Electronic Supplementary Information

Near-infrared AIE phosphorescent iridium(III) complex for mitochondria-targeted photodynamic therapy

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Supplementary experimental methods

Cell lines and culture conditions

The human breast cancer (MDA-MB-231) and mouse breast cancer (4T1) cells were obtained from the Experimental and Animal Centre of Sun Yat-sen University. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM), which was supplemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin and 100 U/mL penicillin, in a humidified incubator at 37 °C with 5% CO_2.

Measurement of cellular ATP levels

The ATP levels in MDA-MB-231 cells were measured using the CellTiter-Glo® Luminescent Cell Viability Assay kit (G7570, Promega, USA) as previously reported.[1] For dark conditions, the cells cultured in 96-well plates were exposed to the tested compounds for 24 h. For light irradiation conditions, the cells were exposed to the tested compound for 12 h and then illuminated with a green LED light (λ_{ir} = 525 nm, 15 mW/cm^2) for 15 min and incubated for a further 12 h. Next, the CellTiter-Glo® reagent was added to each well, then the plate was shaken for 2 min and incubated at room temperature for 10 min. Luminescence was recorded with a microplate reader.

Western blotting

The experiment was done according to previous literature reports[2]. MDA-MB-231 cells were seeded into 10 cm cell culture dishes and incubated for 24 h. The cells were treated with different concentrations (0.25, 0.5 and 1.0 μM) of complex **Ir1** for 12 h, followed by irradiation with a green LED light (λ_{ir} = 525 nm, 15 mW/cm^2) for 15 min and further incubated in the dark for 12 h. Cells were harvested and washed with ice-cold PBS twice, and then lysed in RIPA buffer supplemented with inhibitors of proteases and inhibitor of phosphatases sodium orthovanadate. The protein concentration was quantified using the BCA Protein Quantitation Kit. Equal amounts of cellular total proteins (30 μg) were separated on SDS-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes. Membranes were blocked in QuickBlock™ Blocking Buffer, and then incubated overnight with the primary antibodies CHOP and PARP at 4 °C. After a subsequent washing step, the membrane was incubated with the appropriate horseradish peroxidase-conjugated secondary antibody. Images were captured using a FluorChem M imaging station and analyzed manually with AlphaView software.

Transmission electron microscopy

MDA-MB-231 cells were cultured in 6-cm dishes (Corning) and treated with **Ir1** (1 μM) for 12 h, followed by irradiation with a green LED light (λ_{ir} = 525 nm, 15 mW/cm^2) for 15 min and further incubated in the dark for 12 h. The cells were rinsed with PBS and fixed overnight at 4°C in a phosphate buffer containing 2.5% glutaraldehyde. After treatment with osmium tetroxide as post-fixative, the cells were stained with uranyl acetate and lead citrate, and then observed by a transmission electron microscope.

2 Supplementary figures and tables

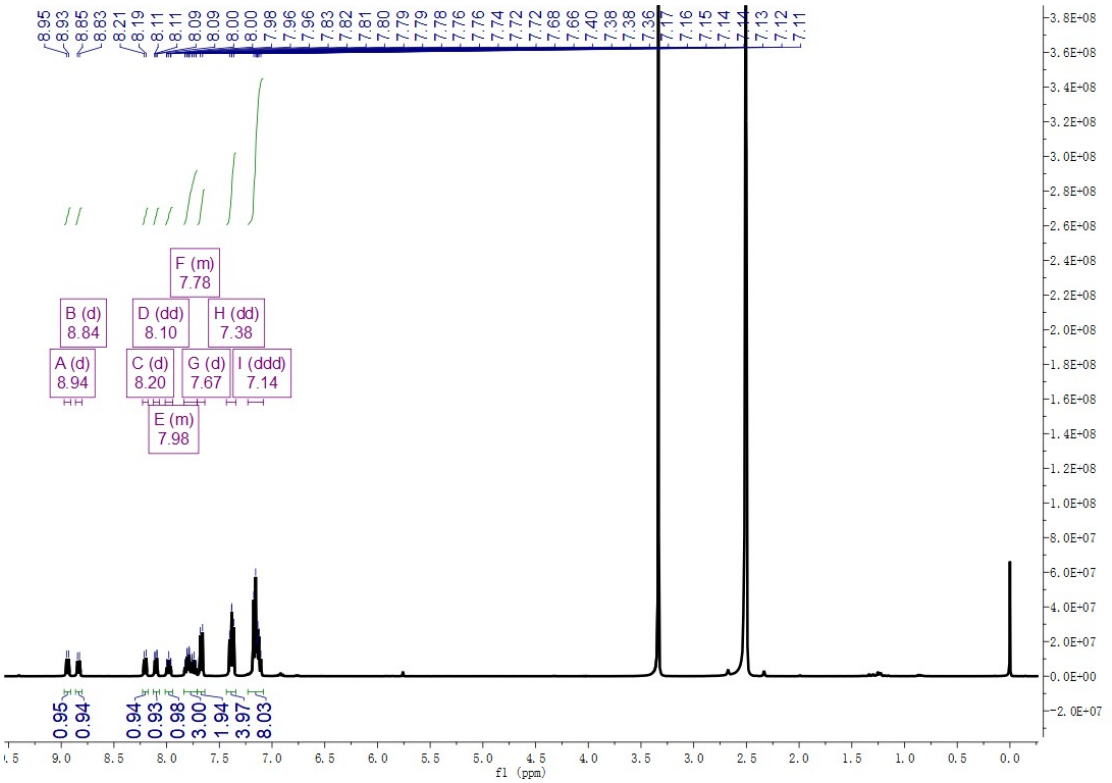

Fig. S1 [1]H NMR spectrum of TPAP in DMSO-d_6 at 298 K.

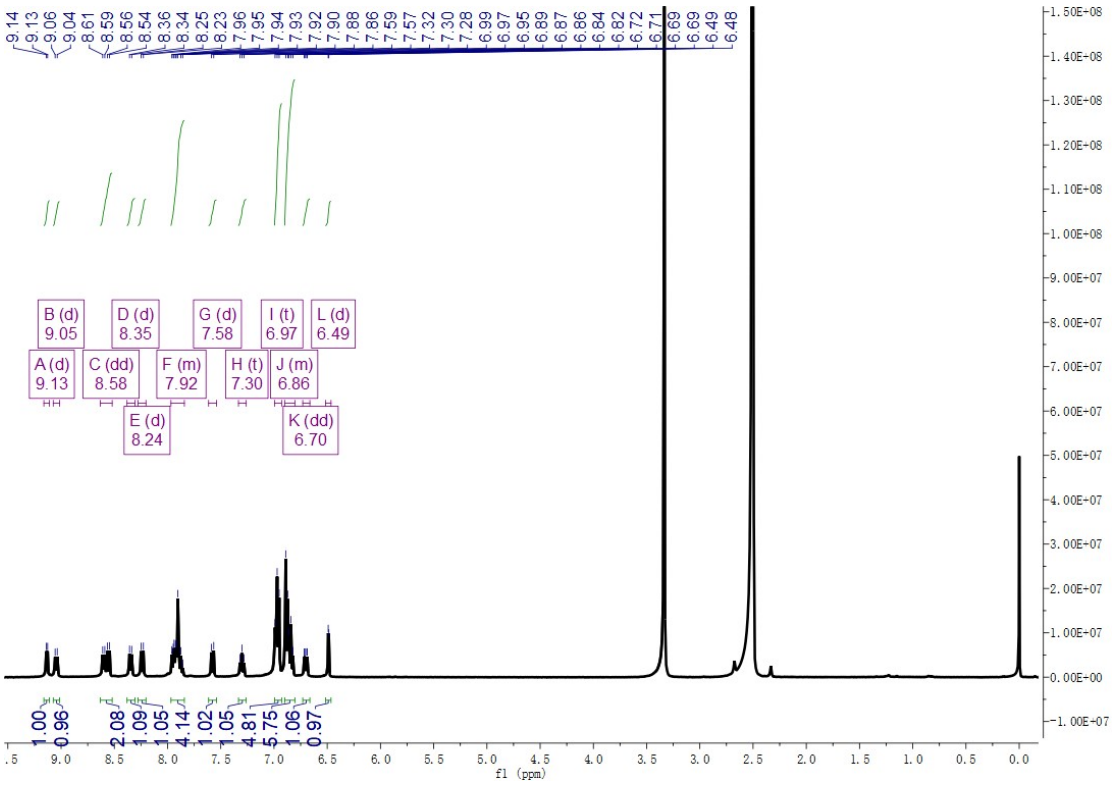

Fig. S2 [1]H NMR spectrum of **Ir1** in DMSO-d_6 at 298 K.

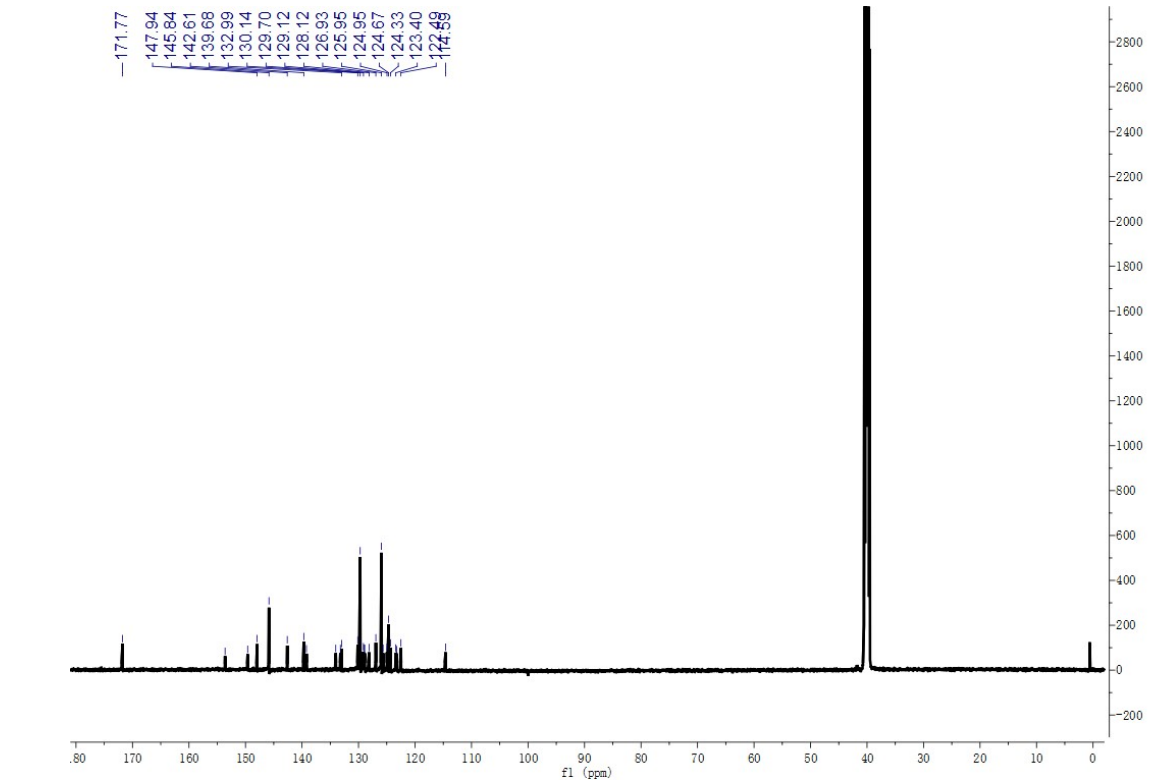

Fig. S3 ^{13}C NMR spectrum of **Ir1** in DMSO-d_6 at 298 K.

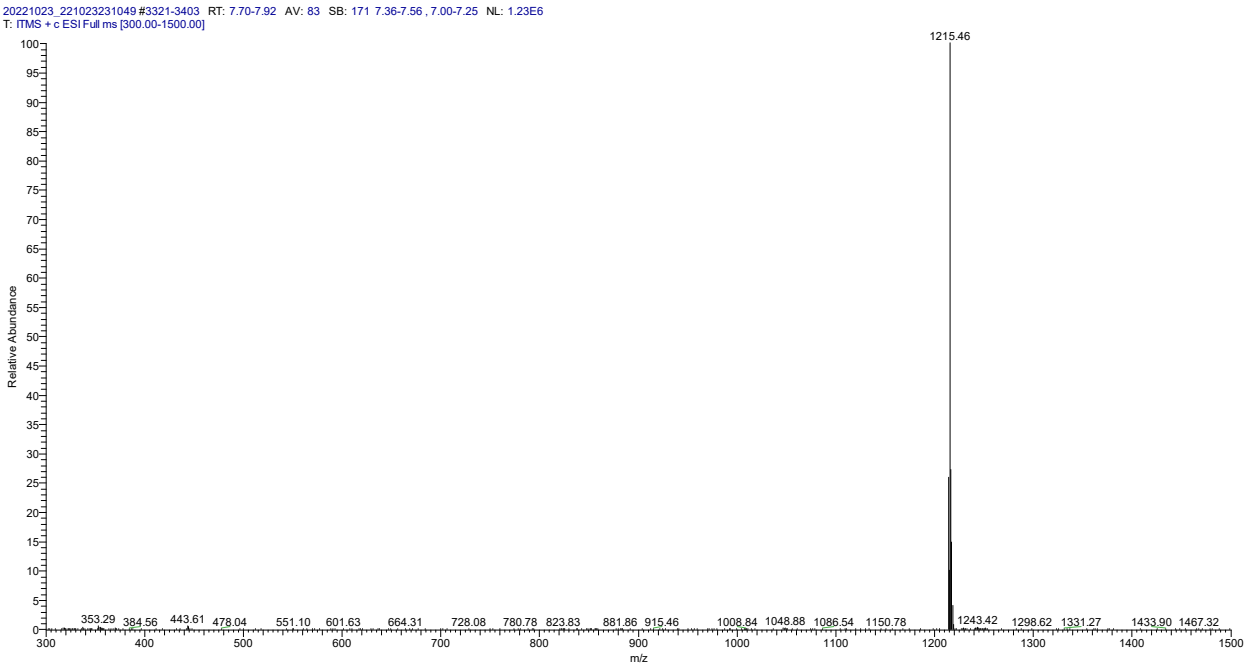

Fig. S4 ESI-MS spectrum of **Ir1** in CH_3OH. $[M-PF_6]^+$, 1215.46.

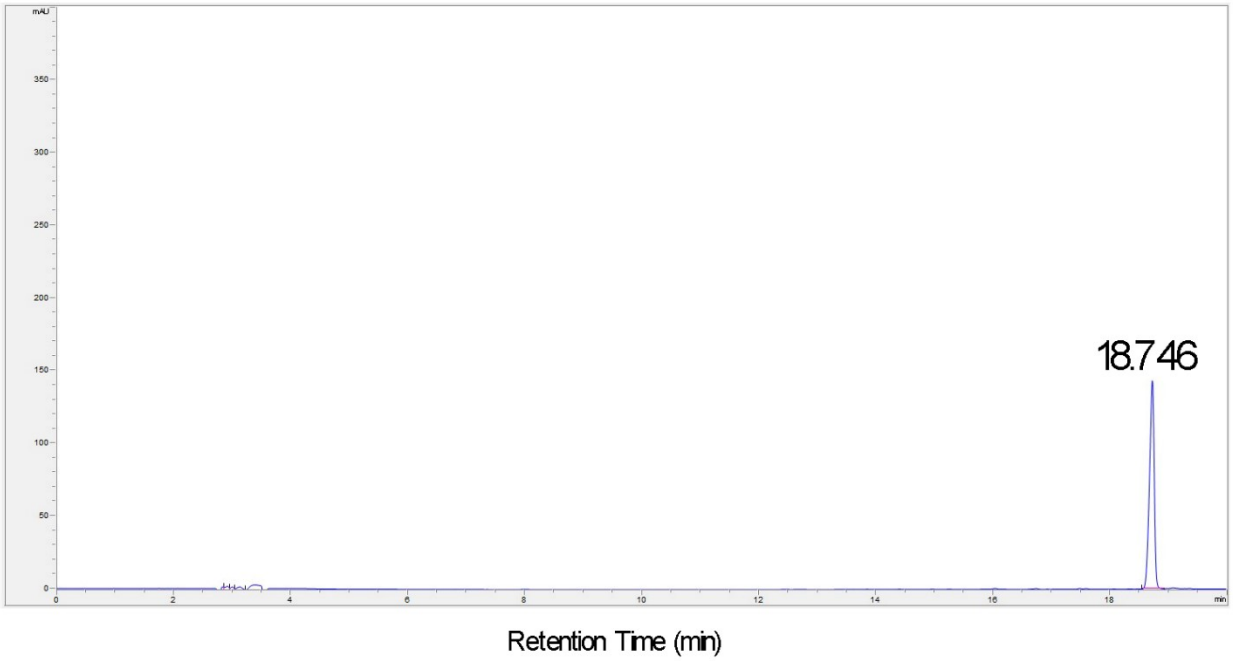

Fig. S5 The purity of **Ir1** analyzed by HPLC.

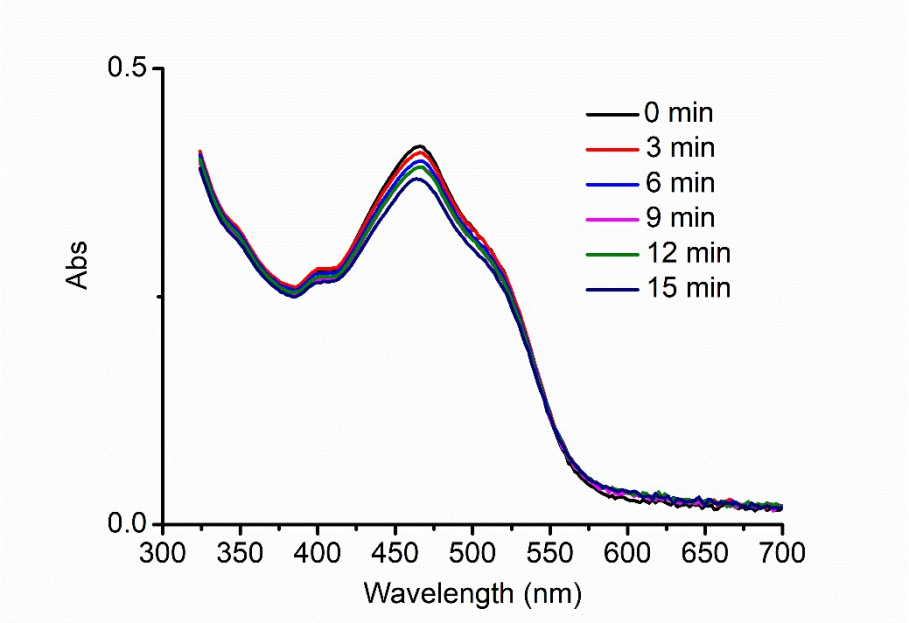

Fig. S6 Photostability of **Ir1** in PBS (20 μM) under light irradiation (λ_{ir} = 525 nm, 15 mW/cm^2) determined by UV/vis absorption spectra.

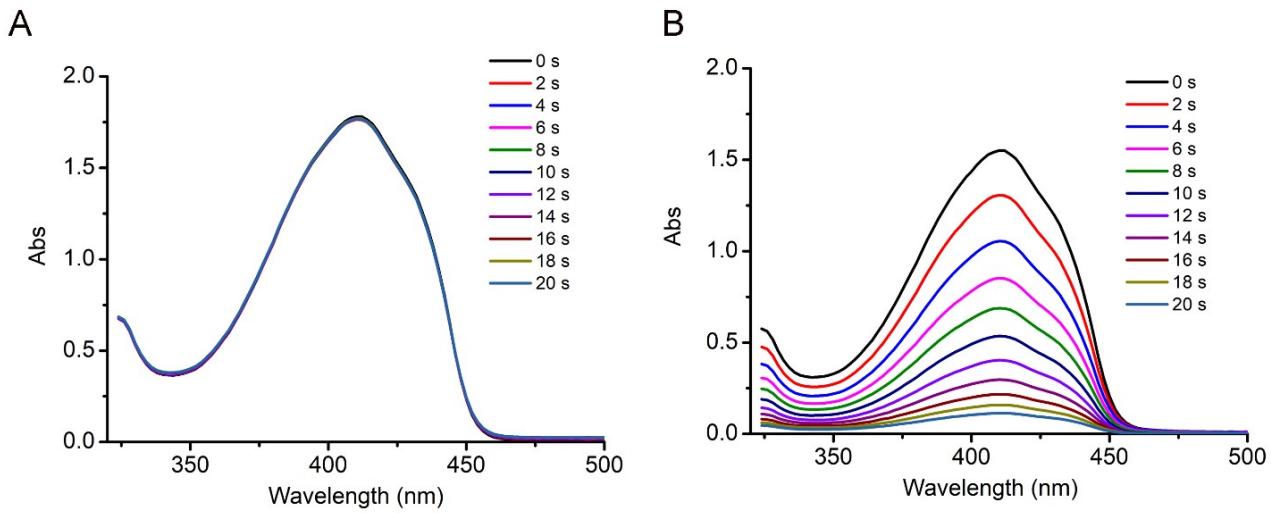

Fig. S7 Photocatalytic oxidation of DPBF upon light irradiation in the presence of (A) control and (B) **Ir1.**

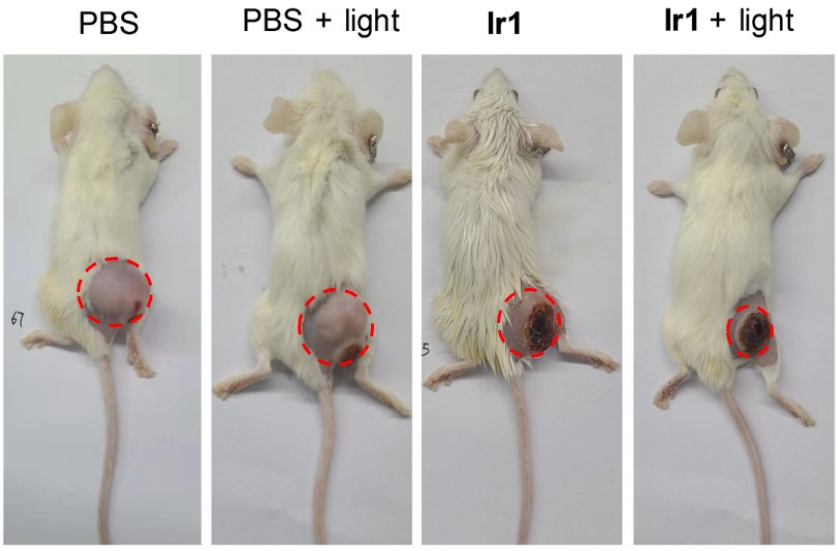

Fig. S8 The images of tumor tissues at the end of the treatment.

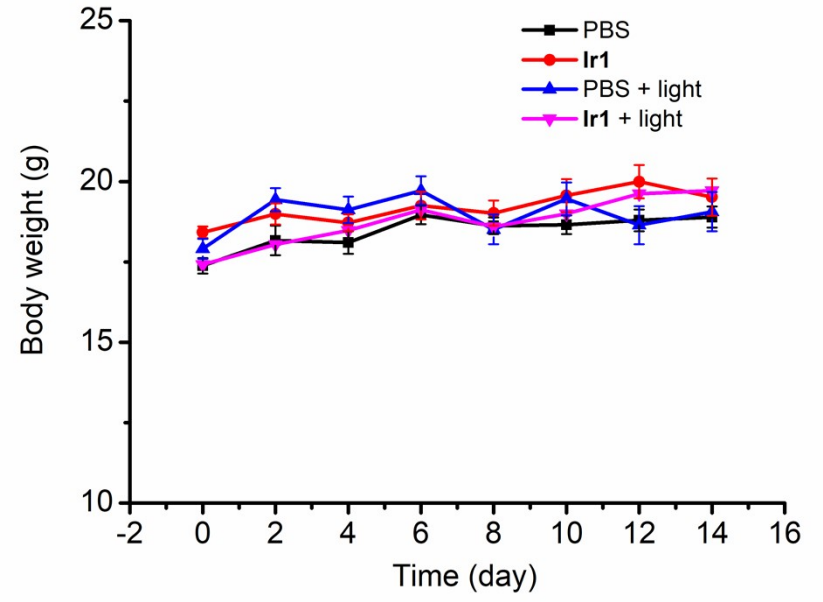

Fig. S9 Body weights of nude mice after treatment with PBS, PBS light, **Ir1** (5 mg/kg) and **Ir1** (5 mg/kg) light.

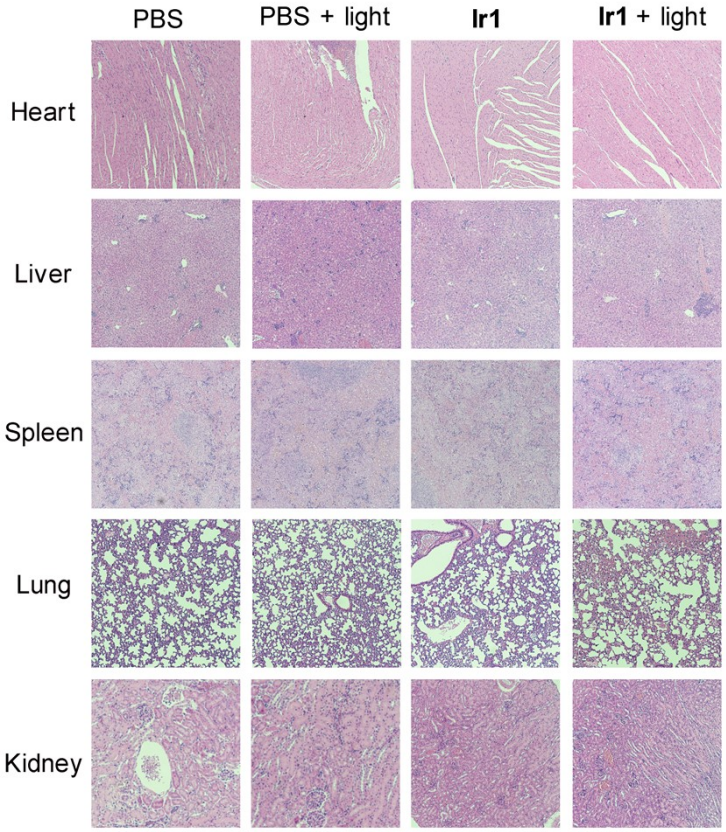

Fig. S10 Hematoxylin-eosin (H&E) staining of organs separated from nude mice after treatment with PBS, PBS + light, **Ir1** (5 mg/kg) and **Ir1** (5 mg/kg) + light. Scale bar: 50 μm.

References

1. L. He, M. F. Zhang, Z. Y. Pan, K. N. Wang, Z. J. Zhao, Y. Li and Z. W. Mao, *Chem. Commun.,* 2019, **55**, 10472-10475.
2. Z. Y. Pan, Y. Y. Ling, H. Zhang, L. Hao, C. P. Tan and Z. W. Mao, *J. Med. Chem.,* 2022, **65**, 14692-14700.