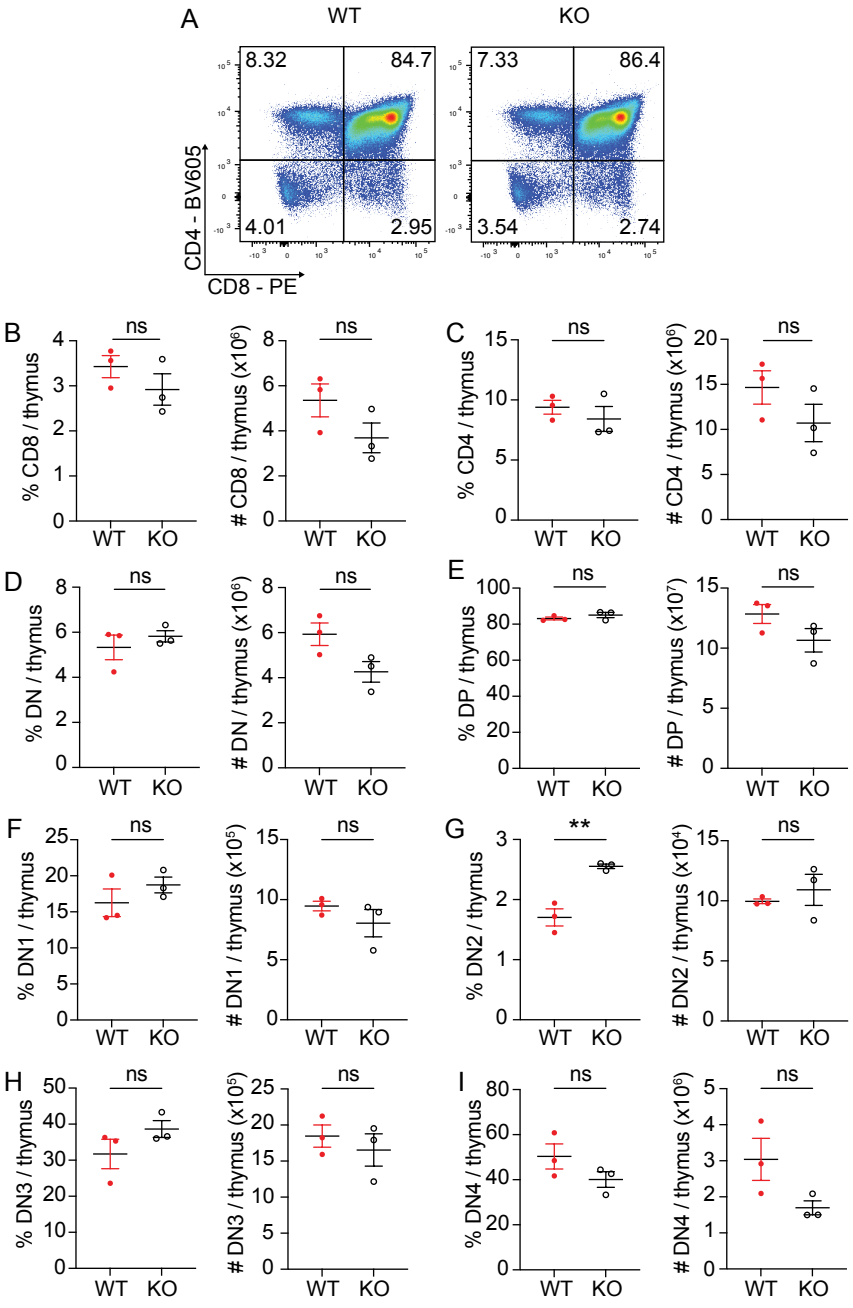
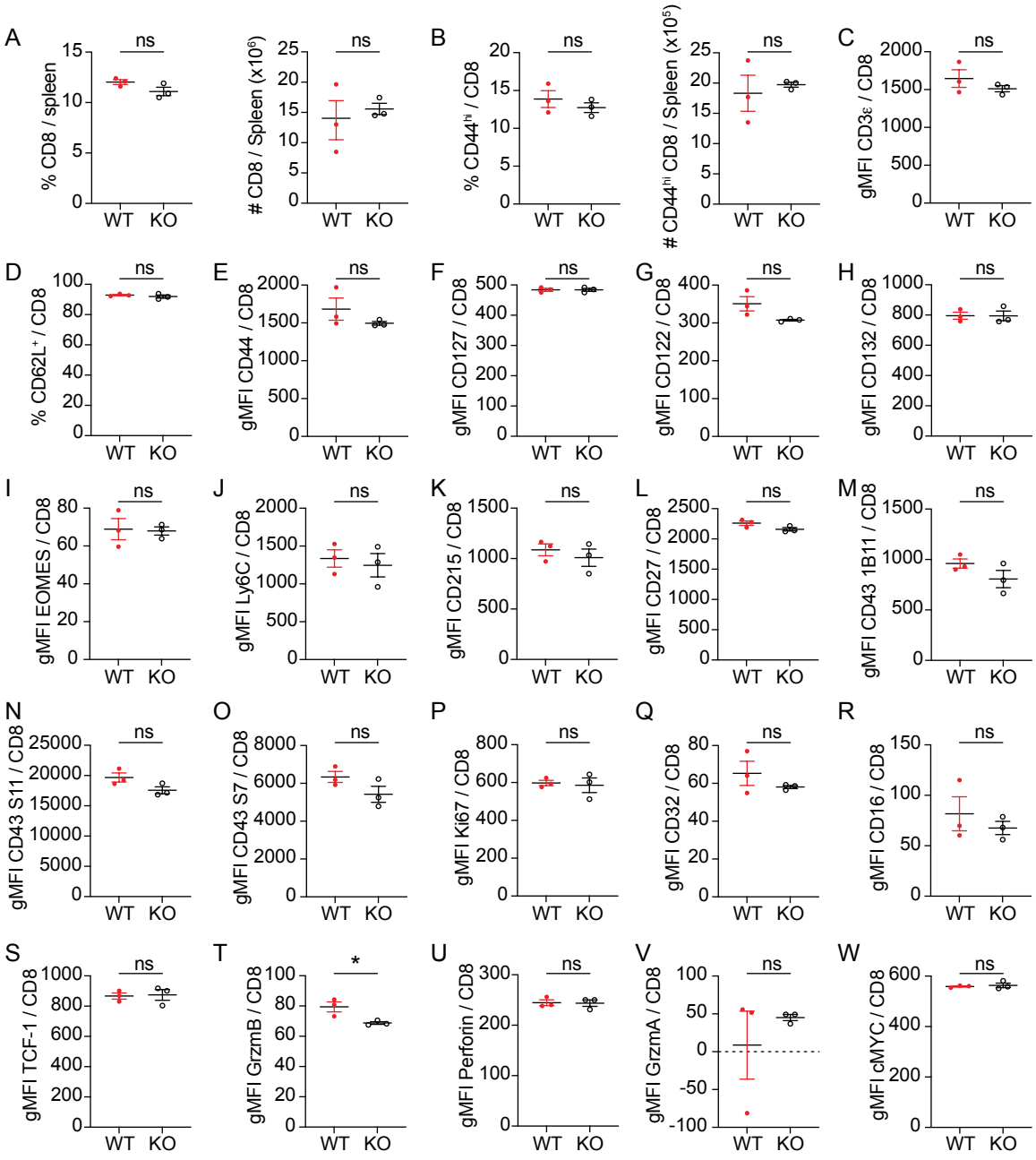
Lumnitzer et al. Sup. Fig. 1



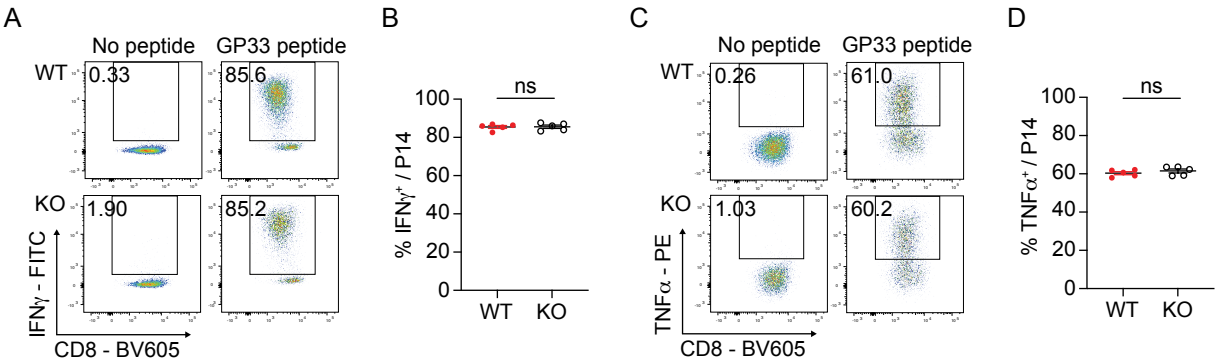
Supplemental Figure 1. *Aff3 deletion does not affect T cell development in the thymus.* Thymi from naïve WT and AFF3 KO mice were harvested and analyzed using flow cytometry to compare T cell development. (**A**) Representative flow plot of CD4 and CD8 expression in thymus from naïve WT and AFF3 KO mice showing single positive (SP), double positive (DP), and double negative (DN) (gated on $CD3^-$ cells) T cell populations. (**B, C**) Frequency and number of single positive CD8 T cells and CD4 T cells from the thymi of naive WT or AFF3 KO mice. (**D, E**) Frequency and number of DN and DP T cells from the thymi of naive WT or AFF3 KO mice. (**F-I**) The frequency and number of the four DN populations from the thymi of naive WT or AFF3 KO mice. (A-I) Groups compared using unpaired student T-test, ** <0.01. Representative of 2 experiments with n=3 mice per experiment. Data represent mean ± SEM.

Lumnitzer et al. Sup. Fig. 2



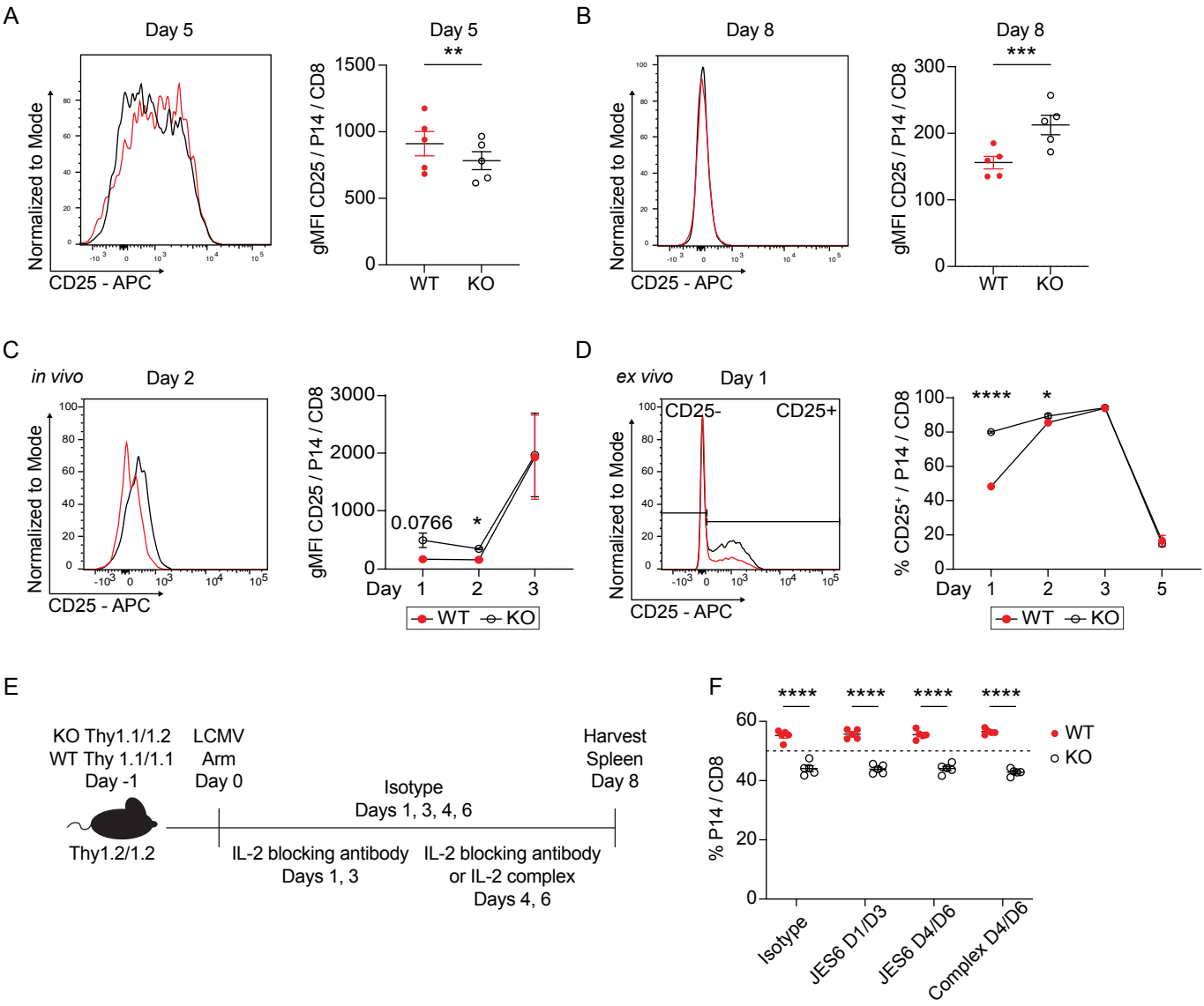
Supplemental Figure 2. *AFF3 KO CD8 T cells maintain a naïve phenotype in the spleen.* Spleens from naïve WT and AFF3 KO mice were harvested and analyzed using flow cytometry to compare the phenotype of naïve CD8 T cells. (**A**) Frequency and number of CD8 T cells in the spleen. (**B**) Frequency and number of CD44^{hi} CD8 T cells in the spleen. (**C**) gMFI of CD3ε expression on the surface of splenic CD8 T cells. (**D**) Frequency of CD62L^{+} CD8 T cells in the spleen. (**E-H, J-O**, **Q-R**) gMFI of the expression of surface proteins on splenic WT and AFF3 KO CD8 T cells, surface protein indicated in the Y axis of the graph. (**I**, **P**, **S-W**) gMFI of the expression of intracellular proteins in WT and AFF3 KO CD8 T cells, protein indicated in the Y axis of the graph. (A-W) Groups compared using unpaired student T-test, * < 0.05. Representative of 2 experiments with n=3 mice per experiment. Data represent mean ± SEM.

Lumnitzer et al. Sup. Fig. 3



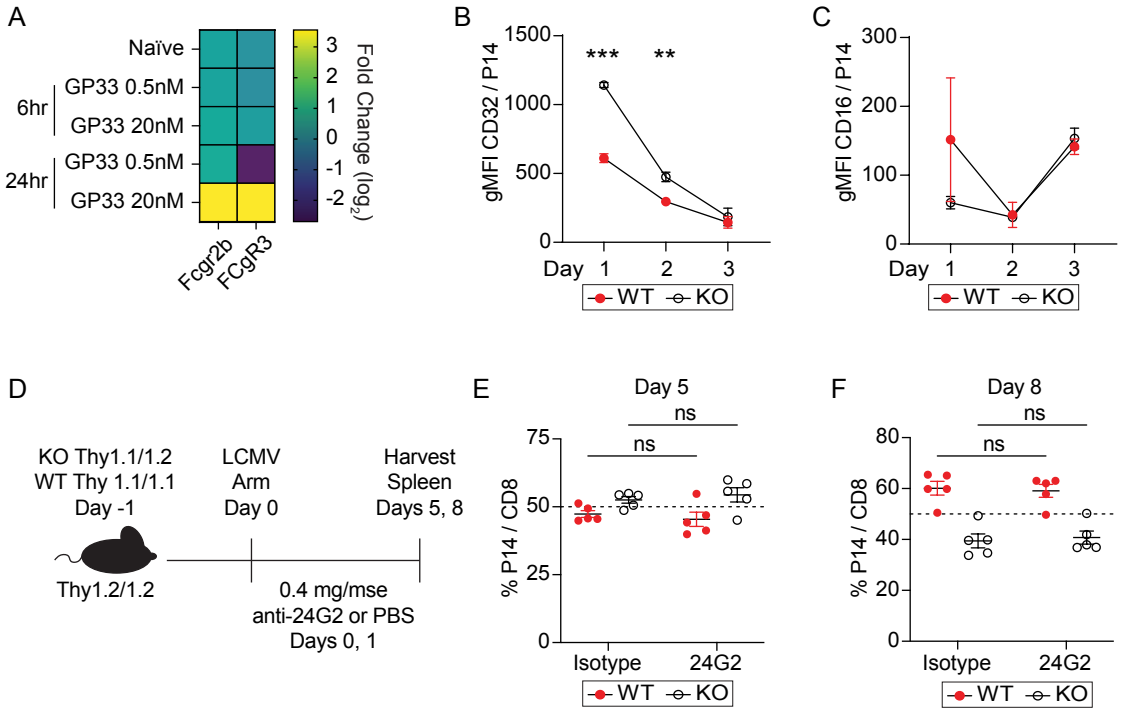
Supplemental Figure 3. *The production of cytokines is not different between WT and AFF3 KO CD8 T cells.* (**A, C**) Representative flow plots of intracellular staining for the indicated cytokine by splenic WT or AFF3 KO CD8 T cells with or without stimulation with GP_{33-41} peptide. Number on the graph represents frequency of cytokine producing cells. (**B, D**) Cumulative frequency of cytokine producing splenic WT or AFF3 KO CD8 T cells. Representative of at least 2 experiments with n=5 mice per group. Groups compared using unpaired student T-test. Data represent mean ± SEM.

Lumnitzer et al. Sup. Fig. 4



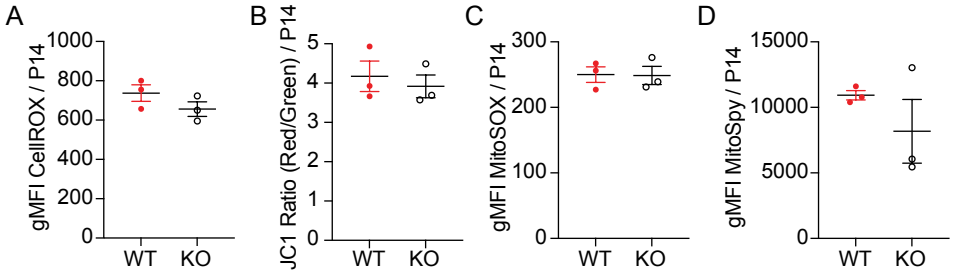
Supplemental Figure 4. *The accumulation defect of AFF3 KO CD8 T cells is independent of IL-2.* Naïve C57BL/6 mice received co-transfer of WT and AFF3 KO CD8 T cells, phenotype of the transferred cells was determined after infection. (**A-C**) Representative histograms and quantification by gMFI of CD25 expression on the surface of splenic WT and AFF3 KO CD8 T cells on 5 dpi, 8 dpi, and 1-3 dpi with LCMV Arm. (**D**) Representative histograms and quantification by gMFI of CD25 expression on the surface of splenic WT and AFF3 KO CD8 T cells 1-5 days after ex vivo activation with GP_{33-41} peptide and anti-CD28 antibody. (**E**) Experimental layout indicating timing of adoptive cell transfer, infection, and treatment of mice. (**F**) Frequency of splenic WT and AFF3 KO P14 CD8 T cells 8 dpi with LCMV Arm, with treatment with anti-IL-2 or IL-2 antibody complexes as indicated. (A-D) Groups compared using unpaired student T-test, p value = 0.05, * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. (F) Groups compared using two-way ANOVA analysis with post hoc test, **** < 0.0001. Representative of 2 experiments with n=5 mice per experiment. Data represent mean ± SEM.

Lumnitzer et al. Sup. Fig. 5



Supplemental Figure 5. *The accumulation advantage of the WT CD8 T cell is independent of CD32/CD16.* Based on RNA-seq analysis C57BL/6 mice received co-transfer of WT and AFF3 KO CD8 T cells and treatment with CD16/CD32 blocking antibody, frequency and phenotype of the transferred cells was determined after infection. (**A**) Heat map of *FCGR2B* and *FCGR3A* expression determined by RNA-seq analysis. (**B, C**) Quantification of the gMFI surface expression of CD32 and CD16 (respectively) on splenic WT and AFF3 KO CD8 T cells 1, 2, and 3 dpi with LCMV Arm. (**D**) Schematic showing experimental design with timing of adoptive transfer, infection, and 24G2 antibody treatment. (**E, F**) Frequency of the transferred cells in the isotype control and treated mice at 5 dpi and 8 dpi with LCMV Arm in the spleen. (B-C, E-F). Representative of two experiments with n=5 mice per group. (B,C) Groups compared using paired student T-test. (E-F) Groups compared using two-way ANOVA with post hoc test. ** < 0.01, *** < 0.001. Data represent mean ± SEM.

Lumnitzer et al. Sup. Fig. 6



Supplemental Figure 6. *AFF3 deficiency does not impact the mitochondrial health of CD8 T cells.* Naïve WT and AFF3 KO CD8 T cells were isolated from the spleen, and their mitochondrial health was determined using flow cytometry. (**A**) Quantification of gMFI of CellROX staining. (**B**) Quantification of ratio of gMFI JC1 polymer/gMFI JC1 monomer indicating membrane polarization. (**C**) Quantification of gMFI of MitoSOX staining. (**D**) Quantification of gMFI of MitoSpy staining. (A-D) Representative of two experiments with n=3 mice per group. Groups compared using unpaired student T-test. Data represent mean ± SEM.

Lumnitzer et al. Sup. Table 1

Age at time of draw	Sex
29	Male
28	Male
25	Female
22	Female
25	Female
27	Female
32	Female
33	Male
25	Female
63	Male
66	Male
65	Female
60	Female
68	Male
66	Male
61	Male
65	Female

Supplemental Table 1. *Information of donors of human PBMC samples.*

Supplemental methods

Antibodies. The antibodies used in this study were as follows in various combination of fluorophores: IFN-γ (XMG1.2, BioLegend), TNF-α (MP6-XT22, BioLegend), CD25 (3C7, BioLegend).

Ex vivo cytokine production. Splenocytes ($1 – 3 \times 10^6$) were stimulated with 200nM of GP_{33-41} peptide (Bio-Synthesis) for 5.5 hours at 37^{o}C in the presence of 1x Brefeldin A solution (420601, BioLegend). Cytokines were stained using fluorescently conjugated antibodies via intracellular staining as described in the methods.

CD32/CD16 blockade. One day before infection, 10,000 AFF3-KO and WT P14 CD8 T cells were cotransferred at a 1:1 ratio into naive C57BL/6 mice via i.v. injection. Mice were treated on with anti-CD16/CD32 (2.4G2, a gift from Dr. John Harty, University of Iowa) (0.4 mg/mse, i.p.) on 0 and 1 dpi and the frequency of the cells were analyzed on 5 and 8 dpi via flow cytometry.

IL-2 blockade and supplementation. One day before infection, 10,000 AFF3-KO and WT P14 CD8 T cells were cotransferred at a 1:1 ratio into naive C57BL/6 mice via i.v. injection. The mice were then given either (0.2 mg/mouse, i.p.) anti-IL-2 (JES6, Bio X Cell) (1 and 3 dpi or 4 and 6 dpi, i.p.) or IL-2 stimulating complex of 50 ug/ml anti-IL-2 (S4B6, Bio X Cell) with 1.5 ug/ml purified mouse IL-2 (Peprotech) (4 and 6 dpi, i.p.). Control mice received injections of isotype control (0.2 mg/mouse, i.p.) (IgG2aκ isotype, 2A3, Bio X Cell) on 1, 3, 4, and 6 dpi. Mice were euthanized on 8 dpi and cell frequency was determined using flow cytometry.