Supplementary figure legends

Supplementary Figure 1. scRNA-seq of young adult CNS compartments show similar profile of resting CNS tissue resident ILCs. (A) Complete flow cytometry gating strategy for identification of ILC in adult CNS WT young adult (8-11 weeks old) mice. **(B)** UMAP visualization of the scRNA-seq from total $CD45^+$ immune cells found within whole brain and micro-dissected CNS barriers (dura mater and choroid plexus). Data are from Van Hove et al., (2019). **(C)** Violin plot represent the distribution of ILC gene expression programs, defined in table 1, grouped by clusters. **(D)** UMAP representation of the lymphocyte subclusters (left), and feature plot of T cell associated markers expressed among the new obtained clusters (right). **(E)** Heatmap displaying the top 15 DEGs among ILC-specific clusters (clusters 1, 2 and 5). Color scale represents average expression. **(F)** Violin plots of lineage-specific cytokines and Ki67. **(G)** Representative gating for identification i.v. CD45-PE^+ immune cells (CD45-$PerCP^+$) in peripheral and CNS compartments. Quantification is represented as mean ± SD, n= 6. **(H)** UMAP plot representation showing average expression of circulating and tissue resident associated markers. **(I)** Dotplot of selected gene expression within clusters. Color scale represents average expression, dot size visualizes fraction of cells within the cluster expressing the gene. **(J)** On the left is the representative gating strategy for the identification of $ST2^+$ ex-ILC3s using the $Rorc(gt)^{cre/wt}$×$R26^{eYFP}$ FM^+ mice 2–3-month-old. On the right, the graph depicts the proportion of $RORgt^-YFP^+$ ex-ILC3s among $ST2^+$ ILCs in brain and dura mater. Graph is depicted as mean ± SEM., n = 9 examined over 2 independent experiments.

Supplementary Figure 2. No gender differences in the CNS ILC compartment of young adult mice. (A) Quantification of the absolute numbers of each ILC subset and the percentage over total ILCs in whole brain and **(B)** dura mater of male and female young adults (8-10 weeks old). Graphs are depicted as mean ± SEM., n = 6-8 / group. Dura mater were pooled in groups of two when necessary. **(C)** Quantification of Ki67 expression among ILCs within the different CNS compartments in male and female mice. Data are depicted as ± SEM., n = 3 / group. Data were analyzed by a Mann Whitney t-test.

Supplementary Figure 3. $Eomes^-$ and $Eomes^+$ cells are present in the brain along ontogeny. (A) Quantification and representative plots of NKp46 and Eomes expression by $CD122^+$T-bet^+ cells along ontogeny. **(B)** Frequency and **(C)** absolute numbers of group 1 ILC subsets identified as $Eomes^-$ (ILC1) and $Eomes^+$ (NK) in the brain along ontogeny. Data are depicted as mean ± SEM., n = 3-7 / timepoint examined over ≥ 2-3 independent experiments. **(D)** Quantification of Ki67 expression in $Eomes^+$ and $Eomes^-$ in brain along ontogeny.

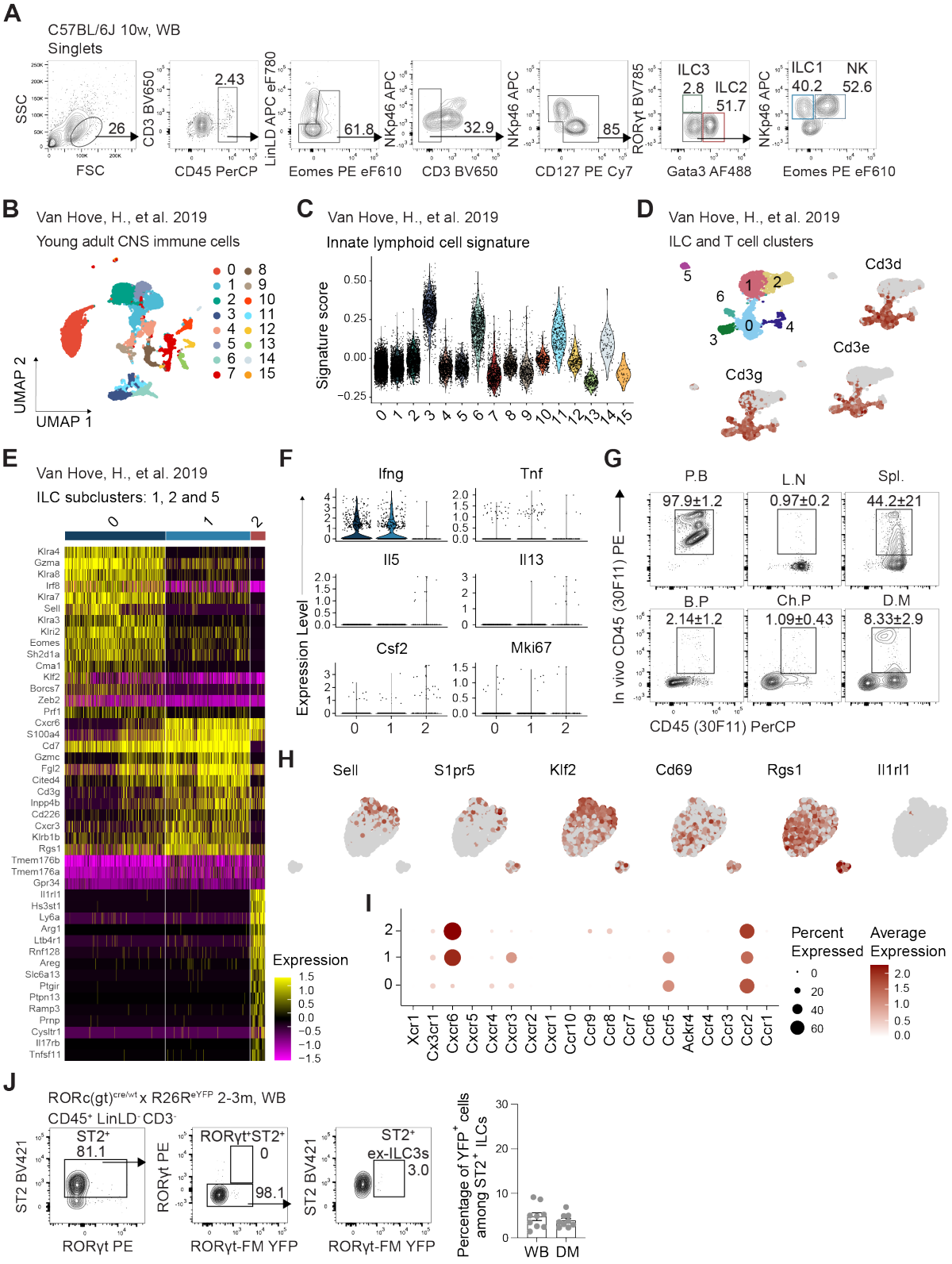
Quantification as mean ± SEM., n = 4-8 / timepoint. Data are representative of ≥ 3 independent experiments. From E16 to P1 whole litter were pooled. From P9 to P15 onwards, animals were pooled in 2-3 when necessary.

Supplementary Figure 4. scRNA-seq analysis of P9.5 brain and dura ILCs identified presence of all ILC subsets. (A) Heatmap displaying the top 15 differentially expressed genes (DEGs) within the identified ILC clusters comparing brain versus dura cells. **(B)** Dot plot and **(C)** UMAP plot representation showing average expression of other brain immune cell, common lymphoid progenitor (CLP) and ILC progenitor (ILCp) cell markers, respectively. **(D)** Violin plot visualization of the expression of effector cytokines within the identified helper-ILC clusters in brain and dura meninges.

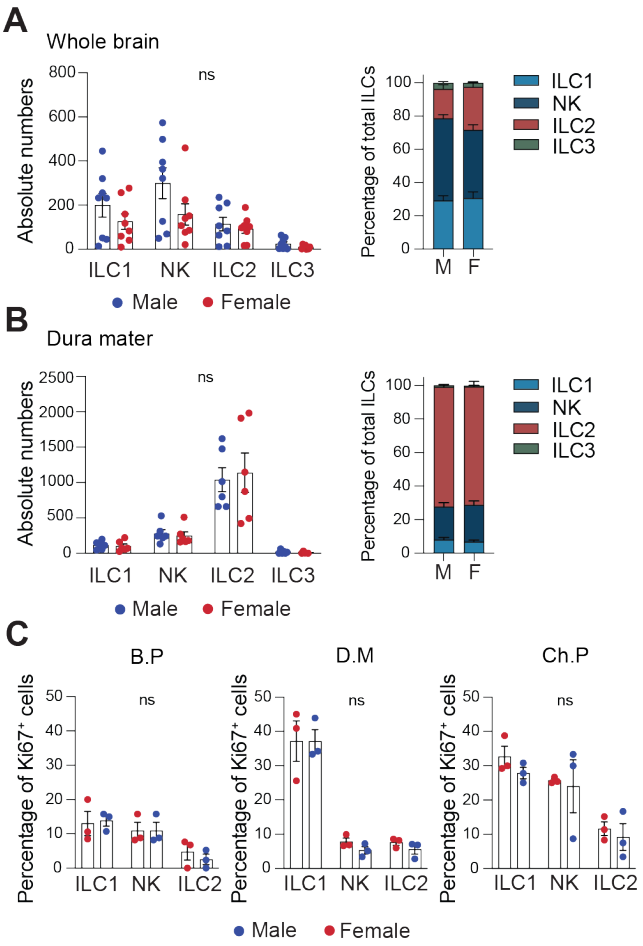
Supplementary Figure 5. Transitory presence of ILC3s during brain ontogeny. (A) Absolute numbers of $CD4^{+/-}CCR6^{+}$, $NKp46^{+}$ and double-negative (DN) $ROR\gamma t^{+}$ ILC3 subsets (n = 3-8 / timepoint, mean ± SEM.). Data are representative of ≥ 3 independent experiments. **(B)** Heatmap displaying the top 15 DEGs as assessed by scRNA-seq within ILC1/ILC3 subclusters. **(C)** Violin plot of selected markers expression within ILC1/ILC3 subclusters. **(D)** Percentage from total analyzed cells of each identified subcluster among ILC1 and ILC3s in whole brain and dura. **(E)** Quantification of Ki67 expression in each ILC3 subset in brain along ontogeny (n = 3-7 / timepoint). From E16 to P1. whole litters were pooled, from P9 to P15 onwards. 2-3 animals were pooled if needed. Data are depicted as mean ± SEM.

Extended data

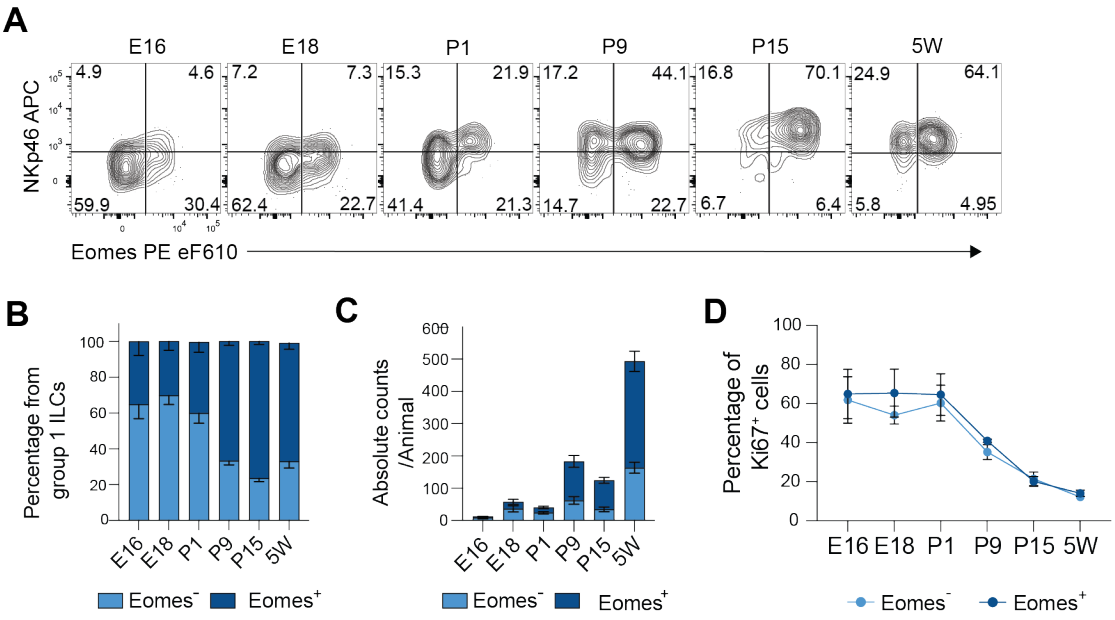
Supplementary Figure 1



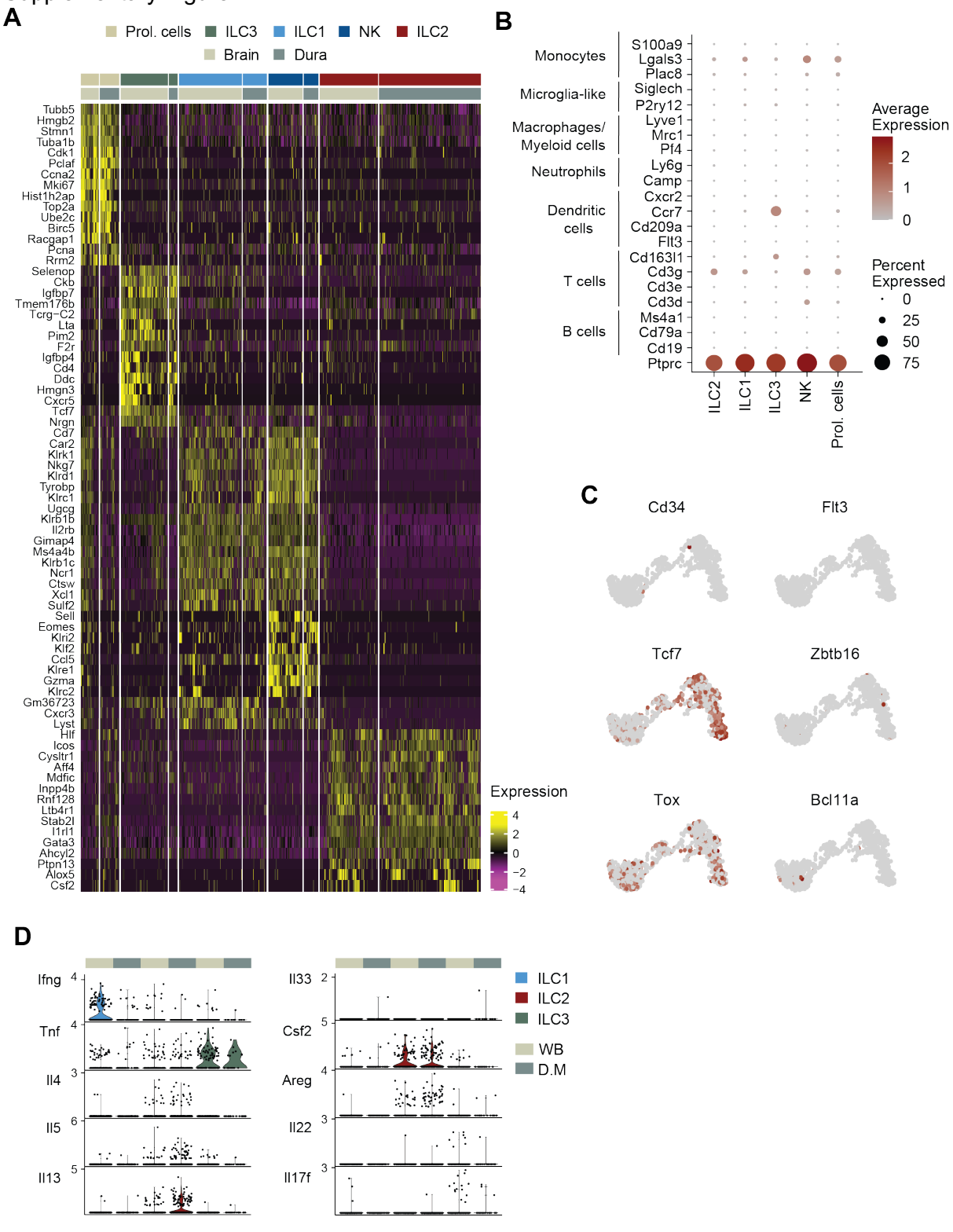
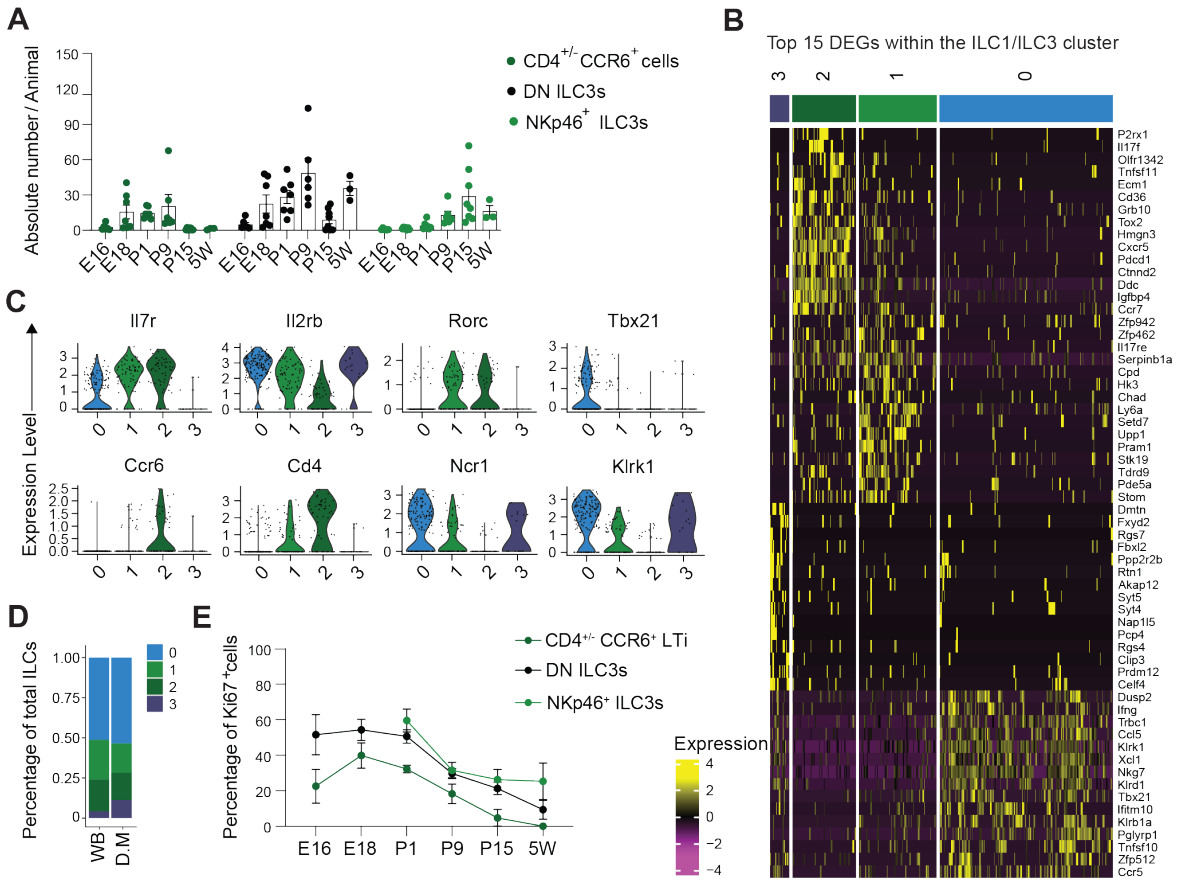
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5