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## Supplemental information

# Vaccine adjuvant-elicited CD8$^+$ T cell immunity is co-dependent on T-bet and FOXO1

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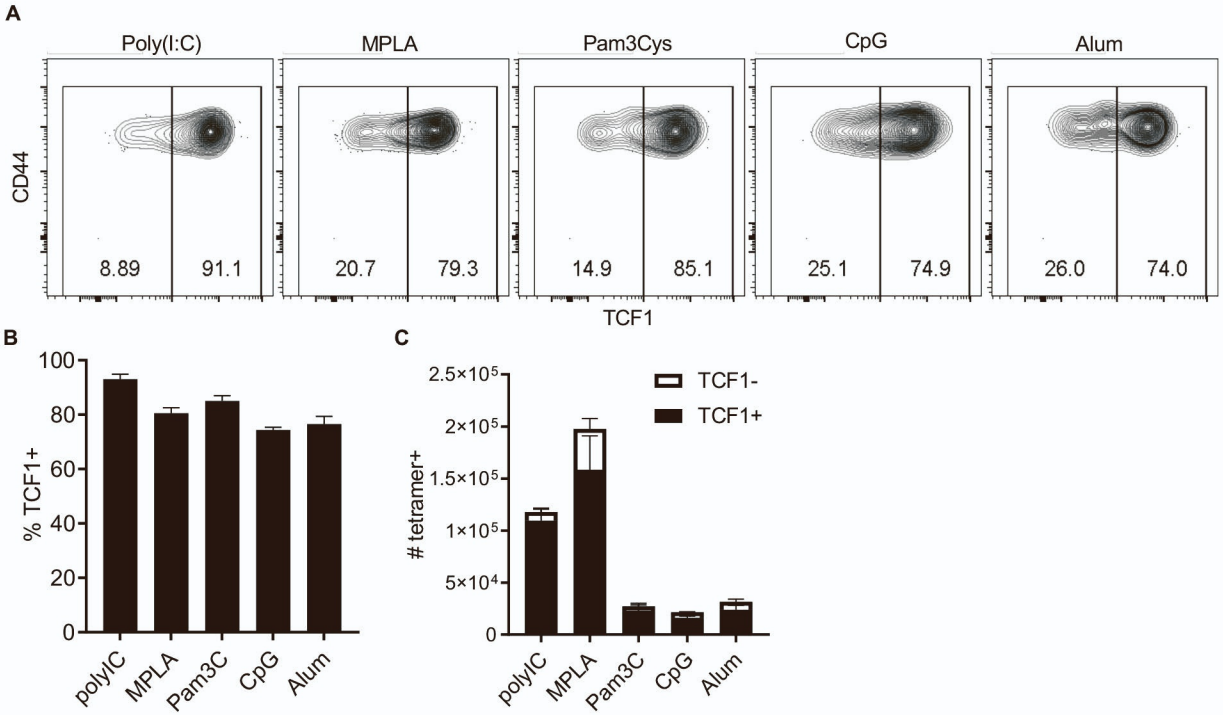



**Supplemental Figure 1. TCF1hi Tvac phenotype applies to adjuvants more broadly, related to Figure 1.**

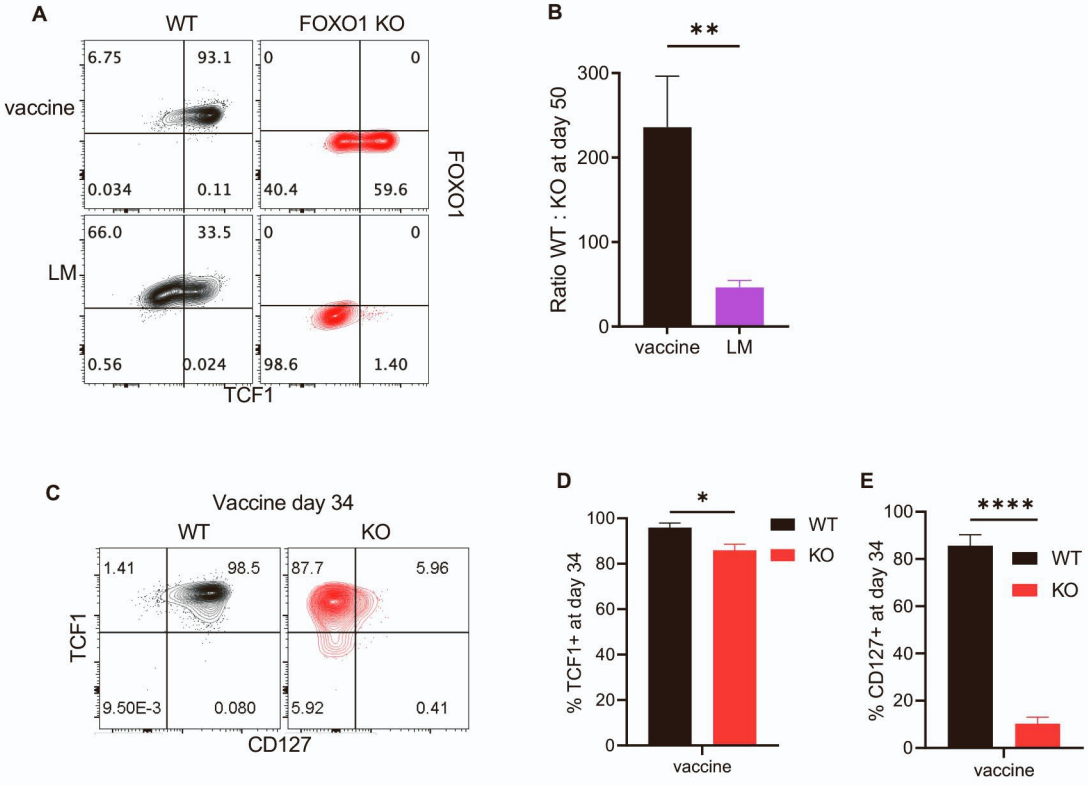
C57BL/6 mice were immunized with single adjuvant (poly(I:C), MPLA, Pam3Cys, CpG, or Alum) and OVA and spleens were harvested 7 days later.

(A) Representative TCF1 staining in tetramer+ CD8+ T cells.

(B) The percent of TCF1+ in tetramer+ cells.

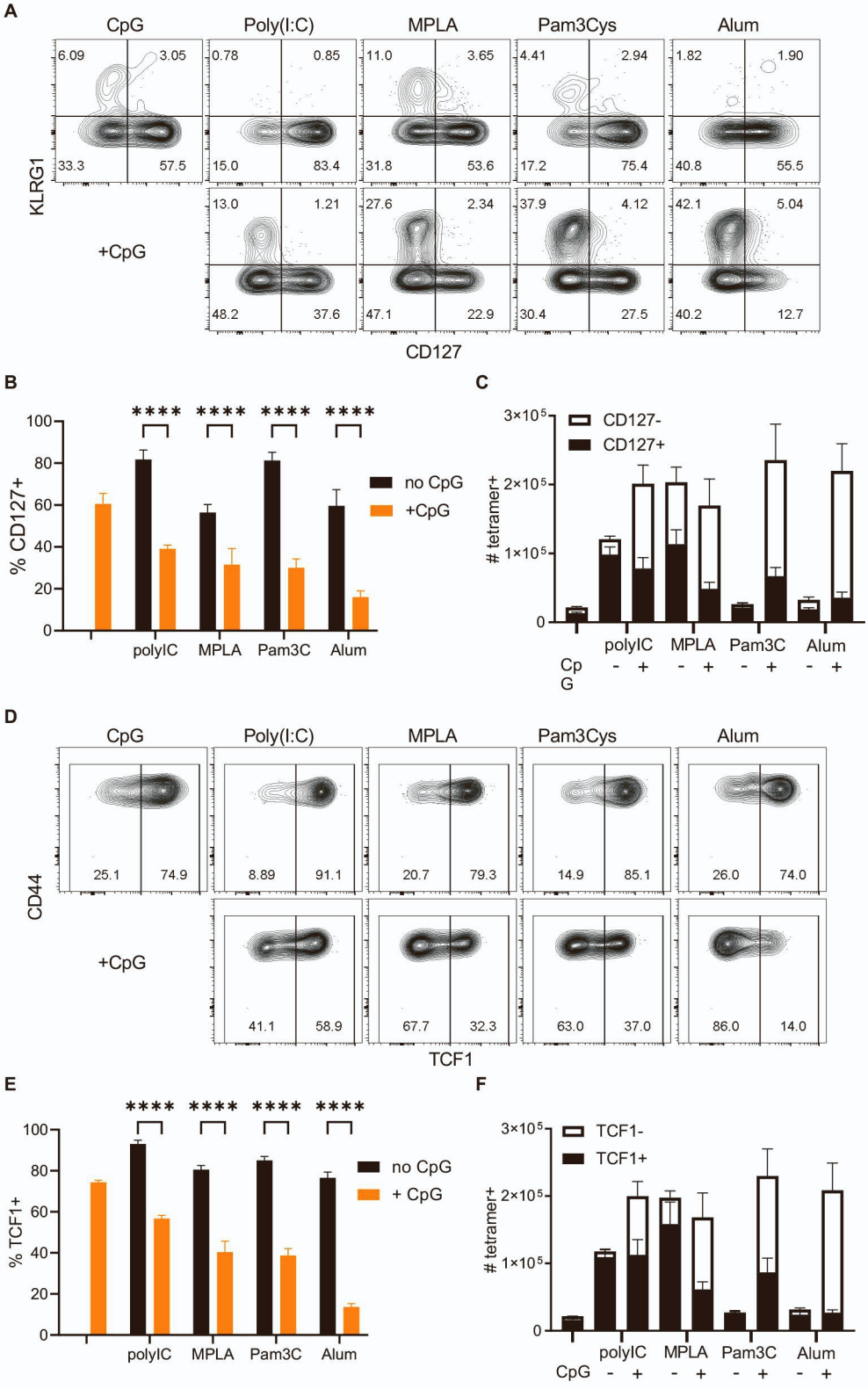
(C) The number of TCF1+ and TCF1- cells in tetramer+ cells.

Data shown are mean ± SEM; n=4 mice per group, one experiment.




**Supplemental Figure 2. Tvacs require FOXO1 for memory response despite FOXO1 independent TCF1 expression, related to Figure 3.**

5×10$^3$ splenic WT OT1 (WT) and 5×103 *foxo1*fl/fl dLck-Cre OT1 (KO) cells were co-transferred into congenically different WT C57BL/6 recipients followed by i.v. administration of combined-adjuvant vaccine (poly(I:C), anti-CD40, and OVA) or infection with LM-OVA. (A). Seven days after immunization/infection, the response of the transferred cells and their FOXO1 sufficient or deficient status were confirmed by flow cytometry. (B) At day 50 post challenges, the spleens were harvested and the donor cells were identified based on the differential expression of FOXO1, CD45.1 and CD45.2. The number of WT cells divided by the number of KO cells normalized to input. Data shown are mean ± SEM; n≥4 mice per group, one experiment. Significance was defined by unpaired t test with Welch's correction, where **p < 0.01.(C) representative TCF1 x CD127 phenotype of WT and FOXO1 KO T cells 34 days post vaccination. (D) The percent of TCF1+ cells within WT and KO donor cells. (E) The percent of CD127+ cells within WT and KO donor cells. Data shown are mean ± SEM; n≥4 mice per group, representative of two experiments. Significance was defined by unpaired t-test, where *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

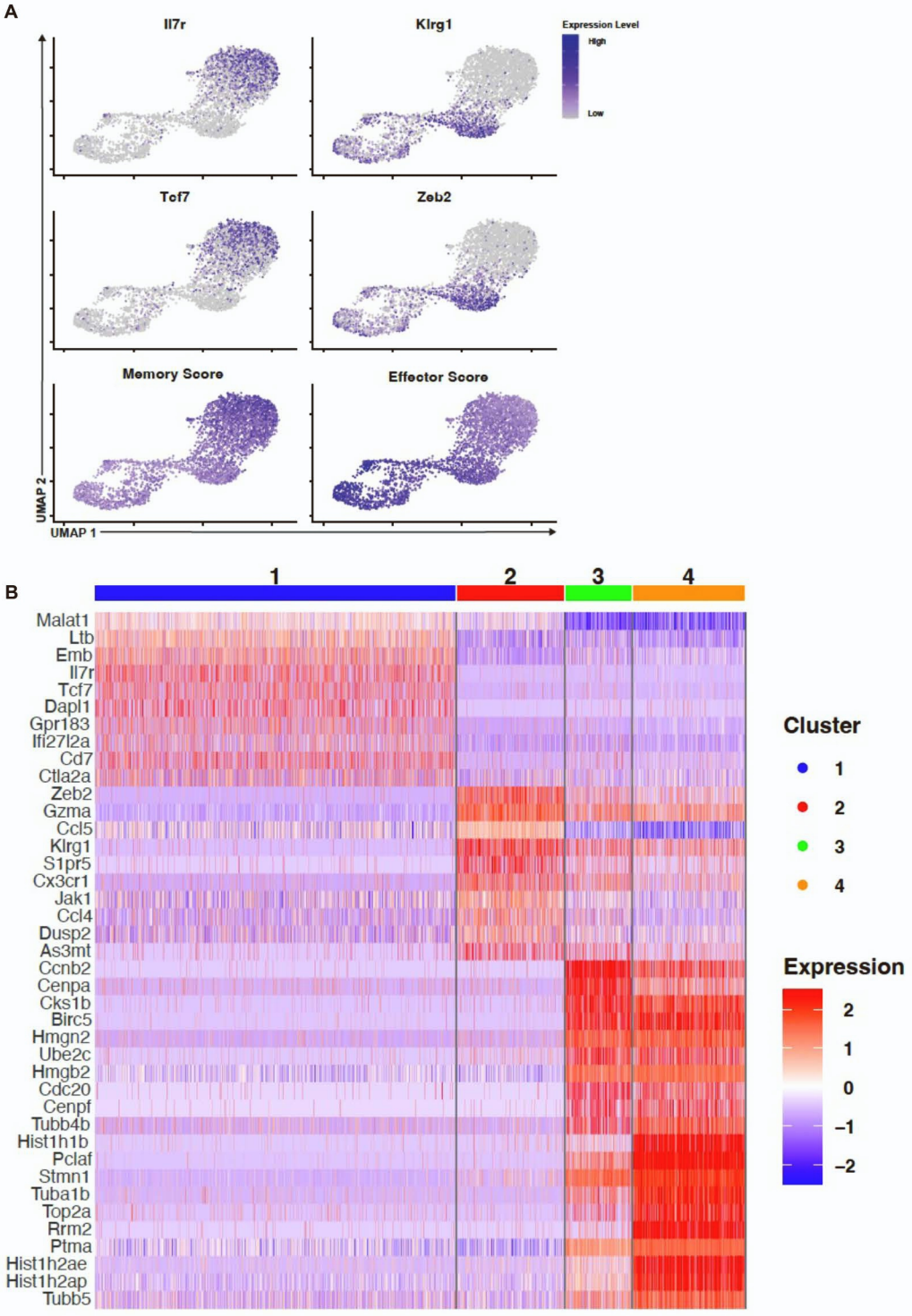



**Supplemental Figure 3. Inflammation re-directs Tvacs into separate effector and memory cell fates, related to Figure 5.**

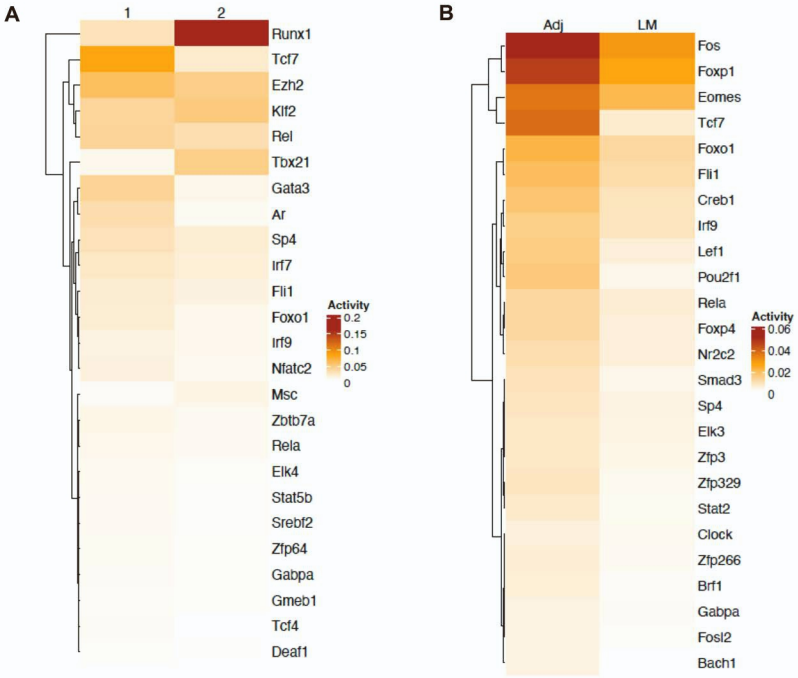
C57BL/6 mice were immunized with single adjuvant (poly(I:C), MPLA, Pam3Cys, CpG, or Alum) and OVA with or without CpG. Seven days later the spleens were harvested and the phenotype of tetramer+ CD8+ T cells was analyzed by flow cytometry.

(A) Representative contour plots for CD127 versus KLRG1 staining.
(B) The percent of CD127+ and CD127- cells.
(C) The number of CD127+ and CD127- cells.
(D) Representative contour plots for TCF1 staining.
(E) The percent of TCF1+ and TCF1- cells.
(F) The number of TCF1+ and TCF1- cells.

Data shown are mean ± SEM; n=4 mice per group, one experiment. Significance was defined by Ordinary two-way ANOVA, where ****p < 0.0001.

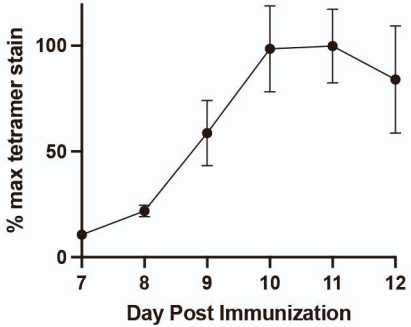



**Supplemental Figure 4. Cluster-defining genes in scRNAseq analysis, related to Figure 6.**
A) UMAP plots of indicated gene expression or module score of cells as described in Fig. 7A. Color indicates relative strength of expression or score. B)Heatmap of the top 10 most differentially expressed genes in each cluster as indicated by colored bars at the top. Each colored line represents the expression of the indicated gene in an individual cell as a z-score relative to the global average expression




**Supplemental Figure 5. Heatmaps of SCENIC analysis, related to Figure 6**

A) Heatmap of average inferred transcriptional activity of top 25 differentially active transcription factors based on SCENIC analysis of cells in clusters 1 and 2 . B) Heatmap of average inferred transcriptional activity of top 25 differentially active transcription factors based on SCENIC analysis of cells in Adj or LM cells within clusters 3 and 4. Lines represent clustering by Euclidean distance. Color represents average SCENIC inferred activity.




**Supplemental Figure 6. Time course of ova-specific CD8+ T cell response to ova-mRNA-LNP, related to Figure 7.**

Mice were immunized with 2ug ova-mRNA-LNP as described in the Materials and Methods. The ova-specific T cell responses was monitored in the blood at the indicated time points by tetramer staining. The highest average response (day 11) of the 4 mice per group was taken as the maximum to which all other values were compared.