

## Supporting Information

### **A ratiometric lysosomal pH probe based on the imidazo[1,5- $\alpha$ ]pyridine–rhodamine FRET and ICT system**

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Scheme S1 Synthesis of Donor of RhMP

Synthesis of donor

Fig. S1 Fluorescence spectra of RhMP in different solution

Fig. S2 UV spectra of RhMP at different pH values

Fig. S3–S6 Some fluorescence and UV spectrum of RhMP about energy transfer

Fig. S7 UV spectra of RhMP containing different metal cations

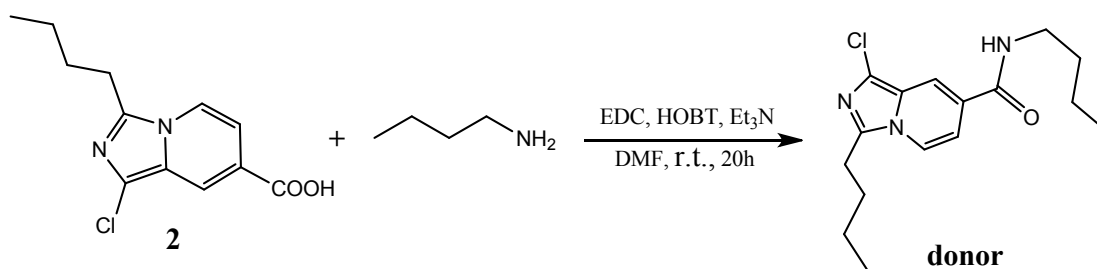
Fig. S8 Fluorescence spectra of RhMP containing different metal

Fig. S9 pH reversibility study of RhMP

Fig. S10 The photostability of the probe

Fig. S11 Effect of the probe on the viability of HeLa cells

Fig. S12–S15 Some MS, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of RhMP and donor



### Scheme S1 Synthesis Donor of RhMP

#### Synthesis of donor

The **donor** was obtained by the synthetic route (Scheme S1). Compound **2** (254.0 mg, 1 mmol), HOBT (162.0 mg, 1.2 mmol), EDC (229.2 mg, 1.2 mmol),  $\text{Et}_3\text{N}$  (202.0 mg, 2.0 mmol) were dissolved in 20 mL of DMF. Then the solution was stirred under  $\text{N}_2$  for 1 h, and n-butylamine was added to the solution. The mixture was stirred under  $\text{N}_2$  atmosphere at room temperature for 20 h. The solvent was then evaporated under reduced pressure, and the crude product was dissolved in 150 mL  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with water (100 mL  $\times$  3), dried over anhydrous  $\text{Na}_2\text{SO}_4$  and filtered. The combined organic phase was concentrated and then purified by column chromatography (silica gel, Ethyl acetate/petroleum ether = 1:1, v:v) to give **donor** in 47% yield. Yellow solid, Mp: 101-103  $^\circ\text{C}$ ,  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm) = 8.58 (t,  $J$  = 4.8 Hz, 1H), 8.25 (dd,  $J$  = 7.5, 0.9 Hz, 1H), 8.01 (s, 1H), 7.10 (dd,  $J$  = 7.5, 1.5 Hz, 1H), 3.28 (q,  $J$  = 6.9 Hz, 2H), 2.98 (t,  $J$  = 7.5 Hz, 2H), 1.66-1.76 (m, 2H), 1.51-1.58 (m, 2H), 1.30-1.39 (m, 4H), 0.91 (t,  $J$  = 7.5 Hz, 6H).

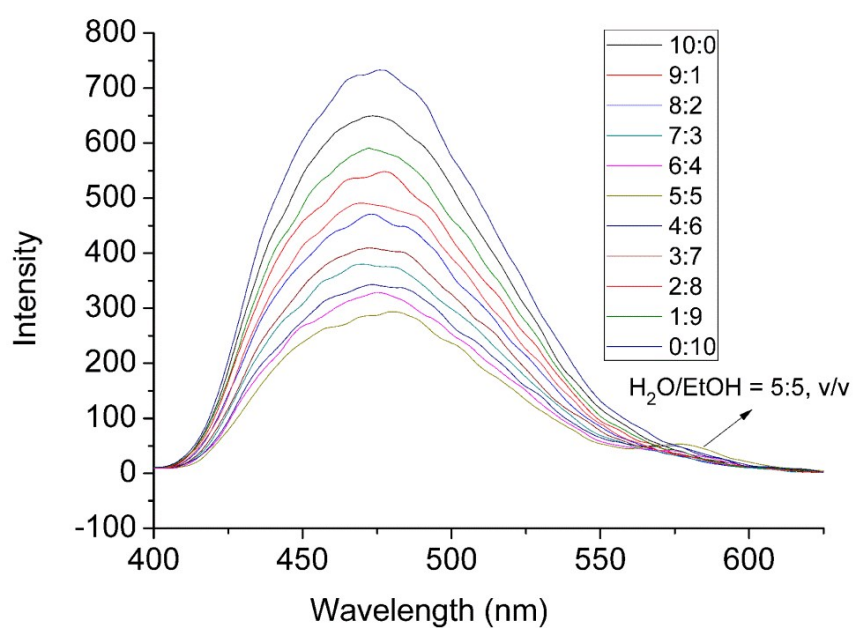


Fig. S1 Fluorescence spectra of RhMP (10 μM) in different volume proportion of B-R buffer/EtOH (10/0-0/10) at pH 4.0.

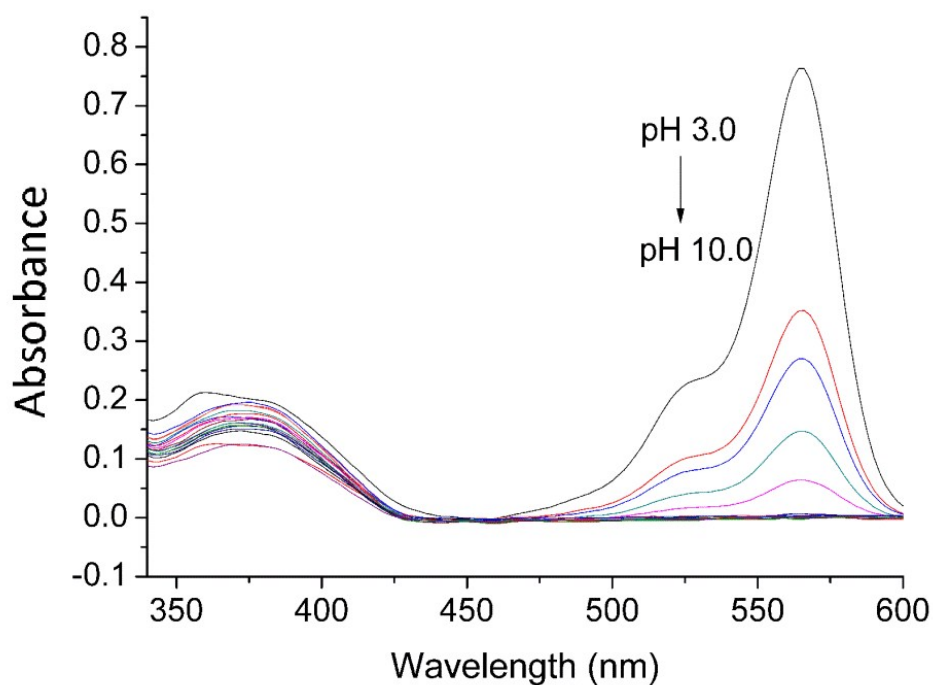
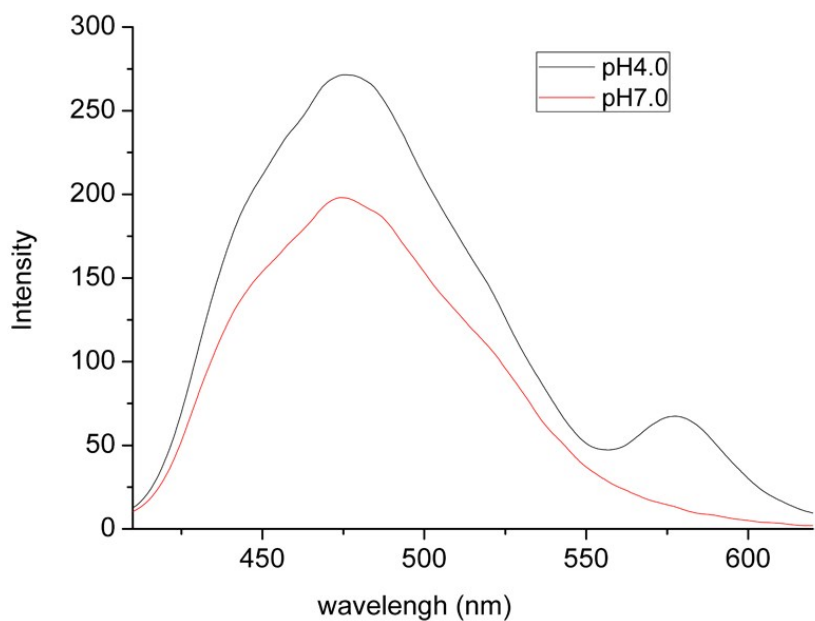
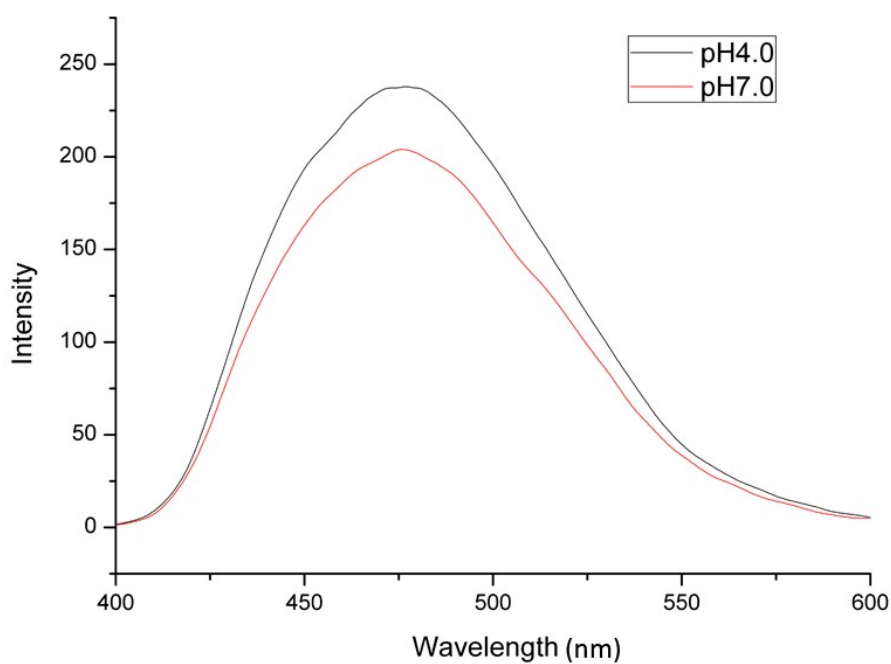


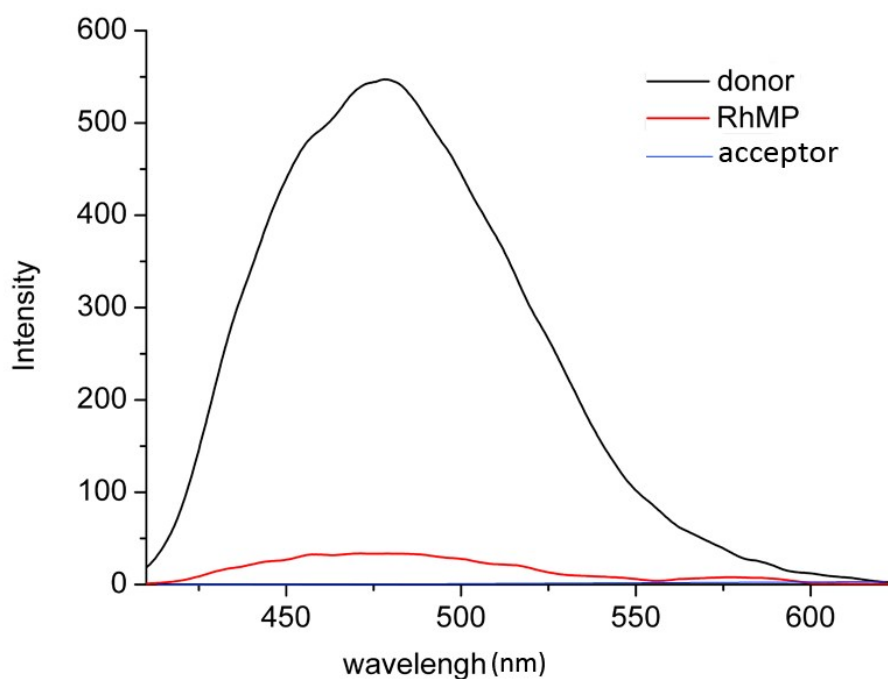
Fig. S2 UV spectra of RhMP (40 μM) in the B-R buffer/EtOH = 1/1, v/v solution at different pH values (3.0-10.0).



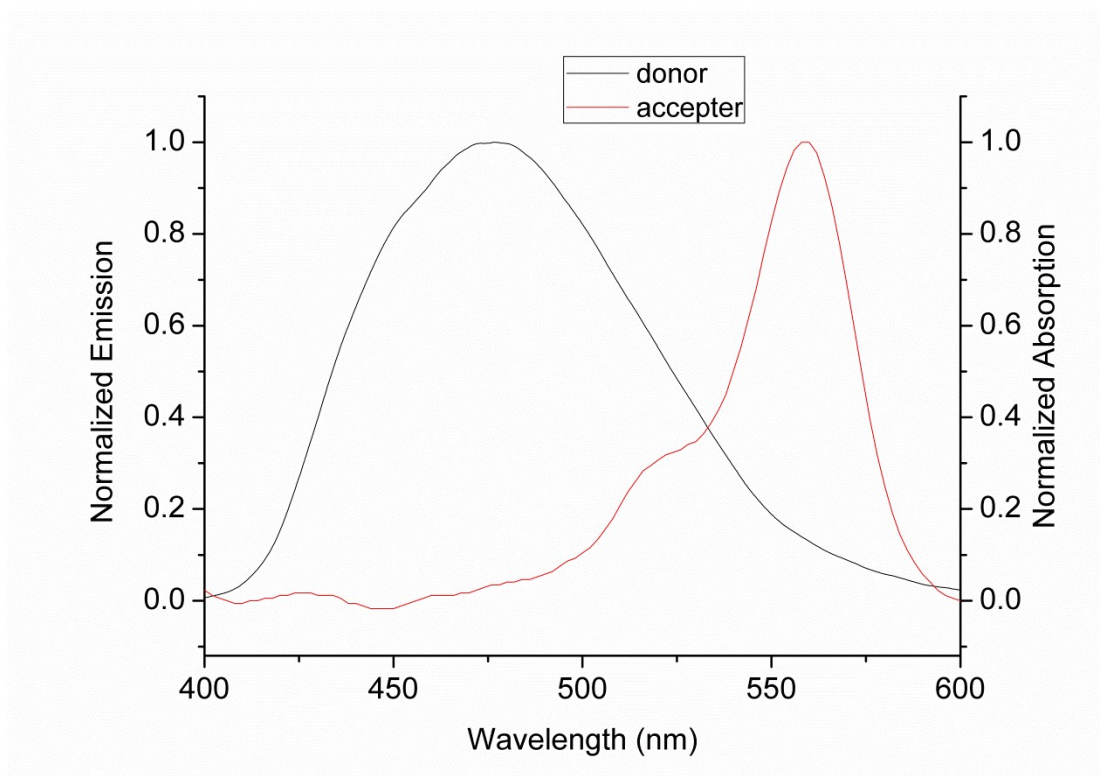
**Fig. S3** Fluorescence spectra of RhMP (10  $\mu$ M in the B-R buffer/EtOH = 1/1, v/v) solution at different pH values (4.0 and 7.0). ( $\lambda_{\text{ex}}$  = 355 nm, slit: 10 nm/5 nm).



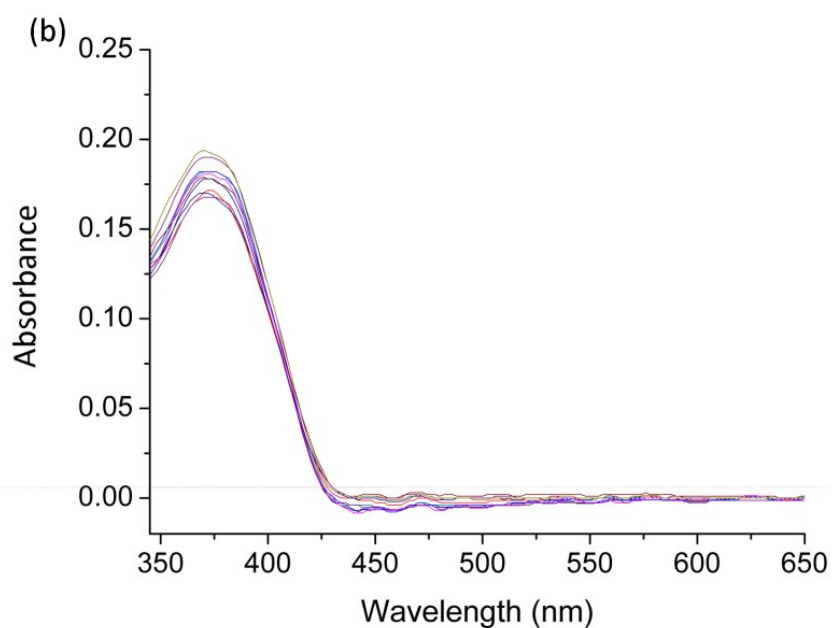
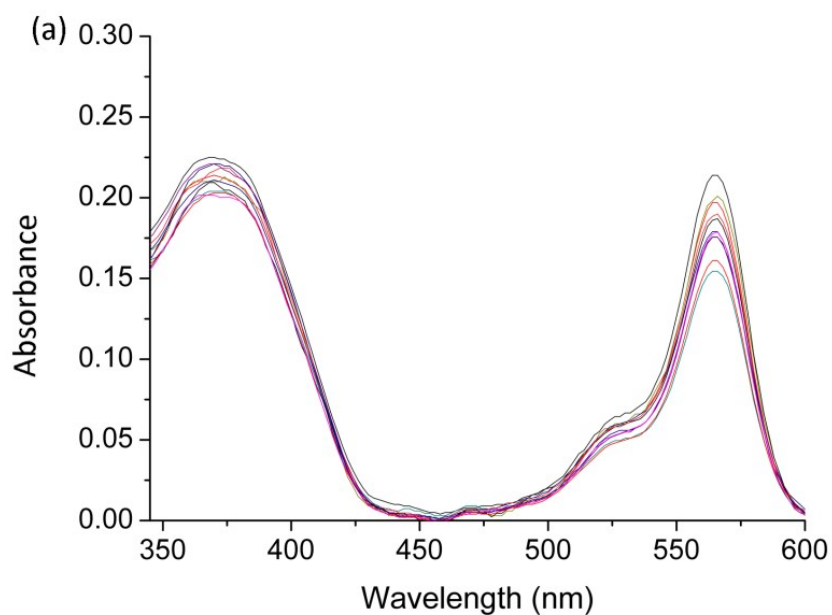
**Fig. S4** Fluorescence spectra of donor (10  $\mu\text{M}$  in the B-R buffer/EtOH = 1/1, v/v) solution at pH values 4.0. ( $\lambda_{\text{ex}}$  = 355 nm, slit: 12 nm/2.6 nm)



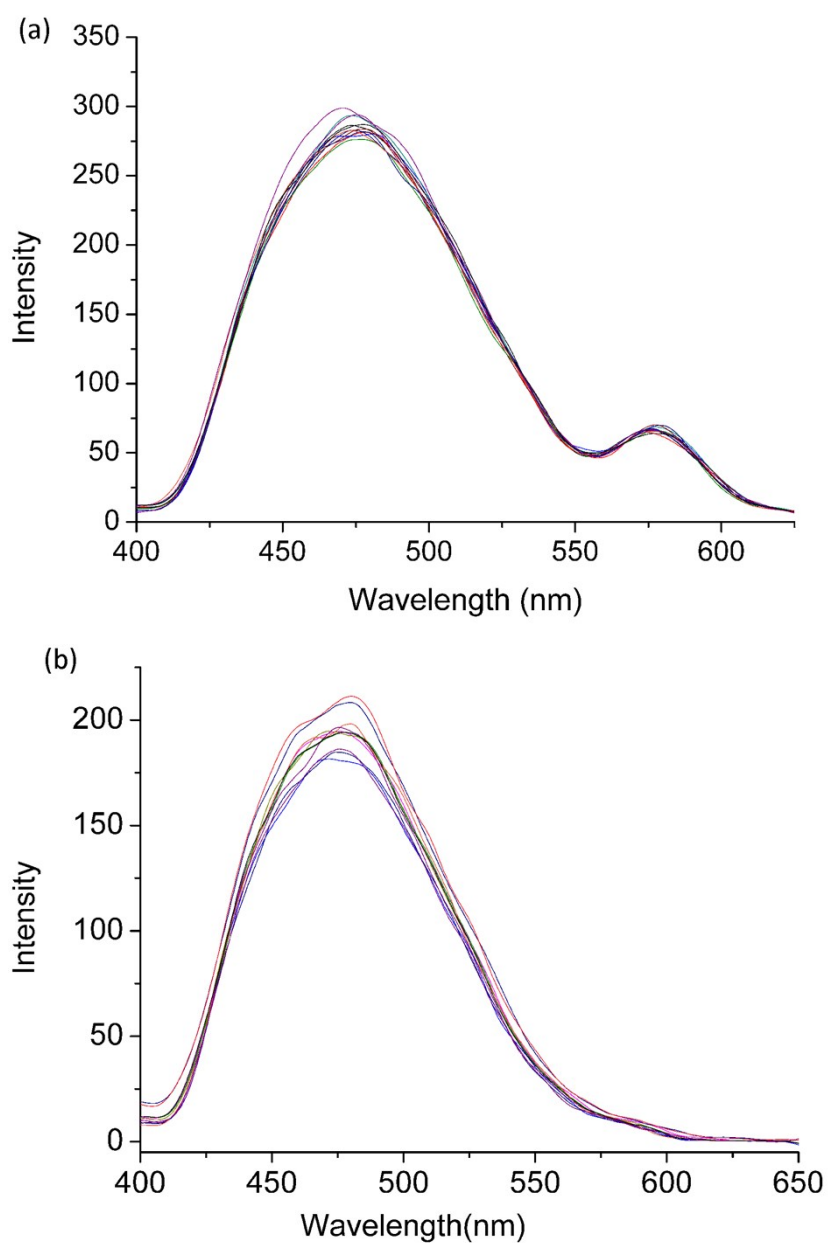
**Fig. S5** The fluorescence emission spectra of the probe (10  $\mu\text{M}$ ) (red), the reference donor (10  $\mu\text{M}$ ) (black) and the acceptor (10  $\mu\text{M}$ ) (blue) (in the B-R buffer/EtOH = 1/1, v/v solution) at pH = 4.0. ( $\lambda_{\text{ex}}$  = 355 nm, slit: 10 nm/2.5 nm).



**Fig. S6** The normalized fluorescence spectrum of the donor (black) and the normalized absorption spectra of the acceptor (red). (in the B-R buffer/EtOH = 1/1, v/v solution) at pH = 4.0.

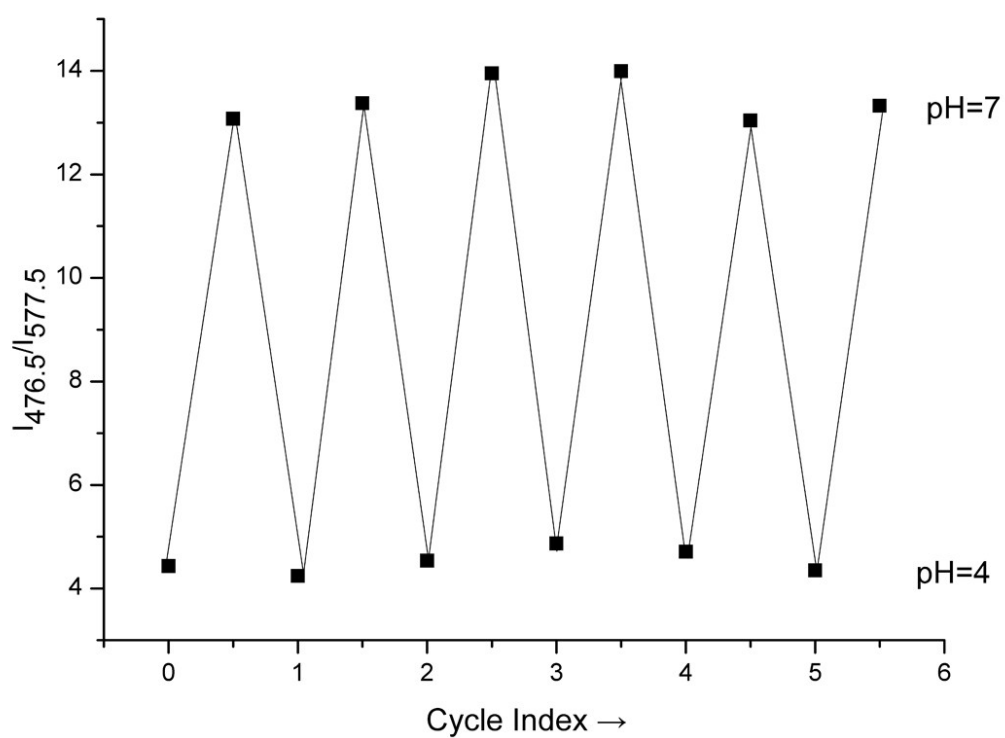


**Fig. S7** UV spectra of RhMP (40 μM) containing different metal cations (blank, Fe<sup>3+</sup>, Ag<sup>+</sup>, Al<sup>3+</sup>, Ba<sup>2+</sup>, Ca<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, Cr<sup>2+</sup>, Cu<sup>2+</sup>, Hg<sup>2+</sup>, K<sup>+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>) in the solution (B-R buffer/EtOH = 1/1, v/v) at pH 4.0 (a) and pH 7.0 (b), All the concentration of the metal cations are 100 μM.

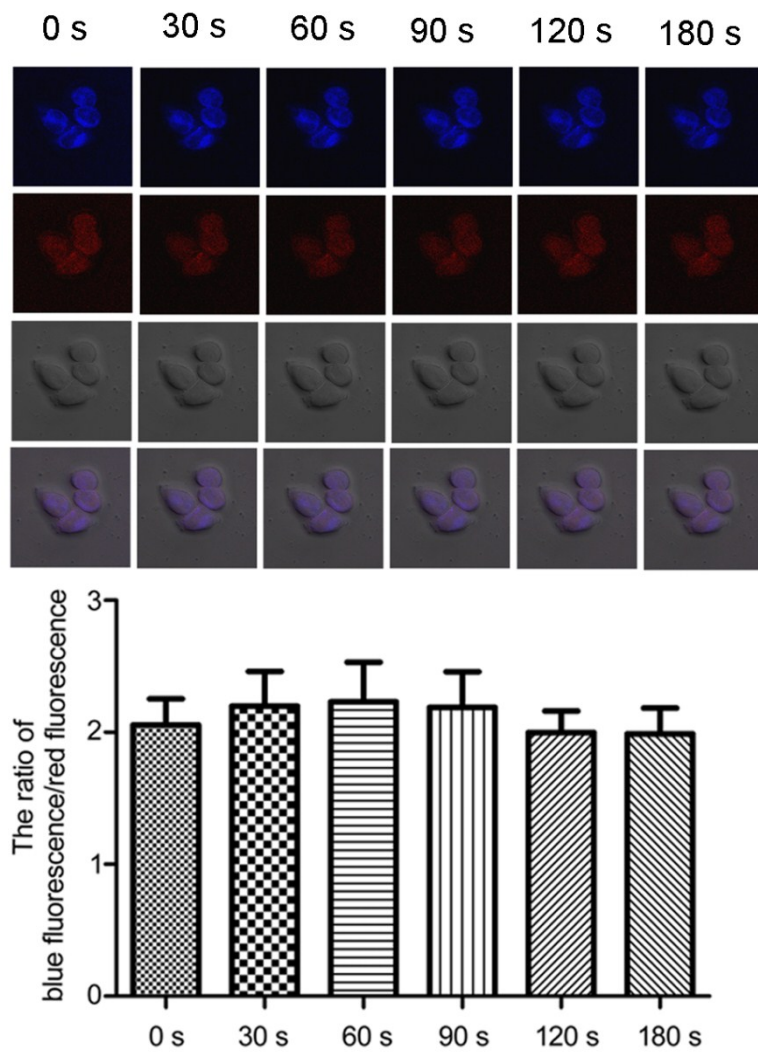


**Fig. S8** Fluorescence spectra of RhMP (10  $\mu\text{M}$ ) containing different metal cations (blank,  $\text{Fe}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ) in the solution (B-R buffer/EtOH = 1/1, v/v) at pH 4.0 (a) and pH 7.0 (b), All the concentration of the metal cations are 100  $\mu\text{M}$ . ( $\lambda_{\text{ex}} = 355 \text{ nm}$ , slit: 10 nm/5 nm).

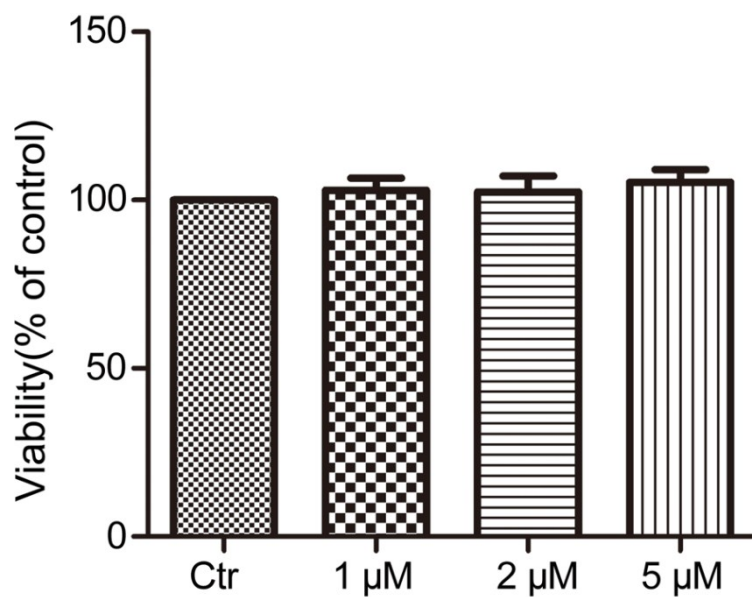




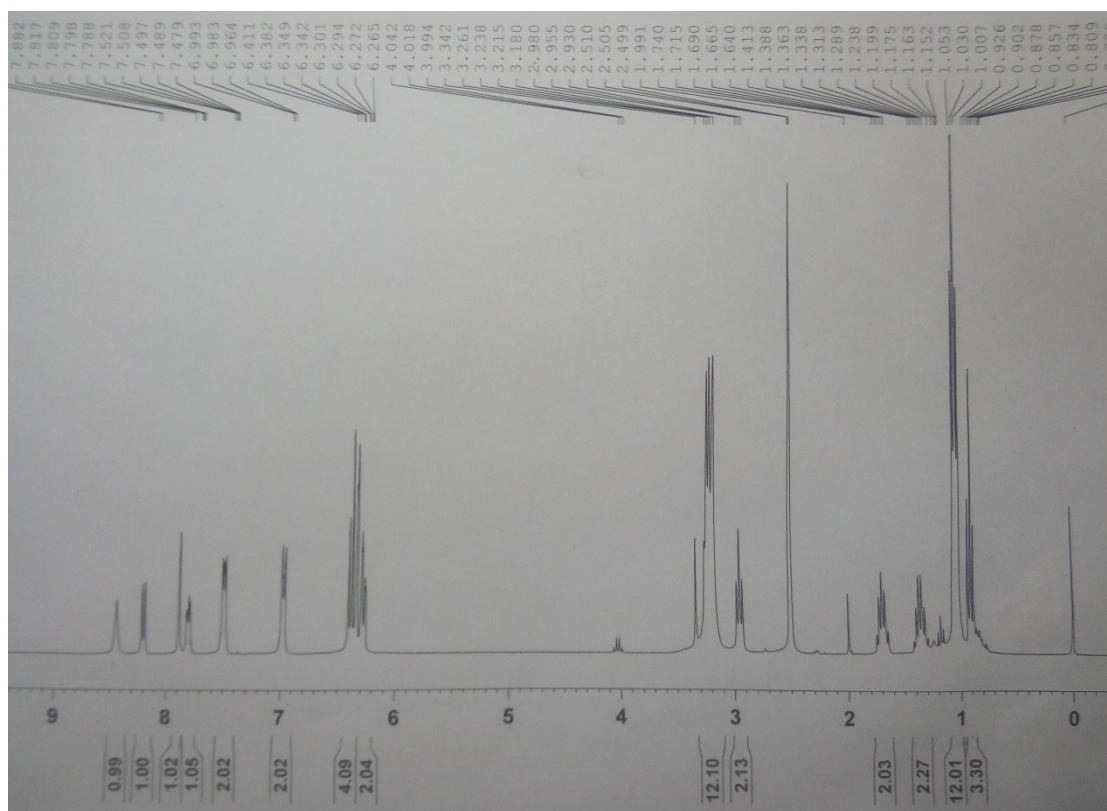
**Fig. S9** pH reversibility study of RhMP (10  $\mu$ M) in the solution (B-R buffer/EtOH = 1/1, v/v) at pH 4.0 and pH 7.0. ( $\lambda_{\text{ex}}$  = 355 nm, slit: 10 nm/5 nm).



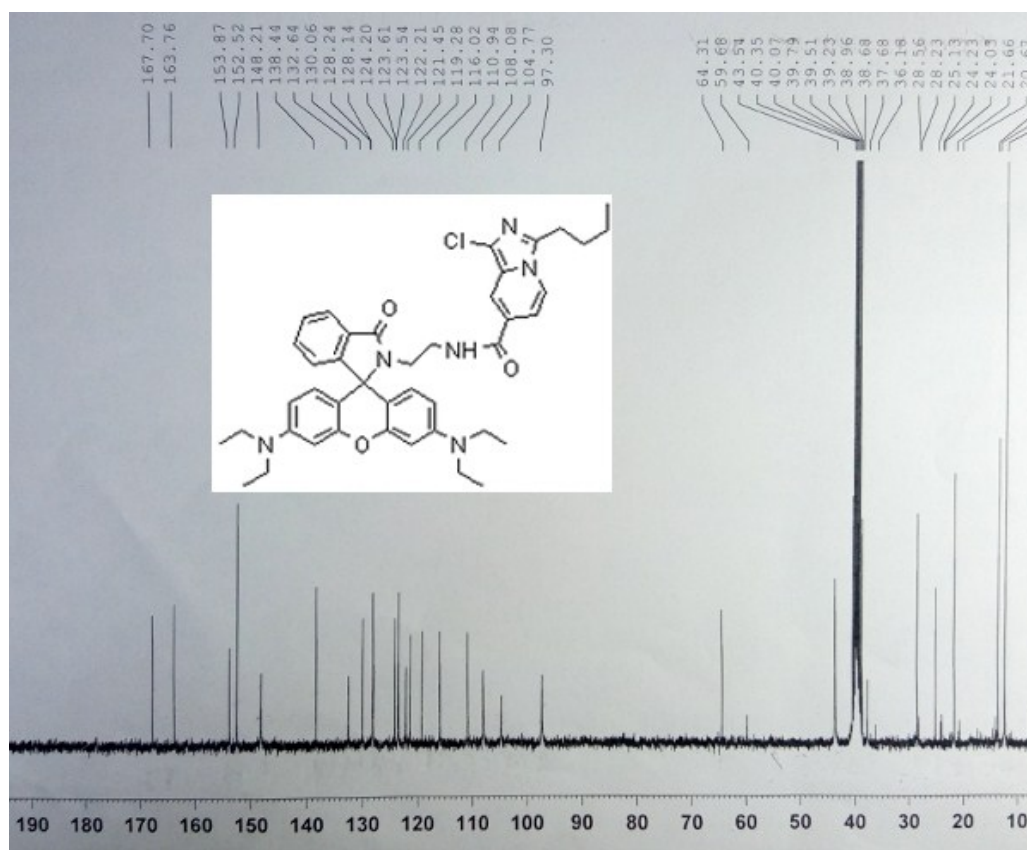
**Fig. S10** The photostability of the probe. After treatment with probe RhMP (2  $\mu$ M) for 3 min, the photostability in HeLa cells was tested under a continuous excitation using  $\lambda = 405$  nm lasers.



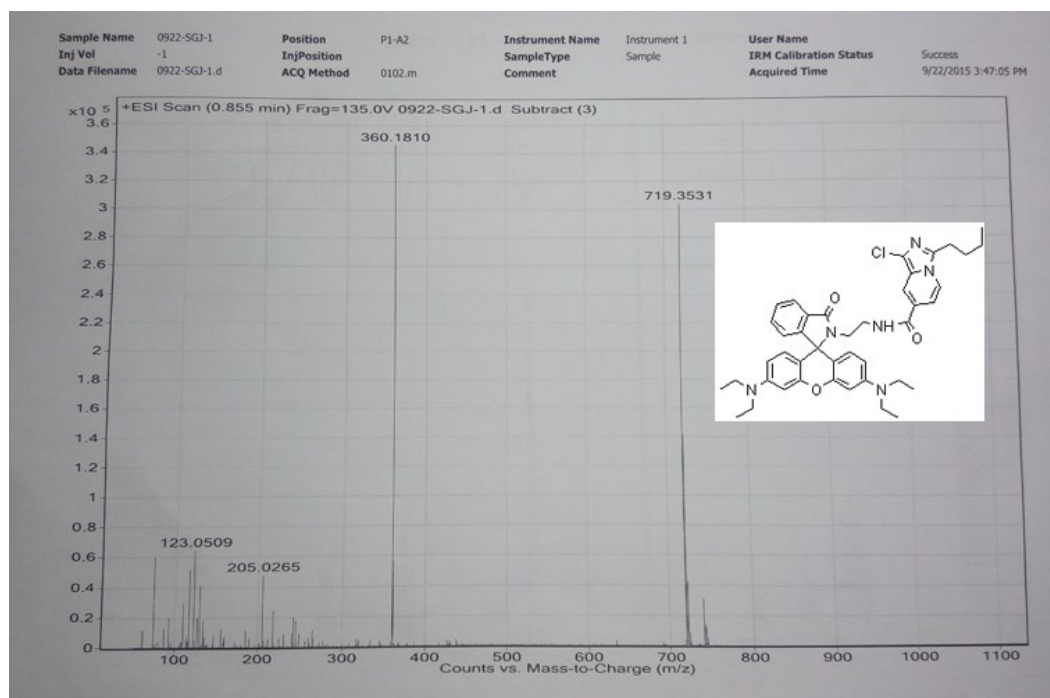
**Fig. S11** Effect of the probe on the viability of HeLa cells. HeLa cells were incubated with indicated concentrations of the probe for 6 h. ( $p > 0.05$  vs control;  $n = 3$ ).



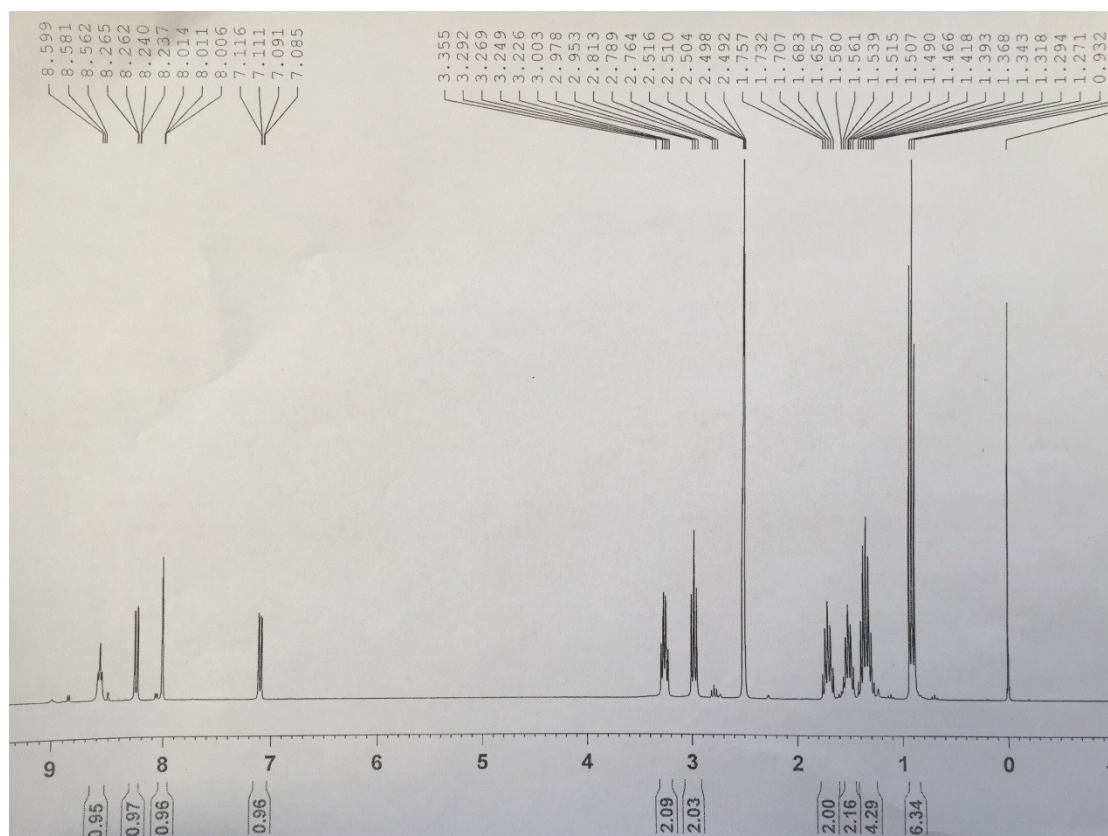
**Fig. S12**  $^1\text{H}$  NMR spectrum of RhMP in  $\text{DMSO-}d_6$ .



**Fig. S13**  $^{13}\text{C}$  NMR spectrum of RhMP in  $\text{DMSO-}d_6$ .



**Fig. S14** HRMS spectrum of RhMP.



**Fig. S15**  $^1\text{H}$  NMR spectrum of donor in  $\text{DMSO-}d_6$ .