Electronic Supplementary Information

Real-Time the Monitoring Mitophagy Process by A Photostable Fluorescent Mitochondrion-Specific Bioprobe with AIE Characteristic

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(LysoTracker Red DND-99), 50 μg/mL (rapamycin). Excitation wavelength: 540–580 nm. All the images share the same scale bar = 20 μm.

Fig. S11 Confocal images of HeLa cells co-stained with (A) MTR (50 nM) and LysoTracker Green DND-26 (150 nM), and (B) TPE-Py-NCS (5 μM) and LysoTracker Red (150 nM) in the presence of rapamycin (50 mg/mL). Scale bar: 20 μm. S11

Experimental Section

Materials

Triphenylphosphine (PPh_3), carbon disulfide (CS_2) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich and used as received. Minimum essential medium (MEM), fetal bovine serum (FBS), penicillin, streptomycin, LysoTracker Red DND-99 (LTR), LysoTracker Green DND-26 (LTG), MitoTracker Red CMXRos (MTR) and rapamycin were purchased from Life Technologies. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was bought from Sigma. Tetrahydrofuran (THF) and dichloromethane (DCM) were distilled from sodium benzophenone ketyl or calcium hydride, respectively under nitrogen immediately prior to use. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States).

Instruments

^1H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS; $\delta = 0$) was chosen as an internal reference. High-resolution mass spectra (HRMS) were recorded on a Finnigan MAT TSQ 7000 mass spectrometer system operating in a MALDI-TOF mode. Absorption spectra were measured on a Milton Roy Spectronic 3000 Array spectrophotometer. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer with a xenon discharge lamp excitation. Particle size analysis was determined at room temperature on a ZetaPlus Potential Analyzer (Brookhaven Instruments Corporation, USA).

Synthesis of TPE-Py-NCS

TPE-Py-N_3 was synthesized according to our previous literature method.[1] Into a 100 mL two-necked round bottom flask equipped a condenser were added a dry DCM solution (10 mL) of TPE-Py-N_3 (200 mg, 0.30 mmol), PPh_3 (160 mg, 0.6 mmol) and CS_2 (17.4 mL, 10 mmol) under nitrogen. After reflux for 24 h, the mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The residue was purified by a silica gel column chromatography using DCM/acetone mixtures (5:1 v/v) as eluent to furnish a yellow powder in a yield of 84%. ^1H NMR

(400 MHz, DMSO-d_6), δ (ppm): 8.92 (d, 2H), 8.20 (d, 2H), 7.91 (d, 1H), 7.51 (d, 2H), 7.43 (d, 1H), 7.18 (m, 9H), 7.07 (d, 2H), 7.00 (m, 6H), 4.52 (t, 2H), 3.73 (t, 2H), 1.99 (m, 2H), 1.66 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 152.9, 145.6 144.2, 143.0, 142.9, 142.7, 141.6, 140.4, 139.9, 133.3, 131.4, 130.7, 130.6, 128.0, 127.8, 127.8, 126.9, 126.8, 123.8, 123.2, 58.9, 44.2, 27.7, 25.8. HRMS (MALDI-TOF): *m*/*z* 549.2360 (M^+, calcd. 549.2364).

Cell culture

Human cervical cancer cell (HeLa) line was provided by American Type Culture Collection. The HeLa cells were cultured in MEM containing 10% heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 °C in a humidified incubator with 5% CO_2. Before experiment, the cells were pre-cultured until confluence was reached.

Cytotoxicity studies

MTT assays were used to evaluate the cytotoxicity of TPE-Py-NCS. The cells were seeded in a 96-well plate at a density of 5000 cells/well. After 24 h incubation, TPE-Py-NCS with a concentration varying form 0–7.5 μM was added into the 96-well plate. After 24 h treatment, 10 μL of freshly prepared MTT solution (5 mg/mL in PBS) was added into the each well. After 4 h incubation, 100 μL of solubilization solution containing 10% SDS and 0.01 M HCl were added to dissolve the purple crystals. After 8 h incubation, the absorbance of MTT at 595 nm was recorded using a Perkin-Elmer Victor plate reader. The cell viability was expressed by the ratio of absorbance of the cells incubated with TPE-Py-NCS to that of the cells incubated with culture medium only. Each of the experiments was performed at least 6 times.

Cell imaging

The HeLa cells were grown overnight on a 35 mm petri dish with a cover slip. The live cells were incubated with 5 μM of TPE-Py-NCS solution (by adding 2 μL of a 5 mM stock solution of TPE-Py-NCS in DMSO to 2 mL of MEM) or 50 nM of MTR solution (by adding 0.5 μL of a 200 μM stock solution of MT in DMSO to 2 mL of MEM) for 15 min. The dye-labelled cells were rinsed with freshly PBS 3 times and

then fixed by using 4% formaldehyde in PBS for 20 min. After fixation, the cells were washed with acetone 3 times. The cells were imaged under a fluorescence microscope (BX41 Microscope) using different combination of excitation and emission filters for each dye. For TPE-Py-NCS: excitation filter = 330–385 nm, dichroic mirror = 400 nm, and emission filter = 420 nm long pass. For MTR: excitation filter = 540–580 nm, dichroic mirror = 600 nm, and emission filter = 610 nm long pass.

Cell imaging with CCCP treatment

The HeLa cells were grown overnight on a 35 mm petri dish with a cover slip. The cells were incubated with 5 μM of TPE-Py-NCS for 30 min. The dye-labelled cells were then treated with 10 μM of CCCP (by adding 1 μL of a 200 mM stock solution of CCCP in DMSO to 2 mL of MEM) for 30 min.

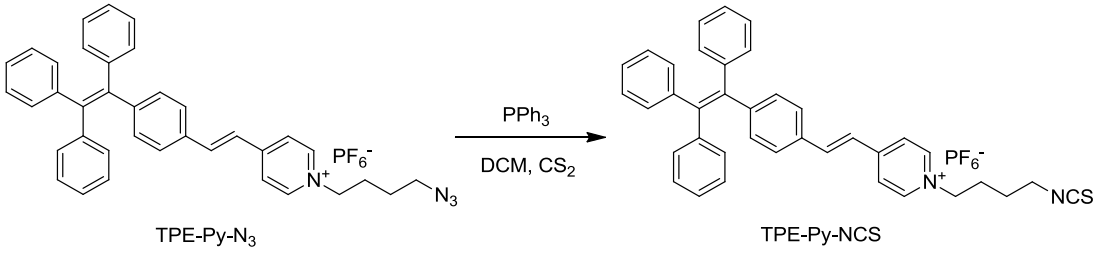
Photostability test

The dye-labelled HeLa cells were imaged by confocal microscope (Zeiss laser scanning confocal microscope LSM7 DUO). Conditions: excitation wavelength = 405 nm and emission filter = 560–620 nm (TPE-Py-NCS); excitation wavelength = 560 nm and emission filter = 580–680 nm (MTR). Laser powers were unified as 50 μW.

Mitophagy induction and monitoring

The HeLa cells were grown overnight on a 35 mm petri dish with a cover slip. The cells were incubated either in TPE-Py-NCS (5 μM) and LTR (150 nM) mixtures or MTR (50 nM) and LTG (150 nM) mixtures for 15 min. The cells were rinsed with PBS for 3 times. Rapamycin (50 μg/mL) was then added to induce mitophagy. Time-dependent confocal images were collected on a laser scanning confocal microscopy. Excitation wavelength: 405 nm (TPE-Py-NCS), 488 nm (LTG) and 560 nm (MTR and LTR); emission filter: 490–580 nm (LTG), 560–620 nm (TPE-Py-NCS and LTR) and 580–620 nm (MTR).

Scheme S1 Synthetic route to TPE-Py-NCS.



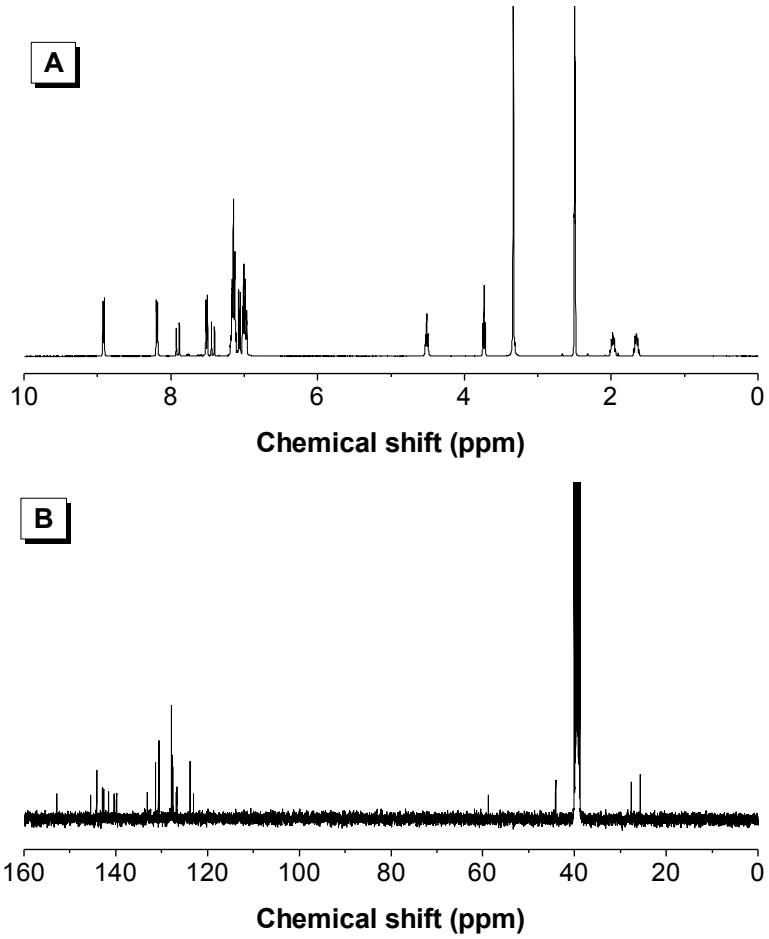


Fig. S1 (A) ^{1}H and (B) ^{13}C NMR spectra of TPE-Py-NCS in DMSO-d_6.

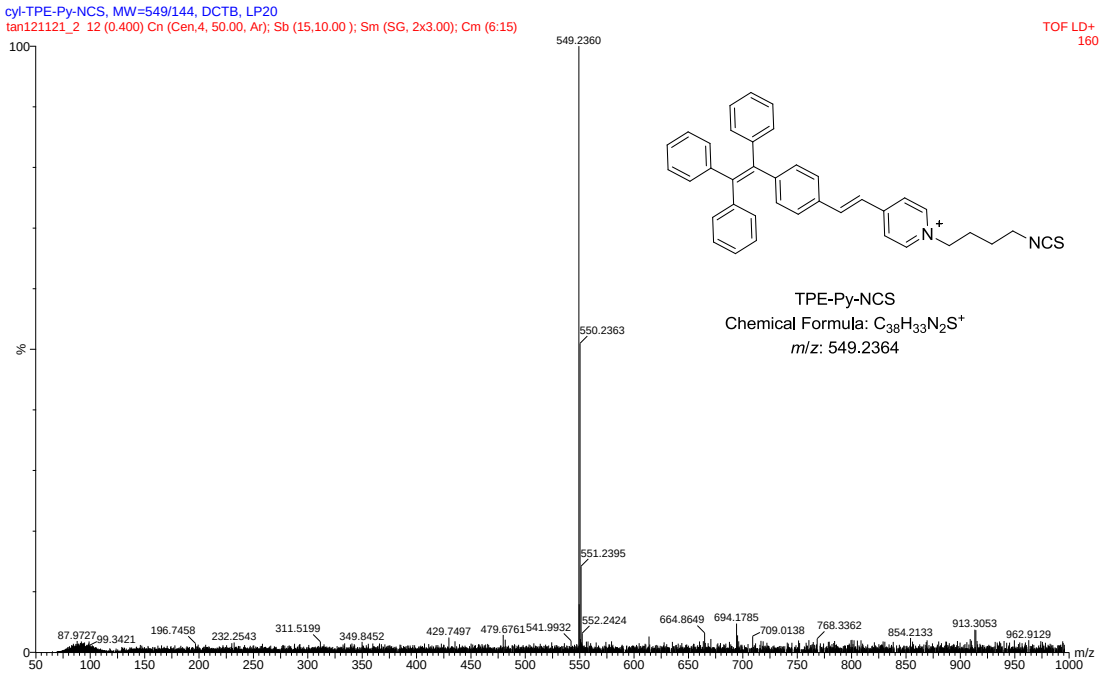


Fig. S2 High resolution mass spectrum of TPE-Py-NCS.

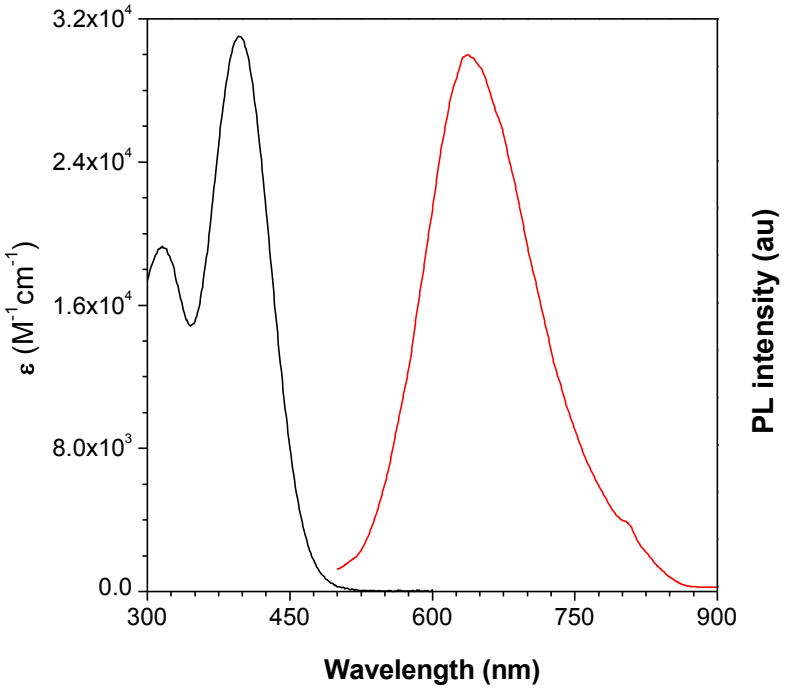


Fig. S3 Absorption and PL spectra of TPE-Py-NCS in DMSO. Concentration: 10 μM.

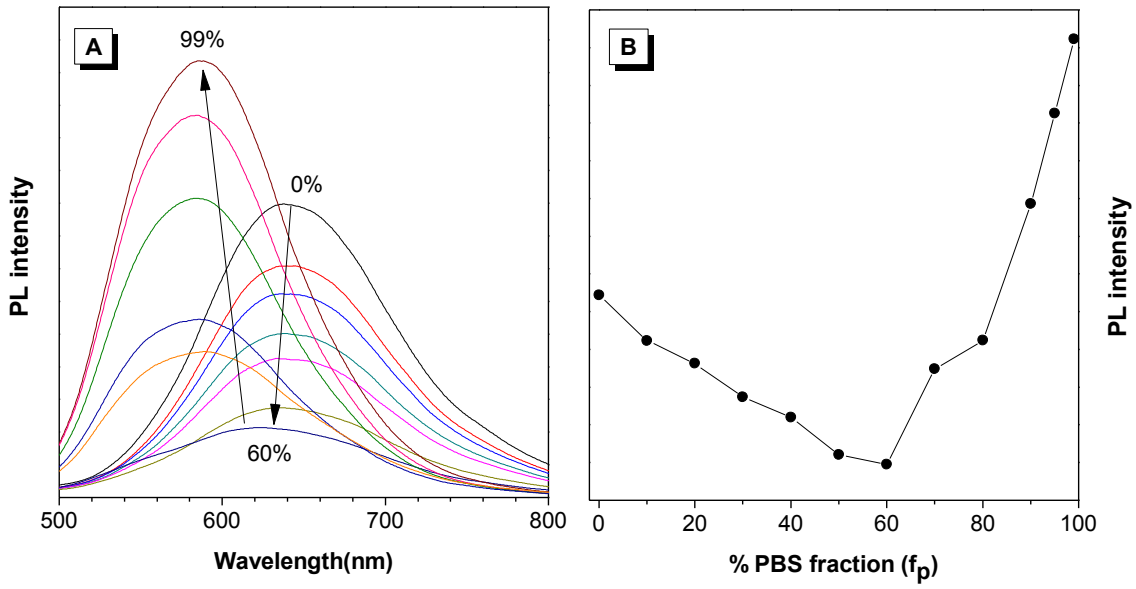


Fig. S4 PL spectra of TPE-Py-NCS in DMSO/PBS mixtures with different PBS fractions (f_p) (B) Plot of PL intensity at 598 nm versus the composition of the DMSO/PBS mixture of TPE-Py-NCS. Concentration: 10 μM; excitation wavelength: 405 nm.

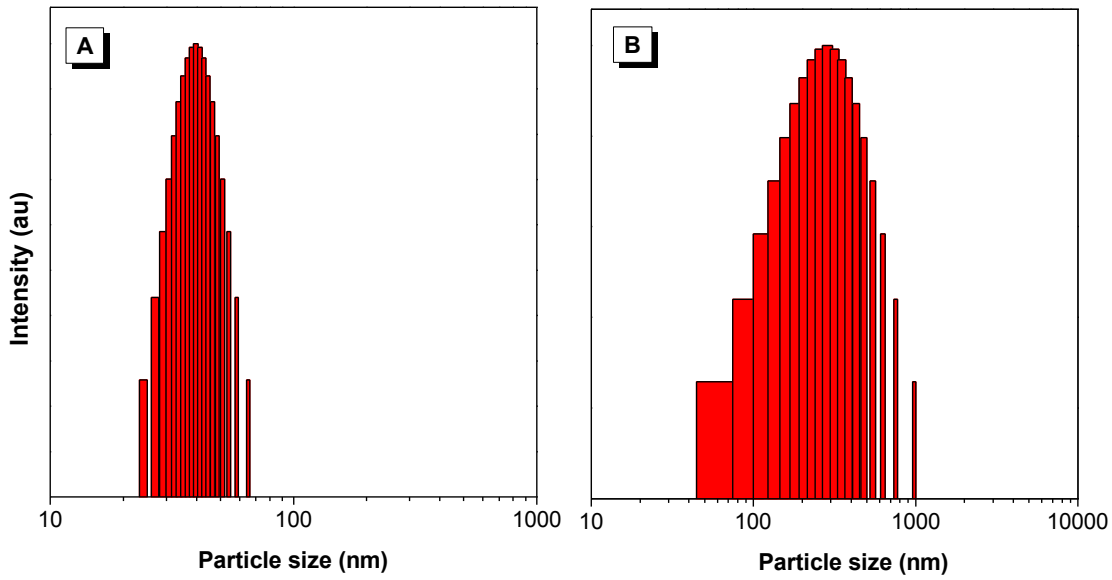


Fig. S5 Size distribution of TPE-Py-NCS aggregates in (A) DMSO/water mixtures with 99% water fraction and (B) DMSO/PBS mixtures with 99% PBS fraction. Concentration: 10 μM.

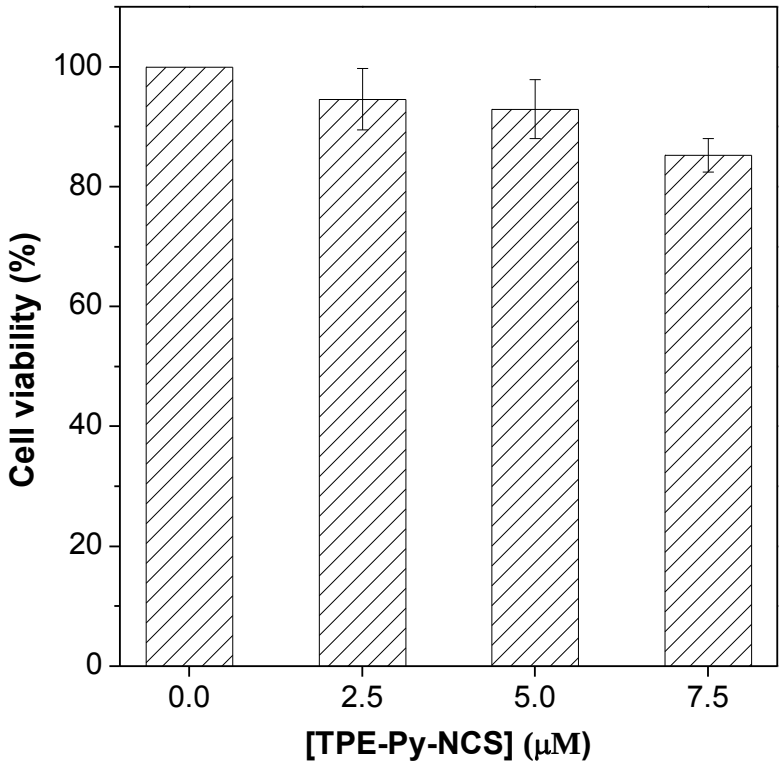


Fig. S6 Cell viability of HeLa cells incubated with different concentration of TPE-Py-NCS.

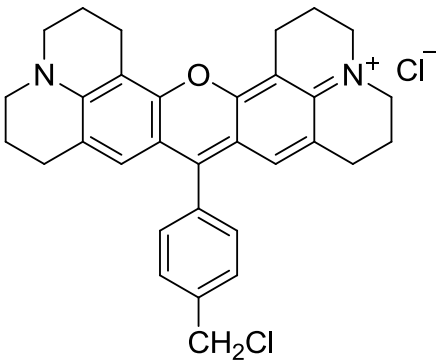


Fig. S7 Molecular structure of MitoTracker Red CMCRos.

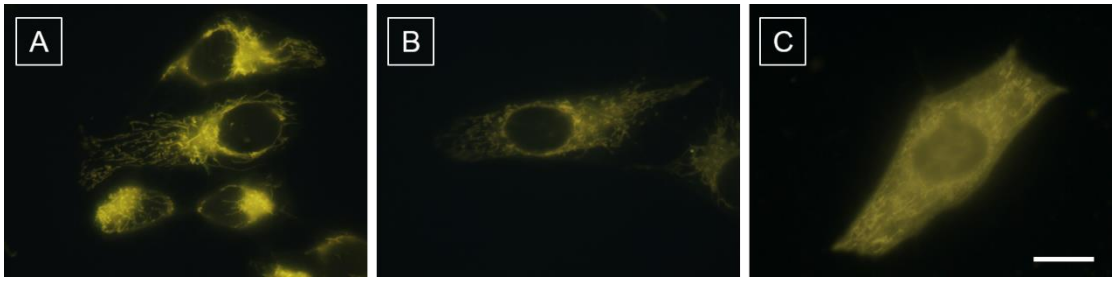


Fig. S8 Fluorescent images of HeLa cell stained with TPE-Py-NCS (5 μM) for 15min. The stained cells (A) are without any treatment, (B) are fixed with 4% PFA and (C) are fixed with 4% PFA followed by washing three times with DMSO. scale bar: 20 μm.

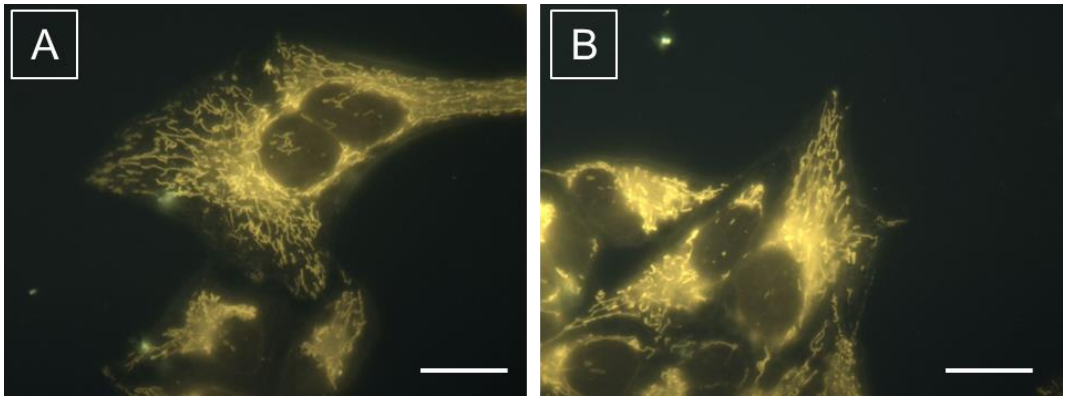


Fig. S9 Fluorescent images of HeLa cells stained with TPE-Py-NCS (A) untreated and (B) treated with CCCP after dye staining. Concentration: 5 μM (TPE-Py-NCS) and 10 μM (CCCP); excitation wavelength: 330−385 nm. All the images share the same scale bar: 20 μm.

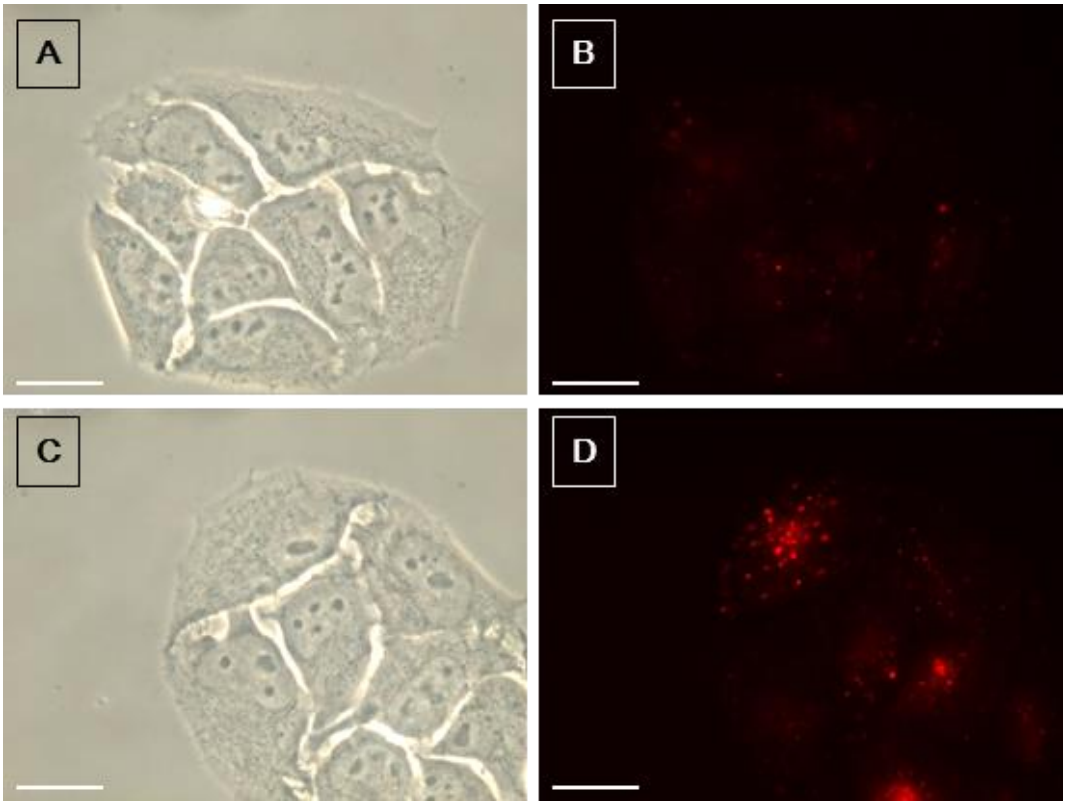


Fig. S10 (A and C) Bright-field and (B and D) fluorescent images of HeLa cells stained with LysoTracker Red DND-99 for 30 min after incubated in (A and B) PBS and (C and D) PBS with rapamycin for 1 h. Concentration: 150 nM (LysoTracker Red DND-99), 50 μg/mL (rapamycin); excitation wavelength: 540–580 nm. All the images share the same scale bar = 20 μm.

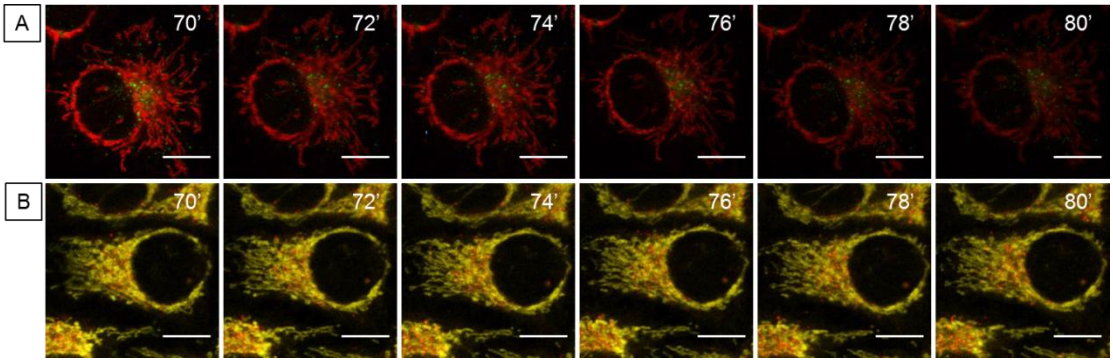


Fig. S11 Confocal images of HeLa cells co-stained with (A) MTR (50 nM) and LysoTracker Green DND-26 (150 nM), and (B) TPE-Py-NCS (5 μM) and LysoTracker Red DND-99 (150 nM) in the presence of rapamycin (50 mg/mL). Scale bar: 20 μm.

Reference

1 H. Shi, N. Zhao, D. Ding, J. Liang, B. Z. Tang, B. Liu, *Org. Biomol. Chem.*, 2013, **11**, 7289.