Supporting Information

A highly specific and ultrasensitive fluorescent probe for basal lysosomal HOCl detection based on the chlorination induced by chlorinium ion (Cl^+)

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1. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of probe **Lyso-HOCl** was measured by five times and the standard deviation of blank measurement was obtained. To gain the slope, the fluorescence intensity at 587 nm was plotted as a concentration of HOCl. So the detection limit was calculated with the following equation (1):

$$\text{Detection limit} = 3\sigma/k \qquad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus HOCl concentration.

2. Determination of quantum yield

The quantum yield of probe **Lyso-HOCl** was determined according to the following equation:

$$\phi_1 = \frac{\phi_B I_1 A_B \lambda_{exB} \eta_1}{I_B A_1 \lambda_{ex1} \eta_B} \qquad (2)$$

Where Φ is quantum yield; I is integrated area under the uncorrected emission spectra; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the subscripts 1 and B refer to probe **Lyso-HOCl** and the standard, respectively. *N*-butyl-4-butylamino-1,8-naphthalimide in absolute ethanol was used as the standard, which has a quantum yield of 0.810.[1]

3. Time courses of probe Lyso-HOCl and HOCl system

The time required for the reaction of **Lyso-HOCl** with HOCl in the completed aqueous solution containing PBS (10 mM, pH = 5.0) was investigated.

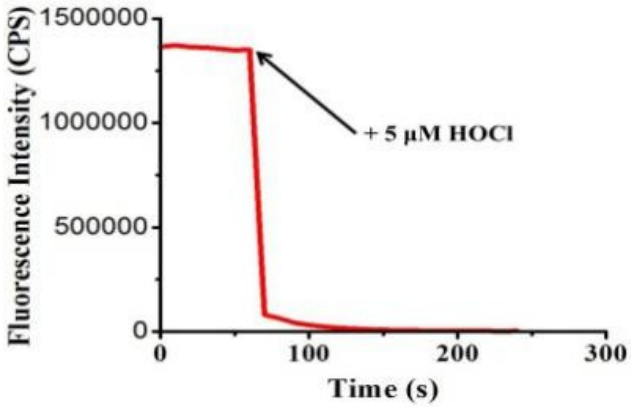


Figure S1. Time-course of fluorescence intensity (at 587 nm) of **Lyso-HOCl** (5 μM) with HOCl (5 μM) in the aqueous solution containing PBS (10 mM, pH = 5.0). Excitation wavelength was 520 nm, excitation and emission slit widths were 3 nm.

4. Effects of pH on probe Lyso-HOCl in the absence and presence of HOCl

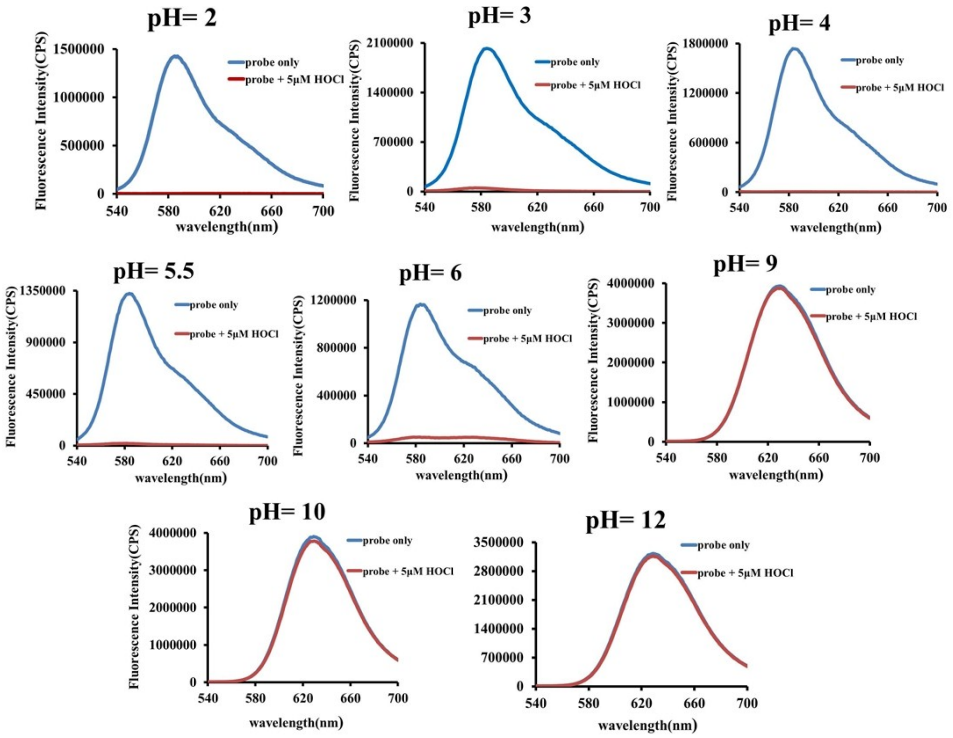
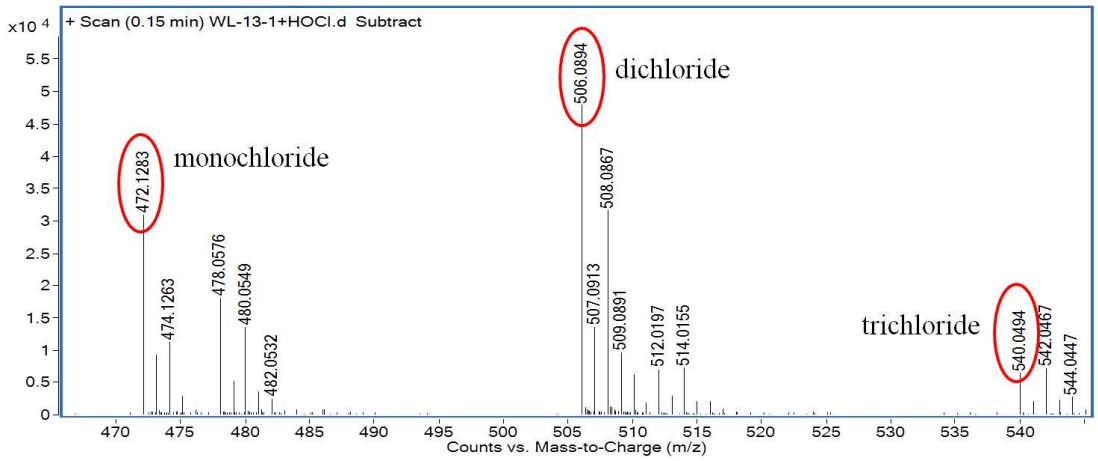


Figure S2. The fluorescence spectra of probe **Lyso-HOCl** (5 μM) in the absence and presence of HOCl (5 μM) under the completed aqueous solution containing PBS (10 mM).

5. HRMS of the reaction of probe Lyso-HOCl with HOCl



Fiure S3. HRMS of the reaction of probe **Lyso-HOCl** with HOCl

6. The absorption spectra of probe Lyso-HOCl in the absence and presence of HOCl

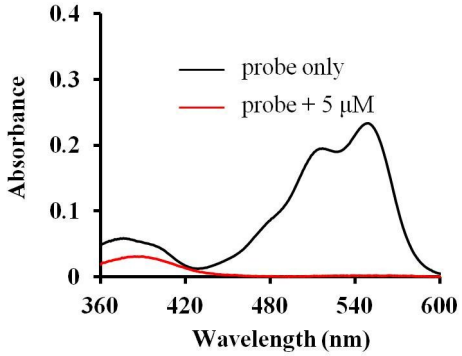


Figure S4. The absorption spectra of **Lyso-HOCl** (5 μM) in the absence and presence of HOCl (5 μM) under the aqueous solution containing PBS (10 mM, pH = 5.0).

7. Cell culture and bioimaging

RAW264.7 macrophage cells were grown on glass-bottom culture dishes using DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 50 μg mL^{-1} penicillin-streptomycin in a humidified 37 °C, 5% CO_2 incubator. Before use, the adherent cells were washed three times with FBS-free DMEM. Cells were incubated with 10 μM **Lyso-HOCl** in culture media for 20 min at 37 °C, and washed with PBS

(pH 7.4) three times. Then the fluorescence imaging of cells was carried out. On the other hand, cells pretreated with PMA (1.0 μg mL^{-1}) or NAC (200.0 μM) for 1 h were incubated with 10 μM **Lyso-HOCl** in culture media for another 20 min at 37 °C, and washed with PBS three times. Then the fluorescence imaging of cells was carried out. Fluorescence imaging of RAW264.7 macrophage cells was observed under an Olympus FV1000-IX81 confocal fluorescence microscope. The excitation wavelength is 488 nm, and the emission wavelength is 500-600 nm. Similarly, the exogenous HOCl of imaging in living RAW264.7 macrophage cells were carried out by the preincubation of NaOCl (10 μM) for 20 min. Referring to the above-mentioned experimental procedures, the co-location of probe **Lyso-HOCl** and Lyso-Tracker Blue (λ_{ex} = 405 nm and λ_{em} = 420-460 nm) in living RAW264.7 macrophage cells were also performed. The green channel for probe fluorescence, and the blue channel for Lyso-Tracker Blue fluorescence.

8. Cytotoxicity assays

RAW 264.7 cells (10^6 cell mL^{-1}) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 24 h. Then RAW 264.7 cells were incubated for 12 h upon different probe concentrations of 5, 10, 20, 30, 50, and 100 μM. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL^{-1}, HEPES) was then added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a Triturus microplate reader.

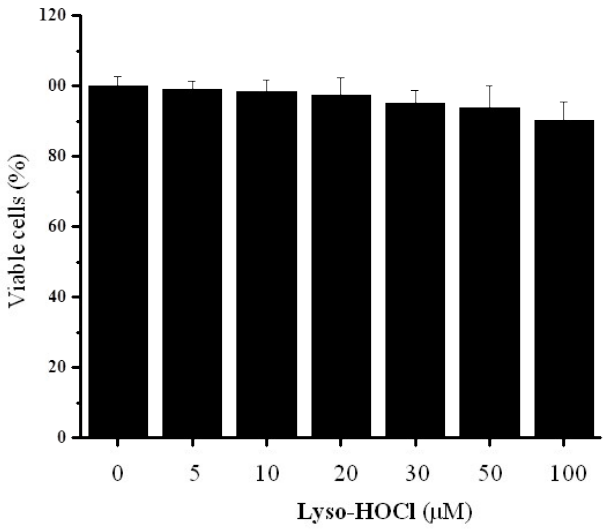


Figure S5. Cytotoxicity assays of **Lyso-HOCl** at different concentrations for RAW264.7 cells.

9. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)[2]

Sodium hypochlorite (NaOCl), H_2O_2 and *tert*-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in ultrapure water. Hydroxyl radical ($^{\cdot}OH$) and *tert*-butoxy radical ($^{\cdot}O^tBu$) were generated by Fenton reactions. Nitric oxide (NO) was generated from potassium nitroprusside dihydrate. Superoxide (O_2^-) was prepared from KO_2 in DMSO. Singlet oxygen ($^1O_2$) was generated from HOCl and H_2O_2, and peroxynitrite ($ONOO^-$) was synthesized from sodium nitrite and hydrogen peroxide in the presence of hydrochloric acid. The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 M^{-1} cm^{-1}). The concentration of $ONOO^-$ was determined from the absorption at 302 nm (ε = 1670 M^{-1} cm^{-1}) in 0.1 N NaOH aqueous solution. The concentration of ^-OCl was determined from the absorption at 292 nm (ε = 350 M^{-1} cm^{-1}).

10. Bioimaging of basal and endogenous HOCl in live RAW264.7 macrophage cells

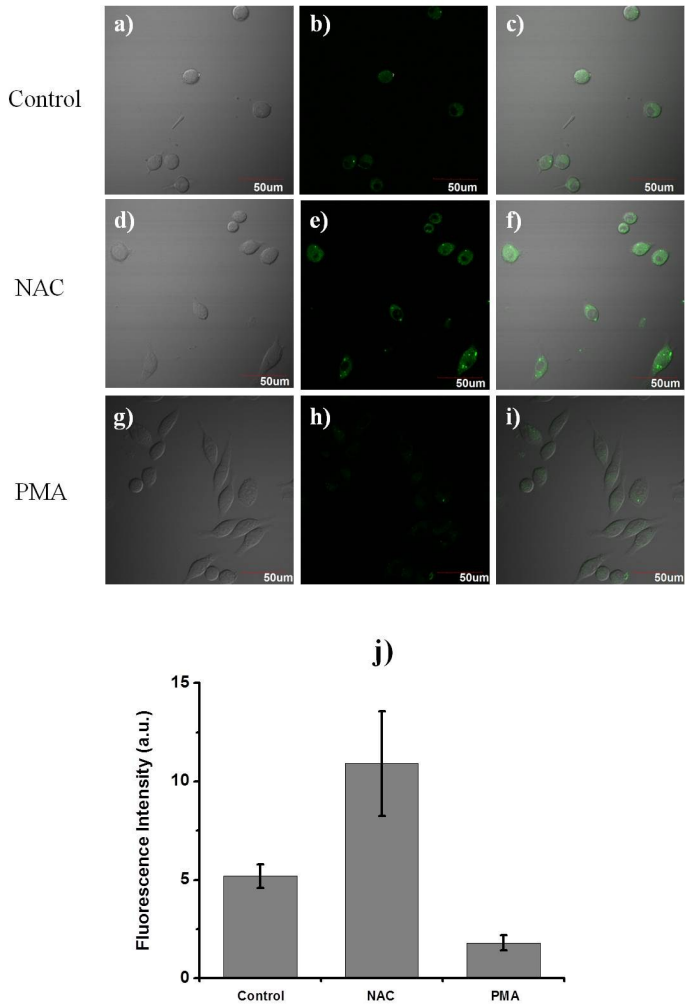


Figure S6. Confocal fluorescence images of live Raw 264.7 macrophages cells: (a-c) control Raw 264.7 macrophages cells incubated with **Lyso-HOCl** (10 μM) for 20 min; (d-f) Raw 264.7 macrophages cells pretreated with NAC (100 μM) for 60 min, and then with **Lyso-HOCl** (10 μM) for another 20 min; (g-i) Raw 264.7 macrophages cells pretreated with PMA (1.0 μg mL^{-1}) for 60 min, and then with **Lyso-HOCl** (10 μM) for another 20 min. (a, d, g) bright-field images; (b, e, h) green channel; (c, f, i) merged images of the corresponding bright-field and green channel. (j) relative fluorescence intensity of cells in panels (b, e and h). The provided images of Raw

264.7 macrophages cells are representative ones (n = 10 fields of cells). Incubation was performed at 37 ℃ under a humidified atmosphere containing 5% CO_2. Fluorescence images were acquired using a 488 nm excitation, and fluorescence emission windows: 500~600 nm. Scale bar is 50 μm.

11. Bioimaging of exogenous HOCl in live RAW264.7 macrophage cells

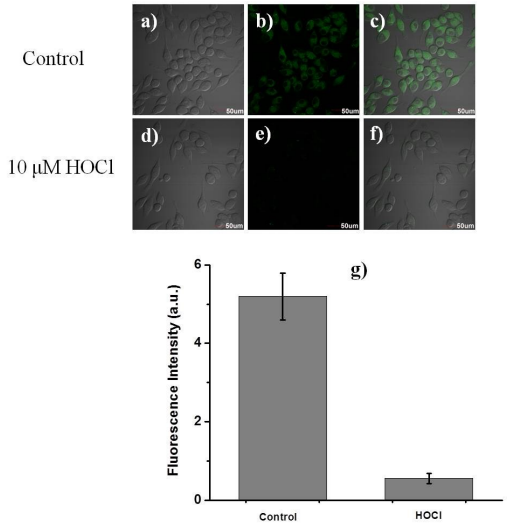


Figure S7. Confocal fluorescence images of live Raw 264.7 macrophages cells: (a-c) control Raw 264.7 macrophages cells incubated with **Lyso-HOCl** (10 μM) for 20 min; (d-f) Raw 264.7 macrophages cells pretreated with HOCl (10 μM) for 60 min, and then with **Lyso-HOCl** (10 μM) for another 20 min; (a, d) bright-field images; (b, e) green channel; (c, f) merged images of the corresponding bright-field and green channel. (g) relative fluorescence intensity of cells in panels (b and e). The provided images of Raw 264.7 macrophages cells are representative ones (n = 10 fields of cells). Incubation was performed at 37 ℃ under a humidified atmosphere containing 5% CO_2. Fluorescence images were acquired using a 488 nm excitation, and fluorescence emission windows: 500~600 nm. Scale bar is 50 μm.

12. Spectra of ^{1}H NMR and ^{13}C NMR for probe Lyso-HOCl

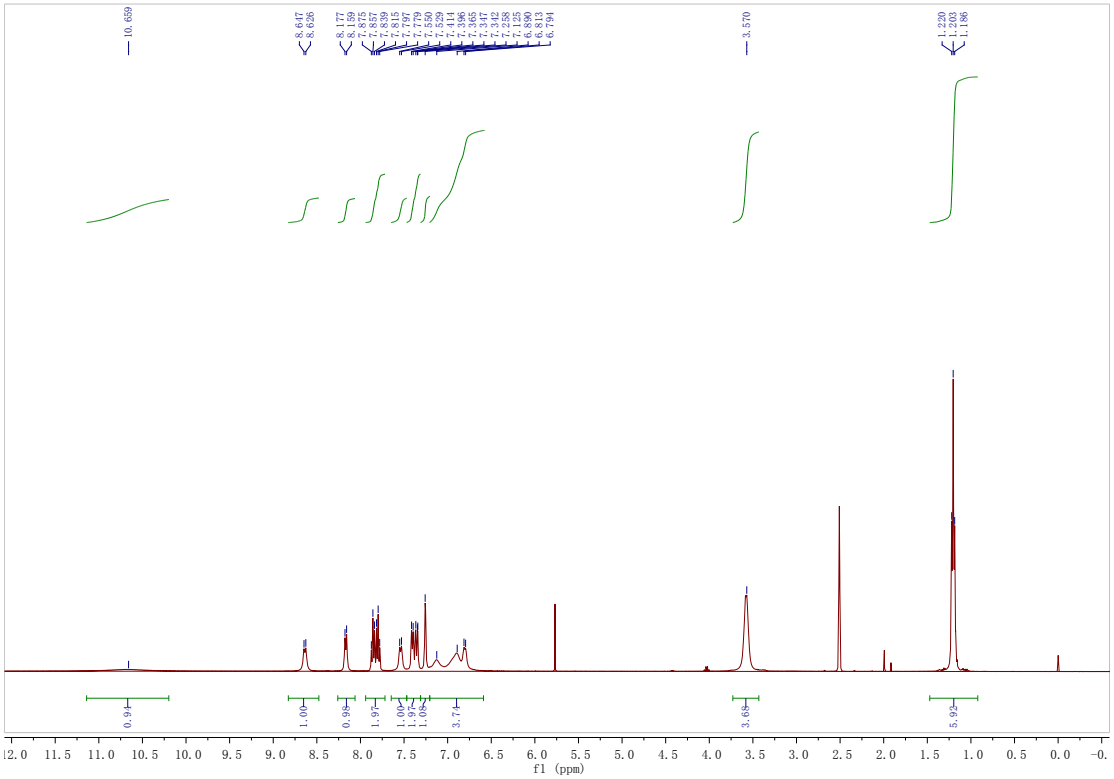


Figure S8. Spectrum of ^{1}H NMR for probe **Lyso-HOCl**

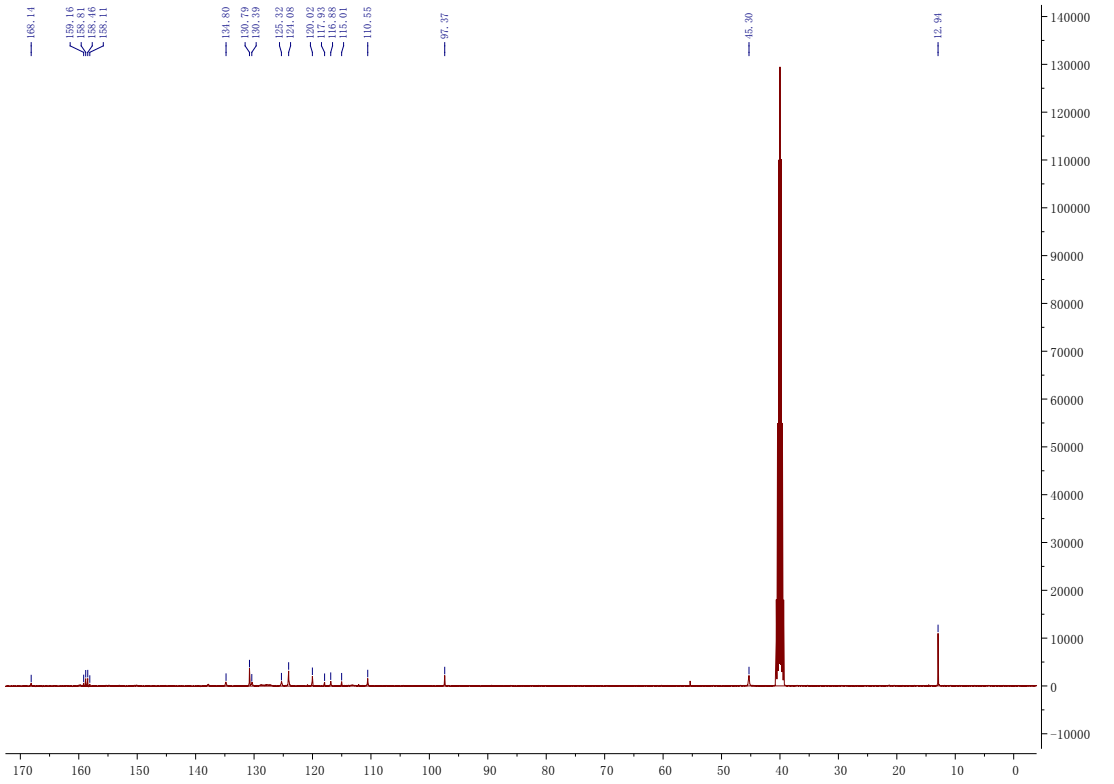


Figure S9. Spectrum of ^{13}C NMR for probe **Lyso-HOCl**

13. Refererences

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2 M. Abo, Y. Urano, K. Hanaoka, T. Terai, T. Komatsu and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629-10637.