ADVANCED MATERIALS

Supporting Information

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X-Ray-Controlled Generation of Peroxynitrite Based on Nanosized $LiLuF_4:Ce^{3+}$ Scintillators and their Applications for Radiosensitization

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Experimental Section

Materials:

Lutetium oxides (Lu_2O_3, 99.9%), $CF_3COOLi·H_2O$ (97%), $Ce(CH_3COO)_3·nH2O$ (99.99%), trifluoroacetic acid (TFA), oleic acid (OA, 90%), 1-octadecene (ODE, 90%), oleylamine (OM, 90%), sodium nitrite ($NaNO_2$, 97%), ammonium sulfide ($(NH_4)_2S$, 48%), iron(II) sulfate heptahydrate ($FeSO_4·7H_2O$, 98%) and ammonium hydroxide (NH_4OH, 28% NH_3) were purchased from Alfa Aesar Ltd. $Lu(CF_3COO)_3$ was prepared as reported in the literature.[1] D-alpha-tocopheryl poly (ethylene glycol 1000) succinate (TPGS) and Nitro blue tetrazolium (NBT) were purchased from Sigma-Aldrich. Cyclohexane and ethanol were supplied from the Beijing Chemical Reagent Company. Hoechst 33342, 3-Amino,4-aminomethyl-2,7-difluorescein, diacetate (DAF-FM DA), 2',7'-Dichlorofluorescein, diacetate (DCFH-DA) and dihydroethidium (DHE) were bought from the Beyotime Institute of Biotechnology. Cell counting kit-8 (CCK-8), Calcein-AM (CA) and propidium iodide (PI) were provided by Dojindo Laboratories in Japan. All the chemical reagents were of analytical grade and used as received without further purification. Poly(ADP-ribose) poerase (PARP), Cleaved-PARP and Phospho-Histone H2A.X (γ-H2AX) antibodies were obtained from Cell Signaling Technology Company (USA). Hypoxia inducible factor-1α (HIF-1α) and Nitro-Tyrosine antibodies were purchased from Abcam Company (USA). Purified water was used throughout.

Synthesis of $LiLuF_4:Ce^{3+}$ (2%) SCNPs:

OA-capped $LiLuF_4:Ce^{3+}$ (2%) SCNPs were synthesized via a successive layer-by-layer injection thermal decomposition strategy.[2] In a typical procedure, a mixture of $Lu(CF_3COO)_3$ (1.96 mmol), $Ce(CH_3COO)_3$ (0.04 mmol) and CF_3COOLi (2 mmol) were added into a 20 mL round-bottom beaker containing 6 mL of OA, 2 mL of ODE and 2 mL of OM. The resulting mixture was heated to 120 ^oC under N_2 flow with constant stirring for 1 h to form a clear

yellowish solution, and then cooled down to room temperature for the following reaction. Then, 3 mL of the above solution was added to a 50 mL round-bottom beaker containing 9 mL of OA, 3 mL of ODE and 3 mL of OM. The flask was subsequently heated to 310 ^{o}C under an argon (Ar) atmosphere and maintained for 2 h. After the homogeneous crystal nucleus created, the residual stock solution was tardily injected into the reaction mixture and ripened at 310 ^{o}C. The entire process lasted about 3 hours until the stock solution was exhausted. After injection, the reaction mixture was naturally cooled to the room temperature. Then, about 20 mL of anhydrous ethanol was added to precipitate the products. The as-obtained SCNPs were separated by centrifugation (12000 rpm, 5 min), washed with ethanol for 3 times, and finally re-dispersed in cyclohexane.

Synthesis of Ligand-Free SCNPs:

The ligand-free SCNPs were obtained by removing the surface oleic acid ligands through acid treatment. In a typical process, 50 mg of the as-synthesized OA-capped $LiLuF_4$:Ce^{3+} SCNPs were dispersed in 20 mL of acidic ethanol solution (pH 1; prepared by hydrochloric acid and absolute ethanol) under ultrasound to remove the oleic acid ligands. After 30 min, the SCNPs were collected by centrifugation (12000 rpm, 10 min), and re-dispersed in the acidic ethanol solution (pH 4) for further purification. The ligand-free SCNPs were washed with ethanol and distilled water several times, and finally re-dispersed in distilled water.

Synthesis of TPGS-Functionalized SCNPs:

The TPGS-Functionalized SCNPs (T-SCNPs) were obtained by the electrostatic interaction between TPGS and the ligand-free SCNPs. In brief, 20 mg of the ligand-free SCNPs were dispersed in phosphate buffer saline (PBS) solution and 1 mL of aqueous solution of TPGS (0.2 mM) was added. The mixture solution was allowed to stir overnight at 4 ^{o}C. The resulting T-SCNPs were washed with distilled water for several times, and then re-dispersed in distilled water.

Synthesis of Roussin's Black Salt (RBS):

Roussin's Black Salt ($[NH_4]$ $[Fe_4S_3(NO)_7]$) was synthesized as reported in the literature.[3] In brief, 20 mL of distilled water containing 5 g of $NaNO_2$ was added to a 250 mL 2-neck flask. Then 15 mL of distilled water containing 5 mL of $(NH_4)_2S$ was added under continuous stirring. The yellow solution was heated to 70 ^oC until the color turned dark red. Next, 80 mL of distilled water containing 10 g of $FeSO_4$ was added to the flask and the solution turned black immediately. Then 12 mL of NH_4OH solution was dropped into the flask. The reaction was heated to 90 ^oC for 10 min and purified by centrifugation. The brownish-black supernatant was collected and kept at 4 ^oC overnight. Finally, the RBS crystalline solid was obtained by centrifuge at 4 ^oC and dried through lyophilization.

RBS Loading and Stability Study:

RBS was loaded on the surface of T-SCNPs by a soaking method. In brief, 10 mg of T-SCNPs was dispersed in 10 mL of RBS aqueous solution with different concentrations (50 ~ 400 μM) and stirred overnight under dark conditions. The obtained product was collected by centrifugation, washed with PBS solution for 3 times and dried via lyophilization. The loading amounts were calculated according to the standard curves of RBS and the UV-vis absorption variation of the supernatant before and after RBS loading. The product was denoted as RBS-T-SCNPs. The stability of RBS-T-SCNPs was researched by detecting the released amount of RBS with time. For determination, 10 mg of RBS-T-SCNPs was re-dispersed in 20 mL of PBS buffer and stirred at 37 ^oC under dark conditions. After certain time intervals, 3 mL of the solution was sucked out to centrifuge and the UV-vis absorption of the supernatant was measured. The amounts of released RBS were calculated from the changes of the UV-vis absorbance.

Characterization:

The morphology and size of the nanoparticles were observed on a transmission electron microscopes (TEM, JEM2100Plus, JEOL) and a filed-emission scanning electron microscope (FE-SEM, S-4800, Hitachi). X-ray diffraction (XRD) patterns of the nanoparticles were collected by a Japan Rigaku D/max-2500 diffractometer using Cu kα radiation (λ = 1.5418Å). Dynamic light scatting (DLS) and zeta potential analysis were provided by Brookhaven instruments, NanoBrook Omni, 280044. The emission spectra of SCNPs were measured by a self-constructed platform converted from a fluorescence spectrophotometer (Horiba Jobin Yvon FluorolLog3) as the detection component and a controllable X-ray tube (Mini-X, AMPTEK Inc.) as the excitation source. The UV-Vis absorption data was acquired by U-3900 spectrophotometer (HITACHI). Fourier transform infrared (FT-IR) spectra were collected using the Fourier transform Bruker EQUINOX55 spectrometer with the KBr pellet technique.

Detection of Superoxide (O_2^{-}•) under X-ray Irradiation:

The production of O_2^{-}• generated under X-ray irradiation was monitored by using nitro blue tetrazolium (NBT), which could be specifically reduced by O_2^{-}• to form the insoluble purple formazan.[4] For this reason, the production of O_2^{-}• was quantitatively analyzed by the absorbance changes of NBT at 260 nm. In a typical experiment, proper concentration of NBT solution was mixed with the T-SCNPs solution (100 μg mL^{-1}) or PBS solution and subjected to X-ray, respectively. The absorbance of both solutions before and after irradiation was recorded for the evaluation of the produced O_2^{-}•, respectively.

Detection of Nitric Oxide (NO) under X-ray Irradiation:

The X-ray triggered nitric oxide release from RBS-T-SCNPs was quantitatively measured using a Free Radical Analyzer (TBR 1025) with ISO-NOP sensor (World Precision Instruments, Inc.). Before detection, the standard curve derived from the corresponding

electrical currents and NO concentrations was constructed by chemical generation of NO. After washing with deionized water for several times, the sensor tip was immersed in 10 mL of RBS-T-SCNPs solution (2 mg mL^{-1}), ensured the stable current under continuous stirring. The solution was irradiated with the mini X-ray tube under diverse voltages (50, 35, 20 kV), the acutely varying electrical current along with time was recorded and transformed into NO concentration.

Detection of Peroxynitrite ($ONOO^-$) under X-ray Irradiation:

The X-ray triggered $ONOO^-$ generation was monitored by using L-Tyrosine as a probe molecule, which could be oxidized by $ONOO^-$ in the presence of CO_2 in a weak alkaline environment.[5] In brief, 10 mL of an aqueous solution containing PBS (0.10 M, pH = 8.2), $NaHCO_3$ (0.015 M), and L-tyrosine (5.0×10^{-4} M) was prepared at room temperature. Then 1 mg of RBS-T-SCNPs was added to the solution and illuminated with X-ray for 10 min. The fluorescence spectrum of both solutions before and after irradiation was recorded for the evaluation of the produced $ONOO^-$, respectively.

Detection of Reactive Oxygen Species (ROS) under X-ray Irradiation:

In order to detect the ROS yield under X-ray irradiation, 2',7'-dichlorofluorescein diacetate (DCFH-DA) was employed. In brief, 10 μL of DCFH-DA in DMSO was reacted with 40 μL NaOH solution (0.01 M) in the dark conditions. After 30 min, 200 μL of the phosphate buffer (PBS, 25 mM, pH 7.2) was added to the solution to stop the reaction. The chemically hydrolyzed DCFH solution was kept in dark, and preserved on ice for subsequent use. In a typical experiment, T-SCNPs solution (100 μg mL^{-1}) or PBS solution was mixed with the DCFH solution (10 μM) and subjected to X-ray, respectively. The fluorescence spectrum of both solutions before and after irradiation was recorded for the evaluation of the produced ROS, respectively.

Cell Culture:

Human lung adenocarcinoma cells (A549) and human umbilical vein endothelial cells (HUVECs) were cultured with RPMI-1640 culture medium which were supplemented with FBS (10%), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}). The cells were cultured at 37 ^{o}C in a 21% O_2 /5% CO_2 /74% N_2 environment. The medium was changed every two days.

Detection of intracellular O_2^{-}• under X-ray Irradiation:

The intracellular O_2^{-}• generation was measured by a commercial fluorescence probe, dihydroethidium (DHE), and then recorded by confocal laser scanning microscopy and flow cytometry. For O_2^{-}• fluorescence image, A549 cells were uniformly seeded in several confocal dishes at 5×10^4 cells per well and cultured for 24 h. After adherent, the cells were incubated with T-SCNPs (100 μg mL^{-1}) for 6 h. The treated cells were rinsed and incubated with the DHE solution for 30 min. After the free probe was clear away, the stained cells were irradiated with X-ray, respectively, and immediately imaged under the confocal laser scanning microscopy. For the quantitative determination of O_2^{-}• level, A549 cells were seeded in 6-well plates with a density of 20×10^4 cells per well. After attachment, RBS-T-SCNPs (100 μg mL^{-1}) were added and co-incubation with cells for 4 h. The cells were incubated with the DHE solution for 30 min and then irradiated with X-ray, respectively. Then all the cells were collected by trypsinization and centrifugation, setting on ice for subsequent flow cytometry experiment.

Detection of intracellular Nitric Oxide under X-ray Irradiation:

The intracellular NO generation was measured by a fluorescence probe, 3-Amino,4-aminomethyl -2', 7' – difluorescein, diacetate (DAF-FM DA), and then recorded by confocal laser scanning microscopy and flow cytometry. For NO fluorescence image,

A549 cells were uniformly seeded in several confocal dishes at 5×10^4 cells per well and cultured for 24 h. After adherent, the cells were incubated with RBS-T-SCNPs (100 μg mL^{-1}) for 6 h. The cells were rinsed with PBS three times and incubated with the DAF-FM solution for 15 min. After the free probe was clear away, the stained cells were irradiated with X-ray, respectively, and immediately imaged under a confocal laser scanning microscopy (A1/LSM-Kit, Nikon). For the quantitative determination of NO level, A549 cells were seeded in 6-well plates with a density of 20×10^4 cells per well. After attachment, RBS-T-SCNPs (100 μg mL^{-1}) were added and co-incubation with cells for 6 h. Then all the cells were collected by trypsinization and centrifugation, and treated with the DAF-FM solution for 15 min. After the free probe was clear away, the stained cells were irradiated with X-ray. The fluorescent intensity and distribution were recorded by flow cytometry (Accuri c6, BD, USA).

Detection of intracellular ROS under X-ray Irradiation:

The intracellular ROS was detected by DCFH-DA and recorded by confocal laser scanning microscopy. In brief, A549 cells were uniformly seeded in several confocal dishes at 5×10^4 cells per well and cultured for 24 h. Then, T-SCNPs (100 μg mL^{-1}) in the culture media (RPMI) were added and co-incubated for 6 h. The treated cells were rinsed and incubated with the DCFH-DA solution for 20 min. Afterward, X-ray radiation was performed on the cells after rinsed with culture media to clear away free DCFH-DA. Finally, the stained cells were imaged under a confocal laser scanning microscopy.

In Vitro Cytotoxicity Study:

The viability of A549 and HUVEC was assessed by a Cell Counting Kit-8 (CCK-8) assay and double Calcein-AM (CA)/Propidium Iodide (PI) staining method. For a CCK-8 assay, the cells were seeded into 96-well plates at a density of 6000 cells per well and cultured for 24 h. Then different concentrations (0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400

μg mL^{-1}) of T-SCNPs or RBS-T-SCNPs dispersed in the culture media (RPMI) were added and co-incubated for 24 h. After treated, 10 μL of Cell Counting Kit-8 (CCK-8) solution was added and further incubated for 1 h. The absorbance was determined with a microplate reader (Thermo Scientific, Multiscan MK3) at 450 nm. For a CA/PI staining, the cells were seeded in 6-well plates at 5×10^4 cells per well and cultured for 24 h. Then, T-SCNPs or RBS-T-SCNPs (100 μg mL^{-1}) were added and co-incubated for 24 h. After treated, the cells were incubated with the CA and PI solution for 15 min, and imaged under an inverted luminescence microscope (Olympus X-73, Japan).

Cellular Uptake Assays:

Cellular uptake was examined using ICP-MS. A549 cells were seeded in 12-well plate with a density of 2×10^5 cells per well and cultured for 24 h. After complete adherent, the cells were incubated with RBS-T-SCNPs (50 μg mL^{-1}). After different incubation times, the cells were rinsed with PBS for 3 times and collected by trypsinization and centrifugation. ICP-MS was used to determine the gadolinium contents in the cell lysis solution.

Colony Formation Assays:

For the clonogenic assay, A549 and/or BEL-7402 cells with different density (250, 500, 1000, 2000, 4000) were seeded in 6-well plate and cultured for 24 h. After complete adherent, the cells were incubated with T-SCNPs or RBS-T-SCNPs (50 μg mL^{-1}) for 24 h. The treated cells were rinsed with PBS for 3 times and changed with new culture medium. Then, the cells with different density were treated with different X-ray irradiation time (0, 20, 40, 60, 80 s) and further cultured for 10 days. Finally. The cell colonies were fixed with 4% paraformaldehyde and stained with Giemsa dye. The effects of different treatments were evaluated by counting the survival fraction of the colonies.

Apoptosis and Necrosis Assay:

The cells' apoptosis/necrosis under X-ray irradiation was evaluated by an annexin V-FITC/PI double staining through flow cytometry. In brief, A549 cells were seeded in 6-well plates at the density of 2×10^5 and cultured for 24 h. After attachment, the cells were incubated with T-SCNPs or RBS-T-SCNPs (100 μg mL^{-1}) for 6 h. Then, the cells were irradiated with X-ray, respectively, and further cultured for 24 h. Finally, all cells were collected by trypsinization and centrifugation, washed with PBS buffer, dyed with Annexin V-FITC/PI kit, and detected by flow cytometry.

Immunofluorescence of DNA Double-Strand Breaks:

The γ-H2AX immunofluorescence analysis was exploited to characterize the DNA double-strand breaks. The experiment was divided into six groups (control, T-SCNPs, RBS-T-SCNPs, X-ray, T-SCNPs + X-ray and RBS-T-SCNPs + X-ray). A549 cells were seeded in several confocal dishes at 3×10^4 cells per well and cultured for 24 h, different approaches were employed with or without nanoparticles and X-ray, respectively. Four hours after X-ray treatment, the cells were fixed with immunostaining fix solution for 10 min. Triton X-100 and bovine serum albumin were used to penetrate the cells and block other nonspecific protein interactions, respectively. Then, the cells were incubated with anti-phospho-histone γ-H2AX rabbit monoclonal antibody overnight at 4 ^oC, and rinsed with PBS. Finally, the cells were incubated with the Cy3 tag goat anti-rabbit IgG (H+L) secondary antibody for 1 h at room temperature. DAPI staining solution was used to stain the cell nuclei and confocal laser scanning microscopy was used to record the fluorescence imaging.

Western Blot:

The expression level of target proteins in A549 cells were measured by Western blot assay. In brief, A549 cells were seeded in 6-well plates at 5×10^4 cells per well and cultured for 24 h. Then the cells were treated with T-SCNPs or RBS-T-SCNPs (100 μg mL^{-1}) for 4 h, and irradiated with X-ray. After 24 h, the cells were collected and lysed by pre-cooled RIPA

buffer on ice. The protein samples were separated by SDS-PAGE (10% gel) and transferred onto nitrocellulose membranes (PVDF). After blocking with 5% non-fat milk, the membranes were incubated with specific primary antibodies (1:1000) overnight at 4 ^{o}C. After 3 times washing, incubations with secondary HRP-linked antibodies were performed at room temperature for 1 h. For detection, the membranes were stained by an ECL detection kit and developed in the FluorChem HD2 Chemiluminescent Imaging System (ProteinSimple, USA).

Photoacoustic Imaging:

To measure the vascular saturated O_2 within A549 solid tumors, the Multispectral Optoacoustic Tomography (MSOT) imaging systems (M-128, iThera) was used. The saturated O_2 around the tumor was measured by the differential optical absorption of oxygenated and deoxygenated hemoglobin at the different wavelength of 850 nm and 750 nm, respectively. The images of the mice before intratumoral injection, after injection and after X-ray irradiation were recorded.

Radiotherapy:

All procedures used in this experiment were under protocols approved by the Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety (Institute of High Energy Physics, CAS). To develop the tumor model, a total of 1.0×10^6 A549 cells were subcutaneously injected into the right hind legs of female BALB/c nude mice. When the tumor volume reached about 80 mm^3, the mice were randomly divided into 6 groups with 5 mice in each group: (i) control, (ii) T-SCNPs, (iii) RBS-T-SCNPs, (iv) X-ray, (v) T-SCNPs + X-ray, (vi) RBS-T-SCNPs + X-ray. The tumor-bearing mice of groups (ii), (iii), (v) and (vi) were intratumorally injected with 20 μL of T-SCNPs or RBS-T-SCNPs (2 mg mL^{-1}), then group (iv), (v) and (vi) were subjected to X-ray (radiation dose = 6 Gy). After treatment, the mice were normally bred, the tumor volume and body weight were recorded after administration for 28 days. The tumor volume was calculated by the following formula V= [(length)

(width)^2]/2. One day after the radiation, one of the mice in each group was sacrificed. The tumors and the major organs (including heart, liver, spleen, lung, and kidney) were stained to monitor the histological changes. Other mice were sacrificed until the end of the experiment. The tumor and major tissues were weighed and stained to assess the therapeutic efficacy of each group. The blood samples were collected after the mice were sacrificed, and the blood hematology and biochemistry analyses were accomplished at the Animal Department of Peking University Medical Laboratory.

Immunohistochemical Staining Analyses in vivo:

The staining was performed on tissue sections excised from the tumors with the different treatments. The antigen retrieval was performed using a pressure cooker containing 10 mM citrate buffer (pH 6.0). The immunohistochemistry was accomplished with the γ-H2AX antibody (dilution 1:200), HIF-1α antibody (dilution 1:800), Cleaved-PARP antibody (dilution 1:1000) and Nitro-Tyrosine antibody (dilution 1:1000), respectively. The staining images were observed by the inverted luminescence microscope.

CT Imaging in Vitro and in vivo:

For in vitro CT imaging, T-SCNPs and iobitridol (commercial CT contrast agent) were dispersed in PBS with different concentrations (0, 1.875, 3.75, 7.5, 15, and 30 mg mL^{-1}) and then placed for CT imaging. To perform in vivo CT imaging, A549 tumor-bearing nude mice were anesthetized and scanned to obtain the CT image of comparison. Then, the mice were intratumorally injected with T-SCNPs (20 μL, 20 mg mL^{-1}) and were scanned at time intervals. The in vitro and in vivo CT images were collected using an in vivo imaging system (Perkins Elmer).

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Table S1. The detailed parameters of all the in-vitro irradiation experiments.

Type	Voltage (kV)	Current (μA)	Distance (cm)	Time (min)	Dose rate (Sv/h)
Controlled Release of NO and O_2^{-}•	50	75	3	10	100
Colony Formation Assays	50	75	2.1	0.1~2	200
Apoptosis and Necrosis Assay	50	75	3	10	100
DNA Damage	50	75	3	10	100
Western Blot	50	75	3	10	100

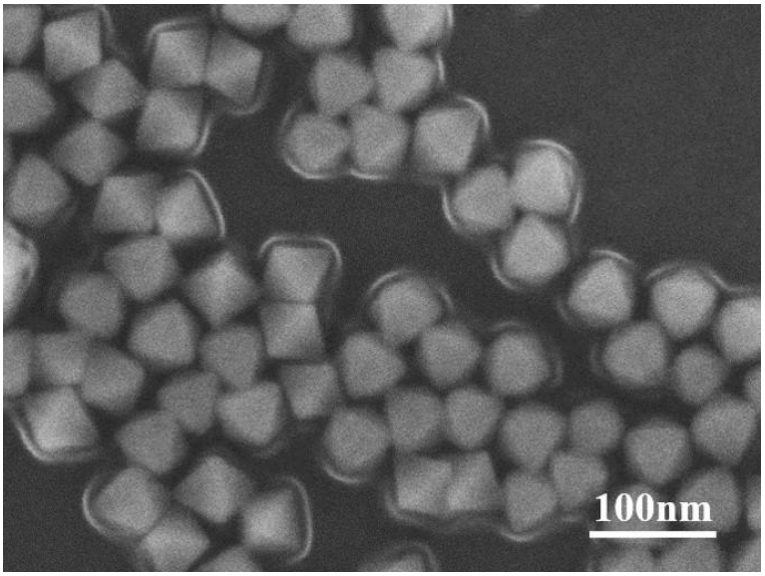

Figure S1. SEM images of the $LiLuF_4:Ce^{3+}$ nanoparticles.

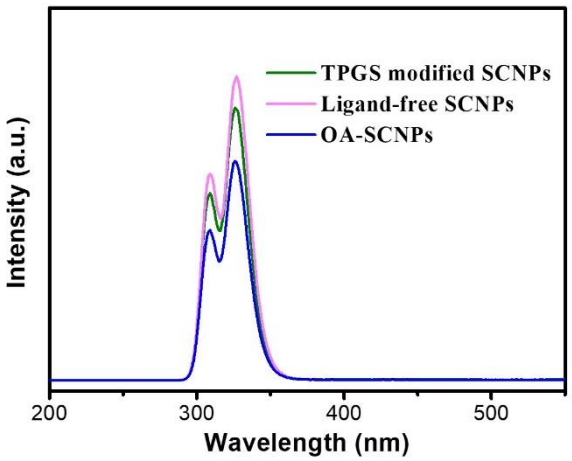

Figure S2. The radioluminescence of SCNPs with different surface treatments.

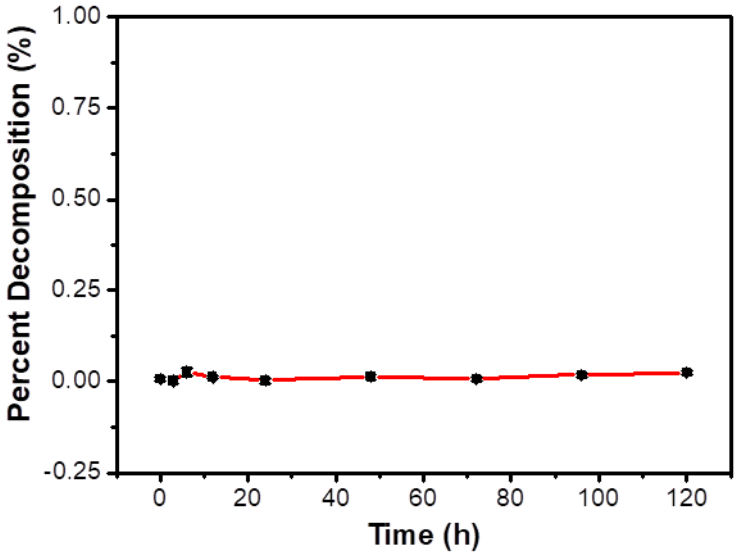

Figure S3. The time-dependent release curve release of Lu element from T-SCNPs.

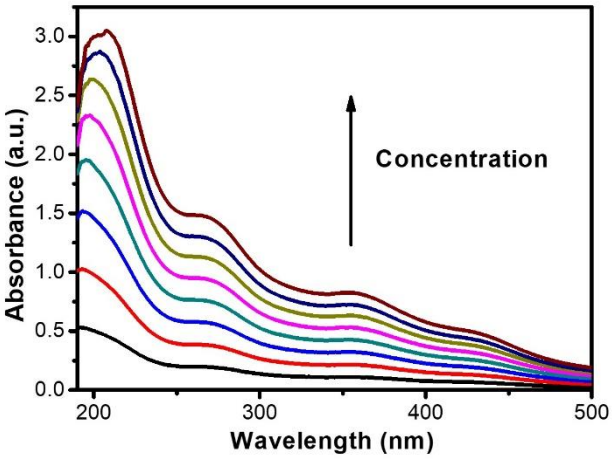

Figure S4. UV-Vis absorption spectra of RBS with different concentrations.

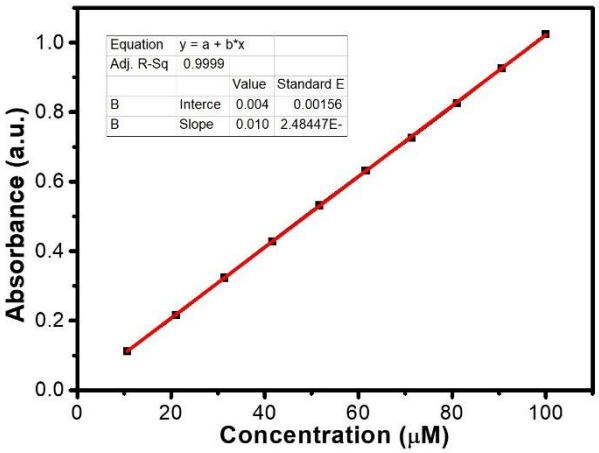

Figure S5. The standard curve of RBS at 430 nm wavelength.

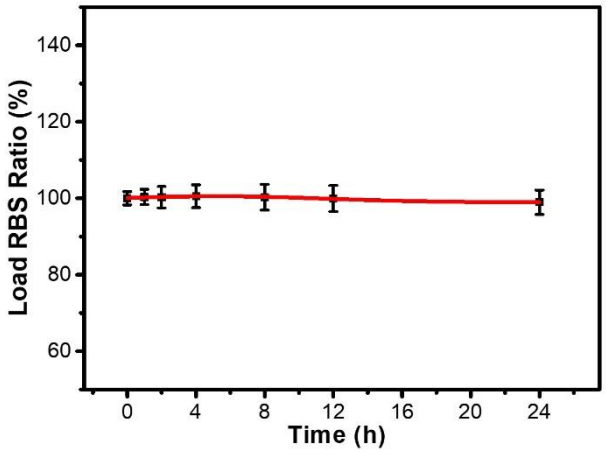

Figure S6. The stability of RBS-T-SCNPs in PBS medium. After 24 h stirring, less than 5% of the RBS was detached from the T-SCNPs, indicating the high stability for RBS-T-SCNPs stored in physiological solutions. Error bars represent standard deviation for n = 3.

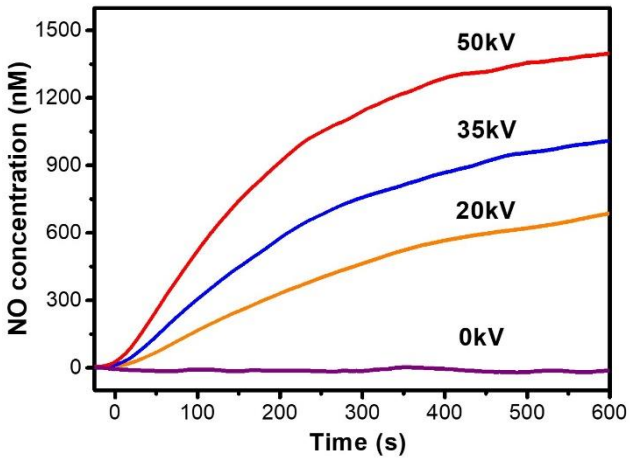

Figure S7. The NO concentration as a function of the irradiation time induced by X-ray under diverse voltages.

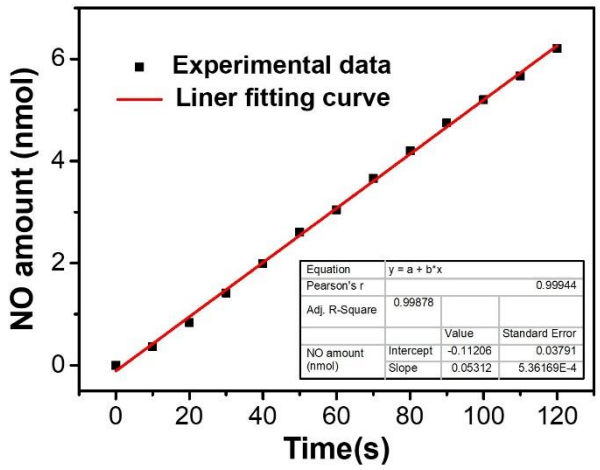

Figure S8. The total amount of NO variation along with time under X-ray irradiation. (2 mg mL^{-1} of RBS-T-SCNPs in 10 mL PBS solution, 100 Sv/h).

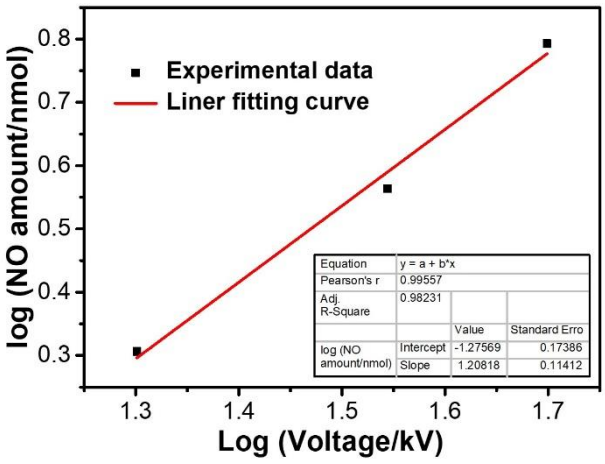

Figure S9. A plot of the quantity of NO generated vs the irradiation voltage of the X-ray tube. In each case the irradiation time was constant.

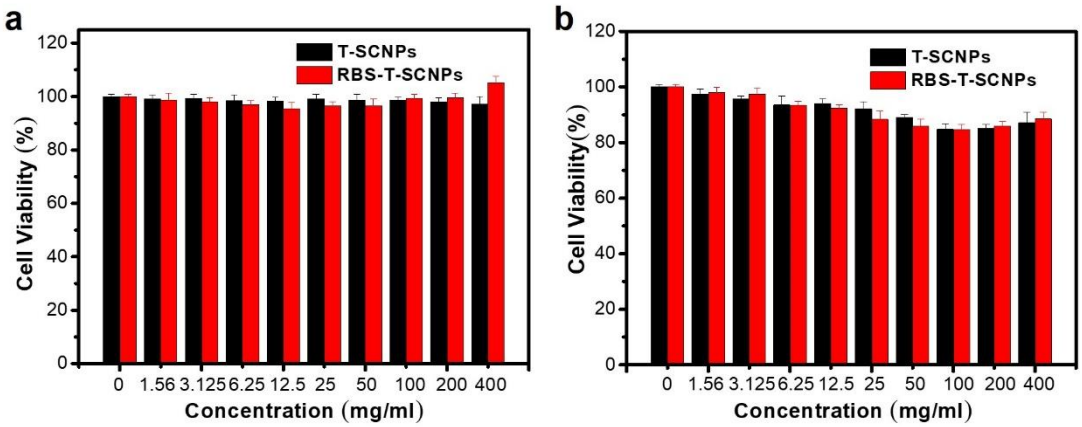

Figure S10. Relative viabilities of A549 **(a)** and HUVEC **(b)** cells that incubated with the T-SCNPs or RBS-T-SCNPs of different concentrations for 24 h. Error bars represent standard deviation for n = 6.

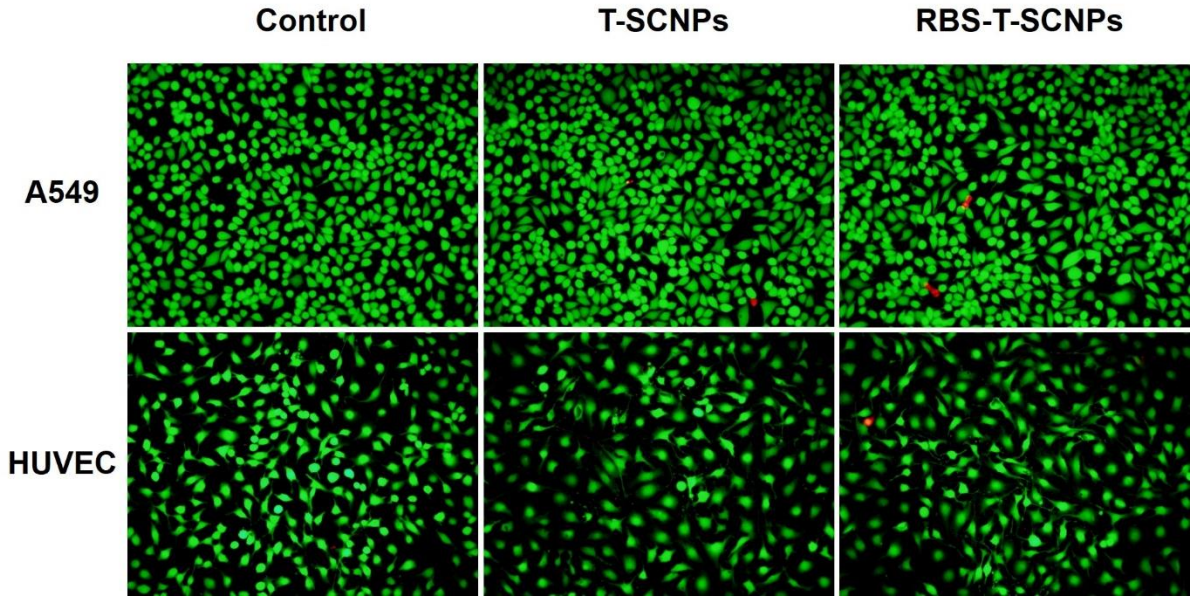

Figure S11. Calcein AM/propidium iodide (PI) double staining of A549 and HUVEC cells that incubated with the T-SCNPs or RBS-T-SCNPs for 24 h.

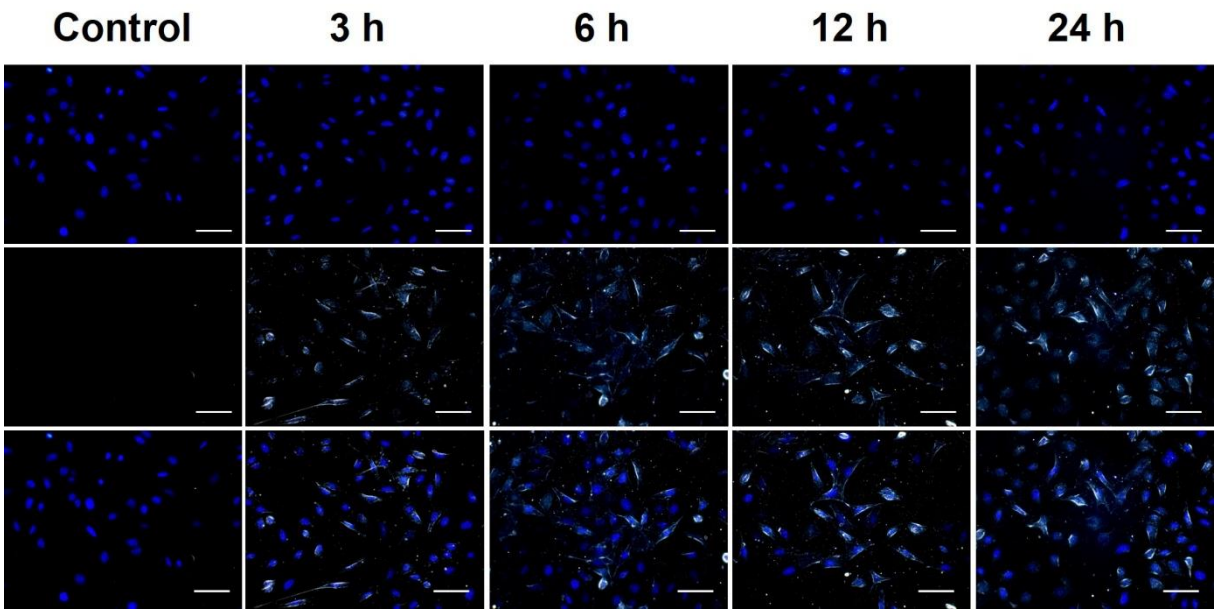

Figure S12. Dark-field optical microscopy images of A549 cells incubated with RBS-T-SCNPs (50 μg mL^{-1}) for different times. Cell nucleus were stained with Hoechst 33342. The scale bar is 100 μm.

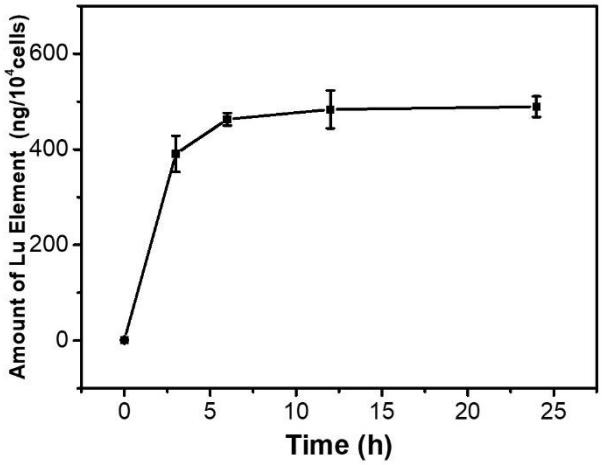

Figure S13. Time-dependent cellular uptake of RBS-T-SCNPs determined by inductively coupled plasma-mass spectrometry (ICP-MS) after incubation. The concentration of SCNPs was 50 μg mL^{-1}).

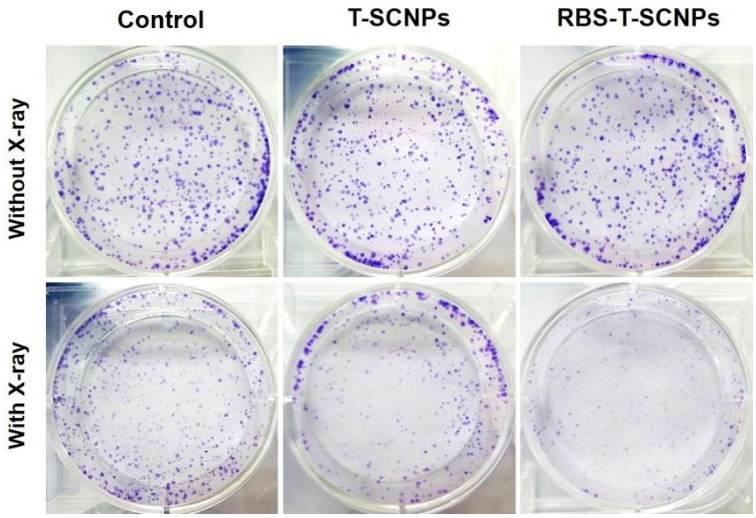

Figure S14. Colony formation assay of BEL-7402 cells incubated with T-SCNPs or RBS-T-SCNPs with or without X-ray irradiation.

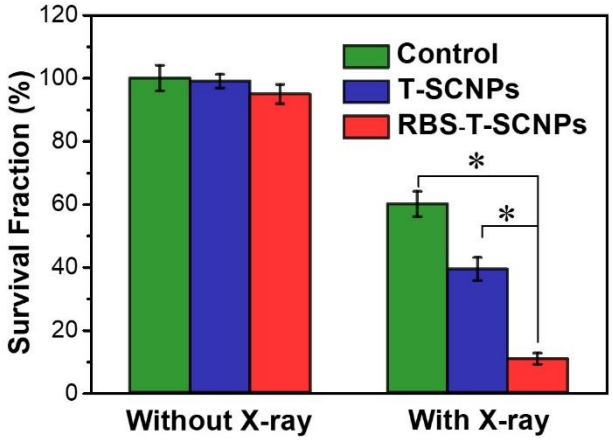

Figure S15. Corresponding surviving fraction of BEL-7402 cells with various treatments. Error bars represent standard deviation for n = 3, P values were based on the Student's *t*-test: *P < 0.001.

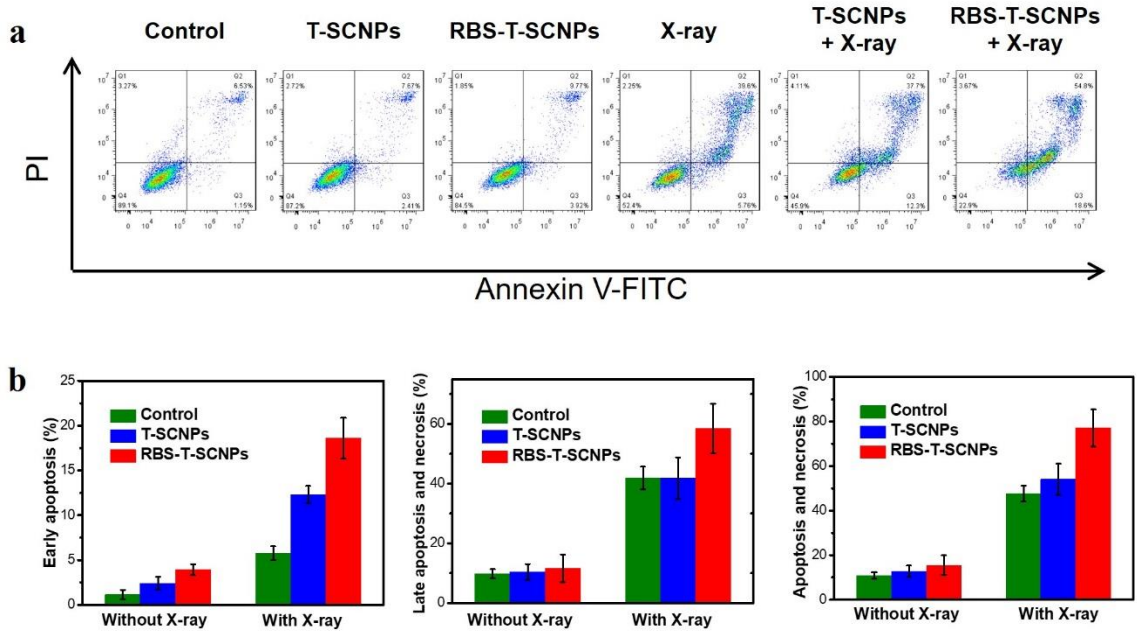

Figure S16. a) The apoptosis/necrosis of A549 cells after different treatment conducted by flow cytometry analysis. b) Statistical data analysis of the percentage of the corresponding early apoptosis, late apoptosis and necrosis, total apoptosis and necrosis cells under diff erent treatments. Error bars represent standard deviation for n = 3.

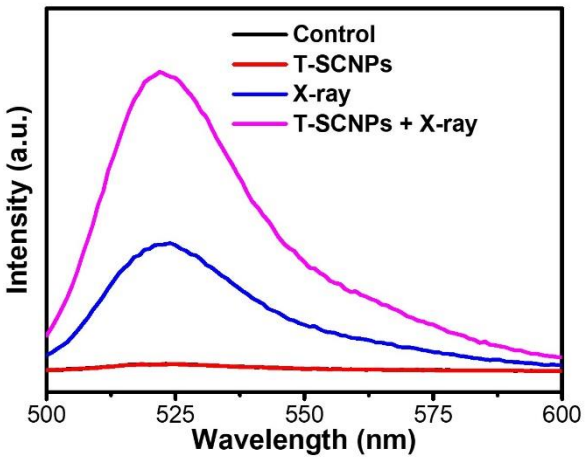

Figure S17. Fluorescence spectra of DCFH in the presence of T-SCNPs (100 μg mL^{-1}) exposed to X-rays.

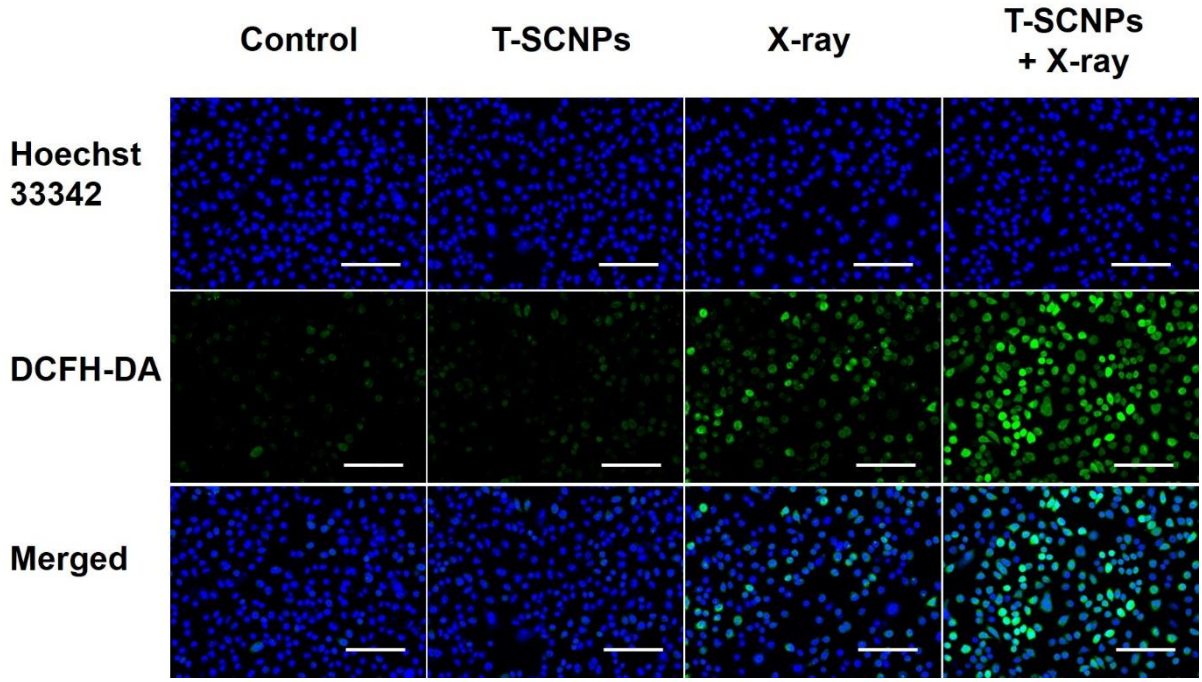

Figure S18. Fluorescence images for A549 cells staining with Hoechst 33342 (blue) and DCFH-DA (a ROS fluorescent probe, green) upon being treated with PBS or T-SCNPs in dark condition or under X-ray irradiation. The scale bar is 100 μm.

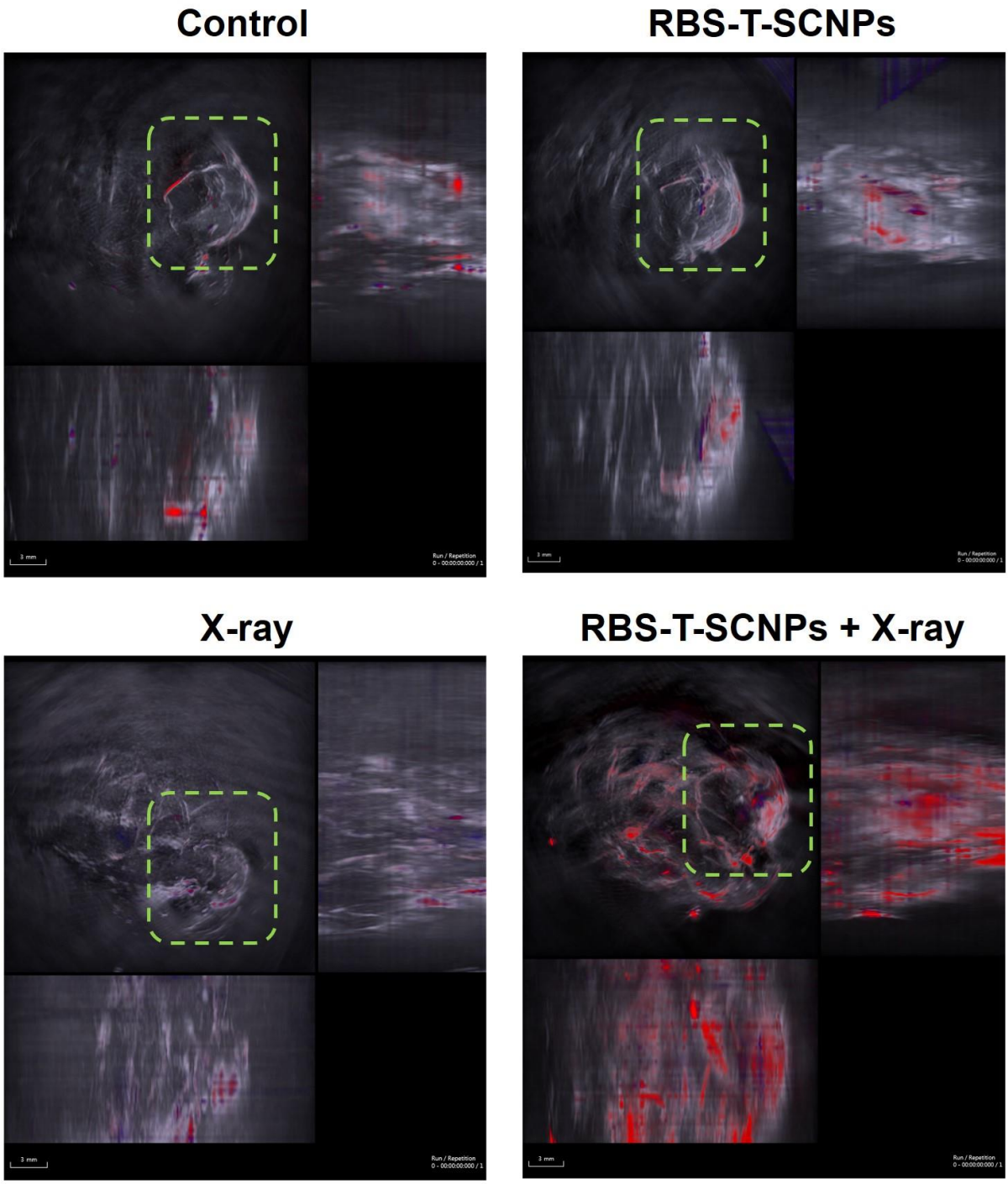

Figure S19. Photoacoustic images of deoxygenated (blue) and oxygenated (red) hemoglobin in A549 solid tumors with different treatment. The control, RBS-T-SCNPs, and RBS-T-SCNPs + X-ray treatment images were acquired from one tumor-bearing mouse, and the X-ray treatment image was obtained from another tumor-bearing mouse.

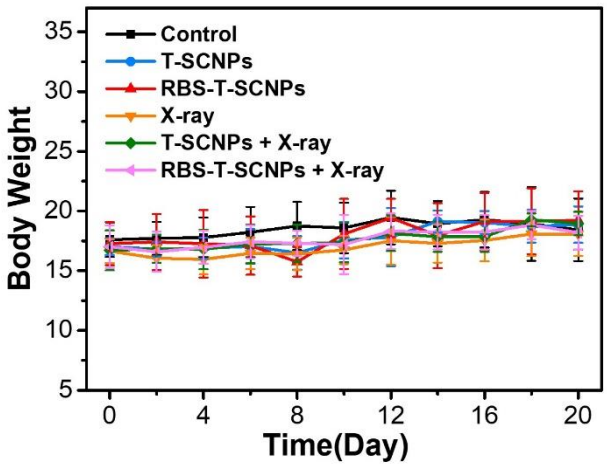

Figure S20. Body weights variation of different groups after various administrations. Error bars represent standard deviation for n = 4.

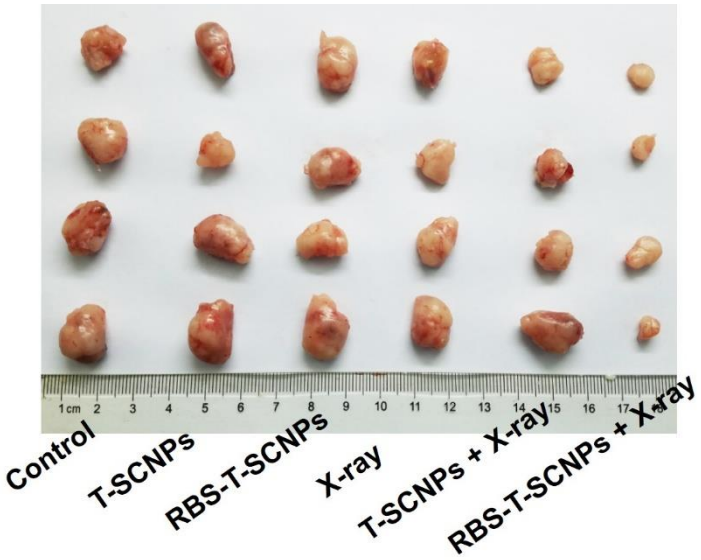

Figure S21. Image of tumors collected on day 20 after various administrations.

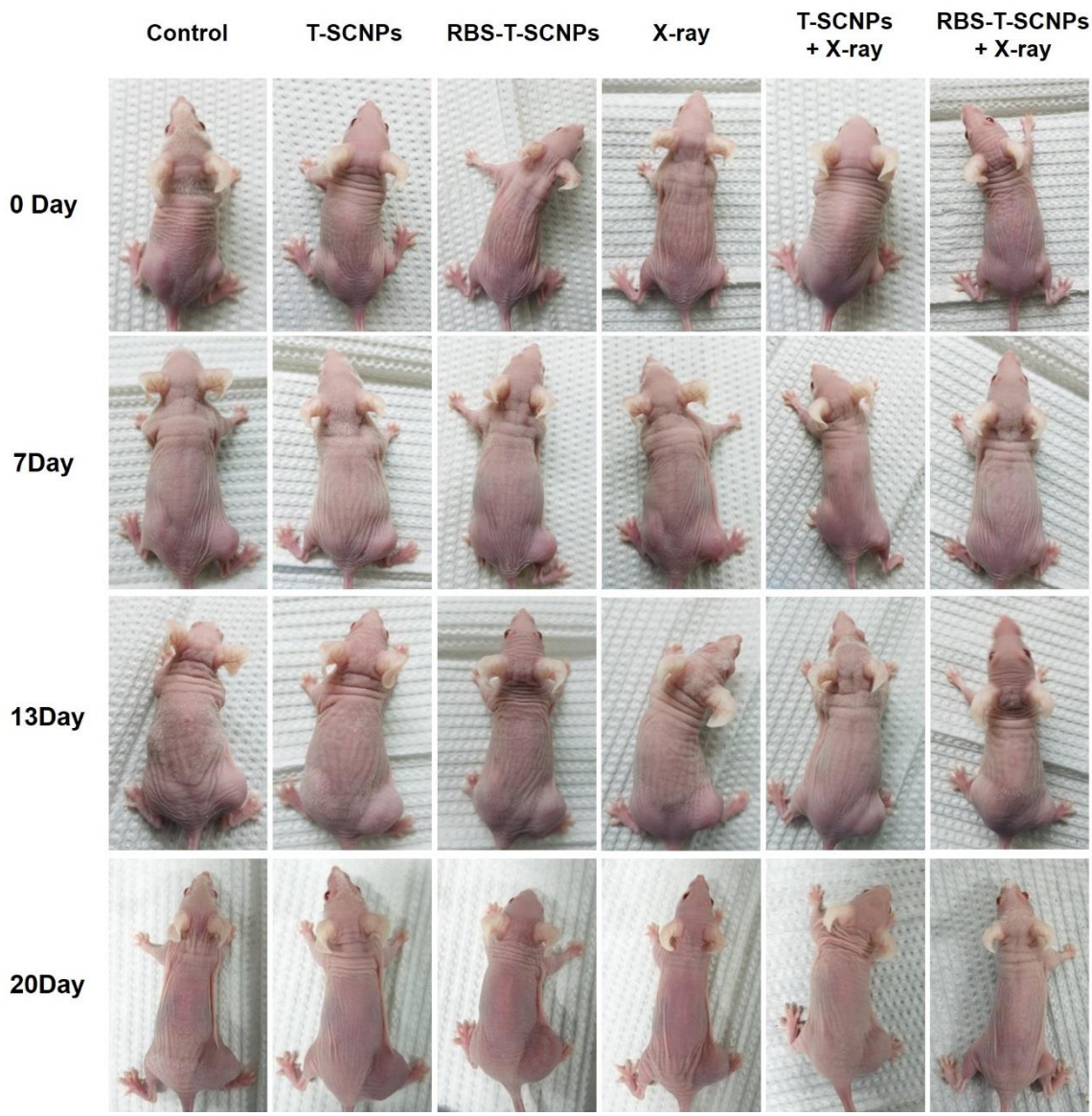

Figure S22. Digital photographs of different groups of mice at 0, 7, 13, and 20 days after treatments, respectively.

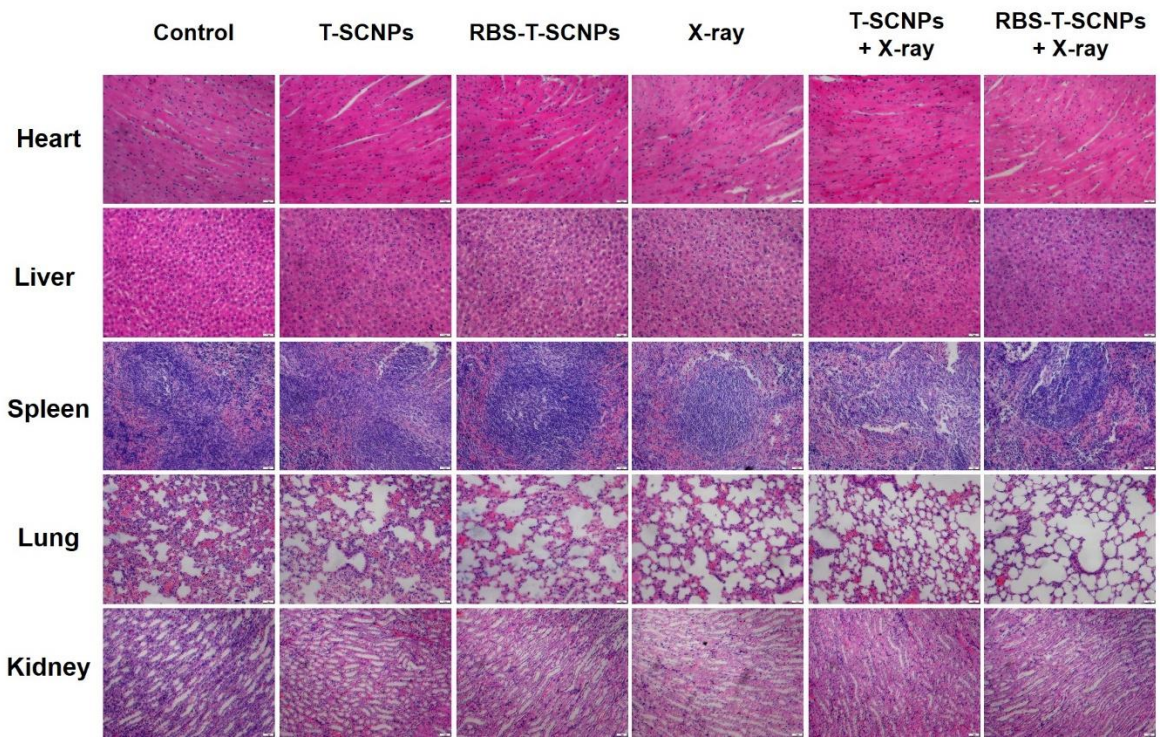

Figure S23. Representative H&E stained images of main organs including heart, liver, spleen, lung and kidney collected from A549 tumor bearing nude mice on the last day.

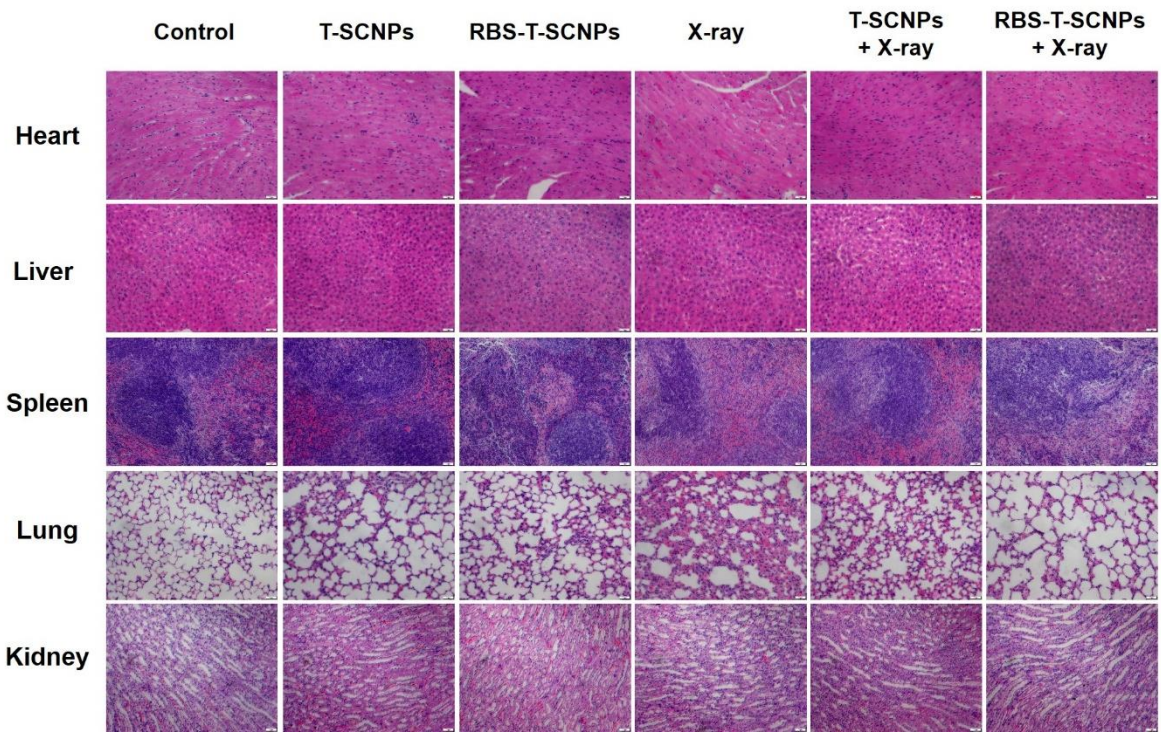

Figure S24. Representative H&E stained images of main organs including heart, liver, spleen, lung and kidney collected from A549 tumor bearing nude mice 24 h after various administrations.

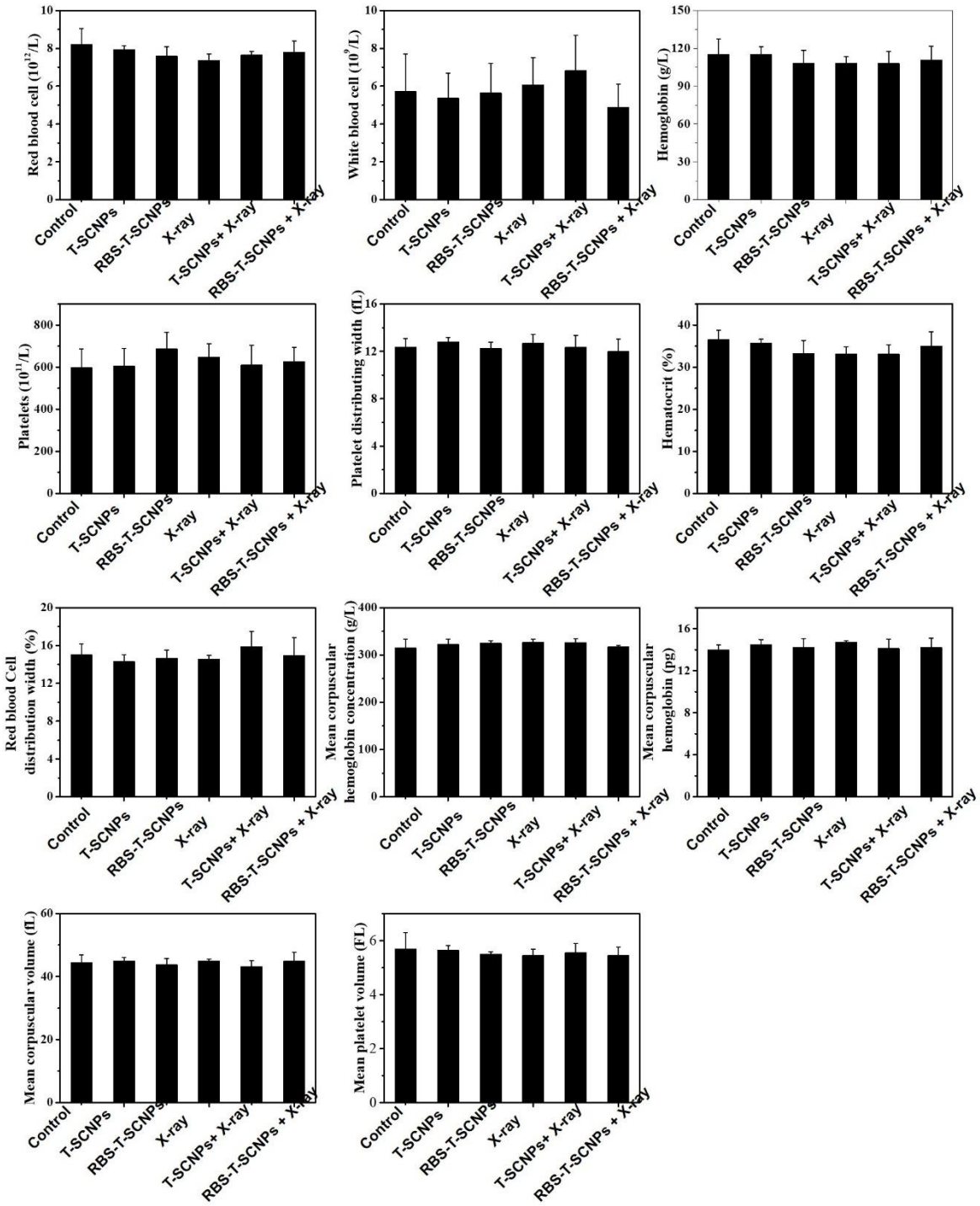

Figure S25. Blood hematology analyses of mice on the last day. Error bars represent standard deviation for n = 3.

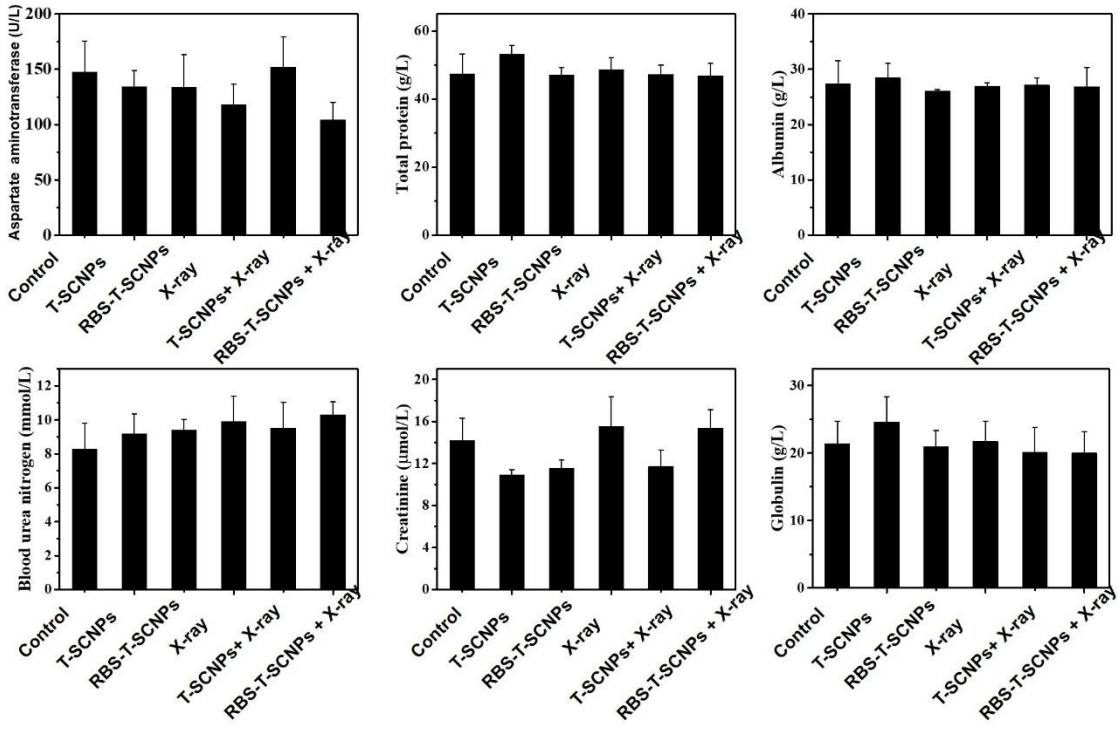

Figure S26. Blood biochemistry analyses of mice on the last day. Error bars represent standard deviation for n = 3.

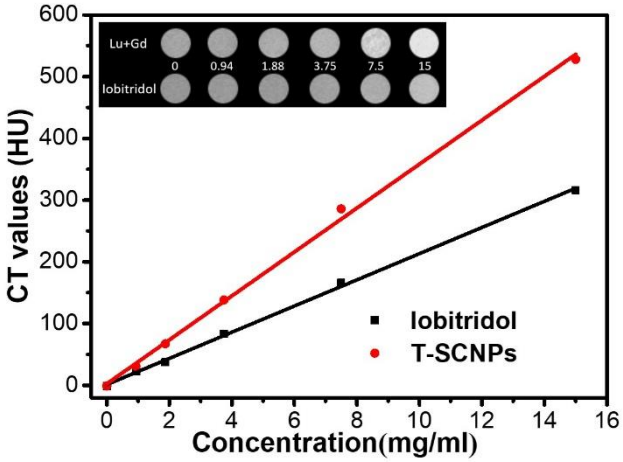

Figure S27. In vitro CT signals of T-SCNPs (upper) and iopromide (bottom) with different concentrations. The plot of the HU values of T-SCNPs and iopromide versus the sample concentrations.

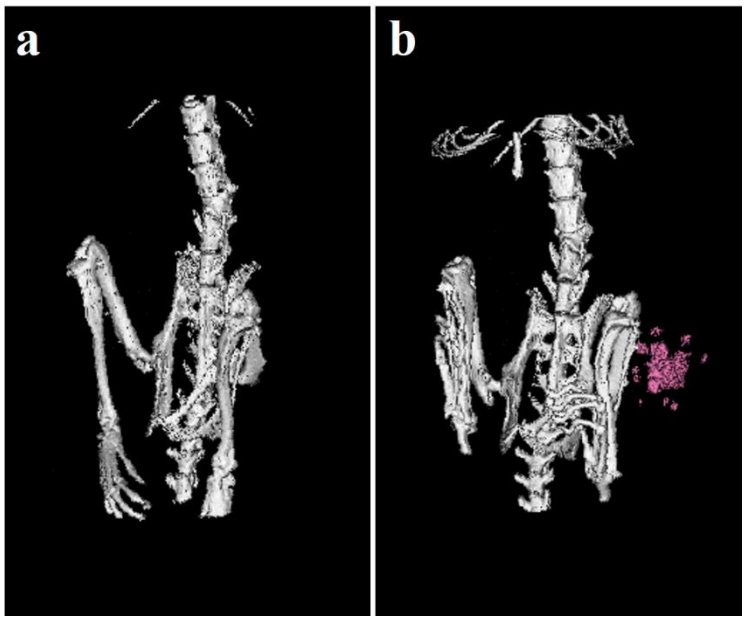

Figure S28. CT images of mice before injection of T-SCNPs (a) and after intratumoral injection of T-SCNPs for 3 h (b).