Electronic supplementary information

Rational Design of Oxygen Deficient TiO_{2-x} Nanoparticles Conjugated with Chlorin e6 (Ce6) for Photoacoustic Imaging-Guided Photothermal/Photodynamic Dual Therapy of Cancer

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Supplementary methods

Materials

Titanium dioxide nanopowder (20 nm) was purchased from Shanghai Macklin Biochemical Co., Ltd (China). Chlorin e6 (Ce6) was ordered from Frontier Scientific, Inc. (USA). Sodium borohydride ($NaBH_4$), (3-aminopropyl)triethoxysilane (APTES), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 1, 3-diphenyl-isobenzofuran (DPBF), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), thiazolyl blue tetrazolium bromide (MTT, 98%), dimethyl sulfoxide (DMSO), propidium iodide (PI), and 1, 3-diphenyl-isobenzofuran (DPBF) were purchased from Sigma-Aldrich (USA). Phosphate buffered saline (PBS), modified Dulbecco's modified eagle's medium (DMEM), 4',6-diamidino-2-phenylindole (DAPI), JC-1 staining kit, calcein AM, propidium iodide (PI) and One Step TUNEL Apoptosis Assay Kit were ordered from Beyotime Biotechnology (China). 2', 7'-Dichlorofluorescein diacetate (DCFH-DA) was acquired from GEN-VIEW Scientific Inc. (USA). The female BALB/c and Kunming (KM) mice (4-6 weeks) were purchase from Chengdu Dossy Experimental Animals Co., Ltd (China). Deionized (DI) water was collected from an ultrapure water system (Milli-Q, Molsheim, France).

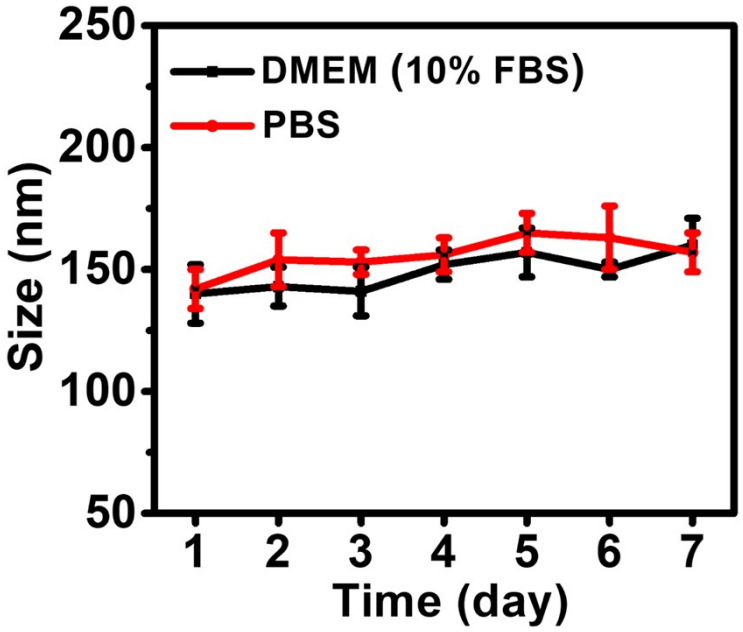

Fig. S1 Variation in the hydrodynamic size of ZPC NPs in PBS or DMEM (with 10% FBS) during the incubation course for 7 days.

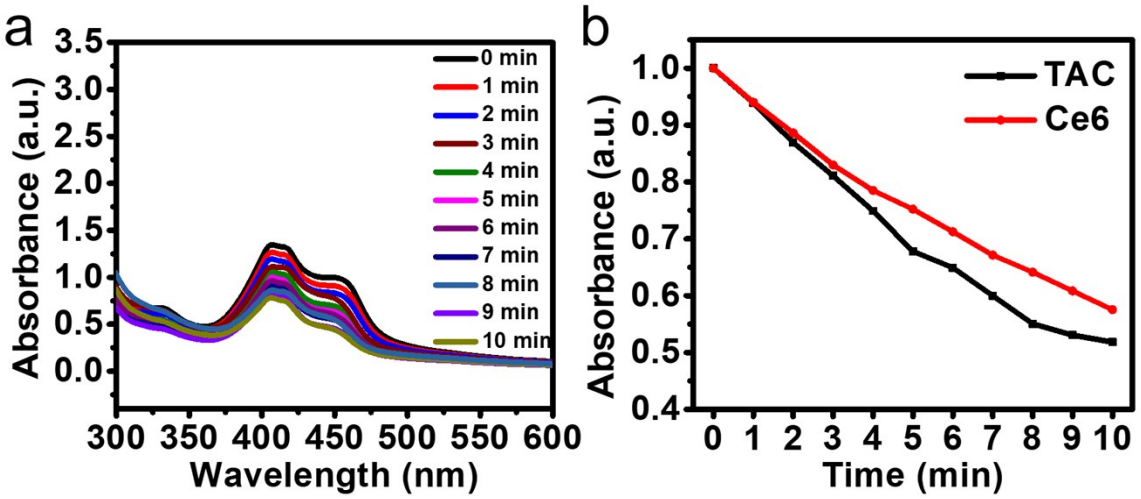

Fig. S2 (a) UV-vis-NIR absorption spectra of Ce6 (5 μg·mL^{-1}) containing DPBF exposed to a 660 nm laser (0.57 W·cm^{-2}) for different periods; (b) decay of the normalized peak absorbance intensity of Ce6 and TAC NPs (equivalent Ce6 concentration: 5 μg·mL^{-1}) at 417 nm over time.

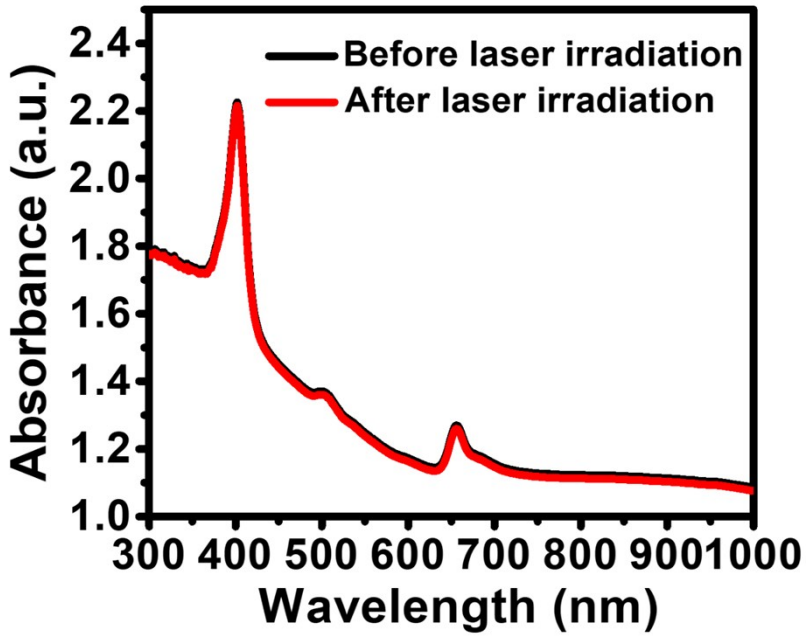

Fig. S3 Optical absorption spectrum of TAC NP dispersion (200 μg·mL^{-1}) before and after periodic laser irradiation corresponding to Fig. 3j.

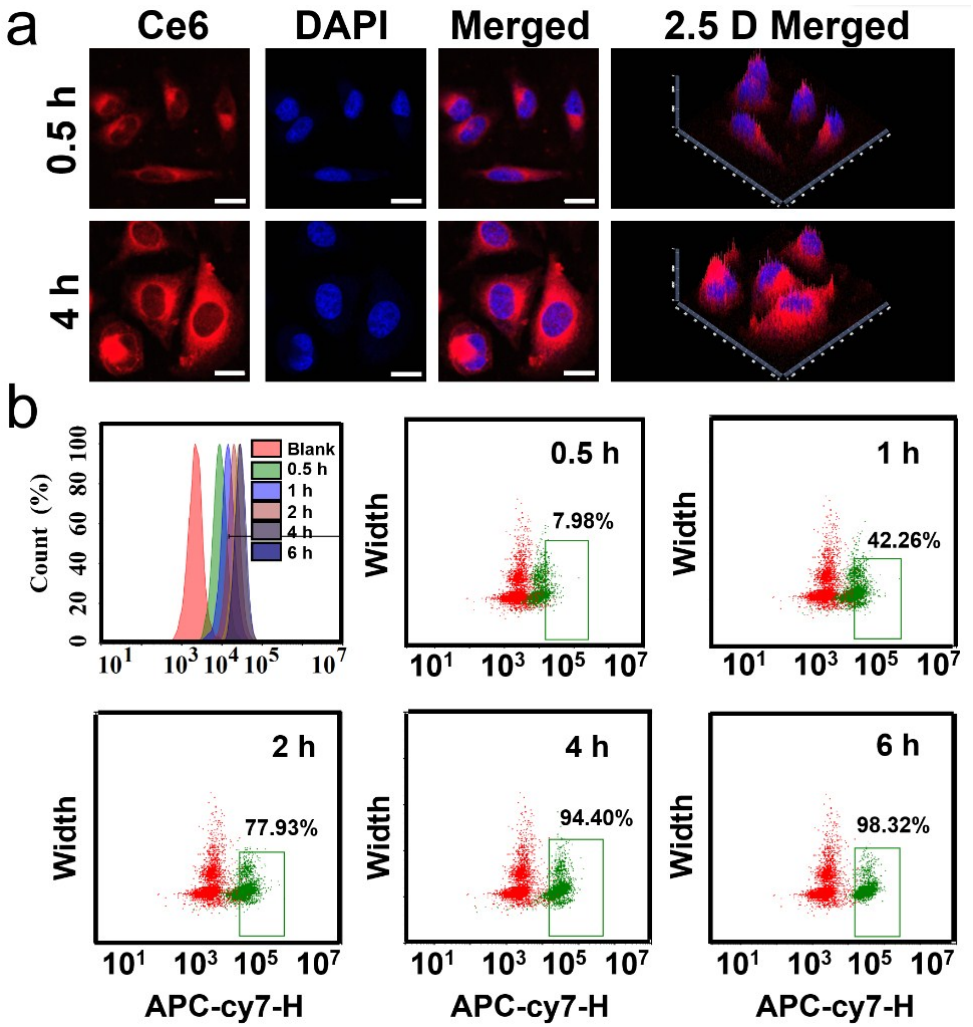

Fig. S4 Cellular uptake of TAC NPs. (a) LSCM images of HeLa cells after treated by TAC NPs (equivalent Ce6 concentration: 10 μg·mL^{-1}) for 0.5 and 4 h (scale bar: 20 μm); (b) flow cytometry assay using HeLa cells being exposed to TAC NPs (equivalent Ce6 concentration: 10 μg·mL^{-1}) for 0.5, 1, 2, 4 and 6 h.

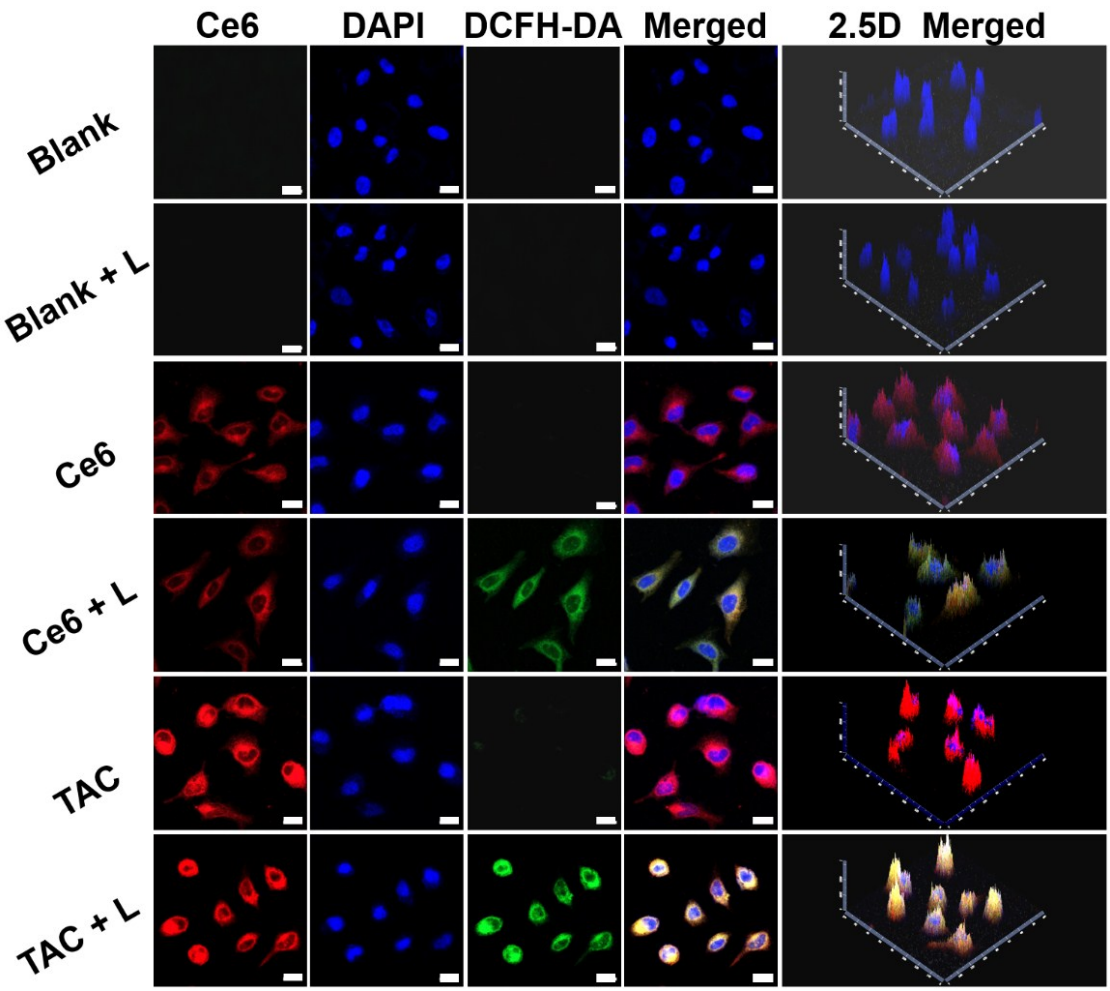

Fig. S5 Intracellular ROS generation of HeLa cells after incubated with Ce6 or TAC NPs (equivalent Ce6 concentration: 10 μg·mL^{-1}) for 4 h subject to laser irradiation (660 nm, 0.57 W·cm^{-2}) where applicable using DCFH-DA probe (scale bar: 20 μm).

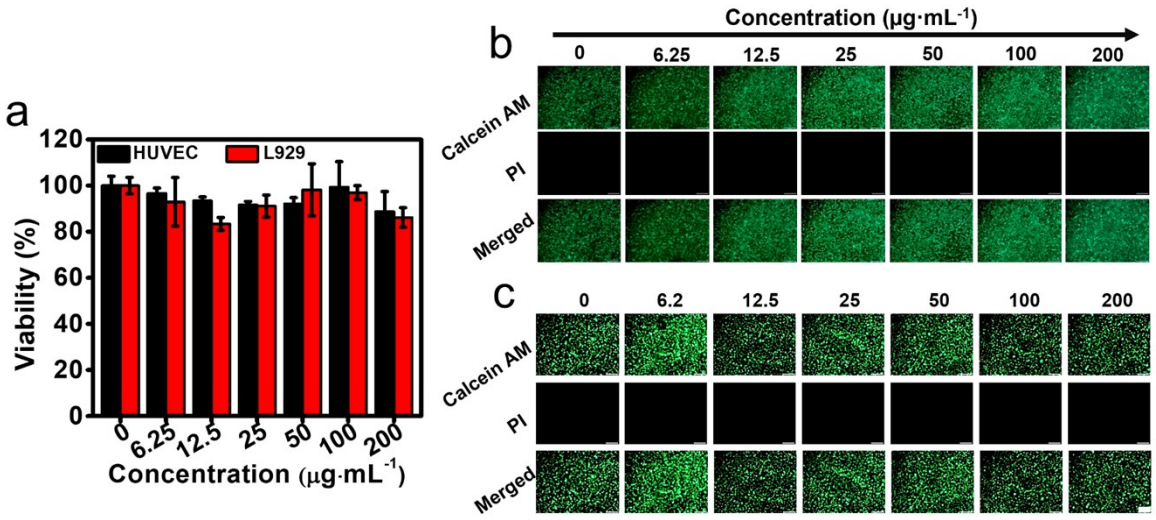

Fig. S6 Biocompatibility assessment. (a) Cell viability of HUVECs and L929s administered with TAC NPs at different concentrations for 24 h; (b) HUVECs or (c) L929s treated by TAC NPs at different concentrations for 24 h and stained by calcein AM and PI (scale bar: 100 μm).

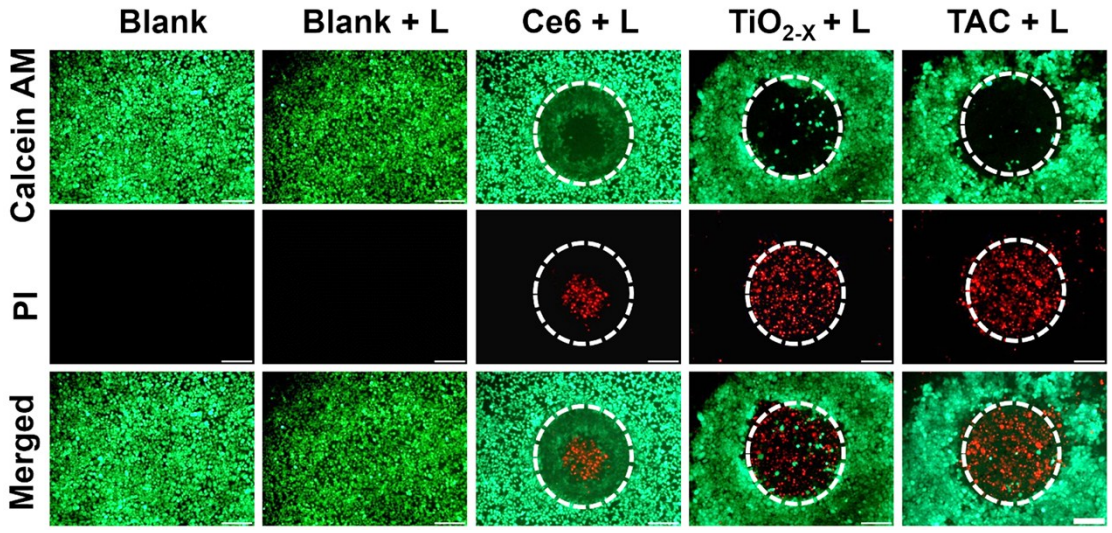

Fig. S7 Fluorescence images of HeLa cells subject to various treatments and stained by calcein AM and PI (scale bar: 200 μm). Enclosed area by white dotted circle denotes the laser irradiation spot. Live and dead cells were labeled with green and red fluorescence, respectively.

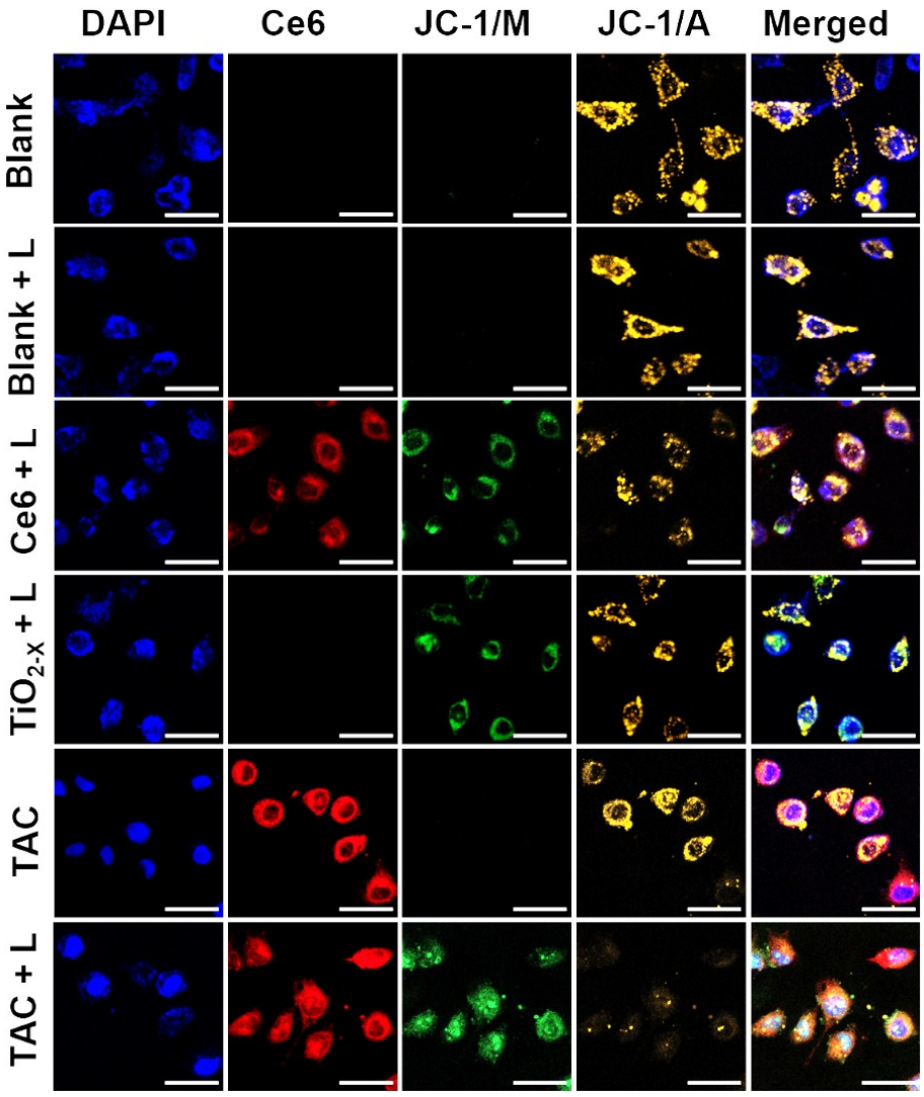

Fig. S8 LSCM images of HeLa cells subject to different treatments, and stained by JC-1 and DAPI (scale bar: 50 μm). JC-1/M and JC-1/A represent the monomer and aggregate form of JC-1, respectively.

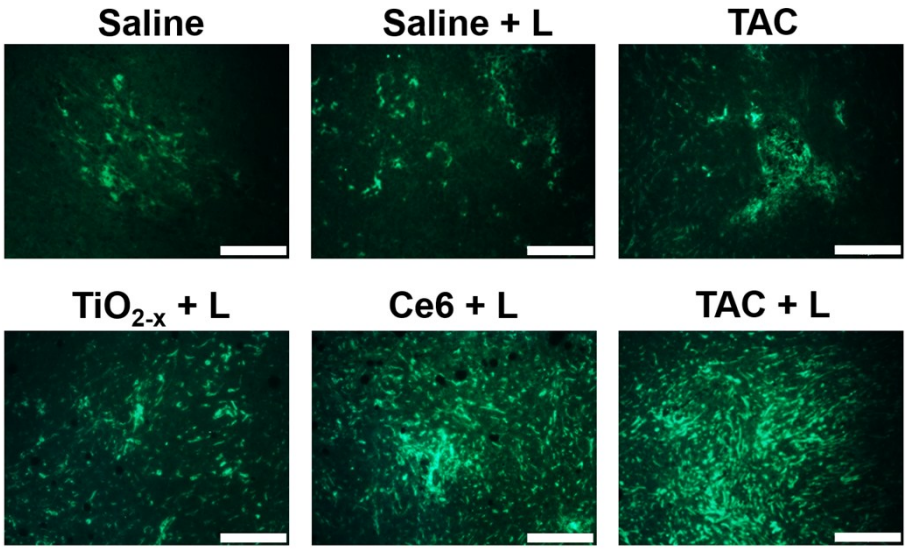

Fig. S9 Histological staining of tumor slices via Hypoxyprobe-1 to indicate the hypoxia level after various treatments (scale bar: 100 μm).

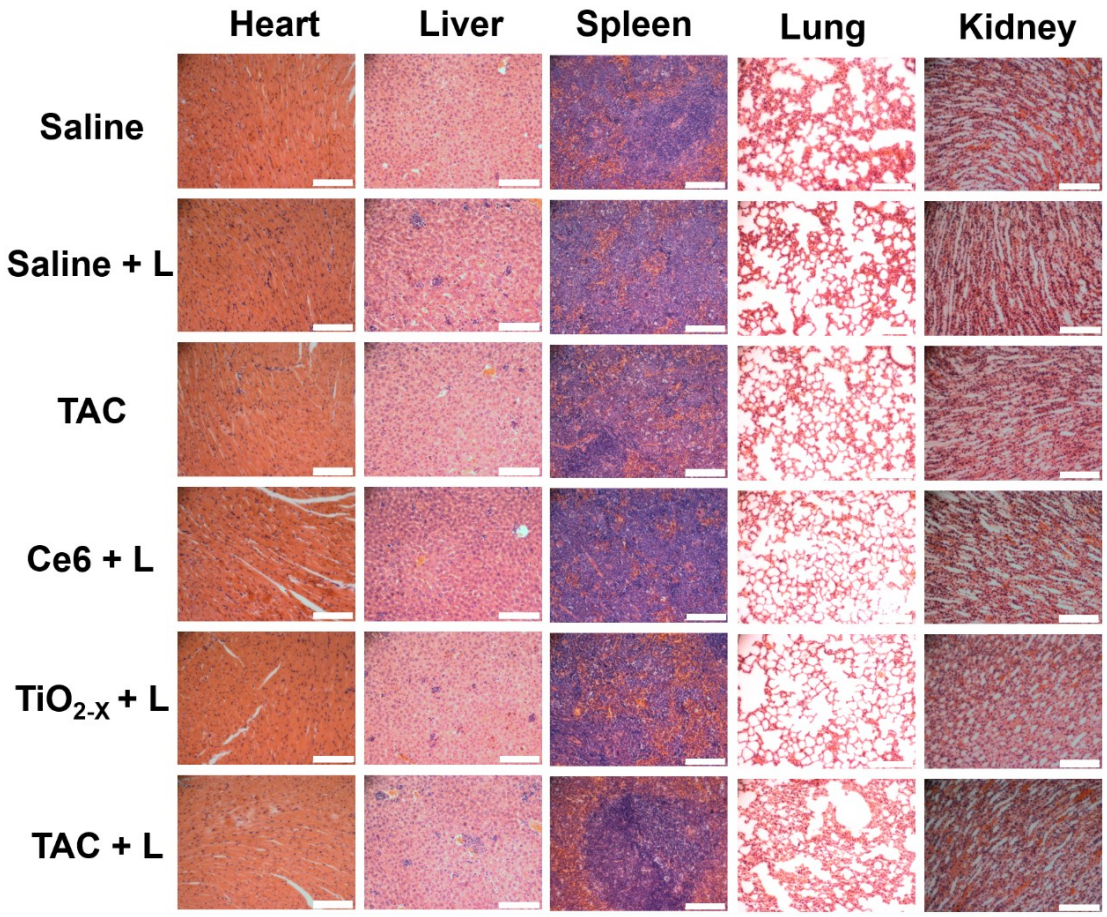

Fig. S10 H&E stained tumor slices from major organs excised from the groups subject to various treatments (scale bars: 100 μm).

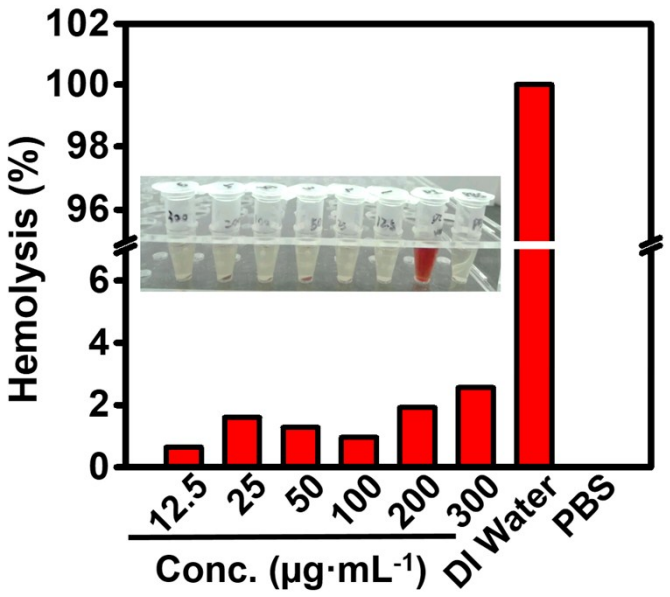

Fig. S11 Hemolysis rate by incubating RBCs with DI water (positive control), PBS (negative control) or TAC NPs under various concentrations. (Inset: corresponding digital images of tubes containing different samples).

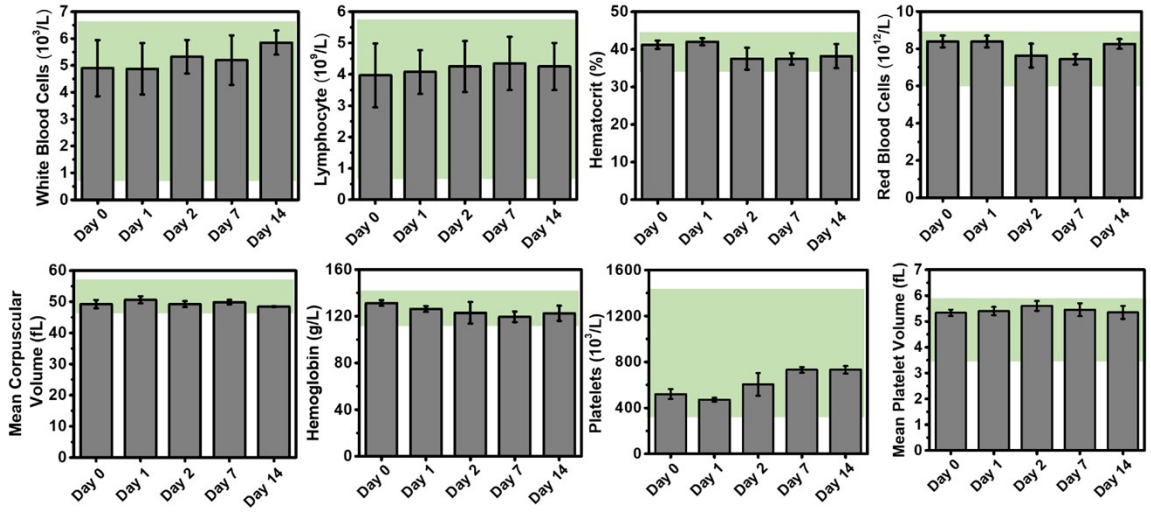

Fig. S12 Key indicators of blood routine test after KM mice being injected with TAC NPs. The olive hatched areas stand for the reference ranges of hematology index of healthy mice.