Electronic Supplementary Information

COX2-targeting cancer-specific fluorescent probe for hydrogen sulfide detection in living cells, *Caenorhabditis elegans*, and zebrafish

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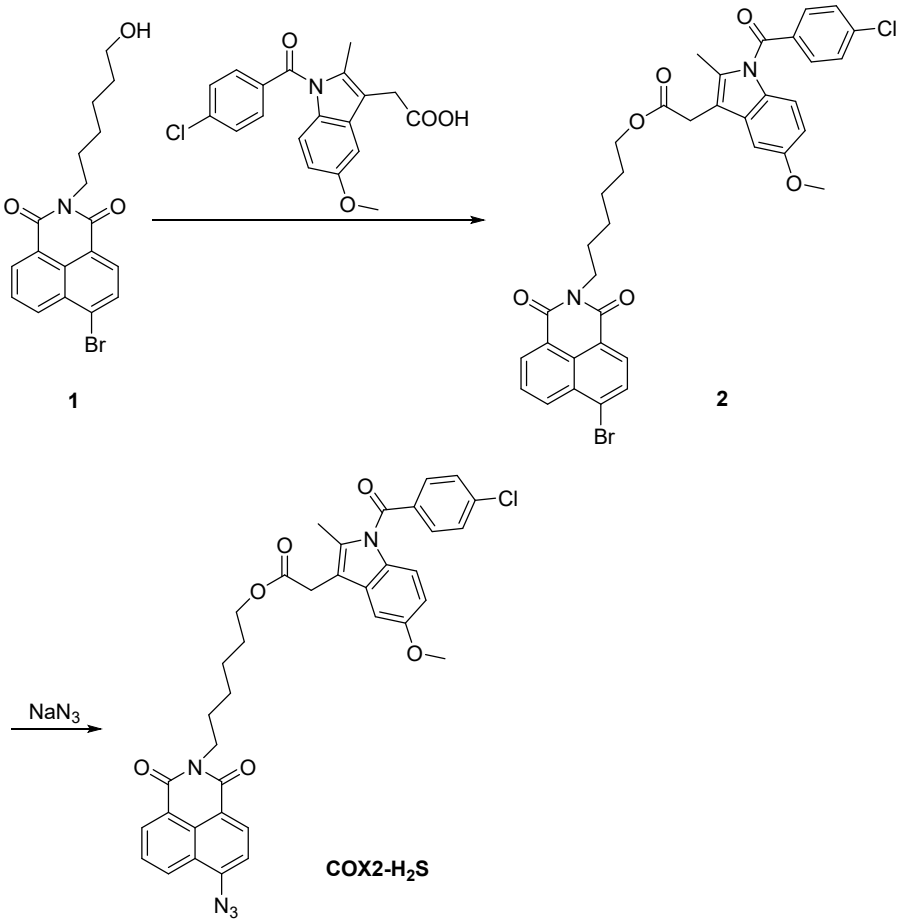
CONTENTS

1. Materials and instruments

Unless otherwise stated, all chemical reagents for synthesis were purchased from commercial suppliers and used without further purification. Flash chromatography was carried out on silica gel (200-300 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz and 125 MHz spectrometer with tetramethylsilane (TMS) as internal standard, respectively (Germany). Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. Mass spectra were performed with Agilent Xevo TQ-S/QTOF (USA). UV-vis absorption spectra were acquired with UV-240ZPC (Japan). Fluorescence spectra were acquired with F97XP spectrophotometer (China). The confocal fluorescence images were analyzed by a confocal laser scanning microscope (Leica SP8 , Germany). The starting material **1**[1] was prepared by the procedures described in literature method.

2. Synthesis of COX2-H_2S



Scheme S1. Synthetic route of **COX2-H_2S**

3. Mass spectrum analysis of COX2-H_2S in the presence of H_2S

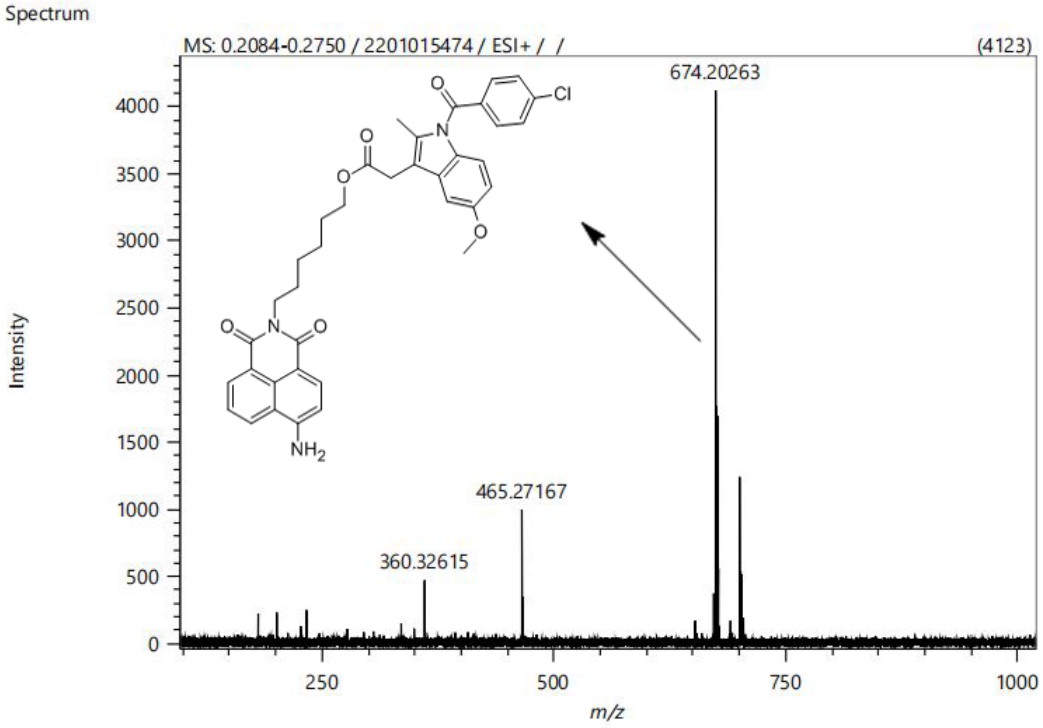


Fig. S1 The mass spectrum after reaction of **COX2-H_2S** with H_2S

4. ^{1}H NMR, ^{13}C NMR and MS spectra of COX2-H_2S

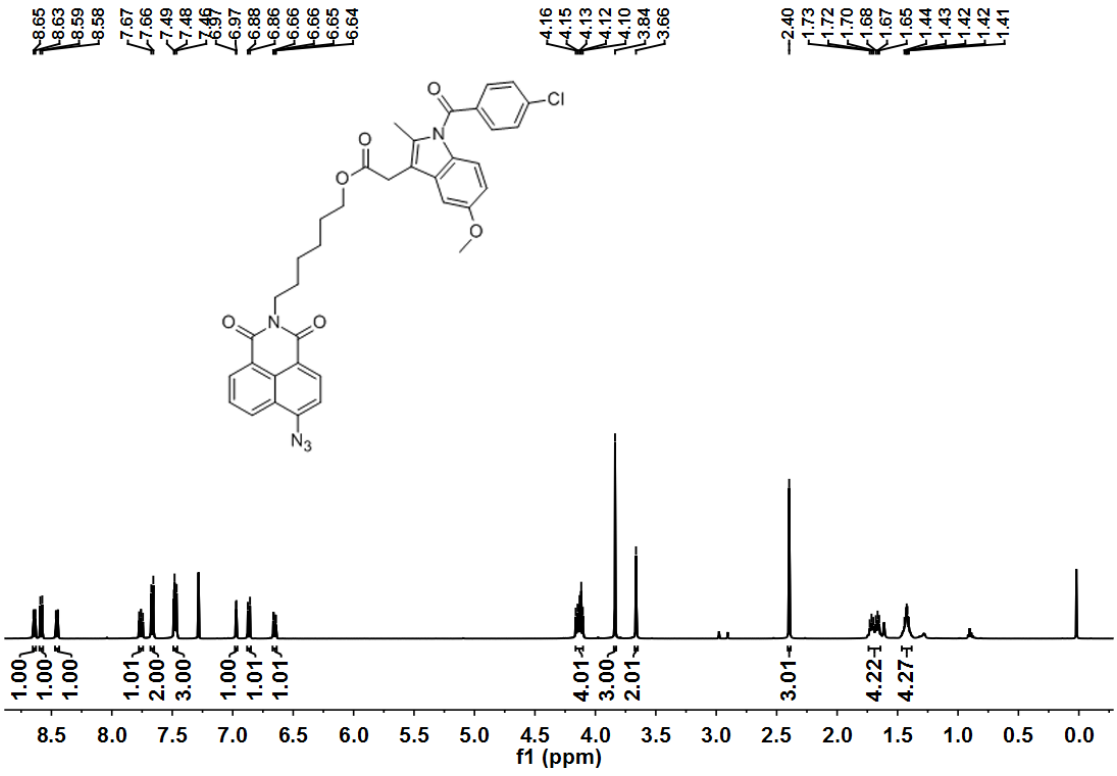


Fig. S2 ^{1}H NMR spectra of **COX2-H_2S** in $CDCl_3$

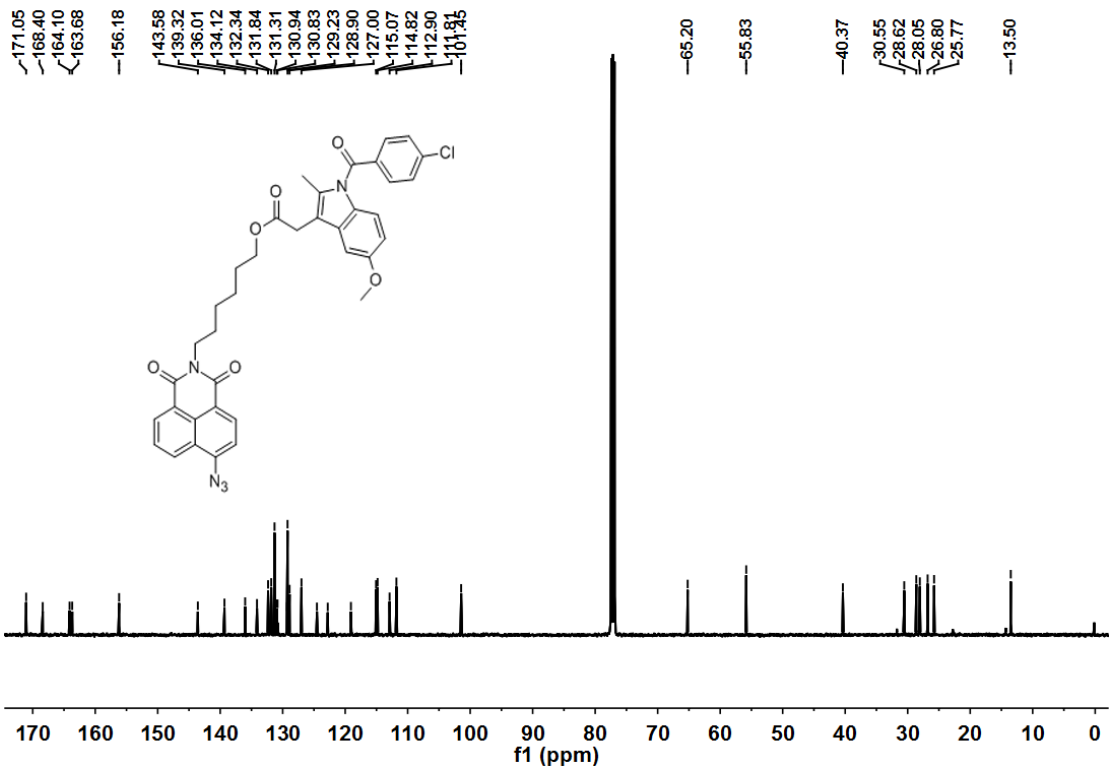


Fig. S3 ^{13}C NMR spectra of **COX2-H_2S** in $CDCl_3$

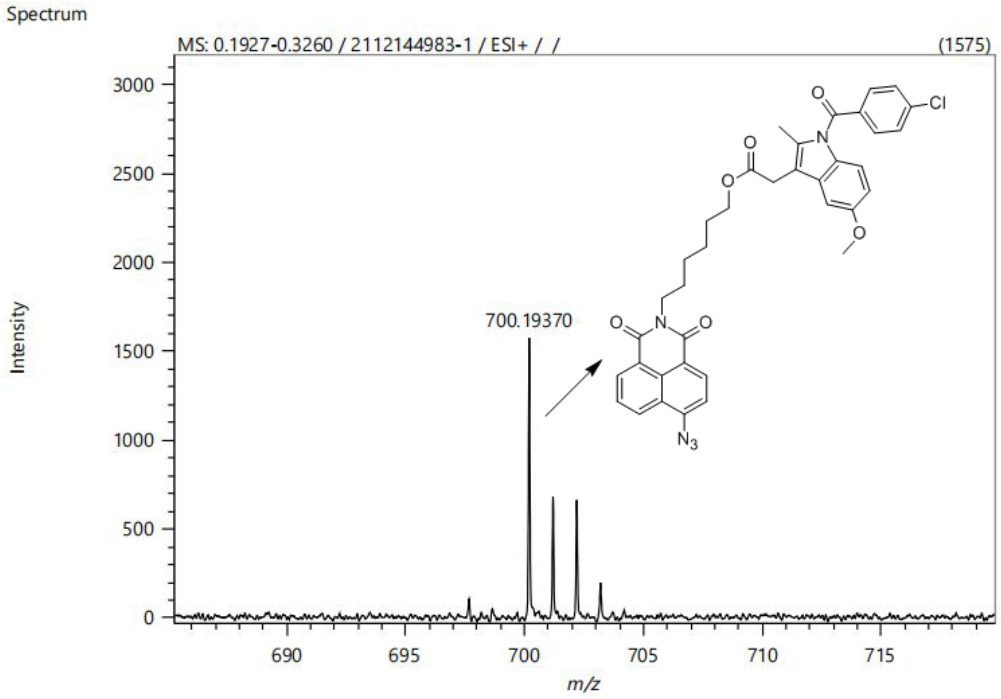


Fig. S4 HRMS spectra of probe **COX2-H_2S**

5. Sample preparation and measurements

A stock solution of **COX2-H_2S** (2 mmol/L) was prepared by dissolving in DMSO. Fluorescence measurements were carried out in PBS (0.01 mol/L, pH 7.4, 60% DMSO)

buffer solution. Various bio-analytes (H_2S, $S_2O_5^{2-}$, H_2O_2, HSO_3^-, NO_2^-, $ONOO^-$, HSO_4^-, $^1O_2$, CH_3COO^-, SO_3^{2-}, ·OH, NO_3^-, Cys, Hcy, GSH) were used for the selectivity tests.

6. The quantum yield (Φ) of COX2-H_2S

Table S1 The Quantum Yield（Φ）of **COX2-H_2S**

	λ_{ex} = 450 nm (refercence: rhodamine B[2])	
Quantum Yield(Φ)	Without H_2S	With H_2S
	Φ = 0.0524	Φ = 0.453

7. Cell viability assay

RAW264.7 cells and 9L/lacZ cells were cultured in DMEM supplemented with 1% penicillin-streptomycin and 10% FBS in an atmosphere of 5% CO_2 and 95% air at 37℃. The cells were seeded in a 96-well plate with a density of 6000 cells per well and allowed to adhere for 24 h. Subsequently, the cells were incubated with various concentrations of the probe (0, 0.5, 1, 2, 3, 4 and 5 μM) at 37℃ in an atmosphere of 5% CO_2 and 95% air for 24 h. After that, 10 μL CCK-8 reagent was added to each well and incubated for 2 h. The absorbance was measured at 450 nm on a microplate reader (Cyation5, BioTek, USA). Cell viability rate was calculated according to following equation: Viability =(mean Abs. of treated wells/mean Abs. of control wells) ×100%. Results are mean ± SD, n = 3.

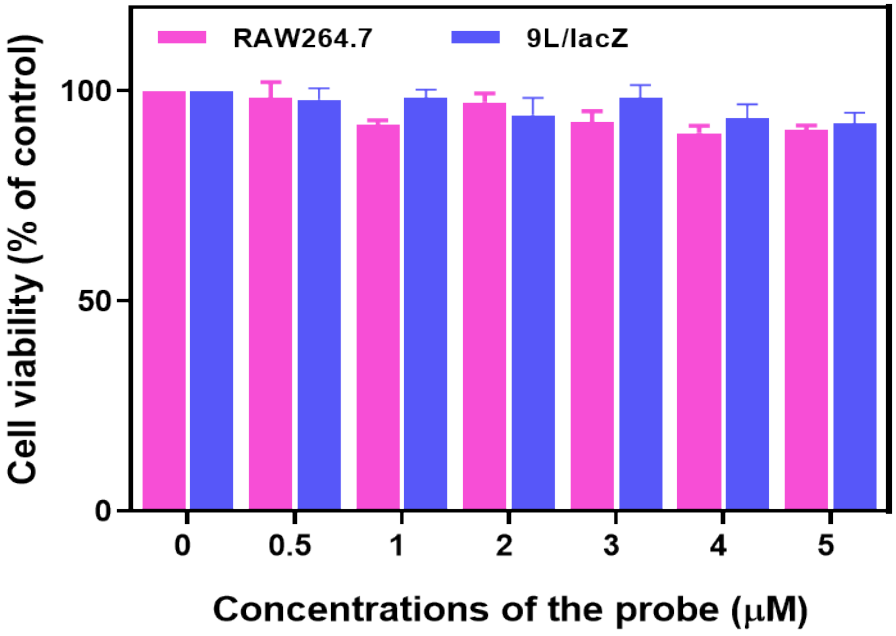


Fig. S5 Cell viability under different concentrations of **COX2-H_2S**.

8. Cell culture and confocal imaging

Cells were from the Affiliated Hospital of Southwest Medical University. RAW264.7, HuH-7, DSL-6A/C1 and 9L/lacZ cells were cultured with DMEM (Gibco) and A549, KYSE150 cells were cultured with RPMI 1640 medium (Gibco), which contained with 10% fetal bovine serum (Hyclone) and 1% penicillin (100 U/mL)-streptomycin (100 μg/mL) (Solarbio) in a humidified incubator containing 5/95 (v/v) of CO_2/air at 37℃. Before imaging experiments, the cells were detached with a treatment of recombinant trypsin digestive fluid (BasalMedia) and suspended in culture media. Then the cells suspension were seeded in 20 mm glass confocal cell culture dishes for 24 h. Note: The cells were washed three times with PBS buffer before proceeding to the next step. The confocal fluorescence images were analyzed by a confocal laser scanning microscope (Leica SP8, Germany). The excitation wavelength was 448 nm and 552 nm, and the emission was collected by a 500-600 nm and 620-680 nm long pass filters, respectively. All fluorescence images were obtained by a confocal laser scanning microscope at magnification (5x, 10x and 63x). Fluorescence images were analyzed using ImageJ software.

9. Time dependent fluorescent imaging of 9L/lacZ cells

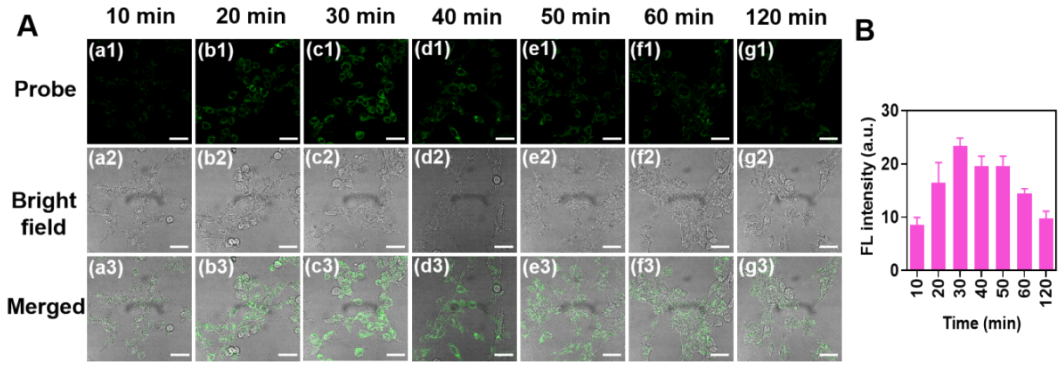


Fig. S6 Time dependent confocal laser scanning microscopy images of 9L/lacZ cells. (A) 9L/lacZ cells were treated with 0.5 μmol/L probe for 10 min, 20 min, 30 min, 40 min, 50 min, 1 h, 2 h, respectively. λex = 448 nm, λem = 500–600 nm. (B) The semi-quantitative analysis of fluorescence images in a1-g1. Scale bar: 25 μm. pH = 7.2.

10. H_2S-concentration fluorescent imaging of 9L/lacZ cells

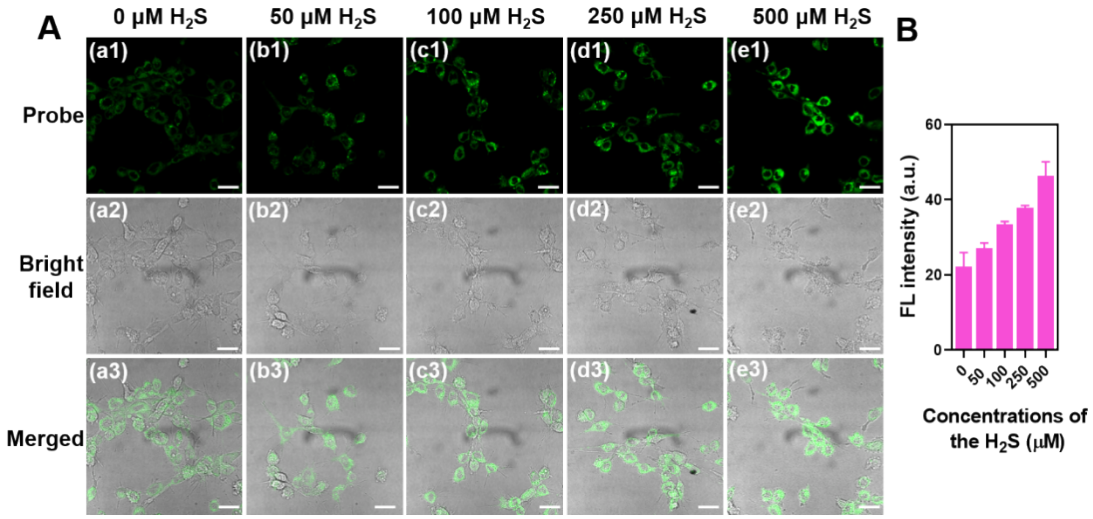


Fig. S7 Fluorescence imaging of H_2S in 9L/lacZ cells. (A) (a1-e1) Fluorescence images, (a2-e2) Bright field images and (a3-e3) Merged images. (a1-e3) Probe-treated (0.5 μmol/L, 30 min) 9L/lacZ cells incubated with H_2S (0 μmol/L, 50 μmol/L, 100 μmol/L, 250 μmol/L, 500 μmol/L) for 1 h, respectively. λex = 448 nm, λem = 500–600 nm. (B) The semi-quantitative analysis of fluorescence images. FL: Fluorescence. Scale bar: 25 μm. pH = 7.2.

11. Intracellular co-localization of COX2-H_2S

Probe and Tracker intracellular co-localization fluorescence imaging of 9L/lacZ cells. 9L/lacZ cells were pre-stained with 100 nmol/L MitoRed (Keygen, KGMP0071, λ_{ex} = 579 nm, λ_{em} = 600 nm) for 30 min, 1 μmol/L ER-Red (Keygen, KGMP016-1, λ_{ex} = 587 nm, λ_{em} = 615 nm) for 30 min and 1 μmol/L LysoRed (Keygen, KGMP006,λ_{ex} = 575 nm, λ_{em} = 600 nm) for 1 h, respectively. Then, the cells were washed three times with PBS buffer, incubated with the probe (0.5 μmol/L, 30 min) and washed with PBS buffer three times again for imaging.

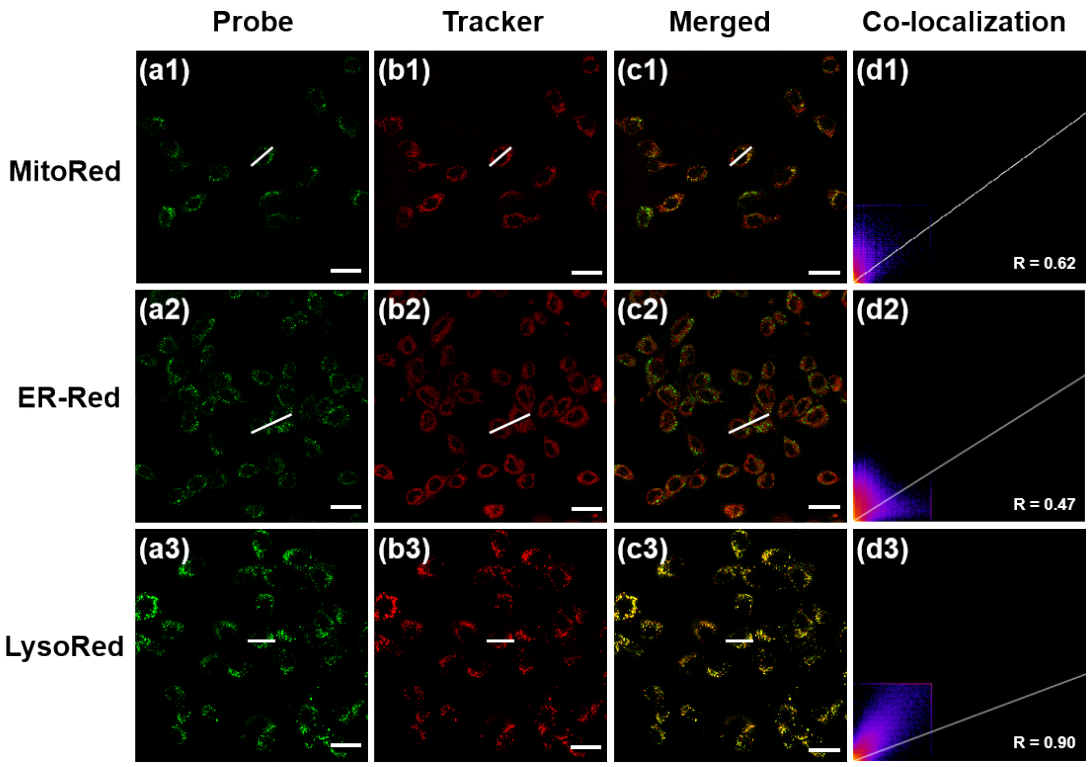


Fig. S8 Co-localization fluorescence images of the probe **COX2-H_2S** and tracker in 9L/lacZ. (a1-a3) Fluorescence images of the probe, (b1-b3) Fluorescence images of tracker, (c1-c3) Merged images. (d) Intensity correlation plot of the probe and commercial dyes. Green channel: λ_{ex} = 448 nm, λ_{em} = 500–600 nm; Red channel: λ_{ex} = 552 nm, λ_{em} = 620–680 nm. Scale bar: 25 μm. pH = 7.2.

12. Targeted imaging of 9L/lacZ cells in the presence of RAW264.7 cells.

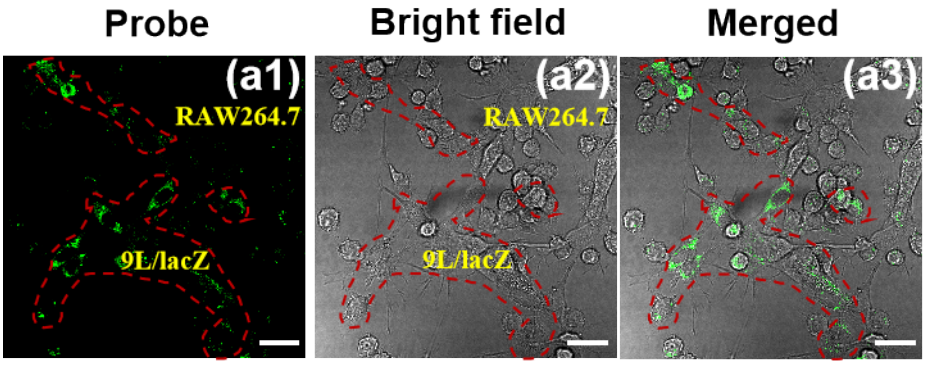


Fig. S9 Confocal laser scanning microscopy images of co-cultured RAW264.7 cells and 9L/lacZ cells. (a1) Fluorescence images, (a2) Bright field images, (a3) Merged images. Scale bar: 25 μm. pH = 7.2.

13. *C. elegans* and zebrafish experiments

5-day-old wild-type *C. elegans* were obtained from the School of Pharmacy at South West Medical University (Luzhou, China). *C. elegans* incubated with the probe (10 μmol/L) for 1 h. *C. elegans* preloaded on the probe and further incubated in PBS

buffer with H_2S (500 μM, 1 h).

2-day-old zebrafish were obtained from the Public Experimental Technology Center at South West Medical University (Luzhou, China). Zebrafish only incubated with 10 μM probe for 1 h. Probe-treated zebrafish incubated with 500 μmol/L H_2S for 1 h.

Reference:

1. J. Lee, H. S. Kim, P. Jangili, H. G. Kang, A. Sharma and J. S. Kim, *ACS Appl. Bio Mater.*, 2021, **4**, 2073–2079.

2. A. M. Brouwer, *Pure Appl. Chem.*, 2011, **83**, 2213–2228.