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Supplemental Information

A Minimum Epitope Overlap between Infections Strongly Narrows the Emerging T Cell Repertoire

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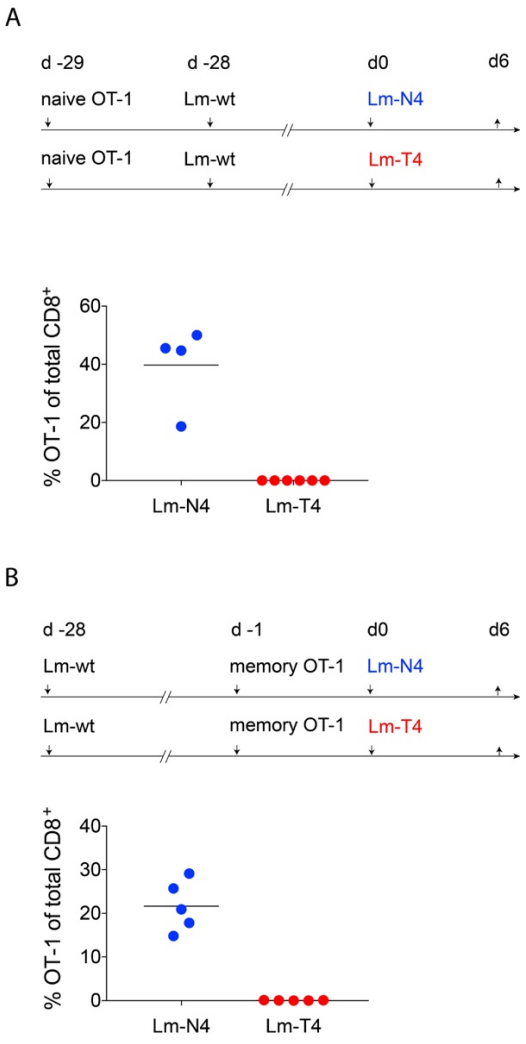
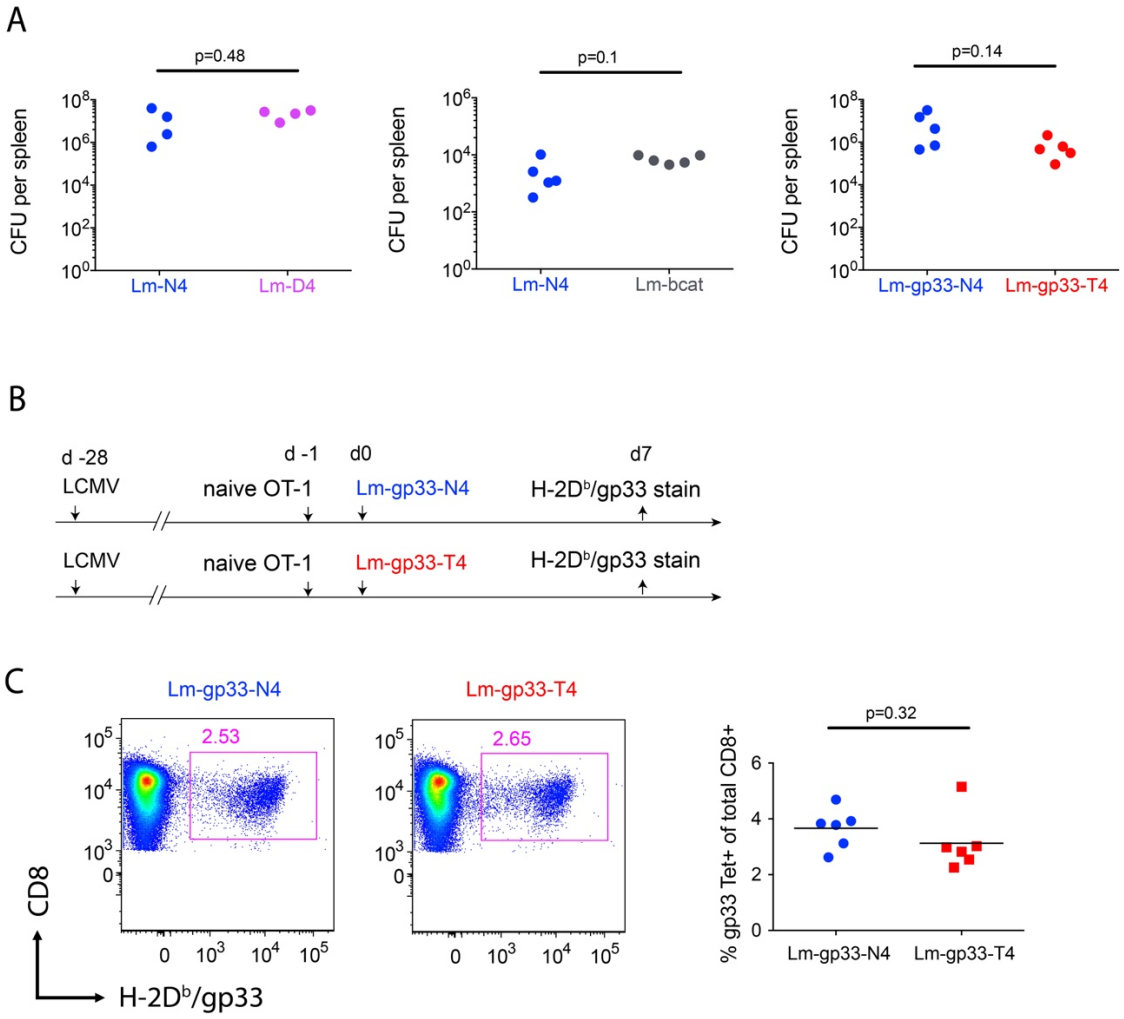
Supplementary Figure 1 (related to Figure 1):



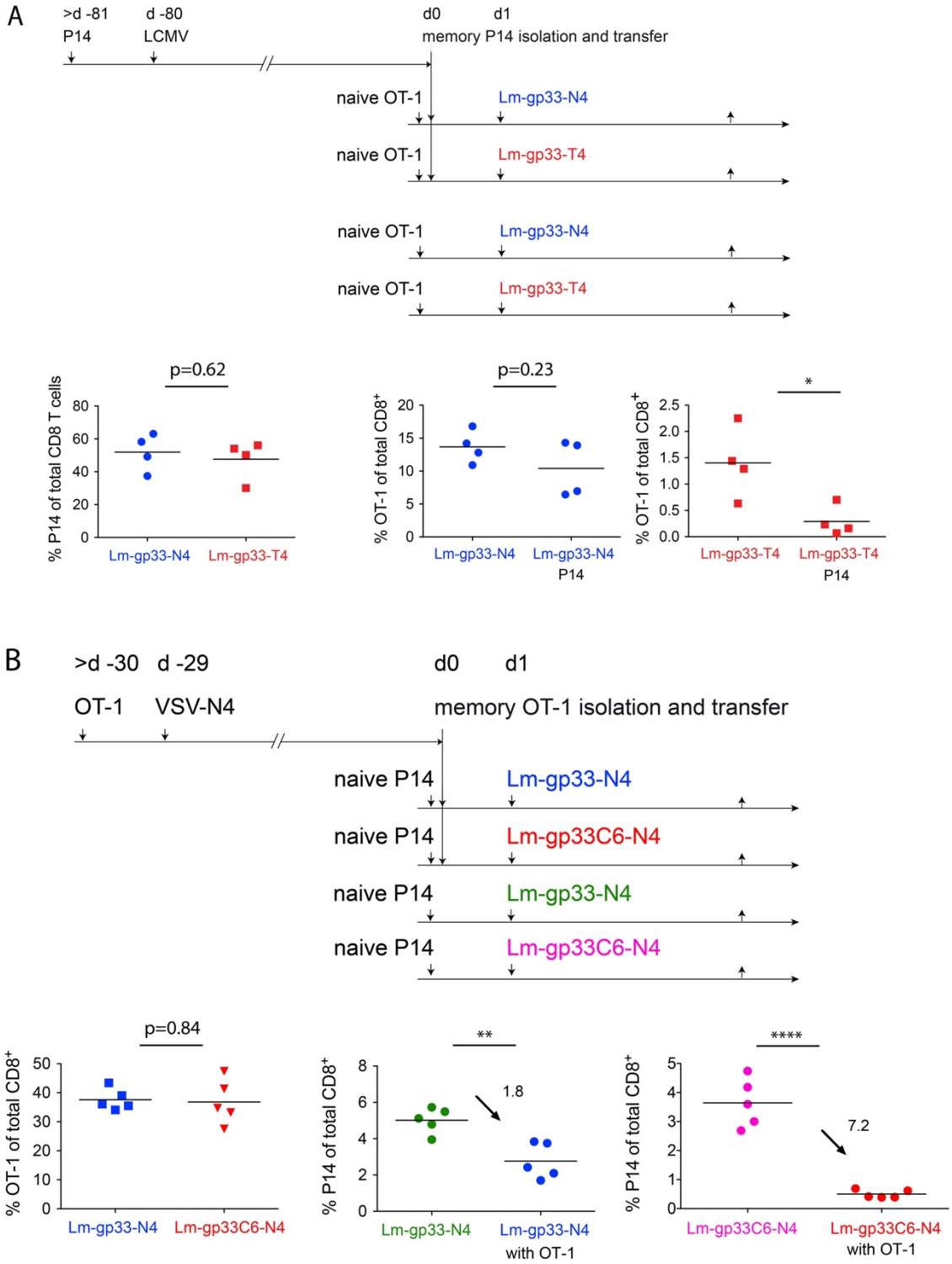
OT-1 transferred prior to the primary Lm-wt infection and memory OT-1 also fail to respond to low affinity de novo stimulation in secondary *Listeria* infections. A: In contrast to the setup in figure 1, the OT-1 T cells were transferred into mice prior to the initial *Listeria* wildtype infection. Four weeks later these mice or uninfected control mice were infected with the indicated *Listeria* strains. Frequency of OT-1 T cells among peripheral blood $CD8^+$ T cells was measured 6 days after infection. **B:** The same setup as in figure 1 has been used except that memory instead of naïve OT-1 were transferred. The memory T cells are derived from mice that had >30 days ago been grafted with OT-1 and infected with Lm-Ova. Shown is the frequency of OT-1 T cells among peripheral blood $CD8^+$ T cells measured 6 days after the infection.

Supplementary Figure 2 (related to Figure 3):



Recombinant Lm-gp33-N4 and Lm-gp33-T4 expressing *Listeria* show similar capacity to stimulate gp33-specific T cells and similar in vivo growth rates. A: Naïve C56BL/6 mice were infected with the indicated strains of *Listeria*. 2-4 days later, the numbers of colony forming units per spleen were enumerated. **B:** Schematic illustration of the experimental procedure. Naïve CD45.1 C57BL/6 mice were infected with LCMV. 28 days later mice received 10^4 naïve CD45.2 congenic OT-1 T cells and were infected with the indicated *Listeria* strains. **C:** Six days later, gp33-MHC multimers were used to determine the frequency of gp33-specific T cells among total endogenous $CD8^+$ T cells in these mice. The dot plots show representative stainings and the graphs data for all mice in the presented experiment.

Supplementary Figure 3 (related to Figure 4):



Transferred memory P14 and OT-1 T cells selectively suppress the response to low affinity ligands but unlike endogenous memory T cells, they fail to completely suppress the

response. A: Similar as in **Figure 4A** and **B** we transferred $5x10^5$ memory CD45.1/1 congenic P14 T cells into naïve C57BL/6 hosts but the memory P14 cells were this time harvested from C57BL/6 hosts that had 80 days ago been transferred with congenic naïve P14 T cells and infected with LCMV. These memory P14 were then transferred along with CD45.1/2 congenic 10^4 naïve OT-1 and the C57BL/6 hosts were infected with the indicated recombinant *Listeria* strains. We enumerated on day 6 post infection the numbers of P14 and OT-1 T cells in the blood. **B:** Naive CD45.1/2 OT-1 T cells were first transferred into naïve C57BL/6 hosts followed by an infection with a recombinant strain of VSV that expresses the Ova antigen (VSV-Ova). Memory OT-1 T cells were harvested 29 days later and transferred into naïve C57BL/6. These hosts were engrafted with naïve CD45.1/1 P14 T cells and infected with the indicated recombinant *Listeria* strains expressing Ovalbumin and a high (Lm-gp33-N4) or low affinity epitope for P14 T cells (Lm-gp33C6-N4). Number of P14 and OT-1 were determined 6 days later in the blood. Statistical analyses are unpaired Student's t tests with * $p< 0.1$, ** $p< 0.01$, and **** $p< 0.0001$.