Supporting information

Nucleic Acid-Driven Aggregation-Induced Emission of Au Nanoclusters for Visualizing Telomerase Activity in Living Cells and In Vivo

Xiang Ran,[a,b] Zhenzhen Wang,[a] Fang Pu,*[a] Enguo Ju,[a] Jinsong Ren,*[a] Xiaogang Qu*[a]

[a] State Key Laboratory of Rare Earth Resource Utilization and Laboratory of Chemical Biology, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China. E-mail: pufang@ciac.ac.cn, jren@ciac.ac.cn, xqu@ciac.ac.cn

[b] School of Pharmacy, Anhui Medical University, Hefei, 230031, China

Experimental Section

Materials and instruments:

L-glutathione (GSH) was purchased from Aladdin (Shanghai, China). $HAuCl_4 \cdot 3H_2O$ was obtained from Sigma-Aldrich. Dialysis bags (molecular weight cut off = 1000 Da) were obtained from Sangon (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagent Co., LTd. (Shanghai, China). All solutions were prepared with Nanopure water (18.2 MΩ; Millpore Co., USA).

All DNA were purchased from Sangon Biotechnology Inc. (Shanghai, China). The sequences are as follows. Telomerase substrate primer (TS primer): 5'-AAT CCG TCG AGC AGA GTT-3'; Strand A: 5'-AGG GTT AAA GAC ATA GGC ATA CAA AAA AAC CCT AAC TCT GCT CGA CGG ATT-$(CH_2)_6$-SH-3'; Strand B: 5'-TTG TAT GCC TAT GTC T-$(CH_2)_6$-SH-3'; Telomerase Reaction Product (TRP): 5'-AAT CCG TCG AGC AGA GTT AGG GTT AGG GTT AGG GTT AGG G-3'; Strand C: 5'-TAT ATA AAA GAC ATA GGC ATA CAA AAA TAT ATA AAC TCT GCT CGA CGG ATT-$(CH_2)_6$-SH-3'. DNA was prepared in MES buffer (20 mM).

TEM images were recorded by a JEOL 1011 transmission electron microscope operating at 200 kV. Fluorescence spectra were measured by JASCO FP6500 spectrophotometer (JASCO International Co. LTD., Tokyo, Japan).UV-Vis absorption spectra were measured by JASCO V-550 UV/Visible spectrophotometer (JASCO International Co. LTD., Tokyo, Japan).

Synthesis of AuNCs and DNA-functionalized AuNCs:

Freshly prepared $HAuCl_4$ solution (20 mM, 1 mL) and GSH solution (100 mM, 0.3 mL) were mixed with ultrapure water (8.7 mL) at room temperature. The mixture was heated to 70 °C under gentle stirring for 24 h. An aqueous solution of AuNCs was obtained. The solution was re-dissolved in nanopure water followed by dialysis using a dialysis bag (retained molecular weight: 1000 Da). The obtained AuNCs solution could be stored at 4 °C for 2 months with negligible change in their optical properties.

2 mL of AuNCs (1.6 mg/mL) solution was mixed with 10 mL of phosphate buffered saline (PBS, 10 mM, pH = 7.4) buffer. Then, 200 μL of DNA strand A (100 μM) solution was added to the above solution and the mixture was incubated for 12 h under constant stirring at room temperature. The obtained DNA-AuNCs were purified by dialysis in nanopure water to remove the unreacted DNA. Finally, the DNA-AuNCs were redispersed in water. Strand B-functionalized AuNCs were prepared in the same way.

Thermal melting:

Absorbance changes of DNA samples at 260 nm versus temperature were recorded at a heating rate of 1.5 °C·Min^{-1}.

Gel electrophoresis:

The samples were incubated for 40 min at room temperature. The final concentrations in the sample were: DNA (20 μM), Tris (100 mM), Boric acid (100 mM), EDTA (2 mM). The samples were loaded on a 20% polyacrylamide gel electrophoresis and electrophoresed at room temperature at 20 Vcm^{-1} for 1 hour. The gel was stained by 0.2% $AgNO_3$ solution and washed by developer containing 3% Na_2CO_3 and formaldehyde.

Telomerase Extract Preparation:

Cells were collected in the exponential phase of growth and 10^6 cells were dispensed in a 1.5 mL EP tube, washed twice with ice-cold PBS, and resuspended in ice-cold CHAPS lysis buffer (10 mM Tris HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 0.5% CHAPS, 10% glycerol) with RNA secure. The lysate was incubated for 30 min on ice and centrifuged for 20 min at 12,000 rpm, 4 ℃. Then the supernatant was carefully transferred to a fresh 1.5 mL EP tube and frozen at -80 ℃ until use.

Quantitation of DNA strands Loaded on AuNCs:

Mercaptoethanol (ME) was added (final concentration of 12 mM) to DNA-AuNCs in 0.3 M PBS. After 18 h at room temperature under intermittent shaking, the solutions containing displaced DNA strands were separated from AuNCs by centrifugation. The supernatant was diluted 2-fold by adding the same amount of PBS (0.3 M pH 7). The absorbance at 260 nm was converted to molar concentrations of the DNA strand by interpolation from a standard linear calibration curve. Standard curves were prepared with standard DNA strand samples. Finally, the average number of DNA strands of each AuNC was obtained by dividing the measured DNA molar concentration by the original AuNCs concentration.

In vitro telomerase assays:

Telomerase activity was detected with our probes. 200 mL of 0.02 mg/mL probes in PBS were incubated with telomerase extracts at 37 ℃ for 2 h. Then the fluorescence of the samples was measured and recorded. The fluorescence responses of AuNCs to telomerase extracts from different cells were evaluated by calculating the change in the fluorescent intensity of AuNCs

Cell culture:

HeLa, HepG2, MCF-7, A549, and HEK-293T cells were cultured with regular growth medium consisting of high glucose DMEM. QSG7701 cells were cultured in RPMI 1640. The cell growth media were supplemented with 10% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin and cultured at 37 ℃ in a 5% CO_2 humidified

environment.

Cell extracts from liver and kidney of mice:

The tissue were removed with ophthalmic scissors and soaked in PBS buffer. The treated tissue blocks were transferred into a new 10 cm culture dish with DMEM medium, followed by cutting up with forceps and ophthalmic scissors. The samples were centrifuged for 2 min (400 rpm) and washed with PBS twice. Then tissue digestive fluid (type I collagenase + hyaluronidase) was added into the samples and incubated in a constant temperature shaker overnight. After that, the digested samples were centrifuged for 5 min (1200 rpm) and washed with fresh DMEM. The cells were counted and diluted to 10000 cells / ml for use.

In vitro cytotoxicity assays:

Methyl thiazolyl tetrazolium (MTT) assays were used to determine cellular viability. HeLa cells were seeded in 96-well plates at a density of 5000 cells/well. Cells were further incubated with strand A/TSP-AuNCs and strand B-AuNCs at the indicated concentrations for 24 h. 10 μL of MTT solution (BBI) was added to each well of the plate and the plate was incubated in the CO_2 incubator for an additional 4 h. Then the cells were lysed by the addition of 100 μL of DMSO. Absorbance values of formazan were determined with a Bio-Rad model-680 microplate reader at 490 nm. The results were expressed as the mean values of three measurements.

Imaging of telomerase activity in living cells:

HeLa cells were seeded into a 20-mm confocal dish and incubated for 24 h at 37 ℃. Then, the cells were incubated with strand A/TSP-AuNCs and strand B-AuNCs (10 μg/mL in culture medium). The fluorescent images at different time periods were recorded. After the 2 h of incubation, the cells were stained with DAPI for another 20 minutes to indicate the nucleus.

Animal tumor model:

Animal Ethics Committee of Anhui Medical University has approved the animal experiments in this study (No.LLSC20200716). All the animal experiments were in accordance with the animal use and ethical policy of Changchun Institute of Applied

Chemistry and Anhui Medical University. Animal experiments were in agreement with the guidelines of the Animal Ethics Committee of Anhui Medical University for Animal Experiments. HeLa tumor-bearing nude mice were used as animal model. The mice were divided into four groups. For group 1, 10^6 HeLa cells (in 100 μL of PBS, pH 7.4) were subcutaneously injected to establish tumor model. When the volume of tumor grew to about 300 mm^3, intravenous injection of 100 μL of the probe was carried out. The tumor-bearing mice in group 2 and group 3 were intravenously injected with 100 μL of the negative control probe using strand C instead of strand A and PBS, respectively. The healthy mice in group 4 were used as a control group and intravenously injected with 100 μL of the probe. Fluorescence imaging of mice was conducted after 6 h. The whole body photoluminescence images were recorded using KODAK In-vivo Imaging System.
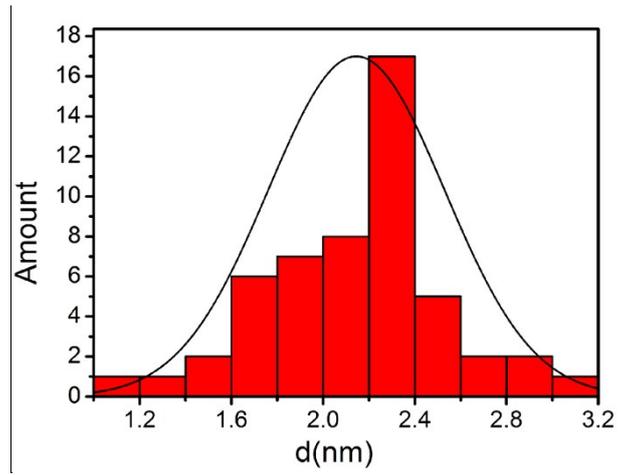
Figure S1 Particle size statistics of the AuNCs before assembling.
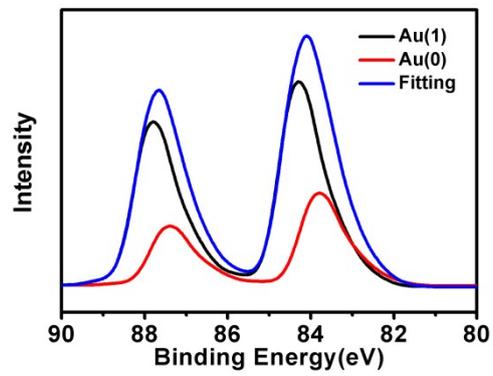
Figure S2 XPS spectra of AuNCs.
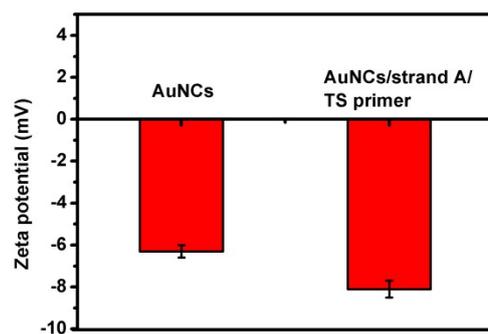
Figure S3 Zeta potentials of AuNCs and AuNCs/strand A/TS primer.
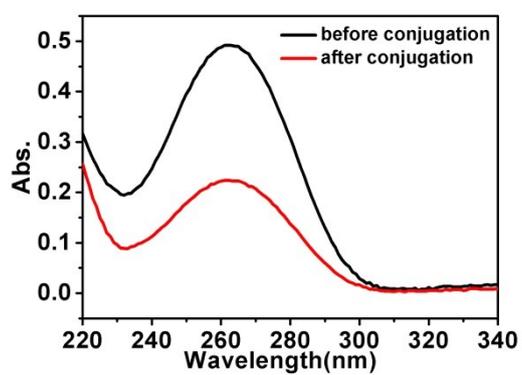
Figure S4 Absorbance spectra of strand A in the supernatant before and after being conjugated to the surface of AuNCs.
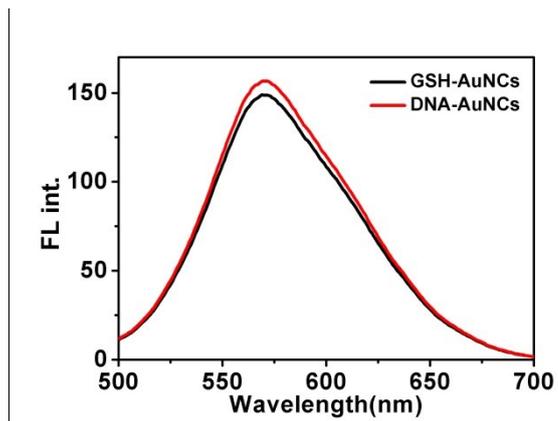
Figure S5 Fluorescence spectra of AuNCs before (GSH-AuNCs) and after (DNA-AuNCs) conjugation of sulfhydryl group-functionalized DNA.
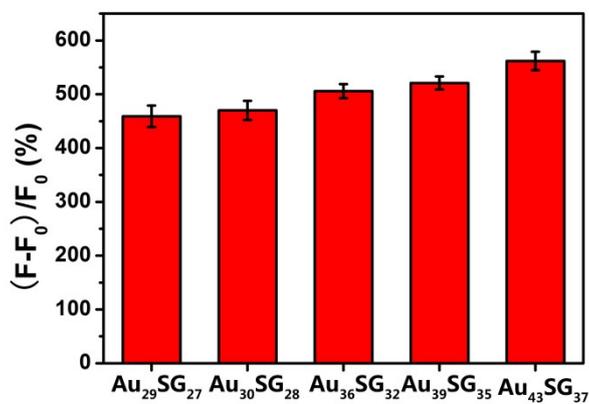
Figure S6 Increase rate of fluorescent intensity of five individual AuNCs.
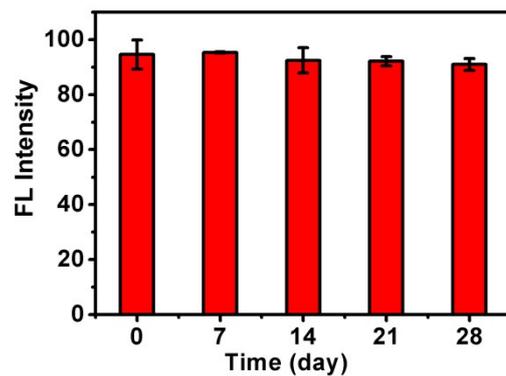
Figure S7 Photostability of AuNCs in aqueous solution.
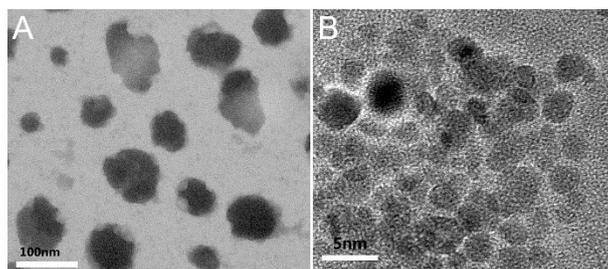
Figure S8 (A) TEM image of the assembled AuNCs. (B) The amplified TEM image of the assembled AuNCs.
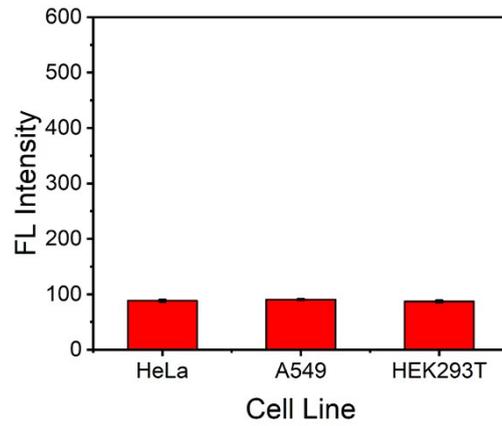
Figure S9 Fluorescence intensity of the probe after telomerase reaction with heated telomerase from different cell lines.
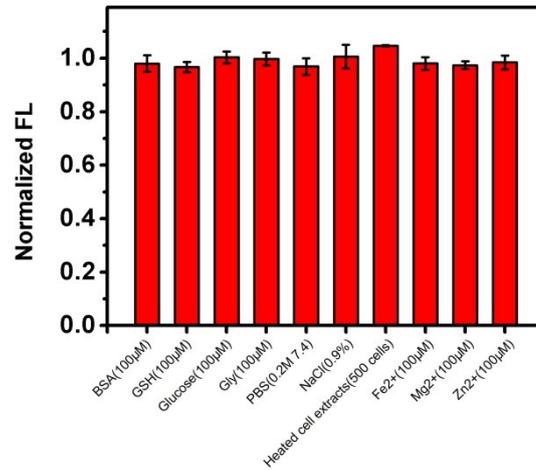
Figure S10 The fluorescence responses of some interference molecules to the nanoprobe.
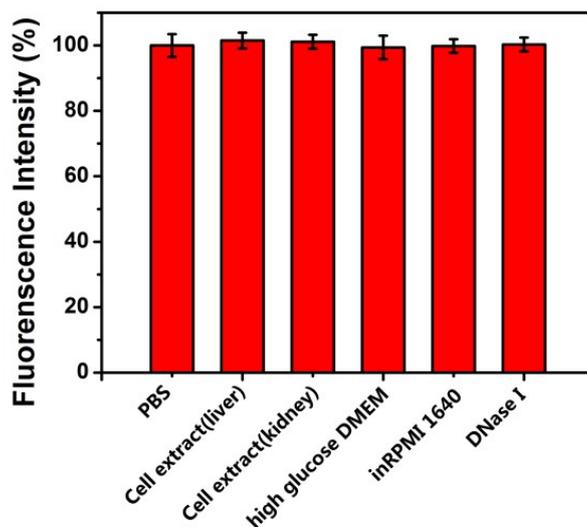
Figure S11 The fluorescence intensities of the nanoprobe in different environments, including cell extracts from liver (100 cells), cell extracts from kidney (100 cells), high glucose DMEM, RPMI 1640 and DNase I (1U).
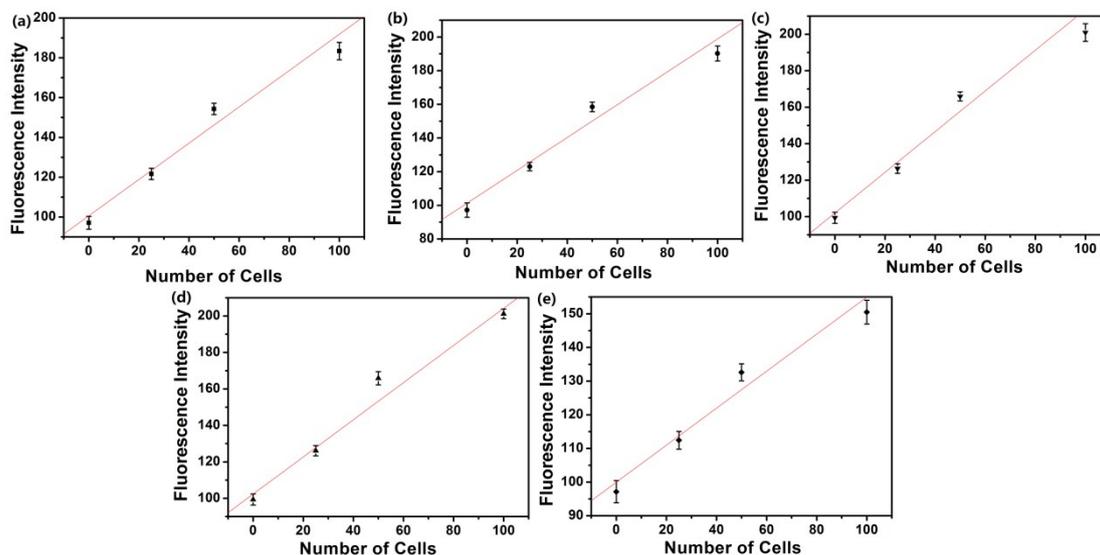
Figure S12 The relationship between fluorescence enhancement and numbers of HeLa cells in different environments. (a) Cell extracts from liver, (b) Cell extracts from kidney, (c) high glucose DMEM, (d) RPMI 1640, and (e) DNase I.
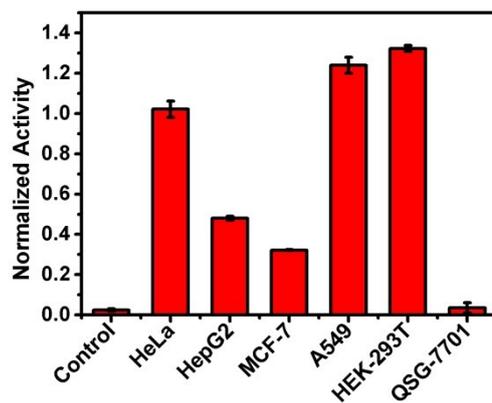
Figure S13 Analysis of telomerase activities in different cell lines using PCR-TRAP kit.
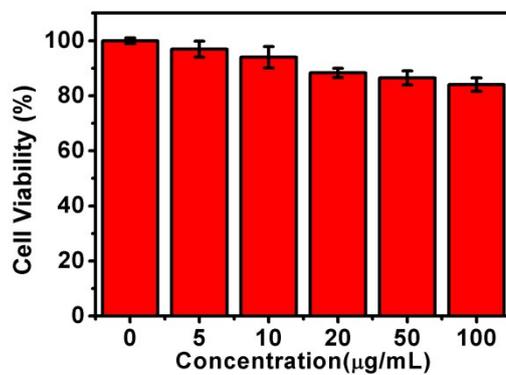
Figure S14 Cytotoxicity of the AuNC-based probe measured by MTT assay.
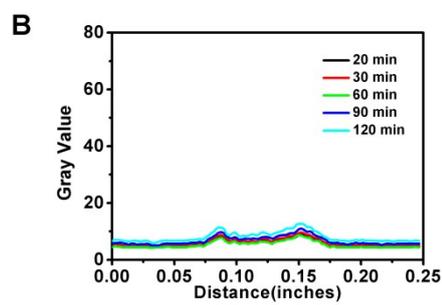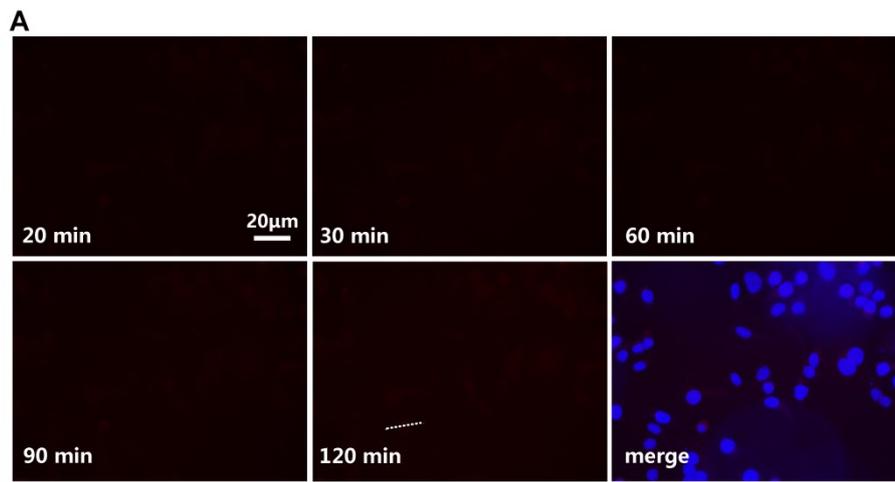
Figure S15 (A) Fluorescence images of HeLa cells after being treated with the negative control probe using strand C instead of strand A at different time points. The nuclei were stained with DAPI and presented blue emission. (B) Linear profile of the fluorescence images at the red channel at different time points. All images share the first scale bar.
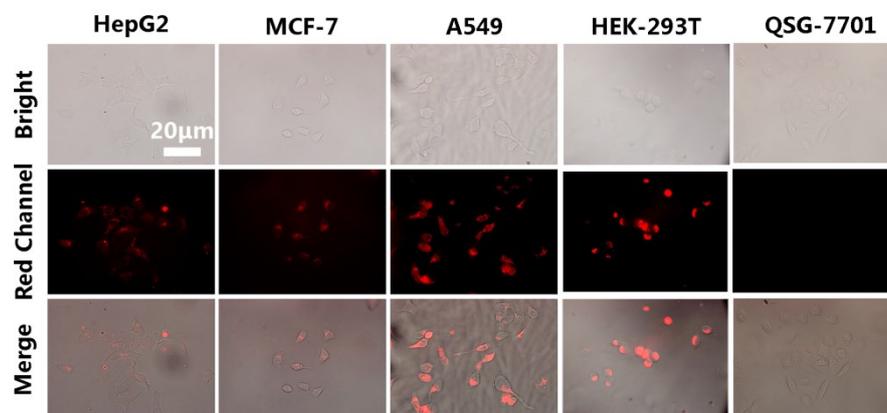
Figure S16 Fluorescence images of different cells after being treated with the AuNC-based probe. All images share the first scale bar.
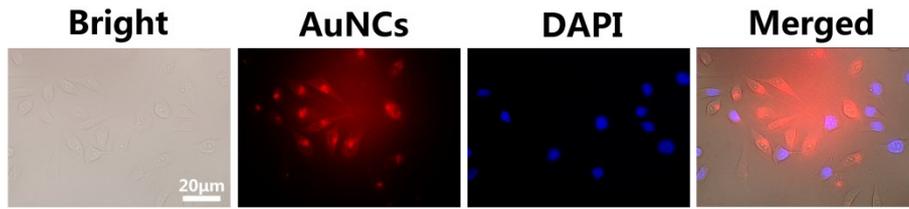
Figure S17 Fluorescence images of the mixture of HeLa cells (without nucleus labeled) and QSG-7701 cells (with nucleus labeled) after being treated with the AuNC-based probe. All images share the first scale bar.
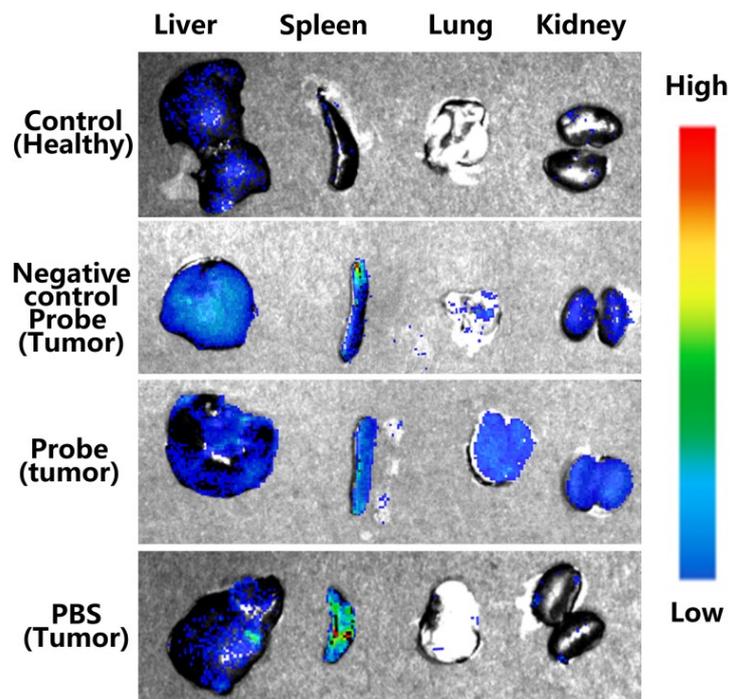
Figure S18 In vivo fluorescence imaging of the liver, spleen, lung and kidney of the treated mice.