Template-jumping prime editing enables large insertion and exon rewriting *in vivo*

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\# Equal contributions to this study

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Fig.S1

a

TJ-PE editing

PCR and gel cut

TA cloning
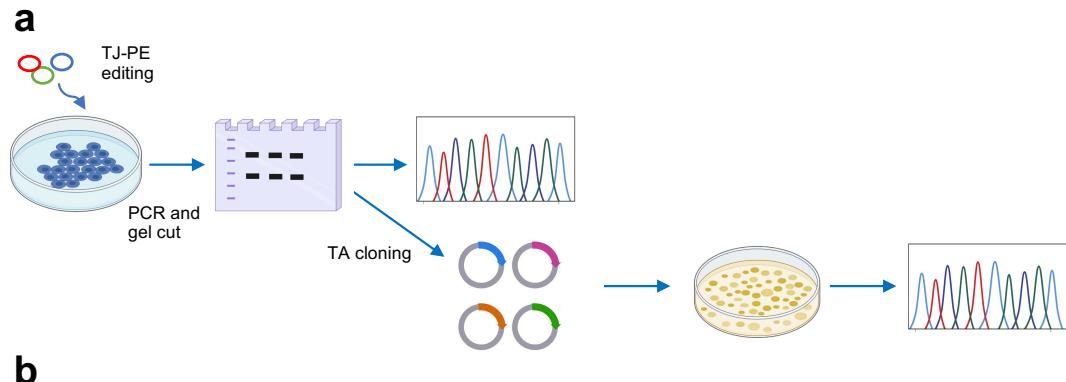
b

Genomic sequence | 200 bp insertion

aacctctaaggtttgcttacgatggagccagagaggaATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACG   Reference
....   Precise insertion 11
                                                                                                  Indel 1

GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGTAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGG
....C...C...........

Genomic sequence

GATTGTTATAACTTCGTATAGCATACATTATACGAAGTTATcccagaacctgagctgctctgacgcggctgtctggtgcgtttcactgatc
.......C...

c

Genomic sequence | 300 bp insertion

aacctctaaggtttgcttacgatggagccagagaggaATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACG   Reference
....   Precise insertion 9
....   Indel 1
....   Indel 1
....   Indel 1

GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG
....
....G.
....
....

CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGG
....
....
....

Genomic sequence

GGGATTGTTATAACT TCGTATAGCATACATTATACGAAGTTATcccagaacctgagctgctctgacgcggctgtctggtgcgtttcactgatc
............T..
...........................ccctc...

d

Genomic sequence   500 bp insertion

aacctctaaggtttgcttacgatggagccagagaggaATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACG Reference
.... Precise insertion 9
....T........................... Indel 1
.... Indel 1
.... Indel 1

GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG
....
....
..............T..⌐‾‾‾⌐

CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAA
....
....
....

GTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACA
....
....
.................................⌐‾‾‾⌐
⌐‾‾⌐

CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGTAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAG
....
....
⌐‾‾‾⌐
⌐‾‾‾⌐

                                                              Genomic sequence

TTGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAAGTTATcccagaacctgagctgctctgacgcggctgtctggtgcgtttcac
....
....
⌐‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾⌐TGGCCACCCTTCGCTACCCTC...
⌐‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾⌐CCTC...

Supplementary Fig. 1. TJ-pegRNA achieved accurate large insertion at *AAVS1* locus. a, Schematic showing procedure for determining accuracy of 200-, 300-, and 500-bp insertions at the *AAVS1* locus. Insertion bands were cut out of the agarose gel and purified for Sanger sequencing and TA cloning. **b-d**, Sanger sequencing of single clone of **b**, 200 bp insertion **c**, 300 bp insertion **d**, 500 bp insertion at *AAVS1* locus. Deleted bases were indicated by blue box and mismatches relative to reference are in red.
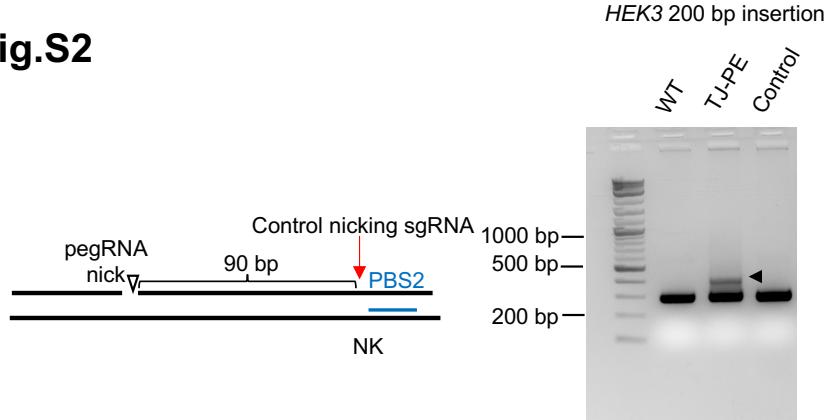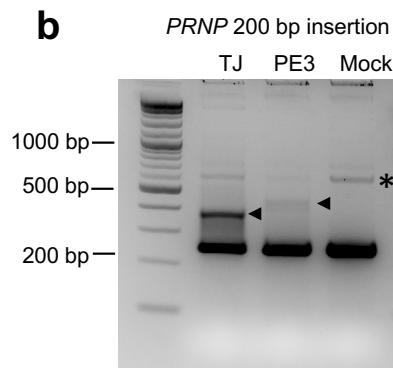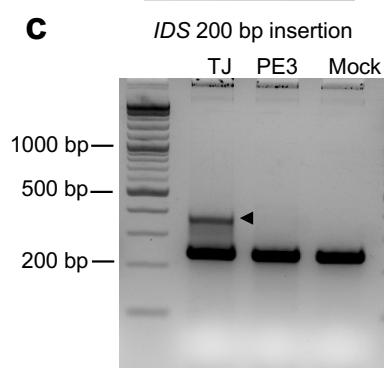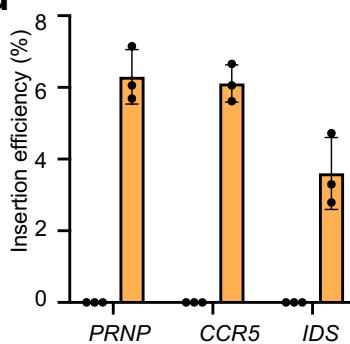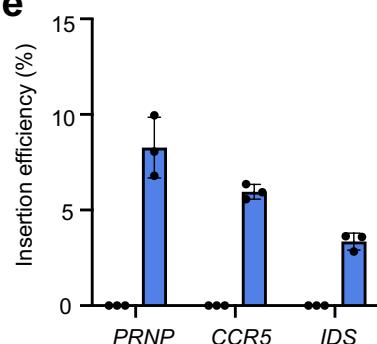
Supplementary Fig. 2. TJ-PE mediates insertions at multiple genomic loci and cell lines. a, New control nicking sgRNA designed to nick at the same position as nicking sgRNA, but on the opposite strand at the *HEK3* locus. The insertion band of the expected size was observed following TJ-PE treatment (arrow), but not controls. Experiments were done three times, and one is shown. **b**, Insertion of a 200-bp DNA fragment with concomitant deletion of 72 bp at *PRNP* locus in HEK293T cells. The insertion bands of expected size are indicated with arrows. The nonspecific bands are indicated with asterisk. Experiments were done three times, and one is shown. **c**, Insertion of a 200-bp DNA fragment with concomitant deletion of 70 bp at *IDS* locus. The insertion bands of the expected size are indicated with arrows. Experiments were done three times, and one is shown. **d,** Insertion of a 200-bp DNA fragment measured by ddPCR at multiple loci in U-2 OS cells. PE plasmid served as negative control. **e**, Insertion of a 200-bp DNA fragment measured by ddPCR at multiple loci in A549 cells. PE plasmid served as control. Results were obtained from three independent experiments, shown as mean ± s.d.

Fig.S3

a

b

c
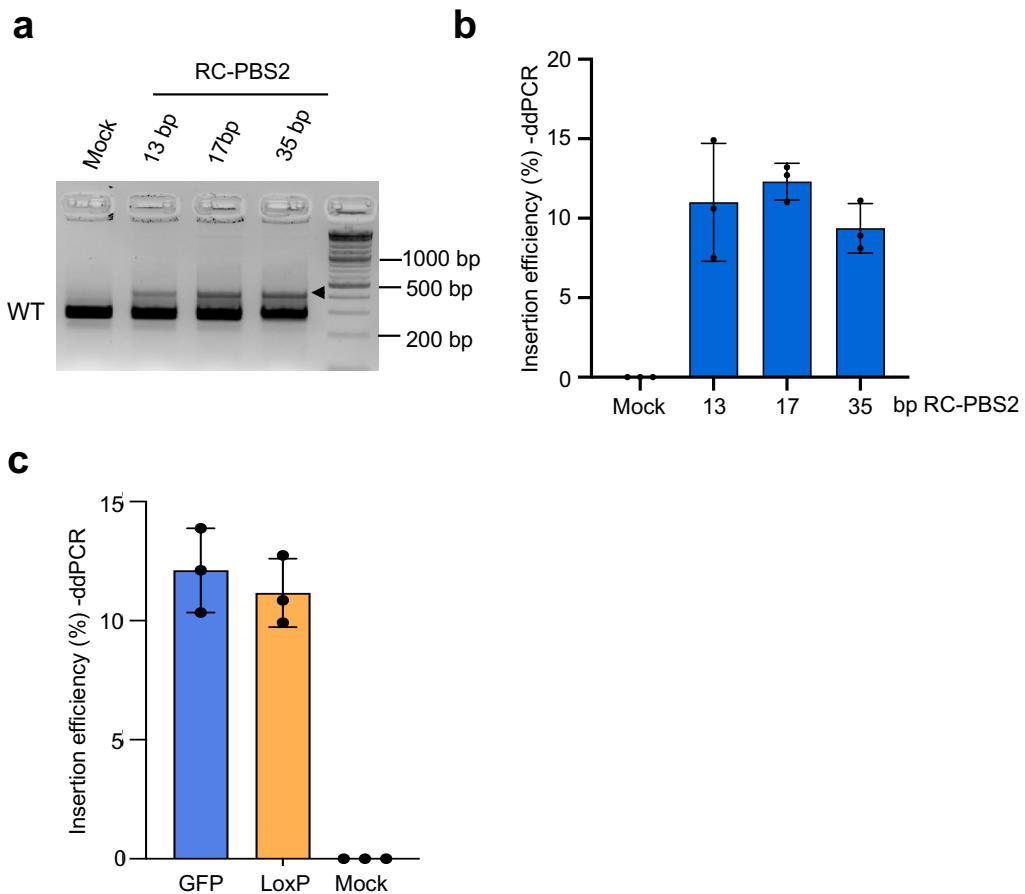
Supplementary Fig. 3. Comparison of the insertion efficiencies of the different lengths of PBS2 sequence or secondary structure. a, Agarose gel shows the insertion band of the expected size for TJ-pegRNA with 13, 17 or 35-bp RC-PBS2 at the *HEK3* locus (arrow). **b**, Insertion efficiency of a 200-bp DNA fragment with various lengths of PBS2 measured by ddPCR. **c**, Comparing insertion of GFP fragments without or with a hairpin (LoxP) at the HEK3 locus. Insertion efficiency was quantified by ddPCR. Results were obtained from three independent experiments, shown as mean ± s.d.

Fig.S4

a

Nicking and PBS1 annealing

NK-TJ-pegRNA

RC-PBS2

PBS1

1st RT

1st strand DNA

PBS2 on DNA flap binds nick

PBS2

Template jump and 2nd RT

Insertion

b

Insertion size: 200 bp   300 bp

Mock   TJ   NK-TJ   TJ   NK-TJ

1000 bp

500 bp

200 bp

WT

c

Insertion efficiency (%) -ddPCR

80

60

40

20

0

mock   TJ   NK-TJ   TJ   NK-TJ

200 bp   300 bp Insertion
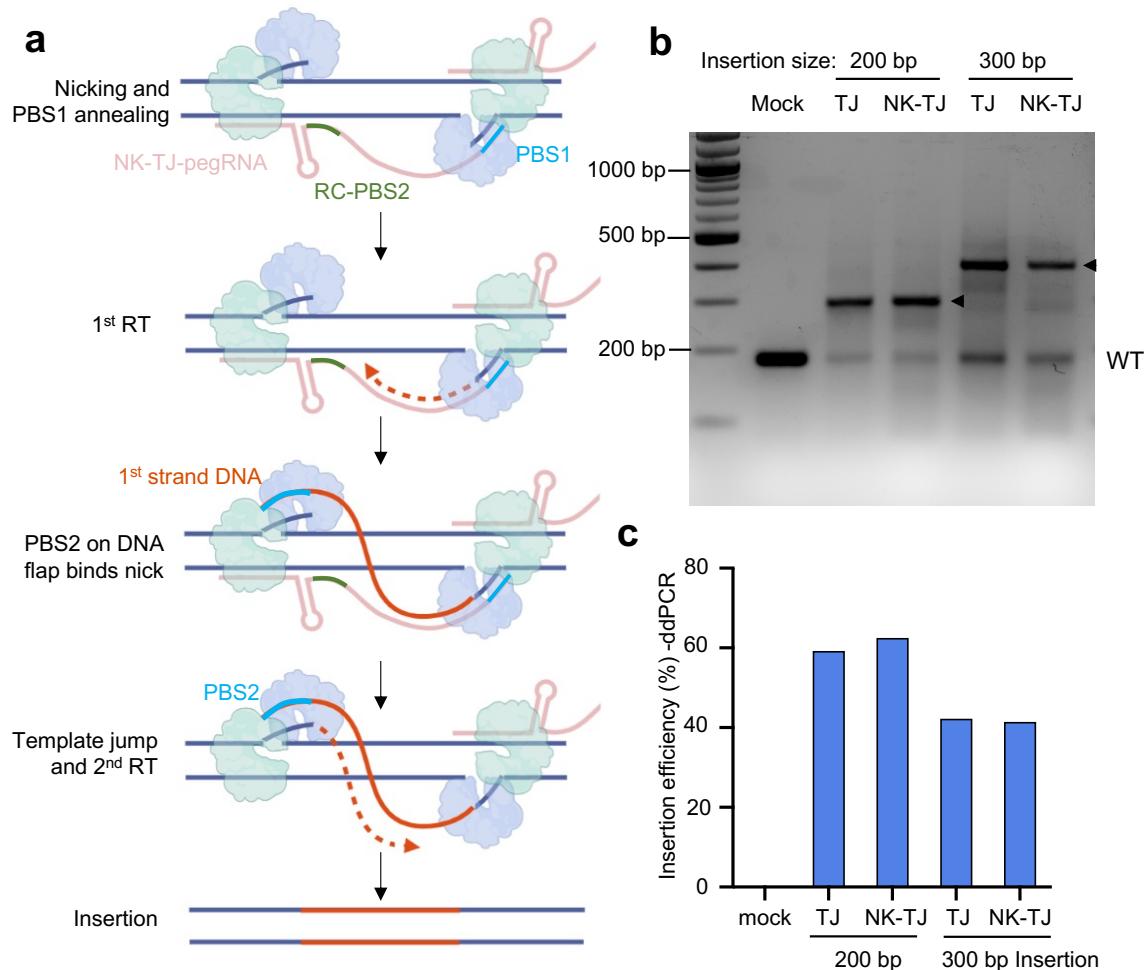
Supplementary Fig. 4. NK-TJ-pegRNA enables comparable insertion efficiency with TJ-pegRNA. a, Illustration of nicking-TJ-pegRNA (NK-TJ-pegRNA), which contains PBS1, RC-PBS2 and an insertion sequence. Compared to TJ-pegRNA, the PBS1 sequence of NK-TJ-pegRNA first hybridizes to the DNA flap generated by the nicking sgRNA. The newly synthesized PBS2 hybridizes to the second nicked site generated by NK-TJ-pegRNA to initiate the second strand synthesis. **b**, Agarose Gel shows insertion bands of expected sizes (200 bp and 300 bp) at *AAVS1* locus. **c**, Insertion efficiency quantified by ddPCR.

Fig.S5
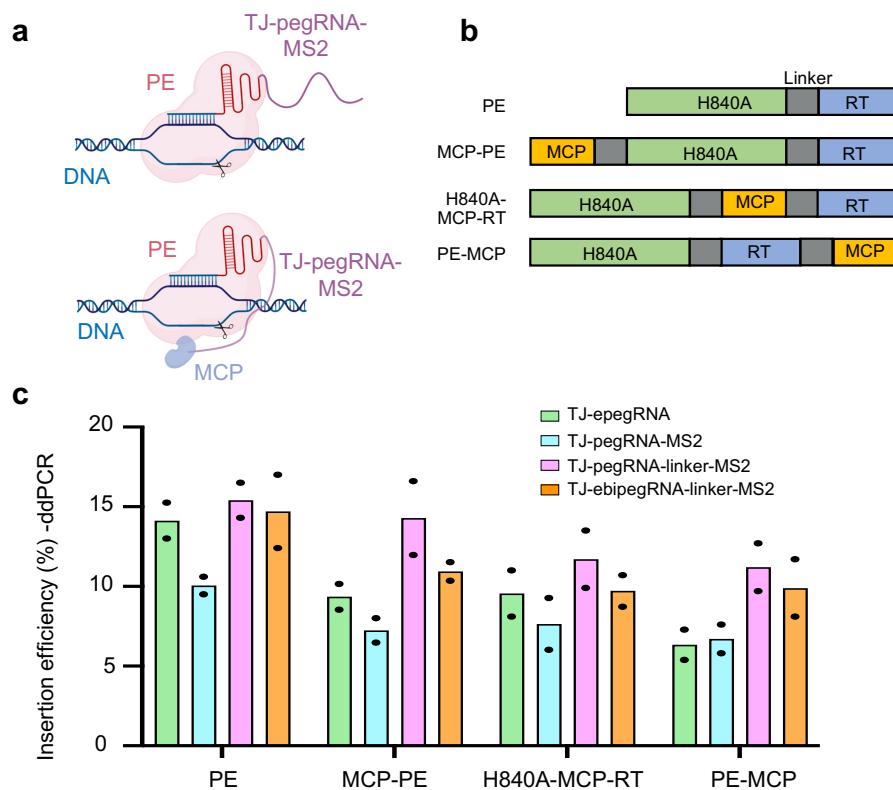
Supplementary Fig. 5. TJ-pegRNA with 3'-RNA aptamer. a, Diagram of pegRNA with 3'-RNA aptamer. **b**, Structure of the MCP and PE fusion protein. Schematic representations of PE-MCP fusion proteins. **c**, Insertion efficiency quantified by ddPCR at *HEK3* locus. Results were obtained from two independent experiments.

Fig. S6

a

TJ-PE

TJ-pegRNA   sgRNA   PBS2   RTT   PBS1

sgRNA

GRAND

pegRNA   RTT   PBS1

pegRNA   RTT   PBS2

b

200 bp insertion

- Mock
- TJ-PE
- GRAND

c

IDS 200 bp insertion

Con   TJ-PE   GRAND

1000 bp
500 bp
200 bp

HEK3 200 bp insertion

Con   TJ-PE   GRAND

1000 bp
500 bp
200 bp

PRNP 200 bp insertion

Con   TJ-PE   GRAND

1000 bp
500 bp
200 bp

d

AAVS1 500 bp insertion

CCR5 400 bp insertion

PRNP 400 bp insertion

IDS 400 bp insertion

- Mock
- TJ-PE
- GRAND

Supplementary Fig. 6. Comparison of insertion efficiencies mediated by GRAND and TJ-PE. a, Illustration of TJ-pegRNA and GRAND pegRNAs. **b,** Insertion of 200-bp DNA fragment with TJ-PE or GRAND editing at *HEK3*, *IDS* and *PRNP* loci in HEK293T cells. **c,** Agarose gel shows insertion bands of expected sizes (arrows). **d,** Insertion of DNA fragment at *AAVS1* (500-bp), *CCR5* (400-bp), *PRNP* (400-bp) and *IDS* (400-bp) loci. Insertion efficiency was quantified by ddPCR. Results were obtained from three independent experiments, shown as mean ± s.d.

Fig.S7

a

b

c
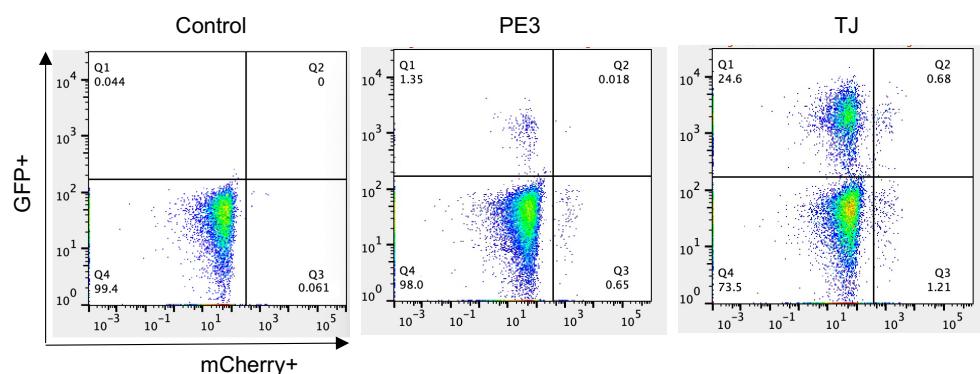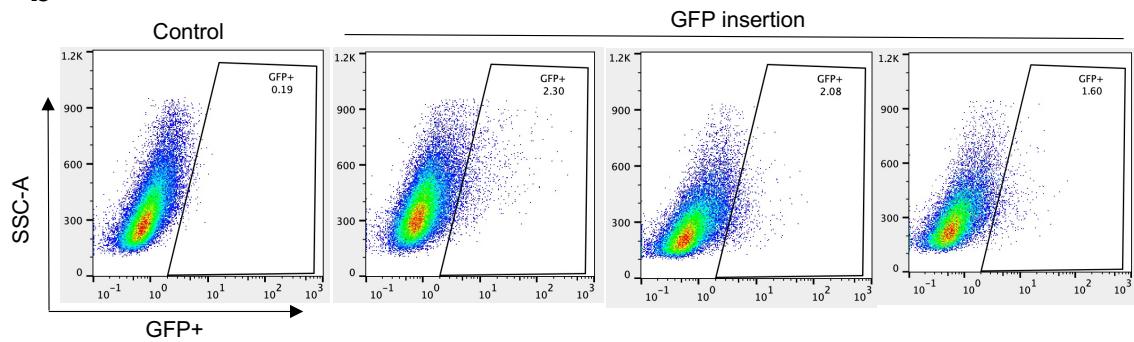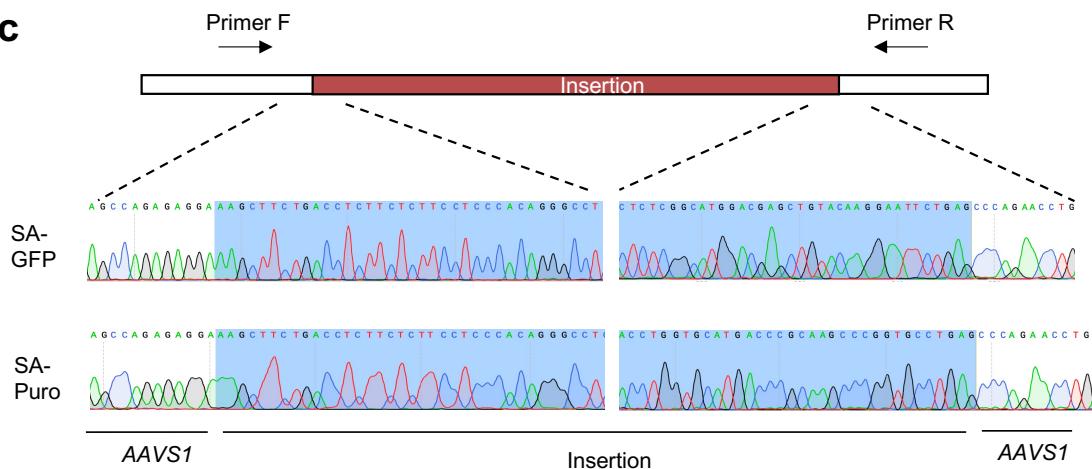
Supplementary Fig. 7. TJ-pegRNA mediates GFP reporter repair and functional gene insertion. a, Flow cytometry plots show the gating of TLR-MCV1 cells in control, PE3 and TJ-PE treated groups. **b**, Flow cytometry plots show the gating of GFP expression cells. **c**, Sanger sequence of SA-GFP and SA-Puro insertion. The insertion bands of predicted size were gel purified. Sanger sequencing shows precise insertions.

Fig.S8

a
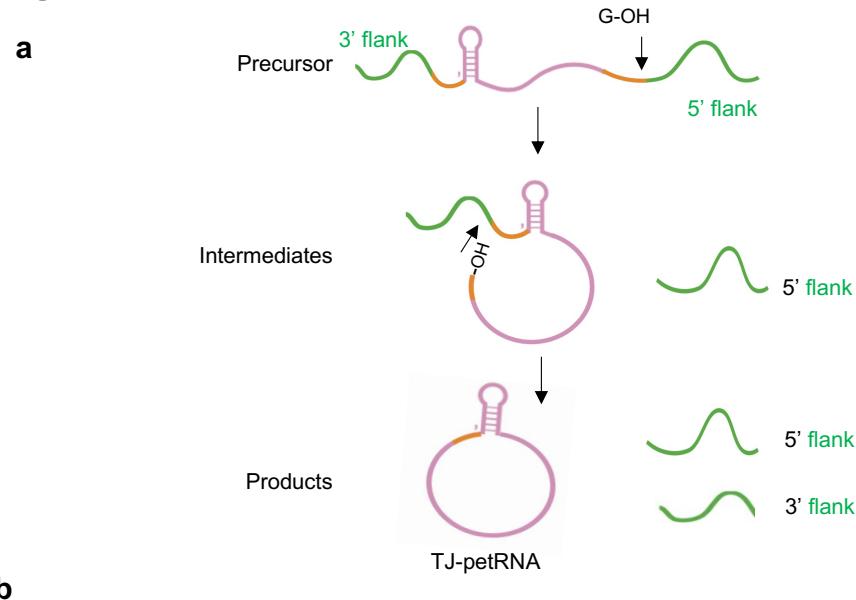
b

Full-length TJ-pegRNA

```
▼ Cut site                                                                                          Cut site▼
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAAGTTATCCCAGAACCTGAG     Insertion reference
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAAGTTATCCCAGAACCTGAG     0.4%   Precise

GGATCCTGGGGAGGGAGAGCTTGGCAGGGGGTGGGAGGGAAGGGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCC                           WT reference
GGATCCTGGGGAGGGAGAGCTTGGCAGGGGGTGGGAGGGAAGGGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCC------                          0.43%  Indels
```

Split circular TJ-petRNA

```
▼ Cut site                                                                                          Cut site ▼
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAAGTTATCCCAGAACCTGAG     Insertion reference
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAAGTTATCCCAGAACCTGAG     41.8% Precise edits
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAAC----------------------------------CTGAG     0.9%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAG-------------------------------------------------AACCTGAG     0.7%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAA-----------CCTGAG     0.7%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATAC-----------------GAACCTGAG     0.5%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATACA------------------GAACCTGAG     0.4%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTC-------------------------------------------------CCAGAACCTGAG     0.4%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATAC--------------------CTGAG     0.3%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAAGTTATCCCAGAACCTGA     0.3%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATA----------------CCAGAACCTGAG     0.3%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCATACATTATACGAAGTTAGCCCAGAACCTGAG     0.3%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGATTGTTATAACTTCGTATAGCA--------------------GAACCTGAG     0.2%
GGATAGGGATAACAGGGTAATGTGCCCCAACTGGGGTAACCTTTGAGTTCTC-------------------------------------------------AGAACCTGAG     0.2%

GGATCCTGGGGAGGGAGAGCTTGGCAGGGGGTGGGAGGGAAGGGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCC                           WT reference
GGAT------------------------------------------------GCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCC              0.38%
GGA-------------------------------AGGGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCC                        0.36%
G----------------CTTGGCAGGGGGTGGGAGGGAAGGGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCC                  0.36%   Indels
-----------------CTTGGCAGGGGGTGGGAGGGAAGGGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCC                  0.22%
```

Imperfect edits

Supplementary Fig. 8. Split circular TJ-PE mediates insertion at *AAVS1* a, Illustration of the circularization pathway to generate split circular TJ-PE. Yellow: circularization sequence. **b**, Sequencing analysis of the 100-bp insertion. All alleles observed with frequency ≥ 0.2% are shown. Green: Insertion sequence. Red: Indels.

Fig.S9
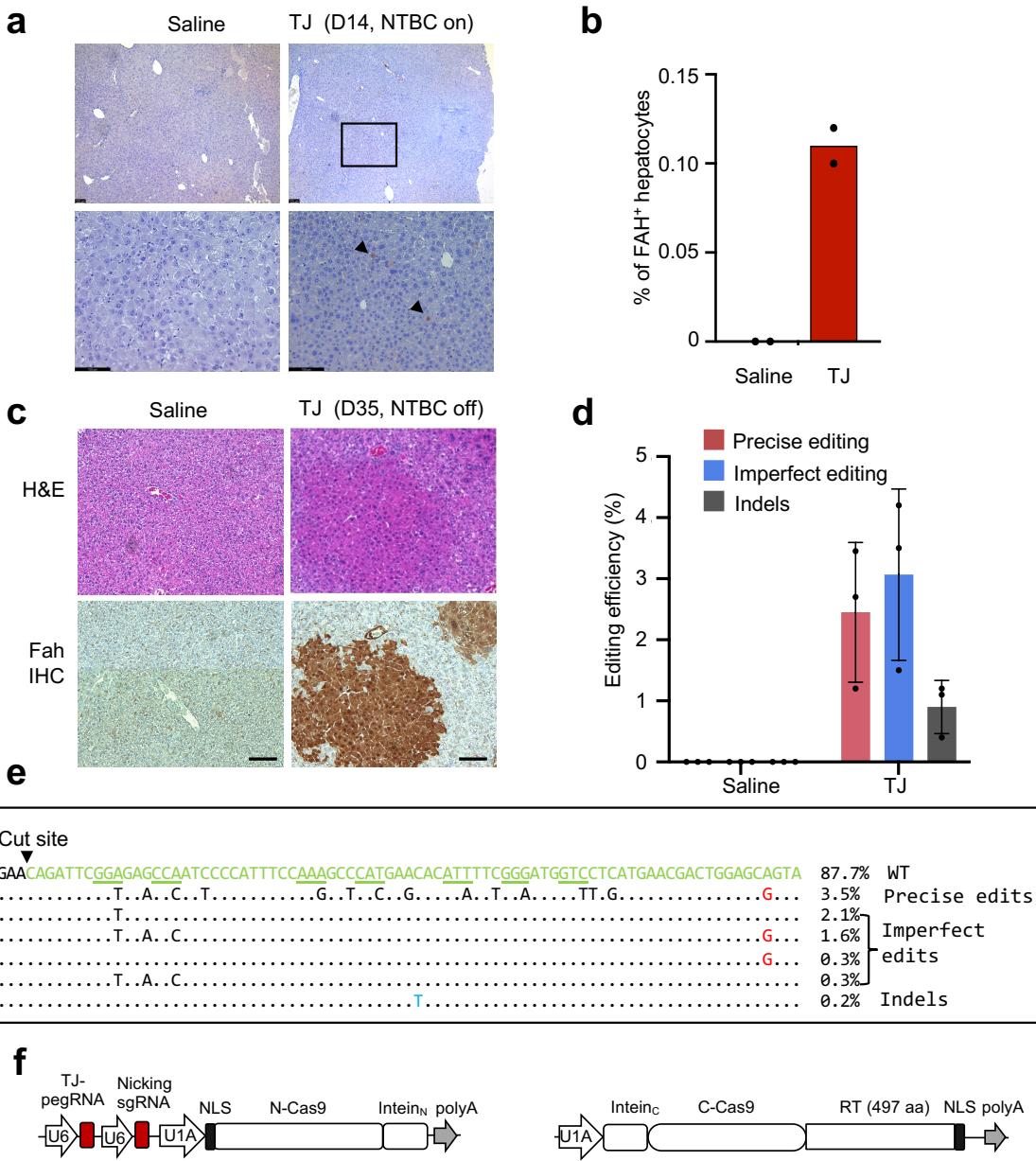
Supplementary Fig. 9. TJ-PE rewrites a correction exon in mouse liver. a, IHC staining of liver sections from *Fah*-mutant mice 14 days after TJ-PE treatment. FAH+ hepatocytes are indicated by arrows; scale bar, 100 μm. The lower panel of TJ is a high-magnification view (box with black line). Experiments were done two times, and one is shown. **b**, Quantification of FAH+ hepatocytes by IHC two weeks after hydrodynamic injection. (n=2 mice). **c**, Hematoxylin and eosin (H&E) staining and FAH Immunohistochemistry (IHC) staining of mouse liver sections 35 days after NTBC withdrawal. Experiments were done three times, and one is shown. Scale bar, 100 μm. **d**, Amplicon sequencing of exon 8 from TJ-PE-treated mouse livers two months after NTBC withdrawal. Results were obtained from three independent experiments, shown as mean ± s.d. **e**, Allele frequencies of a representative liver in **d**. All alleles ≥ 0.2% are shown. Green: Genomic sequence. Codons are underlined. Black: synonymous mutations in the correction exon. Red: correction G. Blue: indel. **f**, Schematic of the split-intein dual AAV8.

Fig.S10
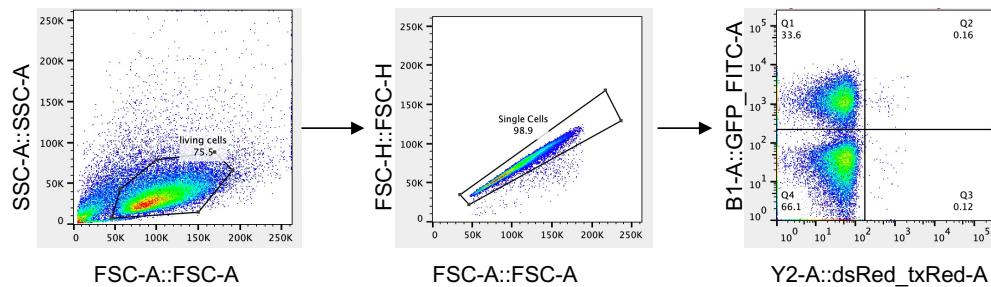
Supplementary Fig. 10. FACS gating examples for GFP-positive or Cherry-positive cells.

Supplementary Table 1. Sequences of pegRNAs and sgRNAs used in this study.

sgRNA scaffold
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCC

RC-PBS2-Insertion-PBS1-Tevopreq1

pegRNA	spacer sequence (5'-3')	3' extension
AAVS1 200 bp insertion TJ-pegRNA	GATGGAGC CAGAGAG GATCC	GCAGCTCAGGTTCTGGGATAACTTCGTATAATGTATGCTATACGAAGTTATAACAATCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACATTACCCTGTTATCCCTACCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATTCCTCTCTGGCTCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
AAVS1 200 bp insertion pegRNA	GATGGAGC CAGAGAG GATCC	CCGGGCAGGTCACGCATATAACTTCGTATAATGTATGCTATACGAAGTTATAACAATCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACATTACCCTGTTATCCCTACCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATTCCTCTCTGGCTCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
AAVS1 300 bp insertion TJ-pegRNA	GATGGAGC CAGAGAG GATCC	GCAGCTCAGGTTCTGGGATAACTTCGTATAATGTATGCTATACGAAGTTATAACAATCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACATTACCCTGTTATCCCTATAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCA

		CCATTCCTCTCTGGCTCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
AAVS1 300 bp insertion pegRNA	GATGGAGC CAGAGAG GATCC	CCGGGCAGGTCACGCATATAACTTCGTATAATGTATGCTATACGAAGTTATAACAATCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACATTACCCTGTTATCCCTATAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATTCCTCTCTGGCTCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
AAVS1 500 bp insertion TJ-pegRNA	GATGGAGC CAGAGAG GATCC	GCAGCTCAGGTTCTGGGATAACTTCGTATAATGTATGCTATACGAAGTTATAACAATCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACATTACCCTGTTATCCCTACCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATTCCTCTCTGGCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
AAVS1 500 bp insertion pegRNA	GATGGAGC CAGAGAG GATCC	CCGGGCAGGTCACGCATATAACTTCGTATAATGTATGCTATACGAAGTTATAACAATCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACATTACCCTGTTATCCCTACCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATTCCTCTCTGGCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
HEK3 200 bp insertion TJ-pegRNA	GGCCCAGA CTGAGCAC GTGA	GTCAACCAGTATCCCGGATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACCGTGCTCAGTCTGTAGACACACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAATTTTTTT
HEK3 200 bp insertion control PBS2 pegRNA	GGCCCAGA CTGAGCAC GTGA	CCTTTCCTCTGCCATCAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACCGTGCTCAGTCTGTAGACACACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAATTTTTTT
PRNP 200 bp insertion TJ-pegRNA	GCAGTGGT GGGGGGCC TTGG	GCATGTTTTCACGATAGATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCA

		CCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTAAGGCCCCCCACCATCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
PRNP 200 bp insertion pegRNA	GCAGTGGTGGGGGGCCTTGG	CCAGCATGTAGCCGCCAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTAAGGCCCCCCACCATCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
IDS 200 bp insertion TJ-pegRNA	GCATTTTCGATTCCGTGACT	ACTGAGGGATGTCTGAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACACGGAATCGAAATCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
IDS 200 bp insertion pegRNA	GCATTTTCGATTCCGTGACT	CGGATCCTCTTCCAAGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACACGGAATCGAAATCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
CCR5 400 bp Insertion TJ-pegRNA	CTCACTATGCTGCCGCCCAG	GGTACCTATCGATTGTCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGTAGGGATAACAGGGTAATGGCGGCAGCATAGTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
IDS 400 bp Insertion TJ-pegRNA	GCATTTTCGATTCCGTGACT	ACTGAGGGATGTCTGAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGTAGGGATAACAGGGTAATCACGGAATCGAAATCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
PRNP 400 bp Insertion TJ-pegRNA	GCAGTGGTGGGGGGCCTTGG	GCATGTTTTCACGATAGATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGT

		AGGGATAACAGGGTAATAGGCCCCCCACCATCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
GFP 89 bp replacement TJ-pegRNA	AGTTCAGCGTGTCCGGCTT	GTCAGGGTGGTCACGAGCGTAGGCCAGGGAACAGGCAGCTTACCCGTTGTGCAAATGAATTTGAGTGTGAGTTTCCCATAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGATAAATATCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
SA-Puro insertion TJ-pegRNA	GATGGAGCCAGAGAGGATCC	GCAGCTCAGGTTCTGGGTCAGGCACCGGGCTTGCGGGTCATGCACCAGGTGCGCGGTCCTTCGGGCACCTCGACGTCGGCGGTGACGGTGAAGCCGAGCCGCTCGTAGAAGGGGAGGTTGTGGGGCGCGGAGGTCTCCAGGAAGGCGGGCACCCCGGCGCGCTCGGCCGCCTCCACTCCGGGGAGCACGACGGCGCTGCCCAGACCCTTGCCCTGGTGGTCGGGCGAGACGCCGACGGTGGCCAGGAACCACGCGGGCTCCTTGGGCCGGTGCGGCGCCAGGAGGCCTTCCATCTGTTGCTGCGCGGCCAGCCGGGAACCGCTCAACTCGGCCATGCGCGGGCCGATCTCGGCGAACACCGCCCCCGCTTCGACGCTCTCCGGCGTGGTCCAGACCGCCACCGCGGCGCAGTCGTCCGCGACCCACACCTTGTCGATGTCGAGCCCGACGCGCGTGAGGAAGAGTTCTTGCAGCTCGGTGACCCGCTCGATGTGGCGGTCCGGATCGACGGTGTGGCGCGTGGCGGGGTAGTCGGCGAACGCGGCGGCGAGGGTGCGTACGGCCCTGGGGACGTCGTCGCGGGTGGCGAGGCGCACCGTGGGCTTGTACTCGGTCATTGGGCCAGGATTCTCCTCGACGTCACCGCATGTTAGCAGACTTCCTCTGCCCTCTCCGCTGCCAGATCTCTCGAGGCCCTGTGGGAGGAAGAGAAGAGGTCAGAAGCTTTCCTCTCTGGCTCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
SA-GFP insertion TJ-pegRNA	GATGGAGCCAGAGAGGATCC	GCAGCTCAGGTTCTGGGCTCAGAATTCCTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACTGGGCCAGGATTCTCCTCGACGTCACCGCATGTTAGCAGACTTCCTCTGCCCTCTCCGCTGCCAGATCTCTCGAGGCCCTGTGGGAGGAAGAGAAGAGGTCAGAAGCTTTCCTCTCTGGCTCTCTCTCTCTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAATTTTTTT
AAVS1 split circular TJ-pegRNA		AGACCCTCGACCGTCGATTGTCCACTGGTCAACAATAGATGACTTACAACTAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCCTAACGTCAAGACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCAAGAGAATGAAAATCCGTTGACCTTAAACGGTCGTGTGGGTTCAAGTCCCTCCACCCCCACGCCGGAAACGCAATAGCCGAAAAACAAAAAAGCACATGAGGATCACCCATGTGCGCAGCTCAGGTTCTGGGATAACTTCGTATAATGTATGCTATACGAAGTTATAACAATCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACATTACCCTGTTATCCCTATCCTCTCTGGCTCCATCGTAAGCAAACCTTAGAGGTTCTGGCAAGCAAAAAACAAAACGGCTATTATGCGTTACCGGCGAGACGCTACGGACTTAAATAATTGAG

		CCTTAAAGAAGAAATTCTTTAAGTGGATGCTCTCAAACTCAGGGA AACCTAAATCTAGTTATAGACAAGGCAATCCTGAGCCAAGCCGA AGTAGTAATTAGTAAGACCAGTGGACAATCGACGGATAACAGCA TATCTAG
Fah TJ-pegRNA-1	GTAGGCCC TGGGAACA GATT	TAAGAACAGAACATCAGAGGAAGCTGGGCCACCAGGCATTACCG CTCCAGTCGTTCATCAAAACCATTCCAAATATGTGCTCGTGAGCC TTGGAAATGGGAATGGGTTCACCGAATCTGTTCCCAGGGCTATAT ATCTAGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGAAT TTTTT
Fah TJ-pegRNA-2	GTAGGCCC TGGGAACA GATT	TCAGAGGAAGCTGGGCCACCACATTACCGCTCCAATCATTCATCA AAACCATTCCAAATATATGCTCGTGAGCCTTGGAGATCGGAATGG GTTCACCAAACCTGTTCCCAGGGCTATATATCTAGACGCGGTTCT ATCTAGTTACGCGTTAAACCAACTAGAATTTTTTT

Nicking sgRNA	spacer sequence (5'-3')
AAVS1 nicking sgRNA	GCAGCTCAGGTTCTGGGAGA
HEK3 nicking sgRNA	GTCAACCAGTATCCCGGTGC
HEK3 control nicking sgRNA	GCACATACTAGCCCCTGTCT
PRNP nicking sgRNA	GCATGTTTTCACGATAGTAA
IDS nicking sgRNA	ACTGAGGGATGTCTGAAGGC
TLR reporter nicking sgRNA	TAGGTCAGGGTGGTCACGA
Fah nicking sgRNA-1	CCCTAAGAACAGAACATCAG
Fah nicking sgRNA-2	CAGAGGAAGCTGGGCCACC

Supplementary Table 2. Sequences of primers used for genomic DNA amplification.

	F (5'-3')	R (5'-3')
AAVS1	CTTGCCAGAACCTCTAAGGT	CCAGGATCAGTGAAACGCAC
HEK3	TCTGCTGCAAGTAAGCATGCATTTG	GCCCCTTCCAGGGACCTC
PRNP	AGTAAGCCAAAAACCAACATG	CTGTACTCATCCATGGGCCT
IDS	ACGTTGAGCTGTGCAGAGAA	GTGCGTATGGAATAGCCCAT

Supplementary Table 3. Sequences of primers used for ddPCR.

Gene	F (5'-3')	R (5'-3')	Probe
AAVS1	CTTGCCAGAACCTCT AAGGT	GCTGAACTTGTGGCC GTTT	Ins CACCACCCCGGTGAACAGCTC
HEK3	GCATGCATTTGTAGG CTTGATG	CAGCCAAACTTGTCA ACCAG	WT CCTGGCCTGGGTCAATCCTTGG Ins CACGGGCAGCTTGCCGGTGG Ins CACCACCCCGGTGAACAGCTC
PRNP	F GTCAGTGGAACAA GCCGAGT F TAGGTCAGGGTGG TCACGAG	ACTTGGTTGGGGTAA C GGTG	WT TGAAGCACATGGCTGGTGCTGC Ins CACGGGCAGCTTGCCGGTGG Ins CACCACCCCGGTGAACAGCTC
IDS	F GCTGAACTTGTGG CCGTTT F TAGGTCAGGGTGG TCACGAG	GTGCGTATGGAATAG CCCAT	Ins CACCACCCCGGTGAACAGCTC Ins CACGGGCAGCTTGCCGGTGG

	TAGGTCAGGGTGGT	TTCCTGGGAGAGACG	Ins CACGGGCAGCTTGCCGGTGG
CCR5	CACGAG	CAAAC	

Supplementary Table 4. Sequences of primers used for high throughput sequencing.

Gene	F (5'-3')	R (5'-3')
AAVS1	AGACGTGTGCTCTTCCGATCTCTTGCC AGAACCTCTAAGGT	CTACACGACGCTCTTCCGATCTCCAGGAT CAGTGAAACGCAC
Fah	CTACACGACGCTCTTCCGATCTACCAA CTTTCTCCATGGCAG	AGACGTGTGCTCTTCCGATCTTGTCCCAT ACCCAACTCCTG