Electronic Supplementary Information (ESI)

Gold nanoparticle cluster/plasmon-enhanced fluorescent silica core-shell nanoparticles for X-ray computed tomography/fluorescence dual-mode imaging of tumors

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Experimental Section

Ethics statement

The study protocol was approved by the Animal Use Committee of the University of Tokushima (Tokushima, Japan).

Materials

Tetrakis(4-carboxyphenyl)porphyrin (TCPP), tetraethoxysilane (TEOS), and 3-aminopropyltriethoxysilane (APTES) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Ammonia water (28%) was obtained from Kishida Chemical (Osaka, Japan). $HAuCl_4$, trisodium citrate, $NaBH_4$ and 80-nm gold NPs were purchased from Sigma–Aldrich (St Louis, MO, USA).

Synthesis of 7-nm gold NPs

Gold NPs were synthesized using the method reported by Gole et al.[22] Typically, a 20 mL aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ and 2.5×10^{-4} M trisodium citrate was prepared; 0.6 mL of ice-cold 0.1 M $NaBH_4$ was added to the solution, and the resulting mixture stirred at room temperature for 24 h. Gold NPs were

collected by centrifugation (20,000×g) of this dispersion, and re-dispersion in water. This sequence was repeated thrice.

Preparation of porphyrin-containing silicon alkoxide

The preparation and structural analyses of porphyrin-containing silicon alkoxide was undertaken as described previously.[21]

Synthesis of c-Au@SiO_2 NPs

Porphyrin-containing silicon alkoxide (2.5×10^{-3} M), TEOS (8.0×10^{-3} M), and APTES (7.5×10^{-3} M) were added to the solution containing 7-nm gold NPs (8.3×10^{-4} M). A total of 6 mL of ammonia water (28%) was added to this solution, which was then stirred at room temperature for 24 h. c-Au@SiO_2 NPs were collected by centrifugation of this dispersion, and re-dispersion in water. This sequence was repeated thrice.

Synthesis of s-Au@SiO_2 NPs

Porphyrin-containing silicon alkoxide (2.5×10^{-3} M), TEOS (8.0×10^{-3} M), and APTES (7.5×10^{-3} M) were added to a solution containing 80-nm gold NPs (8.3×10^{-4}

M). A total of 6 mL of ammonia water (28%) was added to this solution, which was then stirred at room temperature for 24 h. c-Au@SiO_2 NPs were collected by centrifugation of this dispersion, and re-dispersion in water. This sequence was repeated thrice.

Animals

Female CB17/Icr-*Prkdc*^{scid} mice (4 weeks of age) were purchased from Charles River Laboratories (Yokohama, Japan) and maintained in a specific pathogen-free facility in our Animal Resources Center. To eradicate residual natural killer cells, mice were injected (i.p.) with 10 μL of rabbit anti-asialo GM1 antiserum (Wako Pure Chemicals, Osaka, Japan) 1 day before tumor inoculation.[23] Inoculation with human myeloma cells (5×10^6 cells) was accomplished *via* subcutaneous injection into the backs of the mice. PEGylated c-Au@SiO_2 NPs were injected into mice with tumors of diameter 100 mm^3. Tumor volumes were calculated from the formula

$V = AB^2\pi/6$,

where A is the longer and B is the shorter lateral diameter of the tumor.

Characterization

The morphology and size of 7-nm gold NPs, s-Au@SiO_2 NPs, and c-Au@SiO_2 NPs were studied using transmission electron microscopy (TEM) with a Hitachi H-760 machine (Hitachi, Tokyo, Japan). The hydrodynamic diameter and zeta potential of 7-nm gold NPs, c-Au@SiO_2 NPs and PEGylated c-Au@SiO_2 NPs were measured in PBS, using dynamic light scattering (DLS, NICOMP 380 ZLS, Showa Denko, Tokyo, Japan). The absorption bands of c-Au@SiO_2 NPs, s-Au@SiO_2 NPs, TCPP, 80-nm gold NPs and 7-nm gold NPs were measured using an ultraviolet–visible (UV-vis) spectrophotometer (U-3000, Hitachi, Tokyo, Japan). Fluorescence properties were characterized using a fluorescence spectrophotometer (F-2500, Hitachi, Tokyo, Japan). The quantum yield of c-Au@SiO_2 NPs were estimated from the fluorescence spectra of TCPP and c-Au@SiO_2 NPs based on the quantum yield of TCPP (0.044). (Chen et al. *Eur. J. Inorg. Chem.* **2009**, 5494–5505) Porphyrin contents in c-Au@SiO_2 NPs and s-Au@SiO_2 NPs were measured by thermogravimetry (TG, Rigaku, TG 8120, Tokyo, Japan).

CT

CT images of c-Au@SiO_2 NPs and Iopamiron in water, as well as mice injected intravenously with PEGylated c-Au@SiO_2 NPs (15 mg/kg), were obtained using the Aloka Latheta LCT-200 system (Hitachi, Tokyo, Japan) (n = 5 per cohort).

The imaging parameters were as follows: pixel size, 96; thickness, 192 μm; pitch, 192 μm. CT images were obtained using OsiriX (ver3.8.1; 32 bit; OsiriX foundation, Geneva, Switzerland). CT values were estimated using the region of interest (ROI) tool.

In vivo fluorescence imaging

Fluorescence images of the mice injected intravenously with PEGylated c-Au@SiO_2 NPs (300 μg/mouse) were obtained at λ_{ex} = 675 nm and λ_{em} = 740 nm, using the IVIS® 200 Imaging System (Caliper Life Sciences, Hopkinton, MA, USA) (n = 5 per cohort). The fluorescence intensities were estimated using the ROI tool.

Histological Analyses

Tumors were extracted from mice 24 h after intravenous injection of PEGylated c-Au@SiO_2 NPs. Tumor tissue samples obtained for histology were immediately immersed in 4% paraformaldehyde solution for 24 h. Fixed tissues were encased in paraffin blocks. Tissue sections (2 μm) were cut from paraffin blocks and affixed to Superfrost Microscopy Slides. Hematoxylin and eosin (H&E) staining of tissue sections was carried out in a conventional manner. The bright-field images of H&E stained sections and the fluorescence images of unstained sections were obtained

using Nikon E800 microscope (λ_{ex} = 408 nm, λ_{em} = 480/25 nm and 605/75 nm, Nikon, Tokyo, Japan).

Toxicity evaluation

PBS and PEGylated c-Au@SiO_2 NPs (300 μg/mouse) were injected intravenously into mice (n = 5 per cohort). One month after injection, blood samples were collected, and serum obtained by centrifugation of whole blood at 3,000 rpm for 15 min. Liver function was evaluated based on the serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Nephrotoxicity was determined by blood urea nitrogen (BUN). These biochemical parameters were determined using an automated biochemical analyzer (7180, Hitachi).

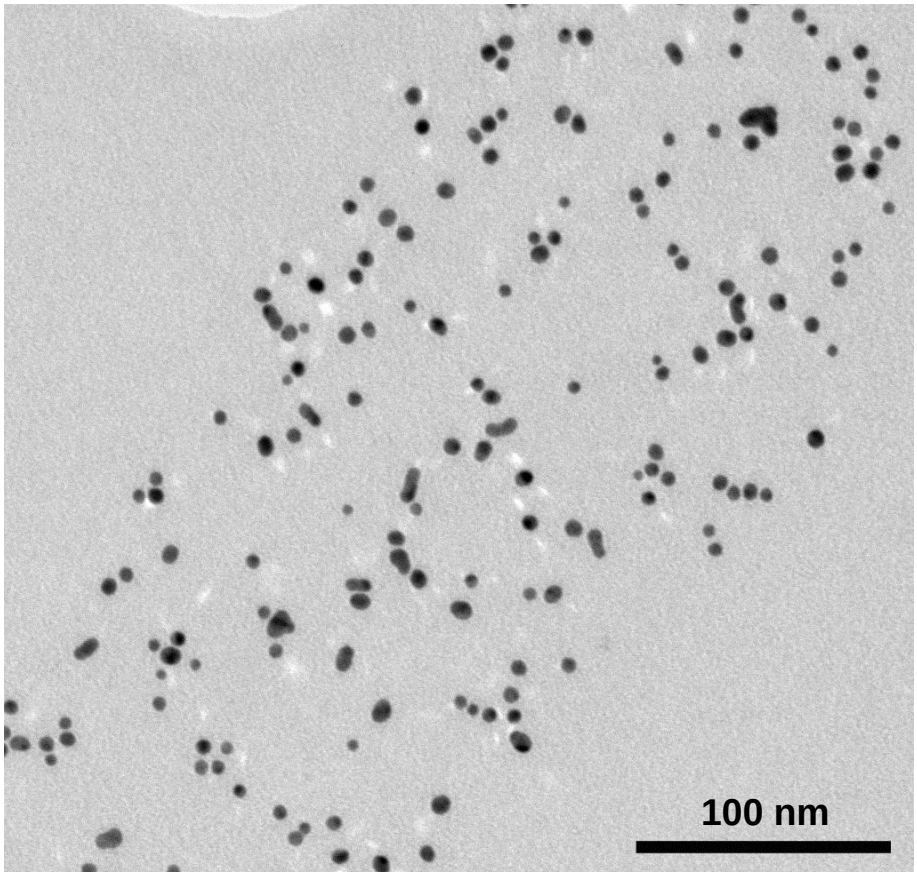

Fig. S1 TEM image of 7 nm-gold NPs.

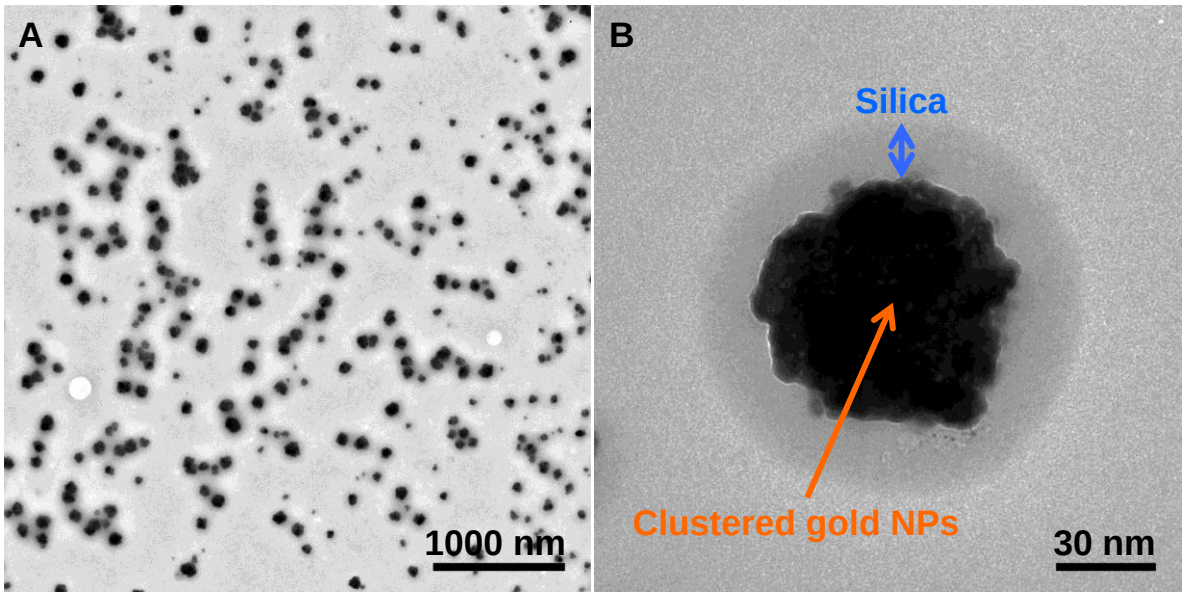

Fig. S2 (A) TEM image of c-Au@SiO_2 NPs. (B) Close-up image of Fig. S2A.

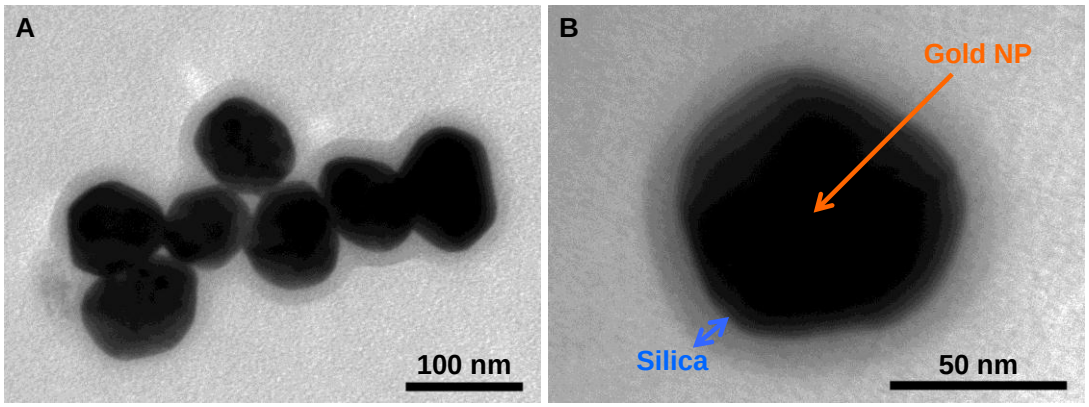

Fig. S3 (A) TEM image of s-Au@SiO_2 NPs. (B) Close-up image of Figure S3A.

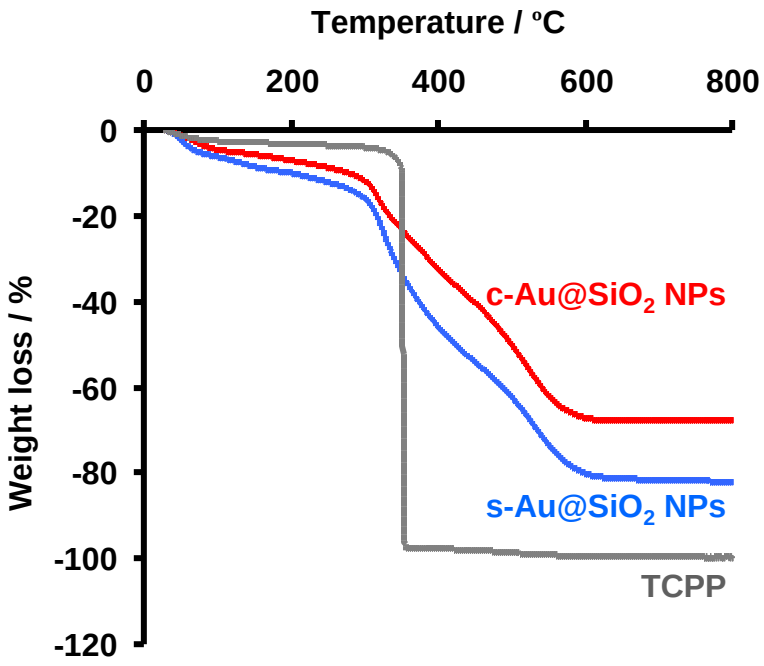

Fig. S4 TG curves of TCPP, s-Au@SiO_2 NPs and c-Au@SiO_2 NPs.

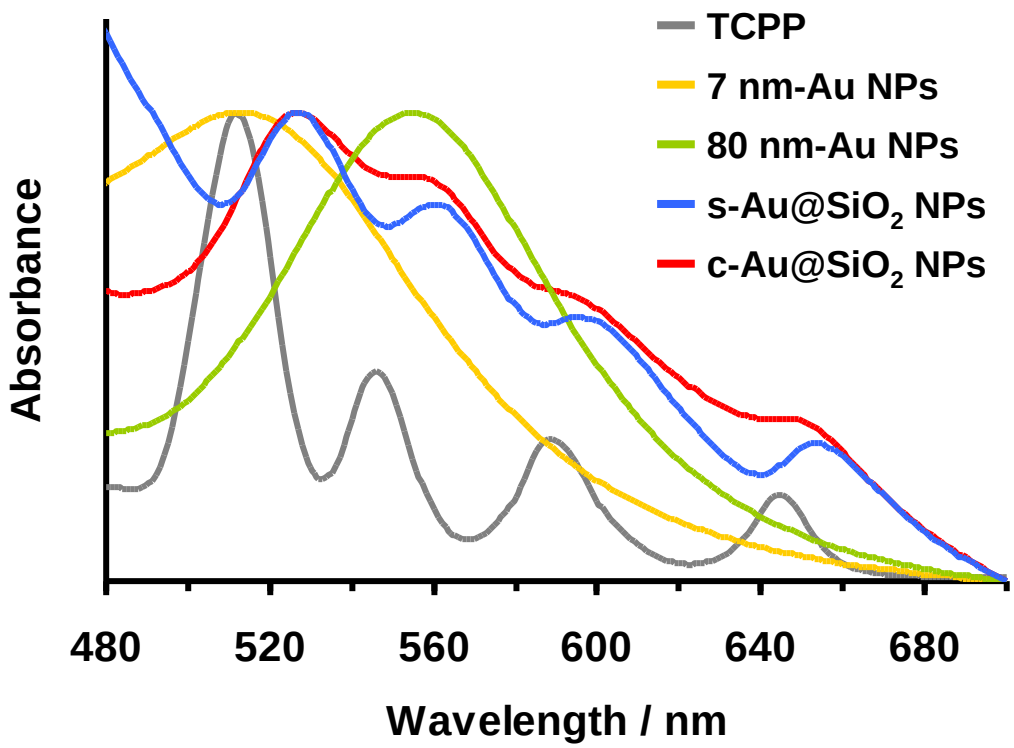

Fig. S5 Absorption spectra of 7 nm-gold NPs, 80 nm-gold NPs, TCPP, s-Au@SiO_2 NPs and c-Au@SiO_2 NPs.

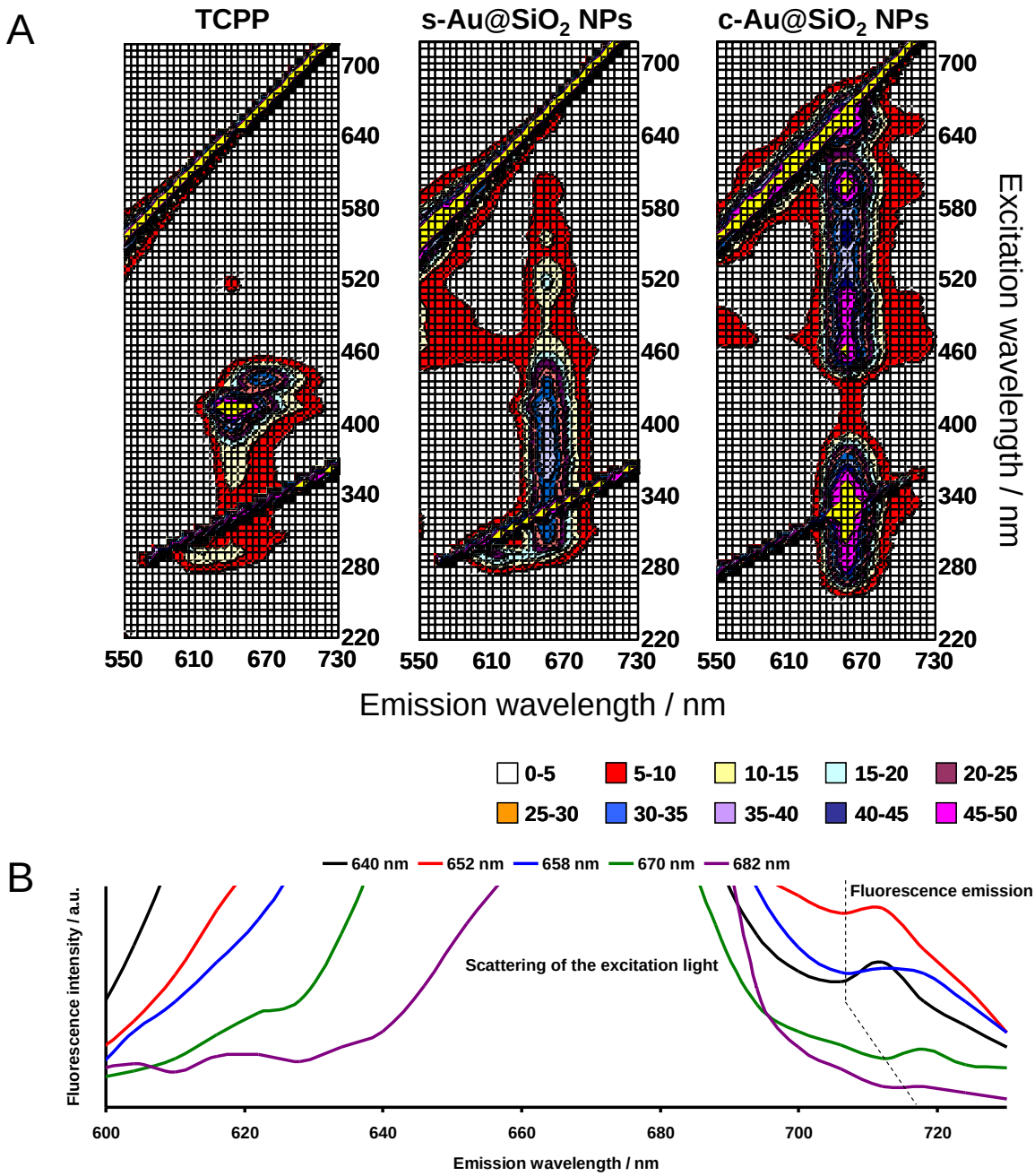

Fig. S6 Fluorescence properties of TCPP, s-Au@SiO_2 NPs and c-Au@SiO_2 NPs. (A) 3D fluorescence spectra of TCPP, s-Au@SiO_2 NPs and c-Au@SiO_2 NPs. (B) The fluorescence spectra of c-Au@SiO_2 NPs at λ_{ex} of 640, 652, 658, 670 and 682 nm.

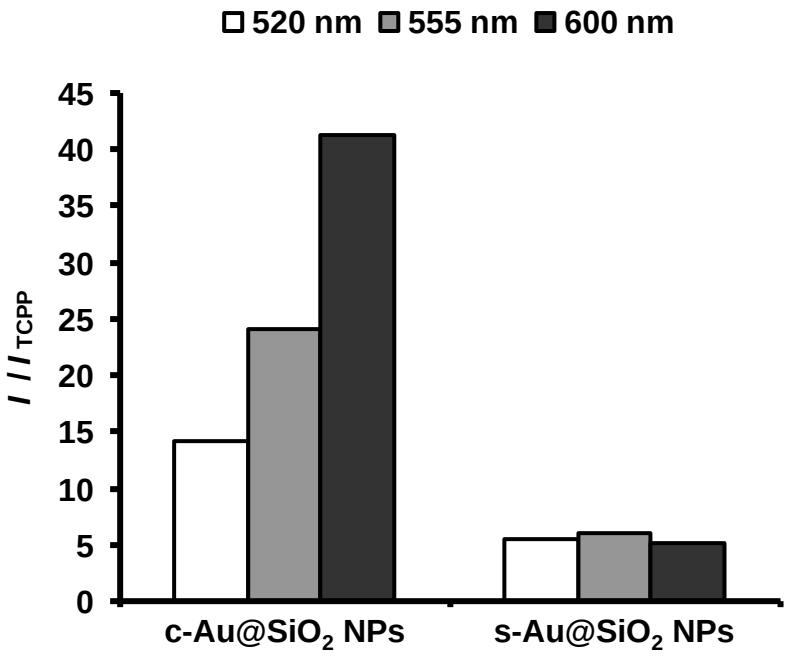

Fig. S7 I/I_{TCPP} of c-Au@SiO_2 NPs and s-Au@SiO_2 NPs at λ_{em} of 660 nm and λ_{ex} of 520, 555 and 600 nm

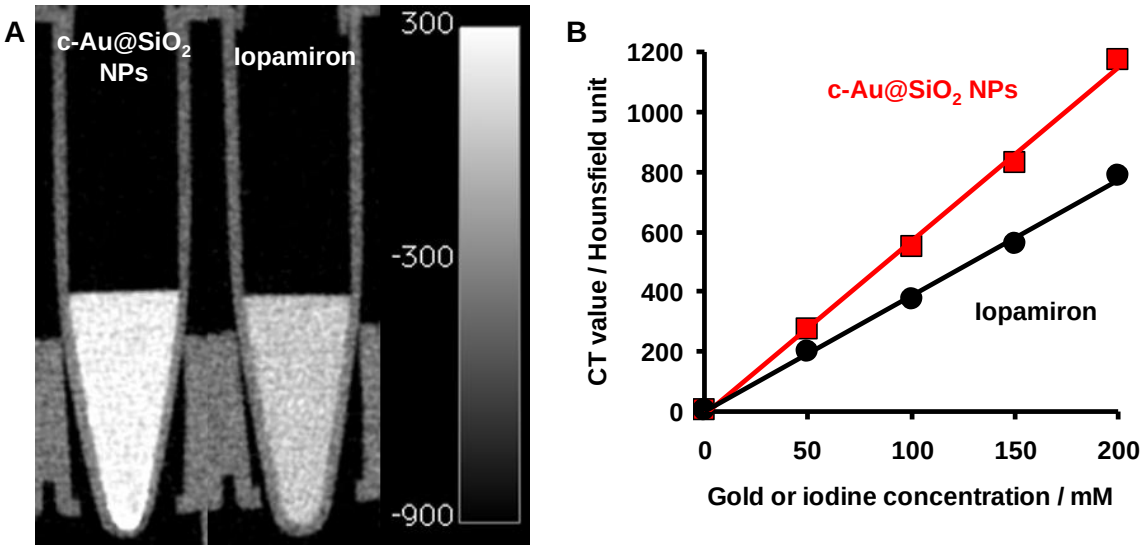

Fig. S8 (A) CT image of c-Au@SiO_2 NPs and Iopamiron in water (50 mM). (B) CT values of c-Au@SiO_2 NPs and Iopamiron at 0−200 mM.

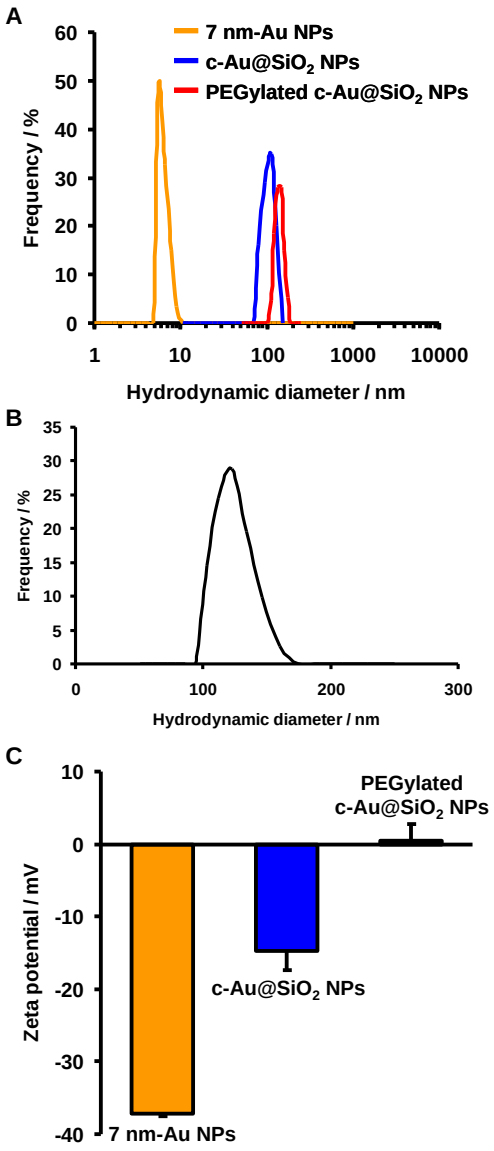

Fig. S9 (A) DLS curves for 7 nm-gold NPs, c-Au@SiO_2 NPs and PEGylated c-Au@SiO_2 NPs. (B) DLS curves for PEGylated c-Au@SiO_2 NPs a month after synthesis. (C) zeta potential for 7 nm-gold NPs, c-Au@SiO_2 NPs and PEGylated c-Au@SiO_2 NPs.

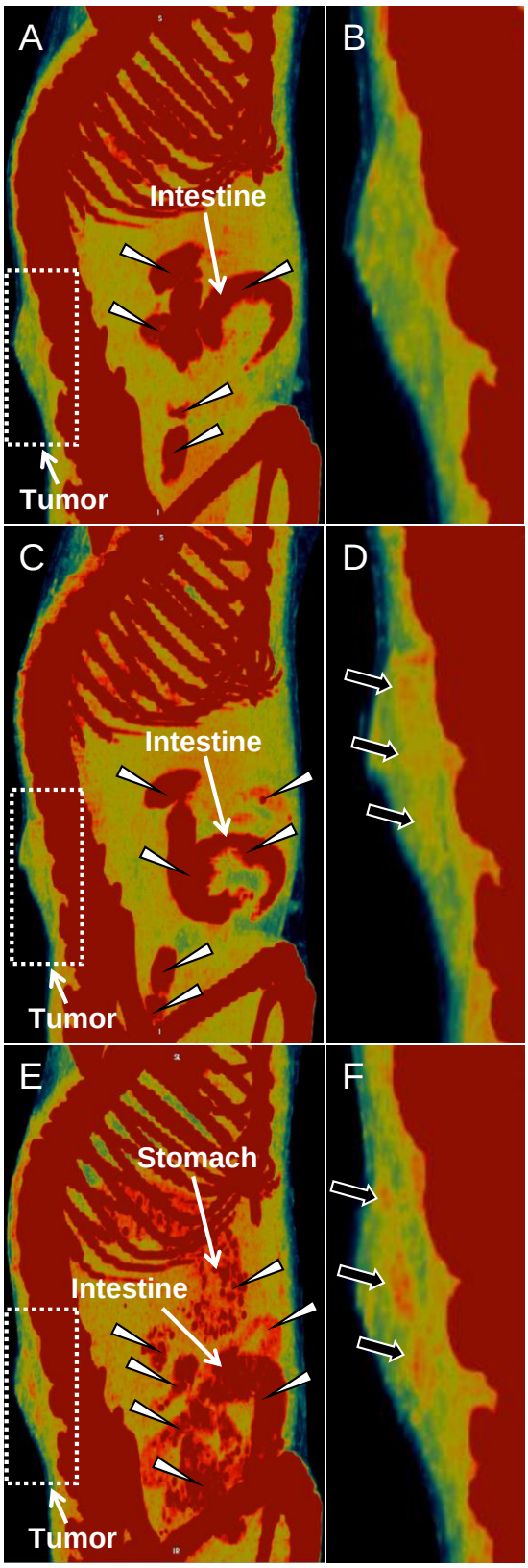

Fig. S10 CT images of tumor-bearing mouse injected intravenously with PEGylated c-Au@SiO_2 NPs: (A) pre-injection, (B) close-up image of the tumor shown in Fig. S10A, (C) 3 h after injection, (D) close-up image of the tumor shown in Fig. S10C, (E) 24 h after injection, (F) close-up image of the tumor shown in Fig. S10E. White triangles indicate food matter that absorbed X-rays.

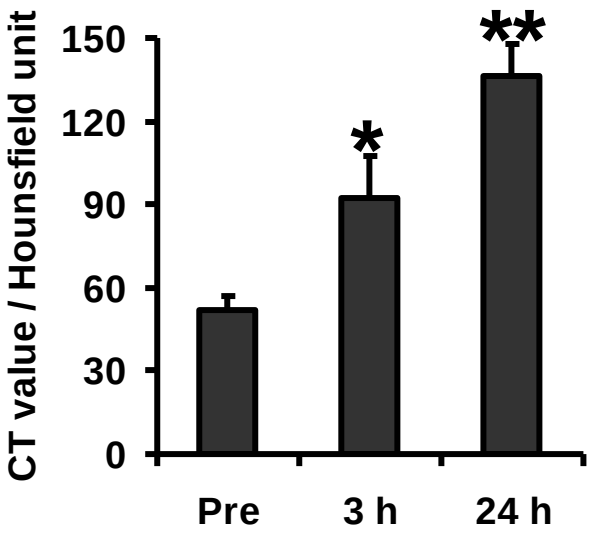

Fig. S11 CT values in the tumor pre-injection, at 3 h post-injection, and at 24 h post-injection. Statistical significance (p) was calculated using a comparison between the values for pre-injection, and 3 h or 24 h post-injection: $^{*}p<5\times10^{-8}$; $^{**}p< 3\times10^{-12}$.

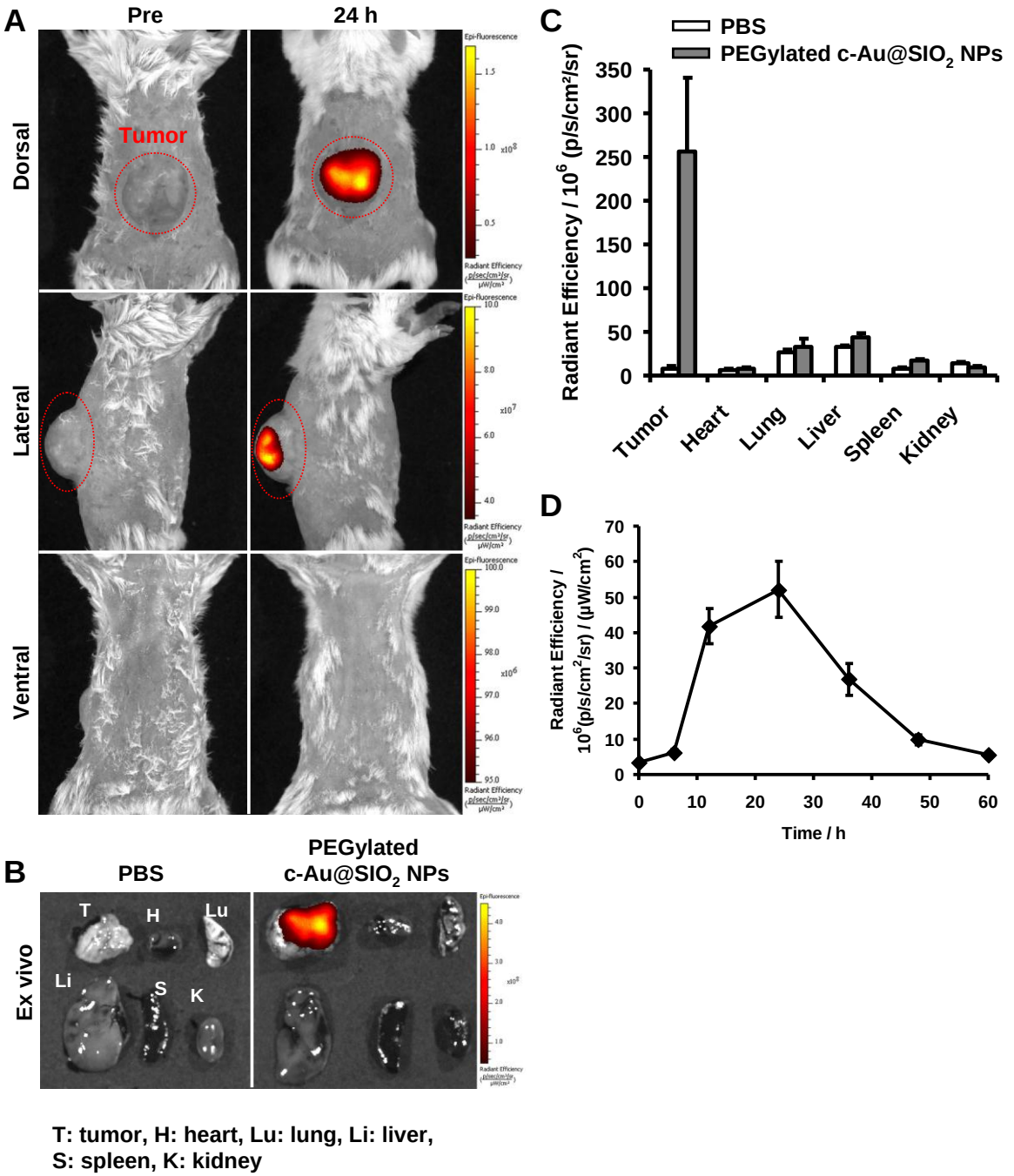

Fig. S12 (A) In vivo fluorescence images of tumor-bearing mice before and 24 h after the intravenous injection of PEGylated c-Au@SiO_2 NPs. (B) Ex vivo fluorescence images 24 h after the injection of PBS and PEGylated c-Au@SiO_2 NPs (T: tumor, H: heart, Lu: lung, Li: liver, S: spleen, K: kidney. (C) Fluorescence intensities of major organs 24 h after the injection of PBS and PEGylated c-Au@SiO_2 NPs. (D) Temporal change in fluorescence intensity of the tumor area.

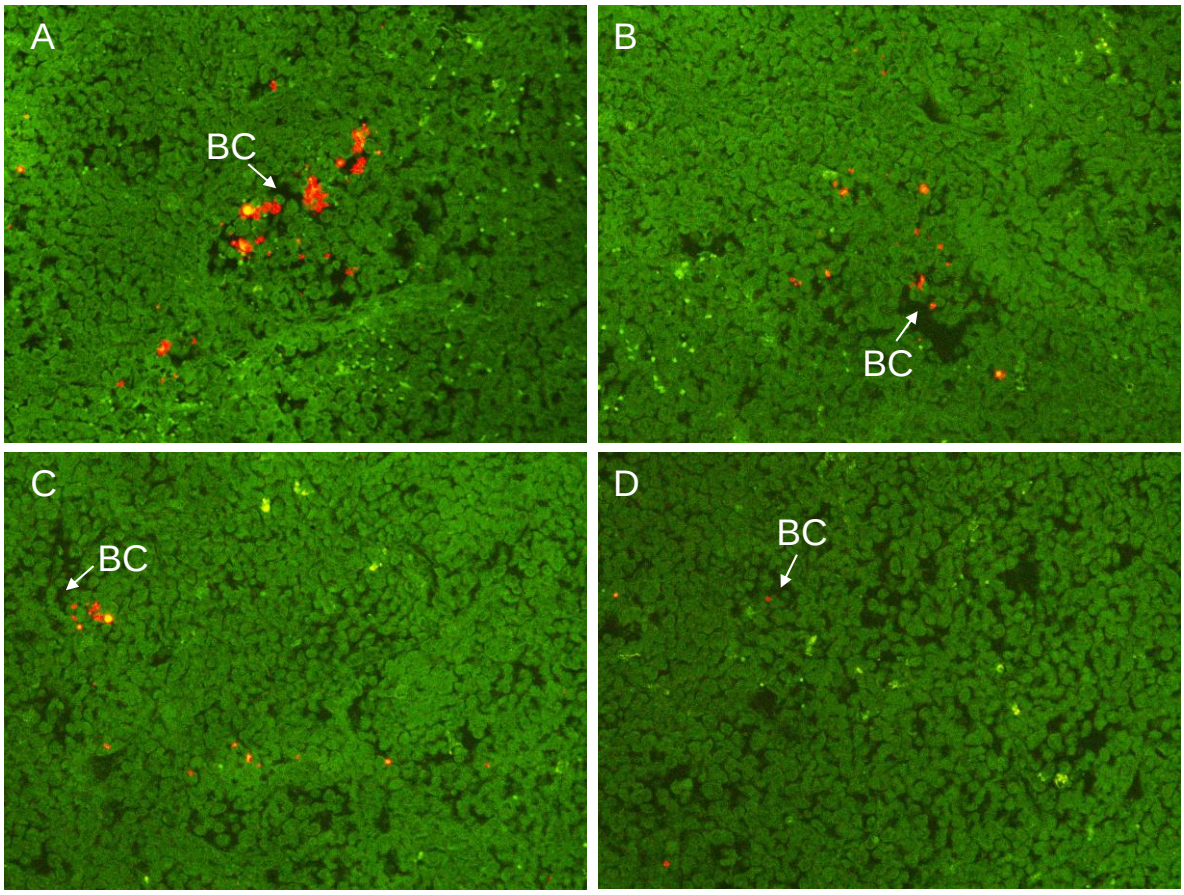

Fig. 13 Fluorescence images in (A) the regions II, (B) III, (C) IV, (D) V in Fig. 1G (red: PEGylated c-Au@SiO_2 NPs, green: tissue autofluorescence, BC: blood capillaries.

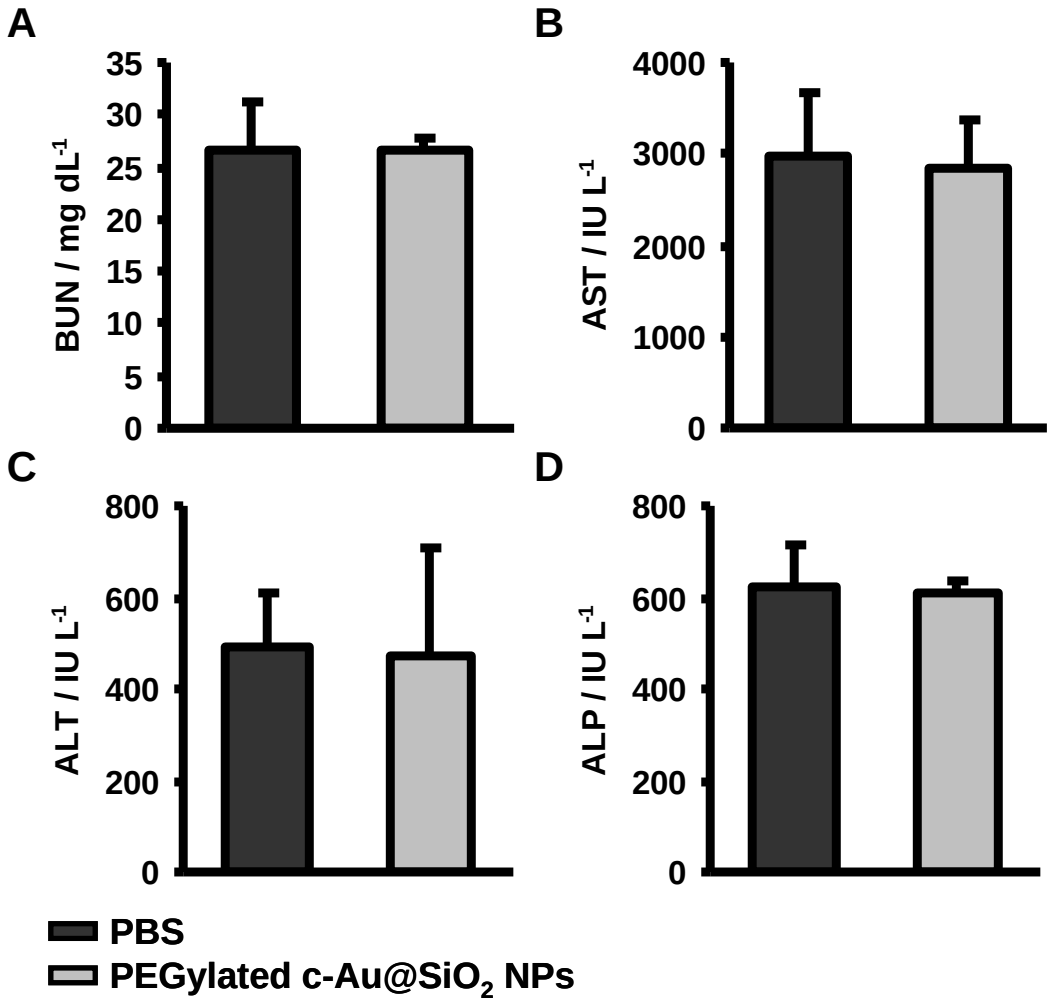

Fig. S14 Biochemical assays in the sera of mice injected with PBS and PEGylated c-Au@SiO_2 NPs. (A) BUN, (B) AST, (C) ALT, and (D) ALP values were measured one month after injection.