

Enhanced anti-PD-1 therapy in hepatocellular carcinoma by tumor vascular disruption and normalization dependent on combretastatin A4 nanoparticles and DC101

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Supplementary Figures

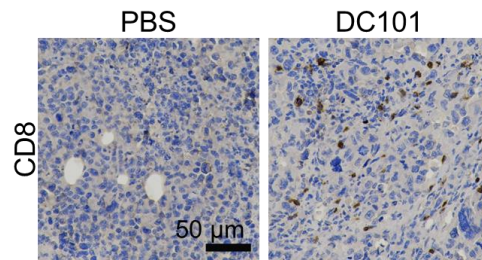


Figure S1. IHC staining of CD8 for tumors in the PBS and DC101 groups on day 11.

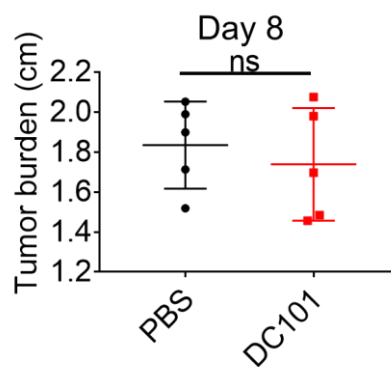


Figure S2. Tumor burden (long axis of tumors) in the PBS and DC101 groups on day 8 ($n = 5$). Data are presented as mean \pm SD (ns, not significant).

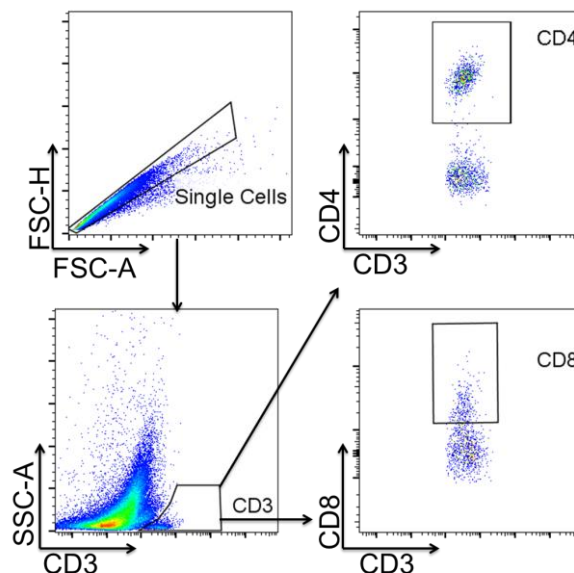


Figure S3. Gating strategies for flow cytometry detecting CD4⁺ T cells and CD8⁺ T cells in the PBS, CA4-NP, DC101, and CA4-NP + DC101 groups on day 10.

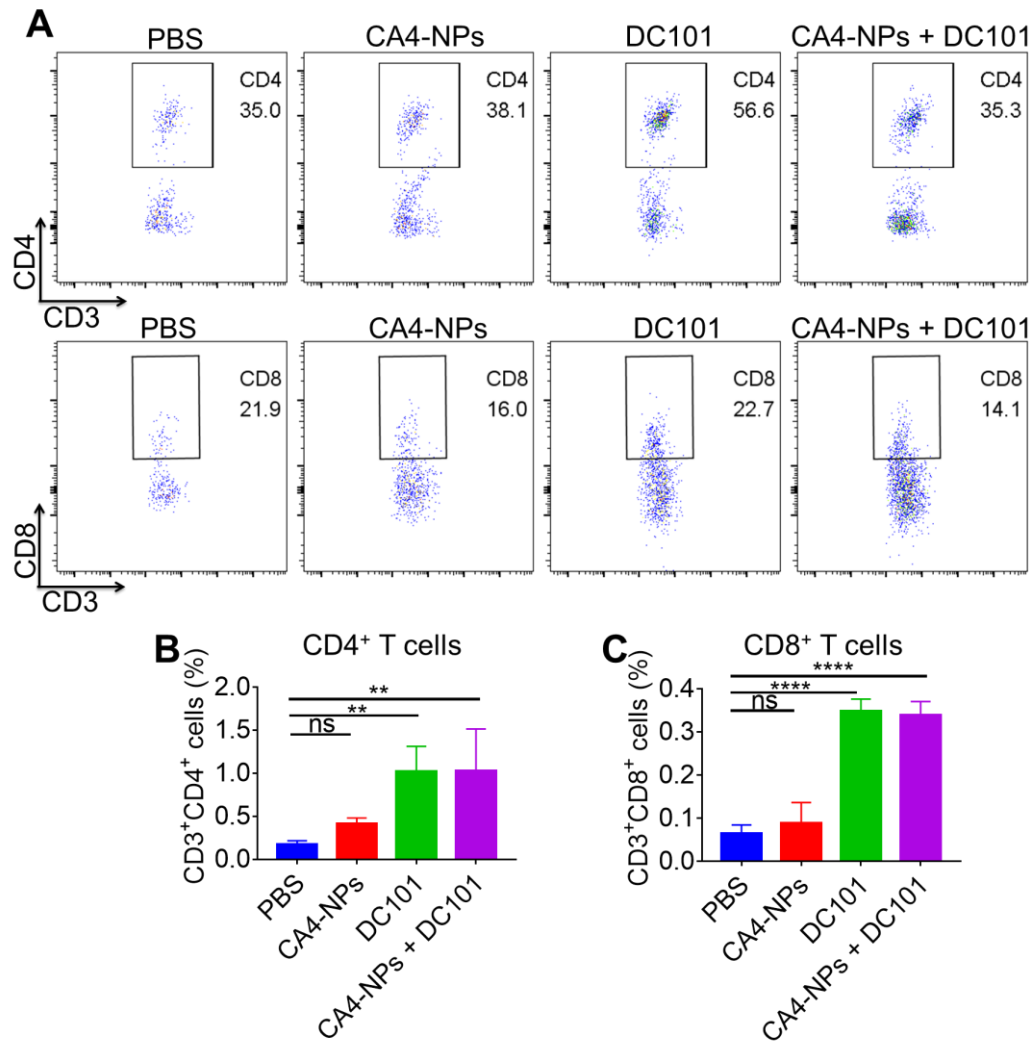


Figure S4. CA4-NPs + DC101 increased the number of intratumoral CD4⁺ T cells and CD8⁺ T cells. (A) Representative flow cytometry plots for intratumoral CD4⁺ T (CD3⁺CD4⁺) cells and CD8⁺ T (CD3⁺CD8⁺) cells in the PBS, CA4-NP + DC101, anti-PD-1, and CA4-NP + DC101 + anti-PD-1 groups on day 10. (B, C) Quantification of intratumoral CD4⁺ T cells (B) and CD8⁺ T cells (C) detected by flow cytometry. Data are presented as mean \pm SD (** P < 0.01, **** P < 0.0001, ns, not significant).

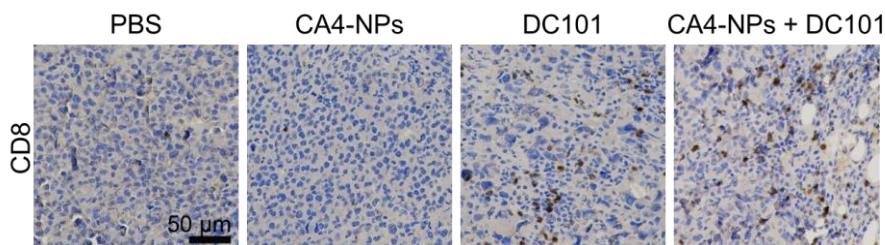


Figure S5. IHC staining of CD8 for tumors in the PBS, CA4-NP, DC101, and CA4-NP + DC101 groups on day 11.

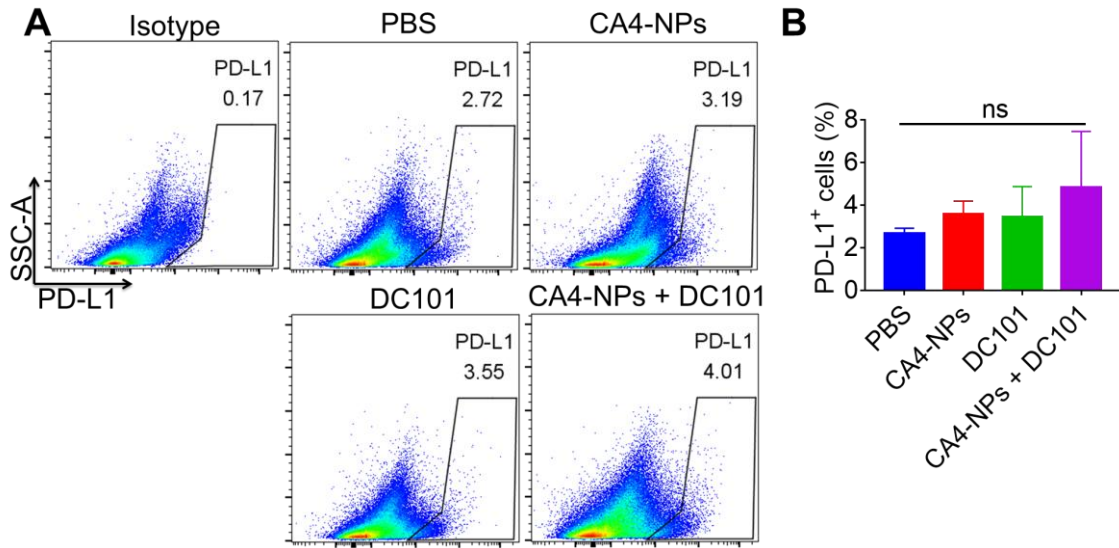


Figure S6. Expression of PD-L1 in H22 tumor after treatment. (A) Representative image for flow cytometry plots showing PD-L1 expression in tumor cells after PBS, CA4-NP, DC101, and CA4-NP + DC101 treatment on day 10. (B) Quantification of PD-L1⁺ cells detected by flow cytometry (n = 3). Data are presented as mean \pm SD (ns, not significant).

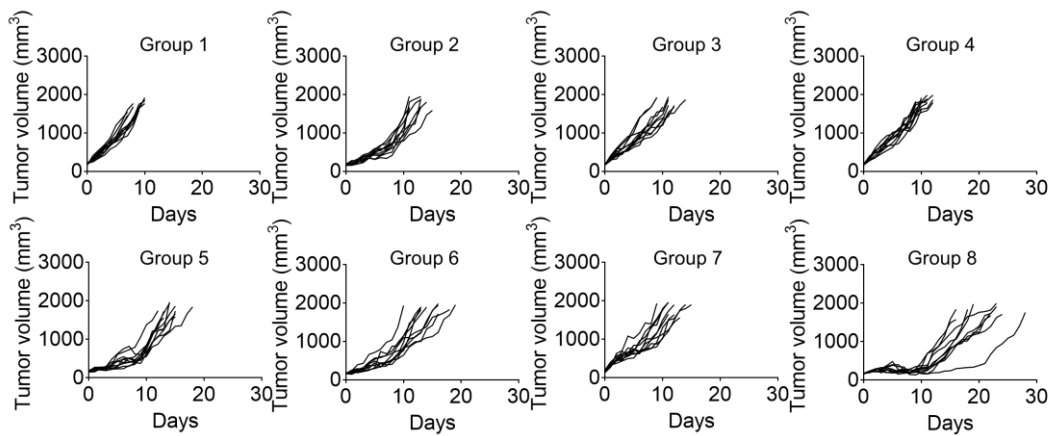


Figure S7. Tumor growth curves for individual H22-bearing mice in the eight groups (n = 10). Note: PBS (Group 1); CA4-NPs (Group 2); DC101 (Group 3); anti-PD-1 (Group 4); CA4-NPs + DC101 (Group 5); CA4-NPs + anti-PD-1 (Group 6); DC101 + anti-PD-1 (Group 7); and CA4-NPs + DC101 + anti-PD-1 (Group 8).

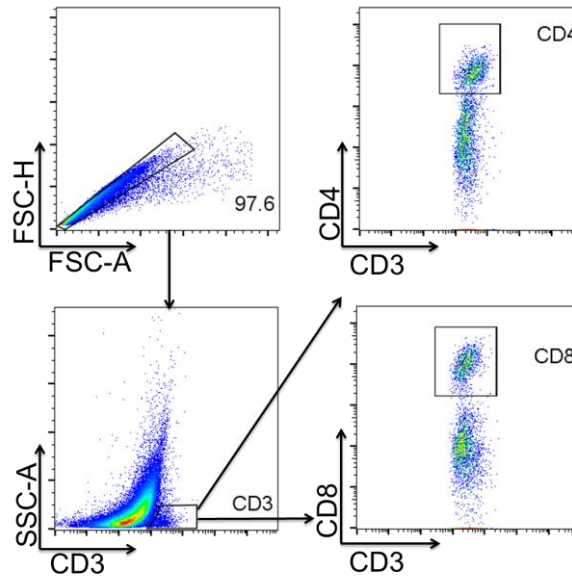


Figure S8. Gating strategies for flow cytometry detecting CD4⁺ T cells and CD8⁺ T cells in the PBS, CA4-NP + DC101, anti-PD-1, and CA4-NP + DC101 + anti-PD-1 groups on day 11.

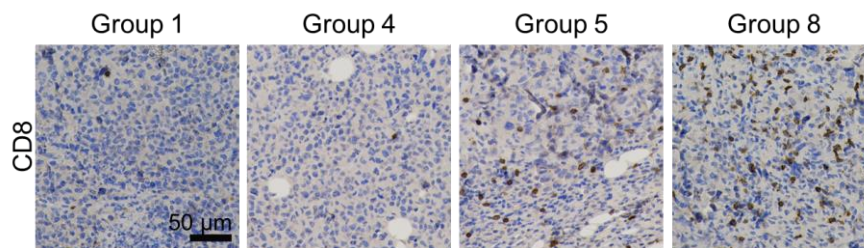


Figure S9. IHC staining of CD8 for tumors in the Group 1, Group 4, Group 5, and Group 8 on day 11. Note: PBS (Group 1); anti-PD-1 (Group 4); CA4-NPs + DC101 (Group 5); and CA4-NPs + DC101 + anti-PD-1 (Group 8).

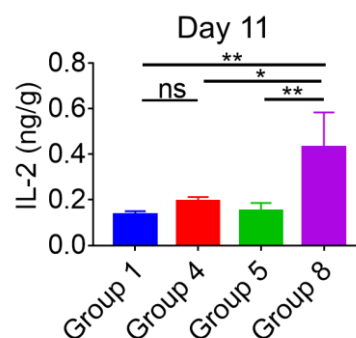


Figure S10. ELISA results evaluating intratumoral IL-2 levels in the Group 1, Group 4, Group 5, and Group 8 on day 11 (n = 3). Data are presented as mean ± SD (P* < 0.05, ***P* < 0.01, ns, not significant). Note: PBS (Group 1); anti-PD-1 (Group 4); CA4-NPs + DC101 (Group 5); and CA4-NPs + DC101 + anti-PD-1 (Group 8).**

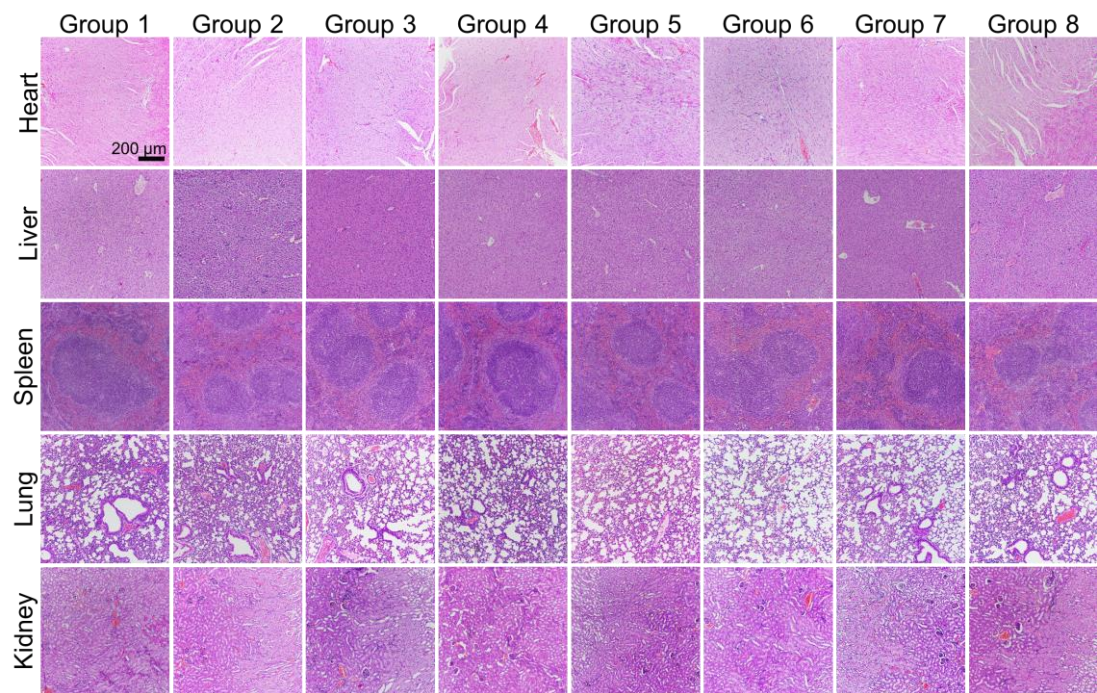


Figure S11. H&E staining of the heart, liver, spleen, lung, and kidney tissues on day 11 in the eight groups. Note: PBS (Group 1); CA4-NPs (Group 2); DC101 (Group 3); anti-PD-1 (Group 4); CA4-NPs + DC101 (Group 5); CA4NPs + anti-PD-1 (Group 6); DC101 + anti-PD-1 (Group 7); and CA4-NPs + DC101 + anti-PD-1 (Group 8).

Supplementary Tables

Table S1. List of antibodies used in the experiments.

Antibodies	Company	Catalog	Application
DC101	BioXCell	BE0060	Treatment
Anti-PD-1	BioXCell	BE0146	Treatment
FITC anti-mouse CD3 Antibody	BioLegend	100204	Flow cyt
PE/Cy7 anti-mouse CD4 Antibody	Invitrogen	25-0042-82	Flow cyt
PE anti-mouse CD4 Antibody	Invitrogen	12-0041-82	Flow cyt
APC anti-mouse CD8a Antibody	BioLegend	100712	Flow cyt
PE anti-mouse PD-L1 Antibody	Invitrogen	12-5982-82	Flow cyt
Ki67 Rabbit Antibody	Servicebio	GB13030-2	IHC
Anti-CD31 Antibody	Abcam	ab182981	IHC
Anti-CD8 Rabbit Antibody	Servicebio	GB13429	IHC
HPR Goat anti-Rabbit IgG	Servicebio	GB23303	IHC
APC anti-mouse CD31 Antibody	eBioscience	17-0311-80	IF
Anti-α-SMA Antibody	Abcam	ab7817	IF
Cy3 Goat anti-mouse IgG	ABclonal	AS008	IF

Flow cyt, Flow cytometry; IF, Immunofluorescence; IHC, Immunohistochemistry

Table S2. Nuclear dye used in the experiments.

Dye	Company	Catalog
DAPI	Sigma	D9542
SYTOX Green Nucleic Acid Stain	Thermo Fisher	S7020

DAPI, 4', 6-diamidino-2-phenylindole dihydrochloride

Table S3. ELISA kits used in the experiments.

ELISA kits	Company	Catalog
Mouse-IFNγ ELISA kit	Anoric	TAE-366m
Mouse-TNFα ELISA kit	Anoric	TAE-569m
IL-2 Mouse Uncoated ELISA Kit	Thermo Fisher	88-7024-88
Mouse-VEGF ELISA kit	Lengton	BPE20260

ELISA, Enzyme-linked immunosorbent assay; IFN, Interferon; TNF, Tumor necrosis factor; VEGF, Vascular endothelial growth factor