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Supplemental Information

A flexible split prime editor using truncated reverse transcriptase improves dual-AAV delivery in mouse liver

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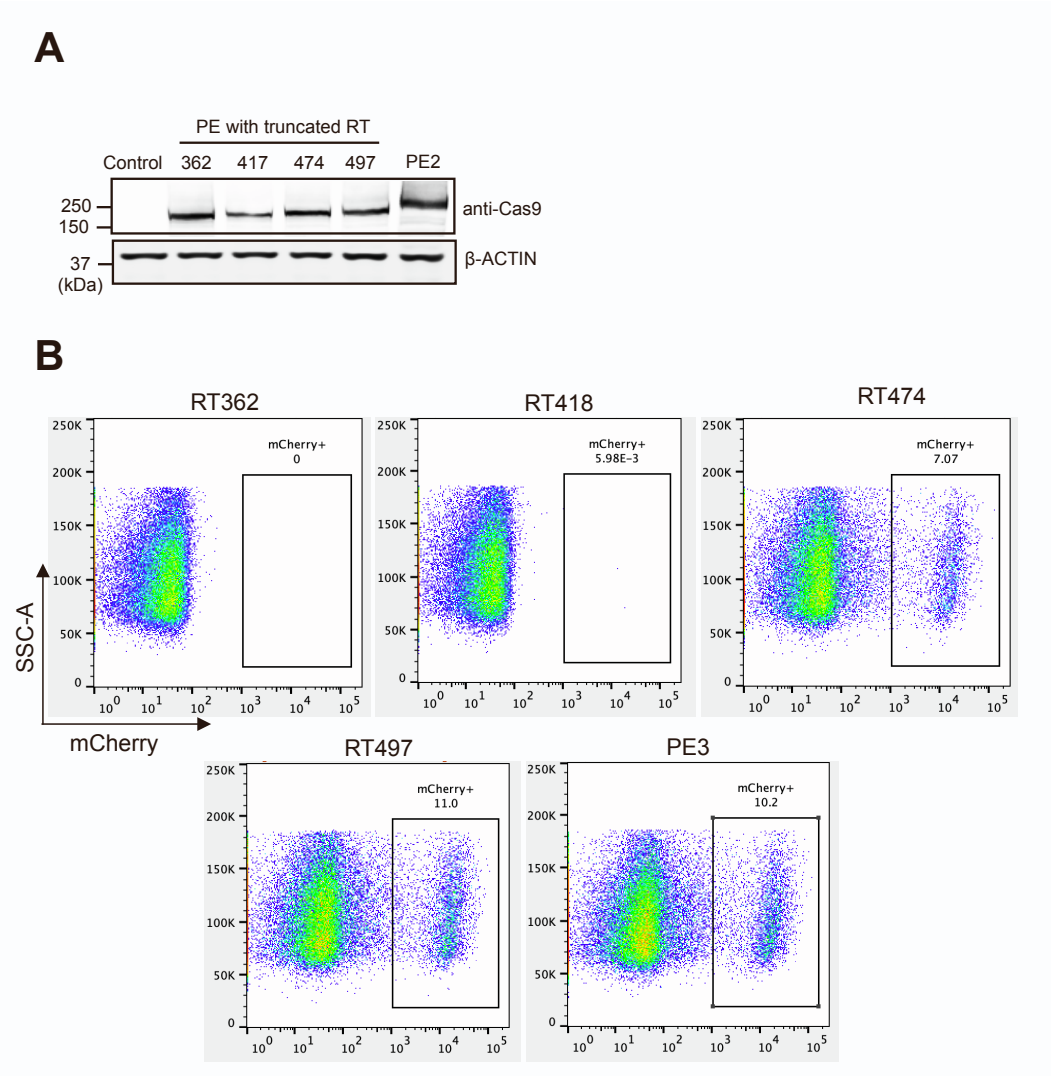
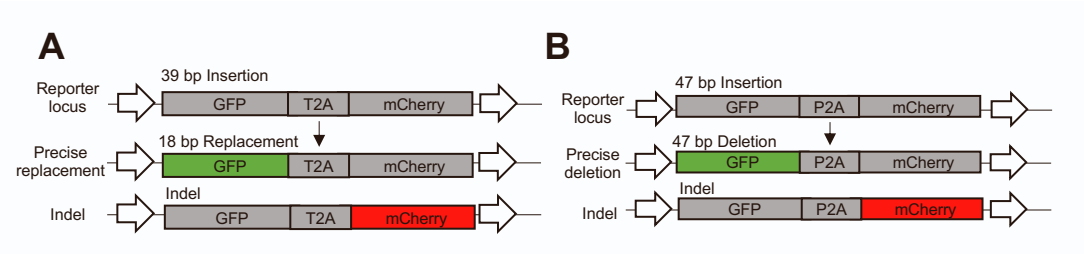
Figure S1



Supplemental Figure 1: Flow cytometry analysis of editing efficiency of full-length PE2 and RT variants.

A Western blot of full-length PE2 and compact PE2 variants. **B** Representative data of flow cytometry analysis of mCherry+ cells. The image data was analyzed by FlowJo 10.0 software. HEK293T cells were initially gated using FSC-A/SSC-A, then sorted for single cell using FSC-A/FSC-H. mCherry-positive cells were gated by SSC-A/Y2-A.

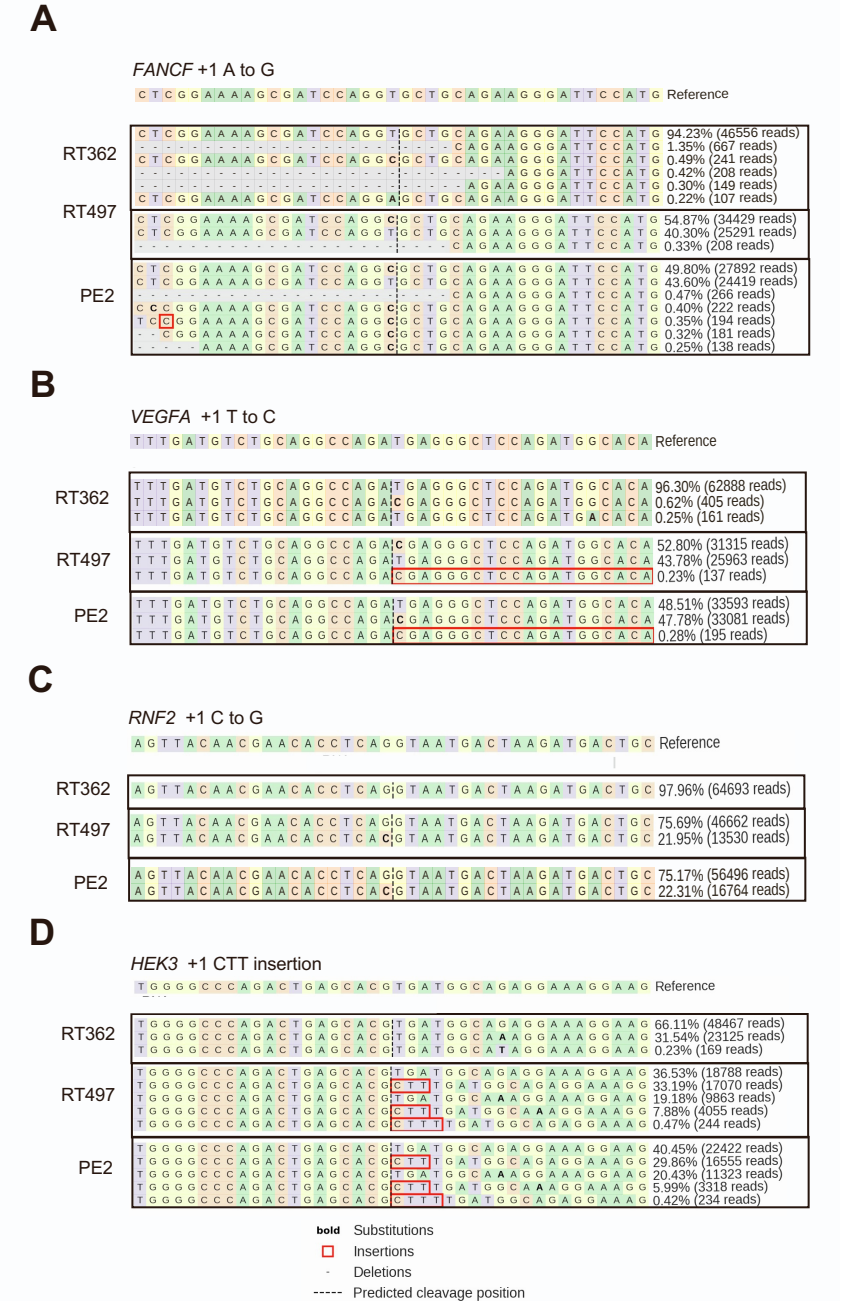
Figure S2



Supplemental Figure 2 Editing frequencies of RT variants in TLR-MCV1 and TLR reporter lines.

A Traffic light reporter multi-cas variant 1 (TLR-MCV1) cells containing a GFP with a 39-bp insertion, P2A, and out-of-frame mCherry. **B** TLR system containing a GFP with 47-bp insertion.

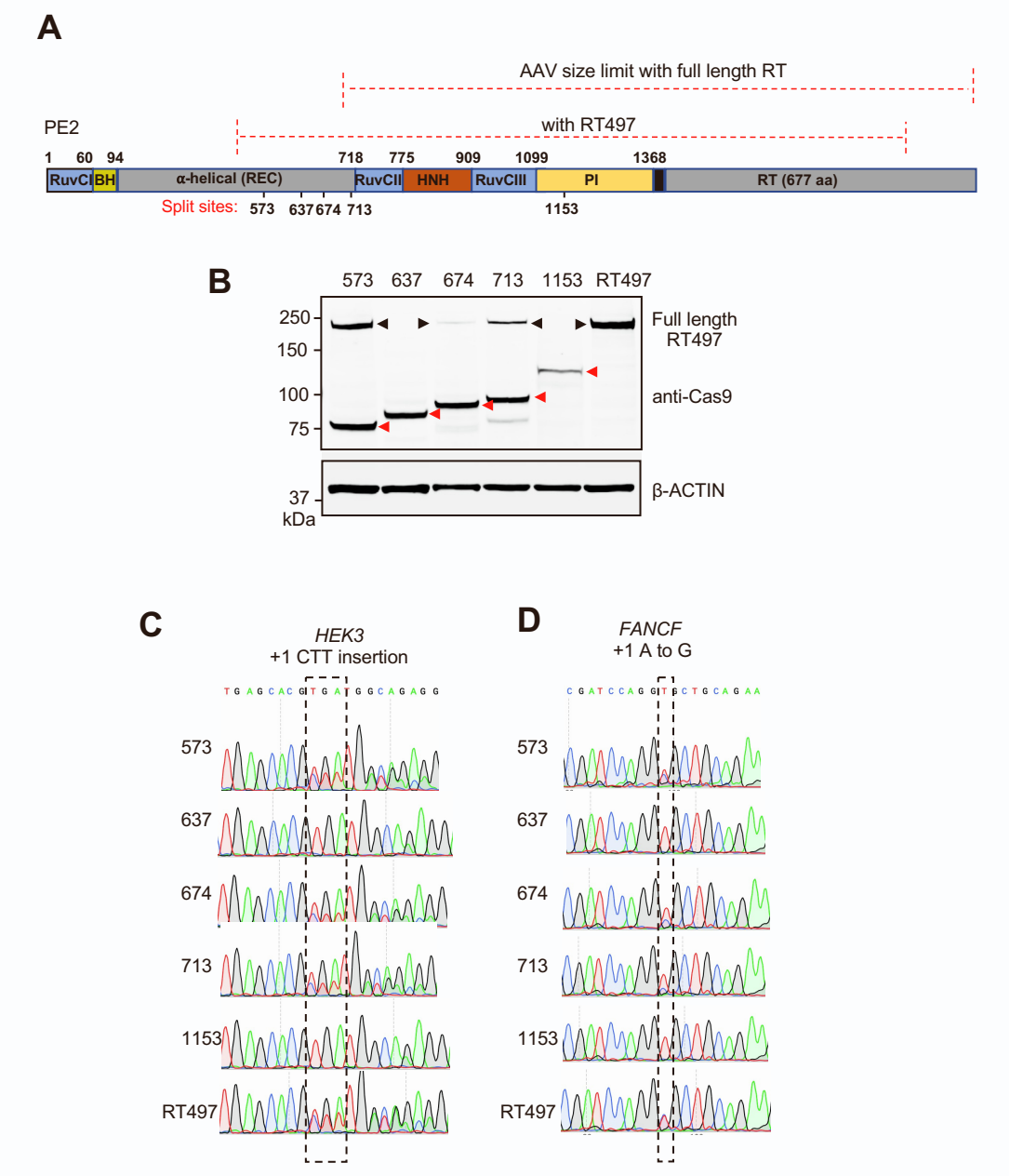
Figure S3



Supplemental Figure 3: Deep sequencing of endogenous loci.

A-D Allele frequencies and corresponding Illumina sequencing read counts are shown for each allele. All alleles observed with frequency ≥ 0.2% are shown.

Figure S4

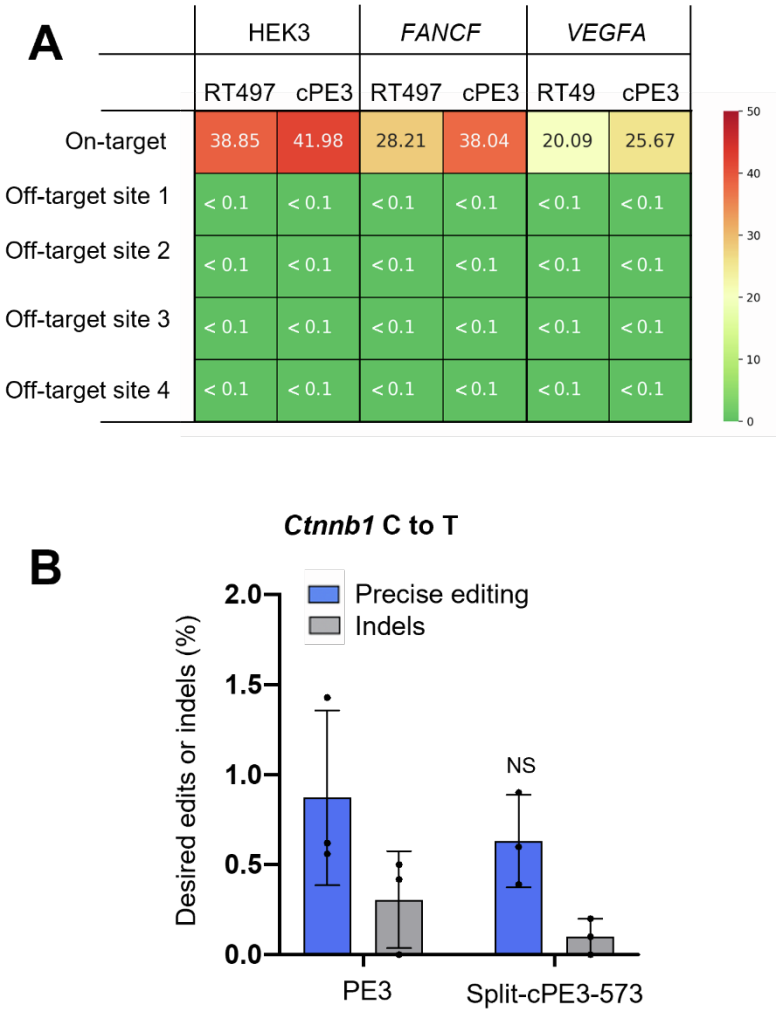


Supplemental Figure 4: Sanger sequencing of endogenous loci of split-cPE3 and RT497.

A Schematic representation of PE2 and RT497 split sites. AAV size limit is ~4.7 kb without ITR sequence. **B** Western blot showing split-cPE2 and unsplit RT497 expression. N-terminal

compact PE2 and C-terminal compact PE2 of each variant were co-transfected into HEK293T cells. As a positive control, unsplit RT497 plasmid was transfected. Cell lysates were probed with anti-GAPDH and anti-Cas9. Unspliced (red arrows) and reconstituted RT497 (black arrows) were detected using anti-Cas9 antibody. β-actin was used as a loading control. Unspliced and reconstituted RT497 were observed after transfection of split-cPE2-573, split-cPE2-674, and split-cPE2-713 (lanes 1,3,4). Only unspliced RT497 (red arrows) was detected after transfection of split-cPE2-637 and split-cPE2-1153 (lanes 2,5). **C-D** Sanger sequencing showed +1 CTT insertion at HEK3 locus **C** and +1 A to G transversion at *FANCF* locus. **D** Split-cPE2-573 supports robust editing efficiency at HEK3 and *FANCF* loci.

Figure S5



Supplemental Figure 5: Comparison of prime editing and off-target editing by cPE3 and split-cPE3-573.

A Average triplicate on-target and off-target editing efficiencies in HEK293T cells for cPE3 or split-cPE3-573 at known Cas9 off-target sites of HEK3, *FANCF* and *VEGFA* using deep sequencing. **B** Editing frequencies of C to T transversion in *Ctnnb1*. PE2 or split-cPE2-573, pegRNA and nicking sgRNA were delivered to the liver of FVB mice via hydrodynamic tail vein injection. Seven days after injection, livers were harvested, and the genomic DNA was sequenced. Mean ± s.d. of n = 3 independent biological replicates.

Supplementary Table 1. Sequences of pegRNAs and sgRNAs used in this study. All sequences are shown in 5' to 3' orientation.

Supplementary Table 2. Sequences of primers used for cloning.

Supplementary Table 3. Sequences of primers used for genomic DNA amplification and high throughput sequencing.

Supplementary Sequences: Sequence of backbone plasmid used for prime editing

Supplementary Table 1. Sequences of pegRNAs and sgRNAs used in this study. All sequences are shown in 5' to 3' orientation.

sgRNA scaffold
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA AGTGGGACCGAGTCGGTCC

pegRNA	spacer sequence (5'-3')	3' extension	PBS (nt)	RT (nt)	Figure
mCherry A to G	CACCTTCA GCTTGGCG GTCT	TACGAGGGCACTCAA ACCGCCAAGCTGAAG	14	16	Figure 1C
GFP-insertion	AAGTTCA GCGTGTCC GGCTT	GTCAGCTTGCCGTAG GTGGCATCGCCCTCG CCTTCG	13	36	Figure 1D
GFP-deletion	GCGGAGA GGGCACC CCCGA	GTTGGTCATGCGACC CTGCTCGGGGGTGCC CTCTCC	14	22	Figure 1E
FANCF +1 A to G	GGAATCC CTTCTGCA GCACC	GGAAAAGCGATCCAG GCGCTGCAGAAGGGA T	14	17	Figure 2A, 3B
VEGFA +1 T to C	GATGTCTG CAGGCCA GATGA	AATGTGCCATCTGGA GCCCTCGTCTGGCCTG CAGA	13	22	Figure 2A
RNF2 +1 C to G	GTCATCTT AGTCATTA CCTG	AACGAACACCTCACG TAATGACTAAGATG	15	14	Figure 2A
HEK3 +1 CTT insertion	GGCCCAG ACTGAGC ACGTGA	TCTGCCATCAAAGCG TGCTCAGTCTG	13	13	Figure 2A, 3B
HEK3 +1 T to	GGCCCAG	TGGAGGAAGCAGGGC	13	34	Figure

A	ACTGAGC ACGTGA	TTCCTTTCCTCTGCCA TCTCGTGCTCAGTCTG			2B
HEK3 +12 G to C	GGCCCAG ACTGAGC ACGTGA	TGGAGGAAGCAGGGC TTCCTTTGCTCTGCCA TCACGTGCTCAGTCTG	13	34	Figure 2B
HEK3 +30 C to G	GGCCCAG ACTGAGC ACGTGA	TGGACGAAGCAGGGC TTCCTTTCCTCTGCCA TCACGTGCTCAGTCTG	13	34	Figure 2B
Ctnnb1 C to T	AGGGTTG CCCTTGCC ACTCA	GCTCCTTTCCTGAGTG GCAAGGGCAA	13	13	Figure 3C
Pcsk9 TGA insertion	GTTGCTGC TACTGTGC CCCAC	AGCGCCGGTTCAGGG GCACAGTAGCA	13	13	Figure 4

Nicking sgRNA	**spacer sequence (5'-3')**	**Figure**
mCherry A to G	GCTGTCCCCTCAGTTCATGTA	Figure 1C
GFP-insertion	GTAGGTCAGGGTGGTCACGA	Figure 1D
GFP-deletion	GAGAAGCCGTAGCCCATCACG	Figure 1E
FANCF +1 A to G	GGGGTCCCAGGTGCTGACGT	Figure 2A, 3B
VEGFA +1 T to C	GATGTACAGAGAGCCCAGGGC	Figure 2A
RNF2 +1 C to G	GTCATCTTAGTCATTACCTG	Figure 2A
HEK3 +1 CTT insertion	GGCCCAGACTGAGCACGTGA	Figure 2A, 3B
HEK3 +1 T to A	GGCCCAGACTGAGCACGTGA	Figure 2B
HEK3 +12 G to C	GGCCCAGACTGAGCACGTGA	Figure 2B
HEK3 +30 C to G	GGCCCAGACTGAGCACGTGA	Figure 2B
Ctnnb1 C to T	GAAAAGCTGCTGTCAGCCAC	Figure 3C
Pcsk9 TGA insertion	GCCATCCTCCTGGGACGGGA	Figure 4

Supplementary Table 2. Sequences of primers used for cloning.

	F (5'-3')	**R (5'-3')**
RT362	CAGATTTGTCTGGCGGCTC AAAAAGAACC	CCGCCAGACAAATCTGGCA ACCCCAGGG
RT418	TTGCCGTATCTGGCGGCTC AAAAAGAACC	CCGCCAGATACGGCAATGG CTGCTACC
RT474	TCGGACCGTCTGGCGGCTC AAAAAGAACC	CCGCCAGACGGTCCGAACT GGACCCGG
RT497	GCCTTGATTCTGGCGGCTC AAAAAGAACC	CCGCCAGAATCAAGGCAGT TGTGTTGCA
N-terminal Split-cPE2-573	TCGAGTCACCAAAGAAGA AGCGGAAAGTCGACAAGA AGTACAGCATCGGC	TGTCAGGATCTCTGTCTCGT AGGACAGGCACTCGATTTT CTTGAAGTAGTCCTC
	TGCCTGTCCTACGAGACAG A	GCCGTCGGCGGTTCTTTTTG AGCCGCCAGAATTAGGCAG

		GTTATCCACTC
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
C-terminal Split-cPE2-573	TTCGAGTCACCAAAGAAGAAGCGGAAAGTCATGATCAAGATTGCTACACG	CACGCCGGAGATTTCCACGGAGTCGAAGCAATTGCTGGCGATAAAGCCA
	TGCTTCGACTCCGTGGAAAT	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATCAAGGCAGTTGTGTTGCA
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
N-terminal Split-cPE2-637	TCGAGTCACCAAAGAAGAAGCGGAAAGTCGACAAGAAGTACAGCATCGGC	TGTCAGGATCTCTGTCTCGTAGGACAGGCATTTCAGCCGTTCCTCGATCA
	TGCCTGTCCTACGAGACAGA	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATTAGGCAGGTTATCCACTC
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
C-terminal Split-cPE2-637	TTCGAGTCACCAAAGAAGAAGCGGAAAGTCATGATCAAGATTGCTACACG	ATTGCTGGCGATAAAGCCAT
	ACCTATGCCCACCTGTTCGA	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATCAAGGCAGTTGTGTTGCA
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
N-terminal Split-cPE2-674	TCGAGTCACCAAAGAAGAAGCGGAAAGTCGACAAGAAGTACAGCATCGGC	TGTCAGGATCTCTGTCTCGTAGGACAGGCACTGCTTGTCCCGGATGCCG
	TGCCTGTCCTACGAGACAGA	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATTAGGCAGGTTATCCACTC
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
C-terminal Split-cPE2-674	TTCGAGTCACCAAAGAAGAAGCGGAAAGTCATGATCAAGATTGCTACACG	ATTGCTGGCGATAAAGCCAT
	GCCCTGAAGAATGGCTTTATCGCCAGCAATTCCGGCAAGACAATCCTGGA	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATCAAGGCAGTTGTGTTGCA
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
N-terminal Split-cPE2-713	TCGAGTCACCAAAGAAGAAGCGGAAAGTCGACAAGAAGTACAGCATCGGC	TGTCAGGATCTCTGTCTCGTAGGACAGGCAcacctgggctttctggatgt

	TGCCTGTCCTACGAGACAGA	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATTAGGCAGGTTATCCACTC
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
C-terminal Split-cPE2-713	TTCGAGTCACCAAAGAAGAAGCGGAAAGTCATGATCAAGATTGCTACACG	ATTGCTGGCGATAAAGCCAT
	GCCCTGAAGAATGGCTTTATCGCCAGCAATtccggccagggcgatagcc	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATCAAGGCAGTTGTGTTGCA
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
N-terminal Split-cPE2-1153	TCGAGTCACCAAAGAAGAAGCGGAAAGTCGACAAGAAGTACAGCATCGGC	TGTCAGGATCTCTGTCTCGTAGGACAGGCACTTGCCCTTTTCCACTTTGG
	TGCCTGTCCTACGAGACAGA	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATTAGGCAGGTTATCCACTC
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG
C-terminal Split-cPE2-1153	TTCGAGTCACCAAAGAAGAAGCGGAAAGTCATGATCAAGATTGCTACACG	ATTGCTGGCGATAAAGCCAT
	CCCTGAAGAATGGCTTTATCGCCAGCAATTCCAAGAAACTGAAGAGTGTG	GCCGTCGGCGGTTCTTTTTGAGCCGCCAGAATCAAGGCAGTTGTGTTGCA
	TCTGGCGGCTCAAAAAGAAC	GACTTTCCGCTTCTTCTTTGG

Supplementary Table 3. Sequences of primers used for genomic DNA amplification and high throughput sequencing.

Gene	**F (5'-3')**	**R (5'-3')**	**Figure**
FANCF on-target	CTACACGACGCTCTTCCGATCTGATGGATGTGGCGCAGGTAG	AGACGTGTGCTCTTCCGATCTAGGCGTATCATTTCGCGGAT	Figure 2A, 3B
VEGFA on-target	CTACACGACGCTCTTCCGATCTAAGCATCCCTGGACACTTCC	AGACGTGTGCTCTTCCGATCTTGGACCCCCTATTTCTGACCT	Figure 2A
RNF2 on-target	CTACACGACGCTCTTCCGATCTACCATAGCACTTCCCTTCCA	AGACGTGTGCTCTTCCGATCTTATCCCAGTTTACACGTCTC	Figure 2A
HEK3	CTACACGACGCTCTTCCG	AGACGTGTGCTCTTCCGATC	Figure

on-target	ATCTTGCTGCAAGTA AGCATGCATTTG	TCTTCCAGCCCAGCCAAACT T	2A, 2B, 3B
HEK3 off-target site 1	CTACACGACGCTCTTCCG ATCTTCCCCTGTTGACCTG GAGAA	AGACGTGTGCTCTTCCGATC TCACTGTACTTGCCCTGACC A	Figure S5A
HEK3 off-target site 2	CTACACGACGCTCTTCCG ATCTTTGGTGTTGACAGG GAGCAA	AGACGTGTGCTCTTCCGATC TCTGAGATGTGGGCAGAAG GG	Figure S5A
HEK3 off-target site 3	CTACACGACGCTCTTCCG ATCTTGAGAGGGAACAGA AGGGCT	AGACGTGTGCTCTTCCGATC TGTCCAAAGGCCCAAGAAC CT	Figure S5A
HEK3 off-target site 4	CTACACGACGCTCTTCCG ATCTTCCTAGCACTTTGG AAGGTCG	AGACGTGTGCTCTTCCGATC TGCTCATCTTAATCTGCTCA GCC	Figure S5A
FANCF off-target site 1	CTACACGACGCTCTTCCG ATCTGCGGGCAGTGGCGT CTTAGTCG	AGACGTGTGCTCTTCCGATC T CTCCTTGCCGCCCAGCCGGT C	Figure S5A
FANCF off-target site 2	CTACACGACGCTCTTCCG ATCTCCAGTGTTTCCCATC CCCAACAC	AGACGTGTGCTCTTCCGATC TCAGGCCCACAGGTCCTTCT GGA	Figure S5A
FANCF off-target site 3	CTACACGACGCTCTTCCG ATCT CCCTGGGTTTGGTTGGCT GCTC	AGACGTGTGCTCTTCCGATC TCACTGGGGAAGAGGCGAG GACAC	Figure S5A
FANCF off-target site 4	CTACACGACGCTCTTCCG ATCTGAATGGATCCCCCC CTAGAGCTC	AGACGTGTGCTCTTCCGATC TGAAGACACAGAAATCACA AACCGGC	Figure S5A
VEGFA off-target site 1	CTACACGACGCTCTTCCG ATCTGTTGCGATGGTTTC ACTCCTG	AGACGTGTGCTCTTCCGATC TGCAGCGTCTCTGATGCGAT	Figure S5A
VEGFA off-target site 2	CTACACGACGCTCTTCCG ATCTCCCTCATGCCCATG AATTGTT	AGACGTGTGCTCTTCCGATC TTGGTGATCGCCTGCCATTT C	Figure S5A

VEGFA off-target site 3	CTACACGACGCTCTTCCGATCTCCCAAGATCATACAGCTCA	AGACGTGTGCTCTTCCGATCTAAGTACCATAGATTGGGTGG	Figure S5A
VEGFA off-target site 4	CTACACGACGCTCTTCCGATCT CCATAGACTGGGTGGCTTA	AGACGTGTGCTCTTCCGATCT GCCTTTGTGAATGGGATCA	Figure S5A
CTNNB1 on-target	CTACACGACGCTCTTCCGATCTCCATGGAGCCGGACAGAAAA	AGACGTGTGCTCTTCCGATCTTGCGTGAAGGACTGGGAAAA	Figure S5B

Supplementary Sequences Sequence of backbone plasmid used for prime editing

Sequence of reverse transcriptase: Finger-Palm domain (1-275 aa) + Thumb domain (276-361 aa) + Connection domain (362 - 496 aa) + RNase H domain (497-671 aa)

TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSI
KQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNK
RVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGIS
GQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQG
TRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT
PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAP
ALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRM
VAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDR
VQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSL
LQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRY
AFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEAR
GNRMADQAARKAAITETPDTSTLL

Sequence of RT497: cmv promoter + N-terminal NLS + Cas9 H840A + Flexible linker + M-MLV reverse transcriptase + C-terminal NLS + Plasmid backbone

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGT
ACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCA

TTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTC
GTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTC
TATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGAGATCCGCGGCCGCT
AATACGACTCACTATAGGGAGAGCCGCCACCATGAAACGGACAGCCGACGGAAGC
GAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTCGACAAGAAGTACAGCATCGGCCT
GGACATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGC
CCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAA
CCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGA
AGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCA
AGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGG
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CCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCC
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CCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGT
ACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATC
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GACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCG
ACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTG
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GGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTCC
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Backbone of pegRNA and Nicking sgRNA: U6 promoter + spCas9-sgRNA scaffold + Plasmid backbone

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