Immunity, Volume *48*

Supplemental Information

Single-Cell RNA Sequencing of Lymph Node Stromal Cells Reveals Niche-Associated Heterogeneity

Lauren B. Rodda, Erick Lu, Mariko L. Bennett, Caroline L. Sokol, Xiaoming Wang, Sanjiv A. Luther, Ben A. Barres, Andrew D. Luster, Chun Jimmie Ye, and Jason G. Cyster

Supplemental Figure 1

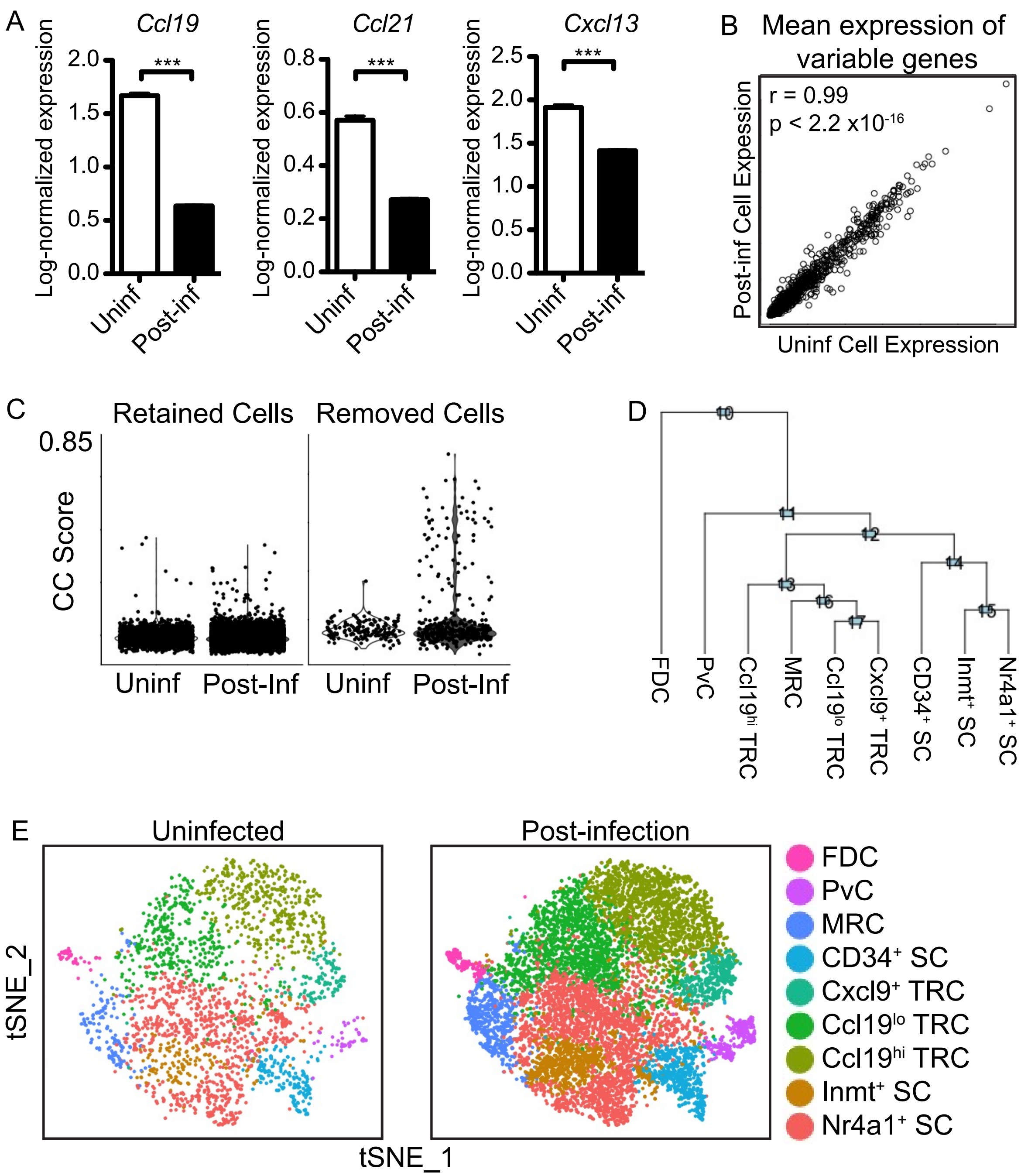

Figure S1. Clustering of pLN non-endothelial stroma is conserved between uninfected and post-infection samples. Related to Figure 1.

(A) Log-normalized expression of *Ccl19*, *Ccl21* and *Cxcl13* by non-endothelial stromal cells from uninfected (Uninf) and day 15 post-LCMV infection pLNs (Post-Inf). Data represents mean and error bars indicate SEM (*** $p < 0.0001$; Mann-Whitney U test). (B) Pearson correlation of mean expression of variable genes between samples. (C) Violin plot of the Cell Cycle Score (CC Score) of cells included in the CCA (Retained) and removed prior to CCA (Removed). Cells plotted by sample affiliation with highest score labeled. (D) Hierarchical clustering of the pLN non-endothelial stromal cells. (E) tSNE visualization of CCA clustering and alignment of uninfected and post-infected pLN non-endothelial stromal cells. Samples are plotted separately and cells are colored by cluster assignment.

Supplemental Figure 2

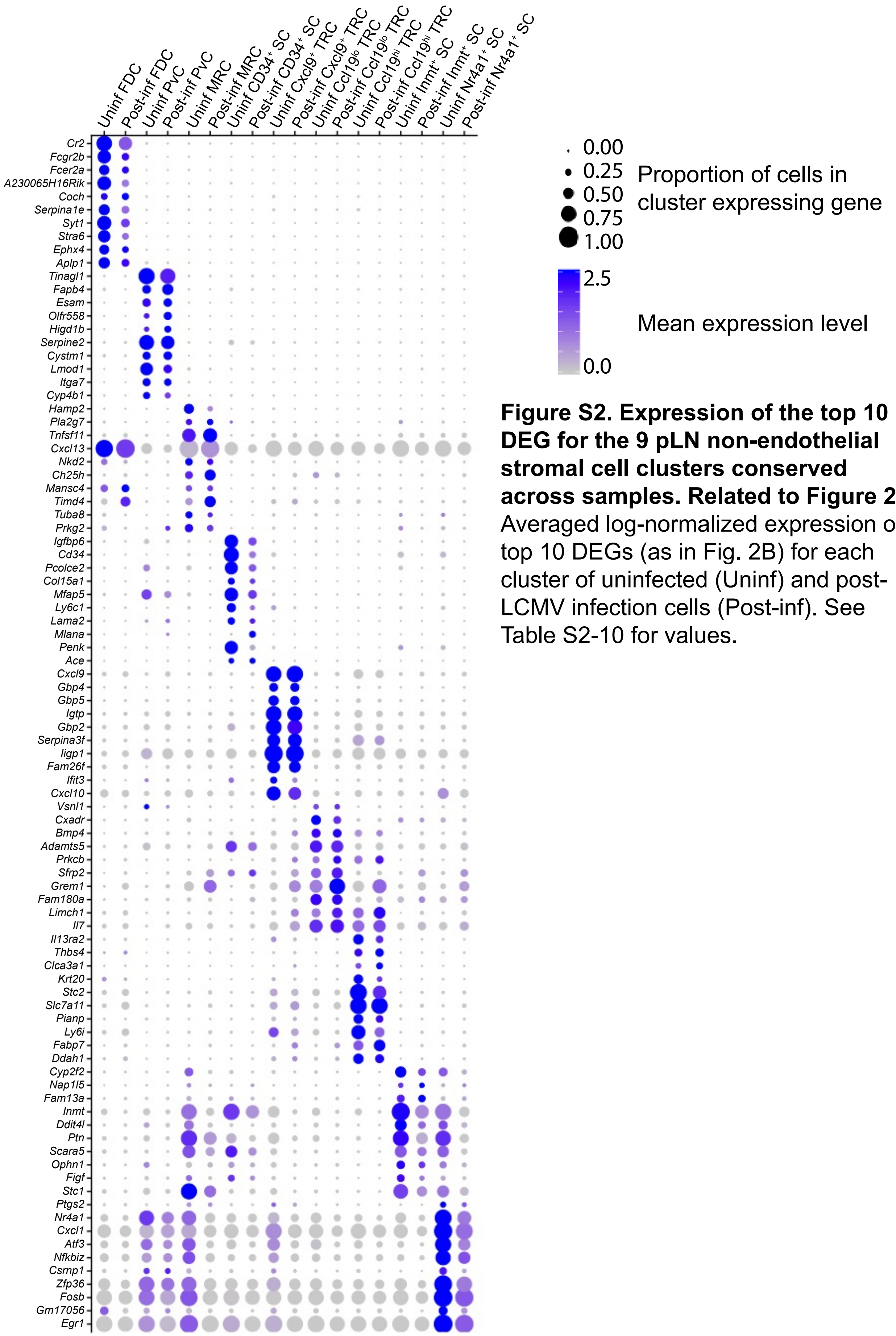

Figure S2. Expression of the top 10 DEG for the 9 pLN non-endothelial stromal cell clusters conserved across samples. Related to Figure 2. Averaged log-normalized expression of top 10 DEGs (as in Fig. 2B) for each cluster of uninfected (Uninf) and post-LCMV infection cells (Post-inf). See Table S2-10 for values.

Supplemental Figure 3

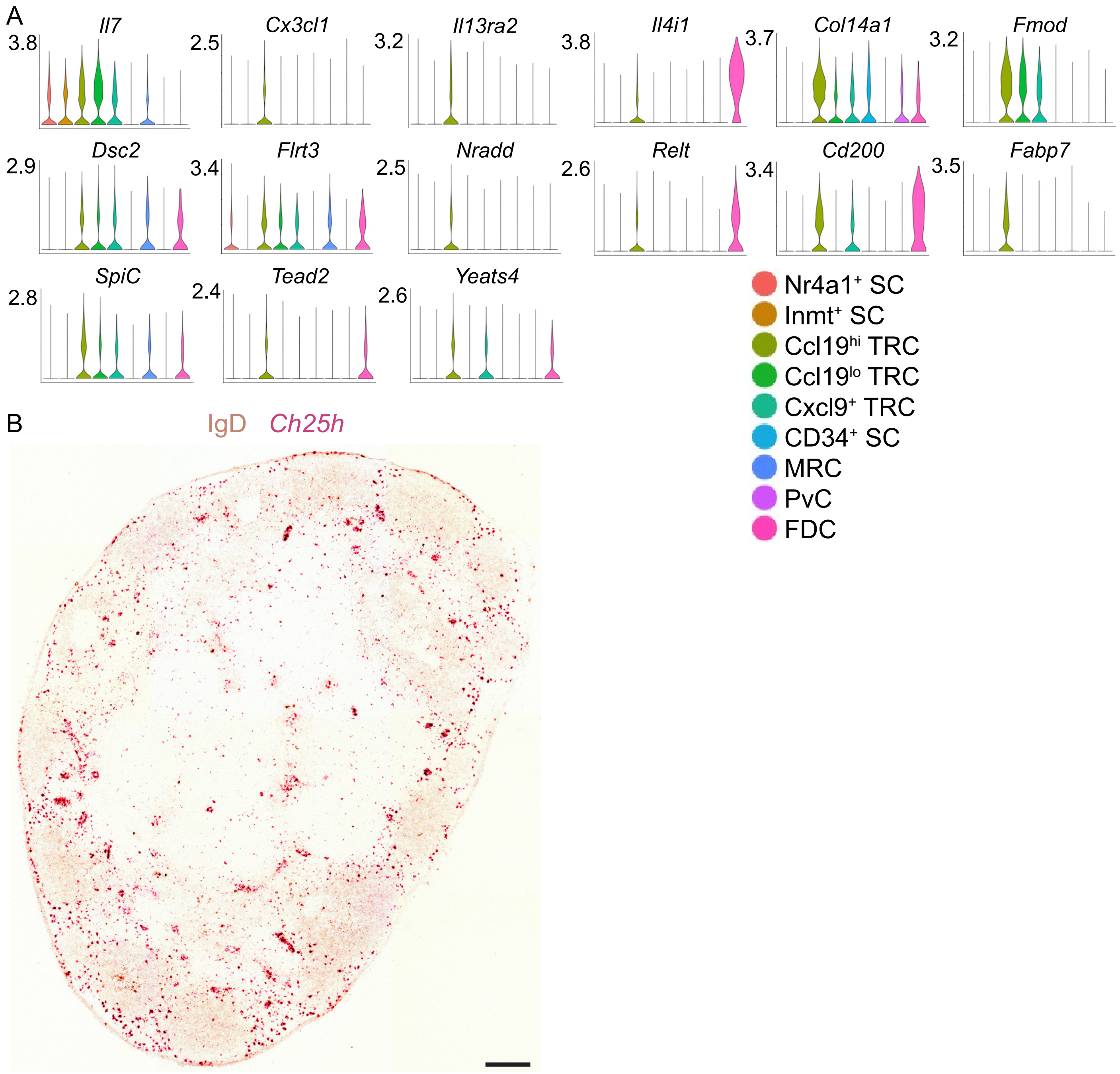

Figure S3. Ccl19^{hi} TRC cluster expression and *Ch25h* expression in mLN. Related to Figure 3.

(A) Violin plots of select TRC gene expression grouped by cluster colored in key. Highest log-normalized expression value is labeled on the y-axis for each gene. (B) RNAscope for *Ch25h* on mLN with IgD counterstain (representative of 3 mice). Scale bar is 200μm.

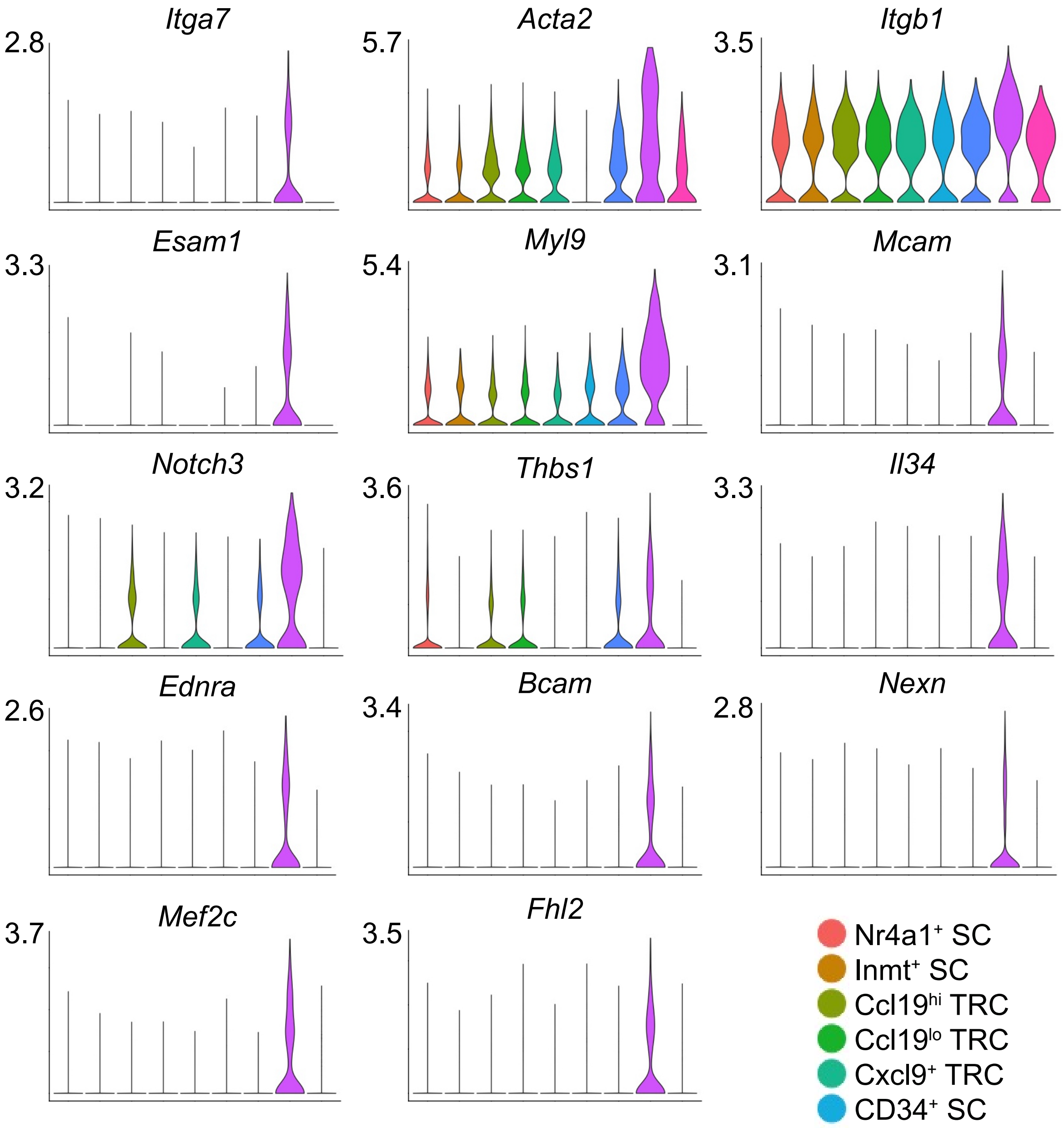

Figure S4. Select single-cell PvC gene expression. Violin plots of select PvC gene expression grouped by cluster colored in key. Highest log-normalized expression value is labeled on the y-axis for each gene.

Supplementary Figure 5

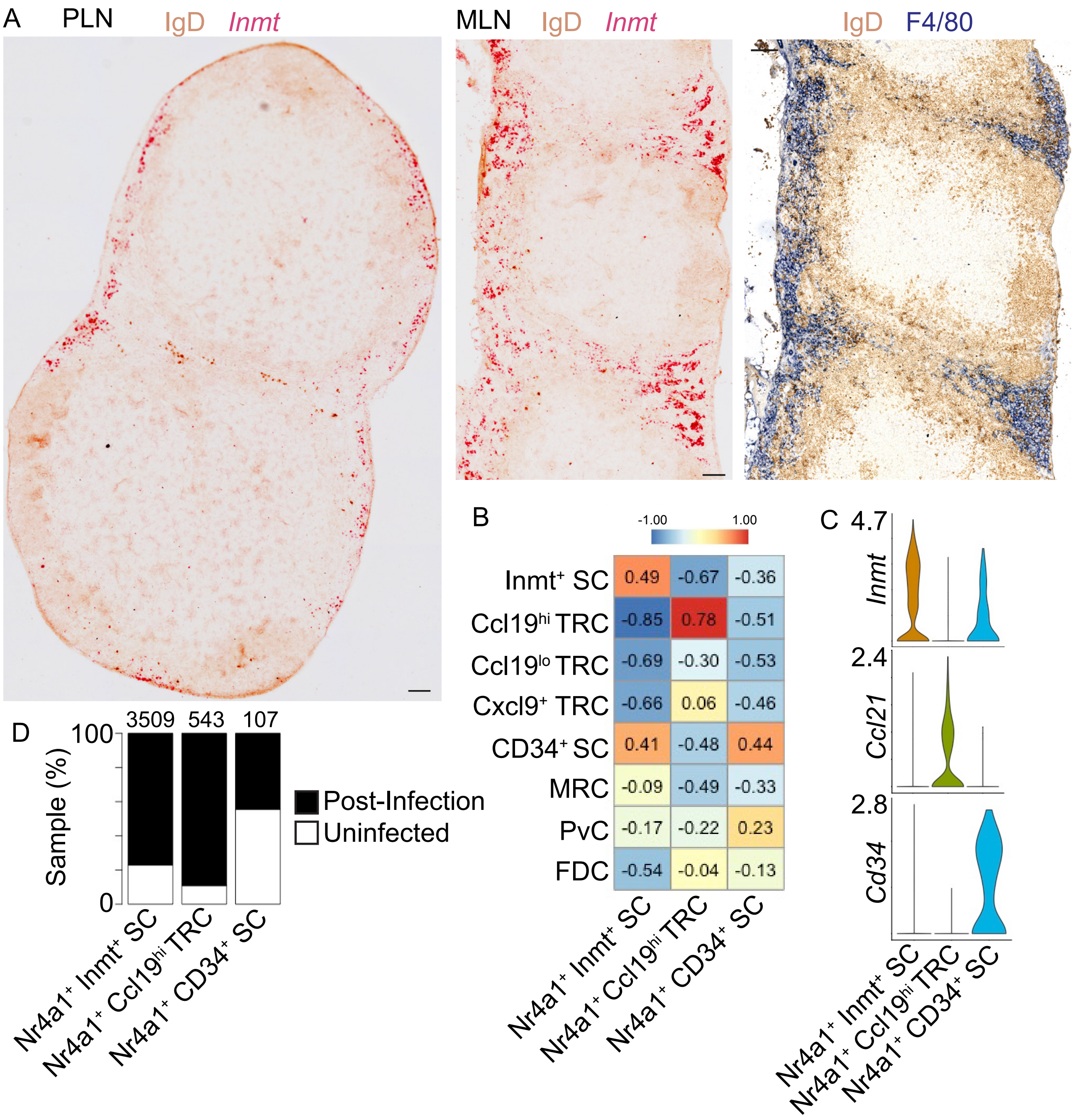

Figure S5. Inmt^{+} SCs populate the medullary cords and Nr4a1^{+} SCs may include activated Inmt^{+} SCs, Ccl19^{hi} TRCs and CD34^{+} SCs. Related to Figure 6.
(A) RNAscope for *Inmt* on pLN (left) and mLN (middle) with IgD counterstain. Sequential stain for F4/80 and IgD on mLN (right). Scale bar is 100µm. (B) Pearson correlation between Nr4a1^{+} SC subcluster differentially expressed genes (DEGs) and the DEGs of the other non-endothelial stromal cell clusters (Nr4a1^{+} Inmt^{+} SC vs. Inmt^{+} SC FDR = 8.9x10^{-31}; Nr4a1^{+} Inmt^{+} SC vs. CD34^{+} SC FDR = 1.1x10^{-20}; Nr4a1^{+} Ccl19^{hi} TRC vs. Ccl19^{hi} TRC FDR = 1.5x10^{-90}; Nr4a1^{+} CD34^{+} SC vs. CD34^{+} SC FDR = 7.7x10^{-17}). (C) Violin plots of Nr4a1^{+} SC subcluster expression of distinguishing genes. Highest log-normalized expression value labeled. (D) Propotional contribution of cells from the uninfected and post-infection samples to each Nr4a1^{+} SC subcluster. Total number of cells in each subcluster labeled on top.

Supplementary Figure 6

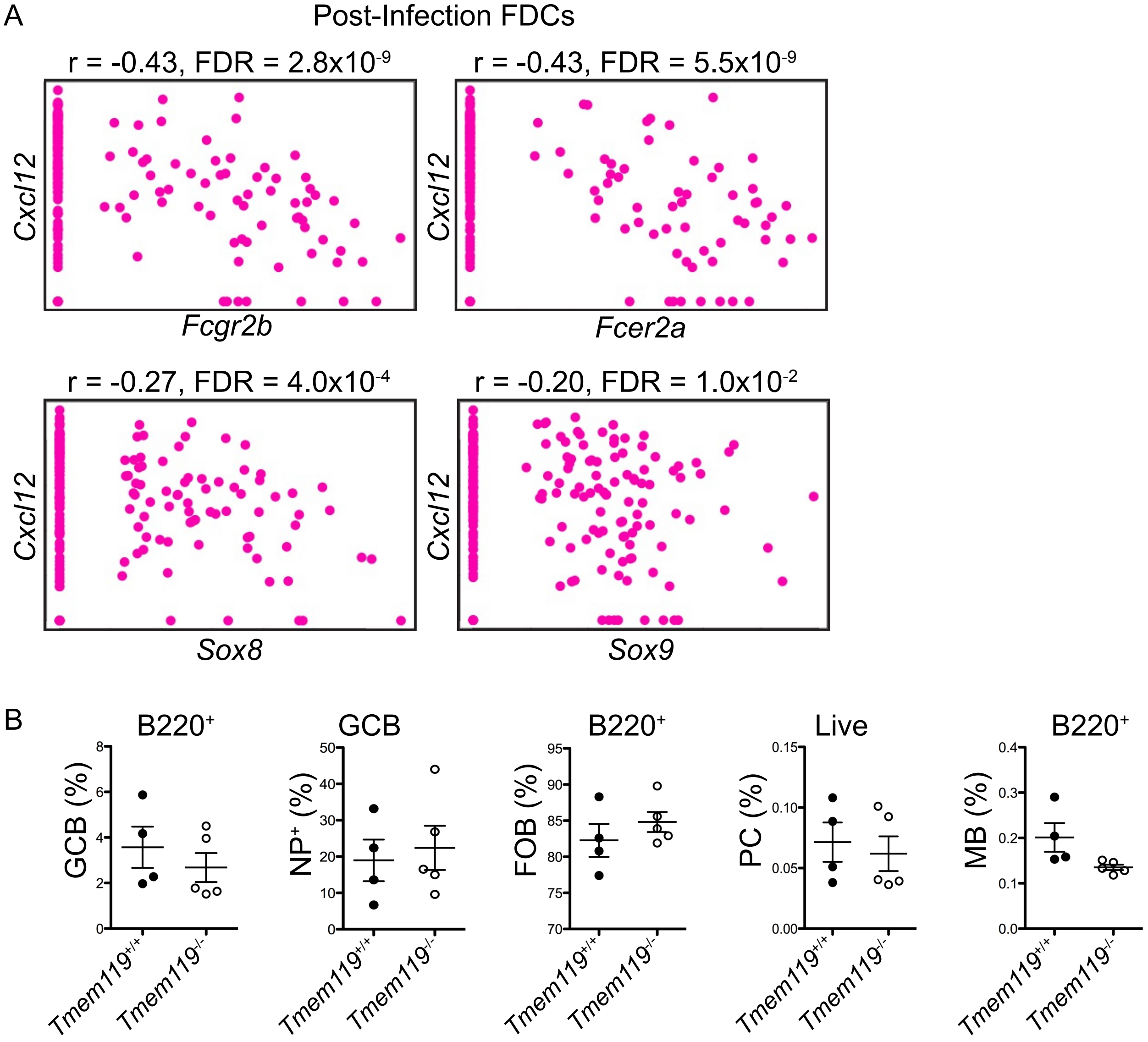

Figure S6. GC stroma heteogeneity and GCs do not depend on FDC *Tmem119*. Related to Figure 7.

(A) Pearson correlation of day 15 post-LCMV infection FDC cluster expression of *Fcgr2b*, *Fcer2a*, *Sox8* and *Sox9* vs. *Cxcl12*. (B) Flow cytometric quantification of the percent of IgD$^-$ CD95$^+$ GL7$^+$ GC B cells (GCB), IgD$^+$ CD95$^-$ follicular B cells (FOB) and IgD$^-$ CD38$^+$ CD73$^+$ CD95$^+$ memory-phenotype B cells (MB) of B220$^+$ B cells and IgD$^-$ CD95$^+$ GL7$^+$ NP$^+$ cells of GC B cells (NP$^+$) and CD138$^+$ B220^{int} plasma cells (PC) of live cells from *Tmem119*$^{+/+}$ (n = 4, black circle) and *Tmem119*$^{-/-}$ (n= 5, white circle) mouse pLNs on day 11 post-immunization with NP-CGG in Sigma Adjuvant. Data represents mean and error bar indicates SEM.