# Supporting Information

*Materials*

Linear polyethyleneimine HCl salt (*l*PEI·HCl, $M_n$ of *l*PEI = 22 kDa) was a gift from Polymer Chemistry Innovations, Inc. (Tucson, AZ). NHS-$PEG_{12}$-SPDP, SYBR™ Safe and AlarmaBlue were obtained from Thermo Fisher Scientific (Waltham, MA). Dithiothreitol (DTT), Hoechst 33342, and all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO), unless specified otherwise. gWiz-Luc plasmid DNA encoding luciferase gene was purchased from Aldevron (Fargo, ND). Human metastatic prostate cancer PC3 cells were a gift from Dr. M. Pomper's lab at Johns Hopkins School of Medicine. Cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in 5% $CO_2$ atmosphere.

*Synthesis of lPEI-g-PEG*

*l*PEI·HCl (8 mg) and NHS-$PEG_{12}$-SPDP (1.1, 3.3, 6.6, 11.1 or 22.1 mg) were dissolved in 1 mL of deionized (DI) water. After tuning the pH to 7.2–7.4 by adding 1 M NaOH solution, the solution was stirred at room temperature for 18 h. The final product was purified by ultracentrifugation using a dialysis tube with molecular weight cut-off (MWCO) of 3,000 Da. PEG grafting density was controlled by tuning the feeding ratios of NHS-$PEG_{12}$-SPDP to *l*PEI. The precise grafting density was determined by measuring the SPDP content of the polymer after adding 50 mM DTT solution. After incubation of 1 h, the concentration of 2-thione pyridine was

measured by UV spectrophotometry at 343 nm following the protocol provided by the manufacturer (Pierce, Rockford, IL).

*Preparation and characterizations of DNA NPs*

The DNA NPs were prepared through gentle mixing of polymer and DNA solutions. One hundred microliter of gWiz-Luc plasmid DNA solution (50 mg/L) was added into an equal volume of *l*PEI or functionalized *l*PEI-*g*-PEG polymer solution to achieve an N/P ratio 8. The mixture solution was then rapidly mixed by vortexing for 10 s and allowed to incubate at room temperature for 10 min to form stable NPs. The NPs were characterized by particle size and zeta potential using a dynamic light scattering (DLS) Zetasizer Nano (Malvern Instruments, Worcestershire, UK). Each sample was measured for three runs and the data are reported as the mean ± standard deviation of these three readings.

*DNA binding and heparin challenge assays*

DNA NPs were mixed with an equal volume of SYBR™ Safe Green dye solution to measure their DNA binding ability. The fluorescence emitted by the complexation of free DNA and SYBR™ dye was measured using a SpectraMax i3x Multi-Mode microplate reader (Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 502 and 530 nm, respectively. The relative fluorescence intensity was calculated using the following formula: Relative fluorescence intensity (%) = $(F_{sample} - F_{blank}) / (F_{free\ DNA} - F_{blank}) \times 100\%$, where $F_{sample}$ represents the fluorescence intensity of DNA NPs, $F_{blank}$ represents the fluorescence intensity of the mixture of water and SYBR™ dye, and $F_{free\ DNA}$ represents the fluorescence intensity of free DNA.[1] Heparin challenge assay was performed using the same method, except that heparin

solutions with different concentrations were mixed with the DNA NPs before adding the SYBR™ dye solution. The relative fluorescence intensity was calculated using the same formula.

*Cell viability assay*

PC3 cells were seeded on a 96-wells plate at a density of $1 \times 10^4$ cells/well. After incubation for 24 h, the cell culture medium was replaced with serum-free DMEM containing DNA NPs (0.25 μg DNA/well). The cells were allowed to incubate for an additional 4 h, and then the DNA-containing medium was changed to fresh cell culture medium. The cells were incubated for another 44 h, and then the cell viability was measured using AlarmaBlue assay according to the manufacturer's protocol.

*Cellular distribution assay*

PC3 cells were seeded onto an 8 well-cover slip borosilicate glass chamber (NUNC) at a density of $4 \times 10^4$ cells/well. After incubation for 24 h, the cell culture medium was replaced with serum-free DMEM containing DNA NPs prepared using YOYO-1 labeled DNA (1 μg DNA/well). After further incubation for 4 h, the cells were washed three times by clod PBS and then incubated with PBS containing 100 IU/mL heparin and 75 mmol/L sodium azide for 30 min to remove any bound extracellular DNA NPs. After staining the nucleus with 10 mg/L Hoechst 33342, the cells were observed under a confocal laser scanning microscope (Zeiss LSM 510).

*Statistical analysis*

All data are expressed as mean ± standard deviation. Student's t-test and one-way ANOVA test are used to determine the significance among groups. The difference between different groups is considered to be statistically significant if $p < 0.05$.

**Supplementary Figures:**

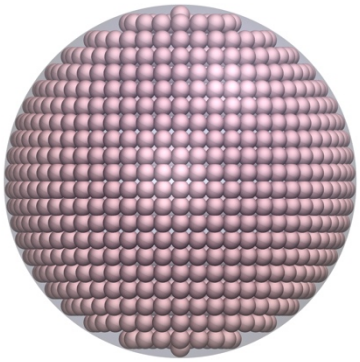


**Figure S1.** Coarse-grained representation of the spherical NP core. The central bead with a diameter of 20$\sigma$ is shown in grey. The other 936 beads with a diameter $\sigma$ are distributed on the surface of the central bead and shown in pink.

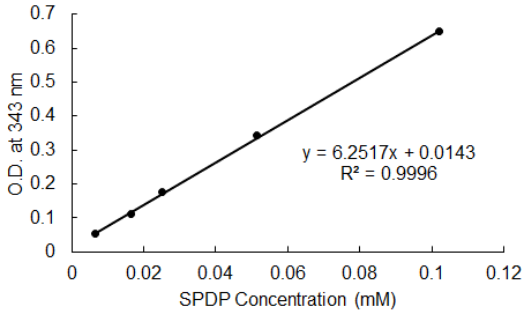



**Figure S2.** Standard curve of SPDP concentration against O.D. reading at 343 nm.

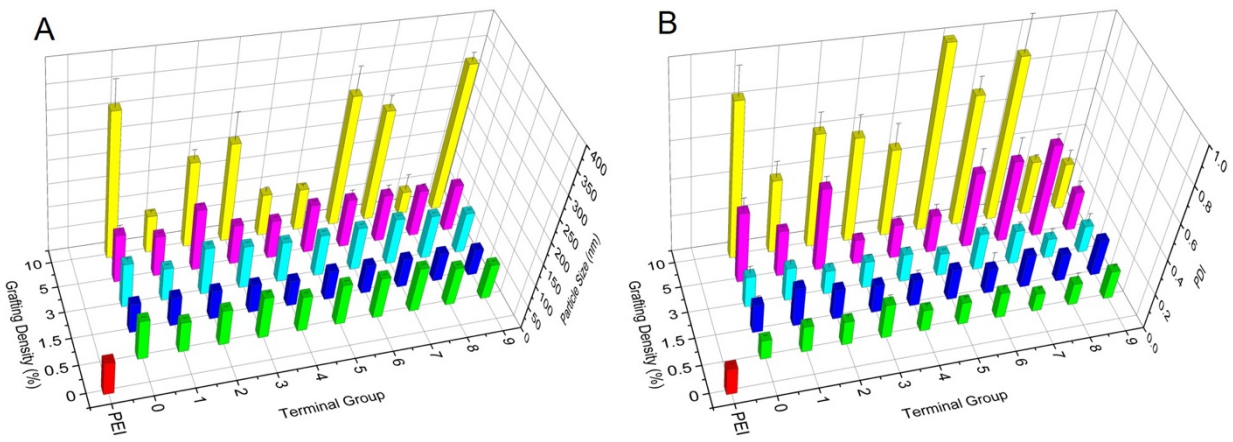



**Figure S3.** Particle size (A) and PDI value (B) of various PEGylated DNA NPs with different PEG terminal groups as measured by DLS. PEG terminal groups are shown in Scheme 1.

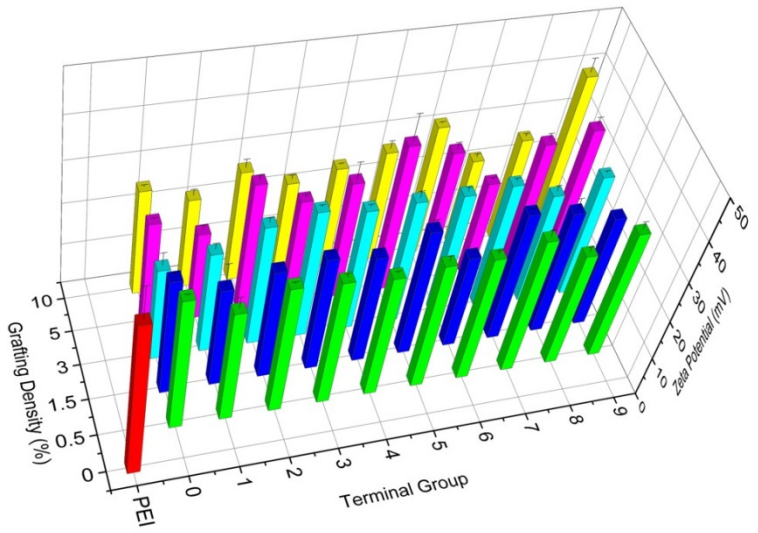



**Figure S4.** Zeta potentials of various PEGylated DNA NPs with different PEG terminal groups measured by DLS. PEG terminal groups are shown in Scheme 1. At a PEG grafting density of 0.5%, all NPs show similar zeta potentials, implying that PEG has a negligible effect on the surface charge at low grafting densities. At higher PEG grafting densities, for **NP0** to **NP5** with different alkyl chain lengths, generally the zeta potential increases with increasing alkyl chain length. For **NP6** to **NP9** with various hydroxyalkyl groups, the zeta potentials first decreases from **NP6** to **NP7** and then increases from **NP7** to **NP9** at grafting densities of 5 and 10%, whereas the trends at grafting densities of 1.5 and 3% are not obvious.

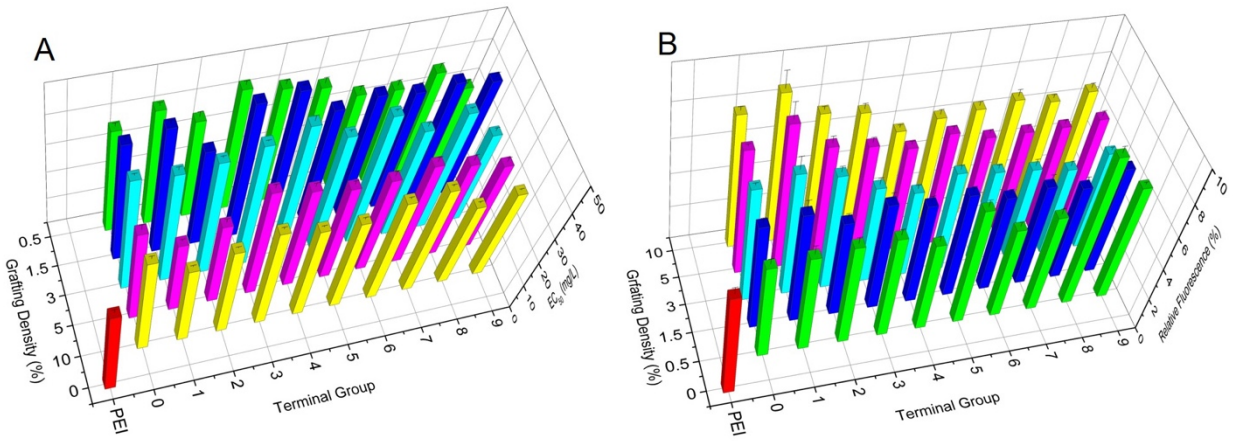



**Figure S5**. $EC_{50}$ values of heparin challenge studies (A) and percentages of free DNA in DNA binding studies (B) of various PEGylated DNA NPs with different PEG terminal groups. PEG terminal groups are shown in Scheme 1. For **NP1** to **NP5** with different alkyl chain lengths at all PEG grafting densities, the $EC_{50}$ values first increase and then decrease with increasing alkyl chain length. The highest values are achieved at **NP3** or **NP4**. For **NP6** to **NP9** with various hydroxyalkyl groups, no significant trend is observed at various grafting densities.

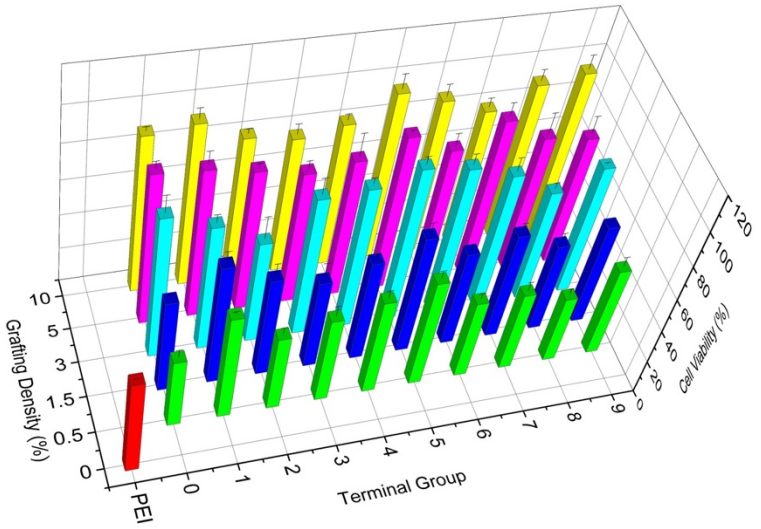



**Figure S6.** Viabilities of PC3 cells after treatment with various PEGylated DNA NPs with different PEG terminal groups. PEG terminal groups are shown in Scheme 1. *l*PEI/DNA NPs are used as control. The N/P ratio is 8 and the DNA dose is 0.25 μg per well.

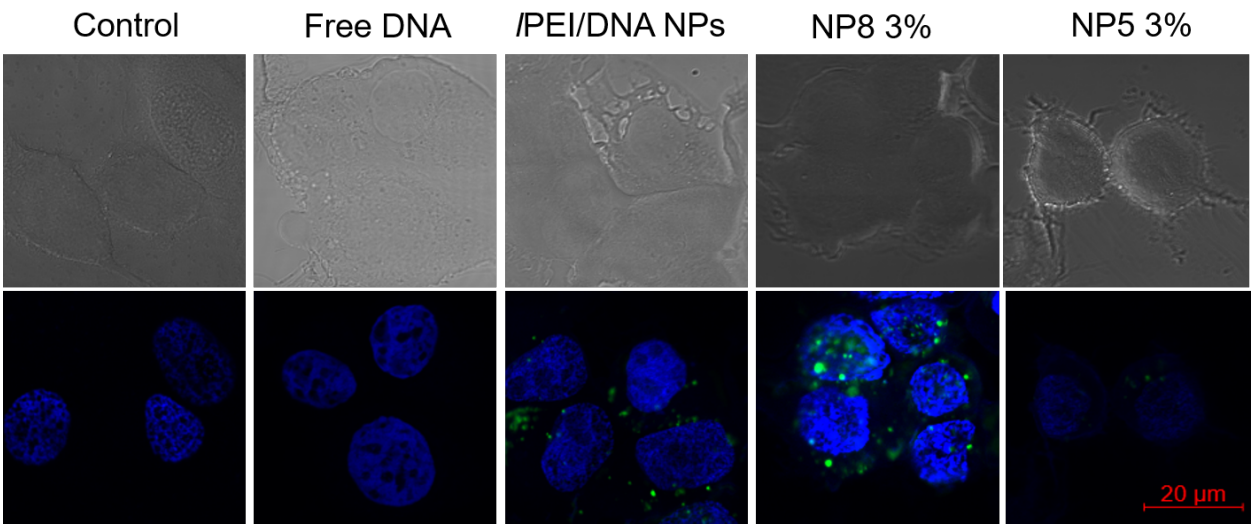
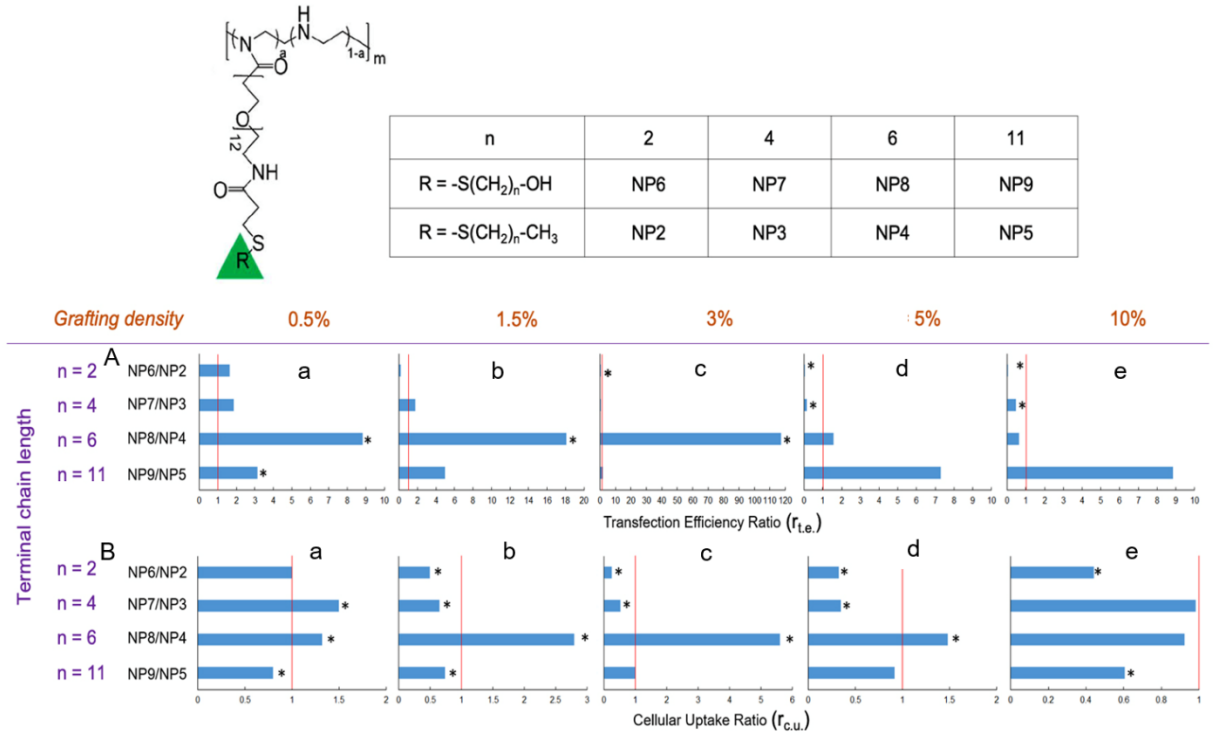



**Figure S7.** Confocal images of PC3 cells after treatment with free DNA, */*PEI/DNA NPs, NP8 (at grafting density 3%), and NP5 (at grafting density 3%) for 4 h. DNA is labeled with YOYO-1 dye, the N/P ratio is 8 and the DNA dose is 1 µg per well.




| n | 2 | 4 | 6 | 11 |
|---|---|---|---|---|
| R = -S($CH_2$)$_n$-OH | NP6 | NP7 | NP8 | NP9 |
| R = -S($CH_2$)$_n$-$CH_3$ | NP2 | NP3 | NP4 | NP5 |

**Figure S8.** Transfection efficiency ratio ($r_{t.e.}$) (A) and cellular uptake ratio ($r_{c.u.}$) (B) of carriers with the same terminal alkyl chain length but different end groups at PEG grafting densities of 0.5% (a), 1.5% (b), 3% (c), 5% (d) and 10% (e). The red line in each graph indicates the ratio of 1.0. A paired statistical analysis with a p value less than 0.05 by student t-test is considered significant.

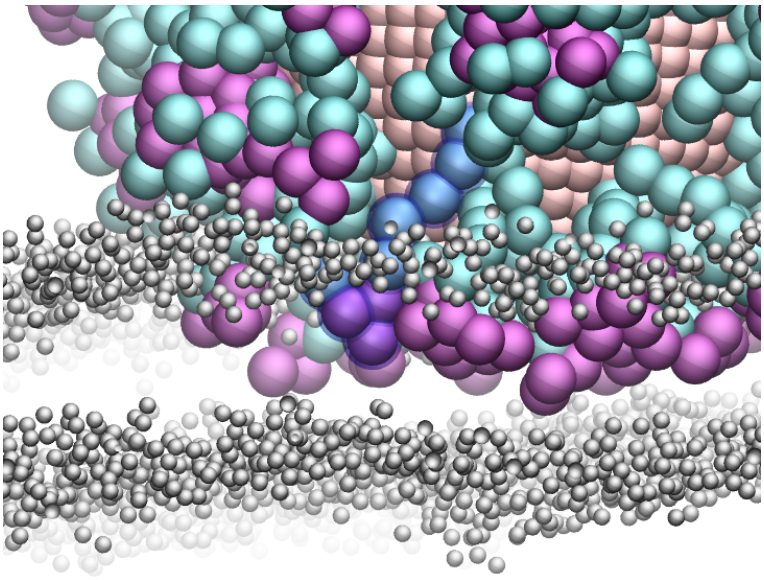


**Figure S9.** Typical configuration of an inserted modified PEG chain (shaded blue) with hydroxyl terminal group. The chain is composed of 5 hydrophilic beads (PEG), 3 hydrophobic beads (alkyl chain) and 1 hydrophilic bead at the end (hydroxyl group). The hydroxyl terminal group is located on the hydrophilic region of the membrane (grey), whereas the alkyl chain is bent to remain in contact with the hydrophobic region of the membrane (the region between the lipid head groups). Color codes are the same as those in Figure 4.

**References**

1. Teo PY, Yang C, Hedrick JL, Engler AC, Coady DJ, Ghaem-Maghami S, et al. Hydrophobic modification of low molecular weight polyethylenimine for improved gene transfection. *Biomaterials* 2013;**34**:7971–9