Supplemental Results

Tumor Penetrating Theranostic Nanoparticles for Enhancement of Targeted and Image-guided Drug Delivery into Peritoneal Tumors following Intraperitoneal Delivery

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Figure S1. Evaluation of nonspecific uptake of uPAR targeted and non-targeted IONPs in normal tissues following i.p. or i.v. delivery.

Mice bearing s.c. and orthotopic (Ortho) tumors received an i.v. or i.p. delivery of 300 pmol of ATF-PEG-IONP or BSA-PEG-IONP for 24 hours. Frozen tissue sections were examined by Prussian blue staining to detect the level of IONP positive cells in the liver and spleen of the mice.

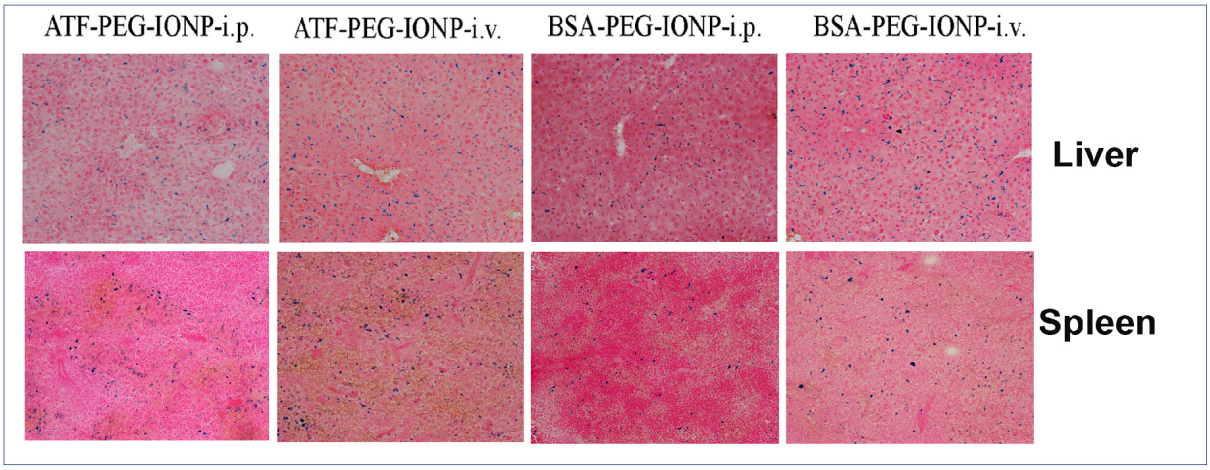

Figure S2. Evaluation of the level and intratumoral distribution of non-targeted BSA-PEG-IONPs following i.p. or i.v. delivery.

Frozen tissue sections were labeled with an anti-CD68 antibody (green) and then Prussian blue staining. Fluorescent images were overlaid with bright field images from the same field. Both orthotopic and s.c. tumors showed low levels of IONP positive cells after i.p. or i.v. delivery of non-targeted BSA-PEG-IONPs.

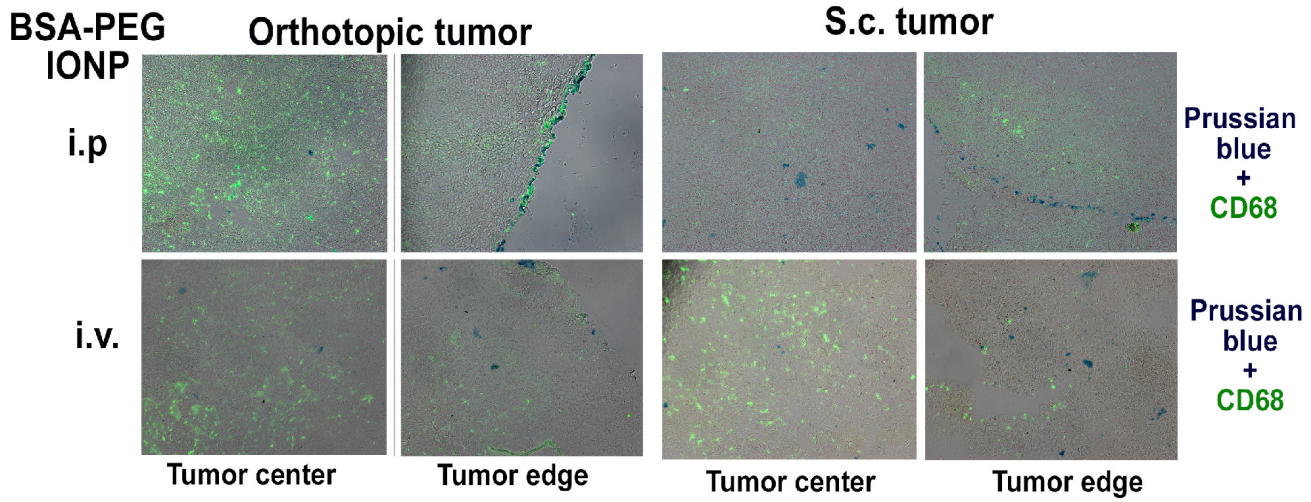

Figure S3. Inhibition of nonspecific uptake of nanoparticles in macrophages using PEG-coated IONPs.

Mouse primary macrophage cells were isolated from femur bones of normal CD1 mice. Cells were then cultured in RPMI-1640 medium supplemented with 20 ng/ml mouse recombinant macrophage colony-stimulating factor for 5 days before conducting the study. Macrophages were cultured on 24-well plates for 2 days. 2.5 pmol of various nanoparticles were added to each well for 24 hours. After washing, cells were fixed and stained with Prussian blue solution to detect the amount of IONPs in the cells.

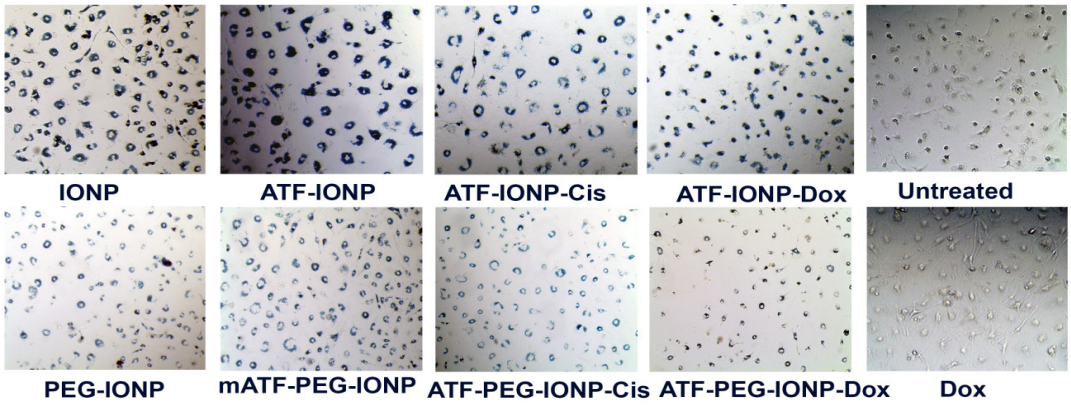

Figure S4. *In vitro* cytotoxicity of ATF-PEG-IONP and BSA-PEG-IONP theranostic nanoparticles carrying Cis or Dox.

The percentage of viable cells following treatment for 72 hours with different drug equivalent concentrations of conventional Cis or Dox, or nanoparticle-Cis or Dox was determined by XTT cell proliferation assay. A, The PANC02 mouse pancreatic cancer cell line showed a relatively low sensitivity to conventional Cis treatment. ATF-PEG-IONP-Cis treatment enhanced cytotoxic effect at a higher Cis concentration (3.3 μM). B, Mouse pancreatic tumor cells had intermediate sensitivity to Dox treatment and 0.85 μM of Dox inhibited cell growth about 50%. In tissue culture, PANC02 tumor cells had a similar sensitivity to conventional Dox or ATF-PEG-IONP-Dox treatment. Bar figure shows the mean and standard deviation of three repeat wells.

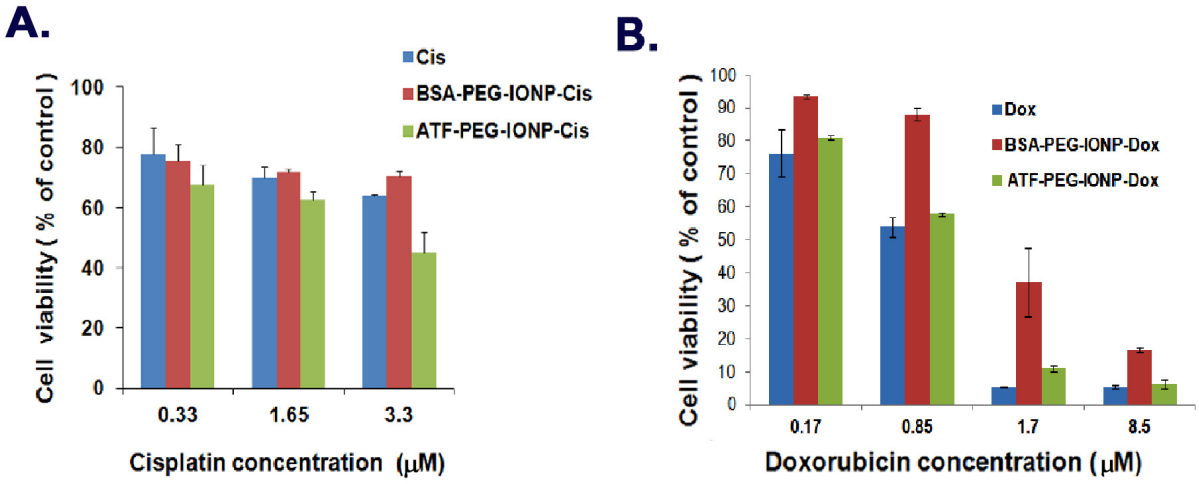

Figure S5. Evaluation of systemic toxicity by measuring body weight changes following IONP treatment.

There was no apparent toxicity as evidenced by body weight of the mice following treatment with theranostic nanoparticles carrying (A) Cisplatin or (B) Doxorubicin.

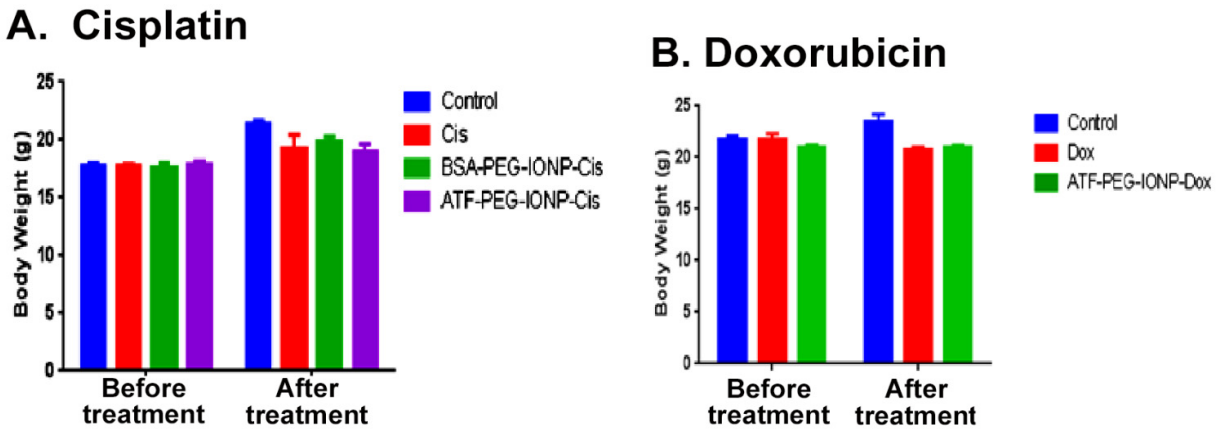

Figure S6. Determination of the effect of theranostic nanoparticle treatment on ascites accumulation in mice bearing pancreatic tumors.

A, I.p. delivery of theranostic IONPs carrying Cis led to a marked decrease in ascites fluid accumulation in the peritoneal cavity of treated mice relative to untreated or conventional Cis treated mice. The total amount of ascites collected from six control mice was considered as 100%. The amount of the total ascites collected from six mice in each group was compared with the control group to determine the percentage of inhibition of ascites production. B. The effect of uPAR targeted IONP-Dox treatment on the production of ascites. N=4 mice/group.