PNAS
www.pnas.org

Supplementary Information for

Chronic virus infection drives CD8 T cell mediated thymic destruction and impaired negative selection

Heidi J. Elsaesser, Mahmood Mohtashami, Ivan Osokine, Laura M. Snell, Cameron R. Cunningham, Giselle M. Boukhaled, Dorian B. McGavern, Juan Carlos Zúñiga-Pflücker and David G. Brooks

Corresponding Author: David Brooks
Email: dbrooks@uhnresearch.ca

This PDF file includes:

Figures S1 to S5
Legends for Figures S1 to S5

www.pnas.org/cgi/doi/10.1073/pnas.1913776117

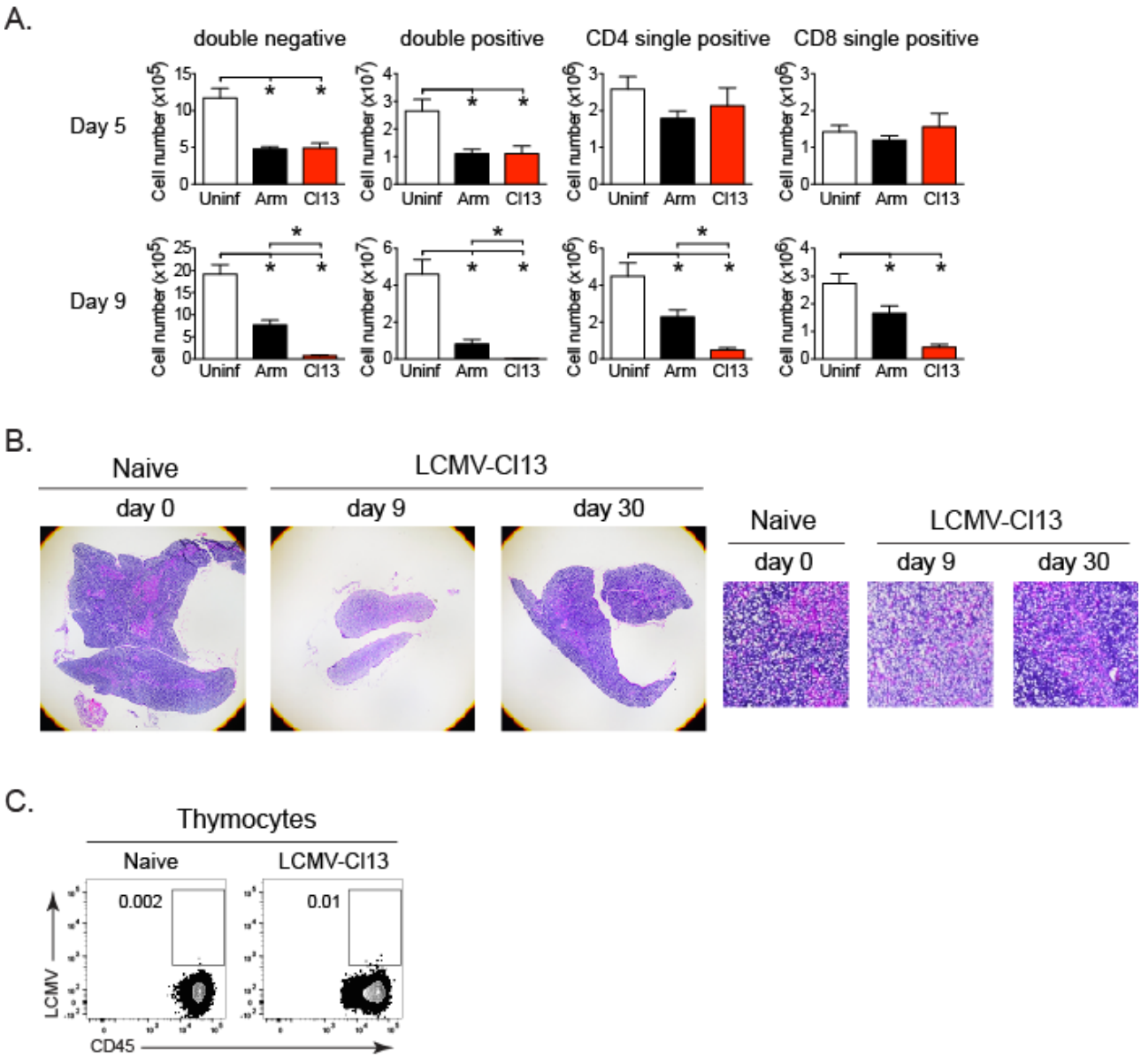

Fig. S1. Thymic depletion during chronic LCMV Cl13 infection.

(A) The number of each thymocyte cell population in uninfected mice and on day 5 and day 9 after LCMV-Arm or Cl13 infection. **(B)** H&E staining of thymus sections from naïve and mice infected with LCMV-Cl13 for 9 or 30 days. **(C)** Flow plots show the frequency of LCMV-nucleoprotein staining in thymocytes from uninfected naïve mice and on day 8 after LCMV-Cl13 infection. Thymocytes include all CD4 SP, CD8 SP and CD4/CD8 DP cells. Data is representative of 2 independent experiments with 3-5 mice per group. Error bars indicate standard deviation (SD). *, $p<0.05$.

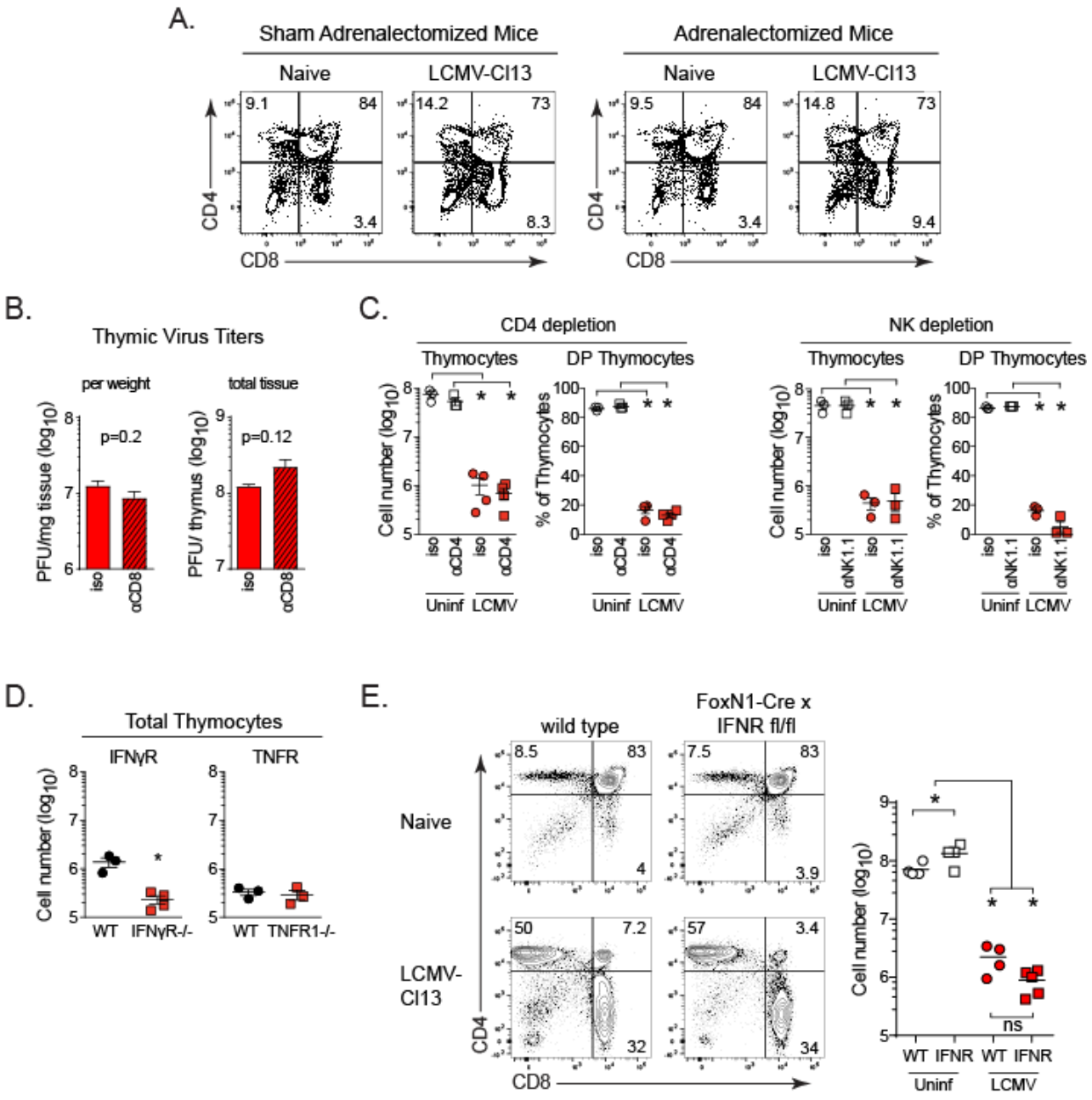

Fig. S2. Cell types and molecules effect on thymic depletion.

(A) Sham adrenalectomized and adrenalectomized mice were left naïve or were infected with LCMV-Cl13 and analyzed 6 days later. **(B)** Thymus virus titers (PFU) from total tissue or normalized per weight of the thymus. Quantification is from isotype control or anti-CD8 depleted mice on day 9 after LCMV-Cl13 infection. **(C)** Mice were treated with isotype, anti-CD4 or anti-NK1.1 depleting antibodies prior to infection. Graphs show total thymocytes and the percent of double positive thymocytes in uninfected mice (black) and in LCMV-Cl13 infected mice (red). **(D)** Graphs show total thymic cellularity on day 9 in

LCMV-Cl13 infected WT mice, IFNγR-/- mice and TNFR1-/- mice. **(E)** WT and FoxN1-Cre x IFNR flox/flox mice (lacking IFNR on thymic stromal cells) were left naïve or infected with LCMV-Cl13. Flow plots show the proportion of thymocytes and the graph shows the total thymic cellularity in each condition. ns; not significant. Data is representative of 2-3 independent experiments with 3-5 mice per group. Error bars indicate SD. *, $p<0.05$.

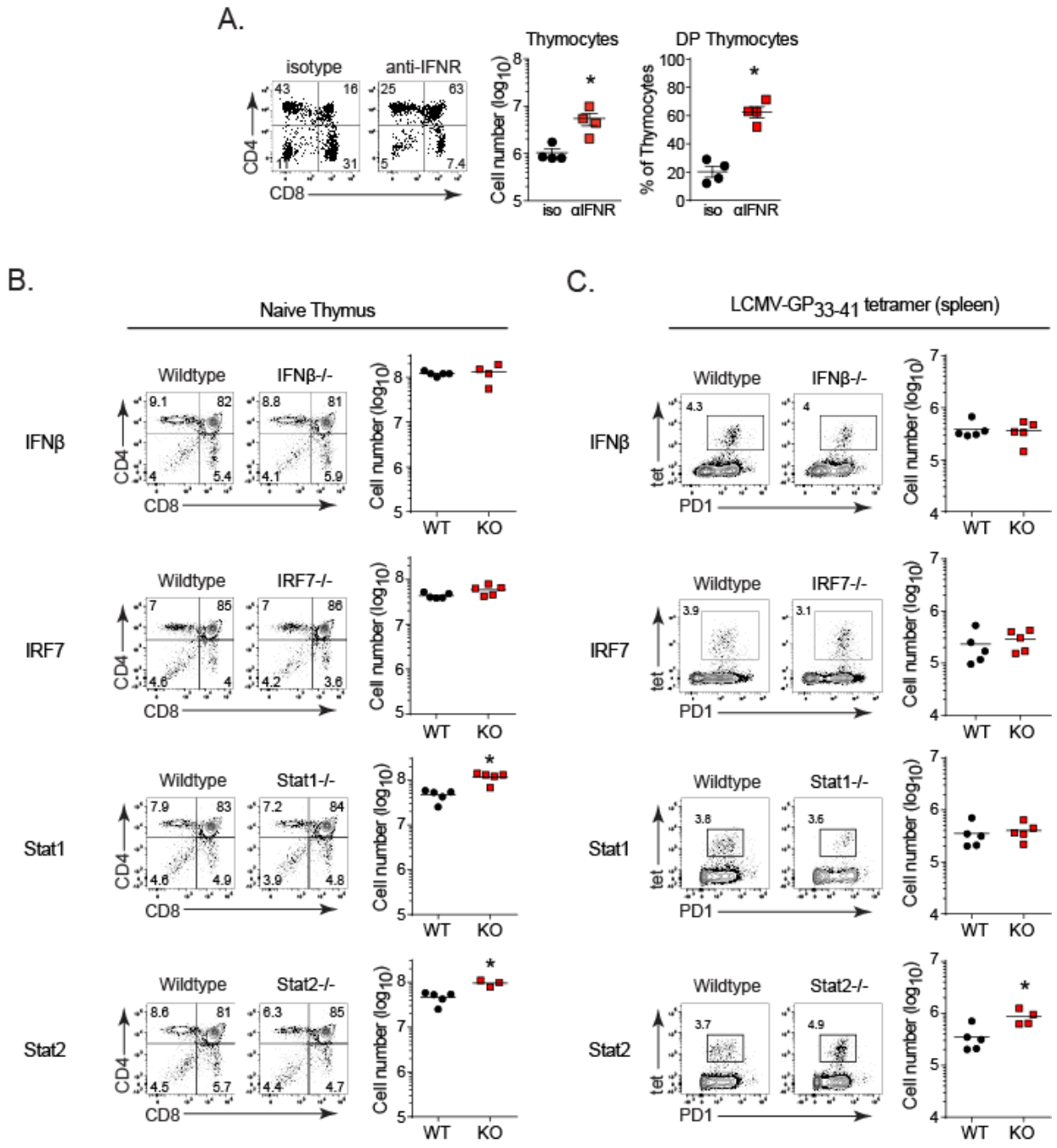

Fig. S3. Lack of thymic depletion in anti-IFNR antibody treated mice and thymocyte profiles and similar thymocyte numbers in naïve wild type, IFNβ-/-, IRF7-/-, Stat1-/- and Stat2-/- mice.

(A) LCMV-Cl13 infected mice were treated with isotype or anti-IFNR blocking antibody beginning 1 day prior to infection and then every other day until day 7. Flow plots show the frequency and bar graphs indicate the number of total and of double positive thymocytes at day 9 after infection. **(B)** Flow plots indicate the CD4 / CD8 distribution and bar graphs the total number of thymocytes in naïve wild type

(WT), or naïve knockout (KO) IFNβ-/-, IRF7-/-, Stat1-/- and Stat2-/- mice. **(C)** The flow plots show the percent and bar graphs the total number of LCMV-GP33-41 tetramer-specific CD8 T cells in the spleen on day 9 after infection of the indicated mouse strain. Data is representative of 2-4 independent experiments with 4-5 mice per group. *, $p<0.05$.

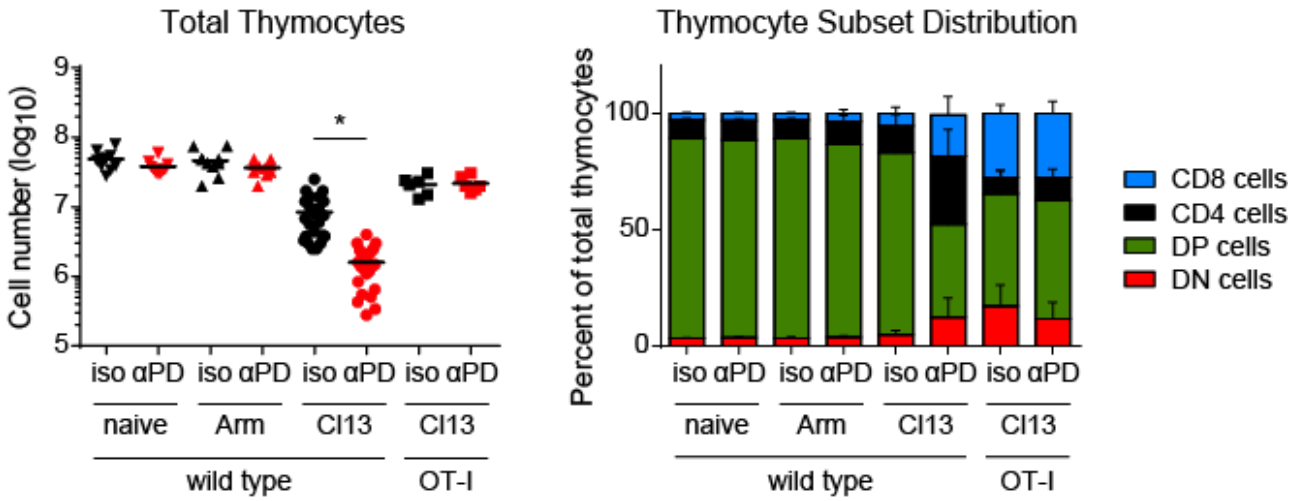

Fig. S4. Anti-PDL1 does not affect thymocyte number or subset distribution in naïve, LCMV-Arm immune or chronically infected OT-I mice.

WT naive mice, WT LCMV-Armstrong immune mice, WT LCMV-Cl13 infected mice or LCMV-Cl13 infected OT-I mice were treated with isotype control (iso) or anti-PDL1 (αPD) blocking antibody beginning 25 days after LCMV-Cl13 infection A total of 3 treatments were given spaced 3 days apart. Graphs indicate (left) total thymus cellularity and (right) the percent of each thymocyte population 2 days following the last treatment. Each symbol in the left graph represents an individual mouse. Data is representative of 2 independent experiments. Error bars indicate SD. * $p<0.05$.

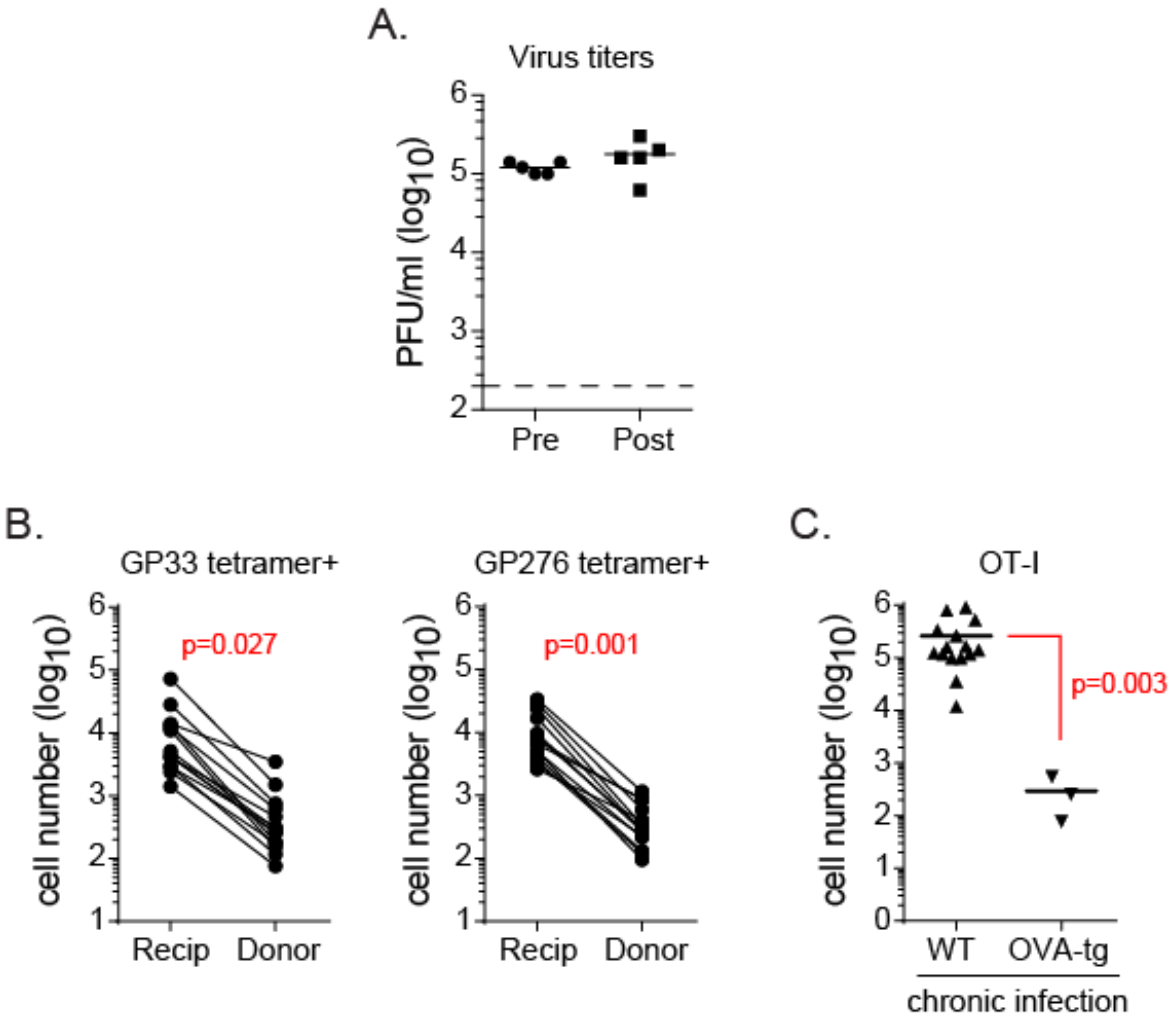

Fig. S5. Busuflan does not alter virus titers in chronically infected mice and chronic infection enables partical escape from negative selection.

(A) Plasma LCMV-Cl13 titers (PFU) were determined two days prior and six weeks after busulfan treatment. These mice received wild type HSC transfer the day after busulfan treatment. Data is representative of 2 experiments with 5 mice per group. **(B)** The number of recipient (CD45.2+) and donor (CD45.1+) LCMV-GP33 tetramer+ (left) or LCMV-GP276 tetramer+ (right) CD8 T cells in the spleen. Data is two experiments combined with experiments containing 5 and 9 mice per group. P-value is shown in each graph. Paired t-test. **(C)** The number of OT-1 CD8 T cells in the spleen of LCMV-Cl13 chronically infected mice. Data is from two experiments combined with experiments containing 3 and 4 mice per group. P-value (paired t-test) is shown in each graph.