## **Electronic Supplementary Information for**

# Three Birds with one stone: A single AIEgen for dualorganelle imaging, cell viability evaluation and photodynamic cancer cell ablation

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<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra analysis of Mito-TTPE

#### **Experimental section**

#### MTT assay

Cell viability was investigated using MTT assay. HeLa cells were cultured in 96well plates (1 × 10<sup>4</sup> cells per well) for 24 h. Different concentrations of **Mito-TTPE** or LD-TTP (0, 1, 2, 5 and 10  $\mu$ M) were incubated with the cells for 30 min, respectively. Then, the cells were cultured for another 24 h after treatment with or without light irradiation (50 mW cm<sup>-2</sup>) for 20 min, respectively. 100  $\mu$ L of fresh DMEM medium containing 10  $\mu$ L MTT (5 mg mL<sup>-1</sup>) was added after the culture medium in each 96-well plate removed completely. The cells were further incubated for 4 h. 100  $\mu$ L of DMSO was added into each well after the DMEM/MTT medium was removed. Then each well was analysed with an ELISA microplate reader and the absorbance was detected at 490 nm. The cell viability was expressed as relative to the control cells taken as 100 % metabolic activity.

#### Zeta Potential Distribution



Fig. S1 Average Zeta potentials of Mito-TTPE.



Scheme S1 The synthetic routine of Mito-TTPE.



Fig. S2 (a) Dynamic light scattering data of Mito-TTPE in EtOH/hexane mixtures with  $f_{\rm H}$  of 95%. (b) Solid-state fluorescence spectra of Mito-TTPE. Inset: Fluorescent photo of Mito-TTPE in solid-state taken under a 365 nm UV irradiation.



**Fig. S3** Fluorescence intensity of Mito-TTPE (5 μM) at 550 nm in PBS buffer (pH 7.4) and various species, with  $\lambda_{ex} = 410$  nm. (1) Blank, (2) K<sup>+</sup>, (3) Na<sup>+</sup>, (4) Cu<sup>2+</sup>, (5) Fe<sup>2+</sup>, (6) CN<sup>-</sup>, (7) Cl<sup>-</sup>, (8) Br<sup>-</sup>, (9) NO<sub>3</sub><sup>-</sup>, (10) ClO<sup>-</sup>, (11) SO<sub>4</sub><sup>2-</sup>, (12) NO<sub>2</sub><sup>-</sup>, (13) S<sup>2-</sup>, (14) Cys, (15) GSH, (16) Gln, (17) Ser, (18) Arg, (19) Phe, (20) Pro, (21) Lys, (22) His, (23) Thr, (24) Trp, (25) Leu, (26) Val, (27) Ala, (28) Tyr, (29) Asn, (30) H<sub>2</sub>O<sub>2</sub>, (31) BSA, (32) nitroreductase, (33) lysozyme, (34) alkaline phosphatase, (35) lipase, (36) CES2.

Solvents	$\lambda_{abs}$ (nm)	$\lambda_{\rm em}$ (nm)	Stokes shift (nm)	ε (M <sup>-1</sup> cm <sup>-1</sup> )	Ф (%)
Toluene	488	516	28	2.13×10 <sup>4</sup>	0.266
THF	486	504	18	2.35×10 <sup>4</sup>	0.086
EtOH	206	548	46	2.36×10 <sup>4</sup>	0.023
EtOH/Hexane = 5/95	482	496	14	1.83×10 <sup>4</sup>	0.054

Table S1 Photophysical properties of Mito-TTPE

 $\lambda_{abs}$  = absorption maximum;  $\lambda_{em}$  = emission maximum;  $\varepsilon$  is molar absorptivity at maximum absorption wavelength;  $\Phi$  = fluorescence quantum yield using XDS 307 as the standard reference with a quantum yield of 0.56 in ethanol.



Fig. S4 Mass spectra of Mito-TTPE and Mito-TTPE reacted with CES2.



Fig. S5 Fluorescence spectra of Mito-TTPE+DCFH (a), LD-TTPE+DCFH (b), DCFH (c), Mito-TTPE (d), LD-TTPE (e) in PBS after white light irradiation for different times.



**Fig. S6** (a) Time-dependent fluorescence images of living A549 cells stained with 5  $\mu$ M **Mito-TTPE**. Red channel:  $\lambda_{ex} = 488$  nm,  $\lambda_{em} = 600 - 710$  nm; blue channel:  $\lambda_{ex} = 405$  nm,  $\lambda_{em} = 540-600$  nm. Scale bar: 20  $\mu$ m. (b) FL intensity changes in red and blue channel in living HeLa cells incubated with **Mito-TTPE** at different time points (± S. D., n = 3).



Fig. S7 EdU assay of living HeLa cells stained with 1  $\mu$ M Hoechst 33342. Red channel:  $\lambda_{ex} = 633$  nm,  $\lambda_{em} = 640$  - 700 nm; blue channel:  $\lambda_{ex} = 405$  nm,  $\lambda_{em} = 430$  - 490 nm. Scale bar: 100  $\mu$ m.



**Fig. S8** Intracellular ROS level using DCFH-DA as indicator in living HeLa cells under white light irradiation (50 mW/cm<sup>2</sup>) for different times. DCFH-DA:  $\lambda_{ex} = 488$  nm,  $\lambda_{em} = 500 - 550$  nm. Scale bar: 20 µm. (b) Normalized fluorescence intensity obtained from a (± S. D., n = 3).



**Fig. S9** Viability of HeLa cells incubated with **Mito-TTPE** (a) or LD-TTP (b) in the dark and under white light irradiation (50 mW cm<sup>-2</sup>) for 20 min.

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra analysis of Mito-TTPE

