Supporting Online Material

Materials and Methods

Mice and viruses. C57BL/6J (B6), B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyJ (CD45.1), B6.129S7-*Rag*^{1tm1Mom}/J (*Rag1*$^{-/-}$), and B6.129S2-*Cd4*^{tm1Mak}/J (*Cd4*$^{-/-}$) mice were purchased from Jackson Laboratory (Bar Harbor, ME). B6;129S5-*Il21*^{tm1Lex} (*Il21*$^{-/-}$) and B6;129S5-*Il21r*^{tm1Lex} (*Il21r*$^{-/-}$) mice were obtained from the mutant mice regional resource center (Davis, CA). *Il21*$^{-/-}$ and *Il21r*$^{-/-}$ mice were backcrossed an additional 3 or 6 generations and 4 or 8 generations respectively, onto the B6 background. All mice were bred and maintained in fully accredited facilities at the University of Alabama at Birmingham. For acute infections, mice were infected by intraperitoneal injection with $2x10^5$ p.f.u. LCMV-Arm. LCMV-Cl 13 infections were established by intravenous inoculation with $2x10^6$ p.f.u. Viral titers were assessed by plaque assays using Vero cell monolayers (*S1*).

Cellular analyses and flow cytometry. Cell preparations, MHC tetramer staining and intracellular cytokine analyses were performed essentially as previously described (*S2*). Samples were acquired using FACSCalibur or LSR II flow cytometers (BD, San Jose, CA), and data was analyzed using FlowJo software (Tree Star, Ashland, OR).

For the detection of IL-21, splenocytes from infected B6 mice were stimulated with the LCMV GP61-80 peptide (10μg/mL) for 5 hours, with Brefeldin A added for the last four

hours of the culture period. Following stimulation, splenocytes were stained with anti-CD4 APC antibodies (RM4-5; eBioscience, San Diego, CA) for 25 minutes at 4ºC and then subjected to two-step intracellular staining procedure (*S3*). Intracellular staining was initially performed using recombinant IL-21R/Fc fusion proteins (0.1μg, R&D Systems, Minneapolis, MN) together with anti-IFN-γ FITC antibodies (XMG1.2; eBioscience). After washing with Perm-Wash buffer (BD Bioscience, San Jose, CA) cells were then stained with $F(ab)_2$ PE-conjugated goat anti-human Fcγ (Jackson ImmunoResearch Laboratories, West Grove, PA). Subsequently, cells were washed, re-fixed and analyzed by flow cytometry.

Follicular helper $CD4^+$ T cells (Tfh) were detected by co-staining splenocytes with anti-ICOS PE (7E-17G9, eBioscience), anti-CD4 PE-Cy7 (RM4-5, eBioscience), and biotinylated anti-CXCR5 (2G8; BD Bioscience) antibodies. Germinal center (GC) B cells were detected by staining with anti-B220 FITC (RA3-6B2, eBioscience), anti-CD19 PE-Cy7 (eBio1D3, eBioscience), anti-CD95 PE (Jo2, BD Bioscience) antibodies, and biotinylated-PNA (Vector). For both Tfh and GC B cell detection secondary staining was performed with Streptavidin-APC (Invitrogen) prior to fixation and flow cytometric analyses.

Antibody Titrations. LCMV specific-antibody levels were measured by ELISA performed essentially as previously described (*S1*). Captured virus-specific antibodies were detected by horseradish peroxidase conjugated goat anti-mouse IgG (H+L) (Caltag

M30207). Plates were developed using tetramethylbenzidine substrate (R&D) with the reaction stopped by the addition of acid prior to measuring OD450 values using a microplate reader.

Mixed Bone Marrow Chimeras. Bone marrow was prepared from the tibias and femurs of B6.SJL (CD45.1) mice, and CD45.2 *Il21r*$^{+/+}$ and *Il21r*$^{-/-}$ littermates. Bone marrow suspensions were depleted of T cells using anti-CD5 (Ly-1) microbeads (Miltenyi Biotec; Auburn, CA). Recipient *Rag1*$^{-/-}$ mice were irradiated twice, 3-4 hours apart, to give a total exposure of ~1000 rads. These mice were then reconstituted by i.v. injection of T depleted bone marrow containing 5-7x10^{6} CD45.1 cells mixed with equal numbers of *Il21r*$^{+/+}$ or *Il21r*$^{-/-}$ (CD45.2) cells. Mice were maintained on chlorinated acidified water containing sulfamethoxazole, trimethoprim, and neomycin. Chimeras were infected with LCMV-Cl 13 ~7 weeks following reconstitution.

IL-21 Treatment. *Cd4*$^{-/-}$ mice were infected with 2x10^{6} pfu LCMV-Cl 13. At 12 hours following infection mice were administered 10 μg recombinant mouse IL-21 (PeproTech; Rocky Hill, NJ) by i.p. injection. The treatments were continued every 24 hours thereafter for a total of 8 days.

Statistical analysis. Two-tailed t-tests and one-way ANOVA were used to determine statistical significance. *P* values were calculated using Prism software (Graph Pad; La Jolla, CA).

Supplementary Figures and Legends

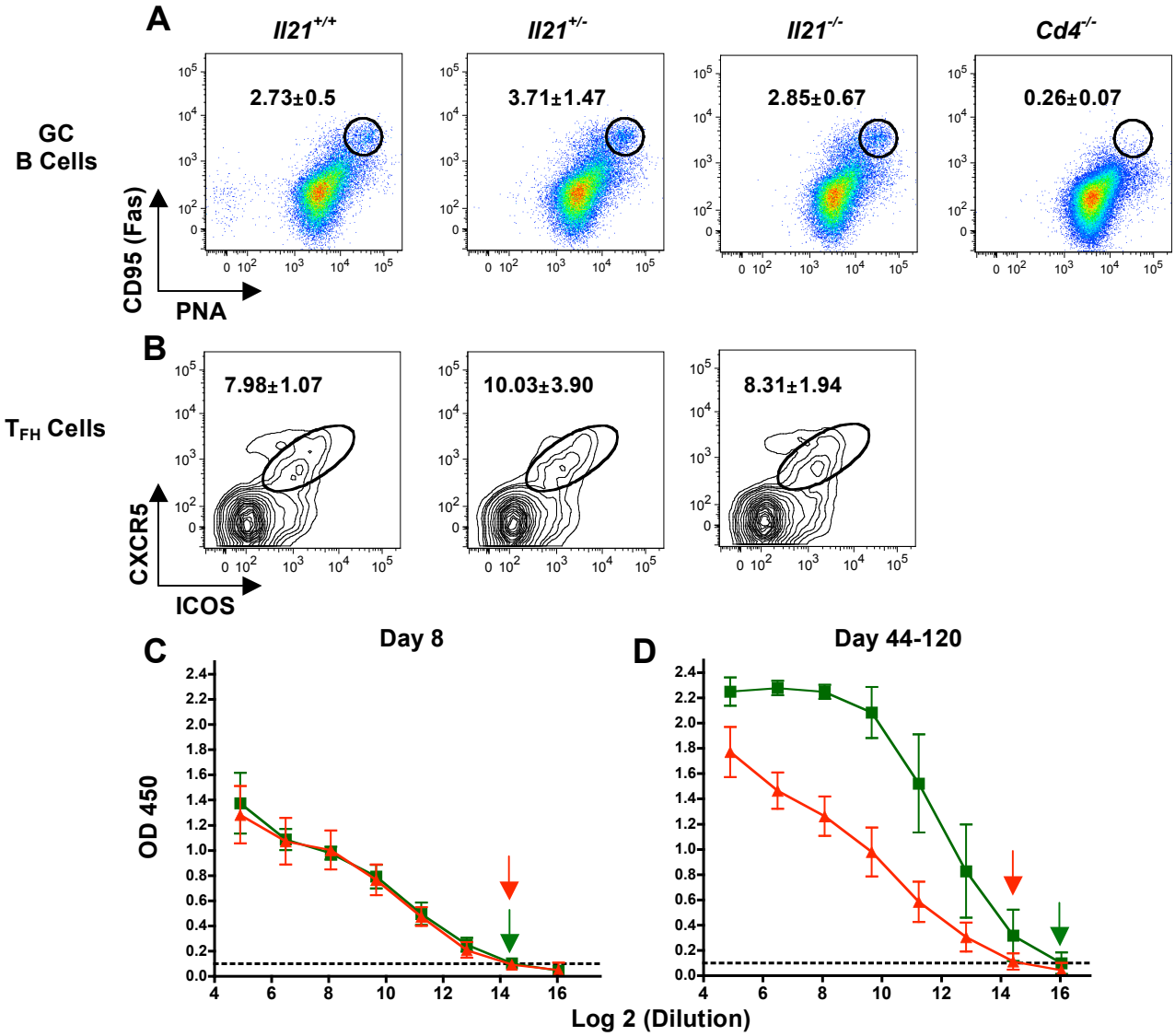

Fig S1. The generation of GC B cells, Tfh $CD4^+$ T cells, and LCMV-specific antibodies in the absence of IL-21. The presence of splenic GC B cells **(A)** and Tfh $CD4^+$ T cells **(B)** was determined by flow cytometric analysis at days 8-9 following LCMV-Arm infection. Gated $B220^+$ $CD19^+$ B cells and $CD4^+$ T cells are shown in panels (A) and (B), respectively. Mean percentages +/- s.d. are reported for cells contained within the regions shown. Representative results are shown from two independent experiments (n=6-8). **(C)** and **(D)** Serum LCMV-specific antibody titers were measured by ELISA at 8 (n=5-6) or 44-120 (n=9-11) days following LCMV-Arm infection of *Il21*$^{+/+}$ (green squares) and *Il21*$^{-/-}$ (red triangles) mice. Mean OD450 values +/- s.d. are plotted; arrows represent endpoint titers.

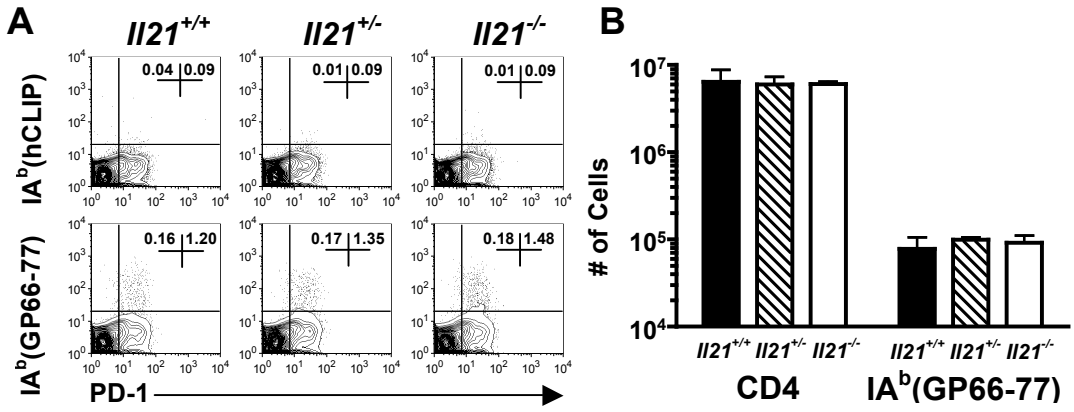

Fig. S2. Induction of LCMV-specific $CD4^+$ T cell responses following LCMV-Cl 13 infection. At 8 days following LCMV-Cl 13 infection of $Il21^{+/+}$, $^{+/-}$, $^{-/-}$ splenic $CD4^+$ T cells were analyzed by staining with either control IA^b(hCLIP) tetramers or with IA^b(LCMV GP_{66-77}) tetramers. Plots show gated $CD4^+$ T cells and the percentage of tetramer+ cells in the upper left and right quadrants are indicated. **(B)** The total numbers of $CD4^+$ T cells and viral-epitope-specific $CD4^+$ T cells were determined by flow cytometric analysis using IA^b(LCMV GP_{66-77}) tetramers. Mean values +/- s.d. are shown. Representative results are shown from two independent experiments (n=3-6).

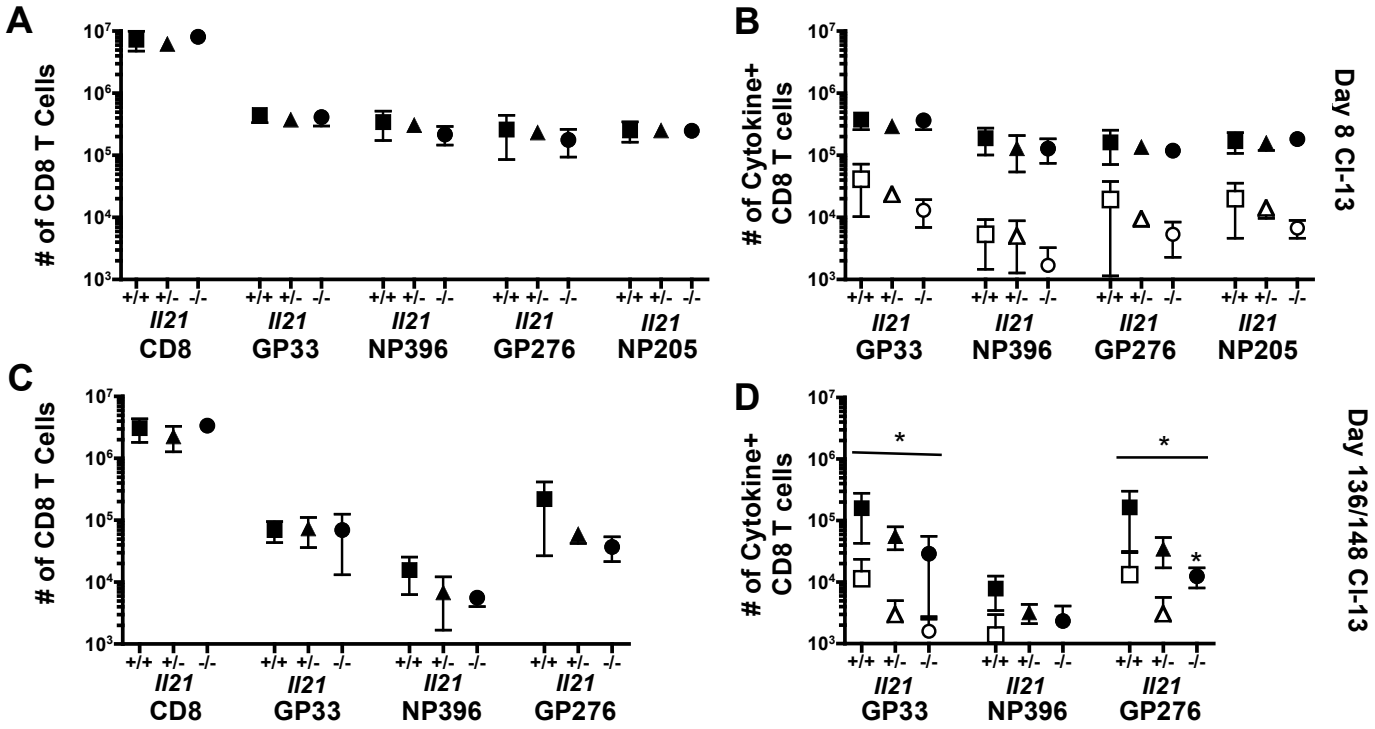

Fig. S3. Enumeration of $CD8^+$ T cell responses indicates a selective functional impairment in the absence of IL-21. Splenic $CD8^+$ T cell responses were enumerated at (**A** and **B**) day 8 following LCMV-Cl 13 infection; and (**C** and **D**) days 136-148 following LCMV-Cl 13 infection. (**A** and **C**) The total numbers of $CD8^+$ T cells and viral-epitope-specific $CD8^+$ T cells were determined by flow cytometic analysis using a panel of MHC class I tetramers. (**B** and **D**) Epitope-specific IFN-γ producing (black symbols) and IL-2 producing (white symbols) $CD8^+$ T cells were enumerated following intracellular cytokine analyses. Results are shown for $Il21^{+/+}$, $^{+/-}$, and $^{-/-}$ groups. Mean values +/- s.d. are shown for composite data from two independent experiments (n=3-6). The limit of detection is 10^3 cells. **P*<0.05 by comparison with $Il21^{+/+}$ cohort.

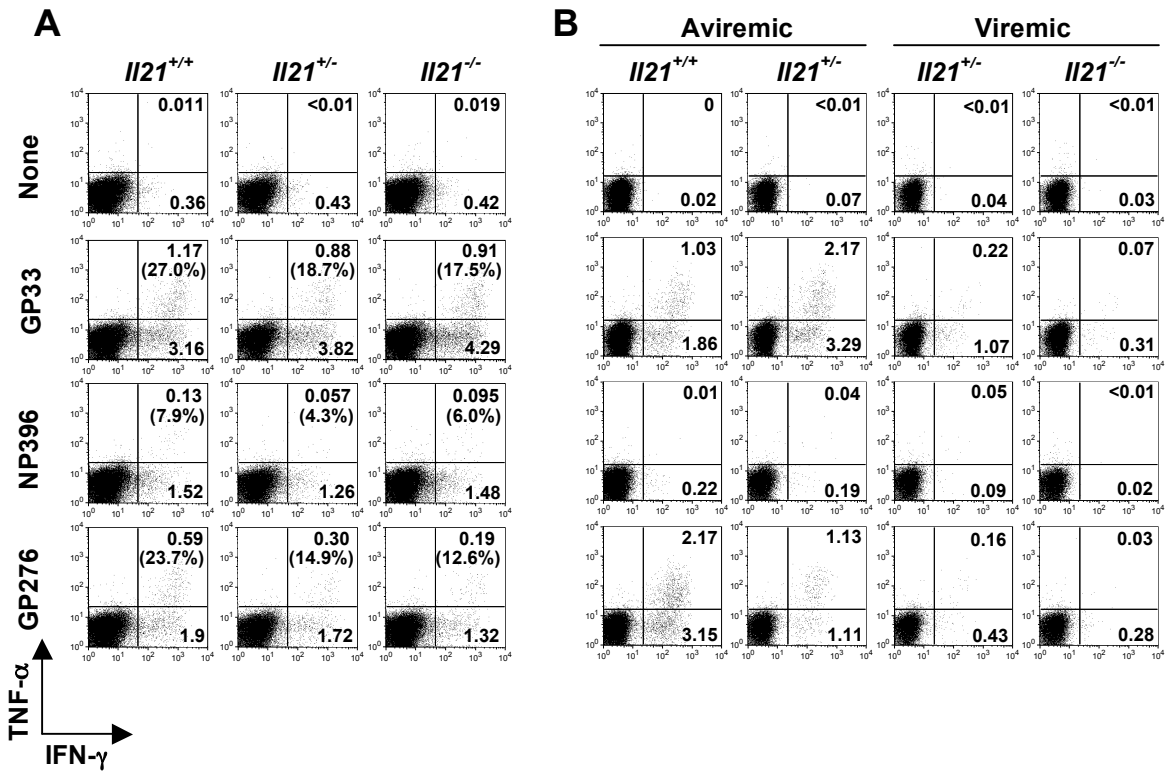

Fig. S4. Severe functional exhaustion of $CD8^+$ T cells in the absence of IL-21. The production of TNF-α and IFN-γ by GP_{33}-, NP_{396}-, and GP_{276}-specific $CD8^+$ T cells was analyzed at 8 **(A)** and 136 **(B)** days following LCMV-Cl 13 infection of *Il21*$^{+/+}$, $^{+/-}$, $^{-/-}$ mice. Gated $CD8^+$ T cells are shown. In (A) the percentage of $CD8^+$, IFN-γ^+ cells that co-produce TNF-α are also reported in parentheses. All mice were viremic at 8 days following infection; however the clearance patterns diverged by day 136 and in (B) data are shown from both aviremic and viremic mice as indicated. Representative data are shown from two independent experiments (n=3-6).

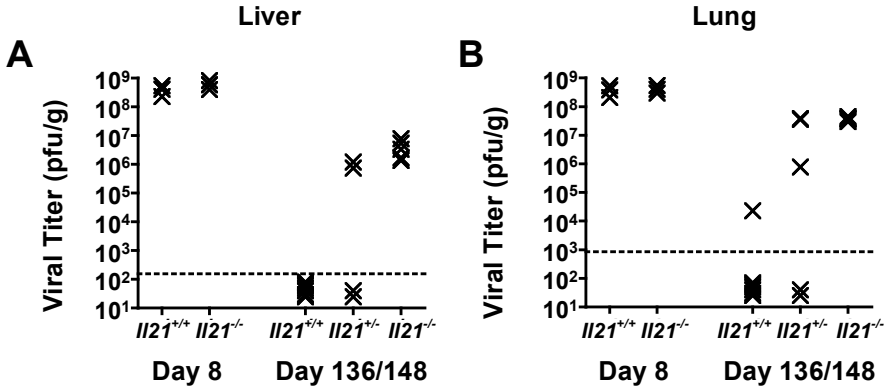

Fig. S5. Divergence in the control of LCMV-Cl 13 in *Il21*$^{+/+}$, $^{+/-}$, and $^{-/-}$ mice. Viral titers in the livers **(A)** and lungs **(B)** of the indicated cohorts of mice were determined by plaque assay at days 8 and 136-148 following infection. Results from individual mice are shown (n=3-6). Limits of detection are indicated by the dashed lines.

References

S1. R. Ahmed, A. Salmi, L. D. Butler, J. M. Chiller, M. B. Oldstone, *J Exp Med* **160**, 521 (1984).

S2. M. J. Fuller, A. Khanolkar, A. E. Tebo, A. J. Zajac, *J Immunol* **172**, 4204 (2004).

S3. A. Suto *et al.*, *J Exp Med* **205**, 1369 (2008).