Electronic Supplementary Information for:

A mitochondria-targeted near-infrared fluorescent probe for imaging viscosity in living cells and diabetic mice model

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1. Experimental.

Materials and Reagents. 1,1,2-Trimethyl-1H-benzo[e]indole, iodoethane and 4-(dimethylamino)cinnamaldehyde were purchased from Energy Chemical. Glycerol was purchased from Yantai Chemical Industry Research Institute (China). Nystatin was purchased from Aladdin Co., Ltd. Glucose was purchased from Tianjin Kermel chemical reagent Co. Ltd. Dimethyl sulfoxide (DMSO) was purchased from J&K Scientific Ltd (Beijing, China). Roswell Park Memorial Institute (RPMI) 1640 Medium, phosphate buffered saline (PBS), penicillin and streptomycin were bought from Solarbio Company. Fetal bovine serum was purchased from Zhejiang Tianhang biotechnology Co. Ltd. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Serva Electrophoresis GmbH (Germany). Streptozotocin (STZ) was purchased from Sigma-Aldrich Co. Ltd. Insulin was purchased from Sanofi Co. Ltd. All other chemical reagents are analytical grade. Ultrapure water used in experiment purified in a Milli-Q reference system (Millipore).

Characterization Methods. Materials and Reagents. ^{1}H NMR and ^{13}C NMR spectra were measured on Bruker Avance III 600 MHz spectrometer. High resolution electrospray ionization mass spectra (HR-ESI-MS) were measured on APEX IV FTMS instrument (Bruker, Daltonics). Fluorescence spectra were measured on Hitachi F-7000 spectrofluorimeter (Tokyo, Japan). Ultraviolet absorption spectra were measured on T10CS spectrophotometer (Beijing Puxi, China). The fluorescence images of cells were obtained by Leica TCS SP8 confocal laser scanning microscope (Germany) with a 63× oil objective lens. The flow cytometry analysis measured on BD Biosciences FACS AriaIII instrument. In vivo imaging was obtained by PerkinElmer IVIS Spectrum.

Synthesis of Probe NIR-V.

The mixture of 1,1,2-trimethyl-1H-benzo[e]indole (200 mg, 0.96 mmol) and iodoethane (224 mg,1.44 mmol) in acetonitrile was refluxed for 12 h at 90 °C under N_2 atmosphere. After the reaction was cooled down to the room temperature, organic layer was evaporated under vacuum. Then the crude product was separated by silica gel column chromatography using CH_2Cl_2/MeOH (v/v, 25:1), affording compound 1 (80 mg, yield: 40%).

Probe **NIR-V**: The mixture of compound 1 (80 mg, 0.34 mmol), 4-(dimethylamino) cinnamaldehyde (119 mg, 0.68 mmol) and sodium acetate (56 mg, 0.68 mmol) in acetic anhydride was stirred for 8 h at 50°C under N_2 atmosphere. After completion of the reaction, the mixture was poured into water (30 mL) and extracted with dichloromethane (4 × 30 mL), Then the organic phase was separated, dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was separated by column chromatography using CH_2Cl_2/ MeOH (v/v, 25:1) to obtain probe as blue solid (45 mg, yield: 56%). The ^{1}H NMR and ^{13}C NMR spectra of probe are shown in Figures S1 and S2, respectively. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 8.17 (s, J = 12.6 Hz 1H), 8.03 (q, J = 16.8 Hz 2H), 7.81 (t, J = 19.5 Hz 1H), 7.68 (t, J = 12.6 Hz 3H), 7.61 (t, J = 12.6 Hz 2H), 7.53 (d, J = 9 Hz 1H), 6.70 (d, J = 13.2 Hz 2H), 4.75 (q, J = 15.9 Hz 2H), 3.10 (s, 6H), 2.03 (s, 6H), 1.64 (s, 3H), 1.61 (d, J = 10.4 Hz 2H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K): δ 179.2, 156.00, 153.18, 131.38, 126.57, 132.99, 128.29, 123.87, 138.17, 132.66, 127.74, 122.50, 136.90, 130.23, 124.82, 111.48, 110.67, 112.23, 52.64, 42.48, 40.20,27.27,13.96. HR-ESI-MS (m/z): calcd for $C_{28}H_{31}N_2^+$, 395.24872 $[M+H]^+$; found 395.24631.

General Procedure for Spectroscopic Detection of Viscosity. The solvents with different viscosity were obtained by mixing water-glycerol systems in different proportions. The stock solution of probe was 1 mM dissolved in DMSO. The final test solution contained probe stock solution (10 μL) and water-glycerol systems (1 mL). Then the mixture was shook and ultrasoniced for 10 min to remove bubbles. The fluorescence intensity was measured at λ_{ex} = 580 nm and the emission wavelength was 600 nm and 700 nm. The voltage was 700V and the emission slit widths was 5 nm.

Cytotoxicity Assay. In order to estimate the cytotoxicity of **NIR-V** on HepG2 cells, we did a routine MTT test, as described previously (Chem. Sci., 2015, 6, 4884).

FL Imaging of Viscosity in HepG2 Cells. HepG2 cells were incubated in RPMI (Roswell Park Memorial Institute) 1640 Medium supple-mented with 10% fetal bovine serum (FBS), and 1% (v/v) penicillin-streptomycin at 37°C under a humidified atmosphere containing 5% CO_2. Then the cells were pretreated with nystatin (80 μM, 160 μM) for 1 h or glucose (40 mM, 80 mM) for 24 h. Then incubated with probe (10 μM) for 30 min at 37°C respectively. Finally, the cells were washed three times with PBS and fluorescence imaging experiments was recorded by Laser Confocal Microscope. The fluorescence excitation was 561 nm and emission was collected at 590-630 nm and 680-750 nm.

Flow cytometry Analysis. The cells were cultured at 2.0 × 10^5 cell in 6-well plates and allowed to adhere for 48 hours. And then treated with nystatin (80 μM, 160 μM) for 1 h or glucose (40 mM, 80 mM) for 24 h. Then incubated with probe (10 μM) for 30 min at 37 °C. Cells were washed three times before harvest. Finally, Cells were resuspended in 500 μL PBS

and analyzed by flow cytometry (BD FACSCalibur). The excitation wavelength was 561 nm and the collected wavelengths was in the ranges of 655-735 nm.

Establishment of type 1 diabetes model (T1DM). All the in vivo experimental procedures were approved by the Animal Ethical Experimentation Committee of Weifang Medical University (2020SDL120) and were performed strictly in accordance with the guidance of the National Act on the use of experimental animals (China). A total of 21 Wistar mice were purchased from experimental animal center of Shandong university medical college. After adaptive feeding for 7 days, 7 mice were randomly selected as the control group, the other mice were fasted for 12 hours and intraperitoneal injected 100 mg/kg streptozotocin (STZ) to establish the type 1 diabetes model (T1DM). Tail venous blood was collected to measure blood glucose 3 days later, and the random blood glucose value of > 16.7 mmo1/L was determined to be a successful T1DM model. Then the T1DM mice were randomly divided into the diabetes group and the insulin group. The insulin group was subcutaneous injection (5 U/kg/d) insulin for 7 days, the blank group and the diabetes group was intraperitoneal injection the same volume saline. Three different mice groups were standby application.

Fluorescence imaging in vivo and biodistribution analysis. The probe (100 μL, 500 μM) was intraperitoneal injected into mouse. After 30 minutes, the mice were anesthetized by intraperitoneal injection of 35 mg/kg 10% chloral hydrate. Then the heart, liver, spleen, lung, kidney and pancreas were removed from the mice. After cleaning with PBS (pH 7.4), the organs distribution imaging analysis was examined. All fluorescence images were taken by IVIS Spectrum in vivo fluorescence imaging system. The excitation was 570 nm and the emission was 700 nm.

H&E staining. The tissues were fixed with 10% paraformaldehyde, embedded in paraffin and sliced. Histological examination was performed with hematoxylin and eosin staining (H&E).

2. Synthesis of probe NIR-V

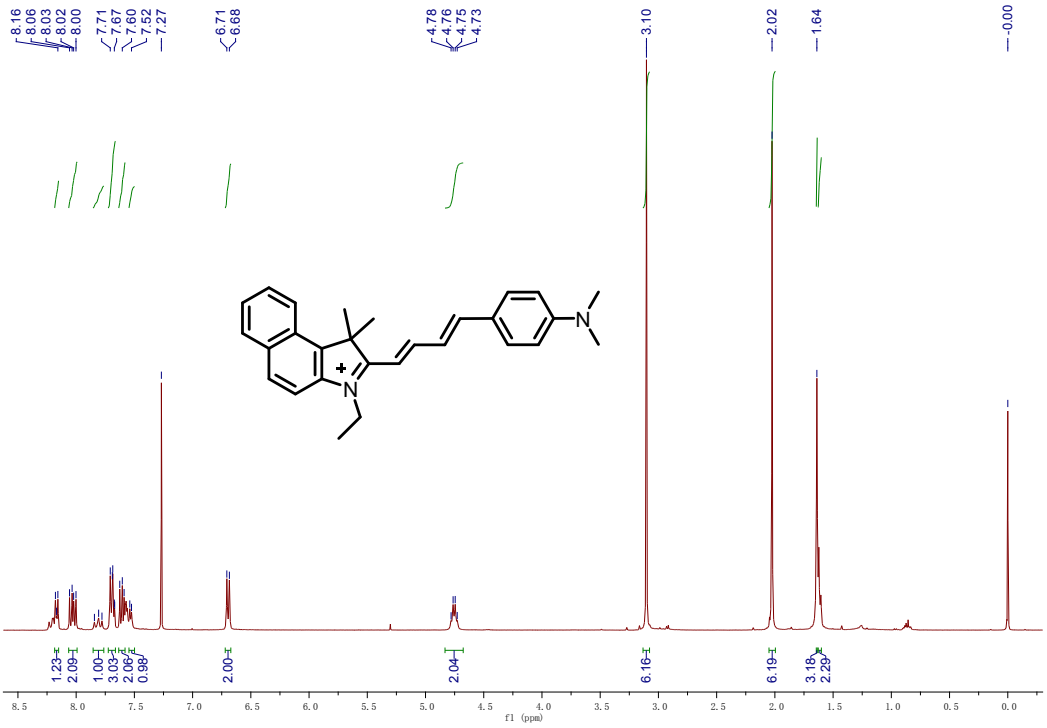


Fig. S1. ^{1}H NMR spectrum of **NIR-V** (600 MHz, $CDCl_3$, 298 K).

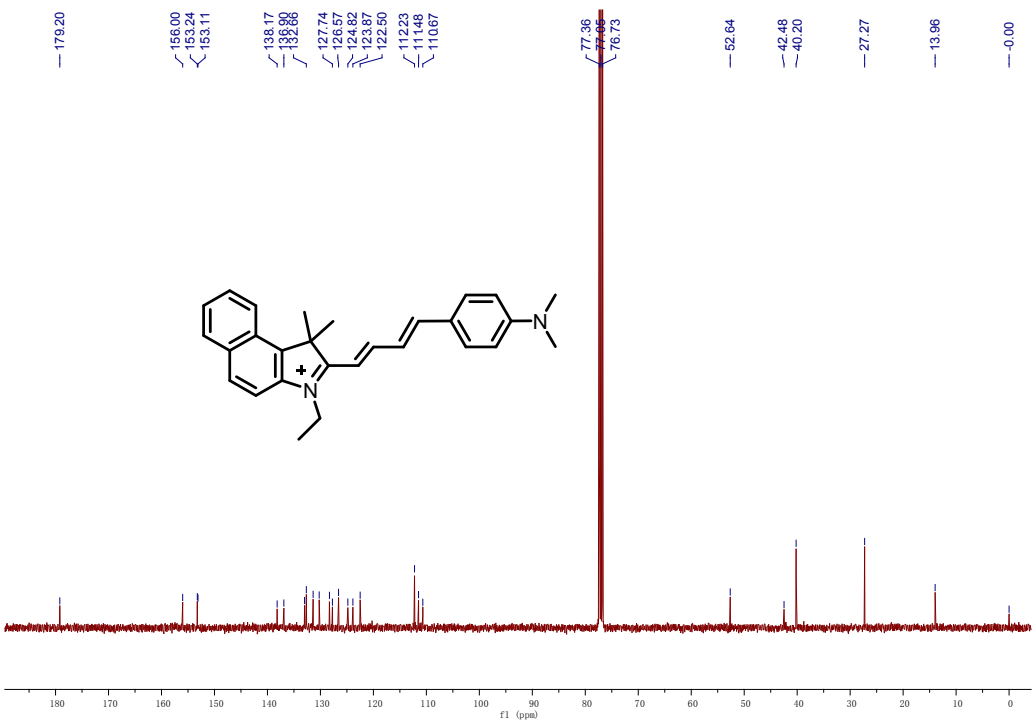


Fig. S2. ^{13}C NMR spectrum of **NIR-V** (150 MHz, $CDCl_3$, 298 K).

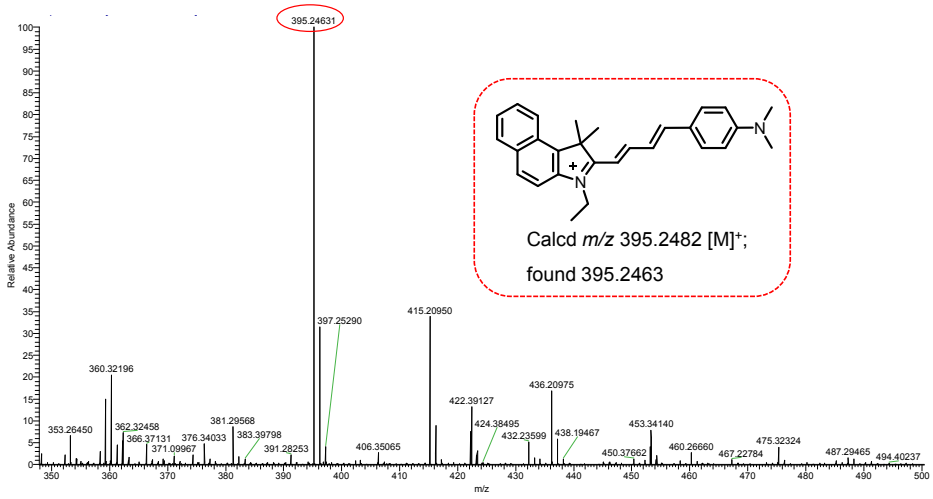


Fig. S3. HRMS result of compound **NIR-V**.

3. Spectral parameters

Table S1. The spectral parameters of **NIR-V** in different glycerol/water mixtures.

Glycerol fraction (%)	0	10	20	30	40	50	60	70	80	90	100
Quantum yields (%)	0.96	1.34	1.70	1.82	2.35	2.81	3.79	6.16	9.01	10.92	12.77
Molar absorption coefficients ($\times 10^4$ M^{-1} cm^{-1})	3.04	6.99	8.08	9.94	10.82	11.6	11.4	11.4	10.9	11.7	10.1

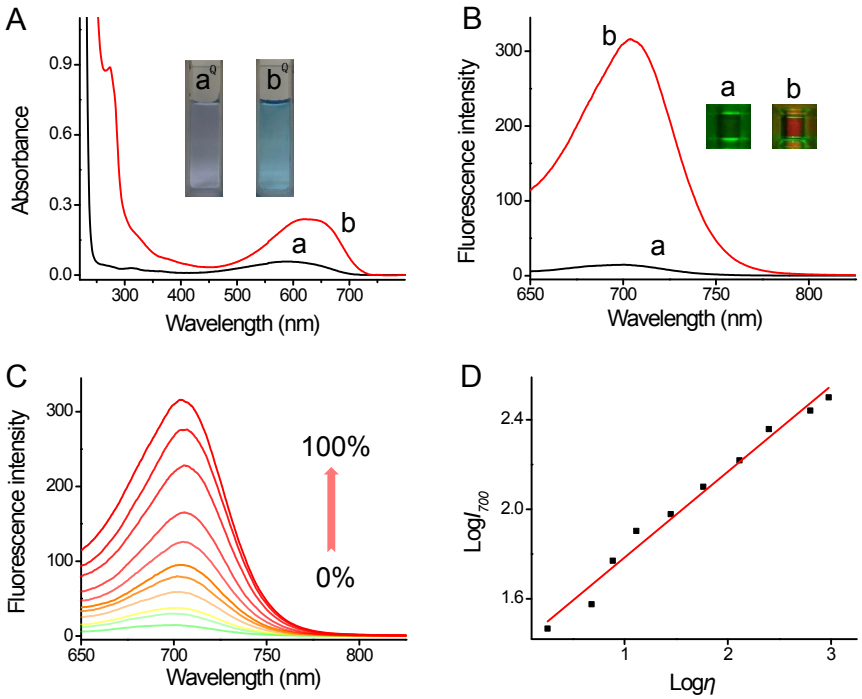


Fig. S4. (A) Absorption spectra of 10 μM **NIR-V** in water (a) and glycerol (b). The color change under the daylight of **NIR-V** in water and glycerol is displayed in the inset. (B) Fluorescence spectra of 10 μM **NIR-V** in water (a) and glycerol (b). The inset shows the corresponding color change of fluorescence under the ultraviolet light of 365 nm. (C) Fluorescence spectra of **NIR-V** (10 μM) with the variation of solution viscosity (the glycerol fractions of water-glycerol system are 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% respectively from the bottom to top). (D) Linear relationship of log I_{700} and log η. $\lambda_{ex/em}$ = 580/700 nm.

4. Optimization of experimental conditions

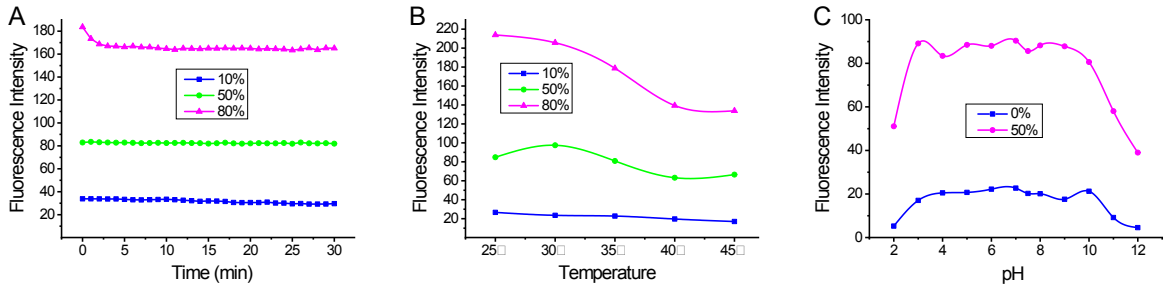


Fig. S5. (A) Plots of fluorescence intensity of **NIR-V** (10 μM) vs. the reaction time in the presence of varied glycerol volumetric ratios. The effects of temperature (B) and pH (C) on the fluorescence intensity of probe **NIR-V** (10 μM) in presence of varied glycerol volumetric ratios.

$\lambda_{ex/em}$ = 580/700 nm.

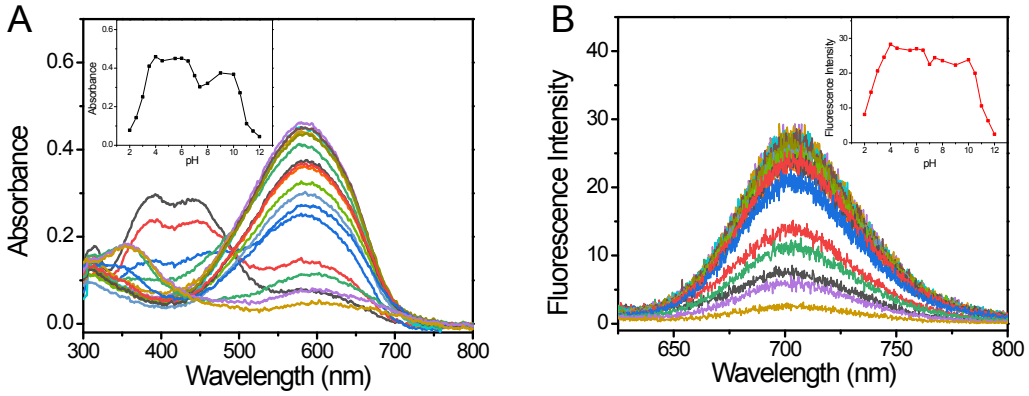


Fig. S6. UV-vis absorption and fluorescence spectra of **NIR-V** (10.0 μM) in aqueous solution of varying pH.

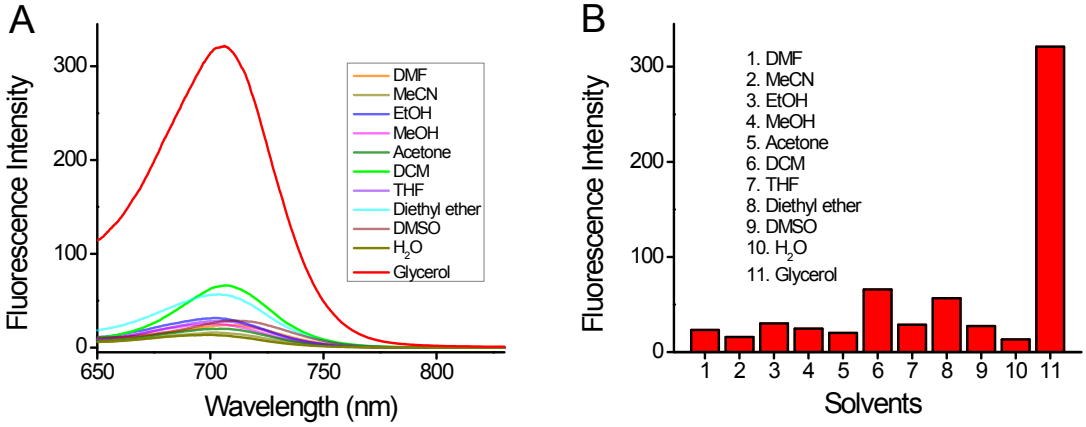


Fig. S7. (A) Fluorescence intensity spectra of 10 μM **NIR-V** in various solvents with different polarities. (B) Bar diagram of panel A. $\lambda_{ex/em}$ = 580/700 nm

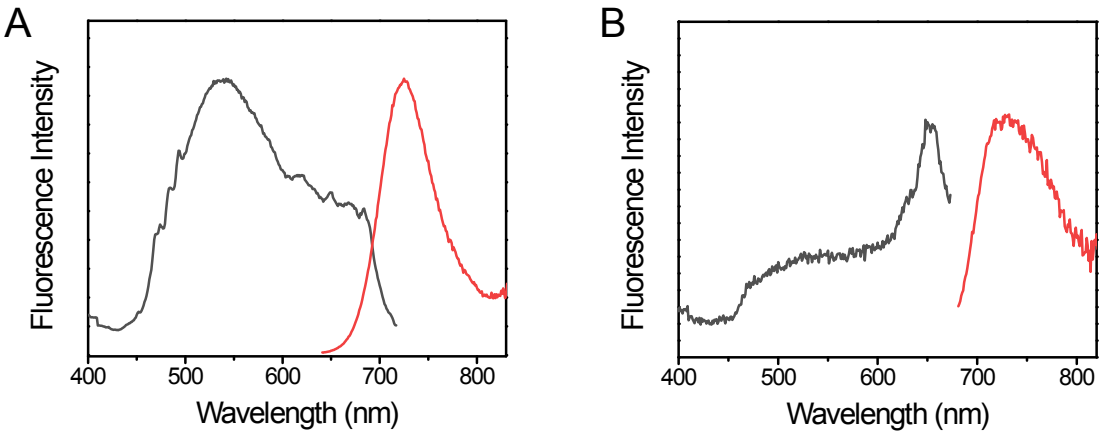


Fig. S8. Fuorescence spectral measurements of **NIR-V** in 2-methyltetrahydrofuran (2-MeTHF)

at room temperature at 298 K (A) and a glassy matrix of 2-MeTHF at 77 K (B).

5. Cytotoxicity assay

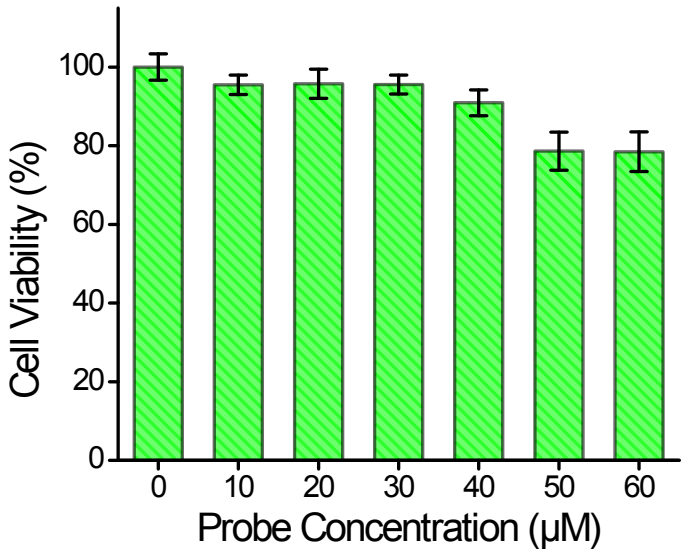
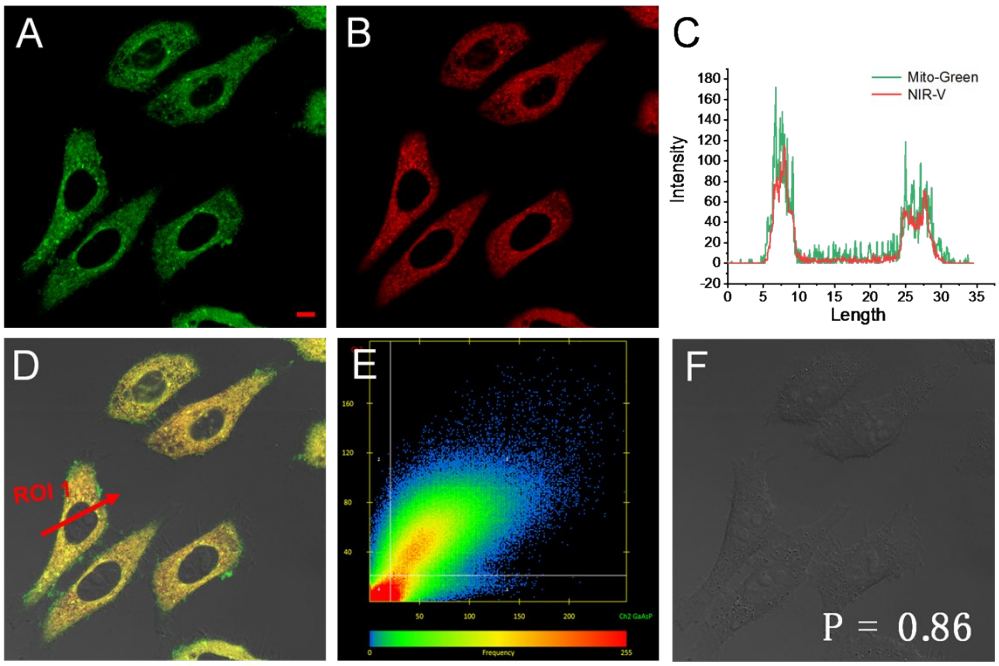
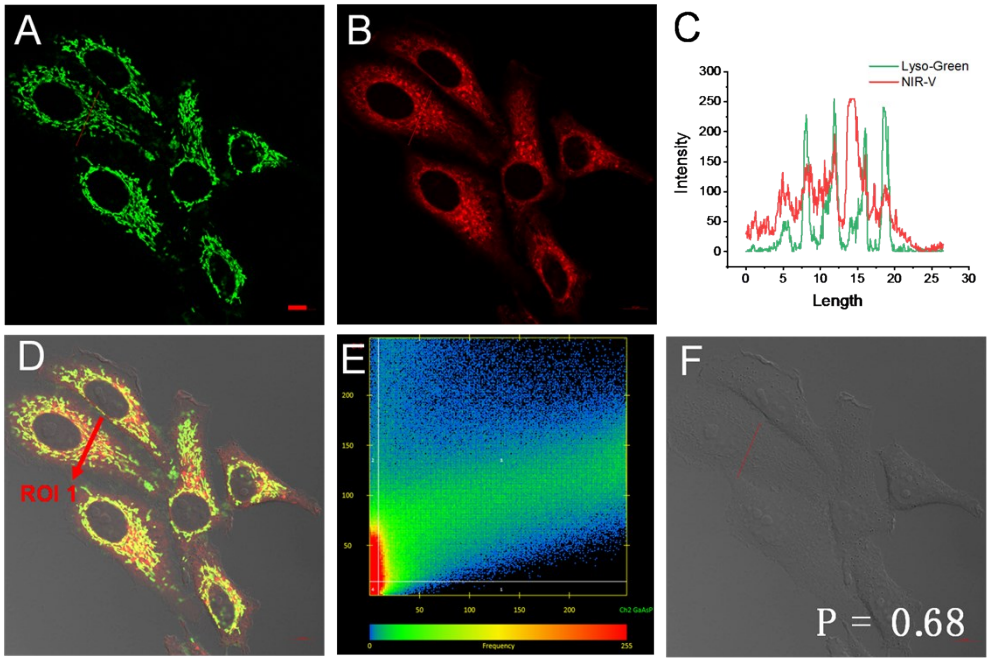


Fig. S9. Effects of **NIR-V** with varied concentrations (0-60 μM) on the viability of HepG2 cells. The viability of the cells without **NIR-V** is defined as 100%. The results are the mean ± standard deviation of five separate measurements.

6. Subcellular localization experiment



Colocalization of **NIR-V** and MitoTracker® Green in HepG2 cells. Cells were co-stained with **NIR-V** (10 μM) and MitoTracker® Green (200 nM) at 37 °C for 30 min. (A) Fluorescence image from MitoTracker® Green channel (λ_{ex} = 488, λ_{em} = 500–540 nm). (B) Fluorescence image from **NIR-V** channel (λ_{ex} = 633 nm, λ_{em} = 650-750 nm). (C) Intensity profile of the linear ROI 1 across the cell (red line in image D). (D) Merged image of images A and B. (E) Intensity correlation plot of NIR-V and MitoTracker® Green. (F) Corresponding DIC image. Scale bar: 10 μm.



R-V and LysoTracker® Green in HepG2 cells. Cells were co-stained with **NIR-V** (10 μM) and

LysoTracker® Green (100 nM) at 37 °C for 30 min. (A) Fluorescence image from LysoTracker® Green channel (λ_{ex} = 488, λ_{em} = 500–540 nm). (B) Fluorescence image from NIR-V channel (λ_{ex} = 633 nm, λ_{em} = 650-750 nm). (C) Intensity profile of the linear ROI 1 across the cell (red line in image D). (D) Merged image of images A and B. (E) Intensity correlation plot of NIR-V and LysoTracker® Green. (F) Corresponding DIC image. Scale bar: 10 μm.