Supplementary Information

Transdermal Therapeutic Polymer: In Situ Synthesis of Biocompatible Polymer Using 5-Aminolevulinic Acid as a Photosensitizer Precursor and a Polymer Initiator

Jaehoon Kim, Eun Woo Seo, Hyunyoung Choi, Hyo In Kim, Jinbong Park, Junyang Jung*, and Dokyoung Kim*

1. Experimental section

1.1. General information

2,2,5,5-tetramethyl-2,5-disila-1-oxacyclopentane (TDOP) was purchased from Gelest (Morrisville, PA, USA). 5-aminolevulinic acid was obtained from TCI (Tokyo, Japan). Citric acid monohydrate, methanol, and acetic acid were sourced from Samchun Chemicals (Seoul, Rep. of Korea). Phosphate buffered saline (PBS, pH 7.4, 10×), Trypsin-EDTA, and penicillin/streptomycin were acquired from Gibco (Waltham, MA, USA). Dulbecco's modified eagle's medium (DMEM), Dulbecco's phosphate-buffered saline (DPBS), and fetal bovine serum (FBS) were procured from Hyclone (Logan, UT, USA). DCFHDA was purchased from Sigma-Aldrich (St. Louis, USA) and used to detect reactive oxygen species in cells. Ki67 (ab16667) was purchased from Abcam (Cambridge, UK). Cleaved caspase 3 (#9661s) was purchased from Cell Signaling Technology (MA, USA). All commercially available reagents and anhydrous solvents were used without further purification. Gel permeation chromatography (GPC) analysis was performed using an Agilent 1100 Series HPLC system (Agilent 1100 S, Agilent, Santa Clara, CA, USA). ^{1}H NMR spectra were obtained using NMR instruments (JNM 500 MHz, JEOL, Tokyo, Japan). For NMR analysis, samples were prepared at concentrations of ~5% (*w/v*) in $CDCl_3$. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy was conducted using a Thermo Scientific Nicolet™ iS™ 5 FT-IR spectrometer (16 scans, Waltham, MA, USA). Contact angles were measured using a contact angle meter (DSA100, Kruss, Hamburg, Germany). The viscosity of the polymers was measured using a rotational rheometer (ARES-G2, TA Instruments Ltd., New Castle, DE, USA). The thermal stability was evaluated using thermogravimetric analysis (TGA, SDT Q600, TA Instruments, DE, USA) at Hanyang University (Seoul, Rep. of Korea). Fluorescence images were captured using a confocal laser scanning microscope (CLSM, LSM800, Zeiss, Oberkochen, Germany), and fluorescence intensity was measured with a fluorescence imaging system (FTIS, VISQUE® InVivo Elite, Vieworks Co., Ltd., Rep. of Korea). Thermal images were captured using HIKMICRO Pocket2 (HIKMICRO, Hangzhou, China).

2. Supporting Figures

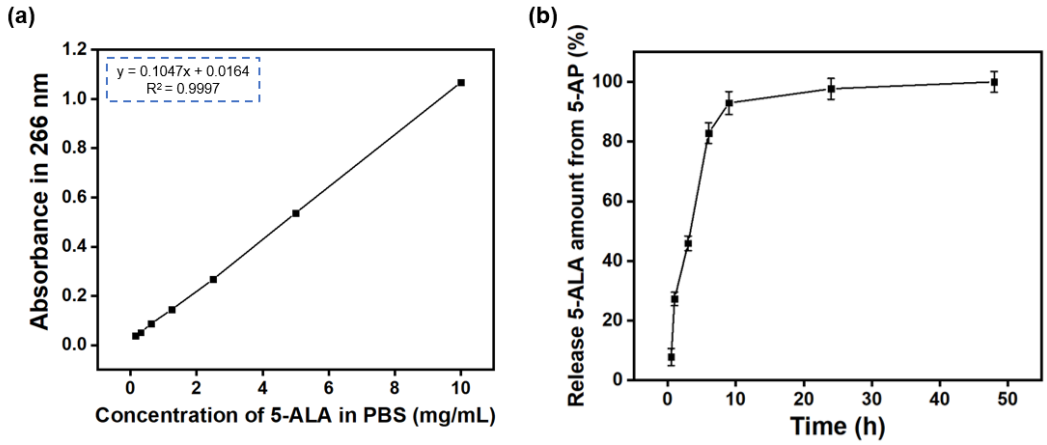

Fig. S1. (a) UV/vis absorption-based standard curve of 5-ALA to analyze the release efficiency of **5-AP**. (b) Release profile of the 5-ALA from **5-AP**. The data are presented as means ± S.E.M. (n = 3).

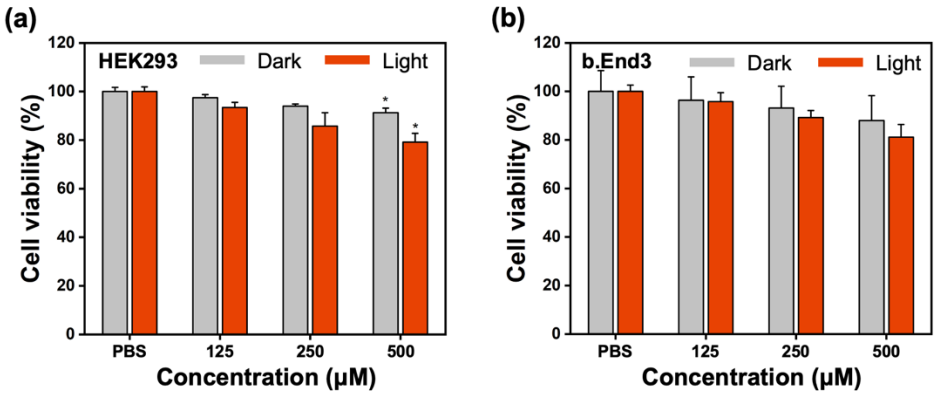

Fig. S2. Cell viability results of 5-AP in (a) HEK293 cells and (b) b.End3 with/without laser irradiation (530 nm) using a CCK-8 assay kit. Incubation time: 4 h (37 °C), irradiation time: 2 min. Data are presented as the mean ± S.E.M. (n = 3). *p<0.05 compared to the PBS group.

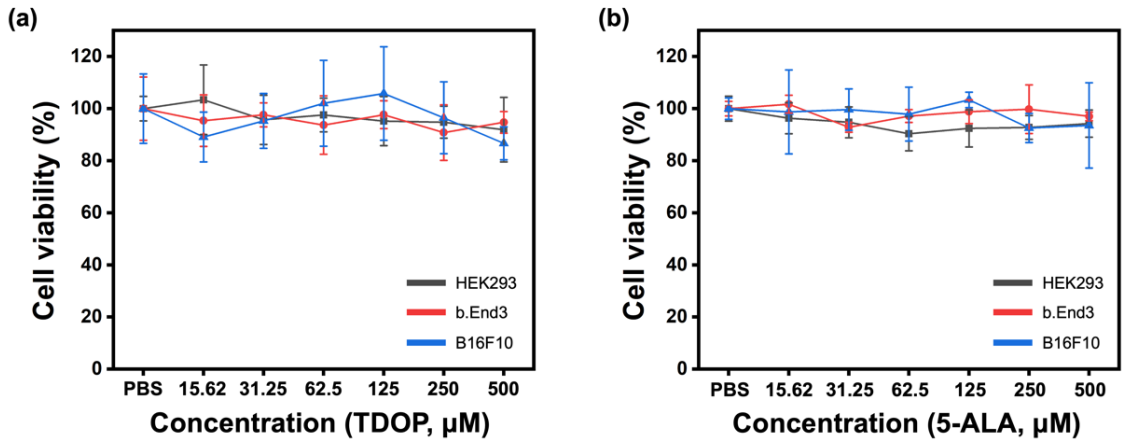

Fig. S3. Cell viability results for (a) TDOP and (b) 5-ALA using a CCK-8 assay kit. Three cell lines (HEK293, b.End3, and B16F10) were used at representative concentrations (15.62–500 μM) of TDOP and 5-ALA. (Incubation time: 4 h at 37 °C). Each error bar represents the mean ± S.E.M., with values calculated from triplicate measurements.

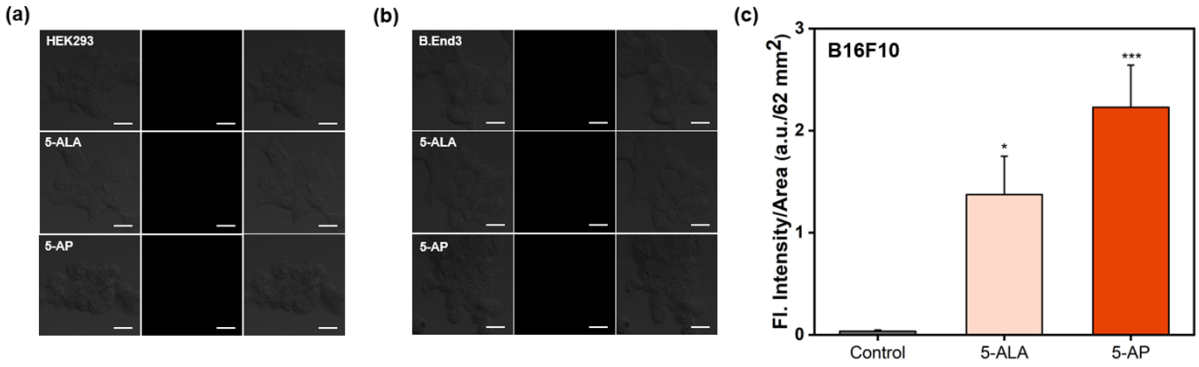

Fig. S4. Confocal laser scanning microscopy (CLSM) images of (a) HEK293 cells and (b) b.End3 cells after treatment with 5-ALA and 5-AP (Concentration: 250 µM). Incubation time: 2 h (37 °C). Scale bar: 20 µm. (c) Fluorescence intensity plot from the 5-ALA treated group in B16F10 of Fig. 3f. Confocal images were obtained under excitation at 488 nm (Laser power: 0.23%) using a detector (GaAsP, Detector gain: 700 V; Detector wavelength: 504–700 nm).

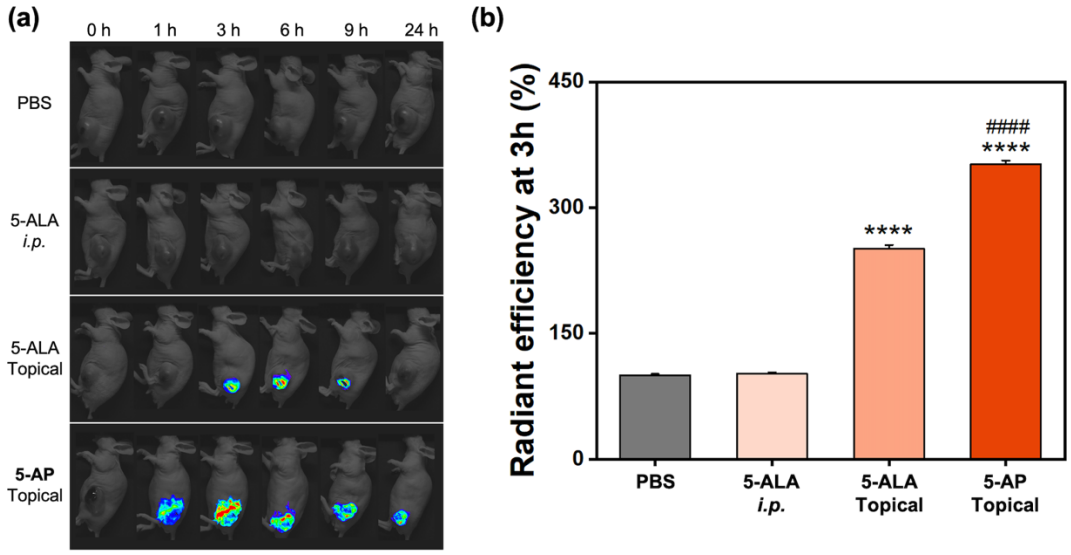

Fig. S5. (a) Biodistribution analysis of 5-ALA and **5-AP** in melanoma xenograft mice over a 24 h period. (b) Radiation efficiency in mice 3 hours after drug administration as shown in FTIS images. Data were acquired by tracking the signals of **5-AP** (Cy5.5 Channel: 390–490 nm excitation, 690–740 nm detection channel). Data are presented as the mean ± S.E.M. (n = 3). ****<0.001 compared to the PBS group; ####p < 0.001 compared to the 5-ALA TDDS group.

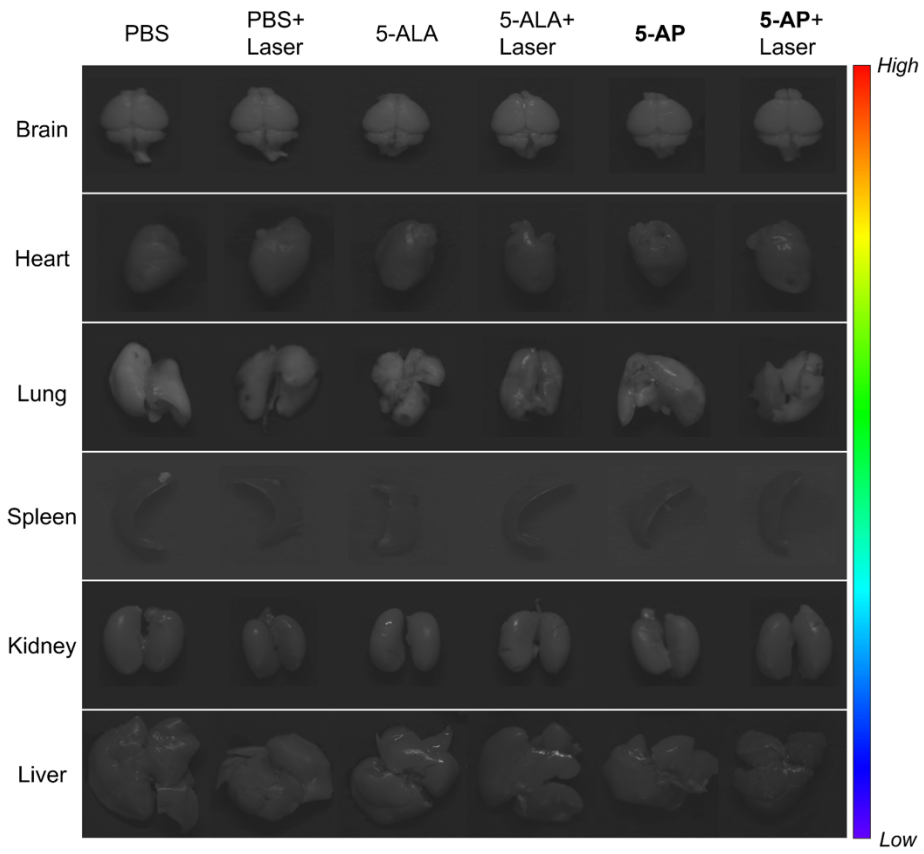

Fig. S6. FTIS image of mouse organs of the melanoma xenografted mouse model (on day 10 after treatment of 5-ALA and **5-AP** with/without laser irradiation (530 nm, 50 mW/cm^2, 3 min)). The images were acquired tracking the signals of PANA **5-AP** (Cy5.5 Channel: 390–490 nm excitation, 690–740 nm detection channel).

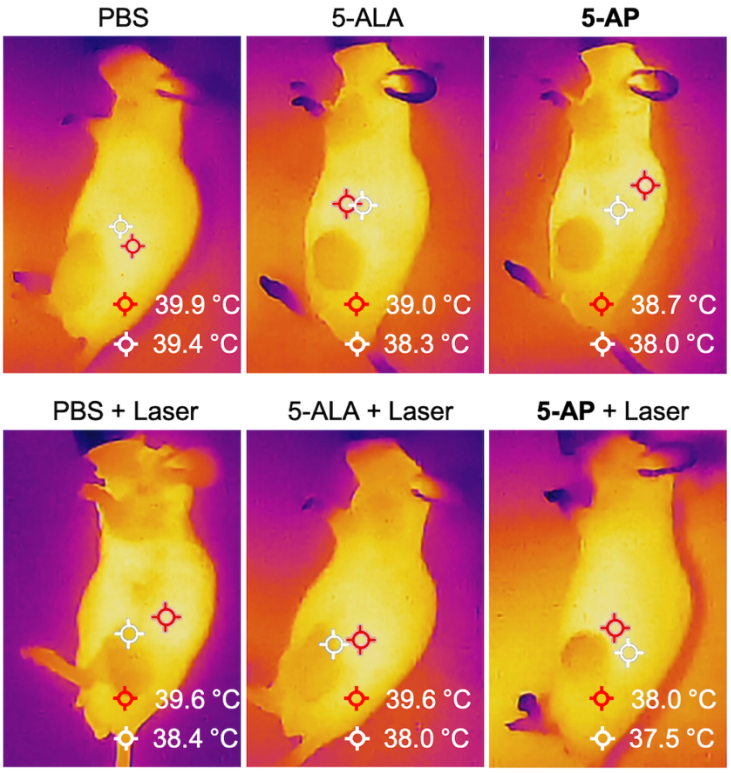

Fig. S7. Thermal imaging of melanoma xenografted mouse model after treatment of 5-ALA or **5-AP** (250 μM, 50 μL/day) with laser irradiation (530 nm, 50 mW/cm^2, 3 min).

^{1}H NMR spectra of TDOP