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Supplemental information

Epithelial organoid supports resident memory CD8 T cell differentiation

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Supplementary Figure 1.

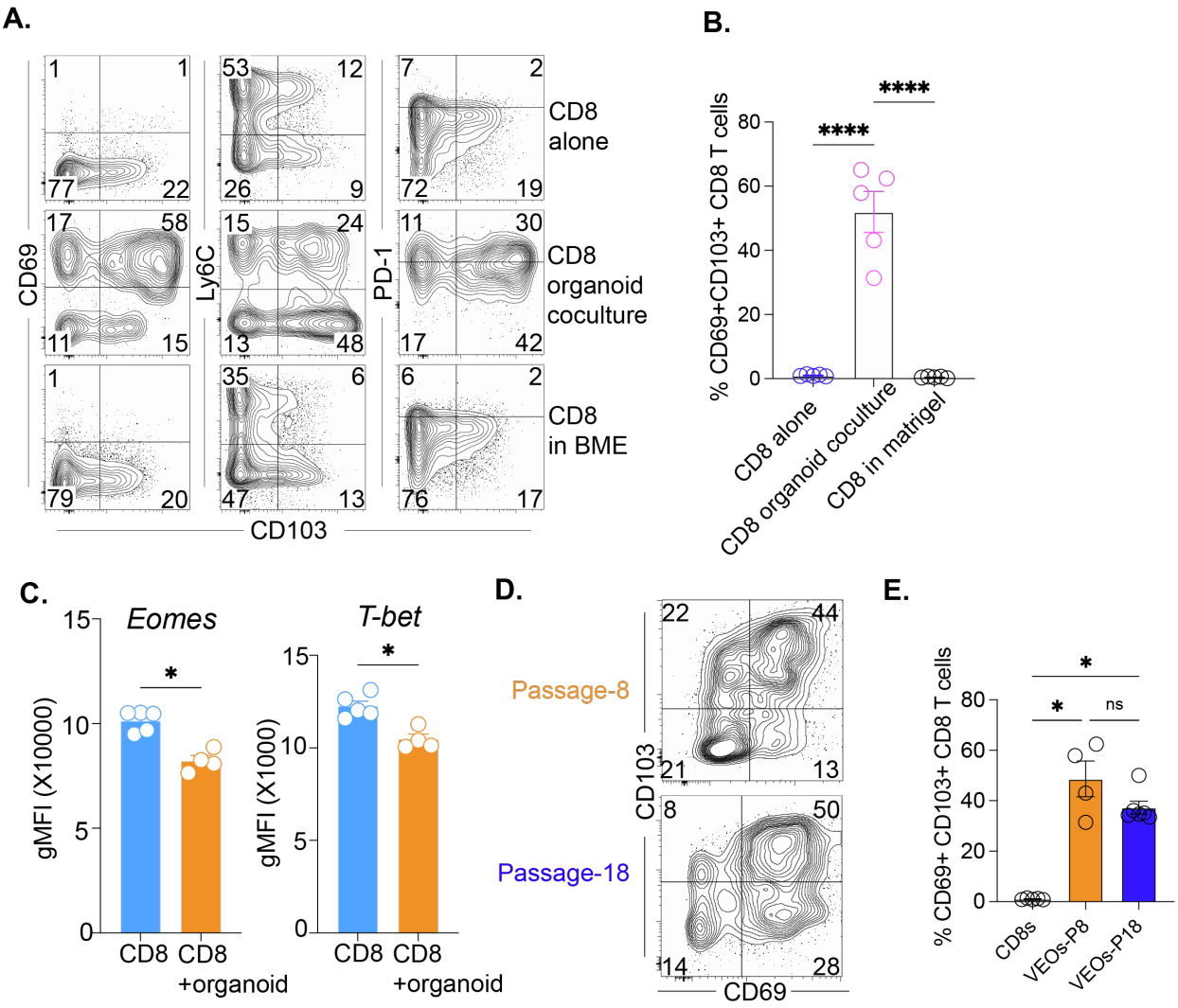

Figure S1. *The extracellular matrix embedding alone is not capable of supporting TRM differentiation and the VEO passage history does not impact TRM formation*, Related to Fig. 2. **A**. CD8 T cells were mixed with VEO-derived epithelial cells and were embedded in BME (middle row) or just embedded alone in BME in the absence of VEOs (bottom row) for 14 days. CD8 T cells maintained as a suspension culture in the absence of VEOs (top row) were included as a control. Representative flow plots depicting expression of various TRM-associated markers are shown. Cells were gated on live congenic marker (CD45.1 or CD90.1) + CD8 T cells. **B**. Bar graph comparing the percentage acquisition of TRM phenotype among different conditions. **C**. Geometric mean fluorescence intensity comparison of transcription factors eomesodermin and T-bet between *in vitro* generated TRM and CD8 T cells maintained alone after 14 days of culture. **D**. Representative flow cytometry plot showing presence of CD69+CD103+ CD8 T cells among cocultured T cells with VEOs that were either at passage-8 or -18. **E**. Bar graph showing percentage of CD69+CD103+ CD8 T cells in CD8 T cells maintained alone or in coculture with VEOs. Data are representative of two repeats with n=4-6/condition. Bars indicate mean ± SEM. One-way ANOVA with Tukey's multiple comparison test (B, E). Student t-test (C). * < 0.05, ****<0.0001

Supplementary Figure 2.

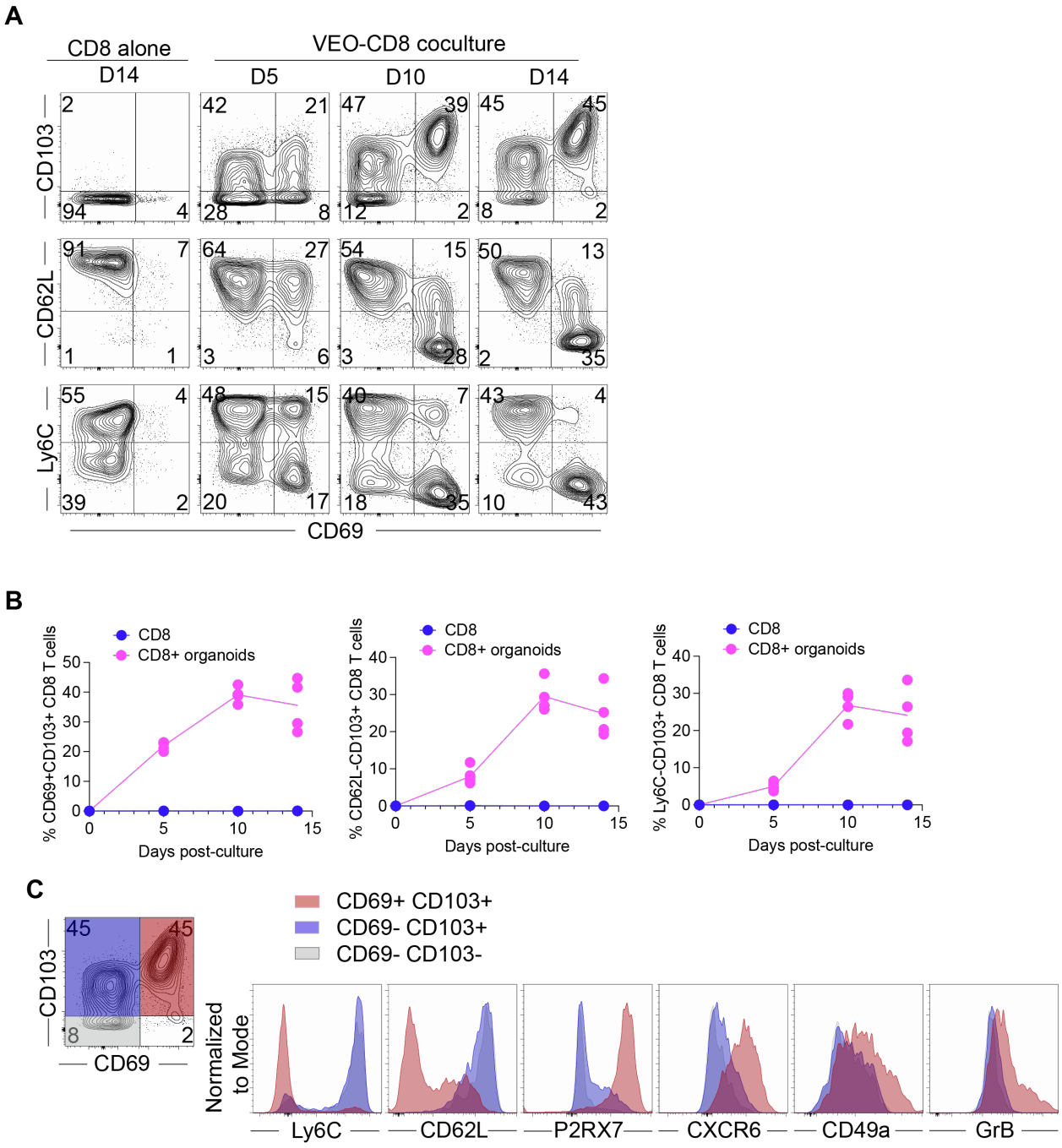

Figure S2. *The induction of TRM phenotype by the VEOs is a gradual process, and among various subsets generated post-co-culture, the CD103+CD69+ subset alone phenotypically resembles true epithelial TRM*, Related to Fig.2. **A.** Phenotype of CD8 T cells cultured with VEOs for indicated time points was assessed by flow cytometry. The left most column represents cells maintained in the absence of VEOs for 14 days. Representative flow plots depicting expression of various TRM-associated markers are shown. Cells were gated on live and congenic marker (CD45.1 or CD90.1). **B.** Scatter plot depicting percentage of CD8 T cells positive for various TRM-associated markers across time. **C.** Flow-based comparison of TRM markers among the 3 subsets generated by the co-culture (CD69+CD103+, CD69-CD103+, and CD69-CD103-) showing that only the phenotype of CD69+CD103+ population aligns with previously described TRM phenotype including CD62L-, P2rx7+, CD49a+, CXCR6+ and a fraction of cells that are granzyme-B+. Data are representative of two repeats with n=4-6/condition.

Supplementary Figure 3.

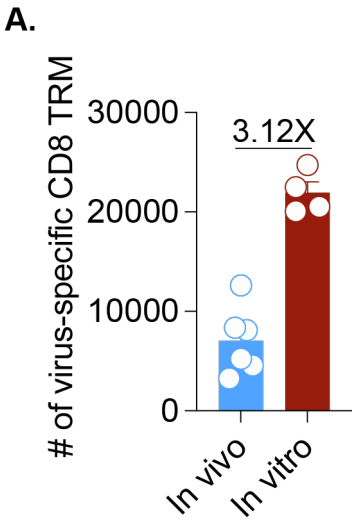

Figure S3. *Comparison of FRT TRM cell yield between in vivo mouse model of intravaginal LCMV infection (infected 50 days prior) and VEO-induced in vitro TRM isolated from a single well of a 96-well plate,* Related to Fig.6. Data are representative of two repeats with n=4-6/condition. Bars indicate mean ± SEM.

Supplementary Figure 4.

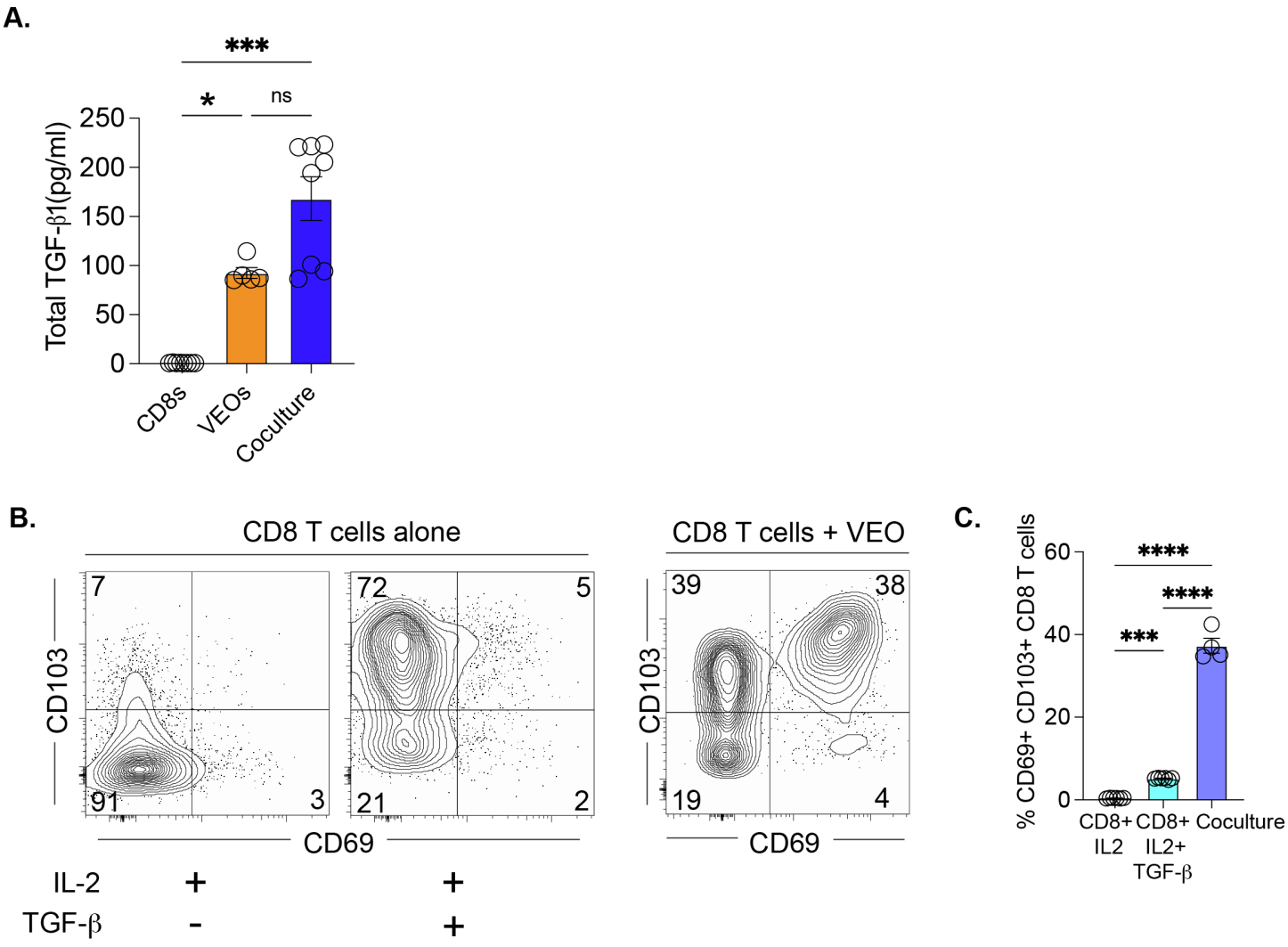

Figure S4. *VEOs are a source of TGF-β1, but TGF-β1 alone is not capable of inducing CD69+CD103+ CD8 TRM differentiation*, related to Fig.7. **A**. Level of total TGF-β1 in cell culture supernatants determined by legendplex assay from wells that contained CD8 T cells alone, VEOs alone or coculture of CD8 T cells and VEOs. **B**. Naïve P14 CD8 T cells were magnetically enriched from mouse secondary lymphoid organs and were stimulated by plate bound CD3ε/B7.1 Fc for three days in presence of IL-2 and IL-12. After three days, cells were washed and exposed to TGF-β1 (10 ng/ml) in presence of IL-2. Another group of cells were maintained in the absence of IL-2 and their phenotype was determined at 48 hr. Representative flow plots (**B**) and bar graphs (**C**) with CD69 and CD103 expression is shown. For comparison phenotype of CD8 T cells cocultured with VEOs for 10 days is also shown. Data is representative of two repeats with at least n=4/condition. Bars indicate mean ± SEM. Kruskal-Wallis ANOVA with Dunn's multiple comparison test (A). One-way ANOVA with Tukey's multiple comparison test (C). ***<0.001, ****<0.0001. *<0.05, ***<0.001.