Supplementary Information

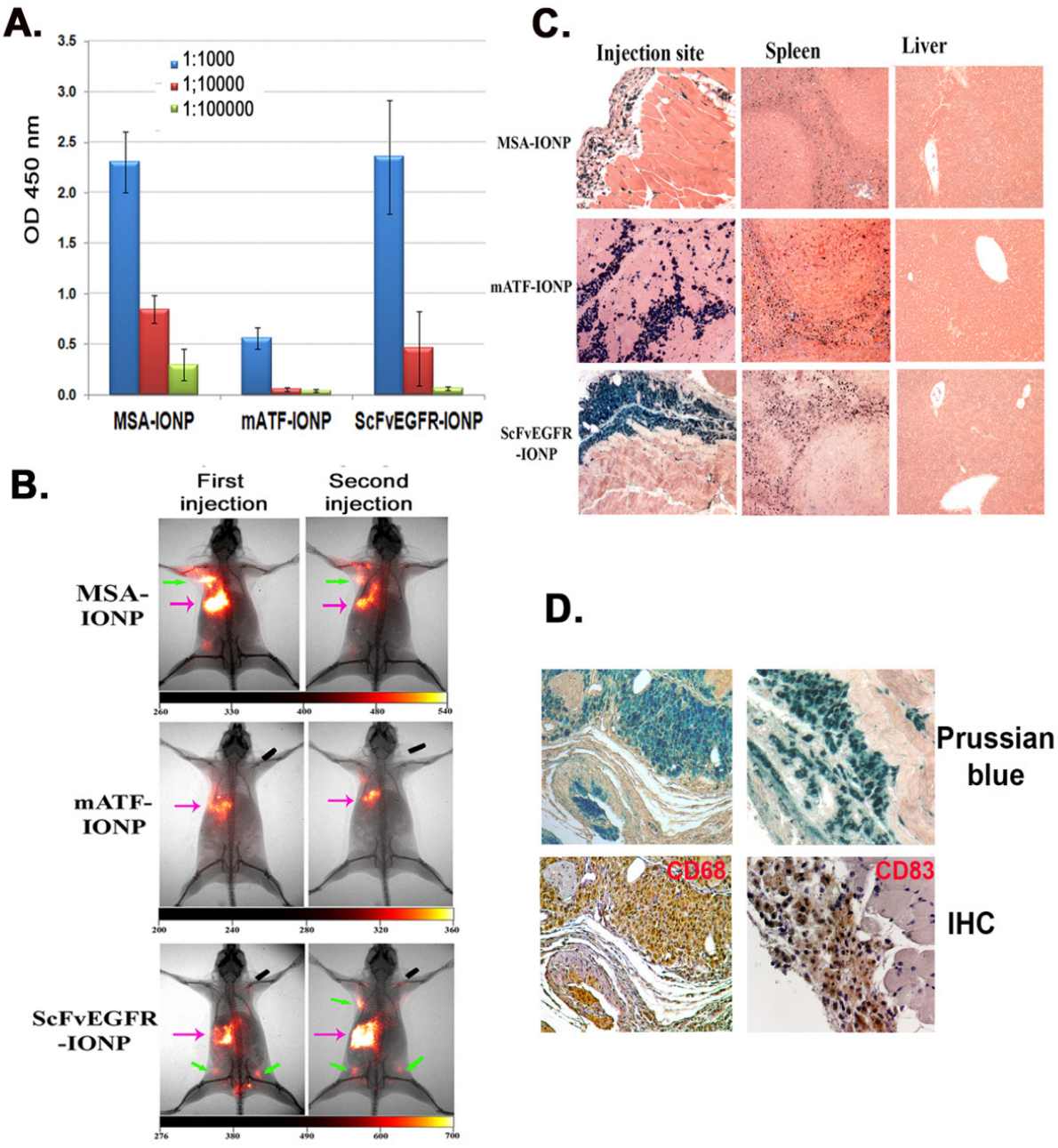

Figure S1 Activation of ligand specific antibodies following s.c. delivery of targeted IONPs and *in vivo* tracking the migration and location of the nanoparticles in normal Balb/c mice.

100 pmol of NIR 830 dye labeled MSA-IONPs, ScFvEGFR-IONPs or mATF-IONPs were injected s.c. into the middle abdominal flank region once per week for two weeks.

A. Comparison of the levels of ligand specific IgG antibodies using ELISA. The mouse serum showed high levels of anti-human ScFvEGFR antibody and a relatively low level of anti-mATF antibody, which was expected since human ScFvEGFR is highly immunogenic in mice. However, a high level of anti-MSA antibody was also found in the mice that received MSA conjugated IONPs. Unconjugated MSA was not immunogenic and the O.D. 450 nm value at a serum dilution of 1:1,000 was 0.096 following three s.c. injections. n=3 mice/group.

B. Optical imaging of the location of the IONPs conjugated with different targeting ligands. NIR optical imaging was performed 48 hours after each injection using the Kodak FX *in vivo* imaging system. Images shown are NIR optical images overlaid with X-Ray images of the mice. Pink arrows: injection site; Green arrows: sentinel lymph node areas. Optical signals were detected at the injection sites 48 hours following injection. However, strong optical signals were detected in the areas of draining lymph nodes of the mice that received ScFvEGFR-IONPs and MSA-IONPs, but not mATF-IONPs.

C. Histological analysis of the paraffin tissue sections using Prussian blue staining. Tissues were collected at the end of two weeks. High levels of the IONP positive cells were found in the injection site. A high level of IONP containing cells was also detected in the spleen but not in the liver tissues, suggesting that IONPs or IONP-containing macrophages circulated back to the spleen through the lymphatic system. Blue: cells with IONPs; Red: nuclear fast red background staining.

D. Immunohistochemical staining of paraffin tissue sections collected from the injection site of the mice that received ScFvEGFR-IONPs showed that all IONPs were located inside the CD68 positive cells, a biomarker presented on both macrophage and dendritic cells. CD83 positive cells (mature dendritic cells) were also detected in the areas with IONP accumulation.

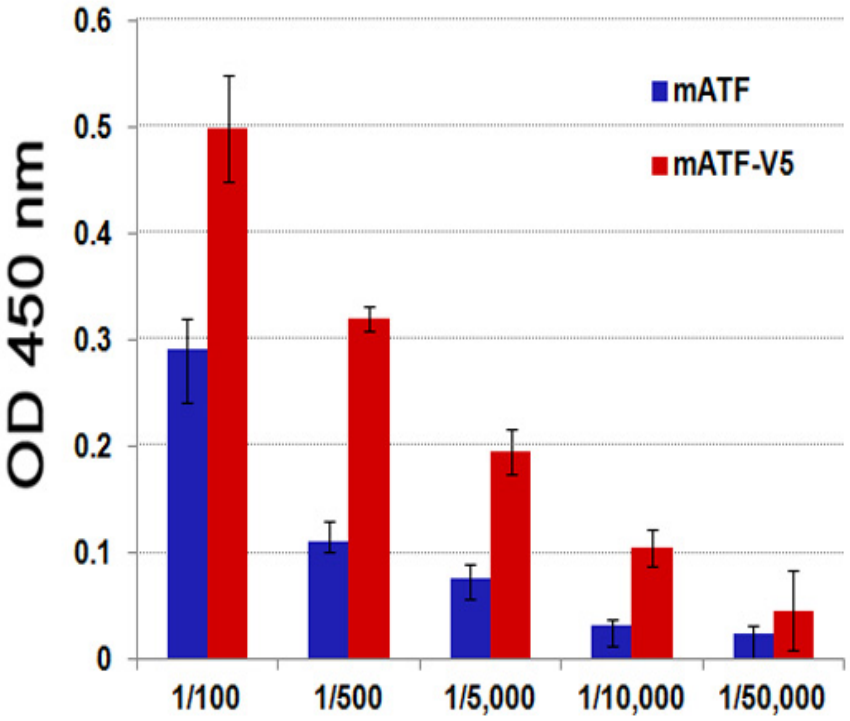

Figure S2 Detection of antibody responses against mATF without or with the V_5 peptide by ELISA

Mice received mATF-V5-conjugated IONP s.c. once per week for two injections. Mouse serum samples were examined using ELISA on a plate coated with mATF without V_5 or mATF-V_5 peptides.

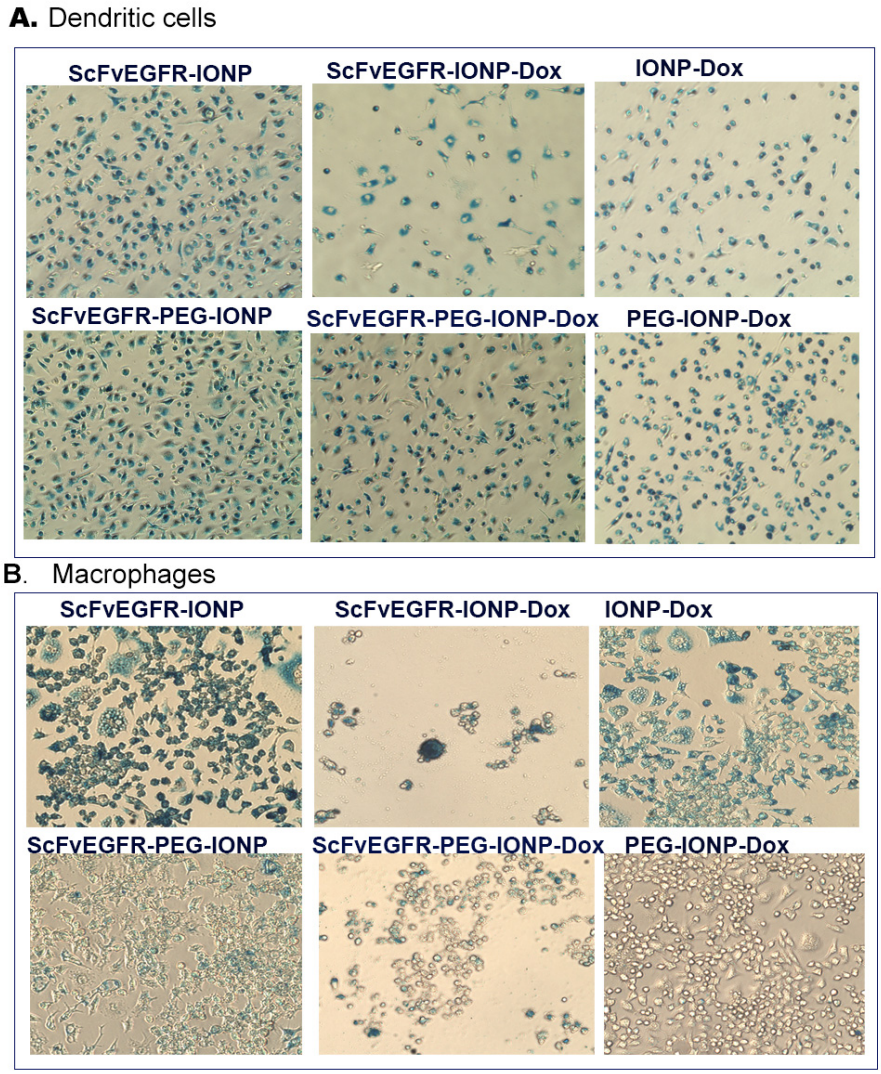

Figure S3 Cytotoxic effects of ScFvEGFR-IONP-Dox theranostic nanoparticles on mouse macrophages and dendritic cells.

Mouse macrophages or dendritic cells were plated in 24 well culture plates at 60 to 70% confluence for 24 hours. 50 nM Dox equivalent IONPs-Dox, PEG-IONP-Dox, ScFvEGFR-IONPs-Dox, or ScFvEGFR-PEG-IONP-Dox, as well as nanoparticle equivalent concentration of ScFvEGFR-IONP or ScFvEGFR-PEG-IONP were added into the wells. 48 hours after treatment, unbound IONPs were washed off with PBS and cells were fixed. Following Prussian blue staining, cells were examined under an inverted microscope to determine uptake of t he theranostic nanoparticles by cells and cytotoxic effects of the nanoparticle-drugs.

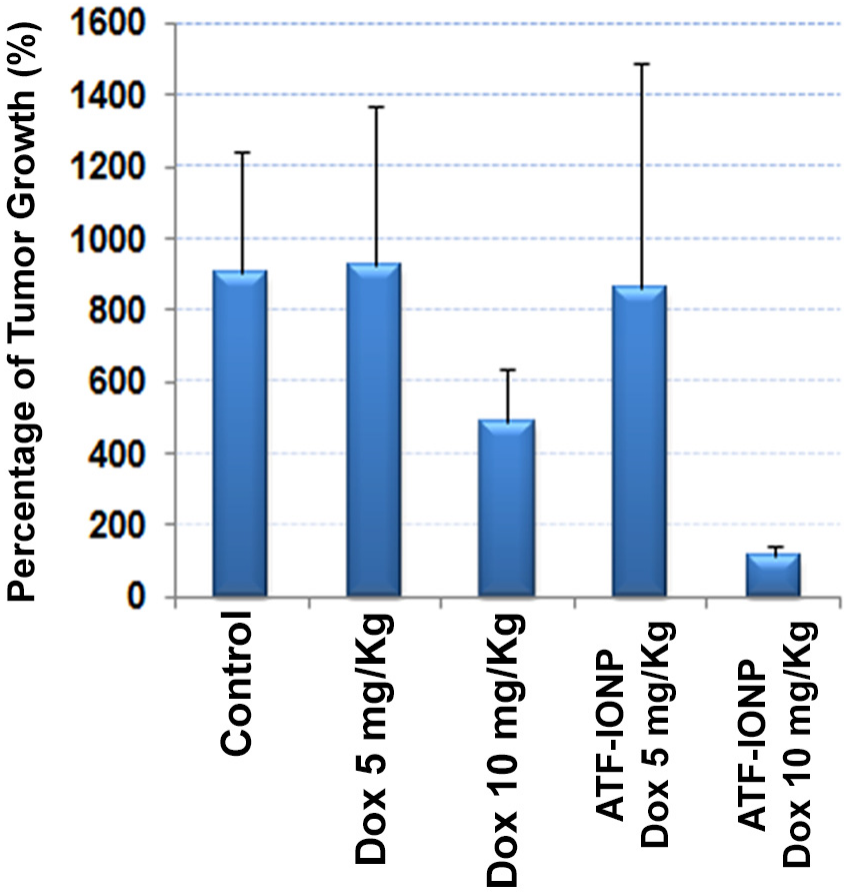

Figure S4 ***In vivo*** **effect of systemic delivery of different Dox doses on the inhibition of the growth of mouse mammary tumors.**

Mice bearing 4T1 mouse mammary tumors were treated with 5 mg/Kg or 10 mg/Kg of Dox, or Dox equivalent IONPs via the tail vein injections once every 5 days for four injections. The percentage of tumor growth inhibition was calculated from mean values of the mammary tumor volumes at 5 days following the last treatment of theranostic IONP-Dox. n=5 mice.

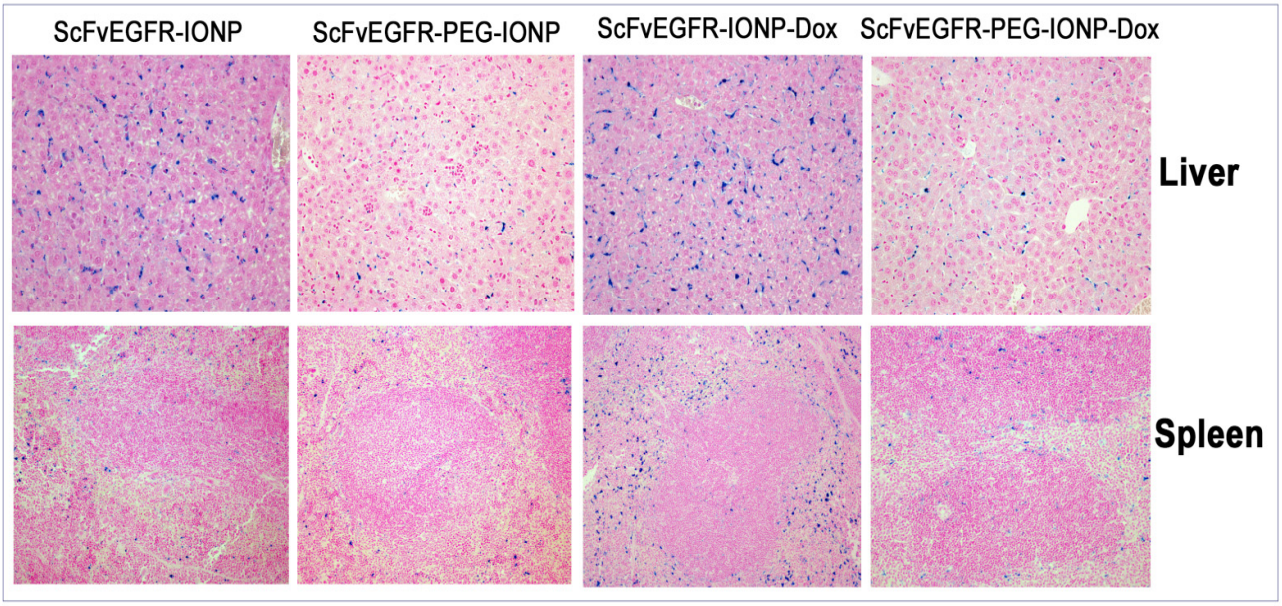

Figure S5 Effect of PEG-modification on non-specific uptake of targeted nanoparticles in the liver and spleen following systemic delivery

Mice bearing 4T1 mouse mammary tumors receive the tail vein injections of 100 pmol of ScFvEGFR conjugated IONP or PEG-IONP, without or with Dox once per week for three injections. Mice were sacrificed 7 days after the last injection and tissues were collected for histological analysis. Paraffin tissue sections of the liver and spleen were examined using Prussian blue staining to determine the levels of IONP accumulation. Bright field microscopic images were taken using 20 x (liver) or 10x (spleen) lens. A low magnification lens was used for spleen tissue sections since the IONPs were distributed unevenly in the spleen. ScFvEGFR conjugated and PEG-modified IONPs or IONP-Dox had significant lower levels of non-specific uptake by the RES in the liver compared to the liver tissues from the mice received the IONPs without the PEG modification. Additionally, changes in the level of the RES uptake in the spleen were apparent in the mice received ScFvEGFR-PEG-IONP-Dox compared with that of ScFvEGFR-IONP-Dox injected spleen tissues. But there was no marked difference in the spleens of mice received either ScFvEGFR-IONP or ScFvEGFR-PEG-IONP.