Supporting Information

Ratiometric detection and imaging of hydrogen sulfide in mitochondria based on a cyanine/naphthalimide hybrid fluorescent probe

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Materials and instruments

IR-780 and Phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich. 4-Bromo-1,8-naphthalic anhydride was purchased from Liaoning Liangang Dye Chemical Co. Ltd. Sodium sulfide was purchased from sigma-aldrich Ltd. 1,2-Bis(2-aminoethoxy)ethane and N,N-diisopropylethylamine (DIEA) were purchased from J&K. Arginine (Arg), lysine (Lys), cysteine (Cys), glutathione (GSH), histidine (His), homocysteine (Hcy), and hexadecyl trimethyl ammonium bromide (CTAB) were purchased from Beijing XinJingKe Biotechnology Ltd. LysTracker Red (LysRed) was purchased from beyotime Ltd. MitoTracker Red (MTRed) was purchased from KeyGEN BioTECH Ltd. All other reagents were purchased from Beijing Chemical Plant. HeLa (human cervical cancer cell line), HEK-293 (human renal epithelial cell line), LoVo (human colon cancer cell line), HCT-116 (human colorectal cancer cell line) were purchased from the Cell Bank of Shanghai Bioscience Center, Chinese Academy of Sciences.

High-resolution mass spectra (HR-MS) were measured on a solarix FT-ICR mass spectrometer (Bruker). ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Avance III 400 and 500WB nuclear magnetic resonance spectrometer (Bruker). Fluorescence spectra were collected on a F-4600 fluorescent spectrophotometer (Hitachi). Absorption spectra were recorded on a UH5300 spectrophotometer (Hitachi). Liquid Chromatography were recorded on a LC-20A high performance liquid chromatography (Shimadzu). Fluorescence images were recorded on an FV3000-IX83 confocal microscope (Olympus). The absorbance for Cell Counting Kit-8 (CCK-8) analysis were recorded on a SpectraMax M5 Reader (Molecular Devices).

Synthesis of L2B (Scheme S1)

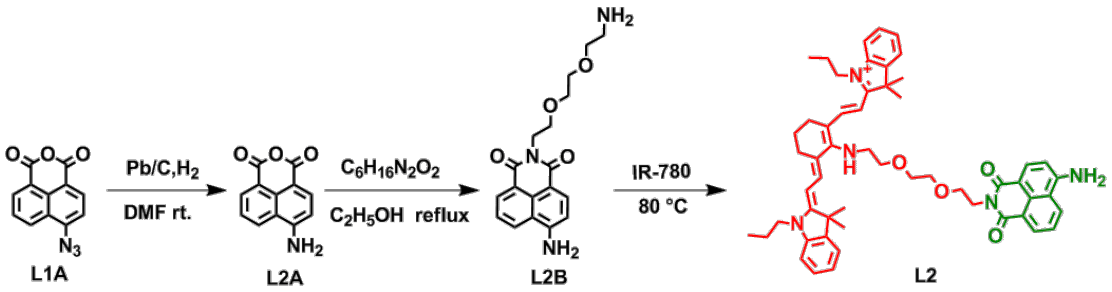
300.0 mg L2A (1.4 mmol) was added in 100.0 mL ethanol, stirred under reflux for 10 min, then 212.0 μL (1.4 mmol) 1,2-bis(2-aminoethoxy)ethane was added and refluxed for 3 hours. The mixture was filtered under reduced pressure, and the supernatant was discarded, the residue was taken to give a pale yellow solid to obtain

L2B. MS (ESI): m/z calcd for $C_{18}H_{21}N_3O_4$ $[M]^+$: 344.15; found, 344.05.

Synthesis of L2 (Scheme S1)

89.6 mg (0.271 mmol) L2B was dissolved in 5.0 mL acetonitrile, then IR-780 (180.8 mg, 0.271 mmol), DIEA (26.6 μL, 0.271 mmol) and 1.3 mL DMF were added. The resulting mixture was heated to 80 °C for 20 hours. After evaporated the solvent under reduced pressure, the residue was purified by silica column chromatography and eluted with dichloromethane/methanol (100:1, v/v) to obtain blue solid L2. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.52 (dd, J = 8.4, 1.2 Hz, 1H), 8.42 (dd, J = 7.3, 1.1 Hz,1H), 8.19 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 13.1 Hz, 2H), 7.50 (dd, J = 8.4, 7.2 Hz, 1H), 7.29(td, J = 7.7, 1.2 Hz, 2H), 7.26 (dd, J = 7.5, 1.2 Hz, 4H), 7.13 – 7.06 (m, 2H), 6.91 (d, J = 7.9Hz, 2H), 6.86 (d, J = 8.3 Hz, 1H), 5.69 (d, J = 13.1 Hz, 2H), 5.29 (s, 5H), 4.39 (t, J = 5.7 Hz,2H), 3.85 (dd, J = 9.9, 6.3 Hz, 5H), 3.74 (s, 4H), 3.67 (s, 4H), 3.19 (qd, J = 7.5, 4.3 Hz, 1H),2.44 (t, J = 6.4 Hz, 4H), 1.90 – 1.76 (m, 6H), 1.59 (d, J = 1.8 Hz, 13H), 1.49 (d, J = 6.7 Hz,3H), 1.25 (s, 11H), 1.03 (t, J = 7.4 Hz, 7H), 0.91 – 0.75 (m, 9H) (Figure S3). HR-MS (ESI): m/z calcd for $C_{54}H_{64}N_5O_4^+$ $[M]^+$: 846.4953; found, 846.4954 (Figure S4).

Scheme S1 Synthesis route of L2.



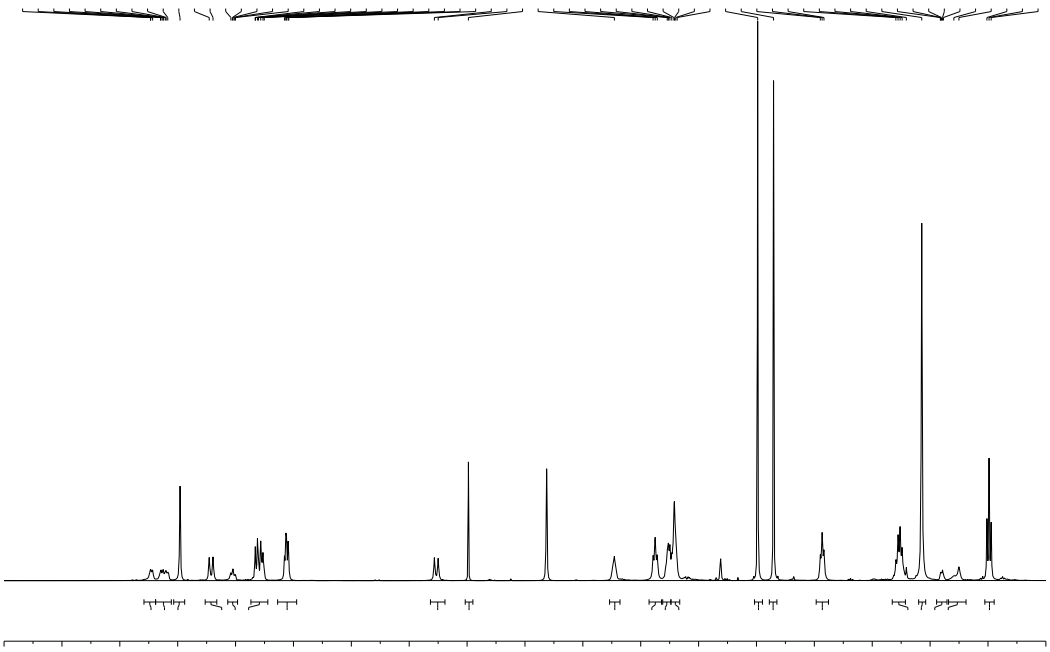


Fig. S1 ^{1}H NMR of L1.

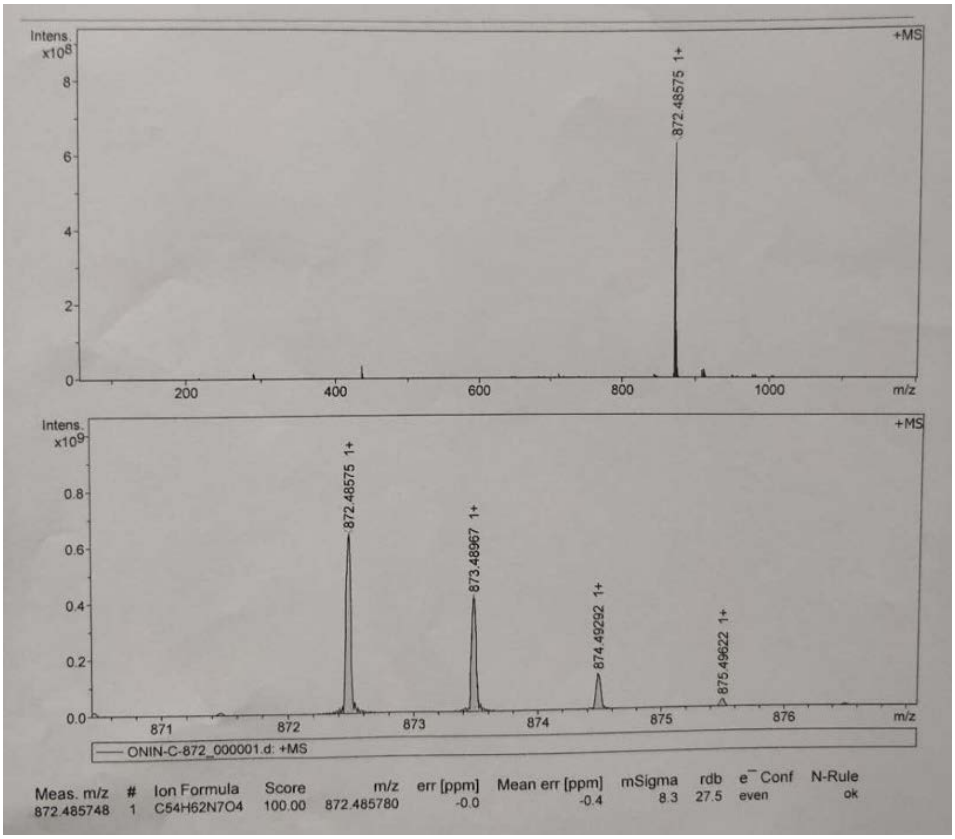


Fig. S2 HR-MS (ESI) of L1.

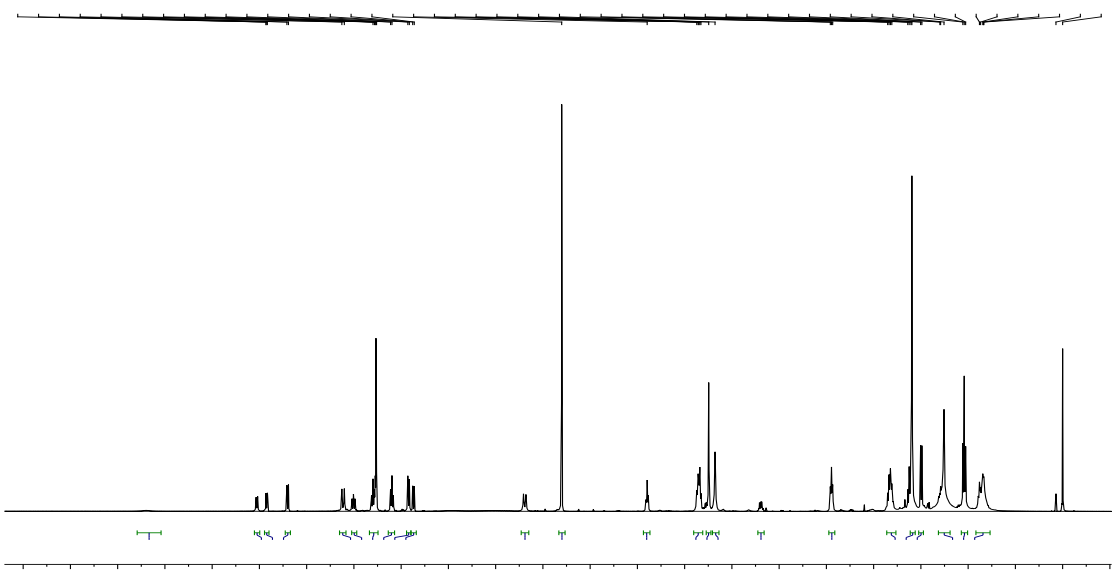


Fig. S3 ^{1}H NMR of L2.

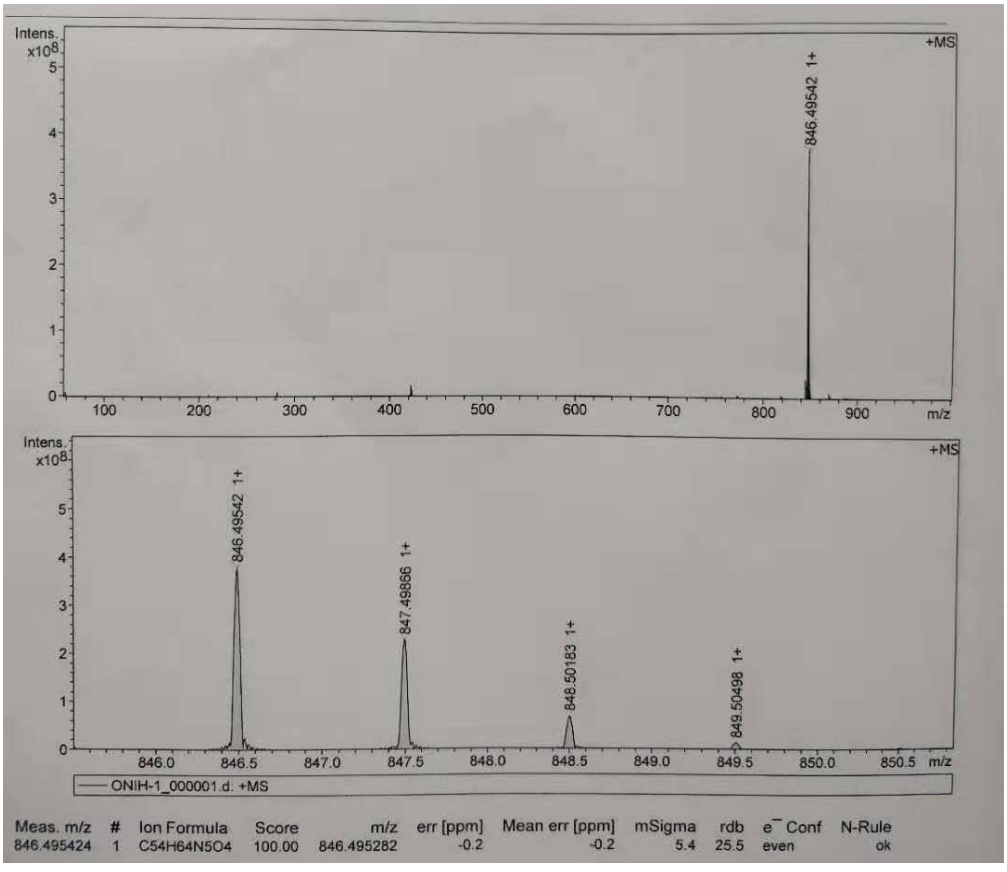


Fig. S4 HR-MS (ESI) of L2.

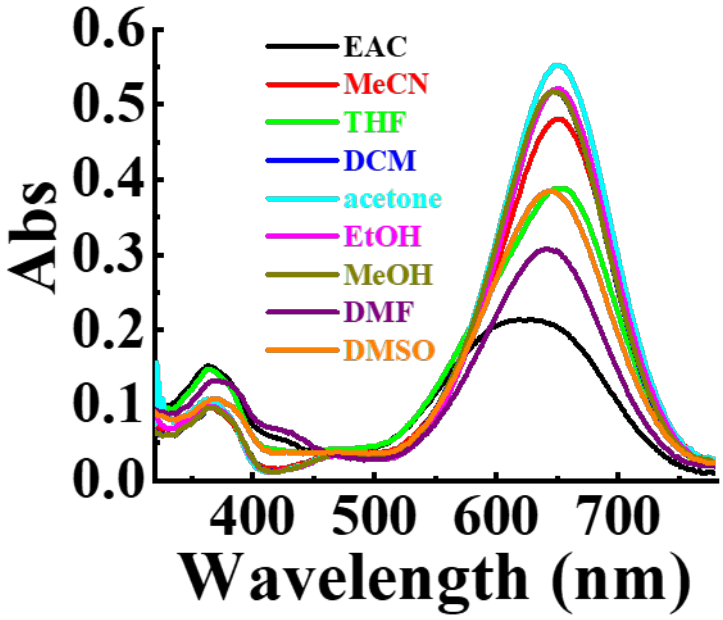


Fig. S5 Absorption of L1 (10.0 μM) in different solvents. Ethyl acetate (EAC), acetonitrile (MeCN), tetrahydrofuran (THF), dichloromethane (DCM), ethanol (EtOH), methanol (MeOH), N, N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO).

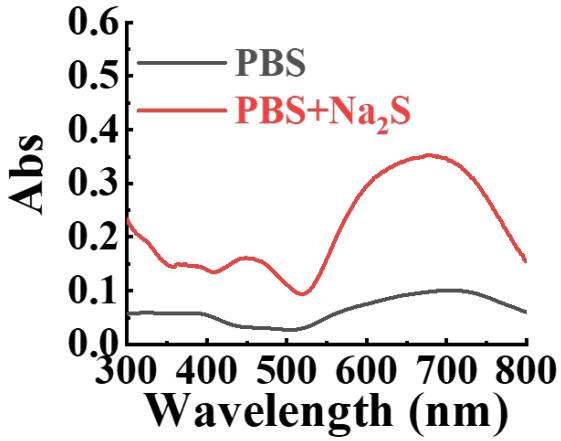


Fig. S6 Absorption of L1 (10.0 μM) in PBS in the absence and presence of Na_2S.

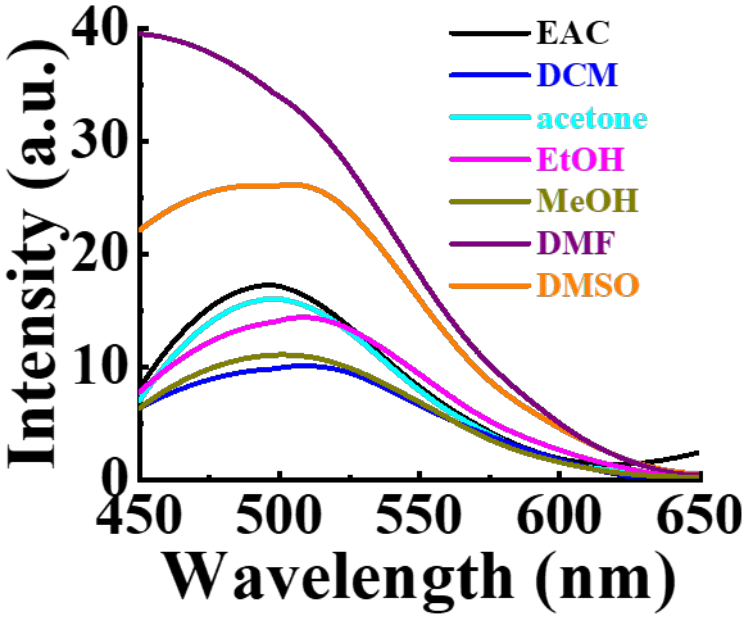


Fig. S7 Fluorescence spectra (λ_{ex} 428 nm) of L1 (10.0 μM) in different solvents.

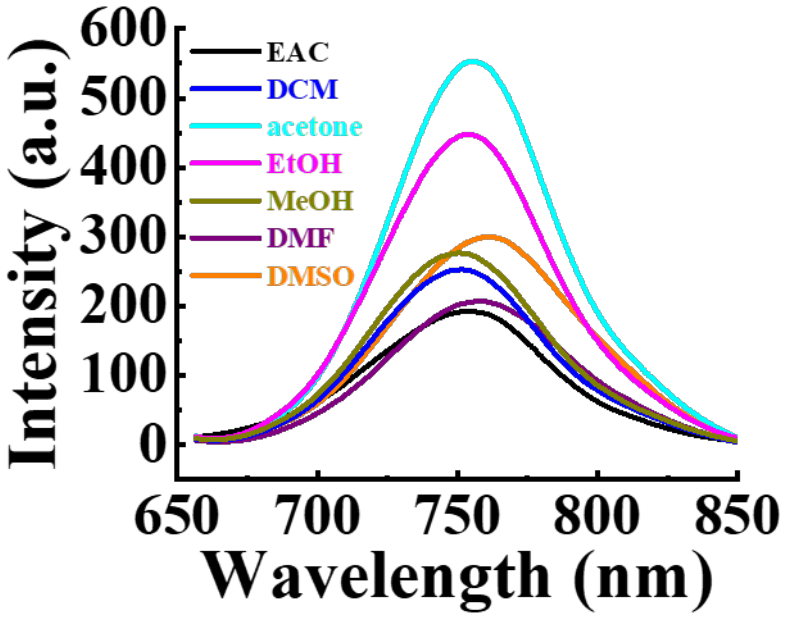


Fig. S8 Fluorescence spectra (λ_{ex} 636 nm) of L1 (10.0 μM) in different solvents.

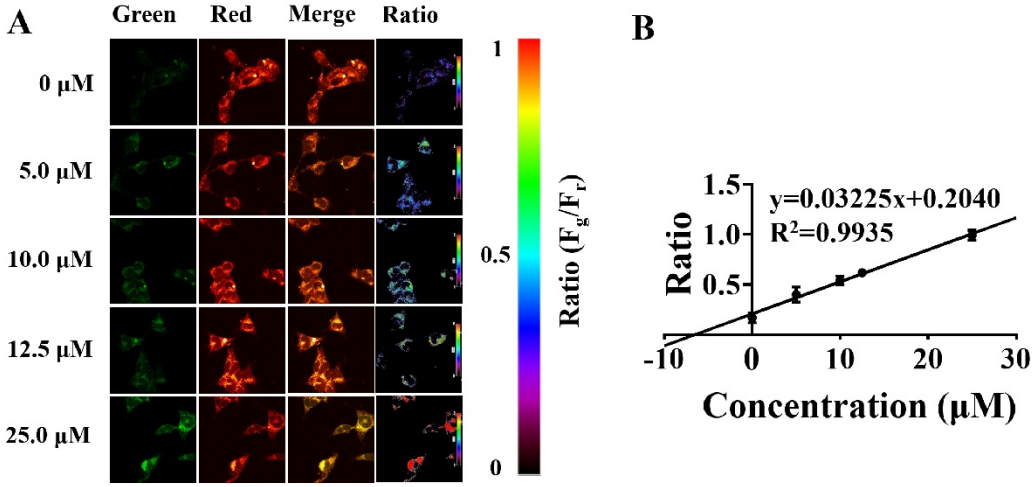


Fig. S9 (A) Confocal images of L1 (10.0 μM) stained HEK-293 cells in the presence of different concentration of Na_2S (0, 5.0, 10.0, 12.5, 25.0 μM), cells were fixed and perforated after L1 staining. (B) Linear relationship of fluorescence ratio with Na_2S concentration.

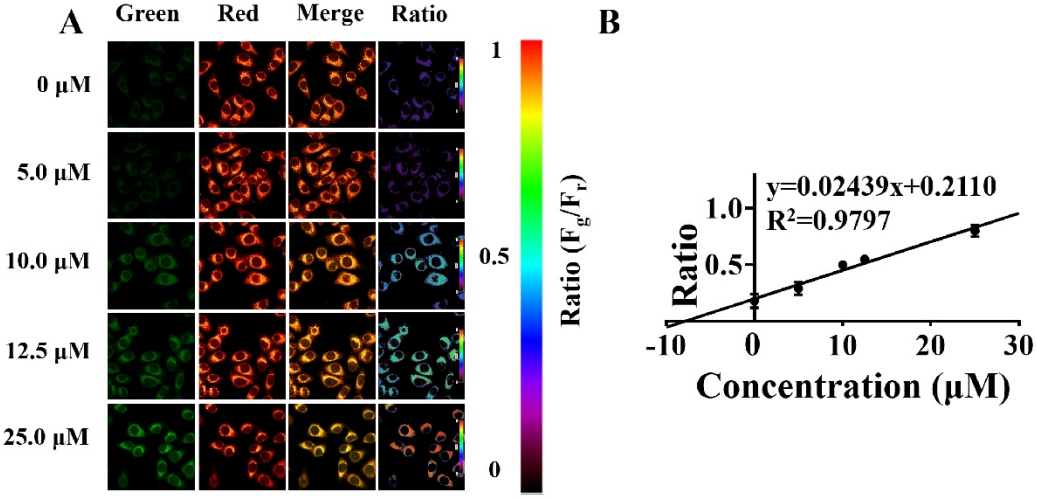


Fig. S10 (A) Confocal images of L1 (10.0 μM) stained LoVo cells in the presence of different concentration of Na_2S (0, 5.0, 10.0, 12.5, 25.0 μM), cells were fixed and perforated after L1 staining. (B) Linear relationship of fluorescence ratio with Na_2S concentration.

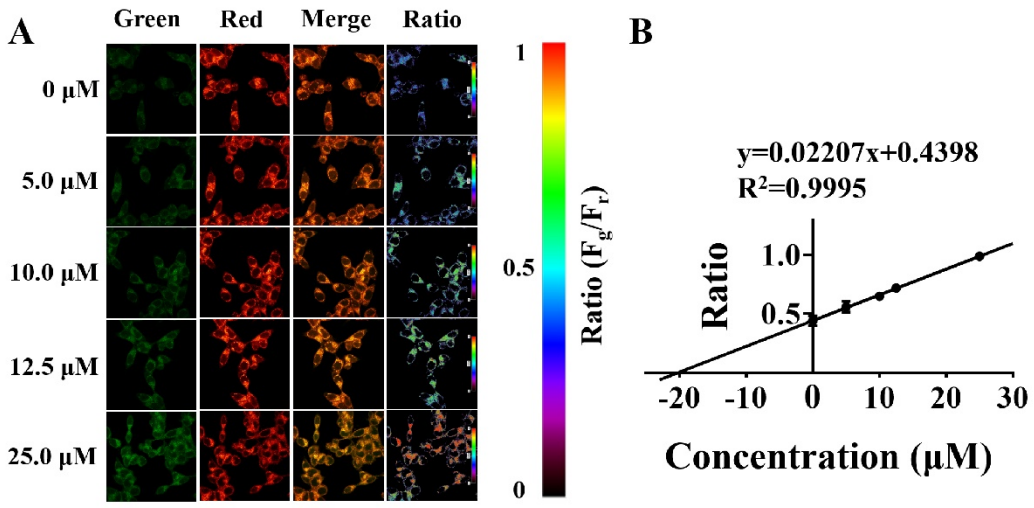


Fig. S11 (A) Confocal images of L1 (10.0 μM) stained HCT-116 cells in the presence of different concentration of Na_2S (0, 5.0, 10.0, 12.5, 25.0 μM), cells were fixed and perforated after L1 staining. (B) Linear relationship of fluorescence ratio with Na_2S concentration.