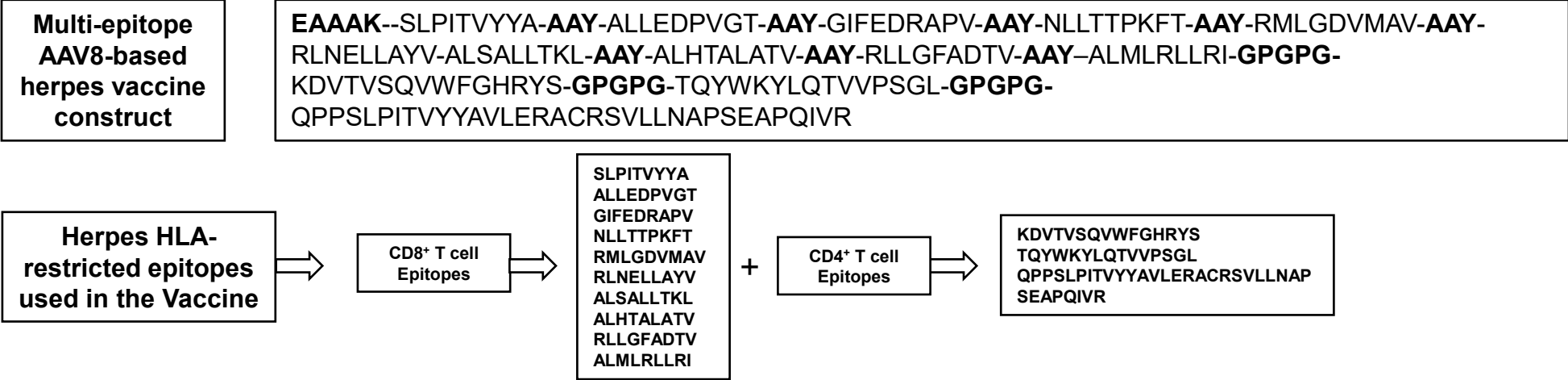


A

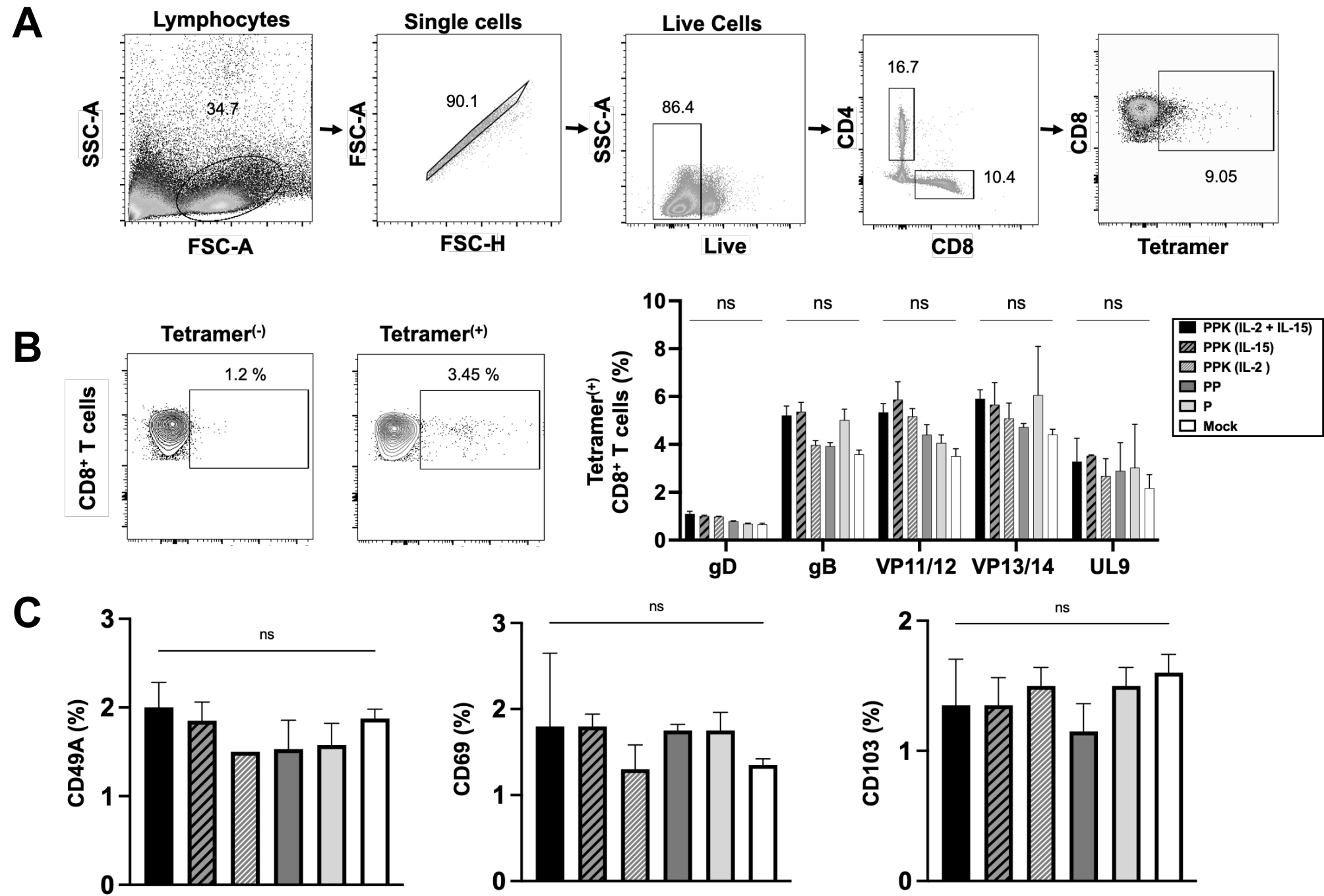


B

- Mock:** AAV8-Null
- Prime:** AAV8-CamKII-CD4/CD8 Epitopes-CamII-Null
- Prime/Pull:** AAV8-CamKII-CD4/CD8 Epitopes-CamII-mCXCL11
- Prime/Pull/Keep:** AAV8-CamKII-CD4/CD8 Epitopes-CamII-mIL2-P2A-mCXCL11
- Prime/Pull/Keep:** AAV8-CamKII-CD4/CD8 Epitopes-CamII-mIL15-P2A-mCXCL11

Supplementary Figure 1. Multi-epitope AAV8-based herpes vaccine construct.

Immunodominant T cell epitopes from HSV-1 major antigens - glycoproteins D and B (gD and gB), viral tegument proteins (VP11/12 and VP13/14), and the DNA replication-binding helicase (UL9) proteins. The prime AAV8 vector encodes only the selected CD8⁺ and CD4⁺ T cell epitopes were separated by appropriate linkers. The prime/pull AAV8 vector co-expresses the epitopes along with mouse CXCL11 chemokine. The prime/pull/keep AAV8 vector expresses the epitopes, CXCL11, and either mouse IL-2, IL-15, or a combination of both cytokines. P represent prime only group receiving antigen priming without subsequent pull or keep phases, serving as a minimal immunization control. PP represent prime/pull group expressing T cell attracting chemokine-CXCL11. PPK (IL-15) represent prime/pull/keep immunotherapy group receiving IL-15 alone. PPK (IL-2) represent prime/pull/keep immunotherapy group receiving IL-2 alone. PPK (IL-2 + IL-15) represent prime/pull/keep immunotherapy group receiving combined IL-2 and IL-15 cytokine to enhance T cell survival, proliferation, and tissue retention. Mock represent non-immunized control group with no treatment or antigen exposure.



Supplementary Figure 2. Example of a representative gating strategy for analysis of T cell phenotypes in HSV-1 infected mice treated with prime/pull/keep T cell immunotherapy. (A) Representative dot plots show the hierarchical flow cytometry gating strategy. Lymphocytes were first identified based on FSC-A vs SSC-A parameters. Doublets were excluded by gating on FSC-H vs FSC-A to select single cells. Lymphocytes were then gated on CD4 and CD8 expression, and CD8⁺ T cells were further analyzed for antigen specificity using tetramers corresponding to HSV-1 epitopes: gD₅₃₋₆₁, gB₁₈₃₋₁₉₁, VP11/12₂₂₀₋₂₂₈, VP13/14₅₀₄₋₅₁₂, and UL9₁₉₆₋₂₀₄. B. Representative flow cytometry plots showing the frequencies of tetramer positive CD8⁺ T cells and the expression of T_{RM}, T_{CM}, T_{EM}, functional, and exhaustion markers in HSV-1 mice treated with PPK immunotherapy compared with untreated controls. Marker gating within tetramer-positive CD8⁺ T cells was performed using fluorescence minus one (FMO) control. PPK represent prime/pull/keep immunotherapy group and Mock represent non-immunized control group with no treatment or antigen exposure. (B) Prime/pull/keep T cell immunotherapy does not enhance HSV-1-specific CD8⁺ T cell responses in the spleen. Frequencies (%) of tetramer positive CD8⁺ T cells specific for multiple HSV-1 epitopes: gD₅₃₋₆₁, gB₁₈₃₋₁₉₁, VP11/12₂₂₀₋₂₂₈, VP13/14₅₀₄₋₅₁₂, and UL9₁₉₆₋₂₀₄ were analyzed in the spleen of PPK immunotherapy treated mice and untreated mice. . Experimental groups (P, PP, and PPK) and vaccination timelines are defined in Figure 1. Data are presented as mean ± SEM. Statistical significance was determined using two-way ANOVA followed by appropriate multiple comparisons tests (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001; ns, not significant). (C) Prime/pull/keep T cell immunotherapy does not promote tissue resident memory (T_{RM}) responses in the spleen. Frequencies (%) of tissue-resident memory (T_{RM}) markers were analyzed on gated CD8⁺ T cells isolated from spleen samples of PPK immunotherapy treated mice and untreated mice. Expression of CD49a, CD69, and CD103 was assessed within gB₁₈₃₋₁₉₁ tetramer positive CD8⁺ T cells. Experimental groups (P, PP, and PPK) and vaccination timelines are defined in Figure 1. Data represent the mean ± SEM. Statistical significance between the experimental groups was determine using one-way ANOVA followed by appropriate multiple comparisons tests (*' *P*<0.05, '**' *P*<0.01, '***' *P*<0.001, '****' *P*<0.0001, 'ns' not-significant).