Electronic Supplementary Information for

UCNP@BSA@Ru nanoparticles with tumor-specific and NIR-triggered efficient PACT activity *in vivo*

Chao Zhang,[ab] Xusheng Guo,[ab] Xuwen Da,[ab] Yishan Yao,[c] Haihua Xiao,[d] Xuesong Wang,*[ab] and Qianxiong Zhou,*[a]

[a]*Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R. China.*
[b]*University of Chinese Academy of Sciences, Beijing 100049, P.R. China.*
E-mail: xswang@mail.ipc.ac.cn (X. Wang); zhouqianxiong@mail.ipc.ac.cn (Q. Zhou)
[c]*Beijing Institute of Pharmacology and Toxicology, Beijing 100850, P. R. China.*
[d]*State Key Laboratory of Polymer Physics and Chemistry, Beijing National Laboratory for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China.*

Experimental section

Materials

All chemicals were analytical grade and used without further purification. Thulium(III) acetate hydrate (99.9%), ytterbium(III) acetate tetrahydrate (99.9%), yttrium(III) acetate hydrate (99.9%), oleic acid (technical grade, 90%), 1-octadecene (technical grade, 90%), and ammonium fluoride (99.99+%), were purchased from Alfa Aesar. Poly(acrylic acid) (PAA MW = 1800) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Ruthenium(III) chloride trihydrate, 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDC) and 4,7-diphenyl-1,10-phenanthroline were purchased from Inno-Chem. Annexin V-FITC/PI apoptosis detection kit and Calcein-AM/PI live/dead cell double staining kit were purchased from Solarbio. Dulbecco's modification of Eagle's medium (DMEM), penicillin, streptomycin, and fetal bovine serum were purchased from Corning.

Instruments

An LED lamp (470 ± 10 nm) and 980 nm laser (Hi-Tech Optoelections Co., LOS-BLD-0980) were used as the light source for the irradiation assays. All UV-visible absorption spectra were acquired in aqueous solutions using Shimadzu UV-1601 spectrophotometer. TEM images were taken on a JEOL JEM2100F microscope operating at 200 kV. Upconverted emission spectra were collected on a Hitach 4600 photospectrometer coupled with a 980 nm laser device. Inductive

coupled plasma emission spectrometer atomic emission spectrometry (ICP-AES) measurements were performed on a Varian ICP-AES, 710-ES, USA. Laser confocal scanning microscope images were collected on an Olympus FV1000. Inductively coupled plasma mass spectrometry (ICP-MS) experiments were conducted on a PerkinElmer ELAN DRC-e.

Methods

Apoptosis assay：The Annexin V-FITC and PI apoptosis detection kit was used to detect apoptosis and necrosis. 4T1 cells were stained with different concentrations of UCNP@BSA or UCNP@BSA@Ru for 4 h. Cell medium was changed for fresh one, and the light group was irradiated with a 470 nm (22.5 mW/cm^2) LED lamp for 30 min, and then placed in an incubator for 10 h. The cells were trypsinized, harvested and washed 3 times with PBS, stained with Annexin V-FITC and PI, then detected by flow cytometry.

Cellular uptake：SKOV-3 and IOSE80 cells were cultured in a 25 cm^2 culture flask for 24 h. The examined agents were added, and cultured for 4 h. The cells were digested with trypsin, and collected with centrifugation after being washed three times with PBS. The uptake level was examined using ICP-MS by measurement of the Ru content or using confocal microscope to quantify the fluorescence intensity.

Assay for the lysosomal membrane permeabilization (Acridine Orange assay)：The SKOV-3 cells were co-cultured with particles for 4 h, then irradiated with 470 nm LED light for 25 min. Then SKOV-3 cells were cultured for 20 min with a

medium containing 5 μM acridine orange. Washed three times with PBS, culture medium was added, and images were collected with confocal microscope.

JC-1 staining assay：The SKOV-3 cells were co-cultured with particles for 4 h, then irradiated with 470 nm LED light for 25 min. Then SKOV-3 cells were cultured for 20 min with a medium containing 5 μM JC-1. Washed three times with PBS, culture medium was added, and images were collected with confocal microscope.

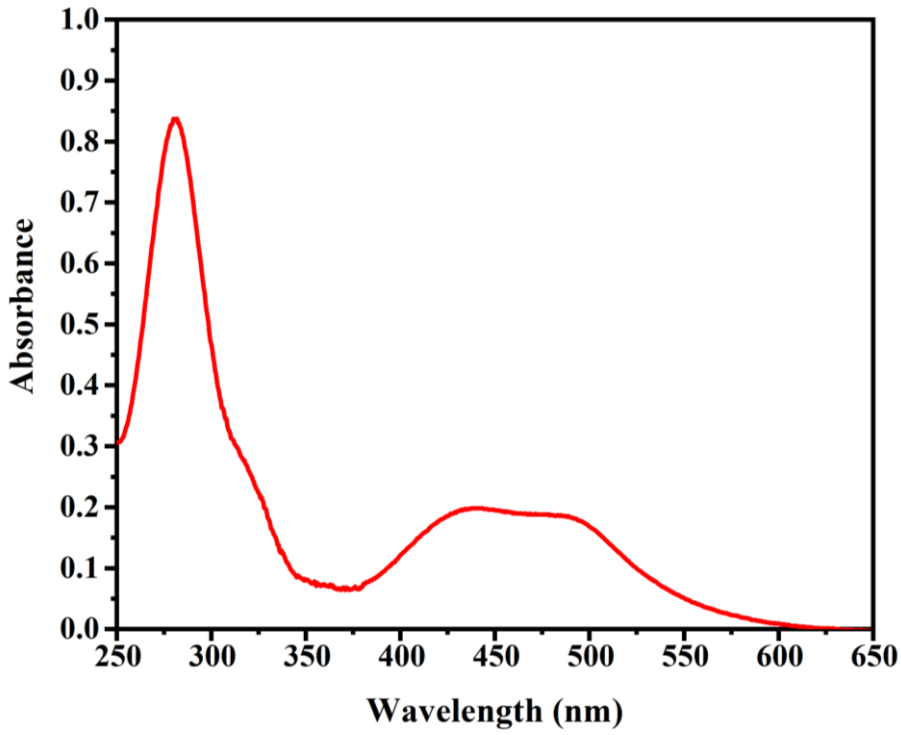

Figure S1 Absorption spectrum of $[Ru(dip)_2(spc)]^+$ (10 μM) in PBS

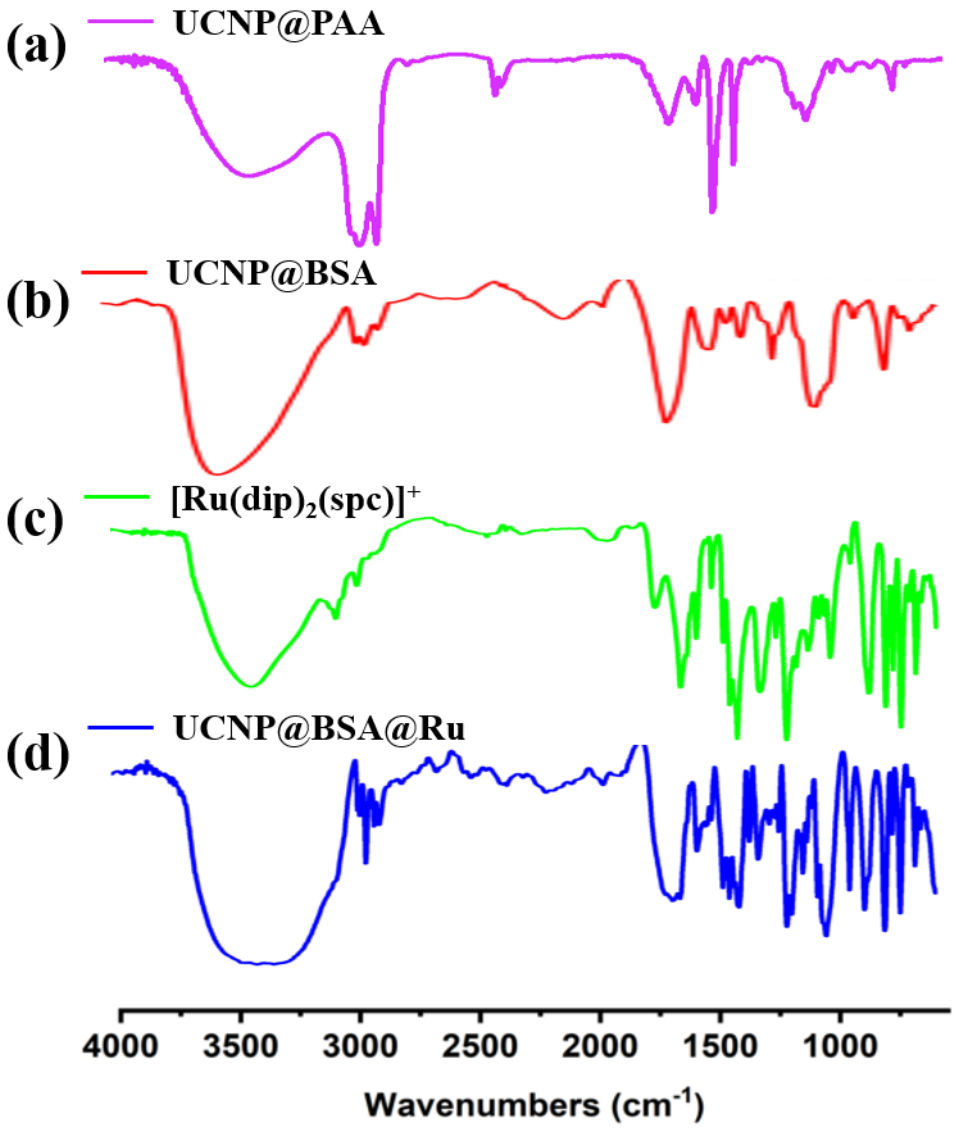

Figure S2 FT-IR spectra of UCNP@PAA (a), UCNP@BSA (b), $[Ru(dip)_2(spc)]^+$ (c), and UCNP@BSA@Ru (d).

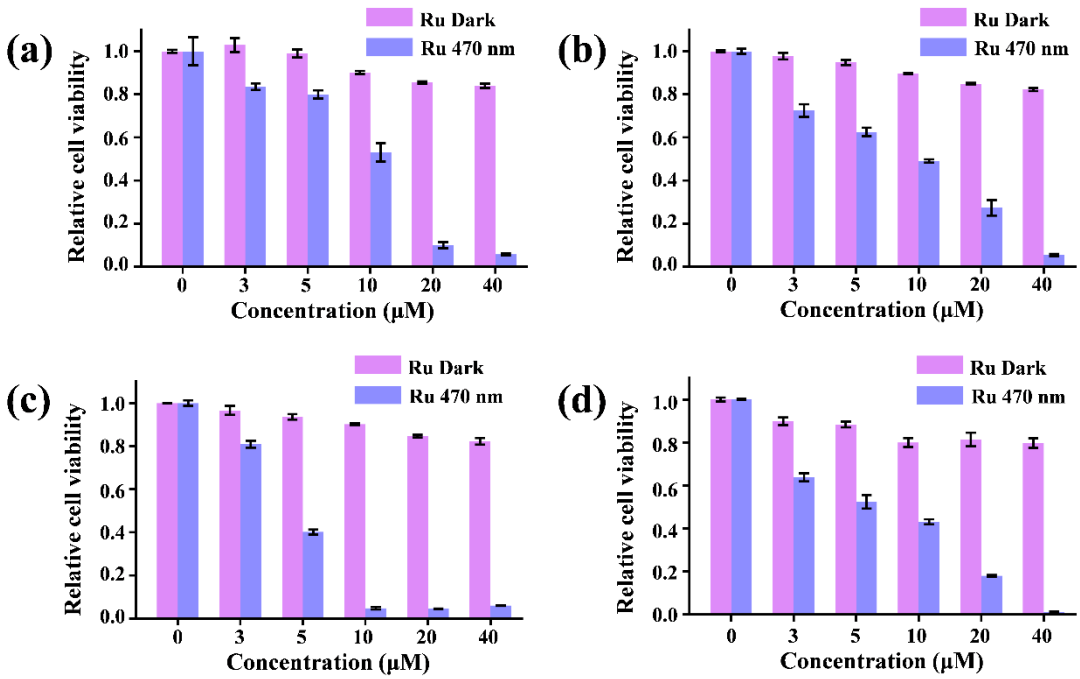

Figure S3 Cytotoxicity of $[Ru(dip)_2(spc)]^+$ (abbreviated as Ru in the legend) towards A549 (a), A549/DDP (b), IOSE80 (c) and SKOV-3 (d) cells in the dark (purple) and with 470 nm (blue) LED irradiation for 30 min (22.5 mW/cm^2).

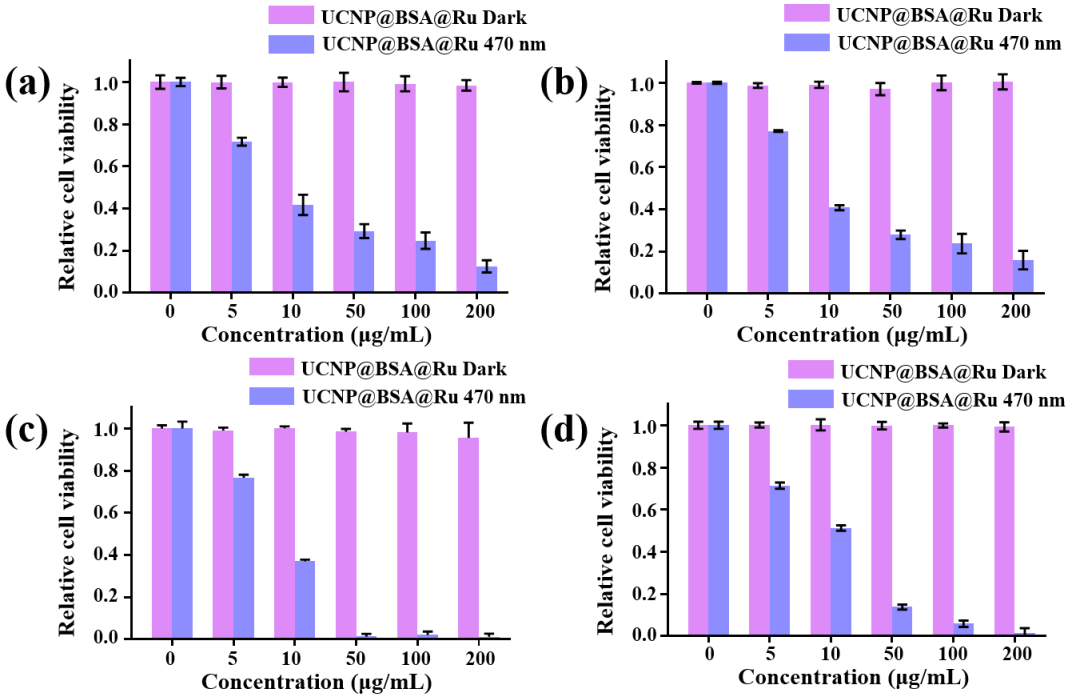

Figure S4 Cytotoxicity of UCNP@BSA@Ru towards A549 (a), A549 in hypoxia (b), A549/DDP (c) and SKOV-3 (d) cells in the dark (purple) and with 470 nm (blue) LED irradiation for 30 min (22.5 mW/cm^2).

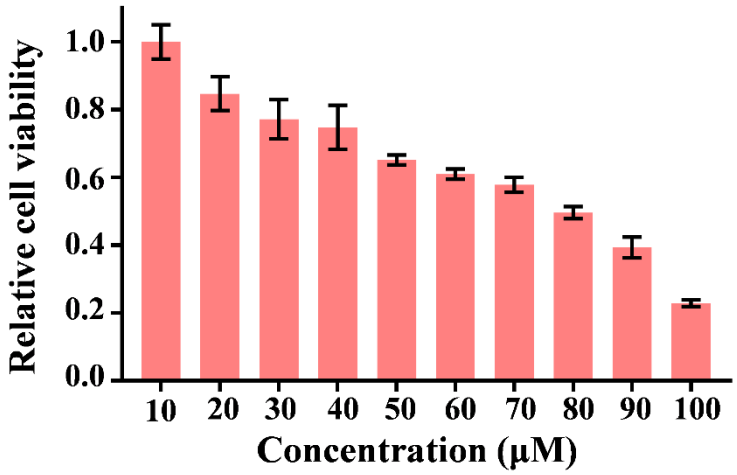

Figure S5 Cytotoxicity of Cisplatin towards A549 cells in the dark.

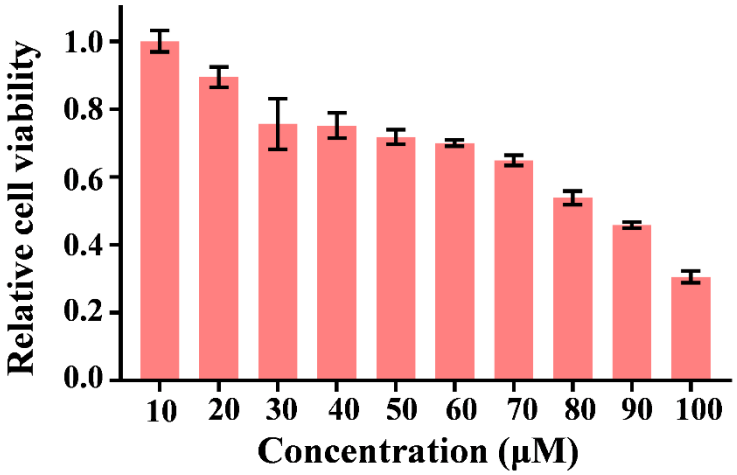

Figure S6 Cytotoxicity of Cisplatin towards A549/DDP cells in the dark.

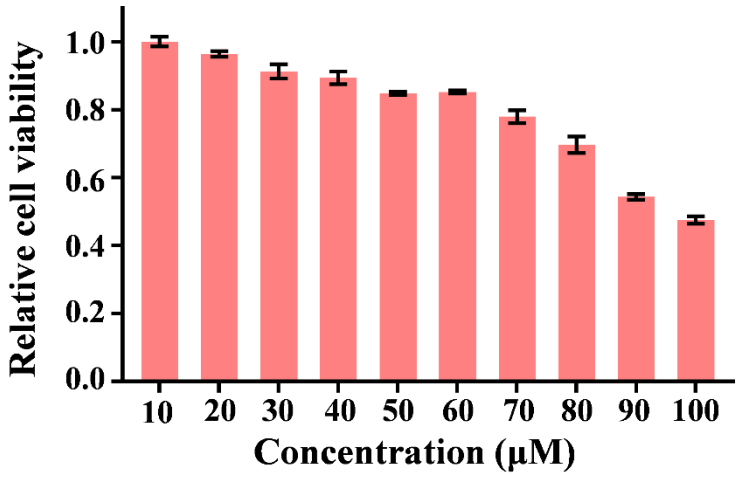

Figure S7 Cytotoxicity of Cisplatin towards SKOV-3 cells in the dark.

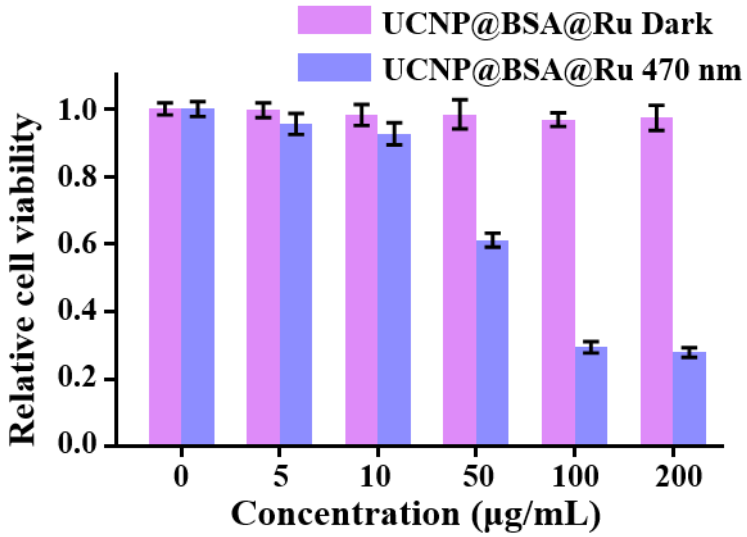

Figure S8 Cytotoxicity of UCNP@BSA@Ru towards IOSE80 cells in the dark (purple) and with 470 nm (blue) LED irradiation for 30 min (22.5 mW/cm^2).

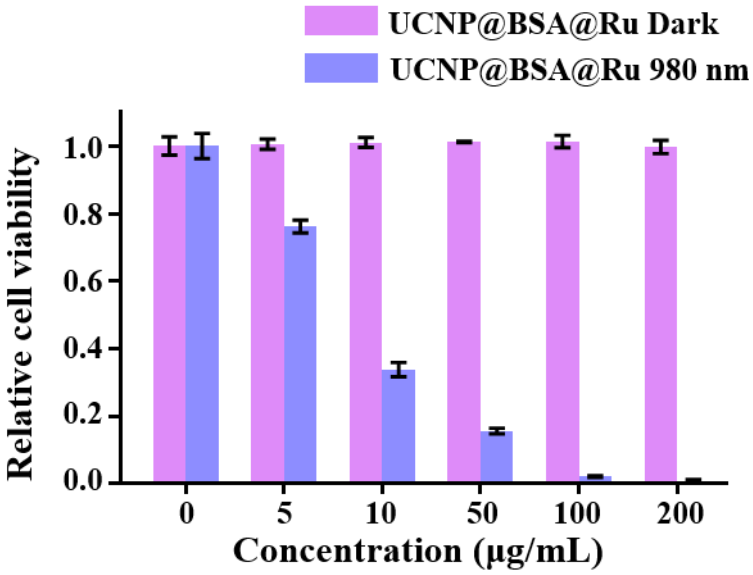

Figure S9 Cytotoxicity of UCNP@BSA@Ru towards A549 cells in the dark (purple) and with 980 nm (blue) laser irradiation for 20 min (3 W/cm^2).

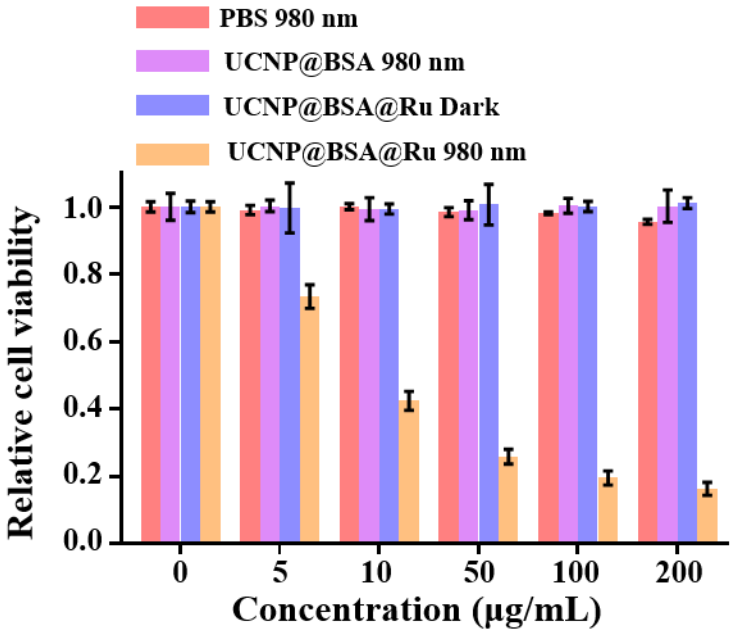

Figure S10 Relative viability of 4T1 cells treated with PBS, various concentrations of UCNP@BSA and UCNP@BSA@Ru under irradiation (980 nm laser lamp for 20 min (3 W/cm^2) or with UCNP@BSA@Ru in the dark.

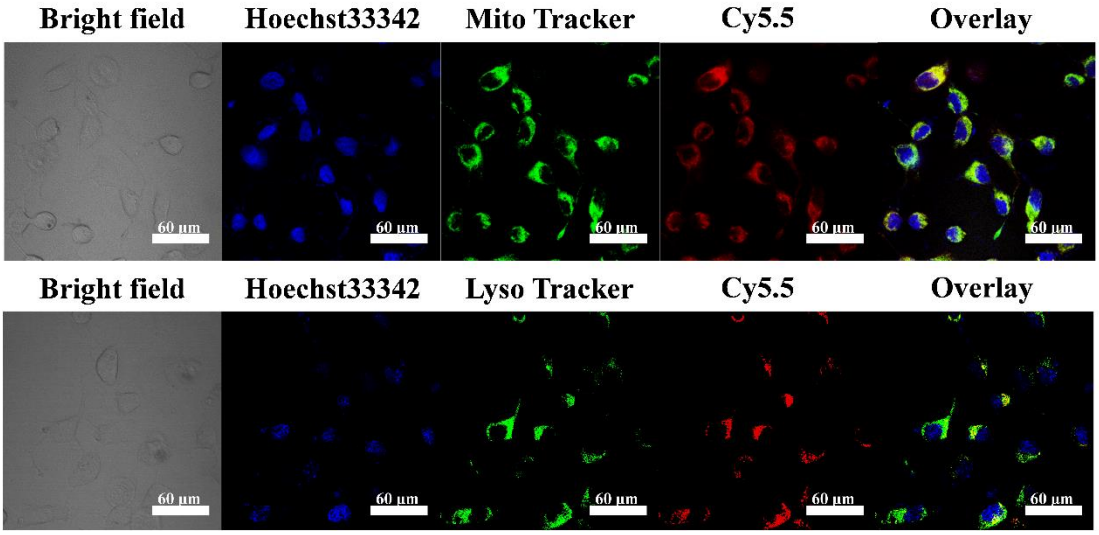

Figure S11 Confocal microscopy images of the live SKOV-3 cells co-labeled with 20 μg/mL the Cy5.5 modified UCNP@BSA@Ru nanoparticles for 4 h and then co-stained with Hoechst 33342, Mito-tracker Green (200 nM) or Lyso-tracker Green (200 nM) for 30 min. Hoechst 33342: λ_{ex} = 403 nm, λ_{em} = 450 ± 20 nm; Mito-tracker Green: λ_{ex} = 488 nm, λ_{em} = 516 ± 20 nm. Lsyo-Tracker Green: λ_{ex} = 488 nm, λ_{em} = 511 ± 20 nm; Cy5.5: λ_{ex} = 641 nm, λ_{em} = 710 ± 20 nm; Scale bars = 60 μm.

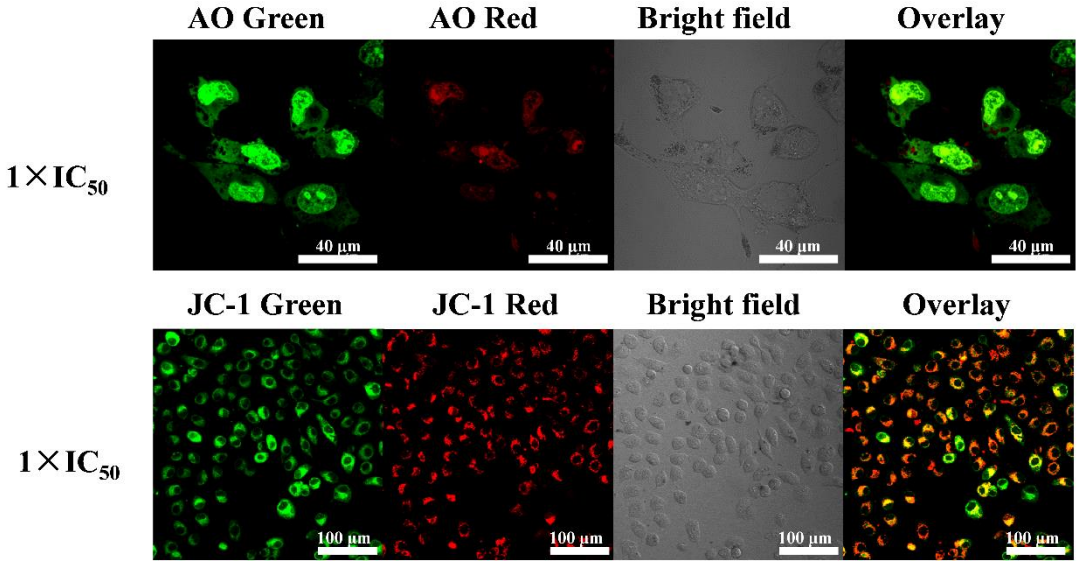

Figure S12 Images of AO (a) or JC-1 (b) stained SKOV-3 cells treated by UCNP@BSA@Ru (1× IC_{50} value) and 470 nm irradiation (22.5 mW/cm^2 for 30 min). (green fluorescence: λ_{ex} = 488 nm and λ_{em} = 510 ± 20 nm; red fluorescence: λ_{ex} = 488 nm and λ_{em} = 625 ± 20 nm).

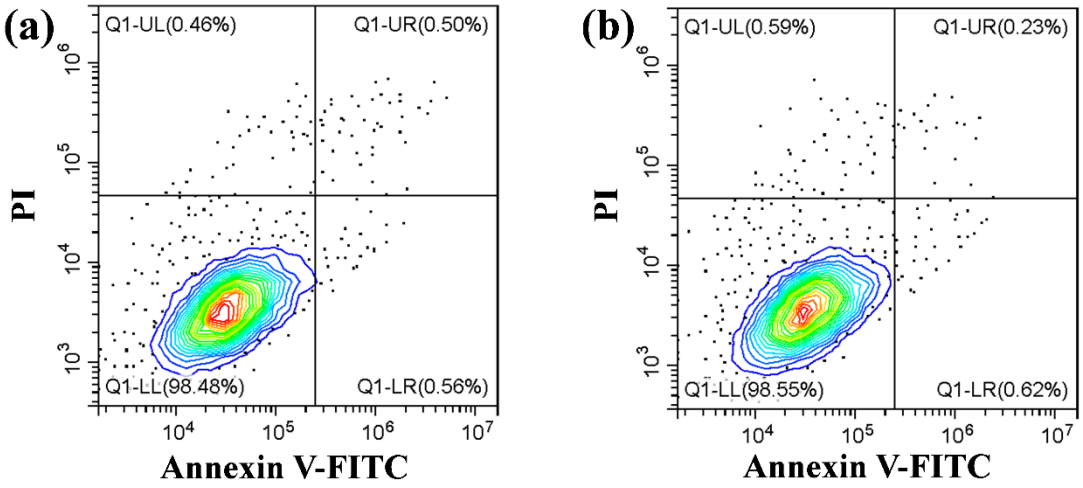

Figure S13 Flow-cytometric analysis of 4T1 cells based on Annexin V-FITC and PI staining. The cells were in the dark (a) or treated with only 470 nm irradiation for 30 min (b) (22.5 mW/cm^2).

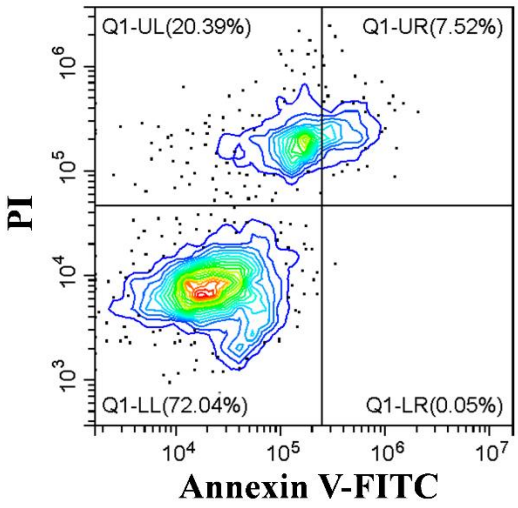

Figure S14 Flow-cytometric analysis of 4T1 cells based on Annexin V-FITC and PI staining. The cells were treated by both UCNP@BSA@Ru and 470 nm irradiation for 30 min (22.5 mW/cm^2).

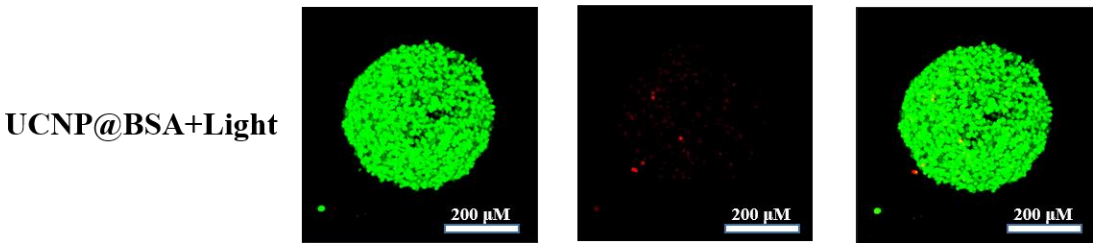

Figure S15 Images of 4T1 3D MCSs treated by UCNP@BSA (50 μg/mL) with 980 nm irradiation (3 W/cm^2 for 20 min), and stained by Calcein-AM and PI. Scale bars: 200 μm.

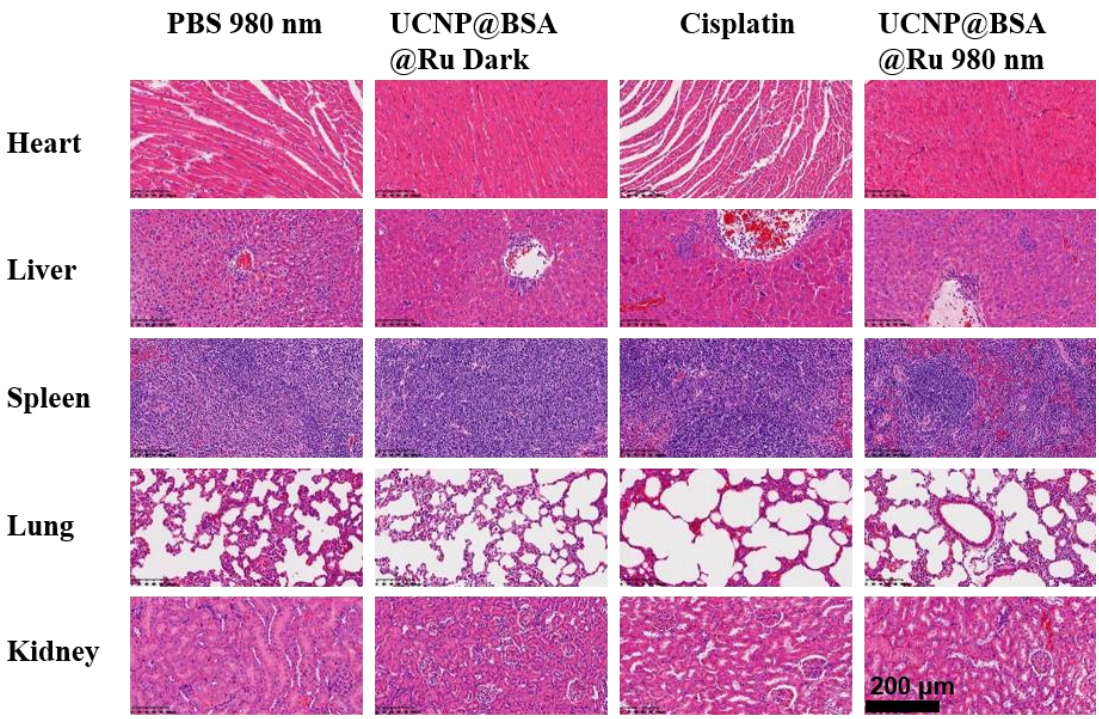

Figure S16 Histological analysis of the organs acquired from mice bearing 4T1 tumors on the 14th day after various treatments as indicated. Scale bars: 200 μm