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Supplemental information

CXCR3 regulates stem and proliferative CD8+ T cells during chronic infection by promoting interactions with DCs in splenic bridging channels

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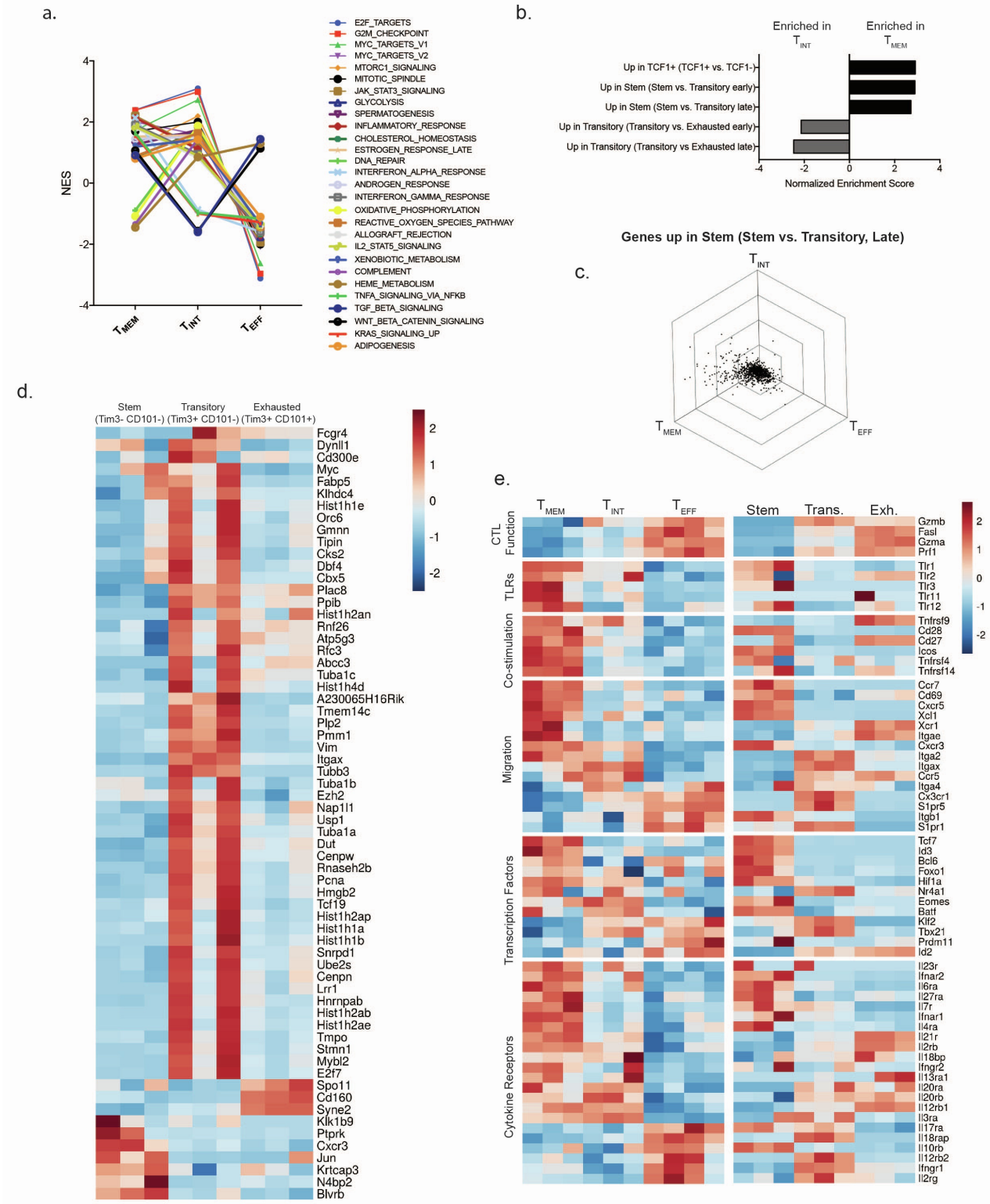
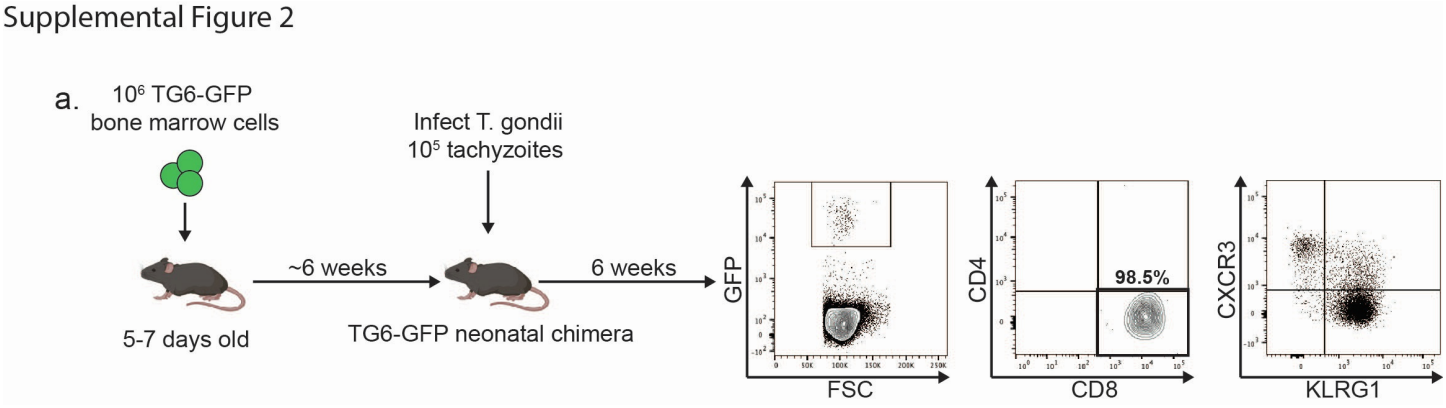
Supplemental Figure 1



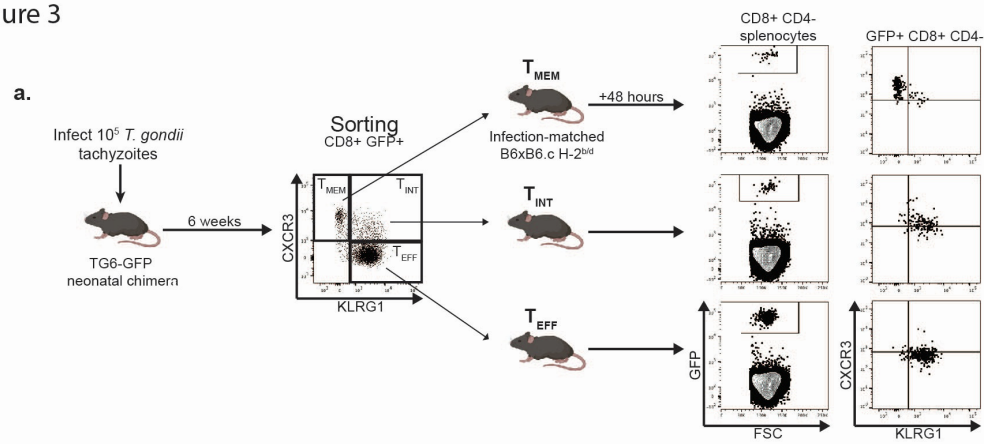
Supplemental Figure 1: Related to Figure 1: RNA-seq analyses of parasite-specific T cell subsets. A) Results of GSEA with Hallmark gene sets using one-versus-all comparisons. Normalized enrichment scores (NES) for each T cell population are displayed for the 28 Hallmark gene sets that exhibited significant enrichment in at least one population (padj < 0.05). B) GSEA was performed on the T_{MEM} versus T_{INT} comparison using signatures generated from published RNA-seq datasets (Utzschneider *et al.*, 2016; Hudson *et al.*, 2019). A positive enrichment score indicates an enrichment of that signature in the T_{MEM} population, a negative score indicates an enrichment in the T_{INT} population. C) Triwise plot visualizing the enrichment for genes between T_{MEM}, T_{INT}, and T_{EFF}. The genes displayed belong to the Stem signature (Hudson *et al.*, 2019). D, E) Heatmaps displaying normalized expression values from published RNA-seq data from Stem, Transitory, and Exhausted cells (Hudson *et al.*, 2019). D) shows expression of genes specifically upregulated by T_{INT} cells compared to both T_{MEM} and T_{EFF}. E) shows the expression of selected genes for T_{MEM}, T_{INT}, T_{EFF} (current study) compared to Stem, Transitory, and Exhausted cells.

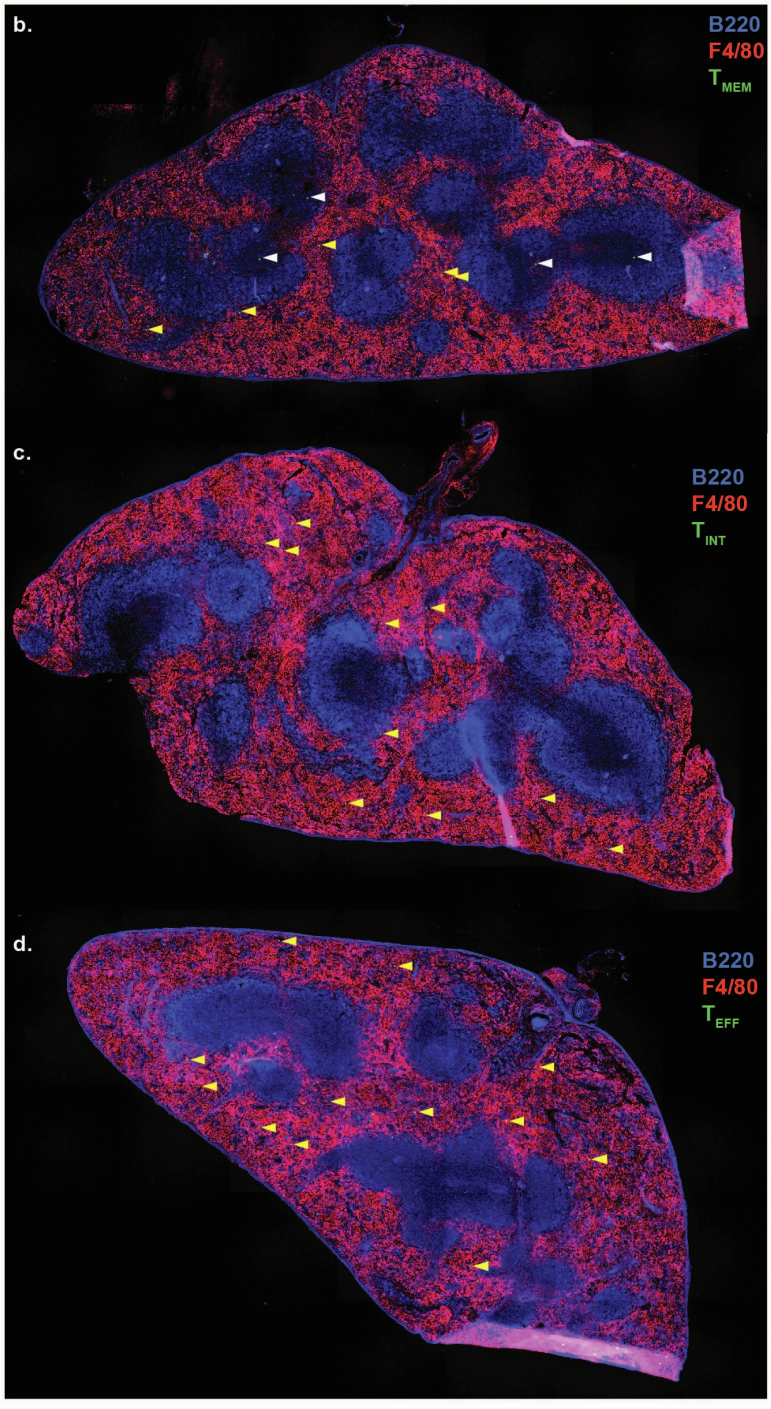
Supplemental Figure 2



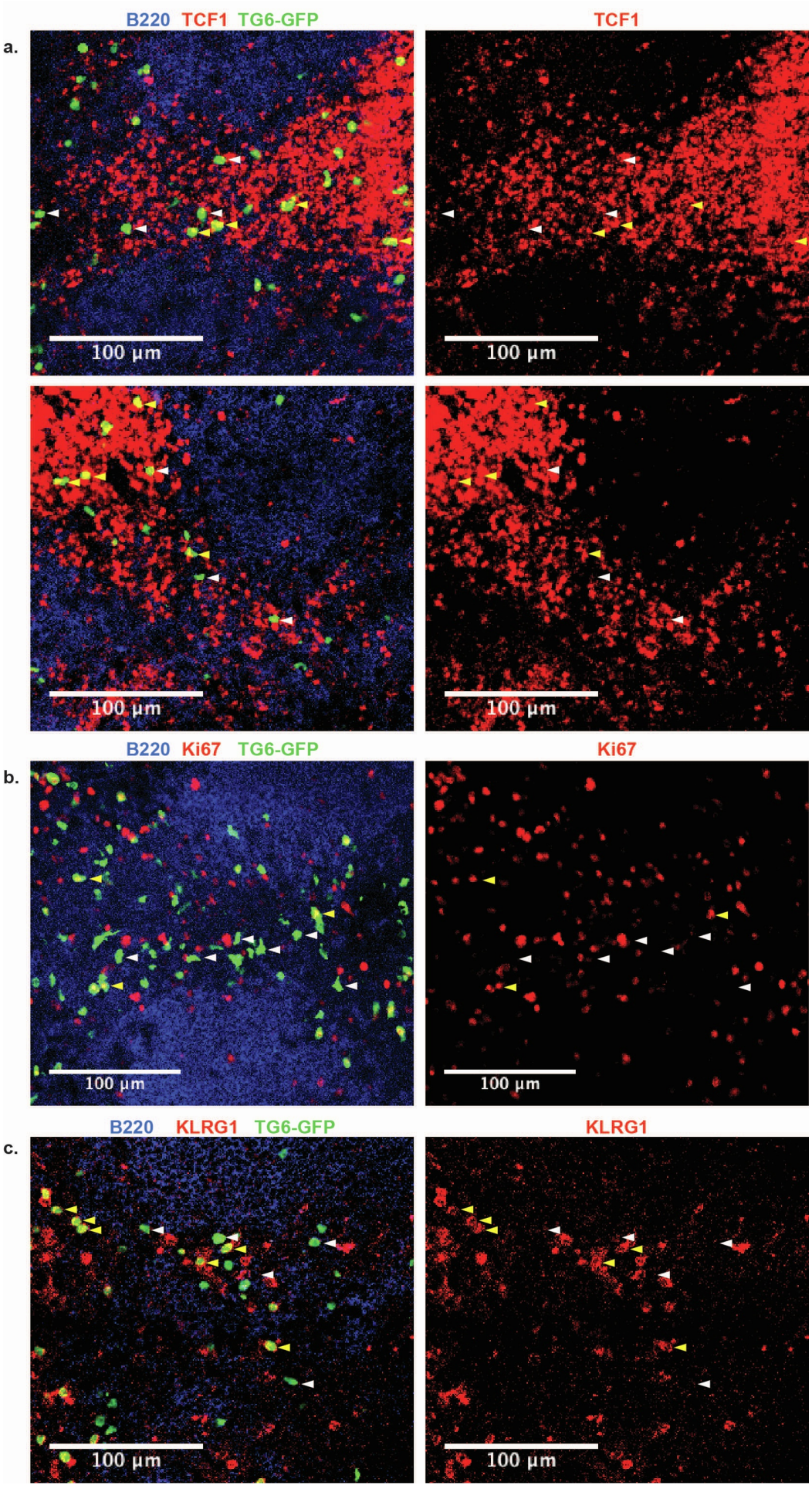
Supplemental Figure 2: Related to Figure 2: TG6-GFP neonatal bone marrow chimeras,. A) Generation and validation of TG6-GFP chimeric mice. TG6-GFP bone marrow was injected into neonatal WT B6xB6.c F1 mice. Adult chimeric mice were infected with *T. gondii*. GFP+ cells expanded during infection and were made up of >90% CD8+ T cells. CD8+ GFP+ cells developed normal T_{MEM}, T_{INT}, and T_{EFF} populations after CXCR3 and KLRG1 staining.

Supplemental Figure 3



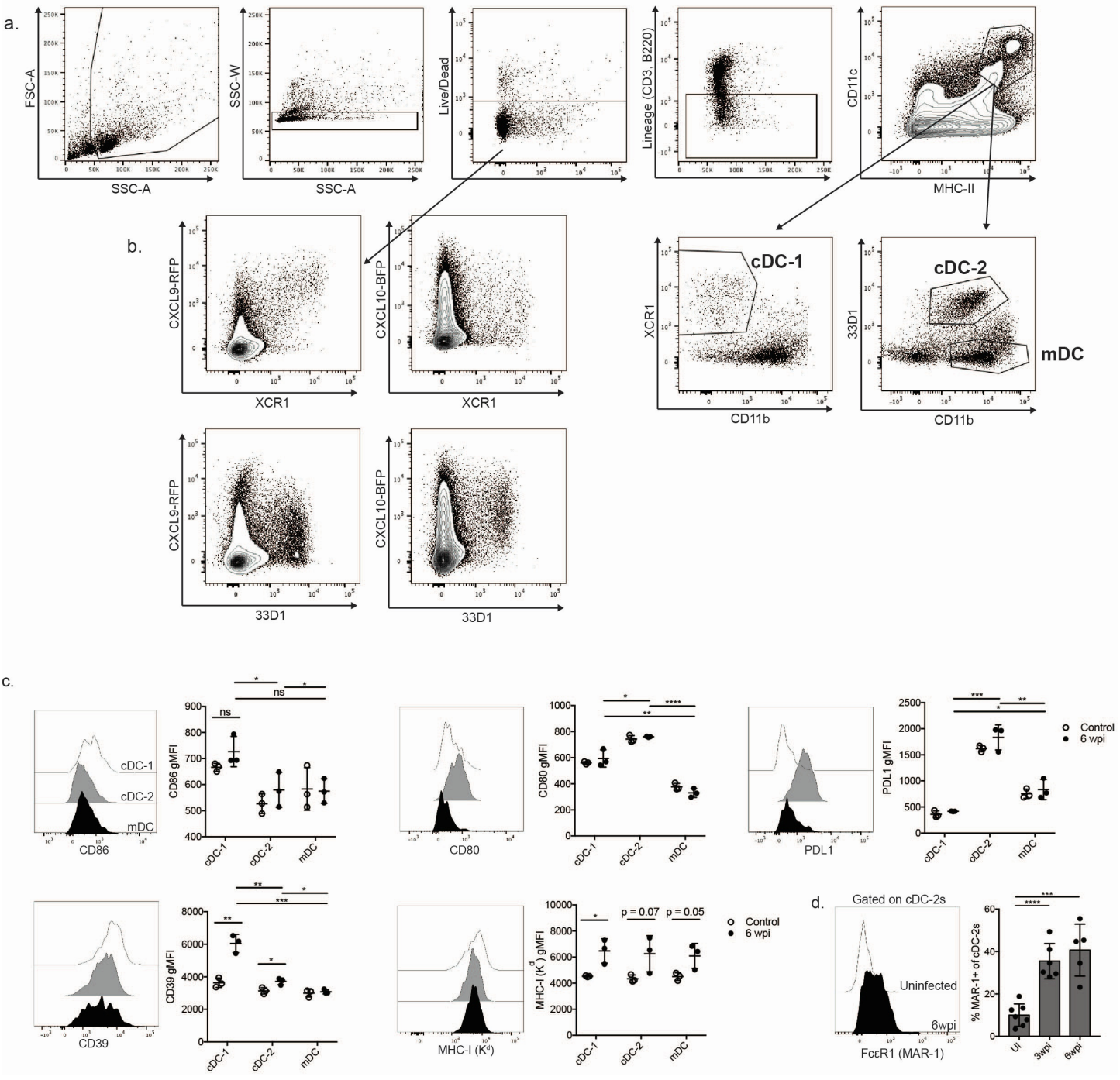


Supplemental Figure 3: Related to Figure 2: TG6-GFP Adoptive transfer results. A) TG6-GFP neonatal chimeric mice were infected with *T. gondii* for six weeks, then GFP+ T_{MEM}, T_{INT}, and T_{EFF} cells were sorted and adoptively transferred into separate infection-matched WT recipients . Two days later spleens were analyzed by flow cytometry and confocal microscopy to determine the identity and location of GFP+ cells. B-D) Representative images from mice receiving T_{MEM} cells (B), T_{INT} cells (C), or T_{EFF} cells (D) are shown. B220 staining (blue) was used to highlight B cell follicles and F4/80 staining (red) was used to highlight the red pulp. Arrows highlight representative samples of scored cells, with white arrows indicating a white pulp-localized cell and yellow arrows indicating a red pulp-localized cell. Images are representative of data taken from three independent experiments (T_{MEM} n=3, T_{INT} n=3, T_{EFF} n=4).


a.
B220 TCF1 TG6-GFP
TCF1
100 µm
100 µm
100 µm
100 µm
b.
B220 Ki67 TG6-GFP
Ki67
100 µm
100 µm
c.
B220 KLRG1 TG6-GFP
KLRG1
100 µm
100 µm

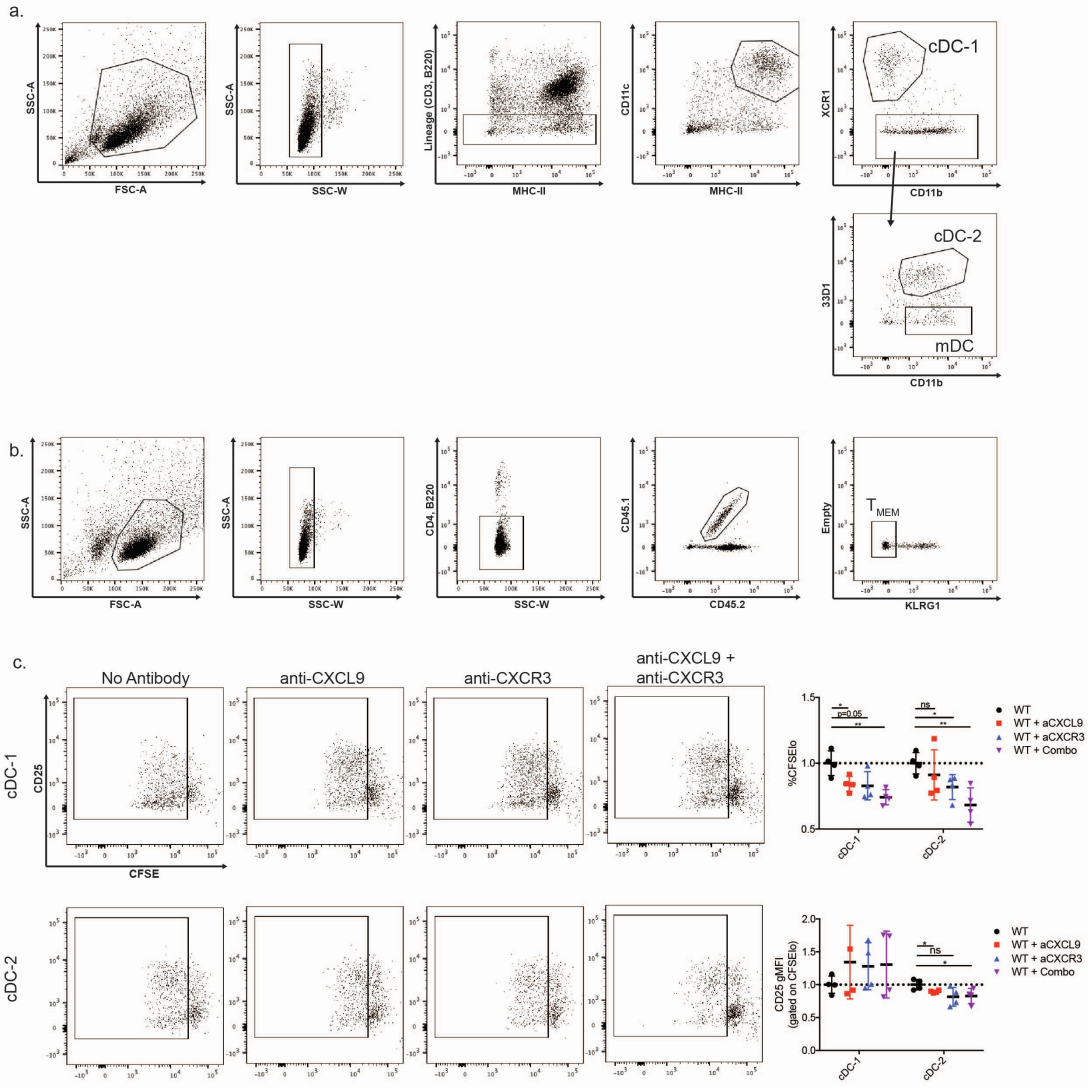
Supplemental Figure 4: Related to Figure 4: Phenotypic analysis of cells within bridging channels.. Spleens of TG6-GFP chimeric mice were imaged, and cells migrating along bridging channels were identified. TCF1 (A), Ki67 (B), and KLRG1 (C) expression was measured. Images are representative of data from four mice across two independent experiments.

Supplemental Figure 5



Supplemental Figure 5: Related to Figure 5: Identification of dendritic cell subsets. A) Gating strategy used to identify cDC-1s, cDC-2s, and mDCs. B) Validation that XCR1 and 33D1 are effective markers of chemokine-producing cells. C) Flow cytometry data evaluating the expression of the indicated activating or inhibitory molecules on cDC-1s, cDC-2s, and mDCs before infection and during chronic infection. Representative histograms are taken from mice six weeks post-infection. Each data point represents an individual mouse (n=3), and data are representative of two independent experiments. D) Representative histograms and summary data of FcεR1 (MAR-1) staining on gated cDC-2s. Each data point represents one mouse and data are combined from three independent experiments.

Supplemental Figure 6



Supplemental Figure 6: Related to Figure 7: Co-cultures of T_{MEM} cells and dendritic cell subsets. A) Gating strategy used to sort cDC-1, cDC-2, and mDC populations for co-culture. Example data shows splenocytes after density column enrichment for dendritic cells. B) Gating strategy used for sorting T_{MEM} cells (CD4- B220- CD45.1+/- CD45.2+/- KLRG1-) after CD8+ T cell negative isolation by magnetic column. C) WT T_{MEM} cells were cultured with either cDC-1s or cDC-2s for 3 days in the presence of the indicated antibodies. T_{MEM} proliferation and activation was measured by CFSE dilution and CD25 expression. Data points are combined from two independent experiments, each with two technical replicates. Data are normalized to the No Antibody condition within each experiment.