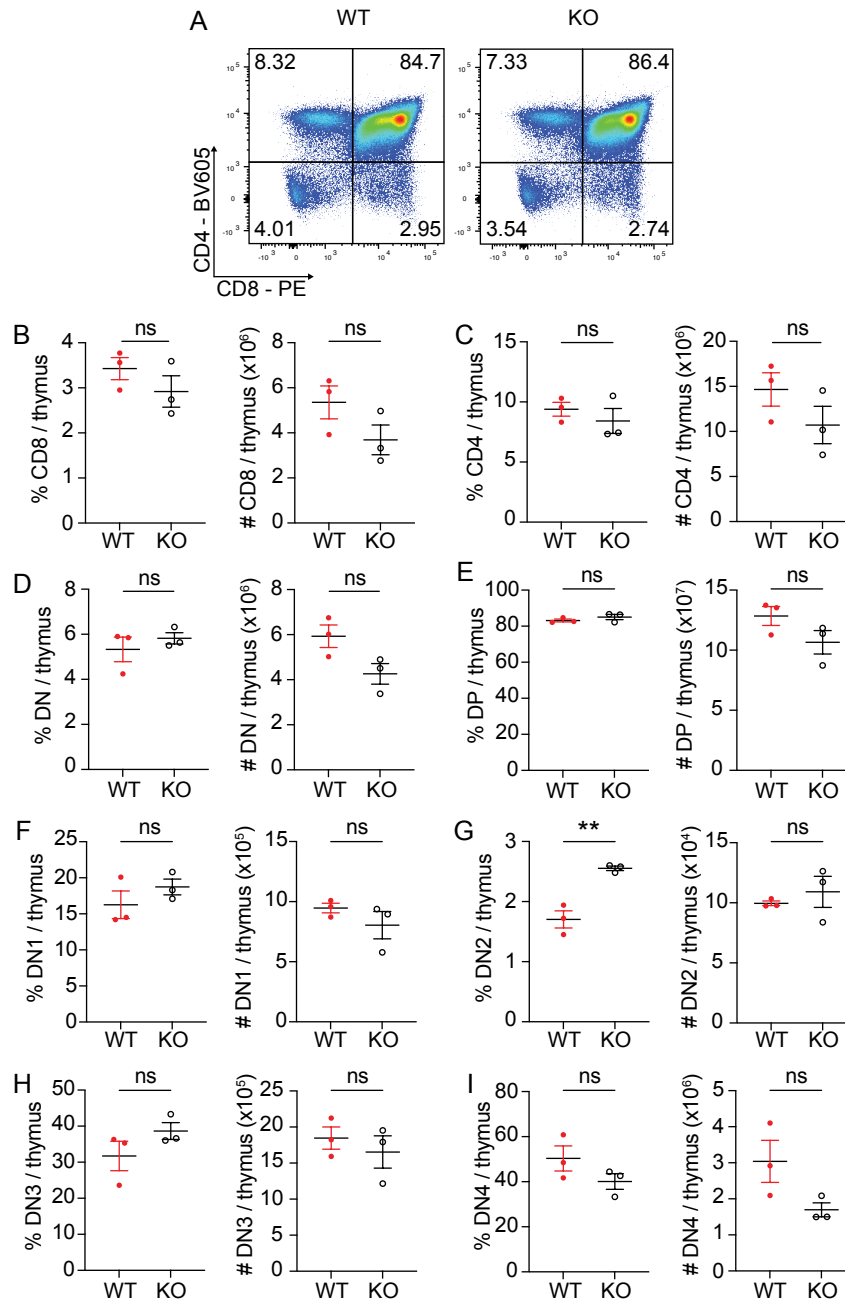
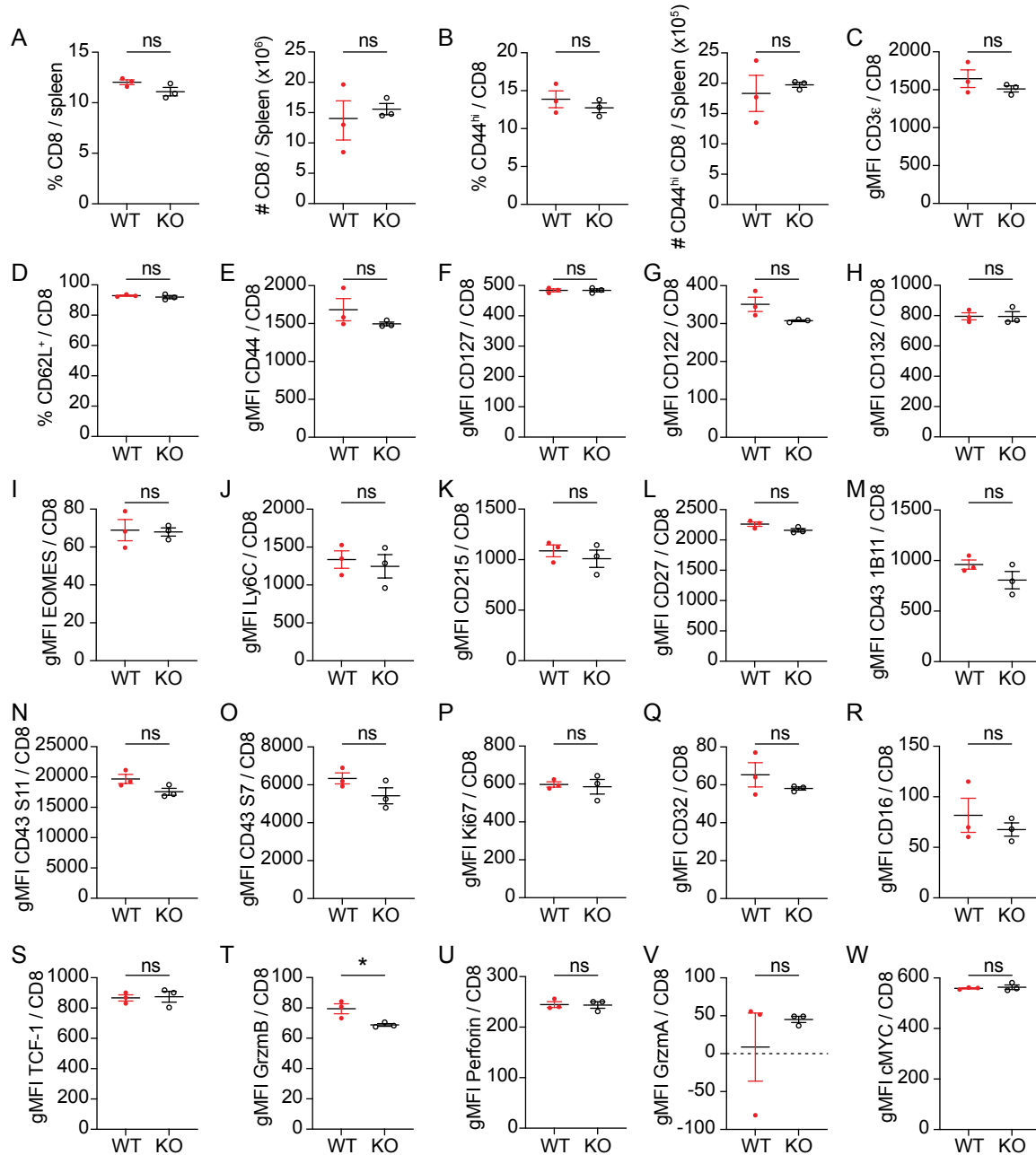
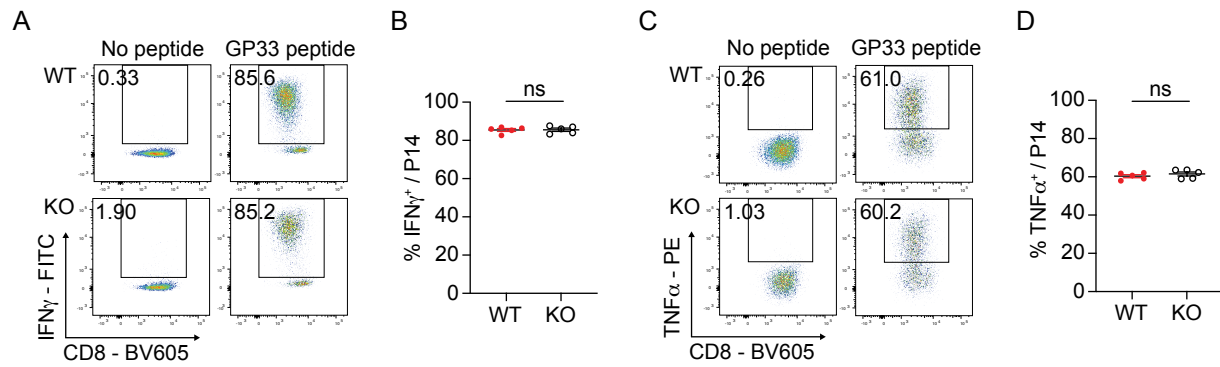




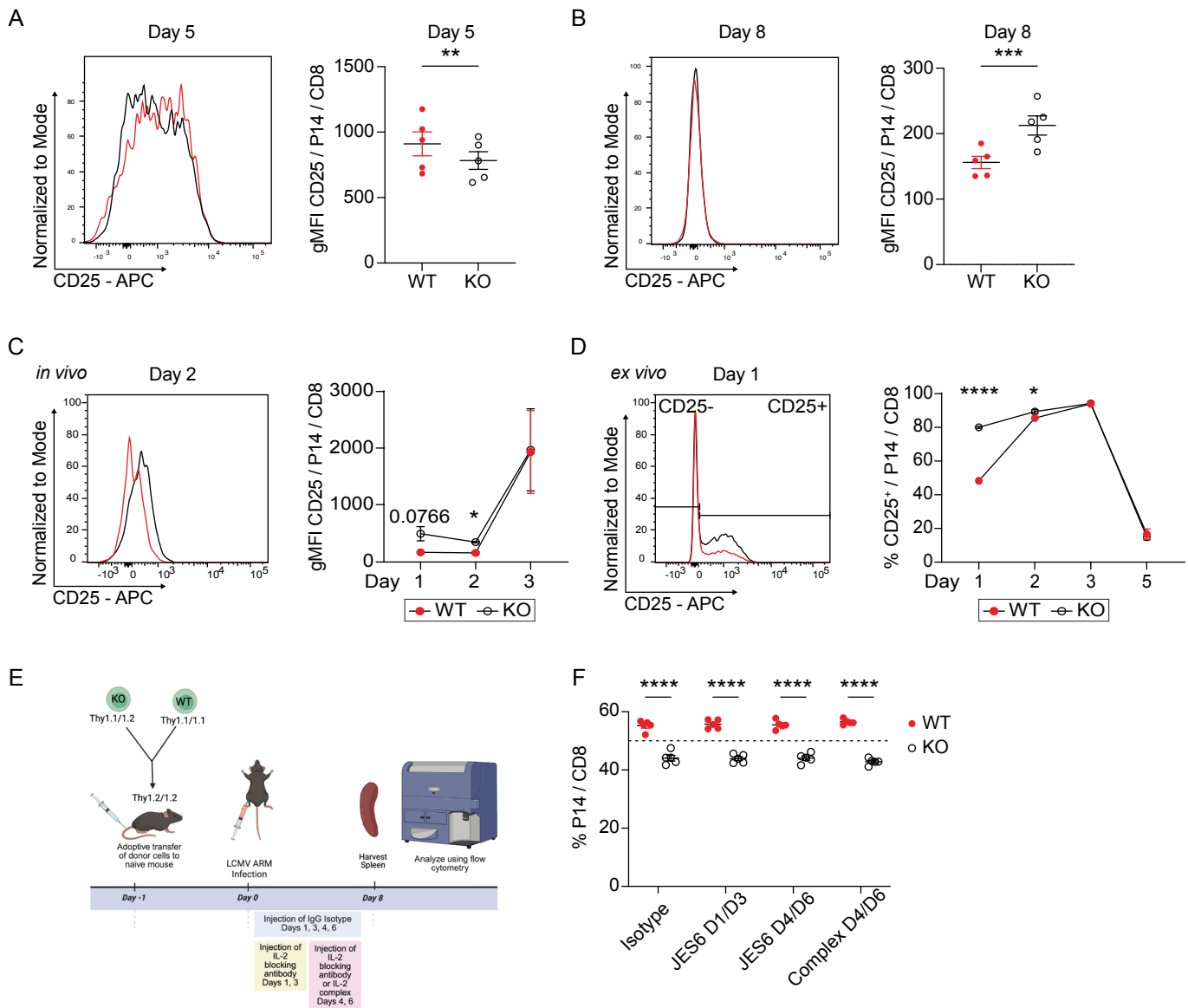
Supplemental Figure 1. *Aff3* deletion does not affect T cell development in the thymus. Thymi from naïve WT and *Aff3* KO mice were harvested and analyzed using flow cytometry to compare T cell development. **(A)** Representative flow plot of CD4 and CD8 expression in thymus from naïve WT and *Aff3* 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 naïve WT or *Aff3* KO mice. **(D, E)** Frequency and number of DN and DP T cells from the thymi of naïve WT or *Aff3* KO mice. **(F-I)** The frequency and number of the four DN populations from the thymi of naïve 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 $\pm$ SEM.



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 CD3 ϵ expression on the surface of splenic CD8 T cells. (D) Frequency of CD62L⁺ CD8 T cells in the spleen. (E-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. (P, S, T-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 $\pm$ SEM.

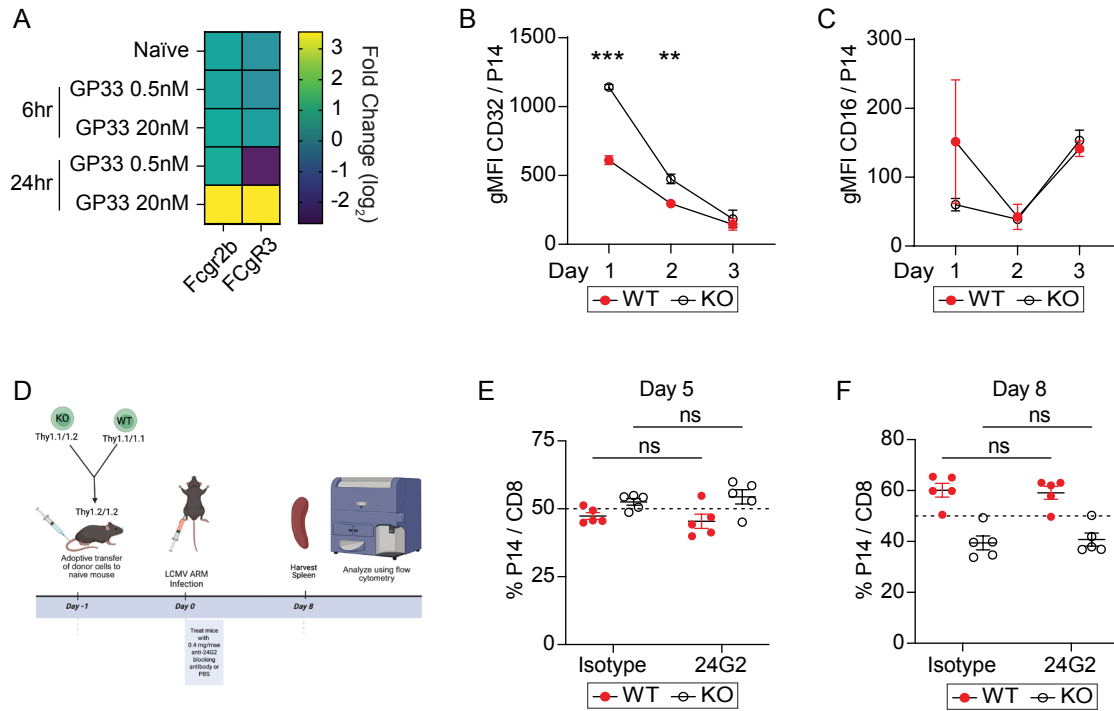


Supplemental Figure 3. *The production of cytokines is not different between WT and AFF3 KO CD8 T cells.* (A, C) Representative flow plots of intracellular staining for the indicated cytokine by splenic WT or AFF3 KO CD8 T cells with or without stimulation with GP₃₃₋₄₁ peptide. Number on the graph represents 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 $\pm$ SEM.

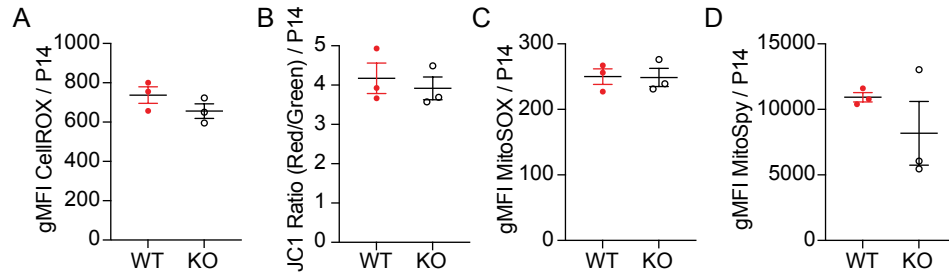


Supplemental Figure 4. The accumulation defect of *AFF3* KO CD8 T cells is independent of IL-2. Naïve C57BL/6 mice received co-transfer of WT and *AFF3* KO CD8 T cells, phenotype of the transferred cells was determined after infection. (**A-C**) Representative histograms and quantification by gMFI of 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 GP₃₃₋₄₁ peptide and anti-CD28 antibody. (**E**) Experimental layout indicating timing of adoptive cell transfer, infection, and treatment of mice, made using Biorender. (**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.

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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 $\pm$ SEM.



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.

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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.*