

Identifying potential genes involved in synaptic pruning activity of Oligodendrocyte Precursor Cells

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Abbreviations

- **OPC** - Oligodendrocyte Precursor Cell
- **scRNA-seq** - Single-cell RNA Sequencing
- **UMI** - Unique Molecular Identifier
- **UMAP** - Uniform Manifold Approximation and Projection
- **LFC** – Log₂ Fold Change
- **GO** - Gene Ontology
- **t-SNE** - t-distributed Stochastic Neighbor Embedding
- **RNA** - Ribonucleic Acid
- **DNA** - Deoxyribonucleic Acid
- **PCR** - Polymerase Chain Reaction
- **NGS** - Next-Generation Sequencing
- **MG** - Microglia
- **ASC** - Astrocyte
- **CPC** - Choroid Plexus Cells
- **CSF** - Colony-Stimulating Factor
- **TK** - Tyrosine Kinase
- **CNS** - Central Nervous System
- ***Adgrb3*** - Adhesion G Protein-coupled Receptor B3
- ***Cx3cr1*** - C-X3-C Motif Chemokine Receptor 1
- ***Trem2*** - Triggering Receptor Expressed on Myeloid Cells 2
- ***Itgb1*** - Integrin Subunit Beta 1

- ***PlxnC1*** - Plexin C1
- ***C1ql1*** - Complement C1q-like 1
- ***Tyrobp*** - TYRO Protein Tyrosine Kinase Binding Protein
- ***Snx3*** - Sorting Nexin 3
- ***Calr*** - Calreticulin
- ***Rgs10*** - Regulator Of G Protein Signaling 10

Abstract

Synaptic pruning is a critical process in which the brain eliminates excess neuronal synapses, optimizing neural circuitry. While microglia and astrocytes are established mediators of this process, the role of oligodendrocyte precursor cells (OPCs) remains underexplored. This study uses single-cell RNA sequencing (scRNA-seq) data from the Aging Mouse Brain dataset to identify key genes involved in OPC-mediated synaptic pruning. Through hierarchical clustering and differential expression analysis, candidate genes such as *Tyrobp*, *Rgs10*, *C1ql1*, *Plxnc1*, *Itgb1*, and *Fyn* were identified, suggesting shared and distinct pathways with other glial cells. These findings offer new insights into OPC contributions to synaptic pruning and assembly and provide insights into potential therapeutic targets for neurodegenerative diseases like Alzheimer's and Parkinson's.

Introduction

Overview of Synapse pruning

Synaptic pruning is a natural process in which the brain eliminates excess synapses or connections between neurons (Feinberg, 1982). This process plays a critical role in both embryonic and postnatal brain development, contributing to the maturation of neural circuits and overall brain function (Irfan et al., 2022). During this process, structural components of synapses, such as presynaptic terminals and postsynaptic membranes, along with small segments of axonal and dendritic branches, are selectively eliminated (Faust et al., 2021).

Many synaptic connections initially form during development, but only a small proportion of these are maintained as the brain matures. This selective elimination occurs due to factors like synaptic weakness or an excessive connections, providing a mechanism to shape the developing brain (Schafer et al., 2012). Synapse elimination is a gradual process that occurs mainly in the first two decades of life (Huttenlocher, 1979; Petanjek et al., 2011), with its timing varying among different brain regions (Huttenlocher, 1979). For instance, thalamic regions undergo synaptic pruning earlier than the other brain regions (Soteros & Sia, 2022).

Mechanisms of Synapse pruning

Synapses are essential connections between neurons that facilitate neuronal communication. Neural circuits are formed from different synaptic connections and perform a specific function (Luo, 2021). A synapse occurs when a neuronal axon contacts a target cell by the synaptic assembly genes. Synapses are strengthened through activity-dependent synaptic plasticity

(Yoshihara et al., 2009), where the presynaptic neurons that activate postsynaptic neurons become stronger (Luo, 2021). However, the synaptic pruning eliminates immature or weak synapses (Yoshihara et al., 2009).

Synaptic pruning may be divided into different mechanism including synaptic/axonal degeneration, synaptic/axonal retraction, synaptic/axonal shedding and synaptic/axonal engulfment (Vilalta & Brown, 2018). This process shares core mechanism with phagocytosis, although it may act with mechanisms other than phagocytosis (Vilalta & Brown, 2018). Phagocytosis generally involves three steps. The first step is recognition, where the “find-me” signals help a phagocyte identify the target sites (Vilalta & Brown, 2018). In the engulfment step, “eat-me” signals trigger the actin cytoskeleton in the responsible cell to initiate phagocytosis. In the digestion step, the phagosome is digested through the fusion with lysosomes (Vilalta & Brown, 2018). For example, in synaptic pruning, the GABA neurotransmitter is secreted from inhibitory neurons and binds to GABA_BR on microglia, recruiting neighboring microglia to form a complex for synapse weakening (Favuzzi et al., 2021).

The complement system is a key biological pathway in synaptic pruning (Bajic et al., 2015). It consists of a series of serine proteases that cleave downstream components (Bajic et al., 2015). The complement system is activated through the classical, lectin, and alternative pathways, all leading to the activation of the C3 molecule, a key player in the process (Bajic et al., 2015). The weaker C3-tagged synapses are phagocytosed via the C3 receptor (CR3) on microglia (Schafer et al., 2012). It also deposits membrane attack complexes (MAC) and opsonin that lyse the pathogen's membrane and tag them for phagocytosis by microglia (Bajic et al., 2015). Complement-mediated synapse elimination occurs in a similar way.

Dopamine, an important neurotransmitter, is associated with many neurodegenerative diseases, particularly Parkinson's disease (Huo et al., 2024). Synaptic pruning through dopamine receptors can be observed in the nucleus accumbens via C3-mediated microglia engulfment (Kopec et al., 2018).

Three main types of brain cells are involved in synaptic pruning: microglia, which are involved in synapse elimination through phagocytosis (Spurgat & Tang, 2022); astrocytes, which help regulate neurotransmission (Spurgat & Tang, 2022) and oligodendrocyte precursor cells (OPCs), which play a role in synaptic plasticity (Akay et al., 2021).

Synaptic pruning is highly regulated by gene expression. Identification of the genes involved in synaptic pruning in the stated three cell types through single cell RNA sequencing can offer a good insight in understanding the underlying mechanism of synaptic pruning and finding potential therapeutic targets in neurodegenerative diseases in future.

Role of Different Cell Types in Synaptic Pruning

Microglia

Microglia, the immune cells of the central nervous system (CNS), account for 5-20% of glial cells in the adult brain (Mecca et al., 2018). They are responsible for the regulation of inflammation (Kettenmann et al., 2011), phagocytosis of cell debris (Fu et al., 2014), synaptic refinement (Hayashi & Nakanishi, 2013; Nizami et al., 2019), mediating immune response (Hayashi & Nakanishi, 2013; Kettenmann et al., 2011) and facilitating programmed cell death (Mecca et al., 2018). These functions are essential for maintaining brain homeostasis and supporting healthy neural development through adulthood (Goel & Lee, 2007).

Microglia exhibit an amoeboid morphology, which allows them to migrate through brain regions such as the corpus callosum. They regulate axon ensheathment by engulfing OPCs via their fractalkine receptors (CX3CR1) and maintaining an appropriate OPC-to-axon ratio for proper axonal coverage (Irfan et al., 2022). This function suggests a homeostatic mechanism that ensures the precise regulation of synaptic connectivity. The CX3CR1 is highly expressed CNS microglia and it is also important in microglia migration (Paolicelli et al., 2011; Ruitenberg et al., 2008).

Recent studies have shown that synaptic pruning by microglia is influenced by neural activity, as demonstrated in mouse models (Mrsic-Flogel et al., 2007; Schafer et al., 2012). So, microglia are essential participants in shaping neural networks in an activity-dependent manner (Mataga et al., 2004; Tremblay et al., 2010). Several receptors on microglia regulate synaptic pruning, acting as either positive or negative modulators of this process.

Complement component 1q (C1q) is an important molecule in the complement system that originates from three genes: *C1qa*, *C1qb*, and *C1qc* (Hammond et al., 2020). C1q is the initiating protein of the classical complement pathway that binds and opsonizes cell debris or pathogens (Stevens et al., 2007). C1q then triggers downstream complement protein C3 that eventually eliminates cells through phagocytosis or the formation of a lytic membrane attack complex (MAC) (Stevens et al., 2007). The role of C1q in the synaptic pruning process may be similar to its role in the complement cascade, but it remains unclear (Stevens et al., 2007).

C1q is upregulated within microglia and neurons in the early stages of neurodegenerative disease. Astrocytes also induce C1q expression in developing neurons, ultimately leading to synaptic pruning. Mice deficient in C1q or C3 show a deficit in CNS synapse elimination (Stevens et al., 2007). However, synaptic pruning is still performed even in the absence of C1q, which

indicates there are other pathways besides the complement pathway that control the pruning process (Stephan et al., 2012).

Other vital positive regulators of synaptic pruning include CX3CR1 (Irfan et al., 2022), CR3 (Irfan et al., 2022; Schafer et al., 2012), TREM2 (Filipello et al., 2018; Irfan et al., 2022), GRP56 (Li et al., 2020) and PYR12 (Sipe et al., 2016) while SIRPa serves as a negative regulator (Ding et al., 2021; Lehrman et al., 2018). These receptors enable microglia to target and remove synapses based on specific signaling selectively.

TREM2 (Triggering Receptor Expressed on Myeloid Cells 2), an important receptor in the synapse pruning process, is responsible for the engulfment of synaptic proteins in the synaptic pruning process (Wilton et al., 2019). Microglia can engulf lower levels of synaptic proteins when the TREM2 is not present, and synaptic pruning is reduced accordingly. TREM2 ligand is still unknown, but similar abnormalities are observed following ablation of DAP12, an adaptor for TREM2 (Wilton et al., 2019).

Astrocytes

One-third of human brain cells are Astrocytes that control synapse development and function, neuronal survival, and blood vessel flow (Barres, 2008). Gene expression analysis of mice astrocytes has shown that astrocytes are enriched in phagocytic genes, suggesting that they can be regarded as phagocytes (Cahoy et al., 2008). One of the discussed pathways is the cell death abnormality pathway, also known as ced, which is shown to participate in axon pruning in *C.elegans* (*Caenorhabditis elegans*). The ced pathways contain enriched genes in astrocytes, namely, ced-1 (Megf10, *Drosophila* Draper, Lrp1/cd91), ced-7 (Abca1), ced-6 (Gulp1), ced-2

(Crk), ced-5 (Dock1), ced-12 (Elmo) complex (Cahoy et al., 2008). The ced genes can activate ced-10 (Rac1) (Cahoy et al., 2008).

Astrocytes are significant players in synapse pruning that continue to engulf synapses in the adult CNS (Barres, 2008). It is shown that the activity-dependent synaptic pruning in astrocytes is via the *Megf10* and *Mertk* pathways (Chung et al., 2013). These receptors start engulfing the target by recognizing phosphatidylserine (Chung et al., 2013). Astrocytes and microglia can signal to each other. Astrocytes signal microglia through pathways such as CSF-1 and ATP, and microglia release signals such as TNF α to signal astrocytes (Barres, 2008). However, astrocyte's role in synapse pruning is independent of C1q in contrast to microglia, which shows they have different phagocytic pathways for synaptic pruning (Chung et al., 2013).

Oligodendrocyte Precursor Cells (OPCs)

While microglia and astrocytes are well-known players in synaptic pruning, recent studies have identified a novel role for OPCs in this process. Studies have recently revealed that another class of cells is a key player in synapse engulfment through phagocytic mechanisms called OPC (Auguste et al., 2022).

Precursor cells are a particular subclass of glial cells that give rise to myelinating oligodendrocytes (Auguste et al., 2022; Buchanan et al., 2023). OPCs first appear in the subventricular zones of the embryonic neural tube. Then, they migrate to different brain and spinal cord regions, followed by proliferation and differentiation to oligodendrocytes (Auguste et al., 2022). As the brain matures, oligodendrocyte production decreases while OPC remains abundant and gives rise to them.

OPCs are one of the heterogeneous populations with distinct subtypes and functions. They have different expression profiles (Simons & Nave, 2015). OPC contributes to antigen presentation in vitro (Falcao et al., 2018), glial scar formation (Kirby et al., 2019), angiogenesis (Bradbury & Burnside, 2019; Kishida et al., 2019; Yuen et al., 2014) and axon remodeling in zebrafish (Yuen et al., 2014) besides contributing to synaptic pruning (Auguste et al., 2022).

Deficits in OPC function lead to disorders caused by synapse loss, including Alzheimer's and multiple sclerosis (Auguste et al., 2022). Multiple sclerosis (MS) is one example of OPC losing their ability to repair the myelination of the damaged axons as they cannot differentiate from oligodendrocytes (Franklin & Ffrench-Constant, 2008). Researchers are focusing on developing and differentiating OPCs to promote their myelination activity (Simons & Nave, 2015).

OPC is mainly detected by expressing various markers such as NG2 and PDGFR α (platelet-derived growth factor receptor- α) that aid in distinguishing them from other glial cells (Franklin & Ffrench-Constant, 2008). OPCs undergo development in a much-regulated way that is affected by signaling pathways such as Notch, Wnt, and Sonic Hedgehog (Shh), as well as extracellular matrix components and cell-cell interactions (Kessaris et al., 2006). OPCs express phagocytic genes to engulf synapses (Bergles & Richardson, 2015; Hughes & Appel, 2016), including LRP1, observed in 30% of synapses phagocytosed by OPCs. OPC-mediated synaptic pruning is influenced by microglial depletion (Auguste et al., 2022).

By analyzing the expression of known phagocytic and pruning genes in OPC, the exact mechanism of OPC involvement in synaptic pruning and its regulation in neurodegenerative diseases become apparent.

Synaptic Pruning in Health and Disease

Synaptic pruning is an evolutionarily conserved mechanism observed in non-human primates (Bourgeois & Rakic, 1993; Rakic et al., 1986) and rodents (Pinto et al., 2013) as observed in *Drosophila* (Tasdemir-Yilmaz & Freeman, 2014). Research indicates that synaptic pruning significantly decreases in neurodevelopmental disorders, such as schizophrenia, during adolescence and adulthood (Penzes et al., 2011). Disruption of synaptic pruning is also associated with Alzheimer's disease (Hong et al., 2016) and multiple sclerosis (Michailidou et al., 2015).

It has been shown that repeated stress induces microglial synaptic pruning through *Cx3cr1* signaling (Milić et al., 2016). Additionally, C3 acts as the tag for excitatory synapses and induces microglia-mediated synaptic pruning (Wang et al., 2023).

Clinical symptoms increase with pathological synaptic loss (DeKosky & Scheff, 1990; Graveland et al., 1985; Terry et al., 1991; Wishart et al., 2006). However, it is still unclear whether the synaptic loss is a consequence of neuronal damage or a trigger for disease development. The excitatory inhibitory ratio is abnormal in neurodegenerative diseases, such as autism and Alzheimer's disease (Ghatak et al., 2021) and targeting microglia for selective synaptic pruning may help restore the balance. In Alzheimer's disease, oAB and C1q cooperate to activate microglia and astrocytes (Hong et al., 2016).

To understand how the synaptic pruning by OPC is altered in neurodegenerative disease, it is first essential to find the key phagocytic genes in OPC contributing to the synaptic pruning process. The scRNA-seq provides a precise tool for identifying of key genes involved in the synaptic pruning process.

Historical Overview of Sequencing Technologies

The first approach in DNA sequencing, known as the first-generation sequencing, was the Sanger sequencing, invented by Frederick Sanger in 1977 (Jovic et al., 2022). This method involves the amplification of the DNA (Deoxyribonucleic Acid) of interest using polymerase chain reaction (PCR) or other amplification methods. Double-stranded DNA is then denatured, and a sequencing primer complementary to a specific region is attached to the DNA template. DNA polymerase then synthesizes the DNA with labeled deoxynucleotides (dNTPs) and terminates synthesis upon incorporating dideoxy nucleotides (ddNTPs). These DNA strands are separated by polyacrylamide gel electrophoresis and analyzed through fluorescence detection methods (Akintunde et al., 2023; Sanger et al., 1977).

Although Sanger sequencing was groundbreaking, it is expensive, labor-intensive, and time-consuming (Mamanova et al., 2010). Next-generation sequencing (NGS) was introduced in 2005 to overcome these limitations. NGS allows parallel sequencing of several DNA molecules with high throughput. This method, known as second-generation sequencing, begins with DNA fragmentation, DNA-end repair, adapter ligation, surface attachment, and amplification (Pfeifer, 2017; Qin, 2019). While NGS significantly improves first-generation sequencing, it still faces challenges such as reassembling sequencing reads for regions with structural variations, low-complexity regions, and genomic repeats (Abbasi & Alexandrov, 2021; Treangen & Salzberg, 2011).

Introduction to Single-cell RNA Sequencing (scRNA-seq)

Recent advancements have led to the discovery of scRNA-seq. While bulk RNA sequencing detects the expression signals from whole tissues/organs, the single-cell method captures signals from one individual cell (Hedlund & Deng, 2018; Jovic et al., 2022). This method was used to discover new cell types, investigate developmental processes, identify gene regulatory mechanisms, and identify random allelic gene expression (Hedlund & Deng, 2018).

Tang *et al.* first introduced the concept of scRNA-seq in 2009, where reverse transcription and amplification of mRNA from single cells were followed by sequencing. Since then, this method has become the state-of-the-art approach to investigating the cellular heterogeneity and complexity of cells, development, differentiation, and disease progression (Jovic et al., 2022; Tang et al., 2009).

Methodologies in scRNA-seq

The scRNA-seq begins with the isolation and capture of individual cells. Several methods are available for single-cell isolation; namely, Fluorescence-activated cell sorting (FACS) (Brown & Wittwer, 2000), Laser capture microdissection (LCM) (Chung & Shen, 2015; Espina et al., 2006), Limiting dilution method (Underwood & Bean, 1988; Ye et al., 2021), Microfluidic Systems (Brouzes et al., 2009), SMART Technology (switching mechanism at the 5' end of the RNA transcript) (Zhu et al., 2001), and 5' Poly A Tail Method.

In the 5' Poly A Tail Method, oligo (dt) primers are included in the reaction that bind to the mRNA's poly-A tails. Unique molecular identifiers (UMI) are incorporated into the primers to

reduce PCR bias by distinguishing between original and duplicate sequences during the process (Aird et al., 2011; Kivioja et al., 2011; Pflug & von Haeseler, 2018). UMIs are short sequences of random nucleotides (typically 8-12 bases) added to the 5' end of the primers during library preparation (Kivioja et al., 2011).

Sequencing and Data Analysis

After cDNA synthesis, the resulting cDNA is amplified through PCR. Sequencing is usually performed by NGS platforms like Illumina (Voelkerding et al., 2009). Shortly, during each sequencing cycle, fluorescent-labeled nucleotides (A, C, G, T) are added to the flow cell and incorporated into the DNA strand, and the emitted fluorescent signals indicate the presence of specific bases (Goodwin et al., 2016).

Some common methods are used in the analysis of scRNA-seq. Clustering algorithms group cells based on similarities in their gene expression profiles in one group (Peng et al., 2020; Petegrosso et al., 2020). The Dimensionality reduction approach reduces the number of variables while preserving the relationship between data (Xiang et al., 2021). Common techniques include the Principal Component Analysis (PCA) (Jolliffe & Cadima, 2016), t-distributed Stochastic Neighbor Embedding (t-SNE), and Uniform Manifold Approximation and Projection (UMAP) (Do & Canzar, 2021).

Applications and Relevance of scRNA-seq in Research

ScRNA-seq has been instrumental in discovering new cell types and cell markers, showing cellular diversity (Treutlein et al., 2014). scRNA-seq has provided significant insights into cancer biology, neurodegenerative diseases, and developmental disorders, aiding in the development of targeted therapies and personalized medicine, microbiology and stem cell research (Huang et al., 2024); (Gupta, 2019).

Despite scRNA-seq advantages, it faces limitations such as technical biases, artificial transcriptional stress responses, data complexity, and high costs. Researchers are actively working to address these challenges by improving isolation techniques, refining amplification methods, and developing advanced computational tools for data analysis (Gross et al., 2015).

Research Gap and Purpose

Although the roles of microglia and astrocytes in synaptic pruning are well-studied, the role of OPCs remains under investigation. Also, the biological need for three different cell classes in the circuit remodeling has yet to be fully elucidated (Buchanan et al., 2023). Addressing this gap is crucial for understanding the mechanisms of synaptic pruning, which are essential for maintaining neural circuit integrity and are often disrupted in neurodegenerative diseases. This study investigates the expression of synaptic pruning genes in OPCs using a scRNA-seq dataset, exploring their potential involvement in synaptic assembly and examining age-related changes in the expression of pruning-related genes.

Methods and Materials

The following methods were employed to investigate the genes participating in synaptic pruning and phagocytosis in OPCs.

Data acquisition and initial gene identification

The genes essential for synaptic pruning and phagocytosis in astrocytes and microglia were identified through literature review and gene ontology database searches. GO:0098883, belonging to the synaptic pruning, was chosen to view the genes contributing to the synaptic pruning in the Gene Ontology database <https://toppgene.cchmc.org/>. To extend the analysis, genes contributing to phagocytosis (GO:0006909) were also candidate for participation in synaptic pruning process since phagocytosis plays a significant role in the synaptic pruning process (Vilalta & Brown, 2018). Synaptic assembly genes were also extracted from the stated database (GO0007416) for the second objective of this paper.

Single-cell dataset utilization

The dataset used for gene expression analysis was obtained from the “Aging Mouse Brain” study by Ximerakis *et al.* (2019), accessible through the Single Cell Portal (<https://singlecell.broadinstitute.org/>). The study by Ximerakis *et al.* (2019) is proper for exploring scRNA-seq data to investigate genes involved in synaptic pruning as it has gone through the pre-processing stage (Ximerakis *et al.*, 2019). The authors employed normalization techniques and quality control measures to ensure the dataset is reliable. They have also used the Seurat

package for data processing, log normalization, and PCA for dimensionality reduction. Additionally, including pooling libraries based on the number of detected cells and conducting multiple sequencing runs reduces technical variability.

Preliminary data handling and filtering

Our gene expression analysis process involved several key steps. We began by evaluating each of the genes included in synaptic pruning and phagocytosis gene ontologies using the single-cell portal <https://singlecell.broadinstitute.org/> using the discussed dataset. The data were then further analyzed using Python. To explore gene expression, the UMIs specific to the target cell clusters (OPC, microglia (MG), and astrocytes (ASC)) were extracted from the supplementary raw data (code/GSE129788_Supplementary_meta_data_Cell_Types_Etc.txt) provided from the Ximerakis et al. study in 2019. Then the gene expression data were filtered to include the genes associated with synaptic pruning and phagocytosis.

First, the mean expression level was calculated for each gene across all UMIs for OPC in the dataset to identify a meaningful threshold for gene expression levels. Genes with zero mean expression across cells were excluded to avoid undefined logarithmic transformations. The logarithmic transformation using $\log_{10}$ was then applied to the filtered data, which normalized the data and highlighted differences in gene expression levels. The transformed data was then visualized using a histogram. Two density peaks were observed, enabling the determination of a threshold that separates genes with negligible expression from those with significant expression and facilitating the determination of an expression threshold for downstream analysis to capture only highly expressed genes.

In addition to the mean expression, the expression frequency of the desired genes was also investigated in each desired cell type (OPC, MG, and ASC) to better compare the expression of genes in the three cell types.

Comparative data analysis and gene interaction network

A combination of Python libraries designed for data manipulation, visualization, and clustering was utilized to analyze gene expression patterns. Utilized libraries included pandas for data handling, scipy, Scikit-learn for statistical and clustering methods, and Scanpy for single-cell analysis (discussed in the following paragraphs).

To identify candidate genes that might be involved in synaptic pruning in OPC, the genes that were highly expressed in OPC, astrocytes, and microglia were determined, considering both the frequency of expression (how often a gene is expressed) and the magnitude of expression (expression level).

Initially, a correlation-based approach was explored that calculated the correlation between the expression levels of each gene in OPC, astrocyte, and microglia. However, this method proved unsuitable for our analysis for several reasons. Firstly, many genes exhibited low or near-zero correlations between the two cell types, even for known synaptic pruning genes in microglia, such as *Trem2* (Irfan et al., 2022; Wilton et al., 2019), *C1qb*, *C1qc*, and *C1qa* (Hammond et al., 2020; Stevens et al., 2007).

Also, the correlation approach does not account for the biological differences between OPC, astrocytes, and microglia. It focuses on how similarly a gene is expressed between the two cell types, which does not necessarily represent the functional importance of a gene among different cell types.

To ensure a robust comparison of gene expression between cell types, the data was filtered as follows:

- Gene Selection: Only genes that were expressed ($\text{UMI} > 0$) in at least one of the three cell types (OPC, MG, ASC) were retained for analysis.
- Mean Expression Calculation: For each gene, the mean expression was calculated by averaging non-zero UMI counts across all cells in each cell type (OPC, MG, and ASC).
- Expression Frequency Calculation: The expression frequency was defined as the percentage of cells expressing each gene ($\text{UMI} > 0$) in each of OPC, MG, and ASC populations.

Thresholds for both mean expression and expression frequency were defined to focus on genes that were highly expressed in each cell type: A mean expression threshold of >0.001 was set based on previous histogram analysis of gene expression. The threshold was set to -2.9 based on the Log10 scale of the gene expression that accounts for 0.00126, set as 0.001 in this stage for the mean expression threshold. A bar chart was plotted to investigate the expression frequency of the pruning and phagocytic genes in the dataset. A frequency threshold of 0.5 was used, capturing genes expressed in at least 50% of cells within each cell type to restrict the identification of essential genes.

To identify genes with similar expressions between OPCs and the other two cell types, an expression similarity threshold of 20% was defined. This threshold was used to capture genes where the difference in mean expression between OPCs and microglia, as well as between OPCs and astrocytes, was fewer than or equal to 20%.

An agglomerative hierarchical clustering algorithm was utilized to review the resulting gene interactions. In this method, different clusters are formed based on their expression patterns, and clusters are then merged until all genes are suited in a single hierarchy. The clustering was

visualized using a dendrogram generated by the scikit-learn library and visualized with Matplotlib. To better identify the critical cluster of genes, several genes were set as referenced genes. *Trem2* (Wilton *et al.*, 2019), *C1qa*, *C1qb*, and *C1qc* (Hammond *et al.*, 2020) were previously shown to be participating in the synaptic pruning activity of microglia (Stevens *et al.*, 2007). So, these genes were selected as reference genes. Genes included in the *C1qa*, *C1qb*, and *C1qc* clusters were also identified to be introduced as candidate genes in the synaptic pruning process in OPC.

A gene interaction network was constructed based on the hierarchical clustering results to explore further the relationships between genes involved in synapse pruning and phagocytosis in OPC cells. Genes were labeled using the I2G dictionary. The I2G dictionary generates numeric labels during clustering that allows tracking gene names through the clustering process.

The study was focused on the genes implicated in synapse pruning and phagocytosis. For each pair of genes in these sets, the shortest paths in the hierarchical clustering tree were computed using Dijkstra's algorithm. Each gene node was annotated with a label and color: blue for synapse pruning genes, red for phagocytosis genes, and purple for genes involved in both processes.

Finally, the resulting gene interaction network was visualized using the networkx library, where the edges represent the shortest paths between gene pairs. This network offers insights into how genes associated with synapse pruning and phagocytosis are interconnected and introduces candidate genes essential in the synaptic pruning process.

Statistical validation of gene expression results

Log₂ Fold Change (LFC) and adjusted p-value were calculated for synapse pruning and phagocytosis genes to confirm the results further. The criteria for selecting differentially expressed genes in this study were based on both adjusted p-values and LFC thresholds similar to the method described by McDermaid *et al.*, (2019), ensuring biological relevance while capturing moderate

expression changes (McDermaid et al., 2019). LFC, defined as the ratio of expression in a target cell cluster to expression in other cell clusters, was set to a threshold greater than 1. This approach facilitated the identification of genes up- or down-regulated in a specific cluster relative to others. In addition, adjusted p-values were calculated to assess the statistical significance of expression differences, with values below 0.05 indicating statistically significant results. In this study, adjusted p-values reported as 0 were interpreted as highly significant and were considered important when supported by biologically relevant LFC values.

The candidate genes were compared across OPCs, microglia, and astrocytes, with the Choroid Plexus epithelial cells (CPC) included as a negative control to validate the results. Candidate genes less expressed in CPC were deemed relevant. Finally, the identified genes were cross-verified with the Gene Ontology database (<https://geneontology.org/>) to ensure their roles in synaptic pruning.

Synaptic assembly genes

After identifying candidate genes in synaptic pruning in OPC, their expression was investigated along with the synaptic assembly genes to examine whether OPCs participate in synapse assembly and whether the synaptic assembly genes are co-expressed with synaptic pruning genes. The assembly genes filtered through the LFC and P-value were used to plot a UMAP with the candidate pruning genes. In addition, the filtered assembly genes were also searched on the Gene Ontology database (<https://geneontology.org/>) to investigate if they participated in any specific gene ontology annotation.

Differential gene expression analysis among young and old OPC

Ximerakis *et al.* and his colleague analyzed 50,212 single cells in the “Aging Mouse Brain” dataset. 24,401 cells among them were young and 25,811 cells were old. The cells were extracted from 8 young (2-3 months) and 8 old (21-23 months) mice. This dataset was segmented into two age groups: young OPCs and old OPCs. Age classifications were based on the metadata provided by Ximerakis *et al.* (2019), distinguishing cells by age markers. A comparative gene expression analysis investigated the differential expression of candidate synaptic pruning genes between young and old OPCs. Specifically, candidate genes associated with synaptic pruning were investigated, including *Adgrb3*, *Fyn*, *Snx3*, *Plxnc1*, *Calr*, *Itgb1*, and *C1ql1*, to determine how aging affects their expression in OPCs. The results of our analysis, presented in a volcano plot, highlight the genes that show significant changes in expression. This provides insights into the potential decline in OPC-mediated synaptic pruning associated with aging.

Results

Characterization of OPCs in Synaptic Pruning

The analysis of the scRNA-seq dataset from the “Aging Mouse Brain” study by Ximerakis *et al.* (2019) showed several clusters of neuronal and glial cells. In this study, different methods were employed to normalize the data and address batch effects. The scRNA-seq data was filtered based on specific parameters to ensure quality. Figure 1 shows the t-SNE clustering performed using shared nearest neighbor modularity optimization with a clustering resolution set to 1.6.

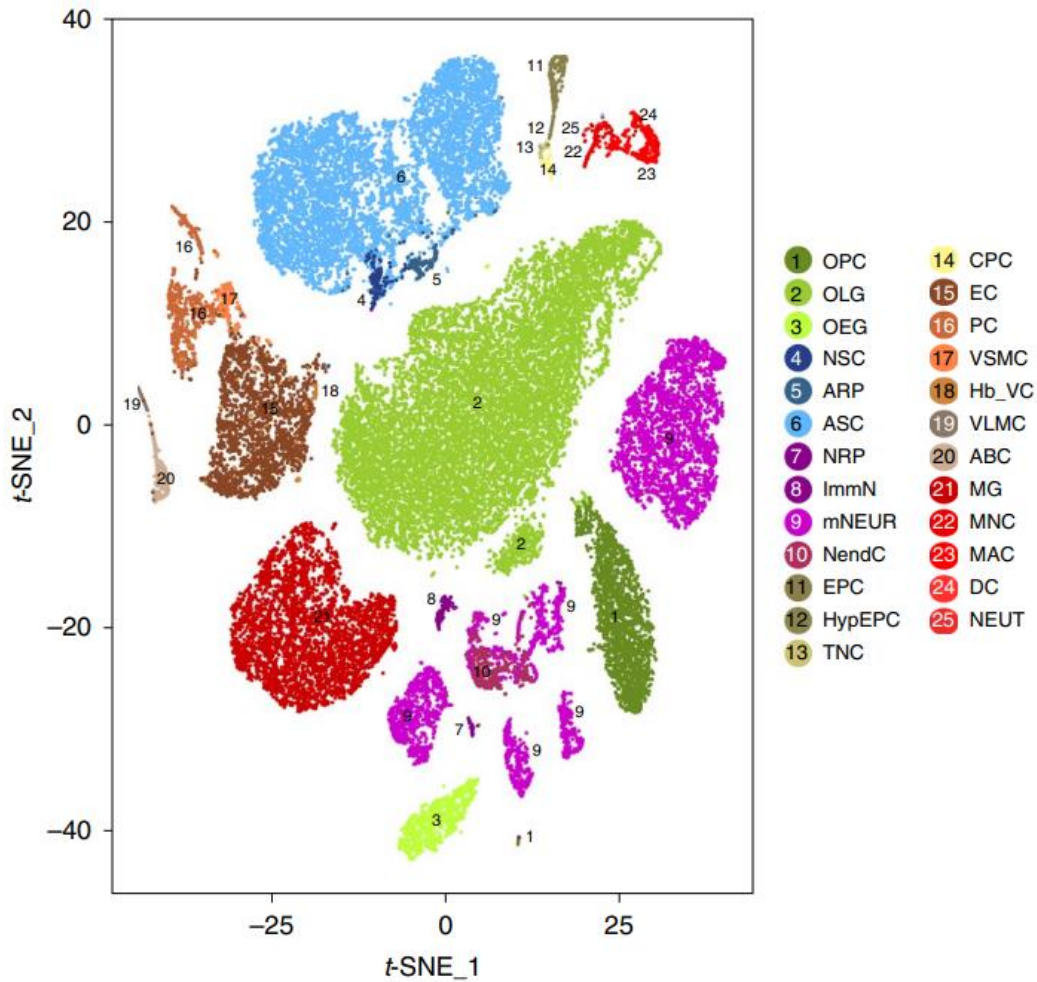


Figure 1: t-SNE Clustering identifies OPCs and other glial cells in the Aging Mouse Brain dataset, illustrating transcriptional heterogeneity. The plot displays 25 distinct transcriptionally defined clusters of 50,212 cells. Each dot represents a cell, colored and labeled according to its assigned cluster identity. Key clusters include oligodendrocyte precursor cells (OPC, green, cluster 1), astrocytes (ASC, purple, cluster 6), and microglia (MG, red, cluster 21). This figure is reproduced from Ximerakis et al. (2019).

Individual cells were filtered based on parameters including mitochondrial RNA content, UMIs, and the number of genes detected, to only capture high-quality cells in the analysis. Additionally, genes detected in less than a predetermined number of cells were excluded from the

dataset. This resulted in a dataset composed of 12,844 cells: 2,187 OPCs, 3,910 microglia, and 6,747 astrocytes. Table 1 summarizes these parameters and their respective thresholds:

Filtering Parameter	Threshold
Mitochondrial RNA Content	Cells with mitochondrial gene expression exceeding 30% of total expression were excluded.
Unique Molecular Identifiers	Cells with fewer than 500 and more than 30,000 UMIs were excluded.
Number of Genes Detected	Cells with fewer than 250 and above 6,000 detected genes were excluded.
Gene Detection Across Cells	Genes detected in fewer than 3 cells were excluded from the dataset.

Table 1. Summary of cell filtering parameters and thresholds used to filter individual cells in the scRNA-seq dataset ensuring high-quality data for analysis. Parameters include mitochondrial RNA (Ribonucleic Acid) content, UMIs, the number of genes detected per cell, and the minimum number of cells in which a gene must be detected to be included. These thresholds were set to exclude low-quality cells and uninformative genes, as described in Ximerakis et al. (2019).

When phagocytosis and synaptic pruning genes were explored in the gene ontology database, 18 genes were included in GO:0098883 for the synaptic pruning biological pathway shown in Table 2, and 287 genes were included in GO:0006909 for phagocytosis, indicated in Appendix-Table A1. When the discussed genes were searched in single-cell data, only 224 genes among them were found in the mouse brain aging dataset.

Gene ID	Symbol	Name
577	ADGRB3	adhesion G protein-coupled receptor B3
54209	TREM2	triggering receptor expressed on myeloid cells 2
10882	C1QL1	complement C1q like 1
3684	ITGAM	integrin subunit alpha M
712	C1QA	complement C1q A chain
3688	ITGB1	integrin subunit beta 1
6376	CX3CL1	C-X3-C motif chemokine ligand 1
713	C1QB	complement C1q B chain
714	C1QC	complement C1q C chain
10154	PLXNC1	plexin C1
718	C3	complement C3
1524	CX3CR1	C-X3-C motif chemokine receptor 1
2043	EPHA4	EPH receptor A4
1020	CDK5	cyclin dependent kinase 5
22943	DKK1	dickkopf WNT signaling pathway inhibitor 1
25791	NGEF	neuronal guanine nucleotide exchange factor

Table 2. Genes associated with synaptic pruning identified through GO:0098883 to be examined in this paper. This table lists the genes identified from the Gene Ontology database as being involved in the synaptic pruning biological pathway (GO:0098883).

Single cell portal database defines a scale mean expression and expression frequency for the input genes. The scale mean expression shows the relative mean expression of a gene in a cell type based on the smallest and largest mean expression of that gene across different cell types in the dot plot (Scale Mean Expression). Expression frequency (also called expression percentage) shows the percentage of cells that show non-zero expression values for the desired gene (Expression Percentage).

The gene expression threshold was defined according to Figure 2. The histogram below shows the gene expression levels across OPC UMIs available in the Ximerakis *et al.* (2019) study. The threshold is assumed as a region that the histogram shows a significant decrease between the two peaks. This accounts for approximately logarithm of -2.9 .

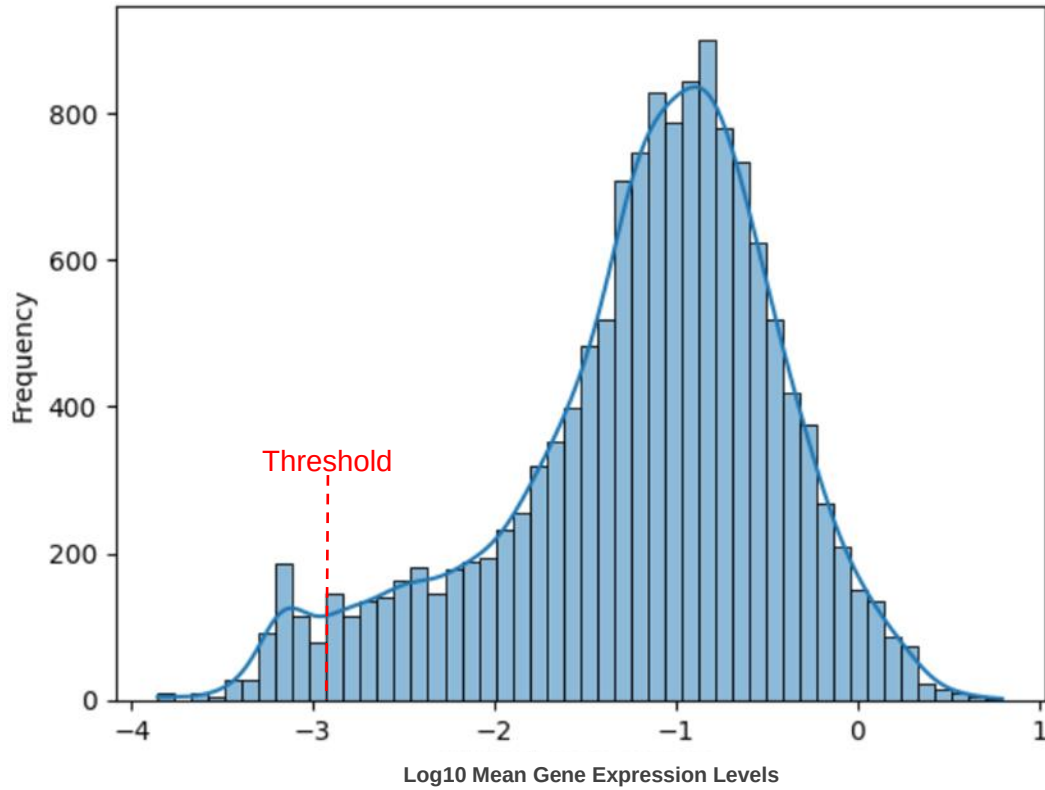


Figure 2: The mean expression levels across OPC UMIs provides a threshold to identify significant genes for downstream analysis. The histogram of mean gene expression level across OPC UMIs, identifies a significant decrease between two density peaks used to set the expression threshold. The x-axis represents the mean expression levels of genes, while the y-axis indicates the frequency of genes at each expression level. A threshold at $\log_{10}(-2.9)$, shown in red dashed line, separates negligible and significant gene expression levels.

After eliminating the genes with total mean expression of zero and below the expression threshold, the frequency of gene's expression was investigated. A frequency distribution analysis via bar chart (Figure 3) showed that most genes were expressed in fewer than 50% of cells in OPC, leading to the selection of a 50% frequency threshold to capture only highly expressed genes in OPC.

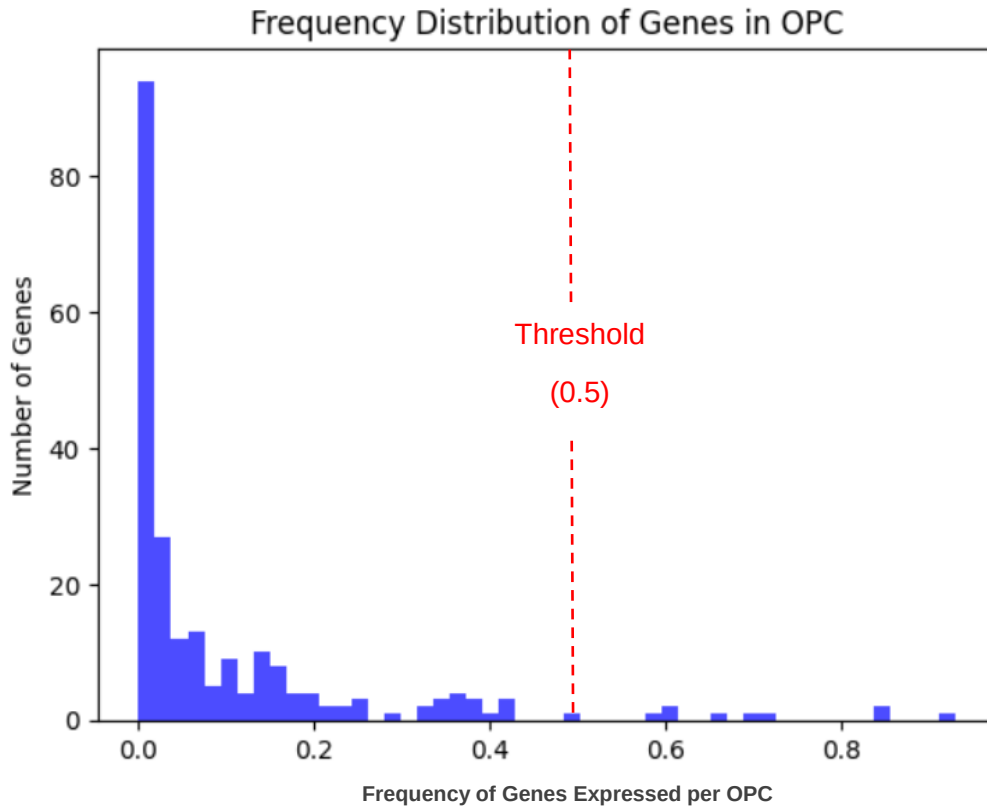


Figure 3: Frequency distribution of gene expression demonstrates that most genes are expressed in fewer than 50% of OPCs. The histogram depicts the distribution of gene expression frequencies in OPCs. The x-axis represents the proportion of cells in which a gene is expressed, while the y-axis shows the number of genes. Most genes are expressed in fewer than 50% of the cells, supporting the selection of a 50% frequency threshold (shown in red) to retain genes that are significantly expressed across the OPC population.

ScRNA-seq Reveals Gene Expression Profiles in OPCs

Using the threshold values for mean expression (>0.001), expression frequency ($>50\%$) and similarity threshold of 20%, 6 genes were found to be highly expressed in both OPC and microglia, and 7 genes in OPC and astrocyte. Notably, 6 genes (*Hspa8*, *Cdc42*, *Calr*, *Rab14*, *Hmgb1*, and *Snx3*) overlapped between the microglia and astrocyte comparison results with OPC, indicating concurrent high expression of these genes across all three cell types. These genes are shown in Table 3 along with their mean expression levels and expression frequency.

Gene	OPC Mean Expression	MG Mean Expression	ASC Mean Expression	OPC Frequency	MG Frequency	ASC Frequency	Similar Expression MG	Similar Expression ASC
<i>Hspa8</i>	2.49	2.39	2.26	0.84	0.83	0.79	TRUE	TRUE
<i>Cdc42</i>	1.95	2.27	1.79	0.60	0.83	0.45	TRUE	TRUE
<i>Calr</i>	2.11	2.12	1.84	0.71	0.72	0.48	TRUE	TRUE
<i>Rab14</i>	1.92	1.97	1.76	0.59	0.68	0.40	TRUE	TRUE
<i>Hmgb1</i>	2.08	1.89	1.79	0.67	0.58	0.43	TRUE	TRUE
<i>Snx3</i>	1.95	1.68	1.79	0.61	0.35	0.45	TRUE	TRUE

Table 3. Differential expression analysis for synaptic pruning and phagocytic genes in OPCs details six genes with similar mean expression levels and frequencies among the three cell types of OPC, microglia (MG) and astrocyte (ASC). The table presents genes that show high mean expression across OPC, MG, and ASC, along with their respective mean expression values and expression frequencies. The columns “Similar Expression MG” and “Similar Expression ASC” indicate whether the genes have similar expression patterns in OPC compared to microglia and astrocytes, respectively. Notably, six genes (*Hspa8*, *Cdc42*, *Calr*, *Rab14*, *Hmgb1*, and *Snx3*) are concurrently highly expressed in all three cell types, indicating a potential role in shared cellular processes. “TRUE” indicates that gene expression is similar in the respective cell type to the levels in OPC.

Hierarchical Gene Clustering Identifies Potential Synaptic Pruning Genes in OPCs

The hierarchical clustering analysis aimed to identify genes potentially involved in synapse pruning within OPCs. Using *C1qa*, *C1qb*, *C1qc*, and *Trem2* as reference genes, which are associated with synaptic pruning in microglia, revealed insight into candidate genes that may participate in the same process in OPC (Appendix-Figure A1: Hierarchical Clustering Network of Genes in OPCs).

The analysis highlighted *Tyrobp* (TYRO Protein Tyrosine Kinase Binding Protein) and *Rgs10* (Regulator of G Protein Signaling 10) as significant candidate genes. Figure 4 illustrates *Tyrobp* and *Rgs10* association with the reference complement genes (*C1qa*, *C1qb*, *C1qc* and

Trem2) suggesting a possible role in OPC-mediated synaptic pruning, like microglial function in synaptic refinement during development. Figures 5 and 6 illustrate that the genes selected based on expression similarity between OPCs, microglia, and astrocytes (*Hspa8*, *Cdc42*, *Calr*, *Rab14*, *Hmgb1*, and *Snx3*) group closely, suggesting their potential shared functions.

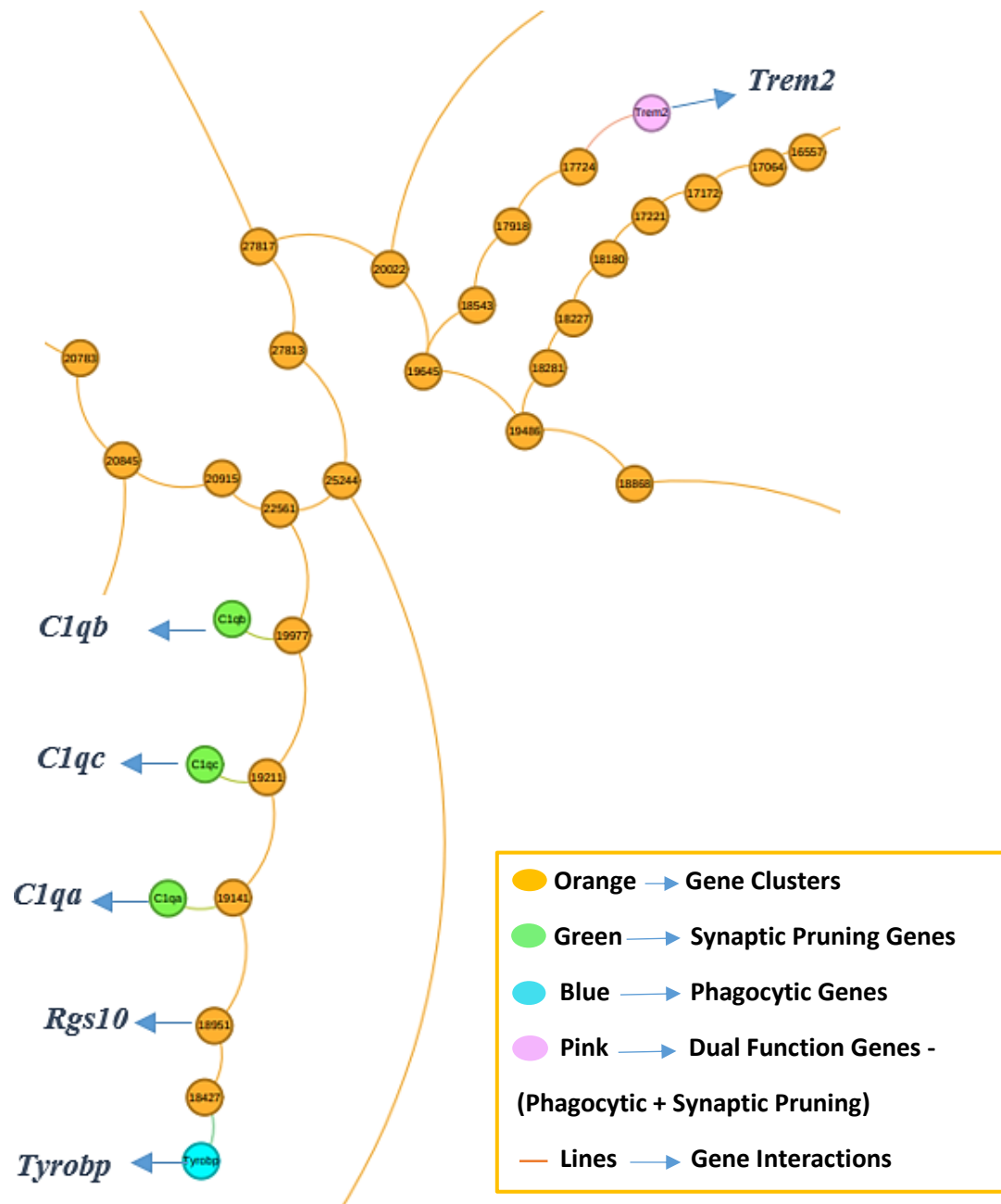


Figure 4: Hierarchical clustering reveals interactions between *Tyrobp* and *RGS10* with other genes involved in synaptic pruning and phagocytosis indicating their probable role in synaptic pruning. The figure emphasizes how *Tyrobp* (cluster 18427) and *Rgs10* (cluster 18951) potentially coordinate with genes like *Trem2*, *C1qa*, *C1qb*, and *C1qc* in neuronal processes.

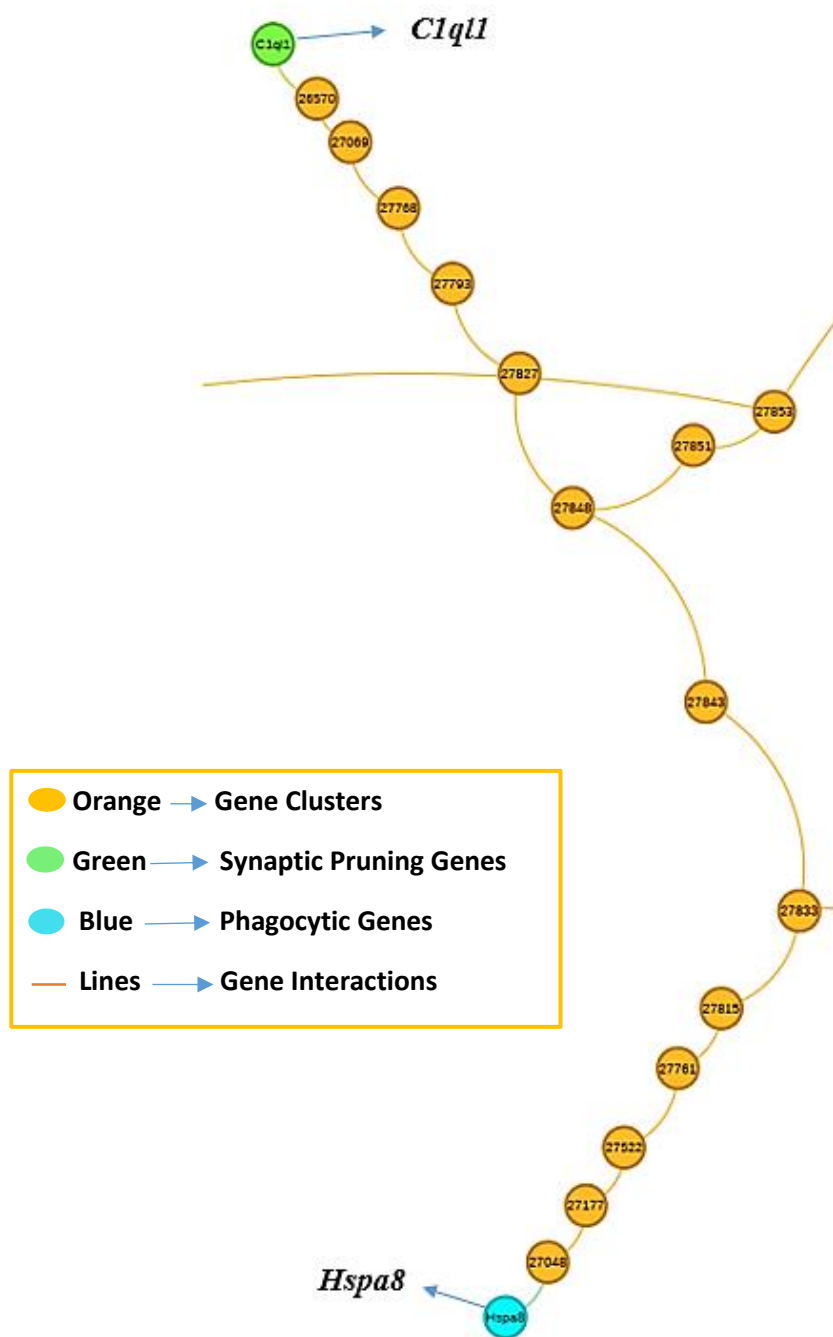


Figure 5: Gene network analysis shows a close relationship between Hspa8 and its interactions with C1ql1 suggesting its involvement in synaptic pruning. The figure focuses on Hspa8 within the hierarchical clustering network, illustrating its connections with C1ql1 and potential roles in shared processes across OPCs, microglia, and astrocytes.

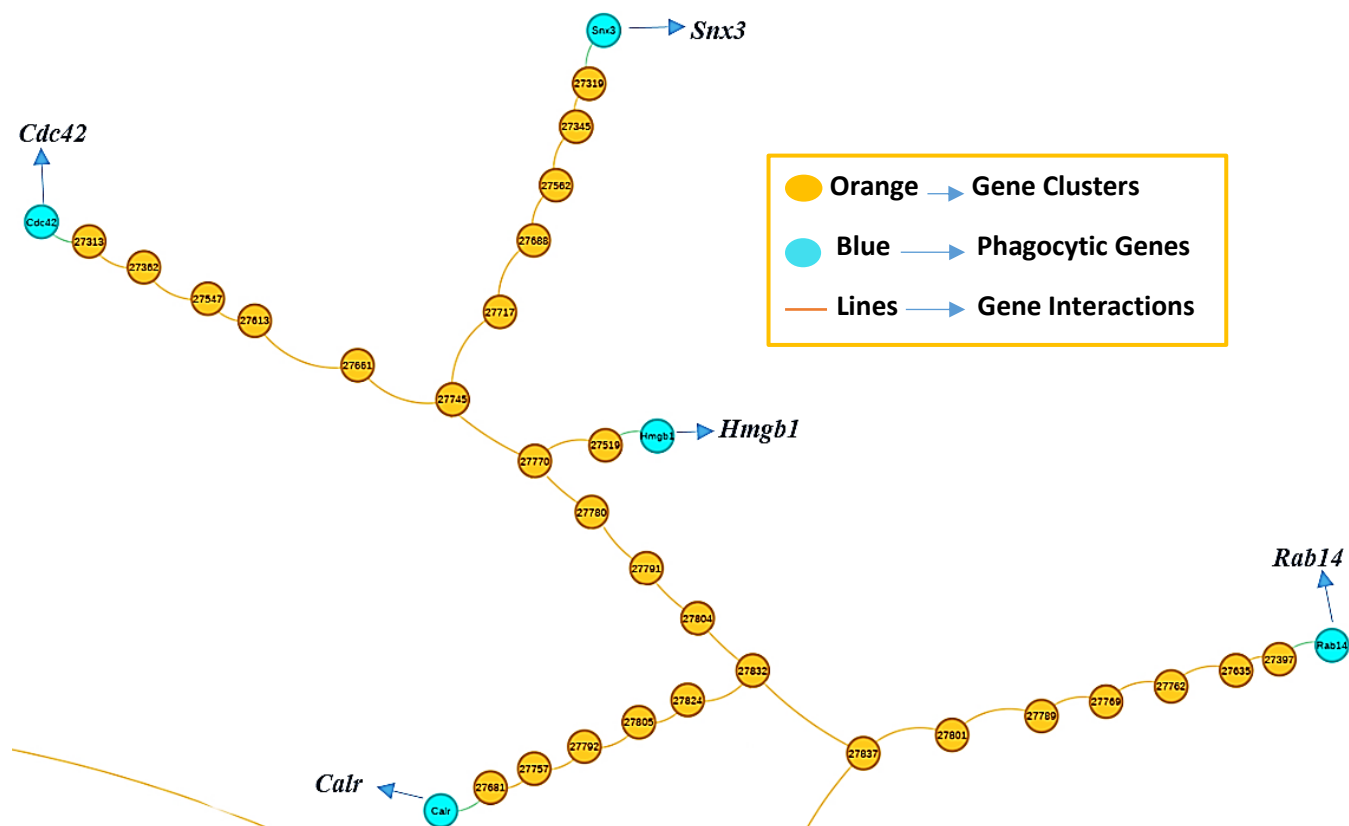


Figure 6: Network highlighting shared highly expressed genes (Cdc42, Calr, Rab14, Hmgb1, and Snx3) across OPC, microglia, and astrocyte, proposing their involvement in synaptic pruning. This figure emphasizes the network connections of genes such as Cdc42, Calr, Rab14, Hmgb1, and Snx3. These genes are concurrently expressed across OPCs, microglia, and astrocytes.

Statistical Validation of Candidate Genes Involved in Synaptic Pruning by OPCs

In addition to the clustering analysis, statistical assessment of synaptic pruning genes revealed a set of promising candidates with significant differential expression through LFC and adjusted P-Value and potential roles in synapse regulation. Overall, 47 genes were filtered based on the acceptance criteria for LFC and adjusted P-Value that are shown in Appendix-Table A2. *C1ql1*, *Plxnc1*, and *Itgb1* were highlighted for their established associations with synaptic pruning,

particularly in microglia and astrocytes, with our findings extending their significance to OPCs. *C1ql1* remains a key gene given its known role in synaptic organization (Kakegawa et al., 2015; Scuderi et al., 2019), while *Plxnc1* and *Itgb1* are implicated in this process based on gene ontology annotations and their expression patterns across cell types. Figure 7 depicts a general overview of the main annotations associated with the filtered synaptic pruning genes.

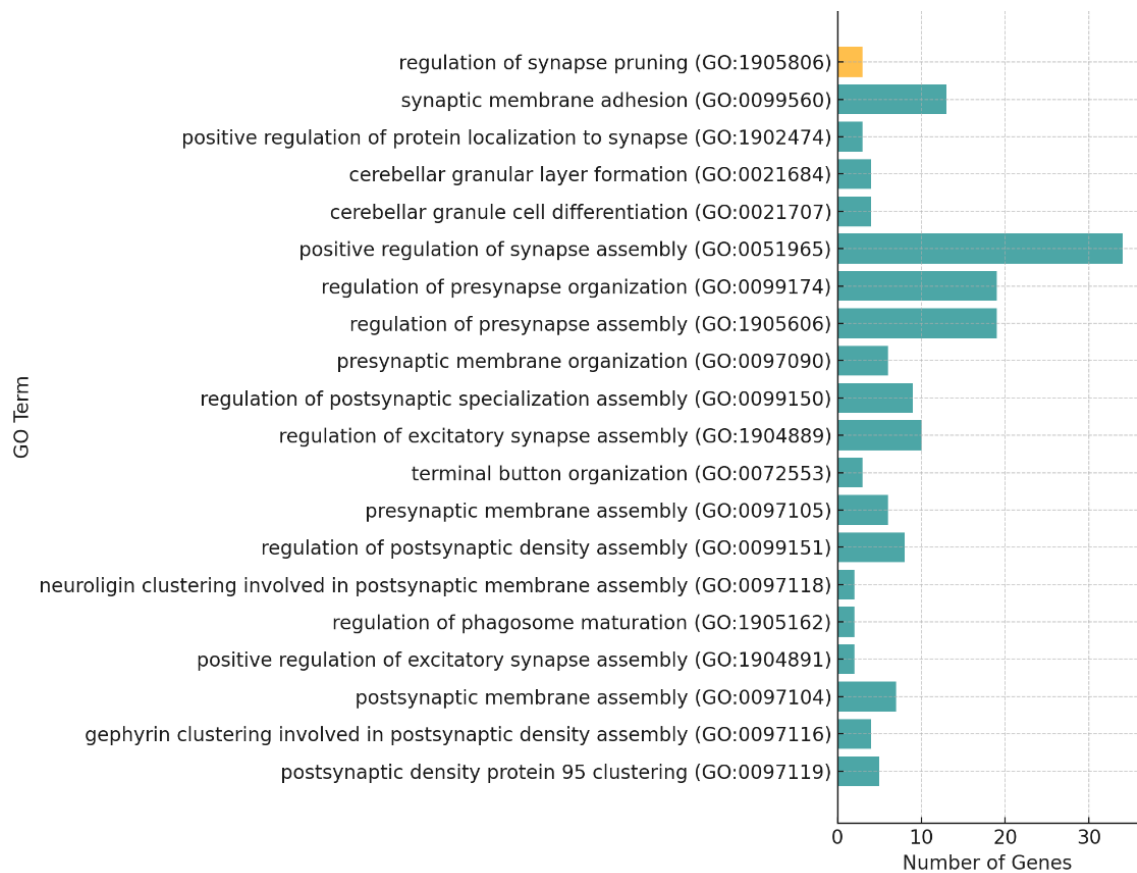


Figure 7: Gene ontology enrichment highlights the role of OPC in synapse organization and pruning. The bar chart displays the number of genes associated with various biological processes, with a specific emphasis on the "regulation of synapse pruning" ontology highlighted in orange.

Moreover, *Fyn* and *Adgrb3* present intriguing cases: although not classically associated with pruning, they are integral to the “regulation of synapse organization, GO:0050807” and “regulation of synapse structure or activity, GO:0050803” processes. Both genes exhibit extremely

significant expression in OPCs, with *Fyn* showing a p-value of 0 and *Adgrb3* demonstrating selective expression that is not as pronounced in MG or ASC. This suggests unique, OPC-specific roles in synaptic organization that may contribute to pruning activities. Additionally, *Snx3* (Sorting Nexin 3) and *Calr* (Calreticulin) were identified as relevant based on prior expression similarity analyses across MG, ASC, and OPCs (Table 3 & Figure 6), further reinforcing their involvement in synaptic processes. Figure 8 shows a heatmap of mean gene expression levels of candidate genes across different cell types (MG, ASC, OPC and CPC (Choroid Plexus Cells) as negative control) highlighting the strong expression of these genes in OPC.

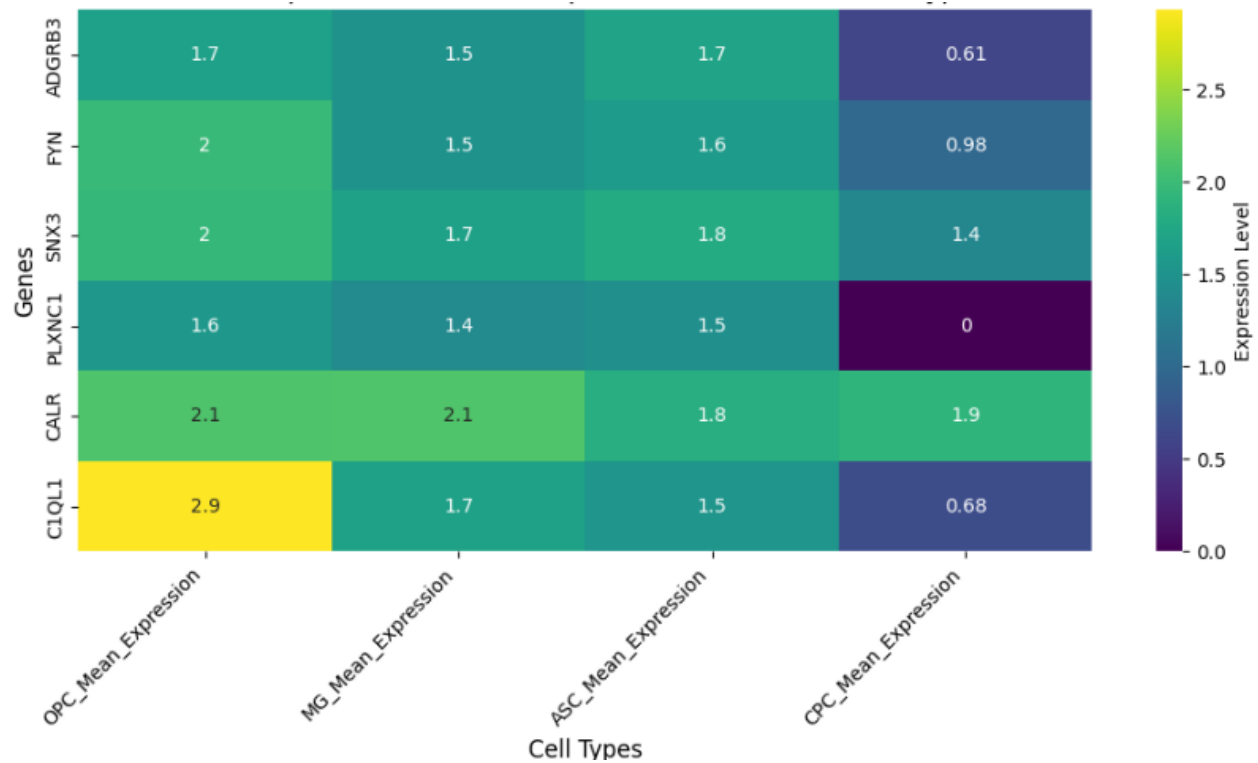


Figure 8: Candidate pruning genes show strong expression in OPCs compared to other cell types. Heatmap displaying the mean expression levels of *C1ql1*, *Plxnc1*, *Itgb1*, *Fyn*, *Adgrb3*, *Snx3*, and *Calr* across OPC (Oligodendrocyte Precursor Cells), MG (Microglia), ASC (Astrocytes), and CPC (Choroid Plexus Cells). CPC works as a negative control. The color intensity represents the relative gene expression with darker shades showing lower expression.

Figure 9 illustrates the logarithmic (Log_2) distribution of gene expression for selected candidate genes involved in synaptic pruning across four different glial cell types: OPC, MG, ASC, and CPC as negative control. The genes examined include *C1ql1*, *Plxnc1*, *Itgb1*, *Fyn*, *Adgrb3*, *Snx3*, and *Calr*. Figure 9 shows higher and broader expression of *C1ql1*, *Snx3*, *Calr* and *Fyn* in OPC.

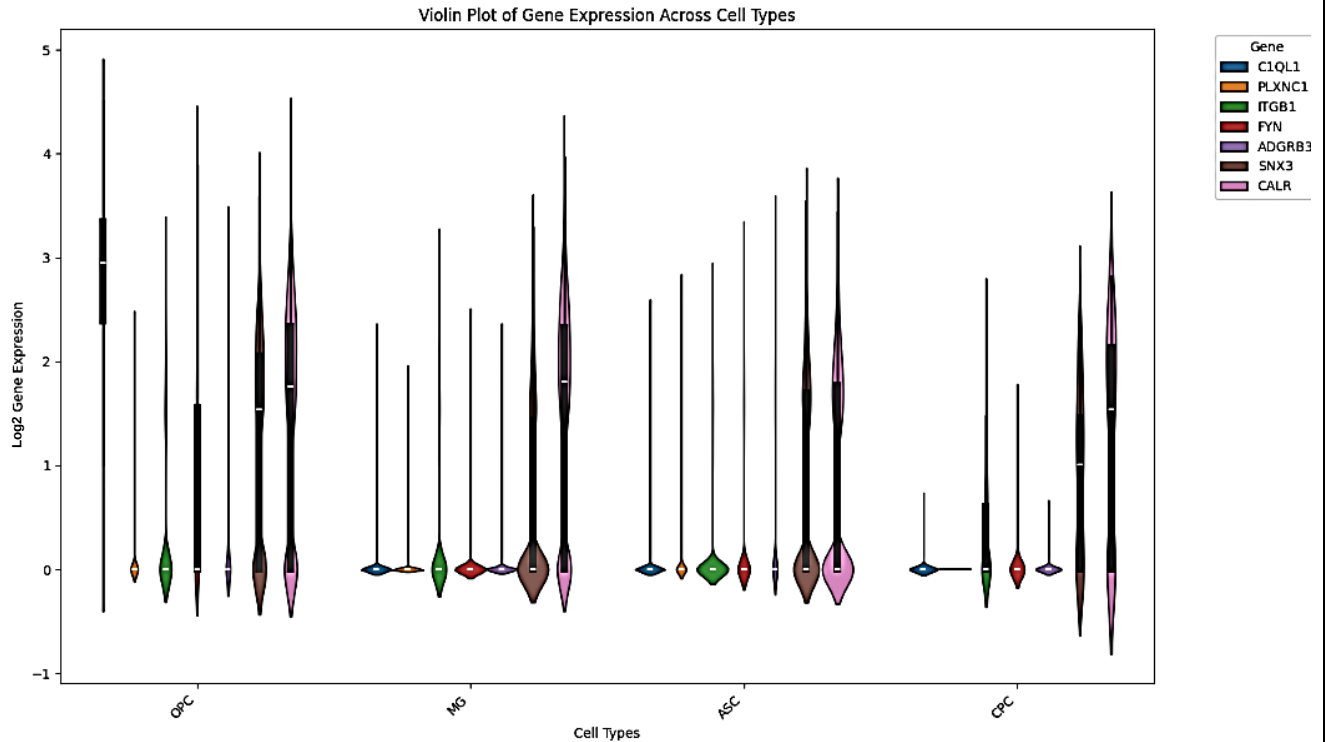


Figure 9: Logarithmic Gene expression distribution confirms OPC similar and specific pruning functions to other cell types. Violin plot of Log_2 gene expression distributions for *C1ql1*, *Plxnc1*, *Itgb1*, *Fyn*, *Adgrb3*, *Snx3*, and *Calr* across OPC (Oligodendrocyte Precursor Cells), MG (Microglia), ASC (Astrocytes), and CPC (Choroid Plexus Cells) shows a broader expression distributions for *C1ql1*, *Fyn*, *Snx3*, and *Calr* in OPCs suggesting their involvement in synaptic pruning.

Concurrent Expression of Synaptic Assembly and Synaptic Pruning Genes in OPC

Our investigation into the expression of synaptic assembly and pruning genes within OPCs revealed several key insights. Using UMAP clustering, illustrated in Figure 10 and Appendix-Figure A2, we observed that both synaptic assembly and pruning genes were evenly distributed across the OPC population, with no evidence of distinct subpopulations. This suggests that OPCs

express the synaptic assembly and pruning genes in a generalized manner, contributing broadly to synaptic functions rather than through compartmentalized subtypes.

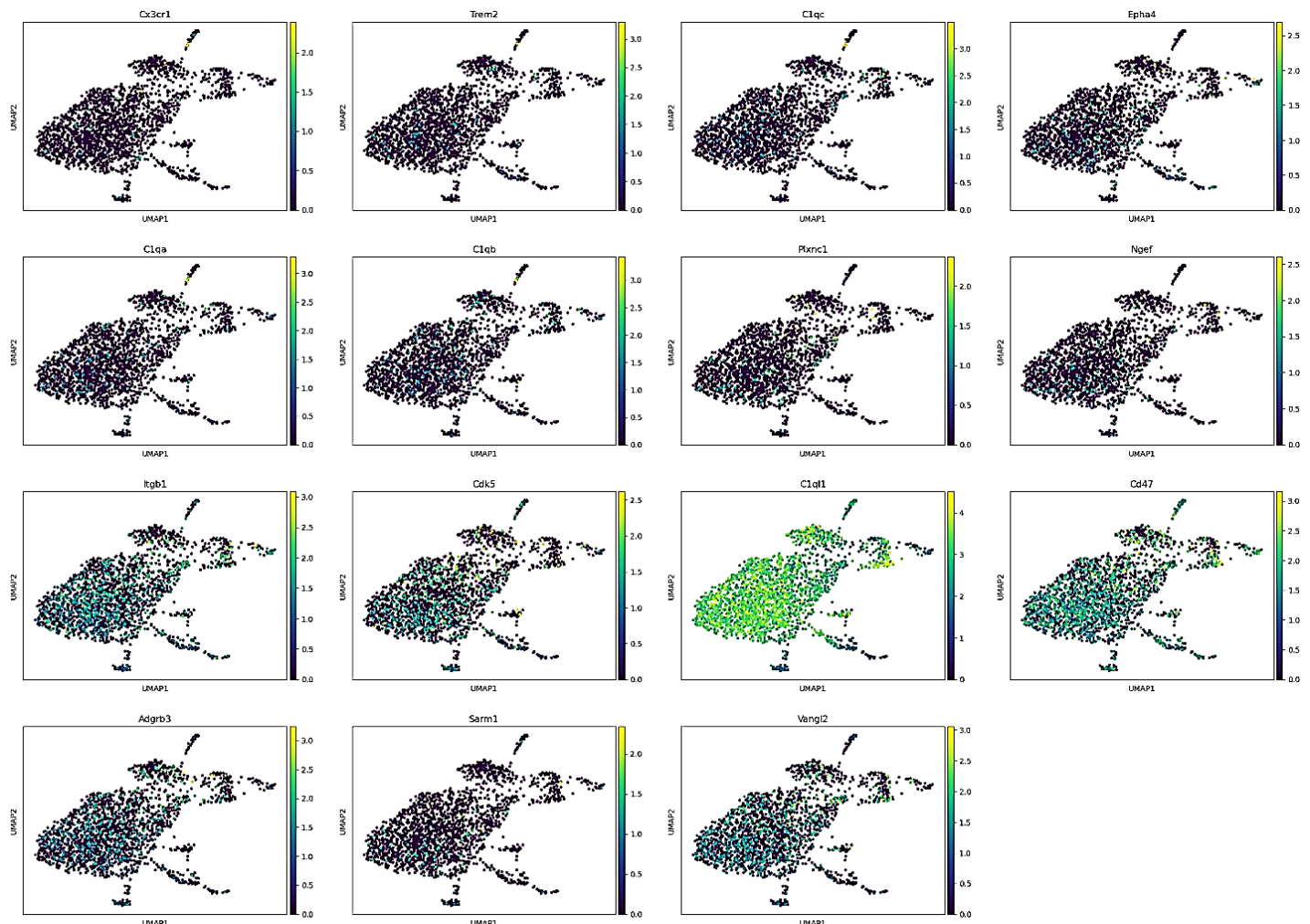


Figure 10: Synaptic assembly genes show uniform expression across OPC populations. Each subplot displays the expression of a single synaptic assembly gene across the OPC population, using UMAP clustering. The consistent color distribution suggests a non-specialized expression pattern of synaptic assembly genes within OPCs. The color intensity represents the relative gene expression with darker shades showing lower expression.

Differential expression analysis highlighted genes with significant LFCs ($LFC > 1$) and extremely low adjusted p-values, some as low as 0, emphasizing their strong differential

expression. Genes with high LFCs and low adjusted p-values, as depicted in Appendix-Table A3, were selected for further analysis, highlighting their robust expression in OPCs compared to other cell types. Notably, Figure 11, the Gene Ontology analysis, revealed that 50 out of the 91 selected genes were involved in 'synapse organization,' reinforcing the idea that OPCs are actively engaged in shaping synaptic networks. Furthermore, many of these highly significant genes were also categorized under 'regulation of cellular process,' pointing to a broader regulatory role for OPCs in cellular and synaptic dynamics.

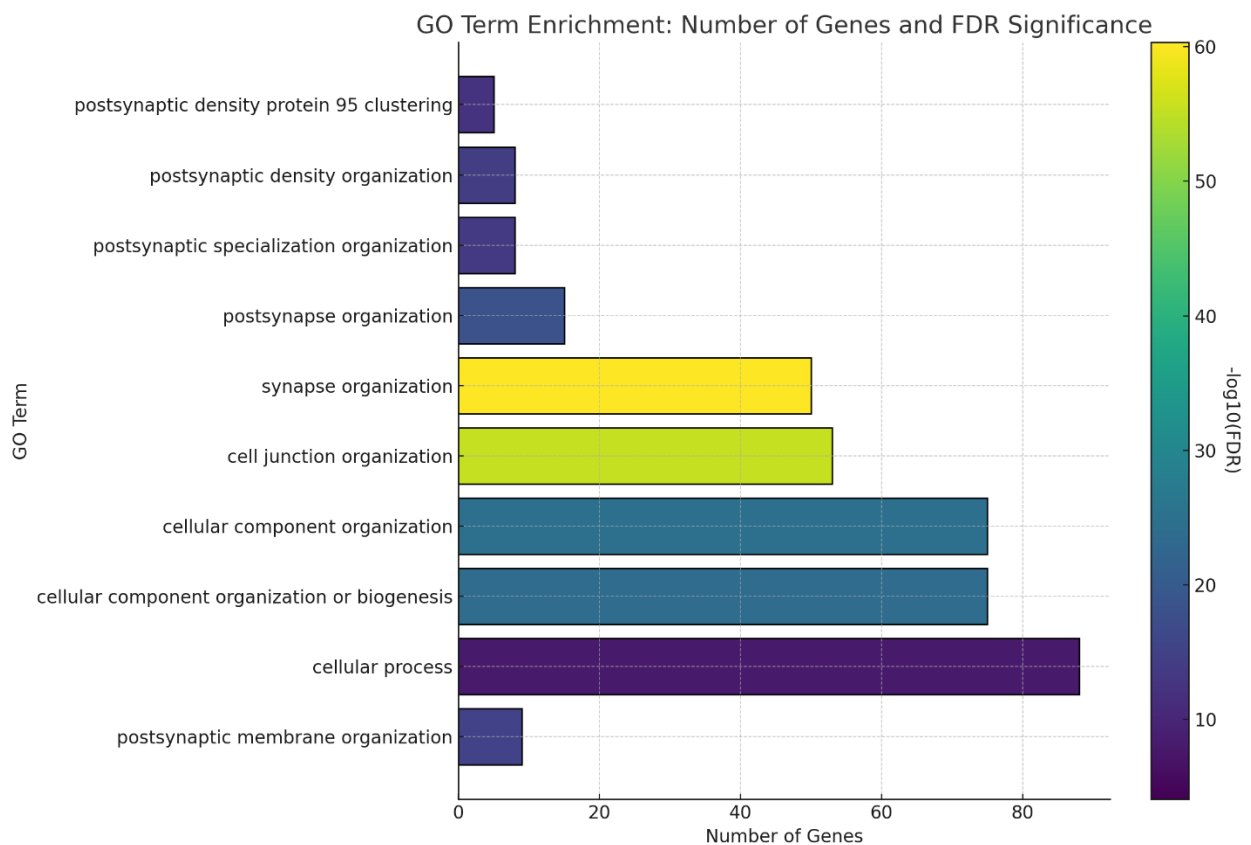


Figure 11: Gene ontology enrichment analysis highlights biological processes related to synaptic assembly in OPCs. A bar chart presenting enriched Gene Ontology terms for synaptic assembly-related genes. The x-axis represents the number of genes mapped to each GO term in the y-axis. Lighter bars indicate higher statistical significance (FDR), with a focus on terms related to OPC contributions to synaptic organization.

Age-Related Variability in Gene Expression of OPCs

Despite some genes, such as *Fyn* and *Snx3*, having relatively low raw p-values (e.g., 0.000073 and 0.00044, respectively), adjusted p-values for other genes were uniformly 1.0 after multiple testing correction. Additionally, the LFCs for all examined genes were minimal, ranging from -0.056601 (for *Adgrb3*) to 0.147065 (for *Fyn*), suggesting negligible expression changes between the two age groups. Figure 12 illustrates that none of the candidate pruning-related genes, including *Adgrb3*, *Fyn*, *Snx3*, *Plxnc1*, *Calr*, *Itgb1*, and *C1ql1* (highlighted in red), exceeded the significance threshold of an adjusted p-value of 0.05, represented by the horizontal blue dashed line. The minimal LFCs clustered near zero, reinforcing the conclusion that transcriptional regulation of these pruning-related genes does not significantly vary with age in OPCs. Consequently, no major transcriptional changes is associated with aging in these genes.

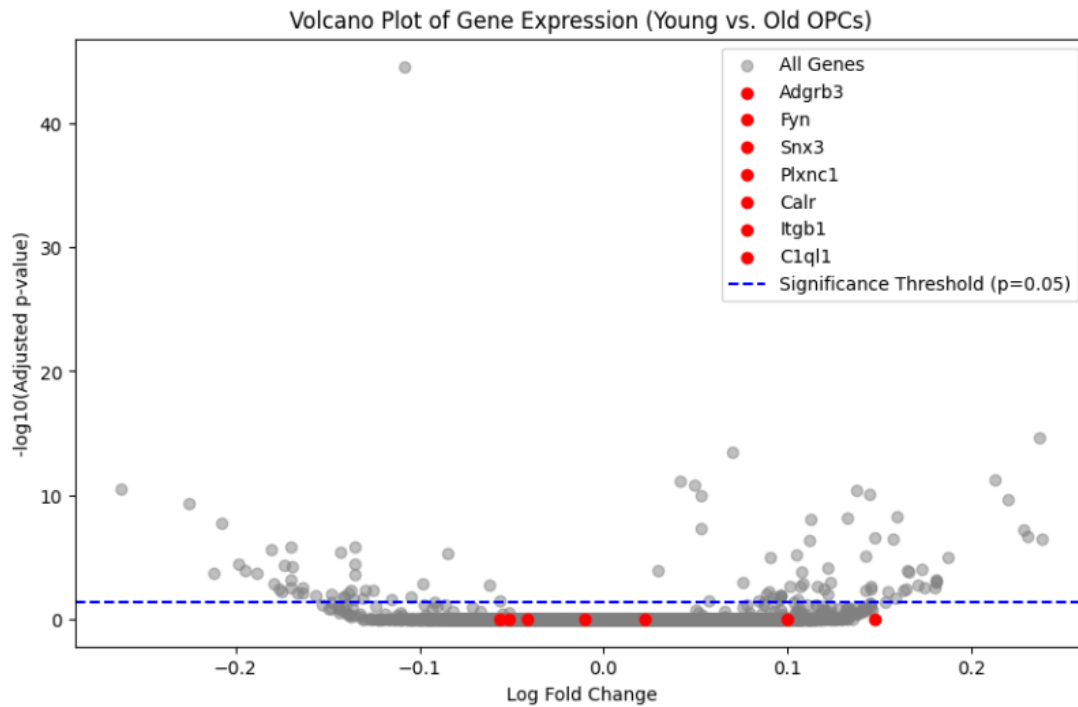


*Figure 12: No significant age-related expression changes was detected in OPC pruning-related genes. Volcano plot showing log₂ fold changes versus adjusted p-value for pruning-related genes in young and old OPCs. None of the candidate genes, including *Adgrb3*, *Fyn*, *Snx3*, *Plxnc1*, *Calr*, *Itgb1* and *C1ql1*, were differentially expressed between populations suggesting age-independent expression.*

Discussion

This study's objective was to identify potential genes involved in the synaptic pruning process of OPCs through statistical analysis and comparison of gene expression with microglia and astrocytes. Our results provide novel insights into the synaptic pruning activities of OPCs and highlight several key genes that may regulate this process.

Gene Identification and Functional Insights

First, our analysis identified a set of genes—*Hspa8*, *Cdc42*, *Calr*, *Rab14*, *Hmgb1*, and *Snx3*—highly expressed in OPCs, microglia, and astrocytes. The concurrent expression of these genes across the three cell types indicates a potential shared role in synaptic regulation. These findings suggest that these genes may play essential roles in facilitating synaptic pruning activities. *Hspa8* was particularly interesting as it formed a cluster close to *C1ql1*, a known synaptic pruning regulator, further supporting its role in synaptic processes.

Identifying *Tyrobp* and *Rgs10* as candidate genes through hierarchical clustering suggests that OPCs might engage in synaptic pruning through similar mechanisms to microglia. TYROBP (DAP12) is an adaptor protein that facilitates signaling through various receptors, such as TREM2. In the absence of TREM2, synaptic elimination is reduced in microglia (Mordelt & de Witte, 2023). Research has shown that TYROBP-deficient mice are associated with an accumulation of C1q, essential in synapse elimination as it marks synapses for elimination (Haure-Mirande et al., 2022). *Rgs10* is highly expressed in microglia and is a regulator of inflammatory response (Alqinyah et al., 2017).

Further statistical analyses highlighted several key genes—*Calr*, *Snx3*, *Fyn*, *Adgrb3*, *Itgb1*, *Plxnc1*, and *C1ql1*—that could be involved in synaptic pruning activities in OPCs. CALR (Calreticulin) works as an “eat-me” signal for phagocytes. It is recognized by receptors on the microglial surface (Vilalta & Brown, 2018). This gene probably does not participate in the OPC synaptic pruning process. Instead, it is related to the OPC phagocytosis by other cells.

Snx3 contributes to endosomal trafficking or protein transportation between cellular compartments (Mladinov et al., 2016). Studies have shown that the *Snx3* level is elevated in

schizophrenia (Mladinov et al., 2016). Trafficking machinery provides recycling endosomes during the process of dendritic remodeling, which can promote dendritic pruning (Vieira et al., 2021).

Fyn is a tyrosine kinase (TK) receptor from Src family kinases (SFKs) that participates in the development of oligodendrocytes (Matrone et al., 2020). Fyn facilitates the activation of semaphorin receptors (plexin), which is important in neuronal migration, synapse formation, and axonal pruning (Matrone et al., 2020). A study by Ohnuma *et al.* revealed that the levels of Fyn mRNA and protein were elevated in patients with schizophrenia, suggesting a potential role for Fyn in neurodevelopmental diseases (Ohnuma et al., 2003). Synapse pruning can include axonal loss or shortening (Vilalta & Brown, 2018). Therefore, Fyn may contribute to synapse and axonal pruning.

ItgB1 (integrin Beta-1), PlxnC1 (plexin C1), Adgrb3 (Brain-specific Angiogenesis Inhibitor 3), and C1ql1 (Complement C1q-like 1) are regulators of synaptic pruning. They play crucial roles in the maintenance and stabilization of synapses (Kakegawa et al., 2015; Kano & Watanabe, 2019). Several significant molecules are involved in the pruning or strengthening of synaptic connections. C1ql1 and Sema7A (Semaphorin 7A) are among these key molecules, specifically regulating synapses on Purkinje cells (PCs) and climbing fibers (CFs) in the cerebellum (Scuderi et al., 2019; Uesaka et al., 2014).

Adgrb3, also known as Bai3, regulates synapse formation and connectivity in mice and interacts with C1ql1 to maintain essential synapses (Kakegawa et al., 2015). Sema7A, a membrane-anchored semaphorin, signals on climbing fibers through its receptors, PlxnC1 and ItgB1. Synapses with Sema7A interactions involving PlxnC1 and ItgB1 are stabilized, while those

lacking these interactions are targeted for elimination through synaptic pruning (Kano & Watanabe, 2019).

These findings suggest that OPCs contribute actively to synaptic pruning alongside microglia and astrocytes, possibly employing distinct but complementary mechanisms. Genes such as *C1ql1*, *Adgrb3*, *Plxnc1*, and *Itgb1* likely play a significant role in the regulation of synaptic pruning, while *Fyn* may facilitate both synaptic and axonal pruning. Additionally, *Snx3* appears to support the pruning process through endosomal trafficking functions.

Age-Related Expression Changes

Our study did not find any significant age-related changes in the expression of the candidate synaptic pruning genes. This finding may imply that OPCs maintain a consistent role in synaptic pruning across different ages or that age-related changes may occur through mechanisms other than transcriptional regulation.

OPCs in Synaptic Assembly

Our investigation into synaptic assembly gene expression in OPCs revealed a generalized and uniform expression pattern across the cell population, as indicated by the UMAP clustering analysis. This finding confirms the multi-functionality of OPCs, suggesting that they are not specialized for a single function but contribute to synapse assembly.

Therapeutic Implications

The genes identified in this study may serve as therapeutic targets to modulate synaptic pruning activities in neurodegenerative and neurodevelopmental disorders. Therefore, targeting these genes could help in regulating synaptic pruning through gene therapy techniques, such as viral vectors (AAV or lentiviral systems). This technique can be used to upregulate or downregulate the expression of candidate genes to restore a balance between synaptic pruning and assembly.

Furthermore, antibody-based therapies could be designed to inhibit or activate receptors associated with synaptic pruning, thereby modulating the process. For instance, antibodies that inhibit or activate Adgrb3 (Bai3) receptor may regulate OPC-mediated synaptic pruning. Also, exposure to light and sound stimuli may influence the synaptic pruning process through modulation of glial cells.

Limitations and Future Directions

One limitation of this study is the reliance on a single scRNA-seq dataset, which might not capture all the gene expression variability present across different biological contexts. Future studies should focus on the datasets from different species and developmental stages to ensure the results are broadly applicable. Complementary approaches such as proteomics can increase the validity of the results.

Moreover, while our computational analysis suggests the involvement of certain genes, functional validation through in vivo experiments is necessary to confirm their roles in synaptic

pruning. Genetic knockout or knockdown techniques and optogenetic tools can be beneficial in the validation of the candidate genes and ensuring their biological relevance.

Conclusion

In conclusion, our findings highlight the involvement of OPCs in synaptic pruning activities. The proposed candidates for further exploration are *Tyrobp*, *Rgs10*, *C1ql1*, *Adgrb3*, *Plxnc1*, *Itgb1*, and *Fyn*. These genes may be novel therapeutic targets for regulating synaptic pruning in neurodegenerative disorders.

Moreover, OPCs are important for synaptic assembly in neural networks. The minimal age-related transcriptional changes observed for synaptic pruning genes highlight the need to explore non-transcriptional regulatory mechanisms underlying the pruning process.

Future work should focus on expanding in vitro analyses using additional datasets, followed by in vivo validation, to confirm the roles of these genes in OPC-mediated synaptic pruning and their broader implications in neurodegenerative diseases.

Appendix

Gene ID	Symbol	Name
64005	MYO1G	myosin IG
4615	MYD88	MYD88 innate immune signal transduction adaptor
375298	CERKL	ceramide kinase like
11277	TREX1	three prime repair exonuclease 1
3600	IL15	interleukin 15
5648	MASP1	MBL associated serine protease 1
3601	IL15RA	interleukin 15 receptor subunit alpha
19	ABCA1	ATP binding cassette subfamily A member 1
4627	MYH9	myosin heavy chain 9
8724	SNX3	sorting nexin 3
535	ATP6V0A1	ATPase H ⁺ transporting V0 subunit a1
25	ABL1	ABL proto-oncogene 1, non-receptor tyrosine kinase
5657	PRTN3	proteinase 3
27	ABL2	ABL proto-oncogene 2, non-receptor tyrosine kinase
1053	CEBPE	CCAAT enhancer binding protein epsilon
29	ABR	ABR activator of RhoGEF and GTPase
389668	XKR9	XK related 9
4647	MYO7A	myosin VIIA
27180	SIGLEC9	sialic acid binding Ig like lectin 9
27181	SIGLEC8	sialic acid binding Ig like lectin 8
558	AXL	AXL receptor tyrosine kinase
9777	TM9SF4	transmembrane 9 superfamily member 4
11314	CD300A	CD300a molecule
56882	CDC42SE1	CDC42 small effector 1
23603	CORO1C	coronin 1C
566	AZU1	azurocidin 1
5175	PECAM1	platelet and endothelial cell adhesion molecule 1
4153	MBL2	mannose binding lectin 2
6714	SRC	SRC proto-oncogene, non-receptor tyrosine kinase
2621	GAS6	growth arrest specific 6
575	ADGRB1	adhesion G protein-coupled receptor B1
2624	GATA2	GATA binding protein 2

23616	SH3BP1	SH3 domain binding protein 1
1089	CEACAM4	CEA cell adhesion molecule 4
64581	CLEC7A	C-type lectin domain containing 7A
11334	TUSC2	tumor suppressor 2, mitochondrial calcium regulator
3146	HMGB1	high mobility group box 1
201294	UNC13D	unc-13 homolog D
5199	CFP	complement factor properdin
4688	NCF2	neutrophil cytosolic factor 2
4689	NCF4	neutrophil cytosolic factor 4
284759	SIRPB2	signal regulatory protein beta 2
10325	RRAGB	Ras related GTP binding B
10326	SIRPB1	signal regulatory protein beta 1
140885	SIRPA	signal regulatory protein alpha
3673	ITGA2	integrin subunit alpha 2
10332	CLEC4M	C-type lectin domain family 4 member M
151647	TAFA4	TAFA chemokine like family member 4
5728	PTEN	phosphatase and tensin homolog
3683	ITGAL	integrin subunit alpha L
8291	DYSF	dysferlin
114786	XKR4	XK related 4
3684	ITGAM	integrin subunit alpha M
613	BCR	BCR activator of RhoGEF and GTPase
3685	ITGAV	integrin subunit alpha V
353376	TICAM2	TIR domain containing adaptor molecule 2
2150	F2RL1	F2R like trypsin receptor 1
3688	ITGB1	integrin subunit beta 1
3689	ITGB2	integrin subunit beta 2
1130	LYST	lysosomal trafficking regulator
3690	ITGB3	integrin subunit beta 3
100302736	TMED7-TICAM2	TMED7-TICAM2 readthrough
7275	TUB	TUB bipartite transcription factor
10347	ABCA7	ATP binding cassette subfamily A member 7
5747	PTK2	protein tyrosine kinase 2
30835	CD209	CD209 molecule
9844	ELMO1	engulfment and cell motility 1
26228	STAP1	signal transducing adaptor family member 1

629	CFB	complement factor B
7287	TULP1	TUB like protein 1
55423	SIRPG	signal regulatory protein gamma
64127	NOD2	nucleotide binding oligomerization domain containing 2
7301	TYRO3	TYRO3 protein tyrosine kinase
729230	CCR2	C-C motif chemokine receptor 2
134	ADORA1	adenosine A1 receptor
135	ADORA2A	adenosine A2a receptor
128646	SIRPD	signal regulatory protein delta
4233	MET	MET proto-oncogene, receptor tyrosine kinase
7305	TYROBP	transmembrane immune signaling adaptor TYROBP
26762	HAVCR1	hepatitis A virus cellular receptor 1
81035	COLEC12	collectin subfamily member 12
78989	COLEC11	collectin subfamily member 11
4240	MFGE8	milk fat globule EGF and factor V/VIII domain containing
23184	MESD	mesoderm development LRP chaperone
343702	XKR7	XK related 7
9364	RAB28	RAB28, member RAS oncogene family
285852	TREML4	triggering receptor expressed on myeloid cells like 4
9370	ADIPOQ	adiponectin, C1Q and collagen domain containing
5788	PTPRC	protein tyrosine phosphatase receptor type C
729238	SFTPA2	surfactant protein A2
1182	CLCN3	chloride voltage-gated channel 3
56990	CDC42SE2	CDC42 small effector 2
2207	FCER1G	Fc epsilon receptor Ig
10399	RACK1	receptor for activated C kinase 1
2209	FCGR1A	Fc gamma receptor Ia
2210	FCGR1BP	Fc gamma receptor Ib, pseudogene
5795	PTPRJ	protein tyrosine phosphatase receptor type J
26276	VPS33B	VPS33B late endosome and lysosome associated
2212	FCGR2A	Fc gamma receptor IIa
2213	FCGR2B	Fc gamma receptor IIb
23208	SYT11	synaptotagmin 11
9897	WASHC5	WASH complex subunit 5
5290	PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

23210	JMJD6	jumonji domain containing 6, arginine demethylase and lysine hydroxylase
2219	FCN1	ficolin 1
2220	FCN2	ficolin 2
8877	SPHK1	sphingosine kinase 1
5806	PTX3	pentraxin 3
4784	NFIX	nuclear factor I X
1201	CLN3	CLN3 lysosomal/endosomal transmembrane protein, battenin
10417	SPON2	spondin 2
177	AGER	advanced glycosylation end-product specific receptor
695	BTK	Bruton tyrosine kinase
6845	VAMP7	vesicle associated membrane protein 7
6850	SYK	spleen associated tyrosine kinase
84162	BLTP1	bridge-like lipid transfer protein family member 1
197	AHSG	alpha 2-HS glycoprotein
199	AIF1	allograft inflammatory factor 1
7879	RAB7A	RAB7A, member RAS oncogene family
5322	PLA2G5	phospholipase A2 group V
8394	PIP5K1A	phosphatidylinositol-4-phosphate 5-kinase type 1 alpha
6347	CCL2	C-C motif chemokine ligand 2
653509	SFTPA1	surfactant protein A1
717	C2	complement C2
718	C3	complement C3
8398	PLA2G6	phospholipase A2 group VI
720	C4A	complement C4A (Chido/Rodgers blood group)
9936	CD302	CD302 molecule
10960	LMAN2	lectin, mannose binding 2
721	C4B	complement C4B (Chido/Rodgers blood group)
722	C4BPA	complement component 4 binding protein alpha
9938	ARHGAP25	Rho GTPase activating protein 25
10451	VAV3	vav guanine nucleotide exchange factor 3
51411	BIN2	bridging integrator 2
1236	CCR7	C-C motif chemokine receptor 7
725	C4BPB	complement component 4 binding protein beta
8406	SRPX	sushi repeat containing protein X-linked
57047	PLSCR2	phospholipid scramblase 2

5336	PLCG2	phospholipase C gamma 2
5338	PLD2	phospholipase D2
2268	FGR	FGR proto-oncogene, Src family tyrosine kinase
10461	MERTK	MER proto-oncogene, tyrosine kinase
90342	FER1L5	fer-1 like family member 5
55529	PIP4P2	phosphatidylinositol-4,5-bisphosphate 4-phosphatase 2
5868	RAB5A	RAB5A, member RAS oncogene family
5359	PLSCR1	phospholipid scramblase 1
84206	MEX3B	mex-3 RNA binding family member B
3312	HSPA8	heat shock protein family A (Hsp70) member 8
1265	CNN2	calponin 2
5873	RAB27A	RAB27A, member RAS oncogene family
7409	VAV1	vav guanine nucleotide exchange factor 1
7410	VAV2	vav guanine nucleotide exchange factor 2
246	ALOX15	arachidonate 15-lipoxygenase
5879	RAC1	Rac family small GTPase 1
1785	DNM2	dynammin 2
5881	RAC3	Rac family small GTPase 3
122618	PLD4	phospholipase D family member 4
375033	PEAR1	platelet endothelial aggregation receptor 1
51454	GULP1	GULP PTB domain containing engulfment adaptor 1
1793	DOCK1	dedicator of cytokinesis 1
1794	DOCK2	dedicator of cytokinesis 2
9474	ATG5	autophagy related 5
89858	SIGLEC12	sialic acid binding Ig like lectin 12
6405	SEMA3F	semaphorin 3F
5906	RAP1A	RAP1A, member of RAS oncogene family
25875	LETMD1	LETM1 domain containing 1
5909	RAP1GAP	RAP1 GTPase activating protein
11031	RAB31	RAB31, member RAS oncogene family
5914	RARA	retinoic acid receptor alpha
57118	CAMK1D	calcium/calmodulin dependent protein kinase ID
91937	TIMD4	T cell immunoglobulin and mucin domain containing 4
146722	CD300LF	CD300 molecule like family member f
10533	ATG7	autophagy related 7
6441	SFTPD	surfactant protein D

811	CALR	calreticulin
301	ANXA1	annexin A1
306	ANXA3	annexin A3
2358	FPR2	formyl peptide receptor 2
311	ANXA11	annexin A11
2359	FPR3	formyl peptide receptor 3
25912	C1orf43	chromosome 1 open reading frame 43
3385	ICAM3	intercellular adhesion molecule 3
22841	RAB11FIP2	RAB11 family interacting protein 2
25913	POT1	protection of telomeres 1
84286	TMEM175	transmembrane protein 175
8512	MBL1P	mannose binding lectin 1, pseudogene
399687	MYO18A	myosin XVIIIa
3394	IRF8	interferon regulatory factor 8
10562	OLFM4	olfactomedin 4
55113	XKR8	XK related 8
2889	RAPGEF1	Rap guanine nucleotide exchange factor 1
10062	NR1H3	nuclear receptor subfamily 1 group H member 3
335	APOA1	apolipoprotein A1
336	APOA2	apolipoprotein A2
80208	SPG11	SPG11 vesicle trafficking associated, spatacsin
10584	COLEC10	collectin subfamily member 10
3929	LBP	lipopolysaccharide binding protein
286046	XKR6	XK related 6
5468	PPARG	peroxisome proliferator activated receptor gamma
55646	LYAR	Ly1 antibody reactive
55647	RAB20	RAB20, member RAS oncogene family
51552	RAB14	RAB14, member RAS oncogene family
8547	FCN3	ficolin 3
23396	PIP5K1C	phosphatidylinositol-4-phosphate 5-kinase type 1 gamma
7525	YES1	YES proto-oncogene 1, Src family tyrosine kinase
6504	SLAMF1	signaling lymphocytic activation molecule family member 1
7529	YWHAB	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta
9066	SYT7	synaptotagmin 7
3949	LDLR	low density lipoprotein receptor

3952	LEP	leptin
3953	LEPR	leptin receptor
2934	GSN	gelsolin
1401	CRP	C-reactive protein
7040	TGFB1	transforming growth factor beta 1
4481	MSR1	macrophage scavenger receptor 1
3458	IFNG	interferon gamma
1411	CRYBA1	crystallin beta A1
200576	PIKFYVE	phosphoinositide kinase, FYVE-type zinc finger containing
4486	MST1R	macrophage stimulating 1 receptor
22918	CD93	CD93 molecule
7052	TGM2	transglutaminase 2
11151	CORO1A	coronin 1A
3984	LIMK1	LIM domain kinase 1
7057	THBS1	thrombospondin 1
63894	VIPAS39	VPS33B interacting protein, apical-basolateral polarity regulator, spe-39 homolog
79767	ELMO3	engulfment and cell motility 3
6556	SLC11A1	solute carrier family 11 member 1
27036	SIGLEC7	sialic acid binding Ig like lectin 7
55198	APPL2	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2
83871	RAB34	RAB34, member RAS oncogene family
929	CD14	CD14 molecule
5027	P2RX7	purinergic receptor P2X 7
1445	CSK	C-terminal Src kinase
64422	ATG3	autophagy related 3
5031	P2RY6	pyrimidinergic receptor P2Y6
55207	ARL8B	ADP ribosylation factor like GTPase 8B
406959	MIR183	microRNA 183
63916	ELMO2	engulfment and cell motility 2
3500	IGHG1	immunoglobulin heavy constant gamma 1 (G1m marker)
196527	ANO6	anoctamin 6
406955	MIR181B1	microRNA 181b-1
3501	IGHG2	immunoglobulin heavy constant gamma 2 (G2m marker)
406952	MIR17	microRNA 17
3502	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)

10670	RRAGA	Ras related GTP binding A
7087	ICAM5	intercellular adhesion molecule 5
3503	IGHG4	immunoglobulin heavy constant gamma 4 (G4m marker)
948	CD36	CD36 molecule (CD36 blood group)
29108	PYCARD	PYD and CARD domain containing
949	SCARB1	scavenger receptor class B member 1
94134	ARHGAP12	Rho GTPase activating protein 12
7097	TLR2	toll like receptor 2
7099	TLR4	toll like receptor 4
406982	MIR20A	microRNA 20a
961	CD47	CD47 molecule
54209	TREM2	triggering receptor expressed on myeloid cells 2
5058	PAK1	p21 (RAC1) activated kinase 1
4035	LRP1	LDL receptor related protein 1
1991	ELANE	elastase, neutrophil expressed
338382	RAB7B	RAB7B, member RAS oncogene family
5580	PRKCD	protein kinase C delta
26060	APPL1	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1
5581	PRKCE	protein kinase C epsilon
5582	PRKCG	protein kinase C gamma
54734	RAB39A	RAB39A, member RAS oncogene family
196051	PLPP4	phospholipid phosphatase 4
7124	TNF	tumor necrosis factor
6614	SIGLEC1	sialic acid binding Ig like lectin 1
27102	EIF2AK1	eukaryotic translation initiation factor 2 alpha kinase 1
3553	IL1B	interleukin 1 beta
4067	LYN	LYN proto-oncogene, Src family tyrosine kinase
998	CDC42	cell division cycle 42
2534	FYN	FYN proto-oncogene, Src family tyrosine kinase
8678	BECN1	beclin 1
3560	IL2RB	interleukin 2 receptor subunit beta
3561	IL2RG	interleukin 2 receptor subunit gamma
8685	MARCO	macrophage receptor with collagenous structure
3055	HCK	HCK proto-oncogene, Src family tyrosine kinase
9711	RUBCN	rubicon autophagy regulator

389610	XKR5	XK related 5
124912	SPACA3	sperm acrosome associated 3
84466	MEGF10	multiple EGF like domains 10
6647	SOD1	superoxide dismutase 1
10747	MASP2	MBL associated serine protease 2
5627	PROS1	protein S
1535	CYBA	cytochrome b-245 alpha chain
3071	NCKAP1L	NCK associated protein 1 like

Table A1: The 280 genes involved in phagocytosis were identified using GO: 0006909. These genes were further analyzed to explore their potential involvement in synaptic pruning in oligodendrocyte precursor cells.



Network.pdf

	from1	from2	to	distance
0	Ifi203	S1pr4	13928	0
1	Kcnmb1	13928	13929	0
2	Ppic	13929	13930	0
3	Mall	Thbd	13931	0
4	Il2rg	13931	13932	0
5	Foxf1	13932	13933	0
6	Apol10b	13933	13934	0
7	Sash3	Tal1	13935	0
8	Cxcl10	13935	13936	0

Figure A1: Hierarchical Clustering Network of Genes illustrates the interaction of candidate synaptic pruning genes within oligodendrocyte precursor cells. This network includes key reference genes, such as C1qa, C1qb, C1qc, and Trem2, known for their roles in microglial synaptic pruning. The analysis highlights candidate genes, including Tyrobp and RGS10, and evaluates their expression similarity to the referenced genes to determine their potential involvement in synaptic regulation. Cluster numbers and their genes can be achieved through double-clicking on the excel worksheet beside the Network.

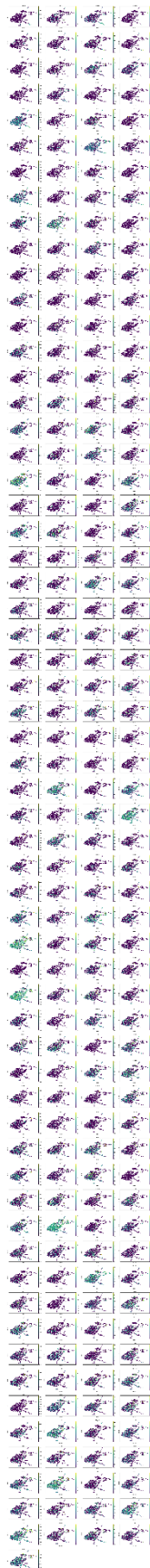


Figure A2: UMAP visualization of each synaptic pruning gene across the OPCs indicating a generalized expression pattern. Similar to the synaptic assembly genes, the distribution indicates a generalized expression pattern, reinforcing the idea that OPCs may not have distinct subtypes specifically dedicated to synaptic pruning.

Gene	MG_lfc	OPC_lfc	ASC_lfc	CPC_lfc	OPC_pvals_adj
ADORA1	-4.60	1.80	-0.03	-2.63	0
CD302	2.07	1.88	1.34	-1.12	0
ITGAV	-0.44	1.73	0.64	0.11	0
SYT11	-5.08	1.28	-0.81	-4.05	0
RAP1GAP	-3.28	3.27	0.14	-3.53	0
TUB	-3.55	2.61	-2.81	-0.42	0
PLPP4	-5.92	5.80	0.20	-25.86	0
FYN	-3.21	2.21	-0.17	-2.60	0
LRP1	0.77	3.06	1.57	-0.08	0
CALR	1.11	0.99	-0.42	0.51	0
RAB34	-1.88	1.43	2.81	0.39	0
C1QL1	-4.41	9.12	-3.98	-4.74	0
RAC3	0.91	2.51	-0.49	-2.00	0
PTPRG	-4.72	1.34	-0.03	-1.80	6.33373E-31
SNX3	-1.12	0.58	-0.42	-0.08	1.36764E-30
P2RX7	1.85	1.57	-0.81	-4.55	8.56208E-28
SRC	-1.25	1.31	1.53	-1.33	1.40267E-23
CLCN3	-1.80	0.69	0.11	-0.57	2.18114E-23
RAB11FIP2	-1.60	0.75	-0.04	-1.56	1.07893E-19
ITGB1	0.56	0.77	-1.44	0.13	6.4343E-19
ABCA1	0.95	0.84	1.67	-0.17	3.15447E-18
CDC42SE1	0.59	0.80	-0.08	-0.66	3.9715E-18
RAB31	-1.00	0.70	-0.40	-2.24	7.96781E-17
MEX3B	-0.15	1.90	-2.32	-1.05	2.46552E-16
TM9SF4	-0.42	0.93	0.34	-1.12	2.53793E-16
PTPRJ	1.32	1.06	-1.39	-1.66	5.2674E-14
ADGRB1	-3.90	0.63	1.36	-3.04	3.23804E-13
SH3BP1	0.47	0.65	-0.79	-2.18	6.60271E-13
CNN2	-0.60	0.94	-3.47	-4.40	3.79791E-10
PIK3CA	-0.66	0.65	-0.24	0.27	1.0524E-09
ATG5	1.31	0.67	-0.29	0.28	3.09945E-09
APPL1	-1.53	0.56	-1.27	0.03	1.61234E-08
ARHGAP12	0.77	0.66	0.88	0.66	4.42702E-07
JMJD6	-0.16	0.53	-0.08	-0.84	1.73252E-06

PIP5K1A	-0.53	0.54	-0.25	0.48	1.71067E-05
TMEM175	0.09	0.55	0.86	-1.75	2.20269E-05
VAV3	-4.11	0.87	3.11	-3.03	0.000156614
YES1	-0.36	0.56	0.22	-0.96	0.000160947
VIPAS39	-0.38	0.55	-0.24	-0.14	0.000231735
PLA2G6	-1.64	0.77	0.90	0.23	0.000242522
VAV2	2.34	0.97	-0.53	-1.22	0.000297235
SRPX	-22.52	2.03	-2.07	-22.36	0.000328525
RAPGEF1	0.02	0.51	-0.49	-1.51	0.000599929
PLXNC1	-3.30	0.85	0.75	-24.77	0.000689982
XKR8	0.76	0.86	0.50	-2.44	0.002771878
RAB27A	2.99	0.91	-3.23	-0.66	0.010143607
PLA2G5	-22.39	1.43	1.14	6.19	0.01678659

Table A2: Differential expression analysis for synaptic pruning and phagocytic genes across OPCs, MG, ASC, and CPC as negative control. The table includes log fold changes (LFC) for each gene in the respective cell types and adjusted p-values (OPC_pvals_adj) indicating statistical significance. Orange colored cells are key genes which show significant expression differences across multiple cell types, suggesting their role in pruning and phagocytic activity.

Gene	MG_lfc	OPC_lfc	ASC_lfc	CPC_lfc	OPC_pvals_adj
DNER	-3.97	2.28	1.43	-2.68	0
C1QL2	-7.26	6.89	-4.92	-1.67	0
C1QL3	-4.03	3.63	-4.24	-25.30	0
SLITRK2	-5.63	1.96	3.37	-26.91	0
NLGN3	-4.27	3.38	0.80	-1.83	0
NLGN1	-4.75	2.65	0.70	-2.52	0
SLITRK3	-4.85	3.38	-4.89	-25.02	0
ADGRL3	-4.63	2.23	1.35	-3.87	0
LRRC4	-3.57	2.52	-0.26	-26.02	0
GRID2	-5.83	2.48	1.22	-2.40	0
LRRTM1	-5.31	4.04	-3.11	-25.01	0
LRRTM4	-4.19	3.15	-3.47	-2.40	0
CHL1	-3.75	3.18	0.22	-3.50	0
LRRN1	-6.52	2.39	-1.48	-4.12	0
LRRC4B	-5.21	1.35	0.38	-5.13	0
NTRK3	-5.82	2.35	0.07	-3.43	0
LRRTM3	-7.52	3.81	-3.44	-4.51	0
ZDHHC2	-4.16	3.67	-2.88	-3.51	0
GPM6A	-3.76	1.75	3.23	0.74	0
CDH13	-5.08	4.08	-3.18	-25.99	0
PCDH17	-4.98	1.91	0.62	-5.90	0
SLITRK1	-4.16	2.94	-2.51	-3.64	0
SLITRK5	-4.98	2.49	-0.97	-2.01	0
NTN1	-7.61	3.79	-1.27	-0.58	0

NLGN2	-3.36	2.90	-0.86	-0.65	0
NTRK2	-3.78	1.32	4.05	0.35	0
GAP43	-1.55	2.24	-1.44	-4.77	0
DSCAM	-4.13	4.27	-5.32	-26.07	0
PTPRS	-2.34	2.33	0.29	-2.29	0
NRXN1	-4.47	2.49	2.45	-3.55	0
LRRTM2	-4.12	2.57	-0.57	0.02	0
PCDHB9	-7.27	4.69	-1.39	-25.23	0
NRXN2	-4.61	2.81	0.72	-1.72	0
ACHE	-1.23	2.48	-2.83	-25.81	1E-45
DNM3	-4.94	0.98	-3.20	-5.27	2.84E-43
FLRT1	-4.88	2.34	1.79	-3.76	6.82415E-39
CACNA1A	-1.61	1.83	-1.10	-2.94	1.29526E-38
GPC6	-6.28	1.72	2.40	-0.70	3.34287E-38
FLRT3	-4.55	2.25	-2.47	0.60	2.1965E-33
SETD5	-0.25	1.08	-0.22	0.56	5.91702E-32
PRKCA	-0.60	0.83	-1.17	-3.20	2.19436E-31
CLSTN1	-1.66	0.82	-0.50	-0.57	4.38022E-30
CADM1	-0.09	0.64	1.86	-3.99	4.00723E-29
NRXN3	-4.49	0.93	-2.65	-5.66	5.97517E-29
LRRN3	-5.31	1.49	-1.69	-3.66	1.79965E-25
LRFN5	-5.24	1.52	-5.27	-3.57	2.37421E-25
MDGA2	-4.97	1.20	0.98	-27.12	4.34458E-25
IL1RAPL1	-4.54	1.49	-0.39	-26.29	3.13404E-22
LRP4	-1.94	1.20	0.90	-27.04	8.12252E-22
CUX2	-1.51	1.93	-1.02	1.78	1.11406E-21
ASIC2	-3.44	1.70	-0.13	-0.21	1.01223E-19
NRG1	-3.73	2.77	-1.08	-24.14	1.7966E-19
ROBO1	-5.42	2.04	0.77	-24.99	2.54217E-19
LRFN1	-1.62	1.91	-0.28	-1.84	6.23613E-18
WNT7A	-4.49	1.43	3.72	-26.01	6.90137E-18
ERBB4	-4.76	1.45	-0.94	0.71	1.56595E-17
GSK3B	-0.95	0.53	-0.28	-0.40	6.27692E-17
STAU2	-0.71	1.21	-0.93	-0.30	1.94472E-16
NRCAM	-4.73	0.84	2.02	-2.10	5.31831E-16
VSTM5	-4.42	3.52	-5.50	-22.91	7.10133E-15
ADGRL1	-3.87	0.68	1.09	-1.32	1.47824E-14
UBE2V2	-0.95	0.53	0.03	0.67	1.72648E-14
PCDHB3	-23.77	2.74	-0.90	-23.62	6.12838E-14
SLITRK6	-5.10	4.74	-5.39	-22.00	1.70847E-13
ADGRB1	-3.90	0.63	1.36	-3.04	3.23804E-13
ADGRB2	-4.13	0.75	0.95	-2.84	1.67452E-12
LRFN3	-2.50	1.75	0.37	-24.70	3.92002E-12

GABRB3	-4.76	0.86	-2.72	-27.01	4.06593E-12
CLSTN3	-4.02	0.92	-3.49	-1.21	1.66183E-11
LRTM2	-4.52	0.90	1.74	-3.39	6.02916E-09
SLITRK4	-5.28	0.98	-4.85	-3.96	9.24576E-09
PPP1R9A	-0.43	0.53	-0.21	0.21	1.53956E-08
RAP2A	0.20	0.69	-1.87	-2.35	2.03969E-08
SDK1	1.23	2.10	-4.40	-23.57	2.21071E-08
NEGR1	-4.23	0.57	0.32	-1.96	2.62673E-08
EPHB3	-2.35	1.57	2.99	-1.63	3.66981E-08
PCDHB10	-3.27	2.20	1.13	-23.05	8.12789E-07
PDLIM5	-0.90	0.59	1.44	1.19	1.10748E-06
SIX4	-3.34	2.77	-2.16	3.69	4.43423E-06
EPHB2	-4.50	1.58	-2.55	-23.83	5.69834E-06
CBLN1	-1.97	1.91	-4.59	-23.21	9.86293E-06
LHFPL4	-2.97	0.60	-1.33	-3.76	3.50141E-05
LRFN4	-5.04	0.80	0.99	-1.49	6.5641E-05
TNC	-5.14	1.39	1.84	-23.33	0.001112008
PCDHB14	-24.95	0.83	1.36	-24.79	0.001270431
NUMBL	-2.09	0.57	-0.76	-1.08	0.00179114
FLRT2	-4.92	0.56	0.66	2.48	0.002095519
VWA5B2	-4.65	0.86	-3.73	-24.22	0.005836493
SYNDIG1	-4.67	0.86	-0.36	-24.01	0.00723763
LGI2	-4.11	0.80	-3.89	-24.17	0.009140064
PCDHB5	-4.42	1.09	-0.78	-23.03	0.020168077

Table A3: Differential expression analysis for synaptic assembly genes in across OPC, MG, ASC, and CPC as negative control. The table includes log fold changes (LFC) for each gene in the respective cell types and adjusted p-values (OPC_pvals_adj) indicating statistical significance.

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