

Supporting Information

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

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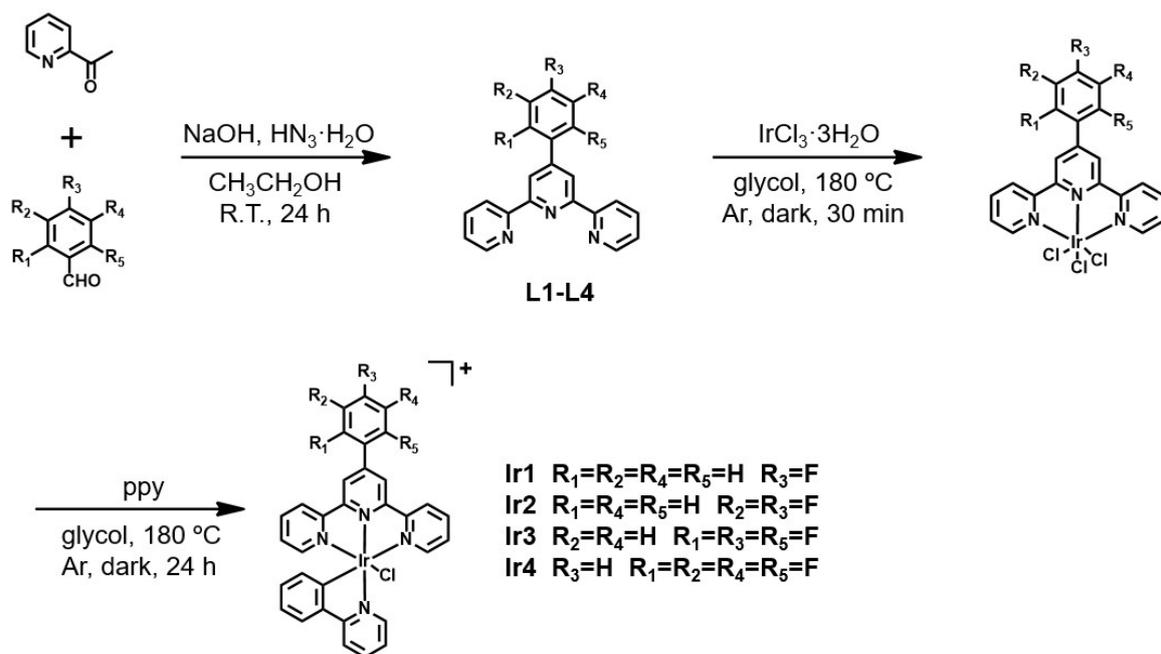
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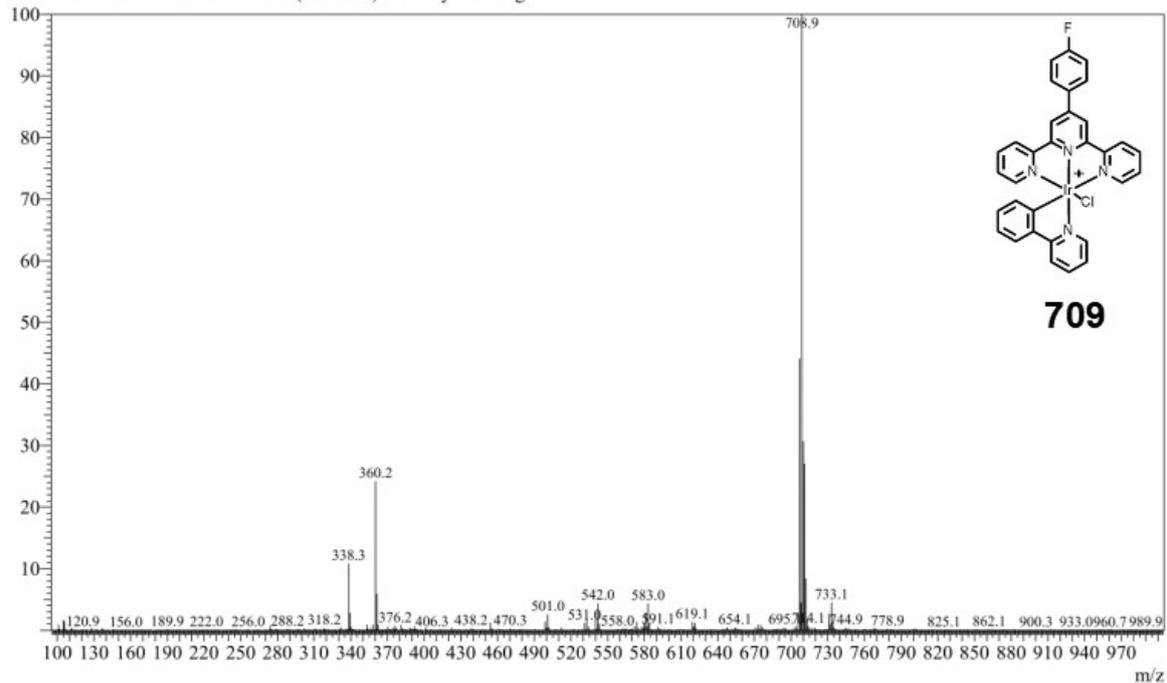
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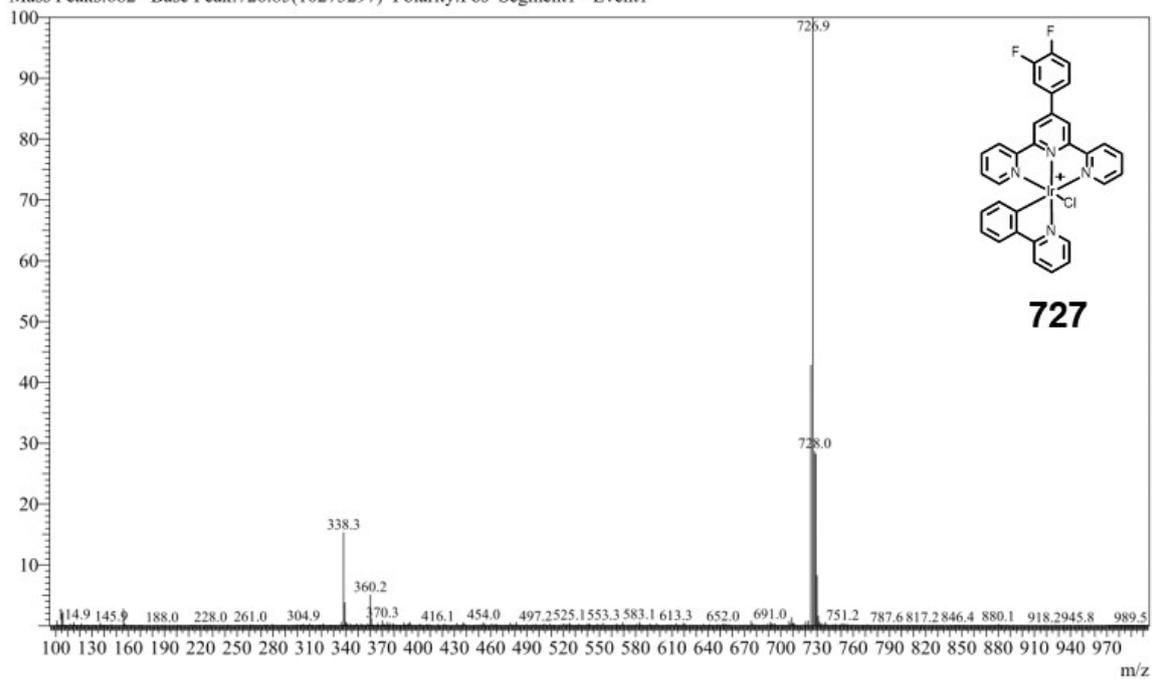
**Scheme S1.** Synthetic route to **Ir1-Ir4**.

BG Mode: Calc 13.183<->13.400(792<->805)  
Mass Peaks:631 Base Peak:708.85(5861333) Polarity:Pos Segment1 - Event1



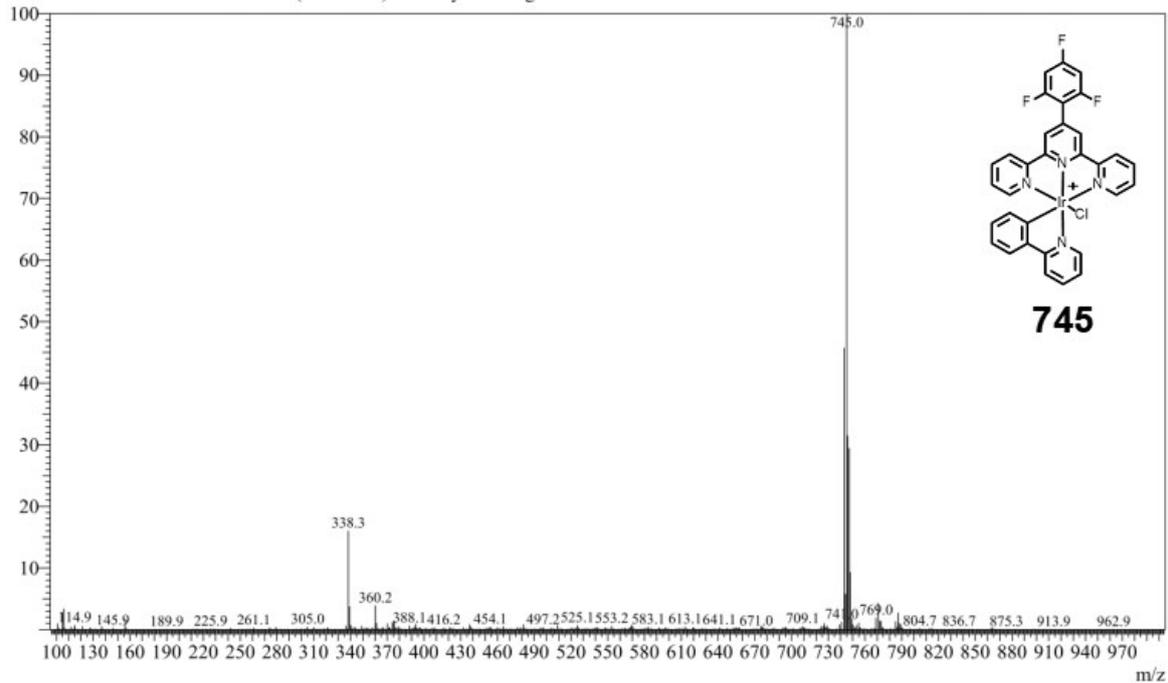
**Fig. S1** ES-MS spectrum of complex **Ir1**.

BG Mode:Calc 7.317<->7.817(440<->470)  
Mass Peaks:682 Base Peak:726.85(10273297) Polarity:Pos Segment1 - Event1

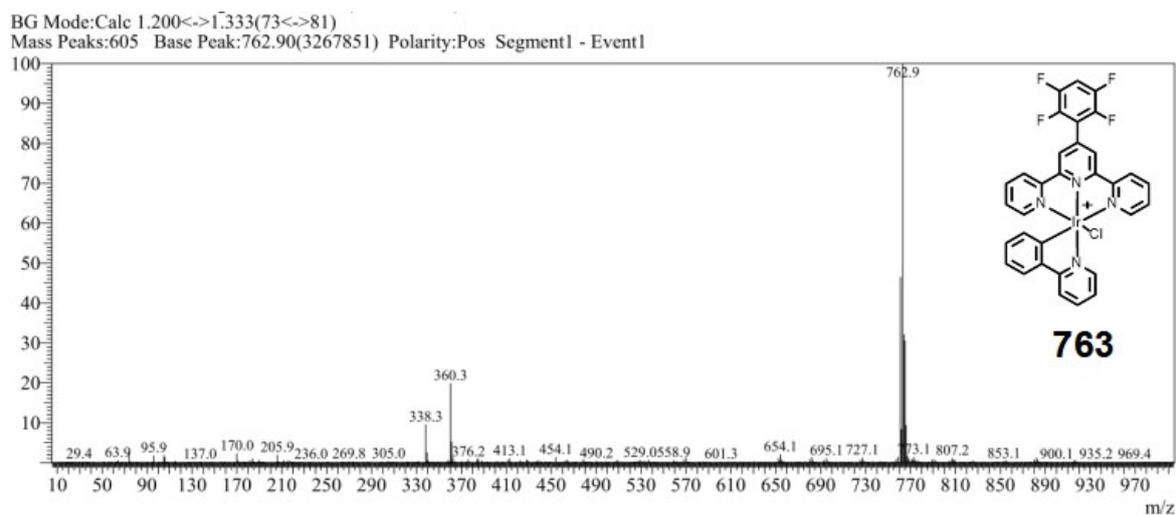


**Fig. S2** ES-MS spectrum of complex **Ir2**.

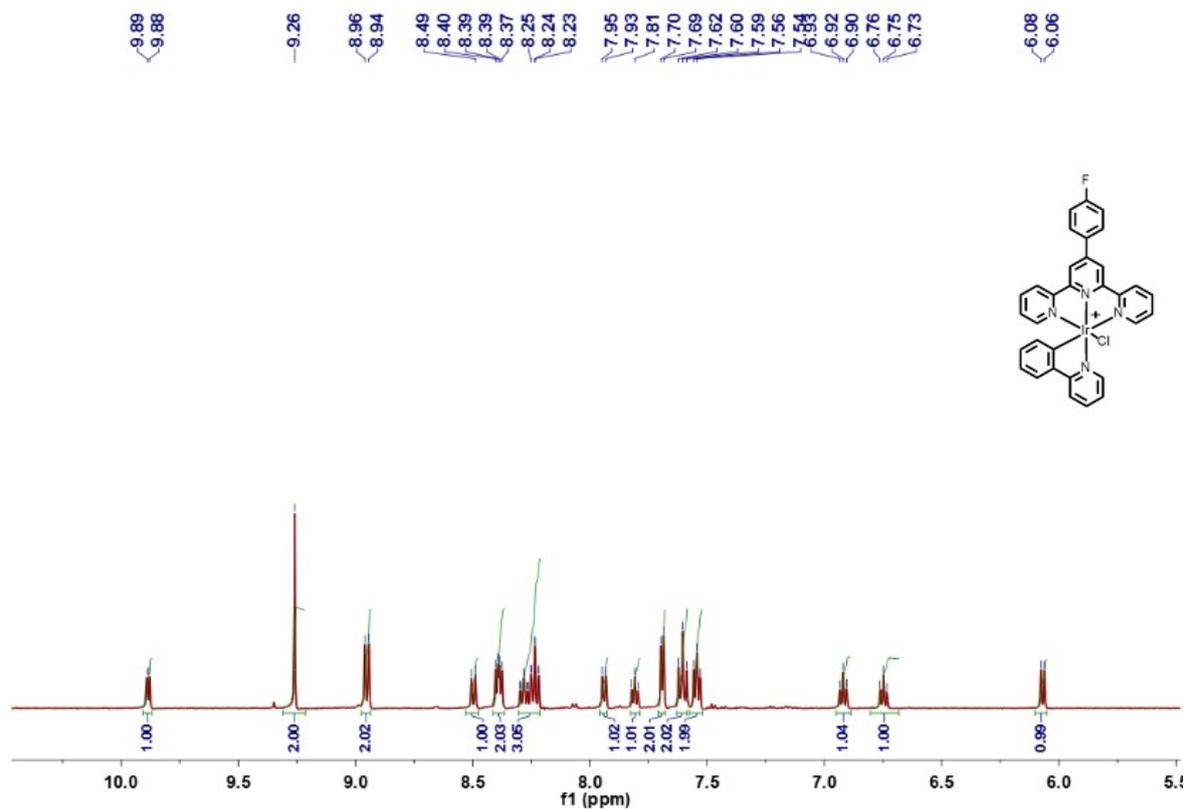
BG Mode:Calc 10.150<->10.817(610<->650)  
Mass Peaks:681 Base Peak:744.95(10659146) Polarity:Pos Segment1 - Event1



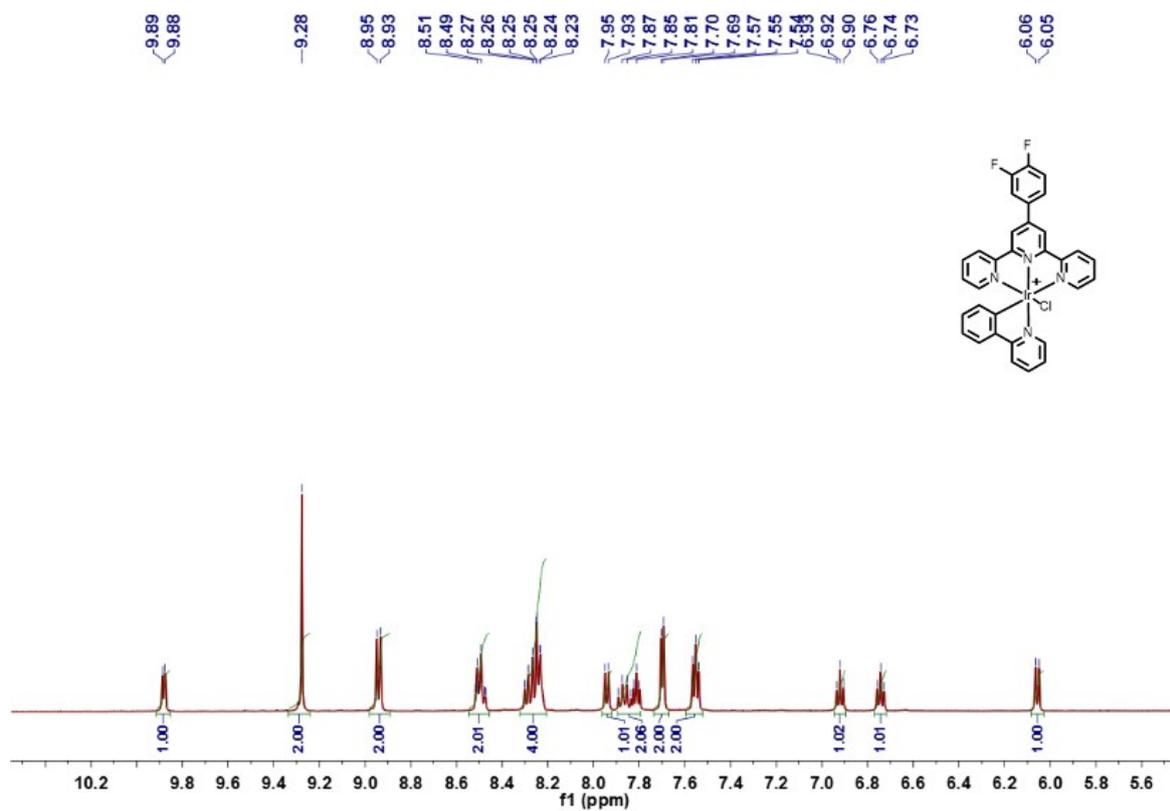
**Fig. S3** ES-MS spectrum of complex **Ir3**.



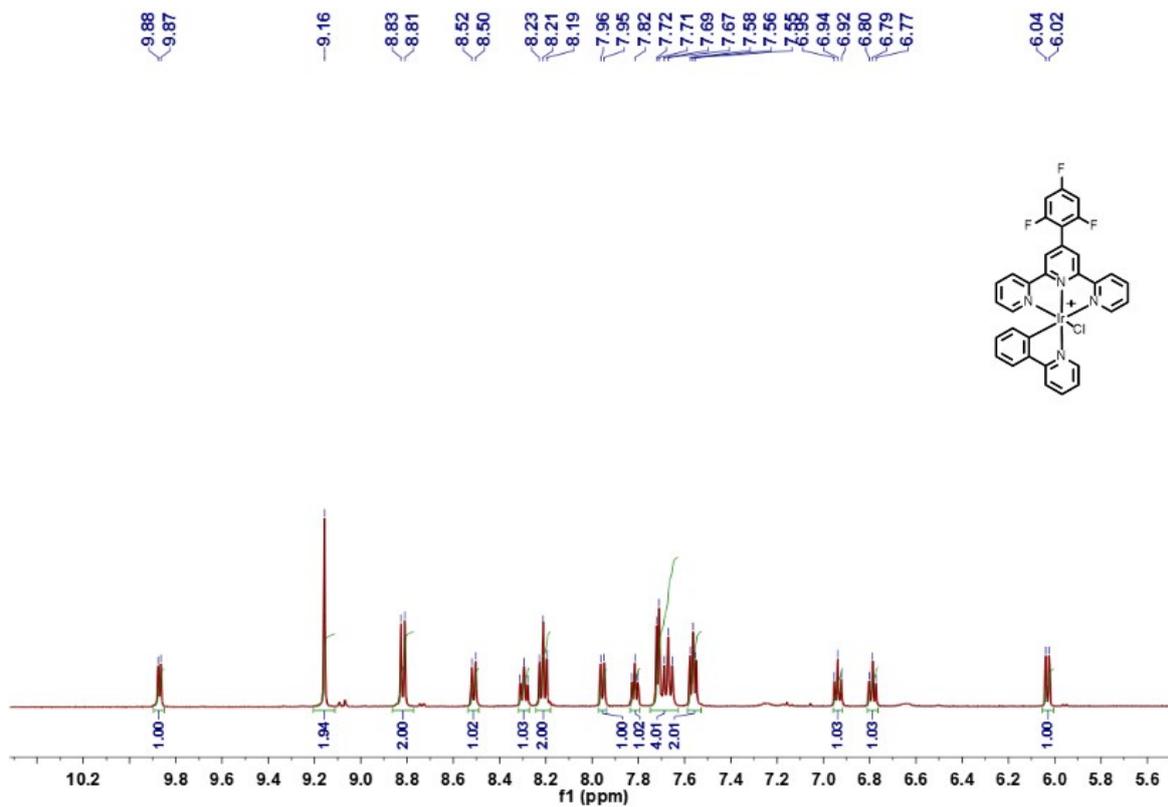
**Fig. S4** ES-MS spectrum of complex **Ir4**.



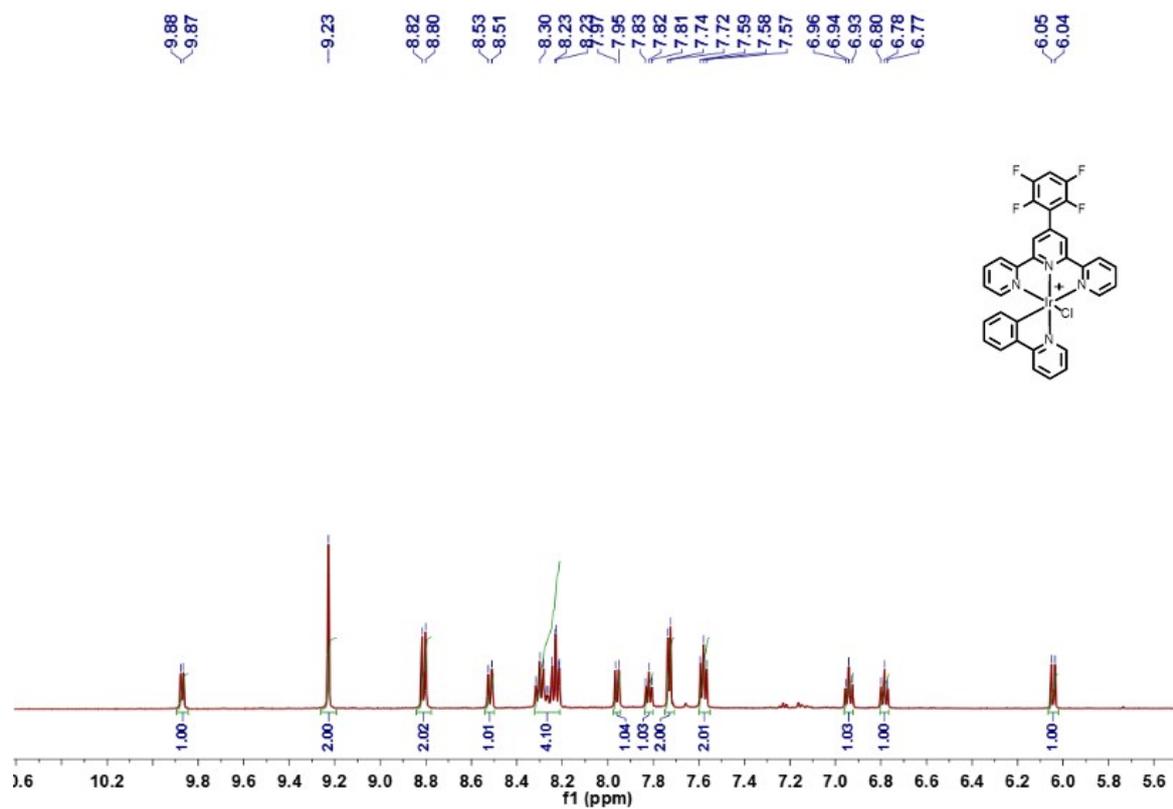
**Fig. S5**  $^1\text{H}$  NMR spectrum of complex **Ir1** in  $d_6$ -DMSO.



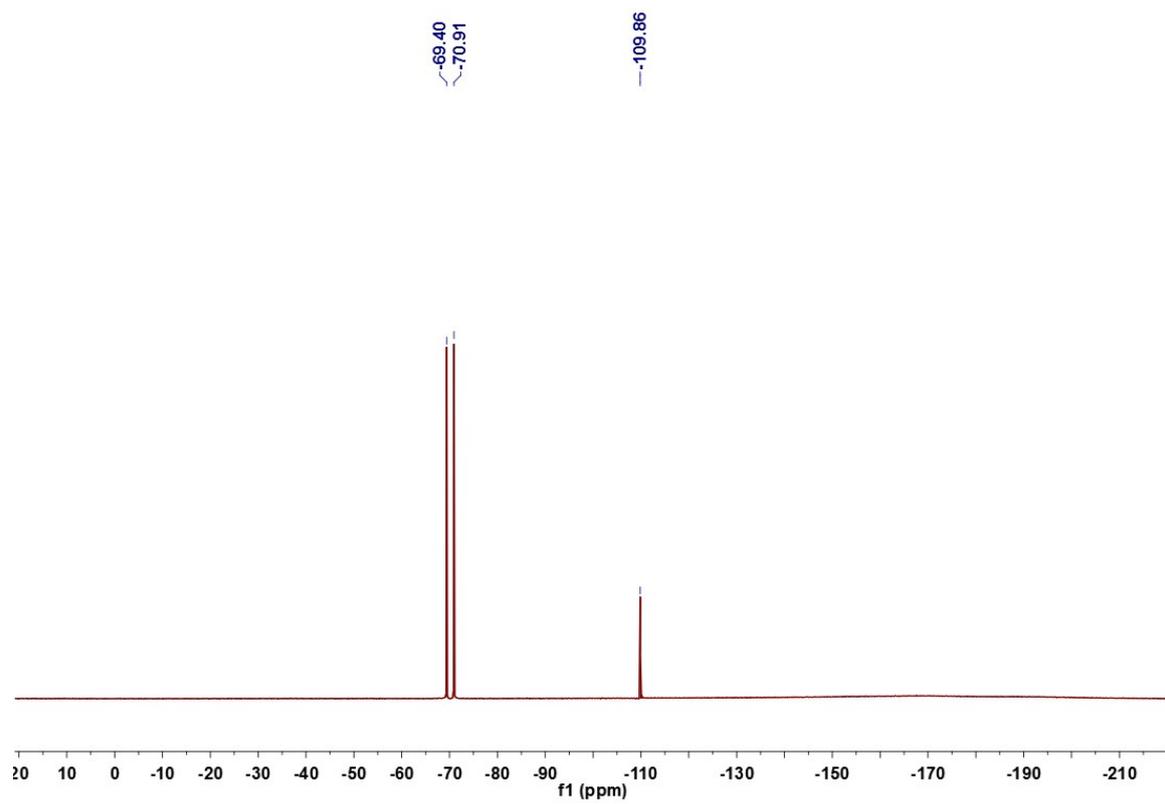
**Fig. S6**  $^1\text{H}$  NMR spectrum of complex **Ir2** in  $d_6$ -DMSO.



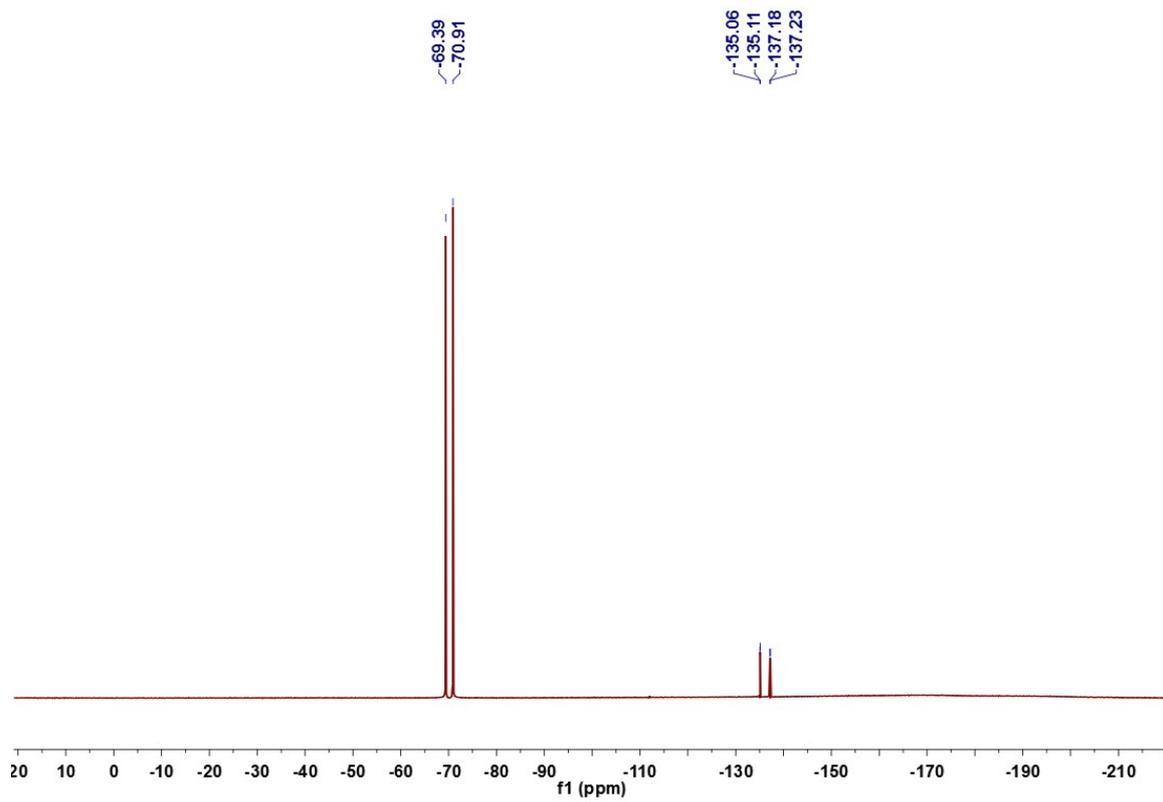
**Fig. S7**  $^1\text{H}$  NMR spectrum of complex **Ir3** in  $d_6$ -DMSO.



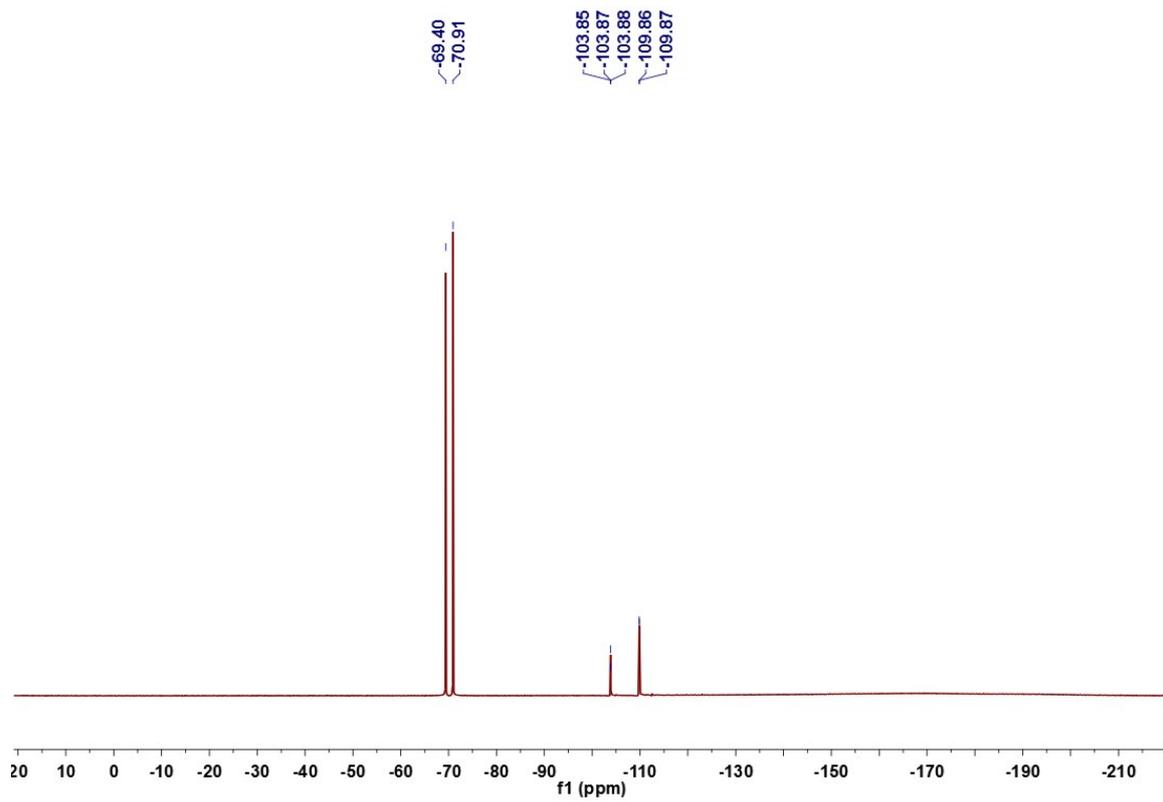
**Fig. S8** <sup>1</sup>H NMR spectrum of complex **Ir4** in *d*<sub>6</sub>-DMSO.



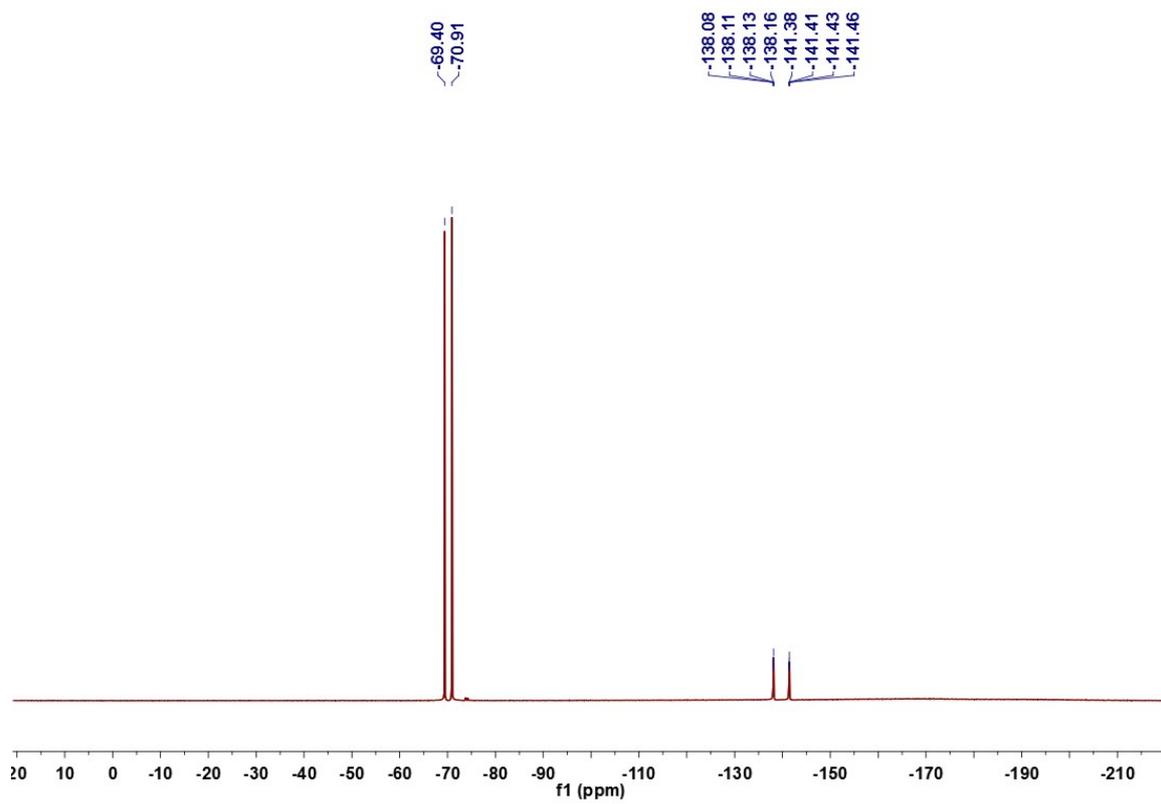
**Fig. S9**  $^{19}\text{F}$  NMR spectrum of complex **Ir1** in  $d_6$ -DMSO.



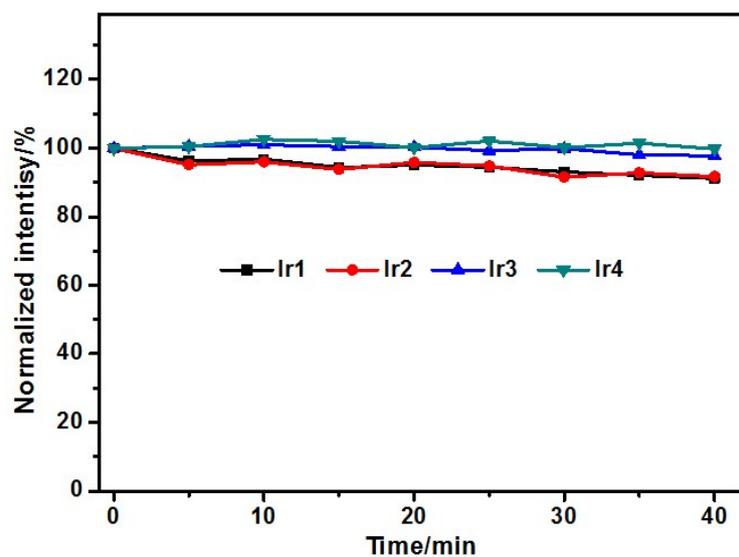
**Fig. S10**  $^{19}\text{F}$  NMR spectrum of complex **Ir2** in  $d_6$ -DMSO.



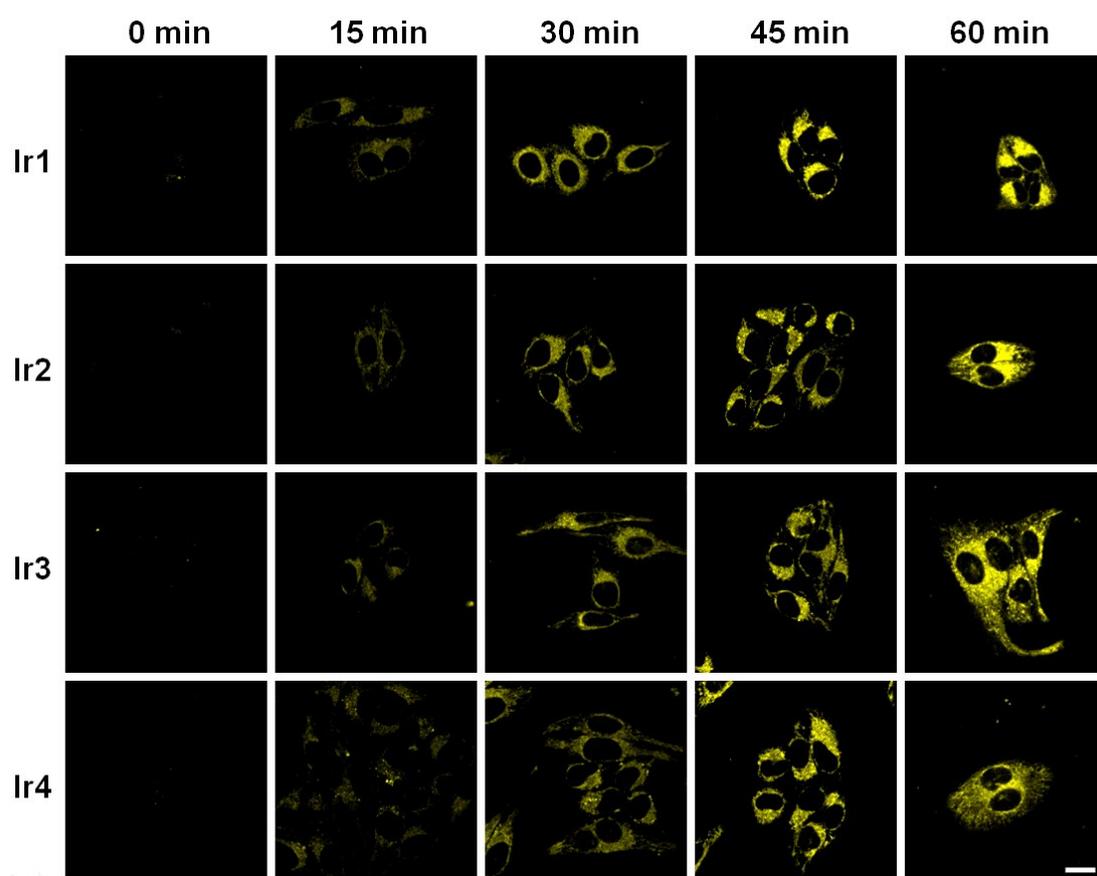
**Fig. S11**  $^{19}\text{F}$  NMR spectrum of complex **Ir3** in  $d_6$ -DMSO.



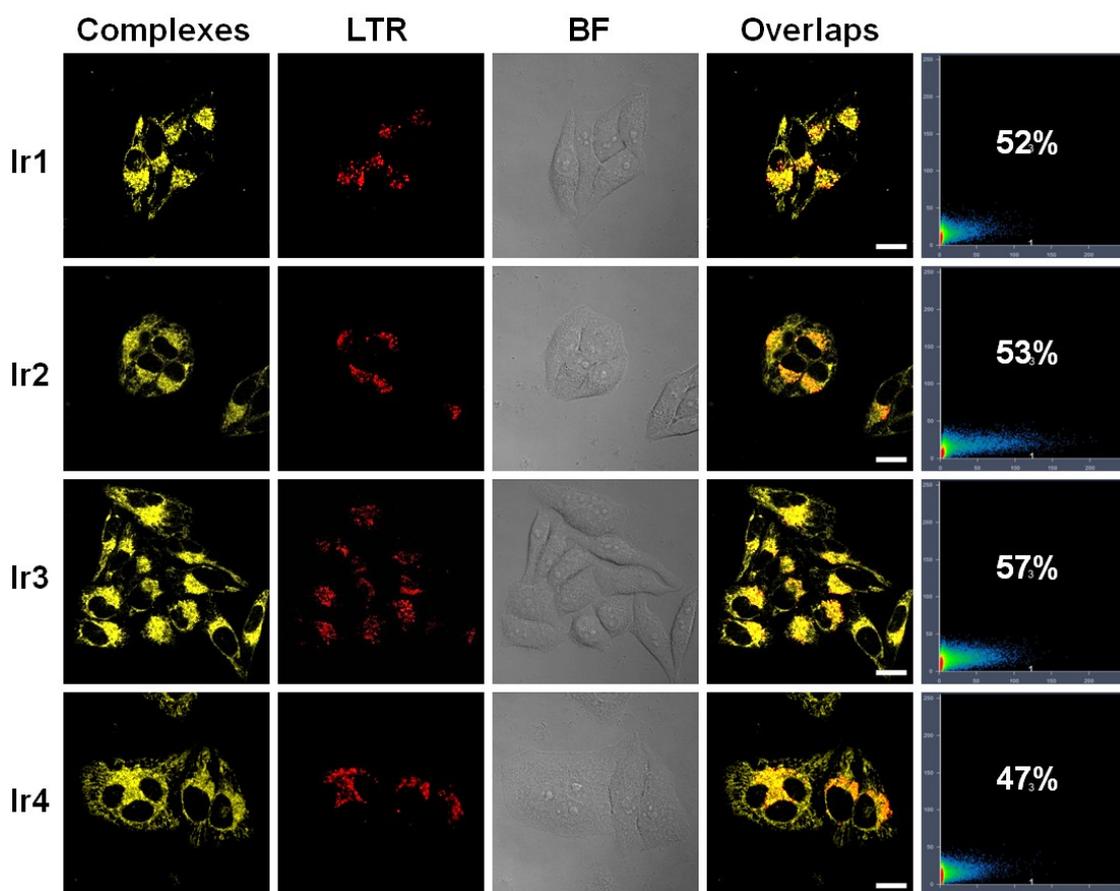
**Fig. S12**  $^{19}\text{F}$  NMR spectrum of complex **Ir4** in  $d_6$ -DMSO.



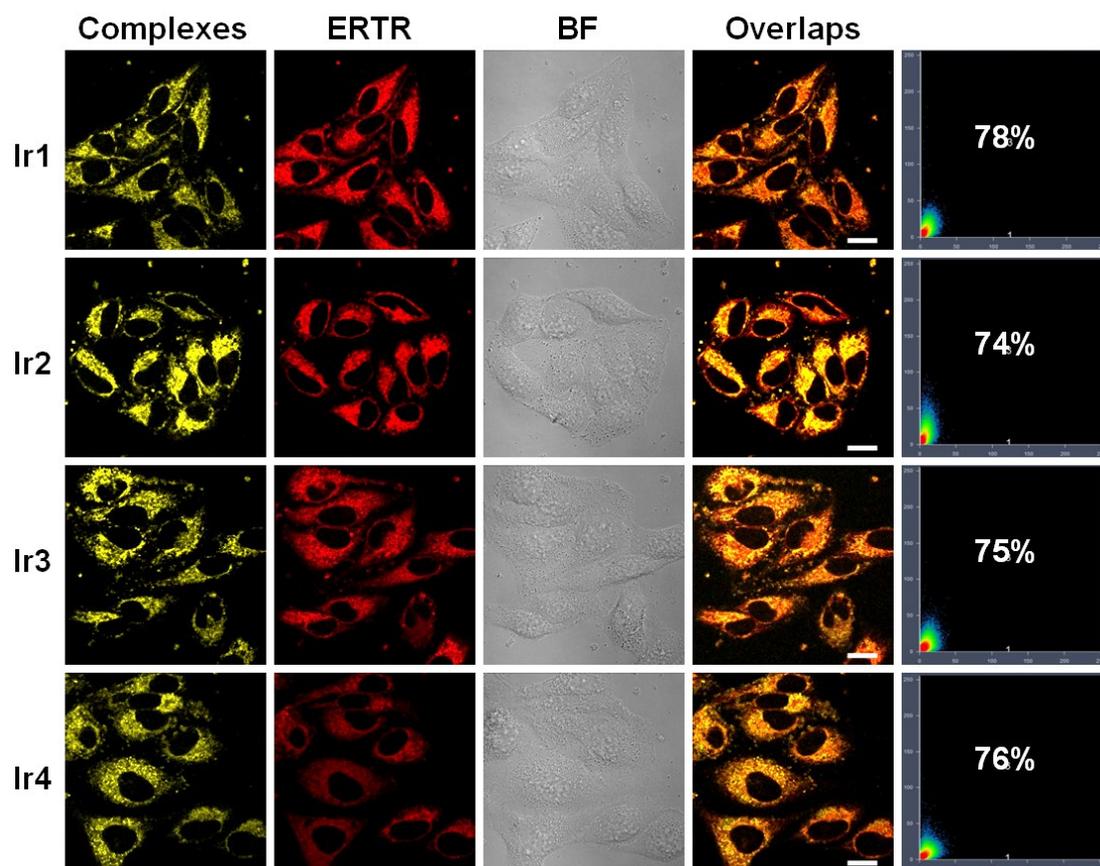
**Fig. S13** Stability of the phosphorescence intensity of **Ir1-Ir4** in PBS under irradiation at 405 nm of 21.2 mW/cm<sup>2</sup>.



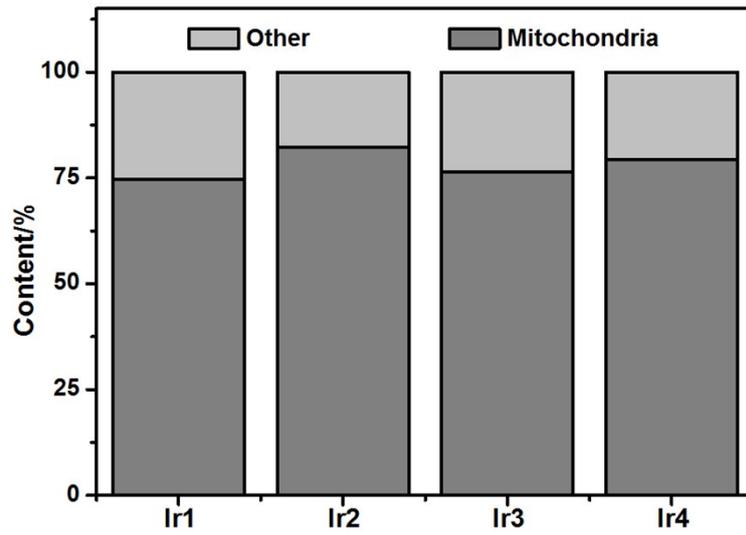
**Fig. S14** Real-time uptake monitoring of the complexes (10  $\mu$ M) in HeLa cells at incubation times of 0-60 min. The complexes were excited at 405 nm. The phosphorescence was collected at  $550 \pm 20$  nm. Scale: 20  $\mu$ m.



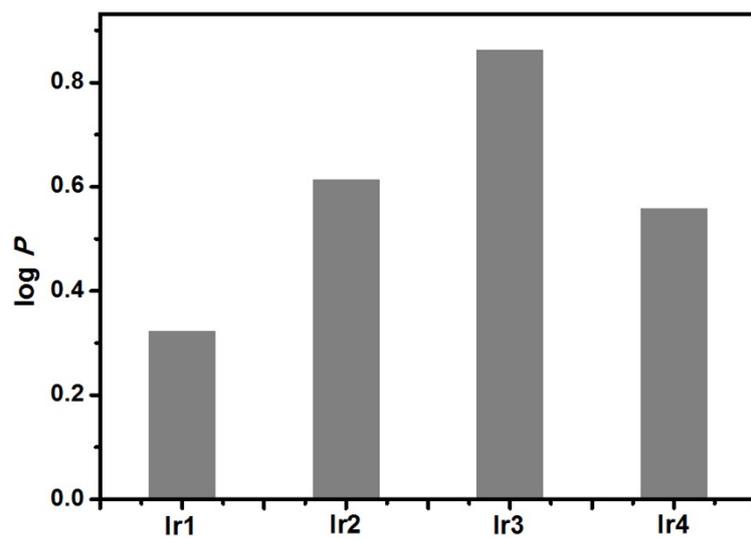
**Fig. S15** Confocal images of HeLa cells co-labeled with the complexes (10  $\mu$ M, 1 h) and the commercial lysosomal imaging agent LTR (50 nM, 0.5 h). The complexes were excited at 405 nm. LTR was excited at 543 nm. The phosphorescence/fluorescence was collected at  $550 \pm 20$  nm and  $620 \pm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



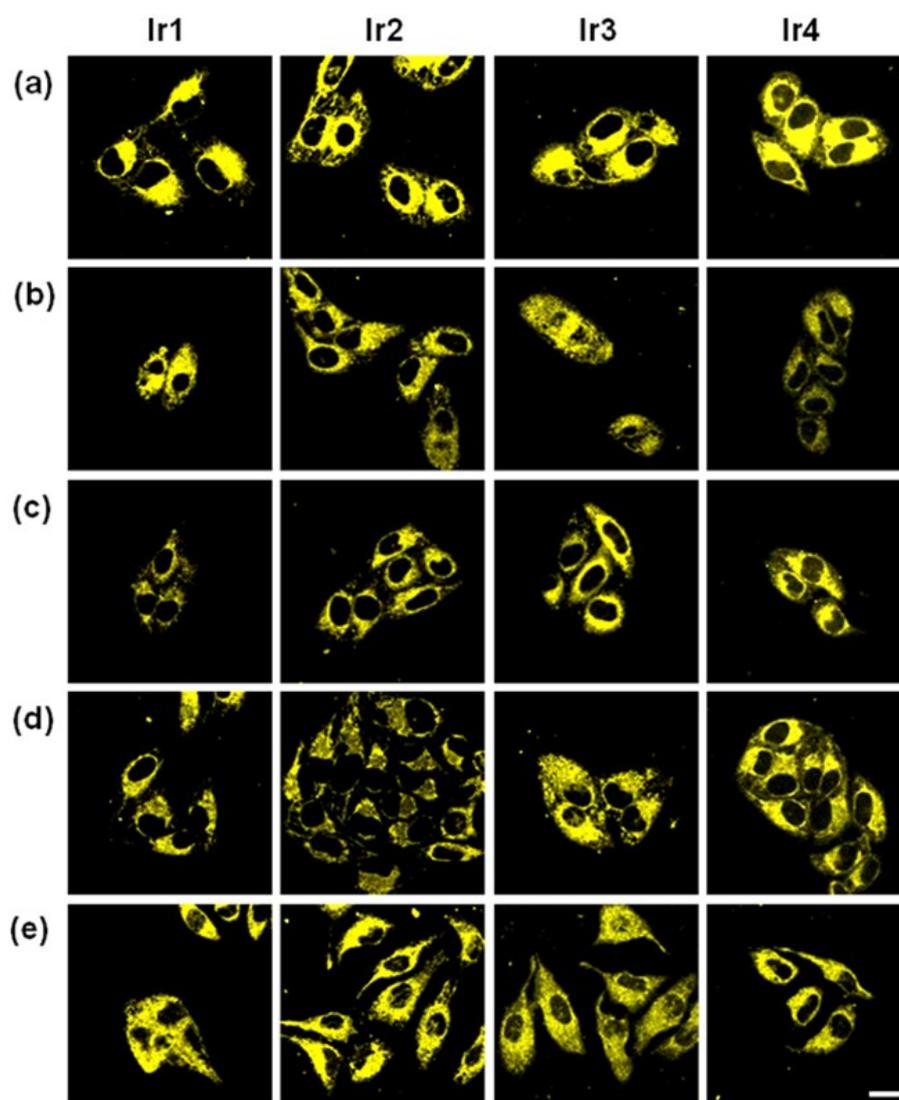
**Fig. S16** Confocal images of HeLa cells co-labeled with the complexes (10  $\mu\text{M}$ , 1 h) and the commercial ER imaging agent ERTR (1  $\mu\text{M}$ , 0.5 h). The complexes were excited at 405 nm. ERTR was excited at 543 nm. The phosphorescence/fluorescence was collected at  $550 \pm 20$  nm and  $620 \pm 20$  nm for the complexes and ERTR, respectively. BF: bright field. The 5<sup>th</sup> column was the Pearson correlation coefficient. Scale bar: 20  $\mu\text{m}$ .



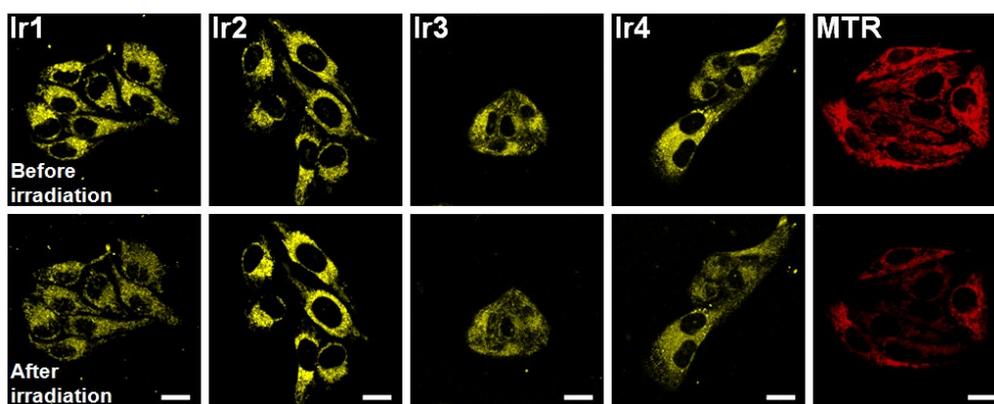
**Fig. S17** Distribution analysis of **Ir1-Ir4** in HeLa cells by ICP-MS.



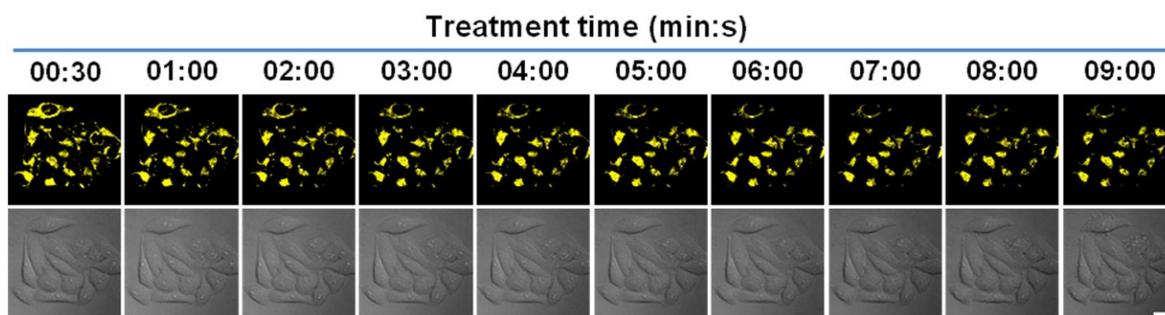
**Fig. S18** The log *P* of Ir1-Ir4 (10 μM).



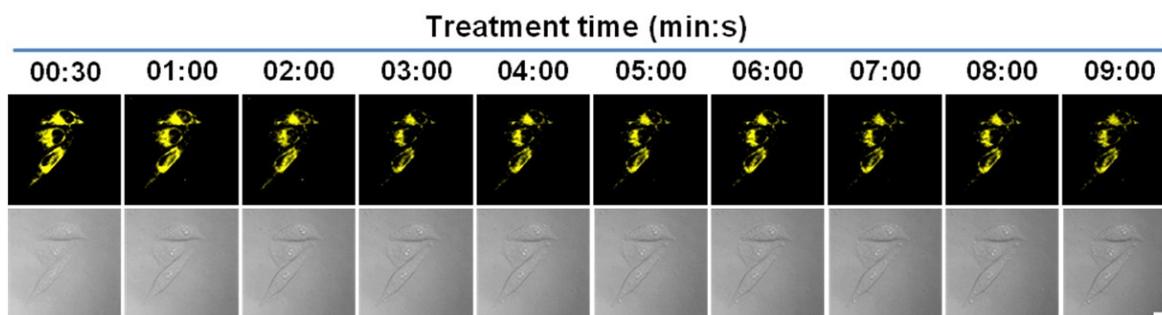
**Fig. S19** Confocal images of living HeLa cells incubated with 10  $\mu\text{M}$  **Ir1-Ir4** ( $\lambda_{\text{ex}} = 405$  nm,  $\lambda_{\text{em}} = 550 \pm 20$  nm) under different conditions. (a) The cells were incubated with 10  $\mu\text{M}$  **Ir1-Ir4** at 37  $^{\circ}\text{C}$  for 1 h. (b) The cells were incubated with 10  $\mu\text{M}$  **Ir1-Ir4** at 4  $^{\circ}\text{C}$  for 1 h. (c) The cells were pretreated with 50 mM 2-deoxy-D-glucose and 5  $\mu\text{M}$  oligomycin in PBS for 1 h at 37  $^{\circ}\text{C}$  and then incubated with 10  $\mu\text{M}$  **Ir1-Ir4** at 37  $^{\circ}\text{C}$  for 1 h. (d and e) The cells were pretreated with endocytic inhibitors  $\text{NH}_4\text{Cl}$  (50 mM), and chloroquine (50  $\mu\text{M}$ ) respectively, and then incubated with 10  $\mu\text{M}$  **Ir1-Ir4** at 37  $^{\circ}\text{C}$  for 1 h. Scale bar: 20  $\mu\text{m}$ .



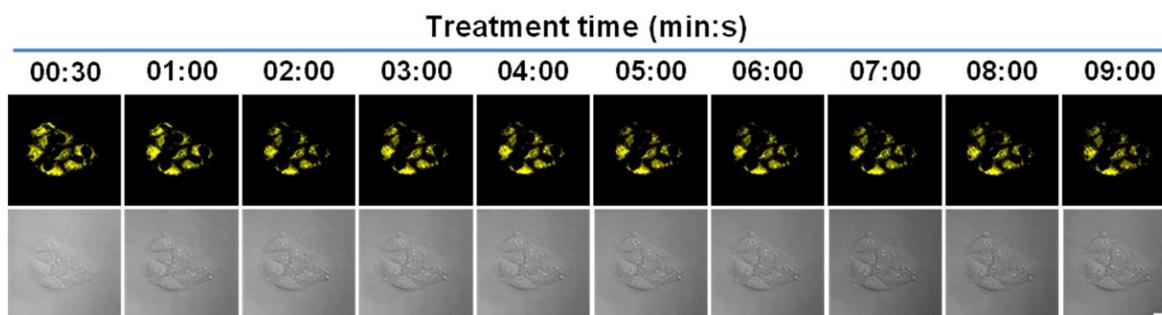
**Fig. S20** Confocal images of **Ir1-Ir4** ( $10 \mu\text{M}$ ,  $\lambda_{\text{ex}} = 405 \text{ nm}$ ,  $\lambda_{\text{em}} = 550 \pm 20 \text{ nm}$ ) and MTR ( $50 \text{ nM}$ ,  $\lambda_{\text{ex}} = 543 \text{ nm}$ ,  $\lambda_{\text{em}} = 620 \pm 20 \text{ nm}$ ) before and after photobleaching in HeLa cells. Scale bar:  $20 \mu\text{m}$ .



**Fig. S21** Real-time imaging of HeLa cells stained with **Ir2** (10  $\mu$ M) for 1 h at 37  $^{\circ}$ C, followed by treatment with 30  $\mu$ M 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  $550 \pm 20$  nm. Scale: 20  $\mu$ m.



**Fig. S22** Real-time imaging of HeLa cells stained with **Ir3** (10  $\mu$ M) for 1 h at 37  $^{\circ}$ C, followed by treatment with 30  $\mu$ M 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  $550 \pm 20$  nm. Scale: 20  $\mu$ m.



**Fig. S23** Real-time imaging of HeLa cells stained with **Ir4** (10  $\mu\text{M}$ ) for 1 h at 37  $^{\circ}\text{C}$ , followed by treatment with 30  $\mu\text{M}$  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  $550 \pm 20$  nm. Scale: 20  $\mu\text{m}$ .

**Table S1** Photophysical data for the complexes at 298 K.

Complexes	$\lambda_{ab}^a$	$\varepsilon^b$	$\lambda_{em}^c$	$\varphi^d$	$t^e$
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

<sup>a</sup>  $\lambda_{ab}$  maximum values of the absorption spectra (nm). <sup>b</sup> Extinction coefficient in ( $1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ ). <sup>c</sup>  $\lambda_{em}$  maximum values of the emission spectra (nm). <sup>d</sup> Phosphorescent quantum yield (%). <sup>e</sup> Phosphorescent life time (ns).