Supporting Information

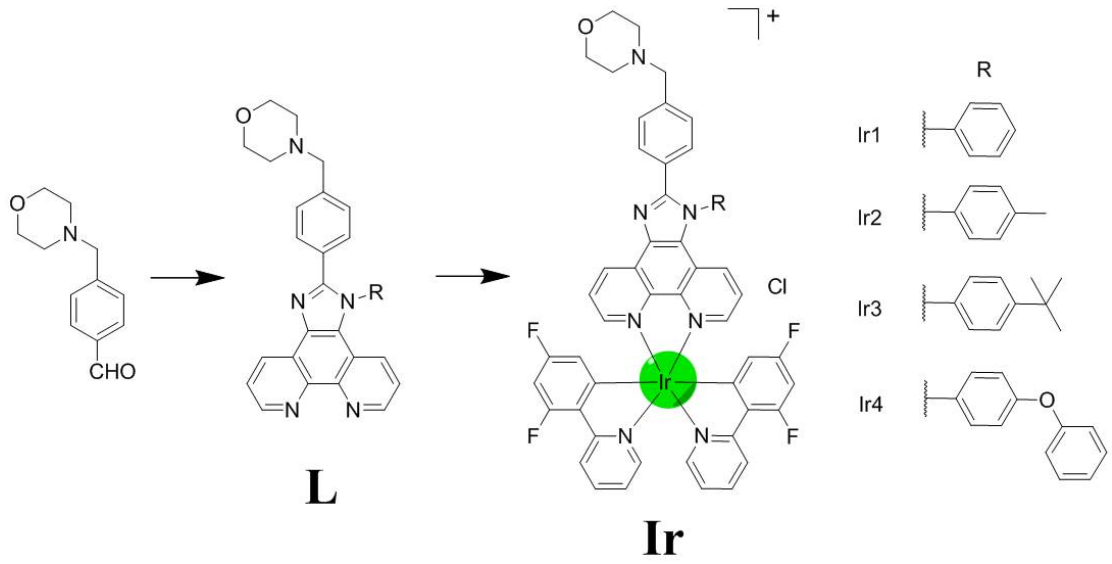
Mitochondria-specific imaging and tracking in living cells with two-photon phosphorescent iridium(III) complexes

Kangqiang Qiu, Huaiyi Huang, Bingyang Liu, Yukang Liu, Pingyu Zhang, Yu Chen, Liangnian Ji, and Hui, Chao*

MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P. R. China

Tel: +86 20 84110613; Fax: +86 20 84112245; Email: ceschh@mail.sysu.edu.cn

Table of Contents



Scheme S1 Synthetic route to **Ir1-Ir4**. (i) CH_3COOH, reflux, 12 h, 70-75%; (ii) CH_3OH-CH_2Cl_2 (1:1, v/v), reflux, 12 h, 50-55%.

Table S1. Photophysical data for Ir1-Ir4 in CH_3CN at 298 K

Complexes	λ_{ab} [a]	ε [b]	λ_{em} [c]	φ [d]	$\tau/\mu S$ [e]	δ/GM [f]
Ir1	390	6.65	519	0.128	0.103	19.1
Ir2	390	6.66	525	0.125	0.109	18.8
Ir3	390	6.68	521	0.129	0.131	19.1
Ir4	390	6.63	522	0.144	0.115	18.4

[a] λ_{ab} maximum values of the one-photon absorption in nm. [b] Extinction coefficient in 1×10^3 $M^{-1}\cdot cm^{-1}$. [c] λ_{em} maximum values of the one-photon emission spectra in nm. [d] Phosphorescent quantum yield. [e] Life time. [f] Two-photon absorption cross section at 760 nm, measured in methanol.

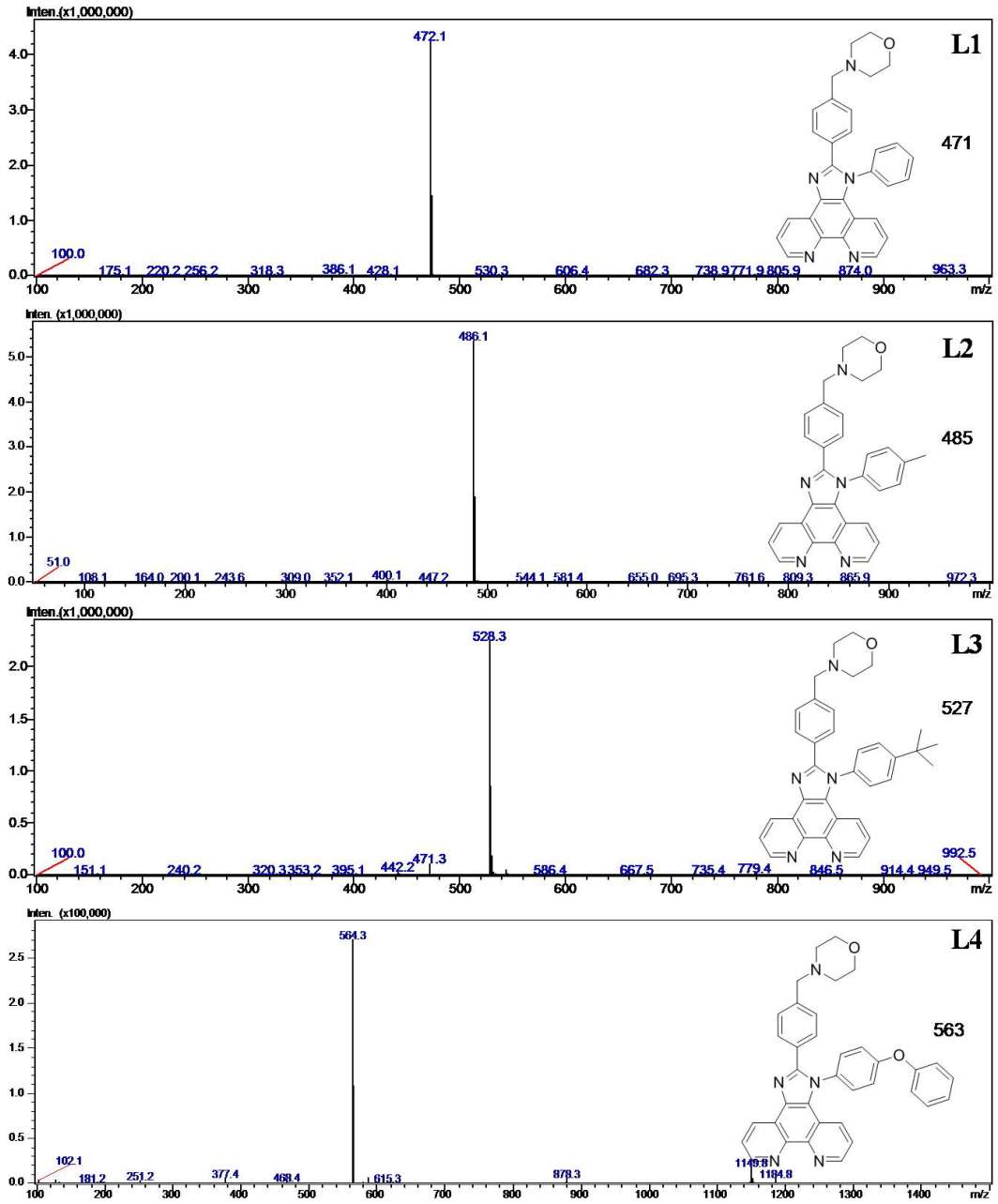


Fig. S1 ESI-MS spectrums of **L1-L4**.

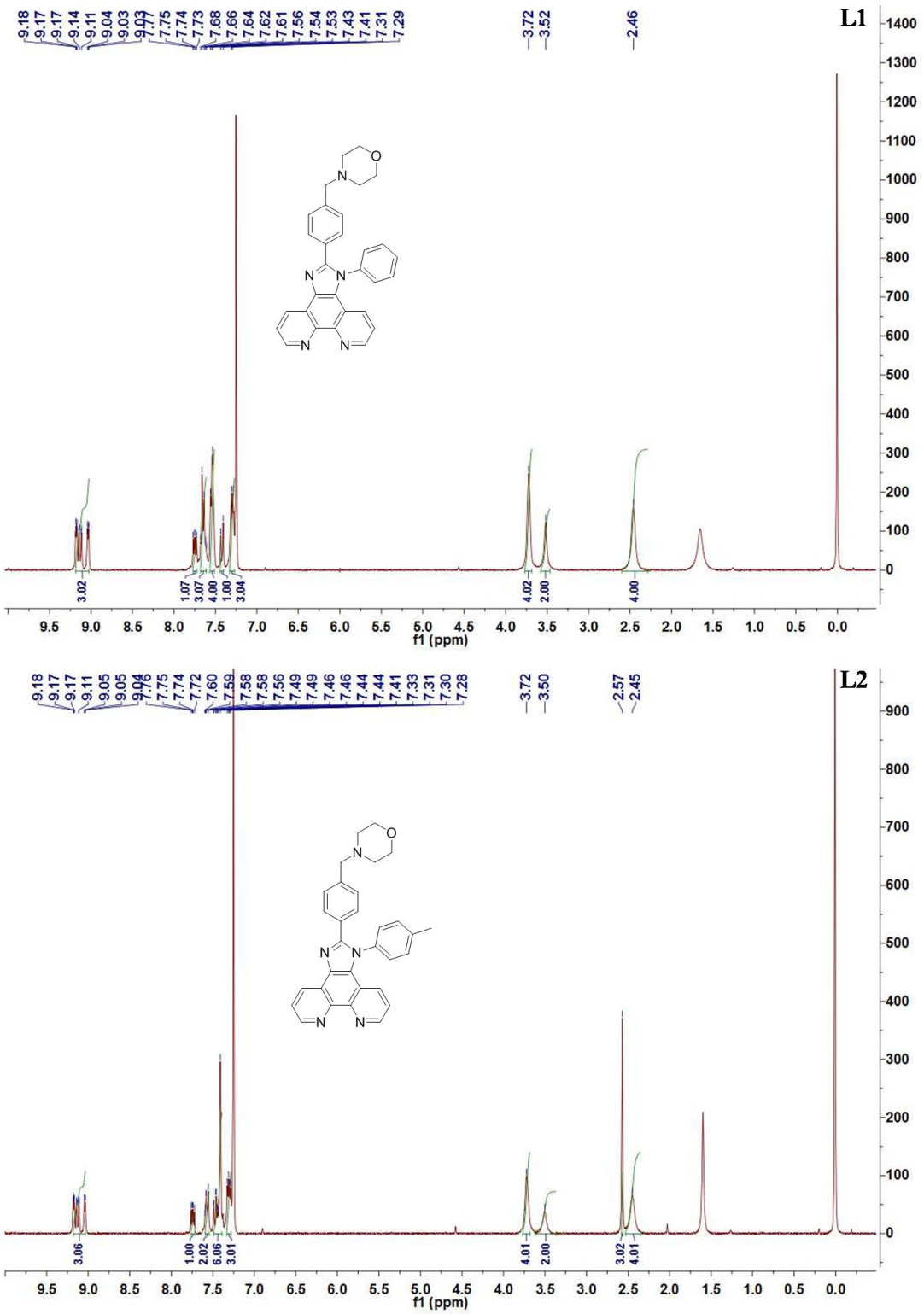


Fig. S2 ^{1}H NMR spectrums of **L1-L2**.

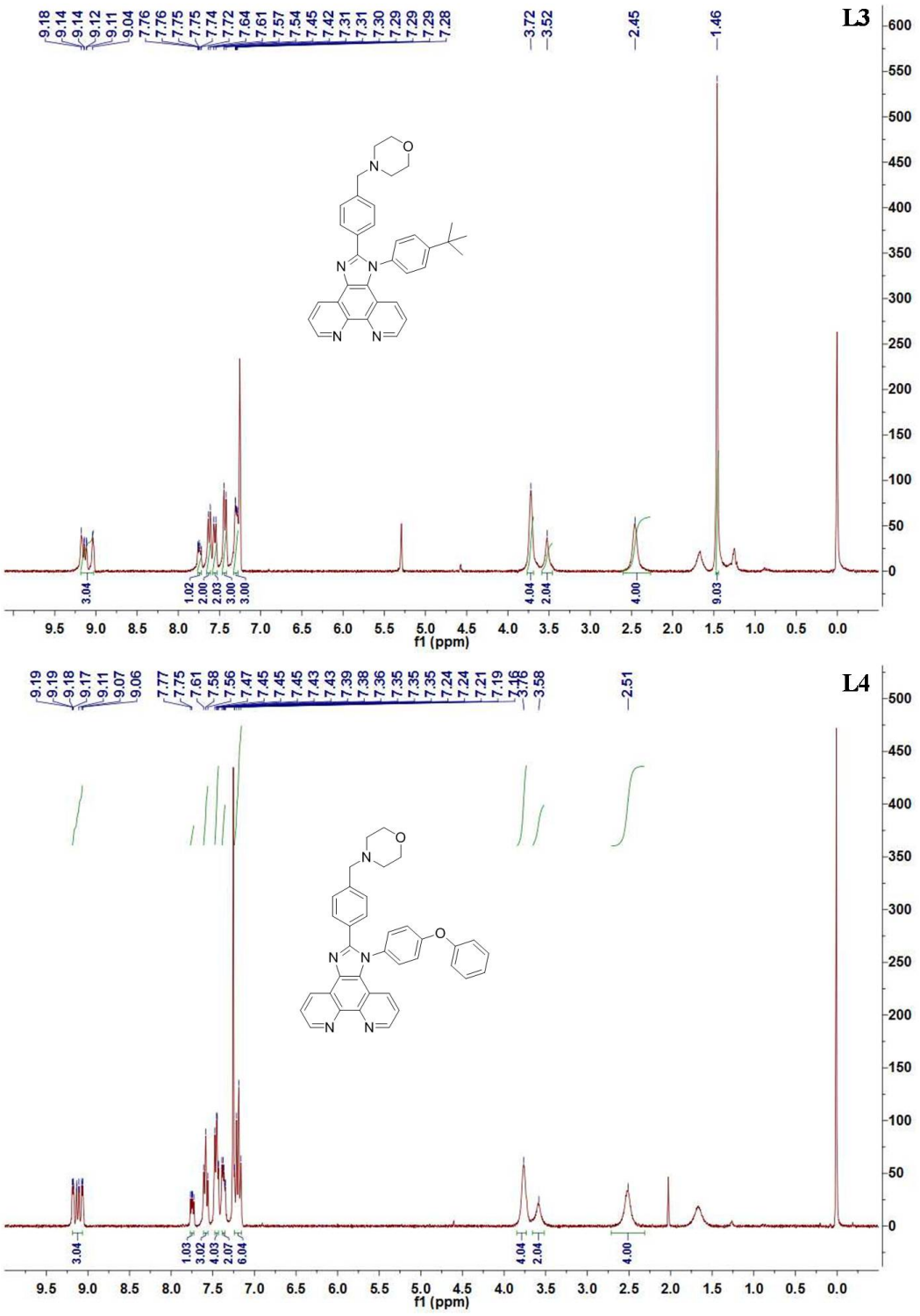


Fig. S3 ^{1}H NMR spectrums of **L3-L4**.

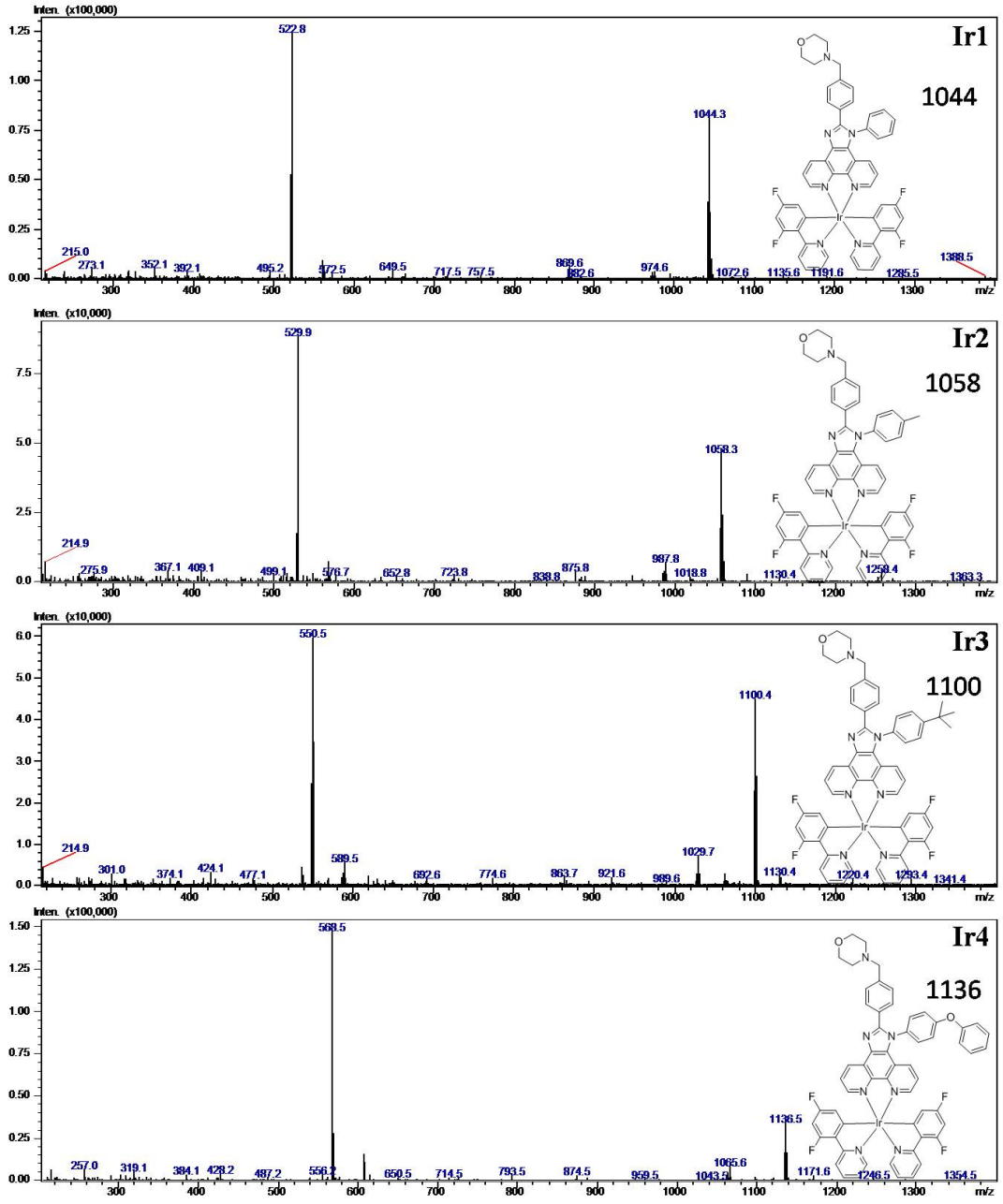


Fig. S4 ESI-MS spectrums of **Ir1-Ir4**.

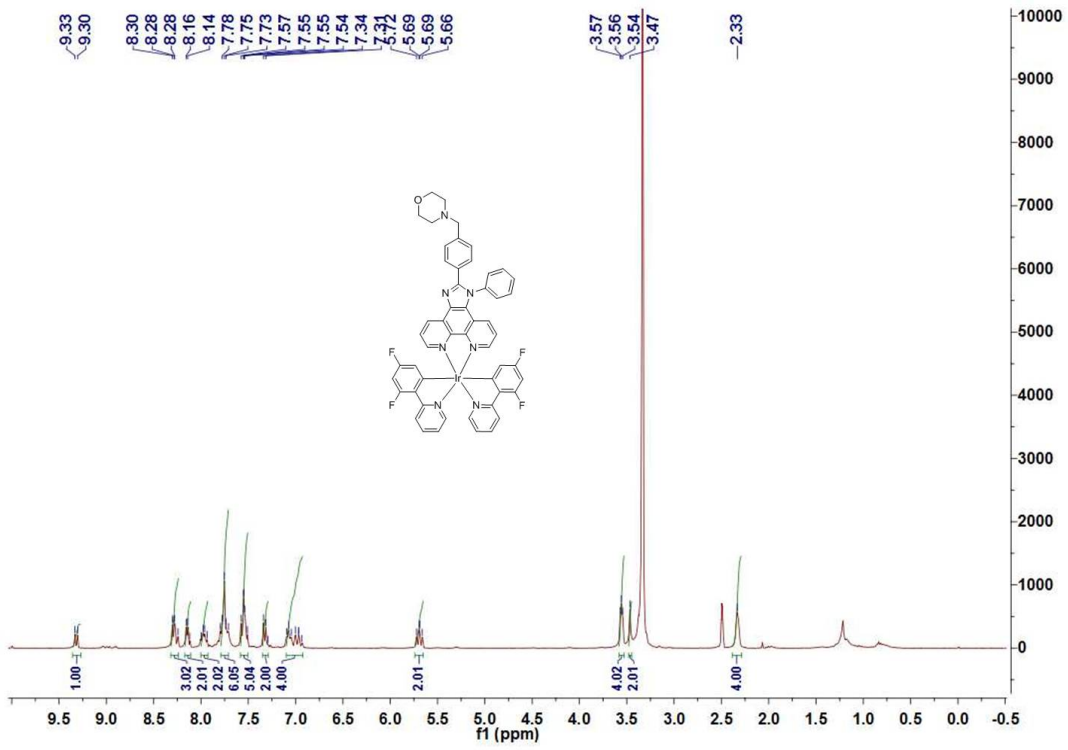


Fig. S5 ^{1}H NMR spectrum of **Ir1**.

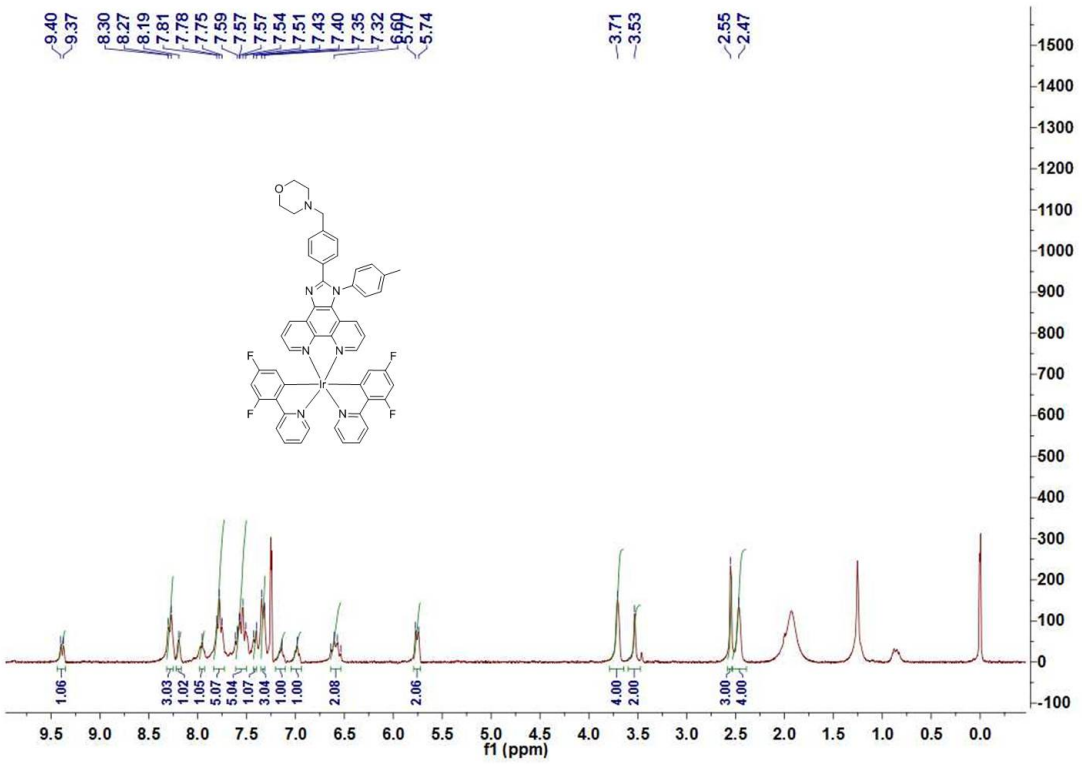


Fig. S6 ^{1}H NMR spectrum of **Ir2**.

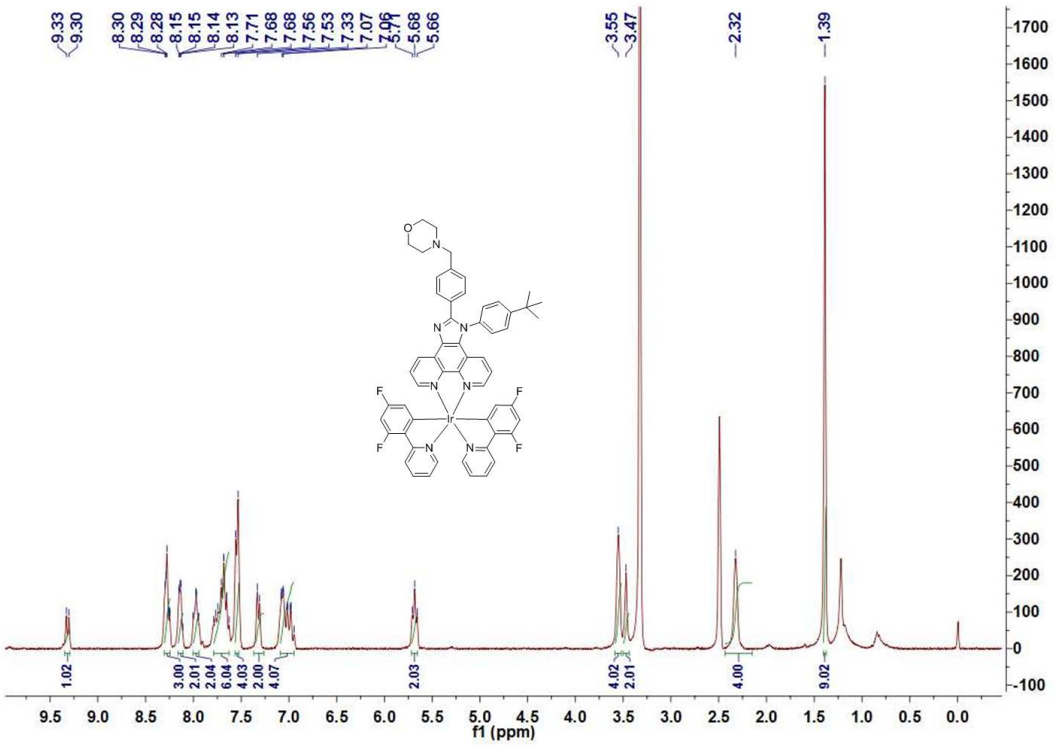


Fig. S7 ^{1}H NMR spectrum of **Ir3**.

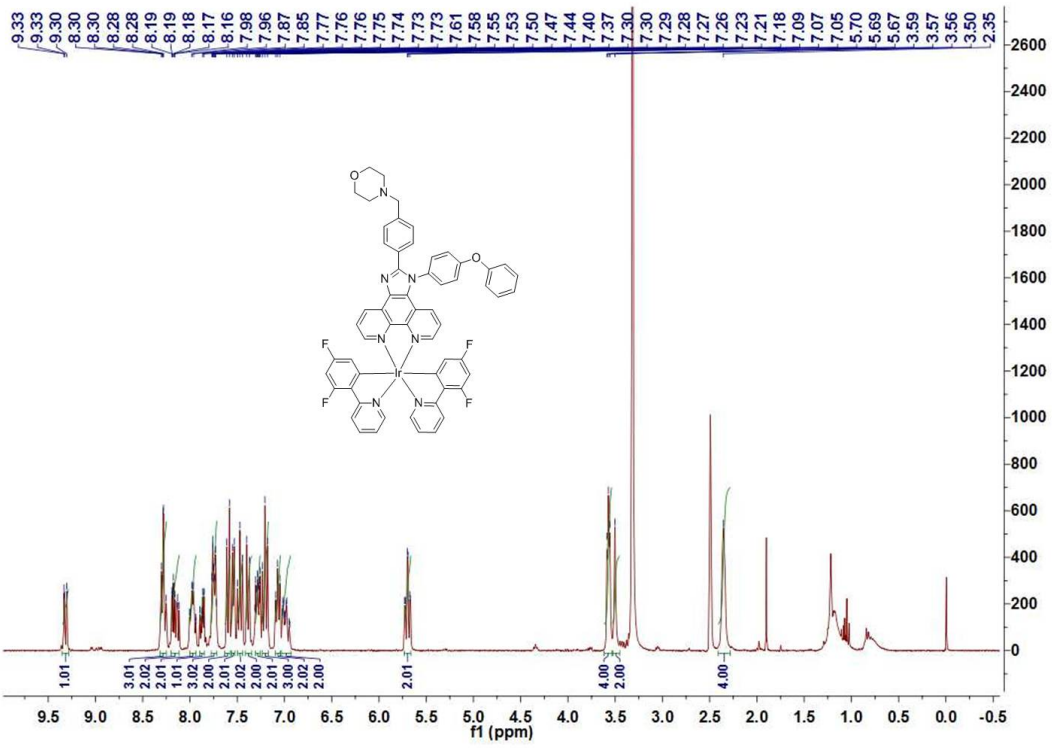


Fig. S8 ^{1}H NMR spectrum of **Ir4**.

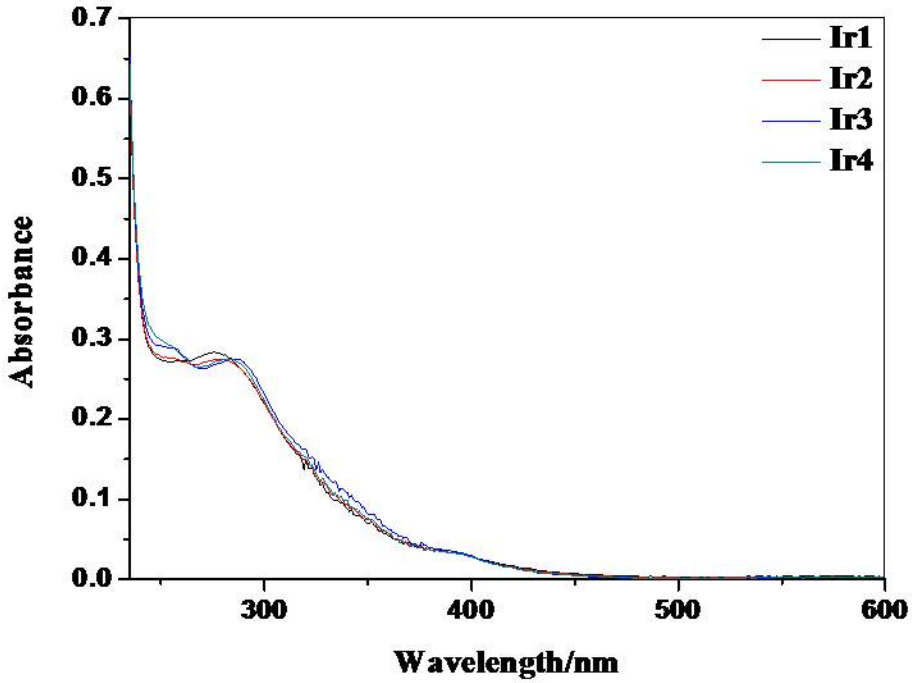


Fig. S9 Absorption spectra of **Ir1-Ir4** (5 μM).

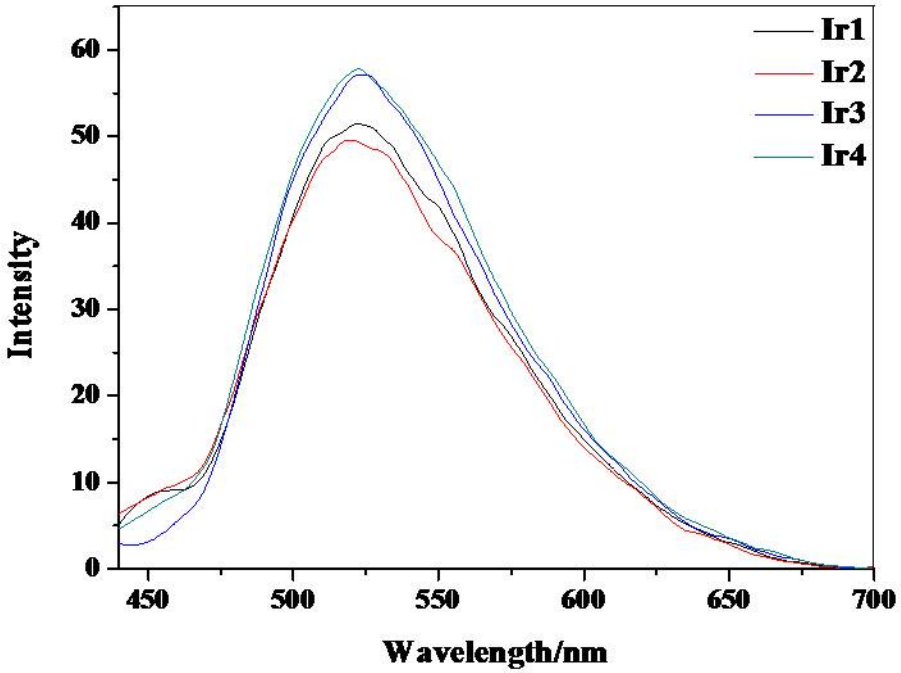


Fig. S10 Emission spectra of **Ir1-Ir4** (5 μM).

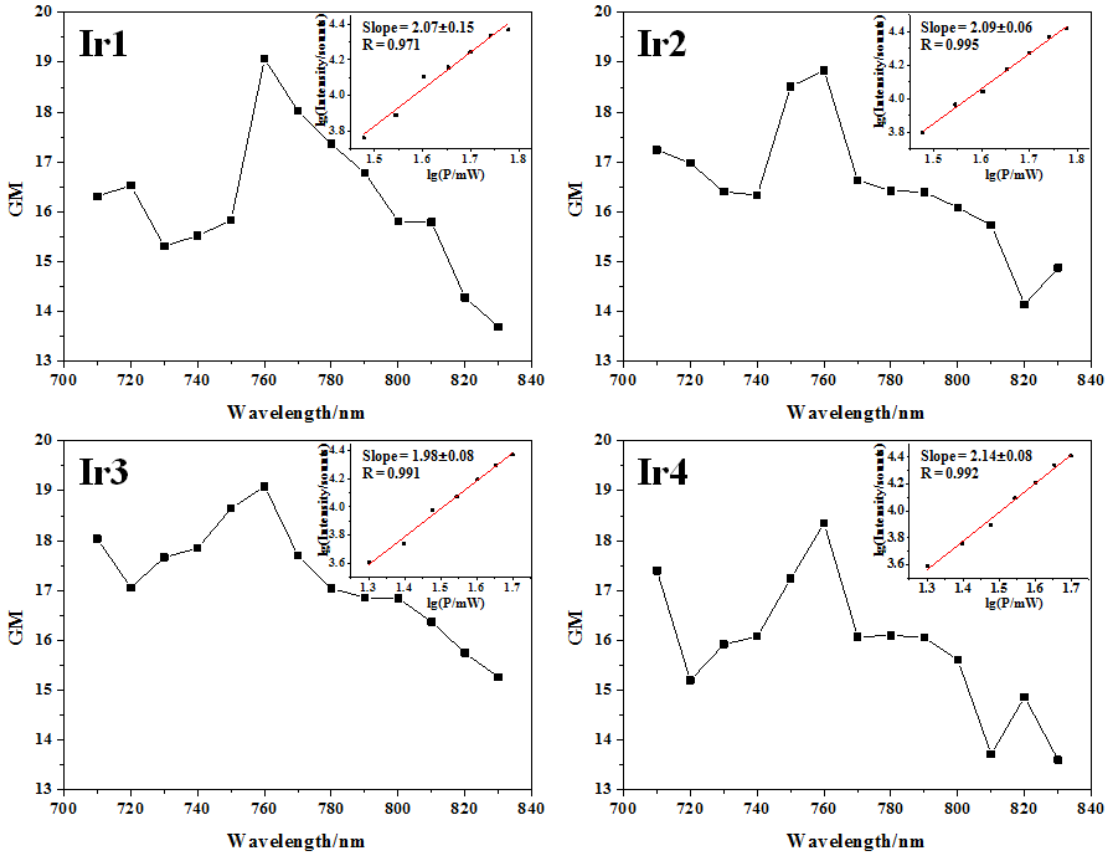


Fig. S11 Two-photon absorption cross-sections of **Ir1-Ir4** at different excitation wavelengths. Insert: The power dependence curve of **Ir1-Ir4** at an excitation wavelength of 760 nm.

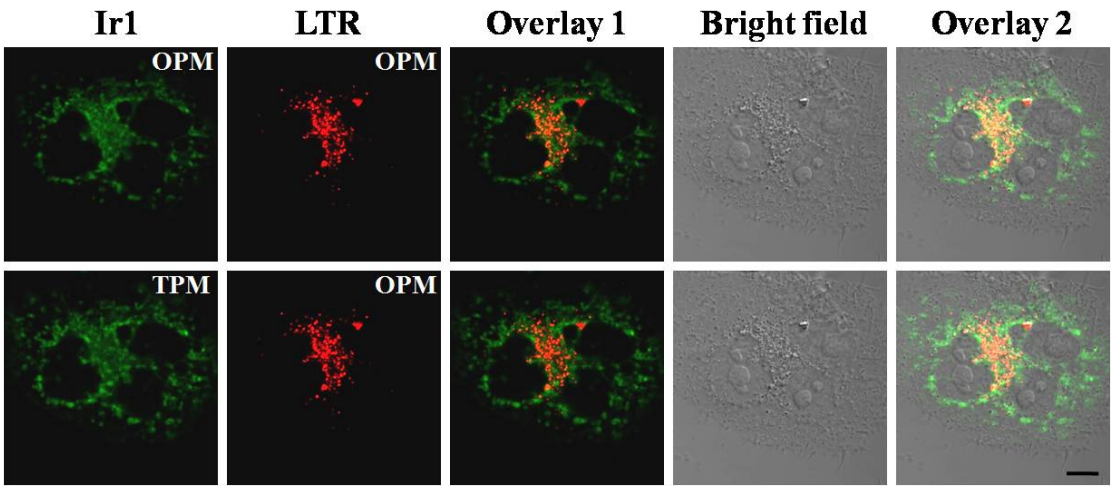


Figure S12. One-photon microscopy (OPM) and two-photon microscopy (TPM) images of HeLa cells co-labeled with **Ir1** (5 μM, 0.5 h) and LTR (50 nM, 0.5 h). **Ir1** was excited at 405 nm (OPM) or 760 nm (TPM). LTR (OPM) was excited at 543 nm. The phosphorescence/fluorescence was collected at 520 ± 20 nm and 620 ± 20 nm for **Ir1** and LTR, respectively. Overlay 1: Overlay of the 1st and 2nd columns. Overlay 2: Overlay of the 3rd and 4th columns. Scale bar: 10 μM.

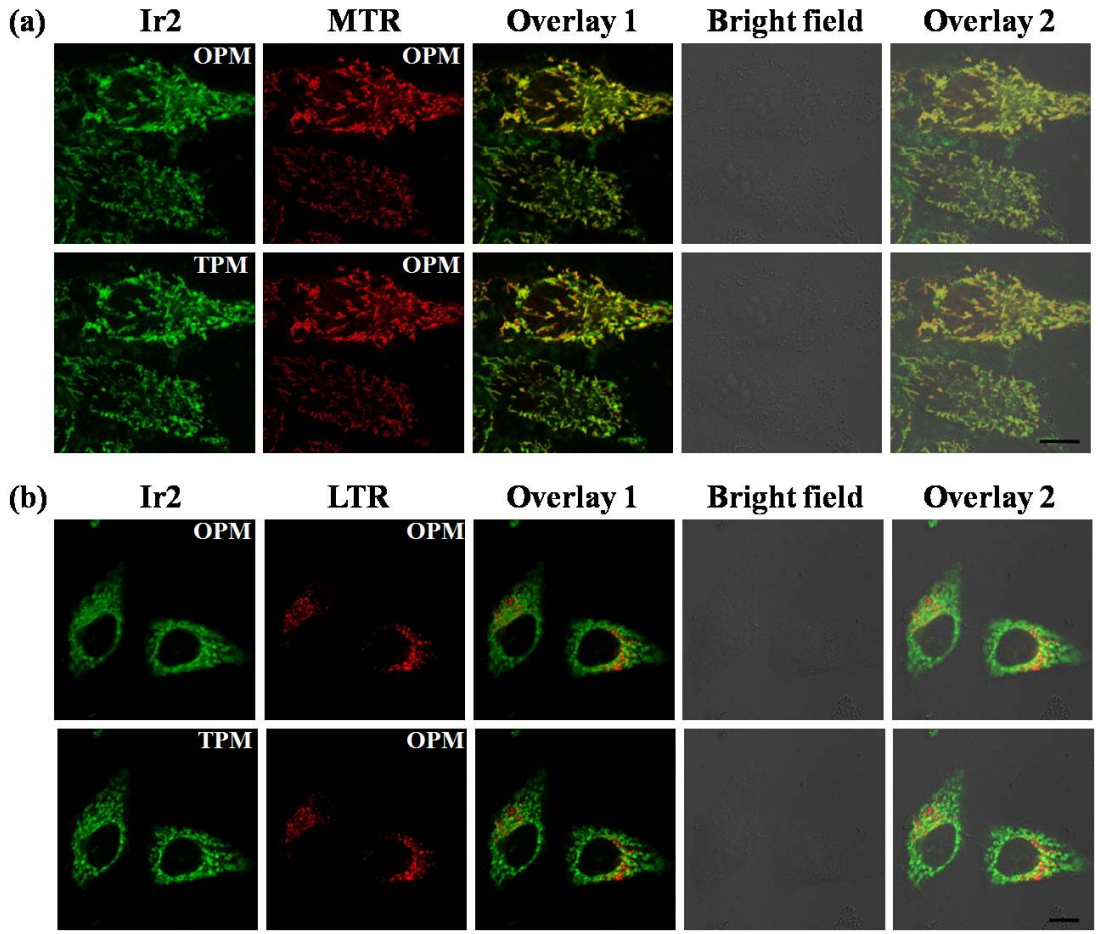


Fig. S13 One-photon microscopy (OPM) and two-photon microscopy (TPM) images of HeLa cells co-labeled with **Ir2** (5 μM, 0.5 h) and (a) MTR (50 nM, 0.5 h) or (b) LTR (50 nM, 0.5 h). **Ir2** was excited at 405 nm (OPM) or 760 nm (TPM). MTR (OPM) and LTR (OPM) were excited at 543 nm. The phosphorescence/fluorescence was collected at 520 ± 20 nm and 620 ± 20 nm for **Ir2**, MTR and LTR, respectively. Overlay 1: Overlay of the 1st and 2nd columns. Overlay 2: Overlay of the 3rd and 4th columns. Scale bar: 10 μM.

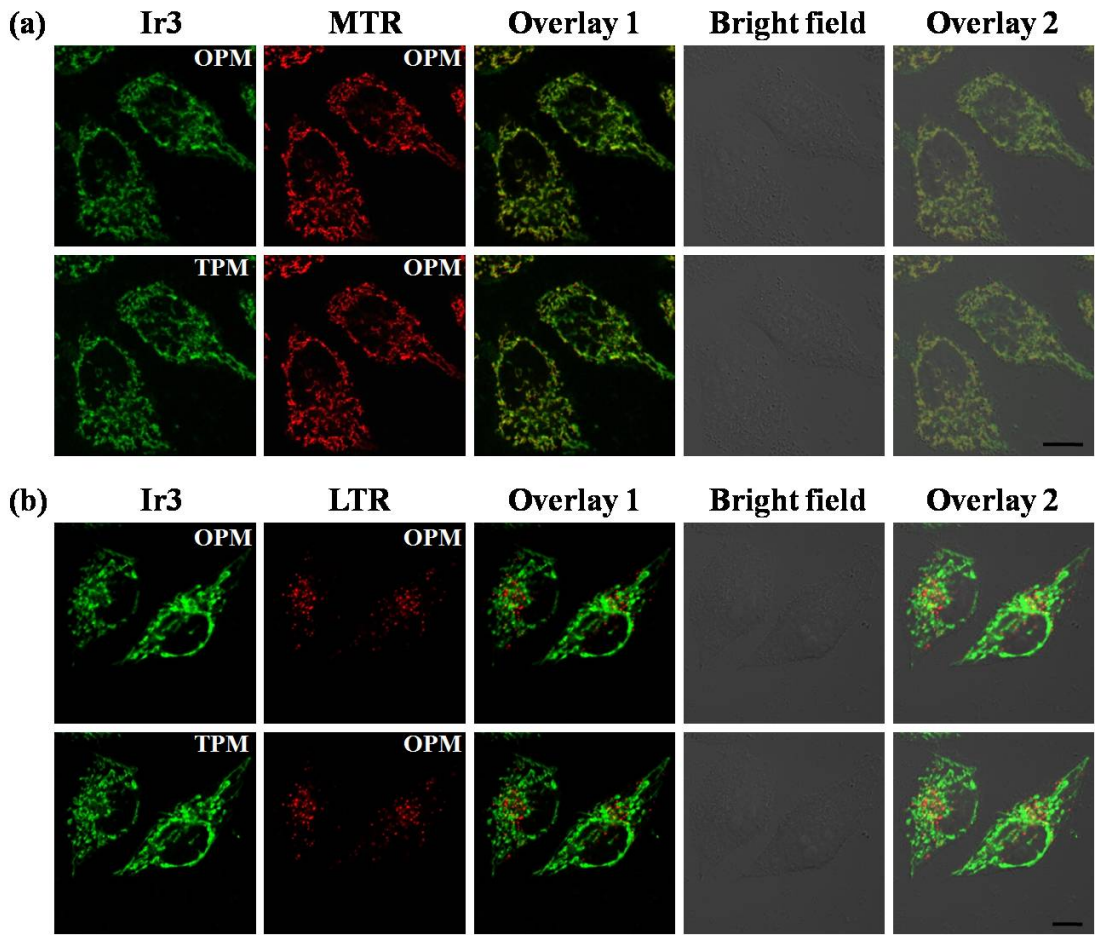


Fig. S14 One-photon microscopy (OPM) and two-photon microscopy (TPM) images of HeLa cells co-labeled with **Ir3** (5 μM, 0.5 h) and (a) MTR (50 nM, 0.5 h) or (b) LTR (50 nM, 0.5 h). **Ir3** was excited at 405 nm (OPM) or 760 nm (TPM). MTR (OPM) and LTR (OPM) were excited at 543 nm. The phosphorescence/fluorescence was collected at 520 ± 20 nm and 620 ± 20 nm for **Ir3**, MTR and LTR, respectively. Overlay 1: Overlay of the 1st and 2nd columns. Overlay 2: Overlay of the 3rd and 4th columns. Scale bar: 10 μM.

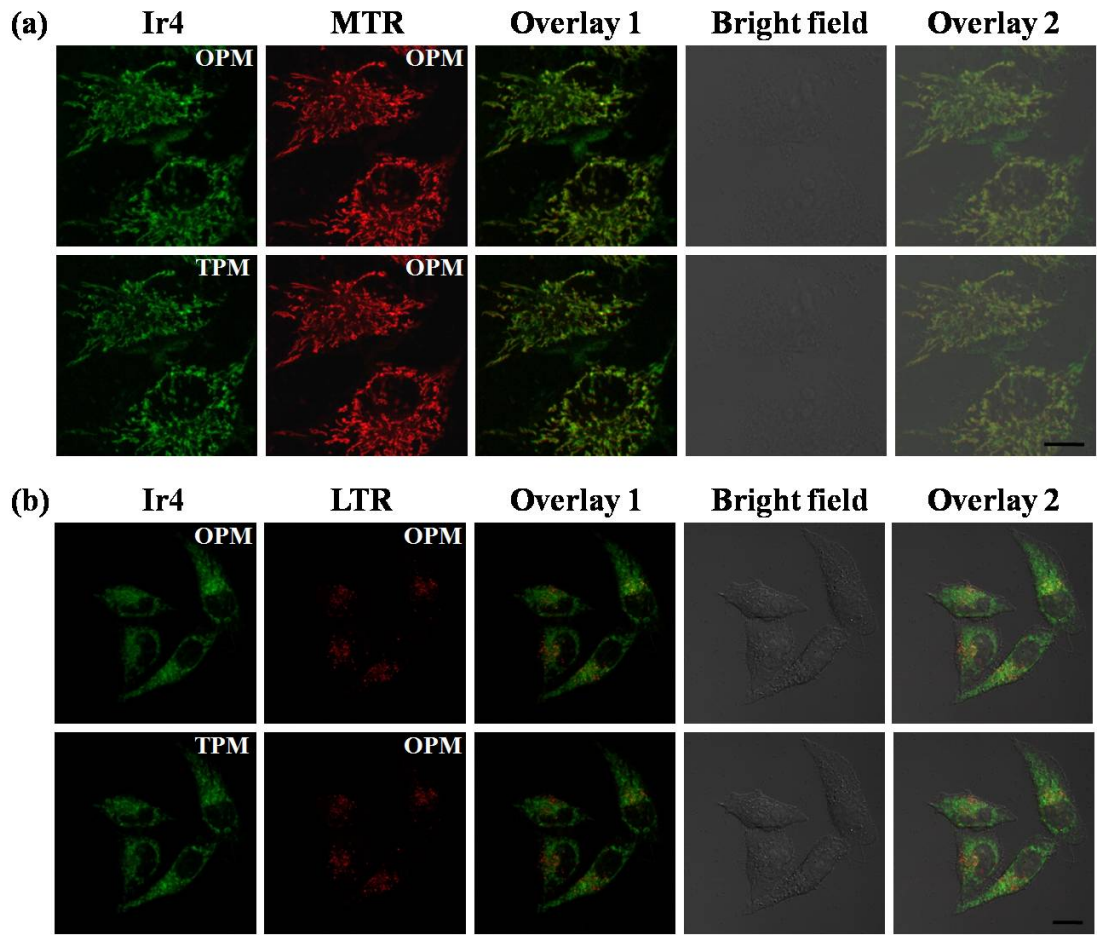


Fig. S15 One-photon microscopy (OPM) and two-photon microscopy (TPM) images of HeLa cells co-labeled with **Ir4** (5 μM, 0.5 h) and (a) MTR (50 nM, 0.5 h) or (b) LTR (50 nM, 0.5 h). **Ir4** was excited at 405 nm (OPM) or 760 nm (TPM). MTR (OPM) and LTR (OPM) were excited at 543 nm. The phosphorescence/fluorescence was collected at 520 ± 20 nm and 620 ± 20 nm for **Ir4**, MTR and LTR, respectively. Overlay 1: Overlay of the 1st and 2nd columns. Overlay 2: Overlay of the 3rd and 4th columns. Scale bar: 10 μM.

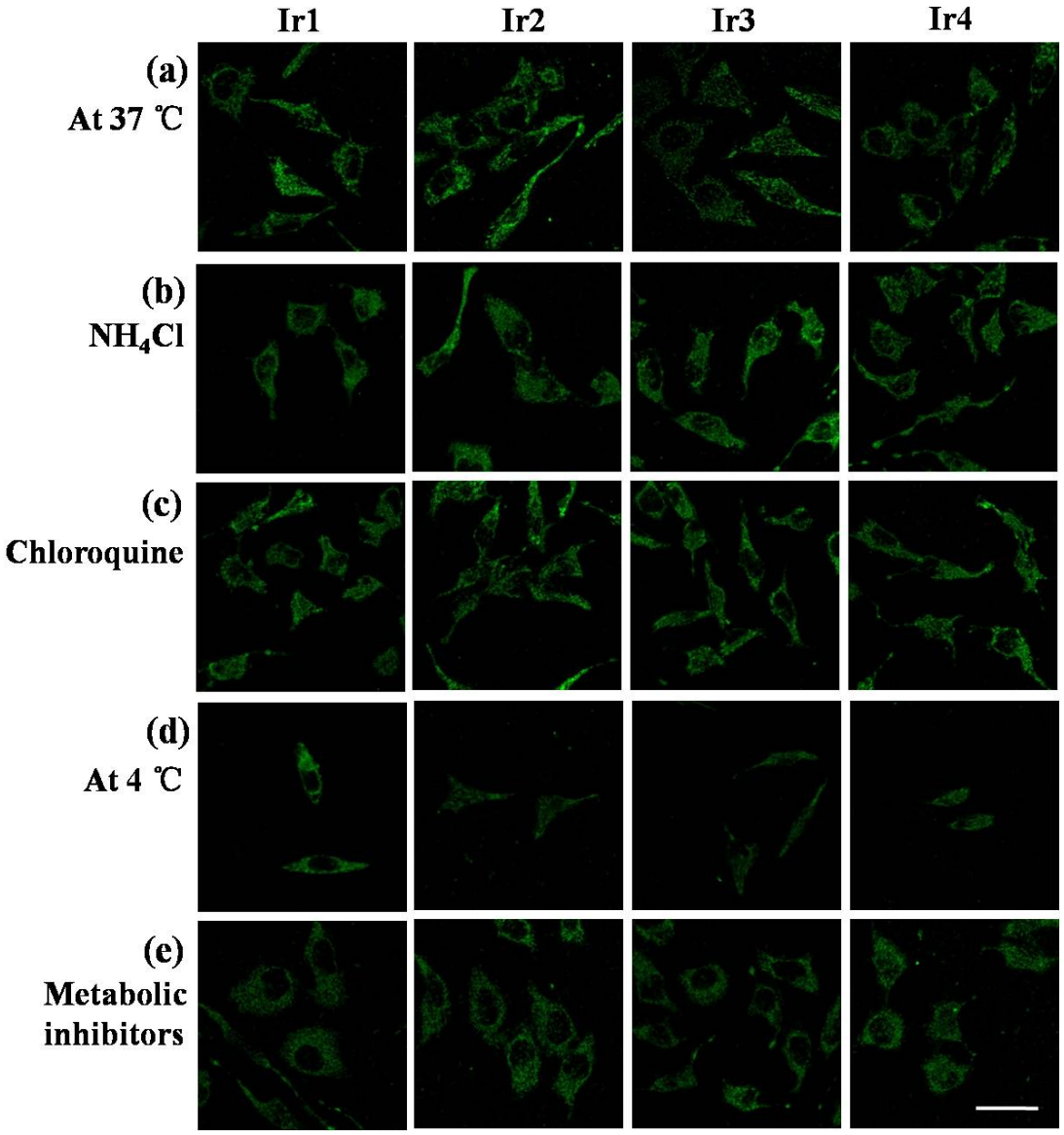


Fig. S16 TPM images of living HeLa cells incubated with 5 μM **Ir1-Ir4** under different conditions. (a) The cells were incubated with 5 μM **Ir1-Ir4** at 37 °C for 0.5 h. (b and c) The cells were pretreated with endocytic inhibitors NH_4Cl (50 mM), and chloroquine (50 μM) respectively, and then incubated with 5 μM **Ir1-Ir4** at 37 °C for 0.5 h. (d) The cells were incubated with 5 μM **Ir1-Ir4** at 4 °C for 0.5 h. (e) The cells were pretreated with 50 mM 2-deoxy-D-glucose and 5 μM oligomycin in PBS for 1 h at 37 °C and then incubated with 5 μM **Ir1-Ir4** at 37 °C for 0.5 h. Scale bar: 10 μm.

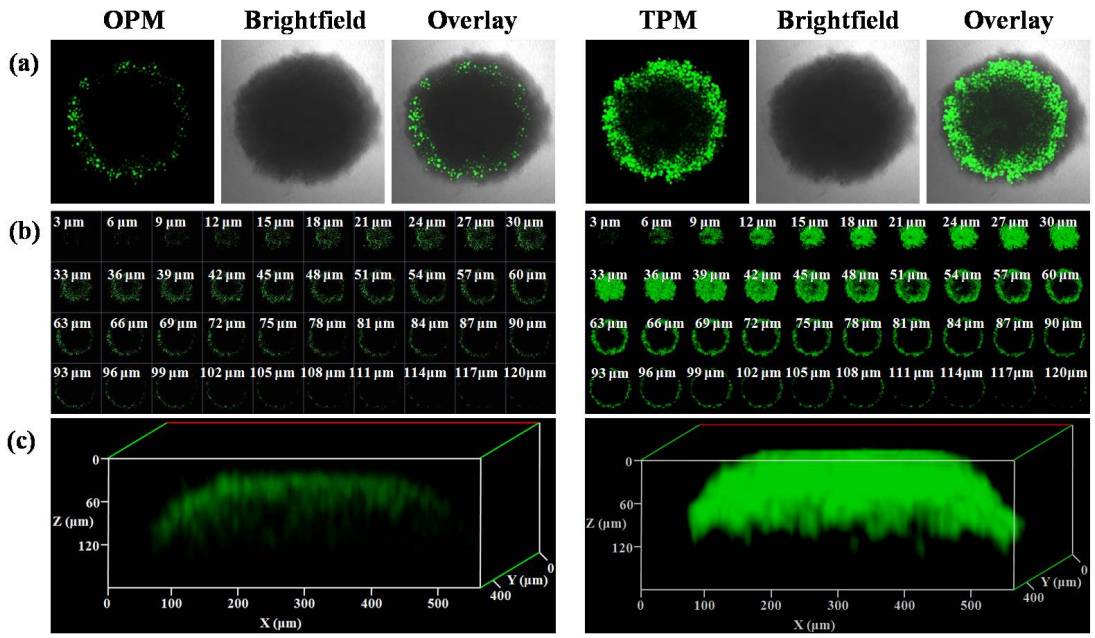


Fig. S17 (a) One- and two-photon phosphorescent images of 3D tumor spheroids after incubation with **Ir2** (5 μM) for 6 h. (b) The one- and two-photon Z-stack images were taken of every 3 μm section from the top to bottom. (c) The one- and two-photon 3D Z-stack images of an intact spheroid. The images were taken under a 10× objective. λ_{ex} = 405 nm (one-photon) or λ_{ex} = 760 nm (two-photon); λ_{em} = 520 ± 20 nm.

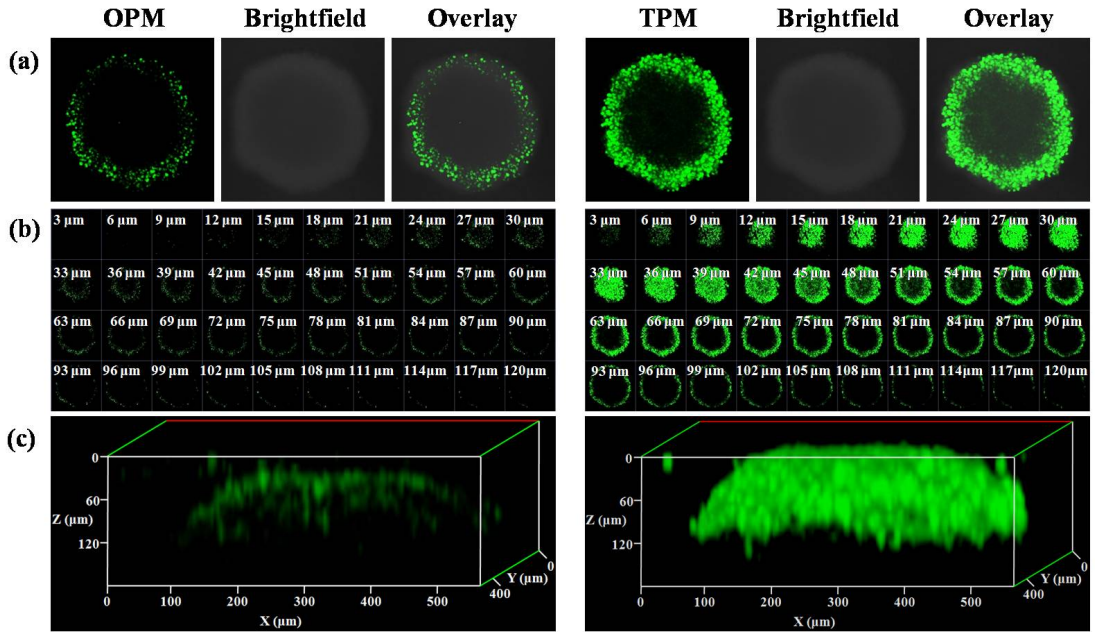


Fig. S18 (a) One- and two-photon phosphorescent images of 3D tumor spheroids after incubation with **Ir3** (5 μM) for 6 h. (b) The one- and two-photon Z-stack images were taken of every 3 μm section from the top to bottom. (c) The one- and two-photon 3D Z-stack images of an intact spheroid. The images were taken under a 10× objective. λ_{ex} = 405 nm (one-photon) or λ_{ex} = 760 nm (two-photon); λ_{em} = 520 ± 20 nm.

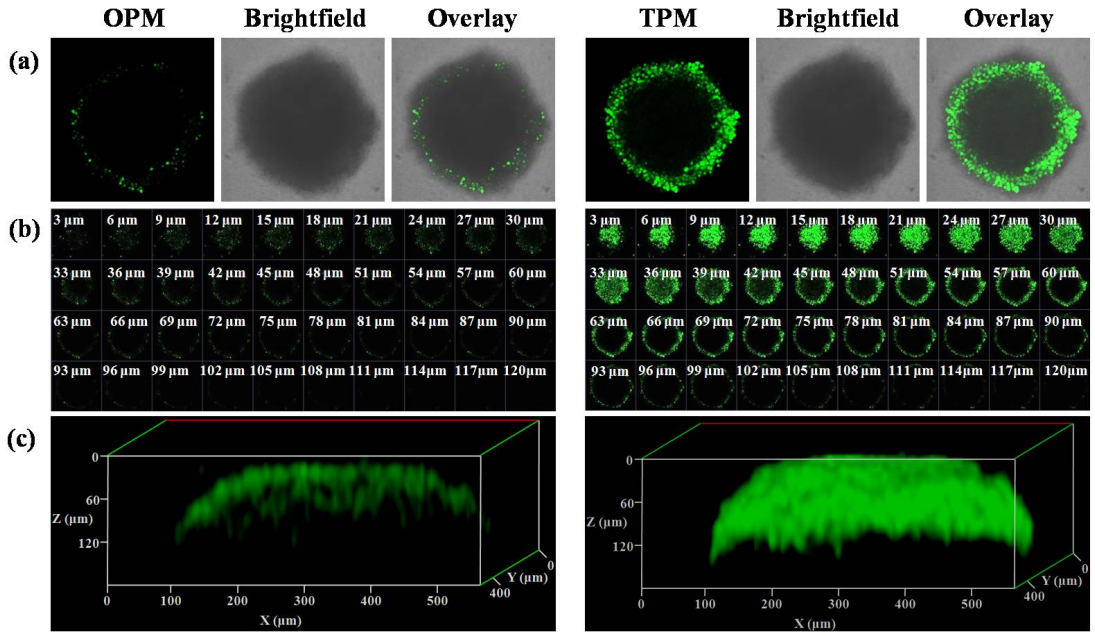


Fig. S19 (a) One- and two-photon phosphorescent images of 3D tumor spheroids after incubation with **Ir4** (5 μM) for 6 h. (b) The one- and two-photon Z-stack images were taken of every 3 μm section from the top to bottom. (c) The one- and two-photon 3D Z-stack images of an intact spheroid. The images were taken under a 10× objective. λ_{ex} = 405 nm (one-photon) or λ_{ex} = 760 nm (two-photon); λ_{em} = 520 ± 20 nm.

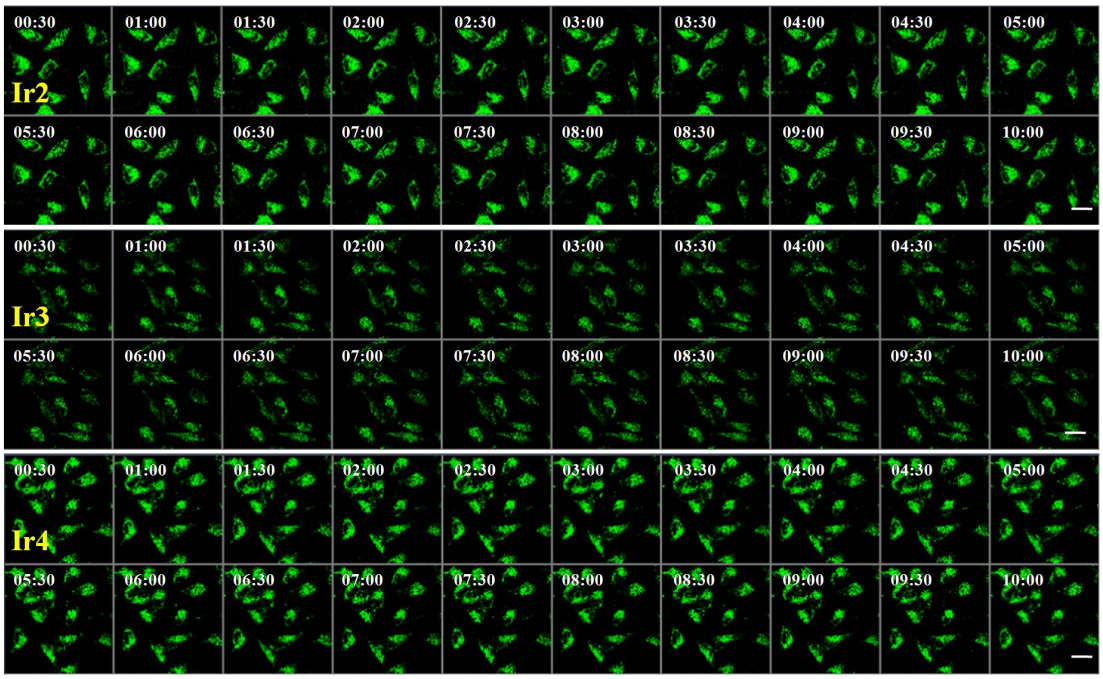


Fig. S20 Two-photon phosphorescent images of live HeLa cells treated with CCCP (20 μM) stained with **Ir2-Ir4** (5 μM, λ_{ex} = 760 nm, λ_{em} = 520 ± 20 nm) with increasing scan time. Scale bar: 10 μm.