

Supplementary Tables and Figures

Silk fibroin-coated nanoagents for acidic lysosome targeting by a functional preservation strategy in cancer chemotherapy

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Time (h)	DOX (μg)		Dose %	C. A. (%)
	Mean	SD		
0-6	4.901	0.774	3.921%	3.921%
6-24	2.786	1.681	2.229%	6.150%
24-36	1.579	0.839	1.263%	7.413%
36-48	1.280	0.555	1.024%	8.437%
48-72	1.028	0.504	0.822%	9.259%
72-96	0.640	0.614	0.512%	9.771%
96-120	0.511	0.331	0.409%	10.180%
120-144	0.279	0.123	0.223%	10.403%
144-168	0.249	0.156	0.199%	10.602%
Sum	13.253	5.576	10.602%	
Dose %	10.602%	4.462%		

*C. A. = Cumulative Amount

Table S1. Cumulative amount of DOX excreted in mice urine after a single intravenous injection of DOX (n=3).

Time (h)	DOX (μg)		Dose %	C. A. (%)
	Mean	SD		
0-6	0.044	0.016	0.035%	0.035%
6-24	0.428	0.350	0.342%	0.377%
24-36	0.999	0.194	0.799%	1.176%
36-48	1.079	0.592	0.863%	2.039%
48-72	1.519	0.150	1.215%	3.254%
72-96	1.244	0.121	0.995%	4.249%
96-120	1.092	0.634	0.874%	5.123%
120-144	0.807	0.156	0.646%	5.769%
144-168	0.751	0.041	0.601%	6.370%
Sum	7.963	2.254	6.370%	
Dose %	6.370%	1.803%		

*C. A. = Cumulative Amount

Table S2. Cumulative amount of DOX excreted in mice urine after a single intravenous injection of ACC-DOX NPs (n=3).

Time (h)	DOX (μg)		Dose %	C. A. (%)
	Mean	SD		
0-6	0.035	0.026	0.028%	0.028%
6-24	0.296	0.169	0.237%	0.265%
24-36	0.539	0.262	0.431%	0.696%
36-48	0.658	0.365	0.526%	1.222%
48-72	1.053	0.595	0.842%	2.064%
72-96	0.569	0.284	0.455%	2.519%
96-120	0.523	0.154	0.418%	2.937%
120-144	0.345	0.107	0.276%	3.213%
144-168	0.376	0.117	0.301%	3.514%
Sum	4.393	2.080	3.514%	
Dose %	3.514%	1.664%		

*C. A. = Cumulative Amount

Table S3. Cumulative amount of DOX excreted in mice urine after a single intravenous injection of ACC-DOX-SF NPs (n=3).

Time (h)	DOX (μg)		Dose %	C. A. (%)
	Mean	SD		
0-6	0.016	0.013	0.013%	0.013%
6-24	0.090	0.088	0.072%	0.085%
24-36	0.118	0.025	0.094%	0.179%
36-48	0.169	0.024	0.135%	0.314%
48-72	0.109	0.016	0.087%	0.401%
72-96	0.061	0.023	0.049%	0.450%
96-120	0.020	0.008	0.016%	0.466%
120-144	0.030	0.008	0.024%	0.490%
144-168	0.015	0.009	0.012%	0.502%
Sum	0.628	0.214	0.502%	
Dose %	0.502%	0.171%		

*C. A. = Cumulative Amount

Table S4. Cumulative amount of DOX excreted in mice faeces after a single intravenous injection of DOX (n=3).

Time (h)	DOX (μg)		Dose %	C. A. (%)
	Mean	SD		
0-6	0.013	0.006	0.010%	0.010%
6-24	0.029	0.016	0.023%	0.033%
24-36	0.059	0.053	0.047%	0.080%
36-48	0.170	0.071	0.136%	0.216%
48-72	0.240	0.077	0.192%	0.408%
72-96	0.301	0.226	0.241%	0.649%
96-120	0.477	0.127	0.382%	1.031%
120-144	0.729	0.037	0.583%	1.614%
144-168	0.577	0.332	0.462%	2.076%
Sum	2.595	0.944	2.076%	
Dose %	2.076%	0.755%		

*C. A. = Cumulative Amount

Table S5. Cumulative amount of DOX excreted in mice faeces after a single intravenous injection of ACC-DOX NPs (n=3).

Time (h)	DOX (μg)		Dose %	C. A. (%)
	Mean	SD		
0-6	0.009	0.006	0.007%	0.007%
6-24	0.014	0.005	0.011%	0.018%
24-36	0.021	0.011	0.017%	0.035%
36-48	0.015	0.006	0.012%	0.047%
48-72	0.048	0.025	0.038%	0.085%
72-96	0.074	0.026	0.059%	0.144%
96-120	0.245	0.071	0.196%	0.340%
120-144	0.251	0.089	0.201%	0.541%
144-168	0.261	0.056	0.209%	0.750%
Sum	0.938	0.295	0.750%	
Dose %	0.750%	0.236%		

*C. A. = Cumulative Amount

Table S6. Cumulative amount of DOX excreted in mice faeces after a single intravenous injection of ACC-DOX-SF NPs (n=3).

	WBC (10 ⁹ /L)	LYMPH (%)	MON (%)	Gran (%)	RBC (10 ¹² /L)	HGB (g/l)	MCV (fL)	MCH (pg)
Reference Range	0.80~6.80	55.80~90.60	1.80~6.00	8.60~38.90	6.36~9.42	110.00~143.00	48.20~58.30	15.80~19.00
Blank Control	4.27±0.46	78.84±8.79	6.33±0.95	25.83±3.68	9.87±0.85	141.67±16.78	52.40±2.10	16.70±0.29
1 h	4.21±1.01	78.20±0.64	4.07±0.66	18.03±1.14	9.14±0.84	140.33±8.18	46.77±0.34	15.10±0.54
3 h	4.23±0.74	57.97±0.54	5.70±0.51	33.80±2.95	10.55±0.31	141.67±1.25	44.33±1.44	15.10±0.33
6 h	3.50±0.94	65.10±6.71	5.73±0.57	26.40±6.55	10.59±1.50	146.33±18.98	47.50±1.69	15.37±0.56
12 h	4.93±0.94	71.30±5.66	5.43±0.25	20.97±6.78	10.56±0.26	142.67±3.09	46.23±0.90	15.40±0.22
24 h	4.63±1.33	79.37±2.43	4.30±0.37	19.46±3.85	10.27±0.26	135.33±3.30	43.40±1.22	14.80±0.21
48 h	4.97±1.86	82.57±1.75	3.43±0.12	15.63±3.03	9.52±0.30	117.67±2.87	43.50±1.35	14.67±0.34
72 h	5.20±0.24	82.40±0.80	3.23±0.12	16.03±2.67	9.07±1.36	128.67±13.47	48.33±0.71	16.23±0.82

	MCHC (g/l)	HCT (%)	PLT (10 ⁹ /L)	BUN (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
Reference Range	302.00~353.00	34.60~44.60	450.00~1590.00	7.00~31.00	40.00~170.00	67.00~381.00	108.00~367.00
Blank Control	322.67±2.87	40.63±4.36	832.67±202.41	32.29±5.15	105.31±33.67	316.62±87.71	154.95±19.70
1 h	325.67±9.74	40.77±4.78	685.67±121.25	19.85±3.80	81.85±23.94	212.10±16.32	180.48±20.66
3 h	332.67±7.41	38.77±0.91	626.00±107.20	23.03±2.63	152.09±30.24	298.24±41.03	158.41±15.00
6 h	320.67±1.70	40.20±5.64	838.00±85.02	30.74±8.87	93.56±14.88	219.99±67.26	165.23±22.83
12 h	325.00±3.74	40.13±1.14	573.00±132.32	30.56±6.00	97.02±30.41	239.32±85.41	197.64±36.36
24 h	339.33±8.50	37.00±1.82	738.33±47.68	28.27±3.97	118.63±31.37	178.87±15.92	174.55±17.93
48 h	340.00±4.55	33.60±3.25	461.33±53.11	21.48±1.60	52.60±7.09	156.55±21.75	167.67±33.57
72 h	335.67±12.76	35.67±5.61	788.33±84.42	27.52±5.69	116.32±58.85	273.50±12.05	142.66±20.08

Table S7. *In vivo* biosafety assay of the ACC-DOX-SF NPs. Healthy Balb/c mice sacrificed at 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, 72 h after intravenous injection of the ACC-DOX-SF NPs.

Hematological analysis, liver and kidney function analysis were measured.

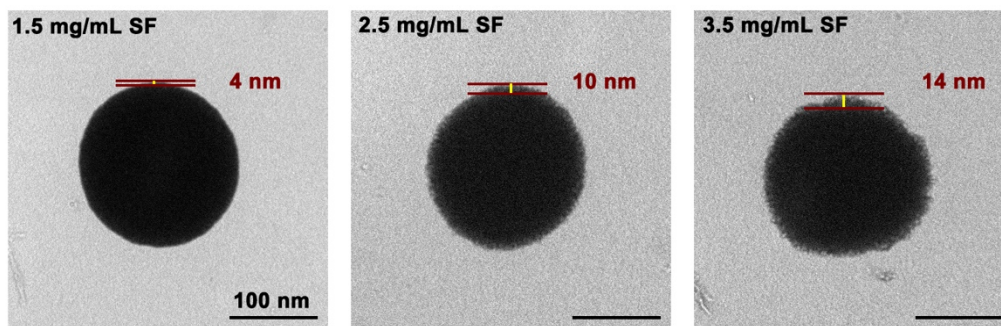


Figure S1. Representative TEM images of ACC-DOX-SF NPs with different amount of silk fibroin (1.5 mg/mL, 2.5 mg/mL and 3.5 mg/mL) at high magnification. The thickness of SF corona was measured as 4 nm, 10 nm and 14 nm, respectively.

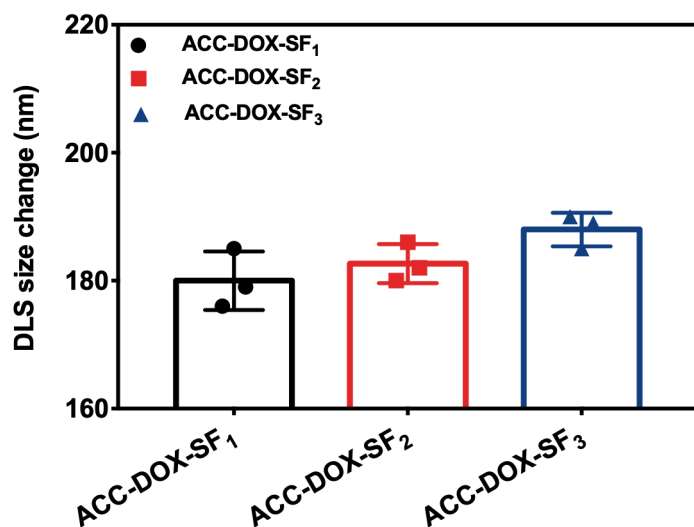


Figure S2. Size variation of ACC-DOX-SF NPs with different amount of silk fibroin. ACC-DOX-SF₁, ACC-DOX-SF₂ and ACC-DOX-SF₃ represented the concentration of SF was 1.5 mg/mL, 2.5 mg/mL and 3.5 mg/mL, respectively.

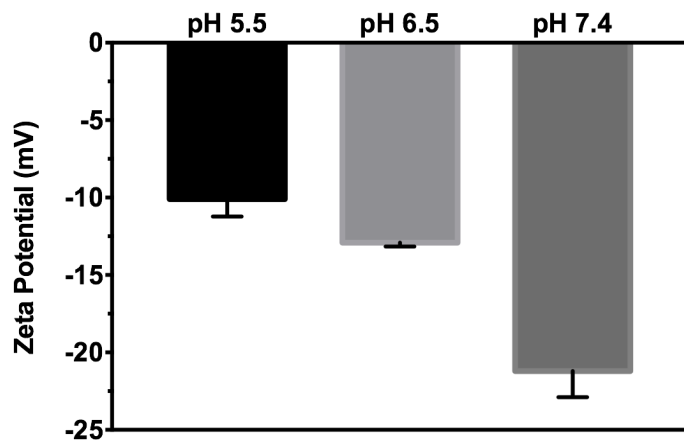


Figure S3. Zeta potential of NPs in acetate buffer solution with different pH values.

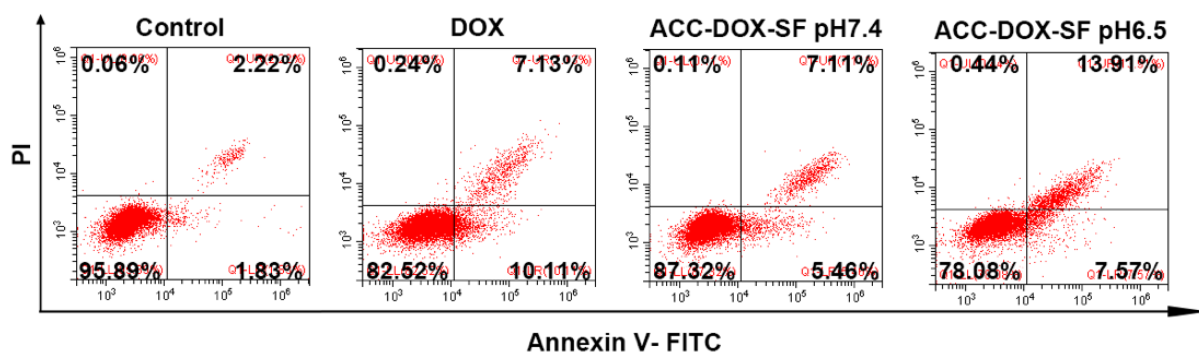


Figure S4. Flow cytometry measurement of 4T1 cell apoptosis induced by various treatments after 12 h of incubation.

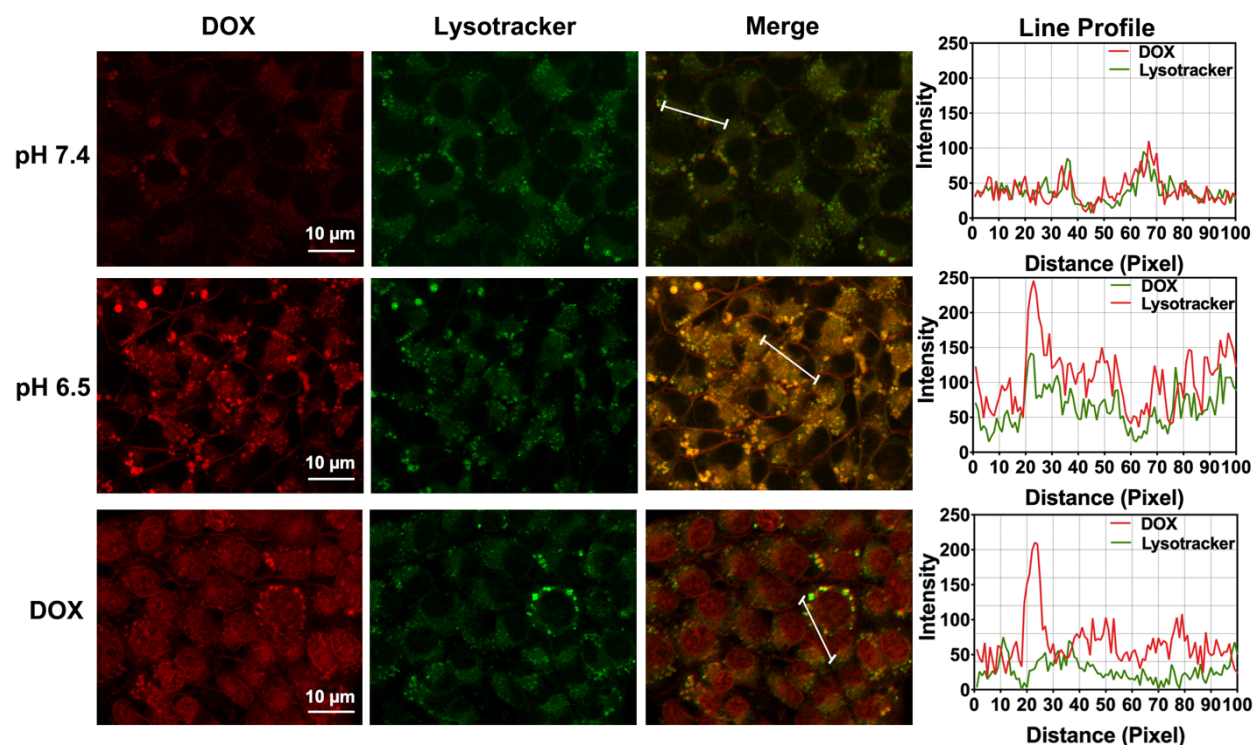


Figure S5. Lysosome colocalization performance of free DOX and ACC-DOX-SF NPs in 4T1 cells. Intensity line profiles of the region of interest (white line) across the 4T1 cells costained with NPs/free DOX and Lysotracker Green.

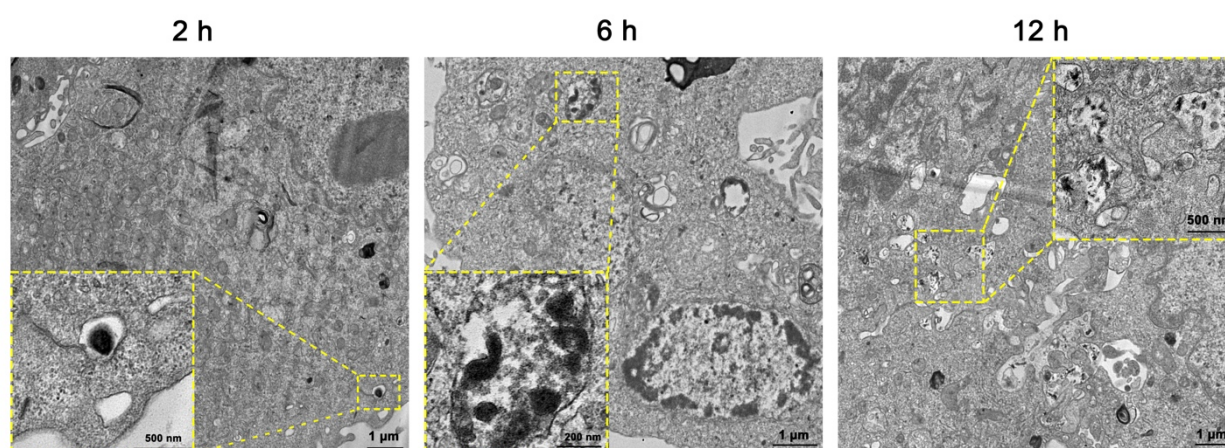


Figure S6. TEM of 4T1 cells incubated with ACC-DOX-SF NPs for 2 h, 6 h, 12 h. The lower left or top right was the magnified ROI area (yellow square in the image).

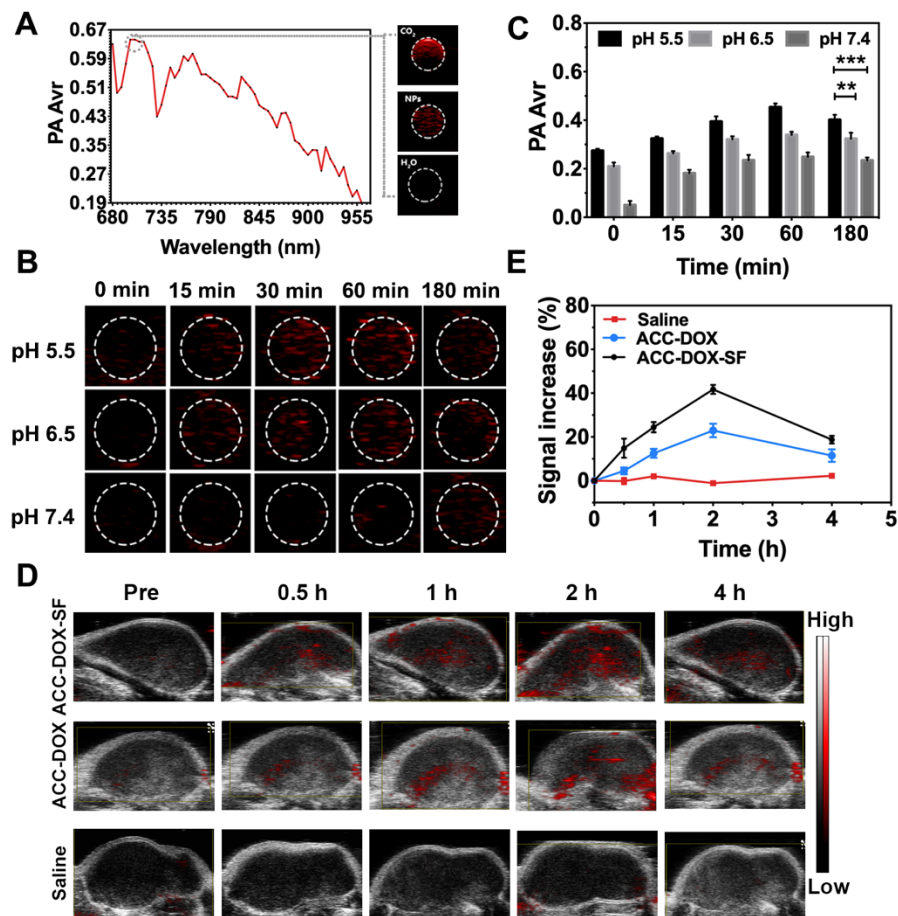


Figure S7. Optoacoustic imaging performance of ACC-SF@DOX NPs. (A) PA-signal changes of pure CO₂, ACC-DOX-SF NPs and H₂O upon irradiation by a laser over the wavelength range of 680-970 nm. The right-hand pictures of various substances were obtained at a wavelength of 700 nm. (B) PA imaging of the ACC-DOX-SF NPs *in vitro* at different pH values. (C) Quantification of the PA-signal intensities of the ACC-DOX-SF NPs at different pH values. (D) PA images and (E) signal increases (%) in the 4T1 tumors with different treatments after 0.5 h, 1 h, 2 h, and 4 h. The results are expressed as the mean \pm standard deviation (SD), *P < 0.05, **P < 0.01, or ***P < 0.001.

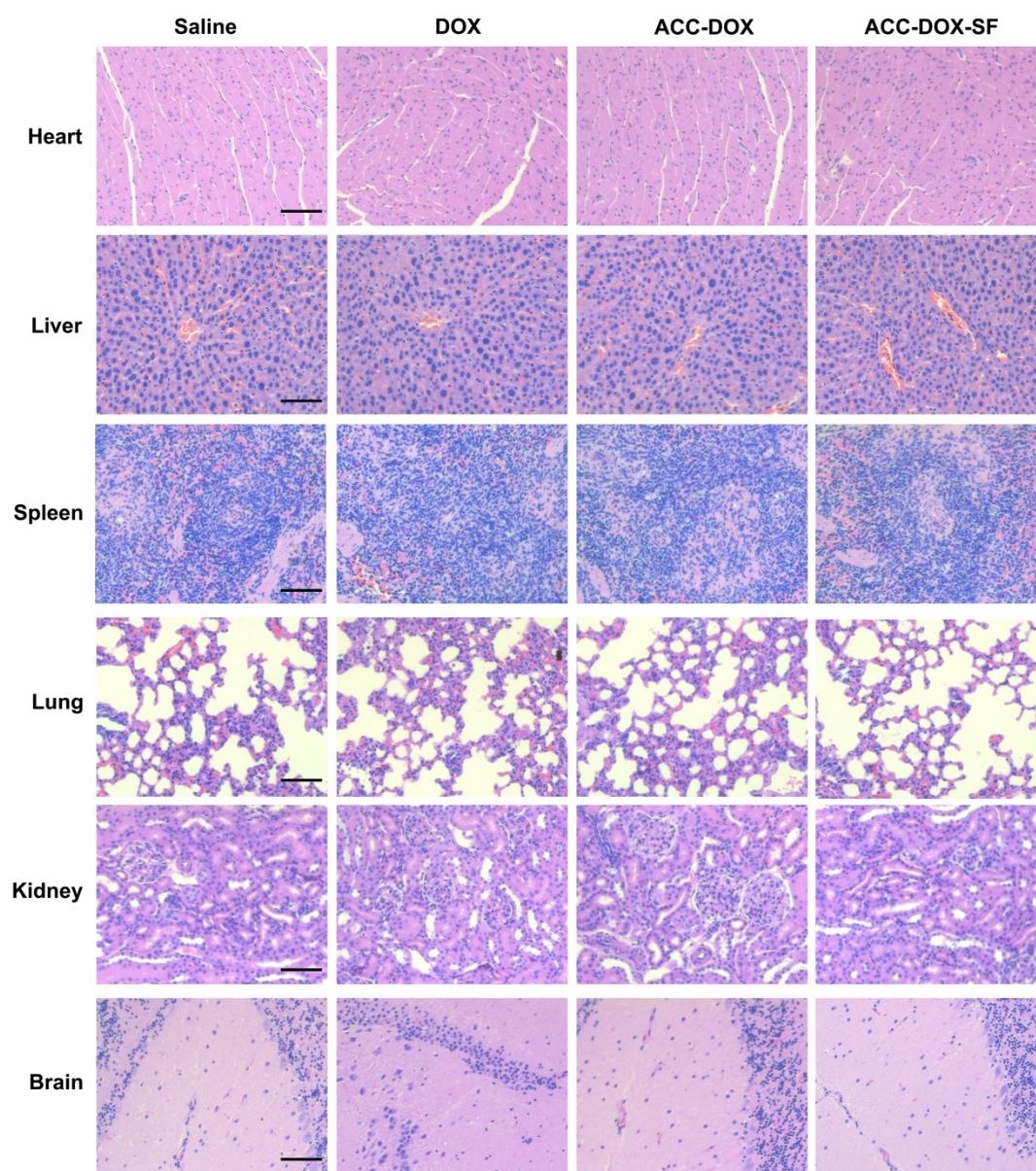


Figure S8. Histological observation of H&E-stained tissue sections of major organs (heart, liver, spleen, lung, kidney and brain) after treatments with saline, free DOX or ACC-DOX-SF NPs at day 21. The scale bars are 50 μ m.

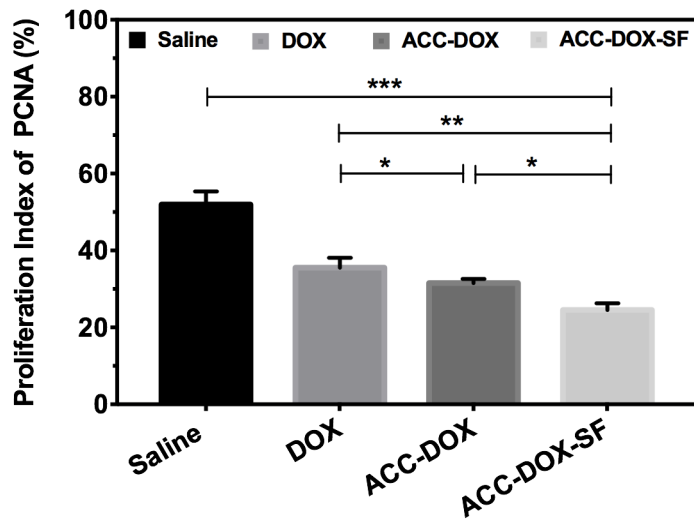


Figure S9. The proliferation index (PI) of PCNA in each group. The results are expressed as the mean \pm SD (n = 5, *P < 0.05, **P < 0.01, or ***P < 0.001).

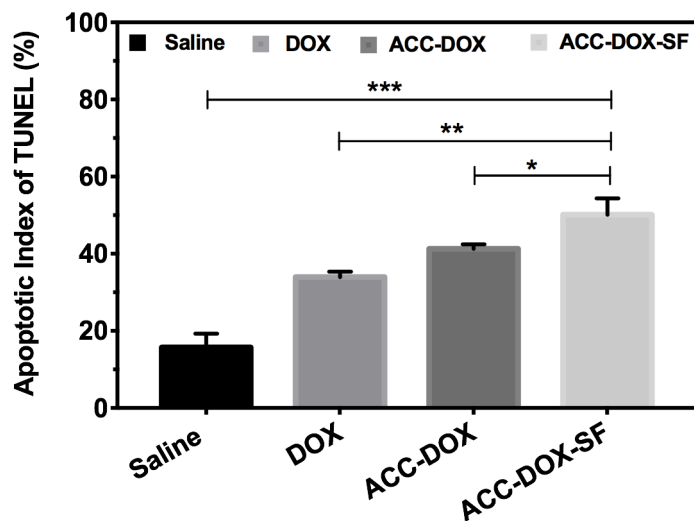


Figure S10. The apoptotic index (AI) of TUNEL in each group. The results are expressed as the mean \pm SD (n = 5, *P < 0.05, **P < 0.01, or ***P < 0.001).

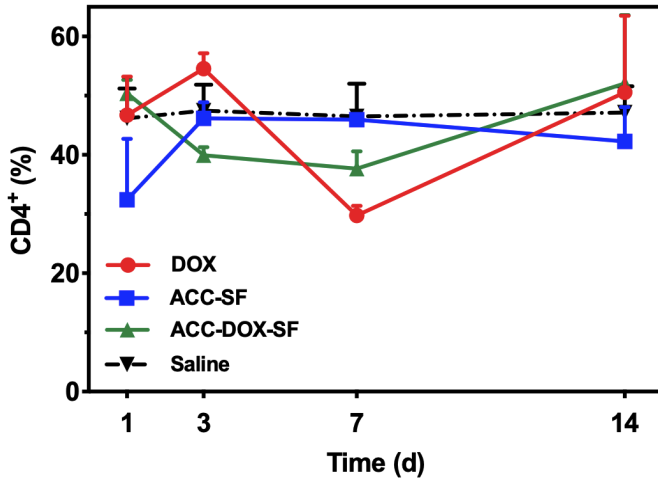


Figure S11. Changes of CD4⁺ T cells subsets after intravenous injection with ACC-DOX-SF NPs, ACC-SF NPs, DOX or saline for 1, 3, 7 and 14 days. The results were expressed as the mean \pm SD (n = 3, *P < 0.05).

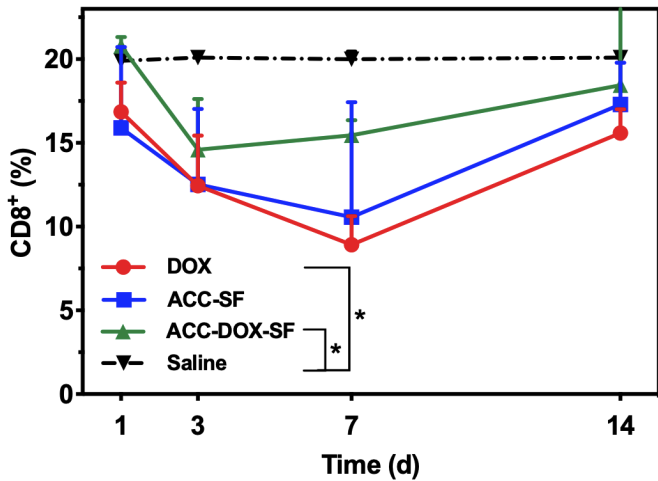


Figure S12. Changes of CD8⁺ T cells subsets after intravenous injection with ACC-DOX-SF NPs, ACC-SF NPs, DOX or saline for 1, 3, 7 and 14 days. The results were expressed as the mean \pm SD (n = 3, *P < 0.05).

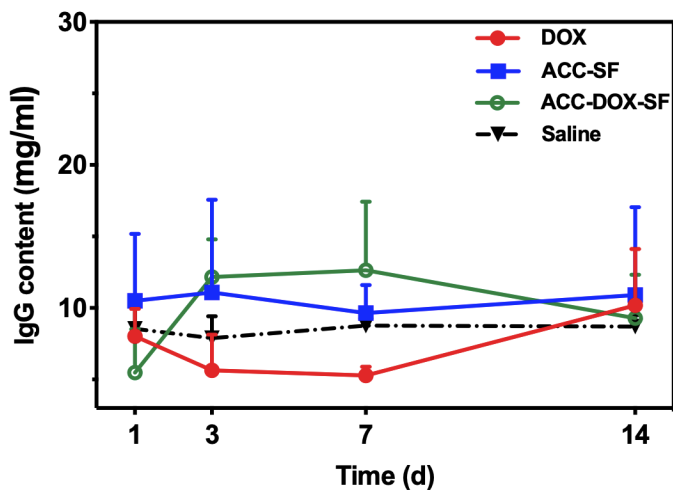


Figure S13. Changes of immunoglobulin G (IgG) levels after treated with ACC-DOX-SF NPs, ACC-DOX NPs, DOX or saline for 1, 3, 7, 14 days. The results were expressed as the mean \pm SD (n = 3, *P < 0.05).

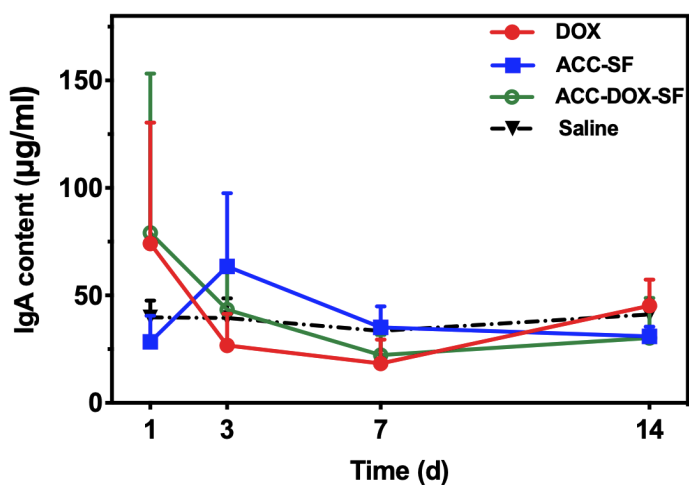


Figure S14. Changes of immunoglobulin A (IgA) levels after treated with ACC-DOX-SF NPs, ACC-DOX NPs, DOX or saline for 1, 3, 7, 14 days. The results were expressed as the mean \pm SD (n = 3, *P < 0.05).

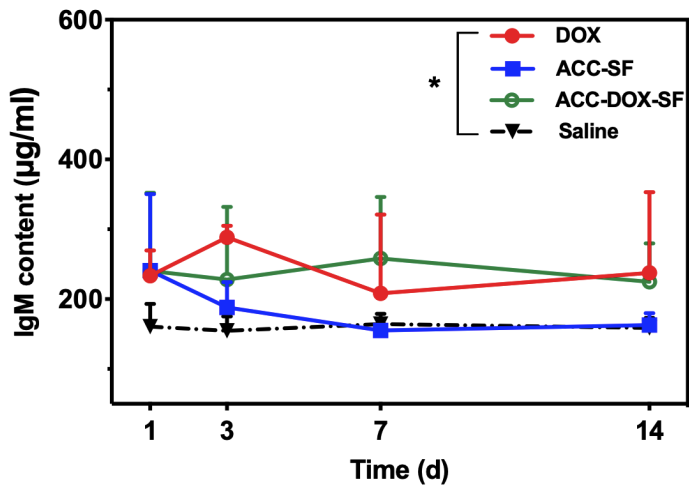


Figure S15. Changes of immunoglobulin M (IgM) levels after treated with ACC-DOX-SF NPs, ACC-DOX NPs, DOX or saline for 1, 3, 7, 14 days. The results were expressed as the mean \pm SD (n = 3, *P < 0.05).