Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Multifunctional FePt-Au Heterodimers: a Promising Nanotheranostic Agent for Cancer Dual-modality MR/CT Imaging Diagnosis and Simultaneously in-situ Therapy

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Supplementary Methods

Chemicals and reagents： All chemicals and solvents used were of analytical grade. Pt(II) acetylacetonate (Pt(acac)2, 98%), Gold(Ⅲ) chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$, ≥47.8%), 1-Octadecene (ODE, 90%), Oleylamine (OAm, 90%), Oleic acid (OA, >85%), toluene (99%), dimethyl sulfoxide (DMSO), DMSA, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC, 97%), N-hydroxysuccinimide (NHS, 99%) were all purchased from J&K Chemical Ltd. Iron pentacarbonyl (Fe(CO)5, 98%) was purchased from Xin Ding Peng Fei Chemical Co. in China. SH-PEG-FA (MW5000) and SH-PEG-COOH (MW5000) were purchased from Nanocs. TLyp-1 (98.29%) was purchased from GL Biochem (Shanghai) Ltd.

Synthesis of FePt seeds： FePt nanocubes of ~5 nm were synthesized through a modified literature method.[1] Briefly, $Pt(acac)_2$ (0.2 g, 0.5 mmol), OAm (5 mL) , OA (5 mL) and ODE (10 mL) were added into a three-necked flask at room temperature. Under a gentle nitrogen (N_2) flow, the mixture was heated up to 70 °C and maintained for 30 min to remove the oxygen. Subsequently, the mixture was heated up to 120 °C and kept at this temperature for 2 min before $Fe(CO)_5$ (0.13 mL,1.0 mmol) was injected into the mixture solution. Then the temperature was quickly raised to 220 °C and maintained for 60min before cooled down to room temperature. After that, hexane and anhydrous ethanol were added into the mixture and the products were collected by centrifugation at 12000 r/min. After rinsed twice with ethanol, the products were dispersed in toluene for the subsequent use.

Synthesis of the Au(I) precursor and 7nm Au NPs： The Au precursor was prepared by the reaction of $HAuCl_4 \cdot 4H_2O$ and OAm forming an Au(I)-OAm complex.[2] $HAuCl_4 \cdot 4H_2O$ (1.0 g, 2.4 mmol) was firstly dissolved in ethanol (5 mL). After the addition of OAm (25 mL), the mixture was evacuated under vacuum for 60 min to remove the ethanol and the color of the solution changed

from orange to light yellow after vigorously stirring for 10h at room temperature. The Au(I)-OAm complex was dissolved in toluene (75 mL) to afford the Au precursor solution (10 mg mL^{-1}).

The 7nm Au NPs were synthesized according to the reported method.[1]

Synthesis of FePt-Au HNPS: To synthesize the heterodimers of FePt-Au HNPs, a typical seed-mediated method was used. Briefly, FePt seeds dispersed in toluene (20 mL) was firstly transferred to a three-necked flask under a gentle N_2 flow at room temperature. Then the mixture was heated up to 50 °C. Subsequently, the solution of the Au precursor (10 mg mL^{-1}) was added drop-wise into the FePt nanocubes suspension. The mixed solution was then heated up to 70 °C and maintained for 120 min under N_2 atmosphere until the color changed from dark black to purple. After that, the solution was cooled down to room temperature and anhydrous ethanol (20 mL) was added. The as-synthesized FePt-Au HNPs were separated out by centrifugation (11000 r/min). The final products were rinsed twice with ethanol and re-stored in hexane for further use.

Preparation of water-soluble FePt-Au HNPS: The as-synthesized hydrophobic FePt-Au HNPs coated with OA and OAm were turned into hydrophilic through a process of ligand-exchange. In brief, 50 mg of DMSA dispersed in DMSO was added to the FePt-Au HNPS solution. After shaking for 60 min, anhydrous ethanol was added into the mixture and followed by centrifugation at 7000 r/min. The products were then rinsed twice with deionized (DI) water to remove the excess DMSA. And the as-prepared FePt-Au-DMSA HNPs were re-dispersed in DI water for the subsequent experiment.

Surface modification of FePt-Au-DMSA HNPs: SH-PEG-FA was immobilized onto the surface of FePt-Au-DMSA HNPs via the robust Au-S bond. SH-PEG-FA (5 mg) was firstly added to the as-prepared FePt-Au-DMSA aqueous solution. After reaction for 24 h, the excess SH-PEG-FA was removed by centrifugation at 14000 rpm. Then the products of FePt-Au-DMSA/PEG-FA

HNPs (named as FePt-Au-FA HNPs), were washed with DI water for three times and dispersed in phosphate buffered saline (PBS, 0.1 M, pH =7.4) for further use. To evaluate the cytotoxicity of the as-prepared HNPs, we also used tLyp-1 instead of FA as a targeting agent. SH-PEG-COOH (5 mg) was firstly added to the FePt-Au-DMSA aqueous solution. After 24 h, the excess SH-PEG-COOH was removed by centrifugation at 14000 rpm for 15 min, and the products of FePt-Au-DMSA/PEG-COOH HNPs were washed with DI water for three times. Then a certain amount of tLyp-1 was added to DI water (20 mL), in which FePt-Au-DMSA/PEG-COOH HNPs were pre-activated by EDC and NHS. After reaction for 24 h, the products of FePt-Au-DMSA/PEG-tLyp-1 HNPs were obtained and the excess tLyp-1, EDC and NHS were removed by centrifugation as described above. Additionally, FePt-DMSA/PEG-FA was prepared as a control in the same way as FePt-Au-FA HNPs apart from the introduction of Au NPs. Finally, all of the resultant solutions (FePt-Au-FA HNPs, FePt-Au-DMSA/PEG-tLyp-1 HNPs and FePt-DMSA/PEG-FA NPs) were filtered through a 0.22-μm syringe filter and stored at 4 °C for further use.

Characterization： TEM and HRTEM images of the HNPs were taken on a JEOL JEM-2100 transmission electron microscopy operated at 200 kV. STEM images and energy dispersive spectrometry (EDS) mapping profiles were collected on a JEOL ARM-200F field-emission transmission electron microscope operated at 200 kV. Magnetic measurement was carried out on a Magnetic Property Measurement System (MPMS SQUID VSM, Quantum Design, USA) at 298K. The concentrations of metals were measured with an inductively-coupled plasma mass spectrometry (ICP-MS (iCAP Q, Thermo Fisher)). X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advance Powder diffractometer with Cu-Kα radiation (λ= 1.54178 Å). UV-vis spectra were obtained by suing a Varian Cary 6000i spectrophotometer. FT-IR data was recorded on a Nicolet 5700 spectrophotometer. Size distribution and zeta potential were performed on a Zetasizer Nano

ZS (ZEN 3600, Malvern).

Cell lines and cell culture： MCF-7 (human breast cancer cell line), HeLa (human cervical carcinoma cancer cell line) and BRL 3A (rat normal hepatocyte cell line) were provided by the Cell Bank of Chinese Academy of Science. L02 (human hepatocyte cell line) was purchased from the American Type Culture Collection. HepG2 (human hepatocellular carcinoma cell line) was obtained from the Type Culture Collection of the Chinese Academy of Science. Cells were cultured in DMEM medium with 10% heat-inactivated fetal bovine serum (FBS) and 1% v/v penicillin/streptomycin in a humidified incubator (Thermo 3111) at 37 °C with 5% CO_2. WST-1 assay was performed with a Biotek Elx 800 Microplate Reader.

In vitro cell cytotoxicity assay： WST-1 assay was performed to evaluate the cytotoxicity of FePt-Au-FA HNPs. The cells were firstly seeded in 96-well plates ($1 \times \sim 10^4$ cells in 100 μL culture medium) and maintained for 24 h. Then cells were incubated for 6 h at various final Fe concentrations of 1 μg mL^{-1}, 2.5 μg mL^{-1}, 5 μg mL^{-1}, 10 μg mL^{-1}, 20 μg mL^{-1}, and 40 μg mL^{-1}. After that, the plates were washed three times with PBS buffer and added fresh DMEM supplemented with 10% fetal bovine serum. The relative cellular viability was examined by the WST-1 assay. In addition, we have also done the WST-1 assay with FePt-Au-FA HNPs under different incubation time (2h, 4h, 6h, 12h, 24h, and 48 h) at the same Fe concentration of 3.0 μg mL^{-1} (IC_{50}) with the other conditions equal. The cell viabilities were all calculated using the following equation

$$\% \text{ cell viability} = \frac{\Delta \text{ Csamples} - \Delta \text{ Cblack}}{\Delta \textit{ Ccontrol} - \Delta \textit{ Cblack}} \times 100\%$$

Where $C_{samples}$ was obtained in the presence of the HNPs, $C_{control}$ was obtained in the absence of the HNPs and C_{black} was obtained in the absence of both the HNPs and cells. Δ means the difference before and after the addition of WST-1.

Meanwhile, the WST-1 assay performed on FePt-Au-DMSA/PEG-tLyp-1 HNPs holds the

same method as well as FePt-Au -FA HNPs.

In vitro cellular uptake assay： Briefly, the cells were firstly seeded in 6-well plates (2×10^5 cells per well) and maintained for 24 h. Then cells were incubated with FePt-Au-FA HNPs for different times (2h, 4h, 6h, 12h, 24h, and 48 h) at the same Fe concentrations of 3 μg mL^{-1} (IC50). After that, the nutrient solution was collected and the cells were washed three times with PBS buffer to completely transfer the free HNPs into the collected solution. Then the HNPs which were not swallowed by cells were separated out by centrifugation. Then the HNPs were nitrified by nitric acid and diluted to 5 mL before the Fe concentration was determined by ICP-MS. The cellular uptake assay performed on FePt-Au-DMSA/PEG-COOH HNPs hold the same method with FePt-Au-FA HNPs.

The relative cellular uptake rates were all calculated using the following equation:

$$\%\ \text{cell viability} = \frac{Acontrol\ -\ Bsamples}{Acontrol} \times 100\%$$

Where $A_{control}$ was obtained in the absence of cells.

In vitro Prussian blue staining and ROS study： For Prussian blue staining, MCF-7 cells seeded in 24-well plates ($1 \times \sim 10^5$ cells in 1 mL culture medium) were incubated with the FePt-Au-FA HNPs for 6h at Fe concentration of 3.0 μg mL^{-1}. Then cells were washed three times with PBS buffer and fixed with 4 % paraformaldehyde for 15 min. After that, the cells were incubated with Pearls agent (4 % potassium ferrocyanide and 12 % HCl, 50:50 v/v) for 30 min and then added in 1% neutral red to stained nucleus. The cells were rinsed well with PBS and examined under an inverted microscope (Olympus IX 53). For ROS study, MCF-7 cells seeded in 24-well plates were incubated with the FePt-Au-FA HNPs for different hours (2h, 4h, 6h, 12h and 24h) at the same Fe concentration of 3.0 μg mL^{-1}. Then cells were loaded with 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA) in dark for 30 min and washed with PBS to remove the free DCFH-DA.

Typically, the fluorescence imaging of the cells after treated with the HNPs for 6 h was obtained on a fluorescence microscope (Olympus IX 53). After that, the cells were dissociated immediately by pancreatic enzymes and collected to test their fluorescence intensity by a fluorescence spectrometer (Hitachi F-4500) excited at 488 nm. Meanwhile, the ROS studies performed on FePt-Au-DMSA/PEG-tLyp-1 HNPs holds the same method as well as FePt-Au -FA HNPs.

In vitro MR/CT imaging study： In a typical MRI measure, MCF-7 cells were incubated with FePt-Au-FA HNPs dissolved in DMEM at different Fe concentrations (0, 1, 2.5, 5, 10, 20 and 40 μg mL^{-1}) at 37 °C for 6 h. Then the cells were washed well with PBS and dissociated by pancreatic enzymes. After that, the cells were collected and centrifuged at 1500rpm/min, and then suspended in 1 mL of agarose gel (0.5%) in 4 mL centrifuge tubes, respectively. In addition, MCF-7 cells treated with FePt-Au-FA HNPs for different hours (3, 6, 12, 24 and 48 h) at the same Fe concentration of 5.0 μg mL^{-1} were also carried out for MRI measurement. MRI imaging was performed by using a 3.0-T whole-body MR scanner. In a typical CT measure, MCF-7 cells were incubated with FePt-Au-FA HNPs dissolved in DMEM at different Pt concentrations (0, 0.1, 0.5, 1 and 3 mg mL^{-1}), corresponding Au concentrations (0, 0.1, 0.5, 1 and 3 mg mL^{-1}), at 37 °C for 6 h. Then the cells were washed well with PBS and dissociated by pancreatic enzymes. After that, the cells were collected and centrifuged at 1500rpm/min, and then suspended in 1 mL of agarose gel (0.5%) in 4 mL centrifuge tubes, respectively. As a control, MCF-7 cells incubated with FePt-DMSA/PEG-FA NPs at different Pt concentrations (0, 0.1, 0.5, 1 and 3 mg mL^{-1}) were treated in the same way as described above. CT scans were performed by a GE LightSpeed VCT clinical imaging system (GE Medical Systems) with the following parameters: beam collimation, 64 × 0.625 mm; table speed, 27 mm per rotation; beam pitch, 1.25; gantry rotation time, 1.0 s.

In vivo MR/CT imaging study： All the animal experiments were complied with guidelines

approved by the institutional ethical committee for animal care, and also in accordance with the policy of the National Ministry of Health. Balb/c mice were obtained from Jinan Peng Yue Experimental Animal Breeding Co Ltd and used under protocols approved by Qilu Hospital Laboratory Animal Center. Tumor-bearing mice were prepared by inoculating 2×10^6 4T1 cancer cells at backside in the female balb/c mice and could be used for experiment after 5~7 weeks. For MR imaging, the mice was firstly anesthetized with an intraperitoneal injection of 20 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, and then a PBS solution containing FePt-Au-FA HNPs (50 μL, Fe concentration, 500 μg mL^{-1}) was injected intratumorally. In addition, another tumor-bearing mouse was also used for MR imaging by intravenously (through the tail vein) injection of the same FePt-Au-FA HNPs. After that, the mice under anesthesia were scanned under a 3.0 T clinical MRI scanner (GE Signa HDx 3.0T MRI, USA) equipped with a custom-built rodent receiver coil using the following parameters: repetition time (TR), 2000; echo time (TE), 6 ms; matrix, 256 × 192; slice spacing, 1.0 mm; slice thickness, 2.0 mm; field of vision (FOV), 7.0 cm × 7.0 cm. T_2 weighted MR images were obtained at various time points of 0 h (before), 0.5 h, 1 h, 2 h, 24 h and 0 h (before), 2 h, 24 h for the mice intratumorally and intravenously injection of FePt-Au-FA HNPs, respectively. After acquiring the T_2-weighted MR images, the signal intensity of the tumor injected HNPs intratumorally was measured within a manually drawn region-of-interest (ROI) for the mouse. For CT imaging in vivo, a similar tumor-bearing mouse was intratumorally injected with a PBS solution containing FePt-Au-FA (100 μL, Fe concentration, 2.5 mg mL^{-1}; Pt concentration, 5 mg mL^{-1}; and Au concentration, 5 mg mL^{-1}) and was anesthetized described as above. A Siemens SOMATOM Force imaging system was used for CT scanning with the following parameters: tube voltage, 70 kV; tube current 8 mAs; slice thickness, 3 mm. CT images were obtained both before (0 h) and after (0.5 h) injection of FePt-Au-FA HNPs, and the corresponding CT images were

reconstructed by a syngo.via advantage workstation. Additionally, the mouse was also used for T_2-weighted MR imaging measurement using the same method described above immediately in the end of CT imaging procedure. After acquiring the CT images and T_2-weighted MR images, the signal intensity of the tumor was measured within a manually drawn region-of-interest (ROI).

In vivo anti-tumor effect： To evaluate the therapeutic effect of FePt-Au-FA HNPs, the similar female balb/c tumor-bearing mice were used, and when the tumor size reached about 100 mm^3, 12 mice with similar tumor sizes were randomly divided into three groups (4 mice per group): (a) PBS injection intratumorally; (b) FePt-Au-FA HNPs injection intratumorally; (c) FePt-Au-FA HNPs injection intravenously through the tail vein; where (b) and (c) using the same PBS solution containing FePt-Au-FA HNPs (Fe concentration, 500 μg mL^{-1}). Every mouse was performed as follows: 50 μL per injection, once every two days and before every injection, the widest width and along the perpendicular length of tumors were measured by a caliper. The tumor volume was calculated as follows:

$$V = L \times W^2/2$$

Where V (mm^3) is the volume of the tumor, L (mm) and W (mm) are the tumor length and width, respectively. The tumor volumes (V) are normalized against the original volumes at 0 day (V_0) for evaluating the tumor growth rates. When the experiments were finished (12 days post-injection), the mice were all sacrificed under an anesthetic status.

Histological study (Haematoxylin and eosin (H&E) staining)： For histology studies, mice after sacrificed were dissected and made into slices to observe the therapeutic efficacy and in vivo toxicity. In brief, the tumors and typical organs (heart, liver, spleen, lung and kidney) were harvested from the mice and immobilized in 4% paraformaldehyde at 4 °C for 24 h, and then embedded in paraffin. The thin sections cut with an ultramicrotome (EMUC6, Leica, Germany)

were placed onto glass slides and stained with hematoxylin and eosin (H & E) to analyze the tissue structure and cell state. The histological sections were observed under a digital microscope (Olympus BX 51).

Reference

[1] J. Zhu, J. Wu, F. Liu, R. Xing, C. Zhang, C. Yang, H. Yin and Y. Hou, *Nanoscale,* 2013, **5**, 9141.

[2] C. K. T. Z. Huo, W. Huang, X. Zhang and P. Yang, *Nano Lett.*, 2008, **8**, 2041.

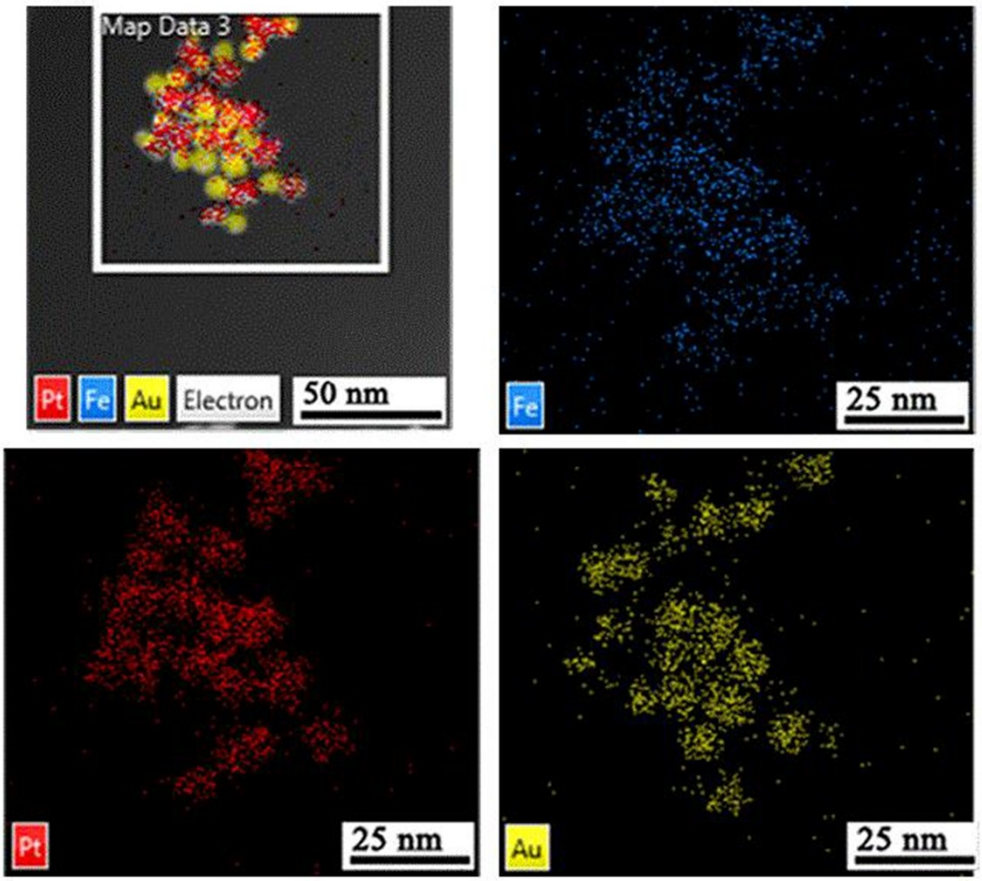

Figure S1 STEM image and EDS mapping profiles of FePt-Au HNPs.

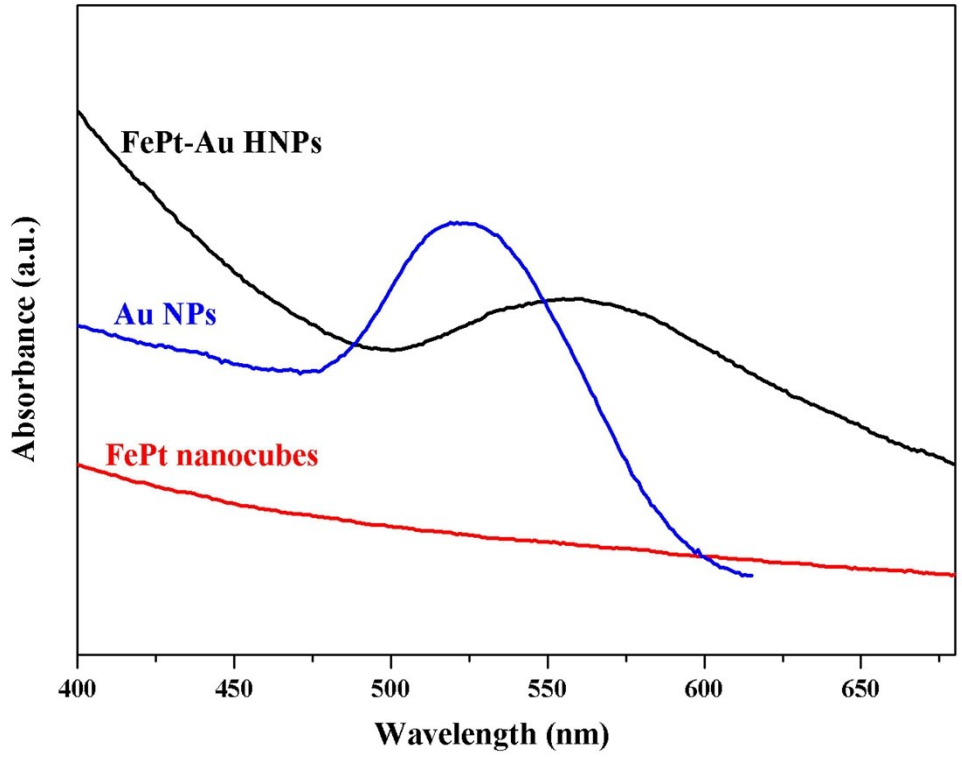

Figure S2 UV-Vis spectra of the as-synthesized FePt seeds, Au NPs and FePt-Au HNPs.

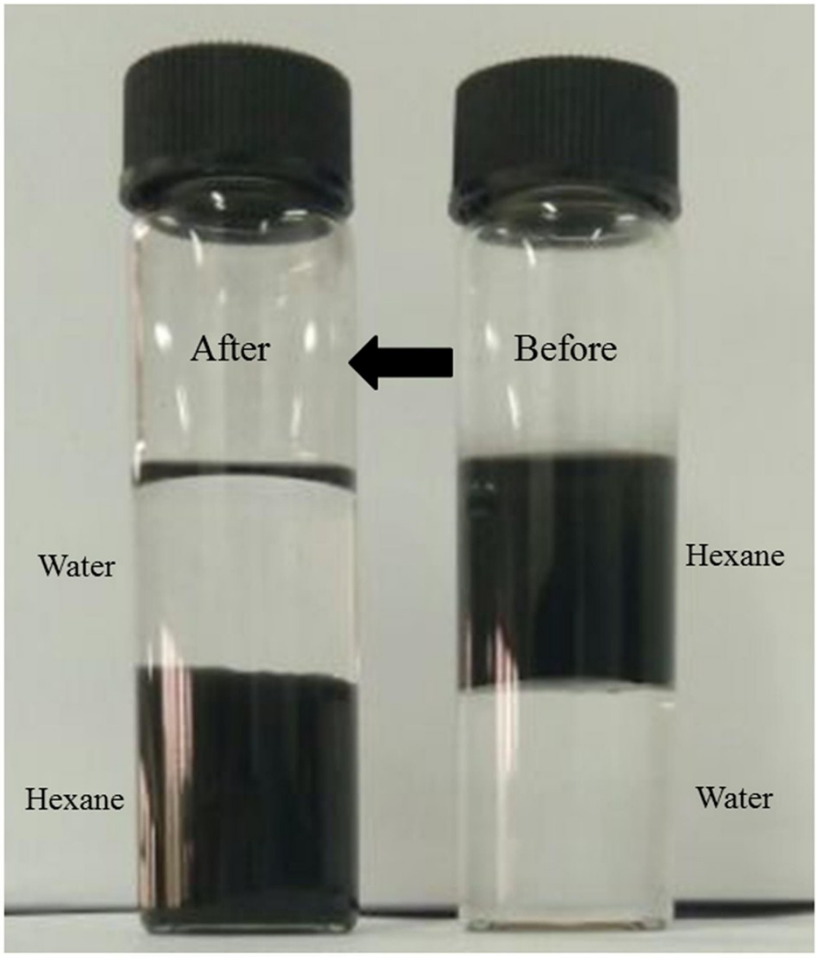

Figure S3 The ligand-exchange process of FePt-Au HNPs by DMSA.

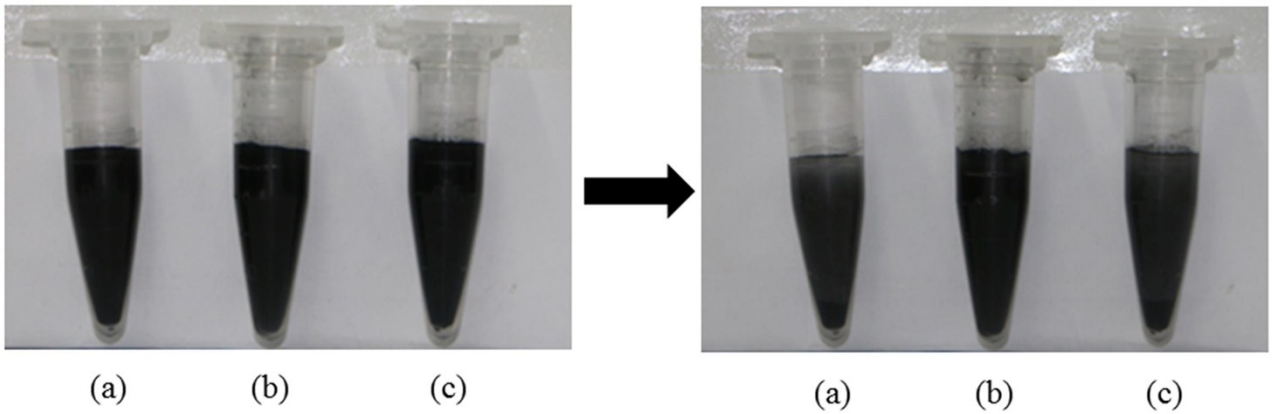

Figure S4 Stabilities of the as-prepared (a) FePt-Au-DMSA HNPs, (b) FePt-Au-FA HNPs and (c) FePt-DMSA/PEG-FA HNPs dispersed in PBS (pH 7.38) for 3 days.

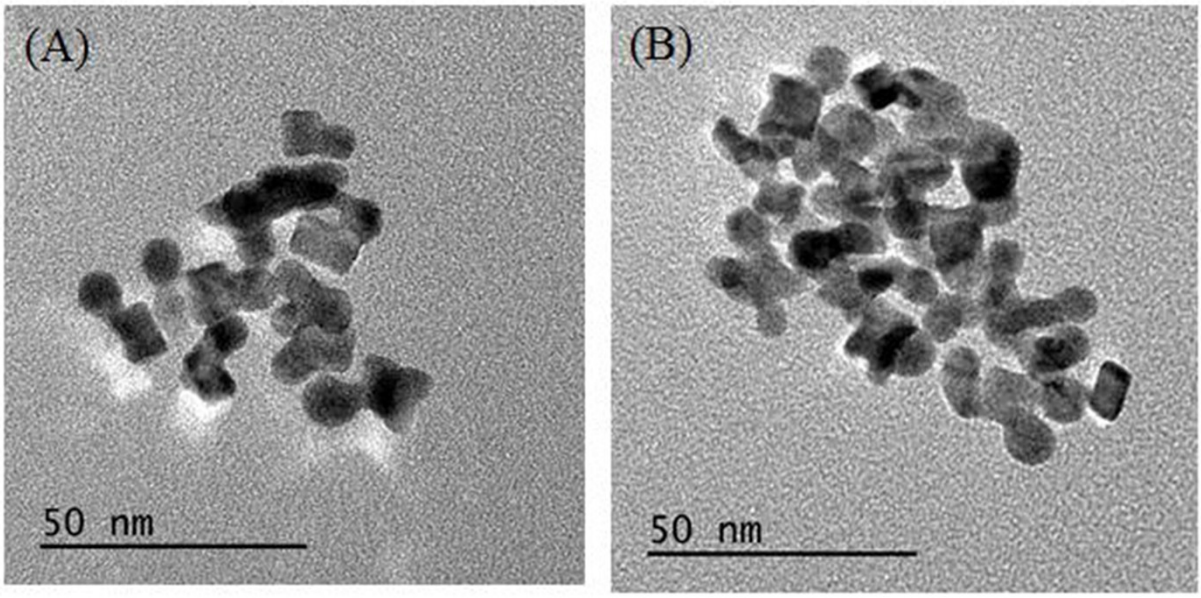

Figure S5 TEM images of (A) FePt-Au-DMSA HNPs and (B) FePt-Au-FA HNPs dispersed in water, respectively.

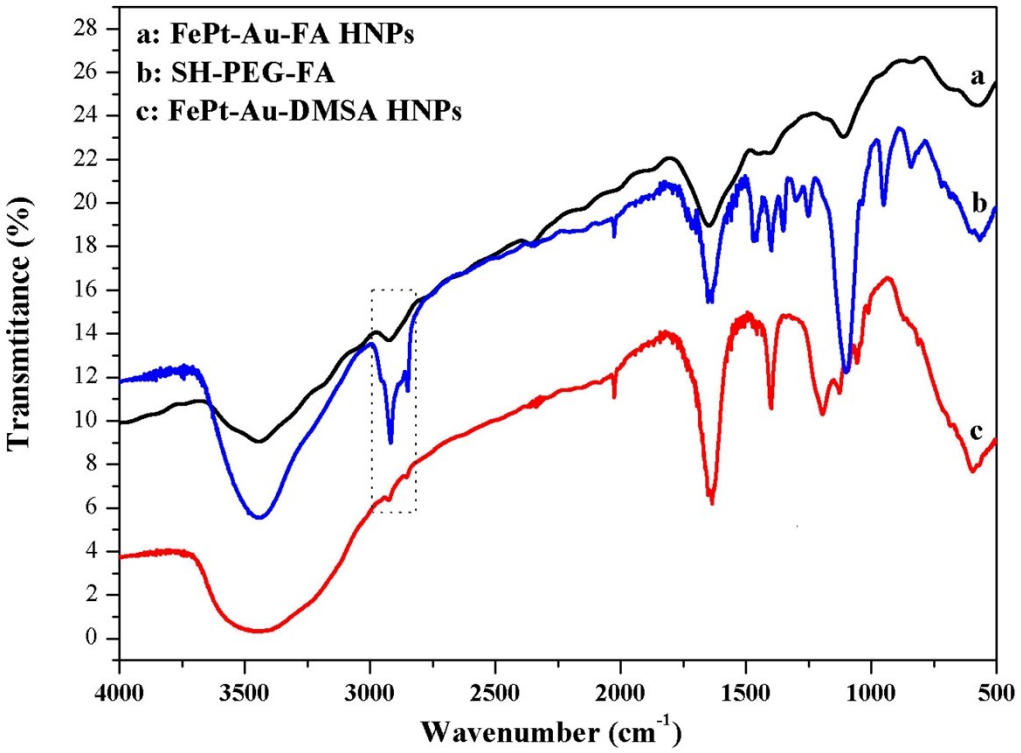

Figure S6 FT-IR of FePt-Au-DMSA HNPs before and after conjugated with SH-PEG-FA.

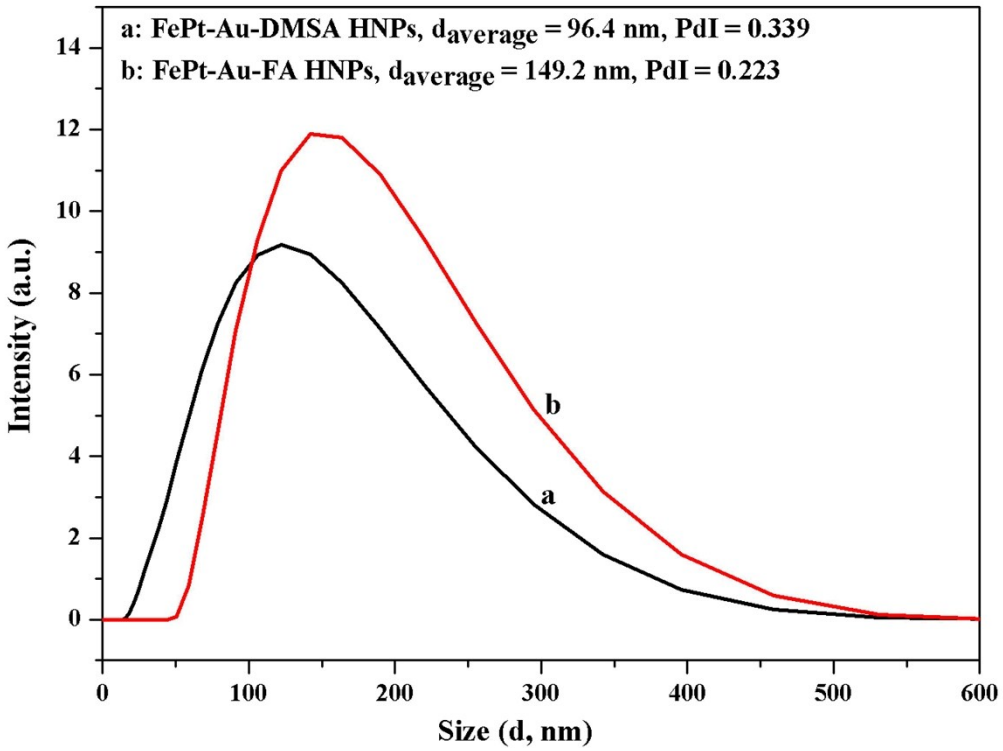

Figure S7 Hydrodynamic sizes of FePt-Au-DMSA HNPs before and after conjugated with SH-PEG-FA.

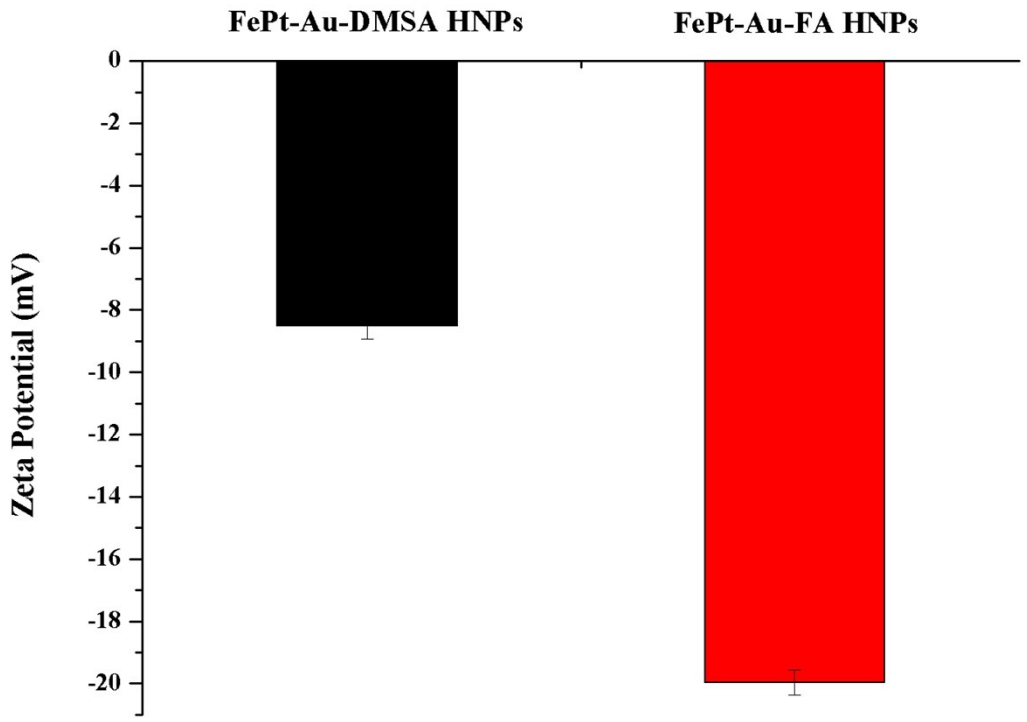

Figure S8 Zeta potentials of FePt-Au-DMSA HNPs before and after conjugated with SH-PEG-FA. Error bars were based on quartet samples.

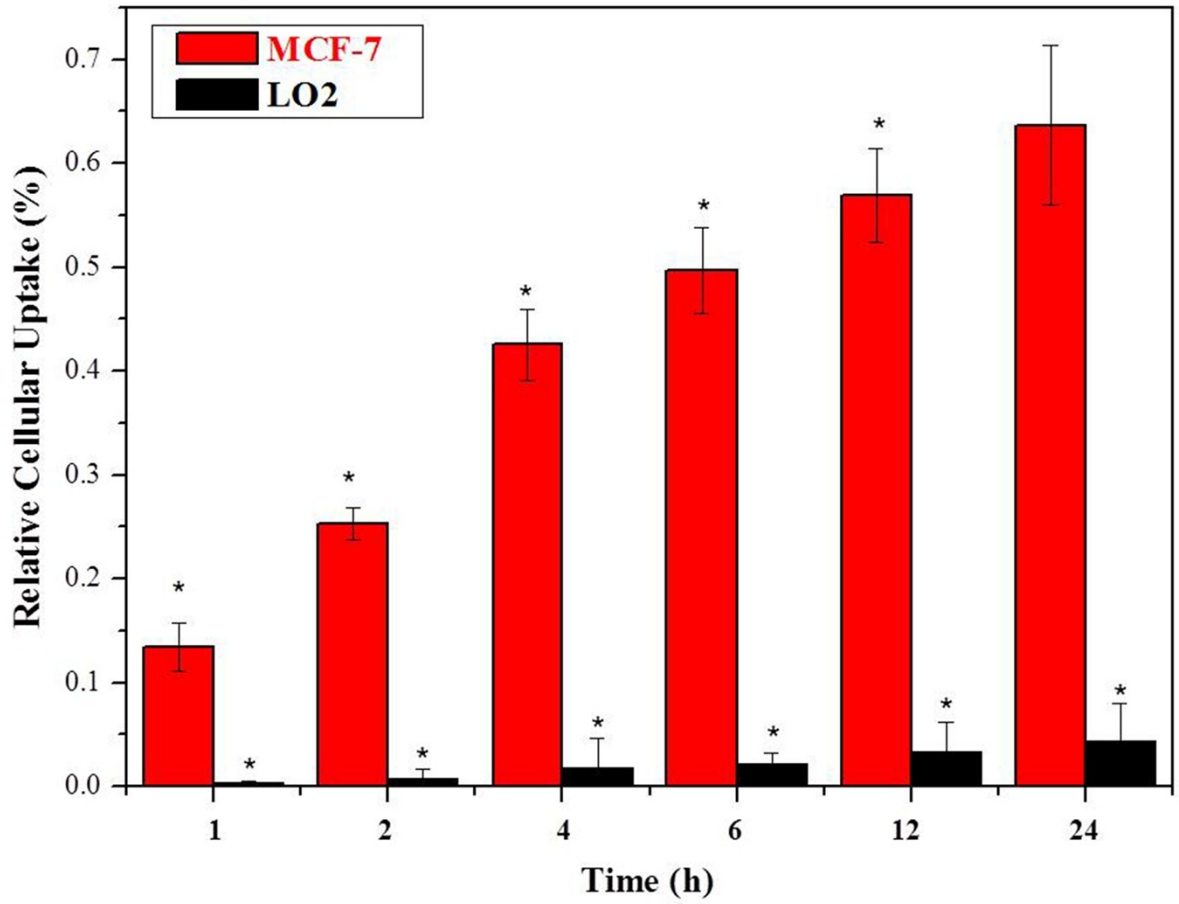

Figure S9 The relative cellular uptake of FePt-Au-FA HNPs toward MCF-7 and LO2 cells, respectively, under different incubation times at the Fe concentration of 3.0 μg mL^{-1}. Error bars were based on quartet samples. The asterisks indicate $P < 0.05$.

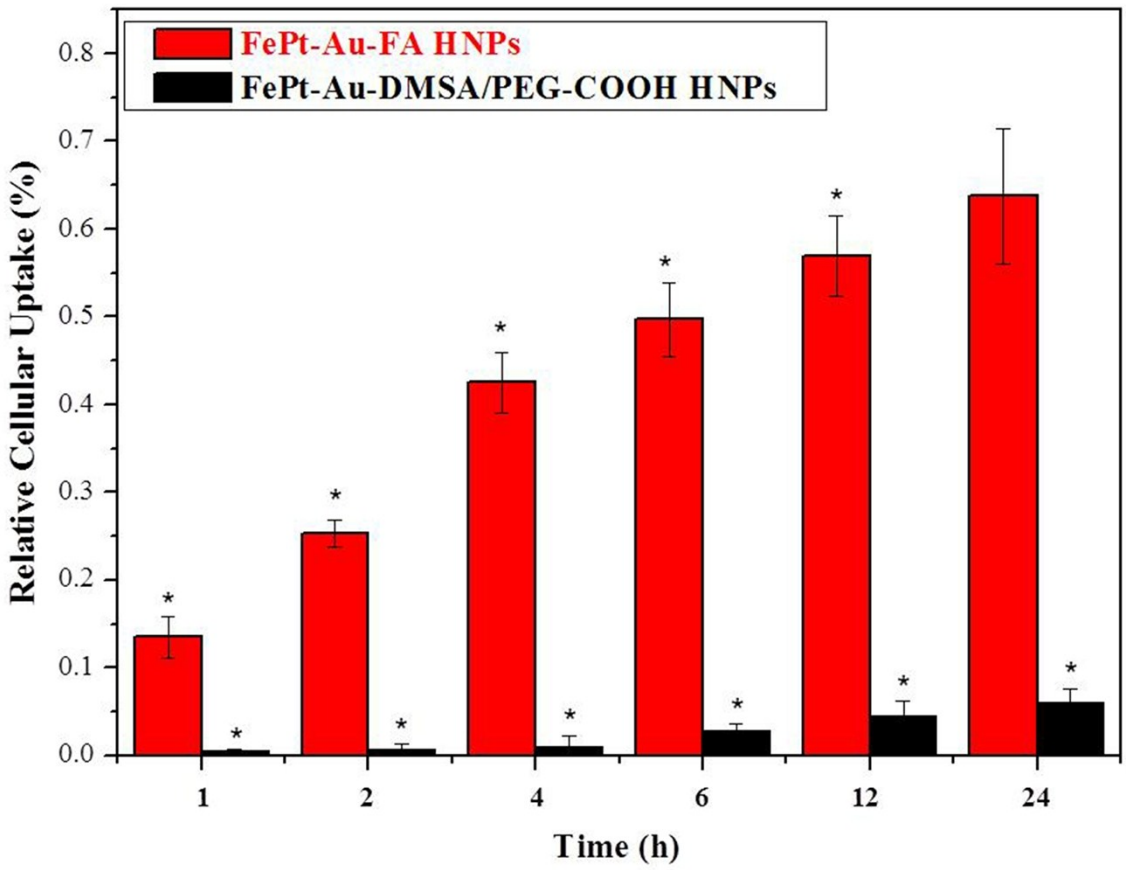

Figure S10 The relative cellular uptake of FePt-Au HNPs with or without FA toward MCF-7 cells under different incubation times at the Fe concentration of 3.0 μg mL^{-1}. Error bars were based on quartet samples. The asterisks indicate $P < 0.05$.

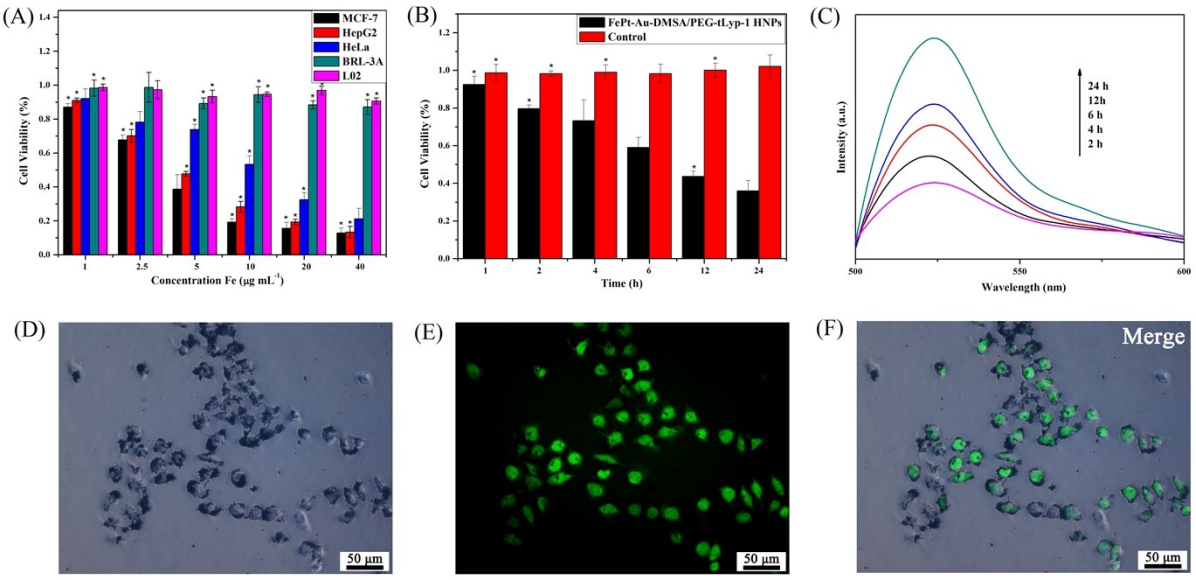

Figure S11 (A) Viabilities of different cell lines treated with FePt-Au-DMSA/PEG-tLyp-1 HNPs for 6 h. Error bars were based on quartet samples. The asterisks indicate $P < 0.05$. (B) Viabilities of MCF-7 cells treated with FePt-Au-DMSA/PEG-tLyp-1 HNPs for different hours at the Fe concentration of 3.0 μg mL^{-1}. Error bars were based on quartet samples. The asterisks indicate $P < 0.05$. (C) Time-dependent fluorescent intensity from DCFH-DA labeled MCF-7 cells treated with FePt-Au-DMSA/PEG-tLyp-1 HNPs at the Fe concentration of 3.0 μg mL^{-1}. (D-F) Typical green fluorescent images of DCFH-DA labeled MCF-7 cells after treated with FePt-Au-DMSA/PEG-tLyp-1 HNPs for 6h at the Fe concentration of 3.0 μg mL^{-1}.

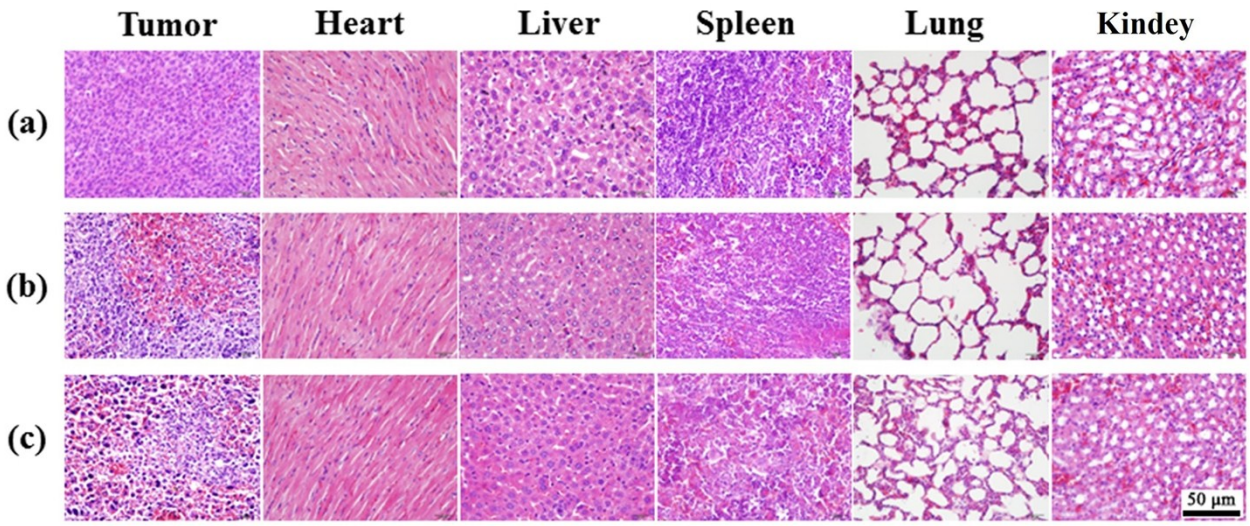

Figure S12 H&E-stained histological slices from mice (a) post-injected with PBS, (b) intratumorally and (c) intravenously post-injected with FePt-Au-FA HNPs for 12 days, respectively.