Supporting Information

Efficient Delivery of mRNA Using Crosslinked Nucleic Acid Nanogel as A Carrier

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Materials

The reagents for DNA synthesis were purchased from DNAchem technology Co., Ltd. (Beijing, China). The Cy3 and Cy5 labelled DNA were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All other reagents and reagent-grade solvents were purchased from Tansoole Co., Ltd (Shanghai, China). PEI MAX (Mw 40 000 g/mol, 24765-1) was bought from Polyscience, Inc. (Warrington, PA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), trypsin-EDTA, and Lipofectamine 2000 were bought from Invitrogen (Carlsbad, CA). EGFP-mRNA (L-7201), Cy5-EGFP-mRNA (L-7701) and Cas9-mRNA (L-7206) were purchased from TriLink BioTechnologies, Inc. (San Diego, CA).

Experimental section.

S1. Synthesis of DNA oligonucleotides.

DNA oligonucleotides and fluorescence-labelled oligonucleotides were synthesized by ABI 3400 DNA/RNA synthesizer (Applied Biosystems), and then purified by reverse-phase high performance liquid chromatography (Waters 2535) with a C18 column (5 μm, 250 mm×19 mm, XBridge) using acetonitrile and 0.1 M triethylammonium acetate (TEAA) aqueous solution as the mobile phase (**Table S1**).

S2. Synthesis of DNA-grafted polycaprolactone (T_{20}-*g*-PCL) brushes.

Amino-DNA (T_{20}) (T_{20}-NH_2) was firstly modified with dibenzocyclooctyl (DBCO)-NHS. DNA (T_{20})-NH_2 (134 OD, 0.825 μmol, 1.0 eqv.) were mixed with DBCO-NHS (33.2 mg, 82.5 μmol, 100.0 eqv.) in DMSO/PBS (*v/v* 4:1) solutions, and the reaction mixture was shaking at rt for 24 h. Then the reaction solutions were extracted with Ethyl acetate and were concentrated by extraction with 2-butanol. DBCO-modified DNA (DBCO-DNA (T_{20})) was obtained after removing the water

(yield >95%). Ethyl-poly(α-N_3-εCL) (PCL-N_3, Mw = 5920 g/mol, each polymer contains ~40 azide groups) was first synthesized according to the literature.[1] Then, ultrapure water (66 μL) containing DBCO-DNA (T_{20}) (200 OD, 1.23 μmol) were mixed with dimethyl sulfoxide (DMSO, 2.64 mL) solution containing ethyl-poly(α-N_3-εCL) (121 μg, ~ 0.82 μmol -N_3), the reaction mixture was shaking at 50 °C for 48 h. The solvent was removed under vacuum, and centrifugal filtration units (Amicon Ultra-4 Ultracel, 30 kDa cutoff, Millipore) were used to remove the excess of DBCO-T_{20}. The grafting efficiency of DNA (T_{20}) to PCL was 91% as determined by UV-Vis spectrophotometer at 260 nm.

S3. Preparation of DNA nanogels (PEI-coated).

Typically, the mRNA-loaded nanogels (EGFP-mRNA was used as an example) were prepared by mixing 0.5 μL mRNA (1 mg/mL, ~3 μM) with 0.70 μL T_{20}-*g*-PCL brushes (71 μM) in 1×TAE/Mg^{2+} buffer (40 mM Tris, 2 mM EDTA•Na•H_2O, 20 mM acetic acid, 12.5 mM $(CH_3COO)_2Mg$•$4H_2O$), then the mixture was incubated at 25 °C for 30 min, followed by the addition of 0.75 uL DNA linker (10 μM, molar ratio, 30%) and incubation for another 30 min at 25 °C (total volume of 5.0 μL). Afterwards, PEI (0.5 mg/mL, 2.5 μL) solution in Tris buffer (pH 8.0, 10 mM) was added and the mixture was incubated at 25 °C for 5 min (with a total volume of 50 μL and mRNA concentration of 10 μg/mL, 30 nM as stock solution for subsequent experiments).

S4. Agarose gel electrophoresis.

S4.1. EGFP-mRNA loaded nanogels with different linker ratio.

The mRNA-loaded nanogels were prepared by mixing EGFP-mRNA (1 mg/mL, 0.5 μL) with T_{20}-*g*-PCL brushes (71 μM, 0.70 μL) in 1×TAE/Mg^{2+} buffer, the mixture was incubated at 25 °C for 30 min, followed by the addition of DNA linker (10 μM, 0.75 uL, molar ratio, 30%) and incubation for another 30 min at 25 °C (with a total volume of 5.0 μL and mRNA concentration of 0.1 mg/mL,

0.3 μM). The nanogels were analyzed by 0.5 % agarose gel (*w/w*) at 4 °C (100 V, constant voltage) in 1×TAE/Mg^{2+} buffer. The agarose gel containing 4S Red plus was visualized in GelRed channel by using a ChemiDoc MP imaging system (Biorad, Hercules, CA).

S4.2. PEI-coated nanogels with different PEI/mRNA ratio.

The PEI-coated nanogels were prepared by mixing Cy5-EGFP-mRNA (1 mg/mL, 0.5 μL) with T_{20}-*g*-PCL brushes (71 μM, 0.70 μL) in 1×TAE/Mg^{2+} buffer, then the mixture was incubated at 25 °C for 30 min, followed by the addition of FAM-labelled DNA linker (10 μM, 0.75 uL, molar ratio, 30%) and incubation for another 30 min at 25 °C (total volume of 5.0 μL). Afterwards, PEI (0.5 mg/mL, 1.0-2.5 μL) solution in Tris buffer (pH 8.0, 10 mM) was added and the mixture was incubated at 25 °C for 5 min (with a total volume of 50 μL and final mRNA concentration of 10 μg/mL, 30 nM as stock solution). The complex was then confirmed by agarose gel electrophoresis using 0.5% (*w/w*) agarose gel, which was then visualized in FAM and Cy5 channels using a ChemiDoc MP imaging system.

S4.3. Cas9-mRNA loaded nanogels.

The nanogels were prepared by mixing Cas9-mRNA (1 mg/mL, 0.5 μL) with T_{20}-*g*-PCL brushes (71 μM, 0.70 μL) in 1×TAE/Mg^{2+} buffer, then the mixture was incubated at 25 °C for 30 min, followed by the addition of DNA linker (10 μM, 0.75 uL, molar ratio, 30%) and incubation for another 30 min at 25 °C (total volume of 5.0 μL). Afterwards, PEI (0.5 mg/mL, 2.5 μL) solution in Tris buffer (pH 8.0, 10 mM) was added and the mixture was incubated at 25 °C for 5 min (with a total volume of 50 μL and final mRNA concentration of 10 μg/mL, 6.6 nM as stock solution for future use). The complex was then confirmed by agarose gel electrophoresis using 0.5% (*w/w*) agarose gel containing 4S Red plus, which was then visualized in GelRed channel using a ChemiDoc MP imaging system.

S5. Particle Size and ζ Potential of mRNA-bearing nanogels.

The EGFP-mRNA nanogels (30% linker ratio) with and without PEI coating (PEI/mRNA weight ratio 2.5:1) were prepared as described in Section S3. Size and ζ potential measurements were determined by dynamic light scattering (DLS), which were performed on Zetasizer Nano ZS Particle Size and Zeta Potential Analyzer (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). All of the size measurements were carried out at a fixed scattering angle of 90° and all samples were measured in triplicate.

S6. AFM and TEM imaging.

S6.1. AFM of mRNA-bearing nanogels.

Both EGFP-mRNA nanogels (30% linker ratio) with and without PEI coating (PEI/mRNA weight ratio 2.5:1) were prepared as described in Section S3. The obtained nanogels (3 μL) were spreaded onto the freshly cleaved mica surface (Electron Microscopy Sciences) and incubated for 30 s to allow nanogels to absorb onto the substrate. The sample drop was then washed off by 10 μL Tris buffer, and dried by compressed air. The nanogels were imaged in the air in tapping mode on a Multimode NanoscopeIIIa AFM.

S6.2. TEM of mRNA-bearing nanogels.

Both EGFP-mRNA nanogels (30% linker ratio) with and without PEI coating (PEI/mRNA weight ratio 2.5:1) were prepared as described in Section S3. 5 μL samples were spread on carbon-coated copper grids. Then the samples were stained by 2% phosphotungstic acid and imaged by transmission electron microscopy (TEM, Talos L120C G2, Thermo Fisher Scientific).

S7. Cell culture.

HeLa cells (human cervical carcinoma), HEK-293 cells (human embryonic kidney), HEK-293 reporter cells stably expressing EGFP and sgRNA that targeting EGFP were cultivated in

Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in humidified environment of 5% CO_2. Subcultivation was done every 2-3 days, and about 60% confluence of the cells was achieved before harvesting for cell experiments.

S8. Cell uptake studies.

S8.1. Uptake analyzed by flow cytometry.

S8.1.1. Uptake of nanogels with different incubation time.

The uptake of nanogels were assessed in HeLa cells. To track the nanogels, a FAM fluorophore labelled linker was used. The cells were seeded at a density of 40,000 cells/well into 24-well plates 24 h before uptake experiment. The medium was replaced by fresh Opti-MEM medium, the nanogels were added to the cells at a final linker-FAM and EGFP-mRNA concentration of 10 nM and 0.33 µg/mL (1.0 nM) (PEI/mRNA 2.5:1), respectively, and incubated for 0.5, 1, 2 and 4 h (PEI/mRNA 2.5:1). The cells were then washed twice with PBS, trypsinized with 0.05% of trypsin for 3 min, followed by the addition of complete medium to stop trypsinization. Then, the cells were collected and washed twice by centrifugation (3 min, 1000 rpm, 4 °C) and resuspended with 300 uL of PBS. Data for 5,000 cells were collected on a BD LSRFortessa Flow Cytometer (BD Biosciences, San Jose, CA) and were analyzed using the FlowJo software (Tree Star, Inc., Ashland, OR). Uptake efficiency was determined by normalizing the mean fluorescence intensity to the control cells.

S8.1.2. Uptake of nanogels in the presence of endocytosis inhibitors.

Ethylisopropyl amiloride (EIPA), chlorpromazine (CPZ) and filipin III are known to inhibit the macropinocytosis pathway, the clathrin-mediated pathway, and the caveolae-mediated pathway, respectively. The EIPA and filipin III were reported to significantly reduce the uptake of the

liposomes by inhibiting the micropinocytosis and caveolae-mediated pathways, and CPZ resulted in about 50% drop in the uptake of the calcium phosphate-based nanoparticles via blocking the clathrin-mediated pathway[2] The uptake of nanogels were assessed in HeLa cells. To track the nanogels, a Cy5 labelled EGFP-mRNA was used (PEI/mRNA weight ratio 2.5:1). The cells were seeded at a density of 60,000 cells/well into 24-well plates 24 h before uptake. The medium was replaced by fresh Opti-MEM medium, and the cells were pre-incubated with endocytosis inhibitors including EIPA (50 μM), CPZ (15 μM) and filipin III (0.5 μg/mL) for 30 min, then, the nanogels were added to the cells at a final Cy5-mRNA concentration of 0.67 μg/mL (2.0 nM) and incubated for 1 h. The collected cells were then analyzed by flow cytometry, and the relative uptake was determined by normalizing the uptake efficiency of cell with the inhibitors to the cells without inhibitors.

S8.2. Uptake analyzed by confocal laser scanning microscopy.

S8.2.1. Uptake of mRNA-bearing nanogels with different incubation time.

The uptake of nanogels were assessed in HeLa cells. The cells were seeded at a density of 60,000 cells/well into 24-well plates containing a clean coverslip in each well 24 h before uptake. To track the nanogels, a Cy5-labelled EGFP-mRNA and FAM labelled linker were used. The medium was replaced by fresh Opti-MEM medium, the nanogels were added to the cells at a final linker-FAM and Cy5-mRNA concentration of 10 nM and 0.67 μg/mL (2.0 nM) (PEI/mRNA weight ratio 2.5:1), respectively, and incubated for 0.5, 1, 2, and 4 h. Then the solutions were removed and the cells were washed with ice-cold PBS (×3), fixed with 4% formaldehyde for 20 min at rt. The fixed cells were further washed with ice-cold PBS (×3) and stained with Hoechst dye (blue) for 10 min. The slides were rinsed with PBS (×3), mounted and observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S8.2.2. Uptake of mRNA-bearing nanogels with long incubation time.

As the delivered EGFP-mRNA would gradually express the EGFP protein inside the cells, which may generate the green fluorescence and affect the tracking of FAM-labelled nanogel, we also use the Cas9-mRNA bearing nanogel for cellular uptake evaluation, especially for longer time incubation. Similarly, the uptake of nanogels were assessed in HeLa cells. The cells were seeded at a density of 60,000 cells/well into 24-well plates containing a clean coverslip in each well 24 h before uptake. Thereafter, FAM-labelled (on linker, 10 nM) and Cas9-mRNA (0.33 μg/mL, 0.22 nM) bearing nanogel with PEI coating (PEI/mRNA 2.5:1) were added to the cells and incubated for 2, 4, 8, and 24 h. Then the solutions were removed and the cells were washed with ice-cold PBS (×3), fixed with 4% formaldehyde for 20 min at rt. The fixed cells were further washed with ice-cold PBS (×3) and stained with Hoechst dye (blue) for 10 min. The slides were rinsed with PBS (×3), mounted, and observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S9. *In vitro* transfection.

S9.1. Transfection analyzed by flow cytometry.

S9.1.1. Transfection of nanogels with different PEI/mRNA ratios.

The transfection efficiency of EGFP-mRNA loaded nanogels with different PEI/mRNA ratios was evaluated in HeLa cells. The HeLa cells were seeded at a density of 40,000 cells/well into 24-well plates 24h before transfection. The medium was replaced by fresh Opti-MEM medium, and the nanogels with PEI/mRNA weight ratio from 1:1 to 4:1 were added to the cells at a final EGFP-mRNA concentration of 1.0 μg/mL (3.0 nM), and incubated for 4 h. Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. The cells were

analyzed by flow cytometry as described above and the transfection efficiency was defined as percentage of EGFP-positive cells.

S9.1.2. Transfection of different mRNA formulations.

The transfection of different mRNA formulations was evaluated in HeLa and HEK-293 cells. The HeLa and HEK-293 cells were seeded at a density of 40,000 cells/well into 24-well plates 24h before transfection. The medium was replaced by fresh Opti-MEM medium, the mRNA, mRNA/Nanogel, mRNA/PEI, mRNA/Nanogel/PEI, and mRNA/Lipo (Lipofectamine 2000) were added to the cells at a final EGFP-mRNA concentration of 1.0 μg/mL (3.0 nM) (PEI/mRNA 2.5:1), and incubated for 4 h. Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. The cells were analyzed by flow cytometry as described above and the transfection efficiency was defined as percentage of EGFP-positive cells.

S9.1.3. Transfection of nanogels with different amounts of mRNA.

The transfection efficiency of EGFP-mRNA loaded nanogels with different amounts of mRNA was evaluated in HeLa cells. The HeLa cells were seeded at a density of 40,000 cells/well into 24-well plates 24h before transfection. The medium was replaced by fresh Opti-MEM medium, the nanogels added to the cells at a final EGFP-mRNA concentration from 0.33 to 1.0 μg/mL (1.0-3.0 nM) (PEI/mRNA 2.5:1), and incubated for 4 h. Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. The cells were analyzed by flow cytometry as described above and the transfection efficiency was defined as percentage of EGFP-positive cells.

S9.1.4. Transfection of nanogels with different incubation time.

The transfection efficiency of Cy5-EGFP-mRNA loaded nanogels with different incubation time was evaluated in HeLa cells. The HeLa cells were seeded at a density of 40,000 cells/well into 24-well plates 24h before transfection. The medium was replaced by fresh Opti-MEM medium, the

nanogels added to the cells at a final Cy5-EGFP-mRNA concentration of 1.0 μg/mL (3.0 nM) (PEI/mRNA 2.5:1), and incubated for 4 h. Then, the medium was removed and fresh complete medium was added and incubated for 24, 48 and 72 h. The cells were analyzed by flow cytometry as described above and the transfection efficiency was defined as percentage of EGFP-positive cells.

S9.2. Transfection analyzed by confocal laser scanning microscopy.

S9.2.1. Transfection of different mRNA formulations.

The transfection of different mRNA formulations was evaluated in HeLa. The cells were seeded at a density of 40,000 cells/well into 24-well plates containing a clean coverslip in each well 24 h before transfection. The medium was replaced by fresh Opti-MEM medium, and the mRNA, mRNA/Nanogel, mRNA/PEI, mRNA/Nanogel/PEI, and mRNA/Lipo (Lipofectamine 2000) were added to the cells at a final EGFP-mRNA concentration of 1.0 μg/mL (3.0 nM) and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. After which, the solutions were removed and the cells were washed with ice-cold PBS (×3), fixed with 4% formaldehyde for 20 min at rt. The fixed cells were further washed with ice-cold PBS (×3) and stained with Hoechst dye (blue) for 10 min. The slides were rinsed with PBS (×3), mounted and observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S9.2.2. Transfection of nanogels with different amounts of mRNA.

The transfection efficiency of EGFP-mRNA loaded nanogels with different amounts of mRNA was evaluated in HeLa cells. The cells were seeded at a density of 40,000 cells/well into 24-well plates containing a clean coverslip in each well 24 h before transfection. The medium was replaced by fresh Opti-MEM medium, and the nanogels added to the cells at a final EGFP-mRNA

concentration from 0.33 to 1.0 μg/mL (1.0-3.0 nM), and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. After which, the solutions were removed, the cells were washed with ice-cold PBS (×3) and fixed with 4% formaldehyde for 20 min at rt. The fixed cells were further washed with ice-cold PBS (×3) and stained with Hoechst dye (blue) for 10 min. The slides were rinsed with PBS (×3), mounted and observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S9.2.3. Transfection of nanogels with Cy5 labelled EGFP-mRNA.

The transfection efficiency of Cy5-EGFP-mRNA loaded nanogels was evaluated in HeLa cells. The cells were seeded at a density of 40,000 cells/well into 24-well plates containing a clean coverslip in each well 24 h before transfection. The medium was replaced by fresh Opti-MEM medium, and the nanogels added to the cells at a final Cy5-EGFP-mRNA concentration of 1.0 μg/mL (3.0 nM), and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. After which, the solutions were removed, the cells were washed with ice-cold PBS (×3) and fixed with 4% formaldehyde for 20 min at rt. The fixed cells were further washed with ice-cold PBS (×3) and stained with Hoechst dye (blue) for 10 min. The slides were rinsed with PBS (×3), mounted and observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S9.2.4. Transfection of mRNA/Nanogel without PEI coating (high concentration).

The high concentration of mRNA-loaded nanogels without PEI coating were prepared by mixing mRNA (0.3 μg, 1.8 nM) with T_{20}-*g*-PCL brushes (10 μM) in 1×TAE/Mg^{2+} buffer (40 mM Tris, 2 mM EDTA•Na•H_2O, 20 mM acetic acid, 12.5 mM $(CH_3COO)_2Mg$•$4H_2O$), then the mixture was incubated at 25 °C for 30 min, followed by the addition of DNA linker (3 μM, molar ratio, 30%) and incubation for another 30 min at 25 °C (total volume of 500 μL). The transfection of the

nanogels was evaluated in HeLa. The cells were seeded at a density of 40,000 cells/well into 24-well plates containing a clean coverslip in each well 24 h before transfection. The medium was replaced by fresh Opti-MEM medium, and the mRNA/Nanogel was added to the cells at a final EGFP-mRNA concentration of 0.33 μg/mL (1.0 nM) and incubated for 4 h. Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. After which, the solutions were removed and the cells were washed with ice-cold PBS (×3), fixed with 4% formaldehyde for 20 min at rt. The fixed cells were further washed with ice-cold PBS (×3) and stained with Hoechst dye (blue) for 10 min. The slides were rinsed with PBS (×3), mounted and observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S9.3. Transfection analyzed by immunofluorescence microscopy.

S9.3.1. Transfection of EGFP-mRNA loaded nanogels.

The indirect immunofluorescence was carried out to characterize the transfection of nanogels in HeLa. The cells were seeded at a density of 40,000 cells/well into 24-well plates containing a clean coverslip in each well 24 h before uptake. The medium was replaced by fresh Opti-MEM medium, and the nanogels were added to the cells at a final EGFP-mRNA concentration of 1.0 μg/mL (3.0 nM), and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for another 24 h. The cells were fixed with 4% formaldehyde for 20 min at rt, permeabilized with 0.2% Triton X-100 (Beyotime, China) for 15 min and blocked with blocking solution (Beyotime, China) for 2 h at rt. Then, they were incubated with mouse anti-GFP (1:100, CST, MA) overnight at 4 °C and Cy3-conjugated secondary antibodies (1:400, Wuhan Servicebio technology, China) for 1 h at rt, and finally stained with Hoechst dye (Thermo Fisher Scientific, MA) for 5 min. The slides were rinsed with PBS (×3), mounted and observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S10. CRISPR/Cas9-mediated genome editing.

S10.1. Genome editing analyzed by flow cytometry.

S10.1.1. Genome editing mediated by different Cas9-mRNA formulations.

The genome editing mediated by different Cas9-mRNA formulations was evaluated in HEK-293 reporter cells stably expressing EGFP and sgRNA that targeting EGFP.[3] The HEK-293 cells were seeded at a density of 150,000 cells/well into 24-well plates (gelatin-coated) 24 h before transfection. The medium was replaced by fresh Opti-MEM medium, the mRNA, mRNA/Nanogel, mRNA/PEI, mRNA/Nanogel/PEI, and mRNA/Lipo (Lipofectamine 2000) were added to the cells at a final Cas9-mRNA concentration of 1.33 μg/mL (0.88 nM), and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for 24 h before the cells were subcultured in a 6-well plate and incubated for 72 h. The cells were analyzed by flow cytometry as described above and the genome editing efficiency was defined as percentage of EGFP-negative cells.

S10.1.2. Genome editing mediated by different amounts of Cas9-mRNA.

The genome editing mediated by different amounts of Cas9-mRNA was evaluated in HEK-293 reporter cells stably expressing EGFP and sgRNA that targeting EGFP. The HEK-293 cells were seeded at a density of 150,000 cells/well into 24-well plates (gelatin-coated) 24h before transfection. The medium was replaced by fresh Opti-MEM medium, the nanogels with concentration of Cas9-mRNA from 0.33 to 1.67 ug/mL (0.22-1.10 nM) (PEI/mRNA 2.5:1) were added to the cells, and incubated for 4 h. Then, the medium was removed and fresh complete medium was added and incubated for 24 h before the cells were subcultured in a 6-well plate and incubated for 72 h. The cells were analyzed by flow cytometry as described above and the genome editing efficiency was defined as percentage of EGFP-negative cells.

S10.2. Genome editing analyzed by confocal laser scanning microscopy.

S10.2.1. Genome editing mediated by different Cas9-mRNA formulations.

The genome editing mediated by different Cas9-mRNA formulations was evaluated in HEK-293 reporter cells stably expressing EGFP and sgRNA that targeting EGFP. The HEK-293 cells were seeded at a density of 150,000 cells/well into 24-well plates (gelatin-coated) 24h before transfection. The medium was replaced by fresh Opti-MEM medium, the mRNA, mRNA/Nanogel, mRNA/PEI, mRNA/Nanogel/PEI, and mRNA/Lipo (Lipofectamine 2000) were added to the cells at a final Cas9-mRNA concentration of 1.33 μg/mL (0.88 nM), and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for 24 h before the cells were subcultured in a 6-well plate and incubated for 72 h. The cells were again subcultured into glass bottom dishes (D35C4-20-1-N, CellVis, CA, gelatin-coated) for 24 h. After which, the solutions were removed and the cells were stained with Hoechst dye for 5 min. The cells were rinsed with PBS and fresh medium was added before they were observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S10.2.2. Genome editing mediated by different amounts of Cas9-mRNA.

The genome editing mediated by different Cas9-mRNA formulations was evaluated in HEK-293 reporter cells stably expressing EGFP and sgRNA that targeting EGFP. The HEK-293 cells were seeded at a density of 150,000 cells/well into 24-well plates (gelatin-coated) 24h before transfection. The medium was replaced by fresh Opti-MEM medium, the nanogels with concentration of Cas9-mRNA from 0.33 to 1.67 ug/mL (0.22-1.10 nM) (PEI/mRNA 2.5:1) were added to the cells, and incubated for 4 h. Then, the medium was removed and fresh complete medium was added and incubated for 24 h before the cells were subcultured in a 6-well plate and incubated for 72 h. The cells were again subcultured into glass bottom dishes (D35C4-20-1-N,

CellVis, CA, gelatin-coated) for 24 h. After which, the solutions were removed and the cells were stained with Hoechst dye for 5 min. The cells were rinsed with PBS and fresh medium was added before they were observed by a laser scanning confocal microscope (Leica TCS SP8 STED 3X).

S10.3. Genome editing analyzed by western blot assay.

S10.3.1. Genome editing mediated by different Cas9-mRNA formulations.

The genome editing mediated by different Cas9-mRNA formulations was evaluated in HEK-293 reporter cells stably expressing EGFP and sgRNA that targeting EGFP. The HEK-293 cells were seeded at a density of 150,000 cells/well into 24-well plates (gelatin-coated) 24h before transfection. The medium was replaced by fresh Opti-MEM medium, the mRNA, mRNA/Nanogel, mRNA/PEI, mRNA/Nanogel/PEI, and mRNA/Lipo (Lipofectamine 2000) were added to the cells at a final Cas9-mRNA concentration of 1.33 μg/mL (0.88 nM), and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for 24 h before the cells were subcultured in a 6-well plate and incubated for 72 h. Thereafter, the cells were washed with PBS (×3) and lysed with 500 μL lysis buffer containing 1×protease and phosphatase inhibitor (Beyotime, China). The cells lysates were incubated on ice for 30 min and shocked repeatedly, followed by centrifugation (12000 rpm, 10 min, 4 °C). The protein concentrations were determined by the BCA protein assay kit (Thermo Fisher Scientific, MA). The lysates containing same amount of total protein were incubated with equal volume of dithiothreitol (DTT)-containing loading buffer. The samples boiled for 5 min and separated by 4-20% Precast gradient gel (Wuhan Servicebio technology, China). The whole gel was transferred to Immobilon-P membrane (ISEQ00010, Millipore), which was incubated in PBST (5% BSA) for 2 h. The membrane was then incubated in PBS (1% BSA) with primary antibodies against GAPDH (Wuhan Servicebio technology, China), EGFP (CST, MA) overnight, followed by incubating in

PBS (1% BSA) with secondary antibodies (1:3000) (HRP-linked antibody, CST, MA) diluted in TBST for 30 min.

S10.4. Genomic mutations analyzed by DNA sequencing.

S10.4.1. Genome editing mediated by Cas9-mRNA loaded nanogels.

To detect the genomic mutations mediated by Cas9-mRNA loaded nanogels. The HEK-293 reporter cells were seeded at a density of 150,000 cells/well into 24-well plates (gelatin-coated) 24h before transfection. The medium was replaced by fresh Opti-MEM medium, and then mRNA/Nanogel/PEI was added to the cells at a final Cas9-mRNA concentration of 1.33 μg/mL (0.88 nM) and incubated for 4 h (PEI/mRNA 2.5:1). Then, the medium was removed and fresh complete medium was added and incubated for 24 h before the cells were subcultured in a 6-well plate and incubated for 72 h. Genomic DNA of HEK-293 (EGFP) cells was collected using GeneJET Genomic DNA Purification Kit (Thermo Scientific, MA) according to manufacturer's instructions. The gRNA targeted genomic locus was amplified with Phusion Hot Start II High Fidelity DNA Polymerase (NEB) using primers (**Table S1**). Touchdown PCR program ((98 °C for 10 s; 66-56 °C with -1 °C/cycle for 15s, 72 °C for 60 s) for 10 cycles and (98 °C for 10 s, 56 °C for 15 s, 72 °C for 60 s) for 25 cycles) was applied to reduce non-specific amplifications. Gel extraction purified PCR amplicons were cloned to the DNA sequencing vectors (Zero Blunt TOPO, Life Technologies, CA). The cloned plasmids were purified by GeneJET Plasmid Miniprep Kit (Thermo Scientific, MA) and sequenced by Sangon Biotech (Shanghai, China).[4]

S11. Cell viability assay.

The viability of cells exposed to the nanogels was determined by a Cell Counting Kit-8 (CCK-8) assay according to the manufacturer's protocol. HeLa cells, cells were seeded in a 96-well plate at a density of 10 000 cells/well in 100 μL complete medium and cultured for 24 h. The medium was

then replaced by 100 μL Opti-MEM, and nanogels with concentration of Cas9-mRNA from 0.33 to 3.33 ug/mL (0.22-2.20 nM) (PEI/mRNA 2.5:1) were added to the wells. After 4 h incubation, the medium was removed and fresh complete medium was added and incubated for 48 h. Then, the medium was replaced with 100 μL complete medium plus 20 μL CCK-8 solution for about 2 h's incubation. The absorbance was measured at 450 nm with a plate reader.

S12. Statistical Analysis

The one-way ANOVA test combined with Tukey's post-hoc test was applied for the pairwise comparison between multiple groups. $p < 0.05$ was considered statistically significant in all analyses.

Supporting results

Table S1. DNA sequences used in this study.

DNA	sequences (5'->3')	Notes
DNA (T_{20})-NH_2	TTT TTT TTT TTT TTT TTT TT-NH_2	Modified with DBCO and conjugated to PCL to produce T_{20}-*g*-PCL
Linker: Strand a	AAA AAA AAA AAA AAA AAA AAG ATT GCC TAA GTG CGT ATC A	Cross-linker
Linker: Strand b	AAA AAA AAA AAA AAA AAA AAT GAT ACG CAC TTA GGC AAT C	Cross-linker
Linker-FAM: Strand a	AAA AAA AAA AAA AAA AAA AAG ATT GCC TAA GTG CGT ATC A-FAM	FAM fluorophore labelling
Linker-FAM: Strand b	AAA AAA AAA AAA AAA AAA AAT GAT ACG CAC TTA GGC AAT C-FAM	FAM fluorophore labelling
sgRNA-EGFP	GGG CGA GGA GCT GTT CAC CGG TTT TAG AGC TAG AAA TAG CAA GTT AAA ATA AGG CTA GTC CGT TAT CAA CTT GAA AAA GTG GCA CCG AGT CGG TGC TTT TTT T	EGFP targeting sequence
CMV-F[a]	CGC AAA TGG GCG GTA GGC GTG	EGFP PCR primer (CMV promoter primer)
EGFP-R	AAG CAC TGC ACG CCG TAG GT	EGFP PCR primer

a, CMV promoter located before the EGFP sequence, and a CMV promoter primer CMV-F was used for the PCR of EGFP.

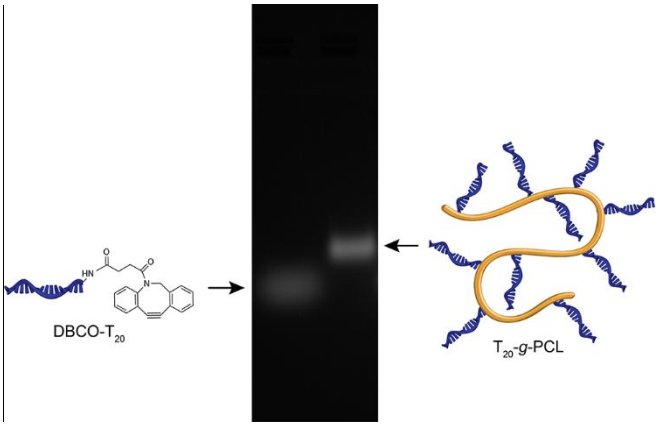

Figure S1. Characterization of T_{20}-*g*-PCL by 1.0 % agarose gel electrophoresis in native condition.

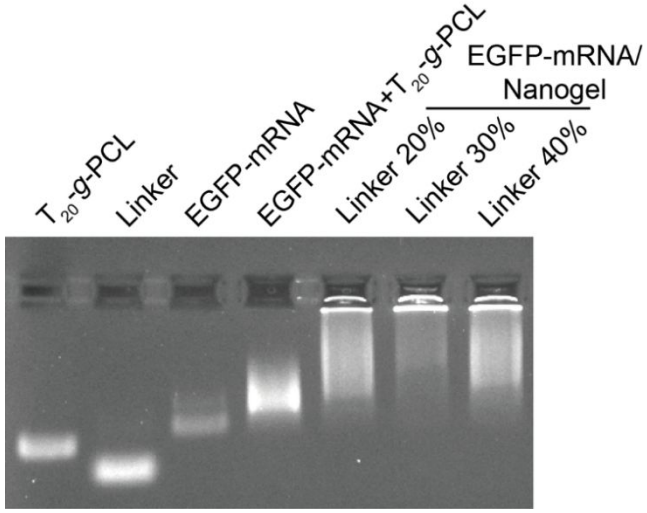

Figure S2. 1.0 % agarose gel electrophoresis of EGFP-mRNA loaded nanogels with different linker ratio. Molar ratio of mRNA to brush DNA (in T_{20}-*g*-PCL) is 3:100.

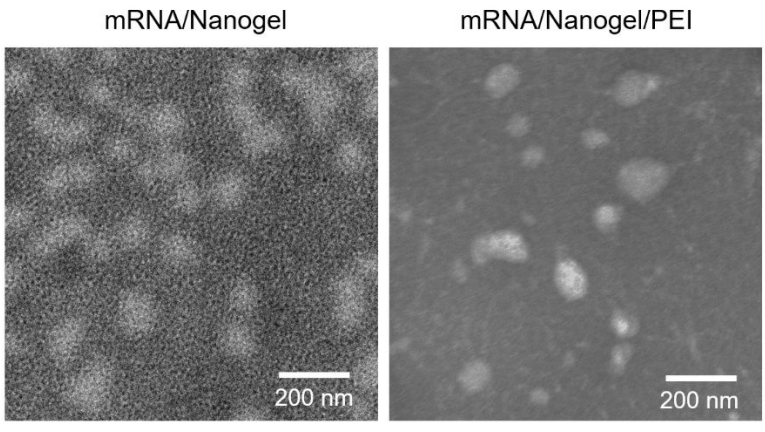

Figure S3. TEM images of the EGFP-mRNA loaded nanogels with and without PEI coating (stained by 2% phosphotungstic acid).

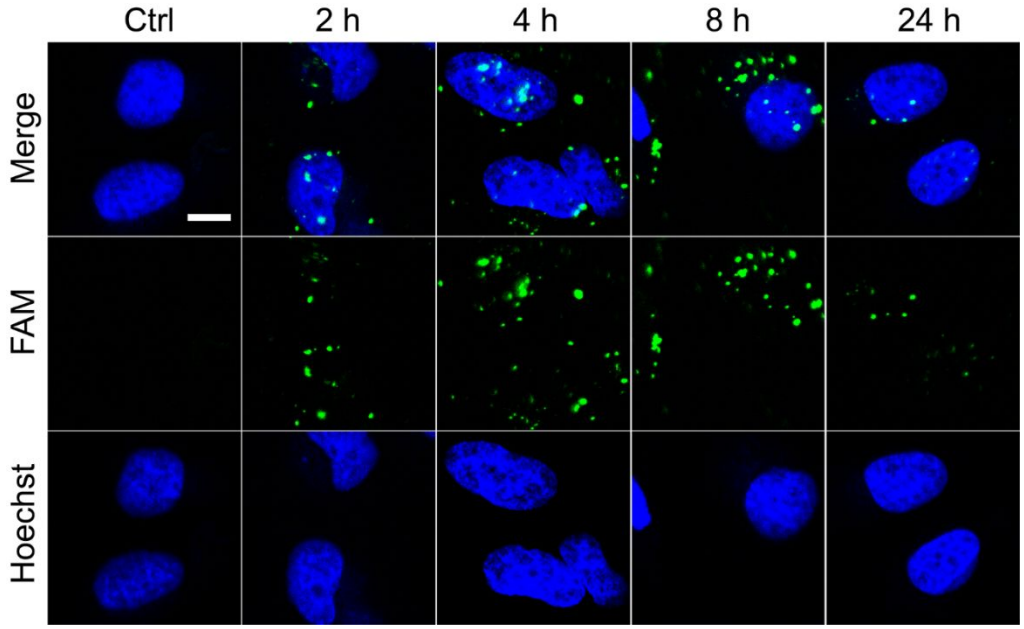

Figure S4. CLSM images of HeLa cells incubated with PEI-coated Cas9-mRNA bearing nanogels for different time (up to 24 h). The weight ratio of PEI to Cas9-mRNA is 2.5:1 and the concentration of mRNA is 0.33 μg/mL (0.22 nM). The linker was labelled with FAM (10 nM, green) with a linker ratio of 30%. Cell nuclei were stained with Hoechst (blue). Scale bars: 10 μm.

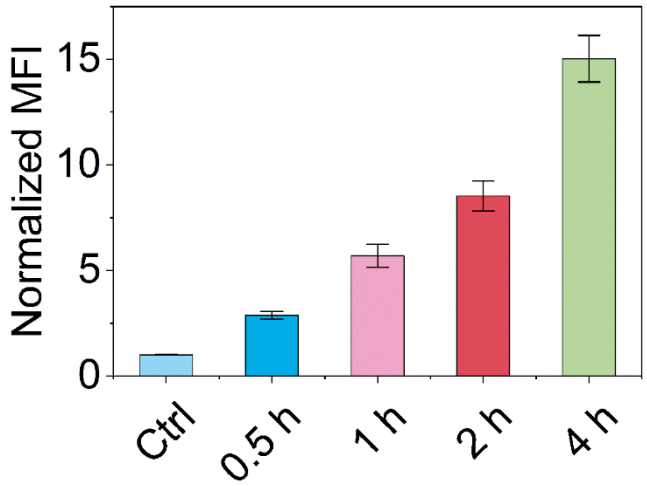

Figure S5. Flow cytometry analysis of HeLa cells incubated with PEI-coated nanogels for different time. The weight ratio of PEI to EGFP-mRNA is 2.5:1 and the concentration of mRNA is 0.33 μg/mL (1.0 nM). The linker was labelled with FAM (10 nM) with a linker ratio of 30%. The mean fluorescence intensity (MFI) of the cells was normalized to untreated cells. Data are presented as means ± SD (n = 3).

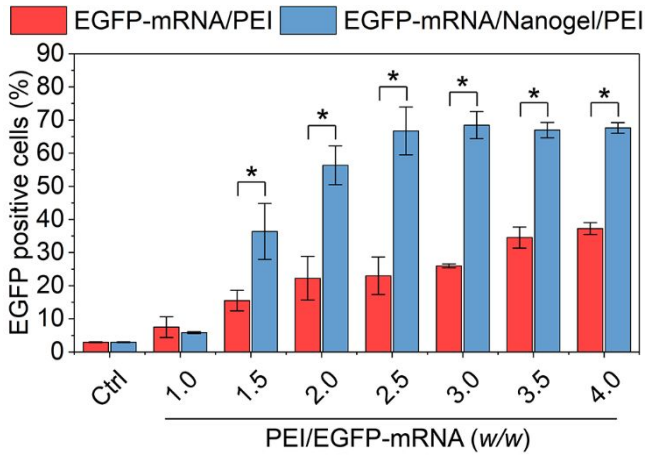

Figure S6. Transfection of nanogels with different PEI/mRNA ratio (*w/w*) in HeLa cells, EGFP-mRNA (1.0 μg/mL, 3.0 nM) was used. The linker ratio is 30%. Transfection efficiency was determined as the percentage of EGFP-positive cells within the total of cells analyzed by flow cytometry. Data are presented as means ± SD (n = 3). **p* < 0.05.

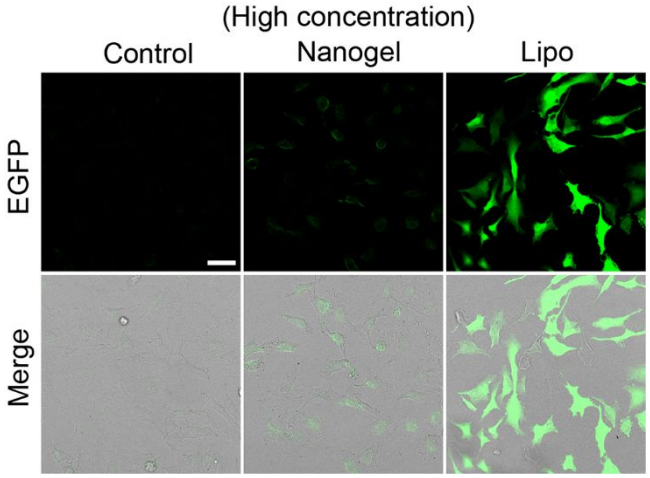

Figure S7. CLSM images of HeLa cells treated with EGFP-mRNA loaded nanogels without PEI coating. The concentration of nanogels used for transfection is 167 times higher than that of the PEI-coated EGFP-bearing nanogels used in this work. The linker ratio is 30%. Scale bar: 75μm.

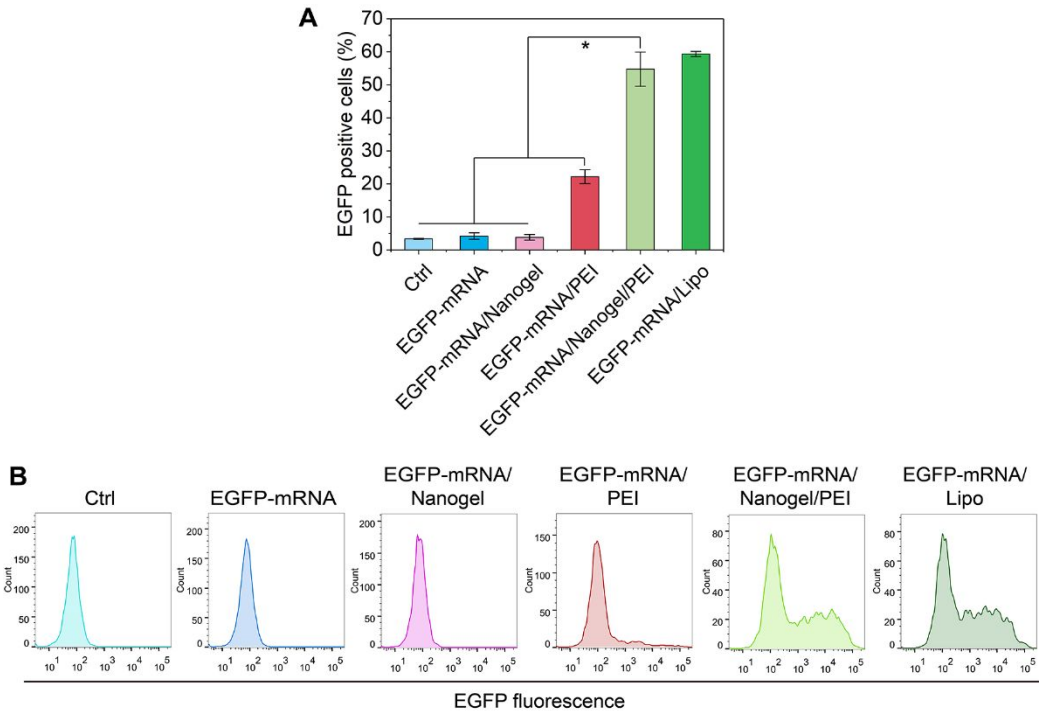

Figure S8. Flow cytometry analysis of HEK-293 cells treated with EGFP-mRNA (1.0 μg/mL, 3.0 nM), mRNA/Nanogel, mRNA/PEI, mRNA/Nanogel/PEI or mRNA/Lipo 2000, EGFP-mRNA was used. The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%. (A) Transfection efficiency was determined as the percentage of EGFP-positive cells within the total of cells. Data are presented as means ± SD (n = 3). *$p < 0.05$. (B) Flow cytometry histogram of cells treated with different mRNA formulations.

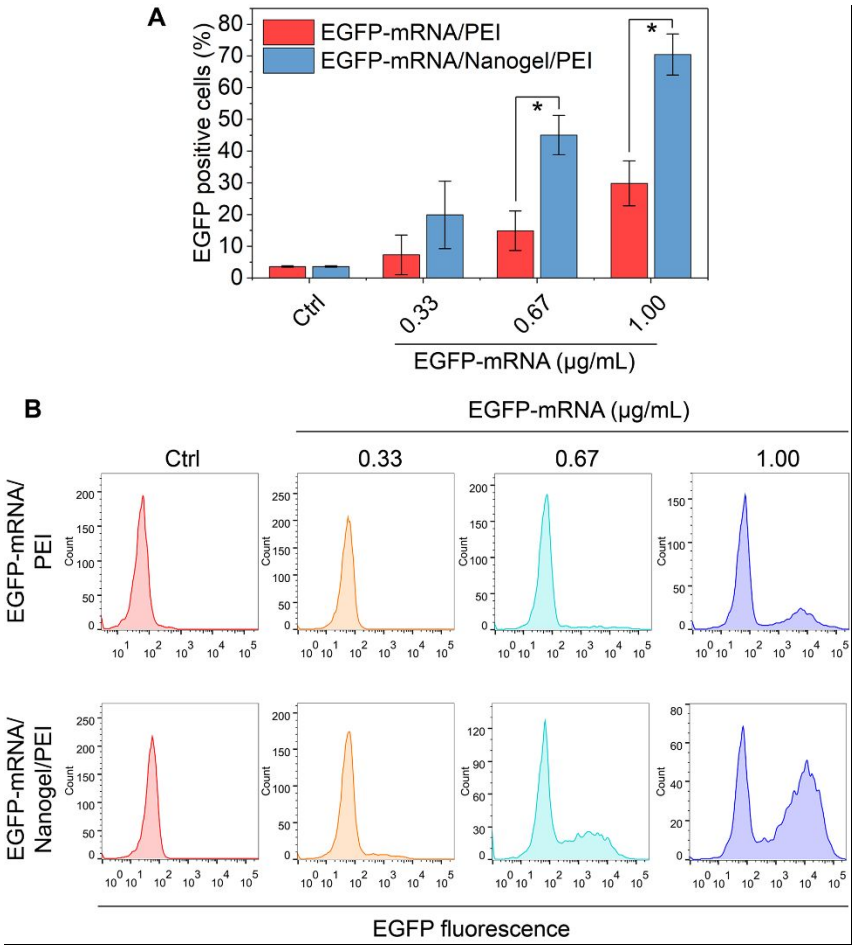

Figure S9. Transfection of PEI-coated nanogels containing different amounts of EGFP-mRNA in HeLa cells (EGFP-mRNA 0.33-1.0 μg/mL, 1.0-3.0 nM). The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%. (A) Transfection efficiency was determined as the percentage of EGFP-positive cells within the total of cells analyzed by flow cytometry. Data are presented as means ± SD (n = 3). *$p < 0.05$. (B) Flow cytometry histograms of cells treated with mRNA/Nanogel/PEI containing different amounts of EGFP-mRNA.

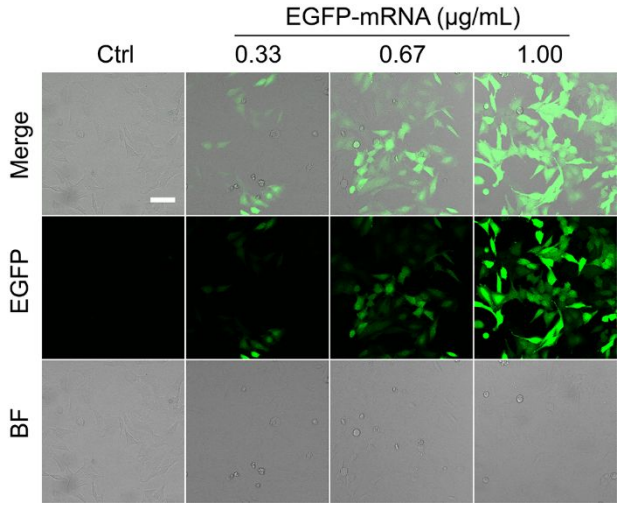

Figure S10. CLSM images of HeLa cells treated with mRNA/Nanogel/PEI containing different amounts of EGFP-mRNA (EGFP-mRNA concentration: 0.33-1.0 μg/mL, 1.0-3.0 nM). The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%. Scale bar: 75 μm.

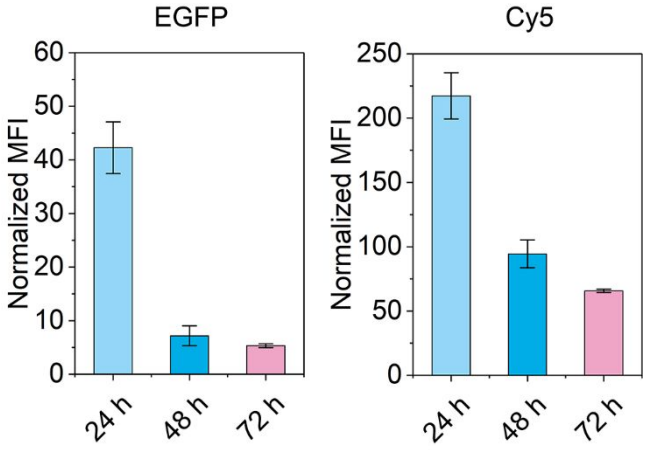

Figure S11. The kinetics of EGFP expression of Cy5-EGFP-mRNA (1.0 μg/mL, 3.0 nM) delivered by DNA nanogels coated with PEI in HeLa cells. The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%. The mean fluorescence intensity (MFI) of the treated cells (Cy5 and EGFP) were normalized to the untreated cells. Data are presented as means ± SD (n = 3).

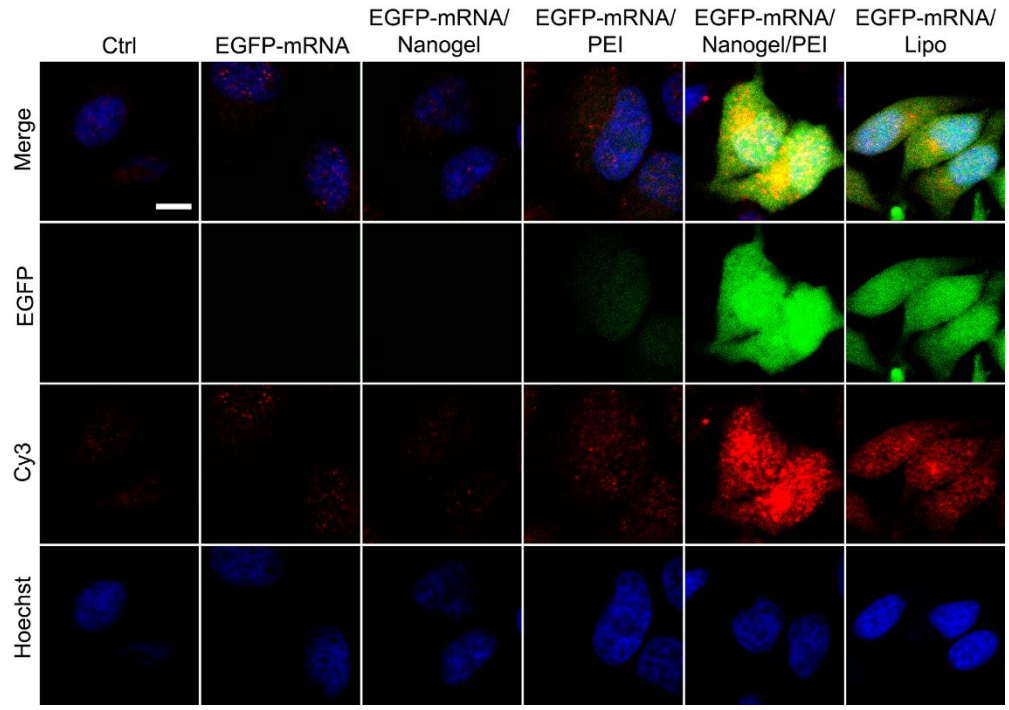

Figure S12. Immunofluorescent staining of HeLa cells treated with different EGFP-mRNA (1.0 μg/mL, 3.0 nM) formulations using antibodies (Cy3 labelled) against EGFP (red). The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%. Cell nuclei were stained with Hoechst 33342 (blue). Scale bar: 10 μm.

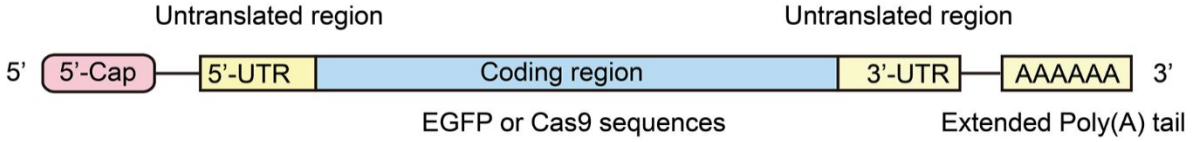

Figure S13. Representative structure of the EGFP or Cas9-encoding mRNA.

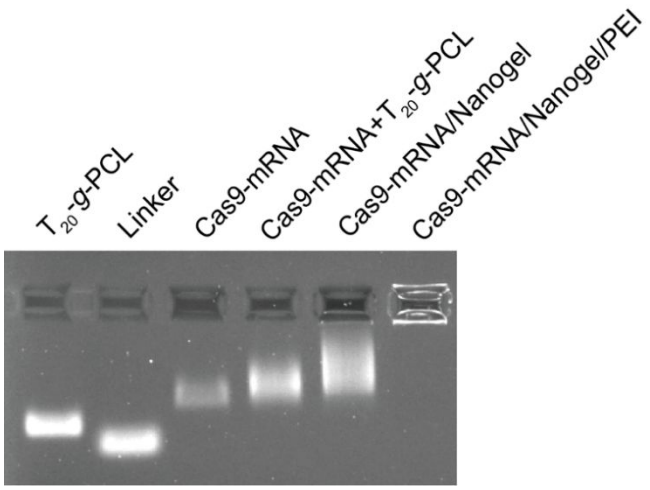

Fig. S14. 0.5 % agarose gel electrophoresis of Cas9-mRNA-loaded nanogels. Molar ratio of mRNA to brush DNA (in T_{20}-*g*-PCL) is 1:100. The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%.

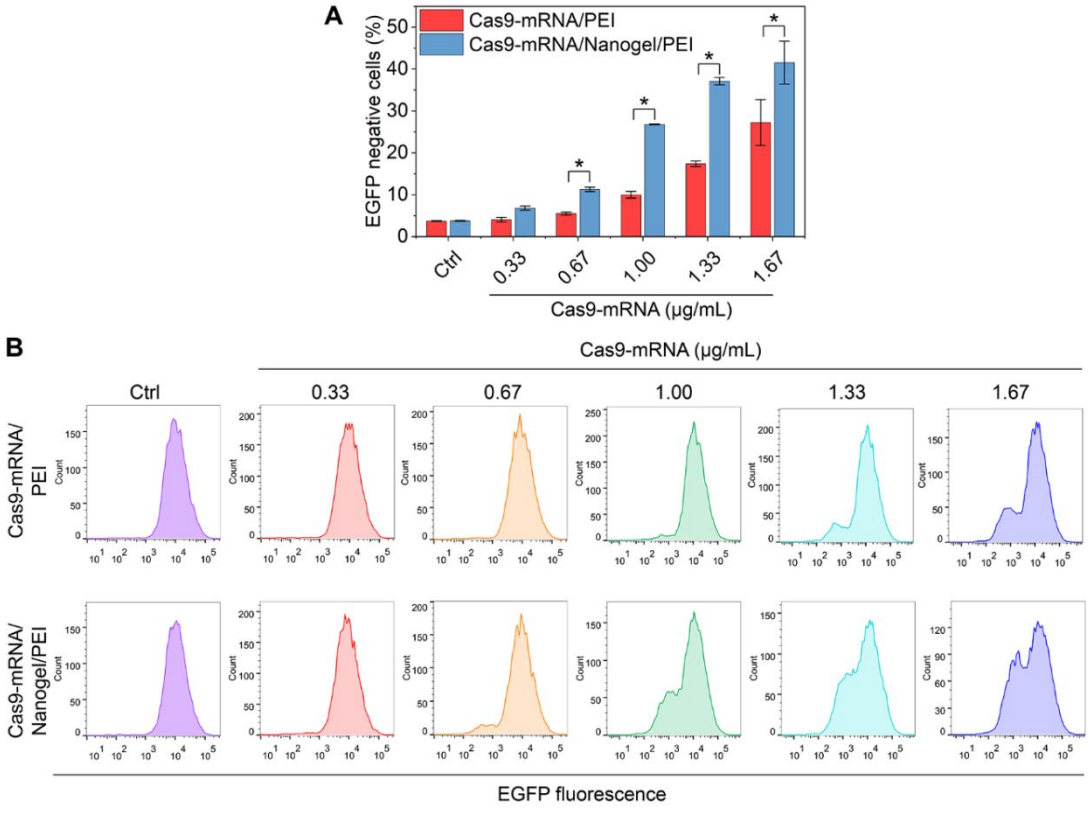

Figure S15. Flow cytometry analysis of EGFP-293 cells treated with mRNA/Nanogel/PEI containing different amounts of Cas9-mRNA (mRNA 0.33-1.67 μg/mL, 0.22-1.10 nM). The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%. (A) The gene editing efficiency was determined as the percentage of EGFP-negative cells within the total of cells. Data are presented as means ± SD (n = 3). *$p < 0.05$. (B) Flow cytometry histograms of cells treated with mRNA/Nanogel/PEI containing different amounts of Cas9-mRNA.

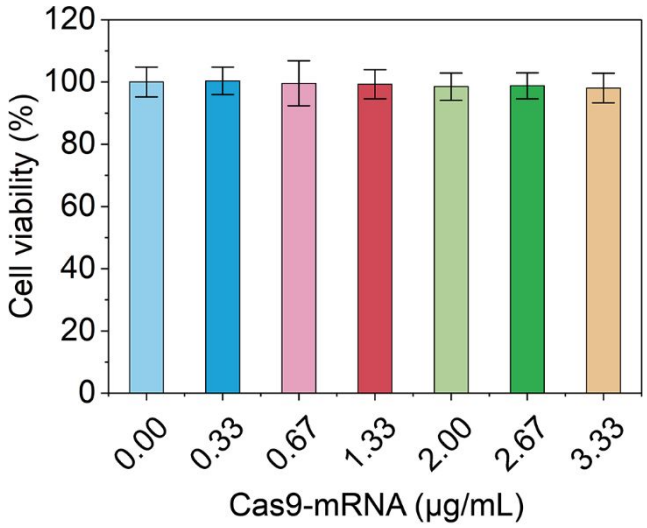

Fig. S16. Cell viability assays of HeLa cells treated with PEI-coated nanogels containing different amounts of Cas9-mRNA (mRNA concentration: 0.33-3.33 μg/mL, 0.22-2.20 nM). The weight ratio of PEI to mRNA is 2.5:1 and the linker ratio is 30%. Data are presented as means ± SD (n = 3).

References of supporting information

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