SUPPORTING INFORMATION

A dual functional AEE fluorogen as a mitochondrial-specific bioprobe and an effective photosensitizer for photodynamic therapy

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Experimental Section.

Materials and Methods

PI and H2DCF-DA were purchased from Sigma-Aldrich and used as received. Minimum essential medium (MEM), fetal bovine serum (FBS), penicillin and streptomycin and MitoTracker® Red FM were purchased from Invitrogen. Pierce™ LDH cytotoxicity Assay Kit was purchased from Thermo Scientific. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl immediately prior to use. $AgBF_4$ was purchased from J&K Scientific. 4-bromobenzophenone, diphenylmehane, *n*-butyl lithium, diphenylacetylene propylamine, dimethylsulfoxide (DMSO), $Cu(OAc)_2$ and other chemicals and solvents were all purchased from Aldrich and used as received without further purification. The rhodium complex $[RhCp^*Cl_2]_2$, named 1,2,3,4-tetramethylcyclopentadienylrhodium (III) chloride dimer, was prepared following the reported procedures.[1,2]

^1H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using chloroform-*d* as solvent and tetramethylsilane (TMS) as internal reference. High-resolution mass spectra (HRMS) were recorded on a Finnigan MAT TSQ 7000 Mass Spectrometer System operating in a MALDI-TOF mode. UV absorption spectra were taken on a Milton Ray Spectronic 3000 array spectrophotometer. Particle sizes were measured on a Zeta potential analyzer (Brookhaven, ZETAPLUS) Photoluminescence (PL) spectra were recorded on a Perkin-Elmer spectrofluorometer LS 55.

Synthesis

A sealed tube containing $[RhCp^*Cl_2]_2$ (2.0 mol %), $AgBF_4$ (0.30 mmol), $Cu(OAc)_2$ (0.30 mmol), aryl aldehyde **1** (0.36 mmol) and internal alkyne **2** (0.30 mmol) was evacuated and purged with nitrogen gas three times. Then, propylamine **3** (0.45 mmol) and *t*-amyl alcohol (2.5 ml) were sequentially added to the system via syringe under a nitrogen atmosphere and the reaction mixture was allowed to stir at 110 ℃ for 3 h. When the reaction was complete, the mixture was cooled and diluted with CH_2Cl_2 (10 mL). The mixture was filtered through a Celite pad and the Celite pad was washed with CH_2Cl_2 (30 mL) and MeOH (20 mL). The combined filtrate was concentrated in vacuo and the residue was purified by alumina column chromatography using CH_2Cl_2/MeOH (100:1 v/v) as eluent to give pure product TPE-IQ as a greenish-yellow solid in 85% yield. ^1H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 9.97 (s, 1H), 8.47 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.37 – 7.28 (m, 4H), 7.15 (m, 14H), 6.95 (d, J = 6.7 Hz, 2H), 6.90 (t, J = 7.3 Hz, 4H), 6.57 (d, J = 7.3 Hz, 2H), 4.48 (t, J = 7.6 Hz, 2H), 1.85 (m, 2H), 0.84 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (TMS, ppm) 153.90, 149.95, 145.99, 143.82, 142.56, 141.95, 139.22, 138.83,

137.13, 134.60, 132.88, 131.58, 131.54, 131.38, 131.30, 130.97, 130.47, 130.28, 128.95, 128.79, 128.68, 128.52, 128.47, 127.96, 127.64, 127.57, 126.22, 60.89, 25.41, 10.91. ^{11}B NMR (128 MHz, $CDCl_3$) δ: -0.81. ^{19}F NMR (376 MHz, $CDCl_3$): δ -151.98. HRMS (MALDI-TOF): *m*/*z* (cation) 578.2854 [M^+, calcd 578.2842]; *m*/*z* (anion) 87.0018 [M^+, calcd 87.0035].

Cell Culture and Imaging

Cell Culture. HeLa cells were cultured in the MEM containing 10% FBS and antibiotics (100 units/mL penicillin and 100 g/mL streptomycin) in a 5% CO_2 humidity incubator at 37 ^{o}C.

Cell Imaging. HeLa cells were grown overnight on a 35 mm petri dish with a cover slip or a plasma-treated 25 mm round cover slip mounted to the bottom of a 35 mm petri dish with an observation window. The live cells were incubated with 200/500/1000 nM of TPE-IQ for 1/10min. In a typical experiment, 2 μL of a 10 mM stock solution of TPE-IQ in DMSO were diluted to 1 mL with cell culture medium, followed by further dilution to desired concentration. For co-staining, the cells were stained with TPE-IQ (200 nM) for 15 min and then MT (50 nM) for 15min. The cells were imaged under an FL microscope (BX41 Microscope) using different combination of excitation and emission filters for each dye: for TPE-IQ, excitation filter = 330–385 nm, dichroic mirror = 400 nm, and emission filter = 420 nm long pass; for MT, excitation filter = 540–580 nm, dichroic mirror = 600 nm, and emission filter = 610 nm long pass.

For laser-scanning confocal microscope mitochondria imaging, the image was collected using 405 nm laser as excitation light, while the spectral collection region was 420–585 nm. For the ROS generation experiment, the cells were firstly incubated with TPE-IQ (1 μM) and H2DCF-DA (1 μM) for 10 min. All the images were collected using 488 nm laser as excitation light and the spectral collection region is 497-579 nm.

For PI staining experiment, after 15 min incubation with the TPE-IQ, the cells were exposed to UV light under the FL microscope for 2 min under the 4× objective, while the control group was put in dark. Both the experimental and the control group were further incubated in dark for 12 h, after which the cells were incubated with 1.5 μM PI for 10 min and imaged with Nikon Eclipse TE2000-U fluorescence microscope. Excitation filter = 510–560 nm, dichroic mirror = 570 nm, and emission filter = 590 nm long pass.

Cytotoxicity Evaluated by LDH Assay. The optimal HeLa concentration was determined to be 5000/ well in a 96-well plate, following the standard method given by Thermo Scientific. To decrease the spontaneous LDH activity, culture medium containing 2% FBS was used. 5000 cells were seeded per well in a 96-well plate. After 24 h culture, various concentrations of TPE-IQ were added into the 96-well plate. After 12 h TPE-IQ treatment in the dark, 10 μL of 10X lysis buffer bromide, was added to untreated cells as Maximum LDH Activity, while 10 μL of ultrapure water was added to other wells. After incubation at 37 ^{o}C, 5% CO_2 for 45 min, 50 μL of each sample medium was transferred to another 96-well plate, followed by addition of 50 μL LDH reaction mix. Positive control was included. After 30 min incubation protected from light, 50 μL stop solution was added to each well and the absorbance was read with plate reader, from which the cytotoxicity could be determined.

For the UV-induced cytotoxicity experiment, after 15 min incubation with the TPE-IQ, each well was exposed to UV light under the FL microscope for 2 min under the 4X objective, while the control group was put in dark. Both the experimental and the control group were further incubated in dark for 12 h and the cytotoxicity was evaluated with regular LDH method as mentioned above.

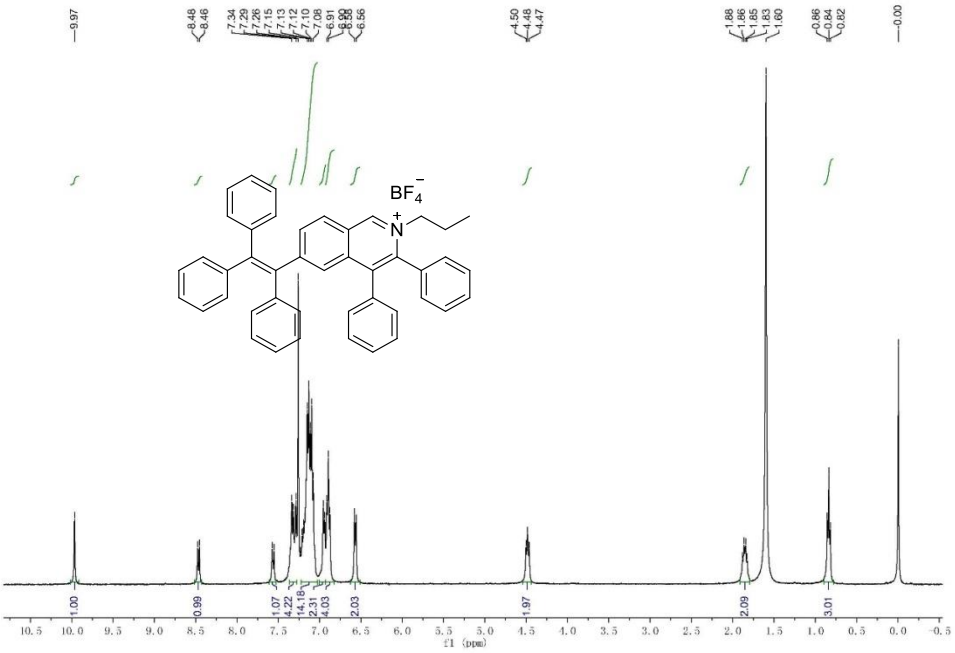

Figure S1. ^{1}H NMR spectra of isoquinolinium salt TPE-IQ in $CDCl_3$.

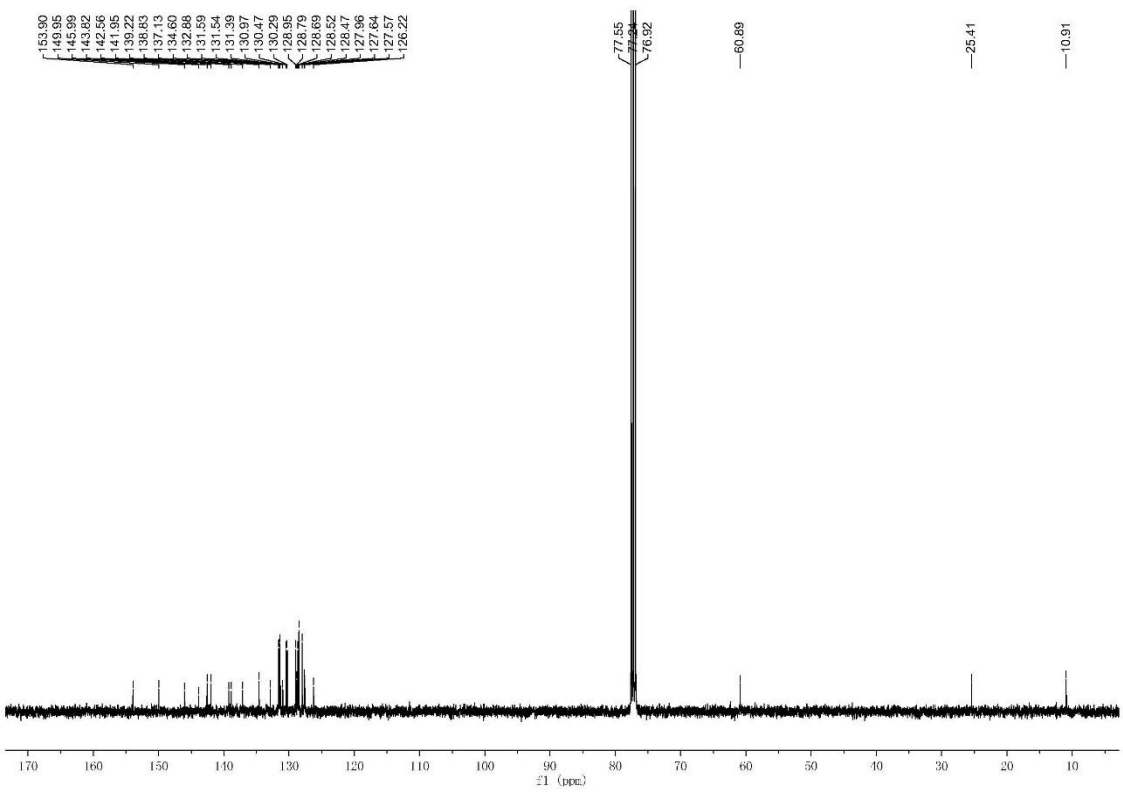

Figure S2. ^{13}C NMR spectra of isoquinolinium salt TPE-IQ in $CDCl_3$.

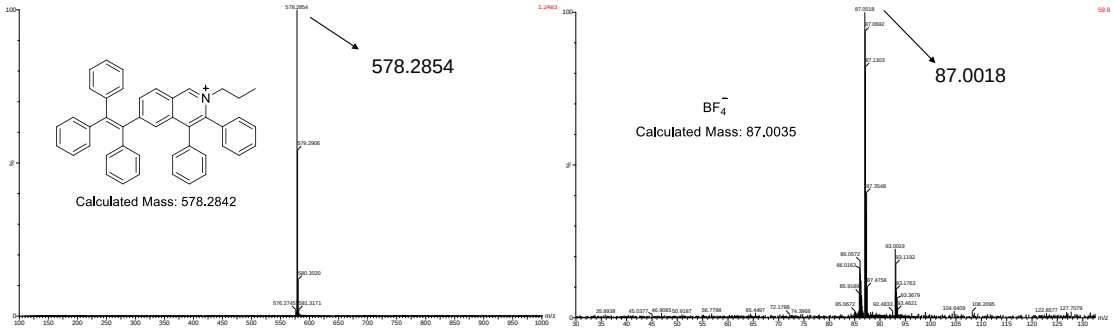

Figure S3. HRMS spectra of (A) the cation and (B) the anion of isoquinolinium salt TPE-IQ .

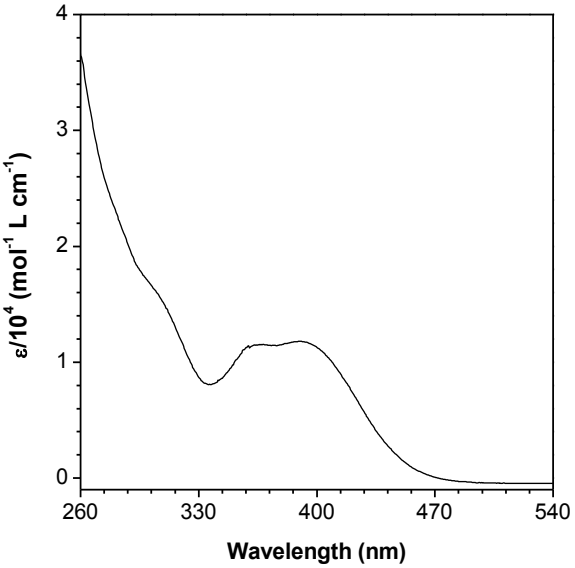

Figure S4. Absorption spectrum of TPE-IQ in DMSO solution. [TPE-IQ] = 10 μM.

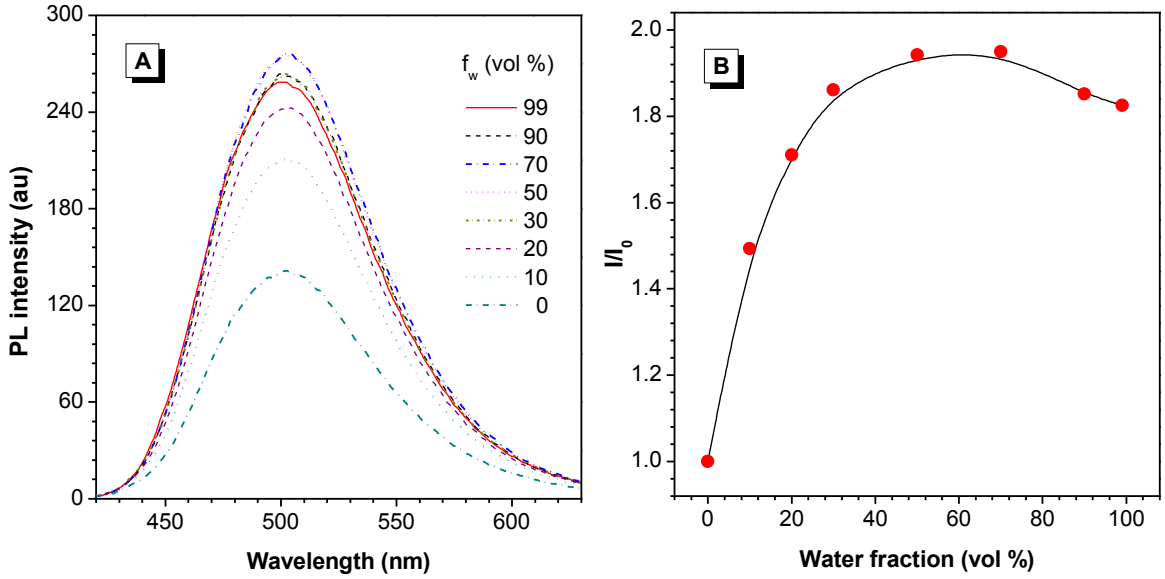

Figure S5. (A) Emission spectra of TPE-IQ in DMSO/water mixtures with different water fraction. (B) Plot of relative emission intensity (I/I_0) of TPE-IQ versus the water fraction of the DMSO/water mixture of TPE-IQ. [TPE-IQ] = 10 μM; excitation wavelength: 390 nm.

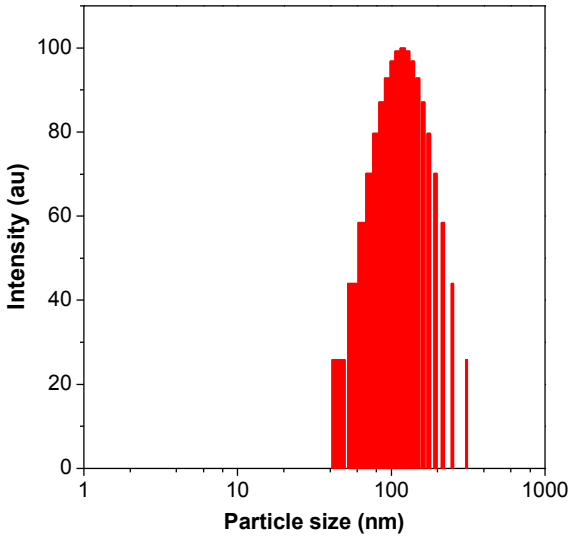

Figure S6. Particle size analysis of TPE-IQ (200 nM) in cell-culture medium.

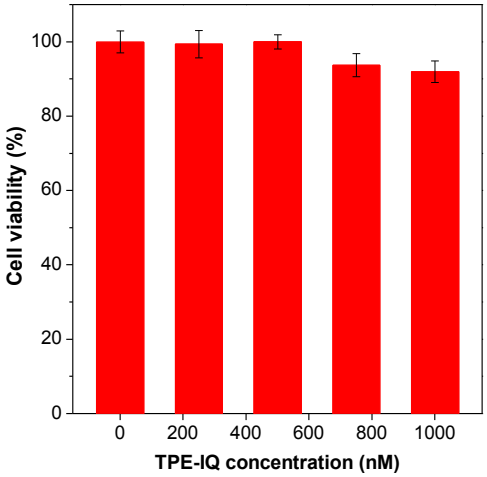

Figure S7. Cell viability assessed by the LDH method. Hela cells are incubated with TPE-IQ for 12 h.

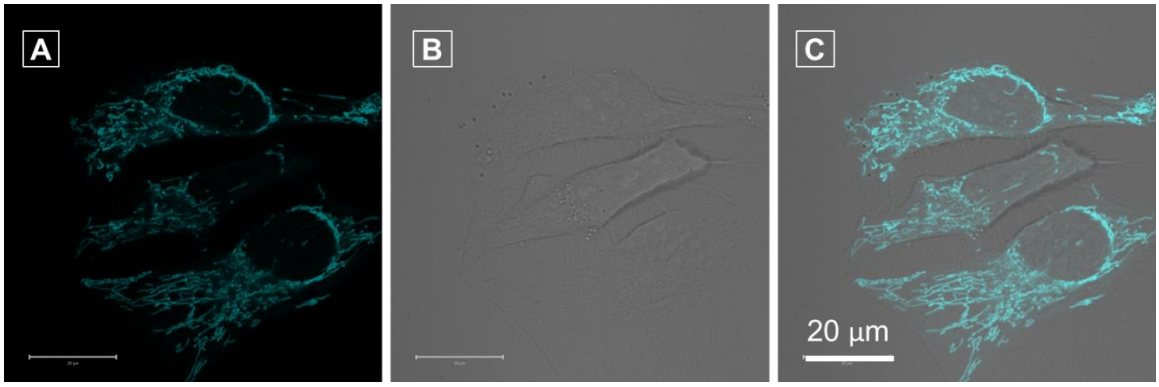

Figure S8. Confocal images of HeLa cells stained with TPE-IQ (200 nM) for 10 min. (A) fluorescence, (B) bright-field and (C) merged images. Excitation wavelength: 405 nm. Emission wavelength: 420–585 nm.

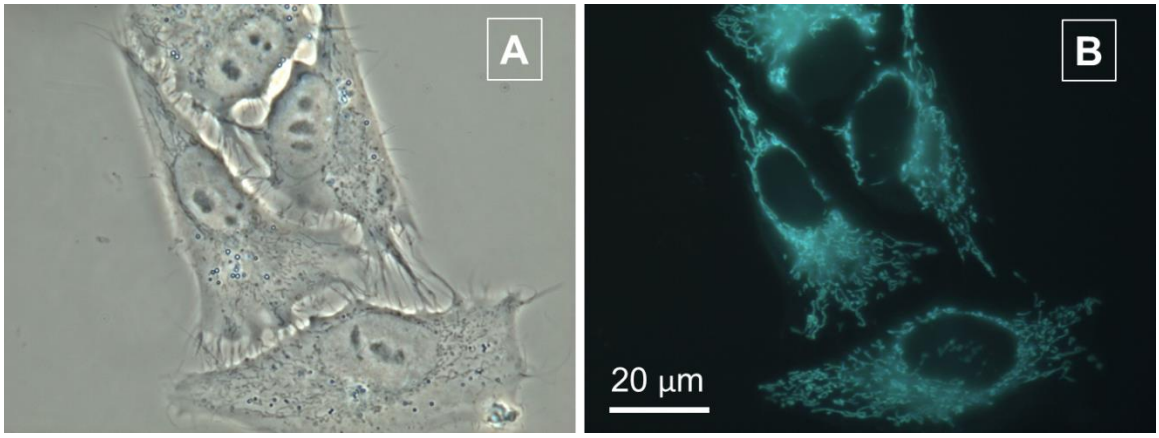

Figure S9. (A) Bright field and (B) fluorescence images of HeLa cell incubated with 500 nM TPE-IQ for 1 min. Excitation wavelength: 330−385 nm. dichroic mirror = 400 nm, and emission filter = 420 nm long pass.

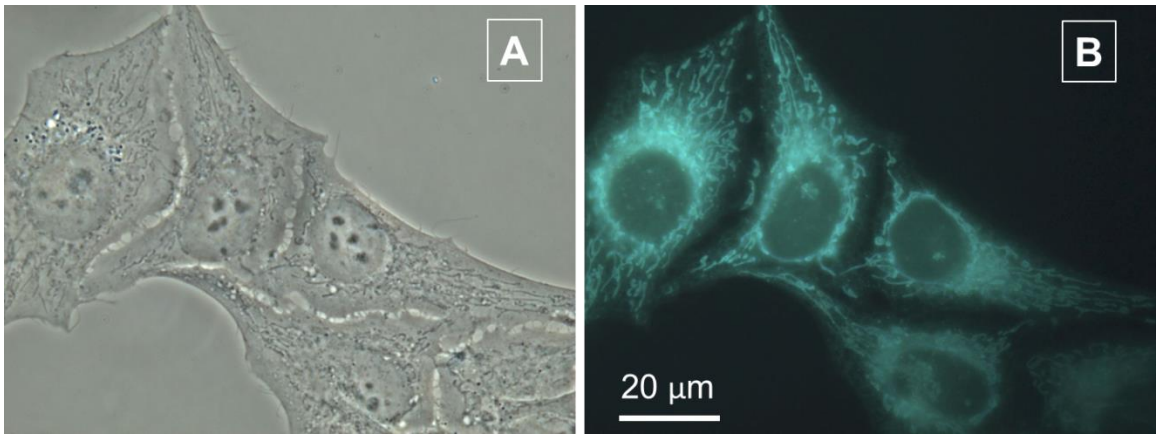

Figure S10. (A) Bright field and (B) fluorescence images of fixed HeLa cells. The cells were pretreated with PFA for 50 min and then incubated with TPE-IQ (500 nM) for 10 min. Excitation wavelength: 330−385 nm. dichroic mirror = 400 nm, and emission filter = 420 nm long pass.

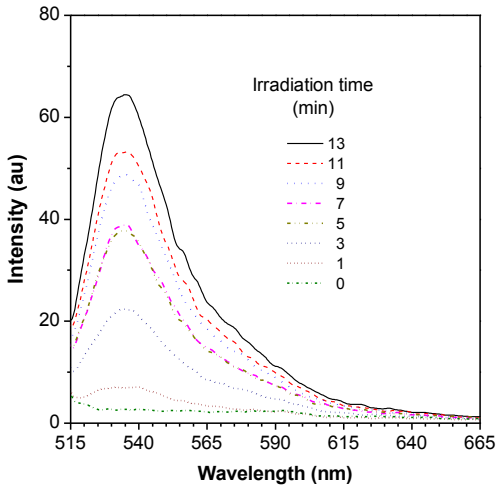

Figure S11. Change in fluorescence spectra of PBS solutions containing TPE-IQ and H2DCF-DA upon UV irradiation for different time. [TPE-IQ]: 10 μM; [H2DCF-DA]: 1 μM. Excitation wavelength: 485 nm.

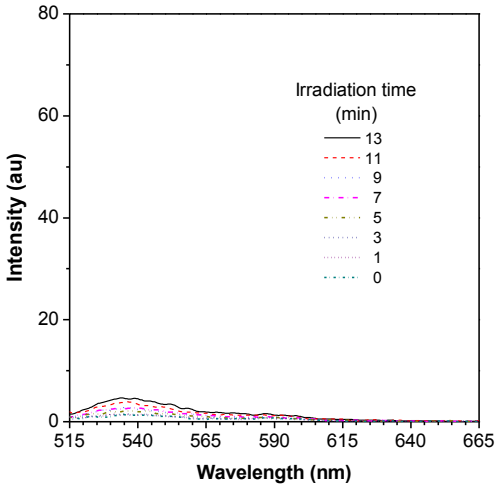

Figure S12. Change in fluorescence spectra of H2DCF-DA upon UV irradiation for different period of time. [H2DCF-DA]: 1 μM. Excitation wavelength: 485 nm.

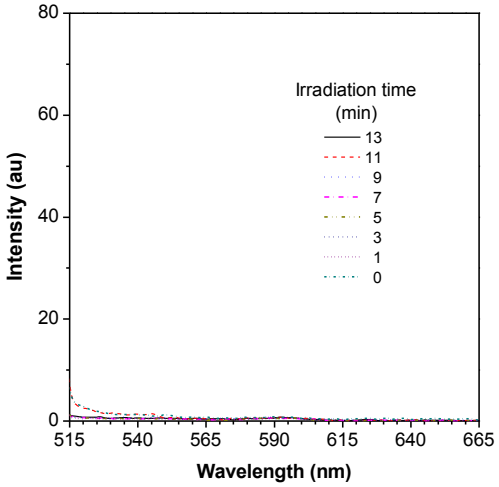

Figure S13. Change in fluorescence spectra of TPE-IQ upon UV lamp irradiation for different period of time. [TPE-IQ]: 10 μM. Excitation wavelength: 485 nm.

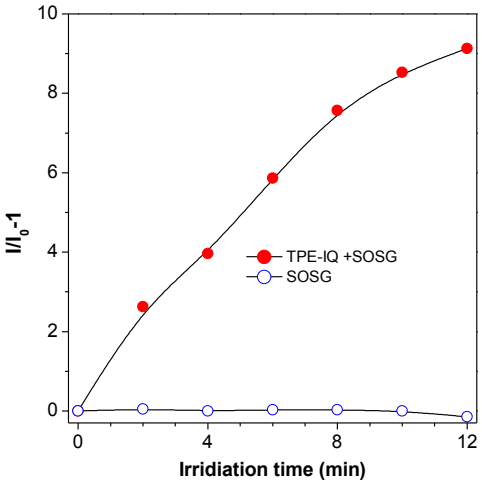

Figure S14. Change in fluorescent intensity at 540 nm of Singlet Oxygen Sensor Green (SOSG) and their mixture in PBS upon UV irradiation (365 nm) for different time. [TPE-IQ] = 10 μM; [SOSG] = 5 μM.

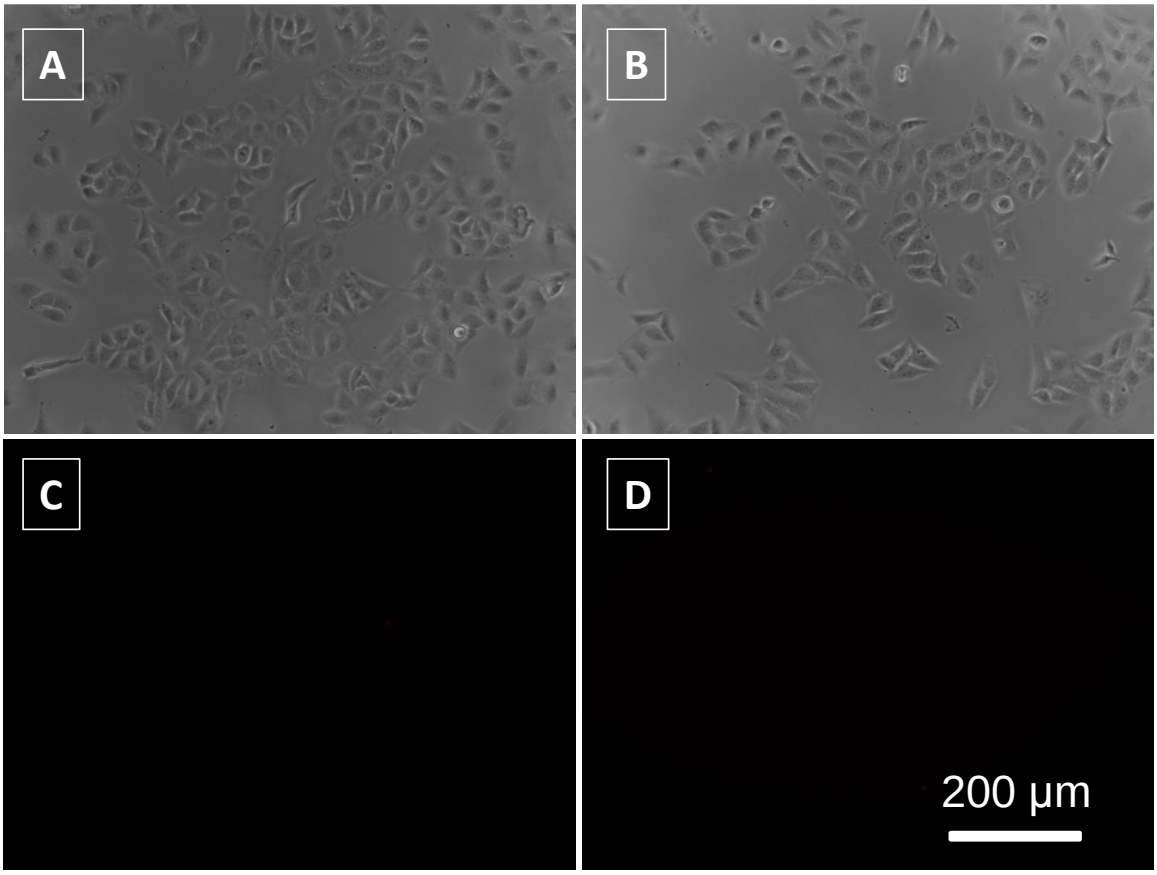

Figure S15. (A, B) Bright field and (C, D) fluorescence images of PI stained (1.5 μM, 10 min) HeLa cells. The cells were pretreated (A, C) without and (B, D) with UV irradiation for 2 min, followed by further incubation at 37 ºC for 12 h.

Reference

1. J. W. Kang, K. Moseley, P. M. Maitlis, *J. Am. Chem. Soc.* 1969, **91**, 5970.

2. M. Gao, J. W. Y. Lam, Y. Liu, J. Li, B. Z. Tang, *Polym. Chem.* 2013, **4**, 2841.