Electronic Supplementary Information

A Dual-Response Fluorescent Probe for Simultaneous Monitoring Polarity and ATP During Autophagy

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1. Characterization of Lyso-NRB.

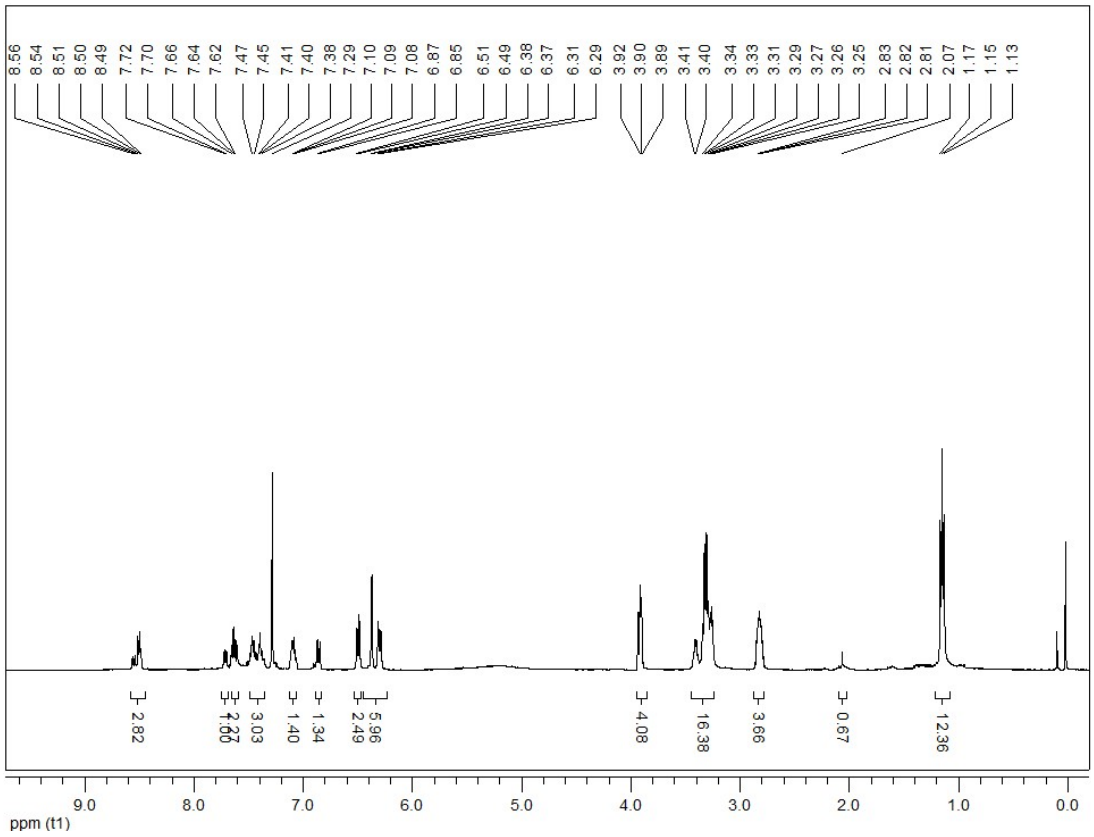

Fig. S1. ^{1}H NMR spectra of compound Lyso-NRB in $CDCl_3$.

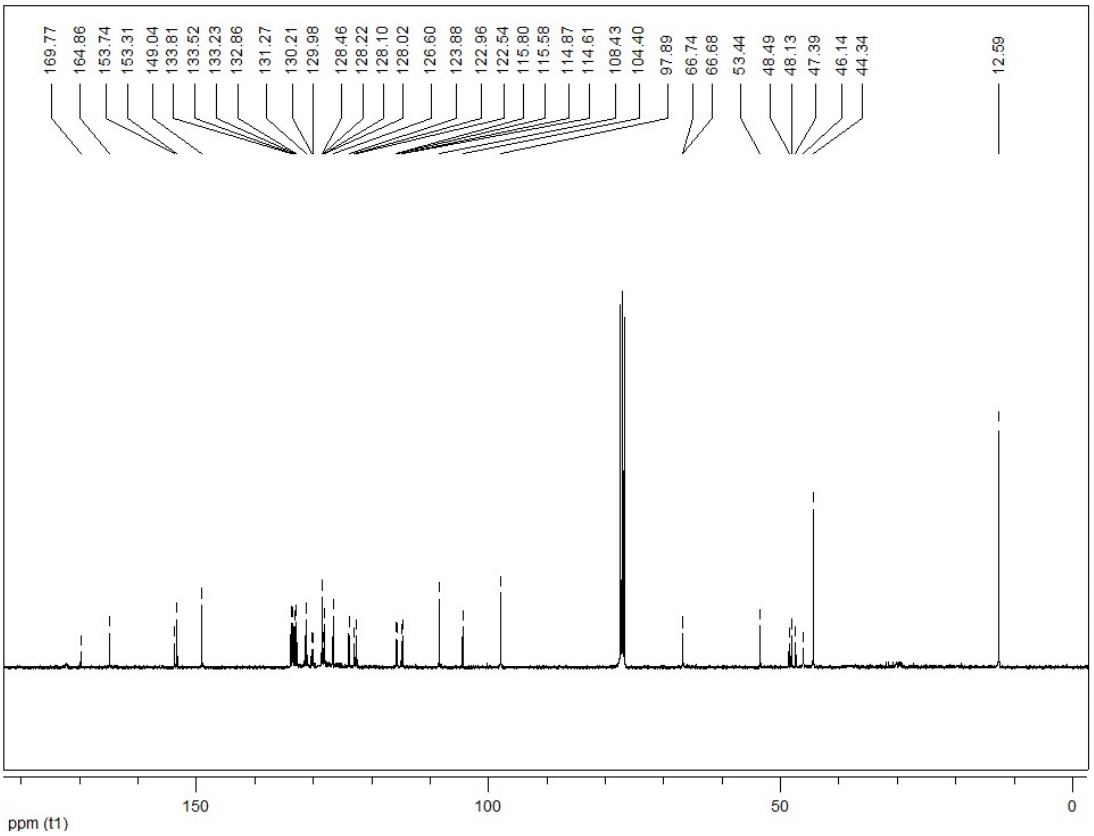

Fig. S2. ^{13}C NMR spectra of compound Lyso-NRB in $CDCl_3$.

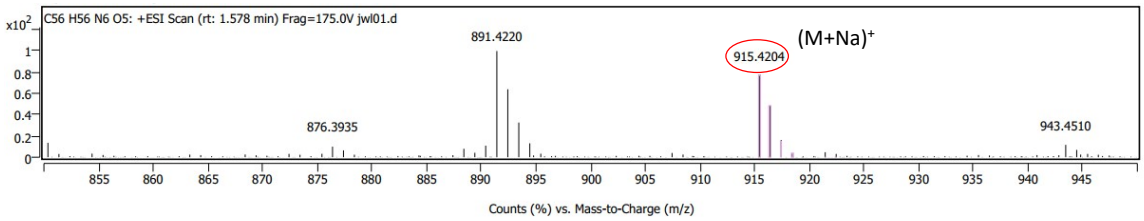

Fig. S3. HRMS spectra of compound Lyso-NRB.

2. Spectroscopic response of Lyso-NRB to polarity and ATP.

Table S1. Spectral properties of Lyso-NRB in various solvents

Solvents	λ_{abs} (nm)	λ_{em} (nm)	$\Delta\lambda$ (nm)	Dielectric Constant (ε)	Refractive Index (n)	Δf
Toluene	399	493	94	2.37	1.497	0.0123
1,4-dioxane	401	508	107	2.21	1.422	0.0205
Trichloromethane	404	527	123	4.81	1.446	0.1483
THF	406	538	132	7.58	1.407	0.2096
DCM	412	544	132	9.08	1.424	0.2183
DMSO	412	540	128	48.9	1.477	0.2645
Ethanol	412	544	132	24.3	1.361	0.2886
ACN	414	539	125	37.5	1.346	0.3047
Methanol	415	537	122	33.6	1.329	0.3091
DMF	416	537	121	37.6	1.333	0.3097
Water	418	638	220	80.4	1.340	0.3174

The dielectric constant data of the solvents were measured at 25 °C. The magnitude of polarity can be calculated by substituting into Lippert-Mataga equation.

$f(\varepsilon) = (\varepsilon-1) / (2\varepsilon+1)$

$f(n^2) = (n^2-1) / (2n^2+1)$

$\Delta f = f(\varepsilon) - f(n^2)$

The ε is dielectric constant, n is refractive index, Δf is polarity values.

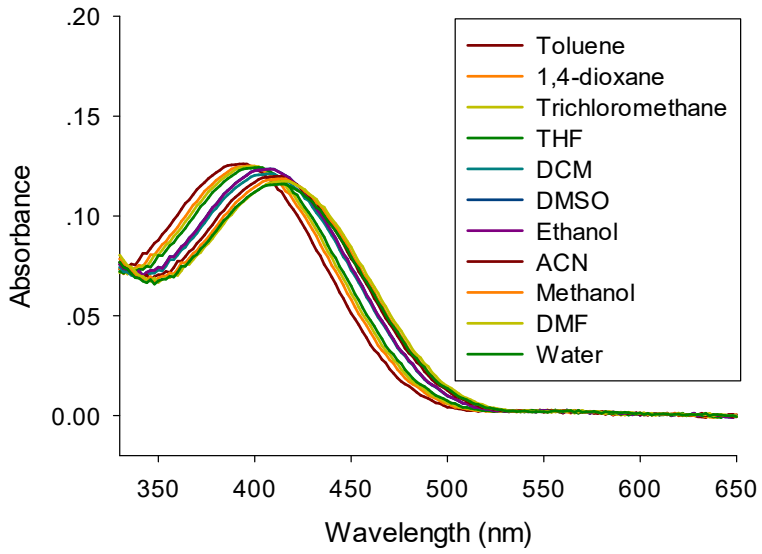

Fig. S4. The absorption spectra of Lyso-NRB (10 μM) in different solvents.

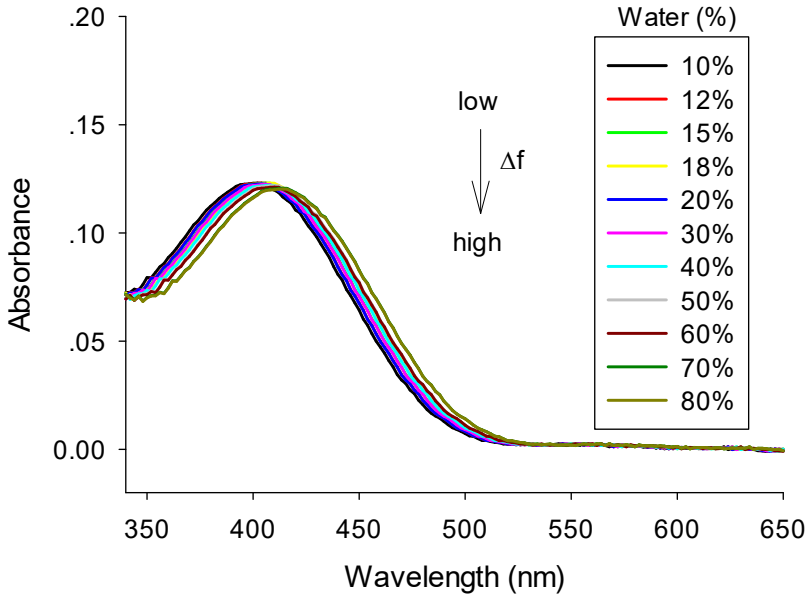

Fig. S5. The absorption spectra of Lyso-NRB (10 μM) upon the addition of different water/1,4-dioxane solvent mixtures (water from 10% to 80%).

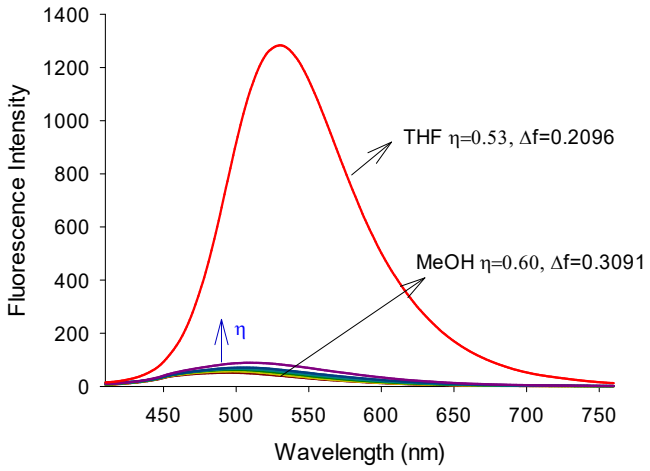

Fig. S6. The fluorescence spectra of Lyso-NRB (10 μM) in methanol-glycerol system under different viscosity (0.60 cP to 100 cP). THF and methanol have almost the same viscosity (0.53 cP vs 0.60 cP) but different polarity (0.2096 vs 0.3091). λ_{ex} = 400 nm.

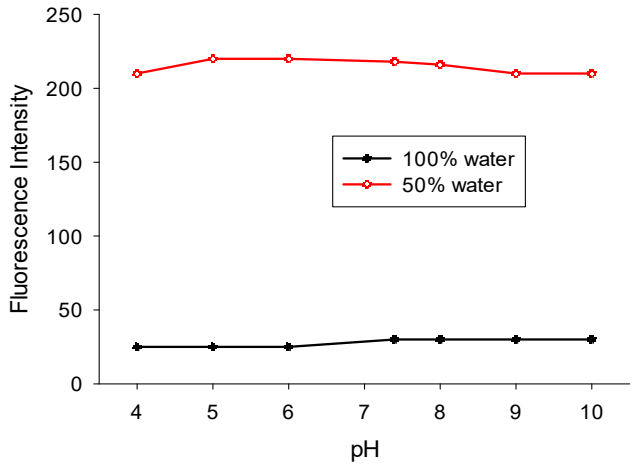

Fig. S7. Fluorescence spectra of Lyso-NRB (10 μM) in different H_2O/1,4-dioxane mixture with different pH values. λ_{ex} = 400 nm.

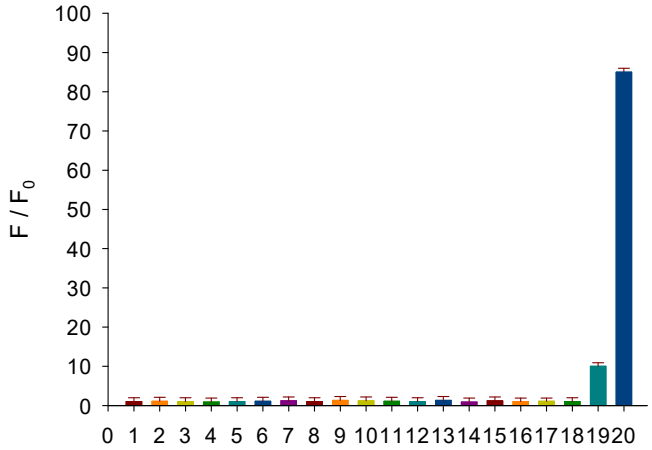

Fig. S8. Fluorescence spectra of Lyso-NRB (10 μM) for various analytes (100 μM). 1: blank, 2: Zn^{2+}, 3: Mg^{2+}, 4: Ca^{2+}, 5: Na^{+}, 6: K^{+}, 7: NO, 8: $ONOO^{-}$, 9: H_2O_2, 10: ClO^{-}, 11: H_2S, 12: SO_2, 13: GSH, 14: Cys, 15: Hcy, 16: $^1O_2$, 17: ·HO, 18: DNase (5.0 KU/L), 19: BSA (20 μM), 20: 1,4-dioxane. λ_{ex} = 400 nm.

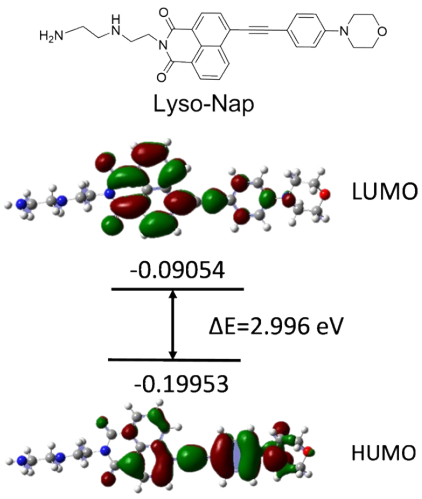

Fig. S9. HOMO and LUMO of Lyso-Nap by DFT calculations at the base level of B3LYP/6-311G via Gaussian 09 program.

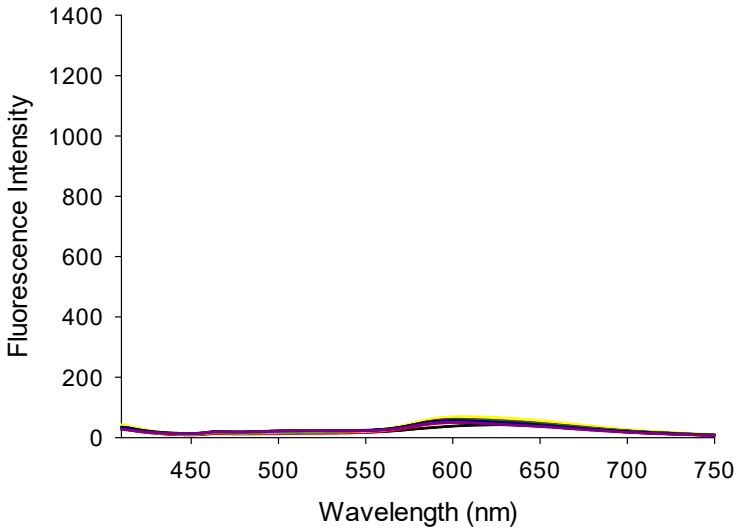

Fig. S10. Fluorescence spectra of Lyso-NRB (10 μM) upon the addition of different concentrations of ATP (0, 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mM). λ_{ex} = 400 nm.

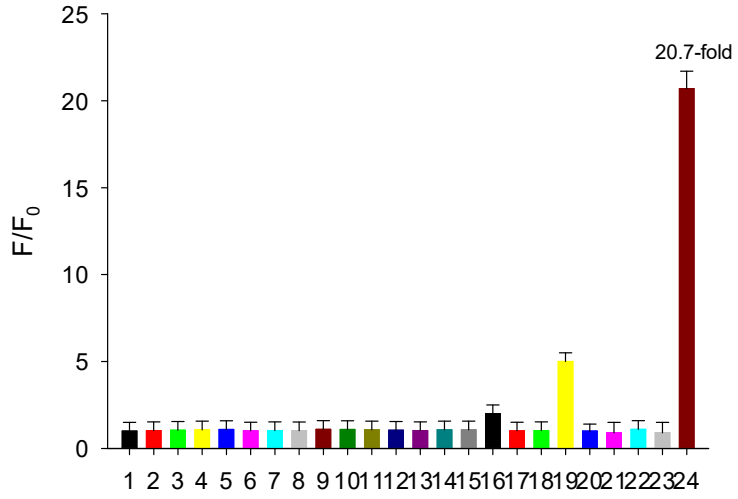

Fig. S11. Fluorescence spectra of Lyso-NRB (10 μM) toward various analytes (10 mM). 1: blank, 2: GSH, 3: Cys, 4: Hcy, 5: H_2O_2, 6: NO, 7: H_2S, 8: K^+, 9: Ca^{2+}, 10: Na^+, 11: Mg^{2+}, 12: Al^{3+}, 13: $P_2O_7^{4-}$, 14: $H_2PO_4^-$, 15: PO_4^{3-}, 16: CTP, 17: GTP, 18: AMP, 19: ADP, 20: $^1O_2$, 21: ·HO, 22: $ONOO^-$, 23: ClO^-, 24: ATP. λ_{ex} = 550 nm.

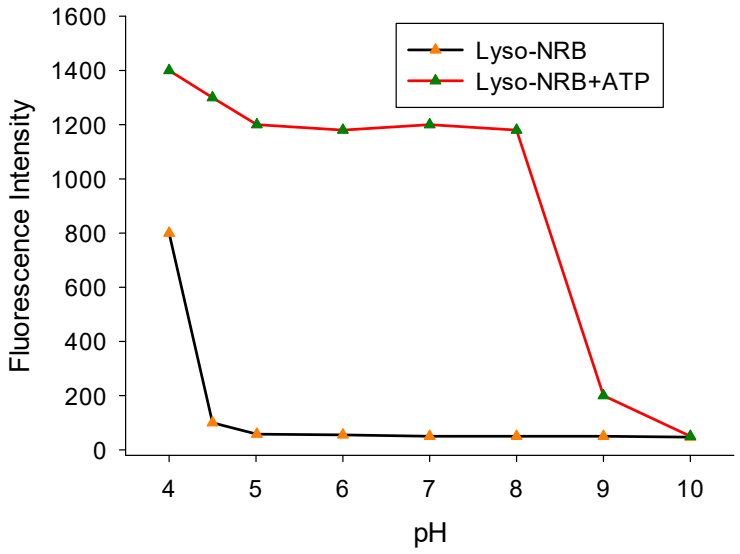

Fig. S12. Effect of pH on the fluorescence intensity of Lyso-NRB (10 μM) before and after the reaction with ATP (10 mM). λ_{ex} = 550 nm.

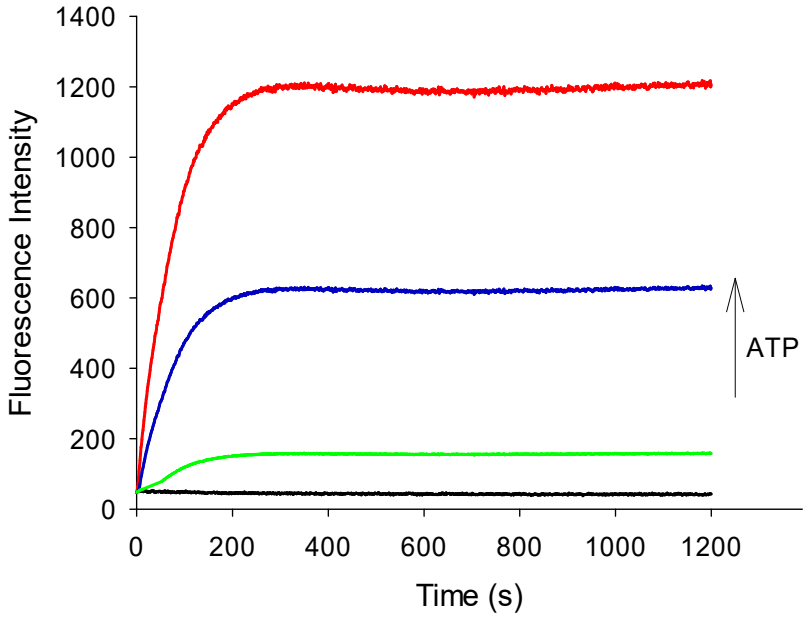

Fig. S13. Time-dependent fluorescence intensity of Lyso-NRB (10 μM) in the absence / prescence of ATP (0, 0.5, 1, 5, 10 mM). λ_{ex} = 550 nm.

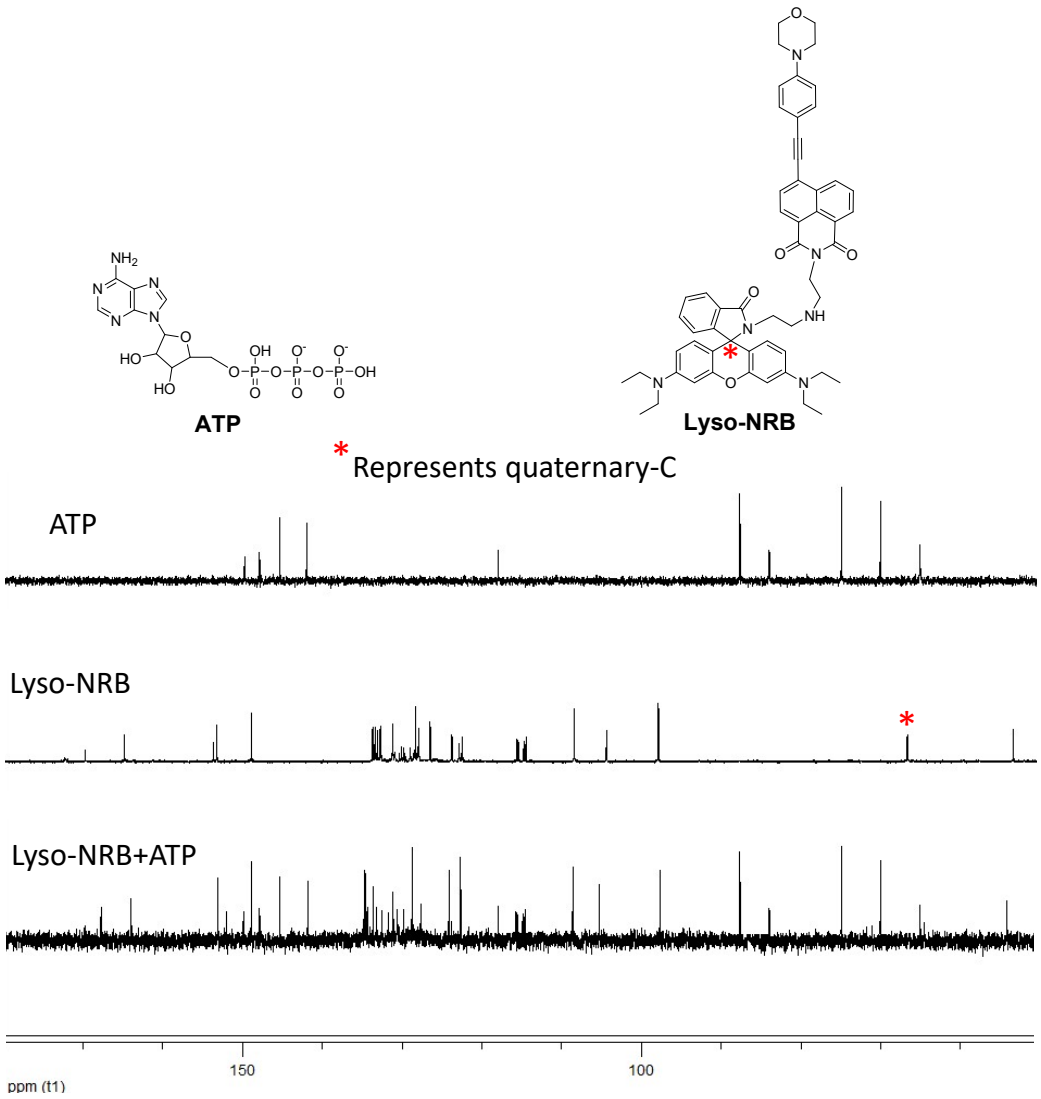

Fig. S14. Partial ^{13}C NMR spectra of ATP, Lyso-NRB and Lyso-NRB+ATP. NMR solvent: 10 % D_2O in d_6-DMSO.

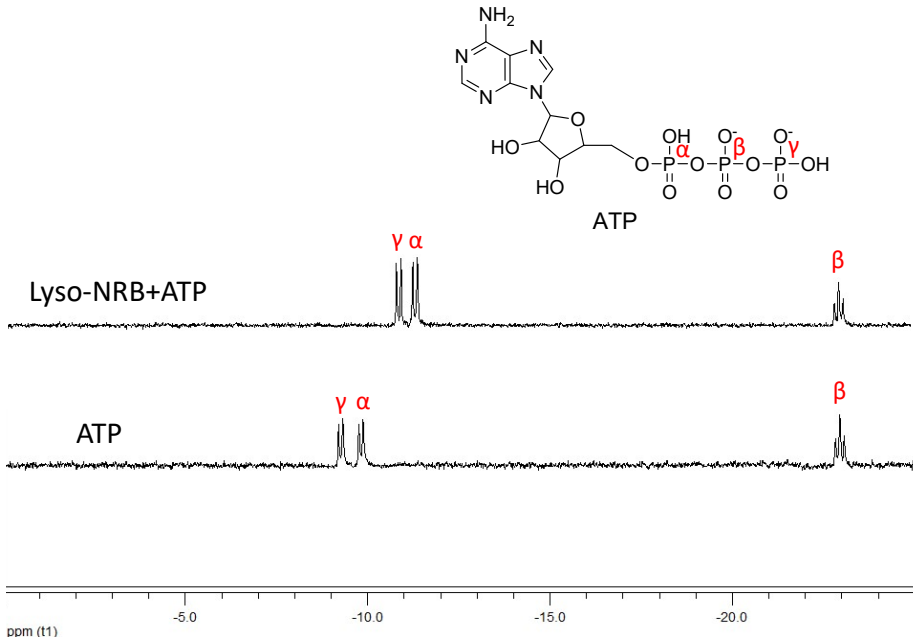

Fig. S15. ^{31}P NMR spectra of ATP and Lyso-NRB+ATP. NMR solvent: 10 % D_2O in d_6-DMSO.

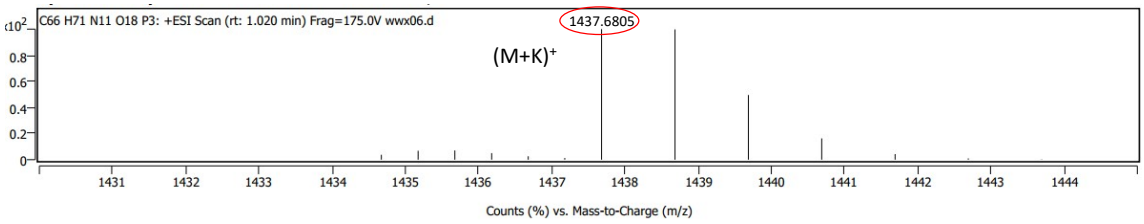

Fig. S16. Mass spectra of Lyso-NRB reacted with ATP.

3. Biological assays.

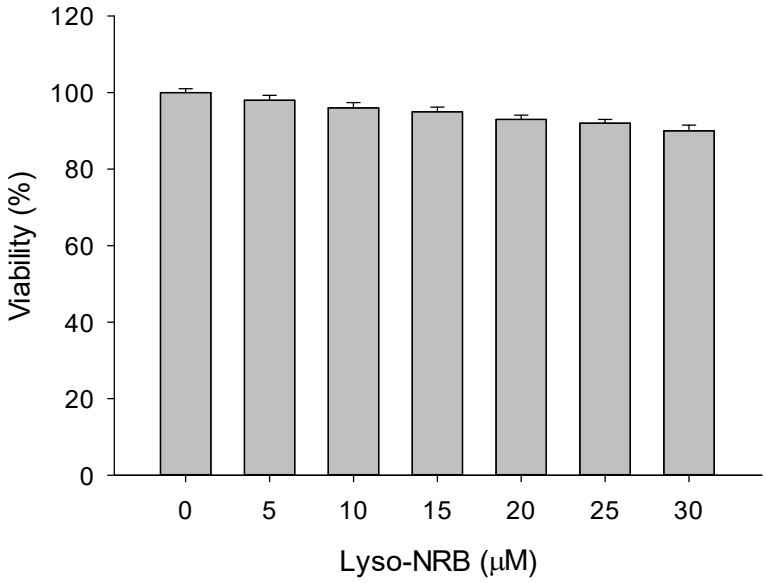

Fig. S17. MTT assay for estimating cells viability (%) in HepG2 cells treated with various concentrations of Lyso-NRB (0-30 μM) after 24 h incubation.

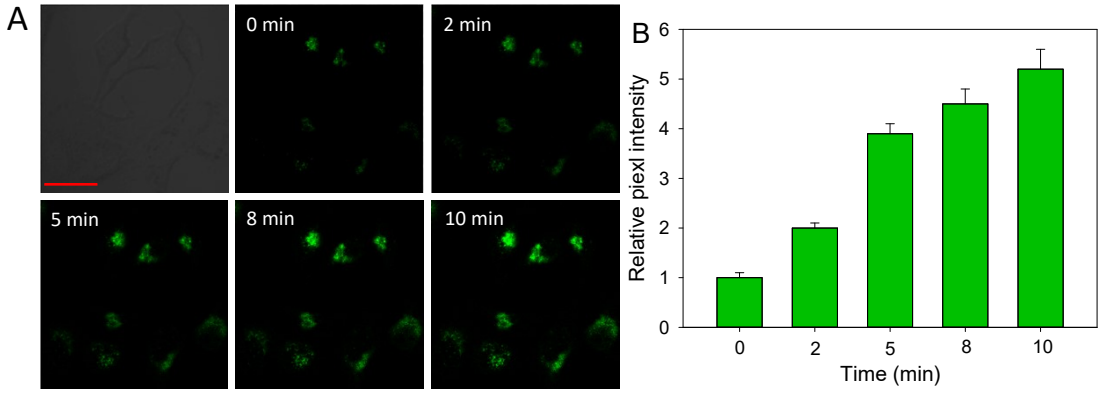

Fig. S18. Fluorescence images of polarity in HepG2 cells. (A) Confocal fluorescence images of HepG2 cells stained with Lyso-NRB (10 μM) in the presence of DMSO (10 μL) at different times. (B) Relative fluorescence intensity quantified from the images in A. Scale bar: 20 μm. Green channel: λ_{ex} = 405 nm, λ_{em} = 430-530 nm.

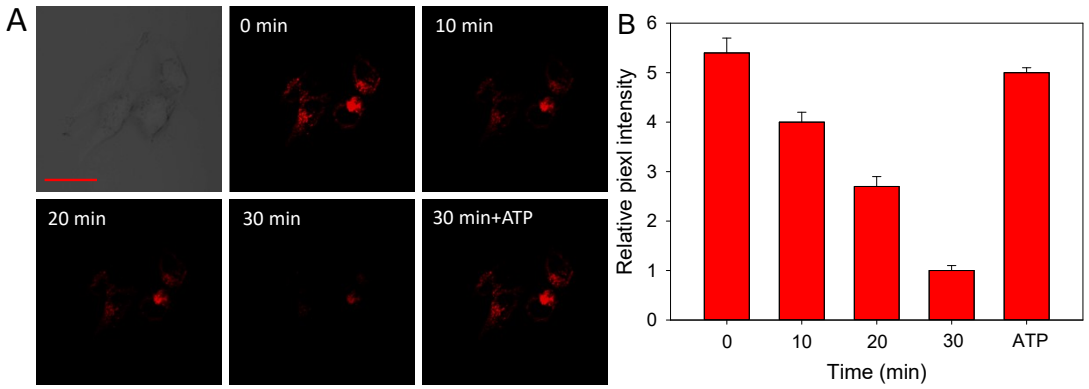

Fig. S19. Fluorescence images of ATP in HepG2 cells. (A) Confocal fluorescence images of HepG2 cells stained with Lyso-NRB (10 μM), and then treated with apyrase (0.5 U/mL) for 0, 10, 20, 30 min. After that, 10 mM ATP was added for another 30 min. (B) Relative fluorescence intensity quantified from the images in A. Scale bar: 20 μm. Red channel: λ_{ex} = 560 nm, λ_{em} = 570-670 nm.

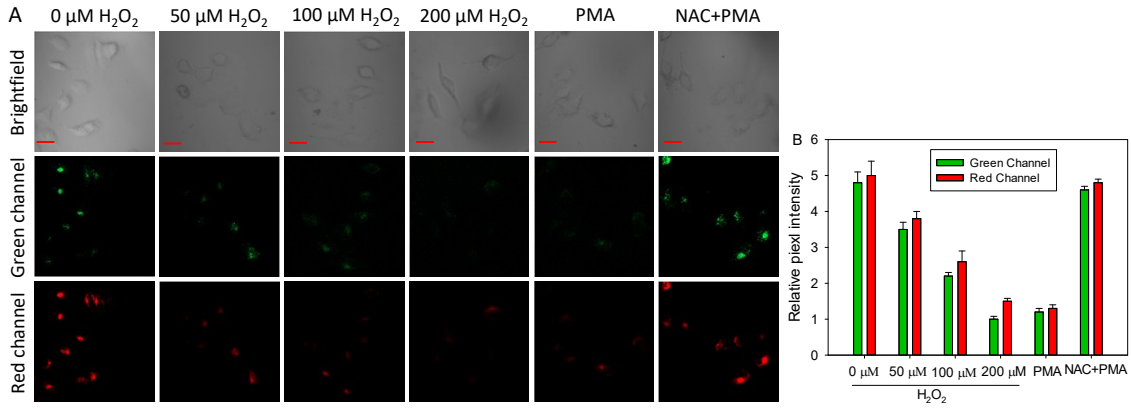

Fig. S20. Fluorescence images of polarity and ATP induced by different concentrations of H_2O_2 stimulation in HepG2 cells. (A) First-Fourth column: the cells were pretreated with H_2O_2 (0, 50, 100, 200 μM), and then cultured with Lyso-NRB (10 μM). Fifth column: the cells were pretreated with PMA (1 μg/mL), and then cultured with Lyso-NRB (10 μM). Sixth column: the cells were pretreated with NAC (1 mM), treated with PMA (1 μg/mL), and then cultured with Lyso-NRB (10 μM). (B) Relative fluorescence intensity quantified from the images in A. Scale bar is 20 μm. Green channel: λ_{ex} = 405 nm, λ_{em} = 430-530 nm; Red channel: λ_{ex} = 560 nm, λ_{em} = 570-670 nm.

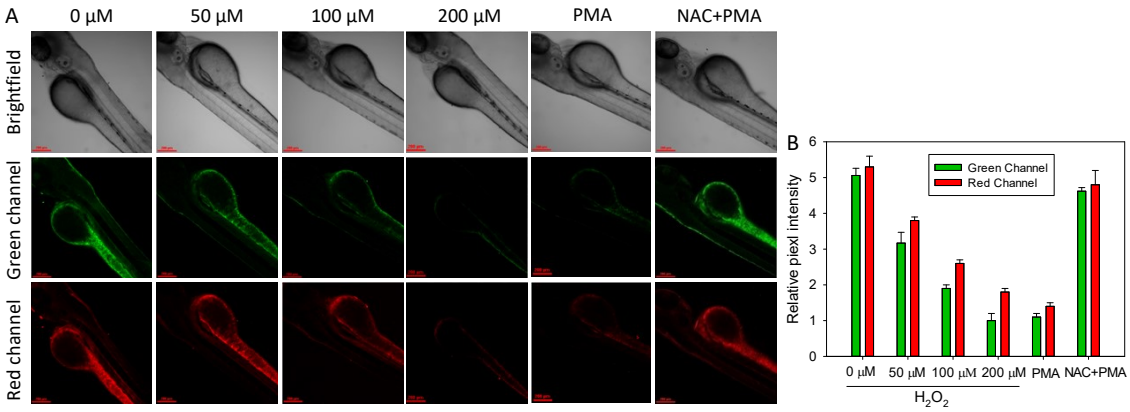

Fig. S21. Fluorescence images of polarity and ATP induced by H_2O_2 stimulation in zebrafish. (A) First-Fourth column: the zebrafish were pretreated with H_2O_2 (0, 50, 100, 200 μM), and then cultured with Lyso-NRB (10 μM). Fifth column: the zebrafish were pretreated with PMA (1 μg/mL), and then cultured with Lyso-NRB (10 μM). Sixth column: the zebrafish were pretreated with NAC (1 mM), treated with PMA (1 μg/mL), and then cultured with Lyso-NRB (10 μM). (B) Relative fluorescence intensity quantified from the images in A. Scale bar is 200 μm. Green channel: λ_{ex} = 405 nm, λ_{em} = 430-530 nm; Red channel: λ_{ex} = 560 nm, λ_{em} = 570-670 nm.