Supporting information

Revealing the Redox status in Endoplasmic reticulum by a Selenium Fluorescence Probe

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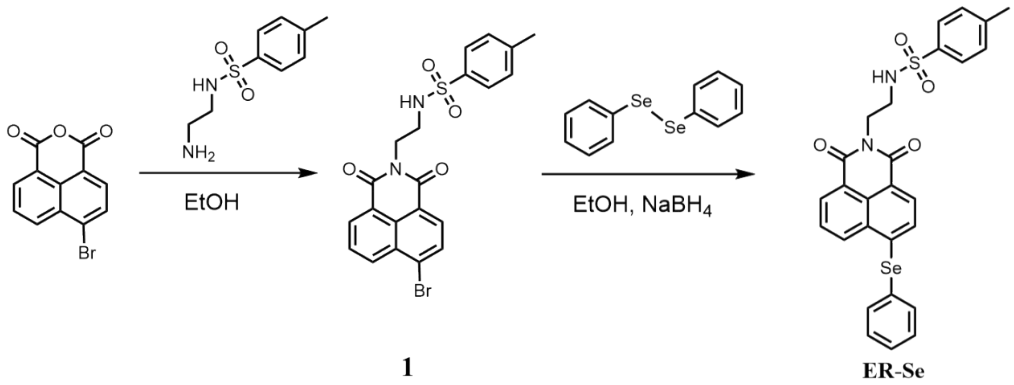
1. Materials and Instruments

All other chemicals used in this paper were obtained from commercial suppliers and used without further purification. Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance at 400MHz or at 100 MHz, δ values are in parts per million relatives to TMS in DMSO-d_6. Mass spectra (MS) was measured with Bruker Apex IV FTMS using electrospray ionization (ESI). Absorption spectra was recorded on a Purkinje TU-1901 spectrophotometer. Fluorescence measurements were taken on a Hitachi F-7000 fluorescence spectrometer with a 10mm quartz cuvette. Fluorescence imaging was observed under an Olympus IX81 confocal fluorescence microscope.

2. The synthesis route of ER-Se



Scheme S1. The synthetic route for **ER-Se.**

3. Determination of the Detection Limit

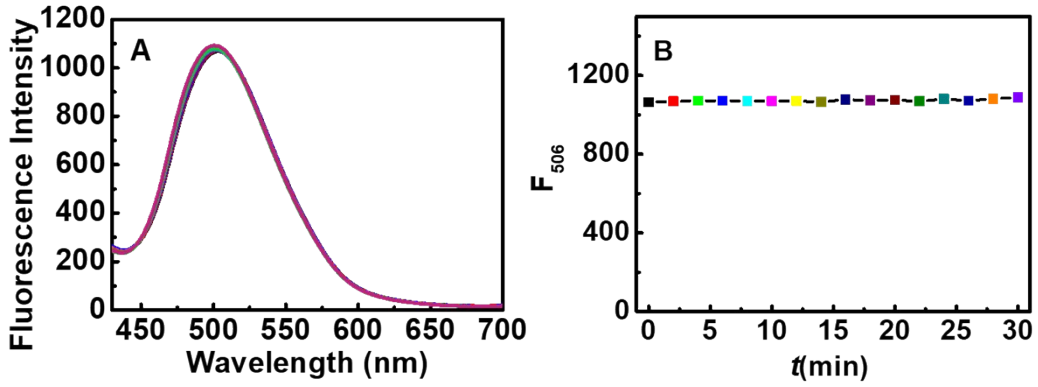


Figure S1. Fluorescence spectra changes (A) and fluorescence intensity (F_{506}) (B) of probe **ER-Se** (10μM) performed in 10 mM PBS, with 1% CH_3CN, v/v at room temperature. λ_{ex} = 410 nm, slit width: d_{ex} = d_{em} = 10 nm.

According to IUPAC, the detection limits were determined based on the fluorescence titrations, carried out in PBS / CH_3CN (9:1, v/v), pH 7.4, using the following equation:

$$\text{Detection limit} = 3\sigma / k$$

where σ is the standard deviation of blank measurements and k is the slope of the plot of fluorescence intensity vs HClO concentration.

The standard deviations σ = 4.8969.

4. The effect of pH

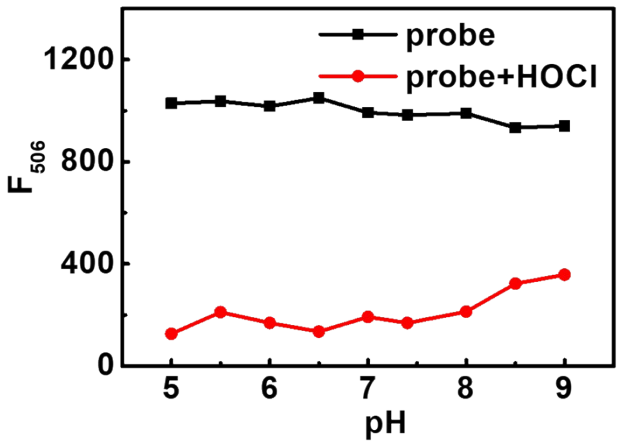


Figure S2. Fluorescence responses of **ER-Se** (10μM) in the absence and presence of HClO (50 μM) under different pHs. All experiments were performed in Na_2HPO_4-KH_2PO_4 buffer (10 mM, with 1% CH_3CN, v/v) at room temperature. Each data was recorded 25 min after mixing. λ_{ex} = 410 nm, slit width: d_{ex} = d_{em} = 10 nm.

5. MTT assay

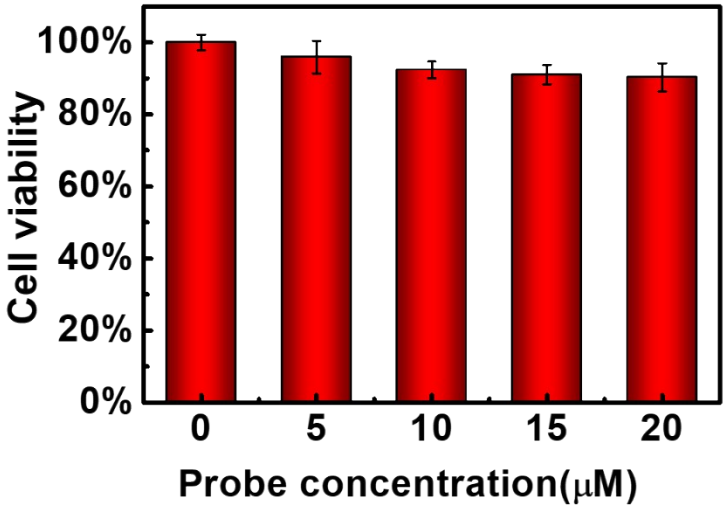


Figure S3. MTT assay for estimating cell viability (%) of HeLa cells were seeded into 96-well plates at a density of 5 × 10^3 cells per well in culture media after treatment with a series concentration of the probe system at 37 °C in an atmosphere of 5% CO_2 and 95% air for 24 h. The concentrations of probe **ER-Se** were used: 1. blank, 2. 5 μM 3. 10μM, 4. 15 μM,5. 20μM, respectively.

6. Structural characterization of compound 1, and ER-Se

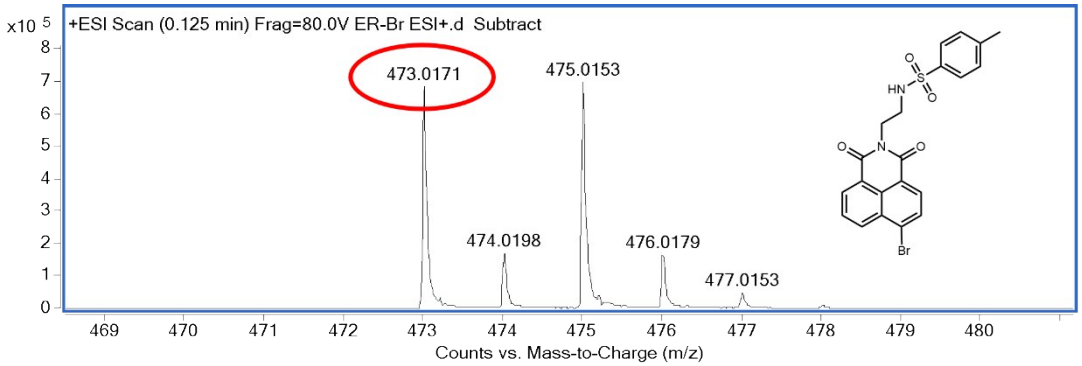


Figure. S4 HR MS spectral of compound 1

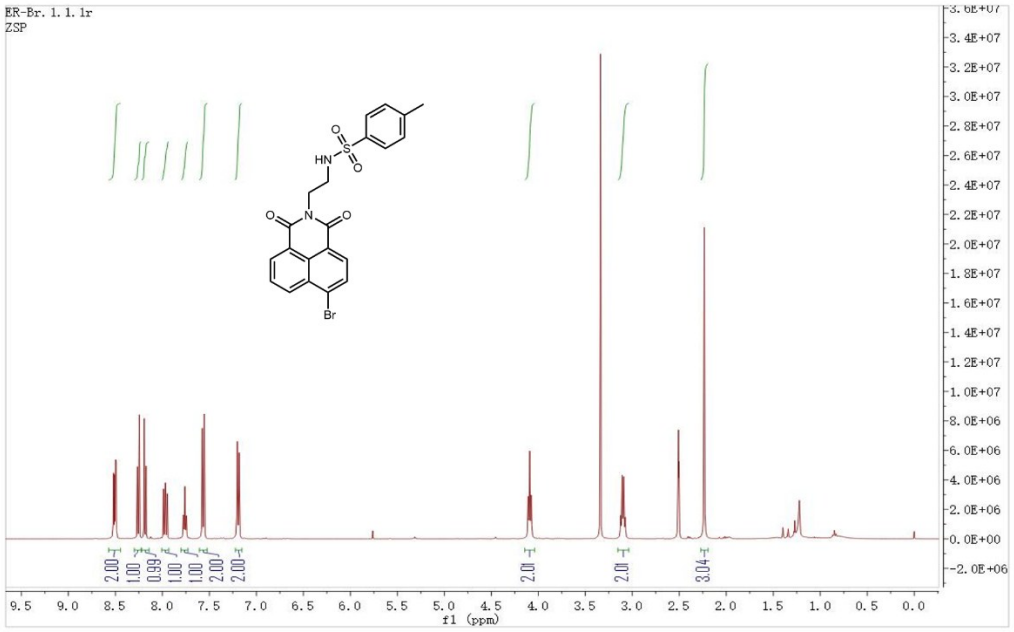


Figure. S5 ^{1}H NMR spectral of compound 1

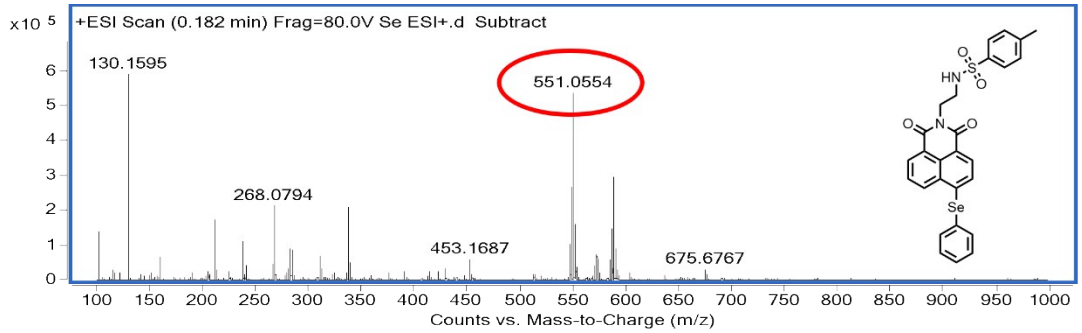


Figure. S6 HR MS spectral of **ER-Se**

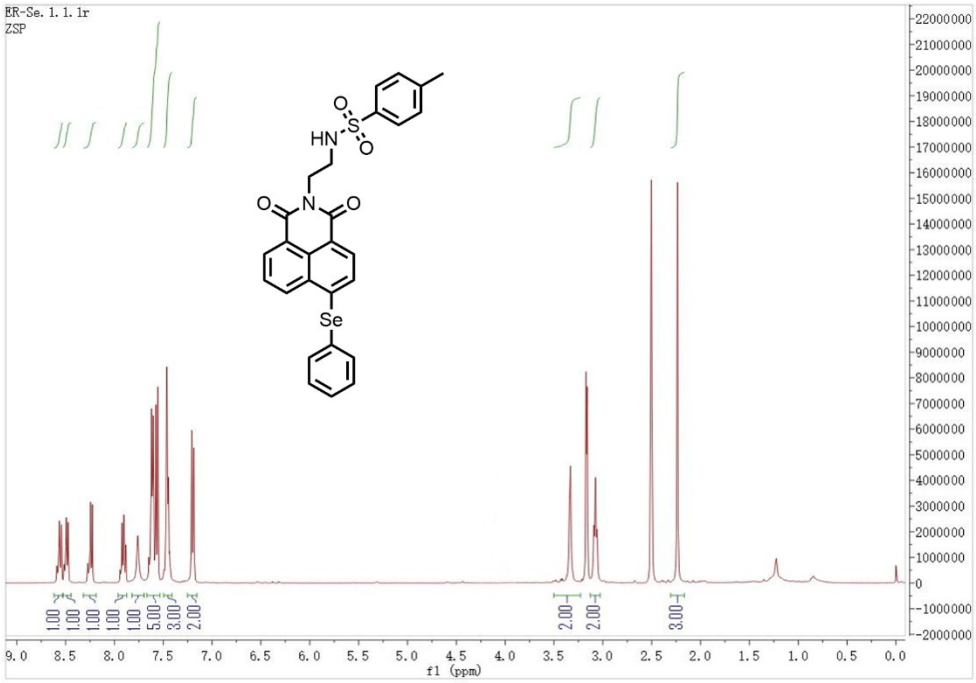


Figure. S7 ^{1}H NMR spectral of **ER-Se**

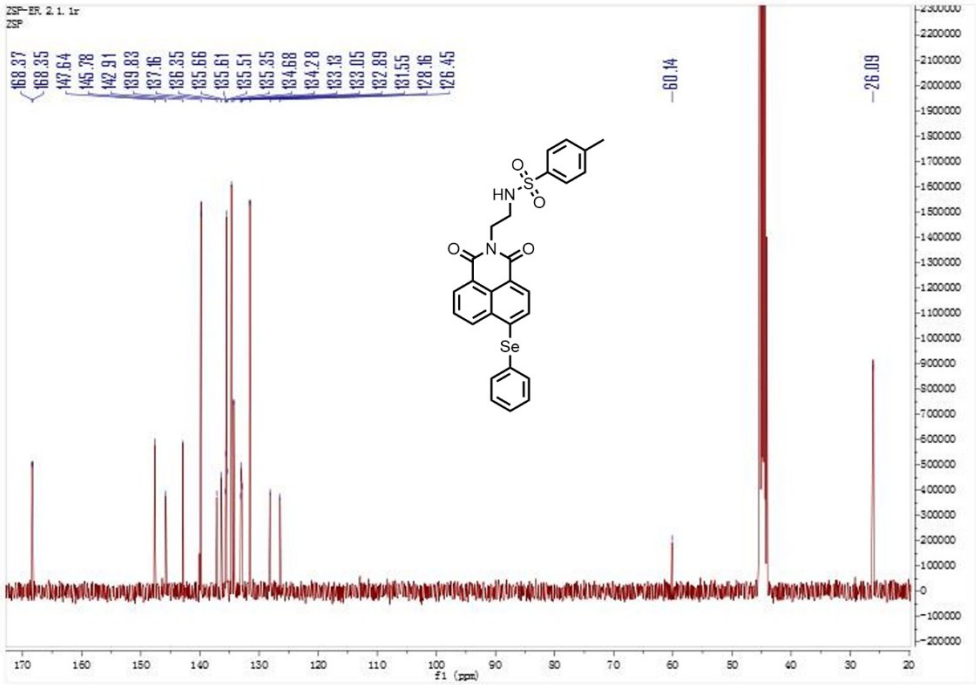


Figure. S7 ^{13}C NMR spectral of **ER-Se**

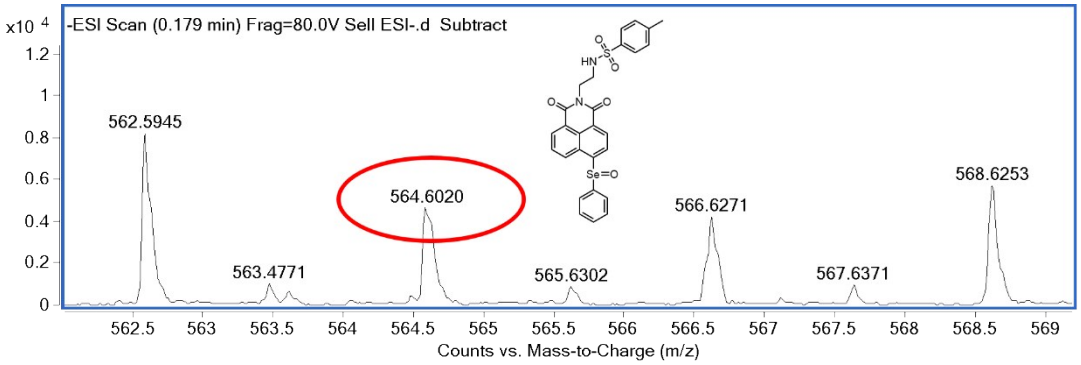


Figure. S8 HR MS spectral of the mixture of **ER-Se** and HClO

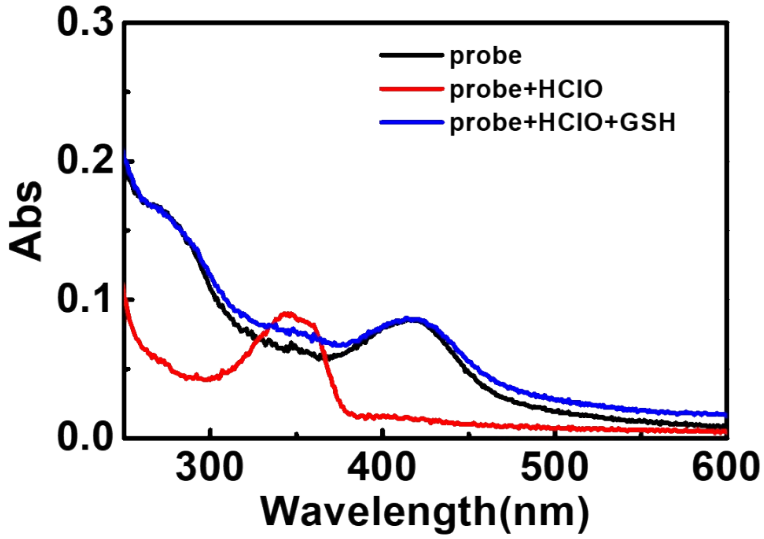


Figure. S9 UV–*vis* spectra of 10 μM probe (black line), the mixture of 10 μM probe and 50 μM HClO (red line) and the mixture of 10 μM probe and 50 μM HClO and then added 50 μM GSH (blue line) 10 mM PBS, pH 7.4, containing 1% CH_3CN, v/v at room temperature.

7. Additional HeLa cell images

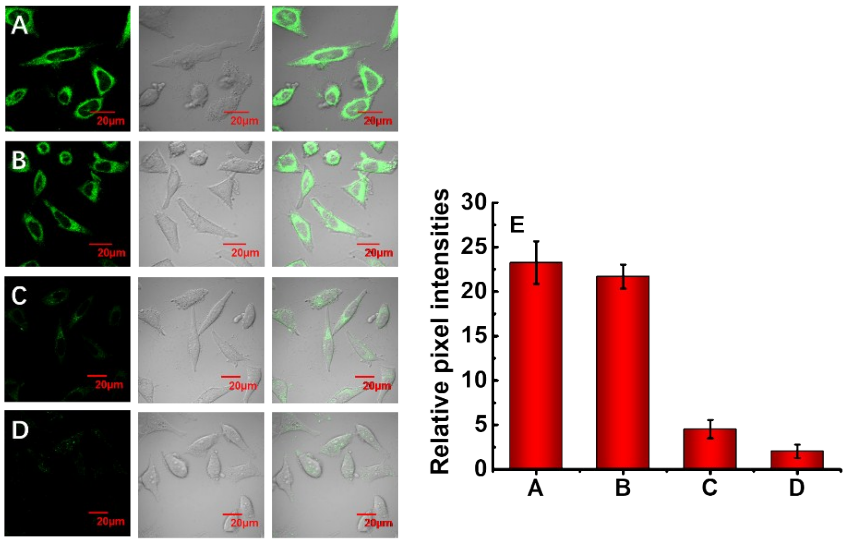


Figure. S10 Fluorescence images in HeLa cells. (A) Cells pretreated by NEM (1 mM) for 30 min and then incubated by **ER-Se** (10 μM) for 30 min. (B) (C) and (D) Cells pretreated by HClO (0 μM, 50 μM and 100 μM) for 30 min and then incubated by **ER-Se** (10 μM) for 30 min. Green channel (450–550 nm), λ_{ex} = 405 nm. (E) Relative pixel intensities for images A-D. Scale bar is 20 μm. Error bars represent standard deviation (±SD).