Supporting Information

Complex Coacervation-Integrated Hybrid Nanoparticles Increasing Plasmid DNA Delivery Efficiency *in vivo*

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Materials

PEG_{5K}-NH_2, branched PEI_{10K}, β-CD, *p*-toluenesulfonyl chloride, 1-adamantanecarbonyl chloride, L-aspartic acid β-benzyl ester, triphosgene, hexane, tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were purchased from VWR (Radnor, PA). Cy7.5 NHS ester was obtained from Lumiprobe company.

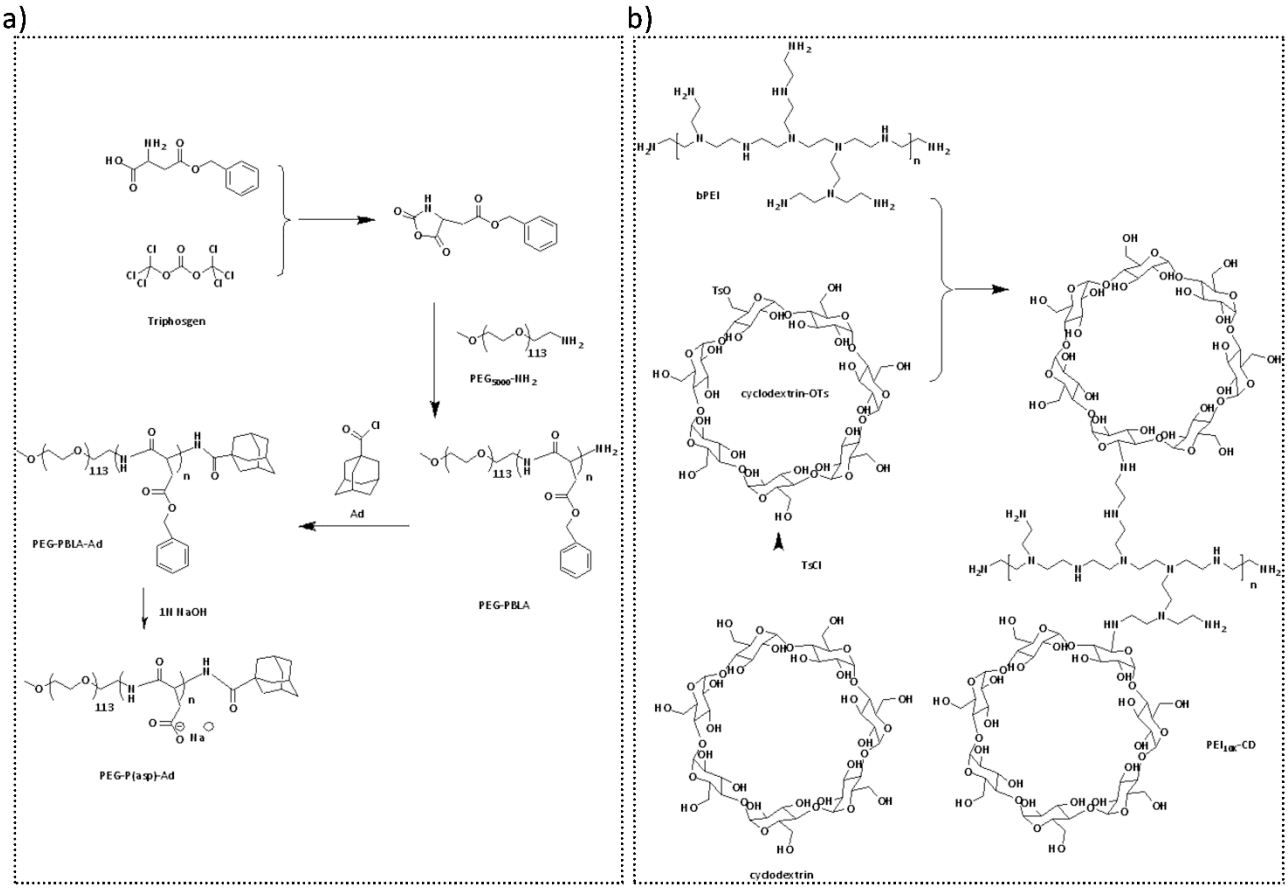

Figure S1. The synthesis scheme for PEG-P(asp)-Ad (a) and PEI_{10K}-CD (b).

Synthesis and characterization of PEG-P(asp)-Ad

The polymer building block PEG-P(asp)-Ad was synthesized via a three-step reaction (Figure S1a). The monomer β-benzyl-L-aspartate N-carboxy-anhydride (BLA-NCA) was synthesized through Fuchs-Farthing method. First, L-aspartic acid β-benzyl eater was cyclized with triphosgene, followed with purification by recrystallization in THF/hexane. Next, amphiphilic polymer

PEG-PBLA was synthesized by the ring-opening polymerization of BLA-NCA initiated by the terminal -NH_2 of PEG-NH_2 as previously described[1]. More specifically, PEG_{5K}-NH_2 was used as a macro-initiator to polymerize BLA-NCA, to yield amine-terminated PEG-PBLA-NH_2. Finally, PEG-PBLA-Ad was prepared by the terminal –NH_2 of PEG-PBLA reacting with 1.1 equivalent 1-Adamantanecarbonyl chloride in anhydrous DMF. The terminal amino groups were substituted by AD groups. The PBLA's degree of polymerization of PEG-PBLA-Ad was finally determined to be 20 by ^{1}H NMR (Figure S2). Chemical shifts were described in ppm, with respect to the deuterated solvent ($CDCl_3$) used.

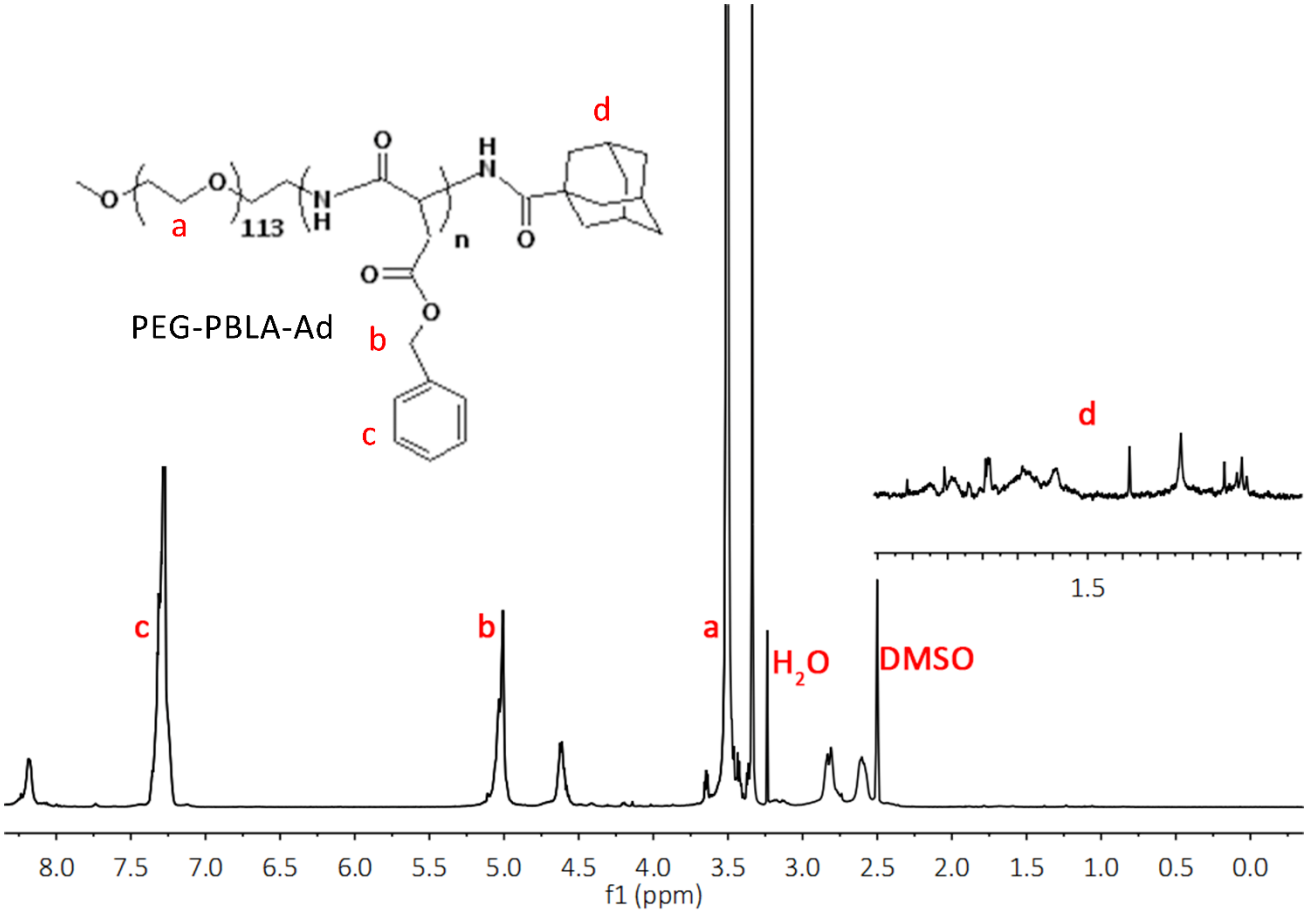

Figure S2. The ^{1}H NMR characterization of PEG-PBLA-Ad.

PEG-P(asp)-Ad was obtained by removing the benzyl groups through the hydrolysis of

PEG-PBLA-Ad in 1N NaOH, and then characterized by ^{1}H NMR. To obtain the final polymer, the benzyl groups on the side chains of PBLA block were removed by an aqueous NaOH solution. The polymer was then dialyzed against double distilled water to remove excess DMF. ^{1}H NMR of the resulting PEG-P(asp)-Ad (Figure S3) revealed that the level of side chain hydrolysis was 100% complete based on the signal disappearance of the aryl protons in the benzyl groups of PBLA. ^{1}H NMR spectra were obtained with an Agilent DDR2 500 MHz NMR. Chemical shifts were described in ppm, with respect to the deuterated solvent (D_2O) used. The polymer of PEG-P(asp)-Ad was estimated to have a total molecular weight of 7700.

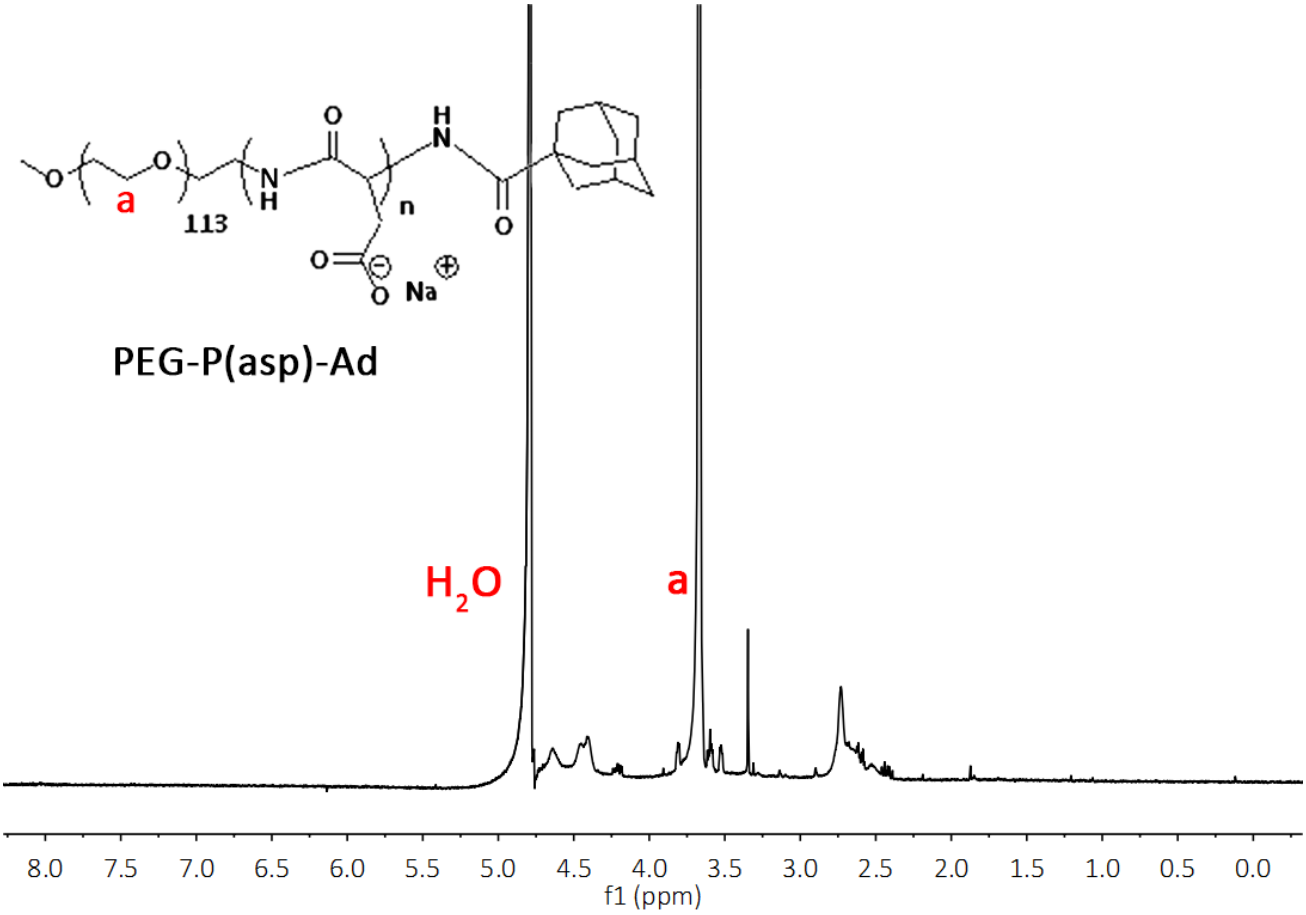

Figure S3. The ^{1}H NMR characterization of PEG-P(asp)-Ad.

Synthesis and characterization of PEI_{10K}-CD

The synthesis of molecular building block PEI_{10K}-CD was prepared by two successive chemical processes (Figure S1b). Firstly, mono-6-deoxy-6-(p-tolylsulfonyl)-β-cyclodextrin (CD-OTs) was synthesized according to the method as described in the literature[2]. Secondly using CD-OTs and PEI_{10K}, PEI_{10K}-CD was successfully synthesized according to the method reported in the literature[3], and characterized by ^1H NMR (Agilent DDR2 500 MHz NMR). Chemical shifts were described in ppm, with respect to the deuterated solvent (D_2O) used. The CD/PEI_{10K} ratio in a PEI_{10K}-CD molecule was calculated based on the proton integration of C_1H of CD versus CH_2 of PEI_{10K}. There were approximately 7-8 CD recognition units finally grafted on a branched PEI_{10K} backbone according to its ^1H NMR spectrum: δ4.92 (C_1H of CD), 2.3-3.0 (CH_2 of PEI_{10K}) (Figure S4). The polymer of PEI_{10K}-CD was estimated to have a total molecular weight of 18000.

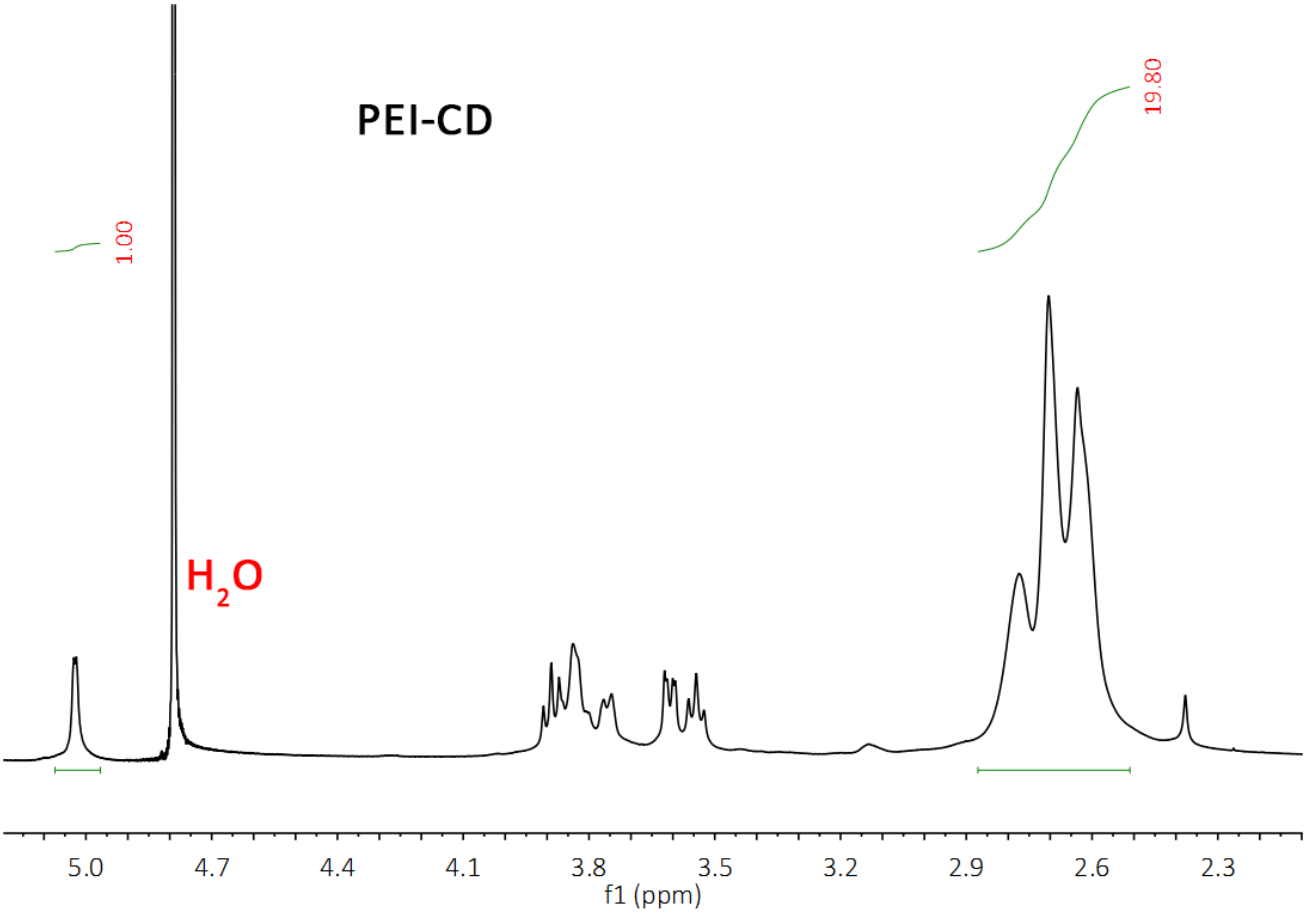

Figure S4. The ^1H NMR characterization of PEI_{10K}-CD.

Synthesis and characterization of PEG-Ad

The polymer of PEG-Ad was synthesized according to Figure S5a. Briefly, 500mg of PEG-NH_2 was dissolved in 3 mL of DMF and then 1.5 equivalent of 1-adamantanecarbonyl chloride was added to the system, which used K_2CO_3 as acid-binding agent. The reaction solution was stirred under 35℃ overnight and then poured into 15 mL of ddH_2O. The excessive 1-adamantanecarbonyl chloride will be removed by dialysis (MWCO 3500) and filtration. After lyophilization, the final product was obtained as yellow white powder and characterized by ^{1}H NMR (Agilent DDR2 500 MHz NMR) as shown in Figure S5b. Chemical shifts were described in ppm, with respect to the deuterated solvent (D_2O) used.

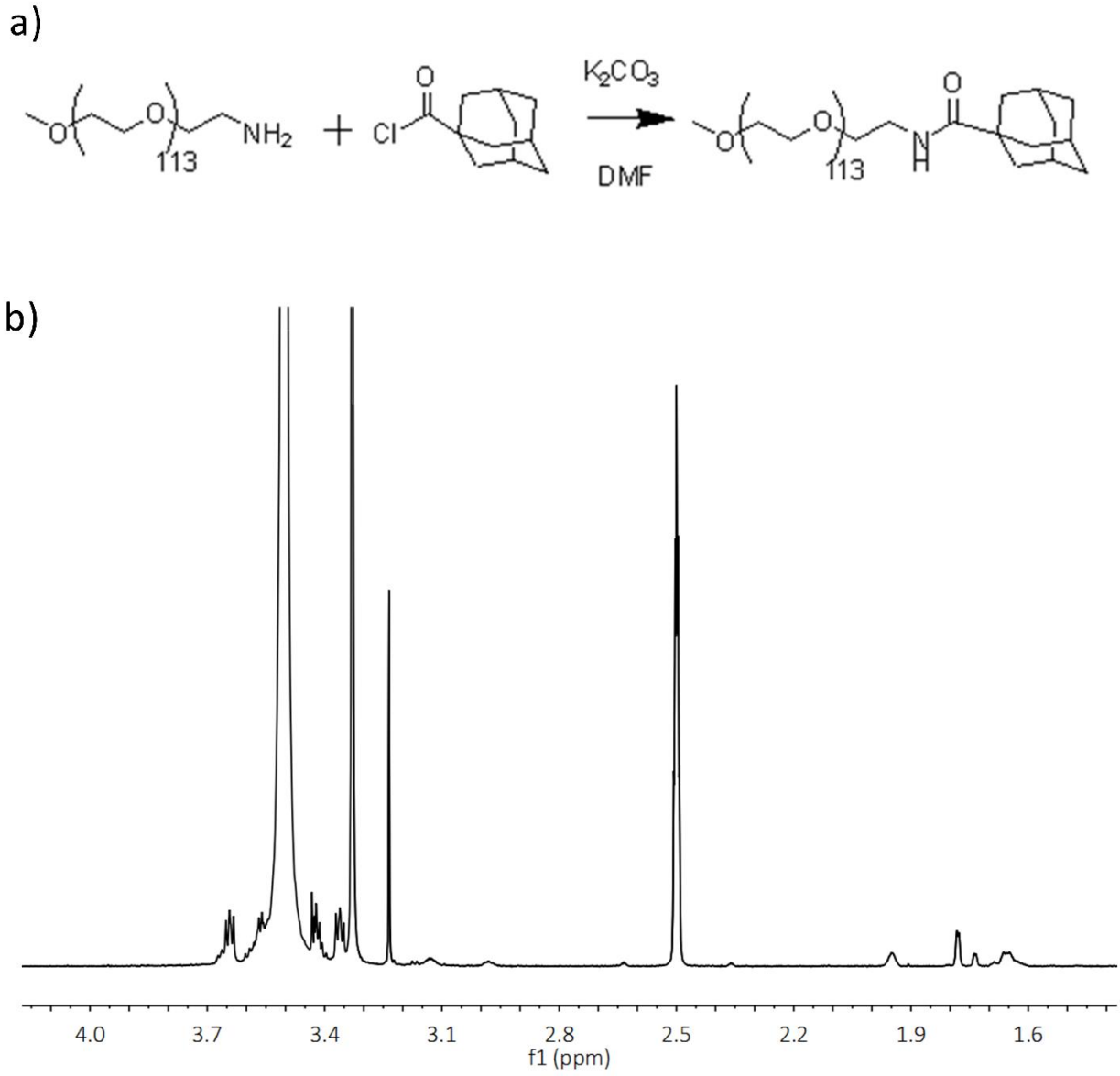

Figure S5. The synthesis scheme (a) and ^{1}H NMR characterization (b) of PEG-Ad.

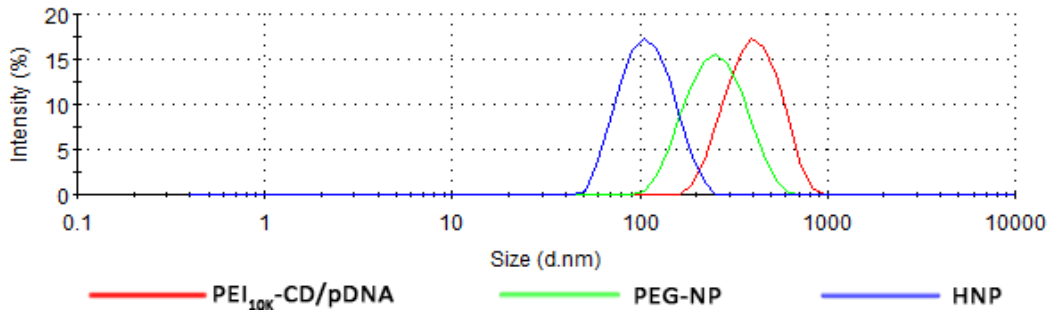

Figure S6 The particle size comparison among PEI_{10K}-CD/pDNA, PEG-NP and HNP in 0.9% saline solution

Synthesis of cy7.5-labeled PEI_{10K}

For cy7.5 conjugation with PEI_{10K}, we referred to the method previously reported[4]. Briefly, 300 mg of PEI_{10K} was first dissolved in 3 mL of anhydrous DMF. Then, 3 mg of cy7.5 NHS ester in 3 mL of anhydrous DMF was added. The reaction mixture was stirred at room temperature for two days. The polymer conjugates were purified by dialysis (MWCO 6000-8000) to remove DMF and centrifugation (15000 g, 15 min) to remove the free dye molecules. The resulting polymer conjugates were lyophilized and stored at -20 °C.

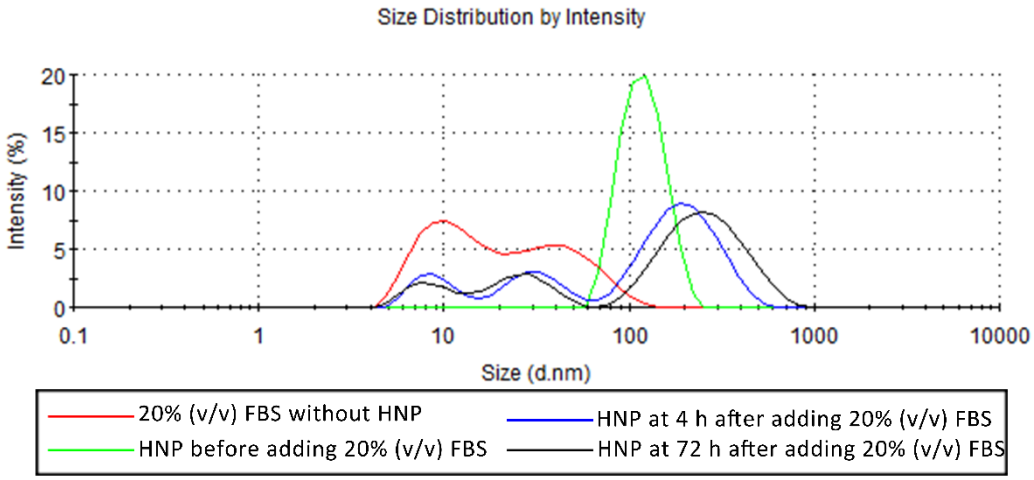

Figure S7. Size distribution curves of HNPs before/after incubation with 20% (v/v) FBS at 37 ℃ (PEG-P(asp)-Ad/PEI_{10K}-CD/PEI_{10K}=9/1/2, N/P ratio=30:1).

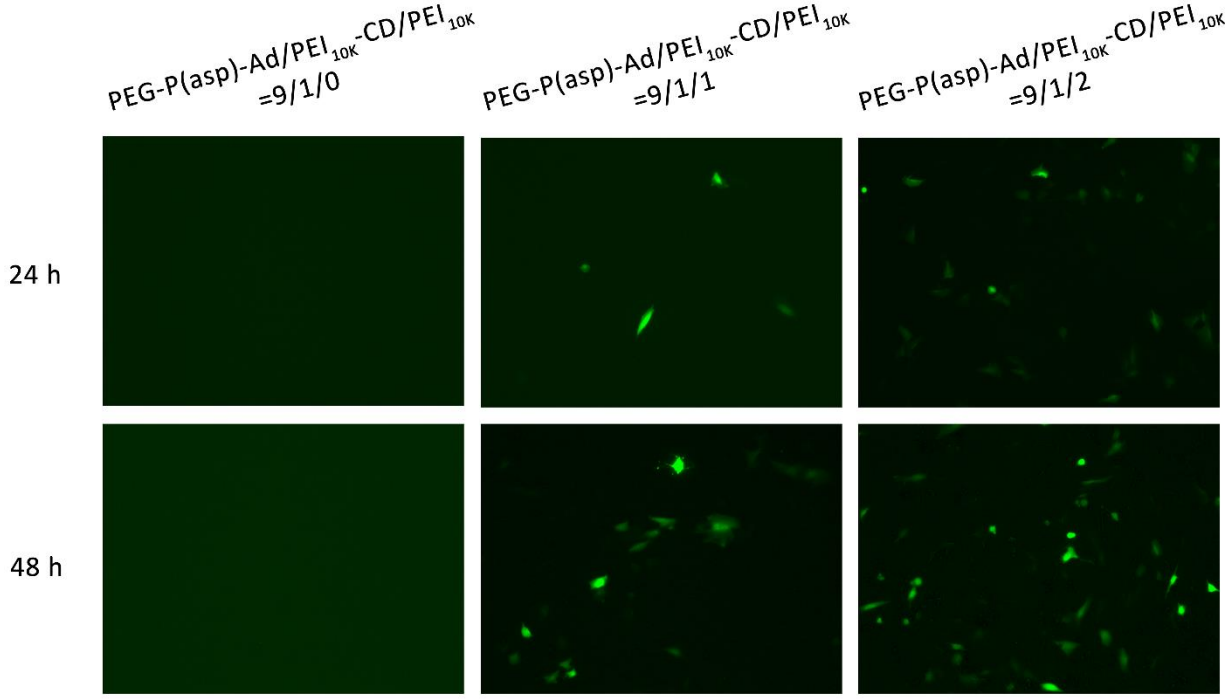

Figure S8. The representative images (100×) of cells transfected with HNPs formulated with different amount of PEI_{10K}. SUM159 cells were transfected with GFP expressing pDNA-loaded HNPs formulated with different PEG-P(asp)-Ad/PEI_{10K}-CD/PEI_{10K} ratios under a N/P ratio of 50/1. The pDNA transfections were performed at a dose of 3 μg of GFP expressing pDNA. Cells were incubated in the transfection solution for 24 h and 48 h, then viewed and photographed under a fluorescent microscope.

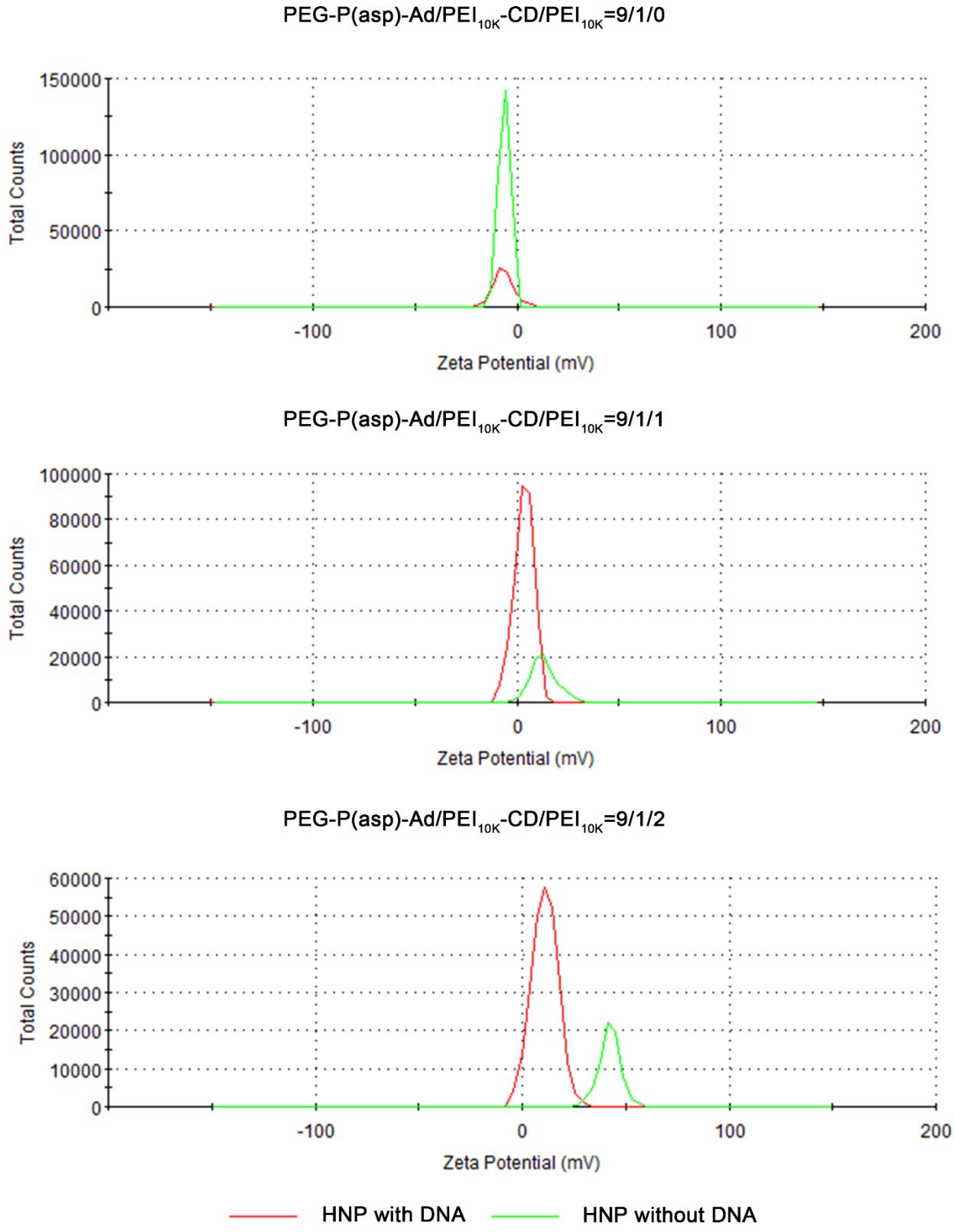

Figure S9. The zeta-potential comparisons of the HNPs formulated with different amount of PEI_{10K} in water.

Reference

1. Zeng, S.; Xiong, M. P., Trilayer Micelles for Combination Delivery of Rapamycin and siRNA Targeting Y-box Binding Protein-1 (siYB-1). *Biomaterials* **2013,** *34* (28), 6882-6892.

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