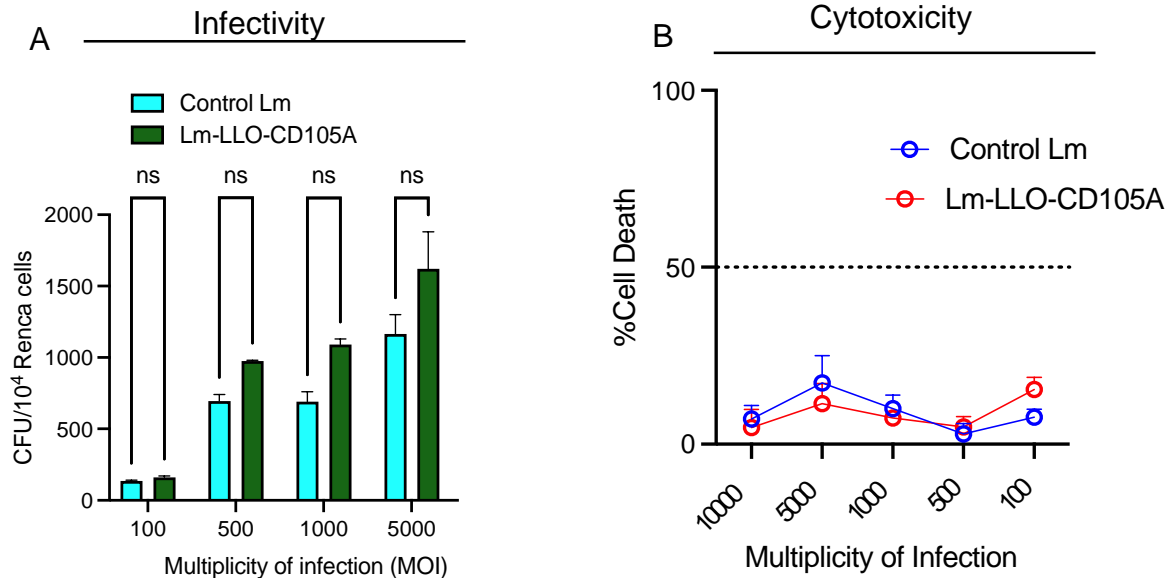


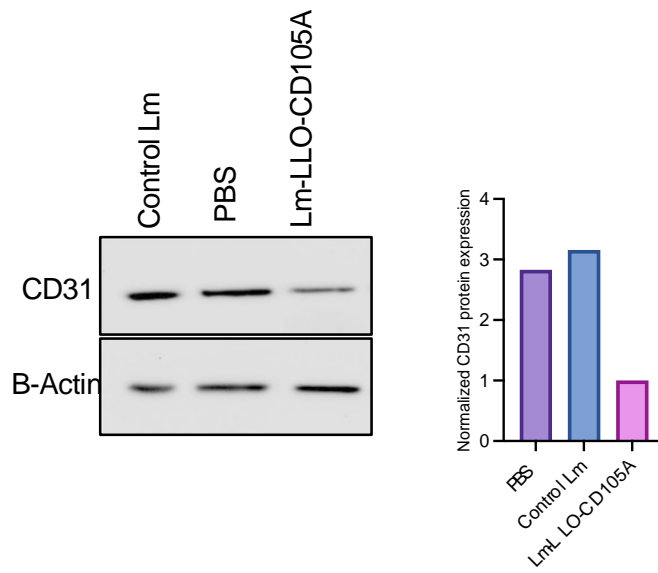
Supplementary Figures

Supplementary Figure S1



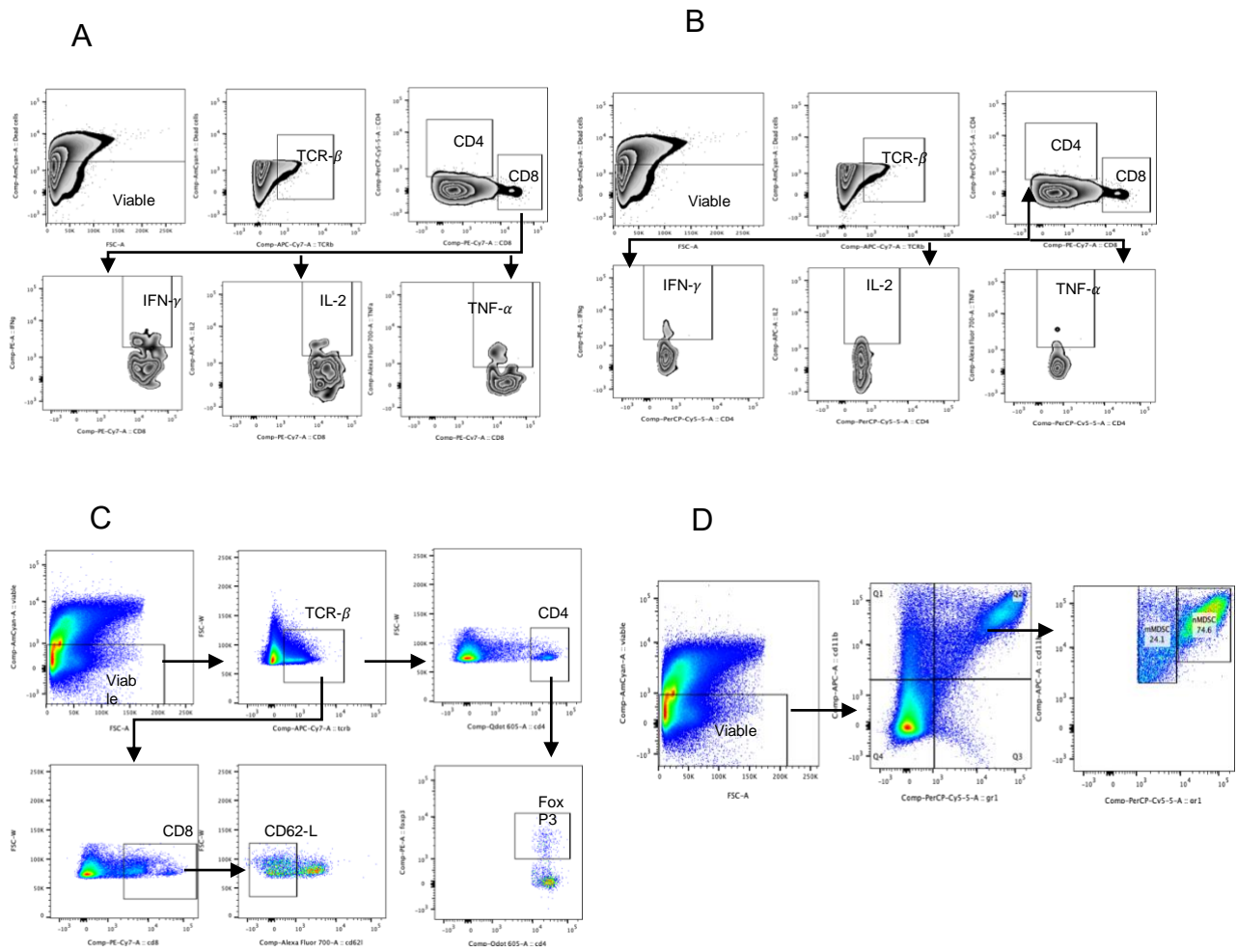
Supplementary Figure S1. Lm-based vaccine does not induce direct tumor cell killing. Renca cells were cultured with varying MOIs of Control Lm or Lm-LLO-CD105A, and infection or cytotoxicity was determined (**A**) Infectivity rate, expressed as CFU/10,000 cells in Renca (**B**) Dose-response curve to Lm-based vaccines in Renca cells

Supplementary Figure S2



Supplementary Figure S2. Protein expression of CD31, a marker of angiogenesis after therapy with Lm-based vaccines.

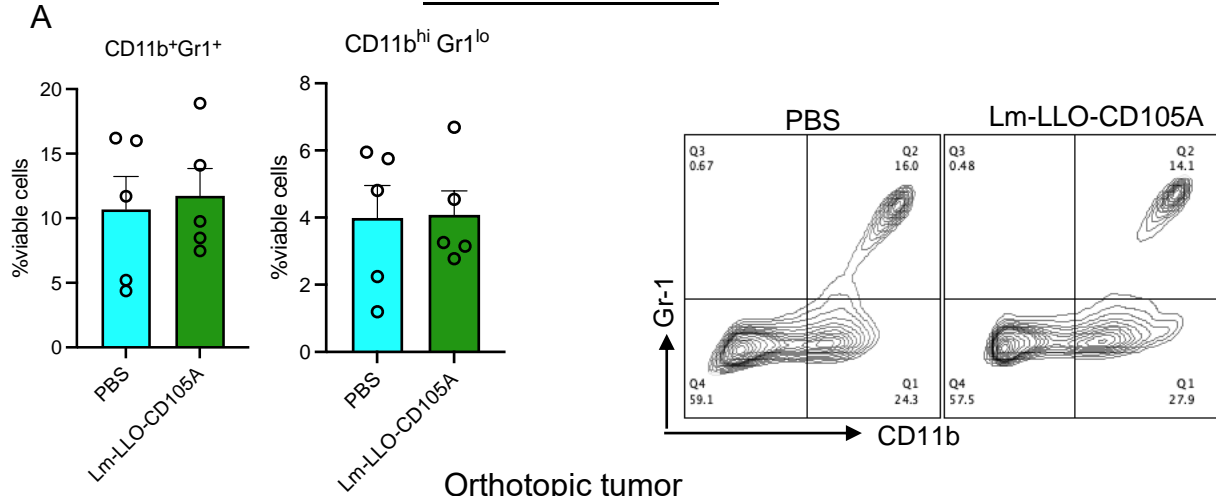
Supplementary Figure S3



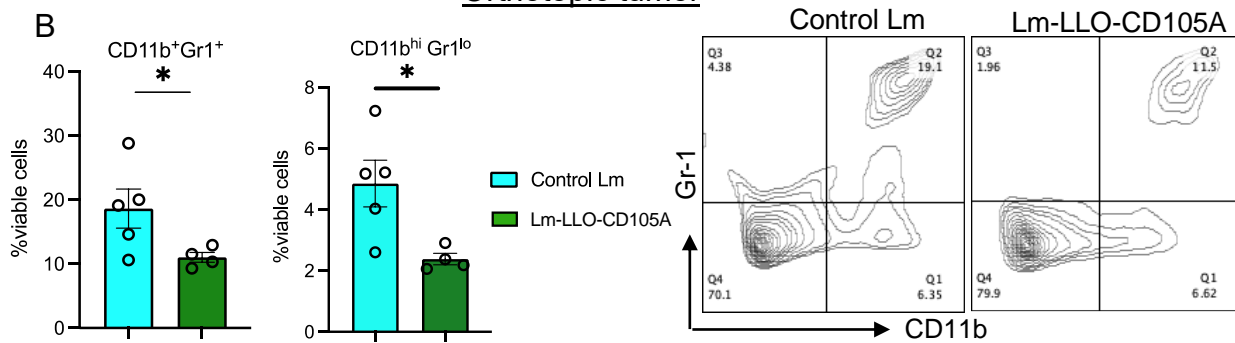
Supplementary Figure S3. Gating strategy for tumor-infiltrating lymphocytes and myeloid cells. (A-D) Following stimulation and staining, viable cells were discriminated with Amcyan gating followed by sub-gating with TCR-b. TCR-b⁺ cells were further sub-gated to CD4 and CD8⁺ T cells. (A) IFN γ , IL2, and TNF-a were then sub-gated on CD8⁺ T cells or (B) CD4⁺ T cells. The combination/Boolean gating function on Flowjo software was used to identify the multicytokine-producing population (C) Dead cells were excluded using Amcyan staining followed by TCR-b selection, CD4 and CD8⁺ T cells were then gated on TCRb⁺ cells. On CD8⁺ cells, the population of CD62L^{lo} cells was gated, and on CD4⁺ cells population of Foxp3⁺ cells were gated (D) Viable cells were discriminated with Amcyan staining, followed by double gating against CD11b and Gr1 to select CD11b⁺Gr1⁺ cells. CD11b⁺Gr1⁺ cells were further sub gated to characterize CD11b^{hi}Gr1^{hi} and CD11b^{hi}Gr1^{lo} cells

Supplementary Figure S4

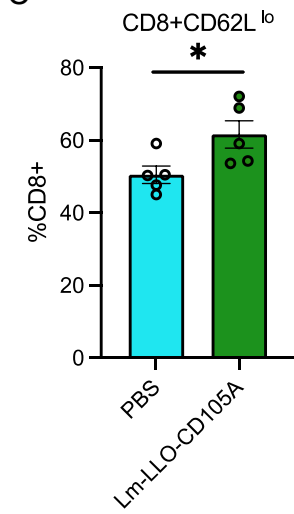
Subcutaneous tumor



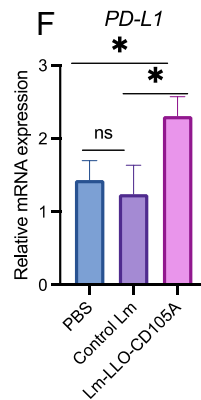
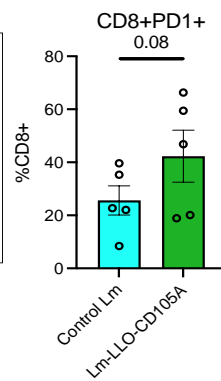
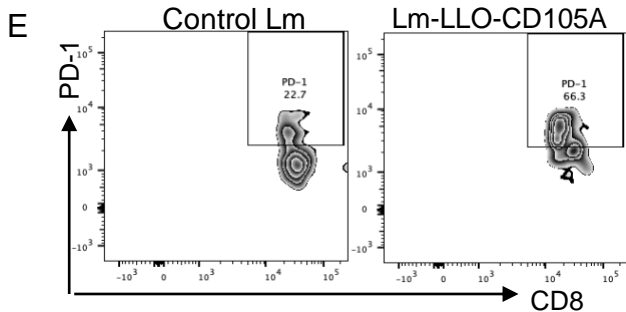
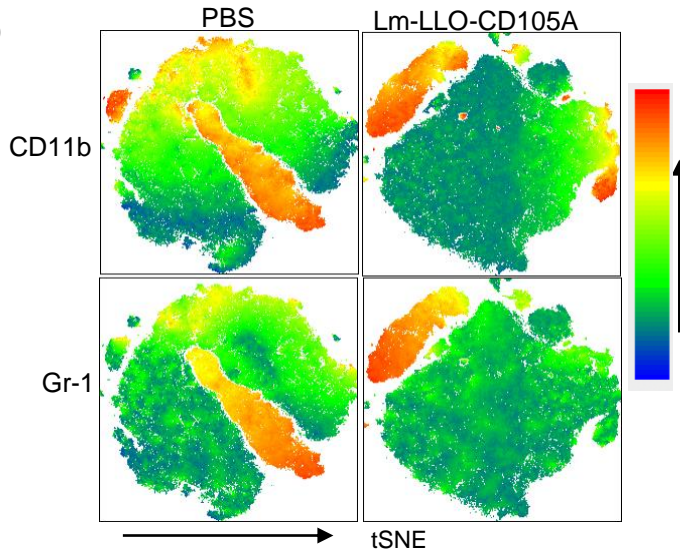
Orthotopic tumor



Orthotopic Tumor

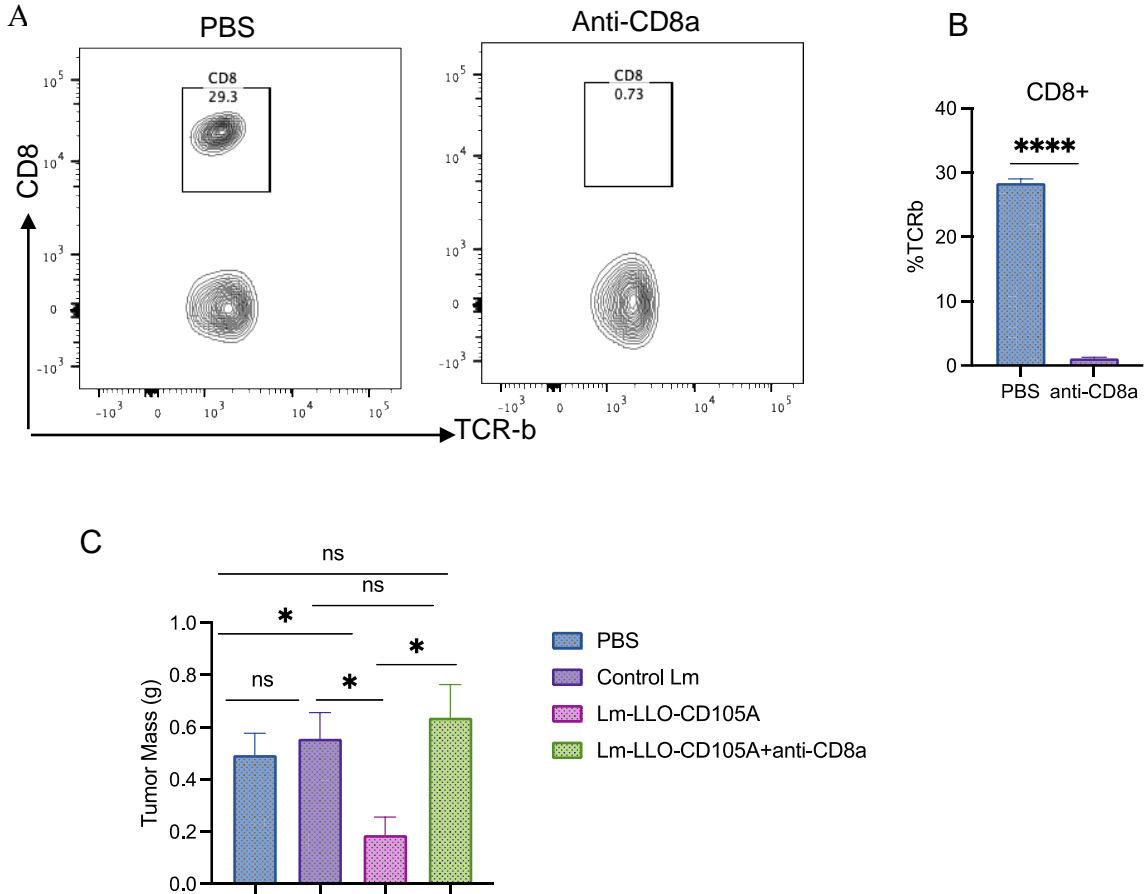


D



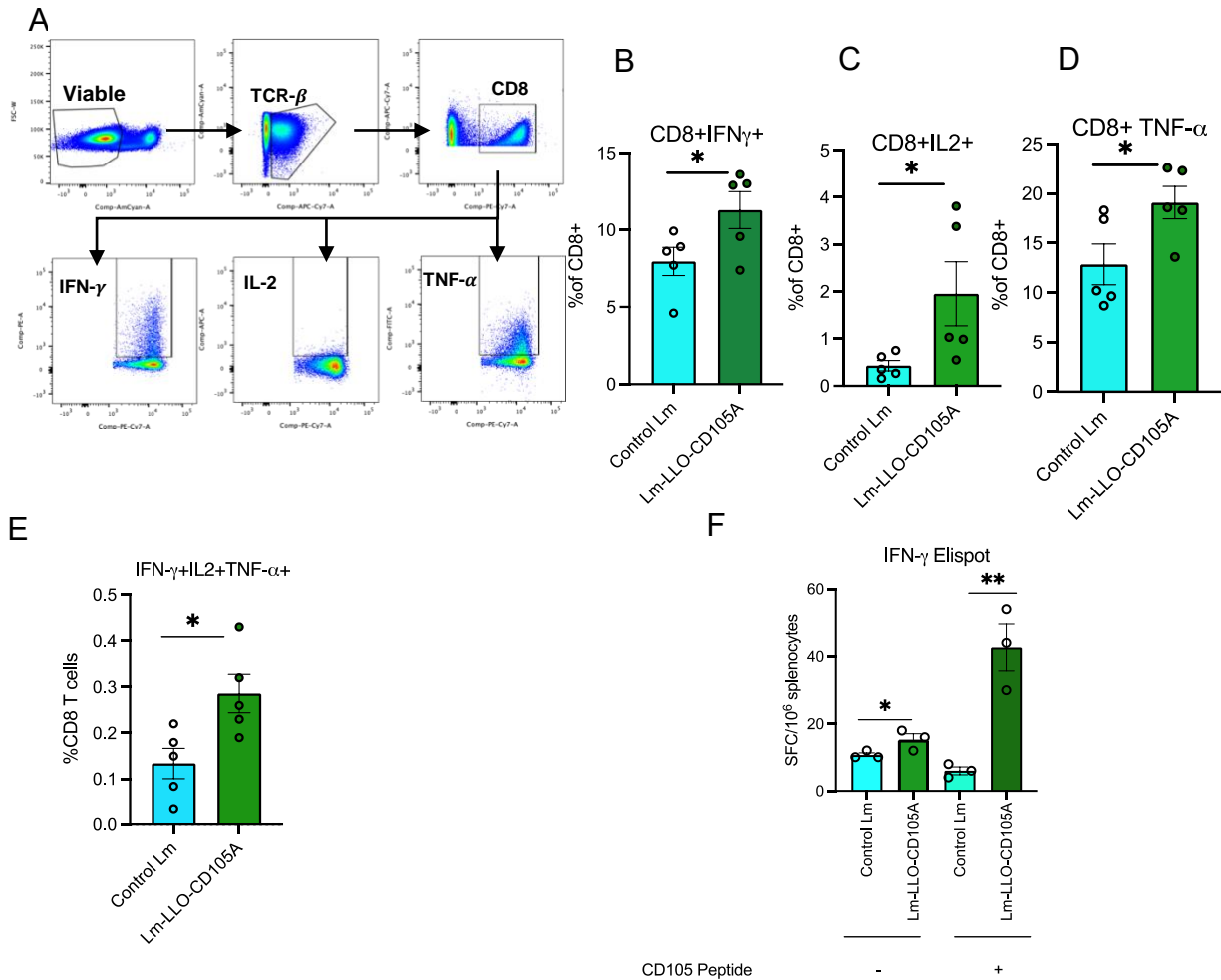
Supplementary Figure S4. Lm-LLO-CD105A reduces MDSCs and moderately increases PD-1/PD-L1 expression (A) MDSC population in the subcutaneous tumor between treatment groups. (B) MDSC population in the orthotopic tumor between treatment groups. (C) CD8⁺CD62L^{lo} effector CD8⁺T cell population between placebo and Lm-LLO-CD105A treatment groups in the orthotopic model (D) tSNE plot of CD11b⁺ and Gr-1⁺ cells (E) PD-1 expression by flow cytometry and (F) *Pd-11* mRNA expression by tumors from Control Lm and Lm-LLO-CD105A treatment groups (n=5/group). Data were analyzed using unpaired t-test. *P < 0.05. All error bars are shown as mean ± SEM.

Supplementary Figure S5



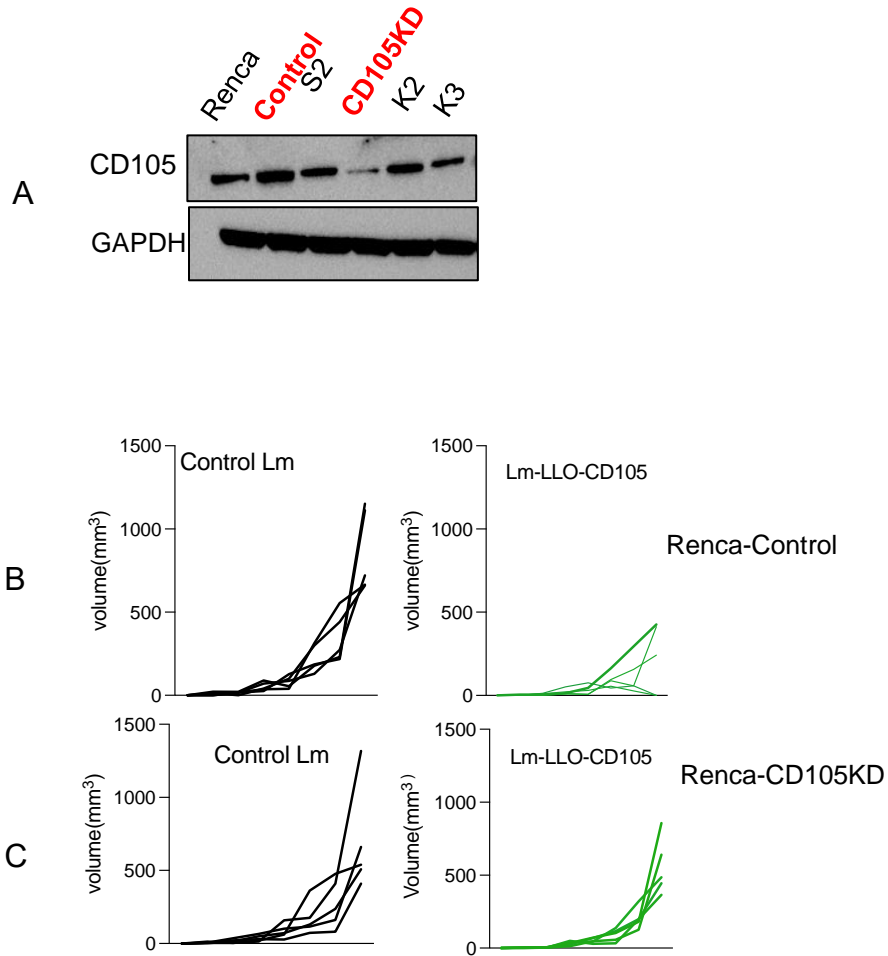
Supplementary Figure S5. Confirmation of CD8⁺ T cell depletion. (A) Representative plots showing confirmation of CD8 T cell depletion. (B) Statistical representation of CD8⁺ population in the spleen (n=3/group). (C) Final tumor mass after CD8 depletion experiments. (n=4-5/group) Data were analyzed using unpaired t-test. *P < 0.05, ****P < 0.0001 ns, not significant. All error bars are shown as mean \pm SEM.

Supplementary Figure S6



Supplementary Figure S6. Lm-LLO-CD105A increased the polyfunctionality of antigen-specific CD8⁺ T cells in the spleens. (A) gating strategy for CD8⁺ T cells, and cytokine production. Production of (B) IFN γ (C) IL-2 and (D) TNF- α by the splenic CD8⁺ T cells after treatment with Lm-LLO-CD105A or Control Lm. (E) IFN γ ⁺IL2⁺TNF α ⁺ CD8⁺ T cell population in the Lm-LLO-CD105A and Control Lm treatment groups (n=5/group). (F) IFN- γ Spot forming cells between Control Lm and Lm-LLO-CD105. *P < 0.05 **P < 0.01 All error bars are shown as mean \pm SEM.

Supplementary Figure S7



Supplementary Figure S7. Ablation of CD105 abrogates Lm-LLO-CD105A's therapeutic efficacy (A) Western blot confirmation of CD105 depletion in Renca cells. (B-C) Individual tumor growth curve of (B) Renca-Control and (C) Renca-CD105KD after vaccination with Lm-LLO-CD105A or Control Lm (n=5/group).