

Supplementary Materials

Materials and methods

Plasmid construction. Modified pegRNAs or split pegRNAs expression plasmids pPB- PE-ALL-RNA, pPB-sPE-ALL-RNA, or pPB-SnPE-ALL-RNA for sPEs tPEs or SnPEs were generated by two steps (Figure S9). First, pPE-pegRNA, psPE-pegRNA, pSnPE- 5'-pRNA, pSnPE-3'-pRNA, pSnPE-circ-pRNA and pSnPE-sgRNA pPE3-sgRNA were constructed separately. For pPE-pegRNA, psPE-pegRNA, pSnPE-5'-pRNA, pSnPE-3'- pRNA and pSnPE-circ-pRNA (Table S1), the oligos were synthesized and annealed with 5' ACCG and 3' AAAA overhangs and cloned into pLH-AAAA backbone plasmid (Sequence S1). For pSnPE-sgRNA and pPE3-sgRNA, the oligos were synthesized and annealed with 5' ACCG and 3' AAAC overhangs and cloned into pLH-sgRNA3 backbone vector (Sequence S1). Second, Golden Gate assembly was used to clone related RNA expression cassettes into one vector pDONOR5.1 (sequence S1) and generate pPB-PE-ALL-RNA, pPB-sPE-ALL-RNA, or pPB-SnPE-ALL-RNA respectively. pCMV-PE2 (#addgene 132775) was used to generate RBP-PE2 (SpCas9H840A-MMLV) expression plasmids. First, T2A-GFP was inserted at the C terminal of pCMV- PE2 resulting in pCMV-PE2-T2A-GFP. Second, RNA binding proteins for stem loop aptamers including MCP, tdMCP, PCP, tdPCP, Com, N22p and Csy4H29A (Sequence S2) were synthesized and cloned to the pCMV-PE2-T2A-GFP using ClonExpress II One Step Cloning Kit (Vazyme, C112-01), resulting pCMV-MCP-PE2-T2A-GFP, pCMV- tdMCP-PE2-T2A-GFP, pCMV-PCP-PE2-T2A-GFP, pCMV-tdPCP-PE2-T2A-GFP, pCMV-Com-PE2-T2A-GFP, pCMV-N22p-PE2-T2A-GFP and pCMV-Csy4H29A-PE2- T2A-GFP respectively.

Cell culture and transfection and genomic DNA extraction. HEK293FT (Thermo Fisher Scientific), HeLa (ATCC, CCL-2), and U2OS (ATCC, HTB-96) cells were cultured in DMEM with high glucose in 10% FBS (fetal bovine serum, Thermo Fisher Scientific). All cells were cultured at 37°C and 5% CO₂ in a humidified incubator. Cells were seeded in 12-well plates and transfected at approximately 60% confluence using Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's protocols. A total of 1.5 µg Prime editor 2 (PE2, dCas9-MMLV) or RBP-PE2, and 500 ng PE-all-RNA, sPE-all- RNA, tPE-all-RNA or snPE-all-RNA expression plasmids were co-transfected into HEK293FT, U2OS or HeLa cells. 72 h after

transfection, Prime editors (GFP+) and PE- all-RNA, sPE-all-RNA, tPE-all-RNA or snPE-all-RNA (BFP+) double-positive cells were collected from flow cytometry (BD FACS Aria III). The genomic DNA of cells was extracted using QuickExtract DNA Extraction Solution (Lucigen) according to the manufacturer's protocols. The isolated DNA was PCR-amplified with Phanta Max Super-Fidelity DNA Polymerase (Vazyme). Primers used are listed in Table S2.

High-throughput DNA sequencing of genomic DNA samples. Genomic sites of interest were amplified from genomic DNA using locus-specific primers containing the Truseq adapters. Equal amounts of PCR products were pooled and gel purified. The purified library was deep sequenced using a paired-end 150 bp Illumina MiniSeq run. All prime editing experiments were analyzed as follows. Demultiplexing and base calling were both performed using bcl2fastq Conversion Software v2.18 (Illumina, Inc.), allowing 0 barcode mismatches with a minimum trimmed read length of 75. Alignment of sequencing reads to the Amplicon sequence (Table S5) was performed using CRISPResso2 (Clement et al., 2019) in standard mode using the parameters “-q 30”. For each amplicon, the CRISPResso2 quantification window was positioned to include the entire sequence between pegRNA- and sgRNA-directed Cas9 cut sites, as well as an additional 10 bp beyond both cut sites. For PE activity quantifications from deep sequenced data, editing efficiency was calculated as the percentage of reads with the desired editing without indels (“-discard_indel_reads TRUE.” mode) out of the total number of reads ((number of desired editing-containing reads)/(number of reference- aligned reads).). For all experiments, indel frequency was calculated as the number of discarded reads divided by the total number of reads ((number of indel-containing reads)/(number of reference-aligned reads). EditR (Kluesner et al., 2018) was used for PE activity quantifications from all sanger sequencing data.

Live cell CRISPR-based DNA imaging. All live-cell imaging was carried out on a DeltaVision Ultra imaging system (GE Healthcare). The cells were cultured on No. 1.0 glass bottom dishes (MatTek). The microscope stage incubation chamber was maintained at 37°C and 5% CO₂. GFP was excited at 488 nm and collected using filter at 498/30 nm (wavelength/bandwidth). Imaging data were acquired by DeltaVision Elite imaging (GE Healthcare Inc.) software. For the representative images, the raw data were deconvoluted by softWoRx (GE Healthcare Inc.) software.

Off-target analysis. Potential off-target sites were predicted in the human genome (GRCh38/hg38) with Cas-OFFinder6 (<http://www.rgenome.net/cas-offinder>); The region around the off-target sites was amplified with Phanta Max Super-Fidelity DNA Polymerase (Vazyme), and subjected to high-throughput sequencing. The amplicons were analyzed with CRIPRESSO2 (V2.0.43) and the off-target sites are listed in Supplementary Table 3. Primers used are listed in Supplementary Table 4.

Data availability

The next-generation sequencing data have been deposited in the NCBI Sequence Read Archive database under the BioProject accession code PRJNA785066. All other relevant data are available from corresponding authors upon reasonable request.

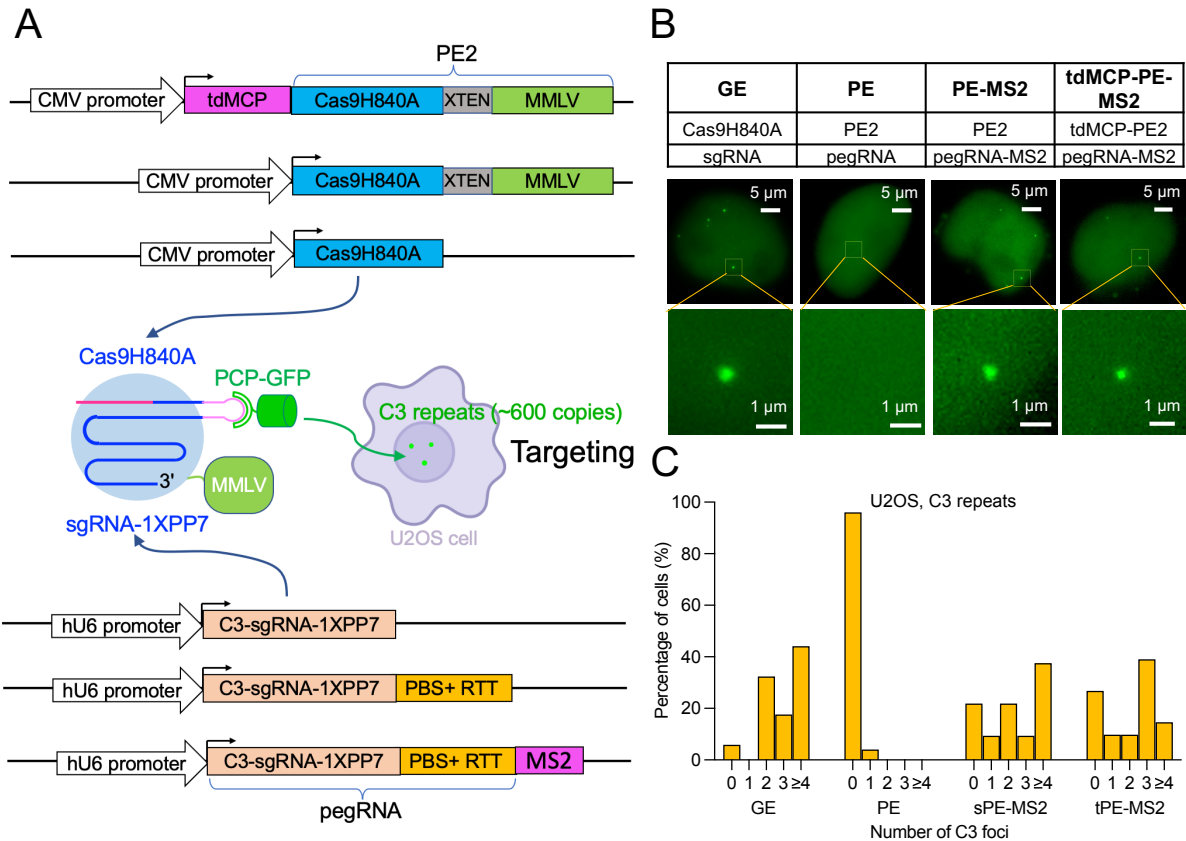


Figure S1 | Increase of targeting efficiency by adding stem-loop RNA aptamers at the 3'-terminal of pegRNAs.

(A) Schematic of the live-cell detection system for targeting efficiency. The detection system consists of Cas9 nickase, PCP-GFP, sgRNA-1XPP7 targeting C3 repeats (~600 copies of target sites on chromosome 3). The expression of Cas9H840A, Cas9H840A-MMLV (PE2) or tdMCP-PE2 was driven by CMV promoter. The expression of sgRNA, pegRNA or pegRNA-MS2 was driven by human U6 (hU6) promoter. (B) U2OS cells were co-transfected PCP-GFP along with expression plasmids as indicated for GE, PE, sPE-MS2, or tPE-MS2. After 48 hours, cells were imaged by collecting z-stack images to capture all foci in each nucleus examined. Representative images show the 2D projection of 3D imaging. (C) Histograms showing the number of C3 foci per cell counts by CRISPR-based labeling, n=50 transfected cells of GE, PE, sPE-MS2 or tPE-MS2.

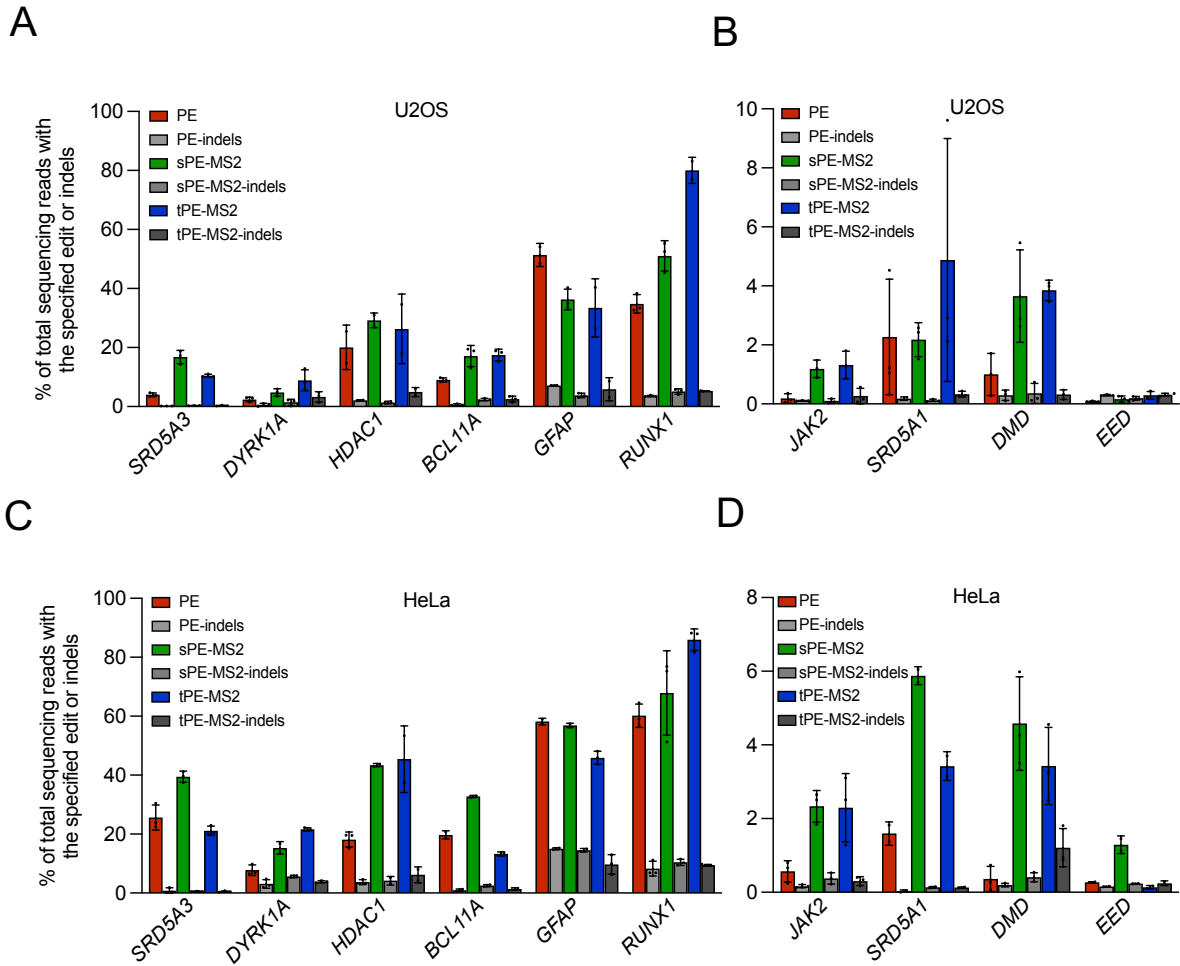


Figure S2 | sPEs and tPEs increase PE efficiency at various loci in different cell lines.

Efficiency of PE, sPE-MS2 and tPE-MS2 mediated point mutation of *SRD5A3*₊₂ C·G to A·T, *DYRK1A*₊₁ C·G to G·C, *HDAC1*₊₁ C·G to G·C, *BCL11A*₊₁ C·G to A·T, *GFAP*_{+1A} T·A to T·A and *RUNX1*₊₅ G·C to T·A using PE3 in U2OS cells (A), HeLa cells (C). Efficiency of PE, sPE-MS2 and tPE-MS2 mediated point mutation of *DMD*₊₁ T·A to C·G, *EED*₊₁ A·T to T·A, *JAK2*₊₁ C·G to T·A and *SRD5A1*₊₁ C·G to A·T using PE3 in U2OS cells (B), HeLa cells (D). Data and error bars in (A-D) indicate the mean and standard deviation of three independent biological replicates.

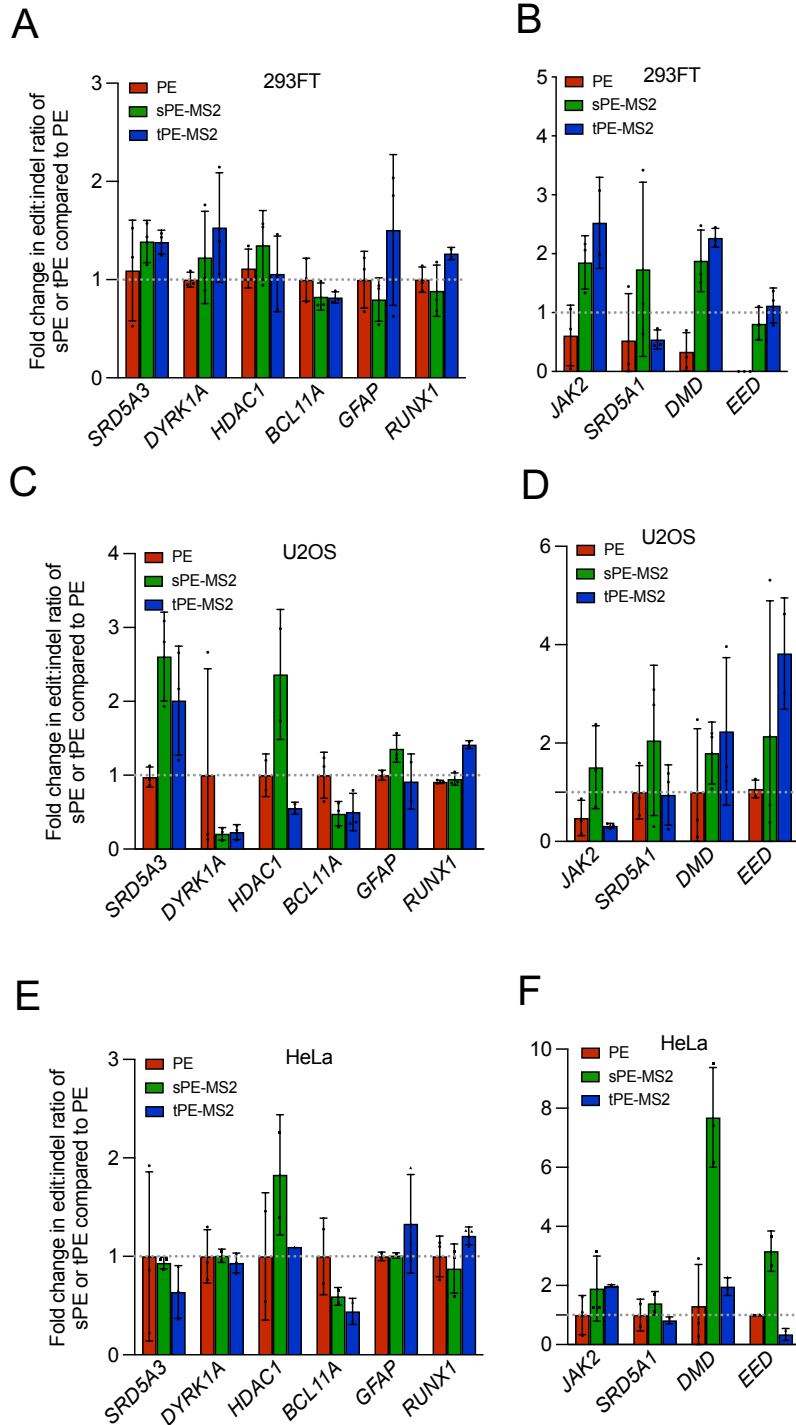


Figure S3 | Fold change in correct editing and Edit:indel ratio for sPE-MS2 and tPE-MS2 at different loci shown in Figure 1 and Figure S2.

Fold-change in the observed edit:indel ratio of PE, sPE-MS2 and tPE-MS2 mediated point mutation for *SRD5A3*_+2 C·G to A·T, *DYRK1A*_+1 C·G to G·C, *HDAC1*_+1 C·G

to G·C, *BCL11A*_+1C·G to A·T, *GFAP*_+1A·T to T·A and *RUNX1*_+5 G·C to T·A in HEK293FT cells (A) U2OS cells (C) and HeLa cells (E). Fold-change in the observed edit:indel ratio of PE, sPE-MS2 and tPEb-MS2 mediated point mutation of *DMD*_+1 T·A to C·G, *EED*_+1 A·T to T·A, *JAK2*_+1 C·G to T·A and *SRD5A1*_+1 C·G to A·T in HEK293FT cells (B) U2OS cells (D) and HeLa cells (F). Values were calculated from the data presented in Figure 1 and Figure S2. Data and error bars indicate the mean and standard deviation of three independent biological replicates.

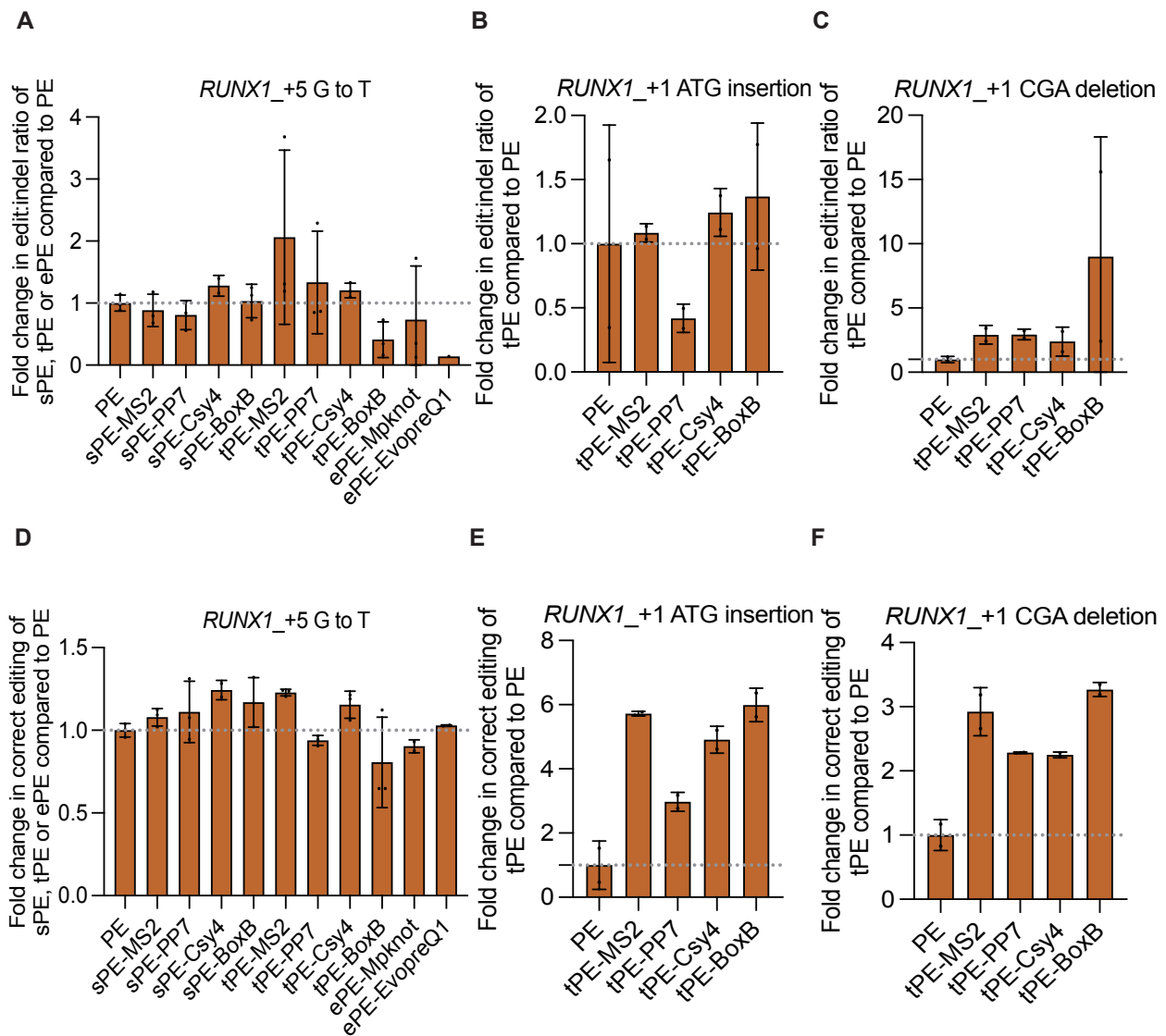


Figure S4 | Fold change in correct editing and edit:indel ratio for sPEs and tPEs shown in Figure 1.

(A) Fold-change in the observed edit:indel ratio of PE, sPE-MS2, tPE-MS2, ePE-Mpknot and ePE-EvopreQ1 mediated with point mutation for *RUNX1*_+5 G·C to T·A in HEK293FT cells. (B) Fold-change in the observed edit:indel ratio of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with insertion for *RUNX1*_+1 ATG in HEK293FT cells. (C) Fold-change in the observed edit:indel ratio of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with deletion for *RUNX1*_+1 CGA in HEK293FT cells. (D) Fold-change in the observed correct editing of PE, sPE-MS2, tPE-MS2, ePE-Mpknot and ePE-EvopreQ1 mediated with point mutation for *RUNX1*_+5 G·C to T·A in

HEK293FT cells. (E) Fold-change in the observed correct editing of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with insertion for *RUNX1*_+1 ATG in

HEK293FT cells. (F) Fold-change in the observed correct editing of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with deletion for *RUNX1*_+1 CGA in HEK293FT cells. Values were calculated from the data presented in Figure 1. Data and error bars indicate the mean and standard deviation of three independent biological replicates.

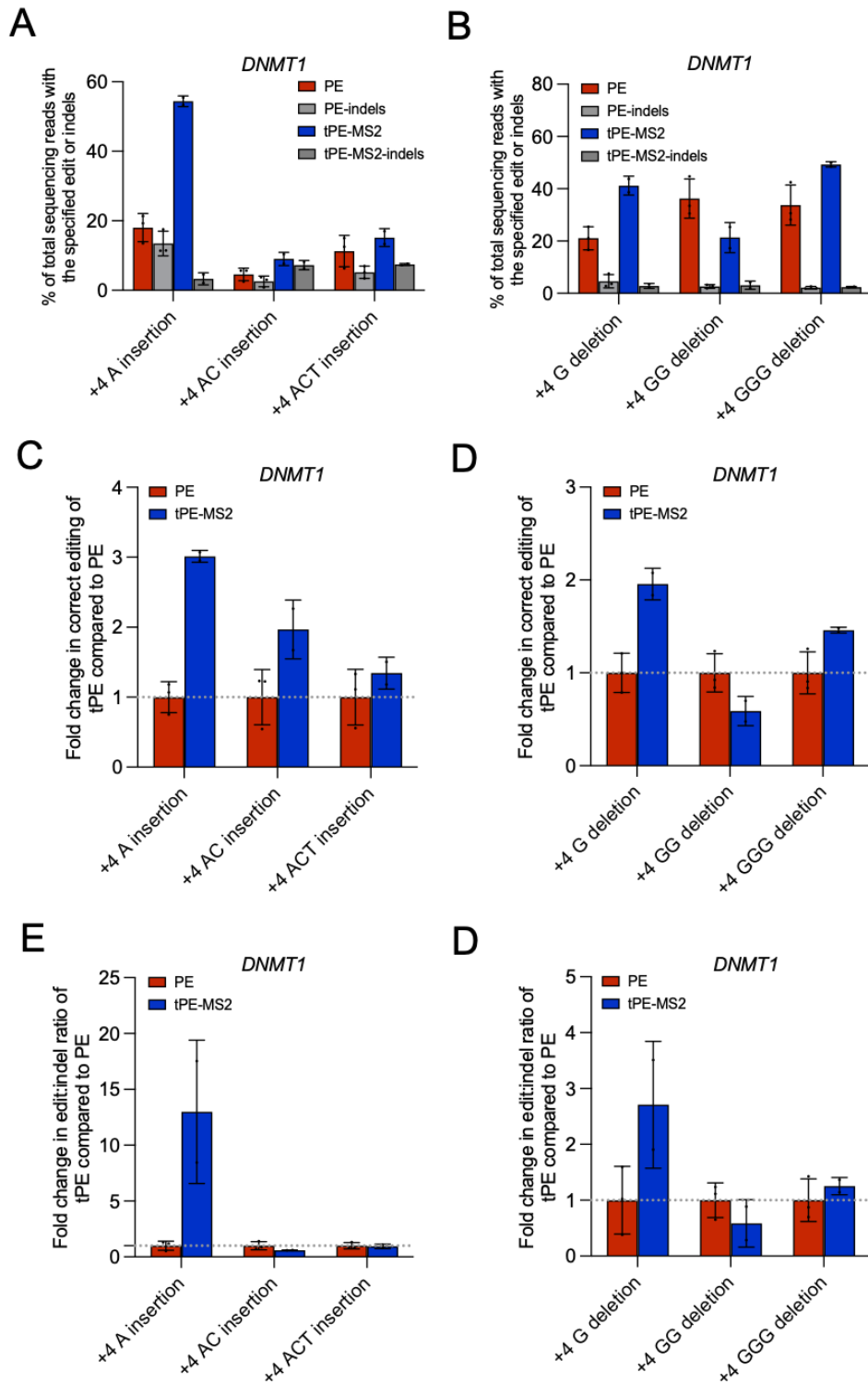


Figure S5 | Insertion and deletion efficiency for *DNMT1* loci and fold change in correct editing and edit:indel ratio.

(A) The efficiency of PE and tPE-MS2 mediated insertion of DNMT1₊₄ A, +4 AC, +4 ACT using PE3 in 293FT cells. (B) The efficiency of PE and tPE-MS2 mediated deletion of DNMT1₊₄ G, +4 GG, +4 GGG using PE3 in HEK293FT cells. (C) Fold-change in the observed correct editing ratio of PE, tPE-MS2 mediated with insertion for DNMT1₊₄ A, DNMT1₊₄ AC, DNMT1₊₄ACT in HEK293FT cells. (D) Fold-change in the observed correct editing ratio of PE, tPE-MS2 mediated with deletion for DNMT1₊₄ G, DNMT1₊₄ GG, DNMT1₊₄GGG in HEK293FT cells. (E) Fold-change in the observed edit:indel ratio of PE, tPE-MS2 mediated with insertion for DNMT1₊₄ A, DNMT1₊₄ AC, DNMT1₊₄ACT in HEK293FT cells. (F) Fold-change in the observed edit:indel ratio of PE, tPE-MS2 mediated with deletion for DNMT1₊₄ G, DNMT1₊₄ GG, DNMT1₊₄GGG in HEK293FT cells. Values were calculated from the data presented in Figure S5A and Figure S5B. Data and error bars indicate the mean and standard deviation of three independent biological replicates.

<i>DYRK1A</i>	PE	sPE-MS2	tPE-MS2
On target	8.0 (1.5)	20.6 (3.7)	28.3 (4.8)
Off-target site 1	<0.1 (<0.7)	<0.1 (2.4)	<0.1 (0.7)
Off-target site 2	<0.1 (<0.3)	<0.1 (<0.7)	<0.1 (0.1)

<i>RUNX1</i>	PE	sPE-MS2	tPE-MS2
On target	70.3 (1.1)	75.9 (1.1)	83.9 (<0.7)
Off-target site 1	<0.1 (5.3)	<0.1 (2.8)	<0.1 (1.0)
Off-target site 2	<0.1 (<0.3)	<0.1 (<0.3)	<0.1 (<0.3)
Off-target site 3	<0.1 (<0.7)	<0.1 (<0.1)	<0.1 (<0.3)

<i>BCL11A</i>	PE	sPE-MS2	tPE-MS2
On target	22.6 (5.9)	34.1 (14.5)	26.6 (10.9)
Off-target site 1	<0.1 (<0.3)	<0.1 (<0.3)	<0.1 (<0.3)
Off-target site 2	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.3)

<i>SRD5A3</i>	PE	sPE-MS2	tPE-MS2
On target	10.2 (<0.7)	17.3 (<0.7)	12.4 (<0.7)
Off-target site 1	<0.1 (0.7)	<0.1 (<0.3)	<0.1 (<0.3)
Off-target site 2	<0.1 (<0.3)	<0.1 (<0.3)	<0.1 (<0.3)
Off-target site 3	<0.1 (<0.1)	<0.1 (<0.3)	<0.1 (<0.3)
Off-target site 4	<0.1 (<0.1)	<0.1 (<0.3)	<0.1 (<0.3)

<i>JAK2</i>	PE	sPE-MS2	tPE-MS2
On target	1.3 (<0.3)	4.1 (<0.3)	5.5 (<0.3)
Off-target site 1	<0.1 (<0.3)	<0.1 (<0.3)	<0.1 (<0.3)
Off-target site 2	<0.1 (<0.3)	<0.1 (<0.3)	<0.1 (<0.3)
Off-target site 3	<0.1 (<0.3)	<0.1 (<0.1)	<0.1 (<0.3)

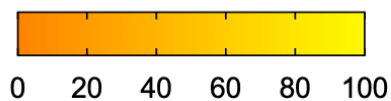


Figure S6 | Comparison of off-target activity between canonical PE, sPE-MS2 and tPE-MS2. on-target and off-target activities of PE, sPE-MS2 and tPE-MS2 at *DYRK1A*, *RUNX1*, *BCL11A*, *SRD5A3* and *JAK2* were detected. PE, sPE and tPE editing is shown as % prime editing alongside % indels (in parentheses).

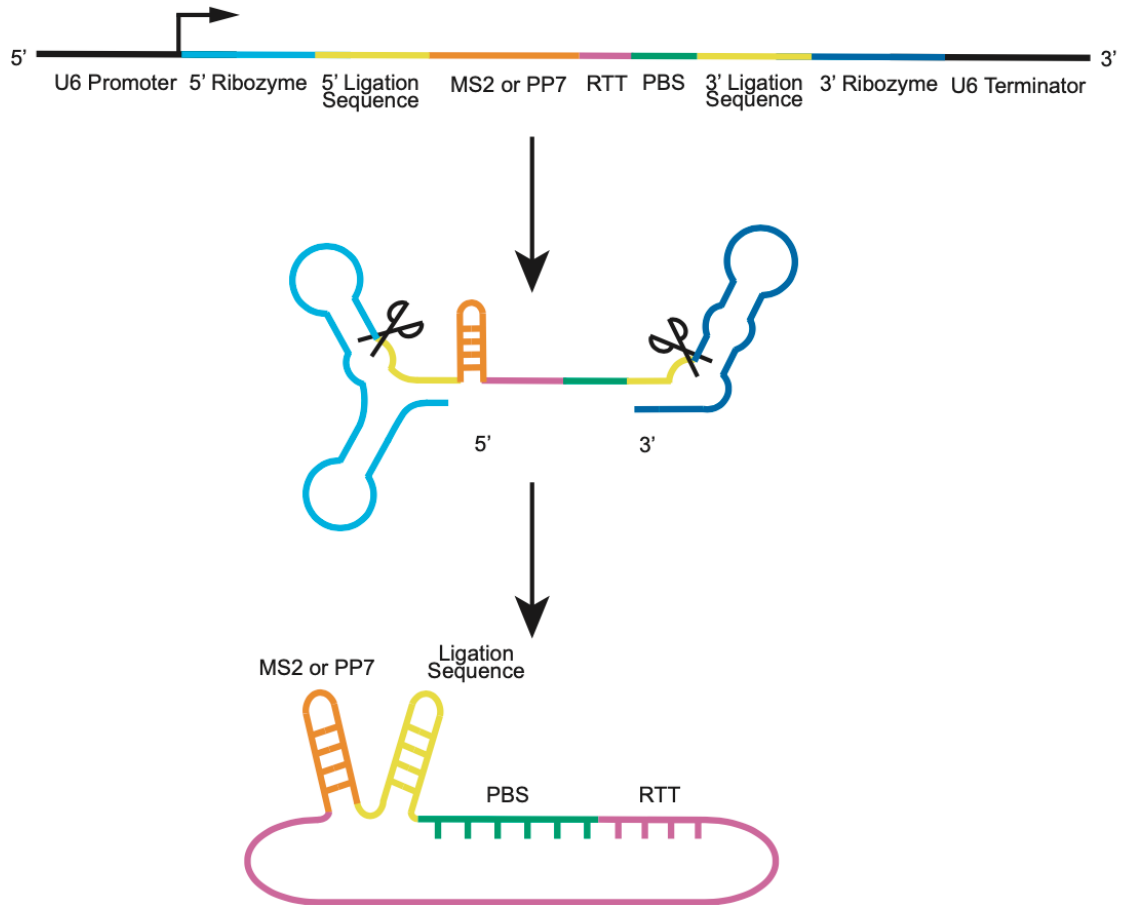


Figure S7 | Circular prime RNA generated by the Tornado expression system used in Figure 2.

Construct design for generating autocatalytically processed circular prime RNA (cpRNA) by the Tornado expression system. The circular prime RNA in the Tornado cassette is driven by U6 promoter. The circular pRNA consists of MS2 or PP7 (orange), RTT (reddish-purple) and PBS (bluish-green). The circular pRNA is flanked by the 5'- and 3'-stem-forming sequences (yellow), each of which is flanked by the 5'- and 3'-self-cleaving ribozymes (sky blue and blue, respectively). The stem-forming by 5'- and 3'-ligation sequences facilitates the circularization of pRNA into circular prime RNA (cpRNA).

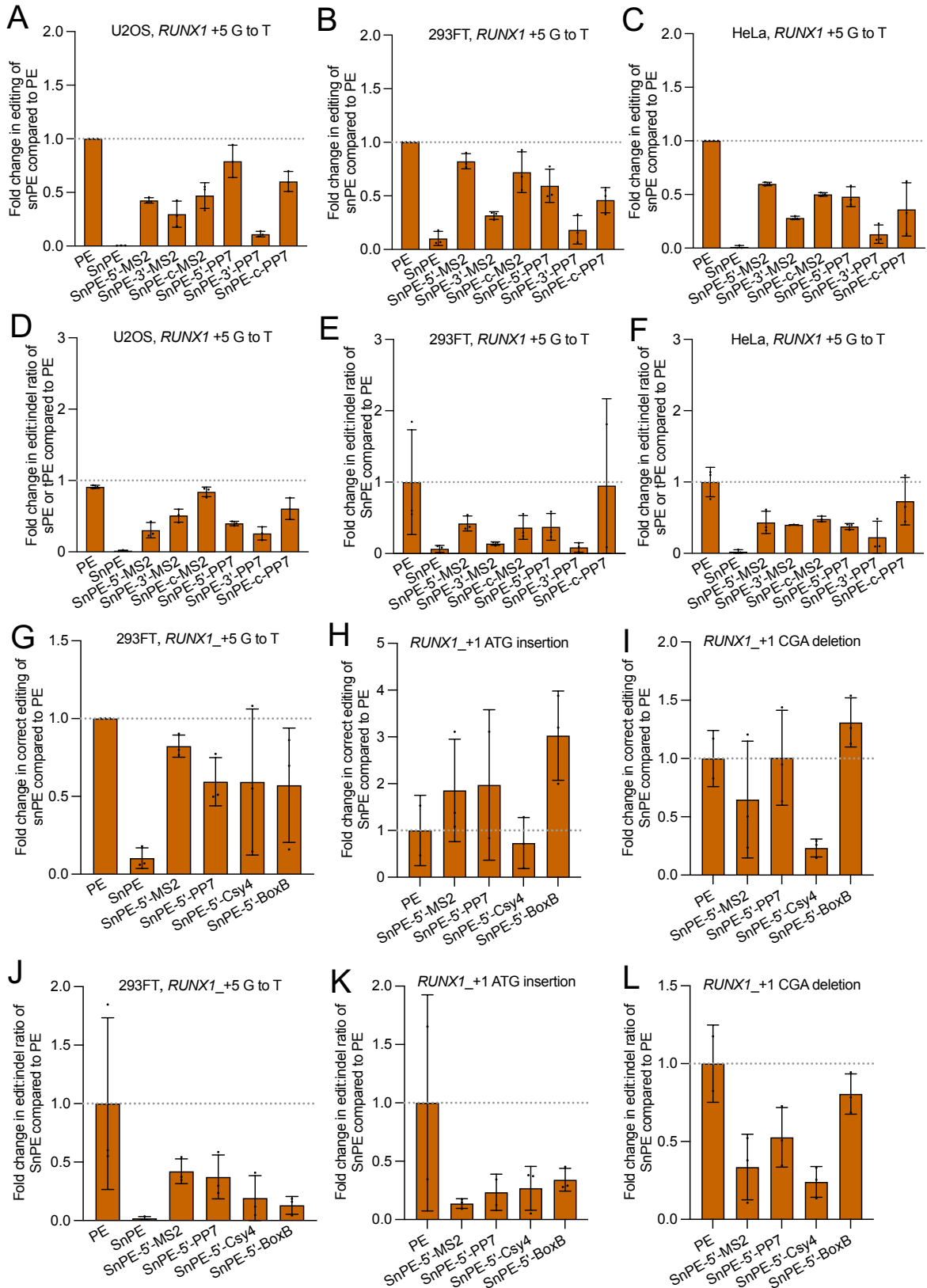


Figure S8 | Fold change in correct editing and edit:indel ratio for SnPEs shown in Figure 2.

Fold-change in the observed correct editing for *RUNX1*₊₅ G·C to T·A transversion of SnPE-5'-MS2 or PP7, SnPE-3'-MS2 or PP7, SnPE-c-MS2 or PP7, compared to canonical PE (dashed line) in U2OS cells (A), HEK293FT cells (B), and HeLa cells (C). Fold-change in the observed edit:indel ratio for *RUNX1*₊₅ G·C to T·A of SnPE-5'-MS2 or PP7, SnPE-3'-MS2 or PP7, SnPE-c-MS2 or PP7, compared to canonical PE (dashed line) in U2OS cells (D), HEK293FT cells (E), and HeLa cells (F). (G) Fold-change in the observed correct editing for SnPE-5'-MS2, SnPE-5'-PP7, SnPE-5'-Csy4 and SnPE-5'-boxB mediated with *RUNX1*₊₅ G·C to T·A in HEK293FT cells. (H) Fold-change in the observed correct editing of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with insertion for *RUNX1*₊₁ ATG in HEK293FT cells. (I) Fold-change in the observed correct editing of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with deletion for *RUNX1*₊₁ CGA in HEK293FT cells. (J) Fold-change in the observed edit:indel ratio for SnPE-5'-MS2, SnPE-5'-PP7, SnPE-5'-Csy4 and SnPE-5'-boxB mediated with *RUNX1*₊₅ G·C to T·A in HEK293FT cells. (K) Fold-change in the observed edit:indel ratio of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with insertion for *RUNX1*₊₁ ATG in HEK293FT cells. (L) Fold-change in the observed edit:indel ratio of PE, tPE-MS2, tPE-PP7, tPE-Csy4 and tPE-BoxB mediated with deletion for *RUNX1*₊₁ CGA in HEK293FT cells. Values were calculated from the data presented in Figure 2. Data and error bars reflect the mean and standard deviation of three independent biological replicates.

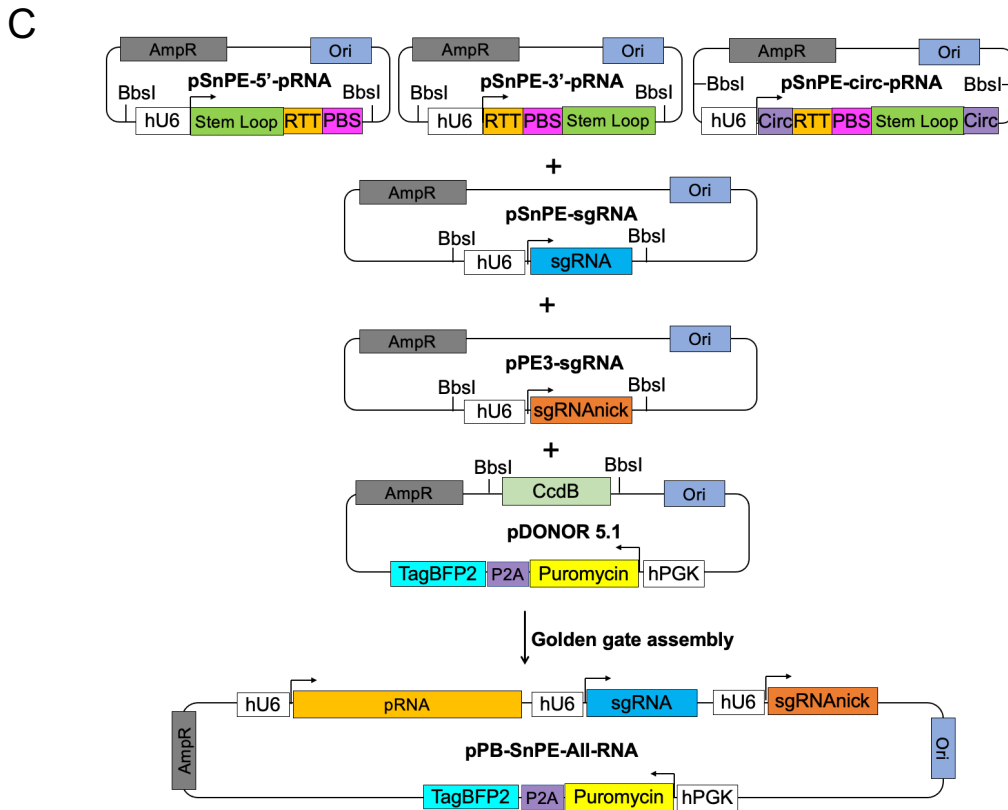
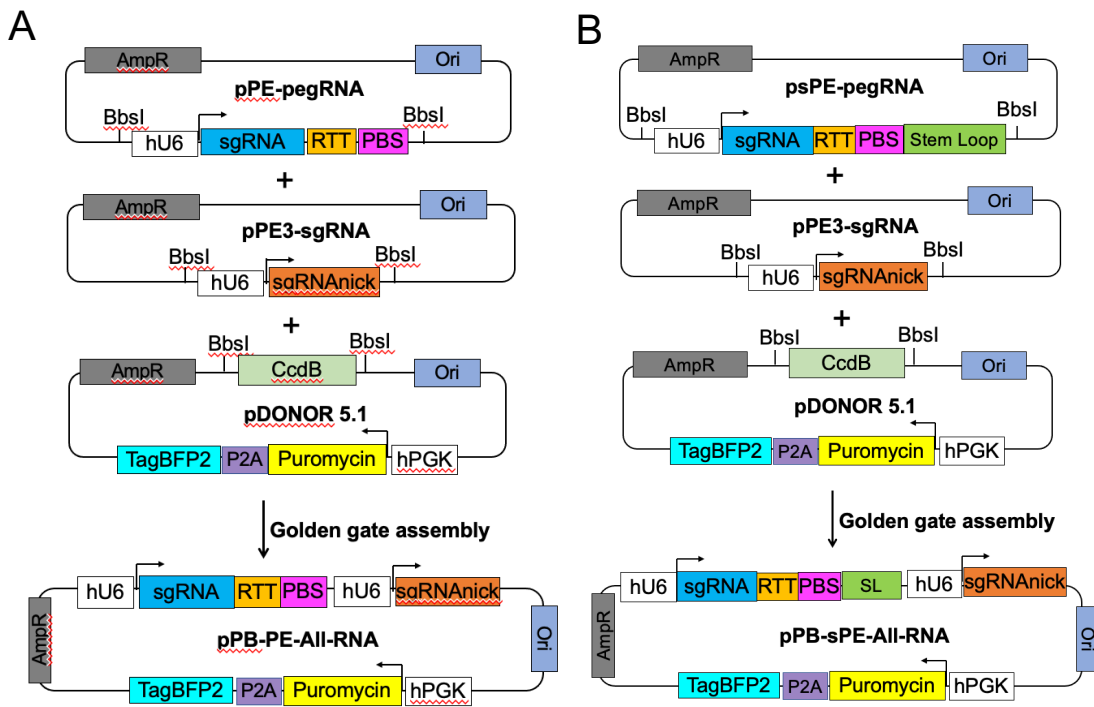


Figure S9 | Cloning strategy for single construct containing all RNA expression components for modified or split pegRNA prime editors.

Construction of pPB-PE-ALL-RNA for canonical PE, pPB-sPE-ALL-RNA plasmids for sPEs and tPEs, and pPB-SnPE-ALL-RNA for SnPEs. (A) pPB-PE-ALL-RNA was reconstituted from pPE-pegRNA, pPE3-sgRNA and pDONOR5.1 by golden gate assembly, pPE-pegRNA consists of sgRNA (blue), RTT (gold) and PBS (Magenta), pPE3-sgRNA contains sgRNA_{nick} (orange), pDONOR5.1 consists of CcdB gene (green), Puromycin (yellow), P2A (purple) and TagBFP2 (Cyan). (B) pPB-sPE-ALL-RNA was reconstituted from psPE-pegRNA, which contains sgRNA (blue), RTT (gold), PBS (Magenta) and stem-loop RNA aptamer (dark green), pPE3-sgRNA and pDONOR5.1 were the same in (A). (C) pPB-SnPE-ALL-RNA contains three types of pRNA: pSnPE-5'-pRNA, pSnPE-3'-pRNA and pSnPE-circ-pRNA. pSnPE-5'-pRNA consists of stem loop at 5'-terminal (dark green), RTT (gold) and PBS (Magenta). pSnPE-3'-pRNA contains stem-loop at 3'-terminal (dark green), RTT (gold) and PBS (Magenta). pSnPE-circ-pRNA contains circular sequence located at both terminals (purple), stem loop (dark green), RTT (gold) and PBS (Magenta). pSnPE-sgRNA was pSnPE-pRNAs, sgRNA (blue), pPE3-sgRNA and pDONOR5.1 that were used in (A) and (B).

Supplementary Table 1 | Sequences of primers used for pegRNA cloning

pPE-pegRNA	Forward	Reverse
JAK2	ataattgaagacATaccgACCTGTGACTCATGAAAC ACgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaCTGTGACTCATGAAATACAGG AAGAATGTgcaccgactcgggtgcc
HDAC1	ataattgaagacATaccgGCACCATGCAAAAGAAGTC CGgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaTGCAAAAGAAGTGCAGGCATC TGGCTgcaccgactcgggtgcc
EED	ataattgaagacATaccgCCGGTTGTTGCAATCTTA CGgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaGTTGTTGCAATCTTTCGTGGAT GCTGATgcaccgactcgggtgcc
BCL11A	ataattgaagacATaccgctcacagATAAACTTCTGCAC gtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaCACAGATAAACTTCTGAACTGG AGGGGCCgcaccgactcgggtgcc
SRD5A3	ataattgaagacATaccgCCTTACTCAATCTCTGTTC Cgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaTACTCAATCTCTGTTACTGGG AGCACCTTTTgcaccgactcgggtgcc
SRD5A1	ataattgaagacATaccgTCCATTCAGATCATATCCT Agtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaCATTTCAGATCATATCATAAGGA ATCTCAGAAAgcaccgactcgggtgcc
DYRK1A	ataattgaagacATaccgagcacatcaaggacattctagttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaaaggacattgtaaggatgatgcaccgact cgggtgcc
DMD	ataattgaagacATaccgCTACCTCTGTGATACTCTT Cgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaACCTCTGTGATACTCTCAGGT GCACCTgcaccgactcgggtgcc
RUNX1	ataattgaagacATaccgcatittcagggaagcaggtttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaatttcagggaagcaggtttgagcagacag caccgactcgggtgcc
GFAP	ataattgaagacATaccgagcctgtgctcatataaagggtttgag gctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaTGTGTCCATATAATGGAGGAG TTGGAAGcaccgactcgggtgcc
psPE-pegRNA	Forward	Reverse
JAK2- <u>MS2</u>	ataattgaagacATaccgACCTGTGACTCATGAAAC ACgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtCTGTGACTC ATGAAATACAGGAAGAATGTgcaccgactcgggtgcc
HDAC1- <u>MS2</u>	ataattgaagacATaccgGCACCATGCAAAAGAAGTC CGgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtTGCAAAAGAA GTGCGAGGCATCTGGCTgcaccgactcgggtgcc
EED- <u>MS2</u>	ataattgaagacATaccgCCGGTTGTTGCAATCTTA CGgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtGTTGTTGCA ATCTTTTCGTGGATGCTGATgcaccgactcgggtgcc
BCL11A- <u>MS2</u>	ataattgaagacATaccgctcacagATAAACTTCTGCAC gtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtCACAGATAA ACTTCTGAACTGGAGGGGCCgcaccgactcgggtgcc
SRD5A3- <u>MS2</u>	ataattgaagacATaccgCCTTACTCAATCTCTGTTC Cgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtTACTCAAT CTCTGTTACTGGGAGCACCTTTTgcaccgactcgggtgcc
SRD5A1- <u>MS2</u>	ataattgaagacATaccgTCCATTCAGATCATATCCT Agtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtCATTTCAGAT CATATCATAAGGAATCTCAGAAAgcaccgactcgggtgcc
PCSK9- <u>MS2</u>	ataattgaagacATaccgcccgcacCTTGGCGCAGCG Ggtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtTGGCGCAG AGGTGGAAGGTGGCgcaccgactcgggtgcc
DYRK1A- <u>MS2</u>	ataattgaagacATaccgagcacatcaaggacattctagttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtcaaggacattgta aggatgatgcaccgactcgggtgcc
DMD- <u>MS2</u>	ataattgaagacATaccgCTACCTCTGTGATACTCTT Cgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtACCTCTGTG ATACTCCTCAGGTGCACCTgcaccgactcgggtgcc
RUNX1- <u>MS2</u>	ataattgaagacATaccgcatittcagggaagcaggtttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtatttcaggagga agcgtattgctcagacagcaccgactcgggtgcc
DNMT1- <u>MS2</u>	ataattgaagacATaccgGATTCTCTGGTGCCAGAAA Cgtttgagagctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtCCTGGTGCC AGAAACAAGGTGACgcaccgactcgggtgcc
GFAP- <u>MS2</u>	ataattgaagacATaccgagcctgtgctcatataaagggtttgag gctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaacatgggtgatcctcatgtTGTGTCCAT ATAATGGAGGAGTTGGAAGcaccgactcgggtgcc
RUNX1- <u>com</u>	ataattgaagacATaccgcatittcagggaagcaggtttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaGATGCTCGCAGGCATTTCAGatttt caggaggaagcattgctcagacagcaccgactcgggtgcc
RUNX1- <u>pp7</u>	ataattgaagacATaccgcatittcagggaagcaggtttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaagcagcagccatctgctgctcattttca ggaggaagcattgctcagacagcaccgactcgggtgcc
RUNX1- <u>csy4</u>	ataattgaagacATaccgcatittcagggaagcaggtttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaCTGCCTATACGGCAGTGAAcatt ttcagggaagcattgctcagacagcaccgactcgggtgcc
RUNX1- <u>boxB</u>	ataattgaagacATaccgcatittcagggaagcaggtttgag agctagaatagcaagttcaaataaggc	ataattgaagacGCaaaaagccctctcagggccatttcaggaggaag cgtattgctcagacagcaccgactcgggtgcc
pPE3-sgRNA	Forward	Reverse
JAK2	accgTGGGGTTTGATCGTTTTCTT	aaacAAGAAAACGATCAAACCCCA
HDAC1	accgATACTTTAGCAGTTCCAGGA	aaacTCCTGGAAGTCTAAAGTAT
EED	accgTGATTAATTTATAAAAGTTT	aaacAAACTTTTATAAATTAATCA
BCL11A	accgCTGCACTCATCCAGGCCTG	aaacCACGCCTGGGATGAGTGCAG
SRD5A3	accgCCAGTAACAGAGATTGAGTA	aaacTACTCAATCTCTGTTACTGG
SRD5A1	accgTCTTATGATATGATCTGAA	aaacTTCAGATCATATCATAAGGA
DYRK1A	accgAAGAGTGAGGAGAAGGCAGG	aaacCCTGCCTTCTCCTCACTCTT
DMD	accgGTCAATGCTCTCCTTTTCAC	aaacGTGAAAAGGAGAGCATTGAC
RUNX1	accgCATGTACTCACCTCTCATGA	aaacTCATGAGAGGTGAGTACATG
GFAP	accgCCAGAATCCAATCTCCCTCA	aaactgaggagattggattctgg
pSnPE-sgRNA	Forward	Reverse

RUNX1	accggcatttccaggaggaagcga	aaactcgcttctcctgaaaatgc
pSnPE-5'(3')-pRNA	Forward	Reverse
RUNX1	accgtgtctgaagcaatcgcttctcctgaaaat	aaacatttccaggaggaagcgattgcttcagaca
RUNX1-MS2	accgtgtctgaagcaatcgcttctcctgaaaatacatgaggatcaccatgt	aaacacatgggtgatcctcatgtatttccaggaggaagcgattgcttcagaca
MS2-RUNX1	accgacatgaggatcaccatgttctgaagcaatcgcttctcctgaaaat	aaactgtctgaagcaatcgcttctcctgaaaatacatgaggatcaccatgt
RUNX1-PP7	accgtgtctgaagcaatcgcttctcctgaaaatggagcagacgatggcgctcctcc	aaacacatgggtgatcctcatgtatttccaggaggaagcgattgcttcagaca
PP7-RUNX1	accgggagcagacgatggcgctcctcctgtctgaagcaatcgcttctcctgaaaat	aaactgtctgaagcaatcgcttctcctgaaaatacatgaggatcaccatgt
COM-RUNX1	accgTGAATGCCTGCGAGCATCgtctgaagccatccttctcctgaaaat	aaacatttccaggaggaagggatggcttcagacaGATGCTCGCAGGCATTCAg
Csy4-RUNX1	accgactgccgtataggcagctaagaaatgtctgaagcaatcgcttctcctgaaaat	aaacatttccaggaggaagcgattgcttcagacatttcttagctgcctatacggcagt
Boxb-RUNX1	accgggacctgaagaaggcctgtctgaagccatccctcctcctgaaaat	aaacatttccaggaggaagggatggcttcagacaggcccttctcaggggcc

Supplementary Table 2 | Primers used for cell genomic DNA amplification and targeted deep sequencing.

gene	Forward primer	Reverse primer	Length of amplicon (nt)
JAK2	ctacacgacgctctccgatctgTGGCGGCATGATTTTGTGCACG	agacgtgtgctctccgatctccaagcatttagataaggagacttaactagat	290
HDAC1	ctacacgacgctctccgatctcatgccagCAAGTGCTGTGAAAC	agacgtgtgctctccgatctgacactcaccgagcacatcctagcc	290
EED	ctacacgacgctctccgatctagtggagagagatgtttctatattgagatga	agacgtgtgctctccgatctaaactagatacaggattctctgggtgatagtg	290
BCL11A	ctacacgacgctctccgatcttatttaaggaaatgtgatactctggttatgatg	agacgtgtgctctccgatctcctcagacttaaaaagtaagggcaattccagaaa	290
SRD5A3	ctacacgacgctctccgatctgctcctgctgttttctttgacagAT	agacgtgtgctctccgatctgccctctgaggagggtgccg	290
SRD5A1	ctacacgacgctctccgatctgaaatttacggttattagccataatctcttgcga	agacgtgtgctctccgatctgatgactacactgaaactgtagctaataactactacg	290
DYRK1A	ctacacgacgctctccgatctctgtcacacacaatgaaacttgcgttcaact	agacgtgtgctctccgatctagtagtaacattttcagacttaactatagccagctat	290
DMD	ctacacgacgctctccgatctAAGCATTAAATCTTAAGACTACAAGACATTACTTGAAGG	agacgtgtgctctccgatctGACAGAGAAGGGTGTAAAA GCTTCTAGC	290
RUNX1	ctacacgacgctctccgatctgtttcgtccgaagGTAAAA GAA	agacgtgtgctctccgatctatagcctcagttgaaatcctctcacaac aag	290
GFAP	ctacacgacgctctccgatcttactgggaaagcagtgagga gca	agacgtgtgctctccgatctaccaccgcttcacagctgtgc	290

Table S3 Sequence of amplicon for deep sequencing analysis

Gene	Sequence
JAK2	gTGGCGGCATGATTTTGTGCACGGATGGATAAAAAGTACCTGTGACTCATGAAACACAGGAAGAATGTCTTGGGATGGCAGTGTAGATATGATGAGAATAGCCAAAGAAAACGATCAAACCCCACTGGCCATCTATAACTCTATCAG gtaatttcttttgc aaatccttacataaagtgtgagtagagatttatataatcgtatataatctctgtgtttaccatgccttttgatttgaatac tagttaagtactccttatctaaaatgcttgg
HDAC1	tcatgccagCAAGTGCTGTGAAACTTAATAAGCAGCAGACGGACATCGCTGTGAATTGGGCTGGGGGCTGCAC CATGCAAAGAAGTCCGAGGCATCTGGCTTCTGTTACGTCAATGATATCGTCTTGGCCATCCTGGAAGTCTGCTAAA gtatgctgctggcctgtctcttgaagagcaccttaggccaggttccatttccctctccctgggcttgcctccctgatttgccttctaccgat gtgctggctaggatgtgctcggtagtgc
EED	agttggagagagatgtttctacttgagatgaaattactgtattttattttcattagGTTACCTTGATGAATGTCATTACAAGG AGAAATCCGGTTGTTGCAATCTTACGTGGATGCTGATgtatccttctctgggttttaatatcttttagtcaaaagaaatattttcaat aagtgcacaaaactttataaataatcatttccctaaagtattgttcttcttaaccgatttcttactatcaccgaagagaatcctgtatctagtt
BCL11A	tatttttaaggaaatgaaatgtgatctctgtggttatgatgcacgttgttttagctgtagtgcttgatttgggtttcttccagATAAACTTC TGCACTGGAGGGGCTCTCCTCCCTCGTTCTGCACATGGAGCTAATCCCCACGCTGGGATGAGTGCAGAA TATGCCCCGAGGGTATTGtaagttgagccttatttcttcaaaatgtccatgtgtatagagatgagaatttctggaaatgaccttactt ttaagtactgaagg

