Supplementary Information

Supplementary Method

Pseudotime analysis of single cells

Pseudotime analysis was performed on microglia when cells transitioned from homeostatic microglia to activated microglia. A matrix of UMAP coordinates along with the cluster labels of microglia cells from subcluster analysis described in the section "Immune cell subcluster analysis" was used as input to Slingshot 2.4.0[1] to obtain pseudo-temporal ordering of the cells along the progression axis. To identify temporally dynamic genes, we fitted a general additive model (GAM) between a loess term of gene expression and pseudotime for each gene. Genes with false discovery rate (FDR) corrected p-value < 0.05 were considered significant. R package ComplexHeatmap[2] was used to perform consensus k-means clustering. The algorithm was set to repeat K-means clustering 100 times (row_km_repeats = 100) and report a consensus result of the 100 iterations as the final consensus k-means clustering result. The number of gene co-expression modules is set to 3 (row_km = 3) because previous publications have shown that DAM activation involves two steps. Therefore, there are 3 groups of genes: down-regulated genes, early-, and late-activated genes[3]. We chose 3 to compare our pseudotime DEGs with the reported DAM pseudotime DEGs.

Comparison with previously published snRNA-seq data

The gene expression data and metadata for Grubman et al.[4] (accession number GSE138852), Lau et al.[5] (accession number GSE157827), and Feleke et al.[6] (accession number GSE178146) were downloaded from the Gene Expression Omnibus (GEO). The snRNA-seq data for Mathys et al.[7] were downloaded at Synapse (https://www.synapse.org/#!Synapse:syn18485175) under the doi 10.7303/syn18485175. The ROSMAP metadata can be accessed at https://www.synapse.org/#!Synapse:syn3157322. We followed the same approach as described above for cell quality control, normalization, clustering, major cell type identification. The astrocyte

and microglia subcluster analyses and pseudotime analyses were performed using the same approaches as described for putamen astrocyte and microglia subpopulation analysis.

RNAscope mRNA in situ hybridization combined with immunohistochemistry

The criteria for enrolling the samples into experimental groups were mentioned above. We randomly selected one case from each group for RNAscope mRNA *in situ* hybridization and immunohistochemistry assays. *CD44, TNC, AIF1, APOC1*, and *TREM2* mRNA in situ hybridization (ISH) combined with immunohistochemistry staining of AQP4 or P2RY12 in the whole striatal slides was performed using the RNAscope 2.5 HD Chromogenic Assay kit and RNA Protein Co Detection Assay (Advanced Cell Diagnostics, Inc. Newark, CA, USA, Cat. #322350 and 323180) with a slightly modified protocol. Major optimizations for success in the snap-frozen human brain included probe incubation, amplification, and non-specific blocking times. Slides were fixed in chilled 10% Neutral Buffered Formalin (NBF; Fisher Scientific, Cat. #22-050-104) and dehydrated in EtOH (50%, 70%, and 100% separately). Primary antibodies were diluted in Co-Detection Antibody Diluent (Abcam Inc. Cambridge, MA, USA, Recombinant Anti-Aquaporin 4 antibody [EPR24281-65] - BSA and Azide free, Cat No. ab282586, diluted 1:200; recombinant Anti-P2Y12 antibody [EPR23511-72] - BSA and Azide free, Cat No. ab274386, diluted 1:100) and incubated on the slides overnight at 4°C, followed by washing 3X for 2 min each in PBS plus 0.01 Tween 20 (PBS-T). The tissue sections were placed in 10% NBF for post-primary fixation, then were pretreated with Protease IV (Universal Pretreatment Reagents, ACD, Newark, CA, USA, Cat. #322380). Briefly, the tissue sections were incubated in the custom human gene-specific RNAscope Hs-CD44 probe (Gene Alias: CDW44; Target Region: 157 - 1435; ACD, Newark, CA, USA, Cat. #311271), Hs-TNC probe (Gene Alias: 150-225; Target Region: 5417 - 6342; ACD, Newark, CA, USA, Cat. #420771), Hs-AIF1 probe (Gene Alias: AIF-1, IBA1, IRT-1, IRT1; Target Region: 8 – 468; ACD, Newark, CA, USA, Cat. #433121), Hs-APOC1 probe (Gene Alias: Apo-C1; Target Region: 2 – 537; ACD, Newark, CA, USA, Cat. #573481), Hs-TREM2 probe (Gene

Alias: TREM-2; Target Region: 5 – 1069; ACD, Newark, CA, USA, Cat. #420491), a positive control probe (human Cyclophilin B (PPIB); ACD, Cat. #476701) and a negative control probe (bacterial dapB; ACD, Cat. #310043); for 1 – 2 h at 40°C in the RNAscope oven (ACD HybEZTM II Hybridization System; ACD, Cat. #321711). Sections were sequentially hybridized to a workflow for amplification molecules, only with modification of incubation with Amp5 for 15 – 45 min at room temperature using the HybEZ humidity control tray and slide rack to maintain humidity. ISH signal was visualized using the Fast RED solution. Applied Co-Detection Blocker to the sections and incubated for 30 min at 40°C for non-specific blocking, followed by washing with PBS-T. Finally, the sections were developed and visualized using a Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (Abcam Inc. Cambridge, MA, USA, Cat. ab64264). The high-resolution images of tissue sections were achieved with a digital whole slide scanner (Nanozoomer 2-HT, Hamamatsu Photonics, Hamamatsu City, Japan) using a 209/0.75 lens (Olympus, Center Valley, PA, USA). We chose the NDP.view2 (Hamamatsu Photonics) software and viewed the digital slides.

_F3_ quantification using RNAscope mRNA in situ hybridization

We chose the RNAscope ISH probe to semi-quantify *F3* gene mRNA expression from the 12 cases studied, which was performed using RNAscope 2.5 HD Chromogenic Assay kit (ACD, Inc. Newark, CA, USA, Cat. #322350) according to the manufacturer's protocol. The sample of PD subject 1654 was replaced with the sample of PD subject 5212 because not enough tissue from subject 1654 was available for the experiment. Briefly, after fixing, dehydrating, and pre-treating, the tissue sections were incubated in a custom human gene-specific RNAscope Hs-F3 probe (Gene Alias: CD142, TF, TFA; Target Region: 275 - 1229; ACD, Newark, CA, USA, Cat. 407611). Then, the tissues were sequentially hybridized to a cascade of amplification molecules, and ISH signal was visualized by incubating with Fast RED solution. The hybridization signals were blindly quantified using FIJI ImageJ version 2.1.0/1.53c. Images were uploaded, and the Colour

Deconvolution function was used to isolate the *F3* signal. The *F3* signal was converted to black and white, and the threshold was adjusted to determine regions of interest. The Analyze Particles function used the pre-determined regions of interest to measure *F3* integrated density on the corresponding non-adjusted image. Four fields of view from each subject (16 images from 4 subjects per diagnostic group) were randomly selected by a lab member not involved in the project. The integrated density of each segmented cell was quantified blindly. Sample IDs were blinded during the data collection and analysis. A total of 1120, 863, and 387 cells in the control, AD, and PD samples were quantified for *F3* expression. A random sampled 387 cells each from control and AD groups together with the 387 cells from PD samples were used for One-Way ANOVA with Tukey's Multiple Comparisons test (*anova* function in R 4.0.2). The analysis was repeated 100 times, and average p-values were calculated and reported. Results with p-value < 0.05 were considered significant.

Statistical analysis.

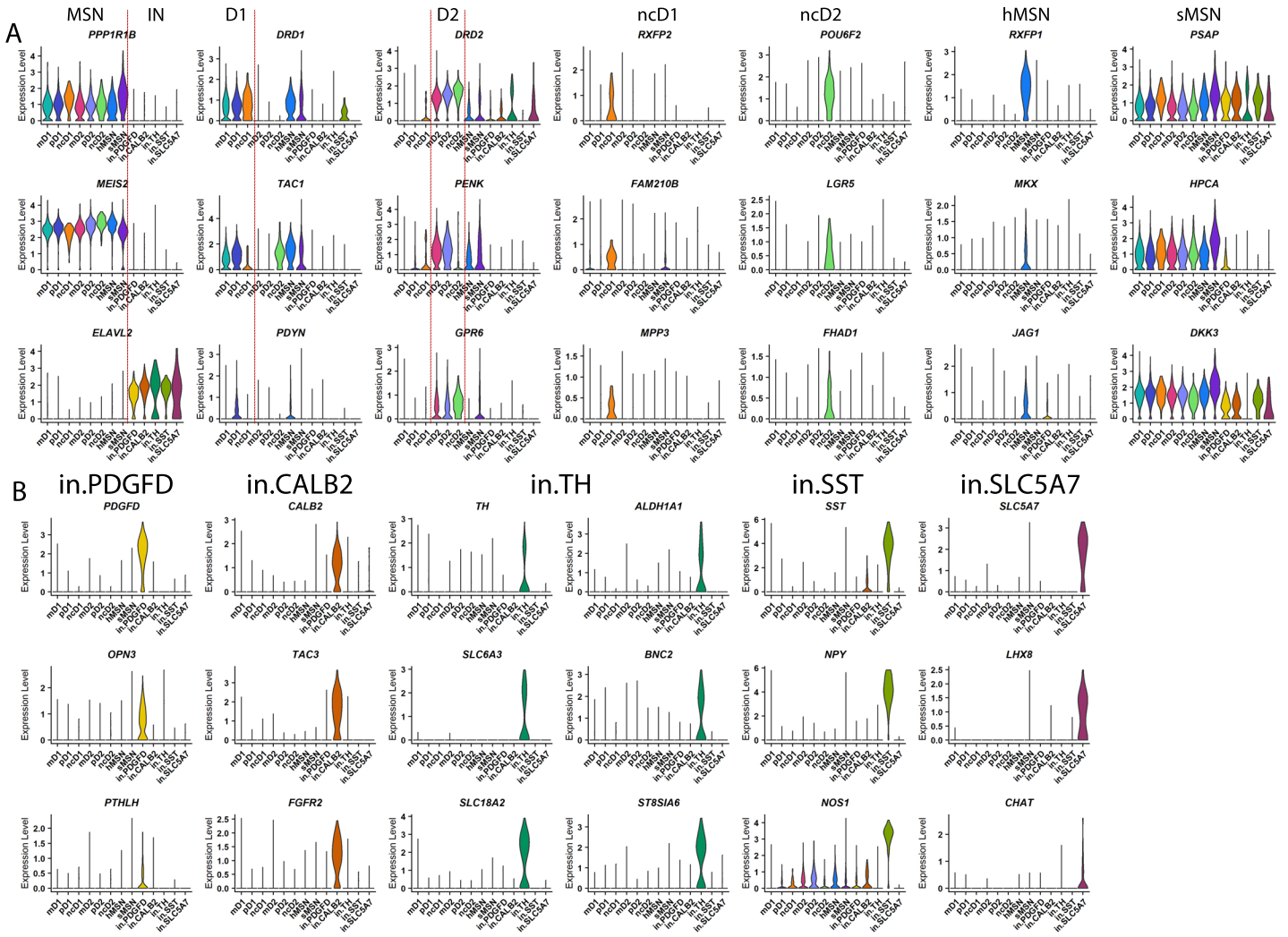
The fraction of the different cell populations (clusters) was separately computed for each subject as the fraction of nuclei in each cluster out of the total number of nuclei in the given subject. The One-Way ANOVA with Tukey's Multiple Comparisons test (*anova* function in R 4.0.2) was used to assess statistically significant changes in the fractions of a specific population. Results with *p*-value < 0.05 were considered significant. Data distribution was assumed to be normal but this was not formally tested.

1 Street, K. *et al.* Slingshot: cell lineage and pseudotime inference for single-cell transcriptomics. *BMC Genomics* **19**, 477, doi:10.1186/s12864-018-4772-0 (2018).

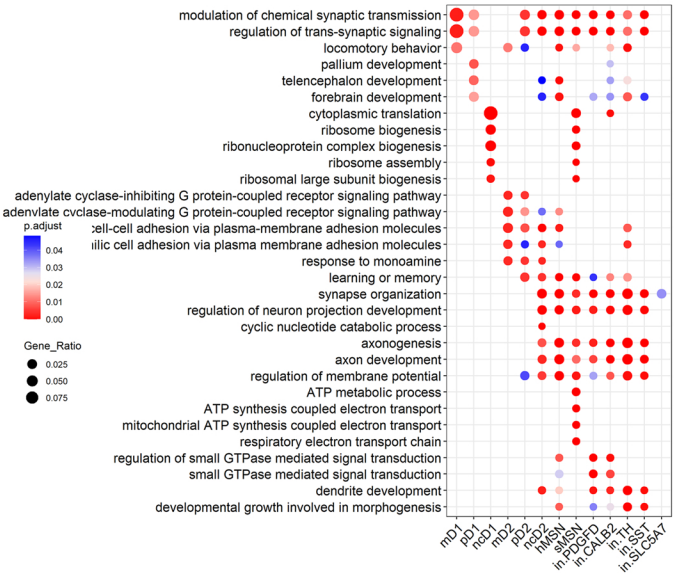
2 Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847-2849, doi:10.1093/bioinformatics/btw313 (2016).

3 Keren-Shaul, H. *et al.* A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* **169**, 1276-1290 e1217, doi:10.1016/j.cell.2017.05.018 (2017).
4 Grubman, A. *et al.* A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nat Neurosci* **22**, 2087-2097, doi:10.1038/s41593-019-0539-4 (2019).
5 Lau, S. F., Cao, H., Fu, A. K. Y. & Ip, N. Y. Single-nucleus transcriptome analysis reveals dysregulation of angiogenic endothelial cells and neuroprotective glia in Alzheimer's disease. *Proc Natl Acad Sci U S A* **117**, 25800-25809, doi:10.1073/pnas.2008762117 (2020).
6 Feleke, R. *et al.* Cross-platform transcriptional profiling identifies common and distinct molecular pathologies in Lewy body diseases. *Acta Neuropathol* **142**, 449-474, doi:10.1007/s00401-021-02343-x (2021).
7 Mathys, H. *et al.* Single-cell transcriptomic analysis of Alzheimer's disease. *Nature* **570**, 332-337, doi:10.1038/s41586-019-1195-2 (2019).

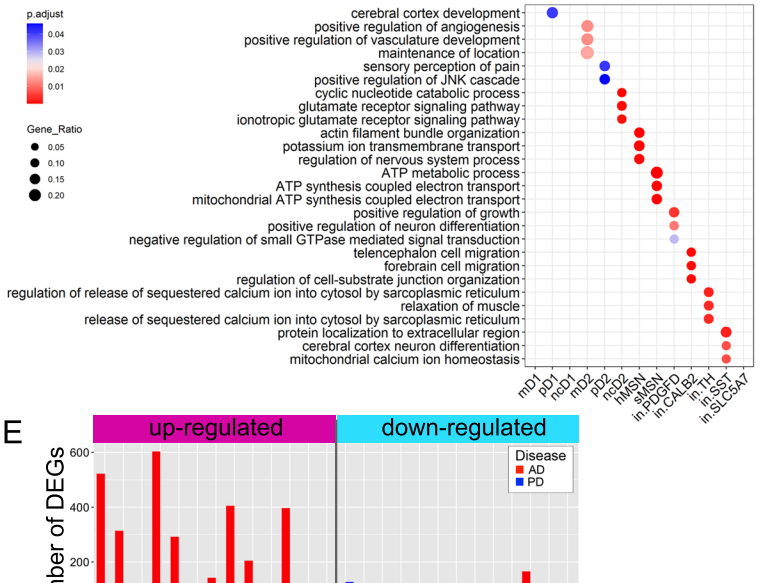
Suppl. Fig. S1



C Top 5 Biological Process terms



D Top 3 cluster-specific unique GO terms



E

Suppl. Fig. S1. Characterization of neuronal subpopulations and conserved marker genes. Violin plot showing the expression of A) known marker genes for MSN, interneuron (IN), D1 neuron, and D2 neuron as well as identified marker genes for ncD1, ncD2, hMSN, and sMSN neurons, and (B) known and identified marker genes for the five interneuron subpopulations. (C) Top 5 GO terms in the Biological Process category enriched in the conserved cluster marker genes of each neuronal subpopulation. (D) Top 3 GO terms in the Biological Process category uniquely enriched in the conserved cluster marker genes of each neuronal subpopulation. (E) The number of differentially expressed genes (DEGs, Wilcoxon Rank Sum test, FDR-adjusted p-value < 0.05, absolute logFC > 0.25) for each neuronal subpopulation comparing cells in the AD (n=4) or PD (n=4) samples with that of controls (n=4). Conserved marker genes were determined by using the *FindConservedMarkers* using Wilcoxon Rank Sum test and *metap* R package with meta-analysis combined p value < 0.05. Pathways with FDR adjusted p-value < 0.05 (hypergeometric test) and at least 5 query genes were considered to be statistically significant. IN: interneuron