

Electronic Supplementary Information

A ratiometric lysosomal pH probe based on naphthalimide-rhodamine system

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Synthesis of N1 and N2

Compound **N1** and **N2** were synthesized according to the literature.^{S1,S2}

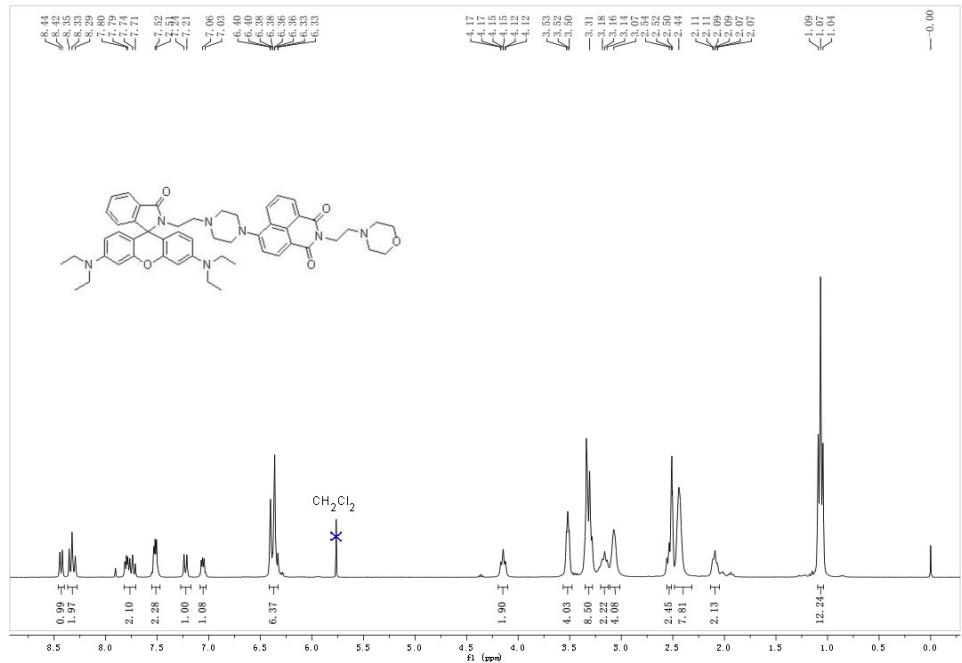


Fig. S1 ^1H NMR spectrum of probe **RNL**.

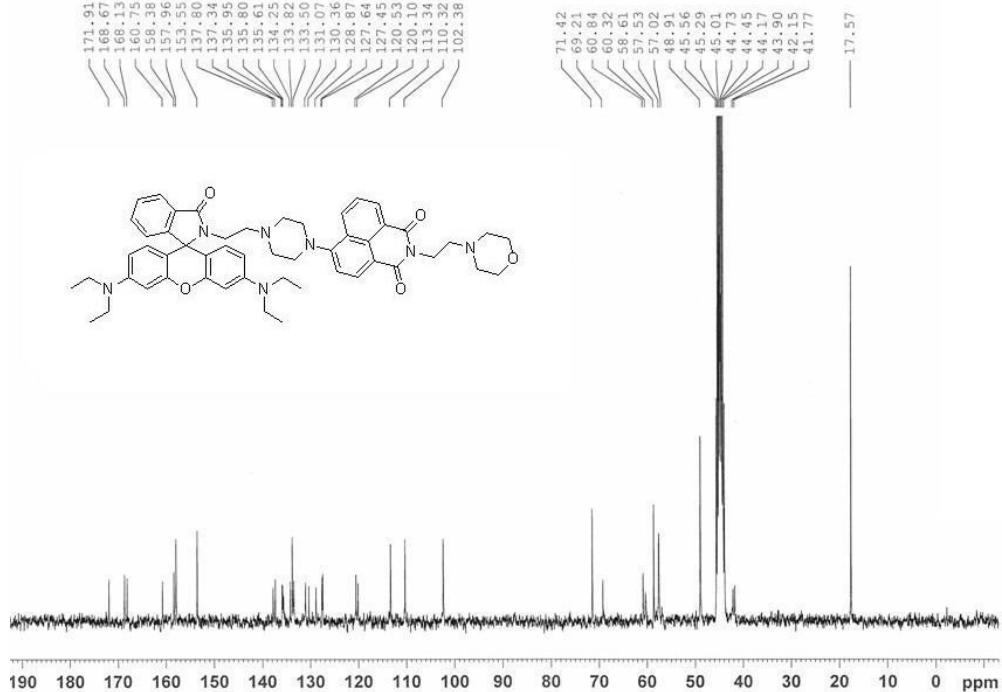


Fig. S2 ^{13}C NMR spectrum of probe **RNL**.

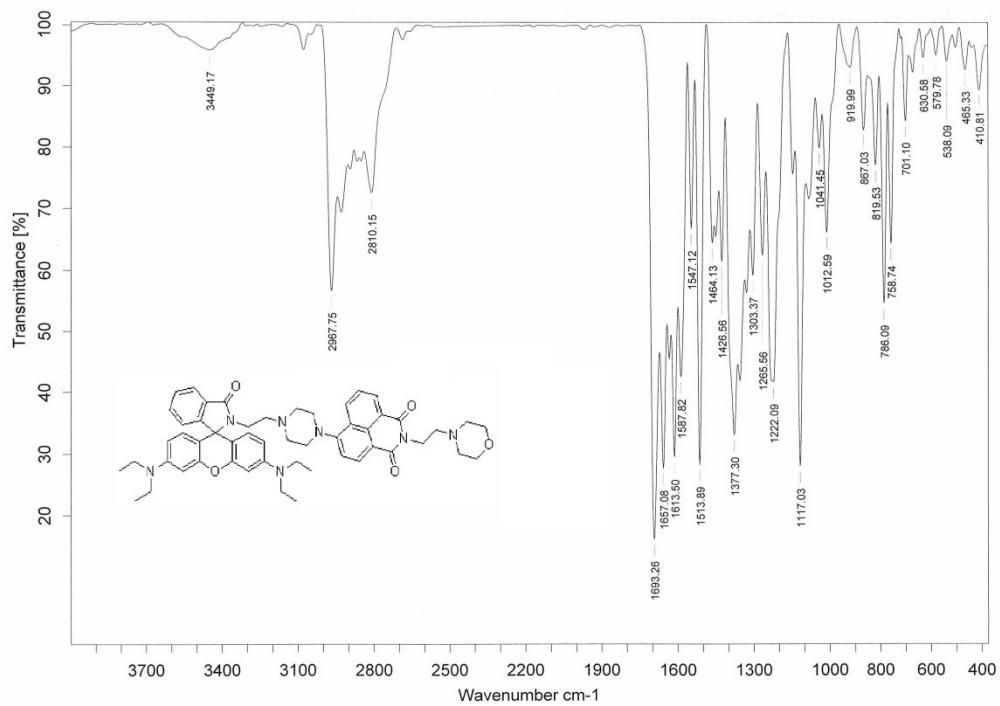


Fig. S3 Infrared spectrum of probe **RNL**.

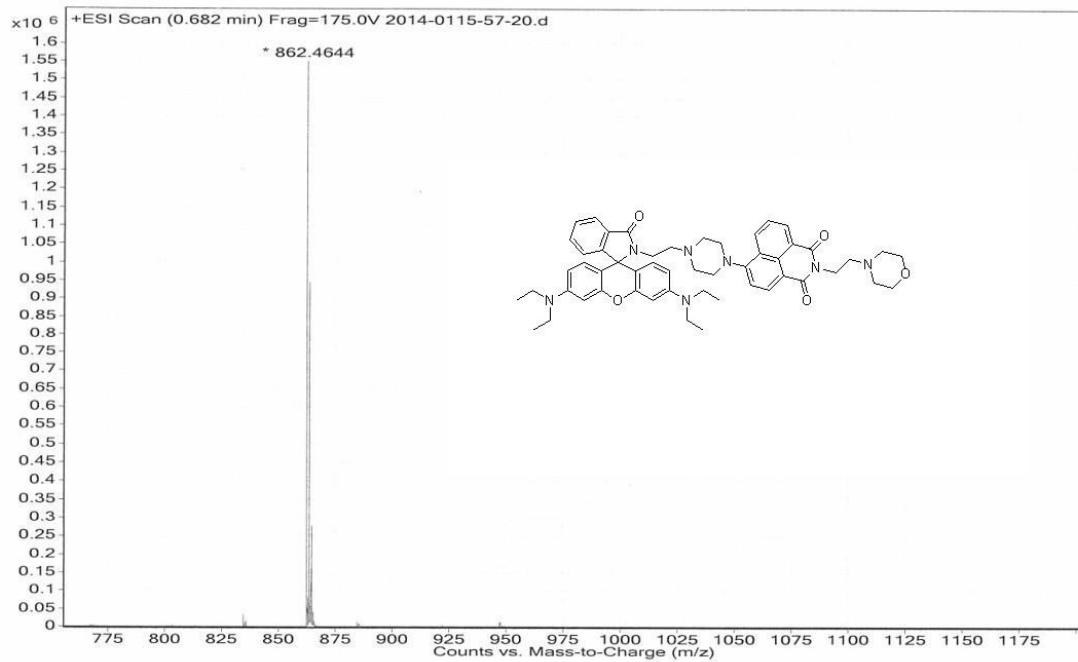


Fig. S4 High resolution mass spectrum of probe **RNL**.

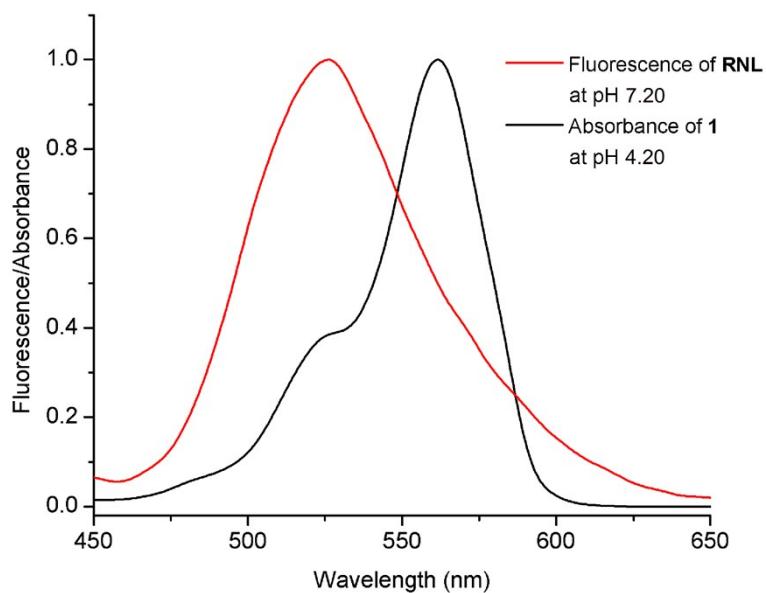


Fig. S5 Normalized emission spectrum of dyad **RNL** (5 μ M) at pH 7.20 ($\lambda_{\text{ex}} = 390$ nm) and absorption spectrum of compound **1** (5 μ M) at pH 4.20 in the B-R buffer/EtOH (9/1, V/V) solution.

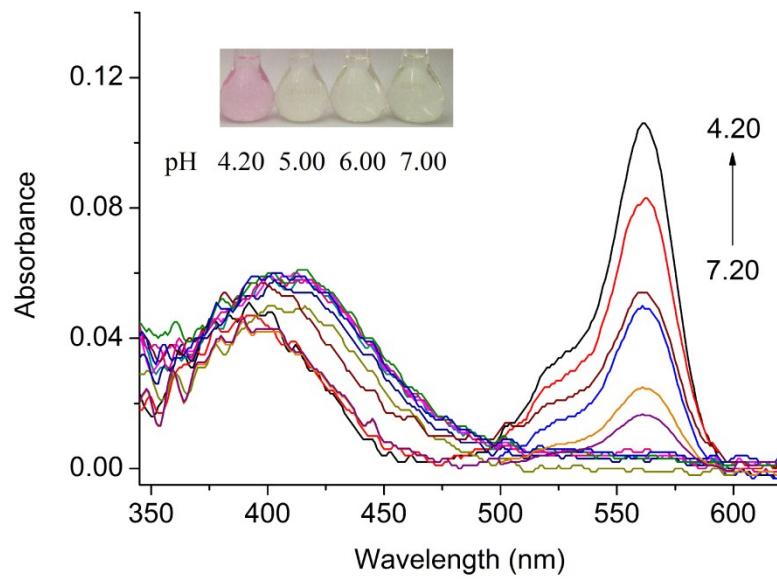


Fig. S6 Absorption spectra of **RNL** (5 μ M) in the B-R buffer/EtOH (9/1, V/V) solution at different pH values (7.20, 7.00, 6.40, 6.00, 5.60, 5.20, 5.00, 4.80, 4.60, 4.40, 4.20).

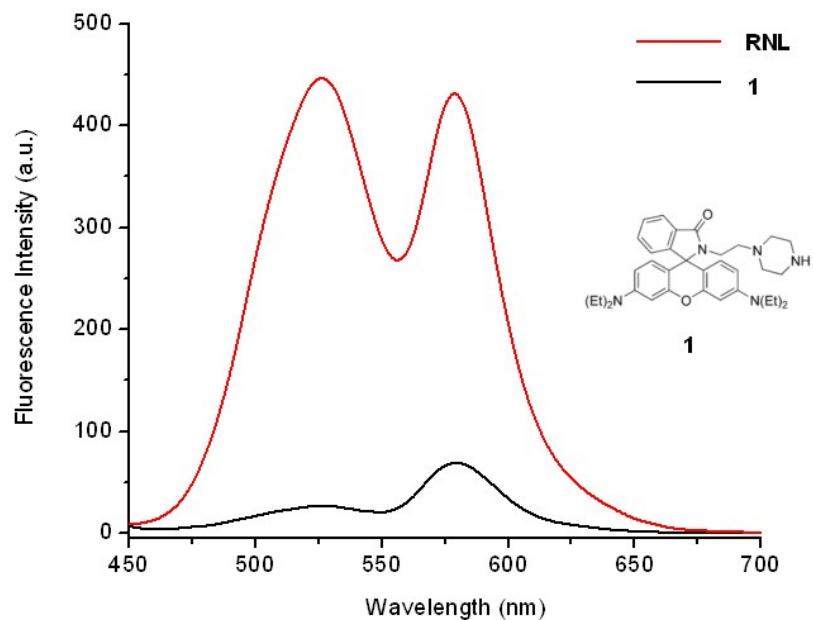


Fig. S7 Emission spectrum of dyad **RNL** (5 μ M) and compound **1** (5 μ M) at pH 4.70 in the B-R buffer/EtOH (9/1, V/V) solution. $\lambda_{\text{ex}} = 390$ nm.

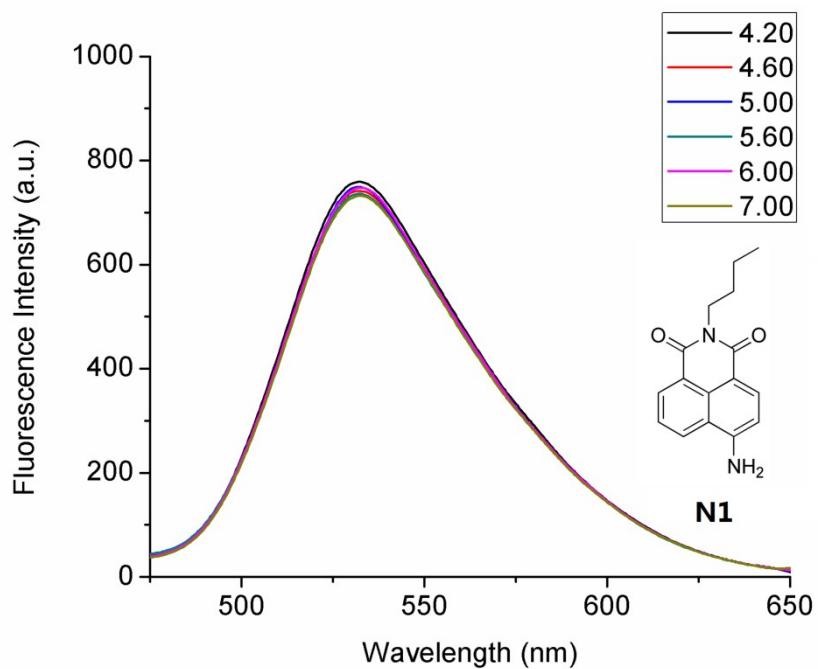


Fig. S8 Fluorescence spectra of **N1** (5 μ M) in the B-R buffer/EtOH (9/1, V/V) solution at different pH values. $\lambda_{\text{ex}} = 438$ nm.

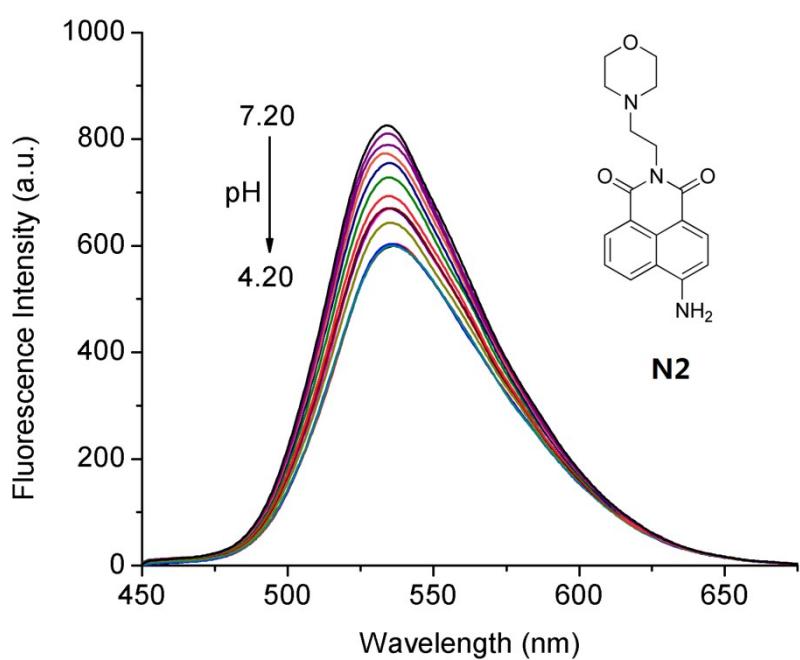


Fig. S9 Fluorescence spectra of **N2** (5 μ M) in the B-R buffer/EtOH (9/1, V/V) solution at different pH values. $\lambda_{\text{ex}} = 438$ nm.

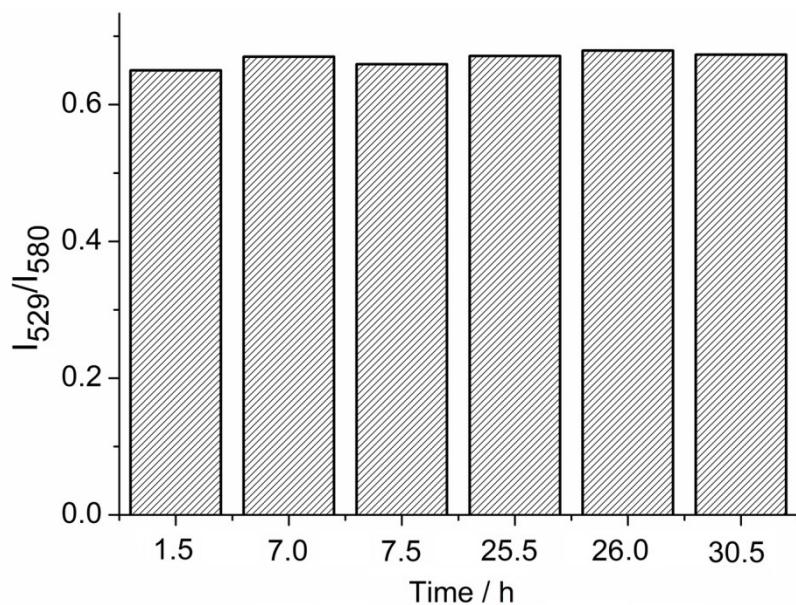


Fig. S10 The ratios of fluorescence intensity of **RNL** (5 μ M) at 529 nm and 580 nm (I_{529}/I_{580}) at pH 4.50 after being incubated in the B-R buffer/EtOH (9/1, V/V) solution for 1.5, 7.0, 7.5, 25.5, 26.0, 30.5 h at room temperature. $\lambda_{\text{ex}} = 390$ nm.

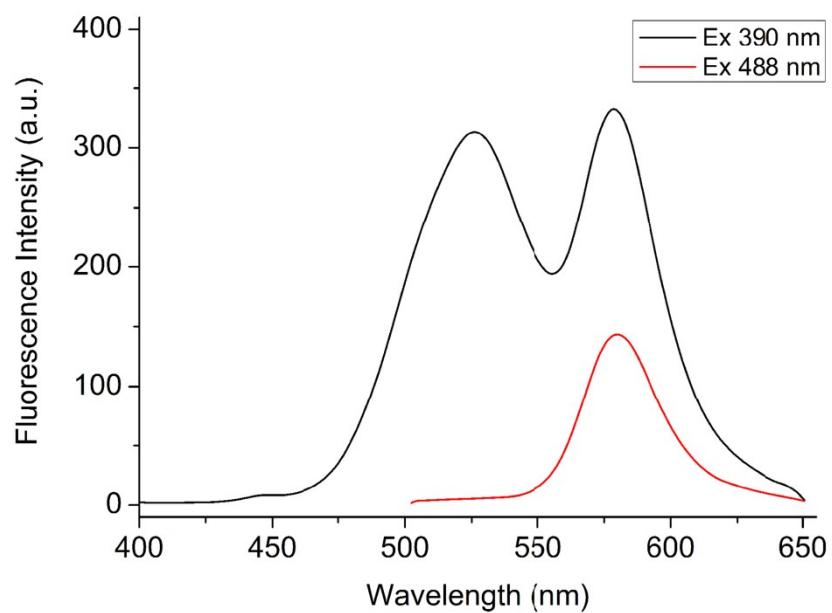


Fig. S11 Emission intensity of **RNL** (5 μ M) at pH 4.70 in the B-R buffer/EtOH (9/1, V/V) solution at $\lambda_{\text{ex}} = 390$ nm and $\lambda_{\text{ex}} = 488$ nm.

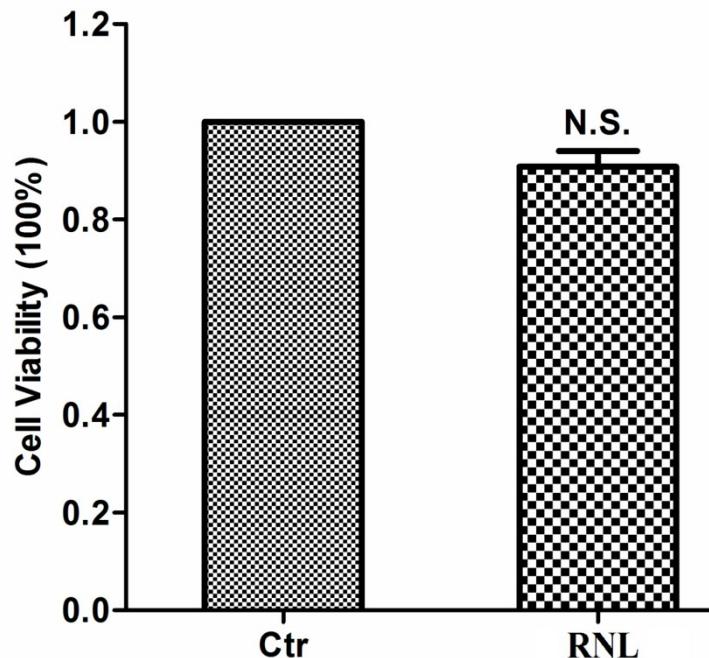


Fig. S12 Effect of **RNL** on the cell viability of HeLa cells. HeLa cells were incubated with 10 μ M **RNL** for 24 h. The cell viability was measured by SRB assays.

Photostability experiment

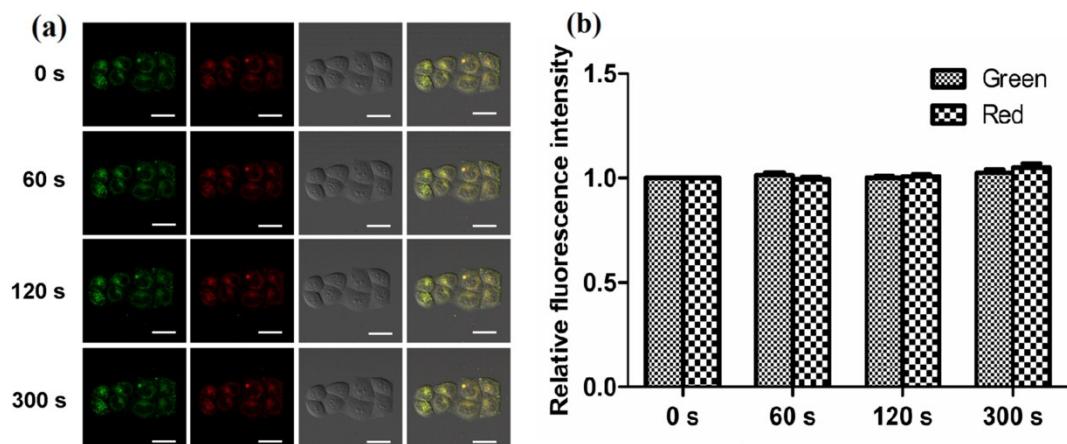


Fig. S13 Photostability experiment of **RNL** (10 μ M) in HeLa cells. (a) Fluorescence microscope images of HeLa cells after 0, 60, 120 and 300 s of continuous irradiation. $\lambda_{ex} = 405$ nm. First column: fluorescence images at green channel (400-555 nm), second column: fluorescence images at red channel (560-700 nm), third column:

bright field images, fourth column: overlay images of first, second and third column.
(b) Relative fluorescence intensity in cells at different periods of time [the initial fluorescence intensity (i.e., at about 0 s) was defined as 1.0]. Fluorescence intensity quantitation was analyzed by the Image J. The results were presented as means \pm SE with replicates $n = 3$. Scale bar, 20 μm .

Reference

S1 L. Cui, Z. X. Peng, C. F. Ji, J. H. Huang, D. T. Huang, J. Ma, S. P. Zhang, X. H. Qian and Y. F. Xu, *Chem. Commun.*, 2014, **50**, 1485.

S2 E. B. Veale, D. O. Frimannsson, M. Lawler and T. Gunnlaugsson, *Org. Lett.*, 2009, **11**, 4040.