## **Supporting Information**

Figure S1. Structure of Nile Red and C-Py.

Scheme. S1. Synthetic route for C-Py.

Figure S2. HR-Mass spectrum of C-Py.

Figure S3. <sup>1</sup>H-NMR spectrum of C-Py.

Figure S4. <sup>13</sup>C-NMR spectrum of C-Py.

Figure S5. Bright field images of untreated HeLa cells.

**Figure S6.** Reconstructed SIM images (A, B and C) stained with **C-Py**, were imaged identically A was acquired with 1.516 (*n*@589.3 nm) immersion oil and B, C with 1.524,1.510 (*n*@589.3 nm) immersion oil.

**Figure S7.** Reconstructed SIM images (A, B and C) stained with **C-Py**, were imaged identically A was acquired with 1.516 (*n*@589.3 nm) immersion oil and B, C with 1.524,1.510 (*n*@589.3 nm) immersion oil.

Figure S8. C-Py tracking LDs in HepG2 cells and A549 cells under SIM.

Figure S9. Cytotoxicity of the C-Py at concentrations of 0.1-50 µmol/L in HeLa cells.

Figure S10. Permeability of the C-Py at concentrations of 0.1-50 µmol/L in HeLa cells.

Figure S11. SIM images of HeLa cells of C-Py under different conditions.

Figure S12. Normalized fluorescence intensity of C-Py in HeLa cells under different stimulations.

Figure. S13. Optical resolution in z of 3D-SIM, Epi-illumination fluorescence microscopy and confocal mroscopy.

Figure. S14. C-Py nanoscopic tracking of the nucleus–LDs interaction in HeLa cells.

Figure. S15. The formation of contact sites between LDs (C-Py) and mitochondria (MTDR).

Figure S16. C-Py and Dil tracking LDs and membranes in HeLa cells under SIM.





Figure S1. Structure of Nile Red and C-Py.



Scheme. S1. Synthetic route for C-Py.







Figure. S4. <sup>13</sup>C-NMR spectrum of C-Py.



**Figure S5.** Bright field images of untreated HeLa cells. (A) Bright field image of C-Py under Confocal. (B) Bright field image of C-Py under SIM.



**Figure S6.** Reconstructed SIM images (A, B and C) stained with **C-Py**, were imaged identically A was acquired with 1.516 (*n*@589.3 nm) immersion oil and B, C with 1.524,1.510 (*n*@589.3 nm) immersion oil.



**Figure S7.** Reconstructed SIM images (A, B and C) stained with **C-Py**, were imaged identically A was acquired with 1.516 (*n*@589.3 nm) immersion oil and B, C with 1.524,1.510 (*n*@589.3 nm) immersion oil.



Figure. S8. C-Py tracking LDs in HepG2 cells and A549 cells under SIM.



Figure. S9. Cytotoxicity of the C-Py at concentrations of 0.1-50 µmol/L in HeLa cells.



Figure. S10. Permeability of the C-Py at concentrations of 0.1-50 µmol/L in HeLa cells.



**Figure. S11.** SIM images of HeLa cells of **C-Py** under different conditions. (A) HeLa cells were incubated with **C-Py** for 2h at 37 °C; (B) HeLa cells were incubated with **C-Py** for 2h at 4 °C. (C) HeLa cells were incubated with the metabolic inhibitors (MI, including 50 mM oligomycin and 5  $\mu$ M 2-deoxy-D-glucose) at 37 °C for 1h and incubated with **C-Py** at 37 °C for 2h. (D) HeLa cells were incubated with 50 mM NH<sub>4</sub>Cl at 37 °C for 1 h and with **C-Py** at 37 °C for 2h.



**Figure. S12.** Normalized fluorescence intensity of **C-Py** in HeLa cells under different stimulations. Data are presented as mean  $\pm$  SEM (n = 20, \*\*\*\*P < 0.0001).



**Figure. S13.** Optical resolution in Z of 3D-SIM, Epi-illumination fluorescence microscopy and confocal mroscopy.

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**Figure S14. C-Py** nanoscopic tracking of the nucleus–LDs interaction in HeLa cells. SIM images at 8 µm at the Z axis of the LDs (**C-Py**) and nucleus (Hoechst 33342). A. z1-39 layers of SIM image, B. 3D SIM images from different angles. The solid white frame represents the LDs in the nucleus.

5 µm

1 µm



Figure. S15. The continuous dynamic image of LDs (C-Py) and mitochondria (MTDR) under SIM



**Figure. S16.** Using **C-Py** and Dil track LD and membranes, respectively, in HeLa cells under SIM. The white dotted line refers to the cell membrane. Scale bar,  $1\mu m$ .