Supporting Information

Chemotherapy based on "Domino-effect" combined with immunotherapy amplifying the efficacy of anti-metastatic treatment

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Chemical synthesis.

In this work, CA was synthesized by 2-pyridinecarboxaldehyde and cyclohexanone (Fig. S1A). Then it was identified by ^{1}H NMR, ^{12}C NMR and the fluorescence spectra. As shown in Fig. S1B, ^{1}H NMR of CA, ^{1}HNMR($CDCl_3$, 300 MHz) δ (ppm): 8.697(m, 2H, arom), 7.673 (m, 4H, arom), 7.442 (s, 2H, -CH=), 7.209 (m, 2H, arom); 3.301 (t, J=6.3Hz, 4H, -CH_2CCH_2-), 1.866 (q, J=6.3Hz, 2H, -C-CH_2-C-); ^{12}C NMR ($CDCl_3$, 75MHz), δ (ppm): 22.25, 28.47, 122.47, 127.11, 134.04, 136.15, 140.12, 149.53, 155.52, 191.39 (Fig. S1D). NMR spectroscopy indicated the successful synthesis of CA. The Fluorescence characteristic of CA was also investigated, it had a clear peak

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at 530 nm (Fig. S1C).

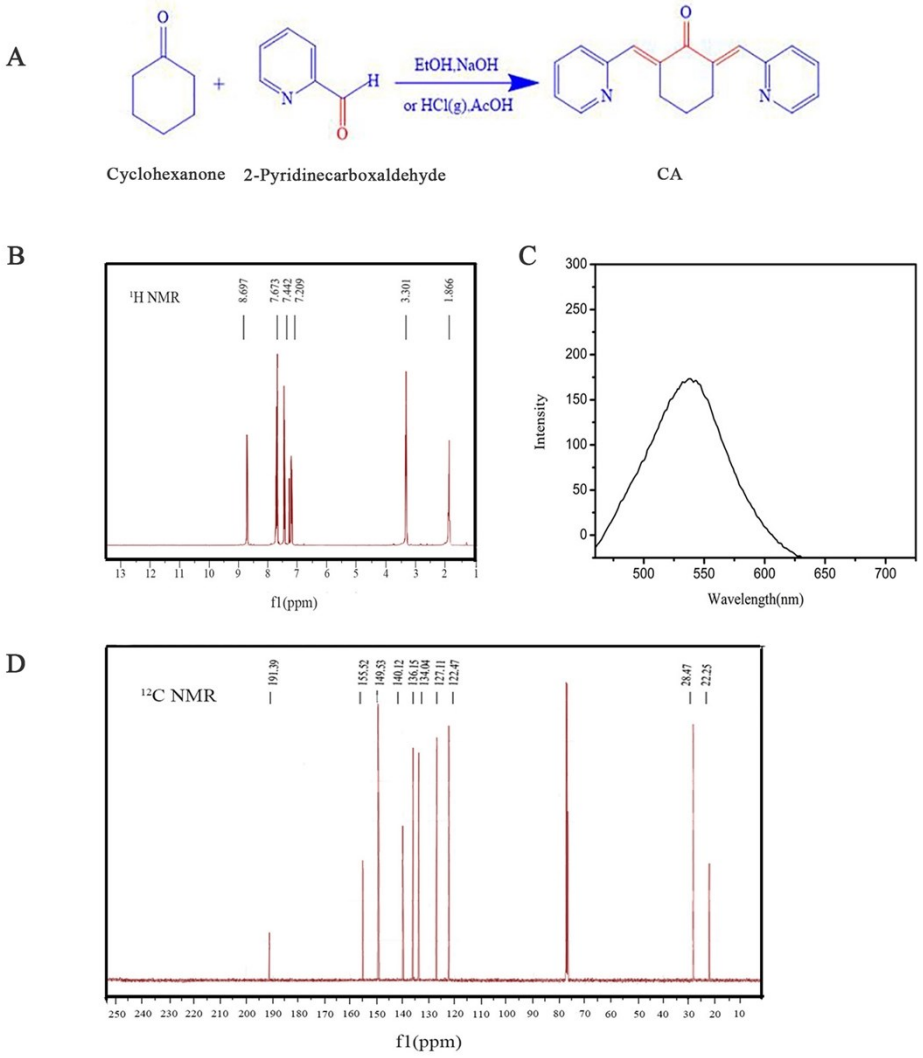

Fig. S1. Characterization of CA. (A) Synthesis of CA. (B) ^{1}H NMR spectrum of CA. (C) Fluorescence spectrum of CA. (D) ^{12}C NMR spectrum of CA.

4T1 cellular uptake of CA

The cell uptake and intracellular distribution of CA@α-LA-P-mAb NPs were carried out to compare the penetration ability by fluorescence microscopy and flow cytometry. Cell nuclei were stained by DAPI with blue fluorescence. CA was observed to show yellow green fluorescence, representing the nanoparticles were up-taken by cells. And the drugs were found to increase significantly in 4T1 cell nuclei, suggesting a high uptake of CA@α-LA-P-mAb NPs in 4T1 cells

(Fig. S2A). After hatching for 4 h, the quantitative analysis of red fluorescence intensity was measured by flow cytometry, the uptake rates have the following trends: CA@α-LA-P-mAb group (78.20%) > CA group (42.20%) > Control group (1.55%), which consistent with fluorescence microscopy (Fig. S2B).

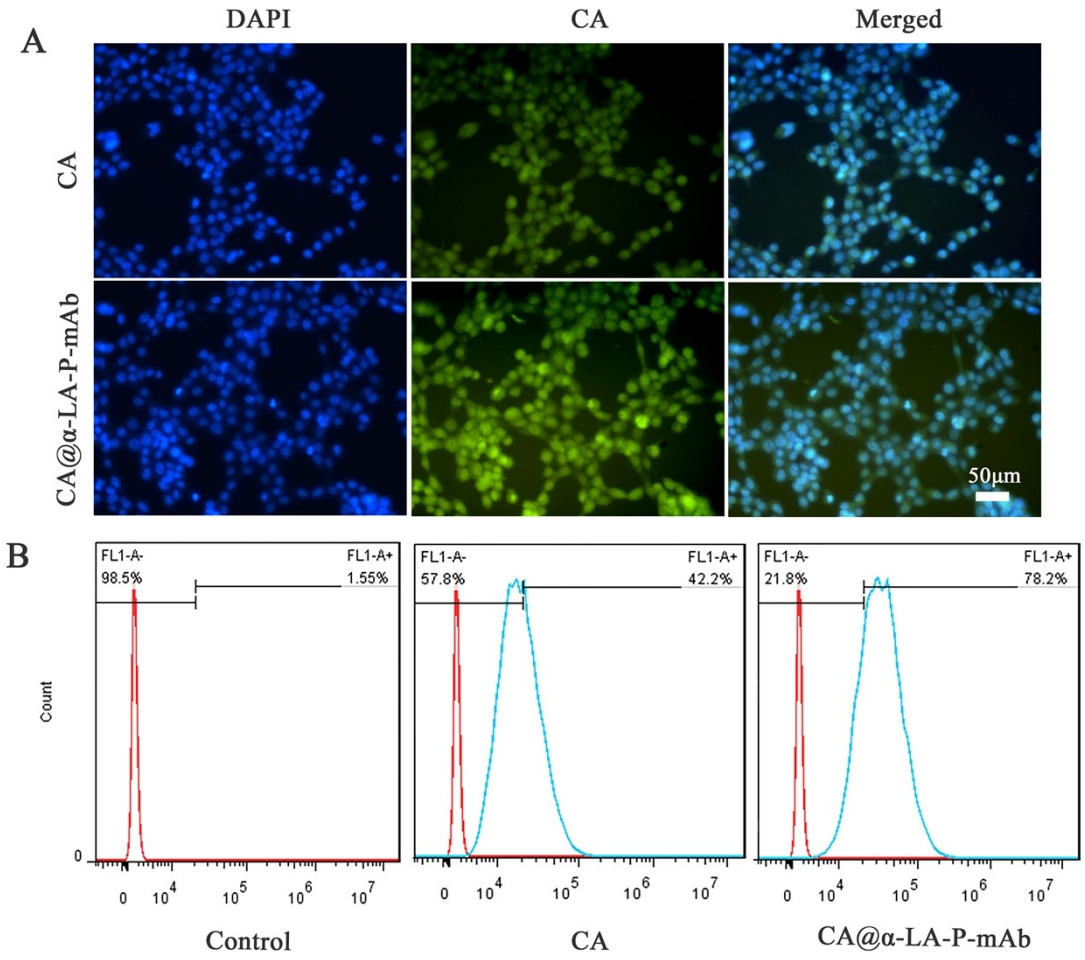

Fig. S2. Cellular uptake and intracellular distribution of 4T1 cells incubated with CA and CA@α-LA-P-mAb NPs. (A) Fluorescence microscopic images at 4 h. (B) The internalization amount of different formulations determined by flow cytometry.

H&E staining and the body weight changes of the mice

As shown in Fig. S3A, the H&E staining results indicated that there were no visible lesions in the

major organs, it demonstrated there were no significant toxic side effects caused by drug treatment. After treatment for 14 days, body weight of the mice was slightly higher, indicating no significant toxicity to survival (Fig. S3B).

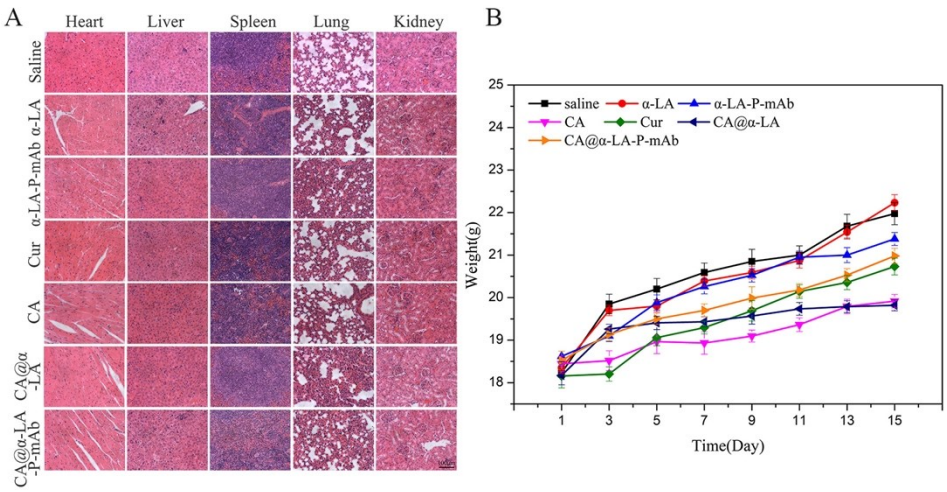

Fig. S3. (A) H&E staining of major organ slices (heart, liver, spleen, lung, and kidney). (B) During the administration, the body weight changes of the mice in different groups.

Materials and methods

BCA protein quantitative method

The series of protein standard (10 μL) and the test protein solution (10 μL) were added to the labeled test tubes individually. Then working solution (0.2 mL) was added to each test tube above. The solution was mixed and put to a 96-well plate. After incubation (37 ℃, 30 min), the absorbance of 96-well plate was measured with a microplate reader (λ = 562 nm). The protein concentration was calculated according to the standard curve. The amount of antibody linked was reflected by the amount of protein.

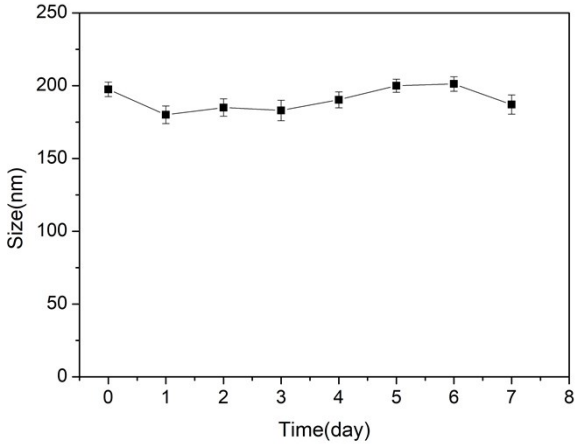

Fig. S4. The stability experiments of CA@α-LA-P-mAb.

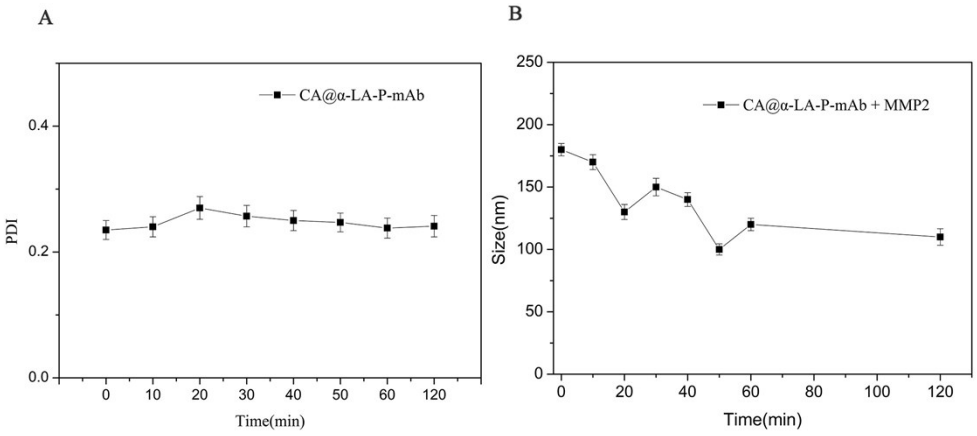

Fig. S5. The stability experiments of CA@α-LA-P-mAb. (A) PDI changes in CA@α-LA-P-mAb solution without MMP-2. (B)The particle size changes during the reaction between CA@α-LA-P-mAb and MMP-2.

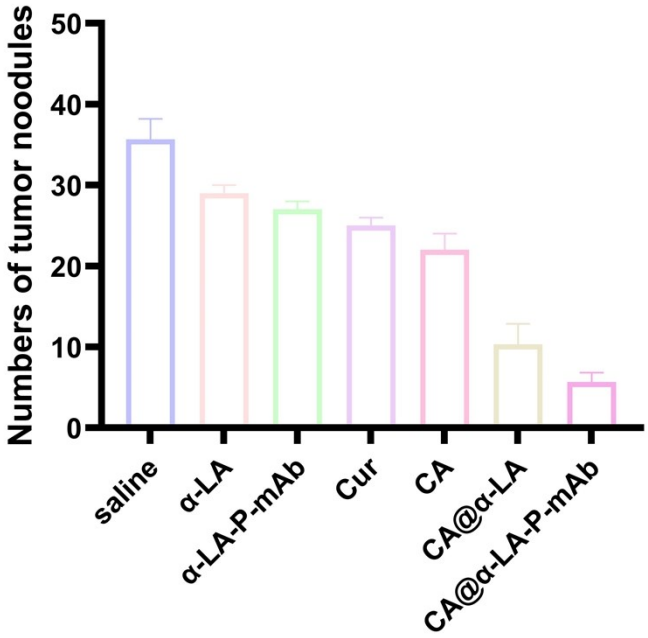

Fig. S6. The quantitative analysis of pulmonary metastatic nodules.