Supplementary data

A novel ratiometric pH probe for extreme acidity based on FRET and PET

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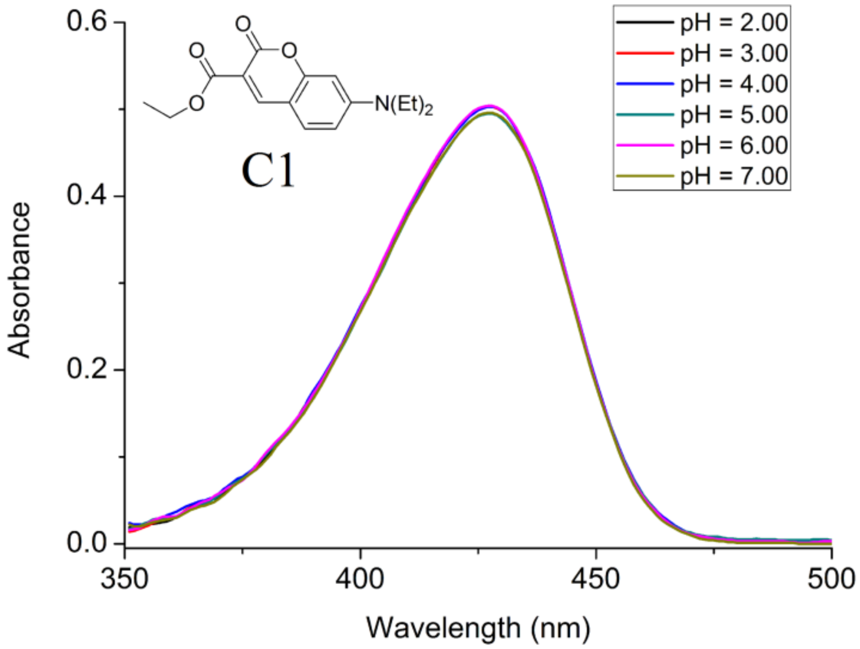

Fig. S-1 The absorption spectra of **C1** (10 μM) in buffer at different pH values. (pH = 7.00, 6.00, 5.00, 4.00, 3.00, 2.00).

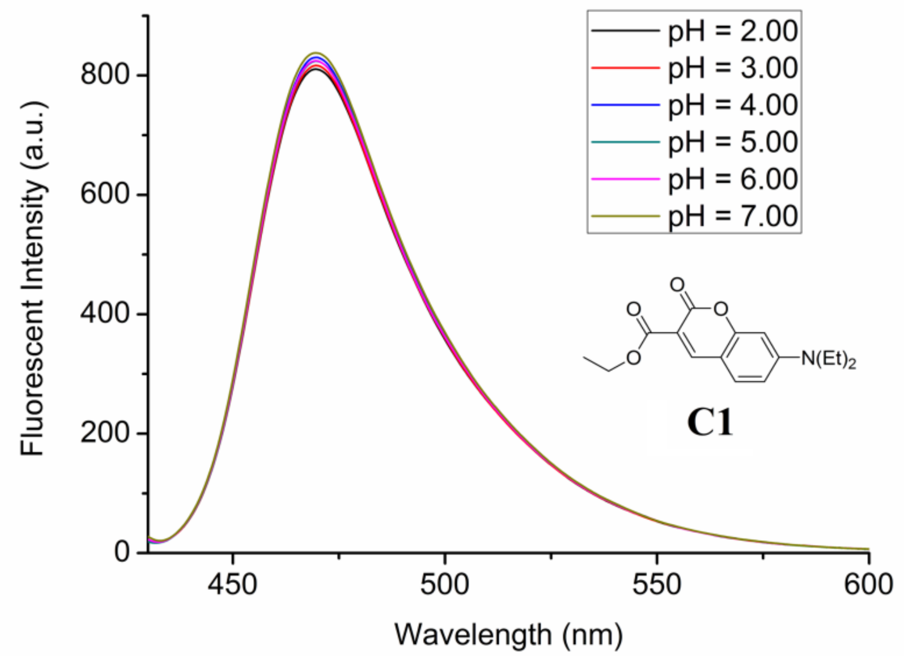

Fig. S-2 The fluorescence spectra of **C1** (10 μM) in buffer at different pH values. (pH = 7.00, 6.00, 5.00, 4.00, 3.00, 2.00, λ_{ex} = 420 nm).

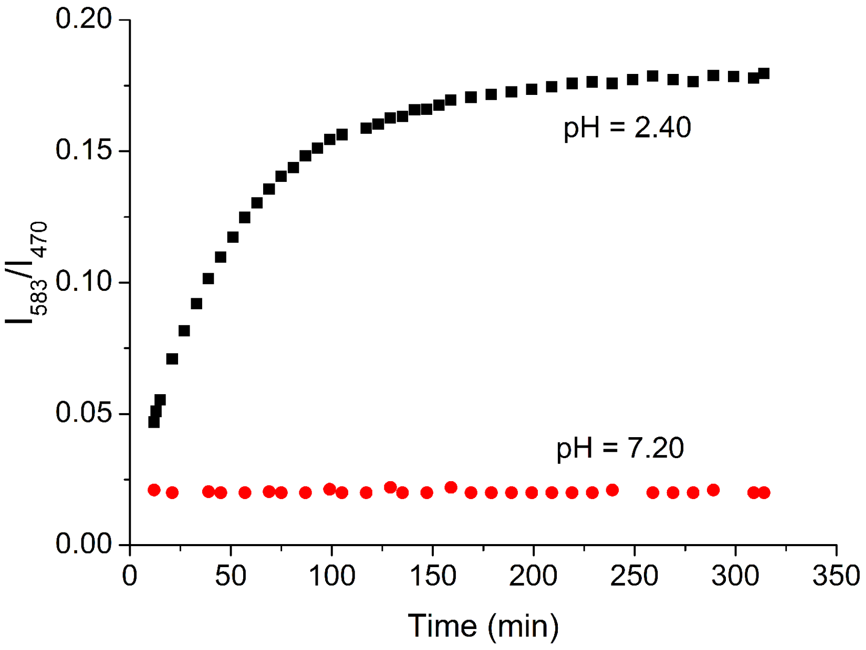

Fig. S-3 The time courses of fluorescence emission ratios (I_{583}/I_{470}) of **RC1** (10 μM) in buffer of various pH values.

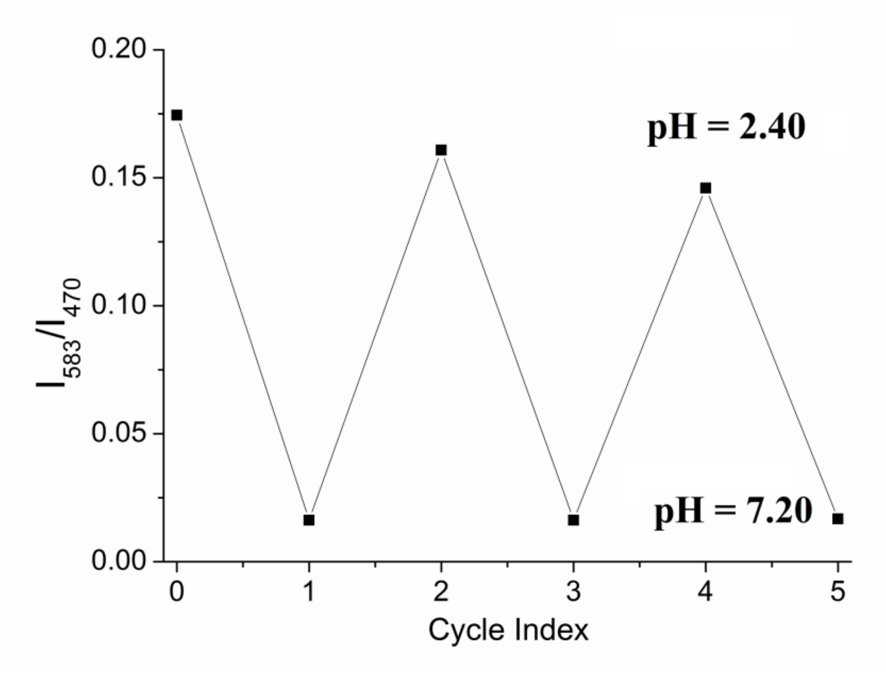

Fig. S-4 pH reversibility study of probe **RC1** (10 μM) between pH 2.40 and 7.20.

Cell culture and cell viability assay

A549 lung cancer cell lines were cultured in RPMI 1640 medium (Gibco, 31800-089) supplemented with 10% (v/v) NBS (Hyclone) at 37 ℃ with 5% CO_2 atmosphere. The cells were seeded in 96 well plates at the density of 12500/cm^2. 24 h later, cells were treated with fresh 1640 medium containing 2 μM **RC1** for 6 h. SRB assay was used to determine the cytotoxicity of the probe in vitro with cells treated only by DMSO solvent as control. The results shown in Figure S5 were the average of triplicate assays.

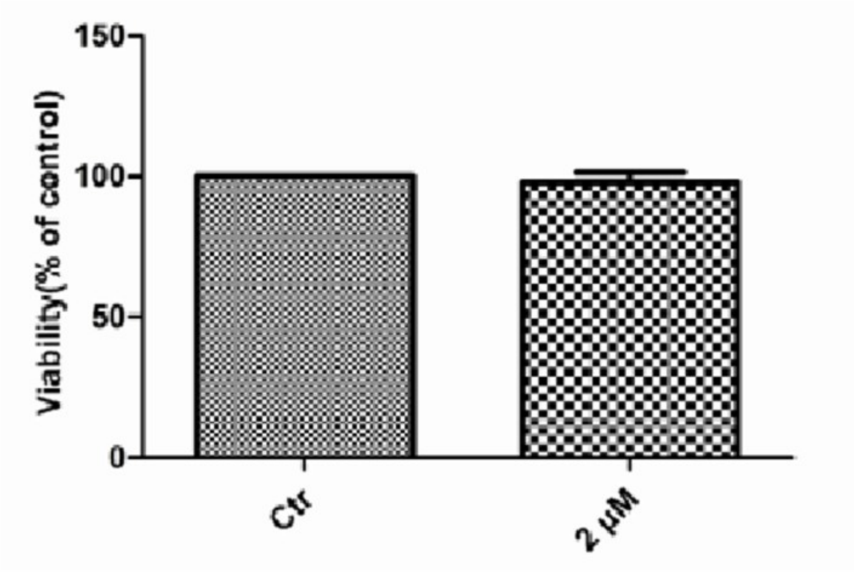

Fig. S-5 Viability of A549 cells after treatment with 2 μM **RC1** for 6 h ($p > 0.05$ vs control; n = 3).