Figure S1

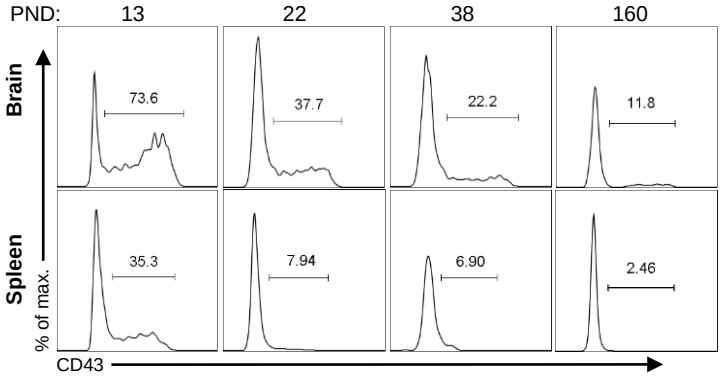

Figure S1 (related to Figure 1). CD8$^+$ T cells are activated in the brain of MCMV-infected newborn mice. Newborn C57BL/6 mice were injected i.p. with 200 PFU of MCMV on PND 2. On indicated time points the expression of CD43 was determined by flow cytometry brain and spleen. Results from one of two independent experiments are shown.

Figure S2

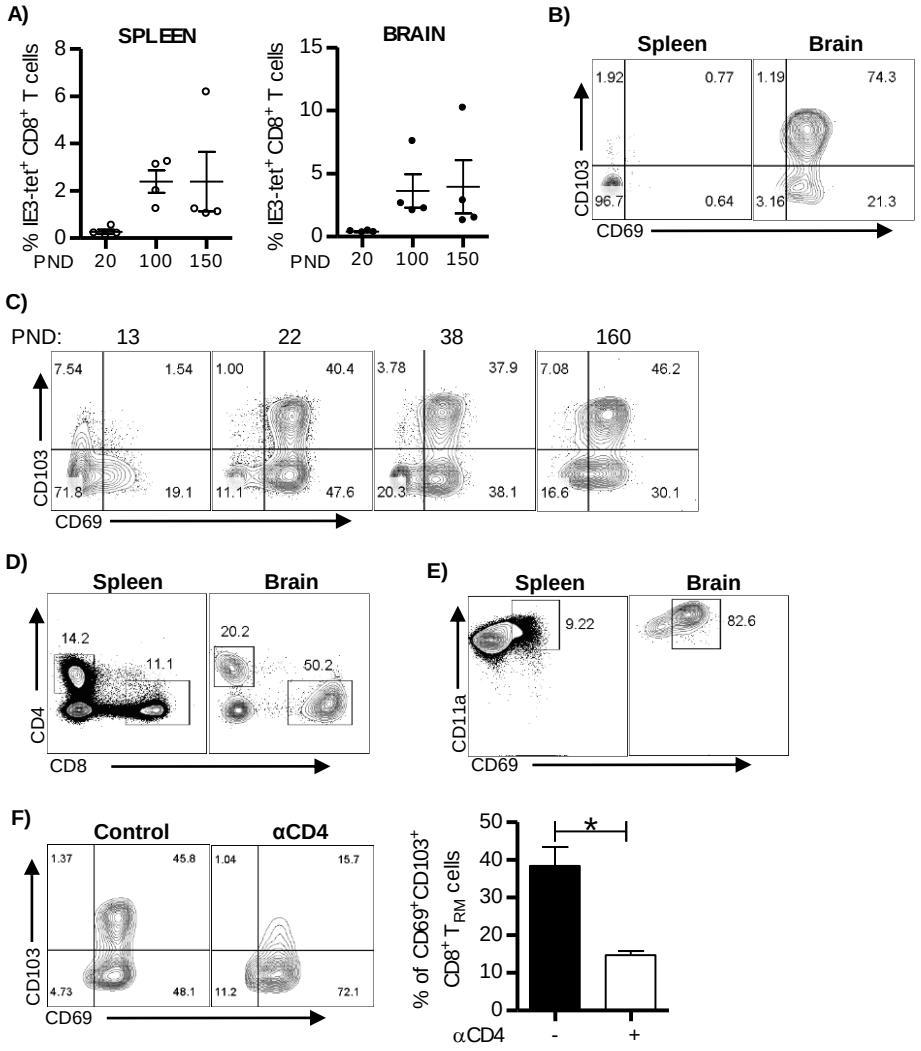

Figure S2 (related to Figure 2). Tissue resident T cells in the brain of MCMV infected newborn mice. Newborn C57BL/6 mice were injected i.p. with 200 PFU of MCMV on PND 1. (A) Frequency of IE3-tetramer positive $CD8^+$ T cells was determined in brain and spleen at indicated time points. Values for each mouse are shown (circles). Mean values +/- SEM are shown (n=4 animals). (B) The expression of CD69 and CD103 is shown on IE3-tetramer positive $CD8^+$ T cells in spleen and brain on PND 100. (C) The expression of CD103 and CD69 on $CD8^+$ T cells in the brain at different time points p.i. is shown. (D) Frequencies of $CD8^+$ T and $CD4^+$ T cells in brain and spleen 4 months p.i. are shown. (E) The expression of CD11a and CD69 on $CD4^+$ T cells in brain and spleen 4 months p.i. are shown. (F) $CD4^+$ T cells were depleted in newborn mice by injecting depleting CD4 antibodies at 3 day-interval. Representative histograms showing expression of CD69 and CD103 (left) and frequency of $CD69^+CD103^+$ $CD8^+$ TRM cells (right) on PND 19 are shown. n=4 animals. Results from one of two independent experiments are shown.

Figure S3

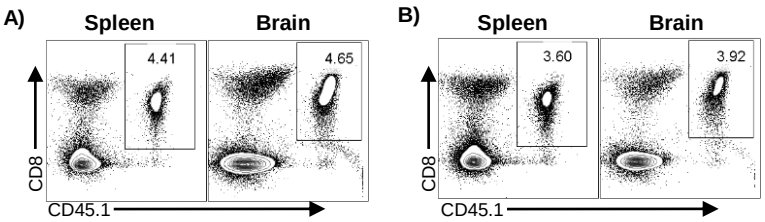

Figure S3 (related to Figure 4). Transferred Maxi CD8$^+$ T cells in the brain of MCMV infected newborn mice. Newborn C57BL/6 mice were infected with 200 PFU of MCMV. Six hours before infection (prophylactic protocol) or on PND 5 (therapeutic protocol) mice were i.p. injected with 10,000 of Maxi CD8$^+$ T cells. Representative plots show Maxi CD8$^+$ T cells in the brain and spleen on PND 14 after prophylactic (A) and therapeutic (B) protocol.. Results from one of three independent experiments are shown.

Figure S4

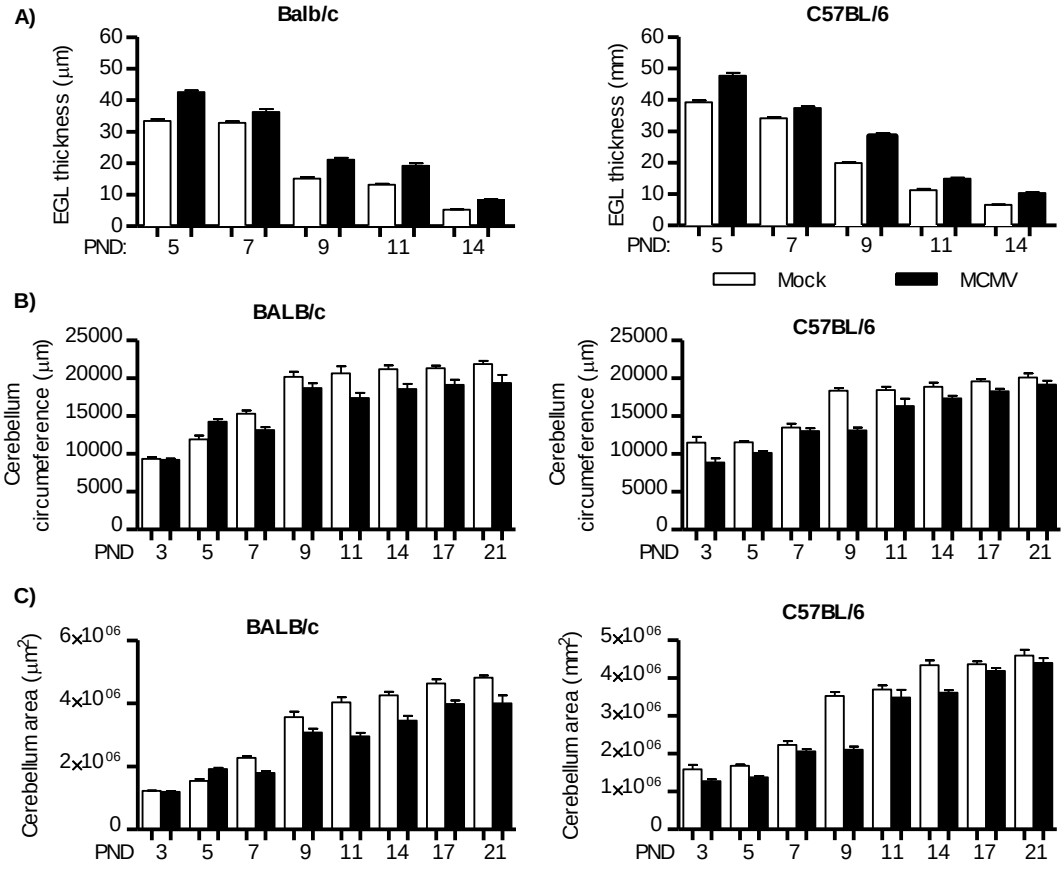

Figure S4 (related to Figure 4). MCMV infection induces neurodevelopmental changes in Balb/c and CD57BL/6 mice. Newborn C57BL/6 and Balb/c mice were injected i.p. with 500 PFU of MCMV on PND 2. On indicated time points (A) the migration of external granular layer (EGL), (B) cerebellum circumference and (C) cerebellum area were measured in brains of both strains of mice. Results from one of two independent experiments are shown.

Figure S5

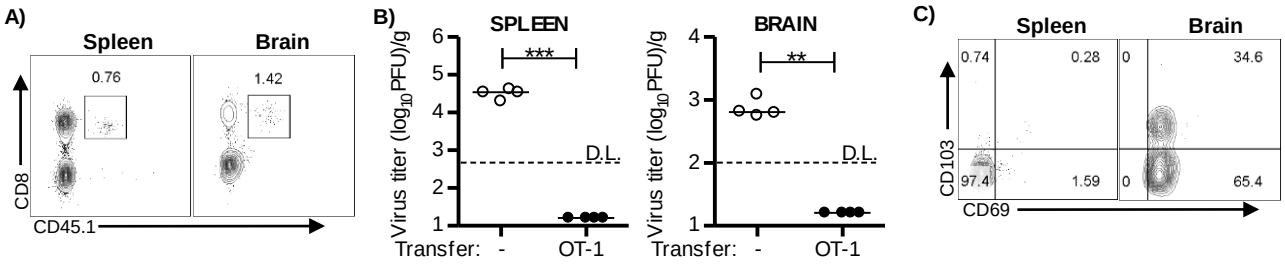

Figure S5 (related to Figure 4). Adoptively transferred OT-1 CD8$^+$ T cells are protective in the brain of MCMV-SIINFEKL infected newborn mice and acquire tissue resident phenotype. Newborn C57BL/6 mice were i.p. injected with 200 PFU of MCMV-SIINFEKL on PND 2 followed by adoptive transfer of 10,000 of OT-I CD8$^+$ T cells on PND 7. (A, B) On PND 14 mice were sacrificed and (A) lymphocytes from brain and spleen were isolated and analyzed for the expression of CD8 and CD45.1 by flow cytometry or (B) viral titers in organs were determined by plaque assay. Titers in organ of individual mice are shown (circles); horizontal bars indicate the median values; D.L., detection limit. (C) On PND 210 lymphocytes from brain were isolated and OT-I cells were analyzed for the expression of CD103 and CD69 markers by flow cytometry. Results from one of two independent experiments are shown.

Figure S6

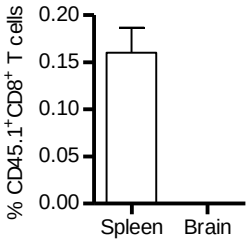

Figure S6 (related to Figure 5). Peripheral memory CD8$^+$ T cells do not repopulate the brain. Newborn C57BL/6 mice were i.p. injected with 200 PFU of WT MCMV on PND 1. Half of the mice received adoptively transferred naive Maxi CD8$^+$ T (CD45.1$^+$) cells on PND 7. Three months p.i. 50 000 of memory Maxi CD8$^+$ T cells were isolated from infected mice and adoptively transferred into mice infected as newborns that did not receive naive Maxi CD8$^+$ T cells upon infection. Two weeks after transfer lymphocytes were isolated from brains and spleens of recipient mice and analyzed for presence of Maxi CD8$^+$ T cells. Shown are frequencies of CD45.1$^+$ cells (Maxi cells) out of total CD8$^+$ T cells. n=4 animals.

Figure S7

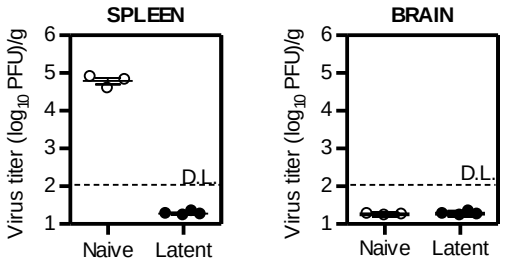

Figure S7 (related to Figure 5). Re-infection of latently infected mice with salivary gland-derived MCMV. C57BL/6 newborn mice were injected i.p. with 200 PFU of MCMV on PND 1, or lef uninfected. Two months old mice were (re-)infected with 50,000 PFU of salivary gland derived MCMV. Five days post infection virus titer was determined in brain and spleen. Titers in organs of individual mice are shown (circles). Mean values +/- SEM are shown. D.L., detection limit. N=1

Figure S8

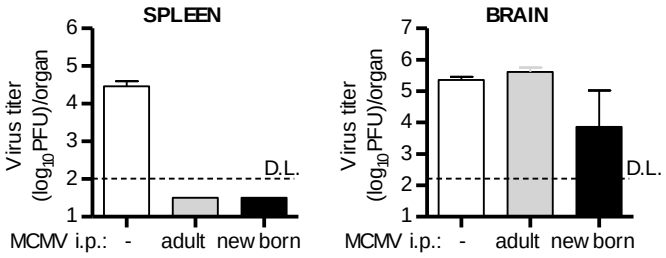

Figure S8 (related to Figure 6). Perinatal MCMV infection protects against intracranial MCMV challenge. C57BL/6 mice were infected as newborns (200 PFU of MCMV) or adults (2×10^5 PFU of MCMV) and left to establish latency. Mice were intracranially challenged with 10^5 PFU of MCMV. Non-infected C57BL/6 mice served as a control. All mice received FTY720 (1μg/g) i.p. daily starting 3 days prior i.c. challenge. Virus titer was determined 3 days post i.c. challenge. (n=3 animals).

Figure S9

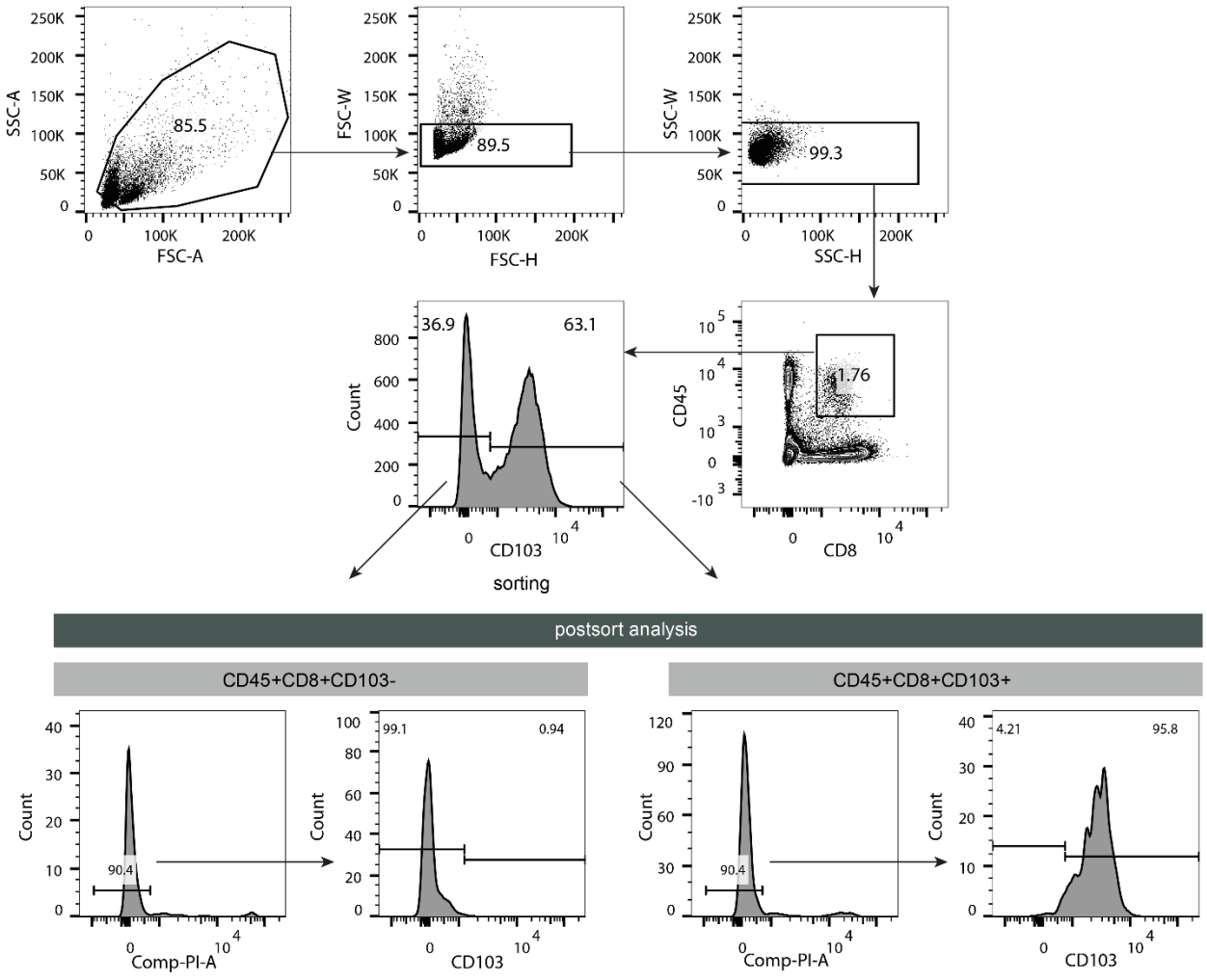

Figure S9 (related to Figure 7). Description of cell sorting procedure. Presort and post-sort analysis of memory Maxi CD8⁺ T cells isolated from the brain of 2 month old mice infected perinatally with MCMV. N=2