

Full-Process Radiosensitization Based on Nanoscale Metal-Organic Frameworks

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Section 1: Methods

UV-Vis spectroscopy. The spectra were determined using SHIMADZU UV-2700 spectrometer. Solid-state spectroscopy was conducted using a diffuse reflectance (DR) attachment including ISR-2600 integrating sphere, with BaSO₄ as the reference (100% reflectance). The sample powder was spread as a thin uniform layer on a layer of BaSO₄ powder. The spectra were given in the form of effective absorbance. The energy gaps for CT transitions were estimated from the Tauc plots of $(F(R)hv)^{1/2}$ against hv , where $F(R)$ is the Kubelka-Munk Function [$F(R) = (1-R)^2/2R$] and R is the diffuse reflectance relative to BaSO₄ (Figure S18b).^{1,2}

Electrochemical measurements. Cyclic voltammetry (CV) was conducted with CHI 604E workstation (CH instruments, China) with a conventional three-electrode system, a MOF-modified glassy carbon electrode (GCE) as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl electrode as the reference electrode. The supporting electrolyte was 0.1 M KCl aqueous solution, and the scan rate was set as 100 mV s⁻¹. The modified GCE was fabricated as follows: 1 mg of a ground nMOF sample was dispersed in 0.5 mL of ethanol and 40 μ L of Nafion solution (5 wt. % in lower aliphatic alcohols and water, contains 15-20% water) by sonication for 20 min. A pre-cleaned GCE was drop-cast with a 10 μ L portion of the dispersion and then dried in air.

Section 2: Biological characterization

Cells and animals. HeLa, A549 and RM-1 cells were purchased from Shanghai Institute of cells, Chinese Academy of Sciences. Culture medium was composed of high-glucose Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS), 1% penicillin (100 U/mL), and streptomycin (100 mg/mL). Kunming mice and Balb/c nude mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. These mice were raised in Laboratory Animal Center of East China Normal University (ECNU), for more than 8 weeks after birth. All the experiments conformed to animal ethics.

MTT assay. The Hela cells were seeded in 96-well plates and incubated with a series of concentrations of **Hf-BPY** or **Hf-BPY-Fe** for 24h. There were 6 wells in each group. 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) cell survival assay was applied to measure the survival rate of cells in each group.

The cytotoxicity of H₂O₂ with nMOFs (5 ppm based Hf⁴⁺ ion) or without nMOFs to Hela cells in 24 h after incubation with the concentration of 0, 12.5, 25, 50, 100 μ M was measured by MTT assay. Based on this, the safe concentration of H₂O₂ used in subsequent cell experiments is 50 μ M.

Cytotoxicity of chemodynamic therapy (CDT) combined with RT was also based on MTT method. Briefly, Hela cells and 50 μ M H₂O₂ were incubated with a series of concentrations of nMOFs (0, 5, 20, 40, 80 ppm based Hf⁴⁺ ion) for 24 h. Then, the cells were irradiated with 0 Gy and 4 Gy of X-rays. After irradiation, cells continued to incubate for 24 h without changing culture medium. MTT cell survival assay was applied to measure the survival rate of cells in each group.

Cellar uptake. FITC and nMOFs were mixed in ethanol and stirred for 6 h to get the FITC@nMOFs. 1×10^4 Hela cells were seeded in 35 mm culture dishes and incubated with FITC@nMOFs for 4 h. After removing culture solution, the cells were washed for 3 times by phosphate buffer saline (PBS) was used to wash. The cells were fixed with 4% formaldehyde, labeled with DAPI, and then imaged by a confocal fluorescence microscope.

Cell cycle analysis. 1 ml culture medium contained 1×10^5 HeLa cells and 50 μM H_2O_2 with 5 ppm (based Hf^{4+} ion) **Hf-BPY**, 5 ppm (based Hf^{4+} ion) **Hf-BPY-Fe** or PBS were seeded in 6-well plates and incubated for 24 h. After incubation, those cells were collected by centrifugation (1000 rpm, 3 min), washed twice with PBS, and then fixed with 70% precooled ethanol at 4 °C for 12 h. Then the cells were incubated with RNase A (100 μg / mL) and propidium iodide (PI, 50 μg / mL) for 30 min at 37 °C in the dark. The flow cytometry assay was employed for cell cycle analysis, and Modfit software was used for fitting analysis.

PCR Analysis. 5×10^4 HeLa cells were seeded in several 35 mm culture dishes and incubated for 24 h. These cells were irradiated with 6 Gy of X-rays. Then, the survival cells were replanted in new culture medium (containing 50 μM H_2O_2) and co-cultured with 5 ppm (based Hf^{4+} ion) **Hf-BPY**, **Hf-BPY-Fe** or PBS for different times (1 h, 6 h, 12 h and 24h), respectively. The ABI 7300 real-time PCR system was used for quantitative PCR measurement. The primer for the target genes were as follows:

Gen	Primer sequences(5'-3')
H-GAPDH-S	GGAAGCTTGTCATCAATGGAAATC
H-GAPDH-A	TGATGACCCTTTGGCTCCC
H-53BP1-S	ACGAGGAGACGGTAATAGTGGG
H-53BP1-A	TGCTTGTCCTGTTTGGCTGA
H-XRCC1-S	TGAACCAAGAAGAAAAGAAGACCC
H-XRCC1-A	GGAAGCCACTCAGCACCCTA
H-RAD51-S	ACCCATTTACGGTTAGAGCA
H-RAD51-A	CTTCTTTGGCGCATAGGCAAC

Colony formation assay. 2 ml culture medium contained 50 μM H_2O_2 and 200, 400, or 800 HeLa cells with 5 ppm (based Hf^{4+} ion) **Hf-BPY**, **Hf-BPY-Fe**, or PBS were seeded in 6-well plates. After incubation for 24 h, 200 cells wells were irradiated with 0 Gy or 2 Gy of X-rays, 400 cells were irradiated with 4 Gy of X-rays, and 800 cells were irradiated with 6 Gy of X-rays. Three parallel experiments were conducted in each group. After irradiation, all the plates were cultivated for 14 days. At last all cells were stained with giemsa stain. A cell colony should contain at least 50 cells. The sensitive enhancement ratio (SER) was evaluated by the multi-target and single-hit model,³

$$y = 1 - (1 - e^{-kx})^N$$

in which x is the dosage of each group, and y is colony formation rate. Then y value of the group treated with 4 Gy and saline was substituted into the equation for the other groups to calculate x . $\text{SER} = 4 \text{ Gy} / x$ for each group. Clonogenic assays of A549 and RM-1 cells were performed using the same procedure.

Comet assay. 1 ml culture medium contained 1×10^5 HeLa cells and 50 μM H_2O_2 with 5 ppm (based Hf^{4+} ion) **Hf-BPY**, **Hf-BPY-Fe**, or PBS were seeded in 6-well plates and incubated for 24 h. Then these cells were irradiated with 0 Gy, or 4 Gy of X-rays. After incubation for 24 h, the cells were centrifuged and washed twice with PBS. Finally, cells were digested and harvested, and the standard single cell gel electrophoresis procedure was employed to observe the DNA damage.

Apoptosis assay. 1 ml culture medium contained 1×10^5 HeLa cells and 50 μM H_2O_2 with 5 ppm (based Hf^{4+} ion) **Hf-BPY**, **Hf-BPY-Fe**, or PBS were seeded in 6-well plates and incubated for 24 h. Then these cells were irradiated with 0 Gy, or 4 Gy of X-rays. After incubation for 24 h, the cells were centrifuged and washed twice with PBS. The suspended cells were added with 500

μ L binding buffer solution, and Annexin V-FITC was added for 15 min incubation in the dark. Before test, PI was added and incubated for 5 min. At last, the flow cytometry assay was employed, and Flowjo software was used for fitting analysis.

Western blotting for detection of protein expression. The cells were seeded and treated the same as cell apoptosis assay. After that, the cells were centrifuged and washed twice with PBS. Then the cells were lysed by pre-cooled RIPA buffer on ice. The protein samples were separated by SDS-PAGE (10% gel) and transferred onto nitrocellulose membranes (PVDF). After blocking with 5% non-fat milk, the membranes were incubated with specific primary antibodies (1:1000) overnight at 4 °C. After 3 times washing, incubations with secondary HRP-linked antibodies were performed at room temperature for 1 h. Lastly, the membranes were detected using the electrochemiluminescence (ECL) reagent kit (Thermo Scientific, USA) according to manufacturer instruction.

***In vivo* toxicity assay.** Kunming mice (7 weeks, female) were intravenous injected with **Hf-BPY-Fe** (Hf, 100 mg/kg) suspended in normal saline and sacrificed at 3 or 30 days to collect blood for hematological assay and tissues (including heart, liver, spleen, lung, and kidney) for hematoxylin and eosin stain (H&E stain). The control group received intravenous normal saline only and sacrificed at 3 or 30 days for the same assays.

***In vivo* radiation therapy.** To establish the bilateral xenograft tumor model, 5×10^6 Hela cells were suspended in 100 μ l PBS and injected subcutaneously into the flanks of Balb/c nude mice (7 weeks, female). As the tumor size reached approximately 100 mm³, the mice were divided randomly into 4 groups (6 mice in each group). Three groups received intratumoral objection of PBS, **Hf-BPY** (Hf, 20 mg/kg), and **Hf-BPY-Fe** (Hf, 20 mg/kg), respectively. After 24 h, tumors in the right flank were irradiated at a dose of 4 Gy, and tumors in the left flank were shielded

from irradiation as the control. The H&E stain and TdT mediated dUTP nick end labeling (TUNEL) of tumor tissues using a commercially available kit were performed at 48 h after irradiation, respectively. Body weight of mice and tumor volume were recorded every 3 days, and the latter was calculated by the following equation: $\text{volume} = \text{length} \times (\text{width})^2 / 2$. The other three groups received intravenous administration of PBS, **Hf-BPY-Fe** (Hf, 20 mg/kg), and **PEG-Hf-BPY-Fe** (Hf, 20 mg/kg), respectively. The tumor volume were recorded every 3 days.

Section 3: Supplementary figures

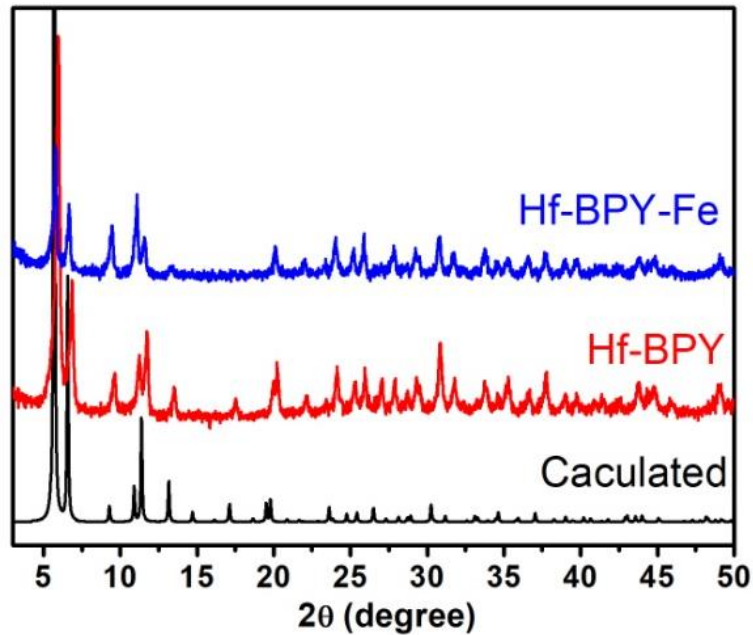


Figure S1. PXRD patterns of nMOFs.

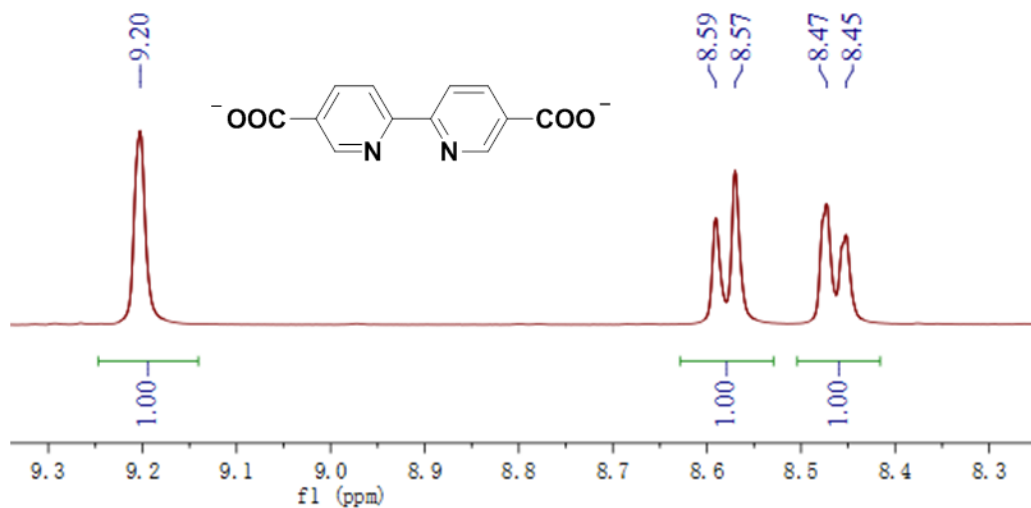


Figure S2. ^1H NMR spectrum of **Hf-BPY**. The spectrum was recorded with the solutions obtained by digesting the solids with HF (aq.)/ d_6 -DMSO (1/40, v/v).⁴ No other miscellaneous peaks were found, indicating the high purity of materials.

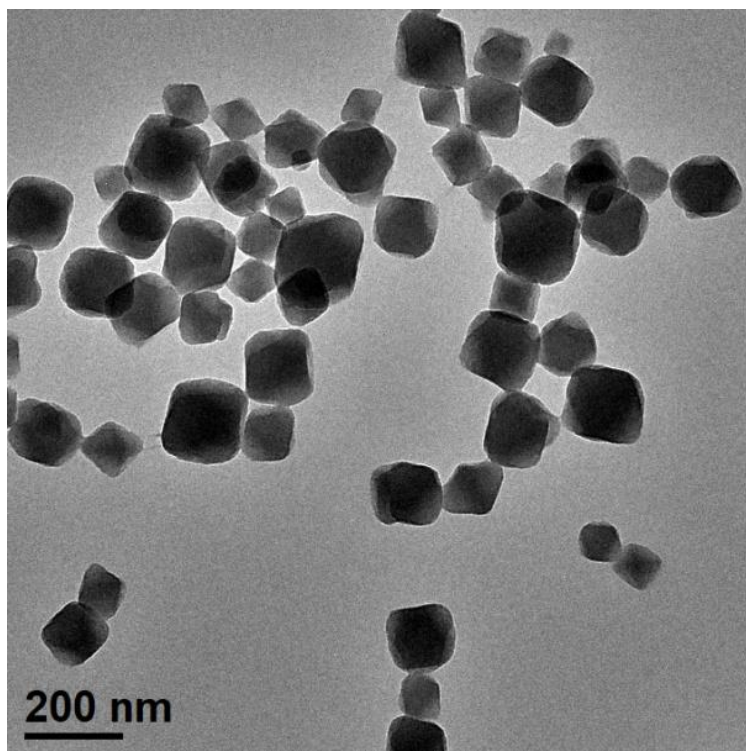


Figure S3. TEM image of **Hf-BPY**.

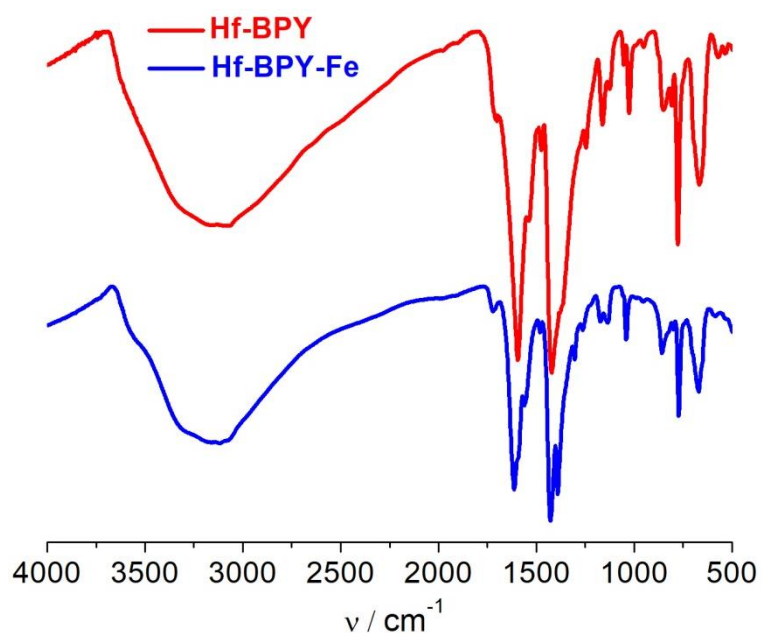


Figure S4. IR spectra of **Hf-BPY** and **Hf-BPY-Fe**.

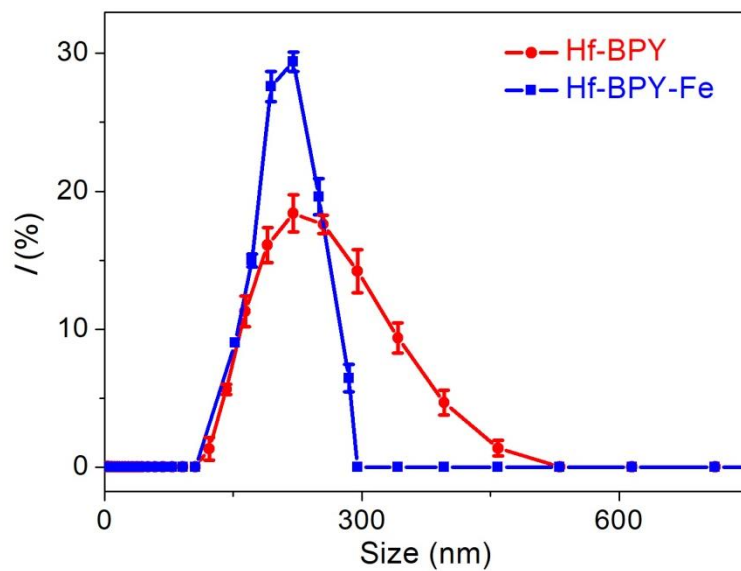


Figure S5. Particle size distributions of **Hf-BPY** and **Hf-BPY-Fe**.

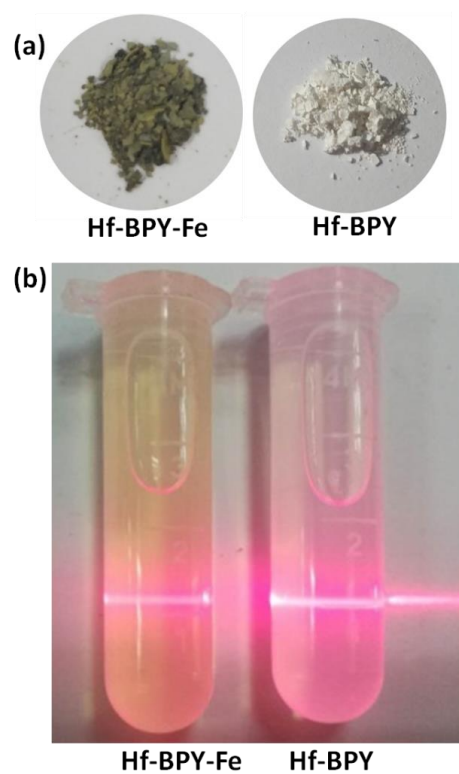


Figure S6. (a) Photographic images showing **Hf-BPY** and **Hf-BPY-Fe** polycrystalline samples.

(b) Photographs of saline dispersion for **Hf-BPY** and **Hf-BPY-Fe**.

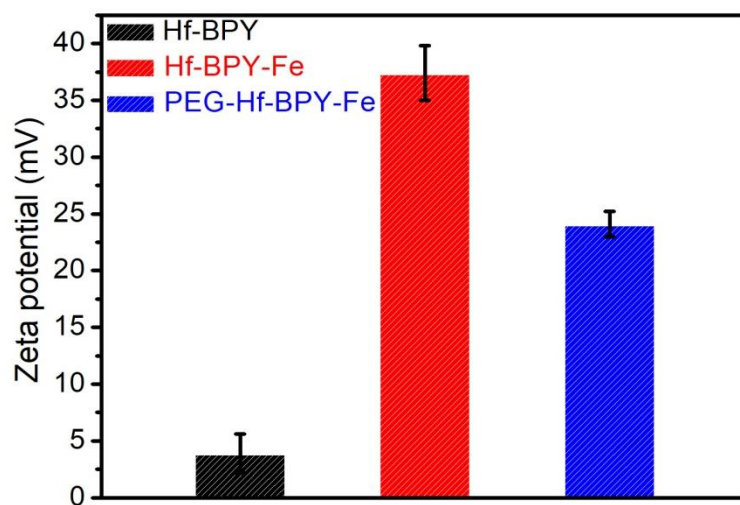


Figure S7. Zeta potentials of **Hf-BPY**, **Hf-BPY-Fe** and **PEG-Hf-BPY-Fe**.

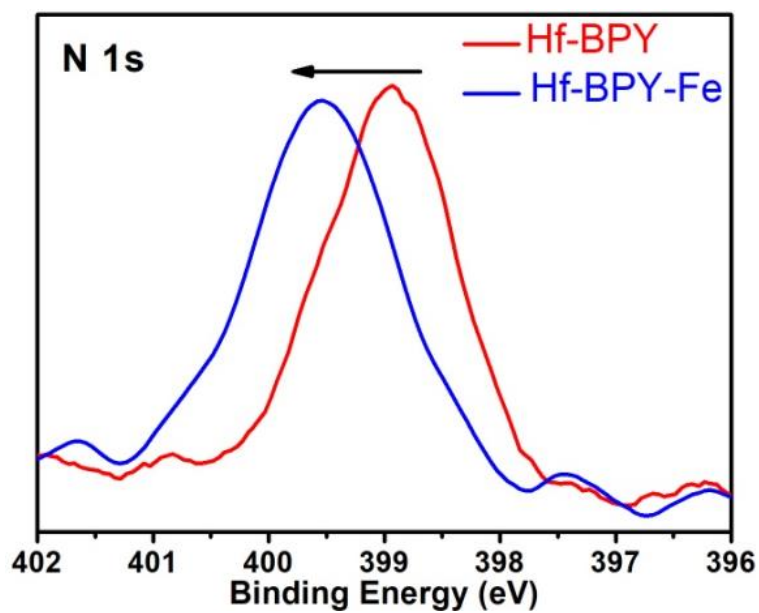


Figure S8. XPS spectra of **Hf-BPY** and **Hf-BPY-Fe**.

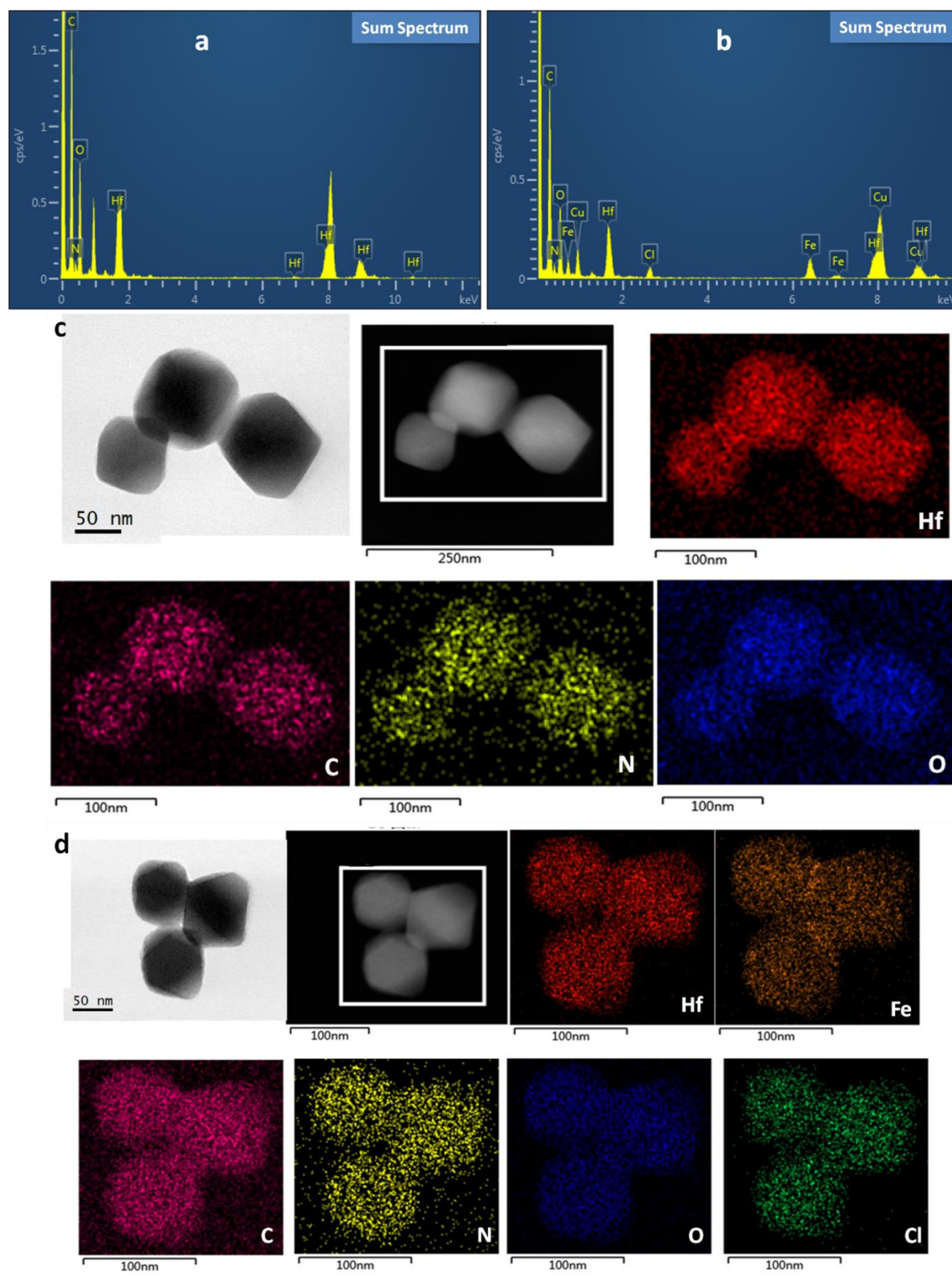


Figure S9. Elemental analysis and energy dispersive X-ray elemental mapping of **Hf-BPY** (a, c) and **Hf-BPY-Fe** (b, d).

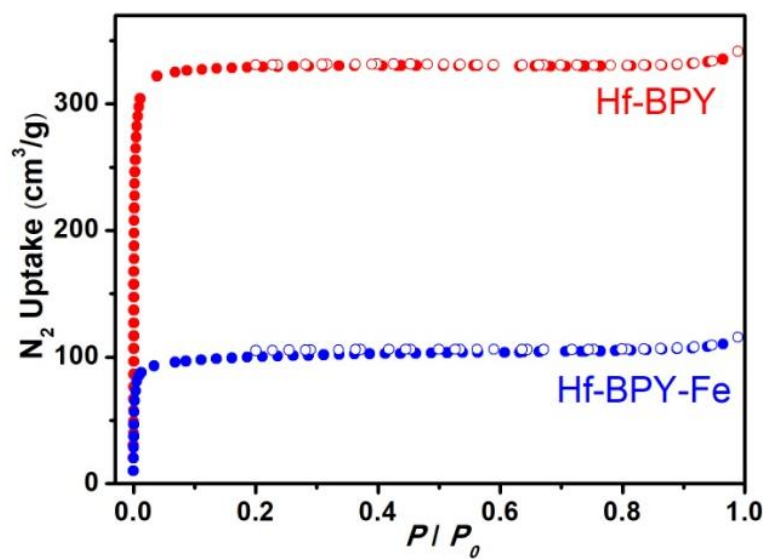


Figure S10. N₂ adsorption/desorption isotherms of **Hf-BPY** and **Hf-BPY-Fe**.

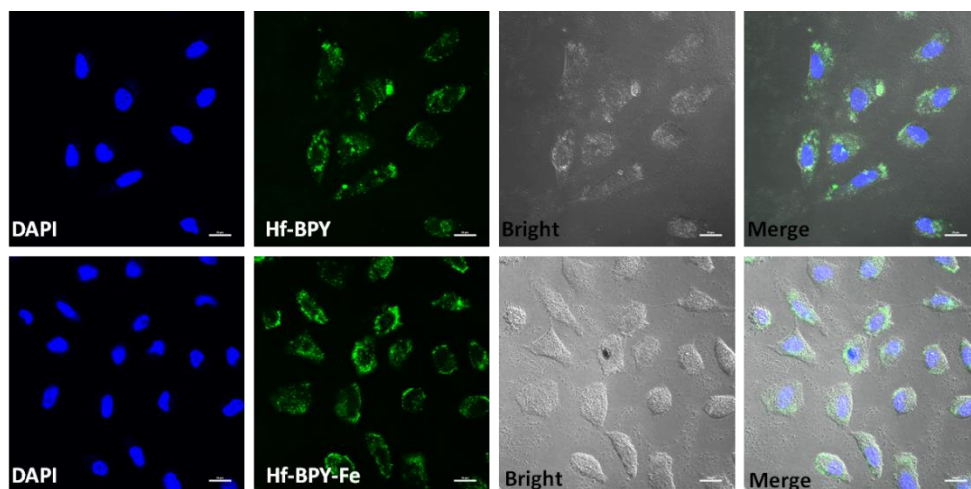


Figure S11. Assessment of cellular uptake of **Hf-BPY** and **Hf-BPY-Fe** (Scale bar = 20 μm).

HeLa cells treated with FITC-labeled **Hf-BPY** and **Hf-BPY-Fe** showed remarkable intracellular green fluorescence spots. Nuclei were stained with DAPI (blue fluorescence).

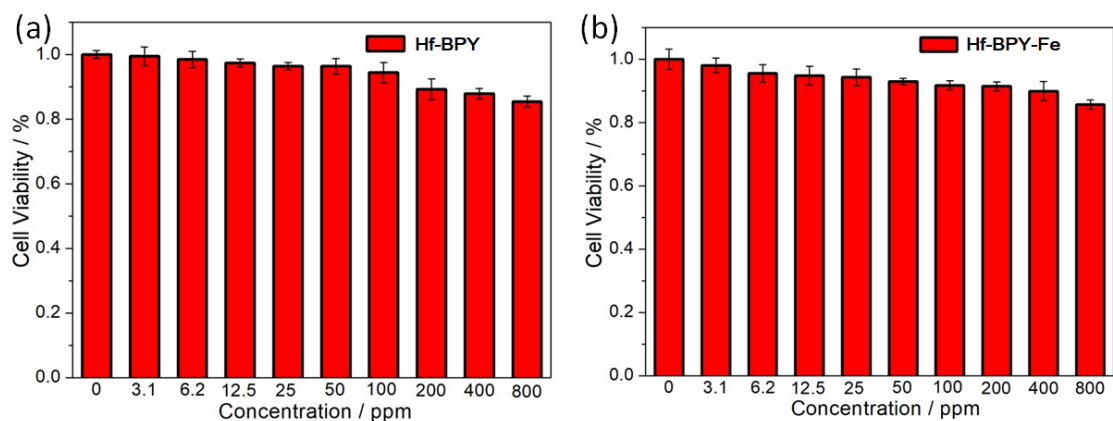


Figure S12. Cell viability of Hela cells after 24 h incubation with a series of concentrations (determined by ICP-OES based on the concentration of Hf^{4+} ions) of **Hf-BPY** and **Hf-BPY-Fe**.

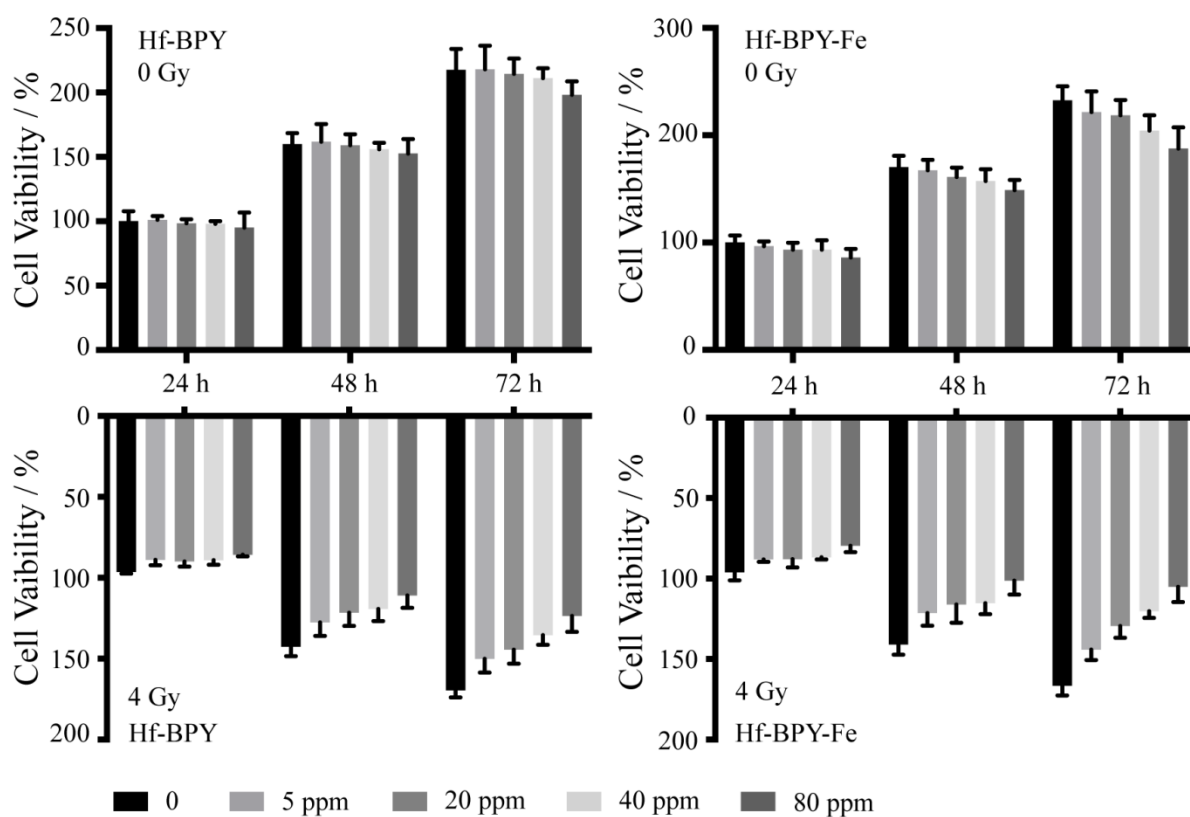


Figure S13. Cytotoxicity test *in vitro* of nMOFs. The Hela cells were seeded in 96-well plates (10000 cells per well) and incubated with a series of concentrations (determined by ICP-OES based on the concentration of Hf^{4+} ions) of **Hf-BPY** or **Hf-BPY-Fe** for 24h, PBS as blank

control group. These cells were irradiated with 0 Gy, or 4 Gy of X-rays. After that, the cells continued to incubate for other different times, 24 h, 48h and 72 h. MTT cell survival assay was applied to measure the survival rate of cells in each group.

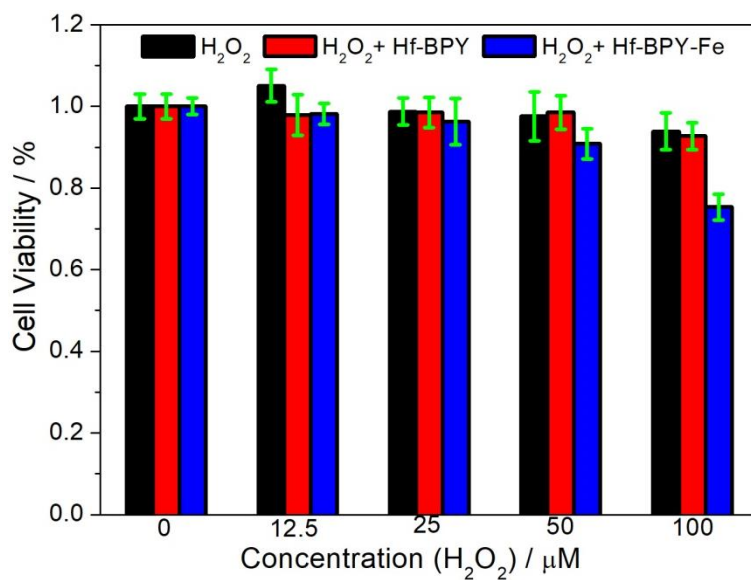


Figure S14. Cell viability of Hela cells after 24 h incubation with a series of concentrations of H_2O_2 and **Hf-BPY** and **Hf-BPY-Fe** (5 ppm based on Hf^{4+} ions). External H_2O_2 was used to simulate the overexpression of H_2O_2 microenvironment in tumor area. The cell survival rate after CDT (induced by 50 μM H_2O_2 and **Hf-BPY-Fe**) was still higher than 80%. Therefore, the safe dosage of H_2O_2 for all cell experiments in this work is 50 μM .

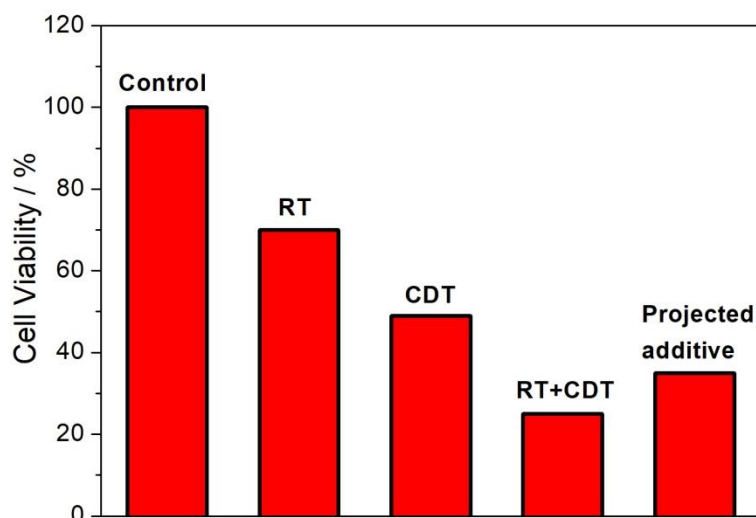


Figure S15. Synergistic therapeutic effect of Hela cells that have taken up **Hf-BPY-Fe** (80 ppm based on Hf^{4+} ions) subjected to RT, CDT, and the combined RT/CDT treatments. The projected additive value is calculated by multiplying the cell viability of RT and CDT group.

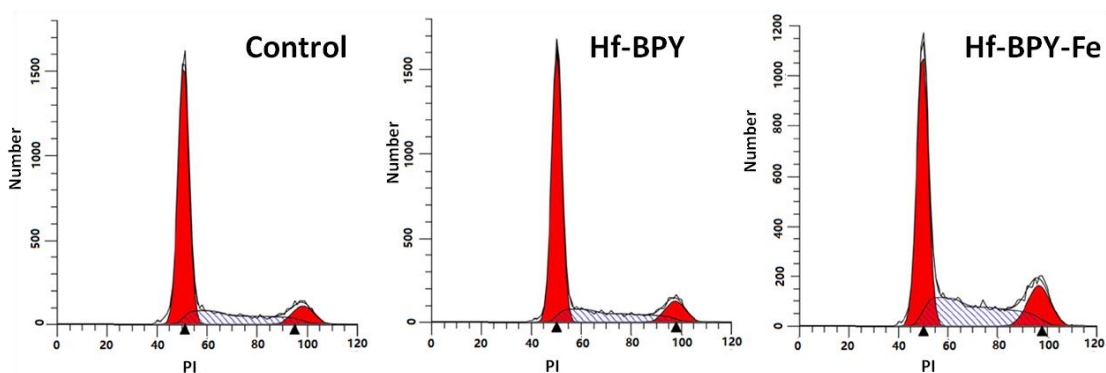


Figure S16. The flow cytometric analysis of cell cycles of Hela cells treated with PBS, **Hf-BPY**, and **Hf-BPY-Fe** for 24 h, respectively.

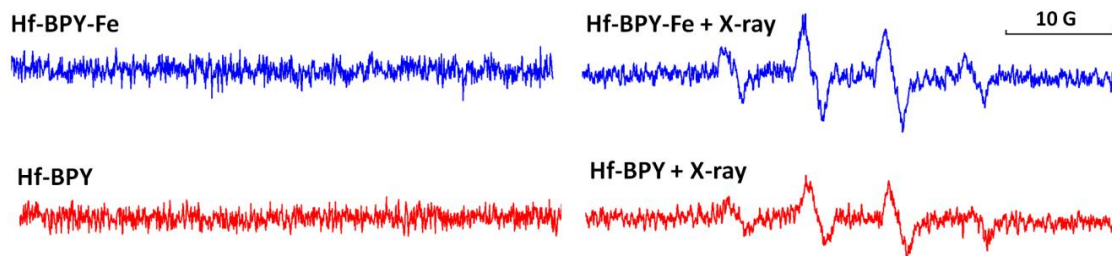


Figure S17. ESR spectra of different groups as indicated with/without X-ray irradiation (4 Gy). DMPO was used as the spin trap. Both of the two nMOFs shows representative $\cdot\text{OH}$ signals after X-ray treatment. The $\cdot\text{OH}$ mainly come from water dissociation induced by strong X-ray attenuation of Hf^{4+} in nMOFs. After irradiation, ESR signal intensity of **Hf-BPY-Fe** is slightly stronger than **Hf-BPY**, which is consistent with the cell growth inhibition of nMOFs with X-ray treatment (Figure S13).

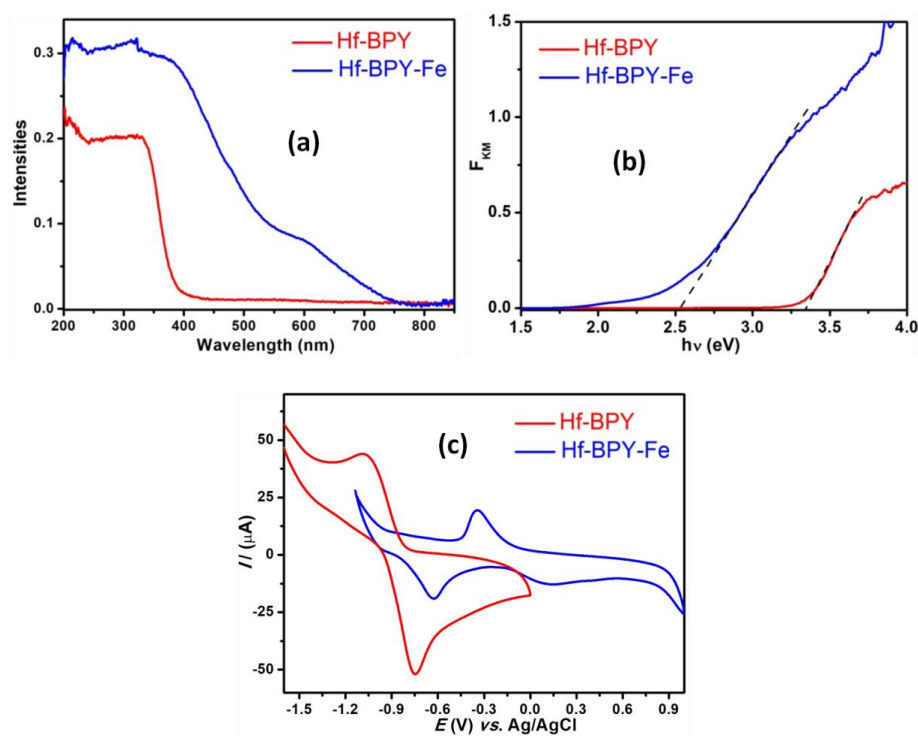


Figure S18. (a) Diffuse reflectance UV-vis spectra of **Hf-BPY** and **Hf-BPY-Fe**. (b) F_{km} spectra of nMOFs from diffuse reflectance UV-vis spectra. (c) Cyclic voltammograms of **Hf-BPY** and

Hf-BPY-Fe. According to E_{LUMO} (LUMO = lowest unoccupied molecular orbital) estimated from the first reduction potential and ΔCT estimated from the charge-transfer absorption in the solid-state UV-vis spectra.^{1, 5}

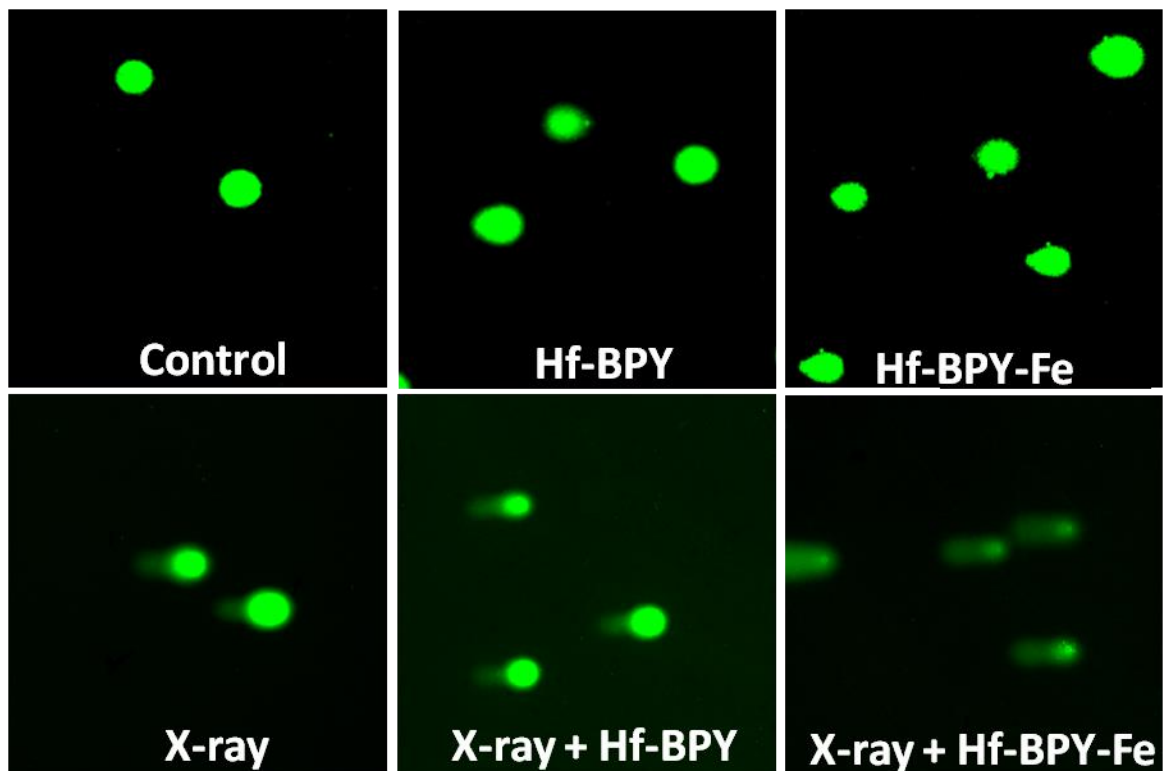


Figure S19. Comet assays after treatment with/without X-ray radiation. Compared to other groups, the clear comet tails in the X-ray + **Hf-BPY-Fe** group suggest severe DNA damage in cells. These results are consistent with the results of the γ -H2AX staining.

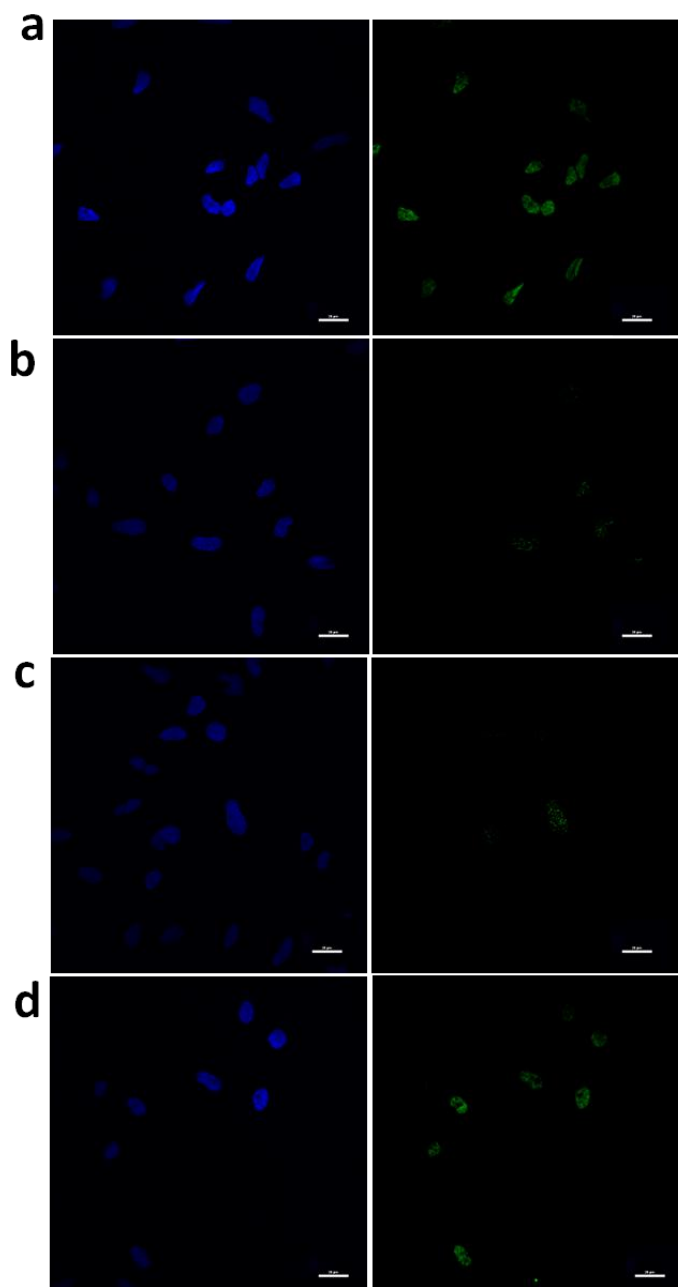


Figure S20. Detection of double-stranded DNA damage repair (Scale bar = 20 μm). The cells were irradiated with 6 Gy X-rays. Immunofluorescence images of $\gamma\text{-H2AX}$ with different treatments: (a) detect immediately; detect after co-cultured for 24 h with PBS (b), **Hf-BPY** (c), or **Hf-BPY-Fe** (d).

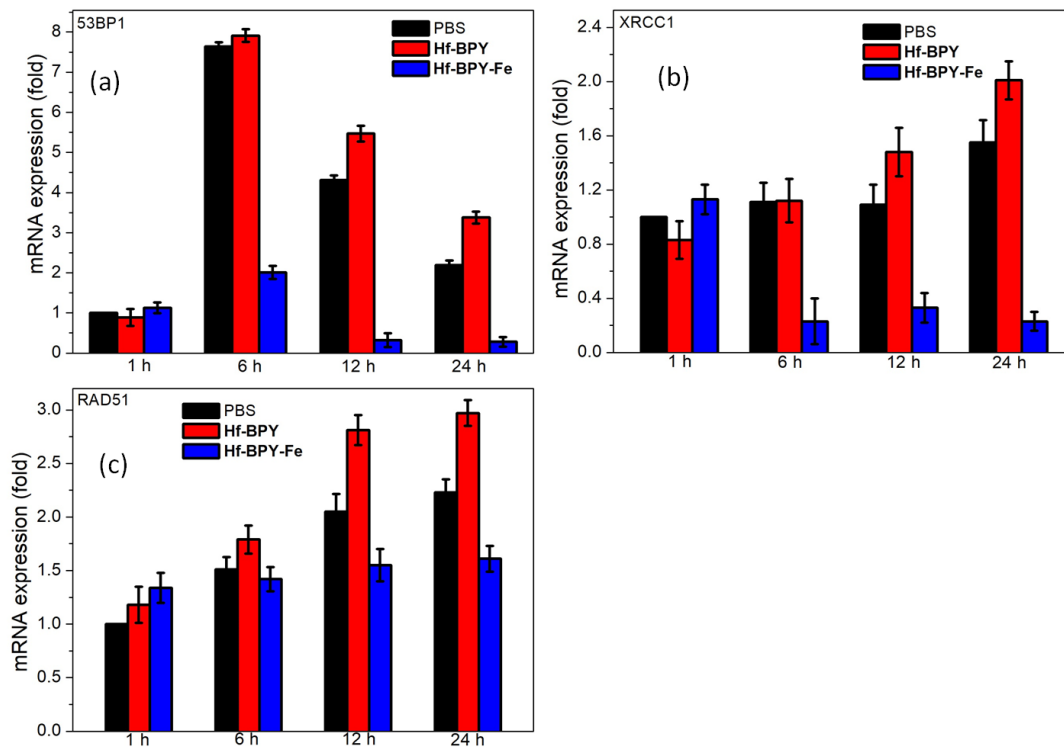


Figure S21. Real time PCR results of different groups including 53BP1, XRCC1 and RAD51.

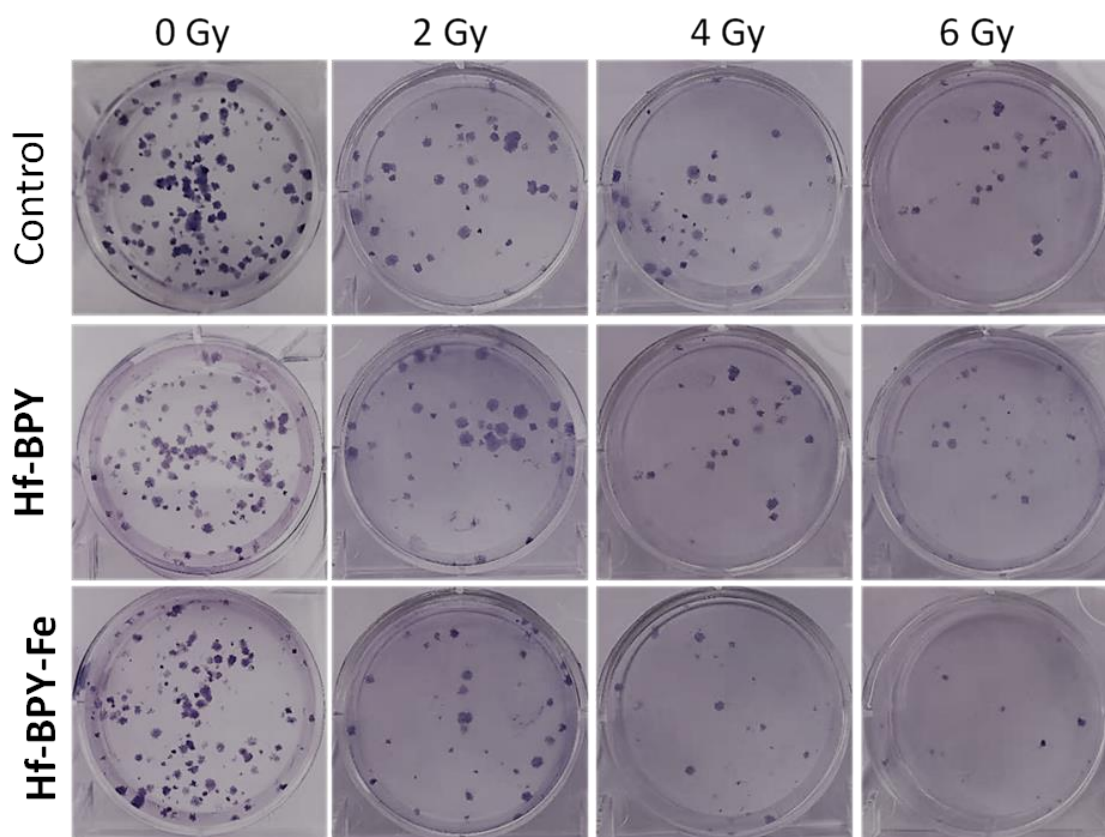


Figure S22. Colony formation assay of Hela cells incubated with PBS, **Hf-BPY** or **Hf-BPY-Fe** with or without X-ray irradiation.

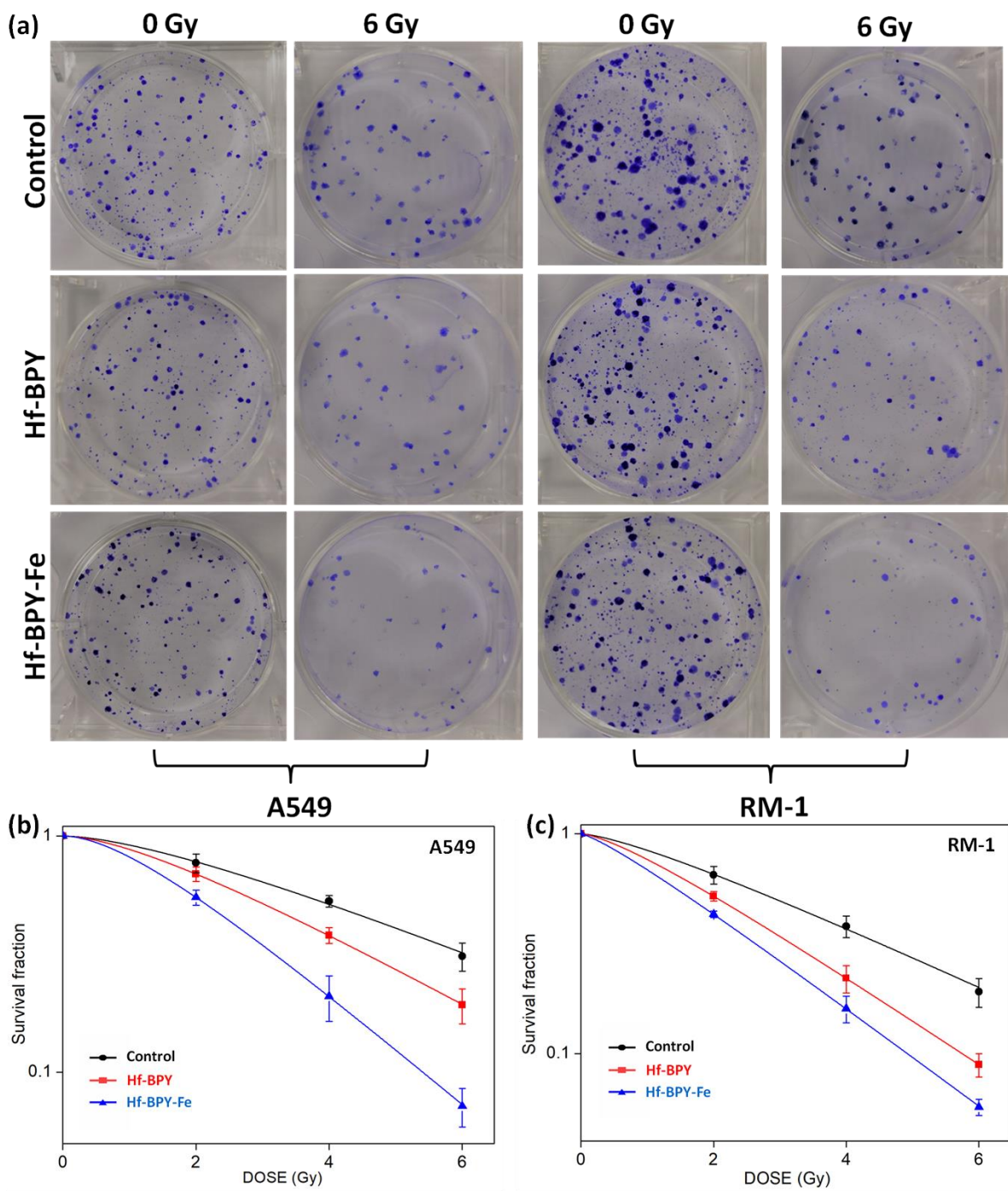


Figure S23. Colony formation assay of A549 and RM-1 cells lines.

Table S1. Sensitization enhancement ratio (SER) values by clonogenic assays in a panel of cell lines upon X-ray irradiation.

SER	Hela	A549	RM-1
Hf-BPY	1.41	1.32	1.43
Hf-BPY-Fe	1.74	1.87	1.75

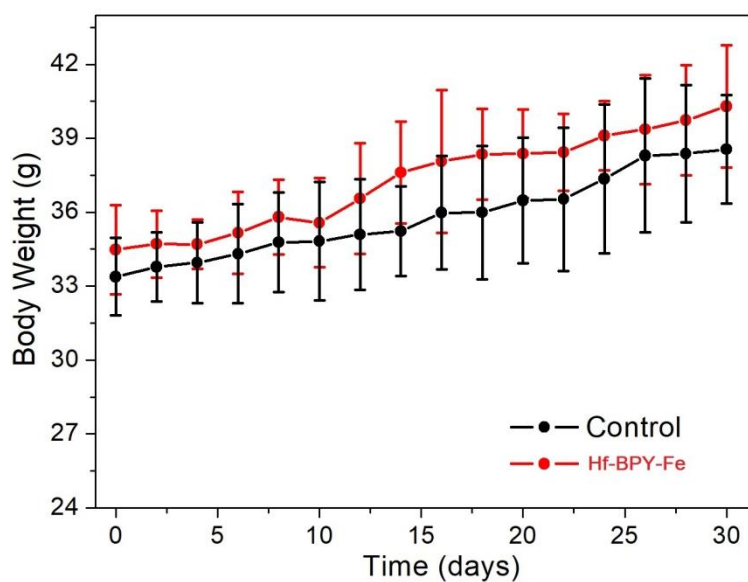


Figure S24. Temporal changes of body weight of Kunming mice after intravenous injection of **Hf-BPY-Fe** (Hf, 100 mg/kg) or PBS.

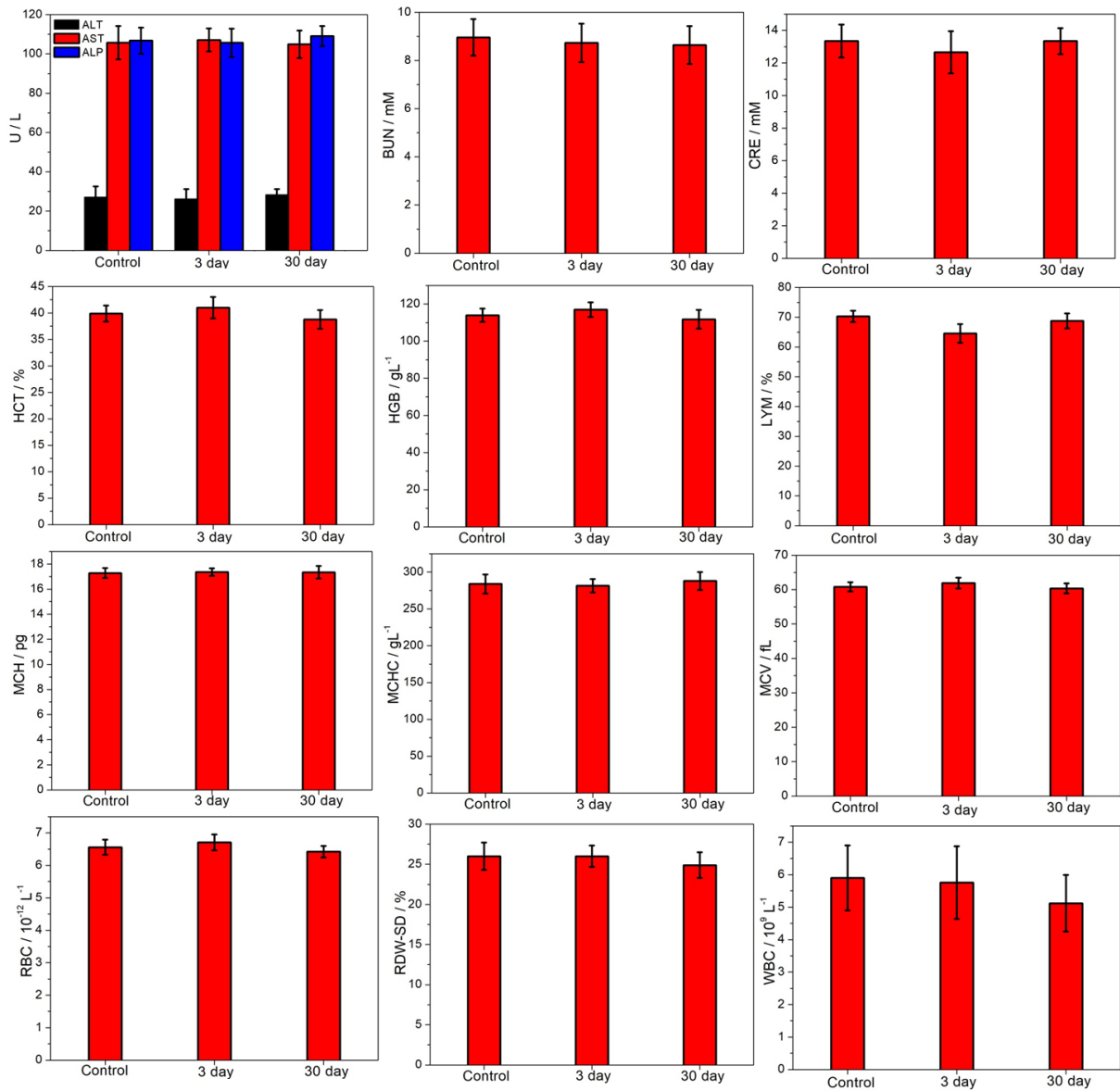


Figure S25. Hematological parameters of Kunming mice after intravenous injection of **Hf-BPY-Fe** (Hf, 100 mg/kg) or PBS.

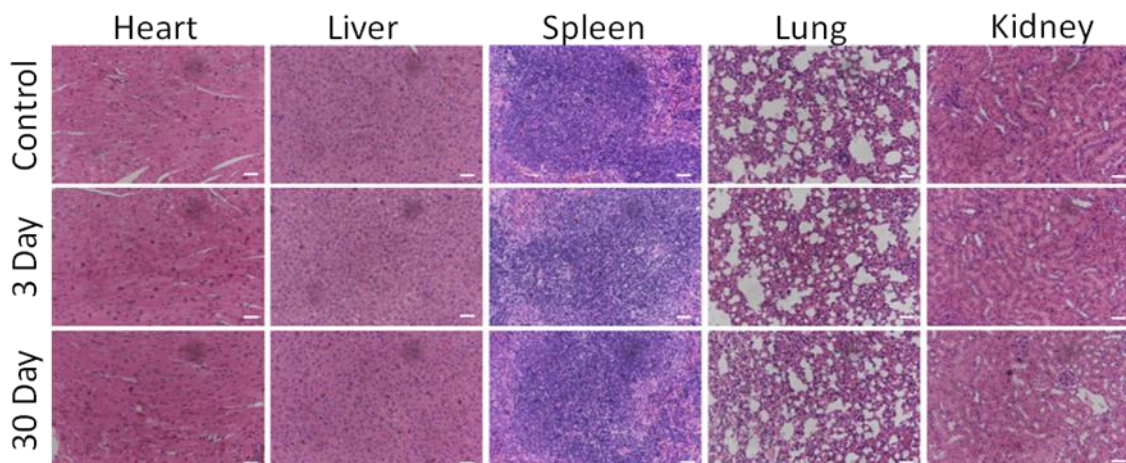


Figure S26. Hematoxylin-Eosin (H&E) stained tissue sections of major organs of Kunming mice after intravenous injection of **Hf-BPY-Fe** (Hf, 100 mg/kg). Scale bar = 50 μ m

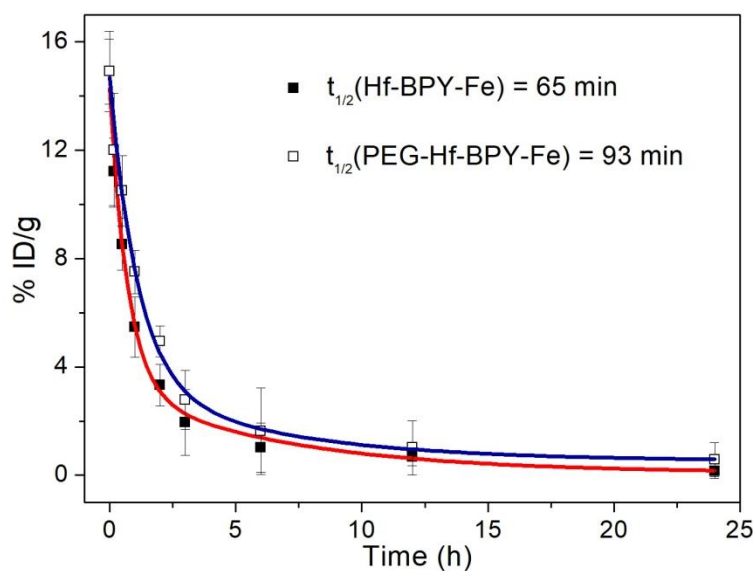


Figure S27. Blood half-time test of Hf element. The blood terminal half-life of **Hf-BPY-Fe** and **PEG-Hf-BPY-Fe** is 65 min and 93 min, respectively, based on the one-component pharmacokinetic model. Three female Balb/c nude mice at 7 weeks were injected intravenously with **Hf-BPY-Fe** and **PEG-Hf-BPY-Fe** (Hf, 100 mg/kg) saline solution, respectively. After that, 20 μ L blood was collected from the tail vein in the given time points (10 min, 30 min, 1 h, 2 h, 3 h, 6 h, 12h and 24 h) and diluted to 4 ml of ultrapure water with 10 mM

ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) as anticoagulant. Then ICP-OES was used to determine the time-dependent concentrations of Hf in blood.⁶ The prolonged circulation time of nanoparticles in blood could be attributed to surface modification of PEG reduces uptake in reticuloendothelial system (RES).⁷⁻⁸

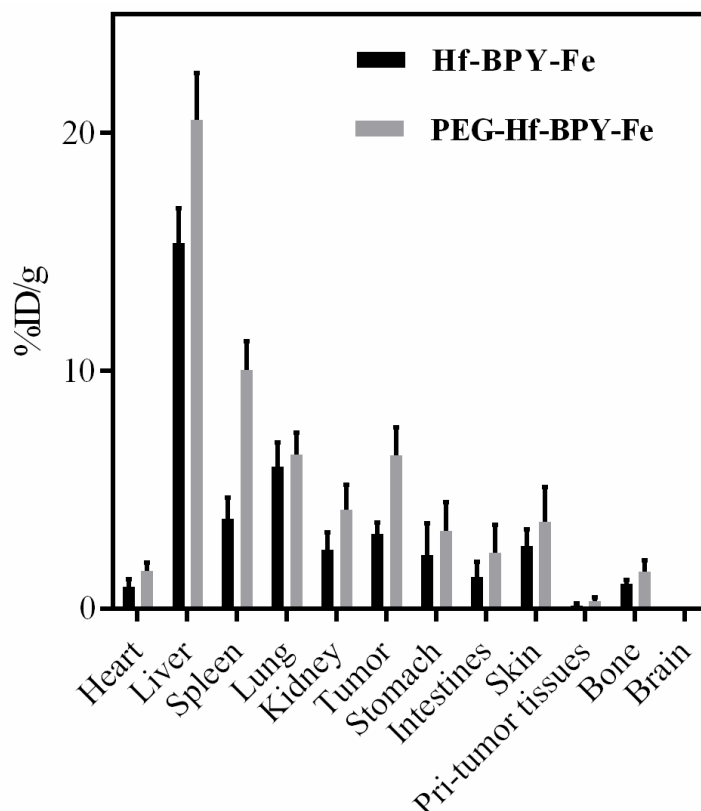


Figure S28. Biodistribution of Hf in Balb/c nude mice. Three female Balb/c nude mice at 7 weeks were injected intravenously with **Hf-BPY-Fe** or **PEG-Hf-BPY-Fe** (Hf, 100 mg/kg) saline solution. After 24h, the mice were sacrificed and the major organs, tumors and muscles adjacent to tumor were collected. These tissues and organs were weighted and then digested with aqua regia for ICP-OES analysis of Hf content. The percentage injected dose per gram (% ID g⁻¹) organ/tissue (*D*) of Hf was calculated by the following equation:⁹

$$D = \frac{M_{\text{Hf}}/M_{\text{organ}}}{M_{\text{ID}}} \times 100\%$$

where M_{Hf} (μg) is the mass in the corresponding organ whose mass is M_{organ} (g), and the constant M_{ID} (μg) is the injected dose of Hf.

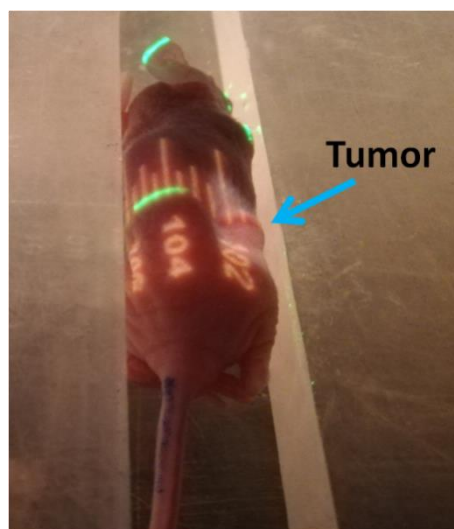


Figure S29. Localization of tumor in the clinical radiation therapy apparatus.

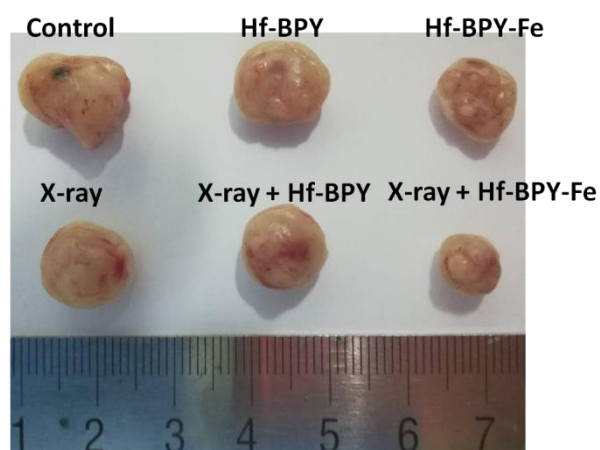


Figure S30. Tumor photos at the end of the observation.

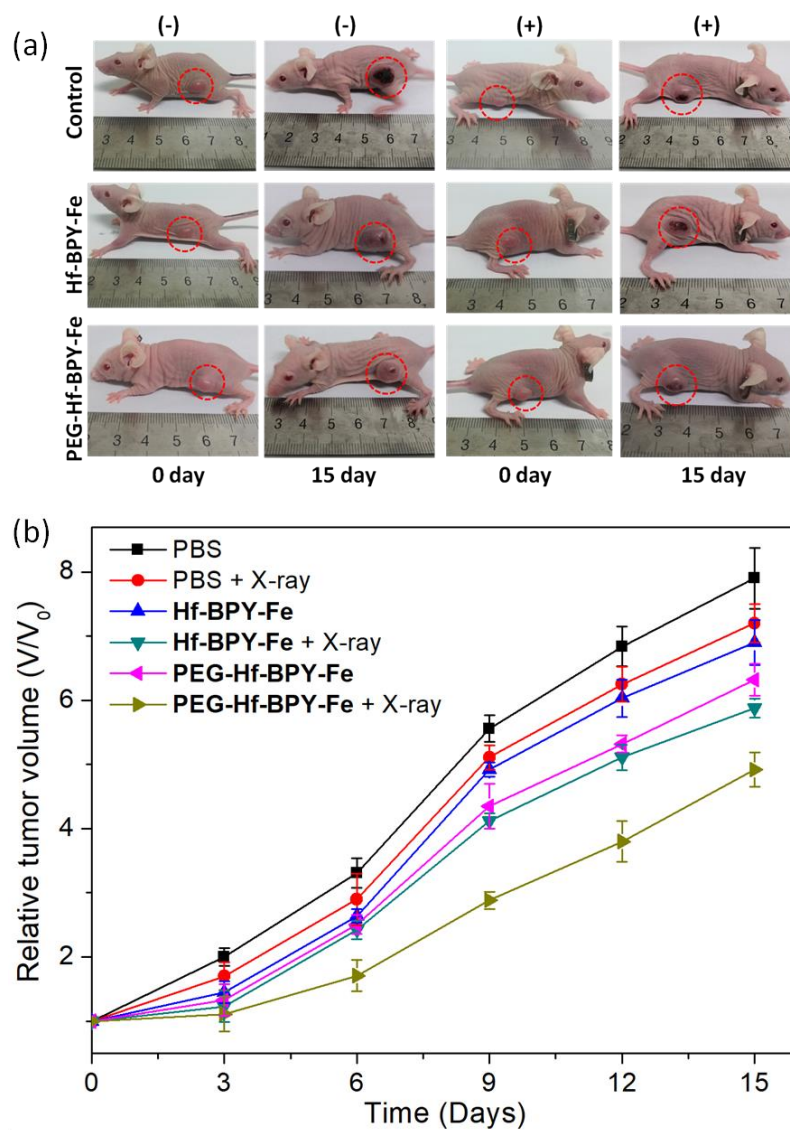


Figure S31. Photographs of xenograft tumors (a) and growth curves (b) after different treatments *via* intravenous injection.

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