Supporting Information for

Dual-channel fluorescent probe for monitoring pH change in lysosomal during autophagy

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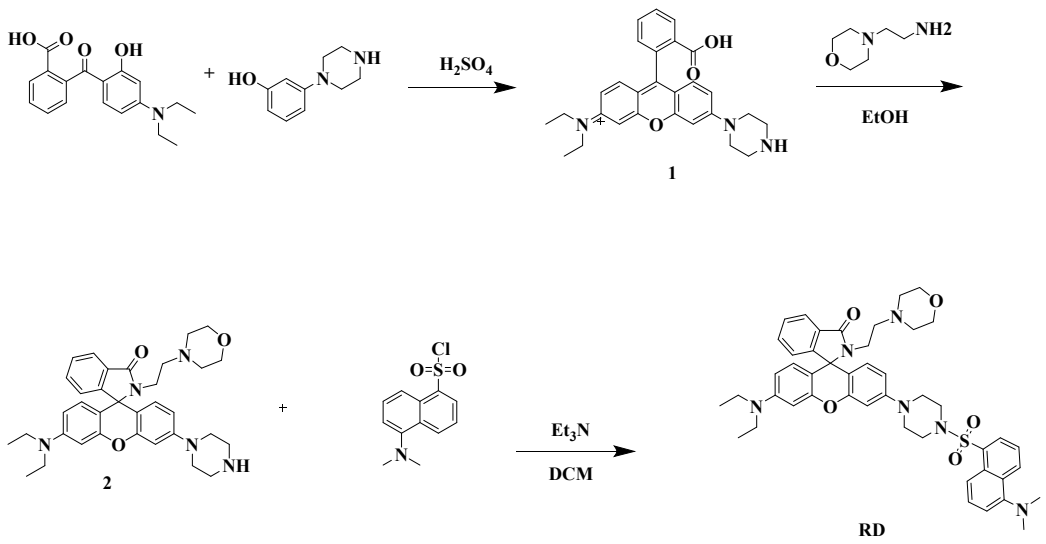

Scheme S1. Structure and synthetic route of **RD**

Synthesis of Compound 1

In 100 mL round bottom flask, 456 mg 4-diethylamine ketoic acid (2.0mmol), 0.3565 g 1-(3-Hydroxyphenyl)–piperazine (2.0 mmol) and 2 mL sulphuric acid were added first. The reaction was heated to 90 °C and refluxed for 3 hours. The solvent was dissolved in 100 mL ice water , and 2 mL perchloric acid was added，then collectedred solid precipitated . The crude product was purified by column chromatography on silica gel (CH_2Cl_2:CH_3OH = 10:1) to afford a red solid (784 mg, yield 86.5%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 7.0 Hz, 1H), 7.60 (t, J = 7.3 Hz, 1H), 7.28 (s, 1H), 7.21 (d, J = 7.6 Hz, 1H), 6.71 (d, J = 2.3 Hz, 1H), 6.65 (d, J = 8.8 Hz, 1H), 6.59 (d, J = 9.0 Hz, 1H), 6.46 (s, 1H), 6.36 (d, J = 2.5 Hz, 1H), 3.38 (q, J = 7.1 Hz, 4H), 3.27 – 3.22 (m, 4H), 3.08 – 3.03 (m, 4H), 1.19 (t, J = 7.0 Hz, 6H), 0.94 (dd, J = 13.8, 7.2 Hz, 1H).

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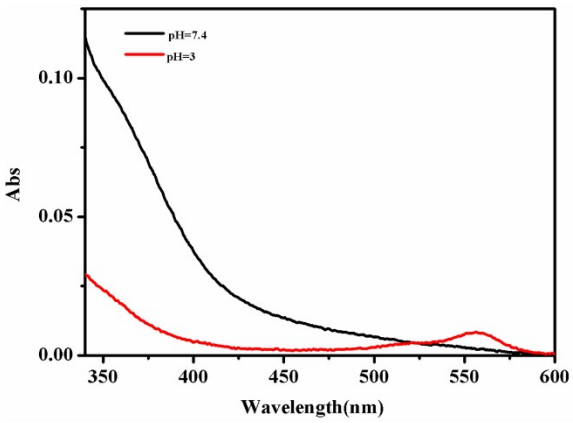

Figure S1. The UV-Vis spectra of the probe **RD** with pH 3.2 and 7 in DMSO/ B-R (1/99, v/v) solution

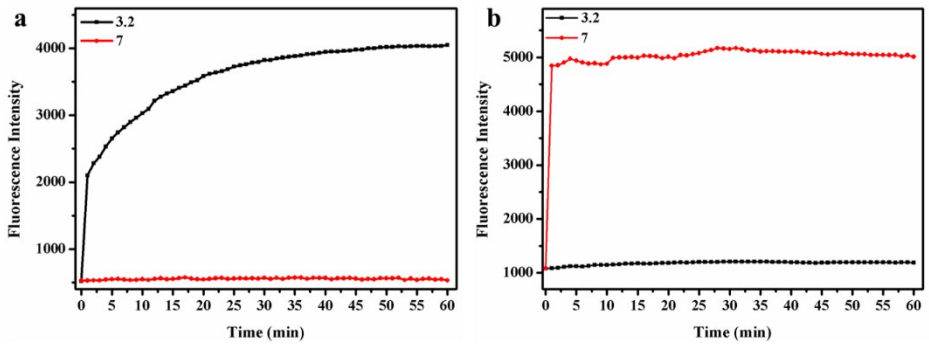

Figure S2. The time courses of reaction of the probe **RD (**10 μM**)** in the presence of pH in B-R solution at room temperature. (a) λ_{ex} 556 nm (b) λ_{ex} 405 nm.

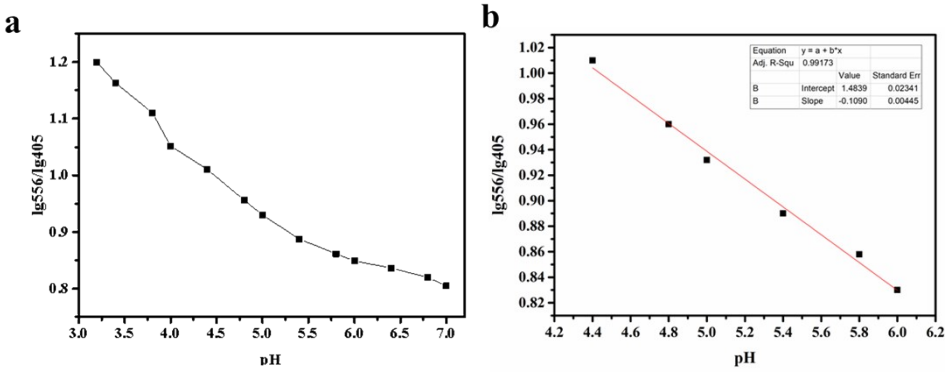

Figure S3. (a) pH titration curves of lg ratios between fluorescence emission of Rhodamine (I_{556} nm) and that of dansyl group (I_{405} nm). (b) The relationship between the value of lg556/lg405 and pH 4.4 - 6.

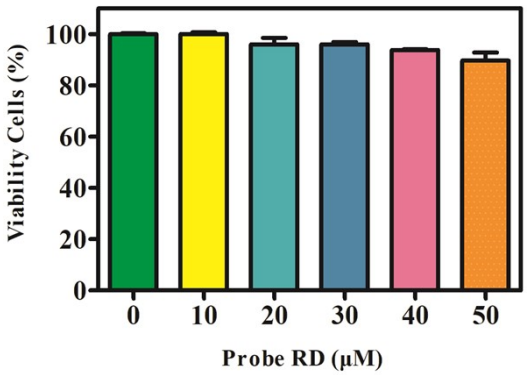

Figure S4. Viability of HL-7702 cells treated with the various concentrations of probe RD for 24h.

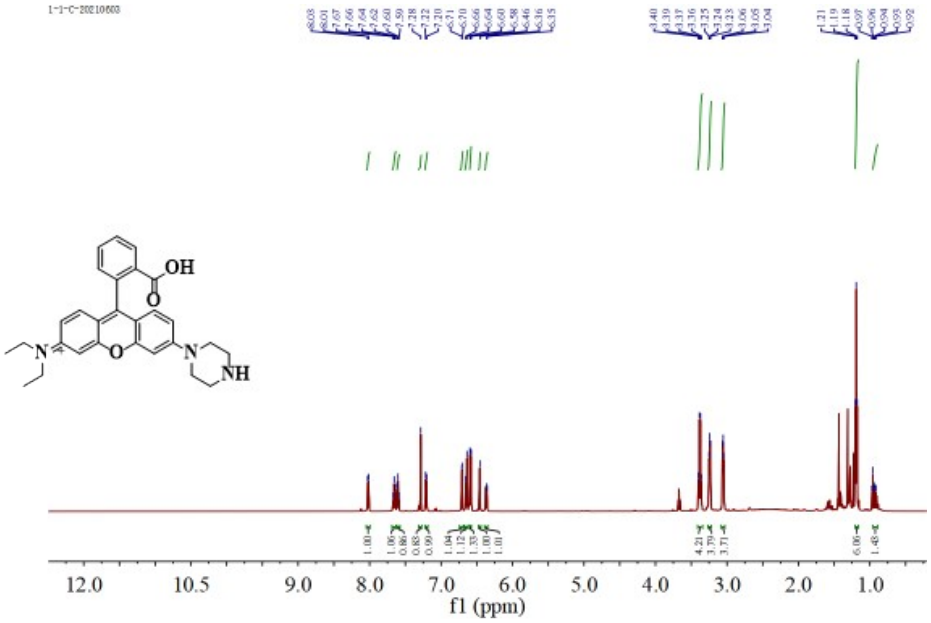

Figure S5. ^{1}H NMR of compound **1**.

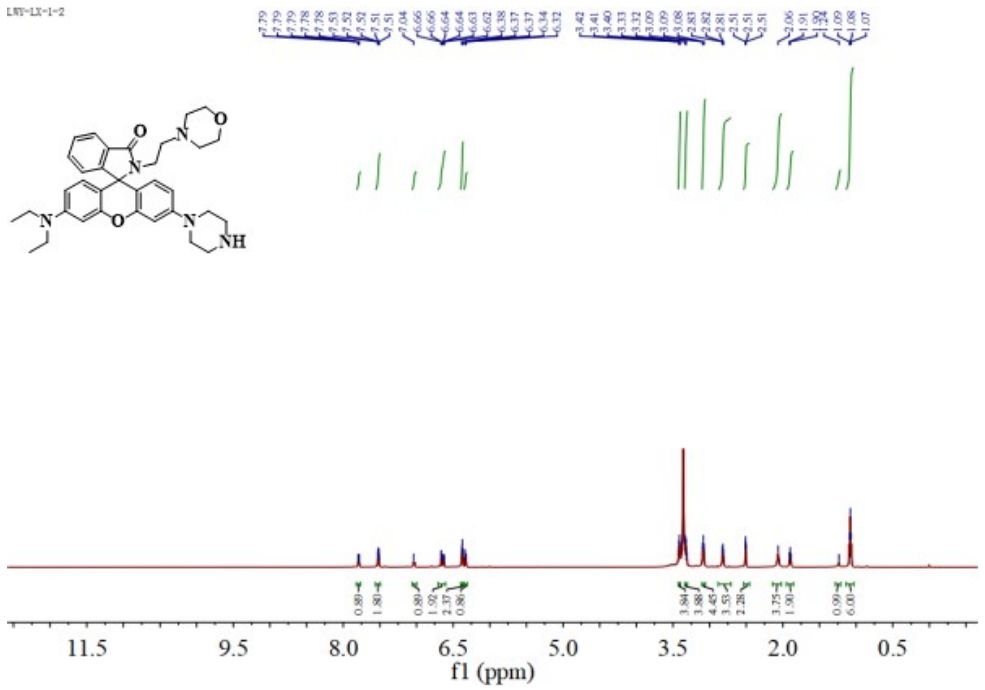

Figure S6. ^{1}H NMR of compound **2**.

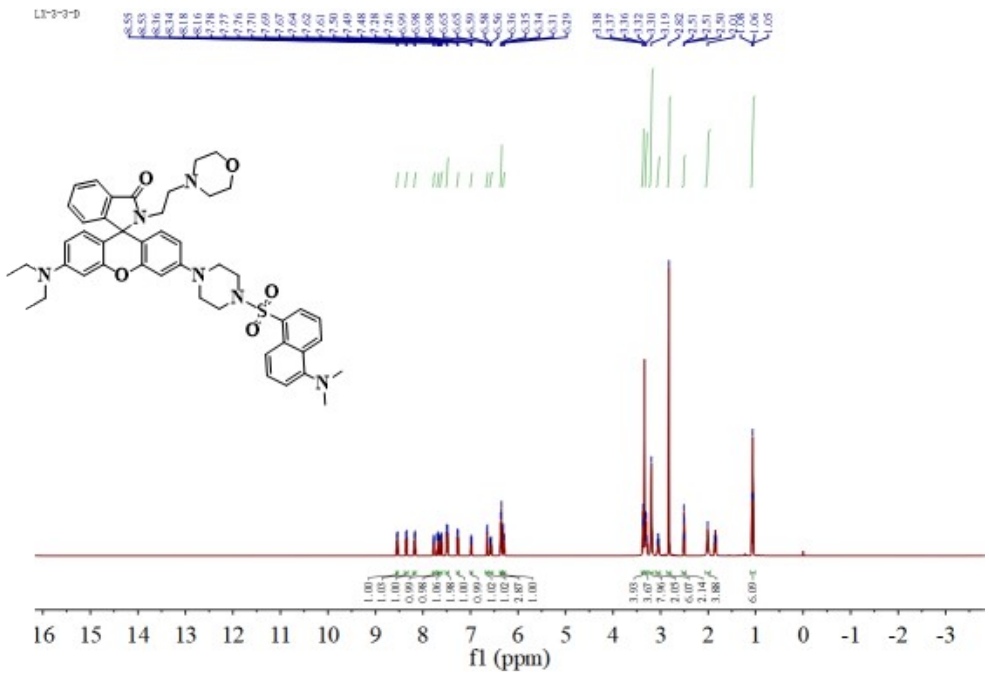

Figure S7. ^{1}H NMR of the probe **RD**

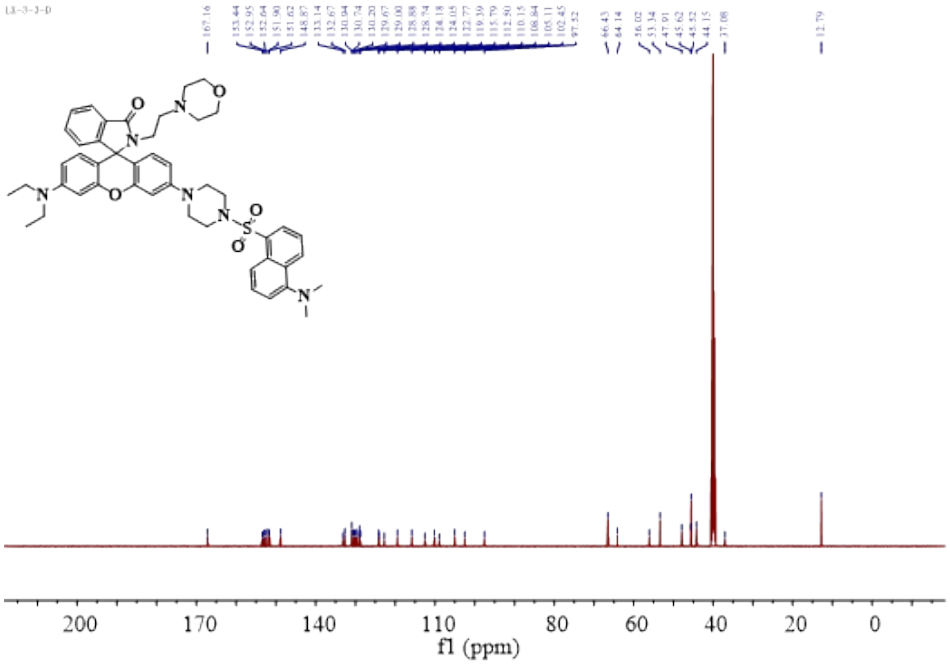

Figure S8. ^{13}C NMR of the probe **RD**.

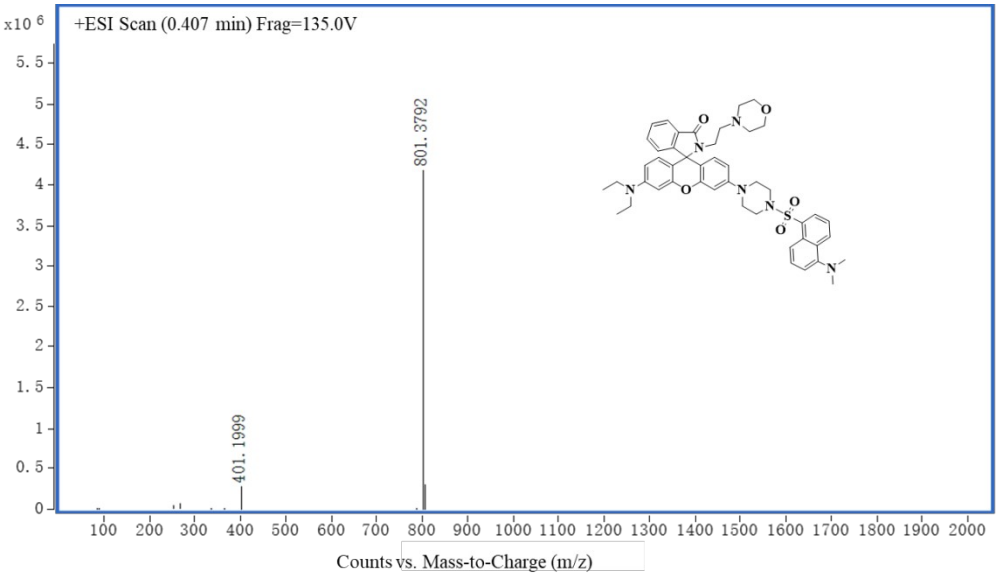

Figure S9. Mass spectrum of the probe **RD**