Supporting information for

A Modified viscosity-sensitive fluorescent probe with a large Stokes shift for simultaneous imaging of lipid droplets and lysosomes in tobacco leaf vein cells and biological system

Yuan He, *[a] Zhi-Hao Zhang, ‡[b] Long-Ke Li, ‡[a] Xun Ji, [b] Guo-Yu Chen, [a] and Jian-Yong Wang *[b]

[a] College of Tobacco Science and Engineering, Zhengzhou University of Light Industry, Zhengzhou 450002, China.

[b] Faculty of Light Industry, State Key Laboratory of Biobased Material and Green Papermaking, Key Laboratory of Paper Science and Technology of Ministry of Education, Qi Lu University of Technology (Shandong Academy of Sciences), Jinan, 250353, P. R., China.

† These authors contribute equally

E-mail: heyuan818@163.com; wjy@qlu.edu.cn

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Materials

Unless otherwise noted, all the solvents, reagents and materials were obtained from commercial companies and used without further purification. The 1HNMR and 13CNMR spectrums were obtained from INOVA-400 MHz nuclear magnetic resonance instruments, respectively. High-resolution electrospray mass spectra (HRMS) were obtained from Bruker APEX IV-FTMS 7.0T mass spectrometer. All the UV–vis absorption spectra and emission spectra were obtained from Shimadzu UV-1800 spectrometer and Shimadzu RF-5301PC spectroscope respectively. The fluorescence images of cells were obtained with Nikon A1MP confocal microscopy with a CCD camera, Both TLC and silica gel were purchased from the Qingdao Ocean Chemicals.

Cell Culture

Unless otherwise noted, the cells used in this article are cancerous HeLa cells.HeLa cells were supplied by Jiangsu Kaiji Biotechnology. HeLa cells were cultured in DMEM containing 1% penicillin-streptomycin and 10% FBS in an incubator at 37°C and 5% CO_2. The medium was changed at 48 h. Cells were collected when they overlapped and treated with 0.25% trypsin-EDTA solution.

Cell viability was then calculated from the absorbance ratio of the sample wells to the control cells. HeLa cells were inoculated into 96-well plates with different concentrations of probes. Subsequently, the cells were incubated at 37 ℃ with 5% CO2 and 95% air for 24 hours. HeLa cells were then washed with PBS buffer and DMEM culture solution (500 μL) was added. Immediately after that, MTT (50 μL, 5 mg/mL) was injected into each well and incubated for 4 hours. The absorbance of the solution was measured at 570 nm using an enzyme marker.

Cellular Imaging and Co-Localization

All cell colocalization experiments were performed under Nikon A1MP confocal microscope. 20μL of the prepared probe reserve solution and the appropriate commercial label dye reserve solution were added simultaneously to 2mL of the cell culture solution for incubation. The final concentration for the probe is generally 10 μ M, and the final concentration for commercial label

dyes is generally 500nM.Tests were performed after an incubation time of 30 minutes. The study employed Lysotracker Red as the commercial lysosomal indicator and Nile Red as the commercial lipid droplet indicator.

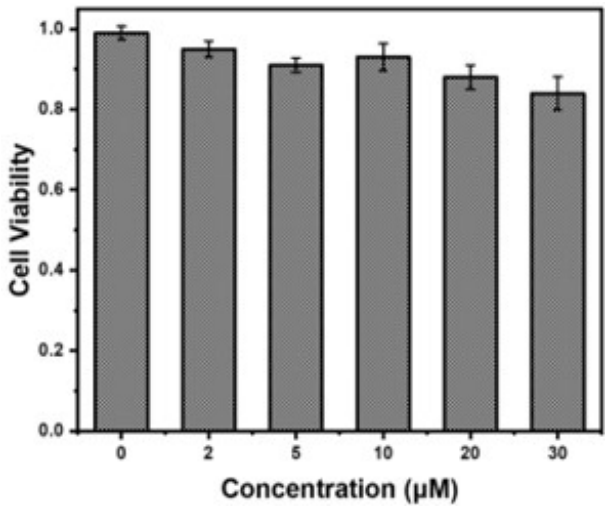

Fig. S1 Cytotoxicity assay of Hela cells at different **Nap-Lyso-Ph-OH** concentrations.

Fig. S2 Colour of Nap-Lyso-Ph-OH in different solvents under 365 nm UV light.

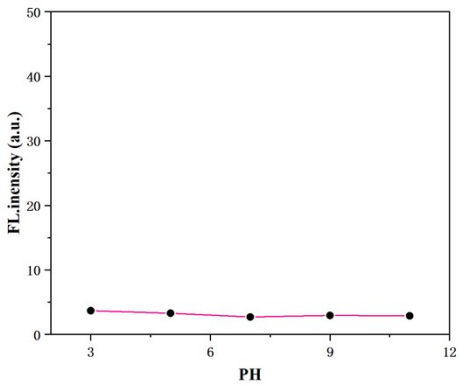

Fig. S3 Fluorescence intensity of **Nap-Lyso-Ph-OH** at different pH values in a high viscosity environment

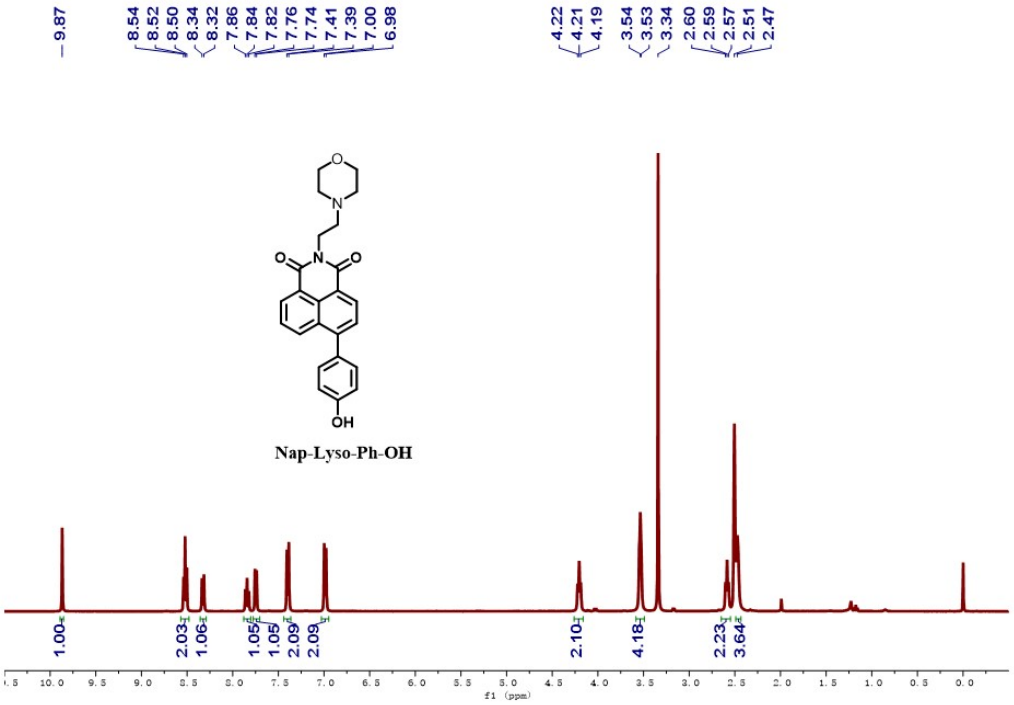

Fig. S4 ^{1}H NMR (DMSO-*d6*) spectrum of compound **Nap-Lyso-Ph-OH.**

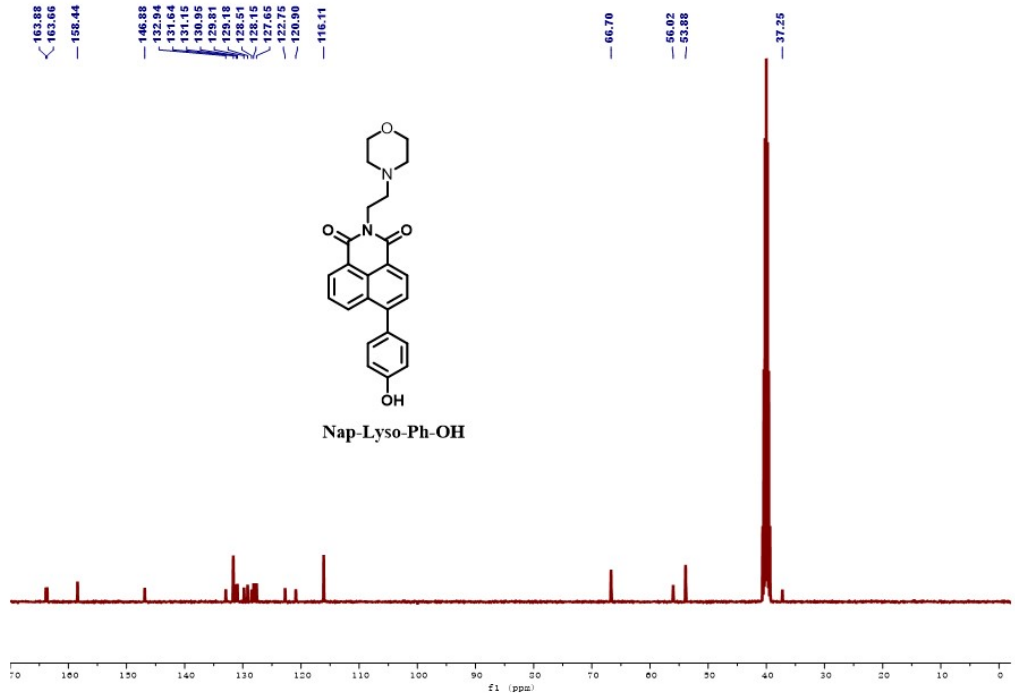

Fig. S5 ^{13}C NMR (DMSO-*d6*) spectrum of compound **Nap-Lyso-Ph-OH**