# Supporting Information

## Self-activating Therapeutic Nanoparticle: A Targeted Tumor Therapy using ROS Self-generation and Switch-on Drug Release

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**This file includes:**

## 1. Materials and Methods

### 1.1 General information

The chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), NanoCS (New York, NY, USA), Merck (Darmstadt, Germany), FluoroChem (Hadfield, UK), AK Scientific (Union City, CA, USA), Carbosynth (Berkshire, UK), and Samchun Chemicals (Seoul, Rep. of Korea). Commercially available reagents and anhydrous solvents were used without further purification. Chemical reaction and centrifugation were performed in an open-air environment at room temperature (25 °C). Calcium chloride (M.W: 147.01, dihydrate) and phenethyl isothiocyanate (Product No. 253731-5G) were purchased from Sigma-Aldrich (St, Louis, MO, USA). Maleimide-PEG-NHS (M.W.=5 kDa) (Product No. PG2-MLNS-5k, USA) was purchased from NanoCS (New York, NY, USA). 3-Aminopropyl-dimethylethoxysilane (APDMES) was purchased from FluoroChem (Product No. S00750, Glossop, UK). Triethoxy(3-isothiocyanatoproypyl)-silane was purchased from AK Scientific (Product No. AMTGC214, Union City, CA, USA). Ethanol was purchased from Samchun Chemicals (Product No. E0223, Seoul, Rep. of Korea). Dimethyl sulfoxide (DMSO, product No. 1.02952.1000) was purchased from Merck (Darmstadt, Germany). 7-Ethyl-10-hydroxycamptothecin (SN-38, product No. FE29579) was purchased from Carbosynth (Berkshire, UK). Roswell Park Memorial Institute-1640 medium (RPMI-1640, Product No. SH30027.01), Dulbecco's phosphate buffered saline (DPBS, Product No. SH30028.02), and fetal bovine serum (FBS, SH30084.03) were purchased from Hyclone (Logan, UT, USA). Trypsin-EDTA (Product No. 25200-056) and Penicillin streptomycin (Product No. 15140-122) were purchased from Gibco (Carlsbad, CA, USA). Cell counting Kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). 4',6-diamidino-2-phenylindole (DAPI, Product No. D1306) was purchased from ThermoFisher Scientific (Waltham, MA, USA). DCFDA/H2DCFDA cellular ROS assay kit (Product No. ab113851) was purchased from Abcam (Cambridge, UK). Primary antibody (Nrf2, HO-1, and β-actin), and secondary antibody (anti-rabbit IgG-HRP, and anti-mouse IgG-HRP) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). DAPI and Alexa-Fluor 555 fluorescence were purchased from Invitrogen (Waltham, MA, USA). Glutathione assay kit (Product No. DG-GLU200) was purchased from DOGEN (Seoul, Rep. of Korea). Chemiluminescence (ECL) solution was purchased from BIOMAX (Seoul, Republic of Korea). EDTA, Glutathione Peroxidase Cellular Activity Assay Kit (Product No. CGP1), 10% formalin, hematoxylin and eosin (H&E) were purchased from Sigma-Aldrich (St, Louis, MO, USA). Bovine serum albumin (BSA) were purchased from GenDEPOT (Barker, TX, USA). Paraffin was purchased from Leica Biosystem (Richmond, IL, USA) and Matrigel Matrix was purchased from Corning Incorporated (Corning, NY, USA). ImmPRESSTM reagent kit and 3,3-diaminobenzidine tetrahydrochloride (DAB) was purchased from Vector Laboratories (Burlingame, CA 94010, USA). Single crystal silicon wafers were obtained from Virginia Semiconductor, Inc. (Fredericksburg, VA, USA). 48% Hydrofluoric acid was obtained from J.T. Baker (Product No. 9564-04, Phillipsburg, NJ, USA). Potassium hydroxide (KOH) was obtained from Sigma-Aldrich (Product No. 484016, St. Louis, MO, USA).

### 1.2. Cytotoxicity analysis of PEITC and TEPITC

The cytotoxicity of PEITC and TEIPTC against BxPC-3 cells, AsPC-1 cells, and PANC-1 cells were analyzed using Cell-Counting Kit-8 (CCK-8) assays according to the manufacturer's instructions. Each cell line ($1 \times 10^4$ cells per well) was seeded on 96-well plates and incubated for 24 h at 37 °C in a humidified 5% $CO_2$ incubator. The cells were then treated with 0.625, 1.25, 2.5, 5, and 10 μM concentrations of phenethyl isothiocyanate (PEITC) and triethoxy(3-isothiocyanatoproypy)-silane (TEPITC) respectively, and the cell toxicity was measured after 24 h incubation. Then, 10% WST-1 reagent was added to each well and followed by 1.5 h of incubation. The absorbance was measured at 450 nm using a microplate reader (Multiskan FC, Thermo Fisher, Waltham, MA, USA). Cell viability is calculated as the relative percentage of untreated cells.

### 1.3. Western-blot

The BxPC-3 cells ($8 \times 10^5$ cells/well) were cultured in a 6-well plate, and PEITC (20 μM) or TEPITC (20 μM) and were treated, and then incubated for 0.5, 1, 2, and 4 h. The cells were treated with TEPITC at respective concentrations of 2.5, 5, 10, and 20 μM for 30 min. To confirm the expression level of HO-1, the cells were treated with TEPITC (5 μM) for 1, 2, 4, and 6 h. Next, the cells were lysed using a cell lysis buffer (Cell Lysis buffer, #9803, Cell signaling Technology, Country, Danvers, MS, USA), and the extracted proteins were quantified by Bradford assay (Bio-Rad Protein Assay Dye Reagent Concentrate, #5000006, Bio-Rad, Hercules, CA, USA). The same amount of protein was separated by loading into SDS-PAGE gels (30% Acrylamide-bis Solution (29:1), A2004-1, Biosesang, Rep. of Korea). The separated protein was transferred to nitrocellulose membranes (BioTrace™ NT nitrocellulose 30 cm × 3 m Roll, 66485, Pall Life Sciences, Ann Arbor, MI, USA) and then incubated with 3% bovine serum albumin (BSA) buffer for 1 h. The blots were further incubated with primary antibodies of Nrf2 (Nrf2 (A-10), sc-365949, Santa Cruz Biotechnology, Dallas, TX, USA) (1:1000) and HO-1 (Heme Oxygenase 1 (A-3), sc-136960, Santa Cruz Biotechnology, Dallas, TX, USA) (1:1000) overnight. After initial incubation, the blots were incubated again with HRP-conjugated secondary antibodies (anti-rabbit IgG-HRP and anti-mouse IgG-HRP, Santa Cruz Biotechnology, Dallas, TX, USA) (1:3000) for 1 h. For the visualization, the blots were spread with enhanced chemiluminescence (ECL) solution (WestGlow™ FEMTO Chemiluminescent substrate, BWF0200, BIOMAX, Seoul, Rep. of Korea) and photographed using an Image-QuantTM LAS500 chemiluminescence (GE Healthcare Bio-sciences AB, Uppsala, Sweden). The blots were rinsed using Tris-buffered saline with 0.1% Tween 20 (TBST) in each step. A western blot was also confirmed the expression of Nrf2 in BxPC-3 cells by PBS, pSiNP, and ROSG-pSiNP (without JS-11) as supplements. The relative expression of nrf2 and HO-1 was quantitatively determined using Image-J software (National Institutes of Health, Bethesda, MD, USA).

### 1.4. Immunocytochemistry for Nrf2

BxPC-3 cells were cultured at amounts of $8 \times 10^4$ cells per well on a 4-well SPL cell culture slide (30104, SPL, Gyeonggi-do, Rep. of Korea) overnight. The cultured cells were treated with TEPITC at respective concentrations of 2.5, 5, and 10 μM for 30 min, and the cells were then fixed using 10% formalin (15 min incubation). After rinsing the cells with PBS, the cells were incubated with PBS (with 0.1% Triton X-100) for a further 20 min for permeabilization and were additionally incubated with 3% BSA in PBS for 1 h. The blocked cells were incubated with anti-Nrf2 (Nrf2 (A-10), sc-365949, Santa Cruz Biotechnology, Dallas, TX, USA) (1:100) overnight at 4 °C. After the incubation, the cells were rinsed 3 times with PBS and incubated with Alexa Fluor® 555 donkey anti-rabbit (1:500) (Donkey anti-Rabbit IgG (H+L), A-31572, Invitrogen, Waltham, MA, USA) for 1 h. After the rinsing, the cells were stained with DAPI solution (1 μg/mL, 3 min incubation) and photographed using the CELENA®S digital imaging system (Gyeonggi-do, Republic of Korea). Excitation and emission channels: DAPI ($\lambda_{ex}$: 358 nm, 461 nm), Alexa-Fluor 555 ($\lambda_{em}$: 555 nm, 580 nm).

### 1.5. JS-11 synthesis

Mefenamic acid (1 eq), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC·HCl, 1.2 eq), 4-dimethylaminopyridine (DMAP, 2.5 eq) was dissolved in dry dichloromethane (DCM) under 0 °C ice bath and stirred for 20 min. After adding 7-ethyl-10-hydroxy-camptothecin (SN-38, 1 eq) to the solution, it was stirred overnight at room temperature (25 °C). The resulting solution was extracted with 1 N HCl and DCM, then the organic layer was dried over $Na_2SO_4$. After removing the solvent under reduced pressure, and the mixture was purified by column chromatography (2% MeOH/DCM) to obtain JS-11. Yield = 95.5%; $^1$H NMR ($CDCl_3$, 500 MHz) δ (ppm) : 9.15 (s, 1H, J = 9.17 Hz), 8.29 (d, 1H, J = 8.68 Hz), 8.26 (d, 1H), 7.92 (s, 1H), 7.69 (d, 1H, J = 8.68 Hz), 7.69 (s,

1H), 7.35 (t, 1H, J = 7.72 Hz), 7.16 (d, 1H, J = 7.24 Hz), 7.12 (t, 1H, J = 7.72 Hz), 7.04 (d, 1H, J = 7.24 Hz), 6.81 (d, 1H, J = 8.68 Hz), 6.76 (t, 1H, J = 7.24 Hz), 5.73 (d, 1H, J = 16.02 Hz), 5.29 (d, 1H, J = 16.02 Hz), 5.25 (s, 2H), 4.19 (broad s, 1H), 3.17 (q, 2H, J = 7.72 Hz), 2.32 (s, 3H), 2.16 (s, 3H), 1.90 (m, 2H), 1.43 (t, 3H, J = 7.72 Hz), 1.02 (t, 3H, J = 7.72 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz), δ (ppm): 173.7, 167.1, 157.5, 151.7, 150.5, 150.1, 149.7, 147.3, 146.7, 145.2, 138.3, 138.1, 135.3, 132.6, 132.0, 131.8, 127.4, 127.2, 127.2, 126.0, 125.8, 123.3, 118.5, 116.2, 114.9, 113.9, 109.0, 98.0, 72.7, 66.2, 49.3, 31.6, 23.1, 20.5, 13.9, 7.8. ESI-MS m/z calc. for $C_{37}H_{33}N_3O_6$: 615.24, found 614.15 [M-1]-. 1H and $^{13}$C NMR spectra were collected in $CDCl_3$ on Bruker 500 MHz spectrometer (MA, USA). Mass spectral analyses were recorded using LC/MS-2020 Series (Shimadzu, Kyoto, Japan).

**1.6. UV/Vis absorption and fluorescence spectroscopic methods**

UV/Vis absorption spectra were measured using a Spectro-photometer (Agilent Technologies Cary 8454, Santa Clara, CA, USA), and fluorescence spectra were recorded on a spectro-fluorophotometer (SHIMADZU CORP. RF-6000, Kyoto, Japan) with a 1 cm standard quartz cell (internal volume of 1 mL, Hellma Analytics, Germany). All absorption and emission spectra were obtained at room temperature (25 °C).

**1.7. ROS responsiveness of JS-11**

The JS-11 stock solution (10 μM) was incubated at 37 °C in 1 mL of phosphate-buffered saline (PBS) or 1 mM hydrogen peroxide ($H_2O_2$). At timed intervals, we measured the fluorescence intensity at 560 nm (emission peak of SN-38, $\lambda_{ex}$ = 365 nm).

**1.8. Degradation rate analysis of pSiNP**

The degradation rate of pSiNP was analyzed by measuring UV/Vis absorbance of pSiNP as a function of time within the $H_2O_2$ solution. pSiNP (0.1 mg/mL) were incubated in 1 mL of $H_2O_2$ (10–1000 μM) at 37 °C. At each interval, the absorbance of pSiNP at 405 nm was measured to analyze the degradation rate of the Si skeleton in the nanoparticles.

**1.9. Drug release and conversion profile**

Release and drug conversion profile (SN-38 from ROSG-pSiNP(JS-11)) was analyzed using a spectro-fluorophotometer. The samples (1 mg/mL) were in PBS with/without $H_2O_2$ at 37 °C, then removed from the aqueous phase by centrifugation at 14,000 rpm for 15 min. The fluorescence (560 nm, $\lambda_{ex}$ = 365 nm) of SN-38 left in the supernatant was measured at the designated time-point to determine the conversion profile.

**1.10 Analysis of cellular total glutathione (GSH) and glutathione peroxidase (GPx) enzyme activity**

A glutathione (GSH) assay kit (Product No. DG-GLU200, Dogen, Seoul, Rep. of Korea) was used to measure total cellular glutathione and a glutathione peroxidase cellular activity assay kit (Product No. CGP1, Sigma-Aldrich St, Louis, MO, USA) was used to measure GPx enzyme activity according to the manufacturer's instructions. The cells ($5 \times 10^5$ cells per well) were seeded on 6-well plates and incubated for 24 h at 37 °C. The cells were then treated with 12.5, 25, 50, and 100 μg/mL of ROSG-pSiNP(JS-11) and further incubated for 6 h. After incubation, the cells were washed with a cold PBS 3 times, and cell extracts were prepared by sonication and freezing/thawing. Total GSH was detected by measuring the absorbance at 405 nm of the product of glutathionylated DTNB using a microplate reader (Multiskan FC, Thermo Fisher, Waltham, MA, USA). GPX enzyme activity (ΔA340 nm/min) was calculated using continuous spectrophotometric rate determination.

**1.11. Cytotoxicity analysis of nanoparticles**

The cytotoxicity of pSiNP, JS-11, pSiNP(JS-11), and ROSG-pSiNP(JS-11) against BxPC-3 cells were analyzed using Cell-Counting Kit-8 (CCK-8) assays according to the manufacturer's instructions. The cells ($1 \times 10^4$ cells per well) were seeded on 96-well plates and incubated for 24 h at 37 °C in a humidified 5% $CO_2$ incubator. The cells were then treated with 0.625, 1.25, 2.5, 5, and 10 μM JS-11 concentrations in the nanoparticles, and the cell toxicity was measured after 24 h incubation. After the incubation, 100 μL of RPMI-1640 (10% WST-1) was added to each well, followed by a 1.5 h incubation period. The absorbance was measured at 450 nm using a microplate reader (Multiskan FC, Thermo Fisher, Waltham, MA, USA). Cell viability is calculated as the relative percentage of untreated BxPC-3 cells.

**1.12. Hemolysis test**

The blood sample was obtained from the hearts of the mice anesthetized with isofluorane. The red blood cells were collected by centrifugation at 4 °C (3000 rpm, 3 min) with a cold 1× PBS (1 mL, 3 times). 0.1 mg/mL of ROSG-pSiNP(JS-11) was treated with 8% (*v/v*) purified red blood cells and further incubated for 1 h at 37 °C. The mixture was then collected using centrifugation at 4 °C (3000 rpm, 3 min). The absorption (492 nm) of supernatant was measured at 25 °C.

**1.13. In vivo clearance of ROSG-pSiNP(JS-11)**

ROSG-pSiNP(JS-11) was were intravenously injected into BALB/c mice (female, n=3 per group) at a dose of 20 mg/kg. For nanoparticle clearance analysis, the mice were sacrificed after 2 h and 1 day. The fluorescence signals from the harvested major organs (lung, heart, liver, spleen, kidneys) were acquired using VISQUE in Vivo Smart LF luminescence and fluorescence animal imaging system (Vieworks Co., Ltd., Rep. of Korea). Excitation: GFP filter ($\lambda_{ex}$: 390–490 nm) and emission: ICG filter ($\lambda_{em}$: 810–860 nm

**1.14. Immunohistochemistry (IHC)**

IHC was performed to confirm that the NCS induced distribution and localization of Nrf2 and HO-1 proteins in tumor tissue. Each female BxPC-3 tumor-bearing mouse was randomly divided into 3 groups (2 mice per group), and i.v. injected (60 mg/kg) with (1) PBS only; (2) pSiNP only; and (3) ROSG-pSiNP. The mice were sacrificed and tumors were dissected 48 h after i.v. injection. IHC detects specific antigens in preserved tissue sections using an appropriate antibody labeling strategy. Therefore, the tissue was fixed by vascular perfusion using a 4% paraformaldehyde fixative solution after removing all blood using a PBS perfusion to preserve tissue morphology and retain the antigenicity of the target proteins. Tumor tissues were fixed in formaldehyde, rinsed under running tap water, and embedded in paraffin wax. Sections of paraffin blocks were cut at the thickness of 5 μm and transferred to a slide. Then, the tissue slides were deparaffinized in xylene, rehydrated in graded ethanol, and finally hydrated in tap water. Antigen was retrieved on a steam slide in 10 mM sodium citrate (pH 6.0) at 100 °C for 30 min. IHC was performed following the manufacturer's instructions (Vector Laboratories ImmPRESSTM REAGENT KIT, 30 Ingold Road, Burlingame, CA 94010, USA). Endogenous peroxidase activity was quenched with 3% $H_2O_2$ for 20 min, and non-specific binding was blocked by incubation, in a blocking solution of normal horse serum in ImmPRESS REAGENT KIT. The tissue slides were then incubated with anti-Nrf2 and anti-HO-1 dilutes 1:200 in a blocking solution overnight at 4 °C. Slides were subsequently rinsed several times in PBS and were incubated with ImmPRESS® Horse Anti-Rabbit and Mouse IgG Polymer KIT solution for 30 min at room temperature (25 °C). Immunoreactive species were detected using 3,3-diaminobenzidine tetrahydrochloride (DAB) as a substrate. Tissue slides were counterstained with Gill's hematoxylin. Finally, the dehydrated tissue slides with ethanol and xylene were mounted under the glass coverslips. Images were taken using an eXcope T500 microscope camera (magnification, 200×, Olympus, Tokyo, Japan).

### 1.15. Histological analysis of AST and ALT

Serum samples (from xenograft mouse model after injection of nanoparticles/JS-11) were collected, and an enzymatic colorimetric method was performed for assessing the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Cobas 8000 c502 (Roche/HITACHI, Japan by Seoul Medical Science Institute, Seoul Clinical Laboratories, Seoul, Korea) was used for the analysis.

### 1.16. Histological analysis (H&E assay)

Each fixated organ was sliced into 4-5 μm thick sections from paraffin blocks as previously described immunohistochemistry (IHC) method. The sliced sections were deparaffinized in xylene and rehydrated absolute ethanol series (100%, 90%, 80%, and 70%). For Hematoxylin and eosin (H&E) staining, the sliced sections were stained in hematoxylin for 5 min and then rinsed in running tap water for 1 min. Then, the sections were stained in eosin for 30 s, dehydrated, and mounted using general methods. Images were taken using an eXcope T500 microscope camera (magnification, 200×, Olympus, Tokyo, Japan).

## Supporting Figures

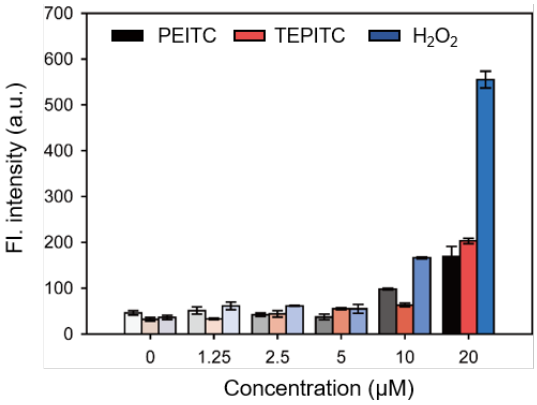


**Figure S1.** PEITC/TEPITC-induced intracellular ROS generation analysis. Intracellular ROS was measured by DCFDA assay after 2 h treatment with PEITC or TEPITC toward BxPC-3 cell line. Different concentrations (0–20 μM) of PEITC/TEPITC were treated to the cells and incubated for 2 h at 37 °C. The cells were then treated with $H_2O_2$ as a positive control. Data are presented as means ± SDs.

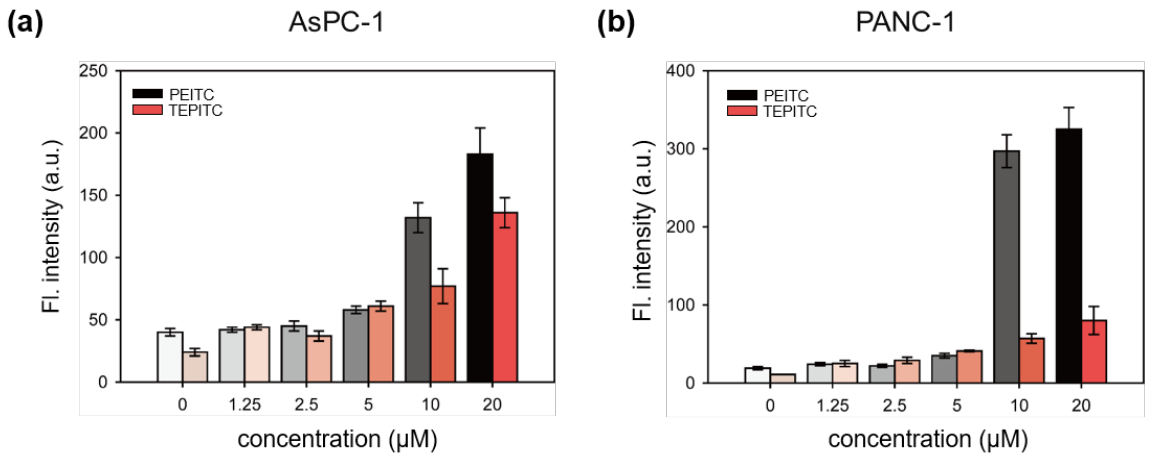


**Figure S2.** PEITC/TEPITC-induced intracellular ROS generation analysis. Intracellular ROS was measured by DCFDA assay after 2 h treatment with PEITC or TEPITC toward (a) AsPC-1 cell line and (b) PANC-1 cell line. Data are presented as means ± SDs.

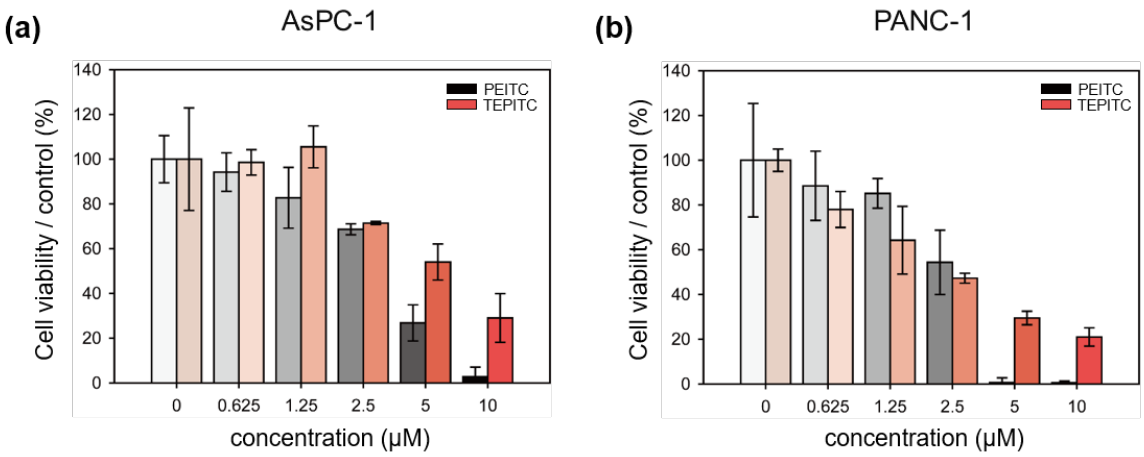


**Figure S3.** Cytotoxicity analysis of PEITC or TEPITC toward (a) AsPC-1 cell line, and (b) PANC-1 cell line. Cytotoxicity of each sample (0–10 μM) was quantified by CCK-8 assay.

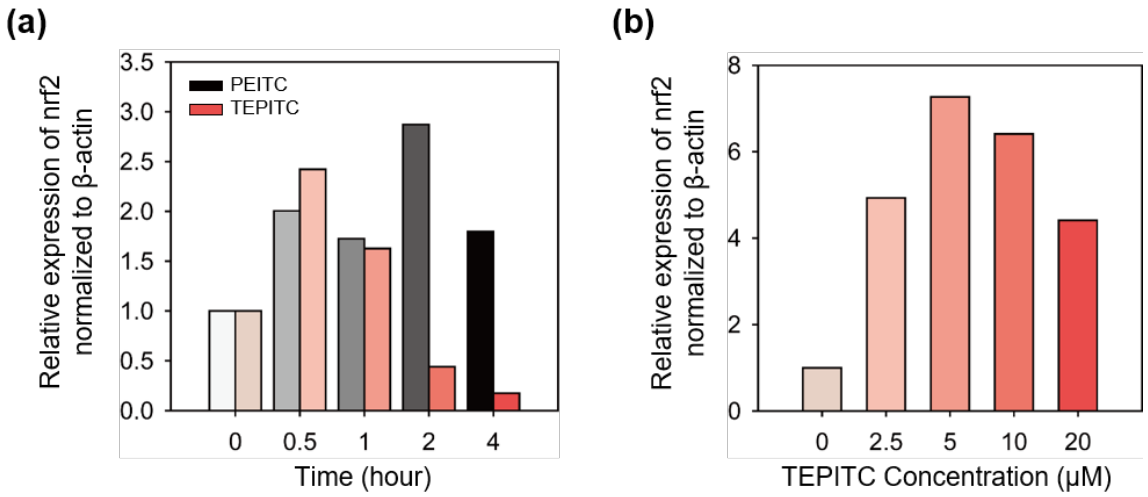


**Figure S4.** PEITC/TEPITC-induced relative nrf2 expression quantification by western blot analysis. Quantitative fold increase of nrf2 expression of BxPC-3 cells (a) at 0.5, 1, 2, and 4 h after treating PEITC and TEPITC with concentration of 20 μM. (b) The cells were incubated with different concentrations (0–20 μM) of TEPITC for 30 min at 37 °C.

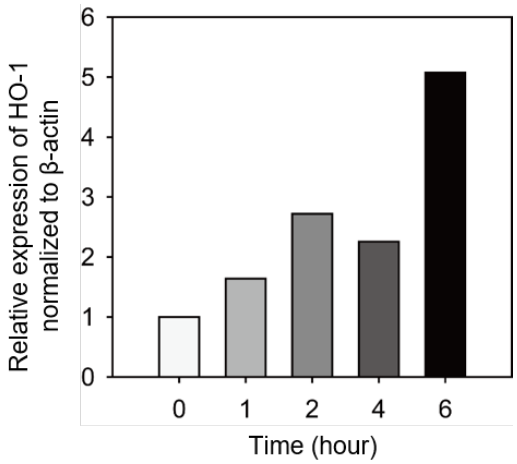


**Figure S5.** PEITC/TEPITC-induced relative HO-1 expression quantification by western blot analysis. The BxPC-3 cells were treated with 5 μM of TEPITC and then incubated at 37 °C for 1, 2, 4, and 6 h.

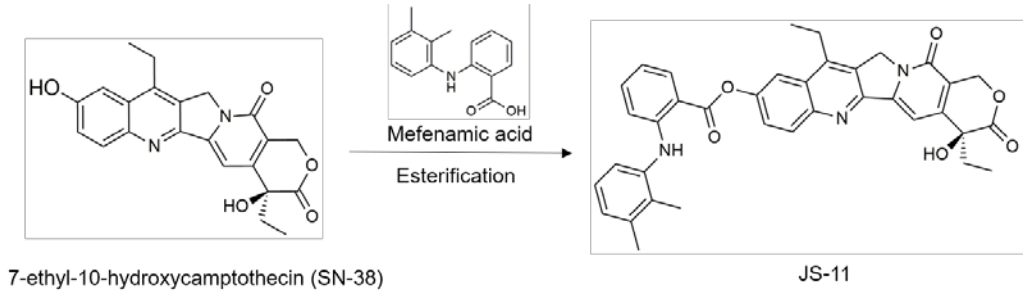


**Figure S6.** Synthesis of JS-11. Reagents and conditions: 7-ethyl-10-hydroxy-camptothecin (SN-38, 1 eq), Mefenamic acid (1 eq), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC·HCl, 1.2 eq), 4-dimethylaminopyridine (DMAP, 2.5 eq), dichloromethane (DCM), 12 h, 25 °C, yield: 95.5%.

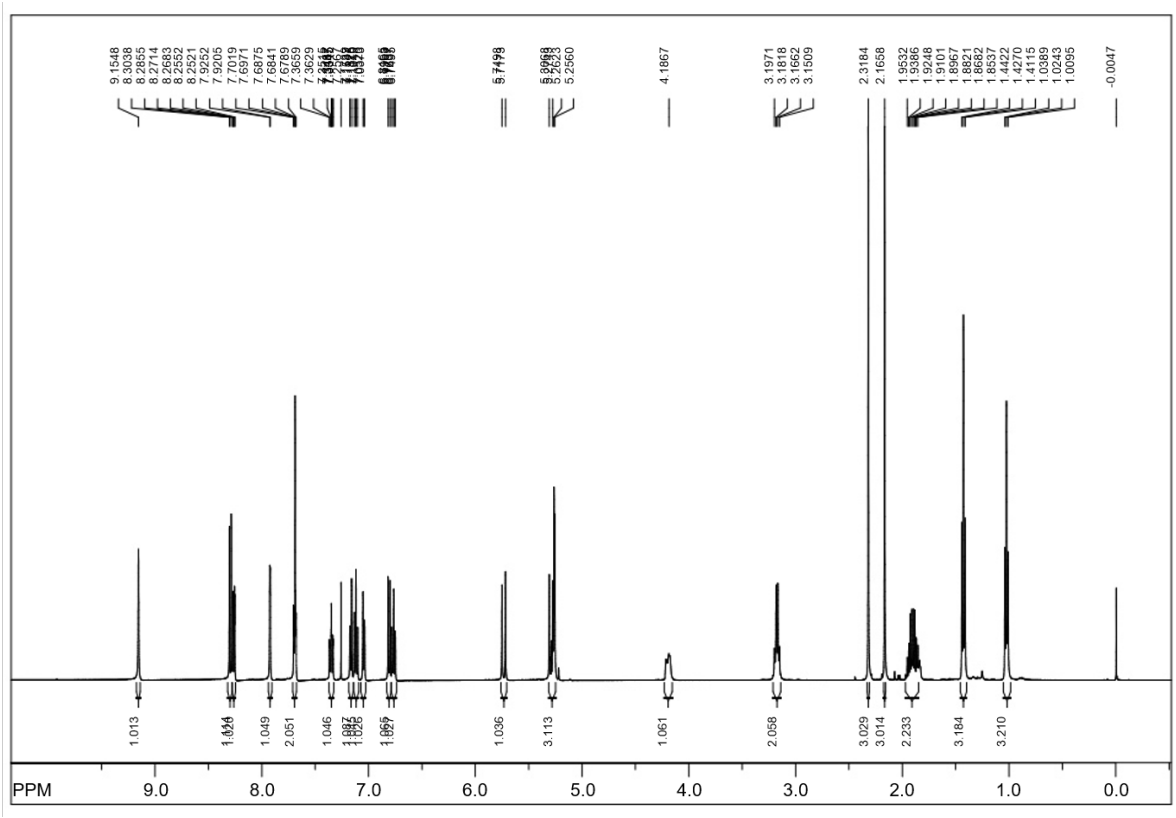


**Figure S7.** $^{1}H$ NMR spectra (500 MHz) of JS-11 in $CDCl_3$

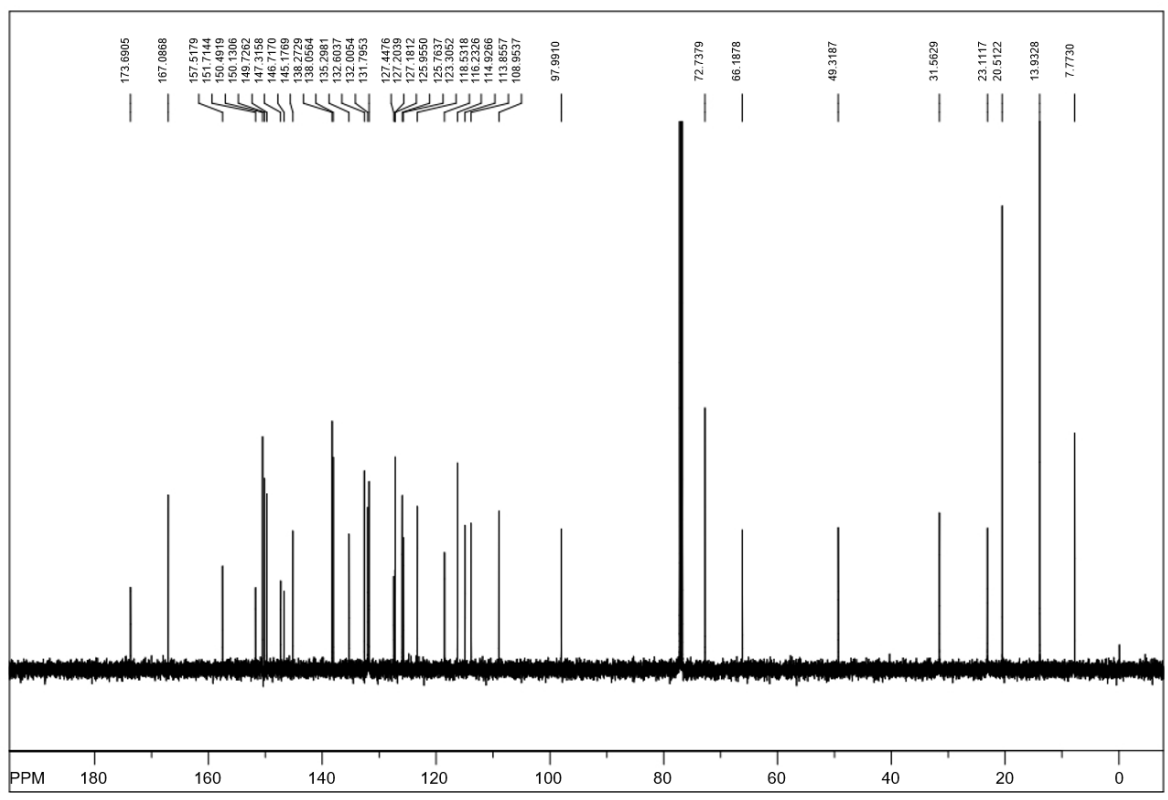


**Figure S8.** $^{13}C$ NMR spectra (125 MHz) of JS-11 in $CDCl_3$.

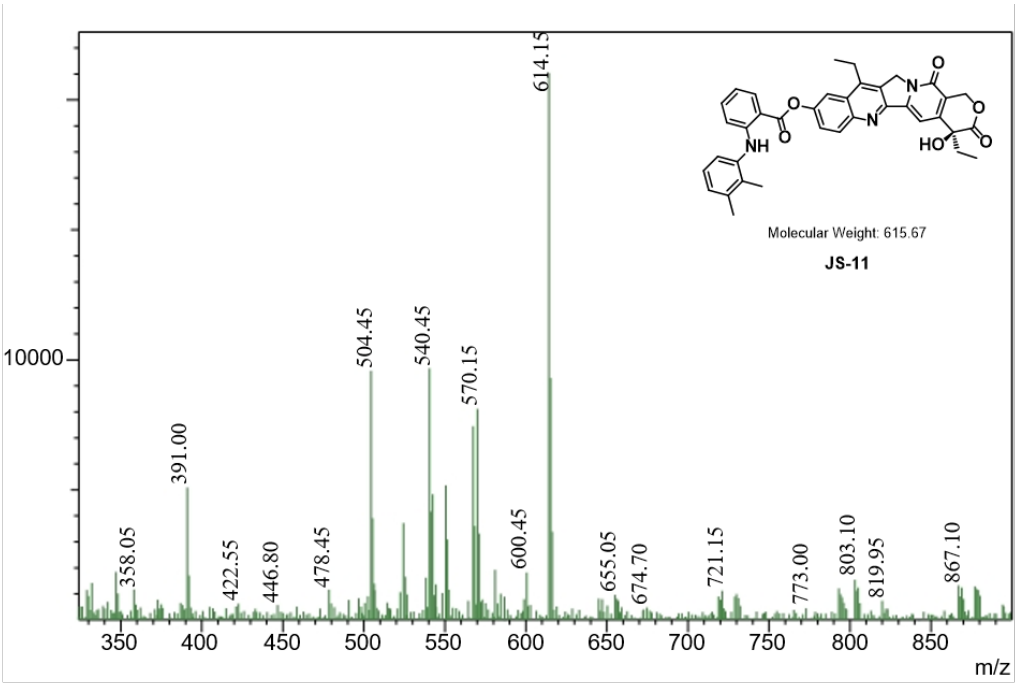


**Figure S9.** ESI-MS spectra of JS-11.

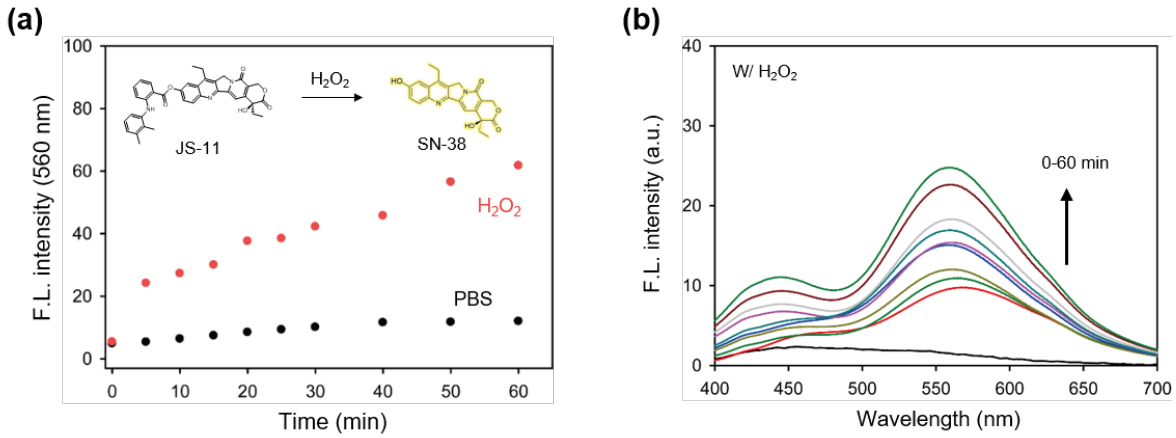


**Figure S10.** Fluorescence intensity response of JS-11 (10 μM) in PBS or $H_2O_2$. (a) Fluorescence intensity plots of JS-11 at 560 nm. (b) Time-dependent fluorescence increases of JS-11 (10 μM) in $H_2O_2$ at 37 °C.

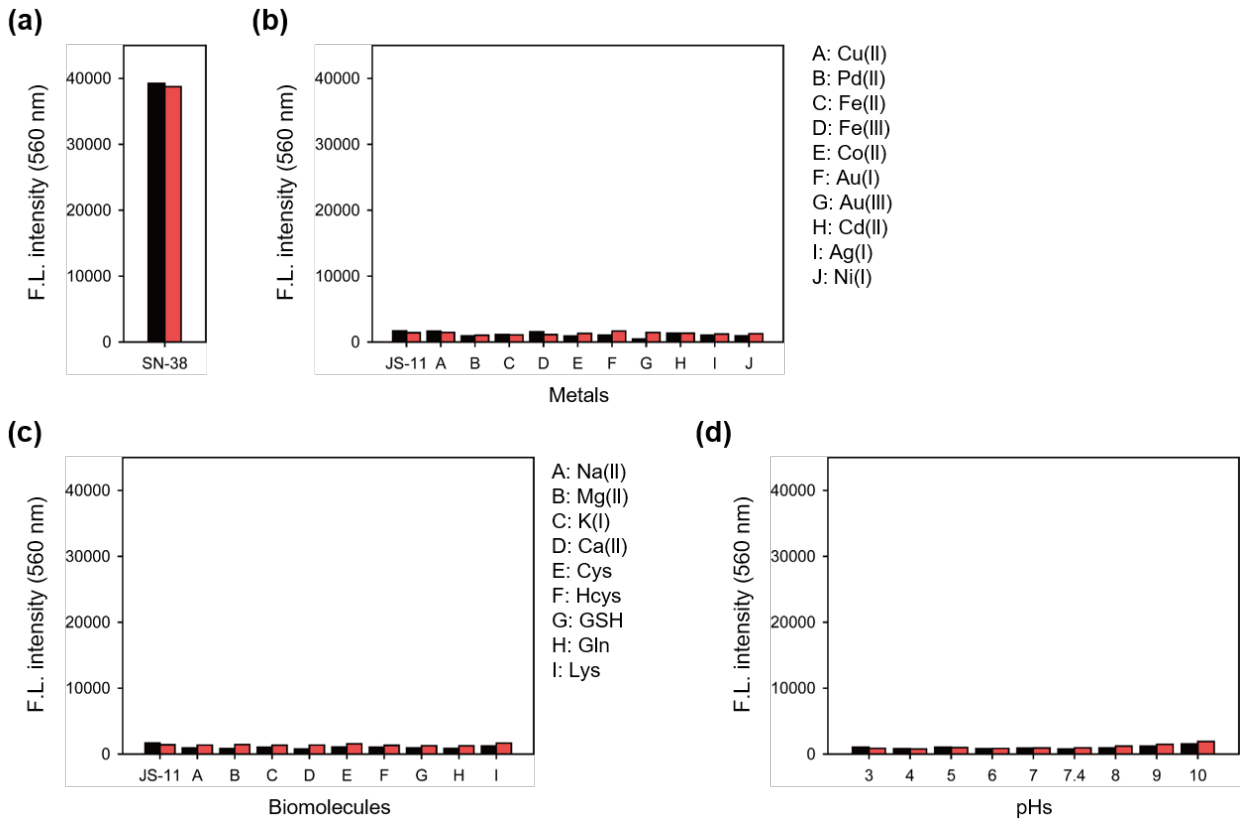


**Figure S11.** Cleavage screening of JS-11. (a) Fluorescence changes (peak height at 560 nm) of SN-38 (10 μM) in PBS, measured after incubating for 10 s (black bar) and 1 h (red bar) at 37 °C. (b) Fluorescence changes (peak height at 560 nm) of JS-11 (10 μM) after adding each metal ion (50 eq) into PBS, measured after incubating for 10 s (black bar) and 1 h (red bar) at 37 °C. (c) Fluorescence changes (peak height at 560 nm) of JS-11 (10 μM) after adding each biomolecule (50 eq) into PBS, measured after incubating for 10 s (black bar) and 1 h (red bar) at 37 °C. (d) Fluorescence changes (peak height at 560 nm) of JS-11 (10 μM) in various pHs (pH 3, 4, 5, 6, 7, 7.4, 8, 9, and 10) measured after incubating for 10 s (black bar) and 1 h (red bar) at 37 °C. The emission spectra were measured under excitation at 367 nm.

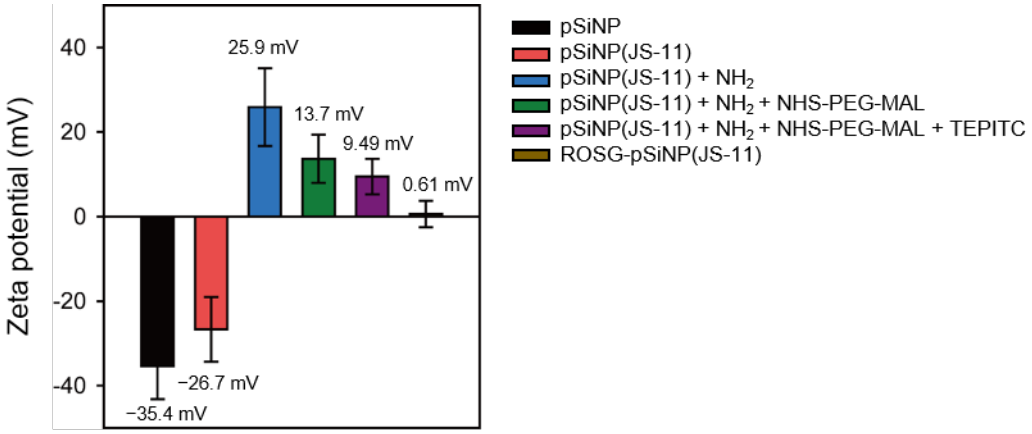


**Figure S12.** The variation of the zeta-potential value of pSiNP and its surface modified formulations. The value of each sample was measured in triplicate for standard deviation calculation.

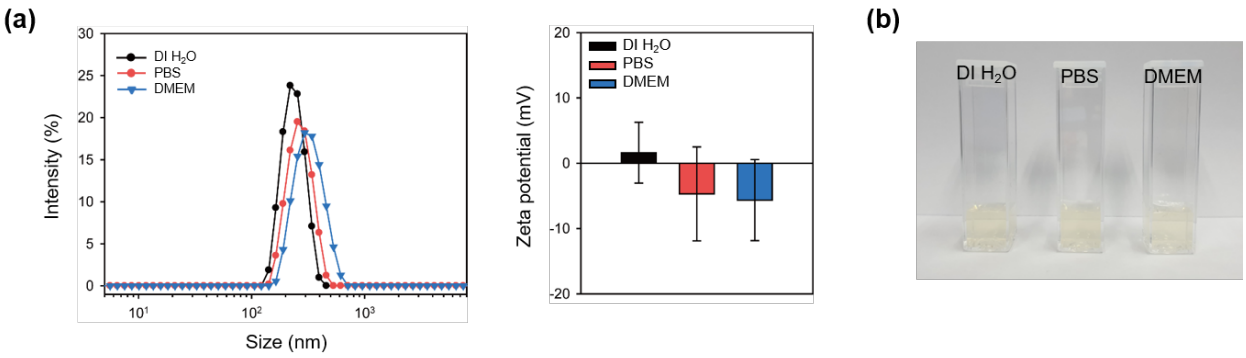


**Figure S13.** The colloidal stability of ROSG-pSiNP(JS-11). (a) Average hydrodynamic size (intensity distribution, left) and zeta-potential value (right) of ROSG-pSiNP(JS-11) in DI $H_2O$, PBS, and DMEM. (b) Photograph of ROSG-pSiNP(JS-11) dispersion in DI $H_2O$, PBS, DMEM.

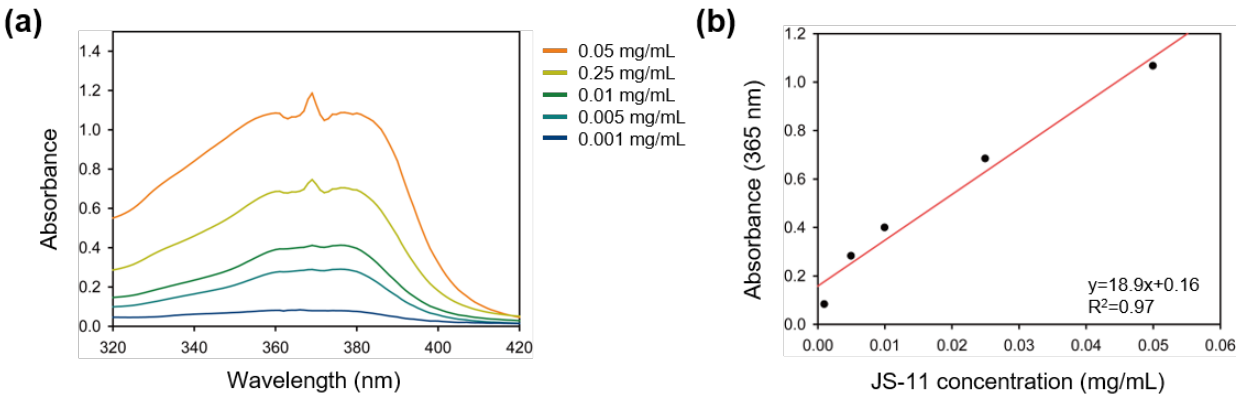


**Figure S14.** Absorption standard curve of JS-11. (a) Absorption spectra of JS-11 (0.001–0.05 mg/mL) in ethanol. (b) Concentration-dependent absorbance intensity plot of JS-11 at 365 nm.

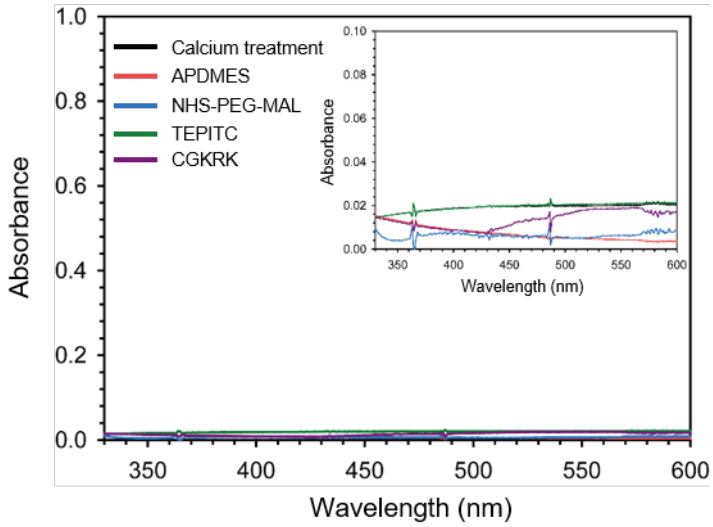


**Figure S15.** The absorption spectra of supernatants of each surface modification step of pSiNP(JS-11) (1 mg/mL).

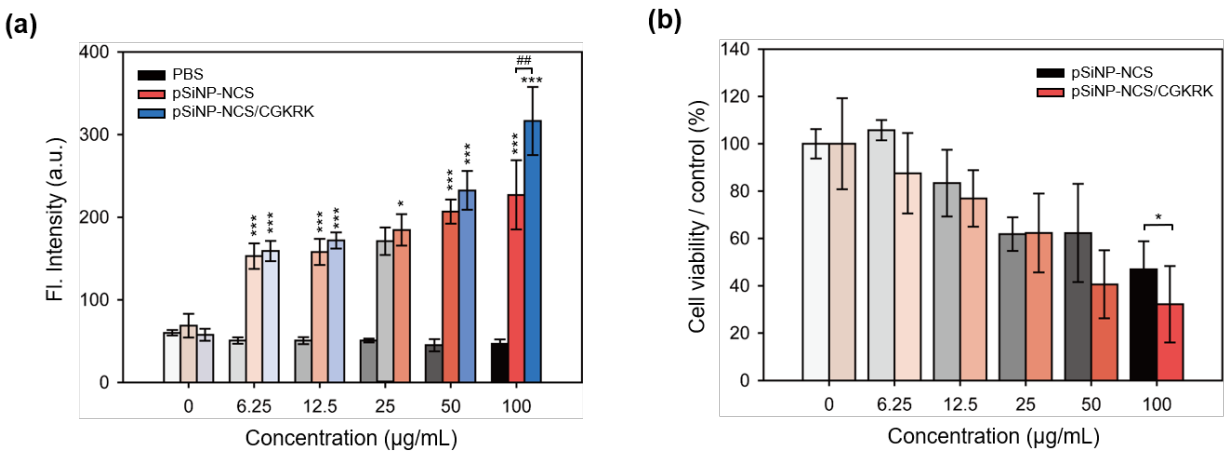


**Figure S16.** Intracellular ROS monitoring and cell viability after treatment of the pSiNP-NCS and pSiNP-NCS/CGKRK (without JS-11). (a) Fluorescent intensity plot ($\lambda_{max}$: 535 nm) of intracellular ROS-sensing probe (DCFDA) within BxPC-3 cells. The excitation wavelength for the monitoring: 485 nm. Different concentrations of nanoparticles (0–100 μg/mL) were treated to the cells and incubated for 5 h at 37 °C. (b) Cell viability of BxPC-3 cells after treatment of nano-formulations incubated for 24 h at 37 °C. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared with PBS control. $^{\#\#}p \leq 0.01$ compared with pSiNP-NCS treated group.

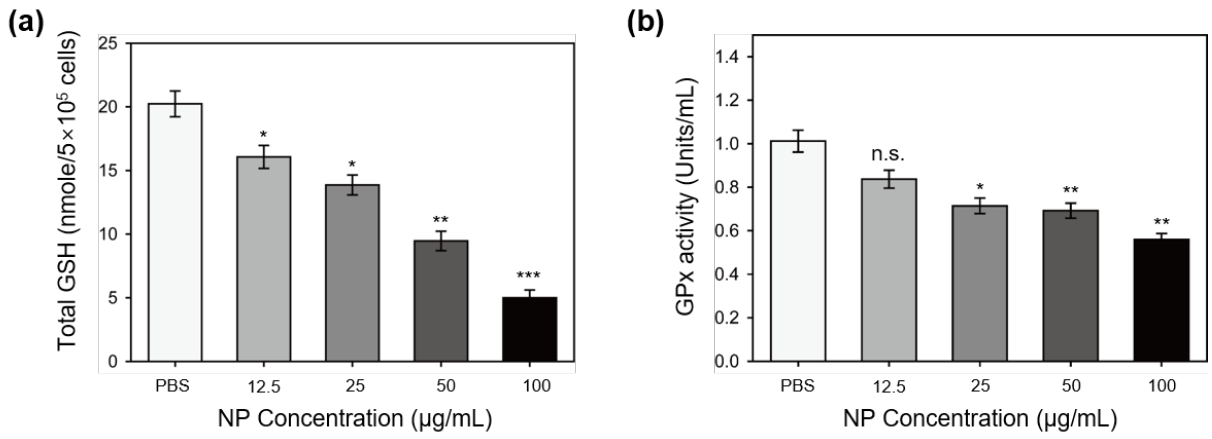


**Figure S17.** Total cellular GSH and GPx enzyme activity depletion by ROSG-pSiNP(JS-11). (a) Total cellular GSH level and (b) GPx enzyme activity in BxPC-3 cells and after treatment with ROSG-pSiNP(JS-11) (concentration: 0–100 μg/mL) for 6 h. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared with PBS control.

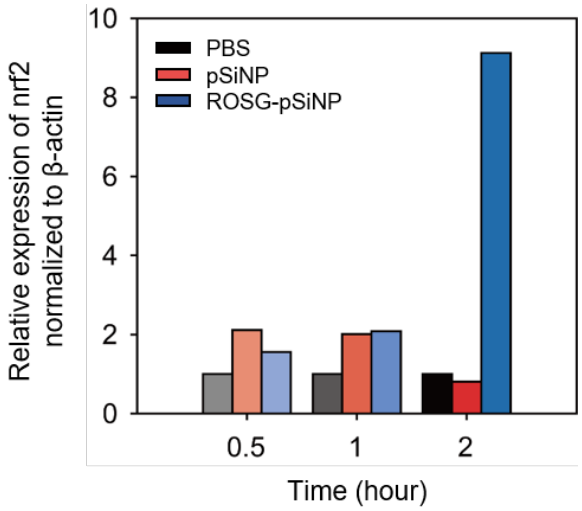


**Figure S18.** ROSG-pSiNP induced-relative level of nrf2 protein. The cells were treated with PBS, pSiNP, and ROSG-pSiNP at 0.5, 1, and 2 h at 37 °C.

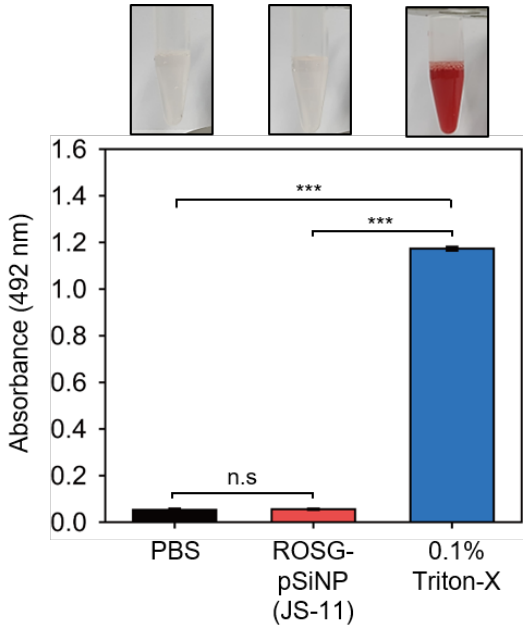


**Figure S19.** Hemolysis anlysis of ROSG-pSiNP(JS-11). The blood samples were incubated with 0.1 mg/mL ROSG-pSiNP(JS-11), 1× PBS (negative control), and 0.1% (*v/v*) triton X-100 (positive control) for 1 h at 37 °C. UV/vis absorption peak ($\lambda_{max}$: 492 nm) was recorded at 25 °C.

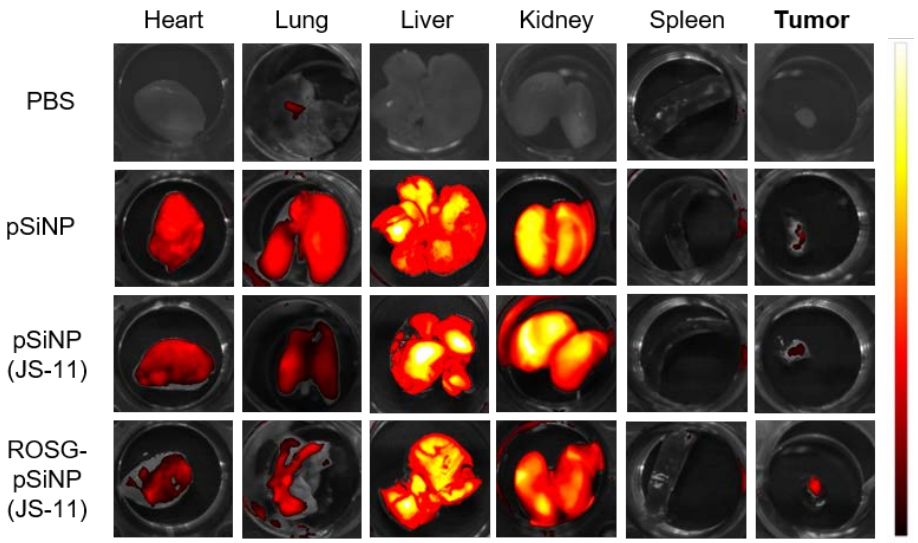


**Figure S20.** In vivo distribution of nano-formulations (pSiNP, pSiNP(JS-11), ROSG-pSiNP(JS-11)) in pancreatic cancer model mouse after 1 h circulation. GFP excitation filter ($\lambda_{ex}$: 390-490 nm) and the ICG emission filter ($\lambda_{em}$: 810-860 nm) were used for the signal collection.

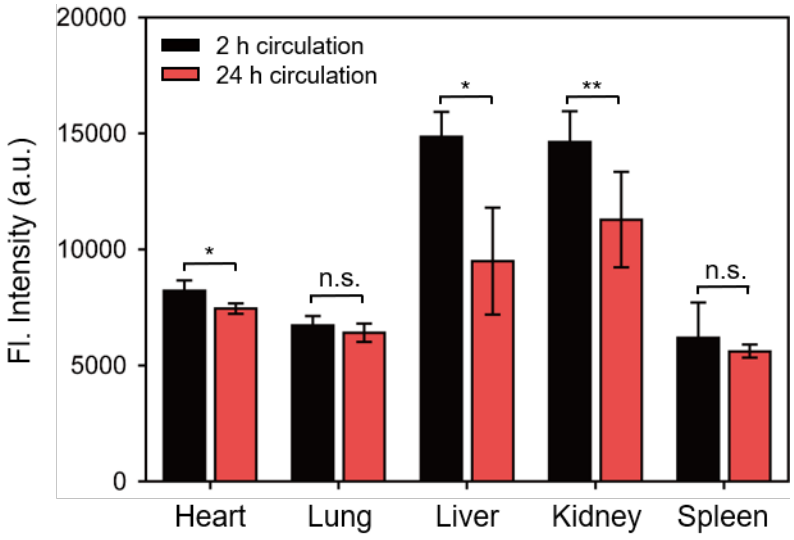


**Figure S21.** In vivo biodistribution and biodegradation of ROSG-pSiNP(JS-11). The ROSG-pSiNP(JS-11) was intravenously injected into the mouse (20 mg/kg, n=3). The major organs (heart, lung, liver, kidney, spleen) were harvested 2 h and 24 h after the injection, respectively. Blue light excitation filter ($\lambda_{ex}$: 390-490 nm) and the ICG emission filter ($\lambda_{em}$: 810-860 nm) were used for the fluorescence intensity measurement.

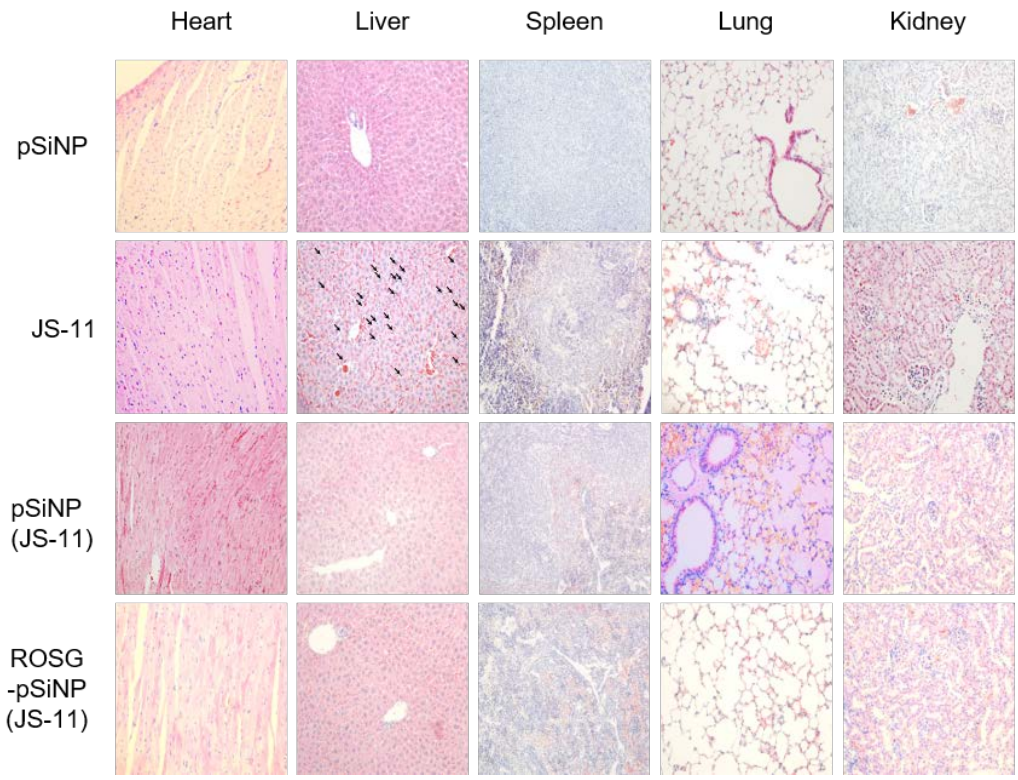


**Figure S22.** Histological analysis. H&E staining of heart, liver, spleen, lung, and kidney after treatment of pSiNP, JS-11, pSiNP(JS-11), and ROSG-pSiNP(JS-11). See details in the Method section. The black arrow indicates nuclear condensation and degenerated forms.