Supporting Information

Erythrocyte Membrane Camouflaged PCN-224 Nanocarriers Integrated with Platinum Nanoparticles and Glucose Oxidase for Enhanced Tumor Sonodynamic Therapy and Synergistic Starvation Therapy

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1. Materials and Methods

1.1 SDS-PAGE analysis

Protein solving liquid was added to protein samples and immersed in boiling water bath for 5 min. After cooling to room temperature, 20 μL mixed samples was added by microsyringe to the sample tanks in gel, which is submerged in electrode buffer solution. The voltage was limited to 60 V before the sample entered the separation glue and was then changed to 150 V for separation. The taken-out gel was dyed by coomassie brilliant blue for 30 min and washed by deionic water for photographing.

1.2 GOx loading studies

GOx and PPE NCs with the weight ratio of 0, 1, 2, 4 were mixed in 1×PBS and agitated for 24 h. Then the suspension was centrifuged and the amount of free GOx in supernatant was determined by BCA kits. The amount of loaded GOx was calculated through subtracting the free GOx in the supernatant from the feeded amount.

1.3 PPGE NCs degradation studies

To study the degradation property of PPGE NCs, 2 mg PPGE NCs were dispersed in 10 mL 1×PBS solution. After 1, 2, 4, 7 days, 1 mL of the solution was extracted and centrifuged, and the absorption of supernatant was detected by UV-Vis spectrum at 519 nm. At the day of 0 and 7, the remained PPGE NCs (precipitate after centrifugation) were also observed by TEM images.

1.4 H_2O_2 production studies

The standard quantitative H_2O_2 concentration curve was first obtained by external addition of H_2O_2 with different concentrations. Then, PGE NCs (1 mg) and GOx (50 μg, calculated equivalent weight) were respectively added to 1 mL pH = 5.0 glucose buffer with different concentrations (0, 1.0, 2.5, 5.0, 7.5 and 10 mM). The reaction was stopped after 30 min, and the PGE NCs were removed by centrifugation. The H_2O_2 in different groups were measured by UV-vis spectrum, and each sample was repeated for three times.

1.5 Cellular acoustic toxicity studies

BxPC-3 cells were cultured in 96-well microplates for 4 h. Then, cells were treated

with planar ultrasound (S = 5 cm^2, f = 3 MHz) with different intensity (namely, 0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.5 2.0 W/cm^2) for 5 min. Then, 10 μL cck-8 reagent was added to each well and was co-incubated with cells for 1 h at 37 ^oC. The absorbance at 450 nm was measured by microplate reader. The results were averaged from three parallel experiments.

1.6 Cellular hypoxia staining studies

0.5 mg PPGE NCs, PE NCs and 1×PBS were respectively incubated with BxPC-3 cells in hypoxia incubator (5% CO_2, 0.3-0.5% O_2). After 24 h incubation, the free NCs were removed, and cells were stained with Hoechst 33342 probe and HIF-1α hypoxia immunofluorescence antibody and observed by a confocal microscope.

1.7 In vivo biosafety studies

To conduct blood biochemical analysis, the blood of sacrificed mice (n = 3) after a single injection of 10 mg/kg PPGE NCs for 15 days was collected. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine (CRE) were measured. In addition, the major organs of mice (including heart, liver, spleen, lung, and kidney) were resected and their indexes were evaluated.

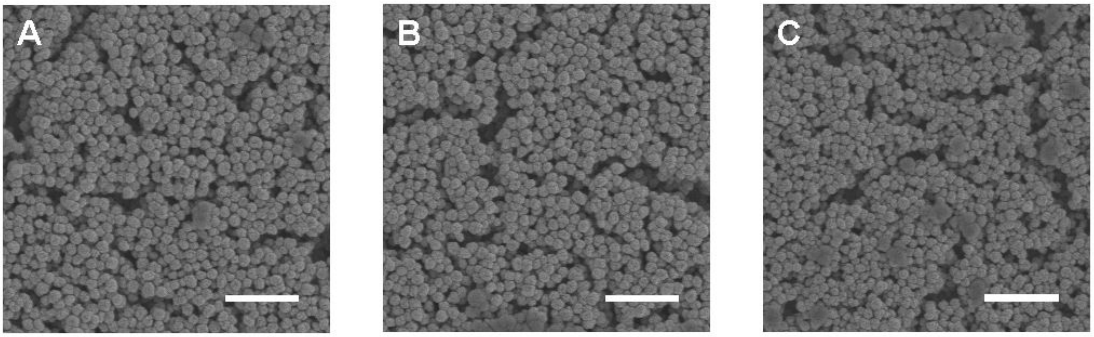

Figure S1. SEM images of (A) PCN-224 NPs, (B) PP NPs, and (C) PPGE NCs. Scale bar: 1 μm.

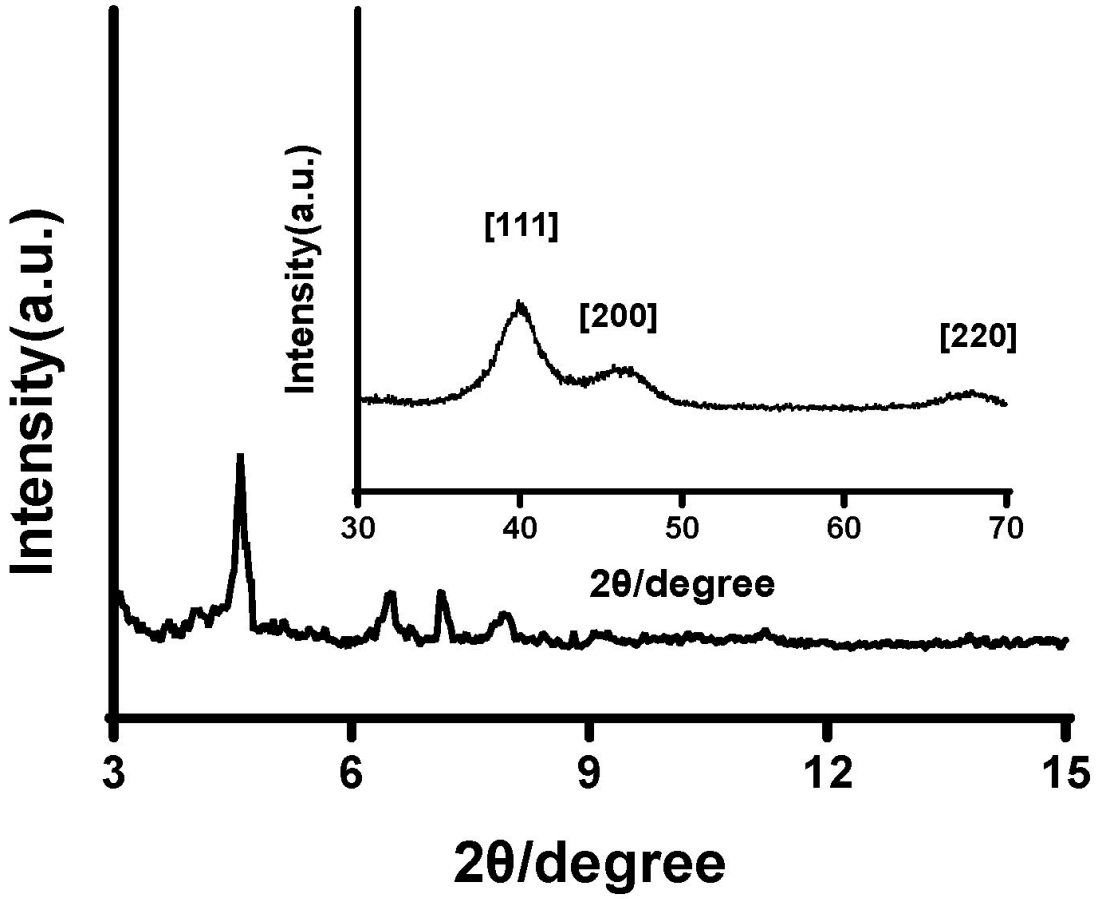

Figure S2. PXRD patterns of PP NPs at the range of 3 - 15 degree (for PCN-224) and 30 - 70 degree (for Pt NPs).

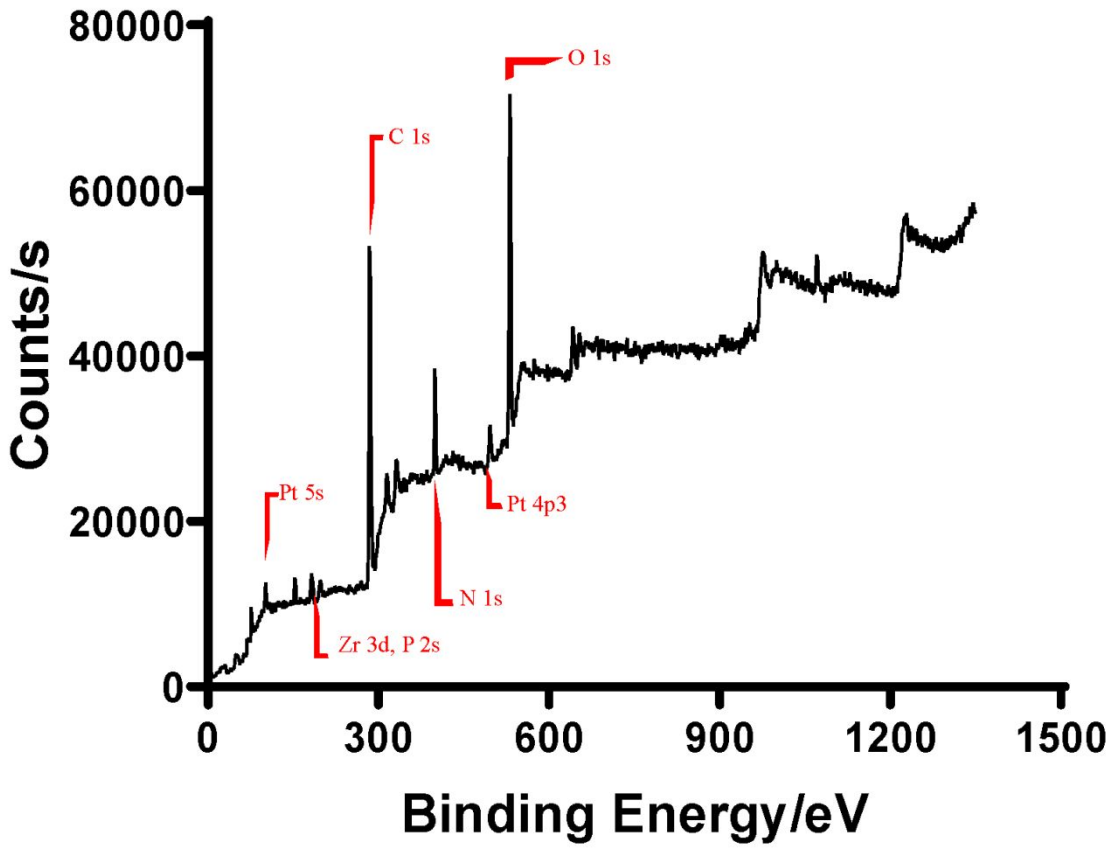

Figure S3. X-ray photoelectron spectroscopy (XPS) analyze of PPGE NCs.

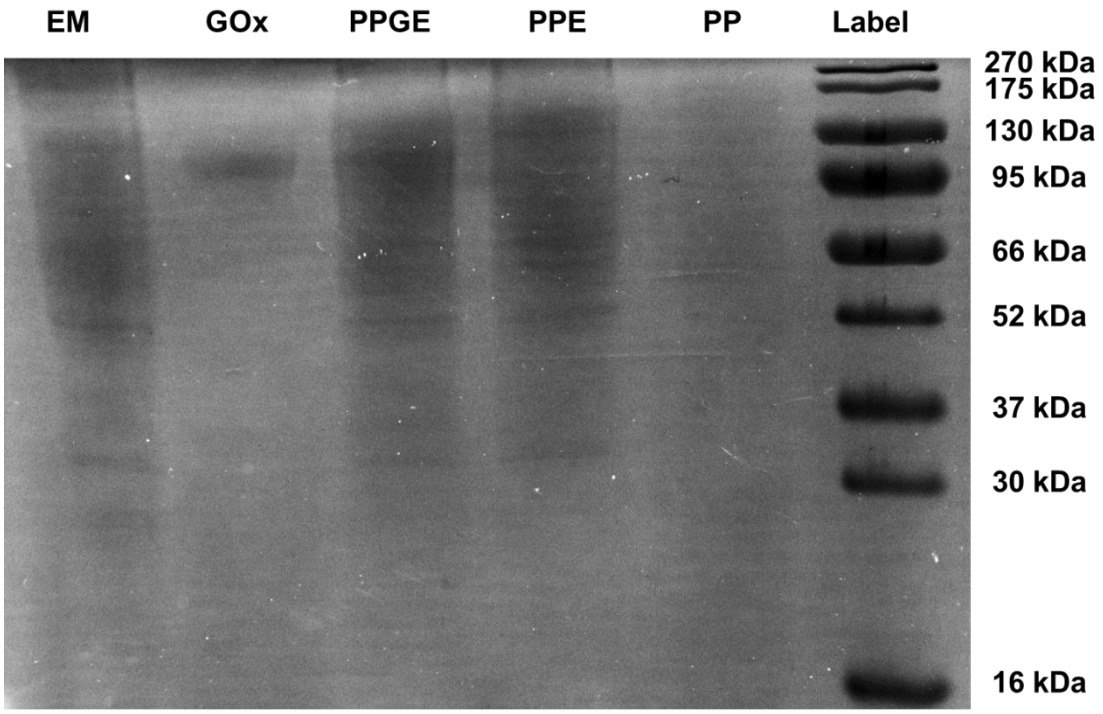

Figure S4. SDS-PAGE analysis of EM, GOx, PPGE NCs, PPE NCs and PP NPs.

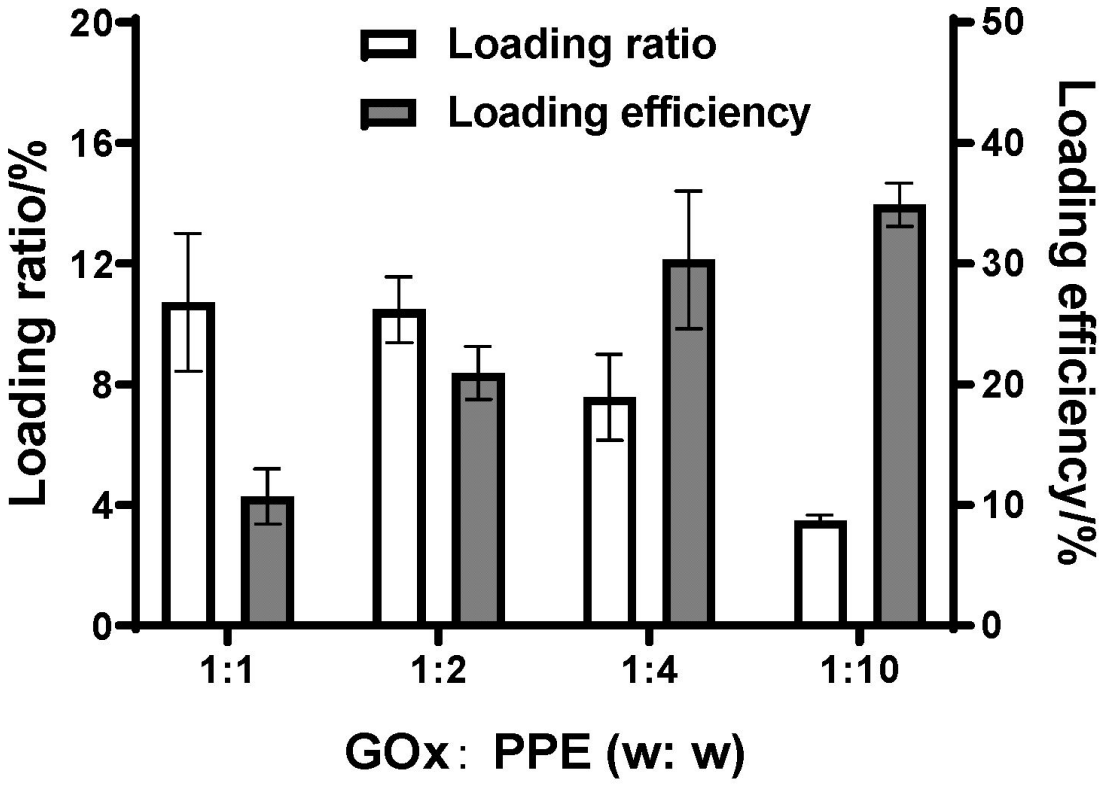

Figure S5. Loading ratio and loading efficiency of GOx at different weight ratio between GOx and PPE by microplate reader. n = 3.

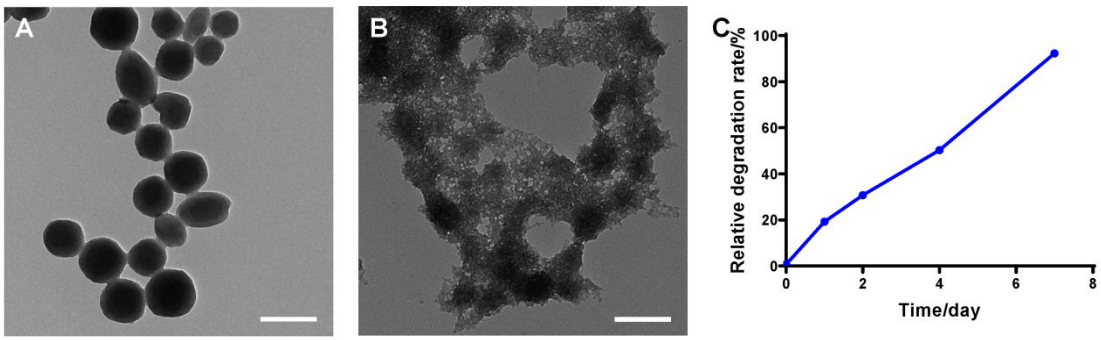

Figure S6. The degradation studies of PPGE NCs in PBS solutions at day 0 (A) and day 7 (B) observed by TEM images and time-dependent degradation monitored by UV-Vis spectrum (C).

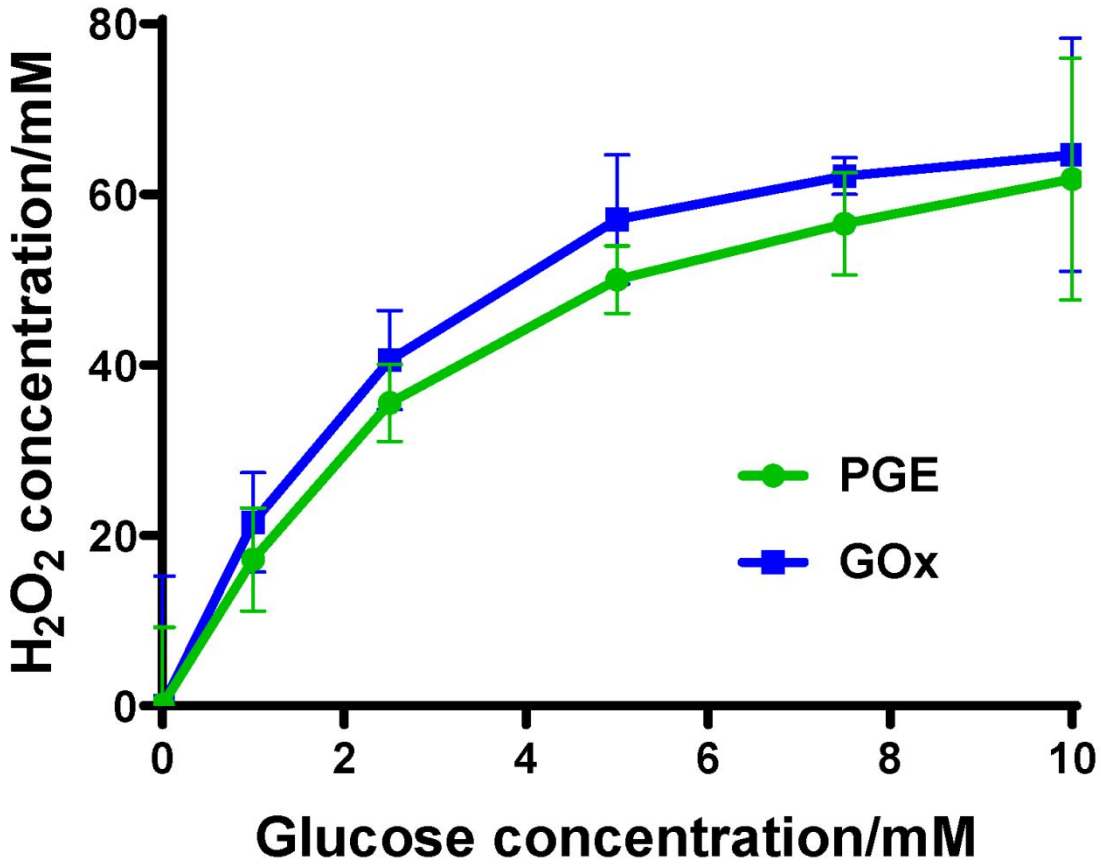

Figure S7. The H_2O_2 production activity of free GOx and loaded GOx in PGE NCs in pH = 5 glucose buffer solutions of different concentrations. n = 3.

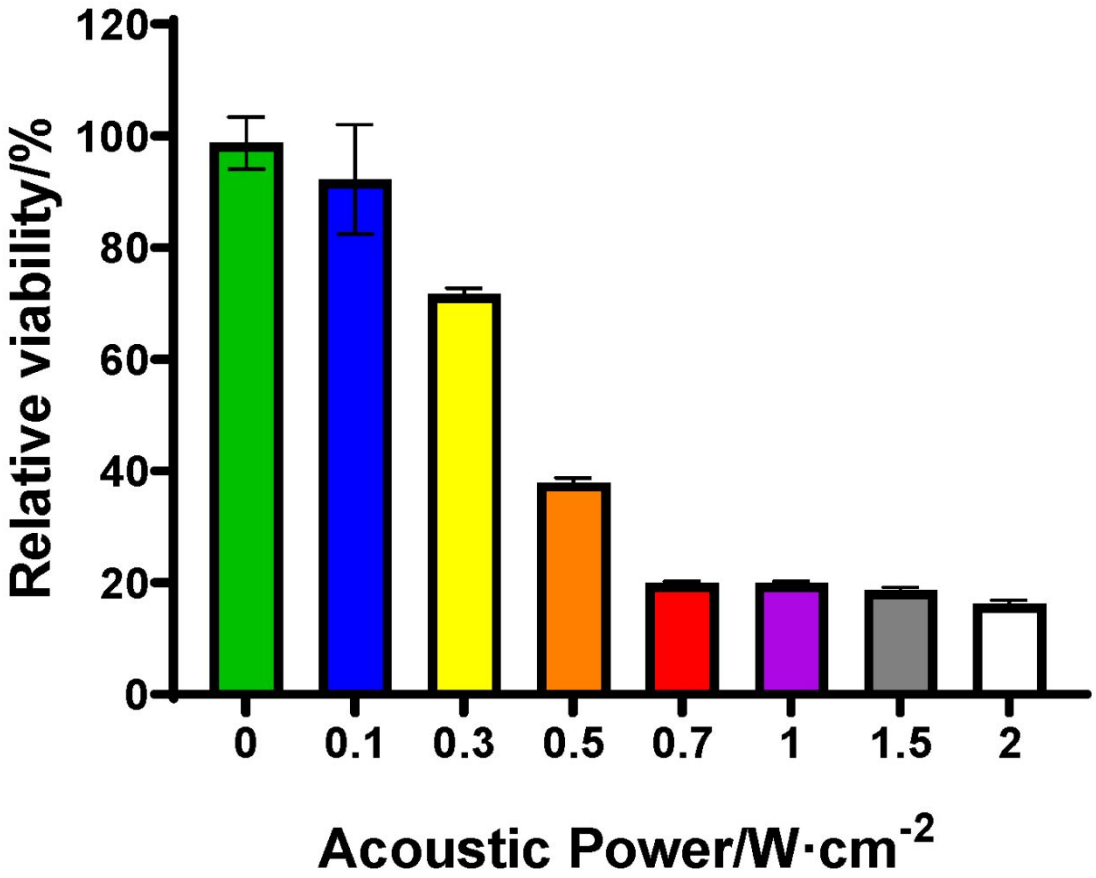

Figure S8. The relative viability of BxPC-3 cells after irradiated by ultrasound at different acoustic powers for 5 min. n = 3.

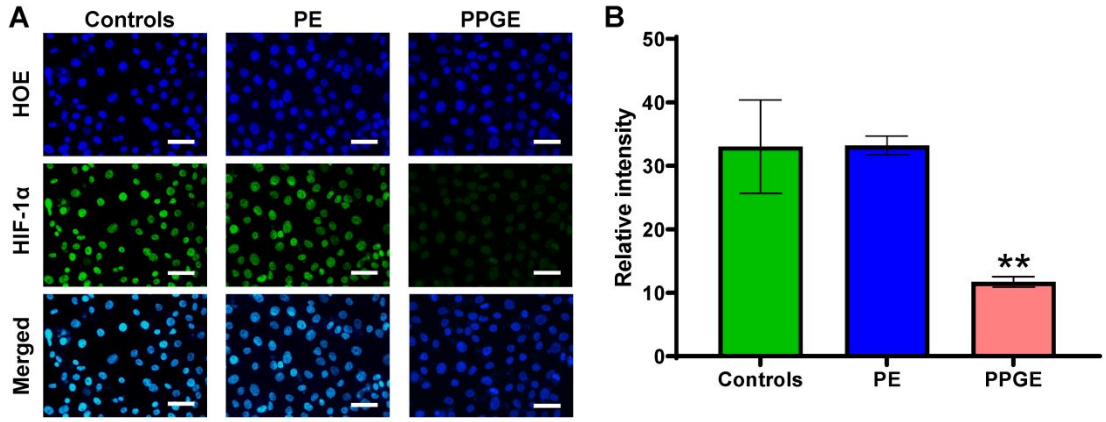

Figure S9. Cellular hypoxia relief property of PE and PPGE NCs demonstrated by HIF-1α immunofluorescence staining (A) and its statistical analysis (B). Scale bar: 50 μm, n = 3, *: $p < 0.1$; **: $p < 0.01$.

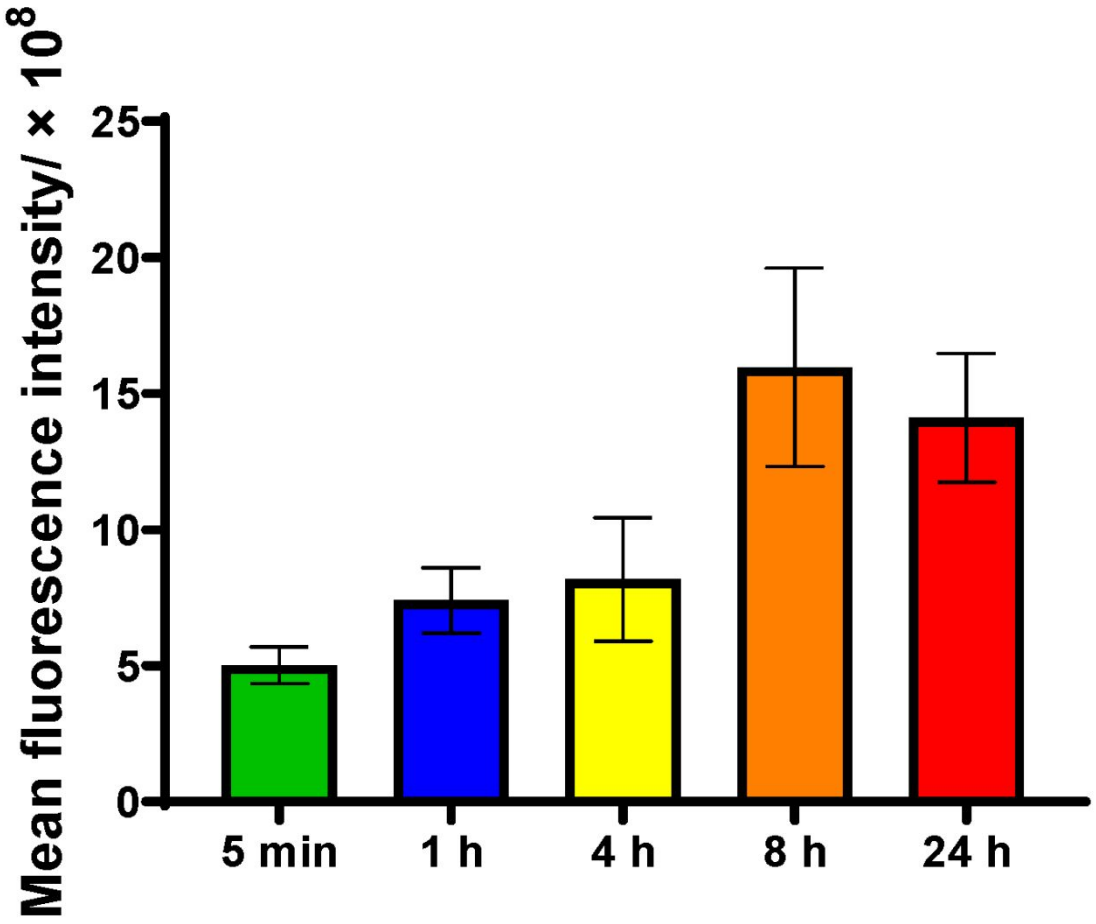

Figure S10. Mean fluorescence intensity of tumor site at 5 min, 1 h, 4 h, 8 h and 24 h after intravenous injection of PPGE NCs to tumor-bearing mice. n = 3.

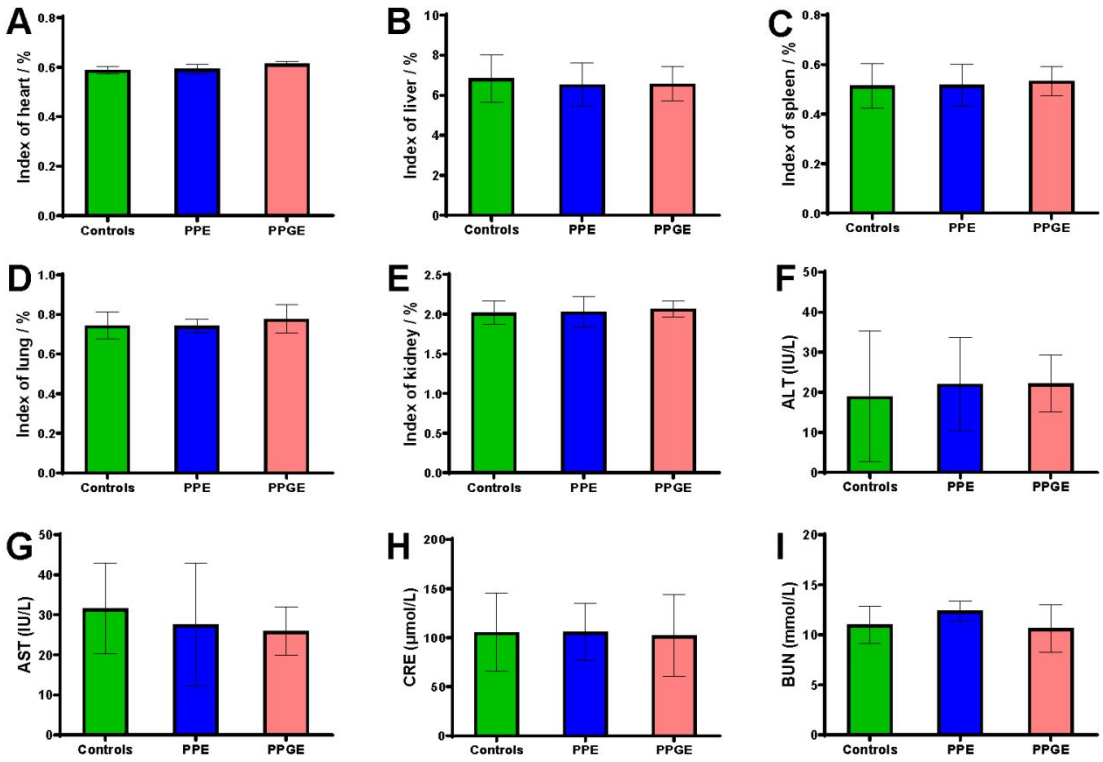

Figure S11. Biosafety analysis of PPE NCs and PPGE NCs. Index of (A) heart, (B) liver, (C) spleen, (D) lung and (E) kidney. Biochemical indexes of (F) alanine aminotransferase (ALT), (G) aspartate aminotransferase (AST), (H) creatinine (CRE), and (I) blood urea nitrogen (BUN). n = 3.

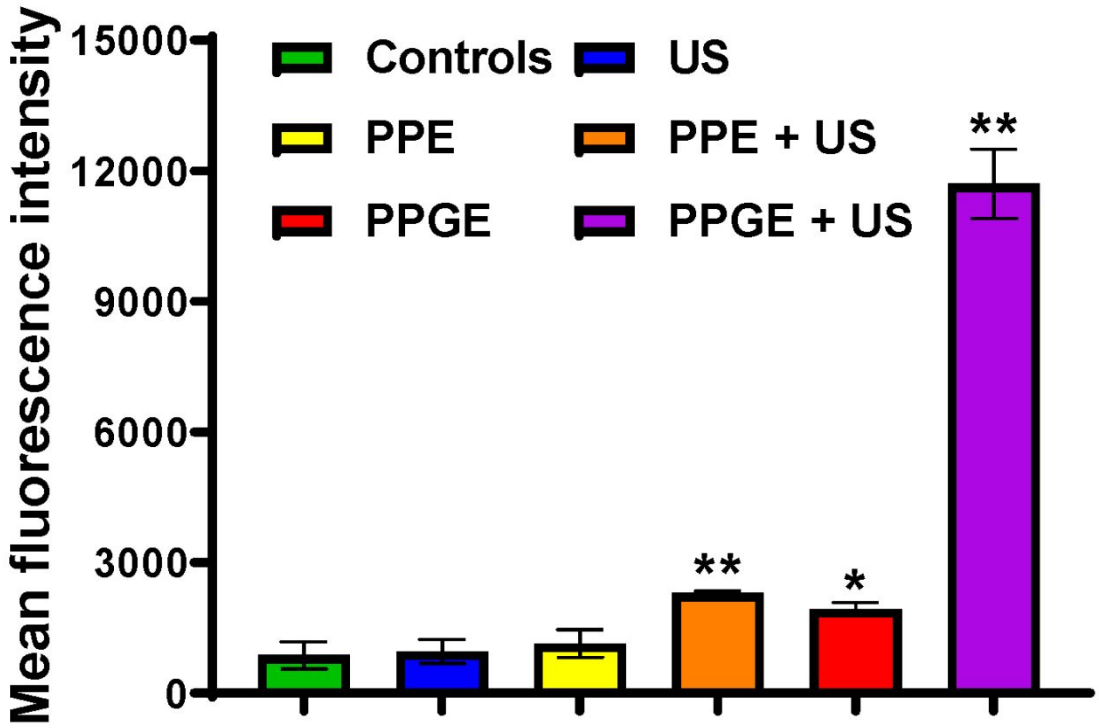

Figure S12. Mean fluorescence intensity of TUNEL immunofluorescence staining of tumor tissue from tumor-bearing mice in different groups. n = 3, *: $p < 0.1$; **: $p < 0.01$.

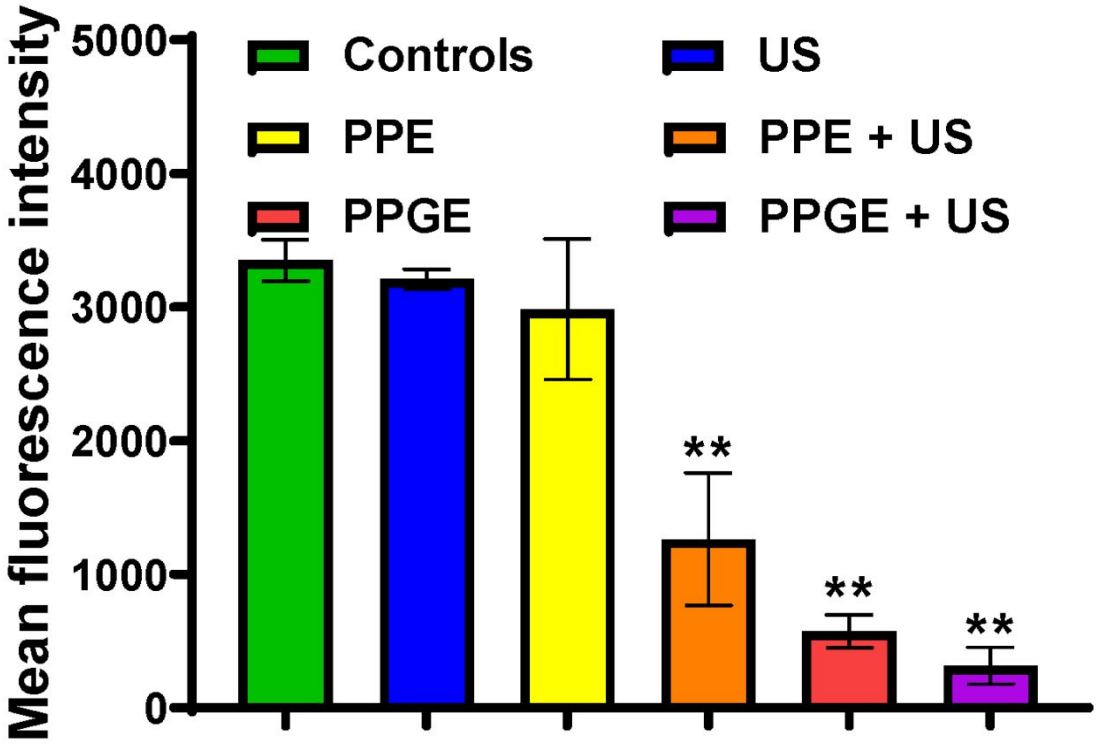

Figure S13. Mean fluorescence intensity of Ki-67 immunofluorescence staining of tumor tissue from tumor-bearing mice in different groups. n = 3, *: $p < 0.1$; **: $p < 0.01$.

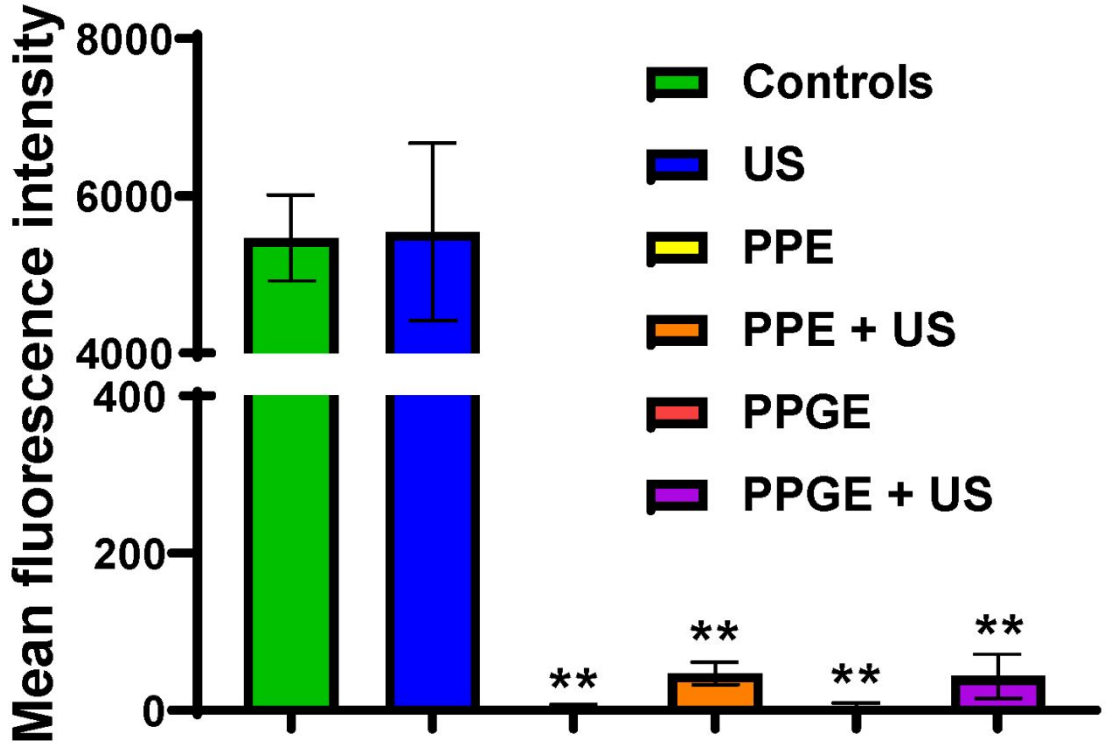

Figure S14. Mean fluorescence intensity of HIF-1α immunofluorescence staining of tumor tissue from tumor-bearing mice in different groups. n = 3, *: $p < 0.1$; **: $p < 0.01$.