

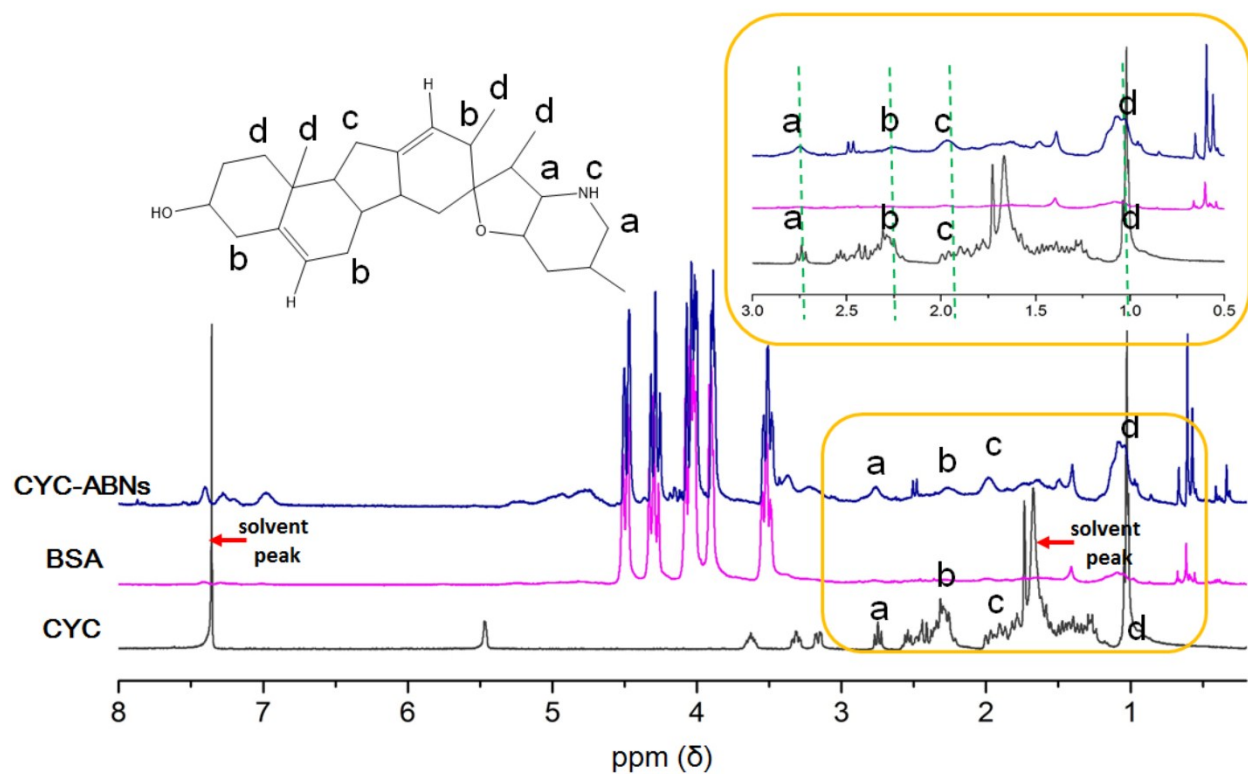
## **Extracellular retention of cyclopamine nanoformulation leveraging larger size and more negative charge for improved breast cancer treatment**

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### **Supporting Information for Publication**

#### **Additional Figures**

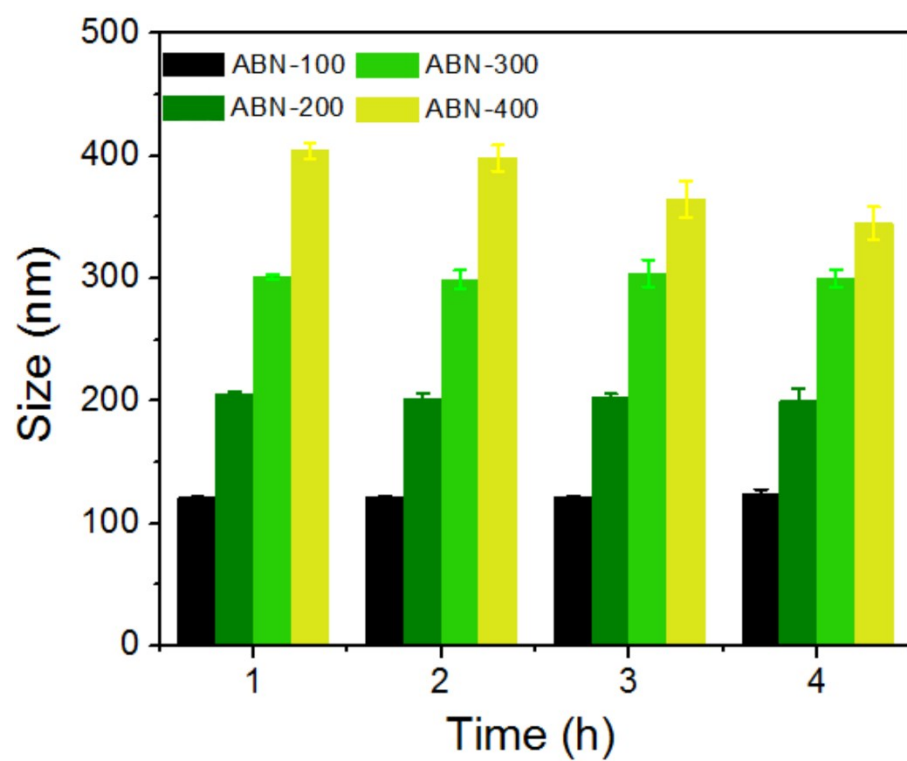
To support the results presented in the main article, additional figures are shown in this supporting document.



**Figure S1.**  $^1\text{H}$  NMR spectroscopy of BSA, CYC and CYC-ABNs.

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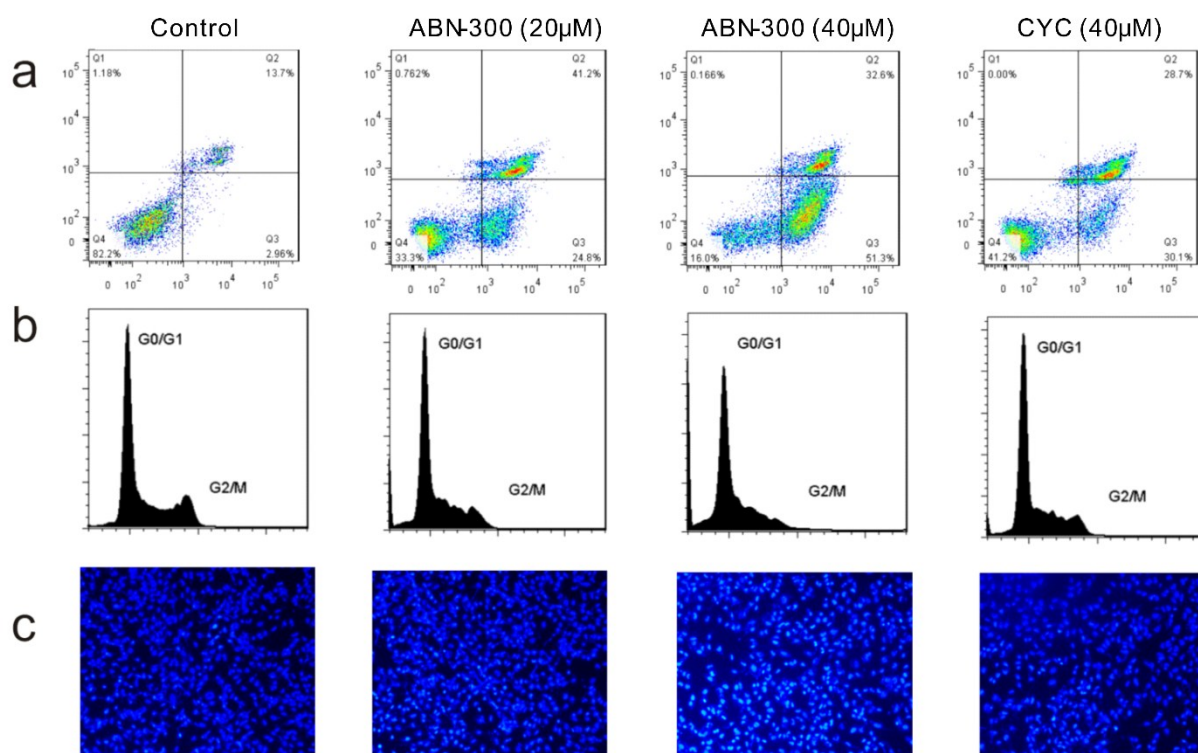
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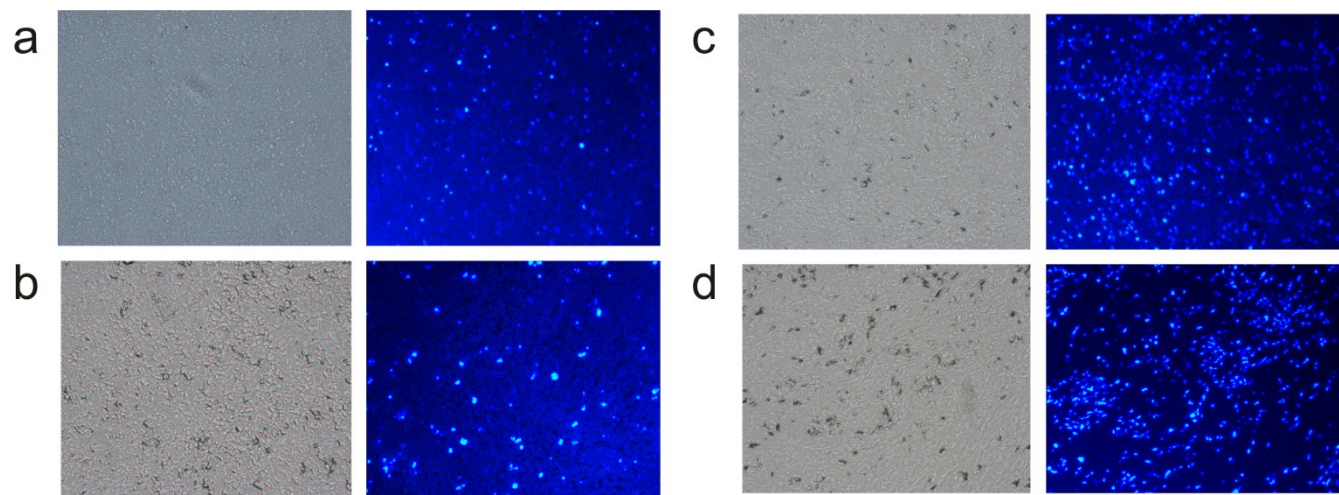
**Figure S2.** Size of CYC-ABNs with different size incubating with PBS with different time.

**Table S1.** Size and charge of CYC-ABNs.

Formulation	ABN-100	ABN-200	ABN-300	ABN-400
Size [nm]	100	200	300	400
Surface potential	$-7 \pm 1$	$-11 \pm 2$	$-13 \pm 1$	$-15 \pm 1$



**Figure S3.** Comparison between the antitumor effect of free CYC and CYC-ABNs. a) Annexin V/PI staining assays of control, ABN-300 (20 μM), ABN-300 (40 μM), and CYC (40 μM). b) Cell cycle assays of control, ABN-300 (20 μM), ABN-300 (40 μM), and CYC (40 μM). c) Hoechst staining of control, ABN-300 (20 μM), ABN-300 (40 μM), and CYC (40 μM).



**Figure S4.** Hoechst staining of tumor tissues dissected from mice after treated with different treatment groups at day 14. a) PBS treated group b) CYC treated group c) ABN-100 treated d) ABN-300 treated.

**Table S2.** Blood biochemical indexes of mice treated with ABNs.

Test	Sex	Normal	Sham	ABNs
GOT/AST [U/L]	M	74–143	61.8 ± 1.1	60.8 ± 1.1
	F	65–203	73.6 ± 0.8	83.6 ± 0.8
GPT/ALT [U/L]	M	18–45	40.4 ± 0.5	45.4 ± 0.5
	F	16–48	22.6 ± 0.8	28.6 ± 0.8
Urea [mg/ dL]	M	12.3–24.6	16.5 ± 0.9	18.1 ± 0.8
	F	13.2–27.1	17.1 ± 1.8	13.3 ± 0.8
Creatinine [mg/ dL]	M	0.2–0.5	0.39 ± 0.1	0.44 ± 0.02
	F	0.2–0.6	0.36 ± 0.04	0.42 ± 0.06