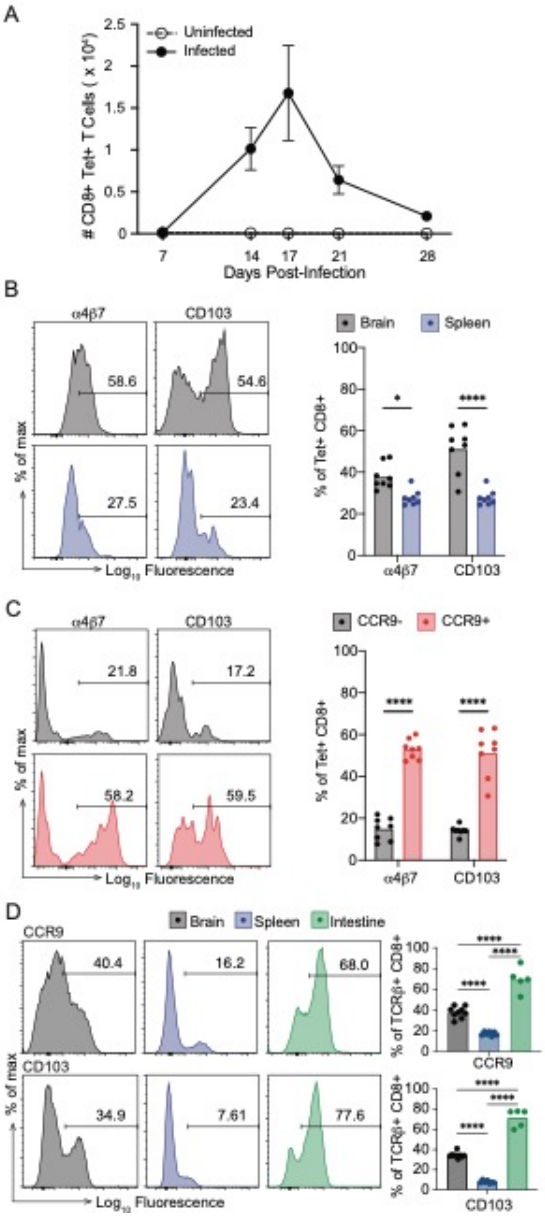
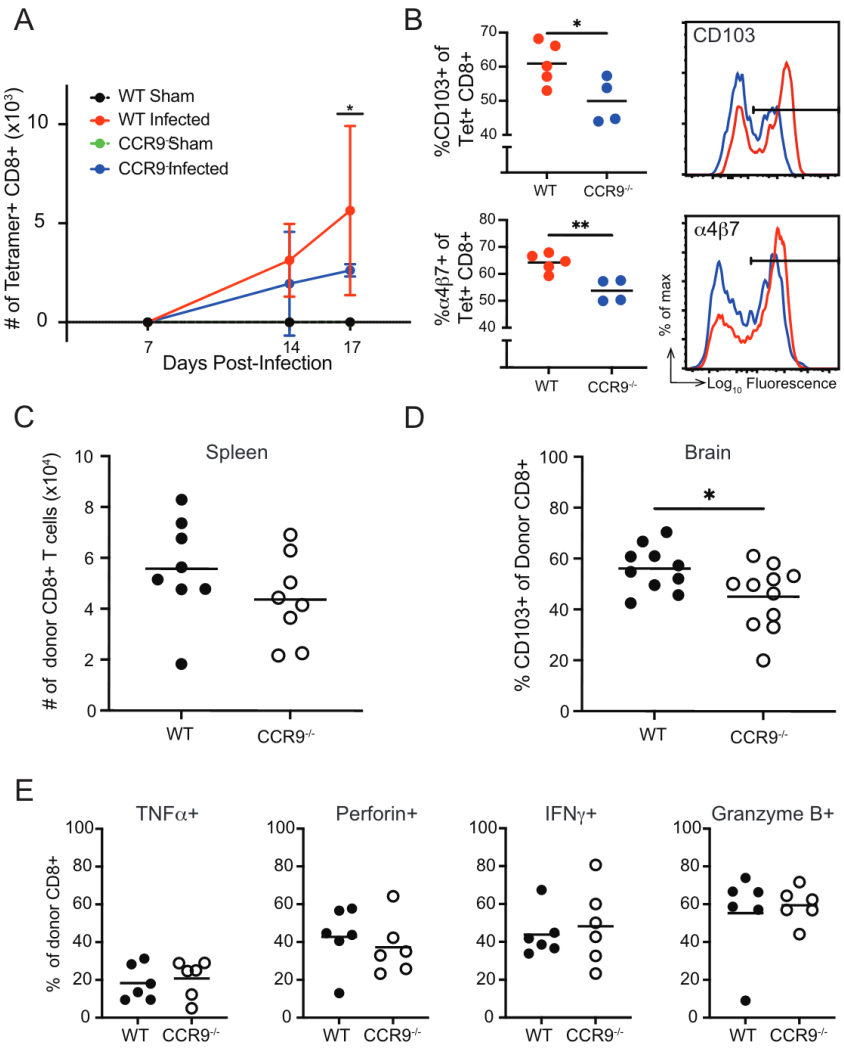
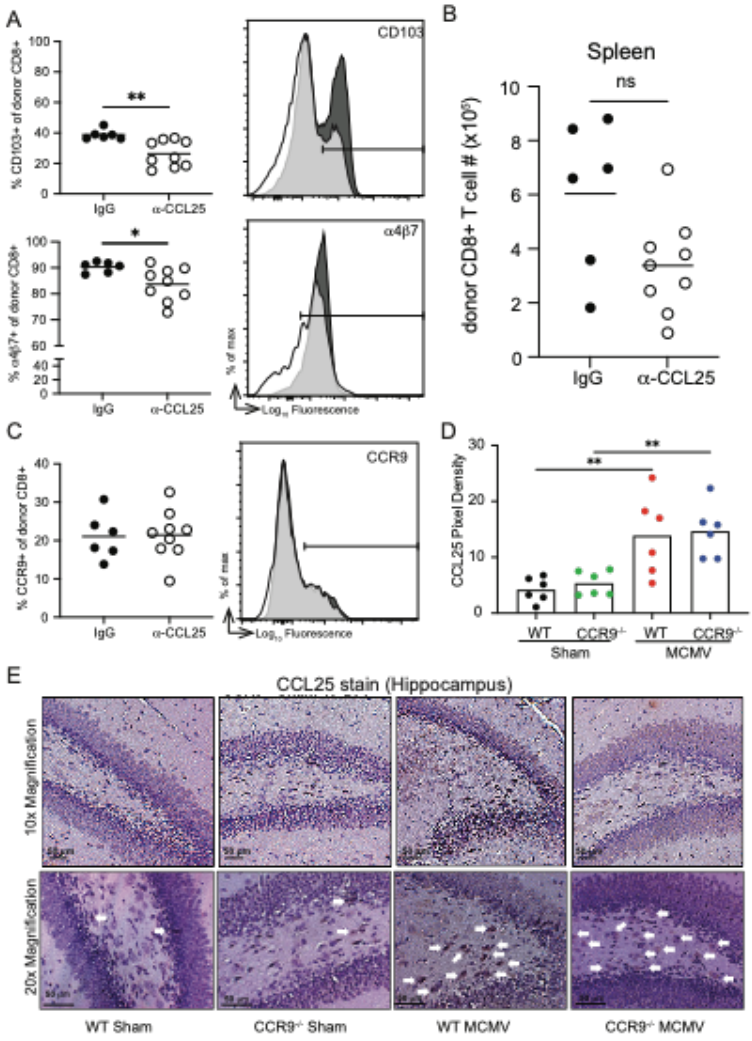


Supplemental Figure 1. CD8+ T cells in the brain express gut-homing receptors. WT mice were either left uninfected or infected with MCMV-gB at 0-1 days post birth. **(A)** Number of Tet+ CD8+ T cells in the brain were quantified by FACS (N = 2-8 mice). Results are shown as mean ± SEM. **(B)** Representative (left) and quantification (right) of FACS staining of surface α4β7 and CD103 in brain and spleen Tet+ CD8+ T cells (N = 8). **(C)** Representative (left) and quantification (right) of FACS staining of surface α4β7 and CD103 in brain CCR9+ Tet+ CD8+ and CCR9- Tet+ CD8+ T cells (N = 8). Results are shown as mean. For statistical analysis of B and C, two-way ANOVA followed by Sidak's multiple comparison test. (D) Representative (left) and quantification (right) of CCR9 and CD103 surface expression in TCRβ+ CD8+ cells of indicated tissues. Statistical analysis performed by ordinary one-way ANOVA with Tukey's multiple comparison test.*, $P < 0.05$; ****, $P < 0.0001$.



Supplemental Figure 2. CCR9$^{-/-}$ CD8+ T cells are distinct by trafficking and surface phenotype but not effector function. WT or CCR9$^{-/-}$ mice were either injected with PBS (Sham) or infected with MCMV-gB at 0-1 days post birth. **(A)** Number of Tet+ CD8+ T cells in the brain (N = 3-6 mice). Results are shown as mean ± SD. For statistical analysis, two-way ANOVA followed by Bonferroni test was performed for multiple comparisons**. (B)** Quantification and representative histograms of CD103 (top) and α4β7 (bottom) expression on Tet+ CD8+ T cells in the brain (N = 4-5 mice). **(C)** Number of donor CD45.2+ CD8+ T cells in the spleen (N = 6 mice). **(D)** Surface expression of α4β7 (left) and CD103 (right) on donor CD8+ T cells (N = 10-11 mice). **(E)** Production of TNFα, Perforin, IFNγ, Granzyme B, and Granzyme A as measured by flow cytometry. Results are shown as mean ± SEM. For statistical analysis, a two-tailed unpaired t test with Welch's correction was performed to compare two groups. *, $P < 0.05$; **, $P < 0.01$.



Supplemental Figure 3. Chemokine CCL25 controls CD8+ T cell trafficking to the brain during MCMV infection. CD8+ T cells from WT gBT-I mice were isolated from the spleen and 200 cells were donated in congenic host at day of birth. MCMV-gB infected pups were treated with either IgG (control) or anti-CCL25 blocking antibody (5 µg/mouse) **(A)** Surface expression of CD103 (top) and α4β7 (bottom) donor CD8+ T cells were analyzed by FACS (N = 6-9 mice). **(B)** Number of donor CD45.2+ CD8+ T cells in the spleen (N = 6-9 mice). **(C)** CCR9 on donor CD8+ T cells were analyzed by FACS (N = 6-9 mice). **(D)** Quantitation of CCL25 pixel density shown in **(E)** Brains were collected 17d post-infection, sectioned, and stained for CCL25 as indicated by brown diaminobenzidine staining. Representative images of the hippocampus (left) and quantification of sections by pixel intensity using ImageJ (right, N = 6 mice). Bar 50 µM, 10x magnification (top) and 20x magnification (bottom). Results are shown as mean. For statistical analysis, one-way ANOVA followed by Bonferroni test was performed for multiple comparisons, a two-tailed unpaired t test with Welch's correction was performed to compare two groups. *, $P < 0.05$; **, $P < 0.01$.