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Supplementary Information

Transition metal doped hydrophilic ultrasmall iron oxide modulate MRI contrast performance for accurate diagnosis of orthotopic prostate cancer

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Materials. All chemical reagents (analytical grade) were used as received without further purification. Iron acetylacetonate ($Fe(acac)_3$), cobalt acetylacetonate ($Co(acac)_2$), nickel acetylacetonate ($Ni(acac)_2$), copper acetylacetonate ($Cu(acac)_2$), zinc acetylacetonate ($Zn(acac)_2$), polyvinylpyrrolidone (PVP-K30) and triethylene glycol (TEG) were supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Cell Counting Kit 8 (CCK-8) was obtained from Bimake (USA).

Preparation of transition-metal doped IONs. Hydrophilic iron oxide nanoparticles (IONs) and Co-, Ni-, Cu-, Zn-doped IONs were synthesis using TEG as solvent by one-pot thermal decomposition method. Briefly, 1 mmol of $Fe(acac)_3$, 0.05 mmol of $Co(acac)_2$, and 0.5 g of PVP-K30 were added into 40 mL of TEG. The mixture was stirred for 10 min at room temperature, and then heated to 100 ^oC in vacuum for 30 min. After that, the system was rapidly heated to 260 ^oC and kept for 1 hour under nitrogen atmosphere. When the system was cooled down to the room temperature, Co-doped ION were successfully synthesized and collected by high-speed centrifugation. Finally, the product was washed with distilled water and ethanol for 3 times. Several other materials were synthesized using similar methods.

Characterizations. The morphology of $M_xFe_{3-x}O_4$ were observed by a transmission electron microscope (TEM, JEM-2100 plus, JEOL, Japan) operated at 200 kV. The crystalline structures of $M_xFe_{3-x}O_4$ were analyzed using X-ray diffractometer (TTR-III, Rigaku Co., Japan). X-ray photoelectron spectra (XPS, Thermo ESCALAB) were used to record the composition of $M_xFe_{3-x}O_4$. Hydrodynamic size of $M_xFe_{3-x}O_4$ were determined with dynamic light scattering (DLS) detector (Nanotrac Wave II, Microtrac Co., USA). The composition of $M_xFe_{3-x}O_4$ was also analyzed using an FT-IR spectrometer (iS10, Nicolet Co., USA). Specific surface areas of $M_xFe_{3-x}O_4$ were

confirmed by an automatic surface area and pore analyzer (Tristar II 3020 M, Micromeritics, USA). Magnetic property of $M_xFe_{3-x}O_4$ was tested on superconducting quantum interference device (SQUID) magnetometer (Bruker Co., Germany). The concentration of metal ions was measured by inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo Fisher Scientific Co., USA) instrument. Positron annihilation lifetime was tested by positron annihilation lifetime spectrometer (time-resolved 189 ps, source intensity 20 μCi).

Cell Culture. HTLE-3, 293T, 4T1, and PC3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone) containing 10% fetal bovine serum (Hyclone) and penicillin-streptomycin (100 U/ml penicillin and 0.1 mg/ml streptomycin) in 5% CO_2 atmosphere at 37 °C. After that, these cells were treated with Fe_3O_4 and $Zn_{0.14}Fe_{2.86}O_4$ at the concentration range from 0-100 μg/mL for 24 and 48 h. Subsequently, the media were removed and the cells were washed with phosphate buffered saline (PBS) and further incubated with 200 μL of 10% CCK-8 solution for 2 h. Cell viability was determined by a microplate reader at a wavelength of 450 nm.

Routine blood examination. All animal experiments were in accordance with the guidelines of the Ethics Committee of Binzhou Medical University. Briefly, the rats were intravenously injected with $Zn_xFe_{3-x}O_4$ and Fe_3O_4 at a dose of 10 mg/kg. At post-injection for 24 h and 48 h, the blood of the rats was collected, and analyzed with an animal blood analyzer (MASCOT, USA).

Histopathology evaluation. BABL/C mice were intravenously administrated using $Zn_xFe_{3-x}O_4$ at a dose of 10 mg/kg. After 48 h, the mice were sacrificed, and the vital organs including heart, liver, spleen, lung, and kidney were excised, cut into slices, and then stained by H&E dyes for histopathological evaluation.

Biodistribution of $Zn_xFe_{3-x}O_4$. 4T1 tumor-bearing BABL/C mice model were

established via subcutaneously injection of 4T1 cells on the left-behind leg. About 7 days, the mice were intravenously administrated using $Zn_xFe_{3-x}O_4$ at a dose of 10 mg/kg. Subsequently, at different time intervals, the mice were sacrificed, and the vital organs including heart, liver, spleen, lung, kidney, and tumor were excised, and weighed. The organs were then nitrated into transparent solution using the concentrated nitric acid by a microwave digestion system. After that, the solutions were further filtered through a 0.45 μm membrane filter, and finally Fe contents of solutions was detected by ICP-OES.

MRI investigation in vitro and in vivo. The r_2 values of transition-metal doped IONs were measured using a 3.0 T and 9.4 T MRI scanner. The longitudinal relaxation time (T_1) was measured through a series of inversion-prepared fast spin-echo images. The series is identical in all respects (repetition time (TR) 2500 ms, effective echo time (TE) 5.6 ms, bandwidth (BW) 25 kHz, slice thickness 1 mm, matrix 96 × 96, 3 averages.).

In vivo MRI experiment, T_2-weighted MR images of 4T1 tumor-bearing mice were acquired at the coronal planes. The following acquisition parameters were used: echo time (TE) = 6.06 ms, repetition time (TR) = 2000 ms, averages: 2, field of view (FOV) = 35 mm × 35 mm, image size = 256 × 256, slice thickness = 1 mm (12 slices, gap = 0), 1 mean, bandwidth (BW) = 50 kHz. In addition, T_2-weighted MR images of prostate cancer-bearing mice were acquired at the axial planes. The following acquisition parameters were used: echo time (TE) = 30 ms, repetition time (TR) = 5000 ms, averages: 8, field of view (FOV) = 30 mm × 30 mm, image size = 192 × 192, slice thickness = 1 mm (12 slices, gap = 0), 1 mean, bandwidth (BW) = 35 kHz.

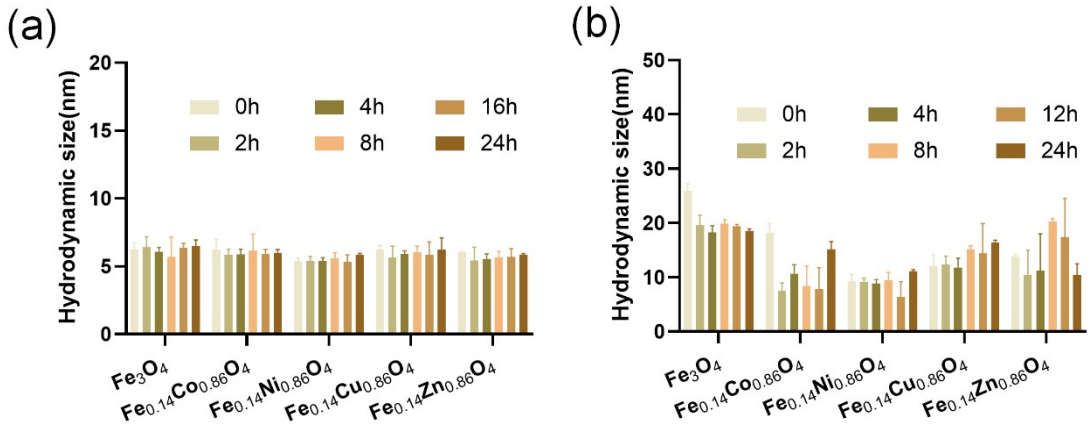

Fig. S1 Hydrodynamic particle size changes of different samples in FBS and deionized water.

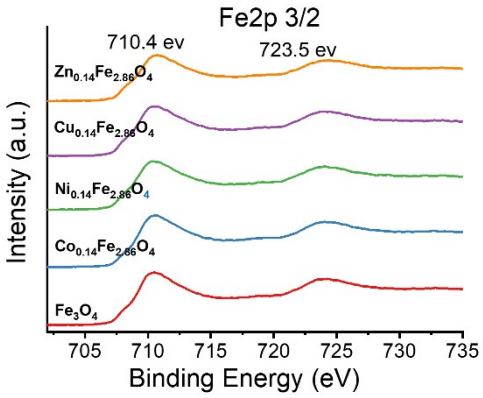

Fig. S2 XPS Fe2p peaks of different samples.

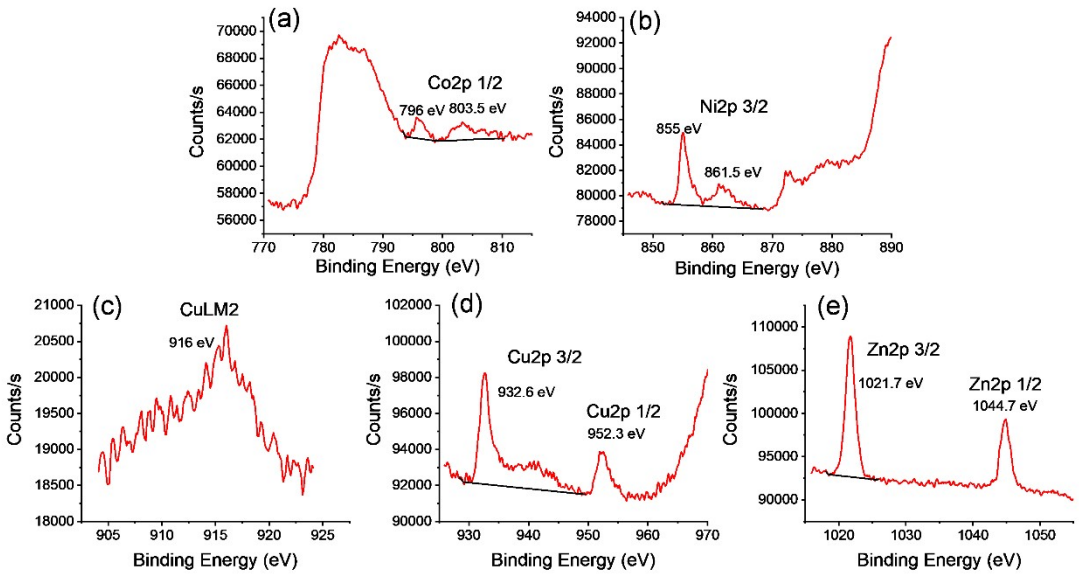

Fig. S3 XPS peaks of Co2p, Ni2p, CuLM2, Cu2p, Zn2p peaks.

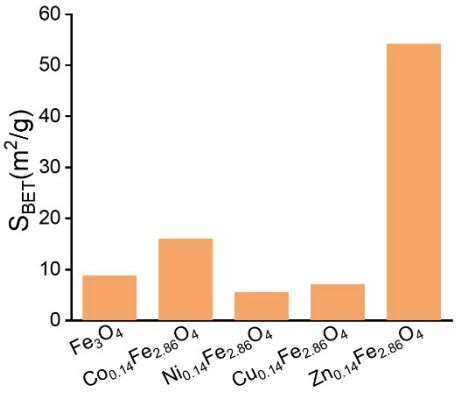

Fig. S4 Specific surface area of different samples.

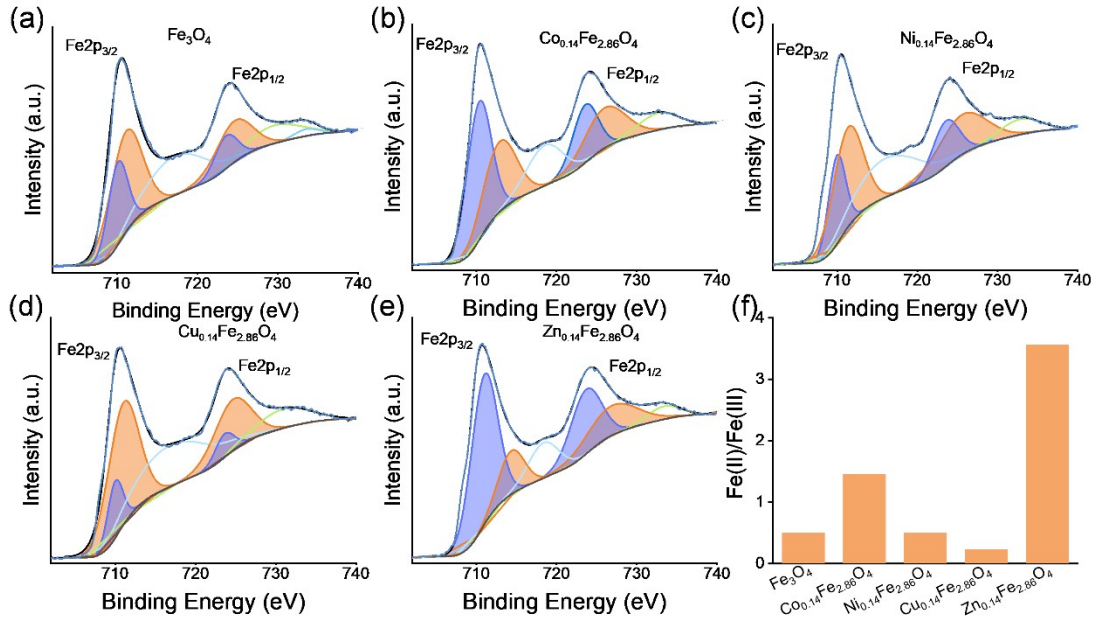

Fig. S5 (a)-(e) Fe 2p XPS spectra different samples (Fe(II), purple; Fe(III), orange). (f) Percentage of Fe(II) in Fe ions in different samples.

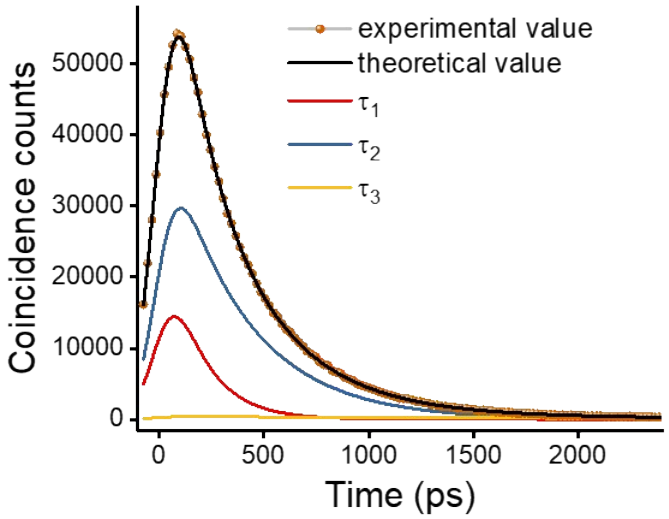

Fig. S6 Positron annihilation lifetime spectrum of $Zn_{0.14}Fe_{2.86}O_4$.

Positron Lifetime (ps)		Corresponding Intensity (%)	
τ_1	166	I_1	22.7
τ_2	358	I_2	73.5
τ_3	1859	I_3	3.9

Table S1 Positron lifetime spectrum characteristic parameters of $Zn_{0.14}Fe_{2.86}O_4$.

Sample	I_2/I_1
$Zn_{0.14}Fe_{2.86}O_4$	3.24
Fe_3O_4	0.49[1]
Fe_3O_4	0.46-1.25[2]

Table S2 I_2/I_1 of $Zn_{0.14}Fe_{2.86}O_4$ and Fe_3O_4.

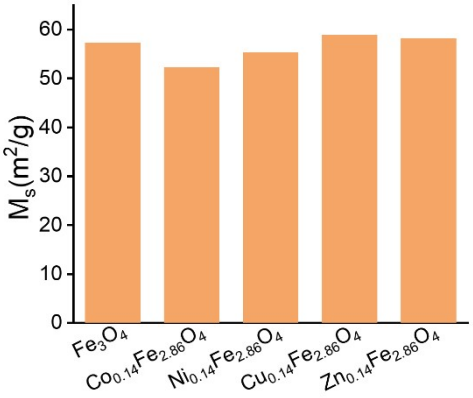

Fig. S7 Saturated magnetization of different samples at 300K.

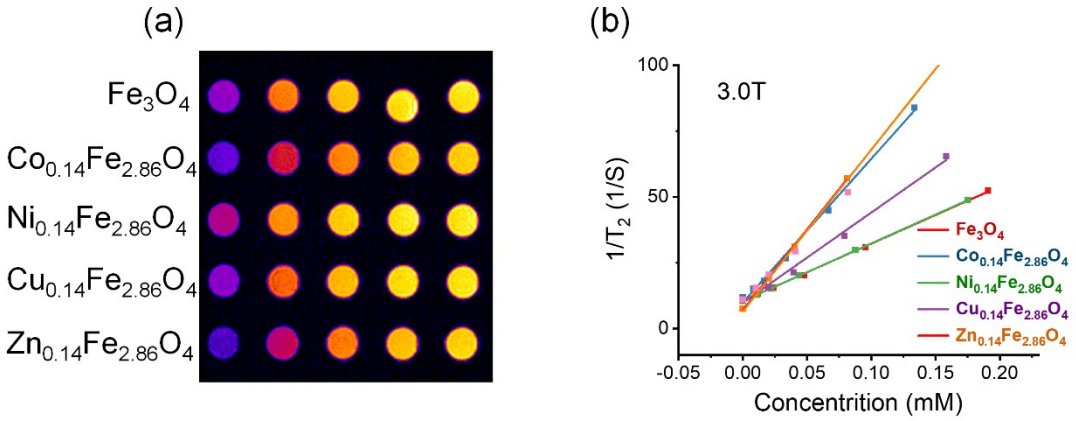

Fig. S8 (a) MRI images of different samples at 3.0T. (b) The transverse relaxation rate of samples through the ratio of 1/ T_2 to concentration.

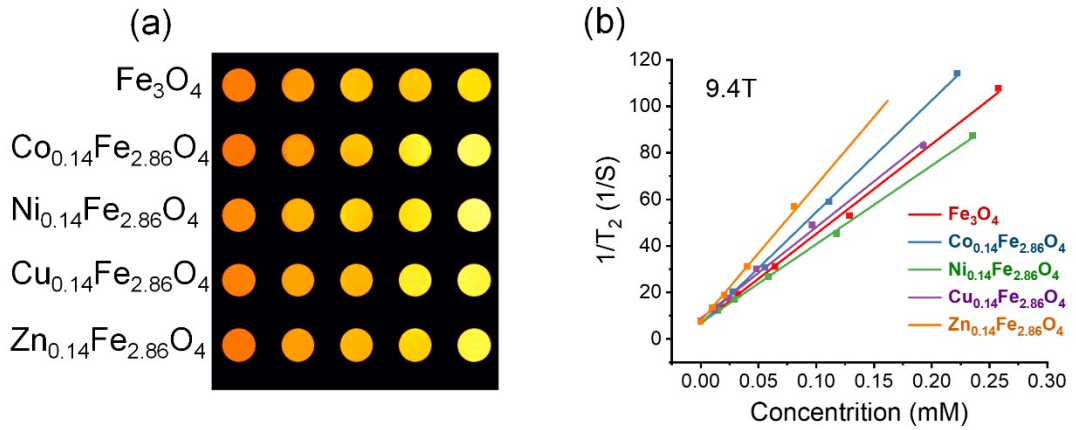

Fig. S9 (a) MRI images of different samples at 9.4T. (b) The transverse relaxation rate of samples through the ratio of 1/ T_2 to concentration.

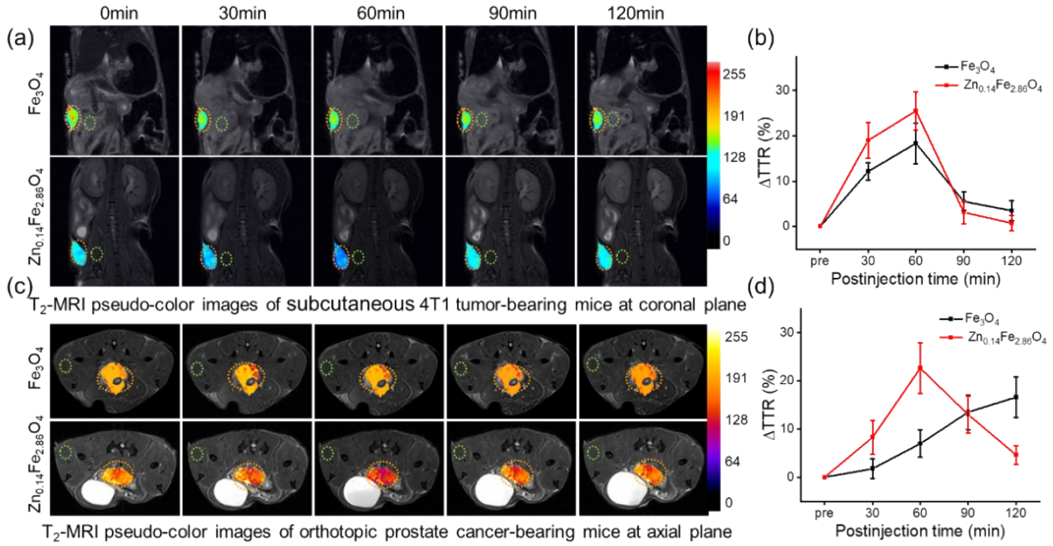

Fig. S10 In vivo MR imaging before and after injection of Fe_3O_4 and $Zn_{0.14}Fe_{2.86}O_4$. (a) T_2-weighted MRI pseudo-color images of 4T1 heterotopic tumor-bearing mice. (Tumor：orange dotted circle；tissue：green dotted circle). (b) Corresponding tumor MRI signal to tissue ratio changes (ΔTTR) from (a). (c) T_2-weighted MRI pseudo-color images of orthotopic prostate cancer-bearing mice. (d) Corresponding tumor MRI signal to tissue ratio changes (ΔTTR) from (c). (mean ± S.D., n = 3).

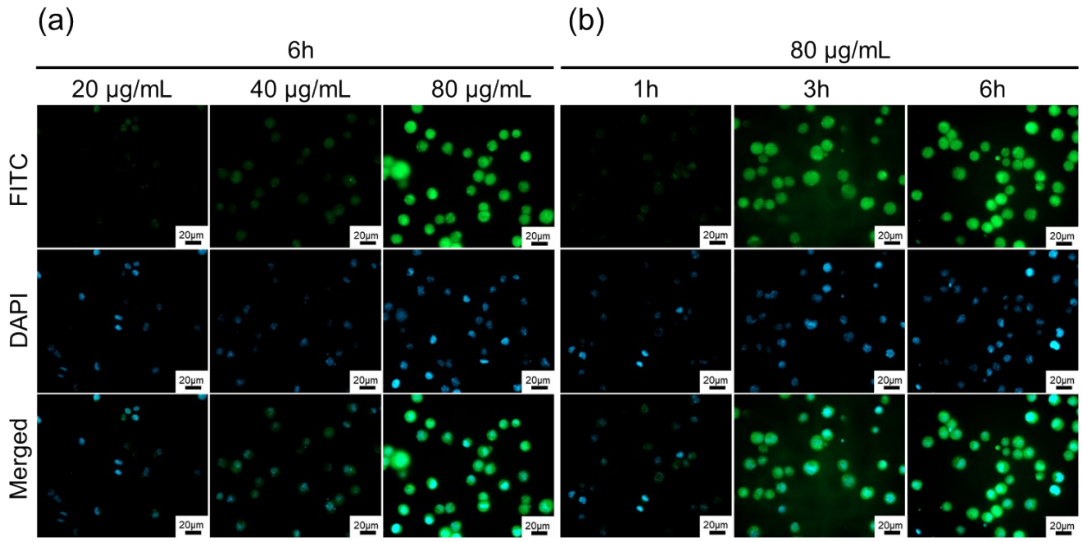

Fig. S11 The internalization of 4T1 cells at (a) different incubation concentrations and (b) different incubation time.

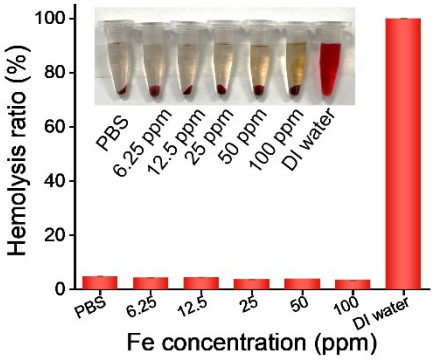

Fig. S12 The hemolysis assay of $Zn_{0.14}Fe_{2.86}O_4$ with different concentrations. Data were presented as mean ± s.d. (n = 3).

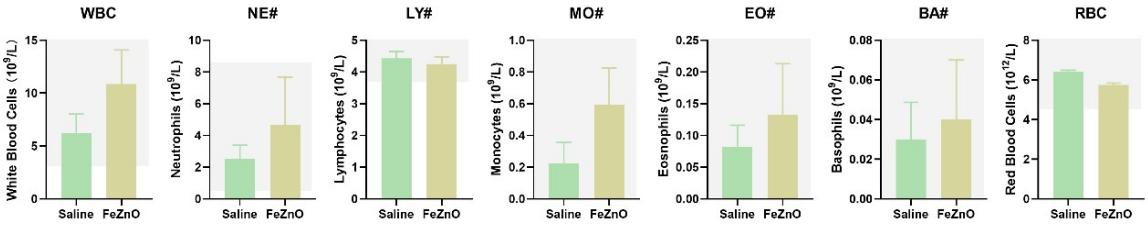

Fig. S13 The main blood routine examination including white blood cells (WBC), neutrophils (NE#), lymphocytes (LY#), monocytes (MO#), eosinophils (EO#), basophils (BA#), and red blood cells (RBC) assays of rats intravenously injected with $Zn_{0.14}Fe_{2.86}O_4$ for 48h.

Reference

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2. H. Xu, Y. Hao, R. Han, H. Weng, H. Du and B. Ye, *Acta Phys. Sin.*, 2011, **60**, 067803.