Supporting Information

Dual drug-loaded metal-phenolic networks for targeted magnetic resonance imaging and synergistic chemo-chemodynamic therapy of breast cancer

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Materials and methods

Materials

Iron (III) chloride ($FeCl_3$) was obtained from Adamas Reagent Co., Ltd. (Shanghai, China). Tannic acid (TA) was from J&K Scientific (Beijing, China). Atovaquone was from Macklin Biochemical Co., Ltd. (Shanghai, China). Cisplatin was from Beijing Bailingwei Technology Co., Ltd. (Beijing, China). EDC was from Macklin Biochemical Co., Ltd. (Shanghai, China). NHS was from SGMA-ALDRICH Co., Ltd. (St. Louis, MO). Hyaluronic acid (HA, Mw = 5830) was from Zhenjiang Dong Yuan Biotechnology Corporation (Zhenjiang, China). 3-aminophenylboronic acid (3-PBA·HCl) was obtained from Sigma-Aldrich (St. Louis, MO). A regenerated cellulose dialysis membrane with a molecular weight cut-off (MWCO) of 7000 was acquired from Fisher Scientific (Pittsburgh, PA).

Phosphate buffered saline (PBS) and Regenerated cellulose dialysis membranes were provided by Shanghai Yuanye Biotechnology Co, Ltd. (Shanghai, China). 4T1 cells (mouse breast cancer cells) and L929 cells (a mouse fibroblast cell line) were from the Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Dulbecco's modified eagle medium (DMEM) was from HyClone Lab., Inc. (Logan, UT). Roswell Park Memorial Institute (RPMI-1640) and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA). Penicillin-streptomycin and trypsin (0.25%) were purchased from Gino Biomedical Technology Co., Ltd. (Hangzhou, China). Cell counting kit-8 (CCK-8) was from 7sea Biotech. Co., Ltd. (Shanghai, China). Reactive oxygen species (ROS) assay kit, GSH/oxidized glutathione disulfide (GSSG) assay kit, Mitochondrial Membrane Potential Assay Kit with JC-1 annexin V-FITC/PI (fluorescein isothiocyanate/propidium iodide) apoptosis detection kit and cell cycle analysis kit was purchased from Beyotime Biotechnology (Shanghai, China). 4’,6-Diamidino-2-phenylindole (DAPI) was acquired from BestBio Biotechnology Co., Ltd. (Shanghai, China). 2, 7-Dichlorofluorescin diacetate (DCFH-DA) was from Shanghai Yeasen Biological Technology Co., Ltd. (Shanghai, China). C11-BODIPY fluorescent probe was from Shanghai Maokang Biotechnology Co., Ltd. (Shanghai, China). The pH fluorescent probe BCECF was from Biyun Tian Biotechnology Co., Ltd. (Shanghai, China). Water used in all experiments was purified using a PURIST UV Ultrapure Water System (RephiLe Bioscience, Ltd., Shanghai, China) with a resistivity higher than 18.2 MΩ·cm. All chemical reagents are commercially available and can be used without further purification unless otherwise stated.

Synthesis and characterization of nanoplatforms

TEM images were obtained by transmission electron microscopy (JEM 2100F, JEOL Ltd., Tokyo, Japan) operated at 200 kV. Zeta potential and dynamic light scattering (DLS) measurements were carried out using a Malvern Zetasizer Nano ZS system (model ZEN3600, Worcestershire, UK) equipped with a standard 633 nm laser. UV-vis spectrophotometer was carried out using a Lambda 25

UV-vis spectrophotometer (Perkin Elmer, Boston, MA). Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 6700 FTIR spectrophotometer (Thermo Electron Corporation, Madison, WI). Samples were mixed with milled KBr crystals and pressed to form 13-mm diameter disks before measurements. XPS spectra were measured by X-ray photoelectron spectroscopy (ESCALAB 250Xi). A thermogravimetric analyzer was used for thermogravimetric analysis (TGA) of the samples under a nitrogen atmosphere in a temperature range of 50-1000 °C. ^{1}H NMR measurements were performed using a Bruker DRX 500 NMR (400 MHz) spectrometer. All samples were dissolved in D_2O before measurements. The concentration of Fe in TFP@ATO-HA was analyzed by Leeman Prodigy inductively coupled plasma-optical emission spectroscopy (ICP-OES, Hudson, NH). The emission slit opening was set at 1.0 mm. T_1-MR relaxometry was performed using a 0.5-T NMI20-Analyst NMR analyzing and imaging system (Shanghai Niumag Corporation, Shanghai, China). The parameters were set as follows: IR sequence, resolution = 156 mm × 156 mm, section thickness = 0.8 mm, and excitation number of 1. The T_1 relaxation times of the as-prepared samples with different Fe(III) concentrations (0.05-1.0 mM) were measured. The r_1 relaxation rate was calculated by linearly fitting the inverse T_1 relaxation time ($1/T_1$) as a function of Fe concentration.

Preparation of TFP@ATO

ATO-loaded TA-Fe/Pt metal-phenolic networks (TFP@ATO) were synthesized according to the literature protocol.[1] In brief, ATO (0.9 mg/mL) was dissolved in DMSO. Then, TA (5.245 mg/mL) was dissolved in deionized water and mixed with the ATO solution at different molar ratios under stirring. Later, cisplatin (2 mg/mL) was added to the mixture under stirring for 2 h. Afterward, an aqueous solution of $FeCl_3$ (0.5 mg/mL) was added to the above mixture under stirring overnight. The unloaded ATO was then removed by centrifugation at 1000 rpm for 10 minutes at 25 °C. The remaining material was re-solubilized and centrifuged at 10000 rpm for 30 minutes at 25 °C to obtain the TFP@ATO network. The obtained TFP@ATO were stored at -20 °C for further use.

Synthesis of HA-PBA

EDC/NHS-coupled amide bond formation was applied to the synthesis of HA-PBA. EDC (57.5 mg) and NHS (34.5 mg) in DMSO (10 mL) were added to HA (96 mg) dispersed in DW (10 mL), and the pH was controlled to 5 with the addition of 1 N HCl. PBA (27.4 mg) in DMSO (5 mL) was put into that mixture, and it was blended by magnetic stirring for 1 day. Then, those samples as contained in the dialysis membrane (MWCO, 10 kDa) were stirred against DW for 2 days. The dialyzed solutions were freeze-dried using a benchtop lyophilizer to obtain the HA-PBA polymer conjugates.

Preparation of TFP@ATO-HA

The aqueous solution of HA-PBA and TFP@ATO solution were mixed at a molar ratio of HA: TA = 2: 1, and the reaction was stirred at room temperature for 24 h, and then centrifuged at 10000 rpm for 20 min to obtain the metal polyphenol network-coated cisplatin/drug TFP@ATO-HA.

Drug loading efficiency

A standard absorbance-concentration calibration curve of ATO was measured and plotted. The encapsulation efficiency of ATO were determined by collecting the free ATO remaining in the supernatant after centrifugation and measured its absorbance at 426 nm using a Lambda 25 UV-visible spectrophotometer (PerkinElmer, Waltham, MA). The Pt content in TFP@ATO was measured using ICP-OES. The drug Loading content (LC%) and Encapsulation efficiency (EE%) of ATO and cisplatin can be calculated according to the following formulas of (1) and (2), where M_t, M_0, and M_L stand for the masses of the encapsulated ATO/cisplatin, the drug-loaded nanoplatforms and the initial ATO/cisplatin, respectively.

$$LC\% = M_t / M_0 \times 100\% \quad (1)$$

$$EE\% = M_t / M_L \times 100\% \quad (2)$$

Characterization techniques

Transmission electron microscopy (TEM) imaging was performed using an FEI Tecnai F20 analytical electron microscopy (FEI, Hillsboro, OR) under an operating voltage of 180 kV to observe the size and morphology of TFP@ATO-HA. The sample dispersed in water (0.1 mg/mL, 5 μL) was dropped onto a carbon-coated copper grid and air-dried before measurements. UV-vis spectra were acquired using a Lambda 25 UV-vis spectrophotometer (Perkin Elmer, Waltham, MA) and all samples were dispersed in water (1 mg/mL) before measurements. Zeta potential and hydrodynamic size were tested using a Malvern Zeta sizer (Nano ZS model ZEN3600, Worcestershire, UK) coupled with a standard 633-nm laser. All samples were dispersed in water (1 mg/mL) before measurements. To study the stability of the TFP@ATO-HA nanoplatforms, the nanoplatforms were dispersed in water, phosphate-buffered saline (PBS), or cell culture medium (DMEM with 10% FBS) to test their hydrodynamic size for seven consecutive days. For DLS measurements, all measurements were performed at room temperature, and three parallel measurements were performed for each sample.

***In vitro* drugs and Fe/Pt responsive release**

In vitro drug release profiles of ATO, Fe, and Pt from TFP@ATO-HA were investigated at pH 6.5 and pH 7.4 buffer solutions. TFP@ATO-HA were dispersed in 1 mL corresponding buffer solution at the concentration of 1 mg/mL, and then sealed in a dialysis bag (cutoff Mw = 1000). The dialysis bag was immersed in 9 mL buffer solution (pH 6.5 or pH 7.4) and placed in the shaker at 37 °C for 48

h. Within a specified times interval (0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h), 1 mL sample solution was taken out from the buffer solution to measure the released amount of ATO (λ_{ATO} = 426 nm) by UV-vis spectrophotometer and to measure the released amount of Fe/Pt by ICP-OES. Then an equivalent amount of fresh buffer solution was added.

Evaluation of •OH generation

Methylene blue (MB) was introduced to detect the hydroxyl radical (•OH) generated by the prepared TFP@ATO-HA complexes according to the literature.[2] Briefly, the TFP@ATO-HA complexes ([Fe] = 25 μg/mL) were dispersed in water, well mixed with an H_2O_2 solution (final concentration = 0.1 mM), a GSH solution (final concentration = 2 mM) and an MB solution (final concentration = 10 μg/mL), and incubated at room temperature for 4 h. The •OH-induced MB degradation was quantified by UV-vis spectroscopy at 665 nm. For comparison, the blank MB solution, MB/GSH/H_2O_2 mixture solution, MB/GSH/H_2O_2/TF mixture solution, MB/GSH/H_2O_2/TF@ATO mixture solution, MB/GSH/H_2O_2/TFP mixture solution, and MB/GSH/H_2O_2/TFP@ATO mixture solution were also tested under the same conditions, the absorbance value of the mixture at 665 nm within 4 h was detected by UV-vis, and the influence of the presence of GSH on ROS generation rate of the nanoplatforms was studied.

***T_1*-Weighted MR relaxometry**

T_1-weighted MR relaxometry of the TFP@ATO-HA and TFP@ATO under different pHs (6.5 and 7.4) was performed on a 0.5 T NMR analyzing and imaging instrument (NMI20, Niumag, Shanghai, China) at room temperature. The samples with different Fe concentrations (0.05, 0.1, 0.2, 0.4, and 0.8 mM, respectively) were measured. The parameters were set as follows: IR sequence, point resolution of 156 mm × 156 mm, section thickness of 0.8 mm. The r_1 relaxivity was obtained through linear fitting of the inverse T_1 relaxation time as a function of Fe concentration. Additionally, the *T_1*-weighted MR images of the above samples were acquired using a clinical MR imaging system (Bruker Biospec 7 T micro-MR imaging system, Karlsruhe, Germany). The detailed parameters were set as follows: TR/TE = 1000.0 ms/6.0 ms, slice gap = 1.0 mm, and FOV = 65 × 85 mm.

Cell culture and cytotoxicity assays

4T1 cells were regularly cultured in DMEM medium containing 10% FBS and 1% penicillin-streptomycin and kept at 37 °C in a Thermo Scientific cell incubator (Waltham, MA) with 5% CO_2. fibrosarcoma cells L929 were cultured in 1640 medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin in a Thermo Scientific cell incubator at 37 °C and 5% CO_2.

The cytotoxicity of TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA were evaluated by CCK-8 assay. Typically, L929 cells were grown in a 96-well plate with a density of 1 × 10^4 cells per well. When the cells were completely adhered to, different Fe concentrations (0.5, 1, 2.5, 5, and 10 μg/mL) of TFP@ATO and TFP@ATO-HA medium solution were added into the plate (n = 6). After being incubated for 24 h, the medium was poured out and the cells were washed with PBS. Finally, the cells were incubated with 100 μL of serum-free medium containing 10 μL of CCK-8 solution at 37 °C for another 4 h in a dark environment. The absorption value at 450 nm was determined by a microplate reader.

To evaluate the inhibition effect of TFP@ATO-HA, 4T1 cells (1 × 10^4 cells/well) were cultured in a 96-well plate overnight. Next, 4T1 cells were incubated with fresh medium containing TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA at different Fe concentrations (0.5, 1, 2.5, 5, and 10 μg/mL) for 24 h. Finally, the absorbance values of different groups were determined by CCK-8 assay, and the cell survival rate was calculated. Furthermore, the combination index (CI) of TFP@ATO-HA towards 4T1 cells was calculated according to the equation of CI = [IC_{50} (ATO in the TFP@ATO)/IC_{50} (ATO)] + [IC_{50} (TF in the TFP@ATO)/IC_{50} (TF)] + [IC_{50} (Cisplatin in the TFP@ATO)/IC_{50} (Cisplatin)].

Cellular uptake

The cellular uptake of TFP@ATO-HA by 4T1 *in vitro* was quantitatively evaluated by measuring the Fe concentration in cells using ICP-OES. 4T1 cells were seeded into 12-well plates at a density of 1 × 10^5 cells per well and cultured overnight to lead cells to adherence. Then, the medium was replaced with fresh medium containing TFP@ATO, TFP@ATO-HA, and TFP@ATO-HA + pre HA at different Fe concentrations (0.5, 1, 2.5, 5, and 10 μg/mL) and cells were further incubated for 4 h. Afterwards, the medium was removed carefully and cells were washed 3 times with PBS, treated with trypsin, suspended in fresh medium. The remaining cells were collected by centrifugation (104 g, 5 min), and treated with 1.0 mL aqua regia solution for 24 h. Finally, the samples were diluted in PBS and the concentration of phagocytic segregants was by ICP-OES.

4T1 cells were seeded at a density of 1 × 10^5 per dish with 1.0 mL of medium in confocal dishes and cultured overnight. Then, the medium in each dish was replaced with fresh DMEM medium containing free TFP@ATO, TFP@ATO-HA, and TFP@ATO-HA + pre HA (TFP@ATO was labelled by Cy5.5). The cells were incubated for another 4 h, and cells were washed three times with PBS and fixed with glutaraldehyde (2.5%) for 15 min, stained with DAPI for 5 min at 37 ℃, and observed by laser scanning microscopy (CLSM).

Cell apoptosis assay

Flow cytometry was applied to analyze the cell apoptosis. The cells were seeded in 6-well plates for 24 h cultivation. Then, the culture medium was replaced by a fresh culture medium containing TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA (with an equivalent Fe concentration of 2.5 μg/mL). After incubating for 12 h, the medium was removed carefully and cells were washed 3 times with PBS, the cells were trypsinized and collected in the centrifuge tube. When redispersed Annexin V-FITC binding buffer, these cells were stained with Annexin V-FITC/PI at room temperature for 15 min, and the cells of each group were finally subjected to flow cytometry analysis.

GSH detection

The GSH and GSSG Assay Kit measured the intracellular GSH. 4T1 cells were seeded into a 6-well plate at a density of 2×10^5 cells per well and incubated for 24 h. Then, the culture medium was replaced by a fresh culture medium containing TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA (with an equivalent Fe concentration of 2.5 μg/mL). After incubating for 12 h, the cells were collected with centrifugation, and the supernatant was discarded. The cell precipitates were resuspended in protein remover (10 mg/30 μL), subjected to 3 cycles of freezing−thawing, and then centrifugated at 1000 g for 10 min at 4 °C. The supernatant was reserved for GSH and GSSG assay according to the manufacturer's protocol.

Intracellular ROS measurements

The total ROS produced by TFP@ATO-HA nanocomplexes inside 4T1 cells was measured by ROS-sensitive DCFH-DA probe through flow cytometry assay. For flow cytometry analysis, 4T1 cells were seeded in a 12-well tissue culture plate at a density of 1×10^5 cells per well with 1.0 mL medium and incubated overnight. Subsequently, the cells were similarly treated with free TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA ([Pt] = 19 μg/mL for all Cisplatin-related groups, and the concentration of TF corresponded to the Cisplatin-incorporated groups) for 6 h. After the cells in each well were treated with 1 mL DCFH-DA probe for an additional 15 min in the dark, the cells were trypsinized, centrifuged, and resuspended in 1 mL of PBS. PBS-treated cells were used as a control. For each sample, 1×10^4 cells were counted and each measurement was repeated 3 times.

Intracellular LPO measurements

The intracellular lipid peroxidation (LPO) level in 4T1 cells after being treated with different nanocomplexes was detected by the C11-BODIPY fluorescent probe. 4T1 cells were seeded in confocal dishes at a density of 1×10^5 cells per dish with 1.0 mL medium and incubated overnight.

Then, the culture medium in each dish was replaced by fresh DMEM medium containing free TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA ([Pt] = 19 μg/mL for all Cisplatin-related groups) and cells were cultured for additional 6 h. After that, the cells were washed with PBS for three times and treated with C11- BODIPY (1 mL, 50 μM, dispersed in serum-free DMEM medium) for another 20 min in the dark. Subsequently, the cells were washed with PBS for three times, fixed with glutaraldehyde (2.5%) for 15 min and stained with DAPI for 5 min at 37 ℃ before CLSM observation.

Western blot analysis

To perform Western blot analysis, the 4T1 cells were seeded in 6-well plates and incubated for 24 h. Next, the cells were subjected to different treatments: TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA (with an equivalent [Pt] concentration of 19 μg/mL). After incubation for 12 h, the treated cells were harvested and washed 3 times with PBS, and then 200 μL of lysis buffer containing phenyl-methane sulfonyl fluoride was added into each tube under an ice bath for 30 min. The lysates were analyzed via Western blotting to detect the GPX-4 (glutathione peroxidase 4), STAT3, p53, and caspase-3 protein expression according to the literature protocols.[3-5]

Mitochondrial membrane potential measurements

The changes in mitochondrial membrane potential (MMP) within 4T1 cells after different treatments were analyzed using an MMP assay kit with a JC-1 probe through CLSM observation. Briefly, 4T1 cells were regularly cultured in confocal dishes (2×10^5 cells per dish) and treated with TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA for 6h afterward, the cells were washed, incubated with a JC-1 probe for additional 20 min in the dark, washed with JC-1 staining buffer for three times, and added with 1 mL of the fresh medium before CLSM observation.

Intracellular and extracellular pH detection

For intracellular pH detection, 4T1 cells were cultured in a single dish overight and treated with TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA for 7 h. After incubation, the cells were washed with PBS, incubated with a pH fluorescent probe (BCECF AM, 5 μM in PBS) for 30 min, and observed using a CLSM.

Hemolysis assay

All animal experiments were performed following the protocols approved by the Animal Care and Use Committee of Donghua University and also in accordance with the policy of the National Ministry of Health. Hemolysis assay was performed to assess the hemocompatibility of the TFP@ATO-HA. Briefly, 1.5 mL of blood collected from the inner canthus vein plexus of mice was

diluted with 3.5 mL of normal saline (NS), and then the pure red blood cells (RBCs) were obtained via repeated centrifugation/redispersion processes (2000 rpm, 10 min, 3 times). The RBCs were then diluted with 5 mL of NS. Afterward, 100 μL of the obtained RBC suspension was mixed with 900 μL NS (negative control), water (positive control), and NS solutions of TFP@ATO-HA with different concentrations (0.5, 1, 2.5, 5, or 10 μg / mL), respectively. After 2 h incubation at 37 ℃, all the samples were centrifuged at 10000 rpm for 5 min. Then, the photos of the samples were taken and the absorbance of the obtained supernatant at 540 nm was measured via UV-vis spectrometry. The hemolysis rate is calculated according to the following formula:

Hemolysis rate (%) = $(D_t - D_{nc}) / (D_{pc} - D_{nc}) \times 100$ %

D_t is the light absorption value of the test sample at 540 nm, and D_{pc} and D_{nc} are the light absorption value of the positive control and the bright control at 540 mm, respectively.

Animal models

Female BALB/c nude mice (3-4 weeks, Shanghai SLAC Laboratory Animal Center, Shanghai, China) were xenografted with 4T1 tumors by inoculating 4T1 cells 2×10^6 cells, 100 μL cell suspension) subcutaneously into the right rear of the mice (the root of the thigh). When the tumor volume reached 100 mm^3 of mice, the tumor models were successfully constructed for the treatment with different reagents.

Bio-distribution *in vivo*

To investigate the biodistribution of the prepared nanocomplexes in tumor-bearing mice, each 4T1 tumor-bearing mouse was intravenously injected with TFP@ATO or TFP@ATO-HA at the same Fe concentration (5 μg, 0.2 mL PBS) *via* tail vein. At different time points post-injection, the mice were sacrificed, and the major organs (heart, liver, spleen, lung, and kidney) and tumors were extracted, weighed, cut into small pieces, and aqua regia digested for two days. After that, each sample was diluted with water to have a volume of 10 mL and analyzed by ICP-OES to quantify the Fe content in the organs and tumors.

***In vivo* MR imaging of tumors**

To establish a xenografted tumor model, female 4-6 weeks BALB/c mice (Shanghai Slac Laboratory Animal Center, Shanghai, China) were subcutaneously injected with 2×10^6 4T1 cells (in 0.1 mL of PBS for each mouse) in the right back leg. When the tumor volume reached about 200 mm, the 4T1-bearing mice were divided into two groups of TFP@ATO and TFP@ATO-HA (n = 3 in each group) for MR imaging. Each tumor-bearing mouse was intravenously injected with TFP@ATO and TFP@ATO-HA ([Fe] = 25 μg/mL, 100 μL) via *the* tail vein. Then, the mice were scanned at 2 h post-injection through a clinical MR imaging system (Bruker Biospec 7T micro-MR imaging system, Karlsruhe, Germany). The instrumental parameters were the same as described above for sample MR imaging

In vivo antitumor activity

4T1 tumor-bearing mice were randomly divided into 8 groups and intravenously injected with PBS, TF, ATO, Cisplatin, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA (100 μL in PBS, [Fe] = 2.5 mg/mL) were intravenously administrated to each mouse via tail vein every 2 days for 3 times. The tumor volumes and body weights were recorded every two days. The tumor volume was calculated according to a formula of $V = W^2 \times L/2$, where W and L represent the width and length of the tumor. The relative tumor volume was calculated based on the tumor volume on the first day. After 14 days, tumors and major organs were excised from euthanized mice, photographed and weighed, followed by hematoxylin and eosin (H&E) staining, and the tumor was also followed by GPX-4 and Ki67 staining. Furthermore, the vital organs were sectioned for H&E staining to observe the histological changes caused by different treatments. Standard TdT-mediated dUTP nick-end labeling (TUNEL) staining tests were performed according to our previous work to confirm the tumor cell apoptosis efficacy.

In vivo blood examinations

To confirm the biosafety of the designed treatment, healthy BALB/C mice (4-6 weeks old) were randomly divided into three groups (n = 3 in each group), including PBS, TF, TFP@ATO-HA (100 μL PBS, [Fe] = 2.5 mg/mL for all Fe-related groups) were intravenously administrated to each mouse via tail vein. The mice treated with PBS were also analyzed for comparison. At 7 days post-injection, the mice were sacrificed and the blood was collected and stabilized with heparin for blood routine and blood biochemical analysis. First, the blood cell counts were performed on an automated blood cell counter (BC-2800 Vet Analyzers, Mindray, Shenzhen, China) for blood routine analysis, including red blood cell (RBC), white blood cell (WBC), platelet (PLT), hemoglobin (HGB), hematocrit (HCT), and mean corpuscular hemoglobin concentration (MCHC). Then, the blood samples were centrifugated at 2000 rpm for 3 min to obtain the serum, and the serum biochemistry markers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA) and creatinine (CREA) were analyzed by Servicebio Technology Co., Ltd. (Wuhan, China).

Statistical analysis

All statistical results were presented as the mean ± standard deviation (SD). The differences between the two groups were calculated using an unpaired Student's t-test. $^*p < 0.05$ was considered as statistically significant, $^{**}p < 0.01$ was considered as moderately significant, $^{***}p < 0.001$ was considered as highly significant.

Table S1. The Fe and Pt mass fractions of TFP with the feed ratios

TA: $FeCl_3$: Cisplatin (mol ratio)	Fe (mass fraction)	Pt (mass fraction)
1: 1: 1	1.8%	5.5%
1: 1: 5	2.7%	15.8%
1: 1: 8	1.9%	16.4%

Table S2. The LC and EE of cisplatin at different feed ratios for TFP

TA: $FeCl_3$: Cisplatin (mol ratio)	Loading content (LC%)	Encapsulation efficiency (EE%)
1: 1: 1	8.5%	50.3%
1: 1: 5	24.3%	32.6%
1: 1: 8	25.2%	24.4%

Table S3. The LC and EE of ATO at different feed ratios for TFP@ATO

TA: $FeCl_3$: Cisplatin: ATO (mol ratio)	Loading content (LC%)	Encapsulation efficiency (EE%)
1: 1: 5: 0.1	0.7%	62.1%
1: 1: 5: 0.2	1.6%	70.0%
1: 1: 5: 0.4	2.0%	52.3%
1: 1: 5: 0.6	2.1%	28.6%

Table S4. The hydrodynamic size of TF, TF@ATO, TFP, TFP@ATO, and TFP@ATO-HA

Sample	Size (nm)	PDI
TF	146.12 ± 3.09	0.17 ± 0.01
TF@ATO	197.63 ± 4.15	0.23 ± 0.02
TFP	229.82 ± 3.25	0.14 ± 0.01

TFP@ATO	267.24 ± 2.35	0.16 ± 0.01
TFP@ATO-HA	361.34 ± 2.47	0.15 ± 0.01

Table S5. IC_{50} value and safety index of TF, ATO, Cisplatin, TF@ATO, TFP, TFP@ATO, and TFP@ATO-HA

Sample	IC_{50} (L929) (μg/mL)	IC_{50} (4T1) (μg/mL)	Safety index
TF	891.4	90.0	9.90
ATO	130.0	27.1	4.80
Cisplatin	248.7	50.4	4.93
TF@ATO	17.5	3.8	4.61
TFP	11.4	2.7	4.22
TFP@ATO	7.4	1.8	4.11
TFP@ATO-HA	4.3	1.3	3.31

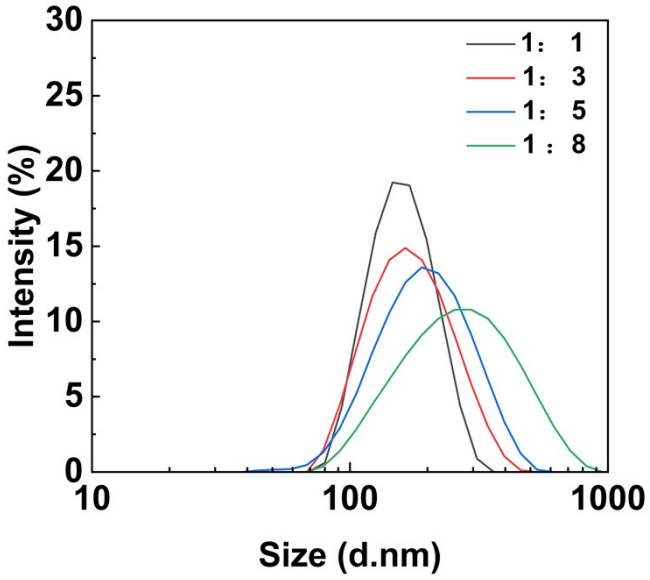

Figure S1 Hydrodynamic size distribution of TF with different molar ratios of TA: Fe.

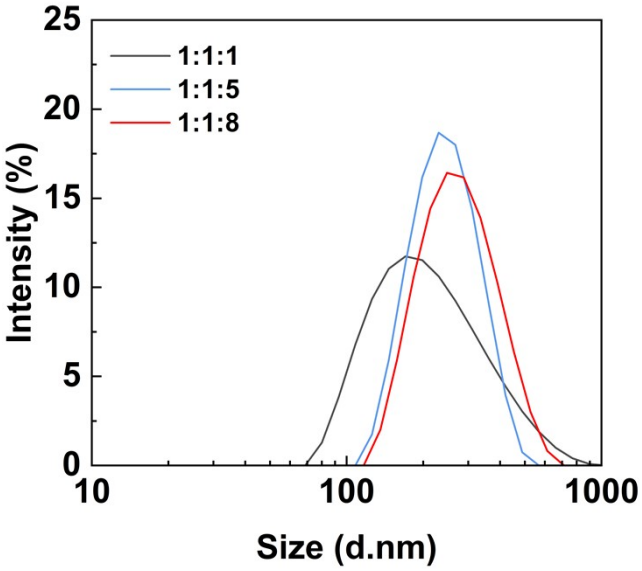

Figure S2. Hydrodynamic size distribution of TFP with different molar ratios of TA: Fe: Cisplatin.

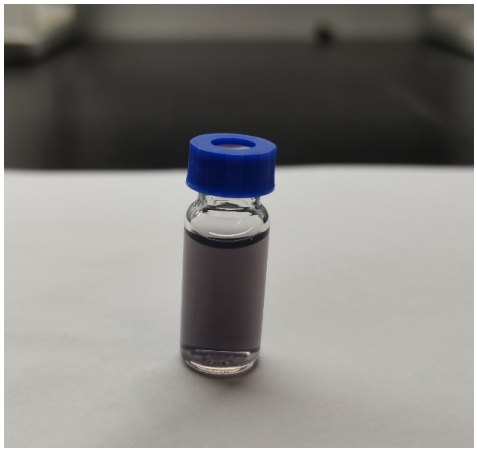

Figure S3. The image of TFP@ATO solution.

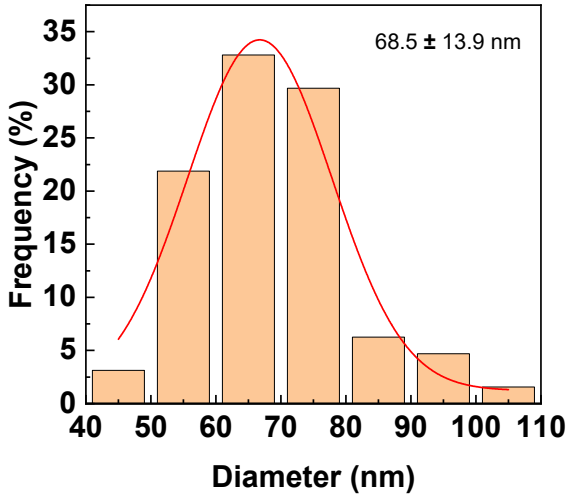

Figure S4. Size distribution histogram of TFP@ATO.

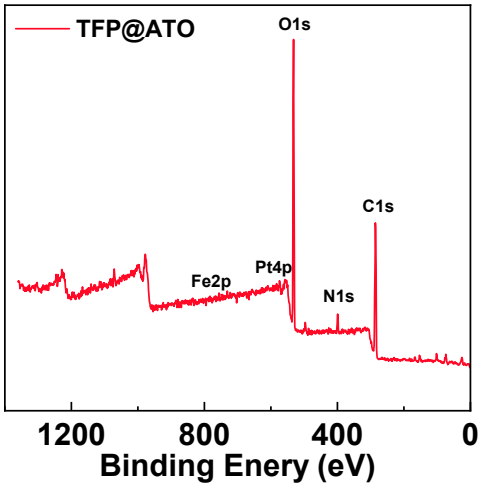

Figure S5. XPS spectra of TFP@ATO.

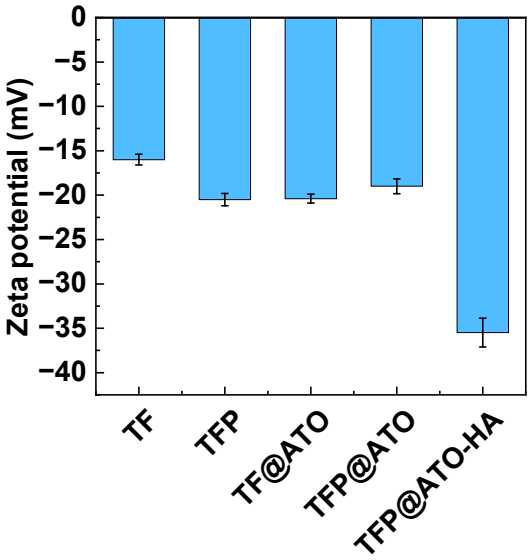

Figure S6. Zeta potential of TF, TFP, TF@ATO, TFP@ATO, and TFP@ATO-HA.

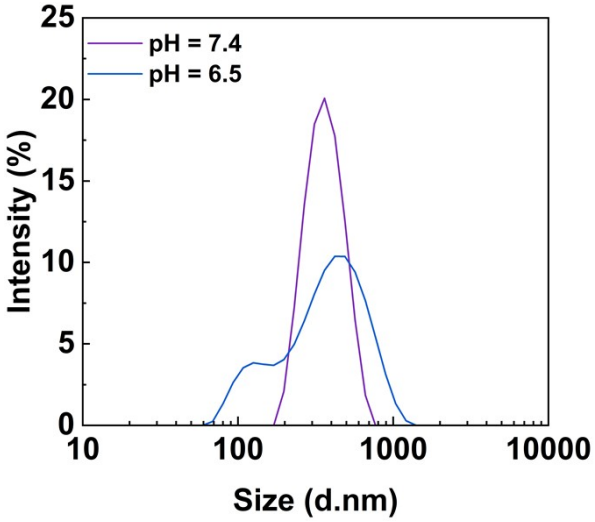

Figure S7. Hydrodynamic size distribution of TFP@ATO-HA with different pH levels.

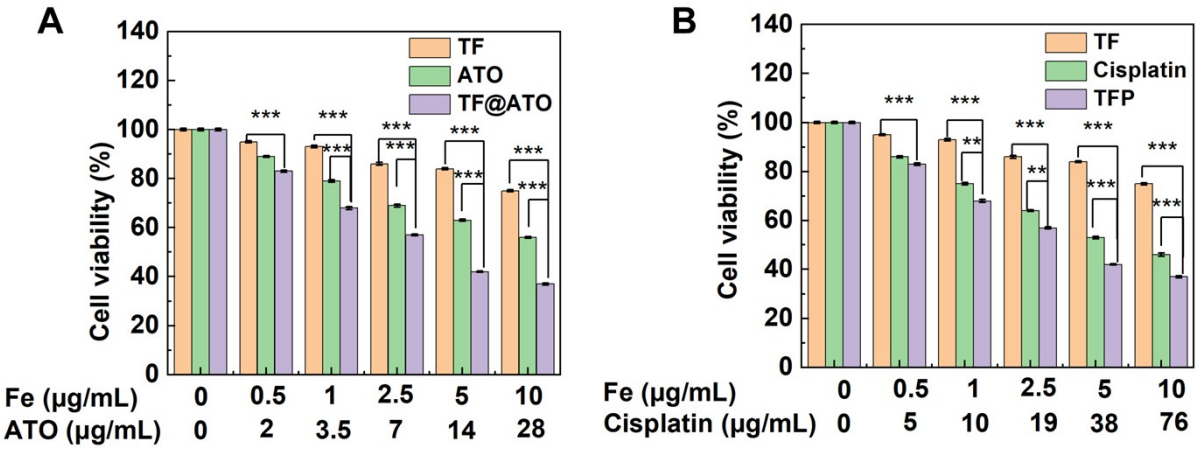

Figure S8. Cell viability of 4T1 cells after incubation with (A) TF, ATO, TF@ATO or (B) TF, cisplatin, and TFP for 24 h.

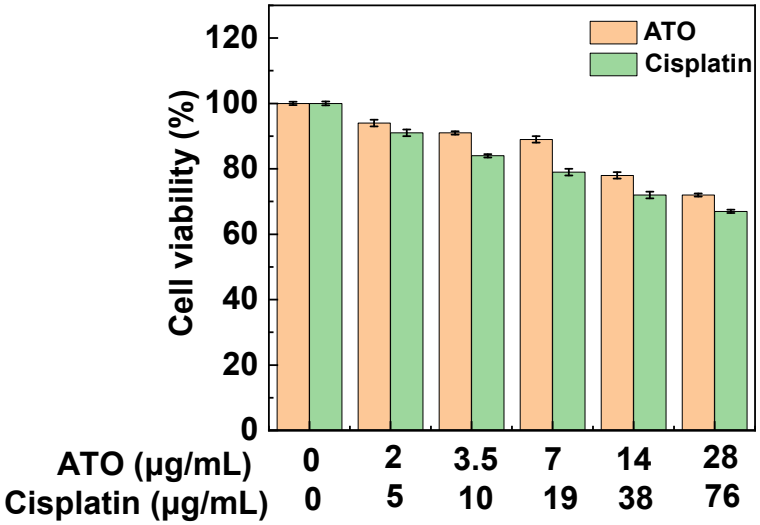

Figure S9. Cell viability of L929 cells after incubation with ATO or cisplatin.

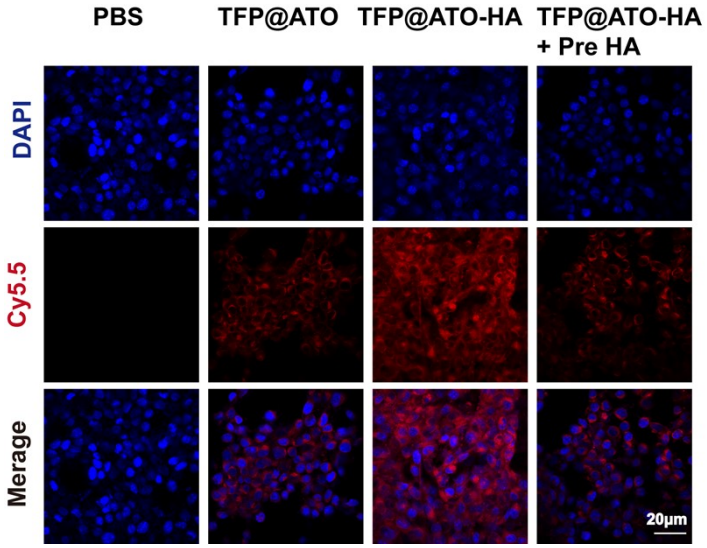

Figure S10. CLSM observation of cellular uptake in 4T1 cells after incubation with PBS, TFP@ATO, TFP@ATO-HA, or TFP@ATO-HA + pre HA.

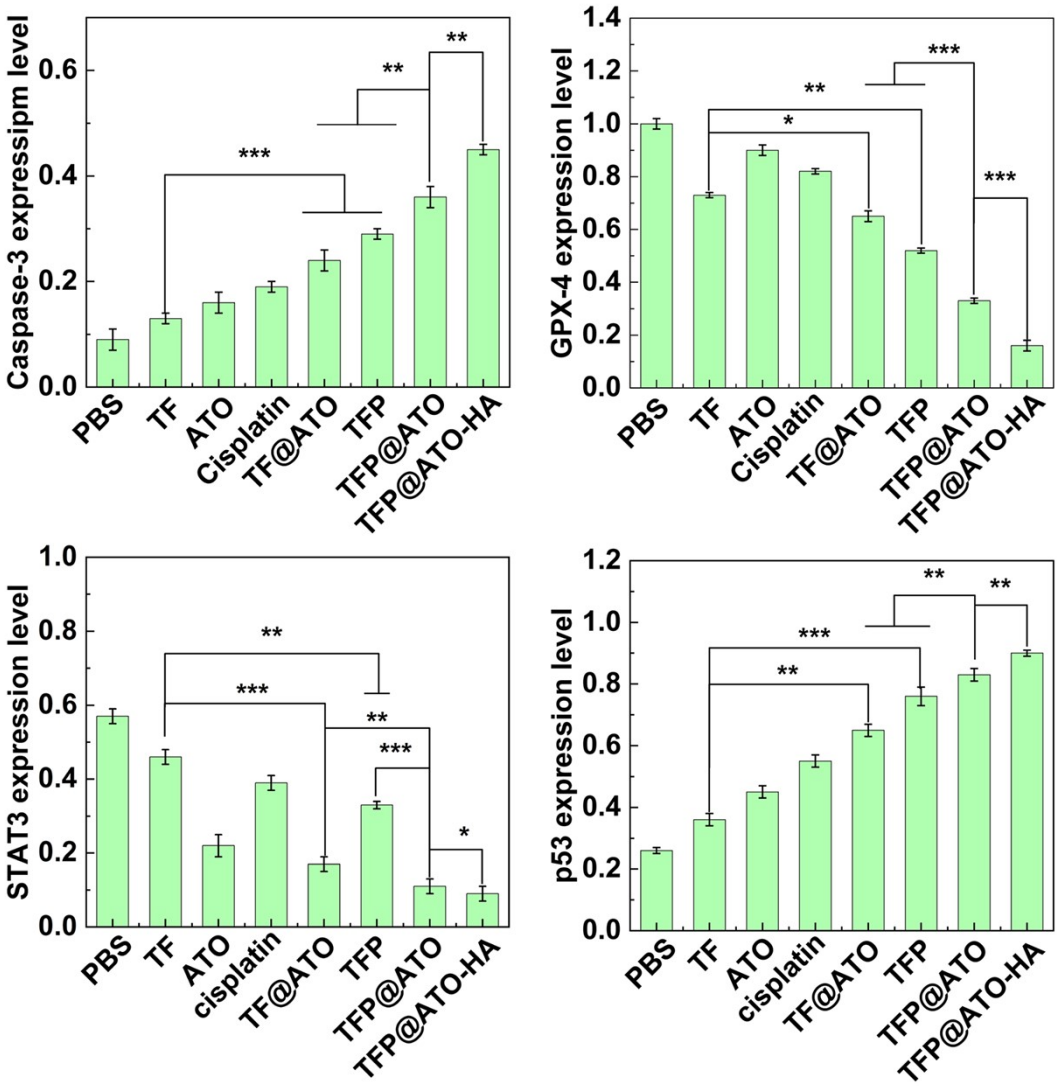

Figure S11. The quantification of the expression level of (A) caspase-3, (B) GPX-4, (C) STAT3, (D) p53 relative to actin (n = 3).

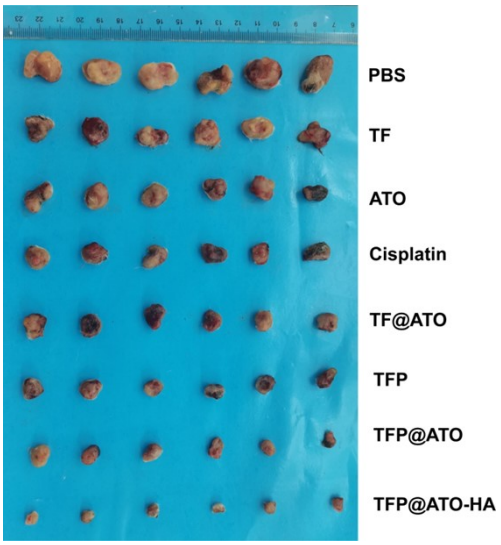

Figure S12. Representative tumor photographs in each group after the mice were treated for 14 days (n= 6).

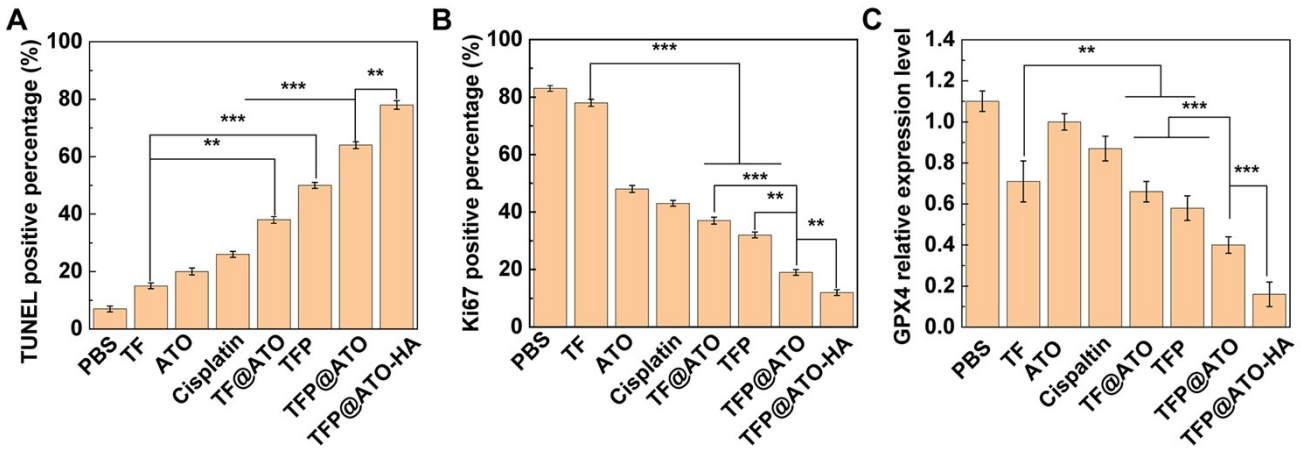

Figure S13. Quantitative analysis of (A) TUNEL-positive cells, (B) Ki67-positive cells, and (C) GPX4 expression level.

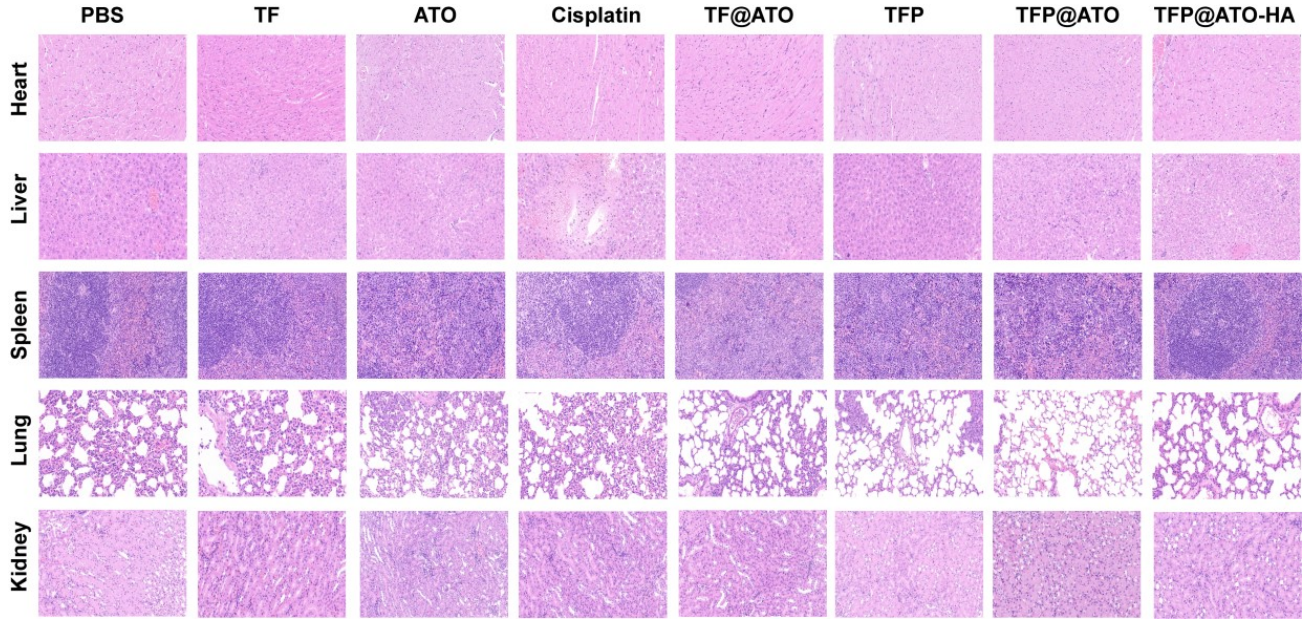

Figure S14. H&E staining of the healthy mouse organs at 14 days post intravenous injection of PBS, TF, ATO, Cisplatin, TF@ATO, TFP, TFP@ATO, and TFP@ATO-HA.

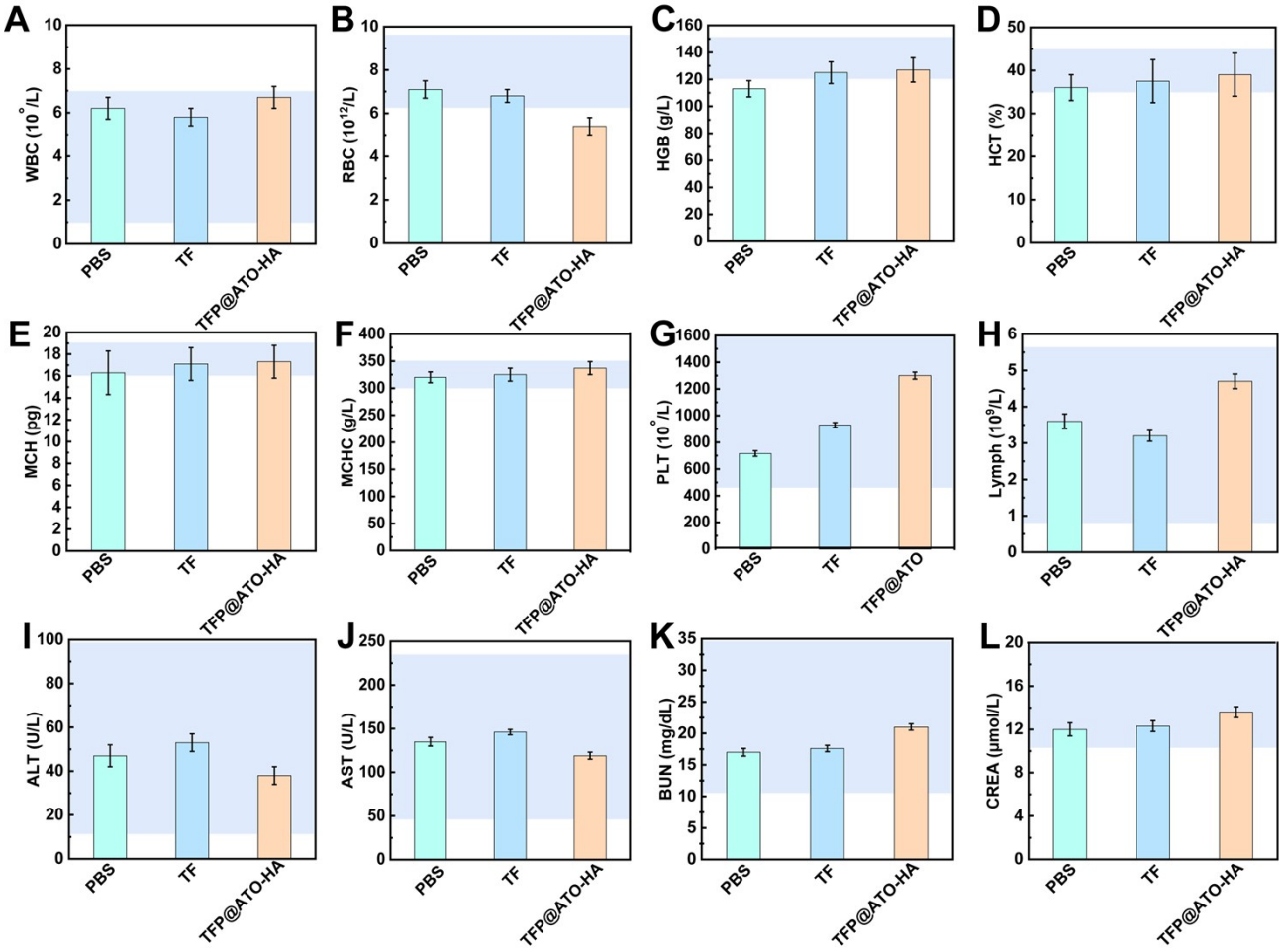

Figure S15. Blood biochemistry analysis of (A) WBC, (B) RBC, (C) HGB, (D) HCT, (E) MCH, (F) MCHC, (G) PLT, and (H) Lymph of mice on the last day of different treatments of PBS, TF, and TFP@ATO-HA. Liver function index of (I) ALT and (J) AST and kidney function index of (K) BUN, and (L) CREA of mice on the last day of different treatments of PBS, TF, and TFP@ATO-HA (n = 3).

References

1 Y. Xu, Y. Q. Guo, C. C. Zhang, M. S. Zhan, L. Jia, S. L. Song, C. J. Jiang, M. W. Shen and X. Y. Shi, *ACS Nano*, 2022, **16**, 984-996.

2 M. S. Taha, G. M. Cresswell, J. Park, W. Lee, T. L. Ratliff and Y. Yeo, *Nano Lett.*, 2019, **19**, 8333-8341.

3 J. Y. Huang, Y. C. Lin, H. M. Chen, J. T. Lin and S. H. Kao, *Pharmaceuticals*, 2022, **15**, 795.

4 Q. Liu and K. Z. Wang, *Cell Biol. Int.*, 2019, **43**, 1245-1256.

5 M. Xiang, H. Kim, V. T. Ho, S. R. Walker, M. Bar-Natan, M. Anahtar, S. H. Liu, P. A. Toniolo, Y. Kroll, N. Jones, Z. T. Giaccone, L. N. Heppler, D. Q. Ye, J. J. Marineau, D. Shaw, J. E. Bradner, T. Blonquist, D. Neuberg, C. Hetz, R. M. Stone, R. J. Soiffer and D. A. Frank, *Blood*, 2016, **128**, 1845-1853.