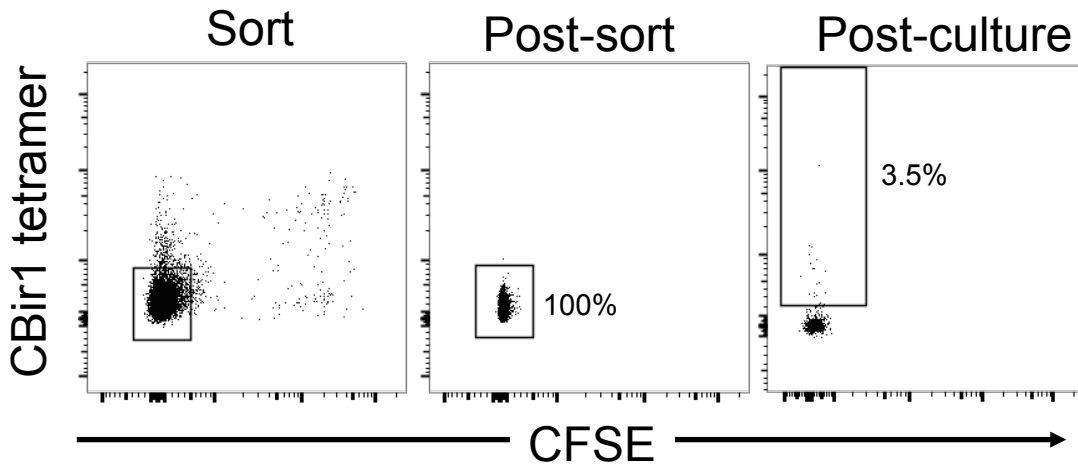


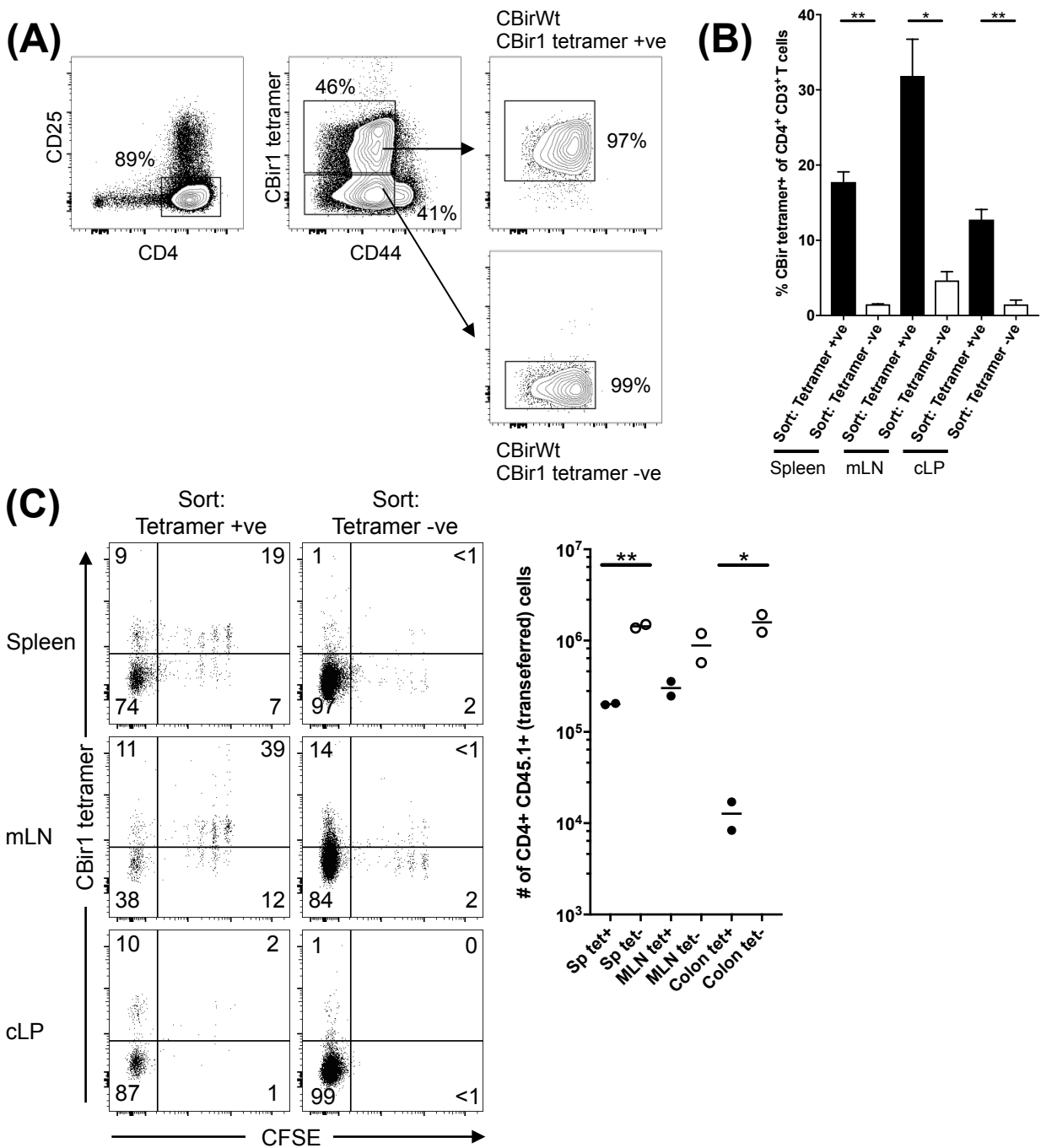
Supplemental Figure 1 CBirRag and CBirWt mice carry both CD4⁺ and CD8⁺ T cells and CBirWT mice possess T cells that express non-transgenic TCR α chains. Peripheral blood was isolated from CBirWt, CBirRag or C57BL/6 mice and stained for flow cytometry to measure **(A)** CD4 and CD8 β expression on TCR β ⁺CD45⁺ T cells or **(B)** TCR V α 2 and TCR V β 8 expression on CD45⁺CD4⁺ cells.

Supplementary Figure 1



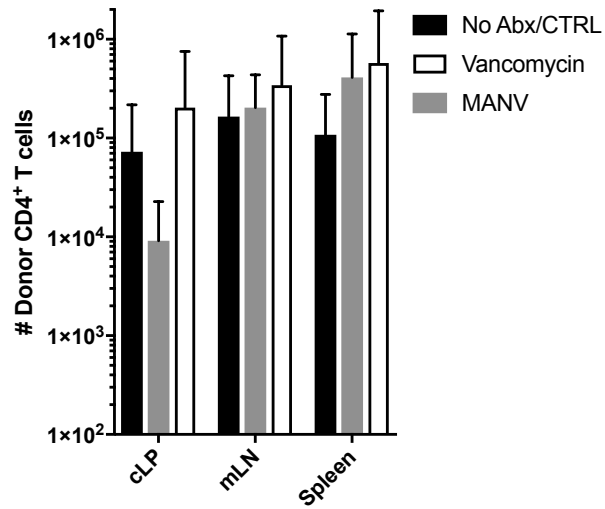
Supplemental Figure 2 Rapidly proliferating CBirWt T cells do not re-express the CBir1 transgenic TCR upon overnight culture with IL-7. 1.5×10^6 FACS-sorted CFSE-labeled naïve $CD4^+$ T cells from spleens and LNs of CBirWt mice were transferred to Rag1^{-/-} recipients. Two weeks later, lymphocytes isolated from pooled recipient spleens and LNs were stained with MHCII CBir1 tetramer and FACS-sorted for CFSE⁺CBir1-CD45.1⁺CD4⁺ T cells. Sorted cells were cultured overnight with 10ng/mL IL-7, stained with tetramer, and quantified for CBir1-specific TCR expression.

Supplementary Figure 2



Supplemental Figure 3 Lymphopenic conditions select for the outgrowth of cells expressing alternative TCR specificities that exclude the transgenic V α chain. (A) CBir1 tetramer positive and negative CD4⁺CD44^{lo}CD25⁻ T cells from CBirWt mice were FACS-sorted, CFSE-labeled and transferred to sex-matched Rag1^{-/-} mice. 13 days later, lymphocytes were isolated from spleens, mLNs and colonic LP of recipient mice and stained with CBir1 tetramer to determine **(B)** the percentage of tetramer positive transferred cells and **(C)** proliferation and accumulation of tetramer positive versus tetramer negative cells in each tissue. Flow cytometry plots were gated on Live CD45.1⁺CD90.2⁺CD4⁺CD8⁻ cells; numbers represent percent of cells in each gate. Bar graph shows mean \pm SD.

Supplemental Figure 3



Supplemental Figure 4 Accumulation of CBir1 T cells in the colon depends on an intact microbiota. Lymphocytes of recipient mice from **Fig. 6A** were quantified for total number of donor CD4⁺ T cells. Graph shows mean \pm SD.

Supplementary Figure 4