Supplementary Materials

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Supplementary Figures

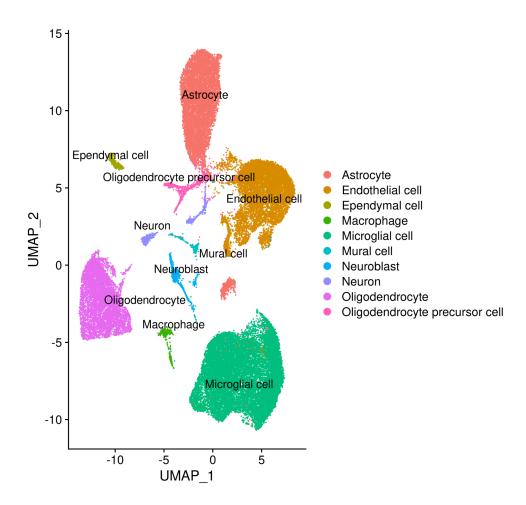


Fig. S1: Visualization of scRNA-seq data clustering at 7 months of age. UMAP visualization of cell clusters representing distinct cell types in cortical tissue from THY-Tau22 and wild-type mice at 7 months of age. Cell type annotation was performed using SCType. The plot integrates data from all mice across both sexes (males and females) and conditions (transgenic and wild type). This figure complements Fig. 2 in the main text which shows the corresponding data for 17-month-old mice.

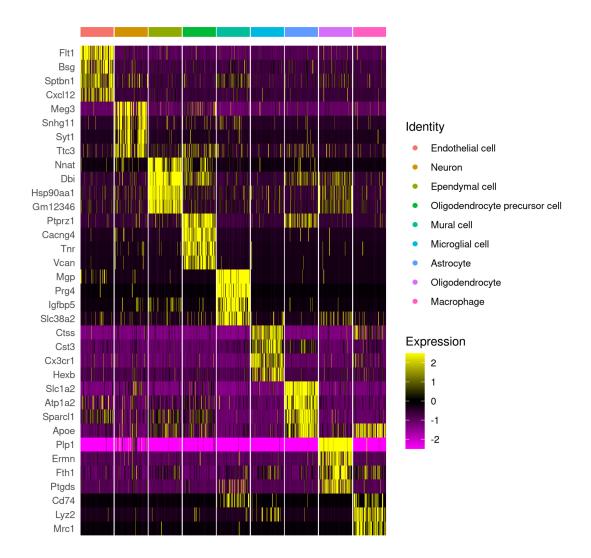


Fig. S2: Heatmap of cell type-specific marker gene expression. Expression patterns of established cell type marker genes across identified cell clusters. Color intensity represents normalized expression levels, with yellow indicating high expression and pink indicating low expression. Hierarchical clustering of both rows (genes) and columns (cell types) reveals distinct expression signatures for each cell population.

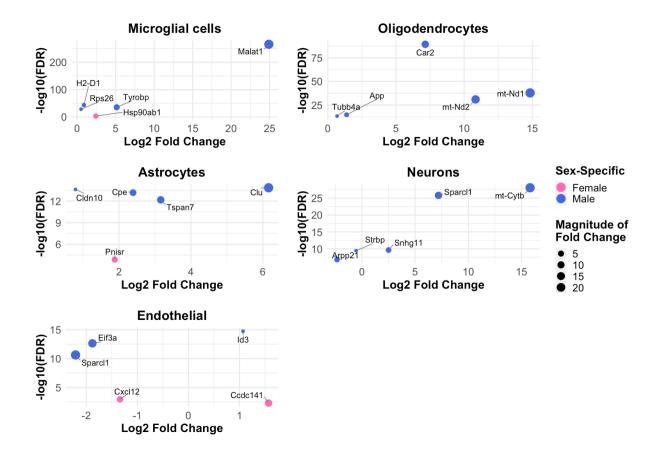


Fig. S3: Sex-specific differential gene expression patterns across five main brain cell types. Volcano plots depicting differentially expressed genes (DEGs) in five main brain cell types. The x-axis shows the log2 fold change (logFC), with positive values indicating higher expression in males and negative values indicating higher expression in females. The y-axis shows statistical significance as -log10(FDR). Point size corresponds to the magnitude of fold change, while colors indicate sex-specific regulation (blue = male-specific, pink = female-specific). Key genes of interest are labeled, including inflammatory response genes in microglia (e.g., Malat1, H2-D1, Tyrobp), protein homeostasis genes in astrocytes (e.g., Clu, Cldn10), mitochondrial genes in neurons (e.g., mt-Cytb) and oligodendrocytes (e.g., mt-Nd1, mt-Nd2), and regulatory genes in endothelial cells (e.g., Id3, Cxcl12).

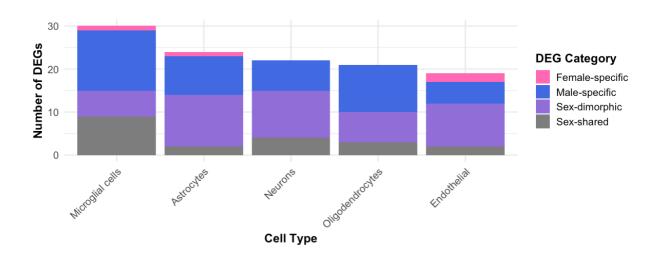


Fig. S4: Distribution of differentially expressed genes across five main brain cell types. Stacked bar plot showing the number and categories of differentially expressed genes (DEGs) in each brain cell type at 17 months of age. The bars are subdivided into four categories: male-specific (blue), female-specific (pink), sex-dimorphic (purple), and sex-neutral (gray) DEGs. Microglial cells show the highest number of total DEGs (55), with a strong bias toward male-specific expression (37 genes). Oligodendrocytes uniquely lack female-specific DEGs but show substantial male-specific expression (17 genes). Neurons and astrocytes display more balanced distributions between sex-dimorphic and sex-specific genes. Endothelial cells show the lowest total number of DEGs (23) but maintain representation across all categories

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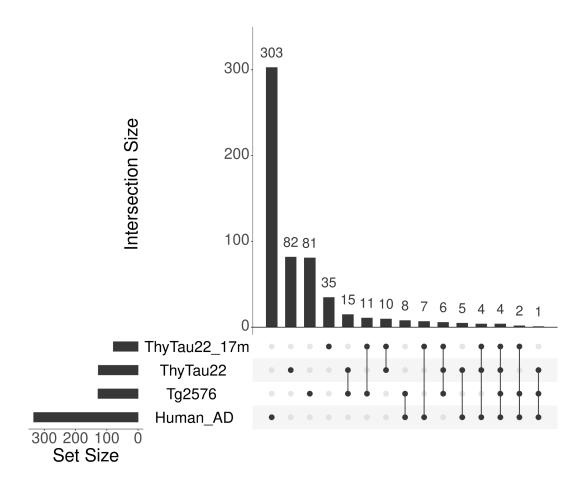


Fig. S5: Overlap of differentially expressed genes between datasets. UpSet plot showing the intersection of differentially expressed genes across four datasets: THY-Tau22 mice at 7 and 17 months, Tg2576 mice, and human AD cortical tissue. The bar chart indicates the size of each intersection set, while the connected dots below show which datasets contribute to each intersection.

Global sex-dependent pathway alterations

We performed pathway enrichment analysis of the sex-dependent DEGs at the level of global changes across all cell types (pseudobulk analysis) for THY-Tau22 mice at 17 months of age (see Methods). Since the number of female-specific DEGs was insufficient to identify cellular process alterations linked to female-specific changes, in the following we focus on identified pathways enriched in male-specific and sex-dimorphic DEGs.

Global male-specific pathway enrichment (THY-Tau22, 17 months)

The pseudobulk enrichment analysis of the male-specific DEGs identified in the combination of cell types revealed distinct patterns of dysregulation in biological processes and molecular functions from the Gene Ontology database (see Fig. 3). The most significantly enriched biological processes centred on RNA processing and splicing pathways, with several related terms showing strong enrichment (adjusted p<0.05). Other enriched processes included erythrocyte and myeloid cell homeostasis and regulation of synaptic potentiation, suggesting broad effects on both cellular maintenance and neuronal function.

At the molecular functional level, the analysis revealed a significant enrichment of chromatin and histone-related activities, in particular protein binding and various methyltransferase functions (adjusted p<0.05). The prominence of RNA processing and epigenetic regulation pathways suggests that male-specific responses to tau pathology at the 17-months time point may particularly affect gene expression control and chromatin modification. A comprehensive list of all enriched pathways and their statistical parameters can be found in Suppl. Table 4.

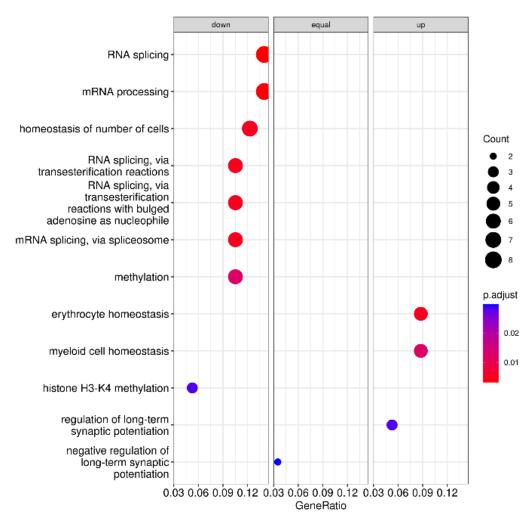


Fig. S6: Dot plot visualization of the gene set enrichment analysis results for global male-specific differentially expressed genes (DEGs) in THY-Tau22 mice at 17 months of age. The plot shows enriched Gene Ontology Biological Process (BP) terms (corresponding enriched Molecular Function (MF) terms are shown in Fig. S7). The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level. RNA processing and splicing pathways dominate the enriched processes. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term.

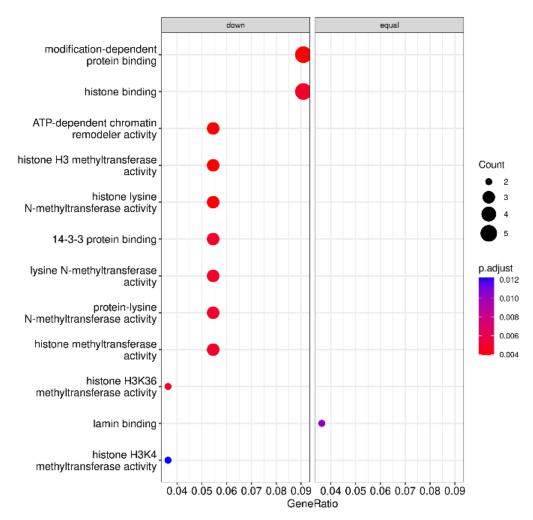


Fig. S7: Dot plot visualization of the gene set enrichment analysis results for global male-specific differentially expressed genes (DEGs) in THY-Tau22 mice at 17 months of age. The plot shows enriched Molecular Function (MF) terms (corresponding enriched Gene Ontology Biological Process (BP) terms are shown in Fig. S6). Left panel shows enriched Gene Ontology Biological Process (BP) terms, while the right panel shows enriched Molecular Function (MF) terms. The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level. Protein modification and histone-related functions are prominent among the enriched functions. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term.

Global sex-dimorphic pathway enrichment (THY-Tau22, 17 months)

The pseudobulk gene set enrichment analysis of sex-dimorphic DEGs in THY-Tau22 mice at 17-months of age revealed distinct patterns of stress response and neurotransmitter signaling pathways (see Fig. 4). The most significantly enriched biological processes centered on cellular responses to salt, gliogenesis, and regulation of neuron death (adjusted p<1e-4), highlighting opposing transcriptional responses between sexes in pathways critical for cell survival and glial function. Several stress response pathways were also prominently enriched, including responses to oxidative stress, metal ions, and reactive oxygen species.

At the molecular function level, the most significant enrichment was found in ubiquitin-related processes and ligand binding (adjusted p<1e-4), followed by acetylcholine

receptor activities and regulatory functions. This suggests fundamental sex differences in protein degradation pathways and cholinergic signaling, both of which are known to be dysregulated in AD. The enrichment of both cellular stress responses and neurotransmitter signaling suggests that male and female mice show divergent adaptations to tau pathology in pathways central to cellular resilience and synaptic function. A comprehensive list of all enriched pathways and their statistical parameters is provided in Suppl. Table 5.

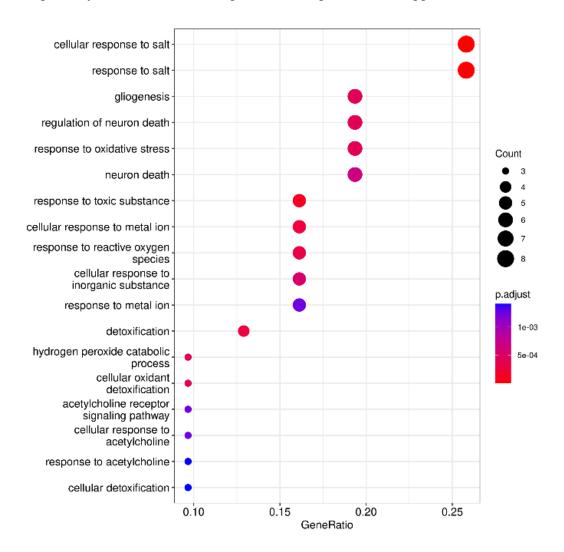


Fig. S8: Dot plot visualization of the gene set enrichment analysis results of global sex-dimorphic differentially expressed genes (DEGs) in THY-Tau22 mice at 17 months of age. The plot shows enriched Gene Ontology Biological Process (BP) terms (corresponding enriched Molecular Function (MF) terms are shown in Fig. S9). The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level (darker purple indicating higher significance). The enriched processes are dominated by stress responses, including cellular responses to salt, oxidative stress, and neuron death regulation. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term

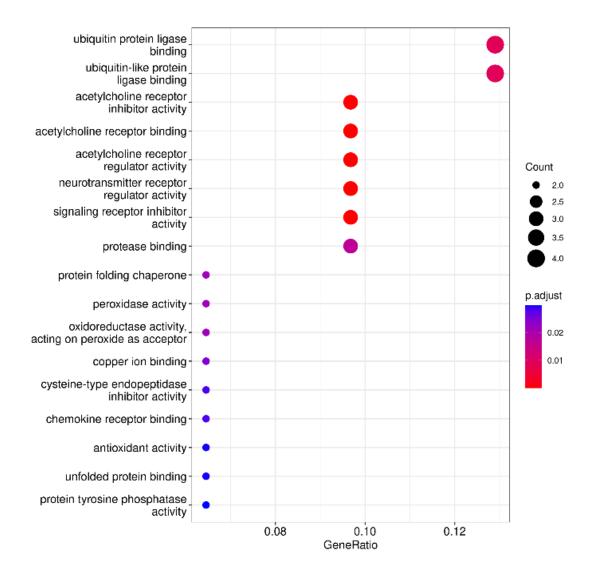


Fig. S9: Dot plot visualization of the gene set enrichment analysis results of global sex-dimorphic differentially expressed genes (DEGs) in THY-Tau22 mice at 17 months of age. The plot shows enriched Molecular Function (MF) terms (corresponding enriched Gene Ontology Biological Process (BP) terms are shown in Fig. S8). The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level (darker purple indicating higher significance). Strong enrichment is observed for ubiquitin-related processes and neurotransmitter receptor activities, particularly acetylcholine receptor pathways. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term.

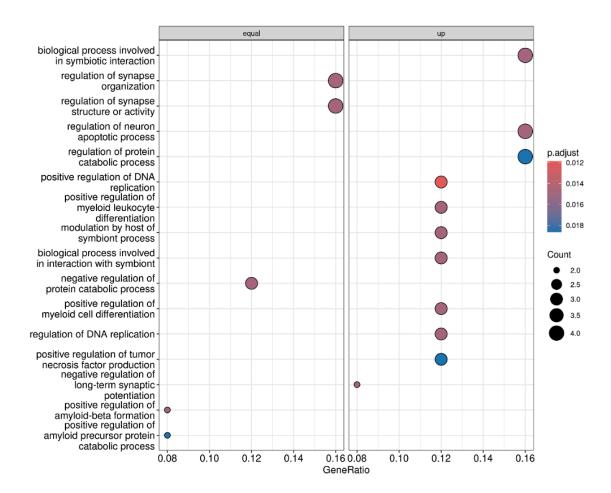


Fig. S10: Pathway enrichment analysis in microglia from 17-month-old THY-Tau22 mice. Dot plot showing enriched Gene Ontology Biological Processes. Dot size indicates number of differential genes in each pathway; color represents statistical significance (red=more significant). Whenever possible, each enriched pathway is classified into three regulatory patterns based on the predominant direction of change in its member genes (up, down, or equal). Gene ratio (x-axis) shows proportion of pathway genes differentially expressed. Only pathways with FDR<0.05 are shown.

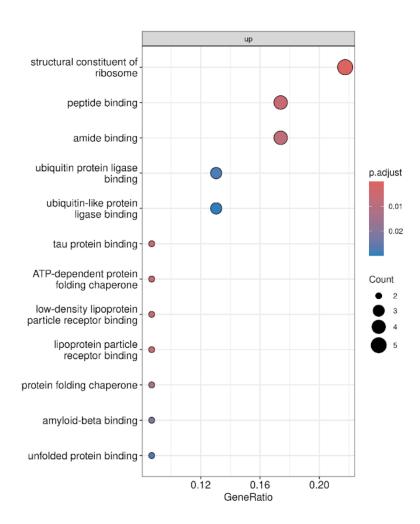


Fig. S11: Pathway enrichment analysis of sex-dependent microglial responses in THY-Tau22 mice at 17 months of age. Dot plot showing enriched Molecular Functions. Dot size indicates number of differential genes in each pathway; color represents statistical significance (red=more significant). Whenever possible, each enriched pathway is classified into three regulatory patterns based on the predominant direction of change in its member genes (up, down, or equal). Gene ratio (x-axis) shows proportion of pathway genes differentially expressed. Only pathways with FDR<0.05 are shown.

positive regulation of DNA replication	negative regulation of protein catabolic	regulation amyloid-	positive regulation of amyloid-beta formation		lation DNA cation	positive regulation of amyloid precursor protein catabolic process	positive regulation of myeloid cell differentiation	positive regulation of myeloid leukocyte	regulation of myeloid leukocyte differentiation	positive regulation natural ki cell activa	n of reg	egative ulation of activation	
positive regulation of tumor necrosis factor production	positive regular of tumor necro factor superfar cytokine produc	regulat regulat of prote	tion ein olic	cytopl transl	asmic lation	interleukin-6 production	positive regulation of osteoclast	ive regulatio	regulation of		cell ci on egulation cell activ		
regulation of amyloid-beta formation	regulation of tumor necrosis factor	amyloid-be metabolic process	an	myloid pre rotein cata proces	abolic	regulation of phosphatase activity	myeloid cell differentiation	myeloid leukocyt differentia	tion myeloid cell	killer cel activation	cell migratio	epithelium migration	
regulation of	production positive regulation of tumor necrosis factor	egulation of DN DNA replication		A replication regulation of interleukin-1		regulation of nitric oxide biosynthetic	positive regulation of hemopoiesis	regulation osteocla differentia	st	of tissue remodelin	tissue migration		
phosphatase activity	superfamily cytokine production	amyloid precur protein metabo process	rsor	I regulation of I		nitric oxide biosynthetic process	regulation of synapse organization	regulation of synapse structure or activity	regulation of neuron apoptotic	neuron apoptotic	negative regulation of long-term nega	ative land ERK2	
amyloid-beta formation	necrosis factor production	regulation of nitric oxide metabolic		positive regulation of DNA metabolic		reactive nitrogen species metabolic process	_	of synapse ization	priregulation a negaproc	poptotic	of long-term regulation regulacynapticulation potentiation K1		
regulation of amyloid precursor protein catabolic process		regulation of protein dephosphorylation		nitric oxide metabolic process		negative regulation of catabolic	regulation of tube	vasodilation	of neuron apoptotic process	of neuron apoptotic process	long-term synaptic potentiation	and ERK2	
regulation of inflammatory response	cellular response to acetylcholine	response to acetylcholine	cellu respo to inorga	ular onse onse in	regulatio of acute oflammate response	regulation of defense	diameter biological process involved in interaction with symbiont biological pro-	symbiont	regulation of cell killing regulat	negative regulation of cell tion of	regula myeloid	itive Ition of Ieukocyte Immunity	
microglial cell activation	regulation leukocyte activation involved in inflammatory response	n of inflam glial cell activation	respo to av inju	onse ne	euroinflamma response	cellular response to calcium ion		interaction modulation	cell k	positive regulation of leukocyte mediated cytotoxicity	ribosome assembly	viral process	

Fig. S12: Microglia pathway enrichment analysis (Biological Processes). Extended visualization of biological process enrichment for microglial DEGs, complementing Fig. 7 in the main text. Includes complete hierarchical organization of enriched terms.



Fig. S13: Microglia pathway enrichment analysis (Molecular Function). Extended visualization of molecular function enrichment for microglial DEGs, complementing Fig. 7 in the main text. Includes complete hierarchical organization of enriched terms.

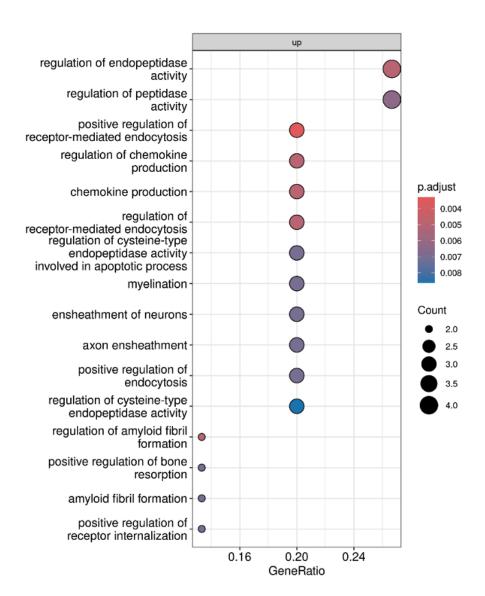


Fig. S14: Pathway enrichment analysis in oligodendrocytes from 17-month-old THY-Tau22 mice. Dot plot showing enriched Gene Ontology Biological Processes. Dot size indicates number of differential genes in each pathway; color represents statistical significance (red=more significant). Whenever possible, each enriched pathway is classified into three regulatory patterns based on the predominant direction of change in its member genes (up, down, or equal). Gene ratio (x-axis) shows proportion of pathway genes differentially expressed. Only pathways with FDR<0.05 are shown.

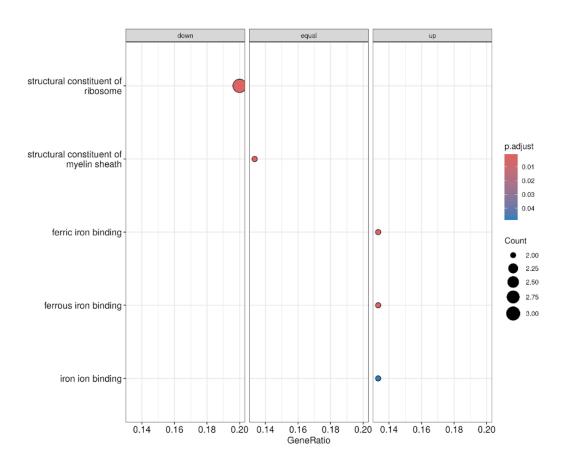


Fig. S15: Pathway enrichment analysis of oligodendrocyte responses in THY-Tau22 mice at 17 months of age. Dot plot showing enriched Molecular Functions. Dot size indicates number of differential genes in each pathway; color represents statistical significance (red=more significant). Whenever possible, each enriched pathway is classified into three regulatory patterns based on the predominant direction of change in its member genes (up, down, or equal). Gene ratio (x-axis) shows proportion of pathway genes differentially expressed. Only pathways with FDR<0.05 are shown.



Fig. S16: Oligodendrocyte pathway enrichment analysis (Biological Process). Extended visualization of biological process enrichment for oligodendrocyte DEGs, complementing Fig. 8 in the main text. Includes complete hierarchical organization of enriched terms.

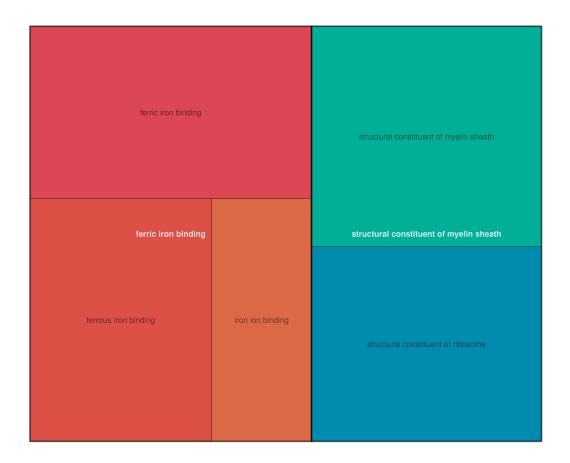


Fig. S17: Oligodendrocyte pathway enrichment analysis (Molecular Function). Extended visualization of molecular function enrichment for oligodendrocyte DEGs, complementing Fig. 8 in the main text. Includes complete hierarchical organization of enriched terms.

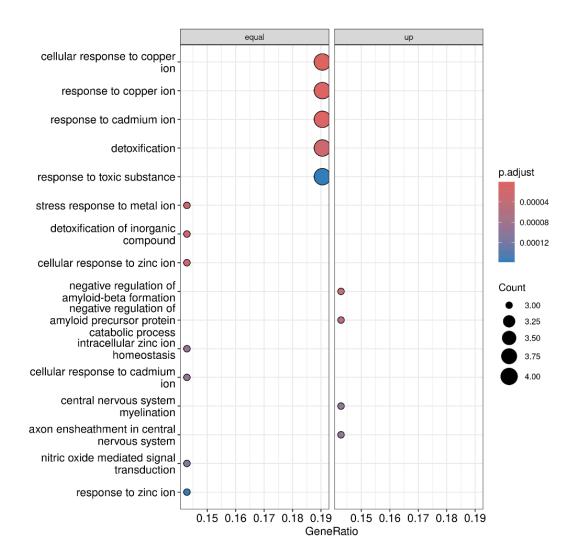


Fig. S18: Astrocyte pathway enrichment analysis. Visualization of Gene Ontology Biological Process (BP) enrichment for astrocyte DEGs. The dot plot shows the enriched pathways with gene ratios and statistical significance. The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level. Regulation of amyloid-beta formation and central nervous system myelination pathways dominate the enriched biological processes. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term.

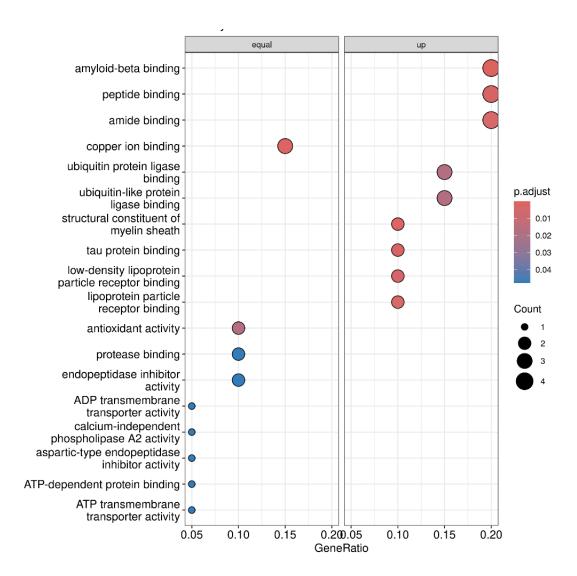


Fig. S19: Astrocyte pathway enrichment analysis. Visualization of Molecular Function (MF) enrichment for astrocyte DEGs. The dot plot shows the enriched pathways with gene ratios and statistical significance. The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level. Amyloid-beta and tau protein binding related functions are prominent among the enriched molecular functions. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term.



Fig. S20: Astrocyte pathway enrichment analysis (Biological Processes). Extended visualization of biological process enrichment for astrocyte DEGs. Includes complete hierarchical organization of enriched terms.



Fig. S21: Astrocyte pathway enrichment analysis (Molecular Functions). Extended visualization of molecular function enrichment for astrocyte DEGs. Includes complete hierarchical organization of enriched terms.

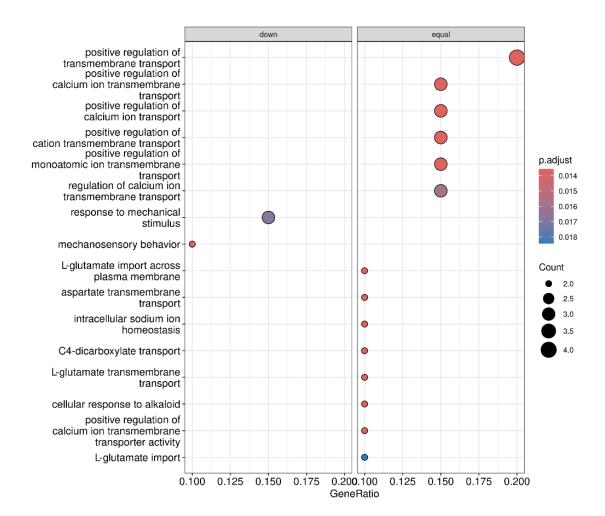


Fig. S22: Neuron pathway enrichment analysis. Visualization of Gene Ontology Biological Process (BP) enrichment for Neuron DEGs. The dot plot shows the enriched pathways with gene ratios and statistical significance. The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level. Transmembrane transport and sodium ion homeostasis pathways dominate the enriched biological processes. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term.

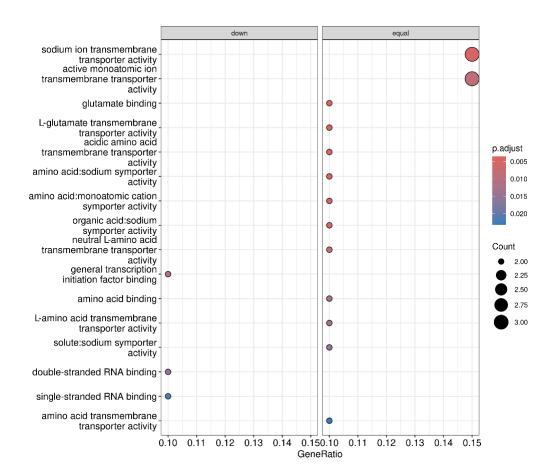


Fig. S23: Neuron pathway enrichment analysis. Visualization of Molecular Function (MF) enrichment for Neuron DEGs. The dot plot shows the enriched pathways with gene ratios and statistical significance. The size of each dot represents the count of genes in each enriched term, and the color scale indicates the adjusted p-value significance level. Transmembrane transporter activity and glutamate binding related functions are prominent among the enriched molecular functions. The gene ratio on the x-axis indicates the proportion of DEGs in each term relative to the total number of genes in that term.

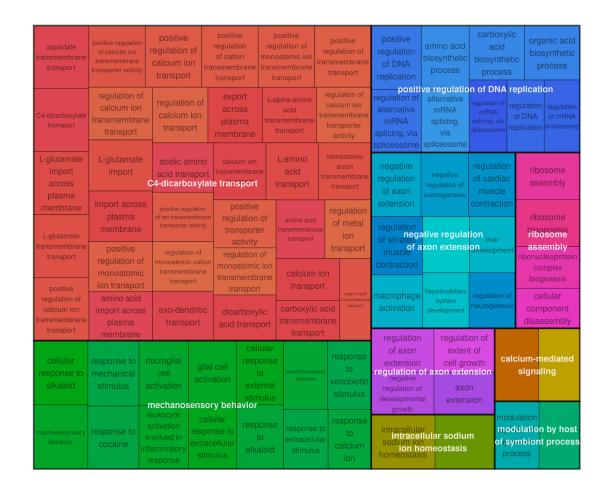


Fig. S24: Neuron pathway enrichment analysis (Biological Processes). Extended visualization of biological process enrichment for Neuron DEGs. Includes complete hierarchical organization of enriched terms.

L-glutamate transmembrane transporter activity	sodium ion transmembrane transporter activity	organic acid:sodiu symporte activity	ım tra	neutral L-amino acid transmembrane transporter activity		active patomic ion membrane nsporter activity	single-stranded RNA binding		cAMP response element binding stranded RNA bi		telomerase RNA binding inding	
acidic amino acid transmembrane transporter activity	L-amino acid transmembrane transporter activity	solute:monoator cation symport activity	er tran	active transmembrane transporter activity		etal ion membrane nsporter ctivity			poly(G) binding		IncRNA binding	
	-glutamate transmembrane transpo						channel	cyclase activator		glutamate bin		binding
amino acid:monoatomic cation	solute:sodium	carboxylic acid transmembrane transporter	P-type potassiu ransmemb transport			symporter activity	inhibitor activity	activity				
symporter activity	symporter activity	activity		ivity			^{cycl} aicium	protein channel atase		amino acid binding		
amino acid:sodium	amino acid	organic acid transmembrane	P-type sodiur	organic ar		secondary active	activity		(ity)tor ctivity	giata		on an ig
symporter activity	transporter activity	transporter activity	transpor activity	and the state of the		ansmembrane transporter activity	phosphatase activator	ATPase activator activity		carboxylic acid binding		organio acid
general transcription initiation	dystroglycan binding	adenyl cyclase b		HMG box domain binding		crotubule binding	activity					binding
factor binding	RNA polymera general transcr domain bindin	ption initiation fact			adina.		chemokine l	emokin ceptor	CONST	ctural uctural tuent of of myelin sheath n sheath		
dynein light chain binding	titin binding	C-C chemokine binding				RNA polymerase Il general transcription initiation factor binding	G protein-coupled chemoattractant receptor activity			protein-cysteir S-acyltransfera activity		

Fig. S25: Neuron pathway enrichment analysis (Molecular function). Extended visualization of molecular function enrichment for Neuron DEGs. Includes complete hierarchical organization of enriched terms.