

**Polyethylenimine mediated magnetic nanoparticles for combined intracellular
imaging, siRNA delivery and anti-tumor therapy**

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This supporting information provides all of the additional information as noted in the manuscript and more detailed discussion of the current study.

Synthesis of γ -Fe₂O₃ magnetic nanoparticles

Magnetic γ -Fe₂O₃ nanoparticles (MNPs) used in this study were synthesized from magnetite (Fe₃O₄) according to methods proposed elsewhere.^{1, 2} First, 4.5 mL FeCl₃ (2 M dissolved in 2 M HCl) was added to 15.5 mL DI water, and then 3 mL Na₂SO₃ (1 M) was added dropwise into the mixture within 1 minute with stirring. When the color of the solution changed from red to light yellow, it was added to 120 mL of NH₄OH solution (0.85 M) with vigorous stirring. A black precipitate quickly formed and was allowed to crystallize completely for another 40 minutes. After washed with deoxygenated water, the black precipitate was diluted to 252 mL (with a mass concentration of 3 mg/mL) and was adjusted to pH 3.0 with HCl (0.1 M). The suspension was then heated to 90 °C in 5 minutes, and was stirred under aeration (with air) for 90 minutes at 110 °C. The color of the suspension slowly changed from black to reddish-brown. After washing with DI water by magnetic decantation, the reddish-brown precipitate was dried to a powder of MNPs.

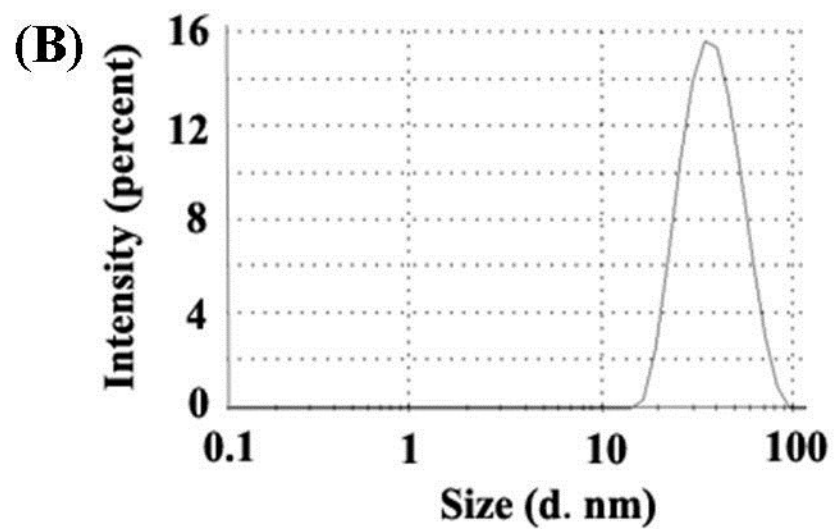
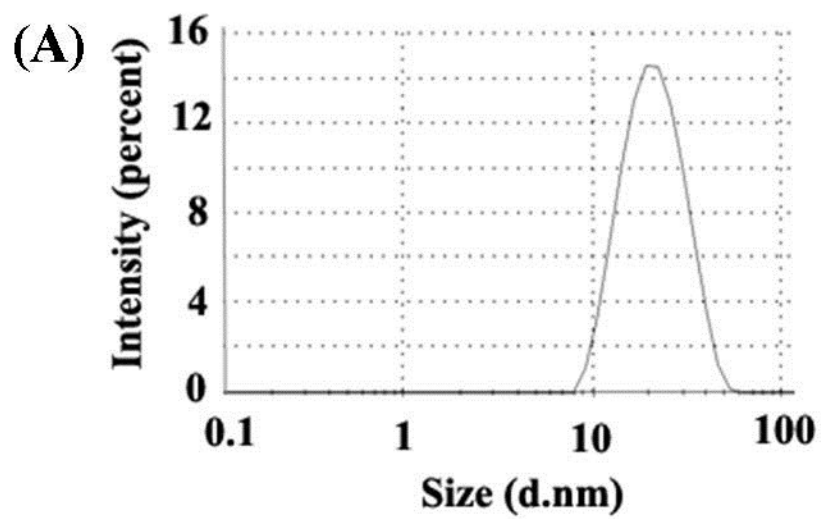


Figure S1. An average particle size distribution graph of MNPs (A), and PEI-MNPs (B).

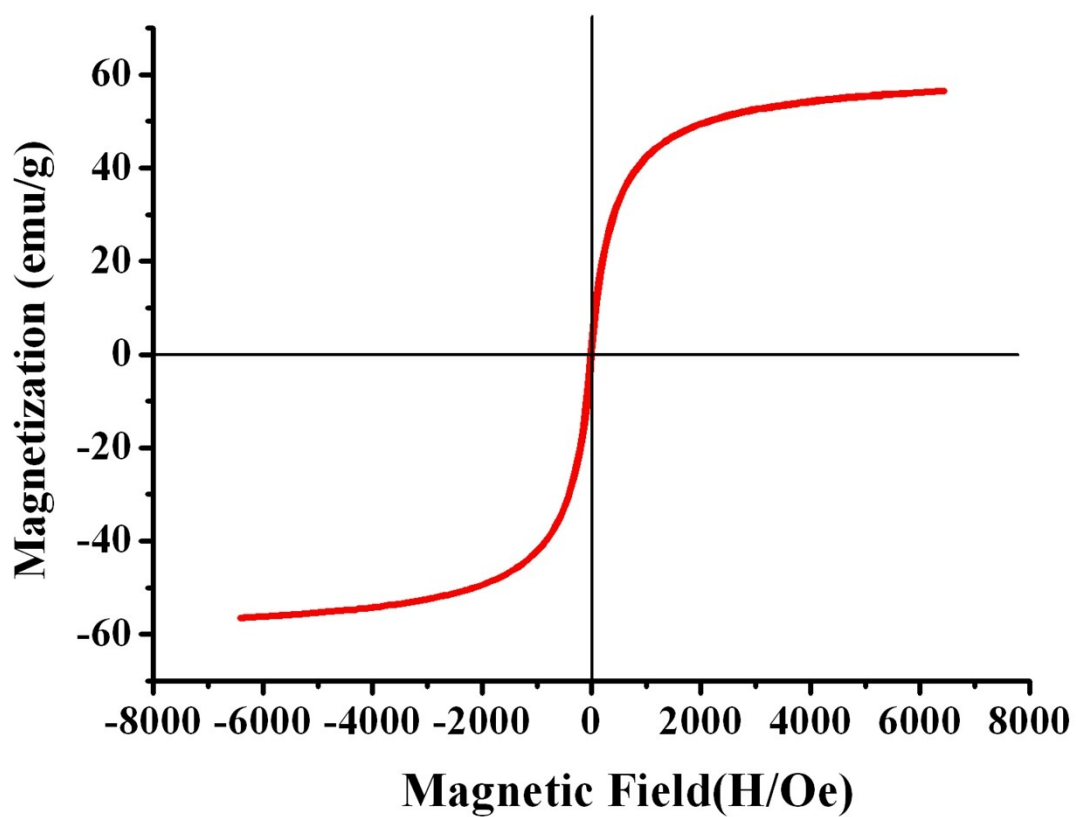


Figure S2. The magnetic hysteresis loop of magnetic γ -Fe₂O₃ nanoparticles (MNPs) at 300 K(A), and the saturation magnetization of the prepared MNPs was 56.26 emu/g.

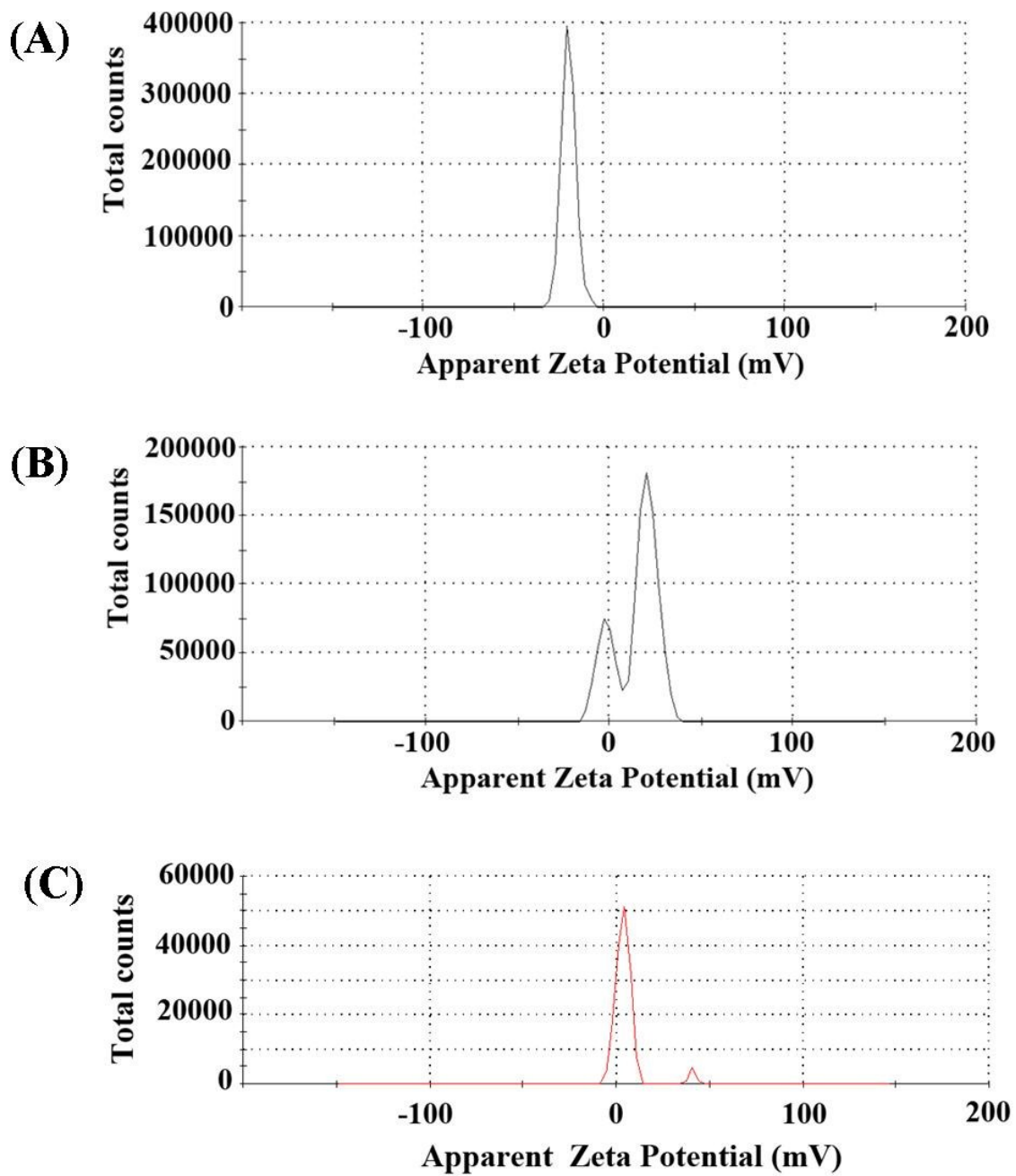


Figure S3. The zeta potential values of MNPs (A), PEI-MNPs (B), and free PEI (C) dispersed in 0.1 M phosphate-buffered saline solution (PBS, pH 7.4).

Assay of cellular uptake, cell viability, cytotoxicity, and apoptosis of PEI-MNPs

Analysis of cellular uptake was then performed by co-culturing U251 cells with the FITC labeling PEI-MNPs. Briefly, the U251 cells were seeded in 24-well plates and cultured for 24 h, the FITC-labeling PEI-MNPs were firstly diluted to 1 mg/mL using RPMI 1640 medium without FBS, and then the FITC-labeling PEI-MNPs with various concentrations (5-80 $\mu\text{g/mL}$) were added to the U251 cells respectively. The culture medium and the residual PEI-MNPs were then replaced and rinsed by PBS for three times after incubation for 4 h, and the cells were counterstained with DAPI dye (100 nM; Sigma-Aldrich) to reveal nuclei. The prepared samples were observed under an inverted fluorescence microscope (Eclipse TE 2000-U, Nikon, Kyoto, Japan) equipped with a high-resolution CCD camera (CV-S3200, JAI Co., Japan). The U251 cells without PEI-MNPs treatment served as controls.

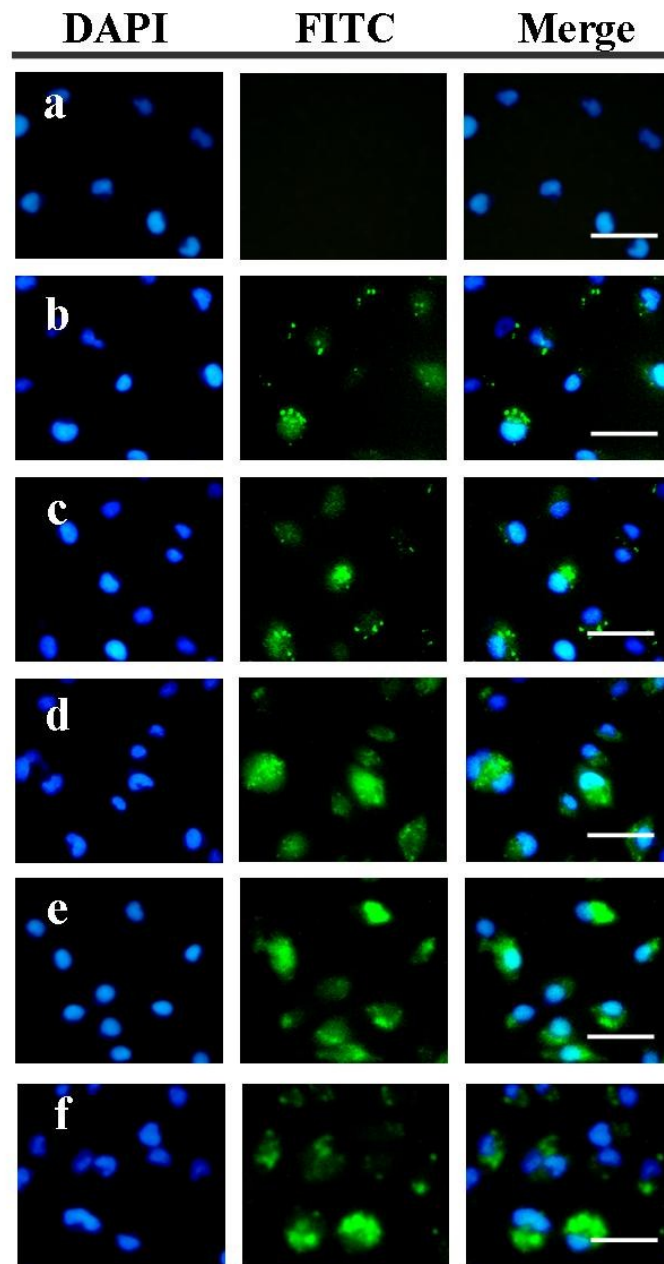


Figure S4. Cellular uptake of the FITC-labeling PEI-MNPs in the U251 cells, and the untreated U251 cells are used as controls (A), and the U251 cells incubated with various concentrations (5, 10, 20, 40, 80 $\mu\text{g}/\text{mL}$) FITC-labeling PEI-MNPs for 4 h (B-F), and the fluorescent PEI-MNPs were located around DAPI-stained nuclei within cells.

The cell viability of the PEI-MNPs treated U251 cells was assessed using a fluorescein diacetate (FDA) (Sigma-Aldrich Co., St. Louis, MO, USA) and propidium iodide (PI) (Sigma-Aldrich Co.) double-staining protocol.^{3, 4} The U251 cells were seeded at a density of 2×10^4 cells/well in a 24-well plate, after incubation 24 h, PEI-MNPs were added to the 24-well plate, and the final concentrations of the PEI-MNPs varied from 5 $\mu\text{g/mL}$ to 80 $\mu\text{g/mL}$. The treated U251 cells with PEI-MNPs was washed three times to remove the residual PEI-MNPs under magnetic field using PBS after cultured for another 48 h. Subsequently, FDA solution with a final concentration of 1 $\mu\text{g/mL}$ and PI solution with a final concentration of 20 $\mu\text{g/mL}$ were successively introduced into the culture plates after being cultured for 48 h. The cellular viability was then analyzed by counting the live and the dead cells after incubation for 10 min at room temperature. The samples were analyzed with an inverted fluorescence microscope (Eclipse TE 2000-U, Nikon, Kyoto, Japan) equipped with a high-resolution CCD camera (CV-S3200, JAI Co., Japan), the living cells were stained green by FDA, whereas the dead cells were stained red by fluorescent dye PI. The U251 cells without PEI-MNPs treatment served as controls.

The 3-(4, 5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay is considered to evaluate cell proliferation for testing cytotoxicity.⁵⁻⁷ We performed a standard MTT method to assess the proliferation capacity of the PEI-MNPs treated U251 cells. After reaching the exponential growth phase, the U251 cells were harvested to prepare cell suspension. Then, the cells were seeded at a density of 1×10^4 cells/well in a 96-well microwell plates, after incubation 24 h, the PEI-MNPs solution were added to the microwell plates, and the final concentrations of the PEI-MNPs were from 5 $\mu\text{g/mL}$ to 80 $\mu\text{g/mL}$. The culture medium was removed after incubating for 48 h, and 200 μL of the MTT solution (final concentration: 0.5 mg/mL; Sigma-Aldrich Co.) was added. The cells were then incubated for 4 h, and 150 μL of dimethyl sulfoxide

(DMSO) was added, the plates were incubated for another 10 min. Finally the absorbance was measured at 570 nm on a microplate spectrophotometer (Bio Tek Instrument Inc., USA). All experiments were performed three times, and the PEI-MNPs untreated U251 cells served as controls.

To visualize apoptotic cells, the PEI-MNPs treated U251 cells were fixed with 4% paraformaldehyde for 15 min, washed thrice with PBS, and then stained with bisbenzimidazole dye Hoechst H33258 solution (2 $\mu\text{g}/\text{mL}$) for 10 min at room temperature. The stained cells were rinsed thrice with PBS and observed using an inverted fluorescence microscope (Eclipse TE 2000-U) equipped with a high-resolution CCD camera (CV-S3200). The untreated and PEI-MNPs-treated U251 cells were used as controls.

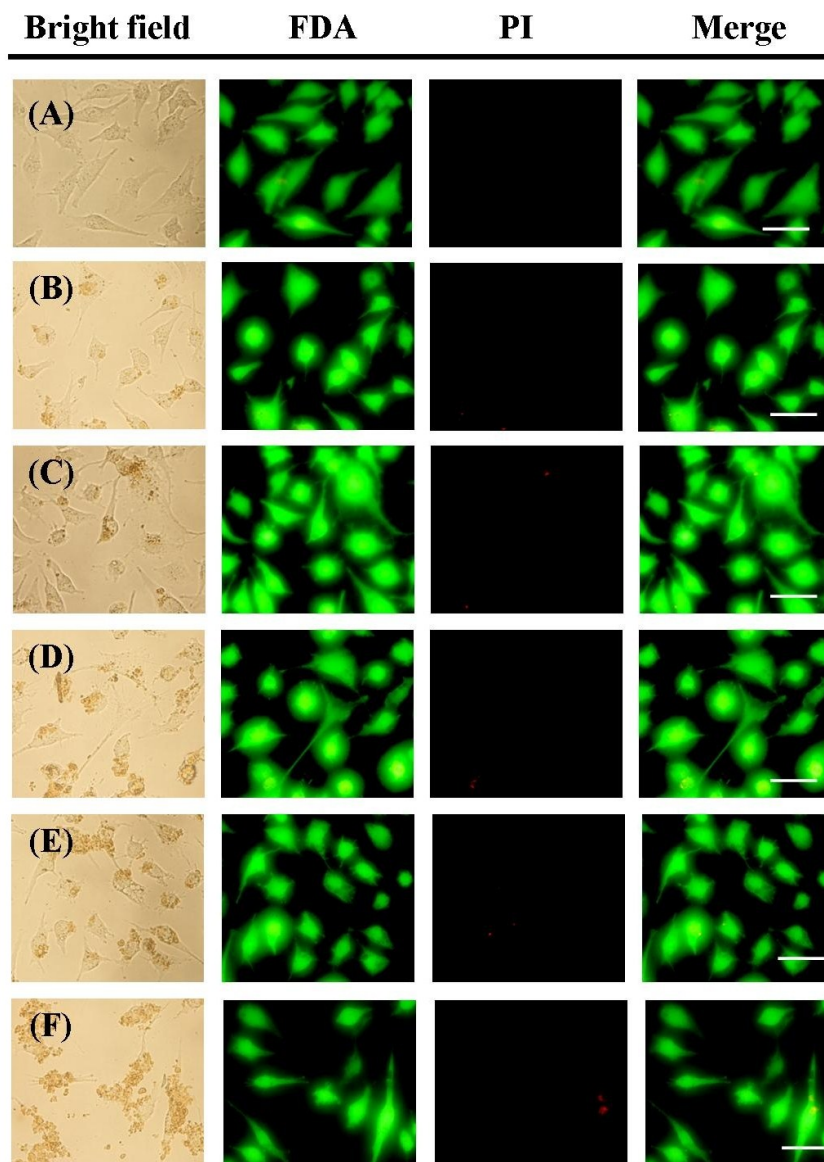


Figure S5. Cell viability of the treated U251 cells with PEI-MNPs assessed with the PI and FDA double-staining protocol. The untreated U251 cells were used as controls (A), and fluorescence images (B-F) of the treated U251 cells with PEI-MNPs of different concentrations (5, 10, 20, 40, 80 $\mu\text{g/mL}$) for 48 h. The data showed that only a few detected cells were dead. Scale bar = 100 μm .

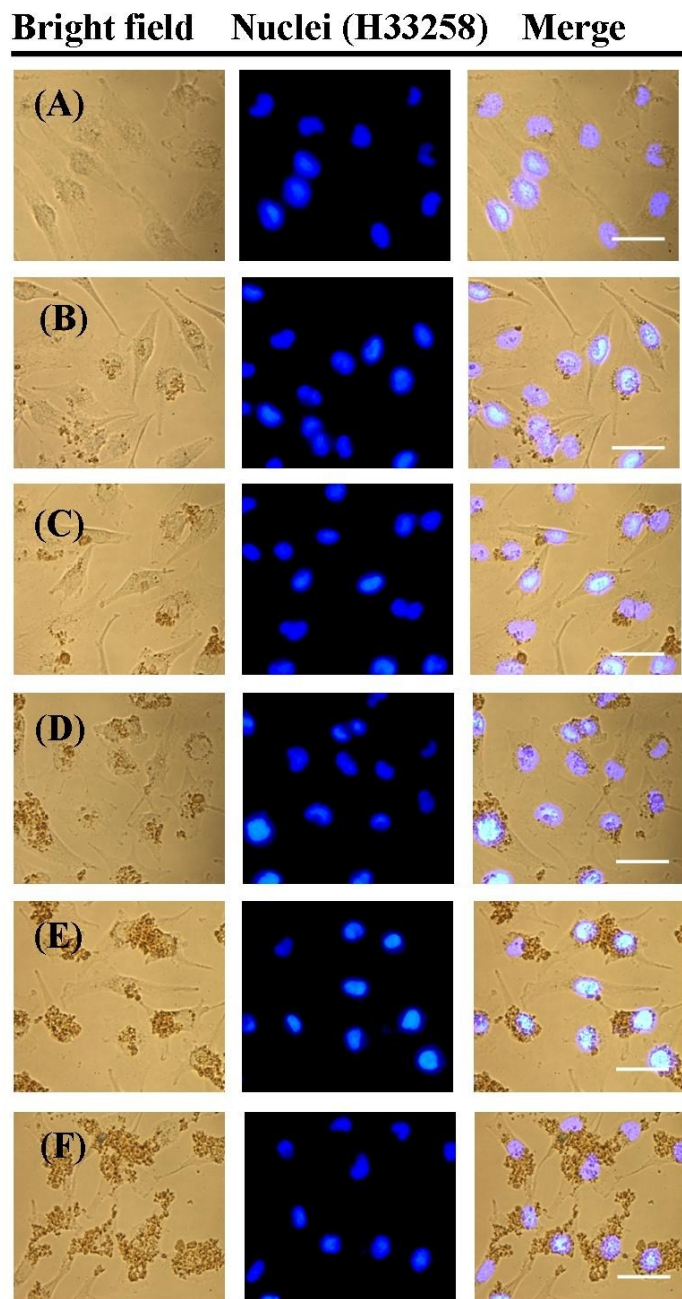


Figure S6. Apoptosis assay of the treated U251 cells with PEI-MNPs using fluorescein Hoechst H33258 staining protocol. The untreated U251 cells were used as controls (A), and fluorescence images (B-F) of treated U251 cells with PEI-MNPs of concentrations (5, 10, 20, 40, 80 $\mu\text{g}/\text{mL}$) for 48 h, the results indicated that the PEI-MNPs did not induce an obvious apoptosis of treated U251 cells. Scale bar = 100 μm .

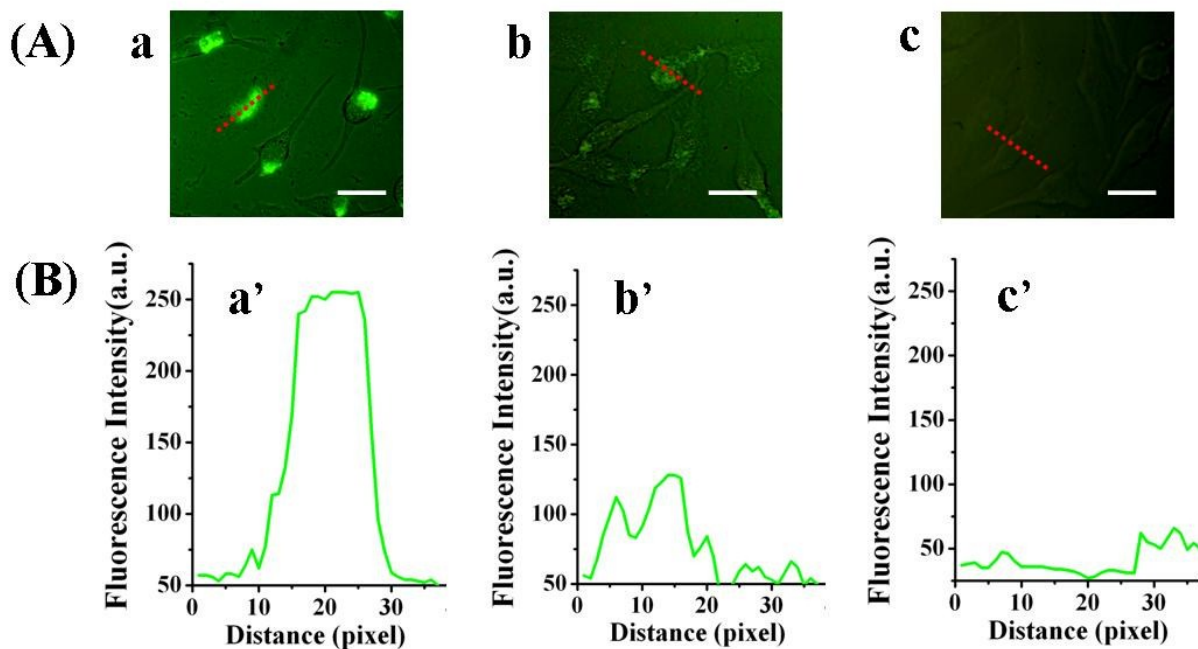


Figure S7. (A) The fluorescence images of U251 cells following incubation with FAM labeling siRNA~PEI-MNPs or siRNA (a, b), green fluorescent FAM-labeled siRNA was used to investigate the intracellular distribution of siRNA, untreated U251 cells were used as blank controls (c), and (a'- c') their fluorescence intensities corresponding to the line profile graphs analyzed using Image-Pro® Plus 6.0 software (B). Scale bar = 50 μ m.

References

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