Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Dynamic Mapping of Spontaneous H_2S in Entire Cell Space and Live Animal by a Rationally Designed Molecular Switch

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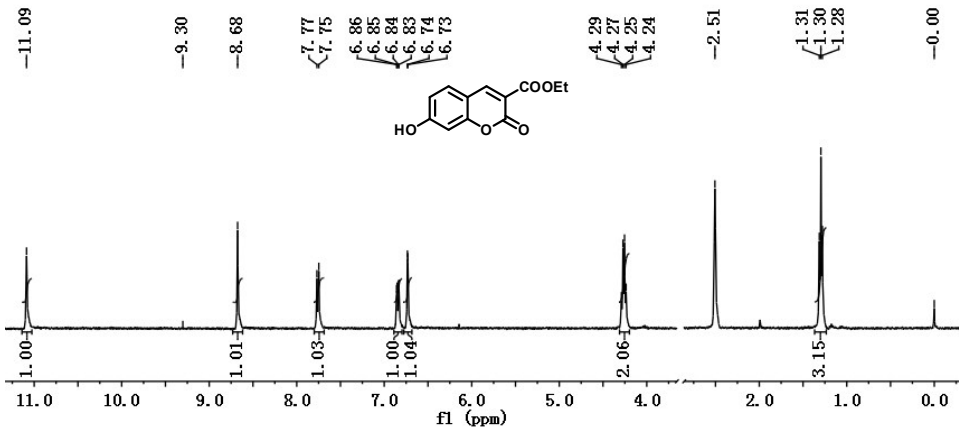

Fig. S1 ^{1}H NMR spectrum of compound C-OH.

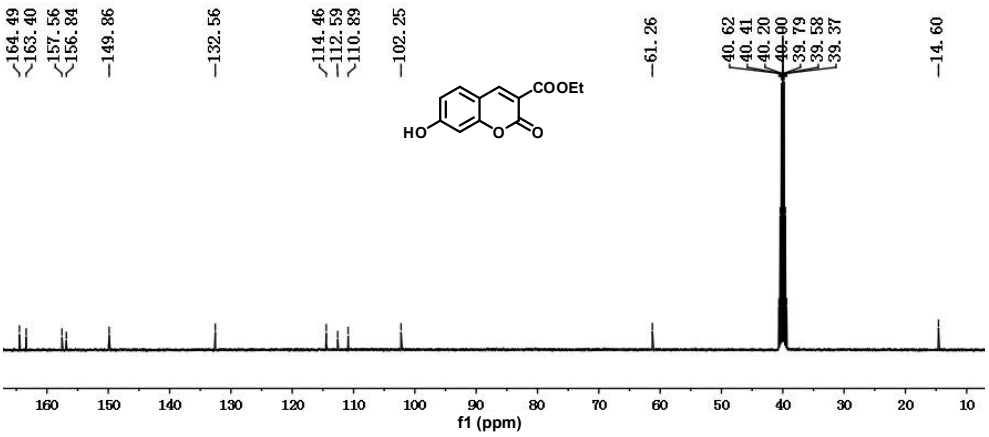

Fig. S2 ^{13}C NMR spectrum of compound C-OH.

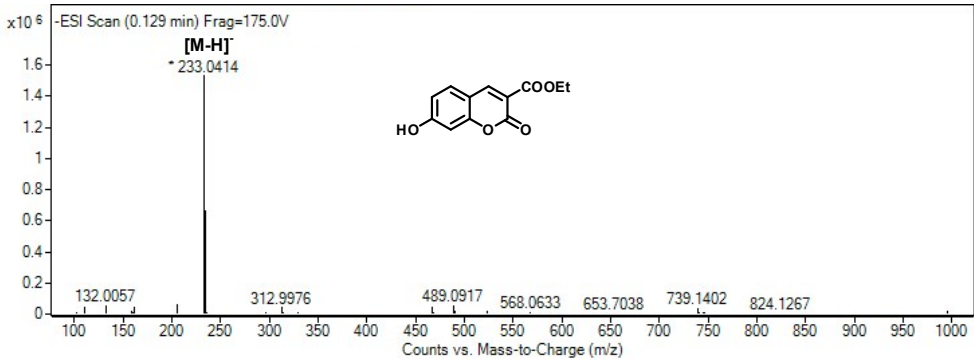

Fig. S3 HR-MS spectrum of compound C-OH (negative mode, calculated for C-OH $[M-H]^{-}$ = 233.0450).

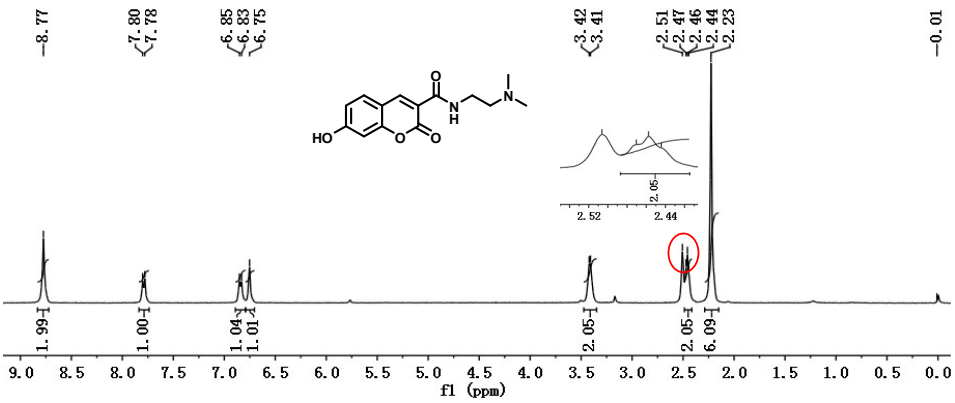

Fig. S4 [1]H NMR spectrum of compound Cda-OH.

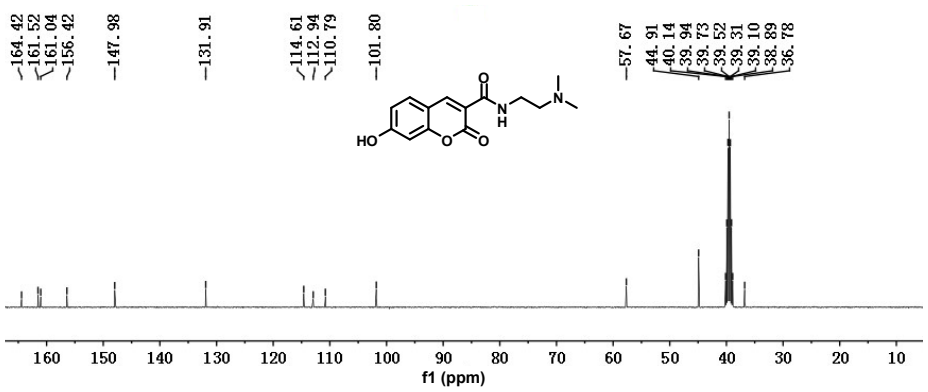

Fig. S5 [13]C NMR spectrum of compound Cda-OH.

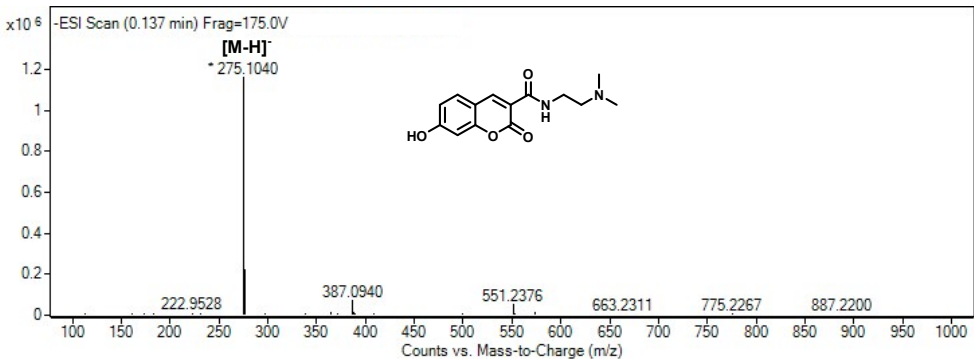

Fig. S6 HR-MS spectrum of compound Cda-OH (negative mode, calculated for Cda-OH $[M-H]^-$ = 275.1032).

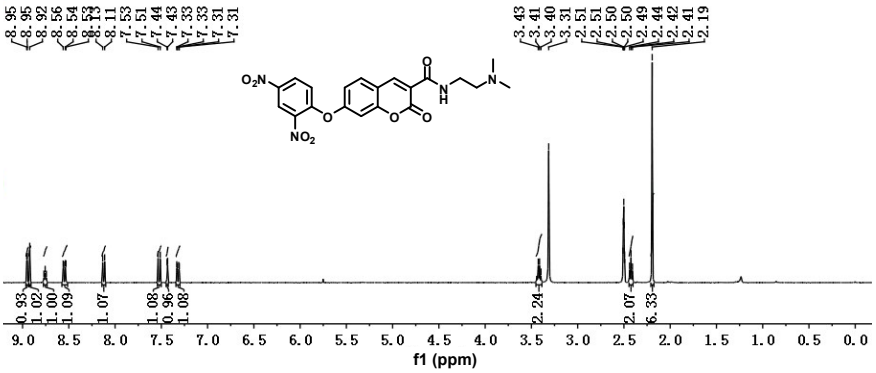

Fig. S7 ^{1}H NMR spectrum of probe Cda-DNP.

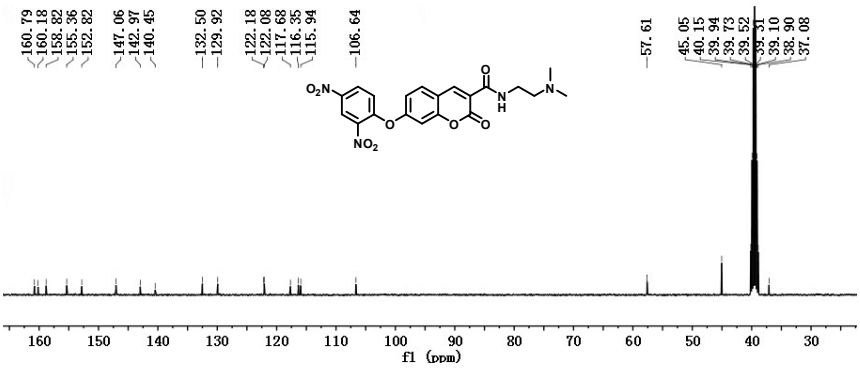

Fig. S8 ^{13}C NMR spectrum of probe Cda-DNP.

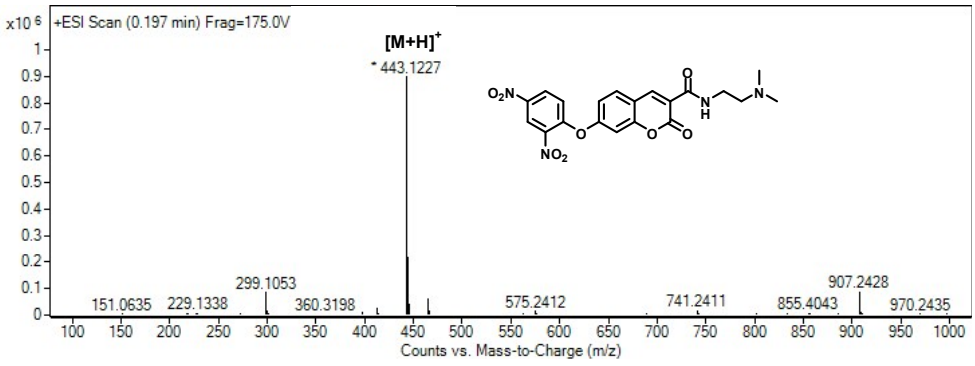

Fig. S9 HR-MS spectrum of probe Cda-DNP (positive mode, calculated for Cda-DNP $[M+H]^{+}$ = 443.1203).

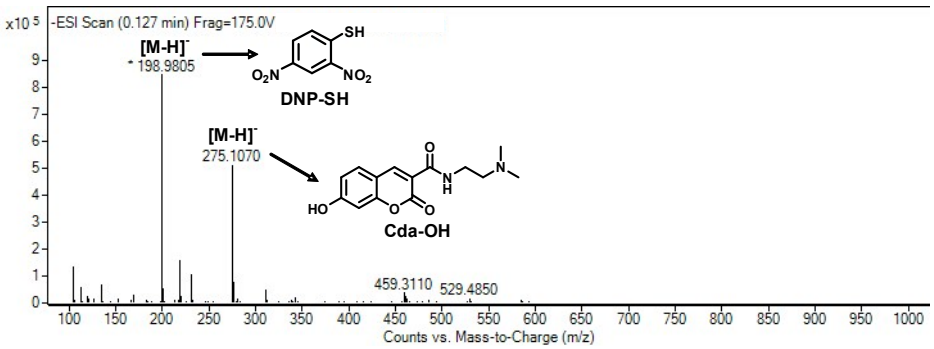

Fig. S10 HR-MS spectrum of the products DNP-SH and Cda-OH resulting from the reaction of probe with H_2S (negative mode, calculated for DNP-SH $[M-H]^-$ = 198.9814 and Cda-OH $[M-H]^-$ = 275.1032).

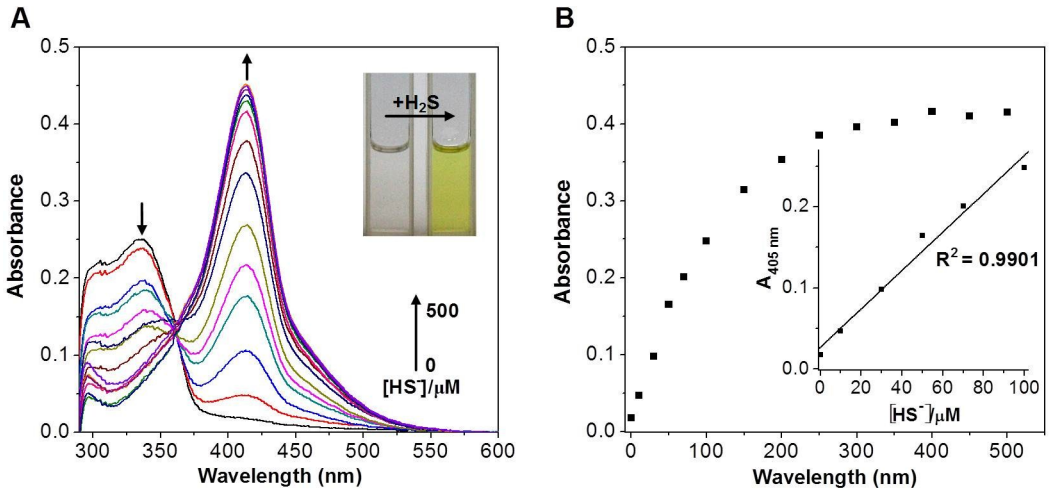

Fig. S11 (A) Absorption spectra of 20 μM Cda-DNP with the addition of HS^- (0-500 μM) at pH 7.4 in 10 mM HEPES/THF (7:3). (B) The plot of absorbances *vs* HS^- concentrations. Inset: the liner relationship between absorbances (at 405 nm) and HS^-concentrations.

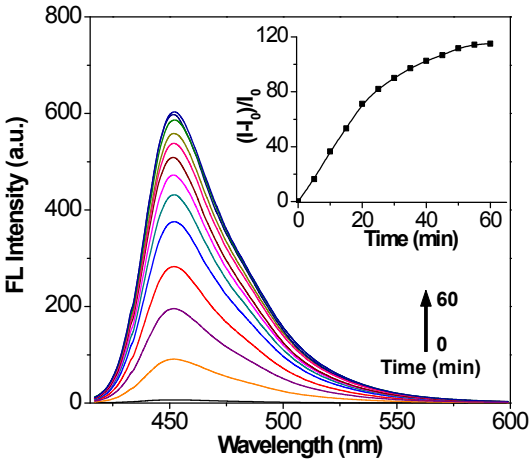

Fig. S12 Time-dependent fluorescence responses of Cda-DNP (1 μM) to 50 μM HS^-. Inset is the plots of fluorescence enhancement *vs* the time of 50 μM HS^- response to Cda-DNP.

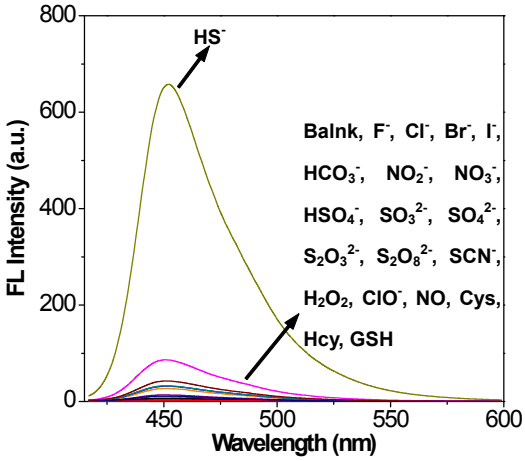

Fig. S13 Fluorescence spectra of Cda-DNP (1 μM) at pH 7.4 in 10 mM HEPES/THF (7:3) in the presence of HS^- (50 μM) and other species (1 mM anions and NO, 200 μM Cys and Hcy, and 2 mM GSH).

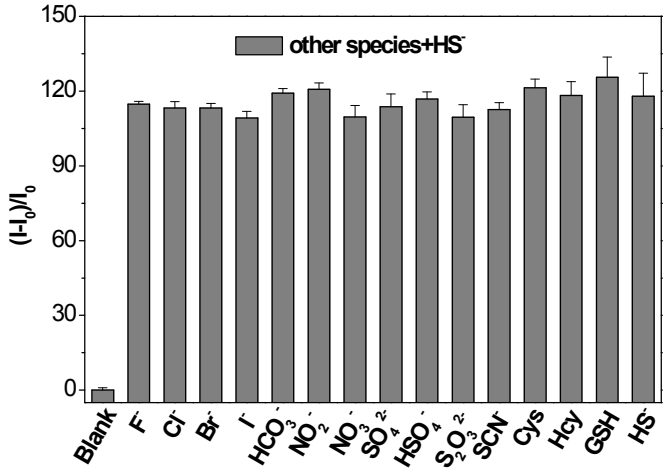

Fig. S14 Interference tests of Cda-DNP (1 μM) in the presence of various species (Blank, 1 mM F^-, Cl^-, Br^-, I^-, HCO_3^-, NO_2^-, NO_3^-, SO_4^{2-}, HSO_4^-, $S_2O_3^{2-}$, SCN^-; 200 μM Cys, Hcy; 2 mM GSH; 50 μM HS^-) in 10 mM HEPES/THF (7:3).

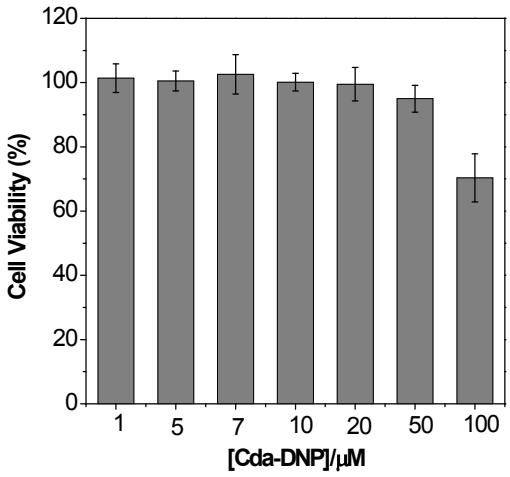

Fig. S15 Viability of A549 cells treated with different concentrations of Cda-DNP for 24 h by MTT assay.

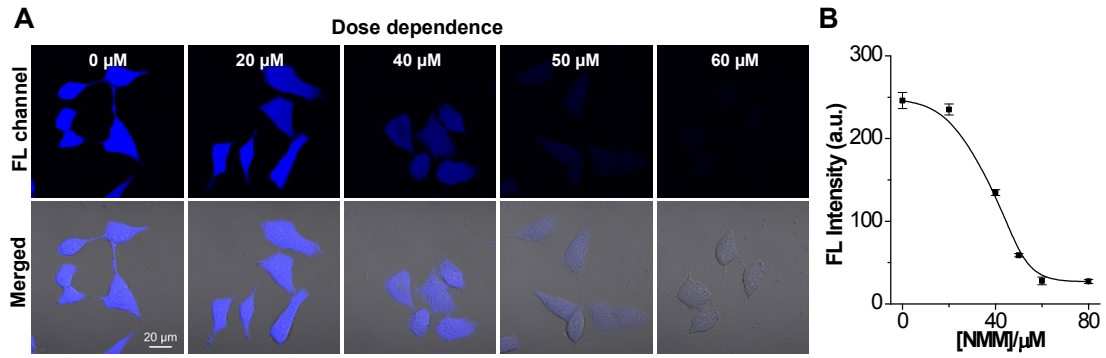

Fig. S16 Screening of the appropriate dosage of H_2S scavenger (NMM) to clean up endogenous H_2S in A549 cells. (A) Fluorescent intensity changes of cells with the addition of different concentrations of NMM. The cells were separately incubated with 0, 20, 40, 50, 60 and 80 μM NMM for 30 min, and then cultured with 5 μM Cda-DNP for another 30 min after cells were washed with medium two times. (B) Dose-dependent evolutions of mean fluorescence intensities. Thirty cells were manually selected to collect the fluorescent intensities, and the mean fluorescent intensities at each concentration of NMM were calculated from the 30 intensities. The error bars represent standard deviation (±SD). All these above quantification experiments were repeated with three batches of cells.

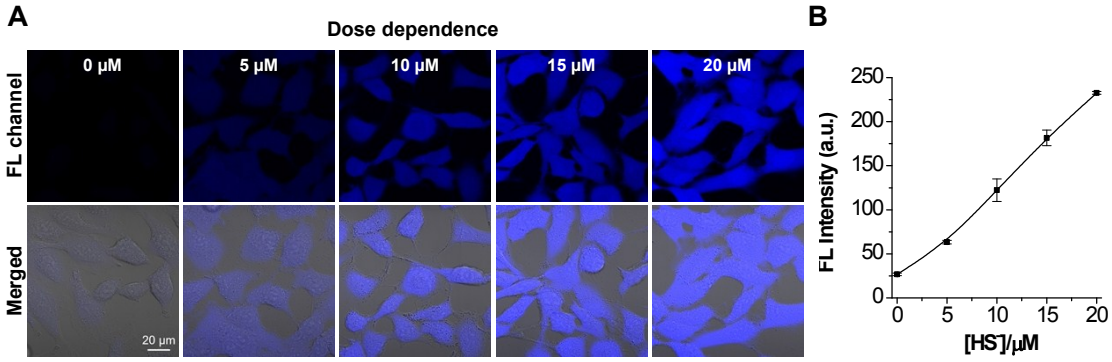

Fig. S17 Quantitative determination of exogenous H_2S in living cells. (A) The fluorescent intensity changes of A549 cells incubated with different concentrations of exogenous H_2S after removed endogenous H_2S. A549 cells were first pretreated with 60 μM NMM, and then incubated separately with 0, 5, 10, 15 and 20 μM NaHS, followed by the addition of 5 μM Cda-DNP. For above tests, all incubation periods were 30 min. (B) Dose-dependent evolutions of mean fluorescence intensities. Thirty cells were manually selected to collect the fluorescent intensities, and the mean fluorescent intensities at each concentration of H_2S were calculated from the 30 intensities. The error bars represent standard deviation (±SD). All these above quantification experiments were repeated with three batches of cells.

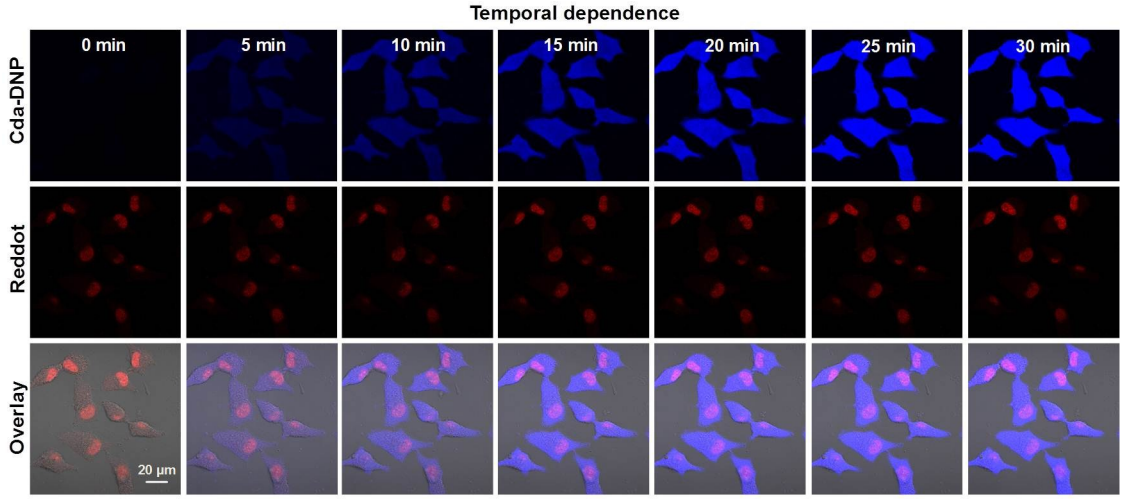

Fig. S18 Time-dependent fluorescence changes of A549 cells. The cells were incubated with 4 μL Reddot (a commercial nucleus tracker) for 30 min, and then the images were obtained from blue and red channel of A549 cells incubated with 5 μM probe Cda-DNP for different times. Blue channel, λ_{ex} = 405 nm, λ_{em} = 410-580 nm; Red channel, λ_{ex} = 633 nm, λ_{em} = 640-750 nm.

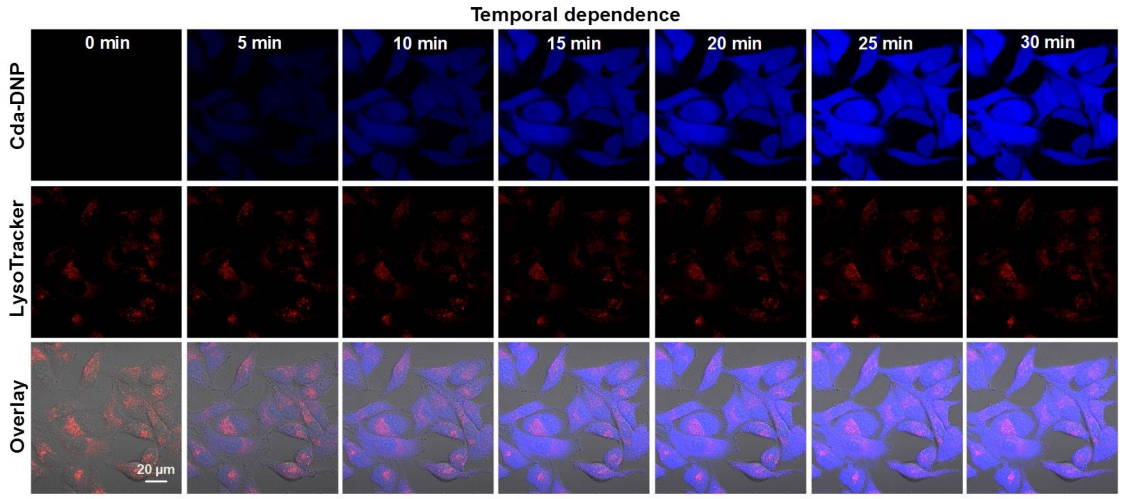

Fig. S19 Time-dependent fluorescence changes of A549 cells. The cells were incubated with 1 μL LysoTracker Deep Red (a commercial lysosome tracker) for 30 min, and then the images were obtained from blue and red channel of A549 cells incubated with 5 μM probe Cda-DNP for different times. Blue channel, λ_{ex} = 405 nm, λ_{em} = 410-580 nm; Red channel, λ_{ex} = 633 nm, λ_{em} = 640-750 nm.

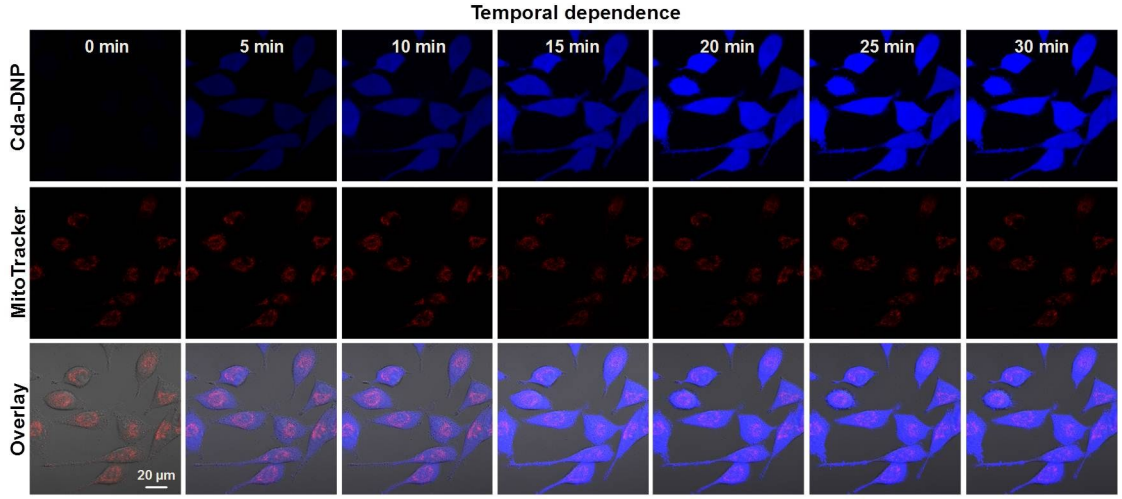

Fig. S20 Time-dependent fluorescence intensity changes of A549 cells. The cells were incubated with 1 μL MitoTracker ((a commercial mitochondria tracker)) for 30 min, and then the images were obtained from blue and red channel of A549 cells incubated with 5 μM probe Cda-DNP for different times. Blue channel, λ_{ex} = 405 nm, λ_{em} = 410-580 nm; Red channel, λ_{ex} = 633 nm, λ_{em}= 640-750 nm.

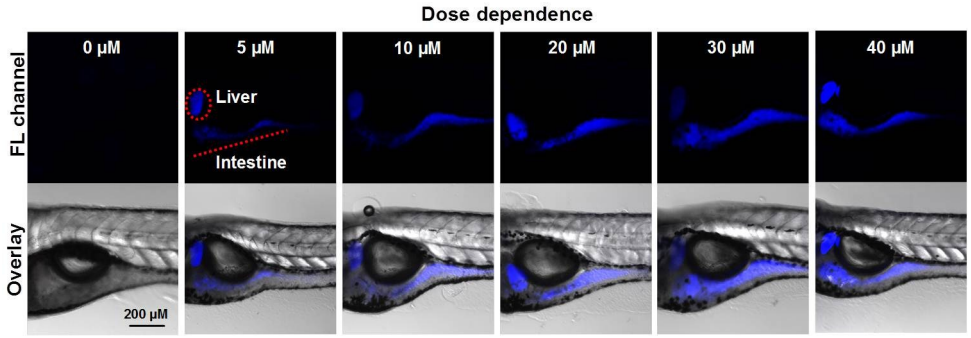

Fig. S21 Dose-dependent fluorescent images of probe for the detection of spontaneous H_2S in normal zebrafish. The seven-day old zebrafish were cultured with 0, 5, 10, 20, 30 and 40 μM Cda-DNP for 60 min, respectively.