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Supplementary Data

Dendritic Cells Regulate Amyloid-β-Specific T-Cell Entry into the Brain: The Role of Perivascular Amyloid-β

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SUPPLEMENTARY MATERIAL

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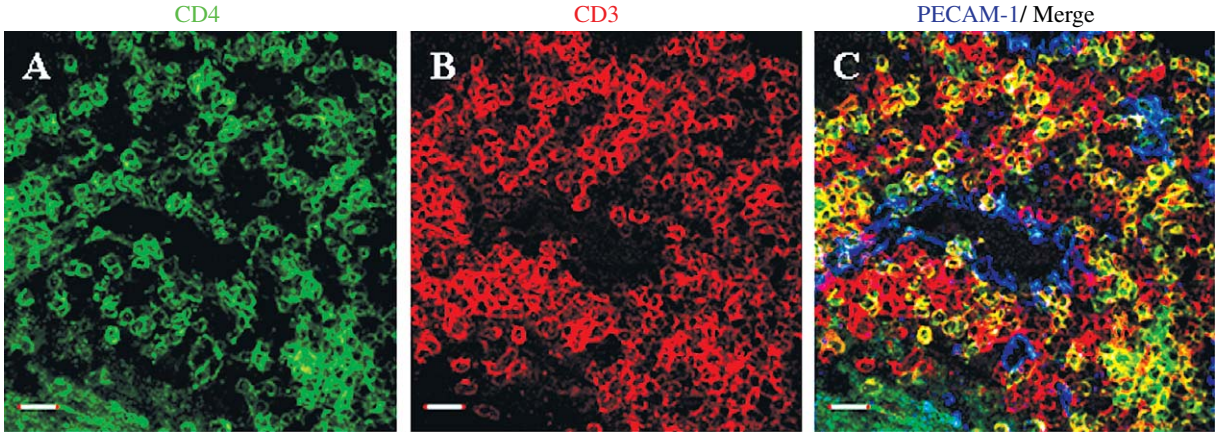
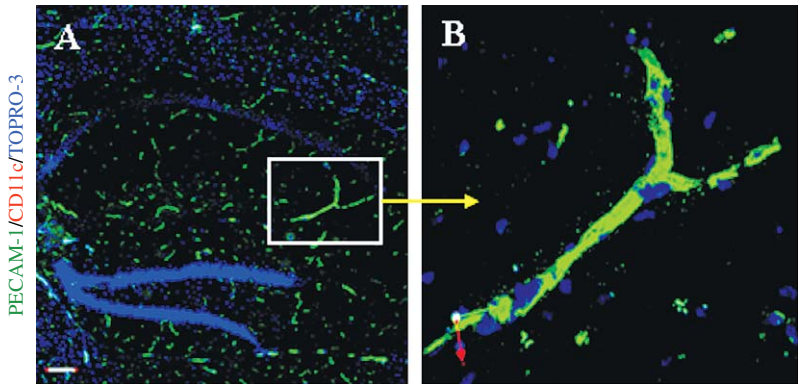
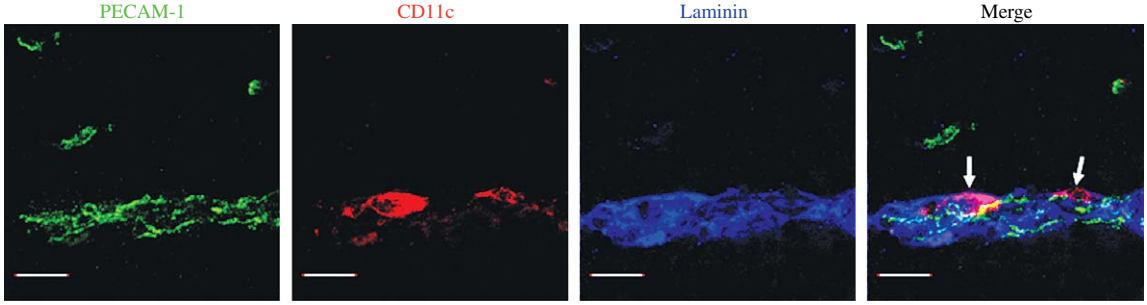


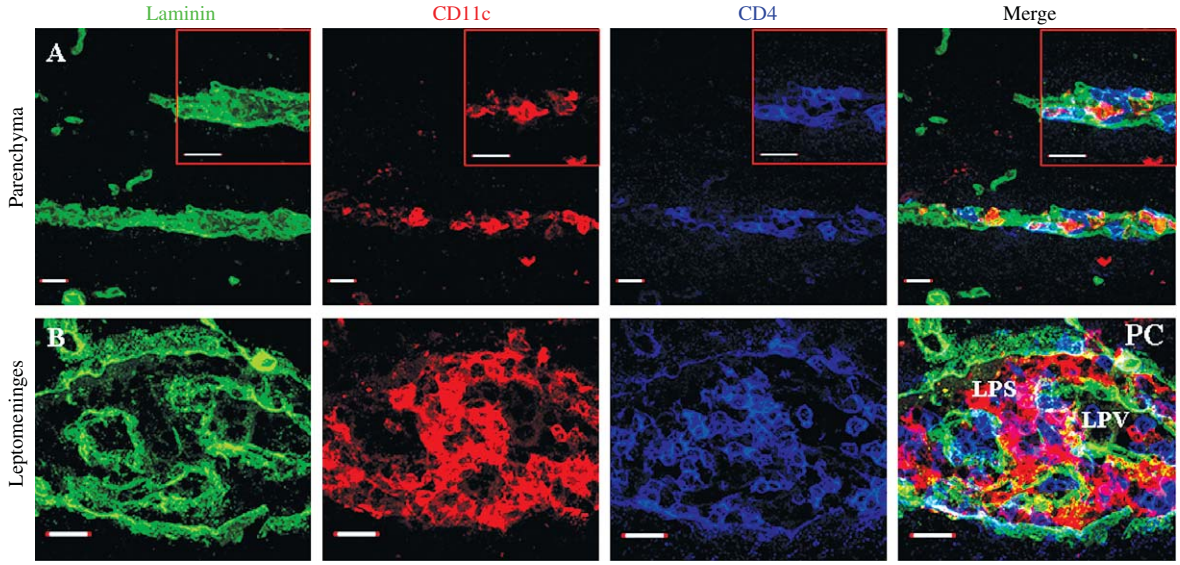
Supplementary Figure 1. $CD4^+$ cells observed in the brains of Aβ-immunized AβPP/IFN-γ Tg mice are T cells. AβPP/IFN-γ Tg mice were immunized with Aβ as described in Materials and Methods and killed 19 days later. To verify that the infiltrating $CD4^+$ cells we observed in the brain were indeed T cells, brain sections were co-stained using anti-CD4 (A, green), anti-CD3 (B, red), and anti-PECAM-1 (C, blue) antibodies. The merge image in panel C shows almost complete co-localization of CD4 and CD3. Bars represent 20 μm.



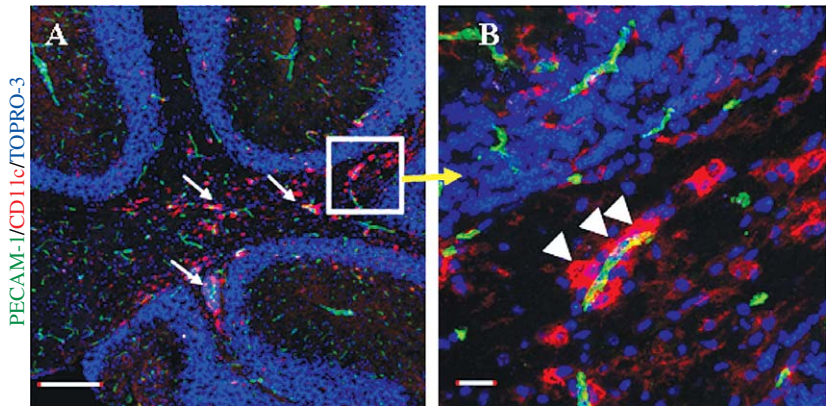
Supplementary Figure 2. $CD11c^+$ cells are not detected in brain sections of untreated AβPP/IFN-γ Tg mice. AβPP/IFN-γ Tg mice aged 9 months were killed and brain sections were prepared for IHC. (A) An overview image of the dentate gyrus area in untreated AβPP/IFN-γ Tg mouse stained for PECAM-1 (green), CD11c (red), and counterstained with TORPO-3 (blue). (B) High magnification of the boxed area in (A) showing a parenchymal blood vessel. Bar represent 100 μm. Image (B) was taken using the 60× objective lens.



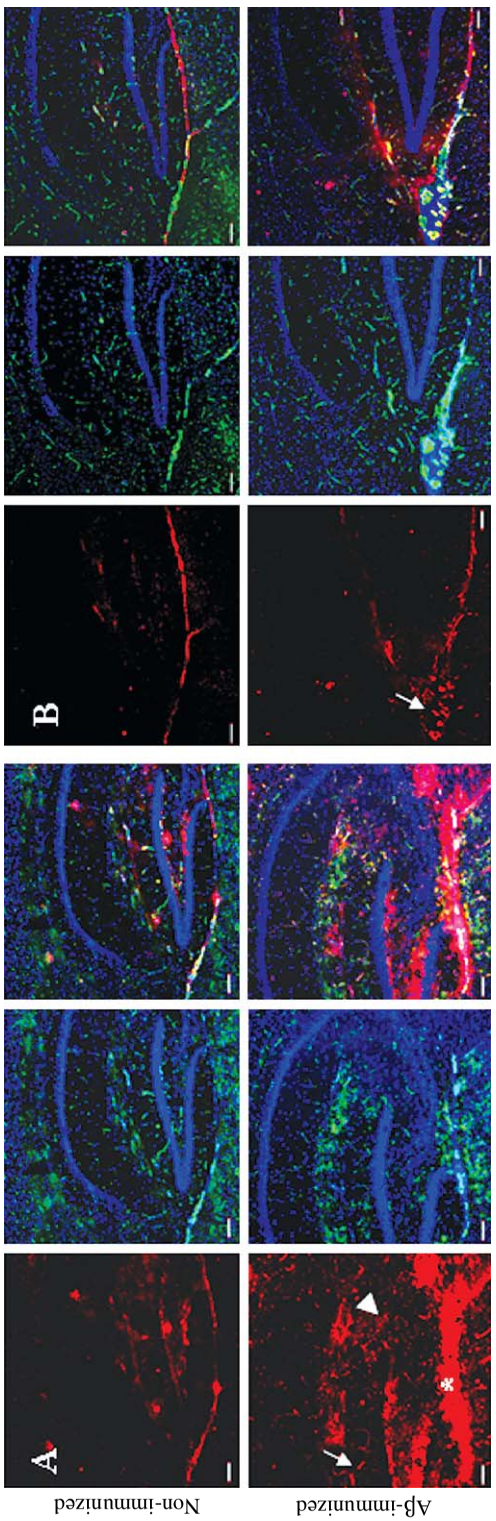
Supplementary Figure 3. $CD11c^+$ cells are localized at the perivascular space of parenchymal vessels. AβPP/IFN-γ Tg mice were immunized with Aβ as described in Materials and Methods and killed 19 days later. Brain sections were immunolabeled using antibodies against PECAM-1 (green), CD11c (red, arrows), and laminin (blue). Bars represent 20 μm.



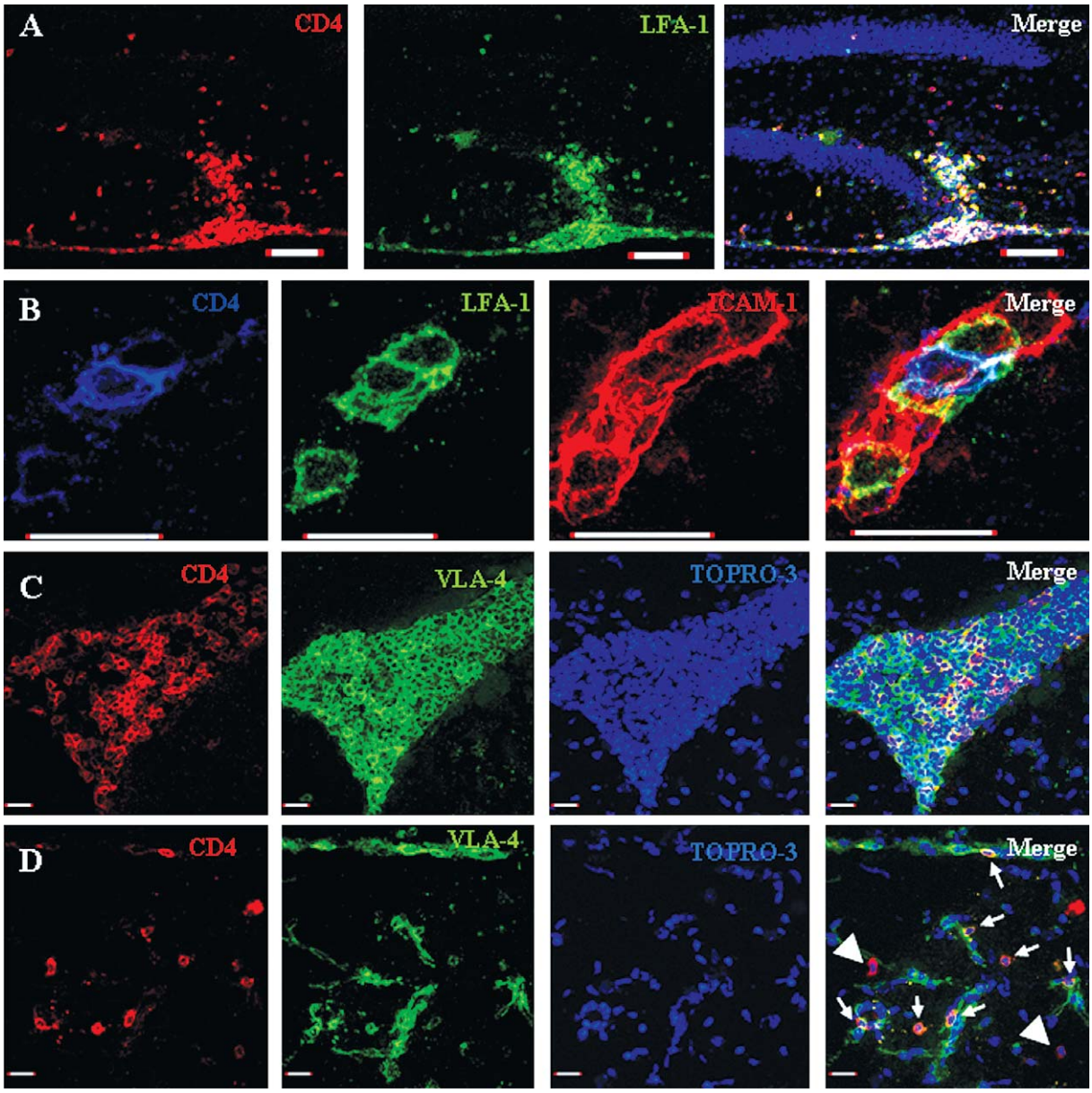
Supplementary Figure 4. CD11c$^+$ cells are co-localized with CD4 T cells at the perivascular space. AβPP/IFN-γ Tg mice were immunized with Aβ as described in Materials and Methods and killed 19 days later. Brain sections were immunolabeled using antibodies against laminin (green), CD11c (red), and CD4 (blue). (A) Parenchymal and (B) leptomeningeal vessels with numerous CD11c$^+$ and CD4 T cells confined to the perivascular space. Higher magnifications are shown in the inlets. Bars represent 20 μm. Abbreviations: parenchyma (PC); leptomeningeal vessel (LPV); leptomeningeal space (LPS).



Supplementary Figure 5. PLP immunization of AβPP/IFN-γ Tg mice results in accumulation of CD11c$^+$ cells on cerebellum vessels. AβPP/IFN-γ Tg mice were immunized with $PLP_{139-155}$ as described in Materials and Methods and killed 19 days later. Brain sections were stained for PECAM-1 (green) and CD11c (red) and counterstained with TO-PRO 3 (blue). (A) Vessels within the white matter cerebellum region loaded with CD11c$^+$ cells. (B) Higher magnification of a representative vessel showing the typical accumulation of CD11c$^+$ cells in the perivascular spaces (arrowheads) of mice under acute EAE. Bars represent 400 μm in (A) and 20 μm in (B).



Supplementary Figure 6. ICAM-1 and VCAM-1 upregulation following Aβ-immunization of AβPP/IFN-γ Tg mice. AβPP/IFN-γ Tg mice were untreated (upper panel) or Aβ-immunized (lower panel), killed 19 days post immunization and brain sections were stained as described in Materials and Methods. (A) A representative brain section stained with antibodies against PECAM-1 (green) and ICAM-1 (red) and counterstained with TO-PRO 3 (blue). ICAM-1 immunoreactivity was seen in both PECAM-1^{+} endothelial cells (arrow) as well as on PECAM-1^{-} leukocytes consisting of cells morphological-resembling resident microglia (arrowhead) as well as on infiltrating immune cells (asterisk). (B) A representative brain section stained with antibodies against PECAM-1 (green) and VCAM-1 (red) and counterstained with TO-PRO 3 (blue).



Supplementary Figure 7. Brain infiltrating CD4 T cells express LFA-1 and VLA-4 adhesion molecules. AβPP/IFN-γ Tg mice aged 9 months were immunized with Aβ and killed 19 days later, and brain sections were immunolabeled as described in Materials and Methods. (A) Brain sections were immunoloabeled with anti-CD4 (red) anti-LFA-1 (green) and counterstained with TO-PRO 3 (blue). (B) High magnification of a parenchymal vessel stained with anti-CD4 (blue), anti-LFA-1 (green) and anti-ICAM-1 (red). High magnification of the leptomeninges (C) and the parenchyma (D) stained with anti-CD4 (red), anti-VLA-4 (green) and counterstained with TO-PRO-3 (blue). VLA-4 expressing (arrows) and not expressing (arrowheads) CD4 T cells are marked. Whereas 85% of the leptomeningeal CD4 T cells expressed VLA-4, only about 50% of the parenchymal CD4 T cells expressed VLA-4. Bars represent 100 μm in (A) and 20 μm in (B, C and D).