SUPPLEMENTARY INFORMATION

A Single-Cell Transcriptome Atlas of Glia Diversity in the Human Hippocampus across the Postnatal Lifespan

Yijing Su, Yi Zhou, Mariko L. Bennett, Shiying Li, Marc Carceles-Cordon, Lu Lu, Sooyoung Huh, Dennisse Jimenez-Cyrus, Benjamin C. Kennedy, Sudha K. Kessler, Angela N. Viaene, Ingo Helbig, Xiaosong Gu, Joel E. Kleinman, Thomas M. Hyde, Daniel R. Weinberger, David W. Nauen, Hongjun Song and Guo-li Ming

Inventory

SUPPLEMENTARY FIGURES

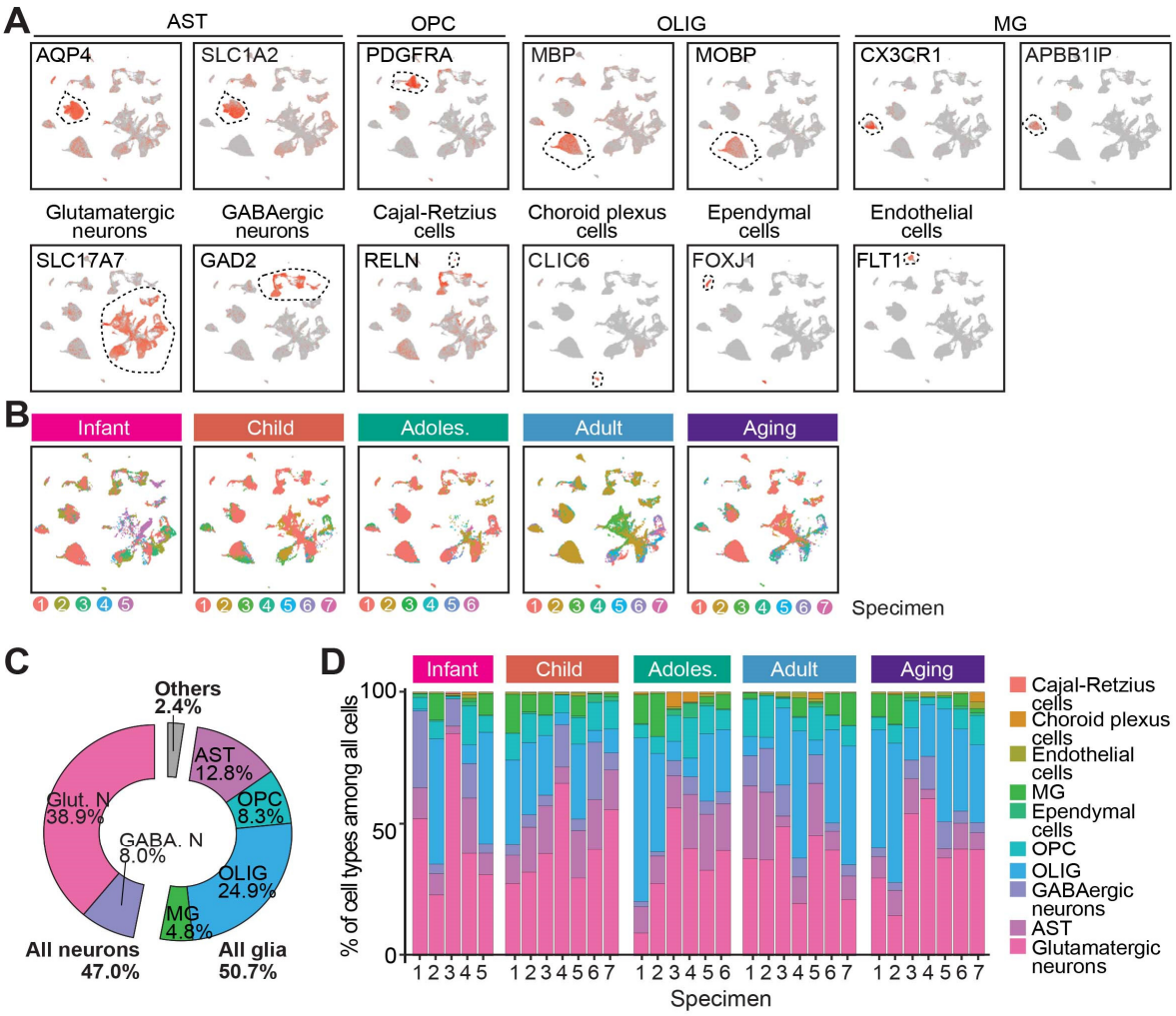

Figure S1. Characterization of the snRNA-seq dataset of the human hippocampus across the postnatal lifespan, related to Figure 1

(A) UMAP visualization of all cells in the integrated human hippocampal dataset across ages, colored by expression of marker genes used to determine cluster identities. Dashed circle highlights the cluster for each major cell type listed. AST: astrocyte; OPC: oligodendrocyte precursor cell; OLIG: oligodendrocyte; MG: microglia.

(B) UMAP visualization of the integrated dataset, shown in aggregate for each age group, colored by specimen within each age group. Adoles.: Adolescent.

(C) Pie chart showing the proportion of each major cell type among all cells obtained. The proportion of all neurons combines glutamatergic and GABAergic neurons, whereas that of all glia combines AST, OPC, OLIG, and MG populations. Others include endothelial cells, ependymal cells, choroid plexus cells, and Cajal-Retzius cells. Glut. N: glutamatergic neuron; GABA. N: GABAergic neuron.

(D) Bar plot showing the proportion of each major cell type among all cells within each individual specimen. Specimens at each age stage are plotted together.

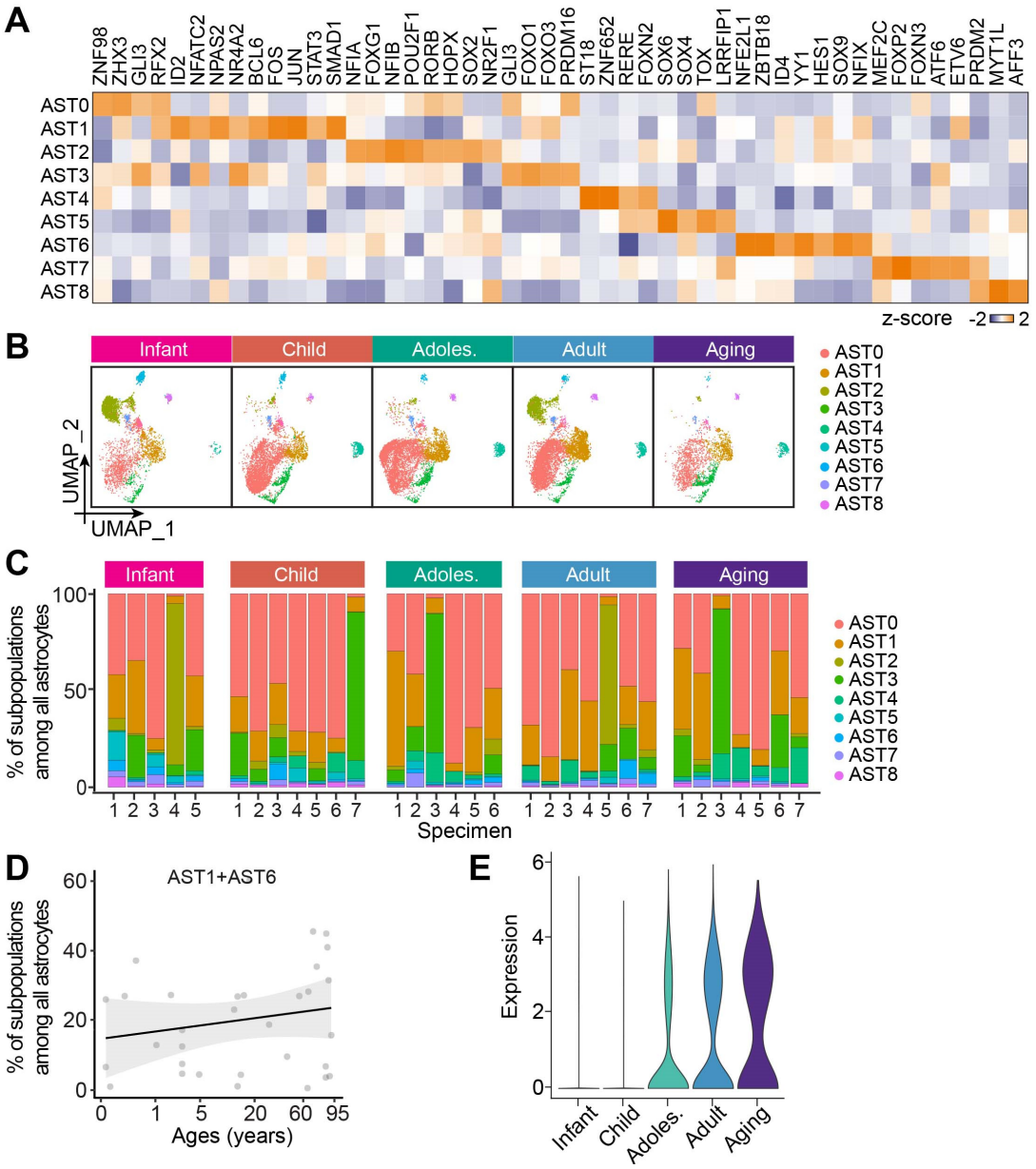

Figure S2. Molecular and cellular characteristics of astrocyte subpopulations, related to Figures 2 and 3

(A) Heatmap showing the expression pattern of exemplary transcription factors enriched in each astrocyte subpopulation.

(**B)** UMAP visualization of all astrocytes re-clustered, shown in aggregate for each age stage and colored by subpopulation.

(C) Bar plot showing the proportion of each astrocyte subpopulation among all astrocytes within each individual specimen. Specimens at each age stage are plotted together.

(D) Dot plot showing the sum of proportions of the GFAP-enriched AST1 and AST6 subpopulations among all astrocytes across ages. Each specimen was plotted as a dot along age (x-axis, calculated on a log-scale). Dots are fitted with linear regression fitting (lines) with 95% confidence interval (grey shades).

(E) Violin plot showing GFAP expression in all astrocytes in our integrated human hippocampal snRNA-seq dataset across ages**.**

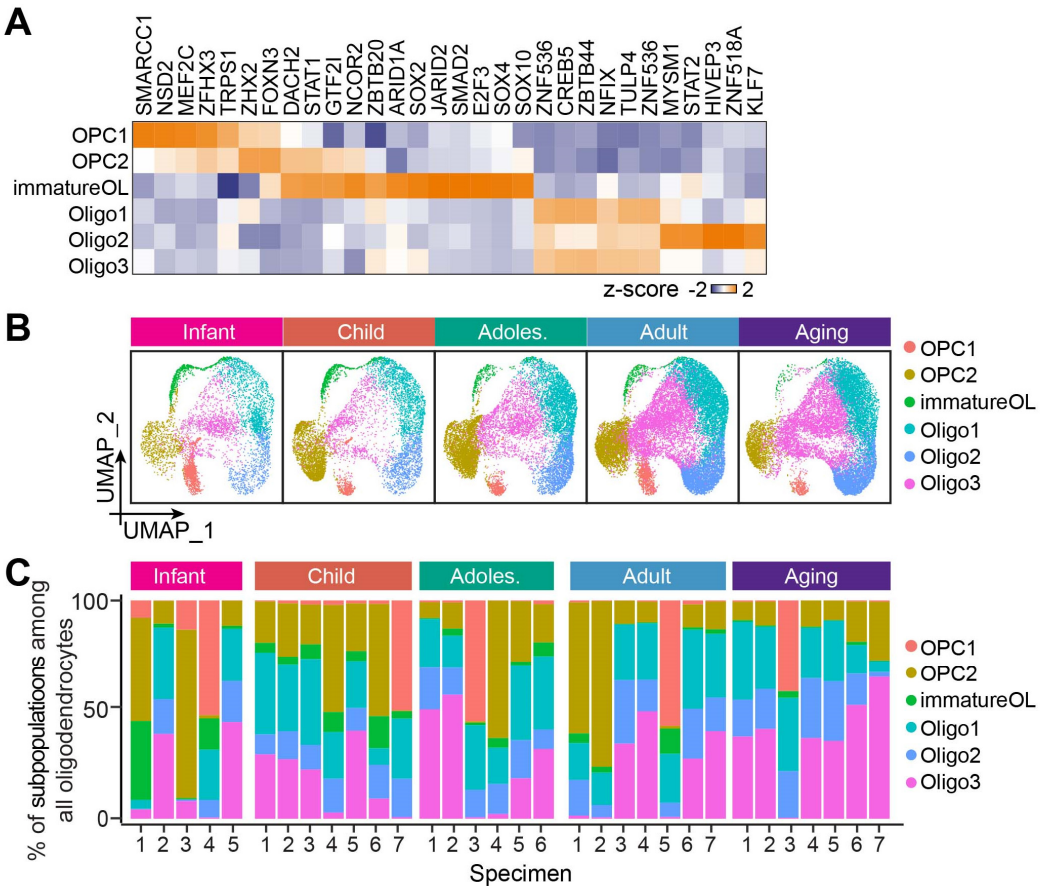

Figure S3. Molecular and cellular characteristics of subpopulations of the oligodendrocyte cell lineage, related to Figure 4

(A) Heatmap showing the expression pattern of exemplary transcription factors enriched in each subpopulation of the oligodendrocyte cell lineage.

(**B)** UMAP visualization of all cells in the oligodendrocyte lineage re-clustered, shown in aggregate for each age stage, and colored by subpopulation.

(C) Bar plot showing the relative proportion of each subpopulation among all cells in the oligodendrocyte lineage within each individual specimen. Specimens at each age stage are plotted together.

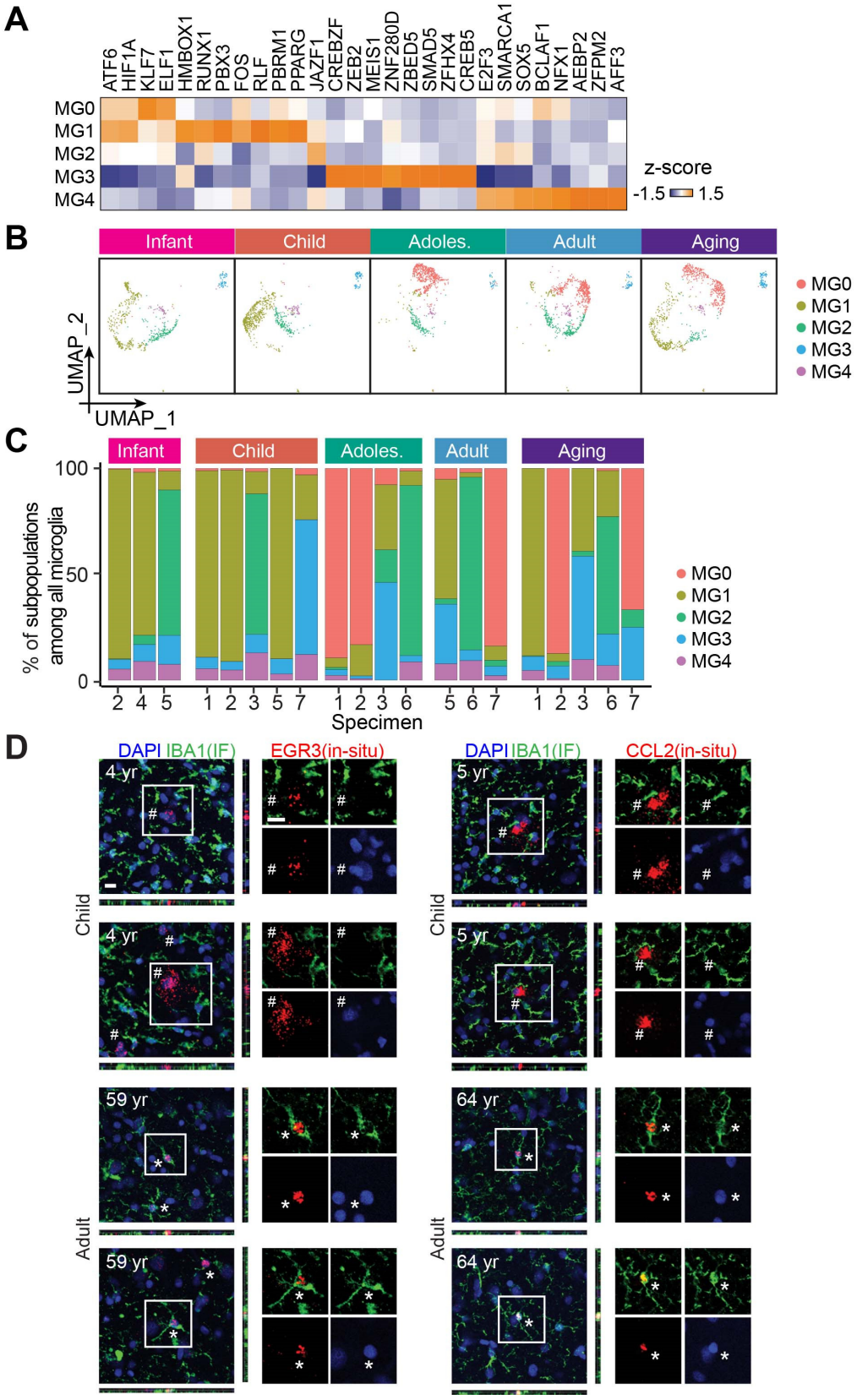

Figure S4. Molecular and cellular characteristics of microglia subpopulations, related to Figure 5

(A) Heatmap showing the expression pattern of exemplary transcription factors enriched in each microglia subpopulation.
(**B)** UMAP visualization of all microglia re-clustered, shown in aggregate for each age stage and colored by subpopulation.
(C) Bar plot showing the relative proportion of each subpopulation among all microglia within each individual specimen. Specimens at each age stage are plotted together.
(D) Sample confocal images showing expression patterns of two genes enriched in MG0, EGR3 and CCL2, in IBA1$^+$ microglia in the child (upper panels) and adult (lower panels) human hippocampus. Asterisks indicate EGR3$^+$ or CCL2$^+$ in IBA1$^+$ microglia and hashtags indicate EGR3$^-$ or CCL2$^-$ in IBA1$^+$ microglia. Scale bars, 10 μm. IF: immunofluorescence.

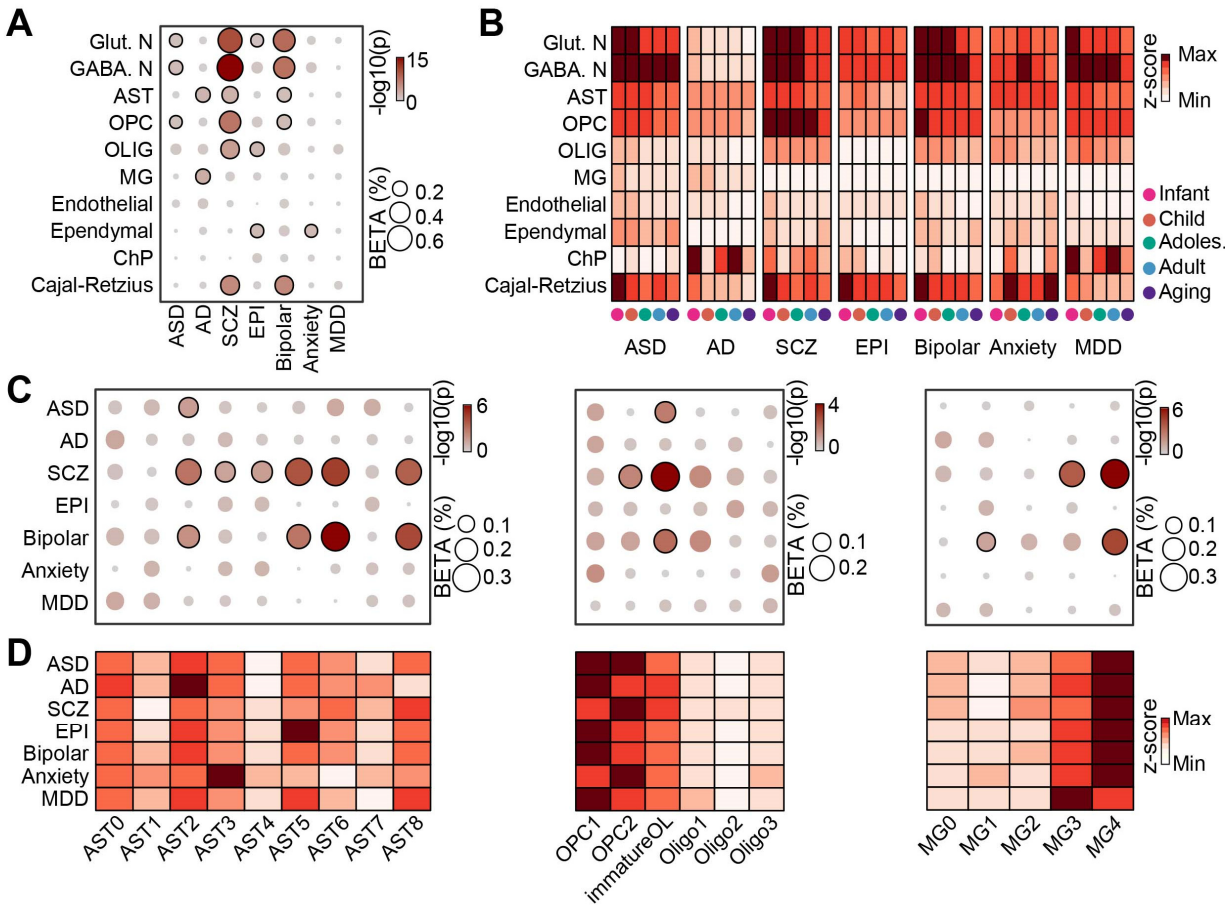

Figure S5. Subpopulation-specific enrichment of expression of risk genes associated with neurological or psychiatric disorders in human hippocampal glial cells, related to Figure 6
(A and C) Cell type-specific enrichment of gene sets associated with various neurological or psychiatric disorders curated from genome-wide association studies (GWAS) (Giannakopoulou et al., 2021; Grove et al., 2019; International League Against Epilepsy Consortium on Complex, 2018; Mullins et al., 2021; Otowa et al., 2016; Trubetskoy et al., 2022; Wightman et al., 2022) in major cell types (**A**) and glia subpopulations (**C**). Gene-based association tests were performed using MAGMA (Bartfeld et al., 2015); black circles indicate an adjusted p-value < 0.05. ASD: autism spectrum disorder; AD: Alzheimer's disease; SCZ: schizophrenia; EPI: epilepsy; MDD: major depressive disorder; ChP: choroid plexus cells.
(B and D) Aggregated gene expression of neurological and psychiatric disease risk genes in all major cell types across ages (**B**) and in glia subpopulations (**D**). Gene lists were from (Yu et al., 2010).
See also **Table S4**.

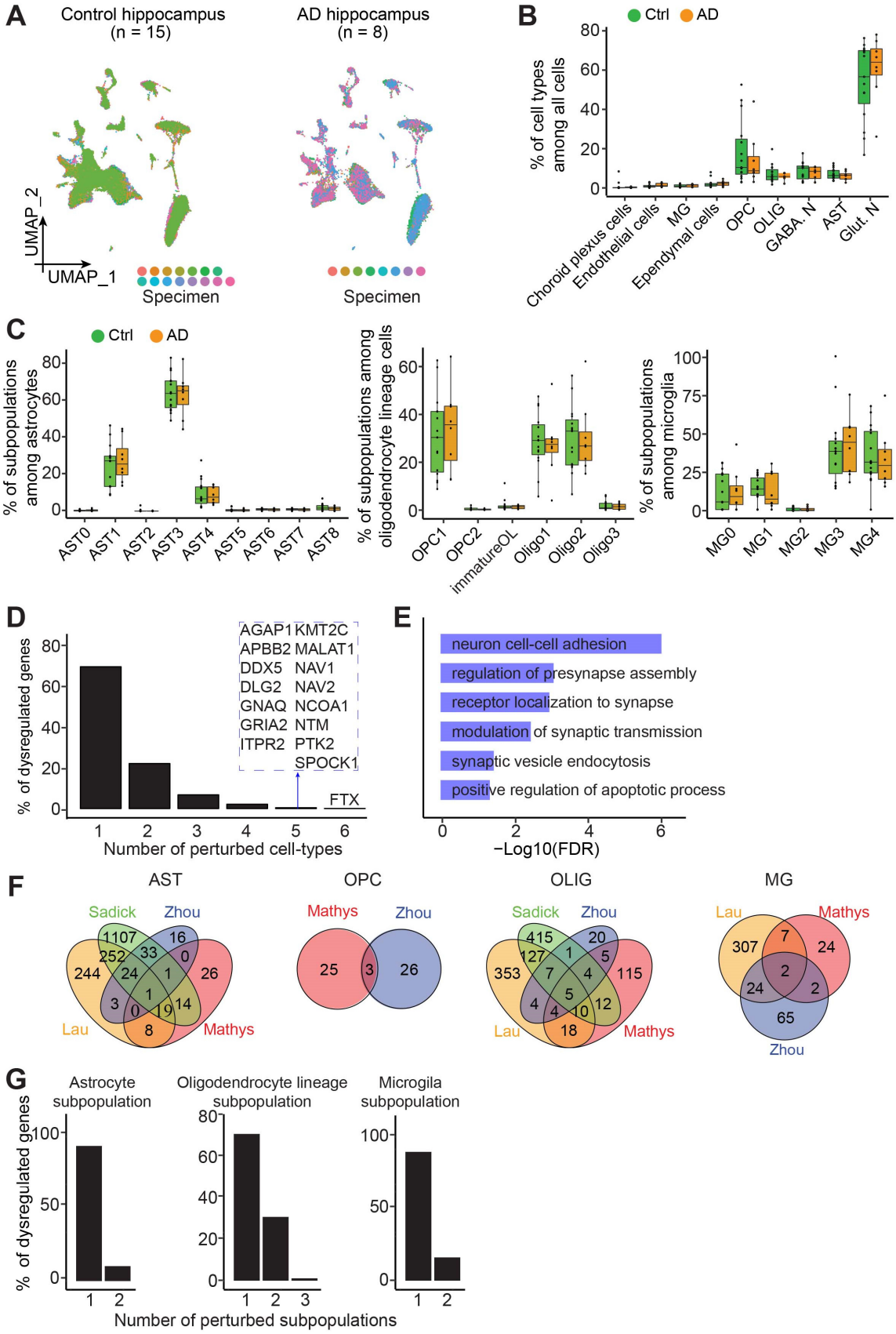

Figure S6. Consistent cellular abundance with divergent patterns of molecular dysregulation

in specific cell types and glia subpopulations of the human hippocampus in Alzheimer's disease (AD), related to Figure 6

(A) UMAP visualization of all nuclei from the integrated human hippocampal dataset of AD and matched controls, shown in aggregate for disease conditions and colored by specimen.

(B and C) Box plots showing the proportion of each major cell type among all cells **(B)** and each subpopulation among its respective glia type **(C)** in each specimen of AD and matched controls. Dots represent value for each specimen and box values represent median ± quantiles with whiskers for the 5^{th} and 95^{th} percentiles. Ctrl: control.

(D) Bar plot showing the proportion of all dysregulated genes in AD (y-axis) as a function of the total number of major cell types where the dysregulation occurs (x-axis). The 16 dysregulated genes shared among five or six cell types are listed.

(E) Bar plot showing the top gene ontology terms associated with the dysregulated genes shared among at least four major cell types in AD (n = 96 genes). Right-tailed Fisher's exact test was applied and a false-discovery rate (FDR)-corrected p-value < 0.05 is considered significantly enriched.

(F) Venn diagram comparing AD-dysregulated genes identified in published studies of the prefrontal cortex (PFC) (Lau et al., 2020; Mathys et al., 2019; Sadick et al., 2022; Zhou et al., 2020). The union of AD-dysregulated differentially expressed genes (DEGs) identified in these studies of the PFC was used for cross-brain-region comparisons for each cell type in **Figure 6C**.

(G) Bar plots showing, for each glia type, the proportion of all dysregulated genes in AD (y-axis) as a function of the total number of subpopulations where dysregulation occurs (x-axis).

See also **Table S5**.

SUPPLEMENTARY TABLES (IN EXCEL FILES)

Table S1. Human brain specimens used in the current study (A) and summary of sequencing characteristics of the snRNA-seq datasets (B), related to Figure 1.

Table S2. Lists of enriched genes for each major cell type and glia subpopulation, related to Figures 1, 2, 4, and 5.

This Table includes lists of significantly enriched genes for all major cell types (**A**), astrocyte subpopulations (**B**), oligodendrocyte lineage subpopulations (**C**), and microglia subpopulations (**D**).

Table S3. Gene Ontology terms related to enriched genes for glia subpopulations, related to Figures 2, 4, and 5.

This Table includes Gene Ontology terms related to enriched genes of astrocyte subpopulations (**A**), oligodendrocyte lineage subpopulations (**B**), and microglia subpopulations (**C**).

Table S4. Glia subpopulation-specific expression patterns across ages of risk genes in brain disorders, related to Figures 6 and S5.

Table S5. Lists of genes dysregulated in AD in major cell types (A) and specific glia subpopulations (B) and their Gene Ontology terms (C), related to Figure 6.

Table S6. Summary of statistical methods used in different studies (A) and lists of genes dysregulated in AD shared among human hippocampus, prefrontal cortex and entorhinal cortex in astrocytes (B), oligodendrocytes (C), and microglia (D), related to Figure 6.