Supplementary information

Part A: Experimental section

1. Synthesis of core and shell materials

1.1 BzPGA

The synthesis scheme was as shown in **Scheme S1**.

1.1.1 Preparation of Cetyltrimethyl Ammonium γ-PGA Salt (γ-PGA-CTA)

γ-PGA-Na (MW 1000 KDa, 10 g) was dissolved in 200 mL distilled water under stirring condition at 40 °C. An aqueous solution of CTA-Br (3% w/v) at 40 °C was then added dropwise into the γ-PGA solution under vigorous stirring until no additional precipitate was formed. The formed white precipitate was filtered off, washed three times with hot distilled water, and finally dried under vacuum. The product of γ-PGA-CTA was stored in a vacuum drier when not use.

1.1.2 Synthesis of BzPGA

2 g of the γ-PGA-CTA was first dissolved in N-Methyl pyrrolidone (NMP) under stirring. 1 g of $NaHCO_3$ and 1.88 mL of benzyl bromide were then added into the γ-PGA-CTA solution. The reaction system was stirred for 8 h at 50 °C. The mixture was cooled to room temperature and then filtered to remove insoluble precipitate. The supernatant was poured into a 400 mL of ice pre-cooled methonal-water mixed solution (*v:v* = 1:1, containing with 0.5% HCl) and continue stirring for 0.5 h in an ice bath. The precipitate was obtained by filtration and washed with ice pre-cooled methonal-water mixed solution (*v:v* = 1:1). The white polymer was dried under vacuum and the product of BzPGA was finally obtained.

1.2 HA-C6-ATRA

The synthesis scheme was as shown in **Scheme S2**.

1.2.1 Synthesis of All Trans Retinoic Acid -6- bromo hexyl ester (ATRA-C6-Br)

0.5 g of ATRA was dissolved in 10 mL tetrahydrofuran (THF) and then K_2CO_3 (0.65 g) was added in the solution. 1, 6-dibromohexane (8.59 mmol) dissolved in 4 ml of THF was added dropwise into the above solution. The reaction system was stirred for 4 h in the dark and at room temperature and then terminated by adding 14 mL water. The product of ATRA-C6-Br was extracted by ethyl acetate and then purified by column chromatography.

1.2.2 Preparation of the salt of tetrabutylammonium of HA (HA-TBA)

HA sodium salt (HA-Na, 9g) was dissolved in 450 mL distilled water and a 732 type cation exchange resin was added. After stirring for 6 h at room temperature, HA solution was obtained by filtration. TBA-OH (Tetrabutylammonium hydroxide, 25% w/v) was then added dropwise into the HA solution under vigorous stirring at room temperature and the pH of the solution was monitored. When the pH changed to 7, stop adding TBA-OH solution. The solution was instantly frozen and freeze-dried. The product of HA-TBA was stored in a vacuum drier when not use.

1.2.3 Synthesis of ATRA graft HA (HA-C6-ATRA)

0.44 g of HA-TBA (0.02 mmol, containing 0.668 mmol of a monomeric unit) was

dissolved in 15 mL DMSO under stirring. In order to obtain 20% grafted ratio HA-C6-ATRA, 62 mg ATRA-C6-Br (0.2 Eq. according to the mole numbers of monomeric unit for HA-C6-ATRA) was added into the HA-C6-ATRA solution with stiring at 40 ºC for 24 h. After reaction, 4 mL of 5% (*w/v*) NaCl aqueous solution was added. The resulting mixture was slowly poured into 80 mL acetone under stirring. A precipitate was formed which was filtered and washed three times with 6 mL acetone/H_2O 5:1 and then three times with acetone. The product of HA-C6-ATRA was finally dried under vacuum and stored in a vacuum drier when not use.

2. CS NP preparation method optimization

2.1 L/P ratio

The BzPGA concentration, Vo/Vw, core material and organic phase were fixed as 1.5 mg/mL, 1:5, BzPGA-100 KDa and acetone, respectively. The L/P ratio was varied (0%, 5%, 10%, 20%, 50%, 100%, 200% and 500%) with HA-C6-ATRA concentration corresponding change (0, 0.015, 0.03, 0.06, 0.15, 0.3, 0.6 and 1.5 mg/mL). The influence of L/P ratio on quality of CS NP and GA/CS NP (formulation I *vs* II *vs* III) were as shown in **Fig. 2** (zeta, size and protective effect against centrifugation caused aggregation), **Fig. S10~S12** and **Tab. S2** (stability in PBS, plasma and FBS), **Fig. 3A** (EE%), **Fig. 3B, Tab. S3** and **Tab. 1** (drug release profile), **Fig. S14** and **Tab. 2** (GA protective effect).

2.2 BzPGA concentration

The L/P ratio, Vo/Vw, core material and organic phase were fixed as 50%, 1:5, BzPGA-100 KDa and acetone, respectively. The BzPGA concentration was varied (0.3, 1.5, 7.5 mg/mL) with HA-C6-ATRA concentration corresponding change (0.03, 1.5, 0.75 mg/mL) to fix the Vo/Vw at 1:5. The influence of BzPGA concentration on quality of CS NP was as shown in **Fig. S5** (zeta, size and PDI).

2.3 Vo/Vw

The L/P ratio, BzPGA concentration, core material and organic phase were fixed as 50%, 1.5 mg/mL, BzPGA-100 KDa and acetone, respectively. The Vo/Vw was varied (1:10, 1:5, 2:5) with HA-C6-ATRA concentration corresponding change (0.075, 0.15, 0.3 mg/mL) to fix the BzPGA concentration at1.5 mg/mL. The influence of Vo/Vw on quality of CS NP was as shown in **Fig. S6** (zeta, size and PDI).

2.4 Core materials MW

The L/P ratio, BzPGA concentration, Vo/Vw and organic phase were fixed as 50%, 1.5 mg/mL, 1:5 and acetone, respectively. The molecule weight (MW) for core material was varied (BzPGA-10 KDa, 100 KDa and 1000 KDa). The influence of core materials MW on quality of CS NP and GA/CS NP (formulation II *vs* IV *vs* V) was as shown in **Fig. S7** (zeta, size and PDI), **Fig. 3A** (EE%), **Fig. 3B**, **Tab. S3** and **Tab. 1** (drug release profile), **Fig. S14** and **Tab. 2** (GA protective effect).

2.5 Organic phase type

The L/P ratio, BzPGA concentration and Vo/Vw were fixed as 50%, 1.5 mg/mL and 1:5, respectively. The organic solvents were DMSO or acetone and the core materials were BzPGA-10 KDa, 100 KDa and 1000 KDa. The influence of organic phase type on quality of CS NP with different core material MW was as shown in **Fig. S8** (zeta,

size and PDI).

2.6 Core material type

The L/P ratio, BzPGA concentration, Vo/Vw and organic phase were fixed as 50%, 1.5 mg/mL, 1:5 and acetone, respectively. The core material was BzPGA and PrPGA, respectively. The influence of core materials type on GA/CS NP (formulation II *vs* VII) was estimated by EE% (**Fig. 3A**).

2.7 Shell material type

The L/P ratio, BzPGA concentration, Vo/Vw and organic phase were fixed as 50%, 1.5 mg/mL, 1:5 and acetone, respectively. The shell material was HA-C6-ATRA and HA-C16, respectively. The influence of shell materials type on GA/CS NP (formulation II *vs* VI) was estimated by EE% (**Fig. 3A**), drug release profile (**Fig. 3B**, **Tab. S3**, and **Tab. 1**) and GA protective effect (**Fig. S14** and **Tab. 2**).

3. Quantitative analysis method development

3.1 HPLC quantitative analysis method development

The HPLC analysis was performed on Shimadzu LC-2010C_{HT} system with an Amethyst C_{18} column (4.6 mm × 250 mm, 5 μm), with 0.1% phosphoric acid-methanol (10:90, *v/v*) as the mobile phase at a flow rate of 1.0 mL·min^{-1}. The detection wavelength was 360 nm. The column temperature was 30 °C, and the injection volume was 10 μL. For each sample containing GA, three ~ five time amount of methanol was used to extract GA from the matrix.

3.2 LC-MS quantitative analysis method development

The LC-MS system comprised an Agilent 1260 series liquid chromatographic system coupled to an Agilent 6420 triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA), which was equipped with an electrospray ionization (ESI) source for ion production. A Hedera ODS-2 column (150 × 2.1 mm, 3 μm) was used for separation, with 0.1% formic acid-methanol (4:96,*v/v*) as mobile phase at a flow rate of 1.0 mL·min^{-1}.

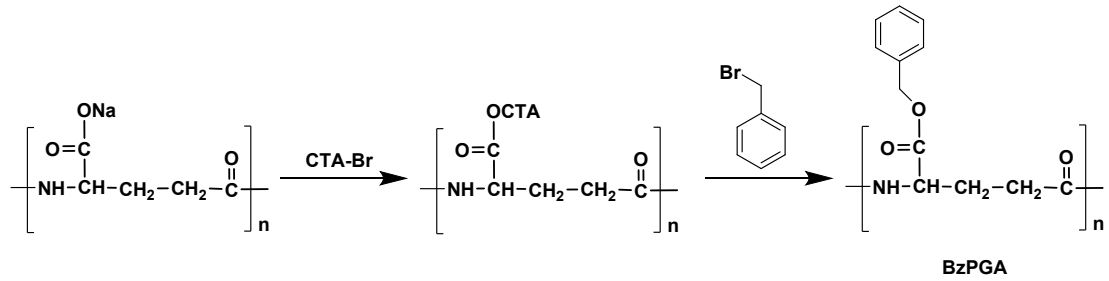
The mass spectrometer was operated in the positive ESI mode with the drying gas (N_2) temperature of 350 °C, drying gas flow rate of 9 L/min, nebulizer pressure of 30 psi, and capillary voltage of 4000 V. Quantification was accomplished in multiple reaction monitoring (MRM) by monitoring the transition of *m/z* 651.0→483.0 for GA, and *m/z* 285.0 → 193.0 for diazepam (internal standard).

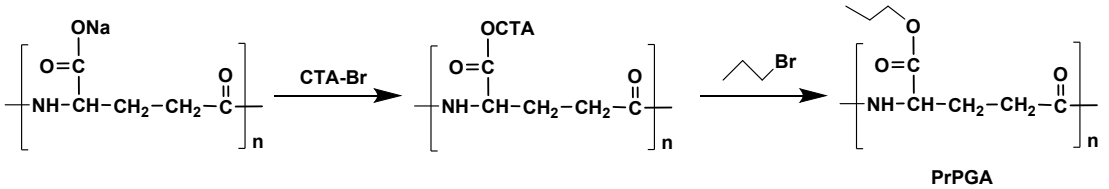
For 50 μL of each biosample, 150 μL of acetonitrile containing 500 ng/ml diazepam were added to precipitate proteins. The mixture was vortexed for 1 min and then centrifuged at 12000 rpm for 10 min. Finally an aliquot of 10 μL supernatants was directly injected into the LC-MS system for analysis.

4. Scratch wound healing assays

B16F10 cells were seeded in a 6-well plate and incubated for 24 h. The monolayer cells were scratched with a p200 pipette tip and washed twice with PBS. The cells were incubated with medium，GA (0.4 μg/mL) and GA/CS NP (GA=0.4 μg/ mL) for up to 48 h. The migrated cells were observed under an inverted microscopy at 24 h and 48 h. Three fields were chosen randomly in each well.

Part B: Supplementary figures and tables.





Scheme S1 Synthesis of core materials (BzPGA and PrPGA).

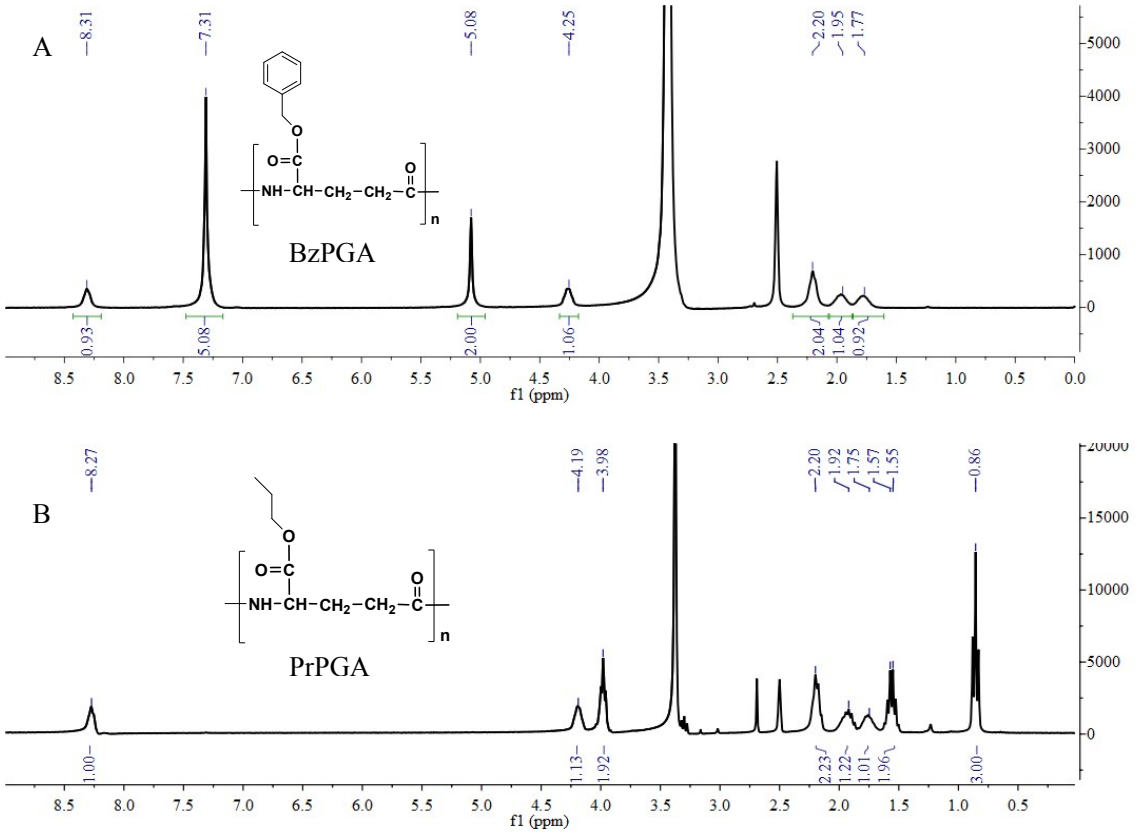
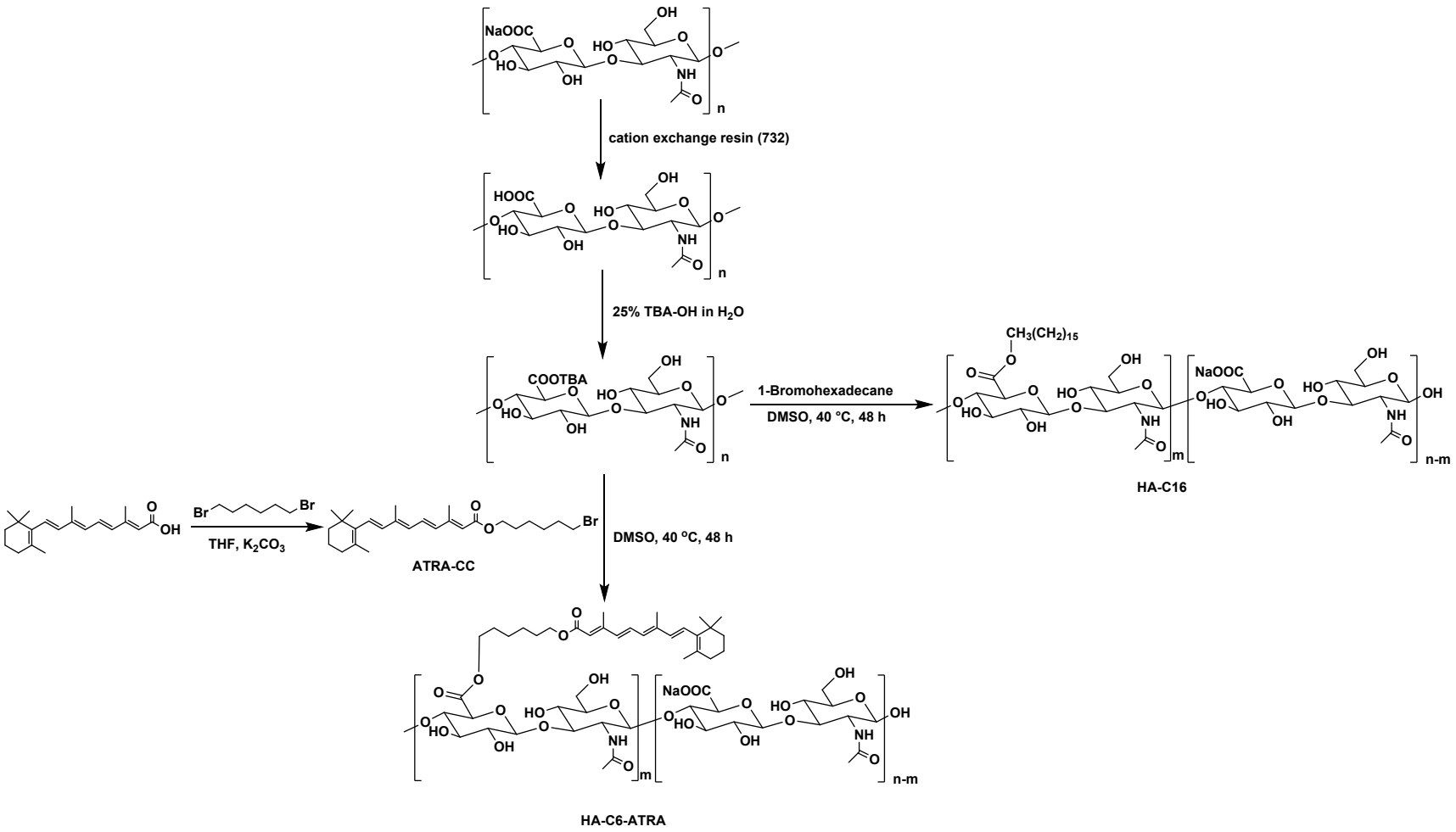


Fig. S1 ^{1}H-NMR spectrum of BzPGA (A) and PrPGA (B) in *d*6-DMSO.



Scheme S2 Synthesis of shell materials (HA-C6-ATRA, HA-C16).

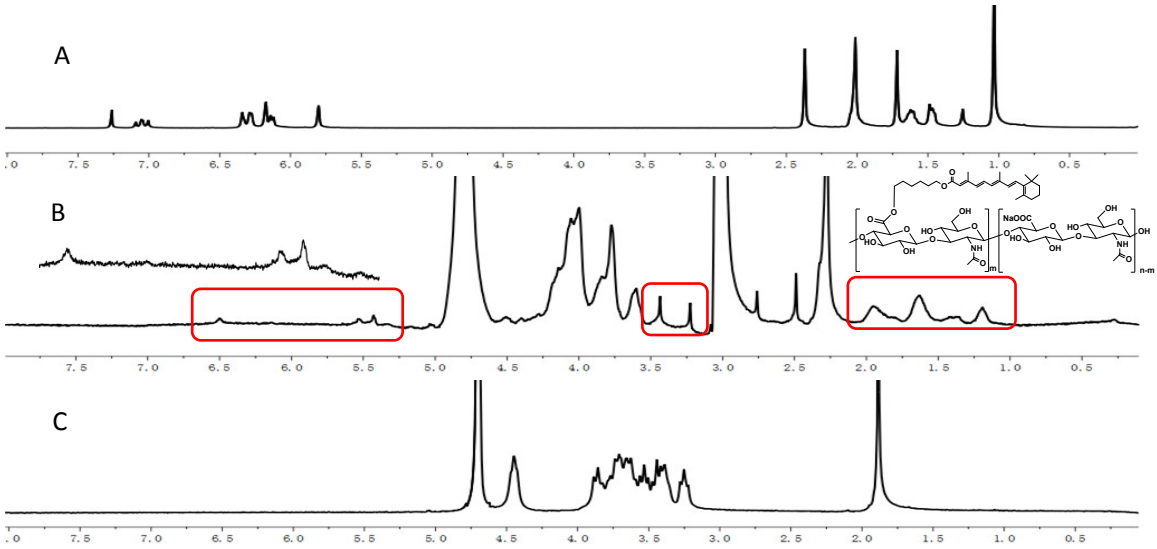


Fig. S2 ^{1}H-NMR spectrum of ATRA (A) in CD_3Cl, HA-C6-ATRA (B) in D_2O and HA (C) in D_2O.

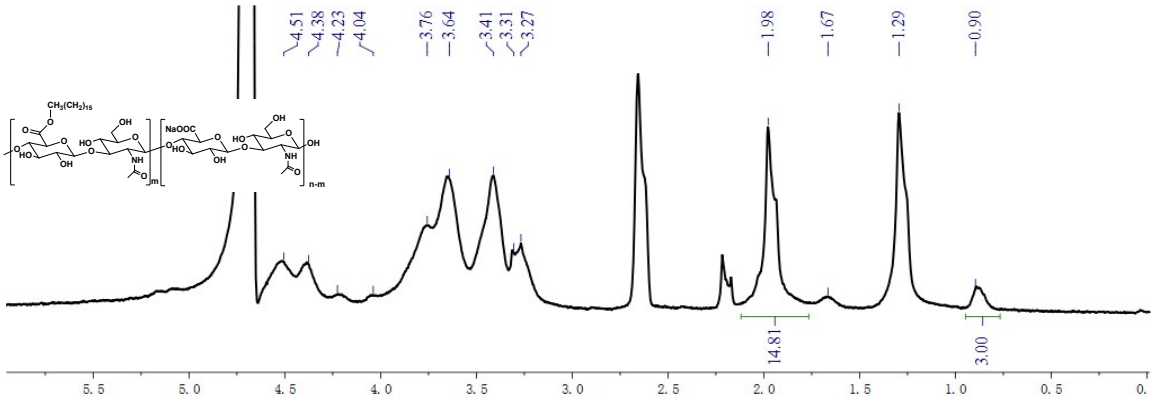


Fig. S3 ^{1}H-NMR spectrum of HA-C16 in *d*6-DMSO:D_2O=1:1.

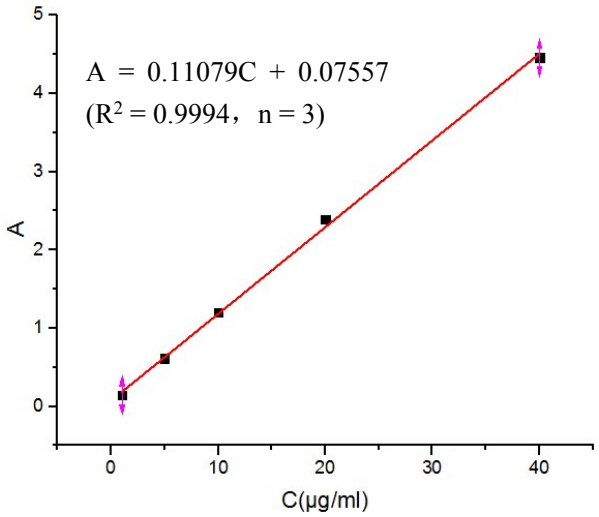


Fig. S4 Standard curve for quantitative determination of ATRA.
C: concentration of ATRA. A: UV absorbance value at 348 nm.

Table S1 Grafting rate of HA-C16 and HA-C6-ATRA.

Sample	Grafting rate (%)
HA-C16	20.26
HA-C6-ATRA	20.40

The degree of grafting rate, defined as the number of ATRA per one HA molecule, was estimated by UV measurements based on a standard curve generated with known concentrations of ATRA in DMSO:H_2O (6:4) (λ = 348 nm).

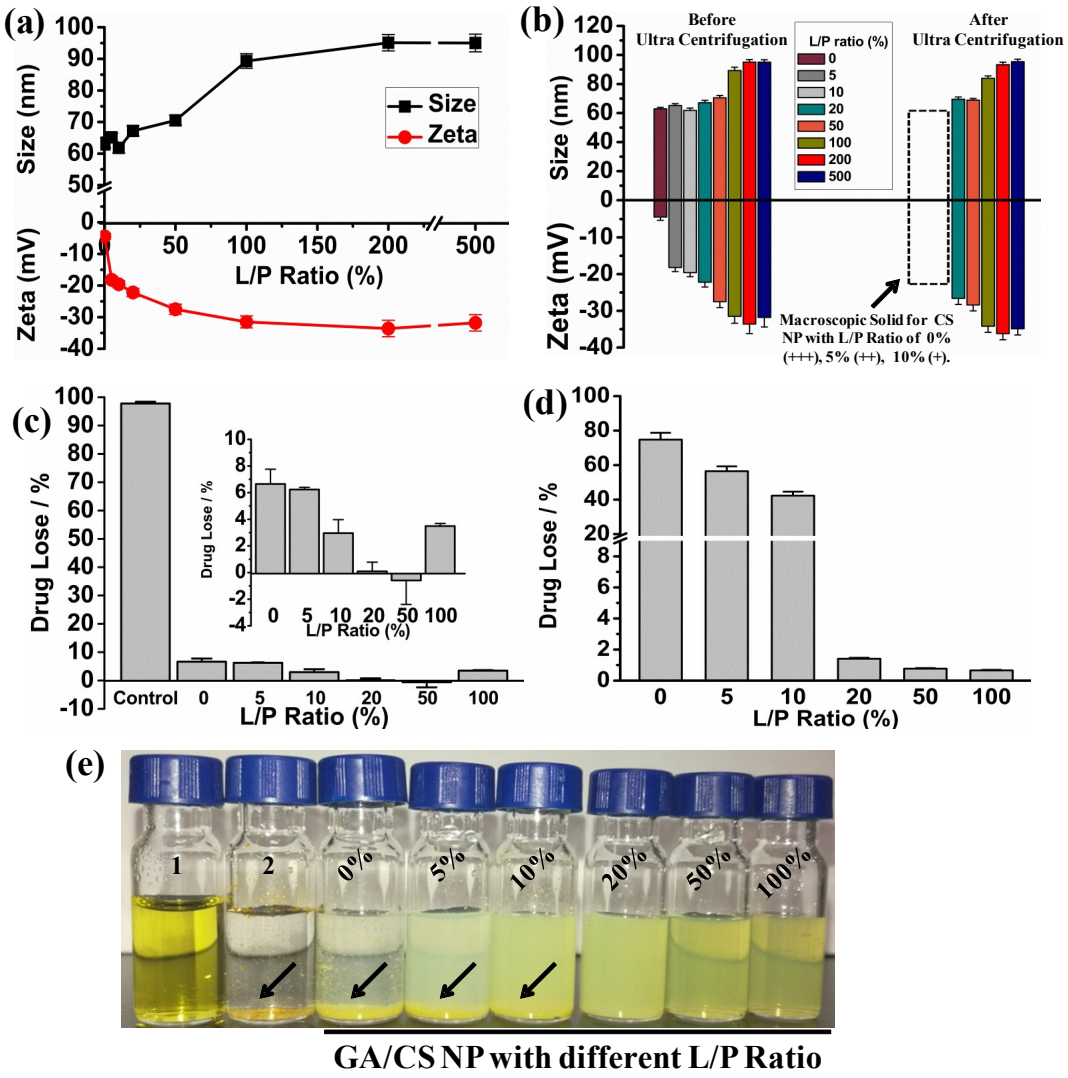


Figure S5. Effect of L/P ratio on GA/CS NL. (a) Size and zeta of CS NP prepared with different L/P ratio. (b) Size and zeta of CS NP before and after ultra centrifugation. "+++", "++" and "+" mean very obvious, obvious and not very obvious macroscopic solid, respectively. (c) Percent drug lose after filtration. After stirring for 2 hours (Step 2 in **Fig. 2A**) to let the organic solvent in mixed solution evaporate, the solution is filtered with a 50 μm filter and the GA content in filtrate is determined to calculate drug lose caused by precipitation formation. Control: GA acetone solution was mixed with water without any core or shell materials and then follow the same preparation process in **Fig 2A**. (d) Percent drug lose after ultra centrifugation. After ultra centrifugation, the solution is collected and filtered with 50 μm filter. GA content in filtrate is determined to calculate drug lose due to the nanoparticles aggregation caused by centrifugation. (e) Phenomenon after ultra centrifugation for GA/CS NP with different L/P ratio. e1: 1 mg/mL of GA acetone solution. e2: 1 mg/mL of GA in water. e3~e8: GA/CS NP with different L/P ratio (0%~100%) after ultra centrifugation and resuspended in water with GA concentration on 1 mg/mL. The arrow points out macroscopic solid.

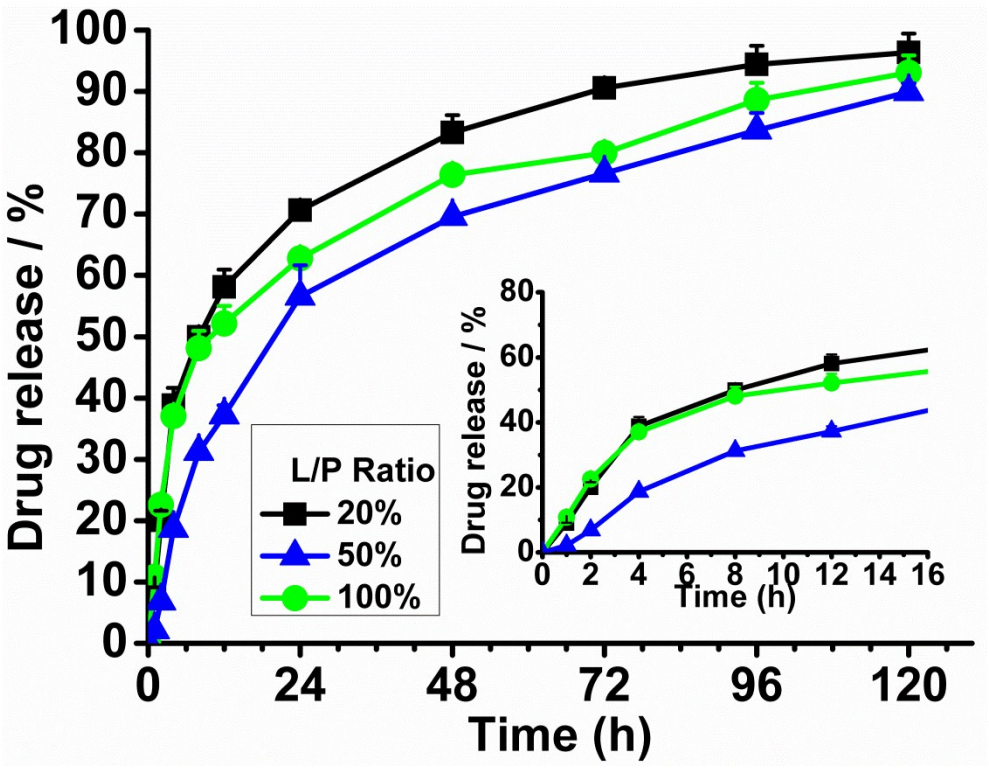


Fig. S6. Drug release profile for GA/CS NP with L/P ratio 20%, 50% and 100%.

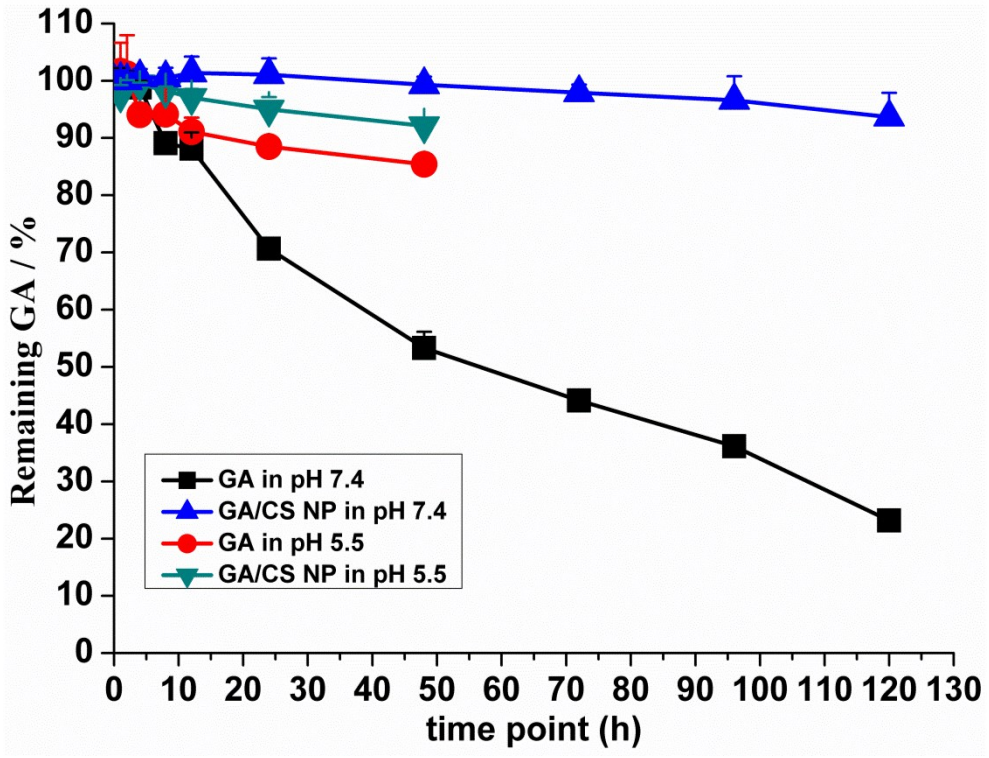


Fig. S7. Stability of free GA and GA/CS NP in buffer with different pH value.

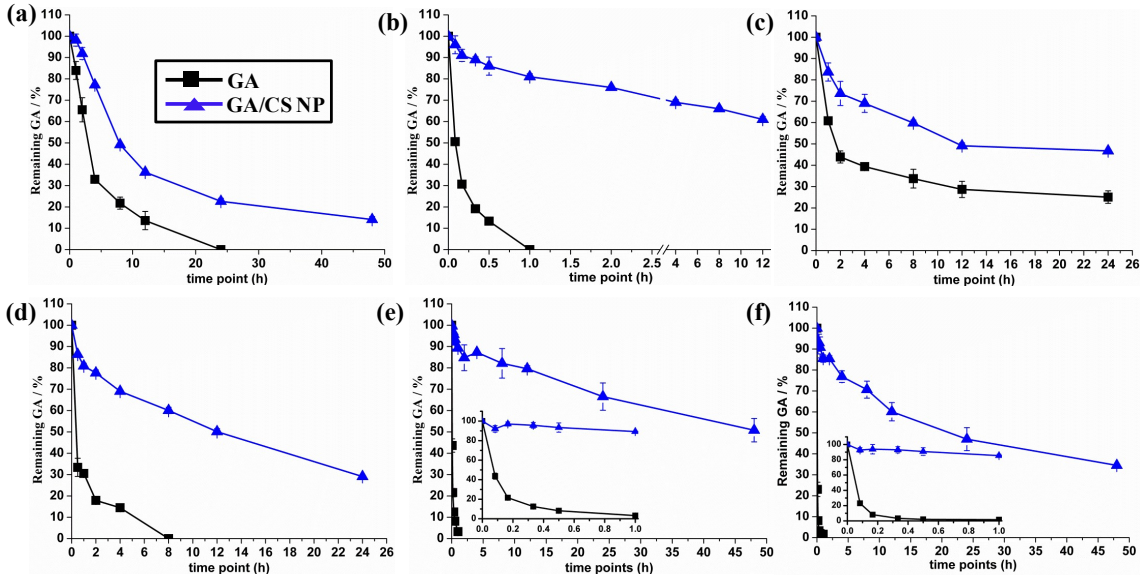


Fig. S8. Stability of free GA and GA/CS NP in different matrix (n=3). (a) 10 mM GSH, (b) 10 mM H_2O_2, (c) fresh rat plasma, (d) fresh rat blood, (e) fresh mouse liver homogenate, (f) Heps tumor homogenate.

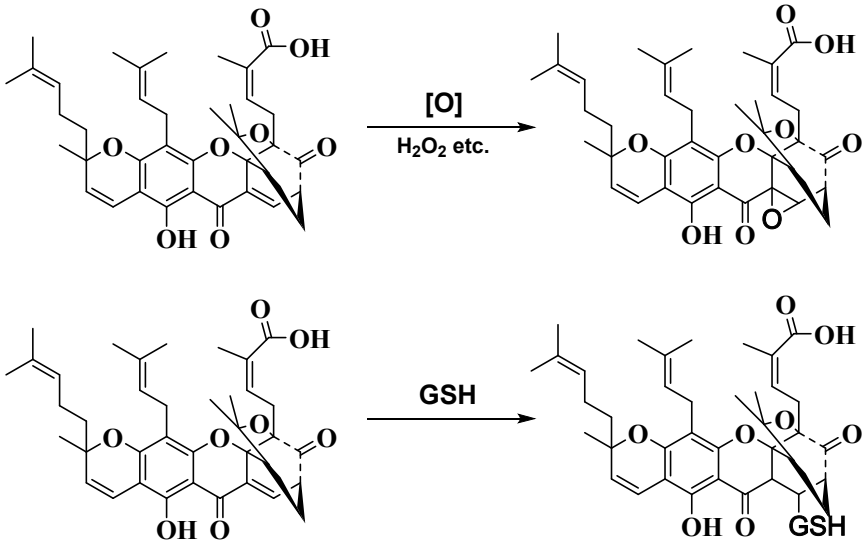


Fig. S9. Possible degradation mechanism by H_2O_2 and GSH.

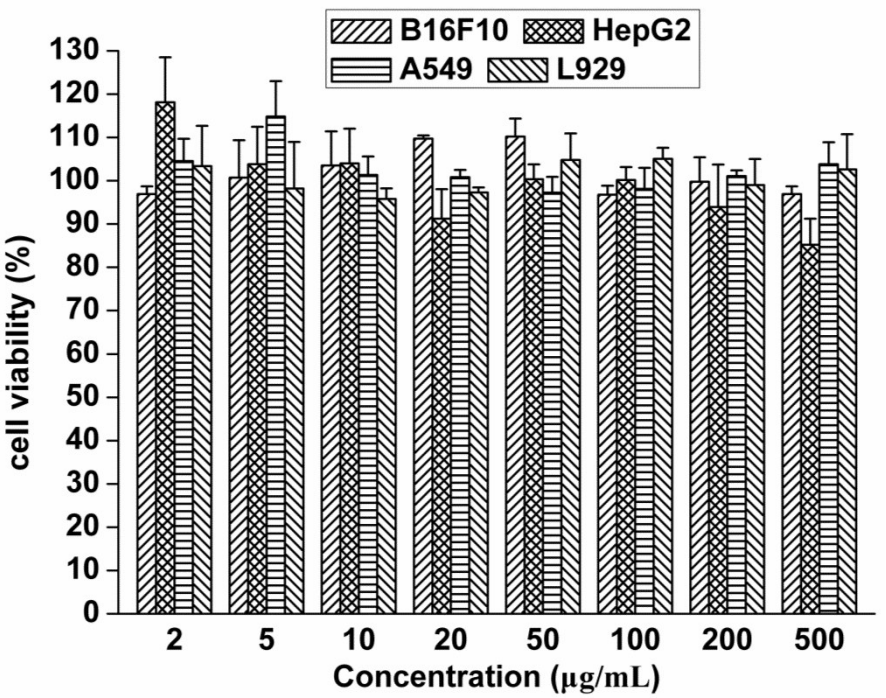


Fig. S10. Cytotoxicity of blank CS NP to B16F10, HepG2, A549 and L929. (n=3).

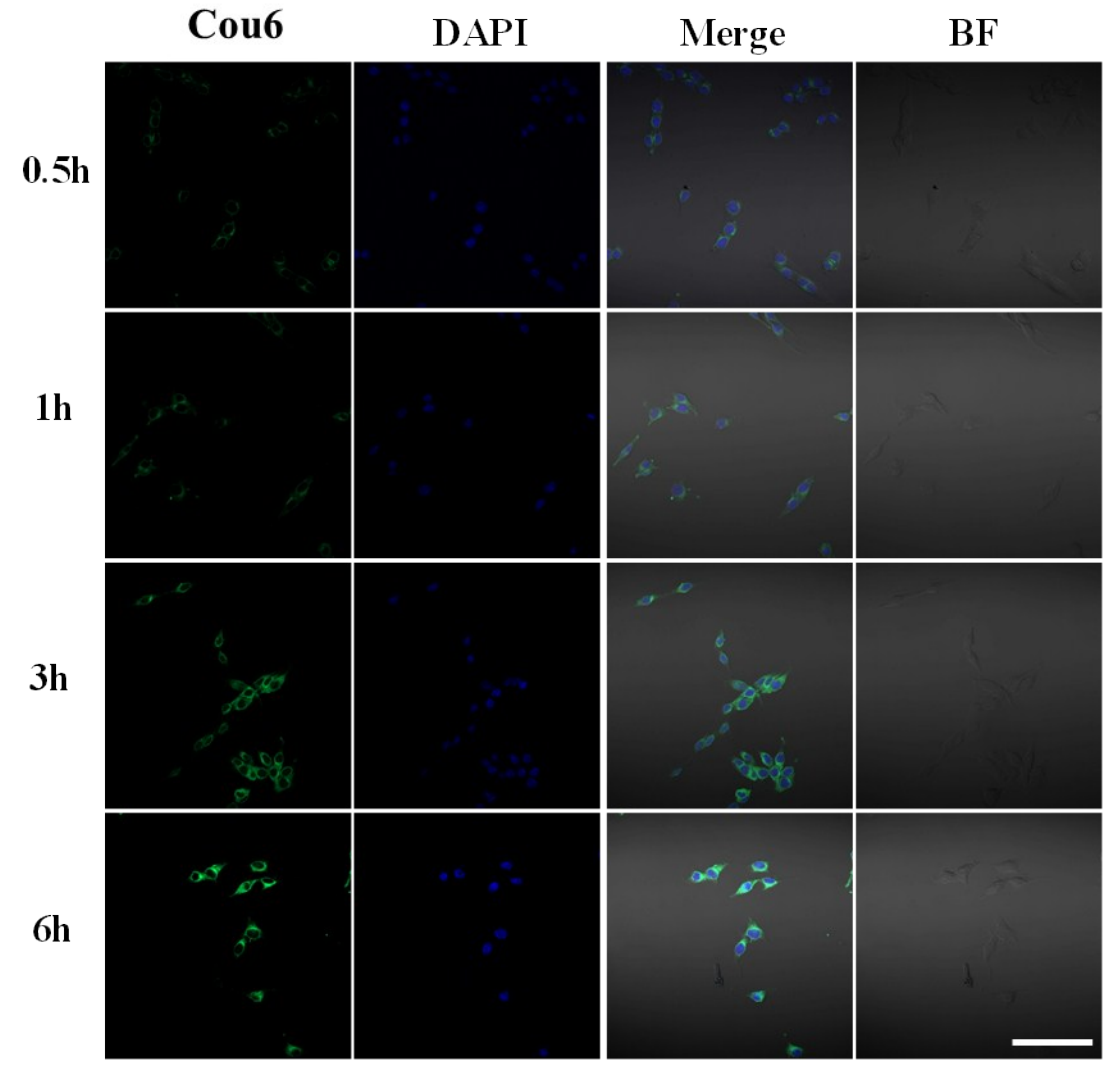


Figure S11. CLSM image of B16F10 cancer cells after co-incubation with Cou6/CS NP for 0.5 h, 1 h, 3 h, 6 h. Cou6 was green fluorescence. After incubation, cell nuclei were stained with DAPI (blue fluorescence). The scale bar is 100 µm.

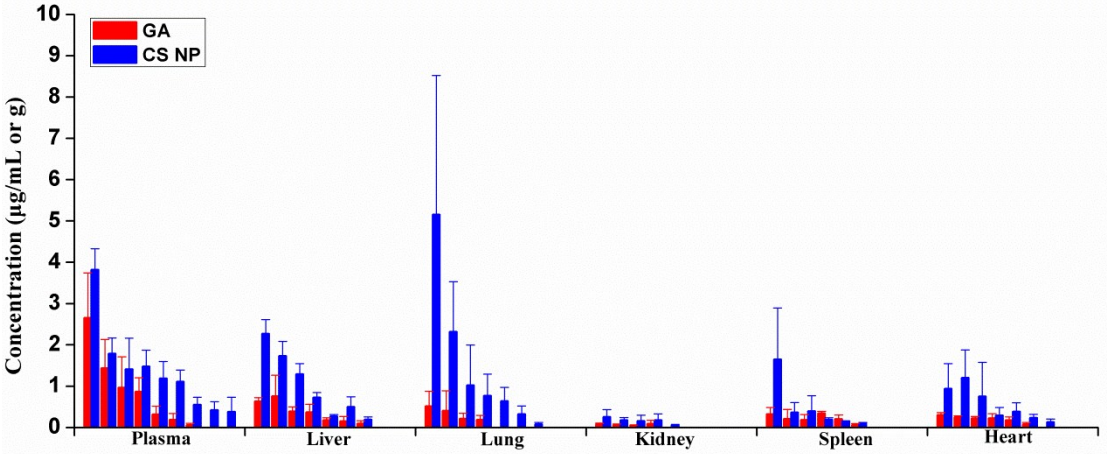


Figure S12. Pharmacokinetic and tissue distribution of GA and GA/CS NP in Heps tumor bearing mice. The time points was same as mentioned in **Fig. 9A**.

Table S2 C_e and r_e of GA/CS NP in organs of normal mice.

Tissue	Liver	Lung	Kidney	Spleen	Heart
C_e	2.99	9.90	2.89	4.85	3.87
r_e	1.94	11.12	5.00	2.04	3.42

C_e, ratio of C_{max}, $C_e=(C_{max})_n/(C_{max})_s$, $(C_{max})_n$ and $(C_{max})_s$ indicated the peak concentration of targeting drug delivery system and common formulation in a certain organ or tissue, respectively. r_e, relative uptake efficiency, $r_e=(AUC_i)_n/(AUC_i)_s$, $(AUC_i)_n$ and $(AUC_i)_s$, represented the AUC of targeting drug delivery system and common formulation in a certain organ or tissue, respectively.

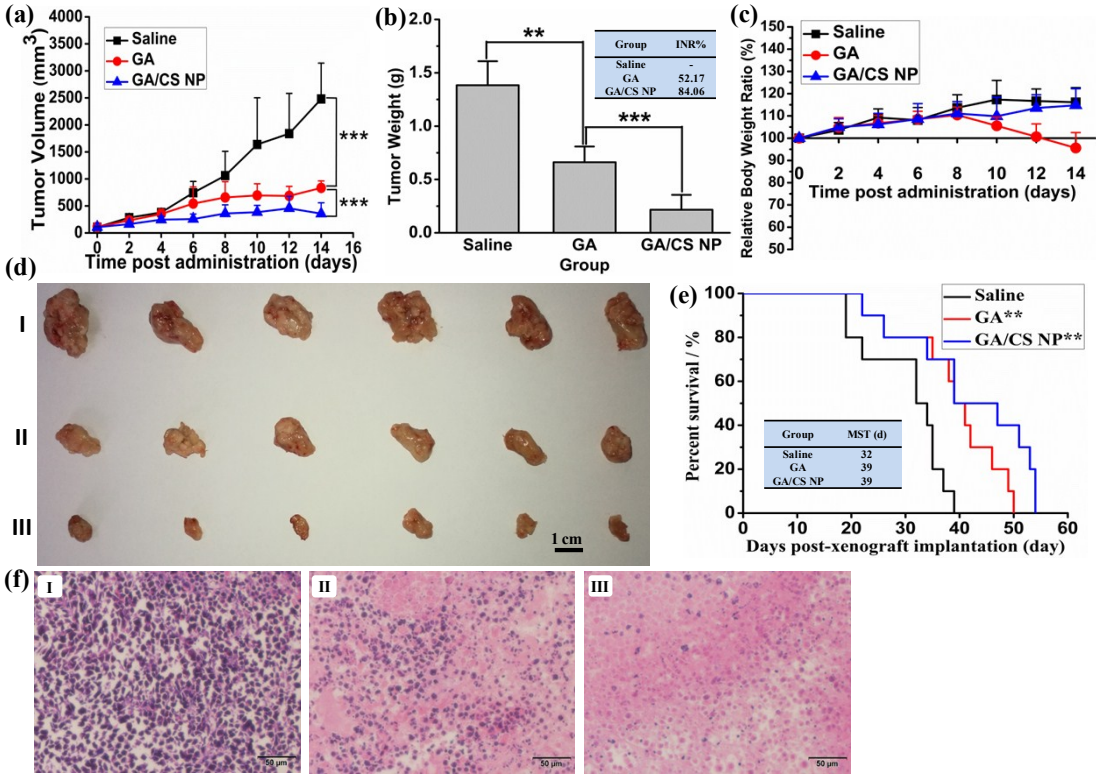


Figure S13. In vivo anti-tumor activity evaluation with Heps tumor bearing mice model. (a) Tumor growth kinetics with dosing every other day for 7 times. Data are expressed as mean ± SD (n=6). "*" Statistical difference for final (14 d) tumor volume between the three group, ***p<0.001. **(b) Final (14 d) tumor weights for mice of saline, GA and GA/CS NP groups.** "*" Statistical difference for tumor weight between the three group, **p<0.01, ***p<0.001. **(c) Relative body weight ratio of tumor bearing mice after treatment. (d) Photographs of typical tumor blocks collected from different treatment groups on day 14.** I: Saline group, II: GA group, III: GA/CS NP group. **(e) Survival time of tumor bearing mice after treatment.** MST: median survival time. "*" Statistical difference for survival in tumor bearing mice after different treatment. **p<0.01. **(f) Representative images of Heps tumor sections after H&E staining.** I: Saline group, II: GA group, III: GA/CS NP group.

Table S3. In vivo anti-tumor activity to Heps and B16F10 tumor bearing mice.

	Group	Dose (mg/Kg)	Weight (g)		Tumor weight (g)	C_t
			Initial weight	Final weight		
Heps	Saline	-	27.17±1.33	31.50±1.05	1.38±0.26	4.38±0.65
	GA	6	27.17±0.98	26.00±2.45***	0.66±0.15**	2.56±0.61***
	GA/CS NP	6	26.83±1.33	30.83±2.99###	0.22±0.14###	0.68±0.41###
B16F10	Saline	-	20.33±1.03	25.67±0.82	8.40±0.84	32.70±2.65
	GA	6	20.50±0.84	22.00±1.26***	4.14±0.66***	18.91±3.62***
	GA/CS NP	6	19.83±0.41	22.83±1.60$$	1.46±0.53###	6.39±2.18###

C_t: tumor coefficient. "*" statistical difference of final weight, tumor weight or C_t between GA and Saline group, $^{**}p<0.01$, $^{***}p<0.001$. There were no difference for initial weight. "$" statistical difference of final weight between saline and GA/CS NP group. "#" statistical difference of final weight, tumor weight or TC between GA and GA/CS NP group, $^{\#\#\#}p<0.001$.

Table S4. Survival time of **Heps and B16F10 tumor bearing mice after treatment.**

	Group	Mean Survival Time					Median Survival Time				
		Estimated value	Standard error	95% confidence intervals		T/C (%)	Estimated value	Standard error	95% confidence intervals		T/C (%)
				Lower limit	Upper limit				Lower limit	Upper limit	
Heps	Saline	30.40	2.38	25.74	35.06	-	32.00	6.32	19.60	44.40	-
	GA	38.80	2.90	33.11	44.49	128.63	39.00	2.37	34.35	43.65	121.88
	GA/CS NL	41.90	3.73	34.59	49.21	137.83	39.00	6.85	25.57	52.43	121.88
B16F10	Saline	24.21	0.54	23.16	25.27	-	24.00	0.62	22.79	25.21	-
	GA	25.71	0.67	24.39	27.04	106.20	26.00	1.25	23.55	28.44	108.33
	GA/CS NL	28.57	0.78	27.04	30.10	118.01	29.00	0.93	27.18	30.81	120.83

[a] T/C%: Survival time prolongation rate, the survival time ratio of GA or GA/CS NP group to Saline group.

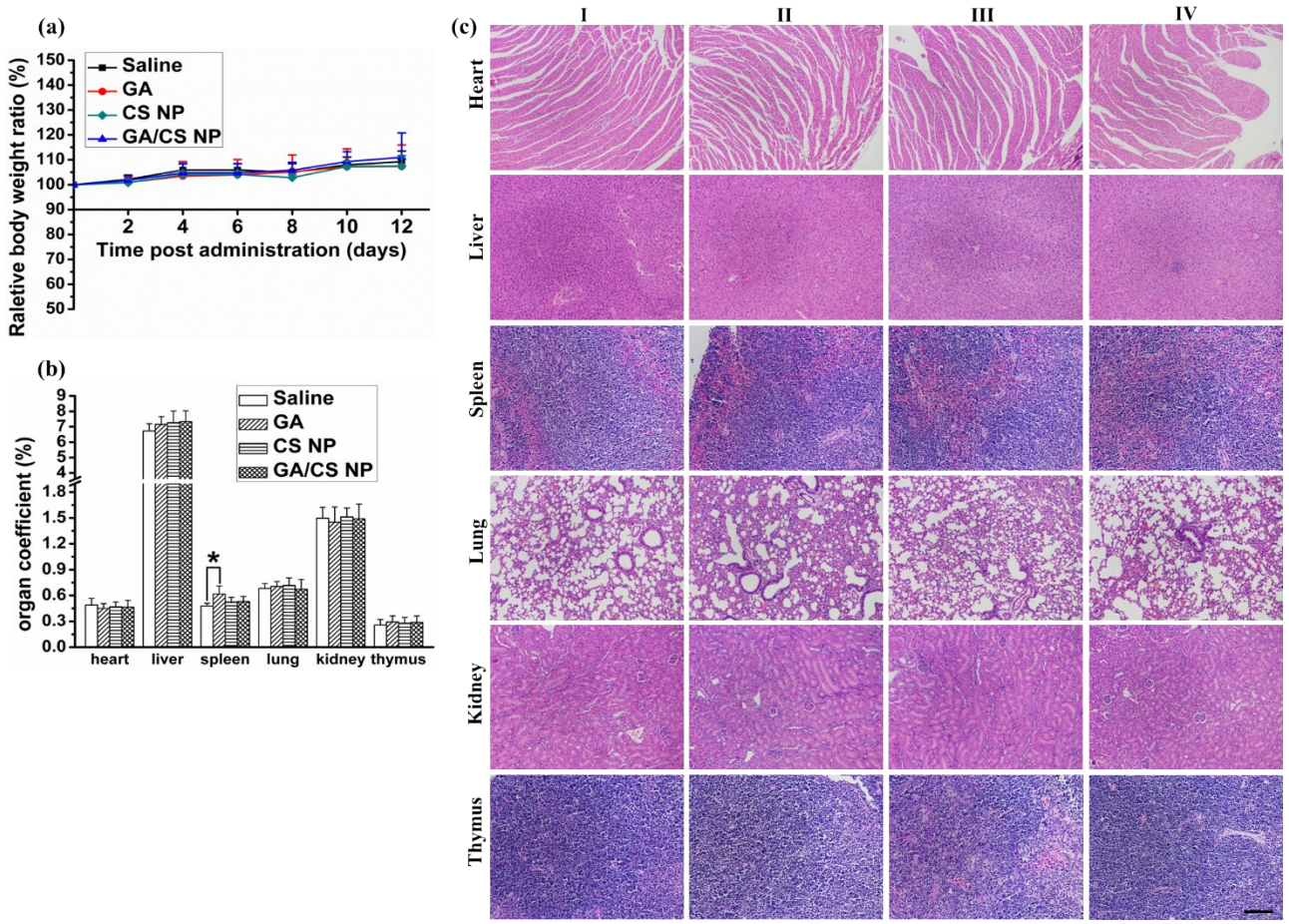


Figure S14. In vivo toxicity of free GA, blank CS NP and GA/CS NP. (a) Relative body weight ratio of mice with different treatment. (b) Main organ coefficient of mice with different treatment. (c) Representative images of organ sections after H&E staining. I: Saline group, II: blank CS NP group, III: free GA group, IV: GA/CS NP group. The scale bar is 50 μm.

Table S5. Result of blood routine examination from mice treated with free GA, blank CS NP and GA/CS NP.

Group	wbc (E^3/μL)	rbc (E^6/μL)	hct (%)	mcv (fL)	mch (pg)	mchc (g/L)	rdw (%CV)
Saline	9.26 ± 1.51	9.22 ± 0.62	48.95 ± 4.28	53.05 ± 2.40	16.17 ± 0.63	305.25 ± 21.48	17.50 ± 0.61
GA 6 mg/Kg	9.9 ± 1.21	9.95 ± 0.95	50.35 ± 3.51	50.75 ± 2.81	15.62 ± 0.60	308.25 ± 12.61	17.48 ± 0.57
CS NL 60 mg BzPGA/Kg	13.18 ± 2.95	10.16 ± 0.47	52.52 ± 2.48	51.72 ± 1.58	15.9 ± 0.34	308.00 ± 15.41	17.62 ± 0.99
GA/CS NL 6 GA mg/Kg	10.825 ± 3.02	9.81 ± 0.58	49.97 ± 2.61	50.97 ± 1.17	16.1 ± 0.47	315.75 ± 2.36	17.90 ± 0.96

continued:

Group	plt (E^3/μL)	mpv (fL)	ne% (%)	ly% (%)	eo% (%)	mo% (%)	ba% (%)
Saline	1091.50 ± 38.97	5.02 ± 0.22	20.52 ± 4.59	76.09 ± 4.90	0.47 ± 0.65	2.87 ± 0.95	0.040 ± 0.07
GA 6 mg/Kg	1060.75 ± 79.38	5.30 ± 0.28	16.24 ± 1.40	79.36 ± 1.66	0.20 ± 0.16	4.18 ± 1.39	0.017 ± 0.02
CS NL 60 mg BzPGA/Kg	1003.25 ± 111.47	5.10 ± 0.22	24.51 ± 11.53	70.64 ± 13.37	0.50 ± 0.15	4.31 ± 1.73	0.037 ± 0.03
GA/CS NL 6 GA mg/Kg	1050.50 ± 101.60	4.72 ± 0.21	20.76 ± 2.66	75.04 ± 2.89	0.40 ± 0.19	3.77 ± 1.19	0.025 ± 0.01

wbc: white blood cell, rbc: red blood cell, hct: hematocrit, mcv: mean corpuscular volume, mch: mean corpuscular hemoglobin, mchc: mean corpuscular hemoglobin concentration, rdw: red blood cell distribution width, plt: platelet, mpv: mean platelet volume, ne%: neutrophil ratio, ly%: lymphocyte ratio, eo%: eosinophil ratio, mo%: monocyte ratio, ba%: basophil ratio.

Table S6. Result of blood biochemistry analysis for mice treated with free GA, blank CS NP and GA/CS NP.

Group	ALT (IU/L)	AST (IU/L)	TBIL (μmol/L)	BUN (mmol/L)	CREA (μmol/L)
Saline	**35.67 ± 1.53**	**93.00 ± 4.58**	**1.33 ± 0.33**	**11.00 ± 1.73**	**13.93 ± 2.15**
GA 6 mg/Kg	**32.25 ± 10.69**	**86.25 ± 17.00**	**1.05 ± 0.56**	**12.52 ± 1.86**	**14.03 ± 1.18**
CS NL 60 mg BzPGA/Kg	**27.25 ± 3.69***	**98.75 ± 18.95**	**1.03 ± 0.45**	**8.95 ± 1.87**	**11.25 ± 1.77**
GA/CS NL 6 GA mg/Kg	**30.50 ± 6.24**	**92.75 ± 11.79**	**1.18 ± 0.36**	**10.05 ± 1.80**	**12.18 ± 3.83**

ALT: alanine aminotransferase, AST: aspartate aminotransferase, TBIL: total bilirubin, BUN: urea nitrogen, CREA: creatinine. "*" Statistical difference between treated groups (free GA, blank CS NP and GA/CS NP) and saline group, *$p<0.05$.