Supporting Information

Hu et al. 10.1073/pnas.1101881108

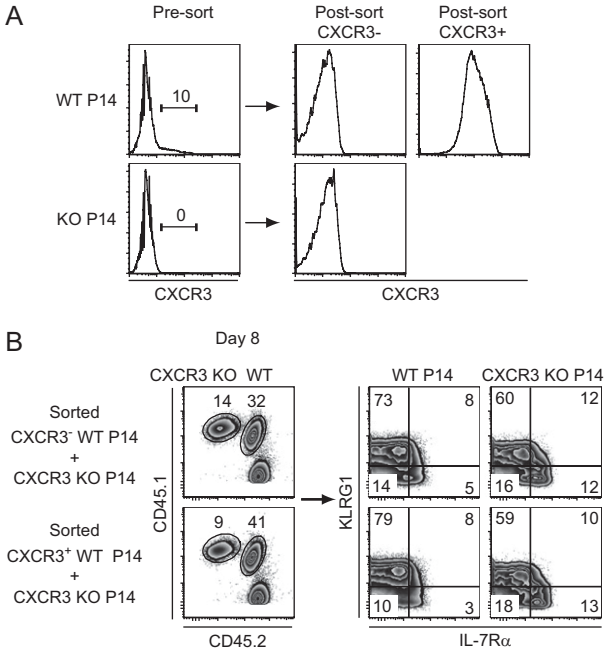

Fig. S1. Early expression of CXCR3 leads to generation of more CD8$^+$ T cells at the peak of expansion. (*A*) Naive WT P14 T cells were sorted based on expression of CXCR3. Histograms are gated on CD8$^+$ TCR Vα2$^+$ P14 T cells and show expression of CXCR3 on presort and postsort populations. As a control, naive CXCR3-deficient cells were sorted using the same mixture of antibodies used for WT cells. (*B*) Equal numbers of either CXCR3$^-$ or CXCR3$^+$ CD45.1/CD45.2 WT P14 cells were cotransferred with CD45.1/CD45.1 CXCR3 KO P14 T cells into C57BL/6 mice that were then infected with LCMV. (*Left*) Proportion of each population within the CD8$^+$ gate in spleen at day 8 postinfection. (*Right*) Proportion of cells within each P14 population that express KLRG1 and IL-7Rα. Data shown are representative of two independent experiments (n = 4 for each time).

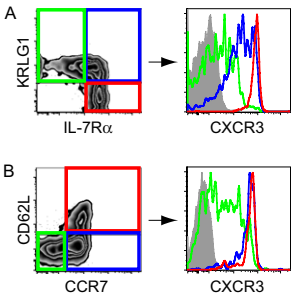

Fig. S2. CXCR3 is differentially expressed on subsets of memory P14 T cells. Memory-cell subsets defined by the expression of KLRG1 and IL-7Rα (*A*) or CD62L and CCR7 (*B*) were gated for expression of CXCR3 in the spleen at day 60 postinfection. The colored histograms (*Right*) show CXCR3 expression within memory cell subsets designated by boxes of the same color (*Left*).

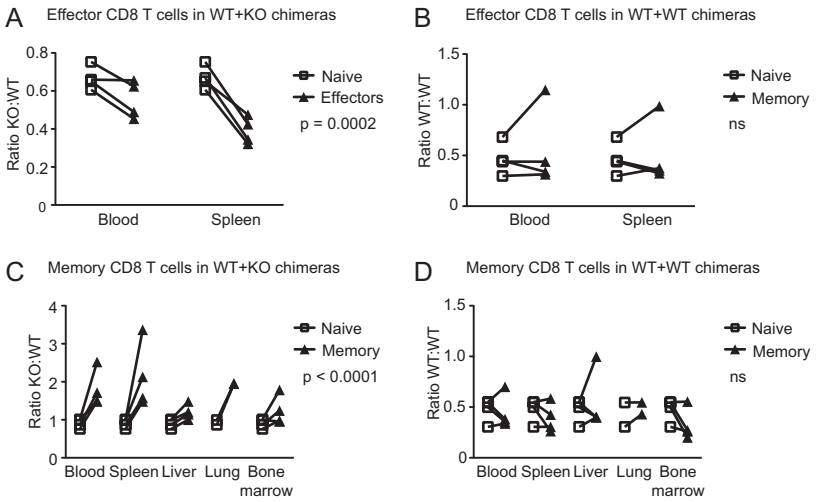

Fig. S3. Non-TCR Tg CXCR3-deficient CD8 T cells generate fewer antigen-specific effector cells and more memory cells after infection. (*A–D*) Irradiated C57BL/6 mice were reconstituted with a 1:1 mixture of bone marrow cells from CD45.1/CD45.2 WT and CD45.1/CD45.1 CXCR3 KO mice or from CD45.1/CD45.2 WT and CD45.1/CD45.1 WT mice as a control. Following reconstitution, chimeric mice were bled to determine the proportions of $CD8^+$ T cells derived from each bone marrow donor population (labeled "Naive"). The mice were then infected with LCMV and analyzed at either day 8 (effectors) or day 220+ (memory) after infection to determine the ratio of tetramer-positive antigen-specific $CD8^+$ T cells derived from each bone marrow donor population in the indicated tissues. The empty squares show data before infection, and the filled triangles show tetramer-positive (GP33, GP276, NP396) effector or memory CD8 T cells after infection. A line connects data from individual mice. Data from one set of chimeras are shown (n = 4 for each group). Statistics were done using two-way ANOVA.

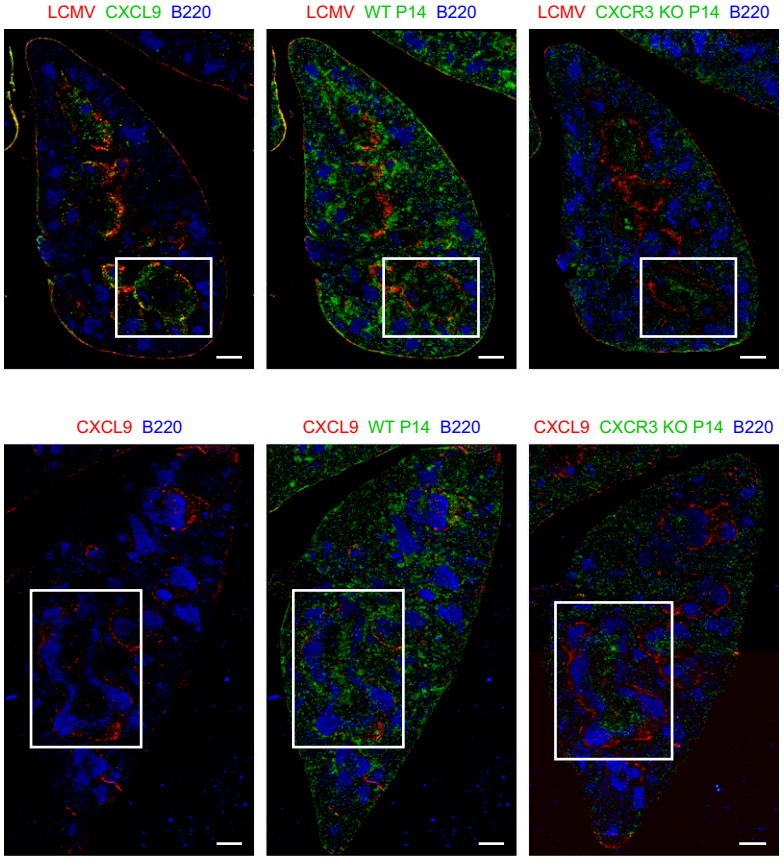

Fig. S4. Effector CD8 T cells colocalize with antigen and CXCL9 in a CXCR3-dependent manner. Equal numbers of $Thy1.1^+$ CD45.2/CD45.2 WT and $Thy1.2^+$ CD45.1/CD45.1 CXCR3 KO P14 T cells were cotransferred into C57BL/6 recipients that were then infected with LCMV. Consecutive sections were prepared from spleens taken on day 4 postinfection and were stained for viral antigen, CXCL9, WT P14 (Thy1.1), CXCR3 KO P14 (CD45.1), and B220. (Scale bars: 300 μm.) Data are representative of two independent experiments (n = 4 for each time). (*Insets*) Delineation of areas shown at higher magnification in Fig. 5.

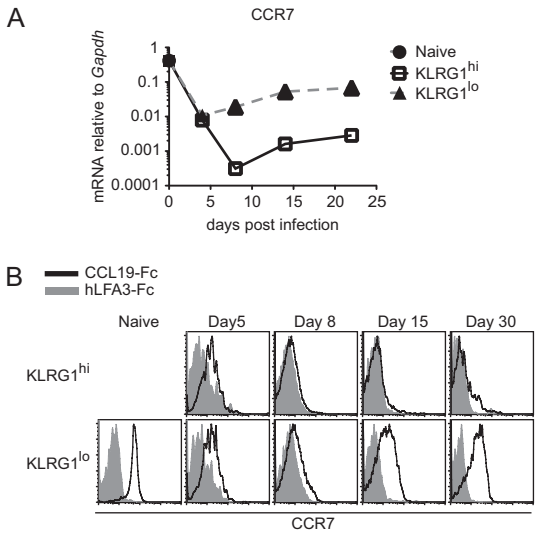

Fig. S5. CCR7 is differentially expressed on KLRG1^{hi} and KLRG1^{lo} effector CD8 T cells. (*A*) Expression of CCR7 mRNA in sorted KLRG1^{hi} and KLRG1^{lo} effector P14 CD8 T cells at the indicated times after infection was determined by quantitative RT-PCR and is expressed relative to *Gapdh* mRNA. Data shown are averages of three independently sorted samples. (*B*) Cell surface expression of CCR7 on KLRG1^{hi} and KLRG1^{lo} CD8^{+} effector P14 T cells at the indicated times after infection was determined by flow cytometry. Histograms are gated on P14 T cells or total CD8 T cells for the naive mouse, with isotype control shown as a shaded histogram. Data shown are representative of two independent experiments (n = 4 for each time).

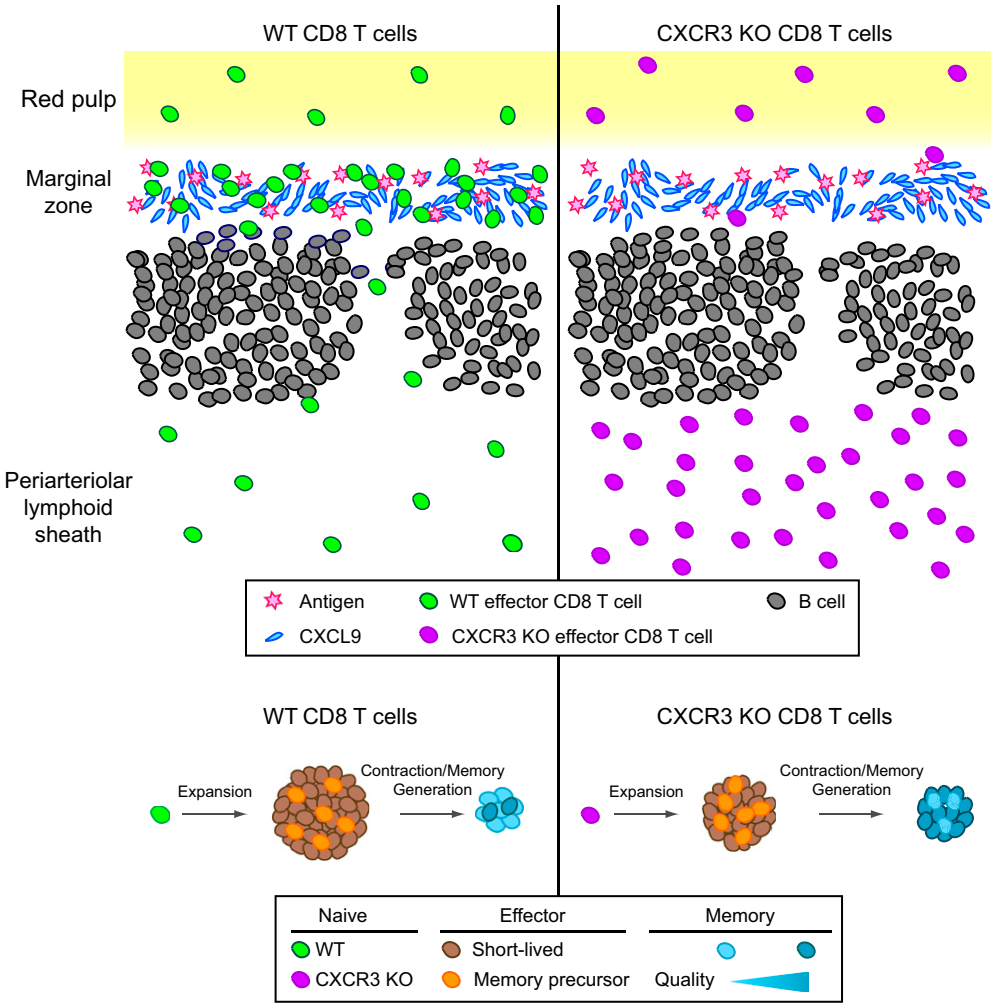

Fig. S6. Model of the contribution of CXCR3 to CD8 T-cell differentiation. In the spleen, marginal zone macrophages and marginal metallophilic macrophages are infected with LCMV and express the CXCR3 ligand CXCL9. Activated effector CD8 T cells express CXCR3 and move toward CXCL9 in marginal zone areas, where they are exposed to antigen. Consequently, WT CD8 T cells encounter more antigen and inflammation than CXCR3-deficient cells. As a result, CXCR3-expressing cells tend to become short-lived effector cells instead of long-lived memory precursors. In contrast, CD8 T cells that lack CXCR3 localize away from marginal zone areas and, as a result, are less exposed to antigen and inflammatory stimuli. This leads to generation of more long-lived memory CD8 T cells that are qualitatively better than WT cells.