Supporting information

Tumor-Penetrating Nanoparticles for Enhanced Anticancer Activity of Combined Photodynamic and Hypoxia-activated Therapy

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Table S1. Nanoparticle characterization. (loading efficiency (LE), encapsulation efficiency (EE))

	Diameter (nm)	ζ (mV)	LE (ICG) (%)	EE (ICG) (%)	LE (TPZ) (%)	EE (TPZ) (%)
iNP/I	103.2	-30.12	12.6	82.8	--	--
iNP/T	96.9	-20.7	--	--	5.5	35.7
iNP/IT	112.4	-34.1	12.1	80.5	6.2	36.9
NP/IT	105.7	-27.3	--	--	--	--

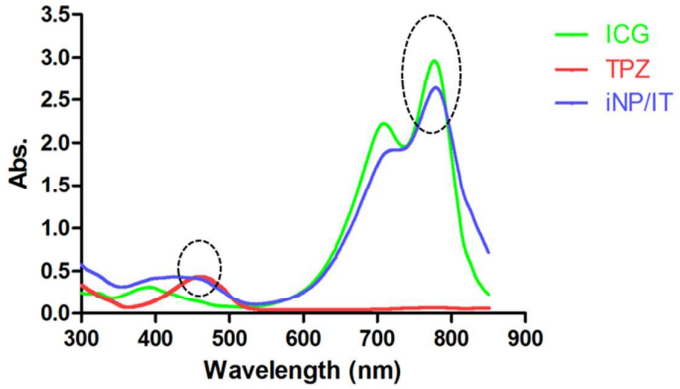

Figure S1. Absorbance spectra of iNP/IT, free ICG, and TPZ.

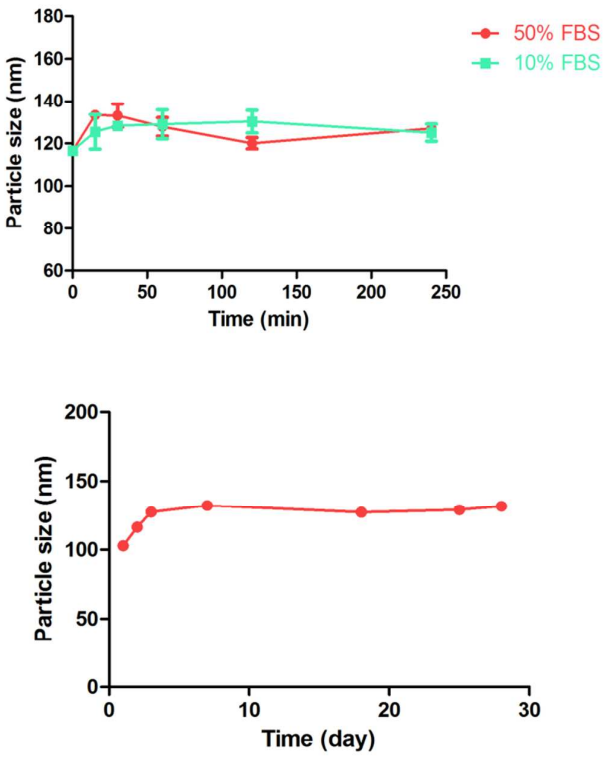

Figure S2. Stability of the iNP/NP in 10% and 50% FBS at 37°C and 4°C.

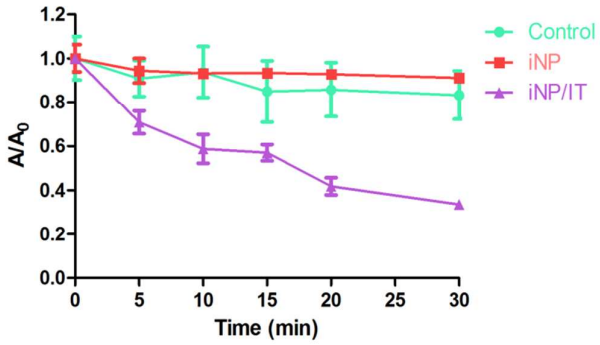

Figure S3. Singlet oxygen generation of control (DPBF only), iNP/I and iNP/IT following irradiation with 808-nm laser (2 W/cm^2). mean ± SD (n = 3).

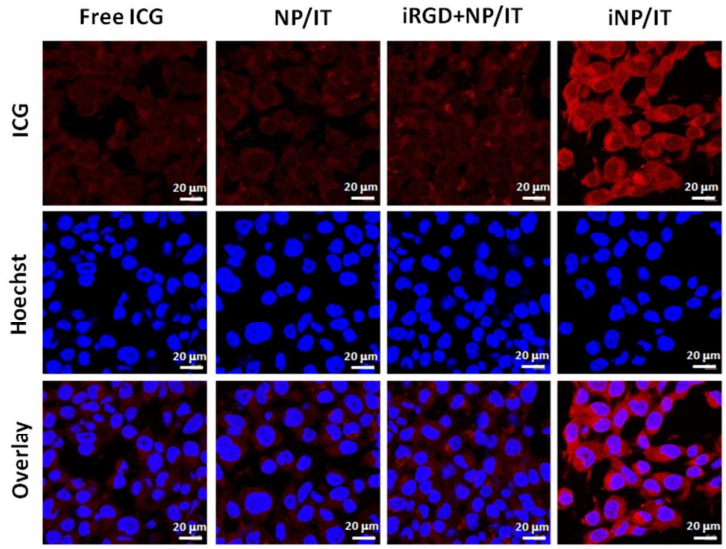

Figure S4. Cellular uptake in 4T1 cells by confocal microscopy. The cells were incubated with the particles or free ICG for 6 h. Nuclei were stained with Hoechst dye. Red fluorescence of ICG. Scale bar = 20 μm.

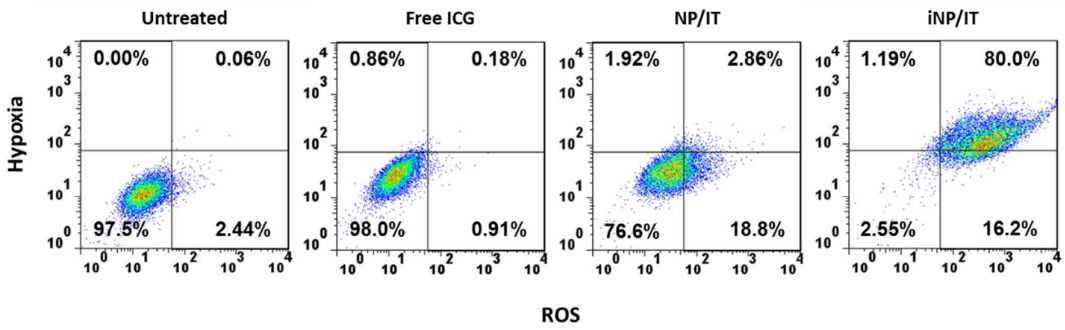

Figure S5. Flow cytometry analysis of ROS generation and hypoxia detection after treatment with nanoparticles or free ICG.

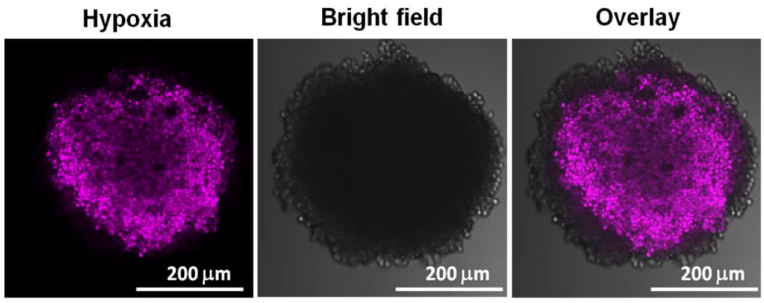

Figure S6. Confocal images of hypoxic condition in 4T1 tumor spheroids detected by ROS-ID® Hypoxia/Oxidative stress detection kit. Scale bar 200 μm. The inner and peri-necrotic rim of 4T1 spheroids was hypoxic, consistent with published literature. [1]

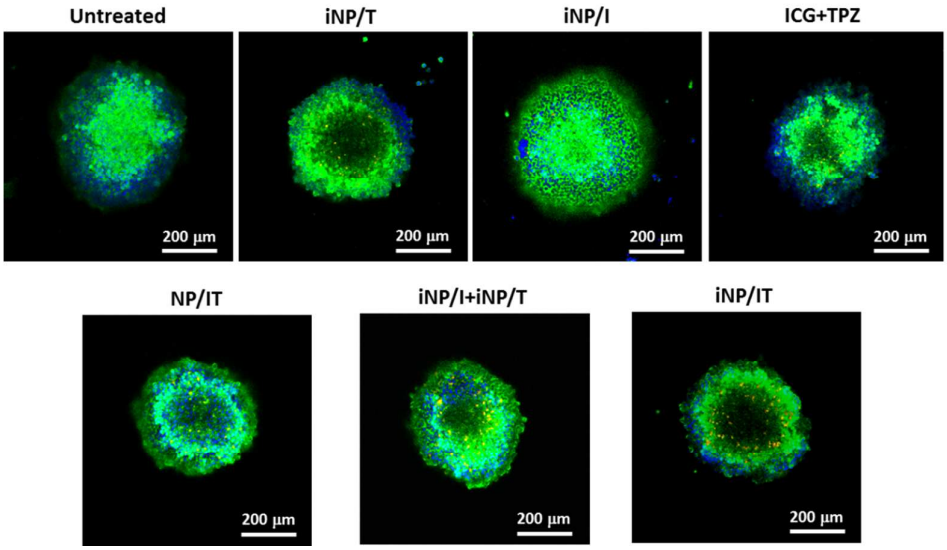

Figure S7. Toxicity of nanoparticles in tumor spheroids without laser irradiation. Confocal images using live/dead viability assay in 4T1 spheroids treated with different formulations for 24 h. Live cells were stained with calcein AM (green) and the dead cells were labeled with EthD-1 (red). Nucleus was stained with Hoechst 33258 (blue).

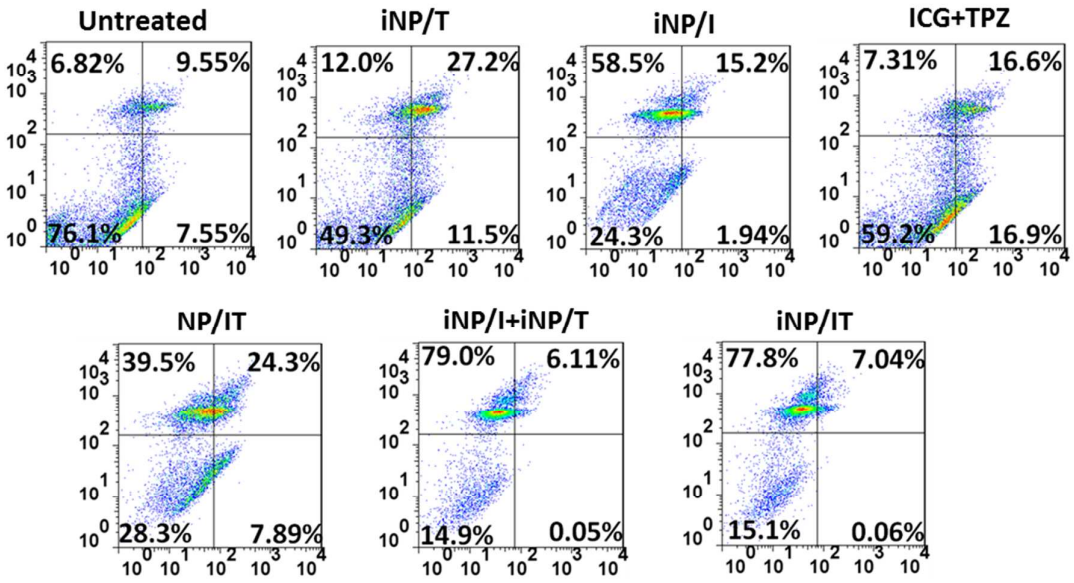

Figure S8. Flow cytometry analysis of cell apoptosis in 4T1 spheroids following the nanoparticle treatment and laser irradiation. Cells were stained with the Annexin V-FITC/PI.

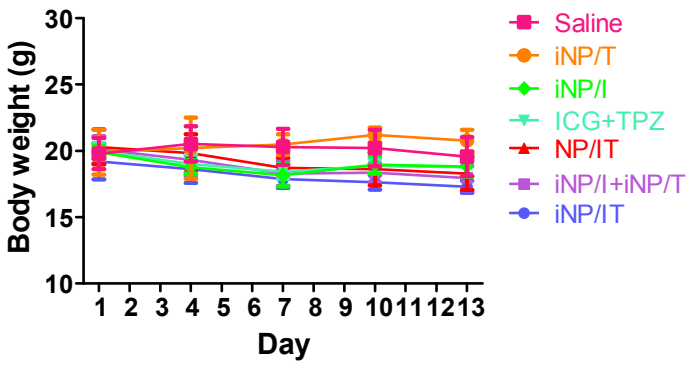

Figure S9. Body weight of mice during anticancer treatment. Data are shown as means ± SD (n = 5).

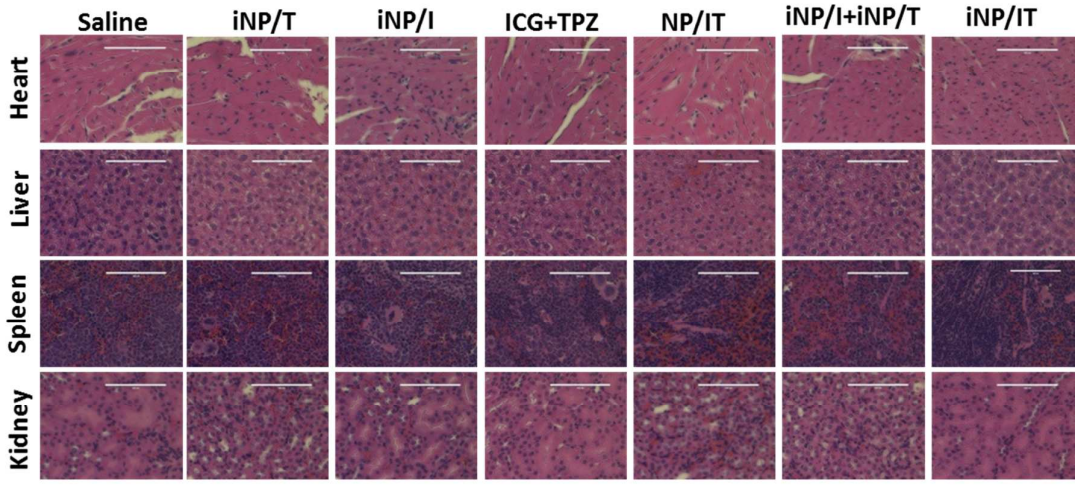

Figure S10. Histological observation of tissue sections from major organs of mice was performed after the treatment. The organ sections were stained with hematoxylin and eosin (HE). Scale bar = 100 μm.

REFERENCES

1. Ryan, R.; Green, J.; Williams, P.; Tazzyman, S.; Hunt, S.; Harmey, J.; Kehoe, S.; Lewis, C., Bacterial Delivery of a Novel Cytolysin to Hypoxic Areas of Solid Tumors. *Gene Ther.* **2009,** *16*, 329-339.