Supporting Information

Acid-Labile Polysaccharide Prodrug via Lapatinib Sensitizing Effect Substantially Prevented Metastasis and Postoperative Recurrence of Triple Negative Breast Cancer

Junhui Sui,[a] Mingda Zhao,[a] Yuedi Yang,[a] Zhihao Guo,[b] Mengcheng Ma,[a] Zhiyi Xu,[a] Jie Liang,[a] Yong Sun,[a] Yujiang Fan,[a*] and Xingdong Zhang[a]*

[a]National Engineering Research Center for Biomaterials, Sichuan University, 29 Wangjiang Road, Chengdu, Sichuan, 610064, P. R. China

[b]Center for Molecular Science and Engineering, College of Science, Northeastern University, Shenyang 110819, China.

Corresponding Author

Yong Sun*

Biomaterials Building, Sichuan University, Chengdu 610064, China

Tel: +86-28-85417654 Fax: +86-28-85417654 e-mail: sunyong8702@scu.edu.cn

Yujiang Fan*

Biomaterials Building, Sichuan University, Chengdu 610064, China

Tel: +86-28-85416196 Fax: +86-28-85410246 e-mail: fan_yujiang@scu.edu.cn

Supplementary Tables

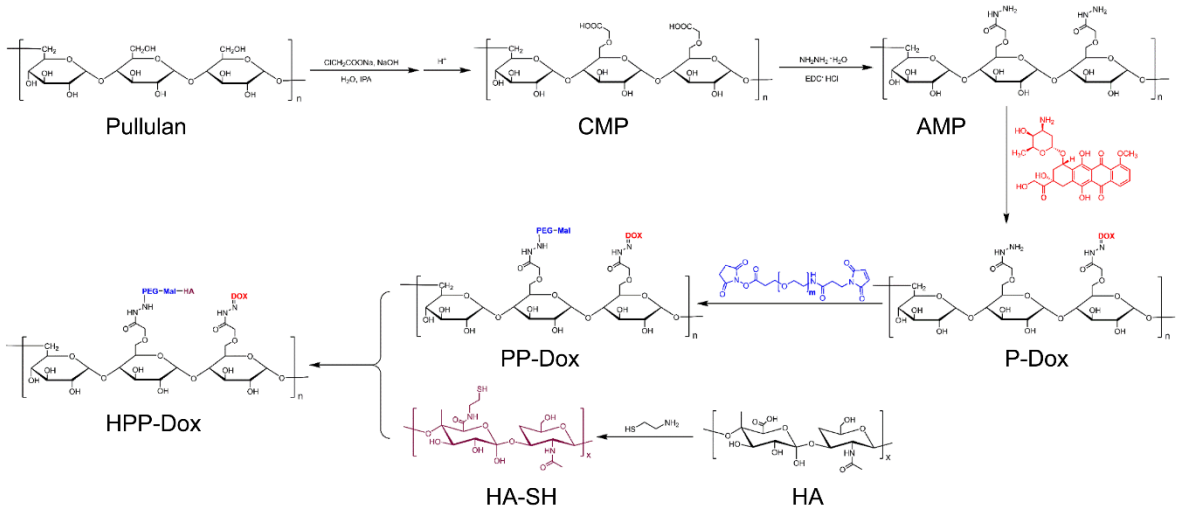

Figure S1. Synthetic routes of HPP-Dox.

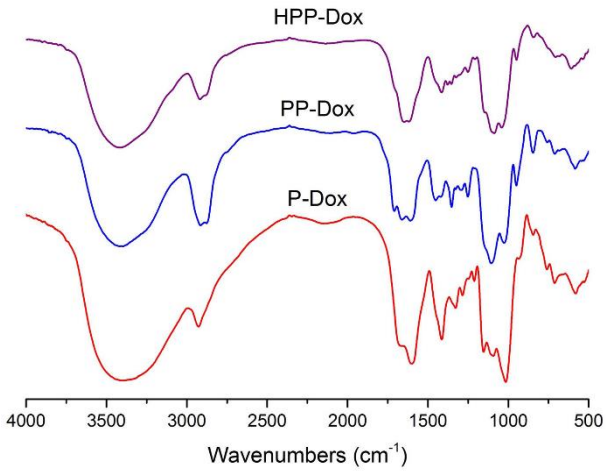

Figure S2. FT-IR spectra of polymers.

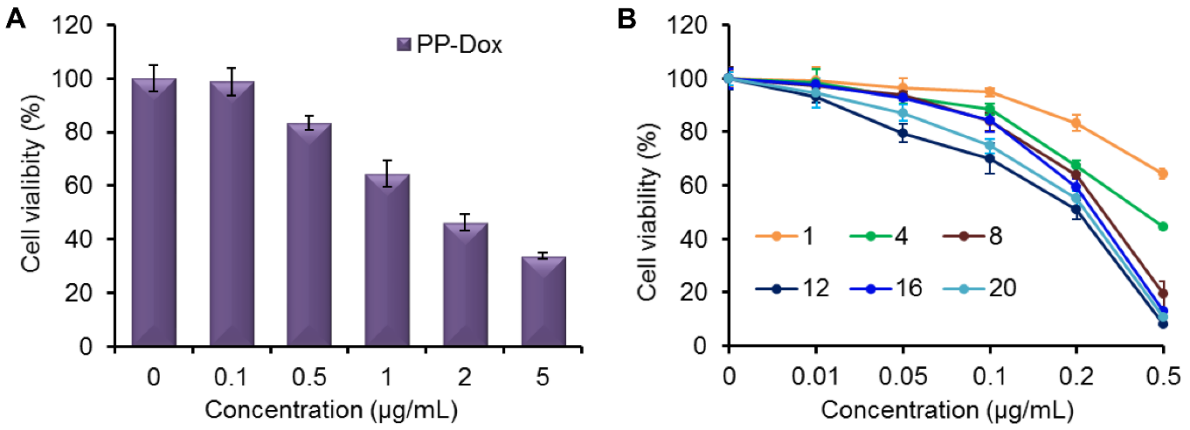

Figure S3. The relative cell viabilities of 4T1 cells incubated with different concentrition of (A) PP-Dox and (B) PP-Dox/Lap with different ratio of Dox/Lap for 24 h. 1, 4, 8, 12, 16 and 20 represented PP-Dox/Lap that the theoretical ratio of Lap with Dox was 1, 4, 8, 12, 16 and 20, respectively.

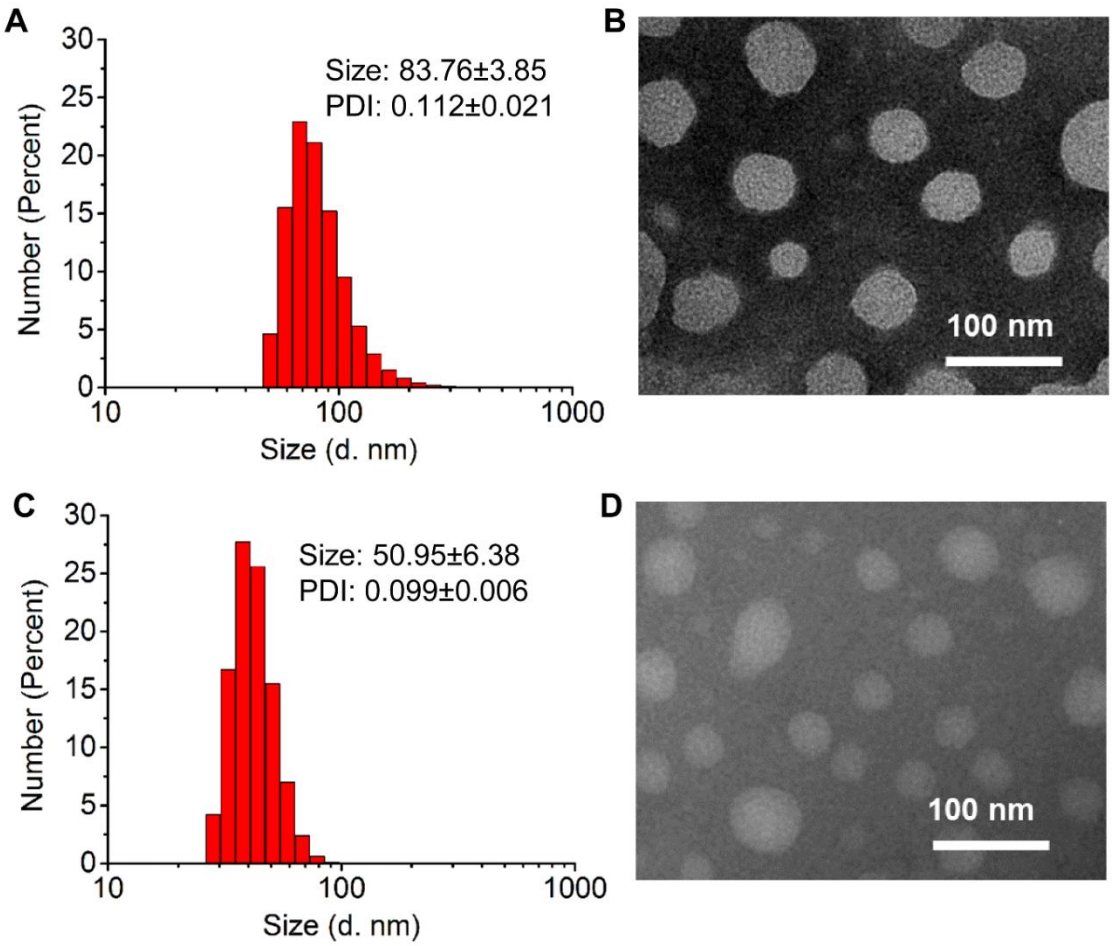

Figure S4. DLS data of (A) PP-Dox/Lap and (C) HPP-Dox/Lap NPs. TEM images of (B) PP-Dox/Lap and (D) HPP-Dox/Lap NPs.

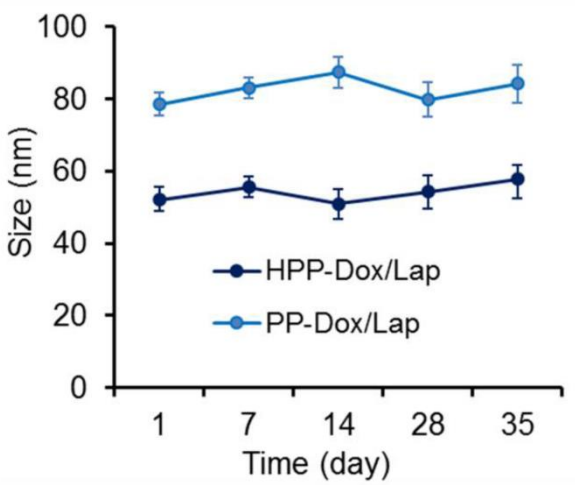

Figure S5. The size changes of PP-Dox/Lap and HPP-Dox/Lap NPs with time at 1.0 mg/mL in PBS.

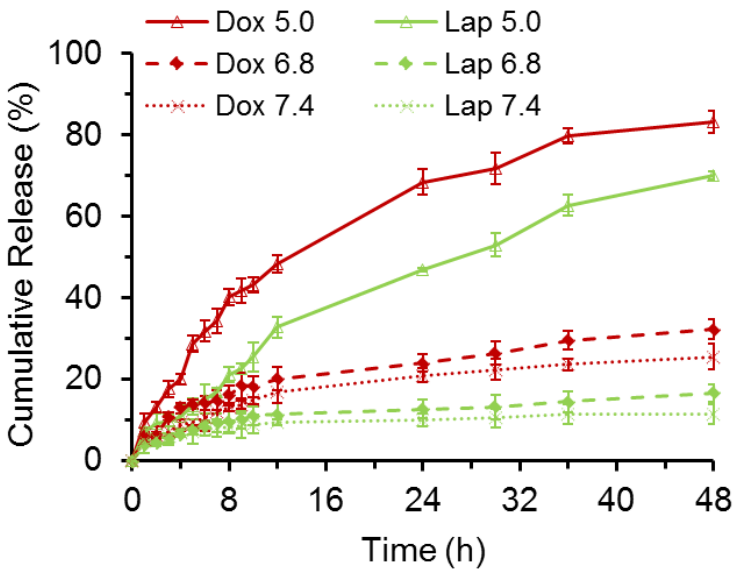

Figure S6. *In vitro* release profiles of Dox and Lap from HPP-Dox/Lap NPs in pH 7.4, pH 6.8 and pH 5.0 media at 37 ℃.

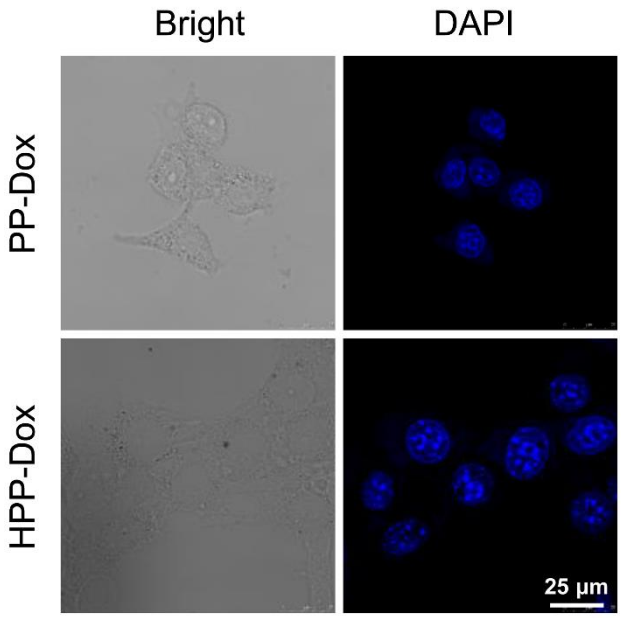

Figure S7. CLSM images of bright and DAPI of 4T1 cells after 4 h incubation with PP-Dox and HPP-Dox.

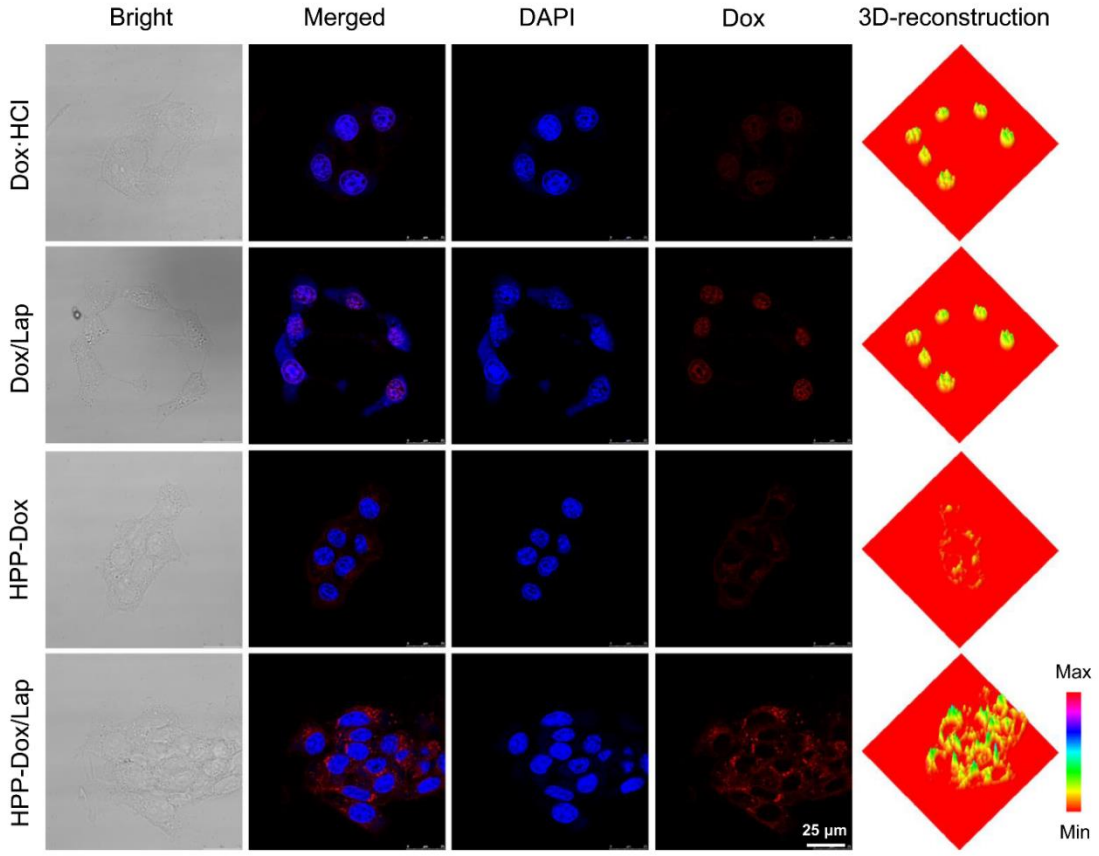

Figure S8. CLSM images and 3D-reconstruction images of Dox penetration into 4T1 cells after 1 h incubation with different formulations.

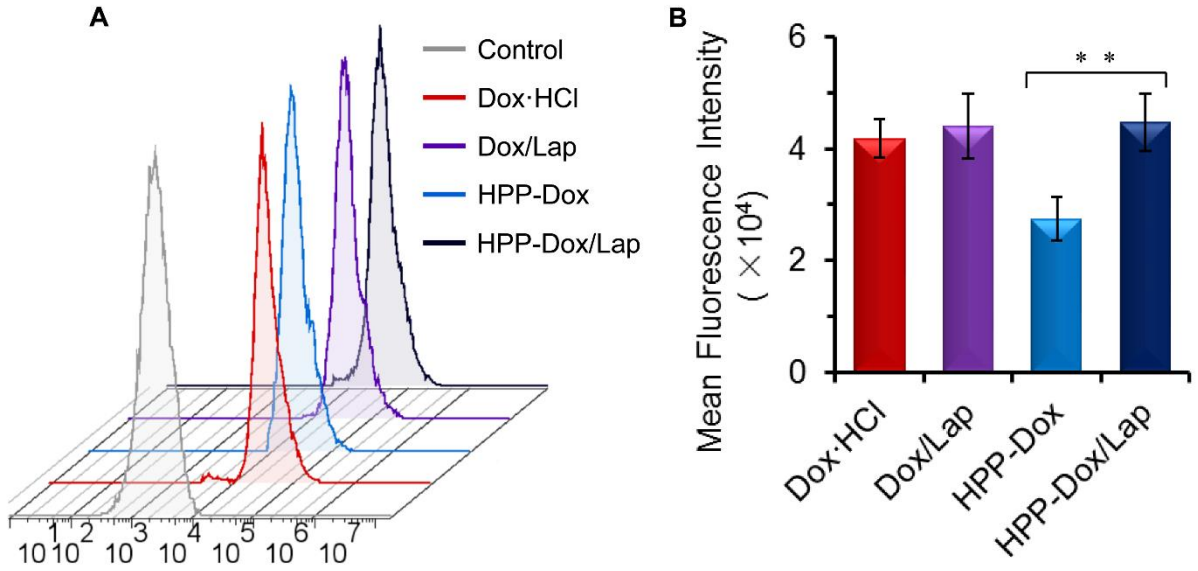

Figure S9. (A) Flow cytometry analysis and (B) Mean fluorescence intensity of intracellular Dox from the flow cytometric results after incubation with different formulations for 1 h.

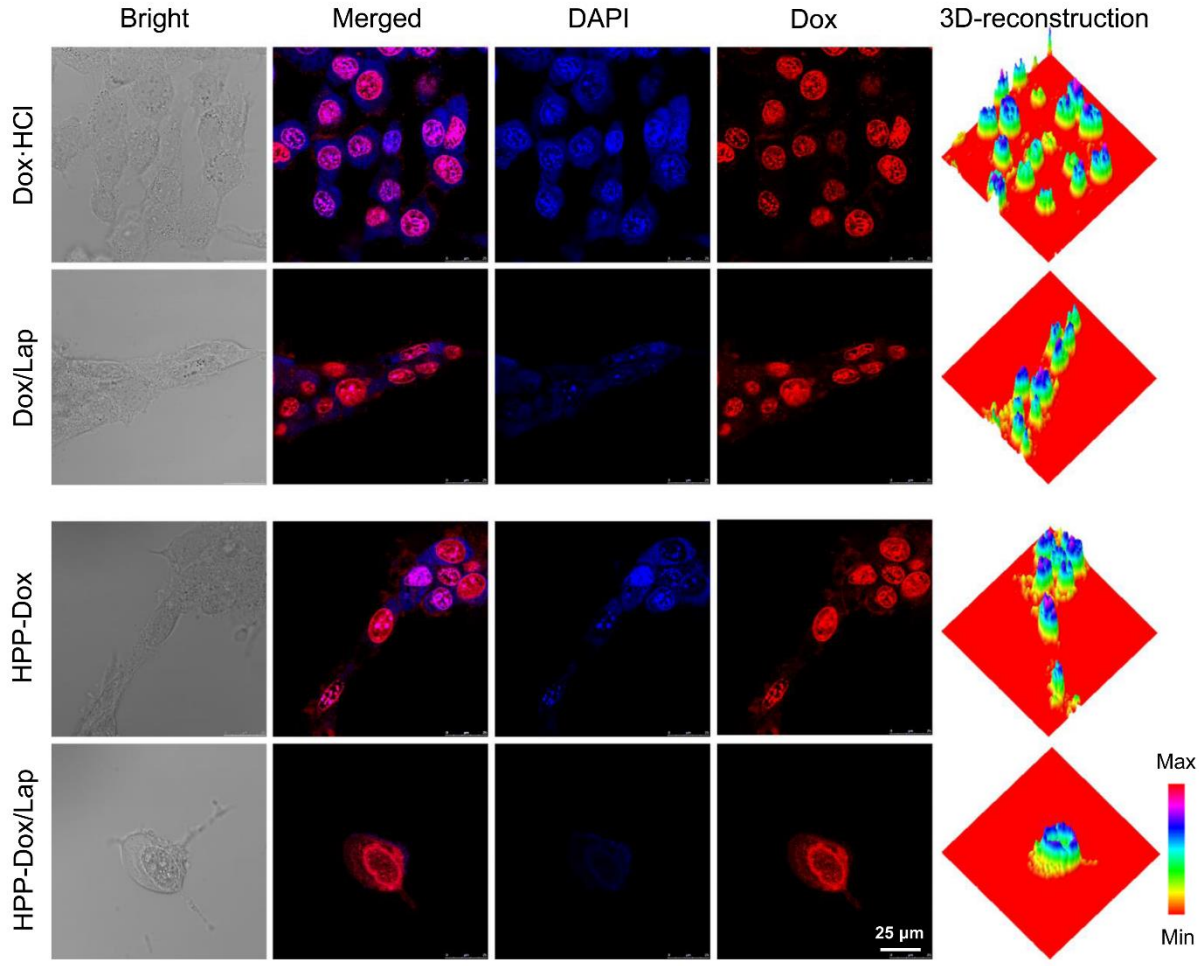

Figure S10. CLSM images and 3D-reconstruction images of Dox penetration into 4T1 cells after 4 and 12 h incubation with Dox·HCl and Dox/Lap, respectively.

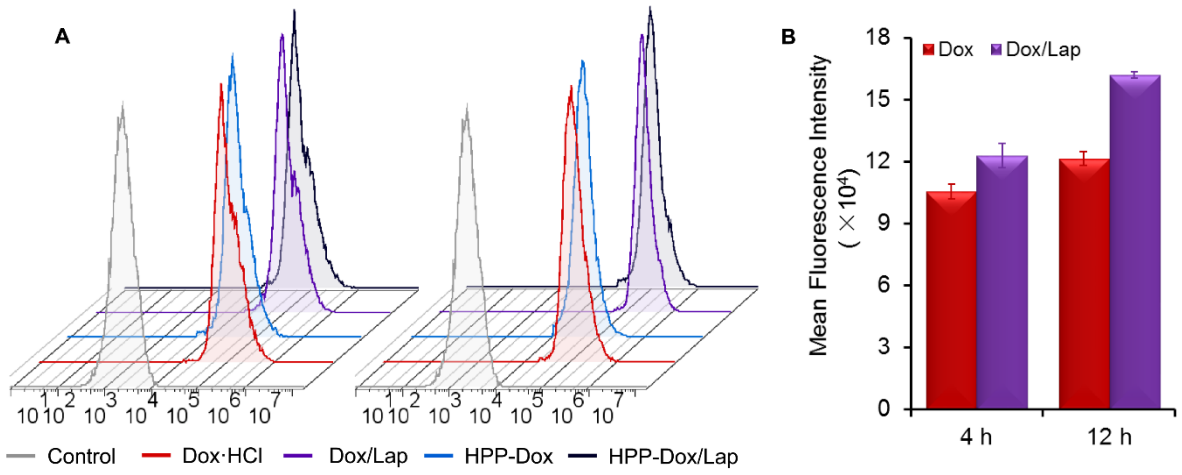

Figure S11. (A) Flow cytometry analysis and (B) Mean fluorescence intensity of intracellular Dox from the flow cytometric results after incubation with different formulations for 4 and 12 h, reapectively.

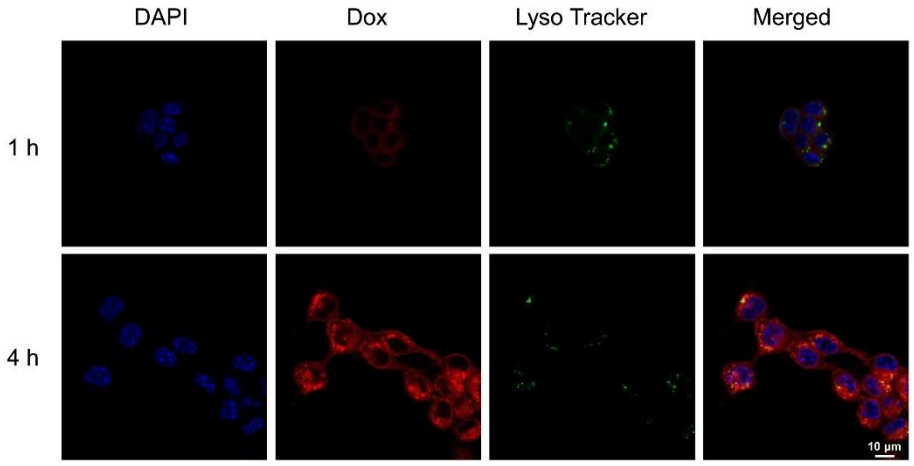

Figure S12. CLSM images of endosomal escape in 4T1 cells treated with HPP-Dox/Lap NPs for 1 h and 4 h, respectively, and then incubated with Lyso Tracker-Green and Hoechst 33342.

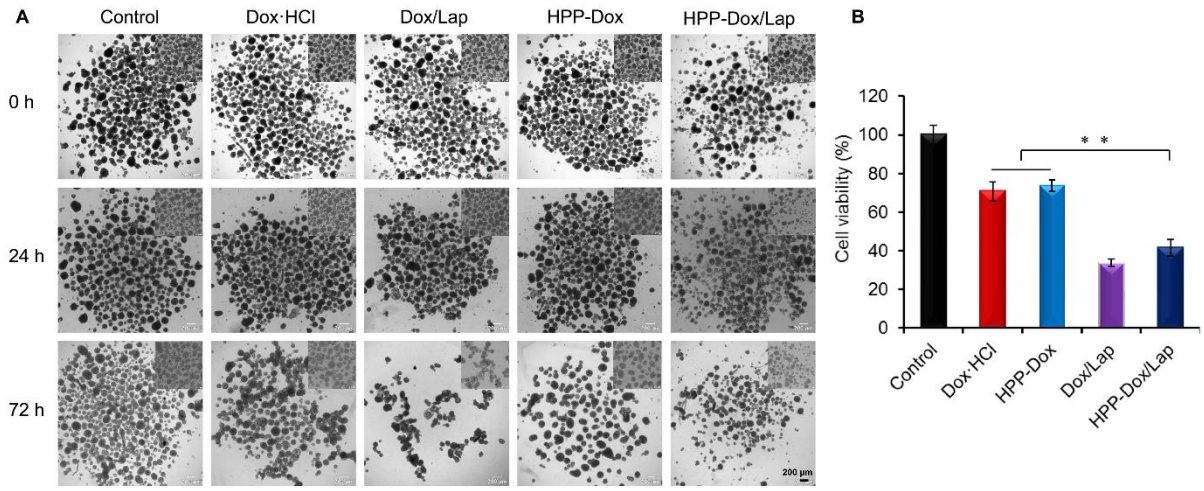

Figure S13. (A) Typical micrographs of 3D-cultured tumor spheres from 4T1 cells after treatment with various formulations for 24 h and 72 h, respectively. (B) The relative cell viabilities of 3D suspension tumor spheres incubated with different formulations for 72 h.

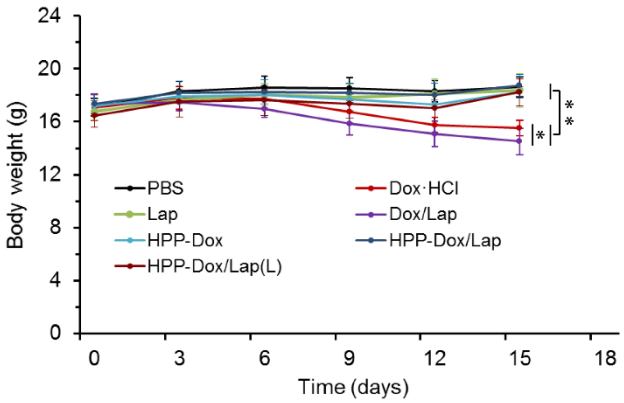

Figure S14. Body weight changes of tumor-bearing BALB/c mice after 15 days following administration with different formulations. n=10, *p < 0.05, **p < 0.01.

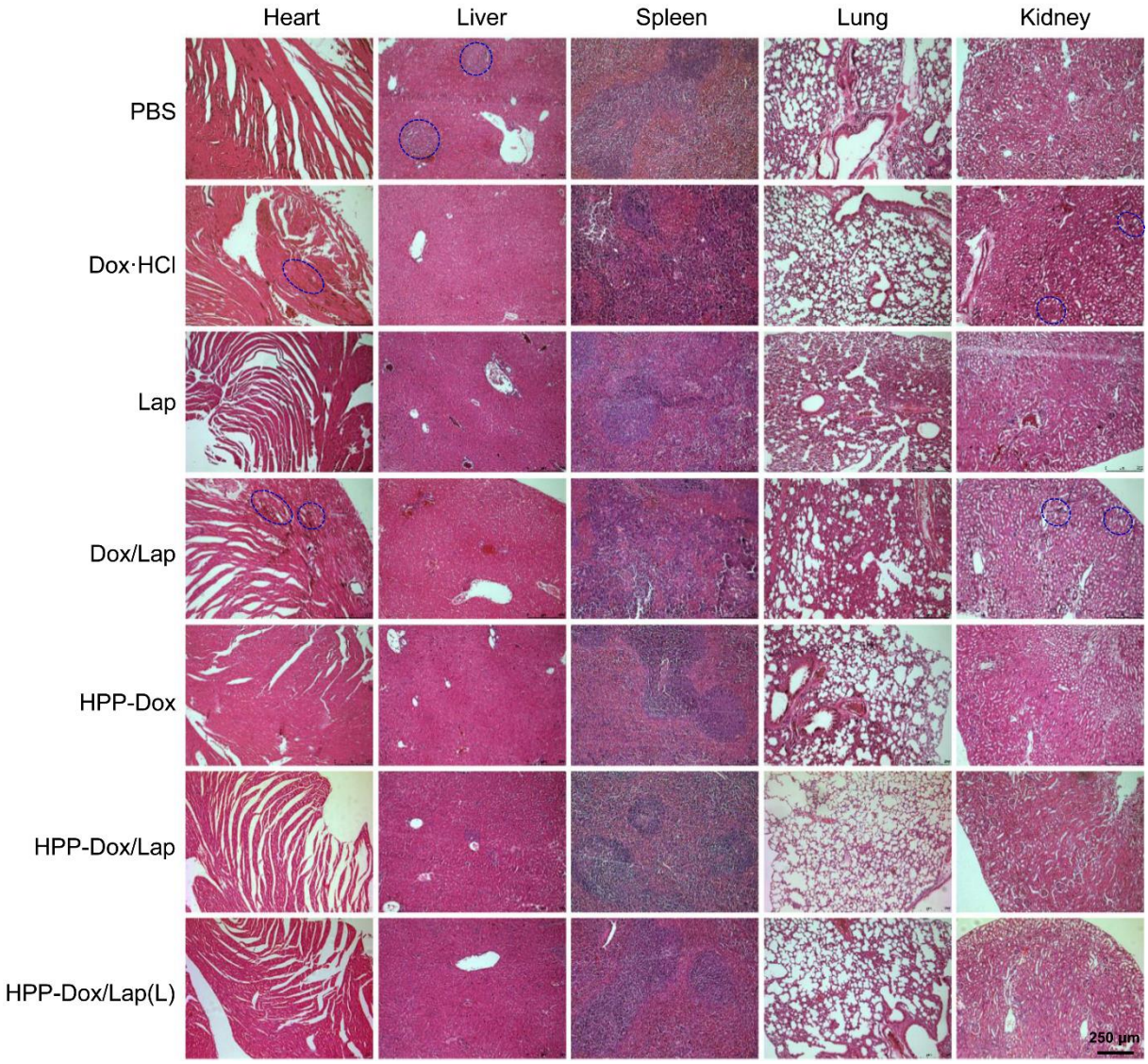

Figure S15. Histological observations after H&E staining for different organs of tumor-bearing BALB/c mice after treatment with different formulations for 15 days. Blue circles indicate the damage of organs.

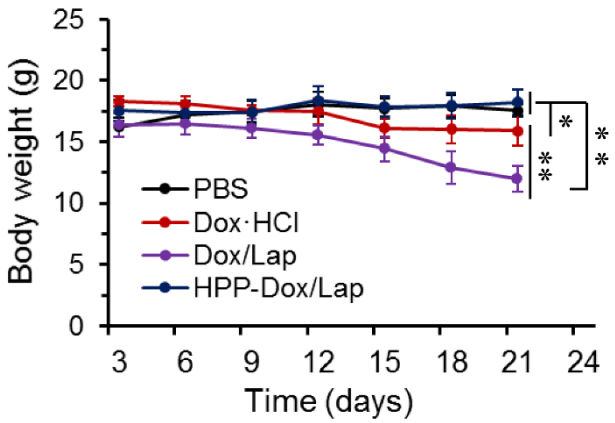

Figure S16. Body weight changes of tumor-bearing BALB/c mice after removal of the primary tumor following administration with different formulations. n=5, *p < 0.05, **p < 0.01.

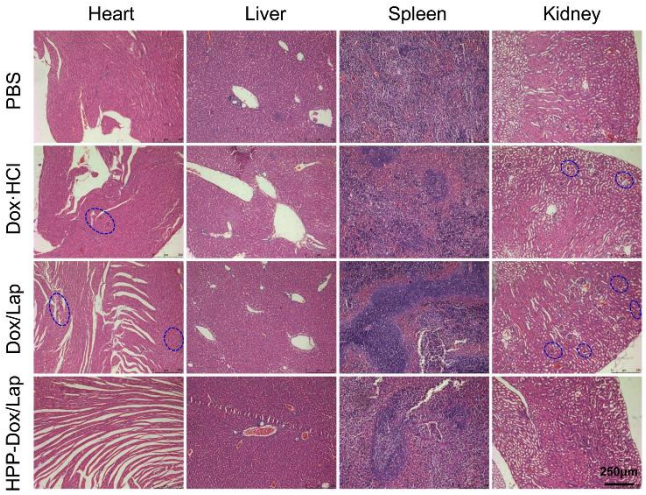

Figure S17. Histopathologic examination of the organs from postoperative 4T1 recurrence and metastasis model after 15 days following administration with different formulations. Blue circles indicate the damage of organs.

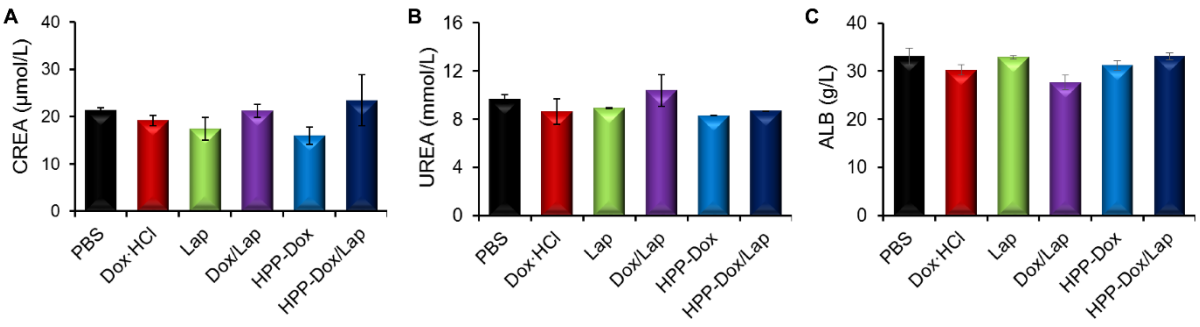

Figure S18. Biochemical markers (A) CREA, (B) UREA and (C) ALB levels in plasma from normal BALB/C mice after 15 days following administration with different formulations.

Supplementary Tables

Table S1. Physicochemical characteristics and IC_{50} value of various formulations.

	Theoretical ratio: Lap/Dox	Quality ratio: Lap/Dox	DLC of Dox (%)	DLC of Lap (%)	EE of Lap (%)	IC_{50} of Dox (μg/mL)	IC_{50} of Lap (μg/mL)
Dox	—	—	—	—	—	1.64 ± 0.17	—
Lap	—	—	—	—	—	—	17.29 ± 3.53
PP-Dox	—	—	5.94	—	—	1.79 ± 0.21	—
PP-Dox/Lap	1	0.89	5.64	5.02	89	1.17 ± 0.18	1.05 ± 0.24
PP-Dox/Lap	4	3.78	4.83	18.2	93.8	0.38 ± 0.03	1.53 ± 0.13
PP-Dox/Lap	8	6.18	4.17	29.8	89.4	0.29 ±0.017	2.31 ± 0.20
PP-Dox/Lap	12	11.09	3.58	39.7	92.3	0.19 ± 0.02	2.30 ± 0.28
PP-Dox/Lap	16	14.72	3.17	46.7	91.9	0.24 ± 0.03	3.83 ± 0.38
PP-Dox/Lap	20	18.35	2.84	52.1	91.4	0.20 ± 0.02	4.12 ± 0.40
HPP-Dox	—	—	3.05	—	—	1.42 ± 0.16	—
HPP-Dox/Lap	12	—	2.35	26.08	—	0.16 ± 0.017	2.0 ± 0.197

Table S2. The sensitizing effect on various formulations to free drugs.

	IC_{50} of Dox (μg/mL)	IC_{50} of Lap (μg/mL)	CI	Sensitization ratio to	
				Dox	Lap
Lap	—	17.29 ±3.53	—	—	1
Dox·HCl	1.64 ±0.17	—	—	1	—
PP-Dox	1.79 ±0.21	—	—	0.92	—
HPP-Dox	1.42 ±0.16	—	—	1.15	—
PP-Dox/Lap	0.19 ±0.02	2.30 ±0.28	0.24	8.63	7.52
HPP-Dox/Lap	0.16 ±0.017	2.0 ±0.197	0.213	10.25	8.65

Table S3. The characterization of size and zeta potential of various formulations.

	Size (d. nm)	PDI	Zeta Potential (mV)
PP-Dox	33.28 ±3.73	0.17 ±0.037	-16.57 ±1.33
HPP-Dox	30.56 ±4.20	0.21 ±0.025	-26.45 ±2.82
PP-Dox/Lap	83.76 ±3.85	0.112 ±0.021	-14.27 ±0.83
HPP-Dox/Lap	50.95 ±6.38	0.099 ±0.006	-28.60 ±2.08