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Supplemental Information

CRISPR/Cas9-*loxP*-Mediated Gene Editing as a Novel Site-Specific Genetic Manipulation Tool

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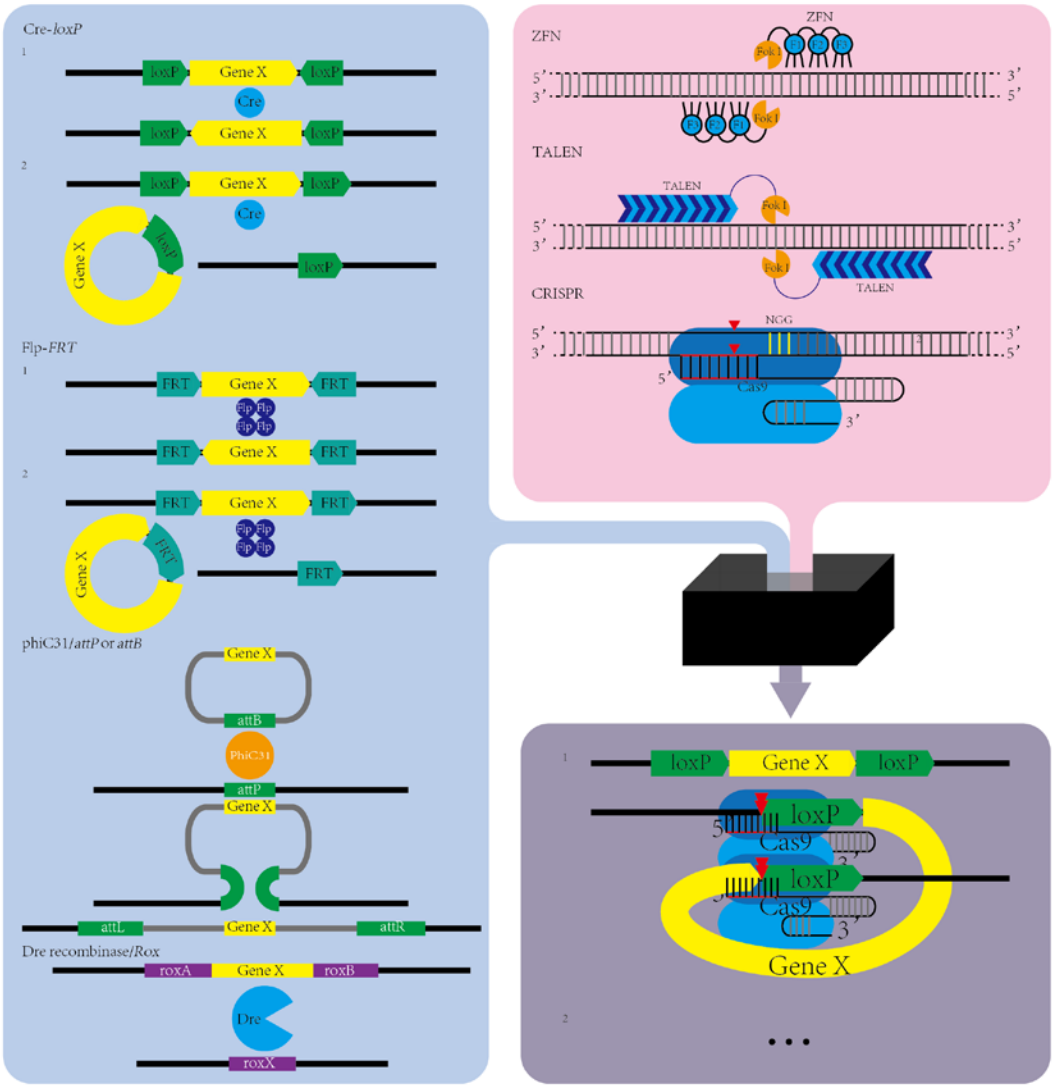

Figure S1. Schematic of the combination of CRISPR/Cas9 and traditional site-specific genetic manipulation tools.

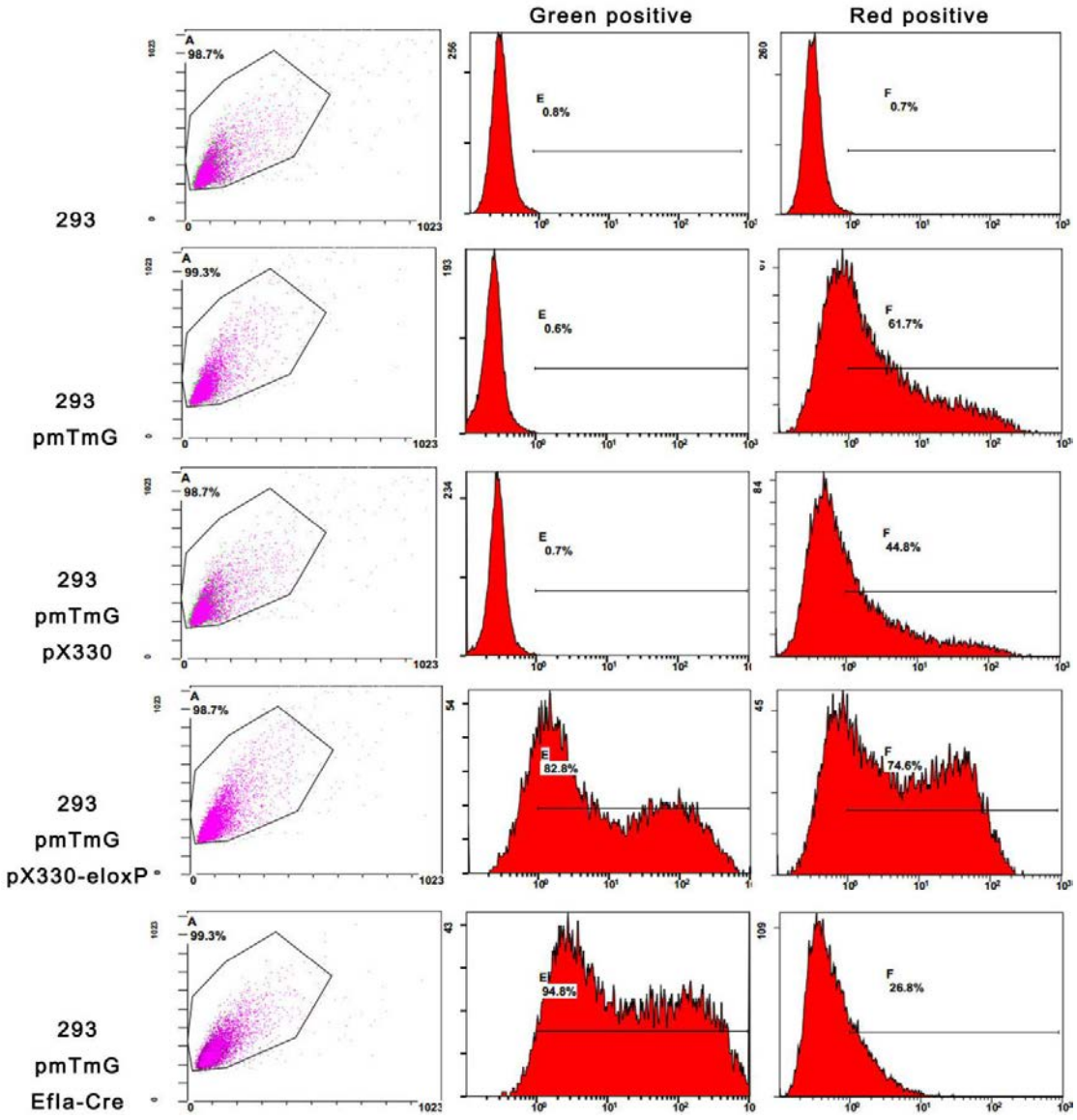

Figure S2. Performance of CRISPR/SpCas9-*eloxP* mediated site-specific genome editing examined with flow cytometry (FCM)

HEK-293 cells were co-transfected with plasmids (pmTmG and CRISPR/SpCas9-*eloxP*,0.75ug each) and flow cytometry were performed at 60h post transfection (Red indicates *mT;* Green indicates *mG*).

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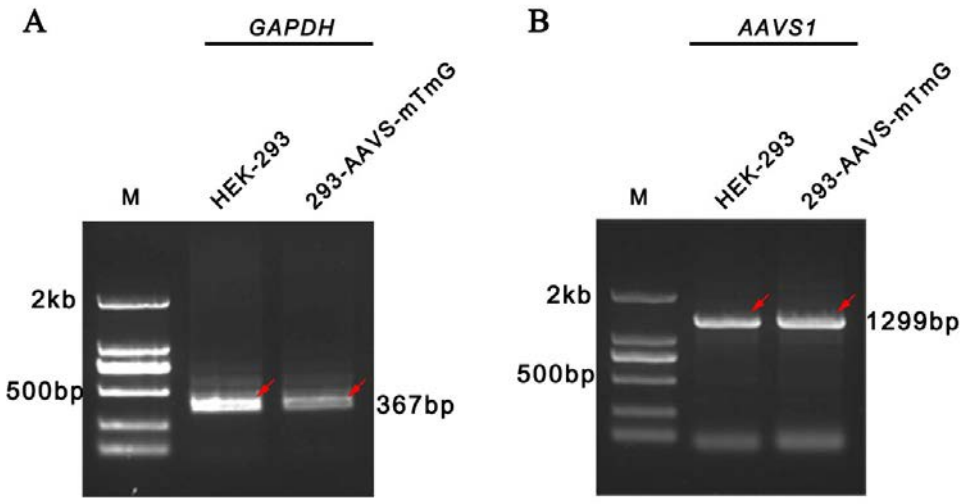

Figure S3. Genotyping of 293-AAVS-mTmG at *AAVS1* locus.

A. PCR products of *GAPDH* gene as control (Primers: F4/R4).

B. Genotyping of the *AAVS1* gene in 293-AAVS-mTmG and parental cells (Primers: F5/R5). The unlabelled bands are the primer dimer.

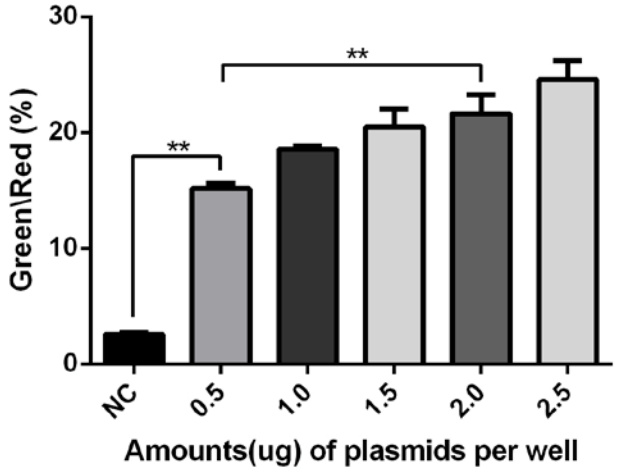

Figure S4. Optimization of transfection conditions of CRISPR/SpCas9 -*eloxP* plasmid to inactivate *mT*.

293-AAVS-mTmG cells were transfected with different amounts of CRISPR/SpCas9-*eloxP* plasmid. The cells were saturated with 2.0 mg of CRISPR/SpCas9-*eloxP* plasmids.

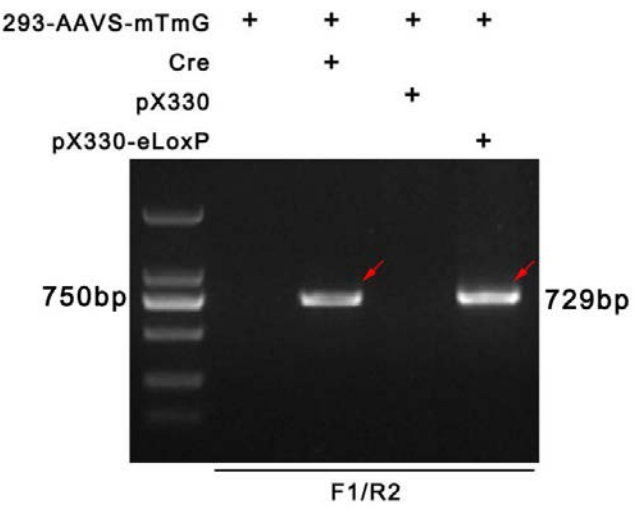

Figure S5. Genotyping of CRISPR/SpCas9-*eloxP* system mediated excision at human *AAVS1* locus.

CRISPR/SpCas9-*eloxP* mediated excision of mTomoto between two *loxP* sites in 293-AAVS-mTmG. Excision bands were detected by PCR (Primers: F1/R2).

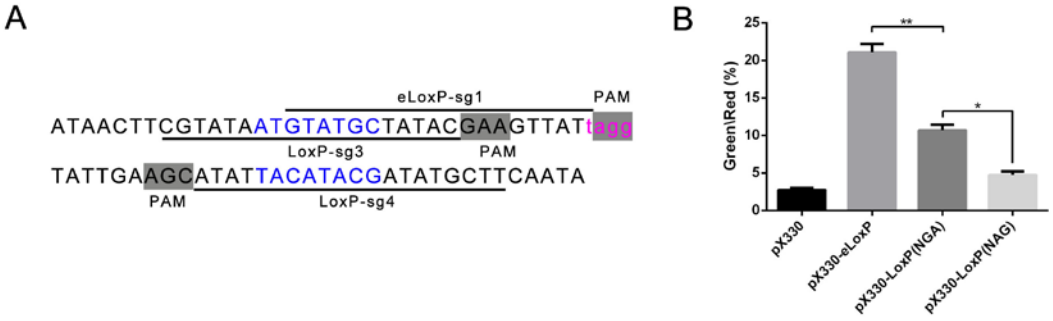

Figure S6. Non-canonical PAM for CRISPR/SpCas9-*loxP* to inactivate *mT*.

A. Targeting sequence and corresponding PAMs for CRISPR /SpCas9.

B. NGA or NAG PAM and the results showed that CRISPR/SpCas9-*loxP* with non-canonical PAM NGA but not NAG could achieve relative highly efficient excision of a specific gene between two *loxP* sites.

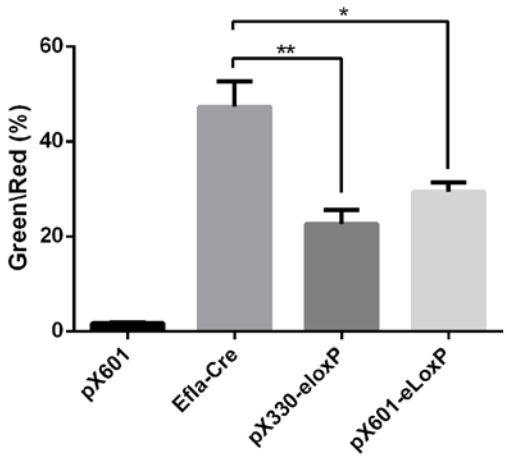

Figure S7. Efficiency comparison of CRISPR/SaCas9-*eloxP* and SpCas9-*eloxP* mediated gene editing.

pX330 and pX601 represent CRISPR /SpCas9 and CRISPR/SaCas9, respectively. Error bars are the standard deviation (SD, n=3).

	Efla-Cre	
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAATATAACTTCGTATAATGTATGCTATACGAAGTTATTAGGTCC 3`		
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAATA -(89bp)- CGTATAATGTATGCTATACGAAGTTATTAGGTC 3`	+89	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAAT -(44bp)- ATAACTTCGTATAATGTATGCTATACGAAGTTAT 3`	+44	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAATATATAACTTCGTATAATGTATGCTATACGAAGTTATTAGGT 3`	+2	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAATATAACTTCGTATAATGTATGCTATACGAAGTTATTAGGTCC 3`	0	6/25(24%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAA - TAACTTCGTATAATGTATGCTATACGAAGTTATTAGGTCC 3`	-2	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTA - - TAACTTCGTATAATGTATGCTATACGAAGTTATTAGGTCC 3`	-3	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAAT - - - CTTCGTATAATGTATGCTATACGAAGTTATTAGGTCC 3`	-4	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAAT - - - - TTCGTATAATGTATGCTATACGAAGTTATTAGGTCC 3`	-5	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAAT - - - - - - - GTATAATGTATGCTATACGAAGTTATTAGGTCC 3`	-8	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAAT - - - - - - - - - ATAATGTATGCTATACGAAGTTATTAGGTCC 3`	-10	2/25(8%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCT - - - - - - - - - - - TAATGTATGCTATACGAAGTTATTAGGTCC 3`	-15	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTAA - - - - - - - - - - - - - - - -TATGCTATACGAAGTTATTAGGTCC 3`	-17	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAAC - - - - - - - - - - - - - - - - - - - TGTATGCTATACGAAGTTATTAGGTCC 3`	-21	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCC - - - - - - - - - - - - - - - - - - - - TGCTATACGAAGTTATTAGGTCC 3`	-23	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTTA - - - - - - - - - - - - - - - - - - - - ATACGAAGTTATTAGGTCC 3`	-24	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCCTT - - - - - - - - - - - - - - - - - - - - TATACGAAGTTATTAGGTCC 3`	-25	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCC - - - - - - - - - - - - - - - - - - - - CTATACGAAGTTATTAGGTCC 3`	-26	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGGAACCC - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - TTATTAGGTCC 3`	-35	1/25(4%)
5` GGCTTCTC- - -(43bp)- -TGATCCGG - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - AAGTTATTAGGTCC 3`	-37	1/25(4%)

Figure S8. CRISPR/ SaCas9-*eloxP* mediated NHEJ pattern at the human *AAVS1* locus.

CRISPR/SpCas9-*eloxP* mediated excision of mTomoto between two *loxP* sites in 293-AAVS-mTmG. NHEJ pattern were detected by sequencing. Pink nucleotides indicate sgRNA, blue nucleotides indicate indels.

Table S1 Target sequence

Target sites for CRISPR/Cas9 System with PAMs

Target site ID	Target sequence (5`-3`)	PAM	Strand
eLoxP-1(pX330)	GTATGCTATACGAAGTTATT	AGG	+
eLoxP-2(pX601)	TACATTATACGAAGTTATATT	AAGGGT	-
AAVS1	TCACCAATCCTGTCCCTAG	TGG	+
LoxP-3(pX330)	CGTATAGCATACATTATACG	NAG	+
LoxP-4(pX330)	TTCGTATAATGTATGCTATA	NGA	-

Oligonucleotide sequences for sgRNA architecture

Primer Name	Primer sequence (5`-3`)
eLoxP-1-F	CACCGGTATGCTATACGAAGTTATT
eLoxP-1-R	AAACAATAACTTCGTATAGCATACC
eLoxP-2-F	CACCGTACATTATACGAAGTTATATT
eLoxP-2-R	AAACAATATAACTTCGTATAATGTAC
AAVS1-F	CACCGTCACCAATCCTGTCCCTAG
AAVS1-R	AAACCTAGGGACAGGATTGGTGAC
LoxP-3-F	CACCGCGTATAGCATACATTATACG
LoxP-3-R	AAACCGTATAATGTATGCTATACGC
LoxP-4-F	CACCGTTCGTATAATGTATGCTATA
LoxP-4-R	AAACTATAGCATACATTATACGAAC

Table S2 Primers sequences

PCR primers

Primer Name	Primer sequence (5`-3`)	Product(bp)	Anneaing(℃)
F1	GGGACTTCCTTTGTCCCAAATC	740bp	58
R1	GGGAAGGACAGCTTCTTGTAATC		
F2	CAGGCATAGAGTGTCTGCTATT	725bp	58
R2	GATGAACTTCAGGGTCAGCTT		
F3	GGGCTATGAACTAATGACGGA	966bp	60
R3	GTCCAGGCCAAGTAGGTG		
F4	CTGGCACCCTATGGACACG	367bp	60
R4	GTCTTCTGGGTGGCAGTGAT		
F5	ACCTCTCACTCCTTTCATTTGG	1299bp	60
R5	TGAGTTTGCCAAGCAGTCA		
F6	CTCTCGTTTCTTAGGATGGCC	150	60
R6	GAAAGCAAGAGGATGGAGAGG		

Sequencing primers

Primer Name	Primer sequence (5`-3`)
U6-F	GAGGGCCTATTTCCCATGATTC
Jet-F	TGAACACCATATCCATCCGGCGTA

Table S3 Off-target analysis for CRISPR/SpCas9-*loxP* System

PCR primers

Primer Name	Primer sequence (5`-3`)	Product(bp)	Anneaing(℃)
F7	GACAGAGAGGCACAAAGTCATA	305bp	58
R7	AGAATACTGGTCCCTGGAAATG		
F8	GAGACGGAGTCTTGTTCCATAG	499bp	58
R8	GTGTTGTTAGTTGGAAGAGTCATTAG		
F9	GCCATGATGACTTCACCTGAT	455bp	58
R9	TTGGTTTGGGCAATACCTTCT		
F10	AGCTGACTTCTCACCACAAAC	374bp	58
R10	GCAATGACACAATTGCCTAACC		
F11	TGGAGATTAAGAGACAAGGGAATG	458bp	58
R11	GTAATATCACAGCTACTTGGAGAGG		
F12	GGCAGAGAAGGGCAAGTAAG	348bp	58
R12	GCCACGTCAGTAGTGGTATTT		
F13	GCATCAGAAAGCACAGAGACTA	441bp	58
R13	GCTTCCTTTACTCTCACCCTTTA		
F14	ACAGTGGTAAAGACCAATCAGG	300bp	58
R14	CTGCTGTGTACCTATGTCAGAAG		
F15	TGGAGATTAAGAGACAAGGGAATG	458bp	58
R15	GTAATATCACAGCTACTTGGAGAGG		
F16	TGGTGCGTGCCTGTAATC	420bp	58
F16	CCAGTTGCCTTTGAAGTCATTC		

Analysis of off-target induced by CRISPR/SpCas9-*loxP* System

Chromosome	Target sequence (5`-3`)	PAM	Off-target
eLoxP-1(pX330)	GTATGCTATACGAAGTTATT	AGG	
Chr 1	GTAGGGTAAAGGAAGTTATT	AGG	No
Chr 3	CTATGACATACTAAGTTATT	AGG	No
Chr 6	GTAT ACTGTTTGAAGTTATT	TGG	No
Chr 8	AAATGCTAACCGAAGTTATT	TGG	No
Chr 9	AAATGCTAAAGGAAGTTATT	TAG	No
Chr 10	AAATGCTTTAGGAAGTTATT	CGG	No
Chr 12	AAATGCTAAAAGAAGTTATT	CAG	No
Chr 13	GTAATGTATACAAAGTTATT	TGG	No
Chr 13	ATATTCTCTAAGAAGTTATT	AGG	No
Chr 18	TAATGCTAAAGGAAGTTATT	TGG	No

Table S4 Off-target analysis for CRISPR/SaCas9-*loxP* System PCR primers

Primer Name	Primer sequence (5`-3`)	Product(bp)	Anneaing(℃)
F17	GCATCACAGTCACAGCATTT	263bp	58
R17	GAAAGGGCAGACCTAGGATATAA		
F18	CATTAGGCTTTATCGACCTAGTGA	373bp	58
R18	CATGTCAATTTAGGCAGGGTTT		
F19	GTAGCCTCTAGCCACATGTTT	320bp	58
R19	TCACCACCTTTGGTGTTCTC		
F20	CCTGTTCCACTCTCCCTTTATAC	386bp	58
R20	ACAGAAGAGTAGACCTATTTGTAAG		
F21	CCTCCTGAATAGCTGGGATTAC	391bp	58
R21	CCCTCCCTGGTTAGAAGAAAC		
F22	GGCAGAATCTGAAAGTGGATACT	551bp	58
R22	GCCTCAATGAGCTCCCAAATA		
F23	AACCCAATTCCCATCAACTAAATC	446bp	58
R23	AGGGAAGAGTCATTGGTAAAGTC		
F24	GTGCCTGGCCAACATTTATC	459bp	58
R24	CCCACAAGAGTGGAGGATTATT		
F25	CTGTTGAGCATTTAATTTCGGTGA	436bp	58
R25	TCACACATGTCTTTGGAGATTGA		
F26	TCCAAGGTAGTGGCATTGATAC	410bp	58
R26	AAGAAAGGAGGGAACTCTTGATAC		

Analysis of off-target induced by CRISPR/SaCas9-*loxP* System

Chromosome	Target sequence (5`-3`)	PAM	Off-target
eLoxP-2(pX601)	TACATTATACGAAGTTATATT	AAGGGT	
Chr 4	TATATTATACAAACATATATT	AAGAAT	No
Chr 4	TTCATTATATGAAGTTCAATT	CAGGAT	No
Chr 7	CACTATCTACGAGGTTATATT	AAGTGA	No
Chr 7	ACTACTTTTAGAAGTTATATT	AAGGCC	No
Chr 8	TTCTTTATAAGAAGATATATT	CTGAGT	No
Chr 11	TACAATCTATGAAGTGATATT	TAGGAT	No
Chr 13	ACATTATACGATGTTACATT	ATATGA	No
Chr 19	TAAACCCAAAGAAGTTATATT	AAGATT	No
Chr X	TACTTTATATAAACTTATATT	TGGAAT	No
Chr X	TCACTTCAAGAATGTTATATT	AAGGGA	No