

Supplementary Materials Figure S1

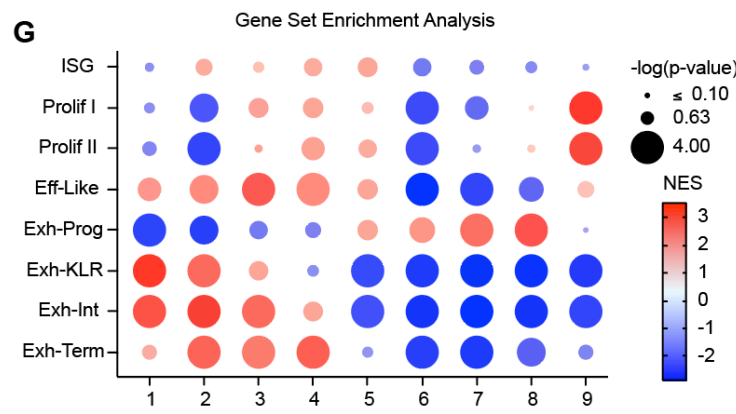
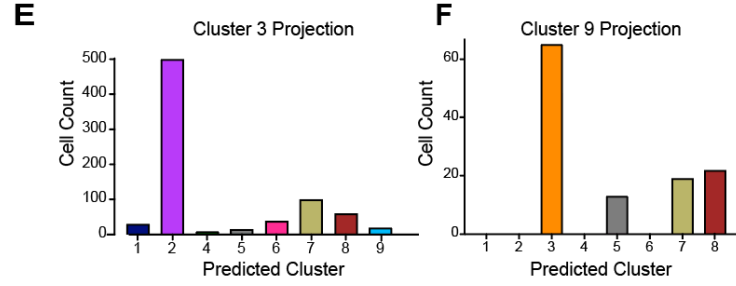
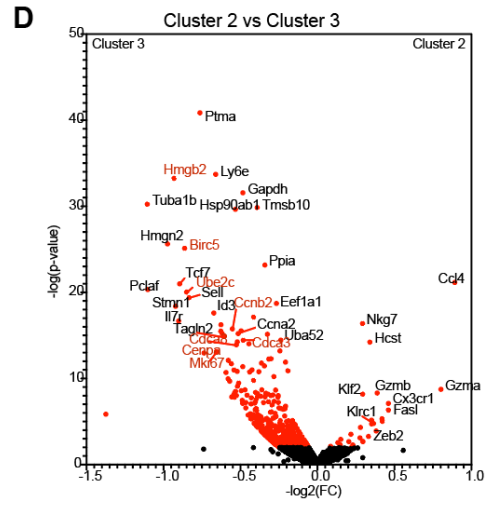
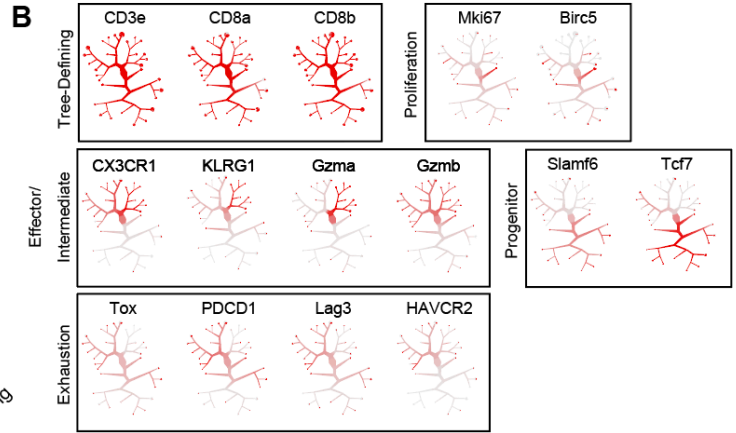
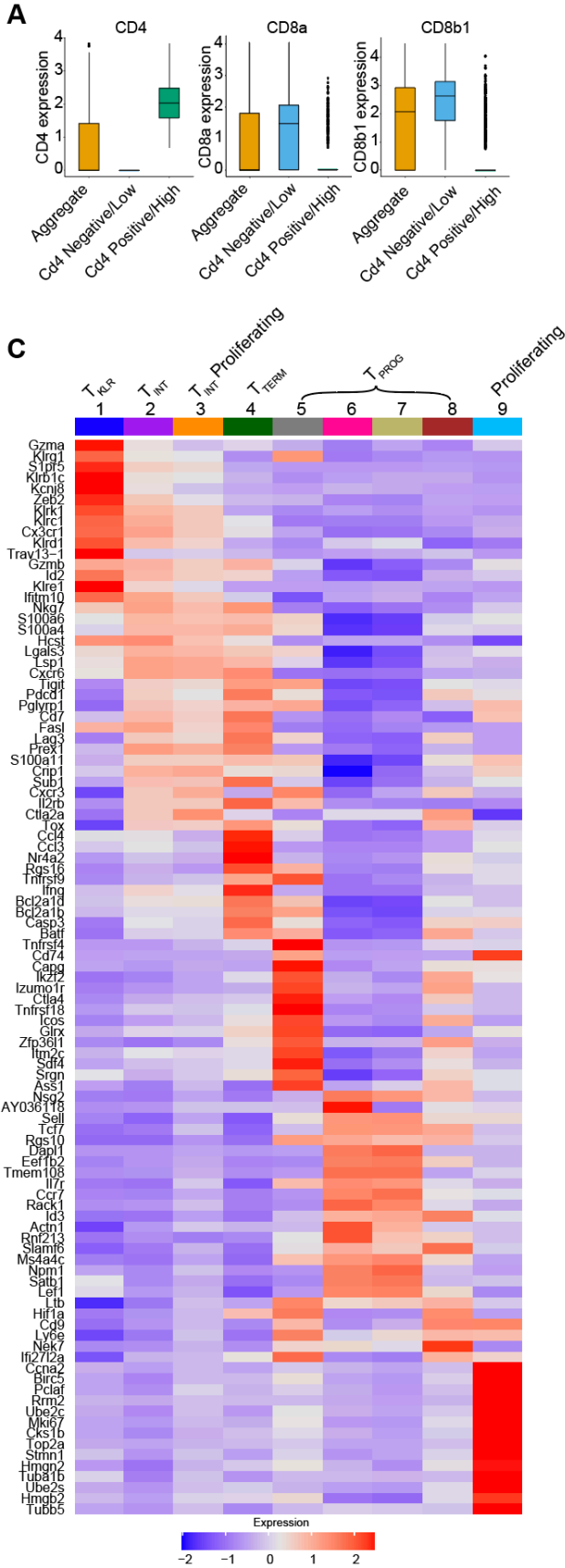


Figure S1: A complete pathway of CD8⁺ T_{EX} differentiation can be identified in both Trib1 WT and Trib1 KO mice. (A) Relative expression of *CD4*, *CD8a*, and *CD8b* mRNA in all aggregate cells sorted for scRNA-seq as referenced in Rome et al¹⁶ (yellow), in the *CD4*-low tree in Figure 1b (blue), or in the *CD4*-positive/high cells which were excluded from the tree in Figure 1b (green). (B) Normalized reference gene expression on the CD8⁺ tree, where red = higher expression and gray/white = lower expression. (C) Heatmap demonstrating top DEGs within each of 9 clusters, where red = higher expression within that cluster and blue = lower expression. (D) Volcano plot of differentially expressed genes between Cluster 2 and Cluster 3. Proliferation genes are highlighted in red font. (E) Predicted identity of cluster 3 cells when proliferating genes were regressed out of analysis using the SCTransform function in Seurat. (F) Predicted identify of cluster 9 cells when proliferating genes were regressed out of analysis using the SCTransform function in Seurat. (F) GSEA analysis comparing the 9 clusters identified in this paper (x-axis) to the 8 populations identified at day 15 of LCMV clone 13 infection by Giles et al⁴ (y axis). Trib1 WT: CD4-cre⁺ Trib1^{+/+}, Trib1 KO: CD4-cre⁺ Trib1^{F/F}

Figure S2

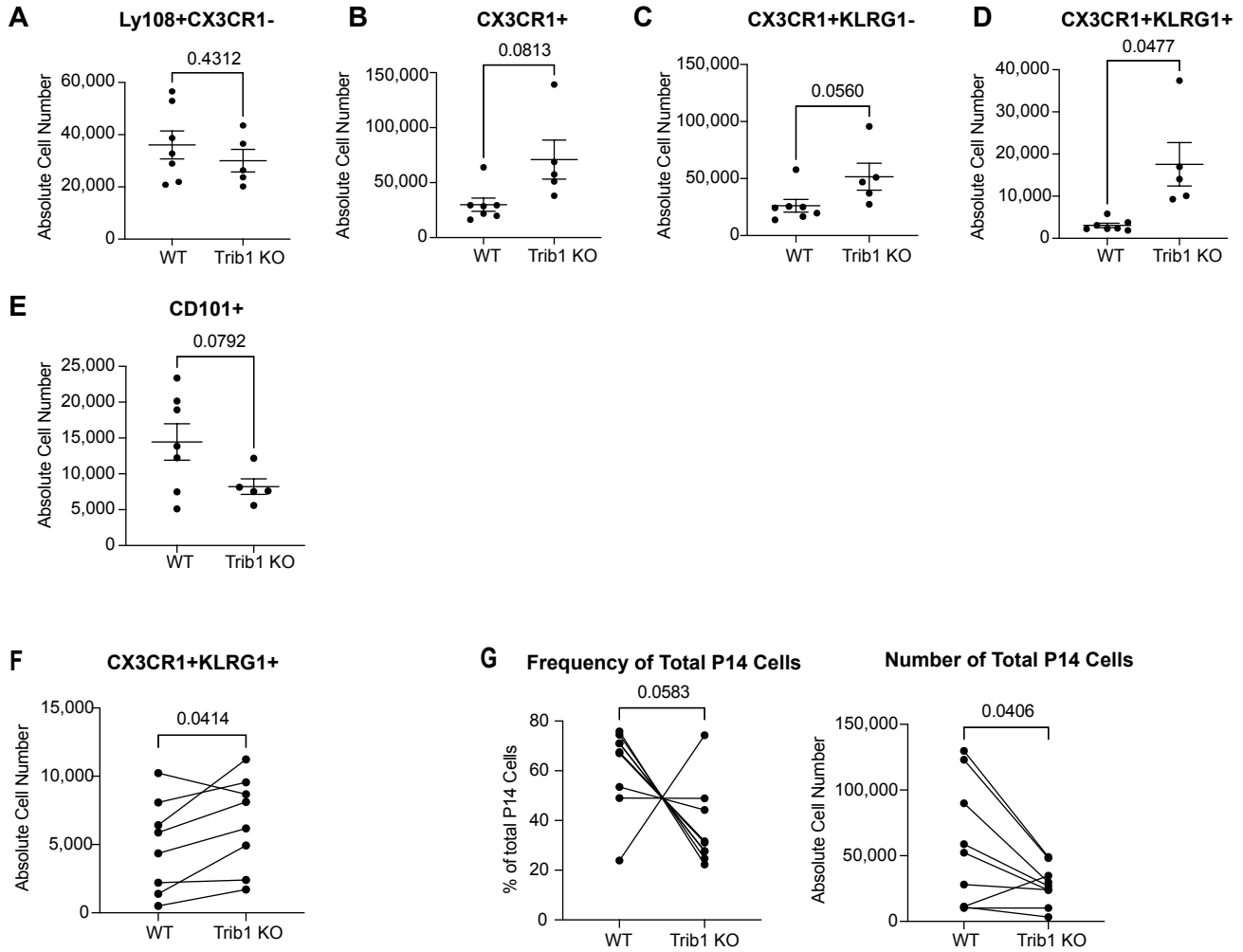


Figure S2: Trib1 restrains the absolute number of T_{KLR} that persist during chronic infection. (A-E)

Absolute numbers of the indicated population from the same experiment shown in Figure 2C-E. For this analysis, cells were gated on CD8⁺CD44⁺D^BGp33⁺ events as shown in Figures 2C-E. (F) Absolute number of CX3CR1⁺KLRG1⁺ P14 cells in the adoptive transfer experiments described in Figure 2G-H. Events are gated on CD8⁺D^BGp33⁺ cells and the appropriate congenic marker for WT (CD4.1/2) or KO (CD45.1) cells to identify genotype-specific P14 cells. (G) Frequency of total P14 cells by genotype as a percentage of total P14 cells or as absolute number. For A-G, absolute cell numbers were calculated by multiplying the frequency of each gated population as a percentage of live cells and by total live splenocytes for each mouse. For A-E, Error bars are mean ± SEM. P values calculated using either Student's t test or unpaired t test with Welch's correction based on variance between genotypes. For F-G, P values were calculated using a paired T test given that WT and KO cells were co-transferred into the same individual recipients. Trib1 WT: CD4-cre⁺ Trib1^{+/+}, Trib1 KO: CD4-cre⁺ Trib1^{F/F}