# Supporting Information

# A NIR-emissive probe with remarkable Stokes shift for CO-releasing molecule-3 detection in cells and in vivo

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**Experiment section:**

**Reagents.** 4-(dimethylamino)cinnamaldehyde, lepidine, methanesulfonic acid and 4-Nitrobenzyl bromide were obtained from Aladdin (Shanghai, China). Tricarbonylchloro(glycinato)ruthenium (III) (CORM-3) was obtained from Sigma-Aldrich (St. Louis, USA). Except for special labels, other chemical reagents were obtained from commercial vendor and employed without further purification. All aqueous solutions were prepared by using ultrapure water.

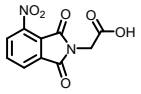
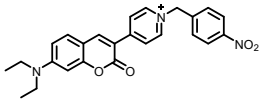
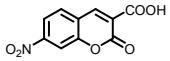
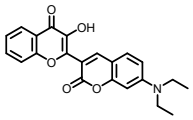
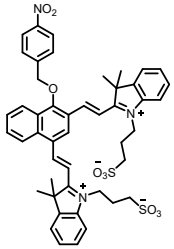
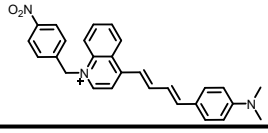
**Apparatus.** The pH of the buffer solution was controlled by a digital pH meter (FE20, MettlerToledo). High-resolution mass spectra (HRMS) were obtained from DIONEX UltiMate 3000 & Bruker Compact TOF mass spectrometer. NMR spectra were recorded on a Bruker AVANCE III HD spectrometer, using TMS as an internal standard; Absorption spectra were measured on a Shimadzu UV 2550 UV-Vis spectrophotometer. The fluorescence spectra were measured on a Shimadzu RF-6000 fluorophotometer. Fluorescence imaging experiments of HeLa cells and HepG2 cells were recorded by Zeiss LSM 880 laser scanning confocal microscope (Carl Zeiss, Germany). Mice were imaged by an IVIS Spectrum in vivo imaging system (PerkinElmer). Image processing was operated with Zen software and ImageJ software.

**Determination of the fluorescence quantum yield.** Fluorescence quantum yield ($\Phi_f$) was determined by using rhodamine B in ethanol solution (10 μM, Φ = 0.69) as the fluorescence standard. The quantum yield was calculated using the following equation.

$$\Phi_{F(X)} = \Phi_{F(S)} \, (A_S F_X / A_X F_S) \, (n_X / n_S)^2$$

Where $\Phi_F$ is the fluorescence quantum yield, $A$ is the absorbance at the excitation wavelength, $F$ is the area under the corrected emission curve, and $n$ is the refractive index of the solvent used. Subscripts $_S$ and $_X$ refer to the standard and to the unknown, respectively.

**Table S1.** Comparison of this probe with other fluorescent probes for CORM-3

| Probe | Chemical structure | $E_x/E_m$ (nm) | Stokes shift (nm) | Response time (min) | Application |
|---|---|---|---|---|---|
| Ref. 21 |  | 420/503 | 83 | 30 min | Cell, zebrafish and living mice |
| Ref. 22 |  | 420/500 | 80 | not mentioned | Cell and zebrafish |
| Ref. 23 |  | 400/450 | 50 | 34 min | Cell imaging |
| Ref. 24 |  | 420/475 | 55 | 20 min | Cell, zebrafish and living mice |
| Ref. 25 |  | 662/743 | 81 | 20 min | Cell and living mice |
| This work |  | 420/670 | 250 | 15 min | Cell and living animal |

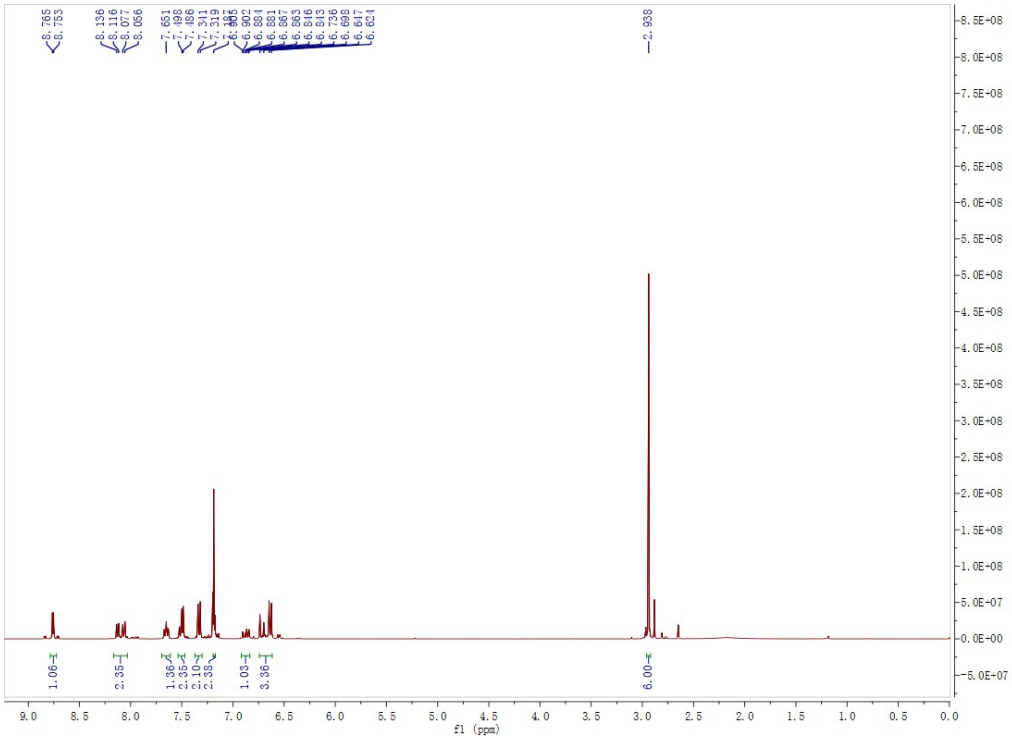



**Fig. S1** $^{1}$H NMR spectra of NIR-Q in $CDCl_3$.

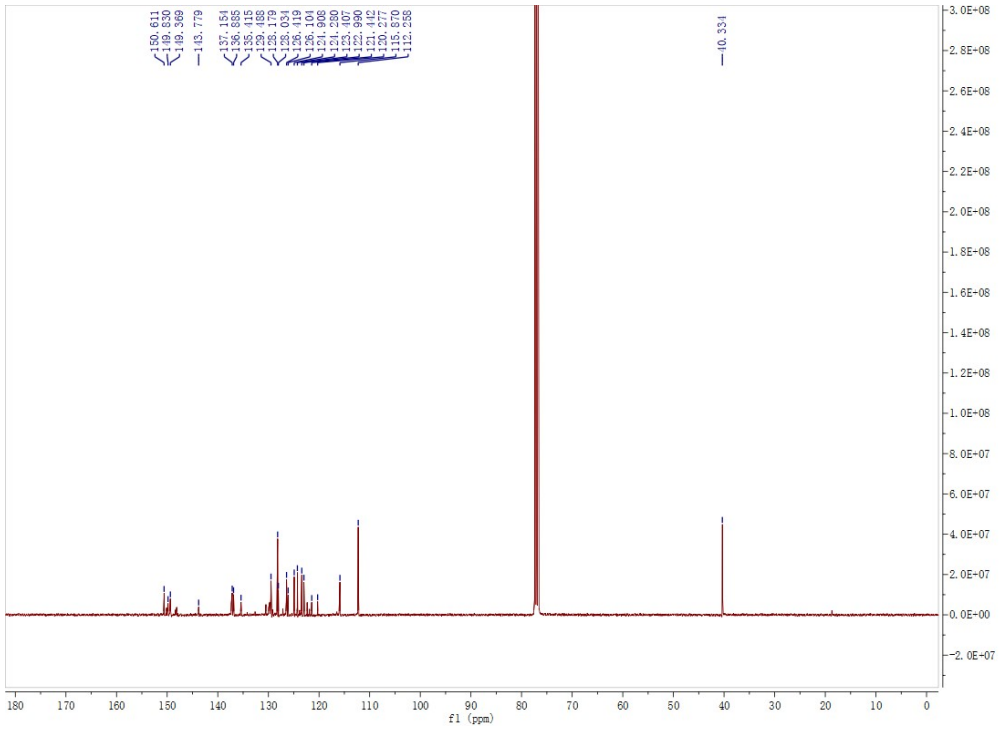



**Fig. S2** $^{13}$C NMR spectra of NIR-Q in $CDCl_3$.

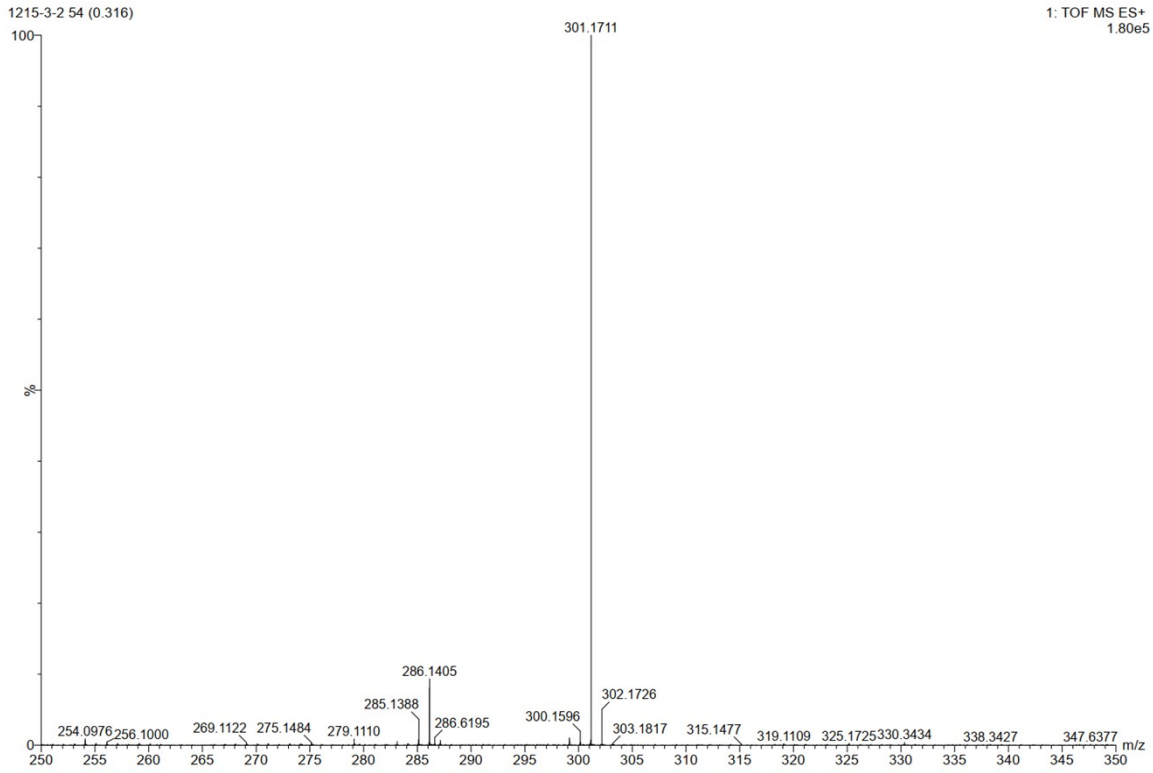



**Fig. S3** HRMS of NIR-Q.

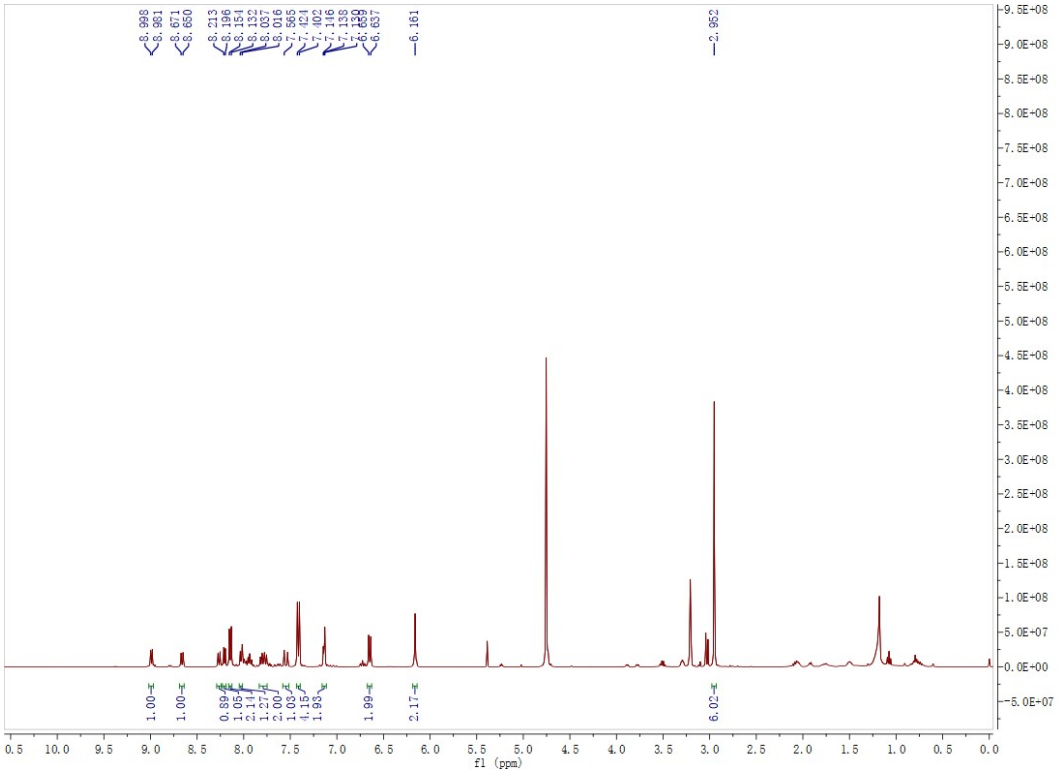



**Fig. S4** $^{1}$H NMR spectra of **NIR-CORM-3** in $CD_3OD$.

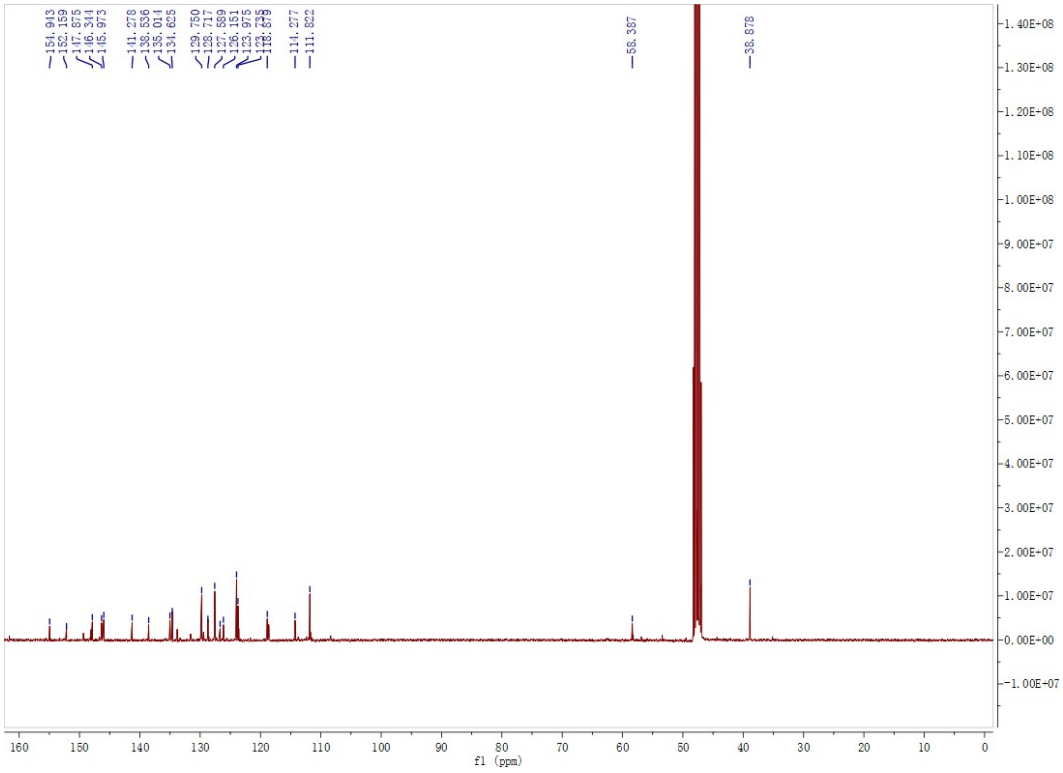



**Fig. S5** $^{13}$C NMR spectra of **NIR-CORM-3** in $CD_3OD$.

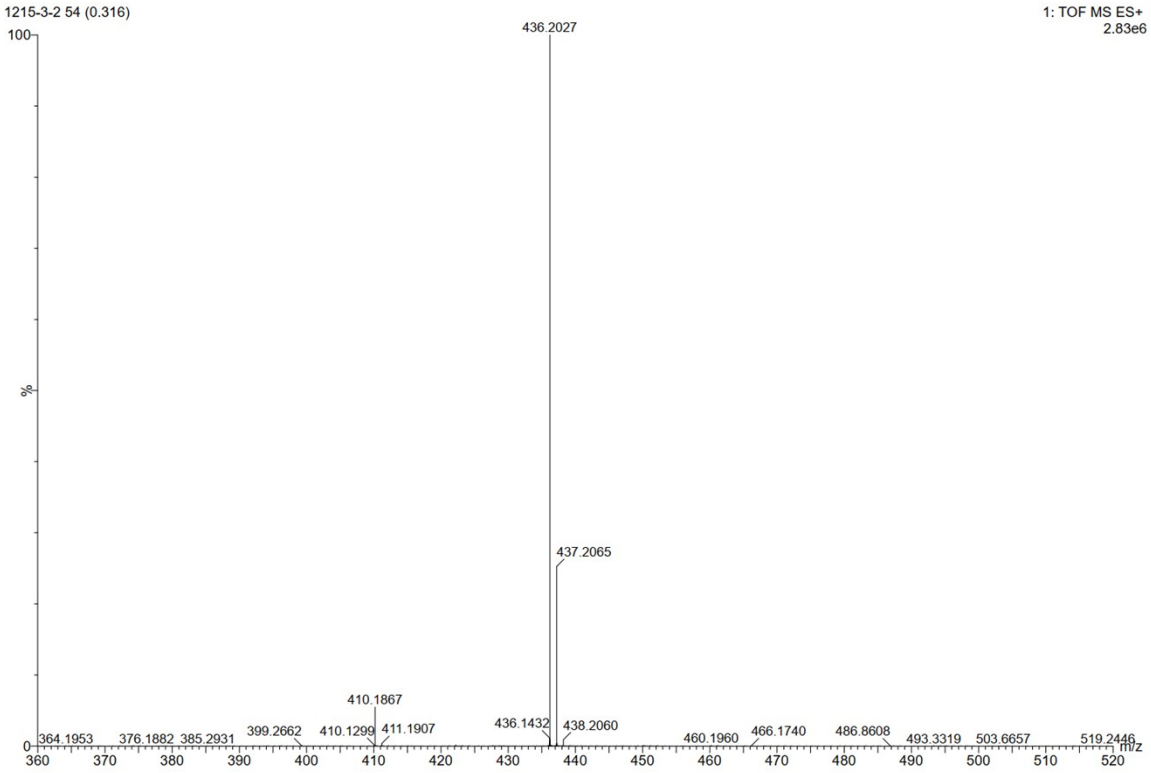



**Fig. S6** HRMS of **NIR-CORM-3**.

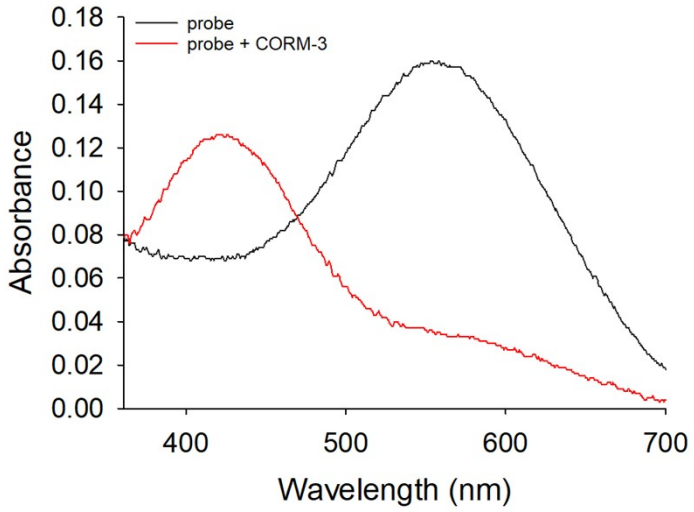



**Fig. S7** UV–vis absorption spectra of **NIR-CORM-3** at absence (black line) and presence of CORM-3 (red line).

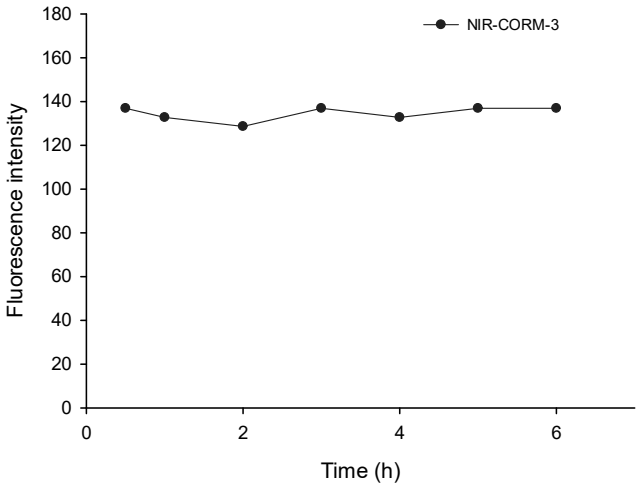



**Fig. S8** The photo-stability of probe **NIR-CORM-3**.

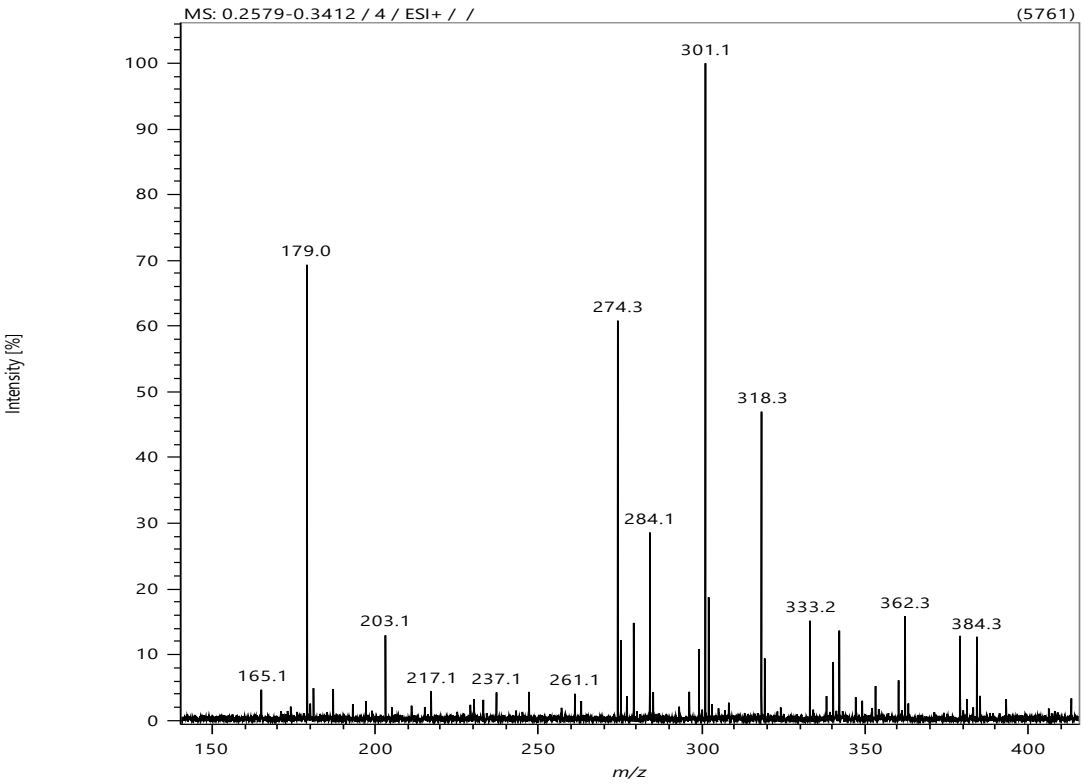



**Fig. S9** Mass spectra of **NIR-CORM-3** reacted with CORM-3 to formed NIR-Q (calcd. 301.1, found 301.1).

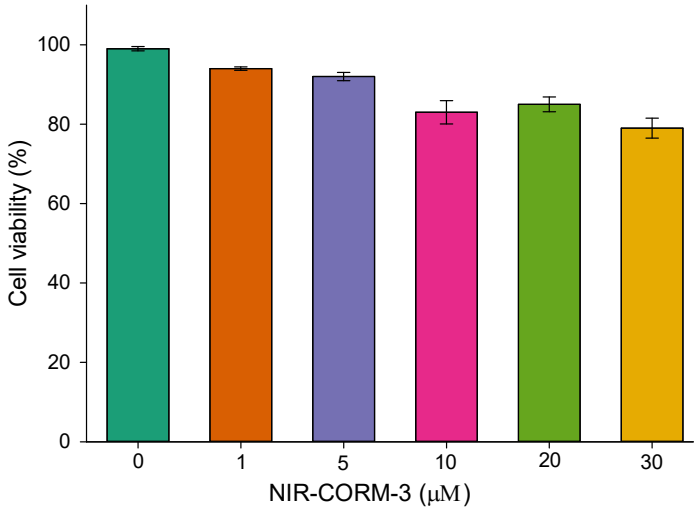



**Fig. S10** Cell viability of HeLa cells treated with different concentrations of **NIR-CORM-3** for 24 h. There is no significance difference of cell viability compared five **NIR-CORM-3** (1, 5, 10, 20, 30 μM) treated cell groups with control group.

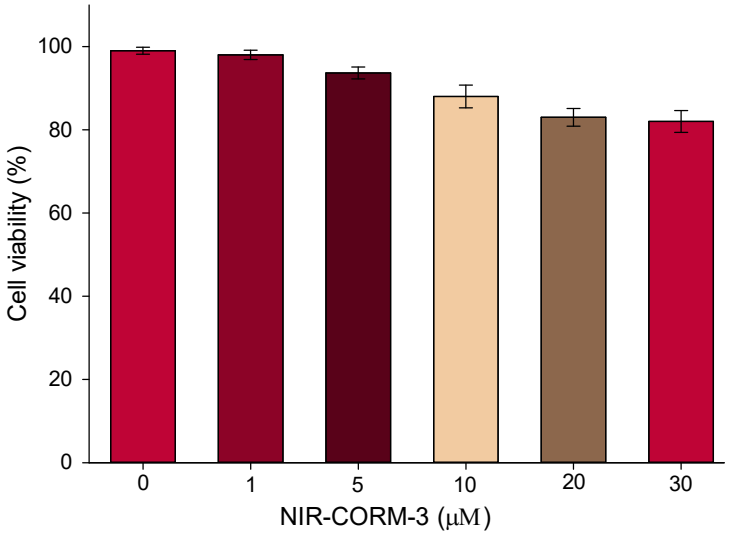



**Fig. S11** Cell viability of HepG2 cells treated with different concentrations of **NIR-CORM-3** for 24 h. There is no significance difference of cell viability compared five **NIR-CORM-3** (1, 5, 10, 20, 30 μM) treated cell groups with control group.

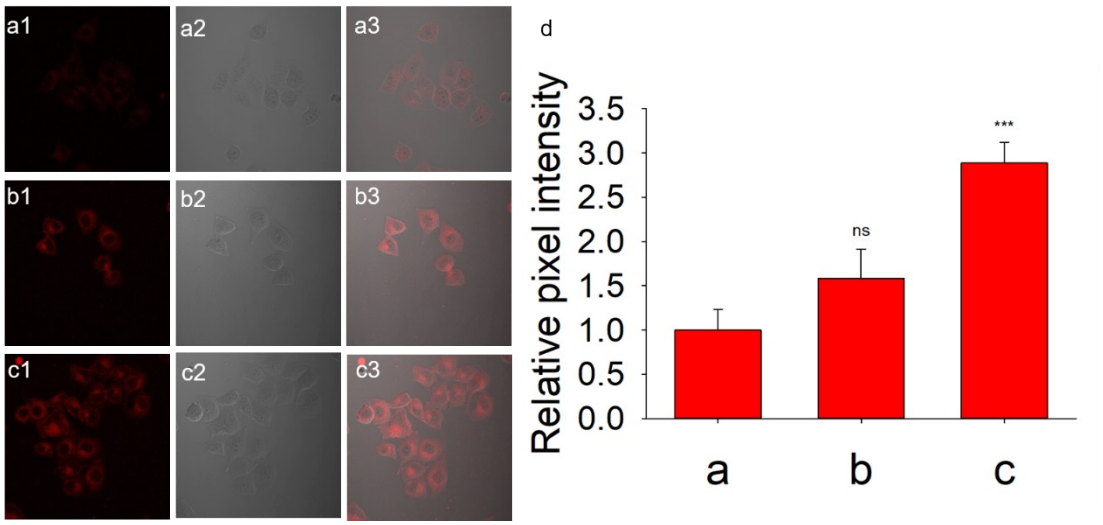



**Fig. S12** Confocal fluorescence images of CORM-3 in HepG2 cells. (a) HepG2 cells incubated with **NIR-CORM-3** (10 μM) for 30 min. (b, c) Hela cells incubated with various concentrations of CORM-3 (10 μM and 30 μM) for 30 min, and then incubated with CORM-3 (10 μM) for 30 min. (Excitation: 458 nm, emission: 600-700 nm, Scale bar: 20 μm). (d) Relative fluorescence intensity of (a)-(c). The results are presented as means ± SE with replicates n = 3. $^{***}p < 0.001$: 30 μM CORM-3 treated cells (c) versus control group (a).