Supporting Information

Mesoporous Nanoparticles Gd_2O_3@mSiO_2/$ZnGa_2O_4$: Cr^{3+}, Bi^{3+} as a Multimodal Probe for Magnetic Resonance Imaging, Drug Delivery and Targeted Bioimaging

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Table of contents

Experimental section

Materials: Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, 99%), gallium nitrate hydrate ($Ga(NO_3)_3 \cdot xH_2O$, 99.9%), chromium nitrate nonahydrate ($Cr(NO_3)_3 \cdot 9H_2O$, 99%), bismuth nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$, 99%), gadolinium nitrate hexahydrate ($Gd(NO_3)_3 \cdot 6H_2O$, 99.9%), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 99.9%), sodium hydroxide (NaOH), and ammonium hydroxide solution (28.0~30.0% NH_3 basis) were purchased from Aladdin (Shanghai, China). Toluene, tert-butylamine, oleic acid (90%, technical grade), chloral hydrate, polyvinylpyrrolidone (PVP, K30), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP) and folic acid (FA) were purchased from Sigma Aldrich. Tetraethoxysilane (TEOS), 3-aminopropyl triethoxysilane (APTES), hexadecyl trimethyl ammonium bromide (CTAB), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethanol, dichloromethane, doxorubicin hydrochloride (Dox) and methyl thiazolyl tetrazolium (MTT) were purchased from Shanghai Chemical Reagent Company. RPMI 1640 and DMEM mediums, fetal bovine serum (FBS) were purchased from Life Technologies. Normal liver cells (L02), human umbilical vein endothelial cells (HUVECs) and hepatic carcinoma cells (HepG2) were purchased from the Committee on Type Culture Collection of the Chinese Academy of Sciences. H22-tumor-bearing mice and Kunming normal mice were purchased from the Shanghai Lab Animal Research Center. All animal procedures were approved by the Animal Ethical Experimentation Committee of Chinese Academy of Sciences according to the requirements of the National Act on the use of experimental animals (China).

Characterizations: Field emission scanning electron microscopy (FE-SEM) experiments were carried out on a Hitachi S-4800. Transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS) experiments were performed on a Hitachi H-7650. The excitation, emission spectra and afterglow decay curves were measured using an Edinburgh FL920 spectrofluorometer. Thermo-luminescence glow curves were measured with a FJ-427A TL meter (Beijing Nuclear Instrument Factory) by heating the irradiated samples from RT to 600 K. The samples were pre-irradiated by using a Xenon lamp for 5 min and then heated at a linear rate of 2 $K \cdot s^{-1}$. Diffuse reflectance spectra were recorded using a Cary 5000 UV-vis-NIR spectrophotometer equipped with a double out-of-plane Littrow monochromator using $BaSO_4$ as a standard reference. X-Ray powder

diffractions (XRD) were performed on a Bruker AXS D8 diffractometer. N_2 adsorption/desorption isotherms were measured using a micromeritics ASAP2020 surface area and porosity analyzer. The specific surface area was calculated by adopting the BET method. Thermal gravimetric analysis (TGA) experiments were performed on a TG209 thermal analyzer (Netzsch, Germany). Fourier transform infrared (FTIR) spectra were recorded on a Vector-33 FTIR spectrometer (Bruker, Switzerland).

Synthesis of Gd_2O_3@$mSiO_2$: 0.5 g $Gd(NO_3)_3 \cdot 6H_2O$, 0.016 g $FeCl_3 \cdot 6H_2O$, 1.260 g CTAB and 0.5 g PVP were dispersed into 25 mL DMF with stirring at 60°C for 6 h. The as-prepared solution was then transferred to the autoclave and sintered at 220°C for 20 h. After the autoclave cooled to RT, the products were washed with ethanol for three times and then dried at 80°C for 7 h to obtain $Gd(OH)_3$.

Under ultrasonication, 0.3 g $Gd(OH)_3$ was dissolved in the mixed ethanol (160 mL) and deionized water (40 mL) solution. Then, 2.0 mL NH_3 basis was mixed to the solution with a rapid stirring at RT for 30 min. Next, 10 mL ethanol containing 48 μL TEOS was added to above solution. After stirring at RT for 6 h, the obtained nanoparticles $Gd(OH)_3$@SiO_2 were dissolved in a mixed solution with ethanol (120 mL), deionized water (160 mL), CTAB (0.6 g) and NH_3 basis (2.0 mL). Subsequently, the mixed solution was continuously stirred at RT for 6 h. After centrifugation, the obtained products were again dried at 80°C for 6 h. Finally, the Gd_2O_3@$mSiO_2$ was obtained by sintering the products at 600°C for 4 h.

Synthesis of Gd_2O_3@$mSiO_2$/ZGOCB: In a typical procedure, the Gd_2O_3@$mSiO_2$ nanoparticles were dissolved in the ZGOCB sol with stirred at RT for 6 h. Subsequently, the mixed product was collected by centrifugation and then dried at 80°C for 6 h. The obtained sample was calcined at 600°C for 1.5 h to get Gd_2O_3@$mSiO_2$/ZGOCB.

Surface modification: The OH^- group was first attached on the surface of Gd_2O_3@$mSiO_2$/ZGOCB to form Gd_2O_3@$mSiO_2$/ZGOCB-OH using 5 mM NaOH solution. Next, the Gd_2O_3@$mSiO_2$/ZGOCB-OH (100 mg) and APTES (100 μL) were dissolved into DMF (100 mL) with stirring for 10 h and then centrifugation to manufacture Gd_2O_3@$mSiO_2$/ZGOCB-NH_2. Finally, to obtain the Gd_2O_3@$mSiO_2$/ZGOCB-FA, 15 mg of Gd_2O_3@$mSiO_2$/ZGOCB-NH_2 was dissolved in 15 mL of dichloromethane with sonication, and then 15 mL of DMSO and 12 mg of FA was added. Then, 15 mg of EDC, 30 mg of NHS and 15 mg of DMAP were added into the solution. After stirring for 36

h at RT, the final mixture Gd_2O_3@$mSiO_2$/ZGOCB-FA was obtained *via* centrifugation.

Cytotoxicity test: For the MTT assay, L02, HUVECs and HepG2 were seeded in 96-well plates (Corning, USA) for 12 h. Then, after the old medium was removed, fresh medium containing Gd_2O_3@$mSiO_2$/ZGOCB-NH_2 nanoprobes with different concentrations was added and incubated for 24 h at CO_2 incubator (Thermo Heracell™ 150i, USA). Subsequently, MTT was added and incubated for 4 h at CO_2 incubator. Then, the medium was removed and DMSO was added to dissolve the formazan for 30 min. The absorbance at 490 nm was monitored using a microplate reader (Molecular devices, Sunnyvale, CA, USA). For cell apoptosis assay, it was performed using the Annexin-V-Fluos and propidium iodide (PI) apoptosis detection kit (Roche, Germany). Briefly, the L02, HUVECs and HepG2 cells were exposed to Gd_2O_3@$mSiO_2$/ZGOCB-NH_2 for 24 h. Next, the cells were collected and redissolved in 100 μL of Annexin-V-Fluos labeling solution. Then, the cells were incubated with the labeling solution at 25°C for 15 min and transferred to 96-well plates using a flow cytometer (Beckman coulter, USA) to analyze.

Drug storage and release: 15 mg of Gd_2O_3@$mSiO_2$/ZGOCB-FA was dissolved in 6 mL of Dox aqueous solution (1 $mg \cdot mL^{-1}$) with stirring for 12 h, and the Dox-Gd_2O_3@$mSiO_2$/ZGOCB-FA was obtained after centrifugation. To measure the Dox loading efficiency, the residual Dox (r_{Dox}) content and the original (o_{Dox}) content were determined by UV-vis measurements at 480 nm. Therefore, the loading efficiency can be calculated as: $[o_{(Dox)} - r_{(Dox)}]/o_{(Dox)} \times 100\%$. The obtained nanoparticles were then transferred to a dialysis tube (molecular weight cut-off 3500 Da, Thermo Scientific) and immersed in 3 mL of phosphoric acidic buffer (PBS, pH = 7.4) at 37°C. At different time intervals, the specimens were centrifuged and PBS was removed and replaced with an equal volume of fresh PBS. Hence, the amount of released Dox in the supernatant solutions was calculated using the data on the absorbance at 480 nm.

***In vivo* MRI:** *In vivo* MRI of Kunming mice (~ 20 g) was performed on 7 T MRI system (BioSpec 70/20 USR, Bruker, USA). In brief, the Gd_2O_3@$mSiO_2$/ZGOCB-FA solution (300 μL, 1 $mg \cdot mL^{-1}$) was injected through tail vein into the anesthetized Kunming mice with 5% chloral hydrate (250 μL). MR images were obtained using a small animal coil before and at 20 min after injection. The fixed parameters were set: TR/TE = 100.0/8.8 ms, FOV = 100×50 mm^2, spin-echo T_1-weighted

MRI sequence, matrix = 256×256, slice thickness = 1.5 mm and at RT.

***In vitro* and *in vivo* luminescence imaging:** For observing *in vitro* imaging of Gd_2O_3@$mSiO_2$/ZGOCB-FA for tumor cell targeting, L02 and HepG2 cells were cultured in RMPI-1640 and DMEM medium containing 10% FBS. The same amounts of cells were firstly seeded in 35 mm culture dishes for 12 h before the treatment day. Then, the seeded cells in serum-supplemented media were treated with Gd_2O_3@$mSiO_2$/ZGOCB-FA (50 $mg \cdot mL^{-1}$) for 2 h at 37°C. Subsequently, the cells were washed three times with PBS and directly imaged by a confocal microscope (LSCM, Zeiss, Germany). Additional, afterglow imaging was collected on an IVIS Lumina II imaging system (Xenogen, USA) after excitation with 254 nm for 2 min and the exposure time was fixed at 1 min. To evaluate the detection sensitivity, HepG2 cells were cultured with 50 $mg \cdot mL^{-1}$ of Gd_2O_3@$mSiO_2$/ZGOCB-FA for 2 h at 37°C. The cells were washed and redissolved in PBS and the cell number was calculated using a hemocytometer and microscope (Zeiss, Germany). To evaluate the capacity of tumor imaging *in vivo*, different numbers of HepG2 cells with the Gd_2O_3@$mSiO_2$/ZGOCB-FA were subcutaneously injected into the abdomen of a mouse after excitation at 254 nm for 2 min. The cell number and luminescence in *vivo* were performed on the IVIS Lumina II imaging system to determine the correlation. Meanwhile, Kunming mice implanted with murine hepatoma H22 tumors overexpressing folate receptors were adopted to investigate tumor targeting with Dox-Gd_2O_3@$mSiO_2$/ZGOCB-FA *in vivo*. The Dox-Gd_2O_3@$mSiO_2$/ZGOCB-FA was dissolved in PBS solution (1 $mg \cdot mL^{-1}$), excited for 2 min under a 254 nm radiation and then injected through the tail vein to a normal and H22 tumor-bearing mice. The luminescence singles were measured using the same system.

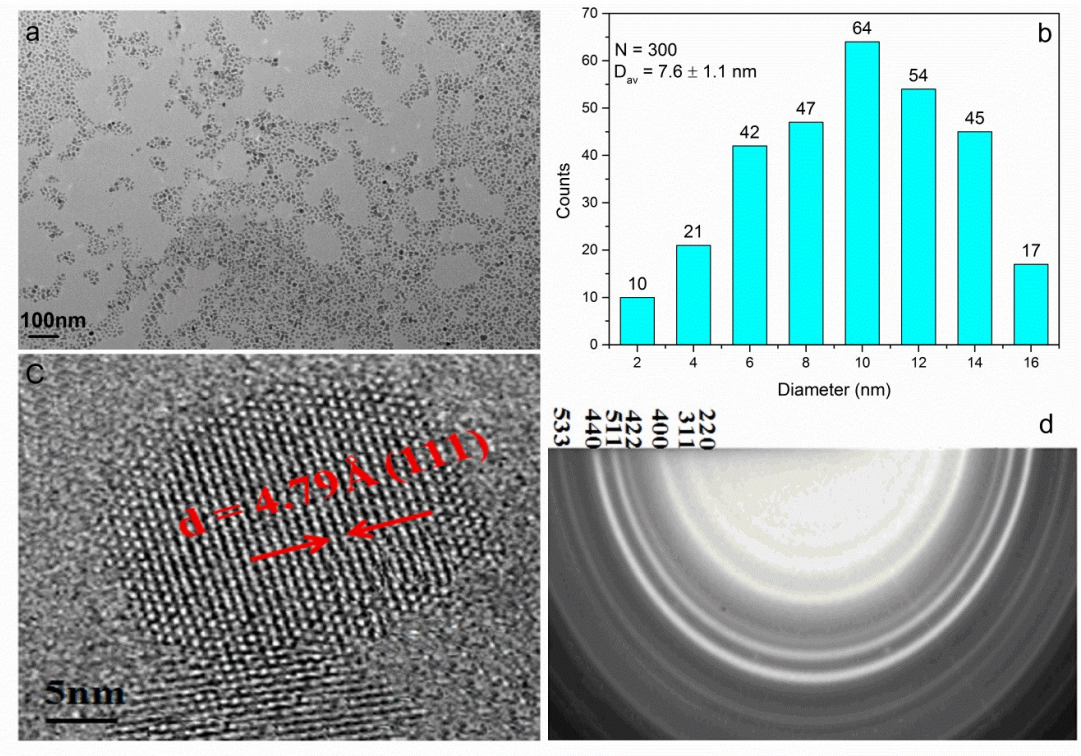

Figure S1. (a-d) TEM image, size distribution, HR-TEM image and electronic diffraction pattern of $Zn_{0.97}Ga_2O_{3.97}$: $0.01Cr^{3+}$, $0.02Bi^{3+}$ NPs, respectively.

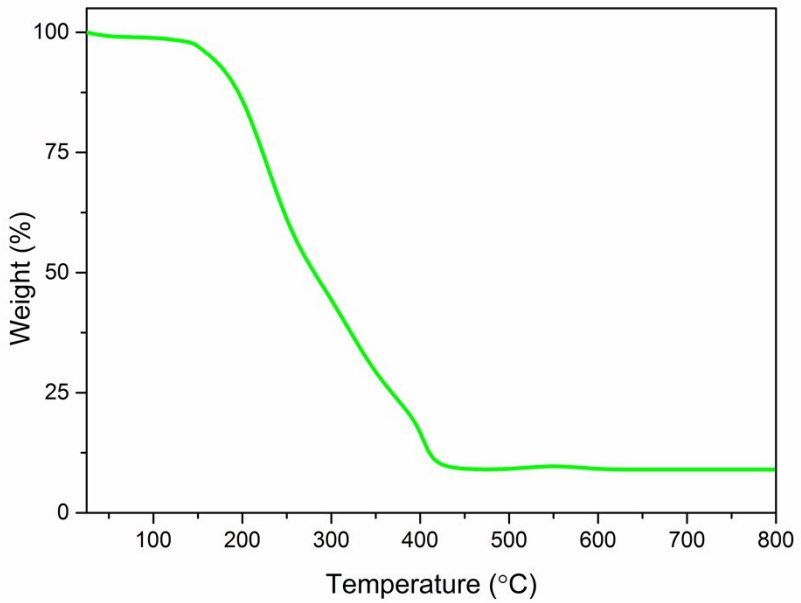

Figure S2. Thermogravimetric analysis of FA.

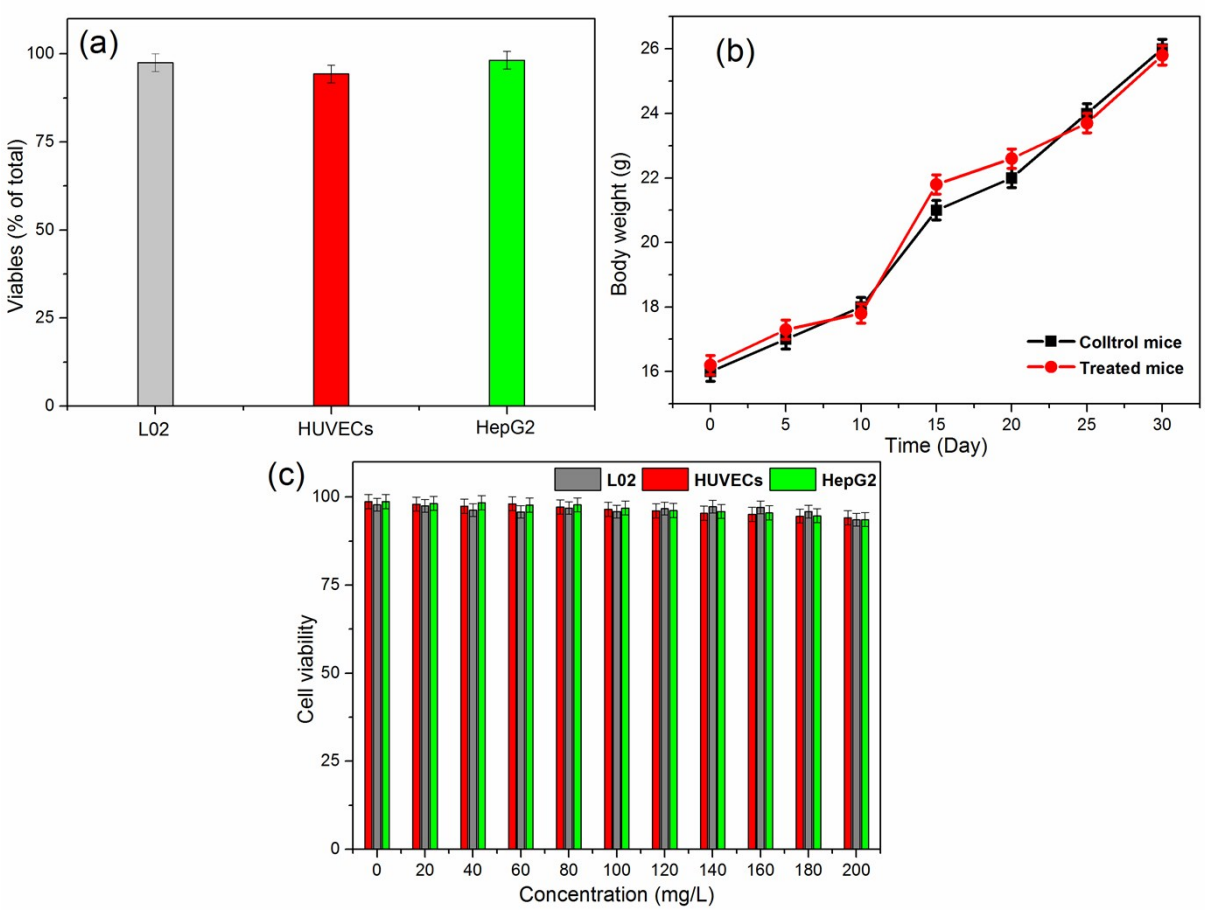

Figure S3. (a) Cell apoptosis assay. (b) Body weight changes of the mice (n = 3) injected with Gd_2O_3@$mSiO_2$/ZGOCB-NH_2 (0.5 mL, 200 mg·L^{-1}) and the control mice (n = 3) injected with PBS. (c) MTT cell viability assay for Dox-Gd_2O_3@$mSiO_2$/ZGOCB-FA.

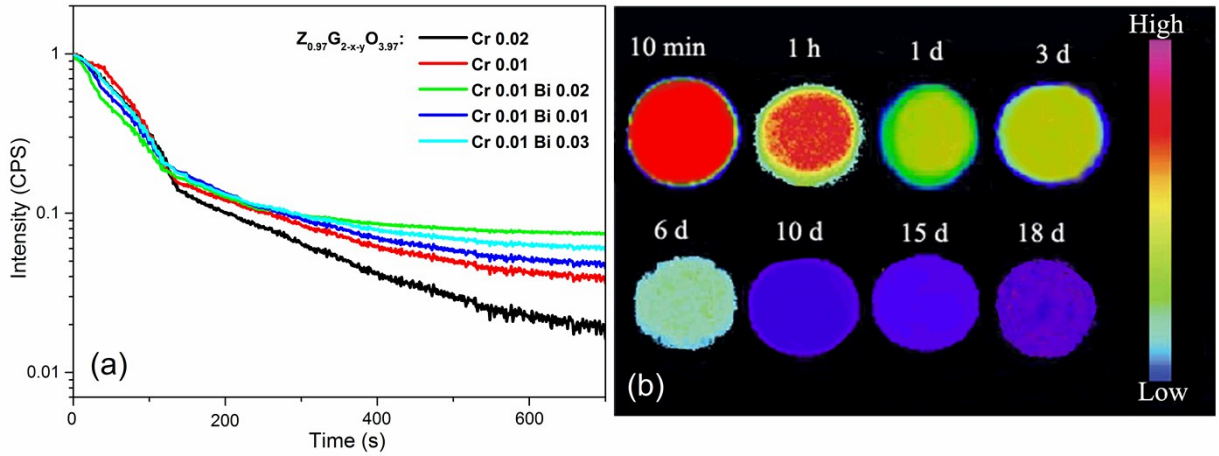

Figure S4. (a) Afterglow decay curves of $Z_{0.97}G_{2-x-y}O_{3.97}$: $x$$Cr^{3+}$, $y$$Bi^{3+}$ NPs after 5 min irradiation with UV lamp. (b) ZGO: 0.01Cr, 0.02Bi NPs afterglow images detected by a CCD camera at different time points after stopping UV irradiation for 5 min.

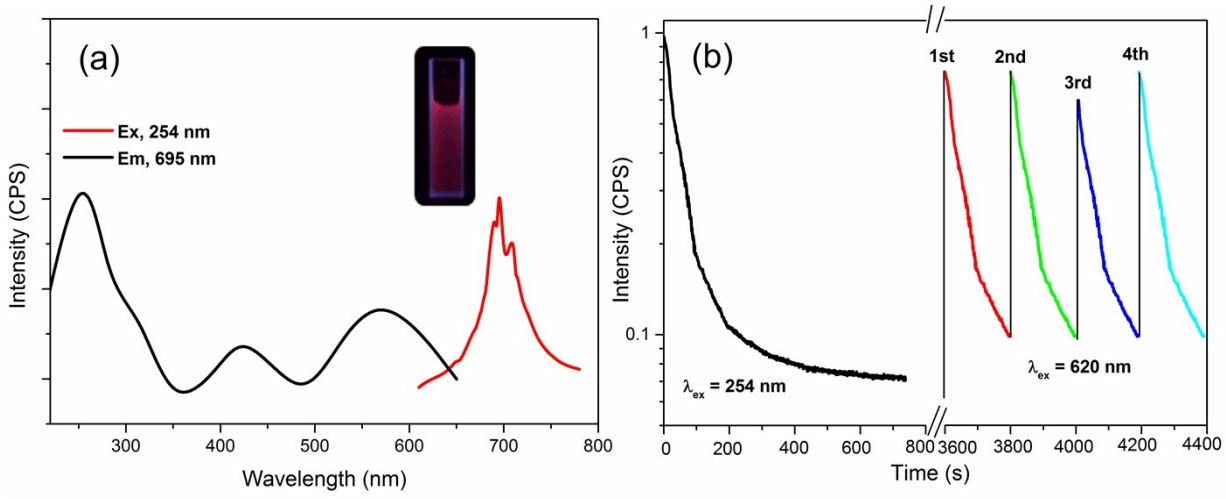

Figure S5. (a) PLE and PL spectra of the aqueous dispersion of Gd_2O_3@mSiO_2/ZGOCB-FA at RT (1 mg·mL^{-1}), the inset shows the digital photo under UV excitation. (b) Charged (λ_{ex} = 254 nm) and recharged decay curves (λ_{ex} = 620 nm).

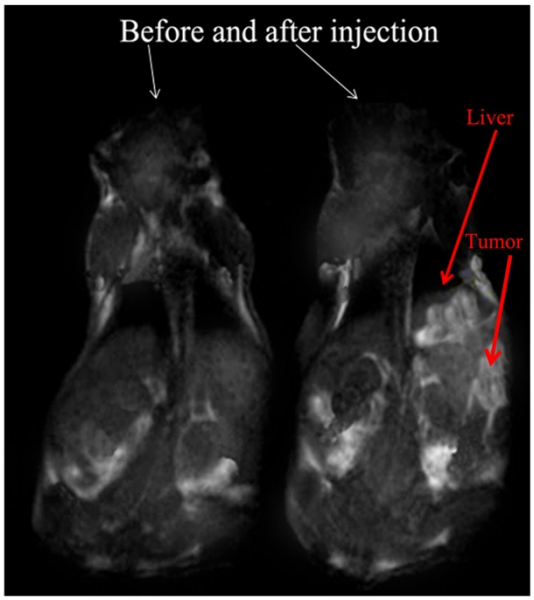

Figure S6. Before and after intravenous injection of Dox-Gd_2O_3@mSiO_2/ZGOCB-FA (0.15 mg) T_1-weighted MR images of the H22 tumor-bearing mice, the red arrow shows the liver and tumor, respectively.

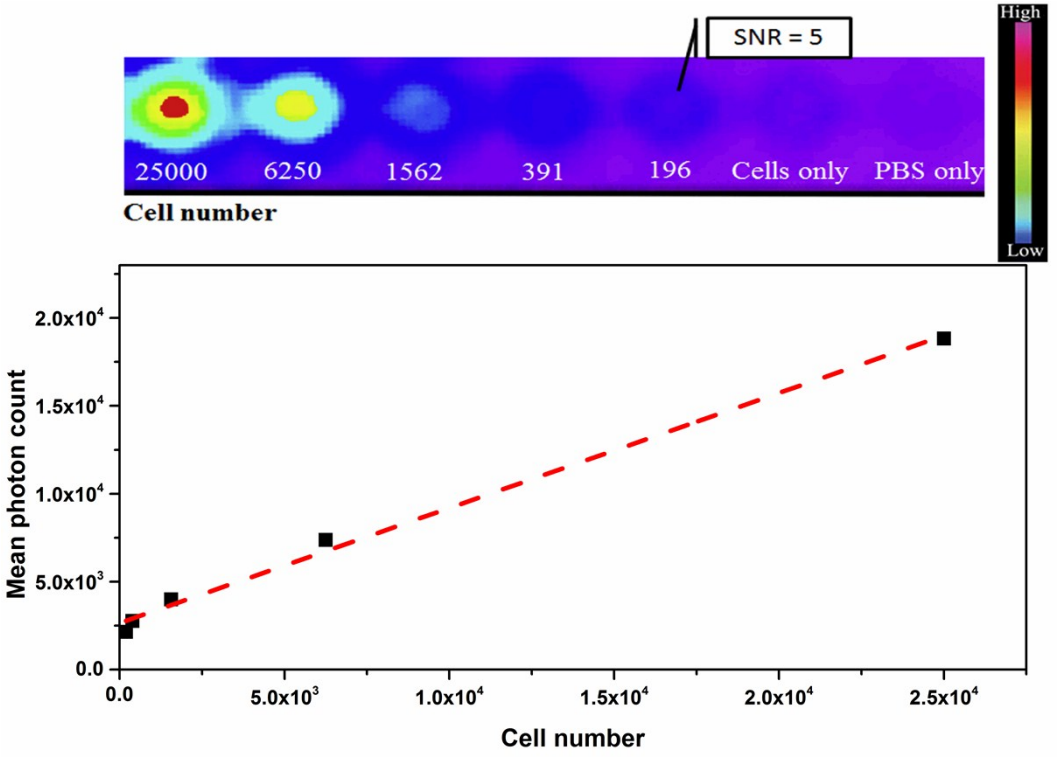

Figure S7. (Top) luminescence imaging of different numbers of HepG2 cells labeled with Gd_2O_3@mSiO_2/ZGOCB-FA. (Bottom) correlation between signal and cell number.

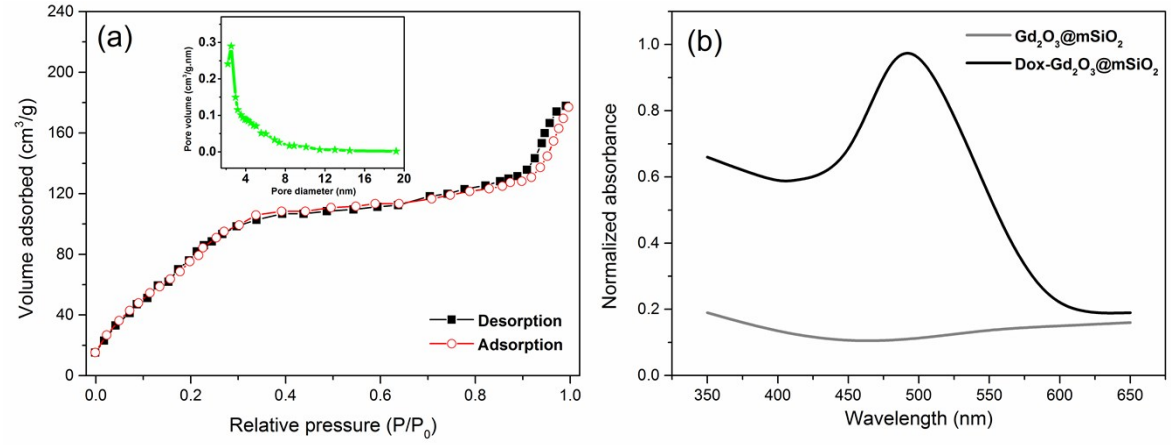

Figure S8. (a) N_2 adsorption-desorption isotherms of Dox-Gd_2O_3@mSiO_2/ZGOCB-FA, the inset shows pore size distribution calculated with the BET method. (b) Influence of Dox loading on the absorbance of Gd_2O_3@mSiO_2.

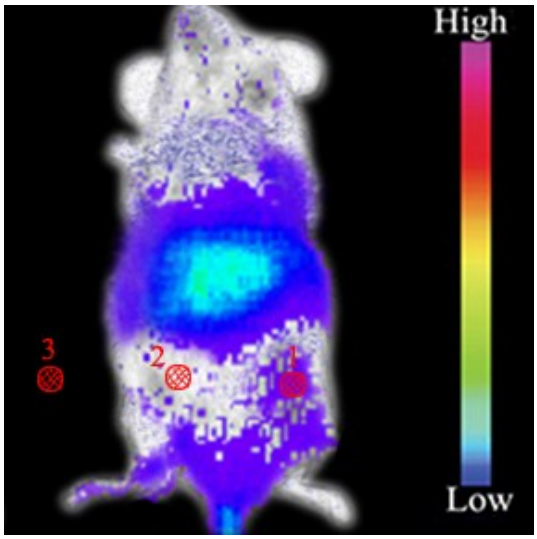

Figure S9. Calculation of SNR from the *in vivo* red luminescence image. SNR [1] = [(mean intensity of the specific uptake, 1) – (mean intensity of background, 3)] / [(mean intensity of the nonspecific uptake, 2) – (mean intensity of background, 3)] = (1274.6 – 25.5) / (255.6 – 25.5) = 5.4285.

[1] Q. Liu, Y. Sun, T. Yang, W. Feng, C. Li, F. Li, *J. Am. Chem. Soc.* **2011**, 133, 17122.

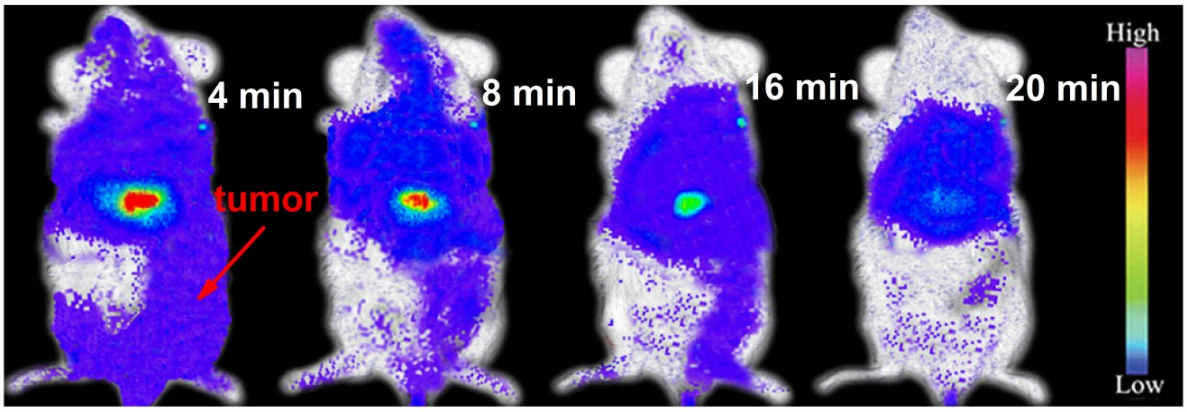

Figure S10. *In vivo* luminescence images for the secondary excitation of the mice with a red LED lamp for 2 min after 3 h intravenous injection of Dox-Gd_2O_3@mSiO_2/ZGOCB-FA.