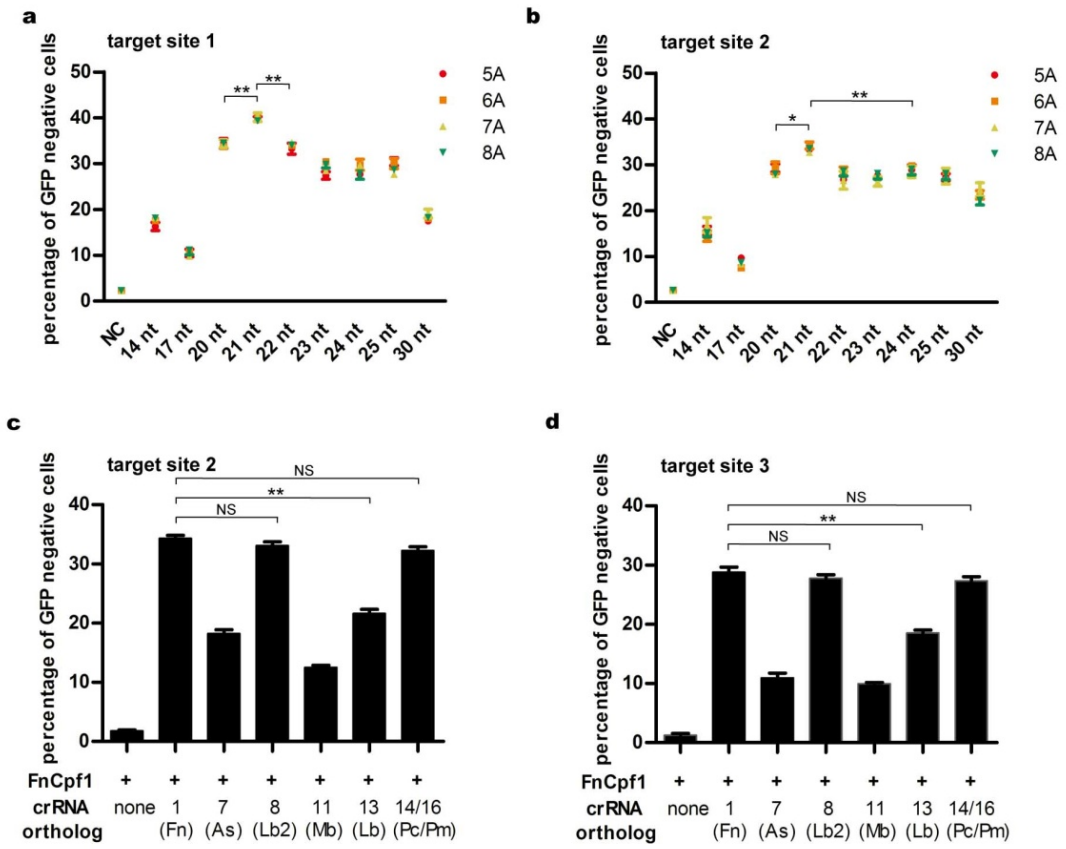

Figure S7.The effects of U length and direct repeats on FnCpf1-mediated gene editing in human cells

(a,b)With different U length of crRNA, no significant difference has been observed. (c,d) Direct repeats from Lb2Cpf1, Pc/PmCpf1 had the same DNA cleavage efficiency as direct repeats from FnCpf1 when they were used with FnCpf1. Error bars, s.e.m.; n=3. **P < 0.01. NS: not significant.

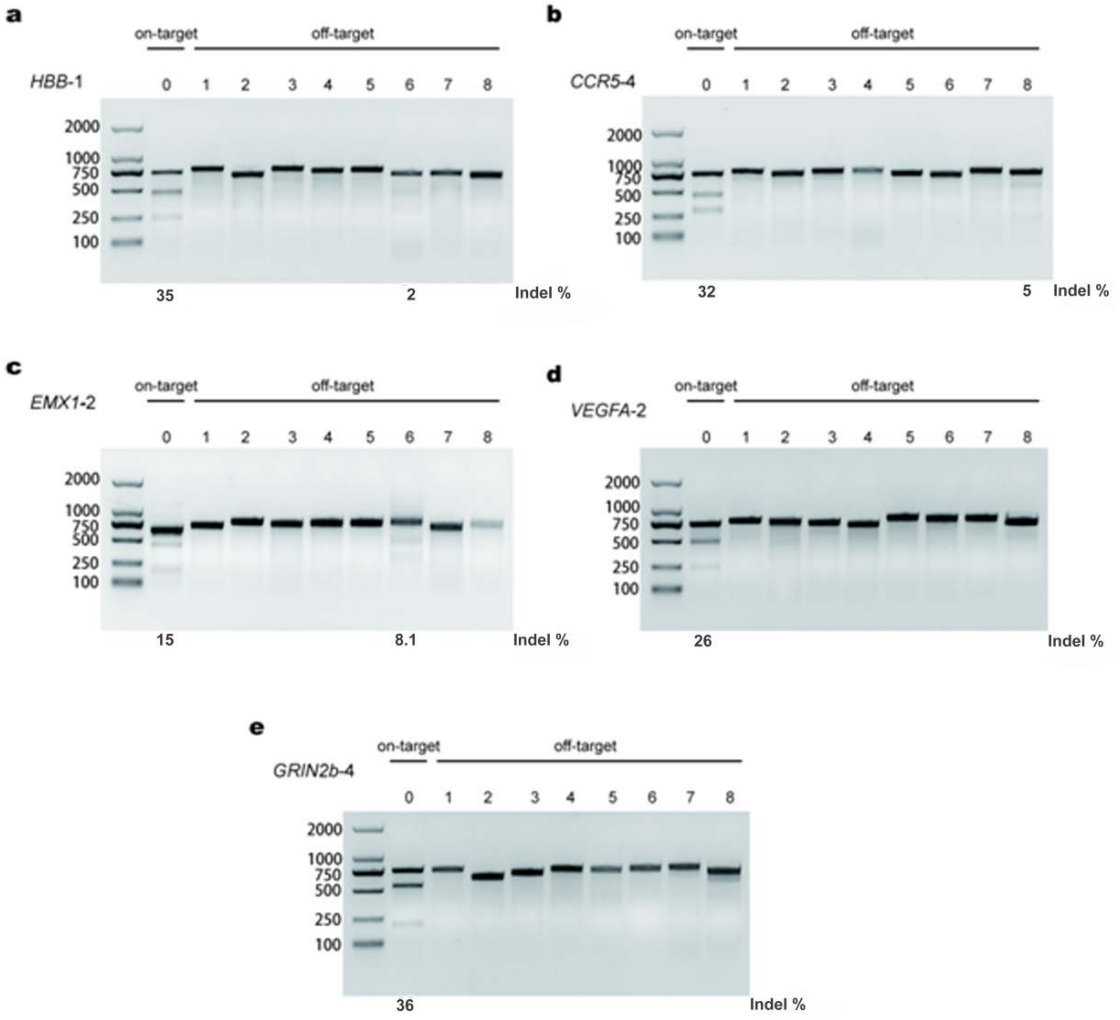

Figure S8.Off-target effects of FnCpf1-mediated gene editing in human cells

The off-target sites were predicted with online software (http://www.rgenome.net/cas-offinder/). Off-targets of five target sequence at different genes were investigated. The results showed that there are detectable off-target effects of FnCpf1 at endogenous genes (*HBB*, Off-target 6; *CCR5*, Off-target 8; *EMX1*, Off-target 6).

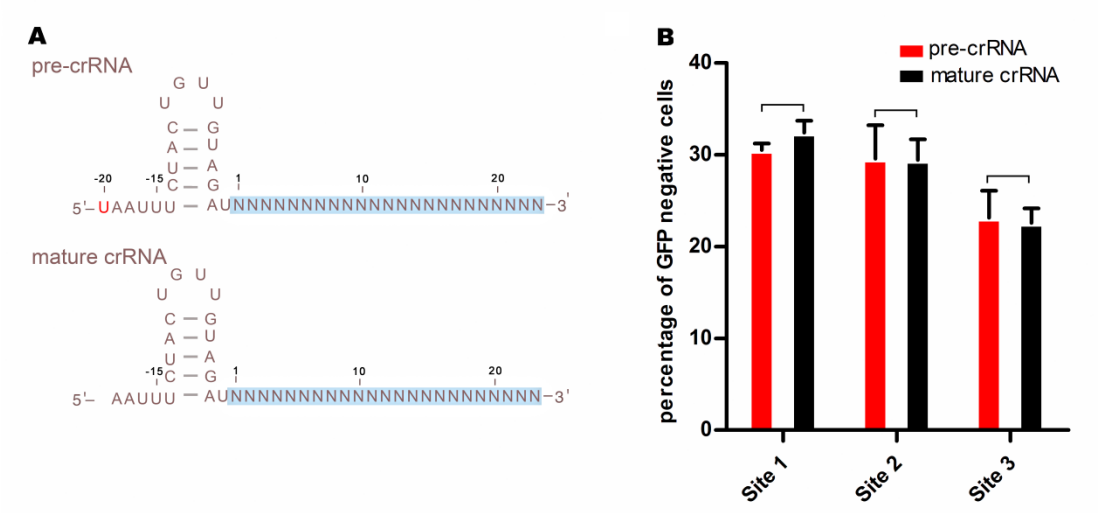

Figure S9.The activities of the pre-crRNA and the mature crRNA.

(a) Schematic representation of pre-crRNA (U+) and mature crRNA (U-) structures. The difference between these two crRNAs is shown in red. (b) Effects of pre-crRNA and mature crRNA on the ability to induce indels at three *GFP* targets. The results show no difference between two groups ($P > 0.05$). Error bars, s.e.m.; n = 3. Significance was calculated using Student's t-test.

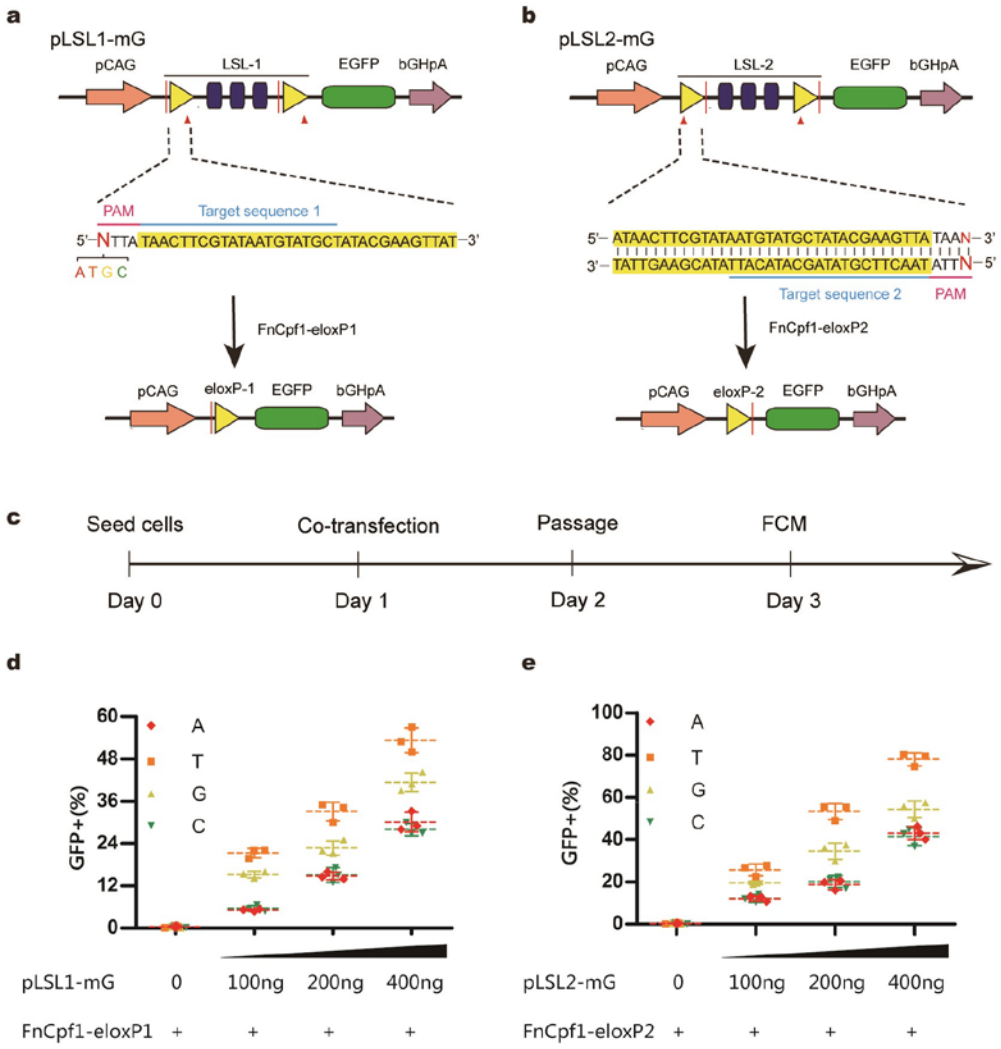

Figure S10. Effects of fourth position of the FnCpf1 PAM for the cleavage

(a,b) Schematic representation and target/PAM information of loxP-STOP-loxP-mG/FnCpf1. After the FnCpf1 mediated DNA double strands break via targeting loxP flanking STOP-cassette, the expression of the *EGFP* gene would be directly driven by the CAG promoter. (c) Illustration of protocol used for loxP-STOP-loxP/FnCpf1. (d,e) Co-transfection of 250 ng plasmids coding for FnCpf1 and 100 ng plasmids coding for crRNA target loxP (labeled these two plasmids as FnCpf1-eloxP) plus different amount of plasmid harboring loxP-STOP-loxP-mG fragment (labeled as pLSL-mG) per well in a 12-well plate. With the increase of the pLSL-mG, more cells in green have been observed. G and T at fourth position of PAM have higher cleavage efficiency, compared with A and C.

Supplementary Table 1. Different crRNA sequences with Cpf1orthologs.

Primer Description	Primer sequence (5`-3`)
AsCpf1 cRNA with TTTN PAM	CAAAAAACCTGGTCGAGCTGGACGGCGACGATCTACAAGAGTAGAAATTCGGTG
AsCpf1 crRNA with GCTN PAM	CAAAAAACGTGCTGCTTCATGTGGTCGGGGATCTACAAGAGTAGAAATTCGGTG
AsCpf1 crRNA with CATN PAM	CAAAAAATGCTTCAGCCGCTACCCCGACCAATCTACAAGAGTAGAAATTCGGTG
LbCpf1 crRNA with TTTN PAM	CAAAAAACCTGGTCGAGCTGGACGGCGACGATCTACACTTAGTAGAAATTCGGTG
LbCpf1 crRNA with GCTN PAM	CAAAAAACGTGCTGCTTCATGTGGTCGGGGATCTACACTTAGTAGAAATTCGGTG
LbCpf1 crRNA with CATN PAM	CAAAAAATGCTTCAGCCGCTACCCCGACCAATCTACACTTAGTAGAAATTCGGTG
FnCpf1 crRNA at *DNMT1*-1	CAAAAAAAGCTGCAGAACATTTCTGTCACTATCTACAACAGTAGAAATTCGGTG
AsCpf1 crRNA at *DNMT1*-1	CAAAAAAAGCTGCAGAACATTTCTGTCACTATCTACAAGAGTAGAAATTCGGTG
LbCpf1 crRNA at *DNMT1*-1	CAAAAAAAGCTGCAGAACATTTCTGTCACTATCTACACTTAGTAGAAATTCGGTG
FnCpf1 crRNA at *RS1*	CAAAAAAGCTCTCGAGGGGATGCCAGCATCATCTACAACAGTAGAAATTCGGTG
AsCpf1 crRNA at *RS1*	CAAAAAAGCTCTCGAGGGGATGCCAGCATCATCTACAAGAGTAGAAATTCGGTG
LbCpf1 crRNA at *RS1*	CAAAAAAGCTCTCGAGGGGATGCCAGCATCATCTACACTTAGTAGAAATTCGGTG
FnCpf1 crRNA at *NRL*	CAAAAAACCCTGCAGCCTGCACTTCCTTGGATCTACAACAGTAGAAATTCGGTG
AsCpf1 crRNA at *NRL*	CAAAAAACCCTGCAGCCTGCACTTCCTTGGATCTACAAGAGTAGAAATTCGGTG
LbCpf1 crRNA at *NRL*	CAAAAAACCCTGCAGCCTGCACTTCCTTGGATCTACACTTAGTAGAAATTCGGTG
FnCpf1 crRNA at *DNMT1*-3	CAAAAAAGAGTAACAGACATGGACCATCAGATCTACAACAGTAGAAATTACGGTG
AsCpf1 crRNA at *DNMT1*-3	CAAAAAAGAGTAACAGACATGGACCATCAGATCTACAAGAGTAGAAATTACGGTG
LbCpf1 crRNA at *DNMT1*-3	CAAAAAAGAGTAACAGACATGGACCATCAGATCTACACTTAGTAGAAATTCGGTG
FnCpf1 crRNA at *EMX1*-2	CAAAAAAGGTGTGGTTCCAGAACCGGAGGAATCTACAACAGTAGAAATTACGGTG
AsCpf1 crRNA at *EMX1*-2	CAAAAAAGGTGTGGTTCCAGAACCGGAGGAATCTACAAGAGTAGAAATTACGGTG
LbCpf1 crRNA at *EMX1*-2	CAAAAAAGGTGTGGTTCCAGAACCGGAGGAATCTACACTTAGTAGAAATTCGGTG
FnCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAACAGTAGAAATTACGGTG
Lb3Cpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGGCATGAGAACCATGCTTTCTCGGTG
BpCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGACCTAATTACTAGGTAATTTTCGGTG
PeCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAAAAGTAGAAATCCCGGTG
PbCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAAAAGTAGAAATTTCGGTG
SsCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGGTCGCGCCCCGCGTGGGCGCGCGGTG
AsCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAAGAGTAGAAATTACGGTG
Lb2Cpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAATAGTAGAAATTCCGGTG
CMtCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAAAGAGTAGAGATTCCGGTG
EeCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAAAGTAGAAATTACGGTG
MbCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAAACAGTAGAAATTTCGGTG
LiCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAAAAGTAGAAATTCCGGTG
LbCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACACTTAGTAGAAATTACGGTG
PcCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAATAGTAGAAATTACGGTG
PdCpf1 crRNA at *GFP* site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACCGAAGTAGAAATTACGGTG
PmCpf1 crRNA at GFP site 1	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAATAGTAGAAATTACGGTG
FnCpf1 crRNA at GFP site 2	CAAAAAAGTTCACCAGGGTGTCGCCCTCATCTACAACAGTAGAAATTCGGTG
AsCpf1 crRNA at GFP site 2	CAAAAAAGTTCACCAGGGTGTCGCCCTCATCTACAAGAGTAGAAATTACGGTG
Lb2Cpf1 crRNA at GFP site 2	CAAAAAAGTTCACCAGGGTGTCGCCCTCATCTACAATAGTAGAAATTCGGTG

MbCpf1 crRNA at GFP site 2	CAAAAAAGTTCACCAGGGTGTCGCCCTCATCTACAAACAGTAGAAATTCGGTG
LbCpf1 crRNA at GFP site 2	CAAAAAAGTTCACCAGGGTGTCGCCCTCATCTACACTTAGTAGAAATTCGGTG
PcCpf1 crRNA at GFP site 2	CAAAAAAGTTCACCAGGGTGTCGCCCTCATCTACAATAGTAGAAATTACGGTG
FnCpf1 crRNA at GFP site 3	CAAAAAACATGTGGTCGGGGTAGCGGCTATCTACAACAGTAGAAATTCGGTG
AsCpf1 crRNA at GFP site 3	CAAAAAACATGTGGTCGGGGTAGCGGCTATCTACAAGAGTAGAAATTACGGTG
Lb2Cpf1 crRNA at GFP site 3	CAAAAAACATGTGGTCGGGGTAGCGGCTATCTACAATAGTAGAAATTCGGTG
MbCpf1 crRNA at GFP site 3	CAAAAAACATGTGGTCGGGGTAGCGGCTATCTACAAACAGTAGAAATTCGGTG
LbCpf1 crRNA at GFP site 3	CAAAAAACATGTGGTCGGGGTAGCGGCTATCTACACTTAGTAGAAATTCGGTG
PcCpf1 crRNA at GFP site 3	CAAAAAA CATGTGGTCGGGGTAGCGGCT ATCTACAATAGTAGAAATTACGGTG
FnCpf1 crRNA at *HBB*-1	CAAAAAATATCATGCCTCTTTGCACCATTCATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *HBB*-2	CAAAAAATTGTATCATTATTGCCCTGAAAGATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *HBB*-3	CAAAAAATTTCTTTCAGGGCAATAATGATAATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *HBB*-4	CAAAAAAGATATTGCTATTGCCTTAACCCAATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *CCR5*-1	CAAAAAACAGCCCAGGCTGTGTATGAAAACATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *CCR5*-2	CAAAAAACTGCTCCCCAGTGGATCGGGTGTATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *CCR5*-3	CAAAAAATCTGTGGGCTTGTGACACGGACTATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *CCR5*-4	CAAAAAATTGACAAACTCTCCCTTCACTCCATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *EMX1*-1	CAAAAAAGGTGTGGTTCCAGAACCGGAGGAATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *EMX1*-3	CAAAAAATTGTACTTTGTCCTCCGGTTCTGATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *VEGFA*-1	CAAAAAACGTCTGCACACCCCGGCTCTGGCATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *VEGFA*-2	CAAAAAAGGACCCCCTATTTCTGACCTCCCATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *VEGFA*-3	CAAAAAATGTACATGAAGCAACTCCAGTCCATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *VEGFA*-4	CAAAAAAGAGAGGGACACACAGATCTATTGATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *GRIN2b*-1	CAAAAAAGTTAAAATAGGATCTACATCACGATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *GRIN2b*-3	CAAAAAAGGCACTTCCGACGAGGTGGCCATATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *GRIN2b*-4	CAAAAAACAATGAAAGGAGATAAGGTCCTTATCTACAACAGTAGAAATTCGGTG
FnCpf1 crRNA at *eloxP*1	**CAAAAAAAGCATACATTATACGAAGTTAATCTACAACAGTAGAAATTCGGTG**
FnCpf1 crRNA at *eloxP*2	**CAAAAAAAATGTATGCTATACGAAGTTAATCTACAACAGTAGAAATTCGGTG**

Supplementary Table 2. Different parameter of crRNA with FnCpf1.

Index	Primer Description	Primer sequence (5`-3`)
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4	#14nt 8A	CAAAAAAAAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
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6	#17nt 5A	CAAAAACGAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
7	#17nt 7A	CAAAAAAACGAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
8	#20nt	CAAAAAAGGTCGAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
9	#20nt 5A	CAAAAAGGTCGAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
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56	#23nt 5A	CAAAAACGGTTCACCAGGGTGTCGCCCTCATCTACAACAGTAGAAATTCGGTG
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74	pre-crRNA at *GFP* site 1	CAAAAAACCTGGTCGAGCTGGACGGCGACGATCTACAACAGTAGAAATTACGGTG
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76	pre-crRNA at *GFP* site 3	CAAAAAATTCATGTGGTCGGGGTAGCGGCTATCTACAACAGTAGAAATTACGGTG

77	crRNA with TTA PAM	CAAAAAATGGTCGAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
78	crRNA with TAA PAM	CAAAAAAACACGCTGAACTTGTGGCCGTATCTACAACAGTAGAAATTCGGTG
79	crRNA with TCA PAM	CAAAAAAGCGATGCCACCTACGGCAAGCATCTACAACAGTAGAAATTCGGTG
80	crRNA with TGA PAM	CAAAAAATGAGCAAGGGCGAGGAGCTGTATCTACAACAGTAGAAATTCGGTG
81	crRNA with ATC PAM	CAAAAAACGGCATCAAGGTGAACTTCAAATCTACAACAGTAGAAATTCGGTG
82	crRNA with GTC PAM	CAAAAAAGTTTACGTCGCCGTCCAGCTCATCTACAACAGTAGAAATTCGGTG
83	crRNA with CTC PAM	CAAAAAAGGGGTGGTGCCCATCCTGGTCATCTACAACAGTAGAAATTCGGTG
84	crRNA with AAC PAM	CAAAAAACGGCGACGTAAACGGCCACAAATCTACAACAGTAGAAATTCGGTG
85	crRNA with AGC PAM	CAAAAAAGGTGAACAGCTCCTCGCCCTTATCTACAACAGTAGAAATTCGGTG
86	crRNA with ACC PAM	CAAAAAAGGTGGTGCAGATGAACTTCAGATCTACAACAGTAGAAATTCGGTG
87	crRNA with GAC PAM	CAAAAAACTTGTGGCCGTTTACGTCGCCATCTACAACAGTAGAAATTCGGTG
88	crRNA with GGC PAM	CAAAAAAGGAGCTGTTCACCGGGGTGGTATCTACAACAGTAGAAATTCGGTG
89	crRNA with GCC PAM	CAAAAAAGAGCTGGACGGCGACGTAAACATCTACAACAGTAGAAATTCGGTG
90	crRNA with CAC PAM	CAAAAAAGAGGAGCTGTTCACCGGGGTGATCTACAACAGTAGAAATTCGGTG
91	crRNA with CGC PAM	CAAAAAAGCCCGAAGGCTACGTCCAGGAATCTACAACAGTAGAAATTCGGTG
92	crRNA with CCC PAM	CAAAAAAGCCGTCCAGCTCGACCAGGATATCTACAACAGTAGAAATTCGGTG
93	crRNA with TTT PAM	CAAAAAAGGTCGAGCTGGACGGCGACGTATCTACAACAGTAGAAATTCGGTG
94	crRNA with TTG PAM	CAAAAAACATCTTCTTCAAGGACGACGGATCTACAACAGTAGAAATTCGGTG
95	crRNA with TTC PAM	CAAAAAACAGGATGGGCACCACCCCGGTATCTACAACAGTAGAAATTCGGTG
96	crRNA with CTA PAM	CAAAAAATGCTGCTTCATGTGGTCGGGGATCTACAACAGTAGAAATTCGGTG
97	crRNA with CTT PAM	CAAAAAAGAGGGCGATGCCACCTACGGCATCTACAACAGTAGAAATTCGGTG
98	crRNA with CTG PAM	CAAAAAAGTGGCCGTTTACGTCGCCGTCATCTACAACAGTAGAAATTCGGTG
99	mismatch crRNA spacer 21nt (C1U)	CAAAAAATGGTCGAGCTGGACGGCGACAATCTACAACAGTAGAAATTCGGTG
100	mismatch crRNA spacer 21nt (G5A)	CAAAAAATGGTCGAGCTGGACGGTGACGATCTACAACAGTAGAAATTCGGTG
101	mismatch crRNA spacer 21nt (C10U)	CAAAAAATGGTCGAGCTGAACGGCGACGATCTACAACAGTAGAAATTCGGTG
102	mismatch crRNA spacer 21nt (U15C)	CAAAAAATGGTCGGGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
103	mismatch crRNA spacer 21nt (C20U)	CAAAAAATAGTCGAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
104	mismatch crRNA spacer 23nt (C1U)	CAAAAAACCTGGTCGAGCTGGACGGCGACAATCTACAACAGTAGAAATTCGGTG
105	mismatch crRNA spacer 23nt (G5A)	CAAAAAACCTGGTCGAGCTGGACGGTGACGATCTACAACAGTAGAAATTCGGTG
106	mismatch crRNA spacer 23nt (C10U)	CAAAAAACCTGGTCGAGCTGAACGGCGACGATCTACAACAGTAGAAATTCGGTG
107	mismatch crRNA spacer 23nt (U15C)	CAAAAAACCTGGTCGGGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
108	mismatch crRNA spacer 23nt (C20U)	CAAAAAACCTAGTCGAGCTGGACGGCGACGATCTACAACAGTAGAAATTCGGTG
109	Upstream loxp-GTTA	GACTAGTCGTTATAACTTCGTATAATGTATGC
110	Upstream loxp-ATTA	GACTAGTCATTATAACTTCGTATAATGTATGC

111	Upstream loxp-TTTA	GACTAGTCTTTATAACTTCGTATAATGTATGC
112	Upstream loxp-CTTA	GACTAGTCCTTATAACTTCGTATAATGTATGC
113	pLSL1 Downstream loxp-GTTA	GGAATTCGTTATAACTTCGTATAGCATACAT
114	pLSL1 Downstream loxp-ATTA	GGAATTCATTATAACTTCGTATAGCATACAT
115	pLSL1 Downstream loxp-TTTA	GGAATTCTTTATAACTTCGTATAGCATACAT
116	pLSL1 Downstream loxp-CTTA	GGAATTCCTTATAACTTCGTATAGCATACAT
117	Plsl2 first Downstream loxp-GTTA	GCATACATTATACGAAGTTATAACATTAAGGGTTCCG
118	pLSL2 first Downstream loxp-ATTA	GCATACATTATACGAAGTTATAATATTAAGGGTTCCG
119	pLSL2 first Downstream loxp-TTTA	GCATACATTATACGAAGTTATAAAATTAAGGGTTCCG
120	pLSL2 first Downstream loxp-CTTA	GCATACATTATACGAAGTTATAAGATTAAGGGTTCCG
121	pLSL2 second Downstream universal primer	GGAATTCATAACTTCGTATAGCATACATT

Supplementary Table 3.Potential off-target sites of four genes.

Gene	Chrom.	Position	Target Sequence (5'-3')	Mismatch	Target Primers (5'-3')
HBB	Chr11	5225953	TTAGAATGGTGCAAAGAGGCATGA	0	F:GTGTGGAAGTCTCAGGATCGT R:AGGAGCTGTGGGAGGAAGAT
OTS-1	chr8	10253144	CTCGAATGGTaaAAAGAGGCATtt	4	F:GCTTCTGCAAATTGGCTTCC R:CCTGTTCCTCAGCAGTGATTTA
OTS-2	chr8	69030676	TTTGAATGGTGCcAAGAGttAaGA	4	F:GGTGGTCAGCTAATTATGTGGTA R:CAGGCCTCTGGCATTAAAGA
OTS-3	chr12	25113068	CTGGAATGGTGCAggGAaGCcTGA	4	F:CTTGGGTGTTTCAATCGTCATATT R:ACAGAGATAGCCTGGAATTCATC
OTS-4	chr3	69071019	TTGGAATGGaaCAAAGAGGgATGg	4	F:CAGTAACAGATGCAAACTTCCTAAA R:CCTCCAGTTGATGTCAGAAGAG
OTS-5	chr7	127142965	TTGGAgTGGTGgAAAGAtGCATGg	4	F:GGACAGCGAGACAGATTGATT R:GCAATGATGGGCTTATGCTTATAC

OTS-6	chr13	31718471	TTAGAATGaTGCAAAaAGGCATat	4	F:GTGCCTTCTTCCCAGACATAG
					R:AGTGACGAAAGGAAAGGATGAG
OTS-7	chr14	86891761	CTTGAATGaTGgAAAGAtGCATaA	4	F:CTGGAGGTGTCTTCACATCATAG
					R:CCCTGCACAACTCCTTATCTAC
OTS-8	chr9	123514207	CTTGAATGGTGCAAtGAGGaATGc	3	F:GGGCAAGGTAGGGTTCTATTT
					R:GACCCACGATAGGCACTTTAT
CCR5	**chr3**	**46374225**	**TTCGGAGTGAAGGGAGAGTTTGTC**	**0**	**F:TGCTGCATCAACCCCATCAT**
					R:CACAAGTCTCTCGCCTGGTT
OTS-1	chr8	110258614	TTAGGAGTGgAGGGAGAGTgTGaC	3	F:GAGTTGTGAGGACCATGAGAAA
					R:GAGACAGATTAATTTAAGGCCTTT
OTS-2	chr2	42804972	CTTGGAGgGgAGGGAGAGTTTGTt	3	F:CAGGATTCAGACACACCAGTAG
					R:ATCCTCCAAACAGGGACATTT
OTS-3	chr15	63193438	TTTGGAGTGAAaGGcGAGTTgGTC	3	F:GCCCAGGAGTTCAAGGTTATTA
					R:ATGCATCCATTTCCCAGAGG
OTS-4	chr6	152746891	TTTGGAGTtAAGGGAGAagTTGTC	3	F:TGTGTGTTCCTGTGATTTCTCT
					R:CTCCTGTTTGGCAGATATTTAAGA
					T
OTS-5	chr8	109541705	CTGGGAGTGAAcGcAGAGTTTccC	4	F:TTGGTGATCTAGAGGGCATTTC
					R:TCCAGTGACTCCATTCAAACC
OTS-6	chr12	41601281	CTGGGAGTGAtGGGAGAcaTTGaC	4	F:AGGATGATTCAAGCCCATTACA
					R:GAAACGGATTTACCCTCCTACC
OTS-7	chr5	138712422	CTAGGAGTGgAGGGAGAGgaTGTg	4	F:TGTTCTGGGCACAAGAAGATAG
					R:GAGTAGCTGGGACTACAGGTAT
OTS-8	chrX	44120268	TTGGGAGTGAAGGGAtAGTgTtTt	4	F:TGACTCACTGCCAGACAATATG
					R:ACAGAGGAGTAGGCTTGTATCT
EMX1	**chr2**	**72933793**	**TTGTCCTCCGGTTCTGGAACCACA**	**0**	**F:CCATCCCCTTCTGTGAATGT**
					R:GGAGATTGGAGACACGGAGA
OTS-1	chr4	23758817	TTTTCCTCCaGTTaTGGAACCAaA	3	F:AGTCACGTGAACCAGAAAGTAG
					R:CTGAAGGGCTTGGGCTTTA
OTS-2	chr10	101134345	TTGTCCgCCGGTTCTGGAACCAgg	3	F:GTGACCATTAGCTCGCCTTAG
					R:GAGAGTGTGCCAACCAGAAA
OTS-3	chr6	134088151	TTCTCCTCaGGTTCTGGAACCAat	3	F:GAACAGTGCAGGTAGAGATCAA
					R:TTTCCTCCTTTCACACCCTAAG
OTS-4	chr5	103120487	TTCTCCTCCGGTTtTGGgcCCtCA	4	F:CCGAGGTGGTCCTAAATTCAA
					R:GTCCCATAACTCGAGGCTAATC
OTS-5	chr1	2051922	CTTTCtTCtGGTTCTGGcACCAaA	4	F:GACCCGTGGGTTTGTTCTT
					R:GGAATTGGACGCCGGAAT
OTS-6	chr19	48509366	CTGTCCTCCtGTTCTGtgtCCACA	4	F:CATACAGCACTCCTTCCACTC
					R:GTGTTGGTAGCACTCAGGAA
OTS-7	chr14	31356145	CTATCtTCtGGTTCTGtAACgACA	4	F:CGTTAGAACCCTACAGTCAGAATA
					G
					R:GACCAGGCACAGTAGTTTACA
OTS-8	chr8	87712058	CTCTCCTCCtGTTCTGaAtCCttA	5	F:AATGGAGCAGGAAGAGGAATG
					R:CTGTTGAGGCAACGATCAATTC

VEGFA	**chr6**	**43769529**	**TTTGGGAGGTCAGAAATAGGGGGT**	**0**	**F:CTCAGCTCCACAAACTTGGTGCC** **R:AGCCCGCCGCAATGAAGG**
OTS-1	chr8	144554313	CTGGGGAtGTCAGgAgTAGGGGGT	3	F:GTGGTCTGAACAGGGATCTTC R:GGTTGTGGCAGGGAATTAGA
OTS-2	chr3	39323102	TTGGGGAGGTCAGtAATAGGGaGT	2	F:CAGCTCAGTTCAATTCTGTGTG R:CAGAGATGGGCTCTTCTGATAAT
OTS-3	chr16	4604192	TTAGGGAGGgCAGAAATtGGGGGc	3	F:CTGGCTTGACTTCTGACTCTC R:ACGGCTTGTTCTGCAAGAT
OTS-4	chr13	67223317	TTGGGGAGGTCAGAAAaAGtGGaT	3	F:GAACATTGGAATACCCATAGGAGA R:CACAGGAAGAAAGGACTTTAATCA
OTS-5	chr6	27374720	CTCcaGAGGTCAGAAATAGGtGGT	3	F:CAGAACACACTCGCTCTTGA R:CAGACAACCCTGGGAAATGTA
OTS-6	chr11	68469846	TTGGGGAGGTCAGAAAgAGGGaag	4	F:GTAGGCACATGCTACTACACC R:TCCCTCCACTCCTTCTGTT
OTS-7	chr14	91353588	TTGGGGAGGTCAGAAgTcGGGccT	4	F:GCACATTCTTGCACCATCTTC R:ATCCCACCGAAGCCATTTAG
OTS-8	chr16	84594983	CTGGGGAGGTCtGAAAgAGGGGaa	4	F:GAGTGTCGTCAGAGCATCAA R:AAACCACGTCCCTCTTTACC
GRIN2b	**chr12**	**13866304**	**TTCAAGGACCTTATCTCCTTTCAT**	**0**	**F:GCATACTCGCATGGCTACCT** **R:CTCCCTGCAGCCCCTTTTTA**
OTS-1	chr5	41757489	CTGAAGGACCaTATCTttTTTCAT	3	F:CCCACACACCAGTGTCTATTC R:TACTTAGCCCATCTGCCTTTG
OTS-2	chr4	169303367	CTAAAGGACCTTAcCTCCTTTCcT	2	F:TTCATCCAGGTGCCTCTAAAC R:GACCAACCACAACCAAAGAAAG
OTS-3	chr2	146415233	TTAAAGGACCTaATCTCtTaTCAT	3	F:CTTCCCATAATACTTCGGGTCTG R:GACTCCATTTCTCAGGCATAGT
OTS-4	chr6	2737740	CTGAAGGACaTTtTCTtCTTTCAT	3	F:GGGAGGAACGAACACATTCT R:GATAGATCAGGAAGGTGGGTAATC
OTS-5	chrX	114935355	CTCAAGGACtTTAcCTCCTTTCcT	3	F:TGTGCAGGCTGTAGAGAAAG R:CAAGTATGAGGGTCAGGAACAA
OTS-6	chr5	95047159	TTCAAGGACaTcATCTaCTTTCAT	3	F:TCTCATCCTGATCCTTCCTCTC R:GCAGTCATGACACACCATGTA
OTS-7	chr11	114648765	CTCAAGGACtTTATCTCCTTTtcT	3	F:TCCAGGAAGAGCCAATGTTT R:TGCTGAAGGCGAAAGGAATA
OTS-8	chr13	46693304	TTTAAGGACCcaAgCTCCTTTCAT	3	F:GTAACTTGCCATTGGTCACATAG R:CTCATGTACAGGTGAGGGAATC

Supplementary Table 4. U6 forward prime and Sequencing Primers.

Locus	Forward Primers (5'-3')	Reverse Primers (5'-3')
U6	GAGGGCCTATTTCCCATGATTCCT	-
GFP	AAGGGCGAGGAGCTGTT	ACTGGGTGCTCAGGTAGTG
DNMT1-1	CCGCAGGTGTTTGAGATTTATG	GAGCGCGATGGCATAATCT
DNMT1-3	CTGGGACTCAGGCGGGTCAC	CCTCACACAACAGCTTCATGTCAGC
EMX1	CCATCCCCTTCTGTGAATGT	GGAGATTGGAGACACGGAGA
RS1	CGGTTATCTGGCTTGACACTTG	GTGAGGATCCCTGAAATCACTTTG
NRL	TTTGCAGACCTTCGCTAGTC	CAGCAGACCGCCTACATAATC
HBB	GTGTGGAAGTCTCAGGATCGT	AGGAGCTGTGGGAGGAAGAT
CCR5	TGCTGCATCAACCCCATCAT	CACAAGTCTCTCGCCTGGTT
VEGFA	CTCAGCTCCACAAACTTGGTGCC	AGCCCGCCGCAATGAAGG
GRIN2b	GCATACTCGCATGGCTACCT	CTCCCTGCAGCCCCTTTTTA