Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

A multi-functional macrophage and tumor targeting gene delivery system for regulation of macrophage polarity and reversal of cancer immunoresistance

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Detailed experimental protocols

X ray diffraction

NP was characterized by X ray diffraction (XRD) analysis (D8 ADVANCE, Bruker, Germany). XRD curve was obtained in the range of 20-90° (2θ) with a scanning rate of $6°\cdot s^{-1}$.

Evaluation on the stability of nanoparticles in the presence of ions

The as-prepared PHNP (with either FITC labeled peptide or cy5.5 labeled HA) was placed in the NaCl solution with a particular concentration (0.1 M, 0.5 M and 1.0 M) for particular times. After that, the nanoparticle containing solution was centrifuged at 4 °C for 1 h at 10000 rpm. After centrifugation, the amount of free cy5.5 labeled HA or free FITC labeled peptide remaining in the supernatant of solution was determined using a spectrofluorophotometer (RF-5301 PC, Shimadzu). The assembly efficiency of HA and peptide was calculated based on the amount of HA and peptide remained in the nanoparticles.

Agarose gel retardation assay

The DNA binding affinity of nanoparticles was studied by Agarose gel retardation assay. The prepared NP, HNP and PHNP were electrophoresed on the 2% (w/v) agarose gel containing GelRed with Tris-acetate (TAE) running buffer at 80 V for 1 h. DNA retardation was visualized using a fluorescence imager (Bio-Rad Pharos FX Molecular Imager). Naked DNA was used as control.

DNase I protection assay was used to evaluate the ability of nanoparticles to protect DNA against enzymatic degradation. The nanoparticles (PHNP) were incubated with

DNase I/Mg^{2+} digestion buffer (50 mM Tris-HCl, pH 7.4, and 10 mM $MgCl_2$) at a final DNase I concentration of 30 U/g plasmid DNA at 37°C for particular times, and then DNase I was inactivated by adding 4 μl of EDTA (250 mM). Finally, electrophoresis was performed to evaluate the integrity of DNA. For comparison, the naked DNA was also studied.

Intracellular Trafficking of PHNP

Confocal laser scanning microscopy (CLSM) was used to study the intracellular delivery of YOYO-1 labeled DNA. J774A.1 cells (1×10^5 cells) in 1 mL of culture medium containing 10% FBS were seeded in a 35 mm glass bottomed culture dish and incubated at 37 °C for several hours. After cell attachment, J774A.1 cells were stimulated by IL-4 and IL-10 for 24 h. Then the culture medium was removed, and 1 mL of fresh medium (with 10% FBS) containing PHNP with a DNA concentration of 2 μg/mL was added. After co-incubation at 37 °C for a particular time (0.5 h, 2 h, 4 h, 8 h and 12 h), the cells were carefully washed with PBS for three times. The cell nuclei were stained with Hoechst 33342 for 15 min and the endosomes/lysosomes were stained with Lysotracker (red) for 45 min at 37 °C. The cells were visualized by CLSM (PerkinElmer UltraVIEW VoX) under magnification of 1000.

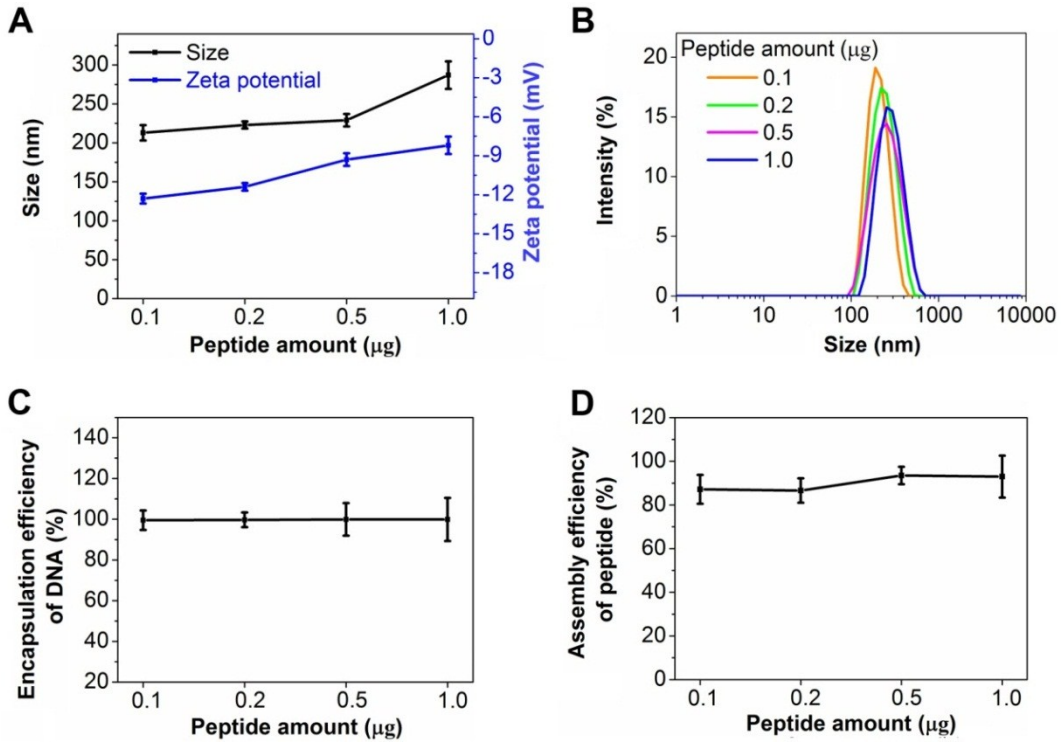

Figure S1. Size and zeta potential (A), size distribution (B), encapsulation efficiency of DNA (C) and assembly efficiency of peptide (D) of peptide/HA/PS/$CaCO_3$/DNA nanoparticles prepared with different peptide amounts.

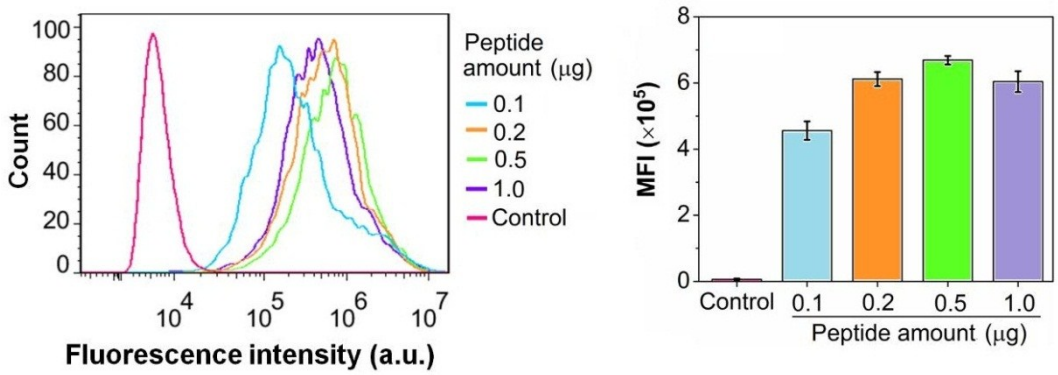

Figure S2. Cellular uptake of peptide/HA/PS/$CaCO_3$/DNA nanoparticles with different peptide amounts in M2 macrophages (J774A.1 cells stimulated by IL-4 and IL-10 for 24 h) studied by flow cytometry. M2 macrophages were co-incubated with PHNP prepared with different peptide amounts for 4 h. M2 macrophages without treatment were used as a control.

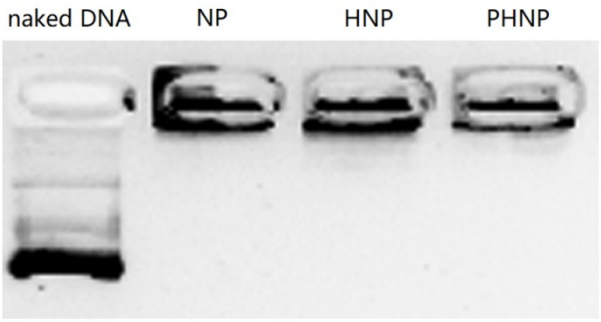

Figure S3. Agarose gel electrophoresis retardation assay on the encapsulation of DNA in nanoparticles. Naked DNA was also used as a control.

As shown in Figure S3, no blots appear in the position of naked DNA, indicating that the prepared NP, HNP and PHNP nanoparticles can effectively encapsulate DNA.

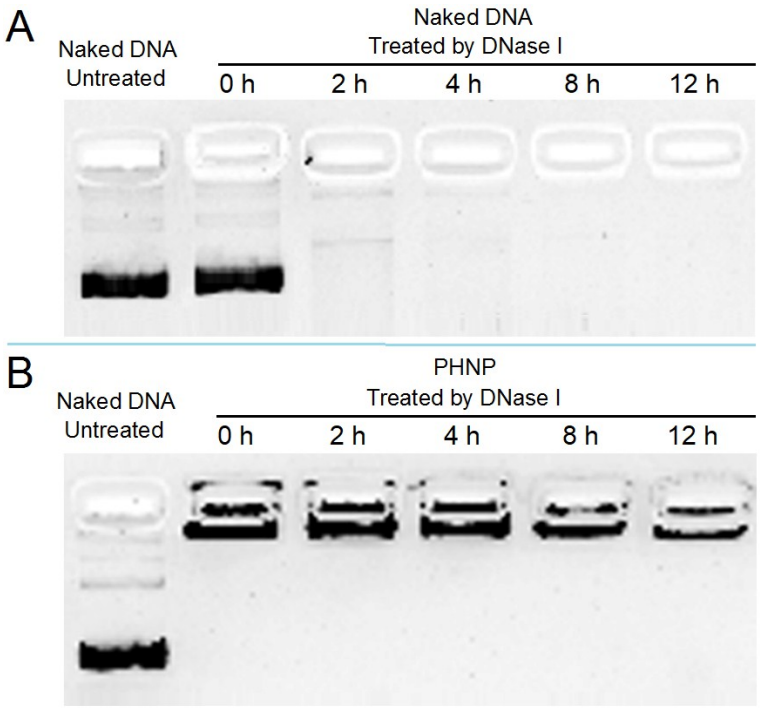

Figure S4. Agarose gel electrophoresis retardation assay on the protection of DNA by PHNP. PHNP was treated by DNase I/Mg^{2+} digestion buffer at a DNase I

concentration of 30 U/g plasmid DNA for different time periods. For comparison, naked DNA was also studied.

As shown in Figure S4, naked DNA is digested completely when treated with DNase I for 2 h, while PHNP can encapsulate DNA efficiently and protect it from digestion by DNase I even after 12 h incubation with DNase I.

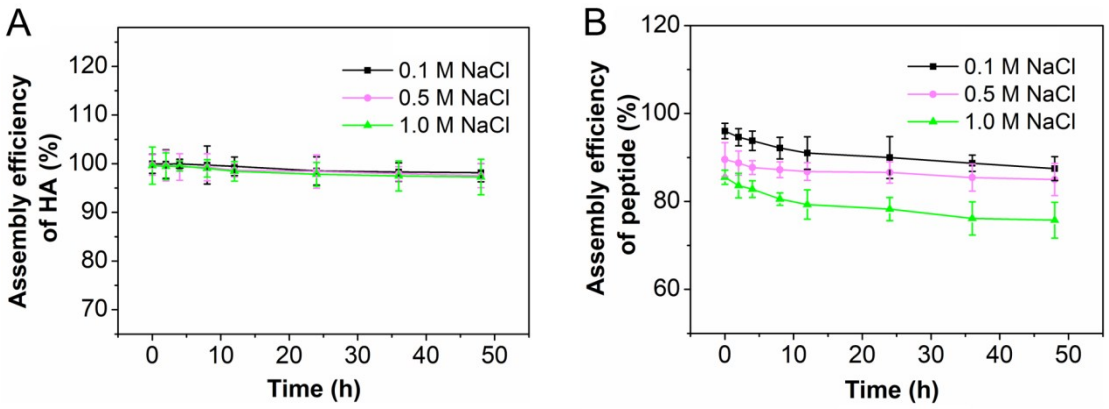

Figure S5. Study on the stability of HPNP in the media with different ion strengths by evaluation of the assembly efficiency of HA (A) and peptide (B) in NaCl solutions.

As shown in Figure S5, the self-assembled nanoparticles are stable in the ion contained media. HA can be stably coated on the nanoparticle surface even at a high ionic strength of 1.0 M since HA has a relatively high molecular weight. More than 85% peptide molecules are coated on the nanoparticle surface in the NaCl solution with a concentration of 0.5 M.

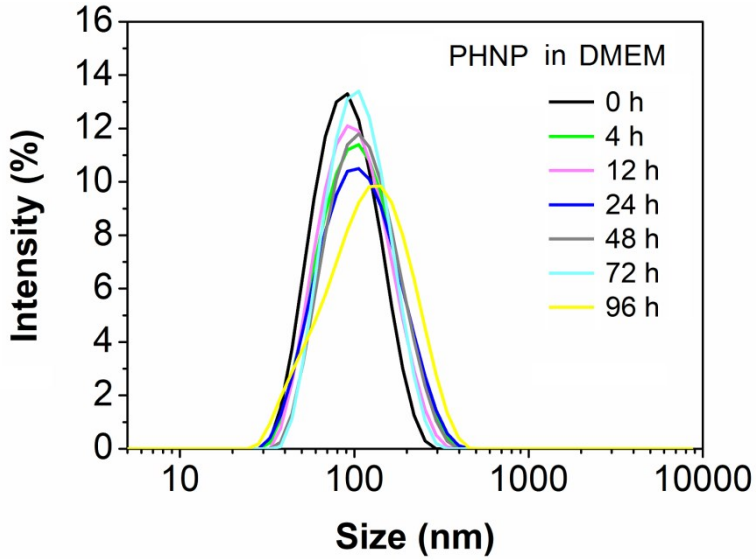

Figure S6. Study on the stability of PHNP in DMEM cell culture medium containing 10% FBS during storage.

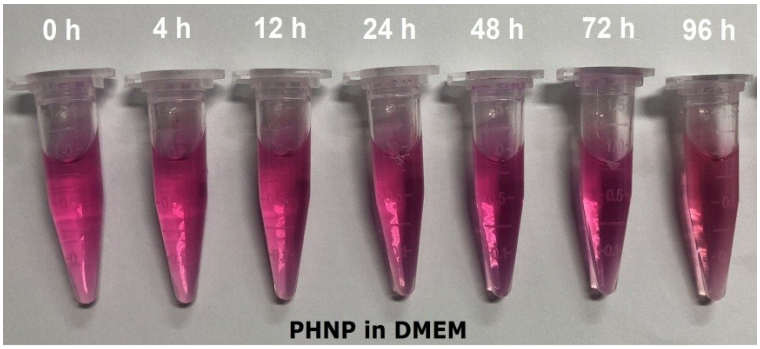

Figure S7. Optical images of PHNP in DMEM cell culture medium during storage.

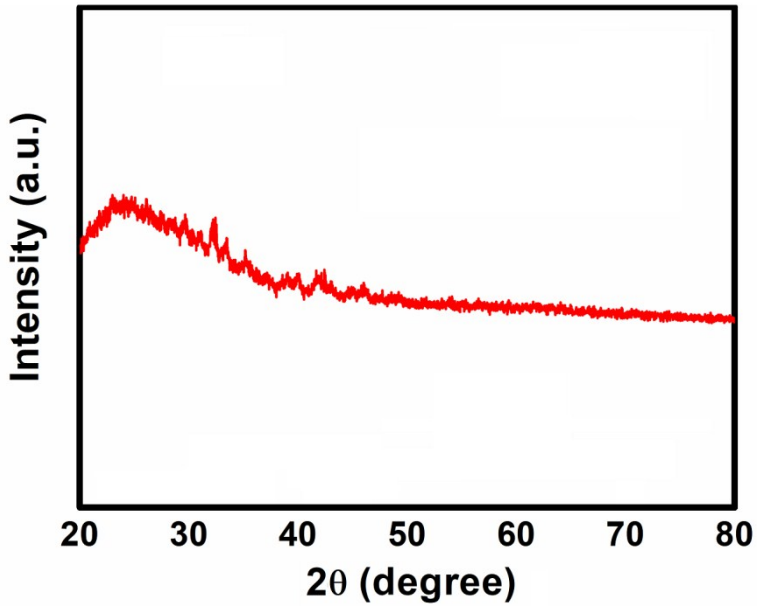

Figure S8. XRD pattern of PHNP.

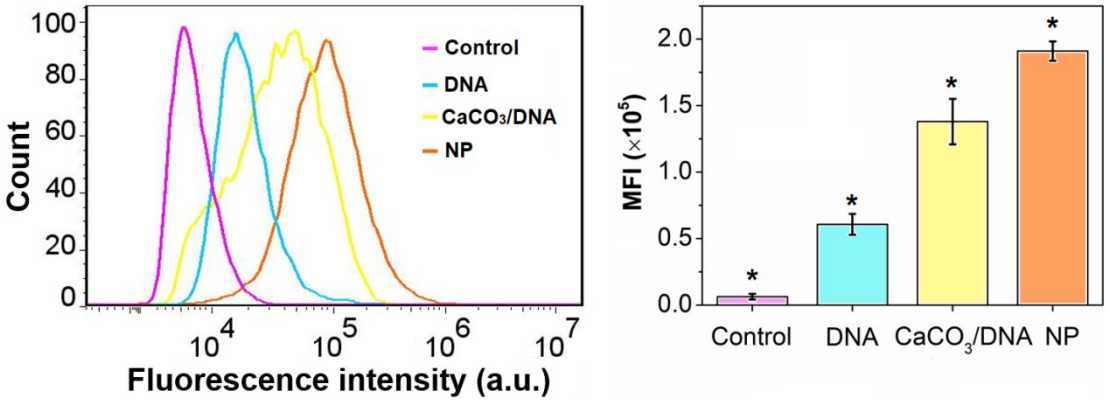

Figure S9. Cellular uptake of $CaCO_3$/DNA nanoparticles as compared with NP (PS/$CaCO_3$/DNA nanoparticles) in M2 macrophages (J774A.1 cells stimulated by IL-4 and IL-10 for 24 h) studied by flow cytometry. M2 macrophages without treatment were used as a control. * $p<0.05$ as compared with all other treatments.

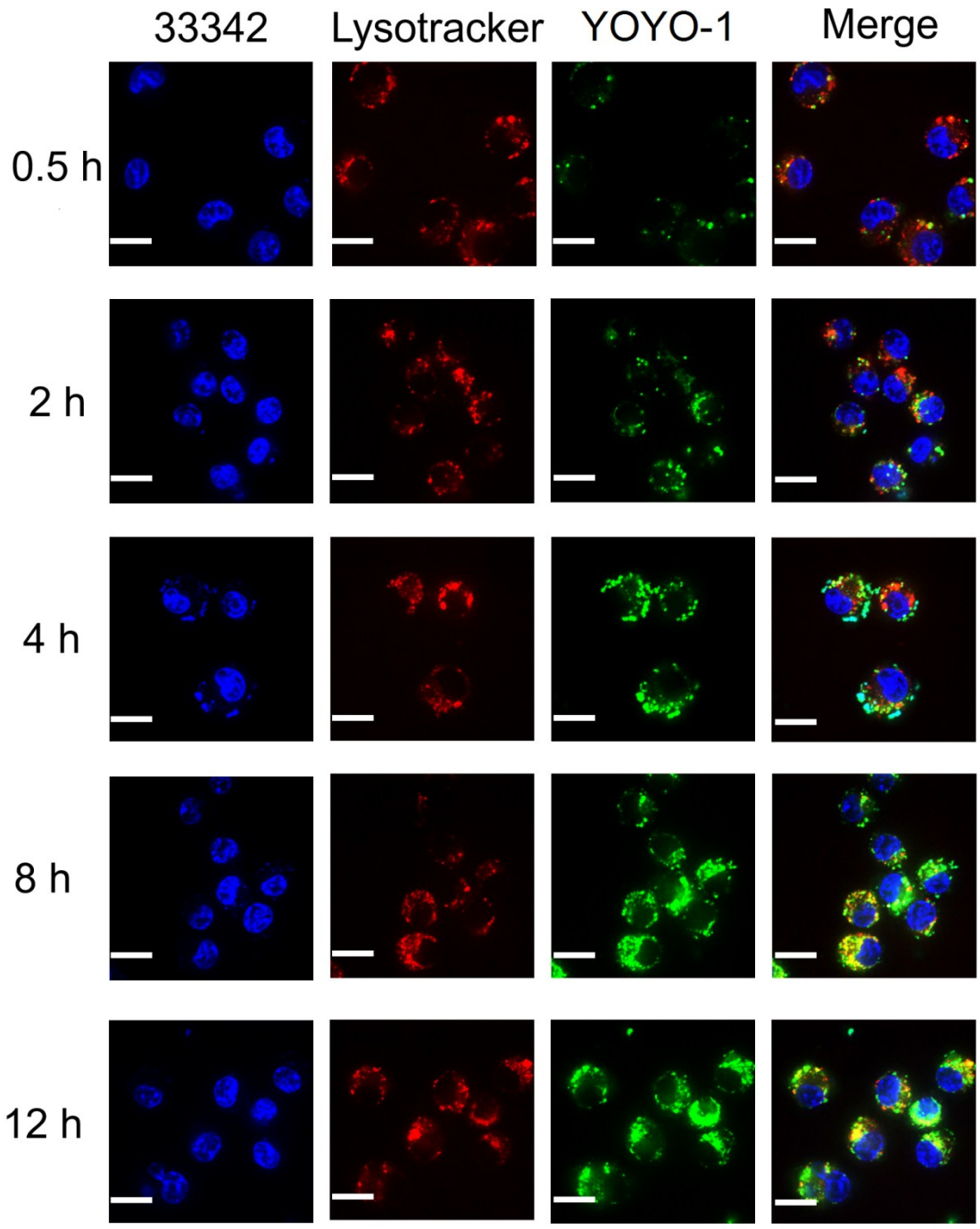

Figure S10. The confocal microscopy images of M2 macrophages (J774A.1 cells stimulated by IL-4 and IL-10 for 24 h) treated with PHNP for different times. DNA was labeled by YOYO-1. Scale bar: 14 μm.

As shown in Figure S10, YOYO-1 labeled DNA co-localized well with endosomes/lysosomes after 30 min of incubation, indicating that DNA loaded nanoparticles were trapped in endosomes/lysosomes after cell uptake. After 2 h incubation, green fluorescence can be observed outside endosomes/lysosomes, indicating that some DNA molecules escape from the endosomes/lysosomes. And the green fluorescence from escaped DNA molecules becomes stronger with an increase in the treatment time. After 12 h incubation, green fluorescence in nuclei can be observed, indicating the successful nuclear transport of DNA with the help of the delivery vector.

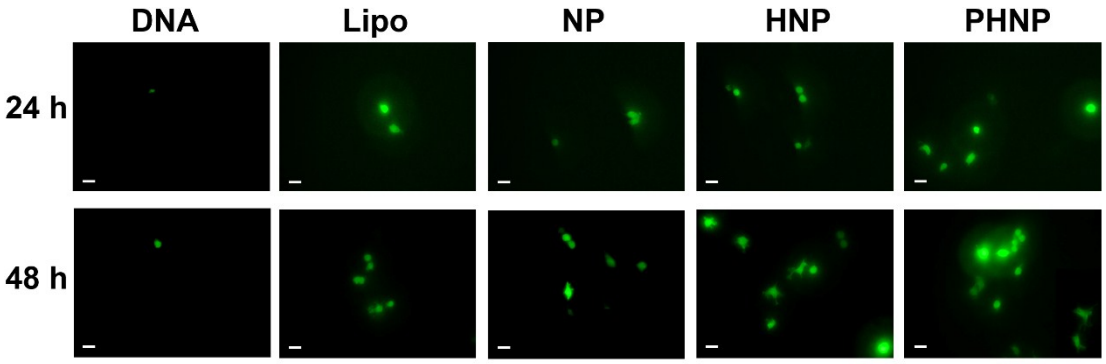

Figure S11. Fluorescence microscopy observation on EGFP expression in M2 macrophages (J774A.1 cells stimulated by IL-4 and IL-10 for 24 h) after being treated with naked DNA (pEGFP-C1) and pEGFP-C1 loaded nanoparticles for 24 h and 48 h. Scale bar: 20 μm.

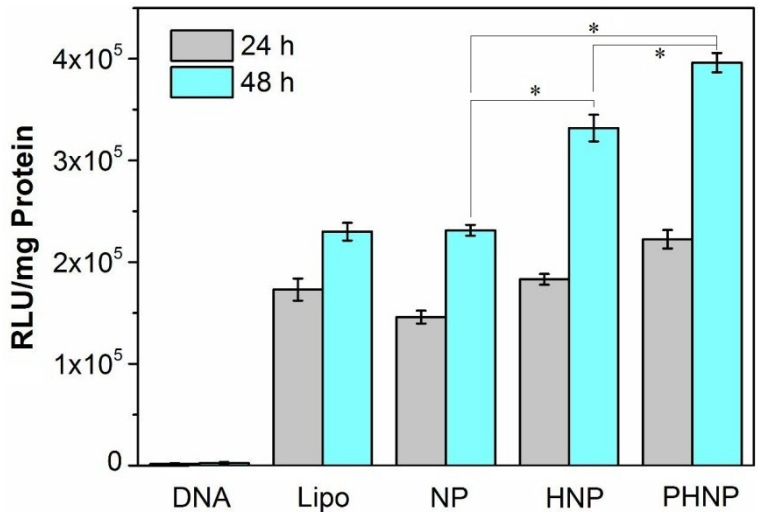

Figure S12. The luciferase expression in M2 macrophages (J774A.1 cells stimulated by IL-4 and IL-10 for 24 h) after being treated with naked DNA (pGL3-Luc) and pGL3-Luc loaded nanoparticles for 24 h and 48 h. * $p<0.05$. Note that the absence of asterisk for other two sets of data does not implies $p \geqslant 0.05$ since it is difficult to mark all significant differences in the figure.

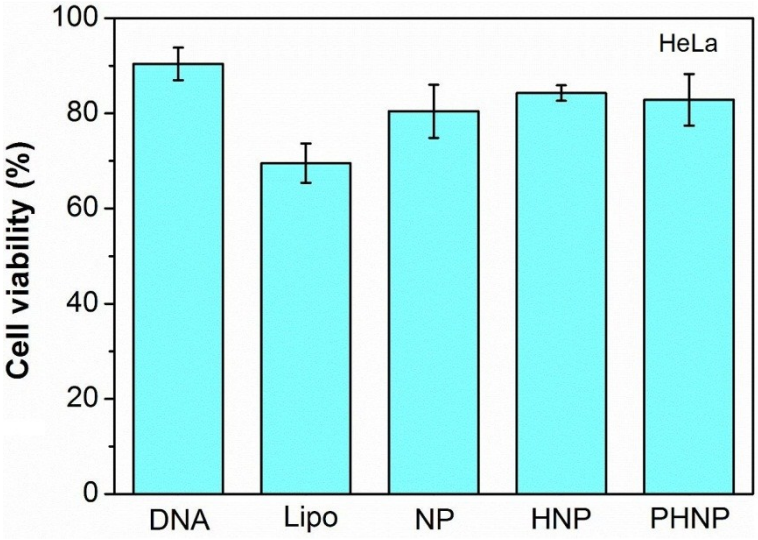

Figure S13. The cell viability of HeLa cells after being treated with naked DNA (pDNA IL-12) and pDNA IL-12 loaded nanoparticles for 48 h.