Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supporting Information

Design and self-assembly of Albumin Nanoclusters as a Dynamic-covalent Targeting Co-delivery and Stimuli-responsive Controlled Release Platform

Wen Liu[a], Jian Dai[a]*, Wei Xue[abc]*

[a] Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Development and Engineering Technology Research Center for Drug Carrier of Guangdong Province, Department of Biomedical Engineering, Jinan University, Guangzhou 510632, China

[b] Institute of Life and Health Engineering, Key Laboratory of Functional Protein Research of Guangdong Higher Education Institutes, Jinan University,Guangzhou 510632, China

[c] The First Affiliated Hospital of Jinan University, Guangzhou 510632, China

*E-mail: Jian Dai, daijian@jnu.edu.cn; Wei Xue, weixue_jnu@aliyun.com

Experimental

Determination of the Content of cRGD peptide conjugated to cBSANCs

The D/R@cBSA NCs-R was removed from the reaction solution by a centrifugal filter device (30 kDa MWCO) (Millipore) for three times. The ultrafiltrate was collected, and the residual peptides were determined by a BCA assay kit. The conjugated content of each peptide (μg/mg cBSA NCs) was calculated by the difference value compared with the amount added.

Characterization of D/R@cBSA NCs-R

The morphology and structure of D/R@cBSA NCs-R was examined by transmission electron microscopy (TEM, Hitachi H-7650) and scanning electron microscopy (SEM, EX-250 system, horiba), respectively. The dynamic light scattering and ζ-potential of the nanoparticles were measured on a Zetasizer Nano ZS particle analyzer (Malvern Instruments Limited). Fluorescence spectra were obtained using fluorescence spectroscopy at 302 nm excitation. The absorption spectra were recorded using a Shimadzu UV-2550 UV vis NIR spectrophotometer. Fourier transform infrared spectroscopy was performed on a FT-IR spectrometer (Bruker VERTEX 70, Germany) with the range of 4000 to 500 cm^{-1}.The encapsulation efficiency of siRNA on the D@cBSA NCs was determined by electrophoretic mobility shift assay (EMSA), D@cBSA NCs loaded with siRNA (1 μg) at various weight ratios (0, 1, 2, 3, 5, 10, 20) for 20 min at room temperature, then was gently mixed with 1 μL of 6 × DNA loading buffer. The nanocomplexes were examined by agarose gel (1%) and ran at a voltage of 130 V for 20 min.

In vitro stability of D/R@cBSA NCs-R

For in vitro stability of D/R@cBSA NCs-R in water, PBS and 100% FBS (Hyclone) solution was monitored at different time intervals using the Zetasizer Nano ZS particle analyzer. Dual programmed stimuli responsiveness of the Nanosystem. Zetasizer Nano ZS particle analyzer was performed to estimate the average size of D/R@cBSA NCs-R at pH 5.0, pH 7.4 and 10 mM GSH solution at pH 5.0 for 2 h. Then, the changes in morphology of D/R@cBSA NCs-R were measured by TEM.

In Vitro DOX Release

1 mL of D/R@cBSA NCs and D/R@cBSA NCs-R was dialyzed against 10 mL of the PBS buffer solution (MWCO = 7500 Da) at pH 5.0 and 7.4 in the presence and absence of 10 mM GSH at 37℃ with shaker at 100 rpm. At predetermined time intervals, 0.1 mL of solution was taken out and the same fresh buffer was added. The concentration of DOX released was determined by fluorescence microplate reader with the excitation and emission wavelengths set as 485 and 590 nm, respectively.

Cell migration and invasion assays

For wound healing assay, Hela cells were seeded into 6-well plates at a density of 2×10^5 cells per well. After culture for 24 h, the cells were scratched wounds by a pipette tip, and the cells were washed and fresh complete medium were added. Then different concentration of D/R@cBSA NCs-R and D/R@cBSA NCs-NR were mixed at 37 ℃ for another 24 h. The images of the cells

were captured by optical microscopy at different times of the experiment. For invasion assays, the 5×10^5 of Hela cells in DMEM without FBS were seeded into the Transwell chamber (Corning, 8 μm pore size) with matrigel-coated membrane. The lower chambers were injected with 600 μL of 10% FBS DMEM. D/R@cBSA NCs-R and D/R@cBSA NCs-NR were added into the upper and lower chambers. After incubated for 24 h, the cells that non-invading the upper chamber membranes were removed by a cotton swab. The invading cells on the lower chamber membrane were fixed with methanol for 30 min, and then stained with 1% crystal violet for 20 min and observed under optical microscopy.

RGD Competition Assay

D/R@cBSA NCs-R and excess amount of free cRGD competed for binding to the integrin αvβ3 on Hela cells. The cellular uptake of D/R@cBSA NCs-R by Hela cells was examined by a microplate reader.

Intracellular Trafficking

The intracellular internalization of D/R@cBSA NCs-R was monitored by Confocal Laser Scanning Microscope. HeLa cells were seeded onto 2 cm confocal microscopy dish. After attached for 24 h, the cells were incubated with D/R@cBSA NCs-Rat 37℃ for different times. Subsequently, the cells were treated with Hoechst 33342 at 37℃ for 10 min. Finally, the cells were washed with PBS and the confocal images were observed by CLSM.

Cellular Uptake Mechanism of D/R@cBSA NCs-R

The cellular uptake mechanism ofD/R@cBSA NCs-R by Hela cells was measured by endocytosis inhibitors. Hela cells were seeded on 96-well plates at a density of 7000 cells/well. After culture for 24 h, the cells were mixed with endocytosis inhibitors, 6 μg mL^{-1} of D/R@cBSA NCs-R was added for another 4 h. Moreover, the influenced of endocytosis inhibitors on the cellular uptake processes were measured by using CCK8 assay.

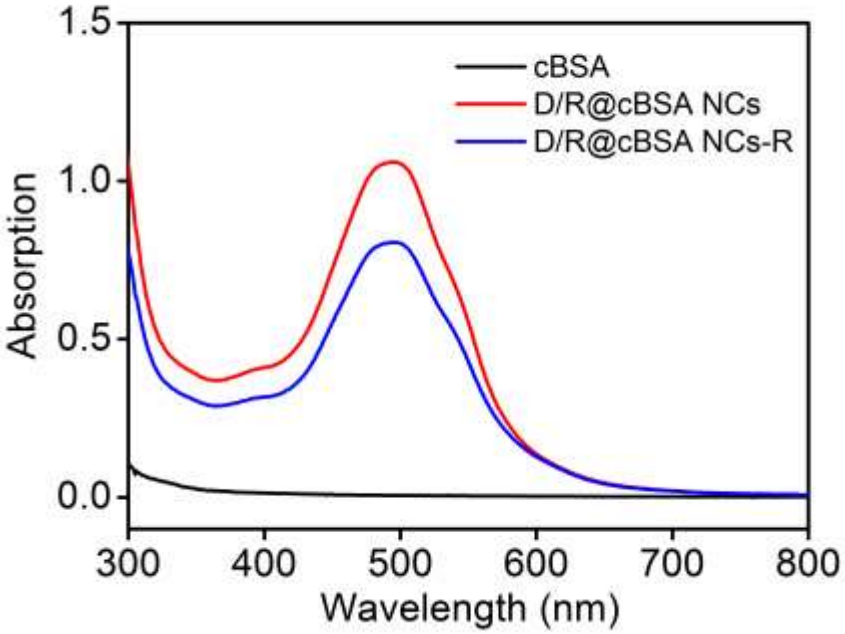

Figure S1. UV-vis-NIR spectra of cBSA, D/R@cBSA NCs, and D/R@cBSA NCs-R.

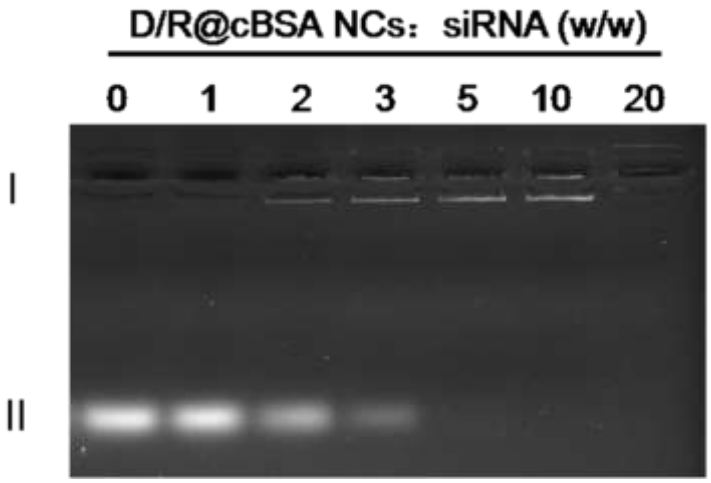

Figure S2. Agarose gel electrophoresis assay of D/R@cBSA NCs with siRNA at different weight ratios.

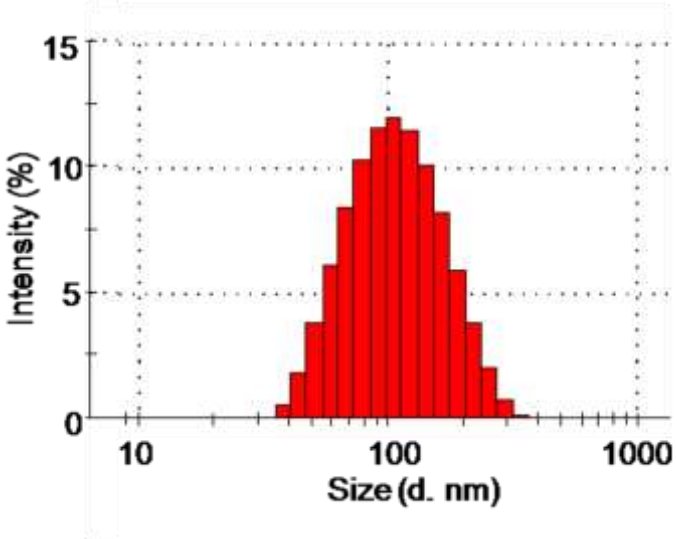

Figure S3. Hydrodynamic size distribution of D/R@cBSA NCs-R.

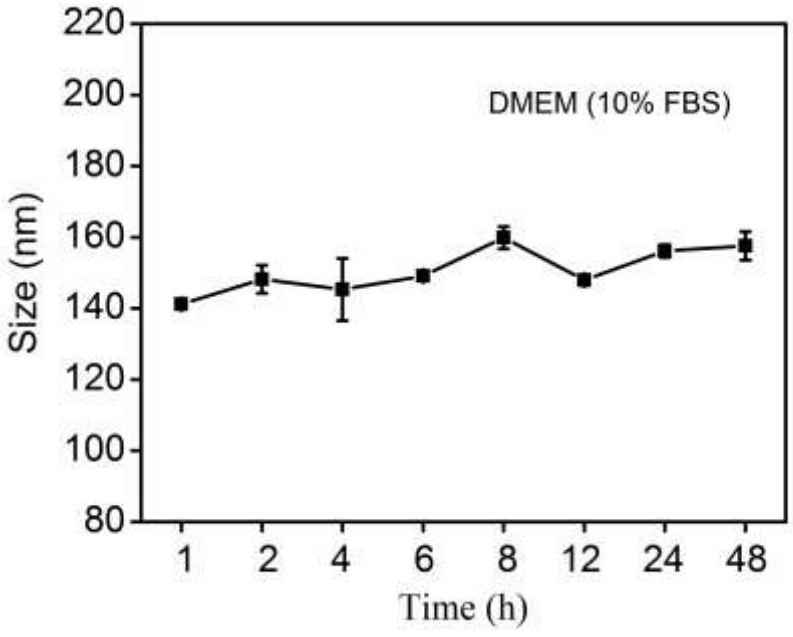

Figure S4. The stability of D/R@cBSA NCs-R in DMEM (10% FBS) for 48 h.

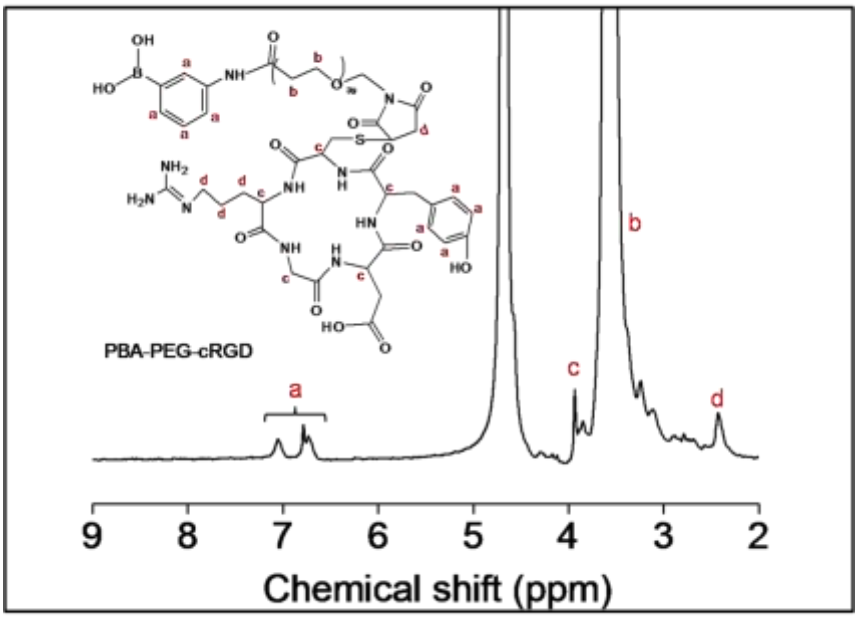

Figure S5. ^{1}H-NMR spectrum of PBA-PEG-cRGDmeasured in D_2O at 25°C.

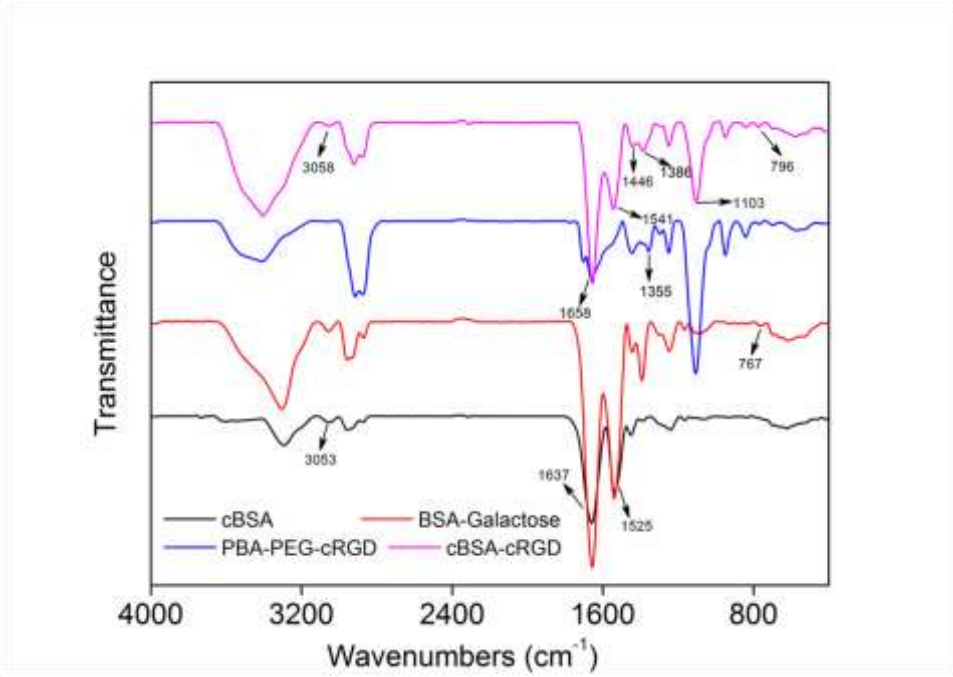

Figure S6. FTIR spectra of cBSA, cBSA with Galactose, PBA-PEG-cRGD,and cBSA-cRGD.

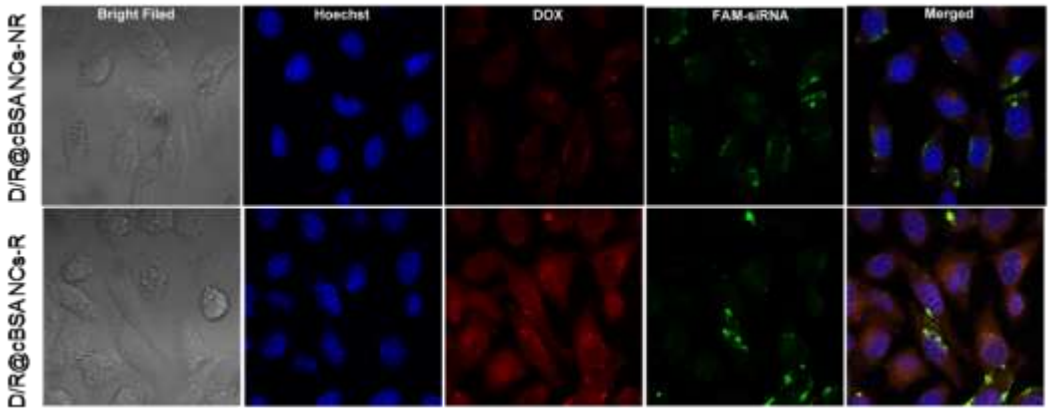

Figure S7. CLSM images of Hela cells incubated with D/R@cBSA NCs-NR and D/R@cBSA NCs-R for 6h. Nuclei of the cells were stained with Hoechst 33342(blue fluorescence).

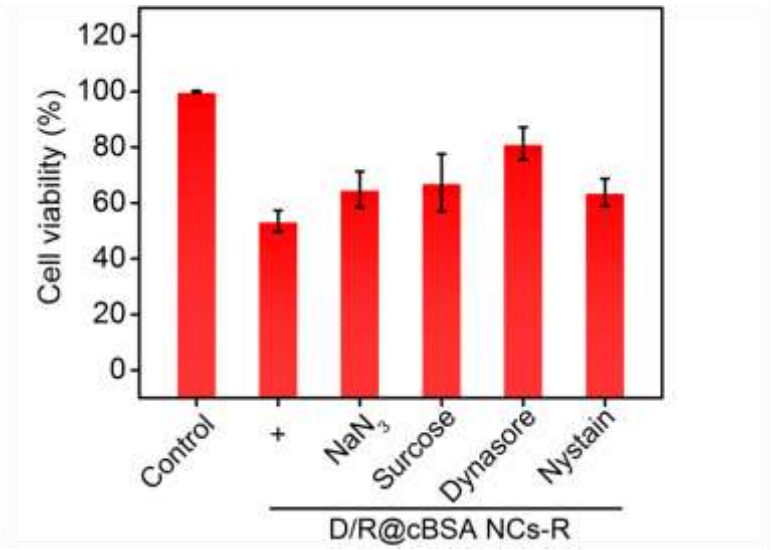

Figure S8. The viability of Hela cells. The cells were treated with specific endocytosis inhibitors (10 mM NaN_3, 225 mM surcose, 16 μM dynasore and 10 μg/mL nystain) under the different periods of time before ncubation with D/R@cBSA NCs-R (0.3 μg/mL). Each value represents means ± SD (n=3).

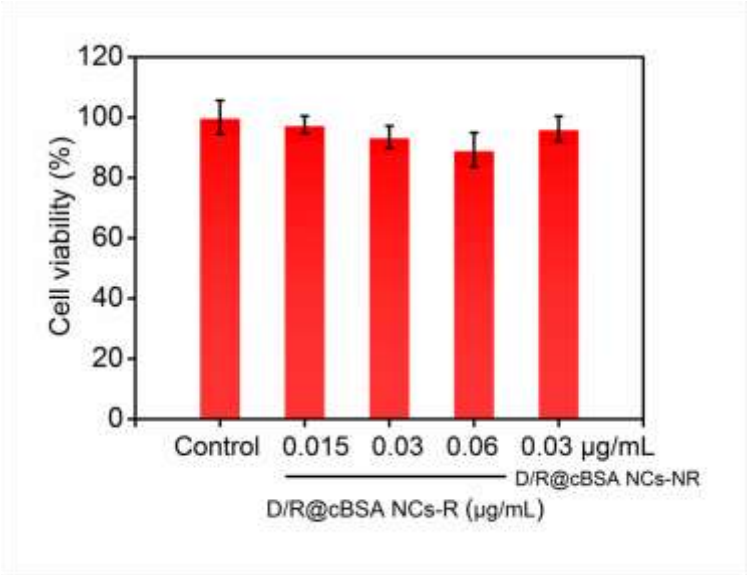

Figure S9. The Hela cells viability after incubation with 0.015, 0.03, 0.06 μg/mL D/R@cBSA NCs-R and 0.03 μg/mL D/R@cBSA NCs-NR for 24 h. Each value represents means ± SD (n=3).

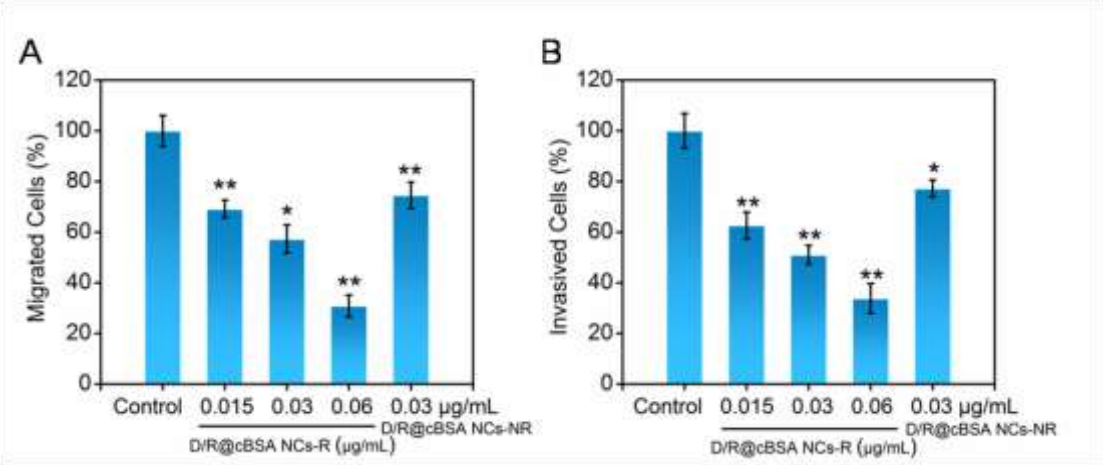

Figure S10. Quantitative analyzes of migration (A) and invasion (B) Hela cells after treatments with 0.015, 0.03, 0.06 μg/mL D/R@cBSA NCs-R and 0.03 μg/mL D/R@cBSA NCs-NR for 24 h. *P < 0.05, **P < 0.01, compared with control. Each value represents means ± SD (n=3).

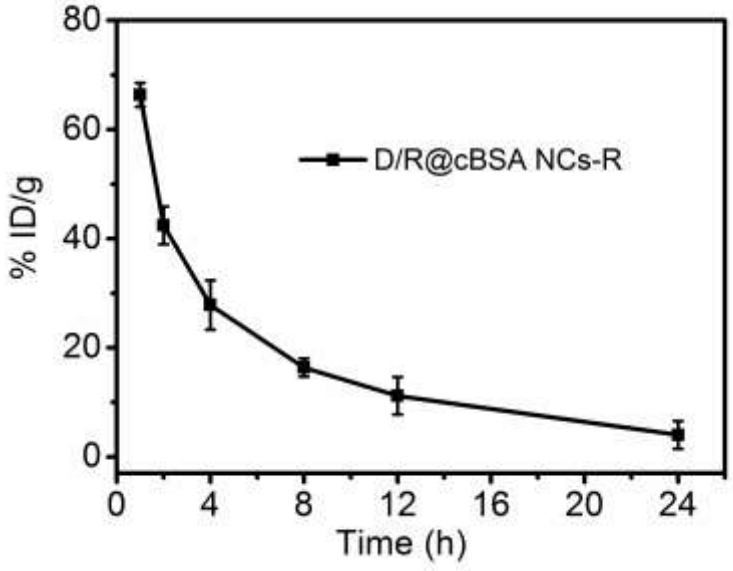

Figure S11. Pharmacokinetic curves of D/R@cBSA NCs-R.

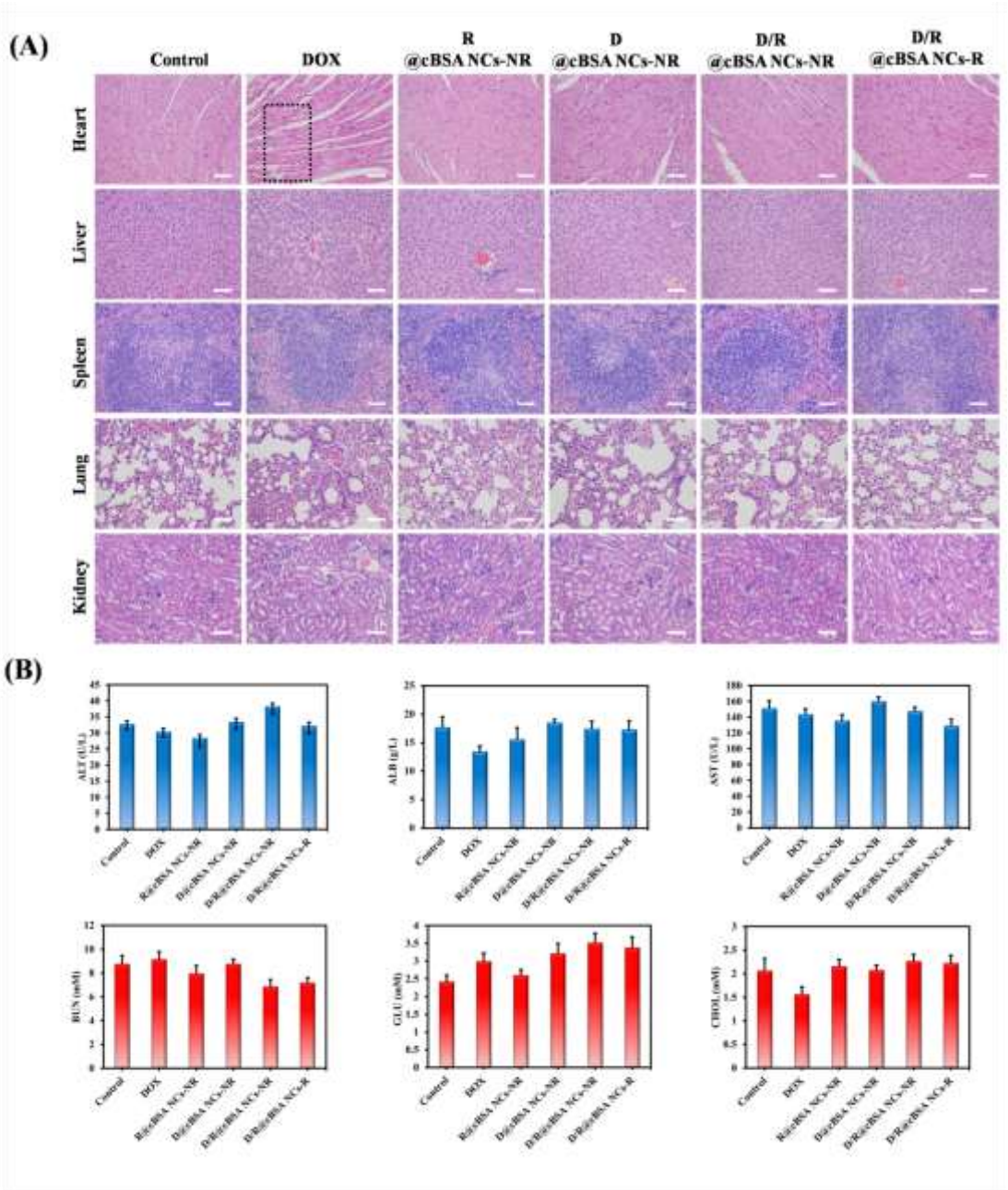

Figure S12. In vivo toxicity evaluation. (A) H&E staining of major organs after treatment with different samples. The scale bar was 50 μm. (B) Blood biochemistry analysis of various groups after 14 days treatment: ALT, ALB, AST, BUN, GLU and CHOL.