

## Electronic Supplementary Information (ESI)

### **Silicon Rhodamine-based Fluorescent Probes for Monitoring Mitochondrial Viscosity Dynamics during Mitophagy**

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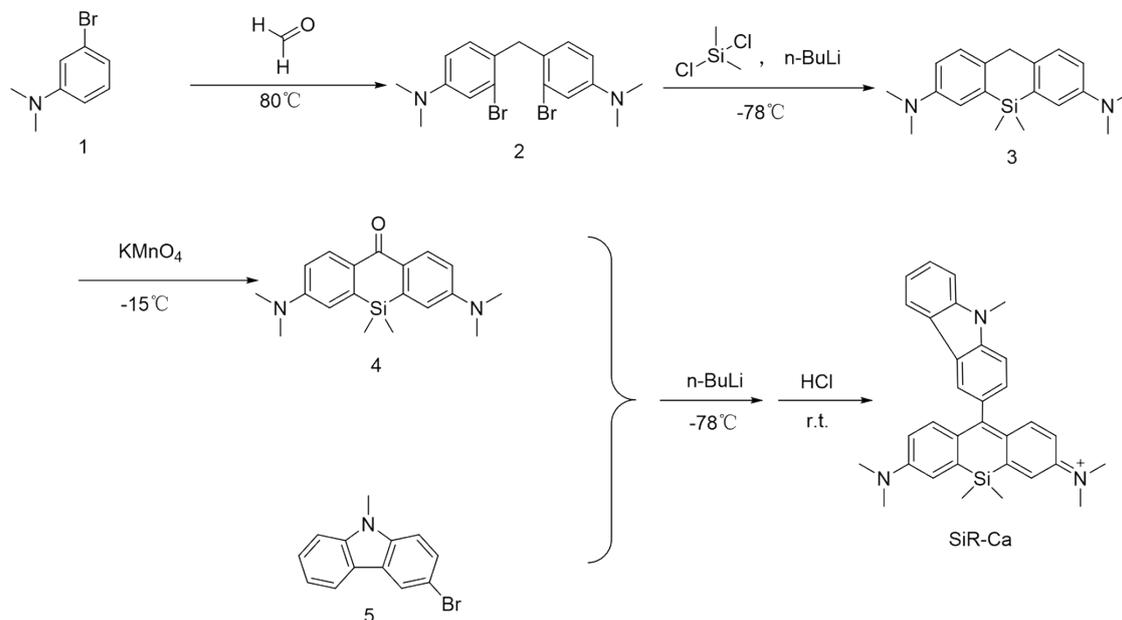
## Table of Contents

<b>1.General information .....</b>	<b>2</b>
<b>2.Synthetic scheme.....</b>	<b>3</b>
<b>3.Synthesis and characterization .....</b>	<b>3</b>
<b>3.1 Synthesis of compound 2 .....</b>	<b>3</b>
<b>3.2 Synthesis of compound 3 .....</b>	<b>3</b>
<b>3.3 Synthesis of compound 4 .....</b>	<b>4</b>
<b>3.4 Synthesis of probe SiR-Ca.....</b>	<b>4</b>
<b>4. Optical experiments.....</b>	<b>5</b>
<b>5. Cell experiments.....</b>	<b>5</b>
<b>6.Additional tables and figures .....</b>	<b>5</b>

## 1. General information

All solvents and reagents were purchased from Shanghai BiDe PharmaTech Co. or J&K Scientific Ltd. and used as received unless otherwise specified. All synthetic steps were performed in sealed containers under nitrogen atmosphere protection. Column chromatography utilized silica gel (200-300 mesh) provided by the Titan Technology Discovery Platform. Liquid nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz or 600 MHz on a Bruker NMR spectrometer. Using TMS as the internal standard,  $^1\text{H}$ -NMR spectra at 400 MHz or 600 MHz and  $^{13}\text{C}$ -NMR spectra at 101 MHz were recorded in deuterated dimethyl sulfoxide and deuterated chloroform. High-resolution mass spectra were obtained using a Thermo Scientific high-resolution mass spectrometer. Ultraviolet-visible absorption spectra were recorded using a Shimadzu WV2550 spectrophotometer. Record the fluorescence emission spectrum using a Shimadzu RF-5301 fluorescence spectrophotometer. The fluorescence lifetime was measured using an Edinburgh FLS980 series fluorescence spectrometer in different solvents. Fluorescent imaging of cells was recorded using a spectral confocal laser scanning microscope (Olympus Fluoview FV-1000). Fluorescence lifetime images of cells were recorded using a super-resolution confocal microscope (TCS SP8 STED 3X).

## 2.Synthetic scheme



**Scheme S1** Synthetic routes of SiR-Ca.

## 3.Synthesis and characterization

### 3.1 Synthesis of compound 2

In a nitrogen-flushed two-neck flask, dissolve 3-bromo-N, N-dimethylaniline (20 g) and 37% formaldehyde solution (30 mL) in glacial acetic acid (153.3 mL). Stir at 80°C for 2 h. Neutralize glacial acetic acid with saturated NaHCO<sub>3</sub> solution, then extract with DCM and H<sub>2</sub>O. Dry the organic layer with Na<sub>2</sub>SO<sub>4</sub> and evaporate. The residue was rapidly purified by silica gel chromatography (DCM/PET = 2:1, v/v), yielding 8.9 g of a white solid as the pure product in 43% yield. <sup>1</sup>H-NMR (400 MHz, Chloroform-d) δ 7.01 (d, J = 2.3 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 6.65 (dd, J = 8.7, 2.6 Hz, 2H), 4.08 (s, 2H), 2.98 (s, 12H).

### 3.2 Synthesis of compound 3

In a nitrogen-flushed two-neck flask, dissolve compound 2 (8 g) in anhydrous THF (200 mL), then cool the solution to -78°C and maintain it for 30 min. Under N<sub>2</sub> atmosphere, n-BuLi (29 mL) was slowly added dropwise from a syringe to the above solution. The mixture was stirred at -78°C for 30 min. dichlorodimethyl-silan (4.5 g) was dissolved in anhydrous THF and slowly added dropwise from a syringe to the

solution. Stirring continued while the mixture was slowly warmed to room temperature. The reaction was allowed to proceed overnight. Terminate the reaction with 2 M HCl, then extract with DCM and H<sub>2</sub>O. Dry the organic layer with Na<sub>2</sub>SO<sub>4</sub> and evaporate the solvent. Unpurified; proceed to the next step.

### 3.3 Synthesis of compound 4

Dissolve compound 3 obtained in the previous step in acetone (100 mL) in a reaction flask. Maintain at 0°C in a low-temperature reactor for 10 min. Add KMnO<sub>4</sub> (5 g, 32 mmol) to the reaction system in small portions over multiple additions, completing the entire addition within 2 h. Then continue the reaction at room temperature, monitoring by TLC until compound 3 is completely converted. Vacuum filtration, solvent evaporation under reduced pressure. The residue was rapidly purified by silica gel chromatography (DCM/PET = 1:1, v/v). A yellow solid weighing 1.9 g was obtained, yielding a 30% yield. <sup>1</sup>H-NMR (400 MHz, Chloroform-d) δ 8.44 (d, J = 8.9 Hz, 2H), 6.89 (d, J = 2.8 Hz, 1H), 6.87 (d, J = 2.8 Hz, 1H), 6.83 (d, J = 2.8 Hz, 2H), 3.13 (s, 12H), 0.51 (s, 6H).

### 3.4 Synthesis of probe SiR-Ca

In a nitrogen-flushed two-neck flask, dissolve compound 5 (120 mg) in anhydrous THF (15 mL), then cool the solution to -78°C and maintain it for 30 min. In an N<sub>2</sub> atmosphere, n-BuLi (0.2 mL), was slowly added dropwise from a syringe to the above solution. The mixture was stirred at -78°C for 30 min. Compound 4 (30 mg), dissolved in anhydrous THF, was then slowly added dropwise from a syringe to the solution. Stirring continued while the temperature gradually rose to room temperature, and the reaction was allowed to proceed overnight. The reaction was terminated with 2 M HCl, followed by extraction with DCM and H<sub>2</sub>O. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated. The residue was rapidly purified by silica gel chromatography (MeOH/DCM = 1:40, v/v), A blue-green solid weighing 20.6 mg was obtained, yielding a 46% yield. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.22 – 8.13 (m, 2H), 7.78 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.53 (t, J = 7.8 Hz, 1H), 7.46 (d, J = 2.8 Hz, 2H), 7.38 (d, J = 8.4 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.08 (d, J = 9.7 Hz, 2H), 6.82 – 6.76 (m, 2H), 3.99 (s, 3H), 3.29 (s, 12H), 0.62 (s, 6H). <sup>13</sup>C-NMR (101

MHz, DMSO-d<sub>6</sub>)  $\delta$  167.47, 154.06, 147.82, 142.20, 132.20, 132.20, 132.09, 129.16, 129.16, 128.32, 122.07, 121.70, 121.69, 114.51, 114.51, 110.01, 67.90, 65.52, 40.90, 38.58, 30.51, 30.30, 28.87, 23.75, 22.91, 19.16, 14.41, 14.06, 11.31.[M]<sup>+</sup>: Calcd. for: C<sub>32</sub>H<sub>34</sub>N<sub>3</sub>Si<sup>+</sup> 488.2517 found: 488.2515.

#### **4. Optical experiments**

A 10 nM SiR-Ca stock solution was prepared using DMSO as the solvent. Working concentrations of SiR-Ca were diluted to 10  $\mu$ M using mixed solvents of varying ethanol-glycerol ratios. Their ultraviolet absorption spectra and fluorescence emission spectra were measured upon excitation by a 633 nm laser. The fluorescence lifetime measurement procedure was identical to the above.

#### **5. Cell experiments**

**Cell culture.** The HeLa cell line originates from the National Cell Line Resource Infrastructure. HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen Corp) supplemented with 10% fetal bovine serum (FBS, Gibco), penicillin (100 U/mL), and streptomycin (100  $\mu$ g/mL). Cells were cultured in a CO<sub>2</sub> incubator at 37°C with 5% CO<sub>2</sub>. Twenty-four h prior to the experiment, cells were seeded into confocal culture dishes.

**Cytotoxicity.** Detection of SiR-Ca cytotoxicity using the CCK-8 assay. HeLa cells were seeded at a density of 1 $\times$ 10<sup>4</sup> cells per well in a 96-well plate containing modified Dulbecco's Eagle Medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100  $\mu$ g/mL). The cells were incubated at 37°C in a 5% CO<sub>2</sub> incubator for 24 h. After removing the culture medium, incubate with medium containing 0, 0.1, 0.2, 0.5, 1, 2 and 5  $\mu$ M SiR-Ca for 12/24 h. Then replace the medium with CCK-8 containing solution (10  $\mu$ L) for 1 h. Measure the absorbance at 450 nm using a Biotek/800TS microplate reader (Berten, USA). Cell viability = (OD value of experimental group - OD value of blank group)/ (OD value of control group - OD value of blank group) \*100%.

#### **6. Additional tables and figures**

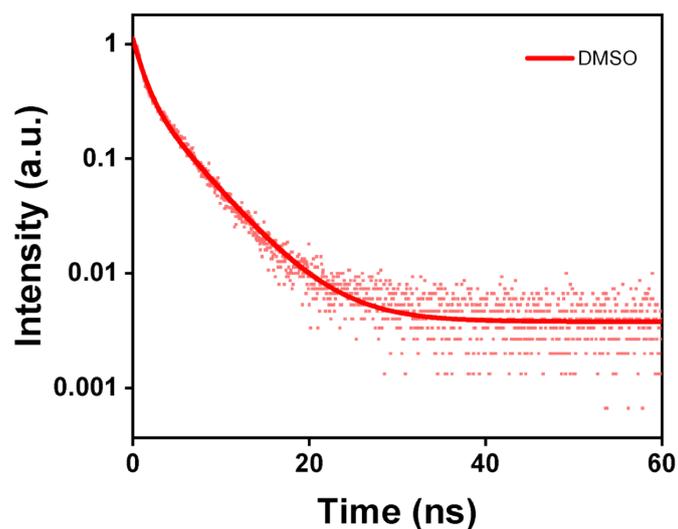


Fig. S1 Fluorescence Lifetime Fitting Plot of SiR-Ca in DMSO

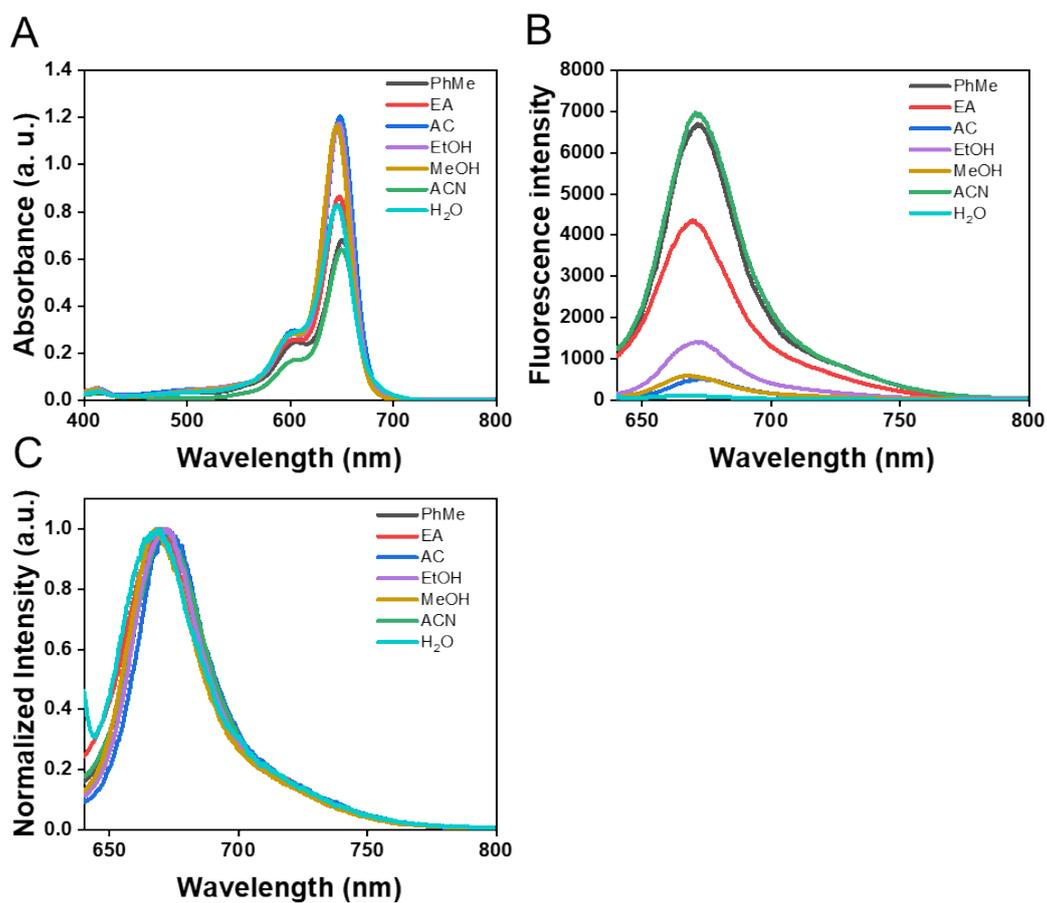
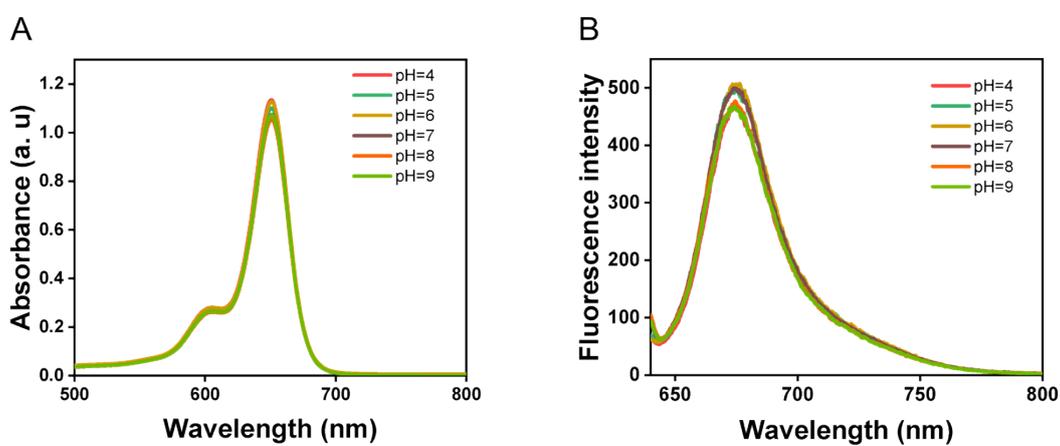
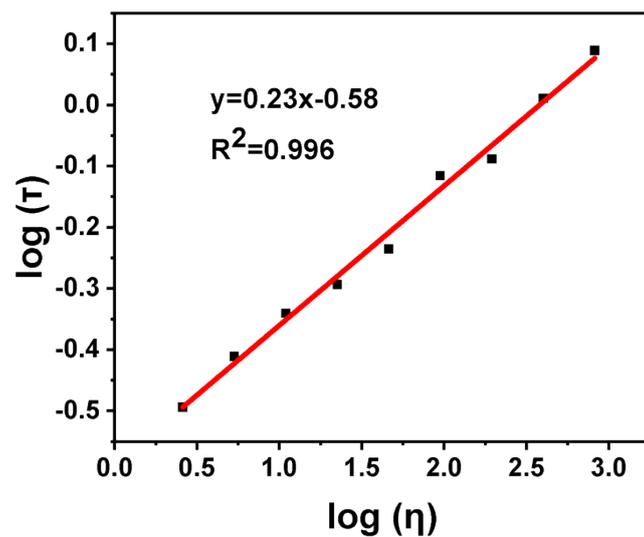


Fig. S2 The absorption (A), emission (B) and normalized emission (C) spectra of SiR-Ca (10  $\mu$ M) in different solvents.  $\lambda_{\text{ex}} = 630$  nm

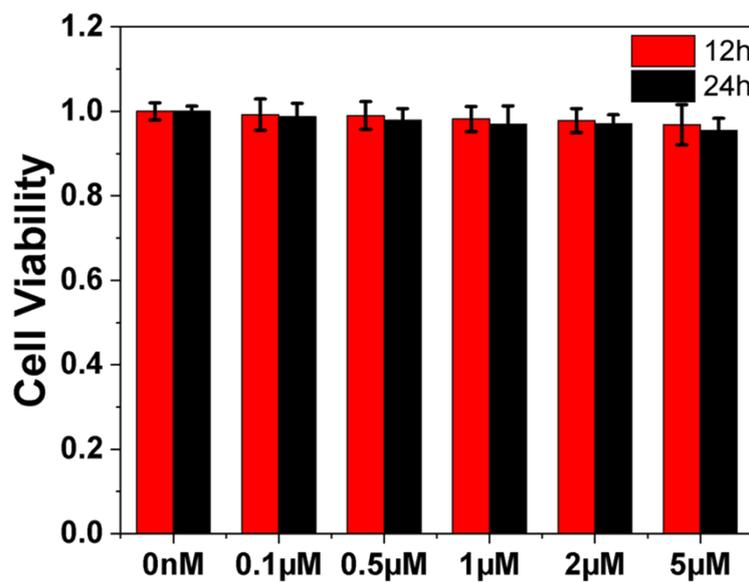
**Table. S1** The photophysical data of probes SiR-Ca

	$\epsilon$ ( $10^5 \text{ M}^{-1} \text{ cm}^{-1}$ )	$\lambda_{\text{abs, max}}$	$\lambda_{\text{em, max}}$	$\Phi(\%)$
PhMe	0.68	650	671.6	0.36
EA	0.86	648	669.4	0.41
AC	1.21	648	672.6	0.35
EtOH	1.17	648	672.4	0.20
MeOH	1.16	650	668.2	0.32
ACN	0.64	650	670.6	0.15
H <sub>2</sub> O	0.83	648	668.6	0.08

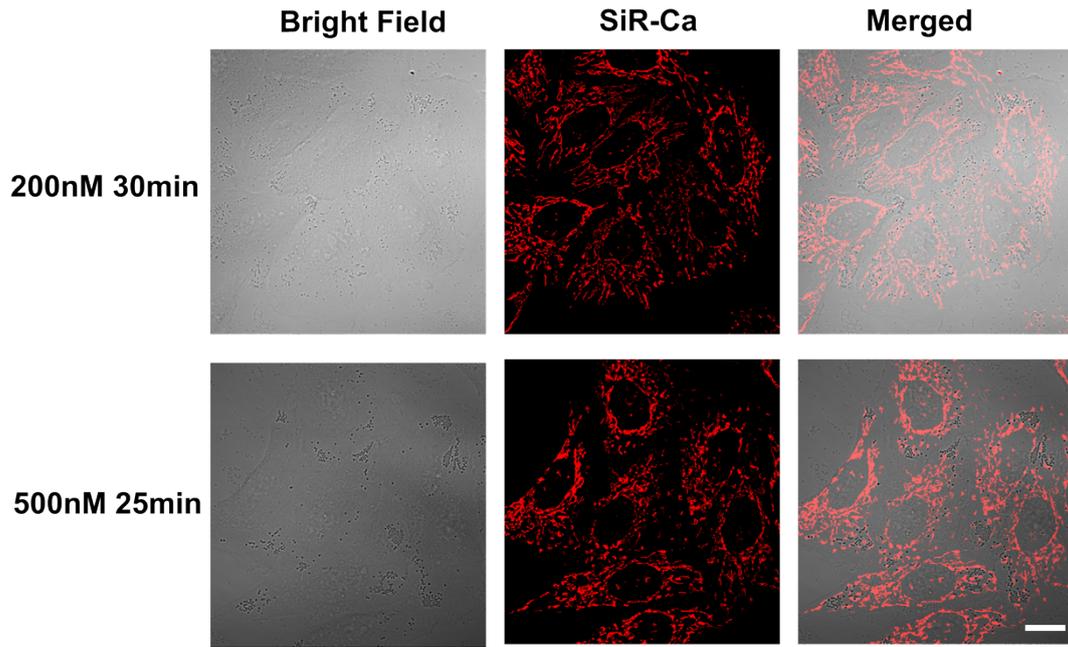
**Fig. S3** The absorption (A) and emission (B) spectra of SiR-Ca (10  $\mu\text{M}$ ) in solvents at different pH values.  $\lambda_{\text{ex}} = 630 \text{ nm}$ .



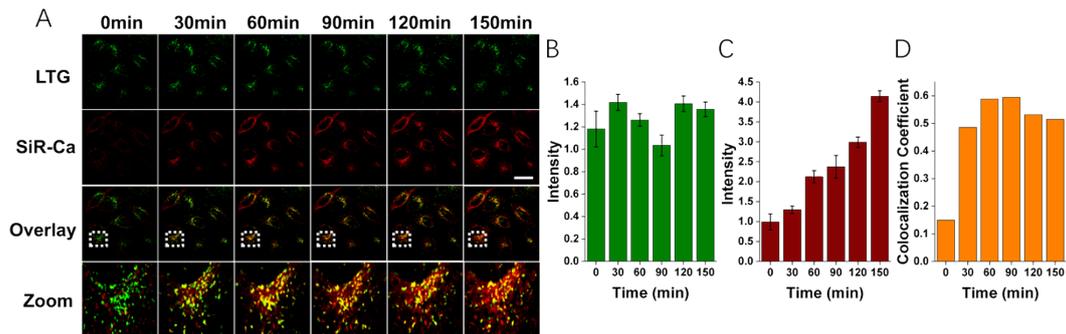
**Fig. S4** Linear plot of fluorescence lifetime versus logarithmic viscosity.



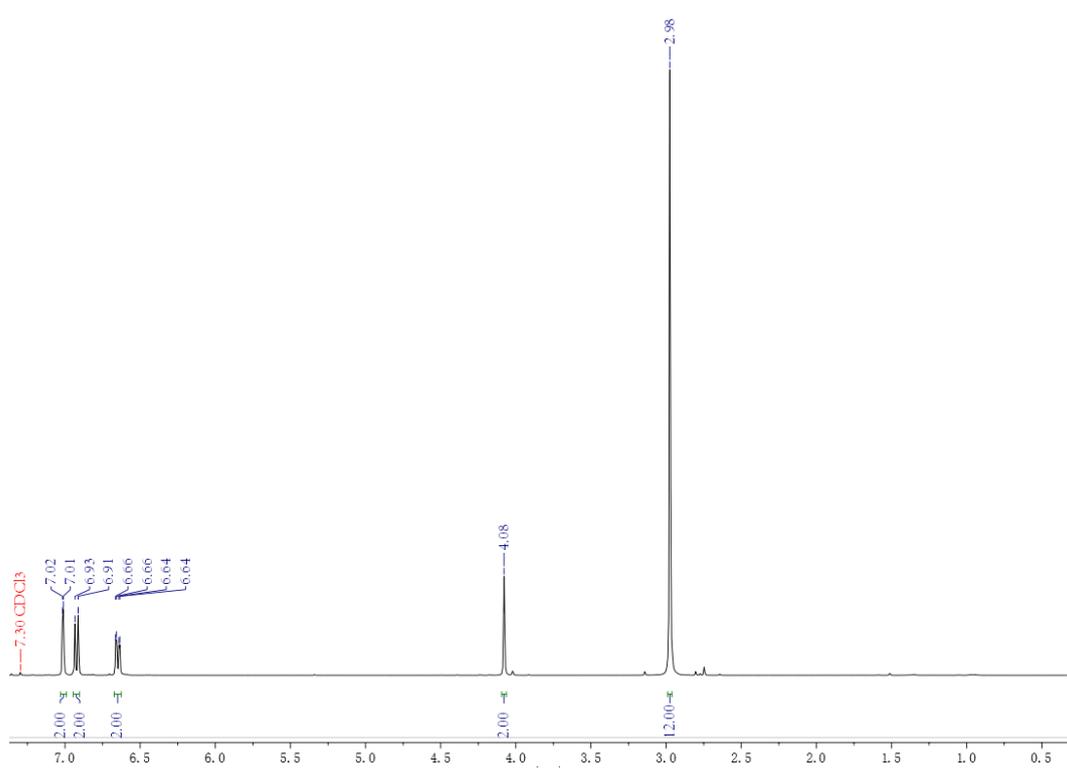
**Fig. S5** Cytotoxicity assay of SiR-Ca after 12 h/24 h incubation quantified by the CCK-8 assay.



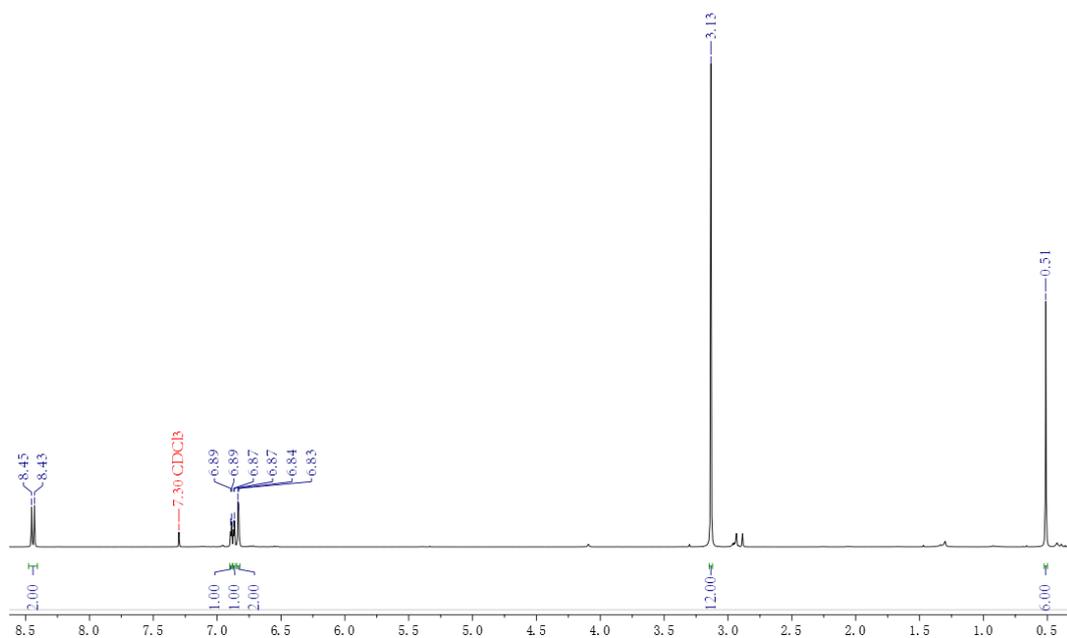
**Fig. S6** Staining of SiR-Ca at different concentrations in HeLa Cells, scable bar 20  $\mu\text{m}$ .



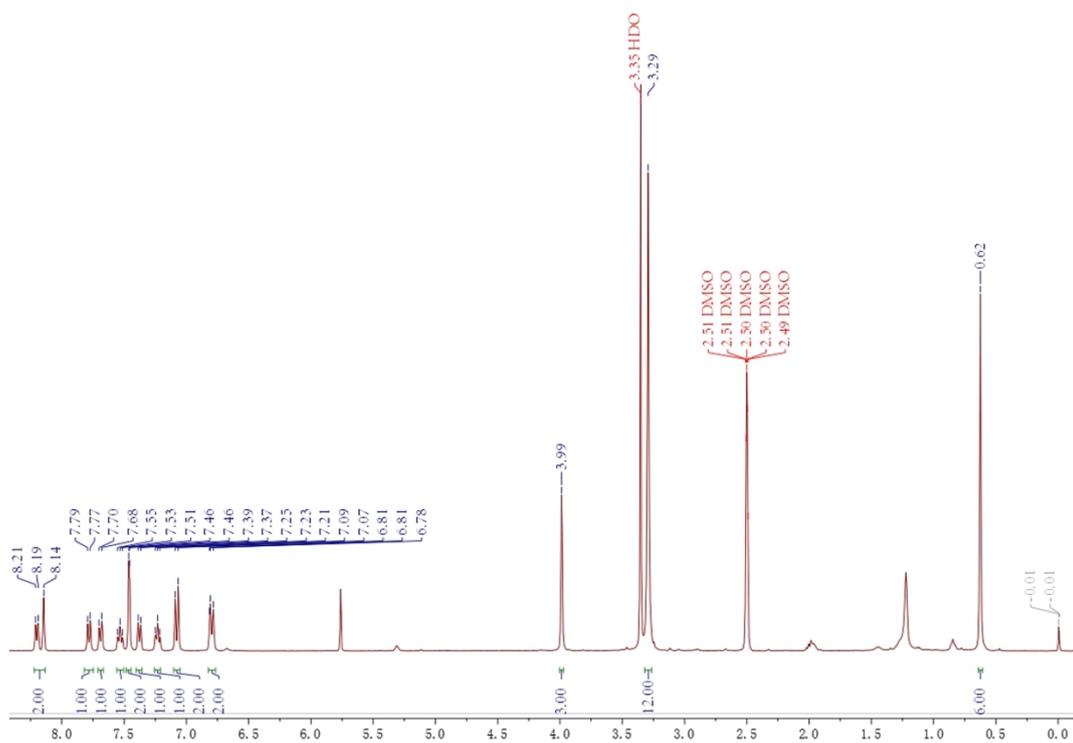
**Fig. S7** Dynamic changes in fluorescence intensity and colocalization during starvation treatment. (A) HeLa cells were costained with  $0.5\mu\text{M}$  LTG and  $0.5\mu\text{M}$  SiR-C8 for 30 min, then subjected to starvation treatment for different times. (B) Time-dependent fluorescence intensity of LTG corresponding to panel (A). (C) Time-dependent fluorescence intensity of SiR-Ca corresponding to panel (A). (D) Colocalization coefficient of LTG and SiR-Ca corresponding to panel (A). Scale bars = 20  $\mu\text{m}$ .



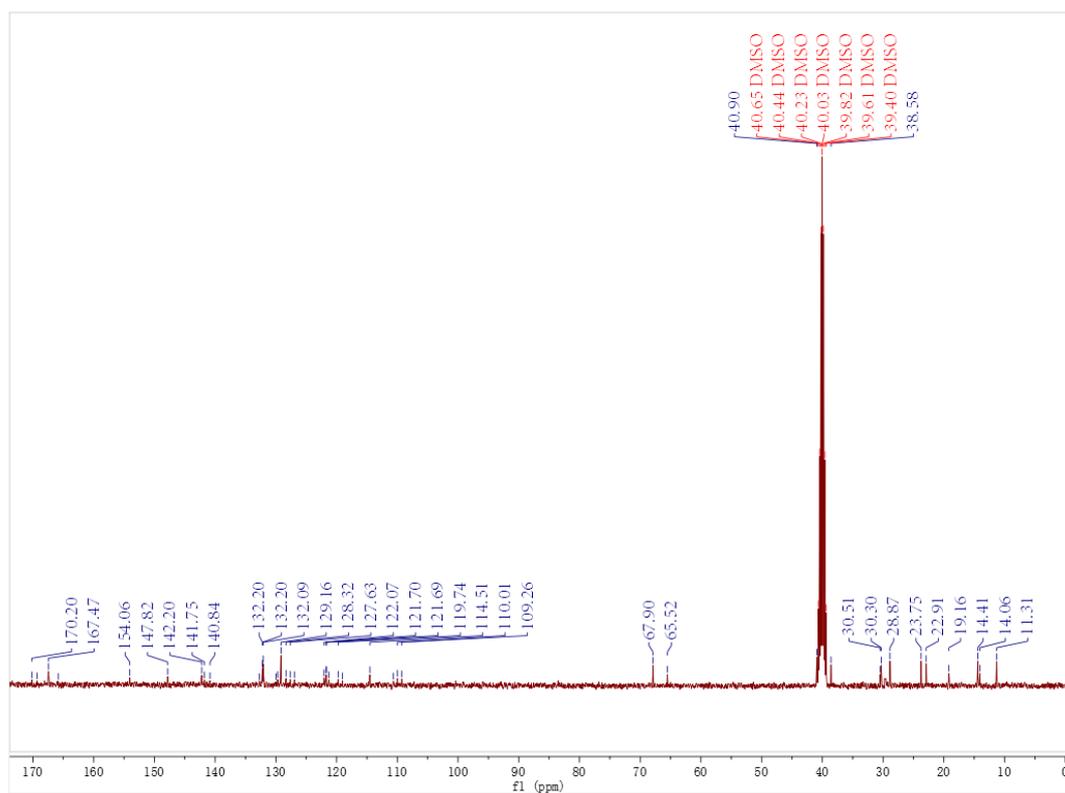
**Fig. S8** <sup>1</sup>H-NMR spectra of compound 2 (CDCl<sub>3</sub>).



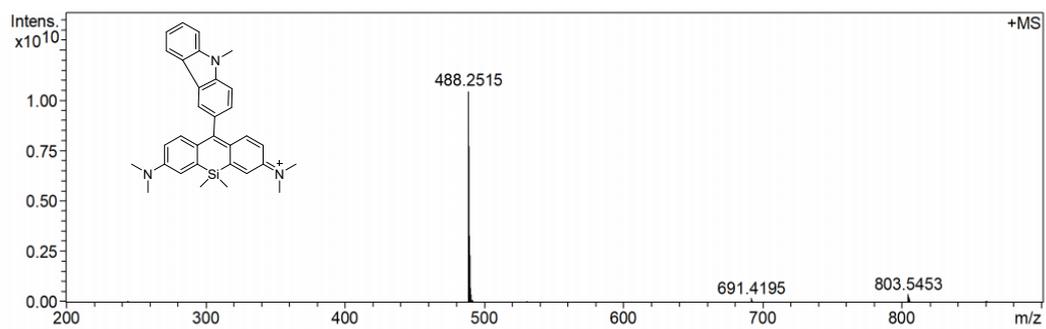
**Fig. S9** <sup>1</sup>H-NMR spectra of compound 4 (CDCl<sub>3</sub>).



**Fig. S10** <sup>1</sup>H-NMR spectra of compound SiR-Ca (DMSO-d<sub>6</sub>).



**Fig. S11** <sup>13</sup>C-NMR spectra of compound SiR-Ca (DMSO-d<sub>6</sub>).



**Fig. S12** HRMS spectra of SiR-Ca.