Supporting Information

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SI Materials and Methods

T-Cell Receptor Transgenic Mouse Generation. The LLO118 and LLO56 T-cell receptors (TCRs) were isolated from the hybridomas and their V regions were engineered into α and β TCR genomic shuttle vectors. The VDJ/VJ sequences from cloned LLO190-205/I-A^b specific TCRs were inserted into the shuttle vector pTα and pTβcass, respectively. The transcription of TCR genes were controlled by both 5′ and 3′ regulatory elements cloned from the TCR-α, -β locus. In mRNA precursors, the VDJ/VJ region of the transgenic (Tg) TCRs was spliced to join the leader region (from 2B4 TCR) and constant region (C1), which were located inside the original shuttle vectors. The sequence of the transgenic inserts was engineered to optimize splicing. These shuttle vectors were coinjected into C57BL/6 mice oocytes and founders were identified by PCR screen of tail DNA. The transgenic lines were were bred to congenic marker lines to facilitate in vivo T-cell transfer experiments (LLO118-Ly5.1 and LLO56-Thy1.1). The two TCR-transgenic lines were bred onto *Rag1*$^{-/-}$ backgrounds with unique congenic markers: LLO118.*Rag1*$^{-/-}$ Ly5.1, LLO56.*Rag1*$^{-/-}$ Thy1.1.

Bacterial Infections. Frozen *Listeria monocytogenes* stocks were grown in brain-heart infusion (BHI) broth to mid-log stage phase colony forming units were determined by counting 10-fold serial dilutions (in triplicate) of three thawed tubes. After stock values were determined they were thawed and diluted in PBS for injection.

Homeostatic Proliferation Assay. LLO118 and LLO56 T cells labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) were transferred into lymphopenic recipients to determine their levels of homeostatic proliferation. Purified $CD4^+$ LLO118 and LLO56 TCR-transgenic T cells were labeled with 5 μM CFSE (Molecular Probes). CFSE-labeled cells (5×10^5 T cells per mouse) were adoptively transferred into B6 *Rag1*$^{-/-}$ mice by retroorbital injection. After 4 d spleen cells were recovered and expression of the congenic markers (Thy1.1 and Ly5.1) and CFSE was analyzed by flow cytometry.

Adoptive Transfer of $CD4^+$ Cells. Single-cell suspensions were made from spleens of LLO118 or LLO56 mice and $CD4^+$ T cells were purified by negative selection using a $CD4^+$ T-cell isolation kit (Miltenyi Biotec) and magnetic bead separation. Cell purity was confirmed by flow cytometry and depending on the experiment either 3×10^3 or 3×10^4 cells were transferred by retroorbital injection into recipient mice. For primary infections mice were infected by retroorbital injection with 1×10^4 CFU *L. monocytogenes.* For recall infections, mice were infected by retroorbital injection with 1×10^5 CFU *L. monocytogenes* 35 d after the primary infection. At the days listed, cells were collected and enriched by staining for 30 min with biotinylated antibodies for their congenic markers (eBioscience CD45.1 clone A20 and CD90.1 clone HIS51). The cells were washed and stained for 30 min with 40 μL Miltenyi Strepavidin beads and then selected on an LS Miltenyi bead column (1). Cell numbers were determined using flow cytometry by Thy1.1 (LLO56) or Ly5.1 (LLO118) staining.

Adoptive Transfer of $CD8^+$ Cells. Single-cell suspensions were obtained from spleens and lymph nodes from Class $II^{-/-}$ mice and $CD8^+$ T cells were purified by MACS negative selection using a $CD8^+$ T-cell isolation kit (Miltenyi Biotec). This purification generated a $CD4^+$ T-cell free cell population with $CD8^+$ T-cell purity >90%; 5×10^5 purified $CD8^+$ T cells were transferred by retroorbital injection into TCR-C$\alpha^{-/-}$ recipient mice either alone, with 3×10^3 LLO56 $CD4^+$ cells, or with 3×10^3 LLO118 $CD4^+$ cells. Mice were infected one day after adoptive transfer with 1×10^3 CFU *L. monocytogenes* expressing a secreted form of the ovalbumin (OVA) (257-264) epitope (LM-OVA) as well as red fluorescent protein. This LM-OVA strain was constructed and provided by the laboratory of Daniel Portnoy (University of California, Berkeley, CA). For rechallenge infections, mice were infected by retroorbital injection with 1×10^5 CFU LM-OVA. At selected timepoints, mice were analyzed by tetramer staining for K^b-OVA–responsive $CD8^+$ T cells in blood and spleen, or by intracellular IFN-γ staining in spleen.

Flow Cytometry. Samples were stained with fluorescence-conjugated antibodies from commercial sources were as follows: Pacific blue (eFluor450)-conjugated anti-CD11b (M1/70; eBioscience), F4/80 (BM8; eBioscience), CD11c (N418; eBioscience), B220(RA3-6B2; eBioscience); PE-conjugated anti-CD5 (Ly-1; 53–7.3;BD Pharmingen); FITC-conjugated anti-Vα2 (B20.1; BioLegend), CD25 (PC61; BioLegend), CD3 (145-2C11; BioLegend), CD44 (IM7; BioLegend), CD62L (L-selectin; MEL-14; Pharmingen), CD8α (53-6.7 BioLegend); Pacific Orange-conjugated anti-CD8 (Invitrogen); PE-Cy7–conjugated anti-CD45.1 (Ly5.1; A20; eBioscience); PerCP-Cy5.5–conjugated anti-CD90.1 (Thy1.1; HIS51; eBioscience); APC-Cy7–conjugated anti-CD4 (GK1.5; BioLegend); APC-conjugated anti-CD8α (53-6.7; BioLegend), IFN-γ (XMG1.2; BioLegend). H-$2K^b$ tetramers bound to residues 257–264 of chicken ovalbumin (K^b-OVA tetramer) were provided by the laboratory of Mark Davis (Stanford University, Stanford, CA).

In Vitro Proliferation Assay. LLO118 or LLO56 splenocytes were plated in 96-well plates (1×10^5 cells per well) and rested overnight. The next day dilutions of peptide or *L. monocytogenes* were added. In the case of infection, bacteria were grown to mid log phase at 30 °C in BHI, measured by optimal density (600-nm absorbance), and washed three times with PBS before infection. Infected cells were washed 3× with PBS at 30 min postinfection and gentamycin (50 μg/mL) was added at 1 h postinfection. Tritiated thymidine was added to wells 48 h after the addition of peptide or *L. monocytogenes* and cultures were harvested and proliferation was assessed 24 h later by the level of tritiated thymidine incorporation.

Intracellular Cytokine Assay. LLO118 or LLO56 T cells were infected with *L. monocytogenes* and 7 d after infection cells were collected and enriched by staining for 30 min with biotinylated antibodies for their congenic markers (eBioscience CD45.1 clone A20 and CD90.1 clone HIS51). The cells were washed and stained for 30 min with 40 μL Miltenyi strepavidin beads and then selected on an LS Miltenyi bead column (1). There are a number of residual antigen-presenting cells still in the samples after enrichment and 10 μM peptide was added to each sample and T cells were stimulated for 5 h at 37 °C; brefeldin A (10 μg/mL) was added for the last 4 h of the incubation. The cells were surface-stained for 30 min, fixed in 2% paraformaldehyde for 20 min at room temperature, and then washed and permeabilized with 0.5% saponin for 10 min. Intracellular staining was performed for 30 min at room temperature using APC conjugated anti IFN-γ (Biolegend; clone XMG1.2), PE-conjugated anti–IL-2 (Biolegend; clone JES6-5H4). A FACSCanto (Becton Dickinson) and FloJo software were used for data collection and analysis.

1. Moon JJ, et al. (2009) Tracking epitope-specific T cells. *Nat Protoc* 4:565–581.

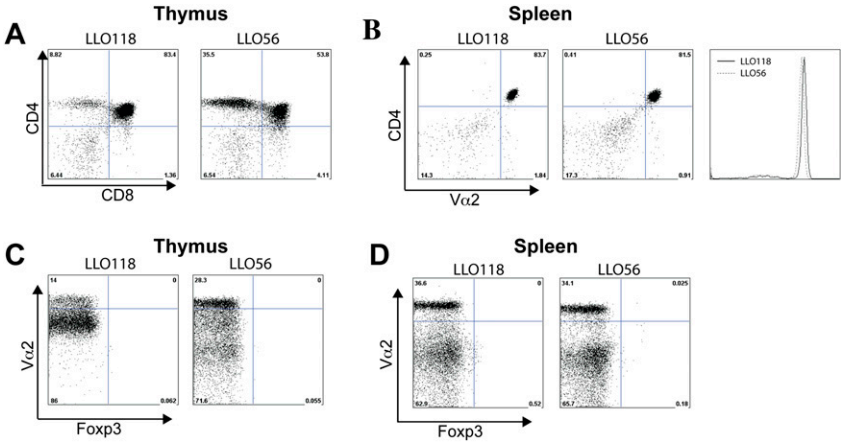

Fig. S1. Characterization of TCR Tg mice specific for an immunodominant epitope in *L. monocytogenes*. (*A*) Flow cytometry analysis of the levels of CD4 and CD8 T cells in the thymus of the $Rag^{-/-}$ LLO118 and LLO56 TCR transgenic mice. (*B*) The levels of TCR (Vα2) in the thymus of $Rag^{-/-}$ LLO118 and LLO56 TCR Tg mice were analyzed by flow cytometry. Data are shown as dot plots as well as a histogram with data overlayed. (*C*) Flow cytometery analysis of the levels of regulatory T cells in the thymus of $Rag^{-/-}$ LLO118 and LLO56 TCR Tg mice. (*D*) The levels of regulatory T cells in the spleen of $Rag^{-/-}$ LLO118 and LLO56 TCR Tg mice was analyzed by flow cytometry. Data represent two (*C* and *D*) or three (*A* and *B*) independent experiments with one to three mice per group.

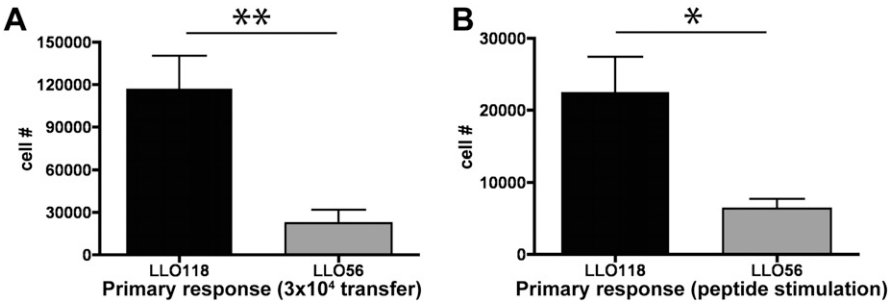

Fig. S2. LLO118 T cells have a better primary response to *L. monocytogenes* infection and peptide stimulation. (*A*) $CD4^+$ T cells from LLO118-Ly5.1 or LLO56-Thy1.1 mice were purified by negative selection and 3×10^4 cells were transferred to C57BL/6 recipient mice. The mice were subsequently infected with 1×10^4 CFU of *L. monocytogenes* and cell numbers were measured 7 d later by flow cytometery. (*B*) $CD4^+$ T cells from LLO118-Ly5.1 or LLO56-Thy1.1 mice were purified by negative selection and 3×10^3 cells were transferred to C57BL/6 recipient mice. The mice were subsequently injected in the footpad with 1 mM $LLO_{190–205}$ peptide in complete Freund's adjuvant and T cells levels were measured 8 d later by flow cytometery. $^*P < 0.05$, $^{**}P < 0.01$ (Student *t* test).

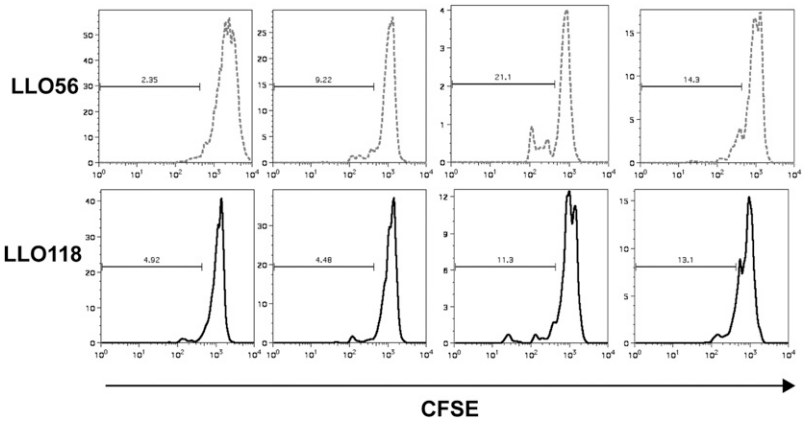

Fig. S3. LLO118 and LLO56 have similar levels of homeostatic proliferation. Levels of homeostatic proliferation of CFSE-labeled LLO56 (*Upper*) and LLO118 (*Lower*) T cells (from four separate mice) 4 d after transfer into B6 $Rag1^{-/-}$ mice. Samples were analyzed by staining for the congenic markers (LLO56-Thy1.1. and LLO118-Ly5.1).

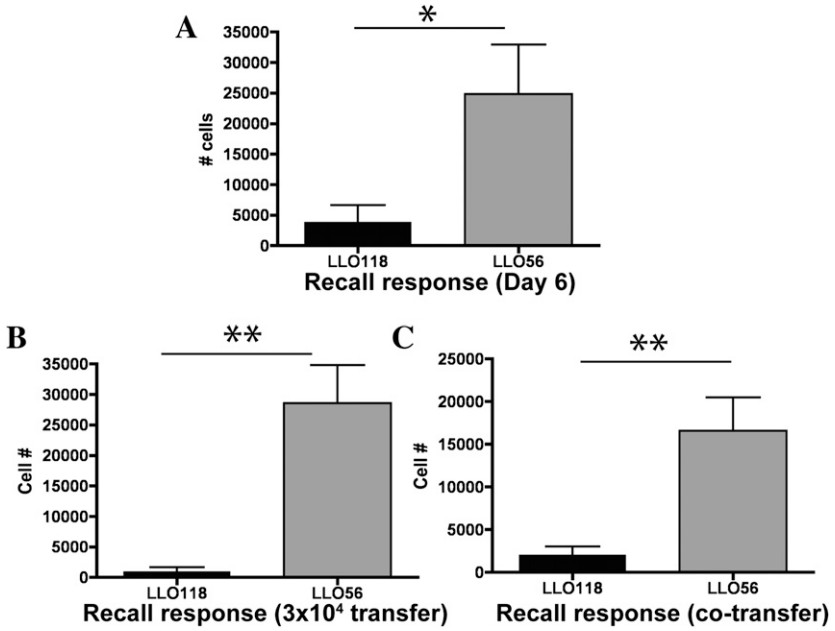

Fig. S4. LLO56 T cells have a better secondary response to *L. monocytogenes* infection. On day 35 after the primary infection, T-cell recipient mice were infected with 1×10^5 CFU of *L. monocytogenes*. Four or 6 d after the secondary infection, spleens and lymph nodes were isolated and analyzed by flow cytometry to determine the total number of cells. (*A*) Analysis of LLO118 and LLO56 response to recall challenge at day 6. These data are consistent with what has been seen at day 4. (*B*) Day 4 postinfection recall response of recipient mice receiving 3×10^4 LLO118-Ly5.1 or LLO56-Thy1.1 cells. (*C*) Day 4 postinfection recall response of a cotransfer of 3×10^4 LLO118-Ly5.1 and LLO56-Thy1.1 cells. Data are representative of three independent experiments (*A*–*C*) with at least three mice per group each experiment. $*P < 0.05$ and $**P < 0.01$ (Student *t* test).

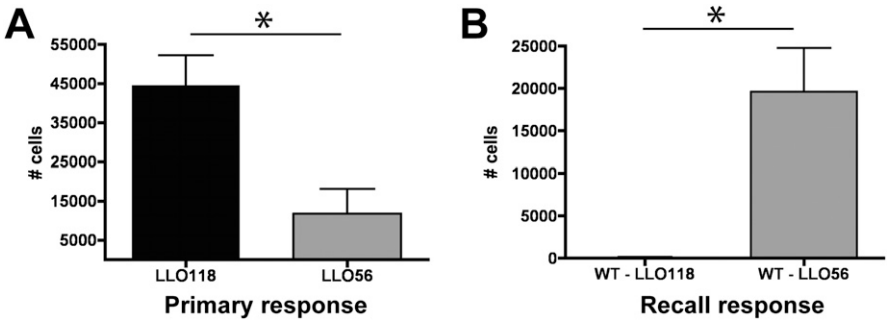

Fig. S5. Second TCR Tg mouse line confirms that LLO56 T cells have a better secondary response to *L. monocytogenes* infection. (*A*) Primary responses of LLO118 and an additional LLO56 TCR Tg mouse line was examined (day 7 postinfection) and LLO118 has significantly higher level of proliferation than LLO56. These results are consistent with what was seen with the original LLO56 TCR Tg mouse line. (*B*) Secondary responses of LLO118 and an additional LLO56 TCR Tg mouse were examined (day 4 postrecall challenge) and LLO56 has significantly higher level of proliferation than LLO118. These results are consistent with what was seen with the original LLO56 TCR Tg mouse.

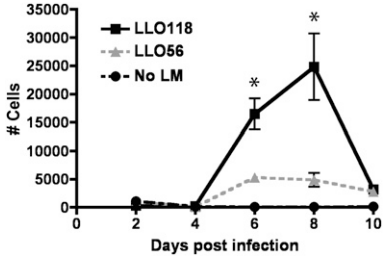

Fig. S6. LLO118 has a greater magnitude of proliferation over the first 10 d compared with LLO56. T-cell proliferation levels over the course of 10 d. At each day indicated, spleens and lymph nodes from three mice per group were isolated and analyzed by flow cytometry to determine the number of cells. $*P < 0.05$ (Student *t* test).

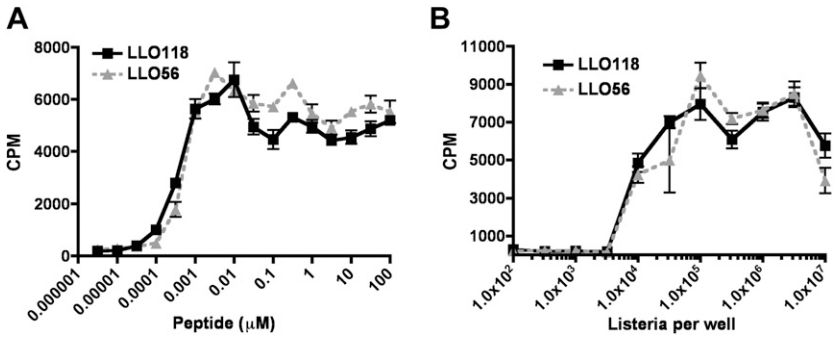

Fig. S7. LLO118 and LLO56 have identical antigen sensitivity when measuring in vitro responses to peptide or *L. monocytogenes* infection. (*A*) LLO118 and LLO56 splenocytes (5×10^5/well) were stimulated for 48 h by addition of $LLO_{190–205}$ peptide and proliferation levels were measured 24 h after the addition of ^{3}H-thymidine. (*B*) LLO118 and LLO56 splenocytes (5×10^5/well) were infected with *L. monocytogenes* and stimulated for 48 h and proliferation levels were measured 24 h after the addition of ^{3}H-thymidine. Data are representative of three independent experiments.

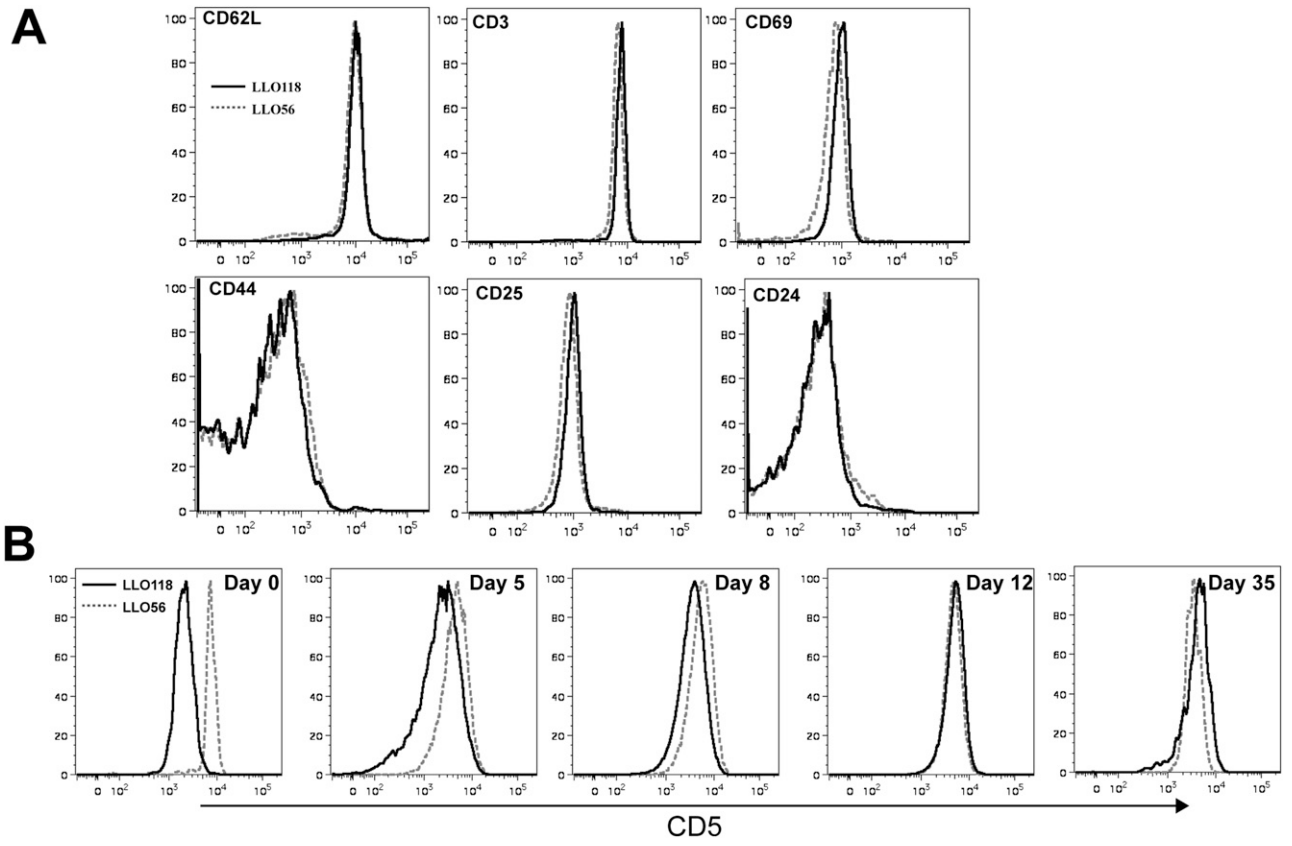

Fig. S8. LLO118 T cells have lower levels of CD5 compared with LLO56. (*A*) Flow cytometry analysis of common TCR surface markers measured before mice had been infected. All of the markers measured are the same except for CD5 (see also Fig. 2). (*B*) 3×10^4 LLO118-Ly5.1 or LLO56-Thy1.1 cells were transferred into B6 mice and they were subsequently infected with 1×10^4 CFU *L. monocytogenes*. CD5 levels were monitored by flow cytometry over the course of 35 d. Data are from three independent experiments with at least three mice per group each experiment.

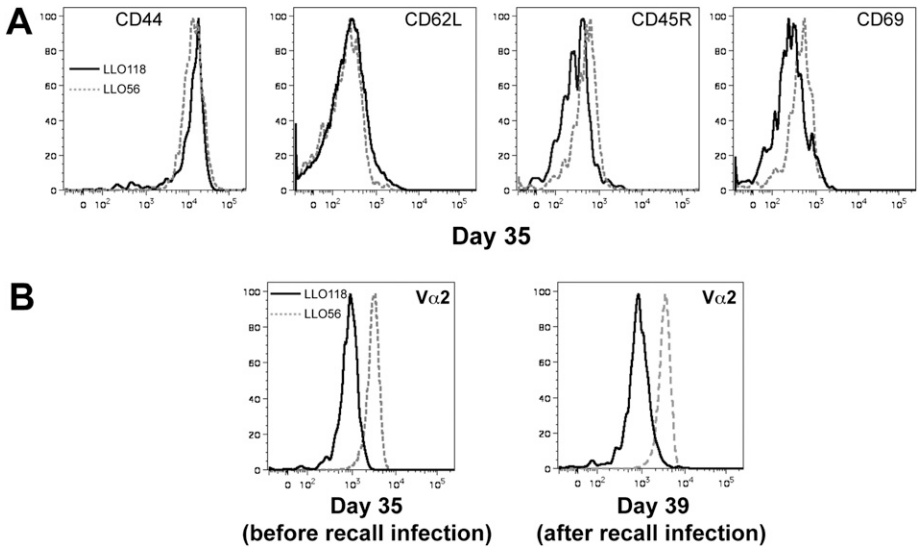

Fig. S9. LLO118 T cells have increased TCR down-regulation compared with LLO56. (*A*) Flow cytometry analysis of common T-cell surface markers at day 35. All of the markers are similar with the exception of Vα2 levels (see also Fig. 5). (*B*) Flow cytometry analysis of Vα2 levels before (day 35) and after recall infection (day 39) (see also Fig. 5).

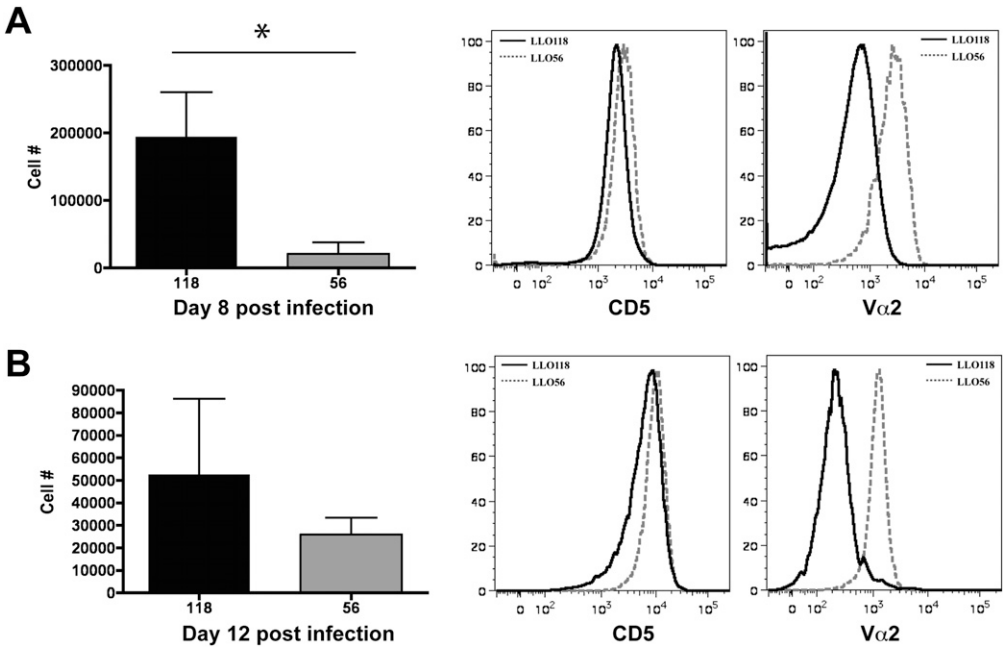

Fig. S10. Additional LLO118 TCR Tg mouse line has robust primary response and TCR down-regulation as seen with the original LLO118 TCR Tg mouse line. (*A*) The primary responses of LLO56 and an additional LLO118 TCR Tg mouse line were examined (day 8 postinfection) and LLO118 has significantly higher level of proliferation than LLO56. Each group contains four mice. CD5 levels are identical and TCR levels are much lower in LLO118 compared with LLO56. These results are consistent with what was seen with the initial LLO118 TCR Tg mouse line. (*B*) Analysis of day 12 cell number, CD5, and TCR (Va2) levels. There is no difference between cell numbers and CD5 levels, but Va2 levels are much lower in LLO118 compared with LLO56. Each group contains four mice. These results are consistent with what was seen with the initial LLO118 TCR Tg mouse line. Data are representative of two independent experiments with two mice per group each experiment. $*P < 0.05$ (Student *t* test).