

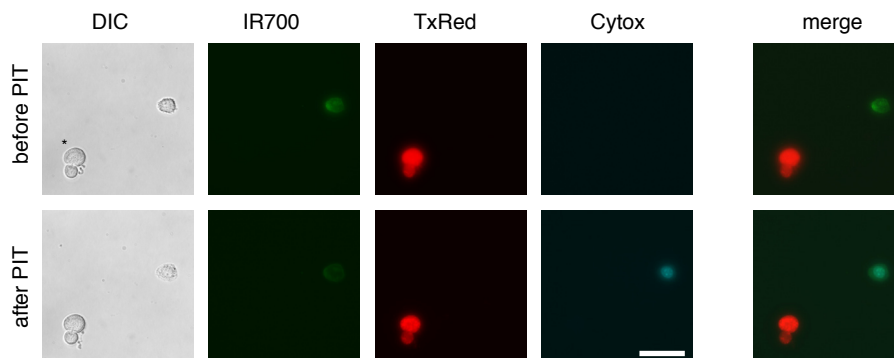
Supplementary video

Time-lapse Imaging of NIR-PIT *in vitro*

Time-lapse sequential images showed shrinkage of the cell and rapid membrane damage detected by PI staining after NIR light irradiation in cells treated with tra-IR700 (50 min observation). Flashing light is NIR light irradiation (2 J/cm^2).

video1: DIC time-lapse image of skov-luc cell treated by NIR-PIT (10sec interval, total 50min)

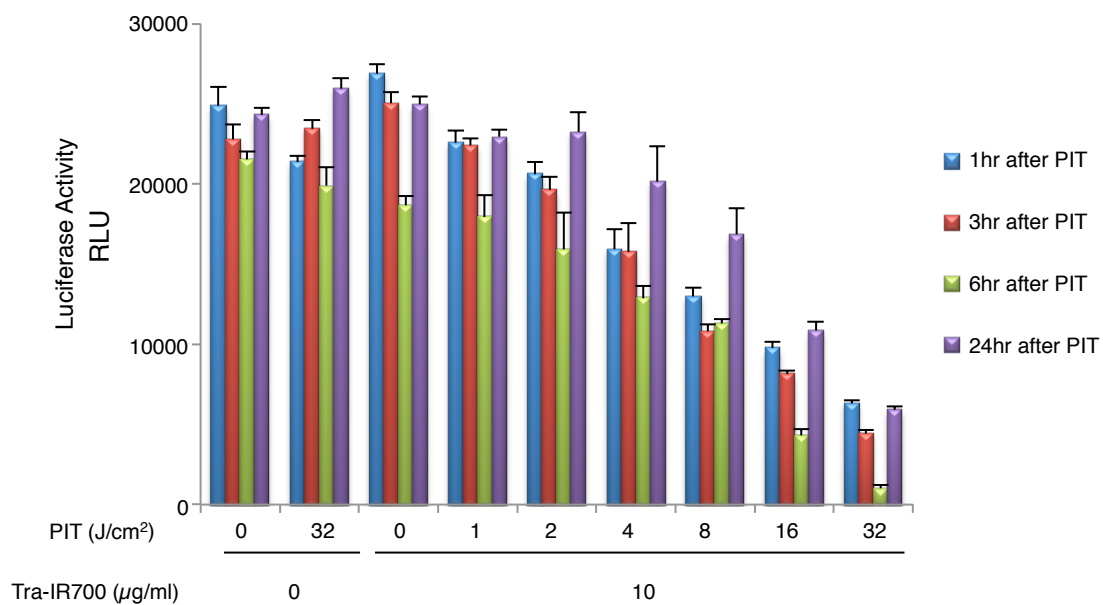
video2: Fluorescence of PI time-lapse image of skov-luc cell treated by NIR-PIT (10sec interval, total 50min)



Supplementary Figure 1.

Targeted necrotic cell death observed after NIR-PIT *in vitro*

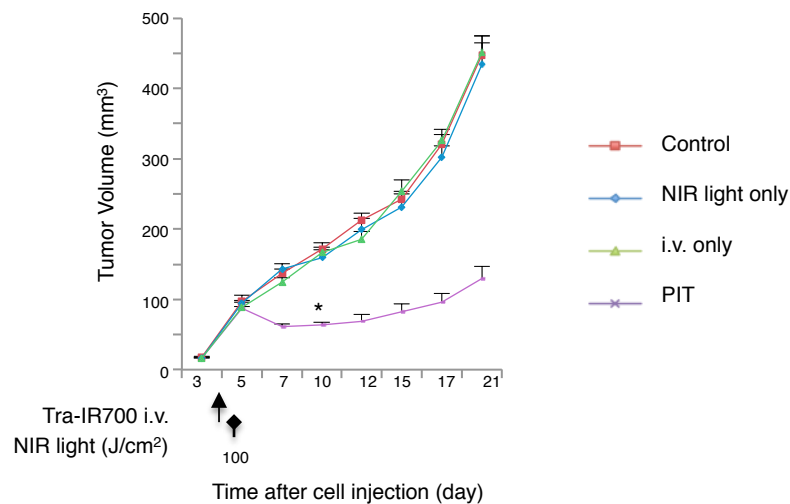
SKOV-luc cells were co-cultured with 3T3/DsRed (non-HER expressing) cells. They were both treated with tra-IR700 and observed by microscopy (before and after irradiation of NIR light). Targeted necrotic cell death was observed upon excitation with NIR light (after 60 min). No damage was showed in non-HER2 expressing 3T3/DsRed cells. * 3T3/DsRed cells, Bar = 50 μ m.



Supplementary Figure 2.

Necrotic cell death evaluated on serial bioluminescence imaging after NIR-PIT

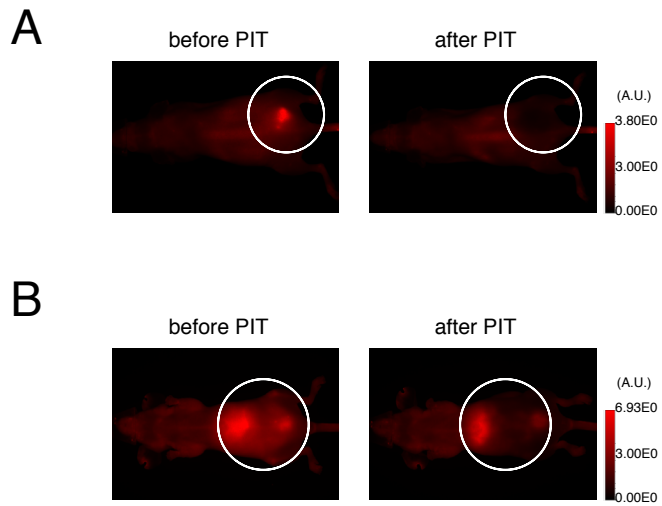
Bioluminescence in SKOV-luc cells was measured as relative light unit (RLU), and decreased in a NIR-light dose-dependent manner over time. 6 hr after NIR-PIT, more cytotoxicity was detected than at 1 or 3 hr. However, at 24 hr after NIR-PIT, bioluminescence increased due to cell-regrowth.



Supplementary Figure 3.

***In vivo* tumor inhibition by NIR-PIT in flank model**

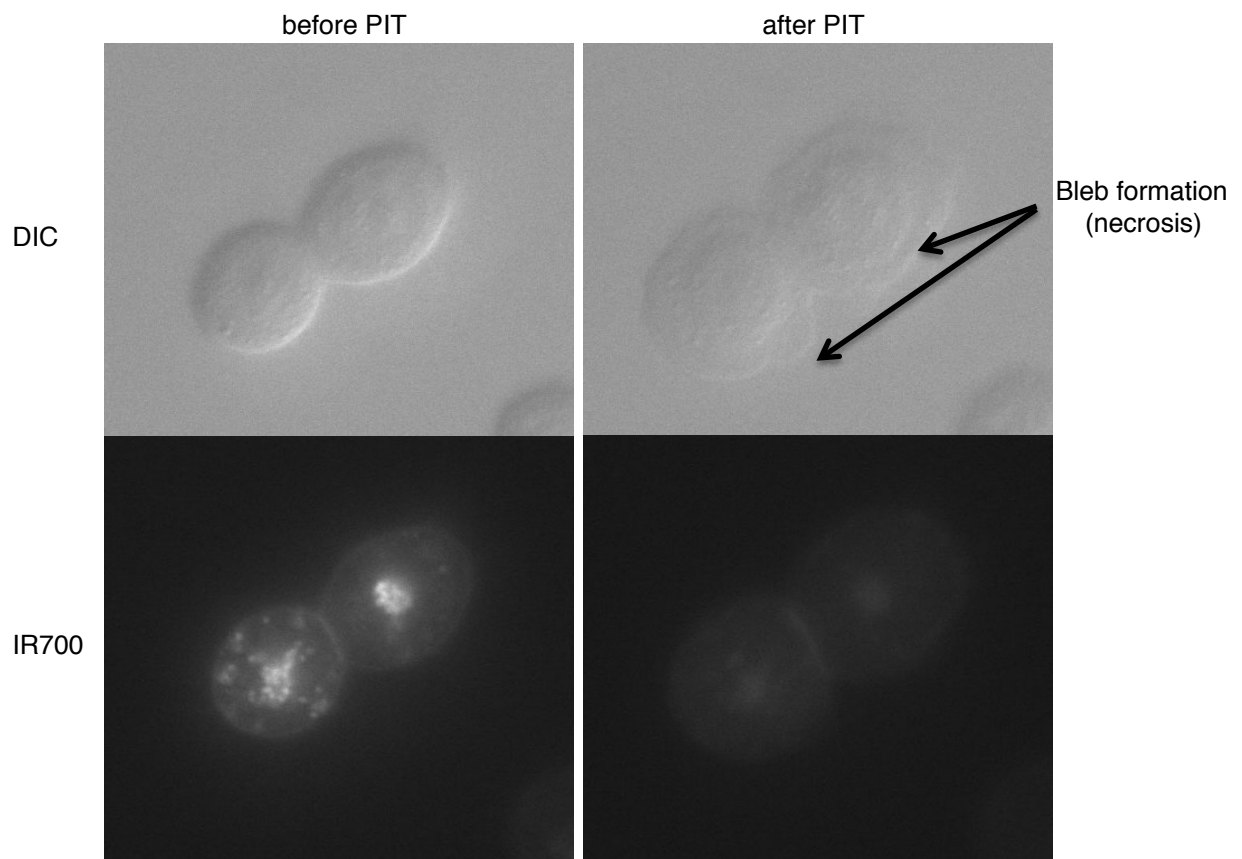
NIR-PIT induced reductions in tumor volume in the flank model (n = 10 mice in each treatment group, (PIT group vs control group at day 10, *p = 0.0001 < 0.001)(PIT group vs light only group at day 10, *p = 0.009 < 0.01)(PIT group vs i.v. only group at day 10, *p = 0.0004 < 0.001), Kruskal-Wallis test with post-test). The treatment regimen is shown below the graph.



Supplementary Figure 4.

***In vivo* fluorescence imaging of tumor bearing mice before and after NIR-PIT**

Serial fluorescence images of the tumor-bearing mice were assessed before and after NIR light exposure. NIR-PIT induced diminishing fluorescence in tumors (A) Flank tumor model. (B) Disseminated peritoneal model.



Supplementary Figure 5.

Mesothelin targeted necrotic cell death observed after NIR-PIT *in vitro*

Under the same condition as shown in Figure 1B and Supplementary video 1, anti-mesothelin-Ab-IR700 induced similar necrotic cell death against A431-k5 mesothelin expressing cells.