Supporting Information for

Nonviral Gene Editing via CRISPR/Cas9 Delivery by Membrane-Disruptive and Endosomolytic Helical Polypeptide

Materials and Cell Lines. Plasmids pSpCas9 (px165) and pSpCas9(BB)-2A-GFP (px458) were gifts from Prof. Feng Zhang (Addgene plasmid # 48137). Plasmid gRNA_GFP-T1 was a gift from Prof. George Church (Addgene plasmid # 41819). Generation of *in vitro* synthesized single guide RNAs (sgRNAs) was done by *in vitro* transcription using T7 High Yield RNA Synthesis Kit (New England Biolabs, NEB). The gene target sequences and polymerase chain reaction (PCR) primers are shown in Table S1. FITC-labelled sgRNAs were generated by the same *in vitro* transcription method involving the FITC-labelled UTP instead of the normal UTP. Lipofectamine 3000 (Lipo3K), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), Lysotracker Red, Fluoromount™ Aqueous Mounting Medium, 4',6-diamidino-2-phenylindole (DAPI), and YOYO-1 were purchased from Invitrogen. Polyethylenimine (PEI, branched, M_W ~25,000), paraformaldehyde, genistein, methyl-β-cyclodextrin (mβCD), wortmannin, and chlorpromazine were purchased from Sigma-Aldrich. All chemicals for the synthesis of polypeptides were purchased from Sigma-Aldrich unless otherwise specified. $_L$-Glutamic acid was purchased from Chem-Impex International Inc. γ-(4-Vinylbenzyl)-$_L$-glutamate *N*-carboxyanhydride (VB-$_L$-Glu-NCA) was synthesized according to previously reported procedure (1).

Human embryonic kidney HEK293T cells, human cervical cancer HeLa cells, mouse fibroblasts NIH3T3 cells and human erythroleukemia K562 cells were purchased from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (FBS, Hyclone), 2 mM L-glutamine (Life Technologies), 1 mM sodium pyruvate (Life Technologies), streptomycin (Life Technologies), 100 U/mL penicillin (Life Technologies) at 37 °C with 5% CO_2. DC2.4 cell, a murine dendritic cell line, was a kind gift from Dr. Kenneth Rock. DC2.4 cells were cultured in RPMI-1640 medium (Life Technologies) supplemented with 10% FBS, 10 mM HEPES (Life Technologies), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 μM nonessential amino acid

(Life Technologies), 100 μg/mL streptomycin, 100 U/mL penicillin, and 50 μM 2-mercaptoethanol (Life Technologies) at 37 °C with 5% CO_2. Human umbilical cord blood derived endothelial progenitor cells (hEPC) were cultured as previously described (2). Normal human dermal fibroblasts (NHDF) (Lonza) were cultured as previously described (3). The U2OS.EGFP cells were a generous gift from Prof. J Keith Joung at Massachusetts General Hospital (4) and cultured in DMEM with 10% FBS, 2 mM GlutaMAX (Life Technologies), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C with 5% CO_2. The A549.GFP cells were a generous gift from Prof. Jun Wang and Prof. Yucai Wang at University of Science and Technology of China and cultured in DMEM with 10% FBS penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C with 5% CO_2.

Synthesis and Characterization of poly(γ-4-((2-(piperidin-1-yl)ethyl)aminomethyl) benzyl-$_L$-glutamate) (PPABLG). PPABLG was synthesized according to the reported procedure (Fig. S1*A*) (5). In a glovebox, VB-$_L$-Glu-NCA was dissolved in anhydrous dimethyl formamide (DMF), into which the DMF solution of hexamethyldisilazane (M/I = 200) and nitrobenzene were added. The polymerization was carried out at room temperature (RT) until the conversion of NCA reached > 99% (monitored by FTIR). The polypeptide precursor, poly(γ-(4-vinyl)benzyl-$_L$-glutamate) (PVBLG), was purified by precipitation in hexane:ether (1:1, v/v). PVBLG was characterized by ^{1}H NMR and GPC (Fig. S1*B*) to confirm its molecular structure and determine its molecular weight and polydispersity index. PVBLG was then dissolved in chloroform, and the side-chain vinyl groups were oxidized into aldehydo groups by bubbling O_3 gas into the solution at -78 ^{o}C. The resulting polypeptide, poly(γ-(4-aldehydo)benzyl-$_L$-glutamate) (PABLG) was purified by precipitation in methanol, and analyzed by ^{1}H NMR to confirm the conversion of side-chain vinyl groups. PABLG was then dissolved in DMF, into which 1-(2-aminoethyl)piperidine and borane-pyridine complex were added sequentially to react with the side-chain aldehyde groups of PABLG. The final product PPABLG was purified by dialysis against DI water and then lyophilized to yield white powder, which was further characterized by ^{1}H NMR (Fig. S1*C*) to confirm its molecular structure and by CD spectrum (Fig. S1*D*) to reveal its α-helical conformation in aqueous solution.

Synthesis and characterization of PEG_{2k}-T_{40} and PEG_{5k}-T_{40}. Methoxyl PEG-azides ($mPEG_{2k}$-azide and $mPEG_{5k}$-aizde) were purchased from Nanocs Inc. The single-stranded

5'-hexynyl-polythymine with a length of 40 nucleotides (T_{40}) was synthesized by Integrated DNA Technologies (IDT), and its molecular weight was confirmed by electrospray ionisation mass spectrometry (ESI-MS). PEG_{2k}-T_{40} and PEG_{5k}-T_{40} were synthesized by Click chemistry. Briefly, the reaction was carried out in 200 mM trithylamiimum buffer (pH 7.0) containing 50% dimethylsulfoxide with T_{40} (50 μM), mPEG-azide (250 μM), L-ascorbic acid (10 mM) and copper(II)-TBTA complex (500 μM) in an argon atmosphere for overnight at RT. The product was purified by reversed-phase high performance liquid chromatography (HPLC). Before use, HPLC-purified PEG_{2k}-T_{40} and PEG_{5k}-T_{40} were reconstituted in molecular biology-graded, ultra-pure water and quantitated by UV-Vis.

Preparation and Characterization of HNPs and P-HNPs. PPABLG, PEG-T_{40} and plasmids/sgRNAs were dissolved in water (molecular biology grade) at 0.2 mg/mL. To form HNPs, PPABLG was added into the plasmid/sgRNA solution at various weight ratios followed by vortex for 30 s and incubation at RT for 20 min. To form P-HNPs, PPABLG was added to the mixed solution containing $PEG_{2k/5k}$-T_{40} and Cas9 expression plasmids/sgRNAs at determined weight ratios followed by vortex for 30 s and incubation at RT for 20 min. To form P-HNP(coating), PPABLG was added into the plasmid solution at the weight ratio 30:1 (PVBLG: plasmid) followed by vortex for 30 s and incubation at RT for 20 min to form HNPs first; Then, PEG_{2k}-T_{40} was added to the HNPs at the weight ratio of 30:1:1 for PVBLG: plasmid: PEG_{2k}-T_{40} followed by vortex for 30 s and incubation at RT for further 20 min. The mean diameter and zeta potential of nanoparticles (0.5 mg/mL) in water and in DMEM with 10% FBS were monitored by a Malvern Zetasizer Nano ZS90.

Cell Uptake. To analyze the cell uptake of HNPs or P-HNPs for Cas9 expression plasmid, plasmids were labeled with YOYO-1 as described previously (6). HEK293T cells were inoculated at 5×10^4 cells/well in a 24-well plate and cultured for 24 h before use. The HNP/YOYO-1-plasmid (w/w 30:1 for PPABLG:plasmid), P-HNP/YOYO-1-plasmid (w/w/w 30:1:1 for PPABLG:plasmid:PEG_{2k}-T_{40}), or PEI/YOYO-1-plasmid (w/w 1:1) were added into the cell-culture well with Opti-MEM (500 μL/well) and sustained for 4 h at 37 °C. The concentration of YOYO-1-plasmid was 1 μg/mL. Then, cells were washed with cold PBS containing heparin (20 U/mL) for 3 times and lysed in 500 μL RIPA lysis buffer. The YOYO-1-plasmid content in the lysate was analyzed by fluorescence spectrophotometer with Ex 485 nm and Em 530 nm and the total protein concentration was determined by BCA assay

(Pierce Biotechnology). Cellular uptake level was shown as per ng YOYO-1-plasmid per mg protein. In the following experiments, we used the same weight ratio to prepare the particles unless otherwise specified.

To observe the intracellular distribution of Cas9 expression plasmids, HEK293T cells were seeded on coverslips in a 24-well plate at a density of 2×10^4 cells/well and cultured for 24 h. Cells were transfected following the same method as above, washed 3 times with cold PBS containing heparin (20 U/mL) and fixed with 4% paraformaldehyde (pH 7.4, in PBS) at RT for 15 min. The cell nuclei were stained with DAPI and the endosomes/lysosomes were counterstained with Lysotracker Red according to the standard protocol provided by the suppliers. The slides were mounted by Fluoromount™Aqueous Mounting Medium and observed by confocal laser scanning microscopy (CLSM, Zeiss-700) with a 40 × objective.

For the cellular uptake of FITC-labelled sgRNAs (FITC-sgRNAs), the HNP/FITC-sgRNA and P-HNP/FITC-sgRNA were formed at weight ratio of 30:1 (PPABLG:FITC-sgRNA) and 30:1:1 (PPABLG:FITC-sgRNAs: PEG) following the method mentioned above. HEK293T cells were seeded at 5×10^4 cells/well in a 24-well plate and cultured for 24 h. The HNP/FITC-sgRNA, P-HNP/FITC-sgRNA or PEI/FITC-sgRNA (w/w 1:1) were respectively added into the cell-cultured well with Opti-MEM (500 μL/well) and sustained for 4 h at 37 °C. The concentration of FITC-sgRNAs was 1 μg/mL. Then cells were washed with cold PBS containing heparin (20 U/mL) 3 times, trypsinized into a single-cell suspension, and then subjected to flow cytometric analysis using a BD FACSCalibur flow cytometer.

Determination of Endocytosis Pathways. Cell uptake inhibition for the P-HNP/YOYO-1-plasmid was carried out at 4 °C, or exposed to the medium including endocytic inhibitors to explore the internalization mechanism of Cas9 delivery through P-HNPs. Briefly, cells were pretreated at 4 °C for 30 mins and the P-HNP/YOYO-1-plasmid was added to perform the uptake study for 2 h at 4 °C wherein the energy-dependent endocytosis was blocked. Otherwise, cells were respectively pre-incubated with medium with 100 μg/mL genistein, 10 μg/mL chlorpromazine, 10 μg/mL wortmannin, or 5 mM mβCD for 30 mins at 37 °C, and followed with 2-h uptake experiment for the P-HNP/YOYO-1-plasmid at 37 °C. Cells were lysed and detected by the same way mentioned above. Inhibition results were shown as percentage uptake level of control group that was incubated with the P-HNP/YOYO-1-plasmid at

37 °C for 2 h. The dose of YOYO-1-plasmid was 1 μg/mL. The FITC-Tris uptake experiment was conducted as described previously (7).

Cas9 Expression Plasmids Transfection. To test the wild-type Cas9 expression plasmid transfection efficiency of HNPs and P-HNPs, the plasmid pSpCas9(BB)-2A-GFP (px458) was used, in which the 2A-GFP was fused to Cas9 to allow the detection of Cas9 expression in the transfected cells. Cells were seeded at 5×10^4 cells/well in a 24-well plate and transfected with HNP/px458, P-HNP/px458, PEI/px458, and Lipo3K/px458 respectively at the dose of 1 μg plasmid/mL. GFP+ cells were counted by flow cytometric analysis at 48 h after transfection.

To observe the GFP expression and intracellular distribution for px458 transfection, HEK293T cells were seeded on coverslips in a 24-well plate at a density of 2×10^4 cells/well and cultured for 24 h. Cells were transfected with HNP/px458, P-HNP/px458, PEI/px458, and Lipo3K/px458 respectively at the dose of 1 μg plasmid/mL. Cells were fixed at 48 h with 4% paraformaldehyde (pH 7.4, in PBS) at RT for 15 min. Then, cells were counterstained with DAPI for cell nuclei and Alexa Fluor® 594 phalloidin (Invitrogen) for the cytoskeleton according to the standard protocol provided by the suppliers. The slides were mounted by Fluoromount™ Aqueous Mounting Medium and observed by confocal laser scanning microscopy (CLSM, Zeiss-700) with a 40 × objective.

***In Vitro* EGFP Gene Disruption.** When using gRNA_GFP-T1 plasmid, U2OS-EGFP cells were seeded at 5×10^4 cells/well in a 24-well plate and transfected after 24 h with HNPs, P-HNPs or PEI, loaded with mixed plasmids containing px165 and gRNA_GFP-T1 plasmids at different weight ratios. The dose of px165 was 1 μg/mL. EGFP cells were analyzed by flow cytometric analysis at 48 h after transfection. When separately delivering EGFP sgRNAs generated by *in vitro* transcription and Cas9 expression plasmids, U2OS-EGFP cells were first treated with HNP/px165, P-HNP/px165, or PEI/px165 at a dose of 1 μg px165/mL for 24 h, and then continued incubate with the same kind of particles loaded with sgRNAs (0.5 μg/mL) for 48 h before flow cytometric analysis or cleavage detection. For codelivery of *in vitro* synthesized EGFP targeting sgRNAs and Cas9 expression plasmids, U2OS-EGFP cells were transfected with P-HNPs loaded mixed nucleic acids containing px165 and EGFP targeting sgRNAs at weight ratio 1:1. The dose of px165 was 1 μg/mL. EGFP cells were analyzed by flow cytometric analysis at 48 h after transfection. The on-target and off-target cleavage was detected by using

GeneArt®Genomic Cleavage Detection (GCD) Kit (Life technology) with PCR primers in Table S1. The percentage of indels was quantitated using the Image J software.

***In Vitro* Endogenous Gene Editing.** HEK293T cells were seeded at 5×10^4 cells/well in a 24-well plate and transfected with P-HNP/px165 for 24 h. The dose of px165 was 1 μg/mL. Cells were then treated with P-HNP/sgRNA loaded with endogenous gene (*AAVS1*, *HPRT1 or Plk1)* targeting sgRNAs for 48 h. For multiplex gene editing, cells were treated with P-HNP to codeliver *AAVS1* targeting sgRNAs and *HPRT1* targeting sgRNAs for 48 h. The sgRNA dose was 0.5 μg/mL each sgRNA. The gene cleavage was detected by GCD assay with PCR primers in Table S1.

Sanger Sequencing to Detect Genomic Mutations. The PCR products of sgRNA targeted genomic locus was generated following the instruction of GCD Kit protocol. The purified PCR products were then cloned into the DNA sequencing vector following the Zero Blunt® TOPO® PCR Cloning Kit instruction (Life technology). The cloned plasmids were sequenced by Eton Bioscience Inc.

***In Vitro* Gene Insertion.** HEK293T cells were seeded at 5×10^4 cells/well in a 24-well plate and transfected with HNP/px165, P-HNP/px165, or PEI/px165 for 24 h. The dose of px165 was 1 μg/mL. The cells were then treated with the same type of particle loaded with a mixture of *AAVS1* targeting sgRNAs and the *AAVS1* homologous recombination (HR) donor (AAV-CAGGS-EGFP plasmid, Addgene Plasmid #22212) at the weight ratio of 1:1. The dose of total nucleic acids was 1 μg/mL. Cells were incubated for 72 h and the HR mediated integration was confirmed by a PCR approach with the primers in Table S1. Then transfected cells were selected by puromycin for two weeks to form clones. Clones with GFP expression in the well treated by P-HNPs were lysed and PCR were done to amplify the DNA sequences at the genome-donor boundary. Then, purified PCR products were cloned into a DNA sequencing vector following the Zero Blunt® TOPO® PCR Cloning Kit instruction. The cloned plasmids were sequenced by the Eton Bioscience Inc.

The pMax-^{VP64}dCas9-BFP^{VP64} Plasmid Construction and *In Vitro* Gene Activation. The ^{VP64}dCas9-BFP^{VP64} cassette was isolated from the TetO-FUW-VdC9BV lentiviral vector plasmid described by us previously (8). The ^{VP64}dCas9-BFP^{VP64} cassette was mobilized as two fragments by digesting with NheI and BstXI, and BstXI and XhoI (New England Biolabs) respectively. The

plasmid vector backbone was isolated from the pMax GFP plasmid (Lonza) by digesting with NheI and XhoI. All the three fragments bearing compatible sticky ends were ligated together by a three-way ligation reaction to form pMax-^{VP64}dCas9-BFP^{VP64}.

NIH3T3 cells were seeded at 5×10^4 cells/well in a 24-well plate and transfected with HNP/pMax-^{VP64}dCas9-BFP^{VP64}, P-HNP/pMax-^{VP64}dCas9-BFP^{VP64}, or PEI/ pMax-^{VP64}dCas9-BFP^{VP64} for 48 h at 1 mg plasmid/mL. dCas9 expression was monitored by flow cytometric analysis to detect the BFP+ cells. To activate mouse *Myod1* expression, NIH3T3 cells were treated as described above for 24 h. Then cells were transfected with HNP/sgRNA, P-HNP/sgRNA or PEI/sgRNA with *Myod1* sgRNAs for 48 h. In order to further enhance gene activation, the transfection was performed three times. The *Myod1* mRNA level was relatively quantified by using SYBR green RT-PCR (delta-delta Ct method).

Measurement of *In Vitro* Cytotoxicity. HEK293T cells were seeded at 1×10^4 cells/well in a 96-well plate and cultured for 24 h. The HNP/px165 (formed at the ratio of w/w 30:1), P-HNP/px165 (formed at the ratio of w/w/w 30:1:1), or PEI/px165 (w/w 1:1) were added into the cell-culture well with Opti-MEM (500 μL/well) and sustained for 4 h at 37 °C. The dose of px165 was 1 mg/mL. The medium was changed to complete DMEM with 10% FBS and the cells were further cultured for 1 day. The cell viability was assessed by the MTT assay as previously described (9). Results were represented as percentage viability of untreated cells.

Animal Experiments. The female BALB/c nude mice (6-week-old) were obtained from Shanghai Laboratory Animal Center, and all animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The procedures were approved by the Animal Care and Use Committee of Soochow University. To set up the tumor model, 1×10^7 A549.GFP or HeLa cells were subcutaneously inoculated into mice. The tumors were allowed to grow to about 100 mm^3 before treatment. The tumor volume (V) was estimated by the equation $V = a \times b^2/2$, where a, b are the major and minor axes of the tumor, respectively.

For GFP in vivo disruption, the mice were intratumorally injected with 50 μL of P-HNPs loaded with 20 μg px165 at the first day and then injected with 50 μL of P-HNPs loaded with 20 μg sgRNAs at the second day. The injection was performed a total of 3 times. At 48 h after the last injection, the mice were euthanized and tumors were collected. Fifty microgram fresh tumor

tissues close to the injection point were lysed and the locus-specific GFP gene cleavage was detected by GCD assay with PCR primers in Table S1. Percentage of indels was quantified using the Image J software. Other tumor tissues were immediately infiltrated into 4% paraformaldehyde/PBS solution overnight, and then dehydrated in 30% sucrose solutions for 6 h. The tumor tissues closed to the point of injections were further sectioned (10-μm) and counterstained with DAPI. Slices were observed by CLSM (Zeiss-700) with a 40 × objective.

For HeLa tumor growth inhibition, mice were intratumorally injected with 30 μL of P-HNPs loaded with 20 μg px165 (P-HNP$_{PCas9}$) on day 7, 9, 11, 13, and 15, and 30 μL of P-HNPs loaded with 20 μg sgPlk1 (P-HNP$_{sgPlk1}$) was intratumorally injected on day 8, 10, 12, 14, and 16. Mice that were administered with PBS, free px165 and free sgPlk1, or P-HNP$_{sgPlk1}$ served as controls. Each group contained seven mice. The tumor inhibition rate (I%) was calculated by 100% × V (P-HNP$_{PCas9+\ sgPlk1}$)/ V (PBS). At 48 h post the last injection, two mice were euthanized and tumors were collected. Remaining mice in each group were continually monitored every other day for survival, and euthanasia was performed when tumor volume reached 1000 mm^3. Harvested tumor tissues (50 mg) were lysed and the locus-specific *Plk1* gene cleavage was detected by deep sequencing. The Plk1 protein level in tumor tissues was detected by western blot using the Plk1 rabbit monoclonal antibody and GAPDH rabbit monoclonal antibody (Cell Signaling Technology, Inc.). Remaining tumor tissues were fixed in 4% paraformaldehyde/PBS solution overnight, and embedded in paraffin. Paraffin-embedded tumor sections (10-μm) were deparaffinized for hematoxylin and eosin (H&E) staining and BeyoClick EdU Cell Proliferation analysis (Beyotime Biotechnology). Slices were observed by CLSM (Zeiss-700) with a 63 × objective.

Statistics. For each data set, results were indicated as mean ± standard deviation (SD). The statistical significance was determined by two-sided Student's *t*-test (*$P < 0.05$,**$P < 0.01$ and ***$P < 0.001$); ns, no significance.

Table S1. Sequences of DNA oligos

		Sequences	Notes
Target Sequences	EGFP/GFP	GTGAACCGCATCGAGCTGAAagg	PAM
	AAVS1	GGGGCCACTAGGGACAGGATtgg	
	HPRT1	GCATTTCTCAGTCCTAAACAggg	
	Myod1	TAGCCAAGTGCTACCGCGTAtgg	
	Plk1	CGGAGGCTCTGCTCGGATCGagg	
Off-Target Sequences	EGFP Off-Target 1	GTGAATCCCAT-GAGCTGAAtgg	PAM Unmatched Sites
	EGFP Off-Target 2	GTGCACCAGCATCGAGCTGGAagg	
Primers for sgRNA Transcription Template	EGFP/GFP-sgRNA-F	GAAATTAATACGACTCACTATAGGTGAACCGCATCGAGCTGAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG	T7 promoter Target Sequence sgRNA Scaffold
	AAVS1-sgRNA-F	GAAATTAATACGACTCACTATAGGGGGCCACTAGGGACAGGATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC	
	HPRT1-sgRNA-F	GAAATTAATACGACTCACTATAGGCATTTCTCAGTCCTAAACAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC	
	Myod1-sgRNA-F	GAAATTAATACGACTCACTATAGTAGCCAAGTGCTACCGCGTACTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	
	Plk1-sgRNA-F	GAAATTAATACGACTCACTATAGCGGAGGCTCTGCTCGGATCGCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	

	Universal Reverse Primer	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGC	
Primers for GCD Assay	EGFP/GFP On-Target-F	GACGTAAACGGCCACAAGTT	
	EGFP/GFP On-Target-R	GCGGATCTTGAAGTTCACCT	
	EGFP/GFP Off-Target1-F	ACAAGCCACCATAACATCATCAG	
	EGFP/GFP Off-Target1-R	AGGCTGATTAACTCCAAAGGCA	
	EGFP/GFP Off-Target2-F	CTAGAGAGGCAGGTCAGCAA	
	EGFP/GFP Off-Target2-R	AGGTCATTTCAGCTCTCCGT	
	AAVS1-F	CACTCCTTTCATTTGGGCAG	
	AAVS1-R	AGAGATGGCTCCAGGAAATG	
	HPRT1-F	AAGGGTGTTTATTCCTCATGGA	
	HPRT1-R	AGCCAGACATACAATGCAAG	
	Plk1-F	CGTGTCAATCAGGTTTTCCC	
	Plk1-R	TTGAGCAGCAGAGACTTAGG	
Primers for HR Detection	HR-AAVS1-F	CTGCCGTCTCTCTCCTGAGT	
	Puro-R	GTGGGCTTGTACTCGGTCAT	
Real-Time PCR Primers	Myod1-F	AGCGACACAGAACAGGGAAC	
	Myod1-R	TCGAAAGGACAGTTGGGAAG	

Reference

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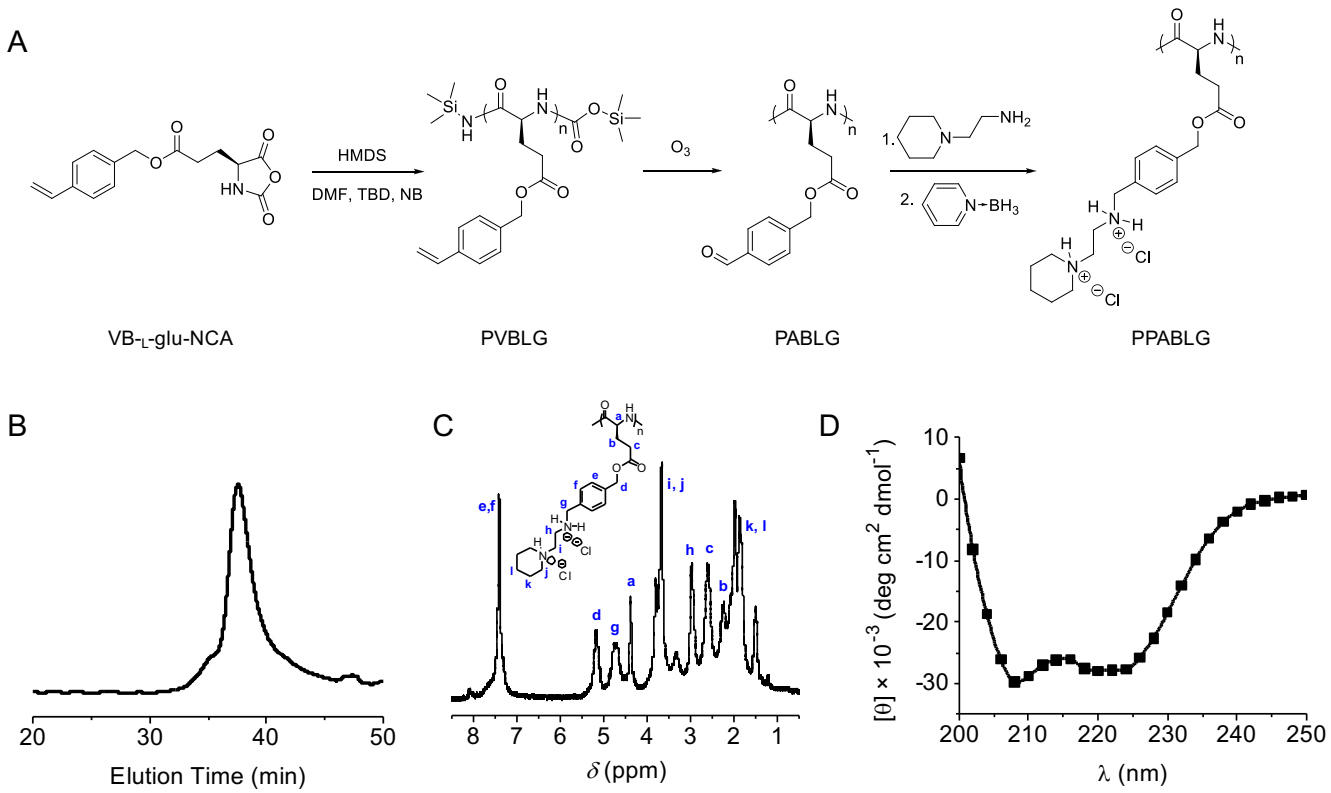

Fig. S1. Synthetic route and characterization of PPABLG. (A) Synthetic Route. (B) GPC trace of PVBLG precursor. (C) ^{1}H NMR spectrum of PPABLG in TFA-*d*. (D) CD spectrum of PPABLG in DI water at pH 7.0.

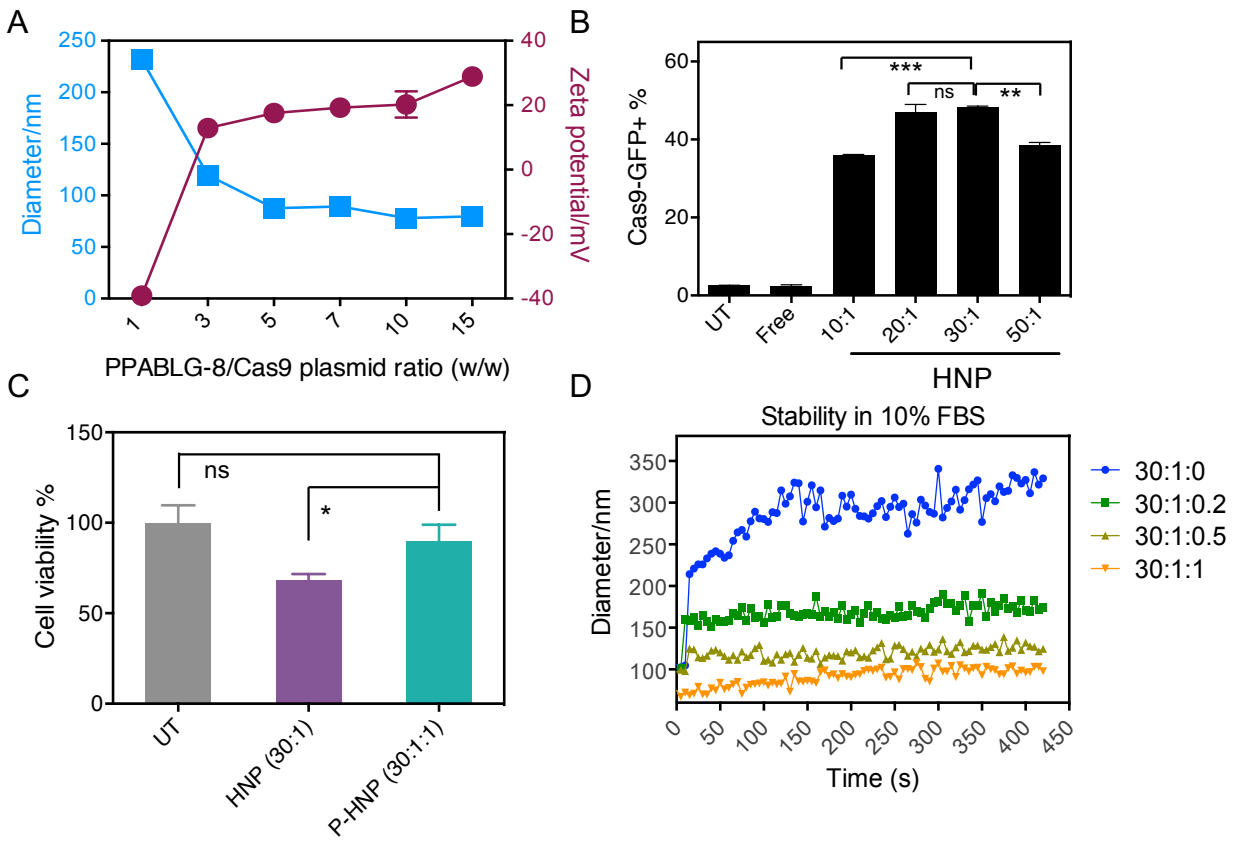

Fig. S2. Optimization of the HNPs and P-HNPs. (A) Size and Zeta-potential of the HNPs with different weight ratios of PPABLG/px458. (B) Transfection efficiency of HNPs for Cas9-GFP expression plasmid px458 in HEK293T cells. (C) Cell viability of U2OS.EGFP cells treated with HNP/px165 or P-HNP/px165. UT, untreated group. (D) The stability of HNPs (blue line) and P-HNPs in DMEM with 10% FBS. The ratios showed in the right are ratios of PPABLG:px458:PEG_{2K}-T_{40}.

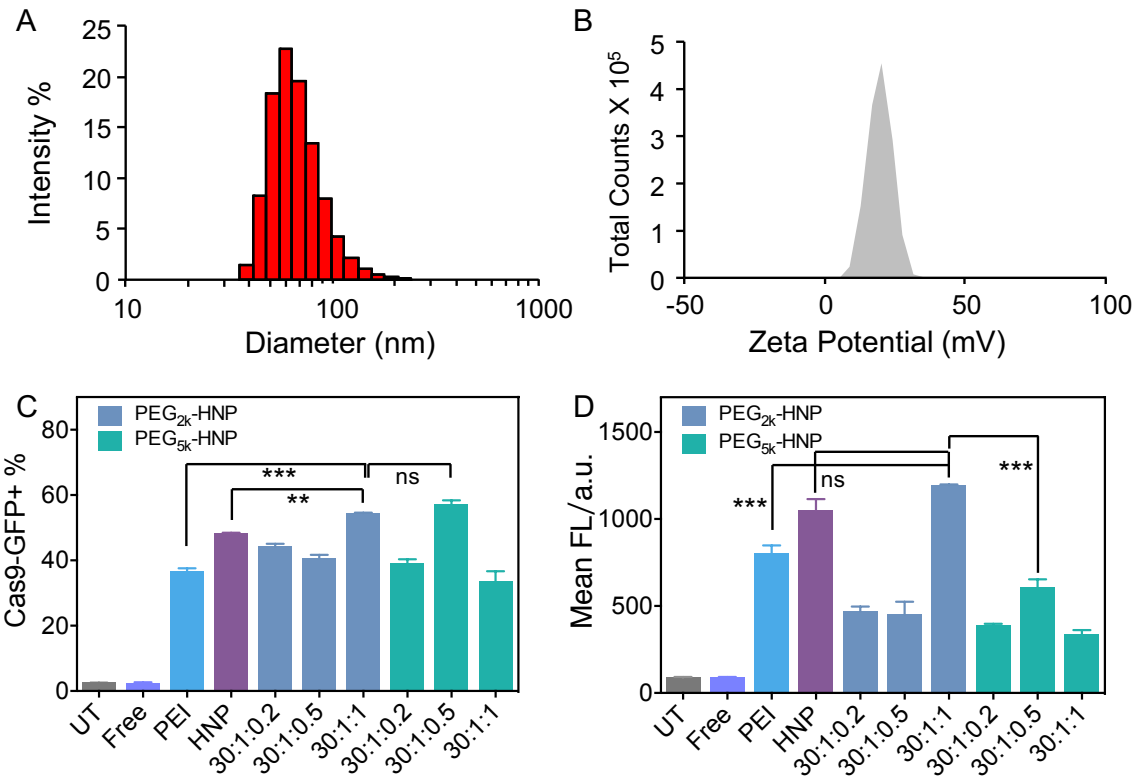

Fig. S3. Characterization and optimization of P-HNPs (A) Size and (B) zeta potential of P-HNPs formed by PPABLG:px458:PEG_{2K}-T_{40} at the ratio of 30:1:1. (C) and (D) Optimization of the transfection efficiency of P-HNPs for Cas9-GFP expression plasmid px458 by detecting the Cas9-GFP positive cell percentage and mean fluorescence intensity. The HNPs were formed by PPABLG/px458 at the w/w ratio 30:1. The ratios of PEG-HNPs were weight ratios of PPABLG:px458:PEG-T_{40}. UT, untreated group; Free, treatment with naked plasmids.

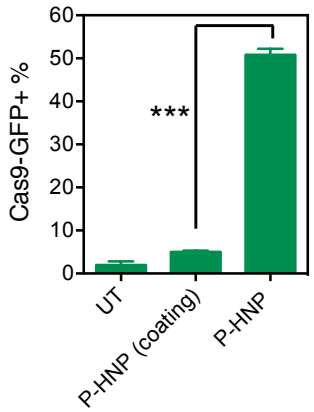

Fig. S4. Comparison of px458 transfection efficiency for P-HNPs, which were formed by mixing the PEG_{2K}-T_{40} with the px458 before forming the complex, versus P-HNPs (coating), which were formed by adding the PEG_{2K}-T_{40} to the PPABLG/px458 complex. UT, untreated group.

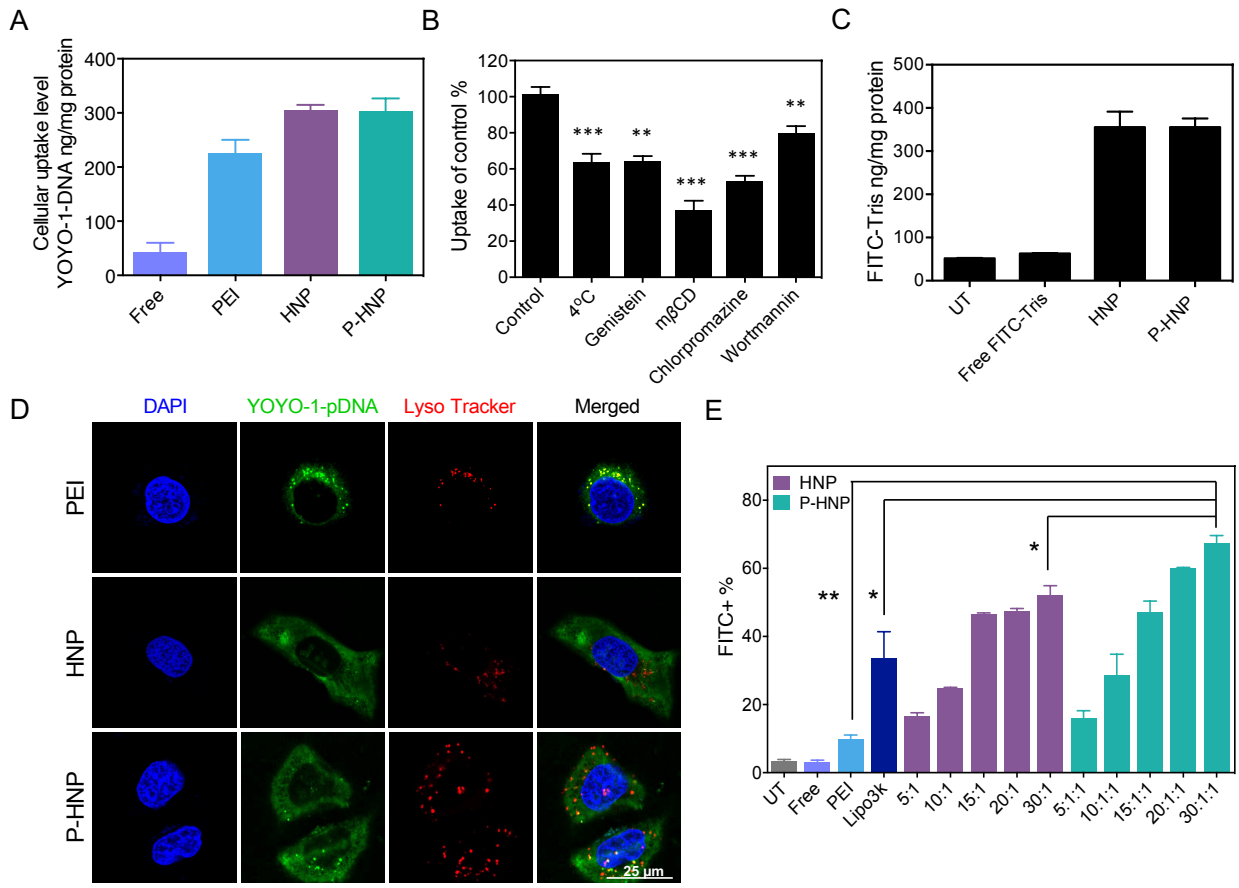

Fig. S5. Cellular Uptake. (A) Cell uptake level of nanoparticles loaded with YOYO-1 labelled px165. (B) Cell uptake level of P-HNPs at low temperature in the presence of various endocytic inhibitors. Control is the group treated with P-HNPs only without inhibitors. (C) Uptake level of FITC-Tris in HEK293T cells following co-incubation with HNPs or P-HNPs. (D) CLSM images of cells treated with PEI/YOYO-1 labelled px165, HNPs and P-HNPs containing YOYO-1 labelled px165. (Scale bar, 25 μm). (E) Cell uptake level of nanoparticles loaded with FITC-labelled sgRNAs. UT, untreated group; Free, treatment with naked plasmids. The results were indicated as mean ± standard deviation (SD) (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001; ns, no significance.

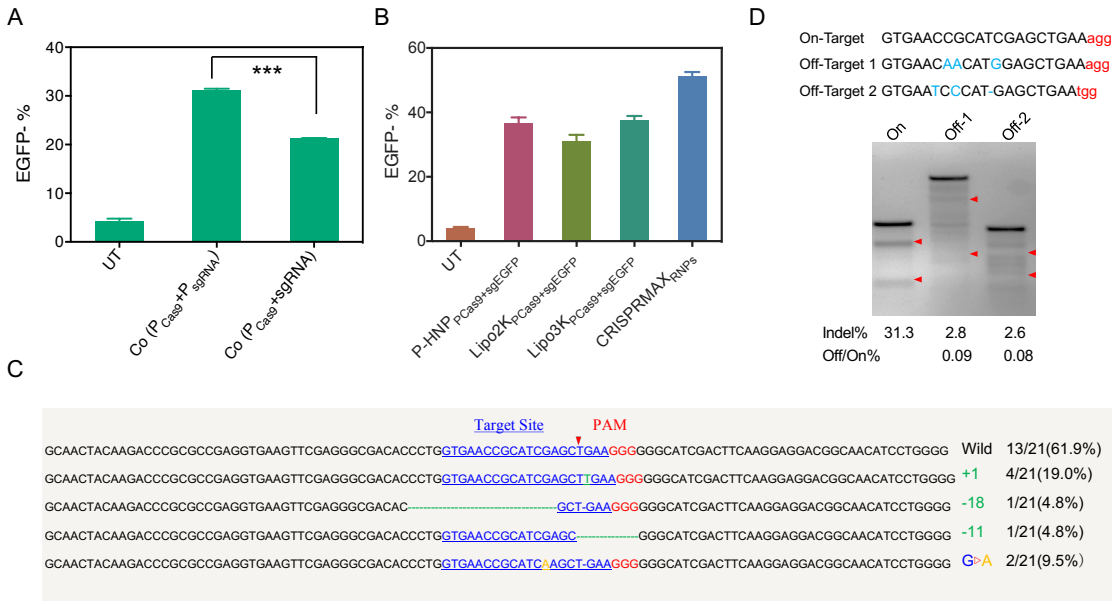

Fig. S6. Supporting results for EGFP disruption assay. (A) EGFP disruption assay based on the codelivery of Cas9 expression plasmid (P_{Cas9}) and sgRNA expression plasmid (P_{sgRNA}) or *in vitro* synthesized sgRNA by P-HNPs. UT, untreated group. (B) Comparation of the EGFP disruption efficiency of P-HNP$_{PCas9+sgEGFP}$, Lipofectamine 2000$_{PCas9+sgEGFP}$, Lipofectamine 3000$_{PCas9+sgEGFP}$ and Lipofectamine CRISPRMAX Cas9 RNP treatment on U2OS.EGFP cells. (C) Sequencing results of EGFP gene editing in U2OS.EGFP cells treated by P-HNPs. (D) GCD assay to detect the on-target and off-target effect of the EGFP targeting sgRNA in the cells treated by P-HNPs delivering Cas9 expression plasmids and synthesized EGFP targeting sgRNAs. PAM sequence is shown in red and unmatched sites are shown in blue. Off/On% is calculated by using the off-target indel% and dividing by the on-target indel%.

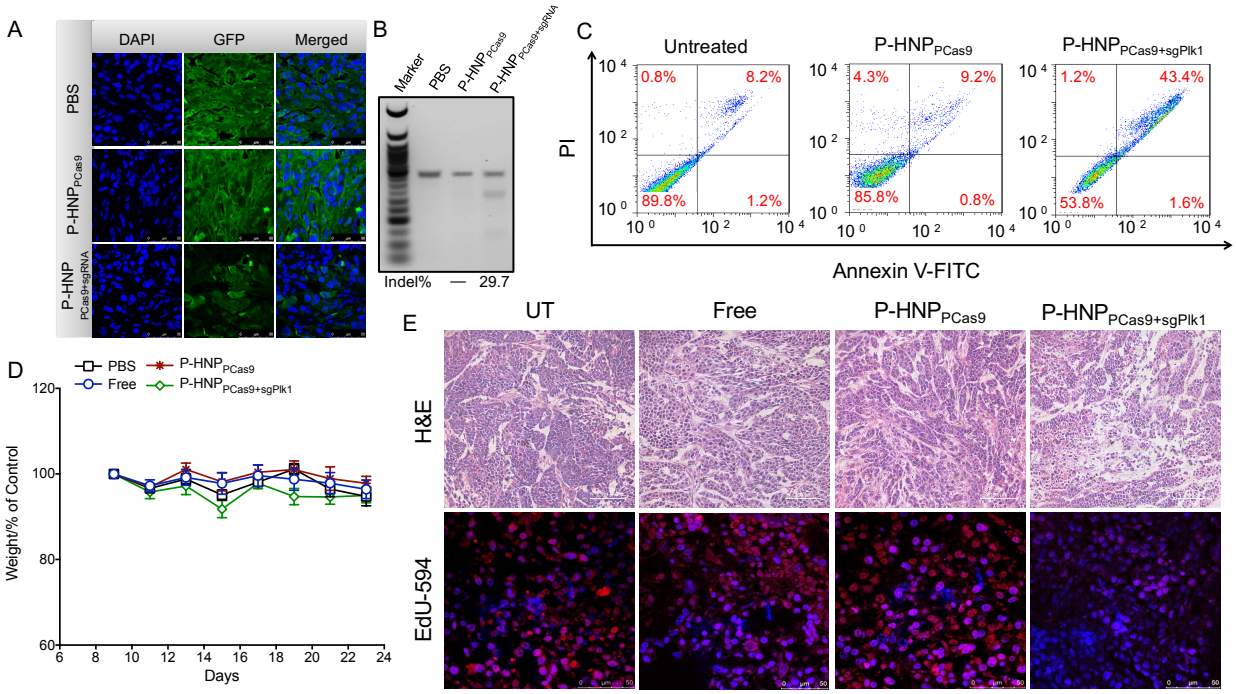

Fig. S7. *In vivo* gene disruption and tumor growth suppression. (A) CLSM images of A549.GFP tumor tissues after intratumoral injection of P-HNPs containing Cas9 expression plasmids only (P-HNP$_{PCas9}$) or P-HNPs containing Cas9 expression plasmids and GFP-targeting sgRNAs (P-HNP$_{PCas9+sgRNA}$). (Scale bar, 50 μm). (B) Indels of GFP gene in the tumor tissue in (A) as detected by the GCD assay. (C) Apoptosis of HeLa cells at 96 h after transfection with P-HNP$_{PCas9}$ or P-HNP$_{PCas9+sgPlk1}$ as determined by the Annexin-V/PI assay. (D) The relative body weight of HeLa xenograft tumor-bearing mice in Fig. 5C. (E) Representative H&E (Scale bar, 100 μm) and EdU-594 staining images (Scale bar, 50 μm) of tumor tissue after different treatments in (Fig. 5C). EdU-594-positive proliferating cells were stained red and cell nuclei were blue.

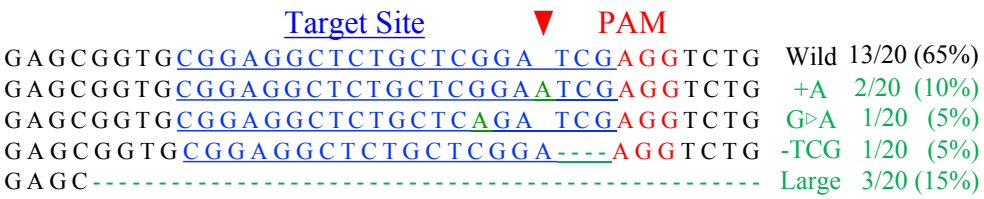

Fig. S8. Sequencing results of Plk1 gene in the tumor tissues following treatment with P-HNP$_{PCas9+sgPlk1}$ in (Fig. 5C).