Supplementary Information

A simple approach for glutathione functionalized persistent luminescence nanoparticles as versatile platforms for multiple in vivo applications

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Experimental Section

Materials.

Magnesium nitrate [$Mg(NO_3)_2{\cdot}6H_2O$] was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Strontium carbonate（$SrCO_3$）was purchased from Aladdin (Shanghai,China). Europium nitrate [$Eu(NO_3)_3{\cdot}6H_2O$], dysprosium nitrate [$Dy(NO_3)_3{\cdot}5H_2O$] were purchased from Jinan Camolai Trading Company (Jinan, China). 6-Aminofluorescein and 5(6)-Carboxyfluorescein were bought from J&K Scientific Ltd. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Alfa Aesar Chemical Ltd (Tianjin, China). L-Glutathione reduced(GSH), MES hydrate and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma Chemical Company. Human hepatocellular liver carcinoma cell line HepG2 was obtained from the Committee on Type Culture Collection of Chinese Academy of Sciences. Cell culture products, unless mentioned otherwise, were purchased from GIBCO. All the chemicals were analytical grade and used without

further purification. Sartorius ultrapure water (18.2 MΩ cm) was used throughout the experiments.

Instruments.

Transmission electron microscopy (TEM) was carried out on a JEM-100CX Π electron microscope. The crystal structure of the samples were determined by Powder X-ray Diffraction (XRD) patterns(Bruker D8, Germany), using Cu Kα radiation(λ=1.54178 Å) at a scanning rate of 0.02 S^{-1} in the 2θ range from 10 to 80. Digital photographs were taken with a Nikon P5000 camera. X-ray photoelectron spectroscopy (XPS) spectra were performed with a PHI 5300 ESCA System (Perkin-Elmer) equipped 5 channeltrons, using an unmonochromated Mg Kα X-ray source (1253.6 eV). Fourier transform infrared (FTIR) spectroscopy spectra were obtained on a Varian 3100 FT-IR spectrometer. Fluorescence spectra were obtained with FLS-920 Edinburgh Fluorescence Spectrometer and 1.0 cm quartz cells at the slits of 15 nm and without in situ excitation. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai LeiCi Device Works, Shanghai, China) with a combined glass-calomel electrode. Absorbance was measured in a microplate reader (RT 6000, Rayto, USA) in the MTT assay. Confocal fluorescence imaging studies were performed with a TCS SP5 confocal laser scanning microscopy (Leica Co., Ltd. Germany). *In vivo* imaging was conducted by Caliper IVIS Lumina III (PerkinElmer, America).

Synthesis of $Sr_2MgSi_2O_7$:1%Eu, 2%Dy Persistent Luminescence Nanoparticles.

The $Sr_2MgSi_2O_7$:1%Eu,2%Dy persistent luminescence nanoparticles (PLNPs) were

synthesized using the typical Sol-Gel synthesis method reported previously with some modifications.[1] 0.7382 g $SrCO_3$ powder was dissolved in concentrated nitric acid under vigorous stirring to get a $Sr(NO_3)_2$ solution. 0.6410 g [$Mg(NO_3)_2 \cdot 6H_2O$], 0.0335 g [$Eu(NO_3)_3 \cdot 6H_2O$] and 0.0658 g [$Dy(NO_3)_3 \cdot 5H_2O$]were dissolved in ultrapure water to obtain a solution with the final volume of 10 mL after the addition of $Sr(NO_3)_2$ solution and then acidified to pH 2.0 by adding concentrated nitric acid. 1.15 mL TEOS was then added rapidly, and the solution was stirred at room temperature until it became transparent. The solution was heated at 70 °C until the sol-to-gel transition occured. To obtain opaque gel, the wet gel was then dried in an oven at 110 °C for 20 h. The resulting opaque dry gel was then fired at 1000 °C for 10 h in a zircone crucible in a weak reductive atmosphere using 10% H_2, 90% Ar to obtain white crystals.

Preparation of PLNPs-GSH

Nanometer-sized particles were obtained by wet grinding of the solid (10 mg) for 20 min with a mortar and pestle in 500 μL of 0.1 M GSH aqueous solution. Then, 1.5 mL water was added into the above solution and the solution was transferred into a 5 mL bottle and stirred overnight. PLNPs-GSH were acquired by centrifugation, and then washed by water for three times. Finally, the nanoparticles were dried in vacuum oven.

Preparation of PLNPs-GSH-6-AF

2 mg PLNPs-GSH were dissolved into 1.4 mL MES (pH=6.0, 20 mM) and then 2 mg EDC was added into the solution. The above solution was stirred for 30 min to activate the carboxyl groups. 100 μL 6-AF (2.3 mM) was added into the above solution and stirred for 24 h. Then PLNPs-GSH-6-AF were acquired by centrifugation and the precipitation was washed for three times to remove residual 6-AF.

Preparation of PLNPs-GSH-5(6)-CF

38 μL 5(6)-CF (2.3 mM), 2 mg EDC and 862 μL MES were mixed and stirred for 30 min to activate carboxyl groups. Then, 2 mg PLNPs-GSH were added into the above solution and the solution was stirred for 24 h. The solution was centrifuged to collect the PLNPs-GSH-5(6)-CF and the precipitation was washed for three times to remove residual 5(6)-CF.

Preparation of PLNPs-GSH-HE

2 mg PLNPs-GSH were dispersed in 1.4 mL MES buffer (pH=6.0, 20 mM) and then 2 mg EDC was added to activate carboxyl groups. The mixture was stirred for 30 min at room temperature. Subsequently, 100 μL HE (3 mM) was added and stirred overnight. The conjugation (PLNPs-GSH-HE) of PLNPs-GSH and HE was acquired by centrifugation and washing with water.

Cell Culture.

HepG2 cells were cultured in Dulbecco's modified Eagles medium (DMEM). The cells were supplemented with 10 % fetal bovine serum and 100 U/ml 1% antibiotics penicillin/streptomycin and maintained at 37 °C in a 100% humidified atmosphere

containing 5% CO_2 at 37 °C.

PLNPs-GSH Imaging in Living Cells.

HepG2 cells were plated on chamber slides for 24 h, then the PLNPs-GSH (0.2 mg/mL)，PLNPs-GSH-6-AF (0.2 mg/mL) and PLNPs-GSH-5(6)-CF (0.2 mg/mL) were separately delivered into the cells in DMEM culture medium at 37 °C in 5 % CO_2. After incubation over night, the cells were washed twice with PBS buffer. The cells were examined by confocal laser scanning microscopy (CLSM) with different laser transmitters. The PLNPs luminscence signal was recorded with 405 nm excitation, 6-AF and 5(6)-CF were recorded by with 488 nm excitation.

MTT Assay.

HepG2 cells were cultured in 96-well microtiter plates and incubated at 37 °C in 5% CO_2 for 24 h. After the original medium was removed, the cells were incubated with PLNPs-GSH (0, 0.2, 0.5, 0.75, 1.0, 1.25, 1.5, 2 mg/mL) over night. Then the cells were washed with PBS for three times. Next, 150 μL MTT solution (0.5 mg/mL) was added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm with a RT 600 microplate reader.

***In vivo* Imaging of PLNPs-GSH**

To demonstrate the applicability of PLNPs-GSH to imaging *in vivo*, animal imaging was conducted as followed. Firstly, one Kunming male mouse was anesthetized using 4% chloral hydrate (250 μL) and then was injected intraperitoneally with PLNPs-GSH (1 mg/mL)，which were excited by 365 nm for 30 min before injection.

Then luminescence signal was collected by Caliper IVIS Lumina III without excitation after the injection and they were taken by every 4 min for 65 min.

Superoxide anion detection *in vivo* imaging of PLNPs-GSH-HE

In order to detect and image the superoxide anion *in vivo* without the excitation source, animal experiment was conducted. Firstly, one Kunming mouse (20 g, male) was injected with LPS (2.5 mg/mL, 400 μL) to cause the acute inflammation and the other was injected saline as control. Four hours later, the two mice were anesthetized using 4% chloral hydrate (250 μL) and then were injected intraperitoneally with PLNPs-GSH-HE (1 mg/mL) at the same spot of LPS and saline injection. PLNPs-GSH-HE were excited by 365 nm UV lamp for 30 min before injection. The imaging result was aquired by Caliper IVIS Lumina III without excitation. The fluorescence of HE were collected at 620±20 nm and the exposure time was 60 s .

Reference

1. N. Li, Y. Li, Y. Han, W. Pan, T. Zhang, B. Tang, *Anal. Chem.* **2014**, *86*, 3924-3930.

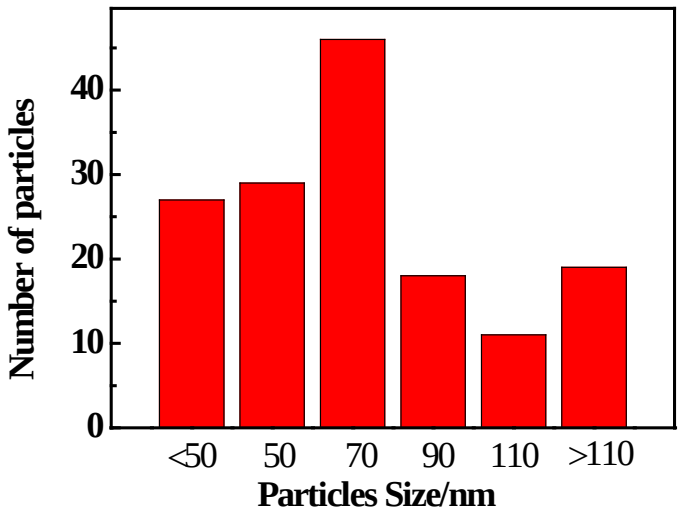

Fig. S1 Hydrodynamic diameter distribution of PLNPs-GSH.

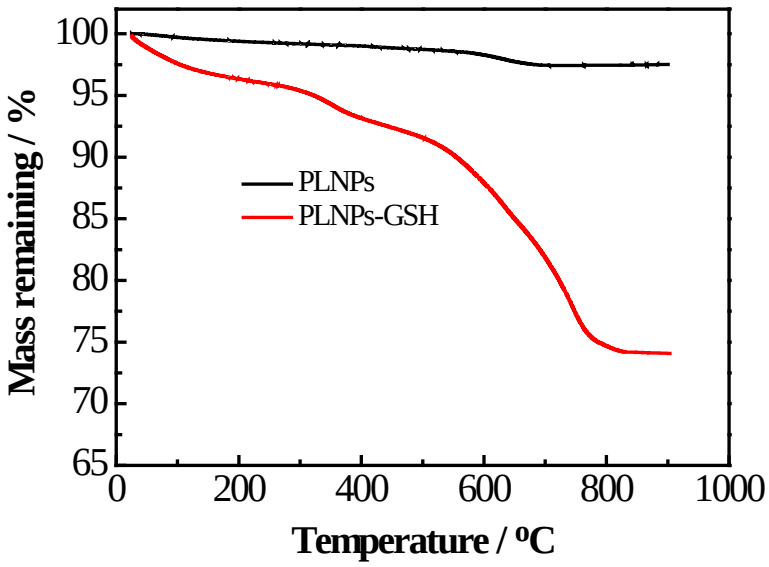

Fig. S2 TGA analysis of PLNPs and PLNPs-GSH.

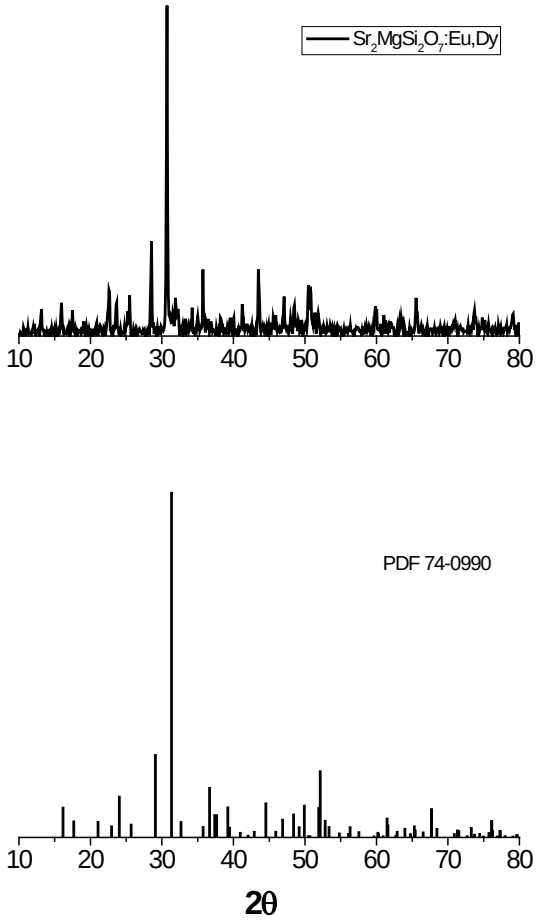

Fig. S3 XRD pattern of PLNPs-GSH (top) and standard sample (bottom).

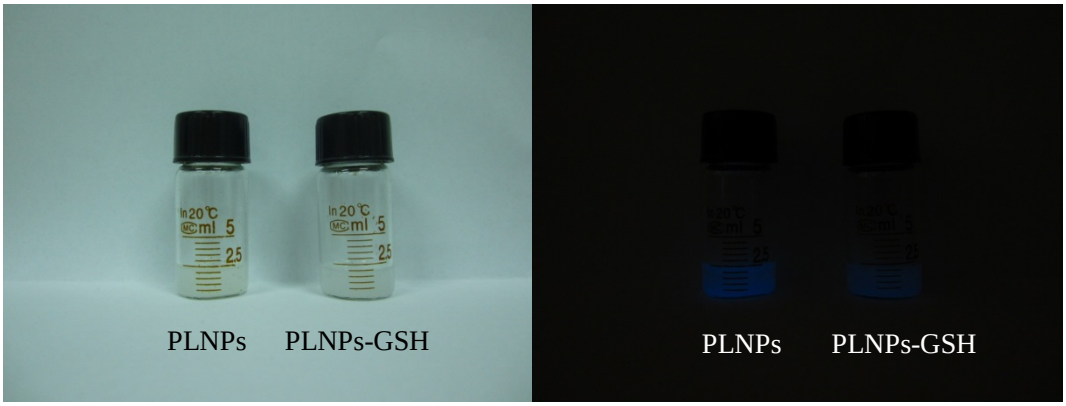

Fig. S4 Photographs of PLNPs and PLNPs-GSH without further excitation after direct exposure under a UV lamp for 10 min.

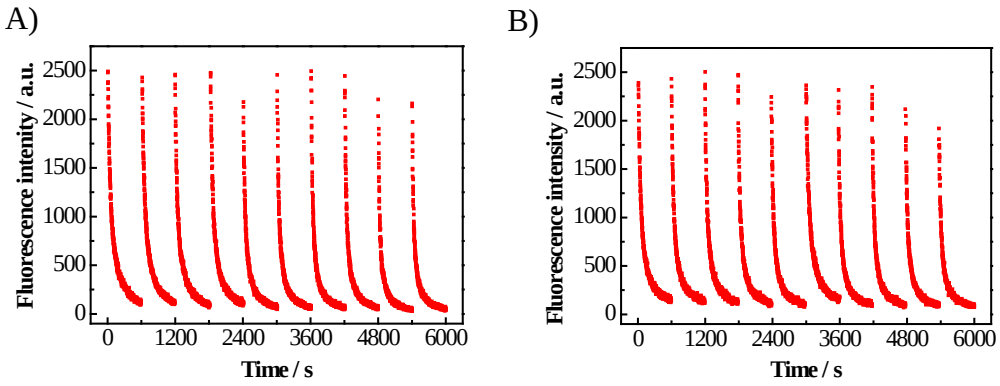

Fig. S5 Kinetic curve of PLNPs-GSH in PBS buffer (A) and DMEM buffer (B) for 10 cycles. PLNPs-GSH was excited at 365 nm for 30 min before each cycle.

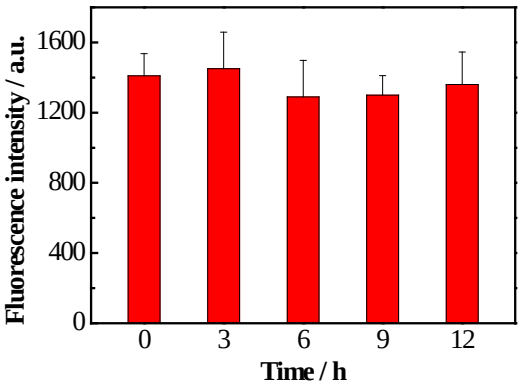

Fig. S6 Stability of PLNPs-GSH in PBS buffer for different time (0, 3, 6 ,9 and 12 h). Before each measurement, PLNPs-GSH was excited at 365 nm for 30 min.

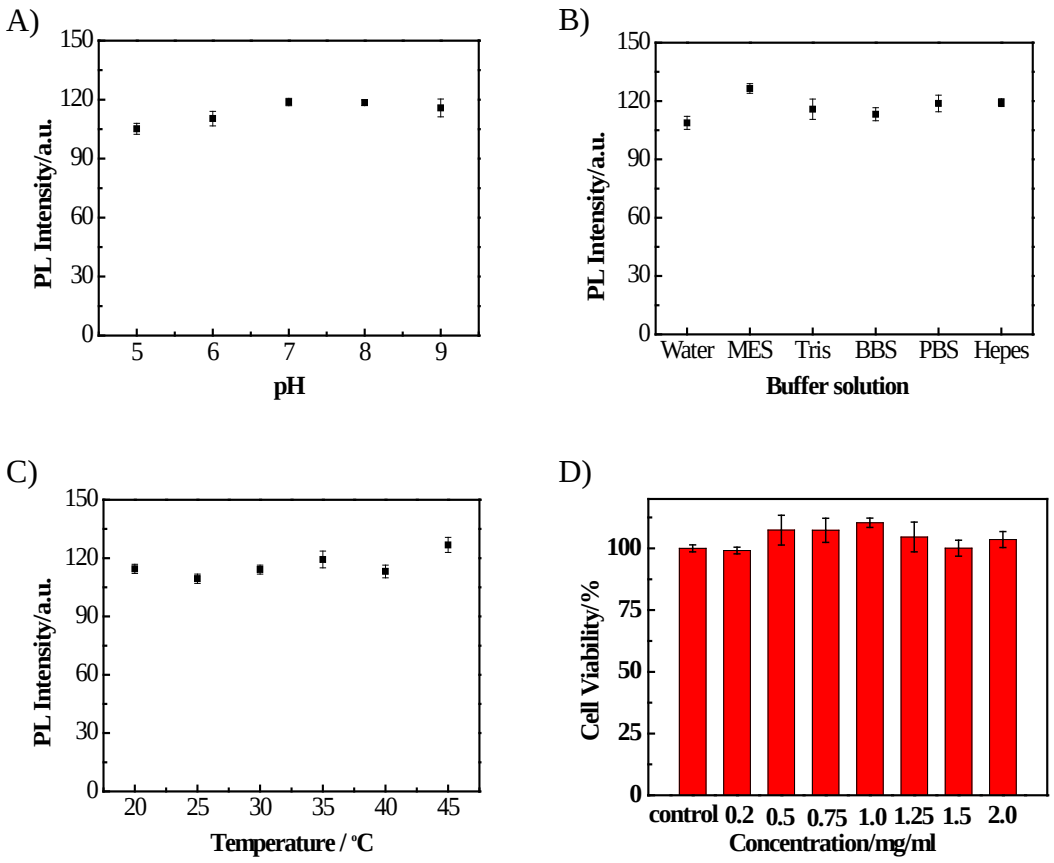

Fig. S7 PL intensity of PLNPs-GHS at different pH (A), buffer solution (B) and temperature (C). (D) Cell viability of MCF-7 cells incubated with PLNPs-GSH with different concentrations.

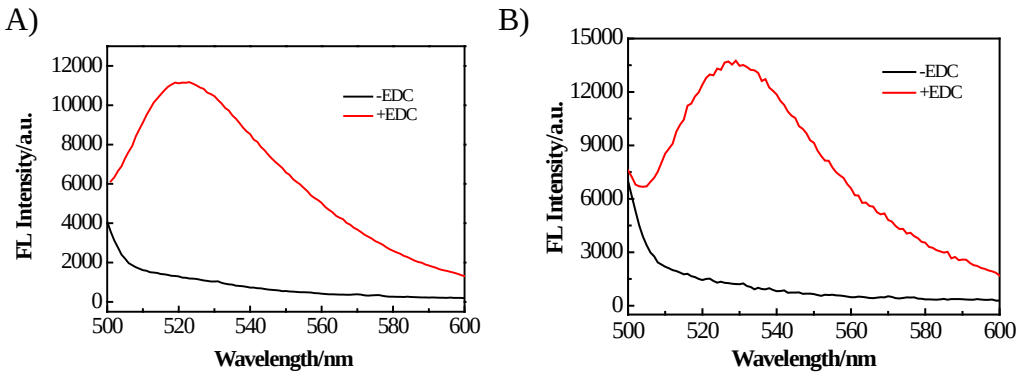

Fig. S8 Fluorescence spectra of PLNPs-GSH-6-AF (A) and PLNPs-GSH-5(6)-CF (B) after the reaction between PLNPs-GSH and 6-AF or 5(6)-CF in the present of EDC or not.

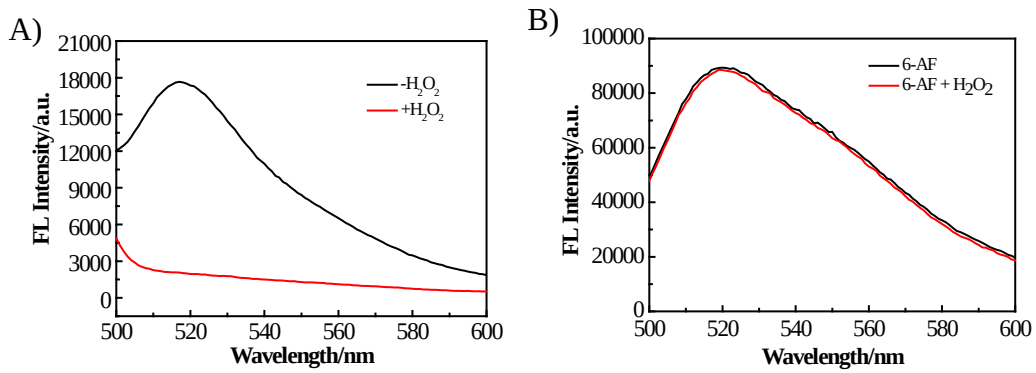

Fig. S9 (A) Fluorescence spectra of PLNPs-GSH-6-AF. GSH was pre-treated with H_2O_2 (red) or not (black) before the coordination. (B) Fluorescence spectra of 6-AF in the present of H_2O_2 (red) or not (black).

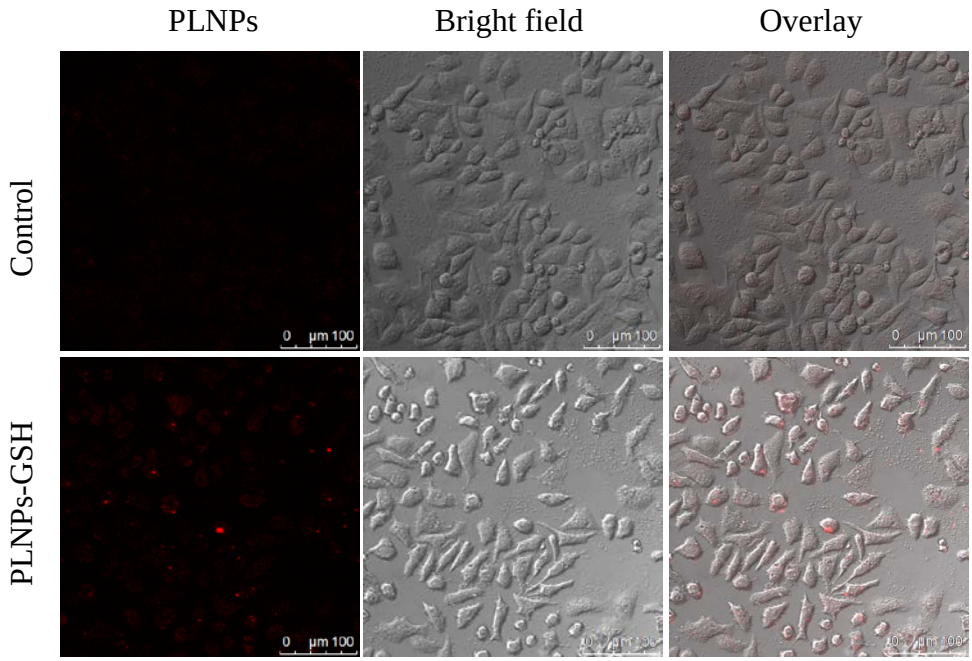

Fig. S10 Confocal images of HepG2 cells incubated with or without PLNPs-GSH. The images of PLNPs-GSH were captured under 405 nm excitation.

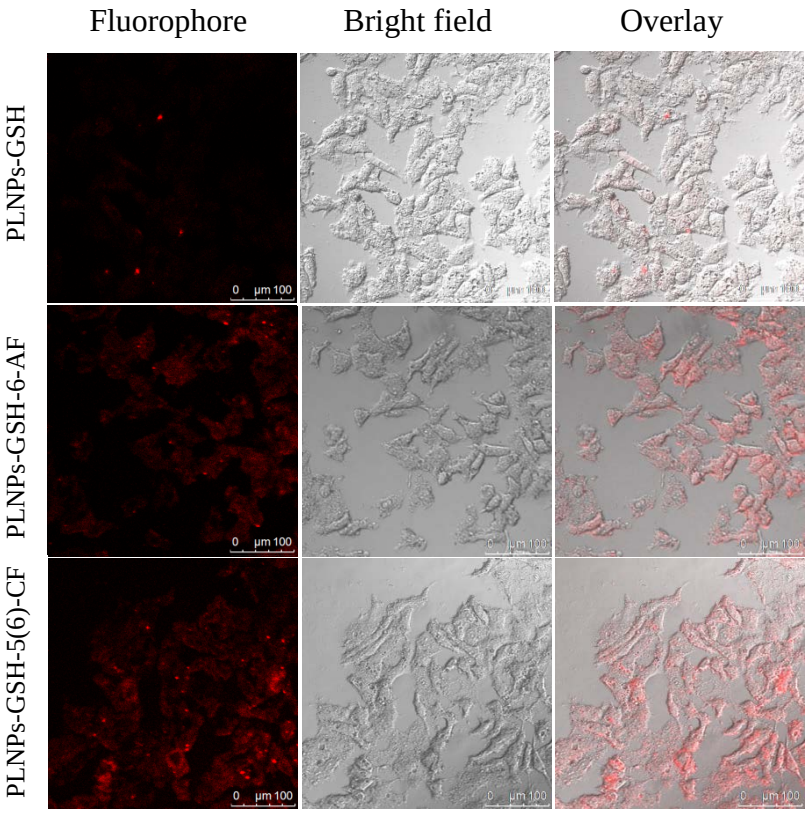

Fig. S11 Cofocal images of HepG2 cells incubated with PLNPs-GSH (top), PLNPs-GSH-6-AF (middle), and PLNPs-GSH-5(6)-CF (bottom). λex=405 nm and λem=510-600.

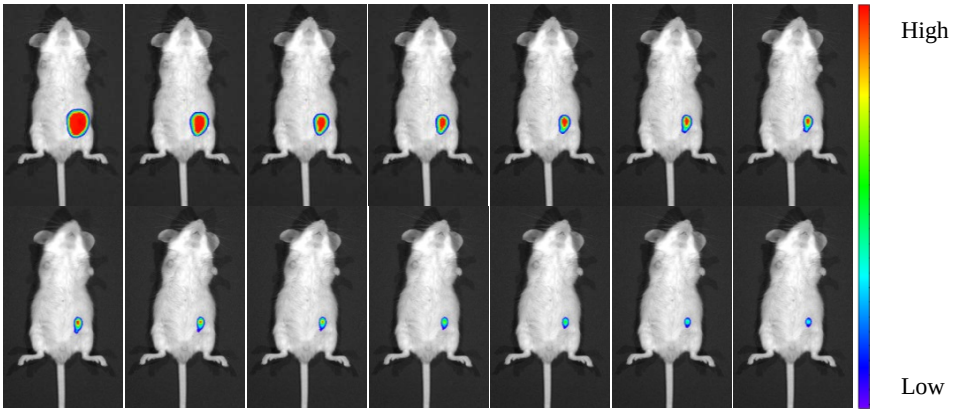

Fig. S12 The PL imaging of PLNPs-GSH *in vivo*. The Kunming mouse was with 4% chloral hydrate (250 μL) and then was injected intraperitoneally with PLNPs-GSH (1 mg/mL)，which were excited by 365 nm for 30 min before injection. Each picture was collected for 1 min and at 4 min interval without extra excitation.