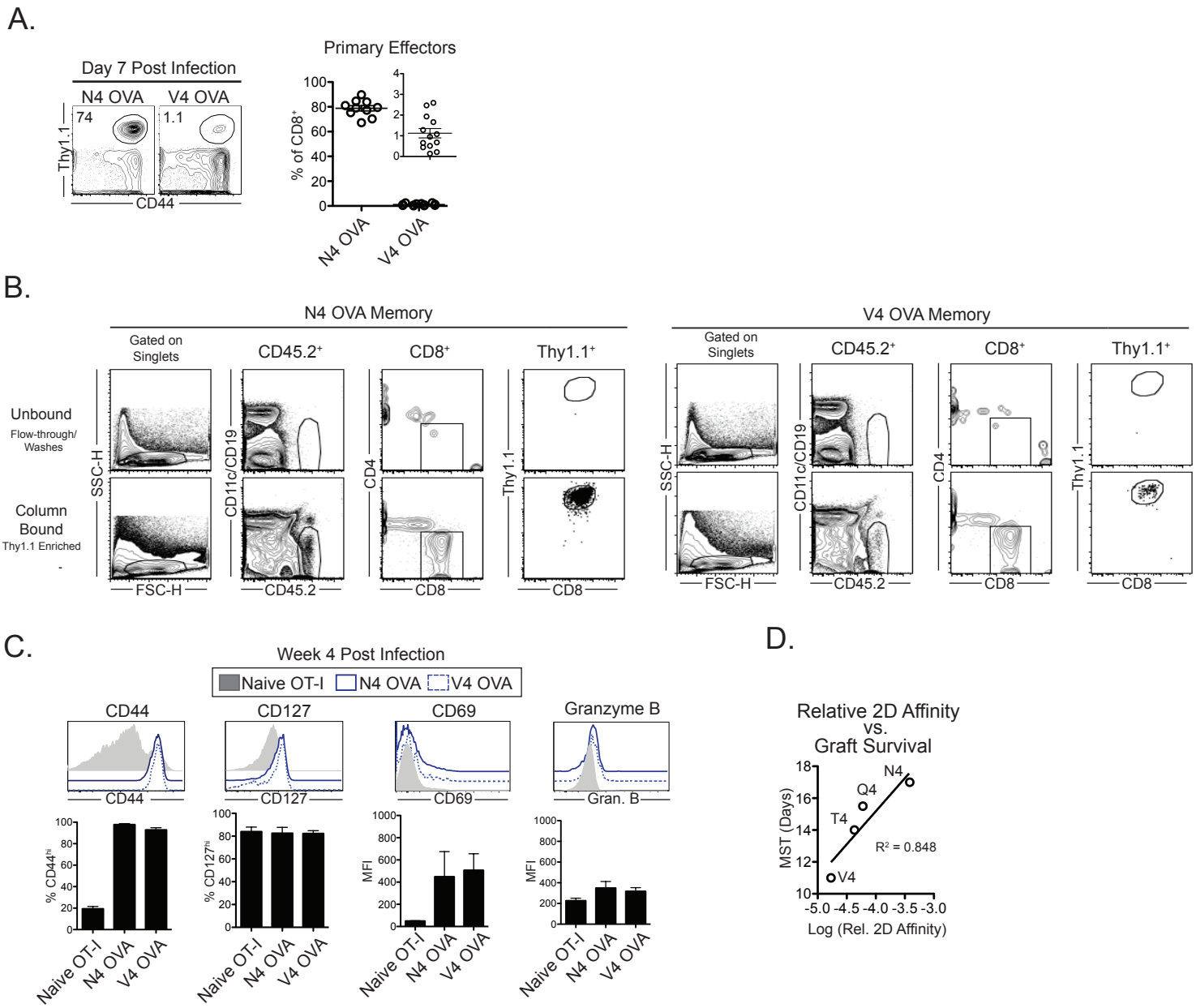
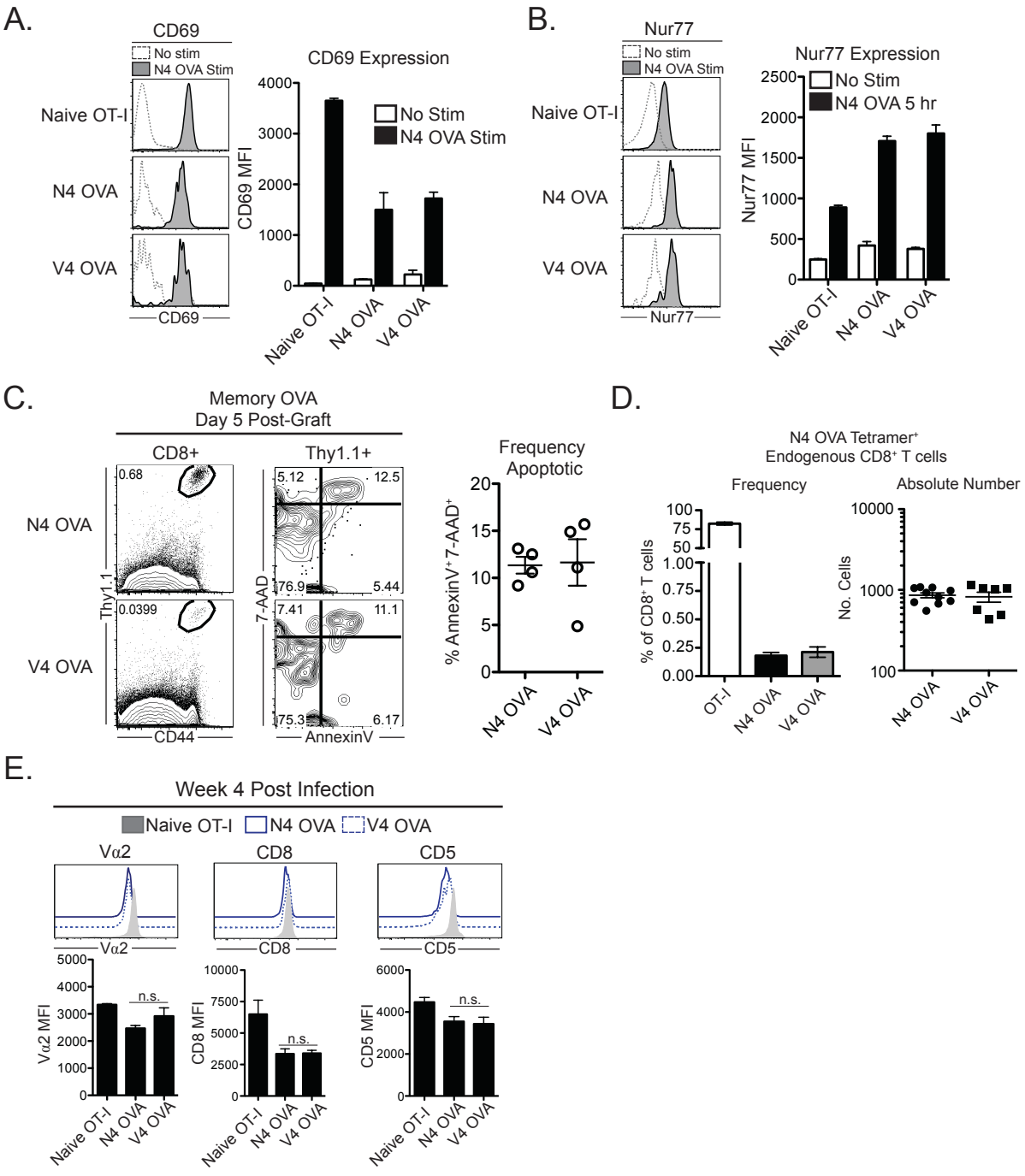
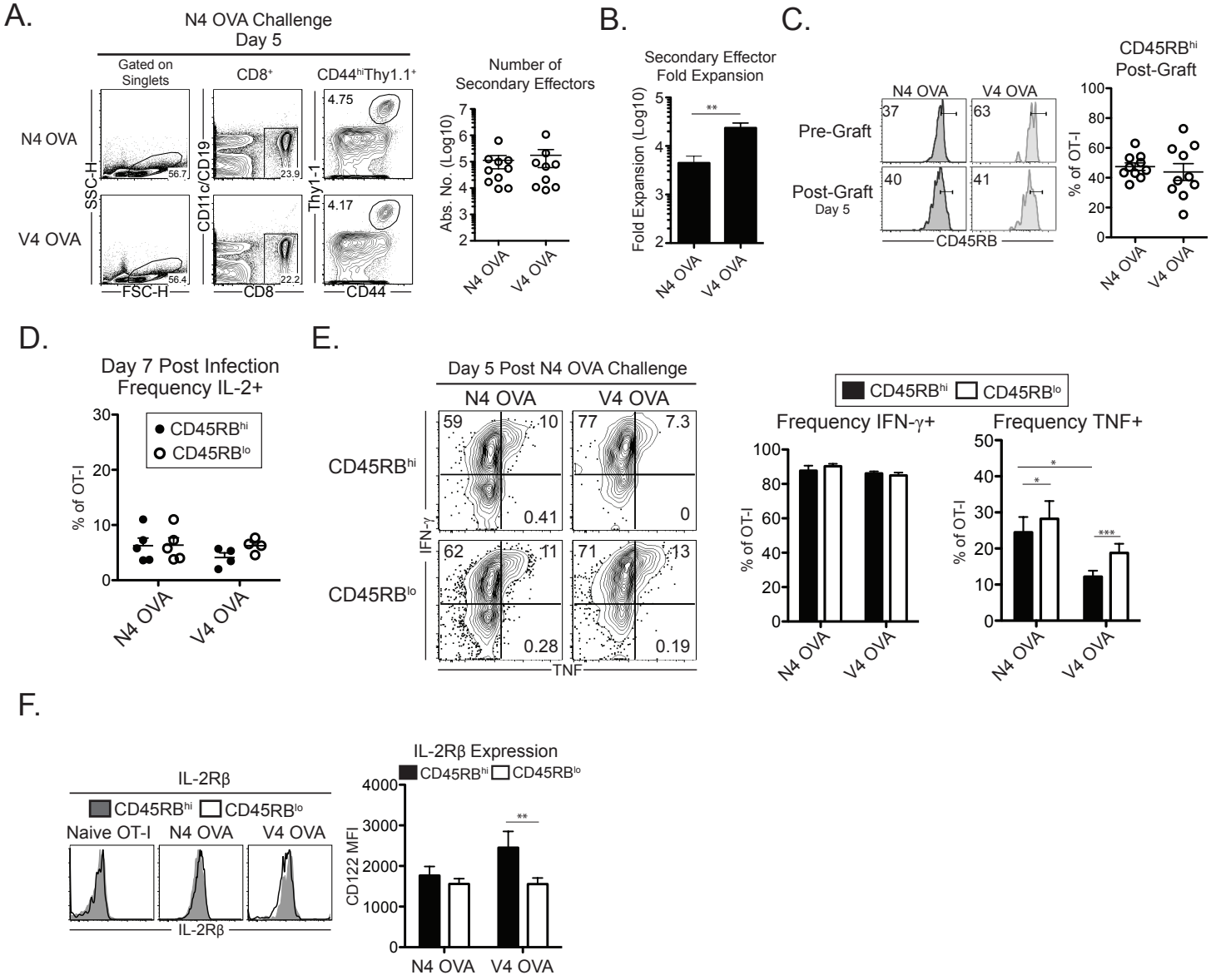


Supplemental Figure 1. High and low affinity priming of OT-I T cells elicits stable memory populations. Naïve mice adoptively transferred with 10^4 OT-I T cells were infected the following day with either LM-N4 OVA or LM-V4 OVA. (A) Thy1.1$^+$ OT-I memory T cells were assessed in the blood on day 7 post infection. (B) Thy1.1$^+$ OT-I memory T cells were assessed in pooled the spleen and lymph nodes 4 weeks post infection using anti-Thy1.1 PE and anti-PE magnetic microbeads. Representative gating of the unbound (column flow-through and washes) fraction devoid of Thy1.1$^+$ cells and the bound (column elution) fraction enriched for Thy1.1$^+$ OT-I memory cells. (C) Expression of CD44, CD127, CD69, and Granzyme B in OT-I T cells in secondary lymphoid organs 4 weeks post infection. (D) Mice infected with LM-N4 OVA, LM-Q4 OVA, LM-T4 OVA, or LM-V4 OVA were transplanted with N4 OVA skin grafts 5 weeks post infection and the correlation between graft mean survival time and relative 2D affinity values of OT-I cells for OVA APLs was assessed (R^2=0.848, N4 OVA MST=17 d, Q4 OVA MST 15.5 d, T4 OVA MST 14 d, V4 OVA MST=11 d). Data from A, C, and D compiled from 2-3 experiments. *$p<0.05$.



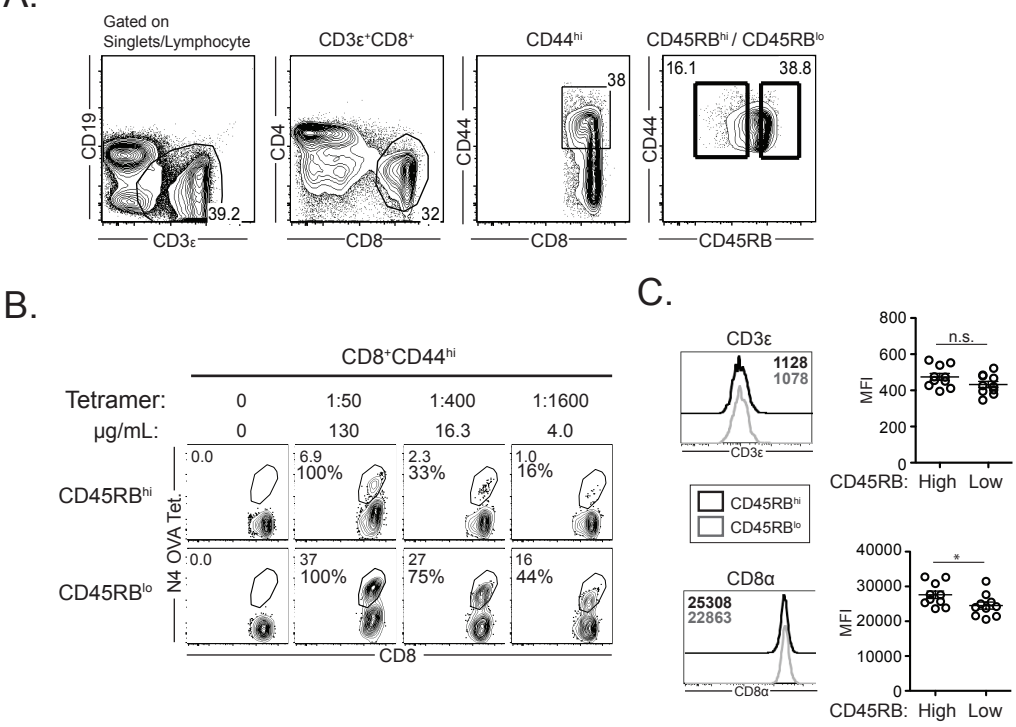
Supplemental Figure 2. High and low affinity primed memory CD8$^+$ T cells express similar levels of TCR signal tuning molecules and undergo similar rates of apoptosis. Naïve mice adoptively transferred with 10^4 OT-I T cells were infected the following day with either LM-N4 OVA or LM-V4 OVA. (A-B) Naïve OT-I, N4 OVA memory, or V4 OVA memory cells were briefly restimulated with N4 OVA peptide and assessed for (A) surface CD69 or (B) intracellular Nur77 expression. (C) Mice containing N4 OVA or V4 OVA primed memory OT-I cells were transplanted with N4 OVA skin grafts 5 weeks post infection and the frequency of apoptotic AnnexinV$^+$7-AAD$^+$ secondary effectors was assessed in the draining lymph node. (D) Frequency and absolute number of N4 OVA specific endogenous CD8$^+$ T cells in mice containing N4 OVA or V4 OVA primed OT-I memory cells 5 weeks post infection. (E) Expression of Vα2, CD8, and CD5 on naïve or memory OT-I T cells 4 weeks post infection. *p<0.05.*p<0.05.

Supplemental Figure 3



Supplemental Figure 3. High and low affinity primed memory CD8$^+$ T cells expand following high affinity peptide immunization. Naïve mice were adoptively transferred with 10^4 OT-I T cells and infected the following day with either LM-N4 OVA or LM-V4 OVA. (A-B) Four weeks post infection mice were challenged with N4 OVA peptide in the foot pad and cells were assessed in the draining popliteal lymph nodes 5 days later. (A) Gating strategy and summary frequency of secondary effector OT-I cells among CD8$^+$ T cells. (B) Fold induction of N4 OVA and V4 OVA primed secondary effectors. (C) Five weeks post infection, mice were grafted with N4 OVA skin and cells were assessed in the draining popliteal lymph node 5 days later. CD45RB expression in resting (pre-graft) memory N4 OVA and V4 OVA primed memory OT-I cells. (D) Expression of IL-2 by N4 OVA or V4 OVA primed effectors on day 7 post infection in the spleen. (E) Representative IFN-γ and TNF expression following brief N4 peptide restimulation in CD45RB^{hi} and CD45RB^{lo} fractions. (F) IL-2Rβ (CD122) expression in CD45RB^{hi} and CD45RB^{lo} fractions of secondary effector OT-I cells. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 4



Supplemental Figure 4. Low affinity CD8+ T cells are CD45RB^{hi}. Naïve mice were infected with LM-N4 OVA mice and cells were assessed in the spleen on day 10-14 post infection. (A) Gating strategy for assessment of relative 2D affinity and functional avidity of CD8^{+}CD44^{hi} CD45RB^{hi} and CD45RB^{lo} cells in the spleen. (B) Frequency of tetramer staining among CD45RB^{hi} and CD45RB^{lo} fractions of CD8^{+}CD44^{hi} T cells. Expression of (C) CD3ε and CD8α among CD45RB^{hi} and CD45RB^{lo} CD44^{hi}CD8^{+} T cells on day 10 post infection. ***p<0.001.