Supporting Information for

Responsive calcium-derived nanoassembly induces mitochondrial disorder to promote tumor calcification

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Experimental Section

Materials: L-Ascorbic acid (L-AA), $CaCO_3$, Na_2HPO_4, NaH_2PO_4 and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co., Ltd. Horseradish Peroxidase (HRP), bovine serum albumin (BSA), Trizol reagent, JC-1 staining kit and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Aladdin Reagents Company (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Ca^{2+} detection probe (Fluo-4 AM), Calcein acetoxymethyl ester (Calcein AM)-propidium iodide (PI) and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Yeasen Co., Ltd. ATP assay kit was obtained from Nanjing Jiancheng Biotech Co., Ltd. Hoechst and DAPI were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Von Kossa staining kit was purchased from Nanjing SenBeiJia Biological Technology Co., Ltd. Deionized water was generated using a Millipore Milli-Q system (Billerica, MA, USA). Deionized water was used throughout the experiments. All chemicals were analytical reagents and used without further purification.

Characterization: The cell pictures of schemes were created with BioRender.com. Transmission electron microscope (TEM) images were obtained from a TEM system (JEOL, JEM-2100Plus) at an accelerating voltage of 200 kV. The hydrodynamic diameter and zeta potential of the as-prepared nanoparticles were measured by dynamic laser light scattering (ZEN 3600, Malvern Instruments). Concentrations of calcium were measured by inductively coupled plasma (ICP-MS, Agilent, 8900). Cells were imaged through a

confocal laser scanning fluorescence microscope (Nikon, Ti-E+A1R MP). The MTT assay was measured using a Spectramax microplate reader (Molecular Devices, iD5). X-ray diffraction (XRD) patterns were used to acquire information on crystallography and measured on an X-ray diffractometer (D8-Advance). Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a FT-IR spectrophotometer (Bruker Vertex 70).

Hemolysis assay: Heparin stabilized mouse blood samples were obtained from female Balb/c mice. First, 3 mL of blood was centrifuged at 1600 rpm for 5 min, and the blood plasma and surface layer were removed. The remaining red blood cell (RBC) pellet was washed several times with 0.9% saline solution and diluted in 25 mL of PBS solution. Then, 0.5 mL of different concentrations of L-AA, CaP and CaP-AA solutions diluted in 0.9% saline were added into 1 mL of RBC suspension, respectively. Meanwhile, positive and negative control samples were prepared by adding 0.5 mL of water and 0.9% saline into 1 mL of RBC suspension, respectively. Then, the samples were incubated at room temperature for 2 h, shaking once slightly every 30 min to resuspend the RBCs. After 2 h, the samples were centrifuged at 1600 rpm, and 100 μL of supernatants were transferred to a 96-well plate. Absorbance of hemoglobin in supernatants was measured with a microplate reader at 570 nm, and the absorbance at 655 nm was recorded as the reference. Hemolysis percentages of the RBCs were calculated using the following formula: Hemolysis% = (abs of sample - abs of negative control) / (abs of positive control - abs of negative control) × 100%. The percent hemolysis values were calculated from three separate experiments.

Statistical analysis: All data are presented as the mean ± SD. Unless stated otherwise, the experiments were performed in triplicate. The significance of the difference was determined through one-way analysis of variance (ANOVA) by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, $^{ns}p \geq 0.05$.

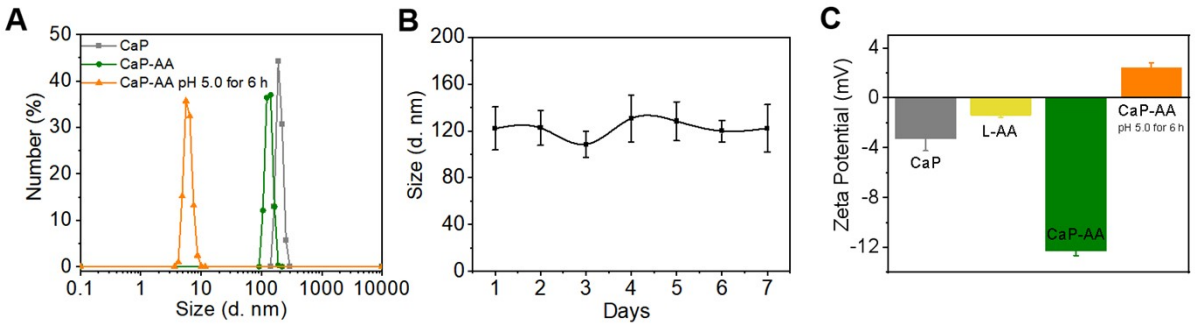

Fig. S1. (A) DLS spectra of corresponding NPs treated under different conditions. (B) DLS data of CaP-AA incubated in serum for various days. (C) Zeta potential of as-prepared NPs.

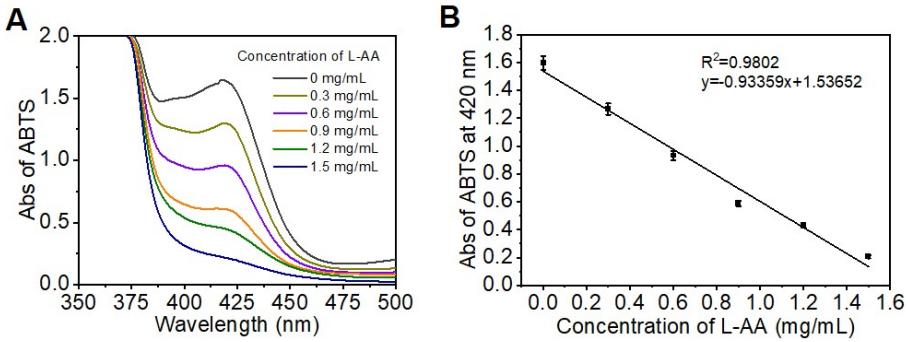

Fig. S2. (A) The Abs changes of ABTS with the addition of different concentrations of L-AA. (B) The corresponding linear trend of A.

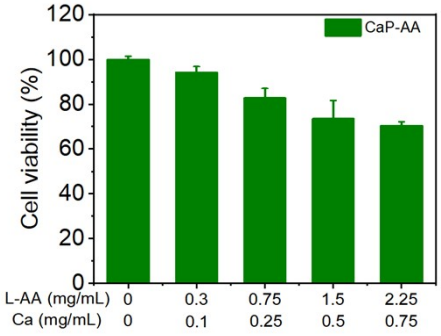

Fig. S3. Cell viability of 293T cells incubated with CaP-AA (quantified by Ca and L-AA, respectively).

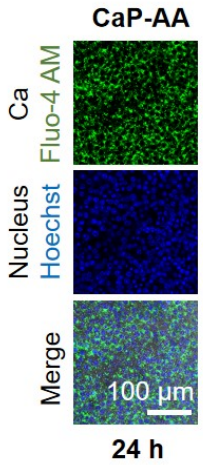

Fig. S4. Calcium generation in 4T1 cells after 24 h incubation with CaP-AA.

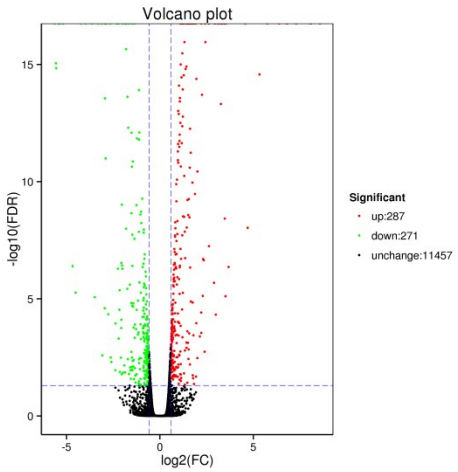

Fig. S5. Volcano plot of 4T1 cells differentially expressed genes after CaP-AA treatment.

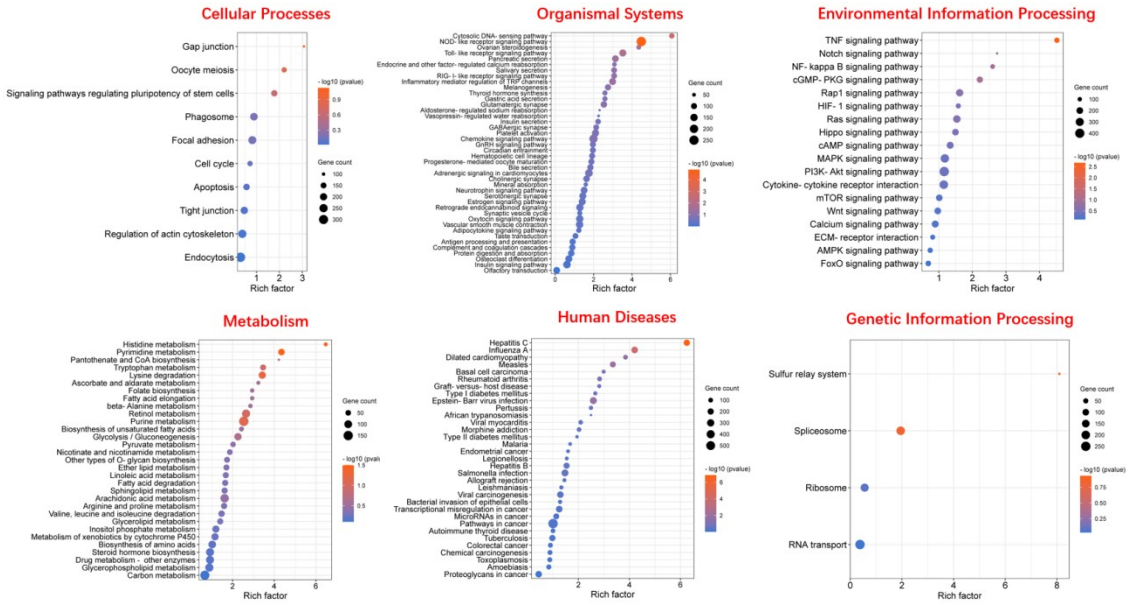

Fig. S6. Classification statistics of KEGG pathway enrichment analysis for differentially expressed genes of 4T1 cells after CaP-AA treatment.

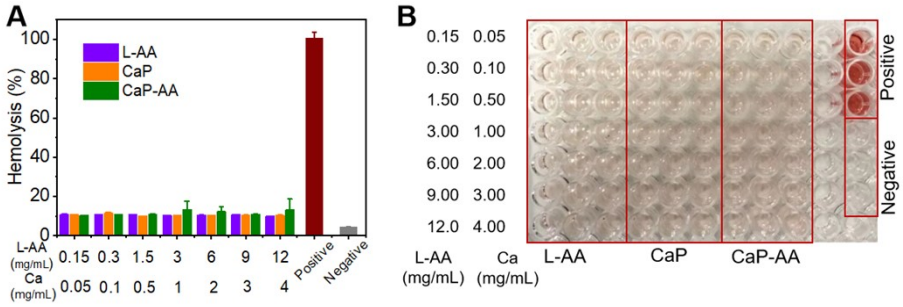

Fig. S7. (A) Hemolysis tests of different concentrations of L-AA, CaP and CaP-AA. (B) Corresponding hemolysis graph of (A).

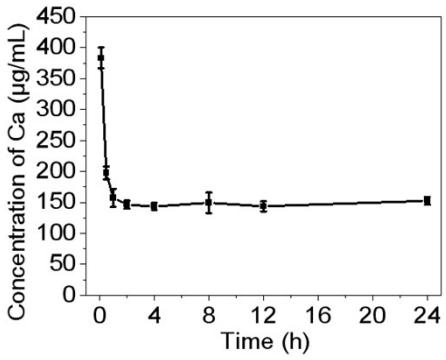

Fig. S8. Pharmacokinetics profile of CaP-AA.

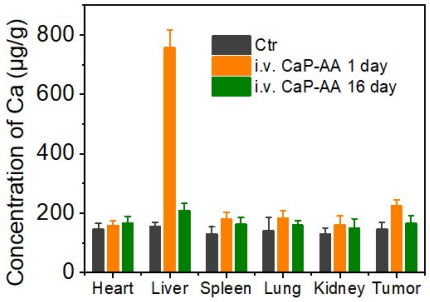

Fig. S9. Quantitative determination of CaP-AA based on ICP-MS (Ca) in different organs at 1 day / 16 days post-intravenous injection.

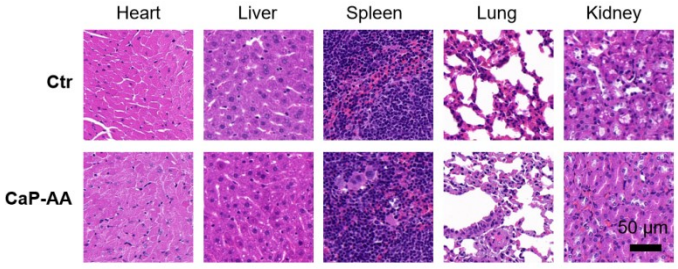

Fig. S10. H&E-stained organ slices at 16 days post-intratumoral injection of corresponding treatment groups.

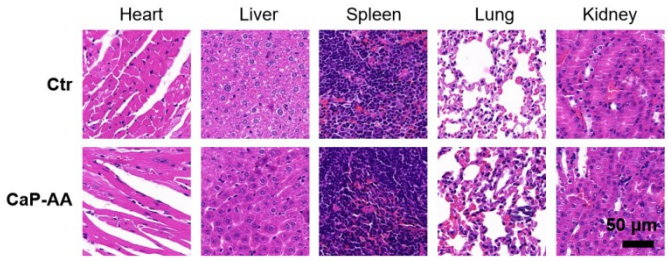

Fig. S11. H&E-stained organ slices at 16 days post-intravenous injection of corresponding treatments.

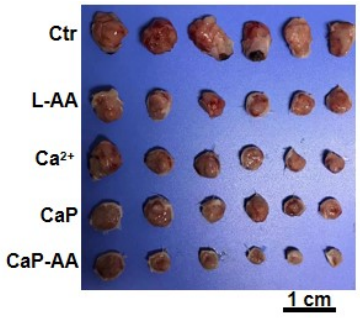

Fig. S12. Images of excised tumors after sacrificing the mice at 16 days post- intratumoral injection.

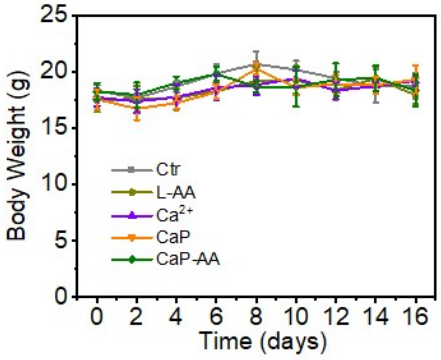

Fig. S13. Average bodyweight curves of mice from different groups recorded after different intratumoral injection treatments.

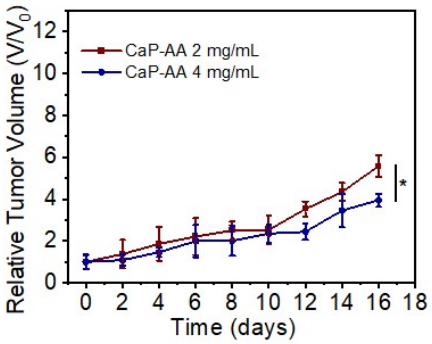

Fig. S14. Tumor growth curves after intravenous injection in different treatment groups.

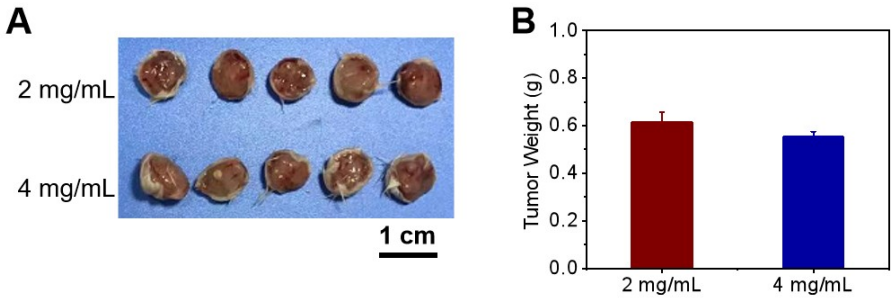

Fig. S15. (A) Images of excised tumors after sacrificing mice at 16 days post- intravenous injection treatment. (B) Average tumor weights of A.

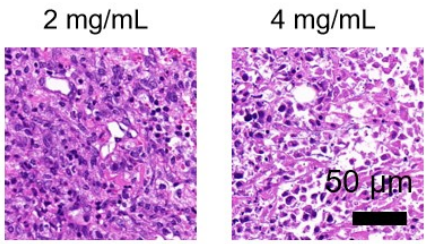

Fig. S16. H&E-stained tumor slices after sacrificing mice at 16 days post-intravenous injection of CaP-AA.

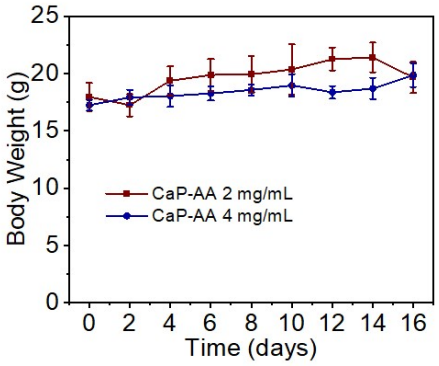

Fig. S17. Average bodyweight curves of mice recorded after different intravenous injection treatments.

Table S1. Complete blood tests of control mice and mice 24 h after intravenous injection of CaP-AA at the therapeutic dose (WBC: white blood cells; Lymph: lymphocyte; Mon: monocytes; Gran: granulocyte; RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: red cell distribution width; PLT: Platelets; WPV: mean platelet volume; PWD: platelet distribution width; PCT: thrombocytocrit). Reference range of mouse parameters was provided by Wuhan Servicebio Technology CO., LTD.

Parameter	Ctr			CaP-AA			Unit	Reference range
WBC	4.8	3.6	4.8	3.4	0.9	1.6	10^9/L	0.8-6.8
Lymph	1.5	5.2	5.4	2.4	0.9	5.0	10^9/L	0.7-5.7
Mon	0.3	0.1	0.1	0.1	0.2	0.2	10^9/L	0.0-0.3
Gran	1.8	1.8	1.6	0.9	1.0	1.2	10^9/L	0.1-1.8
RBC	9.07	9.00	8.15	9.64	8.76	6.59	10^{12}/L	6.36-9.42
HGB	141	110	112	143	121	143	g/l	110-143
HCT	44.3	38.5	37.6	43.8	42.2	44.6	%	34.6-44.6
MCV	50	52.3	49.6	48.5	49.5	58.1	fl	48.2-58.3
MCH	16.2	19	18.2	15.9	15.9	16.0	pg	15.8-19
MCHC	324	313	305	335	352	350	g/l	302-353
RDW	14.1	17	16.3	13.5	16.5	16.0	%	13-17
PLT	963	1550	1059	918	1590	1432	10^9/L	450-1590
MPV	6.0	5.7	5.9	5.5	5.9	5.9	fl	3.8-6.0
PDW	17.3	19.3	18.6	16.4	17.1	17.4		
PCT	0.221	0.175	0.132	0.206	0.168	0.217	%	

Table S2. Blood biochemical parameters of control mice and mice 24 h after intravenous injection of CaP-AA (4 mg/mL, 200 μL) (ALT: aminotransferase; AST: aspartate aminotransferase; DBIL: Direct Bilirubin; TBIL: total bilirubin; ALB: Serum albumin; ALP: alkaline phosphatase; BUN: nephric blood urea nitrogen; CR: creatinine; UA: uric acid). Reference range of mouse parameters was provided by Wuhan Servicebio Technology CO., LTD.

Parameter	Ctr			CaP-AA			Unit	Reference range
ALT	78.894	68.943	71.189	73.681	26.847	68.284	U/L	10.06-96.47
AST	132.714	175.869	164.843	116.18	176.529	132.895	U/L	36.31-235.48
DBIL	28.47	0.4966	1.8631	20.388	26.849	33.121	μmol/L	0.45-33.89
TBIL	41.007	13.846	19.864	35.577	20.846	50.005	μmol/L	6.09-53.06
ALB	20.496	23.596	28.763	38.626	29.562	35.763	g/L	21.22-39.15
ALP	236.052	385.754	463.859	224.914	329.658	423.685	U/L	22.52-474.35
BUN	33.616	19.547	19.749	31.656	31.874	34.586	mg/dL	10.81-34.74
CR	76.656	26.745	96.746	46.483	79.324	46.833	μmol/L	10.91-85.09
UA	128.469	224.869	195.854	217.704	129.852	97.297	μmol/L	44.42-224.77