Electronic Supplementary Information for

Visual monitoring of the lysosomal pH changes during autophagy with a red-emission pH fluorescent probe

Xiaodong Wang[†a], Li Fan[*†a], Yubin Wang[b], Caihong Zhang[b], Wenting Liang[a], Shaomin Shuang[b] and Chuan Dong[*a]

[a]*Institute of Environmental Science, Shanxi University, Taiyuan 030006, P. R. China*

[b]*College of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, P. R. China*

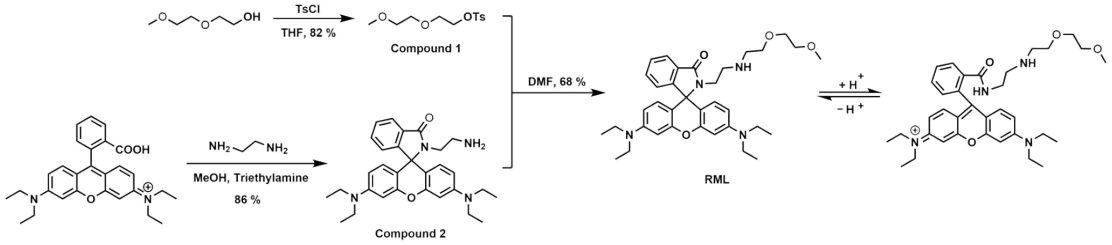
[*]***Corresponding author**:*

dc@sxu.edu.cn; fanli128@sxu.edu.cn

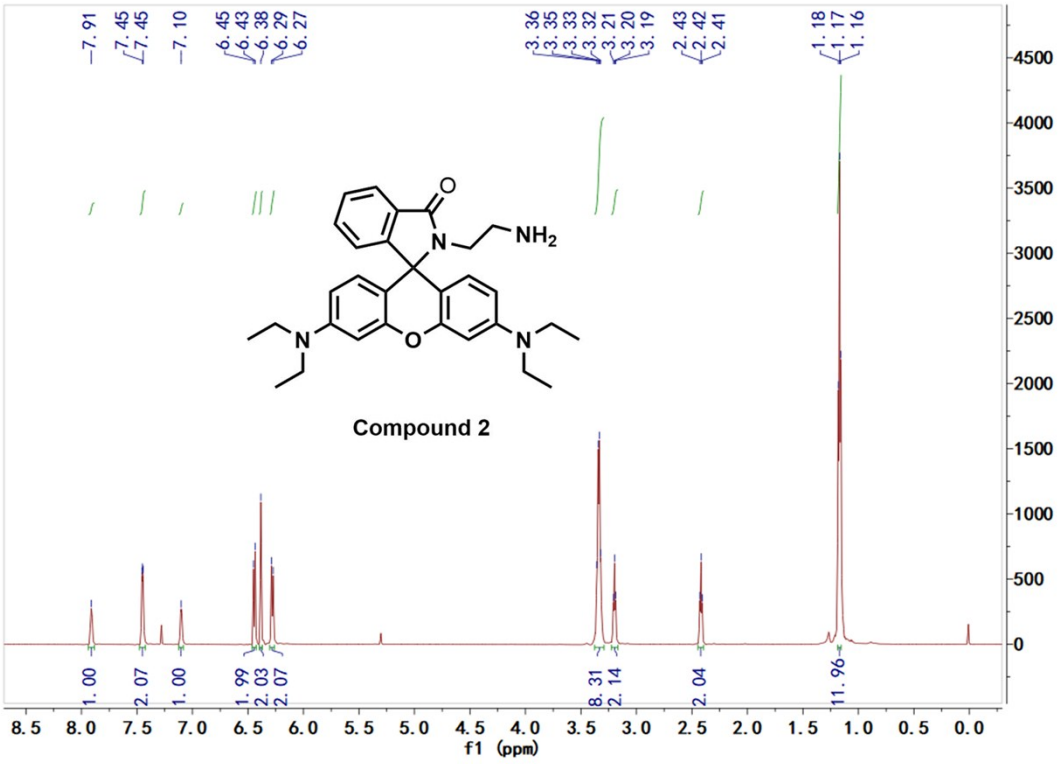
Fax: +86-531-7011011; Tel: +86-531-7011011

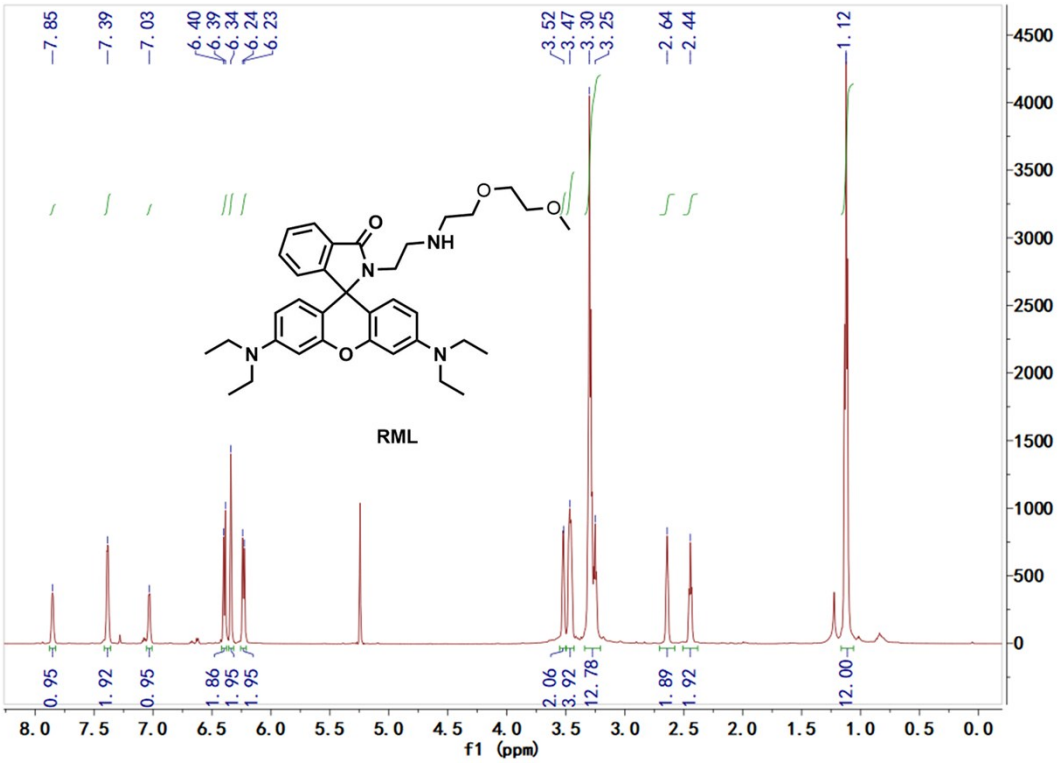
[†]*These authors contributed equally to this work.*

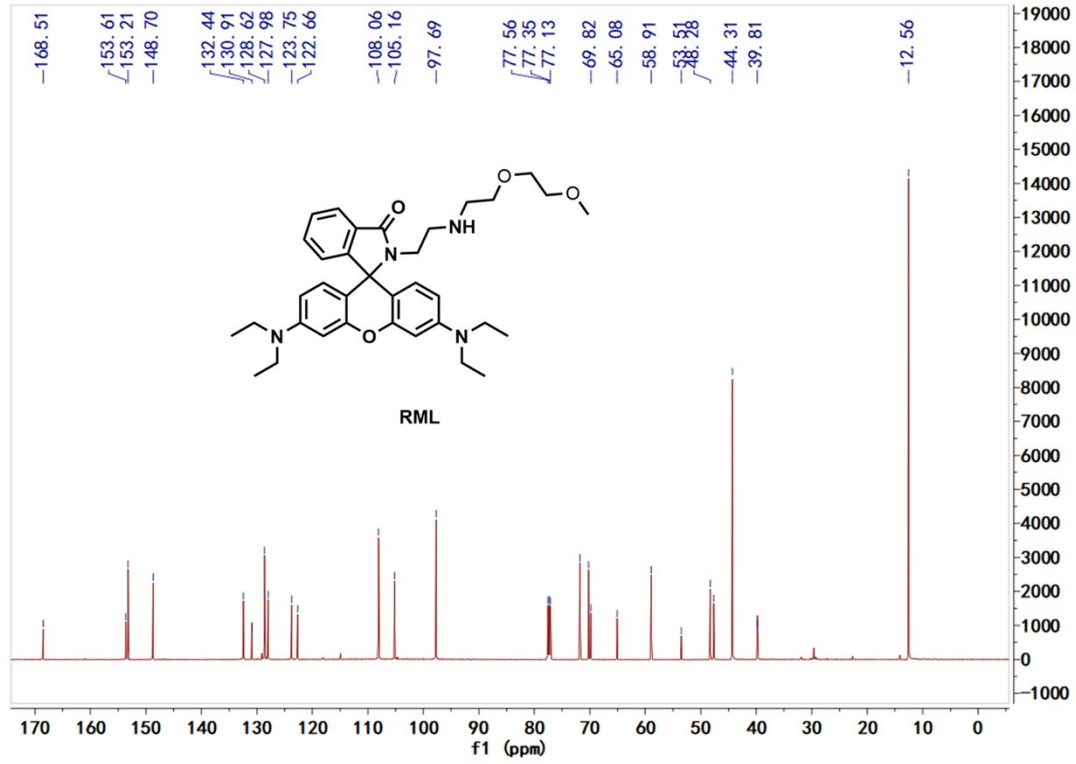
Contents:



Scheme S1 Synthetic scheme of RML and proposed sensing mechanism for pH.


7.91
7.45
7.45
7.10
6.45
6.43
6.38
6.29
6.27
3.36
3.35
3.33
3.32
3.21
3.20
3.19
2.43
2.42
2.41
1.18
1.17
1.16
NH2
Compound 2
1.00
2.07
1.00
1.99
2.03
2.07
8.31
2.14
2.04
11.96
f1 (ppm)


7.85
7.39
7.03
6.40
6.39
6.34
6.24
6.23
3.52
3.47
3.30
3.25
2.64
2.44
1.12
NH
RML
0.95
1.92
0.95
1.86
1.95
1.95
2.06
3.92
12.78
1.89
1.92
12.00
f1 (ppm)



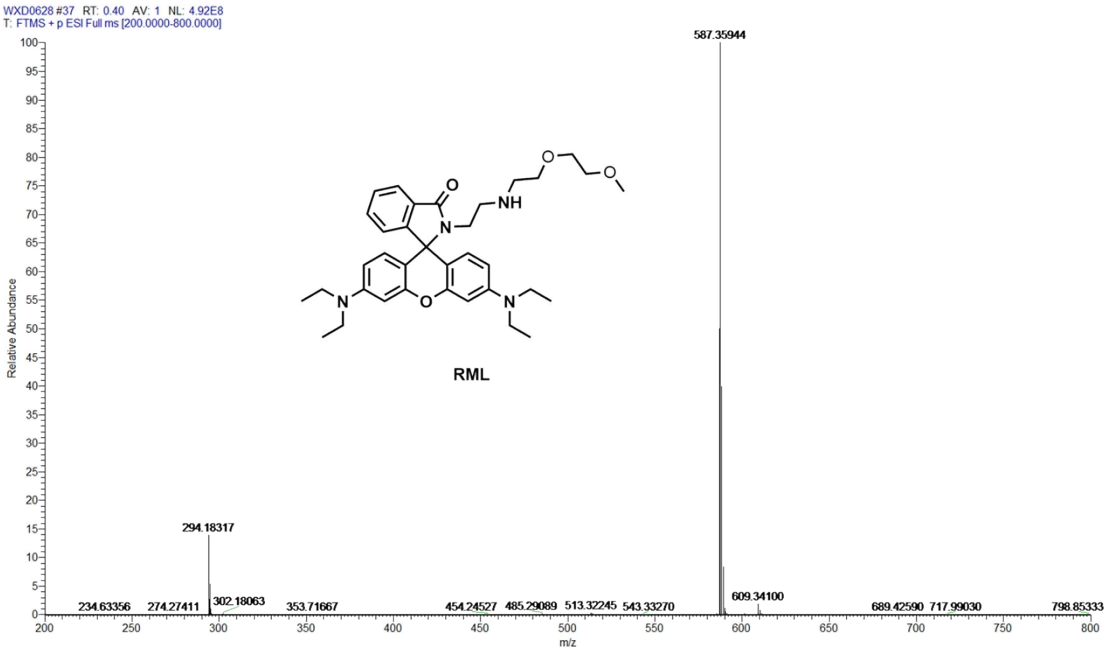


Fig. S1 ^{1}H NMR, ^{13}C NMR spectra and HR-MS analysis of compound 2 and **RML**.

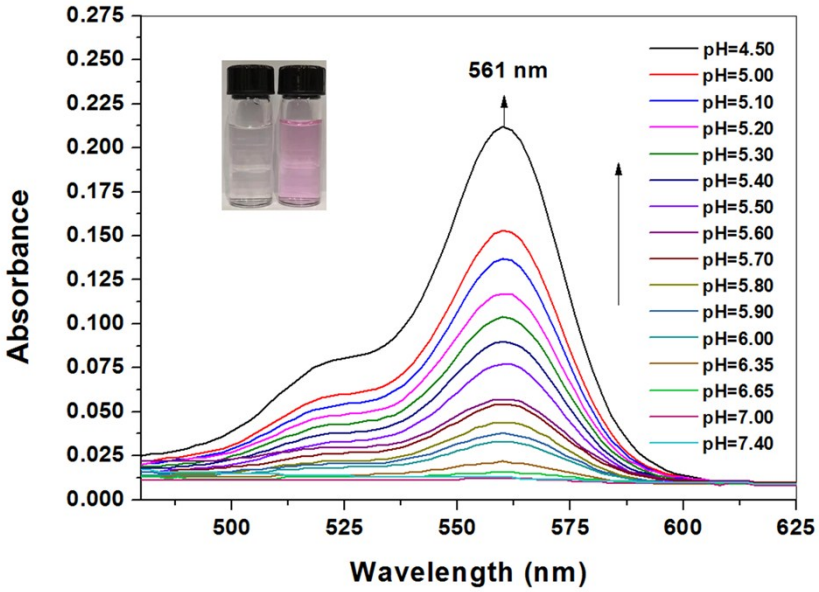


Fig. S2 Absorption spectra changes of **RML** (25 μM) with the pH value reducing from 7.40 to 4.50. Inset: the color of solution changes from colorless to pink with the pH decreasing.

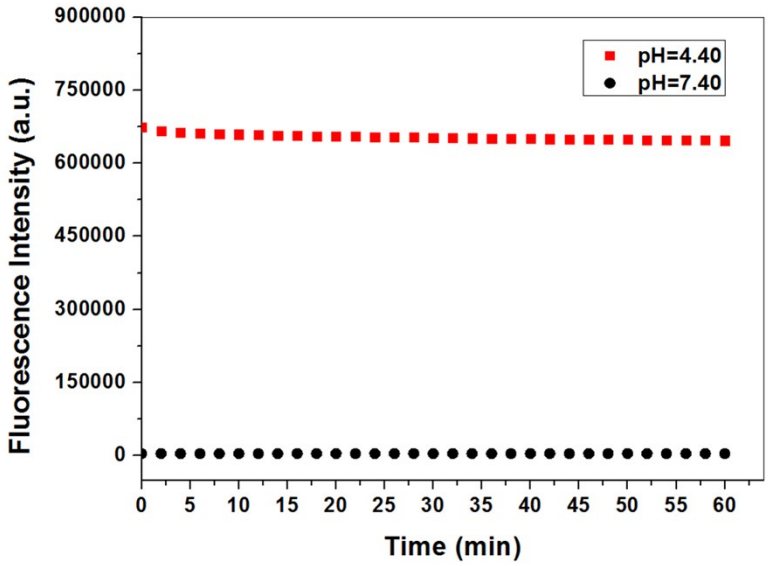


Fig. S3 Changes in fluorescence emission of **RML** with times at pH 4.40 and 7.40, respectively. Conditions: λ_{ex} = 560 nm; λ_{em} = 583 nm.

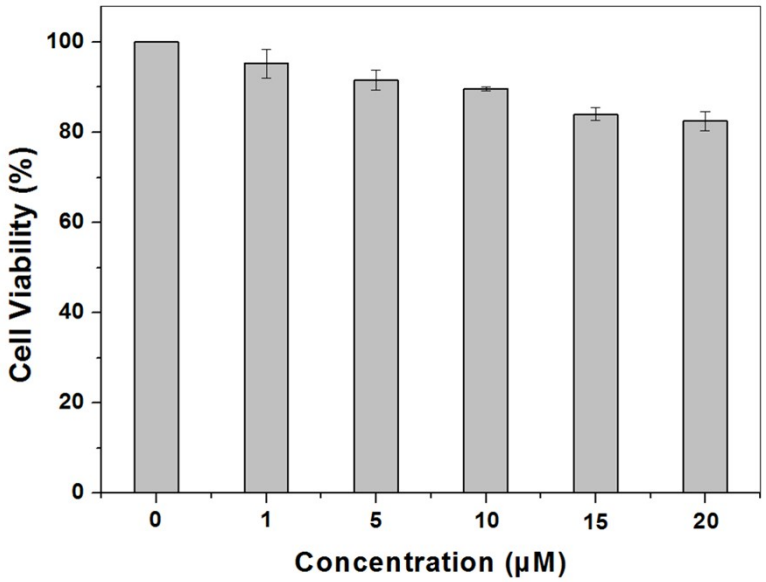


Fig. S4 Cell viability of **RML** on HeLa cells by a standard MTT assay. 1, control; 2, 1 μM; 3, 5 μM; 4, 10 μM; 5, 15 μM; 6, 20 μM. Data are expressed as mean values ± standard error of the mean of three independent experiments, each performed in three triplicate.

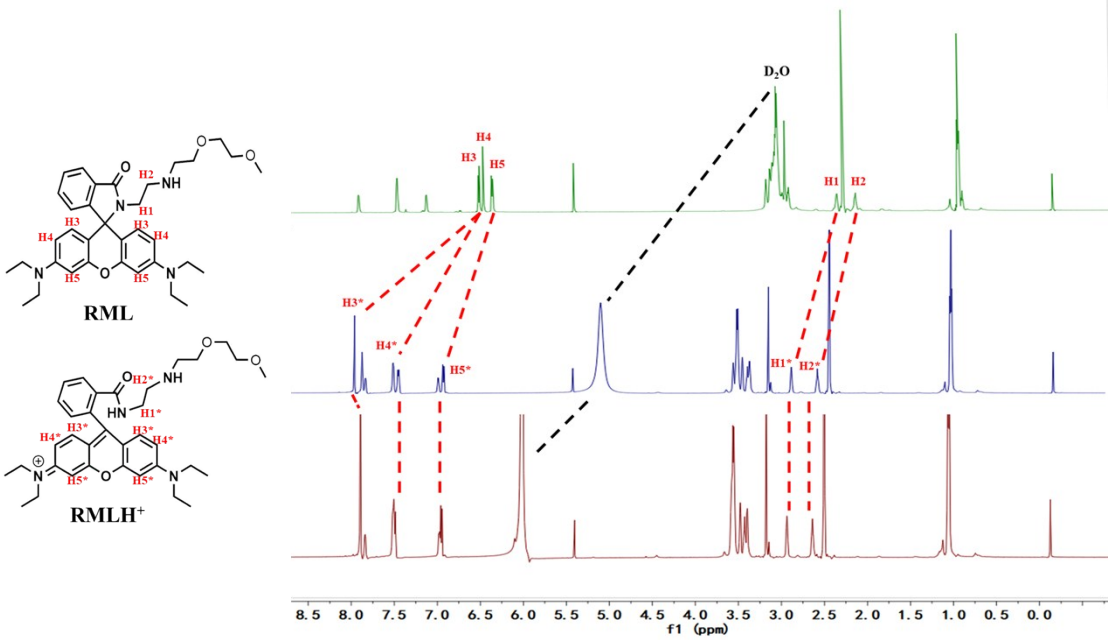


Fig. S5 ^{1}H NMR titration spectra of RML with decreasing pH from 7.40 (bottom) to 4.50 (top).

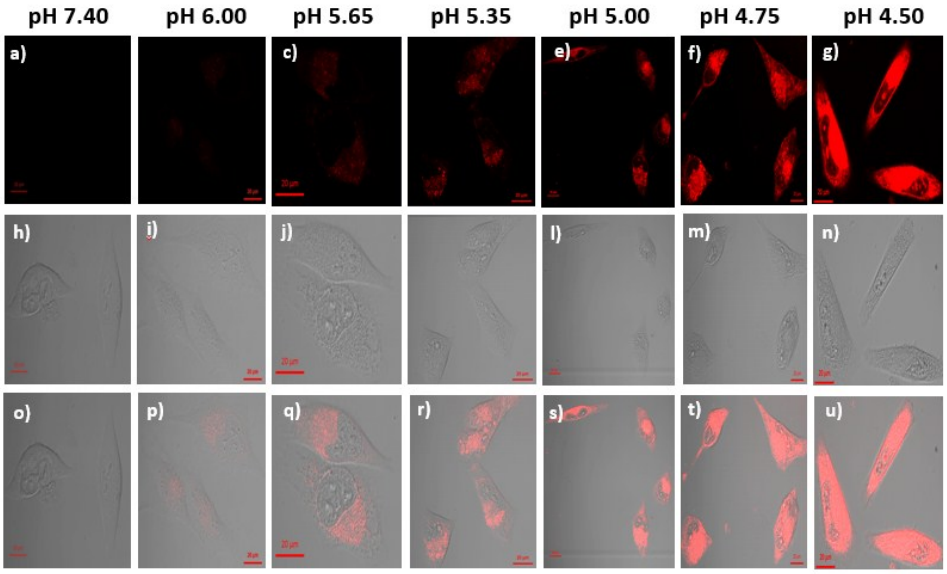


Fig. S6 Fluorescence images of T98G cells incubated with **RML** (10 μM) at pH 7.40 (a), 6.00 (b), 5.65 (c), 5.35 (d), 5.00 (e), 4.75 (f) and 4.50 (g), respectively. (h-n) Bright-field cells images of a-g. (o-u) The corresponding merged cells images. The red emission was collected from 568 to 650 nm (λ_{ex} = 561 nm). Scale bar: 20 μm.

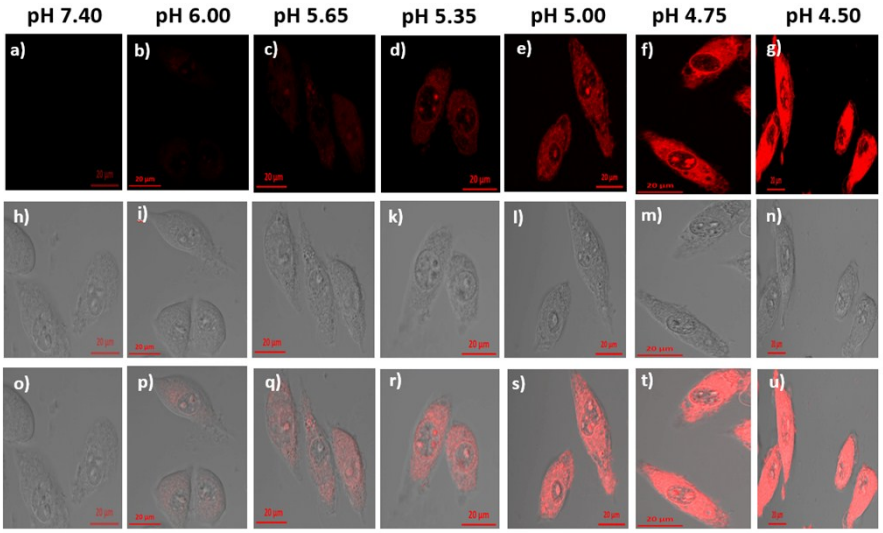


Fig. S7 Fluorescence images of SMMC-7721 cells incubated with **RML** (10 μM) at pH 7.40 (a), 6.00 (b), 5.65 (c), 5.35 (d), 5.00 (e), 4.75 (f) and 4.50 (g), respectively. (h-n) Bright-field cells images of a-g. (o-u) The corresponding merged cells images. The red emission was collected from 568 to 650 nm (λ_{ex} = 561 nm). Scale bar: 20 μm.

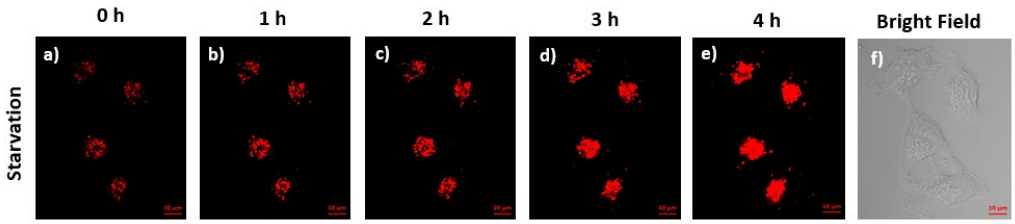


Fig. S8 Real-time visualization of autophagy using RML in HeLa cells. (a-e)Fluorescence imaging of RML in HeLa cells were cultured under starvation conditions (medium of HBSS without bovine serum for inducing cell autophagy) for a certain time (0-4 h). Cells were incubated with RML (10 μM) for 10 min before imaging. (f) Bright-field cells image. The red emission was collected from 568 to 650 nm (λ_{ex} = 561 nm). Scale bar: 10 μm.