

Supplementary Information for

Spatiotemporally Engineered Tumor-derived extracellular vesicles-based scaffold vaccine for personalized cancer immunotherapy

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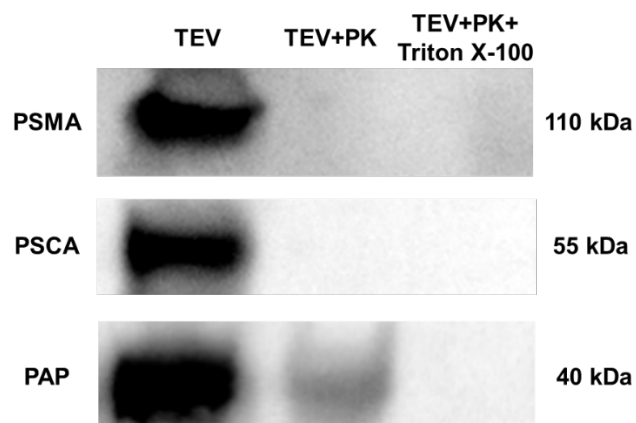
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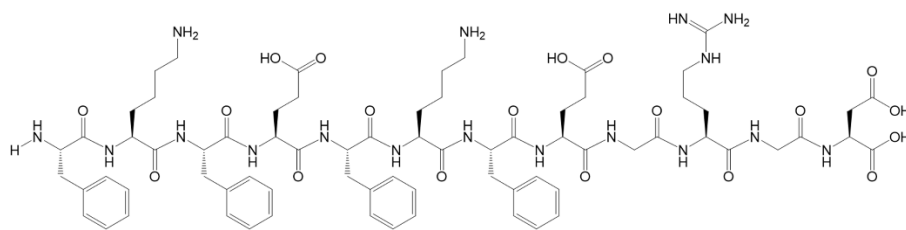
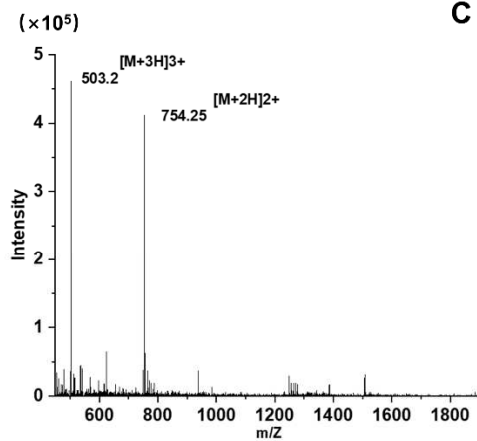
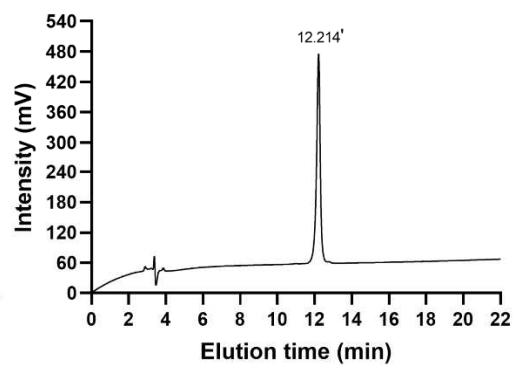
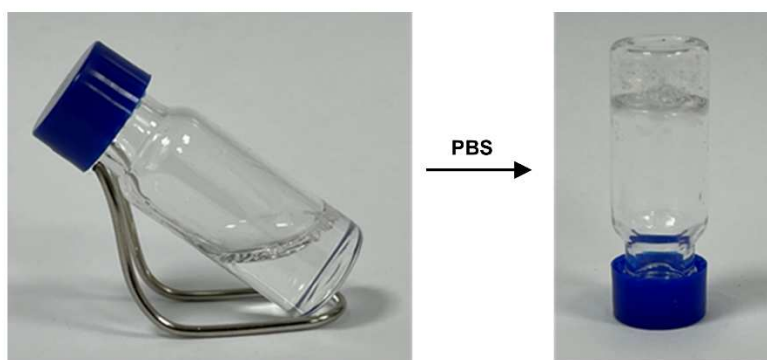
[#]QC and CJ contributed equally to this work.

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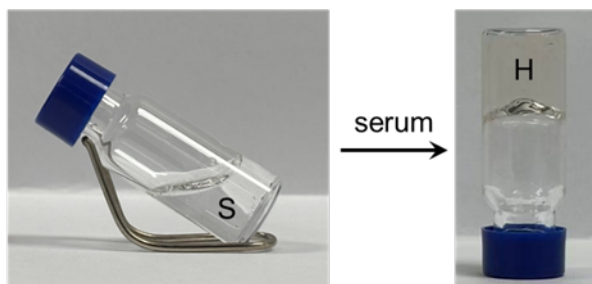
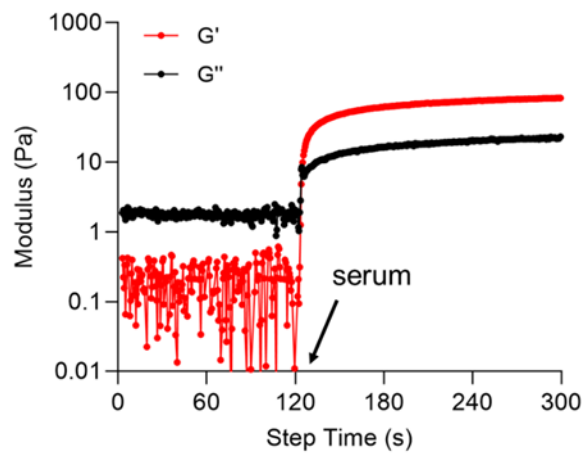
Supplementary Figures



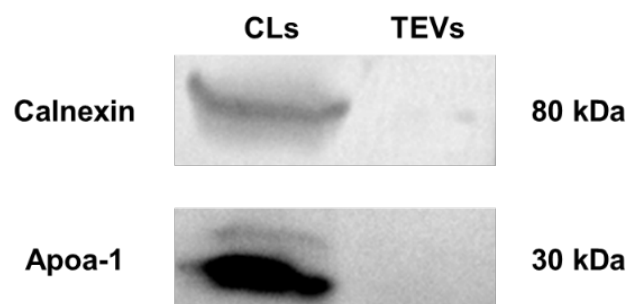
Supplementary Fig. 1. Protease protection assay analyzing the indicated antigens (PSMA, PSCA, PAP) in TEVs. TEVs extracted from human prostate cancer tissue was detected by western blotting after being treated with proteinase K (PK), or PK plus Triton X-100 (n = 3 independent experiments).

A**FKFEFKFEGRGD****B****C****D**

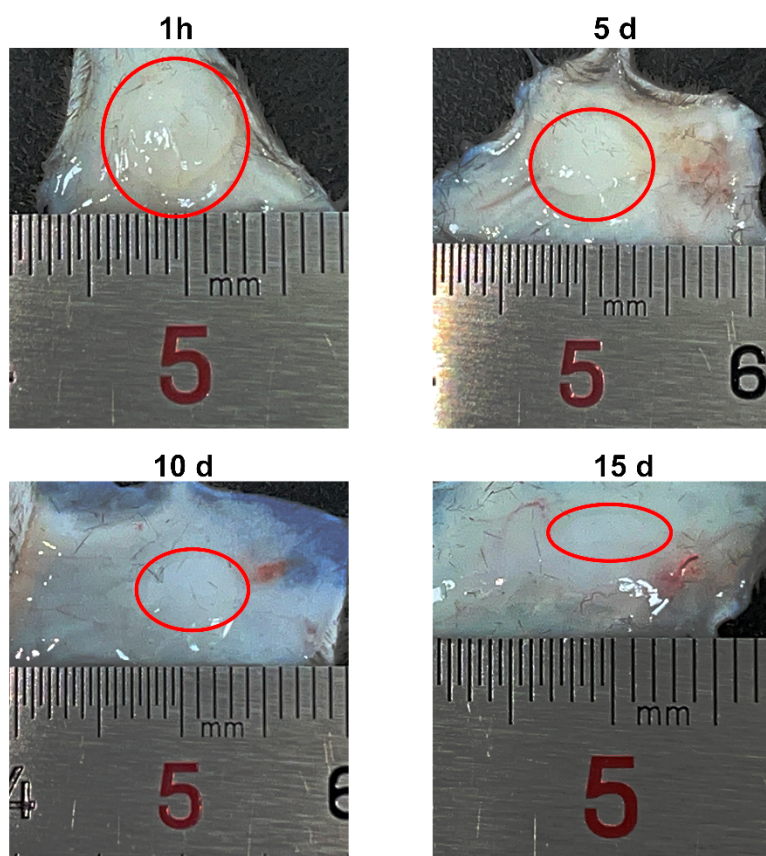
Supplementary Fig. 2. Characterization of the FK12. **A**, Chemical structure of the FK12. ESI MS (**B**) and HPLC trace profile (**C**) of FK12 showing high purity and the expected molecular weight. **D**, Photographs of the solution-to-hydrogel transition of TCG/FK₁₂F triggered by the addition of PBS.

A**B**

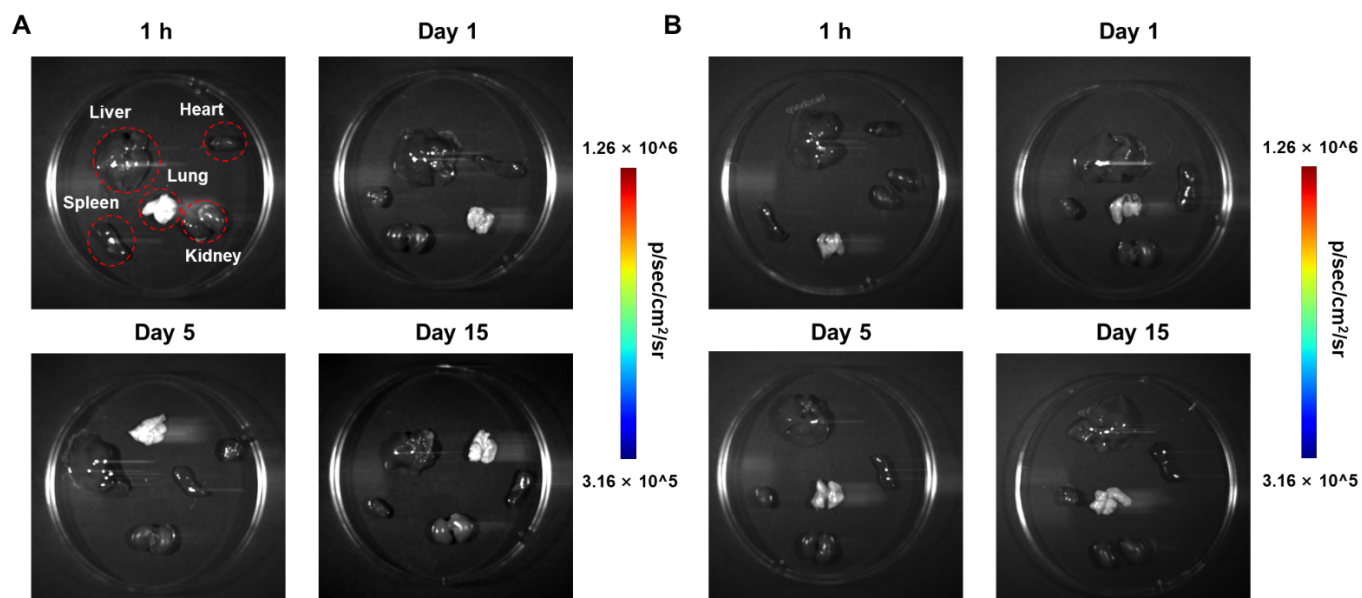
Supplementary Fig. 3. Gelation behavior of FK₁₂F in serum. **A**, Photographic demonstration of the rapid solution-to-hydrogel transition of FK₁₂F upon addition of serum. **B**, Rheological measurement (storage modulus G' and loss modulus G'') of the formed FK₁₂F hydrogel in serum.



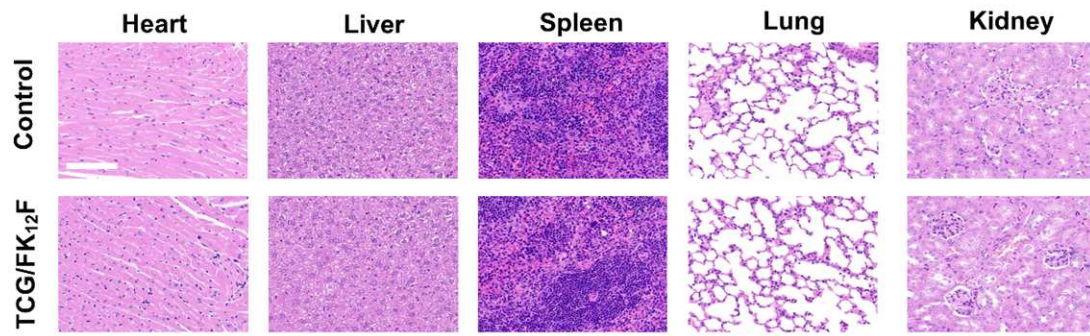
Supplementary Fig. 4. Assessment of TEV purity using negative markers. Western blot analysis of Calnexin and ApoA-1 in cell lysates (CLs) versus isolated TEVs, demonstrating the absence of these negative markers in TEV fractions (n = 3 independent experiments).



Supplementary Fig. 5. In vivo gel formation and retention were assessed following subcutaneous injection of FK₁₂F solution into the back of C57BL/6 mice. The remaining hydrogel in each mouse was photographed at specified time points. The experiments were repeated three times.



Supplementary Fig. 6. Biodistribution of vaccine components. Representative *ex vivo* fluorescence images of major organs (liver, heart, spleen, lung, kidney) collected at the indicated time points following subcutaneous injection of (A) DIR-labeled TEVs or (B) Cy7-labeled CDA within the TCG/FK₁₂F formulation (n = 3 mice per group).

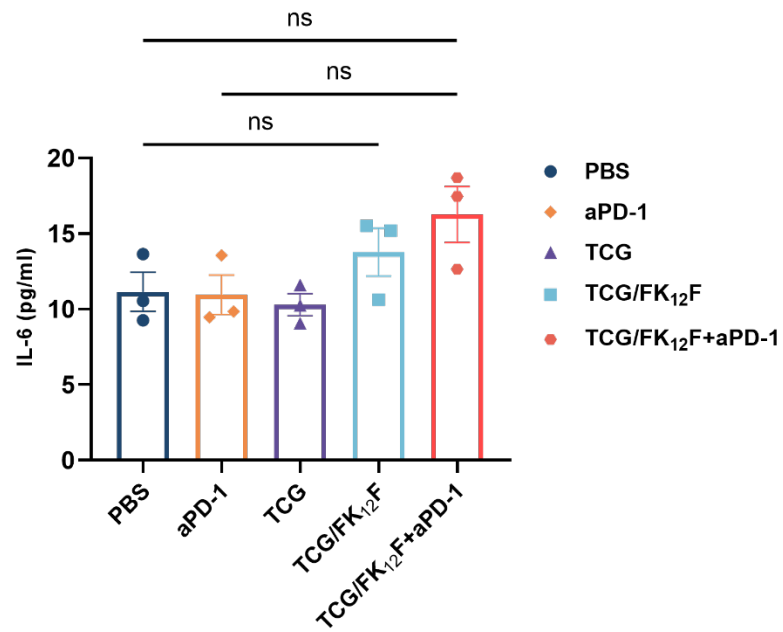
A**B**

	RBC (10 ¹² /L)	WBC (10 ⁹ /L)	PLT (10 ⁹ /L)	HGB (g/L)	HCT (%)	MCV (fL)
Reference range	6.50-11.50	3.90-13.94	545-1849	110-165	37.2-58.0	42.6-55.6
Healthy control	8.92±0.25	10.4±1.04	1294±64	141±5	43.2±1.8	48.4±0.7
Day 15	9.01±0.17	5.6±0.4	989±103	143±2	43.2±0.7	48.0±0.2

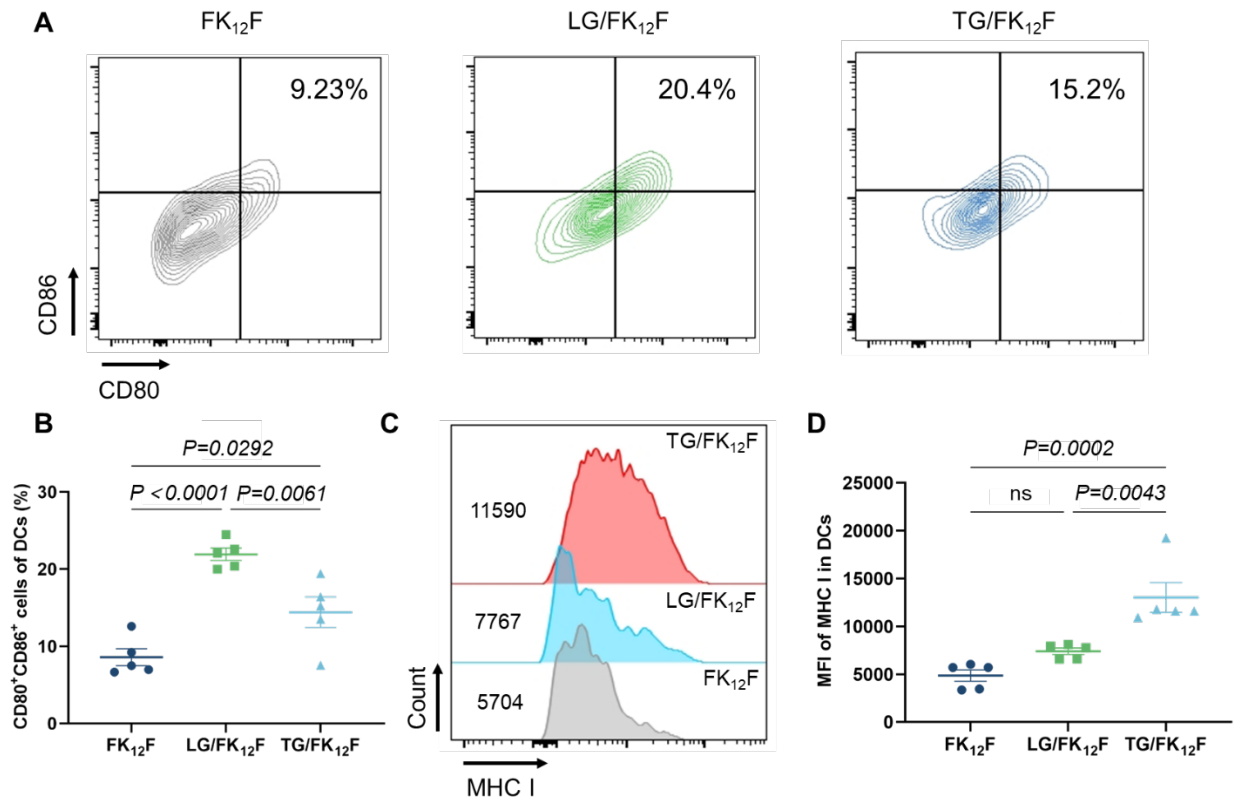
	MCH (pg)	MCHC (g/L)	NEUT (10 ⁹ /L)	LYMPH (10 ⁹ /L)	MONO (10 ⁹ /L)	PDW
Reference range	13.0-16.8	300-360	0.42-2.55	2.88-10.92	0.17-0.69	12.0-17.5
Healthy control	15.8±0.1	326±3	1.5±0.3	8.6±0.9	0.3±0.1	17.0±0.2
Day 15	15.9±0.2	332±3	1.0±0.2	4.4±0.2	0.1±0.1	17.4±0.5

	ALP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CRE (μmol/L)
Reference range	22.52-474.35	10.06-96.47	36.31-235.48	10.81-34.74	10.91-85.09
Healthy control	65.02±9.56	23.95±3.03	190.16±22.60	21.07±1.88	38.76±7.03
Day 15	88.57±4.13	44.82±10.24	177.17±33.74	15.48±1.46	27.81±7.80

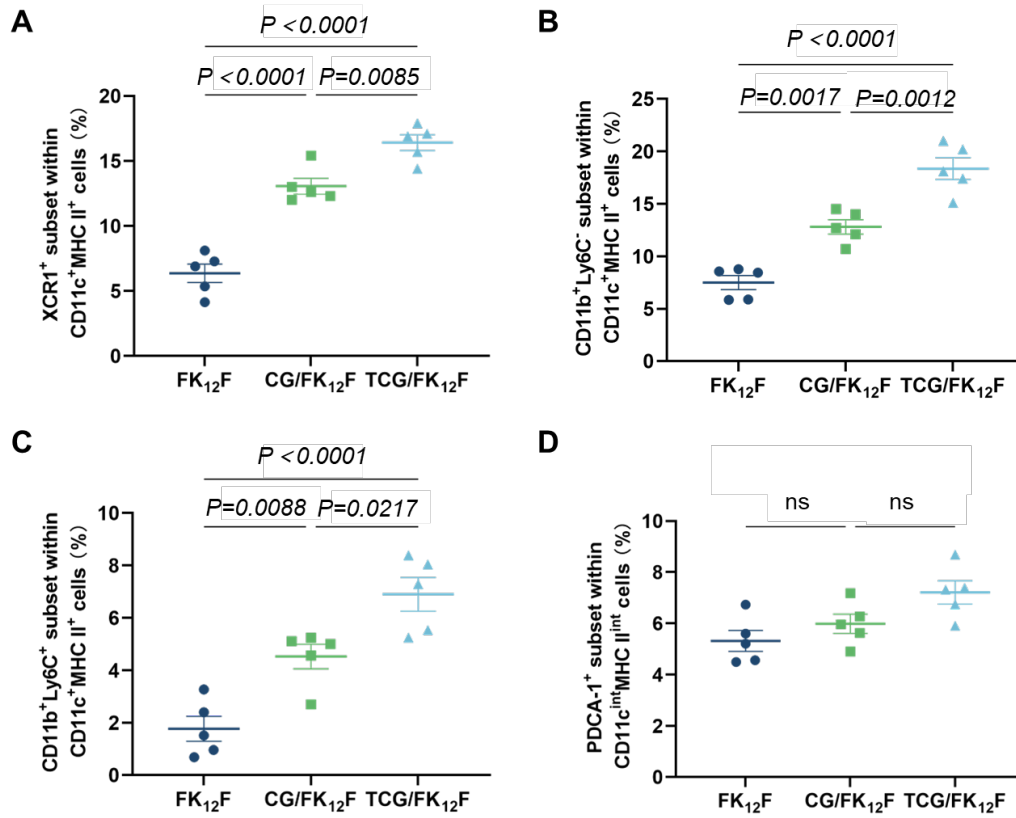
Supplementary Fig. 7. Studies on the biosafety of TCG/FK₁₂F vaccine. **A**, H&E staining of major organs collected from healthy mice and TCG/FK₁₂F treated mice at day 15 (scale bar: 100 μm). Experiments were repeated three times. **B**, Mice that received vaccine treatment had their blood taken on day 15 and healthy mice were used as controls. Experiments were repeated three times. Data are given as mean ± SEM.



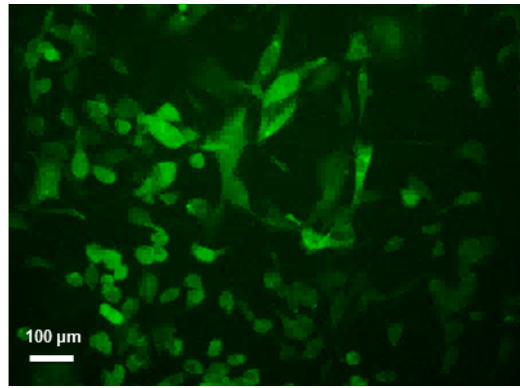
Supplementary Fig. 8. Serum IL-6 levels in mice from different treatment groups. Data are represented as mean $\pm$ SEM (n = 3 mice per group). Statistical significance was determined using one-way ANOVA.



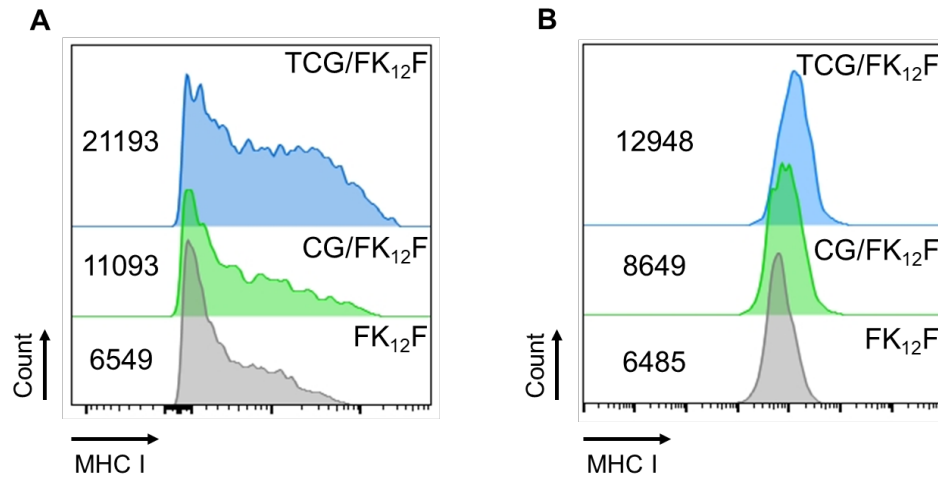
Supplementary Fig. 9. Effects of TL and TEV vaccines on DC stimulation. **A**, Representative flow cytometry plots of CD80⁺CD86⁺ DCs in different treatment groups: LG/FK₁₂F (FK₁₂F + TL + GM-CSF) and TG/FK₁₂F (FK₁₂F + TEV + GM-CSF) (n = 5 mice per group). **B**, Quantification of the percentage of CD80⁺CD86⁺ DCs. **C**, Representative histograms of MHC class I mean fluorescence intensity (MFI) on DCs. **D**, Quantification of MHC class I MFI in DCs (n = 5 mice per group). Data are presented as mean ± SEM. Statistical significance was determined by one-way ANOVA.



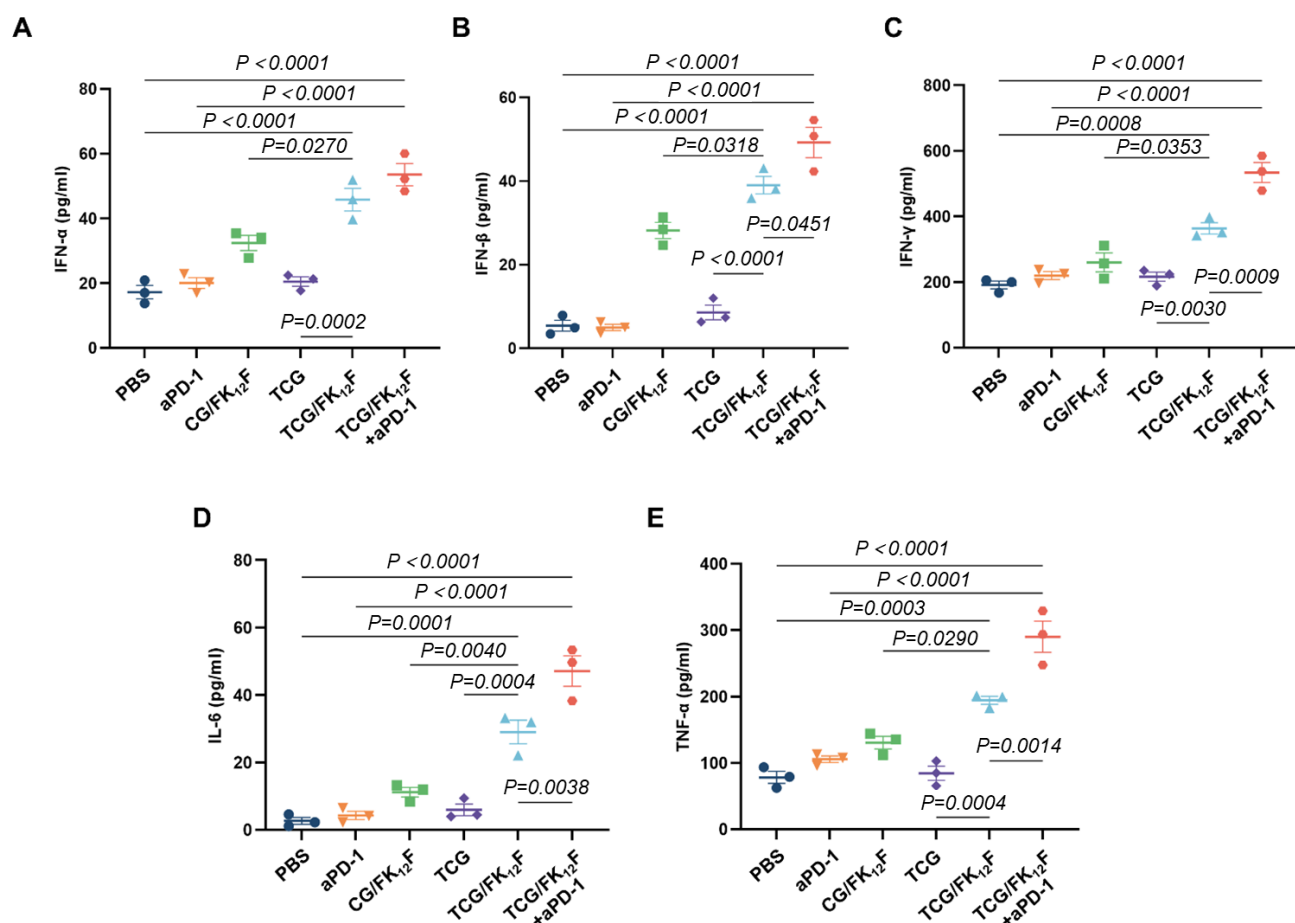
Supplementary Fig. 10. Analysis of DC subsets in the hydrogel post-vaccination. **A**, Quantification by flow cytometry of cDC1, **(B)** cDC2, **(C)** moDC and **(D)** pDC populations within the hydrogel (n = 5 mice per group). Data are mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA.



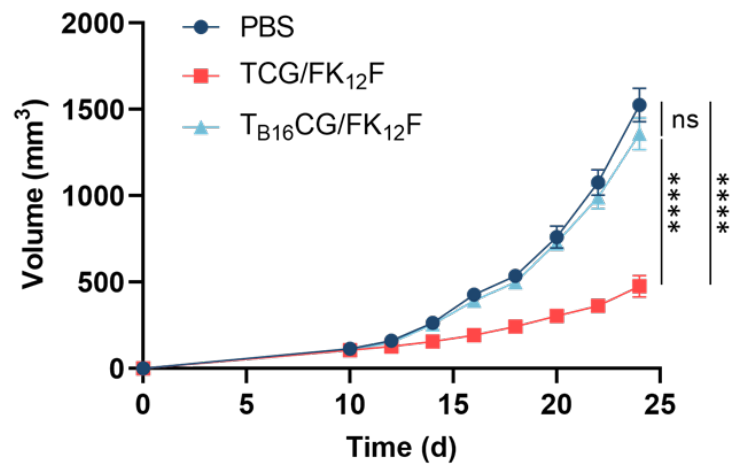
Supplementary Fig. 11. Fluorescence microscopy image of OVA-expressing RM-1 cells (OVA fused with GFP), confirming stable expression of OVA. Scale bar, 100 μm ($n = 3$ independent experiments).



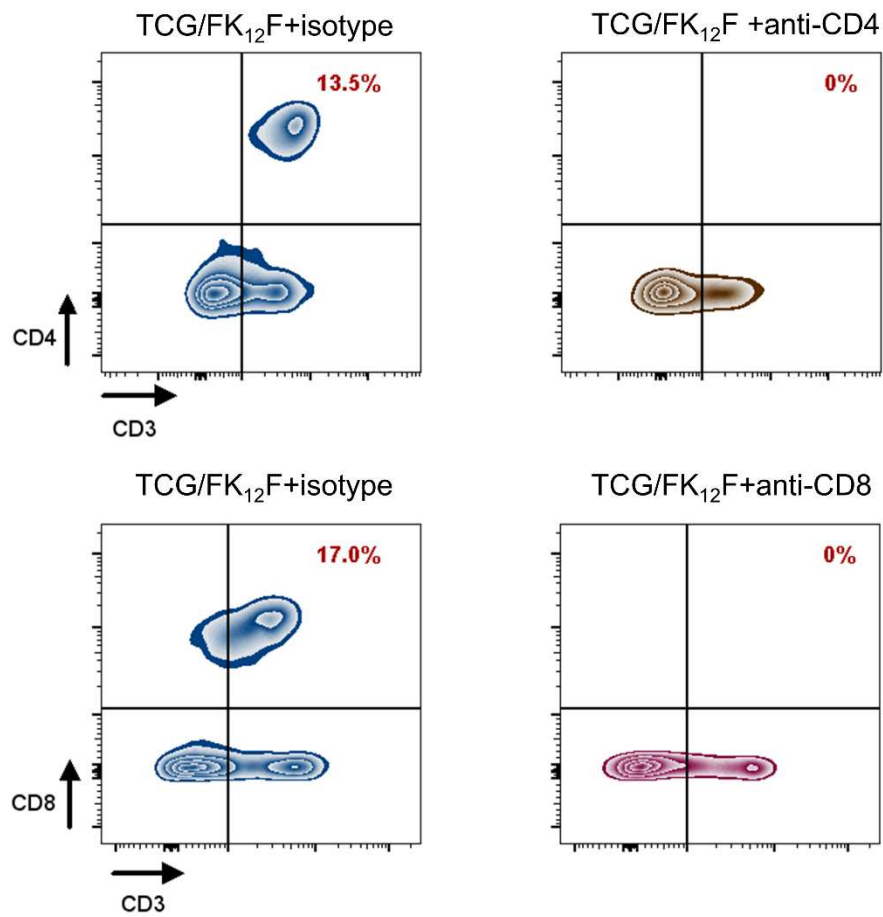
Supplementary Fig. 12. Representative histograms show the mean fluorescence intensity (MFI) of MHC class I. **A**, Gel-infiltrating DCs. **B**, DCs isolated from draining lymph nodes.



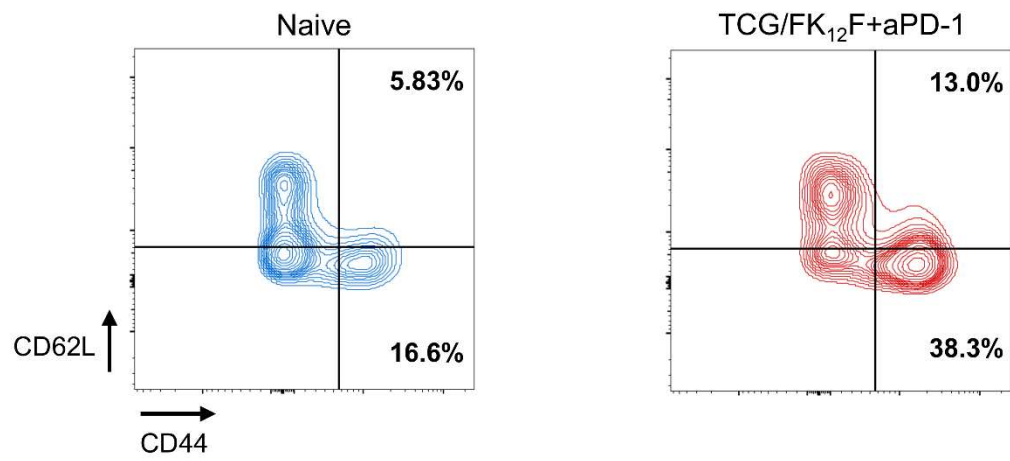
Supplementary Fig. 13. Cytokine detection in different groups of RM-1 subcutaneous tumors after treatment (n = 3 mice per group). Representing IFN-α (A), IFN-β (B), IFN-γ (C), IL-6 (D) and TNF-α (E), respectively. Data are given as mean ± SEM. Statistical significance was determined using one-way ANOVA.



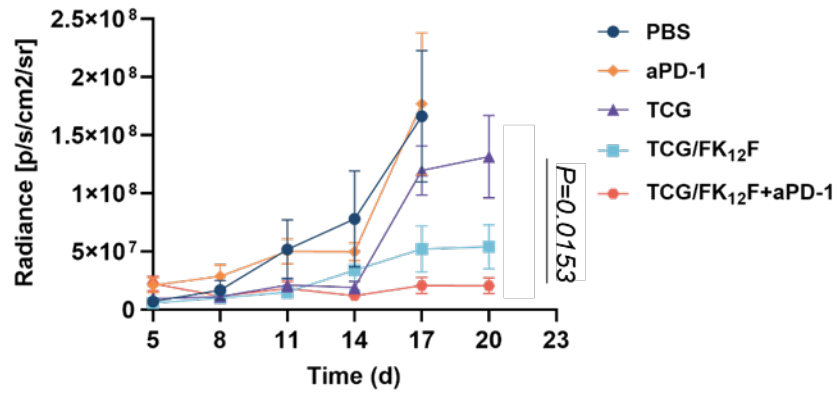
Supplementary Fig. 14. Tumor growth curves following administration of autologous or heterologous TEV vaccines. Mice bearing RM-1 tumors were treated with PBS, TCG/FK₁₂F (autologous TEV vaccine), or T_{B16}CG/FK₁₂F (heterologous, B16-derived TEV vaccine). Data are mean $\pm$ SEM (n = 5 mice per group). Statistical significance was determined using one-way ANOVA.



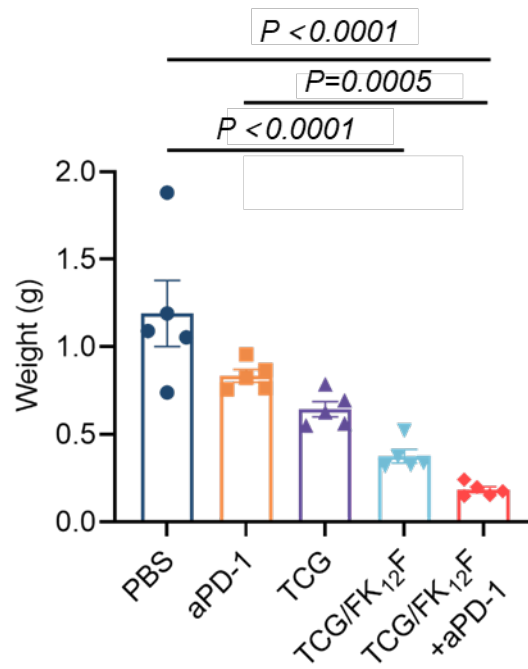
Supplementary Fig. 15. T-cell depletion verified by peripheral blood flow cytometry.



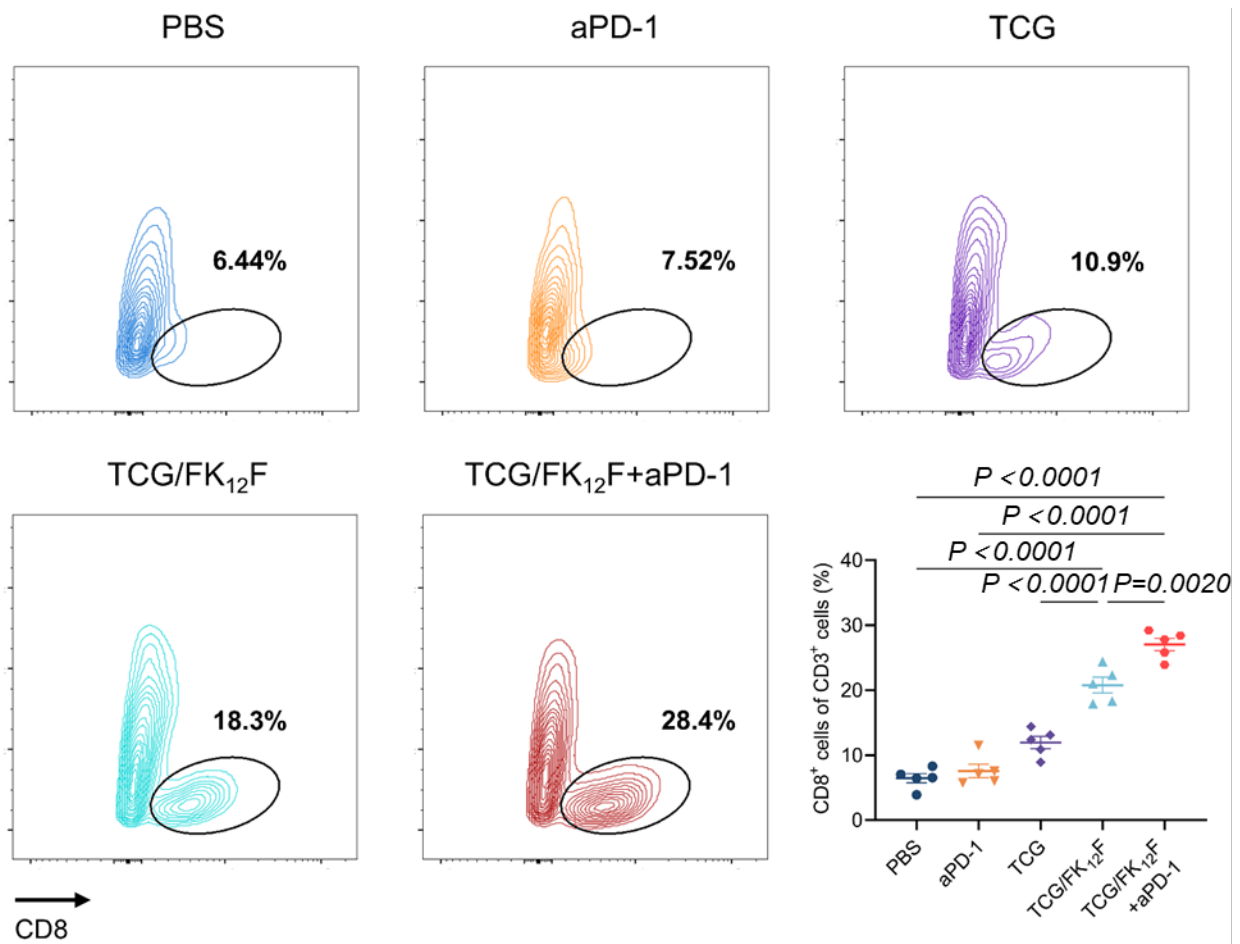
Supplementary Fig. 16. Representative flow cytometry results graph of CD8⁺ memory T cells in Navie and TCG/FK₁₂F groups.



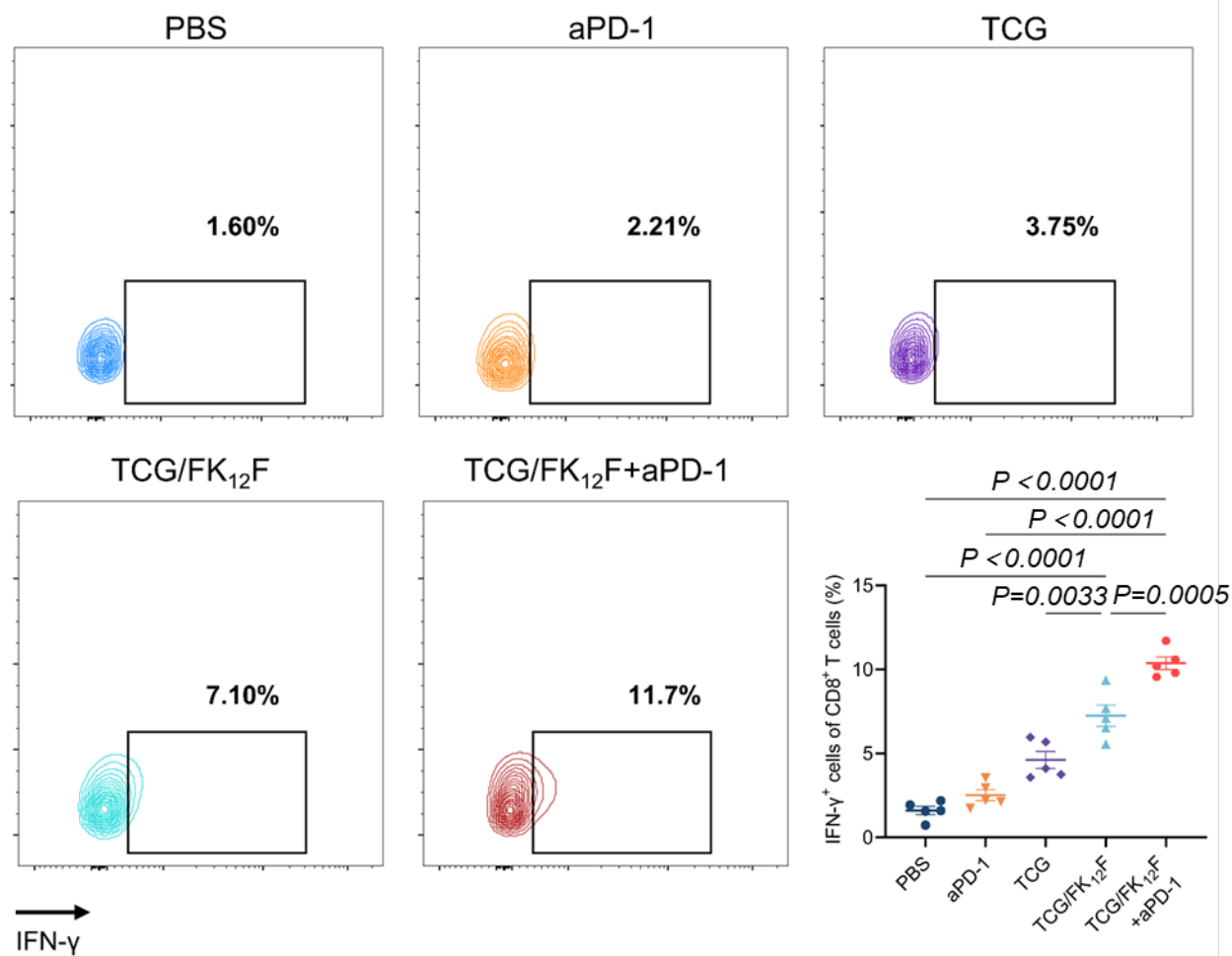
Supplementary Fig. 17. *In vivo* fluorescence quantification curves for RM-1 orthotopic tumors. Data are represented as mean $\pm$ SEM (n = 5 mice per group). Statistical significance was determined using one-way ANOVA.



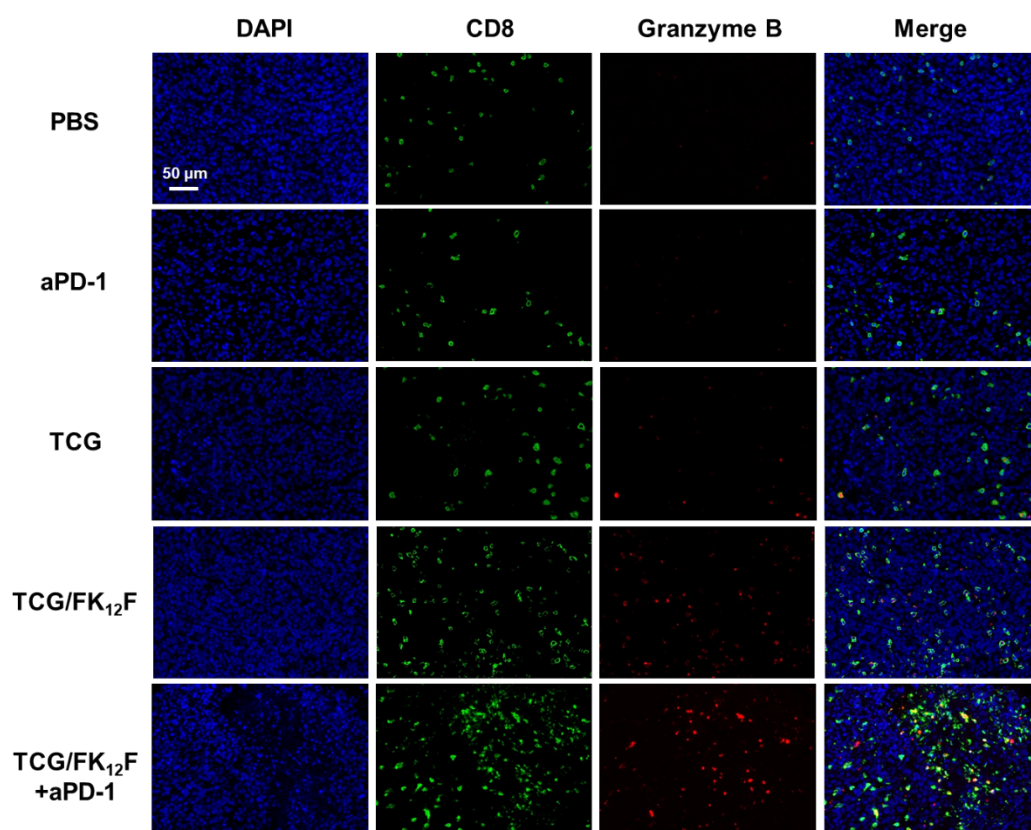
Supplementary Fig. 18. Tumor weight statistics of the RM-1 in situ model of prostate cancer in different treatment groups (n = 5 mice per group). Data are mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA.



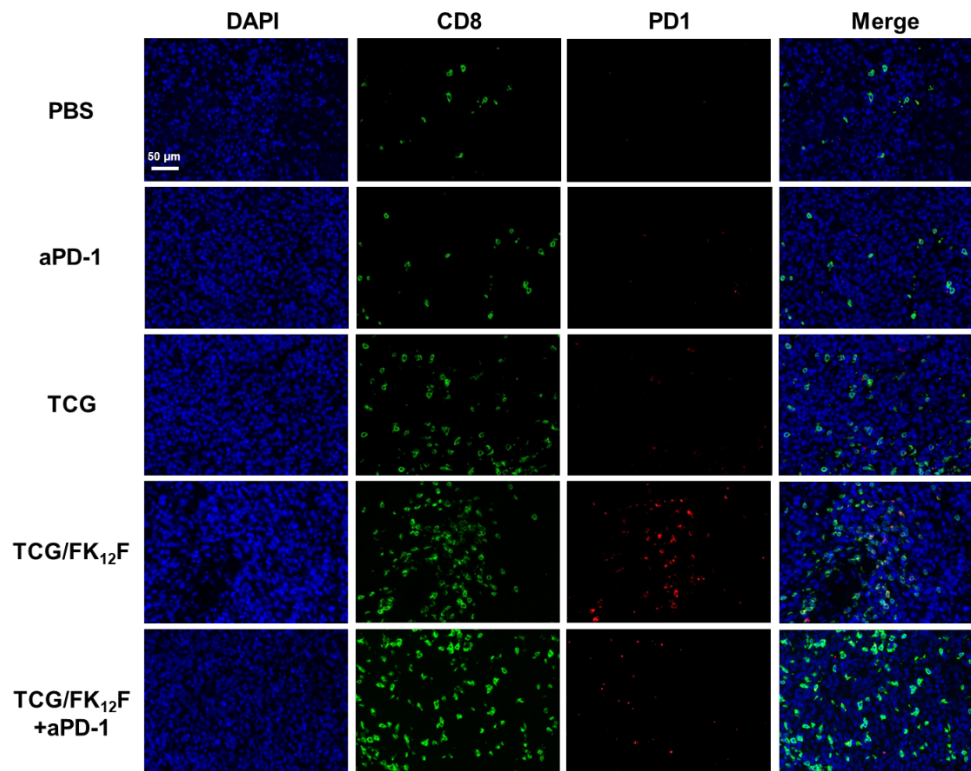
Supplementary Fig. 19. Flow cytometric results of CD8⁺ T cells in tumors of the RM-1 orthotopic model in different treated groups. (n = 5 mice per group). Data are mean ± SEM. Statistical significance was determined using one-way ANOVA.



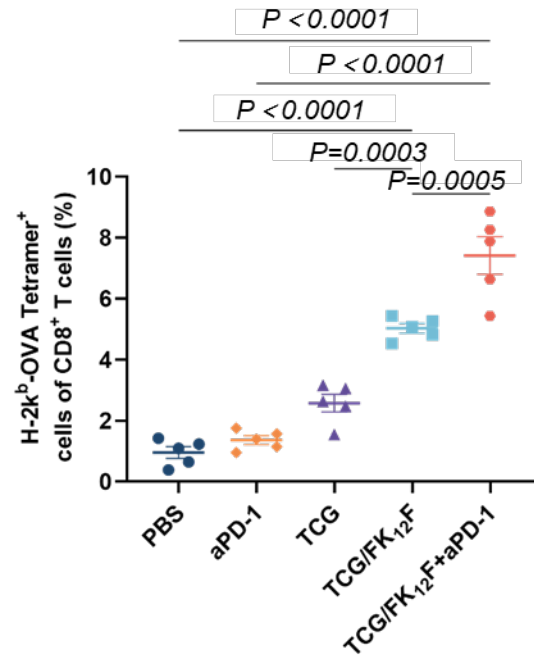
Supplementary Fig. 20. Flow cytometric results of IFN- γ ⁺ cells of CD8⁺ T cells in tumors of the RM-1 orthotopic model in different treated groups. (n = 5 mice per group). Data are mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA.



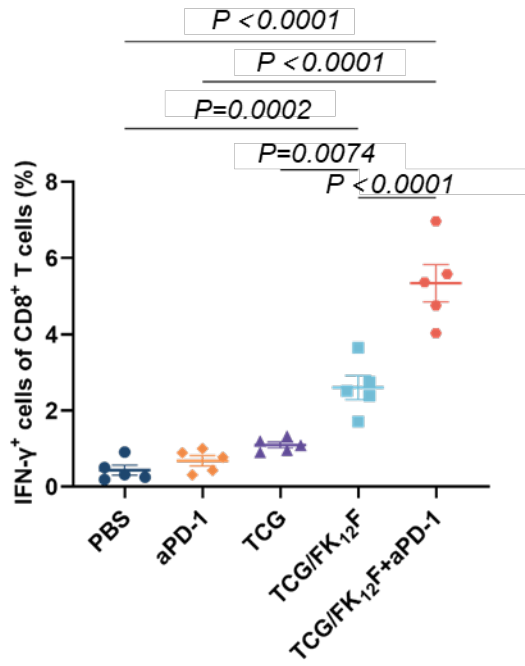
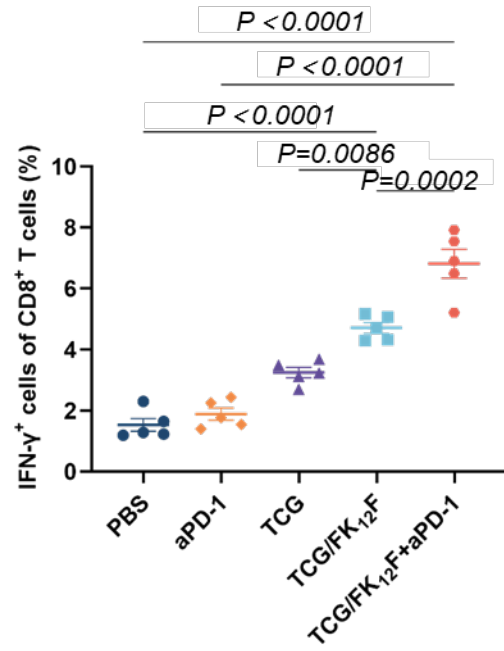
Supplementary Fig. 21. Granzyme B immunofluorescence of RM-1 orthotopic tumors in different treatment groups.



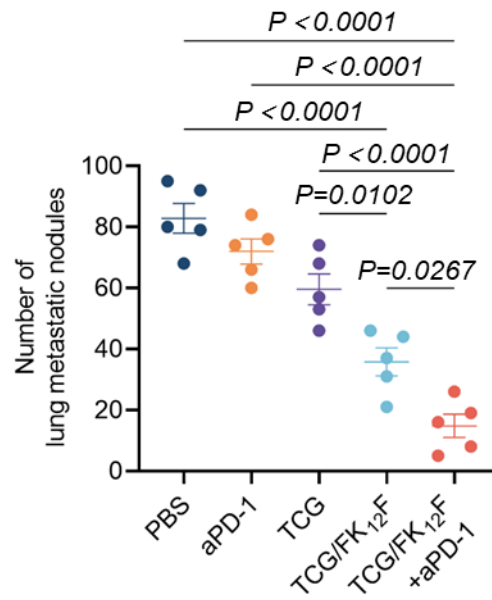
Supplementary Fig. 22. Representative immunofluorescence images showing PD-1 expression in RM-1 orthotopic tumors from the different treatment groups.



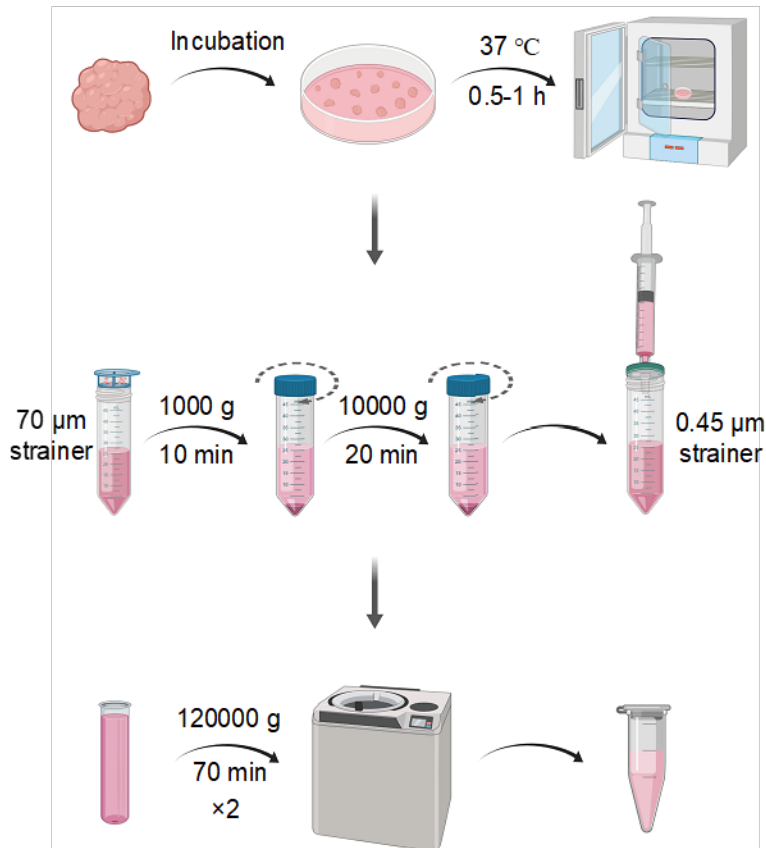
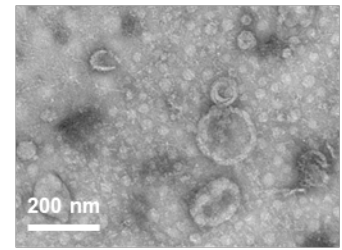
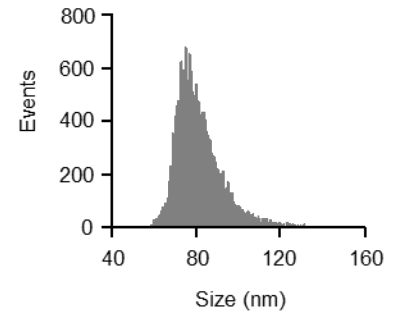
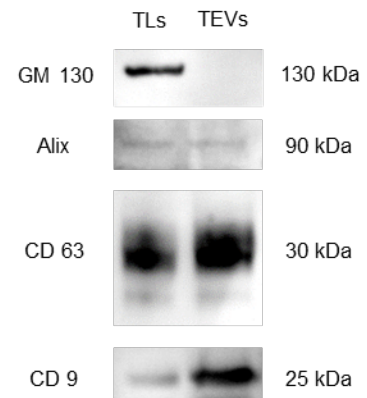
Supplementary Fig. 23. Quantification of SIINFEKL–H-2K^b tetramer⁺ CD8⁺ T cells in orthotopic tumor in different treatment groups (n = 5 mice per group). Data are mean ± SEM. Statistical significance was determined using one-way ANOVA.

A**B**

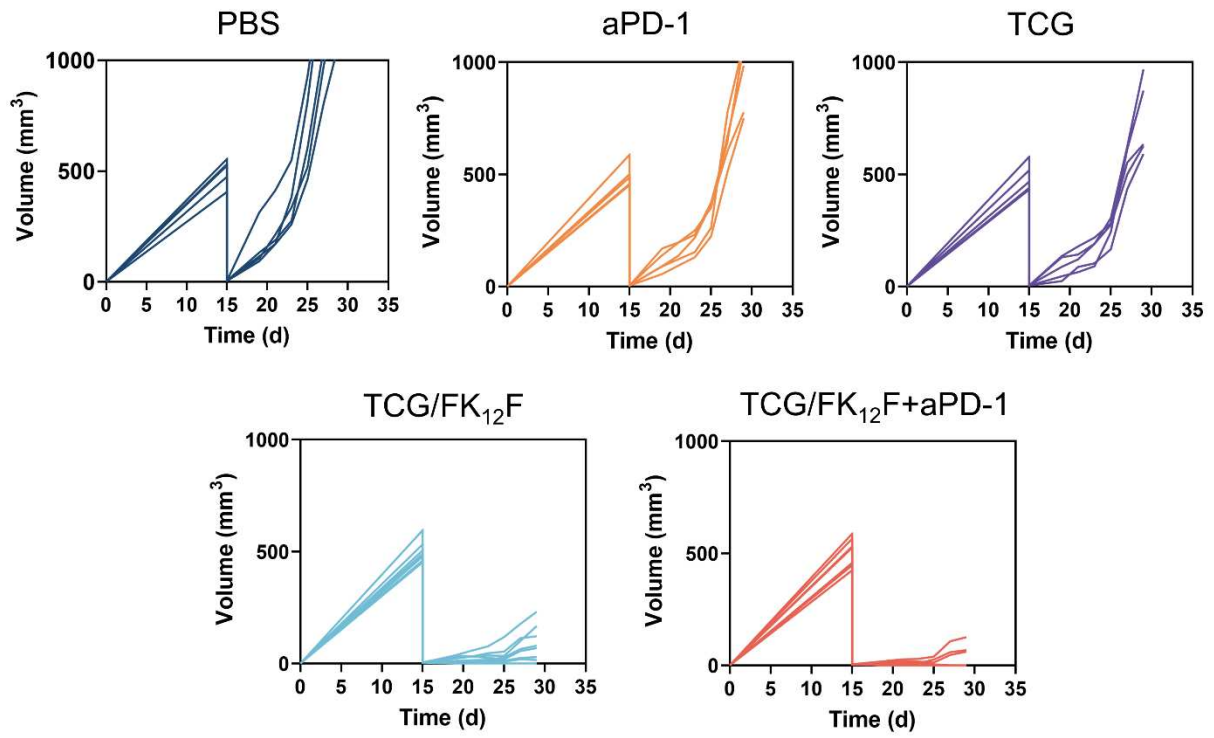
Supplementary Fig. 24. Flow cytometric analysis of systemic immune responses in the 4T1 metastatic tumor model. **A**, Quantification of IFN- γ ⁺ CD8⁺ T cells in peripheral blood in different treatment groups (n = 5 mice per group). **B**, Quantification of IFN- γ ⁺ CD8⁺ T cells in splenocytes after *ex vivo* restimulation with TEVs (n = 5 mice per group). Data are mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA.



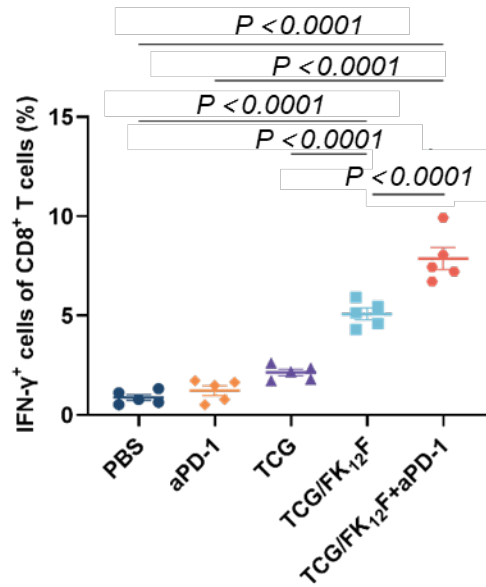
Supplementary Fig. 25. Number of lung nodules in each treatment group (n = 5 mice per group). Data are mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA.

A**B****C****D**

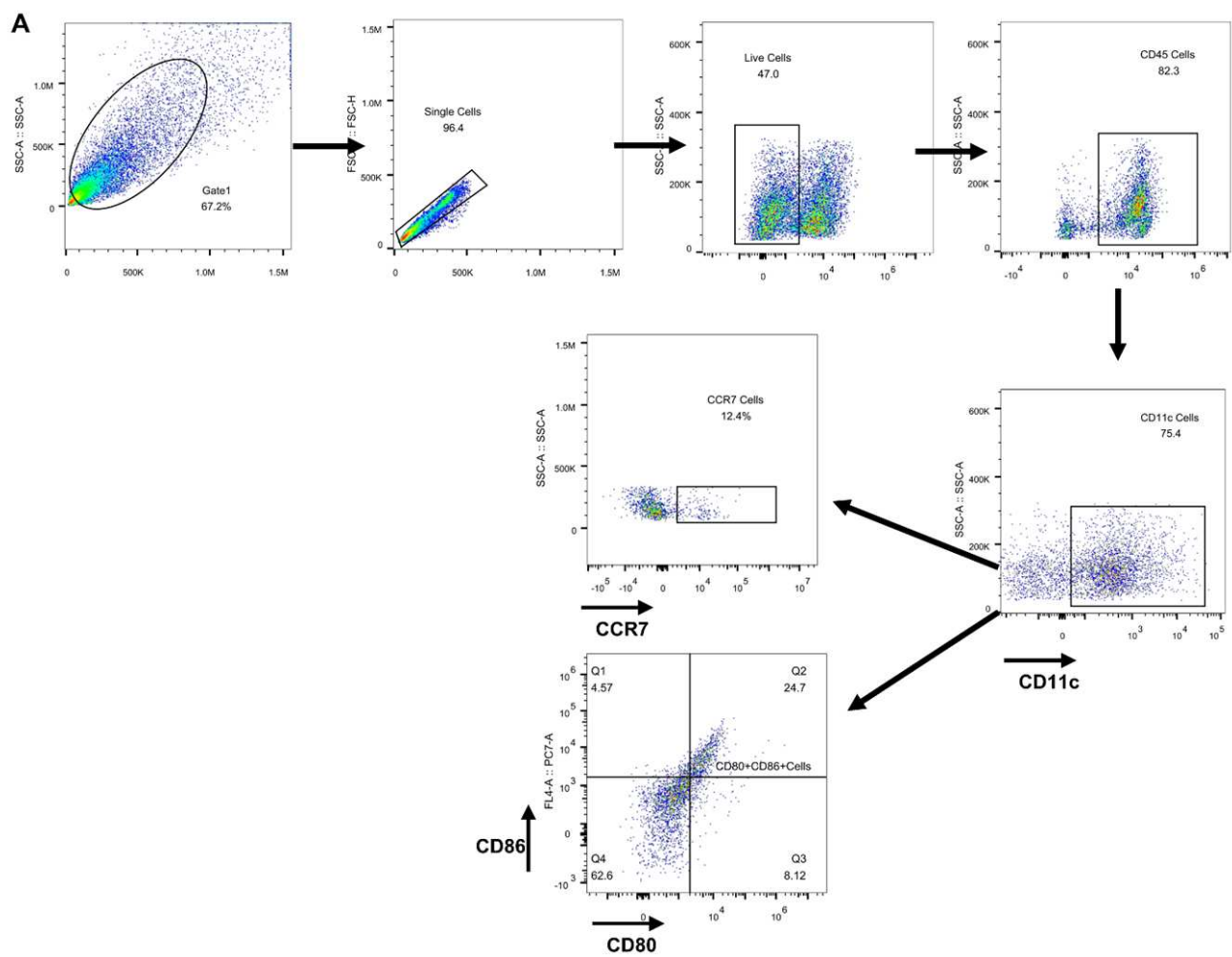
Supplementary Fig. 26. Extracellular vesicle extraction and characterization of tumor tissue sources. **A**, Flowchart of TEVs extraction from tumor tissues. **B**, Representative transmission electron microscopy images of TEVs (n = 3 independent experiments). **C**, Particle size distribution of TEVs (n = 3 independent experiments). **D**, Western blot analysis for extracellular vesicle markers (Alix, CD63 and CD9) and contaminated protein (GM130) expressed in TLs and TEVs (n = 3 independent experiments).

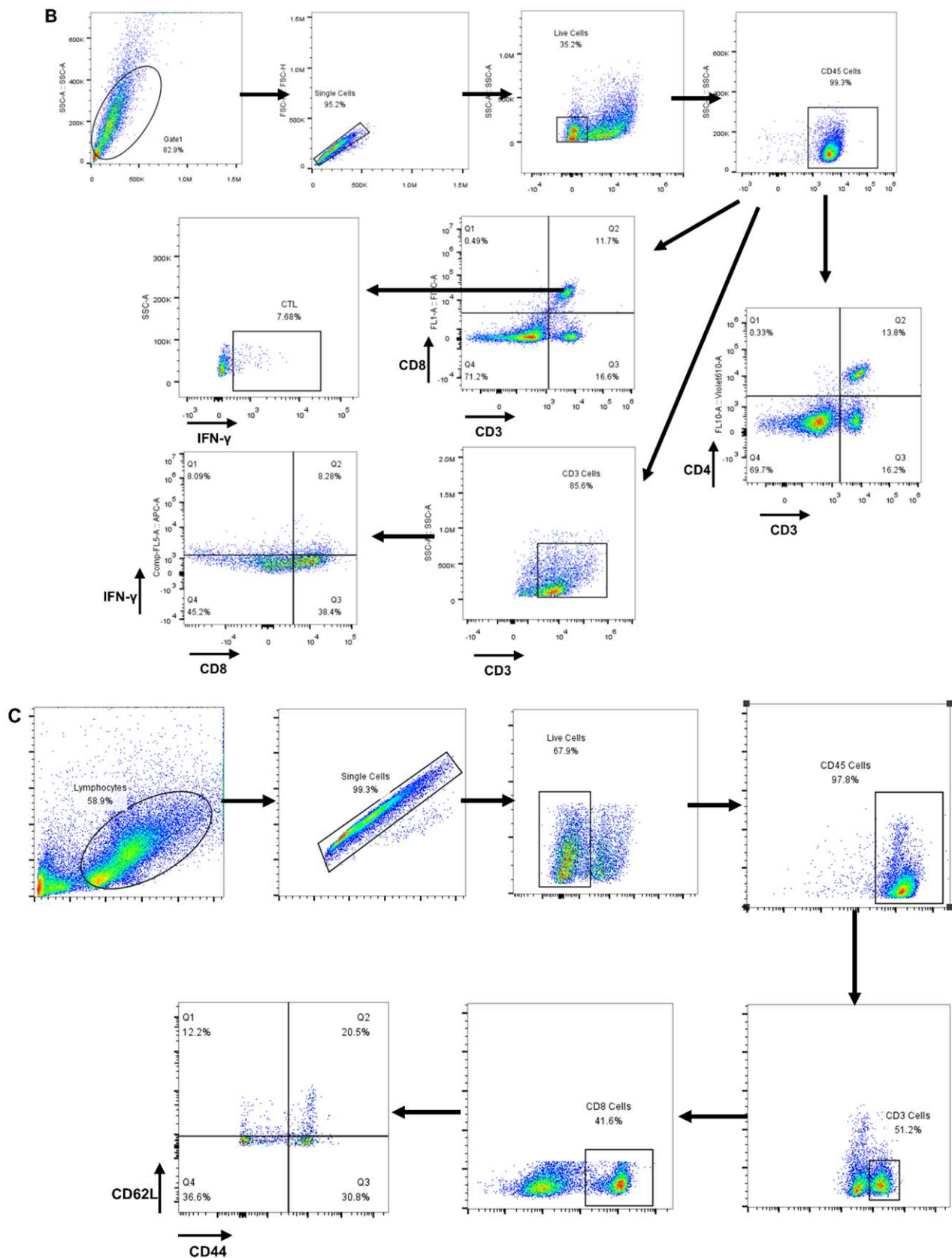


Supplementary Fig. 27. Tumor growth curves per mouse of RM-1 tumor surgical excision model in each group; n=5 mice for PBS, aPD-1 and TCG, and n=8 mice for TCG/FK₁₂F and TCG/FK₁₂F + aPD-1.



Supplementary Fig. 28. Quantification of IFN- γ ⁺ CD8⁺ T cells across treatment groups in 4T1 breast cancer orthotopic resection model (n = 5 mice per group). Data are mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA.





Supplementary Fig. 29. Gating strategies for flow cytometric analysis of different tumoral and peripheral lymphocytes. First remove doublets by FSC-H and FSC-A. Dead cells were subsequently excluded by L/D staining. **A**, Analysis of DCs. **B**, Analysis of CD4 and CD8 cells. **C**, Analysis of T memory cells.