Supplementary Information

ATP-responsive copper(II)-doped ZIF-nanoparticles for synergistic cancer therapy: combining cuproptosis and chemo/chemodynamic therapy

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Experimental sections

Reagents

Zinc acetate and bathocuproinedisulfonic acid (BCS) were purchased from Aladdin Reagent (Shanghai, China). Imidazole-2-carboxaldehyde (2-ICA), doxorubicin hydrochloride, copper chloride dihydrate, adenosine triphosphate (ATP), glutathione (GSH), tetramethylbenzidine (TMB), sodium diethyldithiocarbamate trihydrate (DDTC), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Macklin Reagent (Shanghai, China). GSH kit, oxidized glutathione (GSSG) kit and Calcein-AM/PI double staining kit were purchased from Soleibo (Beijing, China), 4',6-diamidino-2-phenylindole (DAPI) and 2,7-dichlorofluorescein diacetate (DCFH-DA) were provided by Biyuntian (Shanghai, China).

Instruments

The scanning electron microscopy (SEM) imaging and elemental spectroscopy were performed using a Zeiss Sigma 300 (Germany). The transmission electron microscopy (TEM) imaging was performed by a Japanese JEM-2100 transmission electron microscope (Japan). The electron spectroscopic analysis of elements was performed by X-ray photoelectron spectrometer Thermo Scientific K-Alpha (USA). The powder X-ray diffraction pattern (PXRD) was obtained by Bruker D8-Advance X-ray diffractometer (Germany). The FT-IR spectra were recorded by Bruker Fourier transform infrared spectrometer. The zeta potential and hydrodynamic diameter of nanomaterials were measured in a Zetasizer nano ZS90 (UK) analyzer. The thermogravimetric analysis (TGA) was performed by a TGA-50 (Japan). The absorption spectra were carried out on a Perkin

Elmer Lambda 25 UV/vis spectrophotometer (USA). The fluorescence spectra were collected on a Perkin Elmer LS-55 fluorescence spectrometer (USA). The fluorescence imaging of cells was obtained by an Olympus FVl000 fluorescence microscope (Japan). The fluorescence imaging of mice was collected on an IVIS Lumina XR small animal optical in vivo imaging system (USA).

Synthesis of ZIF-90 nanoparticles

A DMF solution (2 mL) of zinc acetate dihydrate (0.2 M) was poured into a DMF solution (2 mL) of 2-ICA (0.4 M) under vigorous stirring at room temperature. After 5 min, DMF (6 mL) was added into the reaction mixture to stabilize the ZIF-90 nanoparticles. The resulting ZIF-90 nanoparticles were then purified by centrifugation (10 000 rpm, 5 min) and washed with DMF once and ethanol in turn for several times. ZIF-90 nanoparticles were then collected and dried under vacuum at room temperature for 24 h.

Synthesis of DOX@ZIF-90 nanoparticles

A mixed solution of zinc acetate dihydrate (0.2 M) in DMF (2 mL) was poured into a solution of 2-ICA (0.4 M) containing doxorubicin (DOX, 4 mg) under vigorous stirring at room temperature. After 5 min, DMF (6 mL) was added to the reaction mixture to stabilize the DOX@ZIF-90 nanoparticles. The resulting DOX@ZIF-90 nanoparticles were then purified by centrifugation (10 000 rpm, 5 min) and washed sequentially with DMF and ethanol several times. DOX@ZIF-90 nanoparticles were then collected and dried under vacuum at room temperature for 24 h.

Synthesis of Cu^{2+}-DOX@ZIF-90 nanoparticles

A mixed solution (2 mL) of zinc acetate dihydrate (0.2 M) and copper chloride

dihydrate (0.067 M) in DMF was poured into a solution of 2-ICA (0.4 M) containing DOX (4 mg) under vigorous stirring at room temperature. After 5 min, DMF (6 mL) was added to the reaction mixture to stabilize Cu^{2+}-DOX@ZIF-90 nanoparticles. Then, the obtained Cu^{2+}-DOX@ZIF-90 nanoparticles were purified by centrifugation (10000 rpm, 5 min), and washed with DMF and ethanol successively for several times. The Cu^{2+}-DOX@ZIF-90 nanoparticles were then collected and dried under vacuum at room temperature for 24 h.

ATP-induced release of DOX and Cu(II) in different cells

In order to study the release ability of DOX and Cu(II) in different cells, 293T, HeLa and HepG2 cells were inoculated on confocal culture dishes (NETS Co.) and cultured for 24 h for adherence. One group of 293T, HeLa, and HepG2 cells were incubated with Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}) for 24 h. Another group of 293T, HeLa, and HepG2 cells were treated with apyrase (0.5 U mL^{-1}) for 2 h and then incubated with Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}) for 24 h. Then, the cells were washed three times with PBS and stained with 4, 6-diamino-2-phenylindole (DAPI) for 30 min. Fluorescence imaging was measured by a confocal fluorescence microscope. DAPI channel: λ_{ex} = 405 nm; λ_{em} = 420-500 nm. DOX channel: λ_{ex} = 488 nm; λ_{em} = 545-645 nm.

Intracellular GSH depletion

In order to study the GSH consumption capacity in different cells, 293T, HeLa, and HepG2 cells were inoculated on 12-well plates and cultured for 24 h for adherence. The three cells were incubated with PBS, ZIF-90 (3.7 mg mL^{-1}), DOX@ZIF-90 (3.8 mg mL^{-1}) and Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}) for 24 h, respectively. Then, the intracellular levels

of GSH and GSSG were tested using reduced glutathione (GSH) kits and oxidized glutathione (GSSG) kits.

In order to confirm that the changes of GSH and GSSG contents in cells were caused by Cu(II), 293T, HeLa, and HepG2 cells were inoculated on 12-well plates and cultured for 24 h for adherence. The cells were divided into two groups. One group was incubated with Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}) for 24 h. The other group was pretreated with BCS (200 μM) for 10 h and then incubated with Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}) for 24 h. Then, the intracellular levels of GSH and GSSG were tested using reduced glutathione (GSH) kits and oxidized glutathione (GSSG) kits.

The effect of intracellular synergistic therapy

In order to study the synergistic therapeutic effect of Cu^{2+}-DOX@ZIF-90 in cells, 293T and HepG2 cells were inoculated on 24-well plates and cultured for 24 h for adherence. The cells were incubated with different concentrations (0~4 mg mL^{-1}) of ZIF-90, DOX@ZIF-90 and Cu^{2+}-DOX@ZIF-90 for 24 h, respectively. Then, cell viability was measured by CCK-8 method.

293T and HepG2 cells were inoculated on confocal culture dishes (NETS Co.) and cultured for 24 h for adherence. The cells were incubated with PBS, ZIF-90 (3.7 mg mL^{-1}), DOX@ZIF-90 (3.8 mg mL^{-1}), Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}) for 24 h. Then, they were stained with calcein -AM and PI for 20 min. Fluorescence imaging was measured by a confocal fluorescence microscope. Calcein-AM channel: λex = 488 nm; λem = 495-555 nm. PI channel: λex = 560 nm; λem = 580-640 nm.

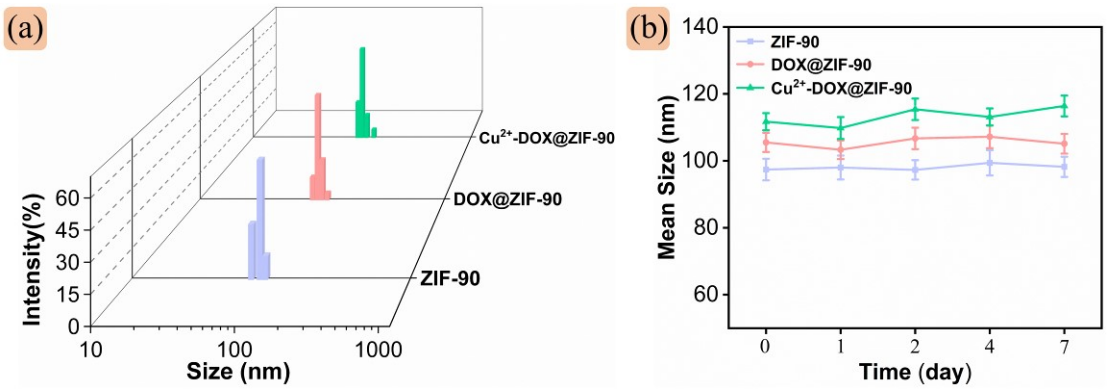

Fig. S1 (a) The dynamic light scattering (DLS) profiles of ZIF-90, DOX@ZIF-90 and Cu^{2+}-DOX@ZIF-90. (b) Hydrodynamic diameters of ZIF-90, DOX@ZIF-90 and Cu^{2+}-DOX@ZIF-90 with times. Data in (a) is shown as mean ± S.D. (n = 3).

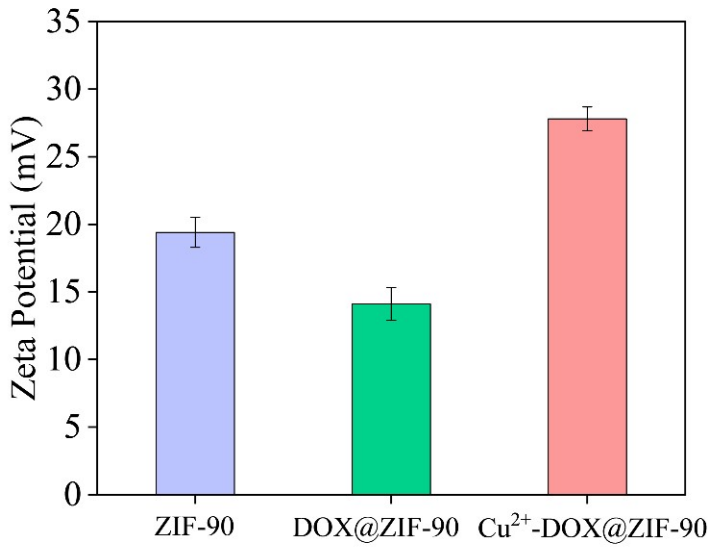

Fig. S2 The Zeta potentials of ZIF-90, DOX@ZIF-90 and Cu^{2+}-DOX@ZIF-90. Data in the figure is shown as mean ± S.D. (n = 3).

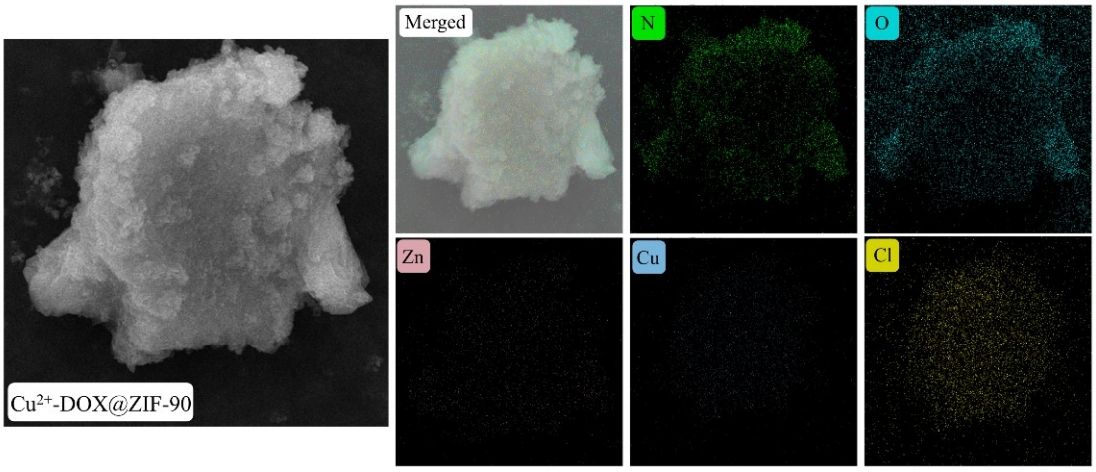

Fig. S3 The corresponding elemental mapping analysis of Cu^{2+}-DOX@ZIF-90.

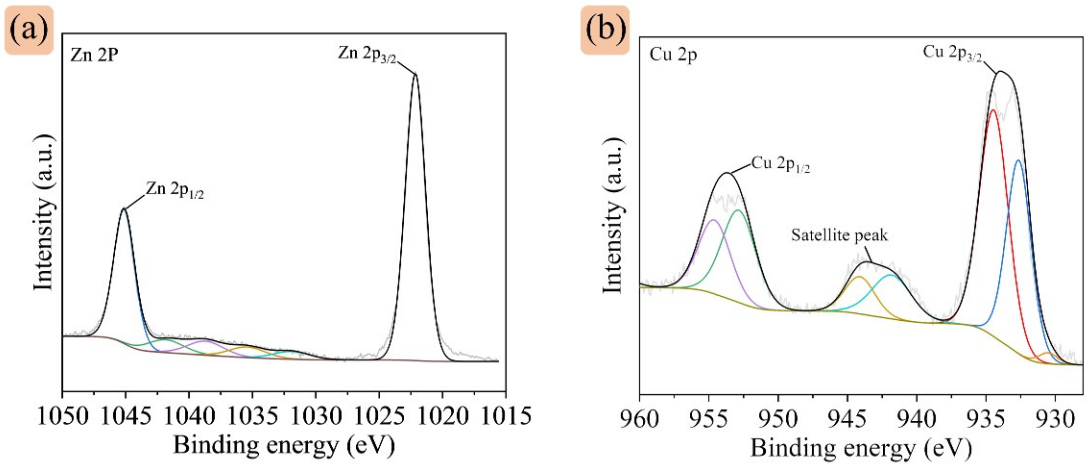

Fig. S4 The high-resolution XPS spectra of (a) Zn 2p region (b) Cu 2p region.

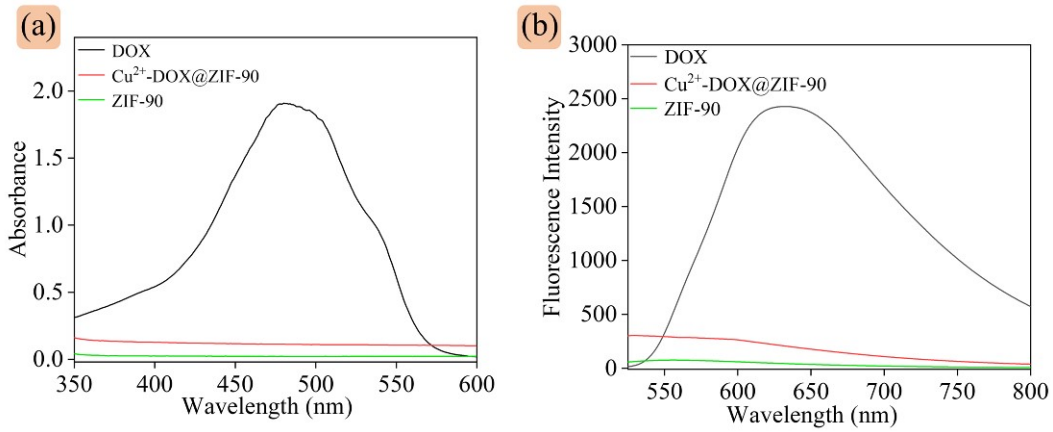

Fig. S5 (a) Absorption spectra and (b) fluorescence spectra of DOX, ZIF-90 (4 mg mL^{-1}) and Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}). λ_{ex} = 480 nm.

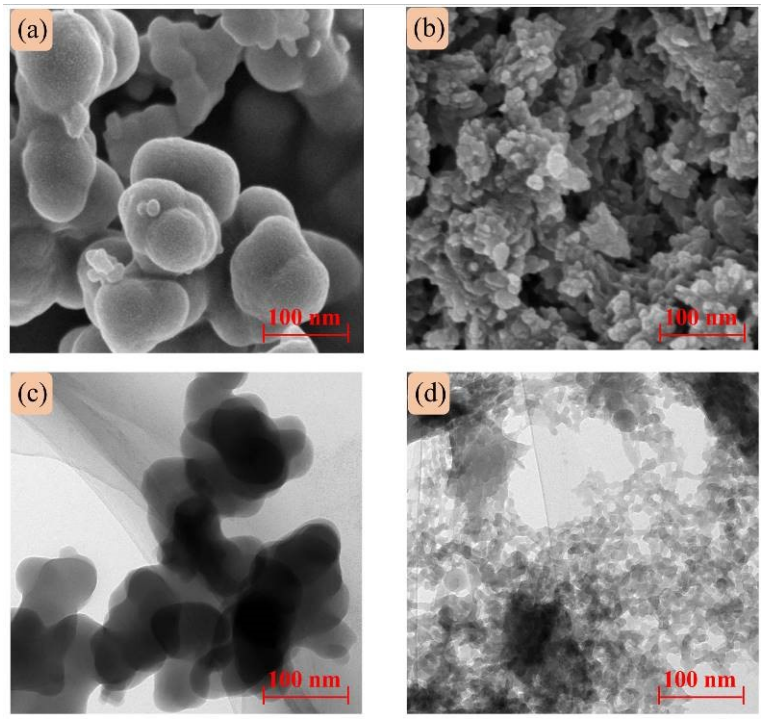

Fig. S6 The SEM images of (a) Cu^{2+}-DOX@ZIF-90 and (b) Cu^{2+}-DOX@ZIF-90 + ATP. The TEM images of (c) Cu^{2+}-DOX@ZIF-90 and (d) Cu^{2+}-DOX@ZIF-90 + ATP.

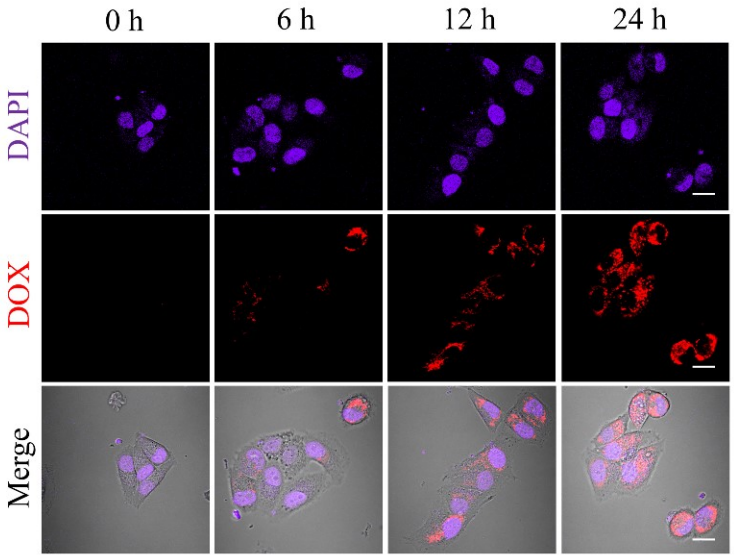

Fig. S7 Fluorescence image of HeLa cells after the incubation with Cu^{2+}-DOX@ZIF-90 (4 mg mL^{-1}) for 0, 6, 12, 24 h. DAPI channel: λ_{ex} = 405 nm; λ_{em} = 420-500 nm. DOX channel: λ_{ex} = 488 nm; λ_{em} = 545-645 nm. Scale bar: 20 μm.

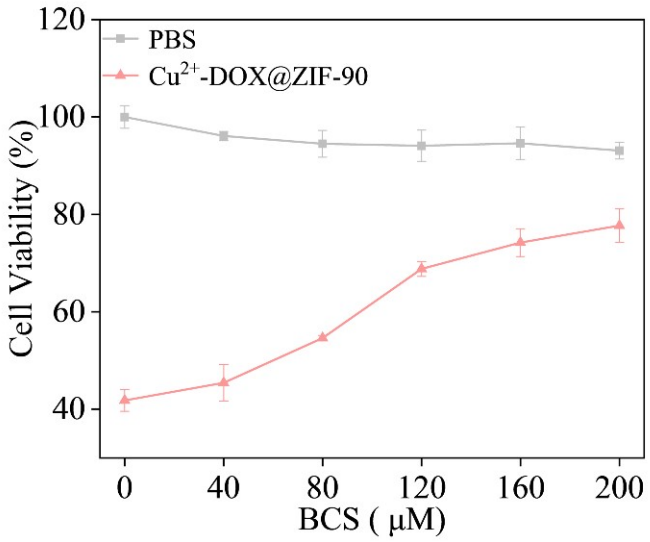

Fig. S8 Cell viability of HepG2 cells at different concentrations of Cu^{2+}-DOX@ZIF-90 (0, 4 mg mL^{-1}) after the pretreatment with different concentrations of BCS (0, 40, 80, 120, 160, 200 μM). Data in the figure is shown as mean ± S.D. (n = 3).

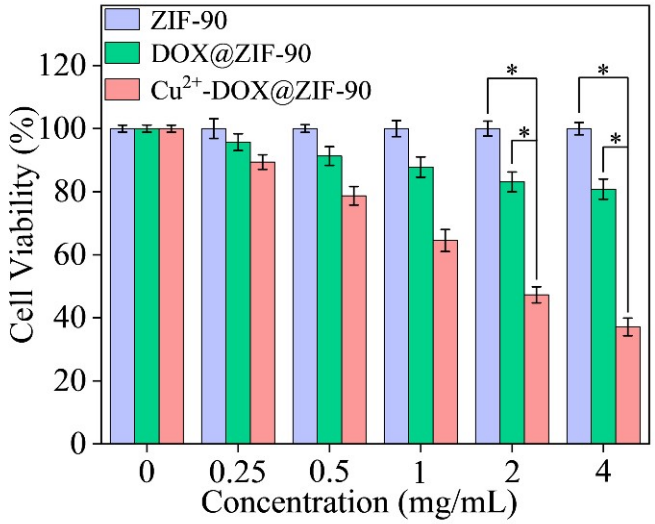

Fig. S9 Cell viability of HeLa cells was treated with different concentrations (0~4 mg mL^{-1}) of ZIF-90, DOX@ZIF-90, and Cu^{2+}-DOX@ZIF-90. Data in the figure is shown as mean ± S.D. (n = 3). *p < 0.05. Statistical significance is calculated using Analysis of Variance (ANOVA).

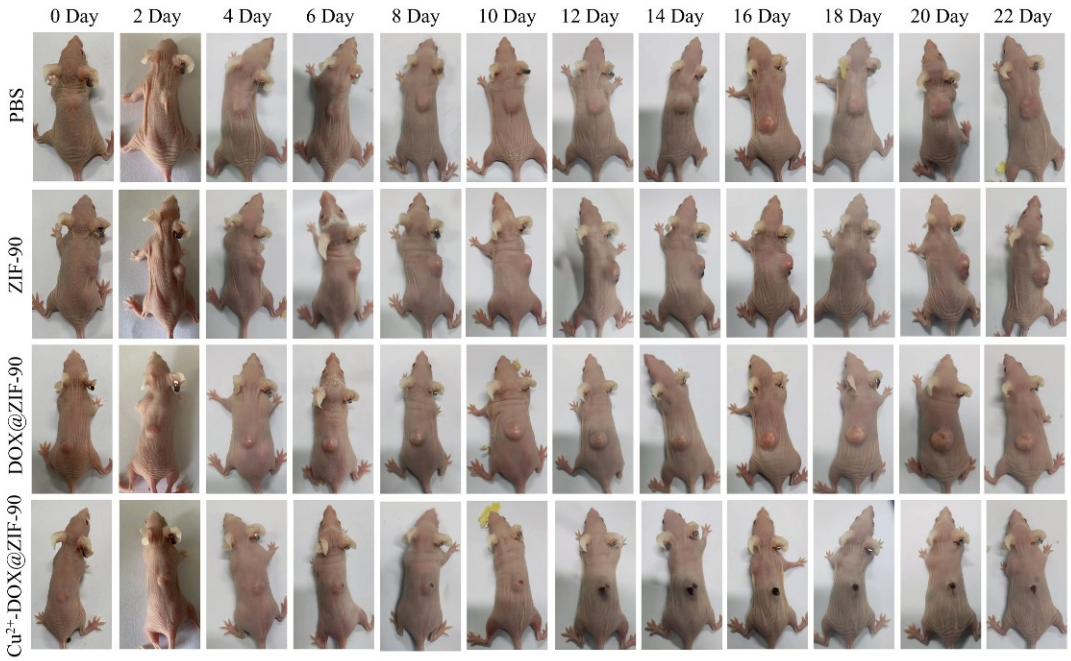

Fig. S10 Photographs of tumor mice treated with PBS, ZIF-90, DOX@ZIF-90, Cu^{2+}-DOX@ZIF-90.

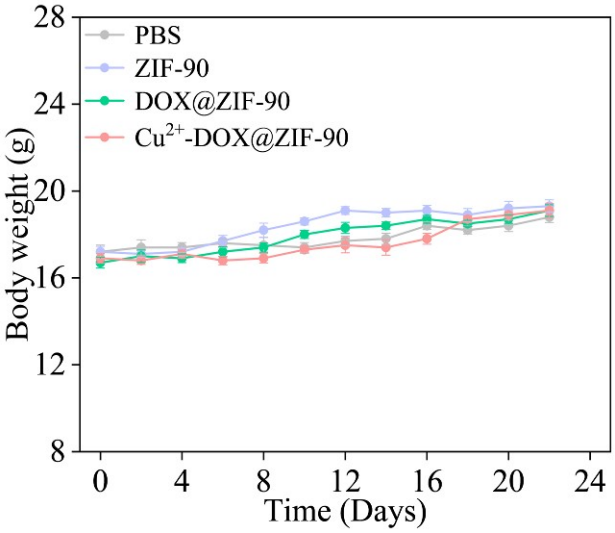

Fig. S11 Body weight of mice treated with PBS, ZIF-90, DOX@ZIF-90, Cu^{2+}-DOX@ZIF-90. Data in the figure is shown as mean ± S.D. (n = 3).

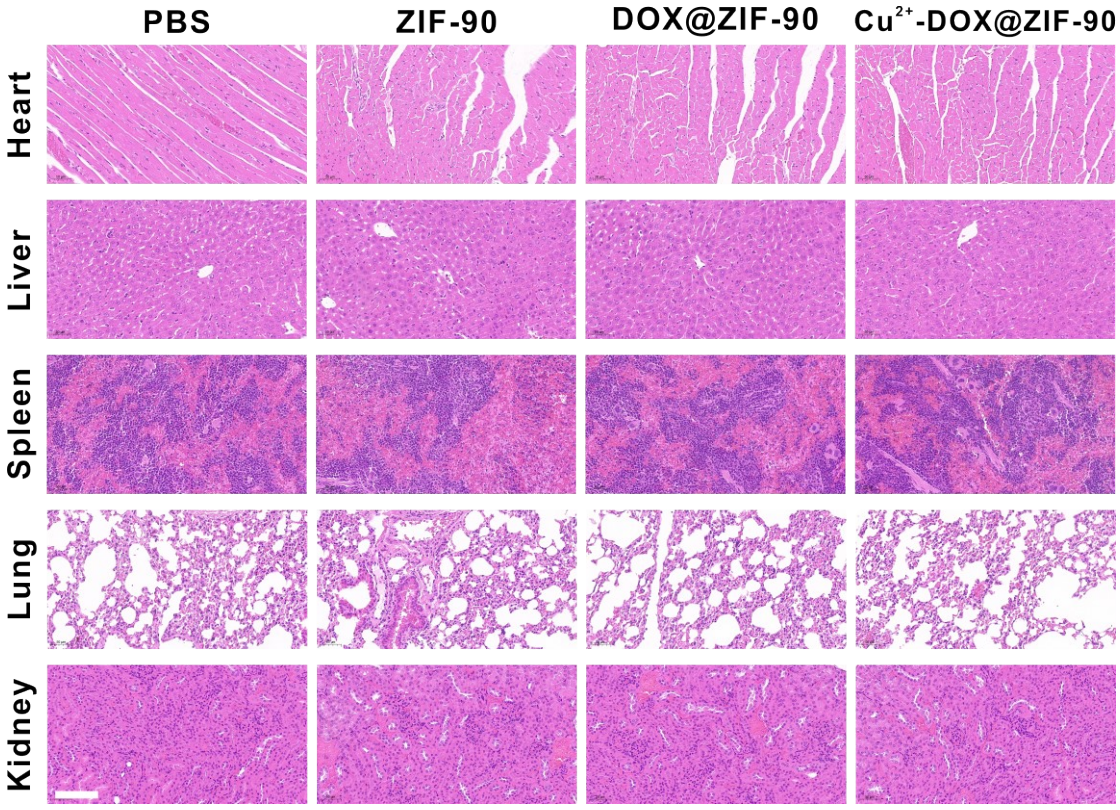

Fig. S12 H&E staining images of major organs (Heart, Liver, Spleen, Lung, Kidney) treated with PBS, ZIF-90, DOX@ZIF-90, Cu^{2+}-DOX@ZIF-90. Scale bars: 100 μm.

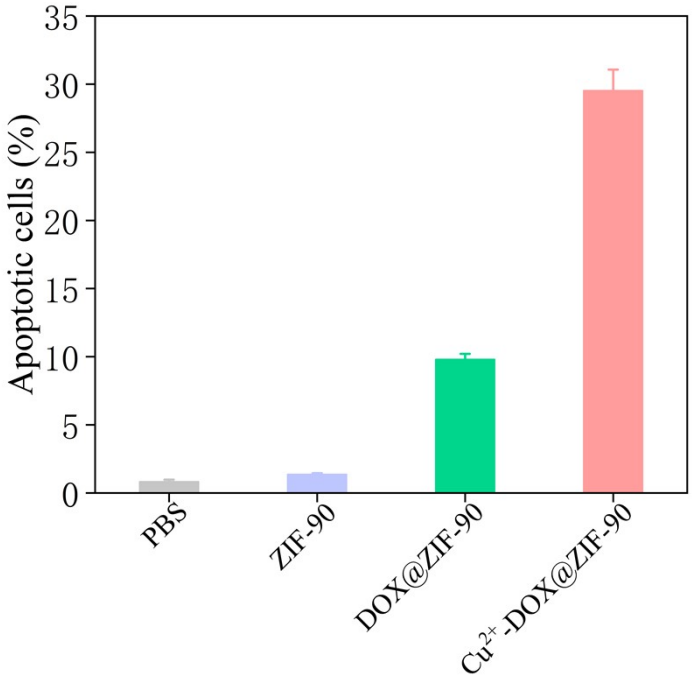

Fig. S13 Tumor cell apoptosis measured by the TUNEL assay. Data in the figure is shown as mean ± S.D. (n = 3).