<Supplementary data>

A nanogel with passive targeting function and adjustable polyplex surface properties for efficient anti-tumor gene therapy

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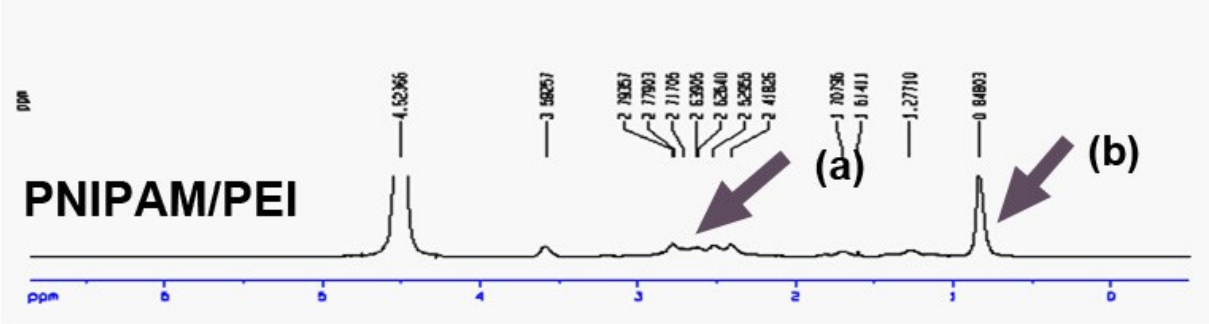
S1, The composition of PNIPAM/PEI nanogel as tested by NMR.



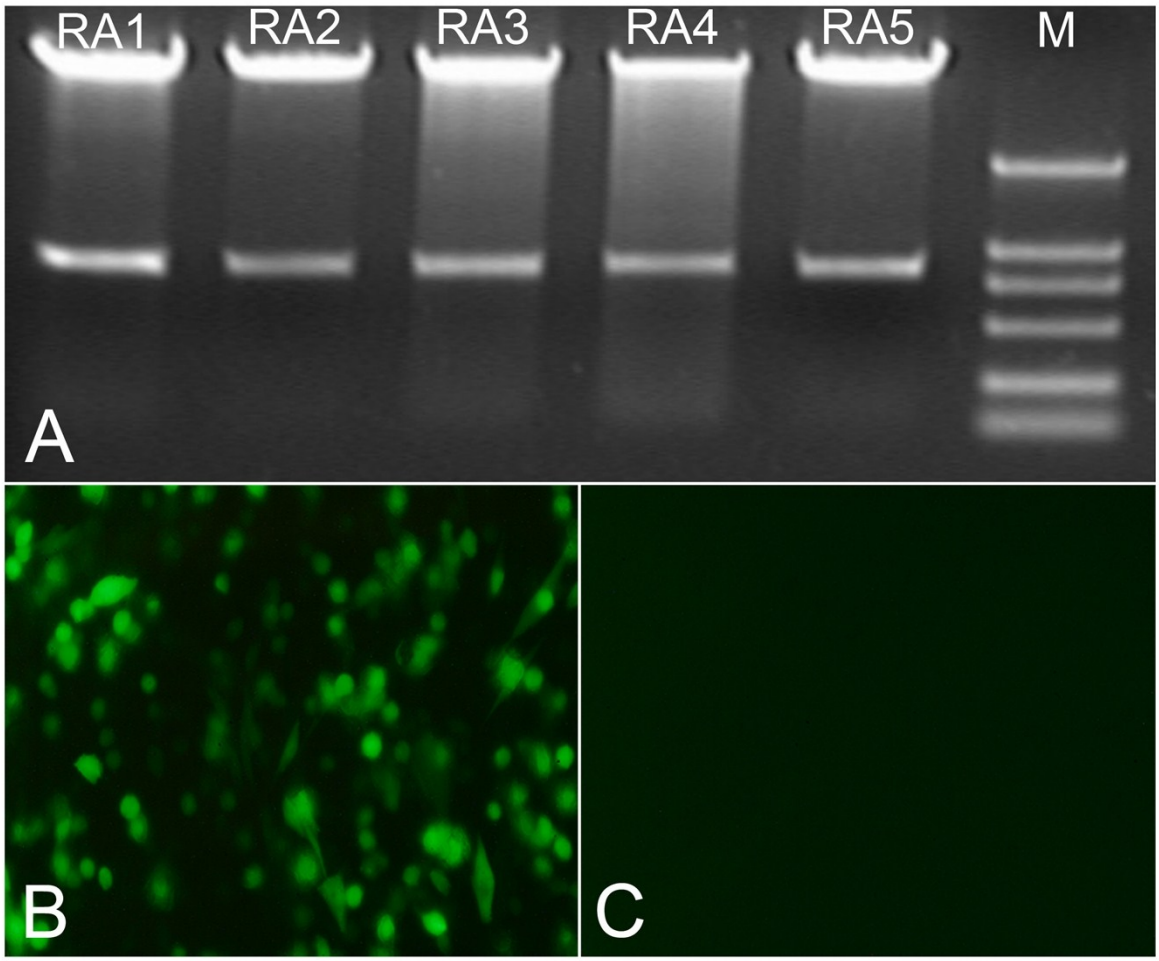
(a) reflecting PEI; (b) reflecting PNIPAM

Supplementary Fig. 1. The NMR characterization of PNIPAM/PEI core shell nanogel as compared with the PNIPAM and PEI.

S2. Amplification and purification of plasmid DNA

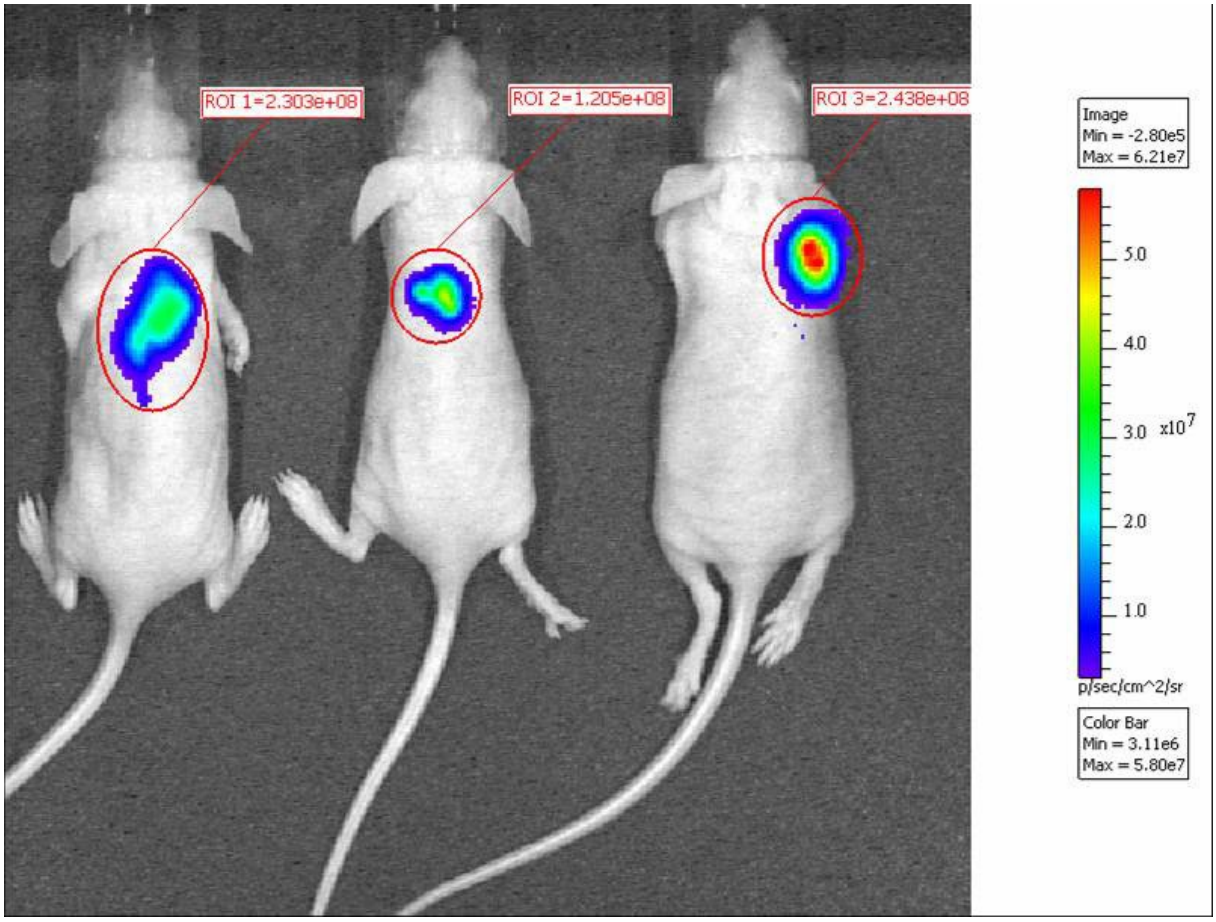
A DNA fragment encoding Ricin A (RA) was cloned by PCR using primers sene:5'-ATTAAGCTTGCCGCCACCATGATCTTCCCGAAACAGTAC-3'; antisense:5'-ATTGAATTCGAACTGGCTGCTCGG-3', and inserted into pEGFP-N1 between the multiclonal sites HindIII and EoR1. So the plasmid containing green fluorescence protein gene, RA gene (pRA-EGFP) can express the firefly luciferase and Ricin A fusion protein. Here, the plasmid DNA encoding a red-shifted variant of wild type green fluorescence protein (GFP) (pEGFP-N1, Clontech Laboratories Inc., USA) was used as control. All the transfection efficiency was evaluated by this pEGFP-N1. Both the pRA-EGFP and pEGFP-N1 plasmid was amplified in transformed bacterium coli cultured 1 mL Luria Bertani medium containing 10 mg/ml kanamycin, and then shake the medium 280 rpm for 16 hours at 37 ^{o}C. The amplified pRA-EGFP was purified using the Concert Rapid Plasmid Miliprep Kit (Life Biotechnologies). The purity of such pDNA was indicated by the A_{260}/A_{280} value which was about 1.8 in the range 1.7 – 2.0. The

concentration of pDNA was then measured by ultraviolet absorbance with a GeneQuant DNA/RNA CalcuLator at 260 nm (Pharmacia). The purity of the pRA-EGFP was further confirmed using double restriction enzyme digestion with BamH1 and HindIII and indicated by 0.7% agarose gel electrophoresis. In this study, a DNA fragment encoding Ricin A (RA) was cloned by PCR. Then its was inserted into the pEGFP-N1 DAN between the multiclonal sites HindIII and EoR1. Such plasmid containing green fluorescence protein gene and Ricin A gene (pRA-EGFP) can express the firefly luciferase gene and Ricin A fusion protein as drived by the SV40 promoter and enhancer. As showed in Supplementary Fig 2 , the pRA-EGFP was successfully constructed as confirmed by the five clones (RA1 to RA5). Here, the band M referred to the marker. The plasmid DNA encoding a red-shifted variant of wild type green fluorescence protein (GFP) (pEGFP-N1) was used as control Supplementary Fig 2 B. In the further experiments, the cytotoxicity of the constructed pRA-EGFP gene was evaluated by the 293T cell line as shown in Figure 3 C. The standard transfection carrier lipo2000 was used in both experiments. From Figure 3 B, we can find that the gene can be successfully transferred in to cell and can express corresponding protein GFP. Comparing Figure 3 B (pEGFP-N1), Figure 3 C showed that cells were almost killed by the RA protein. So the Ricin A was successfully inserted into pEGFP-N1 DNA. Ricin A is an abundant protein component generated from the ricinus seeds (castor beans). It can block protein synthesis once transferred into the cells, consequently causing cell death. This protein is one of the world's deadliest poisons which is also extremely toxic to mammalian cells. The in vitro studies reported that ricin A can attach to tumor antibody directly. This indicated that it is not toxic to the healthy tissue which means that ricin A is powerful in fight against cancer. However, how to decrease its high immunogenicity by nanocarriers is a big problem for ricin A’s clinical application.



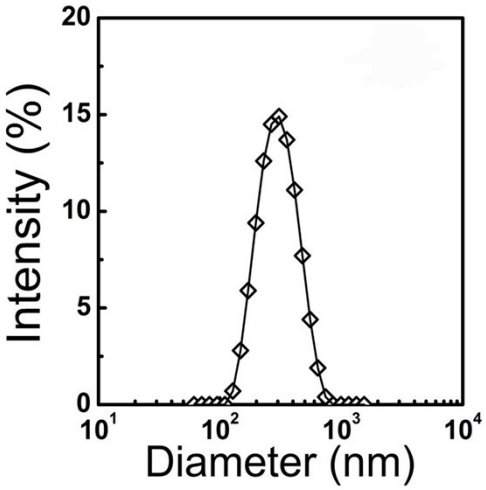
Supplementary Fig. 2. Toxic protein Ricin A (RA) gene and green fluorescent protein EGFP gene encoded plasmid DNA (pRA-EGFR) construction and their properties evaluation. The bands RA1 to RA5 corresponded to five times clones by PCR (A). The pEGFP-N1 proteins was used as control for evaluating the transfection efficiency (B). The ricin A was successfully inserted into PEGFP as confirmed because the cells were almost killed with no fluorescent images (C).

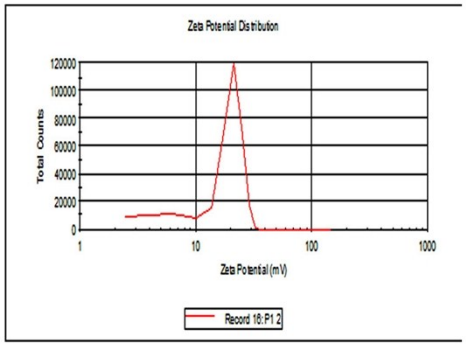
S3. The tumor xenograft model



Supplementary Fig. 3. Successfully established tumor xenograft model which was demonstrated by tumor luminescent images. Mice bearing MDA-MB-231 tumor xenograft were given an intraperitoneal injection of luciferin (Promega) at a dose of 150 mg/kg. The IVIS® Lumina Ⅱ Imaging System (Xenogen) was taken to capture the visible light photograph and luminescent image.

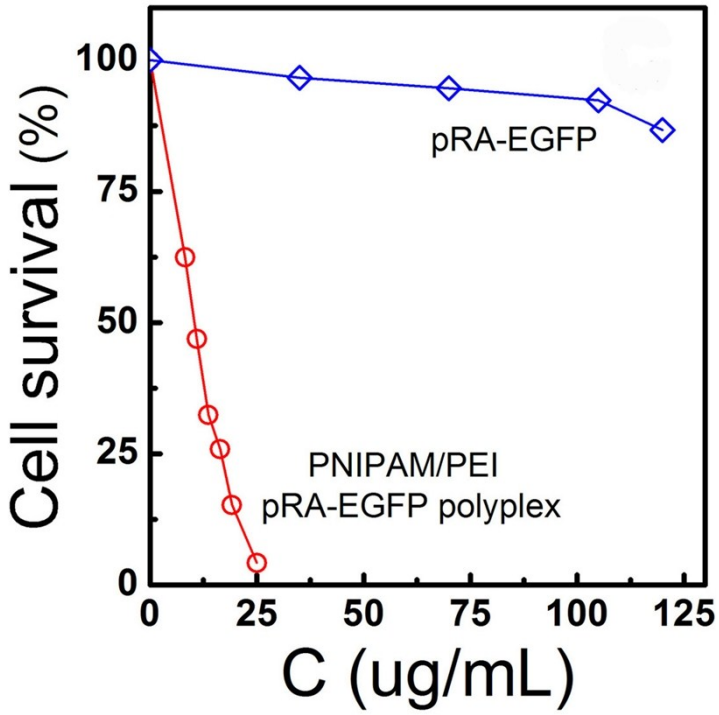
S4. The size and surface potential of PNIPMA/PEI nanogel.





Supplementary Fig. 4. The size distribution of PNIPMA/pEI nanogel with 40% EPI and its surface potential. .

S5. The cytotoxicity profile of nanogel based pRA-EGFP polyplex



Supplementary Fig. 5. The cytotoxicity assay against MDA-MB-231 cells of pRA-EGFP and PNIPMA/PEI-pRA-EGFP polyplex.