Supporting Information

Environment-responsive polydopamine nanoparticles cross-linked phenylboronic-grafted hyaluronic acid with enzymelike properties for tumor synergistic therapy

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Experimental section

Materials

4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), Polyvinyl alcohol, 4-Aminophenylboronic acid hydrochloride (which contains varying amounts of water), Dopamine (DA), folic acid (FA) , N- (3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and Hyaluronic acid (HA) were obtained from Aladdin Reagent Company (Shanghai, China). Potassium permanganate, and sodium hydroxide were purchased from Sinopharm Chemical Reagent (Beijing, China). Cell counting kit-8 (CCK-8), DAPI, CD3, CD8a, CD44, CD62L, CD 11c, CD80, and CD 86 were purchased from Sigma (New York, NY, USA). Phosphate buffered saline (PBS), Fetal bovine serum (FBS), Trypsin, RPMI Medium Modified (RPMI-1640), and penicillin-streptomycin were procured from Hyclone (Logan, UT, USA). Nattokinase was purchased from Yangtze River Delta Health Research Institute (Zhengjiang China). Deionized water was used in the experiments.

Apparatus and procedures

Transmission electron microscopy (TEM) images were obtained using a JEOL 2100 transmission electron microscope. Fourier transform infrared (FTIR) spectra were recorded through a Nicolet Nexus 470 spectrometer (USA). The ζ-potential measurement was acquired through a NanoBrook Omni (Brookhaven, USA). Gelation time was determined by the tube inversion test. The morphology of the NS/FPDA@DOX HA was examined by ZEISS SIGMA scanning electron microscope. Mechanical properties of NS/FPDA@DOX HA were performed through compression testing using a universal material experiment machine (WDW3020, China). The two discs were cut into two semicircles respectively to examine their self-healing ability. NS/FPDA@DOX HA loaded into a syringe and injected into a star-shaped mold through a 25-gauge needle to evaluate its injectability. A TA rheometer (DHR-2) was performed to test rheological property of the NS/FPDA@DOX HA. 808 nm near-infrared light (NIR) was performed through a fiber-coupled NIR laser

(MDL-N-808 nm-10W, Beijing Laserwave OptoElectronics Technology Co., Ltd., Beijing, China). The infrared thermal camera (HT-19, Guangzhou, China) were recorded using an infrared thermal camera of NS/FPDA@DOX HA.

2.1 Synthesis of P&PDA@DOX NAs

The synthesis steps of P&PDA@DOX NAs were adapted from previous literature with appropriate modification.[1-2] First, 50 mg polyvinyl alcohol (PVA) was dissolved in 50 mL ultrapure water and stirred at 80°C for 1 h until completely dissolved. Once PVA solution cooled to room temperature, 500 mg dopamine (DA) was added and stirred for another 30 min. Next, 50 mg doxorubicin (DOX) and 10 mg $KMnO_4$ was added into above solution. The mixture was stirred at room temperature for 12 h. Afterward the supernatant was transferred into a dialysis bag (MWCO 100,000) and dialyzed for 3 d, changing the water every 12 h. Finally, the resulting dialysis solution was freeze-dried in a vacuum freeze dryer to obtain a black powder, which is P&PDA@DOX NAs.

2.2 Synthesis of FA-P&PDA@DOX NAs

The prepared P&PDA@DOX NAs were added into 50 mL ultrapure water to ensure thorough dispersion. Then, 250 mg folic acid was added into the above dispersion and get thorough dispersion. After 30 min, 250 mg N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were added into above dispersion, which was stirred for 12 h. Subsequently, centrifuge the prepared mixture to collect the precipitate (5000 rpm, 10 min) and wash it 3 times with ultrapure water. Finally, the obtained solution was placed in a vacuum freeze dryer to acquire the powder, resulting in FA-P&PDA@DOX NAs.[3]

2.3 Synthesis process of boronic acid-modified hyaluronic acid

0.6 mmol hyaluronic acid (HA) was added to 50 mL deionized water and stirred thoroughly until the HA was completely dissolved. Next, 0.6 mmol 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) was dissolved in 1 mL

deionized water and added into the HA solution. After stirring for 4 h, dissolve 0.6 mmol 4-aminophenylboronic acid hydrochloride in 1 mL deionized water and dropwise into above mixture, then continue stirring for 72 h. Finally, the mixed solution was placed in a dialysis bag (MWCO 100,000) and dialyzed for 3 d. The resulting dialysis solution was then freeze-dried in a vacuum freeze dryer to obtain boronic acid-modified hyaluronic acid (PHA).[4]

2.4 Environmental responsive behavior of DOX

To verify the release behavior of DOX from FA-P&PDA@DOX NAs in simulate acidic lysosomal. FA-P&PDA@DOX NAs was immersed in phosphate-buffered saline (PBS), pH 6.5, pH 6.5 + NIR, pH 5.5, and pH 5.5 + NIR to assess the release behavior of DOX. At designated time points, the absorbance of DOX was measured by UV-vis spectrophotometer to evaluate the environmentally responsive releasing behavior of DOX.[5]

2.5 Cell culture

4T1 cells (mouse breast cancer cells), GES-1 cells (human gastric mucosal epithelial cells) and DC2.4 cells (mouse dendritic cells) were obtained from SUNNCELL Biotechnology Co., Ltd. These cells were cultured in fresh RPMI-1640 medium supplemented with 10 % heat-inactivated fetal bovine serum and 1 % penicillin-streptomycin. All cell lines used in this study were approved by Jiangsu University.

2.6 Animal welfare

All animal experiments were performed by protocols approved by the Institutional Animal Care and Use Committee of Jiangsu University. 5-week-old female BALB/c mice were purchased from Jiangsu University (Zhenjiang, China) and housed under specific pathogen-free conditions. All efforts were made to minimize the animals' suffering and reduce the frequency of animal use.

2.7 DC maturation *in vitro*

DC2.4 and 4T1 cells (each is 8×10^4 cells) were respectively added into the lower and upper chambers of a transwell plate and incubated for 12 h. Then, 50 μL PBS, 50 μL DOX (1 mg/kg), 50 μL NS (500 IU), 50 μL FA-P&PDA@DOX NAs, 50 μL FPDA@DOX HA and 50 μL NS/FPDA@DOX HA were added into 4T1 cells and exposed under NIR (0.75 W/cm^2, 2 min) irradiation. 12 h later, 4T1 cells in the upper chamber were co-incubated with the DC2.4 cells in the lower chamber for 24 h. Finally, the DC2.4 cells were collected and stained with anti-CD11c APC, anti-CD86 PE and anti-CD80 FITC for flow cytometry analysis.[6]

Results

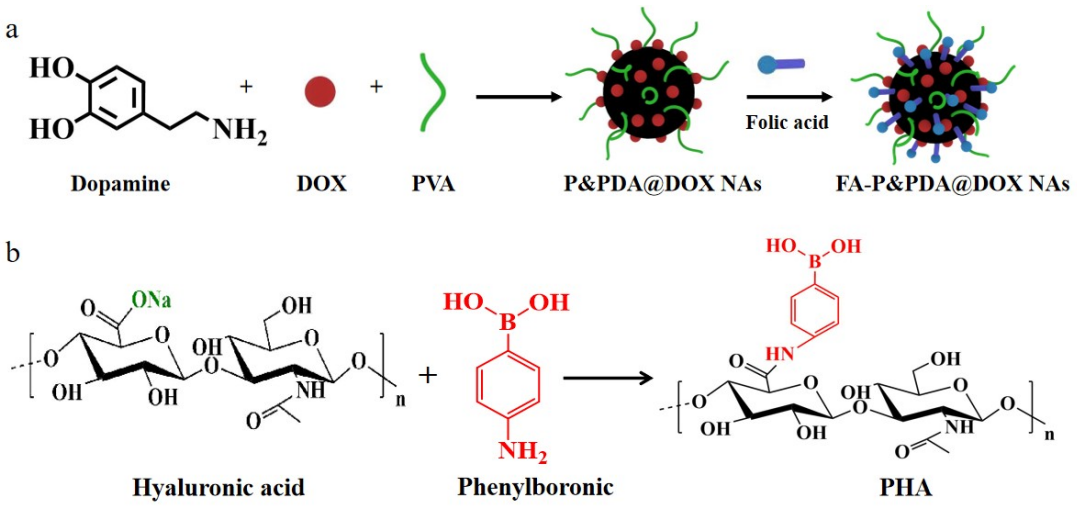

Figure S1. (a) Schematic illustration of the synthesis process of FA-P&PDA@DOX NAs. (b) Schematic illustration of the synthesis process of PHA.

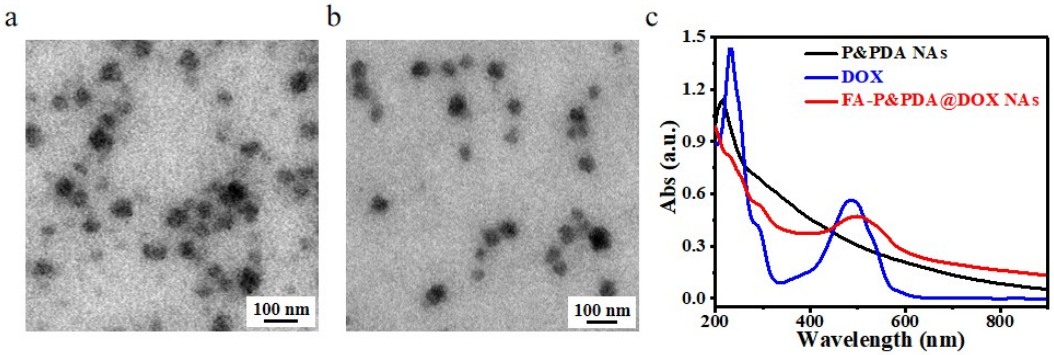

Figure S2. TEM image of (a) P&PDA NAs and (b) P&PDA@DOX NAs. (c) UV-vis absorption of P&PDA NAs, DOX and FA-P&PDA@DOX NAs.

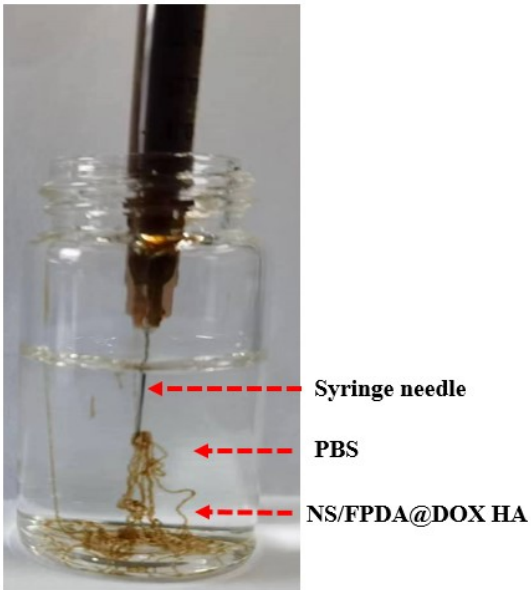

Figure S3. The injectability of NS/FPDA@DOX HA in PBS solution.

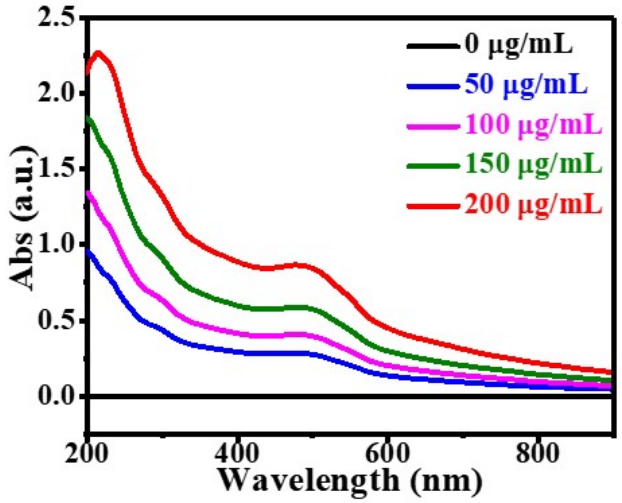

Figure S4. UV-vis absorption of different concentration of FA-P&PDA@DOX NAs.

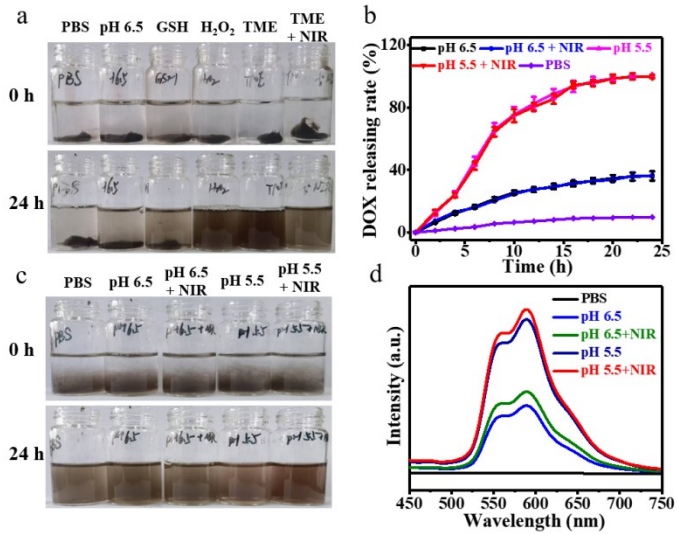

Figure S5. (a) Photograph of NS/FPDA@DOX HA immersed into different solution at 0 and 24 h. (b) DOX releasing behavior from FA-P&PDA@DOX NAs under various conditions. (c) Photograph of FA-P&PDA@DOX NAs immersed into different solution at 0 and 24 h. (d) Fluorescence spectra of DOX once FA-P&PDA@DOX NAs immersed into different solution after 24 h.

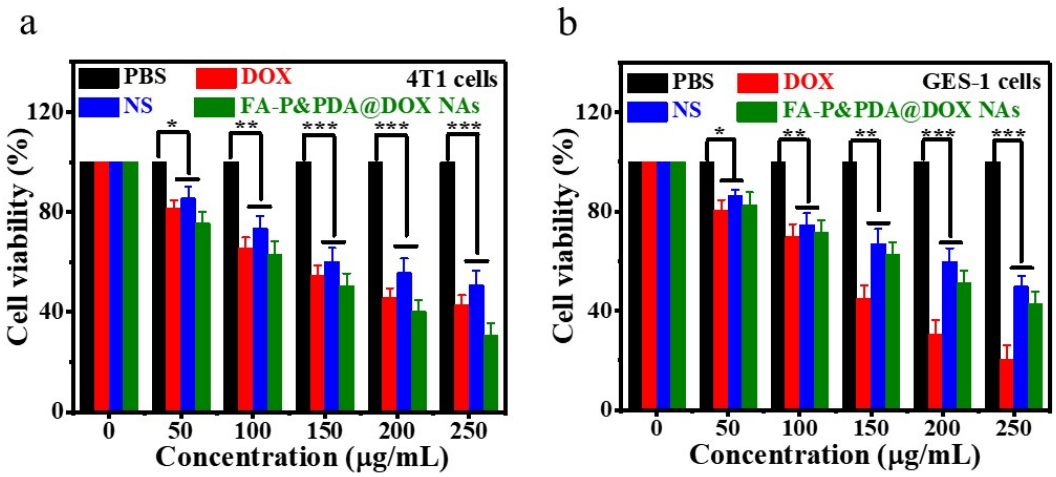

Figure S6. Cell viability of (a) 4T1 cells and (b) GES-1 cells co-cultured with different concentration of PBS, DOX, NS and FA-P&PDA@DOX NAs under NIR irradiation (0.75 W/cm^2, 2 min).

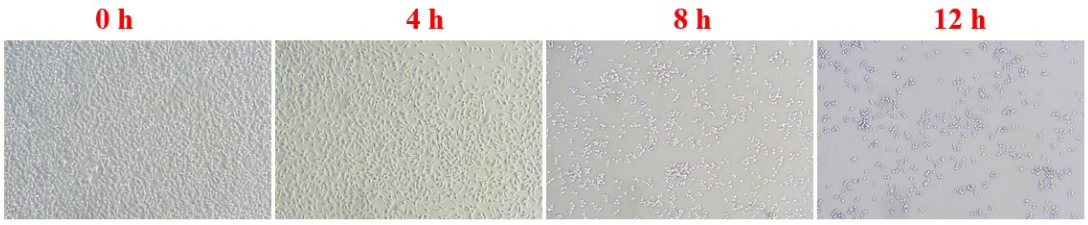

Figure S7. Microscopic images of 4T1 cells incubated with 500 IU NS for 0, 4, 8 and 12 h. Magnification×80.

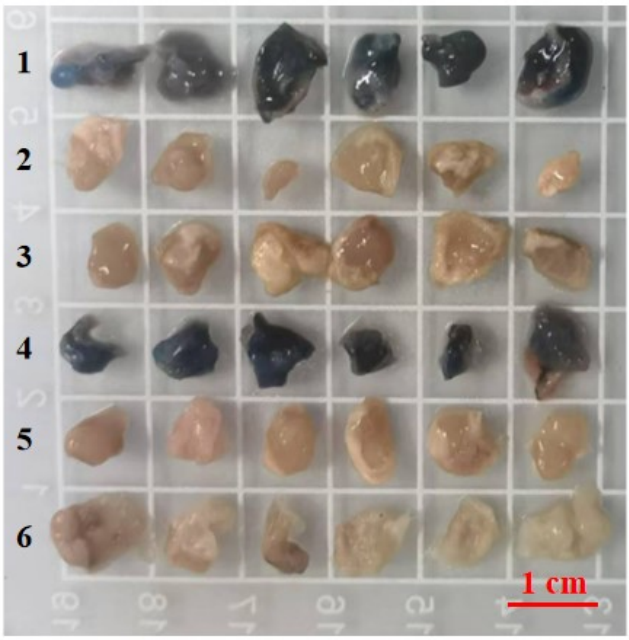

Figure S8. Photographs images shown evans blue in tumors collected from mice after treatment with NS, PBS, DOX, NS/FA-P&PDA@DOX HA, FA-P&PDA@DOX NAs, and FA-P&PDA@DOX HA one times.

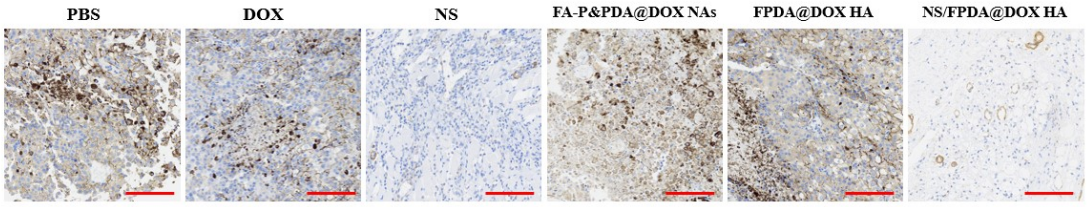

Figure S9. Immunohistochemical images showing α-SMA protein in tumors collected from mice after treatment with PBS, DOX, NS, FA-P&PDA@DOX NAs, FPDA@DOX HA and NS/FPDA@DOX HA one times. Scale bar: 200 μm.

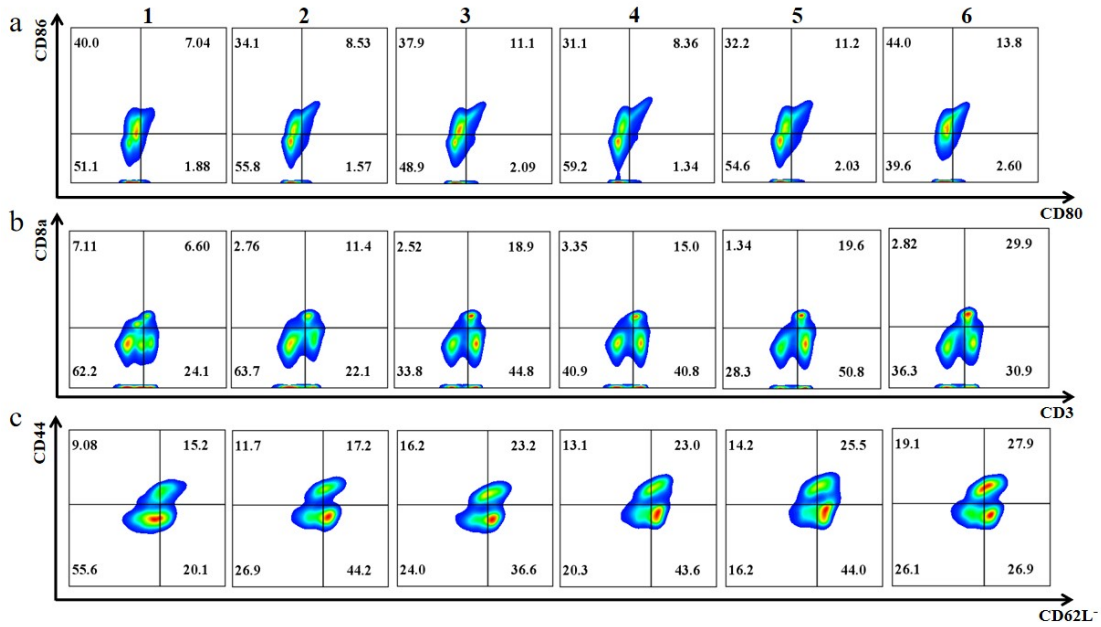

Figure S10. Flow cytometry plots showing (a) the maturation of DC cells identified as $CD11c^{+}CD80^{+}CD86^{+}$ and (b) $CD8^{+}$ T cells identified as $CD3^{+}CD4^{+}CD8a^{+}$, extracted from tumor tissues following different treatments. (c) Flow cytometry plots of memory T cells, defined as $CD44^{+}CD62L^{-}CD3^{+}CD8a^{+}$ T cells, extracted from lymph nodes after various treatments. PBS + NIR (1), DOX + NIR (2), NS + NIR (3), FA-P&PDA@DOX NAs + NIR (4), FPDA@DOX HA + NIR (5) and NS/FPDA@DOX HA + NIR (6).

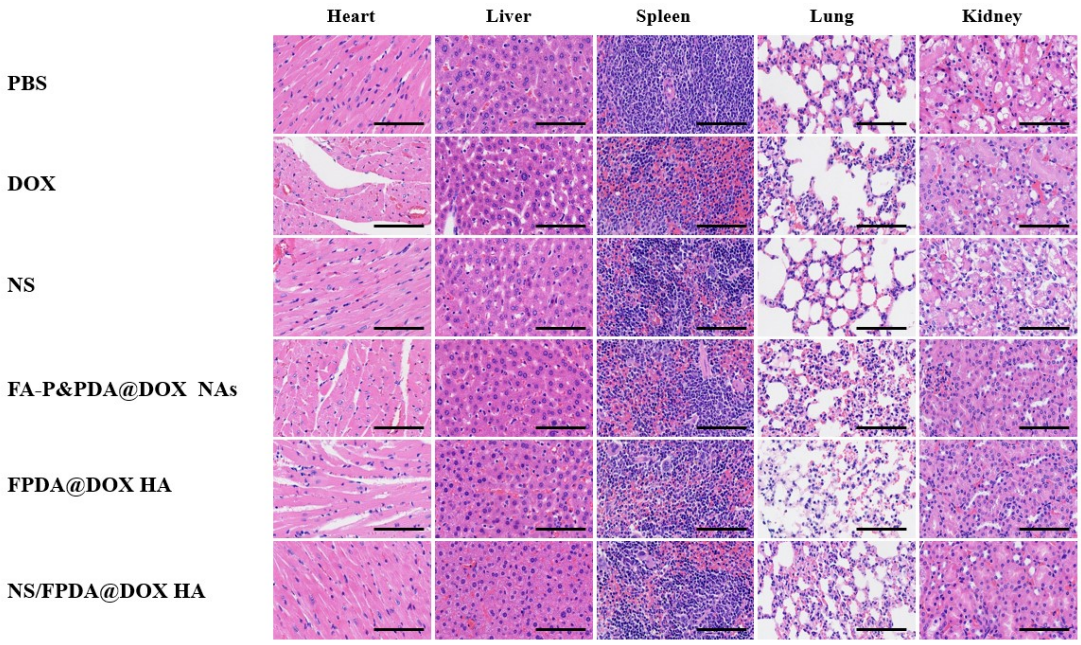

Figure S11. H&E-stained images of the major organs (Heart, Liver, Spleen, Lung and Kidney) in the different groups. Scale bar: 50 μm.

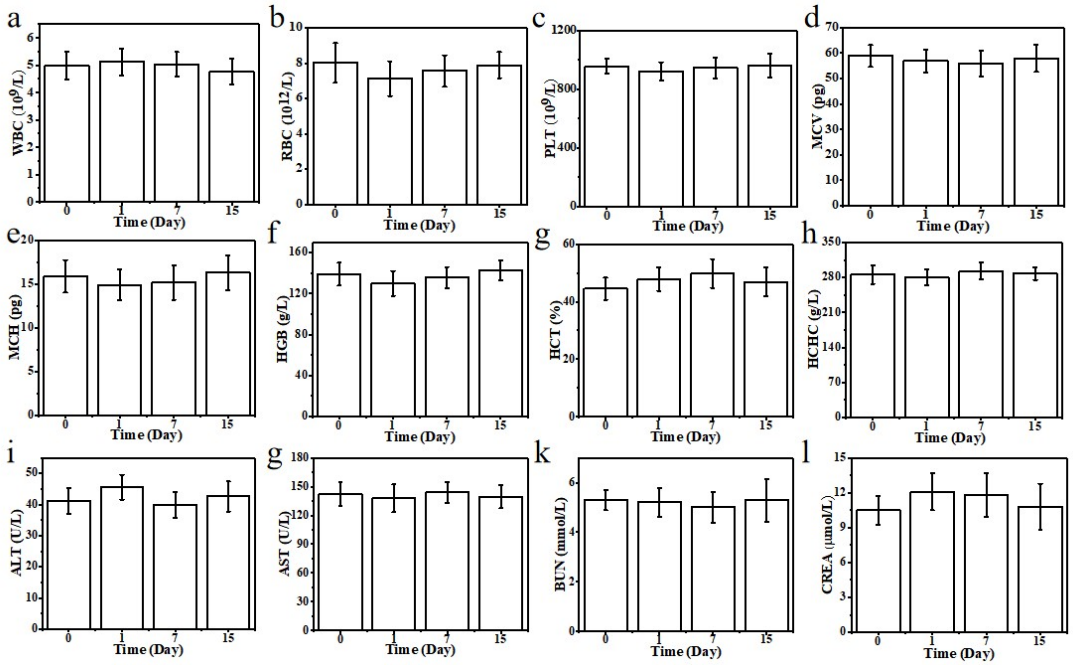

Figure S12. The blood tests (a-h) and the serum biochemistry index (i-l) of the mice on the 0 st, 1st, 7th, and 15th day after the treatment of NS/FPDA@DOX HA.

Reference

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[6] Qianzhe Li, Mengyu Yang, Xin Sun, et al. NIR responsive nanoenzymes via photothermal ablation and hypoxia reversal to potentiate the STING-dependent innate antitumor immunity. *Mater. Today Bio.,* 2023. 19: 100566.