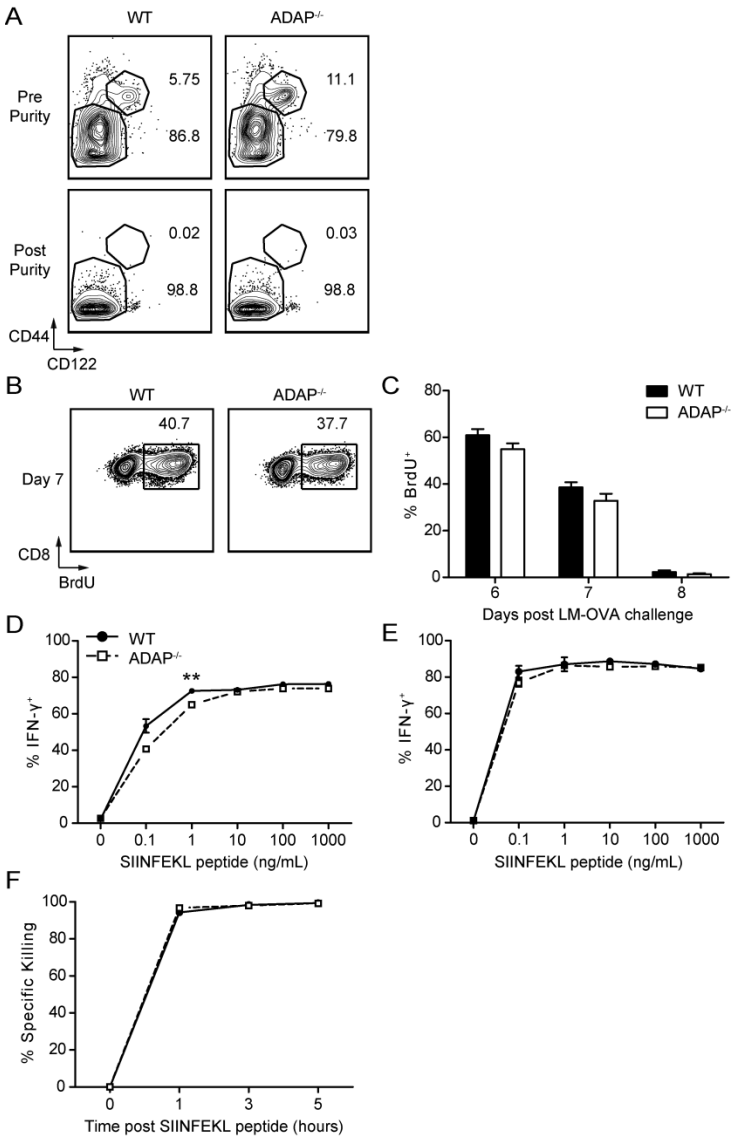
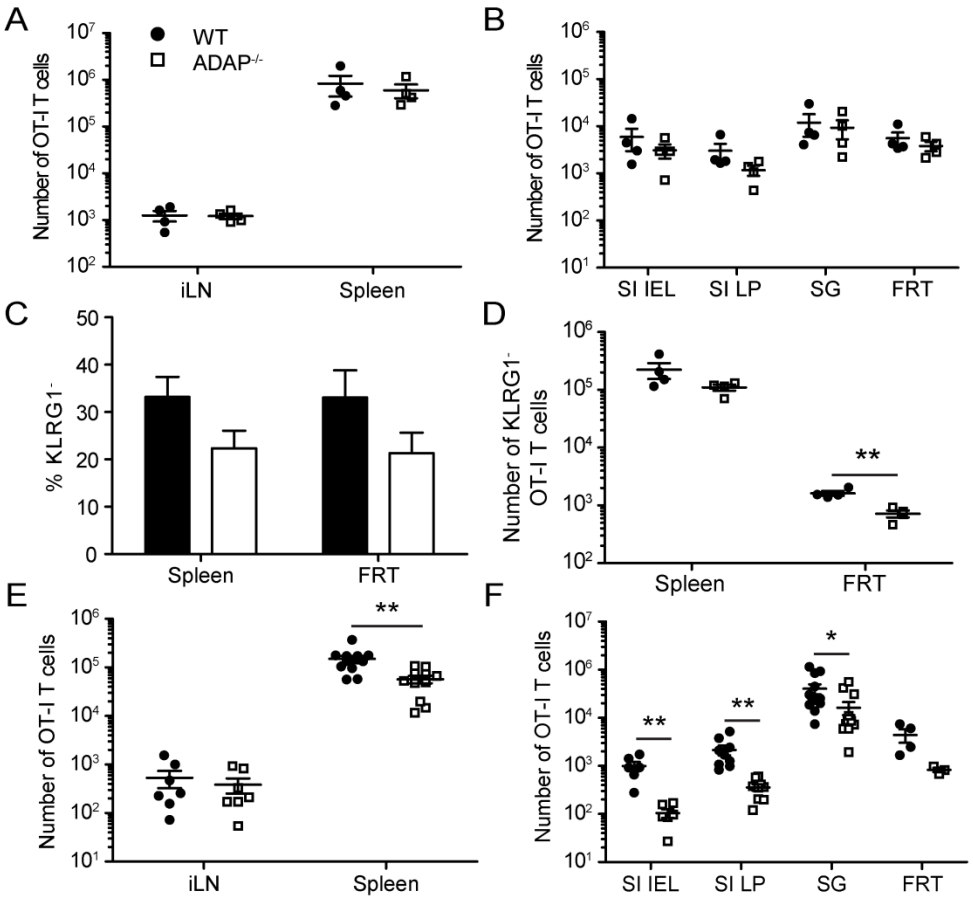
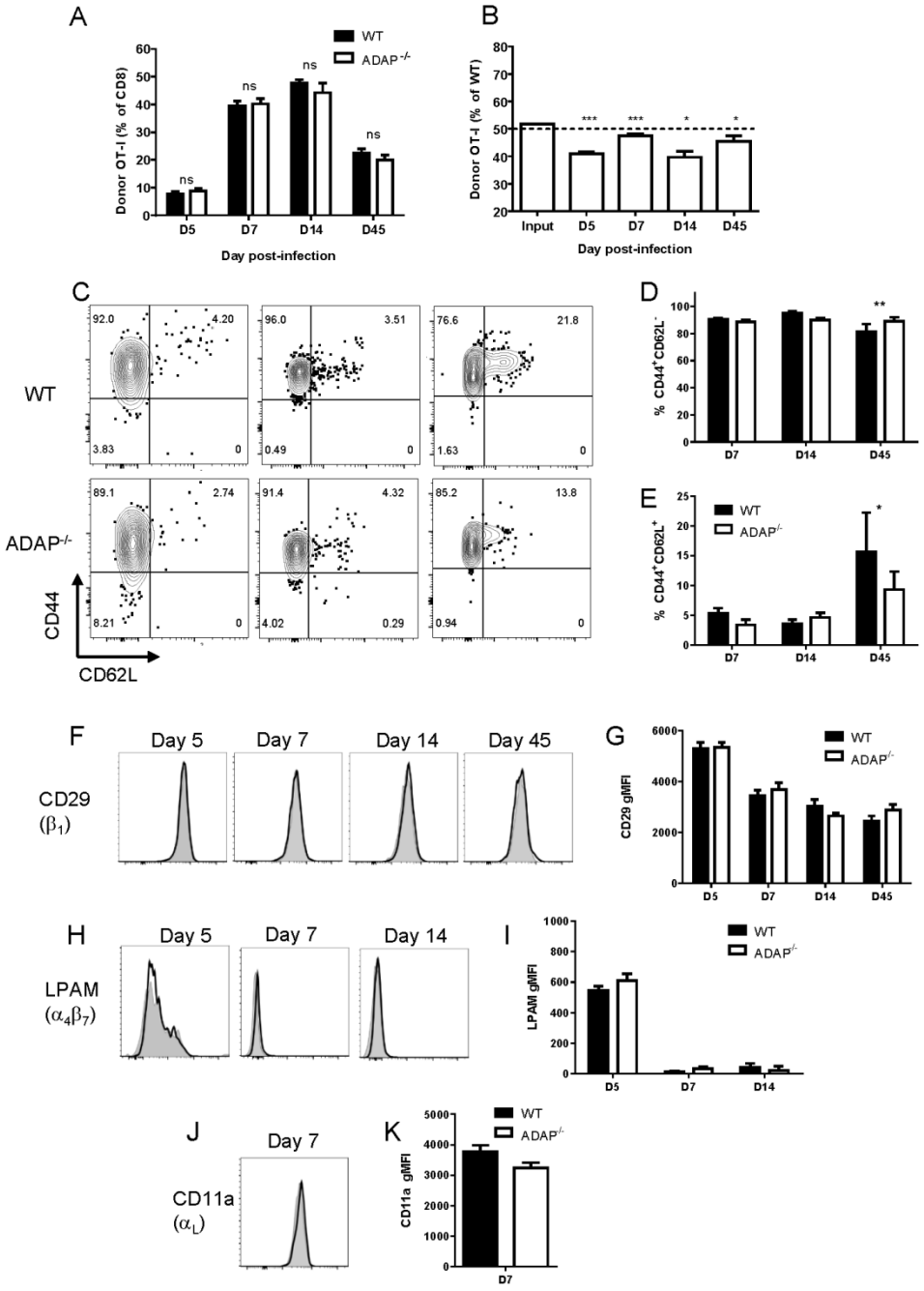


Supplemental Figure 1. Analysis of ADAP$^{-/-}$ OT-I T cells following LM-OVA infection. (A) Example of CD122 and CD44 staining of peripheral lymph node CD8 T cells from WT and ADAP$^{-/-}$ OT-I mice, pre and post purification. Gates indicate the percentage of either CD122^{lo} CD44^{lo} or CD122^{hi} CD44^{hi} populations. (B-E) LM-OVA challenged mice were generated as in Figure 1. (B-C) Mice were pulsed with 1 mg of BrdU i.p. for 5 hours on the day of harvest. (B) Example BrdU staining in the spleen at day 7 post infection. (C) Percentage of BrdU^{+} WT and ADAP$^{-/-}$ OT-I T cells from the spleen. (D-F) Naïve CD45.1 WT or ADAP$^{-/-}$ OT-I T cells were transferred into CD45.2 recipients and infected with LM-OVA as described in Fig. 2. (D and E) Splenocytes were isolated on day 7 post-infection and stimulated e*x vivo* with the indicated doses of SIINFEKL peptide for 3 hours (A) or 5 hours (B) in the presence of Golgi-Plug. The cells were then isolated and stained for cell surface markers and IFN-γ. (F) WT (CD45.1) or ADAP$^{-/-}$ (CD45.1) OT-I T cells were transferred into separate CD45.2 hosts, and challenged with LM-OVA. On day 7 post challenge, an *in vivo* killing assay was performed as described in *Materials and Methods* and in Fig. 2E-F. Spleens were harvested 1-5 hours after transfer of targets and specific killing was determined at each time point.

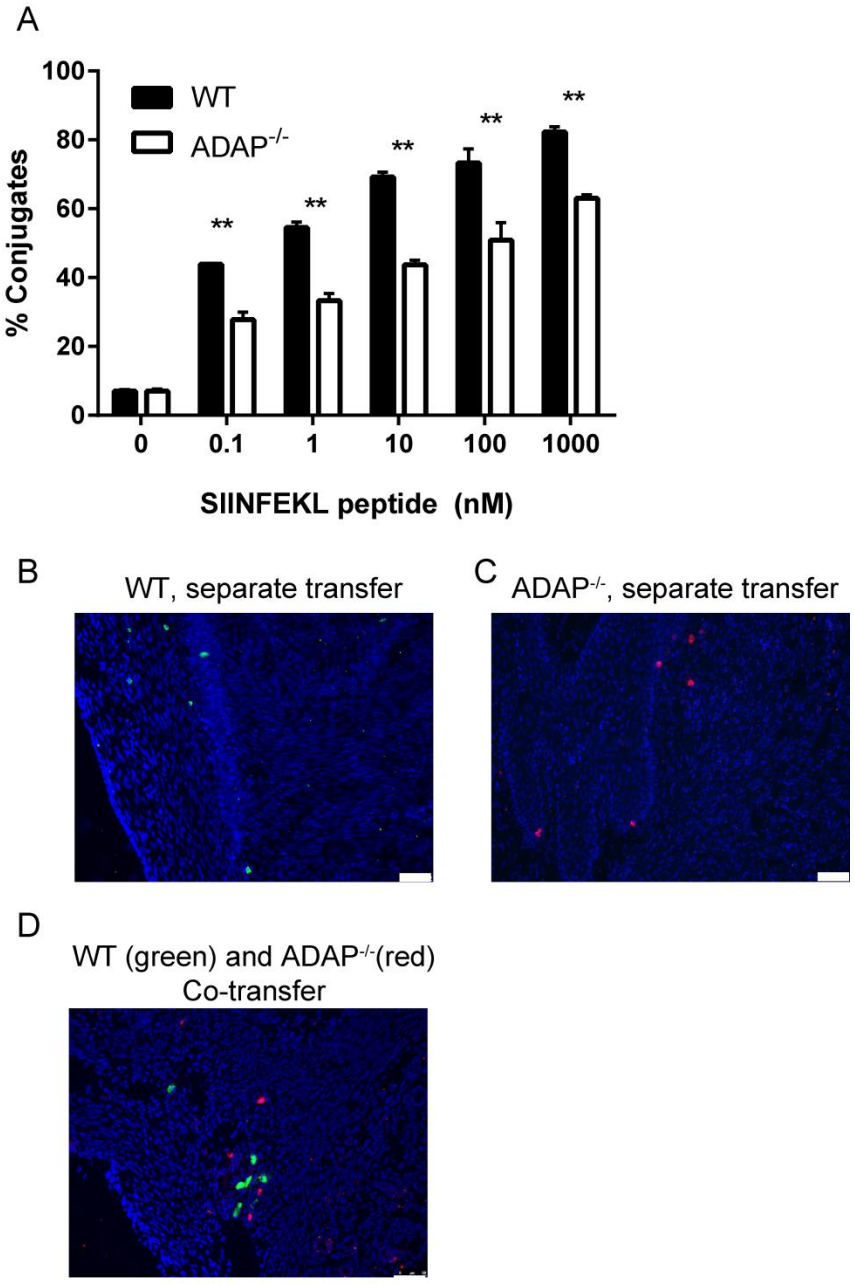


Supplemental Figure 2. CD8 T_{RM} precursors are reduced in the absence of ADAP. LM-OVA challenged mice were generated as in Figure 1. At day 7 (A-D) or day 33^{+} (E-F) animals were harvested and assessed for the number of wild-type and $ADAP^{-/-}$ OT-I T cells. (**A** and **E**) Number of WT (black circles) or $ADAP^{-/-}$ (open squares) OT-I T cells in the iLN or spleen. (**B** and **F**) Number of wild-type and $ADAP^{-/-}$ OT-I T cells from NLTs. (**C**) Percentage or (**D**) number of $KLRG1^{-}$ WT and $ADAP^{-/-}$ OT-I T cells in the spleen or FRT. **, $p < 0.01$.



Supplemental Figure 3. Analysis of circulating ADAP$^{-/-}$ OT-I T cells following VSV-OVA infection. VSV-OVA mice were made as described in Fig 5. Blood was collected at the indicated time points. (A) The expansion and maintenance of donor T cells is expressed as the frequency of total CD8 T cells per mouse. (B) The frequency of ADAP$^{-/-}$ OT-I T cells in the blood at each time point is expressed as a percentage of WT OT-I T cells. (C-E) Blood was stained for the donor T cell populations and for CD44 and CD62L, and analyzed by flow cytometry. (C) Representative flow cytometry plots, with quadrant markers defined based on the position of CD44^{-} and CD62L^{+} endogenous (host) CD8 T cells. (D) Quantification of the

frequency of each donor population with T_{EM} phenotype ($CD44^+CD26L^-$) and (E) with T_{CM} phenotype ($CD44^+CD62L^+$). (F-K) Blood was stained for the donor T cell populations and expression of the indicated integrins was analyzed by flow cytometry. Representative overlays are shown for the indicated integrins (F, H and J). WT cells are depicted with a black line and $ADAP^{-/-}$ cells are shaded gray (G, I, and K). The data (A,B, D, E, G, I and K) is from 5-7 mice per group, per time point, ($\pm$ SEM).



Supplemental Figure 4. Dose response of impaired T:APC conjugate formation by ADAP$^{-/-}$ T_{RM} cells isolated from NLTs and tissue distribution of WT and ADAP$^{-/-}$ memory T cells. Naïve WT (CD45.1 for single-transfer and Thy1.1 for co-transfer) or ADAP$^{-/-}$ (CD45.1) OT-I T cells were co-transferred (A, D) or separately transferred (B, C) into CD45.2 hosts, and challenged with VSV-OVA. (A) On day 120 following infection, animals were sacrificed and the spleen was isolated and processed to generate single cell suspensions and aliquots mixed with DC-enriched splenocytes that were pre-pulsed with the indicated concentrations of SIINFEKL peptide. The frequency of T:APC conjugate formation was determined as described in Figure 8. (B-D) Tissues were isolated from mice 60 days following infection and stained for WT OT-I (B, green) and ADAP$^{-/-}$ OT-I (C, red). (D) Cotransferred ADAP-/- (red) and WT (green) were stained together. Bars in B-D = 50 μm.