Supporting Information

Piezo-Photocatalytic Effect Mediating Reactive Oxygen Species Burst for Cancer Catalytic Therapy

Yong Kang, ‡[a] Lei Lei, ‡[b] Chunfeng Zhu,[c] Hanjie Zhang,[d] Lin Mei,*[d] Xiaoyuan Ji*[a,e]

[a] Academy of Medical Engineering and Translational Medicine, Medical College, Tianjin University, Tianjin 300072, China
Email: jixiaoyuan@tju.edu.cn

[b] State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, No.54 South Xianlie Road, Guangzhou 510060, China

[c] School of Life Sciences,Tianjin University,Tianjin 300072,China

[d] Tianjin Key Laboratory of Biomedical Materials, Key Laboratory of Biomaterials and Nanotechnology for Cancer Immunotherapy, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, China
Email: meilin@bme.pumc.edu.cn

[e] School of Pharmaceutical Sciences (Shenzhen), Sun Yat-sen University, Guangzhou 510275, China

‡ Y. K. and L. L. contributed equally to this work.

Materials

The natural sphalerite (NSH) sample was supplied by a deposit in China. Human cervix cancer cell line HeLa and human breast cancer cell MCF7 were obtained from ATCC. Trypsin-EDTA, PBS (pH 7.4), fetal bovine serum (FBS), RPMI medium, and DMEM medium were purchased from Gibco Life Technologies (AG, Switzerland). Methyl thiazolyl tetrazolium (MTT), 2,7-dichlorofluorescein diacetate (DCFH-DA) , N-methyl-pyrrolidone (NMP), and methylene blue (MB) were supplied by Sigma-Aldrich (St. Louis, MO. USA). Blood biochemical assay kits were bought from Solarbio Science & Technology Co., Ltd. (Beijing, China). The other chemical reagents used in the study were analytical reagent grade and used without further purification.

Preparation of NSH NSs and NSH700 NSs

The strategy coupling ball-milling with probe sonication was applied to prepare NSH NSs. Firstly, the NSH solution with NMP as the solvent (10 mg/mL) was ground via ball mill at 800 rpm for 30 min. After that, the NSH suspension was sonicated for 12 hours. At last, the NSH NSs dispersion solution was centrifuged at 3000 rpm for 5 min and the supernatant was collected and stored at 4 ^{o}C for further use.

The strategy coupling ball-milling, calcination, with probe sonication was applied to prepare NSH700 NSs. Firstly, the NSH solution with NMP as the solvent (10 mg/mL) was ground via ball mill at 800 rpm for 30 min. After that, the NSH suspension was centrifuge at 10000 rpm for 5 min and the precipitates were collected and dry in vacuum drying chamber for 5 h. Calcination of the NMS powders was conducted in furnace from room temperature to 700 °C with a heating rate of 10 °C/min for two hours. Then the NSH700 powders were sonicated for 12 hours with NMP as the solvent. At last, the NSH700 dispersion solution was centrifuged at 3000 rpm for 5 min and the supernatant was collected and stored at 4 ^{o}C for further use.

Synthesis of NSH-PEG NSs and NSH700-PEG NSs

In order to improve the biocompatibility, NSH NSs or NSH700 NSs were further modified with PEG-NH_2. First, PEG-NH_2 (10mg) was added into NSH NSs or NSH700 NSs suspension and then the suspension was ultrasonicated and stirred for 30 min and 12h respectively. To remove the unattached PEG-NH_2, the mixture was washed three times by centrifugation at 2500 rpm (4 ^{o}C) for 30min. Afterwards, the PEGylated NSH NSs or NSH700 NSs were resuspended in PBS and stored at 4 ^{o}C for further use. The quality of PEG-NH_2 loaded on the surface of NSH NSs or NSH700 NSs was measured by thermogravimetric analysis (TGA) according to the thermal decomposition temperature of PEG and NSs.

Characterization

The size and zeta potential of NSs were detected via Dynamic Light Scattering. The morphology of NSs was characterized by transmission electron microscopy (TEM, JEM-2100UHR, JEOL, Japan) and atomic force microscopy (AFM, FASTSCANBIO, Germany). Piezoresponse force microscopic (PFM) measurements were characterized by an AFM (NTEGRA, NT-MDT, Russian) equipped with a ferroelectric test system. The NSs's chemical constituents were detected by energy-dispersive X-ray spectroscope (EDS) (Inca X-MAX, Oxford, UK), X-ray Photoelectron Spectroscopy (XPS, ESCALAB 250Xi, USA), and fourier transform infrared spectrophotometry (FTIR, Nexus 470, Nicolet, Madison, WI, USA). The NSs's chemical structures were characterized by X-ray powder diffraction (XRD, Bruker D8 multipurpose) and Raman spectrum (Renishaw, inVia, UK). The light absorbance spectra were detected by infinite M200 PRO spectrophotometer.

·O_2^- generation of NSH-PEG NSs and NSH700-PEG NSs

Dihydrorhodamine 123 (DHR123) was employed to detect the ·O_2^-, which can react with ·O_2^- and emit strong fluorescence. As a typical process, DHR123 (1mM, 1μL) was dispersed in NSs suspension (0.1 mg/mL, 3mL). Then the mixture was exposed to 650 nm NIR laser (0.3 W/cm^2) and/or US irradiation (0.8 MHz, 0.5 W/cm^2, 50 % duty cycle), and the fluorescence intensity was recorded by fluorescence spectrophotometry every 1 minute. Then, the ·O_2^- signal was also characterized through Electron Spin Resonance (ESR) with the spin trapping agent of DMPO.

·OH Generation of NSH-PEG NSs and NSH700-PEG NSs

The ·OH generation of NSs was determined by methylene blue (MB). Briefly, NSs suspension (1 mg/mL, 100 μL) was mixed with MB (1 mg/ mL, 15 μL) and H_2O_2 (10mM, 150 μL) in PBS. Then the mixture was exposed to 650 nm laser (0.3 W/cm^2) and/or US irradiation (0.8 MHz, 0.5 W/cm^2, 50 % duty cycle). The absorbance of MB was recorded by UV-vis-NIR after different time. Then, the ·OH signal was also characterized through Electron Spin Resonance (ESR) with the spin trapping agent of DMPO.

GSH degradation of NSH-PEG NSs and NSH700-PEG NSs

DTNB (0.2 mg/mL) solution and GSH (final concentration: 0.1 mM) solution was made up, and then kept at 25 °C. NSs (final concentration: 0.1 mg/mL) were mixed with the two solutions aforementioned. Then the mixture was exposed to 650 nm laser (0.3 W/cm^2) and/or US irradiation (0.8 MHz, 0.5 W/cm^2, 50 % duty cycle). The absorbance of supernatant was recorded by UV-vis-NIR after different time

Cell Cytotoxicity of NSH NSs and NSH700 NSs *in vitro*.

MCF7 and Hela cells were seeded into two 96-well plates at a density of 5,000 cells in every well and incubated for 24 h respectively. Afterwards, NSs (0-200 μg/mL) were added to the

cancer cells mentioned above and incubated for another 24h. At last, the viability of cells were detected by MTT Assay (Life Technologies). Detailedly, the PBS was employed to washed cells and fresh medium co-mixed 20 μL of Methyl thiazolyl tetrazolium (MTT 5 mg mL^{-1} dissolved into PBS) was added to 96-well plate. The medium was sucked out and 150 μL of DMSO was added after 4 h of incubation. The optical density (OD) at 570 nm was detected and record by a microplate reader and the relative cell viability was evaluated by the formula: cell viability (%) = OD (sample) × 100/OD (control).

Intracellular ROS generation.

MCF7 cells were incubated in culture dishes for 24h (37 ^{o}C, 5% CO_2). Subsequently, the old culture medium was replaced with the fresh one containing NSs (0-200 μg/mL) and incubation went on for another 12h. Afterwards, the medium culture containing NSs was removed and the cells were washed by PBS for 3 times before the fresh medium culture was added. After that, DCFH-DA (0.2 μM) was added for another 30 min. After washed by PBS for three times, the cells were exposed to a 650 nm (0.3 W/cm^2) or/and US irradiation (0.8 MHz, 0.5 W/cm^2, 50 % duty cycle) and in dark condition respectively. A confocal laser confocal microscope (CLSM, Leica TCS SP5, Germany) was utilized to detect ROS, which emitted green fluorescence.

Combine Therapy *in vitro*

MCF7 and Hela cells were incubated into 96-well plates for 24h (37 ^{o}C, 5% CO_2). Subsequently, the old culture medium was replaced with the fresh one containing NSs (0-200 μg/mL) and incubation went on for another 12h. Afterwards, the medium culture containing NSs was removed and the cells were washed by PBS for 3 times before the fresh medium culture was added. And then, cells mentioned above were irradiated at a 650 nm (0.3 W/cm^2) or/and US irradiation (0.8 MHz, 0.5 W/cm^2, 50 % duty cycle).

Additionally, in order to confirm the intracellular ROS burst is the main reason for cells apoptosis, N-acetyl-L-cysteine, a ROS scavenger, was added to the cell medium for 1 h before US and laser irradiations with a final concentration of 10 mM. Finally, MTT assay was executed to evaluate the cell viabilities.

Xenograft Tumor Model.

MCF7 cells suspension (200 μL, 2×10^6) were subcutaneously injected on the back of naked Balb/c mice. After the tumor grew to about 100 mm^3, *in vivo* anti-tumor and imaging experiments started to perform. The tumor volume was calculated using the eqation "Tumor volume = 0.5 × length × $width^2$''

***In vivo* Therapy of NSH-PEG NSs and NSH700-PEG NSs**

After the tumor growing to ~100 mm^3, mice were randomly divided into 10 groups, 5 mice in each group. Group 1: PBS; Group 2: NSH NSs or NSH700 NSs; Group 3: NSH NSs + 650 nm laser or NSH700 NSs + 650 nm laser; Group 4: NSH NSs + US or NSH700 NSs + US; and Group 5: NSH NSs + 650 nm laser + US or NSH700 NSs + 650 nm laser + US. The injected intravenously dose of NSs was 5 mg/kg and the injection volume was 100 mL for one injection. For the 650 nm laser treatment, the power density was 0.3 W cm^{-2}, and the irradiation time was 10 min. For the US treatments, the frequency was 0.8 MHz, the power density was 0.5 W cm^{-2} with 50% duty cycle, and the irradiation time was 10 min. Both laser or US treatments were carried out at 12 h postinjection. A caliper and electronic scale were employed to measure and record the tumor volumes and body weight of each group every two days for two weeks.

***In vivo* ROS generation**

After 3 h of intravenous injection of different drugs, 100 μL of DCFH-DA with the concentration of 100 μM was injected intratumorally. Laser or/and US irradiation was performed 1 h after injection. Then, the mice were euthanized and the tumor tissues were collected for DAPI staining and made a section for observation.

***In vivo* fluorescence imaging and biodistribution**

Three female Balb/c nude mice were established the MCF7 xenograft tumor model. The NSH700-PEG-Cy 7 NSs were injected at the dosage of 3 mg/kg when volumes of tumor model were reached anout 100 mm^2. The *in vivo* fluorescence images at different time after intravenous injection of NSH700-PEG-Cy 7 NSs were detected and record by animal *in vivo* imaging system (FX Pro, Carestream Health). The fluorescence signals of tumor and major organs after 12 h post-injection were also recorded. Meanwhile, the fluorescence intensity of Cy7 in the blood was also measured at different time after intravenous injection of series of drugs.

***In vivo* Toxicity.**

The *in vivo* toxicity of NSs were performed applied C57BL/6 mice with i.v. syringed NSs (10 mg/kg) as model. After 30 days injection, the main organs were collected and applied to eosin (H&E) and hematoxylin staining to detect any damage of tissues.

The immune response was also detected applied C57BL/6 mice with i.v. syringed NSs (10 mg/kg) as model. After 12 hours and 24 hours i.v. injection, the immunological factors, including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin6 (IL-6) in blood were detected by enzyme-linked immunosorbent assay. After 1 day, 7day, and 14 day i.v. injection, aminotransferase (AST), creatinine (Cr), total protein (TP), urea nitrogen (BUN),

aspartate alanine aminotransferase (ALT), and albumin (ALB) were measured via test the complete blood panel.

Live subject statement.

All animals received humane care, and the Animal Ethics Committee of Tianjin University approved all the animal experiments. All experiments were carried out according to the recommendations of the Committee for the Care and Use of Laboratory Animals, Tianjin University.

Statistical analysis.

All results are reported as the mean ± S.E.M. and comparisons were performed using a two tailed Student's t test. All experiments, unless otherwise stated, were performed in triplicate. Statistical values are indicated in figures according to the following scale: * $P < 0.05$, ** $P < 0.01$, and ***$P < 0.001$.

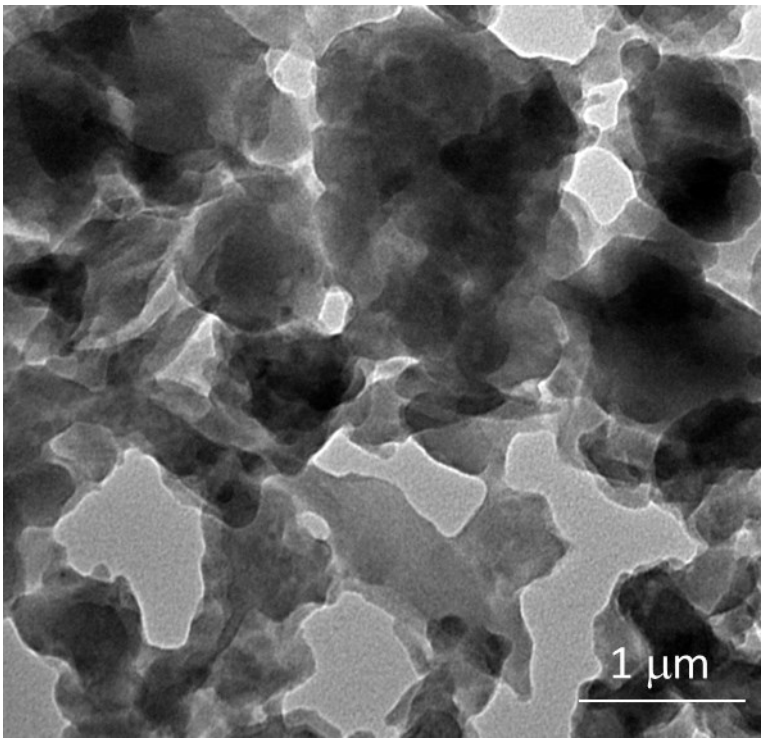

Figure S1. TEM images of NSH after wet grinding in NMP at 1000 rpm for 30 min.

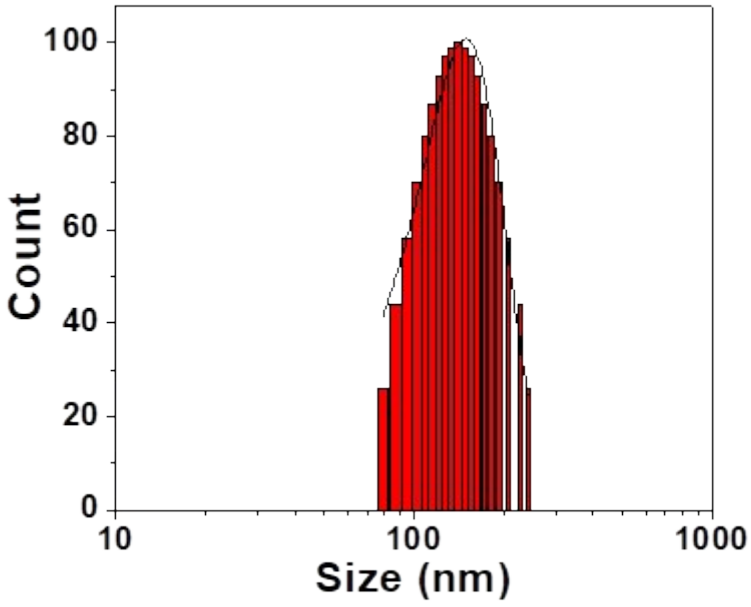

Figure S2. Size distribution of NSH NSs.

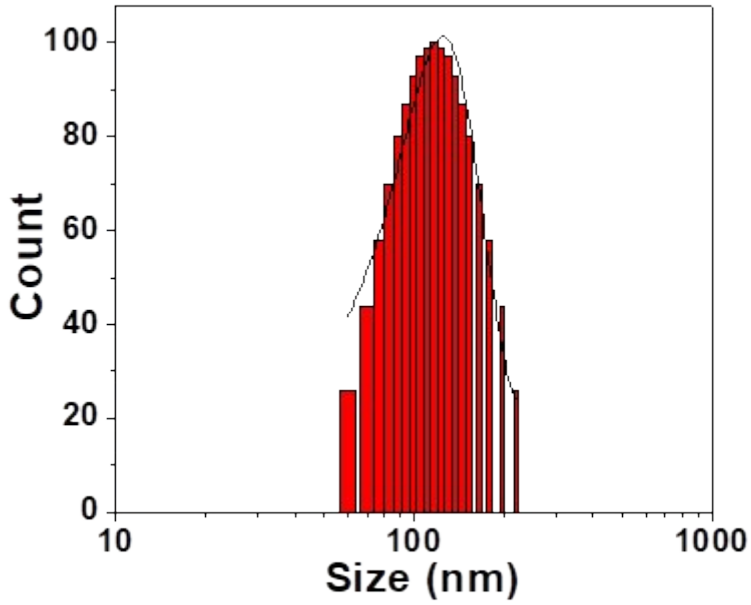

Figure S3. Size distribution of NSH700 NSs.

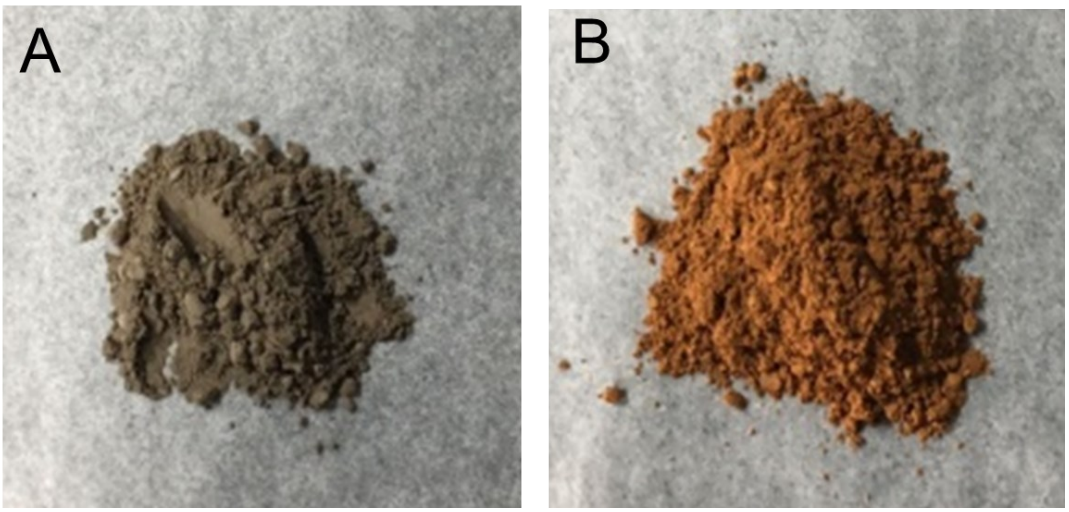

Figure S4. The photo images of NSH (A) and NSH700 (B).

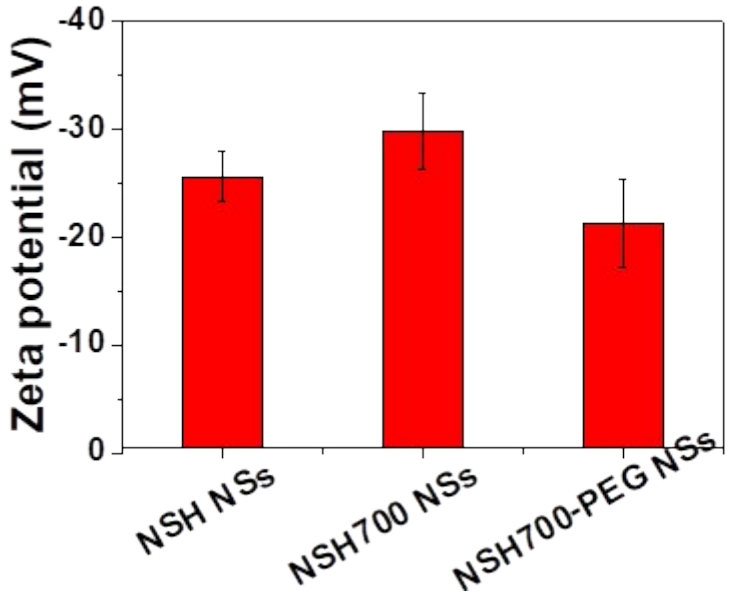

Figure S5. Zeta potential of fabricated NSs.

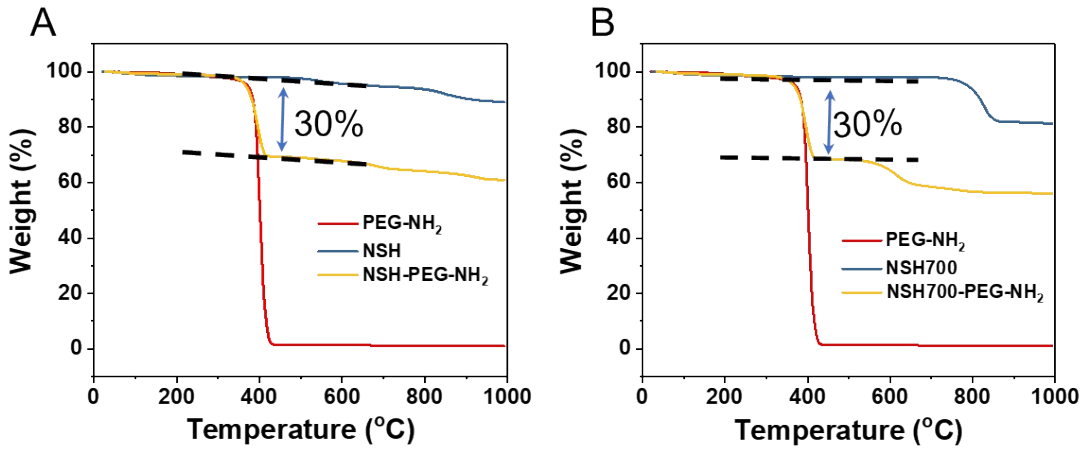

Figure S6. Thermogravimetric analysis (TGA) of NSH NSs, NSH700 NSs, and PEGylated NSH NSs and NSH700 NSs.

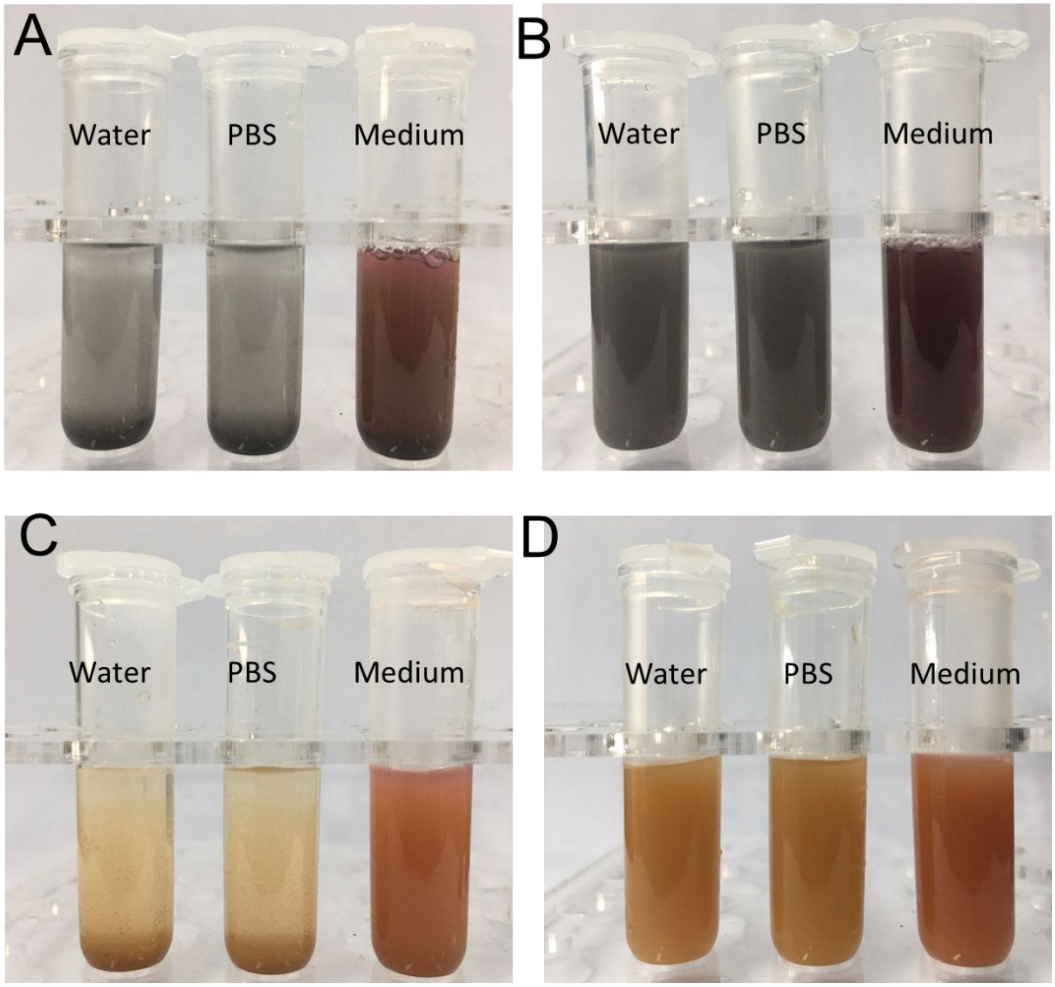

Figure S7. The distribution of (A) NSH NSs and (C) NSH700 NSs in water, PBS, and medium. The distribution of (B) PEGylation NSH NSs and (D) PEGylation NSH700 NSs in water, PBS, and medium.

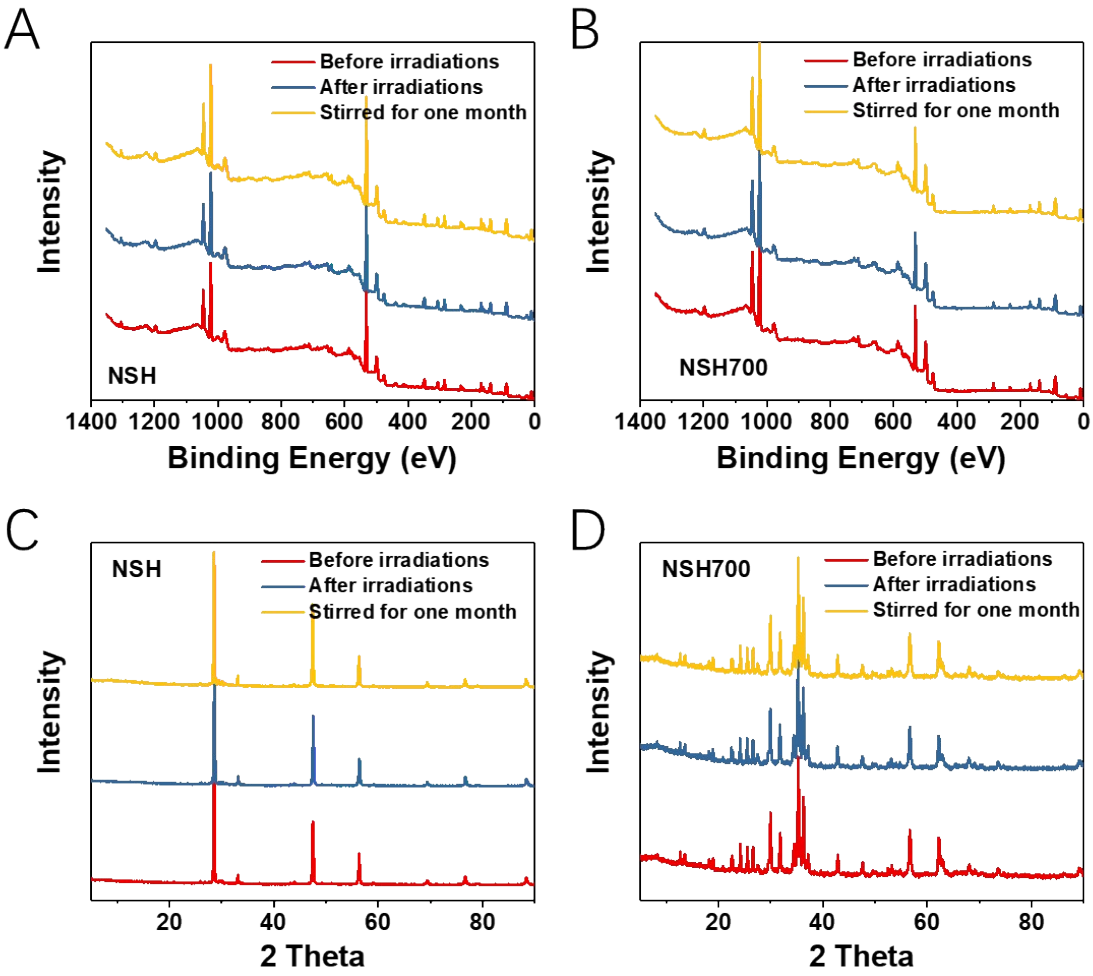

Figure S8. XPS spectra of fresh prapared, long-term stored, and laser and US irradiated (A) NSH NSs and (B) NSH700 NSs. XRD spectra of fresh prapared, long-term stored, and laser and US irradiated (C) NSH NSs and (D) NSH700 NSs.

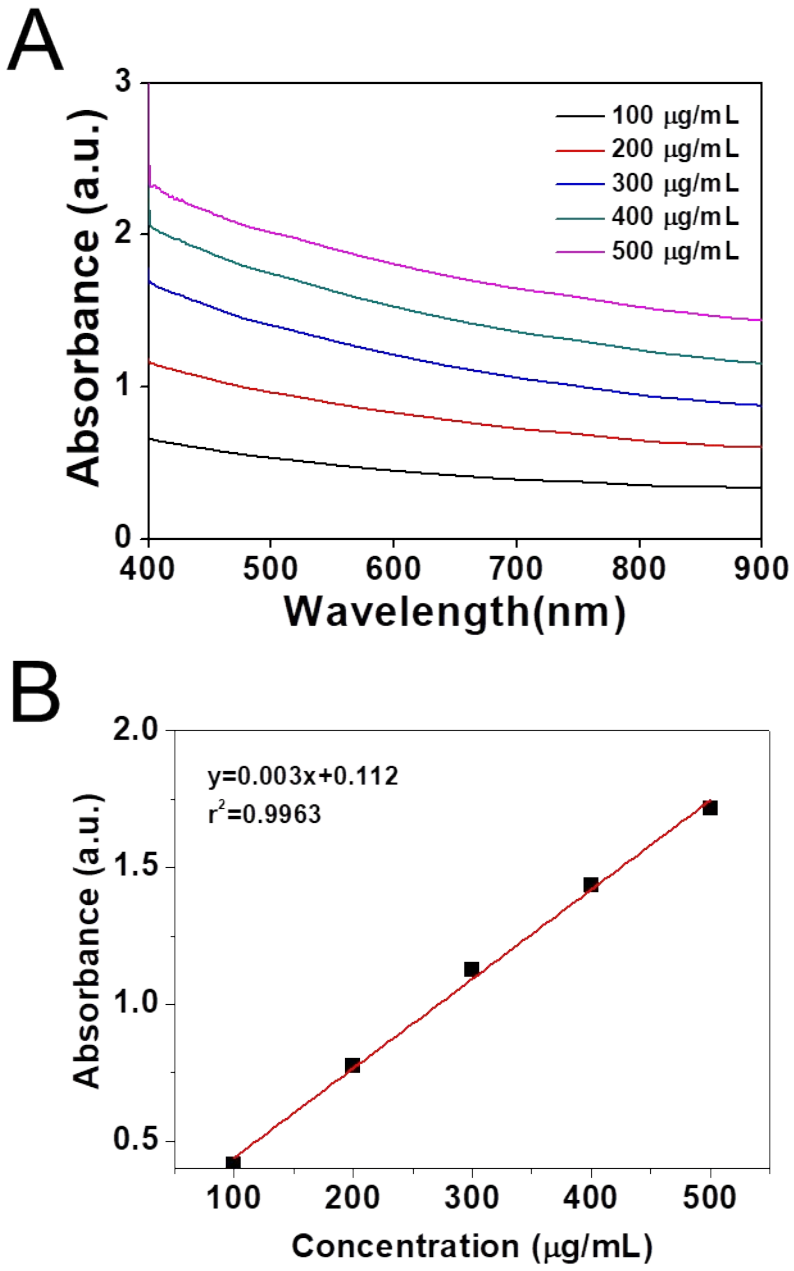

Figure S9. (A) UV-vis-NIR absorbance spectra of PEGylation NSH700 NSs dispersed in water at different concentrations. (B) Normalized absorbance intensity of PEGylation NSH700 NSs divided by the characteristic length of the cell (A/L) at different concentrations for λ=808 nm.

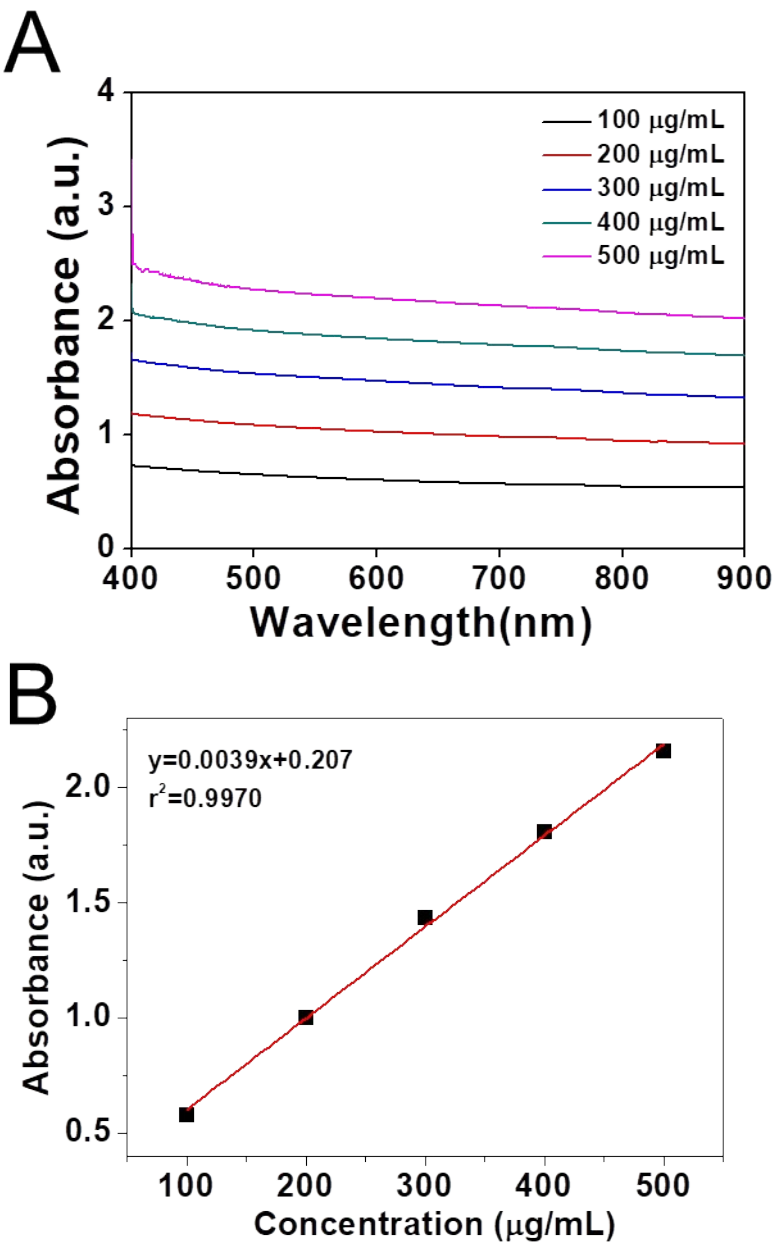

Figure S10. (A) UV-vis-NIR absorbance spectra of PEGylation NSH NSs dispersed in water at different concentrations. (B) Normalized absorbance intensity of PEGylation NSH NSs divided by the characteristic length of the cell (A/L) at different concentrations for λ=808 nm.

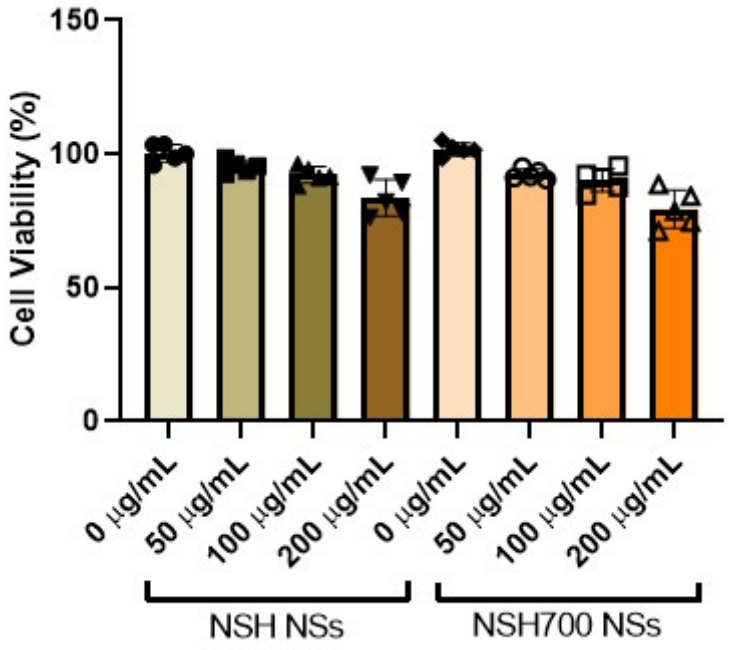

Figure S11. The viability of Hela cells treated with NSH NSs or NSH700 NSs at different concentrations for 24 h.

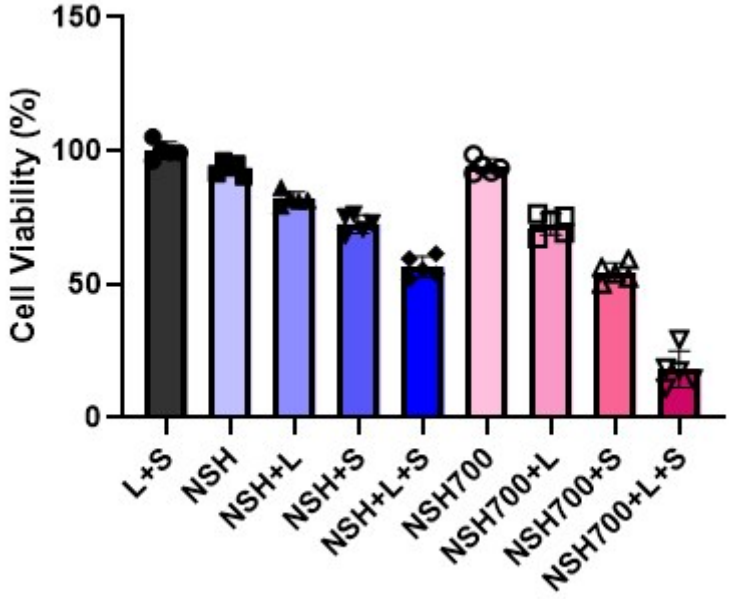

Figure S12. The viability of Hela cells treated with NSH NSs or NSH700 NSs under different excitation energy sources.

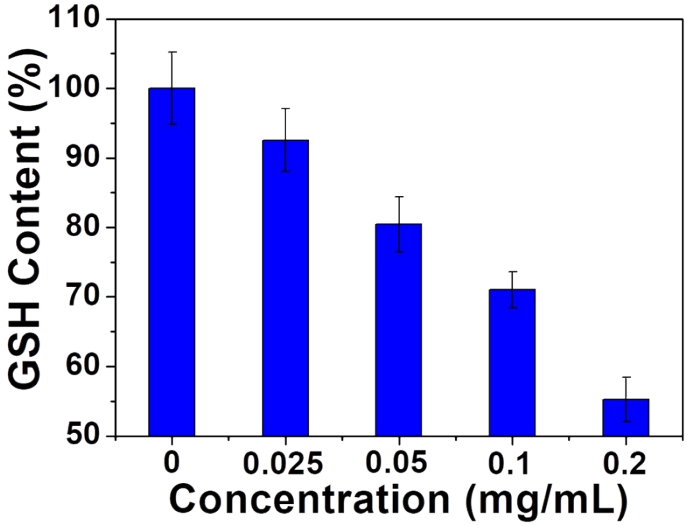

Figure S13. The concentration of GSH in MCF-7 cells treated with different concentrations of NSH700 NSs (0–200 μg mL^{-1}) for 24 h.

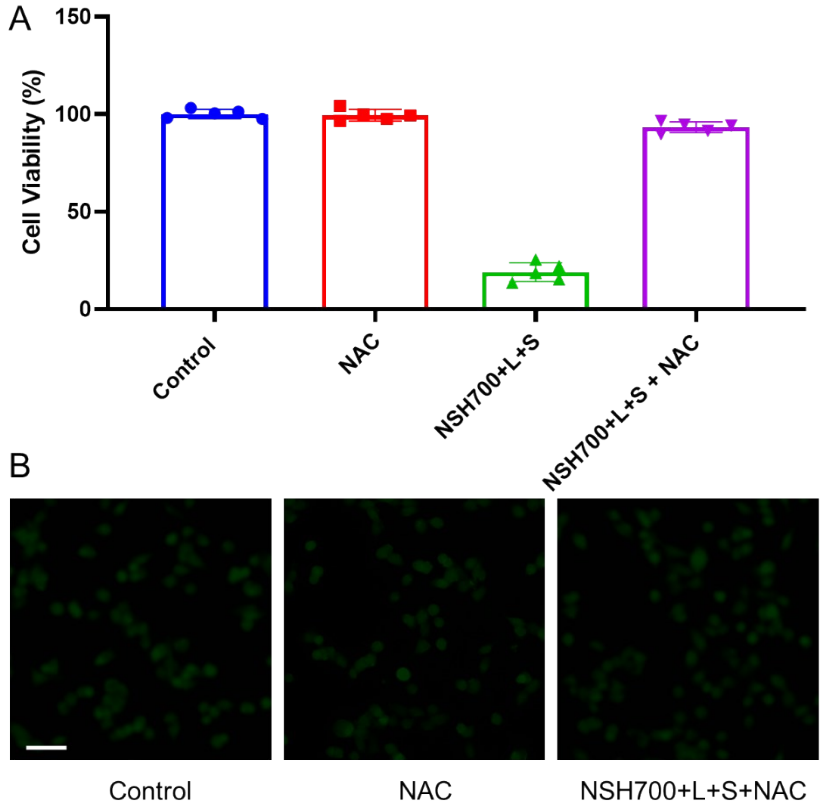

Figure S14. A) The viability of MCF7 cells cocultured with N-acetyl-L-cysteine (NAC) treated with NSH700 NSs under 650 nm laser and US irradiation different excitation energy sources. B) Intracellular ROS generation detected by CLSM after different treatments. Scale bar = 50 μm. The power of 650 nm laser was 0.3 W cm^{-2}. The power of US was 0.8 MHz, 0.5 W/cm^2, 50 % duty cycle. The exposure time was 10 min.

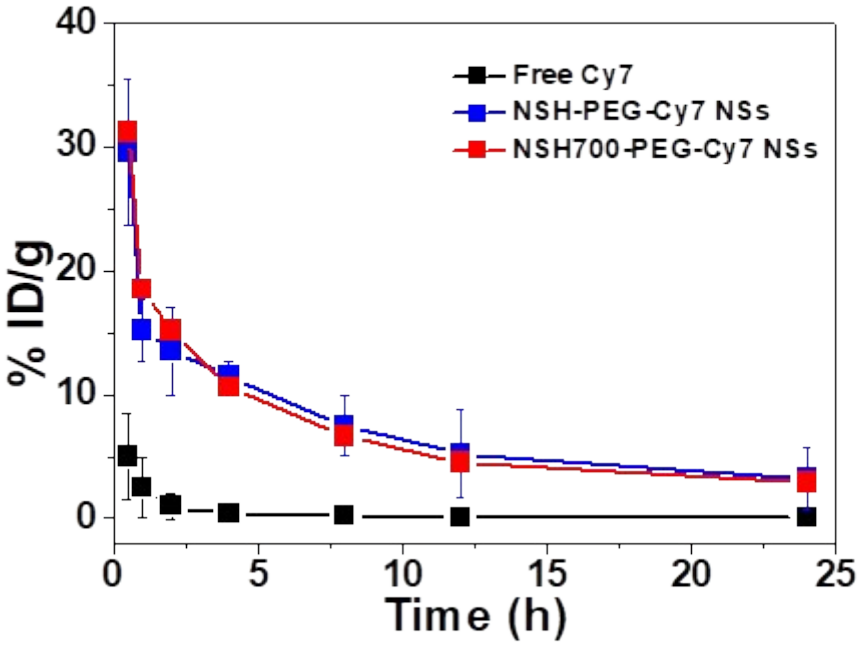

Figure S15. Blood circulation performance of NSH-PEG-Cy7 NSs, NSH700-PEG-Cy 7 NSs, and free Cy7.

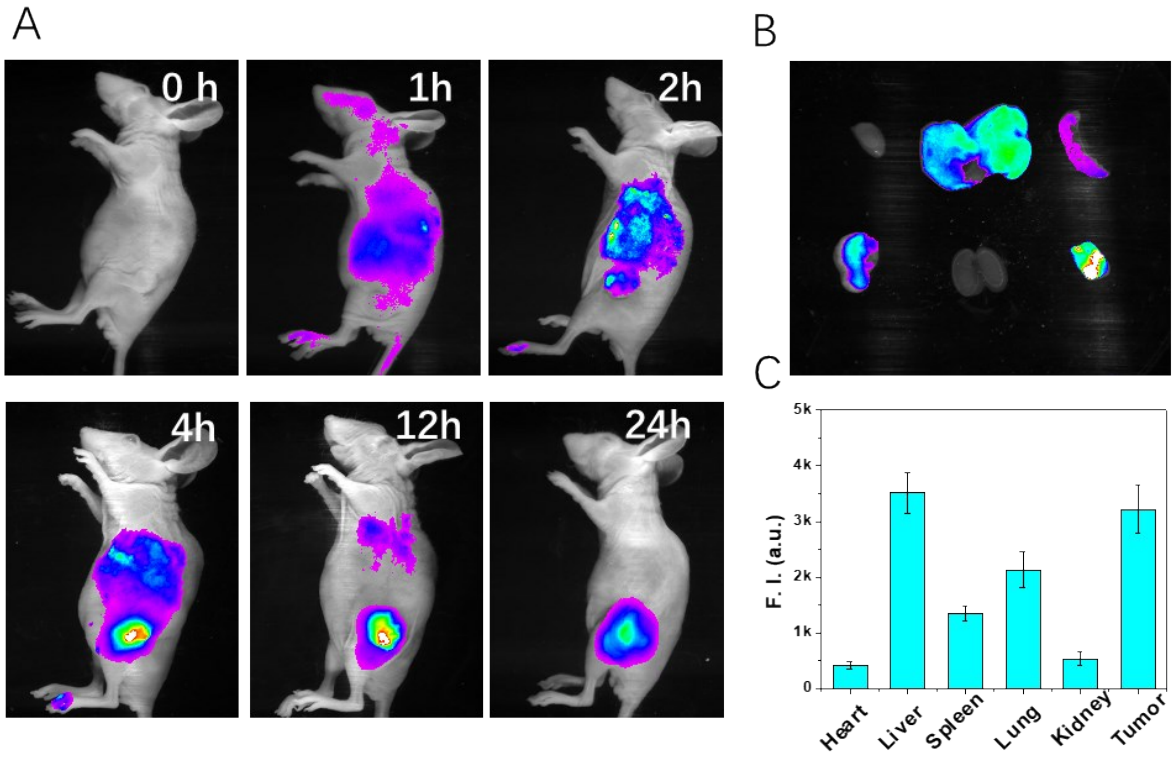

Figure S16. A) and B) Fluorescence images of mice, major organs, and tumors 24 h after injection in vivo and ex vivo. C) Semiquantitative biodistribution of NSH700-PEG-Cy 7 NSs in nude mice.

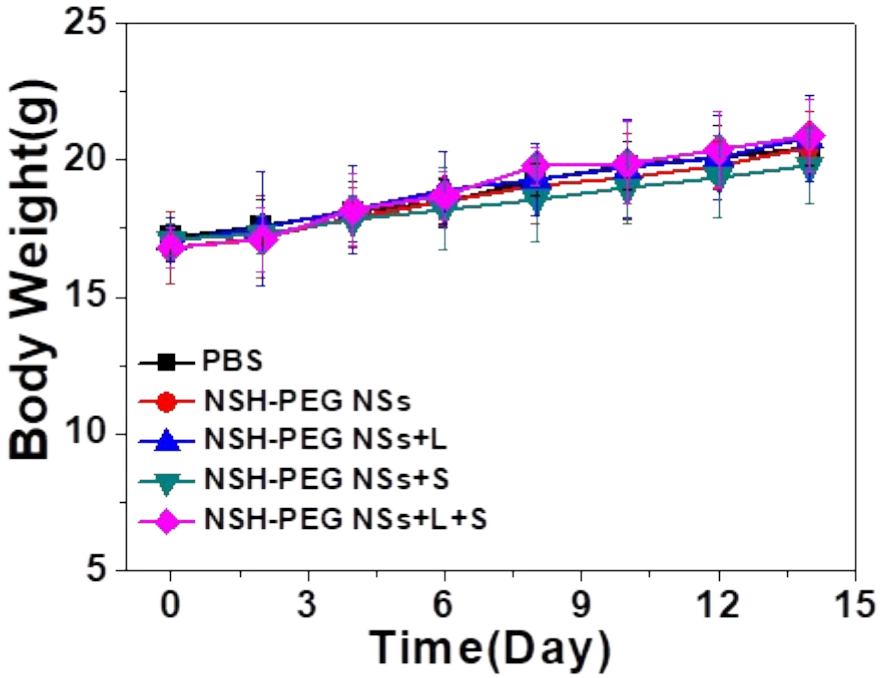

Figure S17. Body weight of mice after different treatments with NSH NSs.

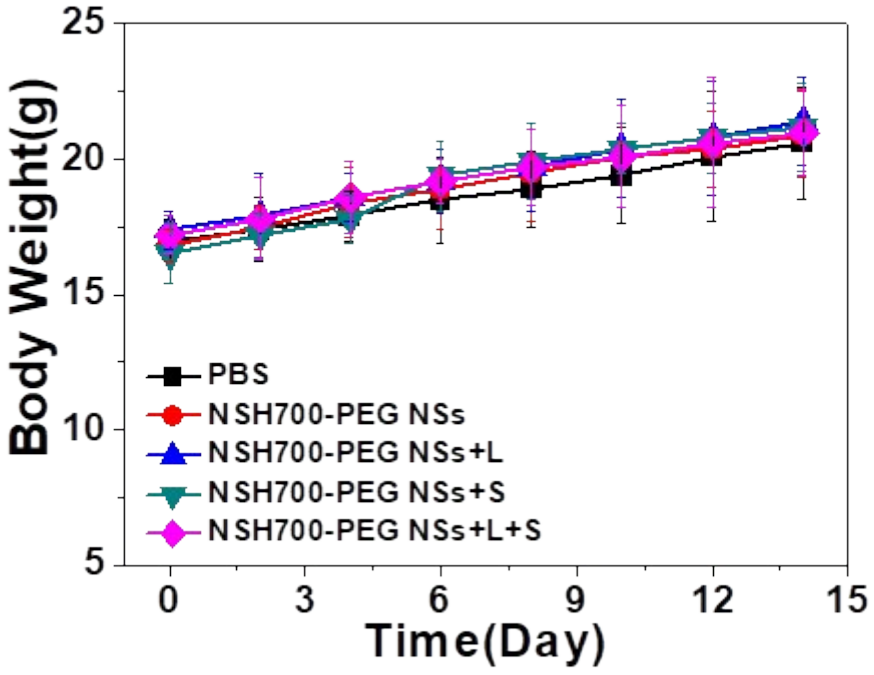

Figure S18. Body weight of mice after different treatments with NSH700 NSs.

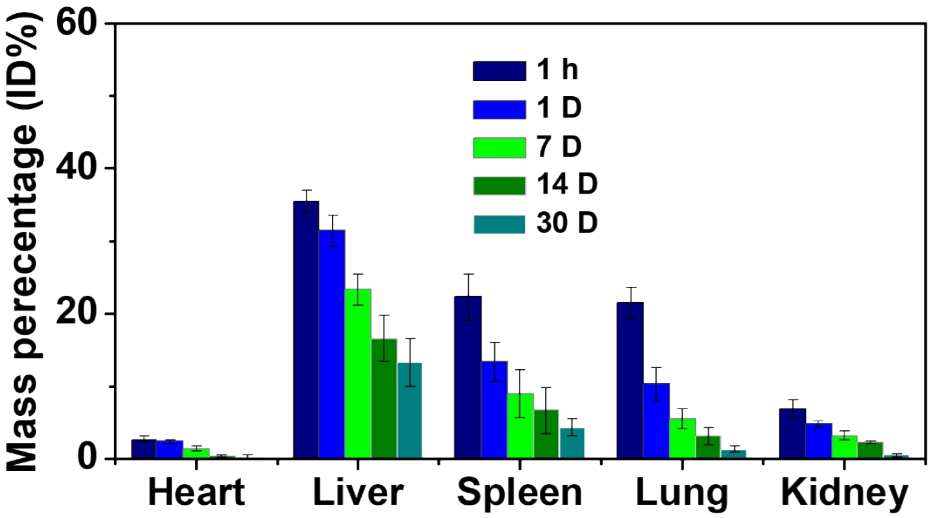

Figure S19. Bio-distribution of NSH700 NSs under different time detected by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

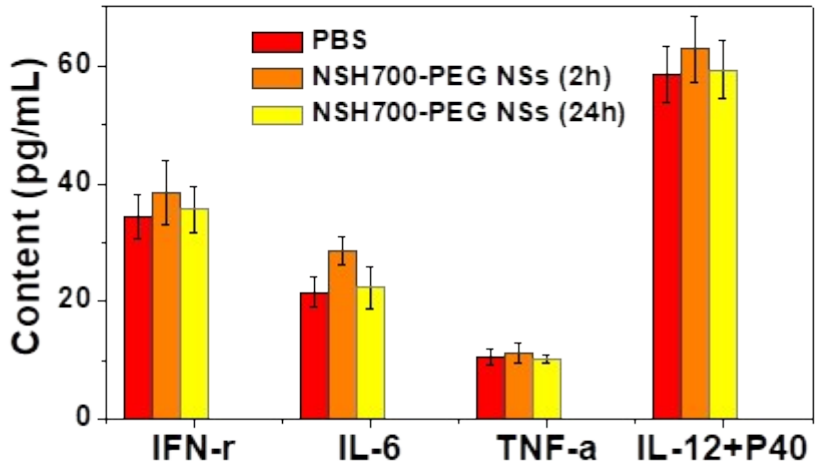

Figure S20. Serum levels of IL-6, IFN-γ, TNF-α, and IL-12+P40 in mice at 2 and 24 h post i.v. injection of PBS versus NSH700 NSs.

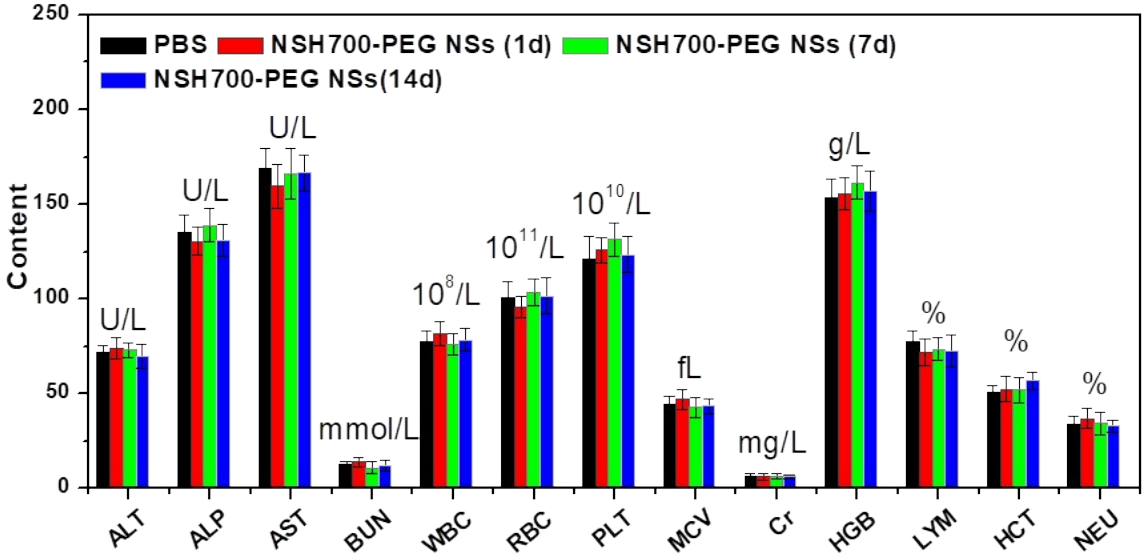

Figure S21. Blood hematology and biochemistry data of Balb/c mice treated with NSH700 NSs.

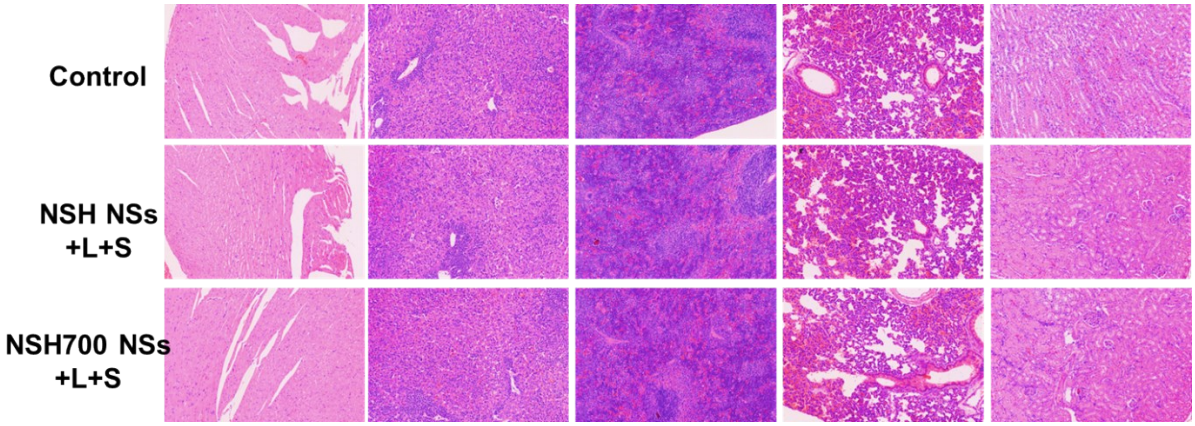

Figure S22. H&E stained histological images of major organs (heart, liver, spleen, lung, and kidney) from mice treated with NSH700 NSs versus saline.