Supplementary material

Engineered lentivirus-derived nanoparticles (LVNPs) for delivery of CRISPR/Cas ribonucleoprotein complexes supporting base editing, prime editing and *in vivo* gene modification

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Supplementary Methods

Cloning of GagPol-IntSpCas9

For construction of LVNP1.0, two fragments encoding (i) FLAG-tagged SpCas9 was PCR amplified from pX330 (Addgene #42230) and, (ii) a C-terminal GagPol fragment from pMDLg/p-PCS-hyPBase(1) and inserted into a BspTI/Kpn2I (Thermo Fisher Scientific) digested pMDLg/p-PCS-hyPBase(1) using NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). Primers are listed in Supplementary Table 6.

Cloning of GagPol-MatSpCas9

For construction of LVNP2.0, FLAG-tagged SpCas9 was PCR amplified from pX330 (Addgene #42230) and inserted into BshTI/Kpn2I (Thermo Fisher Scientific) digested pGFP-PH-GagPol-D64V(2) using NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). Primers are listed in Supplementary Table 6.

Cloning of GagPro-SpCas9/BE/PE

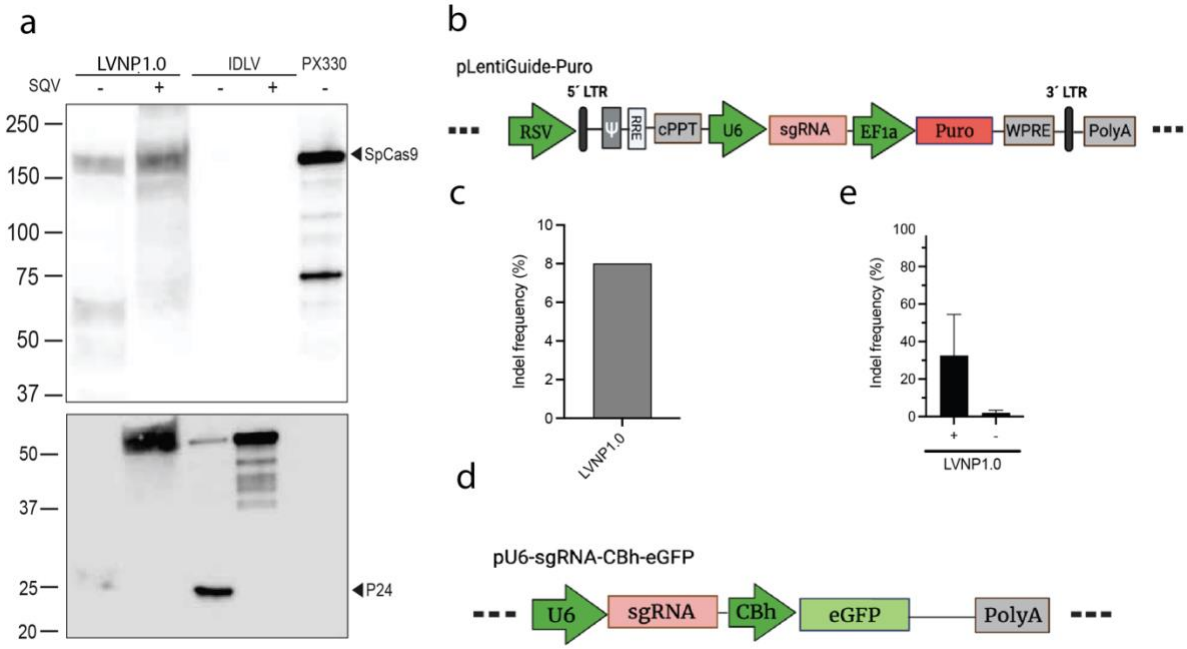
For construction of pGagPro-POI (protein of interest), three fragments were inserted into EcoRI (Thermo Fisher Scientific) digested pMD.2G using NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). Fragment 1 (TWIST Bioscience) encode a MCS and the N-terminal part of Gag. Fragment 2 was PCR amplified from pGagPol-MatSpCas9 and encode the C-terminal fragment of Gag. Fragment 3 (TWIST Bioscience) encodes the HIV-1 protease, a protease cleavage site (SQNYPIVQ), 3xFLAG, cMyc/SV40 NLS, and a XbaI restriction site for insertion of POI. SpCas9, ABE8e, or PE2 was PCR amplified by Platinum SuperFi II DNA Polymerase (Thermo Fisher Scientific) from pLentiCRISPRv2-eGFP (Addgene #82416) and pABE8e (Addgene #138489), respectively, and inserted into XbaI (Thermo Fisher Scientific) linearized pGagPro-XbaI using NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). For generation of GagProPEmax and GagPro-PEmini, two fragments encoding the C- and N-terminal parts of Pemax were amplified form pCMV-Pemax (Addgene #174820) or

pLentiPEmini-eGFP-WPRE and a fragment encoding the *rev*-responsive element (RRE) were amplified form pMDLg-RRE-D64V. The three fragments were then assembled into a XbaI-digested pMD.2G-MCS-GagPro-FLAG-NoNLS-XbaI backbone using NEBuilder. Primers are listed in Supplementary Table 6.

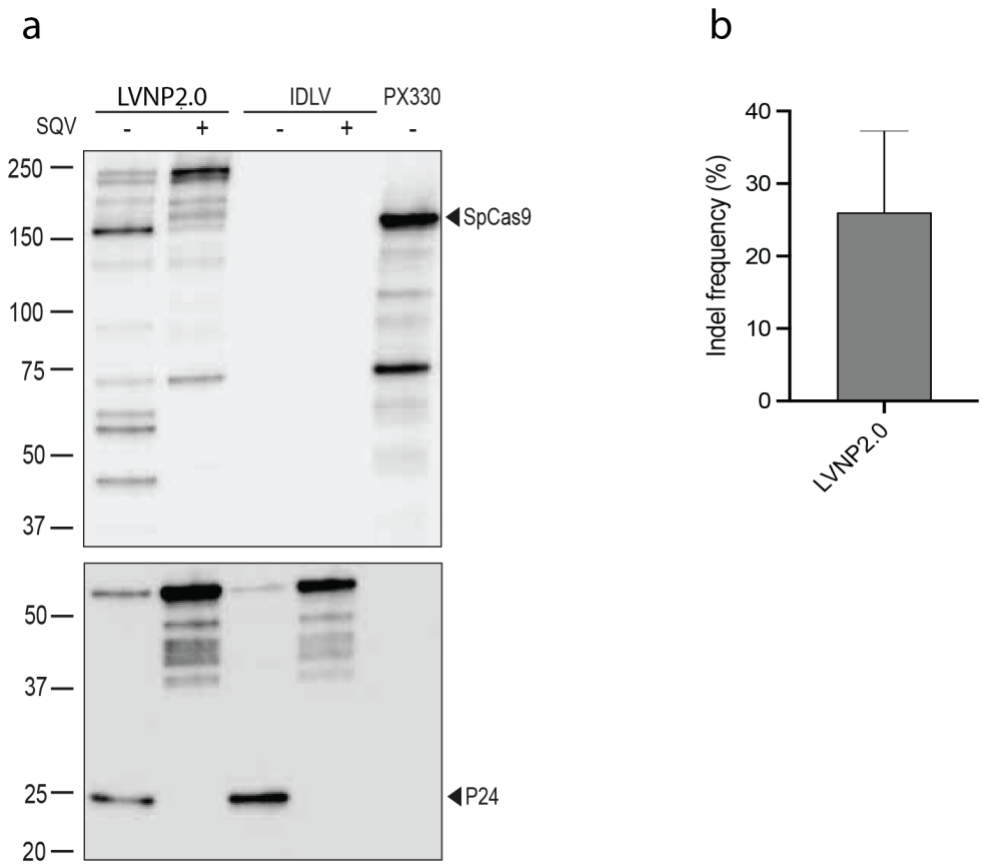
pCCL/PGK-d2eGFP-IRES-puro

A third-generation LV vector encoding pCCL/PGK-d2eGFP-IRES-puro was constructed by digestion of pCCL/PGK-MCS-IRES-puro with BamHI (Thermo Fisher Scientific) following insertion of the d2eGFP fragment amplified from pT2/UASTK-d2eGFP-SV40-neo and assembled by NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). Primers are listed in Supplementary Table 6.

Supplementary Figures

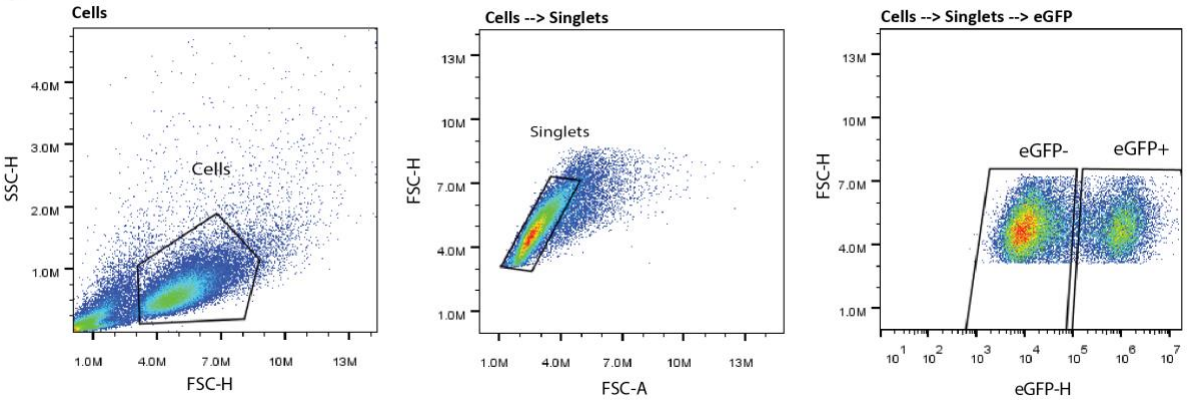


Supplementary Figure S1. Characterization of LVNP1.0. (a) Western blot analysis of FLAG-tagged SpCas9 (FLAG antibody) and p24 loading control of purified LVNP1.0 (90 ng p24) and IDLV (90 ng p24) in the presence or absence of the HIV-1 protease inhibitor saquinavir (SQV). (b) Schematic of the LentiGuide-Puro plasmid. (c) Indel rates in the *AFF1* locus following administration of 300 ng p24 LVNP1 in a 6-well plate. (d) Schematic of the U6-sgRNA-Cbh-eGFP plasmid. (e) Indel rates in the *AFF1* locus following administration of 90 ng p24 LVNP1.0 (+/- VSV-G) in 24 well-plates.

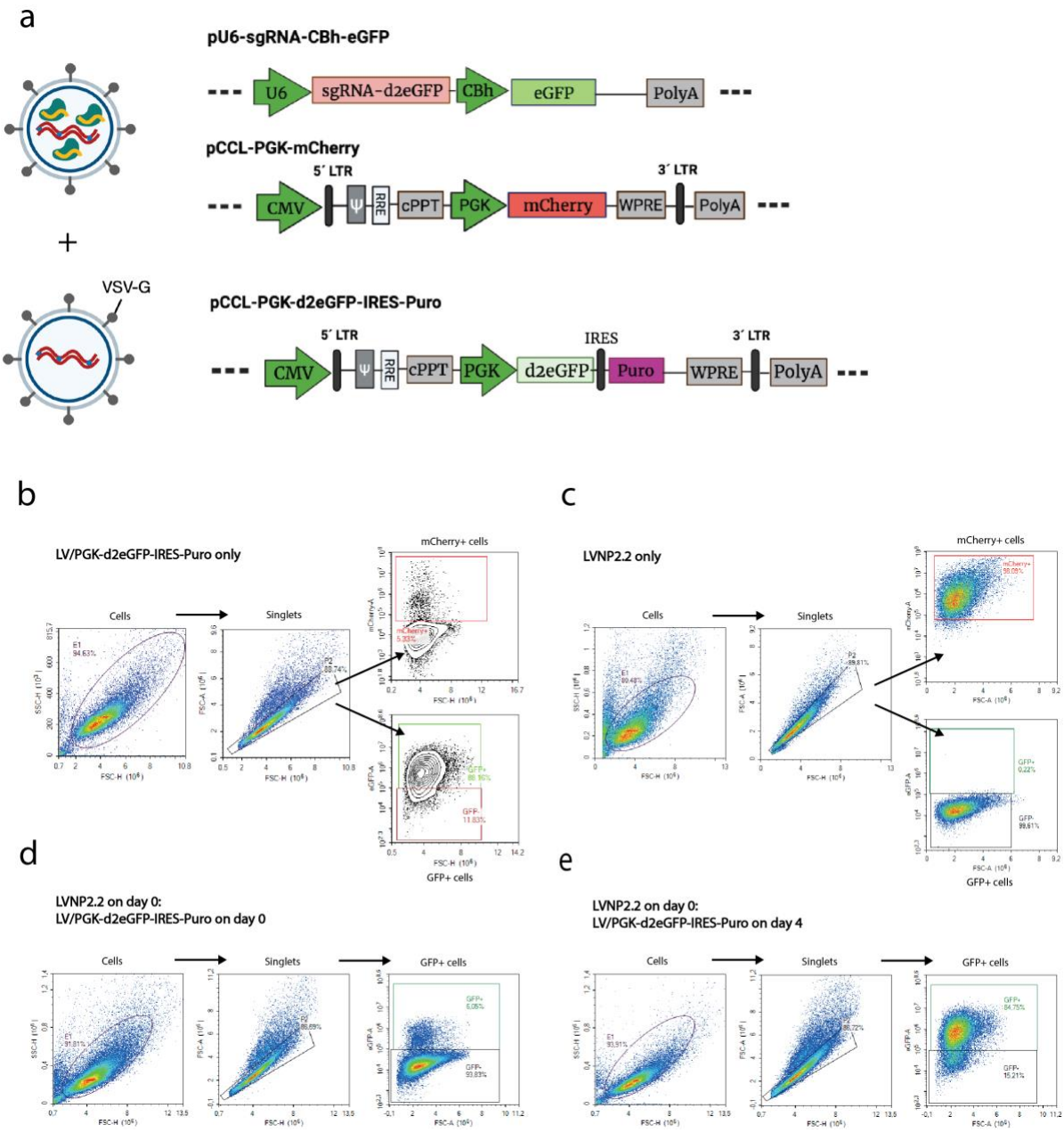


Supplementary Figure S2. Characterization of LVNPs2.0. (a) Western blot analysis of FLAG-tagged SpCas9 (FLAG antibody) and p24 loading control of purified LVNP2.0 (90 ng p24) and IDLV (90 ng p24) in the presence or absence of the HIV-1 protease inhibitor saquinavir (SQV). (b) Indel rates in the *AFF1* locus following administration of 300 ng p24 LVNP2.0 in a 6-well plate. Data is presented as mean of ±SD of triplicates.

a



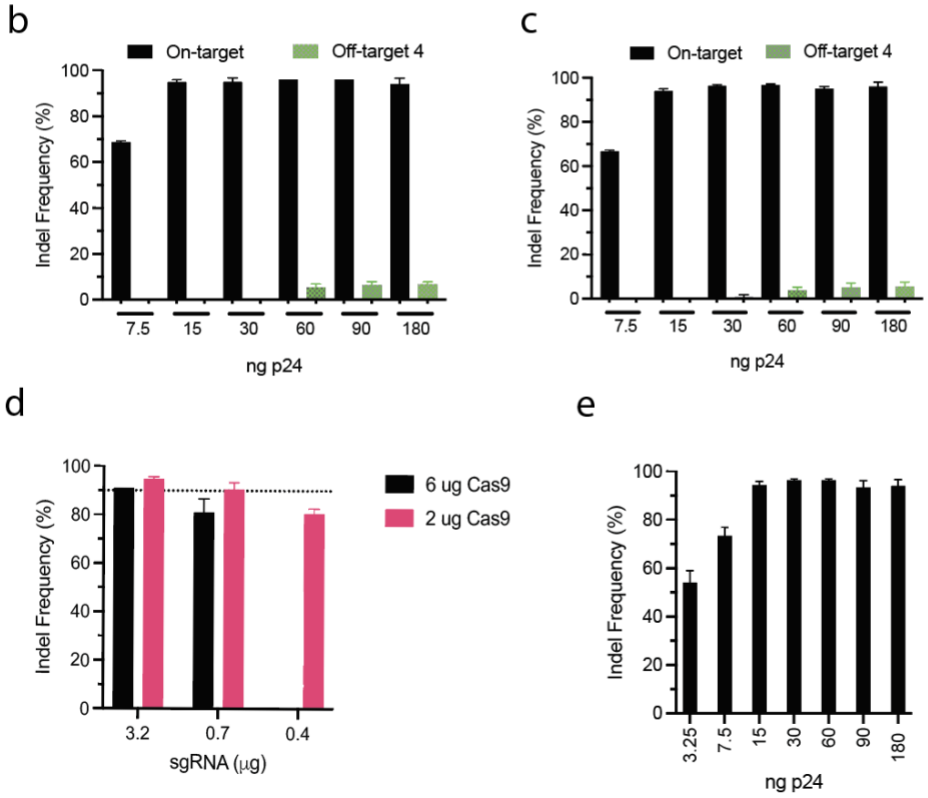
Supplementary Figure S3. Flow cytometry. (a) Representative gating strategy for quantification of eGFP-positive cells following transduction of LVNP2.2 loaded with a sgRNA and a vector encoding the eGFP transgene (15 ng p24). All experiments were performed in triplicates.



Supplementary Figure S4. (a) Upper: Schematic of the U6-sgRNA-CBh-eGFP plasmid, Middle: Schematic of pCCL-PGK-mCherry plasmid that was used to produce LVNP2.2 loaded with a sgRNA targeting d2eGFP and a mCherry transgene. Lower: Schematic of the lentiviral pCCL-PGK-d2eGFP-IRES-Puro backbone. (b) Representative gating strategy to quantify the number of eGFP-positive cells following LV/PGK-d2eGFP-IRES-Puro transduction (90 ng p24). c) Representative gating strategy to quantify the number of mCherry-positive cells following transduction of LVNP2.2 loaded with a sgRNA targeting d2eGFP and a mCherry-encoding vector genome. (d) Representative gating strategy for quantification of double positive mCherry/eGFP cells following co-transduction of LV/PGK-d2eGFP-IRES Puro and LVNP2.2 loaded with sgRNA targeting d2eGFP and a mCherry vector genome at day 0. (e) Same as (d) after 4 days. All experiments were performed in triplicates.

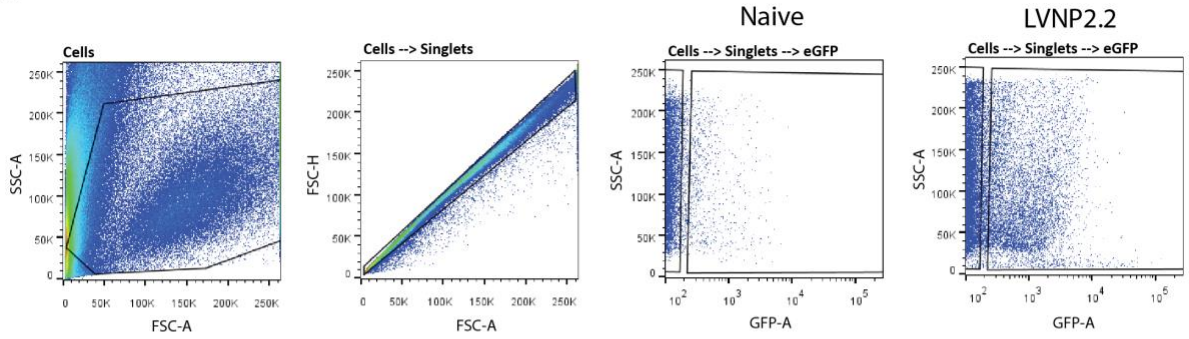
a

		PAM	
On-target	AGCAGCAGCGGCGGCAACAG	GGG	chr4:81612-81635
Off-target 1	AGCAGCAGCAGCAGCAACAG	GGG	chr7:73927327-73927350
Off-target 2	AGCAGCAGCAGCAGCAACAG	GAG	chr15:88958219-88958242
Off-target 3	AGCAGCAGCAACGGCAACAG	CAG	chr19:24013247-24013270
Off-target 4	AGCAGCAGCAGCAGCAACAG	CGG	chr10:37138620-37138643

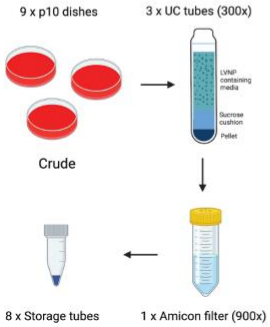


Supplementary Figure S5. Transient LVNP delivery. (a) The genomic sequences (murine) and genomic coordinates (murine) of the tested Pcsk9 off-target sites (b) AML12 hepatocytes were transduced with increasing quantity of LVNP2.2 and the level of Pcsk9 disruption and a well-characterized off-target locus (Off-target 1) after 7, or (c) 14 days. (d) AML12 hepatocytes were nucleofected with decreasing amounts of RNP and synthetic sgRNA to non-saturating conditions, and (e) following LVNP2.2 transduction. Indel frequencies were evaluated in the Pcsk9 locus 3-days post administration. All experiments were performed in triplicates.

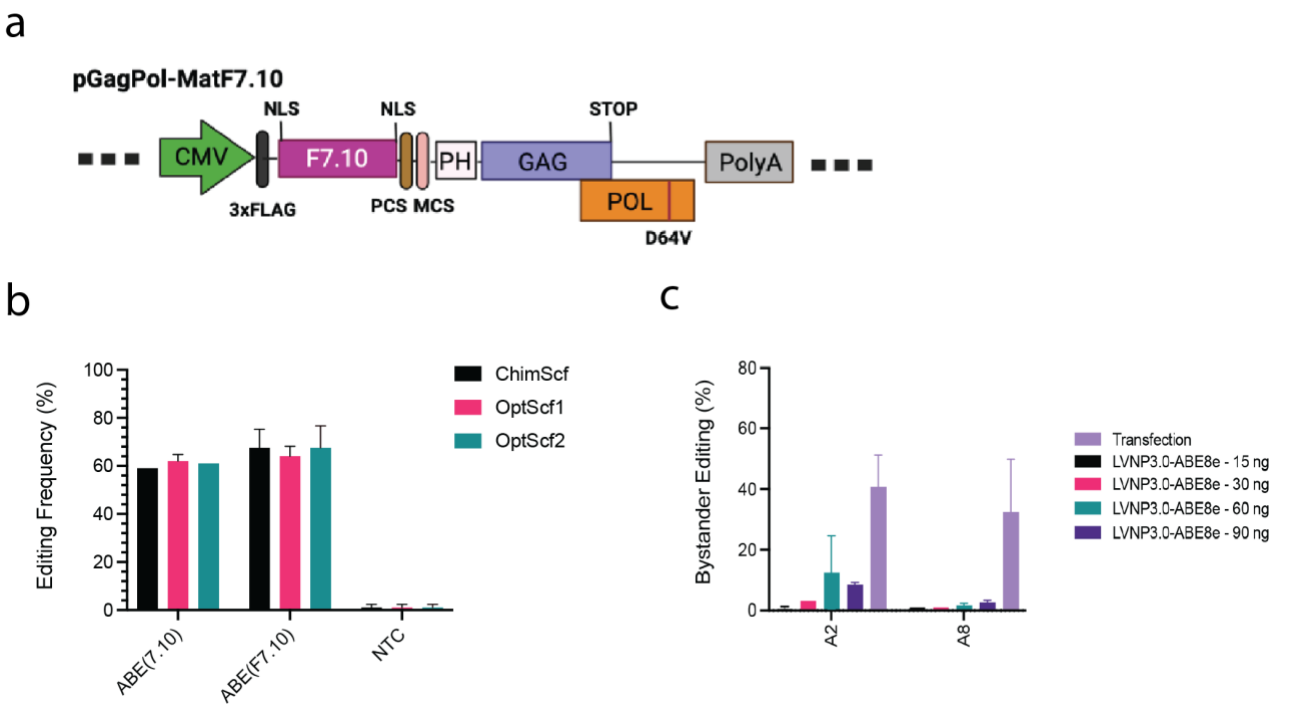
a



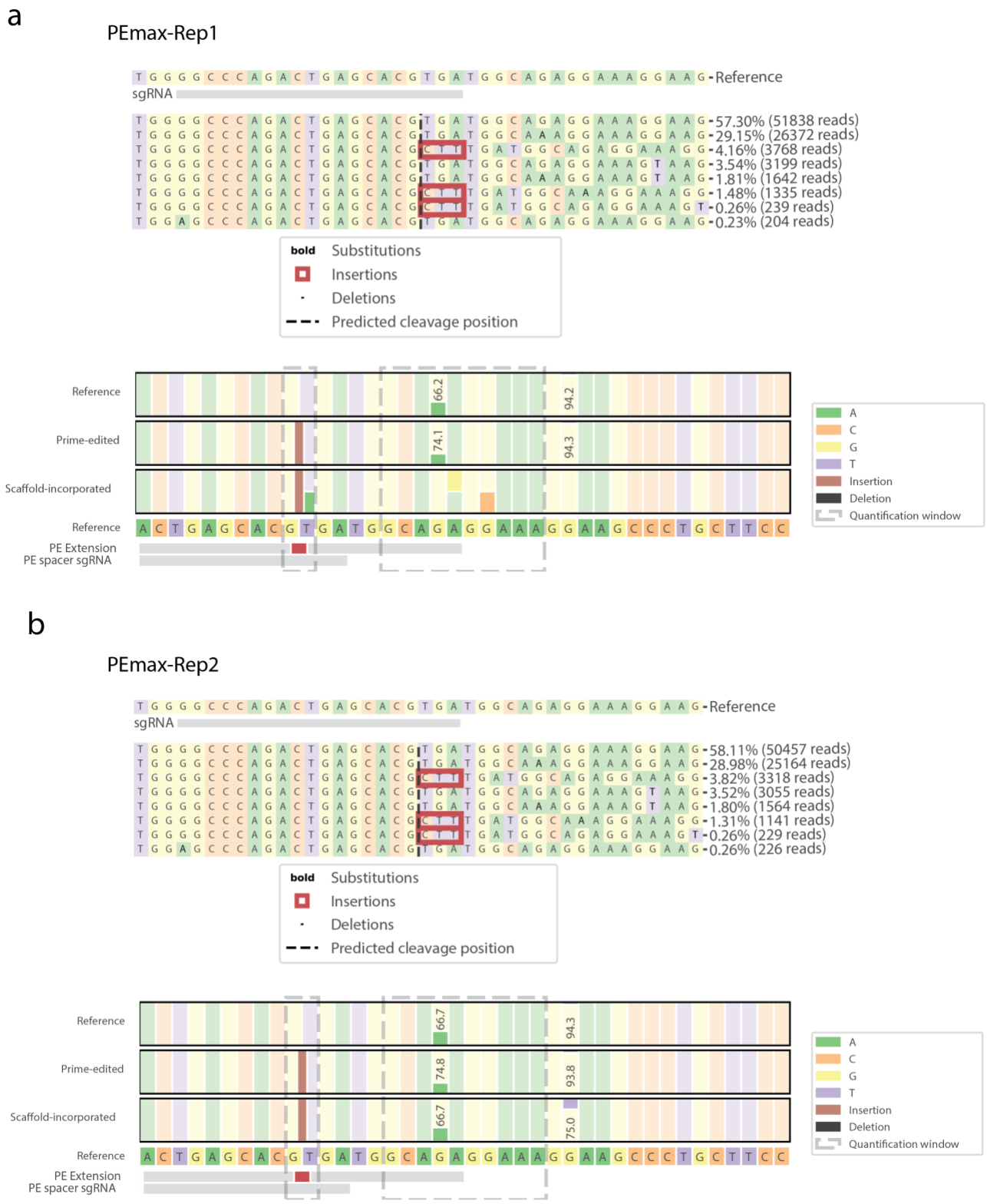
b



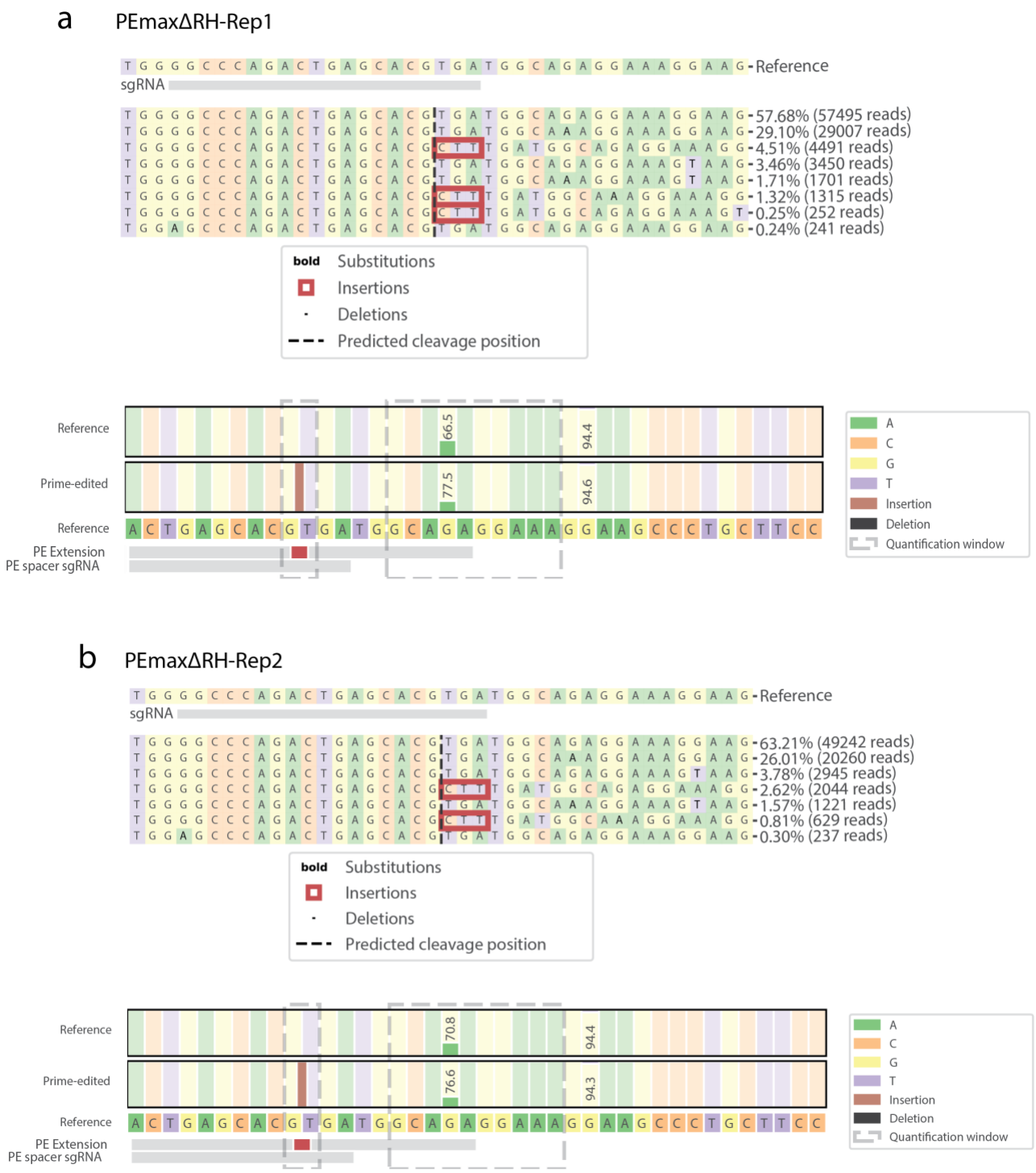
Supplementary Figure S6. *In vivo* studies. (a) Representative gating strategy to sort eGFP-positive and eGFP-negative RPE cells from Naïve or LVNP2.2-treated eyes. (b) Schematics of LVNP3.0 production including an additional purification step by Amicon filtrat



Supplementary Figure S7. Base Editing of ´Site 2´. (a) of Schematics of pGagPol-MatF7.10 base editor composed of FLAG-Tagged 7.10 (F7.10) fused to the N-terminus of Gag harboring an intervening phospholipase C-δ1 pleckstrin homology (PH) domain. (b) Editing frequencies of the base editors ABE7.10 or 3xFLAG-tagged ABE7.10 (ABE(F7.10)) for ChimScf. OptScf1, and OptScf2 in ´Site 2´ in HEK293T 3 days after transfection. (c) The level of bystander editing of plasmid DNA transfection and LVNP-3.0-ABE8e in a dose-escalation manner. Data are presented as +/- SD of at least duplicates.



Supplementary Figure S8. Quantification of desired/undesired prime editing events following LVNP delivery of PEmax. (a) The percentage of reads aligned to the wild-type HEK3 reference sequence or the CTT insertion (red box) in the HEK3 locus (upper). Dashed lines highlights the quantification window of intented/unintended prime editing and unwanted scaffold incorporation. (b) same as (a) for replicate two.



Supplementary Figure S9. Quantification of desired/undesired prime editing events following LVNP delivery of PEmax. (a) The percentage of reads aligned to the wild-type HEK3 reference sequence or the CTT insertion (red box) in the HEK3 locus (upper). Dashed lines highlights the quantification window of intented/unintended prime editing and unwanted scaffold incorporation. (b) same as (a) for replicate two.

Supplementary Table S1. The production parameters of the indicated LVNP configuration. Each value represents the required amount of plasmid (µg) per p10 dish for each configuration.

	LVNP1.0	LVNP2.0	LVNP2.1	LVNP2.2	LVNP3.0-SpCas9	LVNP3.0-BE	LVNP3.0-PE	eVLP
pRSV-REV	3.00	3.00	3.00	3.00	3.00	3.00	3.00	-
*pLVNP backbone**	13.00	8.70	8.70	3.90	3.90	9.80	3.90	-
pMDlg/p-RRE-D64V	-	4.30	4.30	9.10	9.10	3.30	9.10	-
pMD.2G (VSV-G)	3.75	3.75	3.75	3.75	3.75	3.75	3.75	1.28
pU6-sgRNA/pegRNA-Cbh-eGFP	13.00	13.00	13.00	5.20	5.20	5.20	5.20	14.08
*pCCL/PGK-eGFP or mCherry***	-	-	-	7.80	7.80	7.80	7.80	-
pMMLV-Gag-3xNES-Cas9	-	-	-	-	-	-	-	3.60
pMMLV-Gag-Pro-Pol	-	-	-	-	-	-	-	10.80

*pGagPol-IntSpCas9; pGagPol-MatSpCas9/BE; pGagPro-SpCas9/BE/PE

**Titer experiments were performed with 13 µg pCCL-PGK-eGFP and 0 µg sgRNA

Supplementary Table S2. Oligonucleotides of sgRNA/epegRNA including cloning sites.

sgRNA/epegRNA	Forward (5´- 3´)	Reverse (5´- 3´)
sgRNA_Pcsk9	caccgagcagcagcggcggcaacag	aaacctgttgccgccgctgctgctc
sgRNA_AFF1	caccgccttcagctcagtgacagtg	aaacactgtcactgagctgaaggc
sgRNA_Vegfa	caccgctcctggaagatgtccacca	aaactggtggacatcttccaggagc
sgRNA_Fah	caccggatggtcctcatgaacgac	aaacgtcgttcatgaggaccatcc
sgRNA_d2eGFP	caccgggcgaggagctgttcaccg	aaaccggtgaacagctcctcgccc
sgRNA_SERPING1	caccgtttgcaagacagaggcgaa	aaacttcgcctctgtcttgcaaac
sgRNA_Pcsk9- Synthetic	agcagcagcggcggcaacag	n/a
sgRNA_Site2	caccggagtatgaggcatagactgc	Aaacgcagtctatgcctcatactcc
epegRNA-HEK3 CTTins (spacer)	caccggcccagactgagcacgtga	aaactcacgtgctcagtctgggcc
epegRNA-HEK3 CTTins (extension)	gtgctctgccatcaaagcgtgctcagtctgttaaatac	cgcggtatttaacagactgagcacgctttgatggcaga
ChimScf	agagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccga gtcggtgc	gcaccgactcggtgccactttttcaagttgataacggactagccttattttaacttgctatttctagctct
OptScf1	agagctatgctggaaacagcatagcaagtttaaataaggctagtccgttatcaacttgaaaaag tggcaccgagtcg	gcaccgactcggtgccactttttcaagttgataacggactagccttatttaaacttgctatgctgtttccagcata g
OptScf2	agagctatgctggaaacagcatagcaagtttaaataaggctagtccgttatcaactttgctgga aacagcaaagtggcaccgagtcg	gcaccgactcggtgccactttgctgtttccagcaaagttgataacggactagccttatttaaacttgctatgctgt ttccagcatag

Supplementary Table S3. Oligonucleotides for PCR and Sanger sequencing

PCR/Sequencing oligoes	Forward (5´- 3´)	Reverse (5´- 3´)
PCR_Pcsk9	gaggccgaaacctgatcctt	cttagagaccaccagacggc
PCR_Vegfa_Nested-R1	gagcccagggagcaaaggtca	ggttcctggttgtgcttagtggga
PCR_Vegfa_Nested-R2-P1	aaaggtcacgaaagcagatggtcaa	gtgtatatacatagctgtccccgg
PCR_Vegfa_Nested-R2-P2	agagcttcggcagggaagtaca	tatttgatgagtggctgttggcct
PCR_Vegfa_Nested-R2-P3	ttgtaccactgtcctcctgc	gtggagctgtaaggagtggt
PCR_SERPING1	ctcatctgccgcactgtcagaaattactct	gattggtgactcttatgggagtgtccaaca
PCR_Site2	atgtaaaggtggcaagggaacaa	tgacaaggtaaaggaaagaggaggc
PCR_Vegfa (transgene)	aaaggtcacgaaagcagatggtcaa	gtgtatatacatagctgtccccggt
PCR_Fah	gttgccaaattggctccacttac	ccacacccacagagtcagaa
PCR_HEK3	atgtgggctgcctagaaagg	cccagcccaacttgtcaacc
ChIP_Pcsk9	ggctcccgttctctctctct	ctcgggaaggacatggacg
ChIP_Reference	ctgctcctgactgggtaatgaa	attttgtgctgcataacctcct
Vegfa_OT1	ggagactgtttggcctctgt	aatagtcccctgttcgtgcc
Vegfa_OT2	gctggtccctgtggattaca	tgagagacttcggggacaga
Pcsk9_OT	gtacaaggctccacaggtca	ctcctgctctaggagaggtgt

Supplementary Table S4. Oligonucleotides and probes for ddPCR

ddPCR oligoes	Sequence (5´- 3´)
ddPCR_AFF1.FOR	ccttcagctcagtgacagtgg
ddPCR_Pcsk9.FOR	cagcagcggcggcaacaggt
ddPCR_Vegfa.FOR	cctggaagatgtccaccagt
ddPCR_SERPING1.FOR	ttgcaagacagaggcgaagt
ddPCR_sgRNA(universal).BOT	ccgactcggtgccacttt
ddPCR_Probe_universal	FAM-aaataaggctagtccgttatcaactt-BHQ-1

Supplementary Table S5. Oligonucleotides for NGS

NGS oligonucleotides	Sequence (5´- 3´)
HEK3 CTT NGS.FOR	acactctttccctacacgacgctcttccgatctgcaattagtctatttctgctgcaag
HEK3 CTT NGS.REV	gtgactggagttcagacgtgtgctcttccgatctgtcaaccagtatcccggtgc
I5	atgatacggcgaccaccgagatctacacnnnnnnnnacactctttccctacacgac
I7	caagcagaagacggcatacgagatnnnnnnnngtgactggagttcagacgtgt

Supplementary Table S6. oligonucleotides used for NEBbuilder of indicated plasmids

NEBuilder	Sequence (5´- 3´)	Reverse (5´- 3´)
GagPol-IntSpCas9	gagtgctggccgaggccatgagcgactataaggaccacgacg	cctgcggccgctccggaattccatgtgtcacttttttctttttgcctg
GagPol-MatSpCas9	gatccgctagcgctaccggtaccaccatggactataaggac	ctcgagatctgagtccggtctgcacaatcggatagttctggctaaagttctttttctttttgcctggccg
GagPro-Frag1	gcccatcactttggcaaagcacgtgagatctgctcagatctcgagctcaagctt	agtacagggtggccacggtgttgtacaggctcctcagctcct
GagPro-Frag2	gcagaccggcagcgaggagctgaggagcctgtacaacaccgtggccaccctg	cttctggtggggctgttggctctggtctgctctgaagaaa
GagPro-Frag3	ccaccatttcttcagagcagaccagagccaacagccccaccagaa	tgataggcagcctgcactggtggggtgtctagactttccgcttcttctttgggc
GagPro-SpCas9	ggggtagcccaaagaagaagcggaaagtcgacaagaagtacagcatcggcctggac	taggcagcctgcactggtggggtgtttacttttttctttttgcctggccg
GagPro-ABE8e	gtagcccaaagaagaagcggaaagtctctgaggtggagttttcccacgag	ggcagcctgcactggtggggtgtttagactttcctcttcttcttgggct

References to Supplementary Material

1. Skipper, K.A., Nielsen, M.G., Andersen, S., Ryo, L.B., Bak, R.O. and Mikkelsen, J.G. (2018) Time-Restricted PiggyBac DNA Transposition by Transposase Protein Delivery Using Lentivirus-Derived Nanoparticles. *Mol Ther Nucleic Acids*, **11**, 253-262.
2. Cai, Y., Bak, R.O. and Mikkelsen, J.G. (2014) Targeted genome editing by lentiviral protein transduction of zinc-finger and TAL-effector nucleases. *Elife*, **3**, e01911.