Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

SUPPLEMENTARY INFORMATION

Improving Crossing of Multiple Bio-delivery Barriers By a Novel Bio-interface Design Based On Hydrophobic Nanoparticle Surface

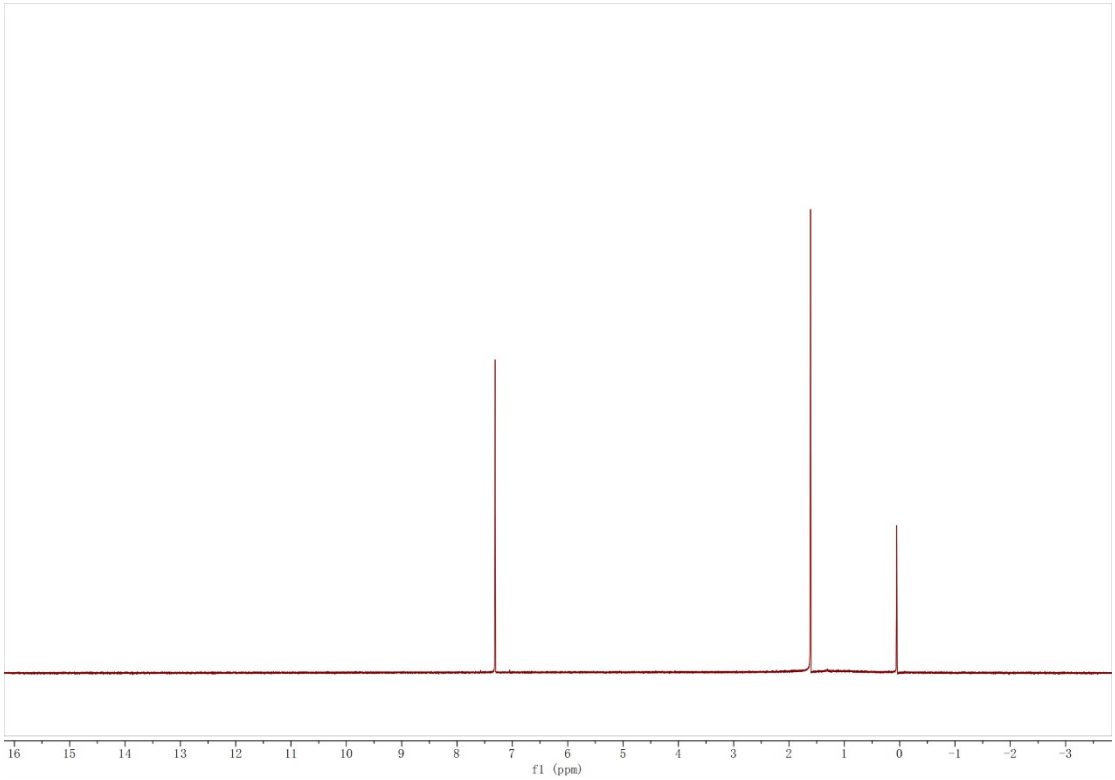
Jie Dai, Zixing Xu, Jinhua Xu, Huoyue Lin, Xuan Yang, Jun Wang, Gang Ruan

Corresponding author: Gang Ruan, Gang.Ruan@xjtlu.edu.cn.

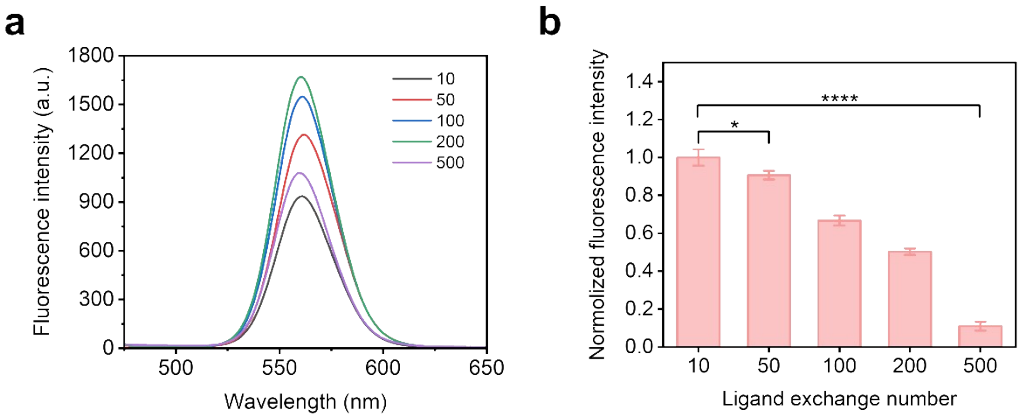
This Supplementary Information file contains the following:

Supplementary Figures 1-9.

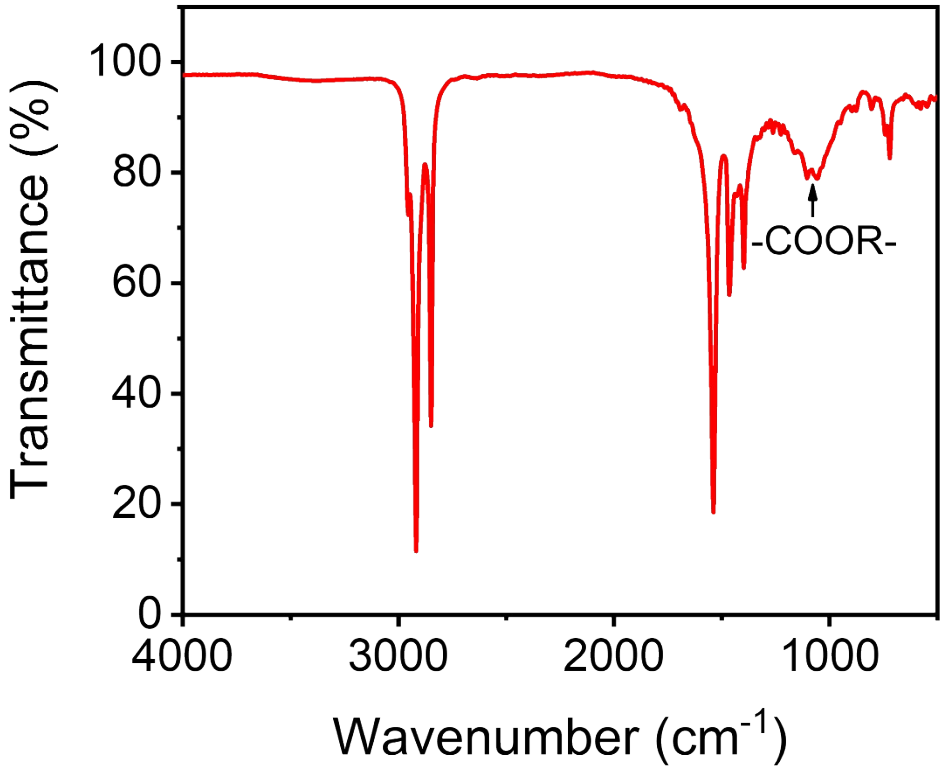
Descriptions of Supplementary Videos 1-5.



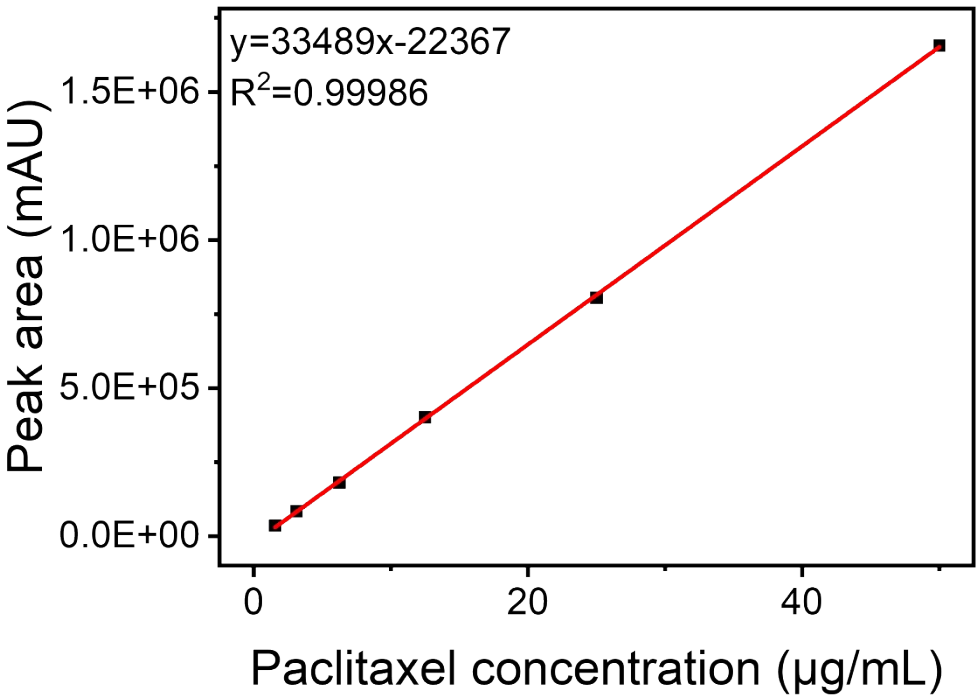
Supplementary Figure 1 NMR spectrum of the solvent used for NMR analysis. The peak of $CDCl_3$ is at 7.26 ppm, the peak of water is at 1.56 ppm.



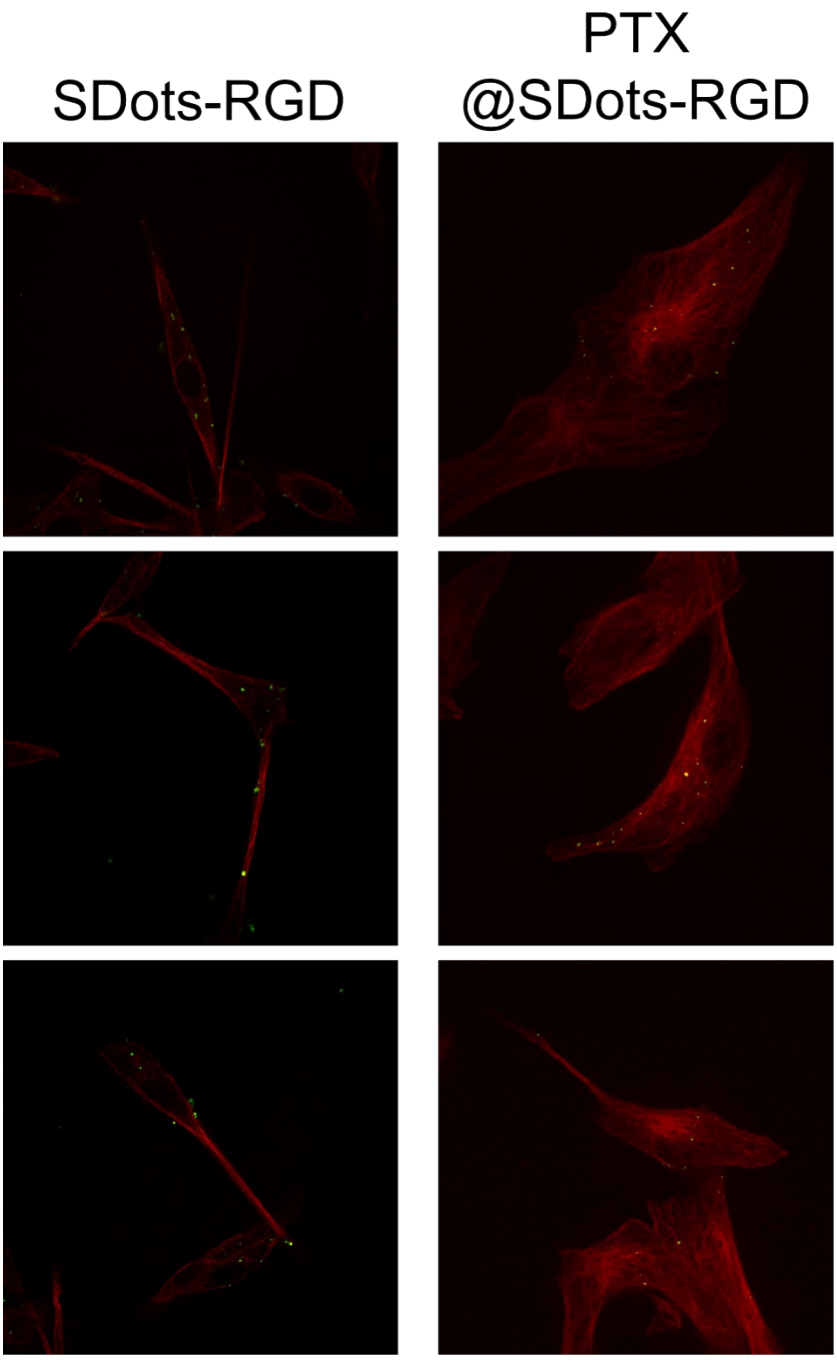
Supplementary Figure 2 Effect of hydrophobic coverage of QDs-RGD on amount of cellular uptake of QDs. U87MG cells were used. Cellular uptake was quantified by flow cytometry using the fluorescence emitted by QDs. 'Ligand exchange number' refers to the number of ligands per QD added to the ligand exchange reaction. Larger ligand exchange number corresponds to less hydrophobic coverage. Ligand exchange number 10 corresponds to the SDots-RGD used in the other parts of this work (*e.g.,* transcytosis, drug delivery). **(a)** shows the fluorescence spectra of QDs-RGD after ligand exchange (before cellular uptake experiment). Solvent: water with 1% DMF. The ligand exchange process changed the fluorescence intensity. This fluorescence change needed to be taken into account when quantifying cellular uptake using the fluorescence intensity of QDs. **(b)** shows the quantification of cellular uptake after taking account of the fluorescence intensity. As can be seen, larger hydrophobic coverage (lower ligand exchange number) yielded greater cellular uptake. *, $P < 0.05$; ****, $P < 0.0001$.



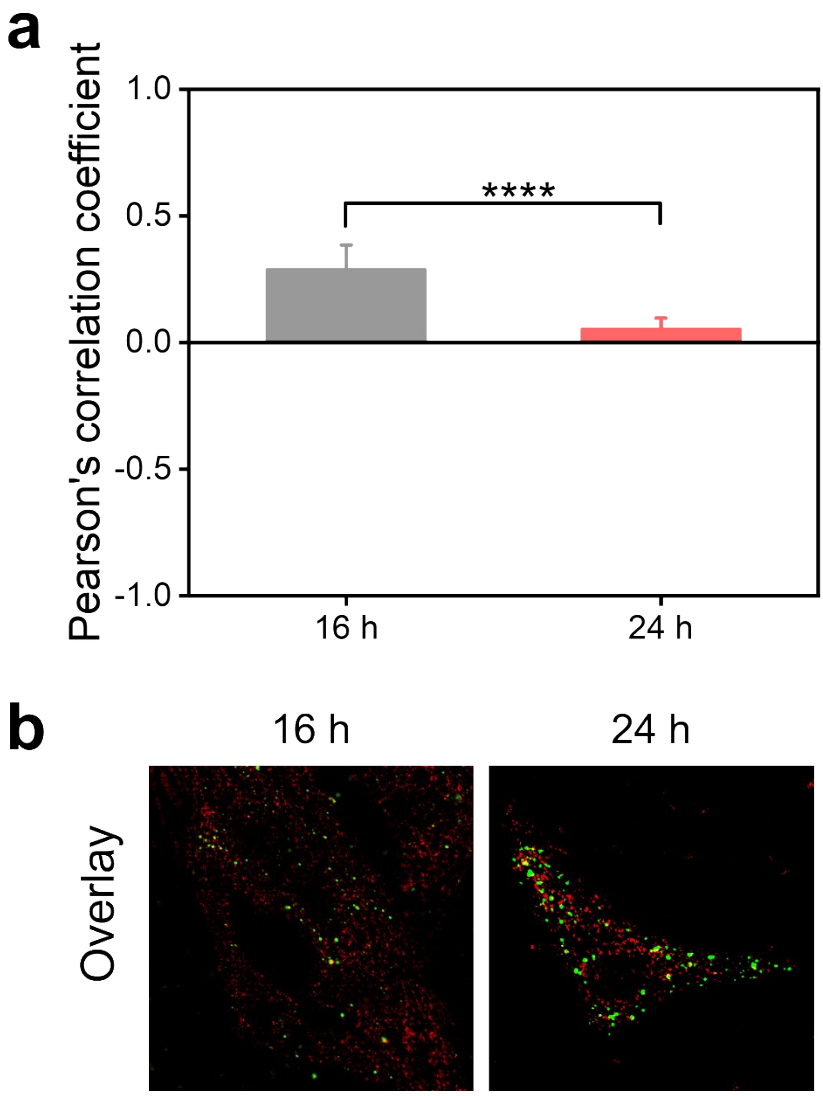
Supplementary Figure 3 Fourier-transform infrared spectroscopy (FTIR) of PTX@SDots-RGD. The presence of PTX in PTX@SDots-RGD is confirmed by the presence of -COOR- peak in the spectrum (only PTX has the peak).



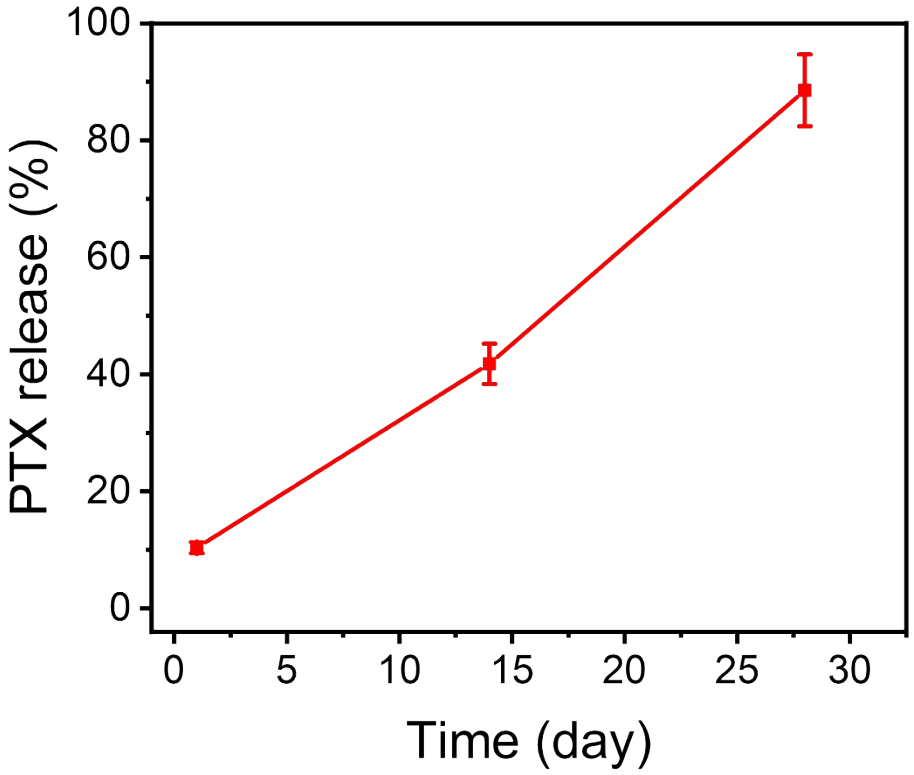
Supplementary Figure 4 Calibration curve for the concentration measurement of paclitaxel (PTX). The measurement was performed by high performance liquid chromatography (HPLC). The experimental parameters used for the HPLC measurements are as follows. Mobile phase 60% Methanol and 40% water (with 5% Acetonitrile); flow rate 0.8 mL/min; temperature 30 ºC; injection volume 1 μL; detection wavelength 227 nm.



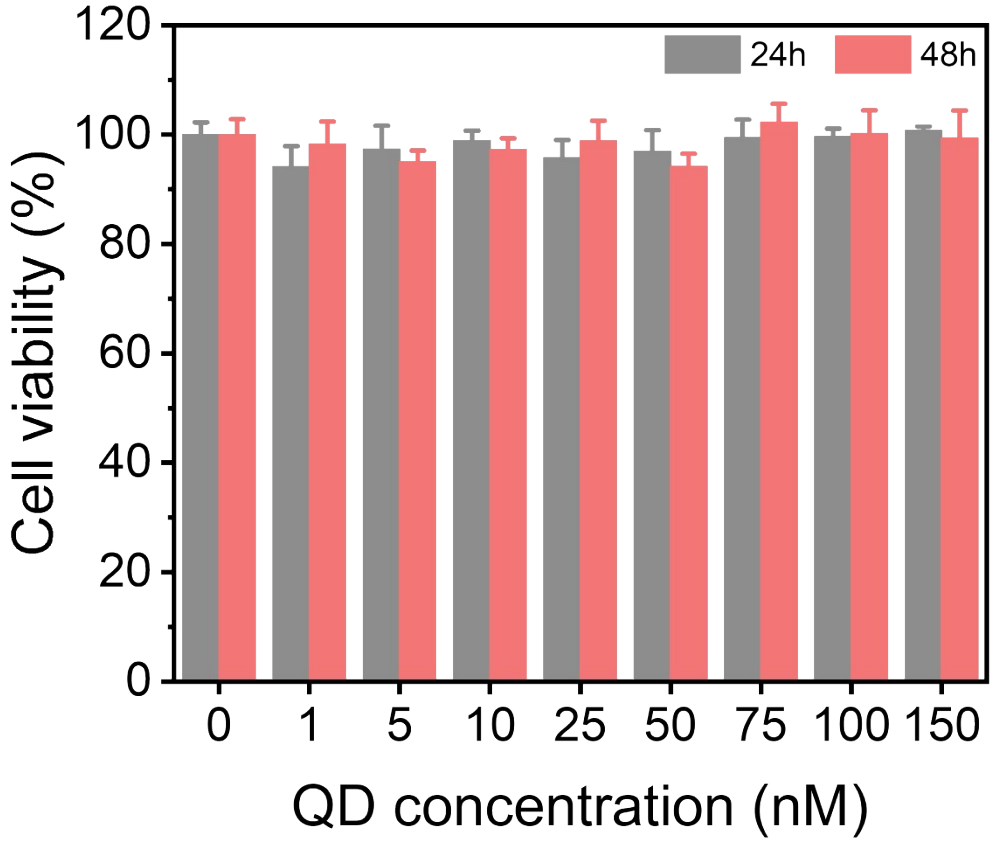
Supplementary Figure 5 Additional results of the colocalization study of microtubules with nanoparticles (SDots-RGD *vs*. PTX@SDots-RGD). The cells were incubated with the nanoparticles for 6 h before being fixed for fluorescent staining of microtubules. Microtubule: green; QD: red. Colocalization between microtubule (green) and QD (red) produced yellow color.



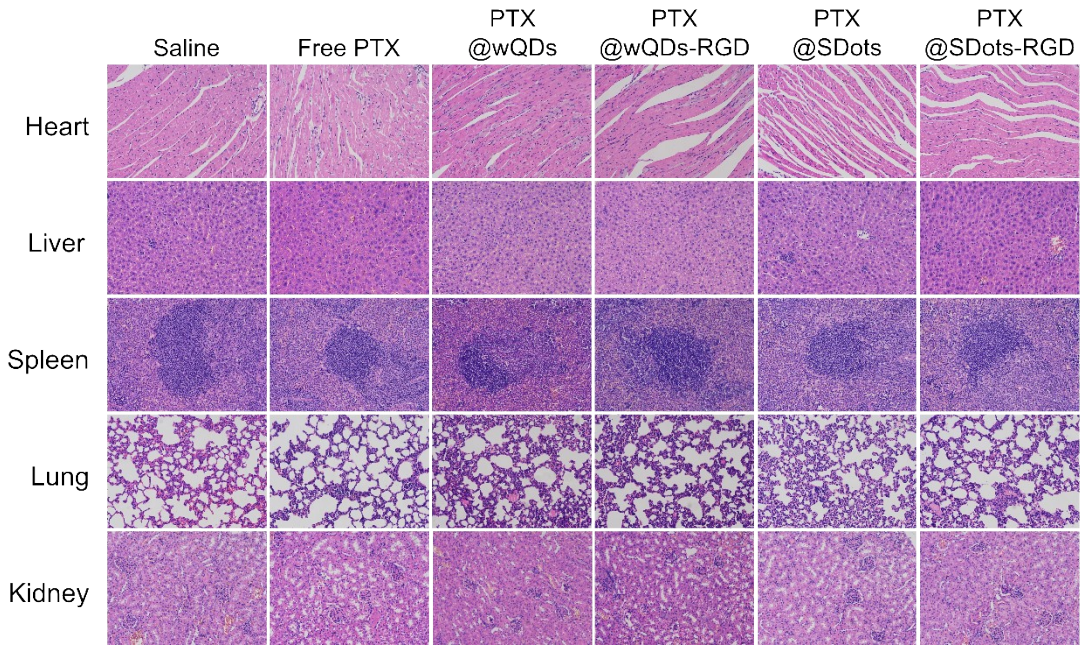
Supplementary Figure 6 Colocalization study of PTX@SDots-RGD with intracellular vesicles. The QDs in PTX@SDots-RGD show green fluorescent color. DiR (general lipophilic vesicle dye) shows red color. **(a)** shows quantification result (Pearson's correlation coefficient) at two different incubation times: 16 h, 24 h. n = 25 cells. ****, $P < 0.0001$. **(b)** shows the representative confocal fluorescent images.



Supplementary Figure 7 *In vitro* PTX release kinetics from PTX@SDots-RGD: from 1 day to 30 days. The result within 1 day is shown in Figure 4c.



Supplementary Figure 8 Cell viability of U87MG cells after incubating with SDots-RGD. Cell viability was assessed by MTT assay. Range of nanoparticle concentration studied: 0-150 nM. Incubation time studied: 24 h, 48 h.



Supplementary Figure 9 Tissue morphology (H&E) analysis of major organs after the treatment of various formulations. The organs were harvested on day 12 of the treatments.

Supplementary Videos

Supplementary Video 1 Fluorescence intermittency (blinking) behavior of SDots-RGD before incubating with cell culture medium. Blinking is a characteristic of single, non-aggregated QDs. Many blinking nanoparticles can be seen.60× objective was used to capture the images. Frame rate of the video 12 frames/second.

Supplementary Video 2 Fluorescence intermittency (blinking) behavior of SDots-RGD after incubating with cell culture medium at 37ºC for 24 h. Blinking is a characteristic of single, non-aggregated QDs. Many blinking nanoparticles can be seen. 60× objective was used to capture the images. Frame rate of the video 12 frames/second.

Supplementary Video 3 Randomly-selected microscopy view #1: Three-dimensional images of SDots-RGD in U87MG cells, based on optical sectioning (confocal imaging), to demonstrate that the fluorescent nanoparticles (SDots-RGD) are indeed inside the cells. SDots-RGD (1 nM) were incubated with the cells for 24 h.

Supplementary Video 4 Randomly-selected microscopy view #2: Three-dimensional images of SDots-RGD in U87MG cells, based on optical sectioning (confocal imaging), to demonstrate that the fluorescent nanoparticles (SDots-RGD) are indeed inside the cells. SDots-RGD (1 nM) were incubated with the cells for 24 h.

Supplementary Video 5 Randomly-selected microscopy view #3: Three-dimensional images of SDots-RGD in U87MG cells, based on optical sectioning (confocal imaging), to demonstrate that the fluorescent nanoparticles (SDots-RGD) are indeed inside the cells. SDots-RGD (1 nM) were incubated with the cells for 24 h.