Supporting Information

Experimental Section

Materials: Gadolinium chloride hexahydrate ($GdCl_3$•$6H_2O$, 99.99%), Diethylenetriaminepentaacetic dianhydride, dimethyl sulfoxide (DMSO, 99.5%), $HAuCl_4$•$4H_2O$, bovine serum albumin (66 000 Da), DTPA-anhydride and MTS assays were purchased from Sigma Aldrich; Hydrochloric acid (HCl, 37%), nitric acid (HNO_3, 65%), sodium hydroxide (NaOH, 99.99%), ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China); phosphate-buffered saline (PBS, pH 7.4), fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco Life Technologies (AG, Switzerland). All other chemicals used in this study were of analytical reagent grade and used without further purification. Superpure water (18.25 MΩ.cm, 25 ^{o}C) was used to prepare all solutions. BALB/c athymic nude mice and BALB/C mice were maintained under aseptic conditions in a small animal isolator. All food, water, bedding and cages were autoclaved before use.

Synthesis of hybrid gold-gadolinium NCs: All glassware was washed with aqua regia (HCl: HNO_3 volume ratio 3: 1), and rinsed with ethanol and superpure water, respectively. In a typical experiment, $HAuCl_4$•$4H_2O$ aqueous solution (25 mL, 10 mM, 37 ^{o}C) was added to BSA solution (25 mL, 50 mg mL^{-1},37 ^{o}C) under vigorous stirring. NaOH solution (0.5 mL, 1 M) was introduced 2 min later, and the reaction was allowed to proceed under vigorous stirring at 37 ^{o}C. The color of the solution changed from light yellow to light brown, and then to deep brown. The reaction was completed in 12 h, and the solution was stored at 4 ^{o}C. The as-prepared gold NCs have relatively high PLQY (~6%). Then, 2 mL 2 M phosphate-buffered saline (PBS, pH 9) was added into 40 mL gold NCs and then 560 mg of DTPA cyclic anhydride (DTPACA) powder was added to the gold NCs solution which was adjusted to pH 6~7 using 5 M NaOH. The solution was stirred for 2 h at room temperature. Subsequently, 600 mg $GdCl_3$•$6H_2O$ was dissolved in 1 mL 1M HCl and added dropwise to the BSA-DTPA

solution to produce hybrid gold-gadolinium NCs, while carefully controlling the pH between 6 and 7.

Purification: Purification of hybrid NCs was performed by centrifugation using Millipore (Centrifugal filter devices, 10K) at 5000 rpm. This step was repeated several times, by filling the tubes with PBS buffer and centrifuging again, until the desired purification rate was reached. Then the solution was filtered through a 0.2 µm membrane to remove the largest impurities.

Spectra assay: UV/vis absorption spectra were obtained with a PerkinElmer Lambda 25 UV-vis spectrophotometer. The PL spectra and the lifetime measurements were obtained by a fluorescent spectrometer (F900, Edinburgh Instruments Ltd.).

Scanning Transmission Electron Microscopy (STEM) image: STEM was used to obtain detailed structural and morphological information about the samples and was carried out using a FEI Tecnai F20 TEM at extraction voltage 4500 V, HT 200 kV. The samples for STEM were prepared by depositing a drop of a diluted colloidal solution on a carbon grid and allowing the liquid to dry in air at room temperature.

DLS Measurements: The hydrodynamic diameter and the potential of the water-soluble gold NCs and hybrid NCs dispersed in water were measured by dynamic light scattering (DLS), using a Malvern Zeta Sizer (NanoZS). The samples have been thoroughly purified with centrifugal filters from Millipore (Centrifugal filter devices, 10K) and dispersed in superpure water prior to the measurements. Given the sensitivity of the instrument, multiple runs (>3) were performed to avoid erroneous results. The data are collected in automatic mode and expressed in number %.

Inductively Coupled Plasma-Atomic Emission Mass Spectrometry (ICP-AES) Analysis: The coupling ratio of DTPA-Gd and BSA was quantitatively determined by ICP-AES analysis (with a Varian 710-ES spectrometer). In vivo biodistribution studies: major organs were removed from mice injected with hybrid NCs 1h and 5h post-injection after complete

anesthesia. Twenty milligrams of purified hybrid NCs was dissolved in double distilled water with the final volume of 20 mL for the ICP-AES test. Tissues were removed, washed, and weighed. For each sample, 30-50 mg of tissue was digested in nitric acid and heated at 120 ^{o}C for several hours. Hydrogen peroxide solution was used to drive off the vapor of nitrogen oxides until the solution was colorless and clear. After the solution volume was adjusted to 2 mL using 2% nitric acid and 1% hydrochloride acid, the Au content was analyzed using ICP-AES.

Cell culture: MCF-7 cells (human breast cancer cells) were cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin, and 1% (v/v) streptomycin. Cells were incubated in a humidified incubator at 37 ^{o}C with 5% CO_2.

Cell viability (MTS) assay: MCF-7 cells were planted into 96-well plates for the cell viability (MTS) assay. All cells in logarithmic growth were treated with hybrid NCs at various final concentrations from 4.25 to 220 μmol mL^{-1} compared to the cells treated with PBS as control. After 24 h and 48 h, 20 mL MTS were added into each well and incubated for 4 h at 37 ^{o}C. A490 was detected to measure the proliferative capacity of each group. Each experiment was repeated at least five times to obtain the average value. The relative viability ratio of treated groups was calculated based on the intensity compared to controls.

Confocal laser microscopy assay: For in vitro studies, 1×10^5 MCF-7 cells were seeded on a glassbottomed culture dish. After 24 h, cells were incubated with hybrid NCs for 0.5 h at 37 ^{o}C. To remove the unbound conjugates, the cells were washed three times for 10 min by using shaking incubation (30 rpm) in Tris buffer and fixed with 3.7% formaldehyde solution (1 mL) and incubated at shaking incubation (20 rpm) each for 20 min. Images of cells were acquired using a Leica DMI6000 inverted microscope with a Leica TCS SP5 confocal scanning system.

Animal Model: All animal experiments were conducted in agreement with the "Principles of Laboratory Animal Care" (NIH publication no. 86-23, revised 1985). The guidelines of the

Institute for Nutritional Science of Chinese Academy of Sciences were also respected. Six-to-seven week old male BALB/c athymic nude mice were maintained under aseptic conditions in a small animal isolator and were housed in a group of five in standard cages with free accessto food and water and a 12 h light/dark cycle. All animals acclimated to the animal facility for at least 7 days before experimentation. All possible parameters that may cause social stress, like group size, type (treated and nontreated), etc., among the experimental animals were carefully monitored and avoided. Animals were observed daily for any behavioral abnormalities and weighed weekly.

NIRF imaging of Albumin-binding gadolinium-gold hybrid NCs: For evaluation of imaging capability of hybrid NCs, it was placed in an eppendorf tube with different concentrations. The tubes were positioned in a home-made rack. The rack was imaged using the Maestro in vivo imaging system (CRI, Inc., excitation, 435–480 nm; emission, 490 nm long-pass). The tunable filter was automatically stepped in 5 nm increments from 600 to 800 nm, whereas the camera captured images at each wavelength interval with constant exposure. Spectral unmixing algorithms were applied to create unmixed images of hybrid NCs.

In vivo NIRF imaging: To perform in vivo NIRF imaging, male nude mice bearing MCF-7 tumors were intravenously injected with hybrid NCs (500 mg/kg). The mouse was imaged using the Maestro in vivo imaging system (CRI, Inc., excitation, 435–480 nm; emission, 490 nm long-pass). The tunable filter was automatically stepped in 5 nm increments from 600 to 800 nm, whereas the camera captured images at each wavelength interval with constant exposure. Spectral unmixing algorithms were applied to create unmixed images of hybrid NCs

CT scanning of hybrid gold-gadolinium NCs: For evaluation of computed tomography imaging capability of hybrid NCs, it was placed in an eppendorf tube with different concentrations. The tubes were positioned in a home-made rack. We placed the rack in a 32 Multislice CT (Siemens). The rack was scanned 4 times under 80 keV, 100 mA at the field of

view (FOV) of 32 cm. The resolution was 512×512 and the slice thickness was 0.625 mm. Under this condition, a voxel is 0.625×0.625×0.625 cm^3, which is isotropic. *CT imaging:* When the tumor size reached a diameter of 5–8 mm, the nude mice received hybrid NCs (500 mg/kg) by tail vein injection. The mice were scanned before injection to determine the CT baseline, as well as one hour post injection. The scans were performed using a clinical 32 detector CT scanner (Siemens), with scanning parameters of 80 kVp and 500 mAs. The contrast enhancement was quantitatively determined according to the differential contrast in CT numbers (in Hounsfield units [HU]), compared to that of the same mouse before injection.

Magnetic resonance (MR): For evaluation of magnetic resonance imaging capability of hybrid NCs, it was dissolved in purified water and placed in an eppendorf tube with different concentrations for in vitro MRI analysis. Purified water was used as negative controls. In vitro MRI experiments were performed on a clinical 3 Tesla horizontal bore magnet (SIEMENS, VERIO). The pulse sequence time parameters (TE/TR) of T1 weighted imaging were as follows: TE =9 ms, TR=500ms.

In Vivo MRI Studies: Athymic nude mice bearing MCF-7 tumors (6-8 weeks) were anesthetized, and then hybrid NCs (500 mg/kg) were injected intravenously. The mice were placed on a mouse bed in the receiver coil and MRI of the whole mice was performed with a 3-T system (SIEMENS, VERIO) equipped with an animal coil. To image the biodistribution, a sagittal T1-weighed spin-echo sequence (TR/TE=500/5.1 ms; 1 mm slice thickness, 27 slices; 40×70mm field of view, 128 × 256 matrix; Tacq=2min2s/image) was performed under a respiratory trigger.

Histology: The excised tumor organs were embedded in Tissue-Tek OTC compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan) and frozen in a deep freezer at -70 ^{o}C. The tissue was sectioned into 10 μm slices. The tissue slides were examined via a fluorescence microscope equipped with a Nuance multispectral imaging system (CRi, Woburn, MA).

For histological assessment, twenty-eight male BALB/C mice of SPF grade were included. The mice were randomly divided into high, middle, low dose groups and PBS group (n=7). 500, 250, 125 and 0 mg/kg hybrid NCs was respectively given by intravenously to mice in the high, middle, low dose groups and PBS group (5 times at intervals of 3 days). Blood samples were collected. The AST and LDH analysis of plasma samples were conducted. Heart, kidney, liver, spleen, and lung were fixed in 10% buffered formalin-saline at 4 ^{o}C overnight. Then, tissues were embedded in Tissue-Tek OTC compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan) and frozen in a deep freezer at -70 ^{o}C. The tissue was sectioned into 10 μm slices for hematoxylin and eosin (H&E) staining.

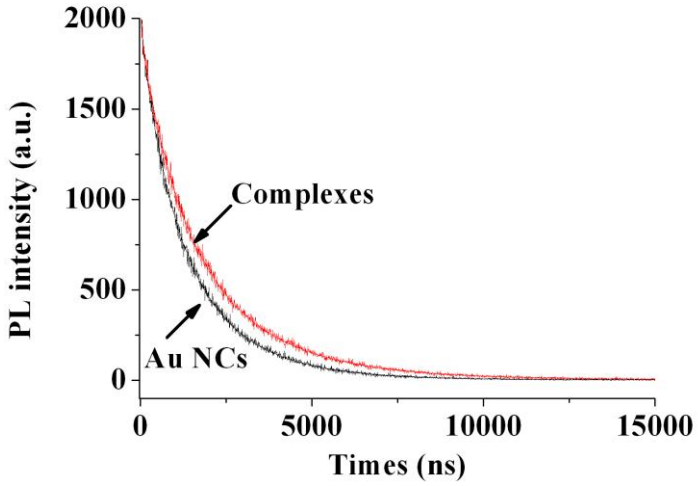

Fig. S1 Time-resolved FL decay curves of gold NCs and hybrid NCs.

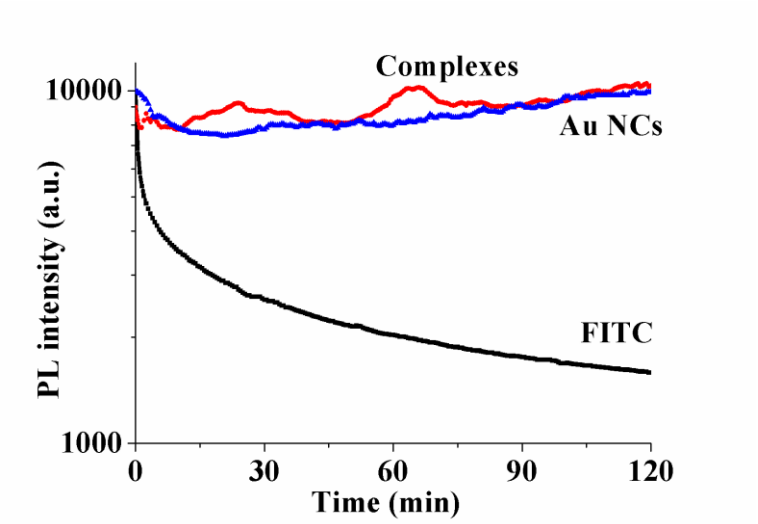

Fig. S2 Photoluminescence stability of gold NCs, hybrid NCs and FITC, respectively.

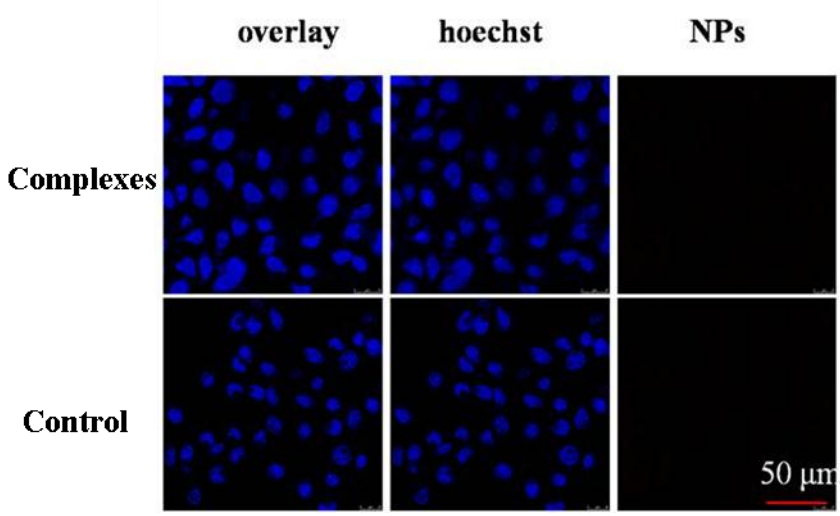

Fig. S3 Confocal microscopy images. Hybrid NCs were used to treat MCF-7 cells. All FL images were obtained under the same conditions and shown at 50 μm of the scale bar.

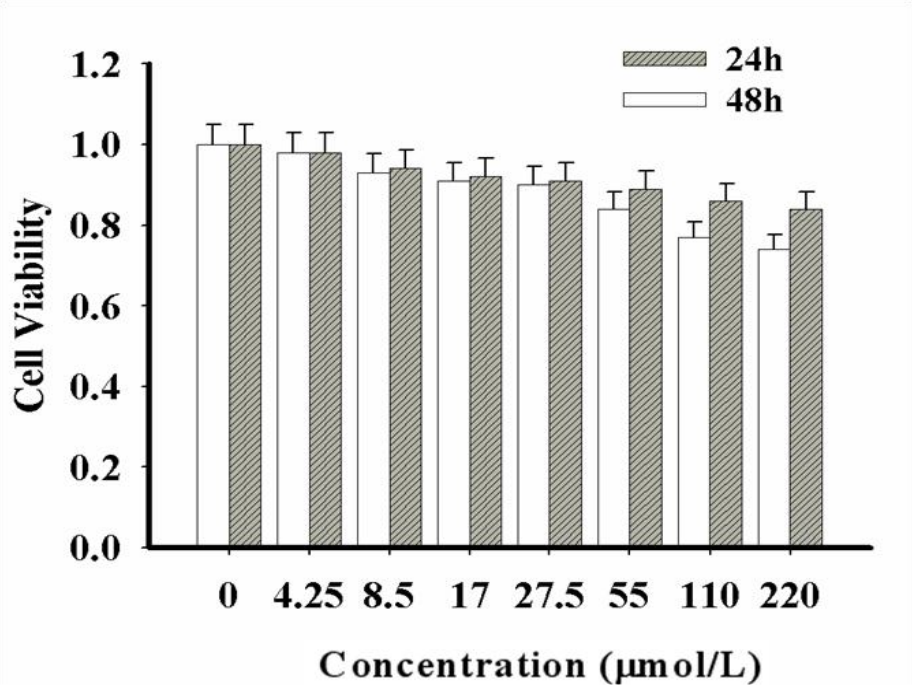

Fig. S4 Cytotoxicity studies of hybrid NCs. MTS assays illustrating cell viability percentage (compared to nontreated cells being arbitrarily assigned 100% viability) upon exposing the cells to different concentrations of hybrid NCs for 24 h and 48 h. Error bars represent the standard deviation of five measurements.

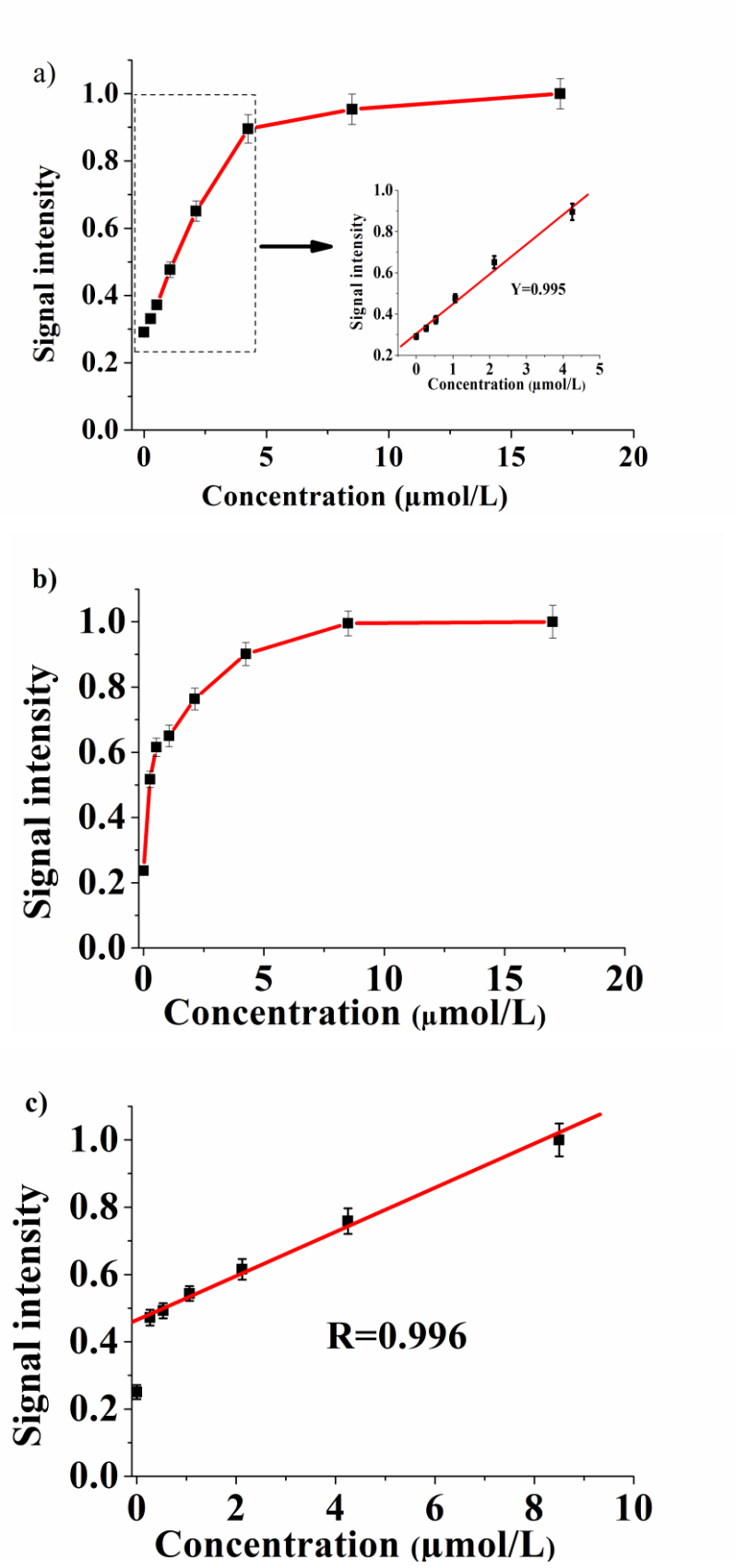

Fig. S5 The NIRF (a), CT (b) and MRI (c) signals intensity vs the concentration of hybrid NCs in PBS. PBS was used as references.

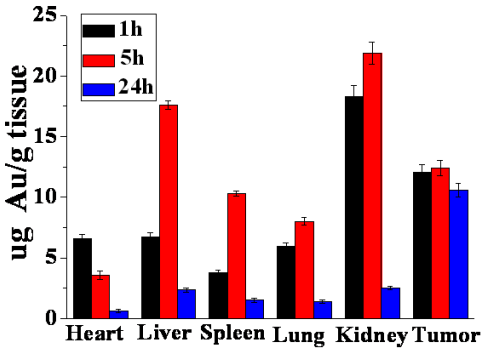

Fig. S6 ICP-AES analysis showing the distribution of Au and specific tissue accumulation in various tissues at 1 h and 5h after injection of hybrid NCs.

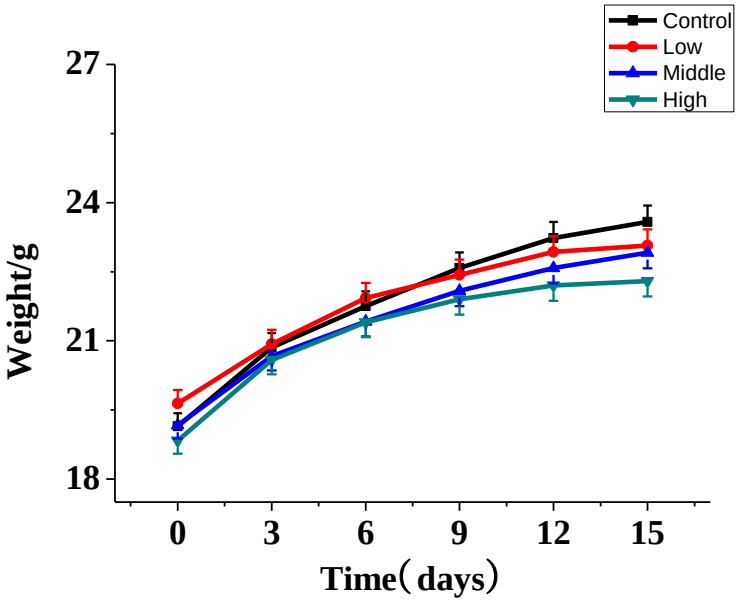

Fig. S7 The effect of treatment with hybrid NCs on the mouse body weight.