This version of the ESI published 10/10/2022 replaces the previous version published 20/04/2020. The authors noted errors in the data reported in figures S16, S18, S36 and S39 and have now supplied updated figures. This correction does not change the conclusions of the main article.

Supporting Information for

Biomimetic Nanoscale Metal-Organic Framework Harnesses Hypoxia for Effective Cancer Radiotherapy and Immunotherapy

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S1. Materials and Methods

All starting materials were obtained from commercial sources (Sigma-Aldrich and Fisher) unless otherwise specified and used as received. Murine colon adenocarcinoma cell MC38 was acquired from the American Type Culture Collection (Rockville, MD, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium (GE Healthcare, USA) containing 10% FBS, 100 U/mL penicillin G sodium and 100 μg/mL streptomycin sulfate in a humidified atmosphere containing 5% CO_2 at 37ºC. C57BL/6 mice (6-8 weeks) were purchased from Harlan-Envigo Laboratories, Inc (USA). All animal experiments were performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985). The Institutional Animal Care and Use Committee at the University of Chicago reviewed and approved the study protocol ACUP 72408.

S2. Synthesis of H_2DBP-Fe and Hf-DBP-Fe

Preparation of H_2DBP-Fe 5,15-di(p-methyl-benzoato)porphyrin (Me_2DBP) was synthesized according to reported protocol.[44] A solution of Me_2DBP (0.582 g, 1.0 mmol) and $FeCl_2 \cdot 4H_2O$ (2.5 g, 12.8 mmol) in 100 mL of DMF was refluxed overnight. The mixture was cooled to room temperature and 150 mL of H_2O was added. The precipitate was separated by centrifugation and washed with 50 mL of H_2O three times. After washing, the solid was suspended in $CHCl_3$, and washed with 1 M HCl three times and with water twice. The organic layer was dried with anhydrous magnesium sulfate and evaporated to afford [5,15-di(p-methyl-benzoato)porphyrin]-Fe^{III}-Cl (Me_2DBP-Fe) as a brown powder in 85% yield (0.543 g, 0.85 mmol). As-synthesized Me_2DBP-Fe (0.510 g, 0.8 mmol) was dissolved in a mixture of tetrahydrofuran (THF) and methanol (100 mL, 1:1 vol/vol). A potassium hydroxide aqueous solution (14 mL, 2 M) was then added. The solution was heated to reflux overnight. THF and methanol was removed with a rotary evaporator before the solution was acidified to pH = 3 with 12 M hydrochloric acid. The dark brown product was collected by centrifugation and washed with water and methanol. The solid residue was dried under vacuum to give the pure [5,15-di(carboxybenzene)porphyrin]-Fe^{III}Cl (H_2DBP-Fe) product in 92% yield (450 mg, 0.74 mmol).

Preparation of Hf-DBP-Fe Hf-DBP was synthesized as previously reported.[1-2] To a 4 mL glass vial, 0.5 mL of Hf-DBP suspension (2 mM based on Hf in ethanol) and 0.5 mL of $FeCl_2 \cdot 4H_2O$ solution (10 mM in ethanol) were added. The reaction mixture was stirred at 70 °C for 48 hours. The dark brown precipitate (Hf-DBP-Fe) was obtained in quantitative yield by centrifugation and washed with ethanol.

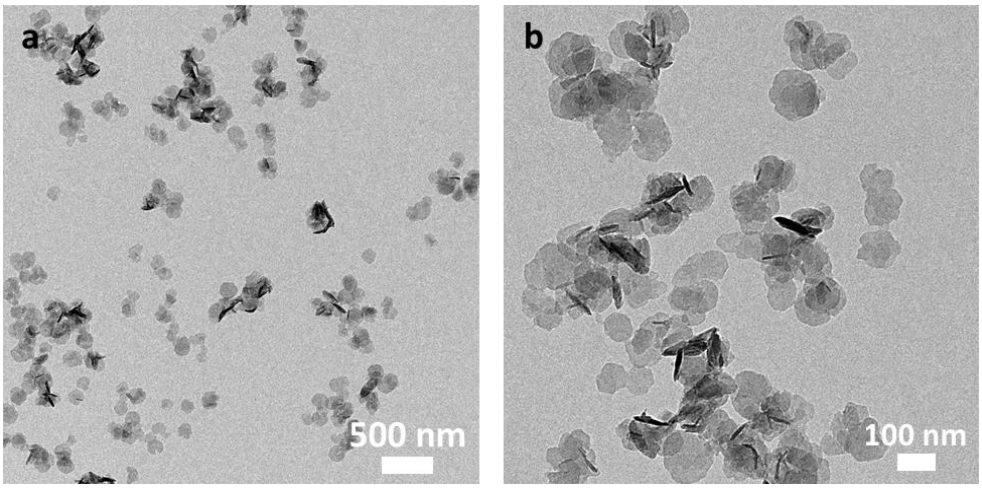
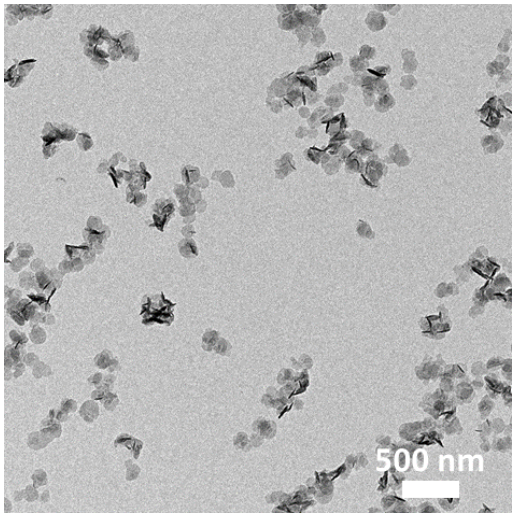
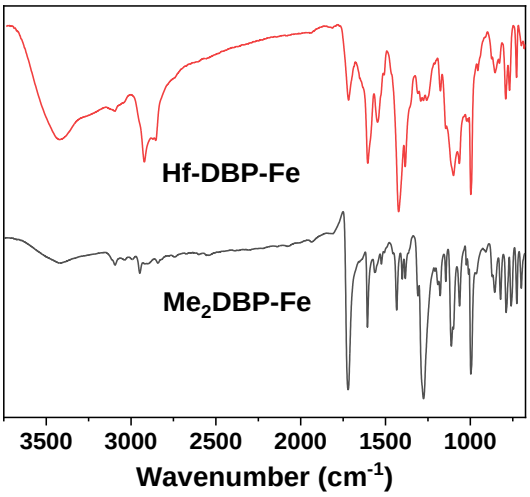


Figure S1 Representative TEM images of Hf-DBP in a large area (a) and zoom in image (b).





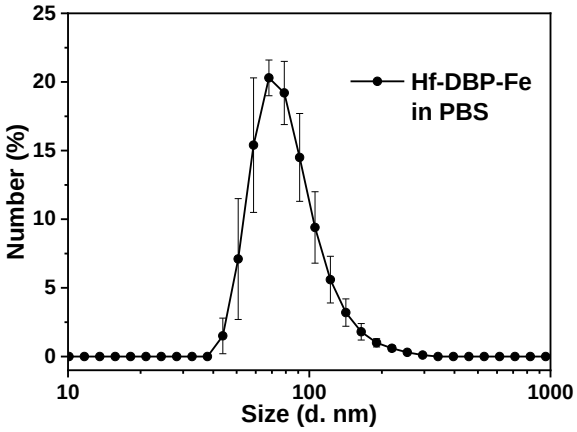


Figure S2 Representative TEM image of Hf-DBP-Fe in a large field (top). FT-IR spectra of Me_2DBP-Fe and Hf-DBP-Fe (middle). Number-averaged diameters of Hf-DBP-Fe in PBS (bottom).

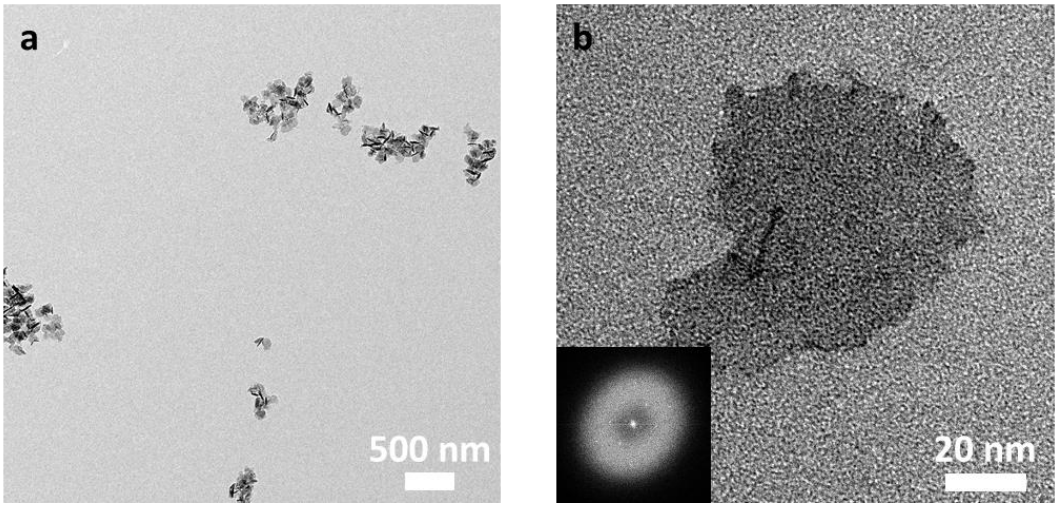


Figure S3 Representative TEM (a) and HRTEM (b) image of Hf-DBP-Fe after 1-day incubation in 6 mM PBS.

S3. X-ray Absorption Spectroscopy

Data collection. Hf-DBP-Fe and H_2DBP-Fe X-ray absorption data was collected at Beamline 10-BM at the Advanced Photon Source (APS) at Argonne National Laboratory. Spectra were collected at the Iron K-edge (7112 eV) in transmission mode. The X-ray beam was monochromatized by a Si(111) monochromator and detuned by 50% to reduce the contribution of higher-order harmonics below the level of noise. A metallic cobalt foil standard was used as a reference for energy calibration and was measured simultaneously with experimental samples. The incident (I_0), transmitted (I_t), and reference (I_r) beam intensities were measured by 20 cm ionization chambers with gas compositions of 98% N_2 and 2% Ar, 83% N_2 and 17% Ar, and 100% N_2, respectively. Data was collected over six regions: -250 to -30 eV (10 eV step size, dwell time of 0.25 s), -30 to -12 eV (5 eV step size, dwell time of 0.5 s), -12 to 30 eV (1.1 eV step size, dwell time of 1 s), 30 eV to 6 $Å^{-1}$, (0.05 $Å^{-1}$ step size, dwell time of 2 s), 6 $Å^{-1}$ to 12 $Å^{-1}$, (0.05 $Å^{-1}$ step size, dwell time of 4 s), 12 $Å^{-1}$ to 15 $Å^{-1}$, (0.05 $Å^{-1}$ step size, dwell time of 8 s). Multiple X-ray absorption spectra were collected at room temperature for each sample. Samples were ground and mixed with polyethylene glycol (PEG) and packed in a 6-shooter sample holder to achieve adequate absorption length.

Data processing. Data was processed using the Athena and Artemis programs of the IFEFFIT package based on FEFF 6. Prior to merging, spectra were calibrated against the reference spectra and aligned to the first peak in the smoothed first derivative of the absorption spectrum, the background noise was removed, and the spectra were processed to obtain a normalized unit edge step.

EXAFS fitting. Fitting of the EXAFS region was performed using the Artemis program of the IFEFFIT package. Fits were performed in the R space, with a k-weight of 3 for the Fe sample. Refinement was performed by optimizing an amplitude factor S_0^2 and Debye-Waller factor (σ^2). The fitting model for Hf-DBP-Fe and H_2DBP-Fe was based on the crystal structure BEDYEQ obtained from Cambridge Crystallographic Database. (Supplementary Table 1 and 2)

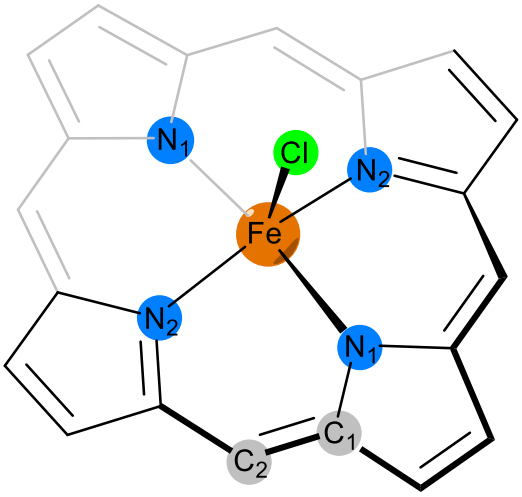


Figure S4 Molecular model of porphyrin-Fe-Cl for EXAFS fitting.

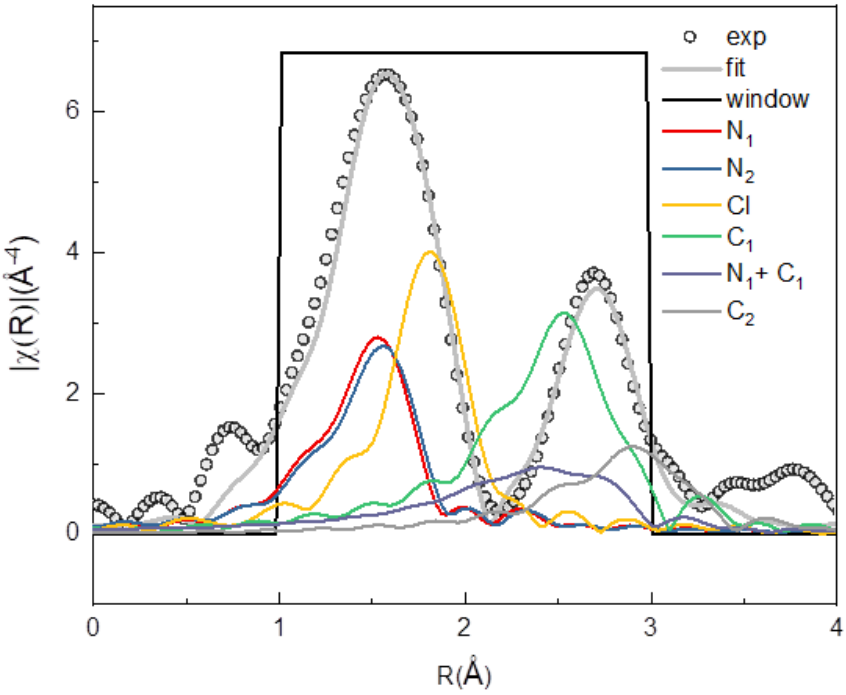


Figure S5 EXAFS fitting for H_2DBP-Fe homo ligand.

Table S1 Summary of EXAFS fitting parameters for Hf-DBP-Fe.

Hf-DBP-Fe		**Fitting Range**	k: 3.0 – 11.0 Å^{-1} R: 1.0 – 3.2 Å
Independent Points	11.0	**Variables**	8.0
Reduced chi-square	520.7	**R-factor**	0.005
ΔE_0(eV)	-9.48	**S_0^2**	1.00

R(Fe-N_1) (2)	2.00 ± 0.05 Å	**σ^2(Fe-N_1)**	0.005 ± 0.001
R(Fe-N_2) (2)	2.04 ± 0.05 Å	**σ^2 (Fe-N_2)**	0.005 ± 0.001
R(Fe- Cl) (1)	2.36 ± 0.14 Å	**σ^2 (Fe- Cl)**	0.003 ± 0.001
R(Fe-C_1) (8)	3.07 ± 0.05 Å	**σ^2 (Fe- C_1)**	0.005 ± 0.001
R(Fe-N_1-C_1) (16)	3.24 ± 0.05 Å	**σ^2 (Fe-N_1-C_1)**	0.005 ± 0.001
R(Fe-C_2) (4)	3.45 ± 0.05 Å	**σ^2 (Fe-C_2)**	0.002 ± 0.001

Table S2 Summary of EXAFS fitting parameters for Hf-DBP-Fe homo ligand.

Fe-homo ligand		**Fitting Range**	k: 3.0 – 13.5 $Å^{-1}$ R: 1.0 – 3.0 Å
Independent Points	13.1	**Variables**	9.0
Reduced chi-square	90.1	**R-factor**	0.011
ΔE_0(eV)	-7.64	**S_0^2**	1.00
R(Fe-N_1) (2)	2.00 ± 0.04 Å	**σ^2(Fe-N_1)**	0.006 ± 0.001
R(Fe-N_2) (2)	2.04 ± 0.04 Å	**σ^2 (Fe-N_2)**	0.006 ± 0.001
R(Fe- Cl) (1)	2.36 ± 0.13 Å	**σ^2 (Fe- Cl)**	0.004 ± 0.001
R(Fe-C_1) (8)	3.07 ± 0.03 Å	**σ^2 (Fe- C_1)**	0.005 ± 0.001
R(Fe-N_1-C_2) (16)	3.24 ± 0.03 Å	**σ^2 (Fe-N_1-C_2)**	0.005 ± 0.001
R(Fe-C_2) (4)	3.45 ± 0.02 Å	**σ^2 (Fe-C_2)**	0.004 ± 0.003

S4. Biomimetic Properties of Hf-DBP-Fe in Test Tubes

H_2O_2 decomposion Hf-DBP-Fe and Hf-DBP were suspended using water at equivalent concentrations of Hf at 20 μM in presence of H_2O_2 with final H_2O_2 concentration of 0, 10, 20, 30, 50, 100, and 200 μM. Water solutions with the H_2O_2 concentrations were used as control. A hydrogen peroxide assay kit was added to these suspensions (0.5 μL kit per 1 mL suspension) 4 hours after particle incubation to detect the remaining H_2O_2. The fluorescence signal of hydrogen peroxide assay kit was then collected with a Xenogen IVIS 200 imaging system.

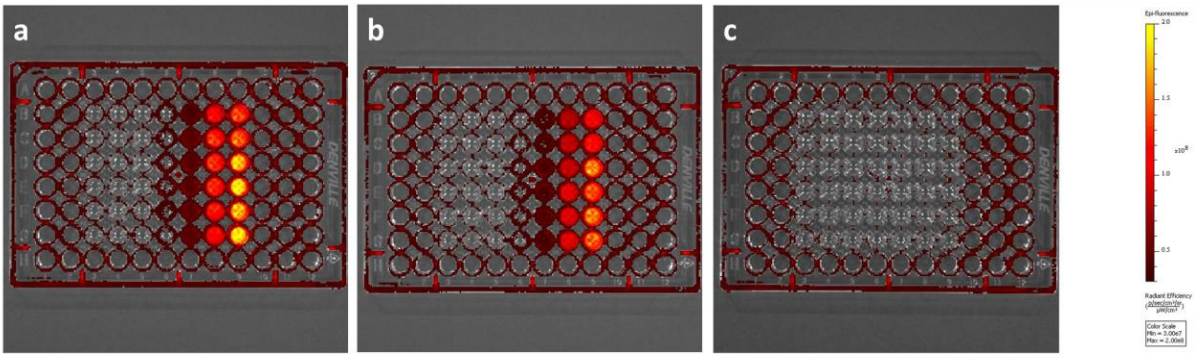


Figure S6 Fluorescence imaging showing the remaining of the H_2O_2 treated with water (a), Hf-DBP (b), and Hf-DBP-Fe (c) for 4 hours with a Hf concentration of 20 μM. The remaining of H_2O_2 was detected with a hydrogen peroxide kit assay. The concentration of H_2O_2 in each plate (from the 3rd to 9th column) was 0, 10, 20, 30 50, 100, and 200 μM, respectively.

Oxygen generation from H_2O_2 150 μM H_2O_2 was incubated with 20 μM Hf-DBP-Fe or Hf-DBP (based on Hf) in oxygen-free water. Dissolved O_2 concentrations were measured with an oxygen meter (Xylem, USA) for 10 mins.

•OH generation from H_2O_2 Hf-DBP-Fe and Hf-DBP were suspended in water at equivalent concentrations of Hf at 20 μM in the presence of 5 μM APF and H_2O_2 with final H_2O_2 concentrations of 0, 10, 20, 30, 50, 100, and 200 μM. Water solutions with the H_2O_2 concentrations were used as control. 4 hours after particle incubation, the fluorescence signal of APF was collected with a Xenogen IVIS 200 imaging system.

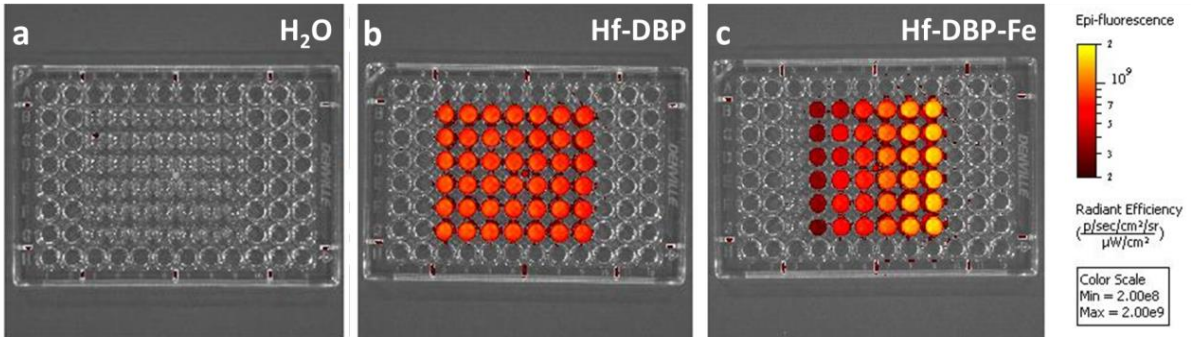


Figure S7 Fluorescence imaging showing the generation of the •OH from H_2O_2 treated with water (a), Hf-DBP (b), and Hf-DBP-Fe (c) for 4 hours with a Hf concentration of 20 μM. The generated •OH was detected by APF assay. The concentration of H_2O_2 in each plate (from the 3rd to 9th column) was 0, 10, 20, 30 50, 100, and 200 μM, respectively.

S5. Biomimetic Properties of Hf-DBP-Fe *in vitro*

Hypoxia treatment MC38 cells were subjected to hypoxic conditions according to reported protocol.[3] MC38 cells were seeded at 2×10^5 cells per well on 6-well plate . A day later, medium was replaced with fresh medium and placed in an anaerobic incubator (Thermo Scientific, USA) filled with 94.5% N_2, 5% CO_2 and 0.5% O_2 to induce hypoxia. MC38 cells were incubated under normoxic condition containing 5% CO_2 and 95% air (about 20% O_2) as control.

Cellular uptake The cellular uptake of Hf-DBP-Fe and Hf-DBP was evaluated on MC38 cells. Using 6-well plates, cells were seeded at 1.5×10^6/well and cultured overnight. Cells were dosed an Hf concentration of 20 μM. After incubation of 1, 2, 4 and 8 hours, the cells were collected, and the cell numbers were counted by a hemocytometer. Cells were digested with 1% hydrofluoric acid and concentrated nitric acid in a microwave reactor (CEM, USA) and the Hf concentrations were determined by ICP-MS (Agilent, USA). Results are expressed as the amount of Hf (ng) per 10^5 cells.

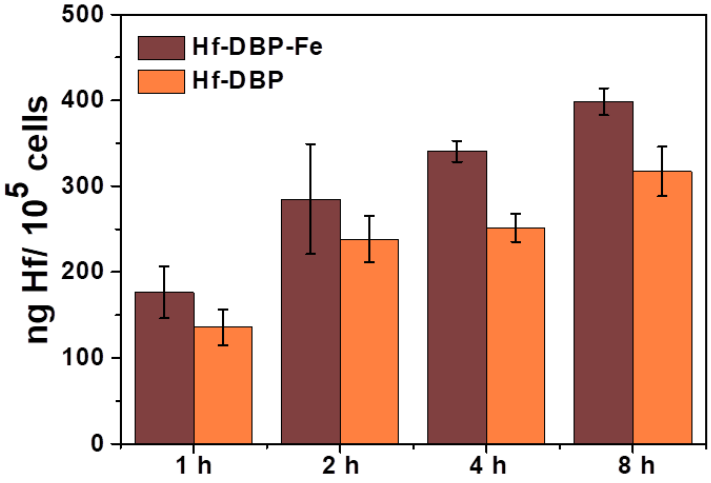


Figure S8 Cellular uptake of Hf-DBP-Fe and Hf-DBP after 1, 2, 4 or 8 h incubation with equivalent Hf concentrations of 20 μM (n = 3). The Hf concentrations were determined by ICP-MS.

Intracellular H_2O_2 assay Intracellular H_2O_2 was detected with a MAK164 intracellular H_2O_2 kit (Sigma Aldrich, USA). For *in vitro* studies, MC38 cells were incubated in normoxic and hypoxic conditions and further incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentration for 4 hours. For hypoxic condition, MC38 cells were cultured in the hypoxic chamber for 12 hours. For the normoxic condition, MC38 cells were treated with 100 μM of H_2O_2 for 12 hours. Then the medium was replaced with assay buffer and incubated for another 1 hour. After washing with PBS, intracellular H_2O_2 was observed under CLSM. For *ex vivo* immunofluorescence staining, MC38 tumor bearing mice were treated with H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe intratumorally at an equivalent dose of 0.2 μmol. Mice treated with PBS served as a control. Following tumor collection, frozen tissue sections with a thickness of 5 μm were prepared using a CM1950 cryostat (Leica, Germany). The sections were air-dried for at least 1 hour and fixed in acetone at 20 °C for 10 min. After staining with MAK164 intracellular H_2O_2 kit and DAPI, the sections were washed 2x with PBS and observed using CLSM.

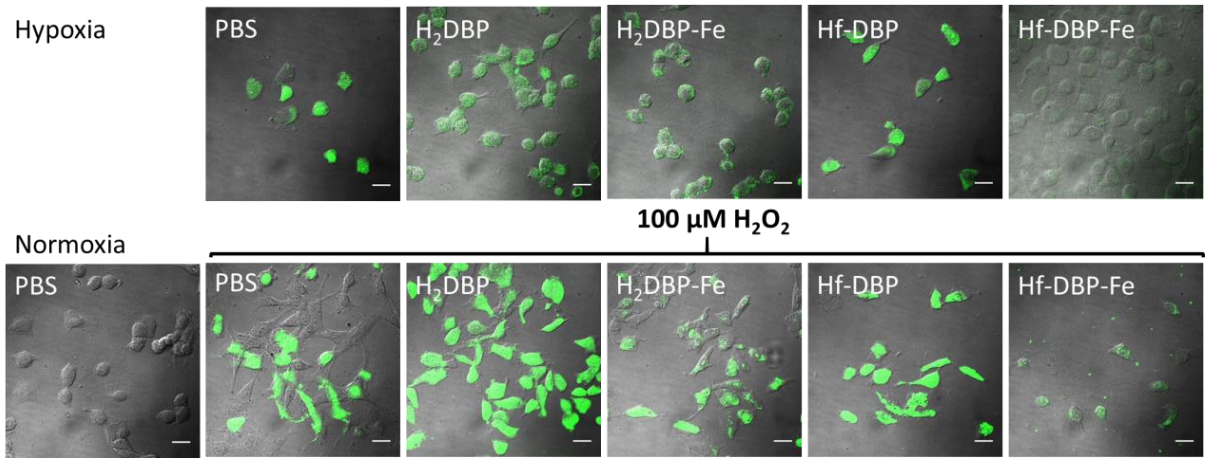


Figure S9 Representative confocal images of intracellular H_2O_2 in MC38 cells incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe under hypoxic condition and normoxic condition with or without 100 μM H_2O_2 treatment. Green fluorescence comes from MAK164 H_2O_2 kit in the cells. First row: cells under hypoxic condition. Second row: cells under normoxic condition; from left to right: PBS without H_2O_2 treatment, PBS with H_2O_2 treatment, H_2DBP with H_2O_2 treatment, H_2DBP-Fe with H_2O_2 treatment, Hf-DBP with H_2O_2 treatment or Hf-DBP-Fe with H_2O_2 treatment, respectively. Scale bar =20 μm.

Intracellular O_2 assay Intracellular O_2 levels were determined with $[Ru(bpy)_3]Cl_2$. MC38 cells were incubated in normoxic and hypoxic conditions and preloaded with $[Ru(bpy)_3]Cl_2$ before further incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentration for 4 hours. Then the cells were washed with fresh PBS and incubated in $[Ru(bpy)_3]Cl_2$ buffer for one additional hour. After washing with PBS, intracellular $[Ru(bpy)_3]Cl_2$ phosphorescence was observed under CLSM.

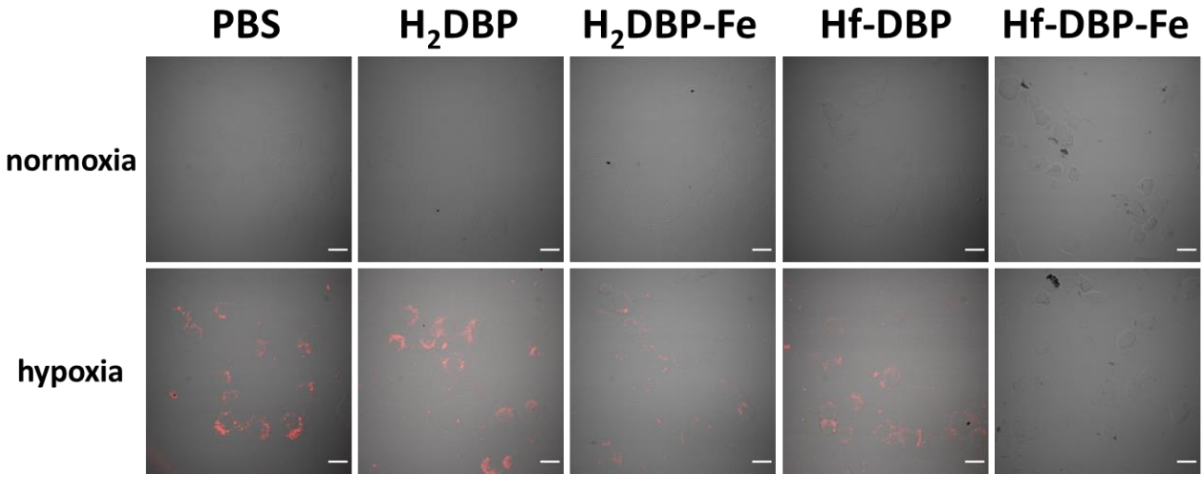


Figure S10 Representative confocal images of intracellular O_2 in MC38 cells incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe under normoxic condition or hypoxic condition. Red fluorescence comes from unquenched phosphorescence of $Ru(bpy)_3Cl_2$ in the cells. First row: cells under normoxic condition. Second row: cells under hypoxic condition; from left to right: PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe, respectively. Scale bar =20 μm.

HIF1-α immunostaining Intracellular hypoxia levels were probed with the ab190197 antibody against HIF1-α that was conjugated with Alexa Fluor 488 fluorochrome (Abcam, USA). For *in vitro*

studies, MC38 cells were incubated in normoxic and hypoxic conditions and further incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentrationfor 4 hours. Cells were then stained with the antibody against HIF-1α and DAPI subsequently then observed under CLSM. For *ex vivo* immunofluorescence staining, MC38 tumor bearing mice were treated with H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe intratumorally at a 0.2 μmol equivalent dose. Mice treated with PBS served as a control. Tumors were collected and frozen tissue sections with a thickness of 5 μm were processed using a CM1950 cryostat (Leica, Germany). The sections were dried in air for at least 1 h and fixed in acetone at 20 °C for 10 min. After stained with Alexa Fluor 488-HIF-1α and DAPI, the sections were then washed twice with PBS before CLSM imaging.

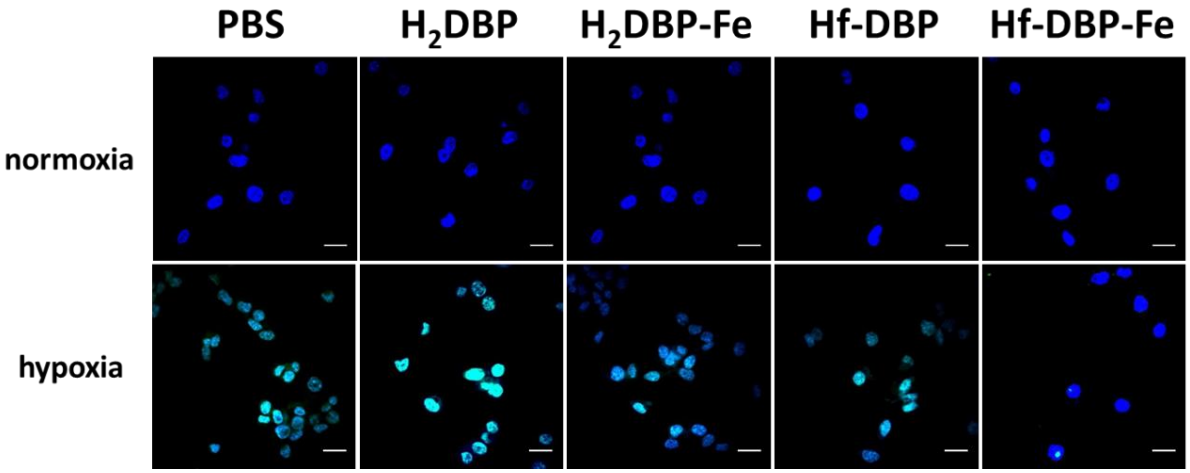


Figure S11 Representative confocal images of intranuclear HIF1-α expression in MC38 cells incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe under normoxic condition or hypoxic condition. Green fluorescence comes from HIF1-α-conjugated FITC fluorophore. First row: cells under normoxic condition. Second row: cells under hypoxic condition; from left to right: PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe, respectively. Scale bar =20 μm.

***In vitro* $^1O_2$ generation** Singlet oxygen sensor green (SOSG) reagent (Life Technologies, USA) was used to detect $^1O_2$ in hypoxic and normoxic conditions. MC38 cells were seeded on cover slides in 6-well plate at 2×10^5 cells per well, further cultured overnight and loaded with SOSG probe. H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe was added to cells at a 20 μM equivalent concentrationfor 4 hours. As a control, cells were incubated with PBS. After 4 hours of incubation, cells were irradiated at 0 and 2 Gy X-ray. The slides were then washed with PBS and observed using CLSM.

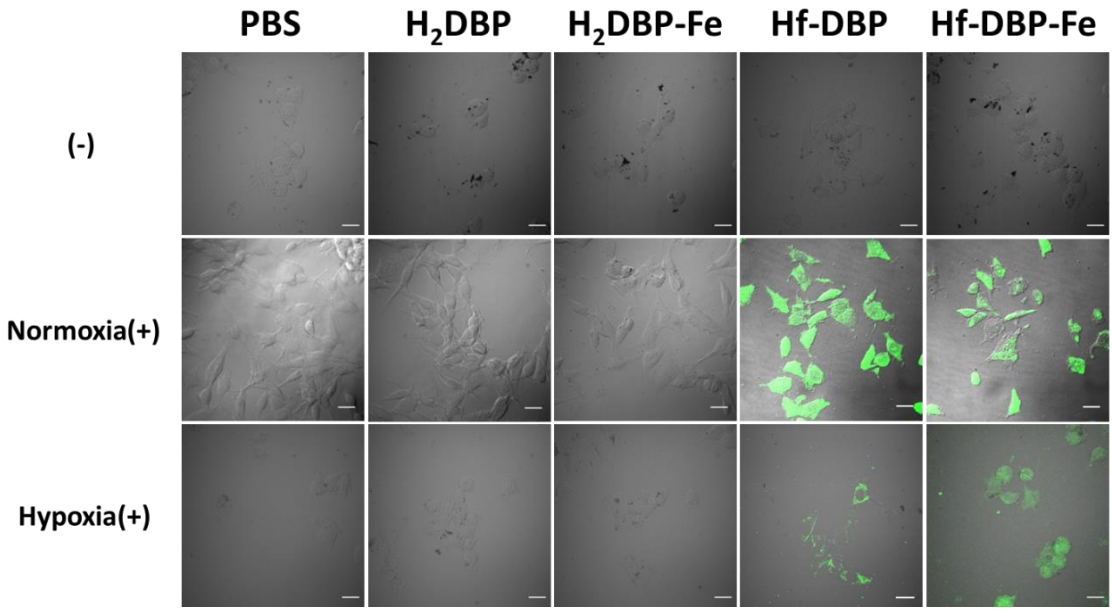


Figure S12 Representative confocal images of intracellular $^1O_2$ generation detected by SOSG. MC38 cells were preloaded with SOSG, treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe, irradiated upon X-ray at 0 (-) or 2 (+) Gy under normoxic condition or hypoxic condition. Green fluorescence from SOSG captured $^1O_2$. Scale bar = 20 μm.

Intracellular ˙OH generation We used coumarin-3-carboxylic acid to probe ˙OH generation in MC38 cells. We seeded MC38 cells on cover slides in 6-well plate at 2×10^5 cells per well. The cells were further cultured overnight and treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentrationfor 4 hours in normoxic or hypoxic conditions. Cells were then immediately stained with 20 μM coumarin-3-carboxylic acid. Following incubation for 20 min, cells were washed using PBS. The ˙OH generated in live cells was visualized by observing the blue fluorescence inside cells under CLSM.

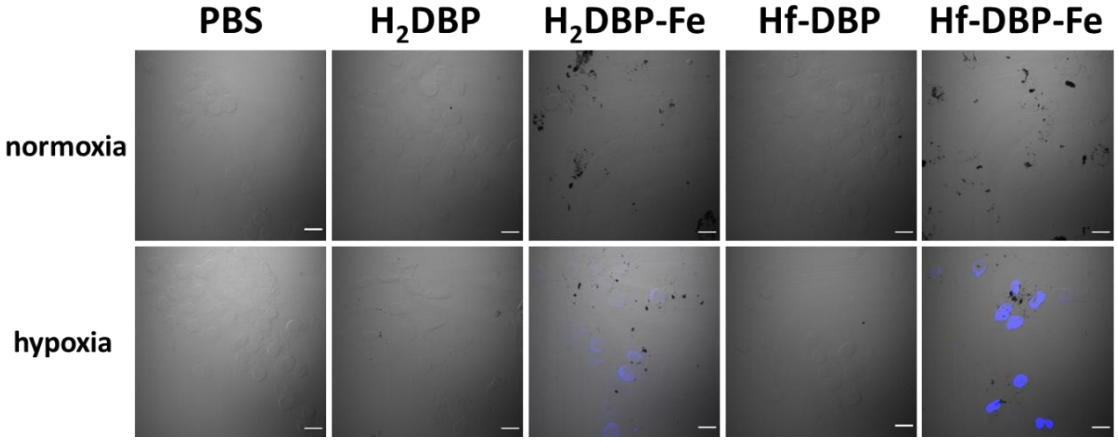


Figure S13 Representative CLSM imaging of coumarin acid assay detecting intracellular ˙OH generation. MC38 cells incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe under normoxic condition or hypoxic condition. Blue fluorescence comes from 7-hydroxy coumarin carboxylic acid (7-OHCCA). First row: cells under normoxic condition. Second row: cells under hypoxic condition; from left to right: PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe, respectively. Scale bar =20 μm.

S6. *In vitro* Anti-cancer Efficacy

Clonogenic assay The clonogenic assay was carried out as per a modified protocol.[4] MC38 cells were cultured in a 6-well plate overnight in normoxic or hypoxic condition and incubated with PBS, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentration for 4 hours and then irradiated with 0, 1, 2, 4, 8 and 16 Gy X-ray (250 KVp, 15 mA, 1 mm Cu filter). Following irradiation, cells were trypsinized and counted. 200-2000 cells were selected and seeded in a 6-well plate and cultured with 2 mL medium for 10 days. Once colony formation was visible (~12 days), culture medium was discarded. Plates were rinsed 2x with PBS stained with 500 μL of 0.5% w/v crystal violet in 50% methanol/H_2O. Using water, the wells were rinsed one time and the colonies were manually counted.

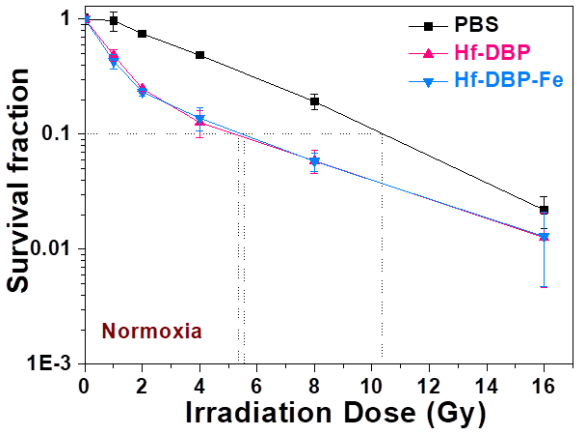


Figure S14 Clonogenic assay upon orthovoltage X-ray of Hf-DBP, or Hf-DBP-Fe against MC38 cells under normoxic condition, n = 6.

Cytotoxicity The cytotoxicity of H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe was tested with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium (MTS) assay (Promega, USA) with or without radiation. MC38 cells were seeded on 96-well plates at 1×10^4/well and cultured overnight in hypoxic or normoxic conditions. H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe was added to the cells at an equivalent dose of 0, 1, 2, 5, 10, 20, 50 and 100 μM and incubated for 4 hours. Following dosage, cells were irradiated with X-rays at a dose of 2 Gy (Philips RT250 X-ray generator, Philips, USA, 250 KVp, 15 mA, 1 mm Cu filter). Cells were incubated for 72 hours and then analyzed for survival by MTS assay.

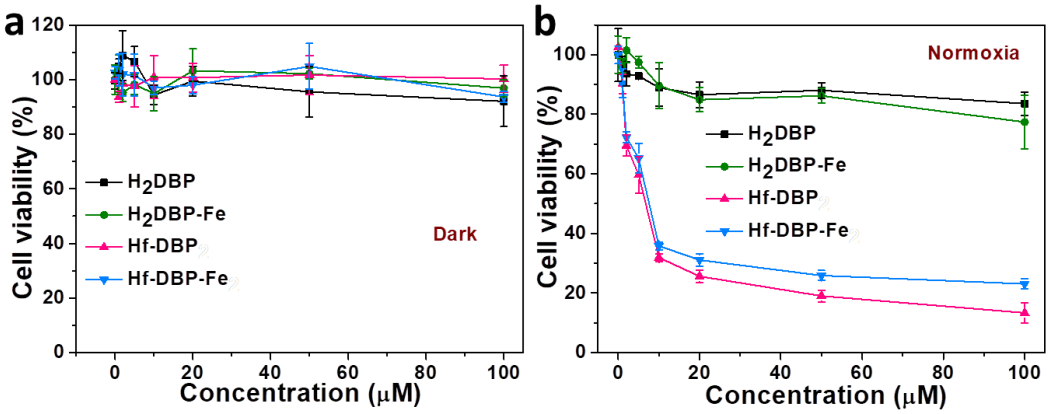


Figure S15 Dark cytotoxicity (a) and cytotoxicity (b) upon orthovoltage X-ray of H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe against MC38 cells under normoxic condition at different concentrations, n = 6.

Apoptosis/necrosis MC38 cells were cultured in a 6-well plate overnight and incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentration for 4 hours in hypoxic or normoxic conditions followed by X-ray radiation at 0 or 2 Gy (250 kVp, 15 mA, 1 mm Cu filter). 24 hours later, the cells were stained according to the AlexaFluor 488 Annexin V/dead cell apoptosis kit (Life technology, USA), imaged under CLSM and quantified by flow cytometry.

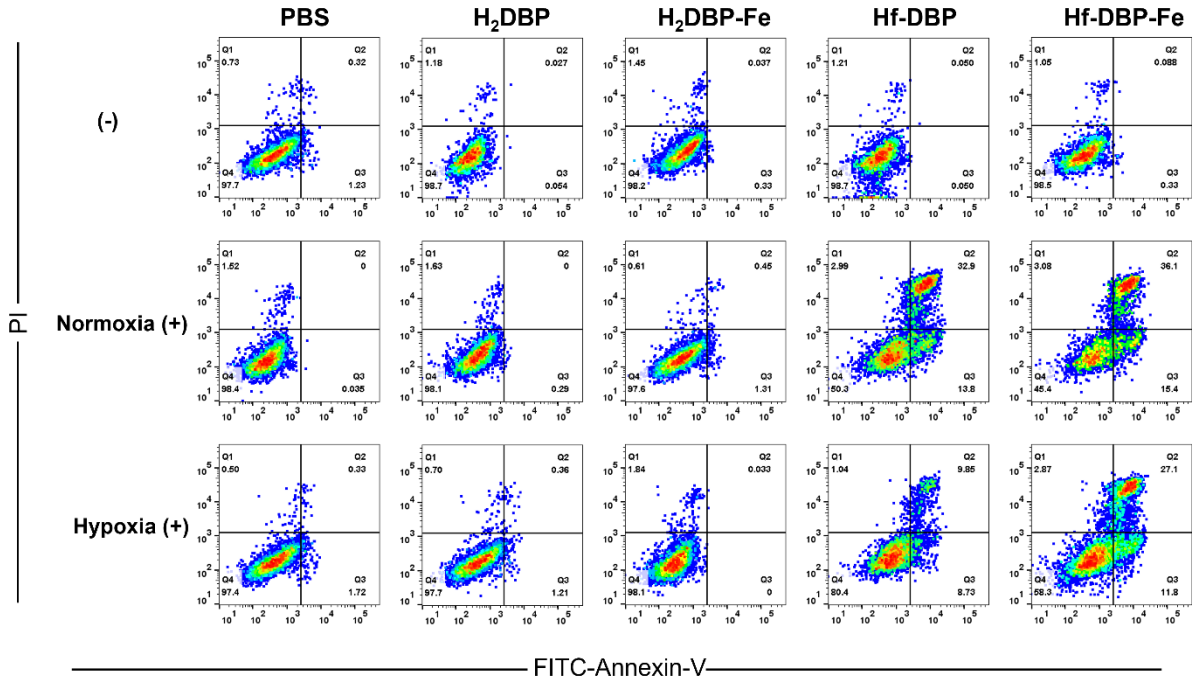


Figure S16 Annexin V/PI analysis of MC38 cells treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe for 4 h and then irradiated upon X-ray at a dose of 0 (-) or 2 (+) Gy under normoxic or hypoxic conditions. The quadrants from lower left to upper left (counter clockwise) represent healthy, early apoptotic, late apoptotic, and necrotic cells, respectively. The percentage of cells in each quadrant was shown on the graphs.

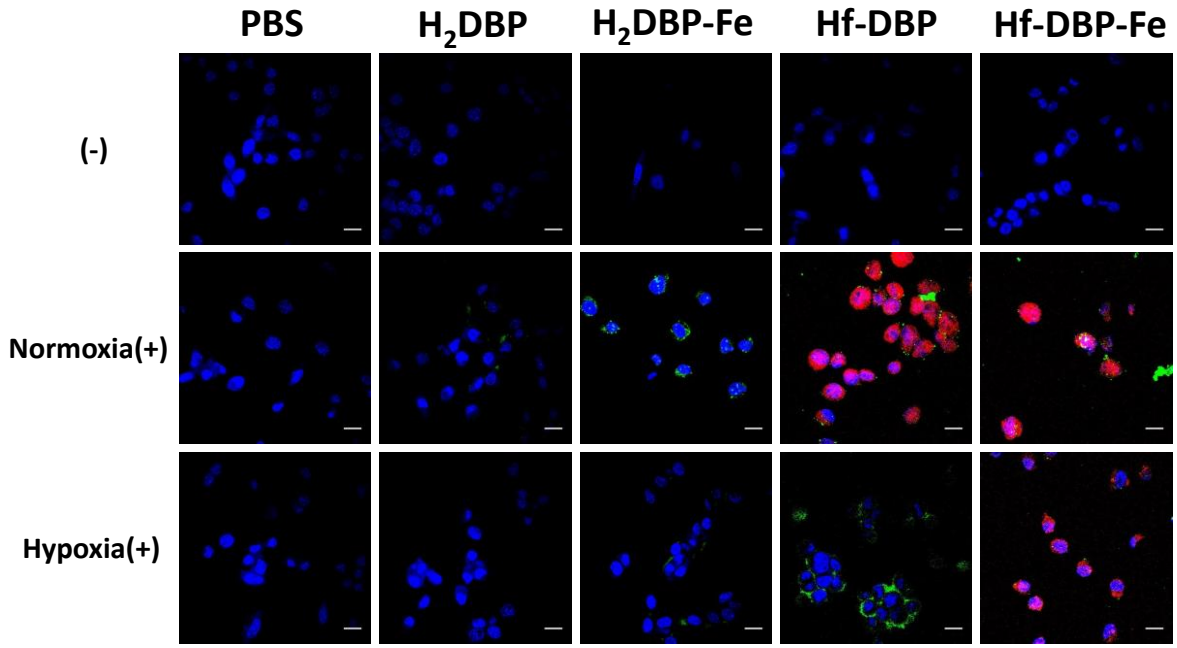


Figure S17 Representative CLSM imaging showing apoptosis and necrosis of MC38 cells treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe and irradiated upon X-ray at a dose of 0 (-) or 2 (+) Gy under normoxic or hypoxic conditions. Blue, red and green fluorescence represent DAPI, PI and Annexin-V-conjugated Alexa-488, respectively. Scale bar = 20 μm.

Live/dead cell analysis The live/dead cell analysis was evaluated with cell permeable dye calcein AM and propidium iodide (PI) kit. MC38 cells were cultured in a 6-well plate overnight and incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentration for 4 hours in hypoxic or normoxic conditions followed by X-ray radiation at 0 or 2 Gy (250 kVp, 15 mA, 1 mm Cu filter). The cells were washed with PBS gently and stained with calcein AM (green) and PI (red) to visualize live cells and dead cells, respectively.

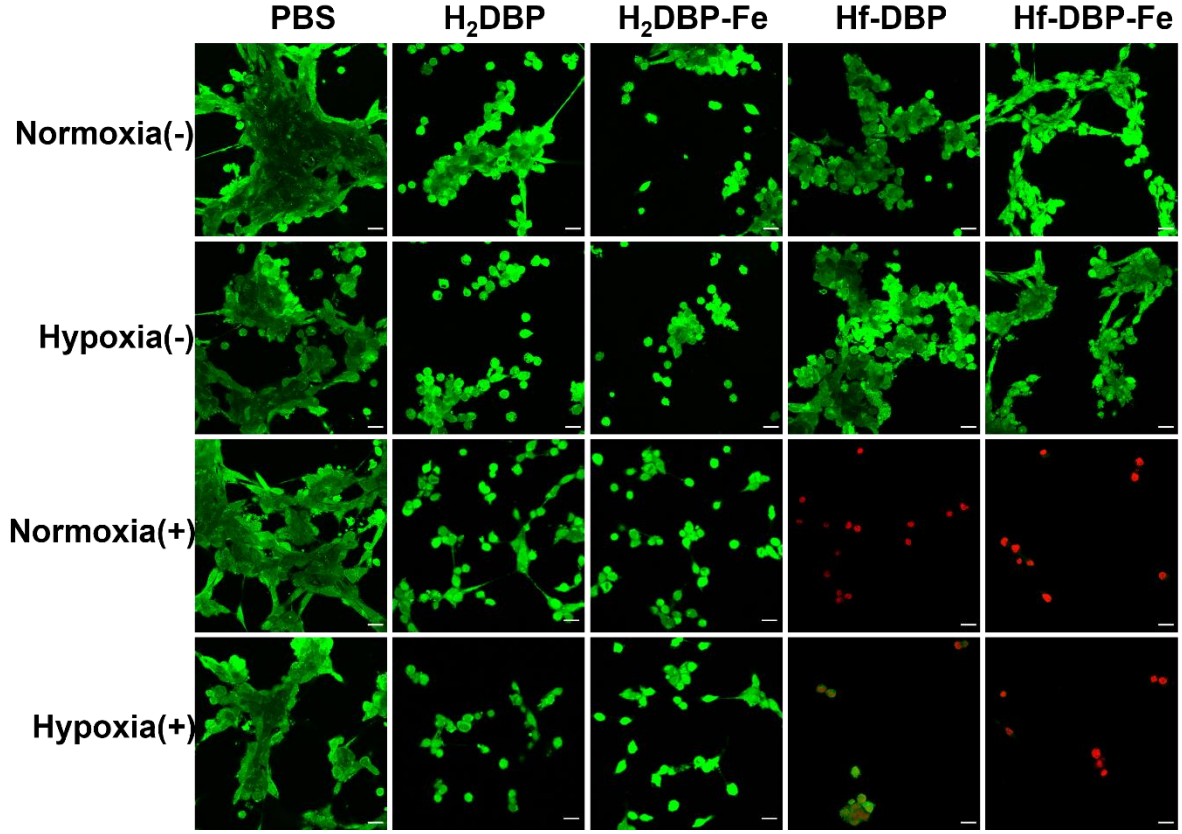


Figure S18 Representative CLSM imaging of Live/dead cell analysis on MC38 cells treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe and irradiated upon X-ray at a dose of 0 (-) or 2 (+) Gy under normoxic or hypoxic conditions. Green and red fluorescence represent Calcein AM and PI signal, indicating live or dead cells, respectively. Scale bar = 50 μm.

DNA double strand break MC38 cells were cultured in a 6-well plate overnight and incubated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentration for 4 hours in hypoxic or normoxic conditions followed by X-ray radiation at 0 and 2 Gy (250 kVp, 15 mA, 1 mm Cu filter). 2 hours after irradiation, cells were stained with HCS DNA damage kit (Life Technology, USA) for CLSM imaging (FV1000, Olympus, Japan).

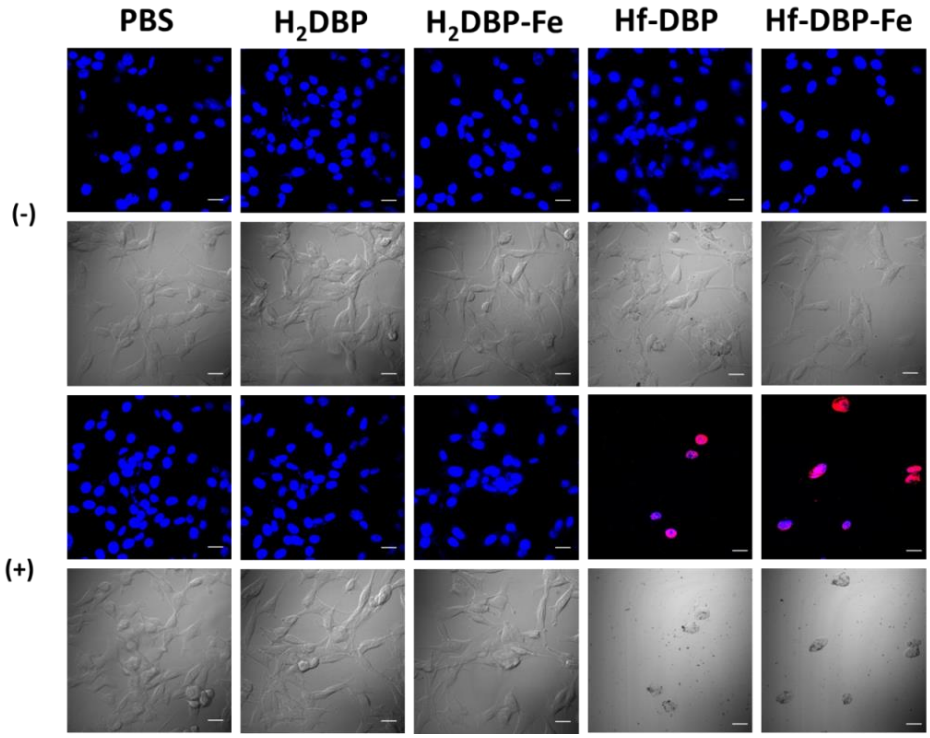


Figure S19 Representative CLSM imaging of γ-H2AX assay showing the DNA double strain breaks (DSBs) in MC38 cells treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe and irradiated with X-ray at a dose of 0 (-) or 2 (+) Gy under normoxic condition. Blue and red fluorescence show DAPI-stained nucleus and antibody-labeled γ-H2AX in the cells, respectively. Scale bar = 20 μm.

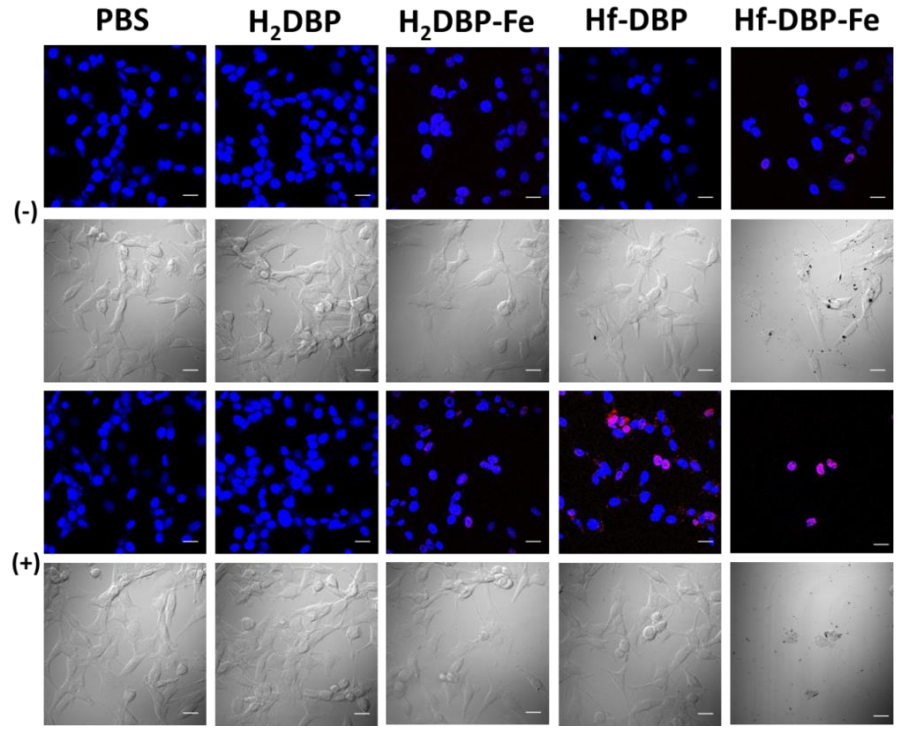


Figure S20 Representative CLSM imaging of γ-H2AX assay showing the DSBs in MC38 cells treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe and irradiated with X-ray at a dose of 0 (-) or 2 (+) Gy under hypoxic condition. Blue and red fluorescence show DAPI-stained nucleus and antibody-labeled γ-H2AX in the cells, respectively. Scale bar = 20 μm.

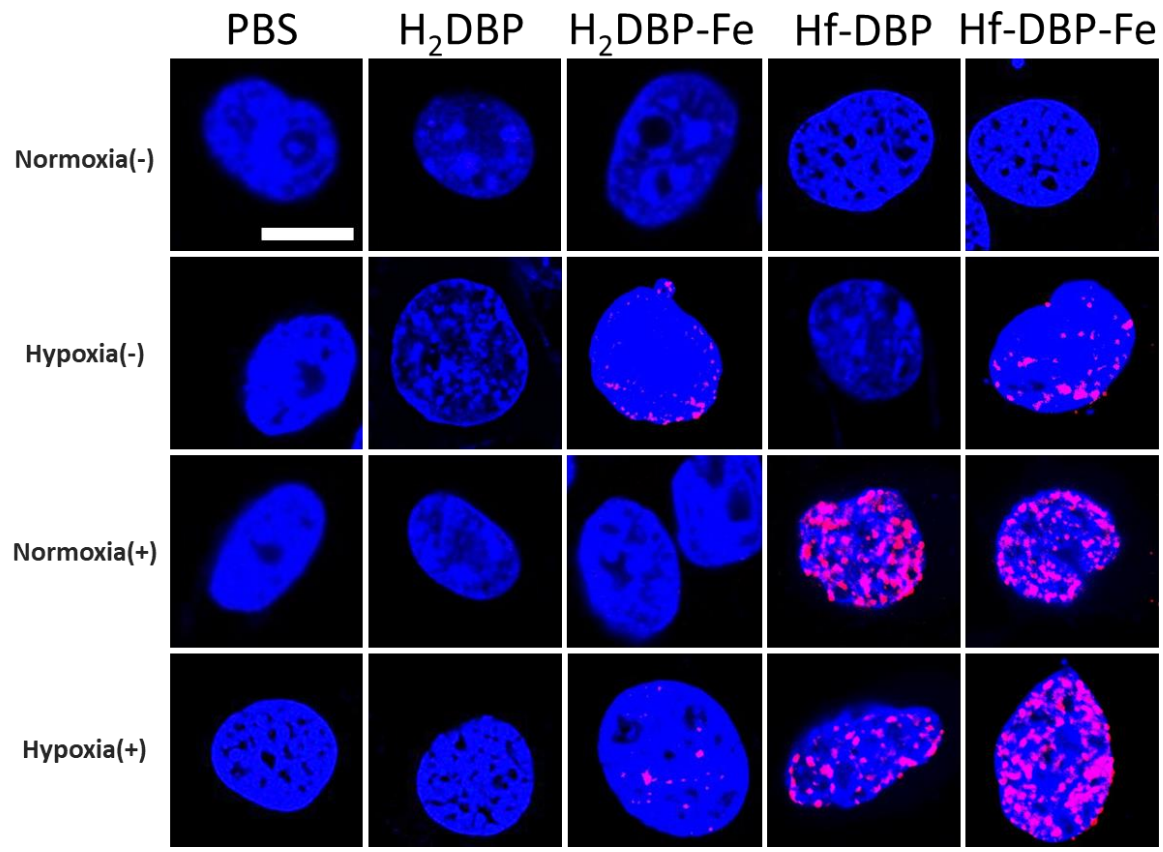


Figure S21 Representative CLSM imaging of γ-H2AX assay showing the DSBs and generated foci in MC38 cells treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe and irradiated with X-ray at a dose of 0 (-) or 2 (+) Gy under normoxic or hypoxic conditions. Blue and red fluorescence show DAPI-stained nucleus and antibody-labeled γ-H2AX in the cells, respectively. Scale bar = 10 μm.

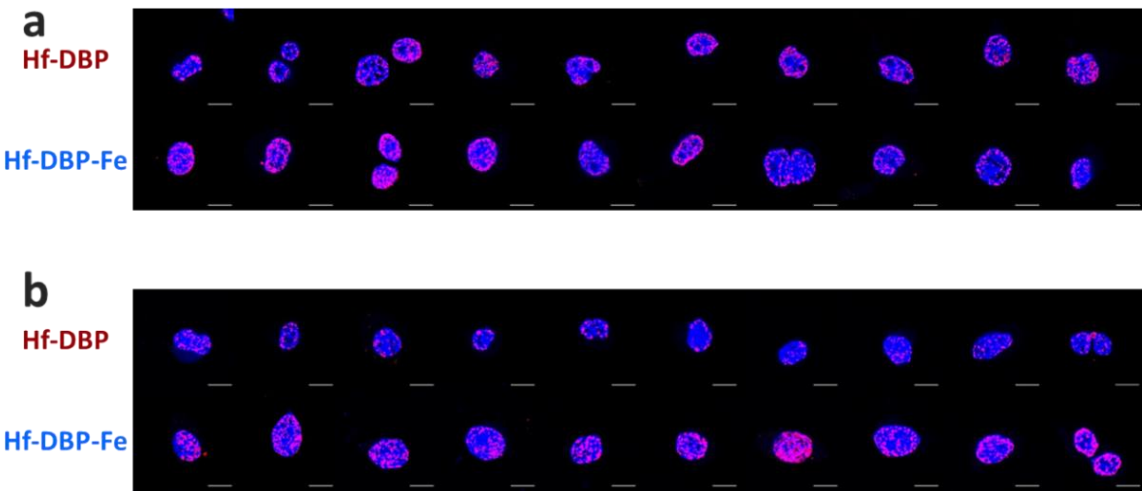


Figure S22 Representative CLSM imaging of γ-H2AX assay showing the DSBs and generated foci in MC38 cells treated with Hf-DBP or Hf-DBP-Fe and irradiated with X-ray at a dose 2 (+) Gy under normoxic (a) or hypoxic (b) conditions. Blue and red fluorescence show DAPI-stained nucleus and antibody-labeled γ-H2AX in the cells, respectively. Scale bar = 10 μm. Each group contained 20 scored nuclei selected randomly for statistical analysis and 10 out of 20 images were shown here.

S7. *In vivo* anti-cancer efficacy

A hypoxic murine colorectal cancer model was established by subcutaneously inoculating 5×10^5 MC38 cells onto C57BL/6 mice. When flank tumors reached 100-125 mm^3 in volume, which took around 14 days, mice were intratumorally injected with H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 0.2 μmol equivalent doseor PBS. 12 hours following injection, mice were anaesthetized with 2% (v/v) isoflurane and the tumors were irradiated with 5 daily fractions of X-ray at 1 Gy X-ray/fraction (225 kVp, 13 mA, 0.3 mm-Cu filter). For i.v. injection, mice were injected intravenously with PBS or Hf-DBP-Fe at a 0.2 μmol equivalent dose or PBS twice at day 13.5 and 15.5 post tumor inoculation. 8 hours after injection, mice were anaesthetized with 2% (v/v) isoflurane and the tumors were irradiated four daily fractions of X-ray at 2 Gy/fraction (225 kVp, 13 mA, 0.3 mm-Cu filter). The tumor sizes were measured every day using a caliper where tumor volume equals (width^2 × length)/2. On day 31, mice were sacrificed and excised tumors were photographed and weighed. Tumors and major organs were sectioned for hematoxylin-eosin staining (H&E) and immunofluorescence analysis for TUNEL and CD31 assays.

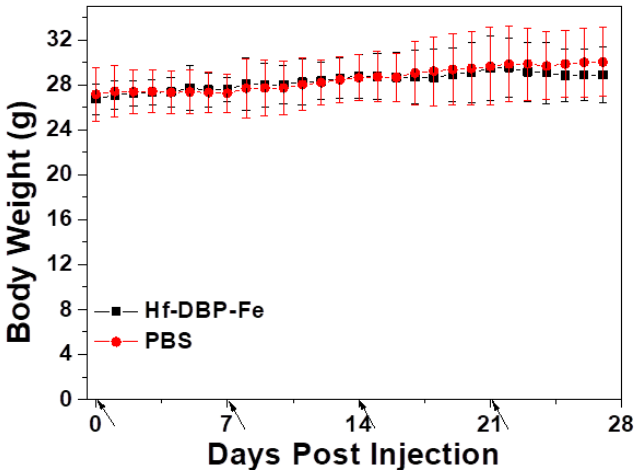


Figure S23 Body weights after subcutaneous injection of Hf-DBP-Fe. C57BL/6 mice were received Hf-DBP-Fe subcutaneously or PBS weekly at a Hf dose of 0.2 μmol with a total of four injections.

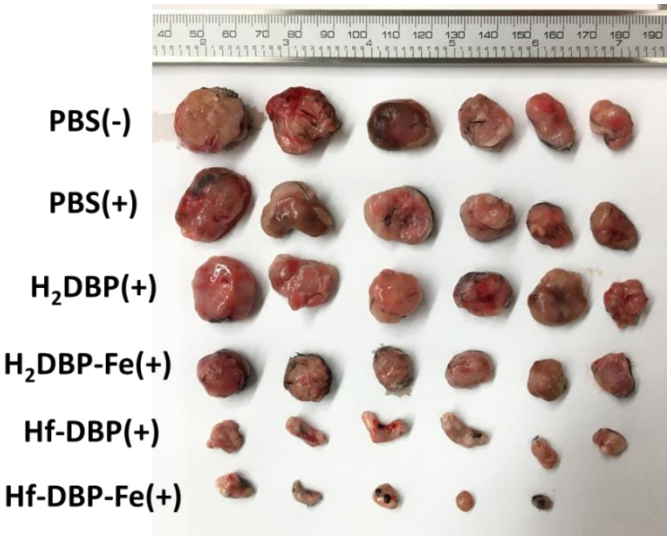


Figure S24 Photos of excised tumors of the MC38-bearing mice, n = 6. From top to bottom: PBS without X-ray irradiation (-), PBS, H_2DBP, H_2DBP-Fe, Hf-DBP and Hf-DBP-Fe with X-ray irradiation (+),

respectively.

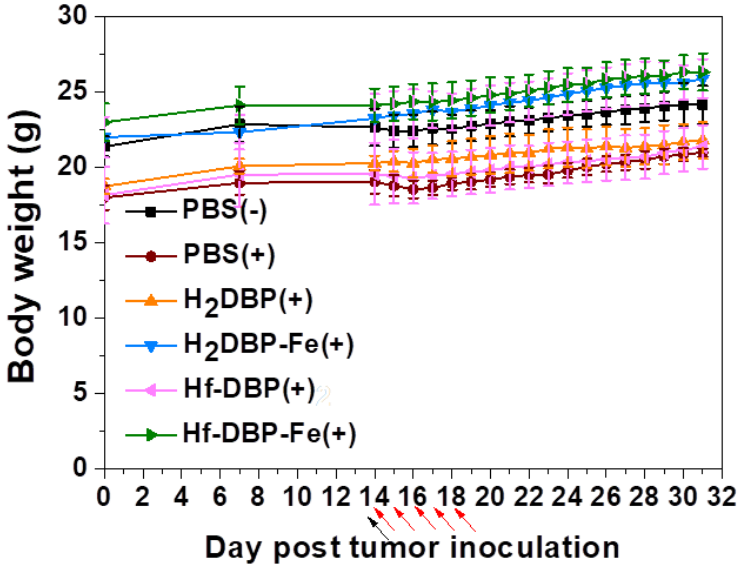


Figure S25 Body weights after tumor inoculation. MC38-tumor bearing mice were received intratumoral injection of PBS with or without X-ray irradiation, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe with X-ray irradiation.

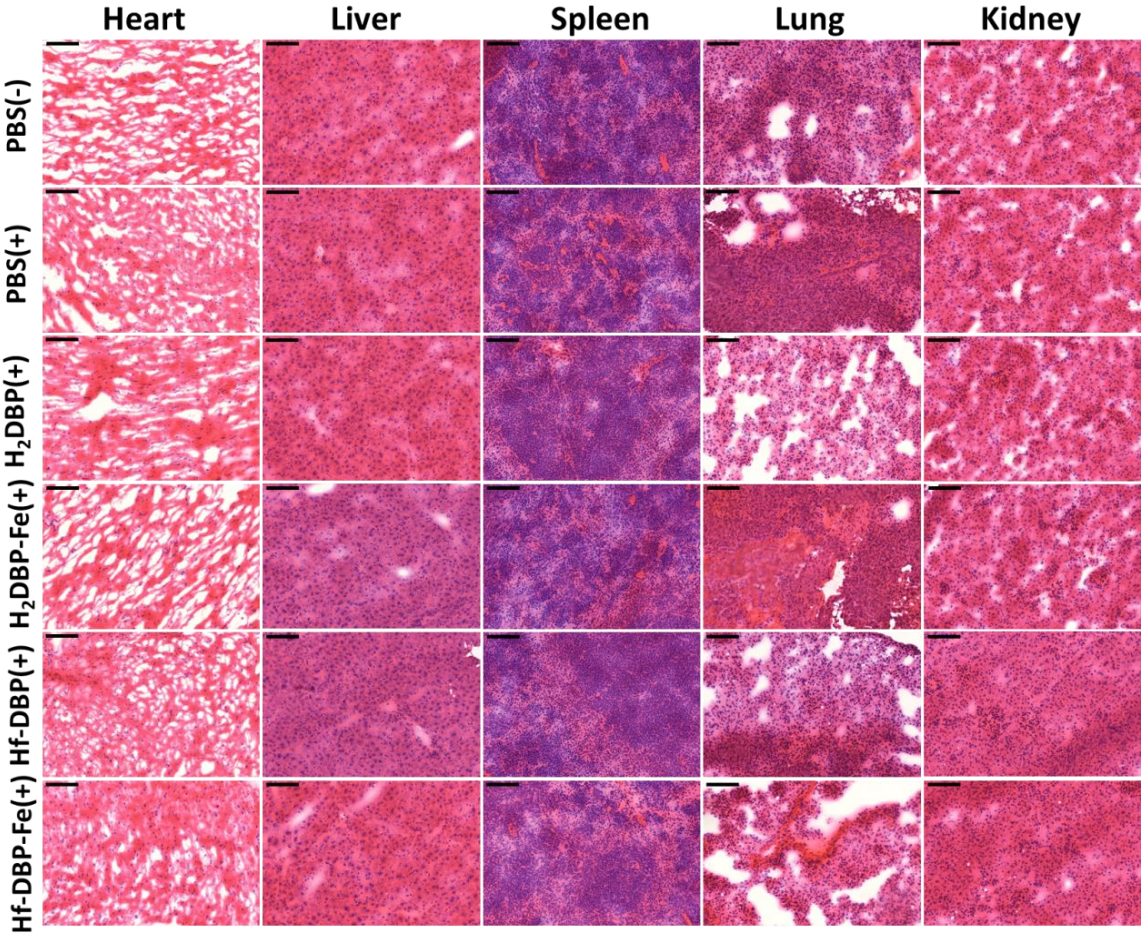


Figure S26 Representative histology of frozen sections of major organs of MC38 tumors-bearing mice receiving intratumoral injection of PBS with or without X-ray irradiation, H_2DBP, H_2DBP-Fe, Hf-DBP, or

Hf-DBP-Fe with X-ray irradiation. Scale bar = 100 μm.

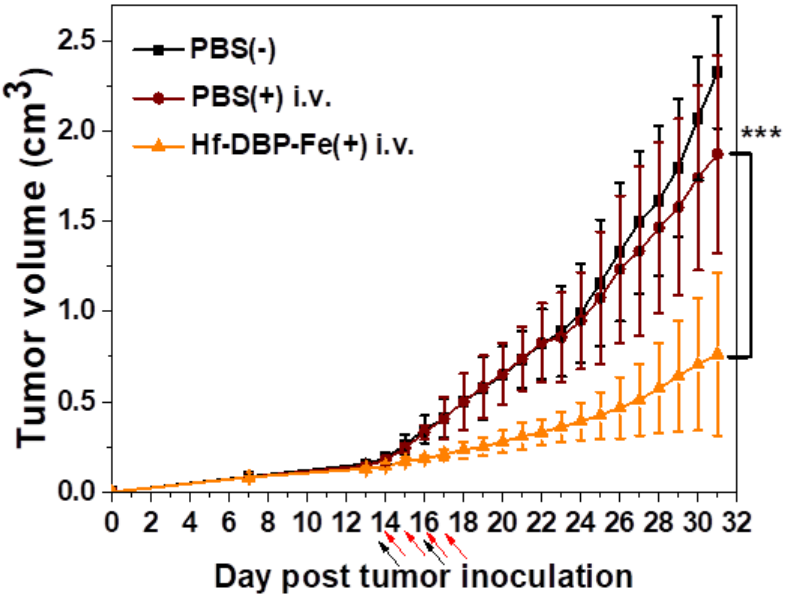


Figure S27 Tumor growth curves in MC38 tumor-bearing mice treated with PBS or Hf-DBP-Fe by intravenous (i.v.) injection with (-) or without (+) X-ray irradiation, n = 5 or 6. Black and red arrows indicate intravenous injection or X-ray irradiation, respectively.

TUNEL assay *In vivo* apoptosis was determined by TdT-mediated dUTP nick end labeling (TUNEL, Invitrogen, USA) assay. Sectioned tumor slices were fixed with acetone and stained sequentially according to standard product protocol. DAPI was used to label the cell nuclei.

S8. *In vivo* Biomimetic Properties of Hf-DBP-Fe

Intratumoral H_2O_2 determination Intratumoral H_2O_2 was detected according to a reported protocol with modification.[5] When flank tumors grew to 100-125 mm^3 in volume, which took around 14 days, mice were injected intratumorally with Hf-DBP, Hf-DBP-Fe at a 0.2 μmol equivalent doseor PBS. 6 hours or 12 hours post-treatment tumors were harvested and homogenized using a Precellys 24 homogenizer (Bertin Technologies, France) with 2 ml of acetone on ice. 1 ml of supernatant was collected after a centrifugation at 10000 rpm for 15 min to remove the suspended tissues or cells. The precipitate was obtained by centrifugation at 5000 rpm for 10 min after the addition of 100 μl of $TiOSO_4$ (0.03 M) and 200 μl of $NH_3 \cdot H_2O$. The precipitate was then dissolved in 3 ml of H_2SO_4 (1 M) to obtain the absorbance at λ = 405 nm using a UV-vis spectrophotometer. The concentration of H_2O_2 was then determined and converted to mass concentration in tumor.

Tumoral blood vessel detection CD-31 was used to probe tumoral blood vessels. After treatment, tumors were collected and frozen tissue sections at thickness 5 μm were prepared using CM1950 cryostat (Leica, Germany). The sections were air-dried for a minimum of 1 hour and fixed in acetone at 20 °C for 10 min. Samples were sequentially stained with CD-31 antibody as primary antibody (2H8, eBioscience, USA, 1:1000 dilution) and FITC-goat anti-rat Ig(G+L) as secondary antibody (eBioscience, USA, 1: 2000 dilution) and DAPI.

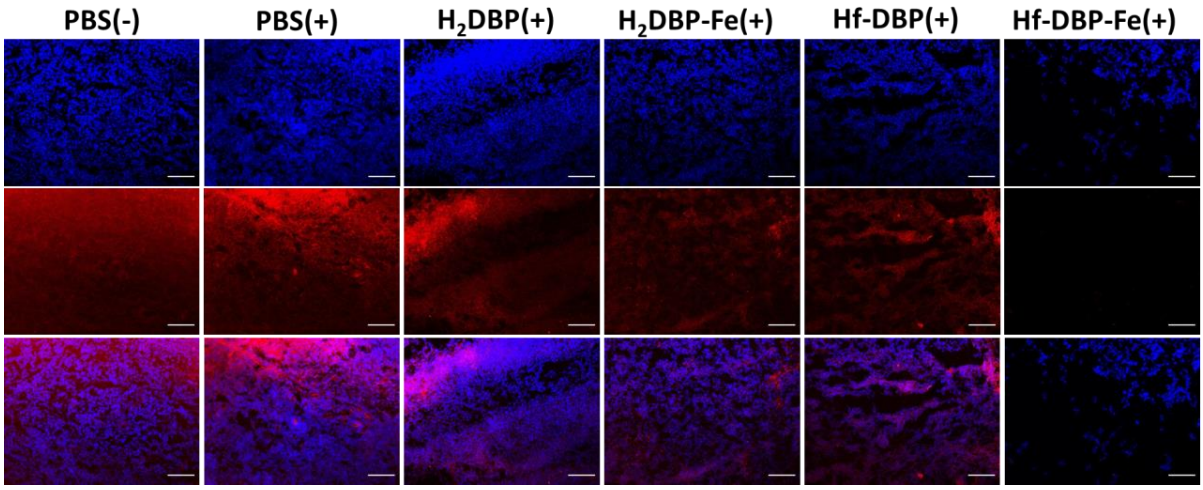


Figure S28 Representative immunofluorescence imaging for H_2O_2 of MC38 tumors-bearing mice receiving intratumoral injection of PBS with or without X-ray irradiation, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe with X-ray irradiation. Blue and red fluorescence indicate tumor cellular nucleus and intratumoral H_2O_2, respectively. Scale bar = 100 μm.

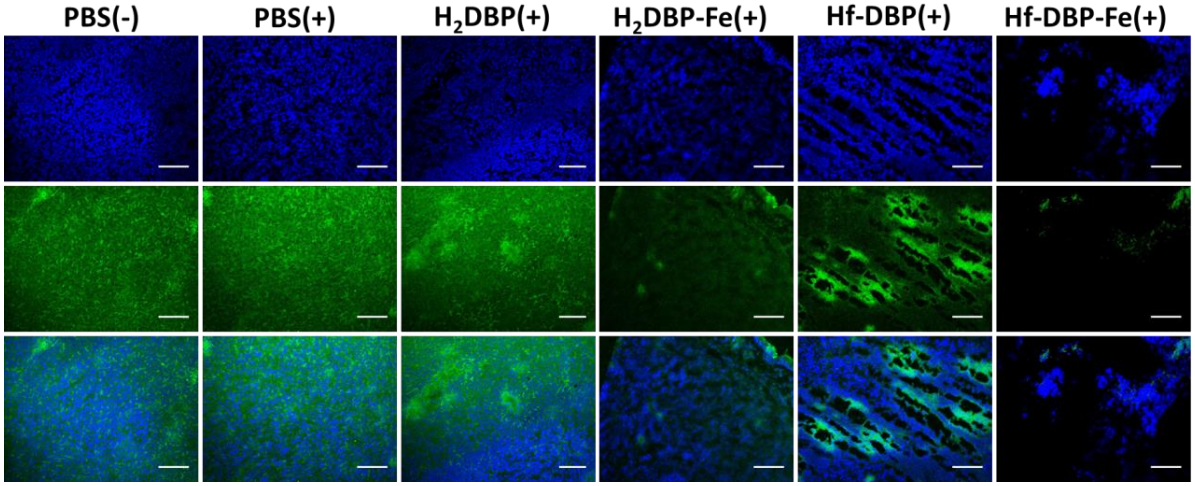


Figure S29 Representative immunofluorescence imaging for HIF1-α of MC38 tumors-bearing mice receiving intratumoral injection of PBS with or without X-ray irradiation, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe with X-ray irradiation. Blue and green fluorescence indicate tumor cellular nucleus and intranuclear HIF1-α, respectively. Scale bar = 100 μm.

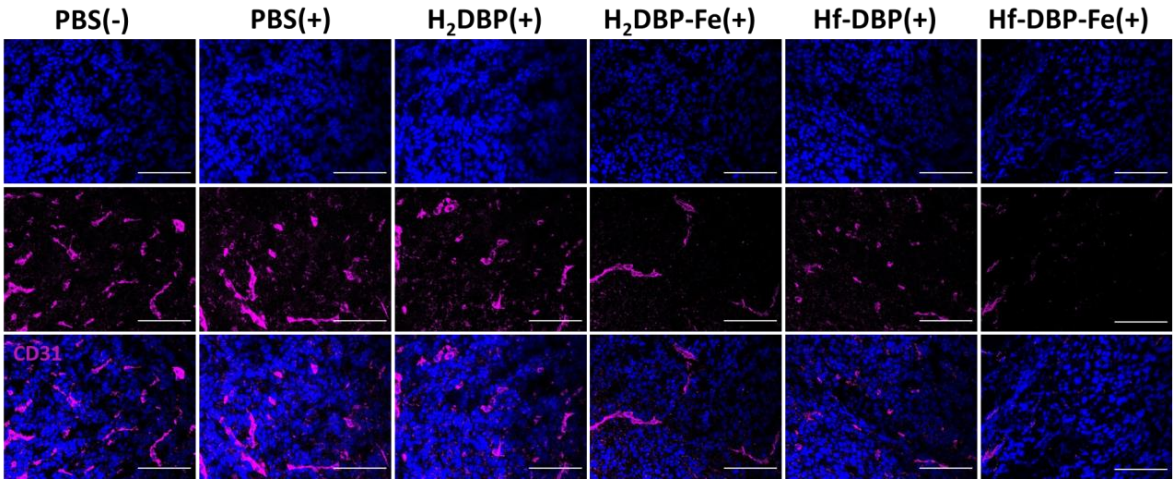


Figure S30 Representative immunofluorescence imaging for CD31 of tumors from MC38 tumors-bearing mice receiving intratumoral injection of PBS with or without X-ray irradiation, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe with X-ray irradiation. Blue and magenta fluorescence indicate nucleus and tumoral blood vessels, respectively. Scale bar = 100 μm.

Immunogenic cell death and PD-L1 upregulation For *in vitro* immunofluorescence staining, MC38 cells were seeded on cover slides in 6-well plates at 5×10^5/well. After culturing overnight and incubation with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe at a 20 μM equivalent concentrationfor 4 hours under hypoxic condition, the cells were irradiated with 0 or 2 Gy X-ray. Following this, slides were observed under CLSM after staining with AlexaFluor 488-CRT antibody (Enzo Life Sciences, USA) at 1: 100 dilution and DAPI for 2 hours. For *ex vivo* immunofluorescence staining, MC38 tumor bearing mice were treated with H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe intratumorally at a 0.2 μmol equivalent dose. Mice treated with PBS served as a control. Tumors were collected 2 days post treatment and frozen tissue sections with a thickness of 5 μm were prepared using CM1950 cryostat (Leica, Germany). The sections were air-dried for a minimum of 1 h and then fixed in acetone at 20 °C for 10 min. After stained with AlexaFluor 488-CRT antibody (Enzo Life Sciences, USA) with 1: 100 dilution and DAPI for 2 hours, slides were observed under CLSM. PD-L1 upregulation of Hf-DBP-Fe treatment was also detected by stained with FITC-PD-L1 antibody (MIH6, Thermofisher, USA) with 1: 1000 dilution and DAPI overnight.

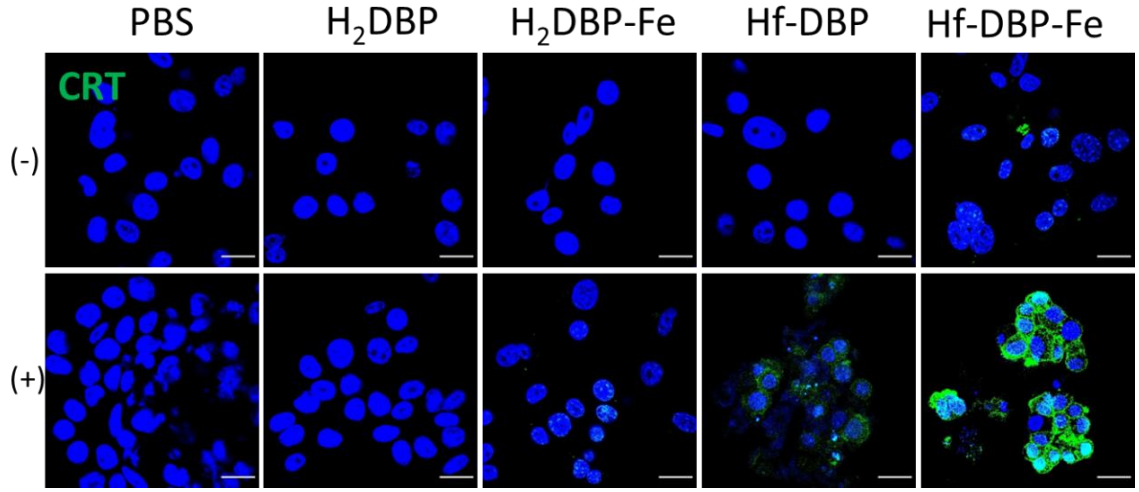


Figure S31 CLSM imaging of CRT exposure on the cell surface of MC38 treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe under hypoxia condition and irradiated with X-ray at a dose of 0 (-) or 2 (+) Gy. Blue and green fluorescence show DAPI-stained nucleus and CRT expression on the cell surface, respectively. Scale bar = 20 μm.

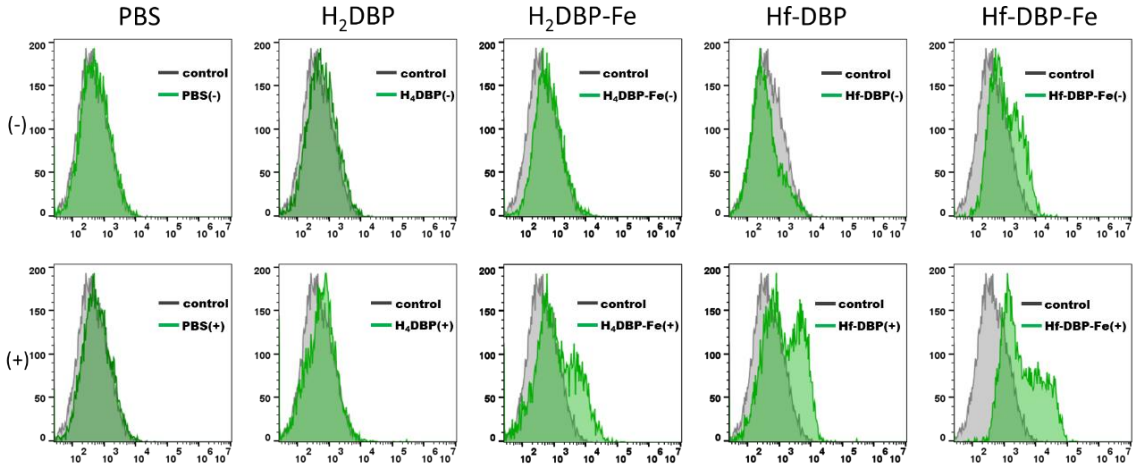


Figure S32 CRT exposure on the cell surface of MC38 treated with PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe under hypoxia condition and irradiated with X-ray at a dose of 0 (-) or 2 (+) Gy quantified by flow cytometric analysis. Grey histogram (control) and green histogram show the difference of CRT level in the cells. From left to right: PBS, H_2DBP, H_2DBP-Fe, Hf-DBP or Hf-DBP-Fe, respectively.

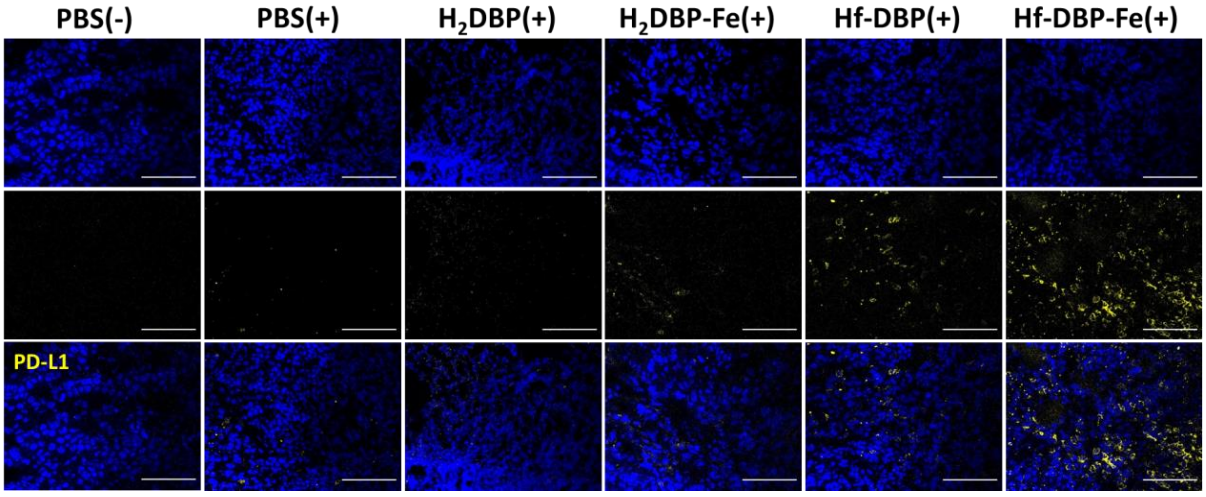


Figure S33 Representative immunofluorescence imaging for PD-L1 of tumors from MC38 tumors-bearing mice receiving intratumoral injection of PBS with or without X-ray irradiation, H_2DBP, H_2DBP-Fe, Hf-DBP, or Hf-DBP-Fe with X-ray irradiation. Blue and yellow fluorescence indicate nucleus and tumoral PD-L1 expression, respectively. Scale bar = 100 μm.

S9. Abscopal Effect and Anti-tumor Immunity

Synergistic bilateral colorectal tumor model MC38 was established to analyze the *in vivo* anti-cancer efficacy of Hf-DBP-Fe(+)/α-PD-L1. 5×10^5 and 2.5×10^5 MC38 cells were inoculated subcutaneously into the right and left flanks of C57BL/6 mice to establish primary and secondary tumors. As soon as primary tumors grew to 100-150 mm^3, mice were intratumorally injected with Hf-DBP-Fe at a dose of 0.2 μmol Hf or PBS. 12 h following injection, mice were anaesthetized with 2% (v/v) isoflurane and the primary tumors were irradiated with 5 daily fractions of X-ray at 1 Gy/fraction. Antibodies were injected every three days by intraperitoneal injection at a dose of 75 μg/mouse. Tumor sizes were measured every day using a caliper where tumor volume equals $(width^2 \times length)/2$. The mice were sacrificed once the tumors on control mice reached 2 cm^3.

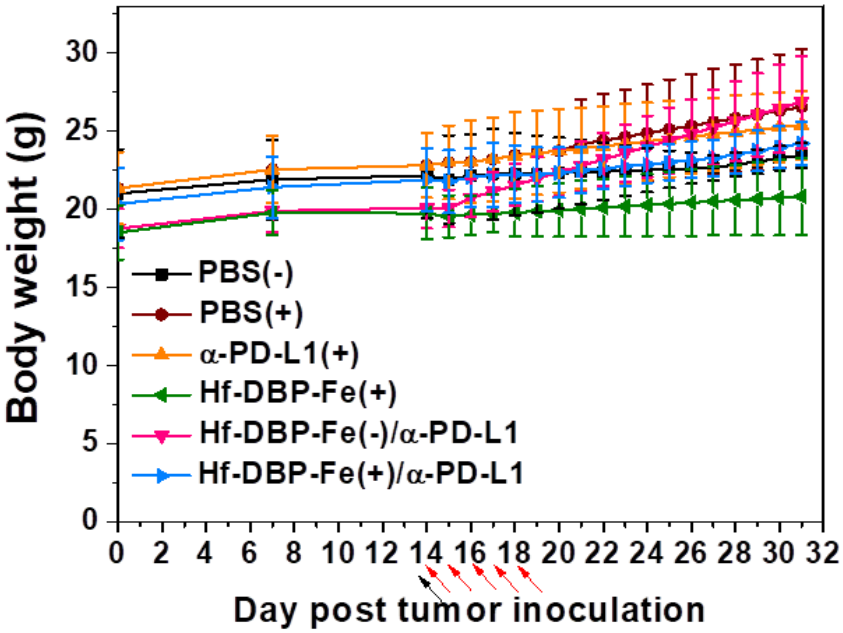


Figure S34 Body weights after X-ray irradiation treatment on bilateral MC38 models treated with PBS (with or without X-ray irradiation), anti-PD-1 antibody with X-ray irradiation, Hf-DBP-Fe with X-ray irradiation, Hf-DBP-Fe with or without X-ray irradiation combined with α-PD-L1 antibody (n = 6). Black, red and green arrows refer to subcutaneous injection, X-ray irradiation or intraperitoneal injection of α-PD-L1, respectively.

Tumor challenge studies As soon as tumors reached 100-150 mm^3, mice were intratumorally injected with Hf-DBP-Fe at a dose of 0.2 μmol Hf/mouse or PBS. 12 hours post injection, mice were anaesthetized with 2% (v/v) isoflurane and primary tumors were irradiated with 5 daily fractions of X-ray at 1 Gy/fraction (120 kVp, 20 mA, 2 mm-Cu filter). 30 days post tumor eradication, mice were challenged with 5×10^5 cells on the contralateral flank. 60 days after challenge, the mice were re-challenged with 1×10^6 B16F10 cells on the contralateral flank. Naive mice had been simultaneously inoculated and served as control. Mice were sacrificed once the tumors on control mice reached 2 cm^3.

ELISpot assay Tumor-specific immune responses to IFN-γ were analyzed *in vitro* by ELISpot assay (Mouse IFN-γ ELISPOT Ready-SET-Go!; Cat. No. 88-7384-88; eBioscience). A Millipore Multiscreen HTS-IP plate was coated with anti-mouse IFN-γ capture antibody at 4 °C overnight. Single-cell suspensions of splenocytes were obtained from the mice bearing MC38 tumors and

seeded onto the antibody-coated HTS-IP plate at 2×10^5 cells per well. Cells were incubated with or without KSPWFTTL peptide stimulation (1×10^4 cells per well) at 37 °C for 42 hours and then discarded. The plate was then incubated with biotin-conjugated anti-IFN-γ detection antibody at r.t. for 2 hours, and then incubated with Avidin-HRP at r.t. for 2 hours. 3-amino-9-ethylcarbazole substrate solution (Sigma, Cat. AEC101) was added to detect cytokine spot at r.t. for 15 mins. Spots were imaged and analyzed using a CTL ImmunoSpot Analyzer (Cellular Technology Ltd, USA).

Lymphocytes profiling Tumors were harvested and then treated with 1 mg/ml collagenase I (Gibco, USA) for 1 hour at 37 °C. Using nylon mesh filters with size 40 nm, cells were filtered washed with PBS. Tumor-draining lymph nodes were collected and then directly ground through the cell strainers. The ensuing single-cell suspension was incubated with anti-CD16/32 (clone 93) to minimize nonspecific binding to FcRs. Cells were further stained with the following fluorochrome-conjugated antibodies: CD45 (30-F11), TCRβ (H57-597), CD4 (GK1.5), CD8 (53-6.7), F4/80 (BM8), , CD11b (M1/70), CD11c (N418), Gr-1 (14-5931-82) and yellow-fluorescent reactive dye. Antibodies were employed at 1: 200 dilution. Representative gating strategies for different immune cells are shown in Supplementary Fig. 40. LSR Fortessa (BD Biosciences) was used for cell acquisition and data analysis was carried out with FlowJo software (Tree Star, Ashland, OR).

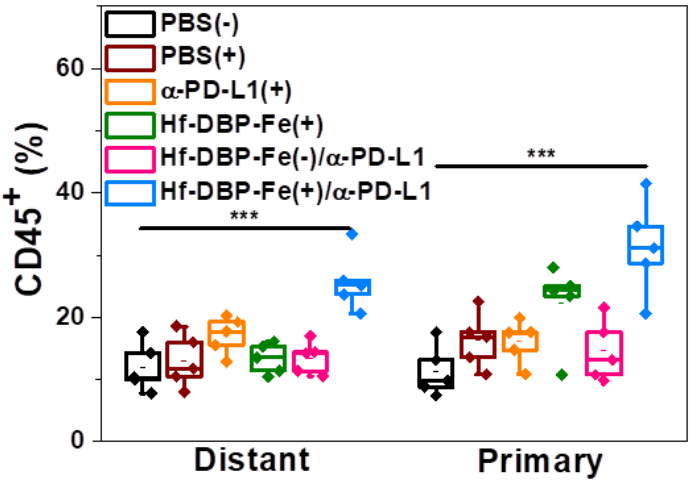


Figure S35 Percentages of tumor-infiltrating leucocytes with respect to the total cells treated with PBS(-), PBS(+), α-PD-L1(+), Hf-DBP-Fe(+), Hf-DBP-Fe(-)/α-PD-L1 or Hf-DBP-Fe(+)/α-PD-L1. Data are expressed as means ± s.d., n=6. ***P<0.005 from control by t-test. Central lines, bounds of box and whiskers represent mean values, 25% to 75% of the range of data and 1.5 fold of interquartile range away from outliers, respectively.

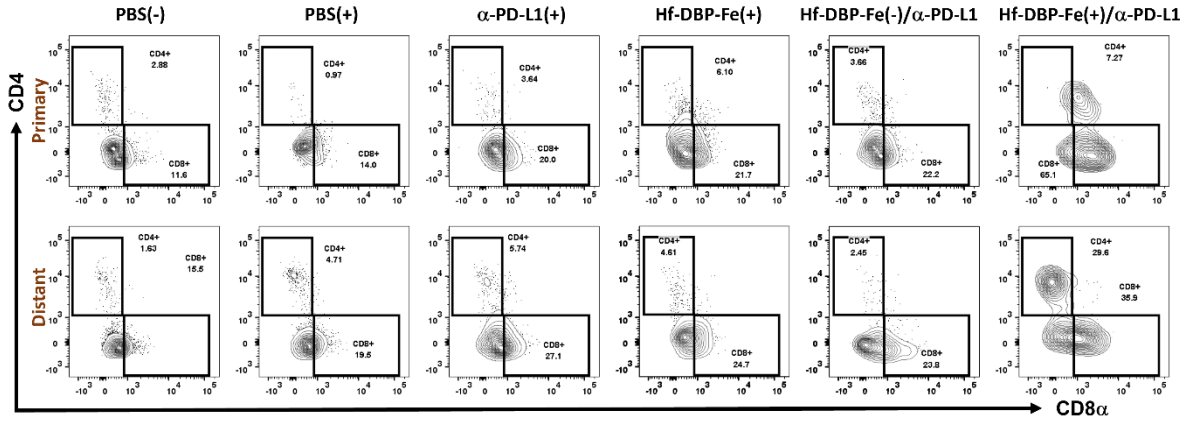


Figure S36 Representative quantitative analysis of T cells (gated on $CD45^+$ $TCR\beta^+$ cells) in both primary (first row) and distant (second row) tumors analyzed by flow cytometry.

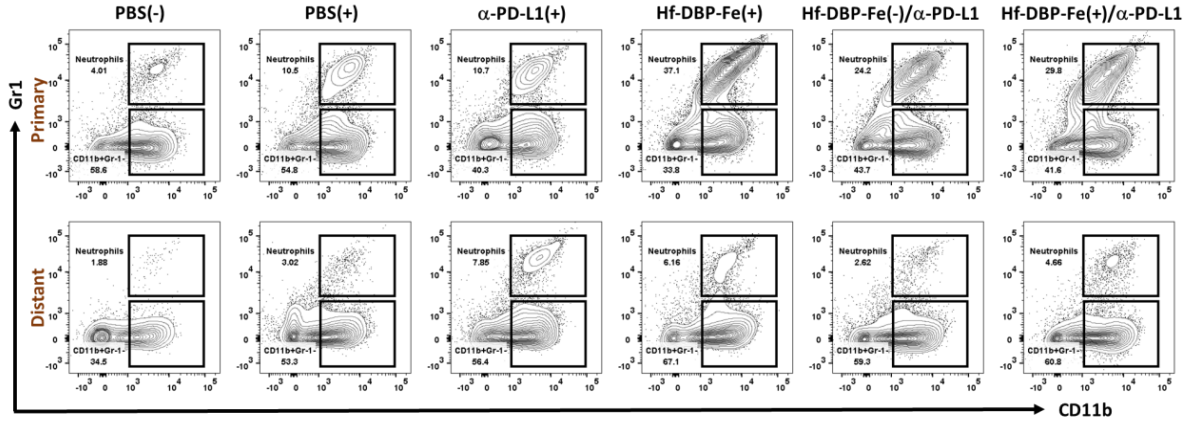


Figure S37 Representative quantitative analysis of neutrophils (gated on $CD45^+$ cells) in both primary (first row) and distant (second row) tumors analyzed by flow cytometry.

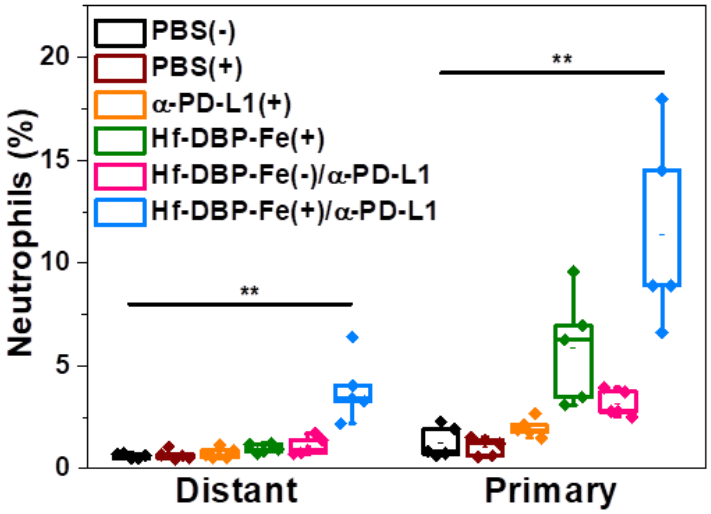


Figure S38 Percentages of tumor-infiltrating neutrophils with respect to the total cells treated with PBS(-), PBS(+), α-PD-L1(+), Hf-DBP-Fe(+), Hf-DBP-Fe(-)/α-PD-L1 or Hf-DBP-Fe(+)/α-PD-L1. Data are expressed as means ± s.d., n=6. **P<0.01 from control by t-test. Central lines, bounds of box, and whiskers represent mean values, 25% to 75% of the range of data and 1.5 fold of interquartile range away from outliers, respectively.

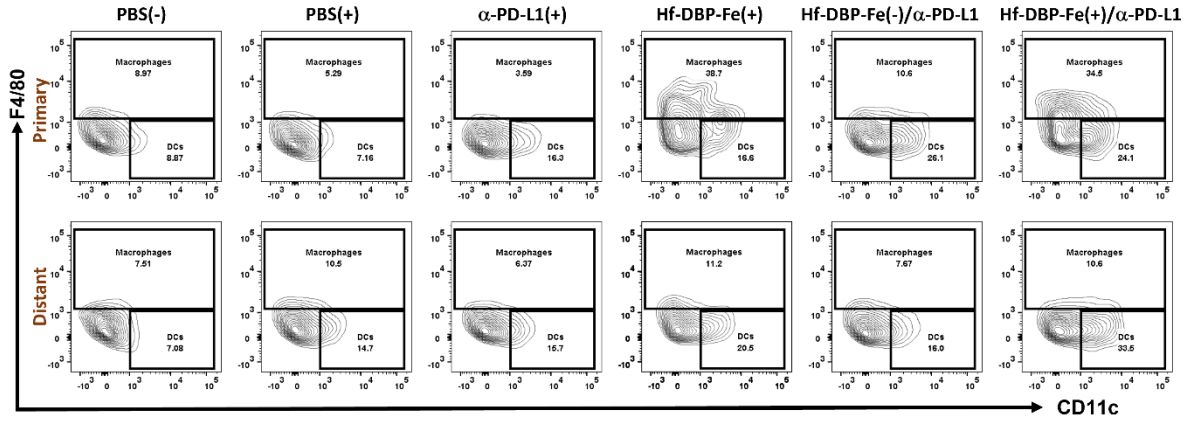


Figure S39 Representative quantitative analysis of macrophages and dendritic cells (gated on $CD45^+$ $CD11b^+$ $Gr1^-$ cells) in both primary (first row) and distant (second row) tumors analyzed by flow cytometry.

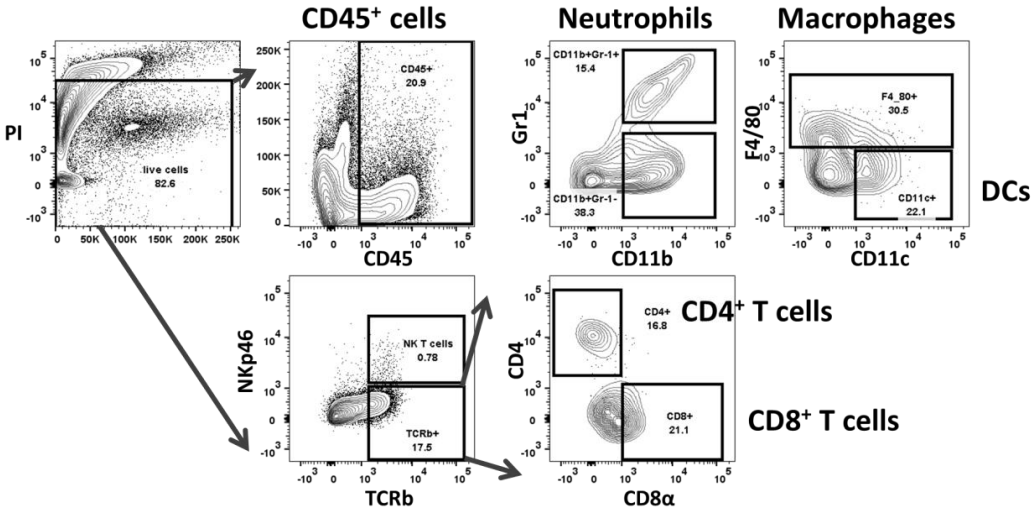


Figure S40 Representative gating strategies for $CD45^+$ cells, neutrophils, macrophages, dendritic cells, $CD4^+$ T cells, and $CD8^+$ T cells.

Immunofluorescence staining Following tumor collection and frozen tissue sections obtained at thickness 5 μm prepared using a CM1950 cryostat (Leica, Germany), sections were air-dried for a minimum of 1 hour and fixed using acetone for 10 min at 20 °C. After blocking with 20% donkey serum, sections were incubated along with individual primary antibodies against CD8 (53-6.7), TCRβ (H57-597) overnight at 4°C, followed by incubating with dye-conjugated secondary antibodies for 1 hour at r.t. The sections were stained with DAPI for another 10 min and then washed twice with PBS and observed under CLSM.

Statistical analysis To ensure proper statistical ANOVA analysis for efficacy studies, group sizes of $n \geq 5$ were chosen. Student's t-tests were used to determine if the variance between groups was similar. All statistical analysis was performed using OriginPro (OriginLab Corp.). Statistical significant was calculated using a two-tailed Student's t-tests with significance levels defined as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Animal experiments were not performed in a blinded fashion, and results are represented as means $\pm$ SD. All immune analysis was performed in a blinded fashion, and results are represented as median $\pm$ SD.

S10. References

1. Lu, K.; He, C.; Lin, W., Nanoscale metal–organic framework for highly effective photodynamic therapy of resistant head and neck cancer. *J. Am. Chem. Soc.* **2014,** *136* (48), 16712-16715.

2. Lu, K.; He, C.; Guo, N.; Chan, C.; Ni, K.; Lan, G.; Tang, H.; Pelizzari, C.; Fu, Y.-X.; Spiotto, M. T., Low-dose X-ray radiotherapy–radiodynamic therapy via nanoscale metal–organic frameworks enhances checkpoint blockade immunotherapy. *Nat. Biomed. Eng.* **2018,** *2* (8), 600.

3. Lan, G.; Ni, K.; Xu, Z.; Veroneau, S. S.; Song, Y.; Lin, W., Nanoscale Metal–Organic Framework Overcomes Hypoxia for Photodynamic Therapy Primed Cancer Immunotherapy. *J. Am. Chem. Soc.* **2018,** *140* (17), 5670-5673.

4. Franken, N. A.; Rodermond, H. M.; Stap, J.; Haveman, J.; Van Bree, C., Clonogenic assay of cells in vitro. *Nat. Protoc.* **2006,** *1* (5), 2315-2319.

5. Zhang, C.; Bu, W.; Ni, D.; Zhang, S.; Li, Q.; Yao, Z.; Zhang, J.; Yao, H.; Wang, Z.; Shi, J., Synthesis of iron nanometallic glasses and their application in cancer therapy by a localized Fenton reaction. *Angew. Chem. Int. Ed.* **2016,** *55* (6), 2101-2106.