Poly-γ-glutamic acid derived nanopolyplexes for up-regulation of gamma-glutamyl transpeptidase to augment tumor active targeting and enhance synergistic antitumor therapy by regulating intracellular redox homeostasis

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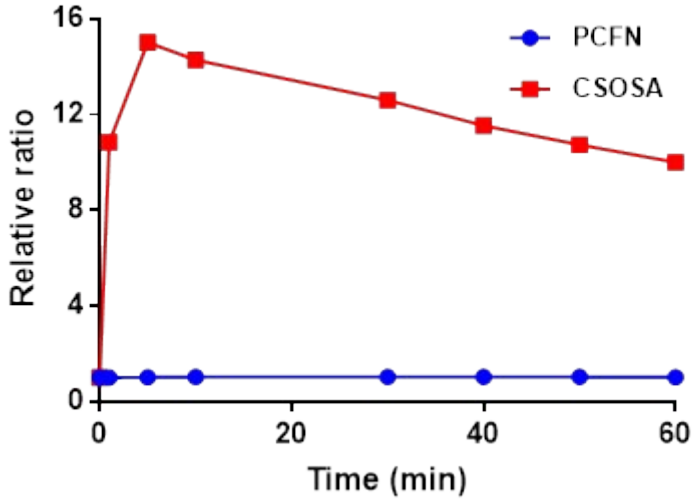

Fig.S1. The stability test result of PCFN and CSOSA nanoparticle. The PCFN and CSOSA were cultured with 3% BSA in vitro, an UV-vis spectrophotometer was employed to further investigate the turbidity of PCFN and CSOSA at 350 nm in vitro, the UV absorption of different preparations was recorded at determined times.

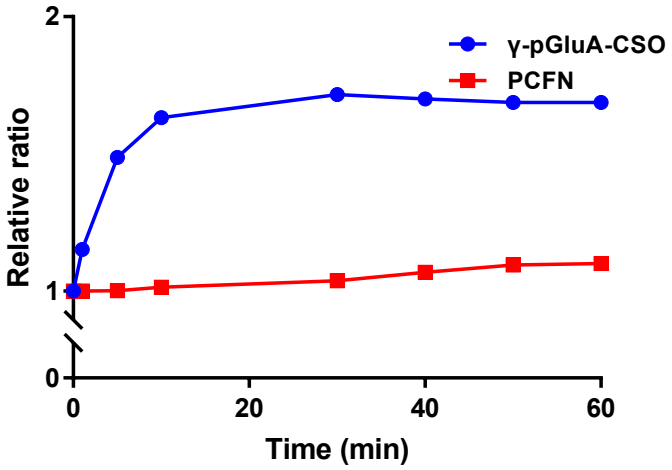

Fig.S2. The PCFN and γ-pGluA-CSO were cultured with BSA in vitro, an UV-vis spectrophotometer was employed to investigate the turbidity of PCFN and γ-pGluA-CSO at 350 nm in vitro, the UV absorption of different preparations was recorded at determined times.

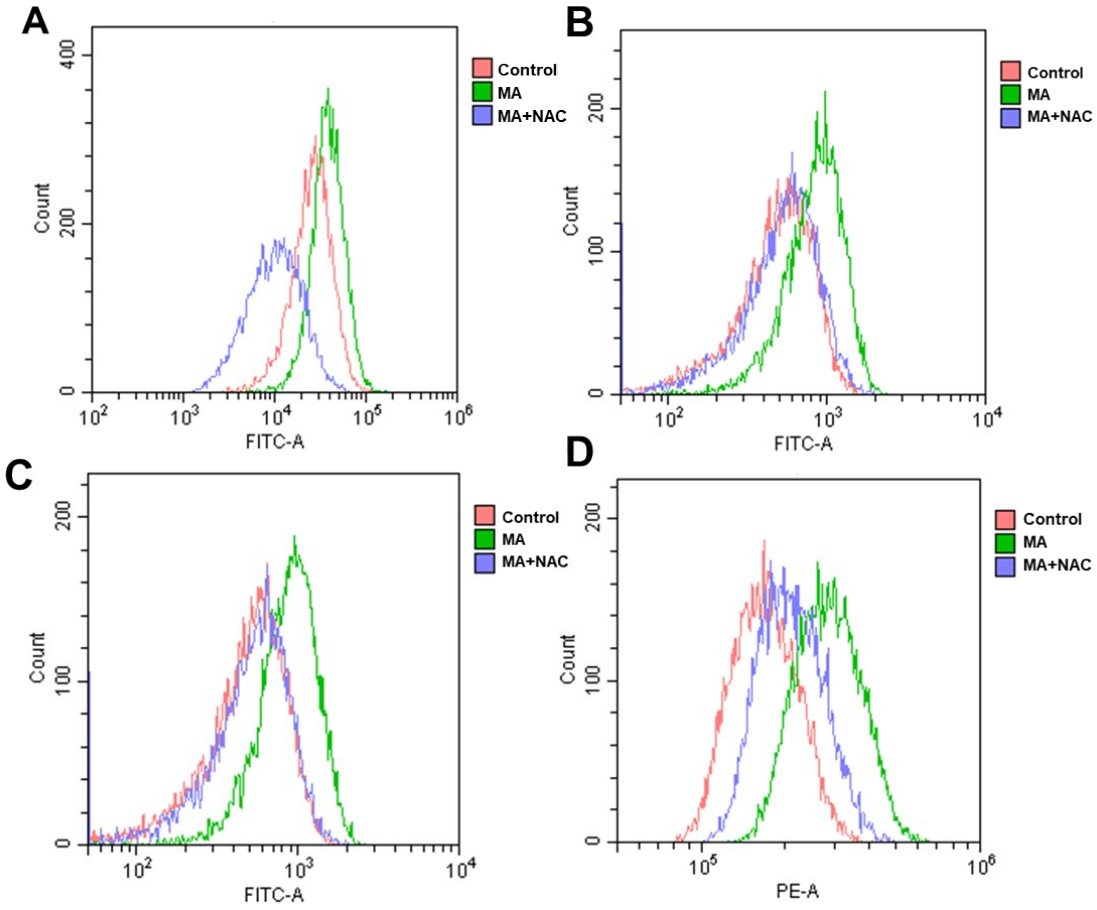

Fig. S3: Evaluation of the mechanism after ROS inhibition. (A) The ROS detection by ROS probe DCFH-DA via flow cytometry (B) GGT1 expression detection via flow cytometry

after MA and NAC intervention. (C) GGT5 expression detection via flow cytometry after MA and NAC intervention. (D)Intracellular PCFN/DOX detection via flow cytometry.

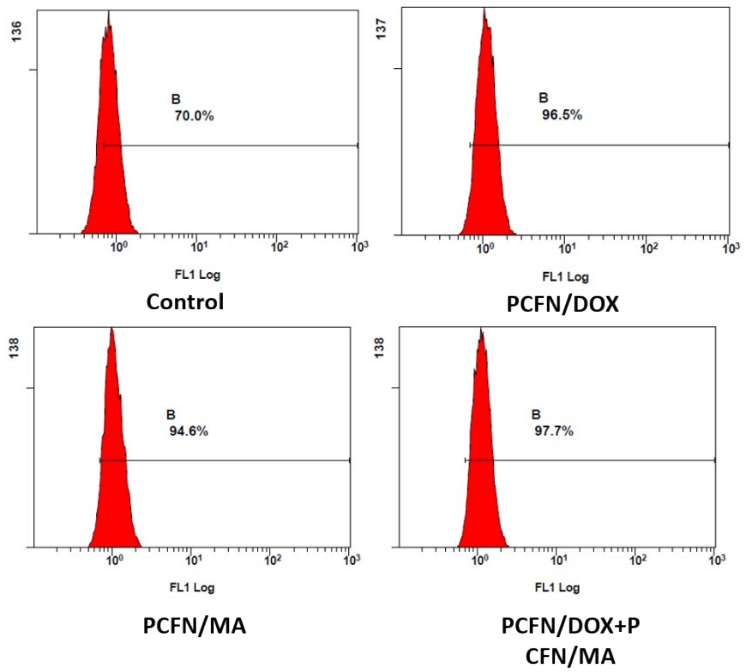

Fig. S4. ROS detection via flow cytometry. MCF-7 was seeded onto 6-well plates and cultured overnight at 37 °C, removed the culture medium, and then cells were treated with PBS, PCFN/MA, PCFN/DOX and PCFN/MA+PCFN/DOX for 8 h. After that, the medium was removed, and cells were washed with PBS three times, and then incubated with 10 mM DCFH-DA dye for 30 min. ROS induced intracellular fluorescence was measured via flow cytometry after cells were harvested in PBS.

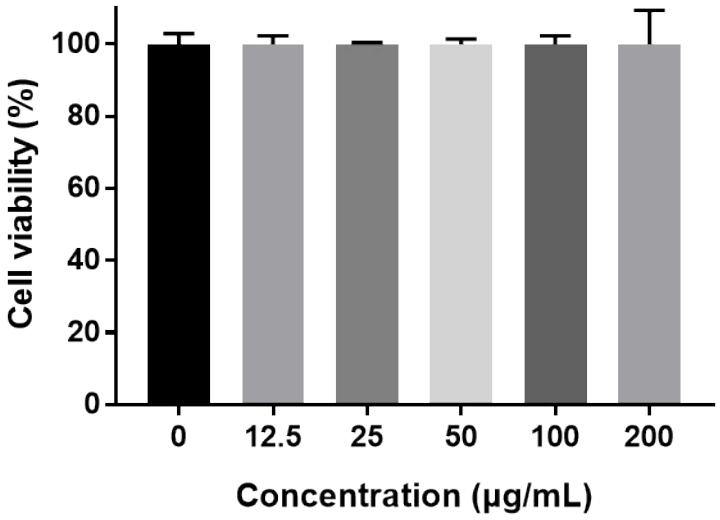

Fig. S5. Cytotoxicity test of PCFN nanoparticles in MCF-7 cells at 12.5, 25, 50, 100, 200

μg/mL.

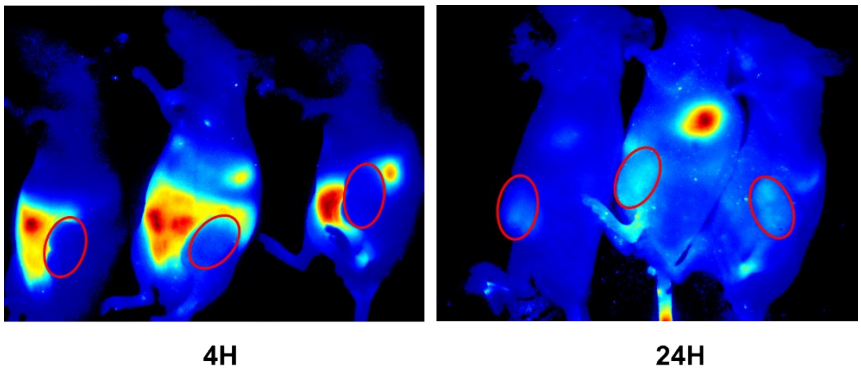

Fig. S6. Fluorescence images of γ-pGluA-CSO/ICG distribution on breast tumor bearing mice in vivo at designed time intervals. The red circle in the image represented the tumor position. (n=3)

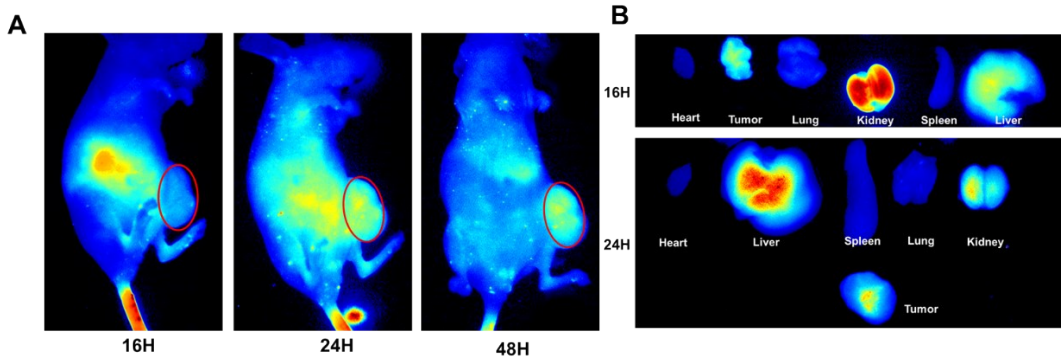

Fig. S7. Biodistribution of ICG-Labeled nanoparticle in Mice at different view (A) and the organ distribution (B) at different times.

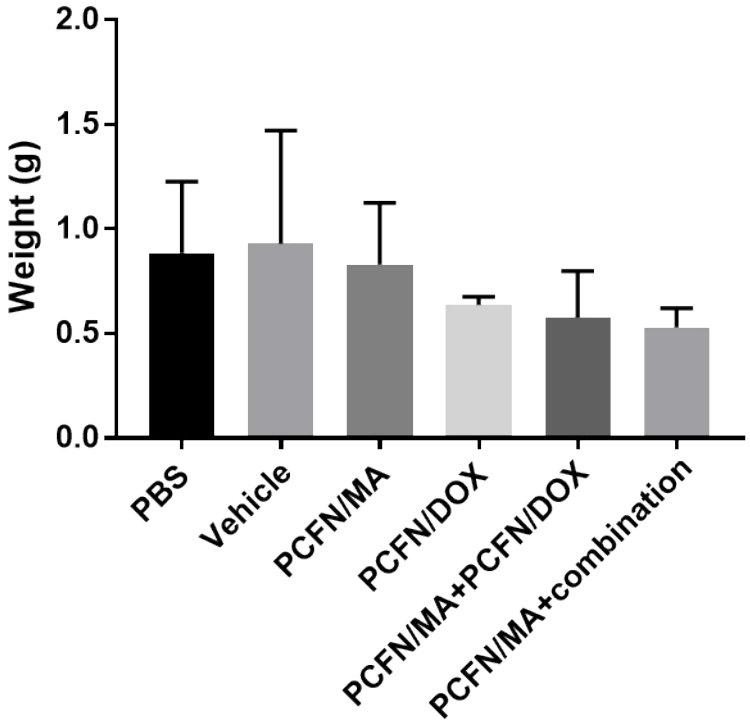

Fig. S8. The weights of excised tumor masses. The mice were sacrificed at the 21st day after treatment, the excised tumor masses were weighted and recorded.

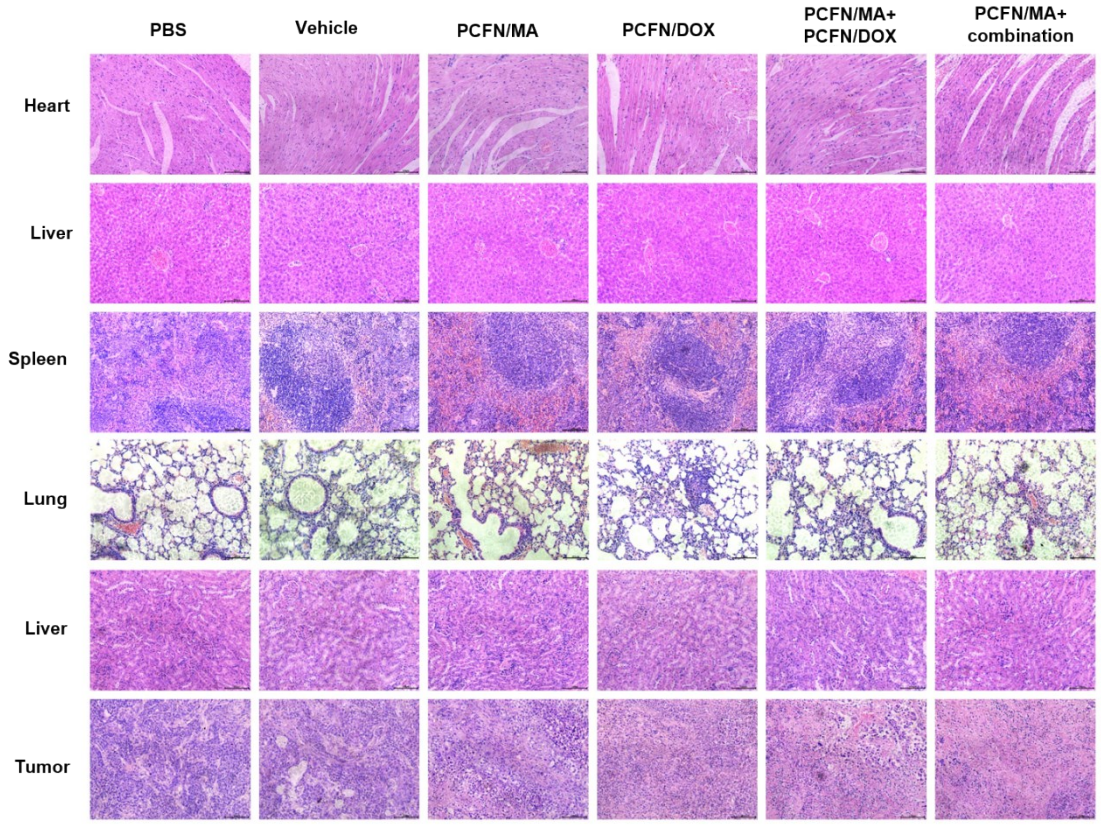

Fig. S9. H&E staining of major organs. All the tumor bearing models were were intravenously injected with 1) Saline; 2) Vehicle; 3) PCNF/MA; 4) PCNF/DOX; 5)

PCNF/MA+PCNF/DOX; 6) PCNF/MA+combination(PCNF/MA and PCNF/DOX at the molar ratio at 5:1). At the end of the treatment, mice were sacrificed and organs were harvested and sliced, followed by H&E staining.