Electronic Supplementary Information for

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

Xiaodong Wang\textsuperscript{a}, Li Fan\textsuperscript{\ast a}, Yubin Wang\textsuperscript{b}, Caihong Zhang\textsuperscript{b}, Wenting Liang\textsuperscript{a}, Shaomin Shuang\textsuperscript{b} and Chuan Dong\textsuperscript{\ast a}

\textsuperscript{a}Institute of Environmental Science, Shanxi University, Taiyuan 030006, P. R. China
\textsuperscript{b}College of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, P. R. China

\textsuperscript{\ast}Corresponding author:
dc@sxu.edu.cn; fanli128@sxu.edu.cn
Fax: +86-531-7011011; Tel: +86-531-7011011

\textsuperscript{\dagger}These authors contributed equally to this work.
Contents:

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

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 with the pH value reducing from 7.40 to 4.50

Fig. S3 Photostability of RML

Fig. S4 Cytotoxic effect of RML on HeLa cells

Fig. S5 $^1$H NMR-spectra of RML at various pH values

Fig. S6 pH-dependent fluorescence images of RML in T98G cells

Fig. S7 pH-dependent fluorescence images of RML in SMMC-7721 cells

Fig. S8 Real-time visualization of autophagy using RML in HeLa cells
Scheme S1 Synthesis scheme of RML and proposed sensing mechanism for pH.
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: $\lambda_{ex} = 560$ nm; $\lambda_{em} = 583$ 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 (\(\lambda_{\text{ex}} = 561\) nm). Scale bar: 10 μm.