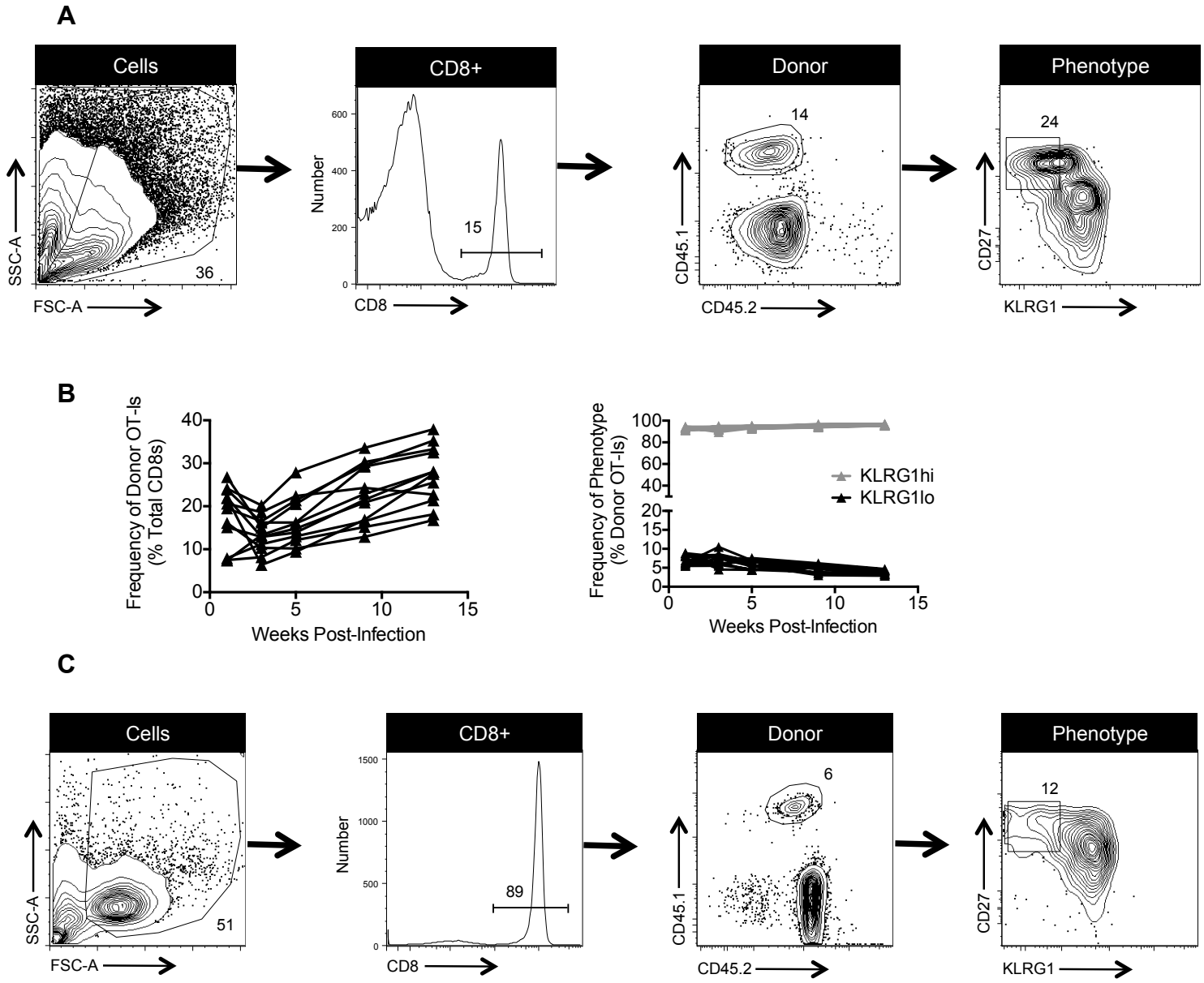
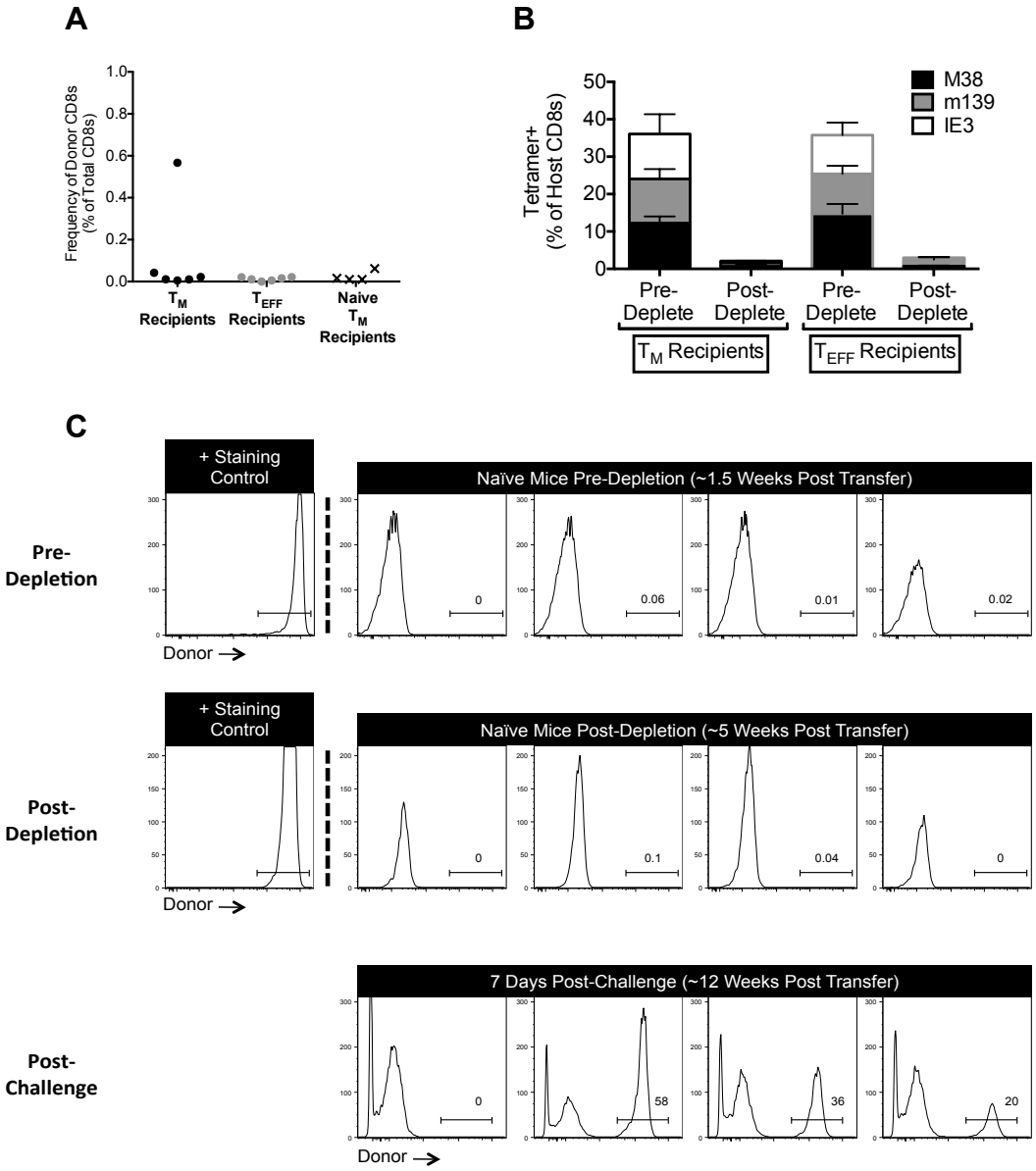
Supplemental Fig. 1



Supplemental Figure 1: Representative plots for repeat transfers and data from secondary stimulated OT-Is

(A) Sorting strategy for isolating OT-I T_M T cells after primary challenge. Briefly, 600 naïve OT-I T cells expressing CD45.1 were transferred into naive B6 (CD45.2) recipients and challenged MCMV-SL8, (i.e. primary challenge). Thirteen weeks after the primary challenge, donor OT-I T cells were sorted using the CD45.1 marker and the T_M subset was identified as $CD27^{hi}$ and $KLRG1^{lo}$. (B) 6,000 sorted T_M OT-I T cells were transferred into naïve B6 recipients and challenged with MCMV-SL8 (i.e. secondary challenge). Shown is the frequency and phenotype of blood-localized OT-Is over time, relative to total $CD8^{pos}$ T cells. Data was collected from two independent experiments (n=7 total). (C) Sorting strategy for isolating OT-I T_M T cells after secondary challenge. Briefly, thirteen weeks after the secondary challenge, donor OT-I T cells were sorted as described in (A).

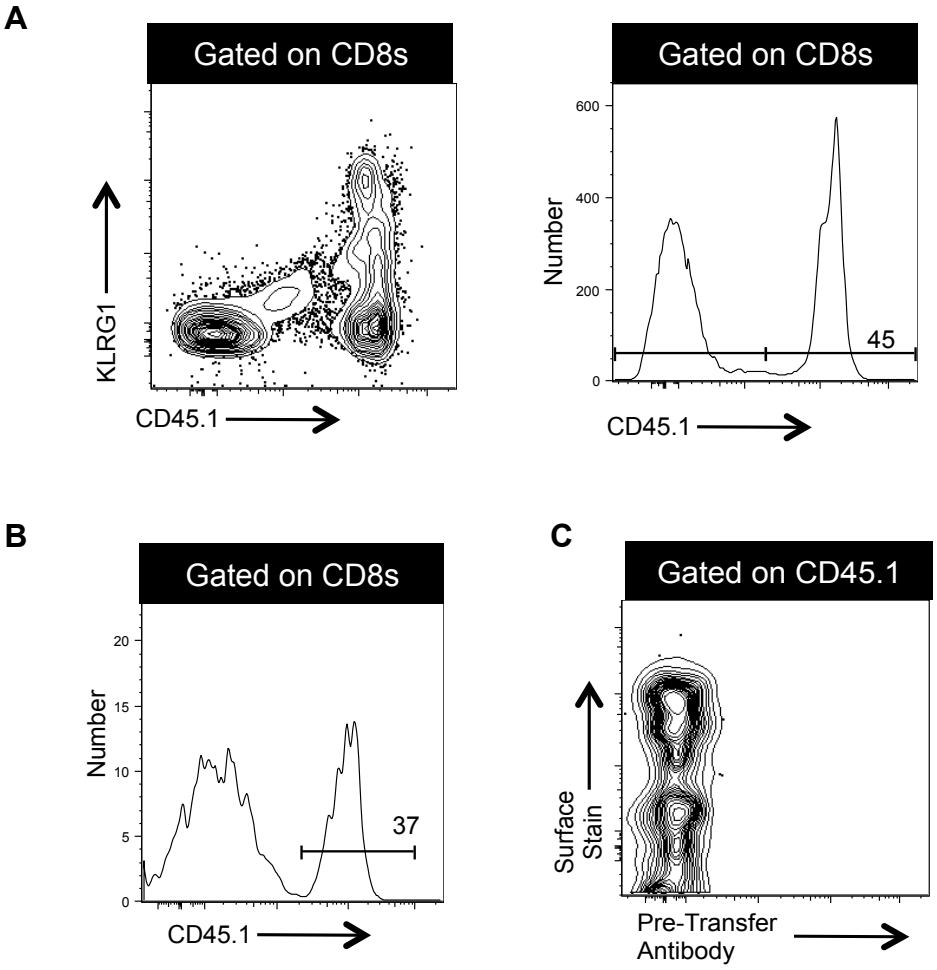
Supplemental Fig. 2



Supplemental Figure 2: Antibody depletion does not induce T_M proliferation in naïve recipients.

(A) Immediately prior to antibody depletion, the presence of donors in the blood was determined by flow cytometry. For T_M and T_{EFF} recipients, data was collected from two independent experiments (*n*=6 total). For naïve recipients, data was collected from one experiment (*n*=4 total) (B) Frequencies of total tetramer-binding $CD8^{pos}$ cells were determined in the blood of the chronically-infected Thy1.1 recipient mice prior to and immediately following the depletion scheduled in Figure 7B. Data was collected from two independent experiments (*n*=6 total) and is displayed as mean ± SEM. (C) Age matched naïve Thy1.1 recipients received T_M cells from a MCMV chronically-infected donor. Recipients were bled "pre-depletion" at 1.5 weeks post-transfer. Mice were started on the antibody depletion regimen immediately following the "pre-depletion" bleed and were bled again "post-depletion" at approximately 5 weeks post-transfer. A B6 mouse ($Thy1.2^{pos}$) was used as the positive staining control. Naïve mice were challenged with MCMV approximately 12 weeks after the adoptive transfer. Data was collected from one experiment (*n*=4 total).

Supplemental Fig. 3



Supplemental Figure 3: The KLRG1-specific antibody used for sorting does not deplete transferred cells.
(A) CD45.1 splenocytes were stained with the anti-KLRG1 antibody (clone 2F1), mixed with unstained CD45.2 splenocytes in a 1:1 ratio and transferred into B6.CB17-*Prkdc*^{scid} (SCID) mice from Jackson. The representative FACS plots show the KLRG1 stain of CD45.1^{pos}, but not CD45.2^{pos} cells (left) and the proportion of transferred cells that were CD45.1^{pos} (right). (B) Six days after transfer, SCID recipients were bled and analyzed by flow cytometry. CD45.1^{pos} cells were still a similar proportion of the transferred T cells (compare to A), suggesting that the large CD45.1^{pos} KLRG1-stained population was not depleted. (C) Anti-KLRG1 staining six days after the transfer showed that there were still KLRG1^{hi} CD45.1^{pos} cells. As KLRG1 expression has been shown to be dependent on antigen stimulation, it is unlikely that the KLRG1^{hi} cells were newly formed. Importantly, the CD45.1^{pos} cells detected 6 days after transfer were no longer positive for the KLRG1 antibody used prior to the transfer (not shown), suggesting that the cells have not been depleted and have cleared the antibody off their surface. Data was collected from a single experiment (n=3 total).