ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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Effective and Selective Anti-Cancer Protein Delivery via All-Functions-in-One Nanocarriers Coupled with Visible Light-Responsive, Reversible Protein Engineering

Hua He, Yongbing Chen, Yongjuan Li, Ziyuan Song, Yinan Zhong, Rongying Zhu, Jianjun Cheng, and Lichen Yin**

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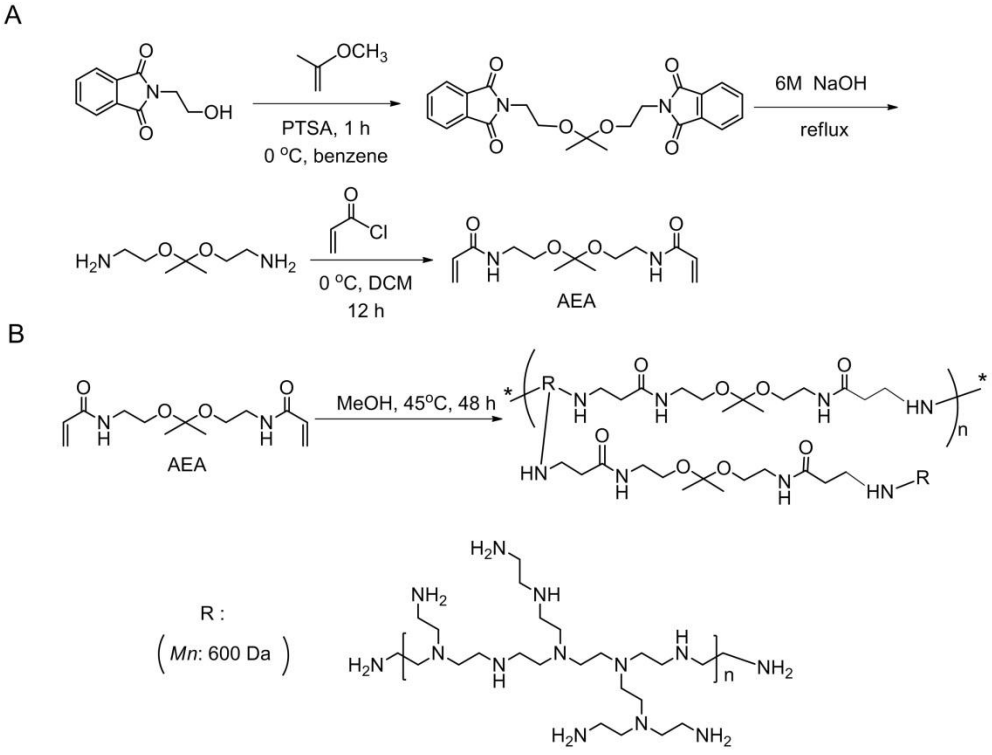
Hua He [a,#], *Yongbing Chen* [b,#], *Yongjuan Li* [a,#], *Ziyuan Song* [c], *Yinan Zhong* [d], *Rongying Zhu* [b], *Jianjun Cheng* [c,*], *Lichen Yin* [a,*]

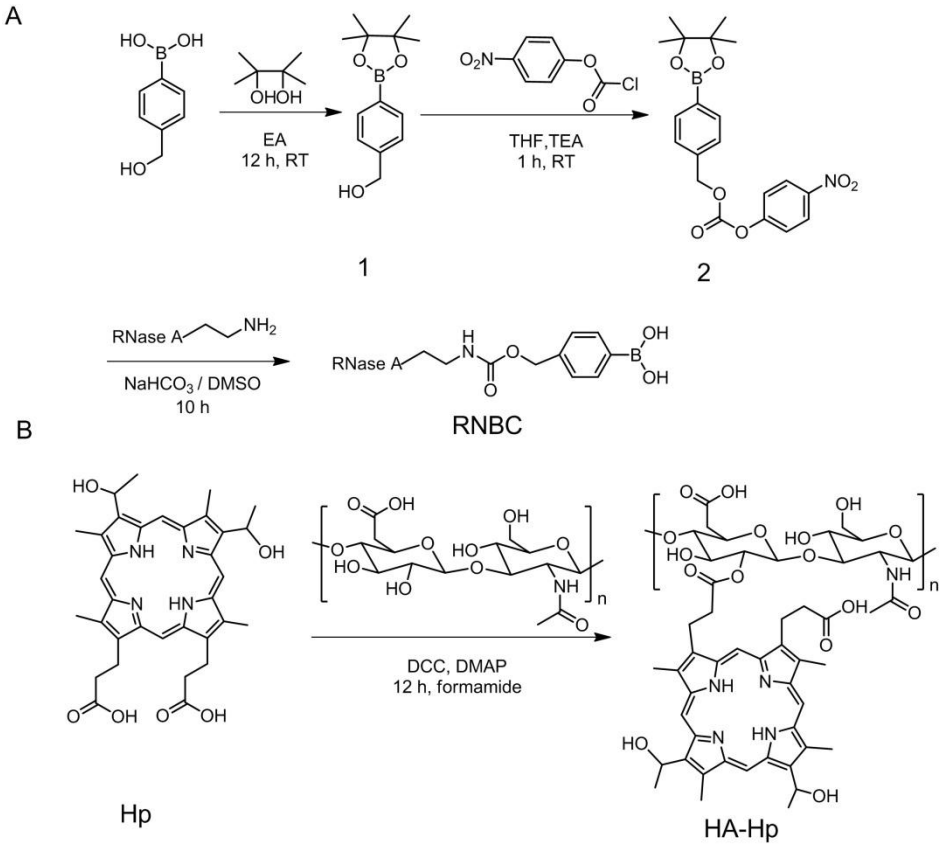
[a] Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Institute of Functional Nano & Soft Materials (FUNSOM), Joint International Research Laboratory of Carbon-Based Functional Materials and Devices, Soochow University, Suzhou 215123, China
[b] Department of Cardiothoracic Surgery, the Second Affiliated Hospital of Soochow University, Suzhou 215004, China
[c] Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana IL 61801, USA
[d] Biomedical Polymers Laboratory and Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China

*Address correspondence to lcyin@suda.edu.cn (L. Yin), jianjunc@illinois.edu (J. Cheng)

[#]These authors contributed equally.



Scheme S1. Synthetic route of AEA (A) and KPEI (B).



Scheme S2. Synthetic route of RNBC (A) and HA-Hp (B).

Table S1. The IC_{50} of RNBC and Hp in various NCs against different cancer cell lines.

	HeLa		B16F10		4T1	
	IC_{50} ($\mu g\ mL^{-1}$)	CI [a]	IC_{50} ($\mu g\ mL^{-1}$)	CI [a]	IC_{50} ($\mu g\ mL^{-1}$)	CI [a]
RNBC (KHR NCs)	3.87		3.61		3.15	
Hp (KHHB NCs)	4.73	0.84	8.5	0.89	12.8	0.95
RNBC (KHHR NCs)	0.58		0.94		1.25	
Hp (KHHR NCs)	3.3		5.4		7.2	

[a] Combination index (CI) between RNBC and Hp in KHHR NCs.

Table S2. The IC_{50} (μg mL^{-1}) of RNBC in KHHR NCs toward B16F10 and 4T1 cells with or without pre-treatment of HA.

	B16F10	4T1
w/ HA pre-treatment	4.70	4.25
w/o HA pre-treatment	3.61	3.15

Table S3. Acronyms of each formulation used.

Component	Acronym
K-PEI/RNBC	KR NCs
K-PEI/HA-Hp/RNBC	KHHR NCs
K-PEI/HA/RNBC	KHR NCs
K-PEI/HA-Hp/BSA	KHHB NCs

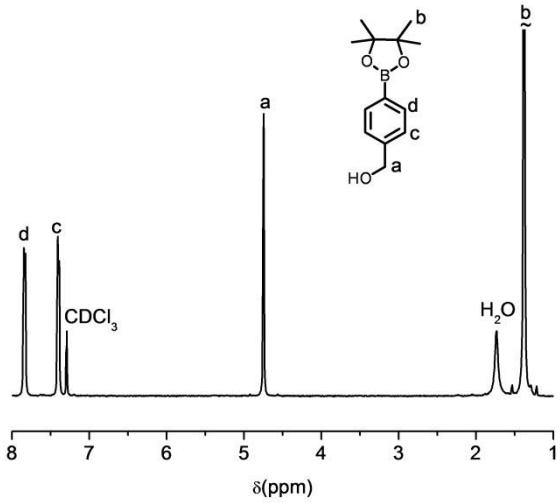


Figure S1. ^{1}H NMR spectrum of **compound 1** ($CDCl_3$, 400 MHz).

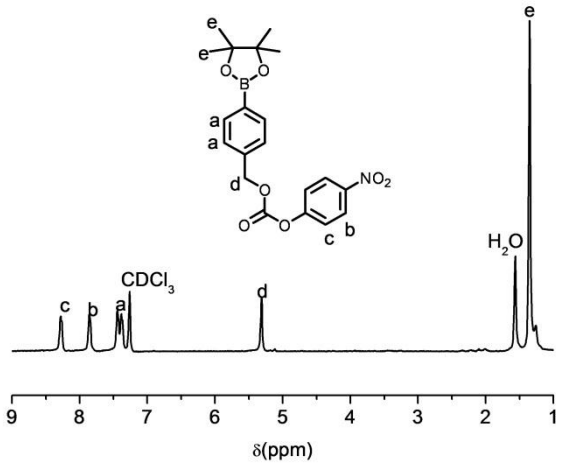


Figure S2. ^{1}H NMR spectrum of **compound 2** ($CDCl_3$, 400 MHz).

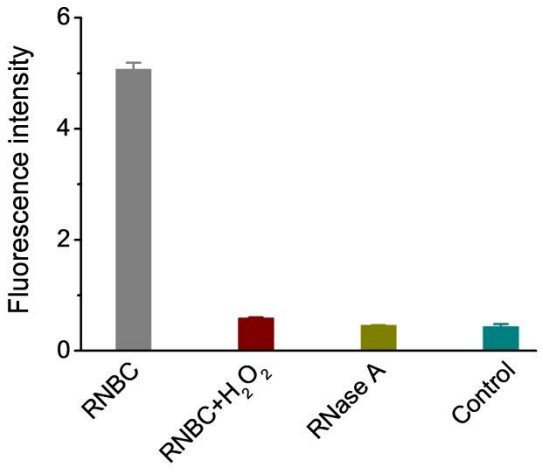


Figure S3. Fluorescence of Alizarin Red S (ARS, 0.025‰, w/v) in the presence of RNBC, H_2O_2-treated RNBC (final H_2O_2 concentration of 300 μM), RNase A, and DI water (control).

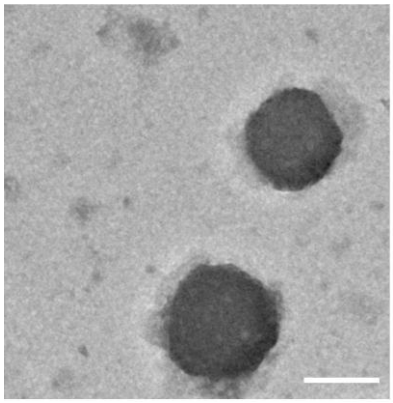


Figure S4. The TEM image of KHHR NCs. Bar represents 100 nm.

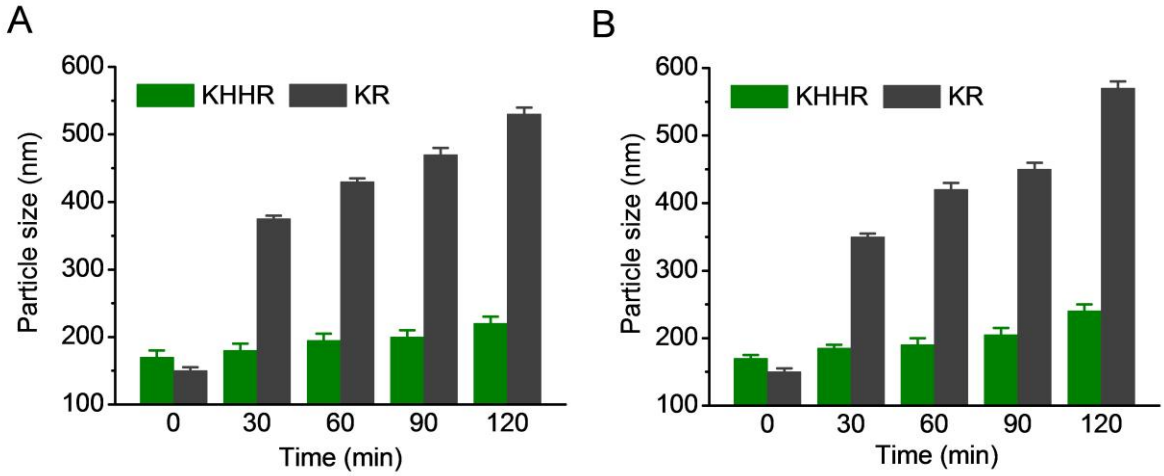


Figure S5. Alteration of particle size of KHHR NCs and KR NCs after incubation with DMEM containing 10% FBS (A) or pH 6.8 PBS (B) for different time.

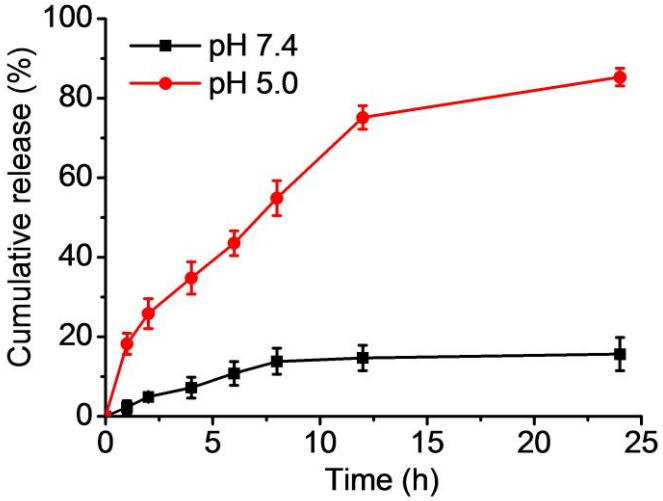


Figure S6. *In vitro* release of FITC-RNBC from KHHR NCs in PBS buffer (pH 7.4) or acetate buffer (pH 5.0) at 37 ^{o}C (n = 3).

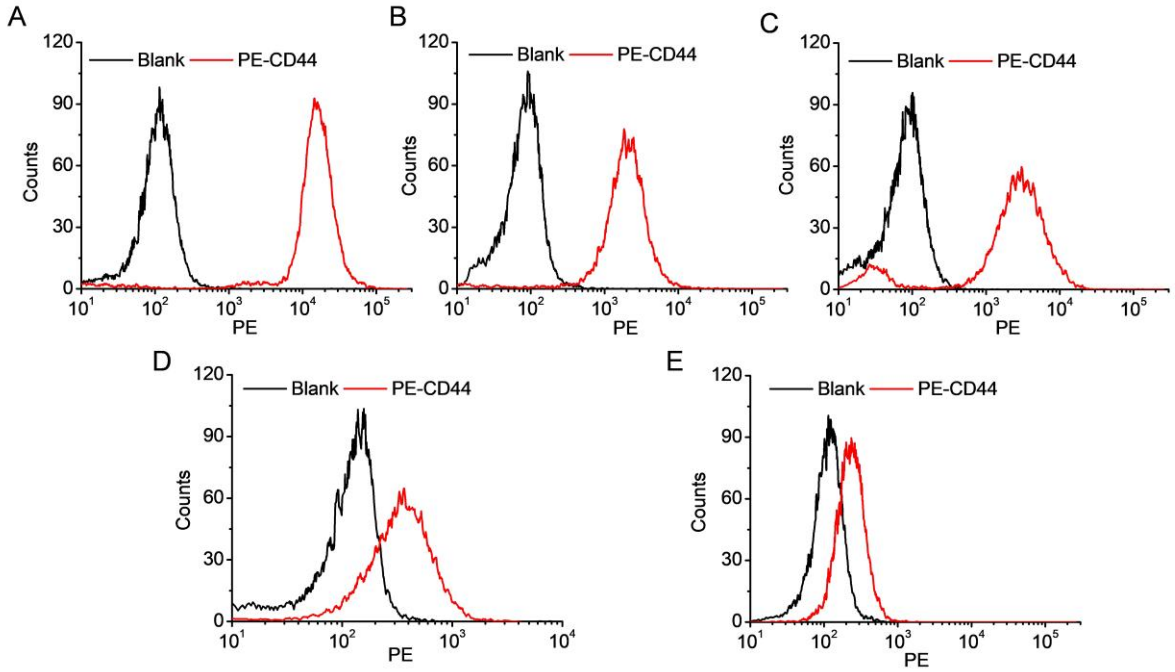


Figure S7. The CD44 expression levels in HeLa (A), B16F10 (B), 4T1 (C), 3T3 (D), and L929 (E) cells as measured by flow cytometry.

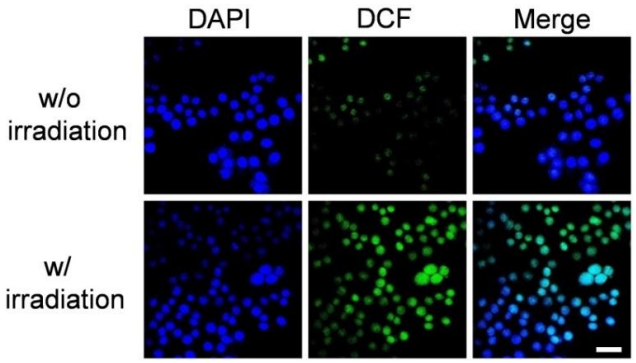


Figure S8. CLSM images of HeLa cells showing the generation of ROS under light irradiation. HeLa cells were treated with KHHR NCs for 4 h, irradiated (635 nm, 5 mW cm^{-2}) for 30 min, and stained with DAPI. Cells without light irradiation served as the control. Bar represents 40 μm.

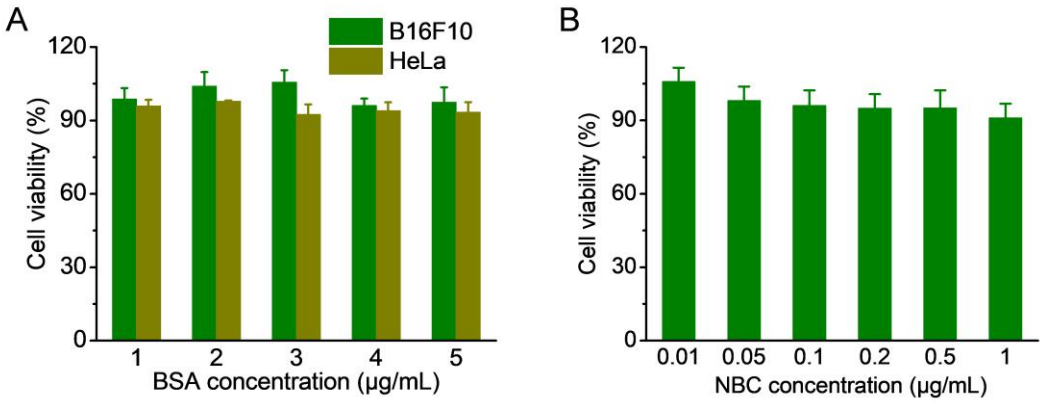


Figure S9. (A) Cytotoxicity of KHHB NCs toward B16F10 and HeLa cells following 24-h incubation at various BSA concentrations as determined by the MTT assay (n = 3). (B) Cytotoxicity of H_2O_2-treated NBC toward HeLa cells following 24-h incubation at various NBC concentrations as determined by the MTT assay (n = 3).

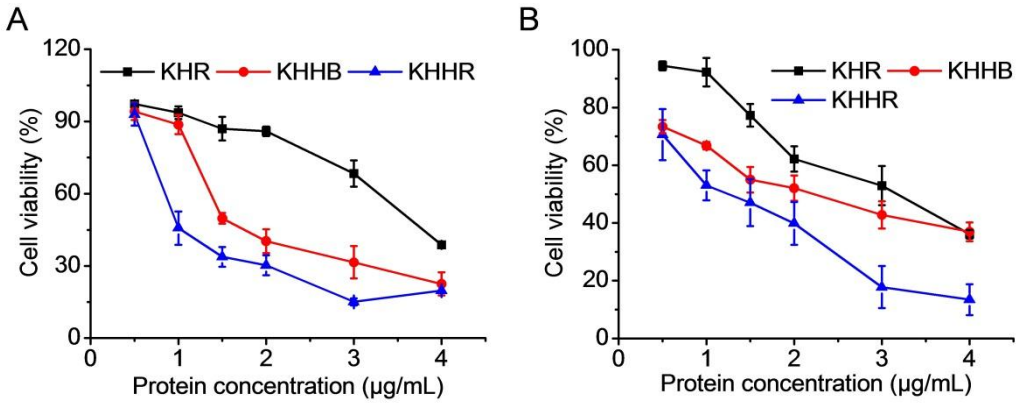


Figure S10. Cytotoxicity of KHR NCs, KHHB NCs, and KHHR NCs toward B16F10 (A) and 4T1 (B) cells as determined by the MTT assay (n = 3). Cells were treated with NCs for 4 h, irradiated (635 nm, 5 mW cm^{-2}) for 30 min, and further incubated in fresh media for 20 h before viability assessment by the MTT assay.

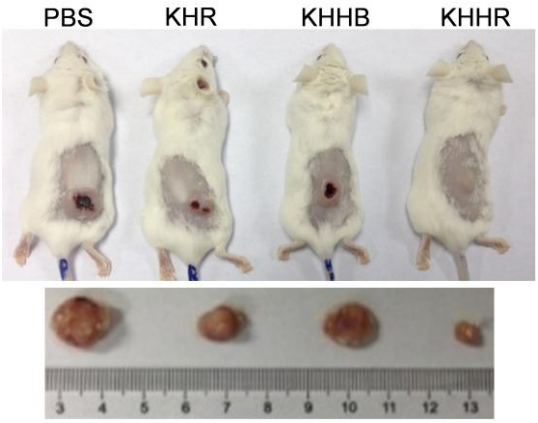


Figure S11. *In vivo* anticancer performance of KHR NCs, KHHB NCs, KHHR NCs, and PBS (control) in 4T1 tumor-bearing BALB/c mice. Mice were treated as described in Figure 5, and photographs were taken on day 12.

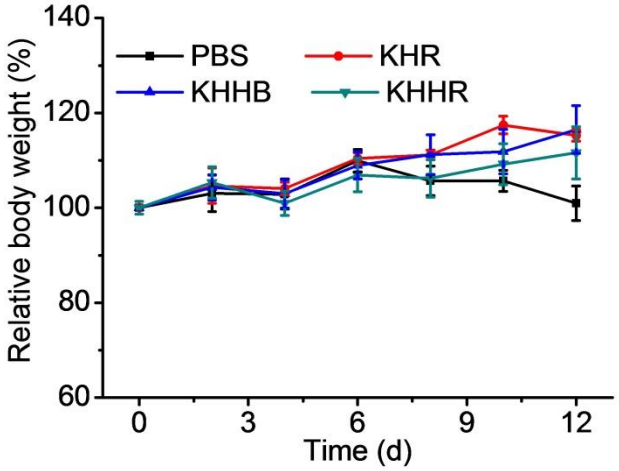


Figure S12. Body weight changes of mice treated as described in Figure 5 within the observation period of 12 d (n = 9).

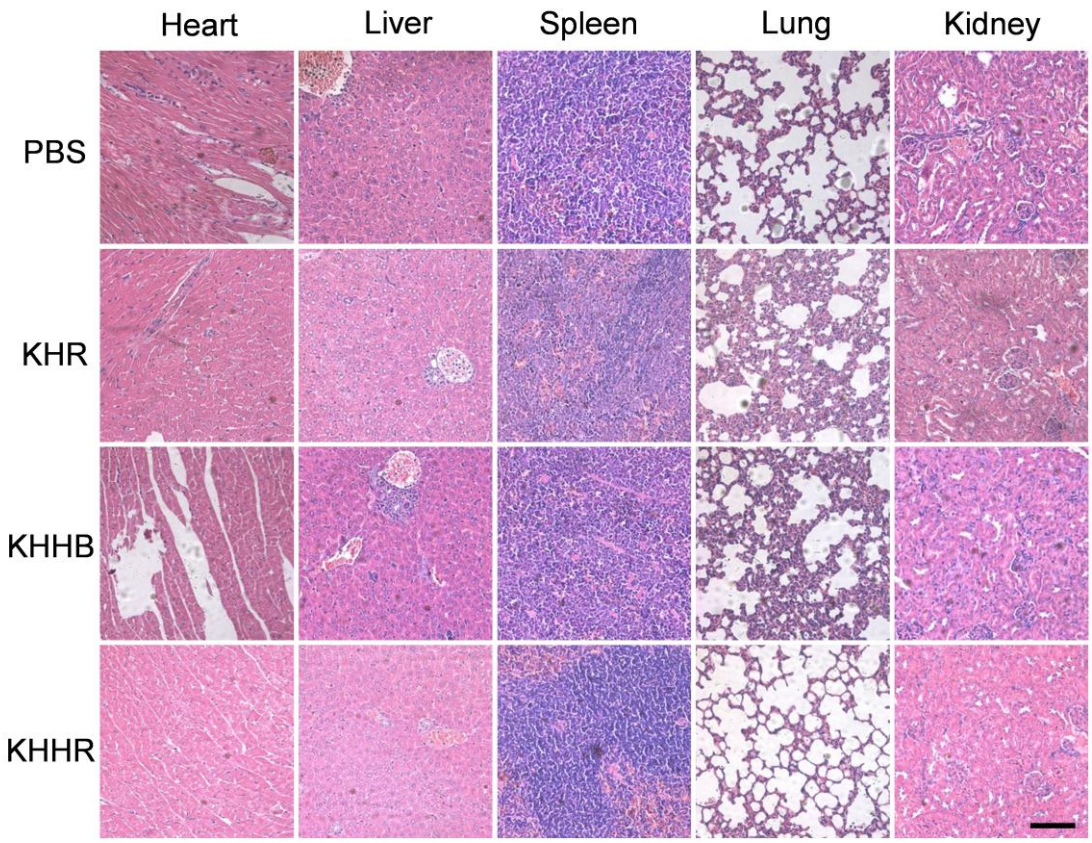


Figure S13. H&E staining of major organ sections harvested from mice on day 12. Bar represents 100 μm.

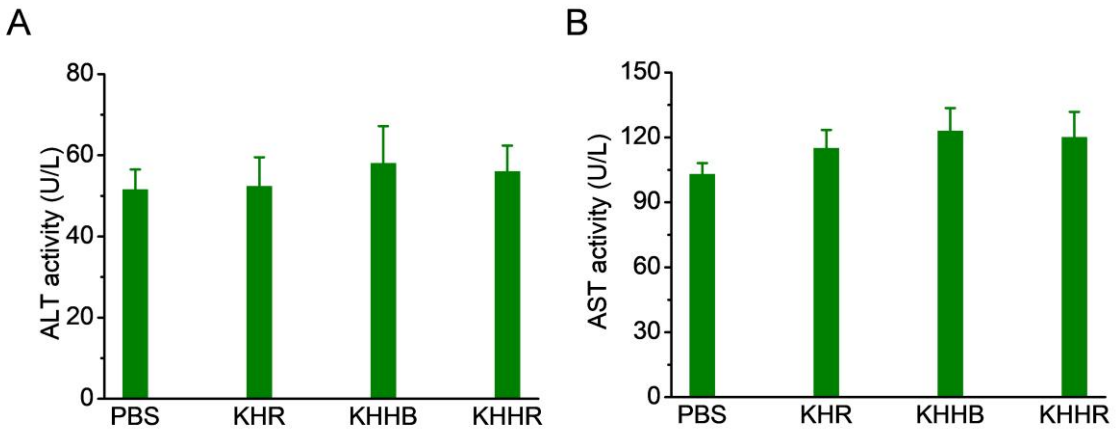


Figure S14. Serum ALT and AST levels in mice at 12 h post the second injection (n = 3). PBS, KHR NCs, KHHB NCs, or KHHR NCs were *i.v.* injected to mice on day 1 and day 4 at 1.75 mg RNBC (or BSA) kg^{-1}.