

Integrating CD4⁺ T Cell Help in Therapeutic Neoantigen Vaccination for Head and Neck Cancer

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Abstract

Head and neck squamous cell carcinomas (HNSCC) are well-suited for cancer vaccination strategies. Peptide vaccine candidates derived from tumor-associated antigens (TAAs) or somatically mutated tumor-specific antigens (TSAs) are typically prioritized by predicted high-affinity between the candidate epitopes and major histocompatibility complex (MHC) class I. However, prioritizing peptide vaccine candidates based on prediction of MHC-II affinity has largely not been considered. Herein, addressing this point, we extend previous studies to evaluate therapeutic vaccination using the carcinogen-induced mouse oral cancer (MOC) 22 model. Two distinct MOC22 derived SLPs were tested—one is the TSA-oriented mutant intercellular adhesion molecule 1 (miCAM1), the second is the ERV encoded antigen p15E. In silico prediction revealed miCAM1 SLP bore strong MHC-I and MHC-II epitopes with a shared mutant residue. By contrast, the p15E SLP had a predicted high-affinity MHC-I epitope but lacked an MHC-II epitope. Notably, therapeutic miCAM1 SLP vaccination attenuated tumor growth more effectively with miCAM1 specific T cells displaying durable IFN- γ production compared with p15E SLP. Furthermore, miCAM1 SLPs carrying weakened MHC-II binding epitopes were unable to control tumor growth. These data underscore the potential value of therapeutic targeting of HNSCC epitopes and highlight the importance of studying distinct antigen classes in this setting. Moreover, they raise the possibility that, at least in part, CD4⁺ T cell help is critical for cancer vaccination in this preclinical HNSCC model and suggest in silico prediction approaches prioritize overlapping MHC-I and MHC-II epitopes to generate potent cancer vaccines.

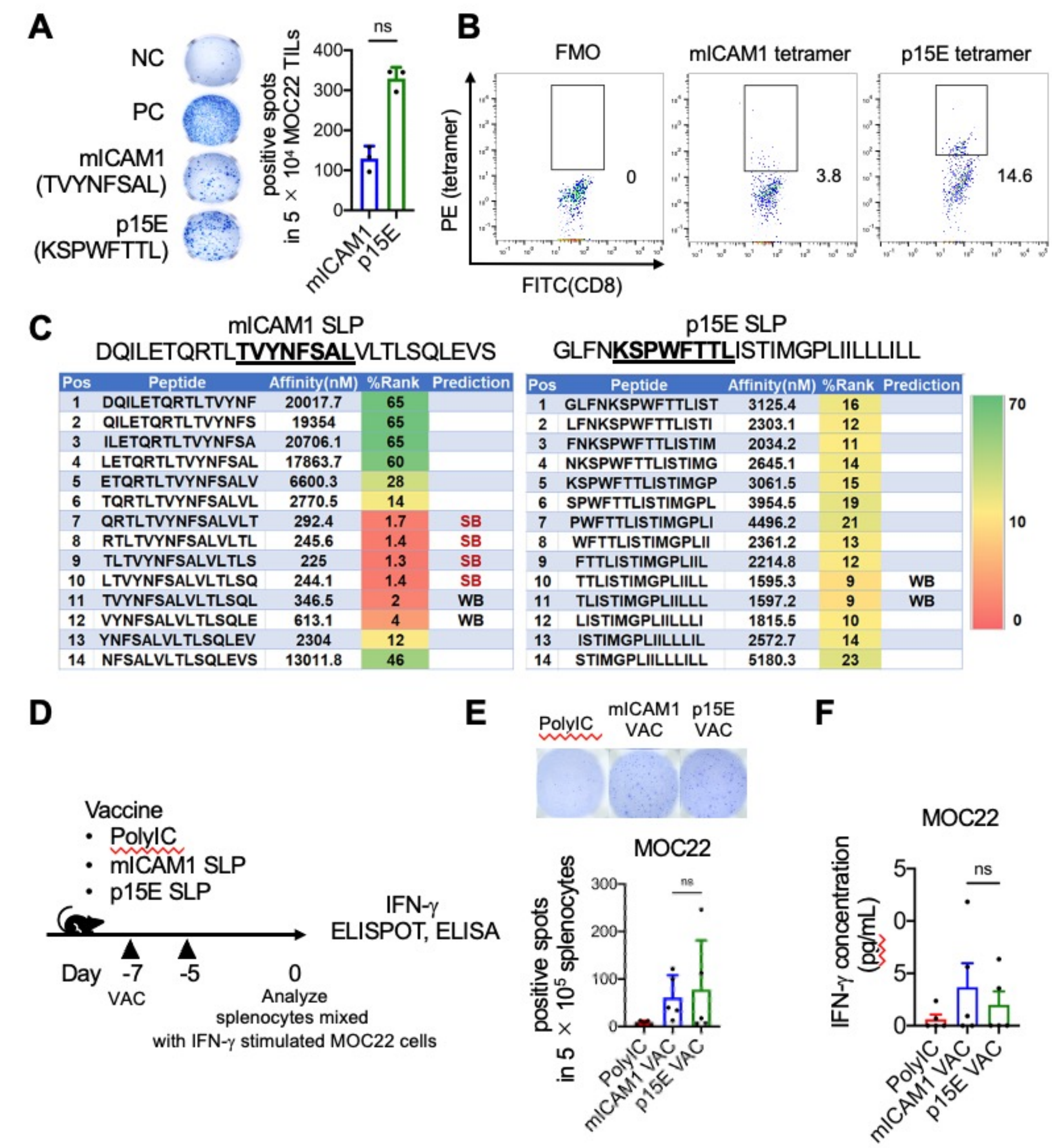


Figure 1. The miCAM1 and p15E SLPs show comparable reactivity against MOC22 tumor.

A) MOC22 TILs ELISPOT and B) tetramers staining show both miCAM1 and p15E antigens have reactivity against MOC22 tumors. C) MHC-II epitope in silico predictions for miCAM1 and p15E SLPs. SB: strong binding candidate, WB: weak binding candidate. D-F) The vaccines of miCAM1 or p15E SLPs induced MOC22 reactive IFN- γ productive antigen-specific T cells. The IFN- γ production level from each vaccinated group does not show significant difference. These data confirm the presence of cell surface miCAM1- and p15E-Class I complexes and suggest that the expression levels are comparable.

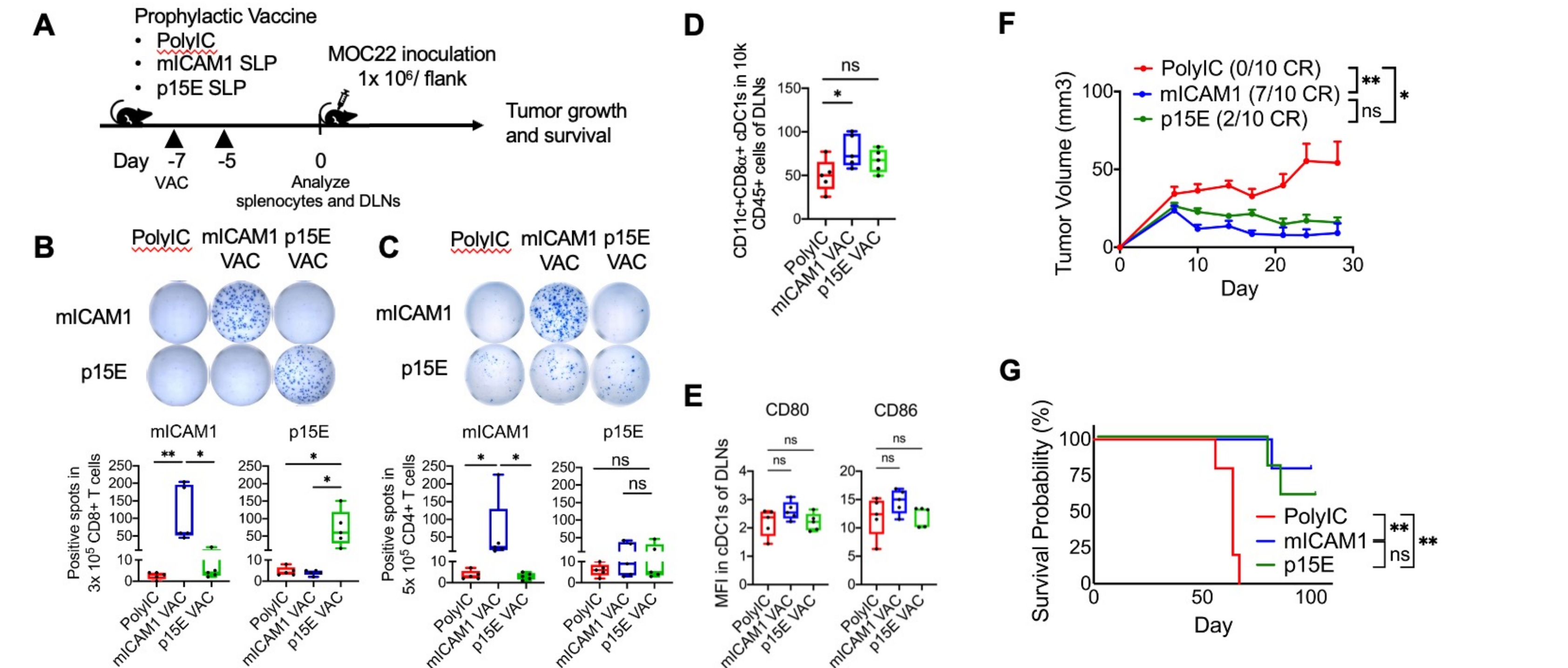


Figure 2. The miCAM1 and p15E SLPs both show preventive anti-cancer effects against MOC22.

A) The prophylactic vaccination protocol. B-C) The miCAM1 SLP VAC activated both CD8⁺/4⁺ T cells while p15E SLP activated only CD8⁺ T cells. F-G) Both SLPs vaccine induced significant reduction of MOC22 tumor size and prolonged survival, suggesting CD8⁺ T cells are main subset to eradicate MOC22 tumor in prophylactic setting.

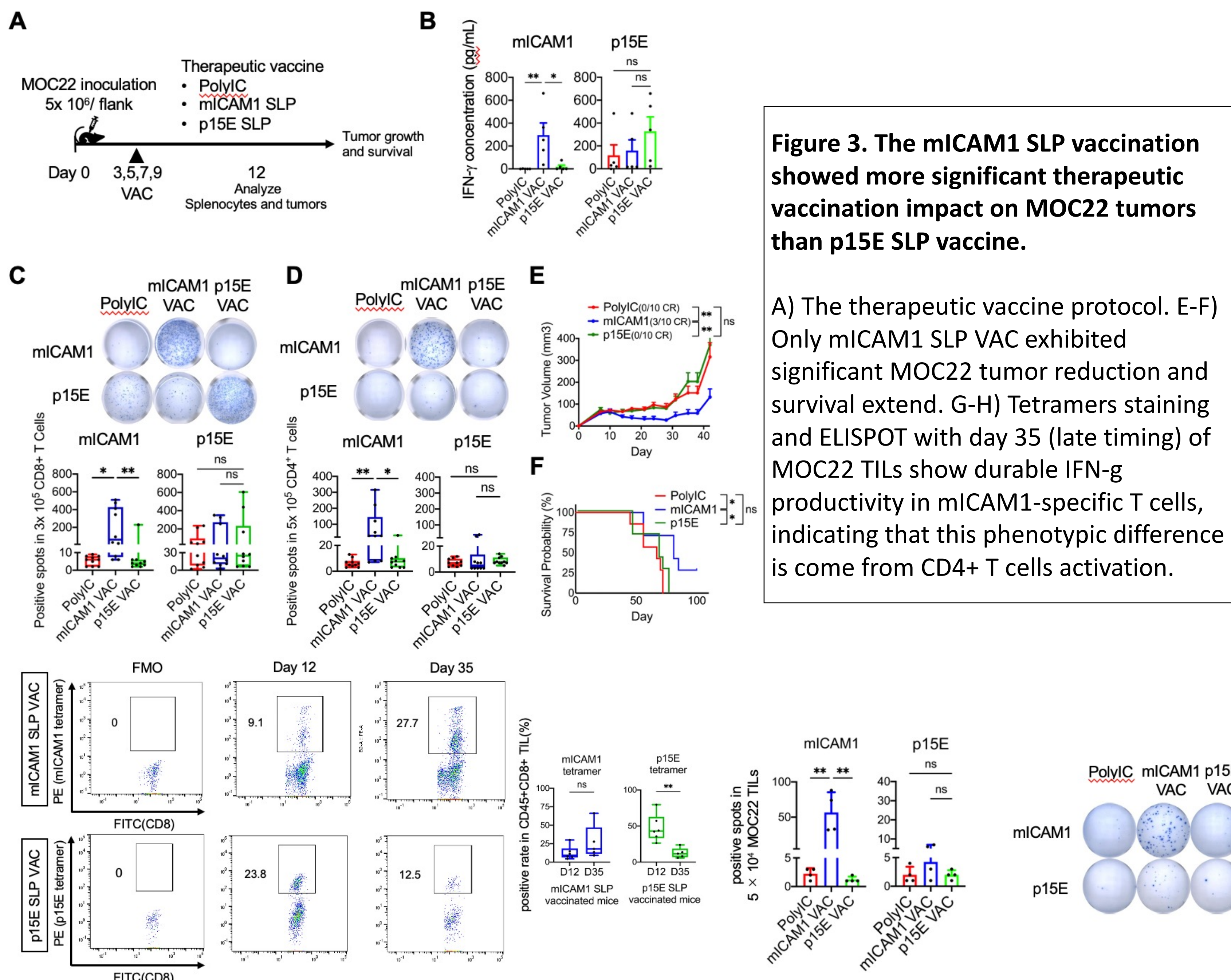


Figure 3. The miCAM1 SLP vaccination showed more significant therapeutic vaccination impact on MOC22 tumors than p15E SLP vaccine.

A) The therapeutic vaccine protocol. E-F) Only miCAM1 SLP VAC exhibited significant MOC22 tumor reduction and survival extend. G-H) Tetramers staining and ELISPOT with day 35 (late timing) of MOC22 TILs show durable IFN- γ productivity in miCAM1-specific T cells, indicating that this phenotypic difference is come from CD4⁺ T cells activation.

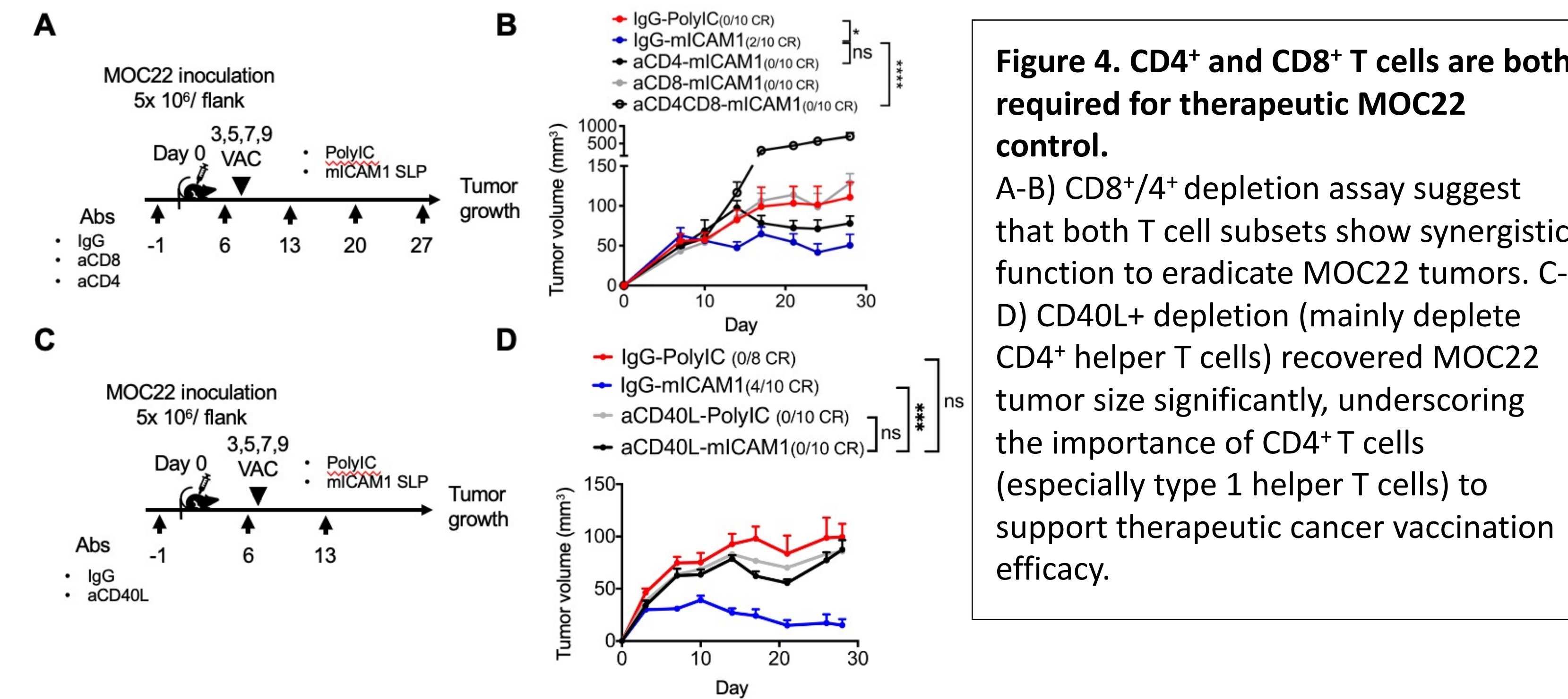


Figure 4. CD4⁺ and CD8⁺ T cells are both required for therapeutic MOC22 control. A-B) CD8⁺/4⁺ depletion assay suggest that both T cell subsets show synergistic function to eradicate MOC22 tumors. C-D) CD40L+ depletion (mainly deplete CD4⁺ helper T cells) recovered MOC22 tumor size significantly, underscoring the importance of CD4⁺ T cells (especially type 1 helper T cells) to support therapeutic cancer vaccination efficacy.

Figure 5. The miCAM1 specific MHC-II epitopes are indispensable for anti-cancer immunity in MOC22.

To further analyze the importance of miCAM1 antigen-specific CD4⁺ T cells for therapeutic vaccine, we synthesized altered miCAM1 SLPs with preserved MHC-I epitope and weakened MHC-II epitopes (B-D). F) The altered miCAM1 SLPs (Alt-1/2 miCAM1 SLPs) exhibited significantly impaired anti-tumor function, suggesting the critical importance of antigen-specific CD4⁺ T cells in therapeutic vaccine setting. G-H) The original miCAM1 SLP increased DCs and IFN- γ producing CD8⁺ T cells in MOC22 TILs, underscoring the durable anti-tumor effect which partly come from antigen-specific CD4⁺ T cells activation capacity.

Conclusion

We extended our previous findings on MOC22 SLP vaccination by identifying that MHC-I and MHC-II overlapping neoepitopes induce robust anti-cancer impact. These data highlight that prioritizing overlapping MHC-I and MHC-II epitopes in therapeutic cancer vaccines may represent an important approach.

