In vivo Bioimaging and Detection of Endogenous Hypochlorous Acid in Lysosome Using a Near-infrared Fluorescent Probe

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Fig. S1 ¹H-NMR spectra of FNIR-HClO fluorescent probe
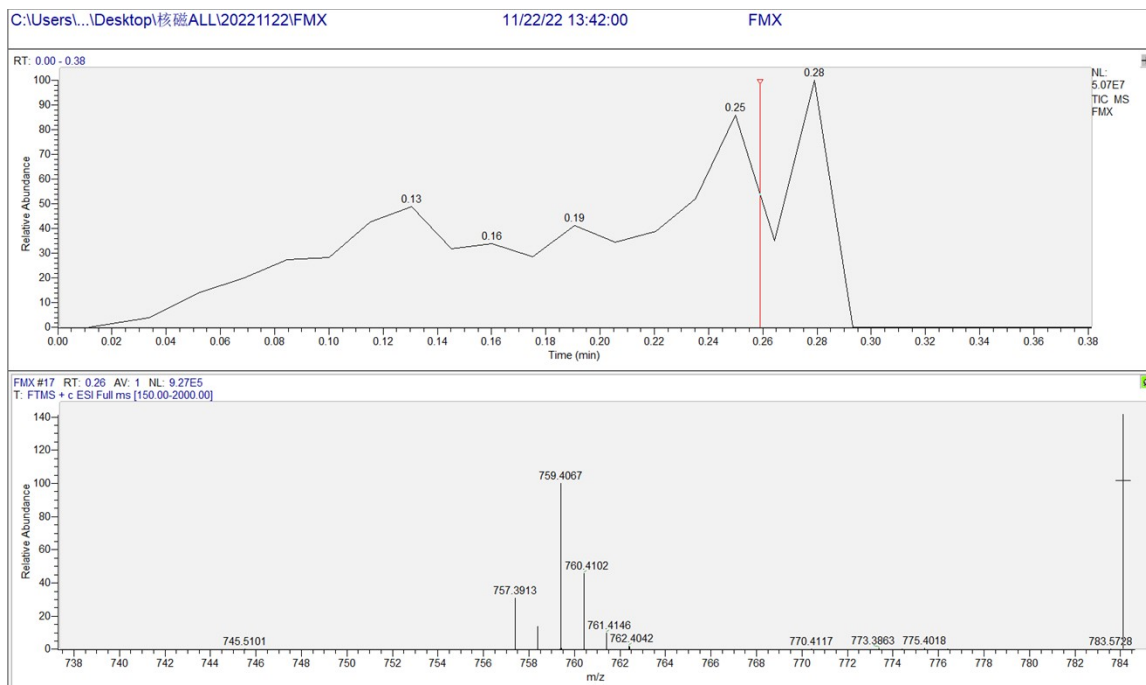
Fig. S2 ^{13}C-NMR spectra of FNIR-HClO fluorescent probe

RT: 0.00 - 0.38

NL:
5.07E7
TIC MS
FMX

0.13

0.16

0.19

0.25

0.28

FMX #17 RT: 0.26 AV: 1 NL: 9.27E5
T: FTMS + c ESI Full ms [150.00-2000.00]

745.5101

757.3913

759.4067

760.4102

761.4146

762.4042

770.4117

773.3863

775.4018

783.5728

Fig. S3 HRMS spectra of FNIR-HClO fluorescent probe

Equation	y = a + b*x
Weight	(=1/ei^2)
Intercept	-0.01248 ± 0.04405
Slope	0.09348 ± 0.00262
Sum of squares of fittin	14.38327
Pearson's r	0.99687
R square(COD)	0.99375
Normalized	0.99296
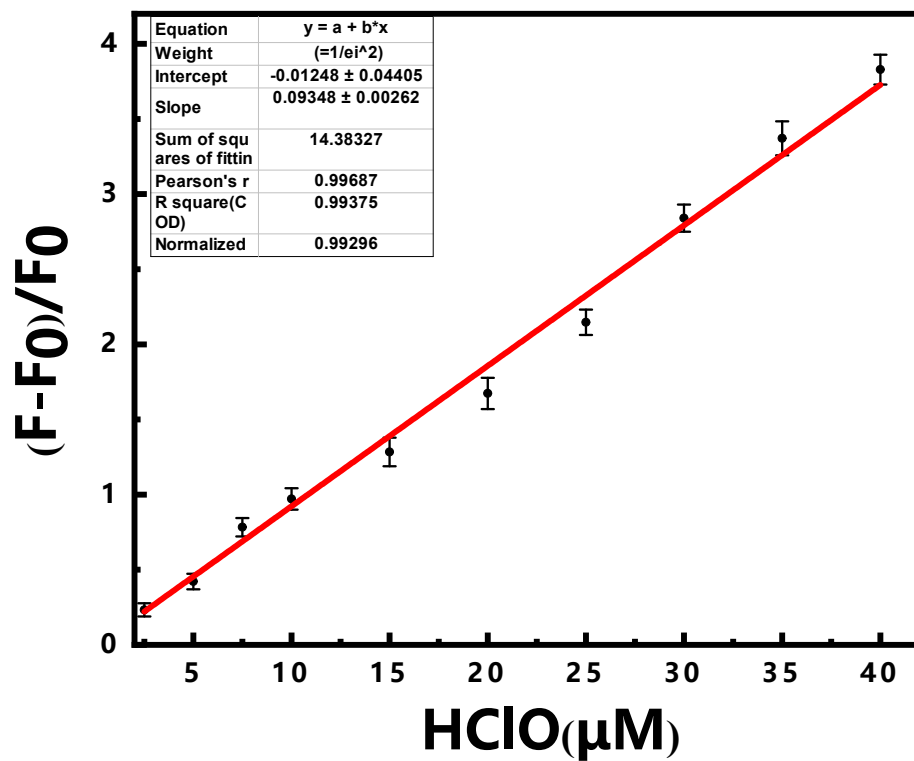
Fig. S4 Steady-state kinetics of reaction between the probe and HClO. The red curve is the linear fitting using linear equation, from which the kinetics parameters were obtained.

LOD was calculated to be $7.019×10^{-8}$ M
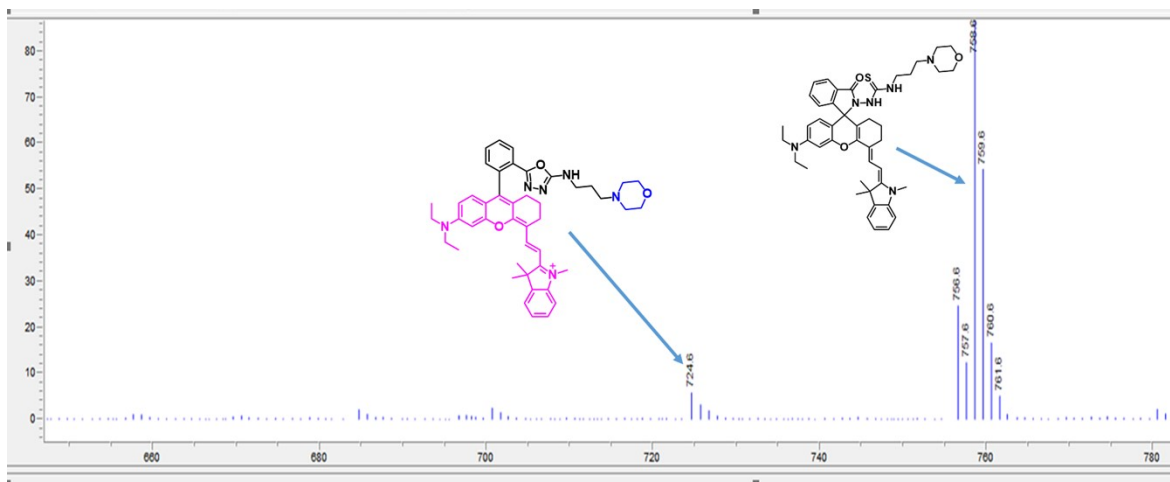
Figure S5. ESI-MS of FNIR-HClO and FNIR-A fluorescent probe

30%MeOH adding 40μM NaClO solution
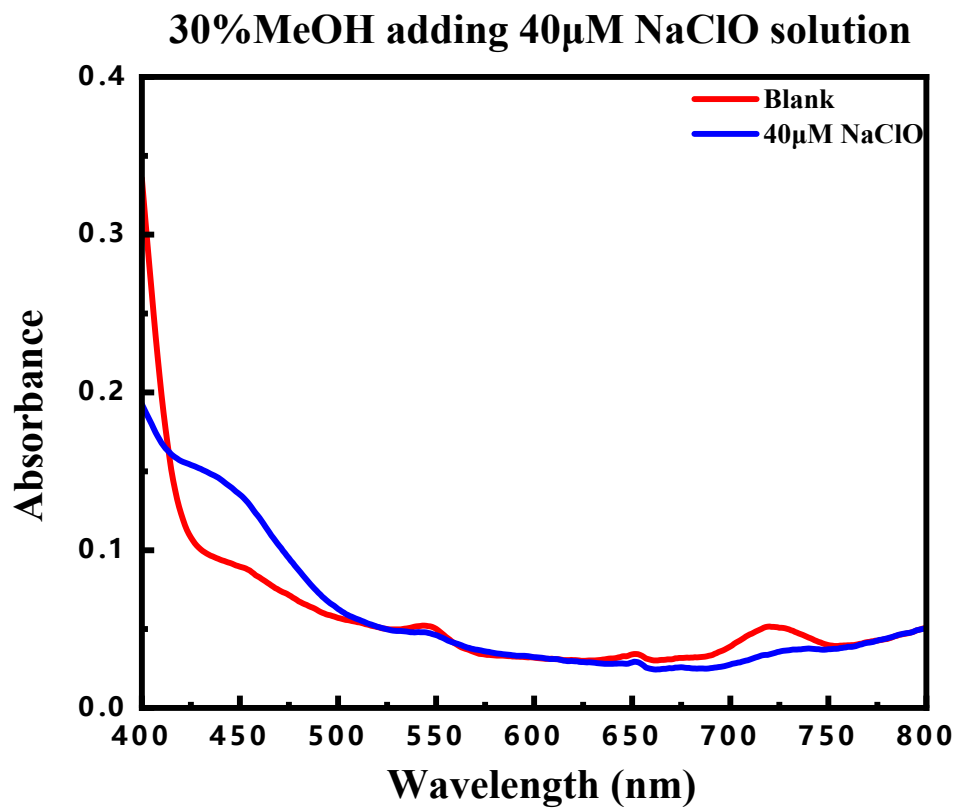
Figure S6. Absorbance of FNIR-HClO in 30% MeOH and PBS solution
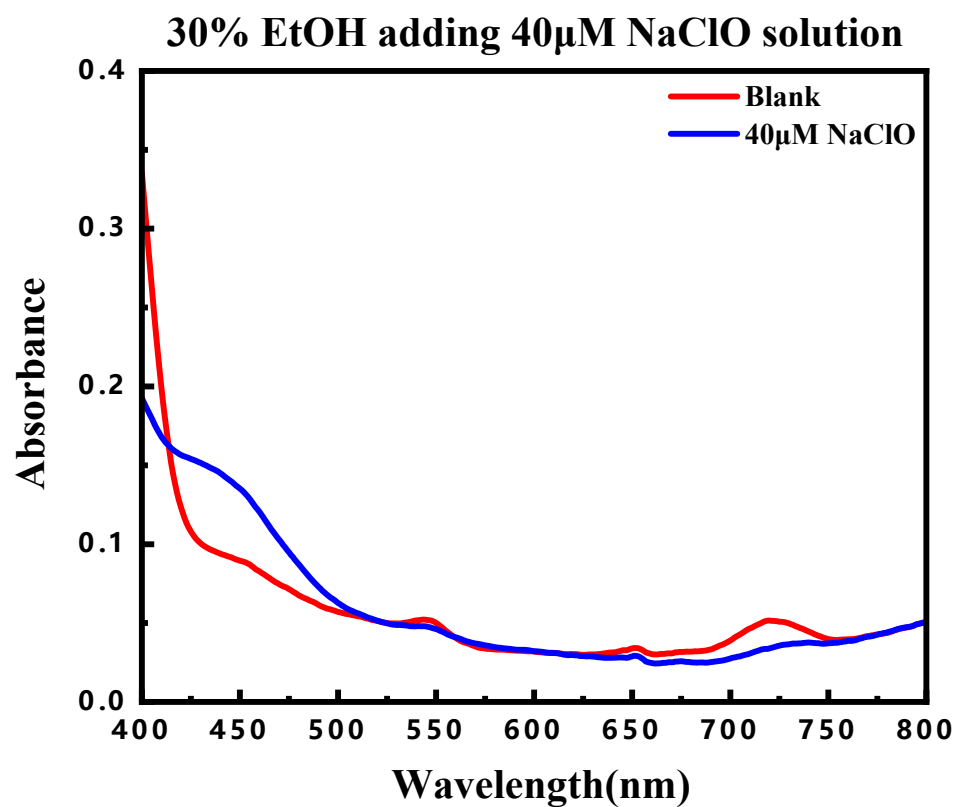
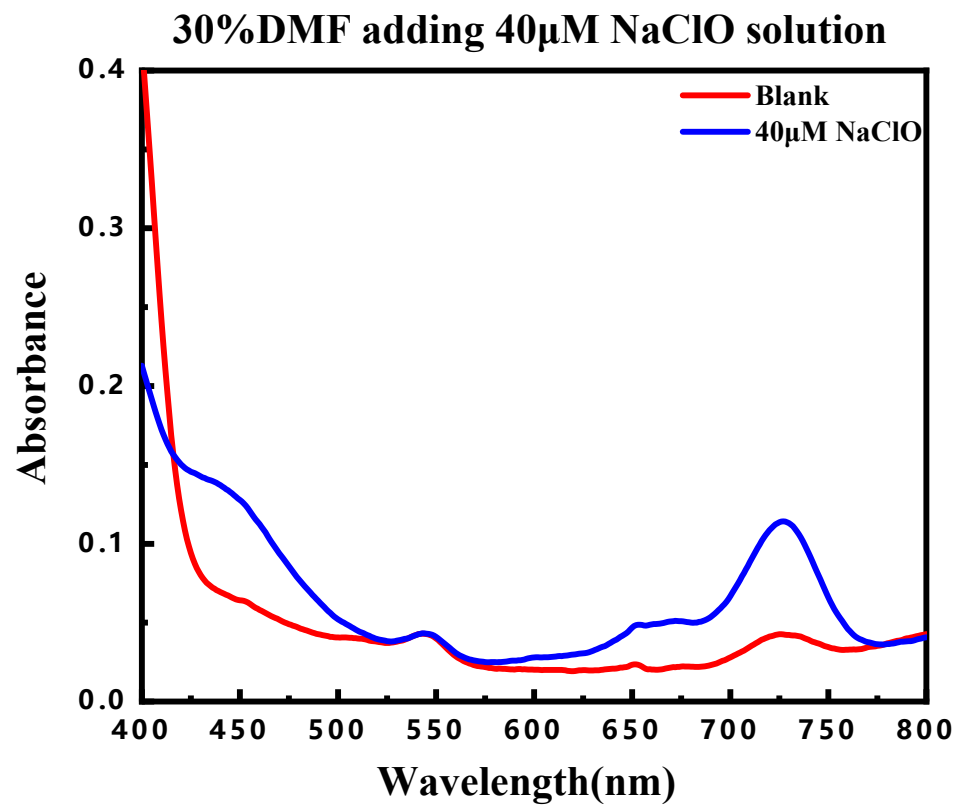**Figure S7.** Absorbance of FNIR-HClO in 30% EtOH and PBS solution

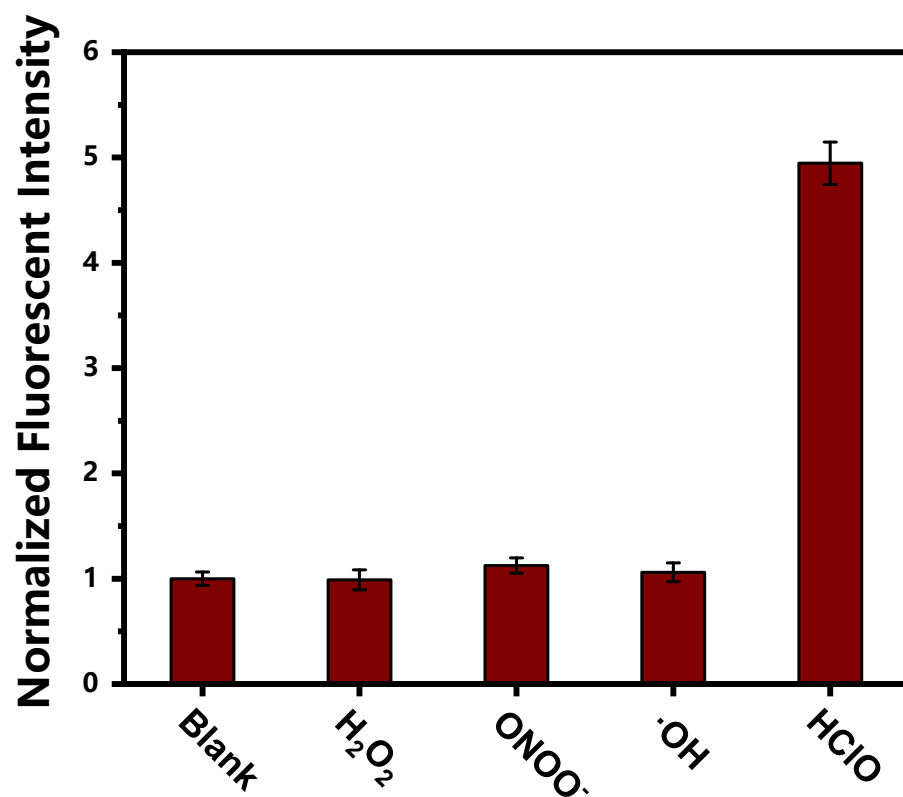**Figure S8.** Absorbance of FNIR-HClO in 30% DMF and PBS solution

Figure S9. Fluorescence comparison of FNIR-HOCl (5 μM) in the presence of different ROS (200 μM) in the mixture of DMF and PBS (v/v = 3:7, PBS = 10 mM, pH 7.4).
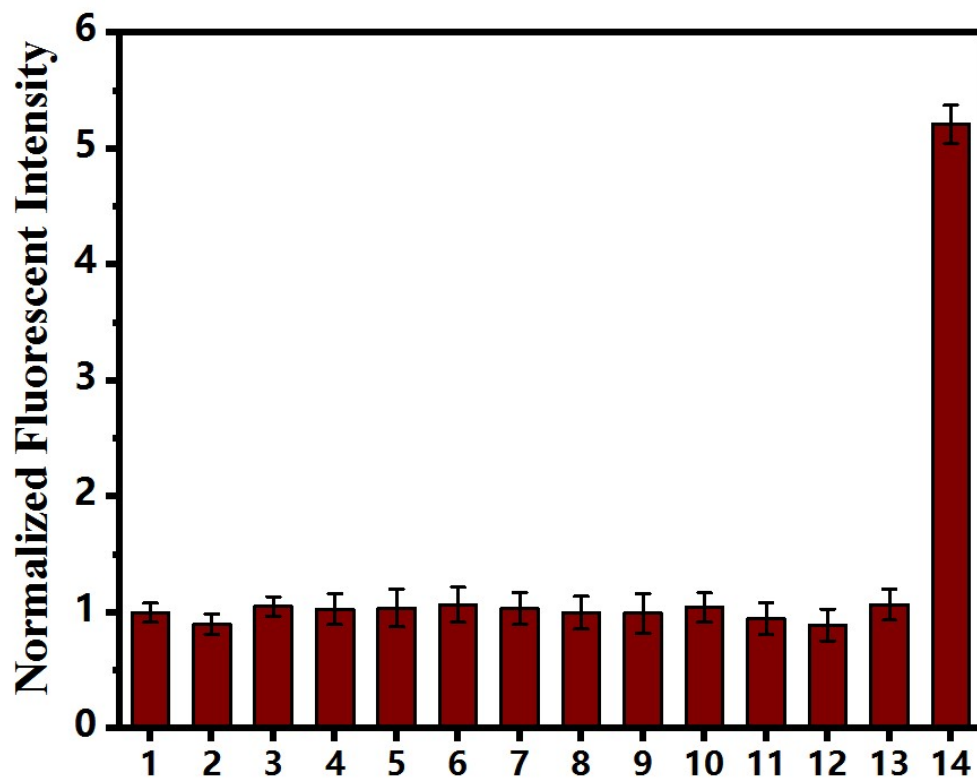
Figure S10. Fluorescence comparison of FNIR-HOCl (5 μM) in the presence of different organic matters (200 μM) in the mixture of DMF and PBS (v/v = 3:7, PBS = 10 mM, pH 7.4). (from 1 to 14: blank, methanol, ethanol, glycerol, isopropanol, triethanolamine, hexane, cyclohexane, trichloromethane, toluene, DMSO, acetic acid, acetone, HOCl)

Optical Measurement:

The UV-Vis absorption spectra of FNIR-HClO were gathered between 300 and 800 nm with 1 nm increments. Their relevant fluorescence spectra were acquired with 1 nm increments at an excitation wavelength of 710 nm. The widths of the excitation and emission slits were each fixed at 10 nm. As a standard reference for determining the fluorescence quantum yields of FNIR-HClO in buffer solutions, Hunan dye $\Phi = 0.41$ with excitation wavelength at 700 nm in methanol)[1] was employed. Both samples and references were newly prepared in the same manner. The quantum yields of fluorescence were computed using the following equation:

$$\Phi_X = \Phi_{st}\left(Grad_X/Grad_{st}\right)\left(\eta_X^2/\eta_{st}^2\right)$$

The subscripts '*st*' and '*X*' stand for standard and test, respectively. Φ is the fluorescence quantum yield, "*Grad*" represents the gradient from the plot of integrated fluorescence intensity versus absorbance, and η is the refractive index of the solvent.

Quantum yield was calculated as 0.256 with excitation wavelength as 710nm in methanol.

References:

1.   Yuan, L.; Lin, W.; Zhao, S.; Gao, W.; Chen, B.; He, L.; Zhu, S., A unique approach to development of near-infrared fluorescent sensors for in vivo imaging. *J Am Chem Soc* **2012,** *134* (32), 13510-23.