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Electronic Supplementary information for

Targeted Cancer Imaging and Photothermal Therapy via Monosaccharide-Imprinted Gold Nanorods

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Experimental

Reagents and materials. 3-Aminopropyltriethoxysilane (APTES, 98%), dimethyl sulfoxide (DMSO, 99%), 2,4-difluoro-3-formyl-phenylboronic acid (DFFPBA), tetraethylorthosilicate (TEOS, 99%), cetyltrimethylammonium bromide (CTAB, 99%), trypan blue dye (0.4%), sodium borohydride ($NaBH_4$ 99%), chloroauric acid ($HAuCl_4 \cdot 3H_2O$ 99.9%), L-ascorbic acid (AA, 98%), silver nitric ($AgNO_3$, 99%), fluorescein isothiocyanate isomer I (FITC) and NIR-797 isothiocyanate were purchased from Sigma Aldrich (St. Louis, MO, USA). Sialic acid (SA, 98%), D-fucose (99%) and D-fructose (99%) were purchased from Aladdin Industrial Corporation (Shanghai, China). D-glucose (99%) was purchased from J&K Chemical (Shanghai, China). D-mannose (99%) was purchased from Alfar Aesar (Tianjin, China). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltertrazolium bromide (MTT) was purchased from BDH Laboratory Supplies (Poole, Dorset, UK). Ammonium hydroxide (28% w/v) was purchased from Sinopharm Chemical Reagent (Shanghai, China). Ten male SPF mice (4-6 weeks, 20-25 g), hepatoma carcinoma cell (HepG-2), normal hepatocyte cell (L-02), phosphate buffer solution for cell culture (1× PBS), parenzyme cell digestion solution (containing 0.25% trypase and 0.02% EDTA) and Dulbecco Modified Eagle Medium (DMEM, containing 4.5 mg/mL glucose, 80 U/mL penicillin and 0.08 mg/mL streptomycin) were purchased from Keygen Biotech (Nanjing, China). Fetal bovine serum (FBS) was purchased from Gibco (Life Technologies, Australia). All other chemical reagents were of analytical grade unless otherwise stated. Water used in all experiments was purified by a Milli-Q Advantage A10 ultrapure water purification system (Millipore, Milford, MA, USA).

Instruments. Transmission electron microscopy (TEM) was performed on a JEOL JEM-1011 TEM instrument (Tokyo, Japan). UV-vis absorption and fluorescence properties characterization and evaluation of boronic acid functionalization and molecular imprinting were performed on a Synergy Mx microplate reader from BioTek

(Winooski, VT, USA). Cell imaging was performed on a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems CMS GmbH, Mannheim, Germany) or an IX71epifluorescence microscope (Olympus, Japan). The *in vivo* distribution of the gold nanorods (AuNRs) in mice was investigated on a Maestro fluorescence imaging system (CRi, USA) (excitation wavelength 797 nm). Photothermal therapy was carried out using a NIR laser of 750 nm (Kaisite Electronic Equipment, Beijing, China). The temperature was monitored by a thermometer (Benetech, Shenzhen, China).

Synthesis of Gold Nanorods. The AuNRs were prepared according to the seed-mediated growth method optimized by El-Sayed and co-workers[1]. First, the gold seed solution was prepared via the chemical reduction of $HAuCl_4$ with $NaBH_4$. Briefly, CTAB solution (5 mL, 0.2 M) was mixed with 5 mL of 0.5 mM $HAuCl_4$. To the stirred solution, 0.6 mL of ice-cold 10 mM $NaBH_4$ was added. The color of the solution changed from dark yellow to brownish yellow under stirring. After stirring, the solution was kept at 30 ℃ for 2 h. Second, AuNRs were prepared from the gold seeds within the growth solution. CTAB solution (50 mL, 0.2 M) was mixed with 1140 μL of 10 mM $AgNO_3$ and 50 mL of 1 mM $HAuCl_4$. After gentle mixing, 550 μL of 100 mM AA was injected quickly under stirring and the solution became colorless immediately. Then, the gold seed solution (120 μL) was added under stirring for 2 min to initiate the growth of AuNRs (6 h). Finally, excess CTAB was removed by centrifuging the samples twice at 11,000 rpm for 15 min, and the AuNRs were re-dispersed in 25 mL pure water.

Synthesis of FITC-doped AuNRs@SiO_2. FITC was incorporated in the silica coating on the surface of AuNRs according to the method reported by Ma and co-workers[17] with some minor modifications. The amino groups of APTES can easily react with the isothiocyanate moieties of FITC molecules to yield thiourea groups. FITC of 10 mg was covalently linked to APTES of 44 μL in 3.52 mL ethanol under dark condition for 24 h. The prepared FITC-APTES stock solution was kept at 4 ℃. A volume of 50 mL of

AuNRs was transferred into 100 mL conical glass flask and the pH was adjusted to ca. 10 by the addition of ammonia. Subsequently, a solution of TEOS/FITC-APTES/ethanol (0.45 mL, 1/0.05/4 v/v/v) was added into the mixture over a period of 3 h at intervals of 30 min under gentle stirring. The resulting solution was further allowed to react for 12 h at 30 ℃. Finally, the FITC-doped AuNRs@SiO_2 was collected by centrifugation (12,000 rpm, 15 min) and re-dispersed in 25 mL ethanol.

Synthesis of SA-imprinted FITC-doped AuNRs@SiO_2. The SA-imprinted FITC-doped AuNRs@SiO_2 was synthesized according to a previous method by our group[2]. First, 30 μL of APTES was injected to 25 mL of anhydrous ethanol solution containing freshly prepared FITC-doped AuNRs@SiO_2, and the mixture was stirred at room temperature for 1 h. After washing, the amino-modified FITC-doped AuNRs@SiO_2 was dispersed in 30 mL ethanol and 300 μL of 5 mg/mL FPBA and 300 μL of 5 mg/mL sodium cyanoborohydride were added. After reaction for 24 h, the solution was centrifuged and the boronic acid-modified FITC-doped AuNRs@SiO_2 was collected, and then washed with ethanol and water for three times each. After that, the boronic acid-modified FITC-doped AuNRs@SiO_2 was dispersed in 9 mL of water. For oriented imprinting, 1 mL of 3 mg/mL SA dissolved in phosphate buffer (100 mM, pH 7.4) was added into 9 mL of boronic acid-modified FITC-doped AuNRs@SiO_2. After incubation for 30 min, SA-bound AuNRs@SiO_2 was collected via centrifuging and re-dispersed into 40 mL ethanol, then added with 0.7 mL ammonium water and a prepolymer solution containing 22.4 μL TEOS and 10 mL ethanol. After reaction for 20 min, the reacting mixture was centrifuged and the precipitates were collected. Finally, the collected precipitates were washed with 0.1 M HAc for 3 h to remove the template. The obtained SA-imprinted FITC-doped AuNRs were collected and stored in water.

To prepare non-imprinted FITC-doped AuNRs@SiO_2 for comparison, the processing procedure was the same except that no template was immobilized onto the boronic

acid-modified FITC-doped AuNRs@SiO_2.

Boronate affinity sandwich assay. In order to evaluate the selectivity of the SA-imprinted FITC-doped AuNRs@SiO_2, the boronate affinity sandwich assay[3] was used. Briefly, a boronic acid-modified 96-well microplate was used as a substrate, and a monosaccharide such as glucose and sialic acid was used as bridge molecule to bind with the substrate and the imprinted or non-imprinted FITC-doped AuNRs@SiO_2. After excessive AuNRs@SiO_2 was removed through washing with 10 mM phosphate buffer (pH 7.4), the fluorescence signal of the formed microplate-monosaccharide -AuNRs@SiO_2 sandwiches were detected by the Synergy Mx microplate reader.

The boronic acid-modified 96-well microplate was prepared according to the method by our group[4], a 150-μL solution (12 mM $HAuCl_4$, 0.5 M $KHCO_3$, and 25 mM glucose) was added, and kept at 30 ℃ (air bath) for 4-5 h. After washing, the gold layer was modified with 4-mercaptophenylboronic acid (MPBA). Briefly, 150 μL of 5 mM MPBA solution in anhydrous ethanol was added to the 96-well microplate and kept open at room temperature for 3-4 h until the solution almost evaporated. Then the microplate was washed with water and anhydrous ethanol 2-3 times and dried by air at room temperature.

The monosaccharide solution (5 mg/mL, dissolved in 0.1 M phosphate buffer, pH 7.4) was added to the boronic acid-modified 96-well microplate and incubated for 30 min. The 96-well microplate was washed with phosphate buffer (10 mM, pH 7.4) for three times. Then, SA-imprinted or non-imprinted FITC-doped AuNRs@SiO_2 was added into the 96-well microplate and incubated for 30 min. The 96-well microplate was washed with phosphate buffer (10 mM, pH 7.4) three times. Finally, the fluorescence signal of the 96-well microplate was detected by the Synergy Mx microplate reader.

***In vitro* cytotoxicity of SA-imprinted FITC-doped AuNRs@SiO_2.** Cell viability was determined by the MTT assay. Briefly, HepG-2 and L-02 cells were respectively seeded on 96-well microplates with a density around 5000 cells per well and allowed to adhere for 24 h prior to the assay. The cells were incubated with the different concentrations of SA-imprinted FITC-doped AuNRs@SiO_2 at 37 °C for 24 h. Then 50 μL of MTT indicator dye (5 mg/mL in PBS, pH 7.4) and the cells were incubated for another 4 h at 37 °C in the dark. The medium was withdrawn and 200 μL DMSO was added in each well and agitated thoroughly to dissolve the formazan crystals. The solution was transferred to 96-well microplates and immediately monitored on a microplate reader. Absorption was measured at a wavelength of 570 nm. The values obtained were expressed as a percentage of the control cells to which no nanoparticle was added, and the cell viability was calculated by the following formula:

$$\text{Cell viability}(\%) = \frac{\text{Abs}(\text{test cell}) - \text{Abs}(\text{backgroud})}{\text{Abs}(\text{controlled cell}) - \text{Abs}(\text{backgroud})} \times 100\%$$

Cell culture and imaging. HepG-2 and L-02 cells were cultured in DMEM medium with 10% fetal bovine serum for 2 to 3 days (37 ℃, 5% CO_2). The cell culture medium was removed and the cells remained on the cell culture dishes were washed with 1× PBS twice. Then the cells were respectively incubated with 1 mL boronic acid-functionalized, SA-imprinted and non-imprinted FITC-doped AuNRs@SiO_2 dissolved in 1× PBS for 20 min. The PBS buffer and free AuNRs@SiO_2 were removed and the remaining cells were rinsed with 1 × PBS for three times and supplemented with 1 mL 1× PBS. The obtained cells were imaged under the confocal laser-scanning microscope.

Flow Cytometry. For flow cytometry assay, the HepG-2 and L-02 cells were stained with SA-imprinted FITC-doped AuNRs@SiO_2 and then digested with parenzyme cell digestion solution (containing 0.25% tryptase and 0.02% EDTA) for 2-3 min. The obtained cells were centrifuged at 1,000 rpm for 3 min. After removing the supernatant,

the cells were washed with 1× PBS for twice and filtrated with 200 mesh sieves. The obtained cell suspensions were injected into cytoanalyzer and the count of cells was set to 20,000.

NIR photothermal treatment of cancer cells. To determine the photothermal heating effect of SA-imprinted FITC-doped AuNRs@SiO_2, solutions (100 μL) of 0.1 mg/mL SA-imprinted FITC-doped AuNRs@SiO_2 were irradiated using a 750-nm NIR laser beam (power density, 1 W/cm^2) for different times, respectively. Aqueous solutions (100 μL) containing 0.1 mg/mL AuNRs and AuNR@SiO_2 respectively, together with water of the same volume, were used as controls, and the solution temperature was monitored by a thermometer.

For photothermal cell therapy, HepG-2 and L-02 cells were precultured for 24 h, followed by the addition of SA-imprinted FITC-doped AuNRs@SiO_2 with a final concentration of 0.1 mg/mL. After incubation for 2 h at 37 ℃, the cells were washed with PBS to remove excess SA-imprinted FITC-doped AuNRs@SiO_2. Then a 750-nm NIR laser beam was used to irradiate cells at a power density of 1 W/cm^2 for different durations (2 min, 4 min, 6 min and 8 min). After staining with 0.4% trypan blue solution for 5 min, microscopic images of the cells were taken on an epifluorescence microscope (Olympus IX71, 40×, 0.65 N.A. objectives) and the cell viability was determined by MTT assay as described above.

NIR797 fluorescence doping and *in vivo* biodistribution of the mice. SA-imprinted NIR797-doped AuNRs@SiO_2 was prepared in the similar way as described above. Briefly, the isothiocyanate moieties of NIR797 dye were allowed to react with the amino groups of APTES, and then the solution of TEOS/NIR797-APTES/ethanol replace the reagent of TEOS/FITC-APTES/ethanol for silica coating. Other procedure was the same as the above labeling process.

All the animal experiments were approved by the Animal Care and Use Committee of Nanjing University. Mice bearing a HepG-2 tumor were kept for 3 days with free access to food and water. After that, the mice were intravenously injected with SA-imprinted NIR797-doped AuNRs@SiO_2 at a dosage of 50 mg/kg and sacrificed at 96 h post injection. The distribution of SA-imprinted NIR797-doped AuNRs@SiO_2 in mice was imaged using the Maestro *in vivo* fluorescence imaging system. The mice were anesthetized with isoflurane and the scans were carried out at desired time after the injection.

***In vivo* photothermal therapy.** Mice bearing a HepG-2 tumor were injected with SA-imprinted AuNRs@SiO_2 (50 mg/kg) in the tail vein (0.1 mL per mouse). At desired time after the injection, the mice were anesthetized with isoflurane and then the entire region of the tumor was irradiated with 750 nm laser at 1 W/cm^2 for 6 min. The laser device has a 200 μm diameter fiber with center wavelength at 750 nm, and the beam diameter was expanded to 10 mm (~0.785 cm^2) by optical lens which can cover the entire tumor area. After laser irradiation, a caliper was applied to measure the volume of the tumor, the tumor volume V was calculated based on the formula, $V=Dd^2/2$, where D and d refer to the length and width and the relative volume (V/V_0) was introduced to describe the change of the tumor size.

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4 X. D. Bi and Z. Liu, *Anal. Chem.*, 2014, **86**, 12382-12389.

Supplementary data

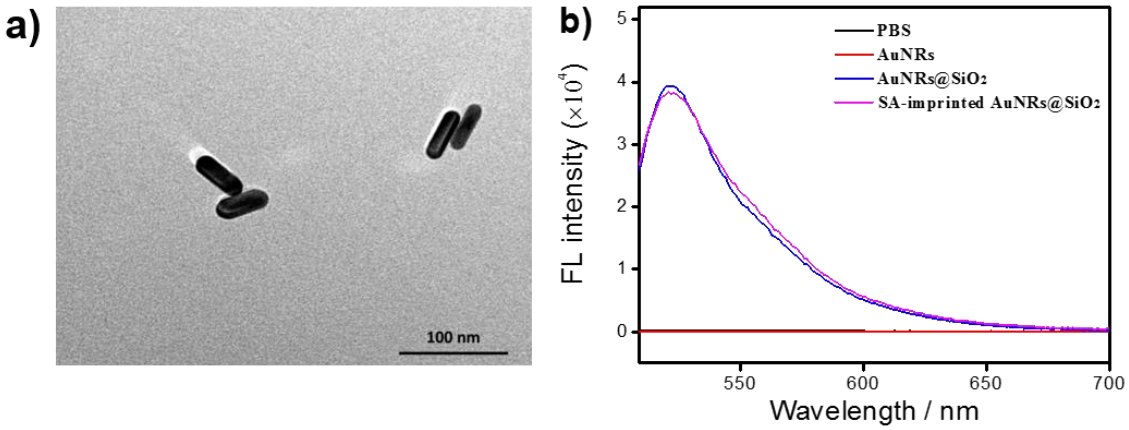

Fig. S1 a) The TEM images of bare AuNRs. b) Fluorescence spectra of FITC-doped AuNRs@SiO_2 and SA-imprinted FITC-doped AuNRs@SiO_2, the PBS and AuNRs were chosen as control. The concentration of AuNRs@SiO_2 was 0.1 mg/mL.

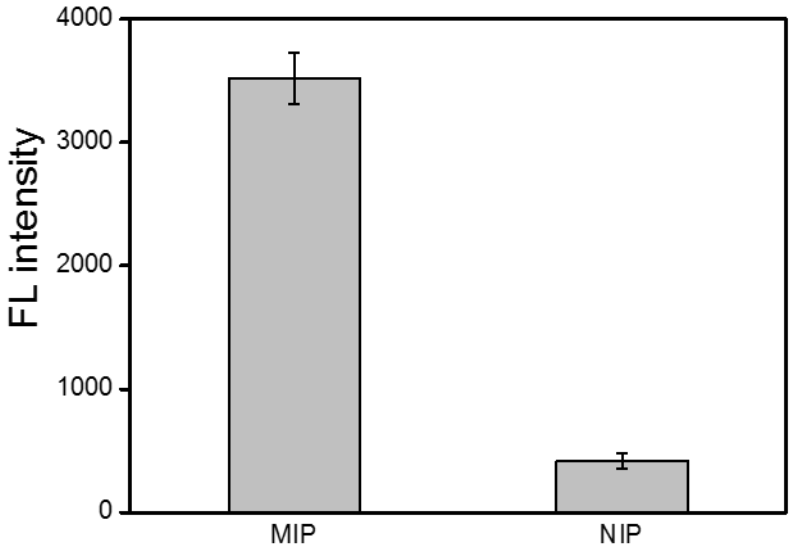

Fig. S2 Affinity of SA-imprinted FITC-doped AuNRs@SiO_2 and non-imprinted FITC-doped AuNRs@SiO_2 to SA. The concentration of AuNRs@SiO_2 was 0.1 mg/mL.

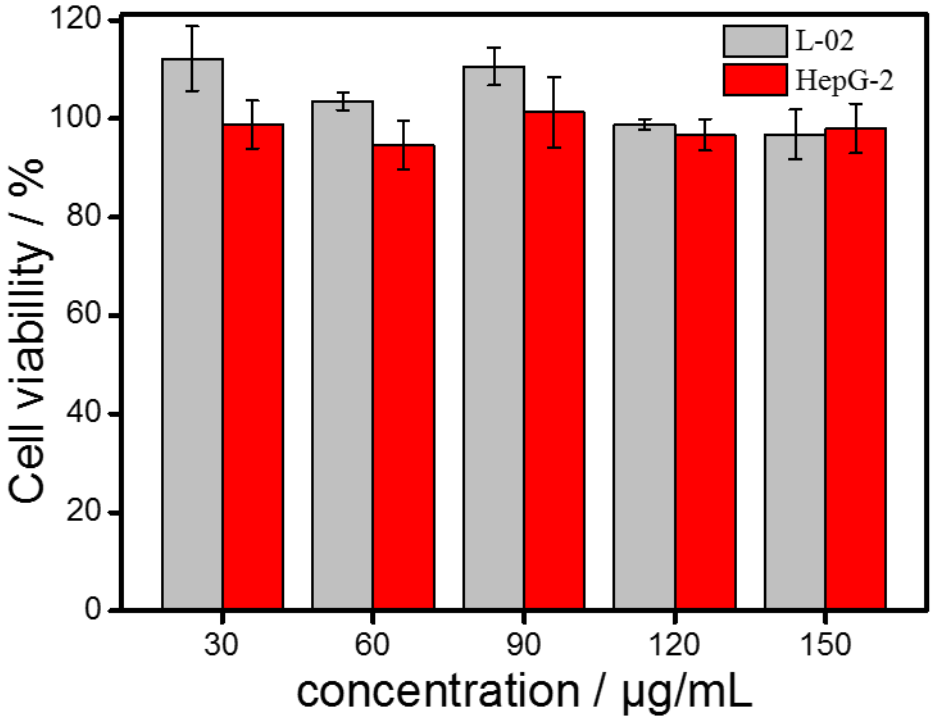

Fig. S3 *In vitro* cytotoxicity of SA-imprinted AuNRs@SiO_2 toward HepG-2 and L-02 cells.

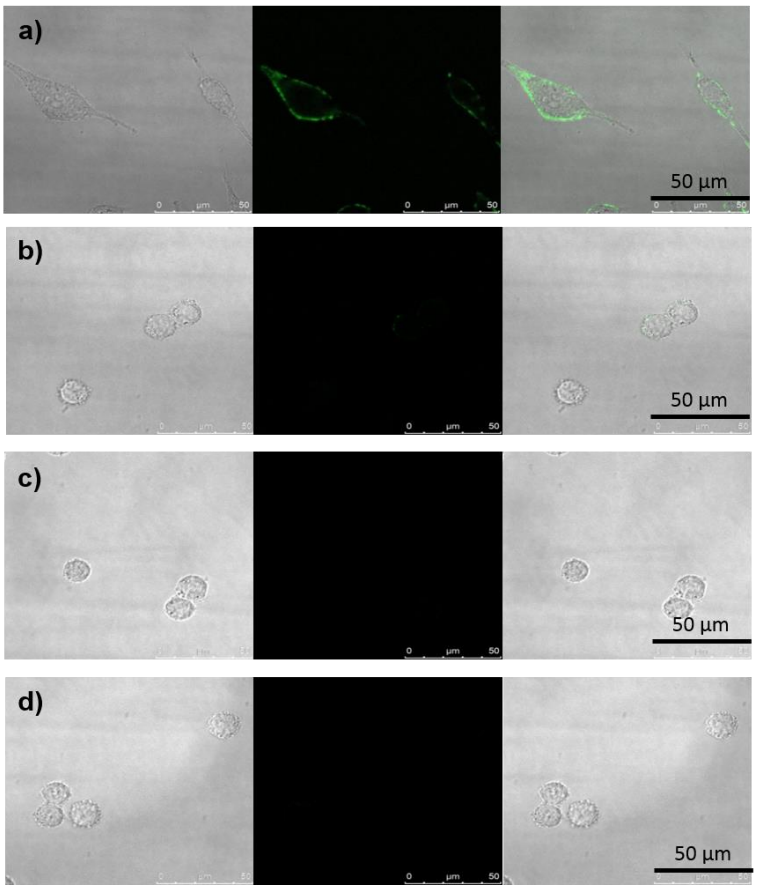

Fig. S4 Confocal fluorescence images of a) HepG-2 and b) L-02 cells after incubation with SA-imprinted FITC-doped AuNRs@SiO_2; c) HepG-2 and d) L-02 cells after incubation with non-imprinted FITC-doped AuNRs@SiO_2. Columns from left to right: bright field, dark field, and fluorescence image. The concentration of AuNRs@SiO_2 was 0.1 mg/mL.

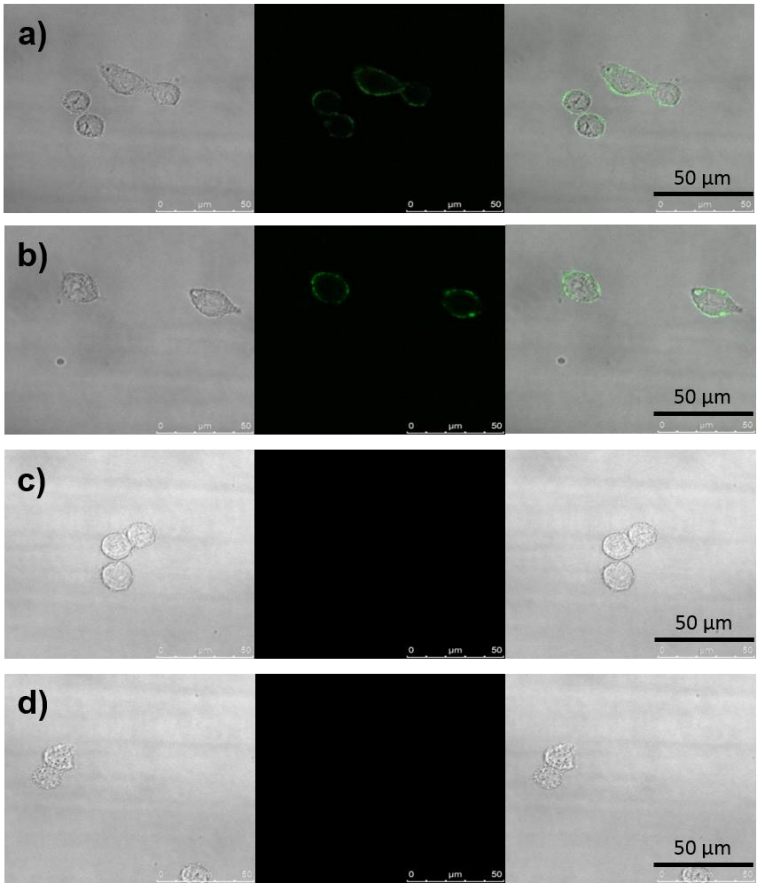

Fig. S5 Confocal fluorescence images of a) HepG-2 and b) L-02 cells after incubation with boronic acid-modified FITC-doped AuNRs@SiO_2; c) HepG-2 and d) L-02 cells after incubation with FITC-doped AuNRs@SiO_2. Columns from left to right: bright field, dark field, and fluorescence image. The concentration of AuNRs@SiO_2 was 0.1 mg/mL.

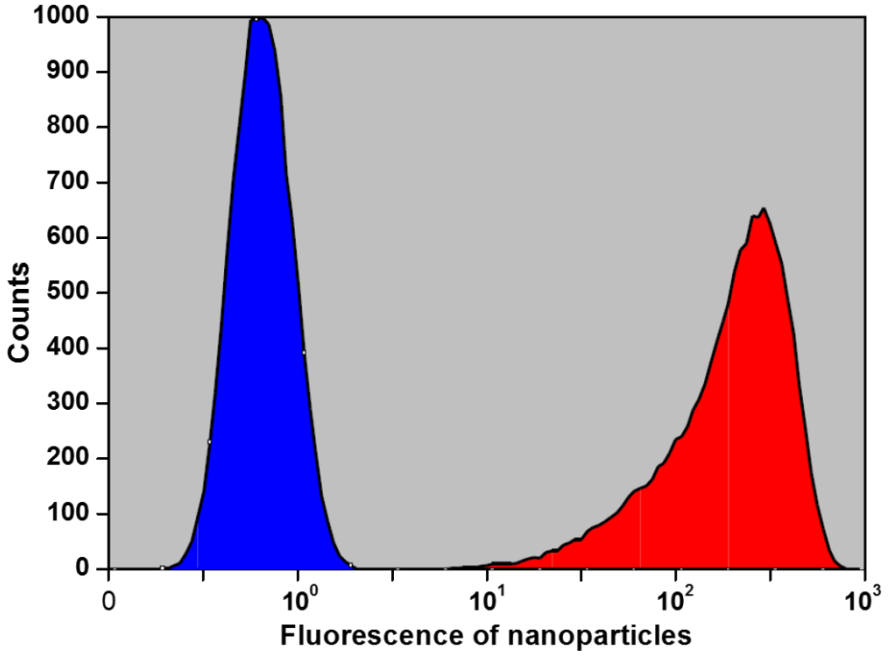

Fig. S6 FCM characterization of L-02 cells (Blue) and HepG-2 cells (Red) after staining with SA-imprinted FITC-doped AuNRs@SiO_2 (0.01 mg/mL).

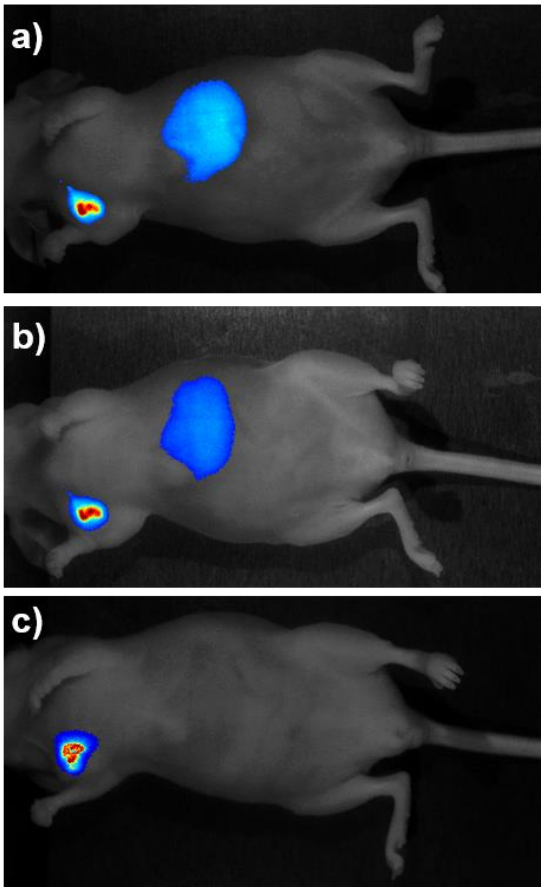

Fig. S7 The NIR fluorescence images of a tumor-bearing mouse at a) 48 h, b) 72 h and c) 96 h after intravenously injection of SA-imprinted NIR797-doped AuNRs@SiO_2. The concentration of AuNRs@SiO_2 was 0.1 mg/mL.

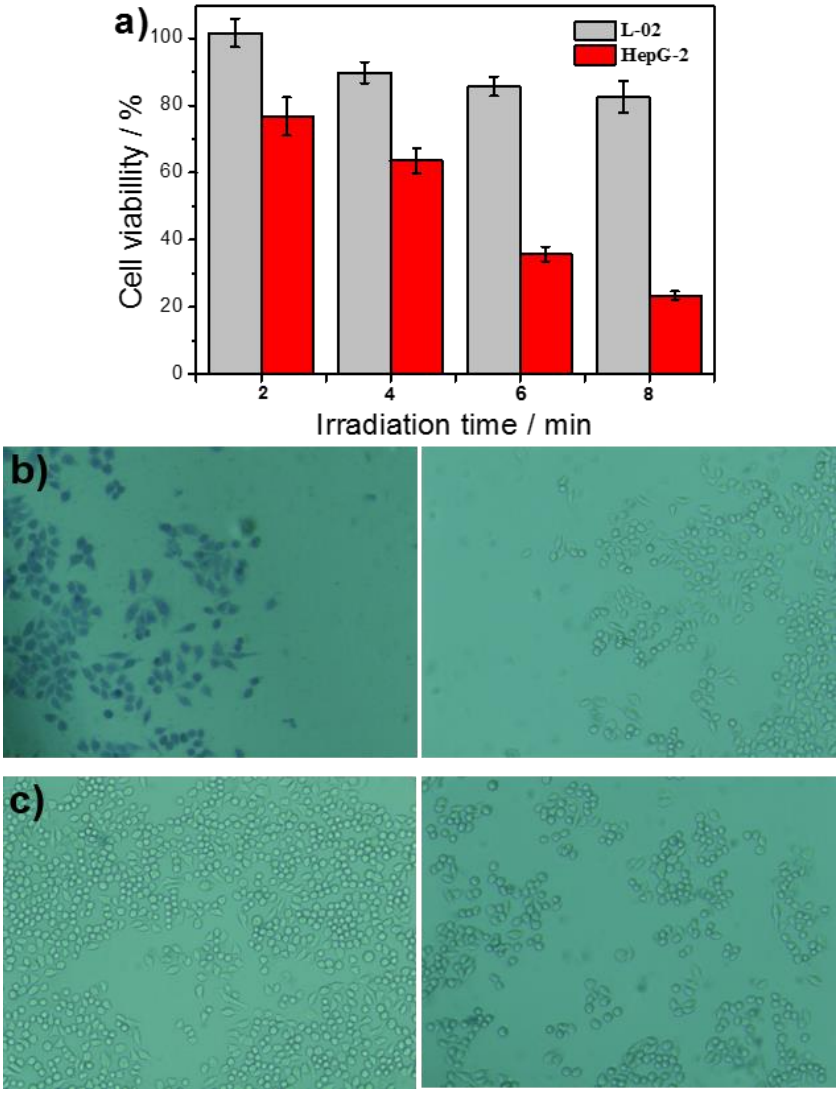

Fig. S8 NIR photothermal effect of SA-imprinted AuNRs@SiO_2. a) Relative cell viability after treatment with a 750-nm laser for different irradiation times; Bright field microscopic images of trypan blue-stained b) HepG-2 cells and c) L-02 cells after incubation with (left) or without (right) SA-imprinted AuNRs@SiO_2 and then 750-nm laser irradiation for 6 min. The concentration of AuNRs@SiO_2 was 0.1 mg/mL.