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Supporting information for

Development of an endoplasmic reticulum-targeting fluorescent probe for the imaging of polarity in living cells and tissues

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Fig. S1. (A) Fluorescence spectra of 5 μ M **NSp** at various pH. (B) Fluorescence intensities at 510 nm of 5 μ M **NSp** at various pH. $\lambda_{ex} = 405$ nm



Fig. S2. Photostability of compound NSp at Dixane and DMF. $\lambda_{ex} = 405$ nm



Fig. S3. The MTT experiments of **NSp** under different concentrations for 4T1 cells; 3T3 cells; HepG2 cells; HL-7702cells.



Fig.S4 (A) Fluorescence images of **NSp** (5 μ M) in live cells at different times after the addition of ER stress stimuli. a1–a5: Fluorescence images of **NSp** in HL-7702 cells after the addition of Tm (50 mg / mL). b1–b5: Fluorescence images of **NSp** in HepG2 cells after the addition of DTT (50 mg / mL). b1–b5: Fluorescence images of **NSp** in HepG2 cells after the addition of DTT (5.0 mM). $\lambda_{ex} = 405$ nm, $\lambda_{em} = 425$ - 475 nm, Scale bar: 20 μ m. (B) Analysis of the quantitative fluorescence intensity in (A) using ImageJ software. (C) The average fluorescence intensity output of **NSp** at different times. Scale bar: 20 μ m.



Fig. S5. ¹H-NMR spectrum of compound 1 in MeOH-*d4*.



Fig. S6. ¹³C-NMR compound 1 in MeOH-*d4*.



Fig. S7. FT-IR data of compound 1.



Fig. S8. ¹H-NMR spectrum of Compound 2 in DMSO-d6.



Fig. S9. ¹³C-NMR spectrum of Compound 2 in DMSO-d6.



Fig. S10. FT-IR data of compound 2.





Fig. S12. ¹³C-NMR spectrum of NSp in DMSO-*d6*.



Fig. S13. HRMS data of the probe NSp.



Fig. S14. FT-IR data of the probe NSp.