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Supplemental Information

Poly(Beta-Amino Ester) Nanoparticles Enable Nonviral Delivery of CRISPR-Cas9 Plasmids for Gene Knockout and Gene Deletion

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Supplemental Information

Supplemental Data:

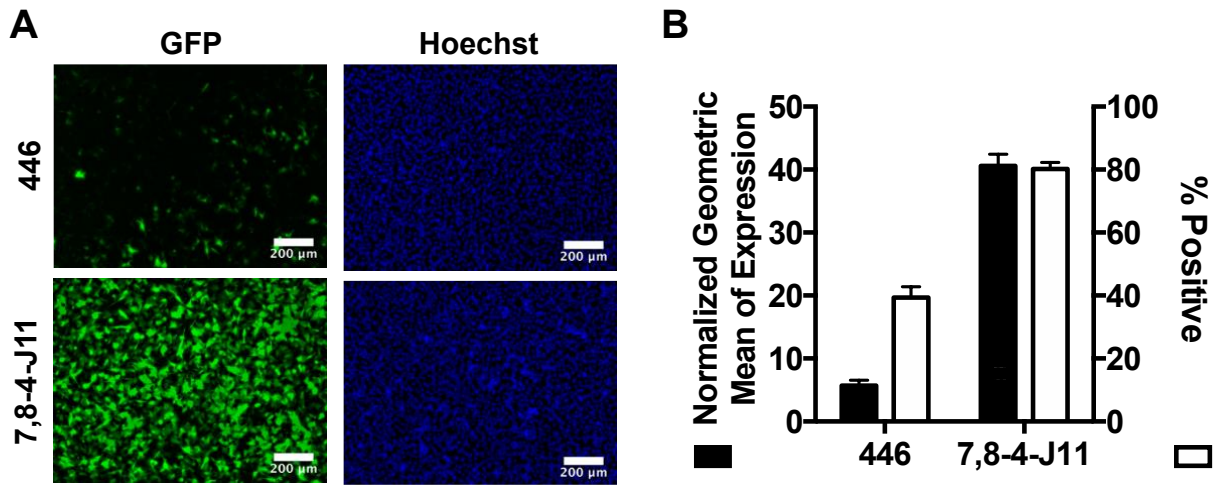

Figure S1. GFP transfection screen results for B16-F10 cells. Fluorescence microscopy images **(A)** and flow cytometry results **(B)** show that branched PBAE polymer 7,8-4-J11 (30 w/w) transfects B16 cells more efficiently than canonical linear PBAE polymer 446 (60 w/w). Data presented as mean + SEM; *N* = 4. Scale bar = 200 μm.

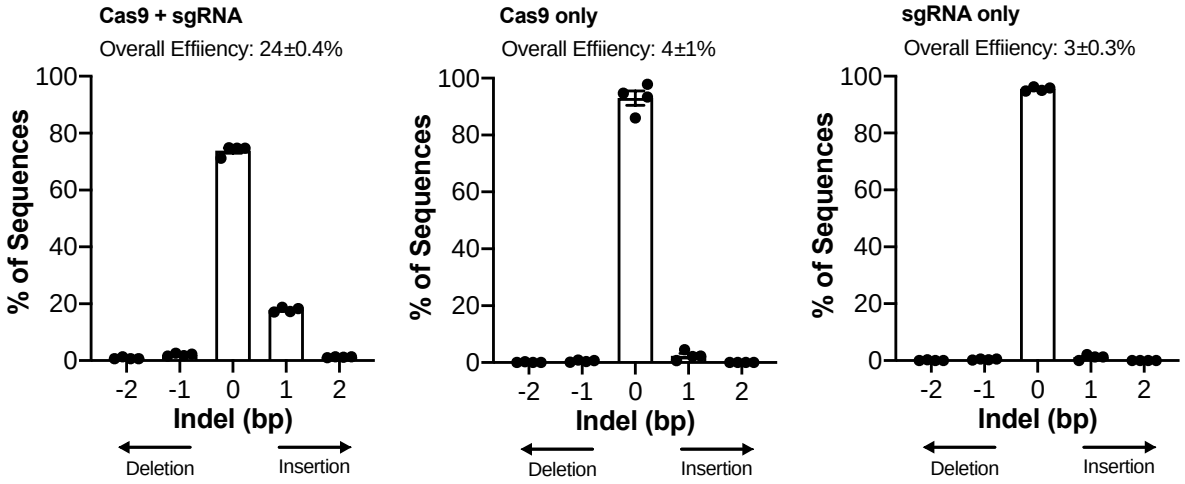

Figure S2. TIDE quantification of indel frequencies. Sanger sequencing data of the genomic DNA of HEK-GFP cells treated with nanoparticles delivering CRISPR plasmids were quantified by TIDE analysis. *N* = 3, data presented as mean ± SEM.

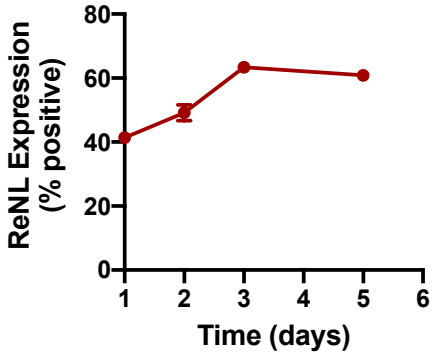

Figure S3. ReNL expression time course after 2-cut edits in HEK cells. HEK cells were transfected on day 0 and gain of ReNL expression was monitored on selected days to determine the expression timeline for ReNL. *N* = 4, data presented as mean ± SEM.

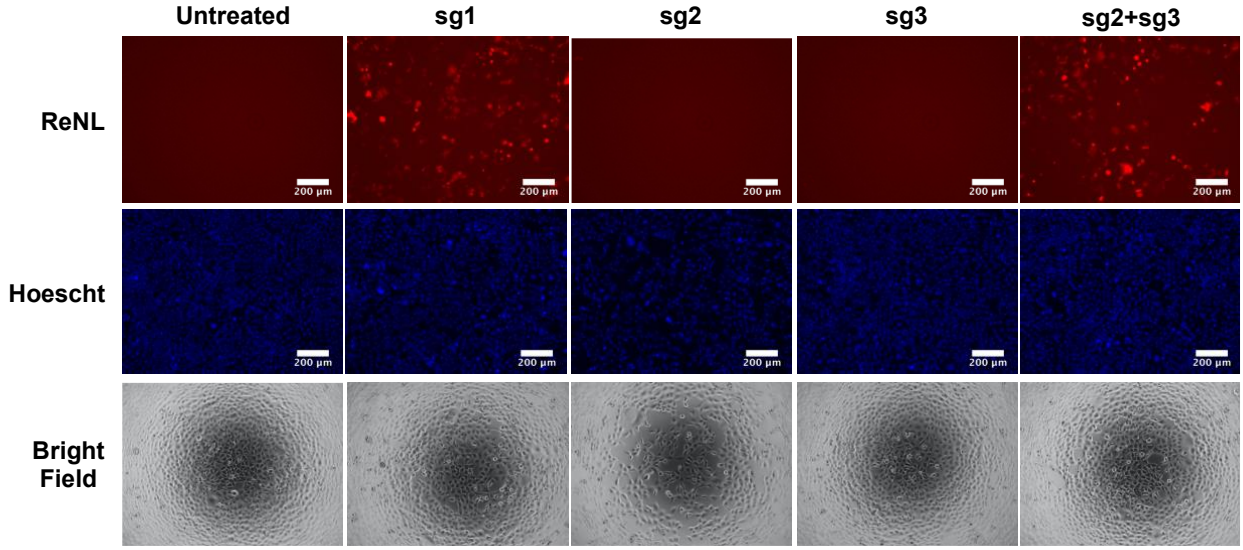

Figure S4. Microscopy images of ReNL gain of expression. 2-cut CRISPR cleavage with sg1 or combination of sg2+sg3 turn on expression of ReNL by removal of two SV40 polyA sequences. Scale bar = 200 µm.

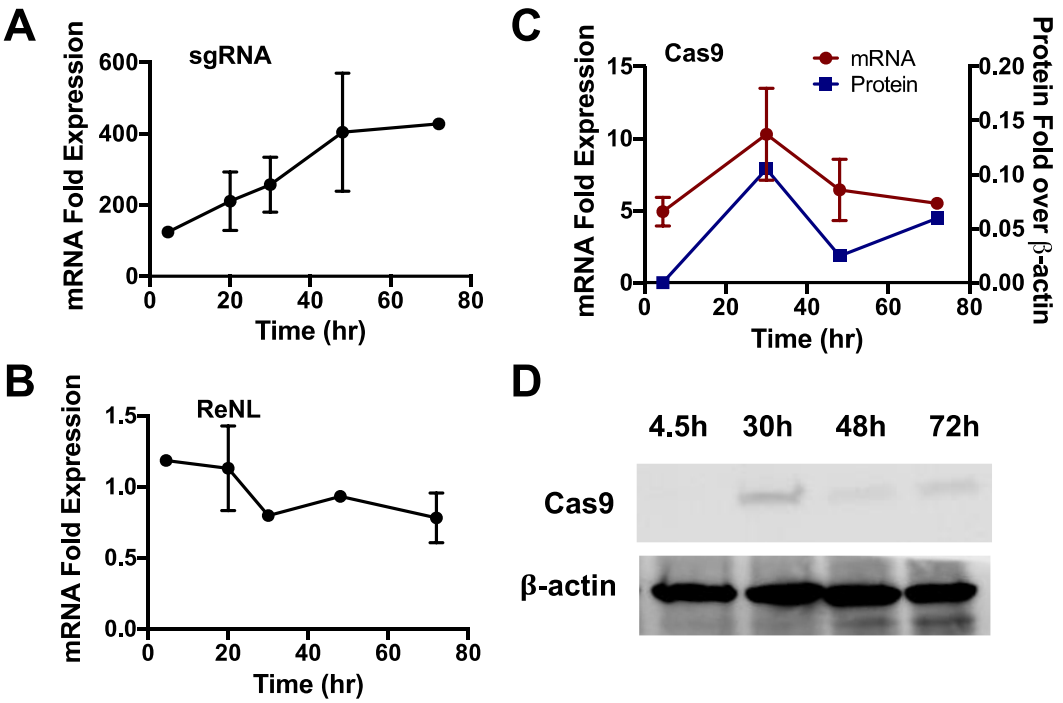

Figure S5. Expression kinetics of CRISPR components in B16 cells. mRNA expression levels of sgRNA **(A)** and ReNL **(B)**. Cas9 mRNA **(C, red curve)** and protein **(C, blue curve; D)** expression levels over time. N = 2 (data shown as mean ± SEM) for qRT-PCR experiments; N=1 for western blots.

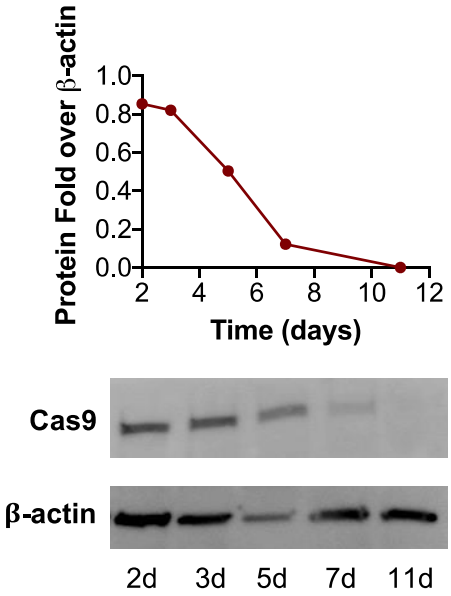

Figure S6. Long-term Cas9 accumulation in 293T cells. Western blotting was performed on indicated days after transfection of Cas9 and sgRNA plasmids in HEK-293T cells and Cas9 protein expression levels were quantified as fold expression over **β**-actin based on image analysis of band intensities (n=1).

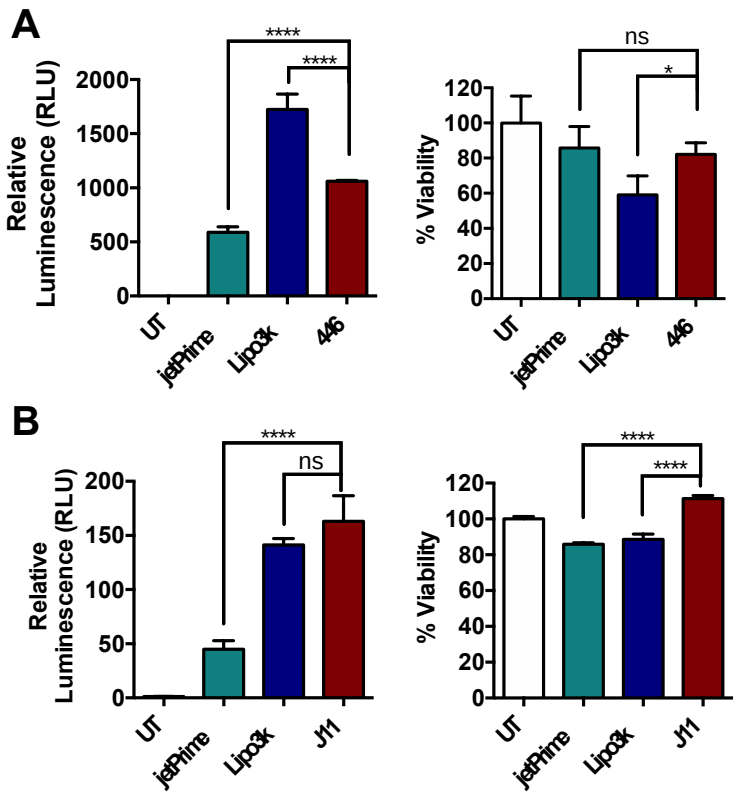

Figure S7. Comparison to commercial transfection reagents. Gain of expression from 2-cut edits and viability of cells using commercial reagents and PBAEs in **(A)** 293T and **(B)** B16 cells as measured by ReNL luminescence. Data presented as mean + SEM; N=4. Statistical significance assessed by one-way ANOVA with Dunnett post-hoc tests as compared to the PBAE treated group (446 or J11, respectively); *p < 0.05, ****p < 0.0001

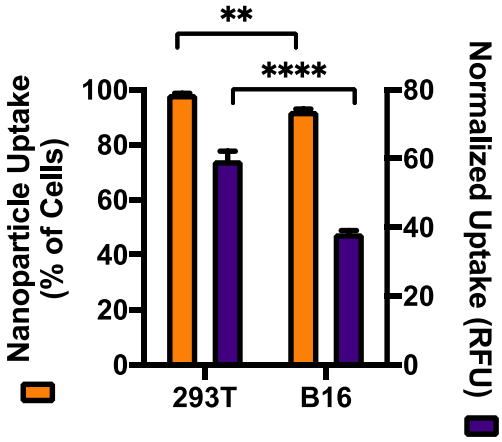

Figure S8. Nanoparticle uptake analysis in B16 and 293T cells. Flow cytometry analysis of cellular uptake of nanoparticles encapsulating 20% Cy5-labeled DNA. Data shown as mean + SEM; N=4. Statistical significance assessed by one-way ANOVA with Sidak's post-hoc tests; **p < 0.01, **** p < 0.0001.

Target	Sequence	Notes
GFP	FWD: CTGGTCGAGCTGGACGGCGACG REV: CACGAACTCCAGCAGGACCATG	Amplicon size: 630 bp
2X-SV40 Stop Cassette	FWD: CGCAAATGGGCGGTAGGCGTG REV: GCCCTTGCTCACCATGAATT	Amplicon size: 755 bp
hCas9	FWD: GGAGTTGACGCCAAAGCAATCC REV: AGATTTAAAGTTGGGGGTCAGCC	Amplicon size: 150 bp
ReNL	FWD: ATCCCGTATGAAGGTCTGAGCG REV: GTCGATCATGTTCGGCGTAACC	Amplicon size: 147 bp
sgRNA1	FWD: ACATTATACGGTTTCAGAGC REV: GACTCGGTGCCACTTTTTCA	Amplicon size: 91 bp
β-actin (human)	FWD: CATGTACGTTGCTATCCAGGC REV: CTCCTTAATGTCACGCACGAT	Amplicon size: 250 bp Primerbank ID: 4501885a1
β-actin (mouse)	FWD: CTGTCCCTGTATGCCTCTG REV: ATGTCACGCACGATTTCC	Amplicon size: 218 bp

Table S1. PCR primer sequences.

Plasmid Name	Addgene ID	Description
PB-iRFP-STOP-ReNL	113965	Piggybac transposon plasmid CRISPR gene deletion activatable fluorescence. Constitutive iRFP670 under EF1A promoter, CMV promoter with two SV40 polyA followed by red-enhanced nanolantern (ReNL)
sg1	113966	Single short guide RNA targeting GTATAGCATACATTATACG
sg2	133967	Single short guide RNA targeting TACCACATTTGTAGAGGTT
sg3	133968	Single short guide RNA targeting CAATGTATCTTATCATGTC
sg1+sg2+sg3	133969	Triple short guide RNA targeting GTATAGCATACATTATACG, TACCACATTTGTAGAGGTT & CAATGTATCTTATCATGTC
sg2+sg3	133970	Double short guide RNA targeting TACCACATTTGTAGAGGTT & CAATGTATCTTATCATGTC
sgiRFP1	133972	Single short guide RNA targeting GATCGAGTTCGAGCCTGCGG in iRFP670 sequence
sgiRFP2	133973	Single short guide RNA targeting GCGCGTTCTTTGGACGCGA in iRFP670 sequence
sgiRFP3	133974	Single short guide RNA targeting CGTGATGTTGTACCGCTTC in iRFP670 sequence

Table S2. Plasmids deposited with Addgene

DNA Sequence	Description and notes
ATTATTGACTAGTAGTGGTTTTAGAGCTAGAAATAG CAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAA AAAGTGGCACCGAGTCGGTGCAACAAAGCACCAGT GGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAG ACCCGGGTTCGATTCCCGGCTGGTGCAGCCAAGCTT GGCGTAA	pGTR sequence SpeI restriction enzyme site HindIII restriction enzyme site gRNA scaffold pre-tRNA
AGTTAGTTtctagaACAAAGCACCAGTGG	tRNA-start_F primer **XbaI restriction enzyme site**
GAACCTCTACAAATGTGGTA	sg2 protospacer sequence; overlapping base pairs used in Golden Gate primers
TAGGTCTCCACAAATGTGGTAGTTTTAGAGCTAGAA	sg2_F primer
ATGGTCTCATTGTAGAGGTTCTGCACCAGCCGGGAA	sg2_R primer
GCAATGTATCTTATCATGTC	sg3 protospacer sequence; overlapping base pairs used in Golden Gate primers
TAGGTCTCCTCTTATCATGTCGTTTTAGAGCTAGAA	sg3_F primer
ATGGTCTCAAAGATACATTGCTGCACCAGCCGGGAA	sg3_R primer
CAATGTATaagcttAAAAAAAAAAGCACCGACTCG	gRNA-end_R primer **HindIII restriction enzyme site**

Table S3. DNA and primer sequences used to generate multiplex tRNA-gRNA plasmid. The pGTR sequence was cloned into a backbone plasmid via restriction enzyme cloning using SpeI and HindIII. The pGTR plasmid was then used as the PCR template for amplifying gRNA-tRNA sequences for Golden Gate assembly. To synthesize a multiplex plasmid containing both sg2 and sg3, PCR amplicons were generated using the following pairs of primers: tRNA-start_F + sg2_R (amplicon 1); sg2_F + sg3_R (amplicon 2); sg3_F + gRNA-end_R (amplicon 3). Amplicons 1-3 were then purified, ligated by Golden Gate assembly, and cloned into a backbone vector containing a single U6 promoter using restriction enzyme cloning with XbaI and HindIII.

Calculation S1. N-P ratios of PBAE/DNA nanoparticles. Gel permeation chromatography was used to measure the molecular weight of polymers 446 and 7,8-4-J11, respectively. The number averaged molecular weight (M_N) was 5935 Da for 446 and 6943 Da for 7,8-4-J11. Using polymer M_N and the molecular weight of individual monomers, the nitrogen (N) weight fraction was calculated to be 0.055 for 446 and 0.046 for 7,8-4-J11. The average phosphate (P) weight fraction for DNA was calculated to be 0.095. Taken together, 446 nanoparticles at 60 w/w had an N-P ratio of 34.5, and 7,8-4-J11 nanoparticles at 30 w/w had an N-P ratio of 14.5.