Supplementary Information

The effective *in vivo* mitochondrial-targeting nanocarrier combined with a π-extended porphyrin-type photosensitizer

Satrialdi[1,2,6], Yuta Takano[3,4,*], Eri Hirata[5], Natsumi Ushijima[5], Hideyoshi Harashima[1] and Yuma Yamada[1,6,**]

[1] *Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan*

[2] *School of Pharmacy, Institut Teknologi Bandung, Bandung, Indonesia*

[3] *Research Institute for Electronic Science, Hokkaido University, Sapporo 001-0020, Japan*

[4] *Graduate School of Environmental Science, Hokkaido University, Sapporo 060-0810, Japan*

[5] *Faculty of Dental Medicine, Hokkaido University, Sapporo 060-8586, Japan*

[6] *These authors are equally contributed to this study as the first author.*

*To whom correspondence should be addressed:

Kita 20, Nishi 10, Kita-ku, Sapporo 001-0020, Japan E-mail tak@es.hokudai.ac.jp

**To whom correspondence should be addressed:

Kita 12, Nishi 6, Kita-ku, Sapporo 060-0812, Japan E-mail u-ma@pharm.hokudai.ac.jp

Cell culturing of EG7.OVA cells

The EG7.OVA cells (American Type Culture Collection, Manassas, VA, USA), murine lymphoma cells transduced with the chicken OVA gene, were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 1 mM sodium pyruvate, 10 mM HEPES, 50 μM 2-mercaptoethanol, 100 units/mL penicillin-streptomycin, and 10% (v/v) FBS. The cells were maintained under an atmosphere condition of 5% CO_2/air at 37°C.

Determination of tumor inhibition rate

The tumor inhibition rate was determined using a simultaneous double implanted tumor model. Female C57BL/6N mice (6 weeks old) were subcutaneously injected with EG7.OVA on the right and left flank at a density of 1 x 10^6 cells/mouse. The tumor on the right side was intratumorally injected with an HBG solution or the rTPA-MITO-Porter followed by a 20 min period of light irradiation, 12 h after administration. Simultaneously, the tumor on the left side received only the sample solution without a further irradiation process. The tumor inhibition rate was then calculated by dividing the tumor volume on the right side with the left side on each observation day.

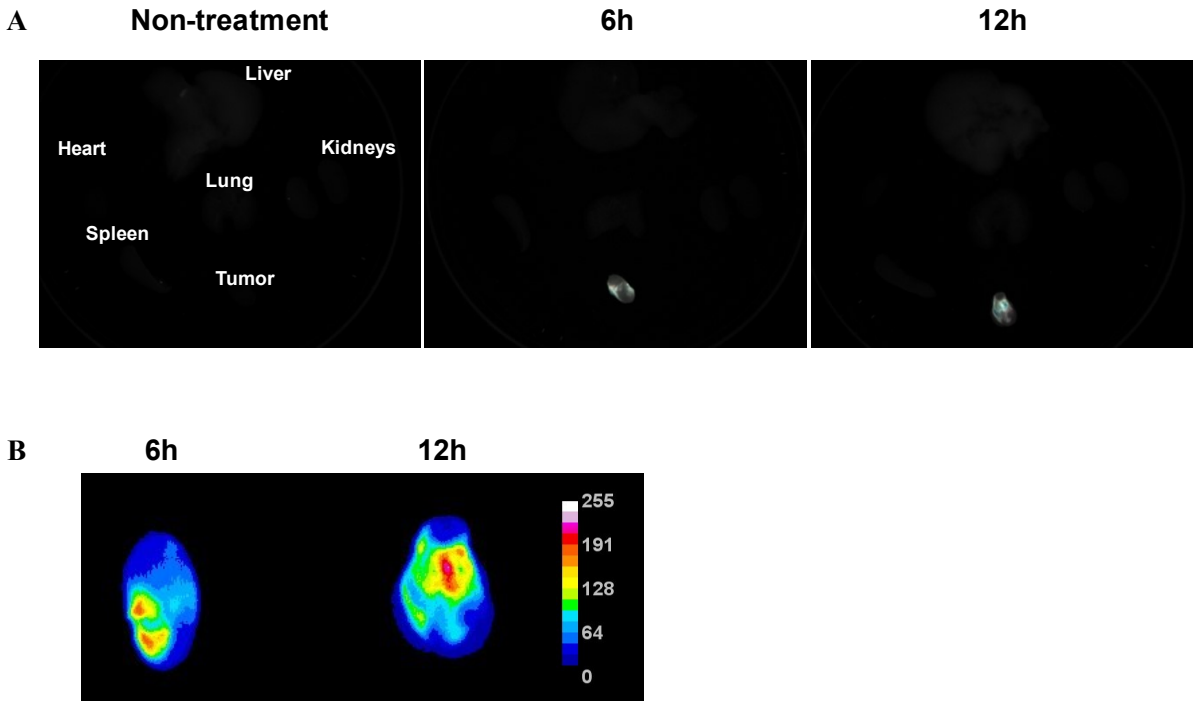

Figure S1. Biodistribution of the DiD-labeled rTPA-MITO-Porter after 6 h and 12 h intratumoral administration (A) biodistribution on several main organs. (B) the pseudo-color image of the particle distribution on the tumor tissues.

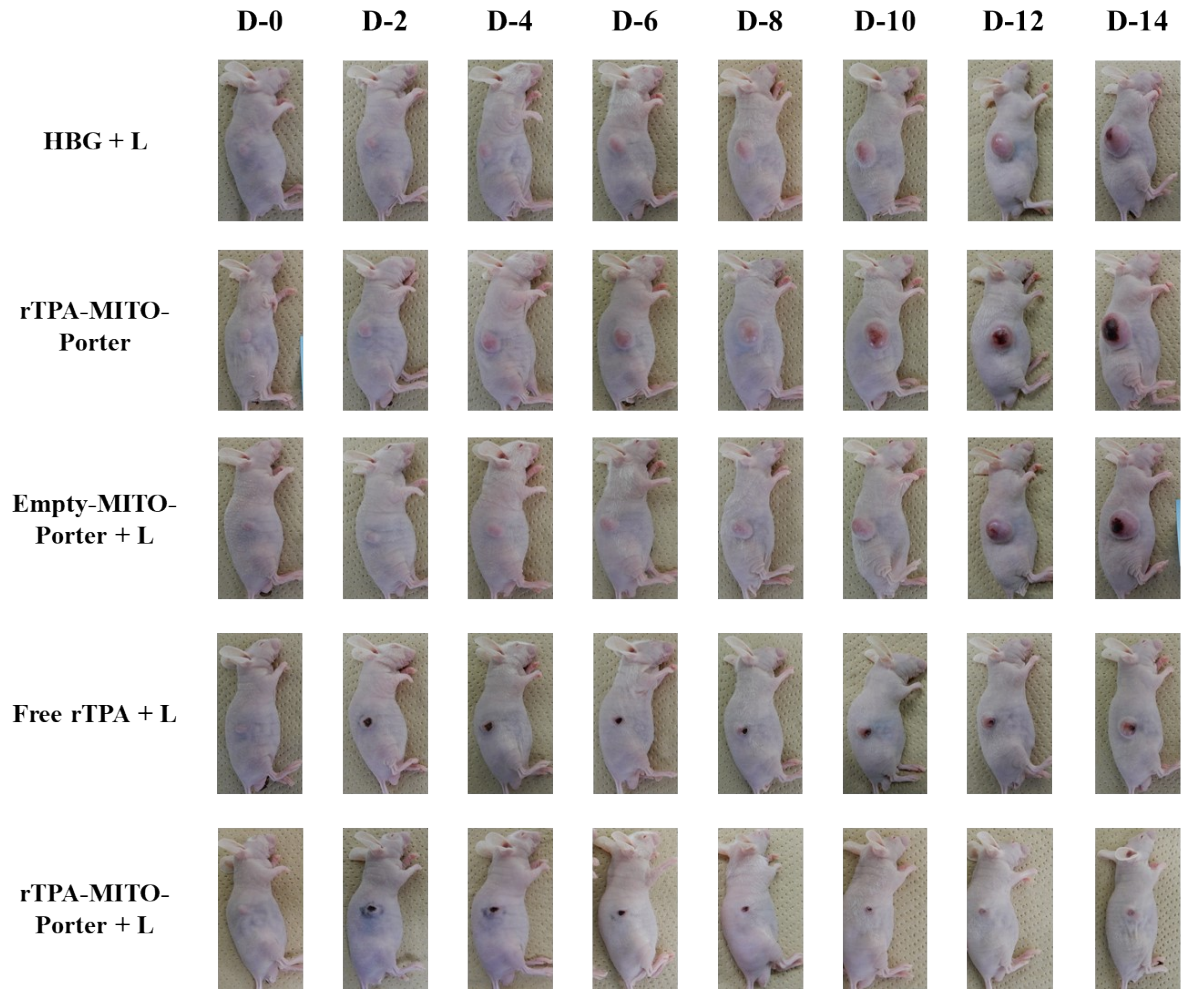

Figure S2. Representative photographs of SAS cell bearing mice at various time points after different treatment modalities.

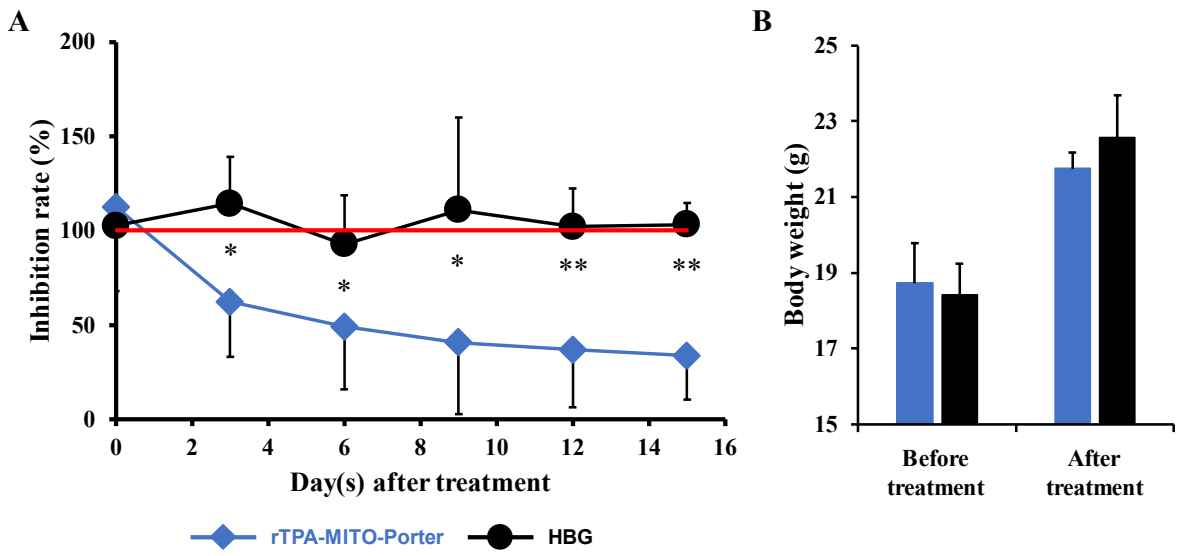

Figure S3. Antitumor activity evaluation on a simultaneous double implanted EG7.OVA tumor model. (A) tumor inhibition rate of the rTPA-MITO-Porter (blue diamond) and HBG (black circle) groups, calculated by dividing the tumor volume on the treated side with the non-treated side. (B) the change in body weight during the treatment using the rTPA-MITO-Porter (blue bars) and HBG (black bars). The error bars indicate S.D. (n = 4; *$p<0.05$; **$p<0.01$ by one-tail unpaired T-test).