Electronic Supplementary Information

An endoplasmic reticulum-targeting fluorescent probe for imaging •OH in living cells

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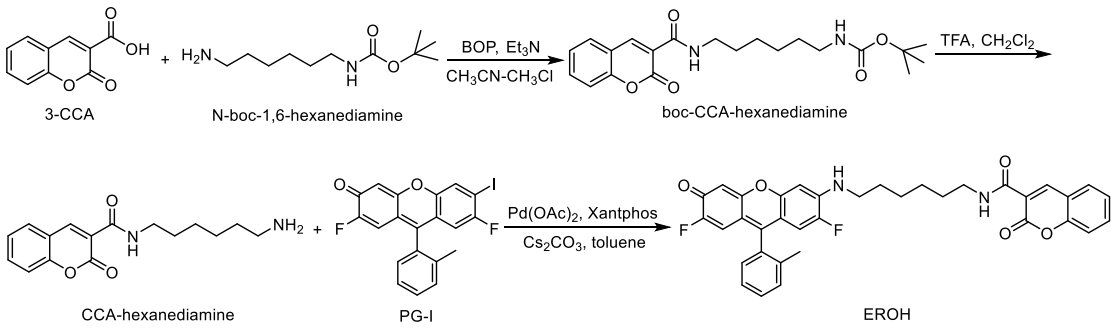
1. Reagents and apparatus

Coumarin-3-carboxylic acid (3-CCA) was obtained from Alfa Aesar. (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), N-Boc-1,6-hexanediamine, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, nile red, phorbol-12-myristate-13-acetate (PMA) and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. HeLa cell strain, Dulbecco's modified Eagle's media (DMEM), ER Tracker Red (ER-Red), Lyso Tracker Red (Lyso-Red), and Mito Tracker Red (Mito-Red) were purchased from KeyGEN BioTECH Co., Ltd, Nanjing, China. Ultrapure water (over 18 MΩ·cm) produced by a Milli-Q reference system (Millipore) was used throughout the experiments.

Fluorescence spectra were recorded on an F-4600 spectrophotometer (Hitachi, Japan). ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker Fourier-400 spectrometer. Electrospray ionization mass spectra (ESI-MS) were measured with a LC-MS 2010A instrument (Shimadzu, Japan). High-resolution electrospray ionization mass spectra (HR-ESI-MS) were measured on an APEX IVFTMS instrument (Bruker, Daltonics). MTT analyses were made on a SpectraMax i3 microplate reader (Molecular Devices, USA). Confocal fluorescence images were recorded on an FV 1200-IX83 confocal laser scanning microscope (Olympus, Japan). All imaging experiments were repeated three times under the same conditions.

2. Synthesis and characterization of probe EROH



Scheme S1. Synthesis of probe EROH.

The synthetic route of EROH is shown in Scheme S1. The iodinate Pennsylvania Green (PG-I) was synthesized according to the previous method.[S1]

Synthesis of CCA-hexanediamine. Coumarin-3-carboxylic acid (1.00 g, 5.26 mmol) and BOP (2.33 g, 5.26 mmol) were dissolved in CH_3CN (50 mL) followed by the addition of Et_3N (1.9 mL, 14 mmol). Then, N-boc-1,6-hexanediamine (1.14 g, 5.26 mmol) in $CHCl_3$ (15 mL) was added, and the solution was stirred at room temperature for 10 h. The product was extracted by ethyl acetate and washed with brine water for 3 times. The solvent was removed by evaporation under reduced pressure, and the crude product boc-CCA-hexanediamine was used directly in the following step.

Boc-CCA-hexanediamine (1.60 g, 4.12 mmol) was de-protected in 30% trifluoroacetic acid in CH_2Cl_2 (120 mL) at room temperature for 3 h. Excess trifluoroacetic acid was removed *in vacuo*. The residue was extracted with CH_2Cl_2 (50 mL) and washed with brine water for 3 times. Then, the product was purified by silica gel column

chromatography (CH_2Cl_2/CH_3OH=10/1) to give CCA-hexanediamine (1.00 g, 3.47 mmol, 87%) as white solid. ^{1}H-NMR (400 MHz, 298 K, DMSO-d_6, Figure S1): δ 8.85 (s, 1H), 8.68 (t, 1H, J=10.8 Hz), 7.99 (d, 1H, J=7.6 Hz), 7.76 (t, 1H, J=16 Hz), 7.63 (s,2H), 7.51 (d, 1H, J=8.4 Hz), 7.45 (t, 1H, 15.2 Hz), 3.34 (t, 2H, J=13.2 Hz), 2.78 (t, 2H, J=14.8 Hz), 1.54 (t, 4H, J=12.8 Hz), 1.34 (t, 4H, J=6.8 Hz). ^{1}C-NMR (100 MHz, 298 K, DMSO-d_6, Figure S2): δ 161.5, 160.9, 154.3, 147.7, 134.5, 130.7, 125.6, 119.7, 118.9, 116.6, 29.2, 27.4, 26.3, 25.9. HR-ESI-MS: m/z calcd for CCA-hexanediamine ($C_{16}H_{21}N_2O_3^+$, $[M+H]^+$), 289.1547; found, 289.1548.

Synthesis of EROH. A mixture of PG-I (180 mg, 0.4 mmol), CCA-hexanediamine (173 mg, 0.6 mmol), $Pd(OAc)_2$ (9 mg, 0.04 mmol), Xantphos (35 mg, 0.06 mmol), and Cs_2CO_3 (261 mg, 0.8 mmol) was refluxed in toluene (50 mL) for 4 h under Ar atmosphere. Then, the mixture was cooled and extracted with CH_2Cl_2 (100 mL×3) and H_2O (50 mL). The combined organic layer was evaporated in vacuo and the residue was purified by silica gel column chromatography (EtOAc/CH_2Cl_2=5/1) to give EROH (32 mg, 0.05 mmol, 13%) as red solid. ^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, Figure S3): δ 8.91 (s, 1H), 8.86 (s, 1H), 7.71-7.66 (m, 2H), 7.51 (t, 1H, J=13.6 Hz), 7.44-7.37 (m, 4H), 7.15 (d, 1H, J=7.2 Hz), 6.79 (d, 1H, J=6.8 Hz), 6.72 (t, 2H, J=23.6 Hz), 3.52-3.47 (m, 2H), 3.41-3.36 (m, 2H), 2.06 (s, 3H), 1.79 (t, 2H, J=14 Hz), 1.69 (t, 2H, J=13.6 Hz), 1.51 (t, 2H, J=9.6 Hz), 1.26 (s, 2H). ^{1}C-NMR (100 MHz, 298 K, DMSO-d_6, Figure S4): δ 161.5, 160.9, 154.3, 152.6, 149.8, 147.7, 136.0, 134.5, 132.5, 132.2, 131.1, 130.7, 130.2, 129.3, 126.7, 125.6, 119.7, 119.0, 116.6, 113.2, 111.4, 111.2, 111.1, 108.9, 108.8, 105.6, 97.0, 97.0, 42.9, 29.4, 28.4, 26.6, 26.5, 21.8, 19.5. HR-ESI-MS: m/z calcd for EROH ($C_{36}H_{30}F_2N_2O_5^+$, $[M+H]^+$), 609.2196; found, 609.2192 (Figure S5).

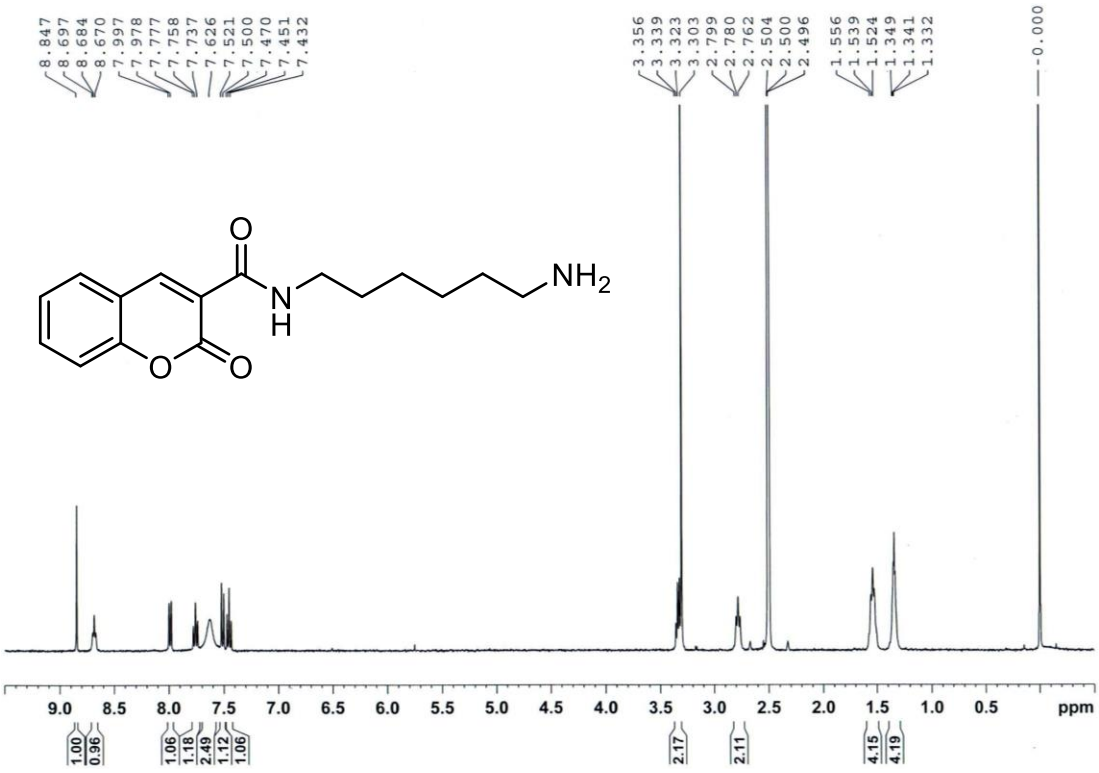


Fig. S1 ^{1}H NMR spectrum of CCA-hexanediamine (400 MHz, DMSO-d_6, 298 K).

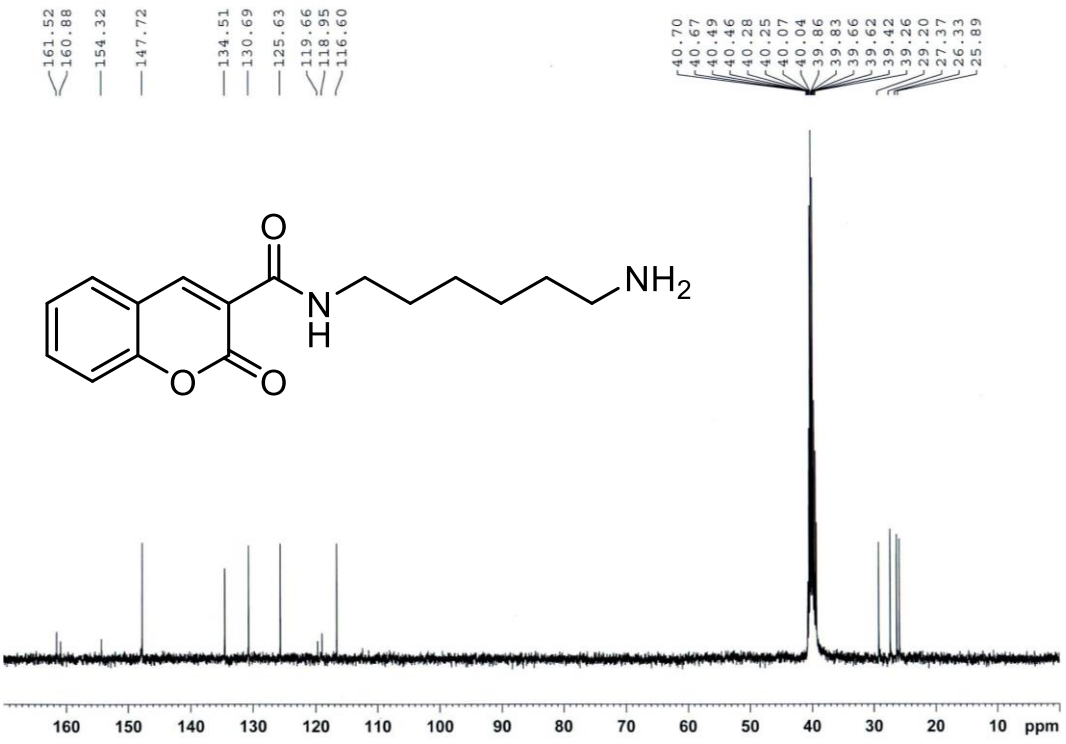


Fig. S2 ^{13}C NMR spectrum of CCA-hexanediamine (100 MHz, DMSO-d_6, 298 K).

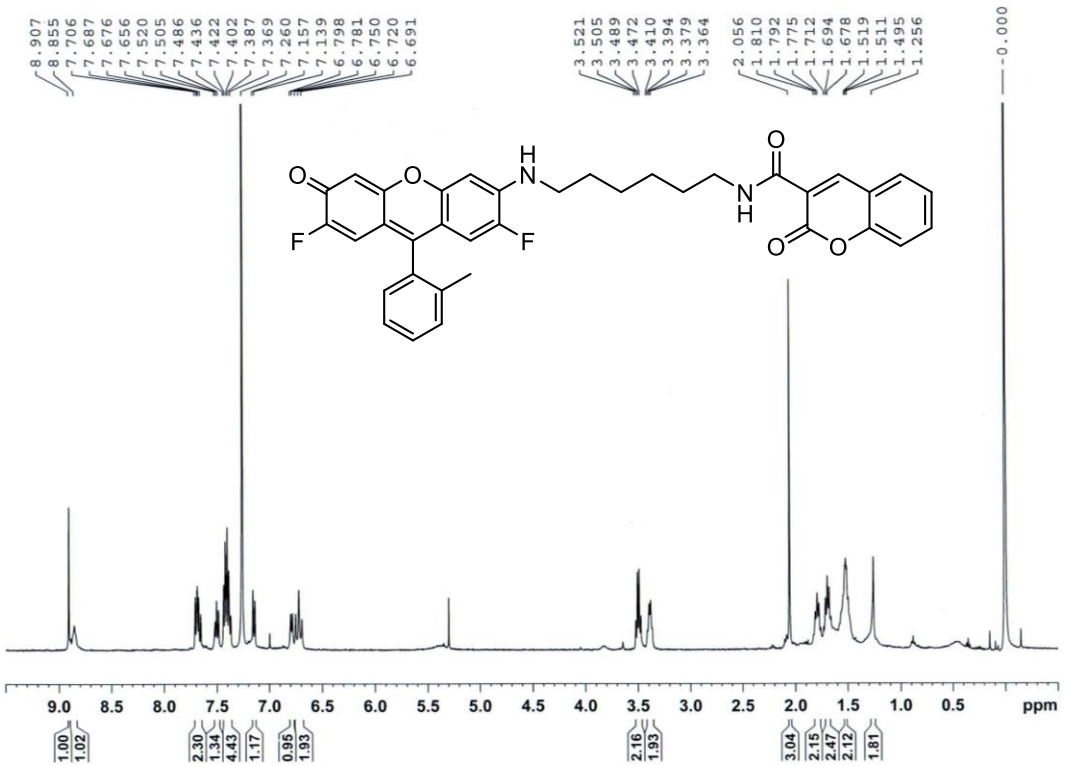


Fig. S3 ^{1}H NMR spectrum of EROH (400 MHz, $CDCl_3$, 298 K).

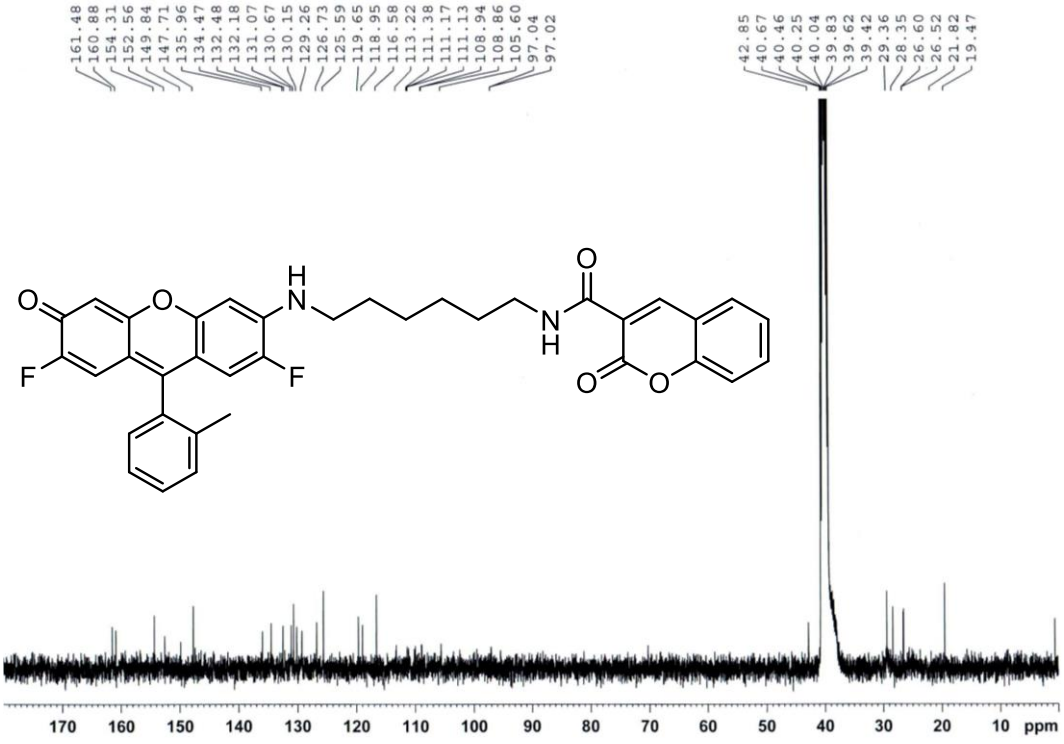


Fig. S4 ^{13}C NMR spectrum of EROH (100 MHz, DMSO-d_6, 298 K).

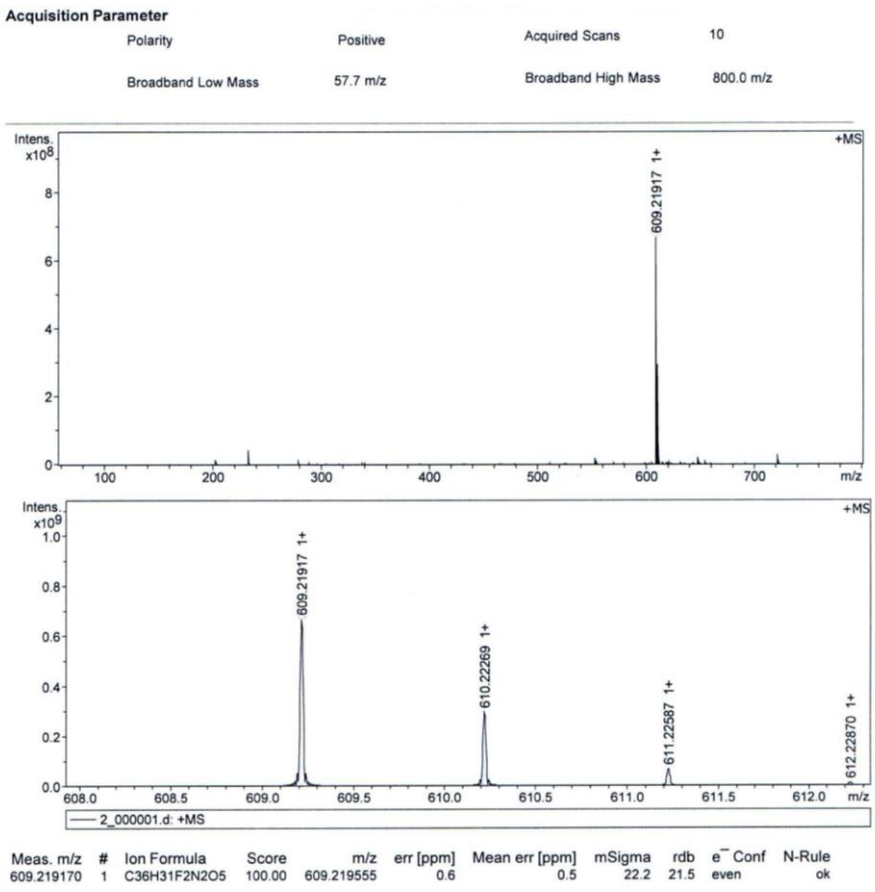


Fig. S5 HR-ESI-MS spectrum of EROH.

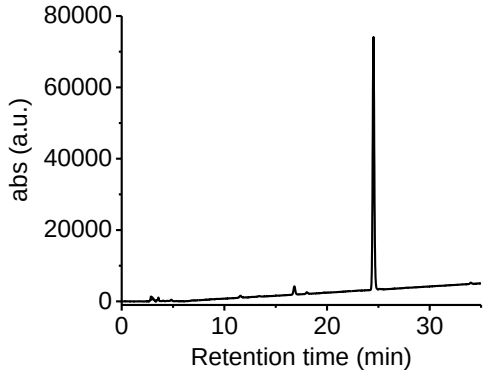


Fig. S6. Chromatogram of EROH (retention time = 24.5 min). The chromatogram was monitored at 254 nm with methanol (flow rate, 0.6 mL/min) and water (flow rate, 0.4 mL/min) containing 0.1% trifluoroacetic acid as eluents.

3. Supplementary method

General procedure for spectral measurements. Unless otherwise specified, the spectral measurements were performed in 20 mM phosphate buffer (pH 7.4) according to the following procedure. In a test tube, 1.5 mL of 20 mM phosphate buffer was added, followed by addition of 30 μL probe stock solution (100 μM in CH_3CN) and an appropriate volume of reactant (•OH or other ROS) solution. Then, the final volume was adjusted to 3 mL by 20 mM phosphate buffer saline (PBS). After incubation at room temperature for 1 h, the fluorescence spectra of the reaction solution were measured.

Preparation of ROS. The preparation of reactive oxygen species and their concentration determinations were following the reported method.[S2]

Co-localization imaging experiments in HeLa cells. HeLa cells were cultured on glass-bottom culture dishes (MatTek Co.) in DMEM supplemented with FBS (10%, v/v) and penicillin-streptomycin (1%, v/v) at 37 ℃ in a humidified 5% CO_2 atmosphere. Before imaging, HeLa cells were seeded in glass-bottom culture dishes and allowed to adhere for 24 h, followed by co-staining with EROH (1 μM) and ER-Red (500 nM) or Lyso-Red (500 nM) or Mito-Red (500 nM) for 30 min at 37 ℃ in DMEM. After washing with DMEM for three times, the cells were subjected to fluorescence imaging experiments. For EROH in the green channel: excitation at 488 nm and emission at 505-545 nm. For ER-Red or Lyso-Red or Mito-Red or Nile Red in red channel: excitation at 559 nm and emission at 580-680 nm.

Imaging exogenous •OH in ER. HeLa cells were incubated with 1 μM EROH for 30 min. Then, the cells were washed with DMEM for 3 times, followed by incubating with different amounts of Fenton reagent. Fluorescence imaging in the blue channel was excited at 405 nm and collected at 425-475 nm.

Imaging PMA-induced •OH in ER. HeLa cells were seeded in glass-bottom dishes and adhered, followed by stimulation with PMA (5 $\mu g \cdot mL^{-1}$) for 1 h. The as-treated cells were washed with DMEM and then incubated with EROH (1 μM) and nile red (500 nm) in DMEM for 30 min at 37 ℃. The fluorescent images were collected in blue/green/red channels as described above.

4. Supplementary figures

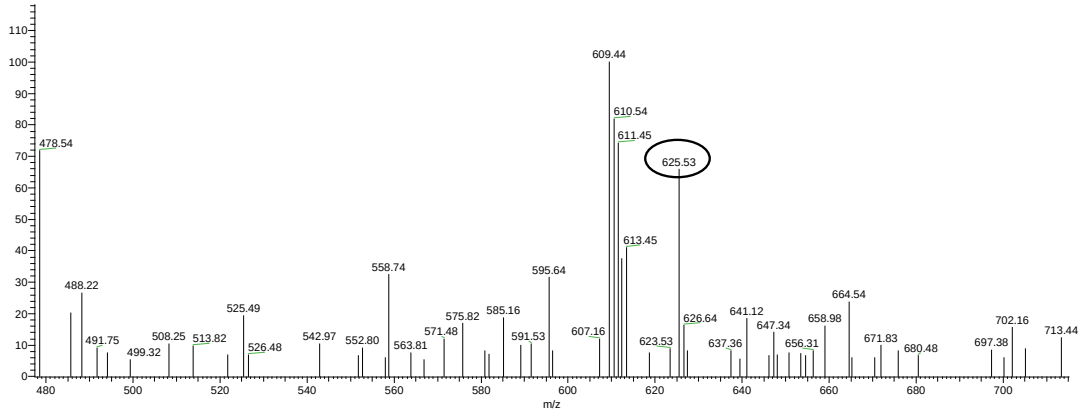


Fig. S7 ESI mass spectrum of the reaction solution of probe EROH (1 μM) with Fenton reagent. The peak at *m/z* = 625.53 indicates the generation of hydroxylation product of the probe; however, the attempt to separate out the hydroxylation product by HPLC-MS was failed due to the low reaction yield of < 5% as reported previously.[S3]

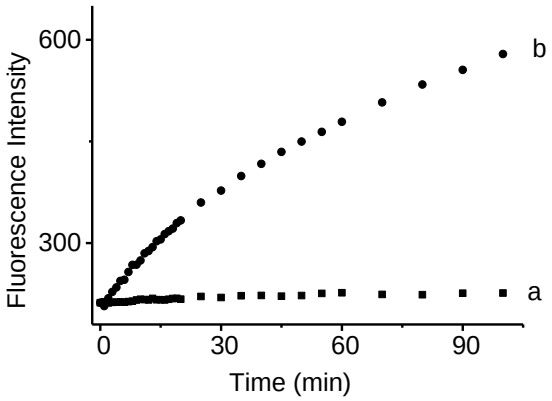


Fig. S8 Time-course curves of the fluorescence change of EROH (1 μM) in the absence (a) and presence (b) of Fenton reagent (500 μM Fe^{2+}-EDTA +500 μM H_2O_2). $\lambda_{ex/em}$=385/446 nm.

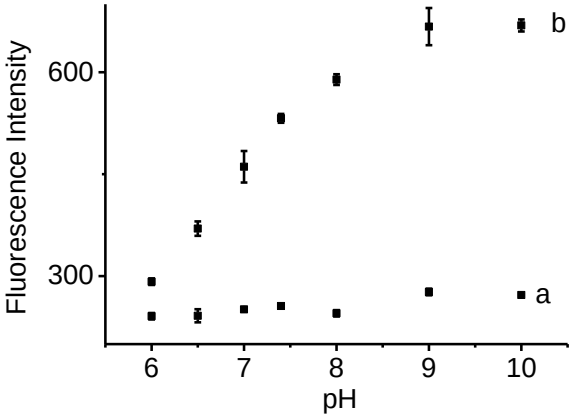


Fig. S9 Effects of pH on the fluorescence intensity of (a) EROH (1 μM) and (b) its reaction system with Fenton reagent (500 μM Fe^{2+}-EDTA + 500 μM H_2O_2) in PBS. $\lambda_{ex/em}$ = 385/446 nm.

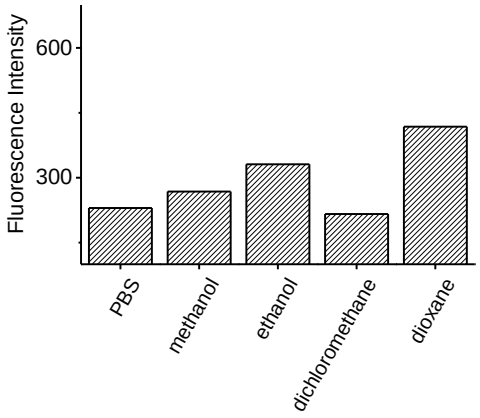


Fig. S10 Fluorescence intensity of EROH (1 μM) in various solvents (PBS, methanol, ethanol, dichloromethane, dioxane). $\lambda_{ex/em}$=385/446 nm.

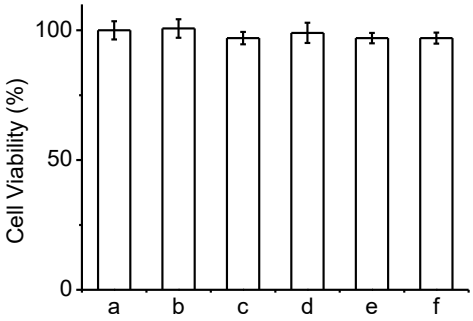


Fig. S11 Viability of HeLa cells after treated with various concentrations of EROH for 24 h. a: Blank; b: 0.25 μM; c: 0.5 μM; d: 1 μM; e: 2.5 μM; f: 5 μM. The viability of cells without EROH is defined as 100%. The results are presented as mean ± standard deviation (n=5).

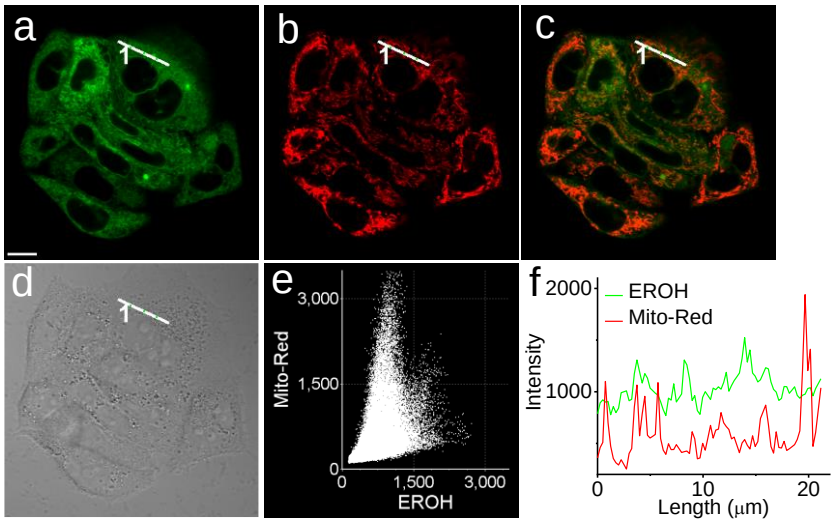


Fig. S12 Co-localization experiments with EROH and Mito-red. (a) Green channel image for EROH. (b) Red channel image for Mito-Red. (c) Merged image of images (a) and (b). (d) Corresponding differential interference contrast (DIC) image. (e) Intensity correlation plot of green and red channels. (f) Intensity profiles of the white crossline in the green and red channels. Scale bar: 10 μm.

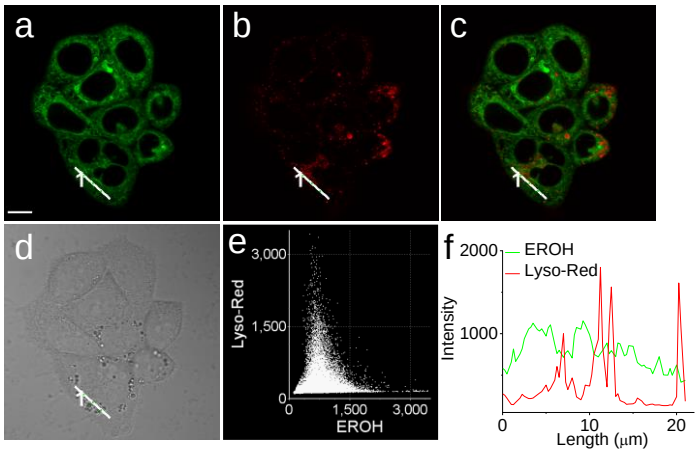


Fig. S13 Co-localization experiments with EROH and Lyso-red. (a) Green channel image for EROH. (b) Red channel image for Lyso-Red. (c) Merged image of images (a) and (b). (d) Corresponding DIC image. (e) Intensity correlation plot of green and red channels. (f) Intensity profiles of the white crossline in the green and red channels. Scale bar: 10 μm.

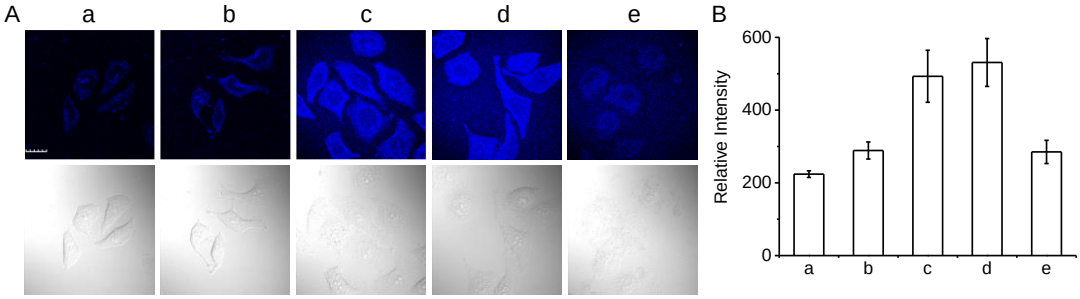


Fig. S14 (A) Confocal fluorescence images of HeLa cells. (a) Cells incubated with EROH (1 μM) for 0.5 h; (b) cells incubated with EROH (1 μM) for 0.5 h, and then treated with 100 μM Fenton reagent (100 μM Fe^{2+}-EDTA +100 μM H_2O_2) for another 0.5 h; (c) cells incubated with EROH (1 μM) for 0.5 h and then treated with 500 μM Fenton reagent (500 μM Fe^{2+}-EDTA +500 μM H_2O_2) for 0.5 h; (d) cells incubated with EROH (1 μM) for 0.5 h and then treated with 1000 μM Fenton reagent (1000 μM Fe^{2+}-EDTA +1000 μM H_2O_2) for 0.5 h; (e) cells pretreated with TEMPOL (1 mM) and EROH (1 μM) for 0.5 h, and followed by incubation with 1000 μM Fenton reagent (1000 μM Fe^{2+}-EDTA +1000 μM H_2O_2) for 0.5 h. The first row is the blue channel for EROH. The second row is the corresponding DIC image. Scale bar: 20 μm. (B) Relative pixel intensity of fluorescence images a-e.

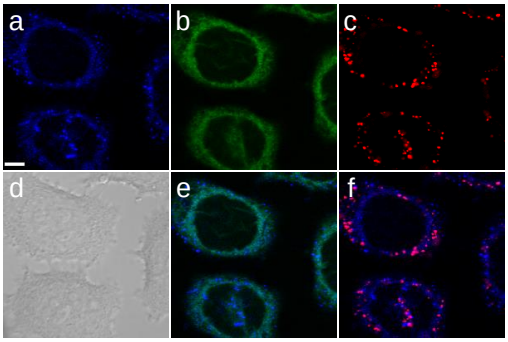


Fig. S15 Co-localization experiments of PMA-treated HeLa cells. Cells were co-stained

with EROH (1 μM) and Lyso-Red (500 nM). (a) Blue channel for •OH sensing with EROH (λ_{ex} = 405 nm, λ_{em} = 425-475 nm). (b) Green channel for ER targeting with EROH (λ_{ex} = 405 nm, λ_{em} = 505-545 nm). (c) Red channel for Lyso-Red (λ_{ex} = 559 nm, λ_{em} = 580-680 nm). (d) Corresponding DIC image. (e) Merged image of images (a) and (b). (f) Merged image of images (a) and (c). Scale bar: 5 μm.

References

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