

Figure S1. Titration of IL-2 injections during chronic LCMV infection. C57BL/6 mice were infected with LCMV clone 13 and beginning on day 23-27 p.i. the mice were treated with 200 μ g of anti-PD-L1 antibody every three days for 12 days (5 total treatments). All IL-2 treated groups were given 1.5X10⁴ IU of IL-2 (i.p.) once (1X) or twice (2X) a day for the last 8 days of anti-PD-L1 treatment. (A) Number of H-2D^b GP33 & GP276 specific CD8 T cells in the blood 1 day post-last treatment. (B) Number of CD8 T cells simultaneously producing IFN- γ and TNF- α in the spleen after ex-vivo re-stimulation with the indicated peptides. (C) Viral titer in the serum 1 day post-last treatment as quantified by plaque assays.

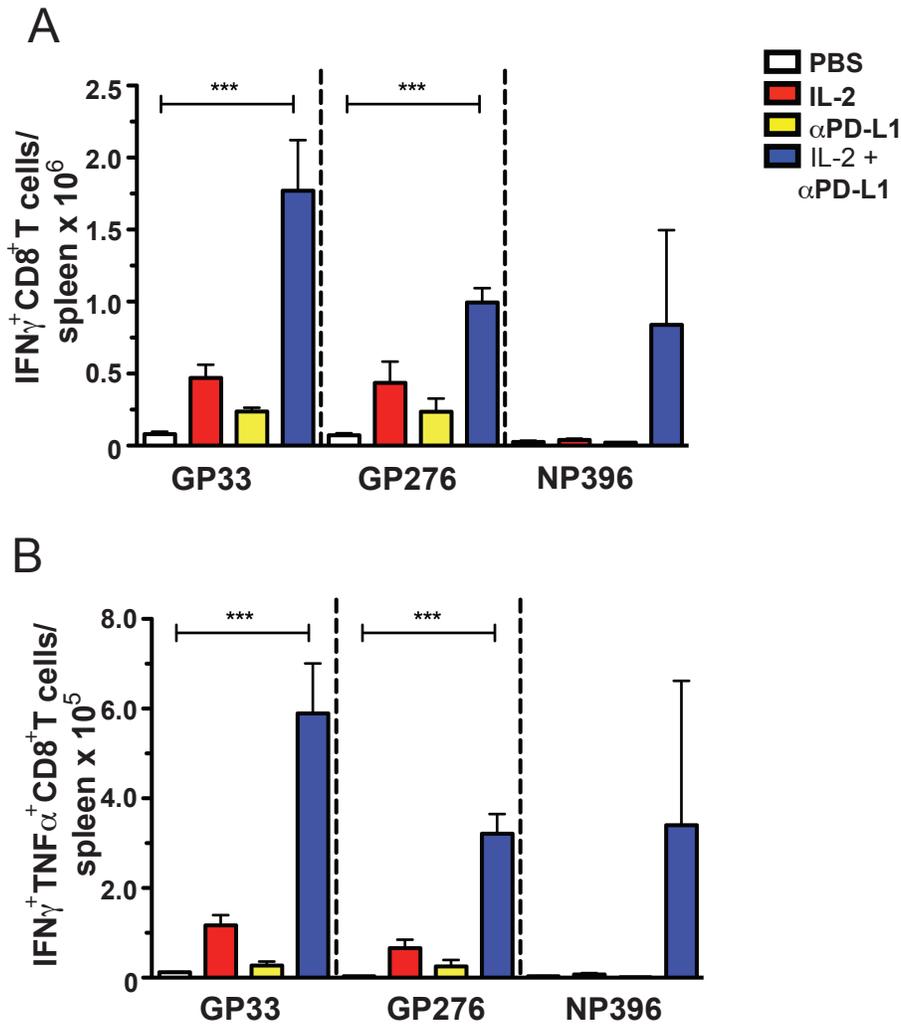


Figure S2. IL-2 therapy combined with PD-L1 blockade enhances antiviral CD8 T cell cytokine production during chronic LCMV infection. C57BL/6 mice were infected with LCMV clone 13 and beginning on day 23-27 p.i. the mice were treated with 200 μ g of anti-PD-L1 antibody every three days for 12 days (5 total treatments). All IL-2 treated groups were given 1.5X10⁴ IU of IL-2 (i.p.) once a day for the last 8 days of anti-PD-L1 treatment. (A) Number of IFN- γ producing and (B) simultaneous IFN- γ and TNF- α producing CD8 T cells in the spleen after ex-vivo re-stimulation with the indicated peptides. Representative results of 3 separate experiments with at least 4 mice per group per experiment.

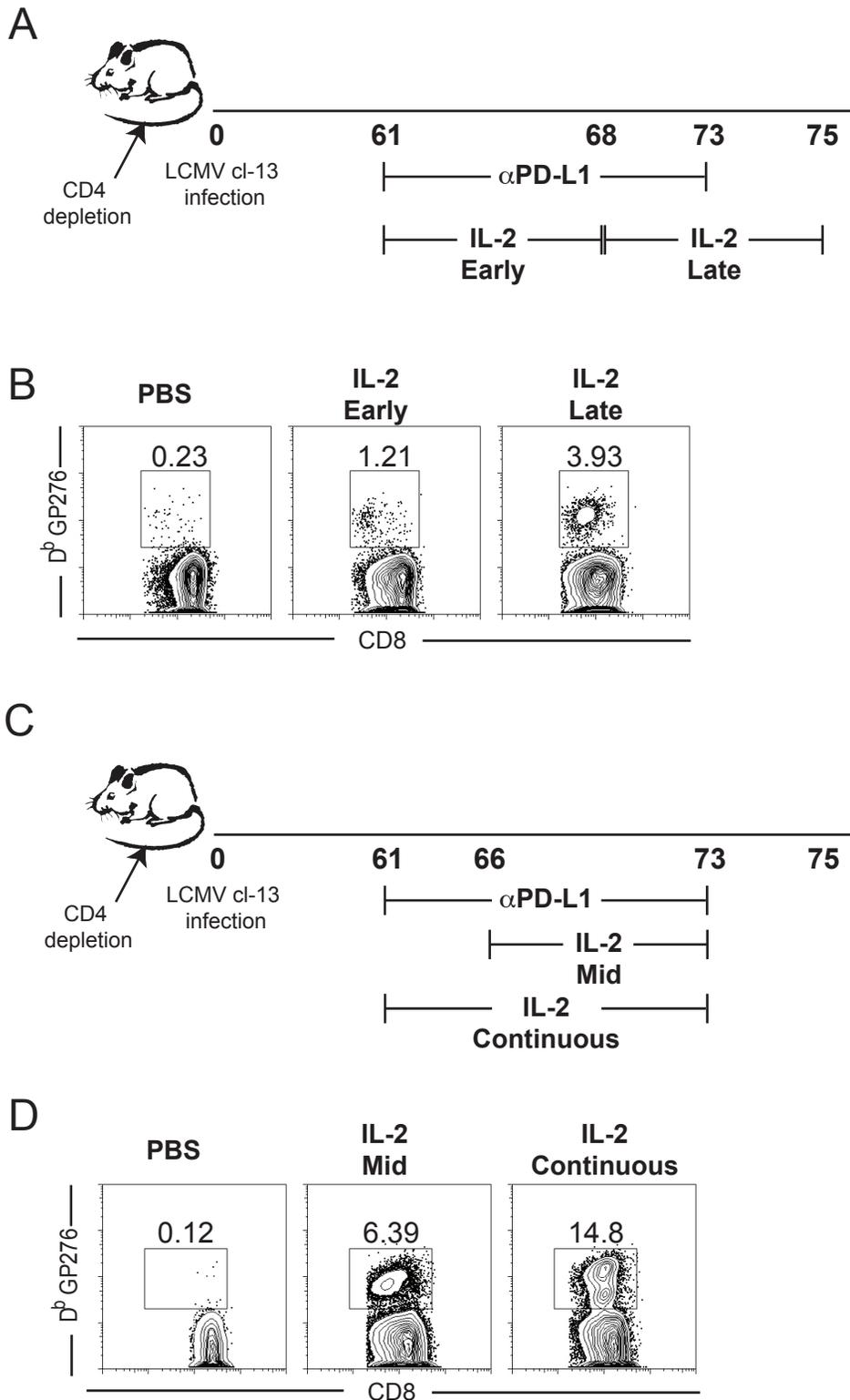


Figure S3. Timing of IL-2 therapy and PD-L1 blockade during chronic LCMV infection. C57BL/6 mice were depleted of CD4 T cells and infected with LCMV clone 13. After day 60 post-infection, the appropriate groups of mice were treated with either PBS/isotype antibody, 200 μ g of anti-PD-L1 antibody every three days for 12 days (5 total treatments), and/or IL-2 (i.p.). IL-2 treated groups were given 1.5 \times 10⁶ IU of IL-2 (i.p.) twice a day for either 8 days or the duration of the anti-PD-L1 treatment (as indicated in (A) and (C)). (A) Experimental set-up for part B. (B) Frequency of H-2D^b GP276 specific CD8 T cells in the blood 3 days after the last IL-2 treatment (gated on CD8 cells). (C) Experimental set-up for part D. (D) Frequency of H-2D^b GP276 specific CD8 T cells in the blood 2 days after the last treatment (gated on CD8 cells).

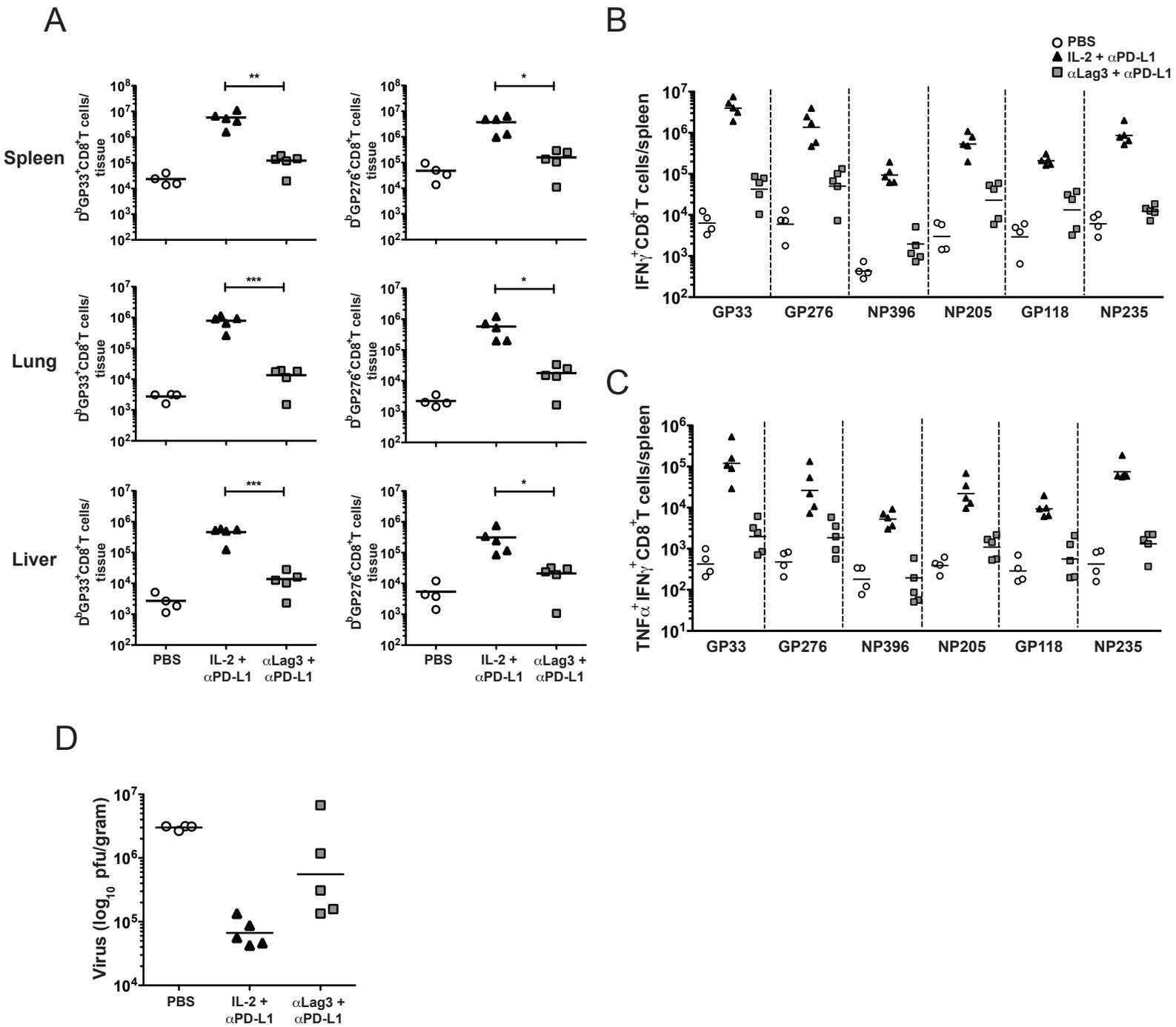


Figure S4. Comparison of combined IL-2 therapy and PD-L1 blockade to co-blockade of Lag-3 and PD-L1.

C57BL/6 mice were depleted of CD4 T cells and infected with LCMV clone 13. After day 100 post-infection, appropriate groups of mice were treated with either PBS/isotype antibody, 200 μ g of anti-PD-L1 antibody every three days for 12 days (5 total treatments), plus either 1.5X10⁴ IU of IL-2 (i.p.) twice a day for the duration of the anti-PD-L1 treatment or 200 μ g of anti-Lag antibody every three days for the 12 days. (A) Number of GP33 and GP276 CD8 T cells tissues 1 day post-last treatment. (B) Number of IFN- γ producing CD8 T cells in the spleen after ex-vivo re-stimulation with the indicated peptides. (C) Number of simultaneous IFN- γ and TNF- α producing CD8 T cells in the spleen. (D) Viral titer in the spleen 1 day post-last treatment as quantified by plaque assays. 4 (PBS group) or 5 mice were used per treatment group. * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = non-significant. Note: in 4B all IL-2 + aPD-L1 group values compared to Lag-3 + aPD-L1 are at least $p < 0.05$.