

Supplementary material

A Tumor-Activatable Theranostic Nanomedicine Platform for NIR Fluorescence-Guided Surgery and Combinatorial Phototherapy

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Characterization:

Eq. 1 Loading efficiency, % = (amount of SiNc (mg) in 1 mL of solution / amount of polymer (mg) in 1
mL solution) *100%

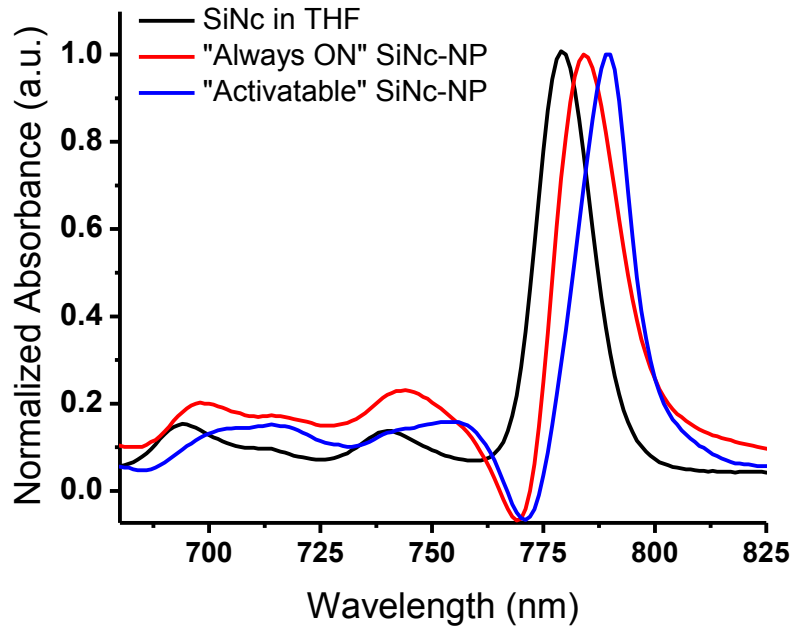


Figure S1. Normalized absorption spectra of free SiNc in THF (black), “Always ON” SiNc-NP (red), and “Activatable” SiNc-NP (blue) in 1X PBS buffer.

Fluorescence quantum yield:

The fluorescence quantum yield for SiNc-PNP was calculated in aqueous solution ($\lambda_{\text{ex}} = 750 \text{ nm}$) with indocyanine green (ICG) as a reference (0.027 in water)^[1] as follows:

Eq. 2
$$\Phi_{\text{SiNc}} = (\text{Abs}_{\text{ICG}}/\text{Abs}_{\text{SiNc}}) \times (\text{Area}_{\text{SiNc}}/\text{Area}_{\text{ICG}}) \times \Phi_{\text{ICG}} \times 100$$

where Φ_{SiNc} is a quantum yield of SiNc-PNP; Abs_{ICG} and Abs_{SiNc} are absorbances of ICG and SiNc-PNP, respectively; Area_{ICG} and $\text{Area}_{\text{SiNc}}$ are fluorescence emission areas of ICG and SiNc-PNP, respectively; where Φ_{ICG} is a quantum yield of SiNc-PNP. Water was employed as a solvent for ICG and SiNc-PNP and, therefore, the refractive index was not used in the equation.

Drug Release:

The drug release profiles of dye (SiNc or IR775) from the PEG-PCL nanoparticles were evaluated in PBS at 37°C at various pH (7.4, 6.5, and 4.5 after different time points). The developed theranostic nanoplatform was dissolved in PBS buffer and placed in a Float-A-Lyzer dialysis tubes (molecular weight cutoff of 50 kDa). The dialysis tubes were immersed in the appropriate medium and incubated at a constant temperature of 37 °C. At fixed time intervals, 200 µL of the samples were withdrawn from the dialysis tubes to record the absorbance of dye. After each absorption measurement, the samples were returned to the appropriate dialysis tubes for further incubation. The dye content in the delivery system at different time points was quantified based on UV-visible absorption spectra of samples, with a prominent dye peak appearing (UV-1800 spectrophotometer, Shimadzu, Carlsbad, CA). The percentage of drug release at different time points was calculated as follows:

Eq. 3
$$\text{Drug release (\%)} = [dye]R/[dye]T \times 100,$$

where $[dye]R$ is the amount of dye released at collection time and $[dye]T$ is the total amount of dye that was encapsulated in the delivery system.

Activatable SiNc-PNP in Media

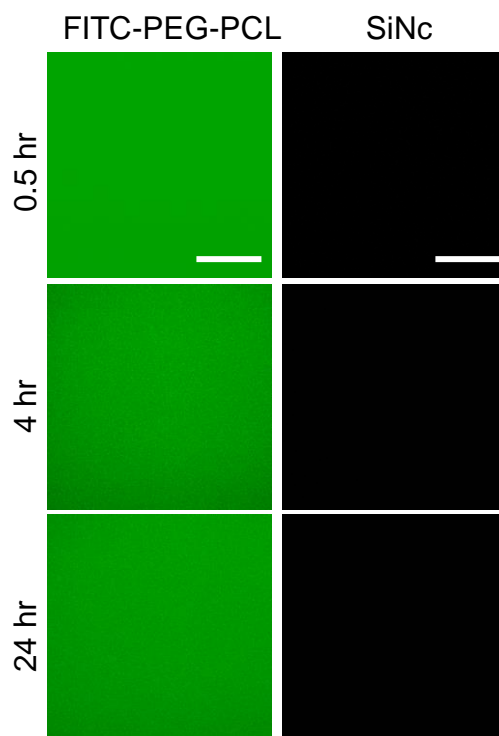


Figure S2. Images of activatable SiNc-PNP prepared with FITC-labelled PEG-PCL in serum containing media incubated at cell culture conditions at corresponding time points. All Images were acquired by 40 × objective, scale bar is 50 μm for all images.

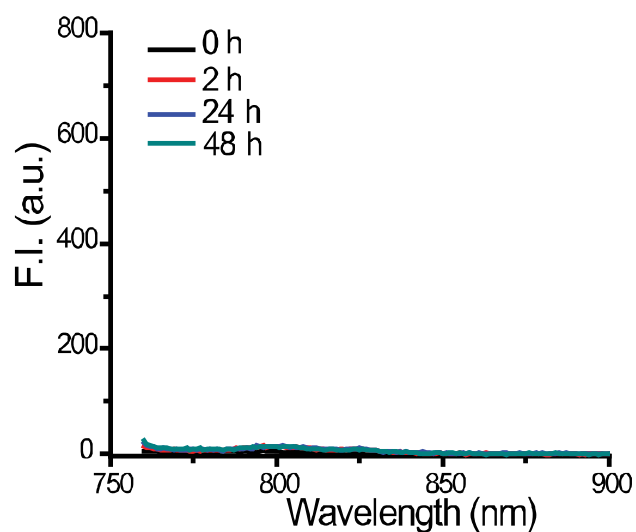


Figure S3. Fluorescence spectra ($\lambda_{\text{ex}} = 750 \text{ nm}$) of activatable SiNc-PNP incubated at 37 °C in 50% human plasma for 0, 2, 24, and 48 h.

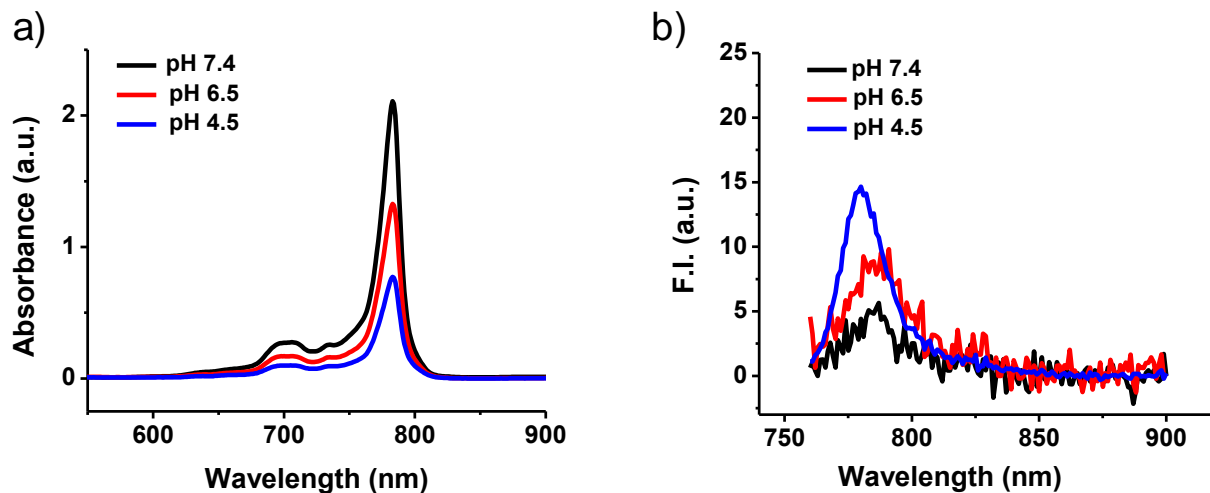


Figure S4. Absorbance spectra of activatable SiNc-PNP incubated at 37 °C in PBS buffer at pH 7.4, 6.5 and 4.5 for 24 hours.

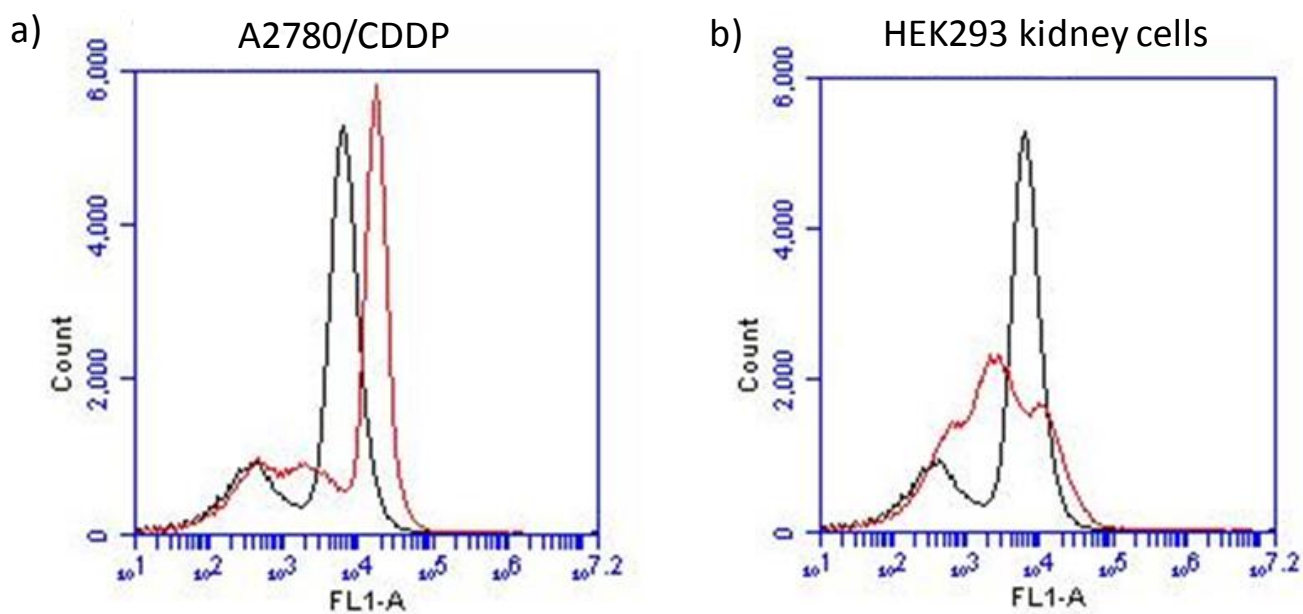


Figure S5. Flow cytometry analysis of A2780/CDDP (a) and HEK293 (b) cells incubated for 24 hours with FITC-labeled SiNc-PNP (red curves). Black curves represent cells treated with media only.

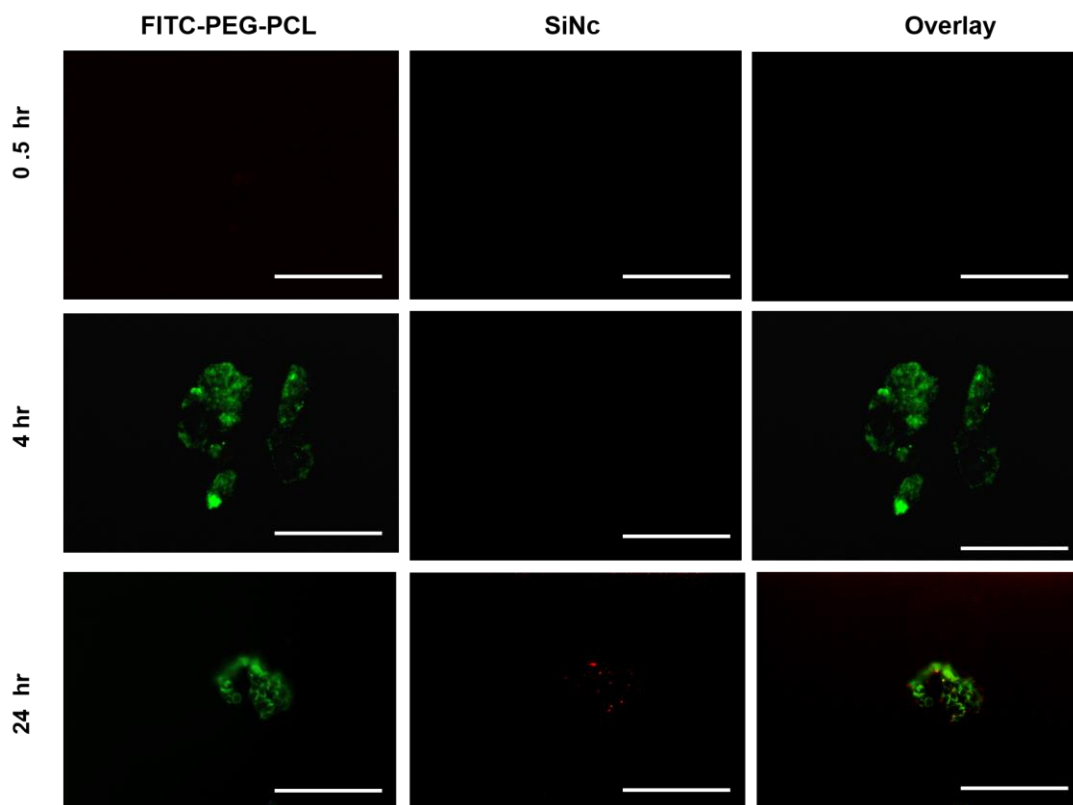


Figure S6. a) Representative fluorescence microscopy images of human embryonic kidney cells 293 (HEK293) at different incubation periods (0.5, 4, and 24 hours) with activatable SiNc-PNP prepared with FITC-labelled PEG-PCL. The left panel shows images of A2780/CDDP ovarian cancer cells obtained at Ex 470/Em 510 nm, representing fluorescent signals from FITC-PEG-PCL; the middle panel show images obtained at Ex 710/Em 775 nm, representing NIR fluorescent signals from SiNc; the right panel is an overlay of first two panels. All Images were acquired by 40 × objective, scale bar is 50 μm for all images.

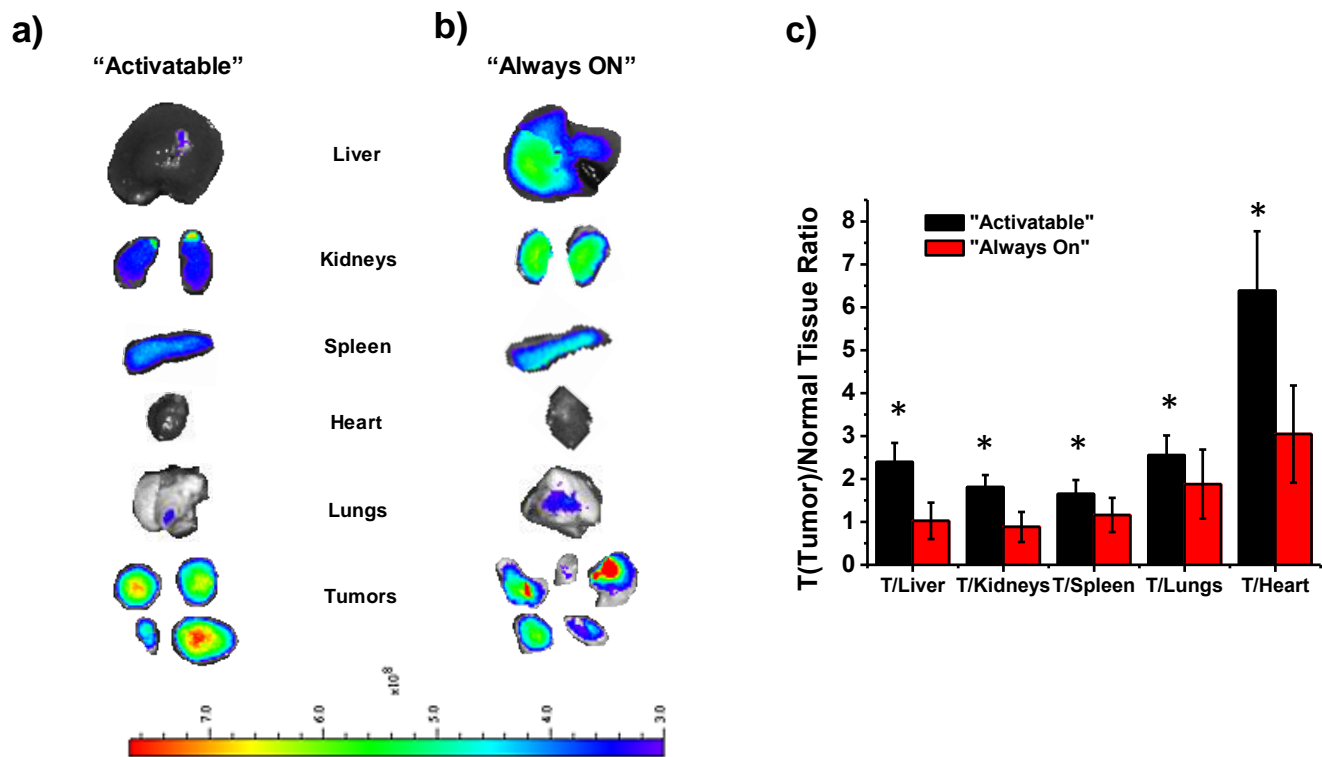


Figure S7. Distribution of NIR fluorescence generated by SiNc in organs and cancer tissues 24 hours after intravenous (*i.v.*) of the “activatable” (a) and “Always ON” SiNc-PNP (b) into mice with intraperitoneal xenograft of luciferase-expressing ES2 ovarian cancer cells. The NIR fluorescence images were recorded with the IVIS imaging system. **Note:** only cancer tissues generate bioluminescence signal in mice injected with D-luciferin. (C) Tumor-to-normal tissue (T/N) analysis of fluorescence intensity in tumors and organs harvested from mice 24 hours after injection with “activatable” and “always ON” nanoparticles. * $P < 0.05$ when activatable nanoparticles are compared with “always ON” nanoparticles.

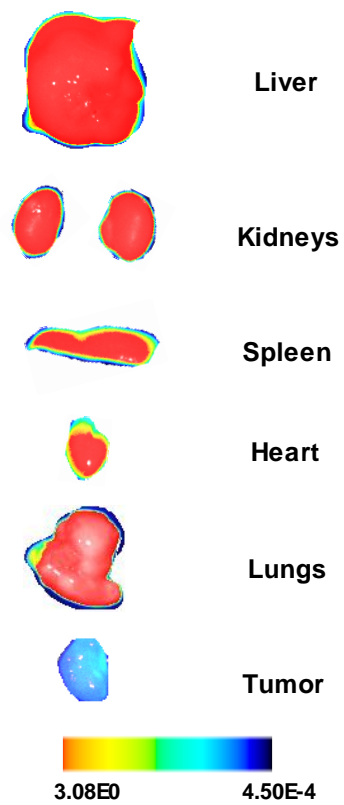


Figure S8. Fluorescence images of various organs and subcutaneous ovarian cancer tumor acquired at 24 h post *i.v.* injection of aqueous solution of the free IR775 dye that contains 5% DMSO.

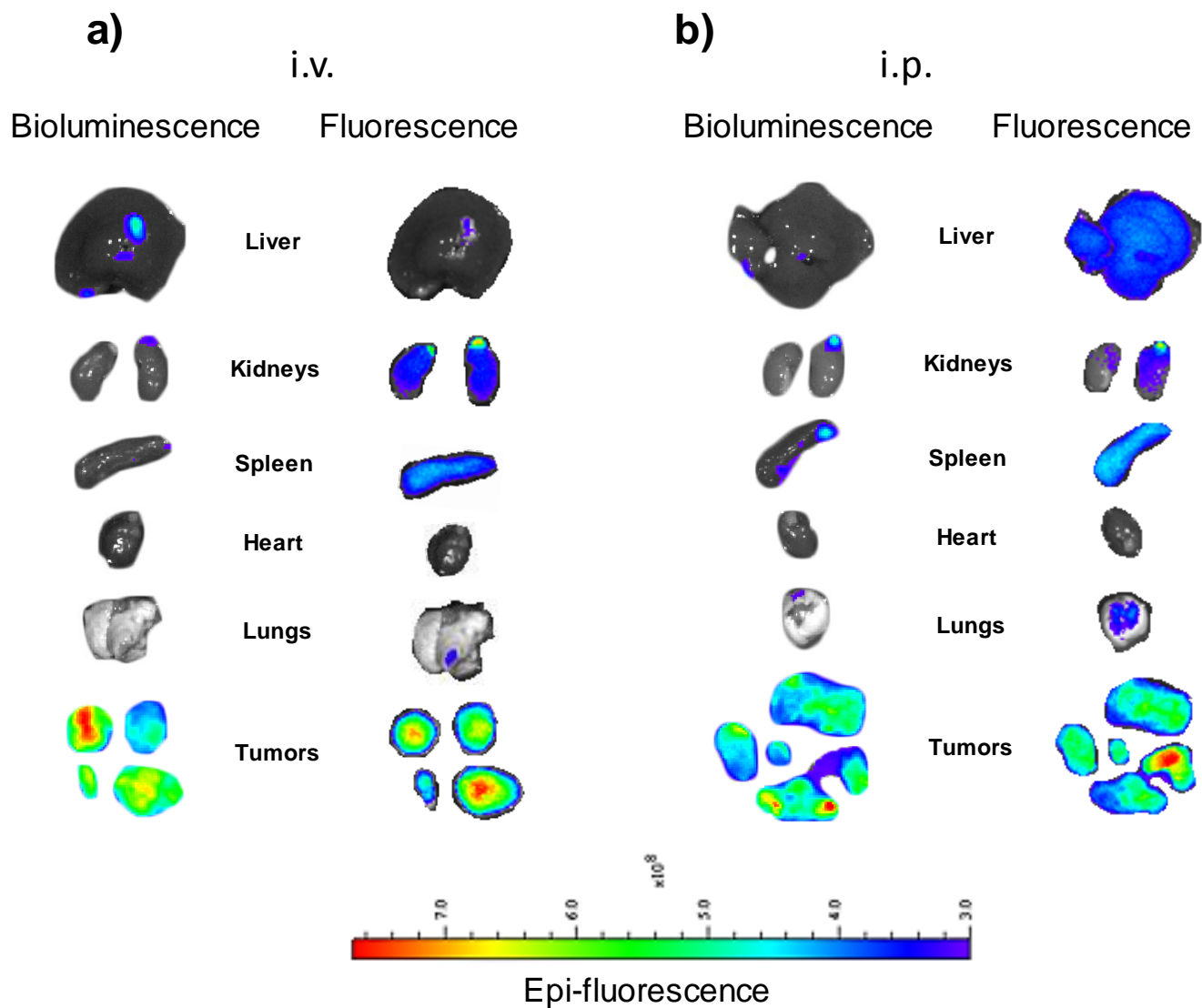


Figure S9. Distribution of NIR fluorescence generated by SiNc in organs and cancer tissues 24 hours after (a) intravenous (*i.v.*) and (b) intraperitoneal (*i.p.*) injection of the activatable SiNc-PNP into mice with intraperitoneal xenograft of luciferase-expressing ES2 ovarian cancer cells. The bioluminescence and NIR fluorescence images were recorded with the IVIS imaging system. **Note:** only cancer tissues generate bioluminescence signal in mice injected with D-luciferin.

Phototherapy on Cell Pellet. The T-25 cell culture flask with A2780/AD cells of 80% confluency was incubated for 36 h with the activatable SiNc-PNP (20 and 50 $\mu\text{g}/\text{mL}$) in RPMI 1640 (10% FBS). Next, 2×10^6 cells were

centrifuged (5 min, 1000 rpm) to give the cell pellet. Samples (in 250 μL of culture media) were photoirradiated (785 nm, 10 min, 1.3 W/cm^2). The temperature was recorded before and after 10 min of light treatment using a fiber optic temperature probe inside of the pellets. After the light experiment, the cells were seeded in a 96-well plate (1×10^4 cells/well). After 24 h, the cell viability was evaluated by Calcein AM assay along with the controls: untreated cells, cells incubated with the activatable SiNc-NP without the light treatment, and untreated cells exposed to the NIR laser only (785 nm, 1.3 W/cm^2 , 10 min).

ROS Measurements. DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) was employed to evaluate intracellular ROS levels. Cancer cells seeded in 96-well plates (1×10^4 cells/well) were incubated with the activatable SiNc-PNP (30 $\mu\text{g}/\text{mL}$) for 36 h. After incubation, cells were treated with 10 μM DCFH-DA for 30 min prior to 10 min light treatment (785 nm, 1.3 W/cm^2). Separately, the untreated cells and activatable SiNc-PNP without light treatment were used as a control and a dark control, respectively. Fluorescence was recorded on a plate reader ($\lambda_{\text{abs}} = 485$ nm and $\lambda_{\text{em}} = 528$ nm).

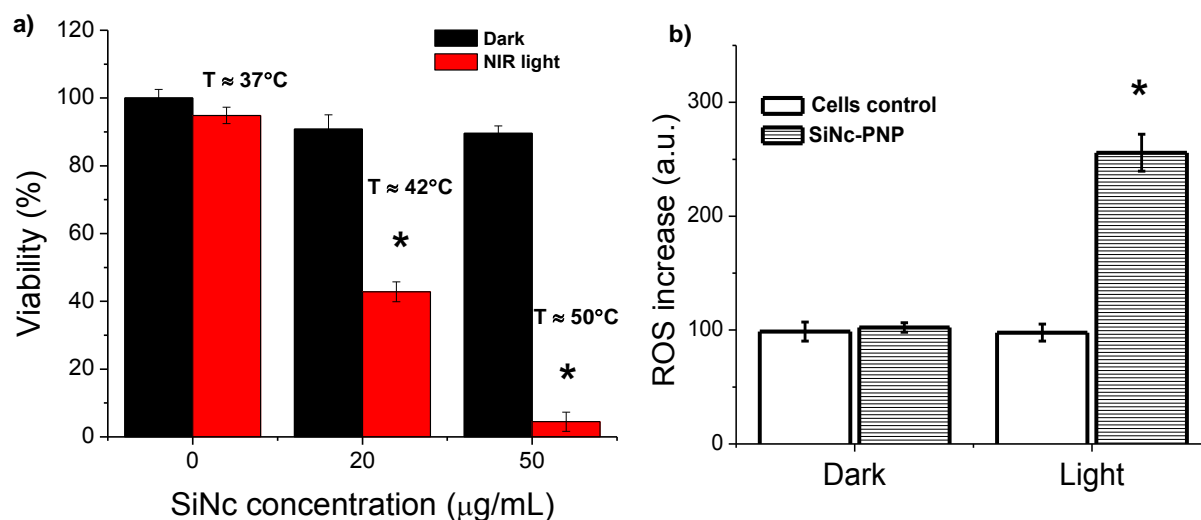


Figure S10. a) Phototherapy against A2780/CDDP ovarian carcinoma cells. The cells were treated with 50 $\mu\text{g}/\text{mL}$ of activatable SiNc-PNP and exposed to a laser light for 10 min (785 nm, 1.3 W/cm^2). Temperature recorded after 10 min of light treatment inside of the pellets for control, 20 $\mu\text{g}/\text{mL}$ and 50 $\mu\text{g}/\text{mL}$ of the activatable SiNc-PNP

was 37, 42 and 50 ° C, respectively. * $P < 0.05$ when cells treated with activatable SiNc-PNP under NIR treatment are compared to dark controls.

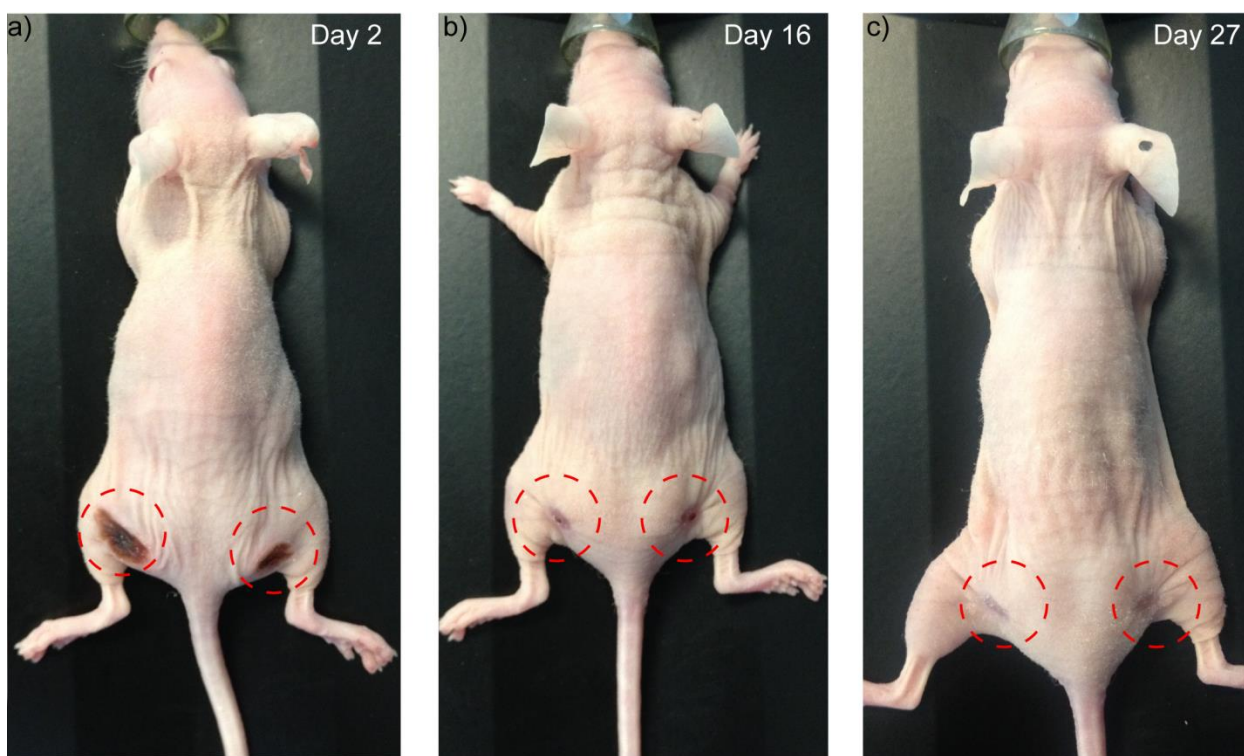


Figure S11. Photos of mice in the activatable SiNc-PNP injected and phototherapy-treated group. (a) Day 2, one-day post-phototherapy, showing the disappearance of tumors on both sides and scarring from the phototherapy burning. (b) Day 16, 15 days post-phototherapy, showing no reoccurrence of tumors on both sides as well as healing of the burn. This was the time point when tumor in control groups reaching the protocol limit. (c) Day 27, 26 days post-phototherapy, showing complete suppression and no reoccurrence of tumors on both sides as well as healing of the burn. This was 11 days after mice in control groups were euthanized due to fast tumor growth.

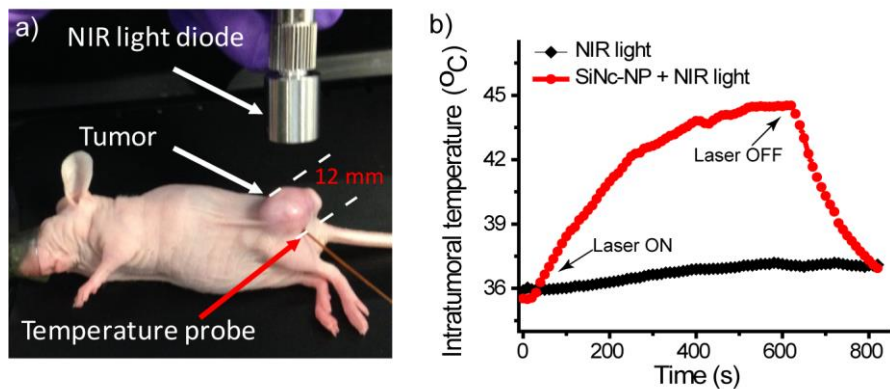


Figure S12. (a) Photo of the 12 mm activatable SiNc-PNP-treated cancer tumor exposed to NIR light. (c) Fluorescence images of tumor cross-section where the temperature was measured. (b) Temperature profile recorded with a fiber optic probe on the opposite side of the tumor where NIR light was applied (distance = 12 mm).

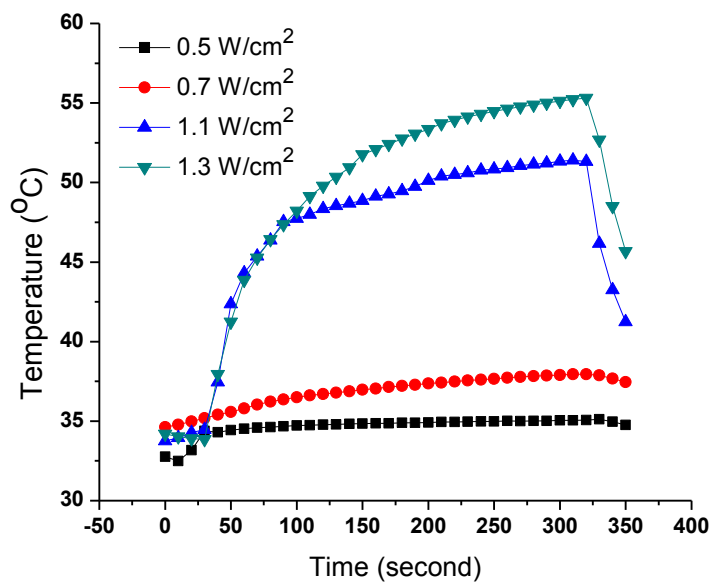


Figure S13. Intratumoral temperature profiles under NIR laser exposure with different laser powers. Experiments were performed one day after injection of the activatable SiNc-PNP (3 mg/kg of SiNc) into mice. Temperature changes are 2.4 °C for 0.5 W/cm², 3.1 °C for 0.7 W/cm², 6.4 °C for 1.1 W/cm², and 18.5 °C for 1.3 W/cm².

The intratumoral temperature during the phototherapy (1.3 W/cm²) studies reached ~47°C for “Always On” sample (1.5 mg/kg SiNc, Chem Mater 2015 27, 6155-6165) and ~ 55°C for “Activatable” sample (3 mg/kg SiNc). The higher intratumoral temperature in case of “Activatable” nanoparticles was expected as the higher dose of SiNc was used (we used the same injection volume with the same amount of polymer but with different SiNc loading: 3% and 6%).

Acute toxicity testing

The safety profile of SiNc-loaded nanoparticles has been evaluated in mice and compared to non-treated mice. None of the mice died or exhibited abnormal behavioral changes during the duration of the study. Changes in the weight, during the course of the study, for the SiNc-loaded nanoparticles as compared to untreated mice as control were monitored. Based on the data, none of the groups in either model demonstrated weight loss $\geq 15\%$ indicating that the SiNc-loaded NPs produces acute toxicity at 1.5 mg/kg dose of SiNc and 50 mg/kg of the PEG-PCL polymer. After 24 hours, 96 hours and 1 week, post *i.v.* injection (1.5 mg/kg of SiNc and 50 mg/kg of PEG-PCL polymer), mice were euthanized and blood samples were collected, centrifuged at 3000 $\times g$ for 7 min and the plasma samples were submitted for complete blood panel chemistry analysis. The analysis was performed at the Oregon State University Veterinary Diagnostic Laboratory. The concentrations of blood urea nitrogen (BUN), creatinine, and alanine transaminase (ALT) values were assessed. BUN and creatinine are surrogate markers for kidney toxicity while ALT is a surrogate marker for liver toxicity. BUN and creatinine levels are indicators of kidney function. ALT is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, and bone and elevated ALT levels are usually indicative of liver toxicity.

Phototoxicity studies

To determine if the SiNc-PNP formulation is photosafe, nude immunodeficient mice were treated by two ways simultaneously (*i.v.* injection: SiNc=1.5 mg/kg and subcutaneous injection at the back: SiNc=1.5 mg/kg). Next, 5

treated mice vs 5 control non-treated mice were exposed to simulated sunlight for 2 hours everyday for 7 days (10000 lux at 2 inches). Body weight and skin conditions in studied mice were monitored.

Cytotoxicity Studies

In vitro micronucleus assay was used to determine the genotoxicity of SiNc-loaded PNPs on CHO-K1 cells with slight modification of previously reported method.^[2] In general, 300,000 cells were seeded in each well of a 6-well plate and cultured for 24 hours prior to treatment. Cells were then treated with 88 µg/mL SiNc-loaded PNPs for experiment group, fresh media for negative control, or 50 µg/mL methyl methanesulfonate (MMS) for positive control. After 24-hour incubation, old media was removed and the cells were fixed by adding 2 mL of cold 100% methanol solution and freezing for 10 minutes. Methanol was removed, and cells were washed with phosphate buffer saline (PBS) for three times followed by addition of 600 nM of 4, 6-diamidino-2-phenylindole (DAPI) for 8 minutes to stain cell nuclei. After staining, cells were washed with PBS containing 0.05% Tween 20 and imaged by a fluorescent microscope. Genotoxicity was then analyzed by counting the number of micronuclei per 1000 cells.

References:

- [1] R. Philip, A. Penzkofer, W. Baumler, R. M. Szeimies, C. Abels, J. Photochem. Photobiol. A: Chem. 1996, 96, 137–148.
- [2] V. Shah, O. Taratula, O. B. Garbuzenko, M. L. Patil, R. Savla, M. Zhang, T. Minko, Curr Drug Discov Technol 2013, 10, 8.