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Supporting Information for:

Supramolecular gelatin nanoparticles as matrix metalloproteinases responsive cancer cell imaging probes

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Experimental section

Materials

Gelatin type B (225 bloom) from bovine skin, glutaraldehyde solution (Grade I, 50%), cadmium oxide (CdO, 98.9%), selenium (99%, powder), sulfur (99.9%, powder), 1-octadecene (ODE, 90%), and mercaptopropionic acid (MPA, 99%) were purchased from Sigma Aldrich and used as received. Oleic acid (OA, 80%) and trioctylphosphine (TOP, 90%) were obtained from Acros Organics. SU-8 2050 photoresist was supplied from MicroChem, USA. RTV615 Silicone Potting Compound was received from Momentive Performance Materials (Waterford, NY, USA). Recombinant Human tissue inhibitor of metalloproteinases 2 **(**TIMP2**)** was purchased from Sino Biological (Beijing, China). HT1080 and LO2 cell lines were purchased from Cell Culture Center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). All the other solvents used in the research were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd.

Synthesis of Quantum Dot

As a typical process, a mixture of 1 mmol CdO, 1.5 mmol Zn(acetate)$_2$ and 3 mL oleic acid (OA) in a 50 mL flask were degassed, filled with Ar (five times) and further heated to 18 ^{o}C to obtain a pale yellow solution, then the temperature was adjusted to 10 ^{o}C. 10 mL 1-octadecene (ODE) was added to the system and degassed for 20 min. Then the temperature was elevated to 300 ^{o}C under Ar flow, yielding a clear solution of Cd(OA)$_2$ and Zn(OA)$_2$. At the high temperature condition, 0.5 mmol Se dissolved in 1.5 mL trioctylphosphine (TOP) was swiftly injected into the system and the reaction flask was heated to 310 ^{o}C for further growth of the Cd_{1-x}ZnxSe core. After reaction duration of 10 min, the reaction system was allowed to cool to 235 ^{o}C, then 1.8 mmol S dissolved in 1.2 mL TOP was swiftly injected into the reaction mixture and the temperature was elevated again to 260 ^{o}C immediately for the growth of the ZnS shell. The reaction was stopped by removing the heating mantle and the solution was allowed to cool down to 60 ^{o}C then 10 mL hexane and excessive acetone was added for the purification of the QDs (twice) and finally the products were re-dispersed in hexane for further treatment. Replacement of the hydrophobic OA ligands by hydrophilic MPA could realize well dispersion of QDs in water. Typically, a mixture of OA capped QDs (10 mg) and excessive of MPA (2 mL) dissolved in 10 mL of chloroform were loaded in a 50 mL round flask. The mixture were refluxed at 60 ^{o}C in inert atmosphere for 1.5 h and then cooled down to room temperature when the solution became cloudy. Afterward centrifugation of the solution at 15000 rpm for 10 min resulted in QDs precipitation (washed twice with chloroform to extract excess MPA and substituted OA). The precipitation was subsequently dispersed in water media and the pH was adjusted to ~7.5, yielding water-soluble MPA coated and negatively charged QDs. The surface charge density was -7.8 ± 1.4 mV was obtained by zeta potential measurement.

Synthesis of Gelatin Nanoparticle

Gelatin nanoparticles were prepared from a modification of the two-step desolvation method[1, 2]. A pretreatment was accomplished as follows. Gelatin type B (0.625 g) was added to 12.5 mL of deionized water and heated at 50 °C until dissolution. The solution was then quickly removed from heat, and 12.5 mL of acetone was added to the solution at 6.0 mL/min while stirring at 300 rpm. After the acetone addition was completed, the stirring was turned off. After

exactly 1 min, the supernatant containing the low molecular weight gelatin fraction was removed. 12.5 mL of QDs solution (83 nmol/L) was added to the remaining precipitate and heated again to 50 °C until dissolution. The pH of the solution was adjusted to 10.0 with a 2M NaOH solution. The obtained nanoparticles were collected by centrifugation and washed three times with deionized water.

Fabrication of microfluidic device

The device was fabricated in polydimethylsiloxane (PDMS) using a standard soft-lithography and replica molding method[3]. Briefly, masters were prepared with features of SU-8 photoresist in bas-relief on silicon wafers. PDMS pre-polymers were poured over the wafers and cured for the microstructured PDMS layers. Then the PDMS layers were lifted off and punched holes for inlets and outlets of the fluids. After an irreversible seal was formed between the PDMS layer and a glass substrate, this assembly produced the required systems of microfluidic channels. The resulting device had one inlet each for water and acetone streams, and one outlet. The acetone stream was split into two in order to achieve two acetone streams at the flow focusing junction. The mixing channel was 50 μm wide, 50 μm high and 2 cm long. The fluidic connections were realized via poly(tetrafluoroethylene) (PTFE) tubes (0.56 mm inner diameter and 1.07 mm outer diameter) connected with 1 mL syringes and 0.4×25 mm stainless-steel needles. The syringes were then mounted on syringe pumps (11plus, Harvard Apparatus Holliston, USA) to complete the fluidic device.

Characterizations

Transmission electron microscope (TEM): The morphology and size of QDs and **QDs⊂SGNs** were examined on a Tecnai G2 20 S-TWIN transmission electron microscope at an acceleration voltage of 200 kV. The TEM samples were prepared by drop 5-μL of QDs and **QDs⊂SGNs** solution onto carbon-coated copper grids. The liquid was removed by a filter paper after 1 min. For **QDs⊂SGNs**, the surface-deposited nanoparticles were negatively stained with 2% uranyl acetate for 45 s before the TEM studies. The quality of the TEM images of **QDs⊂SGNs** was improved by adjusting the brightness and contrast using photoshop CS.

Dynamic light scattering (DLS): Hydrodynamic particle diameter were performed with a Zetasizer Nano instrument (Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.) equipped with a 10-mW helium-neon laser (λ = 632.8 nm) and thermoelectric temperature controller. The measurements were performed at 25°C with a detection angle of 90°. The distribution fits and zeta potential were subsequently correlated by the Dispersion Technology Software (Malvern Instruments Ltd. U.K.) by averaging over three measurements.

Estimation of the effect of flow ratio on mixing time

The mixing time (τ_{mix}) can be estimated from the diffusion timescale as[4, 5]:

$$\tau_{mix} \sim \frac{w_f^2}{4D} \approx \frac{w^2}{9D} \frac{1}{(1+1/R)^2}$$

For w =50 μm, D=2×10^3 $\mu m^2/s$

as flow ratio range from 1-6, the τ_{mix} decreased from 3.48ms to 0.281ms.

Table S1 Estimation of mixing time for different flow ratios

flow ratio	1	2	3	4	6
τ_{mix} (ms)	3.48	1.54	0.869	0.556	0.281

Fluorescence imaging and flow cytometry quantification

All cells were cultured in regular growth medium consisting of high glucose Dulbecco's modified Eagle's medium (DMEM) (Hyclone), supplemented with 10% FBS (Hyclone) at 37 °C in a humidified atmosphere of 5% CO_2. The cell culture media were supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin. Cell internalization studies were assessed via confocal laser scanning microscope (CLSM) (Zeiss LSM710META, Germany). Human fibrosarcoma HT1080 cells were plated on glass-bottom dishes (35 mm, MatTek Corporation) in the day before exposure to QDs**, QDs⊂SGNs, QDs⊂SGNs/MMP** inhibitor. For the inhibition test, 10 μL of TIMP (0.1 mg/mL) was pre-treated to block MMP. After incubation with these nanoparticles for 3 h, cells were washed with PBS for 3 times and then fixed with 4% paraformaldehyde for 15 min. Confocal fluorescence microscope was utilized to monitor the fluorescence signal inside the cells with excitation wavelength of 370 nm. For comparison, human normal liver LO2 cells were also incubated with **QDs** and **QDs⊂SGNs** as described in HT1080 cells. To compare the uptake of HT1080

cells and LO2 cells with QDs and QDs⊂SGNs, the mean fluorescent intensity within the cells was measured using Zeiss software. Three areas were selected and at least 20 cells were measured from each sample.

HT1080 cells were co-incubated with 100 μg/mL of **QDs⊂SGNs** for 3 h, and then washed, stained with 10 μM LysoTracker Green DND-26 (Invitrogen) for 10 min and observed on a CLSM at wavelengths of 370 and 504 nm.

For flow cytometry quantification, the adherent cells were detached by trypsin treatment. The fluorescence from individual cells was detected with a flow cytometer (BD FACS Caliber, CA) equipped with a 488 nm argon laser. At least 10 000 cells were measured from each sample. The mean cell fluorescence intensity was normalized to that of the untreated cells in PBS buffer. Each experiment was performed three times.

MMPs responsive release of QDs from QDs⊂SGNs

The fresh prepared **QDs⊂SGNs** was incubated in the presence of MMP-2 (10 nmol/L) or in the absence of MMP-2 in PBS buffer solution (pH 7.2). The supernatants of the solutions were collected by microcentrifugation and the fluorescence intensity (at 640 nm) of supernatants were measured.

Cytotoxicity of QDs⊂SGNs

HT1080 cells and LO2 cells were cultured as described above. The cytotoxicity of QDs and **QDs⊂SGNs** was assessed by the CCK-8 (cell counting kit-8) assay. HT1080 cells and LO2 cells were seeded at 5×10^3 cells/well in 96-well plates and pre-incubated for 24 h before the assay. Then, the medium was replaced with fresh medium containing QDs or **QDs⊂SGNs** at different concentrations. After 48 h incubation, the solution of CCK-8 (10 μL) was added to the wells followed by 4 h incubation at 37 ℃ in a humified atmosphere containing 5% CO_2. Finally, the absorbance values of the cells per well were determined with a microplate reader at 450 nm for analyzing the cell viability. The background absorbance of the well plate was measured and subtracted. The cytotoxicity was calculated by dividing the optical density values (**OD**) of the treated groups (T) by the **OD** of the controls (C) ([T/C×100%]).

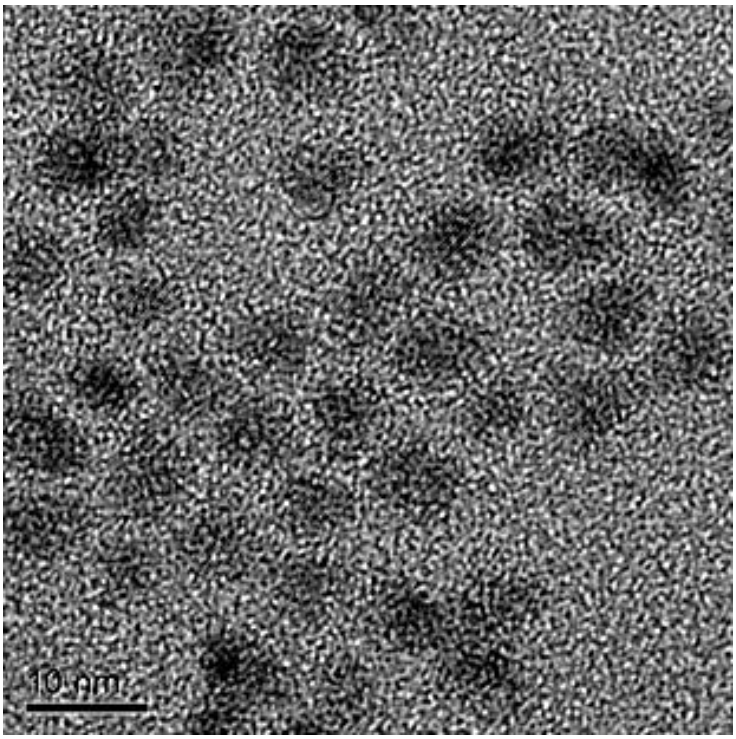

Fig. S1 The TEM image of QDs.

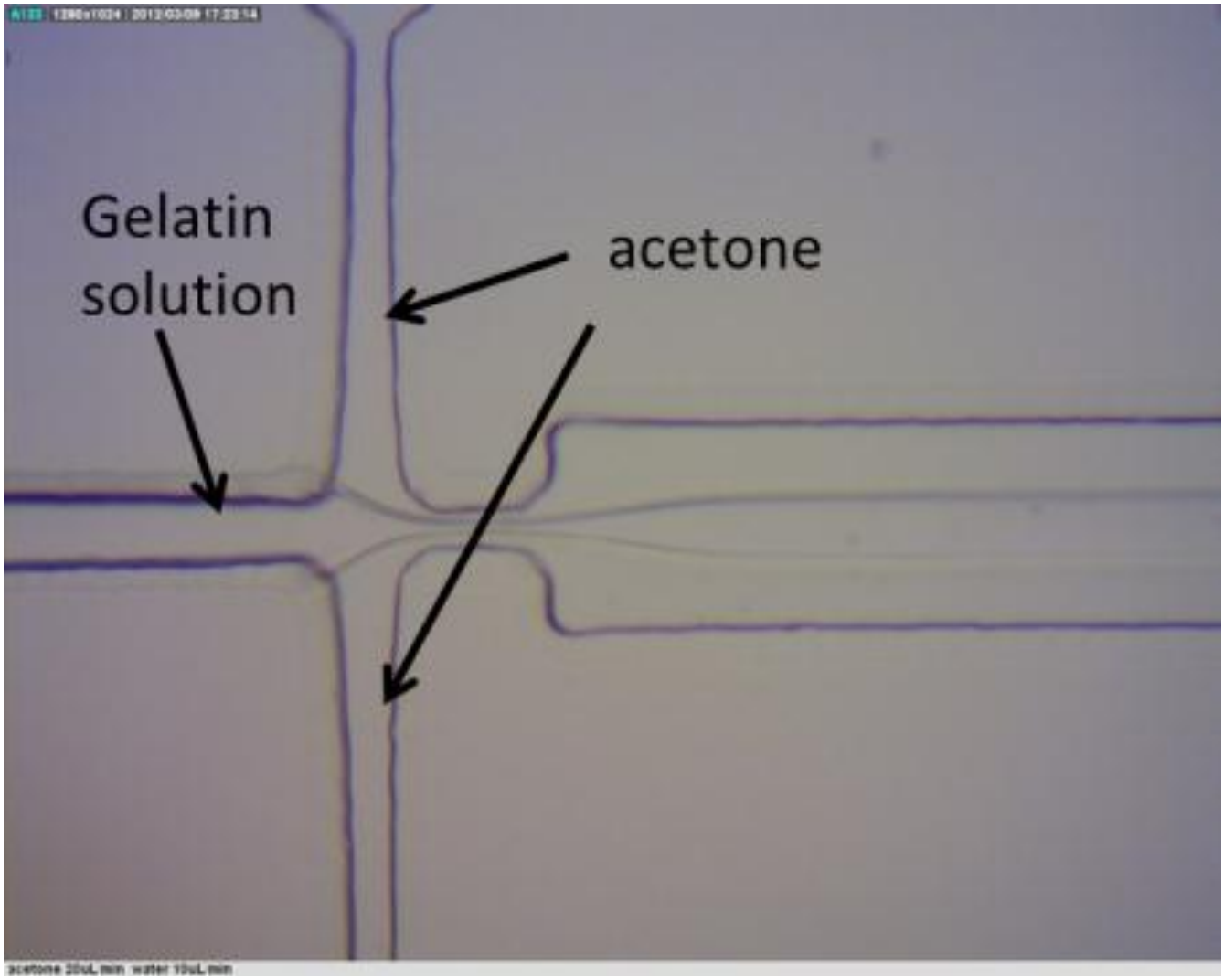

Fig. S2 Photograph of the T-shape microfluidic device.

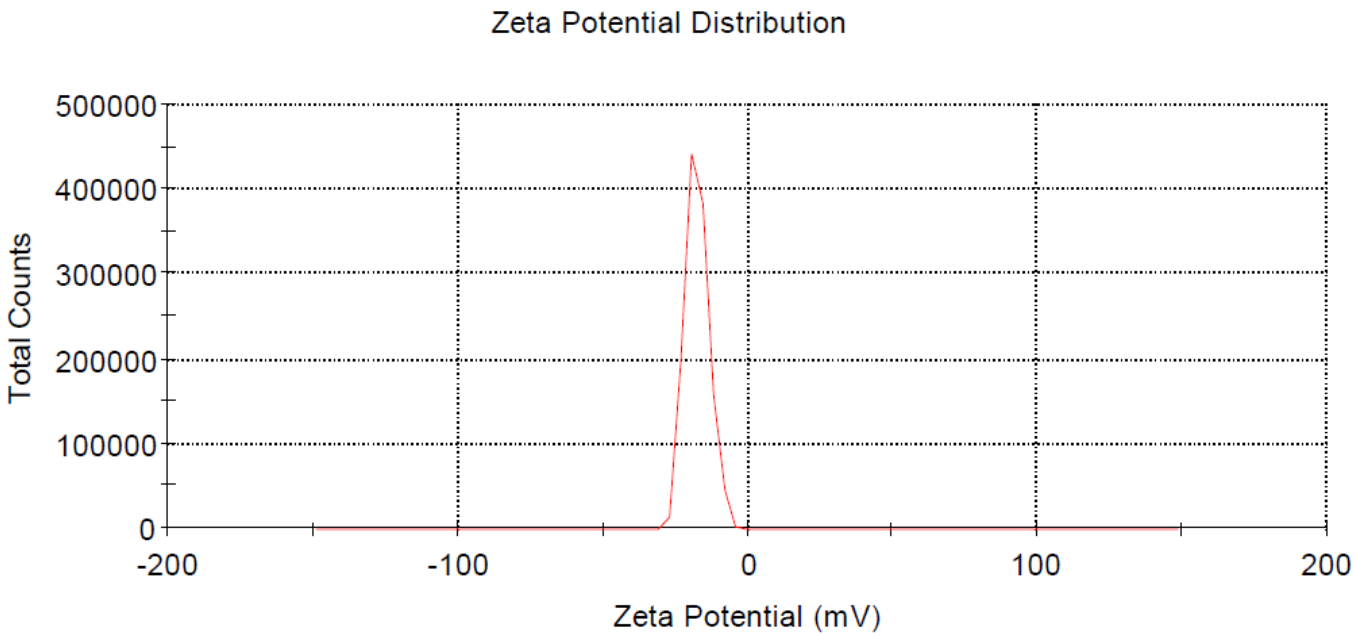

Fig. S3 Zeta potential of **QDs⊂SGNs** in PBS solution (pH 7.2).

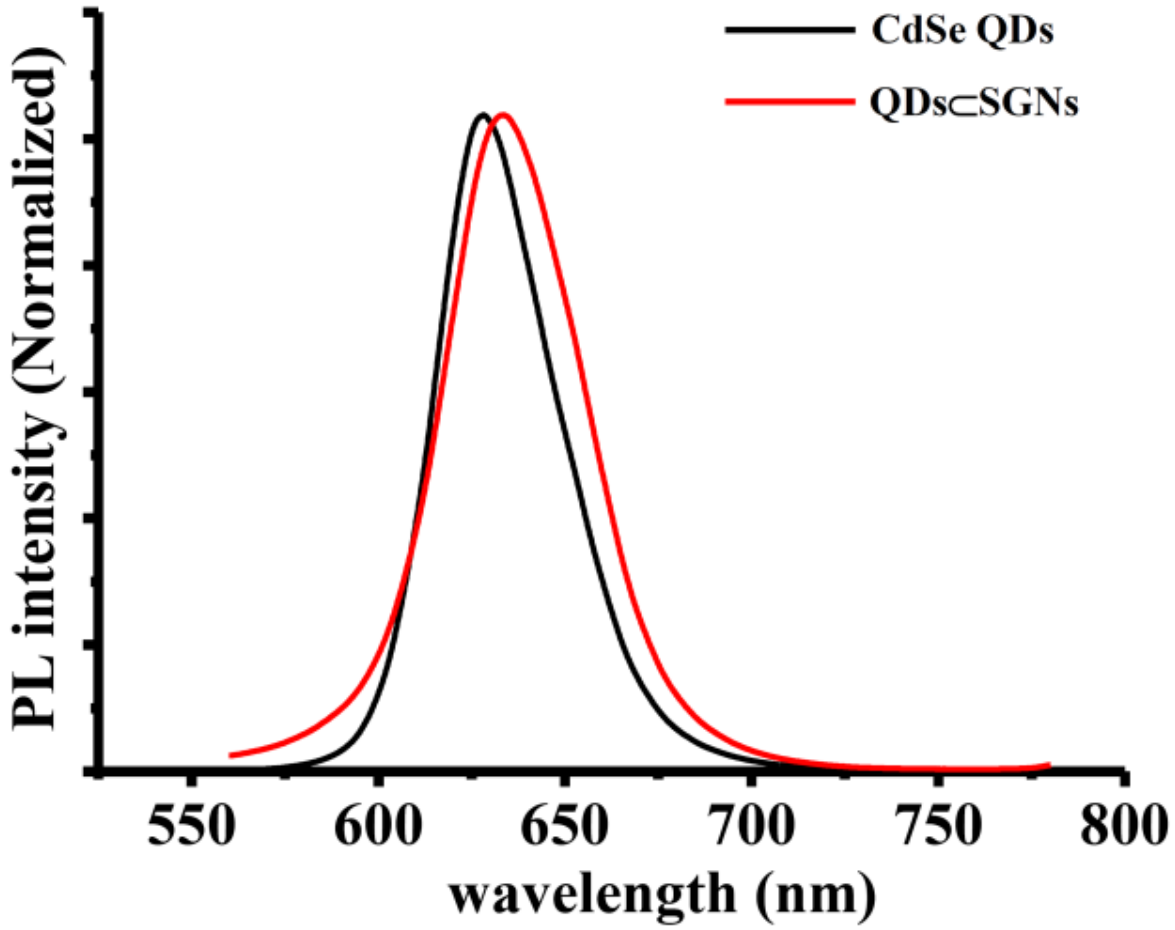

Fig. S4 Normalized fluorescence spectra of CdSe QDs and **QDs⊂SGNs**. The red shift from 635 to 640 nm may comes from a increase of the dipole interaction between immobilized QDs and gelatin macromolecules[6].

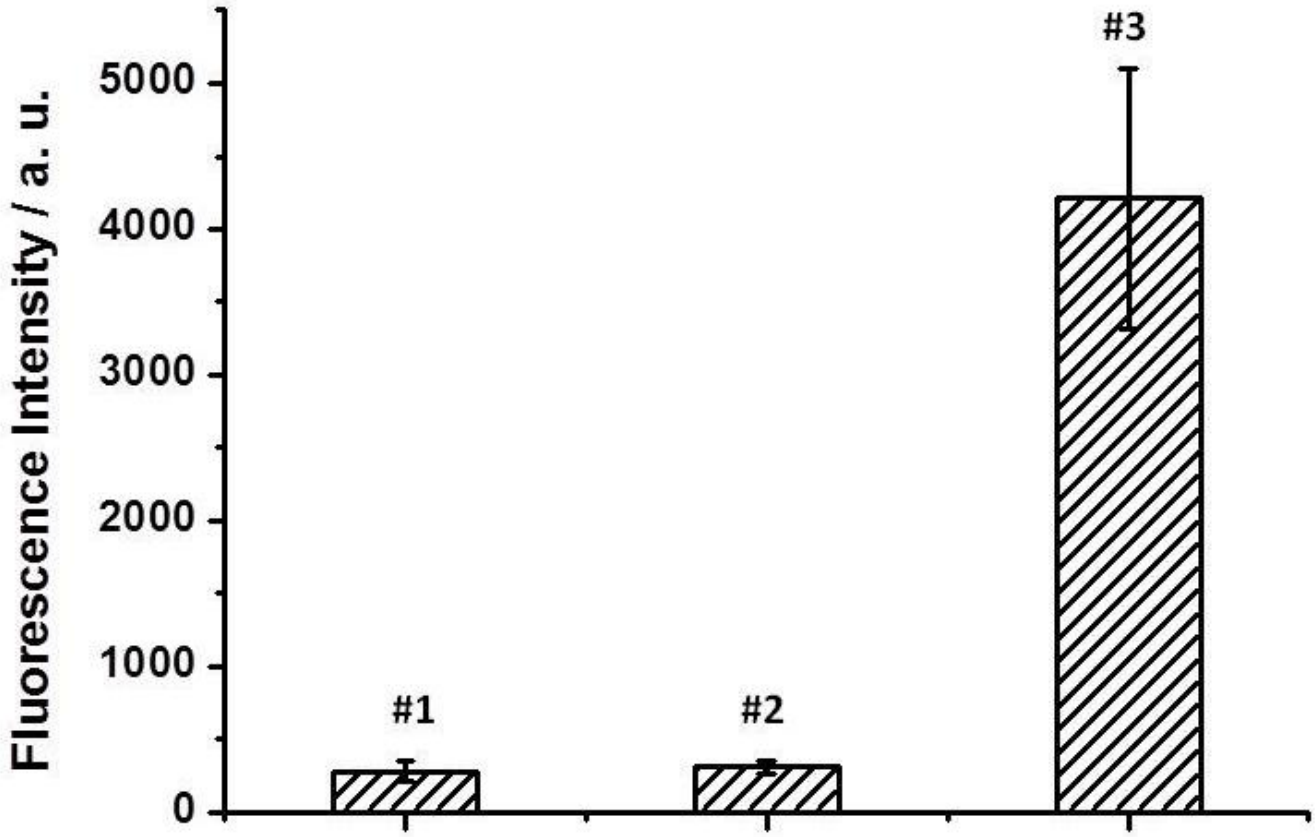

Fig. S5 The leakage experiments of **QDs⊂SGNs** in PBS buffer solution (pH 7.2). The supernatants of the solutions were collected by microcentrifugation and the fluorescence intensity (at 640 nm) of supernatants of (#1) fresh prepared **QDs⊂SGNs**, (#2) 16 h post-incubated **QDs⊂SGNs** and (#3) 16 h post-incubated **QDs⊂SGNs** in the presence of MMP2 (10 nmol/L). Error bars are obtained from three independent experiments.

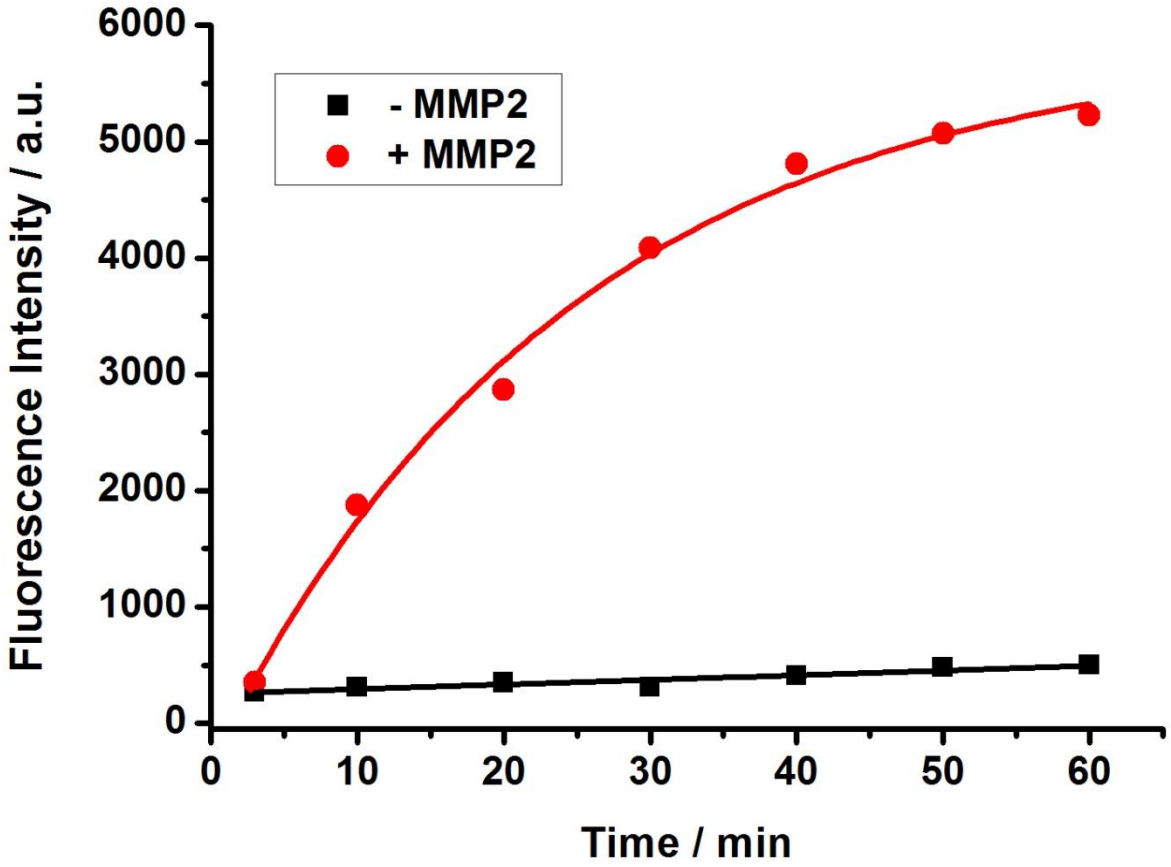

Fig. S6 The time-dependent release of QDs from **QDs⊂SGNs** in the presence of MMP-2 (10 nmol/L, red curve) and in the absence of MMP-2 (black curve) in PBS buffer solution (pH 7.2).

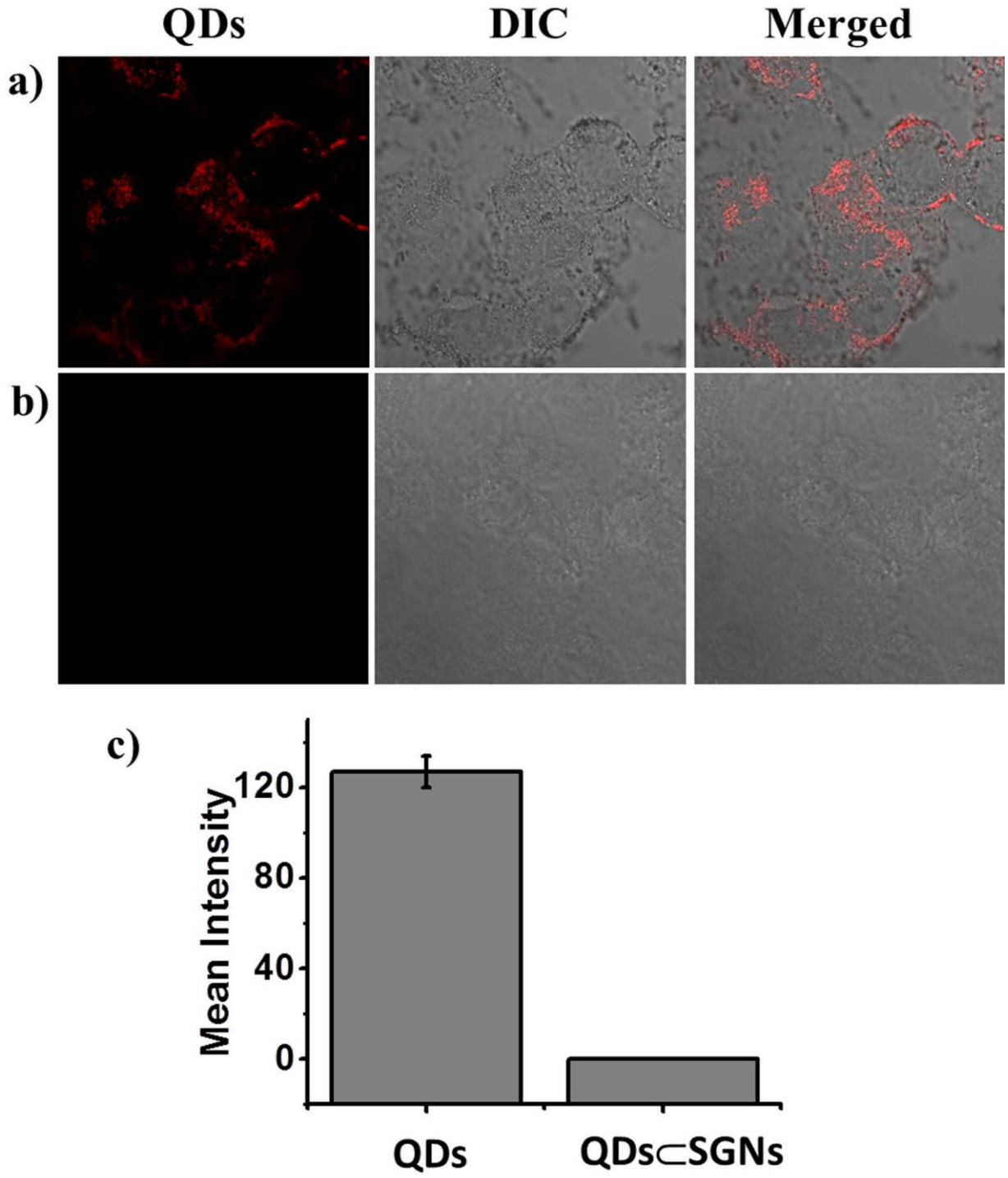

Fig.7 Confocal microscopy imaging of human normal liver LO2 cells cultured with QDs (a) and QDs⊂SGNs (b) for 3 h. c) Corresponding mean fluorescent intensity of LO2 cells measured using Zeiss software.

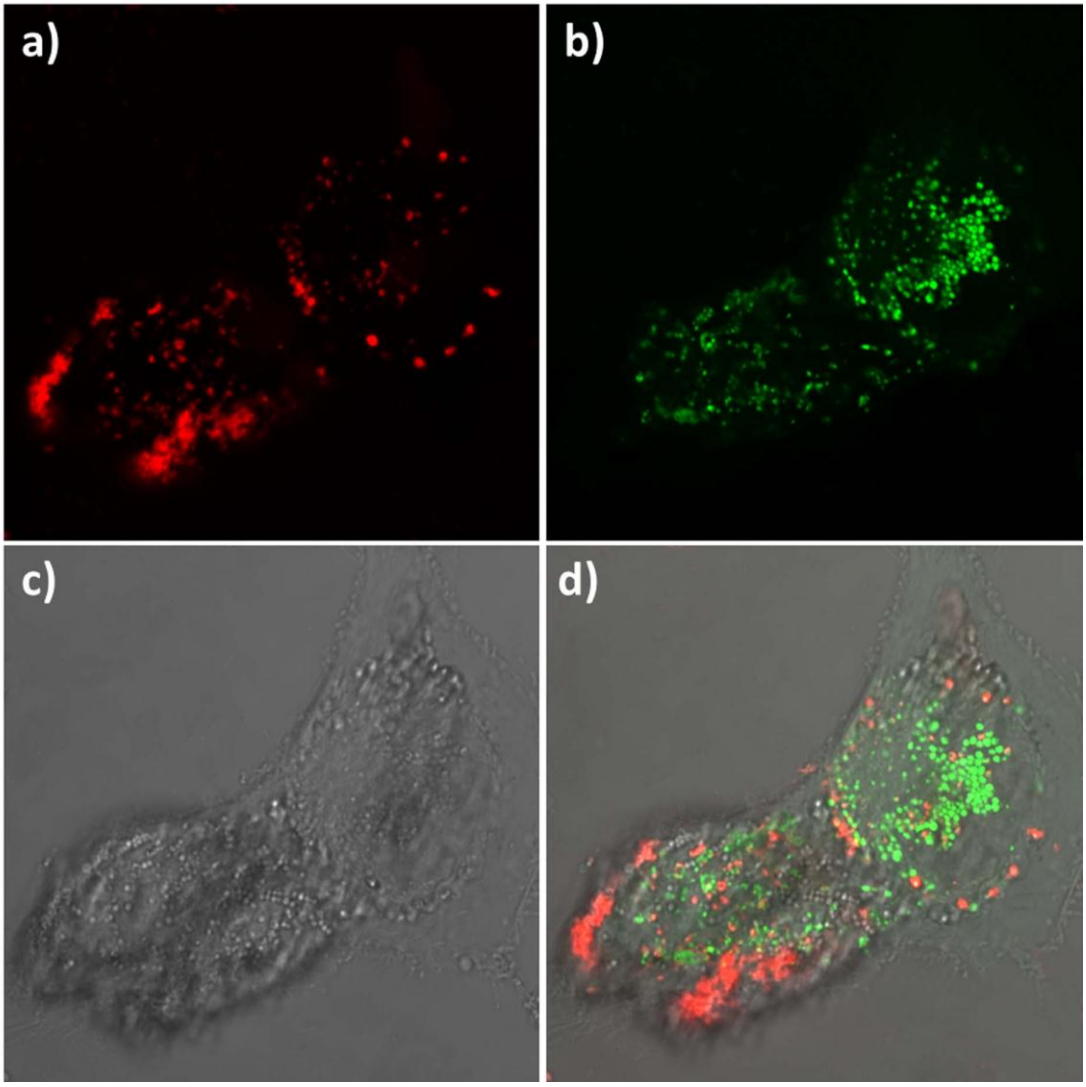

Fig. S8 Colocalization of lysosome with internalized QDs in HT1080 cells after co-incubation for 3 h. a) Red fluorescence shows internalized QDs. b) Green fluorescence shows lysosomes staining with Lyso-Tracker Green. c) Corresponding transmission image. d) Merged image of a), b) and c).

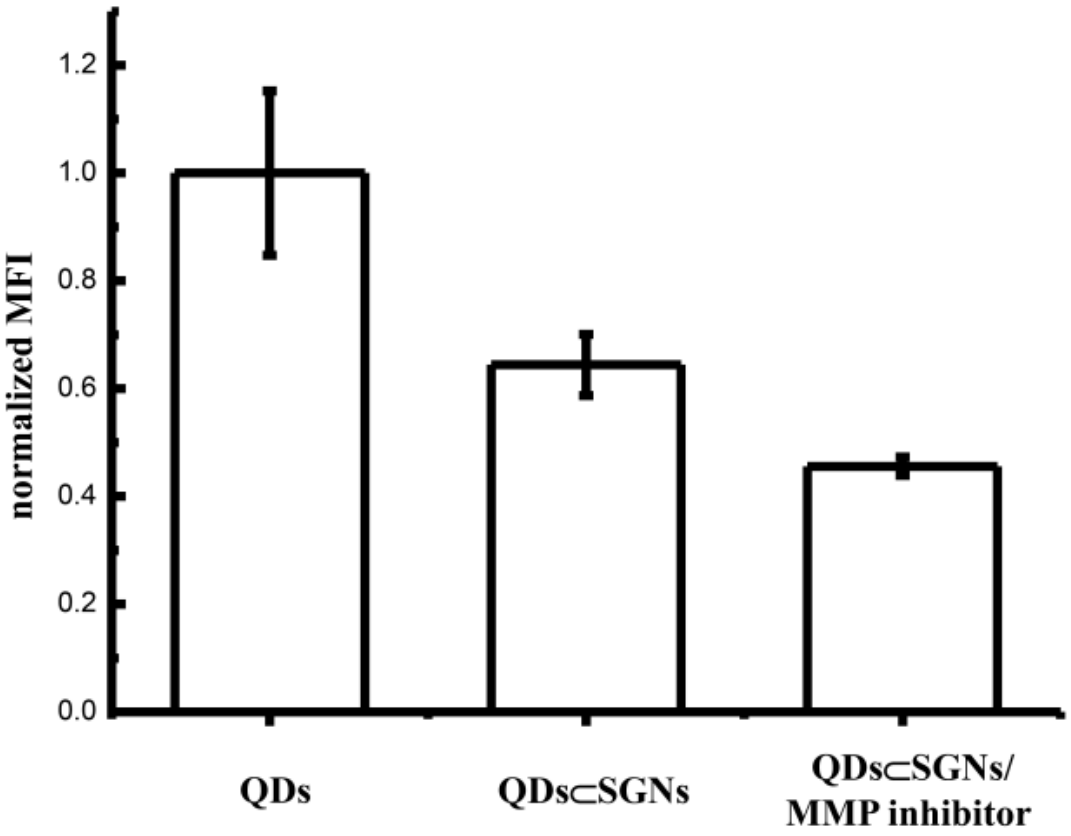

Fig. S9 Mean fluorescence intensity (MFI) of HT1080 cells incubated with **QDs, QDs⊂SGNs, QDs⊂SGNs/MMP** inhibitor . The values were normalized to QDs endocytosis in HT1080 cells (n = 3; mean ± SD).

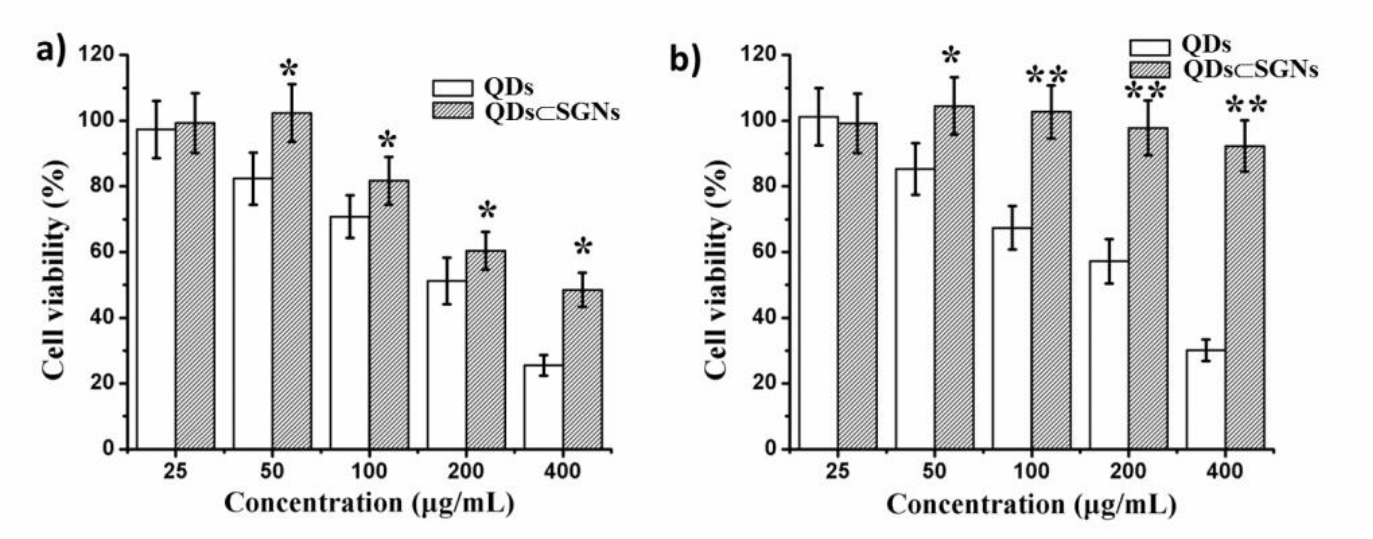

Fig. S10 Cell viability assay of a) HT1080 cells and b) LO2 cells after treatment with different concentrations of **QDs and QDs⊂SGNs** for 48 h. Asterisk (*) or double asterisk (**) denotes statistical significance. *, $p < 0.05$; **, $p < 0.01$.

References

1. K. L. C. J. Coester, H. Von Briesen, J. Kreuter, *J. Microencapsul.*, 2000, **17**, 187-193.
2. C. Wong, T. Stylianopoulos, J. Cui, J. Martin, V. P. Chauhan, W. Jiang, Z. Popovic, R. K. Jain, M. G. Bawendi and D. Fukumura, *Proc. Natl. Acad. Sci.*, 2011, **108**, 2426-2431.
3. X. H. Ji, N. G. Zhang, W. Cheng, F. Guo, W. Liu, S. S. Guo, Z. K. He and X. Z. Zhao, *J. Mater. Chem.*, 2011, **21**, 13380-13387.
4. R. Karnik, F. Gu, P. Basto, C. Cannizzaro, L. Dean, W. Kyei-Manu, R. Langer and O. C. Farokhzad, *Nano Lett.*, 2008, **8**, 2906-2912.
5. T. M. Squires and S. R. Quake, *Rev. Mod .Phys.*, 2005, **77**, 977-1026.
6. H. Döllefeld, H. Weller and A. Eychmüller, *Nano Lett.*, 2001, **1**, 267-269.