Supplementary Information

Combination of tumor vessel normalization and immune checkpoint blockade for breast cancer treatment via multifunctional nanocomplexes

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Isolation and extraction of mouse peripheral blood mononuclear cells (PBMCs)

PBMCs of mice were separated by Ficoll density gradient centrifugation. Peripheral blood from mice was collected. The blood samples were diluted with PBS at 1:1 (v/v). The same amount of Ficoll separation solution was added to the EP tube, and the diluted blood sample was carefully added above the separation solution, which was centrifuged at 450 g for 25 min. Then, the liquid was divided into four layers. The second layer of annular milky white mononuclear cells was carefully pipetted into another EP tube, which was centrifuged at 250 g for 10 min to collect the cells. The cells were inoculated in 6-well plates at a cell density of 2.0×10^6 cells/mL, and added RPMI 1640 complete culture medium. After culturing in a cell incubator for 4 hours, we collected unadherent cells, which were PBMCs. 3 μg/mL anti-CD3 solution was added to the 6-well plates, which were placed at 37 °C for 2 hours, and replaced with new medium. Each well was supplemented with IL-2 (100 μg/mL) to activate and proliferate PBMCs.

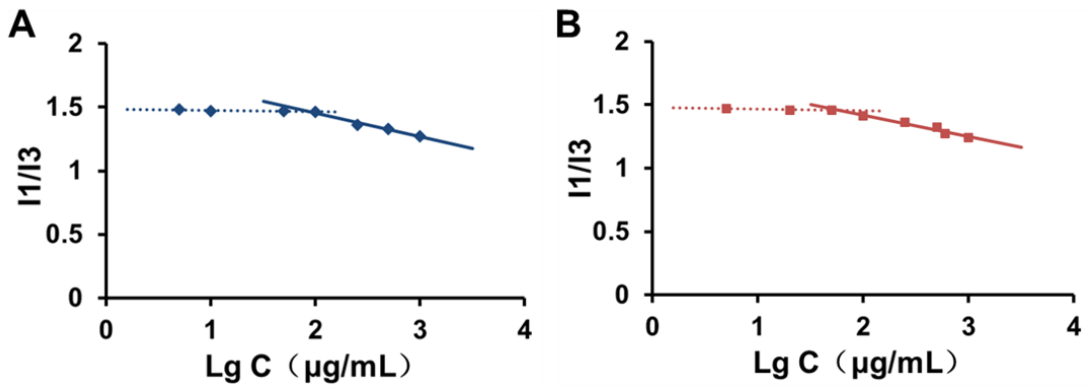


Fig. S1 The measurement of critical micelle concentration (CMC). (A) The measurement of CMC for COSA. (B) The measurement of CMC for FA-COSA.

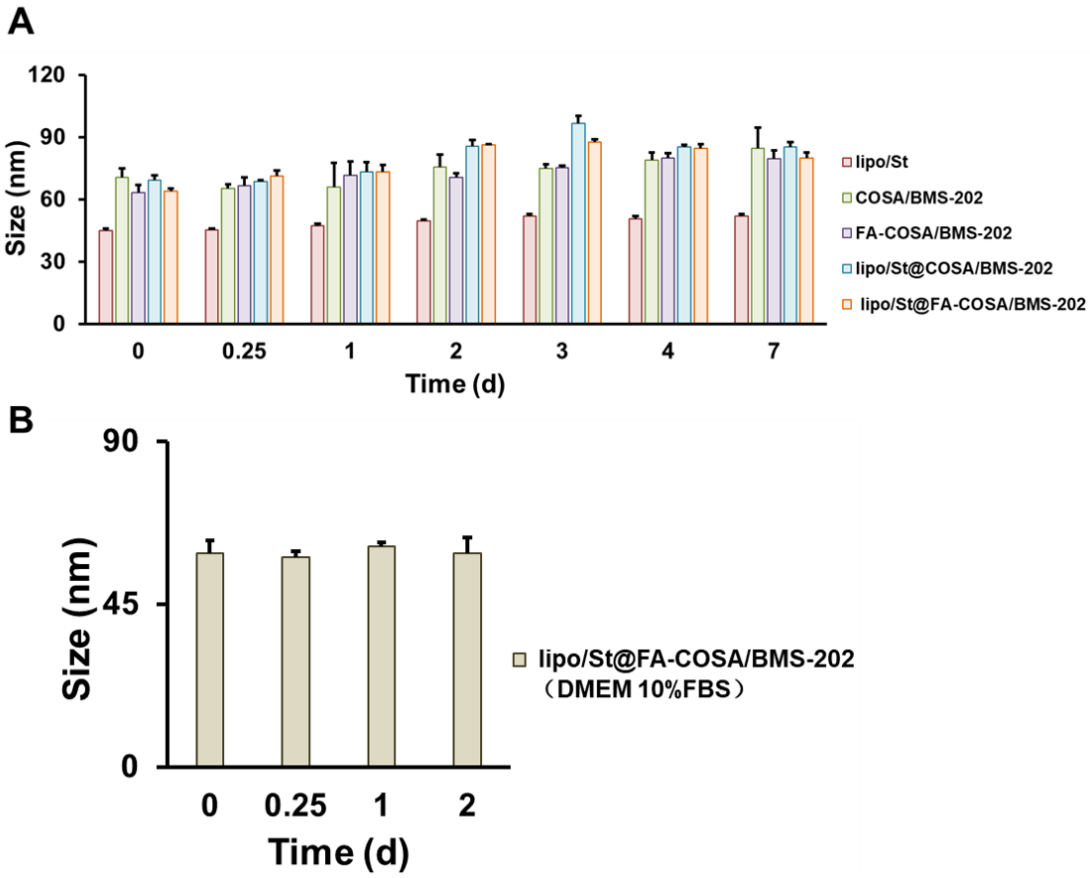


Fig. S2 *In vitro* stability of drug-loaded nanoparticles. (A)The particle size changes of the drug-loaded nanoparticles during 7 days storage at room temperature in PBS. (B)The particle size changes of lipo/St@FA-COSA/BMS-202 at room temperature in DMEM with 10% FBS. Data were expressed as mean ± standard deviation (n = 3).

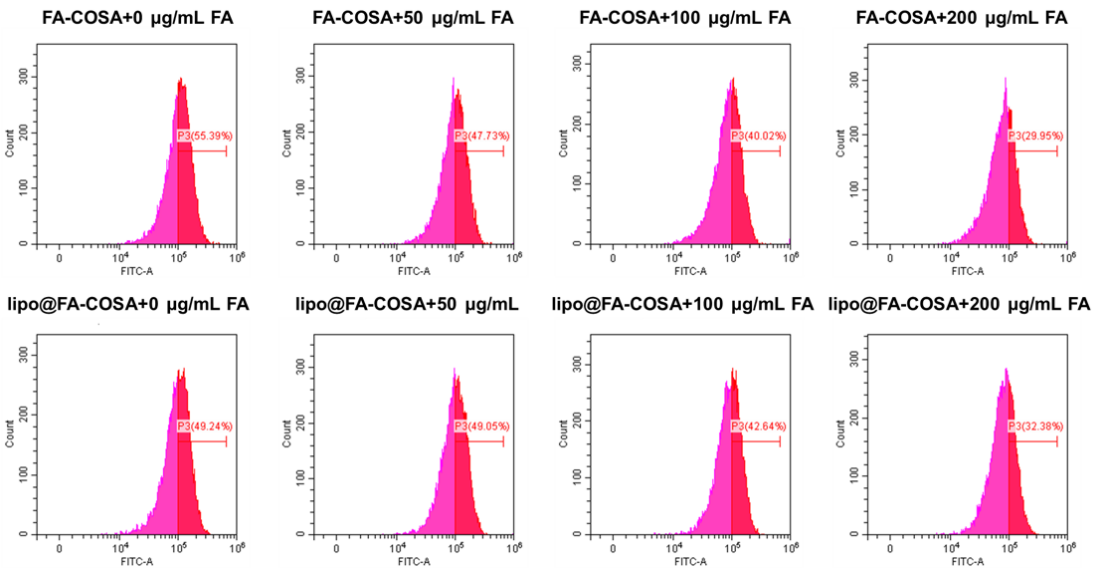


Fig. S3 The uptake of FA-COSA and lipo@FA-COSA by 4T1 tumor cells after FA pretreatment.

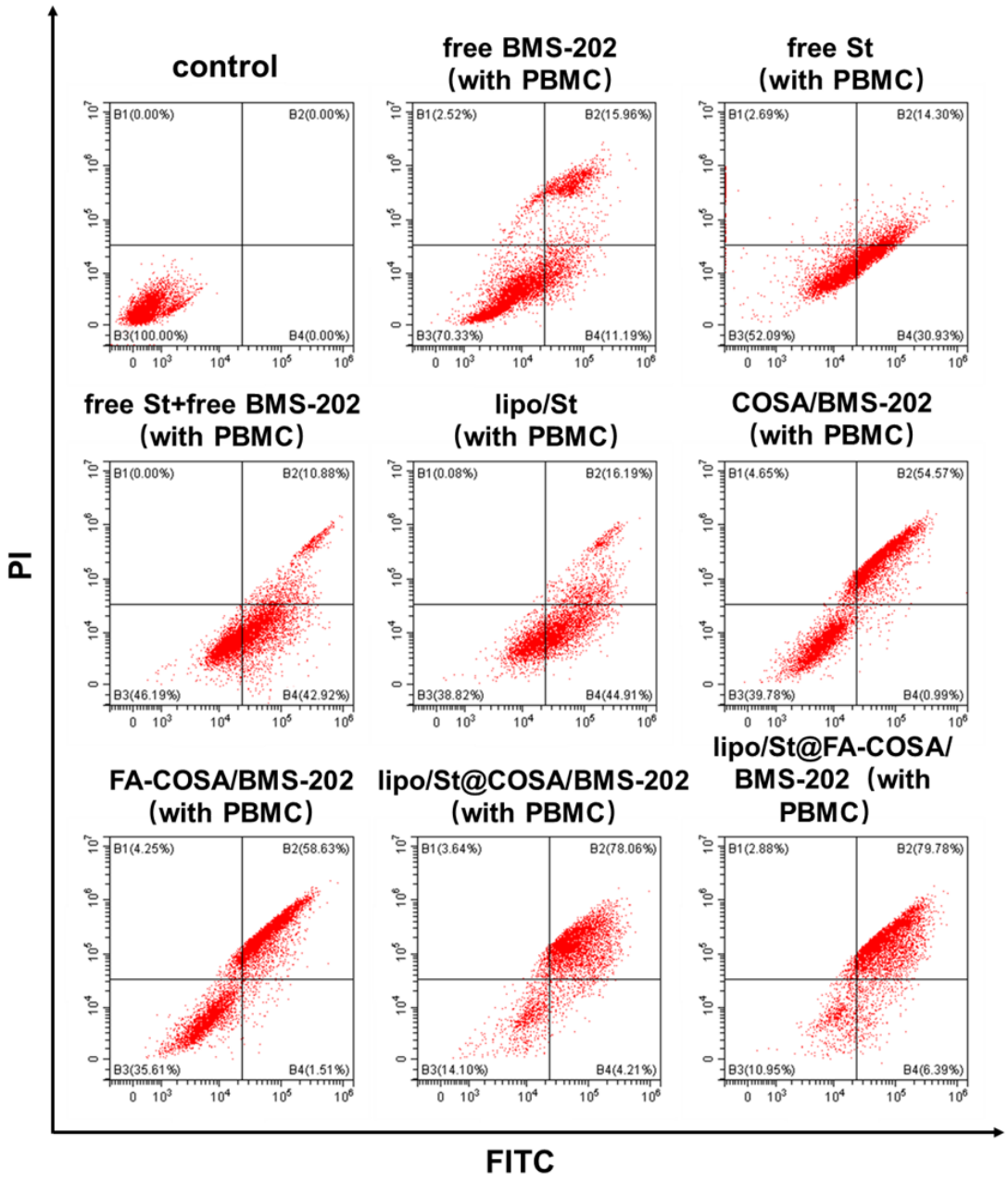


Fig. S4 The ability of different drug-loaded nanoparticles to induce 4T1 tumor cells apoptosis.

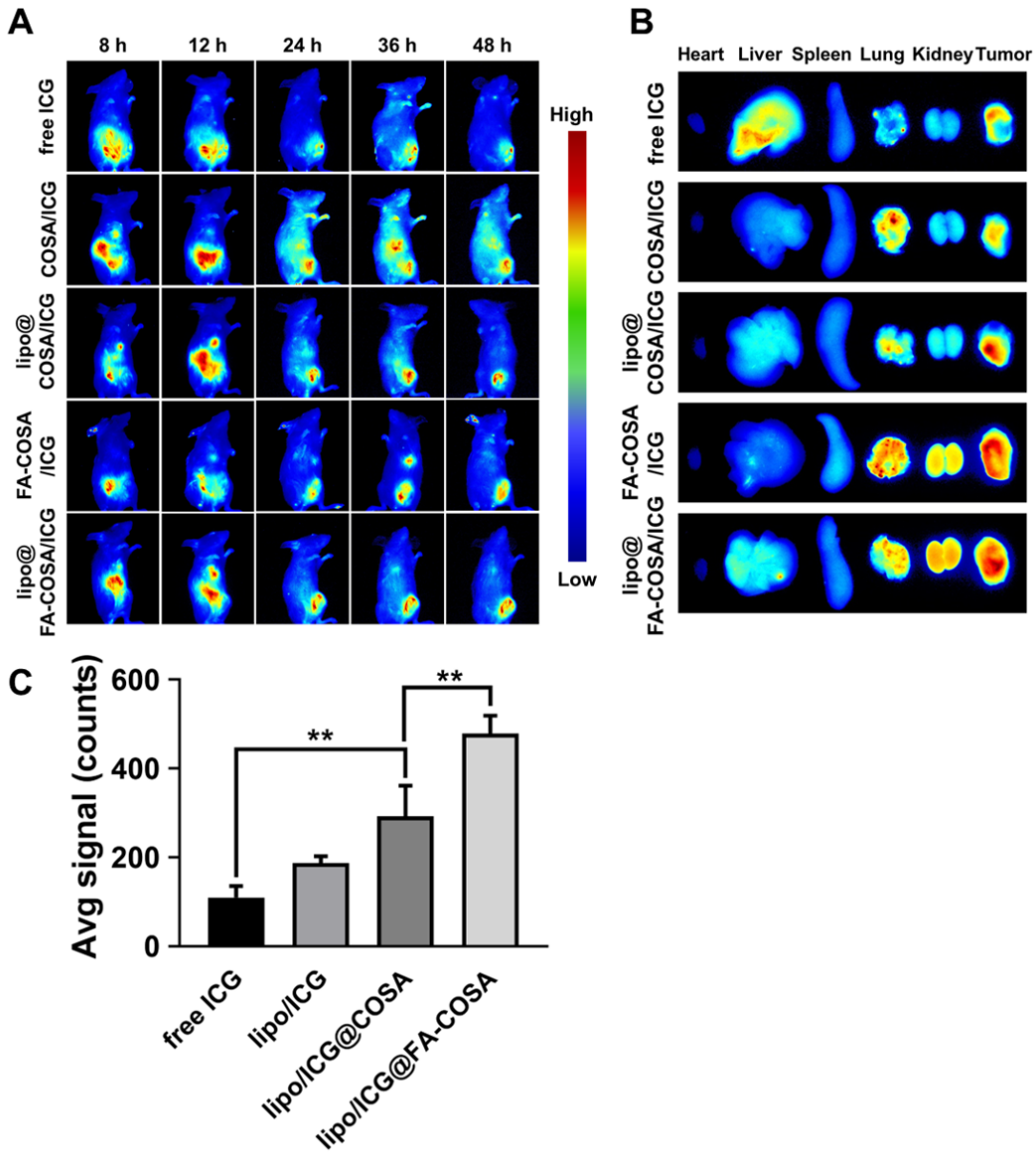


Fig. S5 *In vivo* distribution of nanoparticles in the 4T1 breast cancer mouse model. (A) Fluorescence images of nanoparticles in the 4T1 breast cancer mouse model (ICG-labeled COSA and FA-COSA). (B) *Ex vivo* fluorescence images of major organs and tumors (ICG-labeled COSA and FA-COSA). (C) Analysis of the average fluorescence intensity by Maestro 2.10.0 software (ICG-labeled liposomes). Data were expressed as mean ± standard deviation (n = 3).

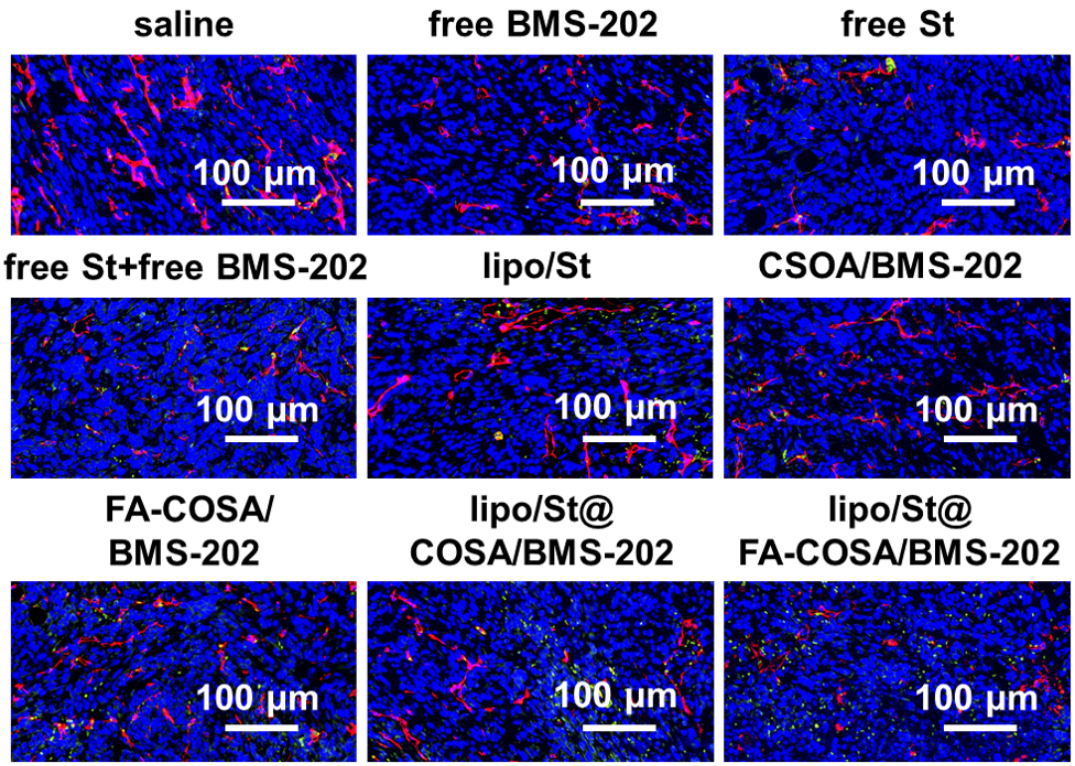
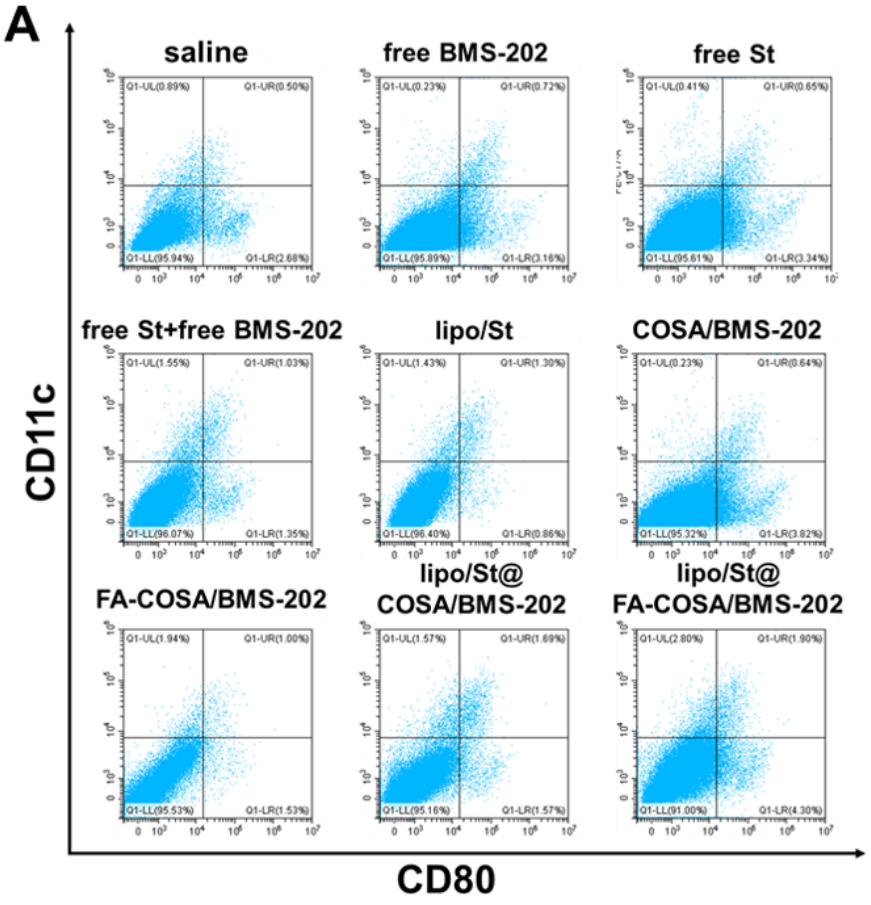
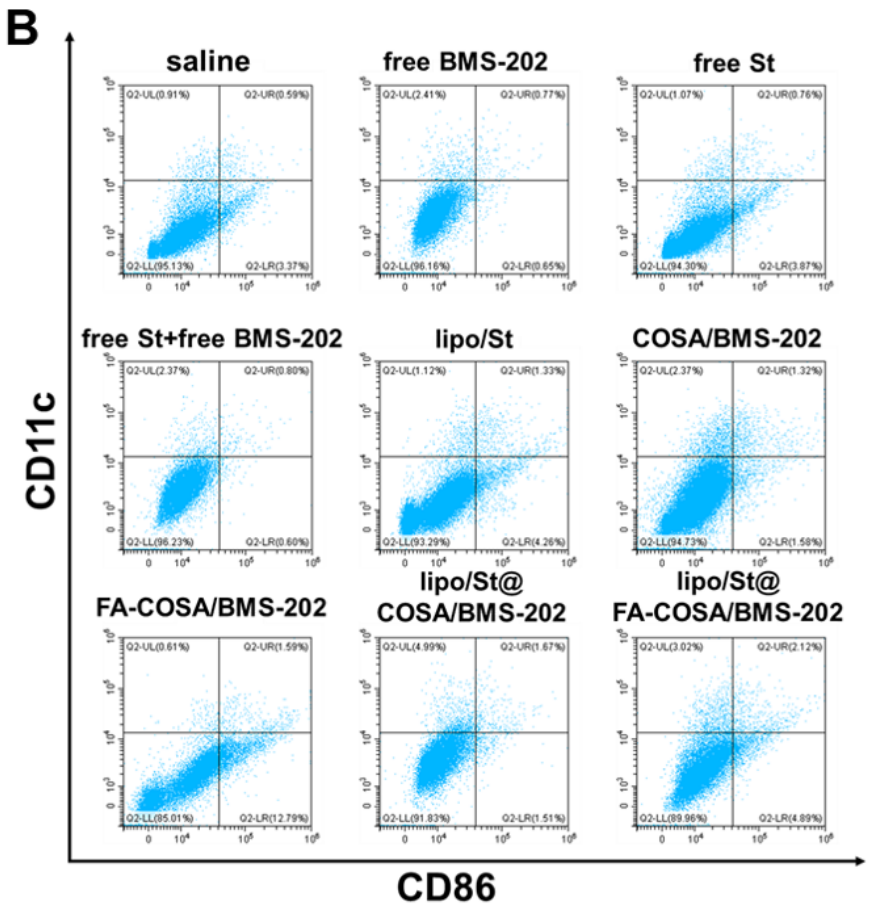


Fig. S6 CD31 and CD8 immunofluorescence staining of tumor tissues in each group. CD31 was marked in red and CD8 was marked in green. (Scale bar: 100 μm).


A
saline
free BMS-202
free St
free St+free BMS-202
lipo/St
COSA/BMS-202
lipo/St@
COSA/BMS-202
lipo/St@
FA-COSA/BMS-202
FA-COSA/BMS-202
CD11c
CD80


B
saline
free BMS-202
free St
free St+free BMS-202
lipo/St
COSA/BMS-202
lipo/St@
COSA/BMS-202
lipo/St@
FA-COSA/BMS-202
FA-COSA/BMS-202
CD11c
CD86

1

Fig. S7 The maturation of intratumoral DCs after treatment. (A) The proportions of $CD11c^+CD80^+$ cells in tumors. (B) The proportions of $CD11c^+CD86^+$ cells in tumors.

Table S1 Particle size and potential of the drug-loaded nanoparticles

Material	Size (nm)	Polydispersity index	Zeta potential (mV)
lipo/St	44.91 ± 1.07	0.210 ± 0.010	-23.6 ± 0.42
COSA/BMS-202	56.31 ± 7.88	0.275 ± 0.010	41.0 ± 1.99
FA-COSA/BMS-202	63.17 ± 3.62	0.258 ± 0.026	34.5 ± 0.84
lipo/St@COSA/BMS-202	69.22 ± 2.33	0.255 ± 0.009	30.3 ± 0.37
lipo/St@FA-COSA/BMS-202	76.97± 8.19	0.225 ± 0.031	26.7 ± 0.90