SUPPLEMENTAL MATERIAL

Fahey et al., http://www.jem.org/cgi/content/full/jem.20101773/DC1

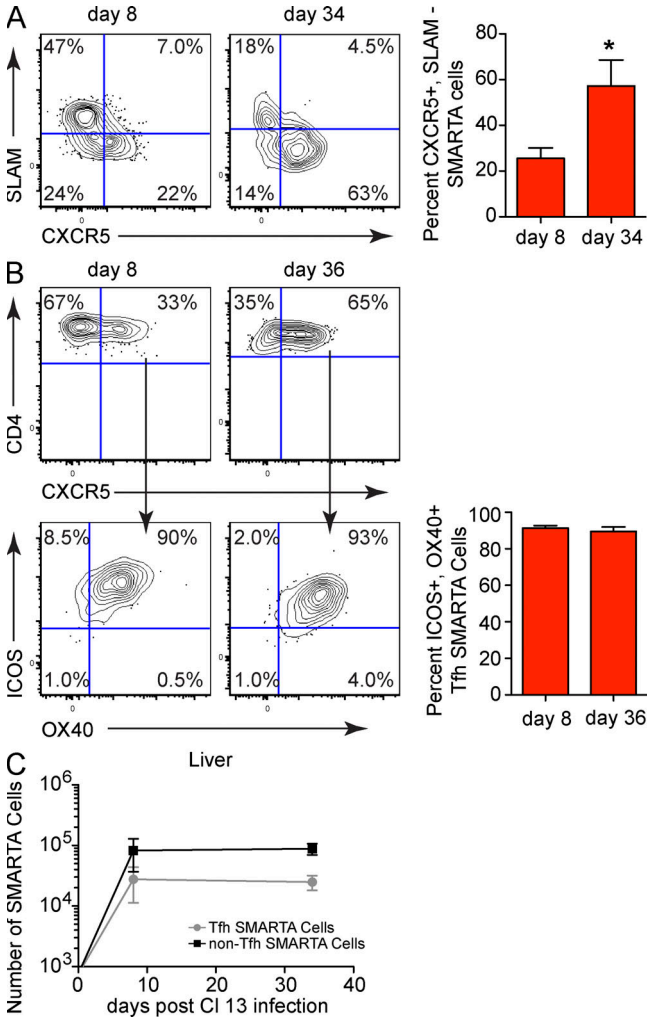

Figure S1. Virus-specific CD4 T cell development in the spleen and liver during persistent viral infection. (A) Flow plots illustrate the frequency of CXCR5 and SLAM expressed by SMARTA cells isolated on day 8 (left) and 34 (right) after LCMV-Cl 13 infection. The bar graph illustrates the percentage ± SD of $CXCR5^+$, $SLAM^-$ cells during LCMV-Cl 13 infection on the indicated days. These data are representative of 3–5 mice per group and of three individual experiments (*, $P < 0.05$). (B) Expression of CXCR5 on virus-specific CD4 T cells (top) and the coexpression of OX40 and ICOS on $CXCR5^+$ virus-specific CD4 T from the top panel (bottom) on day 8 (left) and day 36 (right) after LCMV-Cl 13 infection. The bar graph illustrates the percentage ± SD of $CXCR5^+$ cells that coexpress $ICOS^+$, $OX40^+$ during LCMV-Cl 13 infection. These data are representative of three to five mice per group and of four individual experiments. (C) SMARTA cells were isolated from the liver on day 8 and 34 after LCMV-Cl 13 infection. The line graph indicates the number of Tfh SMARTA cells ($CXCR5^+$; gray) and non-Tfh SMARTA cells ($CXCR5^-$; black) during persistent infection on the indicated days. No significant change in the number of non-Tfh or Tfh was observed in the liver between days 9 and 30. Data are representative of three to five mice per group and two individual experiments (*, $P < 0.05$).

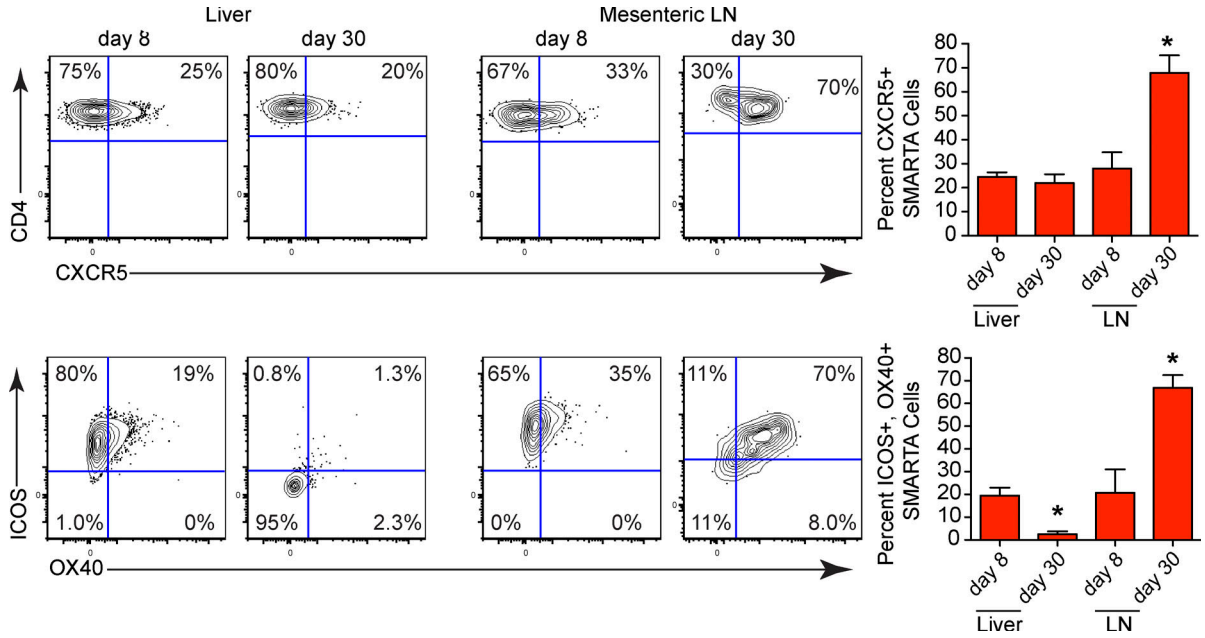

Figure S2. Tfh differentiation in the liver and lymph nodes during persistent viral infection. SMARTA cells from LCMV-Cl 13–infected mice were isolated from the liver (left plots) and mesenteric LN (right plots) on day 8 and 30 after infection. Flow plots represent the expression of CXCR5, ICOS, and OX40 on SMARTA cells. Bar graphs depict the percentage ± SD of CXCR5$^+$ or ICOS$^+$, OX40$^+$ SMARTA cells. Data are representative of three to five mice per group and two individual experiments (*, $P < 0.05$).

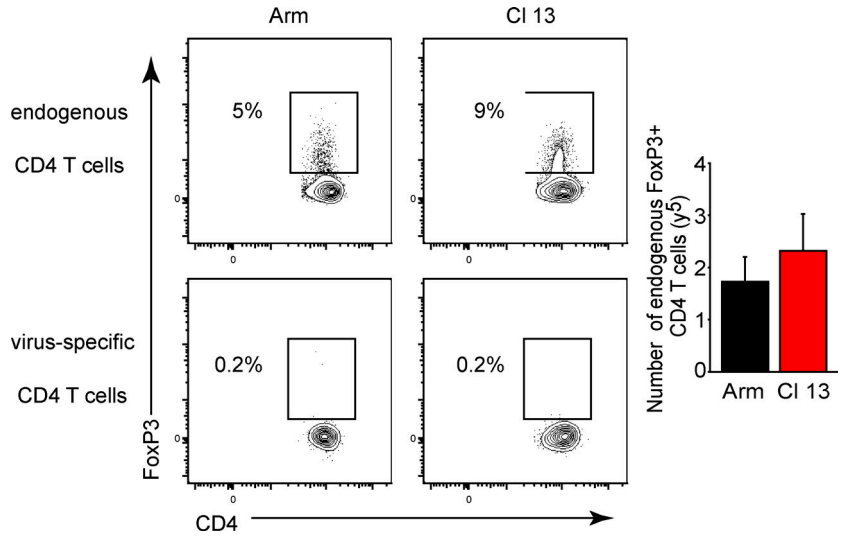

Figure S3. FoxP3 is not expressed by virus-specific CD4 T cells during LCMV infection. Flow plots illustrate the expression of FoxP3 in either endogenous CD4 T cells (i.e., host derived; top) or SMARTA cells (bottom) on day 30 after LCMV-Arm or Cl 13 infection. The bar graph indicates the number ± SD of endogenous FoxP3$^+$ CD4 T cells on day 30 after LCMV-Arm (black) or Cl 13 (red) infection. Data are representative of four to five mice per group and two separate experiments.

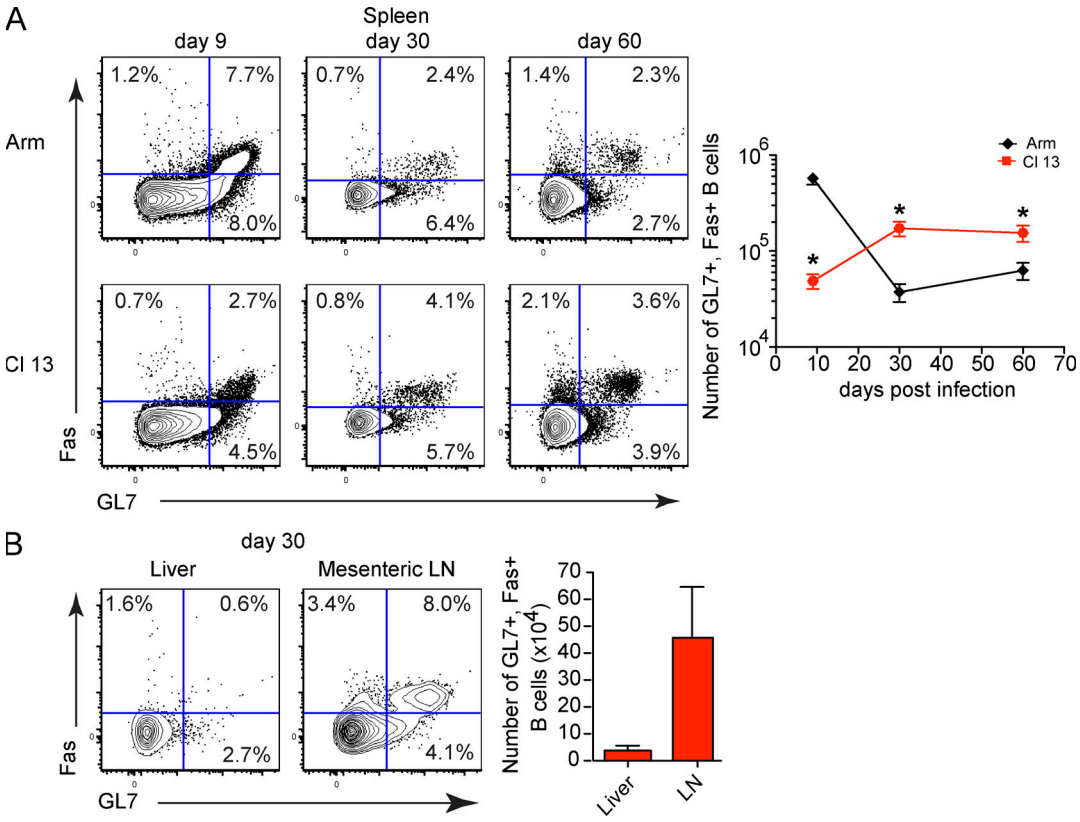

Figure S4. GC B cell development in the lymphoid and nonlymphoid organs during LCMV infection. (A) Flow plots illustrate the frequency of GC B cells ($GL7^+$, Fas^+ B cells) on day 9, 30, and 60 after LCMV-Arm (top) and Cl 13 (bottom) infection. The line graph indicates the number ± SD of GC B cells during LCMV-Arm (black) and Cl 13 (red) infection on the indicated days. Data are representative of four to five mice per group and three individual experiments (*, $P < 0.05$). (B) Flow plots illustrate the frequency of GC B cells ($GL7^+$, Fas^+ B cells) isolated from the liver and mesenteric LNs on day 30 after LCMV-Cl 13 infection. The bar graph indicates the number ± SD of GC B cells. Data are representative of six mice per group and two individual experiments

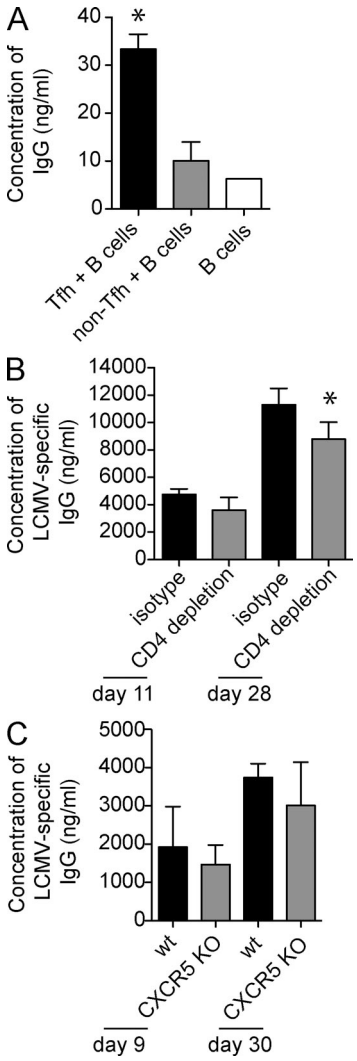

Figure S5. Virus-specific Tfh cells retain functionality during persistent infection. (A) Virus-specific Tfh (CXCR5+) and non-Tfh (CXCR5-) cells were sorted on day 28 after LCMV-Cl 13 infection and co-cultured with naive B cells for 7 d. As a control, B cells were cultured alone. The bar graph indicates the concentration of IgG (ng/ml) ± SD produced after 7 d of co-culture. Data are representative of 3–4 mice per group and two individual experiments (*, $P < 0.05$). (B) Antiviral B cell responses in WT mice during acute LCMV-Arm infection after day 12 isotype antibody or CD4 T cell depletion. The bar graph depicts the serum concentration (ng/ml) ± SD of LCMV-specific IgG produced by day 12 isotype antibody treated (black) or day 12 CD4-depleted mice (gray) on the indicated day after infection. Data are representative of five to six mice per group and two separate experiments (*, $P < 0.05$). (C) Antiviral B cell responses during LCMV-Arm infection in WT (black) versus CXCR5 KO mice (gray). The bar graph depicts the serum concentration (ng/ml) ± SD of LCMV-specific IgG produced by WT mice and CXCR5 KO mice on the indicated day after infection. Data are representative of five mice per group.

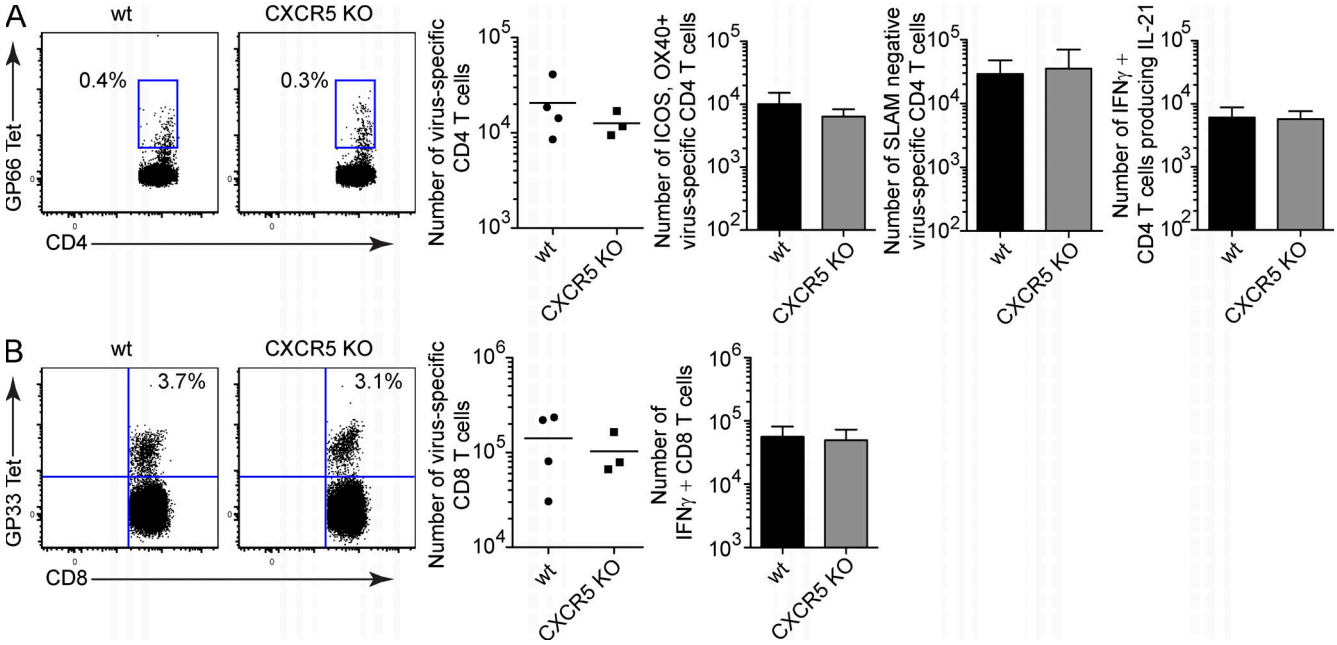

Figure S6. The ability to generate virus-specific CD4 and CD8 T cells is not hindered in the absence of CXCR5 during persistent viral infection. Splenocytes were isolated from day thirty-four LCMV-Cl 13–infected WT and CXCR5 mice. (A) Flow plots depict the percentage of GP_{66-77} tetramer$^+$, virus-specific CD4 T cells. The graphs indicate the number ± SD of virus-specific CD4 T (left), ICOS, OX40$^+$, virus-specific CD4 T cells, (middle, left), SLAM negative, virus-specific CD4 T cells (middle, right) and IFN-γ$^+$ virus-specific CD4 T cells producing IL-21 (right). (B) Flow plots illustrate the percentage of GP_{33-41} tetramer$^+$ virus-specific CD8 T cells in WT and CXCR5 KO mice. The graphs indicate the number ± SD of virus-specific CD8 T cells (left) and IFN-γ$^+$ CD8 T cells (right). Data are representative of three to four mice per group and two individual experiments.

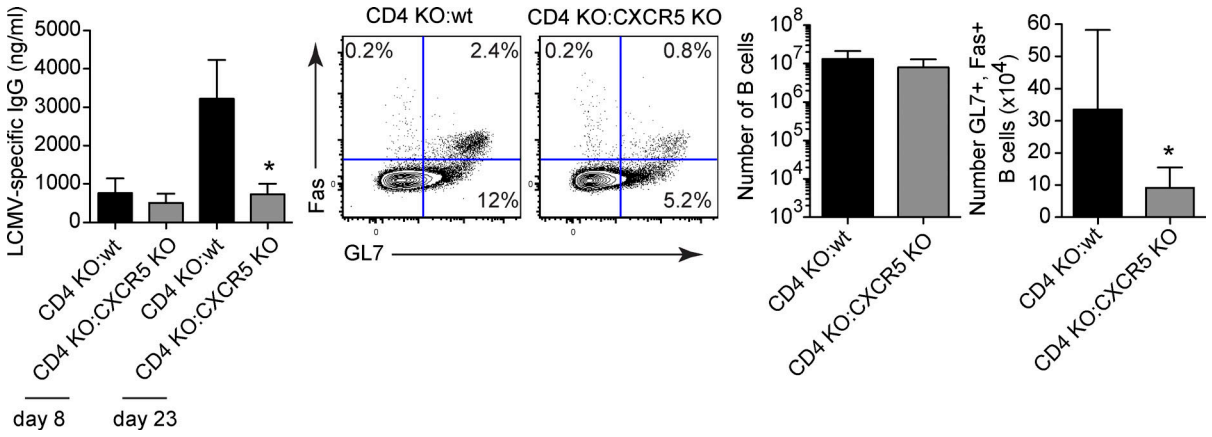

Figure S7. Tfh cells are required to sustain LCMV-specific B cell responses during persistent viral infection. The bar graph depicts the serum concentration (nanogram/milliliter) ± SD of LCMV-specific IgG produced by CD4 KO–WT bone marrow chimera mice (black) versus CD4 KO–CXCR5 KO bone marrow chimera mice (gray) during LCMV-Cl 13 infection. Flow plots illustrate the frequency of GC B cells (GL7$^+$, Fas$^+$ B cells) on day 25 after infection. The bar graphs indicate the number ± SD of total B cells (left bar graph) and GC B cells (right bar graph) during persistent LCMV-Cl 13 infection. Data are representative of a single experiment with seven (CD4 KO:WT) and eight (CD4 KO: CXCR5 KO) mice per group (*, $P < 0.05$).