Supporting Information

Soft X-ray Activated Lanthanide Scintillator for Controllable NO Release and Gas-Sensitized Cancer Therapy

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METHODS

Chemicals and Materials: $GdCl_3 \cdot 6H_2O$ (99.99%), $YCl_3 \cdot 6H_2O$ (99.99%), and $TbCl_3 \cdot 6H_2O$ (99.9%) were purchased from QingDa elaborate Chemical Reagent Co. Ltd (Shandong). NaOH (98%), NaF (99%), oleic acid (OA,90%), absolute ethanol, Igepal CO-520, Tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, 98%), sodium nitrite ($NaNO_2$, 97%), ammonium sulfide ($(NH_4)_2S$, 48%),ammonium hydroxide (NH_4OH, 33% NH_3) and other reagents were purchased from Sinopharm Chemical Reagent Co., China.

Characterizations: Powder X-ray diffraction (XRD) measurements were performed by a Rigaku D/max 2500 X-ray diffractometer with Cu-Kα radiation (λ= 0.15406 nm) at 40 kV and 250 mA. The shape and structure of $NaYF_4$:Gd/Tb and $NaYF_4$:Gd/Tb@SiO_2 samples were characterized by transmission electron microscopy (TEM, FEI Tecnai F20), and high-resolution TEM (HR-TEM) at an acceleration voltage of 200 kV. Photoluminescence spectra of these samples were detected by using a Zolix Analytical Instrument (fluoroSENS 9000 A) at room temperature. The UV-Vis absorption data was acquired by Spectrophotometer system (UV-1800, Hunan Sino-Jewell Electronics Co., Ltd.).

Synthesis of $NaYF_4$:Gd/Tb nanorods: The Ln (40%Gd, 15%Tb) co-doped $NaYF_4$ nanorods were synthesized by a traditional hydro-thermal procedure[s1] as follows: Firstly, 1.2 g of NaOH completely dissolved in 2 mL of deionized water under stirring.

Then, 10 mL anhydrous alcohol and 20 mL OA were added into the above solution stirring for another 20 minutes. After that, A total of 1 mmol $RECl_3$ (RE = Y, Gd, Tb) at designed concentrations/molar ratios and 8 mL of NaF aqueous solution (1.0 M) were added into the aforementioned solution with vigorously stirring. Subsequently, the mixtures were transferred into a stainless Teflon-lined autoclave (50 mL) and maintained at 190 ℃ for 24 h. The resulting products were washed with ethanol and deionized water.

Synthesis of $NaYF_4$:Gd/Tb@SiO_2-NH_3^+: Firstly, $NaYF_4$:Gd/Tb@SiO_2 core-shell nanocomposites were synthesized according to the previous report.[s2] 0.2 mmol $NaYF_4$:Gd/Tb nanorods and 0.1 mL Igepal CO-520 were mixed in 50 mL cyclohexane under stirring for 10 min. Then, 0.8 mL of concentrated ammonia and 0.4 mL Igepal CO-520 were added in above solution and sonicated for 20 min. After that, 0.6 mL TEOS was added drop by drop, and the mixture was stirred for 2 days to obtain $NaYF_4$:Gd/Tb @SiO_2 nanorods. Then, 0.15 mL APTES was added in the mixed solution under stirring for 24 h. The resulting $NaYF_4$:Gd/Tb @SiO_2 products were washed with ethanol several times and finally dispersed in 5 mL deionized water.

Synthesis of RBS: RBS was synthesized as following[s3]: First, the 8 mL deionized water containing 1.8 g $NaNO_2$ was added to 250 mL 3-neck flask. Then, 2 mL $(NH_4)_2S$ solution and 6 mL of deionized water were added into the above solution. The yellow solution was heated at reflux under stirred until the mixed solution turned

to a deep red. Then, 32 mL deionized water containing 4.0 g $FeSO_4 \cdot 7H_2O$ was added to the above solution. After 30 s heating, 10 mL of a 22% NH_4OH solution was dropped into the mixed solution. The solution was heated to 90 ℃ for 10 min and then filtered immediately. The red-brown Fe (OH) $_3$ was discarded, and the black-brown solution was allowed to stand overnight. The black-brown solution was kept in 4 ℃ overnight and the black crystalline solid was collected and freeze-dried.

Synthesis of RBS-loaded $NaYF_4$:Tb@SiO_2: $NaYF_4$:Gd/Tb@SiO_2-RBS nanocomposites were synthesized according to an electrostatic attraction procedure. An aqueous solution of RBS (100 mg) was dropwisely added into the suspension of $NaYF_4$:Gd/Tb@SiO_2 (0.2 mmol) under stirring. After 12 h, the precipitate was obtained by centrifugation at 6,000 rpm. Nanocrystals were centrifuged and washed with water three times.

***In vitro* low dose X-ray induced green emission:** Soft X-ray induced green emission was carried out by a multi-modal *in vivo* imaging system (Bruker *In Vivo* FX Pro) equipped with a detecting CCD (ML4002, Finger Lakes Instrumentation, USA). Different concentrations of $NaYF_4$:Gd/Tb@SiO_2 nanorods (0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL) were transferred into 96-well tubes for X-ray induced optical bioimaging with various irradiation times (1-4 min) and excitation tube voltages (25 - 45 kVp).

Tumor animal models: 8×10^6 Lewis lung cancer (LLC) cells were subcutaneously injected into BALB/c nude mice, after further culturing about two weeks, the tumor-

bearing mouse models were obtained for *in vivo* soft X-ray-activated NO gas therapy experiments. All animal procedures in this study were performed in accordance with the Guidelines for Care and Use of Laboratory Animal Center of Hunan Normal University and approved by the Animal Ethics Committee of Hunan Province.

***In vivo* low dose X-ray activated optical bioimaging:** $NaYF_4$:Gd/Tb@SiO_2 (150 μL, 2 mg/mL) was then subcutaneously injected in Kunming mouse which was anesthetized by intraperitoneally injecting pentobarbital sodium aqueous solution (10 wt%, 100 μL), After that, a multi-modal *in vivo* imaging system was used for soft X-ray induced bioimaging under X-ray (45 kVp, 2 min) irradiation at room temperature.

Measurement of NO release in deionized water: The NO release contents from $NaYF_4$:Gd/Tb -RBS nanocomposites were quantitatively measured by a classic Griess reagent Kit[4]. When in contact with water, the released NO molecules could be converted into nitrate and/or nitrite. After reaction with the Griess agent, the nitrate and nitrite were finally converted into an azo dye that could be quantitatively determined using a microplate reader or UV-vis absorption spectroscopy (λ= 540 nm).

***In Vitro* Cytotoxicity Assay：** A549 cells were pre-incubated in 5% CO_2 at 37 °C on the 96-well microplate. Then the $NaYF_4$:Gd/Tb-RBS nanocomposites were added into the 96-well microplate with different treatments. Subsequently, the cells were incubated for another 24 h with 5% CO_2 at 37 °C. Finally, the cell viability was evaluated by MTT method. In addition, the cells viability after NIR laser exposure was also stained with calcein acetoxymethyl ester (Calcein-AM) and propidium

iodide (PI) and imaged by fluorescence microscopy.

Soft X-ray-activated NO gas therapy: The tumor-bearing mice were randomly into 4 groups: control (group 1); PBS+ X-ray irradiation (group 2); $NaYF_4$:Gd/Tb-RBS(group 3); $NaYF_4$:Gd/Tb-RBS + X-ray irradiation (group 3). After injection of PBS and $NaYF_4$:Gd/Tb-RBS solution (2 mg/mL, 150 μL), tumors were irradiated by soft X-ray with 45 kVp for 2 min every day.

Histology analysis: To obtain histology analysis, the main organs including heart, liver, spleen, lung and kidney from the control and treated mice with 3 and 7 days were collected for hematoxylin and eosin (H&E) staining to examine the potential toxicity.

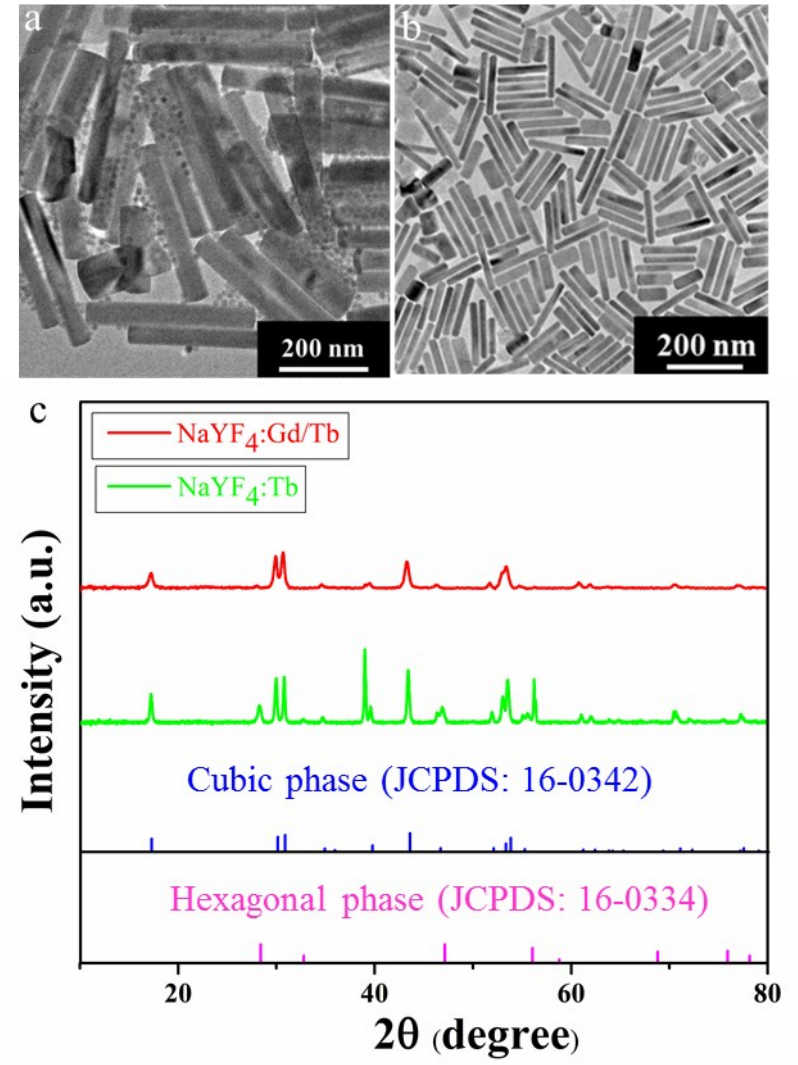

Figure S1. (a) TEM image of $NaYF_4$:Tb (without doping Gd); (b) TEM image of $NaYF_4$:Gd/Tb; (c) XRD patterns of the $NaYF_4$:Gd/Tb (red line), and $NaYF_4$:Tb (green line).

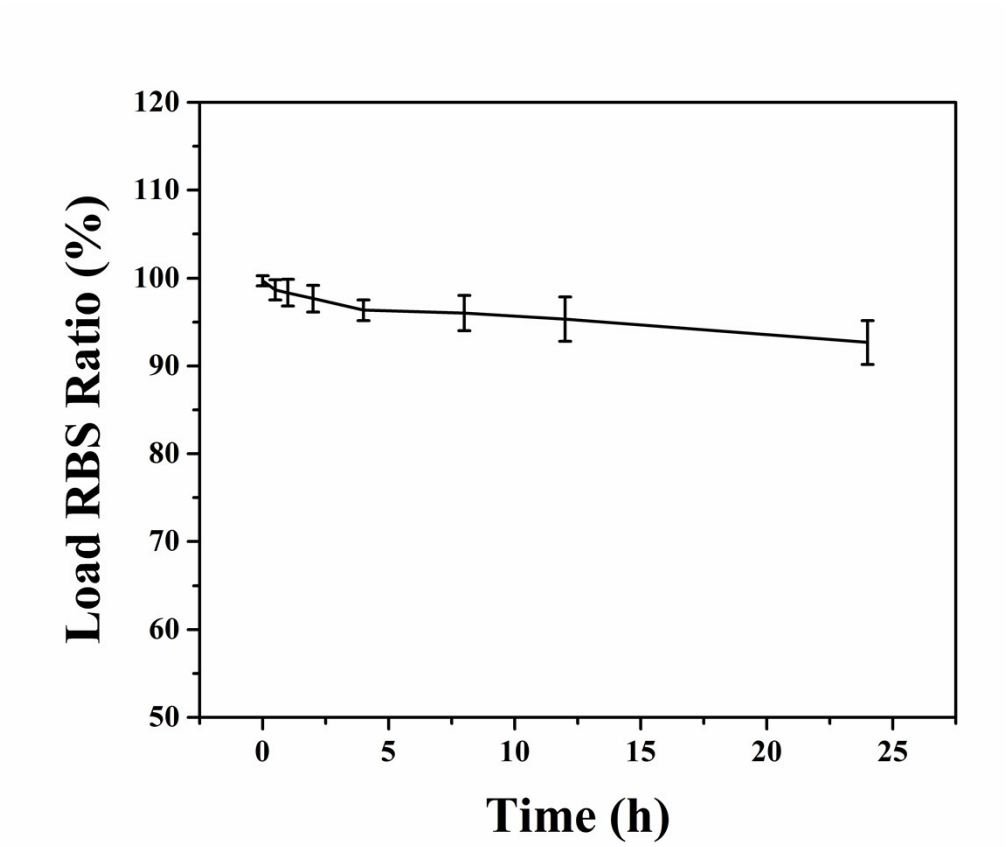

Figure S2. The stability of $NaYF_4$:Gd/Tb-RBS in PBS medium.

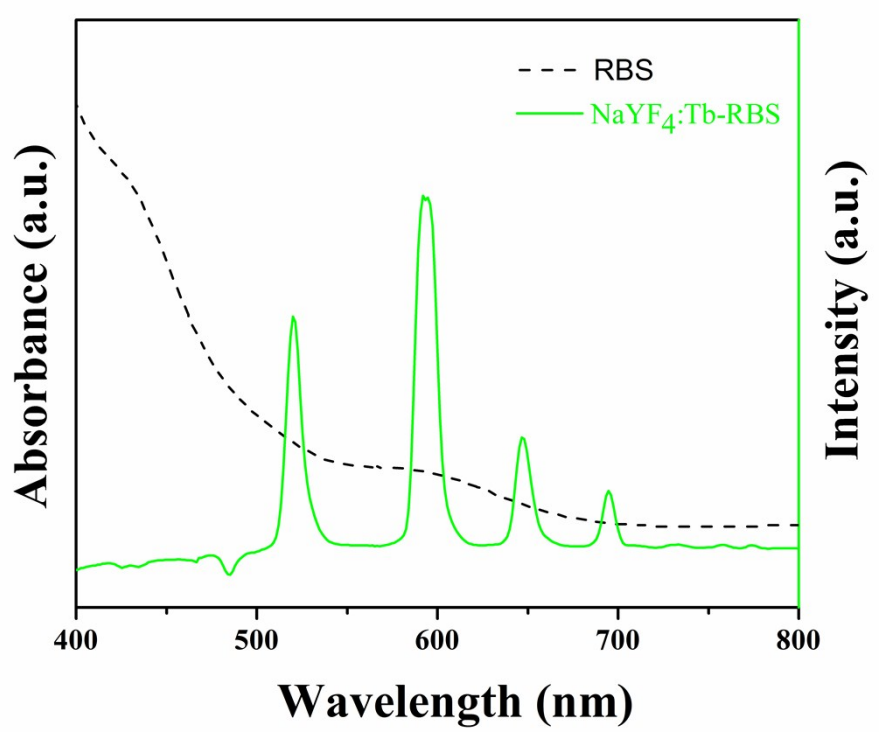

Figure S3. UV/Vis absorption spectra of RBS aqueous solution and the emission spectrum of $NaYF_4$:Gd/Tb@SiO_2 nanorods under the excitation of UV light.

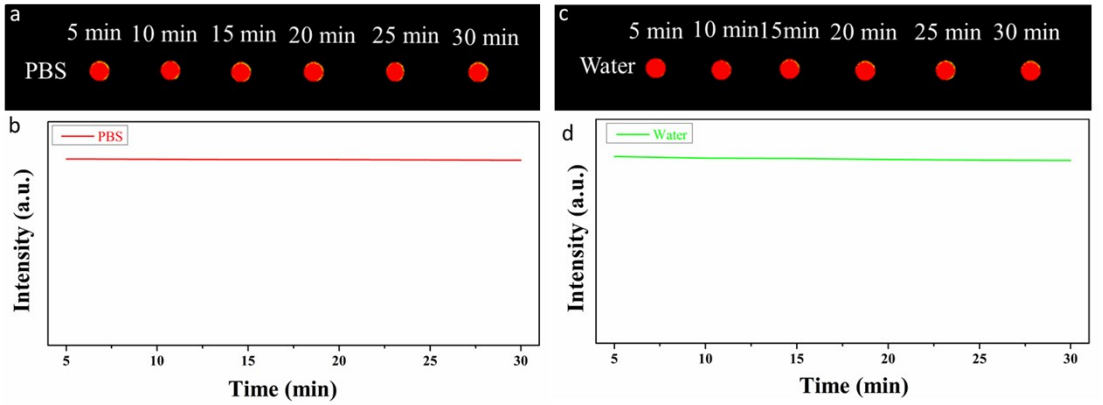

Figure S4. a) The *in vitro* phantom imaging of $NaYF_4$:Gd/Tb-RBS nanocomposites in PBS. b) Photo-stability curve of $NaYF_4$:Gd/Tb-RBS nanocomposites in PBS under soft X-ray irradiation. c) The *in vitro* phantom imaging of $NaYF_4$:Gd/Tb-RBS nanocomposites in water. d) Photo-stability curve of $NaYF_4$:Gd/Tb-RBS nanocomposites in water under soft X-ray irradiation**.**

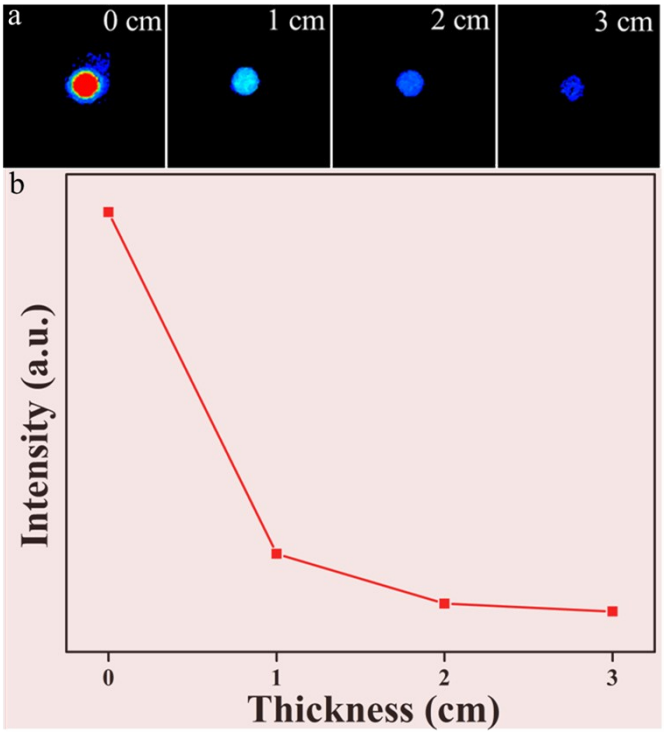

Figure S5. *In vitro* phantom imaging of $NaYF_4$:Gd/Tb@SiO_2 covered with different thicknesses of pork tissues (0, 1, 2, and 3cm) under X-ray (1 min, 45 kVp) excitation.

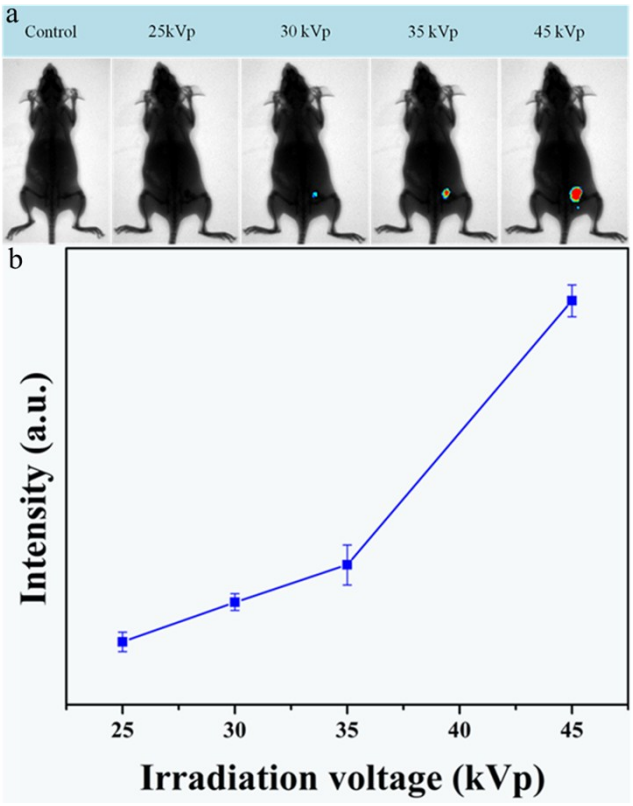

Figure S6. *In vivo* X-ray activated optical bioimaging of mouse based on $NaYF_4$:Gd/Tb@SiO_2: Control (Un-injection) and subcutaneous injection of $NaYF_4$:Gd/Tb@SiO_2 under irradiation at different tube voltages of X-ray.

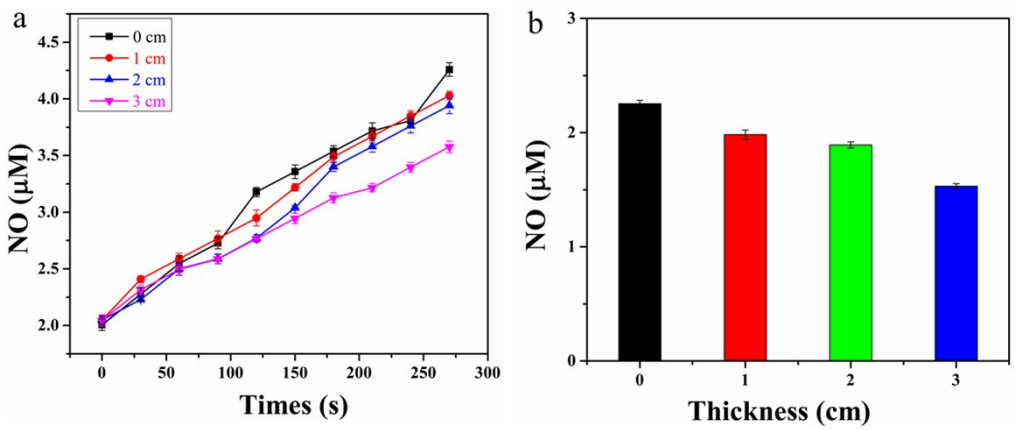

Figure S7. a) Depth-dependent NO-releasing curves of $NaYF_4$:Gd/Tb-RBS irradiated by different irradiation times of soft X-ray at 45 kVp. b) Quantitative soft X-ray induced NO release from $NaYF_4$:Gd/Tb–RBS under different thicknesses of pork slabs (0-3 cm) after 270 s irradiation.

Table S1. Irradiation dosage of soft X-ray (45 kVp) under different irradiation times

	45 kVp			
Exposure time	**1 min**	**2 min**	**3 min**	**4.5 min**
Irradiation dosage	**0.18 mGy**	**0.32 mGy**	**0.59 mGy**	**0.85 mGy**

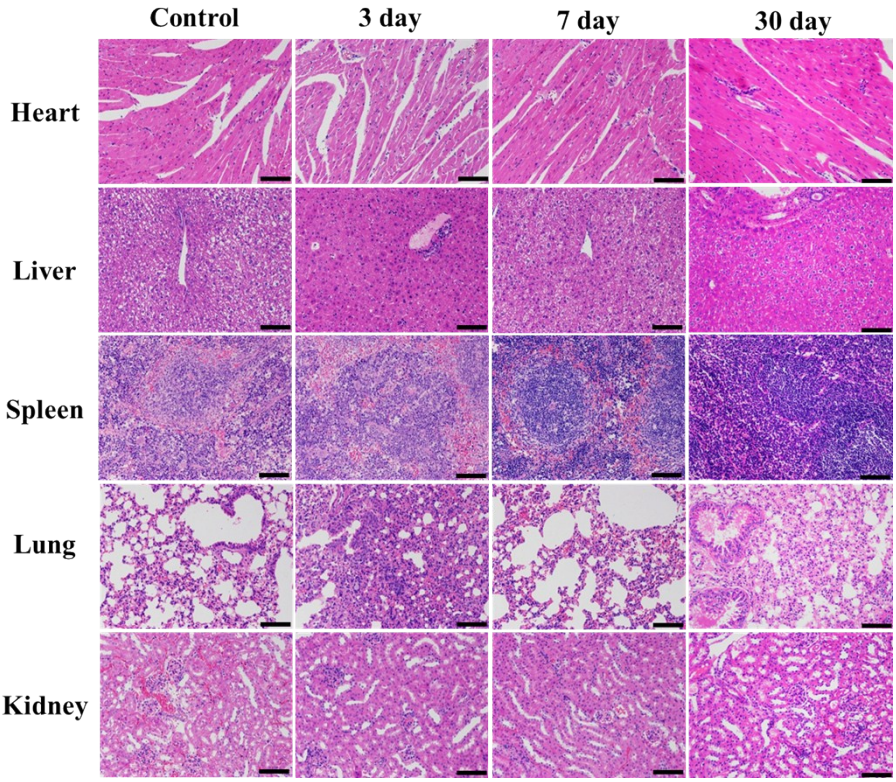

Figure S8 H&E stained images of the heart, liver, spleen, lung, and kidney of the control and test mice (Scale bars: 100 μm).

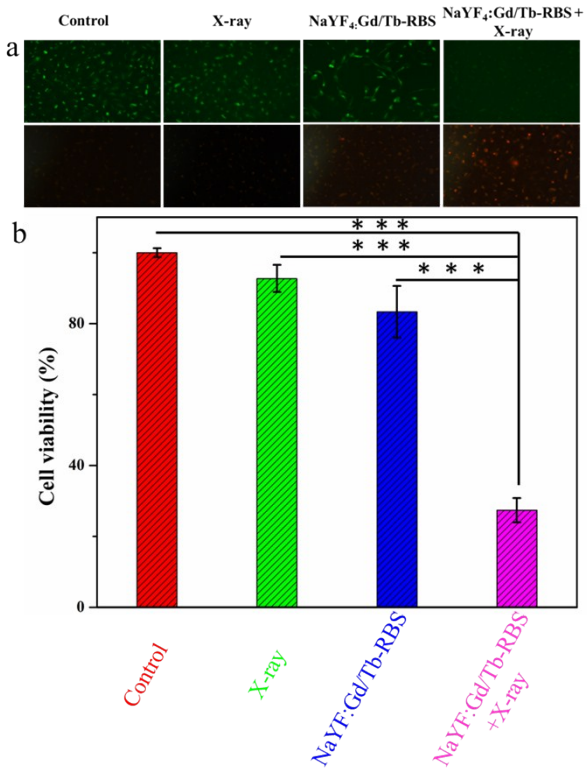

Figure S9. a) Fluorescent images of A549 cells with different treatments after live-dead staining. b) *In vitro* A549 cells viability after various treatments. *** $P < 0.001$.

Supplement References

[s1] Z. L. Xue, S. J. Zeng, J. H. Hao, *Biomaterials* 2018, **171**, 153.
[s2] F. Chen, S. J. Zhang, W. B. Bu, Y. Chen, Q. F. Xiao, J. N. Liu, H. Y. Xing, L. P. Zhou, W. J. Peng, J. L. Shi, *Chem. Eur. J.* 2012, **18**, 7082.
[s3] D. Seyferth, M. K. Gallagher, M. Cowie, *Organometallics* 1986, **5**, 539.
[s4] J. Sun, X. J. Zhang, M. Broderick, H. Fein, *Sensors* 2003, **3**, 276.