Supporting information

IGF1 Receptor Targeted Theranostic Nanoparticles for Targeted and Image-Guided Therapy of Pancreatic Cancer

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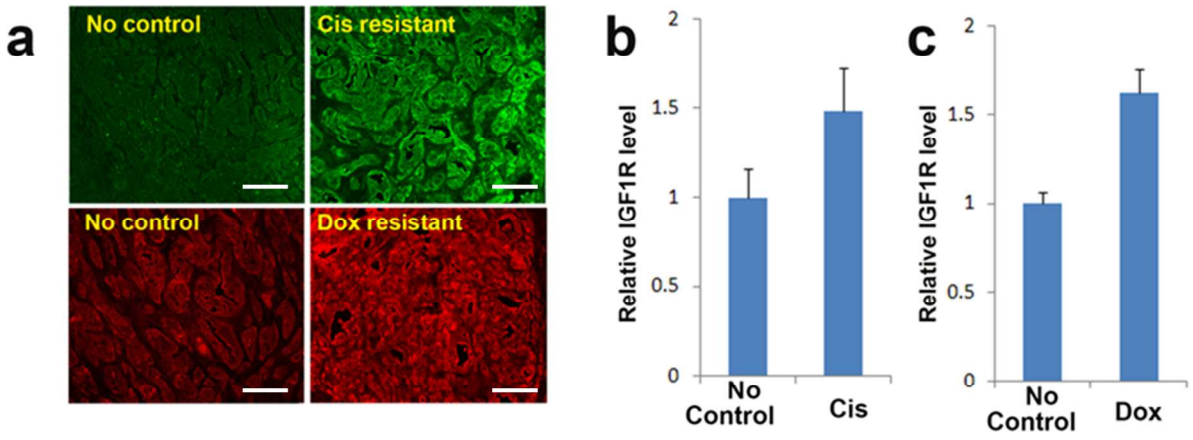

Figure S1. Upregulation of IGF1R in drug resistant human pancreatic PDX tumors.
Pancreatic PDX tumor bearing mice received six weekly treatments of 5 mg/kg dose of chemotherapy drugs, cisplatin or Dox. **a)** Levels of IGF1R expression in Cisplatin treated and control tumor tissues (green, Alexa fluor-488); Levels of IGF1R in Dox treated and control tumors (Red, Alexa fluor-555). **b)** and **c)** Relative levels of IGF1R expression in the control no treatment tumors and chemoresistant tumors determined by measuring fluorescence intensity in tumor cells using ImageJ software. Scale bars are 100 µm.

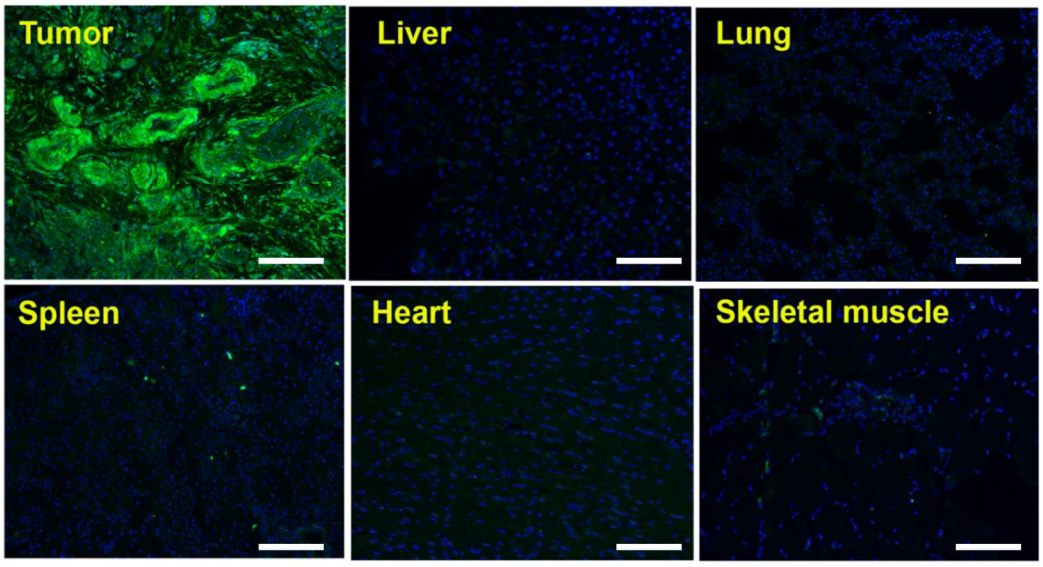

Figure S2. Examination of the levels of IGF1R expression in the first passage of human pancreatic PDX tumor tissues and the major normal organs collected from the tumor bearing SCID mice using immunofluorescence labeling.
In comparison with a high level of IGF1R in pancreatic tumors (green), the levels of IGF1R in major normal organs were relatively low and only low levels of IGF-1R were found in the liver and muscle. Blue: Hoechst 33342 counterstaining. Scale bars are 100 µm.

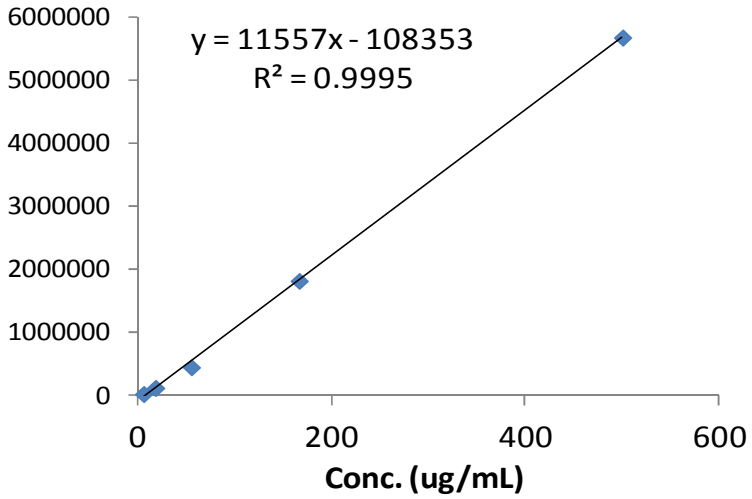

Figure S3. Standard curve of IGF1 ligand by HPLC method with UV detection at 280 nm. It showed a good linear range for IGF1 concentration from 15.6 to 500 µg/mL with R2 = 0.9995. The injection volume was 25 µL.

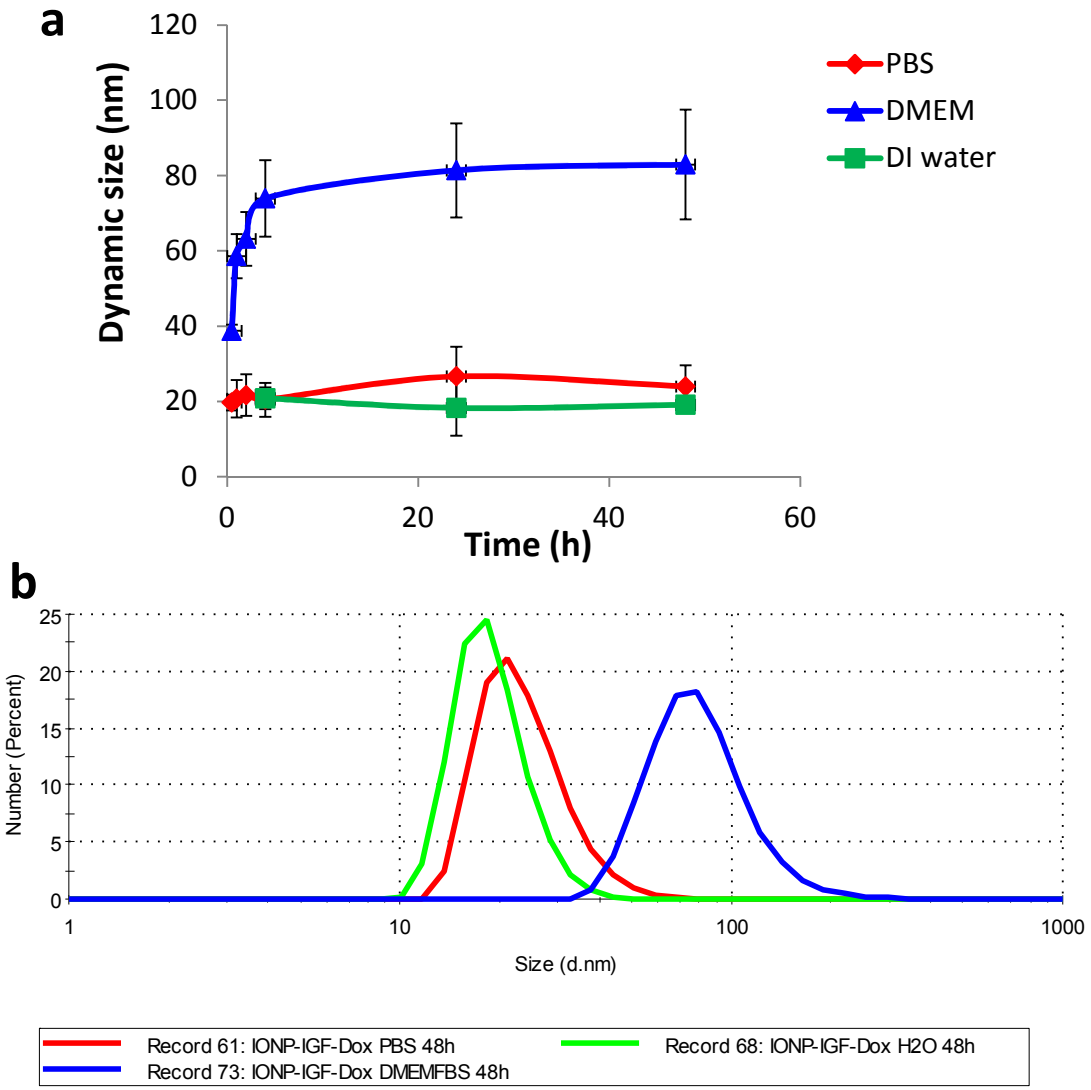

Figure S4. Stability of IGF1-IONP-Dox at different conditions.
a) Stability of IGF1-IONP-Dox in distilled water, PBS and cell culture medium (DMEM with 10% FBS) over 48 h of incubation (n=3, bars represent means ± SD), and **b)** representative dynamic size distribution of IGF1-IONP-Dox in different medium at 48 h of incubation.

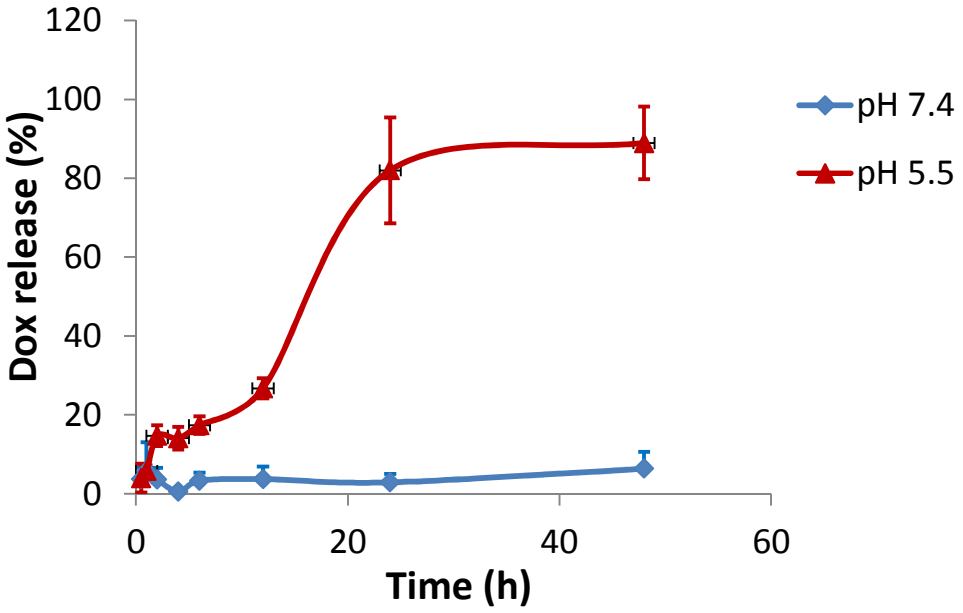

Figure S5. Release profile of Dox from IGF1-IONP-Dox.
IGF1-IONP-Dox was incubated in the borate buffer under different pH conditions for up to 48 h. Dox released from IGF1-IONP-Dox was collected by centrifugation at 3000 rpm for 10 min using Nanosep 100 K column. The amount of released Dox was measured by HPLC. The percentage of release was calculated from the total amount of conjugated Dox molecules on the nanoparticles.

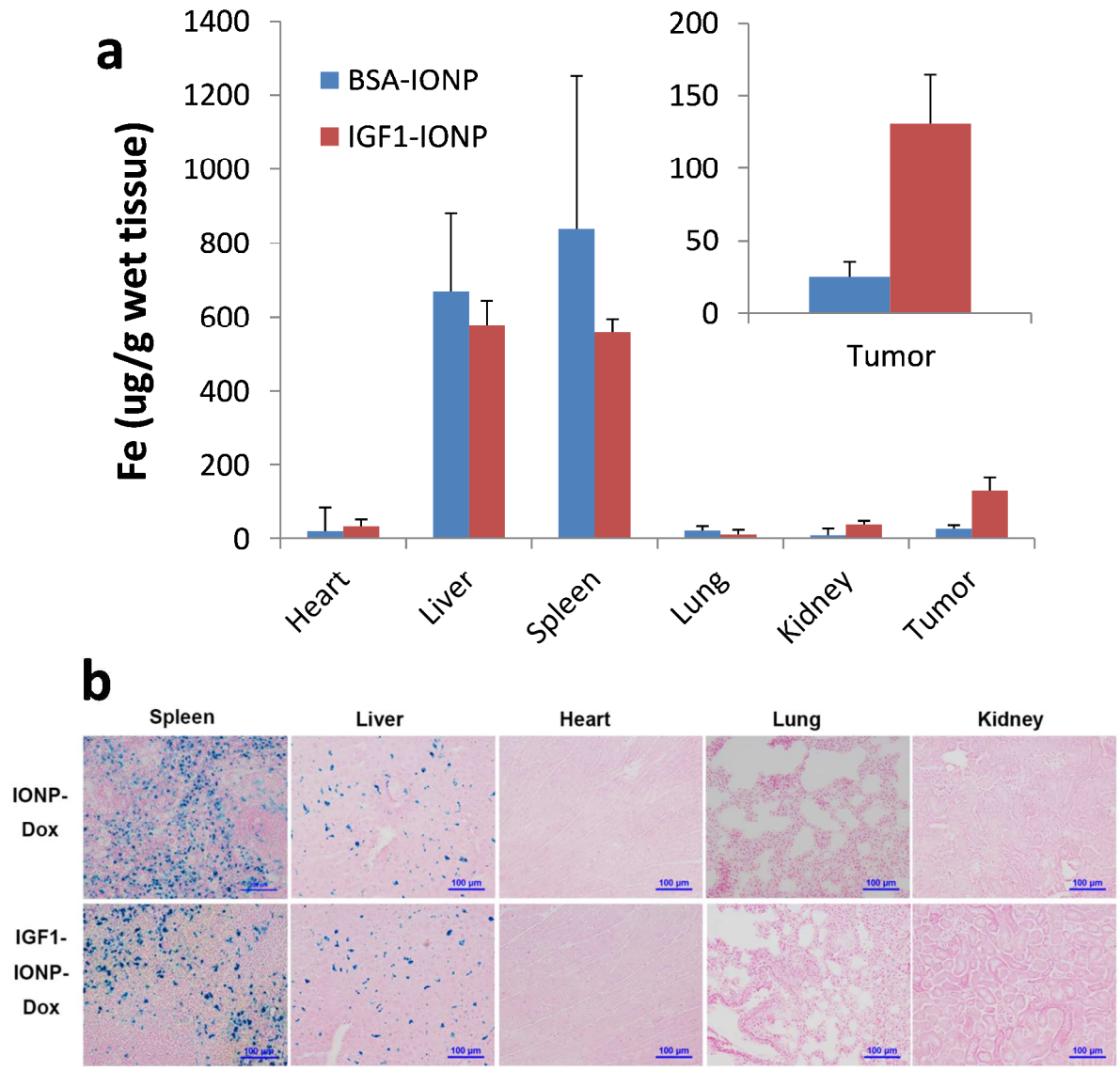

Figure S6. Biodistribution of different IONPs in orthotopic pancreatic PDX tumor and normal tissues following systemic delivery.

a) Tumor and normal organs were collected 24 h after a tail vein injection of 400 pmol of targeted IGF1-IONP or control non-targeted BSA-IONP. Tumor and normal organs were digested using 4 N HNO_3. Iron concentration was measured by Prussian blue colorimetric assay. Iron concentration of tissues was determined by comparing the OD value of the tissue sample with the standard curve produced using known concentration of IONPs. Numbers shown in the bar figure are the mean of three mice in each group. **b)** Prussian blue staining of frozen tissue sections of normal organs and tissues. Scale bars are 100 μm.

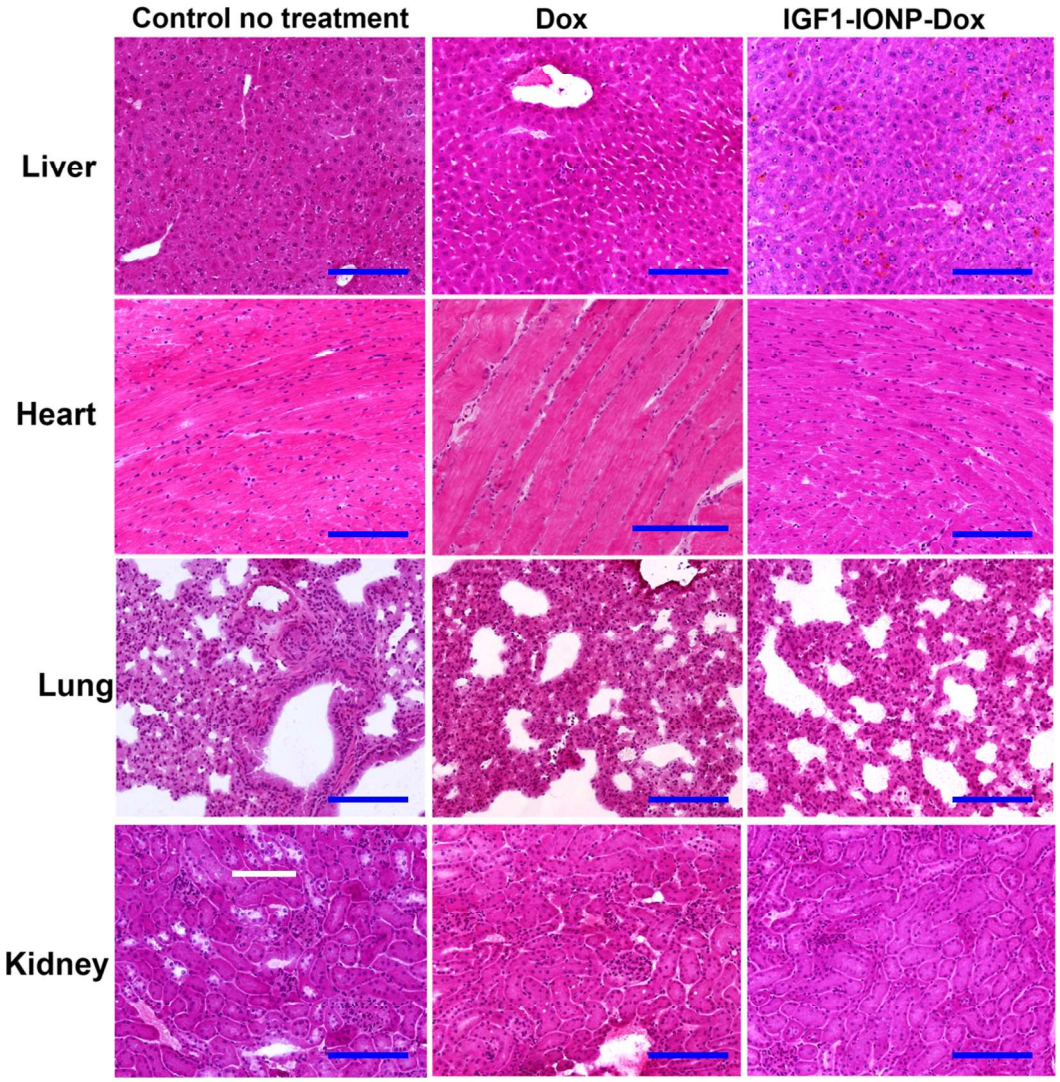

Figure S7. Evaluation of the systemic effect of IGF-1R targeted therapy on normal organs and tissues following six weekly treatments of 5mg/kg of Dox equivalent dose of IGF1-IONP-Dox.

H&E stained frozen tissue sections of normal organs. No apparent morphological and pathological abnormalities were found in normal tissues for all treated mice. Images were taken under 20x lens of a bright field microscope. Scale bars are 100 µm.

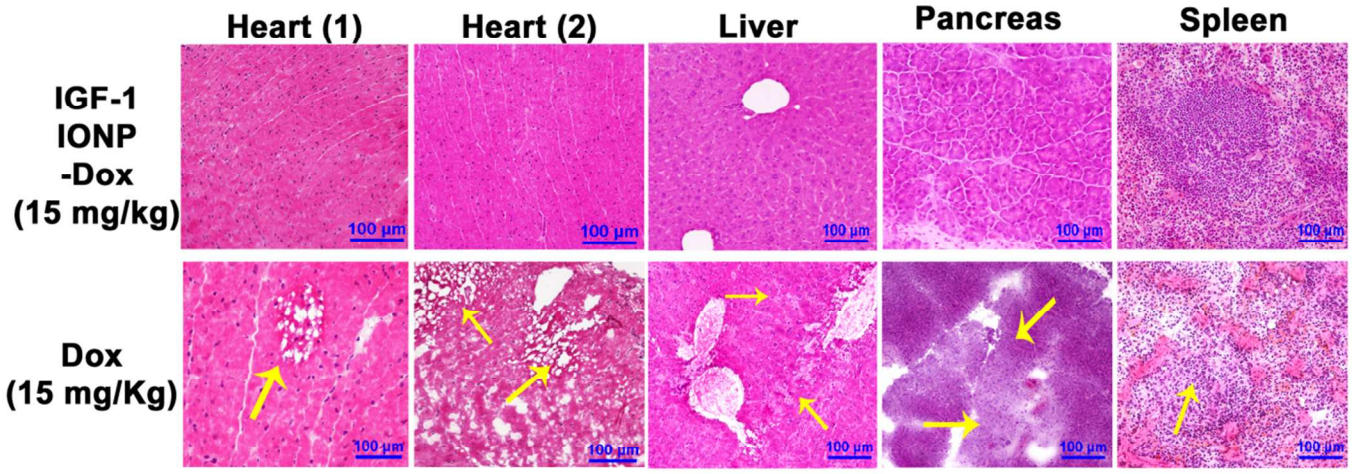

Figure S8. Comparison of systemic toxicity of IGF1-IONP-Dox and conventional Dox administrated at a high dose.
Normal nude mice received tail vein injections of 15 mg/kg of Dox equivalent dose of free Dox (n=2) or IGF1-IONP-Dox (n=2) once every 3 days for three treatments. Mice that received free Dox treatments developed ascites and died 2 days following the third injection. The mean body weight of the mice decreased from 26 to 21 mg, a 20% reduction. However, mice that received three IGF1-IONP-Dox treatments did not show systemic toxicity. The mean body weights before and after treatment were 23 and 23.6 mg, respectively. The mice treated with free Dox also had smaller spleens (90 mg) compared to the mice treated with IGF1-IONP-Dox (200 mg). Histological analysis of frozen tissue sections of normal organs using H&E staining revealed that there was no apparent morphological and pathological abnormality in the mice that received IGF1-IONP-Dox treatments. However, free Dox treated mice showed abnormalities in many normal organs, including necrotic areas in the heart, changes in structure and morphology in the liver and pancreas, and reduced lymphocytes in the white pulp in the spleen. Yellow arrows show abnormal areas. Images were taken under 20x lens of a bright field microscope. Scale bars are 100 µm.

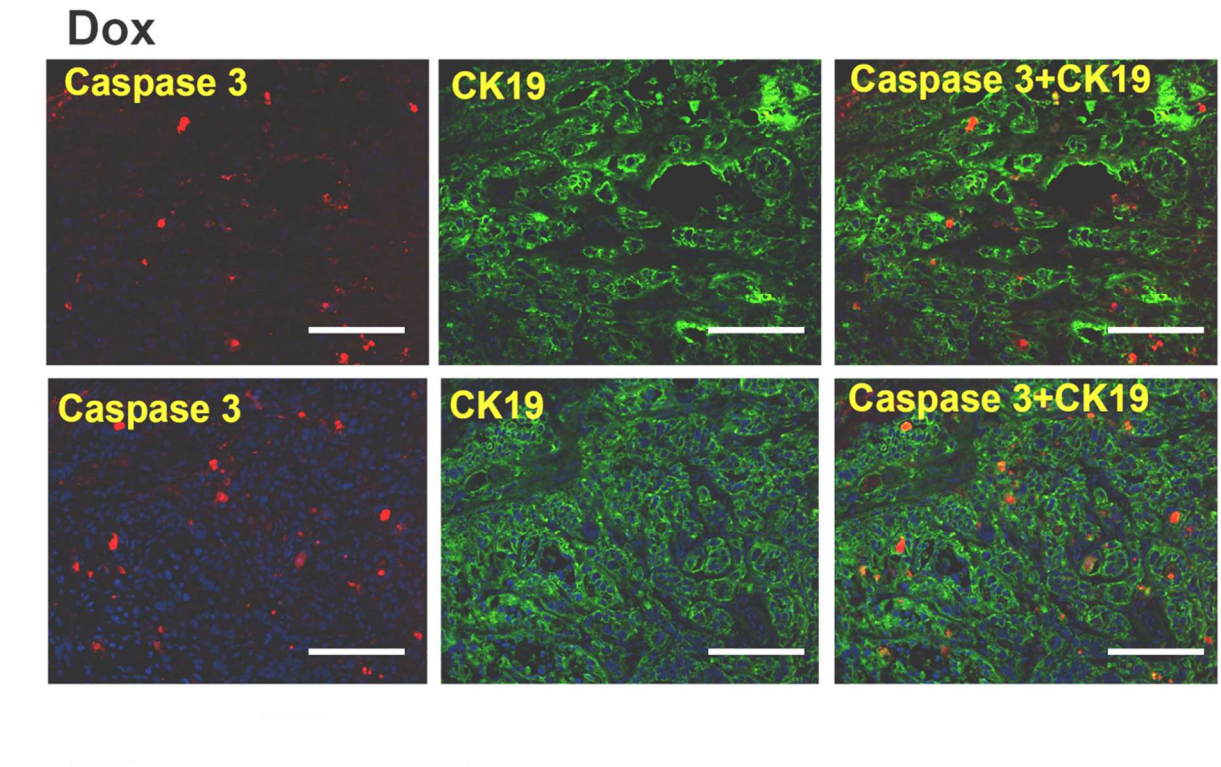

Figure S9. Double immunofluorescence labeling of the apoptotic cells (active caspase-3) and epithelial tumor cells (CK19) in conventional Dox treated PDX tumors.
Low levels of apoptotic cells (active caspase 3, red) were detected in CK19 positive pancreatic tumor cells (green) at the tumor edge (upper panel) and in the tumor center (lower panel) following six weekly treatments of 5 mg/kg of Dox treatments in nude mice bearing pancreatic PDX tumors. Scale bars are 100 μm.