Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Engineering Lipid Droplets Targeting Fluorescent Probe with Large Stokes Shift through Ester Substituent Rotation for In Vivo Tumor Imaging

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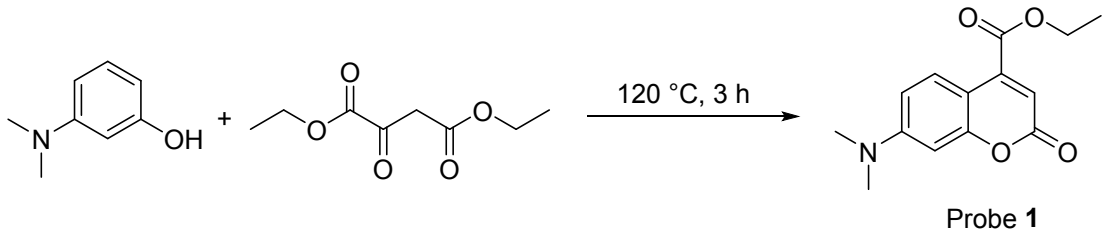
Experimental Section

Synthesis and characterization of Probe 1

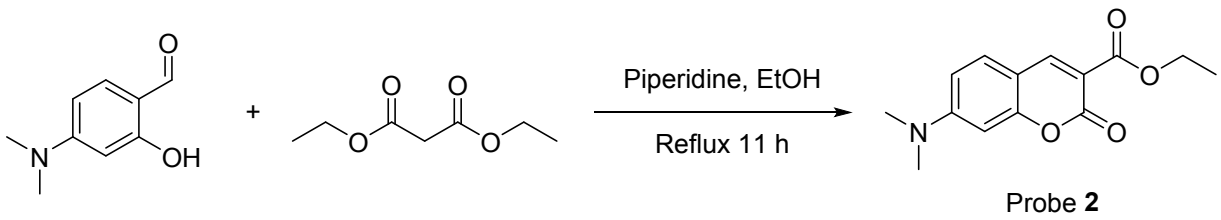
A mixture of diethyl oxalacetate (1.818 g, 10 mM) and 3-(dimethylamino) phenol (1.3718 g, 10 mM) was stirred at 120 °C for 3 hours. After cooling, the reaction mixture was diluted with CH_2Cl_2, which was purified by column chromatography on silica gel (CH_2Cl_2) to afford probe **1** as orange solid (1.07 g, 45% yield). ^{1}H-NMR ($CDCl_3$, 400MHz): δ 8.061 (d, 1H)，δ 6.657 (dd, J_1 = 2.4Hz, J_2 = 11.6 Hz, 1H)，δ 6.586 (s, 1H), δ 6.540 (d, *J* = 2.4 Hz,1H), δ 4.445 (q, *J* = 7.2 Hz,2H), δ 3.095 (s,6H), δ 1.440 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 400MHz): δ 164.629, 161.584, 156.703, 152.954, 142.957, 127.480, 111.94, 109.40, 105.583, 98.179, 62.120, 40.072,14.136. HRMS (m/z): [M + H] $^+$ calcd for, C14H15NO4, 262.1074; found, 262.1074.



Scheme S1. The synthetic route of probe **1**.

Synthesis and characterization of Probe 2

4-(dimethylamino)-2-hydroxybenzaldehyde (200mg, 1.21 mmol), ethyl malonate (273μL, 1.82 mmol), piperidine (10 μL) were stirred in ethanol (3 mL) under 90℃ for 11h. And then, the mixture was cooled and stayed overnight at -4℃. After filtering the mixture, we can obtain a yellow solid probe **2** (95.2 mg, yield: 26.7%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.444 (s, 1H), δ 7.382 (d, *J* = 8.8 Hz, 1H), δ 6.634 (dd, J_1= 2.4 Hz, J_2= 8.8 Hz, 1H), δ 6.461 (d, *J* = 2.4 Hz, 1H), δ 4.379 (q, *J* = 7.2 Hz, 2H), δ 3.125 (s, 6H), δ 1.394(t, *J* =7.2 Hz, 1H); ^{1}C-NMR ($CDCl_3$, 400 MHz): δ 164.18, 158.06, 154.77, 149.30, 130.72, 109.67, 107.99, 97.18, 61.22, 40.23, 14.36. HRMS (m/z): [M + H] $^+$ calcd for, C14H15NO4, 262.1074; found, 262.1074.



Scheme S2. The synthetic routine of probe **2**.

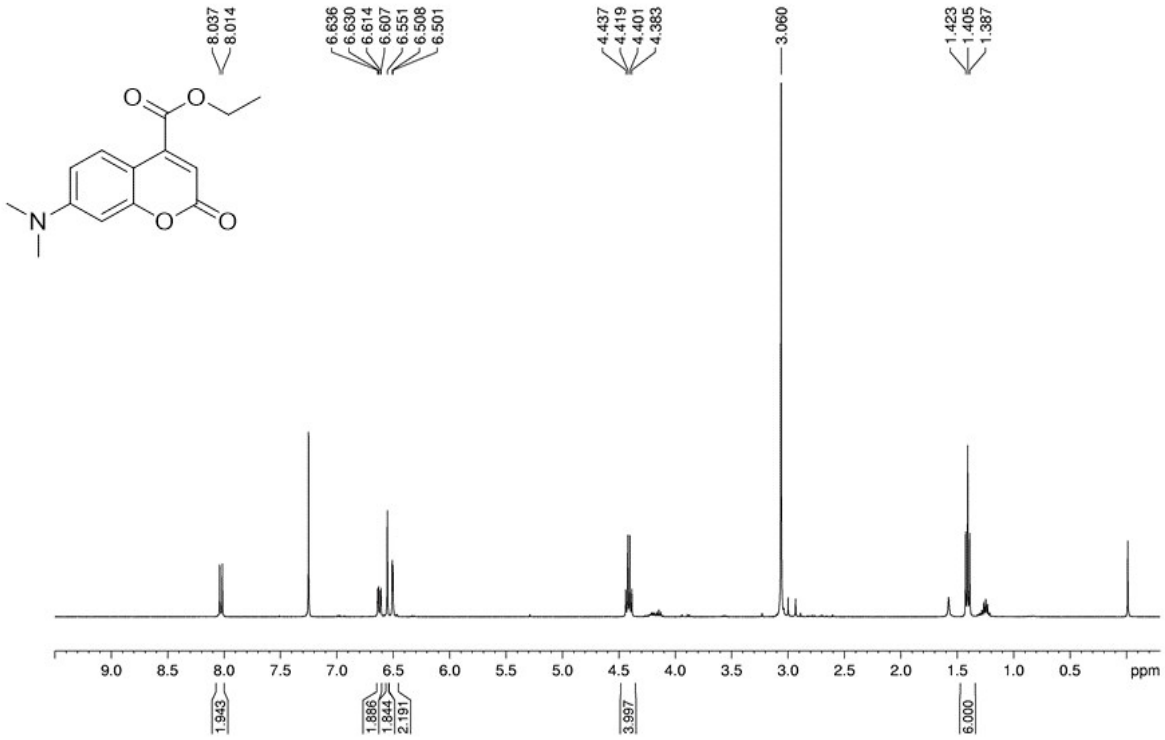


Figure S1. The ^{1}H NMR spectrum of probe **1** in $CDCl_3$.

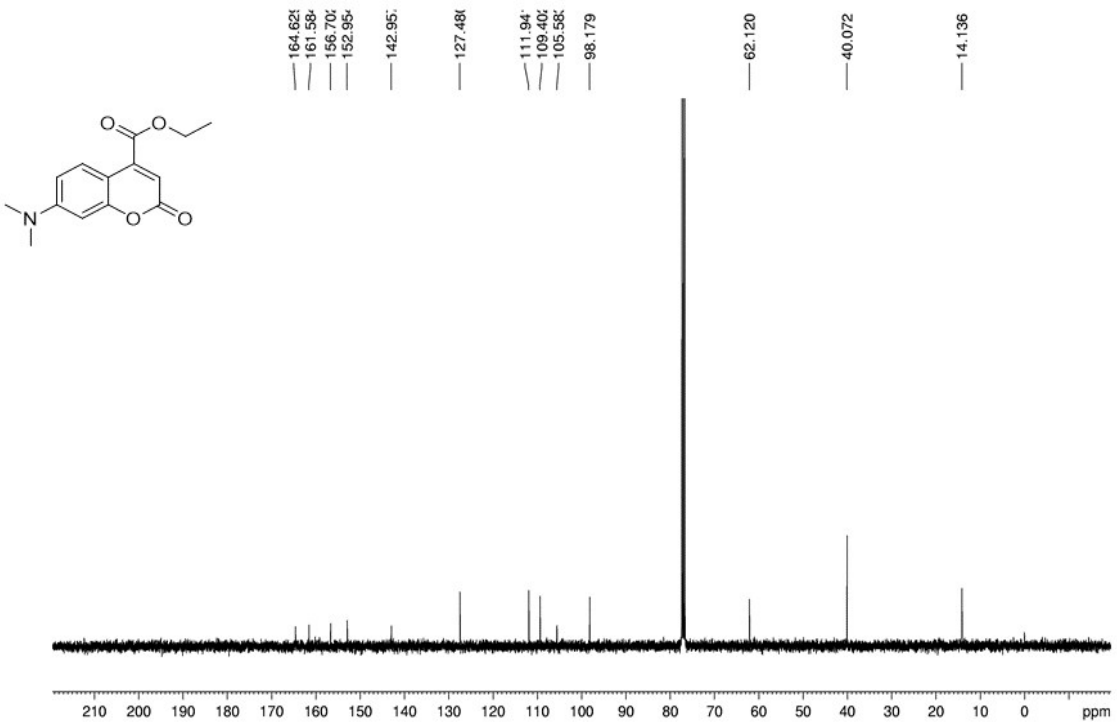


Figure S2. The ^{13}C NMR spectrum of probe **1** in $CDCl_3$.

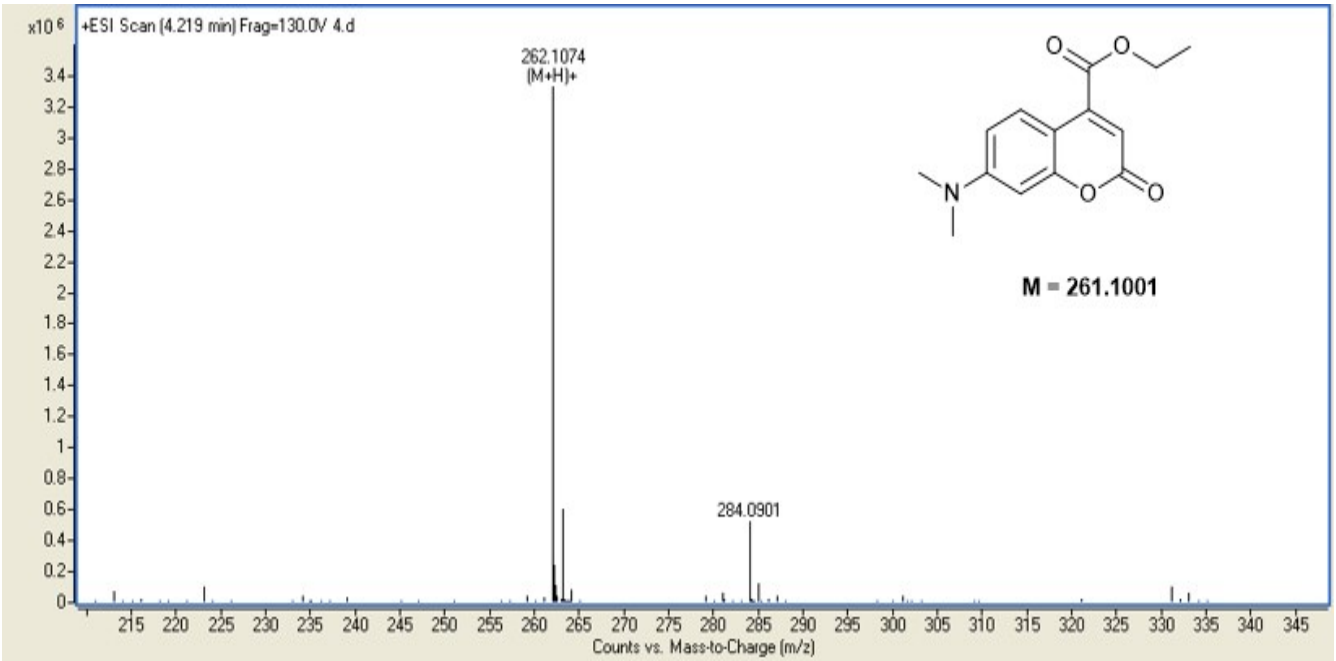


Figure S3. The HRMS spectrum of probe **1**.

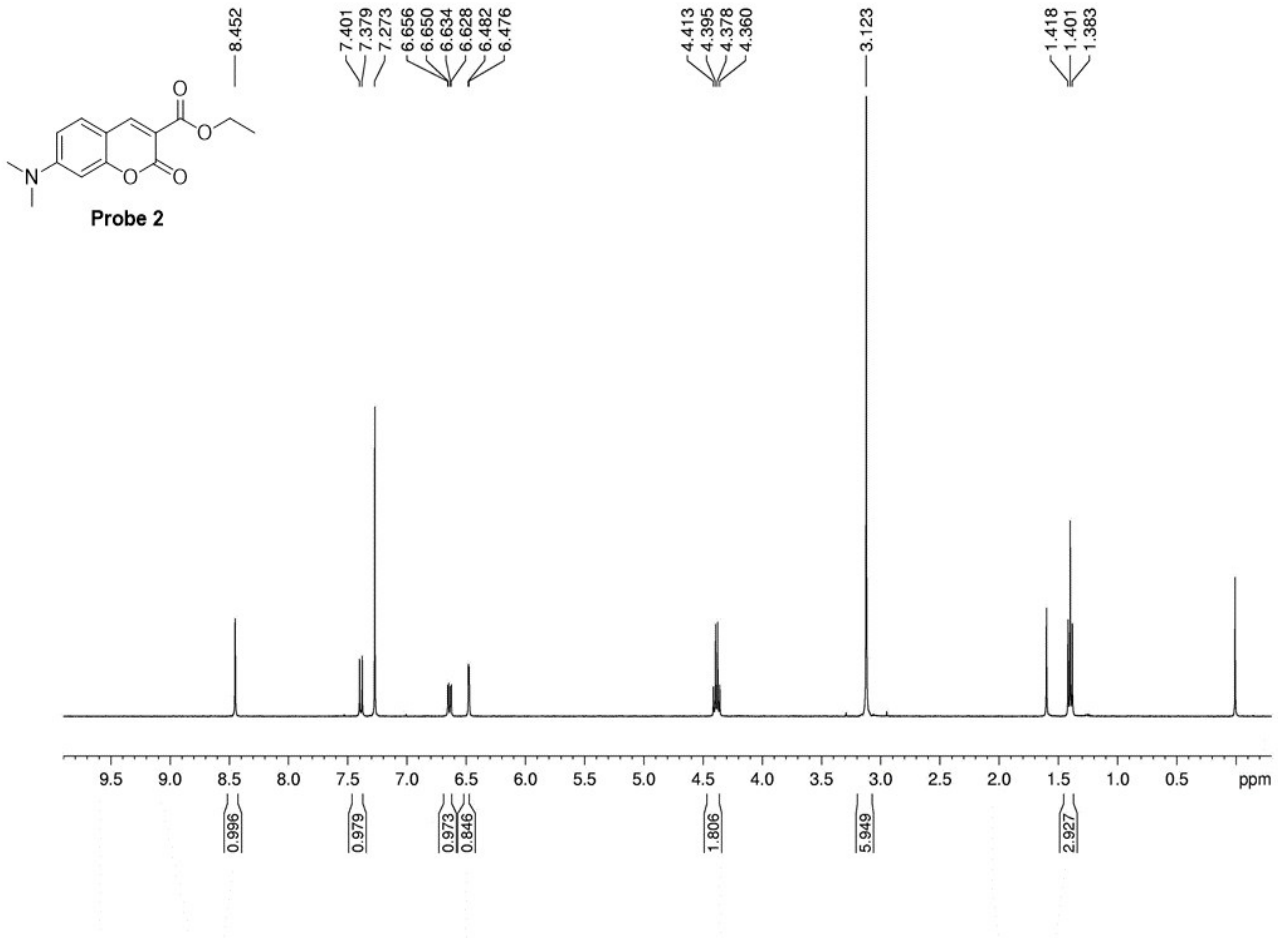
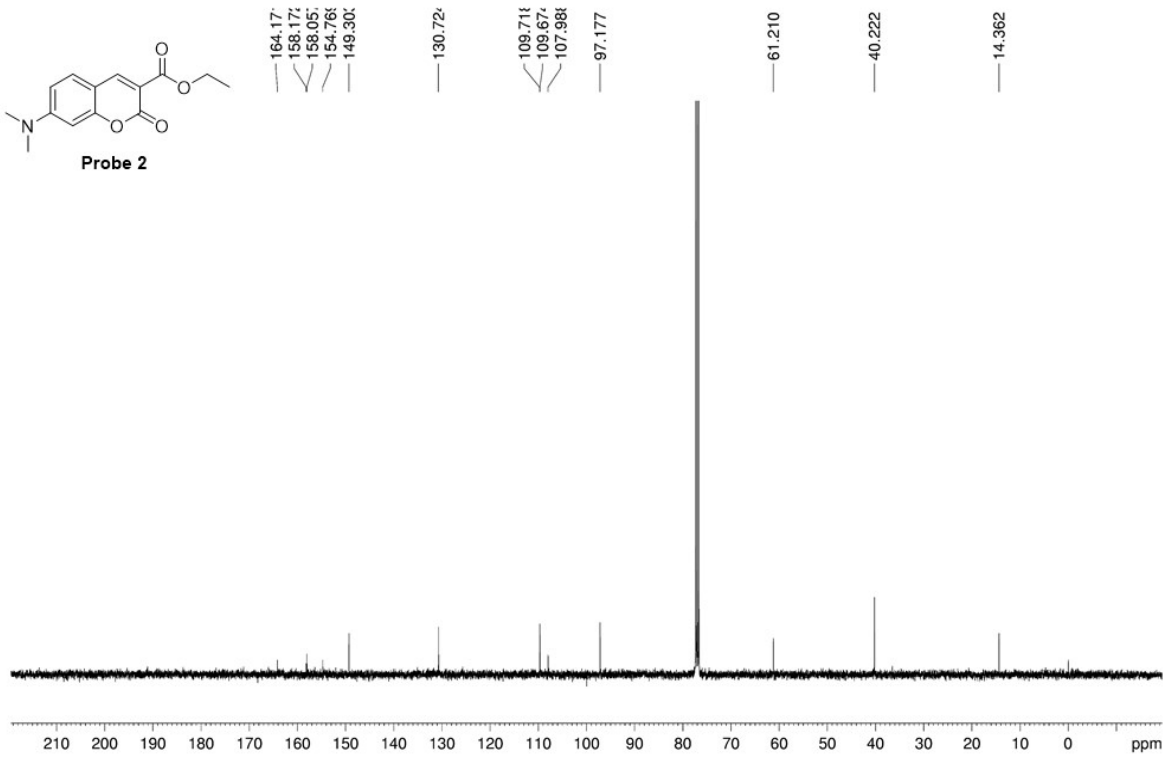


Figure S4. The ^{1}H NMR spectrum of probe **2** in $CDCl_3$.



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igure S5. The ^{13}C NMR spectrum of probe **2** in $CDCl_3$.

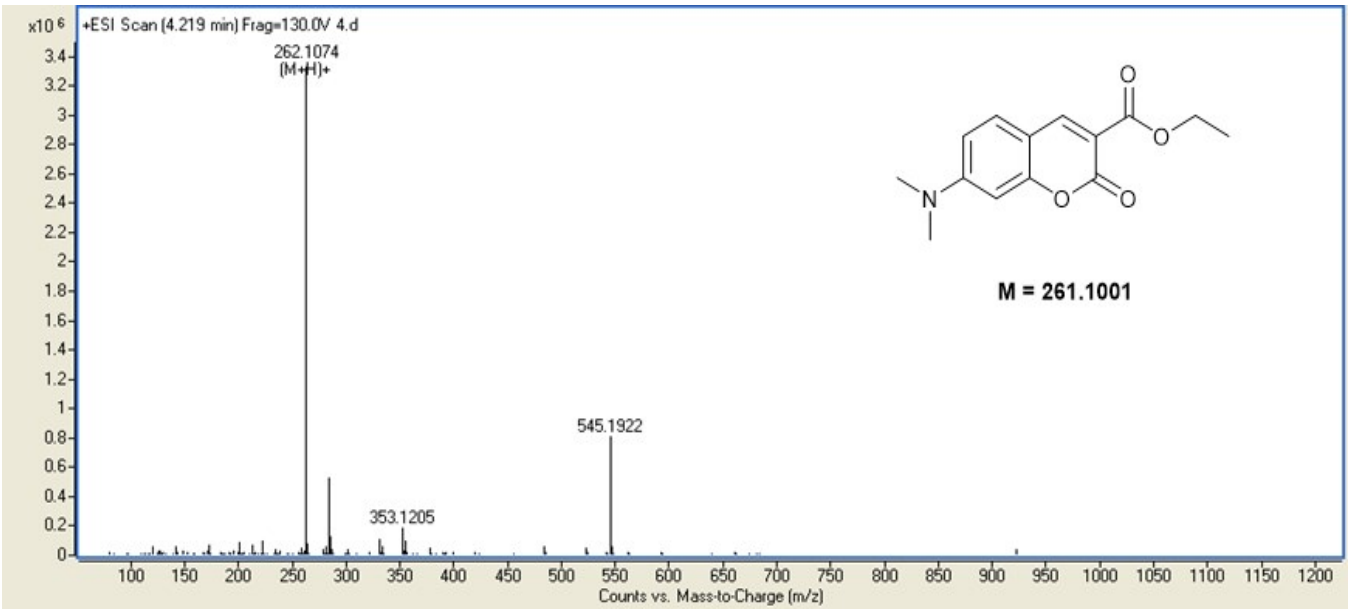

Figure S6. The HRMS spectrum of probe **2**.

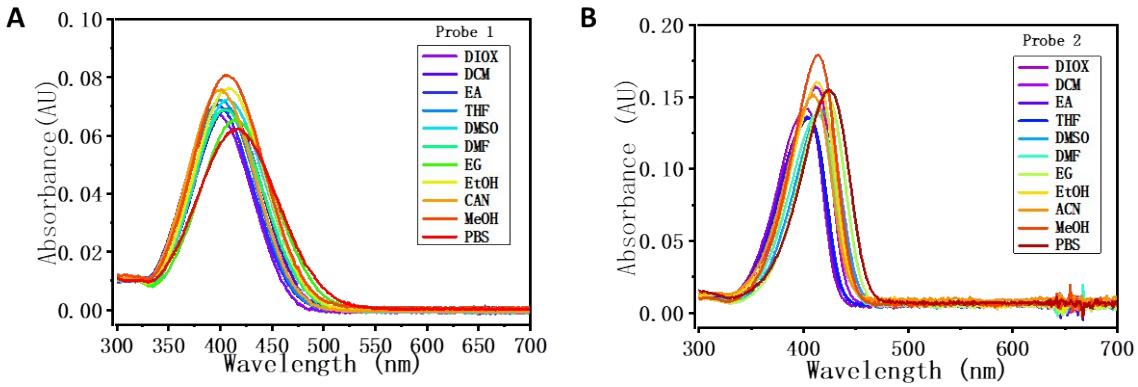

Figure S7. Absorption spectra of 4 μM probe **1** (A) and probe **2** (B) in PBS.

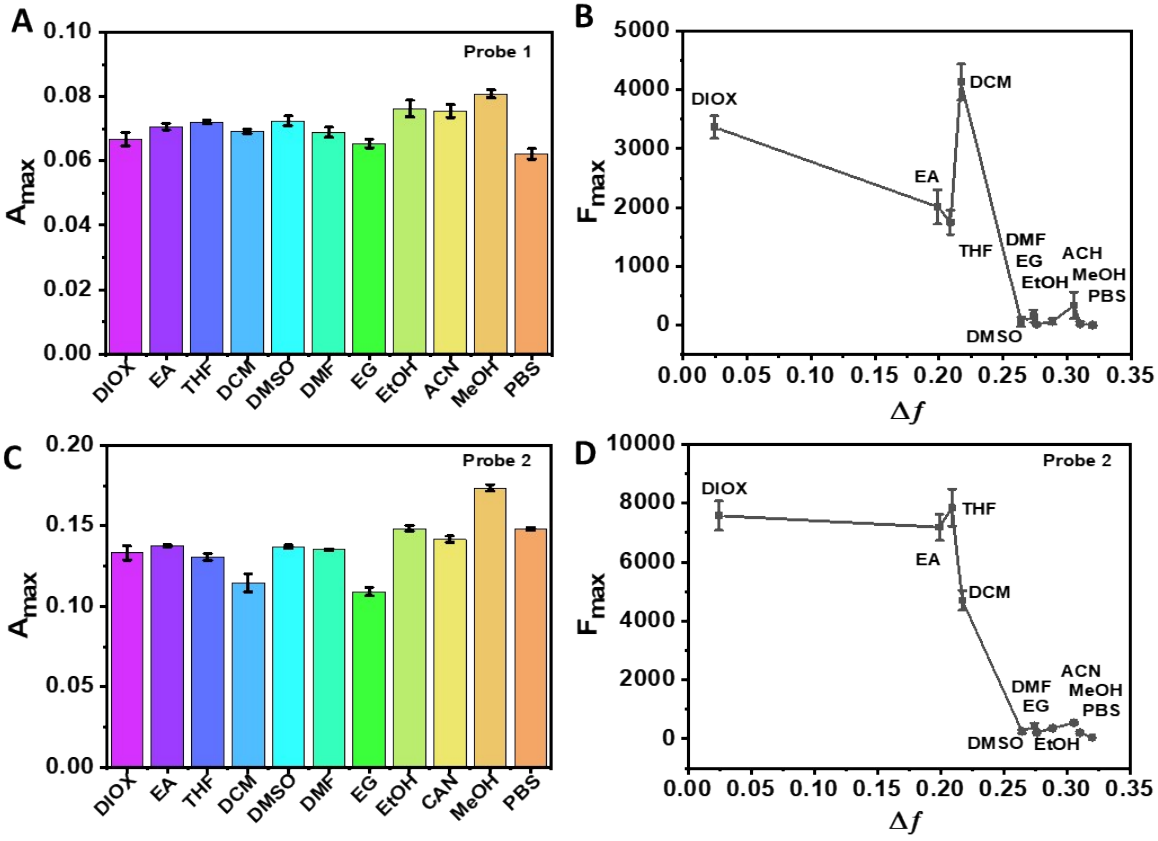

Figure S8. A) The UV-vis spectra and plot of intensity against dye concentration for probe **1** (4 μM) in PBS buffer (pH 7.4, 10 mM); **B)** UV-visible absorption of the probe **1** (4 μM) at 405 nm; **C)** The UV-vis spectra and plot of intensity against dye concentration for probe **2** (4 μM) in PBS buffer (pH 7.4, 10 mM); **D)** UV-visible absorption of the probe **2** (4 μM) at 415 nm; The measured data after the probe was placed for 2 min at 25 °C. (Probe **1**: Ex = 420nm, 5,5 slit/nm, 400 Volta; Probe 2: Ex = 435 nm, 5,5 slit/nm, 400 Volta)

Table S1. Photophysical properties of probe **1** in various solvents. Excitation wavelength: 390 nm. Absolute quantum yield.

Solvent	λ_{Abs} (nm)	ε ($M^{-1}\cdot cm^{-1}$)	λ_{Em} (nm)	Stokes shift (nm)	QY Φ (%)
DIOX	394	16824.25	522	128	52.78
EA	397	17674.5	533	136	33.72
THF	400	18024.5	534	134	26.98
DCM	408	17300.75	530	122	49.30
DMSO	407	18120.5	570	163	4.10
DMF	404	17233	559	155	8.58
EG	414	16356	573	159	1.01
EtOH	408	19067.25	560	152	3.41
ACN	401	18982.25	553	152	12.21
MeOH	405	20215.5	568	163	1.80
PBS	417	15550.25	601	184	0.18

Table S2. Photophysical properties of probe **2** in various solvents. Excitation wavelength: 390 nm.

Solvent	λ_{Abs} (nm)	ε ($M^{-1}\cdot cm^{-1}$)	λ_{Em} (nm)	Stokes shift (nm)	QY Φ (%)
DIOX	404	33226.73	455	51	106.49
EA	404	34328.82	462	58	82.64
THF	404	32507.78	463	59	70.09
DCM	412	26334.64	466	53	101.22
DMSO	417	28884.77	484	67	6.16
DMF	412	30856.73	478	66	9.98
EG	422	19610.29	485	63	6.41
EtOH	413	33432.48	475	62	8.63
ACN	409	34050.7	473	64	7.94
MeOH	413	38644.43	478	65	3.12
PBS	423	25834.2	483	60	0.68

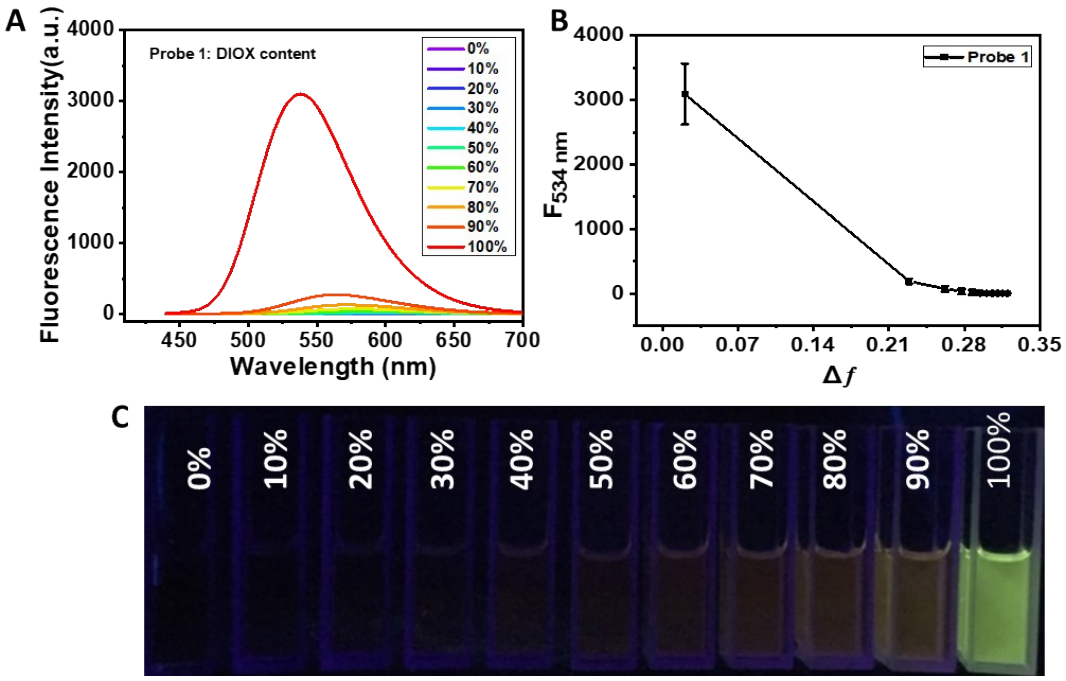

Figure S9. A) Fluorescence emission spectra of probe **1** (4 μM) in 1,4-dioxane-water solution with different 1,4-dioxane content; **B)** Fluorescence emission spectra of probe **1** (4 μM) at 536 nm in different 1,4-dioxane content solutions; **C)** Photograph of probe **1** (4 μM) under different levels of 1,4-dioxane (1,4-dioxane / PBS) under 365 nm violet light. (Ex = 420nm, 5,5 slit/nm, 400 Volta)

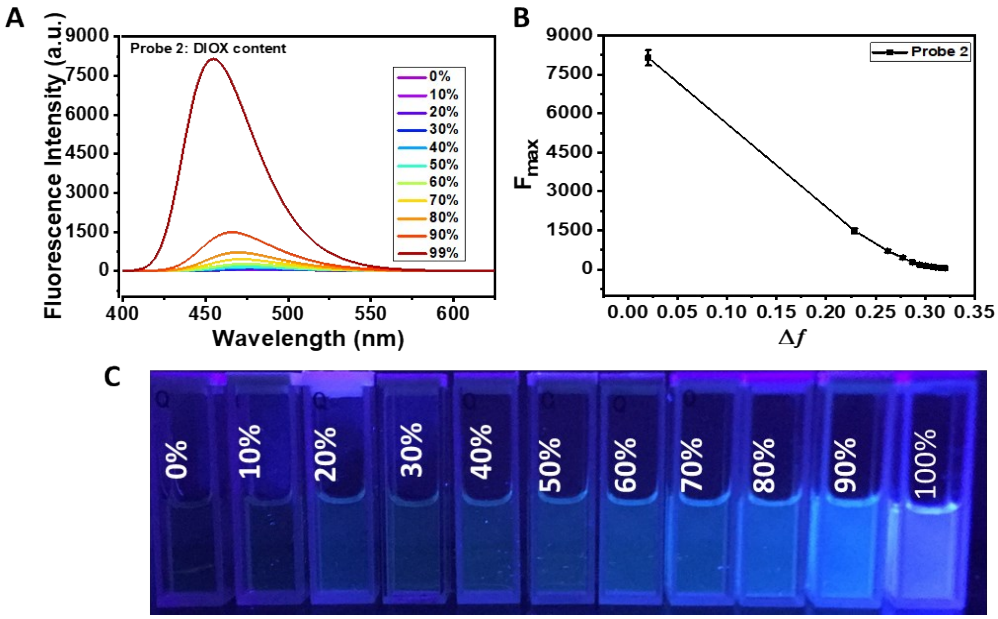

Figure S10. A) Fluorescence emission spectra of probe **2** (4 μM) in different DIOX content solutions; **B)** Fluorescence emission spectra of probe **2** (4 μM) at 488 nm in different DIOX content solutions; **C)** Photograph of probe **2** (4 μM) under different levels of DIOX (DIOX / PBS) under 365 nm violet light. (Ex = 435 nm, 5,5 slit/nm, 400 Volta)

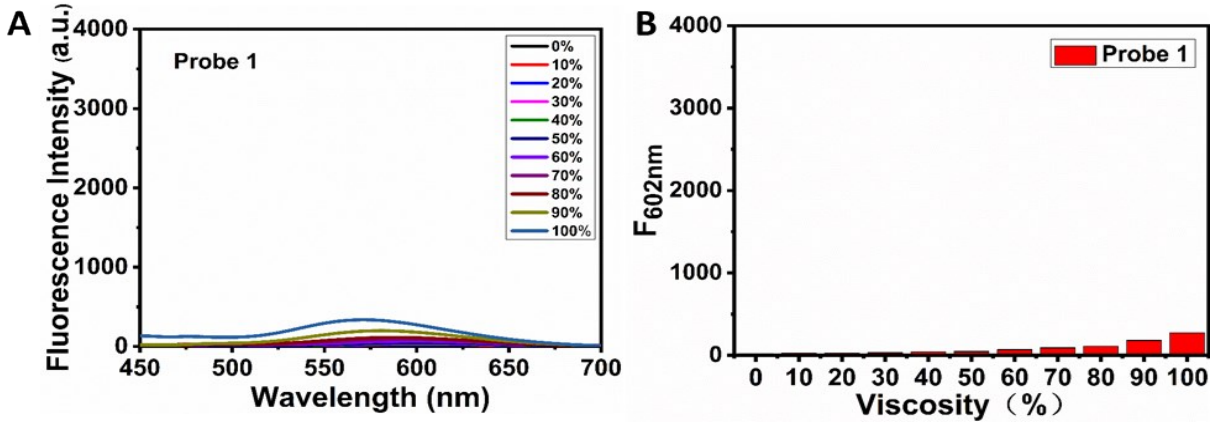

Figure S11. A) Fluorescence emission spectrum of probe **1** (4 μM) at different levels of glycerol at 25 ℃; **B)** Fluorescence emission spectrum of probe **1** (4 μM) at different levels of glycerol at 602 nm. glycerin/PBS (Ex = 420 nm, 5,5 slit/nm, 600 Volta)

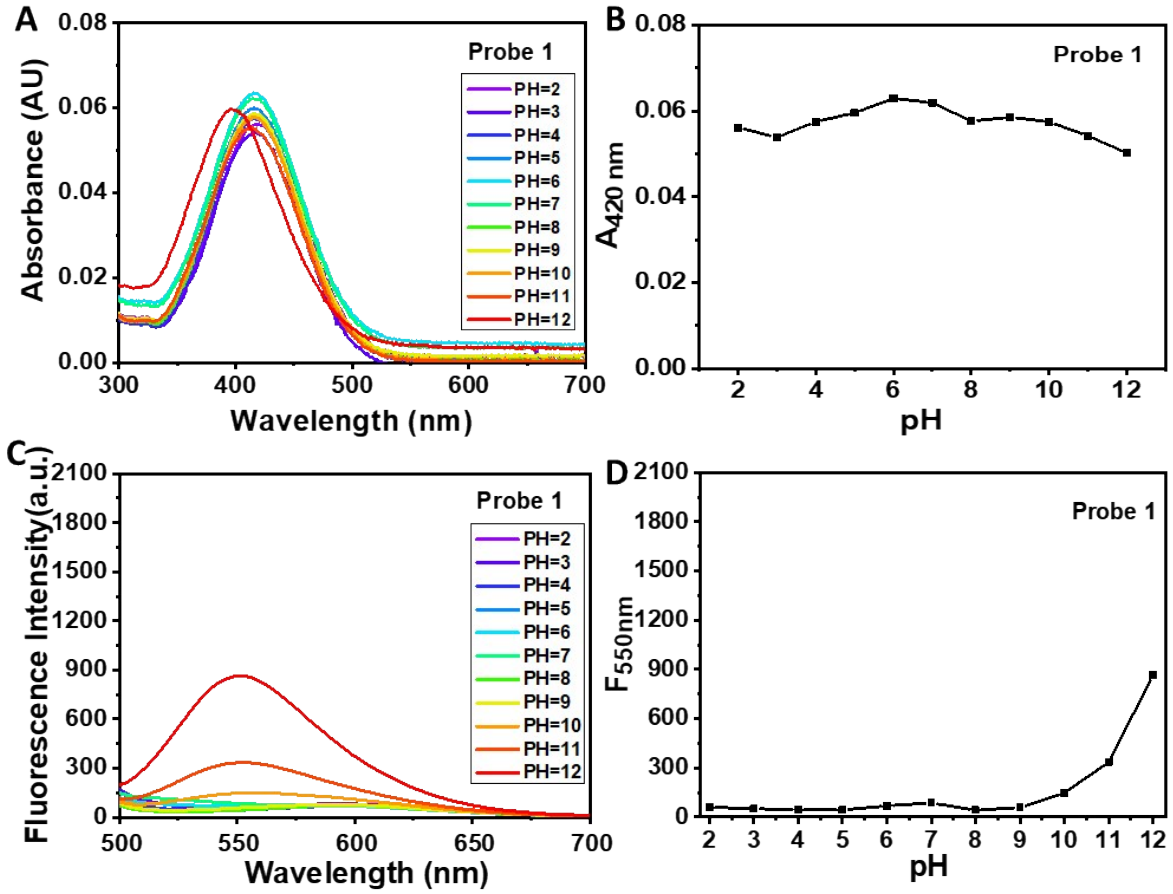

Figure S12. UV-Vis spectra **(A and B)** and fluorescence emission spectra **(C and D)** of probe **1** (4 μM) in different pH solutions (pH 7.4, 10 mM); (Ex = 435 nm, 5,5 slit/nm, 400 Volta)

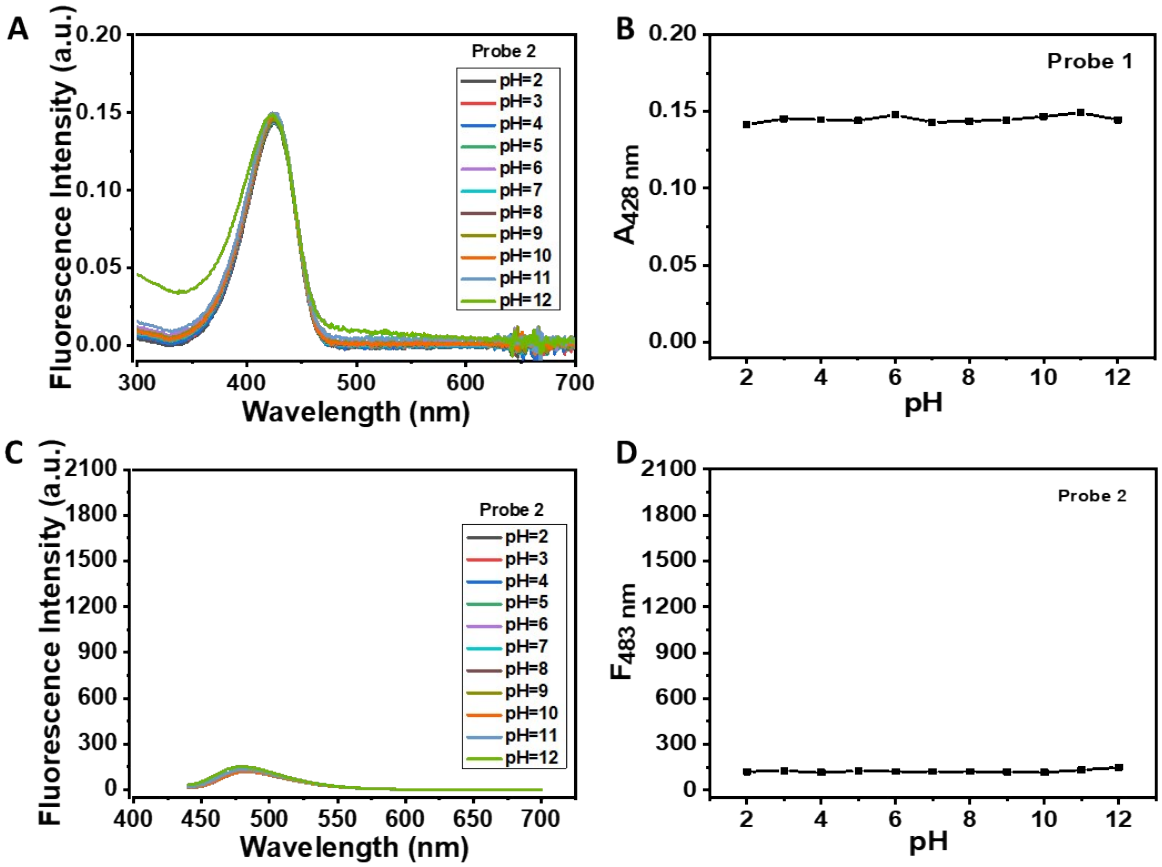

Figure S13 UV-Vis spectra **(A and B)** and fluorescence emission spectra **(C and D)** of probe **2** (4 μM) in different pH solutions (pH 7.4, 10 mM); (Ex = 435 nm, 5,5 slit/nm, 400 Volta)

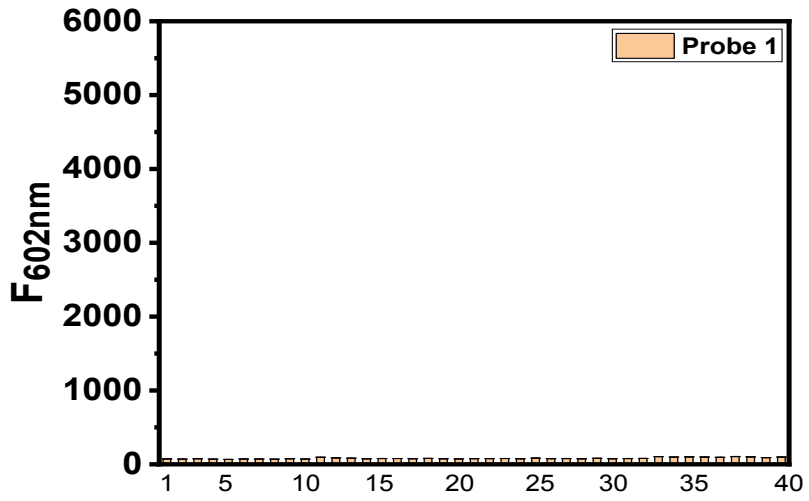

Figure S14. Histogram of fluorescence emission spectra of probe 1 (4 μM) at different Amino acid (PBS、Phe、Thr、Arg、Met、Pro、Lys、Leu、Iso、Ala、His、Asp、Val、Ser、K^{+}、Ca^{2+}、Na^{+}、Mg^{2+}、Al^{3+}、Zn^{2+}、Fe^{3+}、Cu^{2+}、Ni^{2+}、Cr^{3+}、Mn^{2+}、PBS、NaF、NaBr、KI、Na2PO4、Na2CO3、CYS、HCY、GSH、PBS、$NaNO_2$、NaClO、H_2O_2、BrO^{-}、$OH^{\cdot}$ (100 μM) for 5 min at 25 ℃.

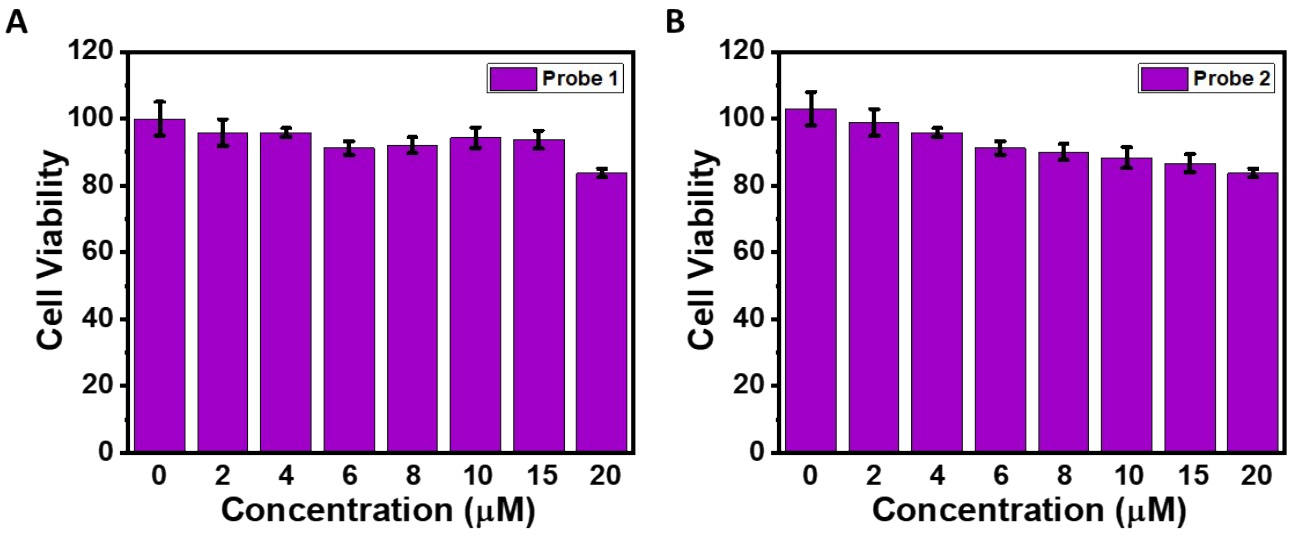

Figure S15. Cell viability of HepG-2 cells with different concentrations of probe **1** and probe **2** respectively. The experiments were carried out for 5 times.

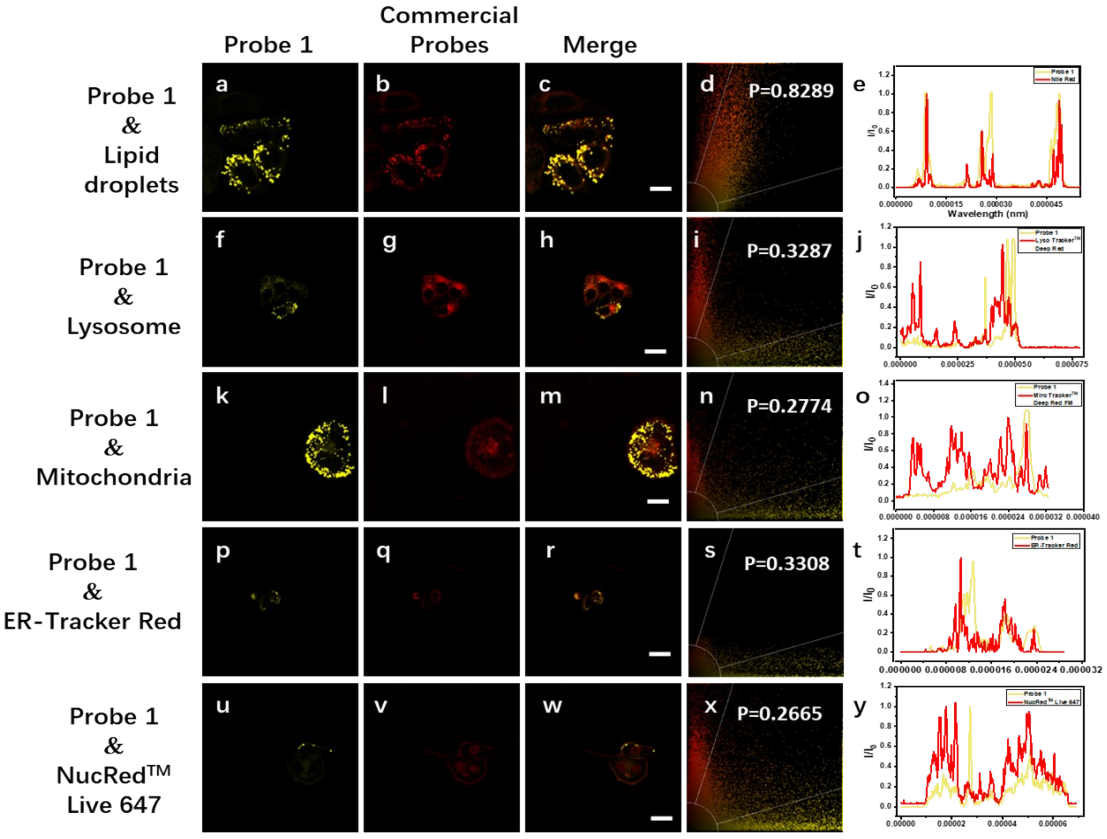

Figure S16. Confocal microscopic images of HepG-2 cells stained with probe **1** and commercial probes. Cells were stained with 4 μM probe **1** for 1 min, or Nile Red (500 nM), or 50 nM LysoTracker™ Deep

Red for 15 min, or 50 nM MitoTracker™ Deep Red FM for 15 min, or 500 nM ER-Tracker™ Red for 15 min, NucRed™ Live 647. Scale bar: 25 μm.

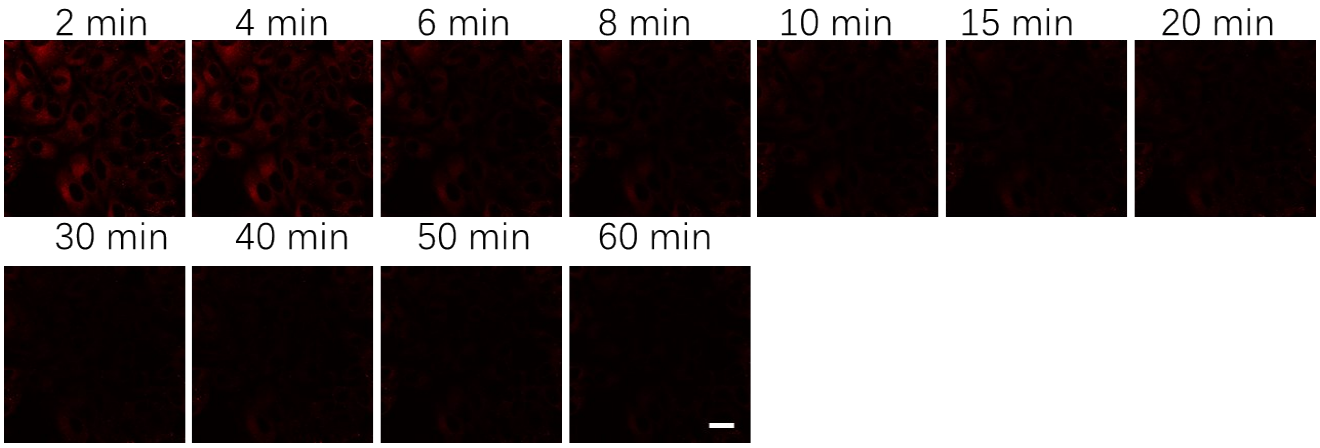

Figure S17. Nile Red incubated HepG-2 cells, fluorescence imaging under confocal microscope illumination for 60 minutes. Scale bar: 20 μm.

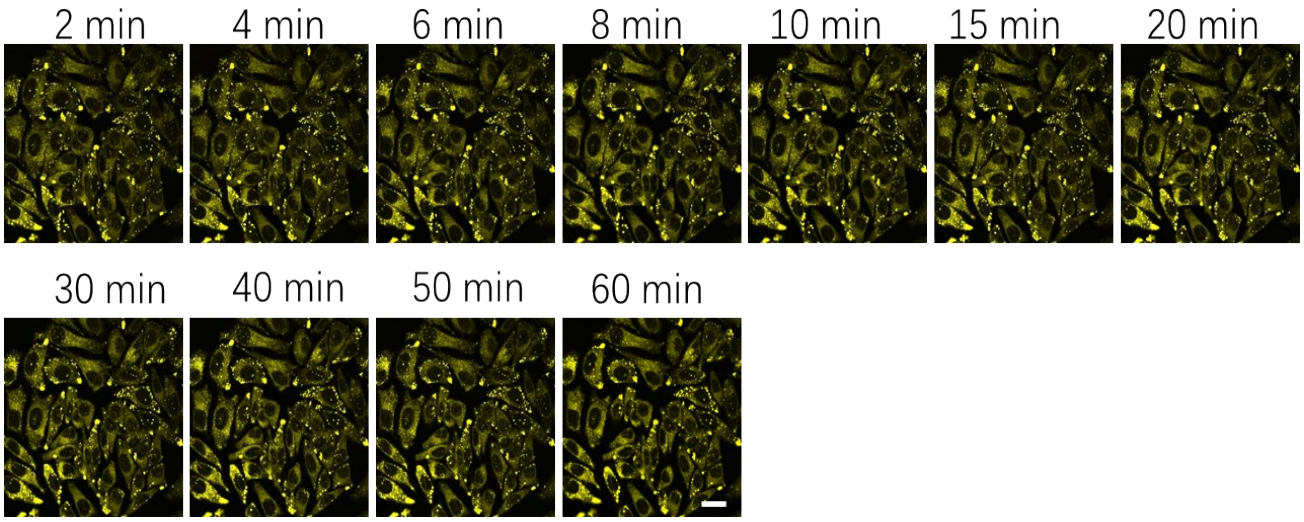

Figure S18. Probe **1** incubated HepG-2 cells, fluorescence imaging under confocal microscope illumination for 60 minutes. Scale bar: 20 μm.

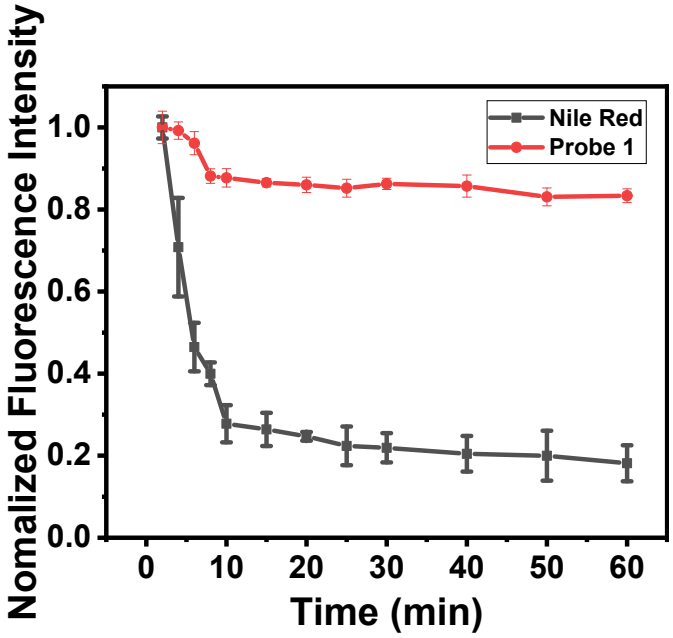

Figure S19. HepG-2 cells were incubated with Nile Red and probe **1**, respectively, and the trend graph of fluorescence changes under confocal microscope illumination for 60 minutes. Mean fluorescence intensities of 50 cells of each sample.