Supporting Information

Tracking mitochondrial dynamics during apoptosis with phosphorescent fluorinated iridium(III) complexes

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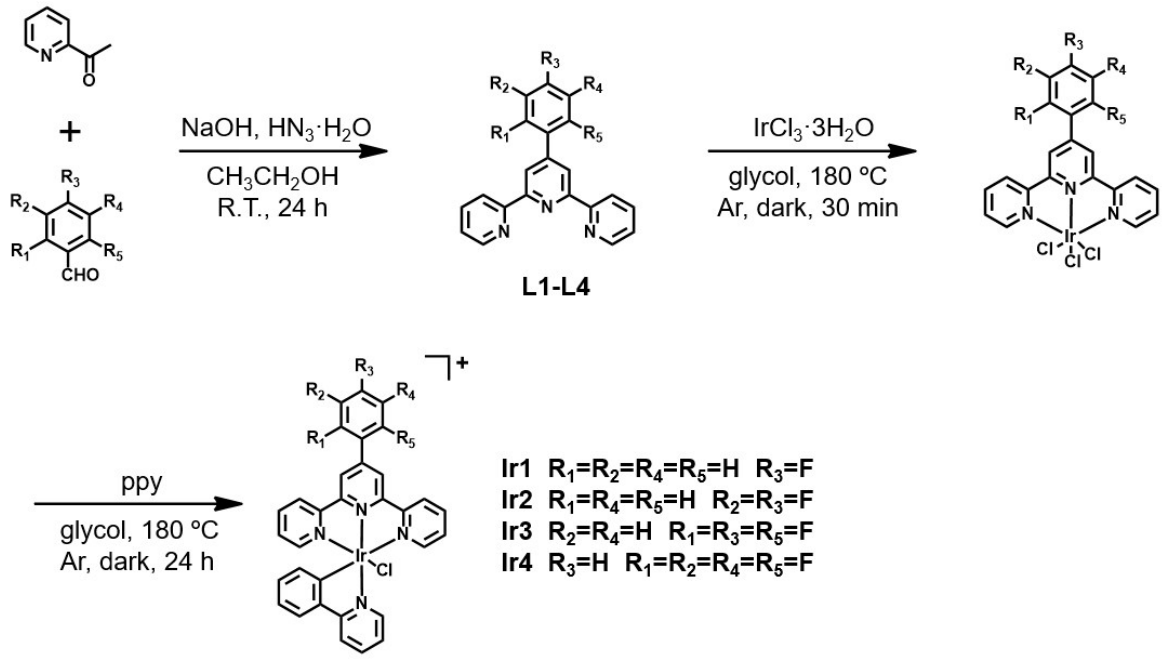

Scheme S1. Synthetic route to **Ir1-Ir4**.

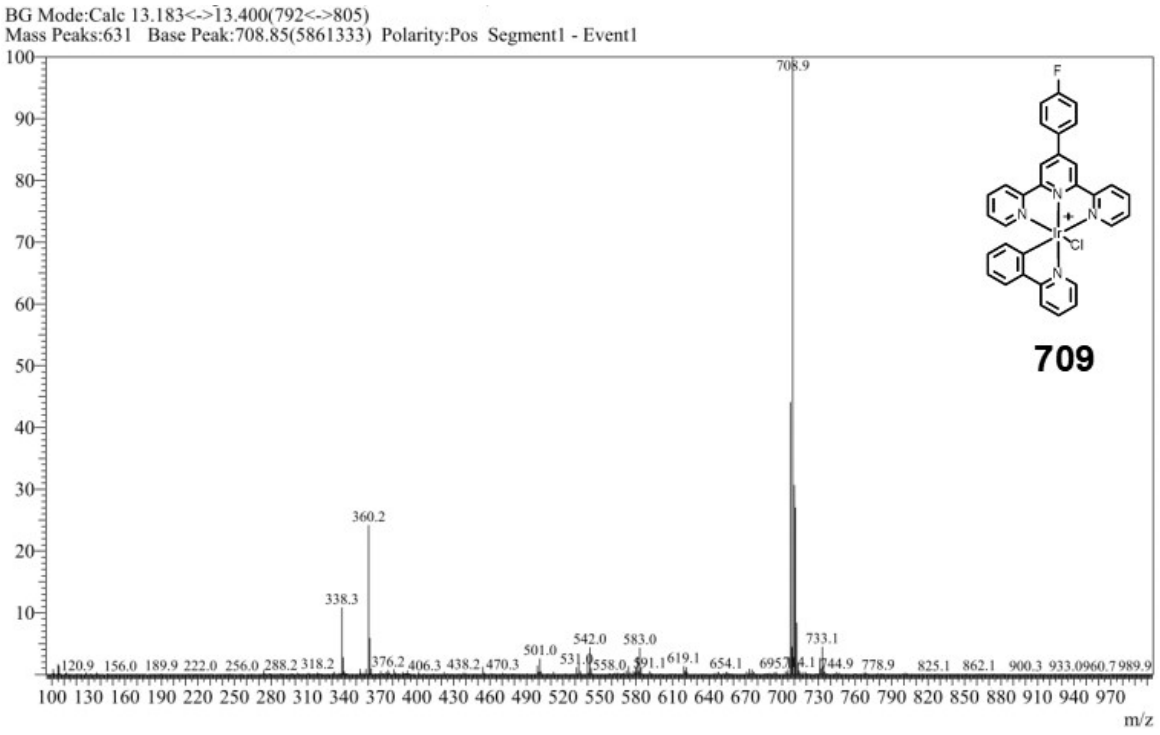

Fig. S1 ES-MS spectrum of complex **Ir1**.

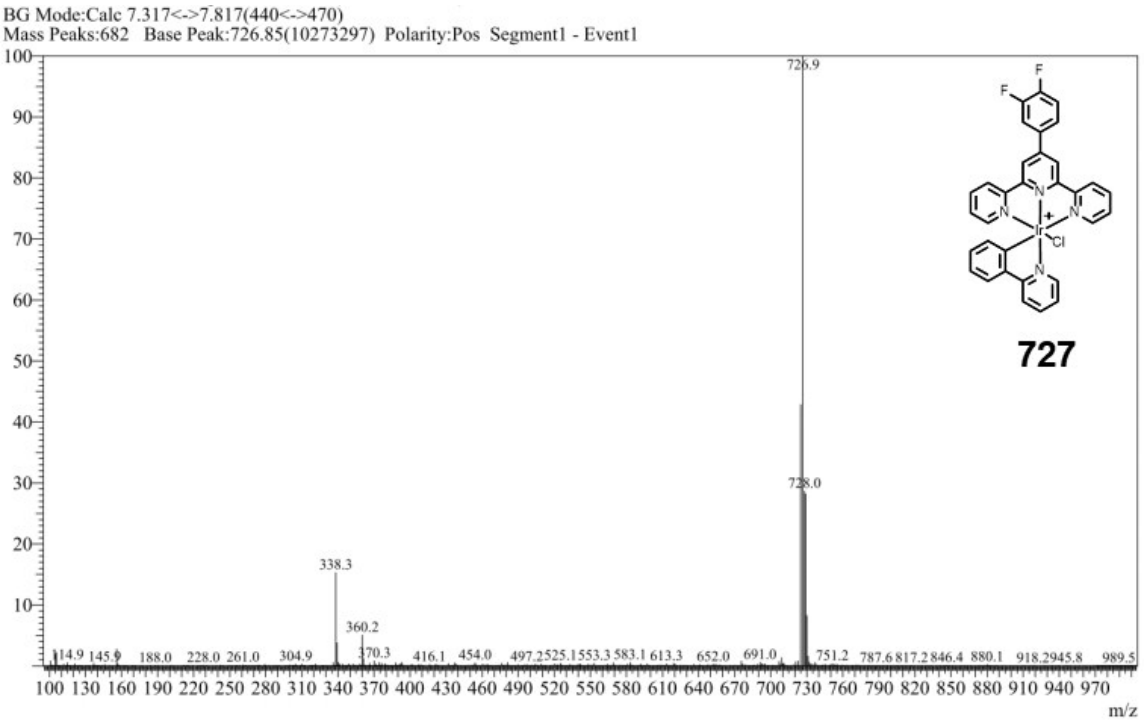

Fig. S2 ES-MS spectrum of complex **Ir2**.

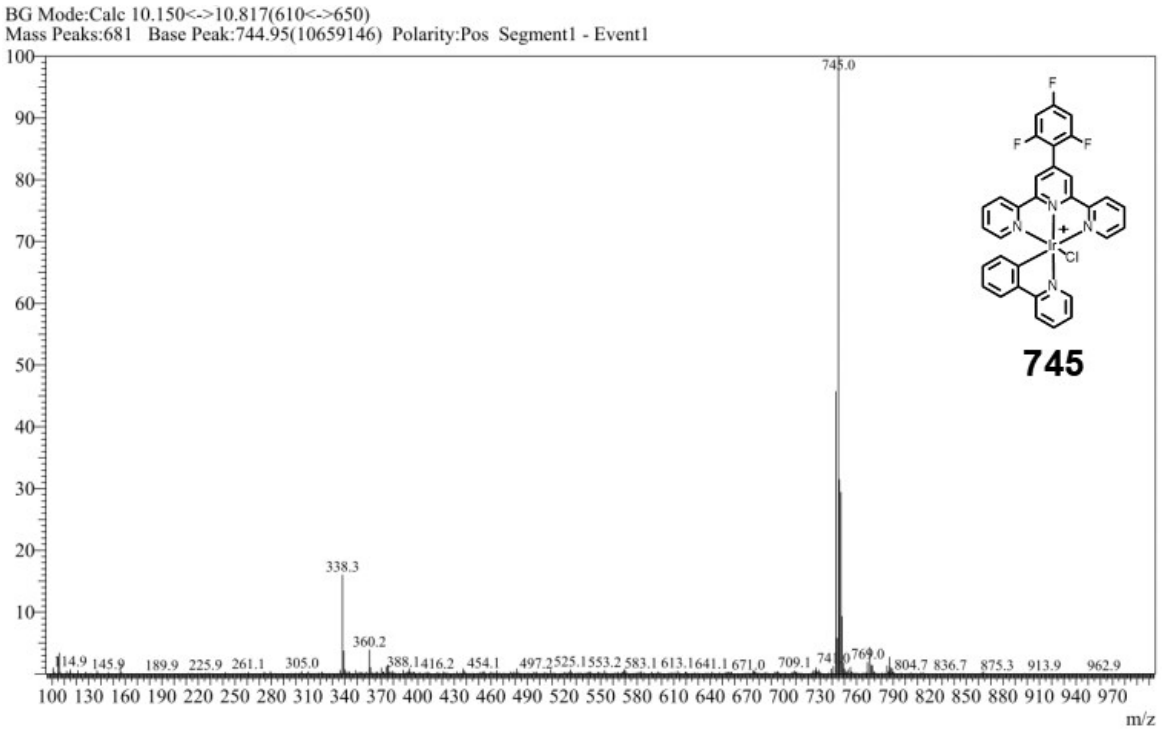

Fig. S3 ES-MS spectrum of complex **Ir3**.

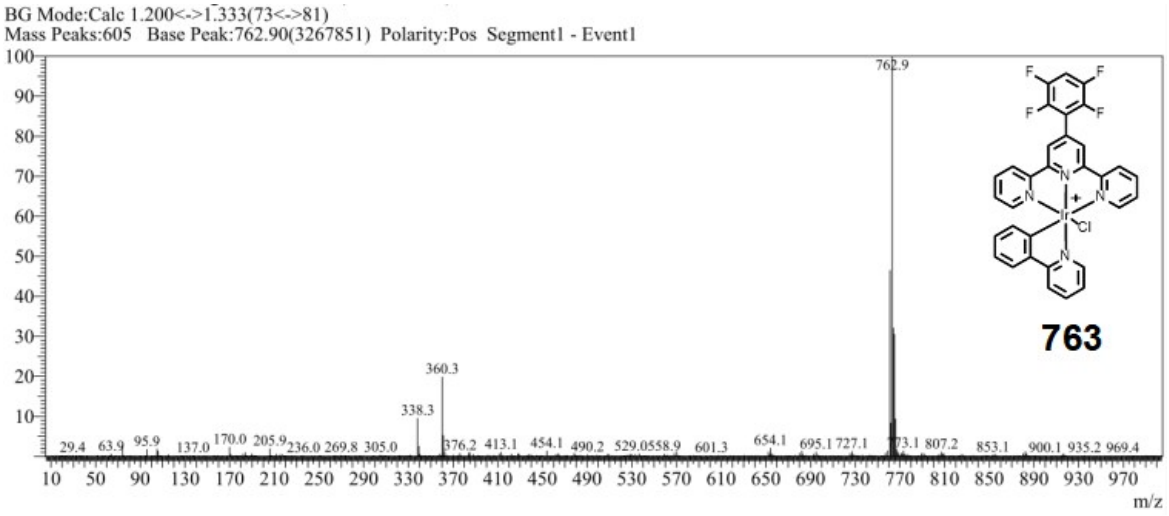

Fig. S4 ES-MS spectrum of complex **Ir4**.

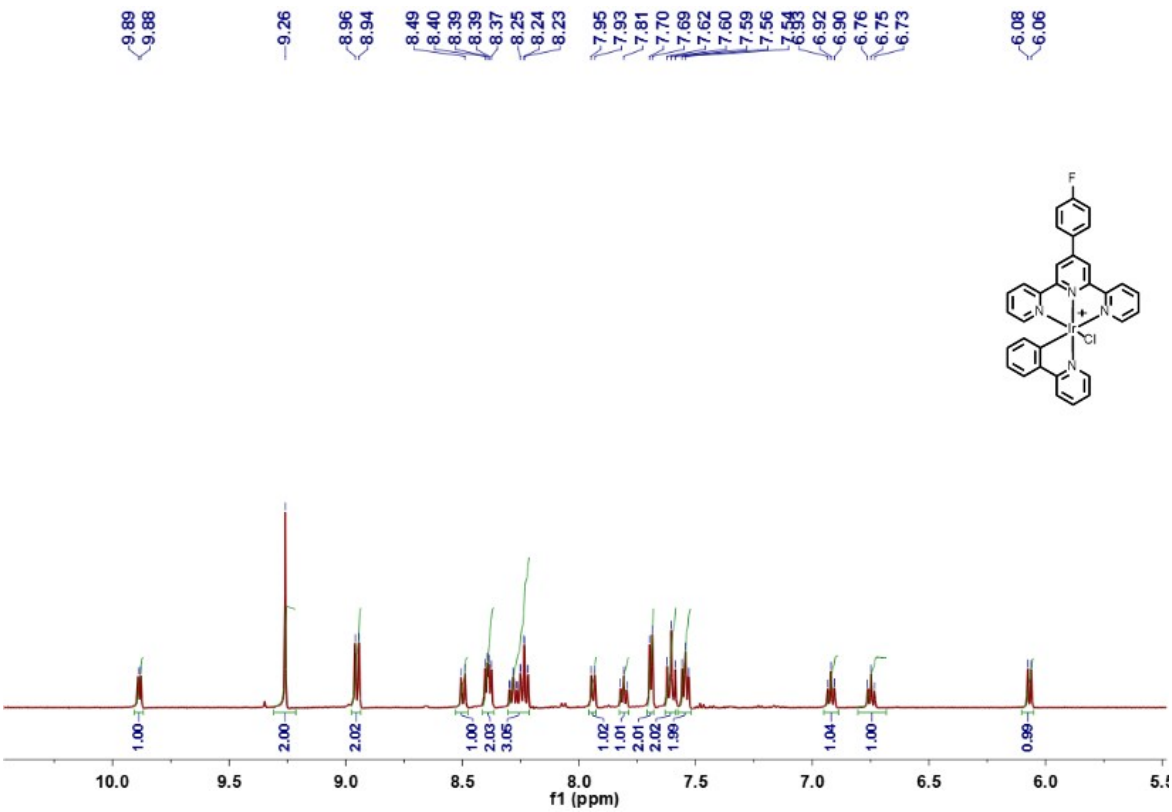

Fig. S5 ^{1}H NMR spectrum of complex **Ir1** in d_6-DMSO.

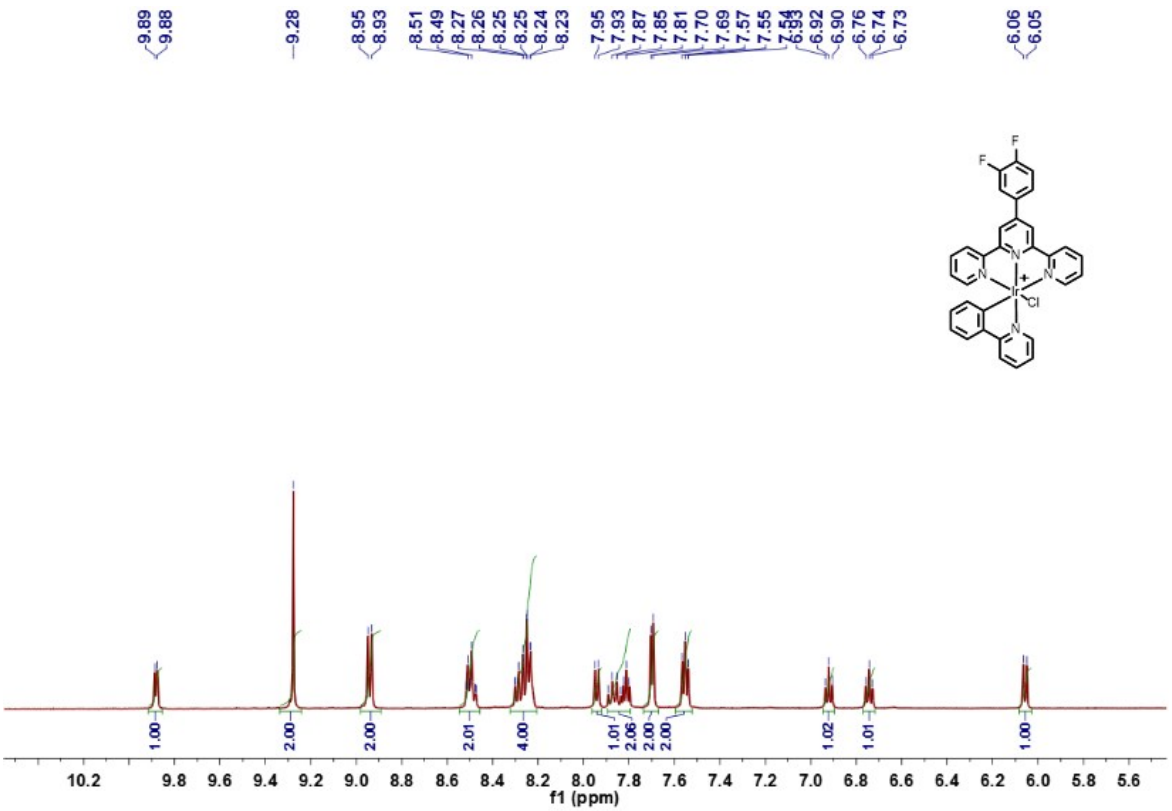

Fig. S6 ^{1}H NMR spectrum of complex **Ir2** in d_6-DMSO.

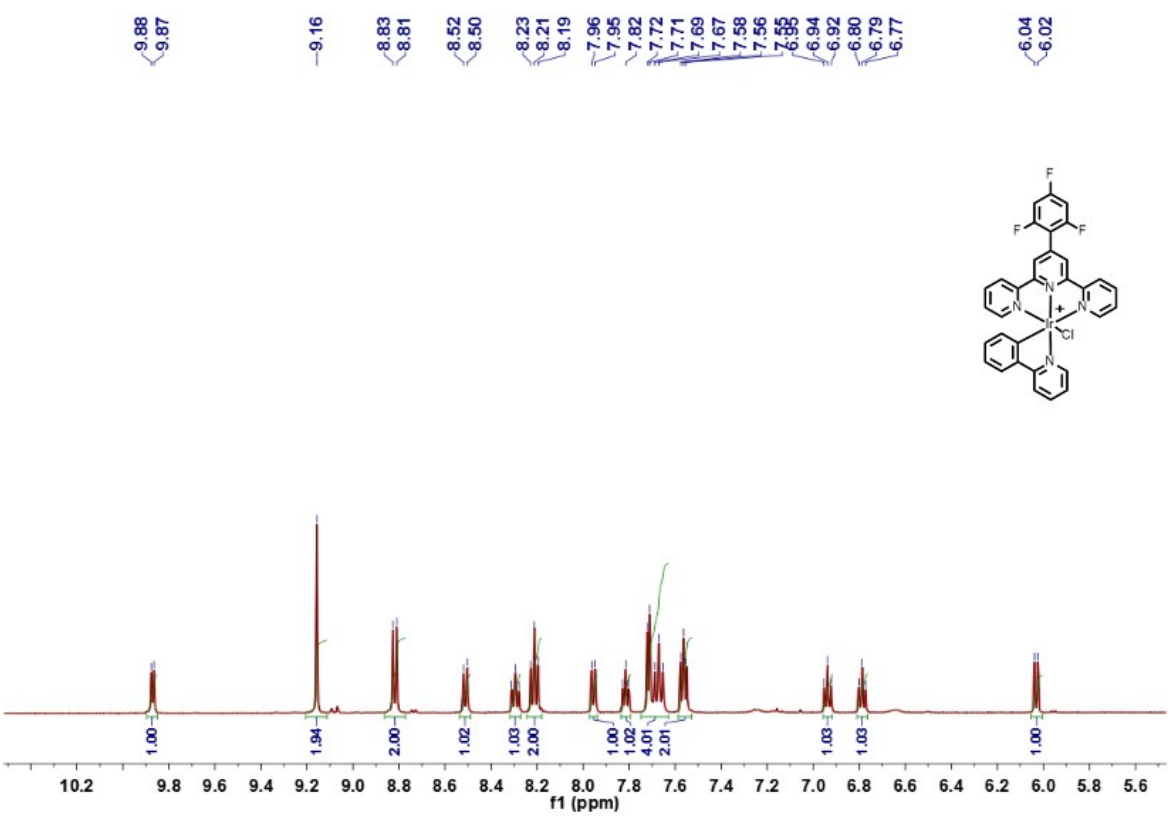

Fig. S7 ^{1}H NMR spectrum of complex **Ir3** in d_6-DMSO.

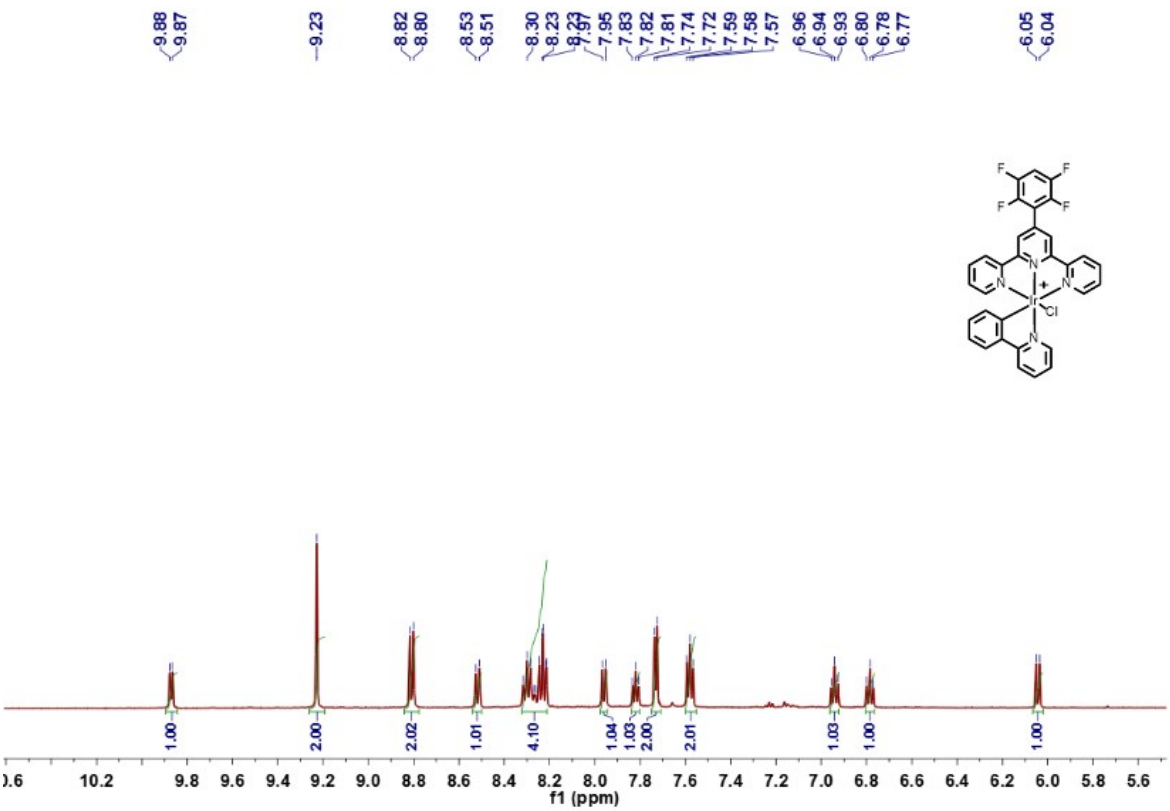

Fig. S8 ^{1}H NMR spectrum of complex **Ir4** in d_6-DMSO.

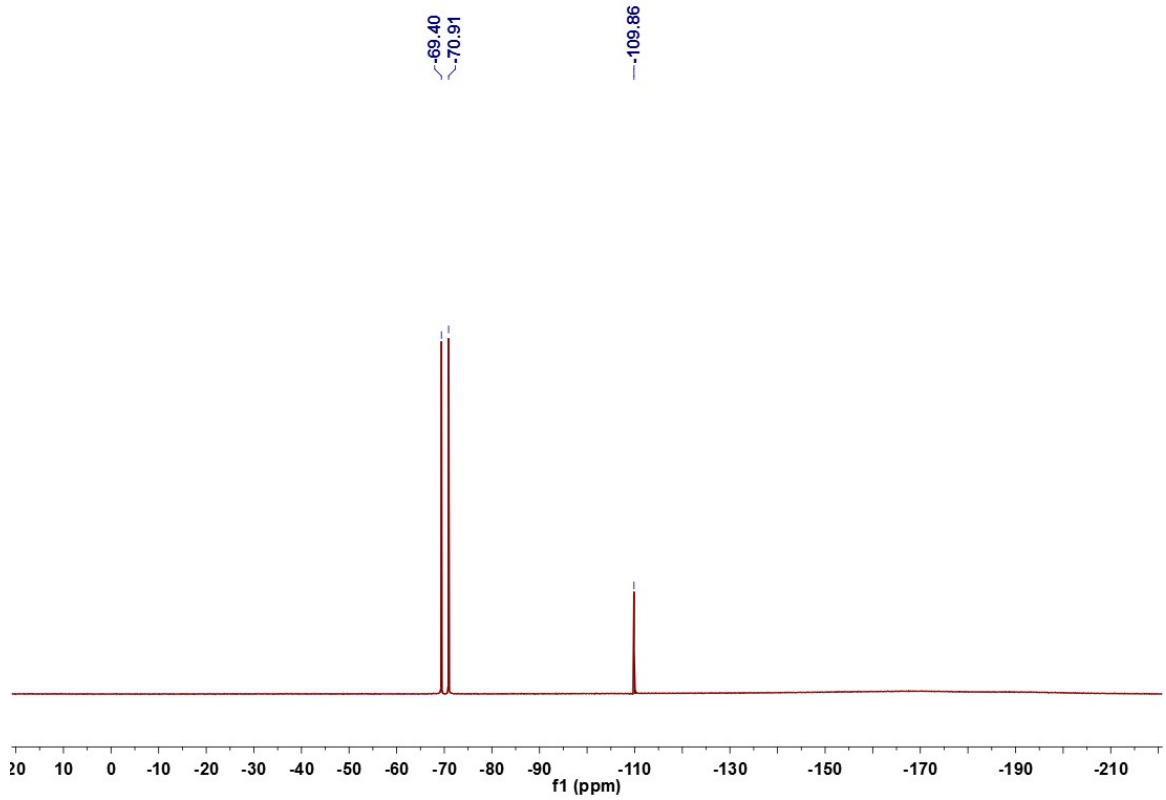

Fig. S9 ^{19}F NMR spectrum of complex **Ir1** in d_6-DMSO.

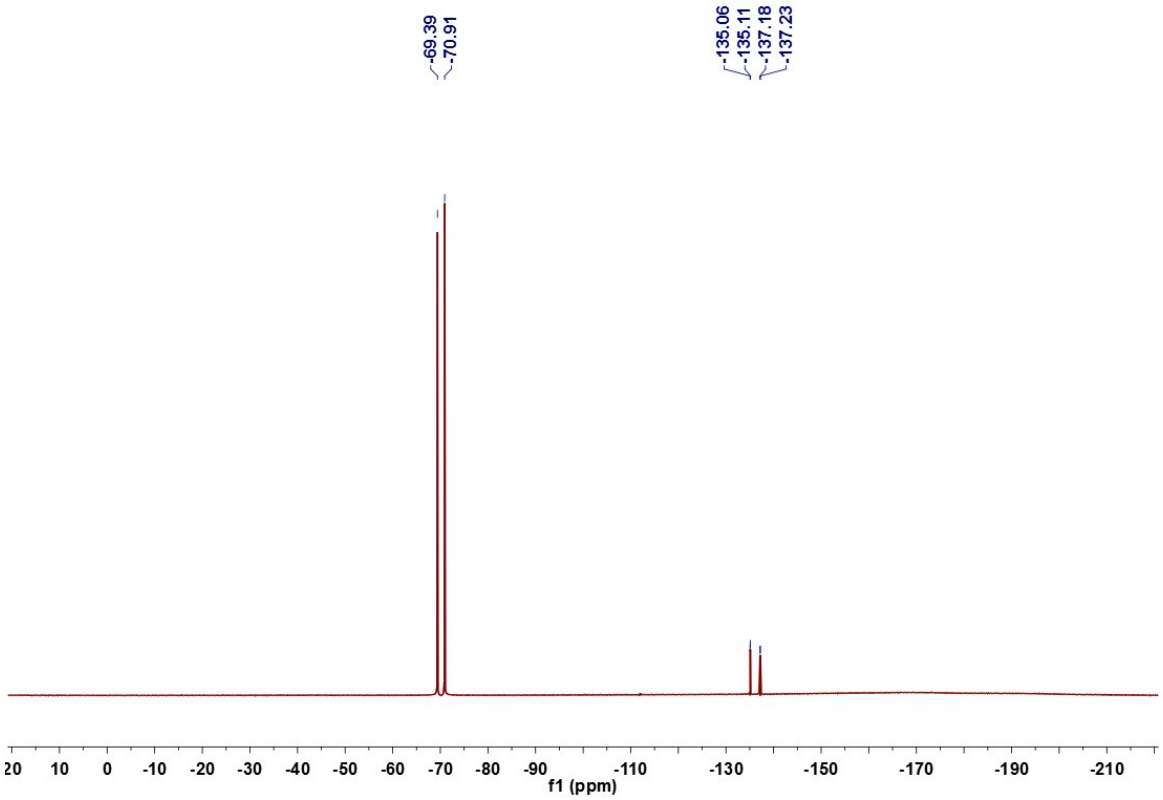

Fig. S10 ^{19}F NMR spectrum of complex **Ir2** in d_6-DMSO.

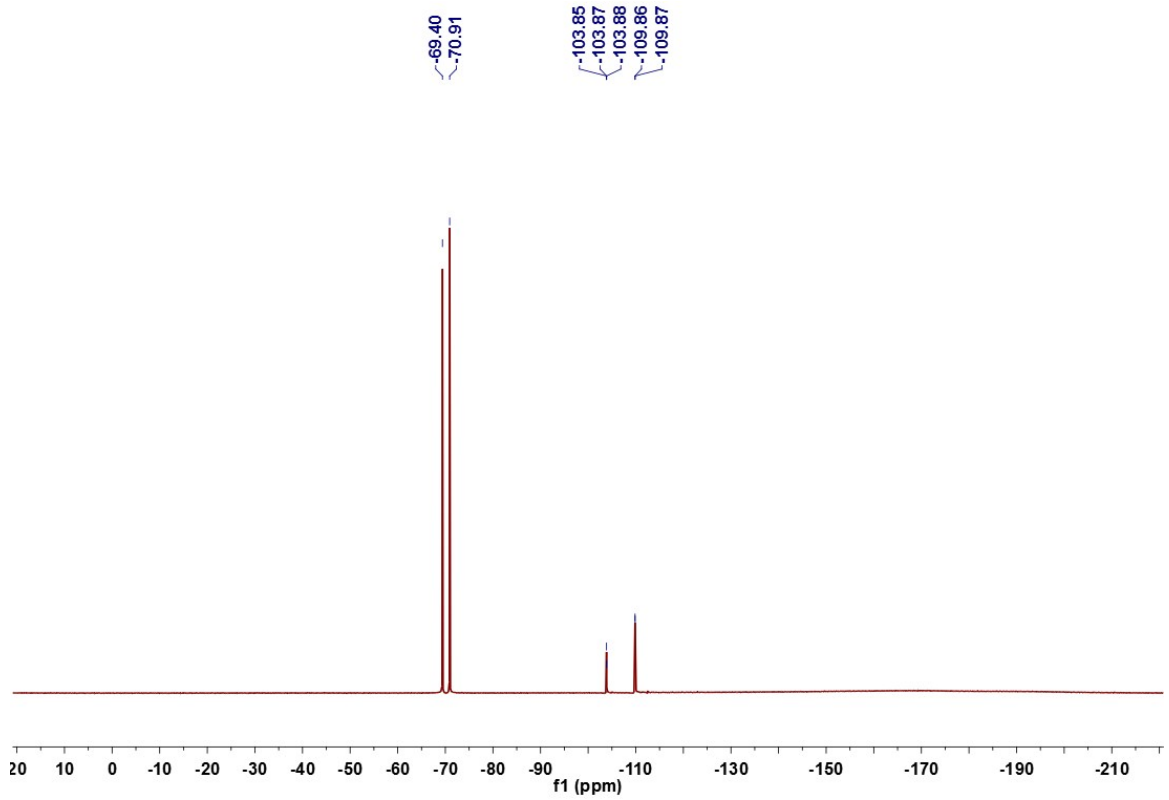

Fig. S11 ^{19}F NMR spectrum of complex **Ir3** in d_6-DMSO.

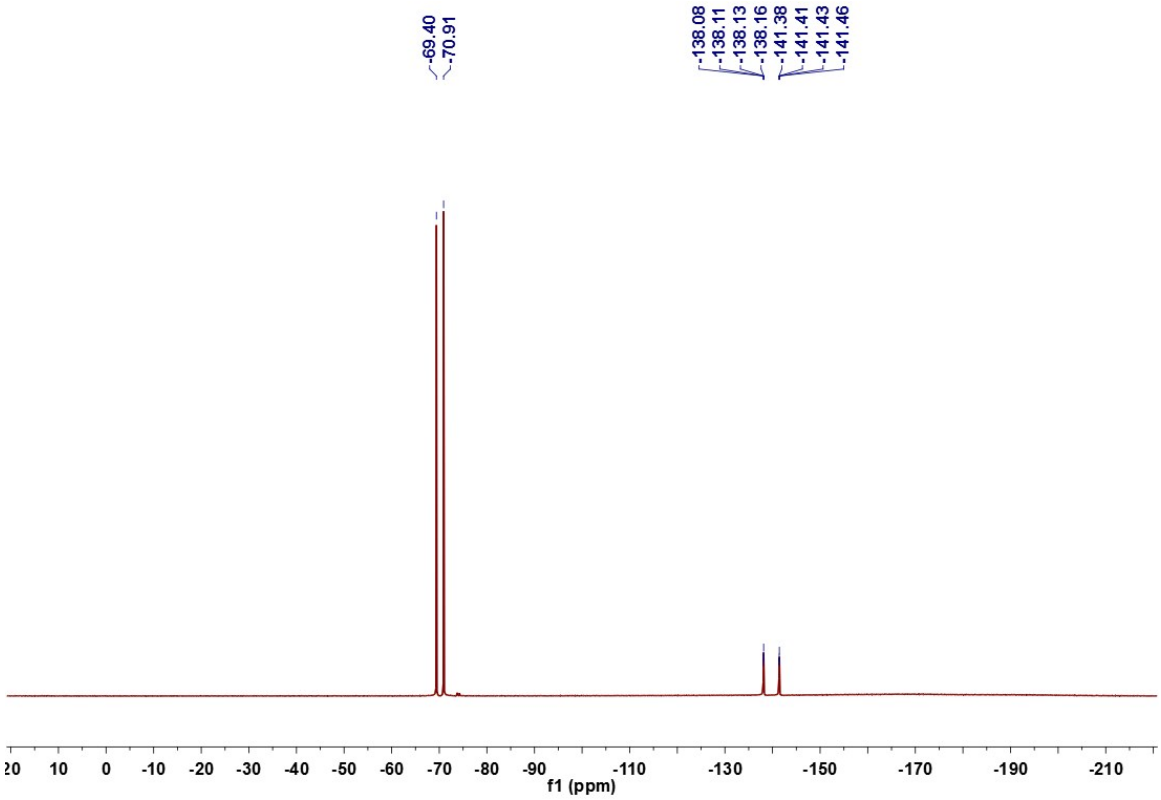

Fig. S12 ^{19}F NMR spectrum of complex **Ir4** in d_6-DMSO.

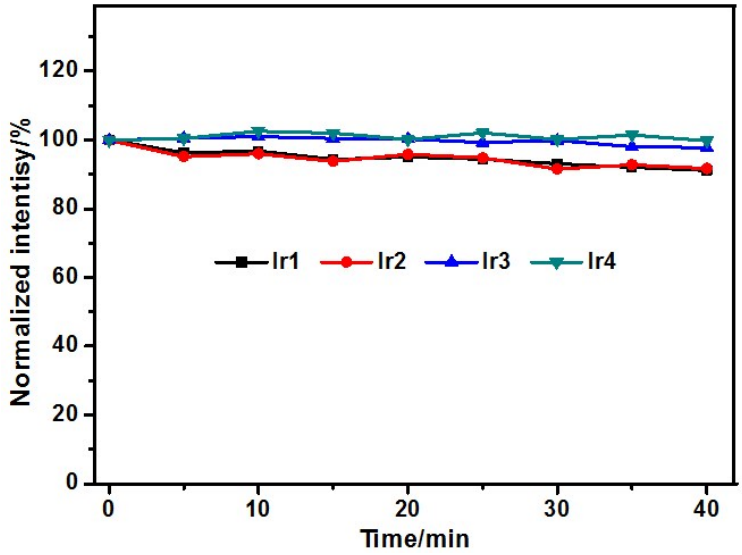

Fig. S13 Stability of the phosphorescence intensity of **Ir1-Ir4** in PBS under irradiation at 405 nm of 21.2 mW/cm^2.

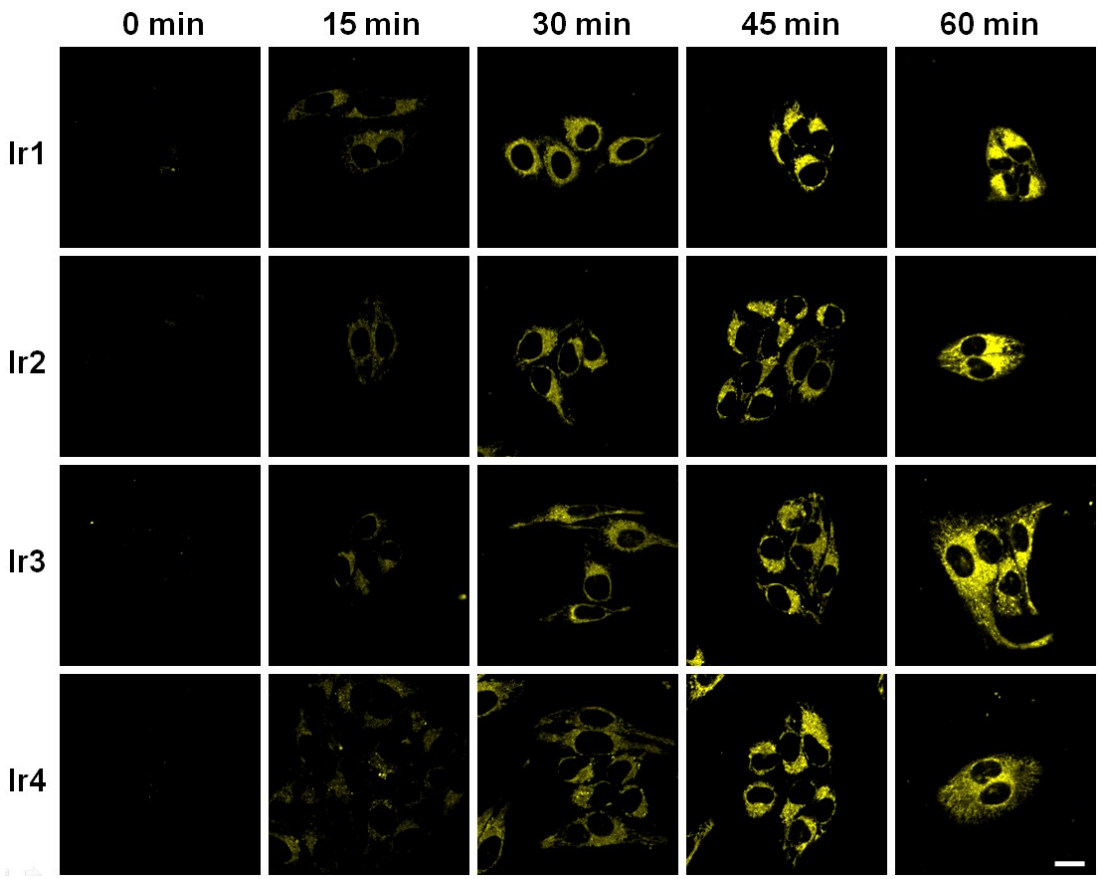

Fig. S14 Real-time uptake monitoring of the complexes (10 μM) in HeLa cells at incubation times of 0-60 min. The complexes were excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.

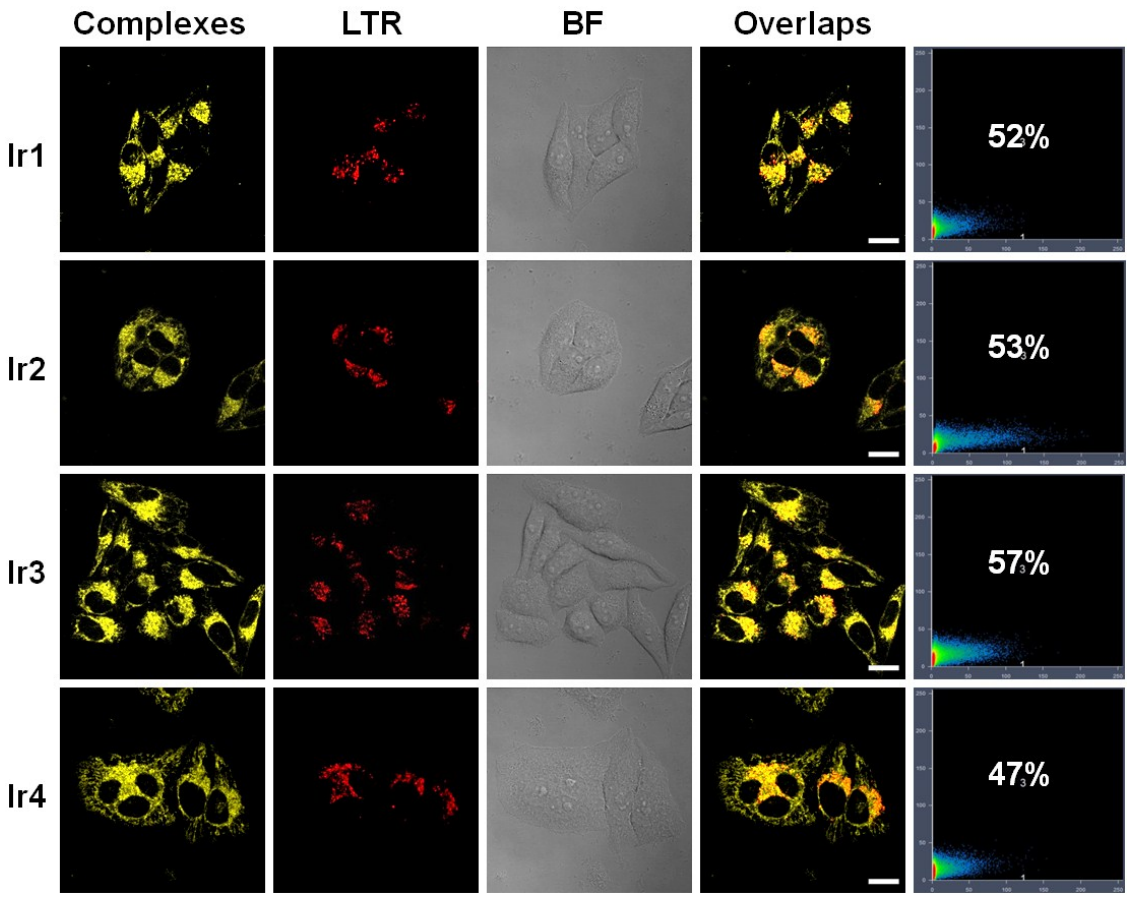

Fig. S15 Confocal images of HeLa cells co-labeled with the complexes (10 μM, 1 h) and the commercial lysosomal imaging agent LTR (50 nM, 0.5 h). The complexes were excited at 405 nm. LTR was excited at 543 nm. The phosphorescence/fluorescence was collected at 550 ± 20 nm and 620 ± 20 nm for the complexes and LTR, respectively. BF: bright field. The 5th column was the Pearson's correlation coefficient. Scale bar: 20 μm.

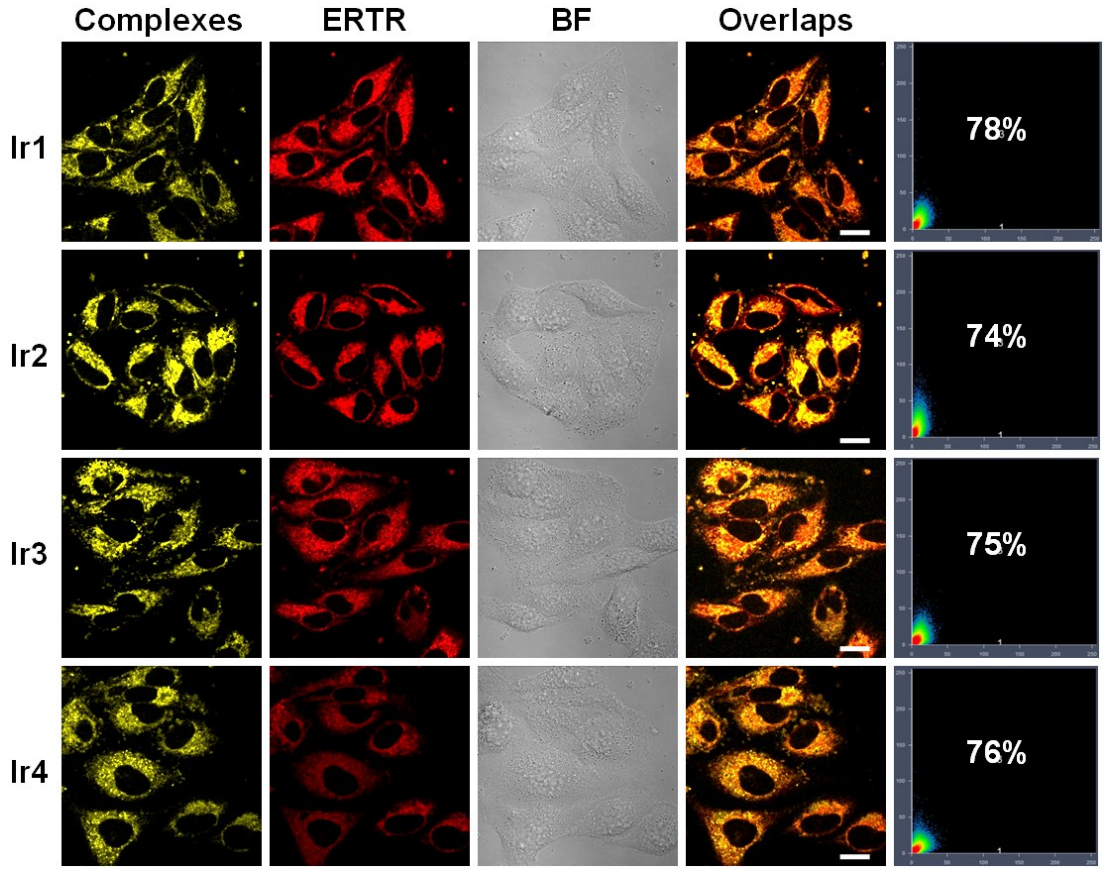

Fig. S16 Confocal images of HeLa cells co-labeled with the complexes (10 μM, 1 h) and the commercial ER imaging agent ERTR (1 μM, 0.5 h). The complexes were excited at 405 nm. ERTR was excited at 543 nm. The phosphorescence/fluorescence was collected at 550 ± 20 nm and 620 ± 20 nm for the complexes and ERTR, respectively. BF: bright field. The 5th column was the Pearson correlation coefficient. Scale bar: 20 μm.

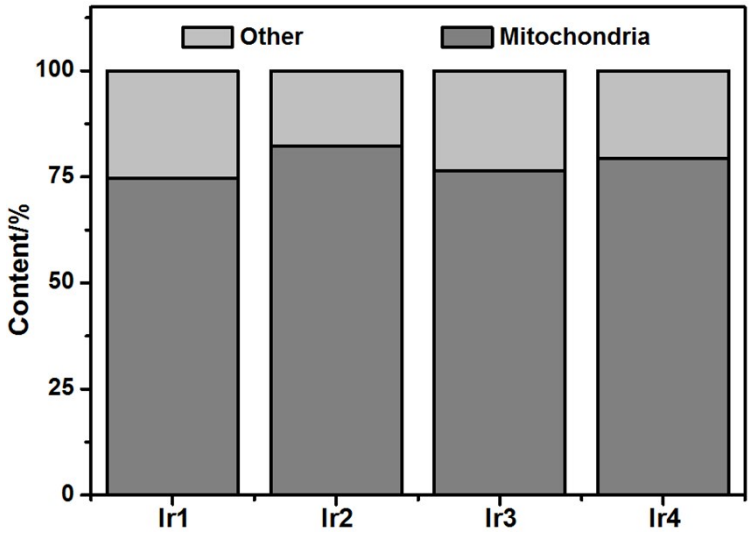

Fig. S17 Distribution analysis of **Ir1-Ir4** in HeLa cells by ICP-MS.

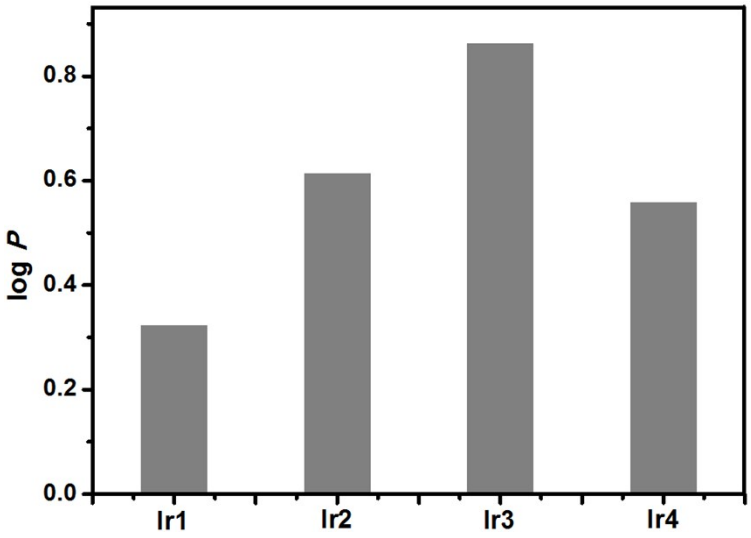

Fig. S18 The log *P* of **Ir1-Ir4** (10 μM).

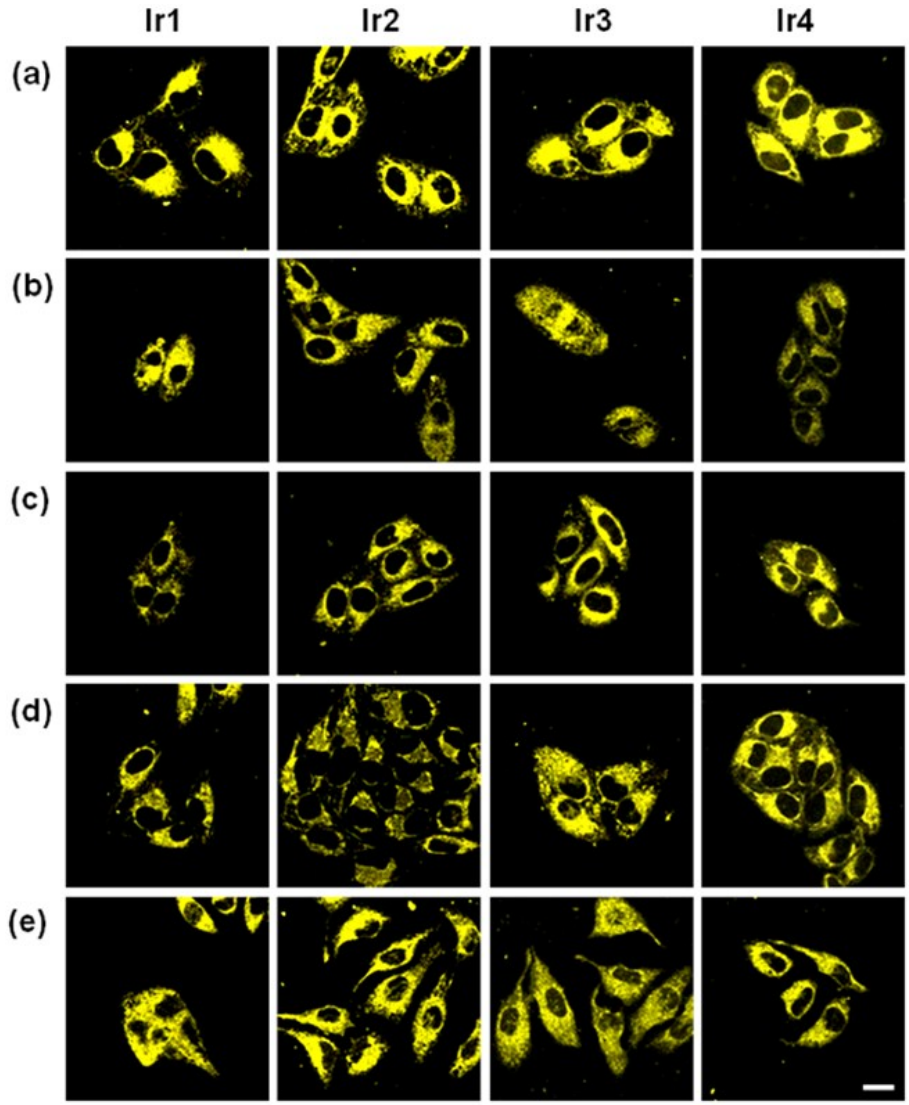

Fig. S19 Confocal images of living HeLa cells incubated with 10 μM **Ir1-Ir4** (λ_{ex} = 405 nm, λ_{em} = 550 ± 20 nm) under different conditions. (a) The cells were incubated with 10 μM **Ir1-Ir4** at 37 °C for 1 h. (b) The cells were incubated with 10 μM **Ir1-Ir4** at 4 °C for 1 h. (c) The cells were pretreated with 50 mM 2-deoxy-D-glucose and 5 μM oligomycin in PBS for 1 h at 37 °C and then incubated with 10 μM **Ir1-Ir4** at 37 °C for 1 h. (d and e) The cells were pretreated with endocytic inhibitors NH_4Cl (50 mM), and chloroquine (50 μM) respectively, and then incubated with 10 μM **Ir1-Ir4** at 37 °C for 1 h. Scale bar: 20 μm.

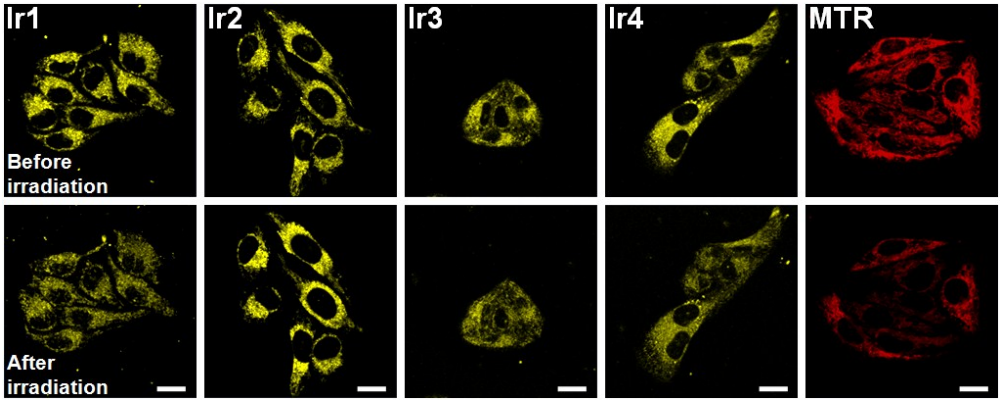

Fig. S20 Confocal images of **Ir1-Ir4** (10 μM, λ_{ex} = 405 nm, λ_{em} = 550 ± 20 nm) and MTR (50 nM, λ_{ex} = 543 nm, λ_{em} = 620 ± 20 nm) before and after photobleaching in HeLa cells. Scale bar: 20 μm.

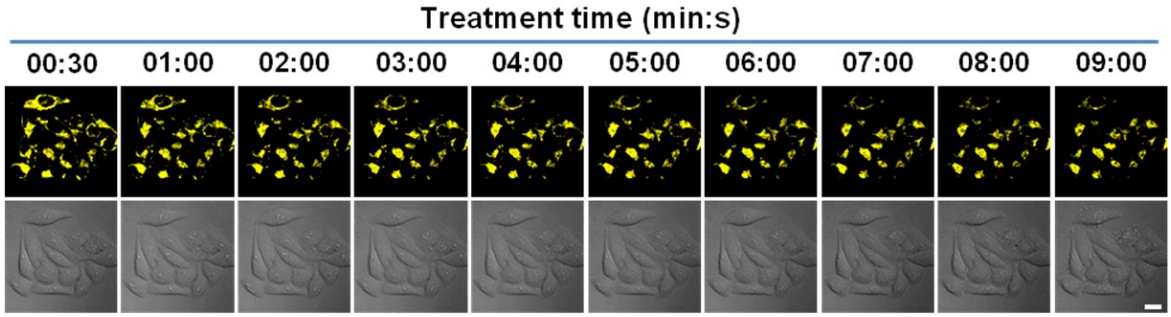

Fig. S21 Real-time imaging of HeLa cells stained with **Ir2** (10 μM) for 1 h at 37 °C, followed by treatment with 30 μM CCCP, with increasing scan time. Phosphorescence images of **Ir2** (upper panels), Brightfield images (lower panels). The complex was excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.

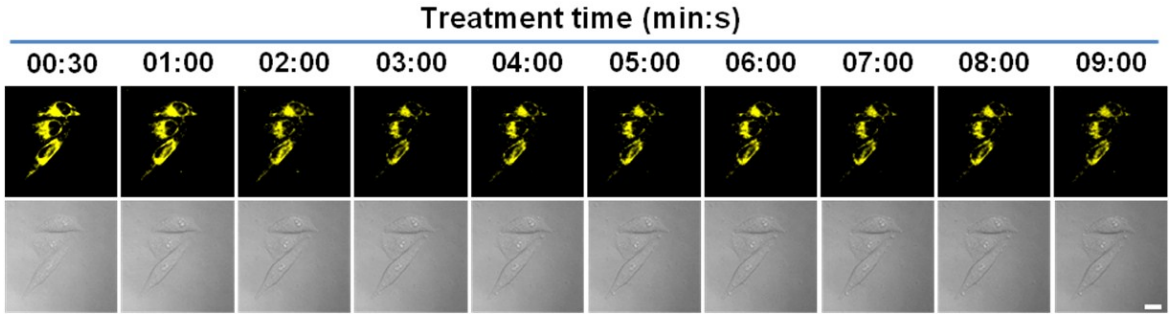

Fig. S22 Real-time imaging of HeLa cells stained with **Ir3** (10 μM) for 1 h at 37 °C, followed by treatment with 30 μM CCCP, with increasing scan time. Phosphorescence images of **Ir3** (upper panels), Brightfield images (lower panels). The complex was excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.

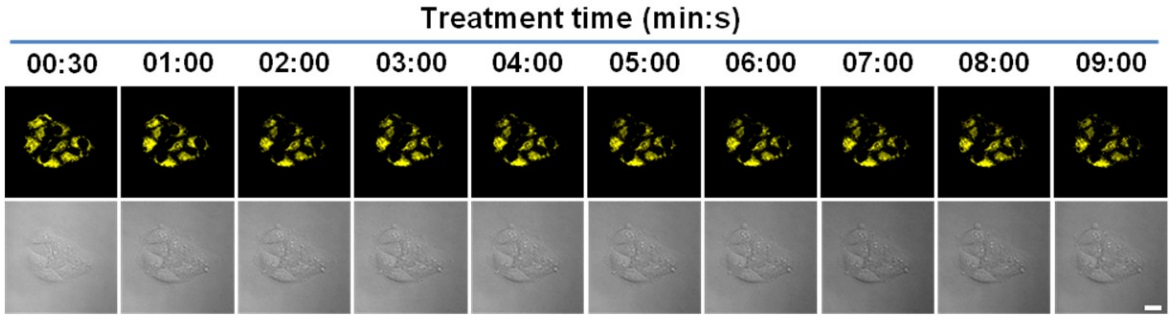

Fig. S23 Real-time imaging of HeLa cells stained with **Ir4** (10 μM) for 1 h at 37 °C, followed by treatment with 30 μM CCCP, with increasing scan time. Phosphorescence images of **Ir4** (upper panel), Brightfield images (lower panels). The complex was excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.

Table S1 Photophysical data for the complexes at 298 K.

Complexes	λ_{ab}[a]	ε[b]	λ_{em}[c]	φ[d]	t[e]
Ir1	457	4.39	549	14.3	731
Ir2	463	7.41	551	12.7	655
Ir3	464	6.40	562	9.23	511
Ir4	468	7.70	569	5.56	400

[a] λ_{ab} maximum values of the absorption spectra (nm). [b] Extinction coefficient in (1×10^3 M^{-1} cm^{-1}). [c] λ_{em} maximum values of the emission spectra (nm). [d] Phosphorescent quantum yield (%). [e] Phosphorescent life time (ns).