Supporting Information

Self-Illuminating ^{64}Cu-Doped CdSe/ZnS Nanocrystals for *in vivo* Tumor Imaging

Xiaolian Sun[†§], Xinglu Huang[†§], Jinxia Guo[†,‡], Wenlei Zhu[I], Yong Ding[II], Gang Niu[†], Andrew Wang[III], Dale O. Kiesewetter[†], Zhong Lin Wang[II], Shouheng Sun[I], Xiaoyuan Chen[†,*]

[†] Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, Maryland 20892, USA

[‡]Center for Molecular Imaging and Translational Medicine, School of Public Health, Xiamen University, Xiamen, Fujian 361102, China

[I] Department of Chemistry, Brown University, Providence, Rhode Island 02912, USA

[II]School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, USA

[III] Ocean NanoTech LLC, Springdale, Arkansas 72764 ,USA

[*]To whom correspondence should be addressed. Email: shawn.chen@nih.gov

[§]Xiaolian Sun and Xinglu Huang contributed equally to this work.

1. Experimental Methods

1.1 Reagents

Quantum Dots (QDs) with emission wavelengths at 526, 580 and 636 nm were obtained from Ocean NanoTech (Springdale, AR). Ascorbic acid (AA) was purchased from Sigma-Aldrich. Amine-poly(ethylene glycol)(PEG)-thiol (molecular weight, M_W, 5000 g/mol) was purchased from Nanocs (New York, NY). ^{64}Cu was produced by the PET Department, NIH. Deionized (DI) water with resistivity of 18.0 MΩ was from a Millipore Autopure system. All other reagents were of analytical grade and used without further purification.

1.2 Instrumentation

Transmission electron microscopy (TEM) images were obtained on a FEI Tecnai 12 (120 kV). Samples for TEM analysis were prepared by depositing a drop of diluted NP dispersion on carbon-coated copper grids. High resolution high-angle annular dark-field scanning TEM (HAADF-STEM) and elemental mapping images were obtained on an aberration corrected JEOL 2200FS microscope with a beam size of ~0.8Å for imaging and ~ 2Å for chemical analysis. Samples for elemental mapping were prepared by first loading QDs onto carbon black (Kejen EC 300J, Sigma Aldrich) via sonicating a mixture of QDs and carbon black at a weight ratio of 1:2 in ethanol for 30 min and then washed five times by centrifugation to remove the excess polymer. Finally, a drop of diluted NP dispersion was deposited on carbon-coated gold grids. UV spectra were obtained by a Genesys 10S UV-vis Spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 spectrofluorometer. The hydrodynamic diameters of the nanoparticles were measured by Malvern Zeta Sizer Nano S-90 dynamic light scattering (DLS) instrument. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) measurements were performed on a JY2000 Ultrace ICP Atomic Emission Spectrometer equipped with a JY AS 421 autosampler and 2400 g/mm holographic grating.

1.3 Experimental procedures

1.3.1 Preparation of ^{64}Cu-doped QDs

In a typical preparation of ^{64}Cu-doped QD580, 2 mg of QD580 and 10 μmol of AA were dispersed in 1 mL of hexane and 0.5 mL of ethanol *via* sonication. As-received $^{64}CuCl_2$ solution was diluted into 0.4 M ammonium acetate buffer (NH_4Ac, pH 5.5). $^{64}CuCl_2$ solution was then added dropwise into the QD solution. The solution was shaken for 1 h at 60 °C before being cooled to room temperature. The ^{64}Cu-doped QD580 was collected via centrifugation (8500 rpm, 8 min). The product was repeatedly dispersed in 0.5 mL of ethanol, and centrifuged after adding 1 mL of hexane until no radioactivity in the supernatant was detected by gamma counting. The yield of radiolabeling was almost 100%. ^{64}Cu-doped QD526 and QD636 were prepared similarly by using QD526 and QD636, respectively.

1.3.2 Surface modification of ^{64}Cu doped QDs

^{64}Cu-doped QDs (2 mg) were dispersed in 1 mL of ethanol. In parallel, 6 mg of amine-PEG-thiol was dissolved in 1 mL of ethanol and the solution was then added dropwise to the above QD dispersion. The mixture was shaken for 1 h before centrifugation upon adding 0.5 mL of hexane. The precipitate was dried under a nitrogen flow and redispersed in DI water for further use.

1.3.3 Radiolabel stability

PEGylated ^{64}Cu doped QDs were incubated in fetal bovine serum and mice blood at 37 ℃ respectively. At certain time points, the QDs were collected by centrifugal filter units (10 K cutoff). The radioactivity of QDs and supernatant were measured respectively via gamma counting.

1.3.4 *In vitro* Cerenkov luminescence imaging

Aqueous suspensions of different samples (100 μL) were placed in a 96-well black plate (Greiner Bio-One, Monroe, NC) in the light-tight chamber. For the comparison study of ^{64}Cu-doped QDs, mixture of ^{64}Cu and QDs, $^{64}CuCl_2$, and QDs without ^{64}Cu, the radioactivity was kept at 46 μCi and the QD amount at 10 μg. Luminescent images were acquired after 5 min scanning with or without different filters. Images were then analyzed via Living Image 3.0 software (Caliper Life Science, Hopkinton, MA) and the signal was normalized to photons per second per centimeter square per steradian (p/s/cm^2/sr).

1.3.5 Cell culture and animal model

All animal work was performed following a protocol approved by the National Institutes of Health Clinical Center Animal Care and Use Committee (NIH CC/ACUC). U87MG human glioblastoma cell line was purchased from the American Type Culture Collection (ATCC) and was cultured in ATCC-formulated Eagle's Minimum Essential Medium (EMEM) with 10% (v/v) fetal bovine serum at 37 °C with 5% CO_2. Athymic nude mice purchased from Harlan (Indianapolis, IN) were subcutaneously implanted with 1 x 10^6 U87MG cells in the front flank. The *in vivo* imaging was performed 3 weeks after the inoculation when the tumor volume reached around 100 mm^3.

1.3.6 Small-animal PET imaging and luminescence imaging

The details of small-animal PET imaging and region-of-interest (ROI) analysis have been reported before.[1,2] The U87MG tumor-bearing mice were anesthetized with isoflurane (Abbott Laboratories) and were injected intravenously with 25 μg of QD580 (250 μCi ^{64}Cu). PET scans and imaging analysis were carried out on an Inveon microPET scanner (Siemens Medical Solutions) at 1, 17, 24, and 42 h after injection. For each PET scan, 3-dimensional ROIs were drawn over the tumor and organs on decay-corrected whole-body coronal images. The average radioactivity concentration was obtained from the mean pixel values within the ROI volume, which was converted to counts per milliliter per minute by using a predetermined conversion factor.[1,2] Given a tissue density of 1 g/mL, the counts per milliliter per minute were converted to counts per gram per minute, and the values were divided by the injected dose to obtain the image

ROI-derived percentage injected dose per gram (%ID/g). At 45 h post-injection, the mice were sacrificed and the tumor as well as the major organs were collected and subjected to an *ex vivo* PET scan.

Luminescence images were obtained using an IVIS Lumina II (Caliper Life Sciences) at 1, 17, 24, and 42 h after injection. Each acquisition took 10 min for all studies and the signal was normalized to photons per second per centimeter square per steradian (p/s/cm^2/sr).

1.3.7 Biodistribution study

The mice were sacrificed at 45 h post-injection after the last PET scan. Organs of interest were collected, weighted and the radioactivity was measured in a well Beckman 8000 gamma counter (Beckman, Brea, CA). The uptake of ^{64}Cu in various organs was calculated as the percentage of the injected dose per gram of tissue (%ID/g) according to the prepared standards.

The organs were then immersed in 5 mL digest solution (HNO_3: HCl = 1: 1). The dispersions were heated to boiling until organs were completely dissolved. H_2O_2 (1 mL) was then added into the solution for continue heating until the solution became clear and transparent. The solution was then cooled down to room temperature, diluted by 2% HNO_3 to 10 mL, and subsequently analyzed by ICP to determine the concentration of Cd, Se and Zn in each sample.

2. Experimental Results

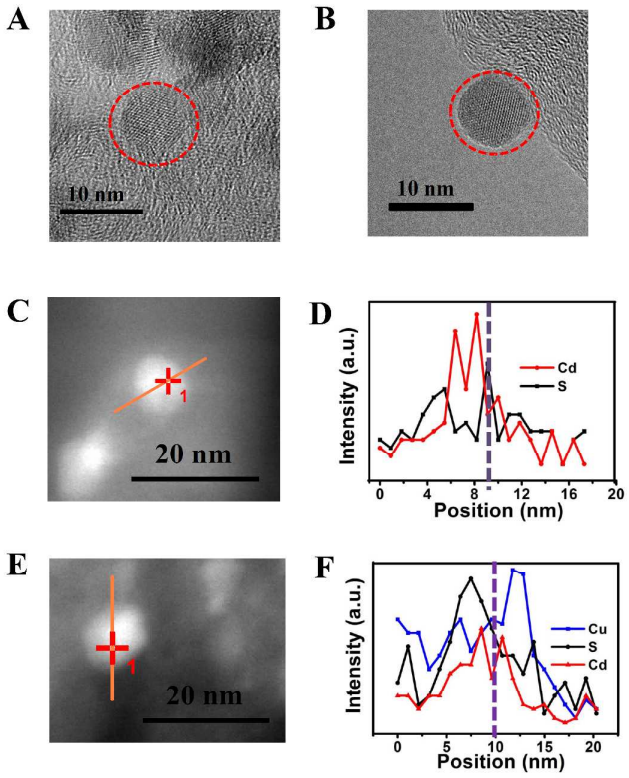

Figure S1. (A) HRTEM of as-received commercial QD636. (B) HRTEM of QD636 after non-radioactive $CuCl_2$ treatment (Se : Cu weight ratio is 2:1). (C) High-angle annular dark-field scanning TEM (HAADF-STEM) image of commercial QD636. (D) High resolution line-scan EDS analysis across a single commercial QD636 indicated in (C). (E) HAADF-STEM image of QD636 after $CuCl_2$ treatment. (F) High resolution line-scan EDS analysis across a single QD636 after $CuCl_2$ treatment indicated in (E). The characterization of QD636 is shown here as a representative of CdSe/ZnS core/shell nanoparticles since it is easy to target the Cu distribution in the core or shell for large sized particles.

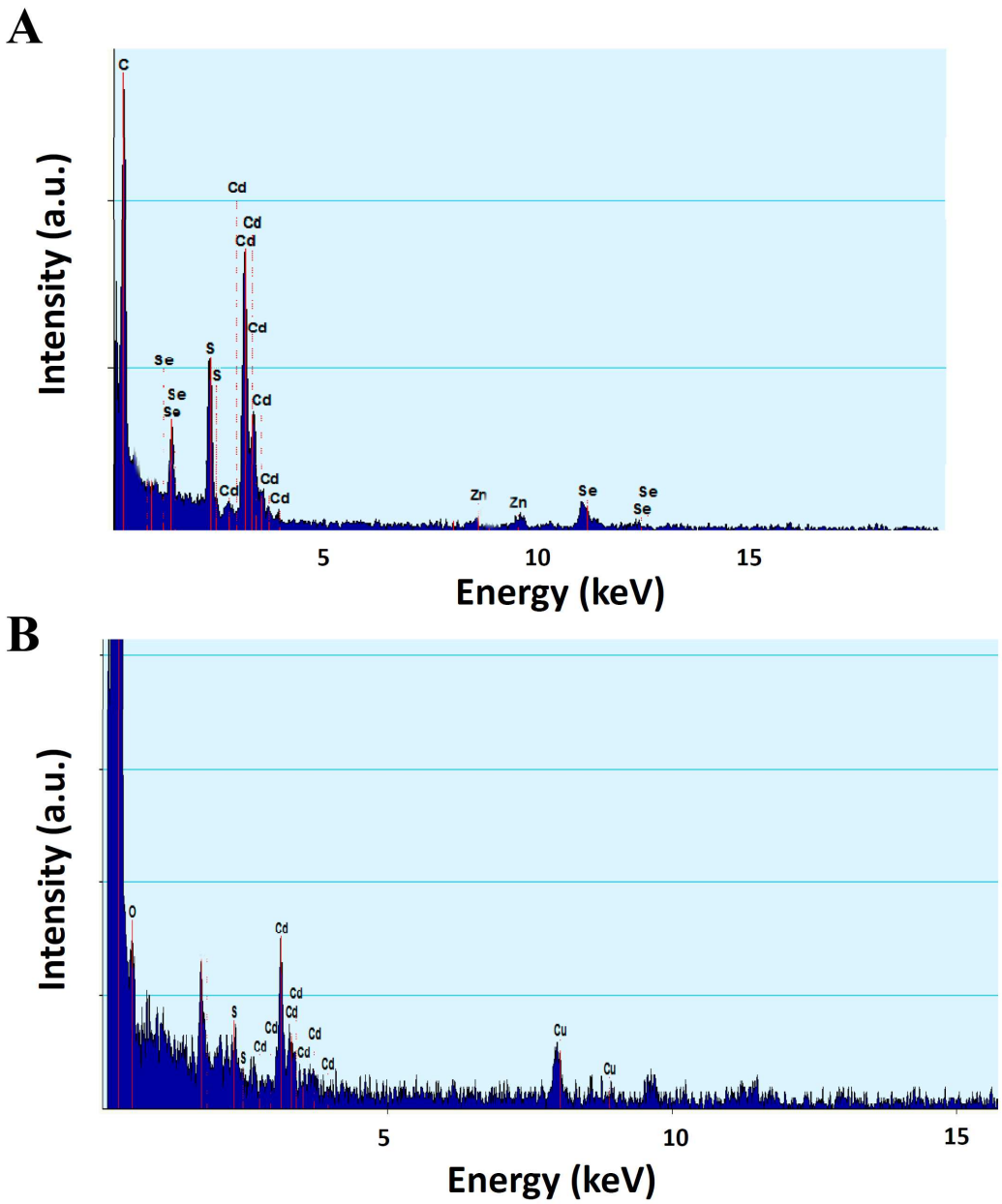

Figure S2. EDS analysis of (A) as-received commercial QD636 and (B) QD636 after non-radioactive $CuCl_2$ treatment (Se : Cu ratio is 2:1).

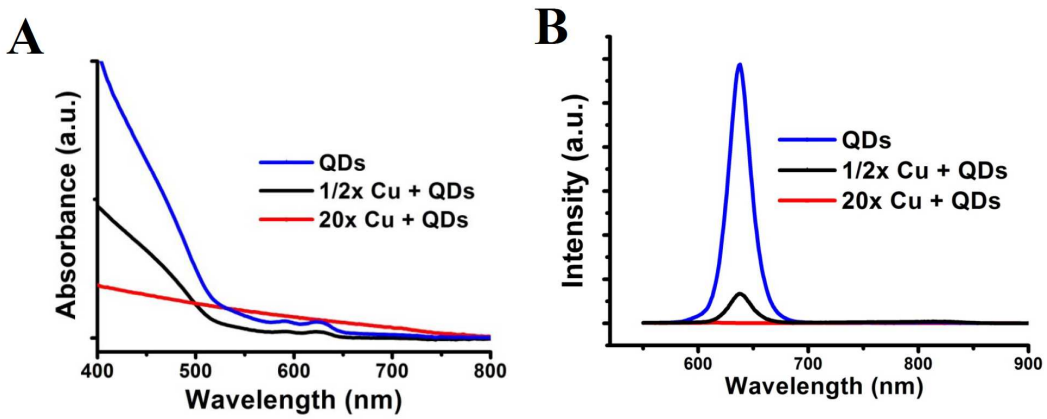

Figure S3 (A) UV absorption and (B) fluorescence emission of as-received QD636 and QD636 after Cu treatment (Se : Cu ratio is 2:1 (black line) and 1 : 20 (red line)).

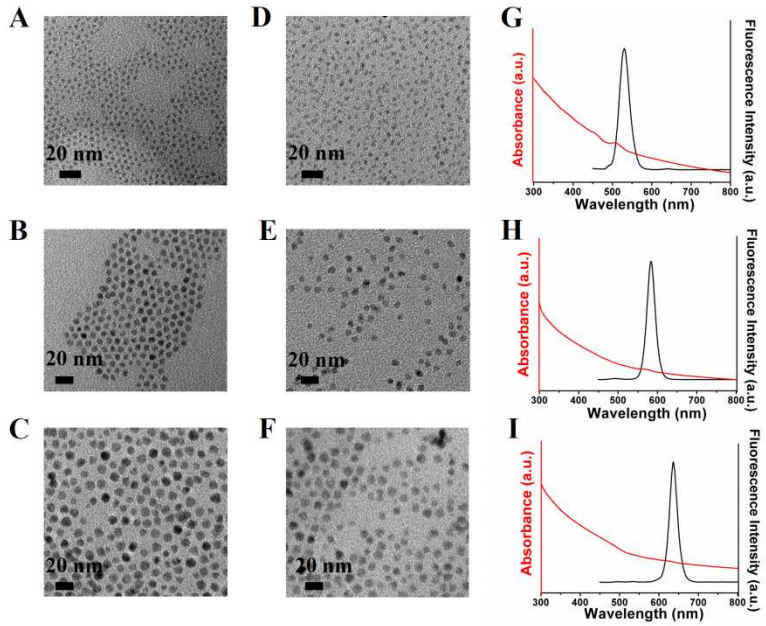

Figure S4. (A-C) TEM of as-received commercial QD526 (A), QD580 (B) and QD636 (C). (D-F) TEM of ^{64}Cu-doped and amine-PEG-thiol modified QD526 (D), QD580 (E) and QD636 (F) after decay. (G-I) Absorption (red line) and fluorescence emission (black line) of ^{64}Cu-doped and amine-PEG-thiol modified QD526 (G), QD580 (H) and QD636 (I) after decay.

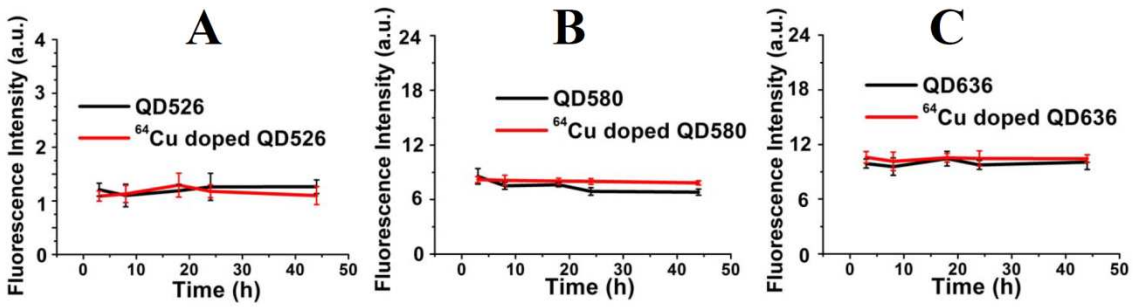

Figure S5. Time-dependent fluorescence intensity of (A) QD526 and ^{64}Cu-doped QD526; (B) QD580 and ^{64}Cu-doped QD580; (C) QD636 and ^{64}Cu-doped QD636.

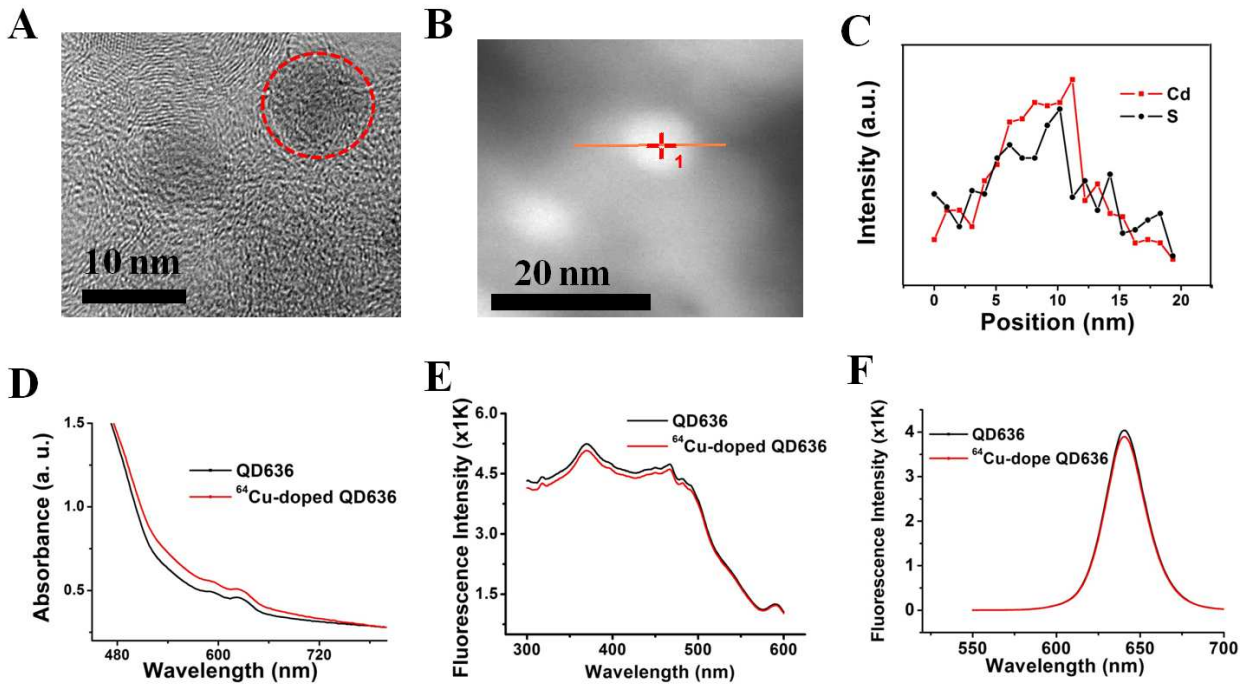

Figure S6. (A) HRTEM and (B) HAADF-STEM images of ^{64}Cu-doped QD636 (Cu amount is negligible compared to QD amount). (C) High resolution line-scan EDS analysis across a single ^{64}Cu-doped QD636 indicated in Figure (B). UV spectra (D), fluorescence excitation spectra (E) and fluorescence emission spectra (F) of QD636 (black line) and ^{64}Cu-doped QD636 (red line).

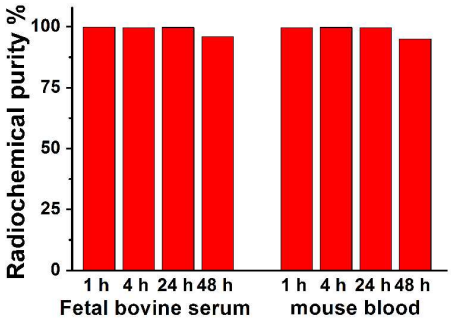

Figure S7 Radiolabel stability of ^{64}Cu-doped QDs in fetal bovine serum and mouse blood incubated at 37 ℃ for 1, 4, 24 and 48 h. Radiochemical purity was calculated as radioactivity of ^{64}Cu-doped QDs divided by the total radioactivity of the ^{64}Cu-doped QDs and the incubation solution.

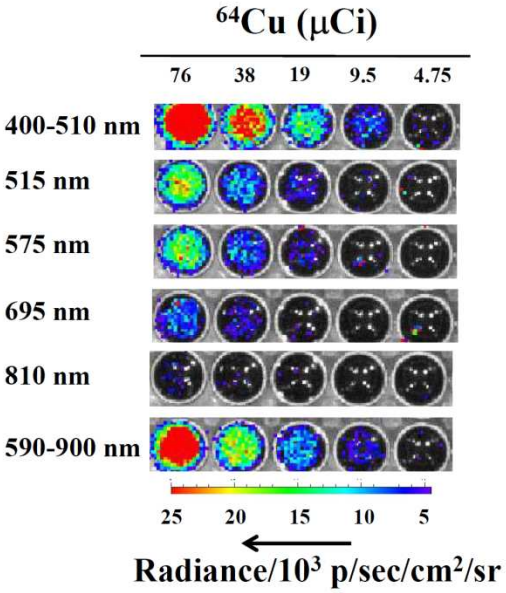

Figure S8. Luminescence intensities recorded from $^{64}CuCl_2$ solution with different filters. The wavelength of each filter is listed on the left of the image.

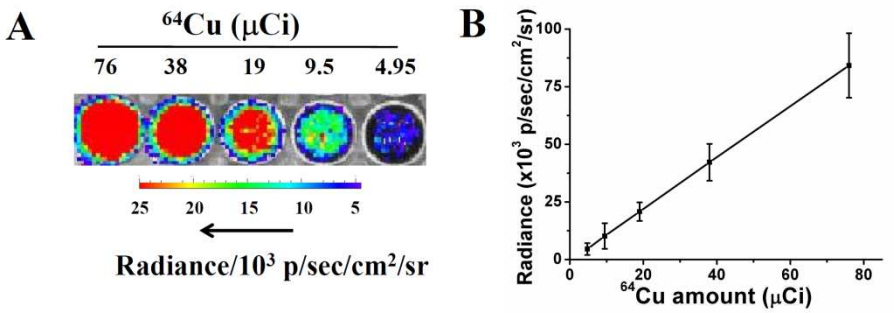

Figure S9. (A) Optical images of aqueous suspension of $^{64}CuCl_2$ with different amount of radioactivity in a 96-well plate. (B) Plot of photon flux versus radioactivity for aqueous suspensions of $^{64}CuCl_2$ in a 96-well plate (n=3 per concentration). R^2=0.99.

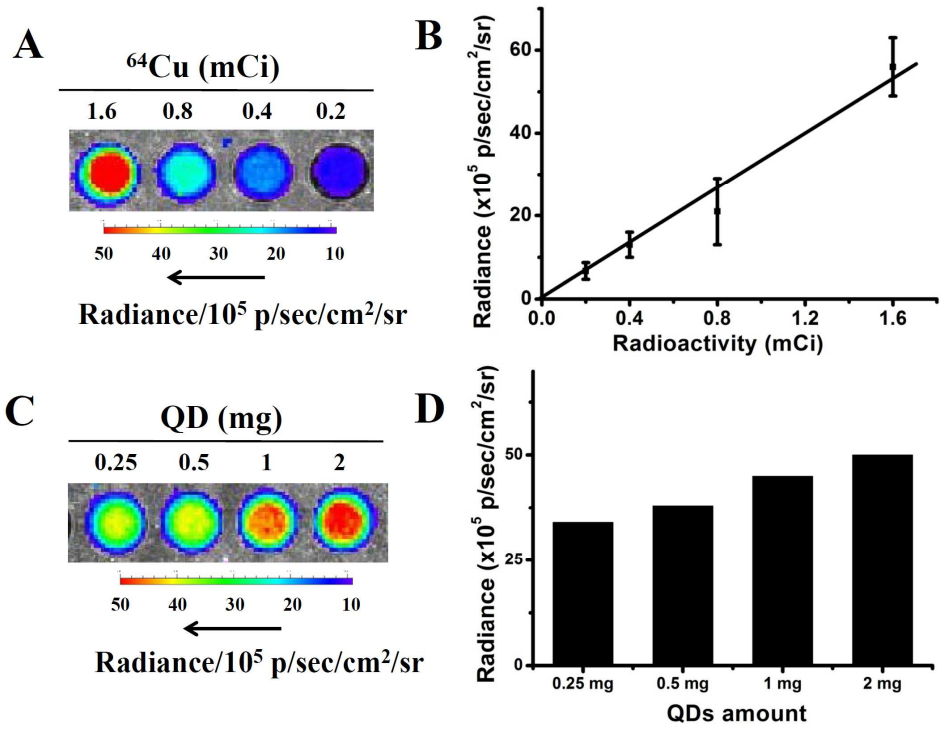

Figure S10 (A) Optical images of ^{64}Cu-doped QDs (0.5 mg) with different amount of radioactivity in a 96-well plate. (B) Plot of photon flux versus radioactivity for ^{64}Cu-doped QDs in a 96-well plate (n=3 per concentration). R^2=0.99. (C) Optical images of ^{64}Cu-doped QDs (1.3 mCi) with different concentrations of QDs in a 96-well plate. (D) Summary of photon flux versus different amount of QDs. More QDs could help convert more photons within the ultraviolet range that are undetectable by the IVIS system into photons at longer wavelengths (via CRET effect) which can be captured by the system.

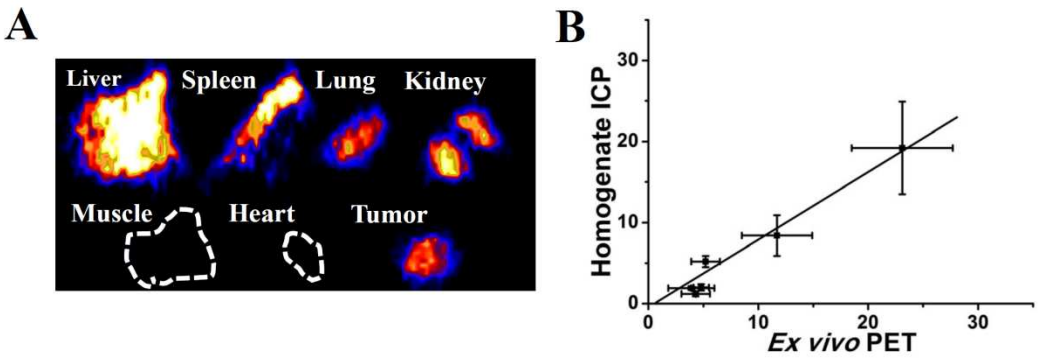

Figure S11. (A) *Ex vivo* PET image of harvested tissues at 45 h after injection of ^{64}Cu-doped QDs. (B) Correlation between heart-, liver-, tumor-, spleen-, lung-, kidney-to-muscle ratios measured by *ex vivo* PET and tissue homogenate ICP. R^2= 0.96.

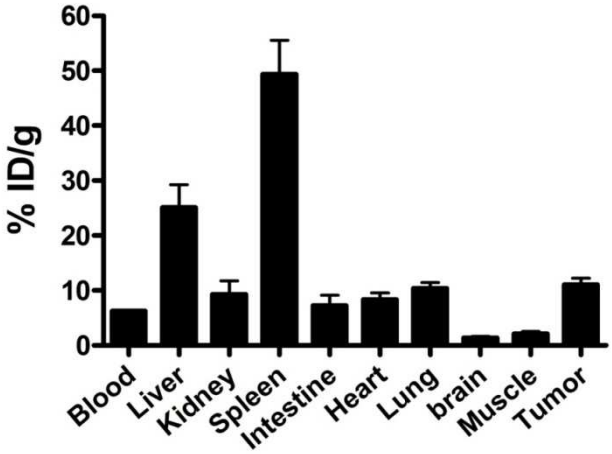

Figure S12. Biodistribution of the ^{64}Cu-doped QDs in mice bearing U87MG tumors at 42 h post-injection.

Table S1. Composition of QD636 after different amount of nonradioactive $CuCl_2$ treatment and 1 mCi $^{64}CuCl_2$ treatment.

Cu to Se weight ratio	Cd: Se: Zn
As-received	13.9 : 1 : 0.75
1: 20	14.0 : 1 : 0.28
1 : 2	11.1 : 1 : 0.1
20 x Cu	0.58 : 1 : 0.08
1 mCi $^{64}CuCl_2$	13.6 : 1 : 0.76
1 mCi $^{64}CuCl_2$ PEGylated	14:1 : 1 : 0.71

Table S2. Inorganic diameter and hydrodynamic size of QDs.

QD sample	Inorganic diameter (TEM)/nm	Hydrodynamic diameter in water (DLS)/nm
QD526-PEG_{5000}	2.1± 0.3	14.1± 1.1
QD580-PEG_{5000}	5.8± 0.5	18.5± 0.9
QD636-PEG_{5000}	11.0± 0.7	28.4± 2.1

Table S3. Tissue-to-muscle ratios measured by different methods

Method	Tissue-to-muscle ratio (SD) (n=3)					
	Heart	Liver	tumor	Spleen	Lung	Kidney
Ex vivo PET	3.9 ± 2.1	11.7± 3.2	5.2± 1.3	23.1± 4.6	4.8± 0.7	4.3± 1.3
Homogenate ICP	1.9 ± 0.2	8.4± 2.5	5.2± 0.7	19.2± 5.7	2.0± 0.4	1.2± 0.3

3. References

(1) Cai, W.; Wu, Y.; Chen, K.; Cao, Q.; Tice, D. A.; Chen, X. *Cancer. Res.* **2006**, *66*, 9673-9681.

(2) Cai, W.; Olafsen, T.; Zhang, X.; Cao, Q.; Gambhir, S. S.; Williams, L. E. Wu, A. M.; Chen, X. *J. Nucl. Med.* **2007**, *48*, 304-310.