Electronic supplementary information (ESI) for Journal of Materials Chemistry C.

Scintillating Nanoplatform with Upconversion Function for Synergy of Radiation and Photodynamic Therapies of Deep Tumor

Yansong Feng, [a, b] *Xiaomeng Liu,* [a] *Qiqing Li,* [b] *Shilin Mei,* [a] *Kefan Wu,* [b] *Jun Yuan,* [b] *Langping Tu,* [e] *Ivo Que,* [d] *Filippo Tamburini,* [f] *Fabio Baldazzi,* [f] *Alan Chan,* [f] *Luis J. Cruz,* [d] *Jing Zuo,** [b, c] *Changjiang Yao* [a] *and Hong Zhang** [b]

[a] State Key Laboratory of Explosion Science and Technology, School of Mechatronical Engineering, Beijing Institute of Technology, 100081, Beijing, China

[b] Van 't Hoff Institute for Molecular Sciences. University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

[c] Key Laboratory of Automobile Materials (Ministry of Education), College of Materials Science and Engineering, Jilin University, 130025, Changchun, China

[d] Translational Nanobiomaterials and Imaging, Department of Radiology, Leiden University Medical Center, 2333 ZA, Leiden, Netherlands

[e] State Key Laboratory of Luminescence and Applications, Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences, 130033, Changchun, China

[f] Percuros B.V., Zernikedreef 8, 2333 CL Leiden, The Netherlands

Corresponding E-mail: jzuo@jlu.edu.cn; h.zhang@uva.nl

EXPERIMENTAL SECTION

Reagents: $Y(CH_3COO)_3 \cdot xH_2O$, $Tb(CH_3COO)_3 \cdot xH_2O$, $Er(CH_3COO)_3 \cdot xH_2O$, $Tm(CH_3COO)_3 \cdot xH_2O$, $Gd(CH_3COO)_3 \cdot xH_2O$, oleic acid (90%), 1-octadecene (ODE, 90%), NaOH, NH_4F, sodium oleate (NaOA), acetic acid, acetic anhydride, rose bengal (RB), poly(allylamine), 20 wt.% solution in water (PAAm), 6-bromohexanoic acid, N,N-Dimethylformamide (DMF), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC-HCl), N-hydroxy-succinimide (NHS), 1,3-Diphenylisobenzofuran (DPBF), folic acid, PEG-NHS, ethanol, acetone and cyclohexane were purchased from Sigma-Aldrich. All the chemicals were of analytical grade and were used without further purification.

Characterization: TEM images, HRTEM images and compositional mapping were conducted with a FEI Talos F200X Transmission Electron Microscope. The XRD pattern was measured with an X-ray powder diffractometer (Bruker D8 Advance). UV−vis absorption spectra were measured with Shimadzu UV-2600. The UCL spectra, X-ray excited optical luminescence spectra and fluorescence spectra were measured with a photoluminescence spectrometer (Edinburgh FLS980) equipped with the ultrafast light source, NIR laser, and X-ray source: RS-2000 Pro (Rad source), the energy was set as 160 kV and 25 mA. The FTIR spectra were analyzed with Bruker TENSOR-27 FTIR spectrometer. The diameter and zeta potential were measured with Dynamic light scattering (Zetasizer Nano ZS).

Synthesis of dry core β-$NaErF_4$:0.5%Tm NPs: 10 mL 1-octadecene and 10 mL oleic acid were heated up to 100 °C and kept under vacuum for 60 min. 0.995 mmol $Er(CH_3COO)_3 \cdot xH_2O$ and 0.005 mmol $Tm(CH_3COO)_3 \cdot xH_2O$ were then added in under nitrogen flow, subsequently, 0.5 mL acetic anhydride was injected in the solution under nitrogen and heating up to 100 °C for 60 min. And acetic anhydride was excepted under vacuum at 100 °C. Thereafter, 2.8 mmol sodium oleate was added under nitrogen flow and

switched to vacuum until the regents fully dissolved. Then, 5.8 mmol NH_4F was added in under nitrogen flow and the solution was heating up at 100 °C under vacuum for 60 min to completely except the water or other H_2O/OH^- sources before forming the NPs. Finally, the solution was heated up to 300 °C with the rate 10 °C min^{-1} and reacted for another 60 min under dry nitrogen. After cooling down to room temperature, the core NPs were obtained through the centrifugation and washed with acetone and ethanol (twice) and finally dispersed in cyclohexane.

Synthesis of α-$NaYF_4$ precursor: 160 mL oleic acid and 160 mL ODE in 500 ml three necks flask were heated up to 100 °C and kept under vacuum for 60 min, 20 mmol $Y(CH_3COO)_3 \cdot xH_2O$ was then added in under nitrogen flow, subsequently, 5 mL acetic anhydride was injected in the solution under nitrogen and heating up to 100 °C for 60 min. And acetic anhydride was excepted under vacuum at 100 °C. Thereafter, 30 mmol NaOA was added under nitrogen flow and switched to vacuum until the regents fully dissolved. Then, 80 mmol NH_4F was added in under nitrogen flow and the solution was heating up at 100 °C under vacuum for 60 min to completely except the water or other H_2O/OH^- sources before forming the NPs. Finally, the solution was heated up to 200 °C with the rate 10 °C min^{-1} and reacted for another 60 min under dry nitrogen. After cooled down to room temperature the precursor was obtained through centrifugation and finally dispersed in 40 mL dry ODE.

Synthesis of α-$NaGdF_4$:15%Tb precursor: The synthesis route for α-$NaGdF_4$:Tb was the same as that of α-$NaYF_4$, except that 17 mmol $Gd(CH_3COO)_3 \cdot xH_2O$ and 3 mmol $Tb(CH_3COO)_3 \cdot xH_2O$ were used.

Synthesis of β-$NaErF_4$:Tm@$NaYF_4$@$NaGdF_4$:15%Tb (1:x:3, x = 0, 1, 2, 3 or 4) multi-layer NPs (Er@Y@Gd/Tb NPs): 0.5 mmol core β-$NaErF_4$:Tm particles in 10 mL 1-octadecene and 10 mL oleic acid were heated up to 100 °C and kept under vacuum for 60

min. The solution was then heated up to 300 °C under nitrogen, followed by injection of 0.5 *x* mmol (*x* = 0, 1, 2, 3 or 4) α-$NaYF_4$ in dry ODE (each time 0.5 mmol at 15 min interval) and then kept in 300 °C for 45 min. Afterwards, 1.5 mmol α-$NaGdF_4$:15%Tb was injected in three times (each time 0.5 mmol at 15 min interval) and reacted for other 45 min. After cooling down to room temperature, the Er@Y@Gd/Tb NPs (1:*x*:3) were obtained by the centrifugation and washed with acetone and ethanol and finally dispersed in cyclohexane.

Surface functionalization of Er@Y@Gd/Tb NPs: The hydrophilic NH_2-functionalized Er@Y@Gd/Tb NPs were obtained via a ligand exchange phase transfer. In detail, 4 mL as-synthesized Er@Y@Gd/Tb NPs (*x*=3) dispersed in cyclohexane and 4 mL 0.1 M HCl were mixed for 4 h. After washed with water for twice, the ligand-free nanoparticles were dispersed in 4 mL DMF and 200 μL PAAm (20 wt.%) aqueous solution was added in. The mixture was then stirred for 24 h. The nanoparticles were washed with water and DMF. Finally, the NPs-NH_2 were obtained and dispersed in DMF. To modify RB and FA covalently to NPs-NH_2, 2 mg RB-HA, 0.5 mg FA, 3 mg NHS and 3 mg EDC-HCl were mixed in 5 mL DMF at 25 °C for 2 h. Afterwards, 10 mg of NPs-NH_2 was added and reacted for 24 h. The obtained NPs were then washed with DMF to remove the unreacted regents. Finally, 10 mg PEG-NHS were reacted with the particles in 15 mL DMSO and 5 mL ethanol for 24 h. After twice washing with water the URIPPs were dispersed in PBS.

Singlet Oxygen Detection ($^1O_2$): $^1O_2$ was determined by DPBF followed reported protocol, each group was with three parallels (n = 3). For the NIR triggered $^1O_2$, 2 mL of UIRPPs water solution and 10 μL (1 mg mL^{-1}) of DPBF-ethanol solution were mixed and kept in dark overnight. The absorption intensity of DPBF at 410 nm was recorded every 5 min (5, 10, 15, 20, 25 and 30 min) after NIR laser irradiation (800 nm, 980 nm or 1530 nm) of 0.7 W cm^{-2}. For X-ray triggered $^1O_2$, different concentration of UIRPPs and 10 μL (1 mg mL^{-1}) of DPBF-ethanol solution were mixed and kept in dark overnight. The absorption

intensity of DPBF at 410 nm was recorded every 5 min (at 5, 10, 15, 20, 25 and 30 min) after X-ray irradiation at 80 kV and 0.5 mA.

UCL imaging and RT&X-PDT in vitro: Breast carcinoma MCF-7 cells were maintained in the DMEM under 5% CO_2 atmosphere at 37°C. The cells were incubated in the special confocal well plate (1×10^4 per well) for 24 h. Afterwards, the UIRPPs were added in the plate with 0 μg mL^{-1}, 50 μg mL^{-1}, 100 μg mL^{-1}, or 200 μg mL^{-1}L, and incubation for 8 h. The nucleus was stained with DAPI, the UCL-imaging was measured by confocal microscopy with the irradiation 980 nm and 650 nm or 540 nm emission. The UCL-RB was measured with 980 nm laser irradiation and 590 nm emission, the RB was measured with 540 nm irradiation and 590 nm emission.

To assess the toxicity of the UIRPPs and lasers, the MCF-7 cells were incubated in the 96-well plate (1×10^4 per well) for 24 h. Then UIRPPs of different concentration groups (0, 0, 0, 0, 50, 100, 200, 400 μg mL^{-1}) were added in the plate, each group was with five parallels (n = 5). The group with 0 μg mL^{-1} of UIRPPs were irradiated with 800 nm, 980 nm or 1530 nm laser for 15 min. Further incubated for 24 h, the cell viability was assessed using a standard MTS assay.

Regarding the PDT effect of the UIRPPs, the cells were separated in 8 groups, each group was with five parallels (n = 5). (G1 to G8) in the 96-well plate, G1 and G5 were incubated with saline, G2, G3, G4 and G8 were incubated with UIRPPs of different concentrations (0, 50, 100, 200, 400 μg mL^{-1}), G6 were incubated with 12% RB (0, 6, 12, 24, 48 μg mL^{-1}L) and G7 were incubated with Er@Y@Gd/Tb NPs. After 8h incubation all groups were washed with PBS. The saline (G1 and G5) were taken as the control groups, the G2, G3 and G4 were irradiated with the NIR laser (800 nm, 980 nm or 1530 nm) for 15 min at 0.7 W cm^{-2}. The G6, G7 and G8 were exposed to X-ray with 1 Gy. After that the cells were cultured for another 48 h, the PDT efficacy of all groups was assessed by MTS assay.

Flow cytometry: For the flow cytometry analysis, the cells were trypsinized and stained by Annexin V-FITC and propidium iodide (Annexin V-FITC Apoptosis Staining/Detection Kit) to measure the cell apoptosis.

Annexin-V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining were performed using an Annexin-V-FITC/PI kit (BD) according to the manufacturer's instructions. Briefly, MCF-7 cells were cultured in 6-well plates and then treated with different reagents (G1: saline, G2: saline, G3: 100 μg mL^{-1} UIRPPs, G4: 100 μg mL^{-1} G5: 100 μg mL^{-1} Er@Y@Gd/Tb NPs, G6: 50 μg mL^{-1}, UIRPPs, G7: 100 μg mL^{-1} UIRPPs, G8: 100 μg mL^{-1}L UIRPPs) for 8 h, each with 3 parallels. The cells treated with saline (G1) were for the control. For the G2 and G4, the cells were exposed to the 980 nm laser for 15 min at 0.7 W cm^{-2}. And for the G5 to G8, the cells were exposed to X-ray with 1 Gy. Then the cells were harvested using 0.05% trypsin after 24 h incubation and washed twice with cold PBS, and suspended in binding buffer. 1×10^5 cells in 100 μL binding buffer were added to a tube and incubated with 5 μL of Annexin-V-FITC and 5 μL of PI. Cells were gently mixed and incubated for 15 min at room temperature. 400 μL binding buffer was then added to each tube. The samples were analyzed by flow cytometry (BD Biosciences). Experiments were repeated three times.

In vivo **experiments**: The mice experiments were performed in accordance with animal regulations and management protocols. The tumors were developed by the subcutaneous injection of MCF-7 cells into the mice. When the tumor volume grew to about 50 mm^3 the mice were randomly separated into 4 groups (n = 3). The volume of tumor was measured as Volume = ($L\times W^2/2$), where L (length) and W(width) are two tumor dimensions, respectively. Mice were administered intravenous injection with same doses (0.65 mg/mouse) of PBS (G1, G3) or UIRPPs (G2, G4). After 24 h, the tumor area of the mice in G3 and G4 were exposed to X-ray irradiation (1.5 Gy dose), and the rest of the body shielded by a thick lead plate. The

mice in the G1 and G2 were not exposed to any laser. The tumor sizes and body weights of the mice were monitored every 2 days during the 14 days of treatment. Finally, the animals were euthanized 15 days after the injection.

Statistical analysis: Statistical data were analyzed applying one-way ANOVA test; C Pns > 0,05; D, P > 0,05, P** = 0,0075, P* = 0,036; E, Pns > 0,05. The shown data are mean ± SEM of all independent measurements.

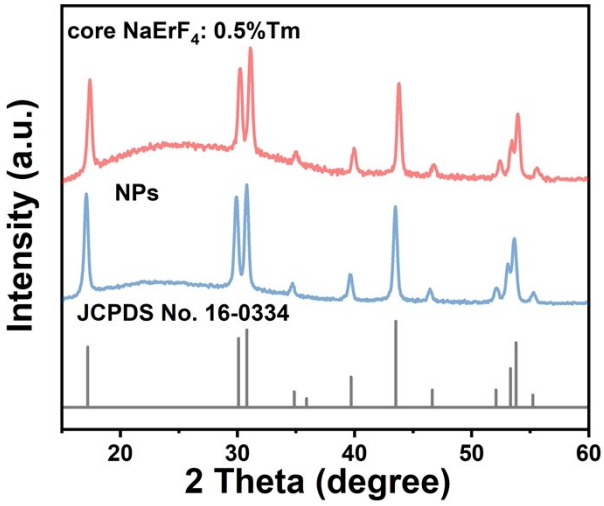

Figure S1. *XRD pattern of the core $NaErF_4$:0.5%Tm and CSS NPs, the standard diffraction pattern of hexagonal phase $NaYF_4$ is for reference. (JCPDS No. 16-0334).*

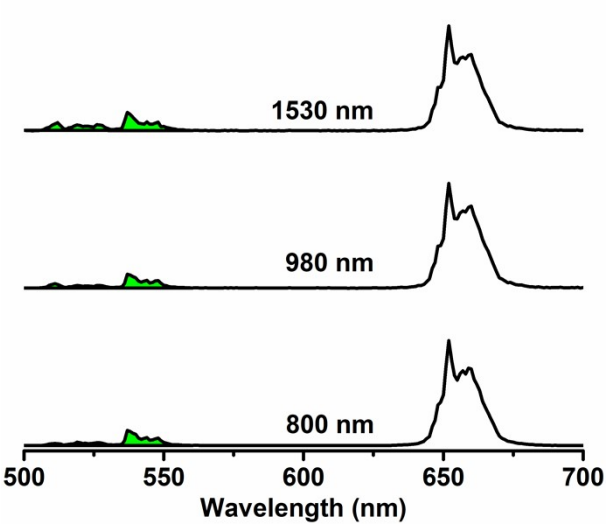

Figure S2. The UCL spectra of $NaErF_4$@$NaYF_4$ under laser excitation at 800 nm (10 W cm^{-2}), 980 nm (10 W cm^{-2}), and 1530 nm (1 W cm^{-2}), respectively.

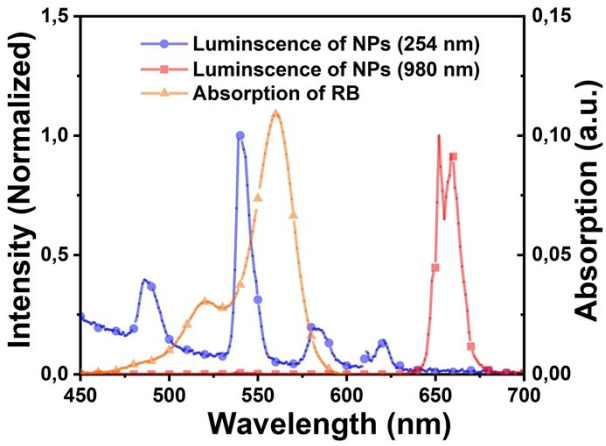

Figure S3. *The luminescence spectra of CSS NPs under UV light (254 nm) or NIR (980 nm) excitation and the absorption spectrum of RB.*

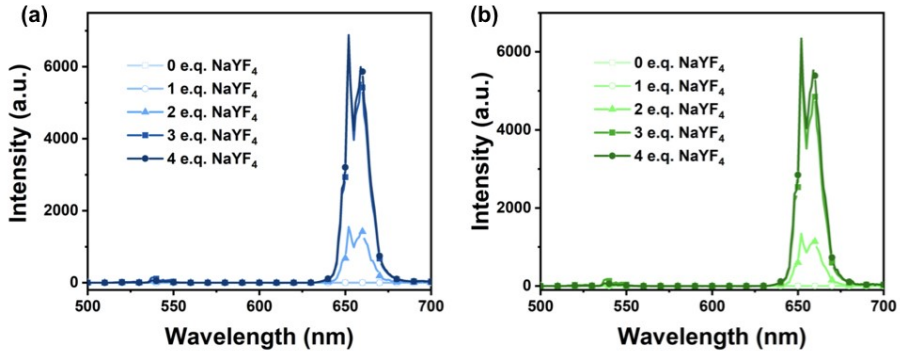

Figure S4. *The UCL spectra of CSS NPs upon (a) 800 nm or (b) 1530 nm laser excitation with inert layer of different thicknesses (the inert layer $NaYF_4$ was 0, 1, 2, 3 or 4 e.q. of the core).*

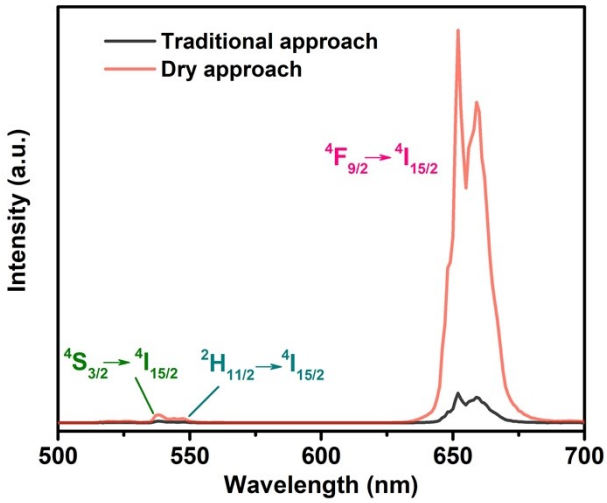

Figure S5. *The UCL spectra of $NaErF_4$:Tm@$NaYF_4$ UCNPs upon 980 nm laser excitation with the traditional solvothermal synthesis approach and dry synthesis approach.*

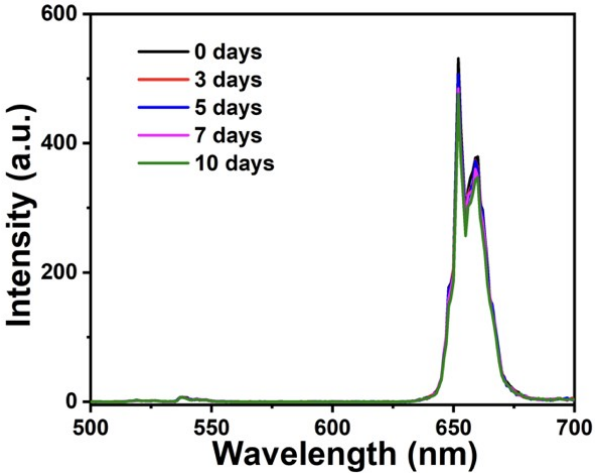

Figure S6*. Ten days track of UCL spectra of UIRPPs after preparation.*

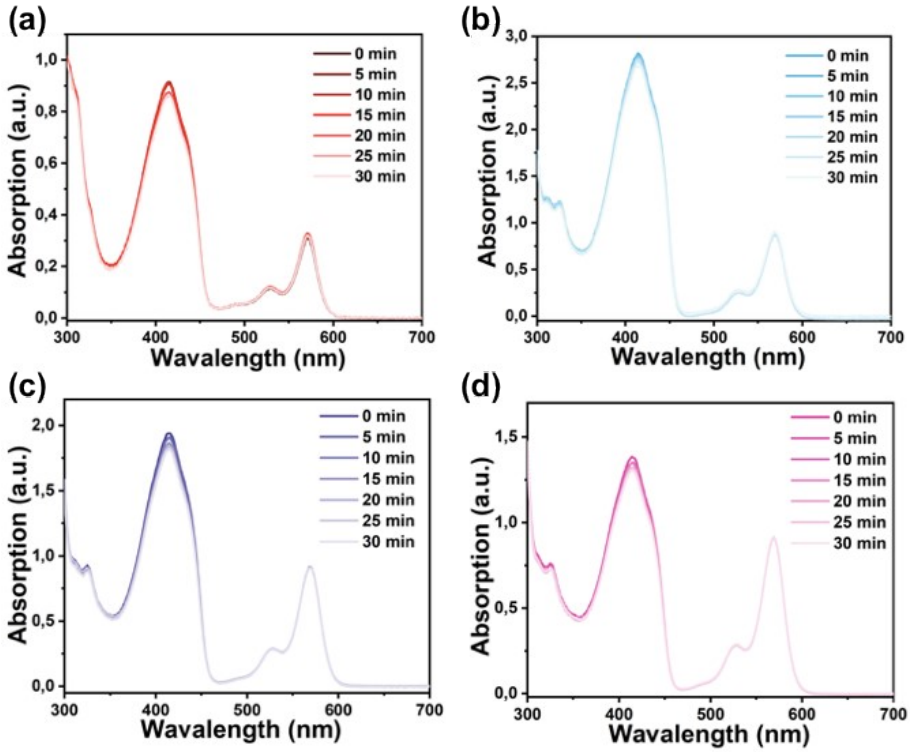

Figure S7*. UV-Vis absorption spectra of the solutions containing DPBF incubated with (a) 0.1 mg/mL RB and (b-d) 2 mg/mL UIRPPs. (b-d) were under irradiation of the laser at 800 nm, 980 nm or 1530 nm, respectively. The irradiation time duration was marked in the figures.*

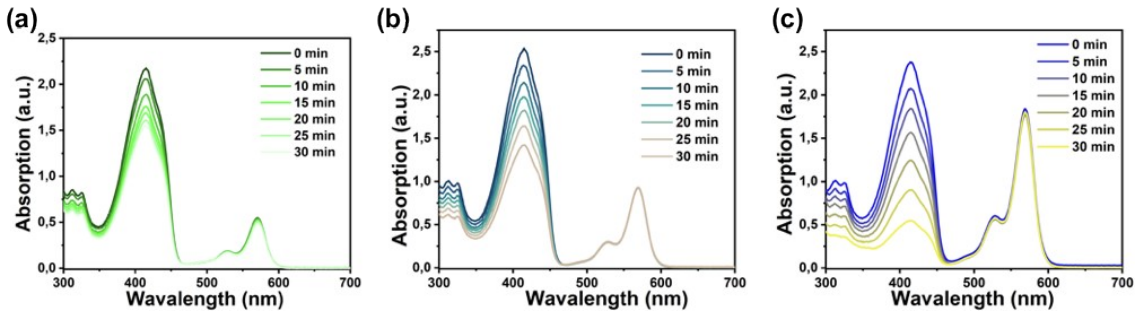

Figure S8. *UV-Vis absorption spectra of solutions containing DPBF incubated with (a) 1.5 mg/mL UIRPPs, (b) 2.5 mg/mL UIRPPs and (c) 5 mg/mL UIRPPs subject to X-ray irradiation with time duration marked in the figures.*

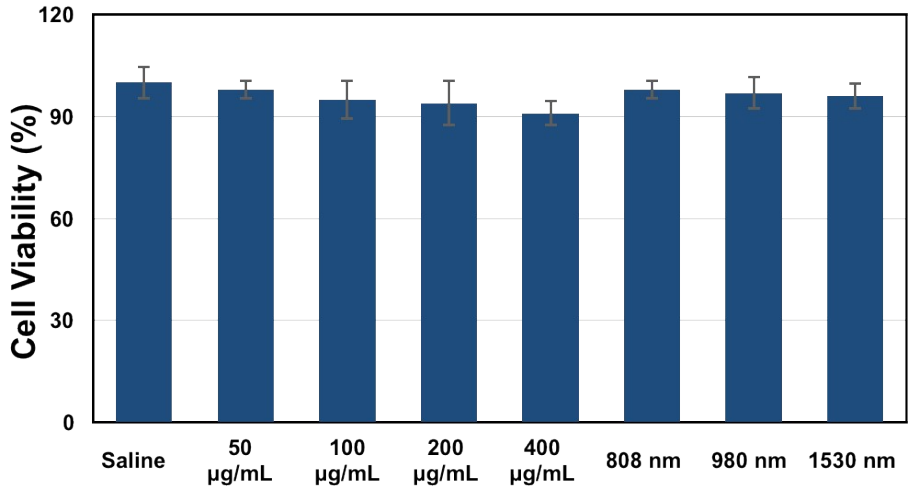

Figure S9. *The cell viability of MCF-7 incubated with different concentration UIRPPs (50, 100, 200 or 400 μg/mL), and the cell viability subject to the NIR laser irradiation (800 nm, 980 nm or 1530 nm).*

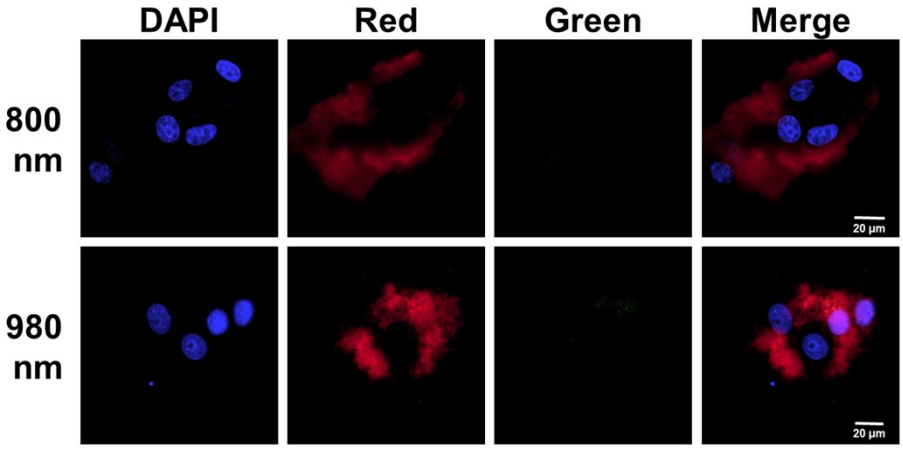

Figure S10. *The confocal images of MCF-7 cells incubated with 100 μg/mL UIRPPs, the nuclei were stained with DAPI; the UCL images with irradiation of 800 nm or 980 nm and emission of 650 nm (red) or green (540 nm), respectively.*

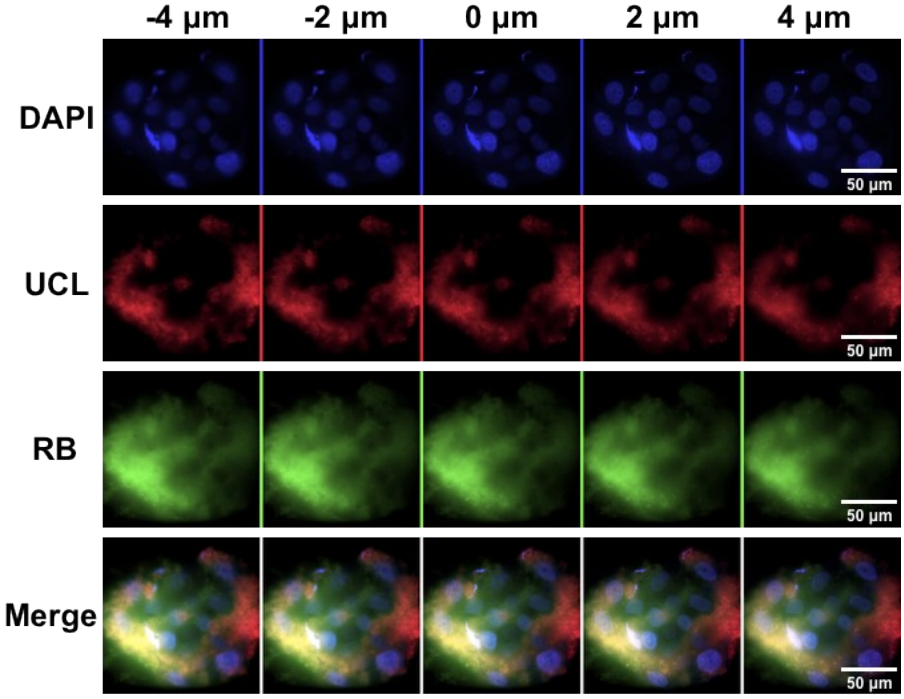

Figure S11. *The Z scan confocal images of MCF-7 cells incubated with 100 μg mL^{-1} UIRPPs, the nuclei were stained with DAPI; the UCL images under irradiation of 980 nm laser and 650 nm emission; the RB images under irradiation of 540 nm and 590 nm emission.*

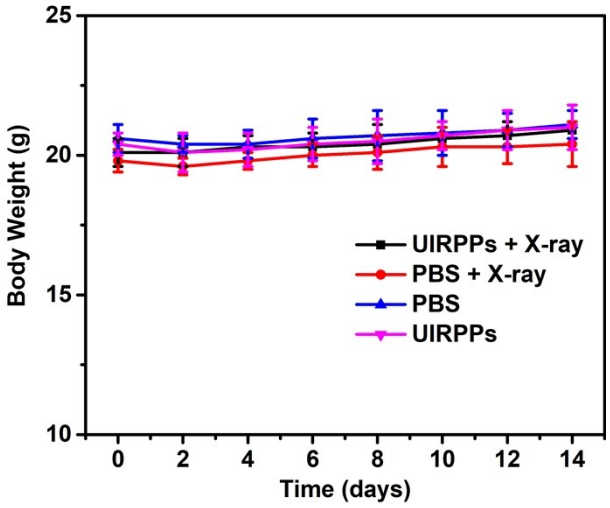

Figure S12. Evolution of body weights of the mice receiving various treatments. Data are reported as means ± SD of n = 3 independent experiments.