# Supporting Information

## A single dual-targeting fluorescent probe enables exploration of the correlation between the plasma membrane and lysosomes

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## 1. Apparatus and Method

**1.1 Apparatus.** The UV absorption and fluorescent spectrum of probes were obtained on a Hitachi U-2500 spectrophotometer and a Hitachi F-2710 spectrofluorimeter equipped with a 450 W Xe lamp, respectively. Confocal fluorescent images were obtained from Leica TCS SP8 and Zeiss 880 confocal fluorescent microscope.

**1.2 Reagents.** The commercial fluorescent probes LysoBrite NIR (Lyso-NIR), Deep Red Tracker FM (MTDR), and DiD were all purchased from Invitrogen, and dissolved by DMSO solution. The cholesterol (CL) and β-cyclodextrin (β-CD) were all purchased from Bailingwei technology co., ltd, and dissolved by water."

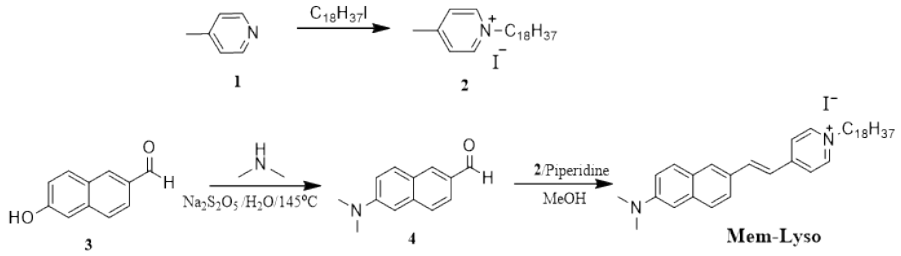
**1.3 Measurements of fluorescence quantum yield.** The fluorescence quantum yields ($\Phi$) can be calculated using equation (1) followed our previous work [1]:

$$\Phi_s = \Phi_r \frac{A_r \lambda_r n_s^2 F_s}{A_s \lambda_s n_r^2 F_r} \quad \text{---------- (1)}$$

The subscripts s and r denote the sample and the reference substance, respectively. $\Phi$ is the fluorescence quantum yield, F is the integrated fluorescent intensity, A is the absorbance, and n stands for the refractive index. In this work, $\Phi$ was measured using fluorescein in aqueous solution of NaOH (pH = 13, $\Phi$ = 0.95) as standard.

**1.4 Cytotoxicity of Mem-Lyso.** The study of the cytotoxicity of Mem-Lyso on the cells was performed by methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. SiHa and HeLa cells were seeded into 96-well plates (ca. $1 \times 10^4$ cells/ well) and adhered for 24 h. Mem-Lyso (200 μL/well) diluted in culture medium at corresponding concentration was added into the wells as the treatment group, and DMSO (200 μL/well) diluted in culture medium with final concentration of 0.2% as the negative control group, respectively. The cells in the 96-well plate were added 2, 4, 6, 8, 10 μM Mem-Lyso for 24 h at 37 °C under 5% $CO_2$, then MTT (5 mg/mL in PBS) was added into each well. After 4 h of incubation, the culture medium in each well was removed and DMSO (200 μL) was added to dissolve the purple crystals. After incubating 30 min, the optical density readings at 492 nm were taken using a plate reader. Each individual cytotoxicity experiment was repeated for three times.

## 2. Synthetic details, NMR and HRMS spectra




**Scheme S1.** The synthesis route of Mem-Lyso.

1. Synthesis of compound 2: 4-methyl-pyridine (0.842 mL, 8.6 mmol) and 1-Iodooctadecane (3.2 g, 8.6 mmol) were added into a three flask, stirred until the white solid precipitated. For 4-methyl-1-octadecylpyridin-1-ium iodide: 3.3 g, yield 80%. $^{1}$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 8.93 (d, $J$ = 4.0 Hz, 2H), 7.99 (d, $J$ = 8.0 Hz, 2H), 4.51 (t, $J$ = 16.0 Hz, 2H), 2.61 (s, 3H), 1.89 (m, 2H), 1.24 (m, 30H), 0.86 (t, $J$ = 12.0 Hz, 3H). $^{13}$C NMR (DMSO-$d_6$, 400 MHz): δ (ppm): 159.23, 143.99, 129.02, 60.39, 31.79, 29.54, 29.50, 29.20, 28.87, 25.61, 22.35, 21.86, 14.44.

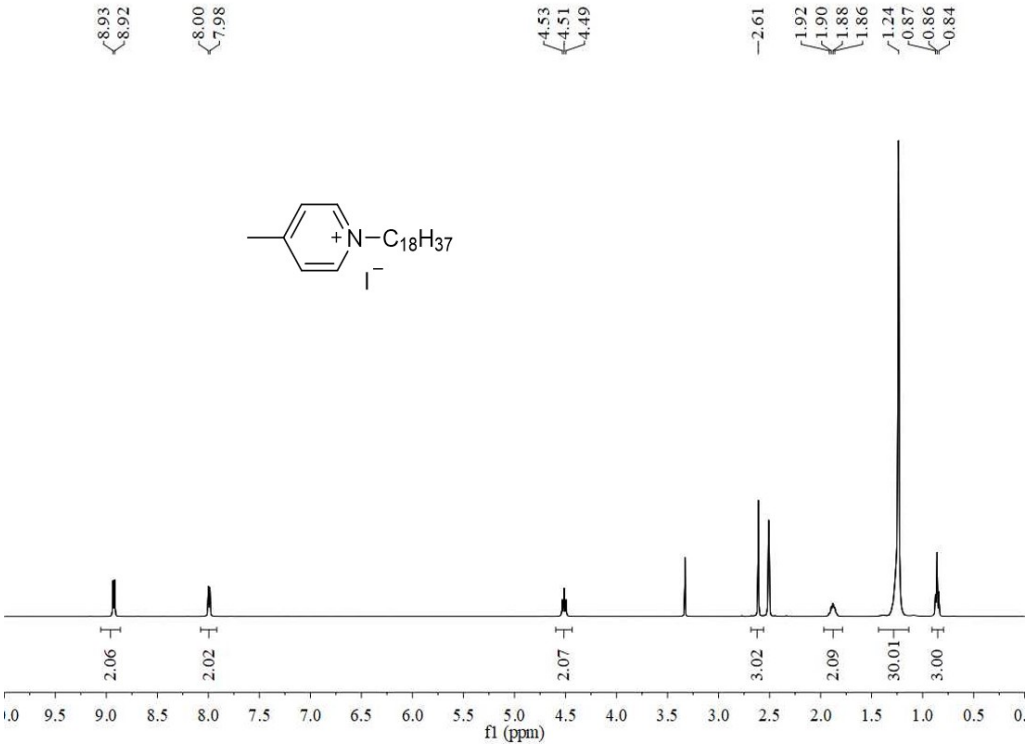



**Fig. S1** The $^{1}$H NMR spectrum of compound 2 in DMSO-$d_6$.

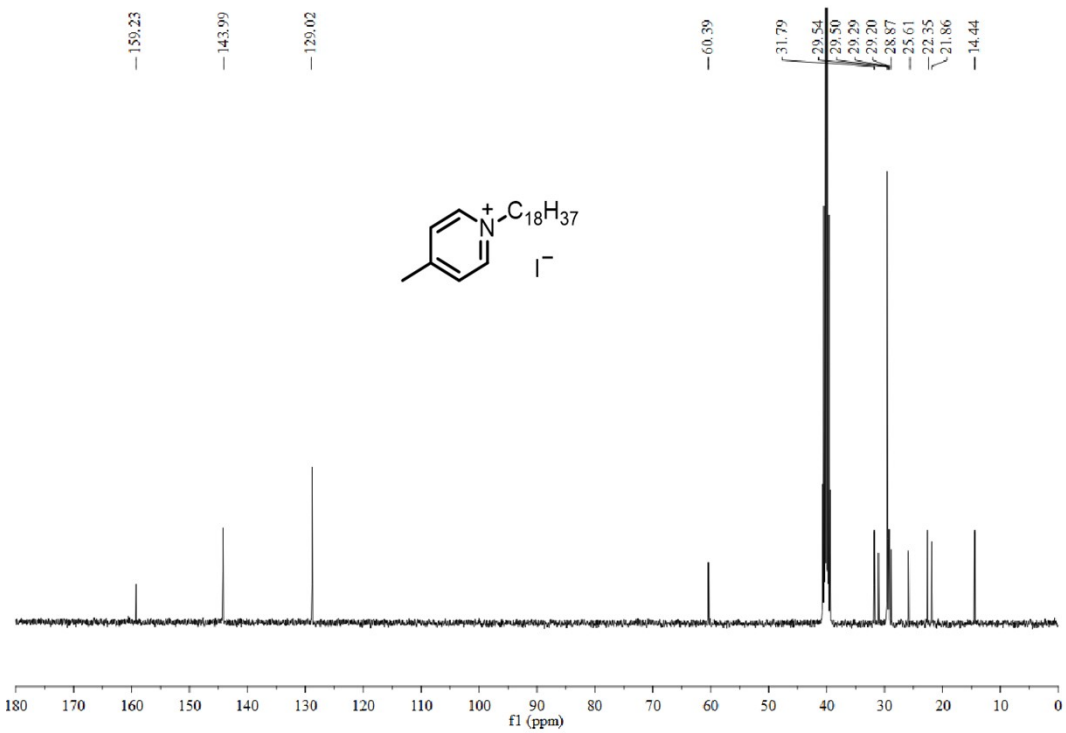



**Fig. S2** The $^{13}C$ NMR spectrum of compound 2 in DMSO-$d_6$.

2. Synthesis of compound 4: The mixture of compound 1, (1.553 g, 9.02mmol), $Na_2S_2O_5$ (4.24 g, 18.1 mmol), NaOH (2.378 g, 45.4 mmol), $Me_2NH$ (3.04 g, 40.6mmol) and $H_2O$ (80 mL) were added in hydrothermal autoclave and stirred at 145°C for 48h. After the reaction was complete, the mixture was extract with dichloromethane, and then the crude product was purified by column chromatography using petroleumether-EtOAc 8: 1 (v/v) as the eluant to yield compound 2: 6-(dimethylamino)-2-naphthaldehyde (1.087 g, 60.5%). $^1H$ NMR (DMSO-$d_6$, 300 MHz): $\delta$(ppm): 9.95 (d, $J$ = 7.56 Hz, 1H), 8.32 (s, 1H), 7.94 (d, $J$ = 9.00 Hz, 1H), 7.71 (m, 2H), 7.31 (q, $J$ = 11.7 Hz, 1H), 6.99 (d, $J$ = 2.7 Hz, 1H),3.10 (s, 6H). $^{13}C$ NMR (DMSO-$d_6$, 400 MHz): δ (ppm): 192.27, 151.03, 138.67, 135.28,127.06, 123.20, 116.94, 105.42.

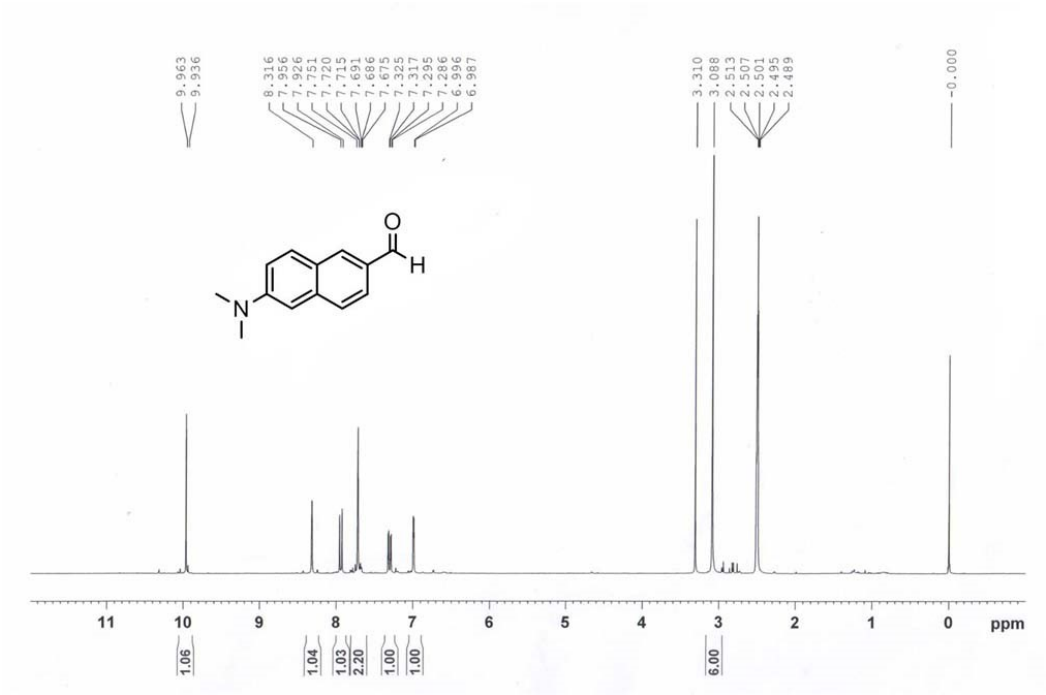



**Fig. S3** The [1]H NMR spectrum of compound 4 in DMSO-$d_6$.

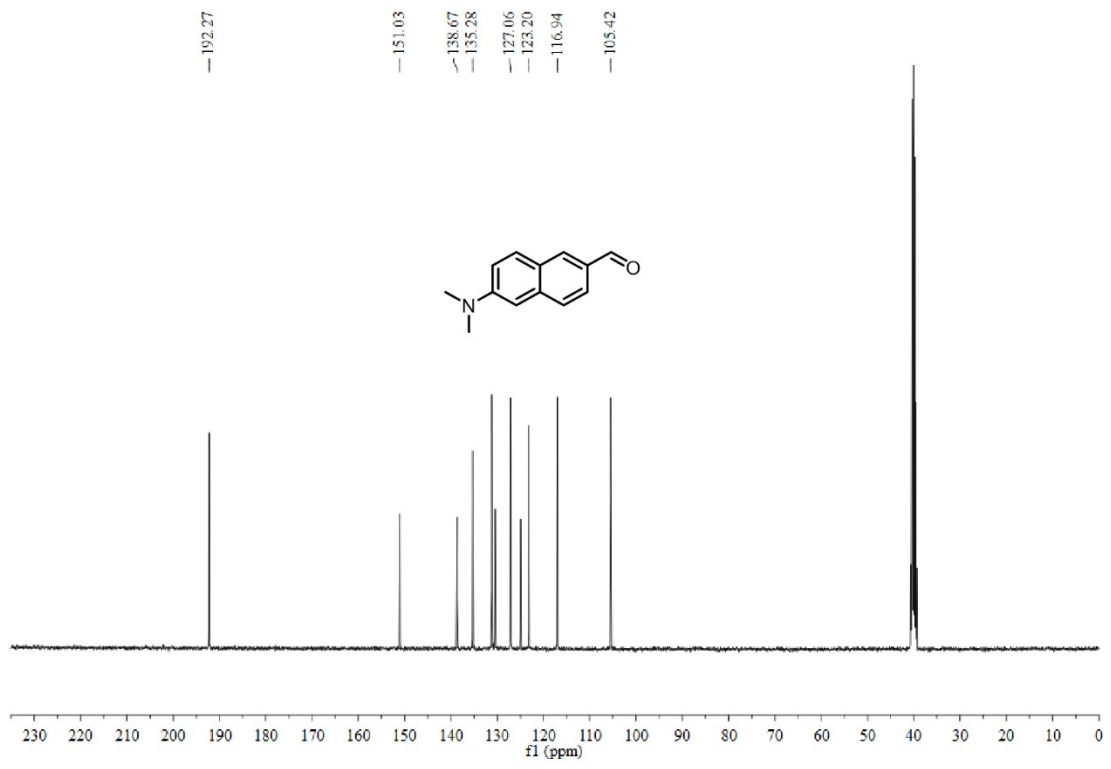



**Fig. S4** The [13]C NMR spectrum of compound 4 in DMSO-$d_6$.

3. Synthesis of Mem-Lyso: The compound 2 (0.357g, 0.754mmol) and anhydrous methanol (25mL) were added into a three flask, heated at 55 °C until they all were dissolved, and then added the 4 (0.177 g, 0.754 mmol) and piperidine (6 drops). On completion of the addition, raise the temperature and reflux until the reddish brown solid precipitated. For (*E*)-4-(2-(6-(dimethylamino) naphthalen-2-yl)vinyl)-1-octadecylpyridin-1-ium iodide, Lyso-Mem: 0.22 g, yield 45%. [1]H NMR (DMSO-$d_6$.,

400 MHz): $\delta$ (ppm): 8.80 (d, *J*=8 Hz, 2H), 8.11 (d, *J*=8 Hz, 2H), 8.02 (d, *J*=16 Hz, 1H), 7.91(s, 1H), 7.67 (m, 3H), 7.39 (d, *J*=16 Hz, 1H), 7.17 (m, 1H), 6.88(s, 1H), 4.37(d, *J*=16 Hz, 2H), 2.98(s, 6H), 1.16 (m, 32H), 0.76 (d, *J*=12 Hz, 3H). $^{13}$C NMR (DMSO-$d_6$, 400 MHz): δ (ppm): 153.74, 150.00，1,4.43, 142.31, 136.45, 136.45, 130.80, 130.16, 128.87, 127.28, 125.96, 124.39, 123.70, 121.18, 116.99, 105.89, 59.96, 31.78, 30.96, 29.53, 29.36, 29.24, 29.19, 25.87, 22.58, 14.45. HRMS (m/z): calcd for $C_{37}H_{55}IN_2$: 654.34; found: 527.44 $(M-I)^+$.

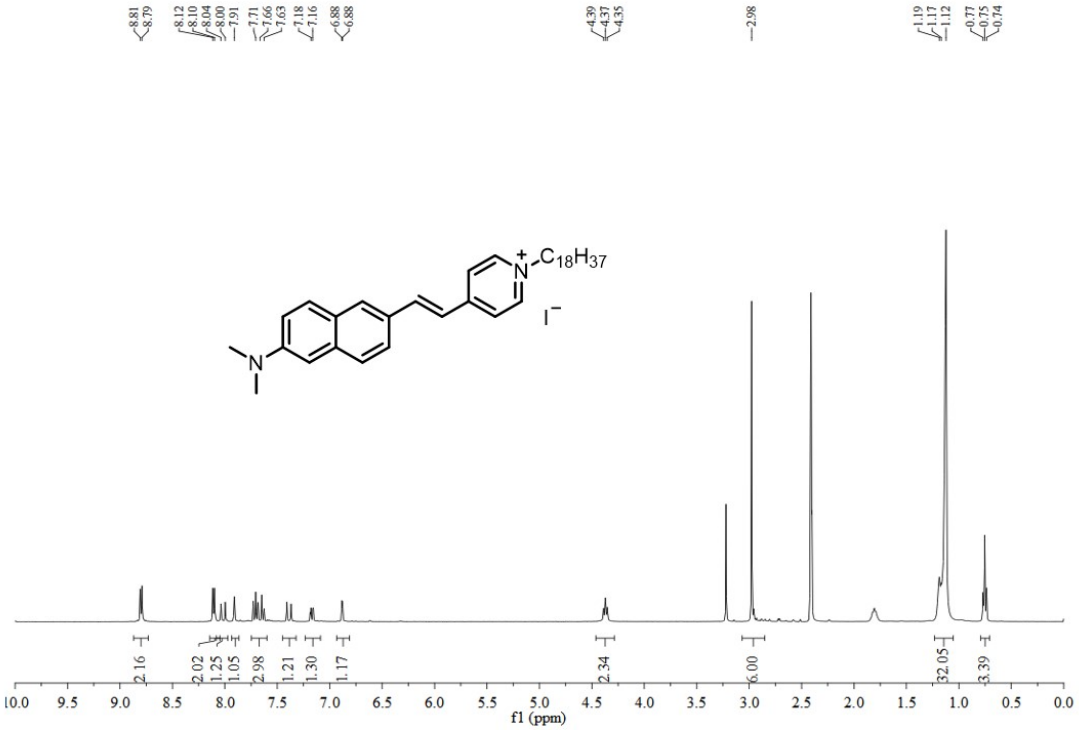



**Fig. S5** The $^1$H NMR spectrum of compound Mem-Lyso in DMSO-$d_6$.

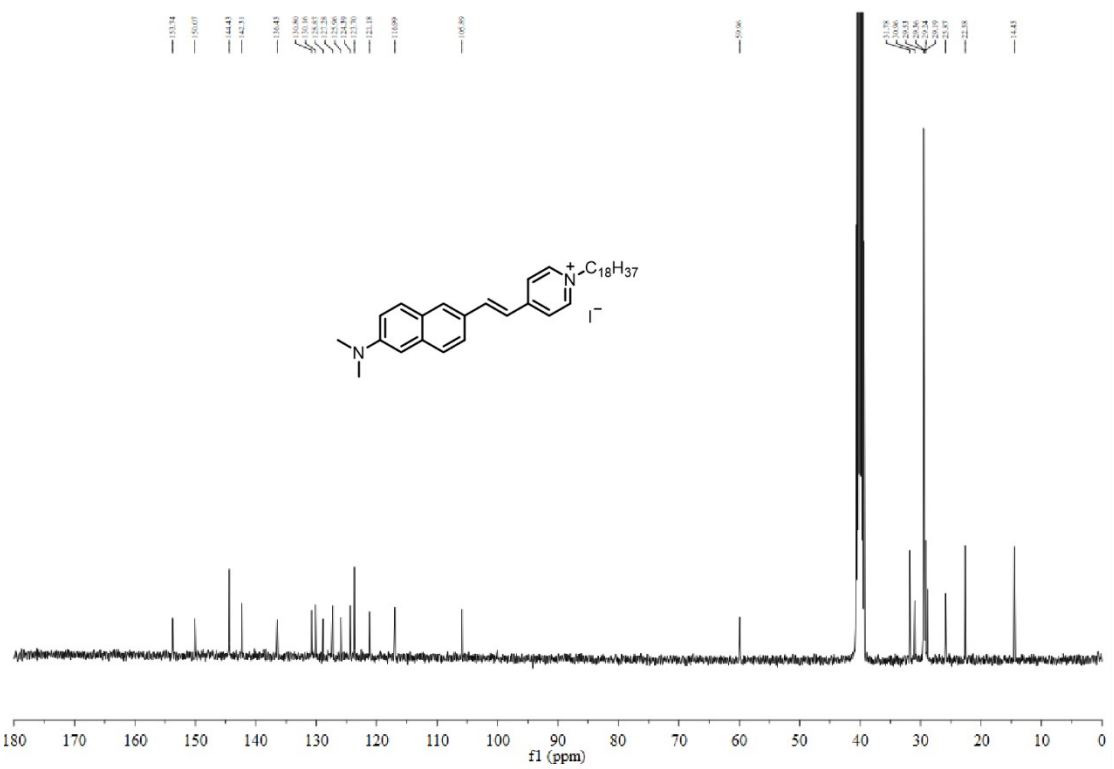



**Fig. S6** The $^{13}C$ NMR spectrum of Mem-Lyso in DMSO-$d_6$.

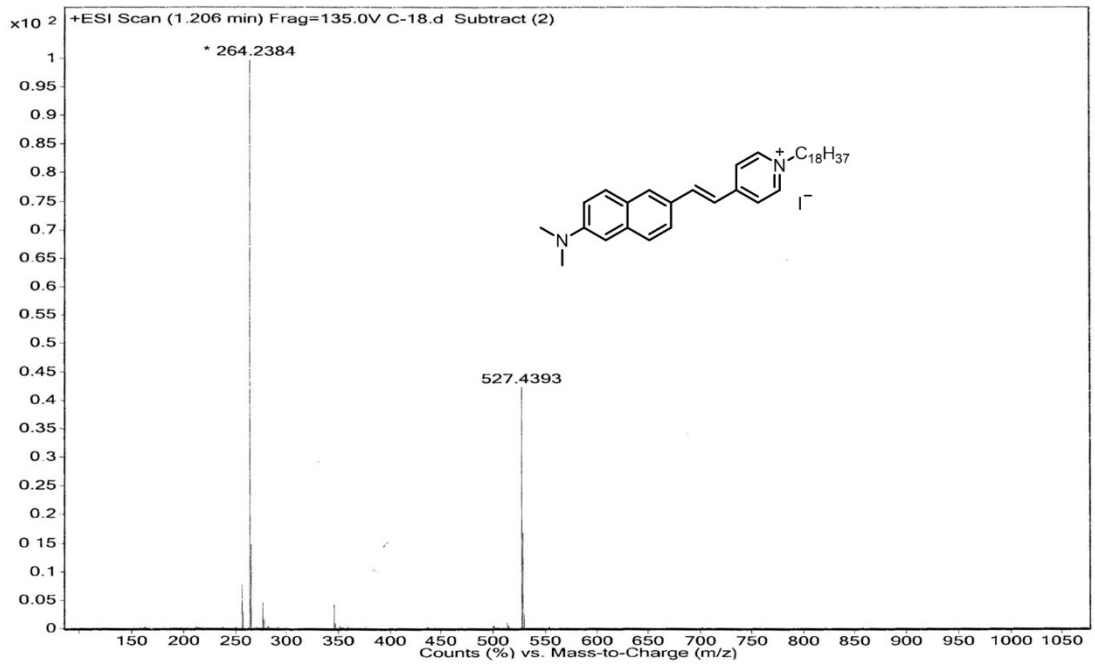



**Fig. S7** HRMS spectrum of Mem-Lyso.

## 3. Photophysical data

**Table S1.** The photophysical properties of Mem-Lyso in different solvents

| **Solvents** | **Maxima $\lambda_{ab}$ (nm)** | **Maxima $\lambda_{em}$ (nm)** | **ε ($M^{-1}\cdot cm^{-1}$)** | ***Φ* (%)** |
|---|---|---|---|---|
| 1,4-dioxane | 456 | 530 | 15000 | 57.78 |
| THF | 474 | 577 | 16500 | 9.77 |
| DMF | 472 | 623 | 16400 | 0.52 |
| DMSO | 474 | 621 | 15600 | 0.66 |
| MeOH | 478 | 630 | 14800 | 0.58 |
| EtOH | 484 | 624 | 18800 | 3.36 |
| $H_2O$ | 489 | 634 | 14400 | 0.05 |

## 4. Cell viability data

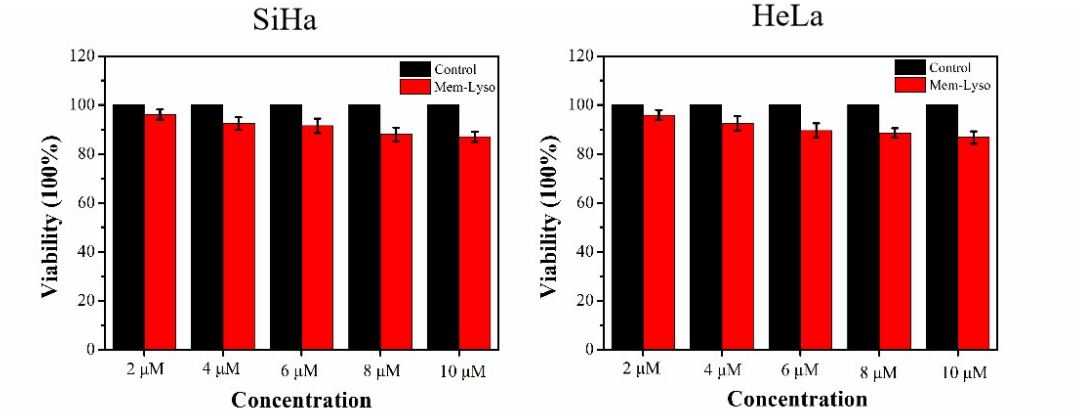



**Fig. S8** MTT results of SiHa and HeLa cell viabilities after incubation with Mem-Lyso for 24 h at different incubation concentrations.

## 5. Spectra data

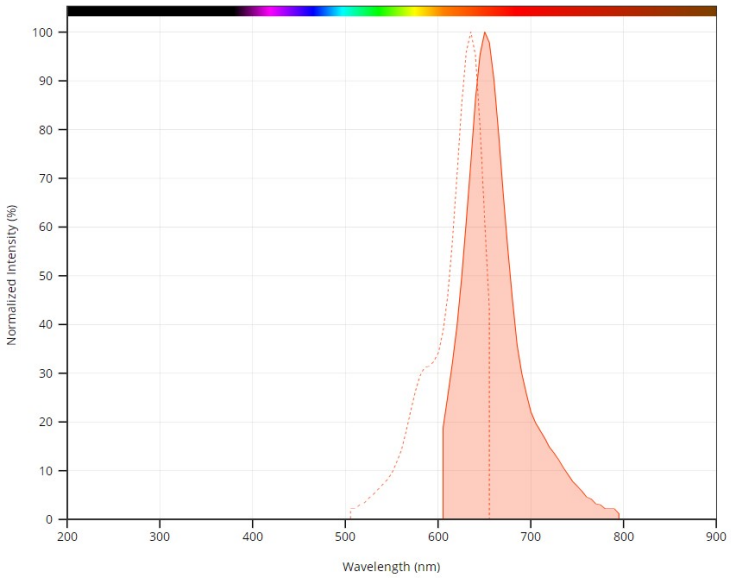



**Fig. S9** The normalized absorption (dotted line) and fluorescent (solid line) spectra of Lyso-NIR. (The figure was cited from the web of https://www.aatbio.com/products/lysobrite-nir?unit=22641)

## 6. Imaging data

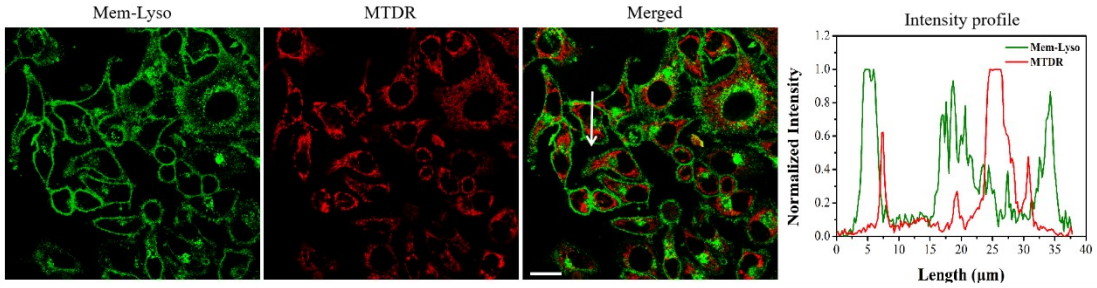



**Fig. S10** Fluorescent images of HeLa cell stained with Mem-Lyso (2 μM, 10 min) and MTDR (0.2 μM, 30 min), their merged image, and relative intensity profile of Mem-Lyso and MTDR in various pixel points along the white arrow in the merged images. $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-560 nm to Mem-Lyso; $\lambda_{ex}$ = 635 nm, $\lambda_{em}$ = 655-700 nm to MTDR, Bar = 20 μm.

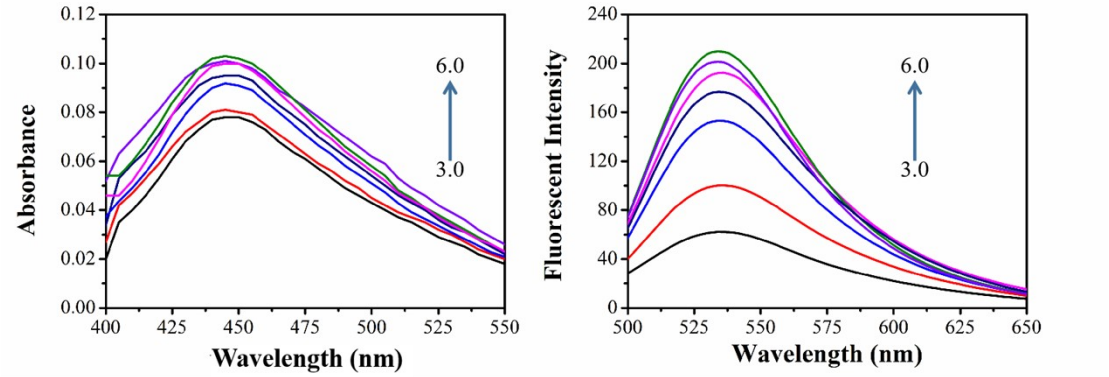



**Fig. S11** The absorption and fluorescent spectra of Mem-Lyso at different pH, which were prepared using phosphate buffer saline (PBS) buffer solution. $\lambda_{ex}$= 455 nm, compound concentration: 10 μM.

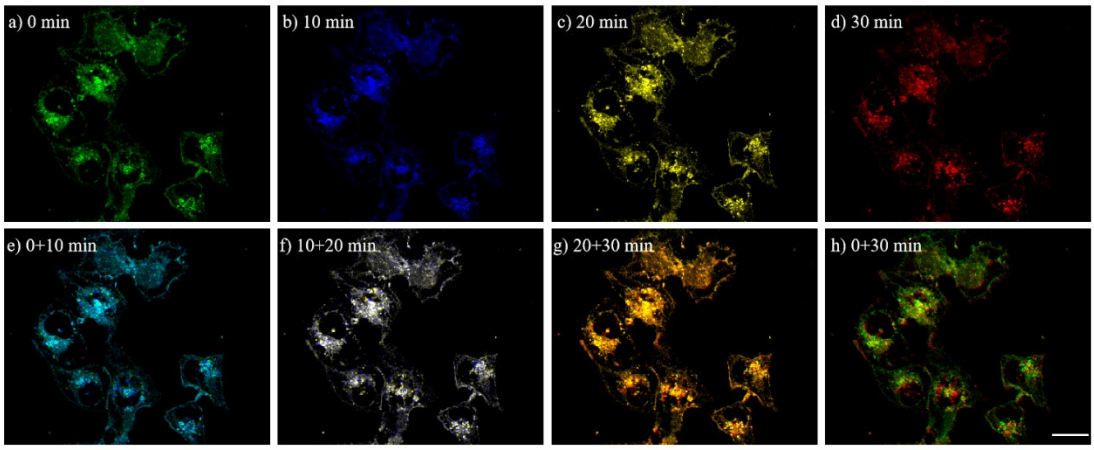



**Fig. S12** a) The fluorescence images of HeLa cells stained with Mem-Lyso (2 μM, 10 min) and observed for different time (b: 10 min, c: 20 min, d: 30 min), and their merged images (e: merged image of a and b, f: merged image of b and c, g: merged image of c and d, h: merged image of a and d), $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-600 nm, bar = 20 μm. (Note: to better observe the dynamic changes of plasma membrane and lysosomes, the pseudo-colors of Mem-Lyso in the figure were adjusted)

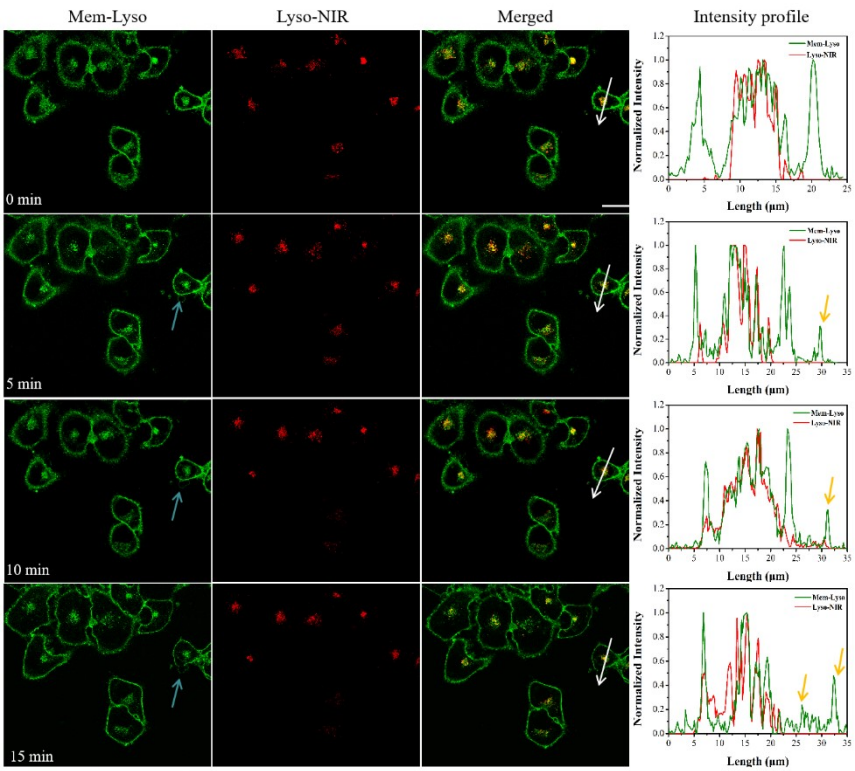



**Fig. S13** Fluorescent images of HeLa cell stained with Mem-Lyso (2 μM, 10 min) and Lyso-NIR (0.2 μM, 30 min) and then incubated with $H_2O_2$ solution for different time, their merged images, and relative intensity profile of Mem-Lyso and Lyso-NIR in various pixel points along the white arrow in the merged images. $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-560 nm to Mem-Lyso; $\lambda_{ex}$ = 635 nm, $\lambda_{em}$ = 655-700 nm to Lyso-NIR, Bar = 20 μm.

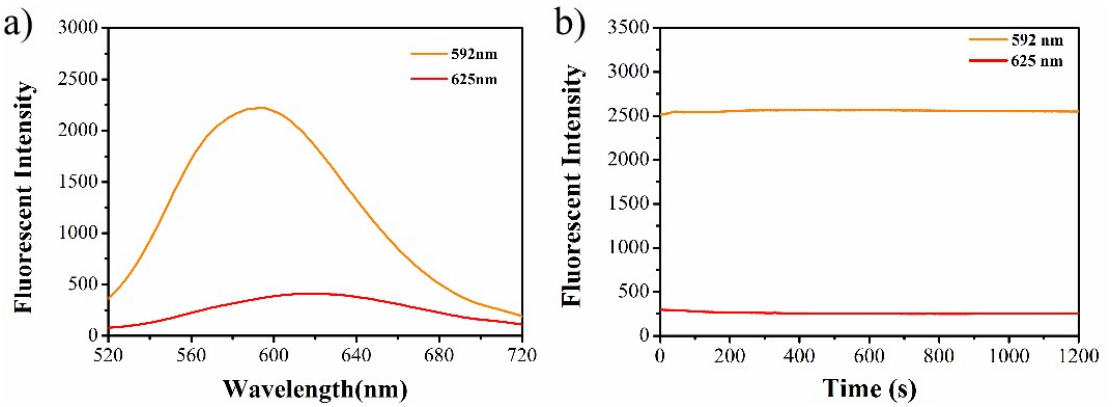



**Fig. S14** a) The fluorescent spectrum of Mem-Lyso (10 μM) in the mixture solutions of 1,4-dioxane and water, $\lambda_{ex}$ = 570 nm, b) the photostability of Mem-Lyso (10 μM) in the corresponding solutions of panel (a) under continuous irradiation of xenon lamp, $\lambda_{ex/em}$ = 570/592 nm (orange line), $\lambda_{ex/em}$ = 570/625 nm (red line).

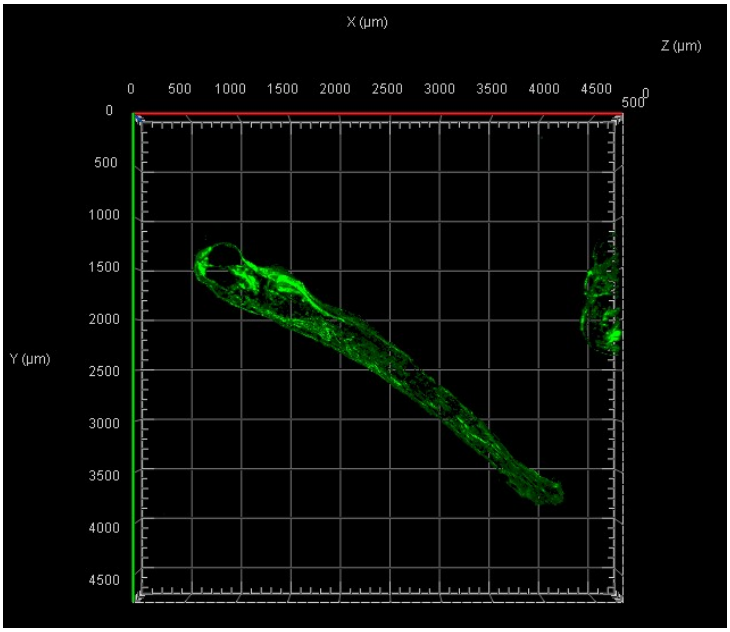



**Fig. S15** The 3D fluorescent image of zebrafish (a week old) stained with Mem-Lyso (2 μM, 2h), $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-600 nm.

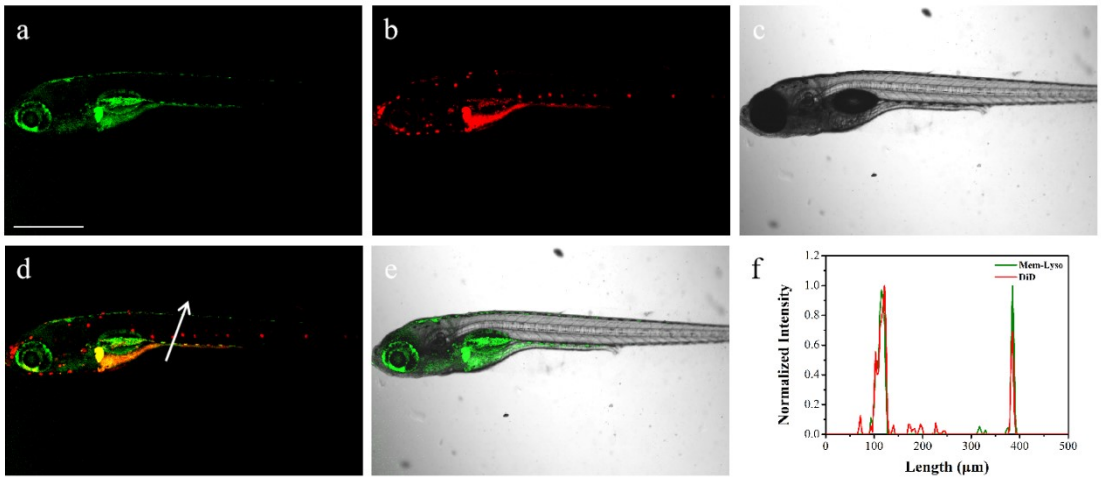



**Fig. S16** a) The fluorescent image zebrafish stained with Mem-Lyso (2 μM, 2h), b) the fluorescent image zebrafish stained with DiD (0.2 μM, 2h), c) the DIC image of zebrafish, d) the merged images of panels a and b, e) the merged images of panels a and c, f) the relative intensity profile of Mem-Lyso and DiD in various pixel points along the white arrow in panel d. $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-560 nm to Mem-Lyso; $\lambda_{ex}$ = 635 nm, $\lambda_{em}$ = 655-700 nm to DiD, Bar = 500 μm.

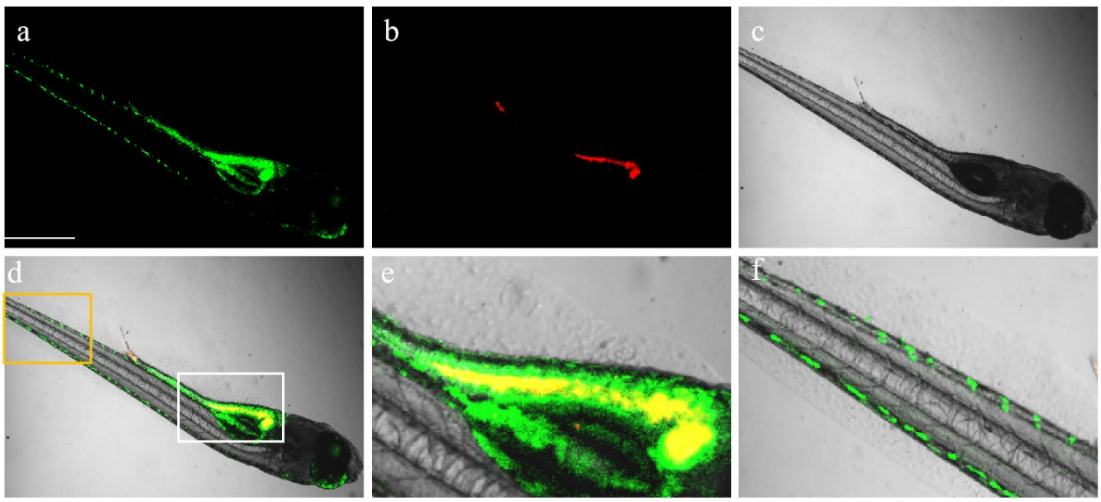



**Fig. S17** a) The fluorescent image zebrafish stained with Mem-Lyso (2 μM, 2h), b) the fluorescent image zebrafish stained with Lyso-NIR(0.2 μM, 2h), c) the DIC image of zebrafish, d) the merged images of panels a, b, and c, e) the enlarged image of white box in panel d, f) the enlarged image of yellow box in panel d. $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-560 nm to Mem-Lyso; $\lambda_{ex}$ = 635 nm, $\lambda_{em}$ = 655-700 nm to Lyso-NIR, Bar = 500 μm.

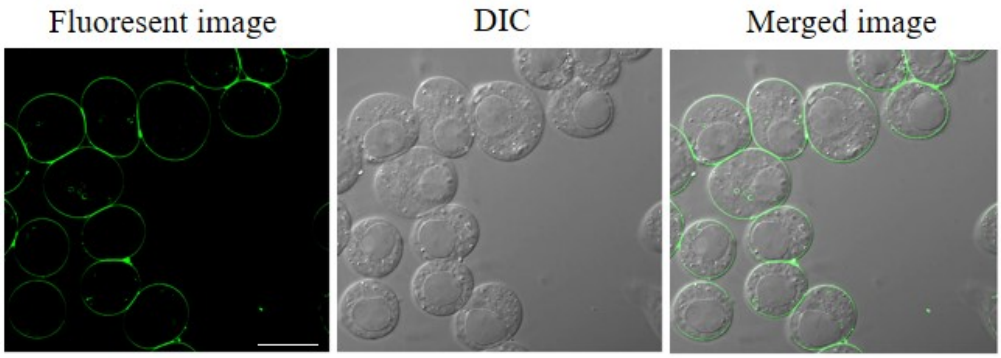



**Fig. S18** Fluorescent images of saturated CL solution pretreated HeLa cells stained with Mem-Lyso (2 μM, 10 min). $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-600 nm, bar = 20 μm.

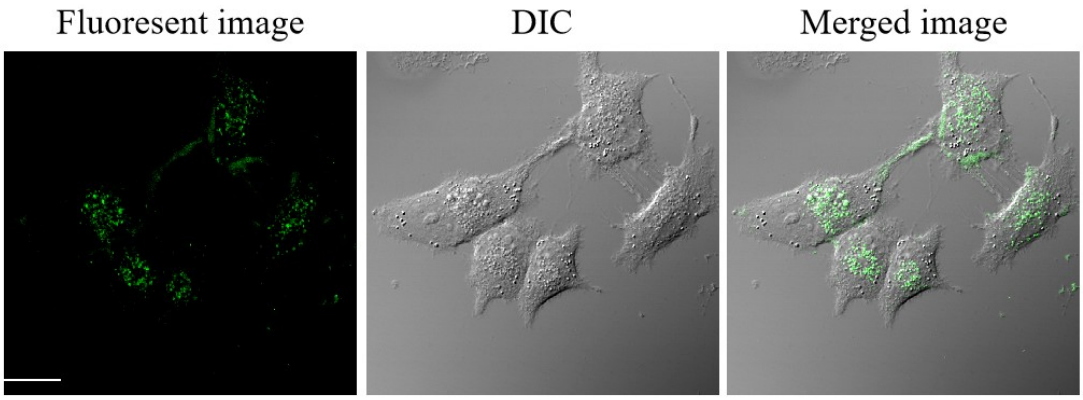



**Fig. S19** Fluorescent images of β-cyclodextrin solution pretreated HeLa cells stained with Mem-Lyso (2 μM, 10 min). $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-600 nm, bar = 20 μm.

## 7. Reference

1. Li, X.; Long, C.; Cui, Y.; Tao, F.; Yu, X.; Lin, W., Charge-Dependent Strategy Enables a Single Fluorescent Probe to Study the Interaction Relationship between Mitochondria and Lipid Droplets. *ACS Sens* **2021**, *6 (4)*, 1595-1603.