Supporting Information

A L-Cysteine hydrochloride Modified Nanoscale Metal–Organic Frameworks to Co-Delivered Valproic Acid/Cisplatin in Drug-Resistant Lung Cancer

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Experimental section

Materials and general information

Zirconium (IV) chloride ($ZrCl_4$, 99.5%) was obtained from Strem Chemicals, Inc. Terephthalic acid (H_2BDC, 99%), L-Cysteine hydrochloride (Cys, 98%) and Sodium valproate (VPA, 98%) were purchased from J&K Scientific Co., Ltd. Polyvinylpyrrolidone (PVP, 95%) was purchased from Shantou Xilong Chemical Factory. Dimethyl sulfoxide (DMSO, 99%), N, N-dimethylformamide (DMF, 99.5%), and ethanol (99.7%) were purchased from Beijing Chemical Reagents Company. PEG-$(NH_2)_2$ (Mw=4000 g mol^{-1}) (96%) was provided by Beijing Kaizheng United Medicine Technology Co., Ltd. Cisplatin was provided by Beijing Huafeng Lianbo Chemical Materials Co., Ltd. RIPA protein extraction buffer was obtained from Beyotime Institute of Biotechnology, Beijing, China. Protease inhibitor were purchased from Roche, Basel, Switzerland. Polyvinylidene fluoride membrane was obtained from EMD Millipore Billerica, MA, USA. We didn't need to further purity these reagents, all that we buy were purely analytical.

Animals

This study was performed in strict accordance with the relevant provisions of the eighth edition of the Guidelines for the Care and Use of Laboratory Animals (International Publication Number: 978-0-309-15400-0) and was approved by the by the Animal Protection and Utilization Committee of the Harbin Medical University Cancer Hospital.

BALB/c nude mice weighing 20±1 g were raised in the experimental animal center of Harbin Medical University Cancer Hospital (temperature 25±1 °C, humidity 45-55%). We dispersed 2×10^7 human lung adenocarcinoma A549/CDDP cells in 100 μL of Dulbecco's Modified Eagle Media (DMEM). The A549/CDDP tumor-bearing model was established by subcutaneous injection into the right axilla of BALB/c nude mice.

Preparation of ZrMOF-Cys Nanoparticles

ZrMOF-Cys nanoparticles were prepared by a modified method as reported in our previous work. Firstly, 80.4 mg of $ZrCl_4$, 57 mg of H_2BDC, 60 mg of PVP and 79.2 ml of DMF were added to a 100 ml glass bottle and dissolved completely by ultrasound. Then 9 mg of Cys was weighed and dissolved in 3 mL of DMSO. Finally, the DMF mixed solution was uniformly dispersed into 6 reactors, and 0.5 mL of DMSO mixed solution was added to each reactor. The 6 reactors were put into an electrothermal constant temperature blast oven at 120 °C for 10 h. After cooling to room temperature, the reaction solution was centrifuged to obtain a precipitate (10000 r/min, 8 min). Finally, the precipitates were washed with DMF, deionized water (dH_2O) and ethanol to obtain ZrMOF-Cys nanoparticles.

Synthesis of CDDP-VPA@ZrMOF-Cys Nanoparticles

Firstly, 10 mg of ZrMOF-Cys nanoparticles, 10 mg of CDDP, 10 mg of VPA, 2 mL of dH_2O, 2 mL of ethanol and 0.5 mL of DMSO were added to a 50 mL erlenmeyer flask. The mixed solution was then ultrasonically dispersed uniformly. The CDDP and VPA were encapsulated into ZrMOF-Cys nanoparticles by vacuum pumping. Finally, the precipitates were washed 3 times with dH_2O to obtain CDDP-VPA@ZrMOF-Cys nanoparticles. The washing supernatant were collected for detecting the drug loading capacity of CDDP (determined by a UV-vis spectrophotometer, 300 nm).

Preparation of CDDP-VPA@ZrMOF-Cys-PEG Nanoparticles

10 mg of CDDP-VPA@ZrMOF-Cys nanoparticles were dispersed in 2 mL of dH2O, then 5 mg of PEG-$(NH_2)_2$ was added to the above mixed solution, and shaken it on a shaking table for 3 h. The precipitates were collected by centrifugation to obtain the CDDP-VPA@ZrMOF-Cys-PEG nanoparticles (CVZP NPs). In this work, the ZrMOF-Cys-PEG nanoparticles (ZP NPs), the CDDP@ZrMOF-Cys-PEG nanoparticles (CZP NPs), the VPA@ZrMOF-Cys-PEG nanoparticles (VZP NPs) were obtained by the same method.

Microwave Heating Properties of ZrMOF-Cys-PEG and CDDP-VPA@ZrMOF-Cys-PEG *in Vitro*

Microwave heating experiment was used to verify the microwave sensitization effect of ZP NPs and CVZP NPs *in vitro*. Dispersed two kinds of nanomaterials at different masses (0 mg, 3 mg, 5 mg, 7 mg, 10 mg) into 1 mL of 0.9% saline solution. 1 mL of saline solution without adding materials (0 mg) as a control group. Then mixed solution was added to the reactor and irradiated by MW at 0.9 W for 5 min. During the irradiation, the infrared imager was used for real-time monitoring and the temperature change was recorded every 10 seconds.

Drug Release at Different pH

We evaluated the drug release results of CVZP NPs using the artificial lysosomal fluids (ALF) at different pH values. First, the CVZP NPs were divided into three groups of the same concentration, and dissolved into ALF of different pH (pH=5.4, 6.5) and the simulated fluids (pH=7.4), respectively. Then, the sample tube containing the different mixed solution was shaken in a 37 °C constant temperature water bath shaking tank. The supernatant was tested for UV at different time points, and the UV absorption peak was used to calculate the CDDP release rate.

Degradation of ZrMOF-Cys-PEG *in Vitro*

In order to verify the biodegradability of ZP NPs, the materials were degraded in ALF (pH=5.4) and the simulated fluids (pH=7.4) *in vitro*. The formulations of simulated lysozyme solutions and simulated body fluids were obtained from a modified method as reported in our previous work. ZP NPs were separately dispersed into simulated lysozyme solutions and simulated body fluids at a concentration of 3 mg/mL. Then, the sample tube was shaken in a 37 °C constant-temperature water-bath oscillation box. The precipitates were taken out at different time points, and then the morphology change was observed by TEM.

Detection of Cell Cycle by PI Staining

The cell cycle changes of A549/CDDP were detected by flow cytometry to verify the effects of VPA and MW on cell cycle. The experiment was divided into six groups: Control group, MW group, VZP NPs group, CZP NPs group, CVZP NPs group and CVZP NPs+MW group. A549/CDDP cells were evenly divided into six dishes with a diameter of 10 cm and incubated for 24 h. Different concentrations (50 μg/mL) of materials were added to the culture dish and incubated for 24 h. Trypsin was used to digest the two groups that needed microwave hyperthermia, which were transferred to containers and irradiated with MW (0.9 W, 5 min). The cells after microwave were transferred to the culture dish and all cells were incubated for 24 h. Cells were digested by trypsin, then centrifuged (1000 rpm, 5 min) to collect cells and washed twice with pre-cooled PBS solution. The cells were uniformly dispersed in a mixed solution of 0.5 ml of pre-cooled PBS solution and 1.5 ml of pre-cooled ethanol. The mixed system was then fixed at 4 °C overnight. After that, cells were collected by centrifugation and washed twice with pre-cooled PBS solution.

1 ml of PBS solution (containing 50 ug/mL ethidium bromide (PI), 100 ug/mL RNase A) was added to suspend cells. Flow cytometry (BD FACSCalibur) was used to detect the cell cycle.

***In Vitro* Verification of Immunofluorescence**

The inhibitory effect of VPA on VEGF was verified by immunofluorescence assay *in vitro*. A549/CDDP cells were evenly divided into four groups and incubated with CVZP NPs (0 ug/mL, 50 ug/mL, 100 ug/mL, 200 ug/mL) for 24 h. The cells were washed with immunostaining detergent and then fixed for 14 min with immunostaining fixative at 4 °C. Immunostaining solution was added and blocked at room temperature for 1 h. Then diluted anti-VEGF antibodies were added and overnight at 4 °C. The diluted FITC-labeled anti-rabbit secondary antibody was added and incubation for 1 h in the dark. Then DAPI staining was added to further stain the nucleus and incubate for 5 min. Confocal fluorescence microscopy (Olympus X71, Japan) was used to collect images. The reagents used in the above experiments were all purchased from the Beyotime Biotechnology.

Western Blot Analysis

We used the RIPA protein extraction buffer (containing protease inhibitor cocktail) to collect cell lysates for Western blot analysis. First, an electrophoresis gel (10% sodium dodecyl sulfate-polyacrylamide gel) was used to separate cell lysates and transferred the separated lysate to a polyvinylidene fluoride membrane. Then, the separated membrane was incubated with the specific antibody overnight (at 4 °C). Furthermore, anti-rabbit antibodies and horseradish peroxidase-conjugated anti-mouse were added, respectively. Finally, we used the ChemiDocTM MP Imaging System to capture the the experimental bands.

Evaluation of Biosafety of CDDP-VPA@ZrMOF-Cys-PEG and ZrMOF-Cys-PEG *in Vitro*

The toxicity of ZP NPs and CVZP NPs were evaluated by MTT assay. Mouse normal fibroblasts cells L929, human lung adenocarcinoma cells A549, and cisplatin-resistant human lung adenocarcinoma cells A549/CDDP were uniformly dispersed into three 96-well plates to incubate for 24 h (37 °C, 5% CO_2). Different concentrations of ZP NPs (0 μg/mL, 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL) were added and co-cultured with three kinds of cells for 24 h. Then 20 μL of MTT (5 mg/mL) was added to each well. After 4 h, the supernatant was removed and 150 μL of DMSO was added. Cell viability was calculated by measuring the absorbance by the enzyme labeling apparatus. Different concentrations of CVZP NPs (0 μg/mL, 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL) were detected toxicity with cisplatin-resistant human lung adenocarcinoma A549/CDDP in the same method.

***In Vivo* Safety Evaluation of ZrMOF-Cys-PEG**

We further validated the toxicity of ZP NPs using ICR mice. After feeding the mice for one week, they were randomly divided into 4 groups (3 mice each group). One group was set as a control, and the other groups were injected with different concentrations of ZP NPs (0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg) through the tail vein. Changes in body weight of the mice were recorded every day. After 14 days of feeding, the mice were sacrificed. Blood was collected to test blood routine and blood biochemistry. Through dissecting the mouse to obtain the main organs and immersed them in a 4% neutral formaldehyde solution for subsequent tissue analysis.

***In Vitro* Evaluation of CDDP-VPA@ZrMOF-Cys-PEG and MW Combined Treatment**

The effect of CVZP NPs and MW combined treatment was verified by cell inhibition experiment *in vitro*. The experiment was divided into 8 groups, (1) Control group, (2) MW group, (3) free CDDP group, (4) ZP NPs group, (5)

ZP NPs+MW group, (6) CZP NPs group, (7) CVZP NPs group, (8) CVZP NPs+MW group, respectively. Human lung adenocarcinoma cells A549 and cisplatin-resistant human lung adenocarcinoma cells A549/CDDP were uniformly dispersed into 6-well plates. Different materials (50 μg/mL) were added after incubation for 24 h. Then continuing to incubate for 24 h, all cells were digested. Several groups requiring microwave hyperthermia were transferred to the reaction under microwave irradiation (0.9 W, 5 min). The rest was directly transferred to 96-well plates. Cells after microwave irradiation were also transferred to 96-well plates and incubated for 24 h. Then 20 μL of MTT was added to each well. After 4 h, the supernatant was removed and 150 μL of DMSO was added. The absorbance was measured with the enzyme labeling apparatus to calculate the cell viability.

***In Vivo* Antitumor Efficacy of CDDP-VPA@ZrMOF-Cys-PEG and MW Combined Therapy**

To further evaluate the therapeutic effects of CVZP NPs, we performed therapeutic experiments with A549/CDDP tumor-bearing BALB/c nude mice. It can be seen from the *in vitro* experiment that the therapeutic effect of ZP group and ZP+MW group is not ideal. And ZP NPs had been tested for acute toxicity *in vivo*. Therefore, these two groups were removed treatment experiments *in vivo*. Nude mice were randomly divided into 6 groups: (1) Control group, (2) MW group, (3) free CDDP group, (4) CZP NPs group, (5) CVZP NPs group and (6) CVZP NPs+MW group. The materials were injected into the mice by tail vein to ensure that each group of materials contained the same concentration of CDDP. Microwave hyperthermia experiments (0.9 W, 5 min) were performed 6 h later and the infrared imager was used for real-time monitoring. Tumor volume and mice body weight were recorded every other day. After 20 days, the mice were sacrificed. The tumor and main tissues were taken out and immersed in a 4% neutral formaldehyde solution for further analysis.

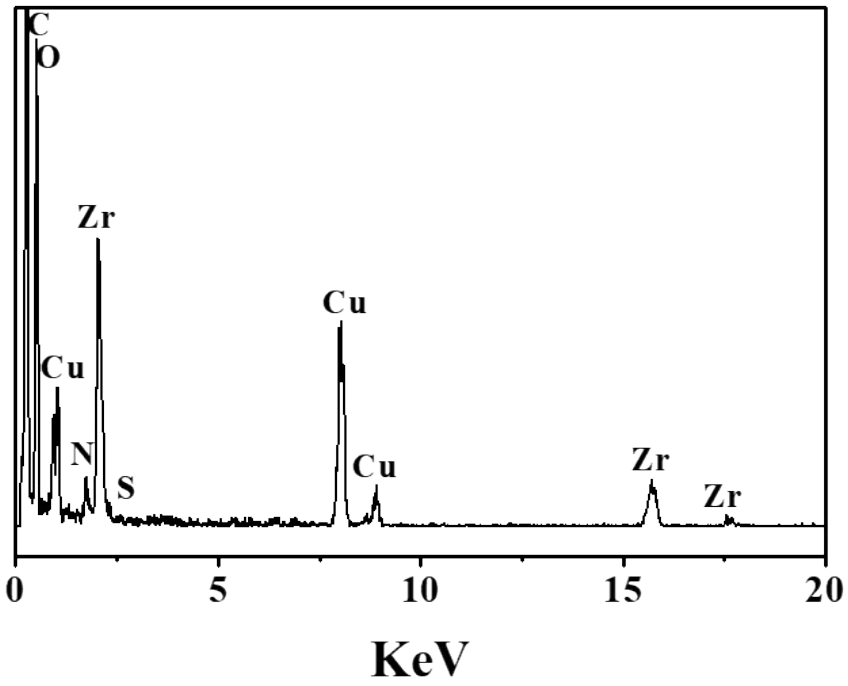

Fig. S1 The EDS of ZrMOF-Cys nanoparticles.

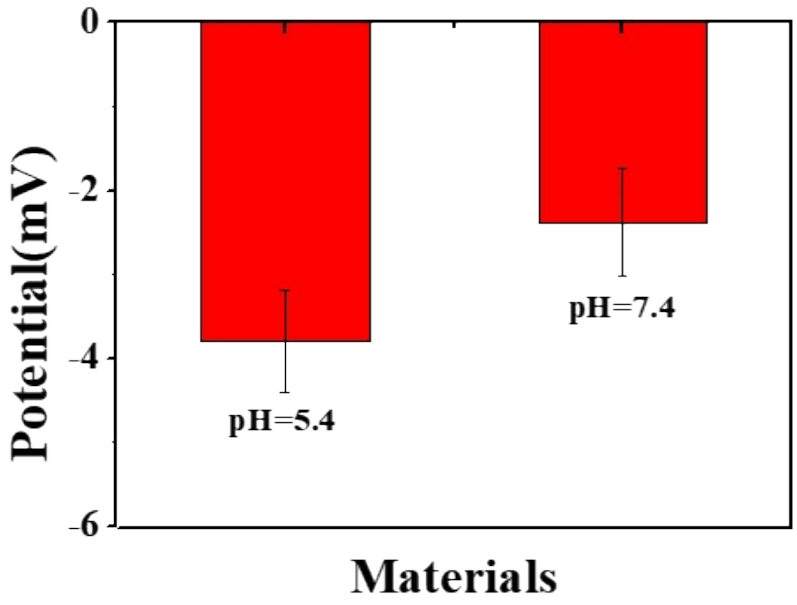

Fig. S2 The zeta potential measurements of CVZP NPs at different pHs in PBS.

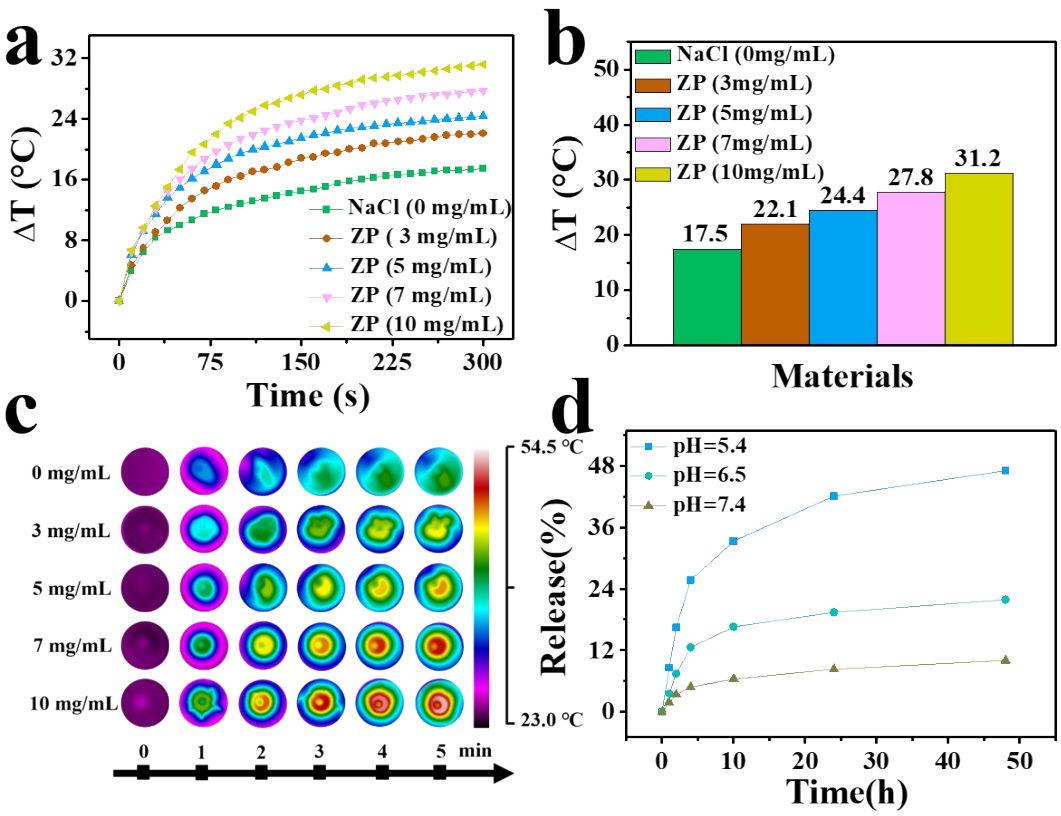

Fig. S3 *In vitro* MW heating image and drug release. (a) Temperature rise diagram of ZP NPs with different concentrations. (b) Temperature difference chart of ZP NPs with different concentrations. (c) FLIR thermal image of ZP NPs with different concentrations. (d) Drug release image of CVZP NPs.

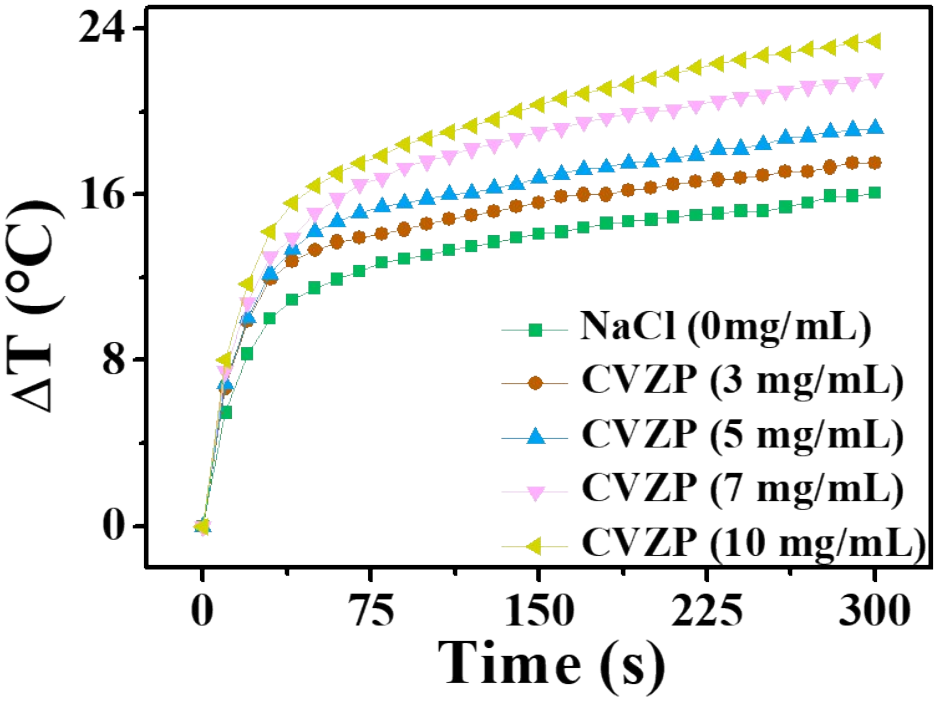

Fig. S4 Temperature rise diagram of CVZP NPs with different concentrations.

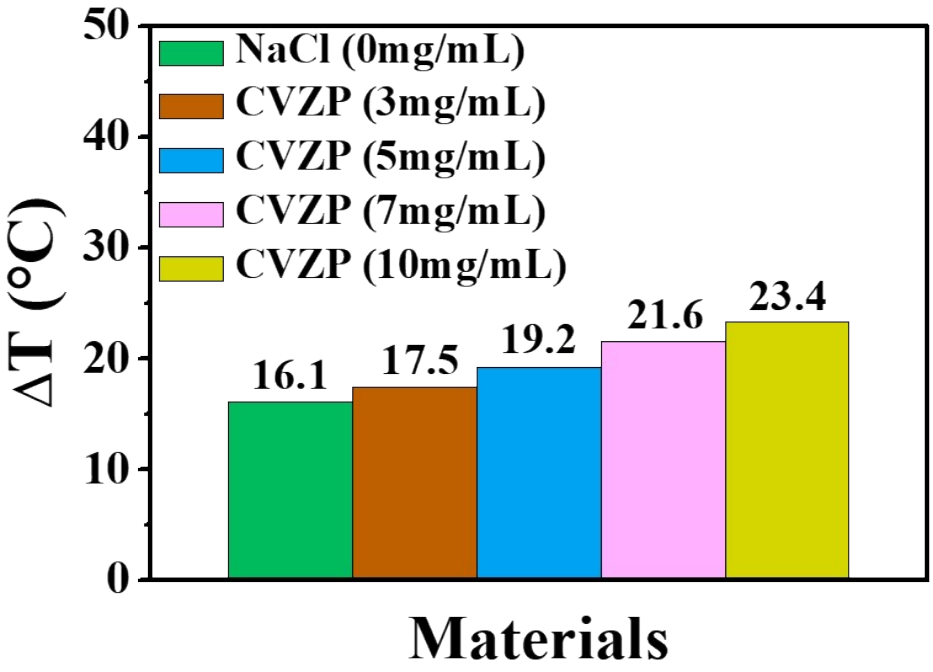

Fig. S5 Temperature difference chart of CVZP NPs with different concentrations.

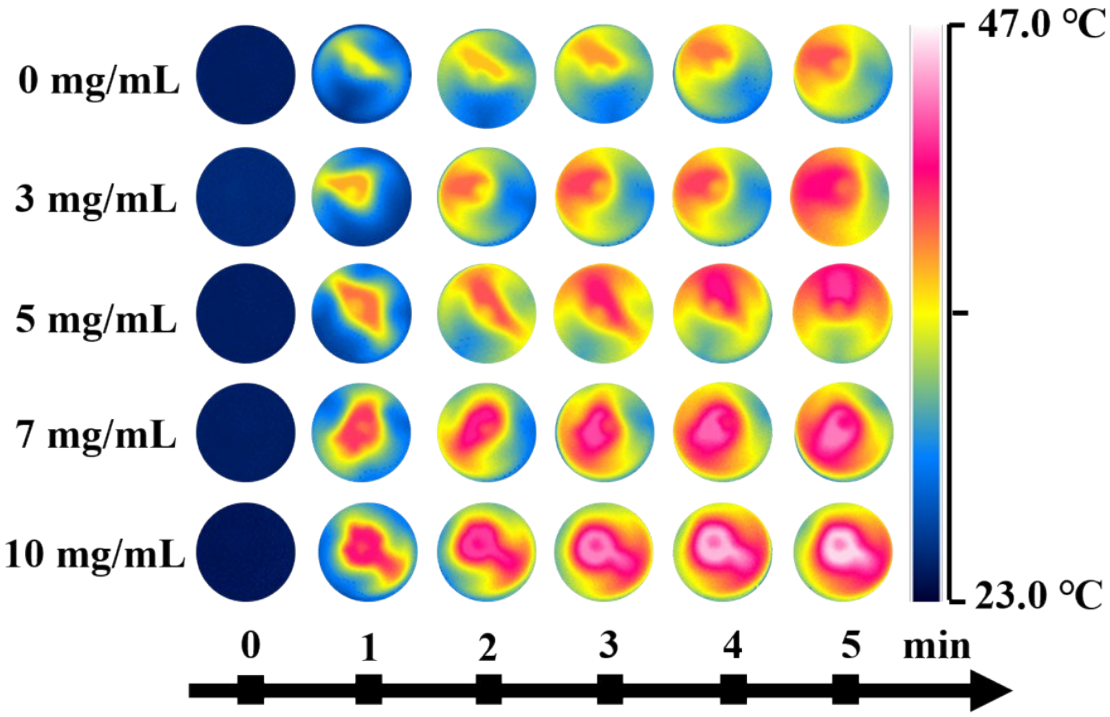

Fig. S6 FLIR thermal image of CVZP NPs with different concentrations.

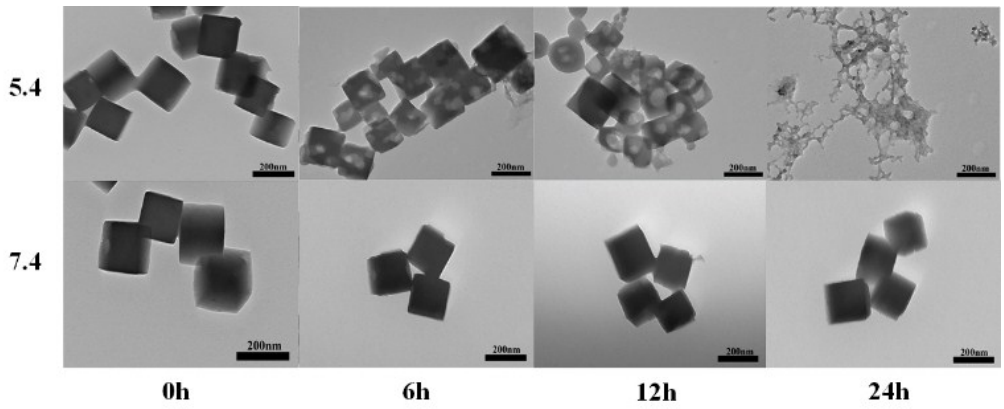

Fig. S7 The TEM images of ZP NPs degradation at 0 h, 6 h, 12 h and 24 h.

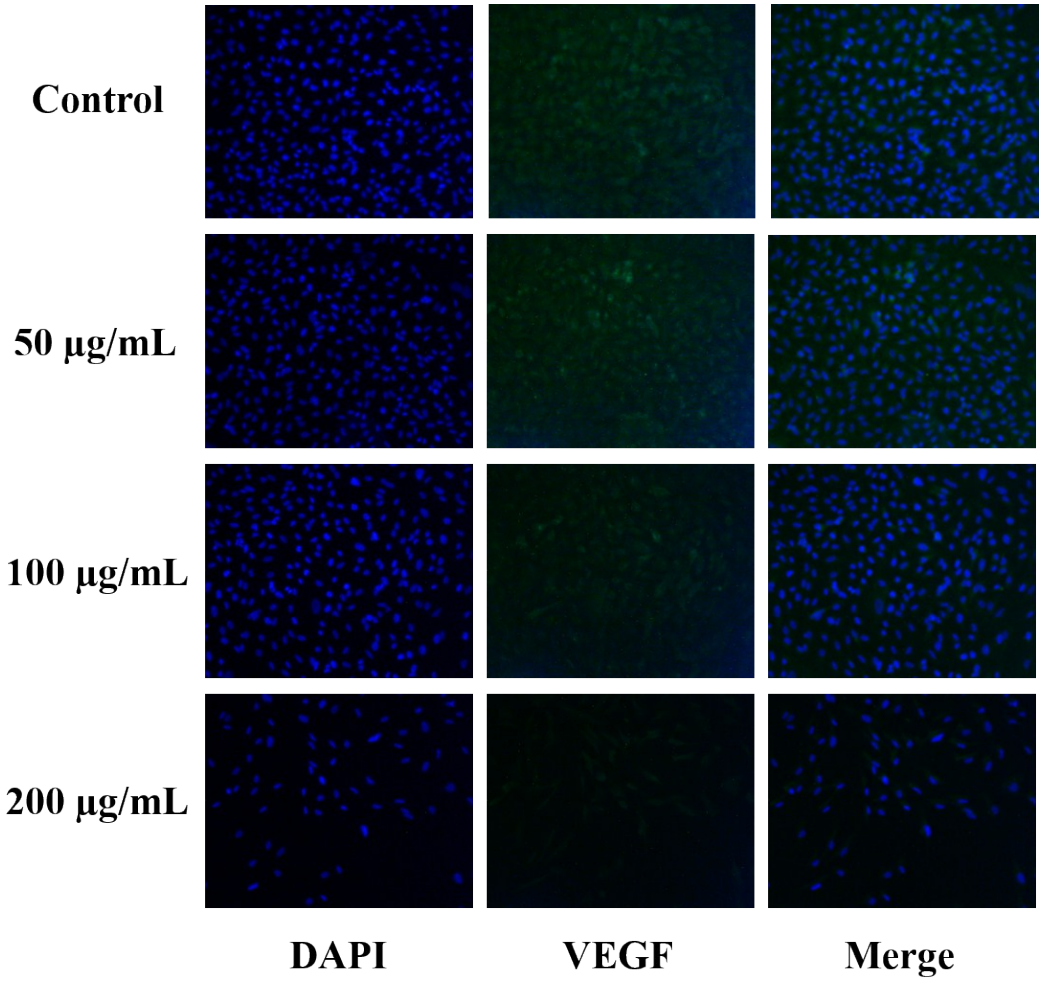

Fig. S8 Immunofluorescence staining of nucleus and VEGF after CVZP NPs treatment (DAPI (blue) and anti-VEGF antibody (green)).

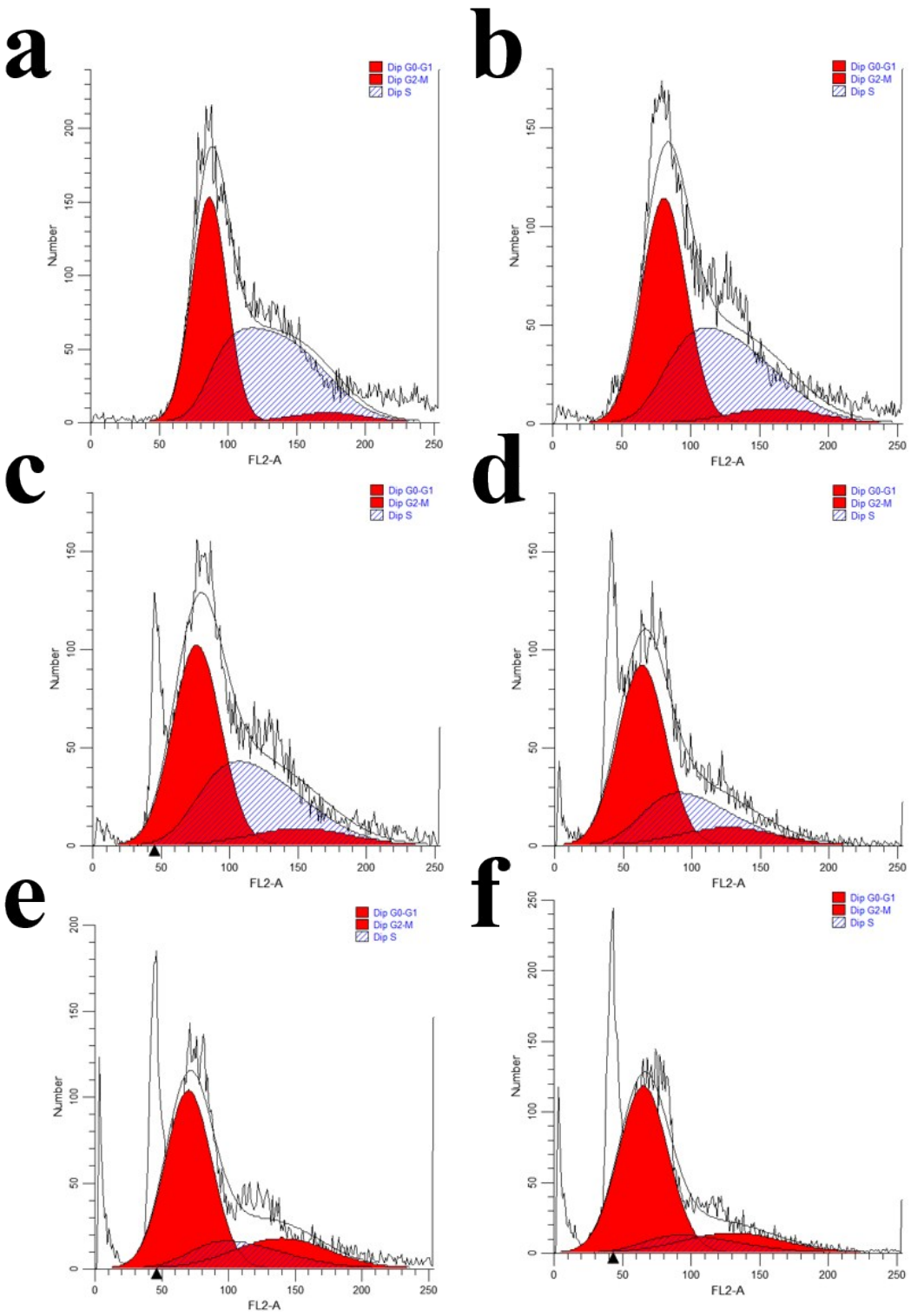

Fig. S9 Cell Cycle detection. (a) A549/CDDP cells. (b) A549/CDDP cells after treated with CZP NPs. (c) A549/CDDP cells after treated with MW. (d) A549/CDDP cells after treated with VZP NPs. (e) A549/CDDP cells after treated with CVZP NPs. (f) A549/CDDP cells after treated with CVZP+MW.

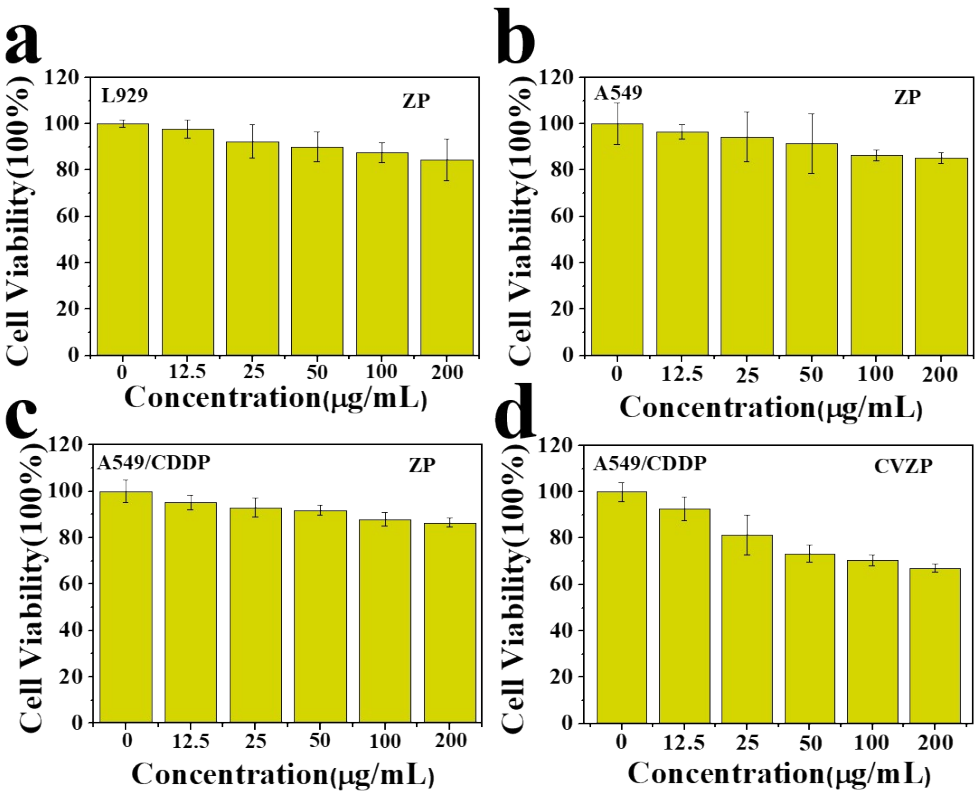

Fig. S10 Toxicity and therapeutic effect in vitro.(a) Cytotoxicity of ZP NPs incubating with L929 for 24 h. (b) Cytotoxicity of ZP NPs incubating with A549 for 24 h. (c) Cytotoxicity of ZP NPs incubating with A549/CDDP for 24 h. (d) Cytotoxicity of CVZP NPs incubating with A549/CDDP for 24 h.

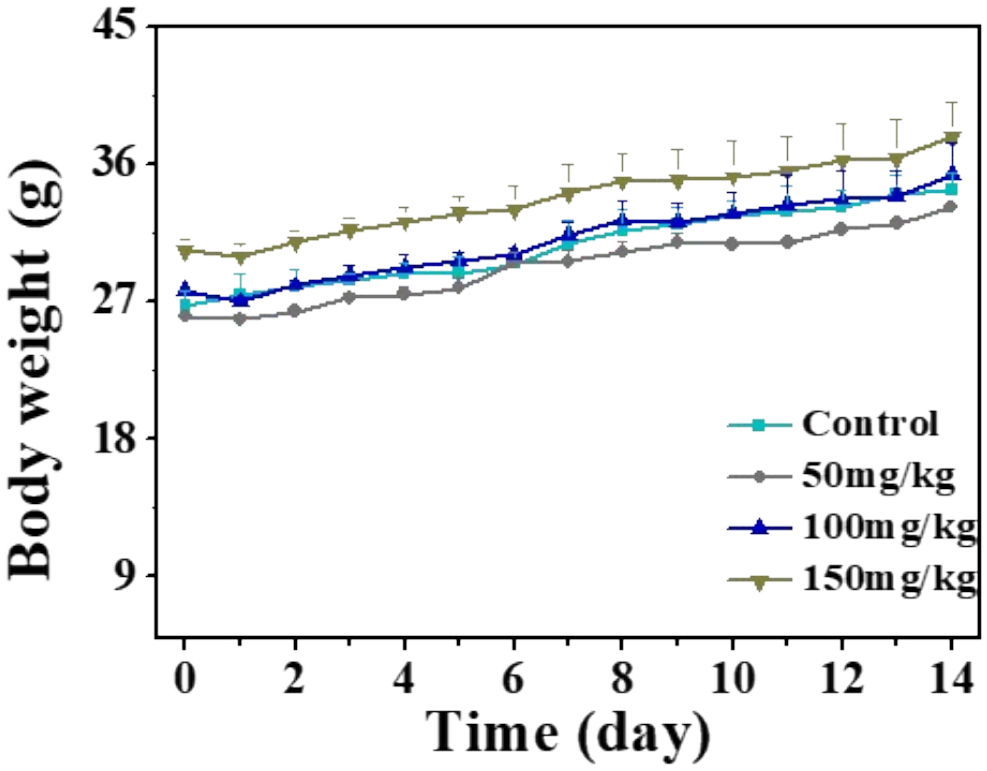

Fig. S11 Weight data of mice in each group with different concentrations of ZP NPs.

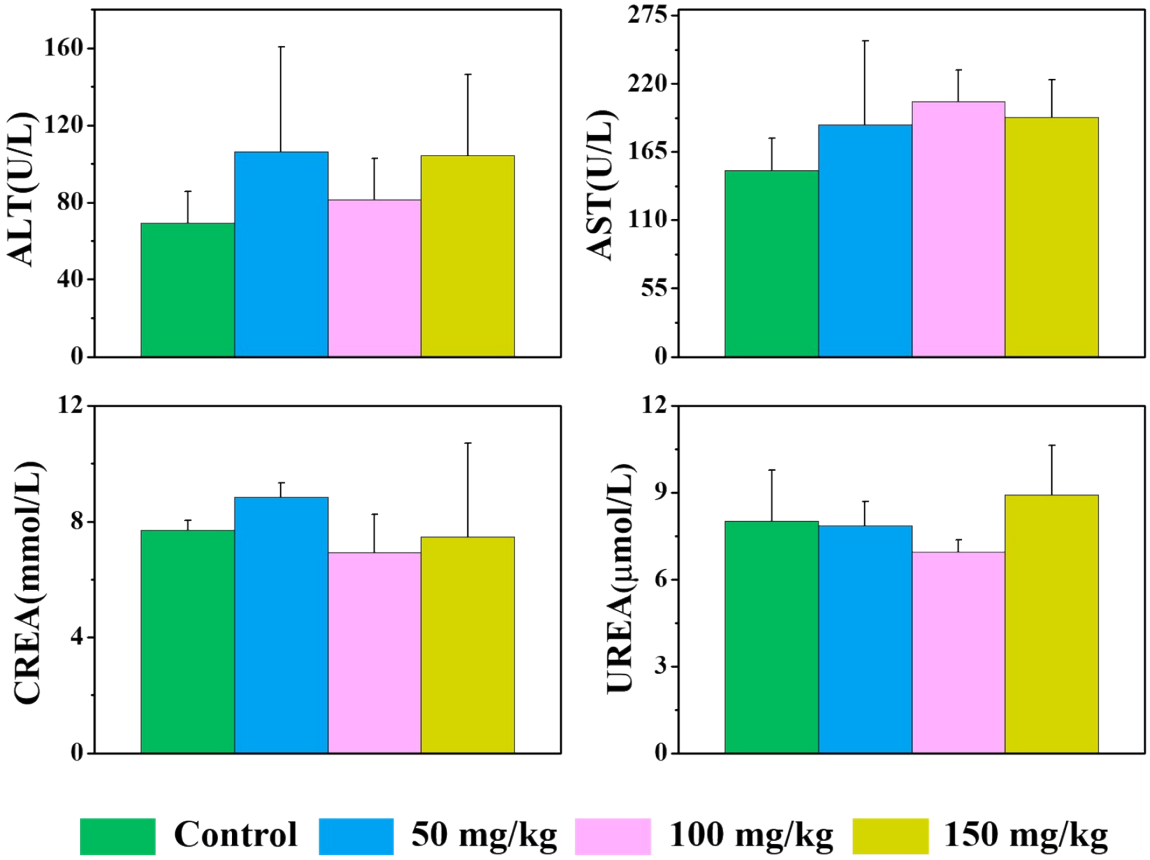

Fig. S12 Blood biochemical data in each group with different concentrations of ZP NPs.

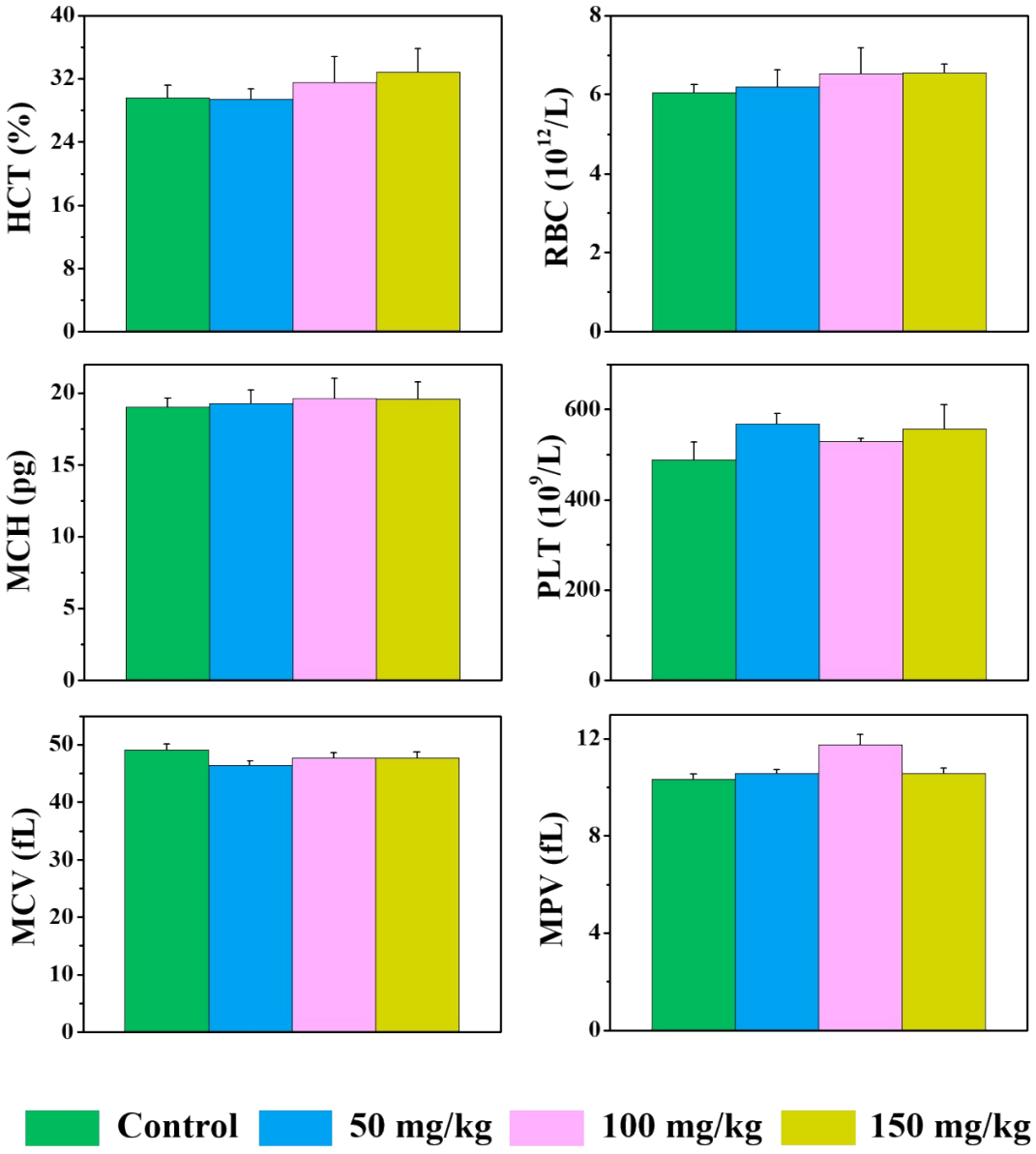

Fig. S13 Blood routine data in each group with different concentrations of ZP NPs.

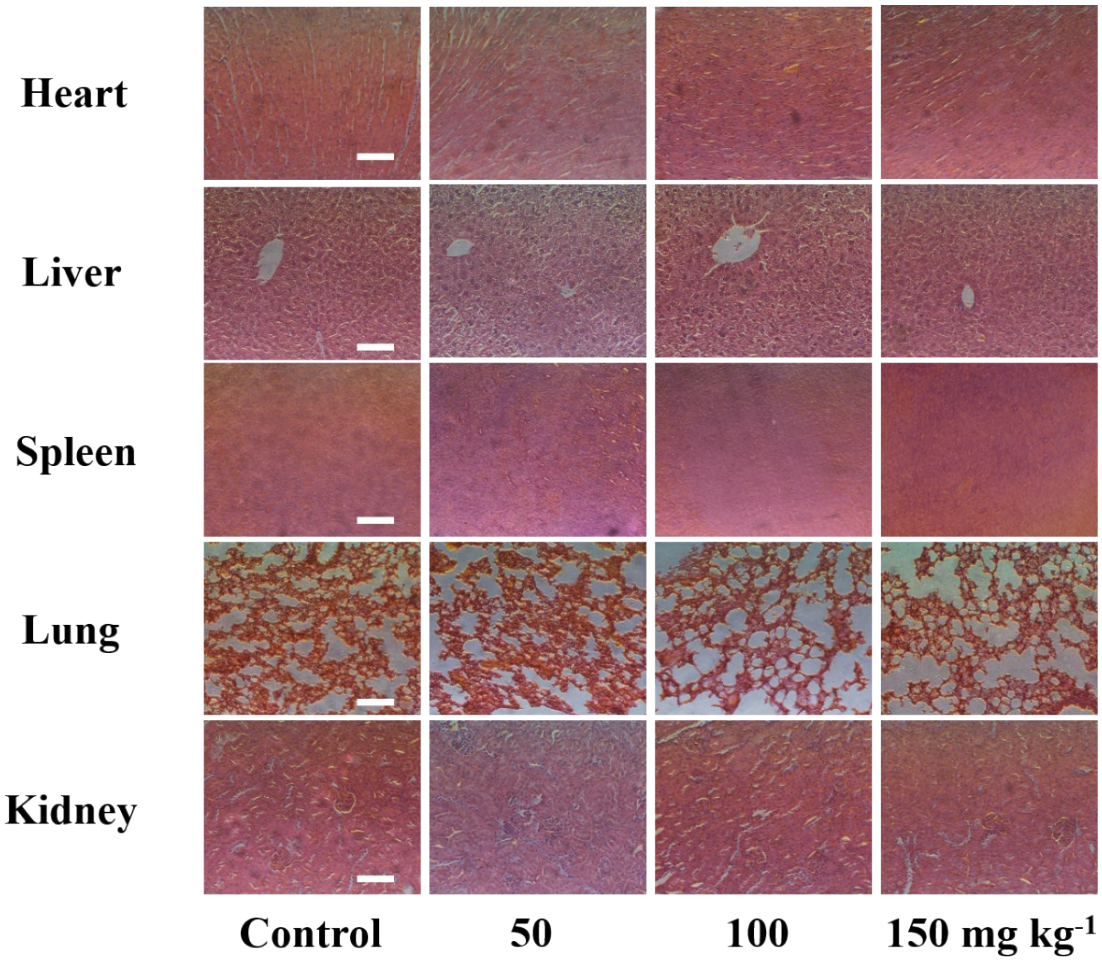

Fig. S14 Different concentrations of ZP NPs after intravenous injection Tissue H&E staining results, the scale bar is 50 μm.

Fig. S15 Image of BALB/c node mice in each group after treatment.