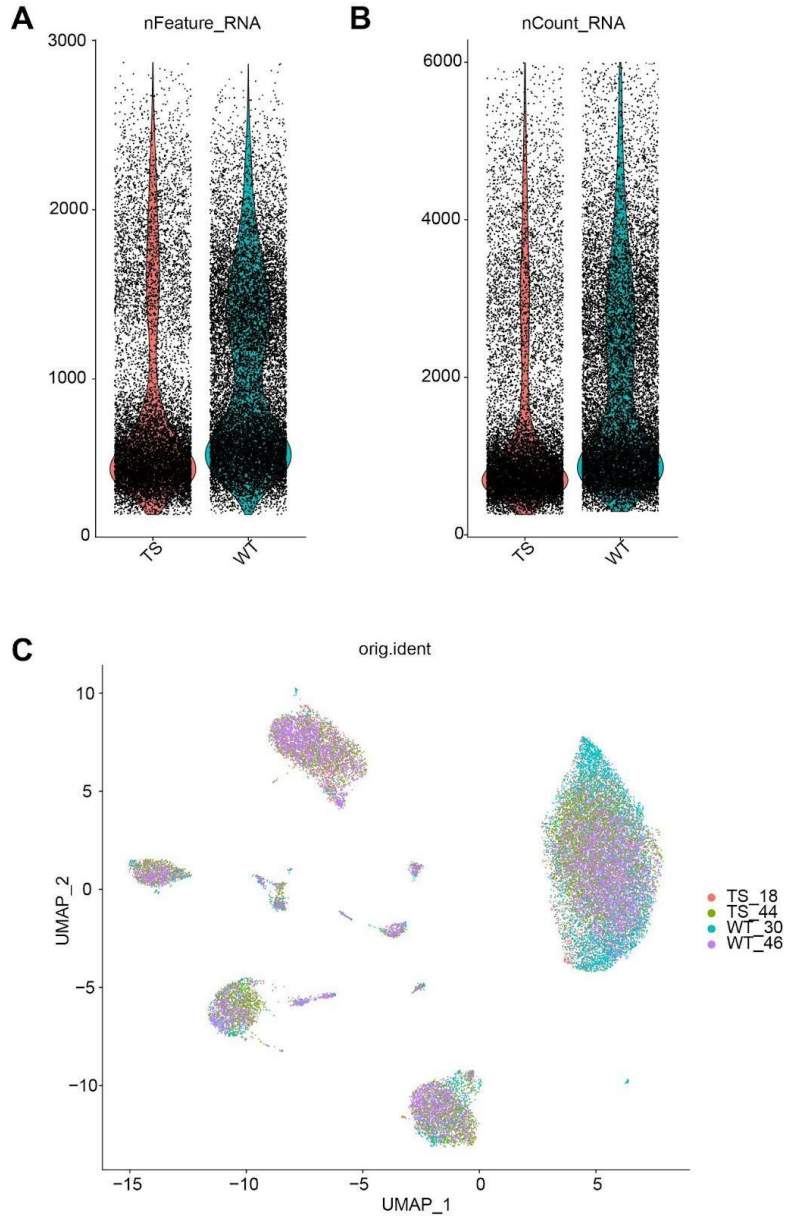
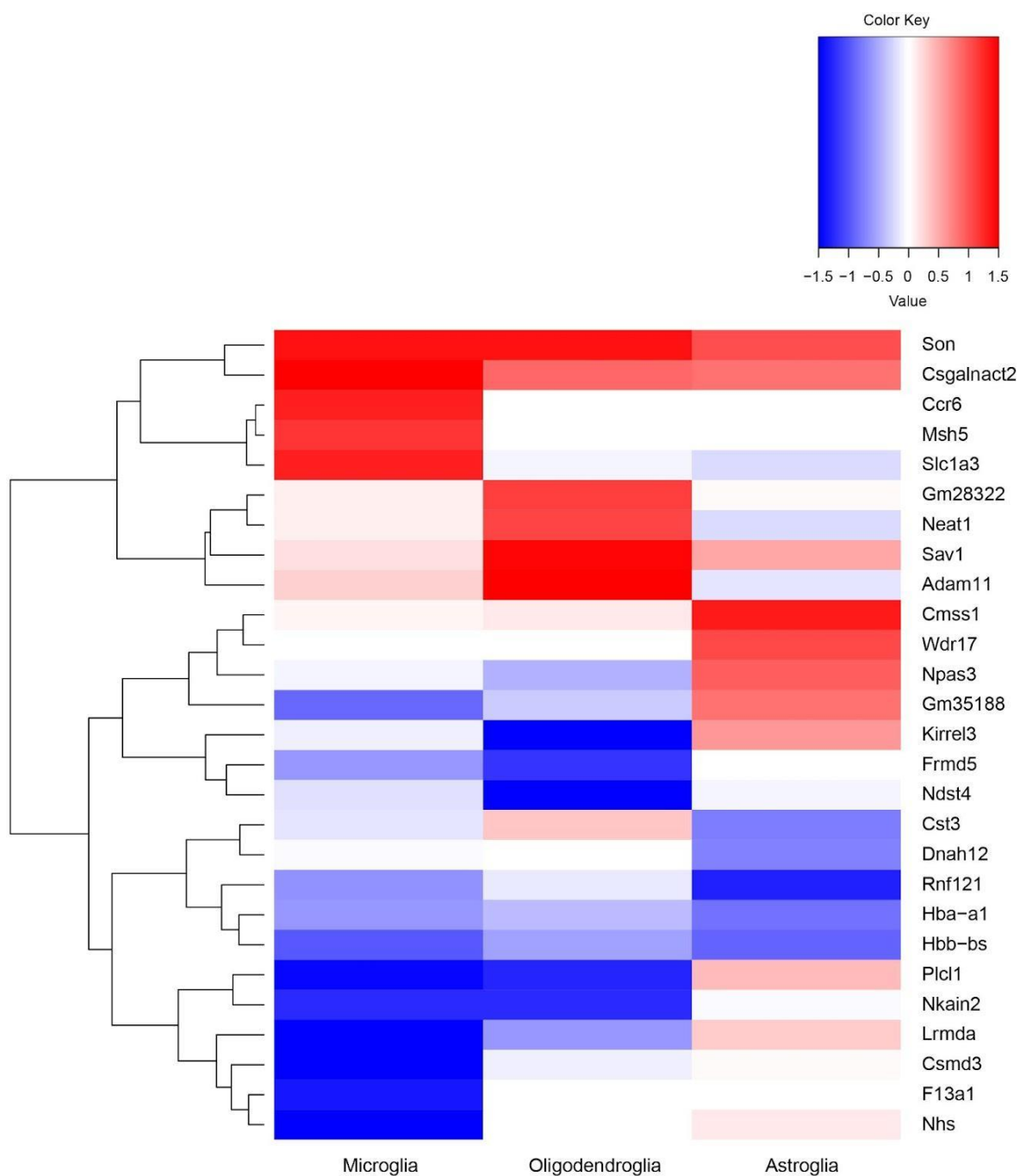


## Supplementary Materials

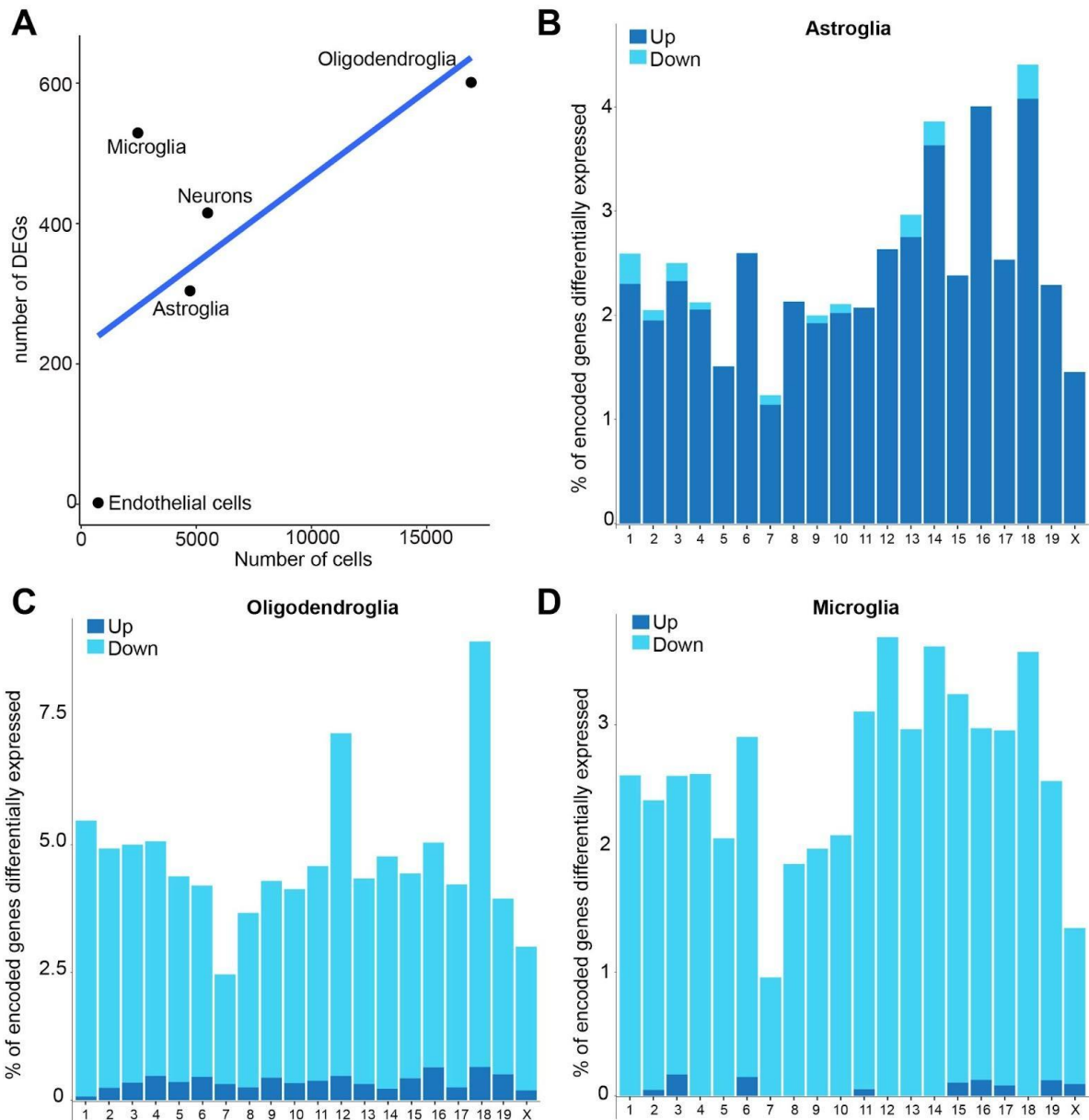
### Supplementary Figures



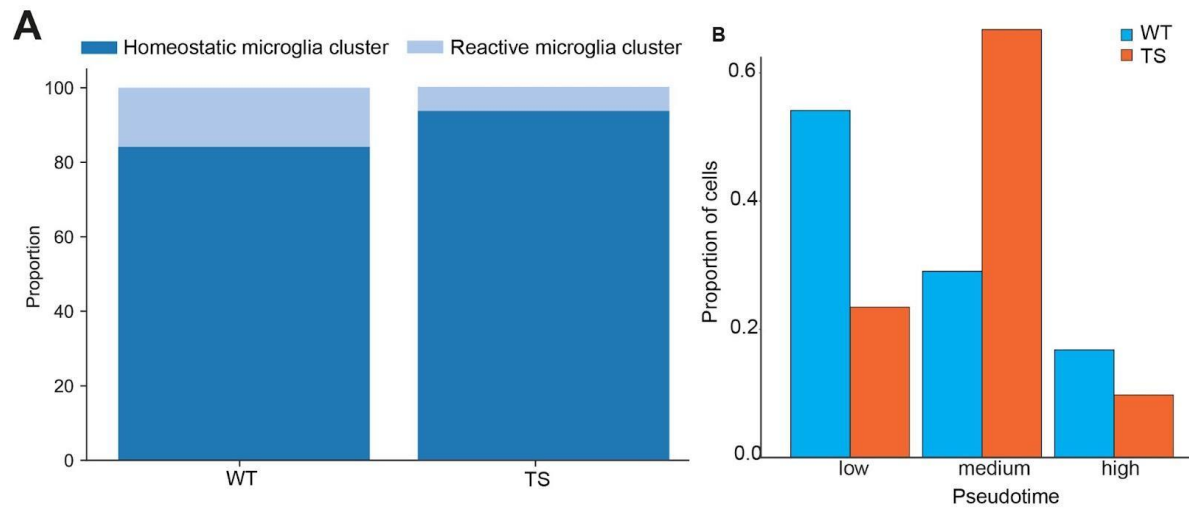
**Supplementary Figure S1.** (A) Violin plot for the number of unique genes detected per cell in each sample. (B) Violin plot for the number of unique transcripts detected per cell in each sample (WT = 2, Ts65Dn = 2). (C) UMAP representation of the nuclei transcriptome representing the sample batch effects on cell clusters. All the clusters are composed of a mixture of cells that originated from each of the four samples. Cells are colored by sample (WT\_30, WT\_46, TS\_18 and TS 44).



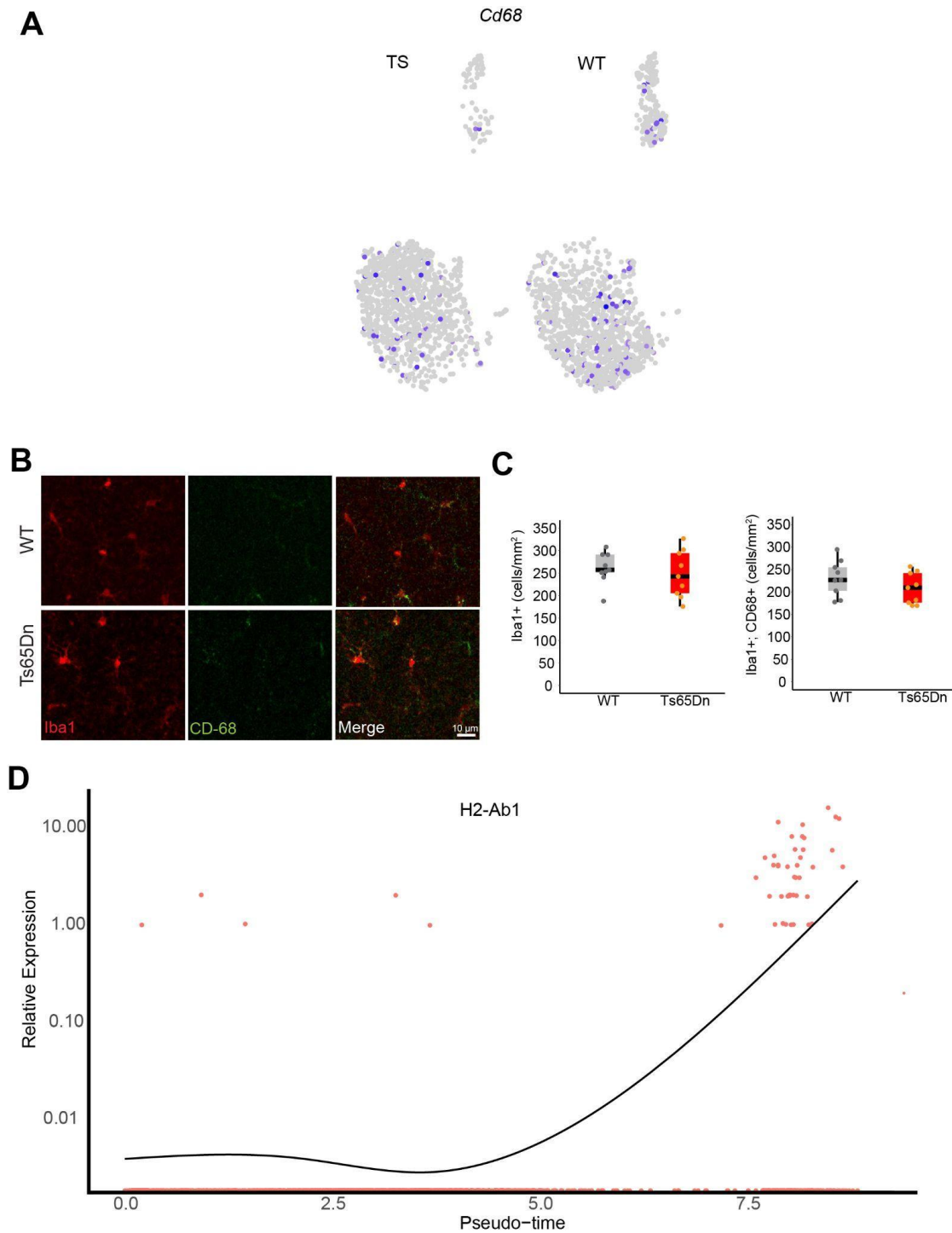
**Supplementary Figure S2.** Heatmap showing log<sub>2</sub> FC values for the top DEGs (rows) in each glial type (columns).



**Supplementary Figure S3. (A)** Correlation between the number of DEGs and the number of cells for the different cellular populations **(B)** Distribution of DEGs along the mouse chromosomes in the astroglia population. **(C)** Distribution of DEGs along the mouse chromosomes in the oligodendroglia population. **(D)** Distribution of DEGs along the mouse chromosomes in the microglia population.



**Supplementary Figure S4. (A)** Proportion of cells that are within homeostatic microglial cluster (MG\_0) and reactive microglial cluster (MG\_1) in WT and in Ts65Dn mice. **(B)** Proportion of cells that are within the different pseudotime scores (low, medium and high) both in WT and in Ts65Dn mice.



**Supplementary Figure S5. (A)** Representation of the two-dimensional embedding of TS and euploid microglial cells by diffusion map representing the expression of *Cd68* marker. **(B)** Representative image of WT (above) and Ts65Dn (below) showing the Iba1+ cells (red), CD-68 signal (green) and merge image. **(C)** Quantification of Iba1+ cells (above) and Iba1+; CD-68+ cells (below) in CA1 stratum radiatum of WT and Ts65Dn mice (WT = 9 sections from 3 mice, Ts65Dn = 9 sections from 3 mice). Two-tailed T test. On the boxplots, the horizontal line indicates the median, the box indicates the first to third quartile of expression and whiskers indicate  $1.5 \times$  the interquartile range. **(D)** Relative expression of H2-Ab1 across the different microglial states in the pseudotime.

## Supplementary Tables

**Supplementary Table S1. Differentially expressed genes in individual cell types.** Statistically significant DEGs are depicted in the Gene column and grouped by cell type (Astrocytes, Microglia, Oligodendrocytes). A negative avg\_log2FC value indicates a downregulation while a positive value indicates upregulation compared to euploid controls. pct.1 is the percentage of single cells in which a gene is expressed for the specific cell type and pct.2 is the percentage expression in all other single cells.

[https://crgcnag-my.sharepoint.com/:f:/g/personal/csierra\\_crg\\_es/Esrwr5mSzk5lvvC2lopxRIMBOtUymZOwxpc1V2HjzVhKdg?e=cJbHIU](https://crgcnag-my.sharepoint.com/:f:/g/personal/csierra_crg_es/Esrwr5mSzk5lvvC2lopxRIMBOtUymZOwxpc1V2HjzVhKdg?e=cJbHIU)

**Supplementary Table S2. Cluster-specific marker genes for the different cellular subpopulations.** Columns A-I give statistics on cell type marker genes. WT and TS\_avg\_log2FC is the log fold change in the expression levels of a gene between the specific cell type and all the other cell types for WT and TS groups, respectively. pct.1 is the percentage of single cells in which a gene is expressed for the specific cell type and pct.2 is the percentage expression in all other single cells. Minimum\_p\_val is the combined p value between WT and TS groups. Columns K-O give the marker gene lists for the hippocampal cell types

[https://crgcnag-my.sharepoint.com/:f:/g/personal/csierra\\_crg\\_es/Esrwr5mSzk5lvvC2lopxRIMBOtUymZOwxpc1V2HjzVhKdg?e=cJbHIU](https://crgcnag-my.sharepoint.com/:f:/g/personal/csierra_crg_es/Esrwr5mSzk5lvvC2lopxRIMBOtUymZOwxpc1V2HjzVhKdg?e=cJbHIU)

**Supplementary Table S3. Identity markers of the homeostatic and reactive microglial clusters.** Columns A and B indicate the specific marker genes of the homeostatic (cluster\_0) and reactive (cluster\_1) clusters, respectively.

[https://crgcnag-my.sharepoint.com/:f:/g/personal/csierra\\_crg\\_es/Esrwr5mSzk5lvvC2lopxRIMBOtUymZOwxpc1V2HjzVhKdg?e=cJbHIU](https://crgcnag-my.sharepoint.com/:f:/g/personal/csierra_crg_es/Esrwr5mSzk5lvvC2lopxRIMBOtUymZOwxpc1V2HjzVhKdg?e=cJbHIU)