Supporting information

ALP-Responsive, Anionic Iridium complex for Specific Recognition of Osteosarcoma Cells

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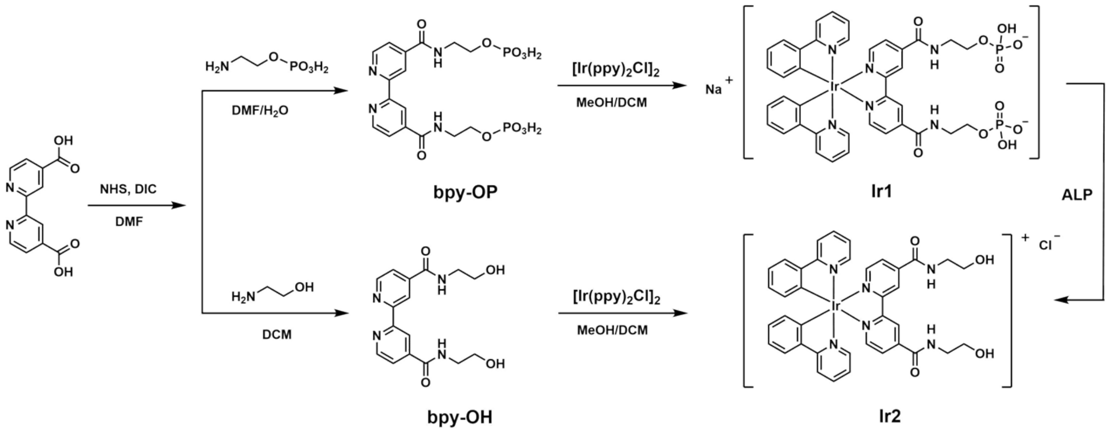

Scheme S1. Synthesis routes of **Ir1** and **Ir2**, and the ALP-mediated convertion reaction. Abbreviations of chemicals are: N-hydroxysuccinimide (NHS); N,N′-diisopropylcarbodiimide (DIC); 2-phenyl pyridine (ppy).

Table S1. Lifetime and fluorescence quantum yield of **Ir1** and **Ir2** in the different media.

Sample	τ_{ave}(ns)	Φ_S
Ir1 in water	74.25	0. 0082
Ir1 in 98%THF	235.78	0.187
Ir2 in water	56.45	0.0056
Ir2 in 98%THF	198.71	0.153

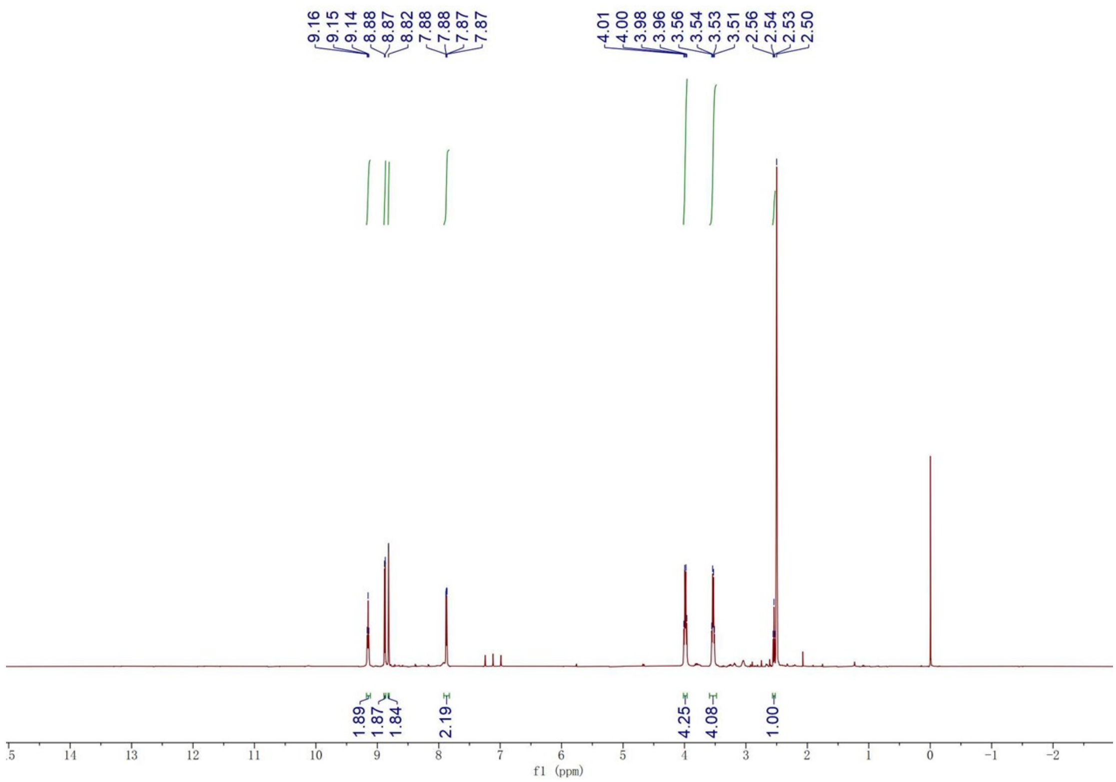

Figure S1. ^{1}H NMR spectrum of ligand **bpy-OP** in D_2O/DMSO-d6 (1:30, v/v).

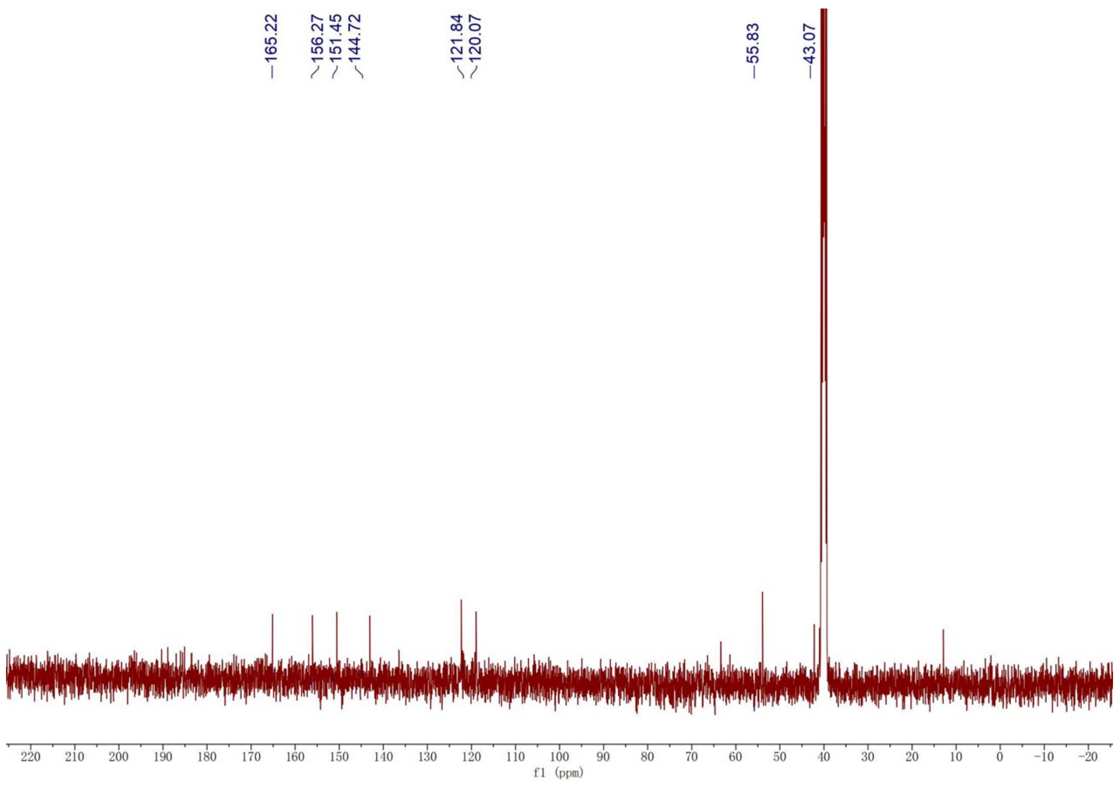

Figure S2. ^{13}C NMR spectrum of ligand **bpy-OP** in D_2O/DMSO-d6 (1:30, v/v).

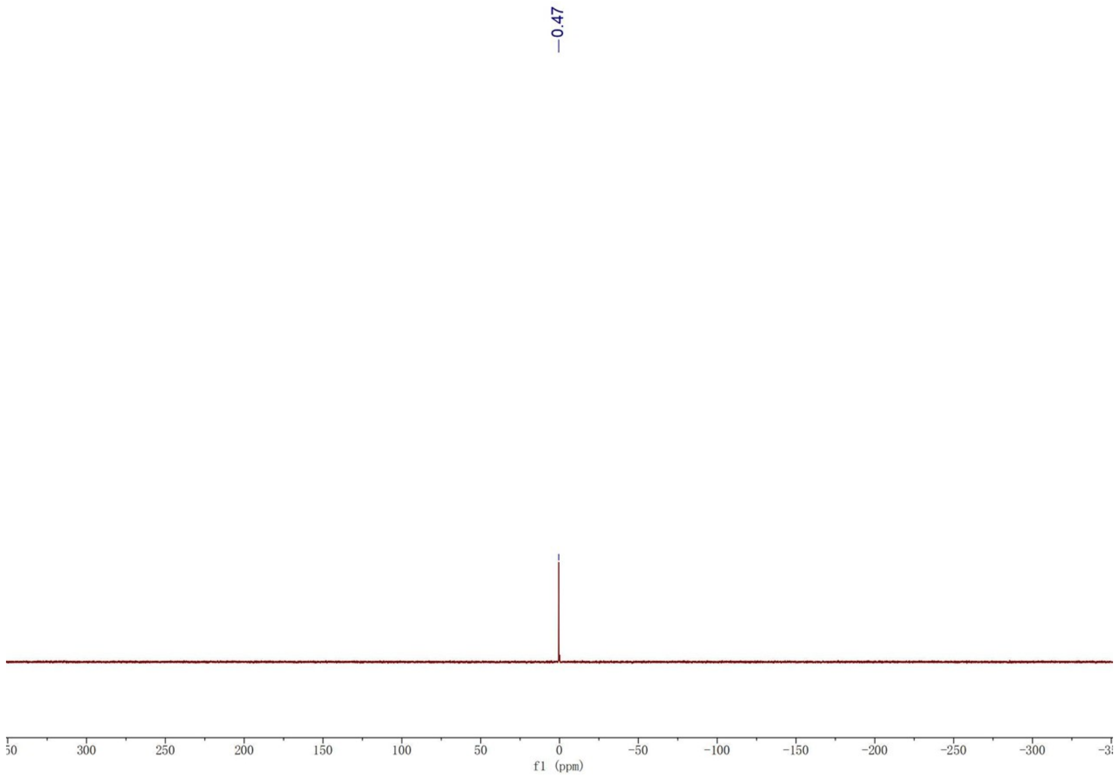

Figure S3. ^{31}P NMR spectrum of ligand **bpy-OP** in D_2O/DMSO-d6 (1:30, v/v).

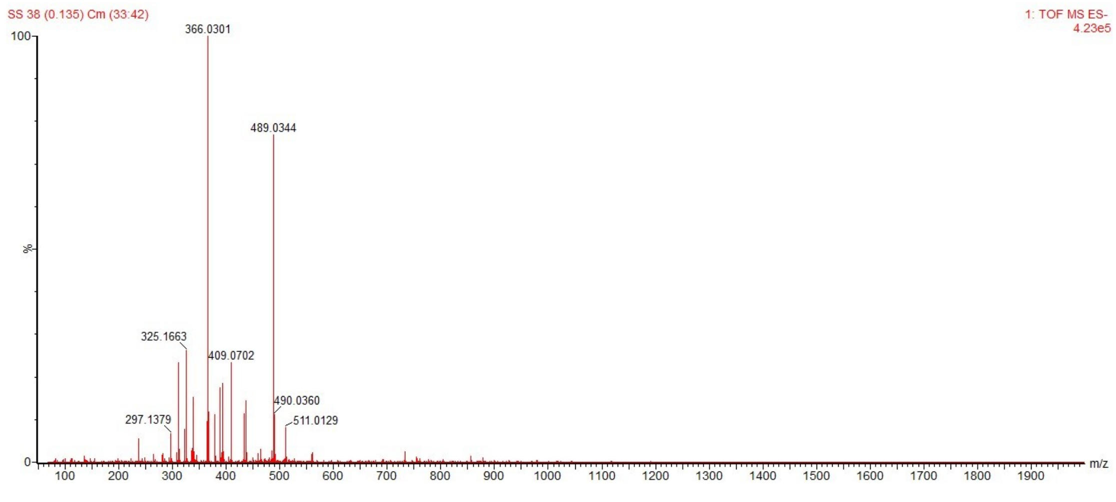

Figure S4. High-resolution mass spectrum of **bpy-OP** in MeOH

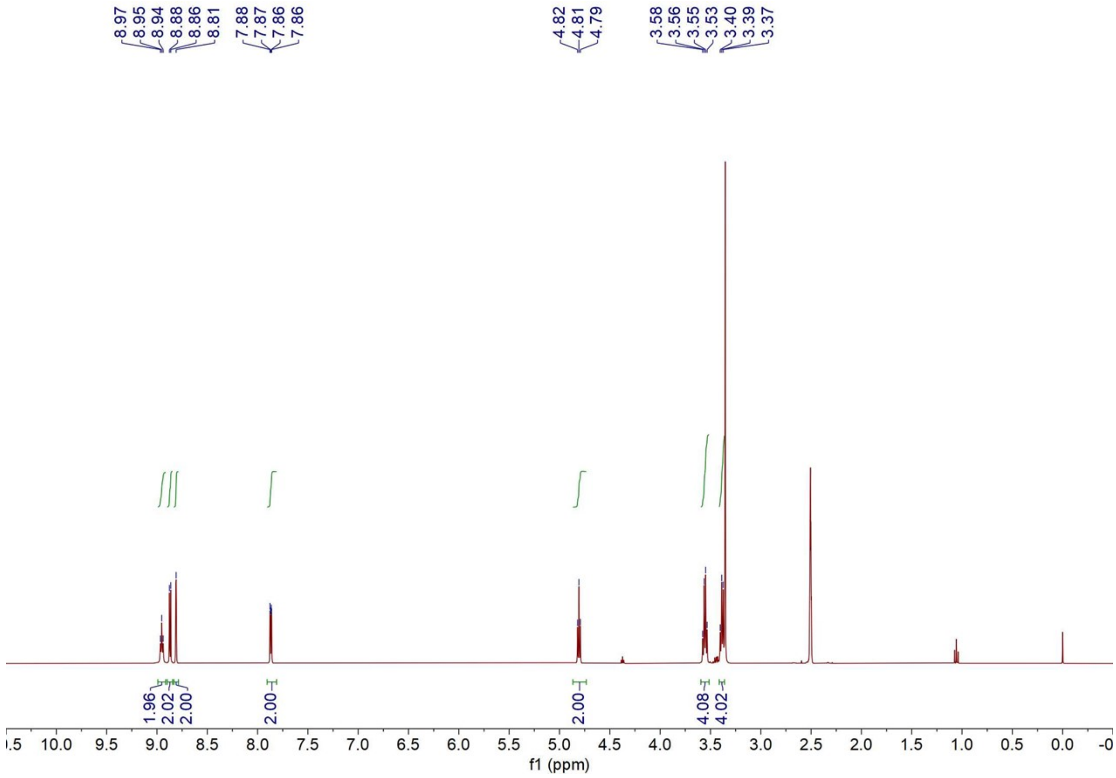

Figure S5. ^{1}H NMR spectrum of ligand **bpy-OH** in D2O/DMSO-d6 (1:30, v/v).

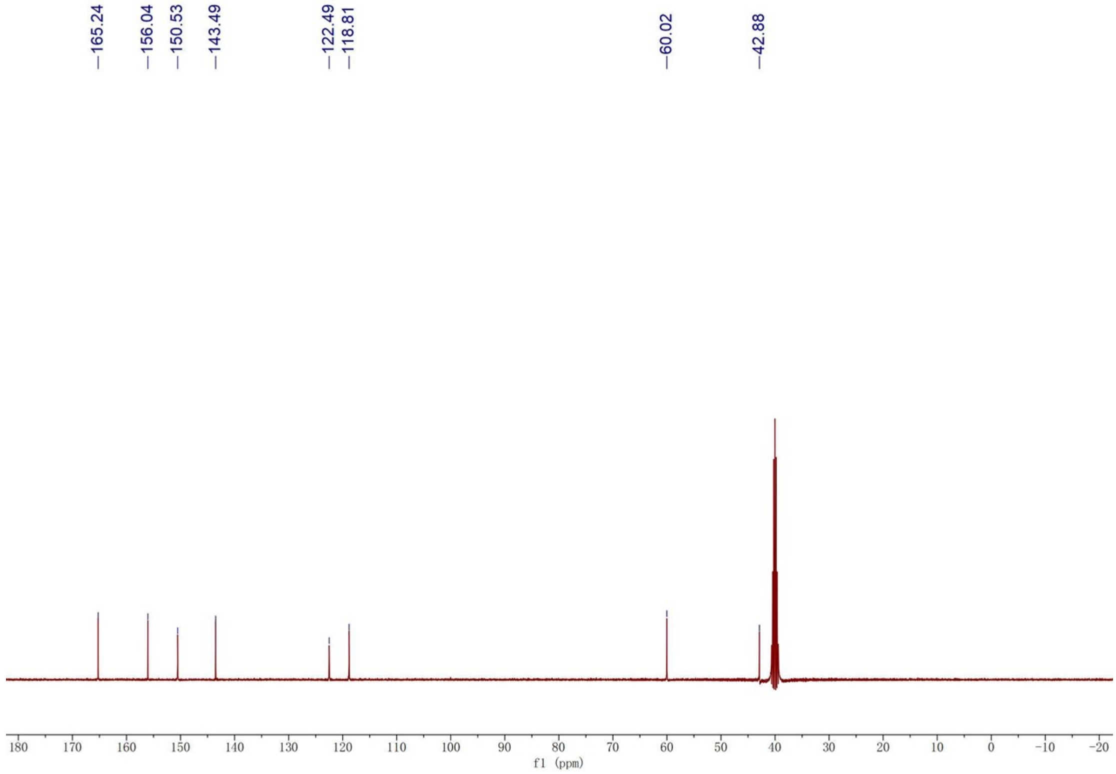

Figure S6. ^{13}C NMR spectrum of ligand **bpy-OH** in D2O/DMSO-d6 (1:30, v/v).

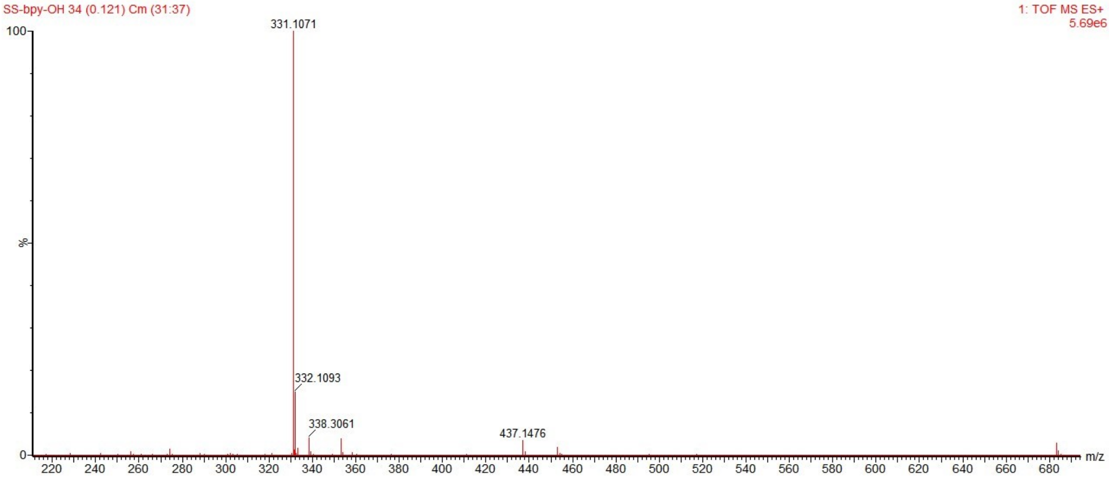

Figure S7. High-resolution mass spectrum of **bpy-OH** in MeOH

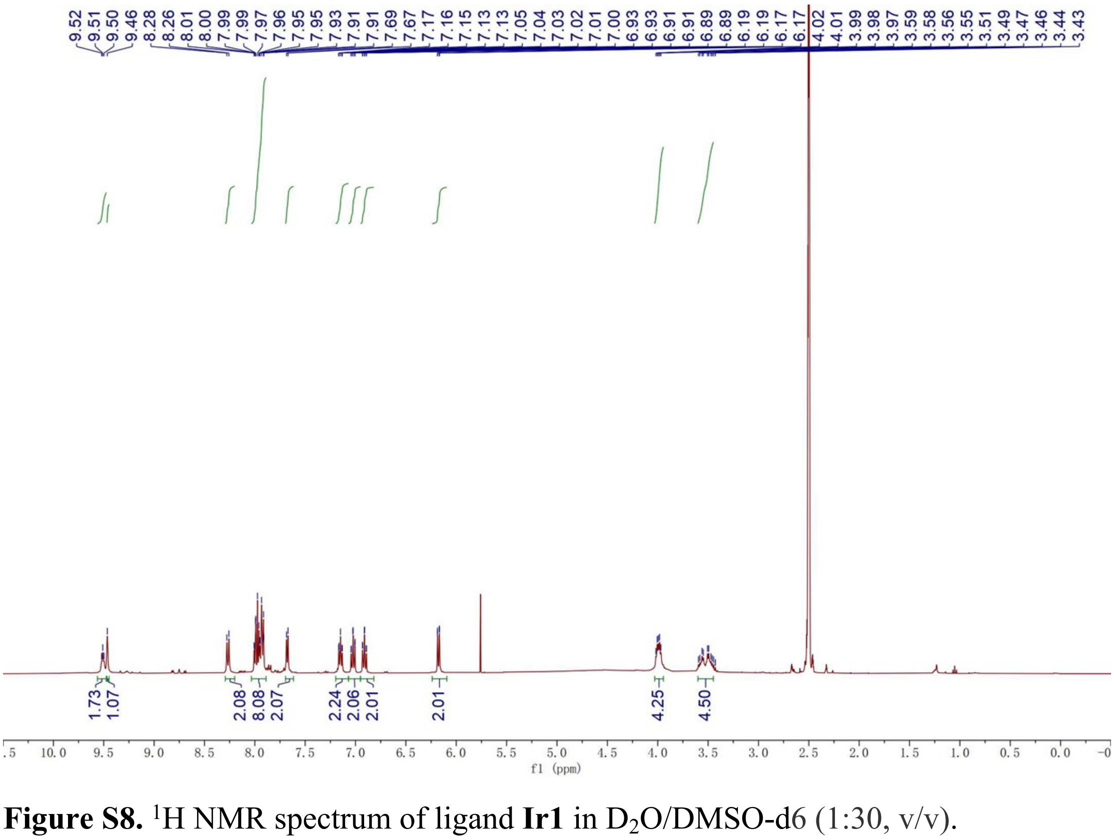

Figure S8. ^{1}H NMR spectrum of ligand **Ir1** in D_2O/DMSO-d6 (1:30, v/v).

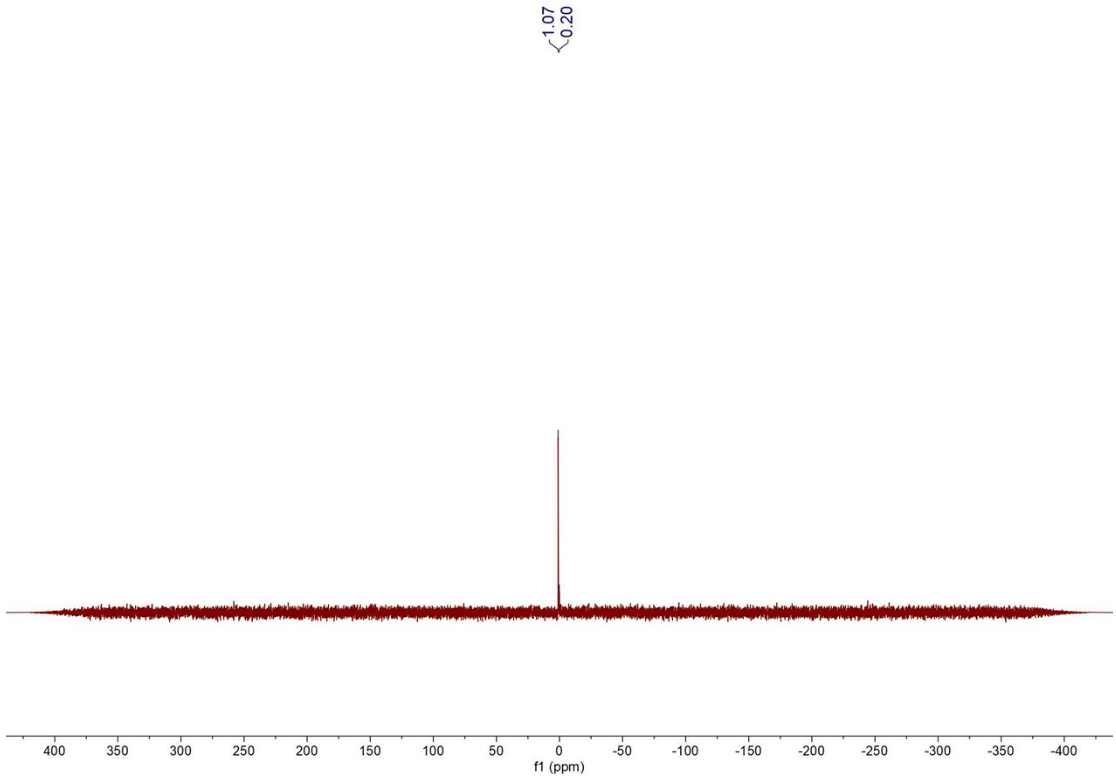

Figure S9. ^{32}P NMR spectrum of ligand **Ir1** in D_2O/DMSO-d6 (1:30, v/v).

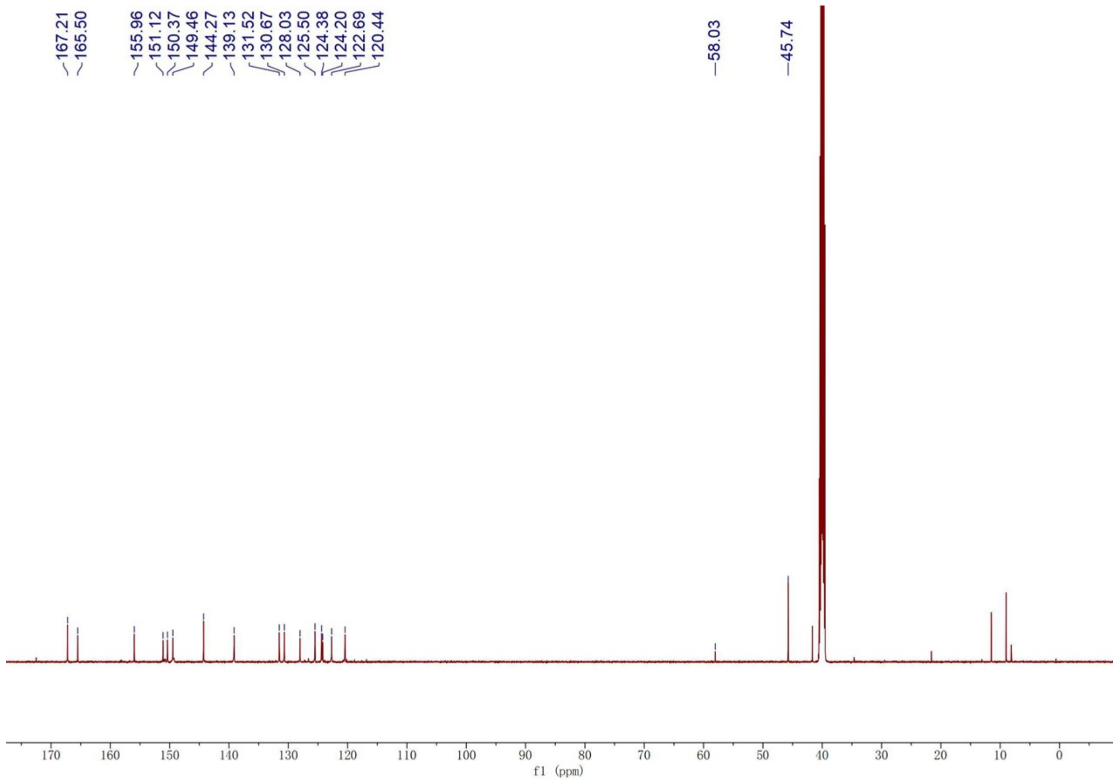

Figure S10. ^{13}C NMR spectrum of ligand **Ir1** in D_2O/DMSO-d6 (1:30, v/v).

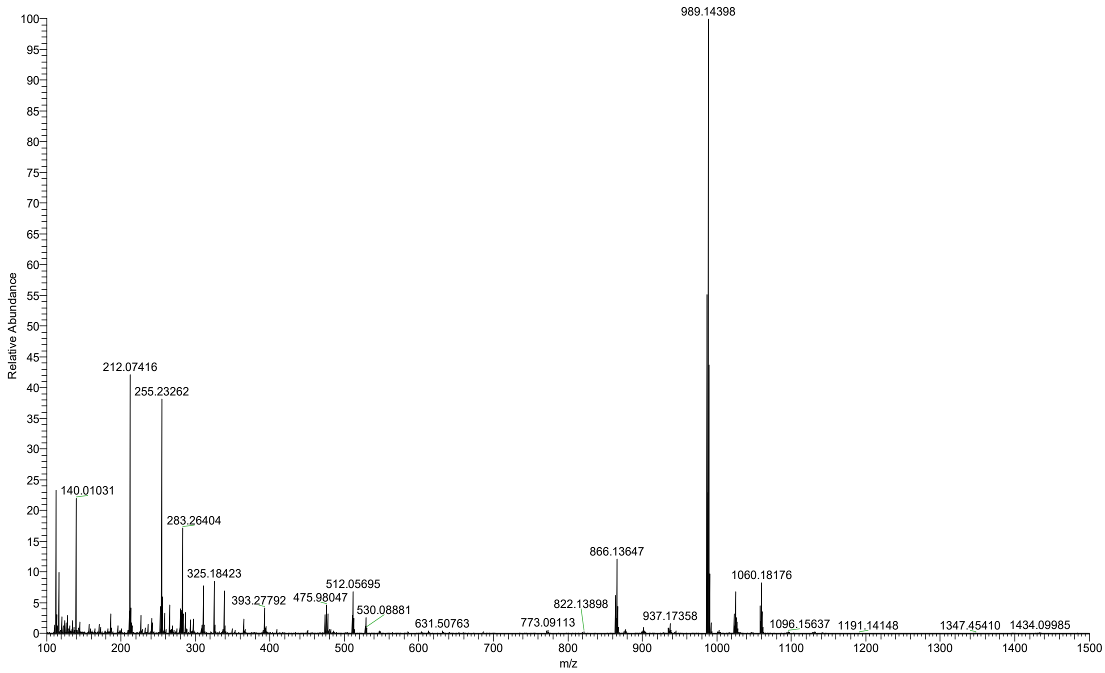

Figure S11. High-resolution mass spectrum of **Ir1** in MeOH.

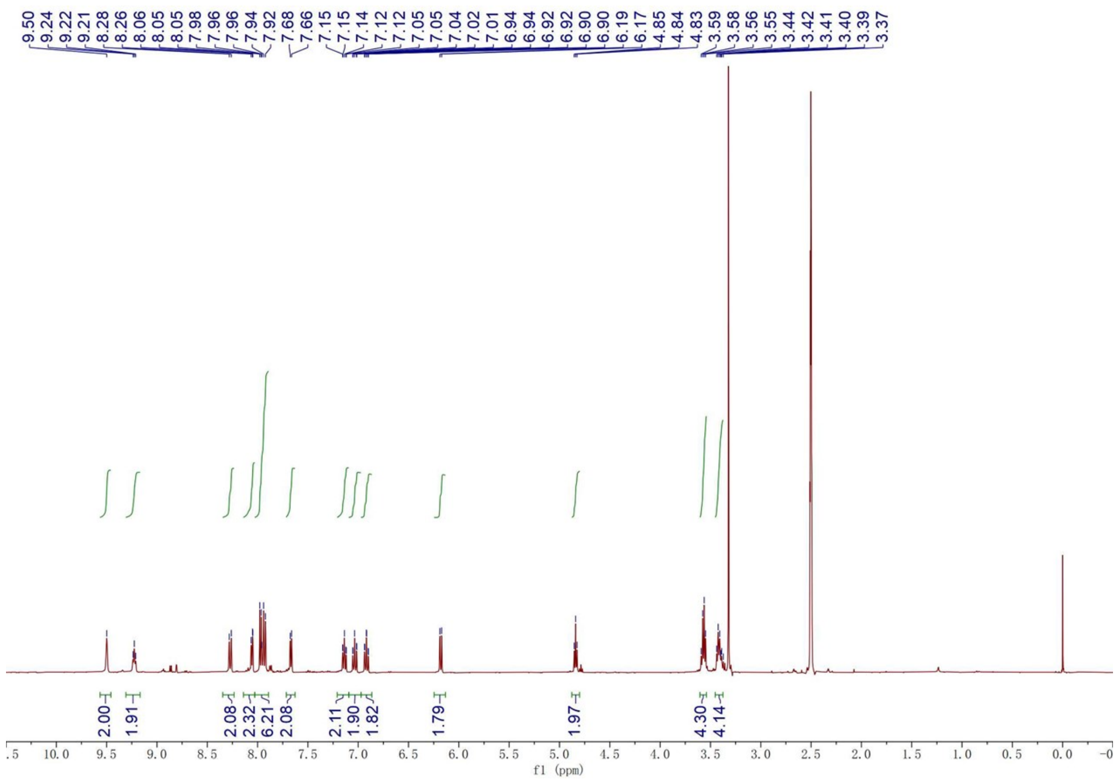

Figure S12. [1]H NMR spectrum of ligand **Ir2** in D_2O/DMSO-d6 (1:30, v/v).

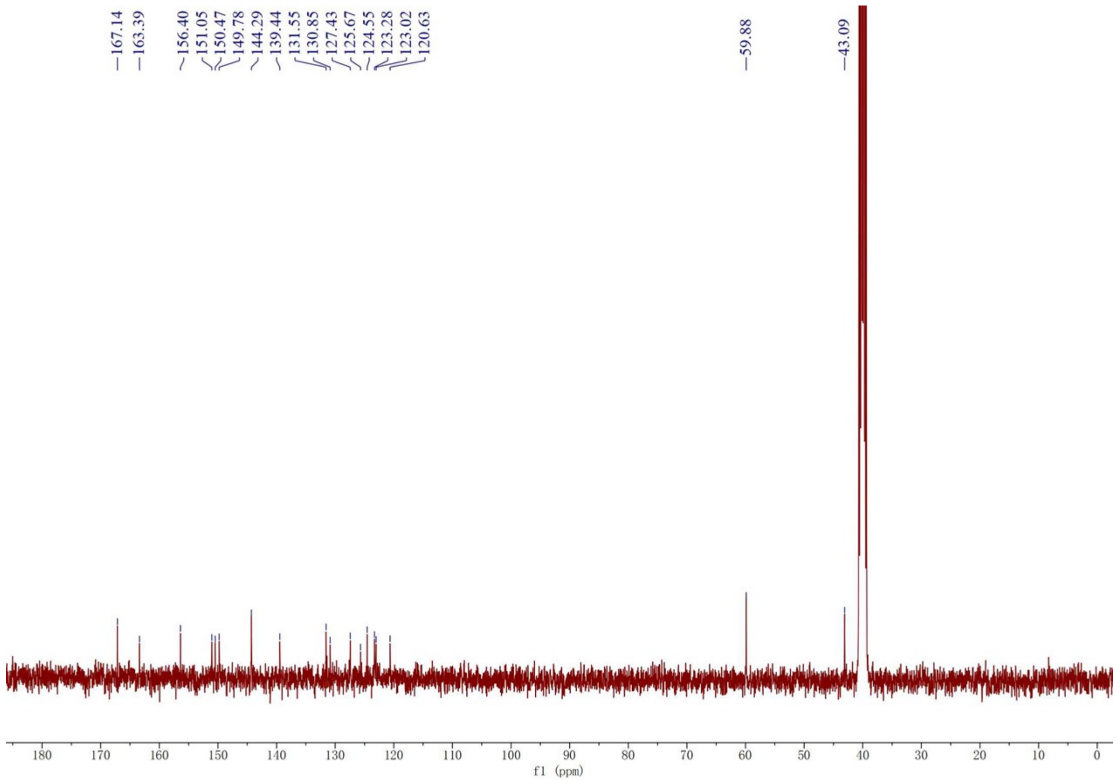

Figure S13. ^{13}C NMR spectrum of ligand **Ir2** in D_2O/DMSO-d6 (1:30, v/v).

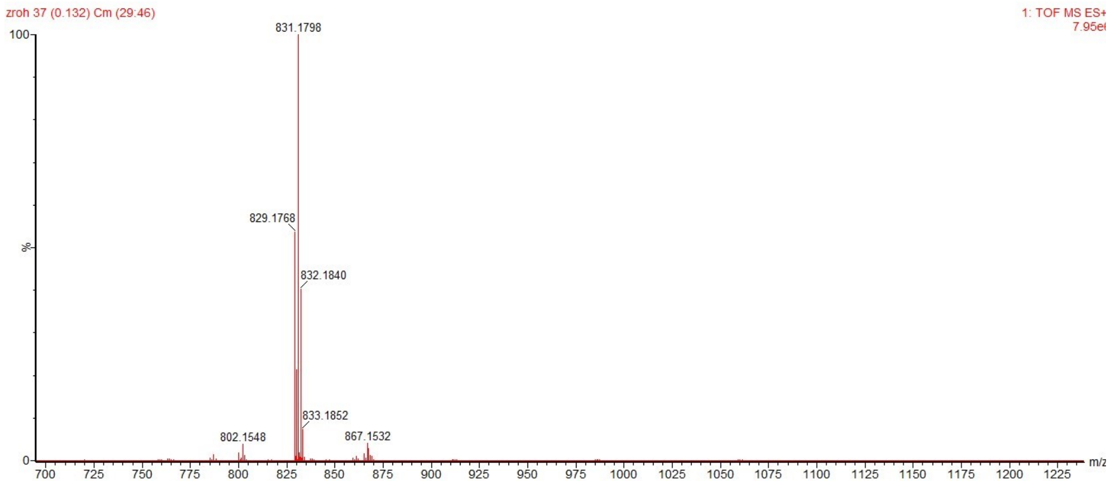

Figure S14. High-resolution mass spectrum of **Ir2** in MeOH

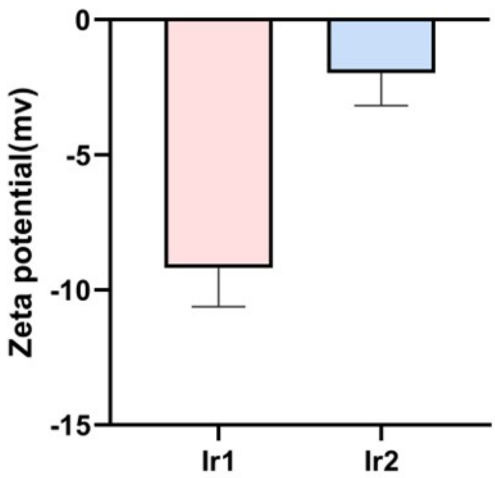

Figure S15. Zeta potential of **Ir1** or **Ir2** solution (20μM).

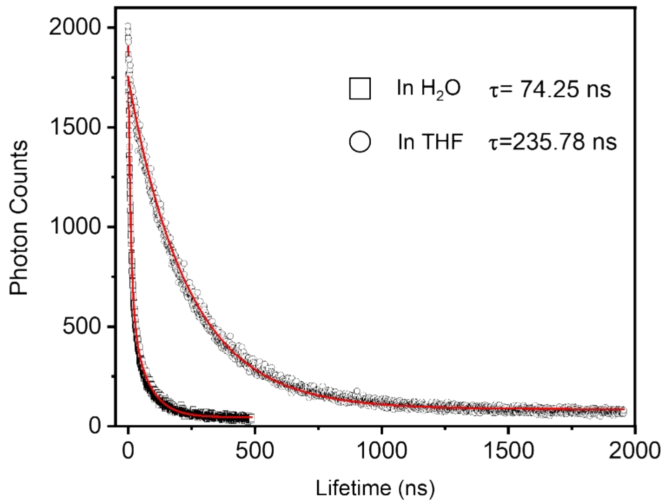

Fig. S16 Fluorescence decay profiles of **Ir1** in water (squares), or in 98% THF (circles) at room temperature (λ_{ex} = 375 nm). Fitting equation: $y = A_1 \times \exp(-x/\tau_1) + A_2 \times \exp(-x/\tau_2)$.

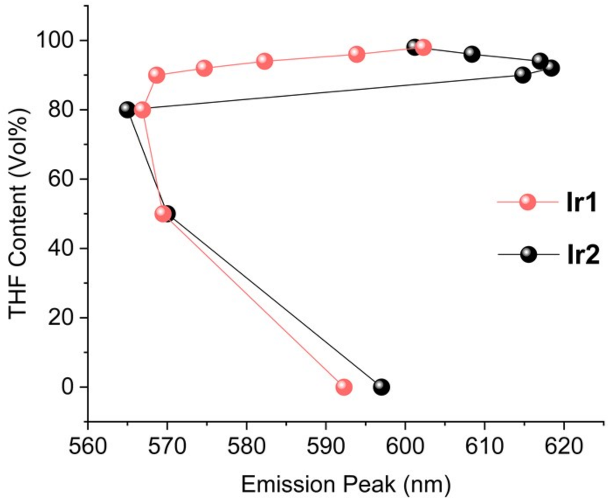

Figure S17. Emission peak of **Ir1** or **Ir2** solution (20μM) under different THF contents. λex = 385 nm.

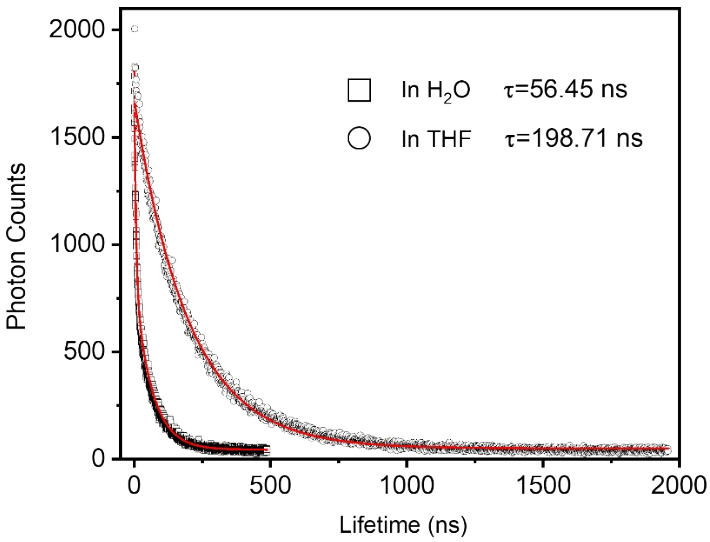

Fig. S18 Fluorescence decay profiles of **Ir2** in water (squares), or in 98% THF (circles) at room temperature (λ_{ex} = 375 nm). Fitting equation: $y = A_1 \times \exp(-x/\tau_1) + A_2 \times \exp(-x/\tau_2)$.

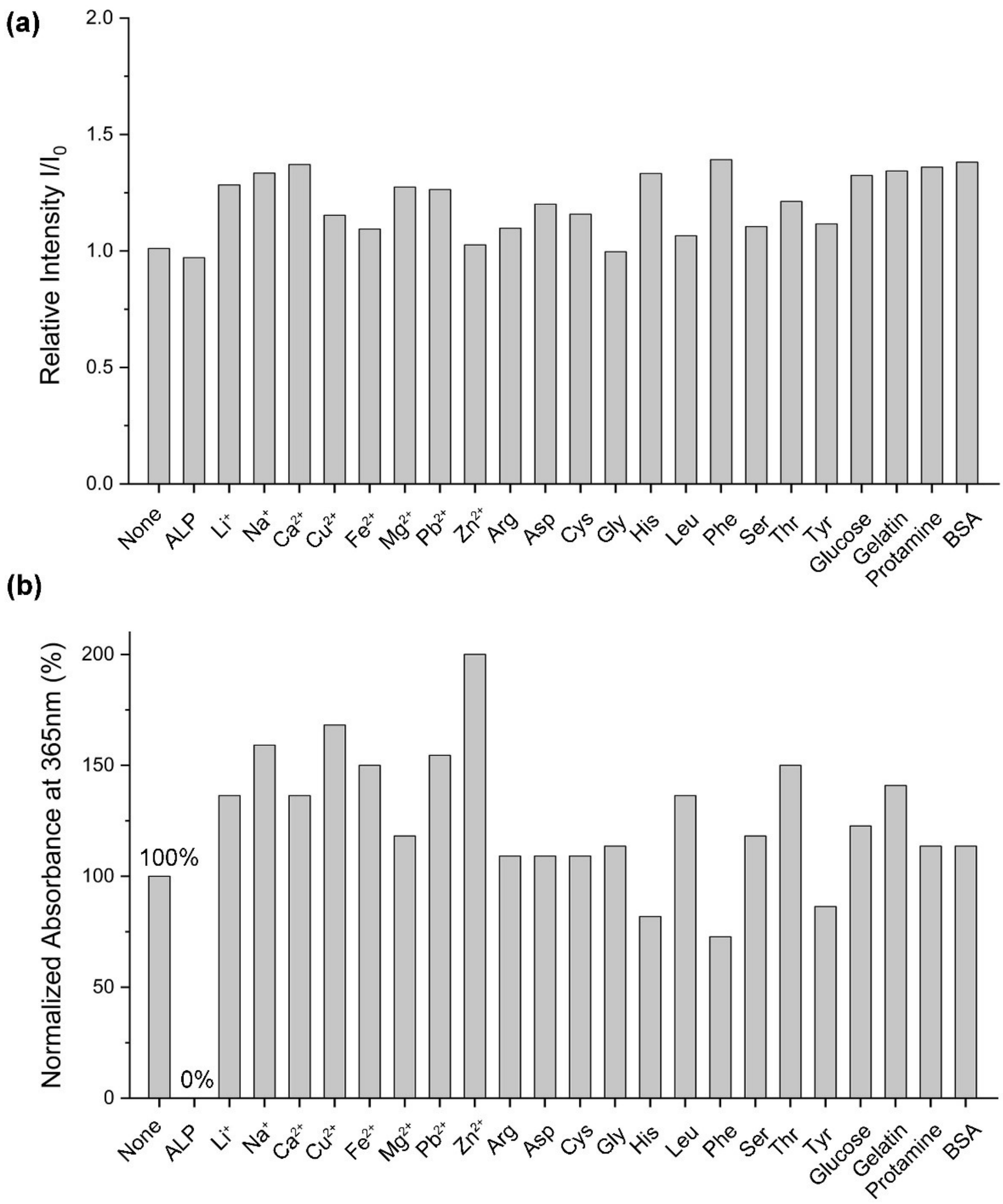

Fig. S19 (a) Relative emission intensity of **Ir1** (10 μM) in response to selective metal ions and biological species in Tris-HCl buffer (pH = 7.0). λex = 385 nm. (b) Normalized absorbance of **Ir1** (10 μM) at 365nm in response to selective metal ions and biological species in Tris-HCl buffer (pH = 7.0). The absorbance was normalized utilizing the formula: $A\% = (A-A_1)/(A_0-A_1)$, where A represents the absorbance of (**Ir1** + analyte), A_1 represents the absorbance of **Ir1** upon ALP cleavage; and A_0 represents the absorbance of **Ir1** without any treatments. Tested analytes and concentrations were: 20 μM selective metal ions (Ca^{2+}, Cu^{2+}, Fe^{2+}, Li^{+}, Mg^{2+}, Na^{+}, Pb^{2+}, and Zn^{2+}), 20 μM amino acids (Arg, Asp, Cys, Gly, His, Leu, Phe, Ser, Thr, and Tyr), glucose (20 μM), gelatin (1 mg/ml), protamine (10 μg/ml), BSA (10 μg/ml), and ALP (1U/mL), respectively.

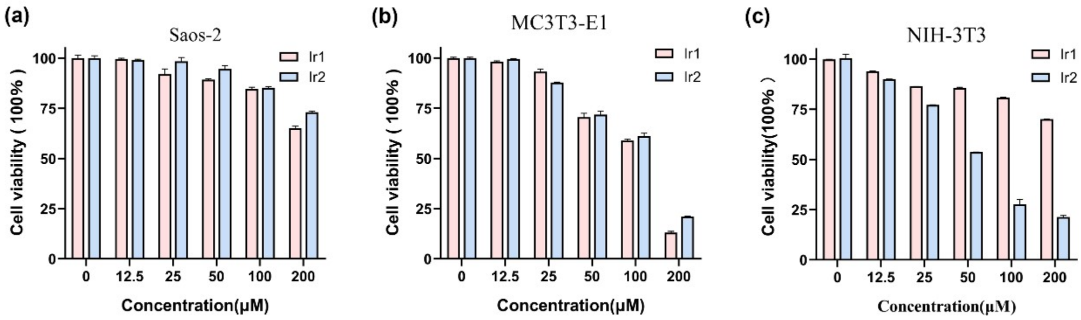

Figure S20. In vitro cell viability of (a) Saos-2 cells, (b) MC3T3-E1 cells and (c) NIH-3T3 cells incubated with 20 μM of **Ir1** or **Ir2** at 37 °C for 48hrs, respectively. Data were reported as mean standard deviation (n = 3).

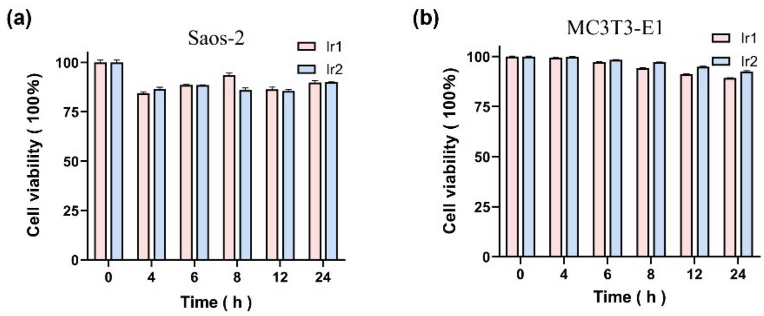

Figure S21. In vitro cell viability of (a) Saos-2 cells and (b) MC3T3-E1 cells incubated with 20 μM of **Ir1** or **Ir2** at 37 °C for different durations of time, respectively. Data were reported as mean standard deviation (n = 3)

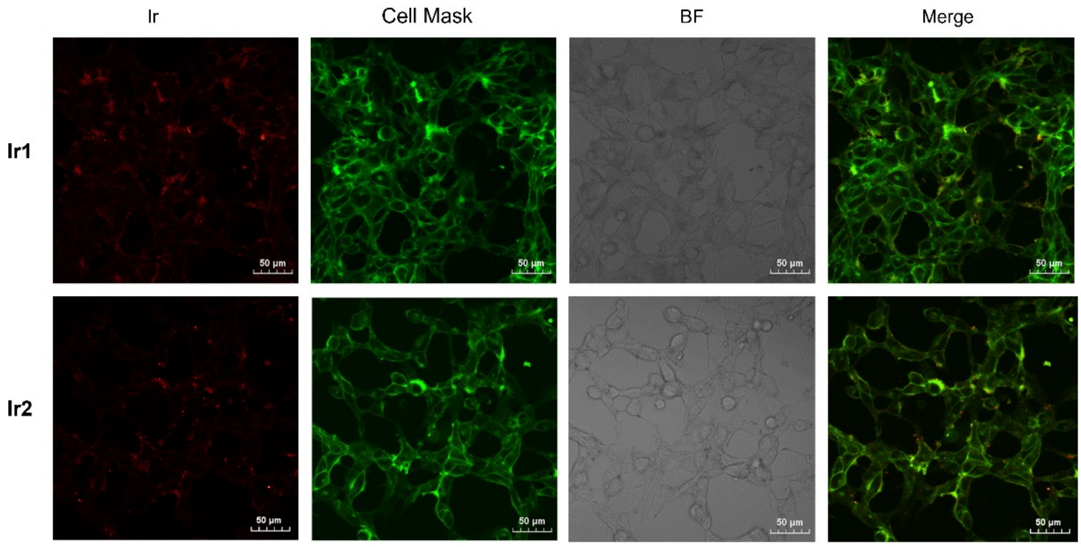

Figure S22. Confocal images of Saos-2 cells after treatment with 20 μM **Ir1** or **Ir2** for 1h. Cell membrane was stained with Cell Mask (green). Scale bars represent 50 μm.