Electronic Supplementary Material (ESI) for RSC Advances.

A new pH/NIR responsive multifunctional theranostic agent based on polydopamine functionalized copper ferrite nanospheres for magnetic resonance imaging guided synergistic therapy

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Part A:Experimental Section

Materials: Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), polyvinylpyrrolidone (PVP 58000) and dopamine hydrochloride (98%) were purchased from Shanghai Aladdin Reagent Co. Sodium acetate (NaAc) was purchased from Acros Organics (USA). Copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), ethylene glycol and polyethylene oxide-polypropylene oxide-polyethylene oxide (PEO-PPO-PEO,P123), tris, tris (hydroxymethyl) aminomethane (Tris-HCl, pH = 8.8) were obtained from Beijing Chemical Reagents Company (Beijing, China). Doxorubicin Hydrochloride was purchased from Dalian Meilun Biotechnology Co., LTD.

Synthesis of CFNs: 0.5 g PVP was dissolved in 30 mL of ethylene glycol was added. After that, 2 mmol of $FeCl_3 \cdot 6H_2O$, 1 mmol of $CuCl_2 \cdot 2H_2O$, and 0.75 g of NaAc were added and vigorously stirred for 2 h. The mixture was added into an autoclave and kept in an oven at 200°C for 24 h. Then, the precipitates were obtained by centrifuging at 12 000 rpm for 20 min. Then, the products were washed three times by deionized water and stored at 4 ° C for further application.

Synthesis of PDA Modified CFNs: 32 mg P123 and 48 mg tris were added into 20 ml deionized water, then 16 mg obtained CFNs which was dispersed in 20 ml deionized water was dropped into 20 ml deionized water, containing 32 mg P123 and 48 mg tris , while vigorously stirring. After stirred 30 min, 20 ml deionized water containing 16 mg dopamine hydrochloride was added drop by drop. After being stirred at room temperature for 12 h, the precipitates were collected by centrifuging at 12 000 rpm for 10 min. Then the products were washed three times by deionized water.

Drug Loading and Releasing: DOX loading onto PDA@CFNs was performed by mixing DOX (10 mL, 1 mg mL^{-1}) with PDA@CFNs in Tris buffer (pH 8.8) (10 mL, 1 mg mL^{-1}) . After being stirred at 37 °C for 24 h in the dark, PDA@CFNs-DOX were separated by centrifugation, then gently washed three times with deionized water. All the washing supernatants were collected for measuring the DOX loading content by UV−vis measurement.

In order to investigate the drug release, PDA@CFNs-DOX was dispersed in 3 mL of different buffer solutions (pH 7.4 and pH 5) and sealed in a dialysis bag (molecular weight cutoff = 8000). The dialysis bag was submerged in 40 mL of respective buffer solutions and stirred at 37 °C for 24 h. The DOX released in the buffer was collected at selected time points and analyzed by UV-Vis spectroscopy. For NIR response controlled drug release experiments, samples in different buffer solutions were exposed to 808 nm NIR laser (1.5 W cm^{-2}) for 10 minutes at selected time points. The DOX released in the buffer was collected before and after the light. The DOX loading efficiency was calculated as follows: $IE\% = m_{loading} / m_{total} \times 100\% = (m_{total} - m_{supernatant}) / m_{total} \times 100\%$ ($m_{loading}$ is the drug loaded into the carrier, m_{total} is the total drug, and $m_{supernatant}$ is the drug in the supernatant). The maximum DOX loading efficiency of PDA@CFNs was calculated to be 64.58%.

Cytotoxicity Assay: CT26 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) culture medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C under 5% CO_2. The cells were seeded into 96-well plates for 24 h (37°C, 5% CO_2). Then, PDA@CFNs (0, 12.5, 25, 50, 100 and 200 μg mL^{-1}) were added into the CT26 cells for 24 h. Then, the CT26 cells were washed with PBS and incubated with CCK-8 solution (100 μL) for 2 h. After that, the plate

reader was used to record the absorbance at 450 nm and the cell viability could be calculated from the average value of six parallel wells.

Chemotherapy and PTT Treatments In Vitro: CT26 cells were seeded in 96-well plates for 24 h, and then incubated with various concentrations of PDA@CFNs, PDA@CFNs-DOX, and free DOX. After 4 h of incubation, the cells incubated with PDA@CFNs, PDA@CFNs-DOX were washed twice with PBS and irradiated using 808 nm NIR laser at a power density of 1.5 W cm^{-2} for 10 min. Then, the cells were incubated for another 24 h. Thereafter, the standard CCK-8 assays were carried out to determine the cell viabilities and proved by the live−dead cell staining technique through CLSM. The cells from group with NIR were washed twice with PBS and irradiated using 808 nm NIR laser at a power density of 1.5 W cm^{-2} for 10 min. Then, the cells were incubated for another 24 h. After that, the cells were carefully washed again with PBS for twice and incubated with Calcein AM (8×10-8 M in PBS) and PI (5×10-7 M in PBS) for 15 min. The cell viability was monitored by a CLSM.

Chemotherapy and PTT Treatments In Vivo: All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Jilin University and experiments were approved by the Animal Ethics Committee of the First Hospital of Jilin University. CT26 cells were implanted subcutaneously into the right armpit of 36 healthy female Balb/c mice with average weight of 20 g. CT26 tumor-bearing mice were randomly divided into six groups (n = 6, in each group): (a) saline (control group), (b) 808 nm laser (NIR group), (c) PDA@CFNs, (d) PDA@CFNs + 808 nm laser, (e) PDA@CFNs-DOX, (f) PDA@CFNs-DOX + 808 nm laser. For each group, PDA@CFNs and PDA@CFNs-DOX (10 mg kg^{-1}) were intravenously injected every 2 days. At 24 h postinjection, the tumors (groups b, d, e) were exposed to an 808 nm laser (1.5 W cm^{-2}) for 10 min. The body weight and

tumor size were measured by an electronic balance and digital calipers every 2 days. The tumor volume was obtained from the equation Volume = (Tumor Length) ×(Tumor Width)^2/2.

In vitro and in vivo MRI: We used 3.0 T clinical MRI instrument to obtain MR images. For in vitro MRI, we put PDA@CFNs solutions with different Fe concentrations (measured by ICP-AES) in a 1.5 mL test tube. The T_2 measurement was performed using nonlinear fitting to adjust the variation of the average signal intensity in each tube with the repetition time. Finally, the value of r_2 was determined by curve fitting of $1/T_2$ relaxation time (s^{-1}) to Fe concentration (mM).

To further study in vivo MRI, the tumor-bearing mice were intravenously injected with PDA@CFNs (2 mg mL^{-1}, 100 μL). T_2 -weighted MRI was obtained at di□erent times (0h, 1h, 2h, and 24 h) after intravenous injection.

Part B: Supplementary figures

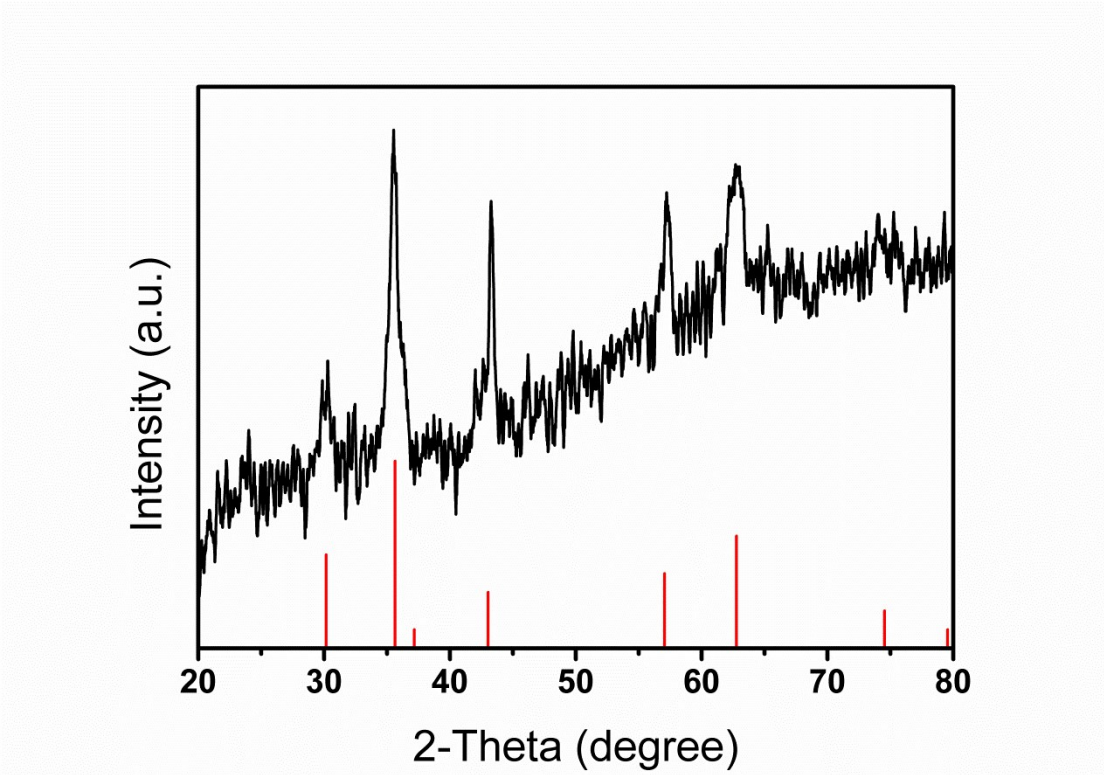

Figure S1. XRD of CFNs and JCPDS 250283

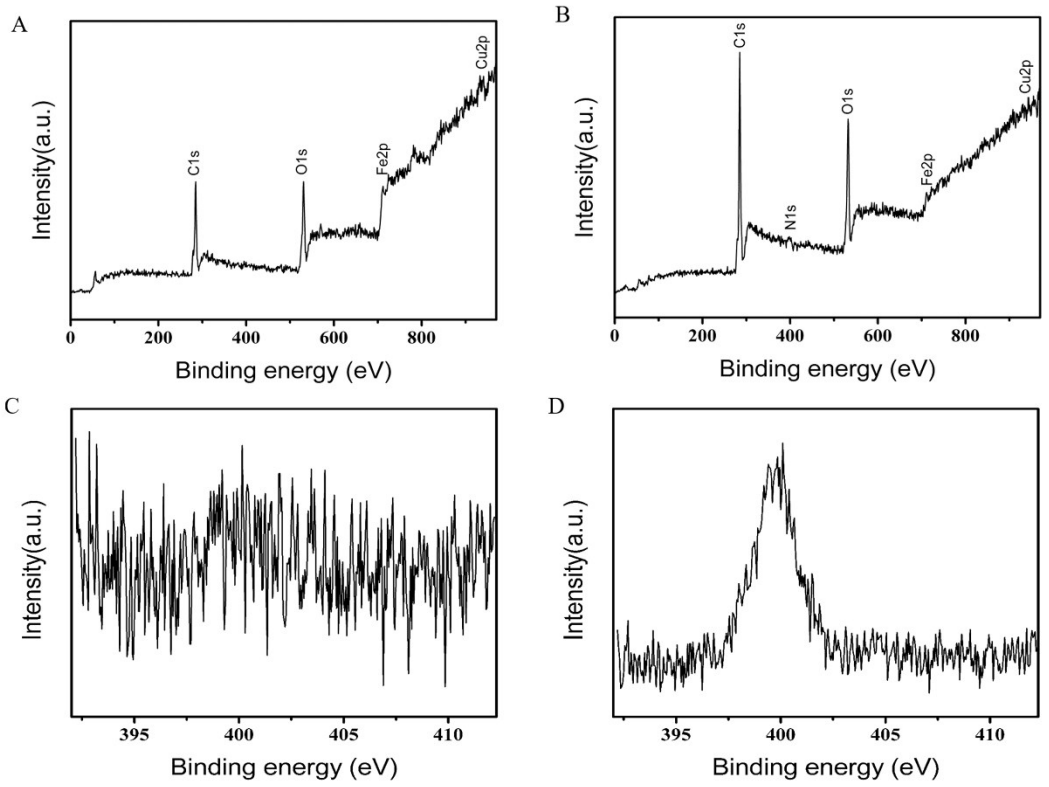

Figure S2. (A) XPS of CFNs, (B) XPS of PDA@CFNs, (C) N peak of CFNs, (D) N peak of PDA@CFNs.

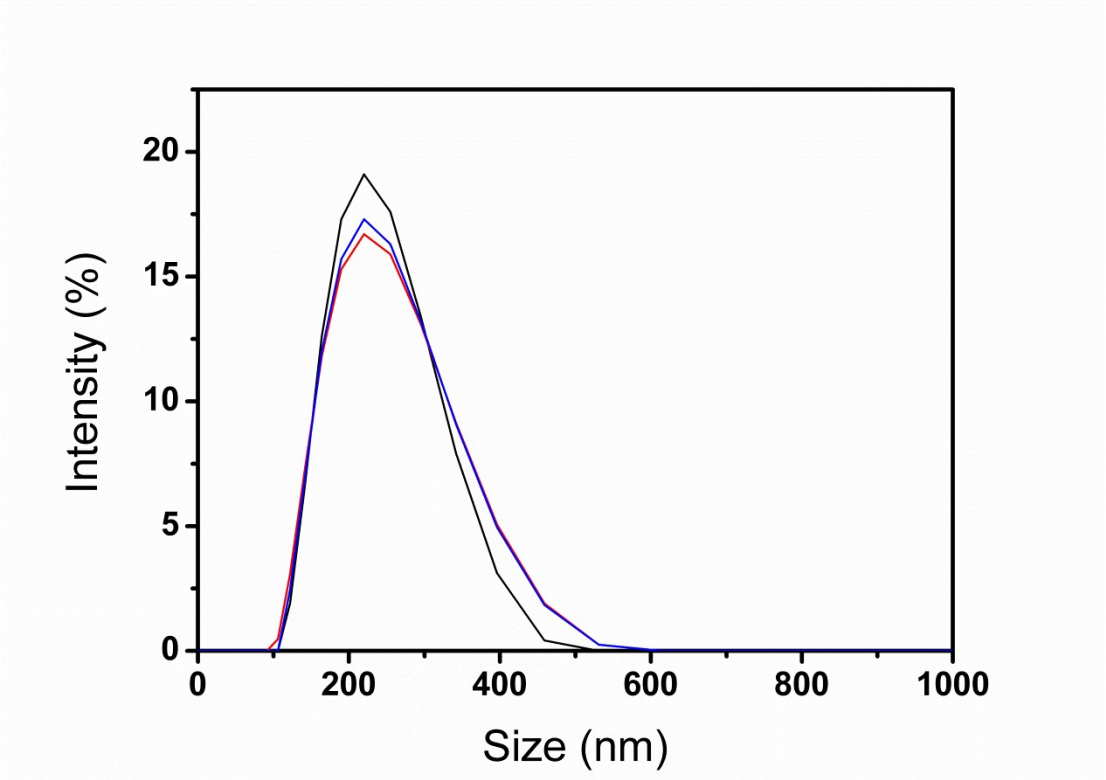

Figure S3. Hydrodynamic size of PDA@CFNs in deionized water.

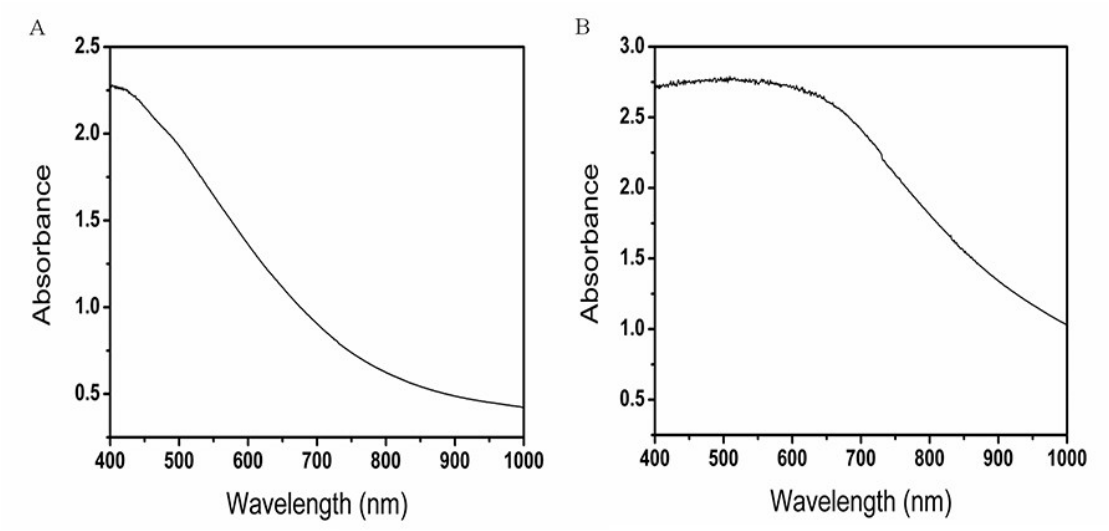

Figure S4. (A) UV-vis absorption spectra of CFNs. (B) UV-vis absorption spectra of PDA@CFNs.

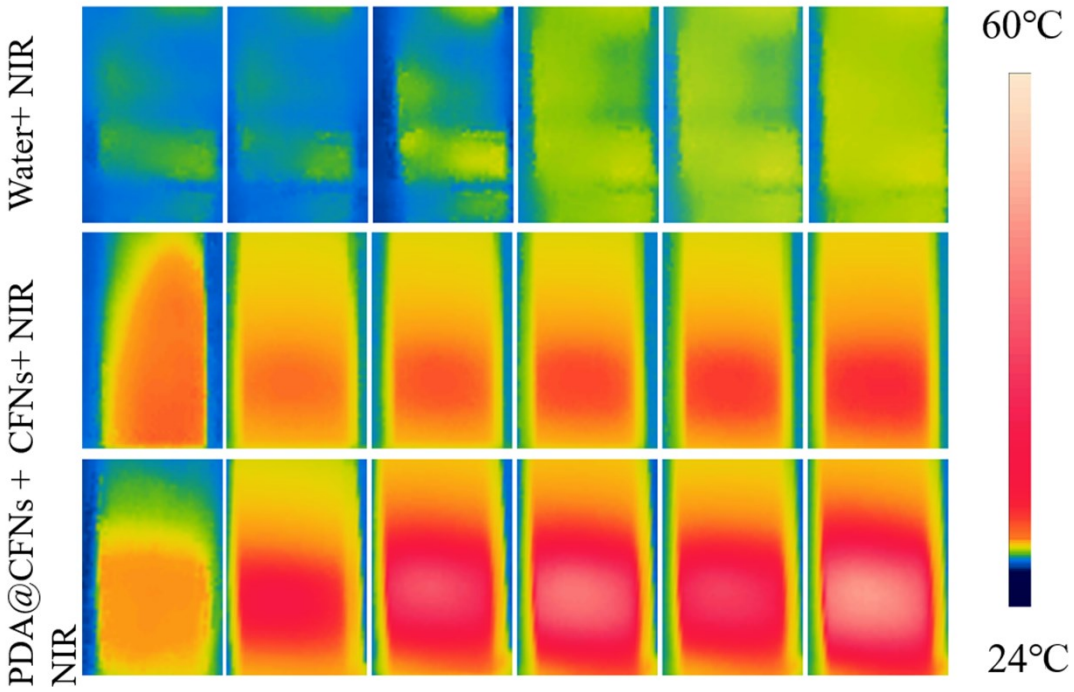

Figure S5. Infrared thermal images of water, 200 μg ml^{-1} CFNs and PDA@CFNs aqueous solutions irradiated with an 808 nm laser (1.5 W cm^{-2}) from 0 to 10 min.

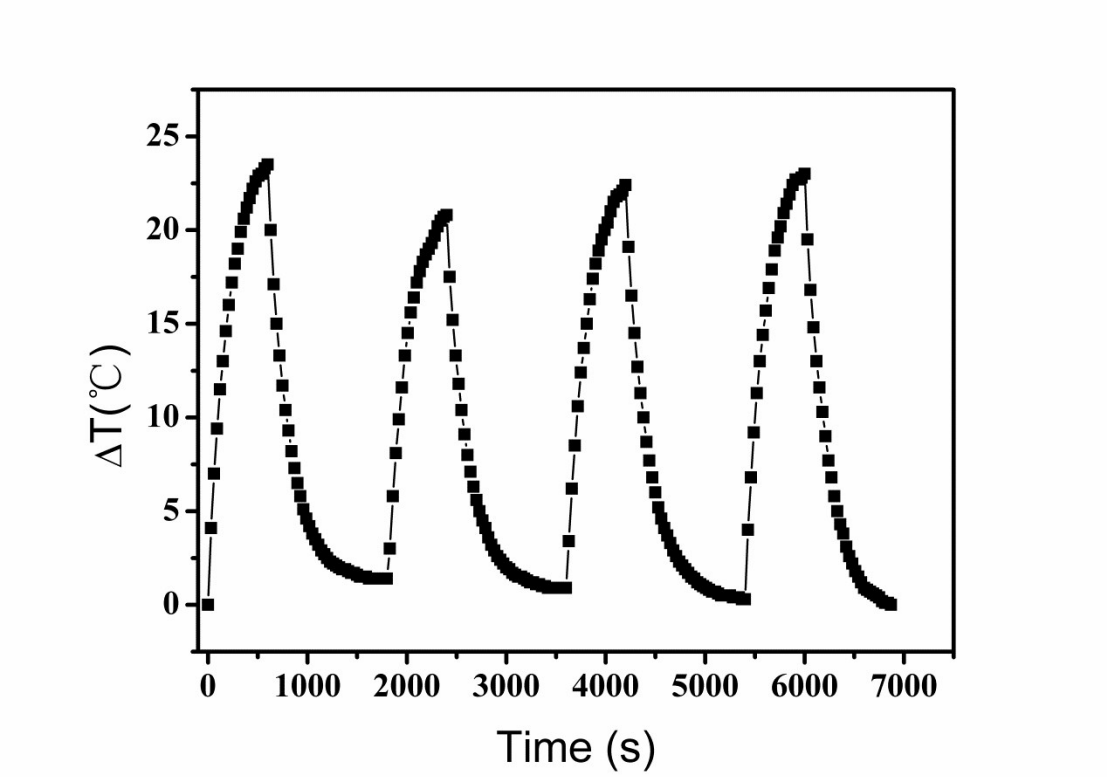

Figure S6. Temperature variations of PDA@CFNs solution (200 μg mL^{-1}). PDA@CFNs solutions were irradiated with 808 nm laser (1.3 W cm^{-2}) and nature cool down to the initial temperature, then repeated four times.

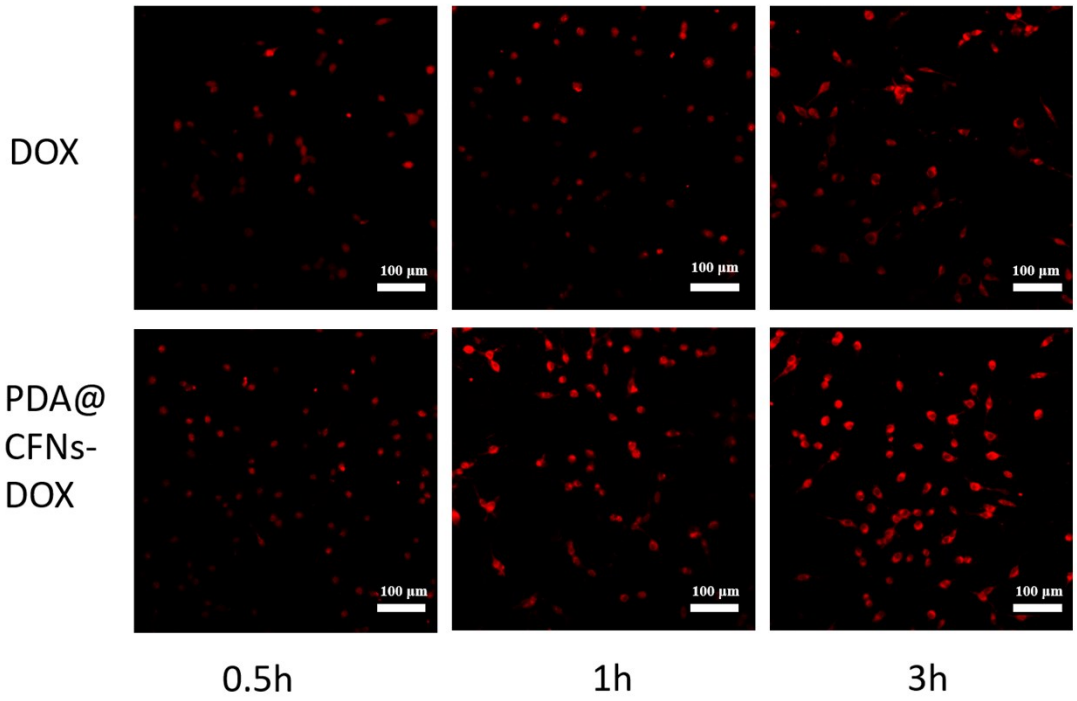

Figure S7. CLSM images of CT26 cells incubated with free DOX and PDA@CFNs-DOX for 0.5 h, 1 h, 3 h at 37 °C.

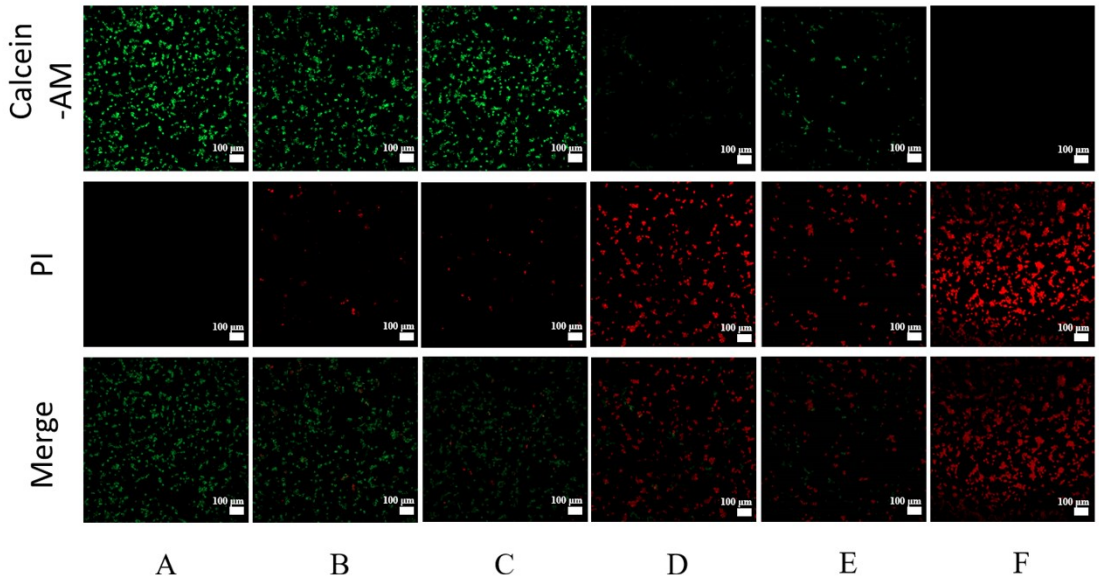

Figure S8. CLSM images of cells viability. A) Control; B) Control + NIR; C) PDA@CFNs; D) PDA@CFNs + NIR; E) PDA@CFNs-DOX; F) PDA@CFNs-DOX + NIR.

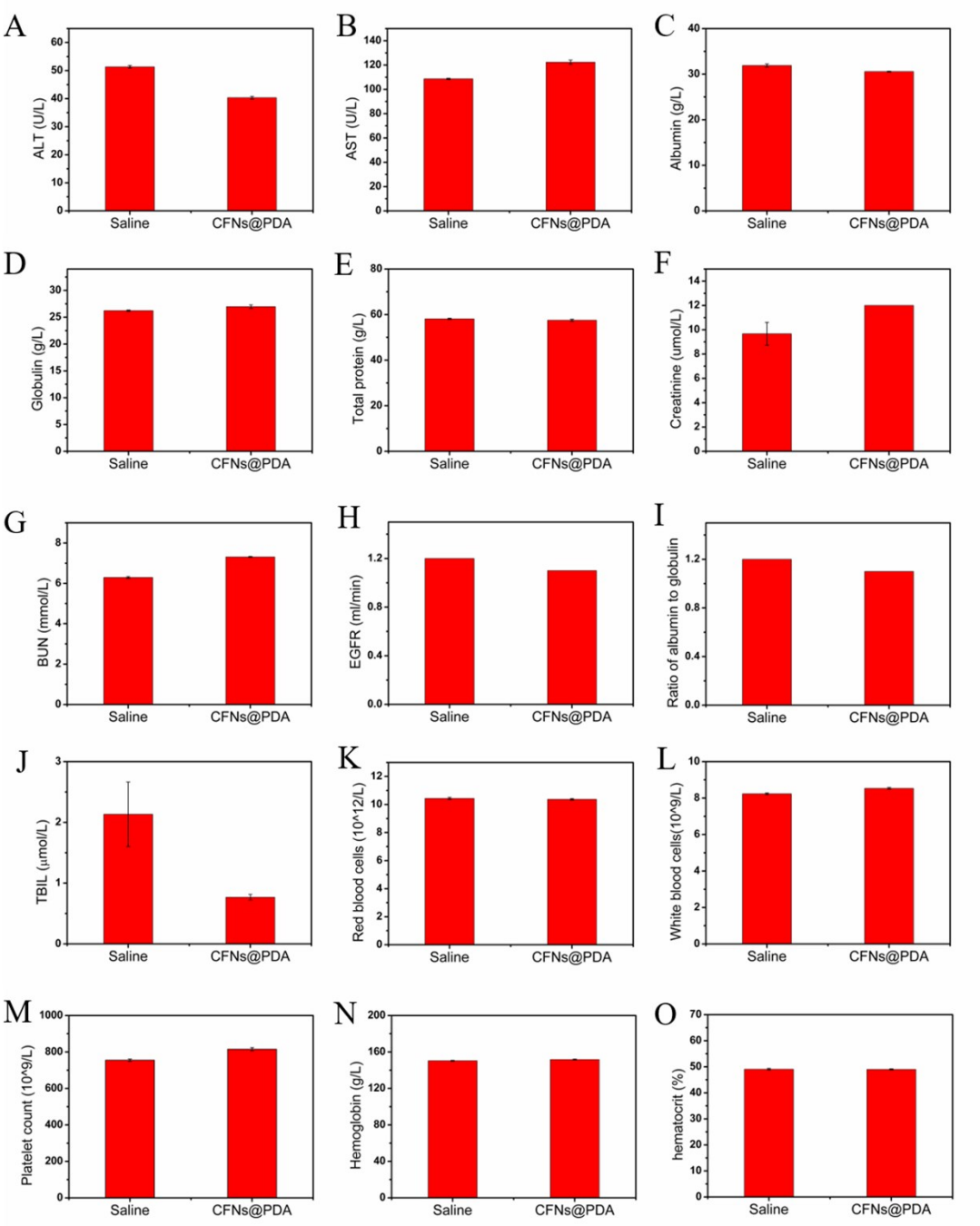

Figure S9. Blood routine and blood biochemical tests on mice 30 days after intravenously injected with saline as control or PDA@CFNs. A) ALT; B) AST; C) Albumin; D)Globulin; E)Total protein; F) Creatinine; G) BUN; H) EGFR; I) Ratio of albumin to globulin; J) TBIL; K) Red blood cells; L) White blood cells; M) Platelet count; N) Hemoglobin; O) hematocrit.

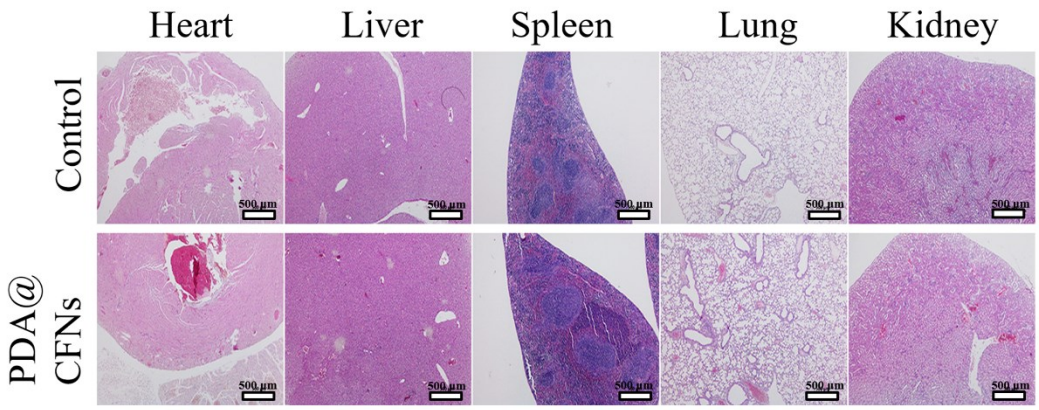

Figure S10. Histological changes of healthy mouse and the mouse after 30 days postinjection of PDA@CFNs, respectively.