

Supporting Information

“Trojan horse” nanoparticle-delivering cancer cell membrane vaccines to enhance cancer immunotherapy by overcoming immune escape

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- 1. Supplementary Figures S1-S13**
- 2. Supplementary Tables S1-S2**

Supplementary Figures

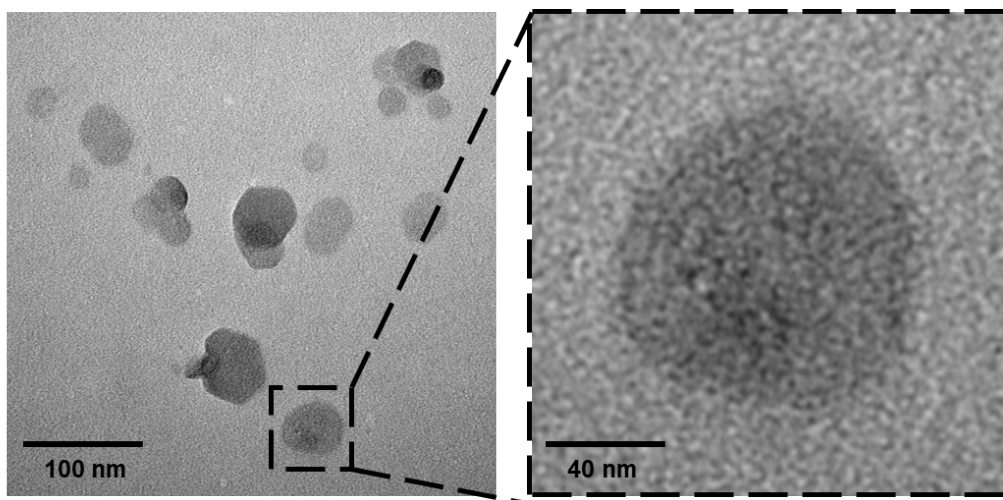


Figure S1. TEM images of LDH stained with 0.4% uranyl acetate.

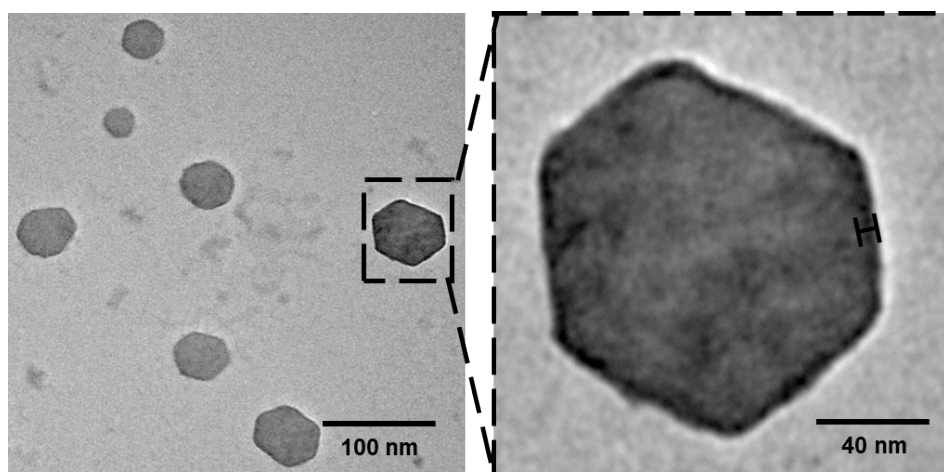


Figure S2. TEM images of LGCMB nano-vaccine with the mass ratio of LDH:CCM being 50:1.

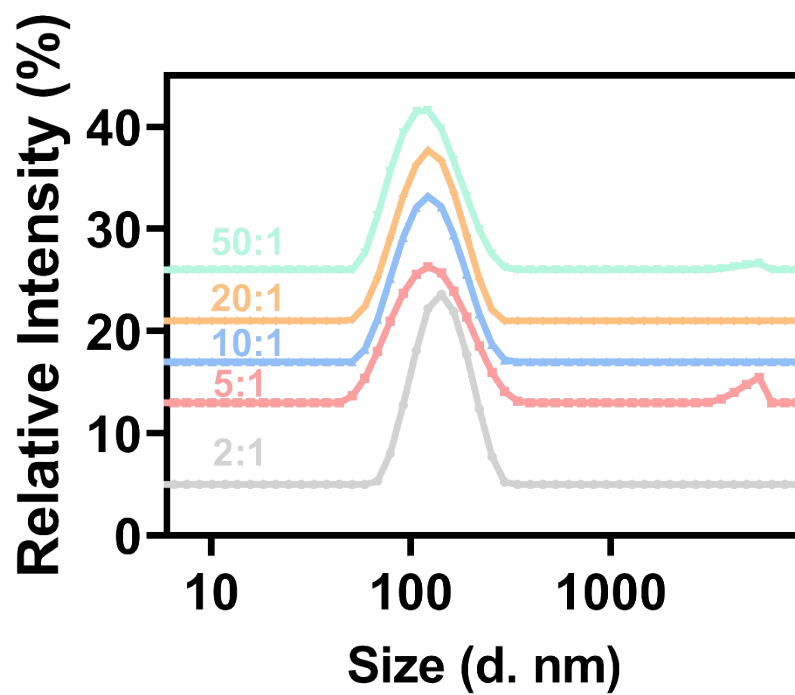


Figure S3. Size distribution of LGCMB with different mass ratios of LDH and CCM (LDH:CCM = 2:1, 5:1, 10:1, 20:1, 50:1).

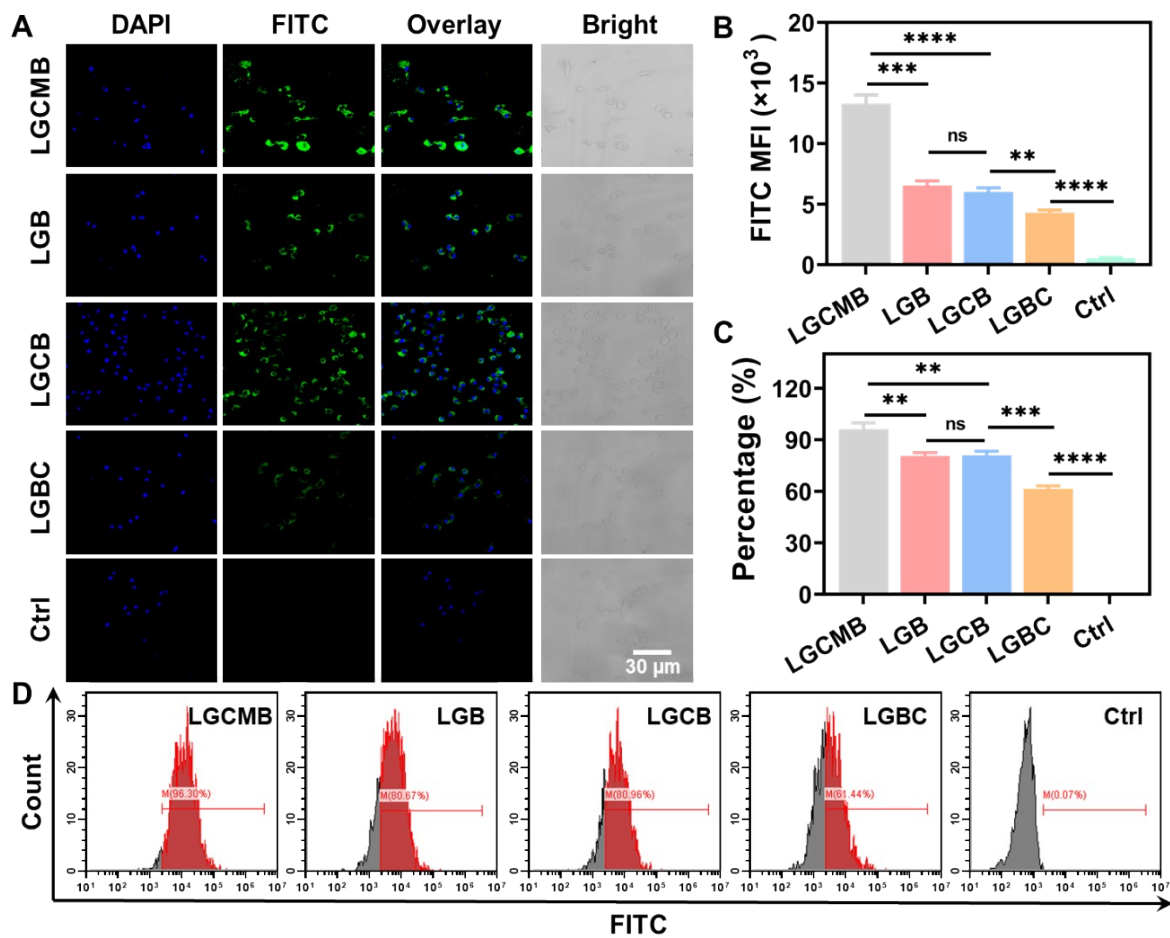


Figure S4. Cellular uptake of the LGCMB nano-vaccine by RAW 264.7 cells. (A) RAW 264.7 cells treated with LGCMB, LGB, LGCB and LGBC for 4 h captured by LSCM. (B) MFI and (C) positive cell percentage in RAW 264.7 cells treated with LGCMB nano-vaccines for 4 h. (D) Cell uptake by RAW 264.7 cells analyzed by FACS.

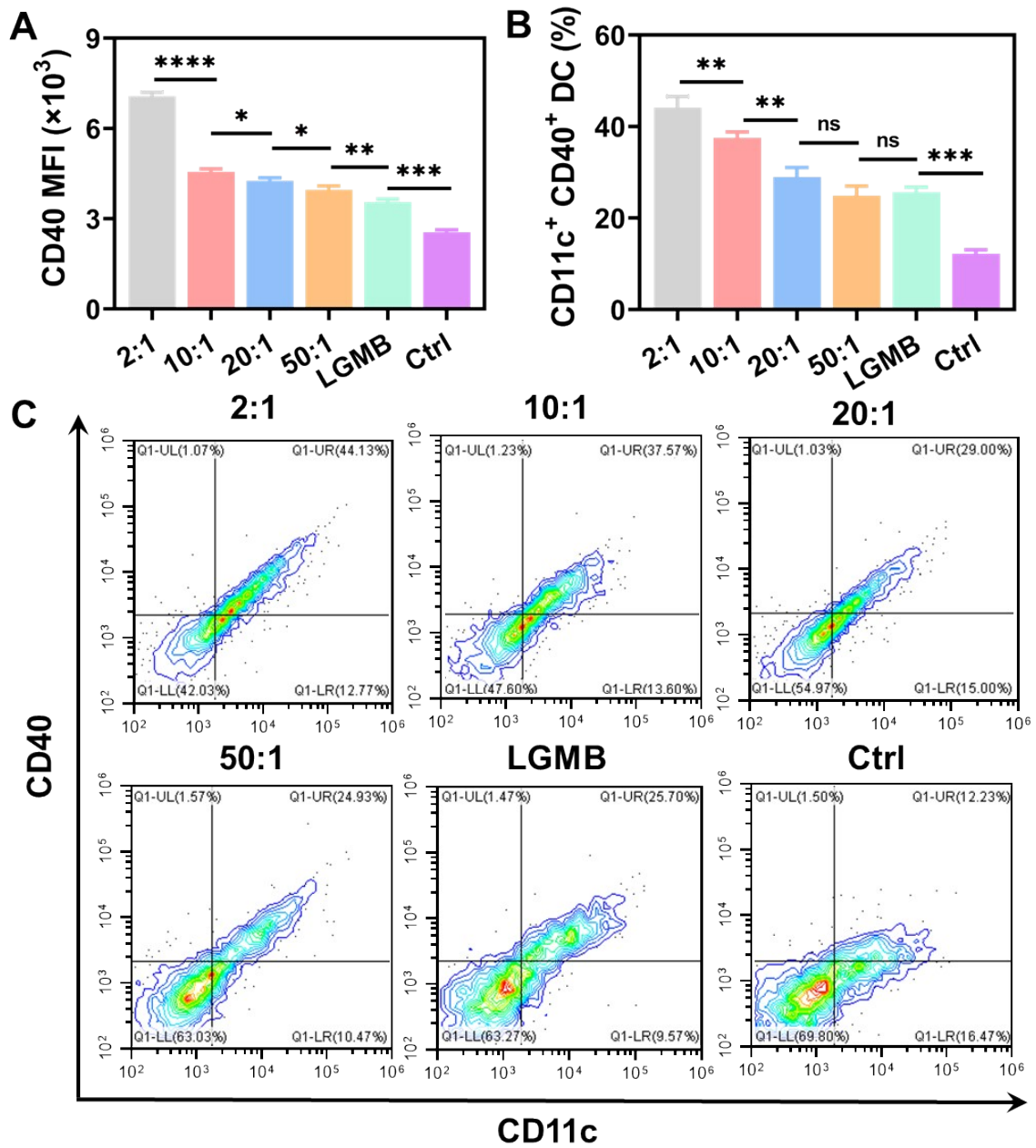


Figure S5. BMDC maturation when treated with LGCMB and LGMB for 24 h. (A-B) MFI and CD11c/CD40 positive cell percentage of DC cells treated with LGCMB (LDH:CCM=2:1, 10:1, 20:1, 50:1) and LGMB for 24 h. (C) CD11c and CD40 co-staining of DC cells treated with LGCMB and LGMB for 24 h.

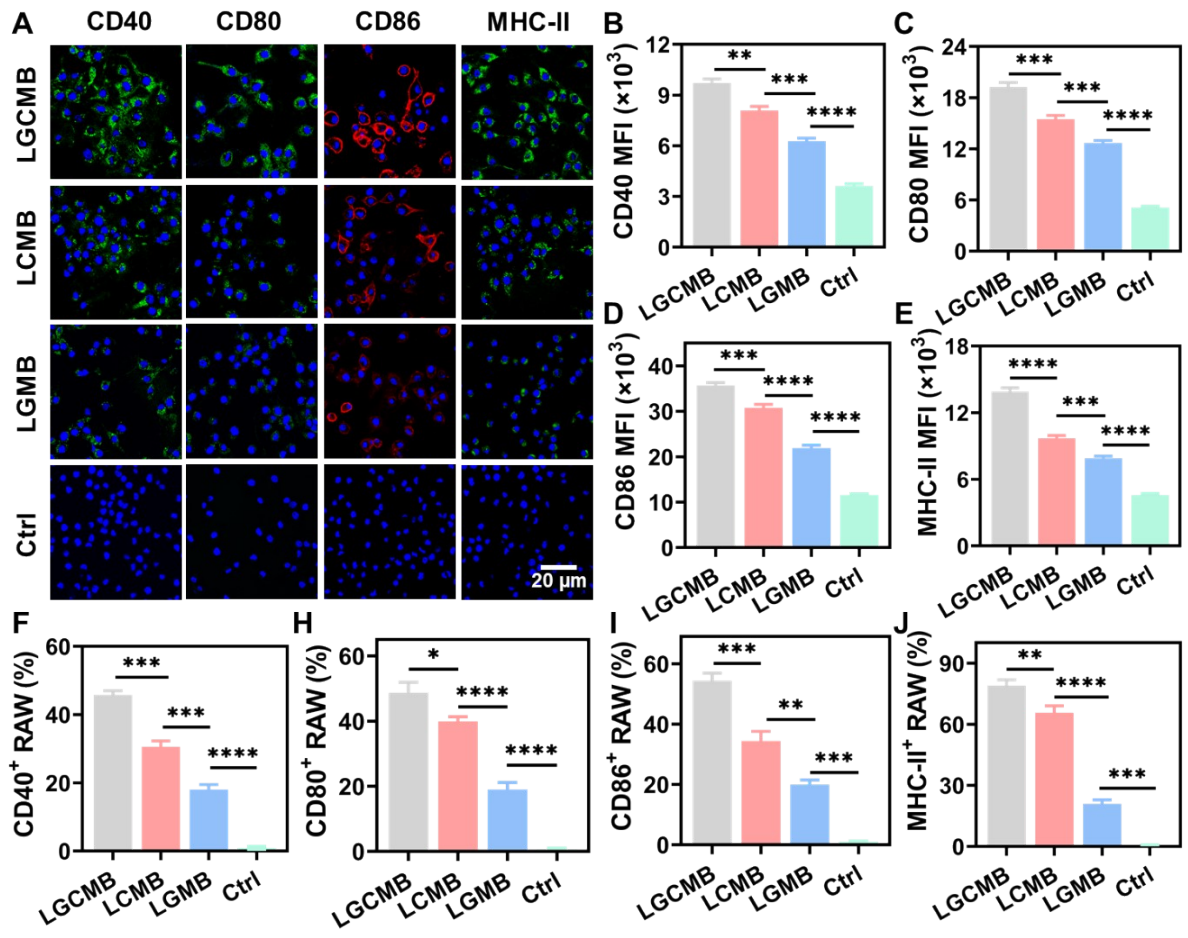


Figure S6. RAW 264.7 maturation stimulated by LGCMB nano-vaccines for 24 h. (A) CD40, CD80, CD86 and MHC-II staining of RAW264.7 cells treated with the nano-vaccines captured by LSCM. (B-E) MFI and (F-J) CD40, CD80, CD86, MHC-II positive cell percentage of RAW 264.7 cells.

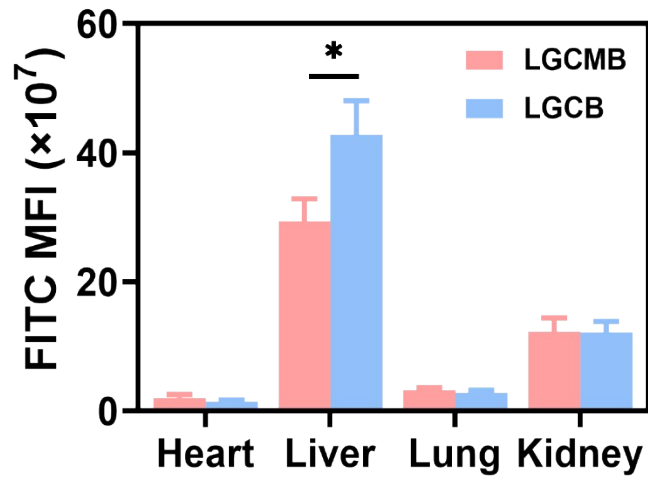


Figure S7. The MFI of FITC in the organs (heart, liver, lung, kidney) at 24 h post-injection of the nano-vaccine.

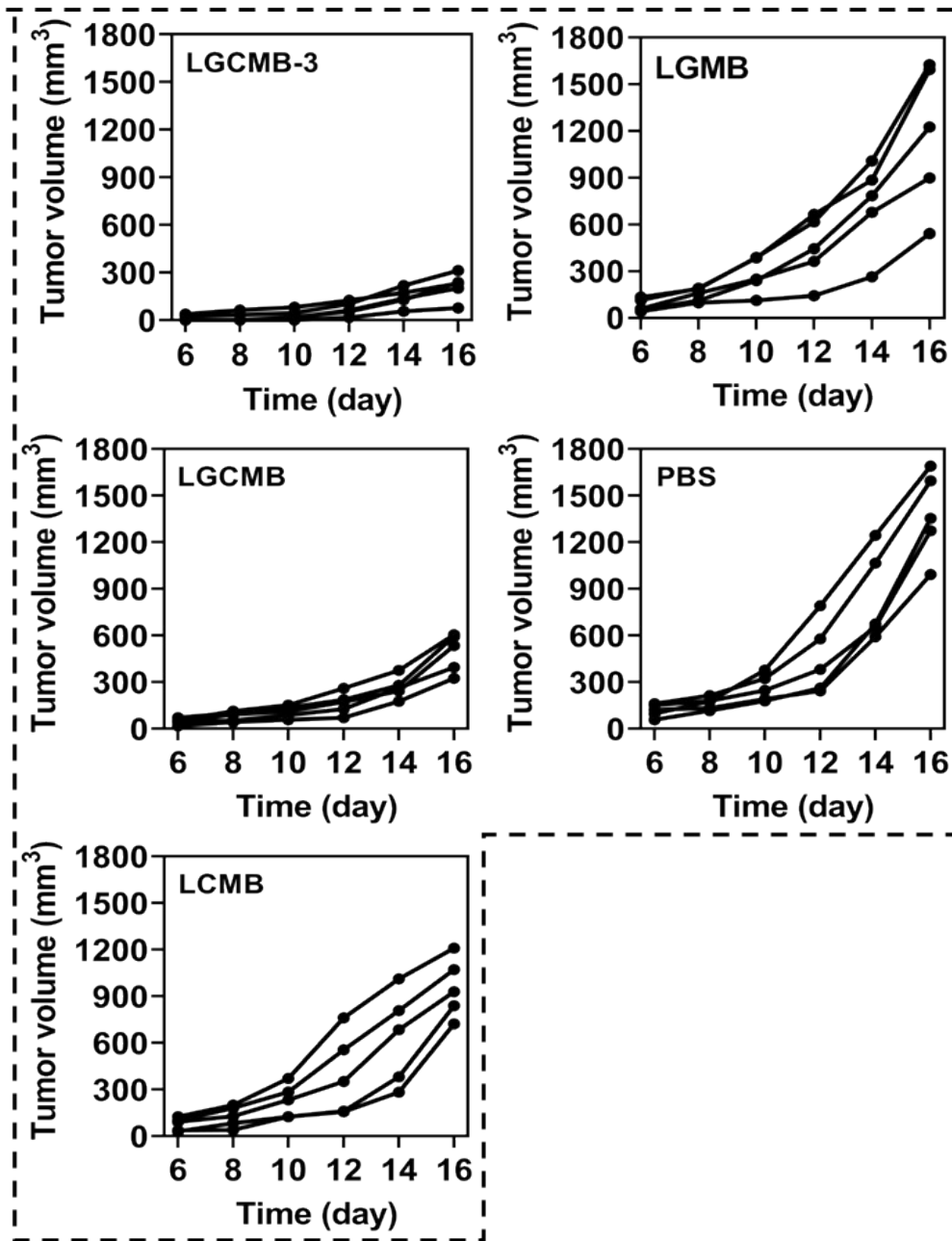


Figure S8. Tumor growth of the mice subcutaneously injected with LGCMB-3SC, LGCMB, LCMB, LGMB and PBS.

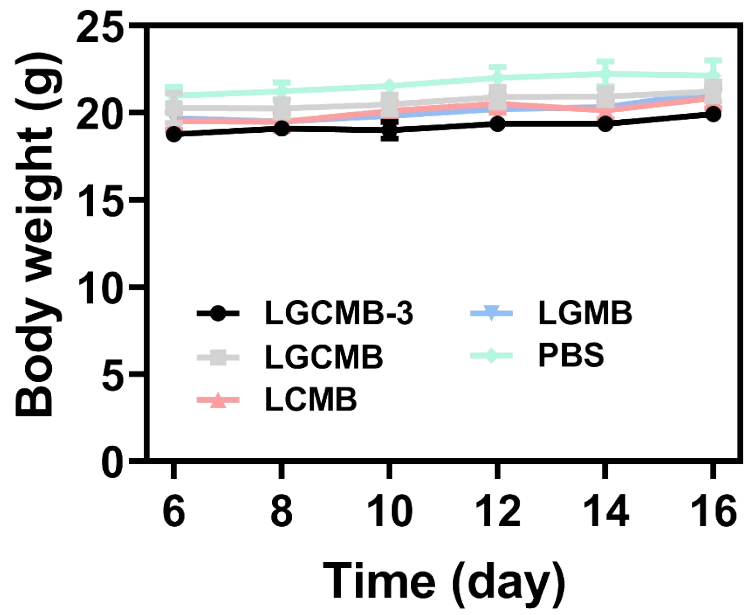


Figure S9. Body weight of the mice.

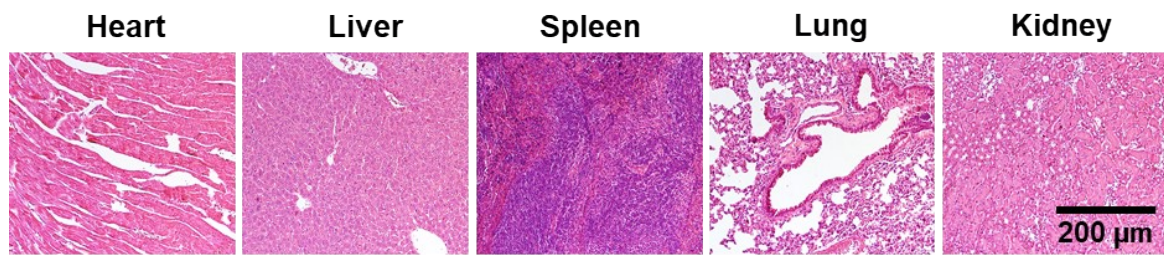


Figure S10. H&E staining of the major tissues collected from LGCMB-3 group.

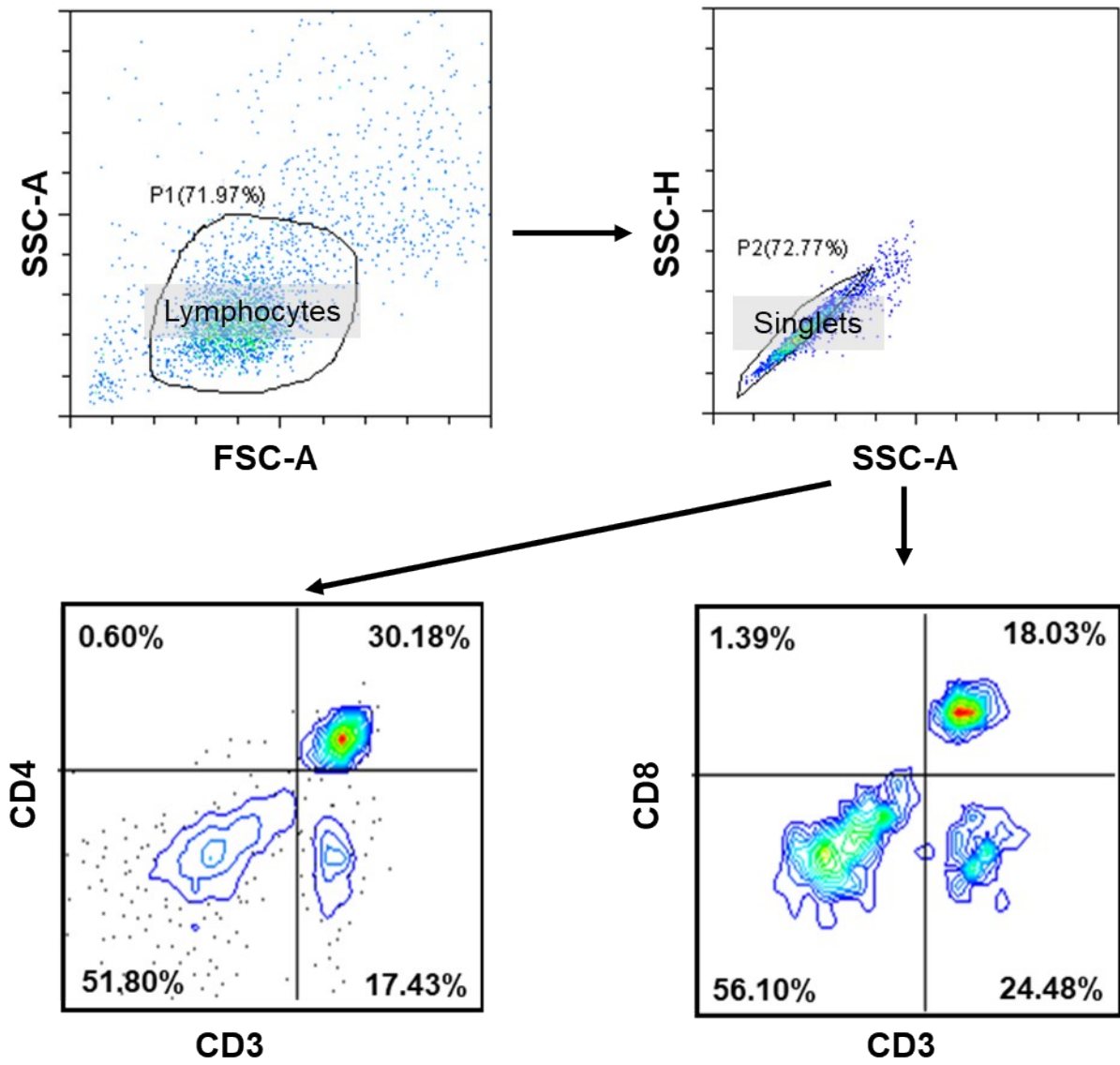


Figure S11: Gating strategy to sort CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells.

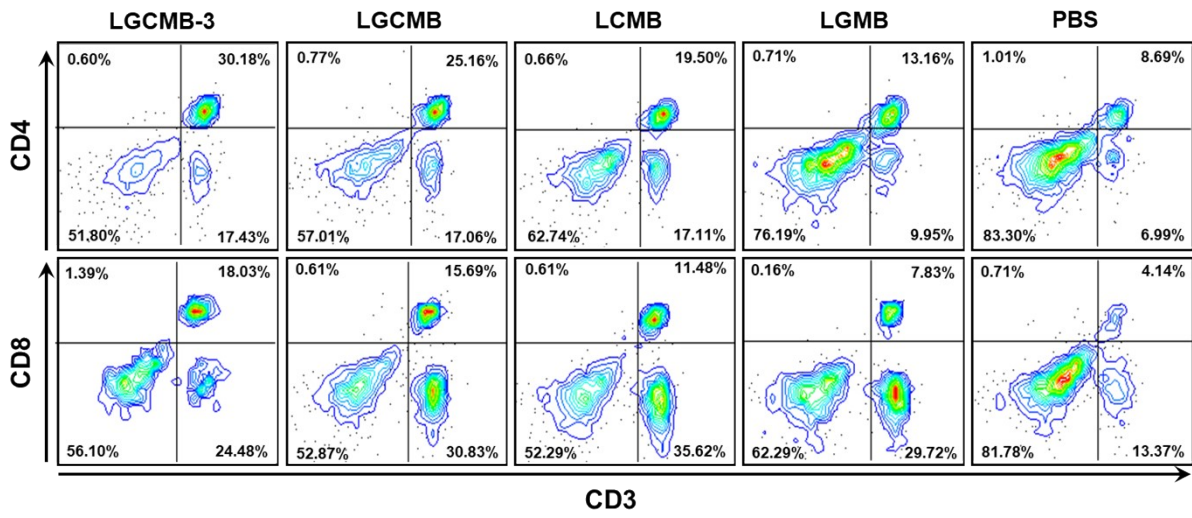


Figure S12. FACS analysis of T cell responses in spleen collected from the mice at day 16.

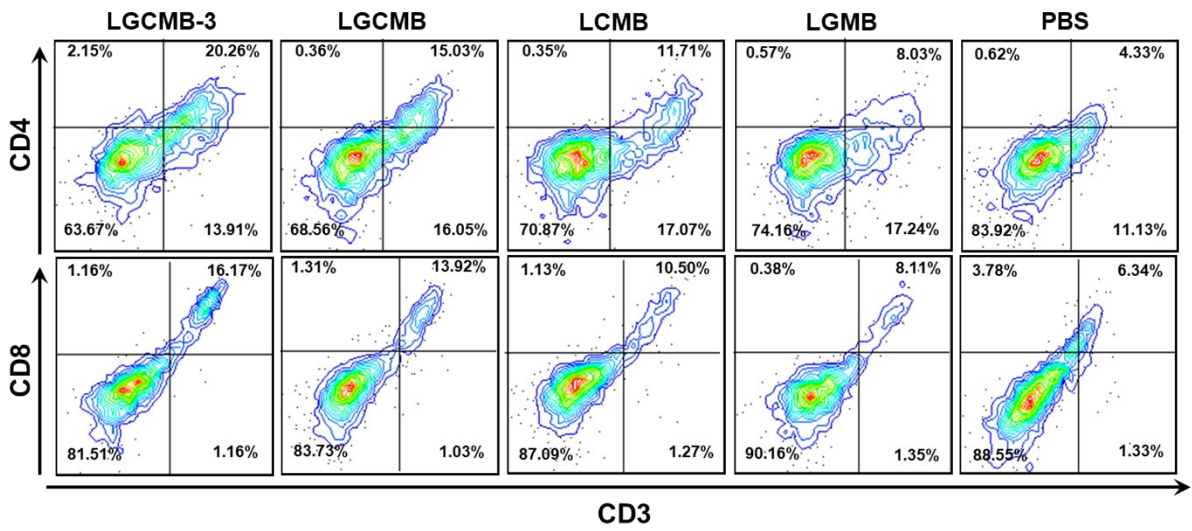


Figure S13. FACS analysis of T cell responses in tumor cells collected from the mice at day 16.

Table S1. Composition of the nano-vaccines.

Composition ($\mu\text{g/mL}$)	LDH	CpG	CCM [#]	Mannose	BSA
LGCMB*	1000	20	500	20	1280
LGB*	1000	20	N/A	N/A	1300
LGCB*	1000	20	500	N/A	1300
LGMB*	1000	20	N/A	20	1280
LCMB*	1000	N/A	500	20	1280
LGBC*	1000	20	500	N/A	1300

* In the LDH-CCM based nano-vaccine, L/G/C/M/B refers to LDH/CpG/CCM/Mannose/BSA.

[#] The amount of CCM on the nano-vaccine was calculated as the amount of CCM added given high CCM loading efficiency.

Table S2. Growth rate of the tumor at day 16 and synergistic effects of LGCMB.

Growth rate*	LGCMB-3 (%)	LGCMB (C) (%)	LCMB (A) (%)	LGMB (B) (%)	PBS (%)	CI [#]	Synergy
Volume	15.4 ± 6.2	35.5 ± 9.0	69.1 ± 13.9	81.3 ± 33.6	100 ± 20.1	1.58 ± 0.43	Moderate
Weight	13.4 ± 2.4	33.8 ± 8.5	57.9 ± 11.9	70.6 ± 13.8	100 ± 21.9	1.21 ± 0.15	Mild

*The growth rate of the tumor after various treatments was calculated based on tumor volume (V) and weight (W) at day 16 using the following formulas:

Growth rate = 1 - Inhibition rate

Inhibition rate (V) = (Tumor V_{control} - Tumor V_{experimental})/Tumor V_{control} × 100%

Inhibition rate (W) = (Tumor W_{control} - Tumor W_{experimental})/Tumor W_{control} × 100%

#The combination index (CI) = A×B/C ratio:

<0.8: asynergy

0.8-1.2: additive synergy

1.2-1.4: mild synergy

1.4-1.6: moderate synergy

>1.6: strong synergy