Supporting Information for

Multistimuli-Response Fluorescent Probe Based on Spiropyran for Visualization of Lysosomal Autophagy and Anticounterfeiting

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Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Experience

The cytotoxicity assays was tested according to the classical procedure. The cell viability assay of **Lyso-SP** and **Lyso-SQ** was assessed by using Hela cells. Using fresh medium the diluted the stock solutions with different concentrations (1, 2, 5, 10 and 20 μM). Cells were then incubated with six gradient concentrations of **Lyso-SP** and **Lyso-SQ** (0, 1, 2, 5, 10 and 20 μM) for 24 hours and then further incubated with 5 mg/mL MTT for 4 hours. The supernatant was then aspirated and DMSO (150 μL/well) was added. Finally, the absorbance was measured at 520 nm using a microplate reader (Infinite M200 Pro). Cytotoxicity was measured by six parallel wells.

Measurement of the fluorescence quantum yields

The fluorescence quantum yields (Φ) were calculated by the **Equation (2)**:

$$\Phi_s = \Phi_r \left(\frac{A_r}{A_s} \right) \left(\frac{n_s^2}{n_r^2} \right) \frac{I_s}{I_r} \qquad (2)$$

Where the subscripts s and r represent the sample and reference molecule, respectively. A stands for the absorbance. I represent the integrated emission intensity, n is the refractive index of the solvent. Φ is quantum yield. In this paper, fluorescence quantum yields were determined by using fluorescein (Φ=0.95 in pH=13 NaOH) as fluorescence standard.

Synthesis of D13

4-bromo-2-hydroxybenzaldehyde (100 mg，0.5 mM), 1-pyreneboronic acid (120 mg，0.5 mM), tetrakis (triphenylphosphine) palladium (58 mg，0.1 mM), THF(10 mL), K_2CO_3 aqueous solution (2 mL, 2 M) was added to the reaction flask, heated at reflux for 24 h with N_2. The obtained products were subjected to column chromatography separation experiments (PE:EtOAc= 5:1).

Synthesis of Lyso-SP

D13 (108 mg，0.33 mM), 1,2,3,3-Tetramethyl-3H-indolium iodide (100 mg，0.33 mM), Tetrahydro pyrrole (200 uL), THF (3 mL) were added to the reaction flask. The reaction was allowed to proceed for 6 h at room temperature. The solid precipitated after adding ethanol to the obtained liquid. Ethanol washed, spun dry. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 9.2 Hz, 1H), 8.19 (dd, *J* = 9.9, 7.4 Hz, 3H), 8.08 (d, *J* = 12.7 Hz, 2H), 8.02 (dd, *J* = 8.5, 5.8 Hz, 2H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 7.7 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.16 – 7.10 (m, 2H), 7.08 (s, 1H), 7.01 (d, *J* = 10.2 Hz, 1H), 6.86 (t, *J* = 7.3 Hz, 1H), 6.58 (d, *J* = 7.7 Hz, 1H), 5.80 (d, *J* = 10.2 Hz, 1H), 2.87 (s, 3H), 1.45 (s, 3H), 1.25 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 154.53, 148.24, 142.82, 137.02, 131.59 - 130.37, 129.21, 127.53, 126.50, 125.98, 125.39 - 124.50, 122.64, 121.54, 119.67, 119.18, 117.89, 117.17, 106.84, 104.53, 51.90, 29.10, 25.94, 20.38.

Synthesis of D15

4-bromo-2-hydroxybenzaldehyde (100 mg，0.5 mM), 3-Quinolineboronic acid (82 mg，0.5 mM), tetrakis (triphenylphosphine) palladium (58 mg，0.1 mM), THF(10 mL), K_2CO_3 aqueous solution (2 mL, 2 M) was added to the reaction flask, heated at reflux for 24 h with N_2. The obtained products were subjected to column chromatography separation experiments (PE:DCM= 5:1).

Synthesis of Lyso-SQ

D15 (83 mg，0.33 mM), 1,2,3,3-Tetramethyl-3H-indolium iodide (100 mg，0.33 mM), Tetrahydro pyrrole (200 uL), THF (3 mL) were added to the reaction flask. The reaction was allowed to proceed for 6 h at room temperature. The solid precipitated after adding ethanol to the obtained liquid. Ethanol washed, spun dry. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (d, *J* = 2.2 Hz, 1H), 8.31 (s, 1H), 8.18 (d, *J* = 8.5 Hz, 1H), 7.85 (d, *J* = 8.1 Hz, 1H), 7.73 (t, *J* = 7.5 Hz, 1H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.21 (s, 3H), 7.15 (s, 1H), 7.10 (d, *J* = 6.8 Hz, 1H), 6.94 (d, *J* = 10.2 Hz, 1H), 6.87 (t, *J* = 7.4 Hz, 1H), 6.56 (d, *J* = 7.7 Hz, 1H), 5.78 (d, *J* = 10.2 Hz, 1H), 2.79 (s, 3H), 1.37 (s, 3H), 1.21 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.15, 149.41, 148.17, 147.16, 139.04, 136.69, 133.15, 129.53, 129.53, 129.53, 129.53, 129.01, 129.01, 129.01, 129.01, 128.04, 127.70, 127.70, 127.44, 127.10, 121.55, 120.07, 119.33, 118.85, 113.67, 106.93, 104.66, 51.92, 29.03, 25.88, 20.23.

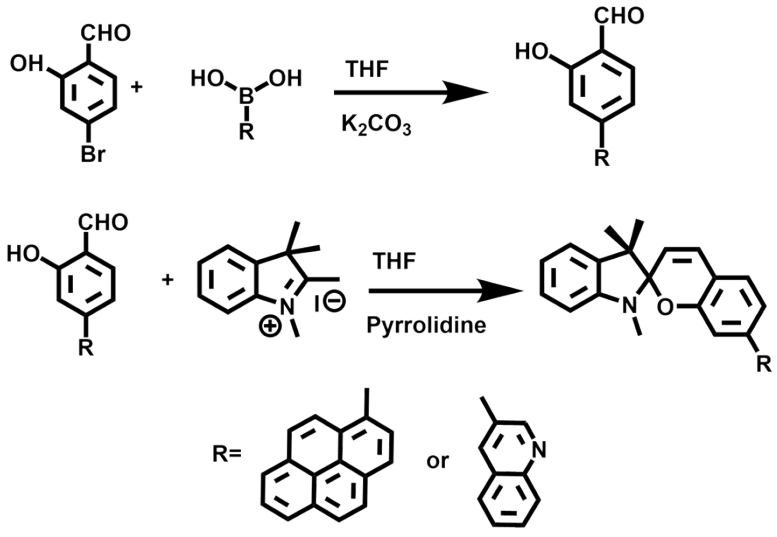

Figure S1. Synthetic routes of **Lyso-SP** and **Lyso-SQ**.

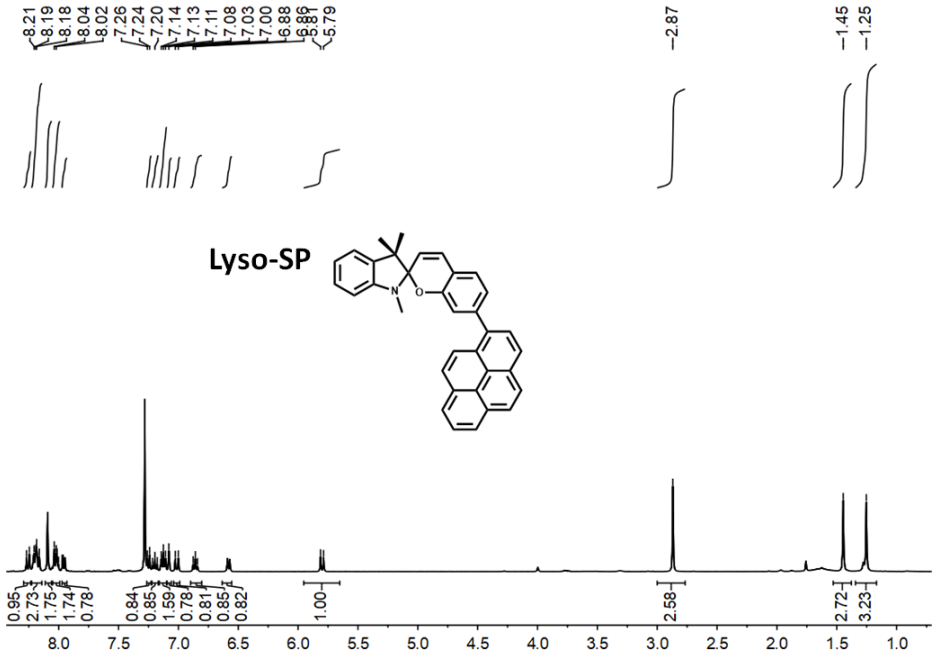

Figure S2. ^{1}H NMR spectrum of **Lyso-SP** in $CDCl_3$.

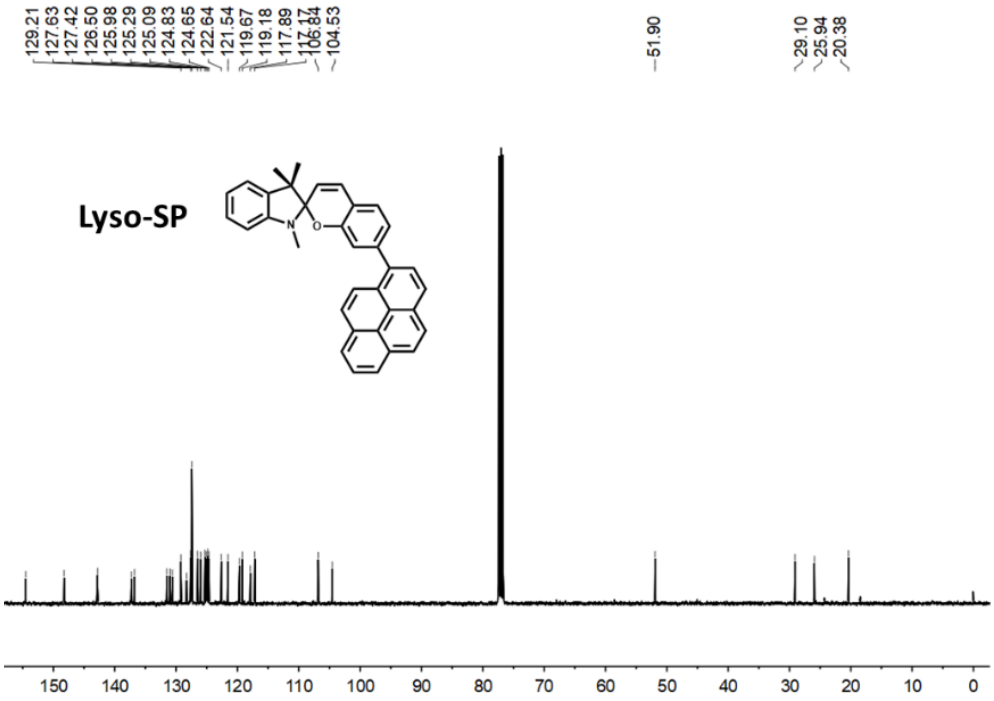

Figure S3. ^{13}C NMR spectrum of **Lyso-SP** in $CDCl_3$

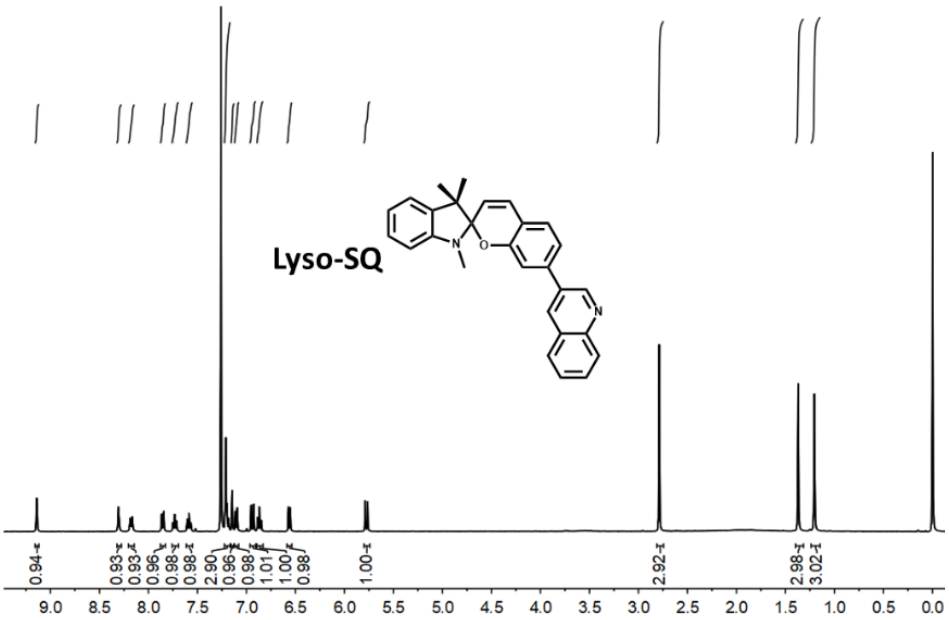

Figure S4. ^{1}H NMR spectrum of **Lyso-SQ** in $CDCl_3$

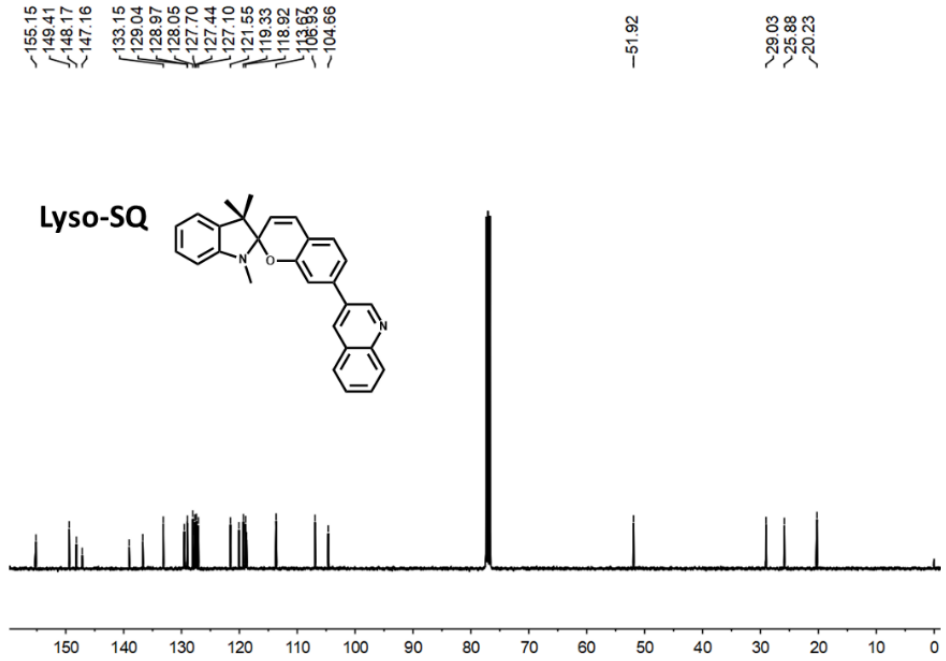

Figure S5. ^{13}C NMR spectrum of **Lyso-SQ** in $CDCl_3$

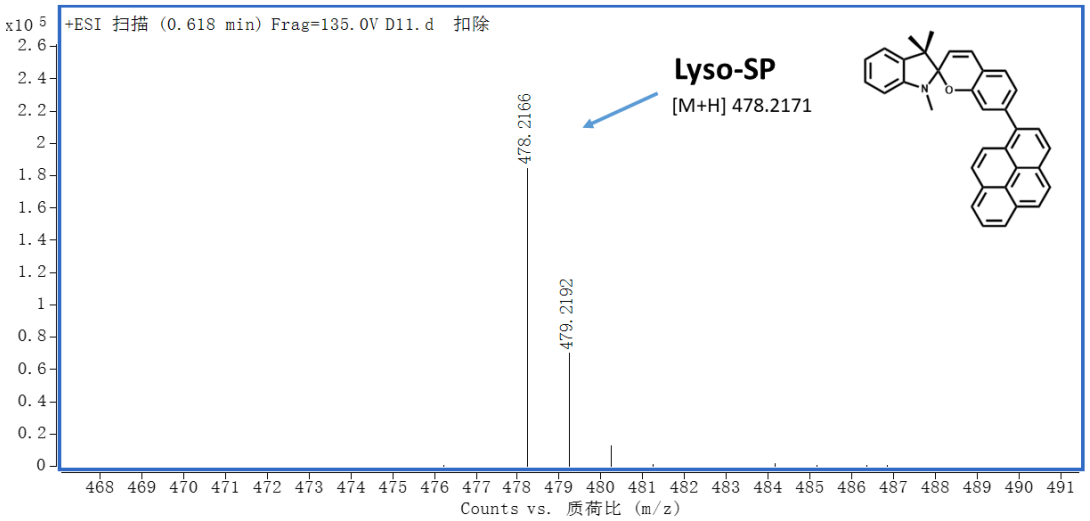

Figure S6. HRMS data of the probe **Lyso-SP** in MeOH.

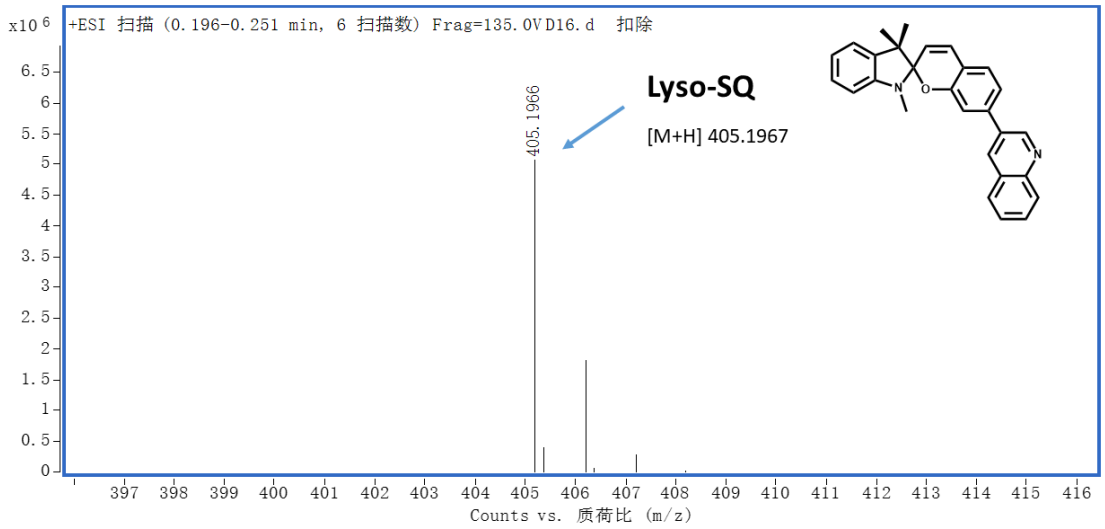

Figure S7. HRMS data of the probe **Lyso-SQ** in MeOH.

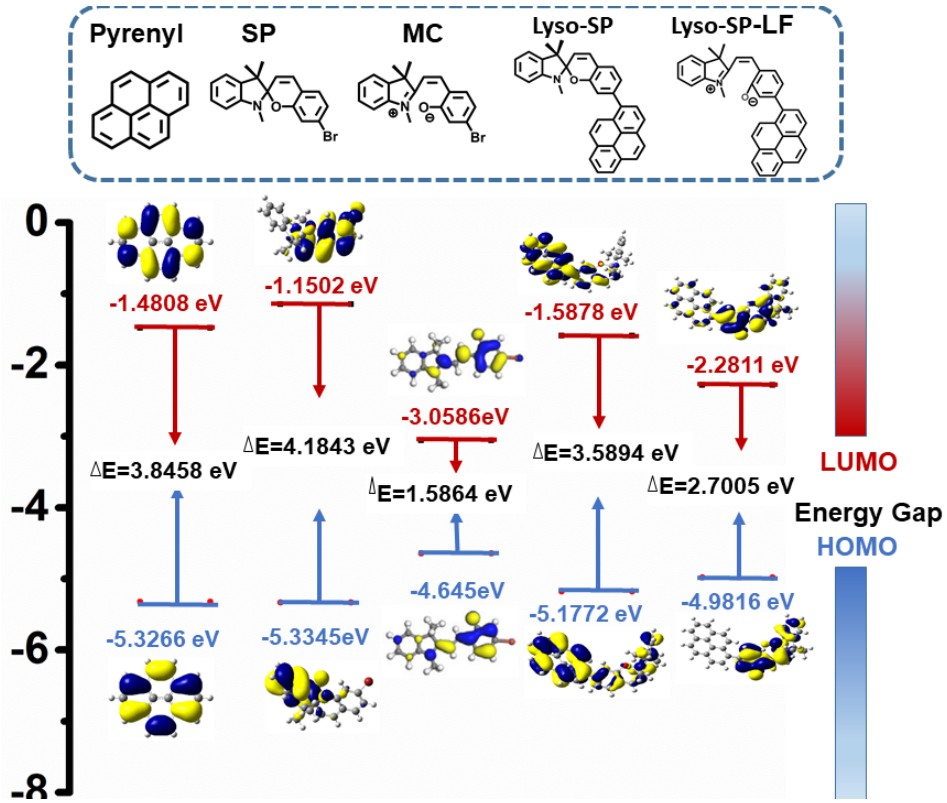

Figure S8. The spatial electron distributions of HOMOs and LUMOs of **SP**, **MC**, **Lyso-SQ** and **Lyso-SQ-LF** in optimized state.

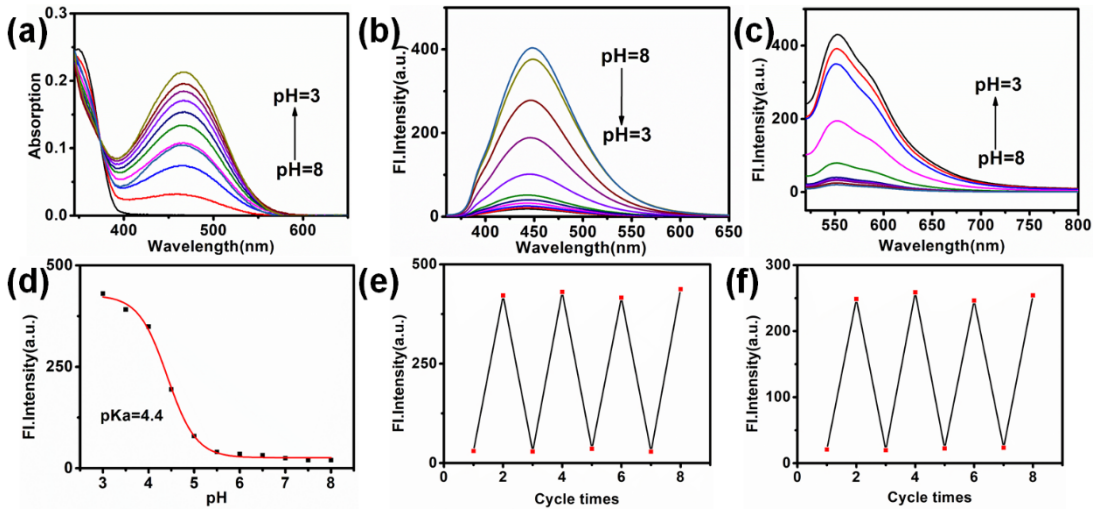

Figure S9. (a) The absorption spectra of **Lyso-SP** (10 μM) at different pH. (b) The fluorescence intensity changes of **Lyso-SP** (10 μM) at different pH (λ_{ex}= 350 nm), (Slit width: EX Slit: 5 nm, EM Slit: 5 nm). (c) The fluorescence intensity changes of **Lyso-SP** (10 μM) at different pH (λ_{ex}= 480 nm), (Slit width: EX Slit: 5 nm, EM Slit: 10 nm). (d) The relationship of fluorescence intensity at different pH values. (e)

Fatigue resistance of **Lyso-SP** in PBS solution after adding HCl or NaOH. (f) Fatigue resistance of **Lyso-SQ** in PBS solution after adding HCl or NaOH. (λ_{ex}= 480 nm). Test solution: PBS:EtOH=9:1.

Table S1. The photophysical properties of **Lyso-SP** and **Lyso-SQ** in the different solvents.

	Solvent	Abs(nm)	Em (nm)	Stokes shift (nm)
Lyso-SP	Hexane	346	420	74
	THF	348	430	82
	EtOH	342	434	92
	MeCN	343	447	104
	DMSO	345	462	117
Lyso-SQ	Hexane	340	404	64
	Toluene	340	415	75
	THF	335	425	90
	EtOH	339	434	95
	DMSO	334	452	118

Table S2 The fluorescence quantum yield of **Lyso-SP** and **Lyso-SQ** in various solvents.

Sample	Solvent	(Φs/ %)	Sample	Solvent	(Φs/ %)
Lyso-SP	Hexane	8.1	**Lyso-SQ**	Hexane	7.9
	THF	11.8		Toluene	8.4
	EtOH	13.9		THF	12.8
	MeCN	16.7		EtOH	14.1

	DMSO	17.9		DMSO	18.3
Φs fluorescence quantum yield (error limit: 8%) determined by using fluorescein (Φ=0.95 in pH=13 NaOH) as the standard.					

Table S3 The fluorescence quantum yield of **Lyso-SP** and **Lyso-SQ** in various pH (3 and 8).

Sample	pH	(Φs/ %)	Sample	pH	(Φs/ %)
Lyso-SP	3	11.3	**Lyso-SQ**	3	11.8
	8	8.1		8	8.8
Φs fluorescence quantum yield (error limit: 8%) determined by using fluorescein (Φ=0.95 in pH=13 NaOH) as the standard.					

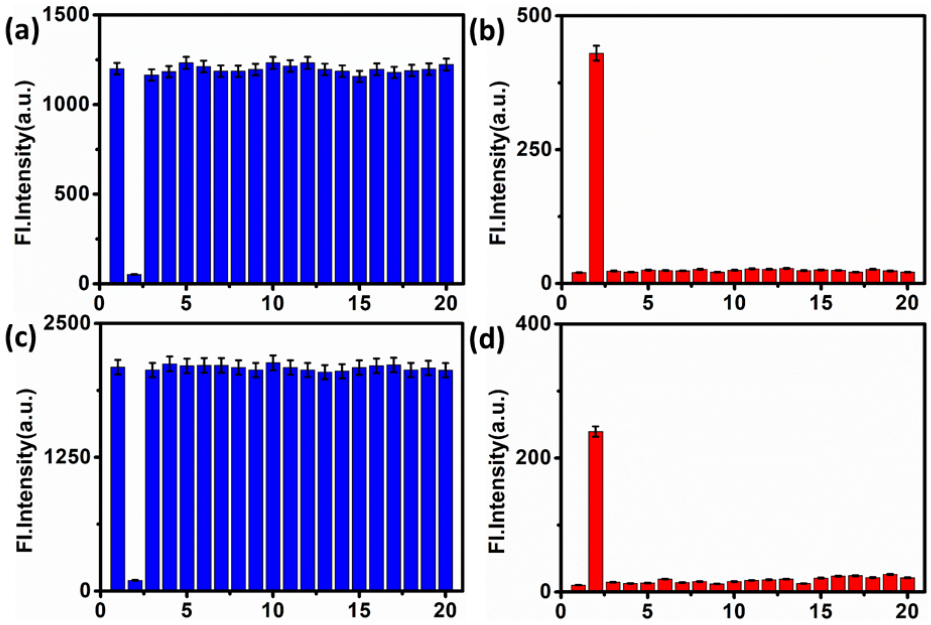

Figure S10. Fluorescence responses of probes to various substances: (a) Blue channel ions selectivity of probe **Lyso-SP** (10 μM) **(**λ_{ex}= 350 nm**)**, (Slit width: EX Slit: 5 nm, EM Slit: 5 nm). (b) Red channel ions selectivity of probe **Lyso-SP** (10 μM) **(**λ_{ex}= 480 nm**)**, (Slit width: EX Slit: 5 nm, EM Slit: 10 nm). (c) Blue channel ions selectivity of probe **Lyso-SQ** (10 μM) (**λ_{ex}**= 350 nm), (Slit width: EX Slit: 5 nm, EM Slit: 5 nm) (d) Red channel ions selectivity of probe **Lyso-SQ** (10 μM) (**λ_{ex}**= 480 nm), (Slit width: EX Slit: 5 nm, EM Slit: 10 nm)**.** Interfering ions: blank; H^+, Al^{3+} , Ba^{2+} , Ca^{2+} , Cu^{2+} , K^+, Fe^{3+}, Li^+, Sn^{2+},

Mg^{2+}, NaCl (1.0 mM); ClO^-, GSH, H_2O_2(1.0 mM)； Cys, Arg, Try, Lys, L-Cys (10 μM). Error bars represent the standard deviation (± S.D.) n=3. Significant difference ($P< 0.05$) are analyzed with two-sided Students's *t*-test. Test solution: PBS: EtOH=9:1.

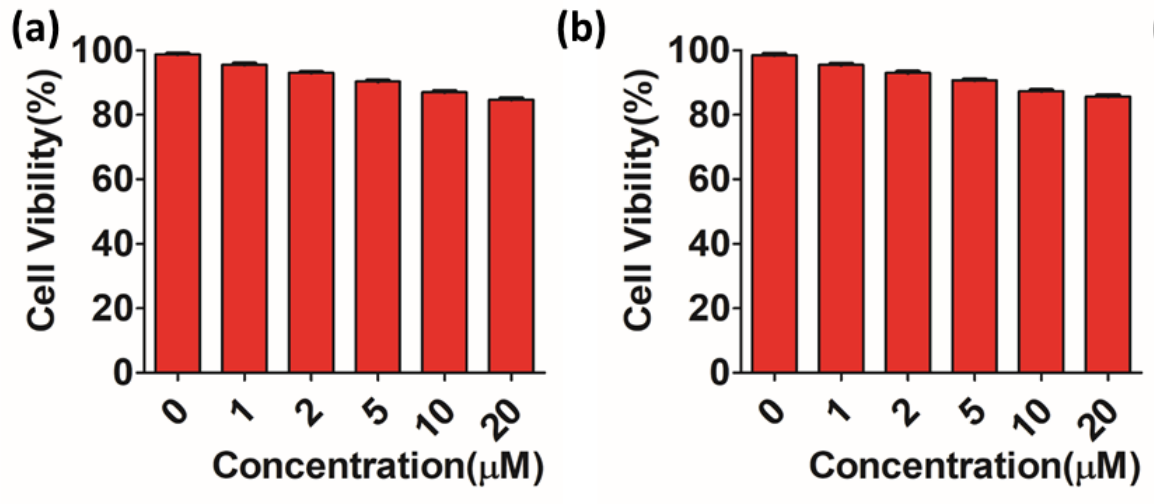

Figure S11. Cytotoxicity assay of **Lyso-SP** and **Lyso-SQ** at different concentrations (1: 0 μM; 2: 1 μM; 3: 5 μM; 4: 10 μM; 5: 20 μM) for HeLa cells. Error bars represent the standard deviation (± S.D.) n=3. Significant difference ($P< 0.01$) are analyzed with two-sided Students's *t*-test.

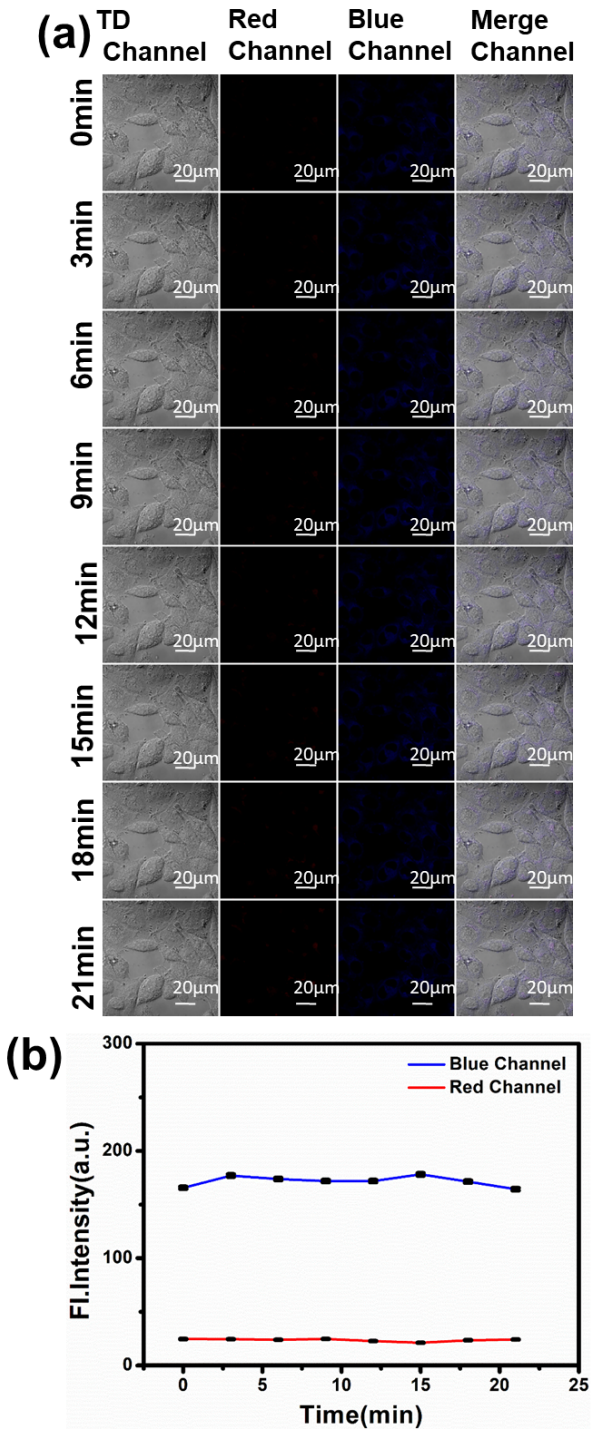

Figure S12. (a) Photostability of the probe **Lyso-SP** (5 μM) in Hela cells, (Blue channel: λ_{ex}=405 nm, λ_{em} = 425-475 nm; Red channel: λ_{ex}=488 nm, λ_{em} = 570-620 nm); (b) Changes in fluorescence intensity of blue channel and red channel. Scale bar = 20 μm. Error bars represent the standard deviation (± S.D.) n=3. Significant difference (P< 0.01) are analyzed with two-sided Students's t-test.

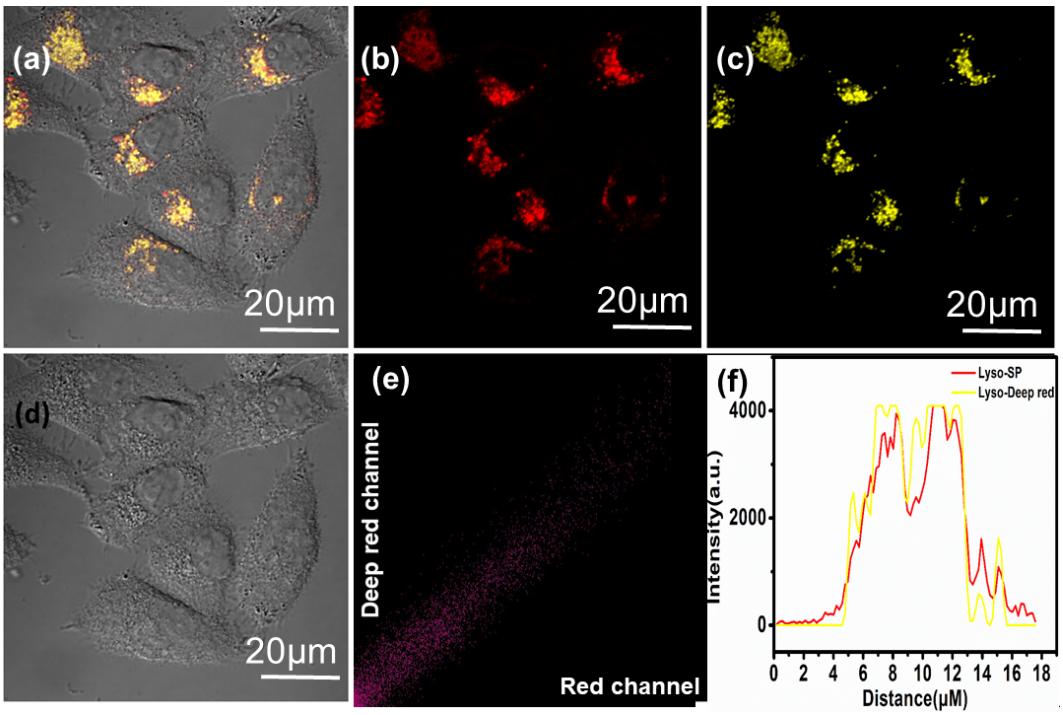

Figure S13. Lysosomal colocation pictures of probe **Lyso-SP** (5 μM) and LysoTracker Deep Red in HeLa cells. (a) Imaging pictures of Merged channel; (b) Imaging pictures of red channel (λ_{ex}= 488 nm, λ_{em} = 570-620 nm); (c) Imaging pictures of deep red channel (λ_{ex}= 647nm, λ_{em} = 663-738 nm); (d) Imaging pictures of TD channel; (e) Intensity profile of Pearson's colocation coefficient in HeLa cells with LysoTracker Deep Red and **Lyso-SP** (5 μM) (The X-axis and Y-axis refer to the red channel fluorescence intensity and the dark red channel fluorescence intensity, respectively). (f) Fluorescence intensity profile of regions of interest. Scale bar = 20 μm.

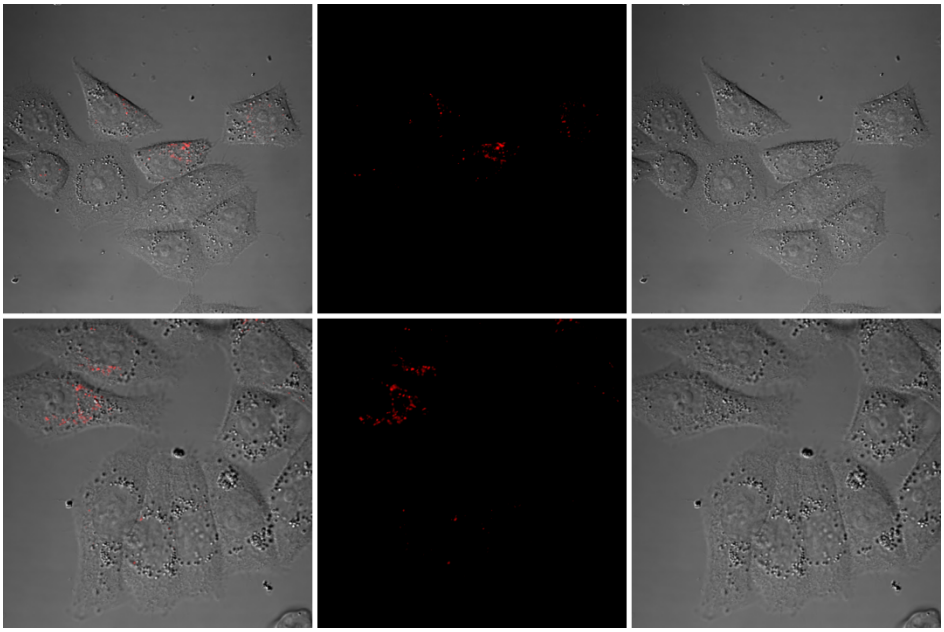

Figure S14. Channel crosstalk experiment of LT-DR.

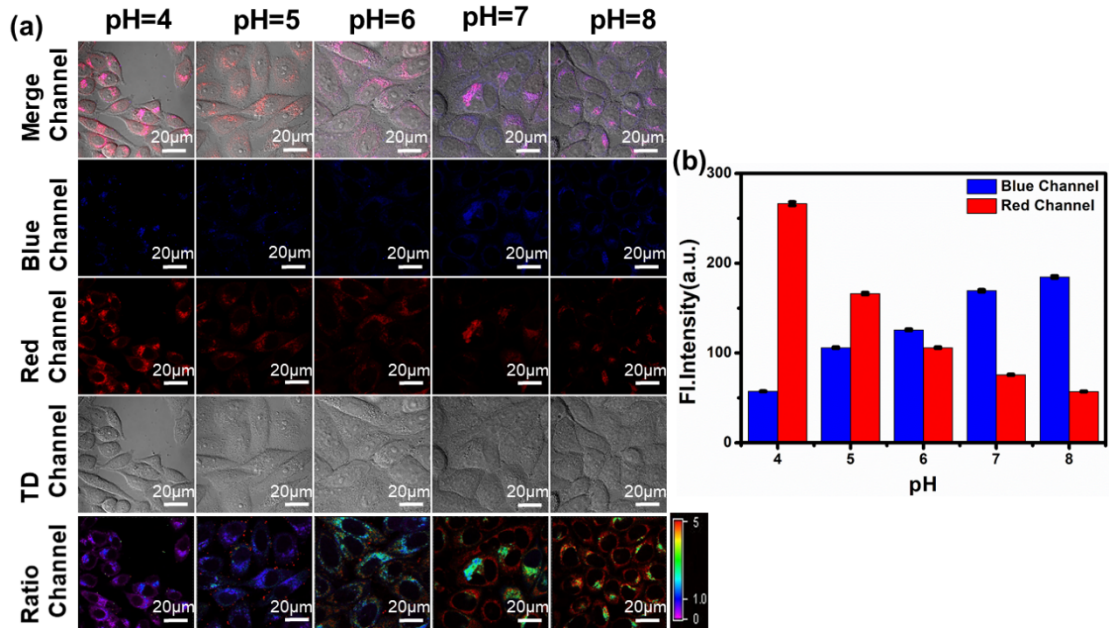

Figure S15. (a)The fluorescent and ratiometric images of HeLa cells pre-treated with cultural medium at different pH values for 10 min then stained with 5 μM **Lyso-SP** for 30 min. Scale bar = 20 μm. (Blue channel: λ_{ex}=405 nm, λ_{em} = 425-475 nm; Red channel: λ_{ex}=488 nm, λ_{em} = 570-620 nm) (b) The fluorescence intensities of blue channel and red channel with different pH. Error bars represent the standard deviation (± S.D.) n=3. Significant difference (P< 0.01) are analyzed with two-sided Students's *t*-test.

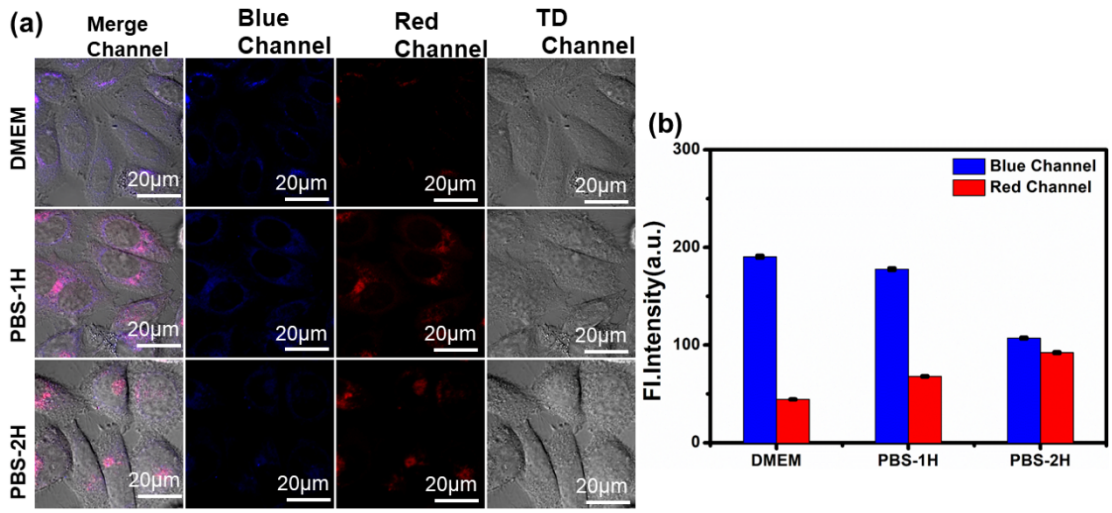

Figure S16. The fluorescence images of Hela cells pre-stained with **Lyso-SP** (5 μM) for 10 min (a) Hela cells incubated with culture medium for 120 min and PBS buffer for 1 h and 2 h. Scale bar = 20 μm. (Blue channel: λ_{ex}=405 nm, λ_{em} = 425-475 nm; Red channel: λ_{ex}=488 nm, λ_{em} = 570-620 nm) (b) The fluorescence intensities of blue channel and red channel. Error bars represent the standard deviation (± S.D.) n=3. Significant difference (P< 0.01) are analyzed with two-sided Students's *t*-test.

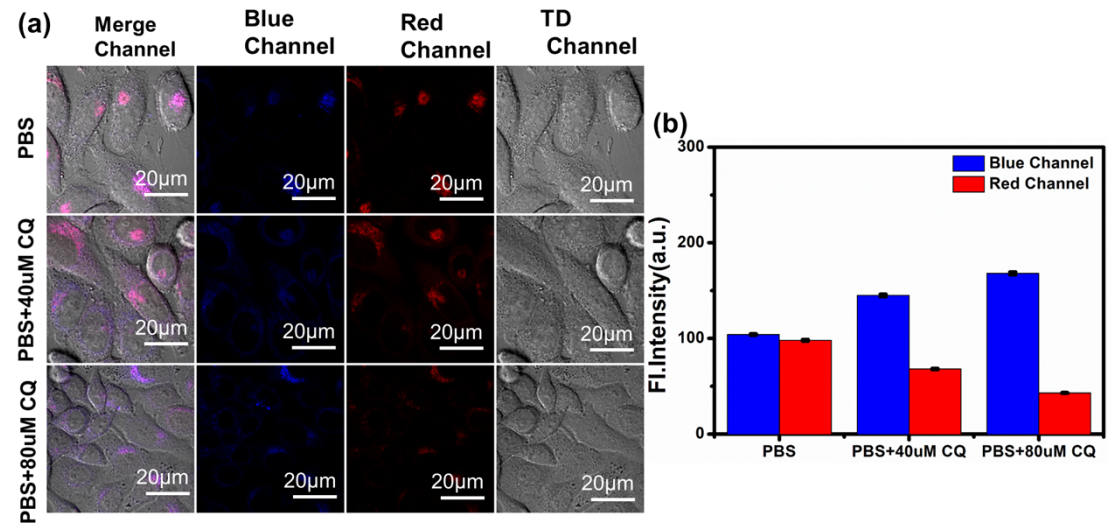

Figure S17. The fluorescence images of HeLa cells pre-stained with 5 μM **Lyso-SP** for 10 min. (a) The HeLa cells were incubated with PBS containing different amounts of CQ for 2 h. Scale bar = 20 μm. (Blue channel: λex=405 nm, λem = 425-475 nm; Red channel: λex=488 nm, λem = 570-620 nm) (b) The fluorescence intensities of blue channel and red channel. Error bars represent the standard deviation (± S.D.) n=3. Significant difference (P< 0.01) are analyzed with two-sided Students's t-test.