Electronic Supplementary Information (ESI)

A Unique Self-Reporting Photosensitizer Enabling Simultaneous Photodynamic Therapy and Real-Time Monitoring Phototheranostic Process in a Dynamic Dual-Color Mode

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Experimental Section

Chemicals and materials: Tris(4-bromophenyl)amine, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde, $Pd(PPh_3)_4$, 4-pyridylacetonitrile, ammonium acetate and iodomethane for synthesizing designed molecules were obtained from Energy Chemical (Shanghai, China). Compound **1** were prepared according to our previous work.[S1] Organic solvents and bovine serum albumin (BSA) were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and propidium iodide (PI) were gained from Macklin Co., Ltd. (Shanghai, China). Calf thymus DNA (ctDNA) was provided by Sigma-Aldrich (Shanghai, China). Lyso-Tracker Green and Mito-Tracker Green were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin were supplied by Wuhan Khayal Biotechnology Co., Ltd. (Wuhan, China). 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was gained from Aladdin (Shanghai, China).

Characterization: Nuclear magnetic resonance (NMR) spectra of all new compounds were obtained from a 400 MHz AVANCE III HD NMR spectrometer (Bruker, Switzerland). Mass spectra were recorded on a Xevo G2 mass spectrometer (Waters, America). Absorption spectra were gained from a Shimadzu UV-3600PLUS UV-vis-NIR spectrophotometer (Japan). Fluorescence spectra were measured on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). Fluorescence quantum yields were obtained from a FL3-111 steady state and transient state fluorescence spectrometer (America). Hydrodynamic diameter was acquired on a ZEN3700 Nano Zetasizer (Malvern, UK). Transmission electron microscopy (TEM) images were recorded on a JEM-2100 transmission electron microscope (120 KV, Japan).

Molecular docking modeling: The crystal structure of DNA (PDB ID: 1BNA) was gained from the protein data bank.[S2] The stable structure of **TPA-3PyA+** was optimized by

Chem3D software. The computation of binding model between DNA and **TPA-3PyA+** was performed using AutoDock Vina.[S3] The docking conformation with the lowest binding energies was further used and analyzed.

Measurement of $^1O_2$ quantum yield: The relative $^1O_2$ quantum yield of **TPA-3PyA+** (2 μM) in aqueous solution was measured using 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) (50 μM) as an $^1O_2$ indicator and a commercial photosensitizer Rose Bengal (RB) (2 μM) as the reference (Φ_{RB} = 75% in water). The changes in the absorbance of ABDA at 378 nm after white irradiation (50 mW cm^{-2}) for 80 s were measured. The $^1O_2$ quantum yield was calculated using the following equation:

$$\Phi_T = \Phi_{RB} \frac{K_T \times A_{RB}}{K_{RB} \times A_T}$$

Where Φ_T and Φ_{RB} represent the $^1O_2$ quantum yield of the sample and RB, respectively. K_T and K_{RB} represent the degradation rate constant of ABDA by the sample and RB, respectively. A_T and A_{RB} are the areas of absorbance peaks of the sample and RB in the range of 400-800 nm, respectively.

Cytotoxicity study: The MTT method was used to evaluate dark-toxicity of **TPA-3PyA+** and Ce6. HeLa or SCC-7 cells were seeded in 96-well plates and cultured in DMEM with 10% FBS and 1% penicillin-streptomycin under a humidified atmosphere containing 5% CO_2 at 37 °C for 24 h. Then, the cells were incubated with different concentrations of **TPA-3PyA+** or Ce6 (0, 10, 20, 30 and 40 μM) for another 24 h. 100 μL MTT solution (0.5 mg mL^{-1}) was added into per well after the cells were washed with PBS. The MTT solution was removed after incubating for 4 h, DMSO (100 μL) was added into per well to dissolve the formazan crystals. The absorbance of MTT at 570 nm was obtained from a Synergy H1 microplate reader (BioTek, America) to evaluate the dark-toxicity of **TPA-3PyA+** and Ce6.

Study of cell phototoxicity: HeLa or SCC-7 cells were cultured in 96-well plates and seeded in DMEM with 10% FBS and 1% penicillin-streptomycin under a humidified

atmosphere containing 5% CO_2 at 37 °C for 24 h. The cells were incubated with different concentrations of **TPA-3PyA+** or Ce6 (0, 10, 20, 30 and 40 μM) for another 8 h. Then, the plates were exposed to white light for 30 min and were further cultured for 12 h. The similar MTT method in the cytotoxicity study was applied to evaluate the cell phototoxicity.

Detection of intracellular ROS generation: HeLa cells were firstly cultured in three confocal dishes at 37 °C for 24 h. After washing with PBS, the solution of **TPA-3PyA+** (40 μM) was only added into two dishes and incubated for 8 h. After washing, the cells of all three dishes were incubated with DCFH-DA (10 μM) for another 20 min. After washing, all three dishes were exposed to white light (50 mW cm^{-2}) for 30 min. Then, the images of cells were acquired on CLSM. The signals of DCF (500-550 nm) was collected under excitation at 488 nm.

Cell imaging: For long-time imaging experiment, HeLa cells were firstly cultured in confocal dishes at 37 °C for 24 h. After washing with PBS, the solution of **TPA-3PyA+** (40 μM) was added into dishes and the cell fluorescence images at different incubation times were captured on a Fluorview FV3000 confocal laser scanning microscope (CLSM) (Olympus, Japan). The signals of green fluorescence (500-600 nm) and NIR fluorescence (> 600 nm) were gained under excitation at 488 nm. For imaging fixed cells, HeLa cells were immobilized by 4% paraformaldehyde for 10 min after being cultured in confocal dishes for 24 h. Then, a **TPA-3PyA+** solution (20 μM) was added to dishes to stain the cells. The fluorescence images at different incubation times were captured on the CLSM. For co-staining experiment, HeLa cells were incubated with **TPA-3PyA+** (40 μM) for 8 h. After washing with PBS, the solution of Lyso-Tracker Green (1 μM) or Mito-Tracker Green (200 nM) was added to dishes and incubated for another 30 min. The images were captured on CLSM after washing with PBS. The signal of Lyso-Tracker Green (500-558 nm) or Mito-Tracker Green (500-558 nm) and **TPA-3PyA+** (> 600 nm) was acquired under excitation at 488 nm.

Cell apoptosis imaging: For real-time PDT imaging experiment, HeLa or SCC-7 cells were cultured in confocal dishes for 24 h. After washing with PBS, A solution of **TPA-3PyA+** (40 μM) was added into dishes and cells were further incubated for another 8 h. Then, the dual-channel images under different irradiation times by a blue laser (488 nm, 4% laser power) were acquired on CLSM. In addition, propidium iodide (PI), a nuclear dye for dead cells was used to further confirm the effective PDT performances of **TPA-3PyA+**. Firstly, HeLa cells were incubated only with PI (100 μg mL^{-1}) to gain images under different irradiation times by a blue laser (488 nm, 4% laser power) on CLSM to evaluate the interference from the laser. Secondly, a solution of PI (100 μg mL^{-1}) was added to dishes after cells being incubated with **TPA-3PyA+** (40 μM) for 8 h to gain images under different irradiation times by a blue laser (488 nm, 4% laser power) on CLSM. The signal of PI (569-619 nm) was obtained under excitation at 561 nm. For dead cell imaging experiment under white light irradiation, HeLa or SCC-7 cells were cultured in the confocal dishes for 24 h. After washing with PBS, cells were further incubated with TPA-3PyA+ (40 μM) for another 8 h. Then, the dishes were exposed to white light (70 mW cm^{-2}) for 30 min and further incubation for 12 h. Finally, the images of cells stained with or without PI (100 μg mL^{-1}) were acquired on CLSM. The signals of **TPA-3PyA+** (500-558 nm) and PI (600-700 nm) were collected under excitation at 488 nm.

Animal Tumor Xenograft Models: All animal experiments were carried out under the permission and guidance of the Animal Ethics Committee of Jiangnan University (Protocol No: JN.No20201115b0240120[297]). Female Balb/c nude mice (5-6 weeks olds) were provided by Changzhou Cavens Laboratory Animal Co., Ltd. SCC-7 cells (1×10^7) suspended in 100 μL PBS were subcutaneously injected into the right rear flank of the mice in order to establish tumor xenograft models. The tumor therapy studies were performed when the tumor volume reached about 85 mm^3.

In vivo photodynamic therapy: SCC-7 tumor-bearing mice were randomly divided into

four groups (three mice for each group): "GS" group: only 5% GS solution; "GS + light" Group: 5% GS solution with light; "**TPA-3PyA+**" group: only **TPA-3PyA+** (1 mg mL^{-1}) and "**TPA-3PyA+** + light" group: **TPA-3PyA+** (1 mg mL^{-1}) with light. Different materials were injected into the mice (100 μL per mouse) at day 0 and day 3. White light irradiation (200 mW cm^{-2}) was only carried out on the "GS + light" and "**TPA-3PyA+** + light" group for the first six days (20 min per mouse per day). The tumor volumes and body weights of mice were measured every day to evaluate the PDT efficacy and biocompatibility of **TPA-3PyA+**. The tumor volume (V) was calculated as $V = L \times W^2/2$ (L and W are the length and width of the tumor, respectively).

Histological analysis: H&E staining was used to stain the major organs (heart, liver, spleen, lung and kidney) and tumors of mice 14 days after treatment according to the manufacturer's protocol and the slices were observed under the microscope.

Figures

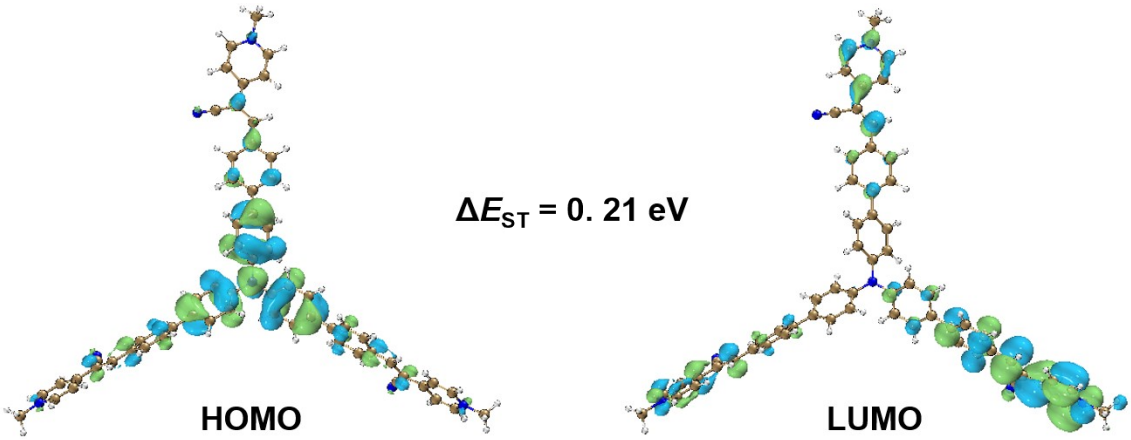

Figure S1. HOMO-LUMO distribution and ΔE_{ST} value of **TPA-3PyA+** calculated based on time-dependent density functional theory (Gaussian 09/B3LYP/6-31G(d)).

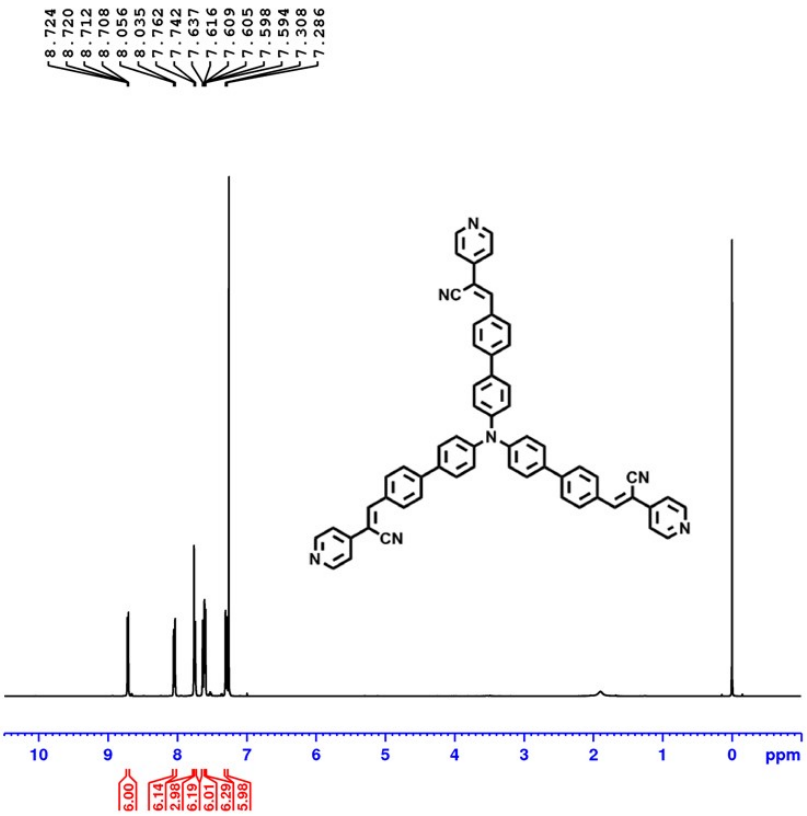

Figure S2. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, ppm) of compound **2**.

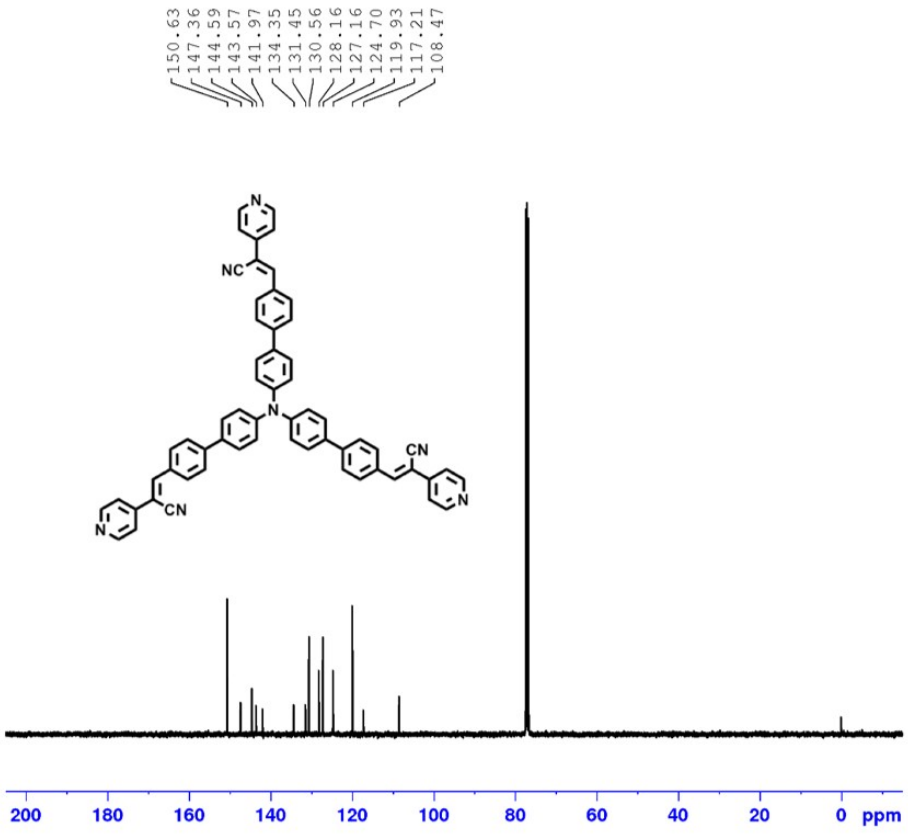

Figure S3. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, ppm) of compound **2**.

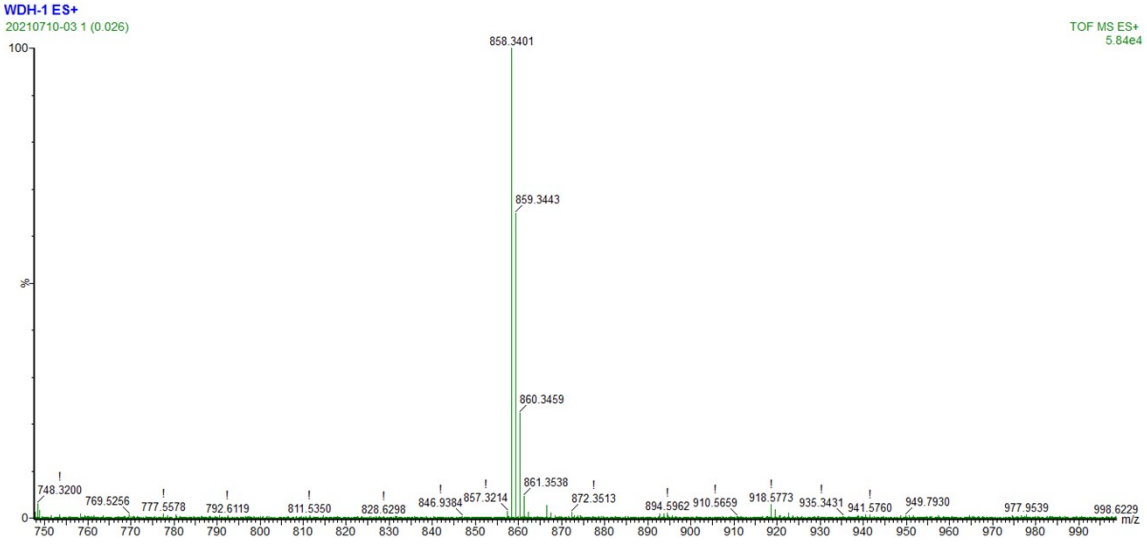

Figure S4. HRMS spectrum of compound **2**.

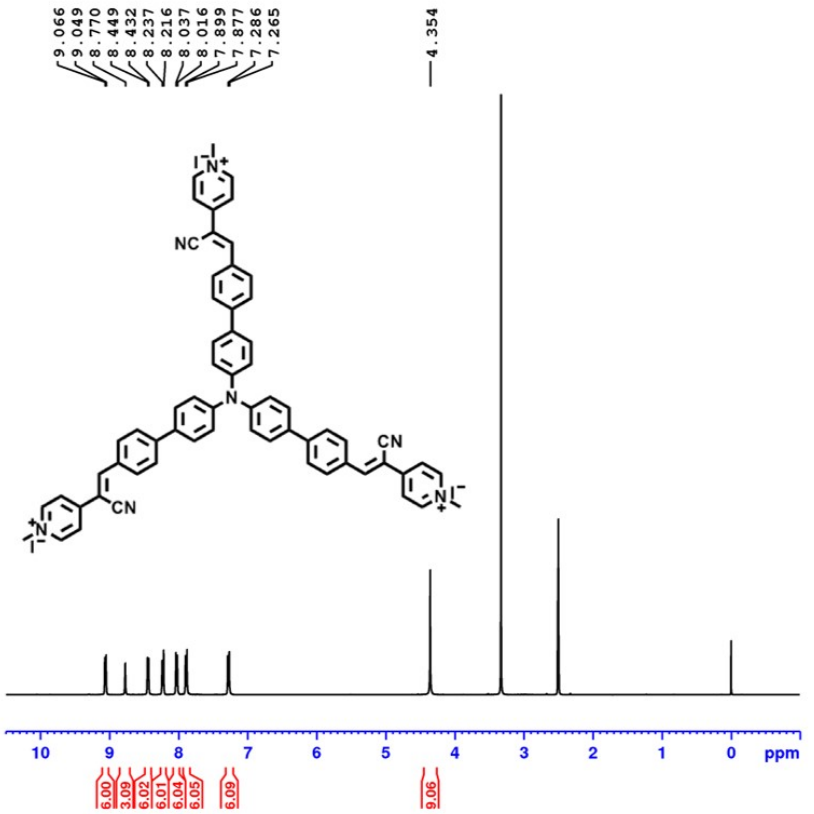

Figure S5. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, ppm) of compound **TPA-3PyA+**.

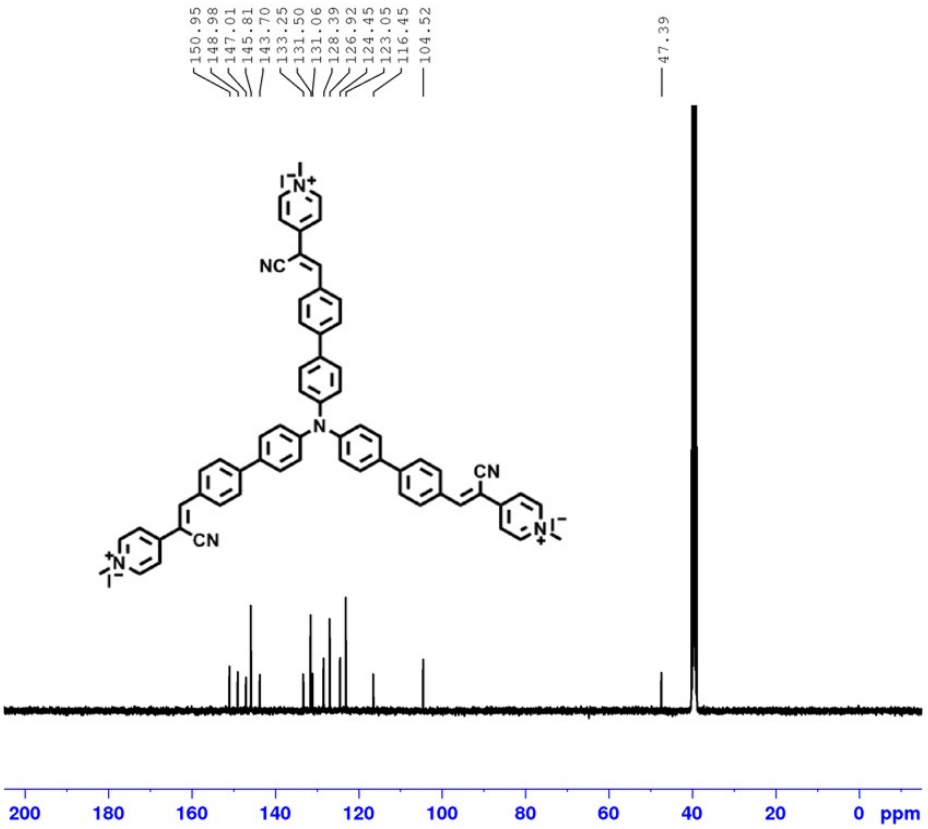

Figure S6. ^{13}C NMR spectrum (100 MHz, DMSO-d_6, ppm) of compound **TPA-3PyA+**.

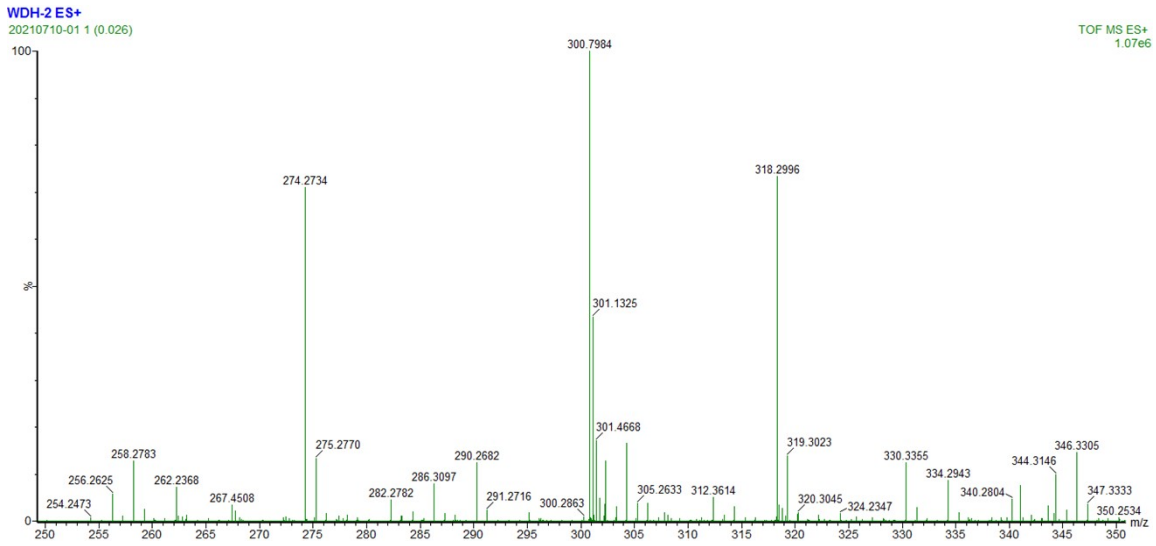

Figure S7. HRMS spectrum of compound **TPA-3PyA+**.

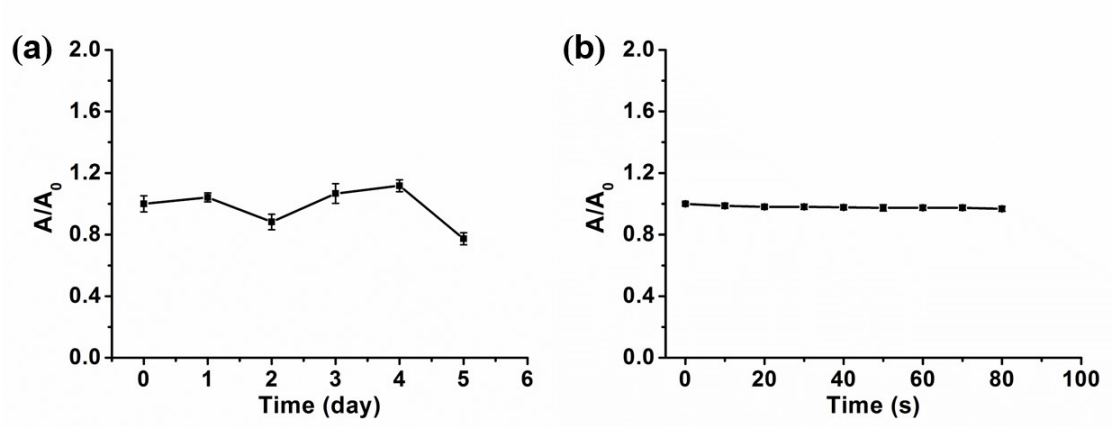

Figure S8. Stability of **TPA-3PyA+** in DMSO at room temperature: (a) in dark; (b) under white light irradiation (50 mW cm^{-2}). A_0 is the initial absorbance at 487 nm and A is the absorbance at 487 nm at different times.

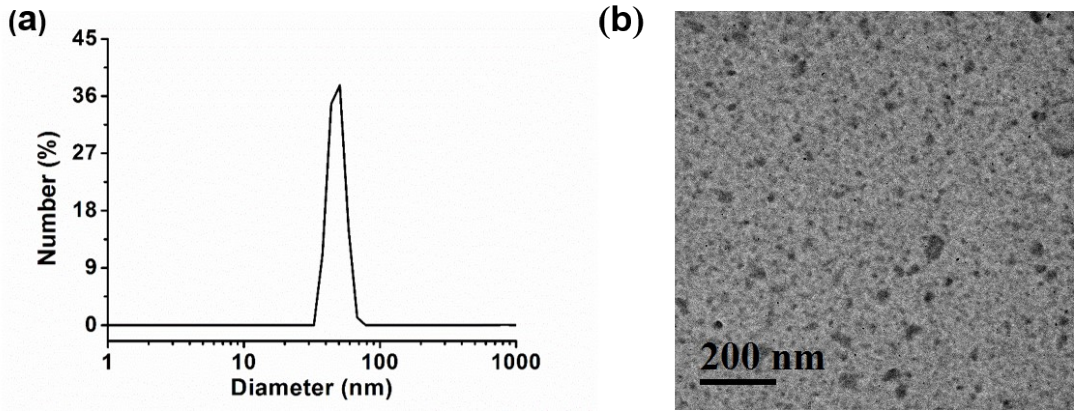

Figure S9. (a) Size distribution of **TPA-3PyA+** (10 μM) in aqueous solution. Nanoaggregates of **TPA-3PyA+** can be formed in this solvent system and the hydrodynamic diameter is about 48 nm. (b) TEM image of nanoaggregates of **TPA-3PyA+**. Scale bar: 200 nm. The image shows that nanoaggregates of **TPA-3PyA+** have spherical morphology.

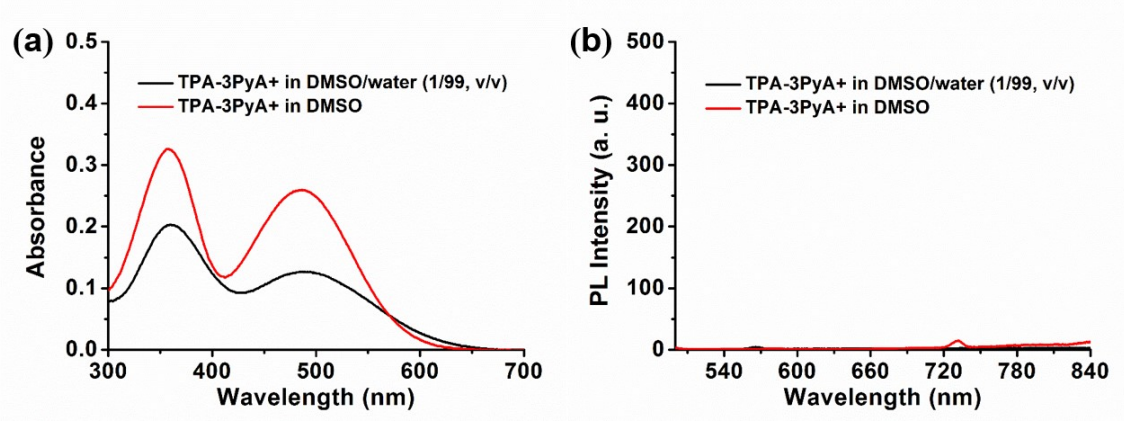

Figure S10. (a) UV-vis absorption spectra of **TPA-3PyA+** (5 μM) in DMSO or DMSO/water mixtures (1/99, v/v); (b) Photoluminescence (PL) spectra of **TPA-3PyA+** (10 μM) in DMSO or DMSO/water mixtures (1/99, v/v).

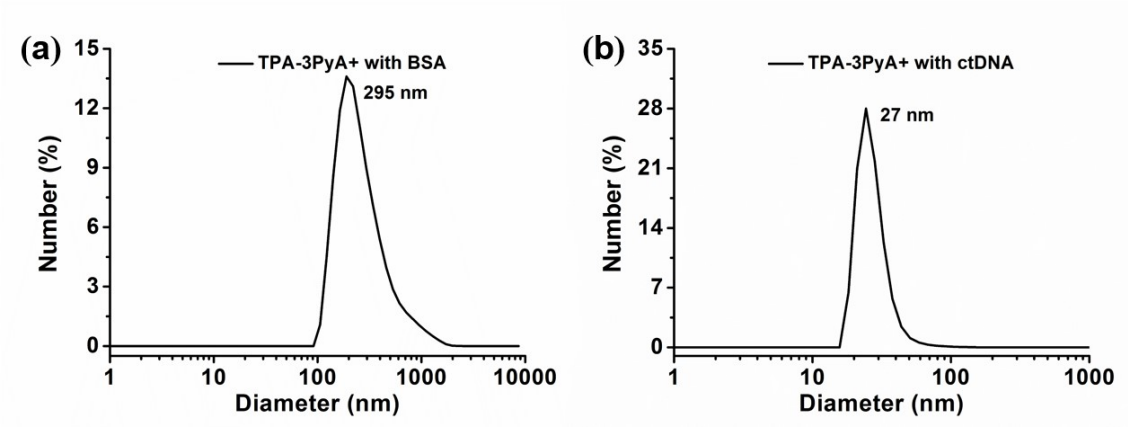

Figure S11. Size distribution of **TPA-3PyA**+ with BSA (a) or ctDNA (b) in aqueous solution.

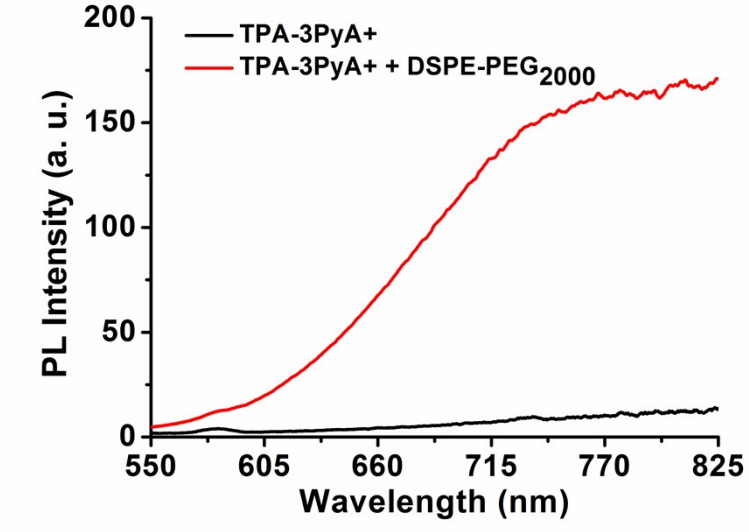

Figure S12. PL spectra of **TPA-3PyA**+ (20 μM) in DMSO/water (1/99, v/v) with DSPE-PEG_{2000} (200 μg mL^{-1}).

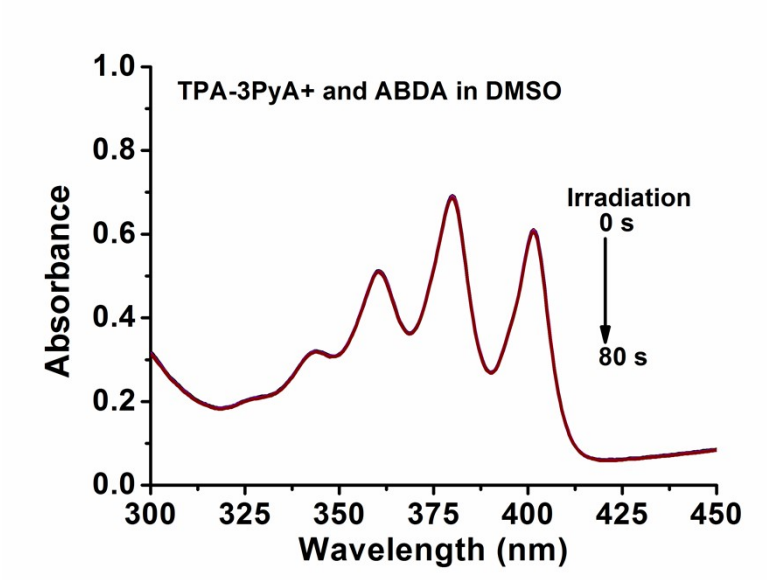

Figure S13. UV-vis spectra of **ABDA** in the presence of **TPA-3PyA+** in DMSO under white light irradiation (50 mW cm^{-2}). [**ABDA**] = 50 μM; [**TPA-3PyA+**] = 2 μM. Time interval for acquiring the UV-vis spectra: 10 s.

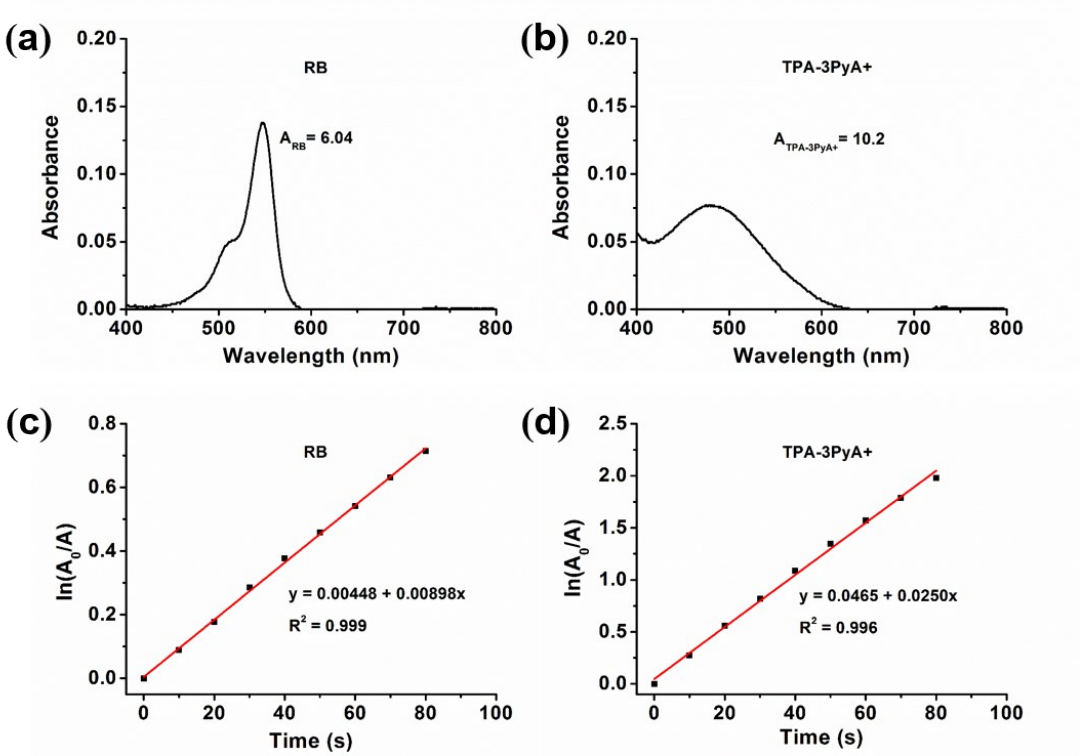

Figure S14. UV-vis spectra of **RB** (a) and **TPA-3PyA+** (b) in DMSO/water mixtures (1/99, v/v); The degradation rate constants of **ABDA** by **RB** (c) and **TPA-3PyA+** (d). [**ABDA**] = 50 μM; [**TPA-3PyA+**] = [**RB**] = 2 μM.

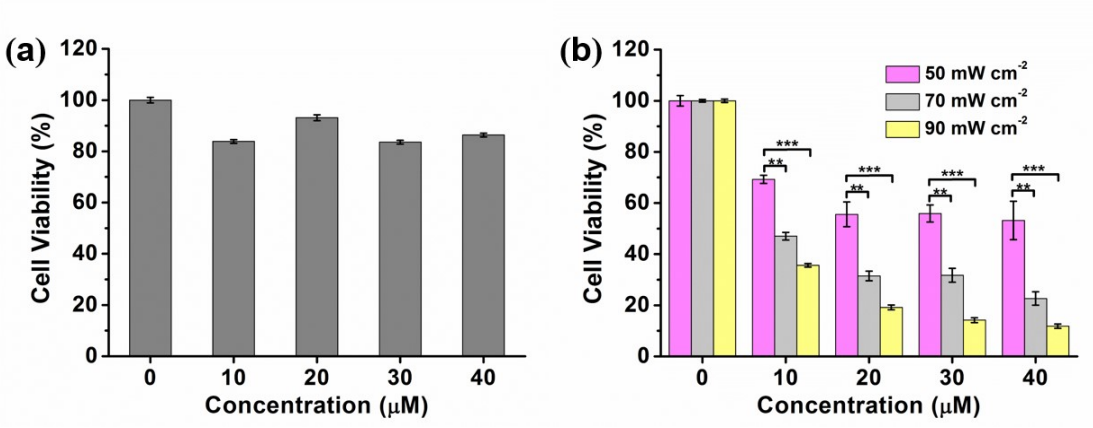

Figure S15. (a) Cell viability of SCC-7 cells treated with different concentrations of **TPA-3PyA+** in dark. (b) Cell viability of SCC-7 cells treated with different concentrations of **TPA-3PyA+** under various doses of light irradiation (The experiments were repeated three times. *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

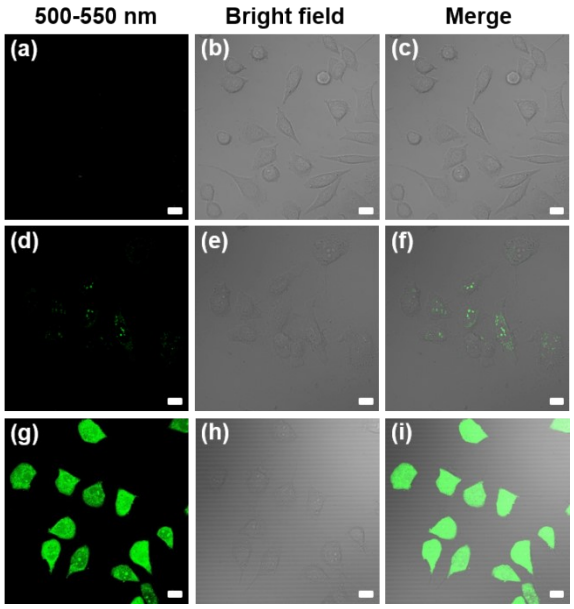

Figure S16. Detection of intracellular ROS generation in HeLa cells under white light irradiation (50 mW cm^{-2}, 30 min): (a-c) HeLa cells treated with DCFH-DA (10 μM); (d-f) HeLa cells treated with **TPA-3PyA+** (40 μM); (g-i) HeLa cells treated with **TPA-3PyA+** (40 μM) and DCFH-DA (10 μM). Scale bar: 15 μm.

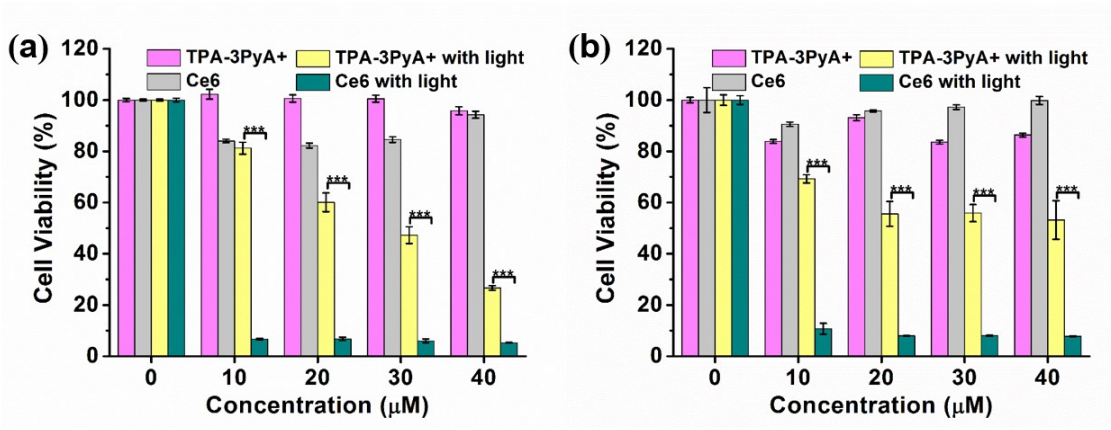

Figure S17. Cell viability of cells treated with different concentrations of **TPA-3PyA+** or Ce6 in dark or under white light irradiation (50 mW cm^{-2}, 30 min): (a) HeLa cells; (b) SCC-7 cells. The experiments were repeated three times. *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

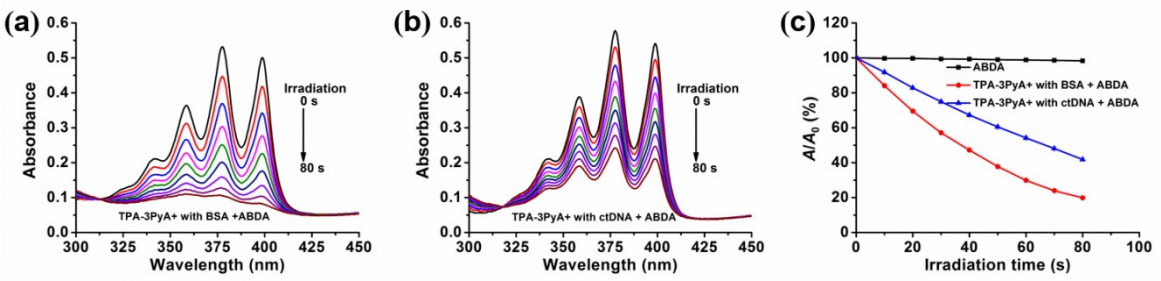

Figure S18. UV-vis spectra of **ABDA** under white light irradiation (50 mW cm^{-2}) in DMSO/water mixture (1/99, v/v): (a) **TPA-3PyA+ with BSA + ABDA** ; (b) **TPA-3PyA+ with ctDNA + ABDA**. (c) Degradation rates of **ABDA** in the presence of different PSs under white light irradiation, where A_0 and A are the absorbances of **ABDA** at 378 nm before and after irradiation, respectively. [**ABDA**] = 50 μM; [**TPA-3PyA+**] = 2 μM; [**BSA**] = 27.5 μg mL^{-1}; [**ctDNA**] = 4 μg mL^{-1}. Time interval for acquiring the UV-vis spectra: 10 s.

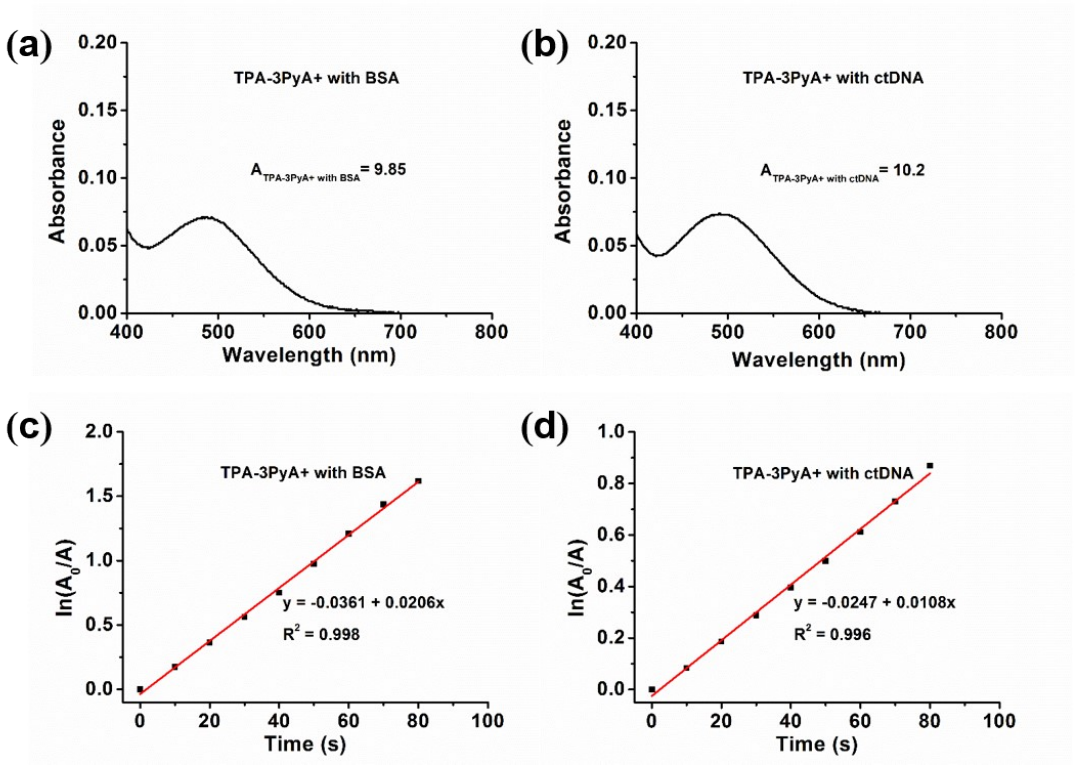

Figure S19. UV-vis spectra of **TPA-3PyA+ with BSA** (a) and **TPA-3PyA+ with ctDNA** (b) in DMSO/water mixtures (1/99, v/v); The degradation rate constants of **ABDA** by **TPA-3PyA+ with BSA** (c) and **TPA-3PyA+ with ctDNA** (d). [**ABDA**] = 50 μM; [**TPA-3PyA+**] = 2 μM; [**BSA**] = 27.5 μg mL^{-1}; [**ctDNA**] = 4 μg mL^{-1}.

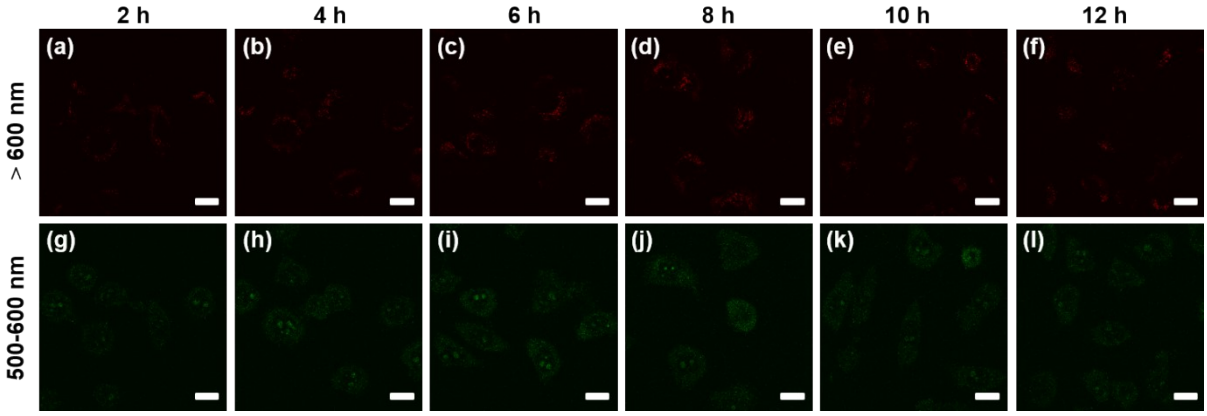

Figure S20. Confocal images of HeLa cells stained with 40 μM **TPA-3PyA+** at different time. NIR channel: > 600 nm; Green channel: 500-600 nm. Scale bar: 15 μm.

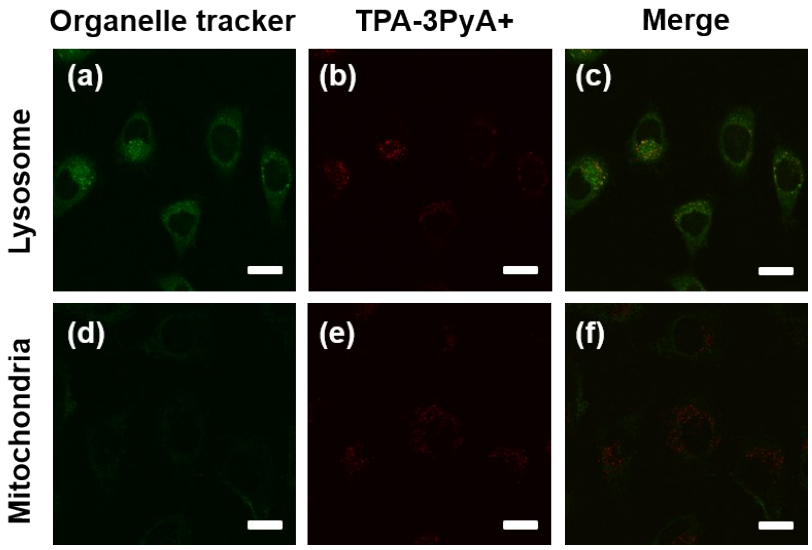

Figure S21. Lysosome and mitochondria colocalization imaging of HeLa cells stained with 1 μM Lyso-Tracker Green (a), 40 μM **TPA-3PyA+** (b and e), 200 nM Mito-Tracker Green (d). (c) Merged images of (a) and (b). (f) Merged images of (d) and (e). Scale bar: 15 μm.

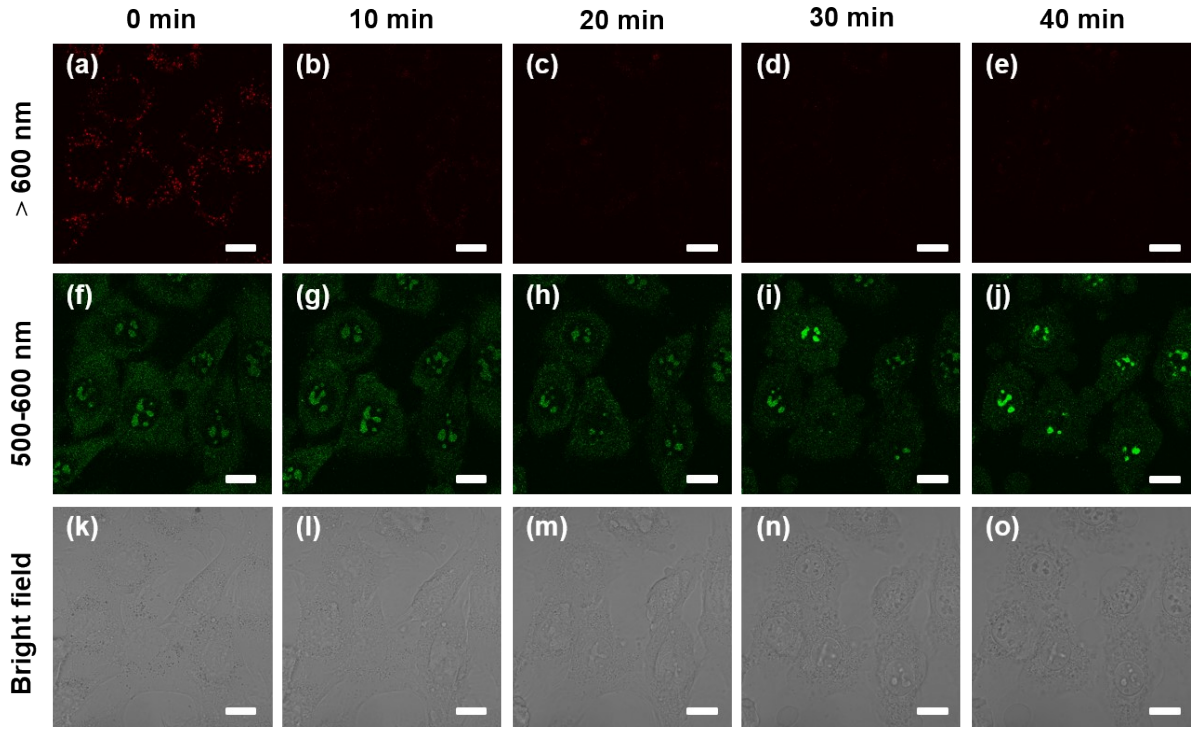

Figure S22. Real-time confocal imaging of SCC-7 Cells under continuous laser irradiation (λ_{ex} = 488 nm, 4% laser power) stained with 40 μM **TPA-3PyA+**. NIR channel: > 600 nm; Green channel: 500-600 nm. Scale bar: 15 μm.

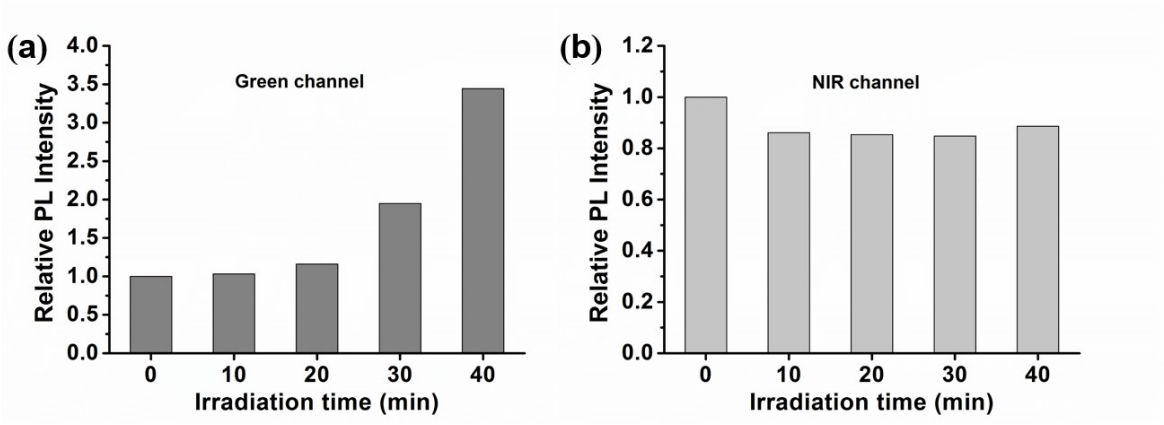

Figure S23. Relative PL intensity of **TPA-3PyA+** (40 μM) in HeLa cells at different irradiation times (λ_{ex} = 488 nm, 4% laser power).

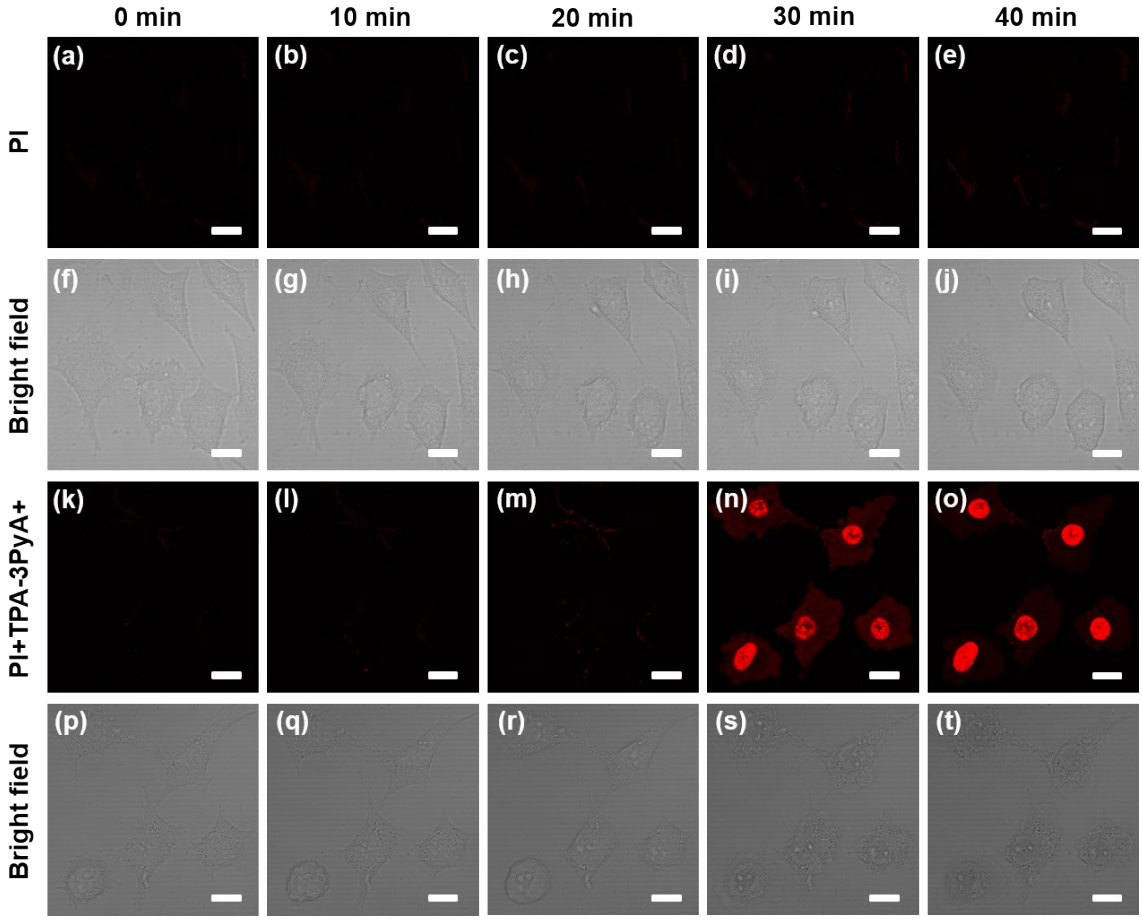

Figure S24. Real-time confocal imaging of HeLa Cells under continuous laser irradiation (λ_{ex} = 488 nm, 4% laser power): (a-j) the cells incubated with PI (100 μg mL^{-1}); (k-t) the cells co-incubated with **TPA-3PyA+** (40 μM) and PI (100 μg mL^{-1}). The signal of PI (569-619 nm) was obtained under excitation at 561 nm. Scale bar: 15 μm.

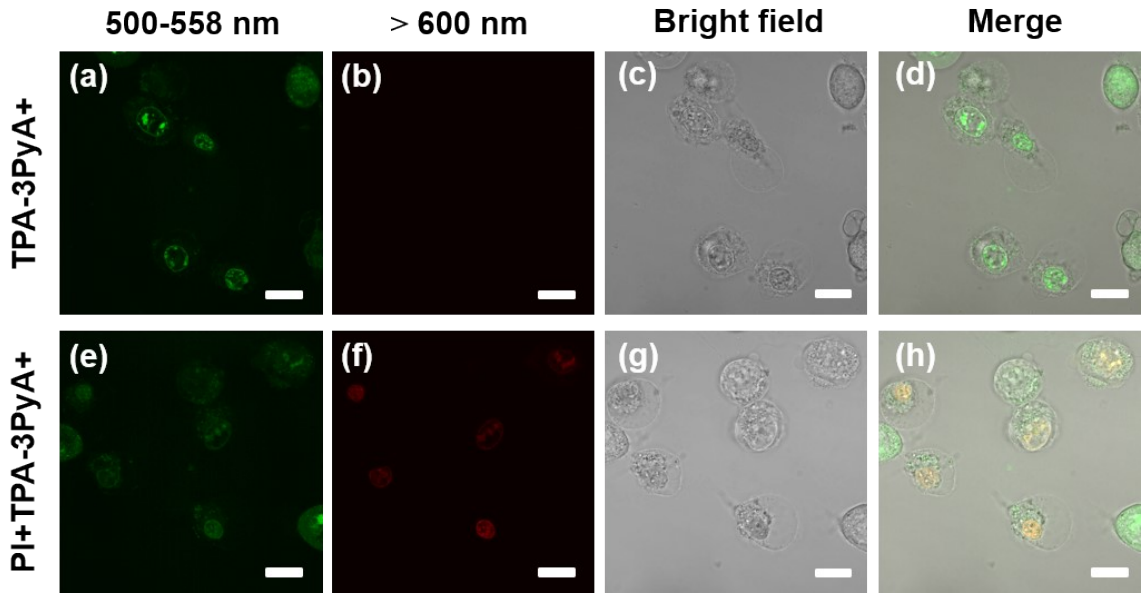

Figure S25. Confocal images of SCC-7 cells after white light irradiation (70 mW cm^{-2}, 30 min): (a-d) the cells incubated with **TPA-3PyA+** (40 μM); (e-h) the cells co-incubated with **TPA-3PyA+** (40 μM) and PI (100 μg mL^{-1}). Scale bar: 15 μm.

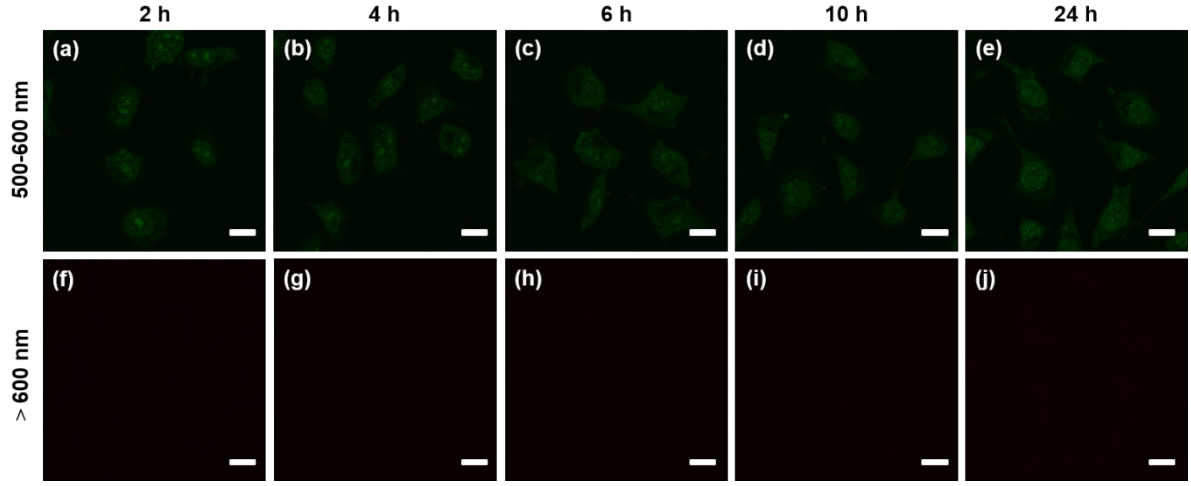

Figure S26. Confocal images of fixed HeLa cells stained with 20 μM **TPA-3PyA+** at different time. NIR channel: > 600 nm; Green channel: 500-600 nm. Scale bar: 15 μm.

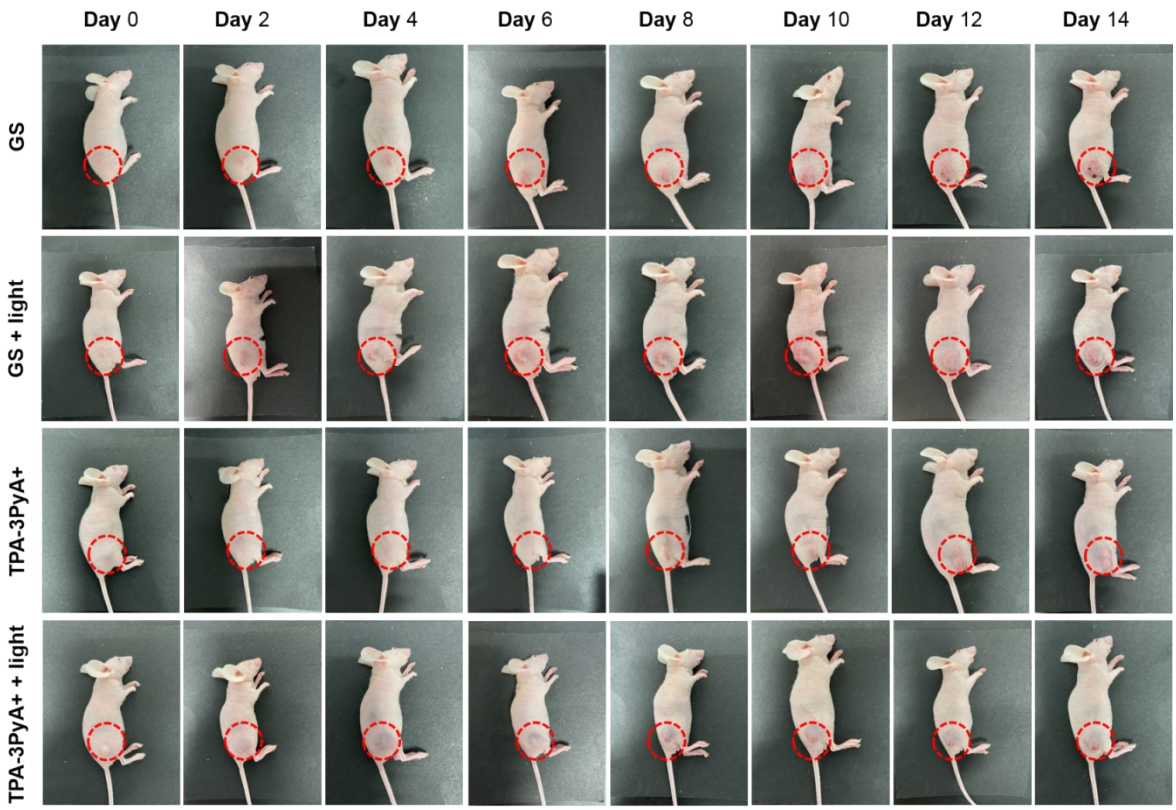

Figure S27. Photos of mice from different groups at 0, 2, 4, 6, 8, 10, 12 and 14 days post-treatment.

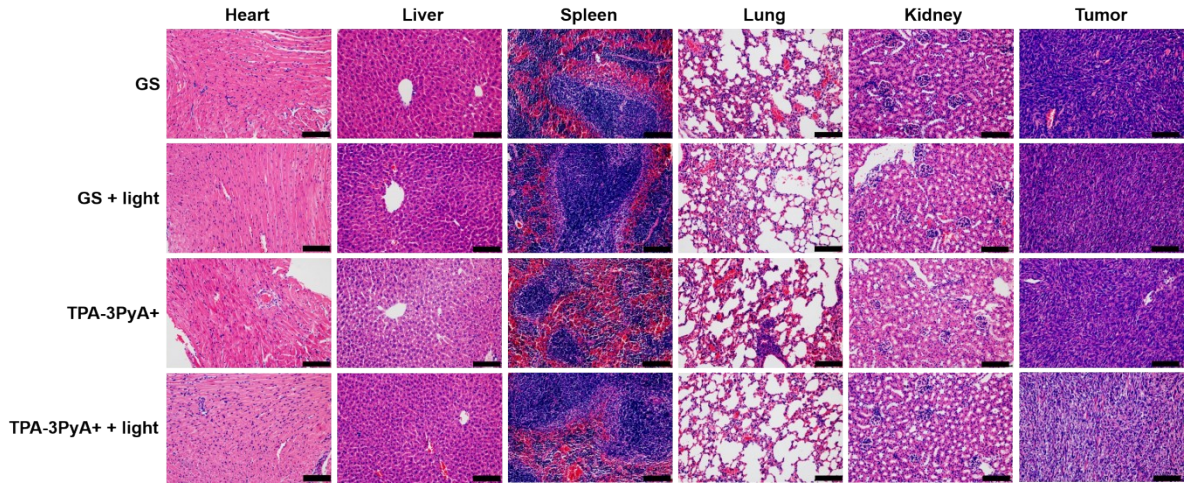

Figure S28. H&E staining of various organ and tumor slices from different groups after corresponding 14 days of treatment. Scale bar: 100 μm.

Tables

Table S1. Fluorescence quantum yields and singlet oxygen quantum yields of **TPA-3PyA+** in various conditions.

Conditions	A	B	C	D
Fluorescence quantum yield (Φ_F)[a)]	N/A	N/A	1.14%	1.71%
Singlet oxygen quantum yield (Φ_T)[b)]	N/A	124%	106%	53%

a) Fluorescence quantum yield determined by a calibrated integrating sphere; b) Singlet oxygen quantum yield was measured using 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) as an $^1O_2$ indicator and a commercial photosensitizer Rose Bengal (RB) as the reference (Φ_{RB} = 75% in water); Condition A: **TPA-3PyA+** in DMSO; Condition B: **TPA-3PyA+** in DMSO/water (1/99, v/v); Condition C: **TPA-3PyA+** with BSA in DMSO/water (1/99, v/v); Condition D: **TPA-3PyA+** with ctDNA in DMSO/water (1/99, v/v).

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