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Supplemental Information

The Major Risk Factors for Alzheimer's Disease: Age, Sex, and Genes Modulate the Microglia Response to Aβ Plaques

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Figure S1

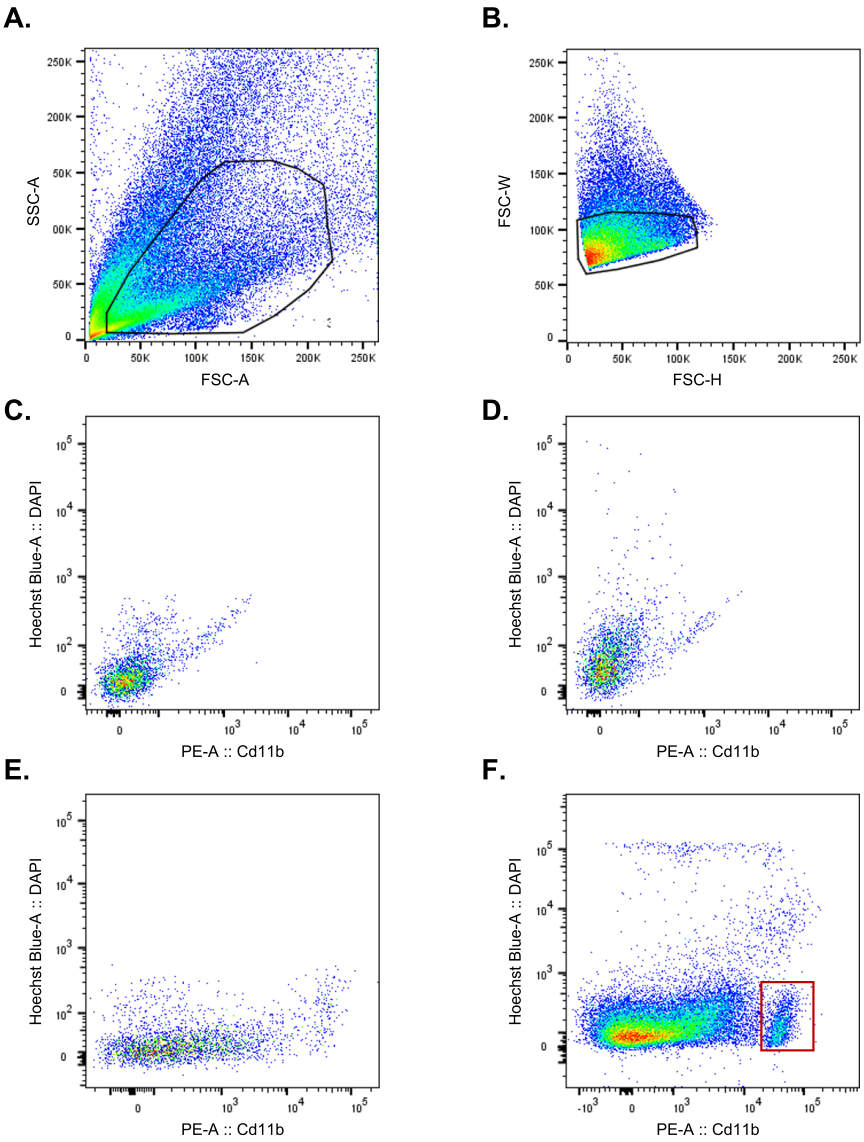

Figure S1, related to Figure 1. Fluorescence activated cell sorting of single live microglia cells. Single cells are identified by drawing gates (black line shapes) when plotting side scatter vs forward scatter (**A**) and forward scatter - width vs forward scatter - height (**B**). Single live microglia cells (CD11b+ / DAPI-) are identified by comparing plots of CD11b-PE signal vs DAPI signal in a non-stained sample (**C**), a DAPI-only stained sample (**D**), a CD11b-PE-only stained sample (**E**), and a double stained sample (**F**).

Figure S2

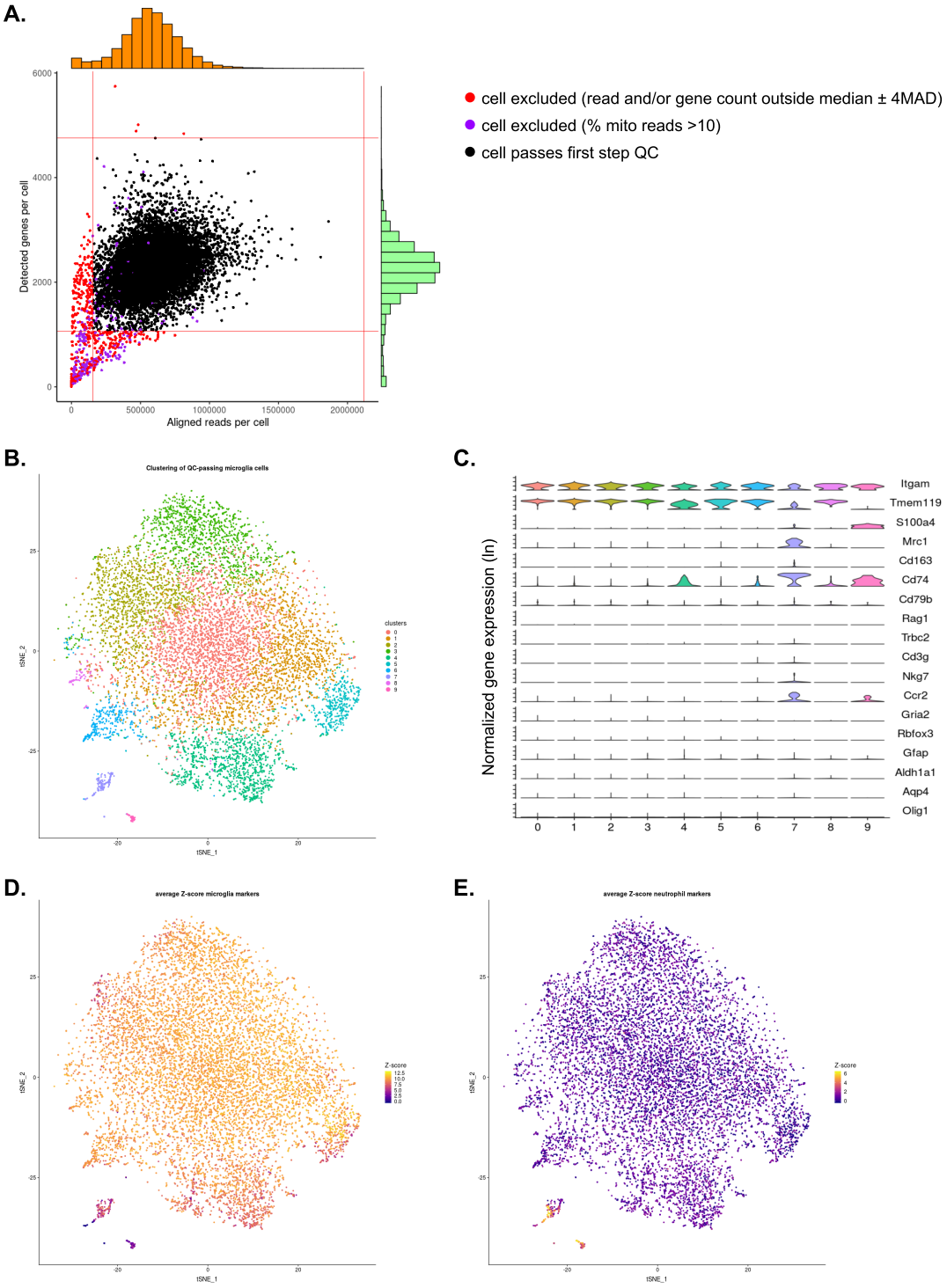

Figure S2, related to Figure 1. Quality control of cells analysed by single cell RNA seq (App^{NL-G-F} & C57Bl/6J dataset). A. From in total 12,024 single cells sequenced,186 cells were excluded because belonging to two outlier plates from a first principal component analysis. For each of the remaining 11,838 single microglia cells the count of reads was aligned versus the number of genes detected. Histograms illustrate the distribution of cells across bins of read counts (top, orange histogram) and genes detected (right, green histogram). We set limits of the median ± 4 * median absolute deviation (MAD) for either read counts or gene counts (red lines) to exclude potential empty wells, doublets and damaged cells; cells outside these limits are coloured in red. Further, we excluded cells (purple) with more than 10% of reads aligning to mitochondrial genes. **B**: t-SNE plot of the 11,038 microglia cells passing quality control, coloured by clusters. **C**: Violin plots of selected marker genes for microglia (*Itgam*, *Tmem119*), monocytes (*S100a4*), perivascular macrophages (*Mrc1, Cd163, Cd74*), B cells (*Cd79b*, *Rag1*), T cells (*Trbc2*, *Cd3g*), natural killer cells (*Nkg7*), neutrophils (*Ccr2*, *Plbd1*), neurons (*Gria2, Rbfox3*), astrocytes (*Gfap*, *Aldh1a1*, *Aqp*4), and oligodendrocytes (*Olig1*). **D**: t-SNE plot as in A, coloured by the Z-score of microglia markers (*Tmem119*, *Trem2*, *Csf1r*, *Cx3cr1*). **E**: t-SNE plot as in A, coloured by the Z-score of neutrophils markers (*Ly6g*, *Ptprc*, *Ccr2*, *Plbd1*).

Figure S3

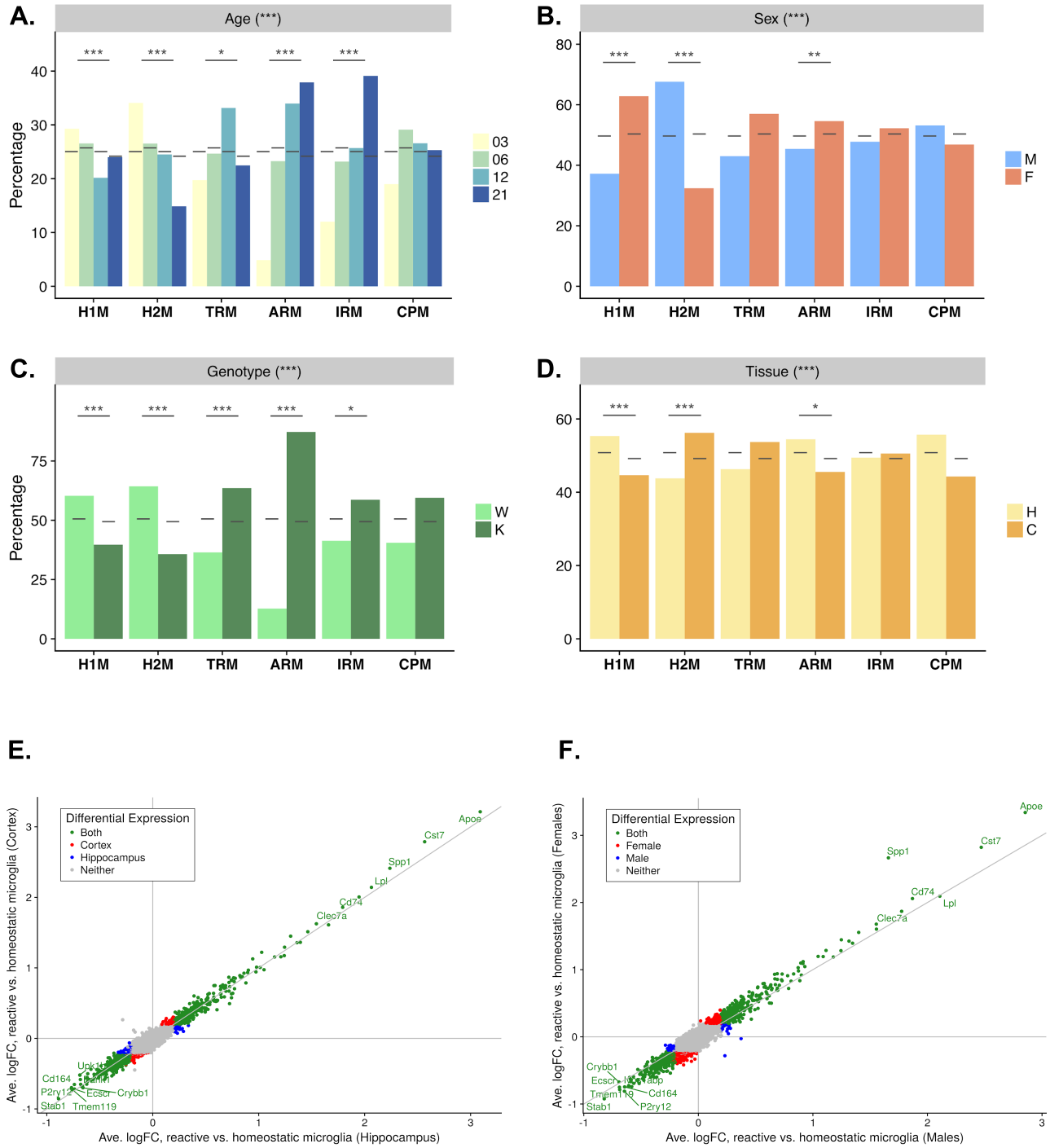

Figure S3, related to Figure 1. Proportions of cells by cluster. Counts of cells per cluster, grouped by age (**A**), gender (**B**), genotype (**C**) and tissue (**D**). Horizontal bars represent the expected amounts of cells expected by chance (χ^2 test), while stars denote range of p value (*: <0.05, **: <0.01, ***: <0.001). **E** and **F**: Comparison of gene expression in reactive cells (TRM, ARM, IRM) vs homeostatic cells (H1M, H2M, CPM). **E.** Comparison between cortex (X axis) and hippocampus (Y axis). **F.** Comparison between female (X axis) and male (Y axis). Genes were deemed to be differentially expressed if they showed an absolute fold change of at least 0.2 (on an ln scale) and an adjusted p value below 0.05.

Figure S4

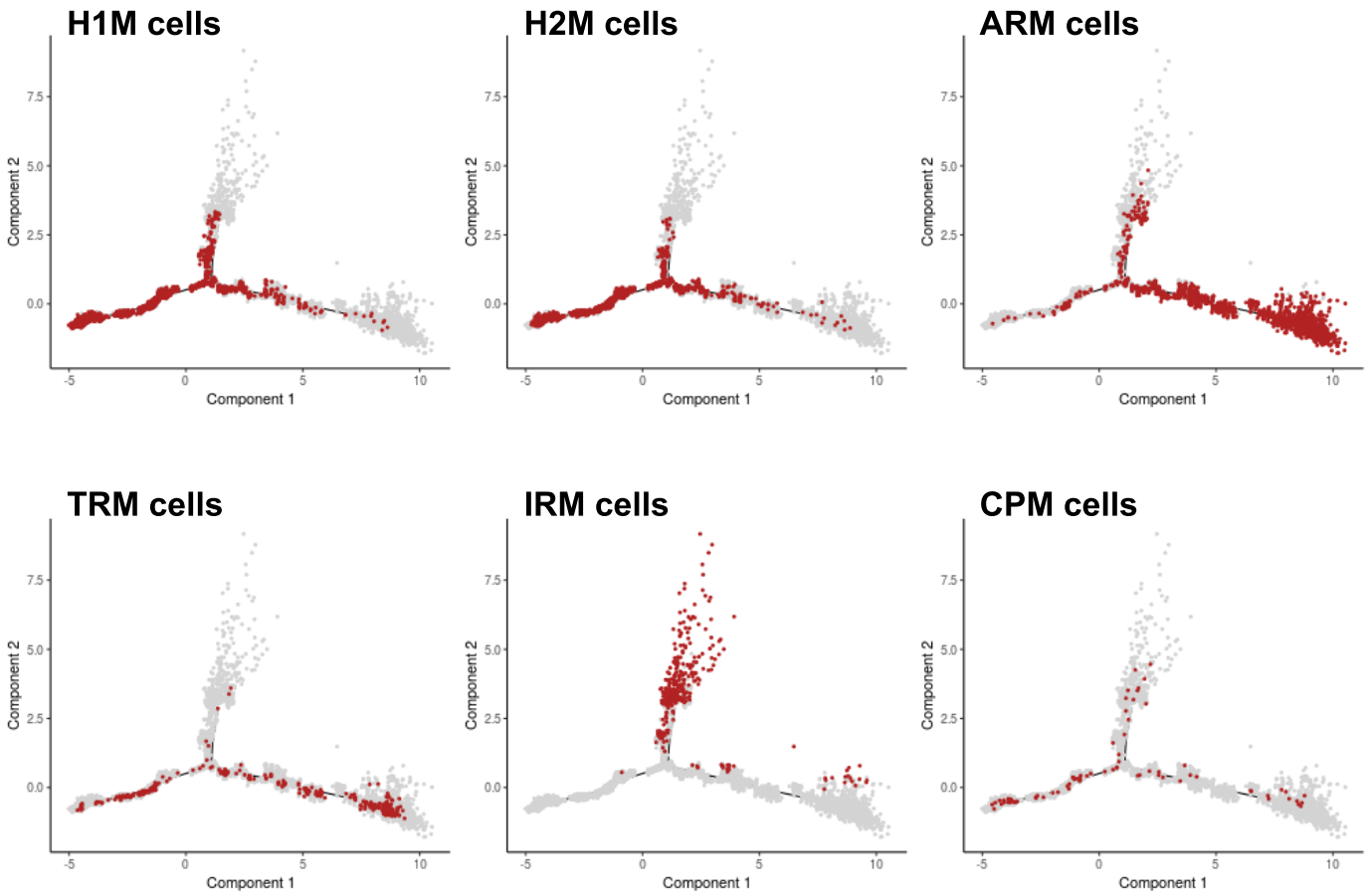

Figure S4, related to Figure 2. Distribution of cells from each cluster over pseudotime trajectories. Cells form each cluster identified (Figure 1B) are plotted over the plot of cell trajectories (Figure 2A).

Figure S5

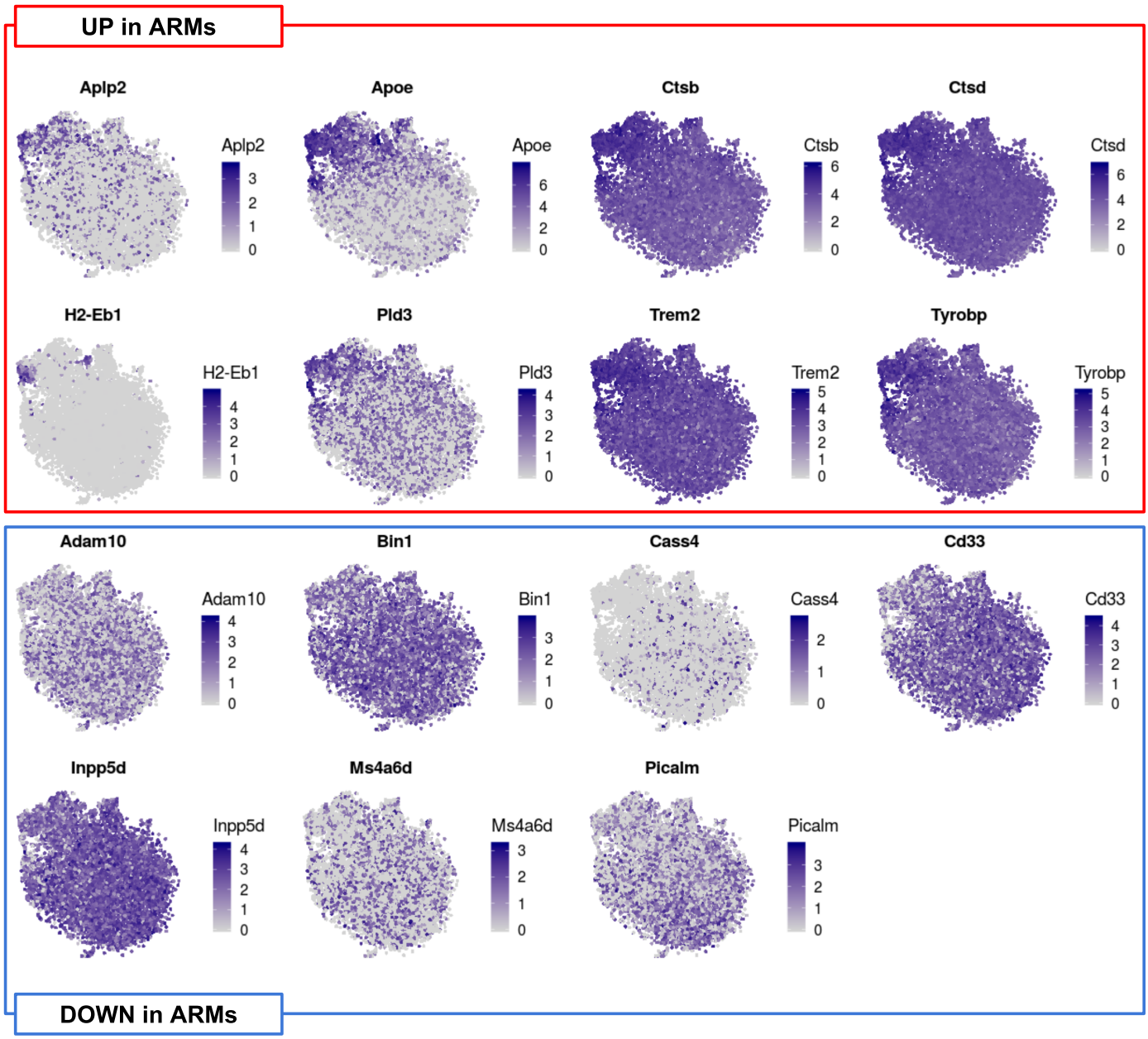

Figure S5, related to Figure 3. Expression of AD-related genes. t-SNE plots as in Figure 1B, coloured by the level of normalized expression (ln scale) of expressed genes related to AD pathology (for a full list of the genes considered see table S2). Genes significantly upregulated in the ARM cluster are highlighted in red, while those significantly downregulated in the ARM cluster are highlighted in blue.

Figure S6

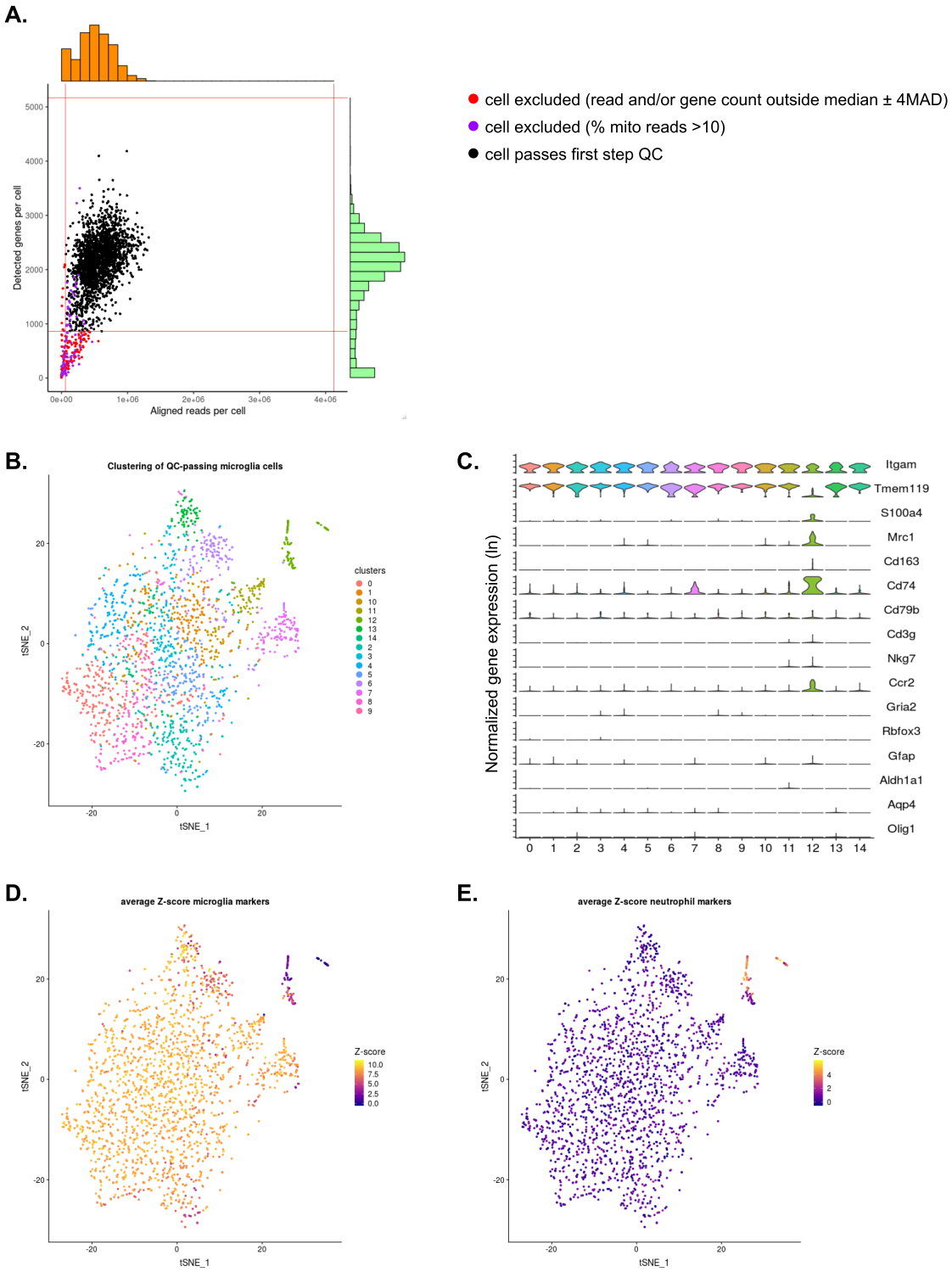

Figure S6, related to Figure 5. Quality control of cells analysed by single cell RNA seq (*APP/PS1* dataset). **A.** Analysis of the count of reads aligned for each of the 2,304 single microglia cells sequenced vs the number of genes detected for each cell. Histograms illustrate the distribution of cells across bins of read counts (top, orange histogram) and genes detected (right, green histogram). We set limits of the median ± 4 * median absolute deviation (MAD) for either read counts or gene counts (red lines) to exclude potential empty wells, doublets and damaged cells; cells outside these limits are coloured in red. Further, we excluded cells with more than 10% of reads aligning to mitochondrial genes (purple dots in scatterplot). We thus excluded 340 cells, and we carried on 1,964 cells for further analysis. **B.** t-SNE plot of the 1,964 microglia cells passing quality control, coloured by clusters. **C.** Violin plots of selected marker genes for microglia (*Itgam*, *Tmem119*), monocytes (*S100a4*), perivascular macrophages (*Mrc1, Cd163, Cd74*), B cells (*Cd79b*, *Rag1*), T cells (*Trbc2*, *Cd3g*), natural killer cells (*Nkg7*), neutrophils (*Ccr2*, *Plbd1*), neurons (*Gria2, Rbfox3*), astrocytes (*Gfap*, *Aldh1a1*, *Aqp*4), and oligodendrocytes (*Olig1*). **D.** t-SNE plot as in A, coloured by the Z-score of microglia markers (*Tmem119*, *Trem2*, *Csf1r*, *Cx3cr1*). **E.** t-SNE plot as in A, coloured by the Z-score of neutrophils markers (*Ly6g*, *Ptprc*, *Ccr2*, *Plbd1*).

Table S1

	App^{NL-G-F}				*C57Bl/6*			
	Female		Male		Female		Male	
	Cortex	Hippocampus	Cortex	Hippocampus	Cortex	Hippocampus	Cortex	Hippocampus
3 months	364	356	273	360	365	368	275	357
6 months	336	357	343	347	361	363	365	363
12 months	336	336	339	354	349	353	335	361
21 months	340	318	342	339	348	337	349	349

Table S1, related to Figure 1. Number of cells post QC. For each experimental condition, we report the number of cells kept for analysis after QC.

Table S2

gene	reason
Apoe	gwas
Trem2	gwas
Tyrobp	gwas
Cr2	gwas
Bin1	gwas
Cd2ap	gwas
Epha1	gwas
Clu	gwas
Ms4a6d	gwas
Picalm	gwas
Abca7	gwas
Cd33	gwas
H2-Eb1	gwas
Sorl1	gwas
Slc24a4	gwas
Dsg2	gwas
Inpp5d	gwas
Mef2c	gwas
Zcwpw1	gwas
Fermt2	gwas
Cass4	gwas
Ptk2b	gwas
Ctsf	exome_seq
Ccl11	modifier
Plcg2	rare_variant
Abi3	rare_variant
Pld3	rare_variant
Mme	abeta_degrading
Mmel1	abeta_degrading
Ece1	abeta_degrading
Ece2	abeta_degrading
Ace	abeta_degrading
Mmp2	abeta_degrading
Mmp9	abeta_degrading
Mmp14	abeta_degrading
Bsg	abeta_degrading
Ide	abeta_degrading
Serpinf2	abeta_degrading
Apeh	abeta_degrading
Mobp	abeta_degrading
Ctsd	abeta_degrading
Ctsb	abeta_degrading
Bace1	functional_relationship
Bace2	functional_relationship
Mapt	functional_relationship
Aplp1	functional_relationship
Aplp2	functional_relationship
App	FAD
Psen1	FAD
Psen2	FAD
Adam10	family_assoc_Marioni
Adamts4	family_assoc_Marioni
Vkorc1	family_assoc_Marioni
Tspoap1	family_assoc_Marioni
Pvr	family_assoc_Marioni

Table S2, related to Figure 3. List of AD-related genes. For each gene, we provide a reason for inclusion.