Supplementary Information

Core-shell FePt-cube@covalent organic polymer nanocomposites: a multifunctional nanocatalytic agentia for primary and metastatic tumor treatment

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1. Experimental section

1.1. Materials

Ferric(Ⅲ) acetylacetonate ($Fe(acac)_3$, 98%) was purchased from Energy Chemical and Pt(Ⅱ) acetylacetonate ($Pt(acac)_2$, 98%) was purchased from Beijing HWRK Chem Co., Ltd. 1,2-Tetradecanediol(TDD), 1-Dodecanethiol(DDT) and Benzidine(BD) were purchased from Macklin Biochemical Co., Ltd. Hexadecyl trimethyl ammonium bromide (CTAB), 1-Octadecene, Oleic acid(OA), Oleylamine(OLA) 1,3,5-triformylphloroglucinol (Tp) and 3-(4-Hydroxyphenyl)propionic acid (DHCA) were all obtained from Aladdin Industrial Co., Ltd. Methylbenzene, n-Hexane, Tetrahydrofuran (THF) and ethanol were all obtained from Sinopharm Chemical Reagent Co., Ltd. HS-PEG-FA and NH_2-PEG-FITC were obtained from Sigma Aldrich Co., Ltd. Ferrostatin-1 (Fer-1) and Ac-DEVD-CHO (Apo) were acquired from MedChemExpress Co., Ltd. Imiquimod (R837, TLR7 ligand) was obtained by Sangon Biotech (Shanghai) Co., Ltd. All ELISA kits were acquired from Beyotime Biotechnology Co., Ltd.

1.2. Sample preparation

1.2.1. Synthesis and surface modification of FePt-cubes

The FePt-cubes were synthesized based on a solvothermal method firstly. In particular, 30 mL Octadecene was mixed with 23.5974 mg $Pt(acac)_2$, 105.95 mg $Fe(acac)_3$, 10.12 mg TDD and 218.6676 mg CTAB in a 100 mL three-neck flask containing a magnetic stir bar. The mixture was heated under a N_2 blanket, subsequently. OA (0.6 mL), OLA (1.3 mL) and DDT (20 μL) were introduced when the temperature raised up to 110 °C. The reaction mixture was then heated to 220 °C and kept for 10 min. Afterwards, methylbenzene (8 mL) was added immediately. Finally, cooling down to room temperature, the product was collected and wished with ethanol and n-hexane three times. In addition, substitution reaction of ligands was utilized to prepare a carboxyl-functionalized FePt-cubes. Firstly, the black dispersion of FePt (100mg) in THF (30 mL) was mingle with DHCA (200 mg) and shaken by constant temperature oscillator for 3 hours. Subsequently, the mixture solution was collected by centrifugation, rinsed with water, ethanol (1:2) and NaOH solution (0.6 mL, 0.5M). Finally, solid product was re-dispersed in ultrapure water for further use.

1.2.2. Preparation of FPCF NCs

Typically, FePt (50 mg) was dispersed in THF (11 mL). Under ultra-sonication, BD (2mg) was

introduced into above solution and gained a homogeneous dispersion solution. When the mixture solution was heated to 50 ℃ and kept for 30 min with rapid stirring, THF solution of Tp (1.5 mg, 4mL) was injected slowly in the above solution. At a temperature of 50 °C, the mixture solution was stirred overnight. Subsequently, the product was obtained by rotary evaporation to remove redundant THF. Lastly, solid product was re-dispersed in ultrapure water for further use.

1.2.3. Targeted modification of FPCF NCs

20 mg HS-PEG-FA was dispersed in a 10 mL flask containing FePt@COP NCs. The solution was blended by ultra-sonication for 5 minutes. Then, the above solution was shaken for 12 hours at room temperature away from light. Subsequently, the FPCF NCs was obtained by removeing redundant HS-PEG-FA. Finally, product was re-dispersed in ultrapure water to further utilize. 20 mg HS-PEG-FA and 30 mg NH2-PEG-FITC were dispersed in a 10 mL flask containing FePt@COP NCs. The solution was blended by ultra-sonication for 5 minutes. Then, the above solution was shaken for 12 hours at room temperature away from light. Subsequently, FePt@COP-FA-FITC NCs was obtained by removeing redundant HS-PEG-FA and NH2-PEG-FITC. Finally, product was re-dispersed in ultrapure water to further utilize.

1.3 In vitro cytotoxicity

1.3.1. Cell culture

All cell lines (L02, MCF-7 and 4T1) were kindly provided by Procell Life Science&Technology Co.,Ltd. All cells were cultured in DMEM culture medium complemented with 2% penicillin/streptomycin and 12% fetal bovine serum at 37℃ under 5% CO_2 atmosphere in wettish cell-incubator (Thermo 3111).

1.3.2. Cytotoxicity assay

Cell viability was evaluated by standard CCK-8 assay. First, L02, MCF-7 and 4T1 cells were cultured into 96-well plates respectively and incubated at 37℃ under 5% CO_2 atmosphere in wettish cell-incubator. After fresh culture medium containing different concentrations of FPCF NCs (0, 20, 40, 60, 80 $\mu g \cdot mL^{-1}$ of Fe) were introduced and further incubated with cells for 10 hours. Subsequently, the reagent of CCK-8 was added into cells. Next, the absorbance of materials was measured with a microplate reader at 450 nm. Similarly, cell viability was evaluated with same method as above. 4T1 cells were treated with FPCF NCs at IC_{50} (Fe of 40 $\mu g \cdot mL^{-1}$) by a single NIR irradiation (808 nm) with different light powers (0, 0.5, 1, 1.25, 1.5 W cm^{-2}). Moreover, 4T1 cells were incubated with FPCF

NCs (IC_{50}, Fe) for 10 hours. The VC (100 μM), VE (100 μM), Glu (5 mM), Cys (5 mM) and GSH (5 mM) were added severally and further incubated for another 30 min.. Similarly, the cell viability of 4T1 cells with Fer-1 and Apo reagent was analysed by CCK-8.

1.3.3. In vitro fluorescence imaging

The FPCF NCs was labeled with FITC defined as FePt@COP-FA-FITC NCs. 4T1 cells were seeded into a 6-well plate at 37℃ under 5% CO_2 atmosphere in wettish cell-incubator (Thermo 3111). When the cell reached nearly 80%, FePt@COP-FA-FITC NCs (IC_{50}, Fe) were cultured with cells for 6 hours. The images were recorded by a fluorescence microscope. DCFH-DA was used as a fluorescent ROS probe to detect ROS produced. 4T1 cells were seeded into a 6-well plate at 37℃ under 5% CO_2 atmosphere in wettish cell-incubator (Thermo 3111). When the cell reached nearly 80%, FPCF NCs (IC_{50}, Fe) were cultured with cells for 6 hours. Subsequently, the cells were stained with DCFH-DA (1 μL) for 30 min, and the ROS fluorescence images were obtained.

1.4. In vivo antitumor therapy

1.4.1. Animal model

6-week female Balb/c mice, with an average weight of 17-22 g, were obtained from Jinan Pengyue Expermental Animal Breeding Co., Ltd. All experiments involving animals were approved by the Principles of Laboratory Animal Care (People's Republic of China), Qilu Hospital of Shandong University Laboratory Animal Center (No. 201905032). All experiments involving mice were obtained from Cell Culture Center of Qilu Hospital of Shandong University. All reagents involving cell culture were purchased from Invitrogen. To establish animal tumor models, 4T1 cells (1×10^6) suspended in PBS buffer were subcutaneously injected into female Balb/c mice at the flank region of the right hind legs.

1.4.2. Living MR imaging

When the tumor volumes reached nearly 180 mm^3, MR image were carried out under a general anaesthetic. Tumor-bearing mice was injected intravenously (i.v.) with FPCF NCs (100 μL in PBS, [Fe] = 500 $mg \cdot mL^{-1}$) through tail vein. Then, MR images were acquired on a MRI instrument of Qilu Hospital of Shandong University.

1.4.3. Antitumor therapy

When the tumor volumes reached nearly 180 mm^3, 4T1 tumor-bearing mice were divided into 3 group (n=5) at random: (1) PBS; (2) FPCF NCs; (3) FPCF NCs+laser. All mice were treated via tail

intravenous injection with the above drug. And drug were injected once every five days for a total of three times. 1 h after drug injection, NIR irritation (808nm, 1.5W cm^{-2}) were performed with tumor tissues of group (3) for 10 minutes. The body weight and tumor size was recorded every two days and calculated by the following equation:

$$V= W^2 \times L \times 0.5 \quad (1)$$

where V was tumor volume , W and L are width and length, separately.

1.4.4. Histology staining

15 days later, each mouse was euthanized and their major organs were recovered and fixed with 4% paraformaldehyde for 24 h at 4℃. After the organs were embedded in paraffin and sectioned at 5 mm, staining procedure with standard hematoxylin and eosin (H&E).

1.5. Nanocatalytic/photothermal/checkpoint blockade combination therapy in vivo

1.5.1. Preparation of PLGA-R837

PLGA-R837 NPs were synthetized by O/W single-emulsion method.31 Firstly, R837 (0.95 mg) was dissolved in 0.38 mL DMSO, and PLGA (500 mg) was dissolved in 10 mL dichloromethane. After the above solution were mixed with 4 mL 5% w/v PVA solution for 10 min by Selecta Sonopuls. Then, 21 mL of a 5% w/v solution of PVA was added to the O/W emulsion. Subsequently, the mixture was stirred slowly to remove the organic solvent. Finally, PLGA-R837 NPs were synthetized after centrifugation at 8000r, 20 min.

1.5.2. Combined treatment with FPCF NCs for activating immune system

To establish orthotopic tumor models, 4T1 cells (1 × 106) were injected into female Balb/c mice at the flank region of the right hind legs. 20 tumor-bearing mice were randomly divided into 4 groups: (1) PBS; (2) FPCF NCs+laser; (3) R837-PLGA; (4) FPCF NCs+laser+R837-PLGA. The mice were treated via intratumoral injection of FPCF NCs (10 μL in PBS, [Fe] = 200 $mg \cdot mL^{-1}$) or PLGA-R837 (1.4 $mg \cdot mL^{-1}$) then irradiated by an 808 nm laserirradiation (1.5 W cm^{-2}) for 10 min. After 4 h, the tumors were collected, grinded and co-stained with CD11c/PI, and then the aggregation of DCs was analysed by flow cytometry. Mice were practised euthanasia 5 days after combined treatment, the groin lymph nodes were collected, grinded and stained with fluorescence-labelled antibodies for assessment using flow cytometry. Before the above mice were practised euthanasia, the mice serum were collected and isolated for analysing cytokines including TNF-α, IL-12p70 and IL-6 by ELISA kit.

1.5.3. Combined treatment with CTLA4 blockade therapeutics

4T1 cells (1×10^6) were subcutaneously injected into female Balb/c mice at the flank of the left hind legs to establish orthotopic tumor models. After 1 week, 4T1 cells (1×10^6) were injected into mice at the flank of the right hind legs as the metastatic tumor models. 20 tumor-bearing mice were divided into 4 group (n=5) randomly: (1) PBS; (2) FPCF NCs+laser; (3) PLGA-R837+anti-CTLA4; (4) FPCF NCs+laser+PLGA-R837+anti-CTLA4. The mice were treated via i.t. of FPCF NCs (10 μL in PBS, [Fe] = 200 mg·mL^{-1}) or PLGA-R837 (1.4mg·mL^{-1}) every five days and then PTT were performed with tumor regions for 10 minutes. Afterwards, the anti-CTLA4 (10 μg per mice) was injected by tail intravenous of mice. The growth of the bilateral tumors were recorded every two days. Mice were practised euthanasia and then dissected after 15 days of treatment, the tumors, spleen and groin lymph nodes were collected to research for cytokine testing and flow cytometry respectively.

2. Calculation of the photothermal conversion efficiency

To determine the photothermal performance of FPCF NCs, previous method was adopted here. The aqueous solution of FPCF NCs ([Fe] = 100 μg mL^{-1}) was irradiated using an 808 nm laser (1.5 W cm^{-2}) for 10 min. After reaching a stable temperature, then the above solution was cooled down naturally to room temperature (Fig. S9a†). And the temperature was recorded every 30 s by IR camera. When the system reaches a thermal equilibrium. The total energy balance for the system is:

$$m_s C_s \frac{dT}{dt} = Q_{NCs} + Q_s - Q_{out}$$

where ms and C_s are the mass and heat capacity of solvent (water), respectively. T is the solution temperature. Q_{NCs} is the heat generation from the FPCF NCs, and Q_s is the heat generation by solvent (water). Q_{out} is the thermal energy lost to the surroundings:

$$Q_{out} = hA\Delta T_{max}$$

where h is the heat transfer coefficient, A is the sample container surface area, $\Delta T_{max}=T_{max}-T_{sur}=23.6$ ℃.

At the maximum steady-state temperature, the heat input is equal to the heat output, that is:

$$Q_{NCs} = Q_{out} - Q_s$$

So, the photothermal conversion efficiency (η) of FPCF NCs was calculated by the following equation:

$$\eta = \frac{hA\Delta T_{max} - Q_s}{I(1 - 10^{-A_\lambda})}$$

where I = 1.5 W cm^{-2}, and A_{808} is the absorbance of aqueous solution of FPCF NCs at 808 nm, λ= 808 nm. The value of hA is determined according to the following equation:

$$hA = \frac{m_s C_s}{\tau_s}$$

where τ_s is the sample system time constant, it can be calculated according to the linear time from the cooling period (Fig. S9b†), and θ is defined as the ratio of ΔT to ΔT_{max}:

$$\theta = \frac{\Delta T}{\Delta T_{max}}$$

$$t = \tau_s(-ln\theta)$$

As can be seen from Fig. S9b†, τ_s is determined to be 104.4 s.

3. Supplemental figures

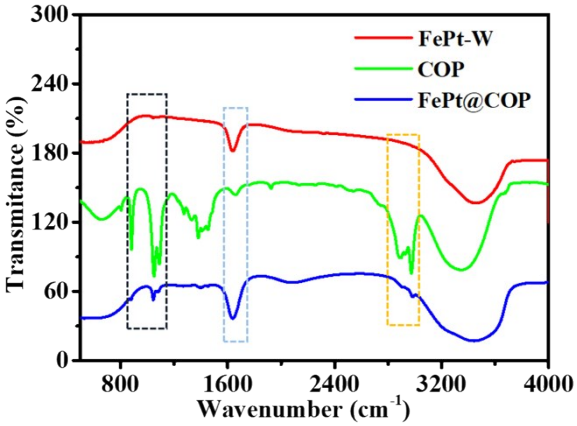

Figure S1. FT-IR spectra of the as-synthesized FePt-W nanocubes, COP and FePt@COP NCs.

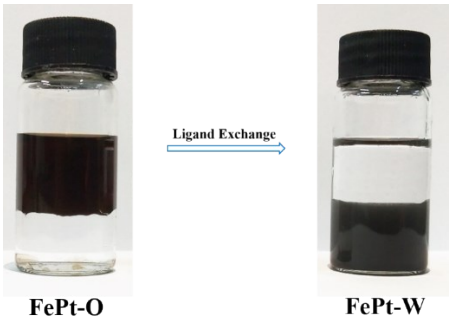

Figure S2. The photograph of hydrophobic FePt nanocubes (FePt-O) transformed into hydrophilic FePt nanocubes (FePt-W) with DCFH (the upper was n-hexane, the lower was the water).

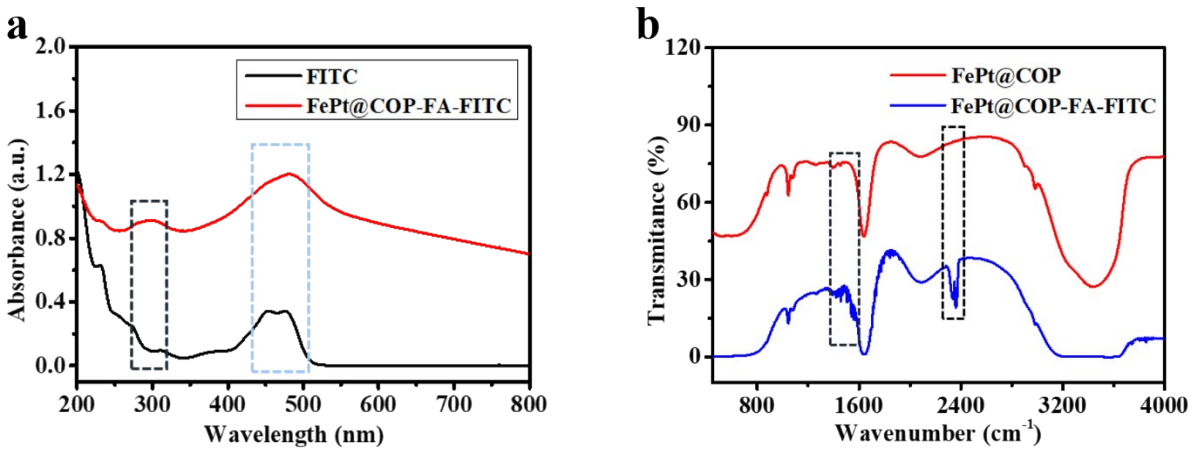

Figure S3. (a) UV-Vis spectra of the as-synthesized FePt@COP-FA-FITC NCs and free NH_2-PEG-FITC. (b) FT-IR of the as-synthesized FePt@COP NCs, FePt@COP-FA-FITC NCs.

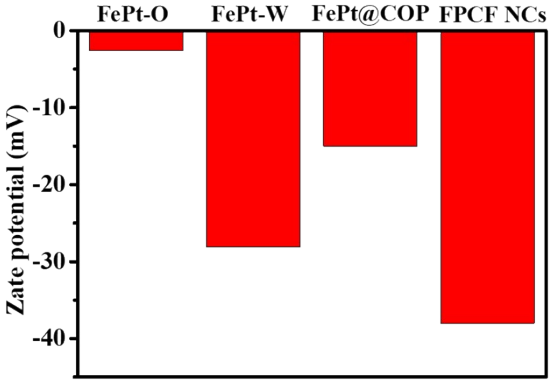

Figure S4. The zeta potential of FePt-O nanocubes, FePt-W nanocubes, FePt@COP NCs and FPCF NCs in aqueous solution.

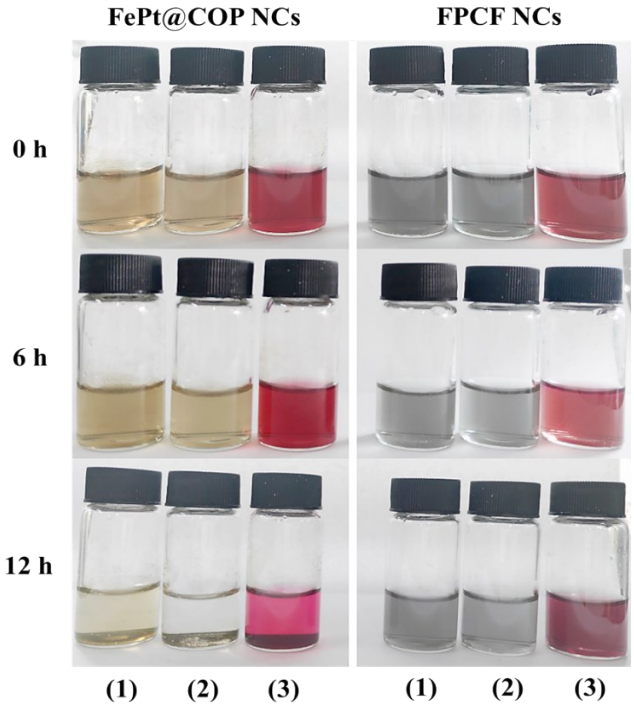

Figure S5. Stability characterization of the FePt@COP NCs or FPCF NCs dispersed in H_2O (1), PBS (2), and DMEM medium (3), respectively.

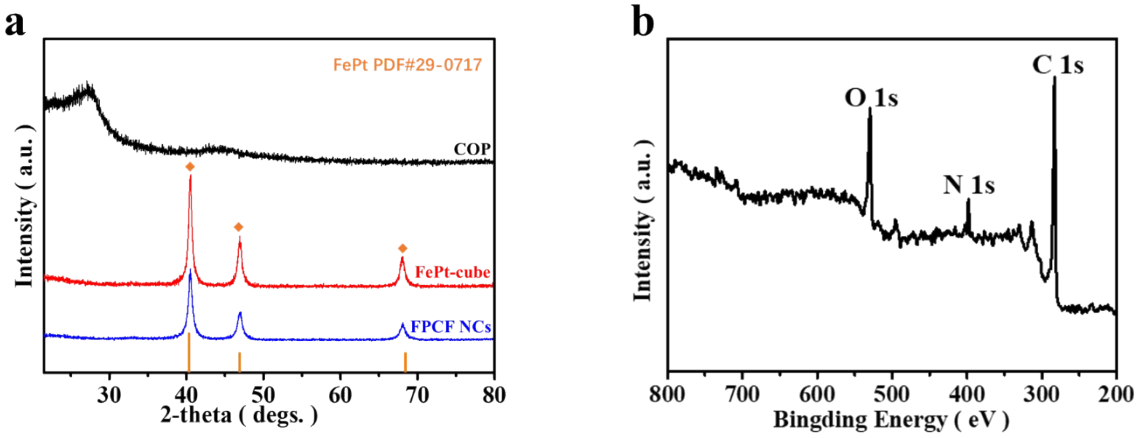

Figure S6. (a) XRD pattern of FePt-cubes, COP and FPCF NCs. (b) XPS survey spectrum of FPCF NCs.

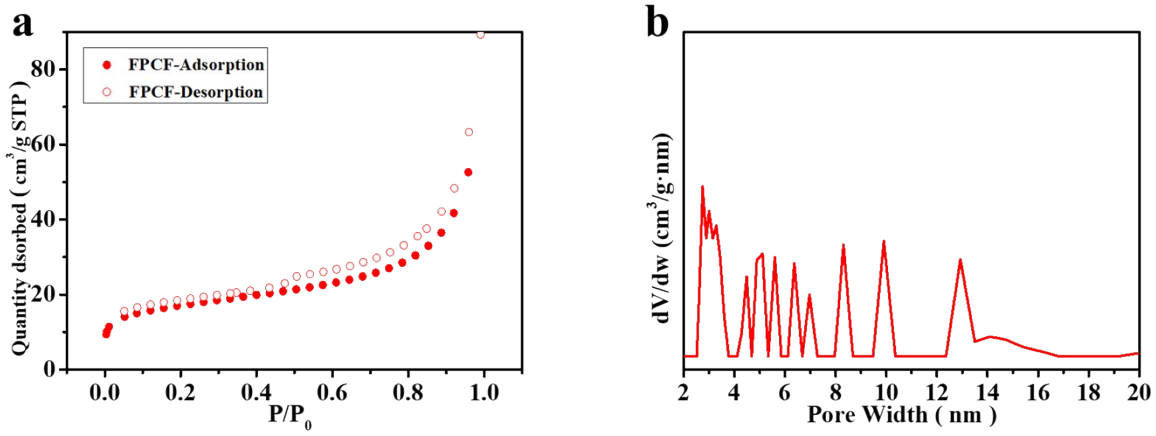

Figure S7. (a) N_2 adsorption-desorption isotherms. (b) pore size distributions.

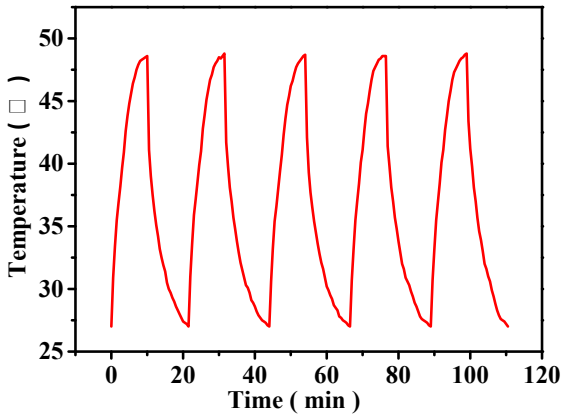

Figure S8. Temperature change of FPCF NCs aqueous solution ([Fe]=100 μg mL^{-1}) irradiated with an 808 nm laser (1.5 W cm^{-2}) over 5 irradiation cycles.

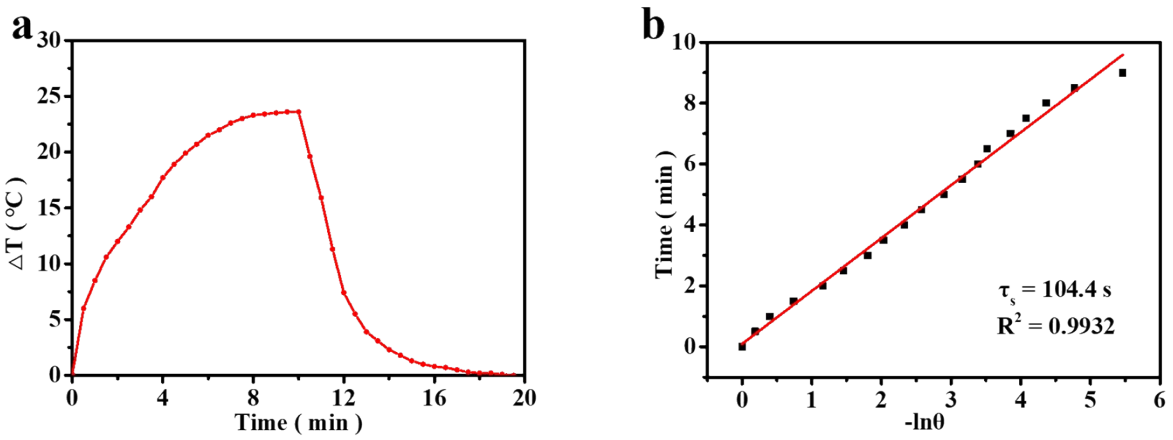

Figure S9. (a) Temperature change of FPCF NCs aqueous solution ([Fe]=100 μg mL^{-1}) irradiated with an 808 nm laser (1.5 W cm^{-2}) for 10 min. (b) Linear fit of time/-ln(θ) obtained during the cooling process.

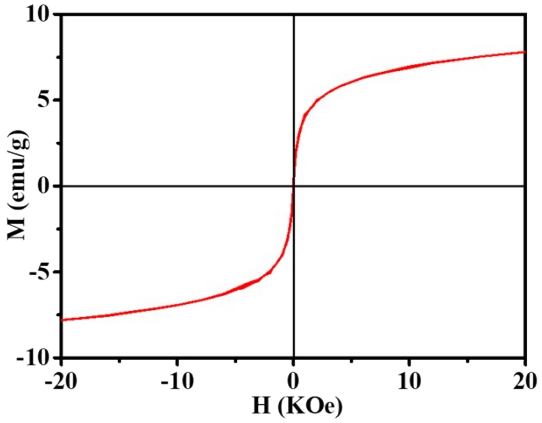

Figure S10. The magnetic hystersis loop of FPCF NCs at room temperature (298K).

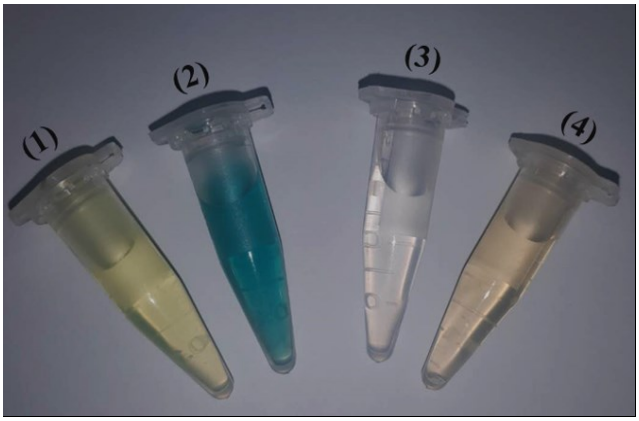

Figure S11. Photo of potassium ferricyanide dispersed in (1) water or (2) FPCF NCs solution at pH 5.8 for 24 h; photo of potassium thiocyanate dispersed in (3) water or (4) FPCF NCs solution at pH 5.8 for 24 h.

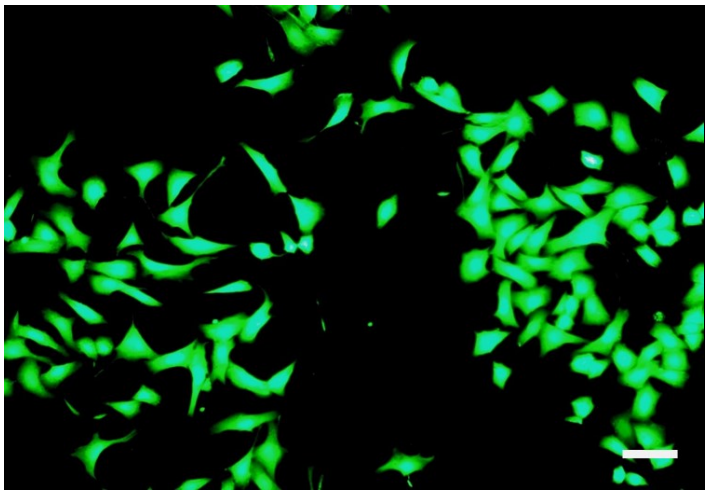

Figure S12. Fluorescence images of 4T1 cells co-incubated with FITC labeled FPCF NCs. (scale bars: 20 μm)

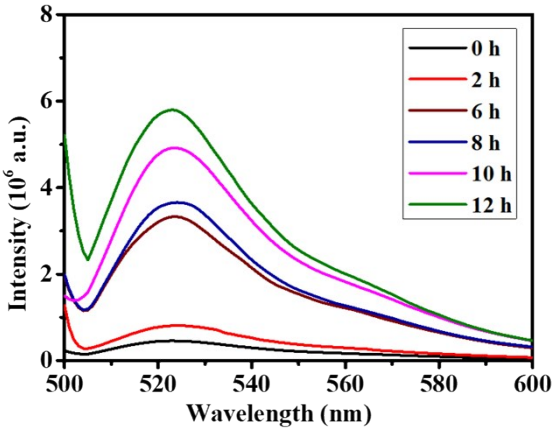

Figure S13. Time-dependent DCF fluorescent intensity from DCFH-DA stained 4T1 cells after being cultured with FPCF NCs at IC_{50} ([Fe] = 40μg mL^{-1}).

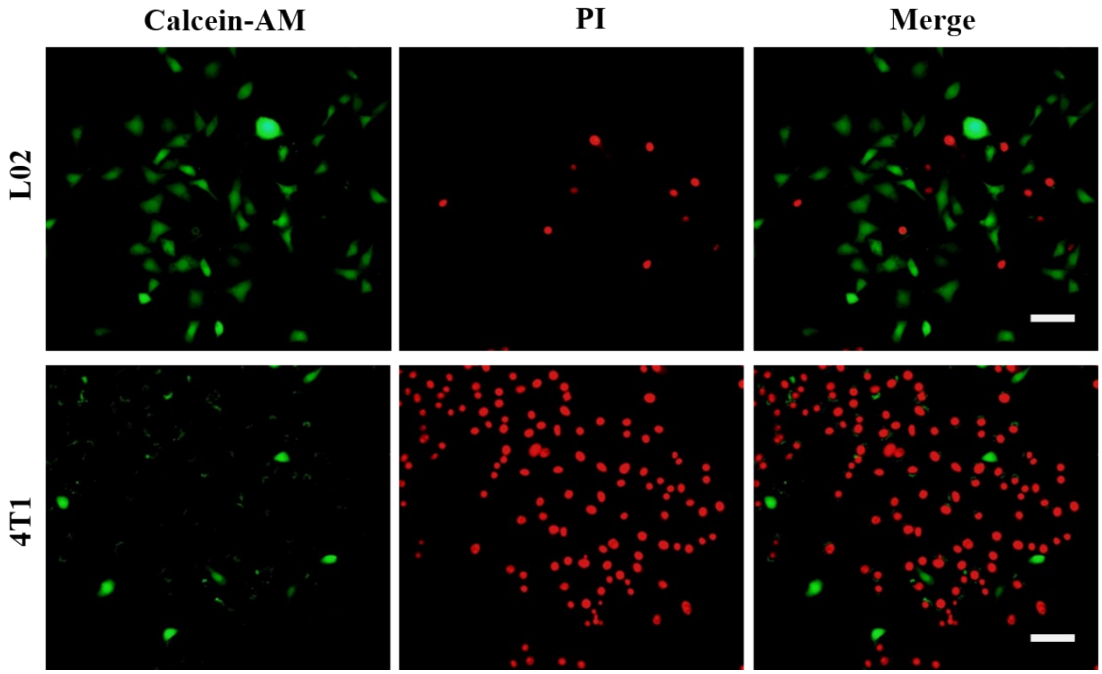

Figure S14. Fluorescence images of Calcein AM (green, live cells) and PI (red, dead cells) costained 4T1 cells and L02 cells after incubation with FPCF NCs for 15 h. (scale bars: 20 μm)

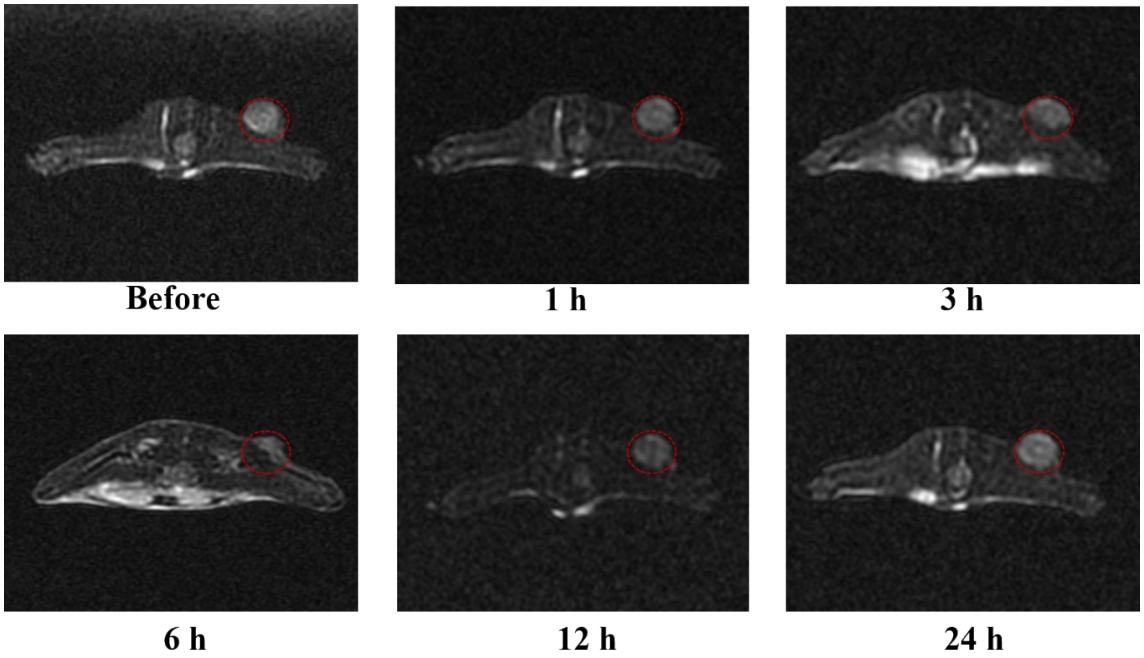

Figure S15. T_2-weighted MR imaging of 4T1 tumor-bearing Balb/c mouse by i.v. injection with FPCF NCs.

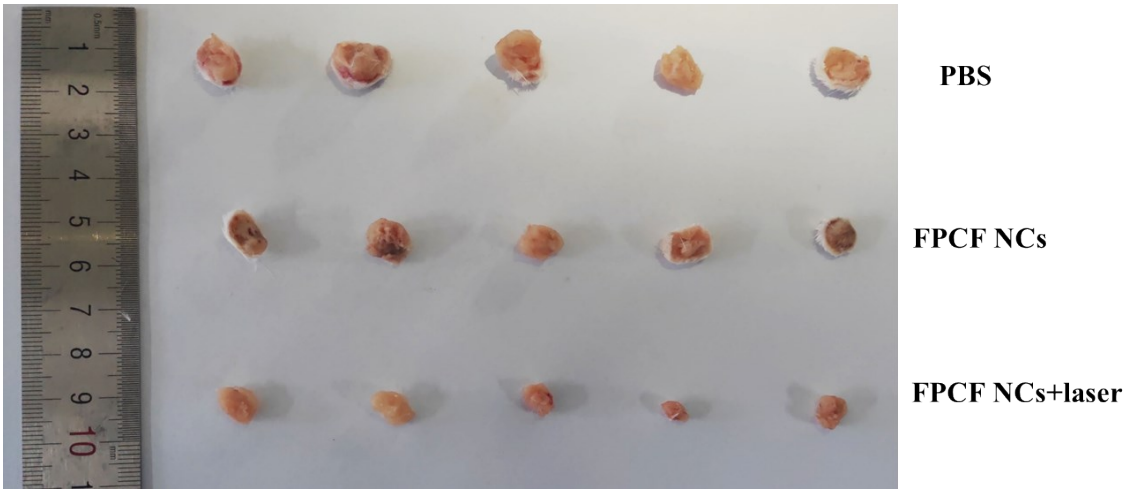

Figure S16. The photographs of tumors after different treatments (n=5).

Figure S17. The photographs of mice after various treatments.

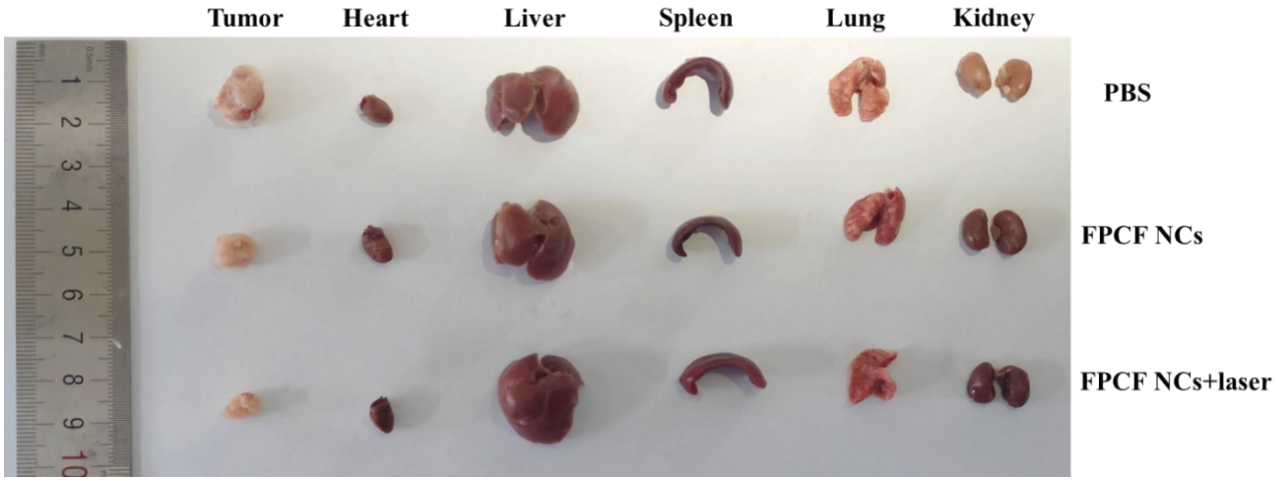

Figure S18. The photographs of tumors and major organs after various treatments.

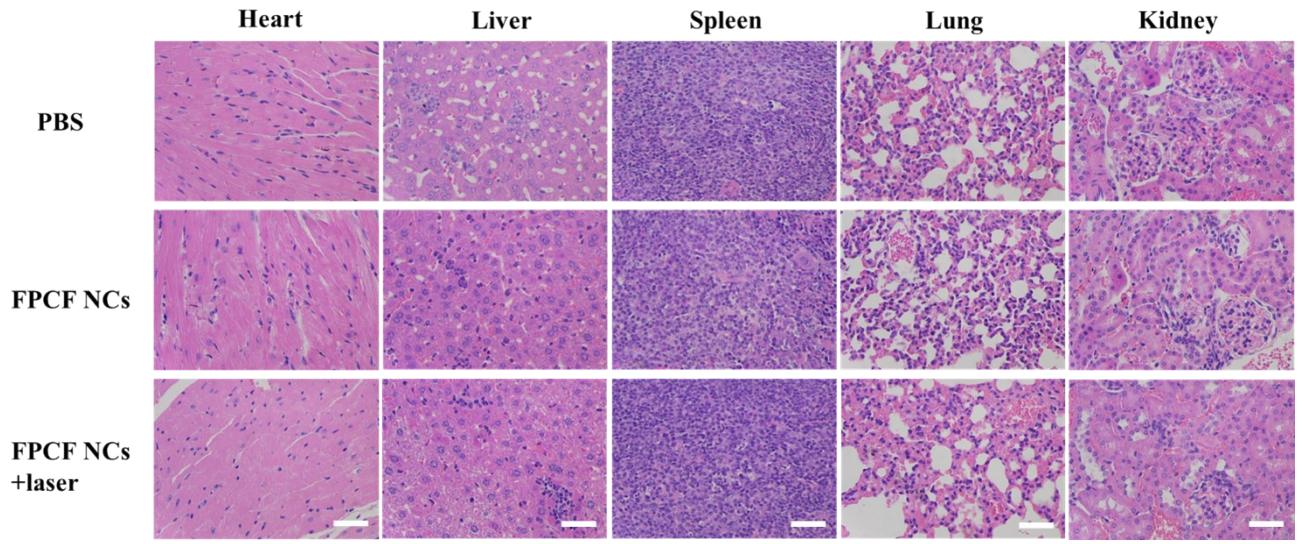

Figure S19. Histological analysis of the major healthy organ via H&E staining after various treatments. (scale bars: 100 μm)

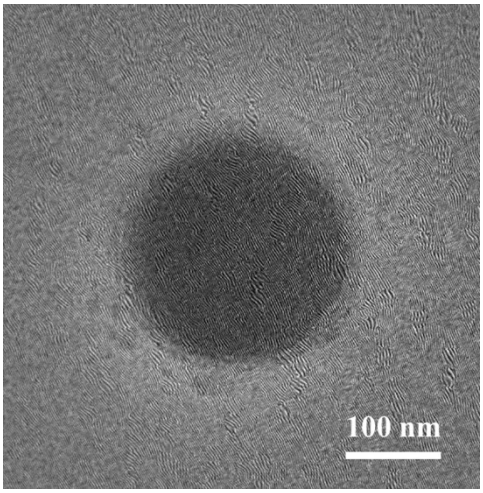

Figure S20. TEM image of R837-PLGA nanoparticles.

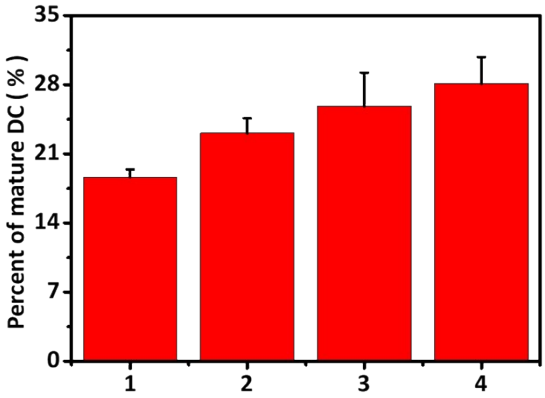

Figure S21. The maturation of DCs in in vivo after treatment by i.t. injected with different drugs. Five days post treatment, the nearby lymph node of mice were collected for assessment by flow cytometry after co-staining with CD80 and CD86.

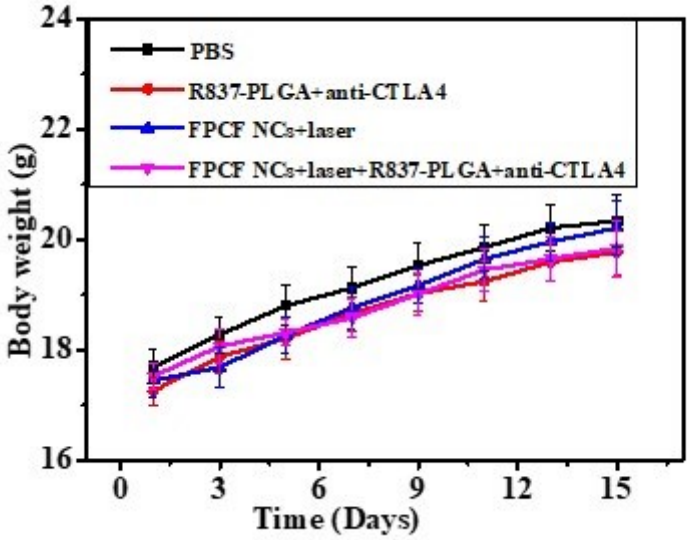

Figure S22. Body weight change of 4T1 tumor-bearing Balb/c mice after various treatments during 15 days.