Supplementary information

Hyaluronic acid hydrophilic surficial rehabilitative curcumin nanocrystals for targeted breast cancer treatment with prolonged biodistribution

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EXPERIMENTAL SECTION

1. Quantitative measurement of the Cur loaded in HA@Cur-NC through UV-vis method.

Accurately weigh Curcumin (Cur) 10.0 mg into a 10 mL volumetric flask, added anhydrous ethanol to the scale, and shaken well to obtain 1000 μg mL^{-1} Cur reserve solution. Then dilute it with anhydrous ethanol to different concentrations (7.5 μg mL^{-1}, 5 μg mL^{-1}, 3.75 μg mL^{-1}, 2.5 μg mL^{-1}, 1 μg mL^{-1}, 0.5 μg mL^{-1}, 0.25 μg mL^{-1}, and 0.125 μg mL^{-1}). The absorbance (A) of different samples at 426 nm wavelength was determined by UV-vis spectrophotometer with anhydrous ethanol as blank control. The obtain standard curve is $y = 0.13391x + 0.00207$, $R^2 = 0.9981$ (y: absorbance value at 426 nm; x: concentration of Cur) (Fig. S1).

2. Quantitative measurement of the Cur in vivo through a high-performance liquid chromatography (HPLC) method.

Samples were analyzed by HPLC (LC-10AT, Japan) with the following conditions: an Inertsil ODS-SP column (4.6 mm × 250 mm, 5.0 μm, Japan); mobile phase: Acetonitrile: 1% acetic acid solution = 55:45 (*v/v*); column temperature 25 °C; detection wavelength 426 nm; flow rate 1.0 mL min^{-1} and injection volume 20 μL. Accurately weigh Cur 10.0 mg into a 10 mL volumetric flask, adding methanol to the mark, shake well, and obtain 1000 μg mL^{-1} Cur reserve solution. After that, dilute to different concentrations (10, 5, 2, 1, 0.5, 0.1, 0.05, 0.025 μg mL^{-1}) using 70% methanol-blank plasma mixed solution, and HPLC obtained the peak area of different

samples. The peak area y was plotted on the ordinate, and the concentration x was plotted on the abscissa. The obtain standard curve is y = 159768x + 953.75, R^2 = 0.99975 (y: peak area value at 426 nm; x: concentration of Cur, R^2 = 0.99975) (Fig. S2).

List of figures

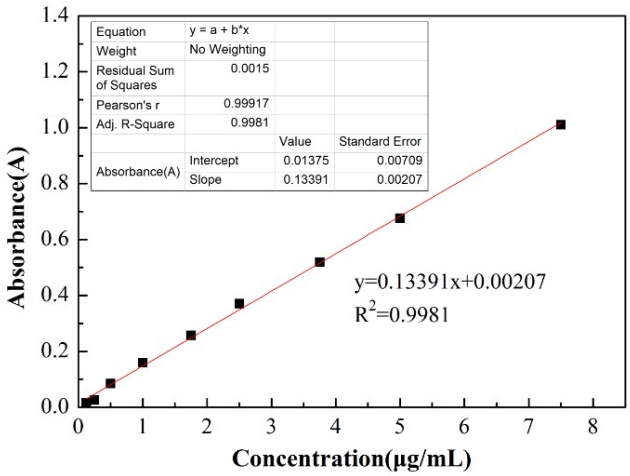

Fig. S1 The quantification of Cur. The absorption spectra of inset: The standard curve for absorbance value at 426 nm. $y = 0.13391x + 0.00207$, $R^2 = 0.9981$

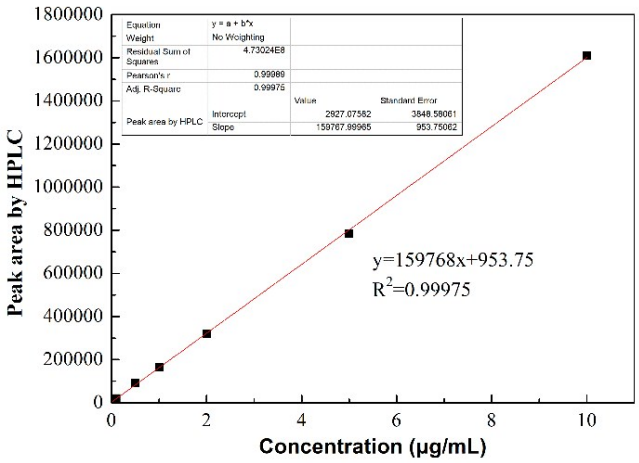

Fig. S2 The obtain standard curve is y = 159768x + 953.75, $R^2 = 0.99975$

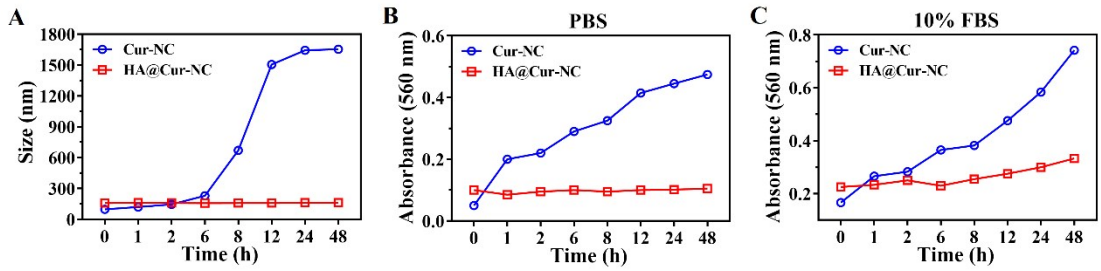

Fig. S3 Long-term stability study of different Cur formulations. (A) Size change was measured in PBS, and change in absorbance at 560 nm was measured in both (B) PBS and (C) 10% FBS.

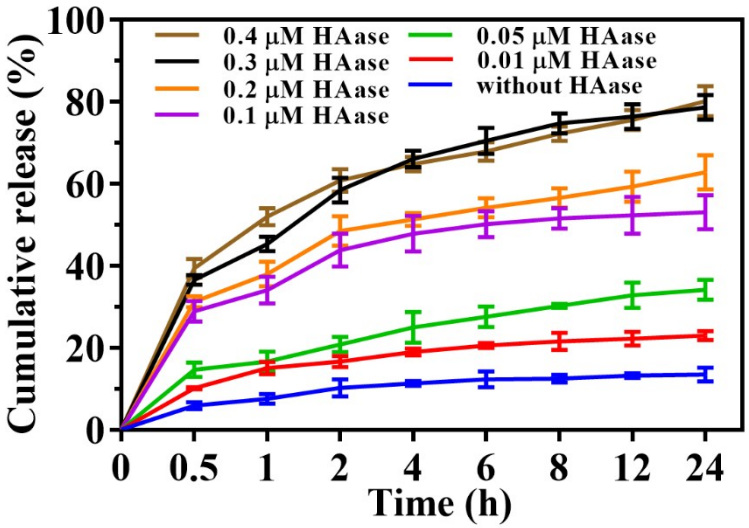

Fig. S4 The release behavior of HA@Cur-NC at pH 5.0 plus 0.5% Tween and different concentrations of HAase.

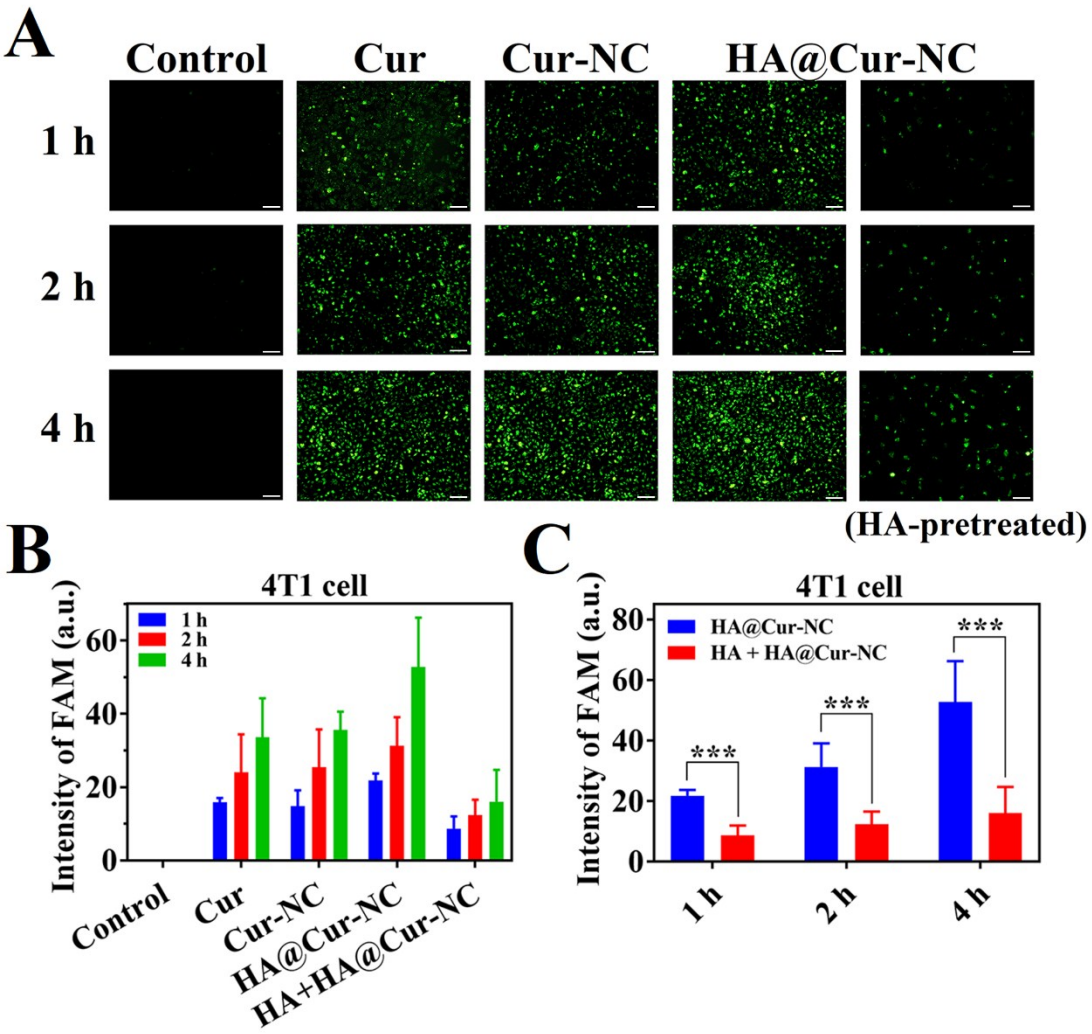

Fig. S5 Uptake in vitro. (A) The fluorescent microscopy images of 4T1 cells after incubating with Cur, Cur-NC, HA@Cur-NC, and HA@Cur-NC (with HA-pretreated) for different hours. Cells without any treatment were as a control group (Scale bar: 100 μm). (B, C) Analysis of FAM fluorescence value according to section A.

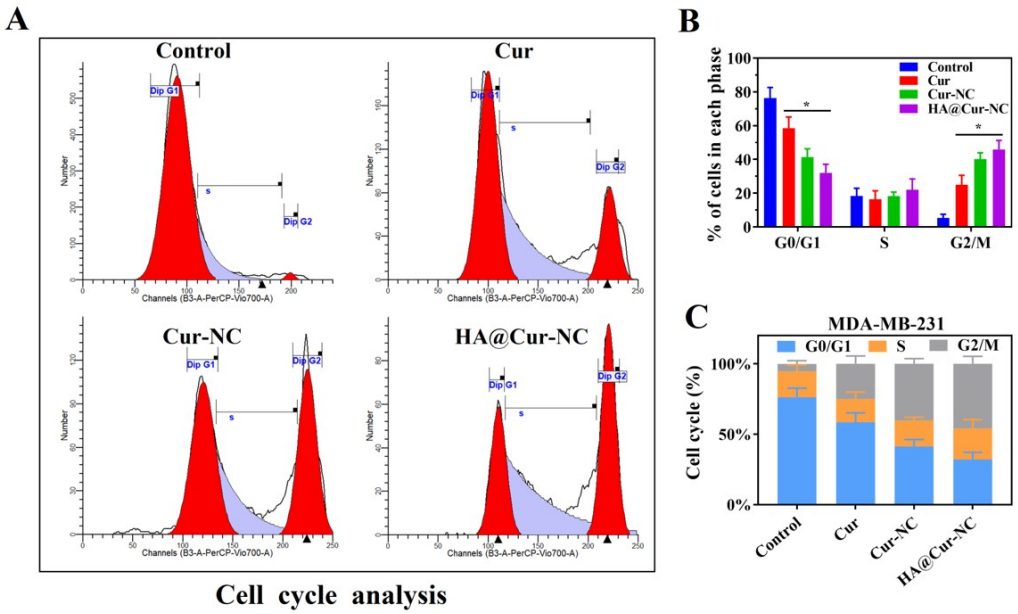

Fig. S6 (A) Flow cytometry analysis of the cycle of MDA-MB-231 cells induced by PBS (control), Cur, Cur-NC, and HA@Cur-NC for 24 h. (B, C) Quantitative analysis of cell cycle profiles of MDA-MB-231 cells according to section A. Data represent the mean of the percentage of cell distribution in each phase ± SD; **P*< 0.05 *vs.* control after one-way ANOVA analysis.

Table S1 Size and PDI of Cur-NC at different weight ratios of Cur to F127 (*w/w*). Data are shown as means ± SD (*n* = 3).

Cur : F27	Sizes (nm)	PDI	Note
1 : 0.25	-	-	instability and precipitate rapidly
1 : 0.5	359.30 ± 10.71	0.71 ± 0.07	
1:1	244.47 ± 7.45	0.65 ± 0.07	
1:2	165.80 ± 3.07	0.47 ± 0.03	
1:4	101.37 ± 7.36	0.33 ± 0.03	
1:5	104.78 ± 7.64	0.34 ± 0.04	

Table S2 Effect of molecular weight of HA to Cur-NC on Size and PDI index of HA@Cur-NC. HA : Cur-NC = 4:1 (*w/w*), Cur : F127 = 1 : 4 (*w/w*). Data are shown as means ± SD (*n* = 3).

Molecular weight of HA (kDa)	Sizes (nm)	PDI
36	396.59 ±19.32	0.43 ± 0.04
100	359.30 ± 10.71	0.35 ± 0.02
570	161.85 ± 1.70	0.25 ± 0.02
770	243.81 ± 9.30	0.29 ± 0.03
1400	302.68 ± 13.18	0.31 ± 0.02

Table S3 Effect of weight ratios of HA to Cur-NC on Size and PDI index of HA@Cur-NC. Molecular weight of HA = 570 kDa, Cur : F127 = 1 : 4 (*w/w*). Data are shown as means ± SD (*n* = 3).

HA : Cur-NC (*w/w*)	Sizes (nm)	PDI
8:1	164.14 ± 5.85	0.29 ± 0.03
4:1	161.85 ± 1.70	0.25 ± 0.02
2:1	143.46 ± 7.59	0.33 ± 0.04
1:1	131.29 ± 9.22	0.32 ± 0.06
2:5	109.92 ± 13.75	0.34 ± 0.05

Table S4 Physicochemical properties of Cur-NC and HA@Cur-NC. Data represented as means ± SD (n = 3).

	Size (nm)	Zeta potential (mV)	PDI	Drug loading (wt%)
Cur-NC	101.4 ± 7.4	-7.1 ± 0.2	0.33	15.3 ± 0.7
HA@Cur-NC	161.9 ± 1.7	-25.0 ± 0.8	0.25	3.3 ± 0.5

Table S5 IC_{50} values of different Cur formulations. Data represented as mean ± SD (n = 5).

Entry	IC_{50} (μg/mL)		
	MCF-7 cells	MDA-MB-231 cells	4T1 cells
Cur	3.90 ± 0.99	3.62 ± 0.75 *	3.18 ± 0.30 **
Cur-NC	3.88 ± 1.70	3.19 ± 0.46 **	2.92 ± 0.26 **
HA@Cur-NC	2.86 ± 0.50	1.92 ± 0.21	2.00 ± 0.17

*P < 0.05 and **P < 0.01 *vs.* HA@Cur-NC.

Table S6 Quantification data of integrated optical density (IOD) of tumor sections from each group with TUNEL staining. Results were expressed as mean ± SD; Student's *t*-test, **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 *vs.* HA@Cur-NC. Data represented as mean ± SD (*n* = 6).

	Control (saline)	Free Cur	Cur-NC	HA@Cur-NC
IOD	0.660 ± 0.131***	1.279 ± 0.507**	1.950 ± 1.262*	3.347 ± 1.602