Electronic Supplementary data for

An endoplasmic reticulum-targeting fluorescent probe for the imaging of GSH in living cells

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Materials and Instruments

All solvents and reagents were commercially available and used without further purification unless for special needs. Twice-distilled water was used in all the experiments. Reactions were monitored by TLC using Yantai Jingyou (China) GF254 silica gel plates. Silica gel column chromatography was performed on silica gel (200–300 mesh) from Qingdao Hailang (China) and on a Biotage Isolera One (Biotage, Uppsala, Sweden). The fluorescence spectra and relative fluorescence intensity were measured with an Agilent Cary Eclipse (Varian, Palo Alto, California USA). UV/vis spectra were made with a Shimadzu UV-2600 spectrophotometer. High-resolution ESIMS data were acquired on an Agilent 6520 Q-TOF LC/MS. ¹H and ¹³C NMR spectra were recorded on Bruker Avance III 600 MHz NMR Spectrometer. Chemical shifts were expressed in *d* (ppm) and coupling constants (*J*) in Hz with residual solvent signals as references (CDCl₃, $d_{\rm H}$ 7.26 ppm and $d_{\rm C}$ 77.0 ppm; DMSO- d_6 , $d_{\rm H}$ 2.50 ppm and $d_{\rm C}$ 39.5 ppm). Cells imaging was performed with a Nikon A1R confocal microscope.



Fig. S1 UV-Vis absorption spectra of 5 μ M ER-G in absence and presence of GSH in PBS (20 mM, 5% methanol, pH = 7.4)



Fig. S2 Linearity between the fluorescence intensity at 558 nm and GSH concentration in the range of 75-300 μ M.



Fig. S3 HRMS (ESI) assay of the reaction products of ER-G with GSH.



Fig. S4 (A) Fluorescence spectra of 5 μ M **ER-G** in absence and presence of 400 μ M GSH at various pH values. (B) Fluorescence intensity at 558 nm of 5 μ M **ER-G** in absence and presence of 400 μ M GSH at various pH values. $\lambda_{ex} = 470$ nm.



Fig. S5 Survivability of HepG2 cells after treatment with 1, 5, 10 and 20 µM ER-G.



Fig. S6 ¹H NMR spectrum of compound 1



Fig. S7 ¹³C NMR spectrum of compound 1



Fig. S8 HRESIMS spectrum of compound 1



Fig. S10 13 C NMR spectrum of compound 2



Fig. S11 HRESIMS spectrum of compound 2



Fig. S12 ¹H NMR spectrum of compound ER-G





Fig. S13 ¹³C NMR spectrum of compound ER-G

Fig. S14 HRESIMS spectrum of compound ER-G