Supporting Information

Multi-stimuli Responsive Nanoparticulate SN38 Prodrug for Cancer Chemotherapy

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Keywords: nanocarrier; stimuli-sensitive; PEG; SN38; CPT-11

Methods

High performance liquid chromatography (HPLC)

A Waters HPLC system consisting of a 1525 binary HPLC Pump, a 2475 multi λ Fluorescence Detector, a 2998 Photodiode Array Detector and a SunFire™ C18 (4.6×250 mm, 5 μm) column. Data were acquired and analyzed using Breeze software. The mobile phase was a gradient of 60-90% of methanol/water solution at a total flow rate of 0.7 mL min^{-1}. 20 μL sample was injected into the column at 35°C. The UV absorption from 200 nm to 400 nm of the elution was recorded for analysis, while the fluorescence detectors were set as 360 nm for excitation and 400-700 nm for emission, respectively. The linear calibration curves of SN38 were also constructed using the peak areas by linear regression analysis.

Size and size distribution measurements

The size and size distribution of the nanoparticles were measured using a Zetasizer Nano-ZS (Malvern Instruments, UK) with a 633 nm laser light at a scattering angle of 173°. The disposable sizing cuvettes were used for measurements. The results were processed with Dispersion Technology Software version 5.1.

Transmission electron microscope (TEM) observation

PEG-S-S-SN38 nanoparticles were dissolved in water at a concentration of 0.5 mg mL^{-1}. Then a copper grid covered with a nitrocellulose membrane was dipped into the solution and air-dried. Observation was carried out on a Hitachi (H-7000) TEM.

Determination of biodistribution

To predict the possible toxicity to normal organs or tissues and evaluate how the nanoparticles

were cleared, we further investigated the biodistribution of PEG-S-S-SN38 nanoparticles. Nine female nude mice were implanted with 1×10^6 BCap37 cells on their right flanks subcutaneously. When the tumors grew up to a mean volume of around 90 mm^3, the tumor-bearing mice were injected *via* tail vein with PEG-S-S-SN38 nanoparticles at a dosage equivalent to 10 mg kg^{-1} SN38. Then mice were sacrificed at 0.5 h, 2 h and 10 h after treatments, respectively (n=3). 50 μL orbital bleeds were collected into tubes, and mixed gently with 50 μL 0.1 N NaOH. Major organs including heart, kidney, spleen, lung, liver and tumors were excised and washed with 0.9% saline before weighed. All the tissues were cut into small pieces and homogenized. 2 mL PBS solution was added into the sample of liver, then mixed with 200 μL 0.1 N NaOH. Other tissues were immersed in 200 μL PBS solution respectively, then 100 μL 0.1 N NaOH was added. SN38 in liver sample was extracted with 6 mL acetonitrile, while those in bloods and other tissues were extracted with 1 mL acetonitrile. The obtained mixture was centrifuged at 14,000×g for 10 min and 100 μL of supernatant was transferred to an eppendorf tube. Then 100 μL 0.1 N HCl was added, and the solution was centrifuged at 14,000×g for 5 min to obtain the supernatant. Finally, 20 μL of the supernatant was injected for HPLC test. The drug concentrations were determined according to the linear calibration curves.

Figures

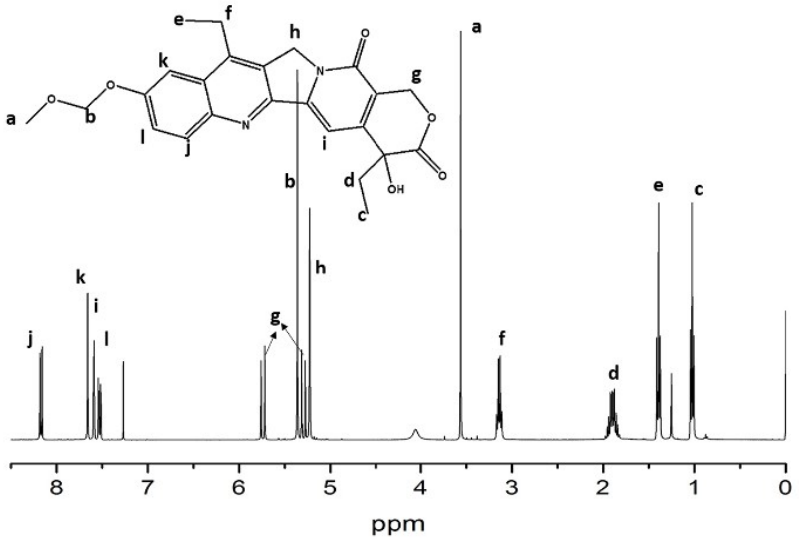

Figure S1. ^{1}H-NMR spectrum of MOM-SN38 (400 MHz, $CDCl_3$, δ): 8.16 (d, J=9.2 Hz, 1H), 7.66 (s, 1H), 7.59 (d, J=2.8 Hz, 1H), 7.54 (q, J1=9.2 Hz, J2=2.4 Hz, 1H), 5.72 (d, J=16.4 Hz, 1H), 5.36 (s, 2H), 5.32 (d, J=16.4 Hz, 1H), 5.23 (s, 2H), 3.56 (s,3H), 3.14 (q, J=7.6 Hz, 2H), 1.9 (m, 2H), 1.39 (t, J=7.6 Hz, 3H), 1.02 (t, J=7.6 Hz, 3H).

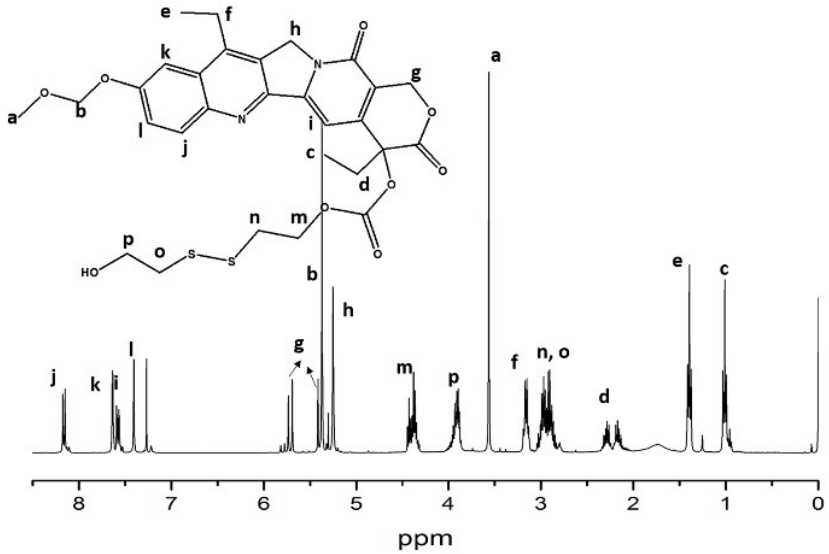

Figure S2. ^{1}H-NMR spectrum of MOM-SN38-S-S-OH (400 MHz, $CDCl_3$, δ):8.15 (d, J=9.2 Hz, 1H), 7.64 (d, J=2.8 Hz, 1H), 7.59 (q, J1=9.2 Hz, J2=2.4 Hz, 1H), 7.4 (s, 1H), 5.7 (d, J=16.4 Hz, 1H), 5.41 (d, J=16.4 Hz, 1H), 5.37 (s, 2H), 5.25 (s, 2H), 4.37 (m, 2H), 3.9 (m, 2H), 3.56 (s, 3H), 3.17 (q, J=7.6 Hz, 2H), 2.96 (m, 4H), 2.28(m, 2H), 1.39 (t, J=7.6 Hz, 3H), 1.01 (t, J=7.6 Hz, 3H).

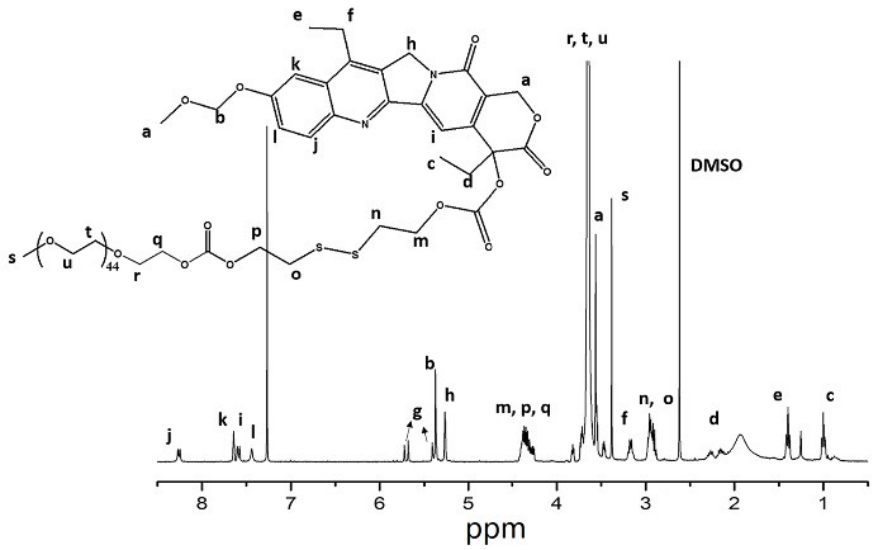

Figure S3. ^{1}H-NMR spectrum of PEG-S-S-SN38 (400 MHz, $CDCl_3$, δ):8.25 (d, J=9.2 Hz, 1H), 7.64 (d, J=2.8 Hz, 1H), 7.59 (q, J1=9.2 Hz, J2=2.4 Hz, 1H), 7.44 (s, 1H),5.7 (d, J=16.4 Hz, 1H), 5.41 (d, J=16.4 Hz, 1H), 5.37 (s, 2H), 5.26 (s, 2H), 4.36 (m, 6H), 3.41-3.85 (m, 178H),3.38 (s, 3H), 3.17 (q, J=7.6 Hz, 2H), 2.94 (m, 4H), 2.25(m, 2H), 1.39 (t, J=7.6 Hz, 3H), 1.01 (t, J=7.6 Hz, 3H).

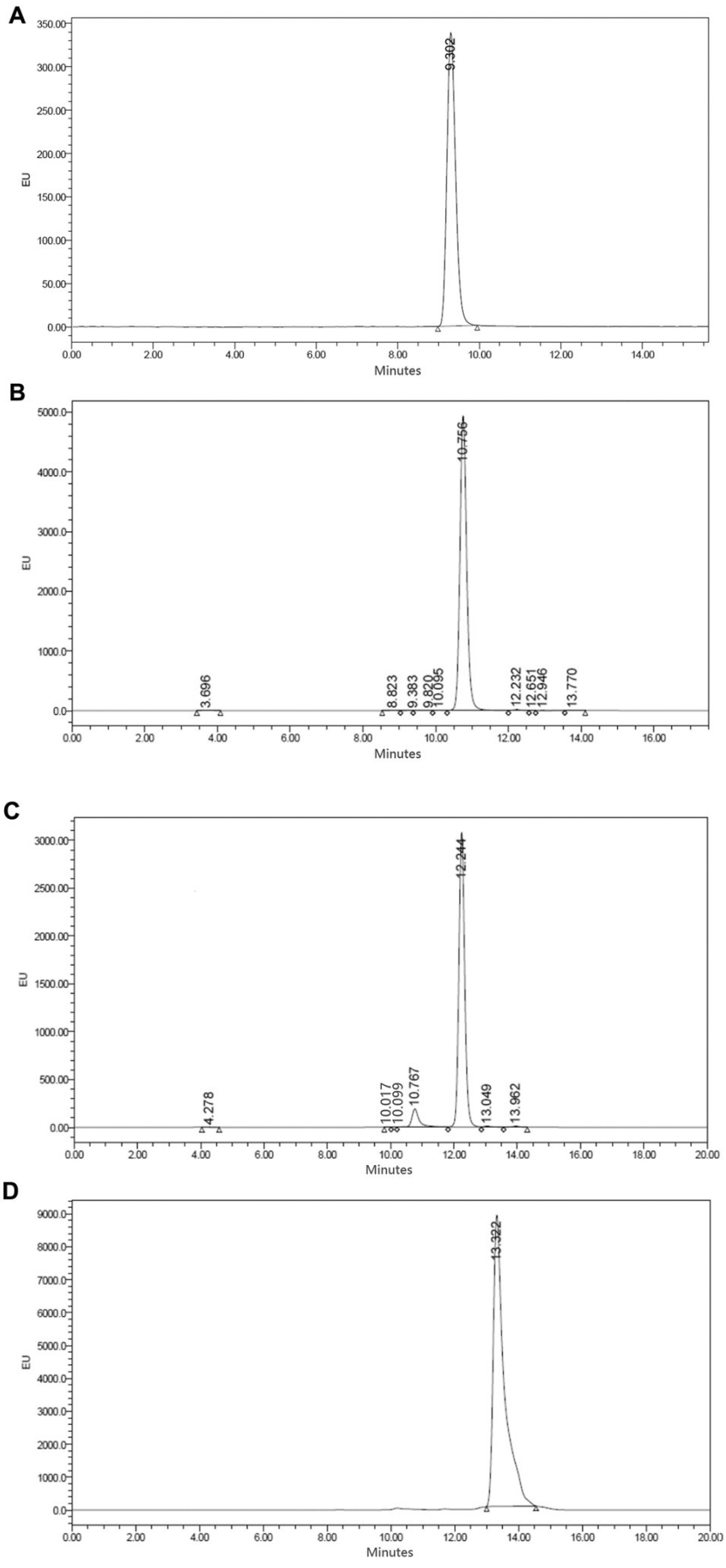

Figure S4. The HPLC traces of SN38 (A), MOM-SN38 (B), MOM-SN38-S-S-OH (C) and PEG-S-S-SN38 (D).

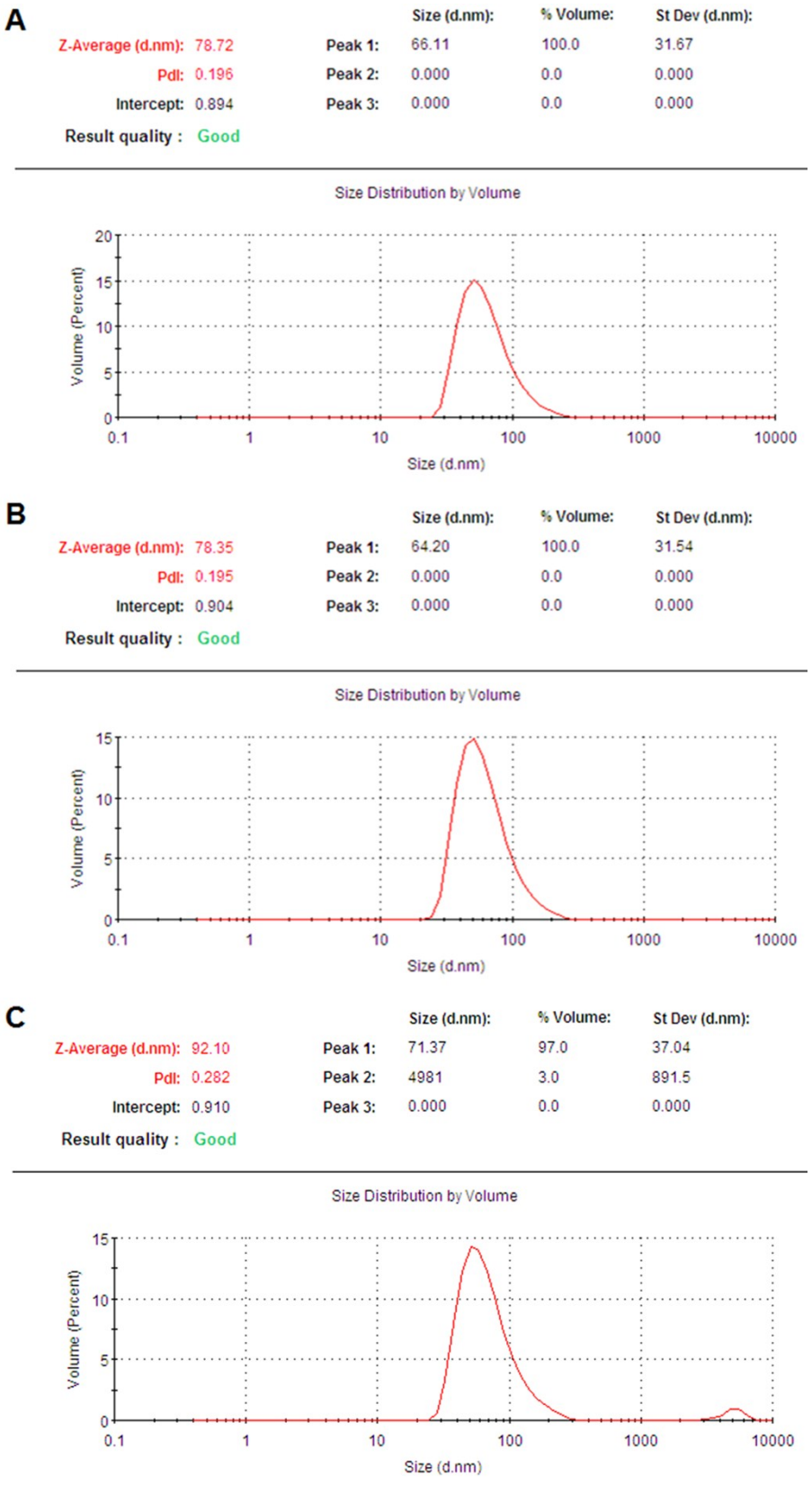

Figure S5. The stability of PEG-S-S-SN38 nanoparticles in 40% fetal bovine serum solution. Samples were analyzed by DLS after incubation at 37°C for 2.5 h (A), 6 h (B) and 32 h (C).

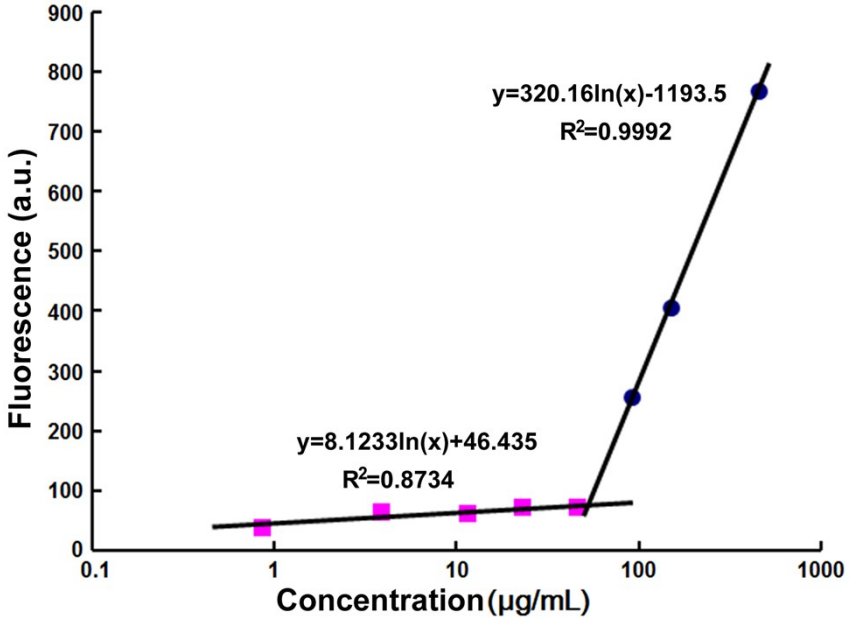

Figure S6. The fluorescence intensity of nile red as a function of the PEG-S-S-SN38 concentration.

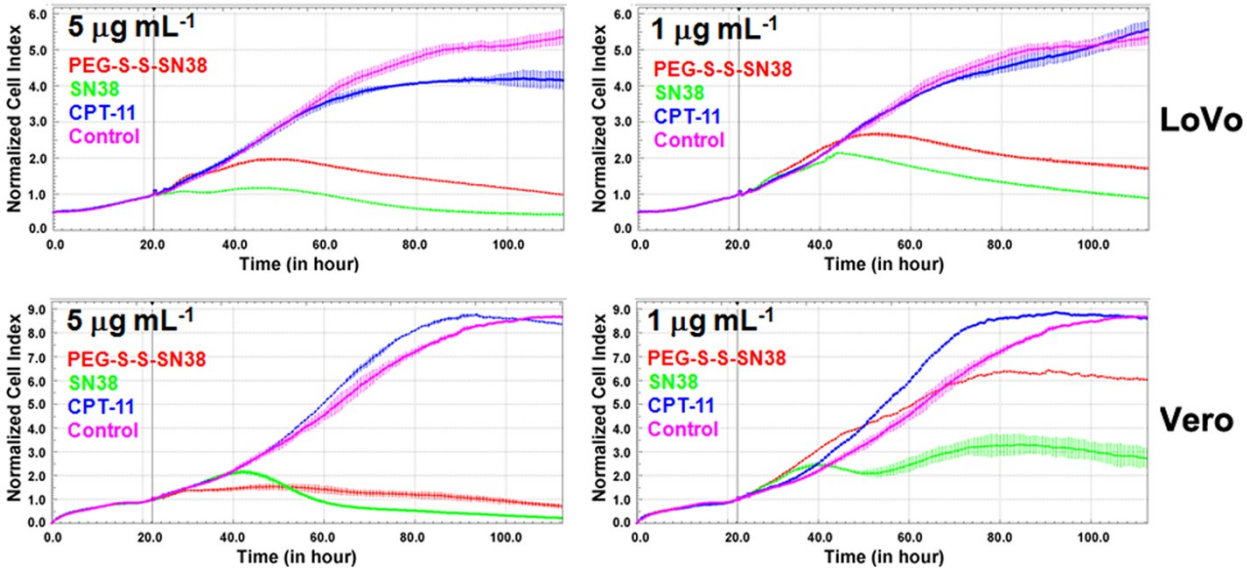

Figure S7. *In vitro* cytotoxicity of CPT-11, SN38 and PEG-S-S-SN38 NPs against LoVo and Vero cell lines evaluated by xCELLigence system real time cellular analysis (RTCA), at representative concentrations equivalent to 5 μg mL^{-1} and 1 μg mL^{-1} SN38, respectively.

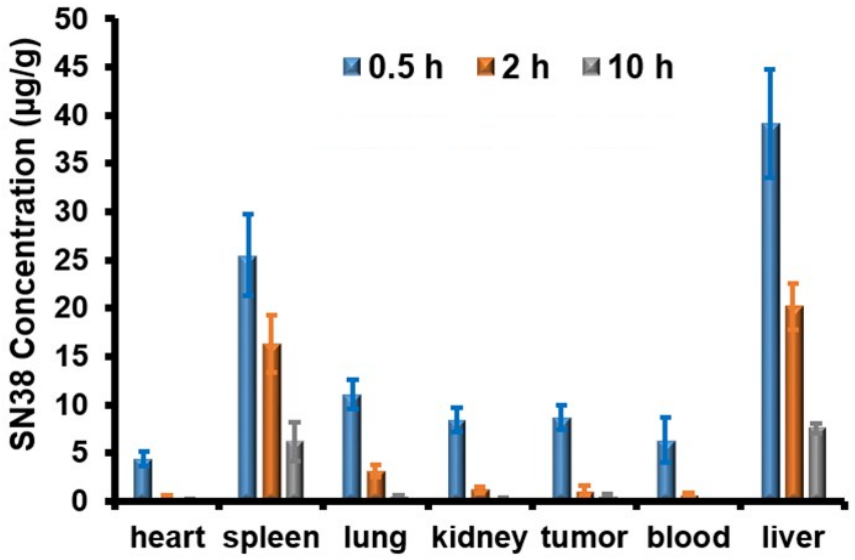

Figure S8. The biodistribution of SN38 in various organs, blood and BCap37 xenograft tumors after tumor-bearing nude mice were administrated with PEG-S-S-SN38 nanoparticles for 0.5 h, 2 h and 10 h, respectively, at a dosage equivalent to 10 mg kg^{-1} SN38.

Table S1. IC_{50} values in a panel of cancer cell lines as determined by MTT assays

IC_{50} (μg/mL)	BCap37	SKOV3	KB	KBv200	MCF-7	MCF-7/ADR
CPT-11	28.43	13.39	50.25	＞100 *	53.75	＞100 *
SN38	0.2707	0.4515	0.1863	0.1435	0.2873	5.49
PEG-S-S-SN38	0.9079	0.5041	0.4511	0.2147	1.598	58.21

* Exact values could not be calculated based on the software used.