

## **Supplementary Information**

**Synergistic Nanotheranostic Platform for Fluorescence/Magnetic Resonance Bimodal Imaging-Guided Near Infrared Photoimmuno-Chemodynamic Thermal Targeting Therapy**

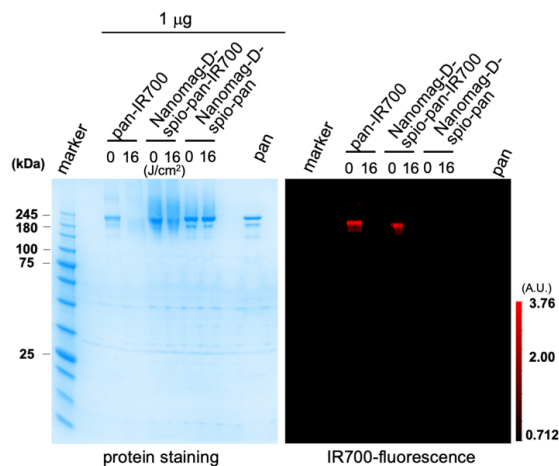
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Figure S1



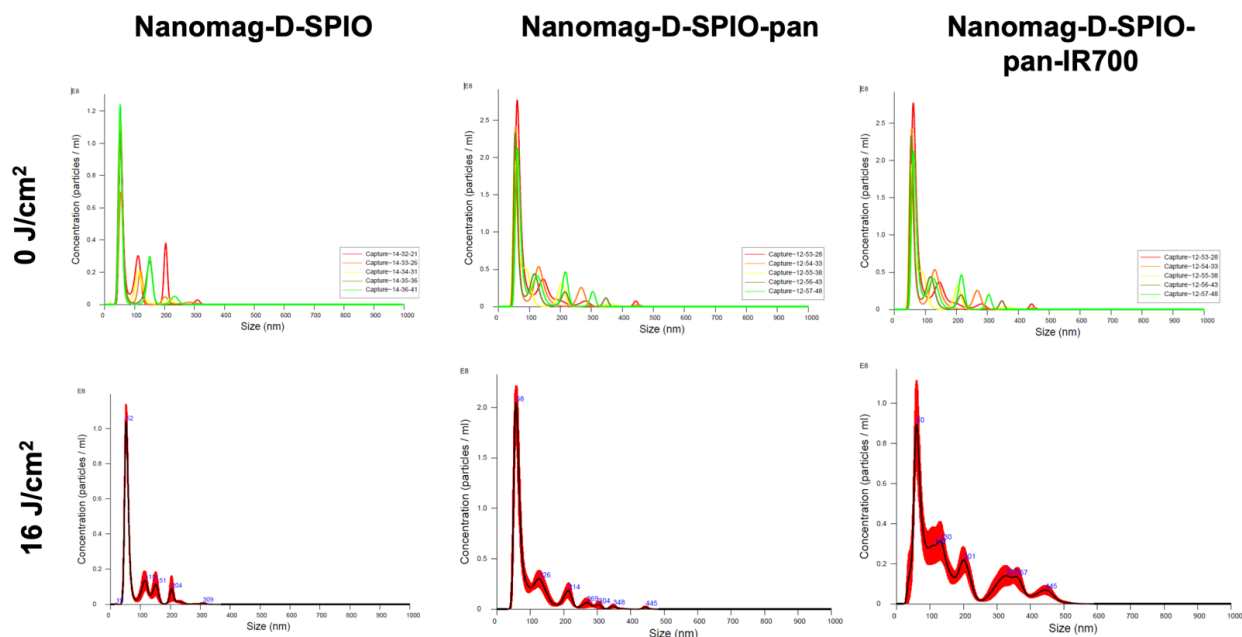
**Panitumumab Conjugation**

## Supplementary Figure. 1

### Production of pan-IR700, nanomag-D-pan-IR700, nanomag-D-pan, and detection of photochemical reaction of IR700 conjugates.

(A) SDS-PAGE analysis of the conjugates. pan-IR700 and Nanomag-D-pan-IR700 showed the IR700-fluorescence in the panitumumab band, suggested successful conjugation. The NIR-light irradiated pan-IR700 and Nanomag-D-spio-pan-IR700 (16 J/cm<sup>2</sup>) got aggregated which is detected with the smear bands and lost NIR-fluorescence. Nanomag-D-spio-pan did not change with or without NIR-light irradiation.

Figure S2

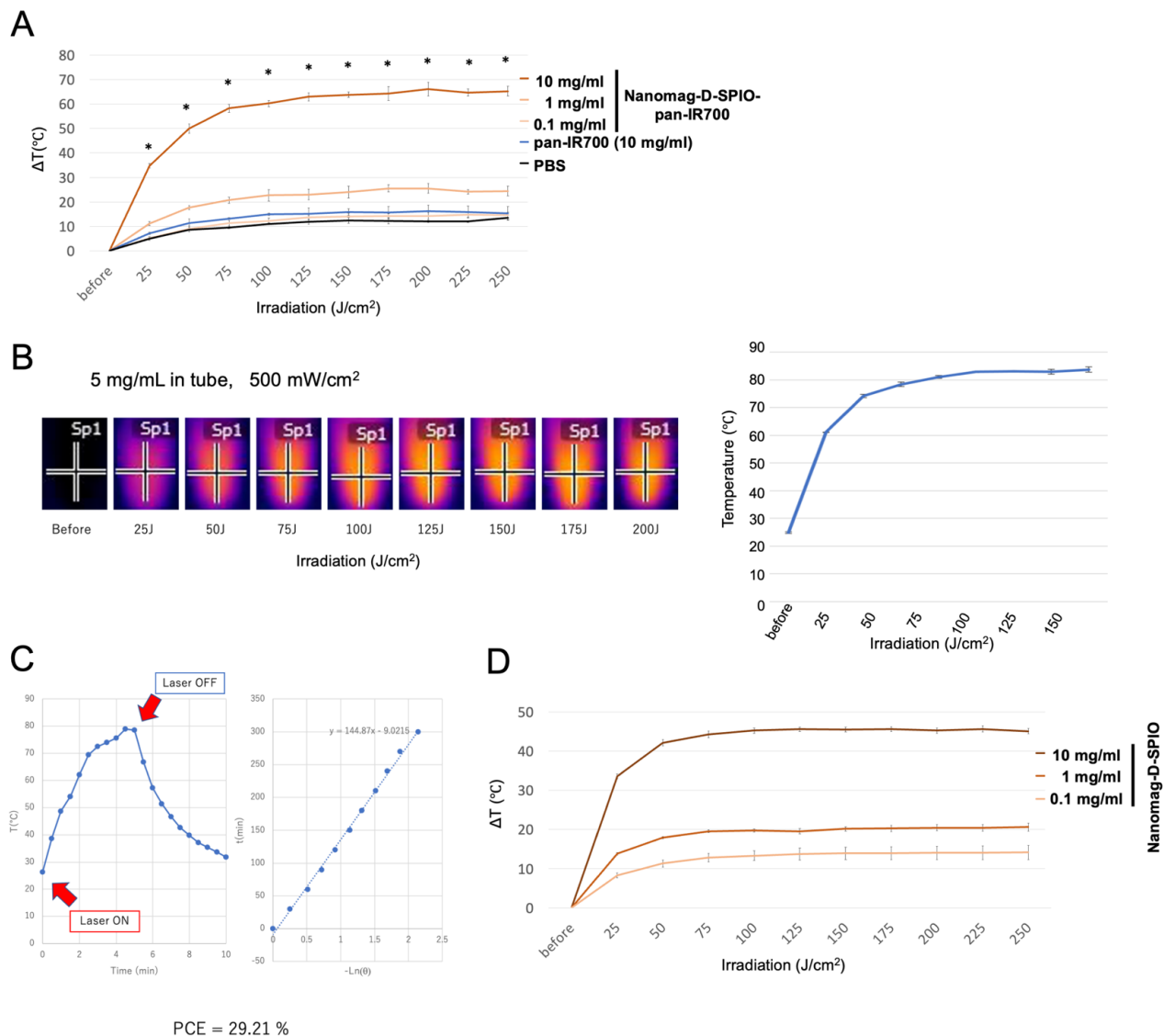


## Supplementary Figure. 2

### Aggregation Sizing of Nanomag-D-SPIO, and conjugates.

Nanomag-D-SPIO, Nanomag-D-SPIO-pan, Nanomag-D-SPIO-pan-IR700 were analyzed with an aggregation sizer, with or without NIR-light irradiation. Without NIR-light, Nanomag-D-SPIO-pan-IR700 showed similar size as Nanomag-D-SPIO-pan. With NIR-light irradiation, Nanomag-D-SPIO-pan-IR700 showed larger aggregations than Nanomag-D-SPIO-pan.

Figure S3



### Supplementary Figure. 3

#### Photothermal effect of nanomag-D-spio (20nm) with 690 nm Laser

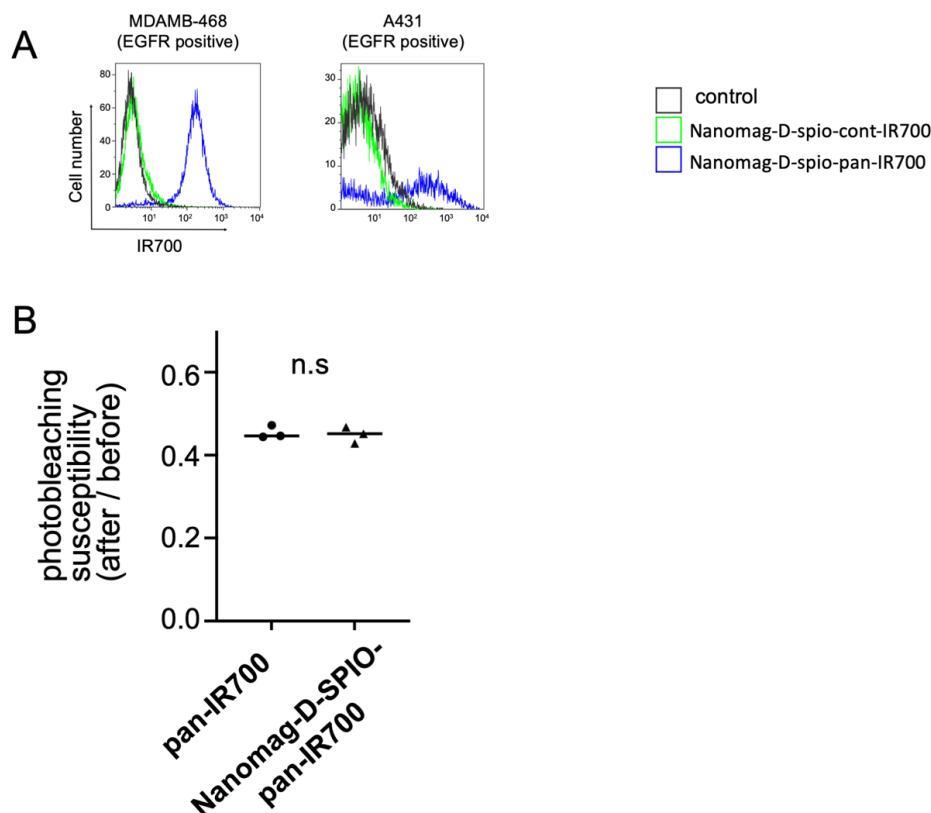
(A) Increase of the temperature with NIR-light irradiated (500 mW/cm<sup>2</sup>) samples in tube. PBS, pan-IR700, nanomag-D-pan-IR700.

(B) The temperature increase with 5 mg/ml nanomag-D-pan-IR700 in tube with NIR-light irradiation (500 mW/cm<sup>2</sup> of 690 nm).

(C) PCE evaluation of nanomag-D. Temperature variation of under 690 nm laser irradiation (0.5 W/cm<sup>2</sup>) for 5 min followed by natural cooling. Measurement of the thermal time constant ( $\tau_s$ ) by linear fit of the time point  $t$  vs  $(-\ln\theta)$  during the natural cooling period. The PCE was calculated as 29.21%.

(D) Increase of the temperature with NIR-light irradiated (500 mW/cm<sup>2</sup>) nanomag-D-spio in tube.

Figure S4

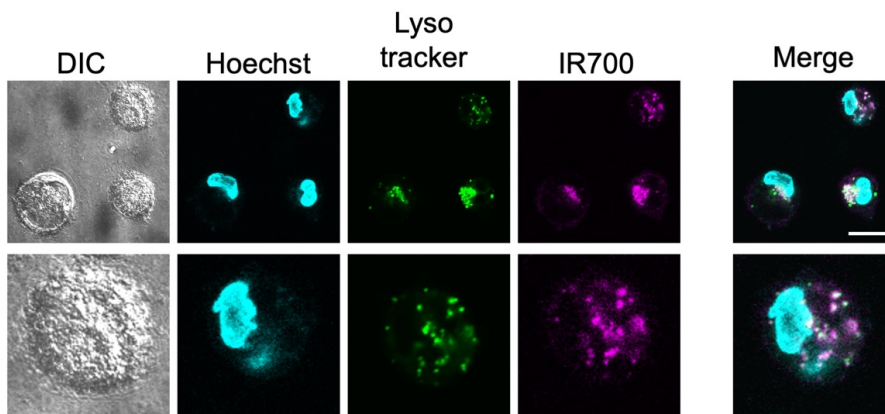


### Supplementary Figure. 4

#### Binding of nanomag-D-pan-IR700 or nanomag-D-cont-IR700 on EGFR overexpressing cancer cells and evaluation of photobleaching susceptibility of pan-IR700 and Nanomag-D-SPIO-pan-IR700

(A) Flow cytometric analysis with nanomag-D-pan-IR700 or nanomag-D-cont-IR700 on EGFR overexpressing cancer cells (A431, mdamb468).  
(B) Photobleaching susceptibility of IR700-fluorescence in pan-IR700 and Nanomag-D-SPIO-pan-IR700. The IR700-fluorescence was evaluated before and after NIR-light irradiation (50 J/ cm<sup>2</sup>) in A431 cells incubated with pan-IR700 or Nanomag-D-SPIO-pan-IR700. (n.s: not significant, Student's T test).

Figure S5

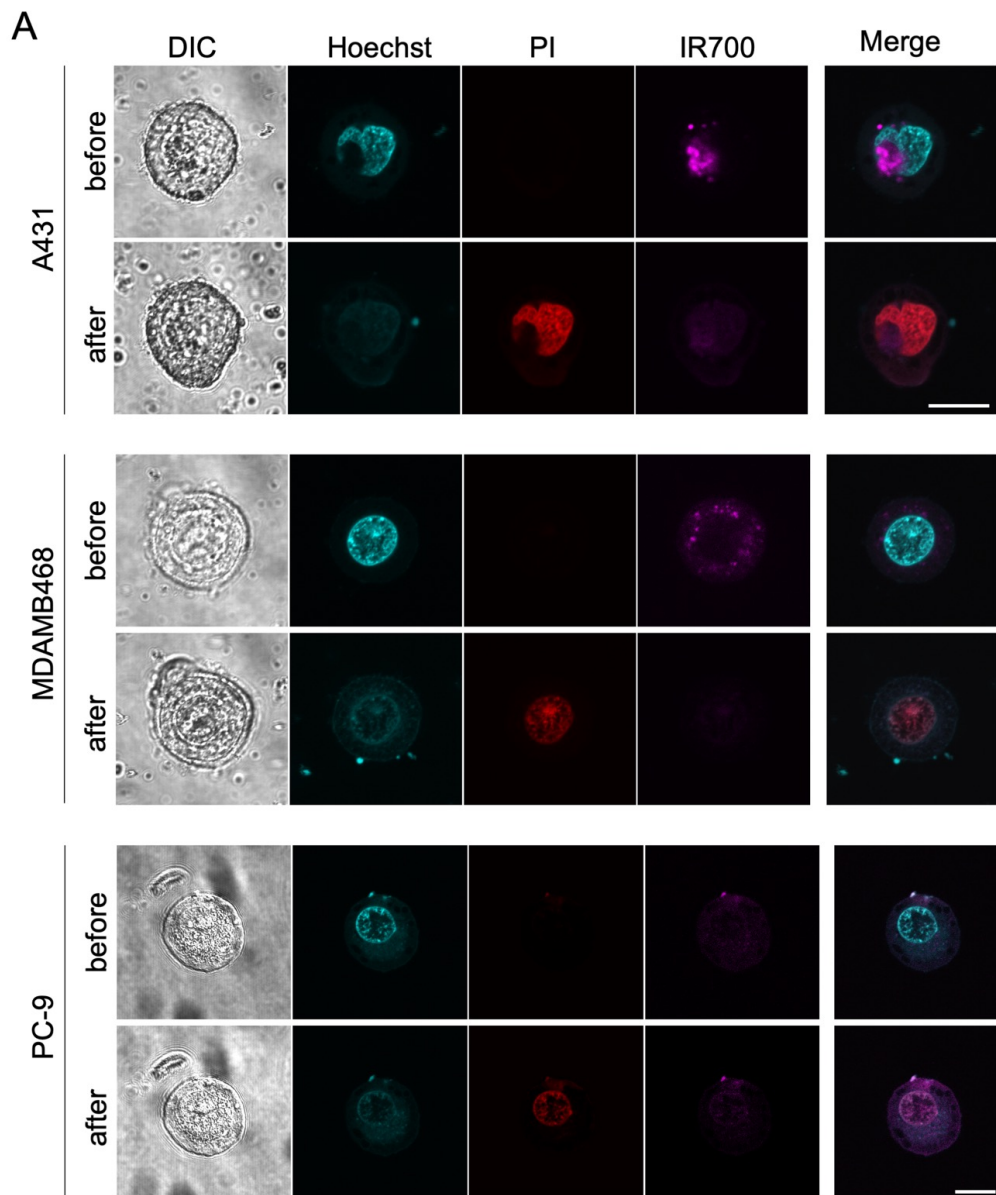


**Supplementary Figure. 5**  
**nanomag-D-pan-IR700 is endocytosed and colocalized with lysosomes.**

Microscopic observation of PC-9 with nanomag-D-pan-IR700. Lyso-tracker stained lysosome in the cell. Co-localize of IR700 and Lyso tracker was observed.

Bar = 20  $\mu\text{m}$ .

Figure S6



## Supplementary Figure. 6

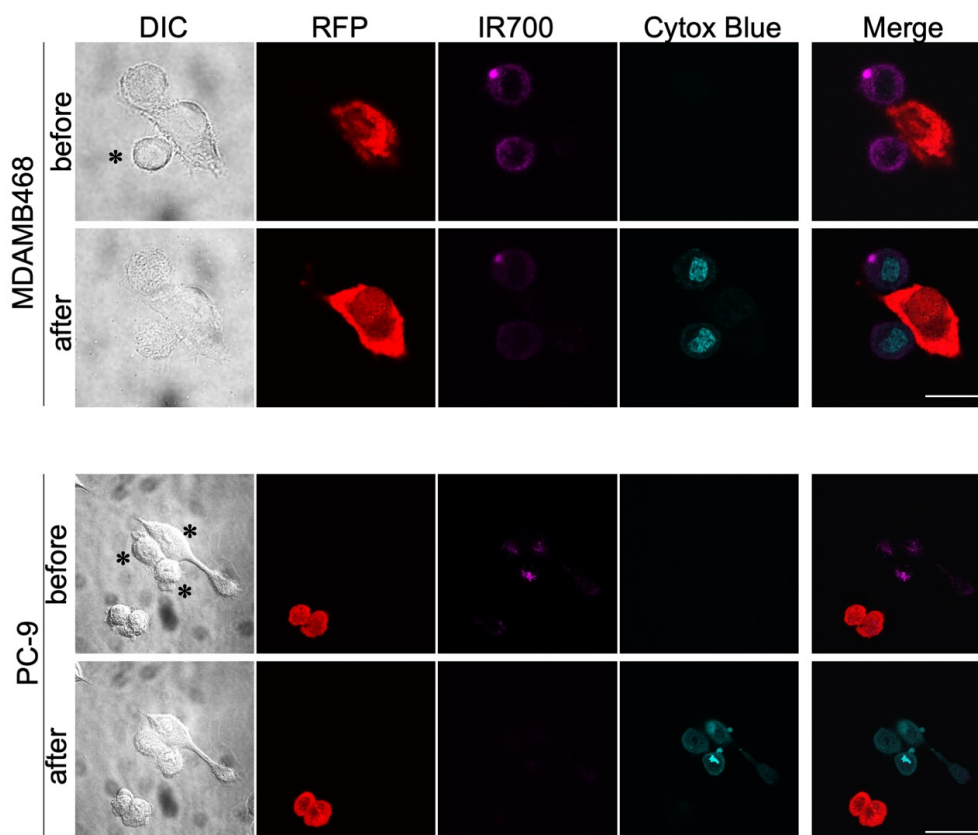
### ***In vitro* NIR-PICT<sup>3</sup> with nanomag-D-pan-IR700**

Microscopic observation of *in vitro* NIR-PICT<sup>3</sup> with nanomag-D-pan-IR700 on EGFR overexpressing cancer cells (A431, mdamb468, PC-9) at before and immediately after NIR-light irradiation. Propidium Iodide (PI) is a dead cell stainer, which showed cell membrane rupture.

Bar = 30  $\mu$ m.

Figure S7

A



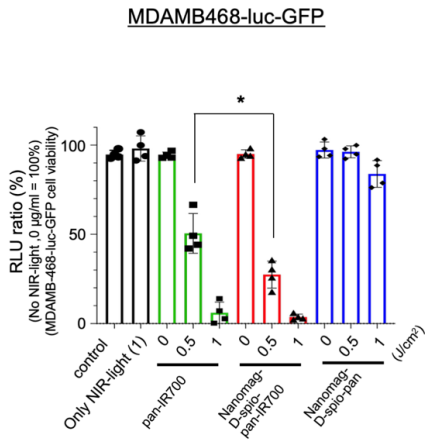
## Supplementary Figure. 7

### *In vitro* EGFR-targeted NIR-PICT<sup>3</sup>

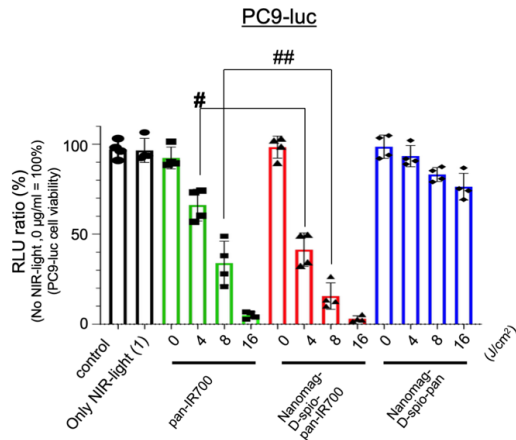
Microscopic observation of *in vitro* NIR-PICT<sup>3</sup> with nanomag-D-pan-IR700 on EGFR overexpressing cancer cells (A431, mdamb468, PC-9) and no EGFR-expressing 3T3-RFP cells at before and immediately after NIR-light irradiation. Cytox Blue is a dead cell stainer, which showed cell membrane damage. Selective cell death achieved *in vitro*. Bar = 30  $\mu$ m.

Figure S8

A



B



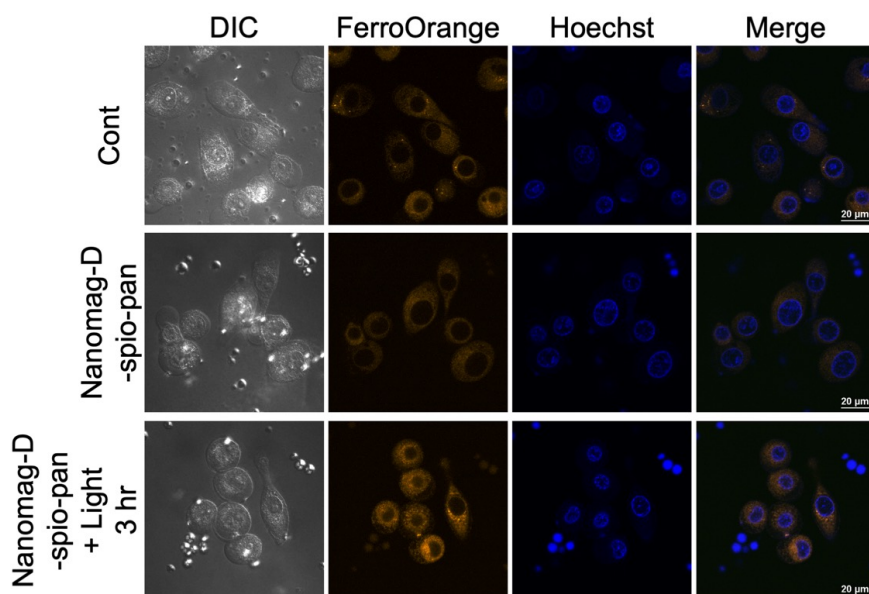
## Supplementary Figure. 8

### Cytotoxic Effect of *in vitro* targeted NIR-PICT<sup>3</sup> with nanomag-D-pan-IR700 on MDAMB468-luc-GFP, or PC9-luc.

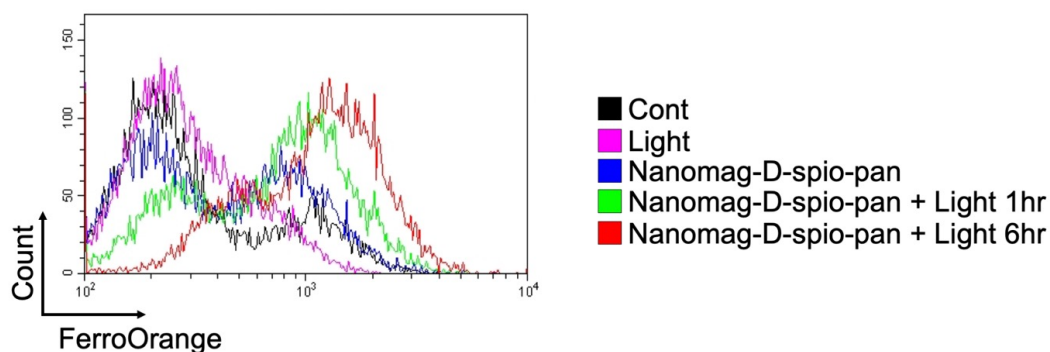
Quantitative evaluation of *in vitro* cytotoxic effect on MDAMB468-luc-GFP (A), or PC9-luc cells (B). The luciferase activity was accessed immediately after NIR-light irradiation (690 nm, 250 mW/cm<sup>2</sup>) (n = 3, \*p = 0.0138 < 0.05, #p = 0.0084 < 0.01, ##p = 0.0424 < 0.05, Student's t-test .)

Figure S9

A



B



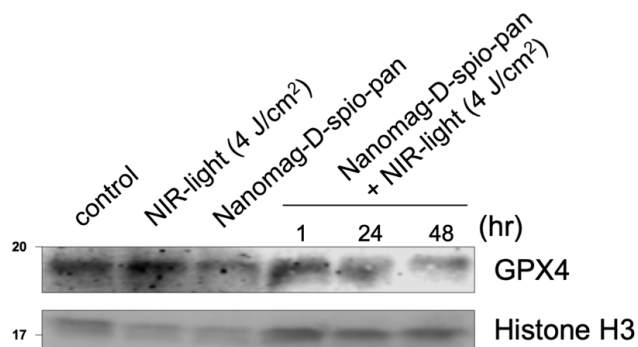
## Supplementary Figure. 9

### “NIR-PICT<sup>3</sup>–Mediated Induction of Ferroptosis”

(A) Confocal laser microscopy images showing intracellular ferrous iron accumulation detected by FerroOrange staining in PC9 cells. Cells were treated with control (Cont), Nanomag-D-spio-pan (0.3 mg/mL), or Nanomag-D-spio-pan followed by NIR-light irradiation (16 J/cm<sup>2</sup>). Images were acquired 3 hours after irradiation. Representative images include differential interference contrast (DIC), FerroOrange (Fe<sup>2+</sup> indicator), Hoechst (nuclei), and merged views. Scale bar = 20 μm

(B) Flow cytometric analysis of FerroOrange fluorescence intensity in PC9 cells. Cells were treated with Cont, Nanomag-D-SPIO-Pan (0.3 mg/mL), Light (16 J/cm<sup>2</sup>), or Nanomag-D-SPIO-Pan + Light. Histograms show the fluorescence distribution at 1 hour and 3 hours post-irradiation.

Figure S10

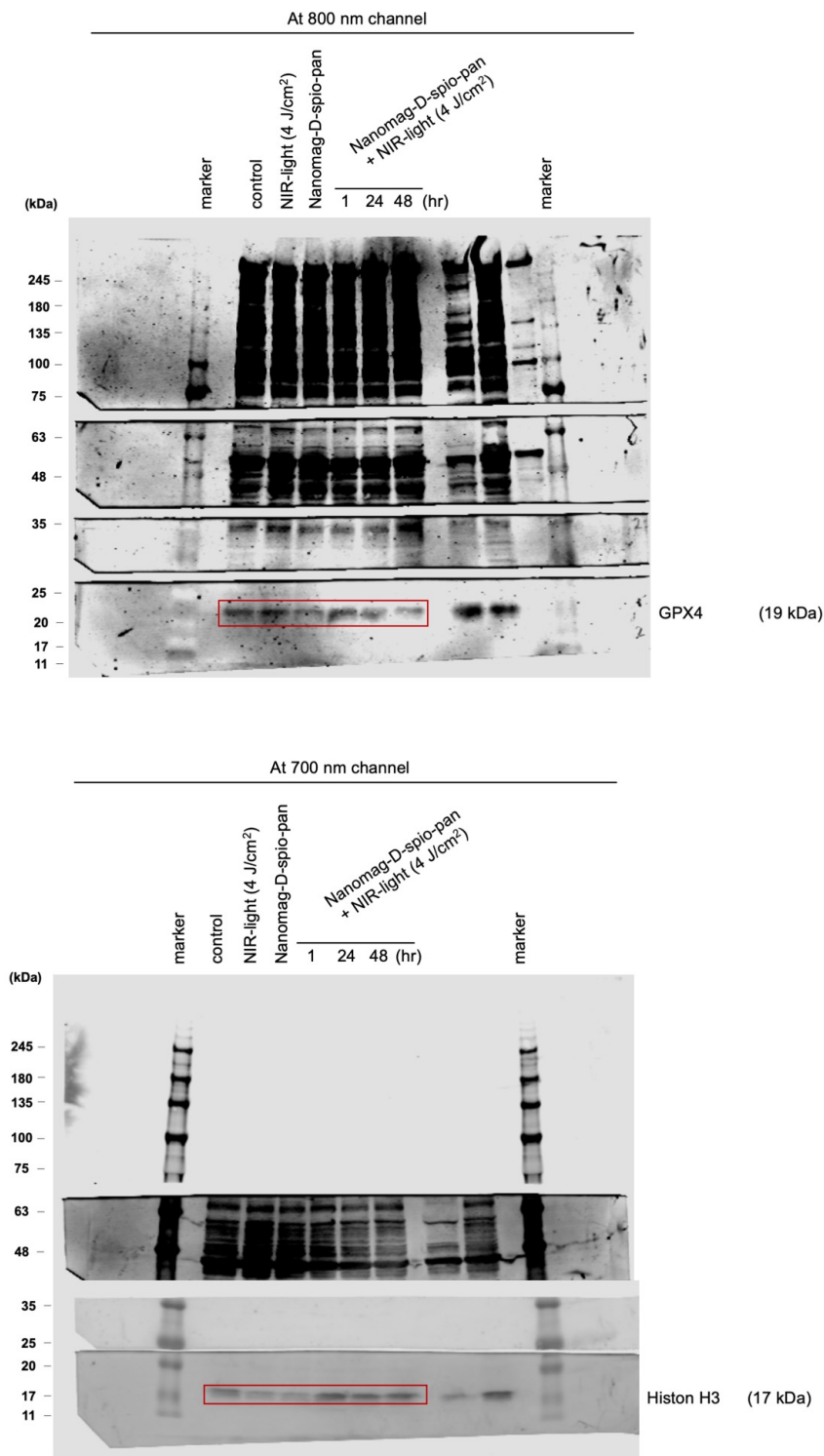


### Supplementary Figure. 10

#### Immunoblot analysis of cellular responses to Nanomag-D-sprio-pan treatment with NIR-light irradiation.

Immunoblot analysis of A431-luc-GFP cells subjected to Nanomag-D-sprio-pan treatment with NIR-light irradiation at 4 J/cm<sup>2</sup>. Cells were incubated overnight with Nanomag-D-sprio-pan before irradiation, and cell lysates were collected at 1, 24, and 48 h after irradiation. Histone H3 was used as an internal control. Full blotting membrane images were shown in Figure S11.

Figure S11

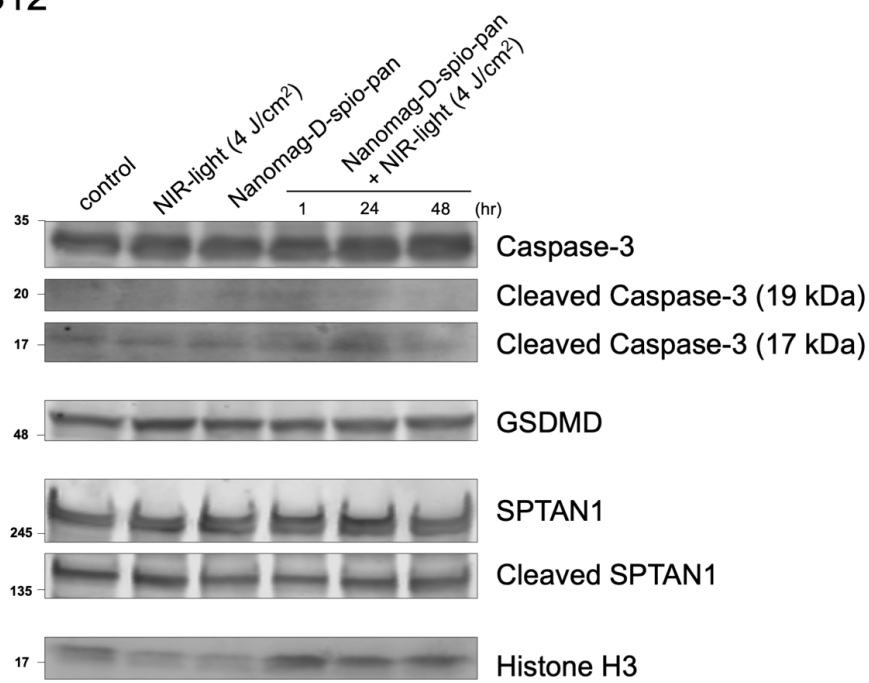


### Supplementary Figure. 11

#### Immunoblot analysis of cellular responses to Nanomag-D-sprio-pan treatment with NIR-light irradiation (Full Blotting).

The regions shown in the supplementary Figure 10 are indicated by red rectangles.

Figure S12

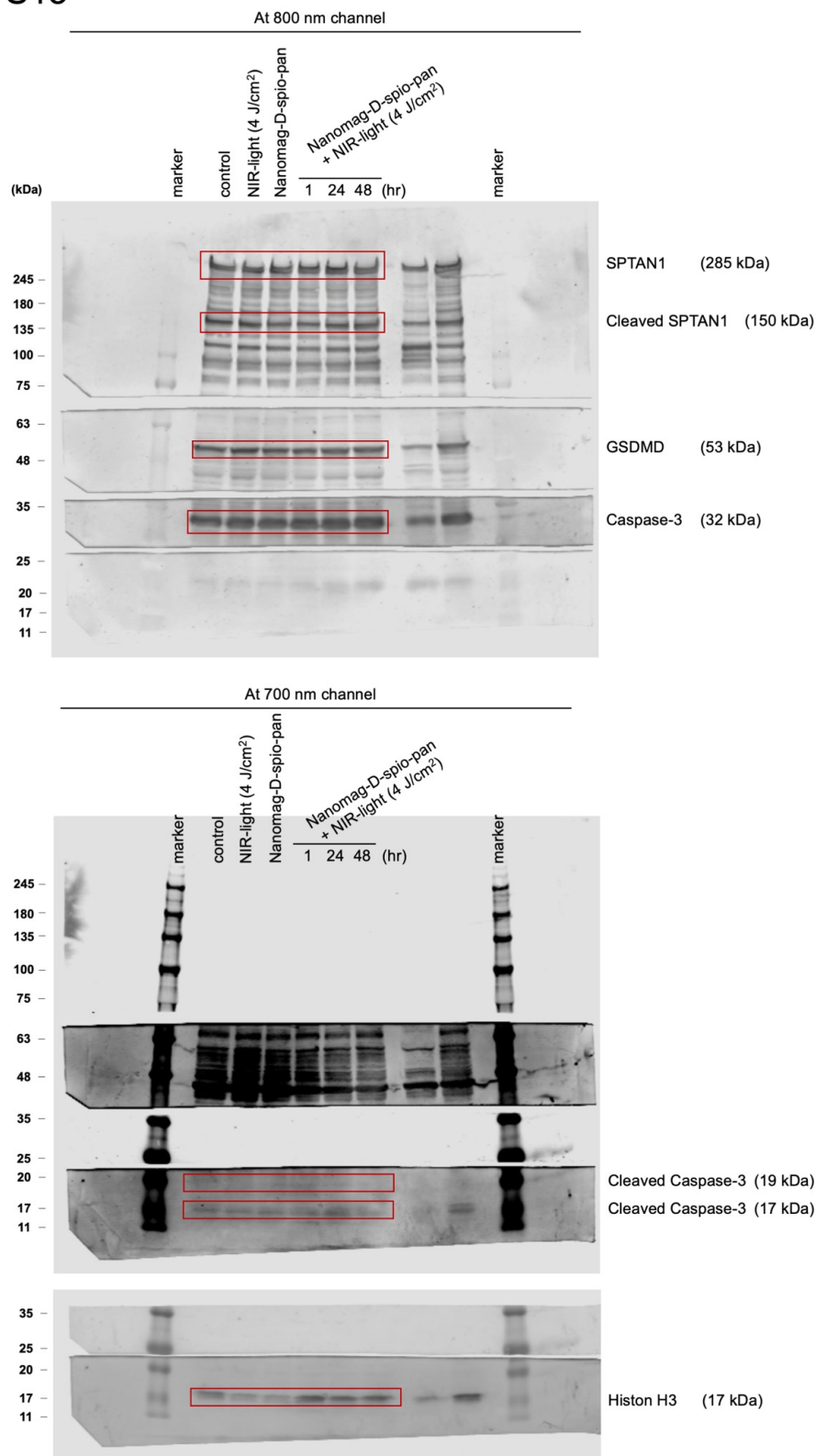


## Supplementary Figure. 12

### Immunoblot analysis of cellular responses to Nanomag-D-sprio-pan treatment with NIR-light irradiation.

(A) Immunoblot analysis of A431-luc-GFP cells subjected to Nanomag-D-sprio-pan treatment with NIR-light irradiation at 4 J/cm<sup>2</sup>. Cells were incubated overnight with Nanomag-D-sprio-pan before irradiation, and cell lysates were collected at 1, 24, and 48 h after irradiation. Histone H3 was used as an internal control. Full blotting membrane images were shown in Figure S13.

**Figure S13**

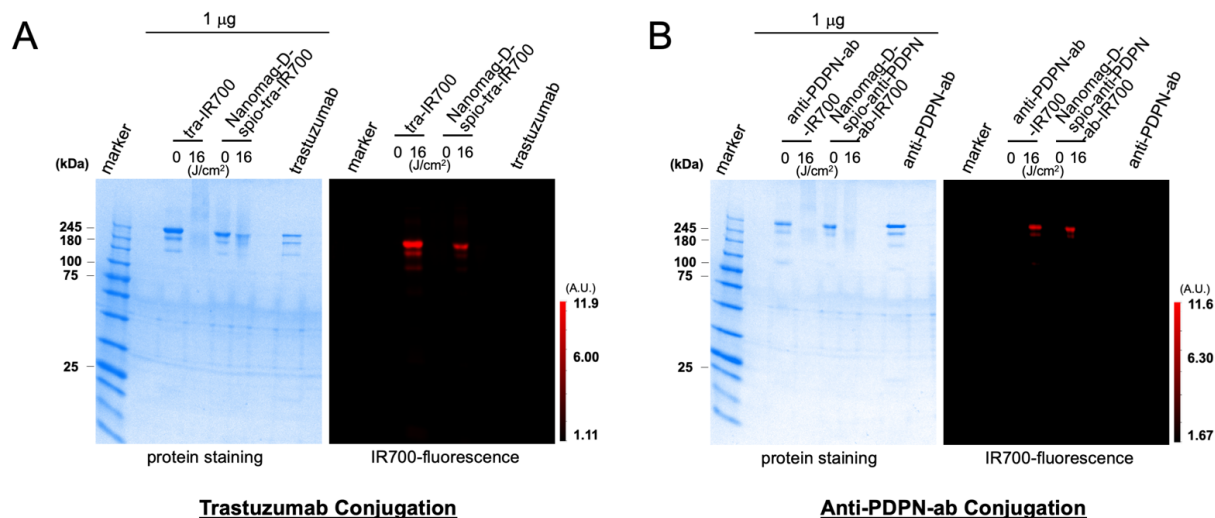


### Supplementary Figure. 13

#### Immunoblot analysis of cellular responses to Nanomag-D-sprio-pan treatment with NIR-light irradiation (Full Blotting).

The regions shown in the supplementary Figure 12 are indicated by red rectangles.

Figure S14

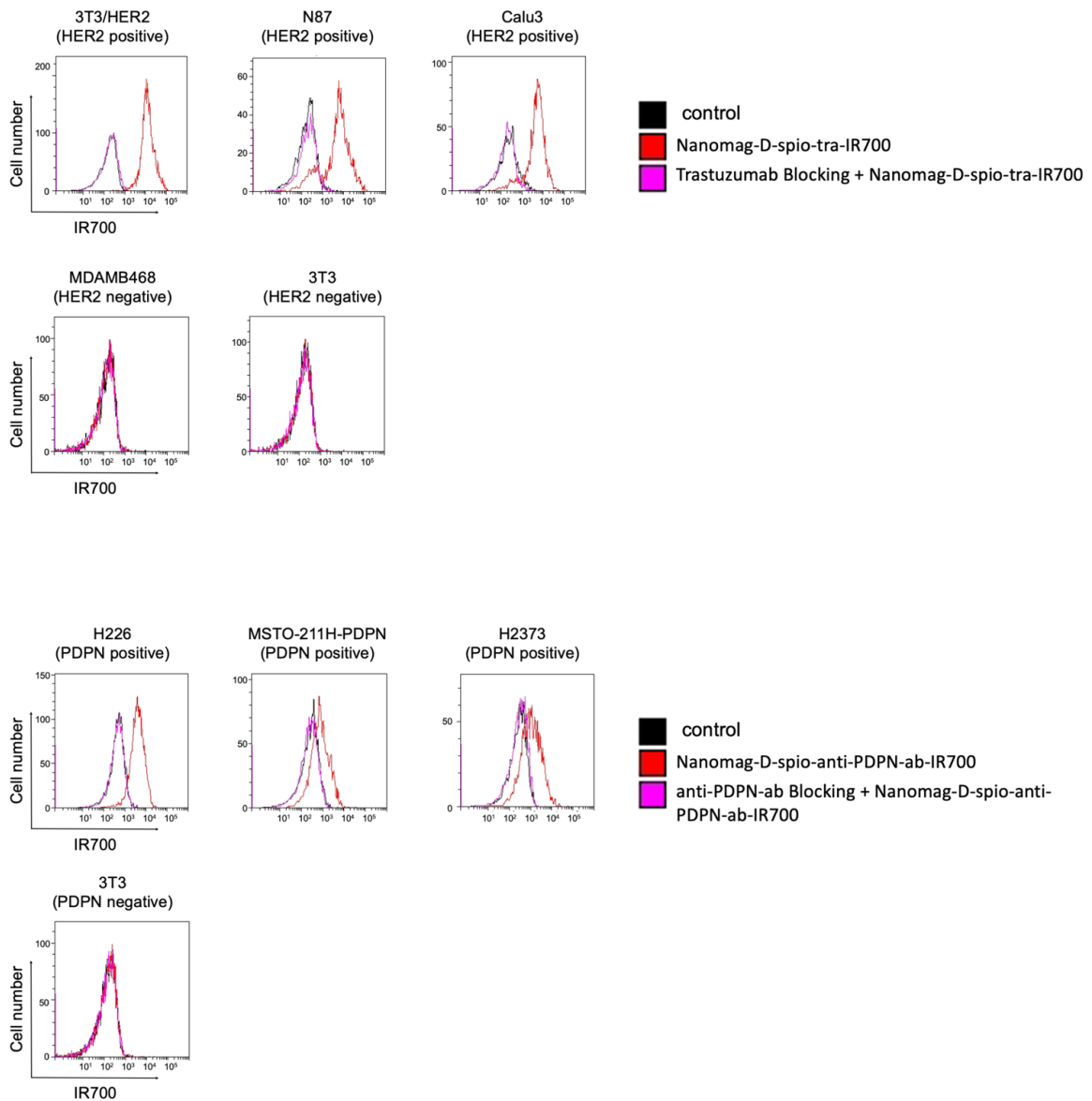


## Supplementary Figure. 14

**Production of tra-IR700, nanomag-D-tra-IR700 (A), anti-podoplanin-ab-IR700, nanomag-D-anti-PDPN(podoplanin)-ab-IR700 (B) and detection of photochemical reaction of IR700 conjugates.**

SDS-PAGE analysis of the conjugates. tra-IR700 and Nanomag-D-spio-tra-IR700 showed the IR700-fluorescence in the trastuzumab (A), anti-podoplanin-ab (B) band, suggested successful conjugation. The NIR-light irradiated tra-IR700 and Nanomag-D-spio-tra-IR700 (16 J/cm<sup>2</sup>) got aggregated which is detected with the smear bands and lost NIR-fluorescence. The NIR-light irradiated anti-podoplanin-ab-IR700 and Nanomag-D-spio-anti-podoplanin-ab-IR700 (16 J/cm<sup>2</sup>) got aggregated which is detected with the smear bands and lost NIR-fluorescence.

Figure S15

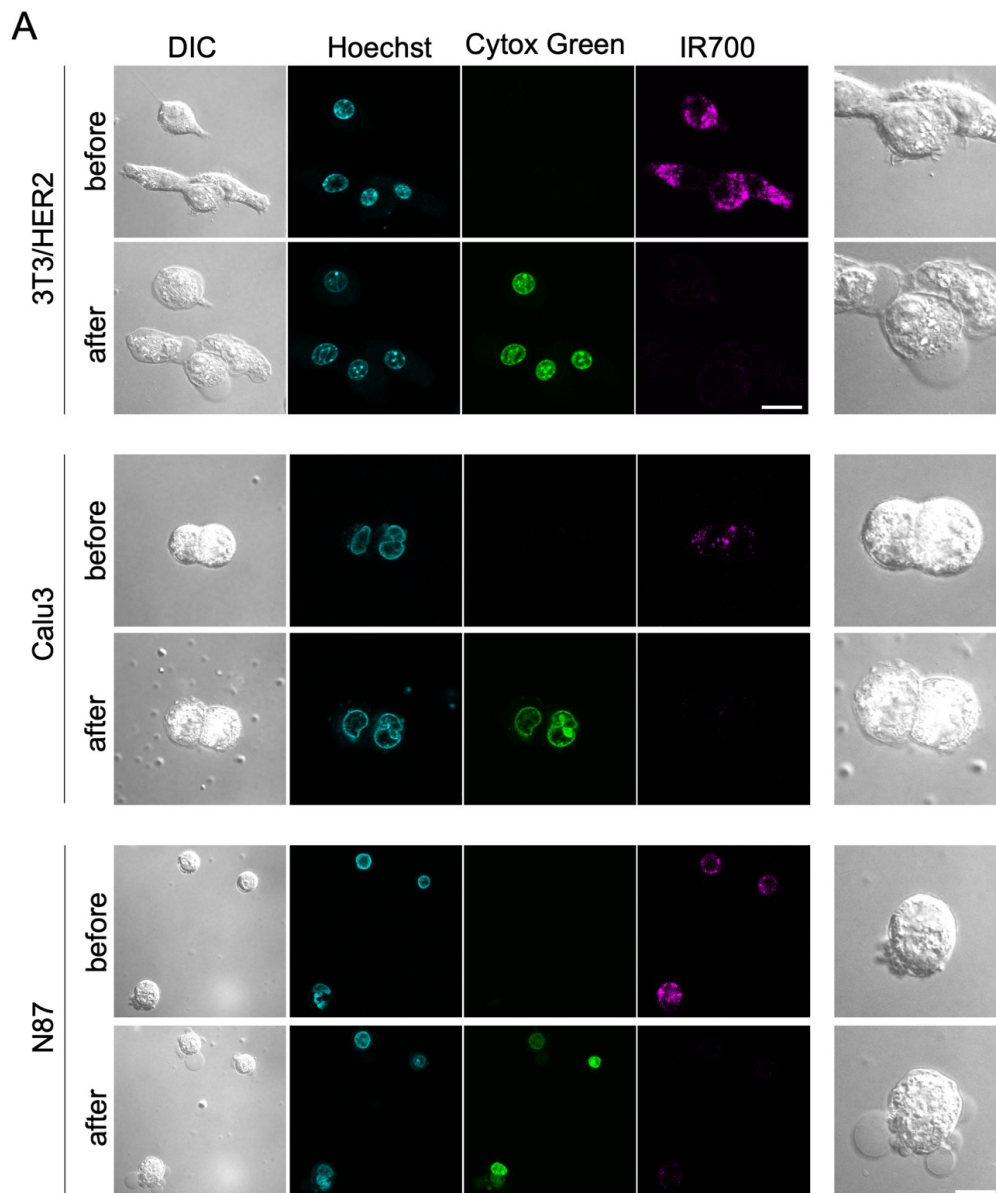


## Supplementary Figure. 15

**Binding of nanomag-D-spio-tra-IR700 on HER2 pverexpressing cancer cells and nanomag-D-spio-anti-PDPN-ab-IR700 on PDPN overexpressing cancer cells.**

Flow cytometric analysis of binding of Nanomag-D-spio-tra-IR700 on HER2 overexpressing cancer cells (3T3/HER2, N87, Calu3) and Nanomag-D-spio-anti-PDPN-ab-IR700 on PDPN overexpressing cancer cells (H226, MSTO-211H-PDPN, H2373).

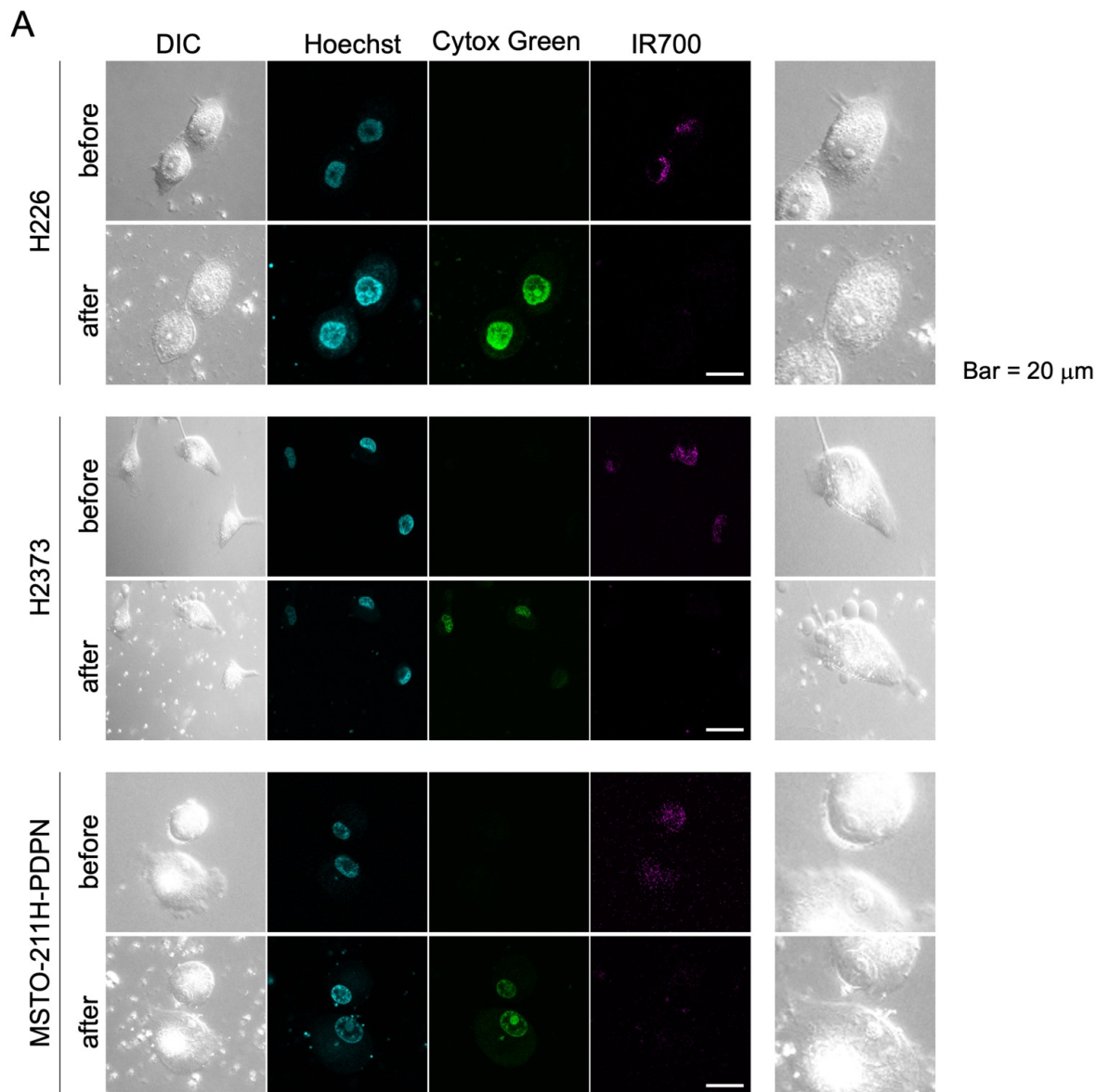
Figure S16



**Supplementary Figure. 16**  
***In vitro* HER2-targeted NIR-PICT<sup>3</sup>**

Microscopic observation of *in vitro* NIR-PICT<sup>3</sup> with nanomag-D-tra-IR700 on HER2 overexpressing cancer cells (3T3/HER2, Calu3, N87) at before and immediately after NIR-light irradiation. Cytos Green is a dead cell stainer, which showed cell membrane damage.

Bar = 30  $\mu$ m.



## Supplementary Figure. 17

### *In vitro* podoplanin targeted NIR-PICT<sup>3</sup>

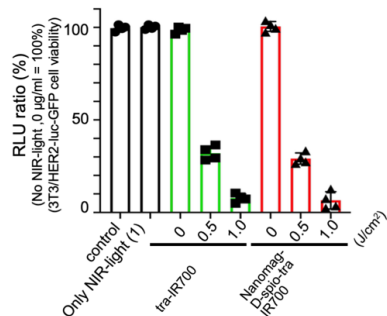
Microscopic observation of *in vitro* NIR-PICT<sup>3</sup> with nanomag-D-anti-podoplanin-IR700 on PDPN overexpressing cancer cell (H226, H2373, MSTO-211H-PDPN) at before and immediately after NIR-light irradiation. Cytox Green is a dead cell stainer, which showed cell membrane damage.

Bar = 20  $\mu$ m.

Figure S18

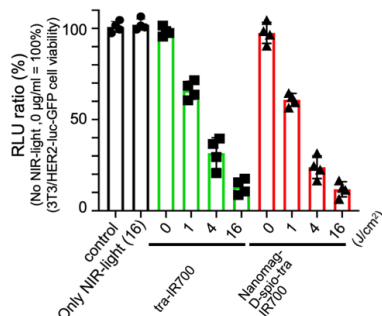
A

3T3/HER2-luc-GFP



B

Calu3-luc-GFP



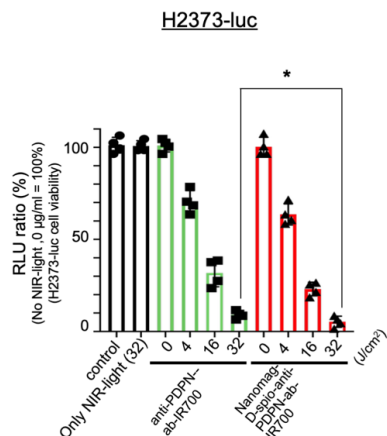
## Supplementary Figure. 18

### Cytotoxic Effect of *in vitro* targeted NIR-PIC<sup>3</sup> with nanomag-D-tra-IR700 on 3T3/HER2-luc-GFP, or Calu3-luc-GFP.

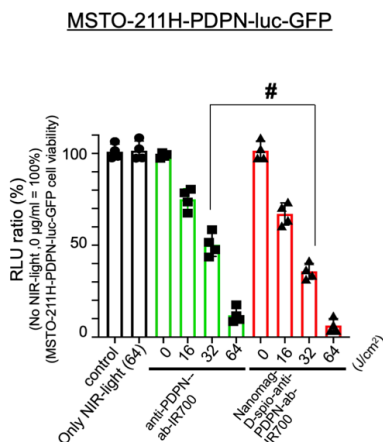
Quantitative evaluation of *in vitro* cytotoxic effect on 3T3/HER2-luc-GFP (A), or Calu3-luc-GFP cells (B). The luciferase activity was accessed immediately after NIR-light irradiation (690 nm, 250 mW/cm<sup>2</sup>) (n = 3).

Figure S19

A



B

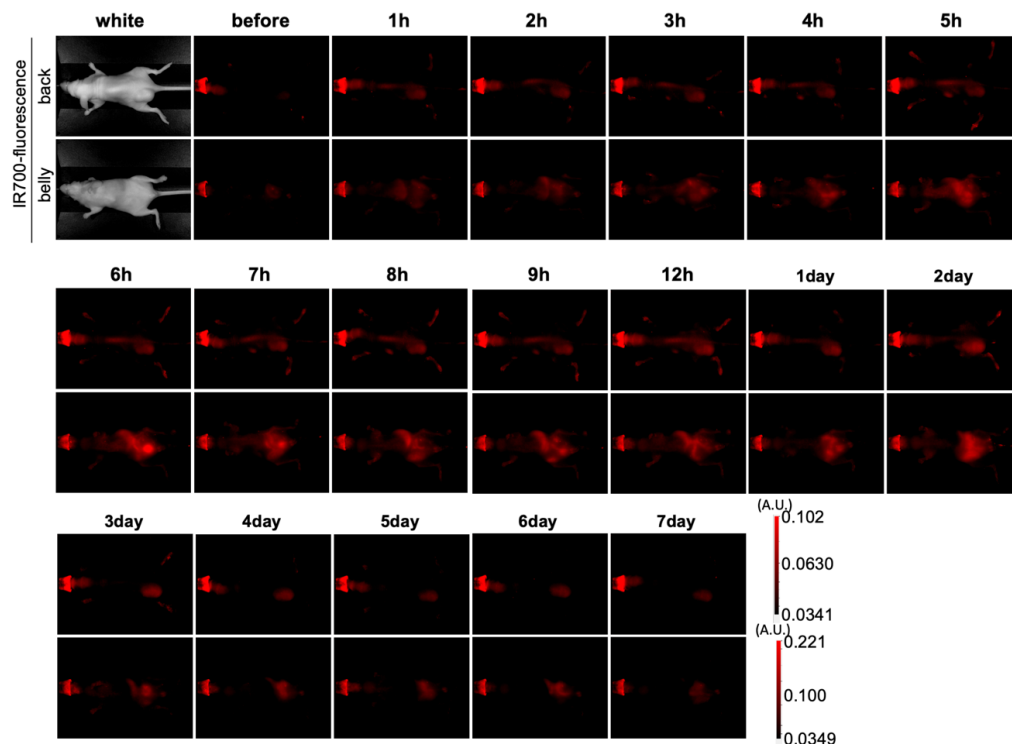


## Supplementary Figure. 19

### Cytotoxic Effect of *in vitro* targeted NIR-PICT<sup>3</sup> with nanomag-D-anti-PDPN-ab-IR700 on H2373-luc, or MSTO-211H-PDPN-luc-GFP.

Quantitative evaluation of *in vitro* cytotoxic effect on H2373-luc (A), or MSTO-211H-PDPN-luc-GFP cells (B). The luciferase activity was accessed immediately after NIR-light irradiation (690 nm, 250 mW/cm<sup>2</sup>) (n = 3, \*p = 0. < 0.042 < 0.05, #p = 0.0094 < 0.01, Student's t-test .)

Figure S20



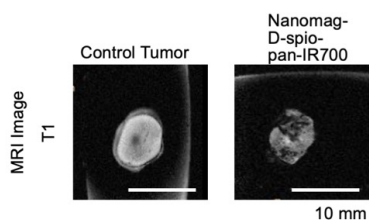
## Supplementary Figure. 20

### Biodistribution of nanomag-D-pan-IR700 in tumor xenografted mice via NIR-fluorescence (more time points showed in Figure 3).

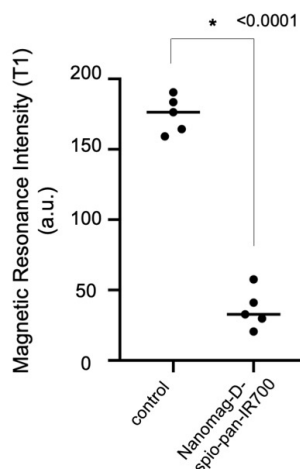
NIR-fluorescence detection of the biodistribution of nanomag-D-pan-IR700 in tumor xenografted mice. 700 nm NIR-fluorescence was gradually accumulated on the xenografted tumor over time.

Figure S21

A



B



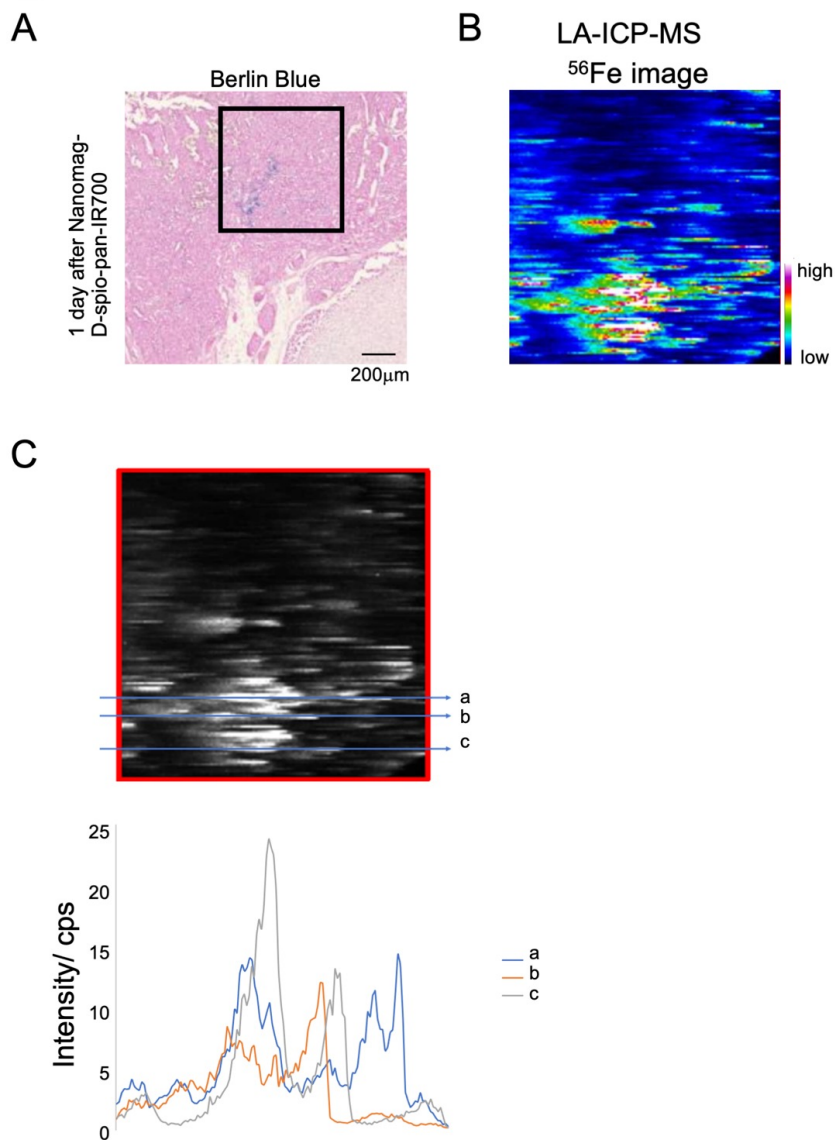
## Supplementary Figure. 21

### Magnetic resonance imaging (MRI) with *ex vivo* tumor.

(A) MRI imaging of *ex vivo* tumors, control and nanomag-D-spio-pan-IR700. At 1 day after intravenous injection of nanomag-D-pan-IR700 or PBS, *ex vivo* tumors were imaged.

(B) Quantitative intensity of *ex vivo* tumors ( $n = 5$ ,  $p < 0.0001$ , Student's t-test).

Figure S22



## Supplementary Figure. 22

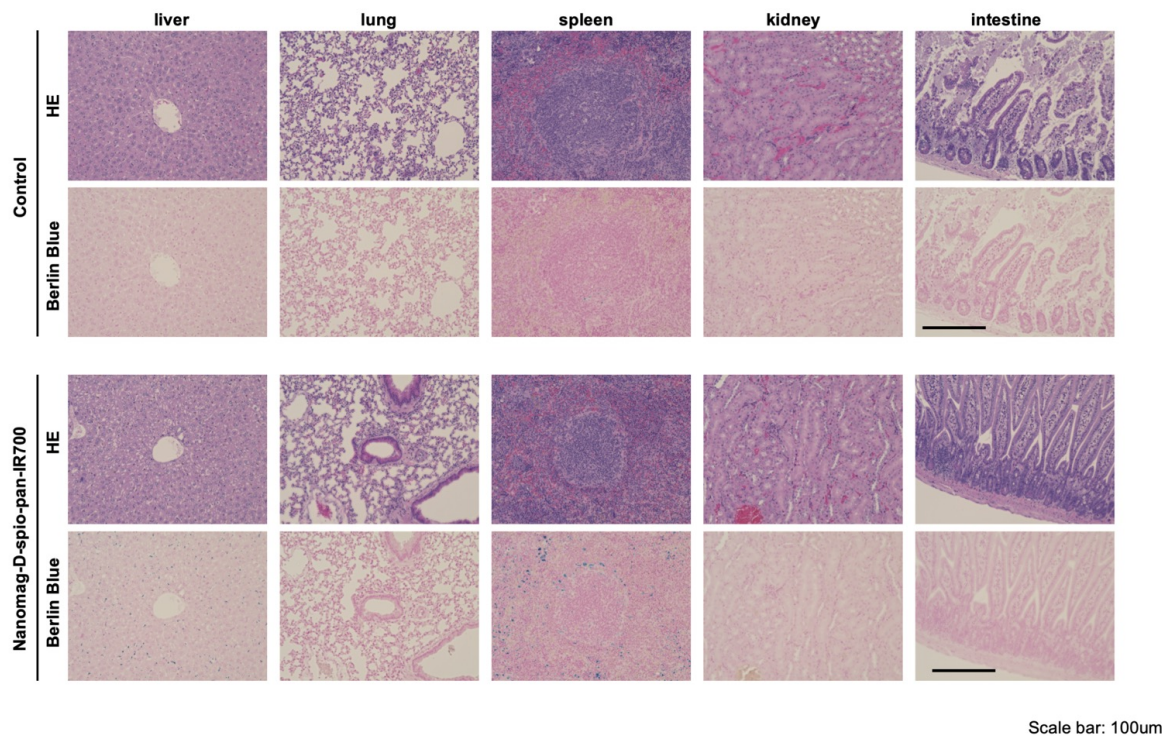
### LA-ICP-MS imaging of tumor specimen for detection Fe.

(A) Berlin Blue (BB) staining of the tumor specimen at 1 day after nanomag-D-sprio-pan-IR700 injection. Bar, 200  $\mu$ m.

(B) LA-ICP-MS within the region of the box in (A).

(C) The Fe intensity of a, b, c, in (B).

Figure S23

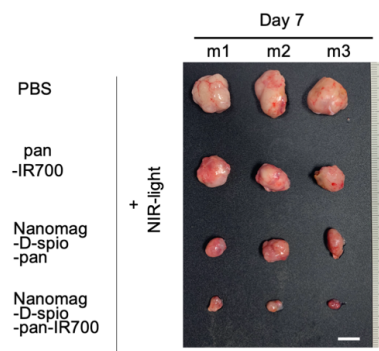


## Supplementary Figure. 23

### HE and BB staining of organs.

(A) HE and Berlin Blue (BB) staining of various organs (liver, lung, spleen, kidney, intestine) of control (PBS injection) mouse and mouse of 1 day after nanomag-D-spio-pan-IR700 injection. Bar, 100  $\mu$ m.

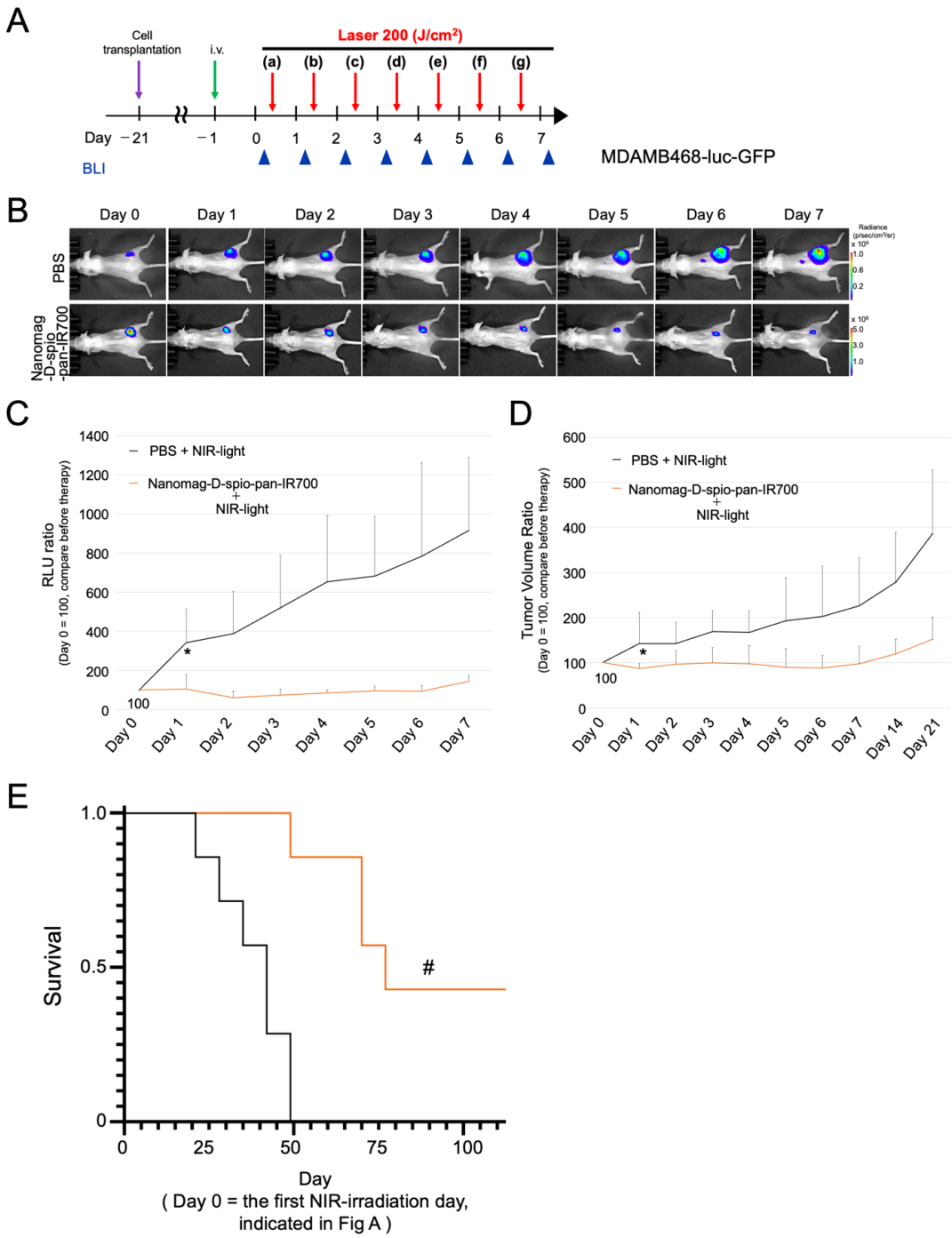
Figure S24



**Supplementary Figure. 24**  
**Appearance of *ex vivo* treated xenografted tumors.**

Appearance of *ex vivo* treated tumors at day7 in Figure4 (n = 3 in each group).

Figure S25



## Supplementary Figure. 25

### ***In vivo effect of Nanomag-D-spio-pan-IR700 on mice xenograft model.***

(A) Therapeutic regimen of *in vivo* targeted phototherapy with pan-IR700, nanomag-D-spio-pan, or nanomag-D-spio-pan-IR700 on mdamb468-luc-GFP xenografted tumors.

(B) Representative images of BLI were demonstrated.

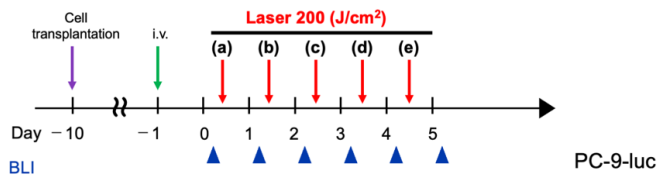
(C) Quantitative RLU on xenografted tumors ( $n = 7$  in each group). RLU ratio was calculated as 100 at before NIR-light irradiation (day0) with same sized ROI on tumors. (the mean  $\pm$  SEM,  $n = 7$ ,  $p < 0.005$ , Student's t-test).

(D) Quantitative tumor volume ratio on xenografted tumors (as 100 at before NIR-light irradiation (day0))( $n = 7$  in each group )(the mean  $\pm$  SEM,  $*p < 0.05$  ; Student's t-test).

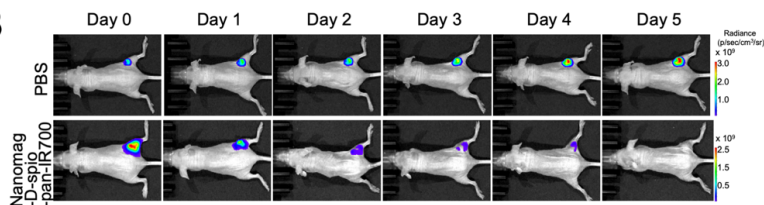
(E) Survival of EGFR-targeted NIR-PIC<sup>3</sup> on mdamb468-luc-GFP tumors ( $n = 7$  mice/group, #  $p < 0.0001$ , log-rank test). 3 mice out of 7 got Complete remission (CR) in nanomag-D-spio-pan-IR700 with NIR-light irradiation group.

Figure S26

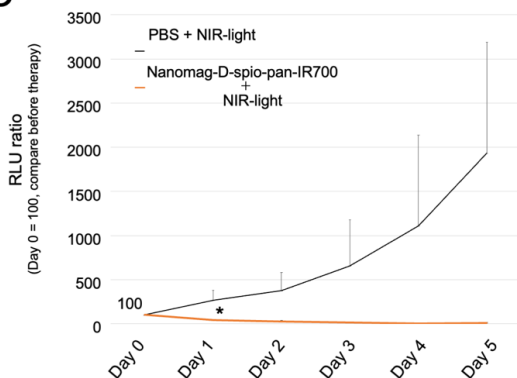
A



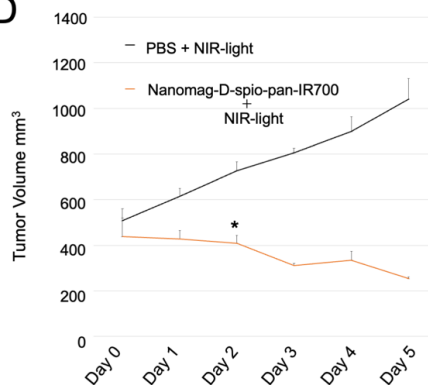
B



C



D



## Supplementary Figure. 26

### *In vivo* effect of Nanomag-D-sprio-pan-IR700 on mice xenograft model.

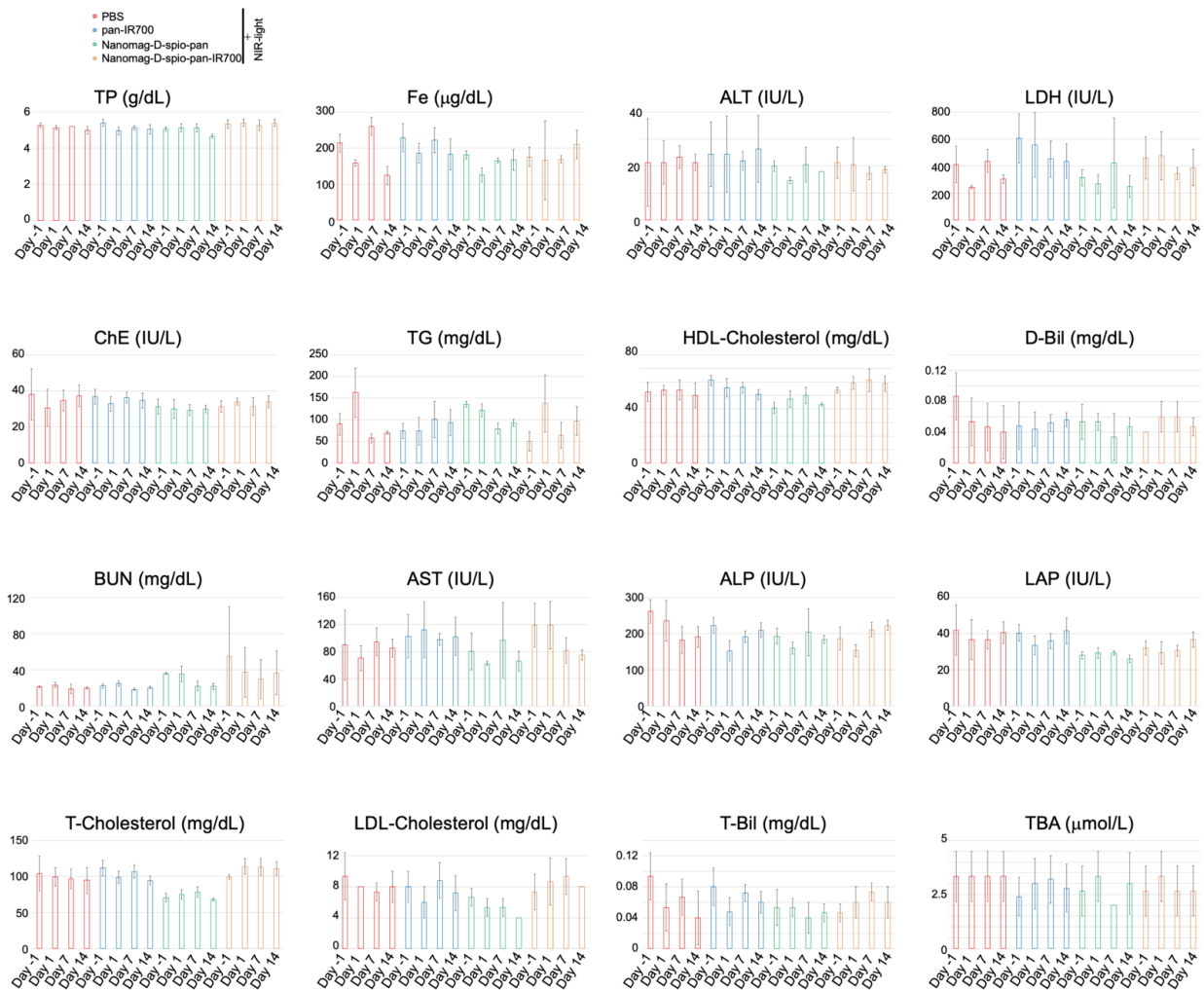
(A) Therapeutic regimen of *in vivo* targeted phototherapy with nanomag-D-sprio-pan-IR700 on PC9-luc xenografted tumors.

(B) Representative images of BLI were demonstrated.

(C) Quantitative RLU on xenografted tumors (n = 3 in each group). RLU ratio was calculated as 100 at before NIR-light irradiation (day0) with same sized ROI on tumors. (the mean ± SEM, n = 3, p < 0.005, Student's t-test).

(D) Quantitative tumor volume on xenografted tumors (n = 3 in each group) (the mean ± SEM, \*p < 0.05 ; Student's t-test).

Figure S27

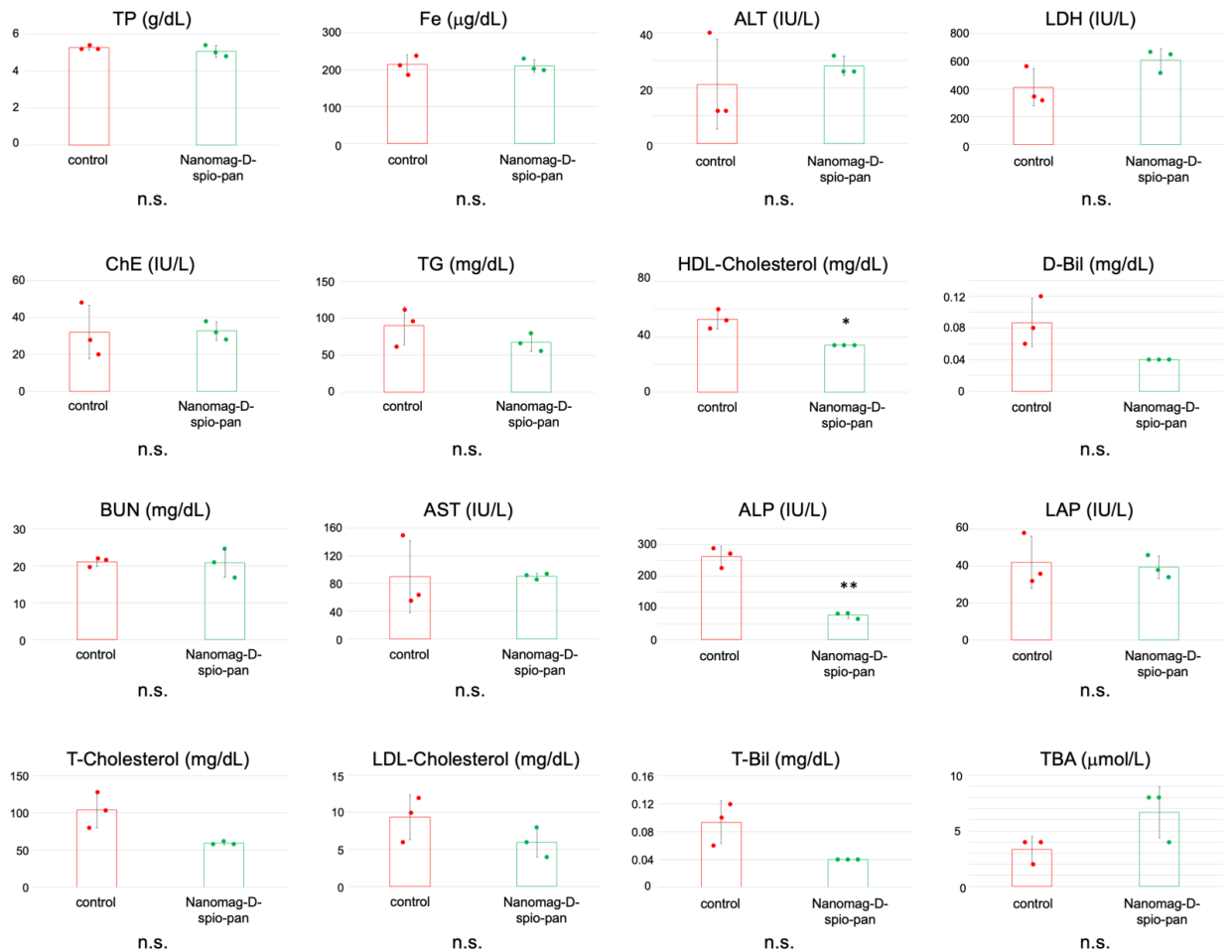


## Supplementary Figure. 27

### Analysis of Serum of treated mice.

(A) Serum analysis of TP, Fe, ALT, LDH, ChE, TG, HDL, D-Bil, BUN, AST, ALP, LAP, T-chol, LDL, T-Bil and TBA before intravenous injection (day-1), and day1 (after once NIR-light irradiation), day7, day14 (n = 3 in each group).

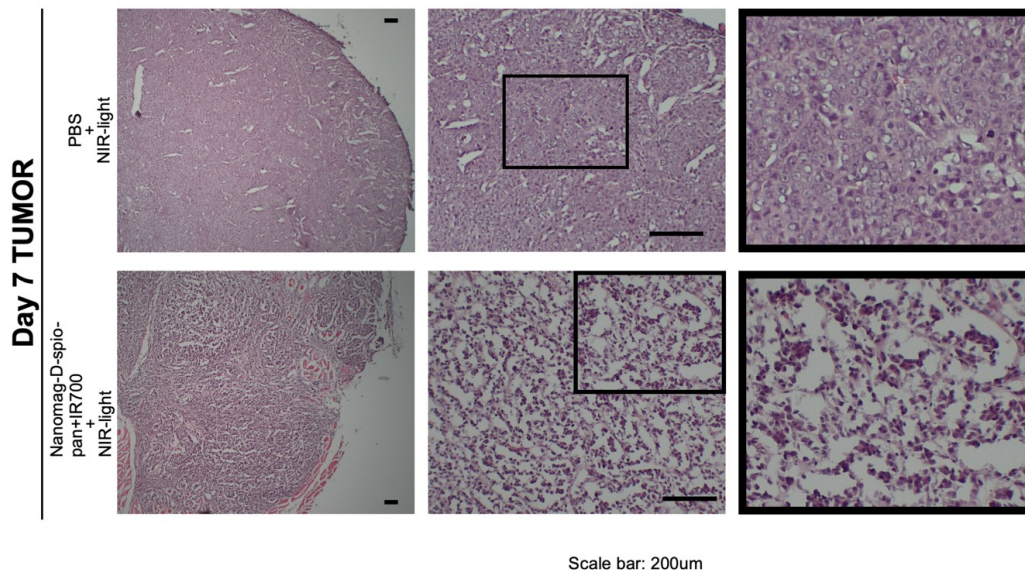
Figure S28



## Supplementary Figure. 28

### Analysis of Serum of mice for Long-Term Toxicity Assessment

(A) Serum analysis of TP, Fe, ALT, LDH, ChE, TG, HDL-chol, D-Bil, BUN, AST, ALP, LAP, T-chol, LDL-chol, T-Bil, and TBA in untreated control mice and mice administered Nanomag-D-spio-pan via intravenous injection ( $n = 3$ , \* $p < 0.05$ , \*\* $p < 0.01$ , Welch's t-test). Serum samples from the Nanomag-D-spio-pan group were collected more than 1 month after intravenous injection.



## Supplementary Figure. 29

### HE staining of Control tumor and EGFR-targeted NIR-PIC T<sup>3</sup> tumor specimen at day7

Histological confirmation (HE stainings) of the EGFR-targeted NIR-PIC T<sup>3</sup> treated tumor specimens at day 7.

The tumor cells were totally destroyed and only the nucleus remained.

Bar = 200 um

