

# Supplementary Materials: Aliphatic Quaternary Ammonium Functionalized Nanogels for Gene Delivery

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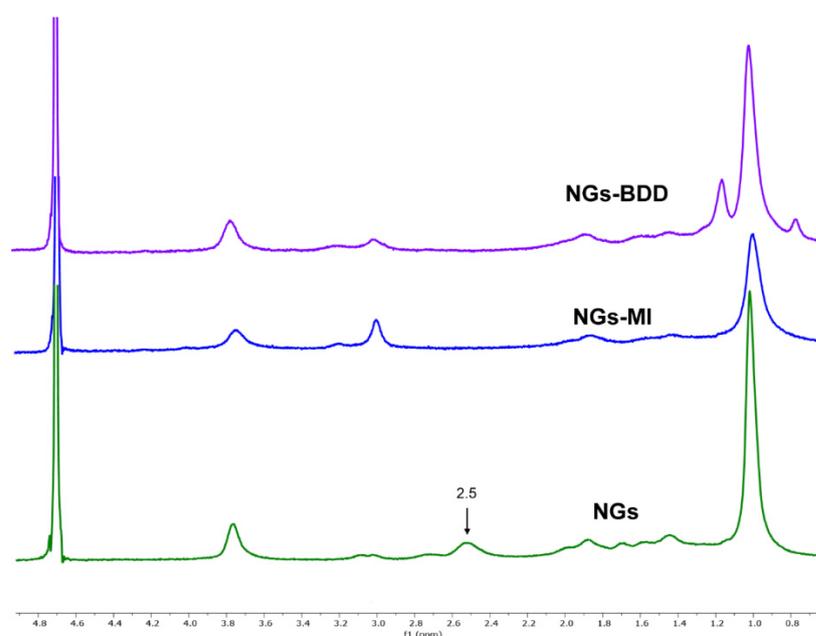


Figure S1.  $^1\text{H}$  NMR spectra of NGs, NGs-MI and NGs-BDD.

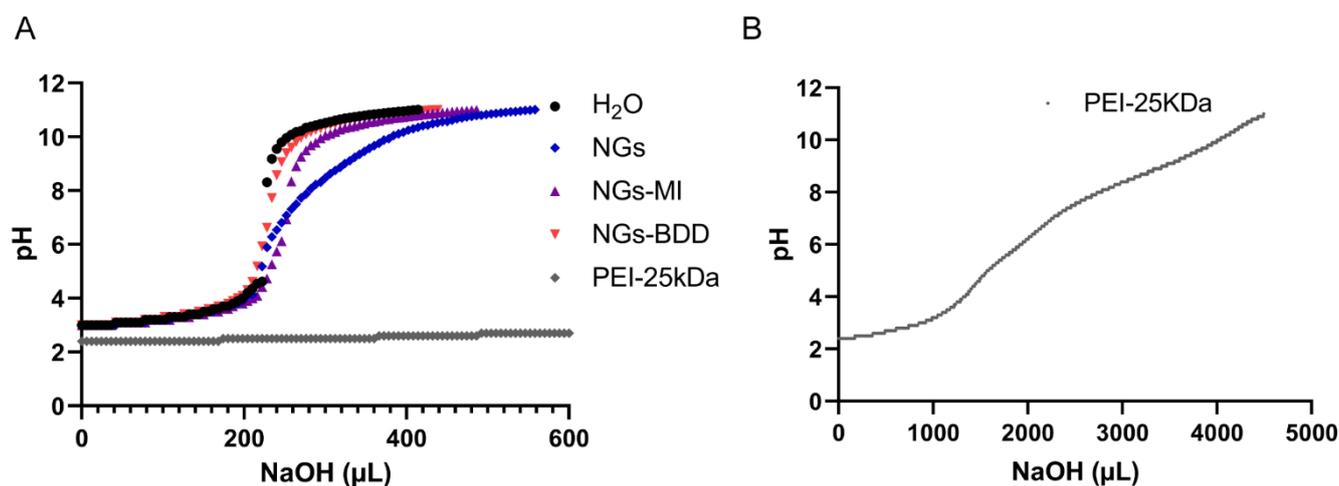
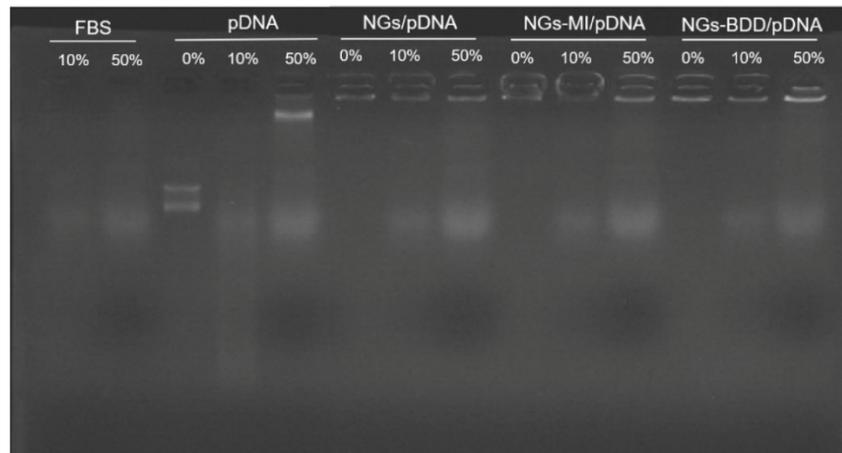
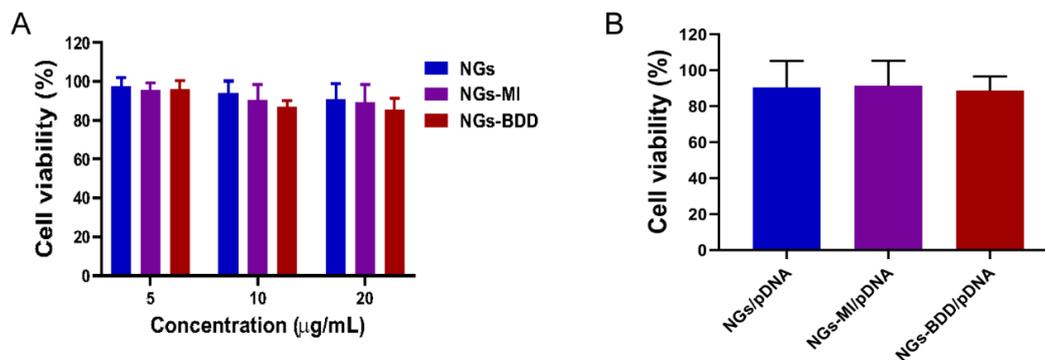


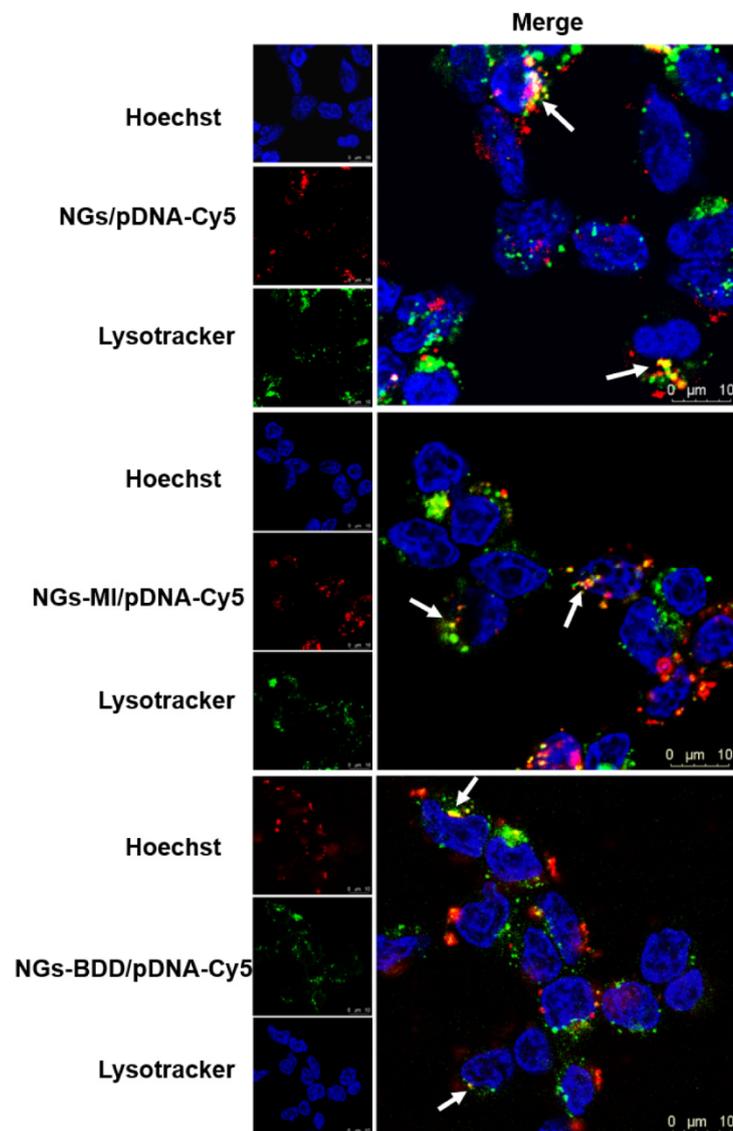
Figure S2. (A) Acid-base titration profiles of nanogels, (B) Acid-base titration profile of PEI-25kDa.



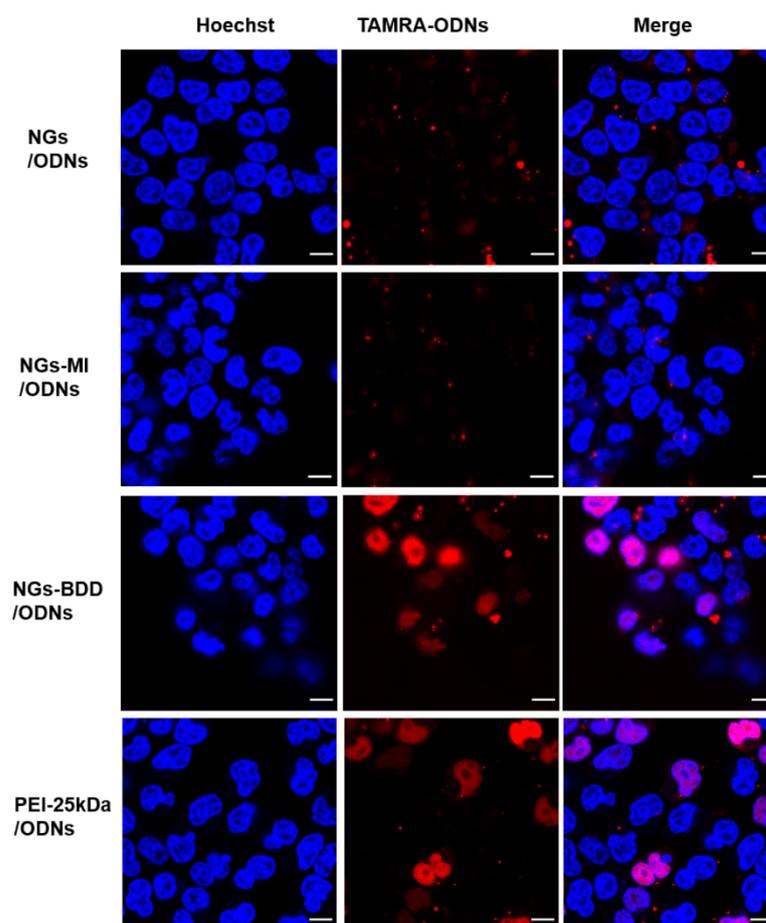
**Figure S3.** DNA protection against serum nucleases in nanogel/pDNA complexes. Agarose gel electrophoresis of NGs/pDNA, NGs-MI/pDNA and NGs-BDD/pDNA treated with 10% or 50% serum for 10 h.



**Figure S4.** Cytotoxicity of nanogels and nanogel/pDNA complexes in HEK293T cells. (A) Relative viability of HEK293 cells after incubation with various concentrations of nanogels for 48 h and (B) Relative viability of HEK293 cells treated with nanogel/pDNA complexes (weight ratio 10) in opti-MEM medium containing 1 µg pDNA for 8 h, followed by culture with cell culture medium for another 40 h (n=3).



**Figure S5.** Intracellular localization of Cy5-labeled pDNA (red) in HEK293 cells incubated with nanogel/Cy5-pDNA complexes, observed by CLSM at 4 h post incubation. The cell nuclei were stained with Hoechst33324 (blue) and lysosomes were stained with LysoTracker Green DND-22 (green). White arrows indicate colocalization between Cy-5 DNA and LysoTracker fluorescence signals. Scale bars indicate 10  $\mu\text{m}$ .



**Figure S6.** CLSM images of HEK293 cells after incubation with fluorescently labeled NGs/ODNs, NGs-MI/ODNs, NGs-BDD/ODNs and PEI-25kDa/ODNs for 4 h. TAMRA-labeled ODNs (red) were used. Cell nuclei were stained with Hoechst33324 (blue). Scale bars indicate 10  $\mu$ m.