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Supplementary Information

The optimization of cancer photodynamic therapy by utilization of a pi-extended porphyrin-type photosensitizer in combination with MITO-Porter

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Synthesis of pi-extended porphyrin-type photosensitizer (rTPA)

Materials: All solvents and chemicals were of reagent-grade quality, purchased commercially, and used without further purification unless otherwise noted. Thin layer chromatography (TLC) and column chromatography were performed with Silica gel 60 F254 (Merck) and SiliaFlash F60 (230 – 400 mesh; SiliCycle Inc.), respectively.

General procedures: ^{1}H and ^{13}C NMR spectra were respectively measured by a JEOL JNM-EX400 NMR spectrometer. FT-IR spectra were recorded on a JEOL FT/IR 660Plus spectrometer. High-resolution mass spectra (HRMS) were obtained with Bruker Ultraflex III (MALDI) or ThermoFisher Scientific Exactive (ESI). UV-vis-near infrared (NIR) absorption spectra were measured with a ThermoFisher Scientific Evolution 220 spectrometer. Steady-state fluorescence spectra were recorded on a Hitachi Spectrofluorometer F4500.

Synthesis:

Compounds **6** [1] (5,15-dibromo-10, 20-di-(4-carboxymethylphenyl)porphyrin) was synthesized according to the literature and characterized based on the spectral data therein.

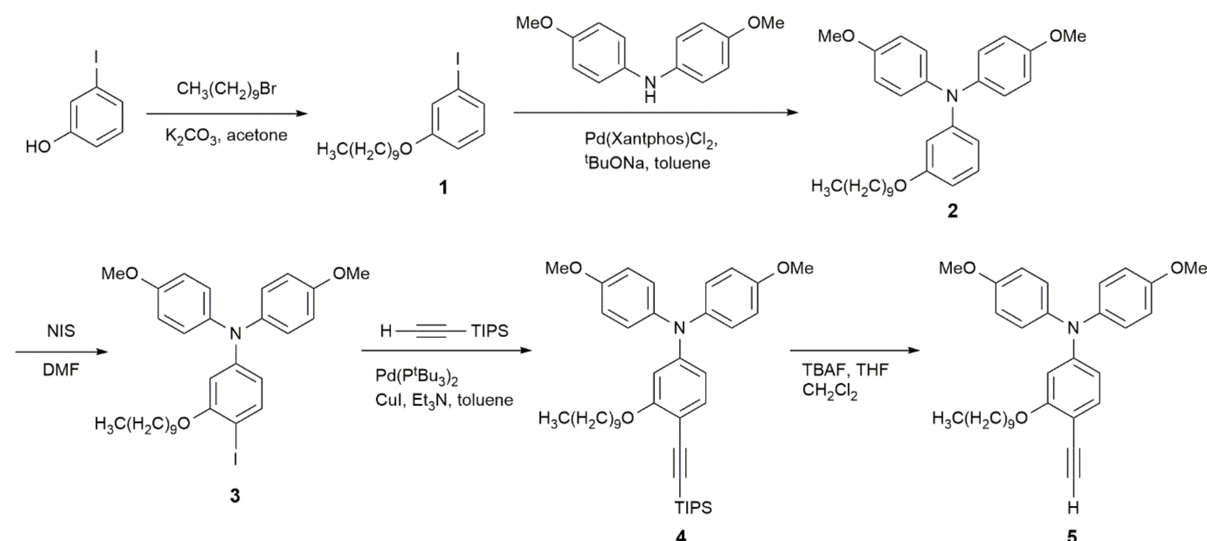

Figure S1. *Synthetic scheme for* ***5***

Compound **1**:

A mixture of *m*-iodonitrophenol (2.22 g, 10.0 mmol), 1-bromodecane (2.20 g, 10.0 mmol), and K_2CO_3 (4.41g, 32.0 mmol) in dry-acetone (50 mL) was refluxed for 44 h. After cooling, the solvent was removed under reduced pressure. Column chromatography on silica gel with hexane/EtOAc (9/1, v/v) as the eluent gave **2** as clear oil. Yield = 3.30 g (8.88 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 2H), 6.98 (dd, J = 8.2 Hz, 1H), 6.85, (dd, J = 8.2 and 1.7 Hz, 1H), 3.91 (t, J = 6.6 Hz, 2H), 1.76 (m,

2H), 1.43 (m, 2H), 1.35-1.25 (m, 12H), 0.88 (t, J = 7.0 Hz, 3H). HRMS (EI): m/z calcd. for $C_{16}H_{25}IO$ ($M^{•+}$), 360.0950; Found, 360.0947.

Compound **2**:

1 (2.24 g, 6.00 mmol), 4,4'-dimethoxydiphenylamine (1.83 g, 8.00 mmol), and NaO^{t}Bu (865 mg, 9.00 mmol) were placed in a 100 mL two-neck flask and filled with argon, and then dry-and-deoxidized toluene (100 mL) was added. Dichloro[9,9-dimethyl-4,5-bis (diphenylphosphino)xanthene]palladium (II) (227 mg, 0.30 mmol) was added to the solution with argon flow. The solution was stirred under Ar atmosphere at 100 °C for 24 h. After cooling, the reaction mixture was washed with water and then dried over anhydrous Na_2SO_4. Column chromatography on silica gel with hexane/EtOAc (9/1, v/v) as the eluent afforded **2** as (2.27 g, 4.92 mmol, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.06-7.02 (m, 5H), 6.83-6.79 (m, 4H), 6.47 (dd, J = 11 and 2.0 Hz, 1H), 6.46 (d, J = 1.4 Hz, 1H), 6.41 (dd, J = 7.8 and 2.4 Hz, 1H), 3.83 (t, J = 6.5 Hz, 2H), 3.79 (s, 6H), 1.70 (m, 2H), 1.43-1.25 (m, 14H), 0.88 (t, J = 7.0 Hz, 3H). HR-mass (ESI): m/z calcd. for $C_{30}H_{39}NO_3$ ($M^{•+}$), 461.2924; Found, 461.2920.

Compound **3**:

A solution of NIS (1.42 g, 6.30 mmol) in dry-DMF (24 mL) was added dropwise into a solution of **2** (2.91 g, 6.30 mmol) in DMF (16 mL), and then stirred at room temperature for 1 h. The mixture was poured onto 40 mL of saturated aqueous $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic layer was washed with saturated $Na_2S_2O_3$ in aqueous solution, dried over anhydrous Na_2SO_4. Then the solvent was removed, and the residue was purified by column chromatography on silica gel with hexane/EtOAc/Et_3N (90:9:1, v/v/v) as the eluent to get **3** (0.765 g, 1.30 mmol, 91%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (d, J = 8.8Hz, 1H), 7.05-7.01 (m, 4H), 6.83-6.79 (m, 4H), 6.40 (d, J = 2.4 Hz, 1H), 6.26 (dd, J = 8.8 and 2.9 Hz, 1H), 3.79 (s, 6H), 3.75 (t, J = 6.3Hz, 2H), 1.71 (m, 2H), 1.45-1.25 (m, 14H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 157.85, 155.94, 150.30, 140.42, 138.73, 126.56, 114.73, 104.95, 74.83, 68.83, 55.32, 31.85, 31.51, 29.49, 29.46, 29.25, 28.93, 25.97, 22.61,22.57, 14.06. IR (film): 2923, 2850, 1580, 1504, 1463, 1328, 1295, 1239, 1179, 1161, 1037, 827, 637, 596, 409 cm^{-1}. HRMS (ESI): m/z calcd. for $C_{30}H_{38}INO_3$ ($M^{•+}$), 587.1891; Found, 587.1889.

Compound **4**:

A mixture of **3** (3.53 g, 6.00 mmol), triisopropylsilylacetylene (1.64 g, 9.00 mmol), $Pd(PtBu_3)_2$ (92.0 mg, 0.21 mmol), CuI (171 mg, 0.90 mmol) and triethylamine (5 mL) in dry-and-deoxidized-toluene (25 mL) was stirred under Ar atmosphere at room temperature for 20 h. The mixture was poured onto 500 mL of brine and extracted with Et_2O. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated. Column chromatography on silica gel with hexane/EtOAc/Et_3N (90:15:1, v/v/v) as the eluent afforded **4** (2.44 g, 3.81 mmol, 64%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.19 (d, J = 8.3Hz, 1H), 7.05-7.01 (m, 4H), 6.83-6.80 (m, 4H), 6.38 (d, J = 2.0 Hz, 1H), 6.35 (dd, J = 8.3 and 2.0

Hz, 1H), 3.77 (s, 6H), 3.74 (t, J = 6.3Hz, 2H), 1.69 (m, 2H), 1.43 (m, 2H), 1.26 (m, 12H), 1.12 (m, 21H), 0.88 (t, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 161.00, 156.08, 150.11, 140.36, 134.04, 126.83, 114.62, 112.00, 104.66, 103.94, 103.86, 92.35, 68.30, 55.39, 53.36, 31.86, 29.59, 29.52, 29.35, 29.33, 26.19, 22.66, 18.71, 14.08, 11.43. IR (ATR): 2925, 2861, 2148, 1598, 1504, 1465, 1331, 1296, 1241, 1180, 1122, 1038, 829, 679, 606, 547, 420 cm^{-1}. HRMS (ESI): m/z calcd. for $C_{41}H_{59}NO_3Si$ ($[M+H]^+$), 641.4259; Found, 641.4253.

Compound **5**:

To a solution of **4** (1.13 g, 1.77 mmol) in dry-CH_2Cl_2 (40 mL), 1 M THF solution of tetrabutylammonium fluoride (10 mL) was added dropwise. The reaction mixture was warmed at 35 °C and stirred for 2 h in a dark. After the solvent was evaporated, the residue was purified by column chromatography on silica gel with CH_2Cl_2/hexane/Et_3N (40:60:1, v/v) as the eluent to get **5** (0.711 g, 1.44 mmol, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.18 (d, J = 8.3Hz, 1H), 7.07-7.04 (m, 4H), 6.84-6.81 (m, 4H), 6.38 (d, J = 2.0 Hz, 1H), 6.35 (dd, J = 8.8 and 1.9 Hz, 1H), 3.78 (m, 2H), 3.16 (s, 6H), 1.71 (m, 2H), 1.39 (m, 2H), 1.26 (m, 12H), 0.88 (t, J = 6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 160.87, 156.27, 150.53, 140.06, 134.22, 127.13, 114.67, 111.48, 103.34, 102.69, 80.81, 79.19, 68.49, 55.37, 31.85, 29.51, 29.48, 29.32, 29.28, 28.92, 25.83, 22.63. IR (film): 3286, 2925, 2853, 1600, 1504, 1465, 1438, 1332, 1297, 1241, 1180, 1117, 1036, 829, 648, 570, 541, 506, 409 cm^{-1}. HRMS (ESI): m/z calcd. for $C_{32}H_{39}NO_3$ ($M^{\bullet+}$), 485.2924; Found, 485.2931.

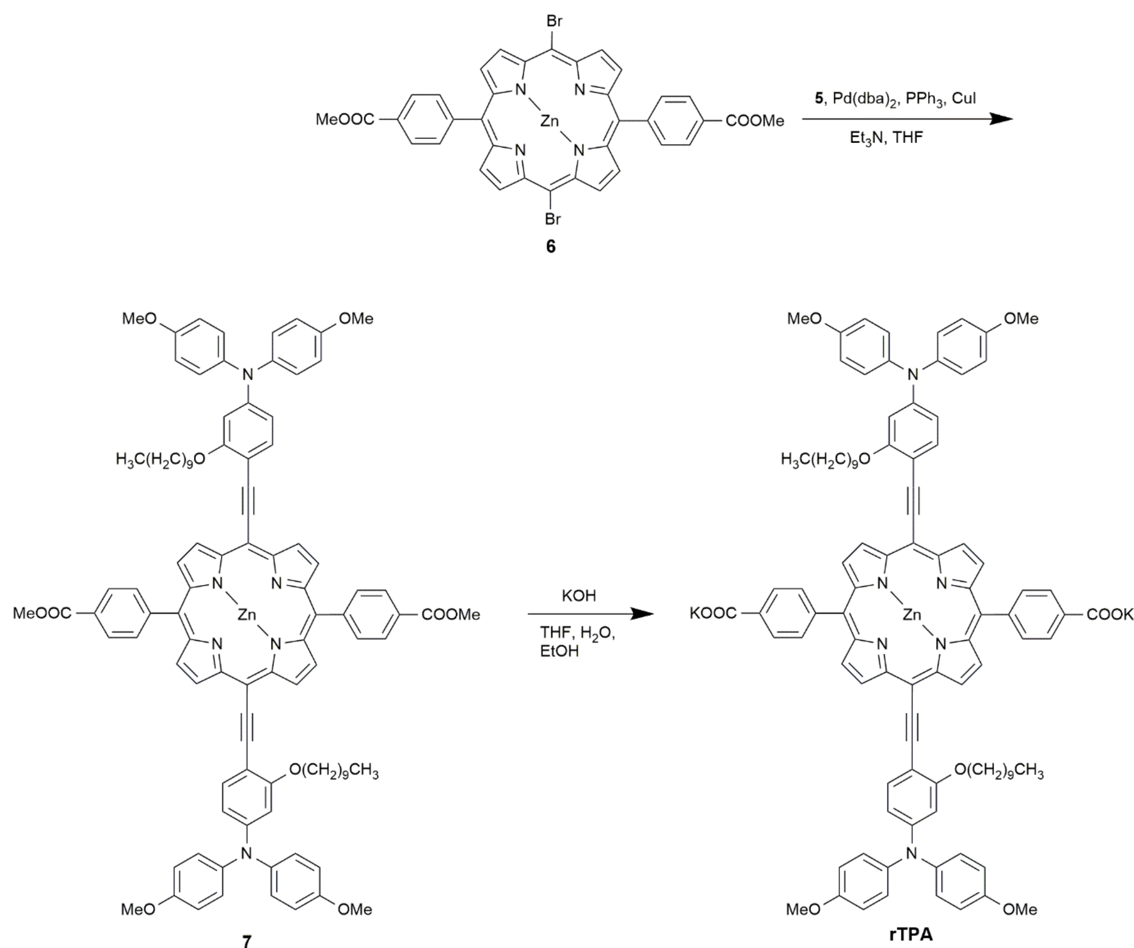

Figure S2. *Synthetic scheme for* ***rTPA***

Compound **7**:

To **6** (5,15-dibromo-10, 20-di-(4-carboxymethylphenyl)porphyrin) (258 mg, 0.323 mmol) in anhydrous THF (30 mL) were added triethylamine (10 mL), $Pd(dba)_2$ (92.9 mg, 0.162 mmol), triphenylphosphane (63.4 mg, 0.242 mmol), CuI (61.4 mg, 0.323 mmol) and **5** (486 mg, 1.00 mmol). The reaction mixture was stirred at r.t. for 21 h and then the solvent was removed under reduced pressure. Column chromatography on silica gel with CH_2Cl_2/Et_3N (100:1, v/v) as the eluent afforded **7** (461 mg, 0.287 mmol, 88 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.74 (d, J = 4.5 Hz, 4H), 8.73 (d, J = 4.5 Hz , 4H), 8.42 (d, J = 7.8 Hz, 4H), 8.26 (d, J = 7.8 Hz, 4H), 7.66 (d, J = 8.8 Hz, 2H), 7.15 (d, J = 8.8 Hz, 8H), 6.86 (d, J = 8.8 Hz, 8H), 6.59 (s, 2H), 6.58 (d, J = 8.8 Hz, 2H),4.10 (s, 6H), 4.01 (t, J = 5.8 Hz, 4H) 3.81 (s, 12H), 2.03 (m, 4H), 1.63 (m, 4H), 1.38 (m, 4H), 1.25-1.00 (m, 20H), 0.71 (t, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 167.43, 160.83, 156.30, 152.03, 150.46, 149.02, 147.50, 140.26, 134.46, 133.24, 131.60, 131.35, 129.25, 127.83, 127.24, 120.99, 114.80, 112.00, 104.57, 103.44, 103.18, 95.41, 95.04, 68.61, 55.42, 52.27, 31.65, 29.58, 29.49, 29.46, 29.40, 29.14, 26.28, 22.46, 13.88. IR (film): 2922, 2850, 2175, 1716, 1595, 1500, 1462, 1432, 1335, 1273, 1234, 1176, 1099, 1034, 999, 822, 795, 717, 648, 629, 606, 586, 571, 544, 521, 482, 459, 425 cm^{-1}. HRMS (ESI): m/z calcd. for $C_{100}H_{98}N_6O_{10}Zn$ ($M^{\bullet+}$), 1606.6630; Found, 1606.6624.

rTPA:

To a solution of **7** (461 mg, 0.287 mmol) in THF/ethanol (1:1, v/v) (200 mL) was added potassium hydroxide (1.5 g) in water (15 mL) and the reaction mixture was refluxed for 1 h. After cooling, the solvent was evaporated, the residue was diluted with water, and the desired porphyrin dipotassium salt was filtered. The deposit was dissolved in THF, the solution was poured onto brine and extracted with THF. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Then the solvent was removed under reduced pressure to get **rTPA** (394 mg, 0.250 mmol, 87% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.68 (d, J = 3.9 Hz, 4H), 8.70 (d, J = 3.9 Hz, 4H), 8.24 (d, J = 8.3 Hz, 4H), 8.00 (brs, 4H), 7.80 (d, J = 8.3 Hz, 2H), 7.20 (d, J = 8.1 Hz, 8H), 7.00 (d, J = 8.1 Hz, 8H), 6.53 (s, 2H), 6.45 (d, J = 8.3 Hz, 2H), 4.03 (m, 4H), 3.79 (s, 12H), 1.96-0.96 (m, 32H), 0.61(t, J = 5.8 Hz, 6H). IR (film): 3066, 2918, 2850, 2187, 2160, 2137, 1687, 1595, 1501, 1434, 1327, 1236, 1176, 1095, 1032, 999, 938, 824, 790, 712, 628, 590, 563, 518, 494, 470 cm^{-1}. HRMS (MALDI): m/z calcd. for $C_{98}H_{94}N_6O_{10}Zn$ ($M^{\bullet+}$), 1578.6317; found 1578.6313.

Materials and Methods

Materials: 1,2-Dioleoyl-sn-glycero-3-phosphatidyl ethanolamine (DOPE) and sphingomyelin (SM) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Non-hydrogenated egg phosphatidyl choline (EPC) was purchased from NOF Corporation (Tokyo, Japan). Stearylated-octaarginine (R8) was obtained from Toray Research Center, Inc. (Tokyo, Japan). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Wako (Osaka, Japan) and Sigma Aldrich Corp. (St. Louis, MO, USA), respectively. SAS cells, human oral squamous carcinoma cells, were received from National Institutes of Biomedical Innovation, Health, and Nutrition JCRB Cell Bank (Osaka, Japan). HeLa cells were collected from Riken BRC (Tsukuba, Japan). The premix WST-1 Cell Proliferation Assay System kit was obtained from Takara Bio Inc. (Shiga, Japan). All additional chemicals and solvents used were purchased as commercially available reagent-grade products.

Incorporation of rTPA into MITO-Porter system: The incorporation of the **rTPA** molecules into the MITO-Porter system was carried out using the hydration method. Briefly, the lipid films were prepared by mixing 550 µM lipids in ethanol in a molar ratio of 9:2 of DOPE:SM and 5 mol% of **rTPA** in chloroform followed by solvent evaporation. The resulting lipid films were hydrated using 10 mM HEPES buffer supplemented with 290 mM glucose (pH 7.4) for 15 min at room temperature. The hydrated lipid film was sonicated for 45 sec to obtain a uniform particle. To remove the non-encapsulated drug, the suspension was centrifuged at 20,600 x g for 5 min; then the supernatant was collected. The R8 solution (10 mol% of the total lipids) was added to the suspension to produce **rTPA**-MITO-Porter. We also incorporated **rTPA** into the non-mitochondrial fusogenic nanoparticles consisting of EPC and SM with a molar ratio of 9:2 and 10 mol% R8 (**rTPA**-EPC:SM-R8) using a similar method as described above. The particle size and their distribution were examined using dynamic light scattering (DLS) method, while zeta potentials were measured using the patented technique of phase analysis light scattering (M3-PALS technology) (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). The encapsulation efficiency (EE) was calculated using the following equation:

$$EE\ (\%) = \frac{C_{1-rTPA}/C_{1-lipid}}{C_{0-rTPA}/C_{0-lipid}} \times 100$$

Where $C_{1\text{-rTPA}}$ and $C_{1\text{-lipid}}$ are the recovered amount of **rTPA** and lipid after liposomal formation, $C_{0\text{-rTPA}}$ and $C_{0\text{-lipid}}$ are the initial amount of **rTPA** and lipid.

Photoinduced $^1O_2$ generation: The detection of $^1O_2$ level using a Singlet Oxygen Sensor Green (SOSG) reagent (Thermo Fischer Scientific Inc., Waltham, MA, USA) was carried out following the manufacture's protocol. In brief, a mixture of 2 µM **rTPA** in nanocarrier and 5 µM of SOSG solution

were irradiated using a Xenon lamp (MAX-303, Asahi Spectra, Tokyo, Japan) at the wavelength of 700 ± 6 nm (20 mW/cm^2). The change in fluorescence intensity of the solutions at 530 nm was measured using spectrofluorometer with the excitation wavelength of 490 nm.

Cell culture: HeLa cells and SAS cells were cultured in DMEM supplemented with 10% (v/v) FBS and penicillin-streptomycin under an atmosphere condition of 5% CO_2/air at 37°C. The cells passage was performed at 90% confluency.

Detection of ROS level in mitochondria: Si-DMA (Dojindo Molecular Technologies Inc., Kumamoto, Japan) and MitoSOX™ Red (Thermo Fischer Scientific Inc.) were used to detect the $^1O_2$ and superoxide level in mitochondria of HeLa cells, respectively. The cells were seeded on 35-mm glass base dish (Iwaki, Osaka, Japan) 24 h before the experiment. The **rTPA-**nanocarriers with 0.1 μM **rTPA** were transfected for 1 h in serum-free medium followed by 2 h in medium containing 10% FBS. The probe solution was added, followed by a 10 min and 45 min period of incubation for MitoSOX™ Red and Si-DMA, respectively. The fluorescence signal of the probe was detected using confocal laser scanning microscopy (CLSM, Olympus FV10i-LIV, Olympus Corporation, Tokyo, Japan) equipped with a water-immersion objective lens (UPlanSApo 60x/NA. 1.2) and a dichroic mirror (DM405/473/559/635). The cells were excited with a 559 nm light from an LD laser with the fluorescence detection channel at 570-620 nm to detect the MitoSOX™ Red signal, whereas, for Si-DMA, the excitation wavelength was set at 635 nm with the fluorescence detector at 660-710 nm. The CLSM observation was conducted before and after light irradiation (Xenon lamp, $\lambda = 700 \pm 6$ nm, 68.5 mW/cm^2). The mean fluorescence intensity of Si-DMA was calculated using Image J software.

Cellular uptake analysis: The cells were implanted on 6-well plates (Corning Inc., Corning, NY, USA) and maintained under an atmosphere of 5% CO_2/air at 37°C for 24 h. The NBD-labelled **rTPA**-nanocarrier was used as the sample and was transfected into the cells for 1 h in serum-free DMEM. The transfected cells were washed twice with phosphate-buffer saline (PBS (-)) containing 20 units/mL of heparin followed by trypsinization. The detached cells were centrifuged at 700 x g at 4°C for 3 min, and the supernatant removed. The cells pellet was washed with PBS (-) containing 0.5% (w/v) bovine serum albumin and 0.1% (w/v) sodium azide (FACS Buffer) followed by centrifugation with the same conditions. The supernatant was discharged, and the cell pellet was resuspended in the FACS Buffer. Cellular uptake was determined using flow cytometer (Gallious, Beckman Coulter, Brea, CA, USA). The NBD fluorescent probe was excited by a 488 nm laser and detected with a fluorescent light sensor at 525 nm (FL1). The measurement results were expressed as the mean fluorescence intensity (MFI). At least four independent analyses were conducted.

Intracellular trafficking analysis: The cells were seeded on 35-mm glass base dishes 24 h before transfection. Cells were transfected with the NBD-labelled **rTPA**-nanocarrier in phenol red-free DMEM without serum for 1 h followed by an additional 2 h in phenol red-free DMEM supplemented with 10% serum. The cells were washed using PBS (-) containing 20 units/mL heparin, and the medium was replaced with fresh phenol red-free DMEM supplemented with 10% (v/v) serum and fluorescent reagents followed by 20 min of incubation. The observation was performed by CLSM (Olympus FV10i-LIV) equipped with a water-immersion objective lens (UPlanSApo 60x/NA. 1.2) and a dichroic mirror (DM405/473/559/635). The cells were illuminated with 473 nm light and 635 nm light to excite the nanocarrier and the MitoTracker™ Deep Red FM (Thermo Fischer Scientific Inc.), respectively. The two fluorescence detection channels were set using a filter at a bandpass 490-540 nm to detect nanocarrier and bandpass 660-710 nm for MitoTracker™ Deep Red FM. The quantification of the mitochondrial colocalization degree was performed from randomly-selected CLSM images using Image J software [2] by calculating Pearson's correlation coefficient. The Pearson's correlation coefficient has a value range from -1 to +1. The -1 value indicates a negative correlation, while the 0 value and +1 value mean no correlation and the perfect correlation between two objects, respectively[3].

In vitro PDT evaluation: Cell viability was measured using the WST-1 assay. Briefly, the cells were cultured on a 48-well plate (Corning Inc.) for 24 h. The sample was then transfected into the cells in serum-free DMEM for 1 h followed and then for 2 h in DMEM containing serum. The cells were washed using DMEM with serum followed by the photoirradiation process using a Xenon lamp at 700 ± 6 nm. The WST-1 reagent was added to the cells immediately after irradiation, then incubated for 2 h. The change in the reagent absorbance was measured at 450 nm with the reference at 630 nm using a microplate photometer (EnSpire® Multimode Plate Reader, Perkin Elmer, Waltham, MA, USA). The non-treatment cells without irradiation were employed as the standard for 100% cell viability. At least three independent evaluations were conducted.

Validation of cell death mechanism: The direct CLSM observation was used to validate the cell death mechanism by staining the mitochondrial compartment using MitoTracker™ Deep Red FM. The NBD-labelled **rTPA**-nanocarrier was used and transfected for 1 h in phenol red-free DMEM without serum followed by 2 h in phenol red-free DMEM containing serum. Changes in cell morphology were observed using a live imaging system during irradiation at a suitable excitation wavelength of **rTPA** sourced from a High-Speed Laser Confocal Microscopy Nikon-A1Rsi (Nikon Corporation, Tokyo, Japan) instrument. The microscope equipped with an oil-immersion objective lens (Plan Apo VC, 60x, NA 1.4) and the dichroic mirror

(DM405/488/561/640). The cells were irradiated using the diode lasers with 487 nm light to excite the **rTPA**-nanocarrier and 636 nm light to excite the MitoTracker^{TM} Deep Red FM. The two fluorescence detection channels were set at 525/50 nm and 700/75 nm to detect the **rTPA**-nanocarrier and MitoTracker^{TM} Deep Red FM, respectively.

Statistical analysis: The quantitative data are represented as the mean value with the standard deviation (S.D.) for the indicated experiments. The T-test was employed to determine significant differences between two treatment groups. For multiple comparisons, one-way ANOVA was used, followed by the Student-Newman-Keuls test (SNK-test) to evaluate the differences between each group and Dunnett test to determine the significance of the treatment in comparison with the non-treated sample. The significance of the mitochondria delivery of photosensitizer was evaluated using Two-way ANOVA analysis followed by Bonferroni correction with the levels of $p<0.001$ were considered as significant.

Table S1. Particle characteristics of the rTPA-loaded nanocarriers

Liposome type	Composition (molar ratio)	Diameter (nm)	Polydispersity index	ζ-potential (mV)	Encapsulation efficiency (%)
rTPA-LPs	DOPE:SM (9:2)	134 ± 3	0.24 ± 0.03	(-)24 ± 4	41 ± 7
rTPA-MITO-Porter	DOPE:SM-R8 (9:2-10 mol%)	157 ± 7	0.23 ± 0.02	(+)32 ± 3	41 ± 7
rTPA-EPC:SM-R8	EPC:SM-R8 (9:2-10 mol%)	163 ± 6	0.24 ± 0.02	(+)27 ± 2	44 ± 14

DOPE, 1,2-dioleoyl-sn-glycerol-3-phosphatidyl ethanolamine; SM, sphingomyelin; EPC, non-hydrogenated egg phosphatidyl choline; R8, stearylated- octaarginine. Data are represented as mean with S.D. from at least three independent experiment.

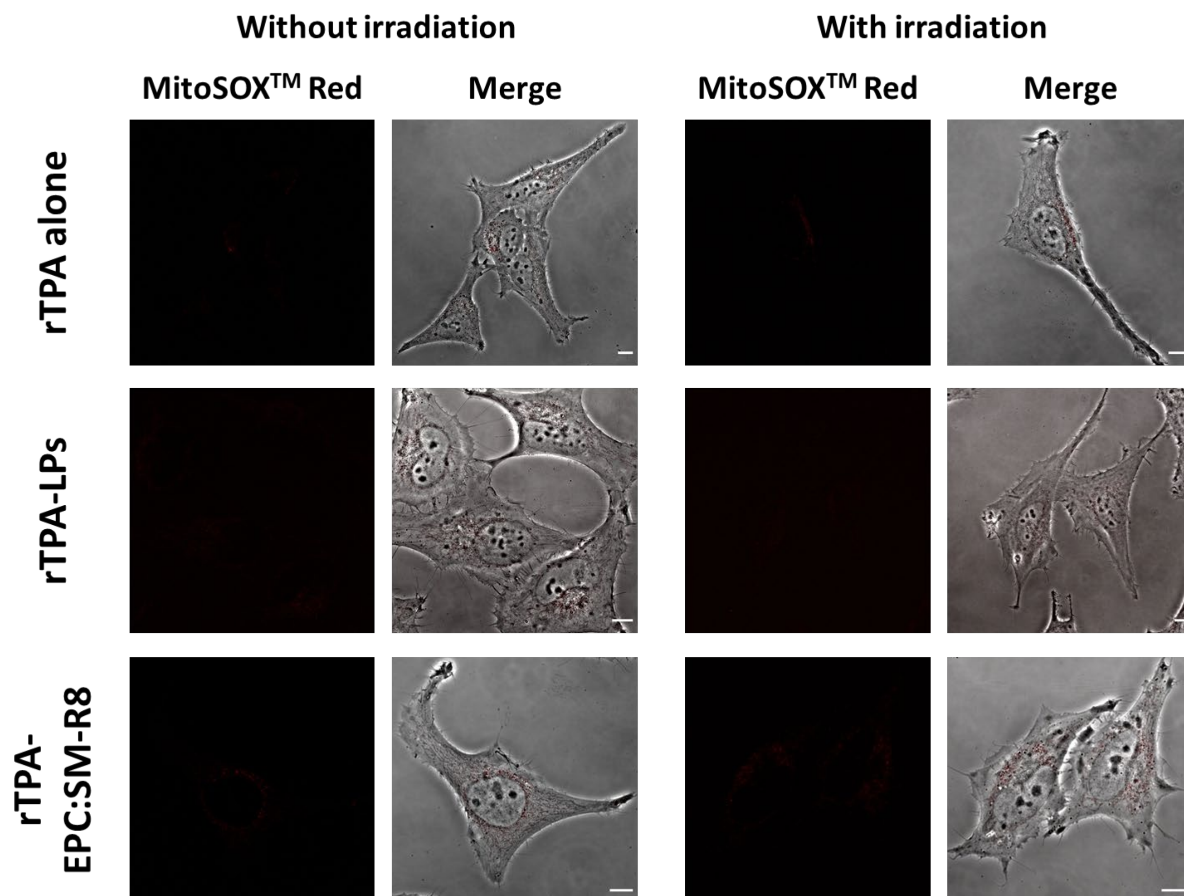

Figure S3. *The mitochondrial superoxide level of the HeLa cells treated by several rTPA formulations in the absence and presence of light irradiation (λ = 700 ± 6 nm, 3 min, 68.5 mW/cm^2) detected by MitoSOX™ Red. Scale bars: 10 μm.*

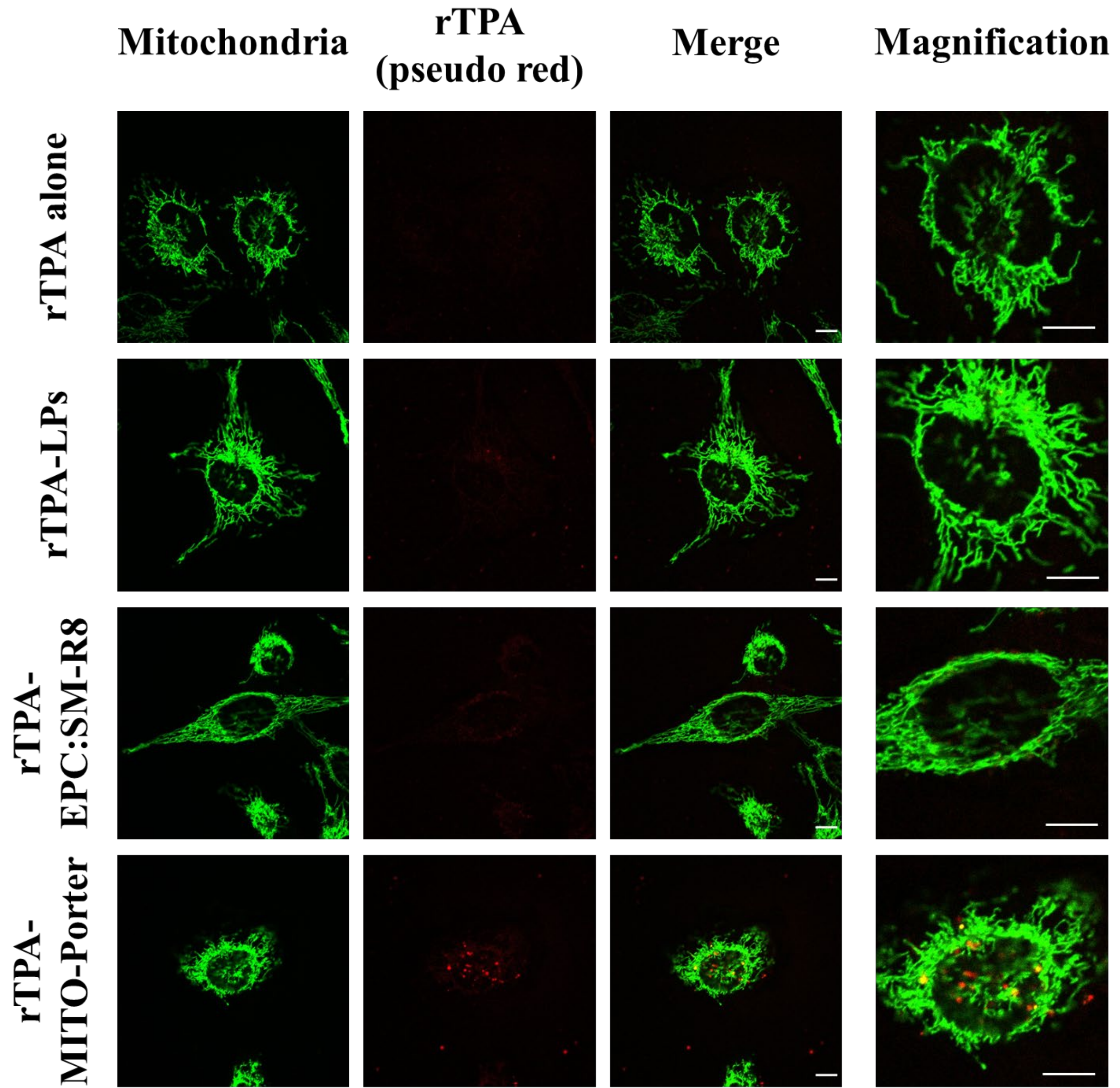

Figure S4. *The representative CLSM images of HeLa cells that had been treated by several rTPA formulations. The mitochondrial compartment was stained with MitoTracker® Orange CM-H2TMRos. The yellow spot shows the colocalization of rTPA in the mitochondrial compartment. Scale bars: 10 μm.*

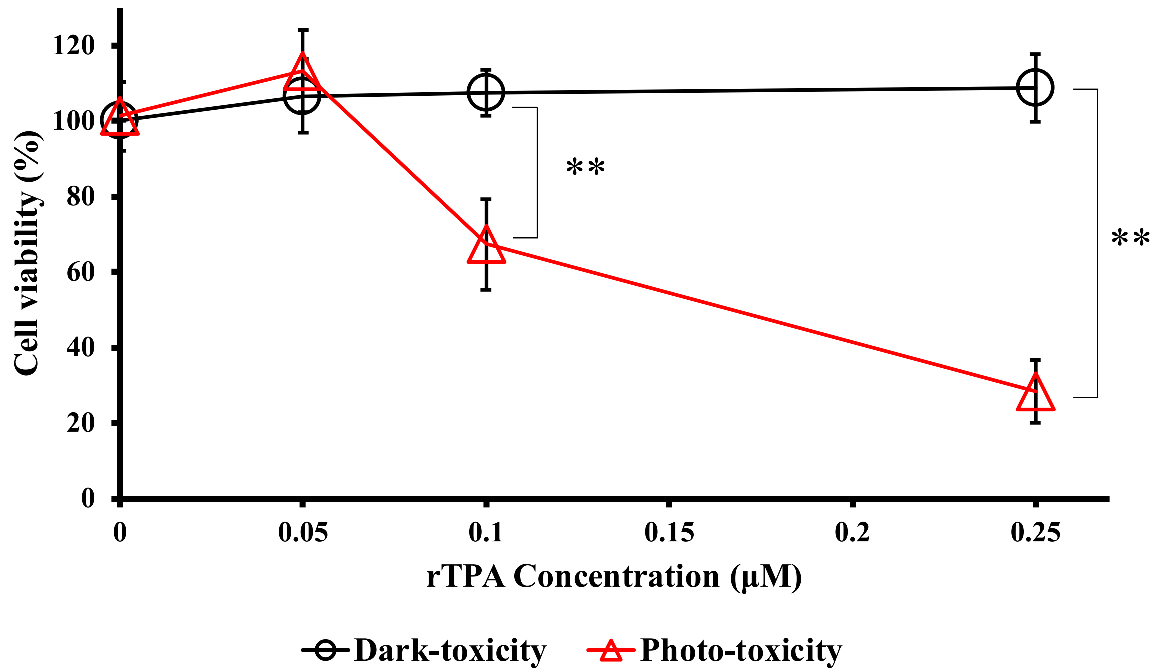

Figure S5. *The cytotoxicity profiles of rTPA-MITO-Porter against HeLa cells in the absence (Dark-toxicity) and presence (Photo-toxicity) of photoirradiation process (λ = 700 ± 6 nm; 3 min; 68.5 mW/cm^2). Value = mean ± S.D. (n = 3; **p<0.01 by Unpaired T-test).*

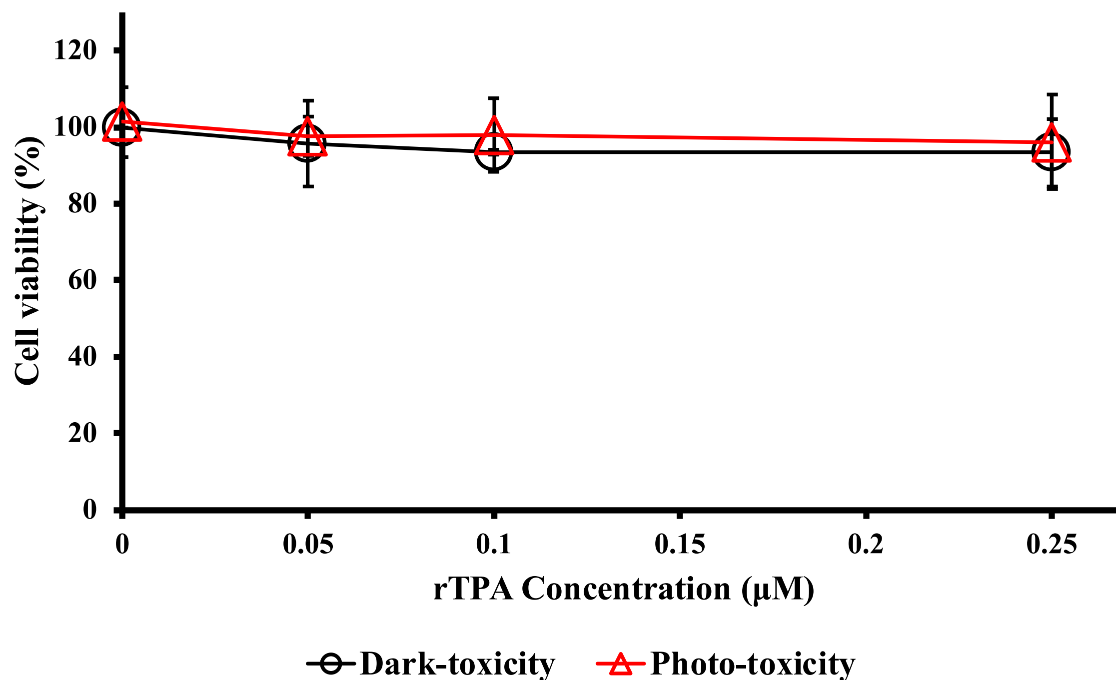

Figure S6. *The cytotoxicity profiles of rTPA-EPC:SM-R8 against HeLa cells in the absence (Dark-toxicity) and presence (Photo-toxicity) of photoirradiation process (λ = 700 ± 6 nm; 3 min; 68.5 mW/cm^2). Error bars indicate S.D. (n = 3).*

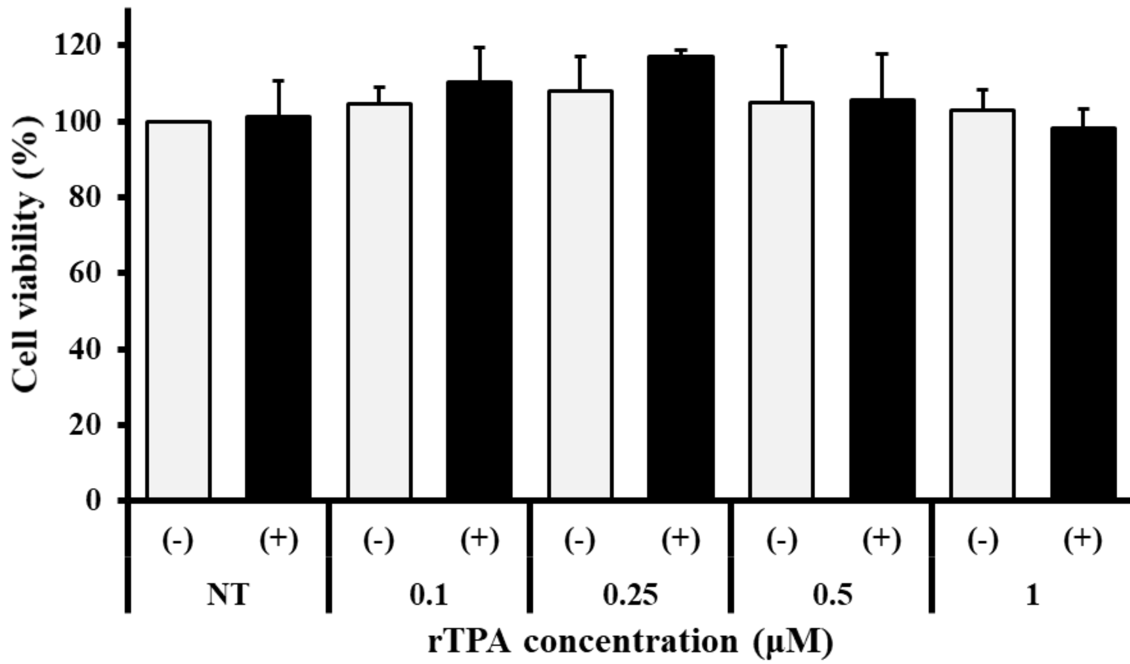

Figure S7. *The cytotoxicity profiles of rTPA alone against HeLa cells in the absence (-) and presence (+) of photoirradiation process (λ = 700 ± 6 nm; 3 min; 68.5 mW/cm2). Error bars indicate S.D. (n = 3).*

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