Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

An Integrated Single-cell Multi-Omics Atlas of the Human Optic Nerve

Rui Chen

ruic20@hs.uci.edu

University of California Irvine School of Medicine

Jun Wang

Baylor College of Medicine

Ye Zheng

Gavin Herbert Eye Institute - Center for Translational Vision Research, Department of Ophthalmology, University of California Irvine School of Medicine

Jin Li

University of California Irvine School of Medicine

Xuan Bao

University of California Irvine

Ismail Yaman

Baylor College of Medicine https://orcid.org/0000-0002-0534-2996

Tingting Yang

Baylor College of Medicine

Gerda Cristal Villalba Silva

Baylor College of Medicine

Jianming Shao

Baylor College of Medicine

Jinjing Jian

Baylor College of Medicine

Jean Li

Gavin Herbert Eye Institute - Center for Translational Vision Research, Department of Ophthalmology, University of California Irvine School of Medicine

Lei Qi

University of California Irvine

Leah A. Owen

Cincinnati Children's Hospital

Ivana K. Kim

Harvard Medical School

Emil Kriukov

University of Pittsburgh

Andrea Rivera
Wellcome sanger institute

Melissa L. Cooper
NYU Grossman School of Medicine

Susannah Waxman
Université de Montréal

Gulab Zode
University of California Irvine School of Medicine

David Gutmann
Washington University in St. Louis https://orcid.org/0000-0002-3127-5045

Carl Sheridan
University of Liverpool

Dong Feng Chen
Harvard Medical School

Zhigang He
Harvard University https://orcid.org/0000-0001-6080-6880

Xian-Jie Yang
University of California, Davide Geffen School of Medicine, Los Angeles

David Osumi-Sutherland
Wellcome sanger institute

Ajith Pankajam
NIH https://orcid.org/0000-0002-4457-9523

Richard Scheuermann
National Library of Medicine, National Institutes of Health

Ayellet V. Segrè
Harvard Medical School

Pei Tan
University of California Irvine School of Medicine

Treefa Shwani
State University of New York, University at Buffalo

Han Chen
New York University

Petr Baranov
University of Pittsburgh

Margaret DeAngelis
State University of New York at Buffalo

John Timothy Stout
Baylor College of Medicine

Yumei Li
University of California, Irvine

Article

Keywords:

Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11587599/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: There is **NO** Competing Interest.

An Integrated Single-cell Multi-Omics Atlas of the Human Optic Nerve

Jun Wang[1*], Ye Zheng[2*], Jin Li[2*], Xuan Bao[2], Ismail Yaman[1], Tingting Yang[1], Gerda Cristal Villalba Silva[1], Jianming Shao[1,3], Jinjing Jian[1], Jean Li[2], Lei Qi[2], Leah A. Owen[4,5,6], Ivana K Kim[7,8], Emil Kriukov[7,8,9], Andrea Rivera[10], Melissa L. Cooper[11], Susannah Waxman[12], Gulab Zode[2], David H. Gutmann[13], Carl Sheridan[14], Dong Feng Chen[7,8], Zhigang He[8,15], Xian-Jie Yang[16], David Osumi-Sutherland[10], Ajith V. Pankajam[17], Richard H. Scheuermann[17], Ayellet V. Segrè[8], Pei Tan[18], Treefa Shwani[5], Han Chen[19], Petr Baranov[7,8,9], Margaret M. DeAngelis[4,5,20], John Timothy Stout[3], Yumei Li[2#], Rui Chen[2#]

1.Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, United States.
2.Brunson Center for Translational Vision Research, Gavin Herbert Eye Institute, Department of Ophthalmology and Visual Sciences, University of California Irvine School of Medicine, Irvine, California, United States.
3. Department of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, Texas, United States.
4. Department of Ophthalmology and Visual Sciences, Moran Eye Center; Department of Population Health Sciences, University of Utah School of Medicine, The University of Utah, Salt Lake City, UT USA
5. Department of Ophthalmology, Ross Eye Institute, Jacobs School of Medicine and Biomedical Sciences, State University of New York, University at Buffalo, NY, USA
6. Division of Ophthalmology, Department of Surgery, Cincinnati Children's Hospital, Cincinnati OH, USA
7. Schepens Eye Research Institute of Massachusetts Eye and Ear, Harvard University, Boston, MA, United States.
8. Department of Ophthalmology, Harvard Medical School, Boston, MA, United States.
9. Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA, United States
10. Wellcome sanger institute, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SA. United Kingdom.
11. Institute for Translational Neuroscience, NYU Grossman School of Medicine, New York, NY, United States.
12. Department of Neuroscience, Université de Montréal, Montreal, QC, Canada
13. Department of Neurology, Washington University School of Medicine, St. Louis, MO, United States.
14. Department of Eye and Vision Science, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, United Kingdom.
15. F.M. Kirby Neurobiology Center, Boston Children's Hospital, Boston, MA, United States.
16. Stein Eye Institute, Department of Ophthalmology, University of California, Davide Geffen School of Medicine, Los Angeles, CA, United States.
17. Division of Intramural Research, National Library of Medicine, National Institutes of Health, Bethesda, Maryland, United States.
18. Department of Anatomy and Neurobiology, University of California Irvine School of Medicine, Irvine, California, United States.
19. Rory Meyers College of Nursing, New York University, New York, New York, United States.
20. Department of Biochemistry; Program in Neuroscience; Program in Genetics, Genomics and Bioinformatics; Jacobs School of Medicine and Biomedical Sciences, State University of New York, University at Buffalo, Buffalo, NY, USA
*. These authors contributed equally.

#Correspondence and requests for materials should be addressed to Yumei Li (yumeil3@hs.uci.edu) and Rui Chen (ruic20@hs.uci.edu). Rui Chen is the Lead Contact.

Abstract

The optic nerve is a critical central nervous system (CNS) tract that conveys visual information from the retina to multiple brain regions and is affected by neurodegenerative and neuroinflammatory disorders, making it an important model for studying CNS biology and disease. Here, we present the first spatially resolved multi-omics human optic nerve cell atlas (HONCA), integrating single-cell transcriptomic, chromatin accessibility, and spatial data from ~2.1 million cells profiled by snRNA-seq, snATAC-seq, and Xenium spatial transcriptomics from 108 donors, as part of the Human Cell Atlas. We identified 10 major cell classes comprising over 60 distinct cell populations and characterized their cis-regulatory elements, transcription factor regulons, and spatial organization, revealing region-specific spatial distributions and longitudinal gradients along the optic nerve. We reconstructed lineage trajectories of oligodendroglia lineage cells and astrocytes in human adults, identifying distinct myelinating oligodendrocyte and astrocyte populations with precursor-like transcriptional signature. Age-associated changes were pronounced in fibroblasts and glial cells, marked by reduced abundance of putative progenitor-like glia populations and microglia, diminished cell-cycle and biogenesis programs, and remodeling of metabolic, mechanosensory, neuro-supportive, and intercellular communication programs. Integration with genome-wide association studies yielded novel insights into the genetic and molecular basis of glaucoma and related neuro-ophthalmological disorders. Together, HONCA provides a high-resolution framework for human optic nerve biology and disease, establishing a foundation for developing therapeutic strategies in CNS disorders.

Introduction

As a specialized white matter tract of the central nervous system (CNS), the optic nerve consists of 1.0-1.2 million retinal ganglion cell (RGC) axons, together with supporting glial cell populations, connective tissue, and vascular components that coordinately maintain axonal support, myelination, immune surveillance, and tissue homeostasis[1,2]. Importantly, optic nerve axons convey visual information from the retina to multiple brain regions, including projections to the primary visual cortex for visual processing, the afferent pathway for pupillary and accommodation reflexes, and input to the suprachiasmatic nucleus for regulation of circadian rhythm[1,3,4]. The optic nerve is the only CNS tract that exits the cranial cavity, providing a clinical window into brain pathology and making it a critical model for studying CNS neurodegeneration, neuroinflammation, and axonal injury[1,5,6].

Surrounded by the meninges and cerebrospinal fluid, the optic nerve is structurally subdivided into fascicles by connective tissue and glial septa[1]. Along its longitudinal axis, it is organized into sequential segments, including the intraocular (optic nerve head, ONH), intraorbital, intracanalicular, and intracranial segments[4]. Damage to the optic nerve leads to a wide range of neurodegenerative and neuroinflammatory disorders, including glaucoma—a major cause of irreversible blindness in aging populations worldwide—as well as optic neuropathies, optic nerve cancers, and optic neuritis[7–10]. Glaucoma is marked by progressive loss of RGCs and degeneration of the optic nerve[8], thought to originate at the optic nerve head, where it may involve disrupted axoplasmic transport, ischemia, biomechanical stress, and glia cell remodeling[8]. Although intraocular pressure (IOP)-lowering treatments remain the only established intervention for glaucoma, elevated IOP is not always associated with glaucoma, and effective neuroprotective strategies are still lacking[8,11].

Despite its clinical and biological importance, a comprehensive characterization of the cellular composition and molecular architecture of the optic nerve in humans remains lacking. While recent advances in single-cell multi-omics and spatial transcriptomics have enabled high-resolution mapping of cellular diversity and spatial organization across tissues, existing studies of the optic nerve and ONH have been limited in scale, resolution, or modality, mostly relying on single data types, small cohorts, or model organisms[2,12–17]. Furthermore, aging is a major risk factor for optic nerve degeneration and impaired remyelination, but the underlying cellular and molecular mechanisms remain poorly understood[8,18,19].

To address these important knowledge gaps, we generated a comprehensive human optic nerve atlas (HONCA) by integrating approximately 0.9 million single cell transcriptomic (snRNA-seq), 0.9 million chromatin accessibility (snATAC-seq), and 0.3 million spatial transcriptomic (Xenium) profiles, as part of the Human Cell Atlas. These data were collected

from more than 108 donors spanning diverse ancestries and ages from birth to 90 years. We systematically characterized and significantly expanded cellular heterogeneity, inferred gene regulatory programs and cell lineage trajectories, mapped spatial cellular organization, and identified population-level and age-associated cellular and molecular variation in the optic nerve. Integration of this comprehensive cell atlas with genome-wide association studies (GWAS) data also identified cell types enriched for glaucoma-related traits, and fine-mapped risk loci to nominate candidate causal variants, genes and relevant cell types.

Taken together, HONCA offers an unprecedented, high-resolution view of cellular diversity, regulatory landscapes, and spatial organization across the optic nerve, revealing potential mechanisms underlying susceptibility to age-related diseases and the decline in regenerative capacity. This critical resource establishes a foundation for understanding CNS biology and disease and offers new insights into the cellular and molecular mechanisms underlying neurodegeneration, with important implications for developing therapeutic strategies.

Results

Single cell transcriptomic atlas of the human optic nerve

To construct a comprehensive cell atlas of the human optic nerve, we performed single-nucleus RNA sequencing (snRNA) on 114 optic nerve and ONH samples from human donor eyes (Fig. 1a. Supplementary Table 1,2). By integrating these newly generated, unpublished datasets (83.8% of cells) with a landmark public dataset[2] (16.2% of cells), we obtained transcriptomic profiles of 865,854 nuclei from 83 donors after quality control (Fig. 1a, Supplementary Table 1, 2). This cohort captured broad population diversity, comprising 41% European, 28.9% African, 24.1% Hispanic, and 6% Asian individuals, with 65.1% males and 34.9% female donors, spanning a wide range of ages, ranging from 0 to 90 years (Extended Data Fig. 1a-d, Supplementary Table 2).

Integrative analysis identified 10 major cell classes across the ONH and optic nerve, dominated by oligodendrocytes (37.9%) and astrocytes (28.4%), followed by fibroblasts (15.3%), microglia (6.5%), endothelial cells (4.6%), mural cells (2.3%), oligodendrocyte precursor cells (1.9%), macrophages (1.3%), melanocytes (1.1%), and immune cells (0.5%) (Fig. 1b,c, Extended Data Fig. 1e-j, Extended Data Fig. 2). Cell annotations were supported by class-specific canonical marker gene expression[2] (Fig. 1d), and annotations of cells from publicly available datasets were consistent with previously reported classifications (Supplementary Table 3). Several cell classes exhibited sex-associated and ancestry-associated differences in cell proportions with nominal significance (Extended Data Fig. 3a-d,

Multivariable linear regression, controlled for age, ancestry, and tissue region). Further refinement of each major cell class identified distinct cell types and states, including diverse populations of astrocytes, oligodendrocytes, fibroblasts, endothelial cells, mural cells, microglia, and macrophages, totaling 62 cell populations in the optic nerve and 35 cell populations in the retina and related tissues (Supplementary Table 4). Only cell types identified in the optic nerve were retained for further analyses.

To validate these cell classes and resolve their spatial organization, we generated spatial transcriptomic profiles of 289,155 cells (after QC) from seven ONH and optic nerve sections across two donors using the Xenium in situ platform (Supplementary Table 1,5). By co-embedding the spatial and snRNA-seq datasets, we annotated all major cell classes in situ (Fig. 1e,1f), with each class displaying specific expression of canonical marker genes (Extended Data Fig. 4a, Fig 1g-j). The spatial distribution of cell classes matched known anatomical organization (Fig. 1f,1j Extended Data Fig. 4b-h). Astrocytes, microglia, endothelial and mural cells span from the optic nerve proper (ONP) to the retina. In the ONH, astrocytes and fibroblasts predominated, followed by endothelial cells and mural cells, whereas oligodendrocytes and oligodendrocyte precursor cells were present only posterior to the ONH. Posterior to the ONH, oligodendrocytes, astrocytes, and fibroblasts were most abundant, followed by microglia, endothelial cells, and mural cells, and tissue-resident macrophages (Fig. 1f,1j). The overall distribution of cell class proportions in Xenium data was consistent with that in snRNA-seq data (Extended Data Fig. 4i).

Chromatin accessibility atlas of the human optic nerve

To investigate gene regulatory mechanisms underlying optic nerve cell types, we profiled 937,278 snATAC-seq nuclei from 103 optic nerve and ONH samples across 86 donors spanning diverse ancestries and a wide age range (Supplementary Table 6). Integration of these data with snRNA-seq datasets (Fig. 1b) revealed a high degree of concordance between chromatin accessibility and transcriptional profiles across ten distinct cell classes (Fig. 2a). Similar to the cell composition in the snRNA-seq data, the snATAC-seq data was predominantly composed of oligodendrocytes (336K nuclei) and astrocytes (303K nuclei), followed by other cell classes (Fig. 2b). These cell identities were validated by correlating marker gene activity scores with corresponding mRNA expression; for example, *GFAP* and *RNF220* exhibited highly specific accessibility and expression in astrocytes and oligodendrocytes, respectively (Fig. 2c). The observed epigenetic landscape was defined by thousands of cell-type-specific open chromatin regions (OCRs), which varied in number across cell classes, ranging from approximately 93,405 in immune cells to 197,592 in

astrocytes (Fig. 2d). Most OCRs were located in intronic genomic areas followed by distal elements, while promoter and exonic regions represented a smaller fraction of the regulatory landscape (Fig. 2d). By comparing chromatin accessibility across cell classes, we identified regulatory elements that exhibit differential accessibility between cell classes, likely driving lineage-defining gene expression (termed differentially accessible regions, DARs; Supplementary Table 7, Supplementary Fig. S1). By correlating OCR chromatin accessibility with gene expression, as well as promoter chromatin accessibility, we assigned 158,361 OCRs to genes and identified putative cis-regulatory elements (CREs) linked to gene expression (Fig. 2e, Supplementary Table 8). Finally, we characterized core transcriptional regulators potentially shaping cell type identity and ranked the cell type specificity of the identified transcription factor (TF) regulons across major cell classes (Fig. 2f, Supplementary Table 9). We identified 79 transcription factor (TF) regulons (Fig. 2g). These regulon TFs were classified as activators or repressors based on the enrichment patterns of TF gene expression and their associated regulatory genomic regions across cell classes (Fig. 2g). This analysis identified key lineage-specific factors, such as *SOX5* and *SOX6* for oligodendrocyte precursor cells[20,21], *NKX6-2*[22,23] and *SOX2*[24] for oligodendrocytes, *ERG*[25,26], *FLI1*[27,28] and *FOXO1*[29] for endothelial cells, *FOXC1*[30] and *KLF5*[31] for fibroblasts, *SOX9* for astrocytes[32,33], *IRF8*[34,35] and *SPI1*[36,37] for microglia, and *STAT3*[38,39] for mural cells, illustrating the coordinated transcription factor activity required to maintain the functional diversity of cells in the human optic nerve (Fig. 2g).

The lineage trajectory of oligodendrocyte precursor cells and oligodendrocytes in adults

Oligodendroglial lineage cells comprise the most abundant cell classes in the optic nerve, serving as glial cells essential for regulating myelination, axonal function, metabolic support, and tissue repair[40,41]. From 13,919 oligodendrocyte precursor cells (OPCs), we identified six transcriptionally distinct precursor populations: OPC_GLIS3+, OPC, OPC_Cycling (proliferating OPC), COP_NFOL (likely a transitional state bridging committed precursors with newly formed oligodendrocytes, expressing marker genes of both cell types), NFOL_UTRN+, and MFOL (myelin-forming oligodendrocytes) (Fig. 3a, Supplementary Fig. S2, S3). In parallel, we resolved four oligodendrocyte populations from 278,509 oligodendrocytes, including OLIGO2_LRRC7+, OLIGO1, OLIGO1_SVEP1+, and OLIGO1_RBFOX1+ (Fig. 3b, Supplementary Fig. S4,S5a-d). These cell populations express both shared lineage markers and cell type-specific marker genes (Fig. 3c) and were observed across multiple donors (Supplementary Fig S2c, S4c), significantly expanding the annotations

of precursor and mature oligodendrocyte populations previously identified in the human optic nerve[2].

By co-embedding of snRNA-seq and snATAC-seq data, we identified all four oligodendrocyte cell populations in the snATAC-seq data, each exhibiting a distinct chromatin accessibility landscape (Fig. 3d). Consistently, gene regulon analysis identified cell type-specific transcription factors and gene regulatory networks that could potentially drive cell identity of these populations (Fig. 3e, Supplementary Table 10). Notably, several identified transcription factors have been previously linked to oligodendrocyte lineages, such as *YY1*[42,43], *NKX6-2*[22,23], *KLF7*[44,45] *CREB5*[46,47], whereas others provide candidate cell type-specific regulators for future investigation.

Transcriptomic trajectory inference revealed that oligodendrocyte precursor populations occupy earlier developmental states than mature oligodendrocytes, with MFOL as the terminal precursor stage (Fig. 3f). The distribution of latent times across precursor populations mirrored the established developmental progression, transitioning from OPC → COP_NFOL → NFOL_UTRN^{+} → MFOL cells (Fig. 3g). Whereas the four mature oligodendrocyte cell types also exhibited distinct latent times aligned with maturation trajectory, with OLIGO2_LRRC7+ representing the earliest state and OLIGO1_RBFOX1+ the most mature state (Fig. 3f, h). Consistent with this ordering, *LRRC7* expression was detected in both precursors and OLIGO2_LRRC7+ (Fig. 3c). In contrast, *PIEZO2* expression was markedly lower in OLIGO2_LRRC7+ compared with other oligodendrocyte populations but was comparable to the levels observed in precursors (Supplementary Fig. S5e). Together, these findings suggest that OLIGO2_LRRC7+ may represent an early state among myelinating oligodendrocytes, potentially retaining precursor cell functional characteristics.

We further validated oligodendrocyte precursor cells in situ using Xenium (Fig 3i). Interestingly, the precursor cells showed a significant increase along the longitudinal axis from the ONH to ON (Fig 3j). This pattern is consistent with the known inhibition of oligodendrocyte lineage cells entering the lamina cribrosa in the ONH. We also validated OLIGO2_LRRC7+ in situ using the Xenium dataset (Fig 3k, Extended Data Fig. 5). Notably, OLIGO2_LRRC7+ showed elevated abundance in regions proximal to the ONH (Fig. 3l-n, Extended Data Fig. 5e-k), which may suggest a potentially distinct origin than other oligodendrocyte populations (as discussed below).

Function roles and age-associated changes in oligodendrocytes and their precursors

To characterize the functional properties of precursor and mature oligodendrocyte populations, we leveraged snRNA-seq data to interrogate their associated biological pathways.

Among precursor populations, OPCs were enriched for KRAS_signaling_DN, WNT-Beta-catenin, and Notch_signaling pathways, indicating a possible progenitor-maintained state (Fig. 4a). OPC_GLIS3+ were enriched for multiple immune and inflammation-related pathways, suggesting a reactive state (Fig. 4a). OPC_Cycling were consistently enriched in G2M checkpoint and mitotic spindle pathways, likely representing a proliferating population (Fig. 4a). COP_NFOL were enriched for oxidative phosphorylation, WNT-beta-catenin, and cholesterol homeostasis pathways, which points to a potentially metabolically active, differentiation-primed state (Fig. 4a). NFOL_UTRN+ were enriched for apical junction, TGF-beta signaling, and hedgehog signaling pathways, likely representing an early newly formed oligodendrocyte state (Fig. 4a). MFOL were enriched for cholesterol homeostasis and fatty acid metabolism pathways, suggesting a myelination-forming state (Fig. 4a). Together, the functional properties of these precursor populations provide additional evidence supporting their transcriptomic classification.

Similarly, the four oligodendrocyte populations were also active in a variety of pathways (Fig. 4b). Surprisingly, OLIGO2_LRRC7+ displayed enrichment of G2-M checkpoint, MYC-TARGETS-V1, and cholesterol homeostasis pathways, indicating a potentially proliferating and highly dynamic state marked by concurrent cell-cycle activity and metabolic priming (Fig. 4b). This functional profile aligns with OLIGO2_LRRC7+ occupying the earliest state among myelinating oligodendrocytes and retaining precursor-like features, as inferred from the analyses above.

These functional and trajectory programs prompted us to examine how oligodendrocyte lineage composition and transcriptional programs changed with aging. Remarkably, several precursor populations, including OPC, OPC_Cycling, COP_NFOL, and NFOL_UTRN+ populations, decreased with age (Multivariable linear regression, controlled for ancestry, sex and tissue region, $P.adj < 0.05$, Fig 4c). The reduction in precursor cells could potentially contribute to the well-documented age-related decline in remyelination capacity. In contrast, within oligodendrocyte populations, the proportion of OLIGO1_SVEP1+ decreased in older adults, whereas the proportion of OLIGO1 increased (Multivariable linear regression, $P < 0.05$, Fig 4d).

In addition to cell proportion changes, age-dependent gene modules and associated pathways were well noted (Supplementary Table 11). In precursor populations, we observed coordinated repression of developmental and cell-cycle programs—including MYC targets (V1), E2F targets, G2-M checkpoint, and mitotic spindle genes—from childhood to adulthood, while PI3K/AKT/mTOR signaling was progressively activated at later ages (Fig 4e,f).

Interestingly, among the different oligodendrocyte populations, OLIGO2_LRRC7+ exhibited the highest number of differentially expressed genes (DEGs) during aging (Linear mixed model, FDR <0.05, Fig 4g, Supplementary Table 12). In OLIGO2_LRRC7+, genes up-regulated during aging were enriched in the pathways related to regulation of GTPase activity, cell projection organization (Fig. 4h, FDR < 0.05), and RNA metabolism, whereas down-regulated genes were associated with modulation of synaptic transmission and biogenesis (Fig. 4i, FDR < 0.05).

Region-specific distribution of astrocyte populations

Following oligodendrocytes, astrocytes are the second most abundant cell class in the optic nerve, which regulate axonal homeostasis, metabolic and vascular support, extracellular matrix organization, mechanosensory signaling, and injury responses[48–50]. From 208,786 astrocytes, we resolved extensive astrocyte heterogeneity across anatomical regions of the optic nerve. We identified 11 transcriptionally distinct astrocyte populations distributed across four regions: the retina (referred to as Astro_retina, 2 populations), the ONH (Astro_ONH, 2 populations), the ON/ONH (located in the optic nerve but proximal to the ONH, Astro_ONHON, 2 populations,), and the ONP (located in the optic nerve but away from the ONH, Astro_ON, 5 populations) (Fig. 5a, Supplementary Fig. S6, S7). These astrocyte populations were observed across multiple donors (Supplementary Fig. S6c). These classifications are supported by both sample collection regions and marker gene expression (Fig. 5b, Supplementary Fig. S6h, S7a-c). Notably, astrocyte populations in the retina and ONH exhibited greater transcriptomic similarity, while astrocyte populations in the ONH/ON and ON appeared to be more closely related (Fig.5a).

Integration of snRNA-seq and snATAC-seq data demonstrated that each region-specific astrocyte subclass was governed by a distinct chromatin accessibility landscape and gene regulatory program (Fig. 5c). Consistently, we identified region-specific transcription factors and regulons driving these programs (Fig. 5d, Supplementary Table 13). For example, *SOX4*, *RAX*, and *VSX2* were identified as transcription factors for Astro_retina; *MEIS1* and *RAD21* for Astro_ONH; *SOX2*[51,52] and *KLF8* for Astro_ONHON cells; and *NFIA*[53–55], and *NFIC* for Astro_ON (Fig. 5d). These transcription factors may cooperate with established astrocyte-lineage regulators to drive region-specific gene expression programs.

We further validated the spatial distribution of the four region-specific astrocyte subclasses in situ, by projecting snRNA-seq-derived astrocyte annotations onto the Xenium spatial transcriptomic data (Fig. 5e). These subclasses exhibited unique transcriptional

signatures and region-specific spatial distributions corresponding to their transcriptomic identities (Fig 5e-i, Extended Data Fig. 6a-k).

Trajectory inference revealed a continuous transcriptional gradient extending from the ONP toward the retina (Fig. 5j). The latent times of the 11 astrocyte populations increased along this axis, following the order Astro_ON < Astro_ONHON < Astro_ONH < Astro_retina (Fig. 5k). Notably, the latent times of the Astro_ONH and Astro_retina appeared to be longer than those of Astro_ONHON and Astro_ON, consistent with the transcriptomic similarity within the two groups (Fig. 5k). These observations prompted us to identify the potential transcription factors driving the two groups (Fig. 5l). Indeed, we identified distinct sets of putative transcription factors associated with the two astrocyte groups, including *PAX2*, *SOX4*, and *SOX8*[56] for Astro_retina/Astro_ONH populations, and *NFIA*[53–55], *SOX2*[51,52], and *ZNF846* for Astro_ONHON/Astro_ON populations (Fig. 5l, Supplementary Table 14). The cellular specificity of these transcription factors is consistent with the region-specific transcriptional programs identified above and may further reflect the cooperative transcriptional regulation shaping the reginal identities of astrocyte populations.

Age-associated changes in astrocytes and identification of a progenitor-like astrocyte population

The 11 astrocyte populations were enriched in shared and distinct pathways, revealing their functional properties (Fig. 6a). Notably, Astro_ON5_DPP10+, Astro_ON4_AFF3+DMGDH+, and Astro_ON2_ACTN1+SERPINA3+, were enriched for immune response and inflammation pathways, consistent with an immune-reactive astrocyte state. In contrast, Astro_ONHON2_CST3+APOE+ were enriched for adipogenesis, cholesterol homeostasis, apical junction (often seen in ventricular or niche astrocytes)[57,58], oxidative phosphorylation (energetic maturity)[59], hedgehog signaling (characteristic of developmental regulation and stem-cell niche maintenance)[59,60], suggesting potential roles in niche support.

Previous studies have shown that specialized astrocyte-like cells lining the lateral ventricles may function as adult neural progenitor cells (NPC)[61,62]. Additionally, one study identified a NPC niche near the lamina cribrosa region of the optic nerve in mouse, rat and human, through immunostaining, SOX2 transgenic reporter mice, and cell culture[63]. Cells within this NPC niche exhibited an astrocyte-like identity[63]. To assess whether astrocyte populations in our dataset exhibited NPC-associated transcriptional signature, we examined the expression of genes associated with neural stem/progenitor states and astrocyte identify. Astro_ONHON2_CST3+APOE+ showed the highest expression of *SOX2*, *SOX9*, *ID1*, *TUBB*,

APOE, *GJA1,* and *VIM* (Fig 6b, 6c) and was identified across multiple donors (Supplementary Fig. S6c). Its inferred localization posterior to the lamina cribrosa in the anterior optic nerve (Fig5a,b,e), together with its gene expression profile (Fig 6b), is consistent with the location and features of the NPC identified in the previous study[63], suggesting a potential association with the previous reported NPC population. However, future studies are necessary to validate and establish the potential progenitor role of Astro_ONHON2_CST3+APOE+ cells.

We further characterized age-related changes across astrocyte populations (Fig 6d). Interestingly, Astro_ONHON2_CST3+APOE+ showed a decrease in older adults (Multivariable linear regression, P =0.020, Fig 6d). This finding is also consistent with the previously reported age-related depletion of the NPC niche near the lamina cribrosa of the human optic nerve[63]. In contrast, Astro_ON2_ACTN1+SERPINA3+ were increased in older adults, aligned with their putative roles in inflammation (Multivariable linear regression, $P <$ 0.05, Fig 6d). Consistently, Astro_ONHON1_SLC4A11+MARCH1+ and Astro_ONHON2_CST3+APOE+ exhibited the highest numbers of age-related DEGs among astrocyte populations (Fig 6e, Linear mixed model, FDR < 0.05, Supplementary Table 15,).

Interestingly, age-related DEGs of astrocyte populations were enriched in biological pathways that aligned with their spatial distribution and functional characteristics. Among astrocytes proximal to the ONH (i.e. Astro_retina and Astro_ONH), pathway analyses revealed that genes related to mechanosensory, stress responses and immune regulations were upregulated with age, including detection of mechanical stimulus, cell-cell adhesion, vesicle-mediated transport between endosomal compartments, and positive regulation of microglial activation (Fig 6f, FDR < 0.05). Whereas genes related to extracellular matrix (ECM) integrity and tissue repair were downregulated with age, including regulation of substrate adhesion-dependent cell spreading and cell substrate junction assembly, and response to transforming growth factor beta (Fig. 6g, FDR < 0.05).

Among astrocytes in the optic nerve (i.e. Astro_ONHON and Astro_ON), genes up-regulated during aging were enriched in pathways associated with impaired axonal homeostasis and metabolism, including regulation of lipoprotein lipase activity, TORC1 signaling, calcium ion transport, and trans-synaptic signaling (Fig 6f, FDR < 0.05). In contrast, genes associated with metabolic, biosynthetic and neuro-supportive functions were down-regulated, including hippo signaling, retinoid metabolic process, cytoplasmic translation, proton motive forces-driven ATP synthesis, negative regulation of amyloid precursor protein biosynthetic process, and cholesterol biosynthetic process (Fig 6g, FDR < 0.05). Taken together, these findings suggest that aging is accompanied by broad remodeling of astrocyte signaling, metabolic and mechanosensory responses, and neuro-supportive functions.

Moreover, cell-cell communication analysis revealed age-related changes in cell interactions between astrocytes and other cell types. Notably, LAMININ-mediated signaling was enhanced in adults (Age 20—40 years vs. Age ≤ 12 years, Fig 6h) but markedly reduced in elderly adults (Age ≥ 70 years vs. Age 20—40 years, Fig 6i), particularly among ONH and ONHON astrocyte populations. These patterns coincided with the selective expression of *PIEZO2* in ONH and ONHON astrocytes (Extended Data Fig. 6l), as well as reduced expression of LAMININ ligands from fibroblast populations and their receptors from astrocyte populations, such as LAMA2 to ITGAV_ITGB8 and LAMC1 to CD44 (Extended Data Fig. 7). These findings suggest an age-associated, region-specific decline in the ability of astrocytes to respond to mechanical stress and mediate extracellular matrix remodeling, which could potentially contribute to increased susceptibility to glaucomatous damage in older adults.

Age-related decline in fibroblasts and microglia

Similar to oligodendrocytes and astrocytes, we uncovered cellular diversity across other cell classes, including fibroblasts (Fig. 7a,b, Supplementary Fig. S8,S9), microglia (Supplementary Fig. S10,S11), macrophages (Supplementary Fig. S12,S13), endothelial cells (Supplementary Fig. S14,S15), and mural cells (Supplementary Fig. S16,S17).

Fibroblasts in the ONH and optic nerve are major structural and regulatory cells that maintain ECM, tissue biomechanics, vascular support, and meningeal integrity, increasingly recognized as important contributors to aging and glaucoma pathogenesis. From 112,466 fibroblasts, we identified 11 populations with distinct transcriptomic signatures (Fig 7a,b, Supplementary Fig. S9a-d) that aligned with anatomical locations (Supplementary Fig. S8g) and were observed across multiple donors (Supplementary Fig. S8c). Based on cell type labels from the integrated published datasets and marker gene expression (Supplementary Fig. S9a-d)[2], two fibroblast populations, Fibro_sclera_dura_KCNMA1+ and Fibro_sclera_dura_NOX4+, were inferred to originate from the sclera, potentially extending into the dura mater of meningeal layers. Several fibroblast populations correspond to the meningeal layers, including one dura mater population (Fibro_dura_SLC47A1+), three arachnoid mater populations (Fibro_arachnoid_CLDN11+, Fibro_STXBP6+, and Fibro_arachnoid_TRPM3+), one pia mater population (Fibro_pia_ABCA10+). In addition, Fibro_ATP10A+ expressed marker genes characteristic of vascular-associated fibroblasts, including high expression of *IGFBP7*, *ENPP1,* and *DCN* and the absence of canonical mural cell markers (Supplementary Fig. S9a,b). We also identified Fibro_x cells, a population previously reported in other ocular cell atlases[2]. Notably, one population, Fibro_HMCN1+, exhibited the highest expression of *ACTA2* and *TPM1* among all fibroblast populations,

suggesting that these cells may represent arachnoid trabeculae-associated fibroblasts (Supplementary Fig. S9e). Together, these annotations provide unprecedented resolution for dissecting fibroblast diversity within the meningeal layers and optic nerve.

Notably, Fibro_HMCN1+, were reduced in older adults (Fig. 7c, Multivariable linear regression, P=0.018), while pia mater fibroblasts (Fibro_pia_ABCA10^{+} cells) exhibited the highest number of DEGs associated with aging (Supplementary Fig. S9f, Supplementary Table 16, FDR < 0.05). Genes up-regulated with age were enriched in pathways related to stress response, structural remodeling, and lipid trafficking, such as DNA damage response, negative regulation of plasma membrane bounded cell projection assembly, regulation of cell motility, lipid localization, and cell adhesion (Fig. 7d, FDR < 0.05). In contrast, genes down-regulated with age were enriched in ECM integrity, biogenesis and homeostasis, including extracellular matrix organization, monatomic anion homeostasis, collagen fibril organization, translation, and gene expression (Fig. 7e, FDR < 0.05).

Microglia and tissue-resident macrophages are key regulators of immune surveillance, debris clearance, synaptic remodeling, axonal support, and injury responses in the central nervous system[64–67]. We identified diverse microglia and macrophage populations reflecting distinct cellular origins and transcriptional states. In the ONH and optic nerve, a single microglial lineage comprised five populations: a predominant homeostatic population, followed by an activated population (characterized by expression of *NAMPT*, *CD83*, *HIF1A*, *PFKFB3*), as well as stress-response, proliferative, remodeling, and NFκB–driven inflammatory populations (Fig. 7f, Supplementary Fig. S10, S11). Remarkably, microglia were reduced with age (Multivariable linear regression, *P*=0.0093, Fig. 7g), potentially reflecting impaired immune surveillance and diminished neuroprotective capacity in the aging CNS and likely contributing to increased susceptibility to age-related neurodegenerative diseases.

In contrast, we identified macrophages originated from two major lineages, including tissue-resident populations (five populations) and monocyte-derived populations (two populations), with no significant age-associated changes in cell proportions detected (Fig. 7h, Supplementary Fig. S12, S13, Extended Data Fig. 8a). Overall, among all the major cell classes, fibroblasts exhibited the highest number of age-related differentially expressed genes, followed by endothelial cells, suggesting that these cell classes undergo the most pronounced transcriptomic alternations during aging (Linear mixed model, FDR < 0.05, Extended Data Fig. 8, Supplementary Table 17).

Spatial cellular niches across the optic nerve

After resolving cellular heterogeneity, we investigated interactions among distinct cell populations within the optic nerve microenvironment using Xenium spatial transcriptomics. We identified 10 cellular niches enriched for specific cell classes and anatomical compartments (Fig. 7i–k, Extended Data Fig. 9). Niche 1, enriched in oligodendrocyte precursor cells, exhibited a decreasing gradient from the ONP toward the ONH, consistent with the restricted distribution of myelination. Niche 6, enriched in oligodendrocytes, astrocytes, and endothelial cells, was distributed throughout the optic nerve, suggesting coordinated interactions among myelinating glia, neural support, and vascular networks. Niche 7 was enriched in infiltrating immune cells, indicating localized immune surveillance, whereas niche 8, enriched in fibroblasts and tissue-resident macrophages, was primarily localized to the meningeal layers, highlighting stromal–immune interactions at the tissue boundary. Niche 9 was enriched in microglia and extended from the ONP to the retina, consistent with their roles in CNS homeostasis and responses to axonal stress. Niche 10 corresponded to the neurovascular compartment and was enriched in mural and endothelial cells. These spatially defined niches recapitulate the anatomical organization of the optic nerve, while further revealing coordinated multicellular microenvironments underlying axonal homeostasis, myelination, immune surveillance, ECM organization, and vascular regulation.

Cell type- and region-specific genetic regulation contributes to glaucoma traits

By integrating GWAS data with the multimodal HONCA atlases, we prioritized candidate causal variants, genes, and relevant cell types underlying ocular GWAS traits. Cell classes enriched for GWAS signals were identified using gene expression-based MAGMA.Celltyping analysis and chromatin accessibility-based LDSC method respectively, generating consistent results (Fig 8a-b, Supplementary Table 18, 19). Glaucoma and related traits[68–71] were enriched in oligodendrocyte precursor cells, oligodendrocytes, astrocytes, endothelial cells, fibroblasts, and mural cells (Fig 8a, FDR < 0.1), whereas age-related macular degeneration loci[72] showed moderate enrichment in endothelial cells, macrophages, and melanocytes (Fig 8a, FDR < 0.1). Remarkably, these GWAS traits showed cell type-specific enrichment patterns within individual cell classes (Fig 8a, FDR < 0.1). For example, GWAS loci associated with primary open-angle glaucoma (POAG), optic disc area (POAG_DA), and vertical cup-to-disc ratio (POAG_VCDR) were enriched in subsets of venule and capillary endothelial cells, pericytes, vascular smooth muscle cells, and several meningeal fibroblast populations (Fig 8a, FDR < 0.1). Interestingly, astrocytes showed region-specific enrichment of glaucoma traits that aligned with their spatial distribution. Astrocytes proximal to the ONH

were enriched for optic disc traits, such as POAG_VCDR and POAG_CA, whereas astrocytes in the ONP were preferentially enriched for IOP and POAG (Fig 8a, FDR < 0.1).

We further fine-mapped GWAS loci associated with glaucoma and related traits, including IOP[70], POAG[68], POAG_CA[69], POAG_VCDR[69], POAG_DA[69], and PACG[71], by leveraging statistical method and functional genomic annotation. For each linkage disequilibrium block, we identified 95% credible sets of GWAS variants using the 'Sum of Single Effects' (SuSiE) statistical model and calculated the posterior inclusion probability (PIP) for each variant[73]. By integrating with genomic annotation, we further refined the fine-mapped GWAS variants to functional genomic regions, including those derived from HONCA, such as OCRs, DARs, and CREs (Supplementary Table 20). Notably, a subset of fine-mapped variants were localized to CREs, DARs, and OCRs, suggesting their potential regulatory roles (Fig. 8c). To further characterize the relevant cell types, we mapped the expression of reported GWAS genes associated with glaucoma and IOP regulation across optic nerve cell types (Fig. 8d, Extended Data Fig. 10).

Fine-mapped variants nominated putative causal candidates and generated hypotheses regarding the regulatory mechanisms underlying GWAS loci. For example, we localized a fine-mapped POAG GWAS variant, rs7636574 (PIP=0.63), to a CRE linked to *ARHGEF3* (Fig. 8e). This CRE was specifically accessible in astrocytes, consistent with the high expression of *ARHGEF3* across astrocyte populations (Fig. 8e). Notably, rs7636574 has a CADD score of 12.8 and was predicted to disrupt binding of *SOX9* (allele effect size = -0.077), which is a astrocytes transcription factor identified in both this study (Fig. 2f) and previous studies[32,33], further supporting its functional role (Fig. 8e, Supplementary Fig. S18). *ARHGEF3* encodes a Rho guanine nucleotide exchange factor that plays key roles in cytoskeletal remodeling, cell contractility, mechanotransduction, migration, and differentiation. Together, these findings support a cell type-specific regulatory role for rs7636574 in mediating POAG risk.

Discussion

In this study, we present HONCA, the first comprehensive single-cell multi-omics atlas of the human optic nerve, comprising approximately 0.9 million snRNA-seq, 0.9 million snATAC-seq, and 0.3 million Xenium cells from more than 108 donors with diverse ancestries and ages. Integrated analysis of the snRNA-seq data identified approximately 62 cell populations in the optic nerve and ONH, nearly doubling the number previously reported, along with 35 cell types in retina and related tissues[2]. HONCA enabled the identification of rare cell populations and revealed extensive cellular heterogeneity across compartments

common to the CNS, including glial populations (such as oligodendrocyte, astrocytes, and microglia), stromal populations (e.g. the sclera and meningeal layers), vascular populations (spanning arterioles, capillaries, and venules), and immune populations (including both tissue-resident macrophages and infiltrating immune cells). Chromatin accessibility and spatial transcriptomics atlases provided orthogonal support for the identified cell types and further uncover underlying cis-regulatory elements, gene regulation programs, as well as region-specific and gradient-like spatial organization. For example, astrocytes exhibit region-specific distribution, transcriptome signatures, and gene regulatory programs along the longitude axis of the optic nerve, consistent with previous studies in rodents that revealed differential responses of region-specific astrocytes to optic nerve crush and glaucomatous injury [74–78].

Remarkably, we reconstructed the lineage trajectories of oligodendrocyte precursor cells, oligodendrocytes, and astrocytes in human adults. The transition of oligodendrocyte precursor populations recapitulated the established developmental progression and were resolved at a higher resolution than previously reported in the human brain[79,80]. Among myelinating oligodendrocyte populations, we identified OLIGO_RBFOX1+ as the most mature state, consistent with a previous study of oligodendrocytes in the human brain[80].

Although the adult mammalian brain was once thought incapable of generating new neurons, adult neurogenesis is now known to persist in restricted niches such as the dentate gyrus and the ventricular-subventricular zone (vSVZ). Specialized astrocyte-like cells in the mouse vSVZ can function as neural stem cells (NSCs)[61,62]. A previous study also identified a neural progenitor cell (NPC) niche near the lamina cribrosa region of the optic nerve in mouse, rat and human, with the NPC exhibiting astrocyte-like features[63]. Here, we identified OLIGO2_LRRC7+ as a myelinating oligodendrocyte population that may retain precursor-like features, supported by gene expression signatures, inferred latent time and lineage trajectories, and pathway analyses. We also identified the Astro_ONHON2 population expressing SOX2+ and SOX9+. Both Astro_ONHON2 and OLIGO2_LRRC7+ were enriched immediately posterior to the lamina cribrosa region in the anterior optic nerve, suggesting a possible association with the previously reported NPC niche near the optic nerve lamina region in mice, rats and humans[81]. Further studies are warranted to determine whether Astro_ONHON2 cells share molecular and functional properties with astrocyte-like NPC, including the potential to give rise to astrocytes, oligodendrocytes, and, more rarely, neuron progenitors. Together, these findings nominate candidate cell populations, associated marker genes, and testable hypotheses for future investigation of neural regeneration.

Furthermore, HONCA captured broad human population diversity, enabling the identification of molecular and cellular variation among individuals. We detected age-related

decreases in multiple cell populations, including oligodendrocyte precursor cells, progenitor-like astrocytes, microglia, and several fibroblast types, along with age-related declines in astrocytes-fibroblast interactions proximal to the ONH. These changes were accompanied by transcriptomic and pathway alterations, characterized by diminished expression of genes involved in cell-cycle programs and biogenesis, and broad remodeling of signaling, metabolic, mechanosensory, neuro-supportive and cell-cell interaction programs. These findings provide new insights into the molecular and cellular mechanisms that may contribute to increased susceptibility to age-related neurodegenerative diseases, including POAG and declining remyelination capacity.

Finally, by integrating with GWAS data, HONCA enabled in-depth functional annotation of disease-associated variants and dissection of the regulatory mechanisms underlying causal genetic variants. We demonstrated that GWAS loci associated with glaucoma and related traits were enriched in the optic nerve cell populations, including glia cells, fibroblasts, and vascular cells. Notably, while most astrocyte populations are enriched for POAG and IOP GWAS loci, astrocyte populations proximal to the ONH region were specifically enriched for GWAS loci associated with POAG optic disc traits, aligning spatial distribution and revealing region-specific and cell type-specific genetic regulation underlying complex diseases. Additionally, we nominated candidate causal variants, genes, relevant cellular contexts for glaucoma and related traits, including fine-mapping a POAG GWAS locus to *ARHGEF3* acting in astrocytes, implicating astrocyte-mediated mechanotransduction and biomechanical stress responses as potential mechanisms underlying disease risk. Together, our work provides single cell multi-omic resources and candidate genetic variants and genes for future studies of the genetics and molecular basis of glaucoma.

HONCA is publicly accessible through interactive platforms, including CELLxGENE, the Single Cell Portal, and the Cell Annotation Platform. Collectively, this foundational atlas establishes an unprecedented, high-resolution resource for elucidating cellular heterogeneity, gene regulation mechanisms, aging dynamics, and spatial organization, and serves as a reference for advancing research on human eye and CNS health and diseases.

Ethics

This study was conducted in compliance with all applicable ethical regulations and the principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Boards (IRBs) of Baylor College of Medicine and University of California, Irvine. Human donor tissues were obtained through the Lions World Vision Institute and the Lions Eye Bank of Texas, both of which operate under independent IRB oversight for donor

recruitment and tissue procurement. Informed consent was obtained from all donors or their legal representatives, and all samples were de-identified in accordance with HIPAA Privacy Rules prior to distribution.

Methods

Human optic nerve sample collection

Optic nerve and optic nerve head tissues were obtained within 12 hours post-mortem from the Lions Eye Bank of Texas at Baylor College of Medicine and Utah Lions Eye Bank. Detailed donor information is provided in Supplementary Table S1. Donor medical histories were reviewed, and only individuals without evidence of retinal disease were included in the study.

Orbital fat and surrounding connective tissue were carefully removed to expose the optic nerve. The optic nerve was transected close to the scleral surface and immediately placed into cryomold. The tissue was flash-frozen in liquid nitrogen and stored at −80 °C until nuclei isolation.

Following optic nerve collection, the anterior segment was separated from the posterior segment by a circumferential incision on the sclera approximately 5 mm from the limbus, and the anterior cup was removed. The posterior eyecup was placed scleral side down and cut radially into four lobes, forming a butterfly shape to allow access to the posterior tissues. The vitreous body was then carefully removed using fine scissors to minimize contamination.

The optic nerve head was identified as the circular, pale region corresponding to the optic disc and was dissected at the final stage of posterior segment processing using a 2 mm punch, after all macular and peripheral retinal/RPE-choroid samples had been collected. The isolated tissue was immediately flash-frozen in liquid nitrogen and stored at −80 °C until nuclei isolation.

Nuclei extraction and single nuclei RNA and ATAC sequencing

For snRNA-seq, tissues were processed for nuclei isolation using the GentleMACS Octo Dissociator with C-tubes in ice-cold Nuclei Extraction Buffer. The resulting suspension was filtered through 70-μm MACS SmartStrainers and centrifuged at 300 × g for 5 min at 4 °C. For optic nerve head (ONH) samples, the nuclei pellet was resuspended in wash buffer (10 mM Tris–HCl, 10 mM NaCl, 3 mM $MgCl_2$, 1% BSA), gently triturated, passed through 30-μm filters, and centrifuged again at 500 × g for 5 min at 4 °C. The final pellet was resuspended in PBS for downstream processing.

For optic nerve (ON) samples, nuclei were further purified using the Anti-Nucleus MicroBeads protocol. Briefly, the pellet was resuspended in nuclei separation buffer consisting of Nuclei Extraction Buffer (14%), MACS BSA Stock Solution (0.04%), and RNase inhibitor (0.2 U/μL) in PBS. The suspension was gently triturated, passed through 30-μm filters, and incubated with 50 μL of Anti-Nucleus MicroBeads for 15 min at 2–8 °C.

During incubation, LS Columns were prepared according to the manufacturer's instructions. After incubation, 2 mL of nuclei separation buffer was added to the sample, which was then applied to the magnetic column. The column was washed, and magnetically labeled nuclei were immediately flushed out. The collected nuclei were centrifuged at 500 × g for 5 min at 4 °C and resuspended in PBS for downstream processing.

Single-nucleus Gene Expression libraries were generated using the Chromium Next GEM Single Cell 3′ v3.1 Reagent Kit (10x Genomics), and single-nucleus ATAC libraries were prepared using the Chromium Next GEM Single Cell ATAC v1.1 protocol. All single-nuclei RNA and single-nuclei ATAC sequencing was conducted at the Single Cell Genomics Core at Baylor College of Medicine in this study.

Tissue processing and paraffin embedding

Ocular tissues were fixed for 48 hours to preserve both tissue morphology and RNA integrity. Donor samples were fixed in Modified Davidson's Fixative (Catalog# 64133-50). To facilitate fixative penetration, several small incisions were introduced at the pars plana prior to immersion fixation. After fixation, tissues were temporarily stored in either 1% PFA with 5% sucrose or 70% ethanol before paraffin embedding.

For embedding, tissues were dehydrated through a graded ethanol series consisting of 70%, 80%, 95% ethanol (three changes), and 100% ethanol (two changes). Samples were subsequently cleared with three changes of HistoClear and infiltrated with two changes of molten paraffin. Each dehydration, clearing, and infiltration step was performed for 1 hour. Following paraffin infiltration, tissues were trimmed to fit Xenium slides, embedded in paraffin blocks, and stored at –20°C until sectioning.

Xenium spatial transcriptomic profiling

FFPE tissues were processed according to the 10x Genomics Xenium protocol (CG000578_RevE). Samples were horizontally sectioned at a thickness of 5 µm using a Leica RM2255 microtome and mounted onto Xenium slides. Tissue sections were deparaffinized and subjected to RNA decrosslinking following protocol CG000580_RevE.

Probe hybridization was performed for 16–24 hours using a customized Xenium Human Eye Panel targeting 478 genes (Design ID: G33B7Z). Enzymatic ligation and rolling circle amplification were subsequently performed to generate spatially resolved transcripts. Following amplification, cell segmentation staining and background fluorescence quenching were conducted in accordance with the manufacturer's instructions (CG000749 Rev B, 10x Genomics). Imaging and transcript decoding were performed on the Xenium Analyzer, producing cell-by-gene expression matrices and transcript-level spatial coordinates for each tissue section.

After Xenium data acquisition, the same tissue sections were stained with hematoxylin and eosin (H&E) following the manufacturer's protocol (CG000613 Rev B, 10x Genomics). Post-Xenium H&E images were acquired using a Keyence BZ-X810 microscope and were used to evaluate tissue morphology, support quality control, and refine image-based cell segmentation.

snRNA-seq data processing and cell type annotation

The data underwent rigorous quality control processing through the same publicly accessible pipeline, located at https://github.com/lijinbio/cellqc[13]. Raw sequencing reads from both published and newly generated datasets were analyzed using Cell Ranger[82] (v7.0.1) against the hg38 genome reference. SoupX[83] (v1.6.2) was used to correct ambient RNAs, and DoubletFinder (v2.0.4)[84] estimated and excluded doublets. Low-quality cells were filtered based on counts (<500), features (<300), or high mitochondrial read percentages (>5%) using Seurat[85] (v4.4.0). The resulting cells are high-quality and retained for HONCA construction. The cell x gene raw count matrices from all samples were concatenated into a unified count matrix using AnnData[86].

The combined cell x gene count matrix was normalized and log1p-transformed using Scanpy[87]. Highly variable genes were selected using scanpy.pp.highly_variable_genes(), with n_top_genes=2000, 5000, or 10000 tested and batch_key=sampleID. scVI[88] (v1.2.0) was used for integrating optic nerve cells across all samples without initial cell type labels and removing batch effects by setting batchID=sampleID. Based on the low-dimensional representation generated by scVI (with n_layer=2, n_latent=30), class-specific cells were clustered using the Leiden algorithm implemented with scanpy.pp.neighbors(use_rep = "X_scVI") and scanpy.tl.leiden(). After cell class annotation(as mentioned below), cells from each cell class were isolated and re-clustered independently using scVI[88] (v1.2.0) with similar parameter settings.

To determine the optimal number of clusters, Leiden resolution values ranging from 0.1 to 2.0 were evaluated, and a resolution that avoided over-clustering was selected. Clusters representing contamination from other cell types, doublets, or low-quality cells were removed, based on the expression patterns of marker genes from all major cell classes, as well as the distributions of gene counts and UMI counts within each cluster. Additionally, cell clusters that were dominated by a single sample were also excluded to ensure that each cluster contained cells from multiple samples and minimize potential artifacts. The remaining clusters were retained for cell type annotation.

Cell classes and types were annotated based on the following data: 1) the top-ranking genes per cell cluster; 2) marker genes reported in previous studies; and 3) the prior cell type labels from the integrated public dataset. After cell type annotation, marker genes for each cell type were further identified using NSForest[89]. Based on the top-ranking genes and NSForest-identified marker genes, the optimal marker genes for each cell type were selected. Each cell type or population was further evaluated based on whether they exhibited distinct marker gene expression patterns.

Benchmarking of integration methods

To evaluate snRNA-seq data integration methods, an adapted integration workflow from scAtlasTb (https://github.com/HCA-integration/scAtlasTb/tree/main) was used to conduct benchmarking of current state-of-art and scalable integration methods, including scVI[88], scANVI[88], and Harmonypy[90] with default parameters. The parameter settings include 10000 highly variable genes, sample ID as the batch key, and major class and cell type as the label key. The metrics to evaluate each method are divided into two categories: (1) batch effect removal and (2) biological variance conservation. The first category metrics include ASW (batch), graph connectivity, and graph iLISI. The second category metrics include ARI, ASW (label), Cell Cycle Conservation, graph cLISI, isolated label ASW, isolated label F1, and NMI. For each method, a batch correction and a bio conservation score were calculated based on these metrics. An average of the batch correction and bio conservation scores were used to rank the methods, labeled the overall score. scANVI requires prior cell type labels for dataset integration, whereas scVI and Harmonypy do not require prior cell type labels. As cell type identities were not known a priori, scVI showed the best integration performance among the three method both at both cell class and cell type levels when cell type labels are unknown (Extended Data Fig.2).

Lineage trajectory analysis and latent pseudo-time inference from snRNA-seq

Velocyto[91] (v0.17.17) was used to generate spliced and unspliced transcript counts, based on sequencing read alignment BAM files generated by Cell Ranger[82] (v7.0.1), the corresponding filtered barcode lists, GRCh38 gene annotation, and repeat-mask annotation. Sample-level loom files were filtered to retain the cells that are shared with the processed snRNA-seq atlas and subsequently were concatenated into a unified spliced/unspliced transcript count matrix. We then applied scVelo[92] (v0.3.3) to infer RNA velocity based on the spliced/unspliced transcript count matrix and UMI count matrix. The pre-trained scVI latent representations were used to compute first- and second-order moments for velocity estimation using scvelo.pp.moments() with n_pcs=30 and n_neighbors=30. RNA velocities were then inferred using scvelo.tl.velocity() under the dynamical model (mode = "dynamical"). The resulting velocities were used to construct a velocity graph via scvelo.tl.velocity_graph(). This velocity graph was subsequently used to compute cell-level pseudotime using scvelo.tl.velocity_pseudotime(). Latent time, representing the inferred internal transcriptional dynamics across cells, was estimated using scvelo.tl.latent_time(). The model fit likelihood was used to identify highly ranked genes for each cell type using scvelo.tl.rank_dynamical_genes(). The transition structure among cell populations were summarized using PAGA[93] after transferring the precomputed nearest-neighbor graph into a scVelo-compatible format using scvelo.tl.paga().

Pathway enrichment analysis of individual cell clusters

To identify the pathways enriched in individual cell clusters (i.e. transcriptionally distinct cell populations), the escape[94] package (v2.7.3) was used to conduct single-cell based pathway enrichment analysis. Escape leverages gene sets from the Molecular Signature Database v7.0 and implemented the ssGSEA[95] algorithm through runEscape().

Cell proportion changes associated with demographic variables

To assess cell proportion changes associated with sex and ancestry, separate multivariable linear regression analyses were performed for ONH and optic nerve samples respectively. The R function lm(cell proportion ~ sex + ancestry + age) was applied with sex and ancestry treated as categorical variables and age as a continuous variable to estimate the association of each factor with cell proportion while adjusting for the others.

To evaluate age-associated changes in cell proportions, multivariable linear regression analyses were performed. Cell proportion was modeled using the R function lm(cell_proportion ~ age + sex + ancestry + tissue_region), with sex, ancestry, and tissue region treated as categorical variables and age as a continuous variable, to estimate the

association of each factor with cell proportion while adjusting for the others. To further assess non-linear age effects, samples were grouped into age ranges and analyzed using lm(cell_proportion ~ age_range + sex + ancestry + tissue_region), with age range, sex, ancestry, and tissue region treated as categorical variables.

Differential gene expression analysis during aging

Because oligodendrocyte precursor cells represented a rare cell population, the Hotspot[96] package (hotspotsc v1.1.3) was used to identify age-associated gene modules. Briefly, oligodendrocyte precursor cells from the same age group were pooled and randomly shuffled to generate meta-cells, with each meta-cell containing 200 cells. This procedure generated a unified meta-cell-by-gene UMI count matrix. Genes with mean counts equal to zero were excluded. A Hotspot object was then initialized using the Hotspot() function with model = "danb". Subsequently, create_knn_graph() and compute_autocorrelations() were applied to identify informative genes exhibiting non-random expression patterns associated with age. The identified informative genes were further grouped into gene modules using create_modules() with min_gene_threshold = 200, fdr_threshold = 0.05, and core_only = TRUE.

For other cell populations, age-related differentially expressed genes were identified using a pseudo-bulk analysis approach. First, raw read counts were aggregated in pseudo-bulk level per gene per donor for each cell class. We only considered the samples containing at least 20 cells in the corresponding cell class. Additionally, samples that have a correlation in read counts <0.75 in >65% of the other samples were considered as outliers and were excluded from subsequent analyses. After quality control and filtering, a linear mixed model (LMM) was used to identified genes significantly correlated with aging through edgeR[97] (v4.4.0) and variancePartition[98] (v1.36.2) R packages. Gene expression was normalized using the trimmed mean of M-values method. The confounding factors, such as ancestry, sex, library size and batch effect were controlled with LMM. The formula we applied were—~age + sex + ancestry + library size + (1|batch). The Benjamini-Hochberg (BH) procedure was used to estimate the False Discovery Rate (FDR). Genes with Log2FC < 0 and FDR < 0.05 were considered downregulated with age, whereas genes with Log2FC > 0 and FDR < 0.05 were considered upregulated with age.

Gene set and biological process enrichment analyses were performed using the Enrichr[99] R package (v3.4) and the associated databases, "GO_Biological_Process_2023" and "MSigDB_Hallmark_2020". Multiple test correction was applied. The top five Gene

Ontology terms with FDR ≤ 0.05 for each cell type were visualized in a heatmap using the ComplexHeatmap R package (v2.18.0) [100].

Cell-cell communication analysis

CellChat v2.1[101,102] was used to perform cell-cell communication analysis at the subclass level. Cells were grouped into three age categories: childhood (≤ 12 years), adulthood (20—40), and older age (≥ 70 years). CellChat analysis was performed for each age group respectively.

The gene expression matrix and subclass annotation were used to construct CellChat objects for each age group. To reduce computational cost, only ligand receptor genes included in CellChatDB.human were retained using subsetData()[101,102]. Overexpressed genes and ligand-receptor interactions were identified using identifyOverExpressedGenes() with thresh.pc = 0.1 and identifyOverExpressedInteractions(). computeCommunProb() with default parameters was used to infer communication probabilities. filterCommunication(min.cells = 10) was used to filter out cell groups containing fewer than 10 cells. computeCommunProbPathway() was used to calculate pathway-level communication. probabilities. aggregateNet() was used to aggregate interaction networks.

To compare age-associated changes in cell-cell communication, mergeCellChat(). was used to merge CellChat objects from childhood and adulthood groups, as well as from the adulthood and older age groups. Changes in cell-cell interaction signaling were identified by comparing the two merged age groups using netAnalysis_signalingChanges_scatter(). The expression of the ligand and receptor genes in the LAMINA signaling pathways were plot based on the sample-level pseudo bulk snRNA-seq data, and gene expression was estimated with count per million (CPM).

Data processing and annotation of snATAC-seq datasets

Raw snATAC-seq reads were processed using the Cell Ranger ATAC pipeline (cellranger-atac-2.0.0)[103] and the ArchR package (v1.0.3)[104]. Cells with ⩽1,000 fragments and fragments with a TSS enrichment score ⩽4 were removed. Doublets were further identified and filtered using the ArchR functions *addDoubletScores()* and *filterDoublets()* using default parameters. To correct for sample batch effects, the *addHarmony()* function was applied to compute batch-adjusted low-dimensional representations using the “Sample” variable[90]. These corrected embeddings were then used to generate UMAP visualizations and to cluster ATAC cells with the *addClusters()* function, with max 50 clusters. Cluster resolution parameters ranging from 0.1 to 1.0 were tested, and the resolution yielding an optimal number of clusters was selected without over-clustering based on UMAP visualization. Major cell

classes were annotated by transferring labels from the HONCA snRNA-seq reference using the *addGeneIntegrationMatrix()* function without constraining correspondence between RNA reference cell types and ATAC clusters, where canonical correlation analysis (CCA) from the Seurat package[85] was used on gene features from the snRNA-seq reference and gene scores derived from the snATAC-seq data. The transferred labels were compared against ATAC clusters in a contingency table. Each ATAC cluster was annotated with the Seurat-predicted label if more than 90% of its cells were assigned to the same class.

To refine the annotation of clusters with mixed class labels, a higher-resolution integration was applied using scGLUE[105] package between the subset of cell types from the snRNA-seq data and clusters from the snATAC-seq data. For this, pseudo-bulk ATAC replicates were calculated for the identified clusters using the *addGroupCoverages()* function, and the union reproducible peak set was calculated using the MACS2[106] package and the *addReproduciblePeakSet()* function. The snRNA-seq data were preprocessed with 10,000 highly variable genes and 100 principal components for dimensionality reduction. The snATAC-seq data were preprocessed to retain the top 20% of highly variable peak features. The processed RNA and ATAC cell-by-gene and cell-by-feature matrices were used in the scGLUE integration with *latent_dim=30* and *h_depth=2* for the model initialization arguments, and *data_batch_size=128*, *graph_batch_size=128*, *align_burnin=32*, *max_epochs=200*, *patience=16*, *reduce_lr_patience=8* for the model fitting arguments. The trained latent representations were used to train Random Forest classifiers with 1,000 trees from the RNA cells with class labels using the *RandomForestClassifier* class[107] from the scikit-learn package[108], and the classifiers were used to predict the classification labels for the ATAC cells. Cells with classification probability $\leqslant$90% were labeled as “Unassigned” and filtered. The refined major class labels from scGLUE integration were combined with major class labels obtained using Seurat integration to form the final annotation for the ATAC cells. The updated union reproducible peak set, calculated based on annotation labels (*maxPeaks=500,000*), formed the chromatin accessibility reference from the snATAC-seq data.

Identifying enhancer-driven regulon from snATAC-seq data

Annotated snATAC-seq and snRNA-seq reference were combined to identify enhancer-driven regulons (eRegulons) for optic nerve cell classes using the SCENIC+ package (v1.0a2)[109]. To enable efficient analysis across all cell classes, both RNA and ATAC references are downsampled with 2,000 cells per class. Cis-topics were first calculated on the union peak feature sets from the ATAC data using the *run_cgs_models_mallet()* function[110]. Various numbers of topics were tested, and the optimal number was selected using the

evaluate_models() function of the pycisTopic package[109]. For each identified topic, two region sets were generated: the top 3,000 regions per topic and binarized topic-specific regions determined by the Otsu method[111]. Cell class-specific differentially accessible regions (DARs) were also identified using the *find_diff_features()* function. The precomputed cisTarget database for humans was downloaded from SCENIC+[109]. The SCENIC+ Snakemake pipeline was then executed to perform the eRegulon analysis. Target gene enrichment was calculated and classified as activators or repressors for each cell class. Finally, class-specific regulons were selected, and heatmaps of the calculated eRegulon Specificity Score (RSS) were plotted using the *heatmap_dotplot()* function.

Analyses of Xenium spatial transcriptomic data

Xenium bundles were generated for each sample, including four-channel morphology images, decoded transcripts for predefined genes, and negative control, using the Xenium Onboard Analysis pipeline (version xenium-3.2.0.7, 10x Genomics)[112]. The four morphology channels consist of DAPI nuclear staining, a cell boundary stain (ATP1A1/E-cadherin/CD45), an interior RNA stain (18S rRNA), and an intracellular protein stain (alphaSMA/Vimentin). The multimodal segmentation algorithm implemented in the Xenium Onboard Analysis pipeline was used to perform cell segmentation. Briefly, the DAPI channel was used to first conduct nucleus segmentation. Then boundary and interior staining signals were used for whole-cell segmentation.

Xenium Ranger (v3, 10x Genomics)[112] was used to conduct the spatial transcriptomic profiling with a customized human eye panel comprising 478 genes. Post-Xenium H&E staining and manual annotation guided the delineation of anatomical regions. Seurat (v5.3.0)[113] was used to analyze the Xenium output. Low quality cells that have fewer than 20 detected transcripts were excluded. Normalization and scaling were performed on the remaining data, followed by dimensionality reduction using principal component analysis (PCA).

For annotation of major cell classes, a approach reciprocal PCA (RPCA)-based from the Seurat package[113] was used to integrate datasets from different samples and account for sample-to-sample variation. Harmony[90] (v1.2.3) was used to align a down-sampled integrated snRNA-seq reference dataset with the integrated Xenium data. A shared nearest neighbor (SNN) graph was constructed for community detection, and UMAP was used to embed the integrated data in a low-dimensional space[113]. A modularity optimization algorithm was used for clustering cells. The annotation of each cell cluster was determined using a majority-voting strategy based on the cell type labels of snRNA-seq reference cells within the same cluster. Established marker gene expression patterns were used to confirm each cell cluster. For

annotation of subclasses and cell types, the Robust Cell Type Decomposition (RCTD) pipeline from the spacexr[114] package (v2.2.1) was used to predict the cell type label for each Xenium cell with the snRNA-seq data as the reference.

To identify spatial neighborhoods among Xenium cells, we applied the BuildNicheAssay() function from the Seurat package[113] to the annotated Xenium cell types together with their spatial coordinates. We set the parameters neighbors.k = 30 and niches.k ranging from 1 to 16. To determine the optimal number of niches, we applied multiple approaches, including the clusGap() function and fviz_nbclust() function, as well as manual inspection. Hence, each Xenium cell was assigned a "niche" label based on the composition of its surrounding cellular microenvironment, thereby defining local spatial neighborhoods. The resulting niche annotations were visualized on tissue maps using the ImageDimPlot() function.

Cell-type enrichment of GWAS loci

Both single-nuclei chromatin accessibility and gene expression were used to examine cell class enrichment underlying GWAS loci. For gene expression, MAGMA.Celltype[115,116] (version 2.0.8) R package was used to assess linear positive correlation between gene expression cell class specificity and gene-level genetic association with GWAS studies. The MungeSumstats[117] R package was used to format GWAS summary statistics based on SNPs in –35 kb/+10 kb of each gene and the 1000 genome (1000G) population. The EWCE[118] (version 1.16.0) R package was used to format snRNA-seq expression data. MAGMA.Celltype R package was used to detect linear enrichment. For chromatin accessibility, we partitioned the heritability of GWAS traits into cell class-specific snATAC–seq peaks with stratified LD score regression through LDSC (version 1.0.1)[119]. We first annotated GWAS SNPs that overlapped with HapMap3 SNPs based on whether they were in OCRs in each cell class. Subsequently, we calculated LD scores of these SNPs within 1-cM windows based on the 1000G data. The LD scores of these SNPs were combined with those from the baseline model, which included noncell-type-specific annotation (1000G_Phase3_baselineLD_v2.2_ldscores, downloaded from https://alkesgroup.broadinstitute.org/LDSCORE/). Finally, we estimated and compared the heritability in the annotated genomic regions with the baseline model to determine whether regions in each cell class were enriched with the heritability of the corresponding GWAS trait. To correct for multiple testing, the Benjamini–Hochberg method was applied to the enrichment P value based on chromatin accessibility and gene expression, respectively, considering the number of cell types tested.

Fine mapping of GWAS variants

GWAS summary statistics was used to fine-map GWAS loci. For each GWAS study, the SNPs with $P < 5 \times 10{-}8$ and present in 1000G (Phase 3) were selected and were grouped into the LD blocks identified by a previous study[120]. For each LD block, credible sets of SNPs and posterior inclusion probability of each SNP were calculated with the 'Sum of Single Effects' model through the susieR[121] package (L = 10). Genomic functional annotations, including OCRs, DARs and CREs from this study, were integrated to enhance statistical fine-mapping analysis.

Data availability

The landing page of the HONCA data resources is accessible at https://rchenlab.github.io/resources/human-atlas.html. Raw sequencing data files, processed Cell Ranger data files, and sample metadata information files of the HONCA have been deposited in the HCA Data Portal (https://data.humancellatlas.org/hca-bio-networks/eye). The datasets used in the HONCA are summarized in Supplementary Table 1. The raw sequencing reads of the newly generated data have been deposited in GEO (GSE325040). Additionally, raw and normalized count matrices, cell type annotations, and embeddings are also publicly available through the CELLxGENE collection (https://cellxgene.cziscience.com/collections/05e3d0fc-c9dd-4f14-9163-2b242b3bb5c2) and Cell Annotation Platform (https://celltype.info/project/621).

Code availability

All code used for the HONCA project can be found in the HONCA reproducibility GitHub repository (https://github.com/RCHENLAB/HONCA_reproducibility).

Acknowledgements

This project was funded by the Chan Zuckerberg Initiative (CZI) 2019-002425 and 2021-239847 to R.C., and CZI 2021-237885 to T.S. Additionally, next-generation sequencing (NGS) was performed on instrument supported by the National Institutes of Health (NIH) shared instrument grant S10OD023469 to R.C. The authors acknowledge support to the Gavin Herbert Eye Institute at the University of California, Irvine from an unrestricted grant from Research to Prevent Blindness and from NIH core grant P30 EY034070. This research was also supported in part by the Intramural Research Program of the National Institutes of Health (NIH) to R.H.S. (ZIA-LM202401). The contributions of the NIH authors are considered Works of the United States Government. The findings and conclusions presented in this paper are those of the author(s) and do not necessarily reflect the views of the NIH or the U.S.

Department of Health and Human Services. We are grateful to Jennifer Zamanian and the Lattice team at Stanford for their support with data dissemination. We thank Mary Futey, Evan Biederstedt, and the Cell Annotation Platform (CAP) team at Harvard University for their support with data decomposition for community annotation. This publication is part of the Human Cell Atlas—http://www.humancellatlas.org/publications/. We would also like to acknowledge the feedback and discussions from the members of the HCA Eye Biological Network and the HONCA curation expert panel.

Author contributions

J.W., J.L. and R.C. conceptualized and designed the study. R.C. supervised the work. M.M.D and J.T.S. provided the optic nerve and optic nerve head samples. X.B. and Y.L. and I.Y. developed the protocols for sample collection and processing. I.Y., X.B., Y.Z. collected and processed the samples. Y.L. conducted the snRNA-seq and snATAC–seq experiments. Y.Z. conducted the Xenium experiments. J.W. performed snRNA-seq, spatial transcriptomics, and GWAS related data analyses. J.L. performed snATAC-seq data analyses. T.Y., C.V., J.S., and J.J. contributed to the data analyses. Jean Li performed the benchmarking study of integration methods. Q.L. conducted HE staining of the Xenium data. L.A.O. and I.K.K. contributed to sample collection. P.B., M.M.D., E.K., A.R., M.L.C, S.W., G.Z., D.H.G., C.S., D.F.C, Z.H., X.J.Y., D.O., A.V.P., R.H.S., A.V.S. P.T., T.S. and H.C. provided input to cell type annotation and data analysis. J.W. and J.L. wrote the first draft of the paper. All authors reviewed the paper and contributed to the critical revisions of the paper.

Competing interests

Authors declare no competing interests.

Main figures

Figure 1. Integrated transcriptomic and spatial cell atlases of the human optic nerve. **a**, Schematic overview of sample collection for the human optic nerve multi-omic single cell atlases. **b**, UMAP of the transcriptomic cell atlas derived from snRNA-seq data, with cells colored by major cell classes. **c**, Bar plot summarizing the cell abundance of each major cell class. **d**, Dot plot showing expression of marker genes across major cell classes in the snRNA-seq data. **e**, UMAP showing the co-embedding snRNA-seq (left) and Xenium (right) cells, with cells colored by major cell classes. **f**, Bar plot displaying cell proportional composition of major cell classes across the optic nerve head and optic nerve in situ derived from Xenium spatial transcriptomic data. **g**, Validation of oligodendrocytes (labeled by CERCAM transcripts) and

astrocytes (labeled by SLC4A11 transcripts) in situ with Xenium explorer browser. **h**, Validation of mural cells (labeled by NOTCH3 transcripts) and endothelial cells (labeled by PTPRB transcripts) in situ with Xenium explorer browser. **i**, Validation of microglia (labeled by CD74 transcripts). **j**, ImageDimPlot showing the spatial distribution of major cell classes cross the optic nerve head (left) and the optic nerve (right) in situ through Xenium spatial transcriptomics.

Figure 2. Chromatin accessibility of the human optic nerve cell atlas

a. UMAPs showing the co-embedding of cells from snATAC-seq (left) and snRNA-seq (right) datasets, colored by major cell classes. **b.** Pie chart showing proportions of major cell classes based on chromatin accessibility profiling. Numbers within each pie chart indicate the number of ATAC-seq cells per class. **c.** Dot plots showing comparison of marker gene activity scores from snATAC-seq (left) and marker gene expression from snRNA-seq (right) across corresponding cell classes. **d.** Bar plot showing the distribution of open chromatin region (OCR) types across major cell classes, colored by genomic regions. X-axis labels indicate cell classes, with total OCR counts shown in parentheses. **e.** Heatmap of 151,001 peak-to-gene links identified across cell classes in the optic nerve. **f.** Scatter plot showing regulon specificity scores of TF regulons across cell classes. The top ten TFs are highlighted in red. **g.** Heatmap showing the 79 identified regulons, grouped as Activators and Repressors. The color key represents TF–gene enrichment scores, and dot size indicates TF–region enrichment.

Figure 3. Identification of oligodendrocytes and their precursors.

a, UMAP of oligodendrocyte precursor cells derived from snRNA-seq data, with cells colored by distinct cell populations. **b,** UMAP of oligodendrocytes derived from snRNA-seq data, with cells colored by distinct cell populations. **c**, Dot plot showing expression of marker genes across precursors and mature oligodendrocytes derived from snRNA-seq. MOL: myelinating oligodendrocyte. **d**, Co-embedding of cells from snATAC-seq (left) and snRNA-seq (right) datasets for oligodendrocytes, colored by cell type labels. **e,** Heatmap showing the identified regulons associated with oligodendrocyte cell types. **f,** PAGA graph with velocity-directed edges showing the lineage progression from precursor cells to mature oligodendrocytes. **g,** Violin plot showing latent times of the precursor populations inferred from transcriptional velocity. **h,** Violin plot showing latent times of the oligodendrocytes inferred from transcriptional velocity. **i,** Validation of oligodendrocyte precursor cells (labeled by *SMOC1* transcripts) with Xenium Explorer. **j,** Scatter plot showing the decline in oligodendrocyte precursor cell proportion along the longitudinal axis of the optic nerve. **k,** In situ validation of

OLIGO2_LRRC7+ cells (labeled by co-localization of *AC110285.1* and *CERCAM* transcripts) in Xenium spatial transcriptomic data visualized with Xenium Explore. **l.** Dim plot showing distribution of OLIGO2_LRRC7+ cells and other oligodendrocytes in situ across the optic nerve. **m**. Bar plot displaying cell proportional composition of OLIGO2_LRRC7+ cells and other oligodendrocytes across optic nerve in situ derived from Xenium spatial transcriptomic data.

Figure 4. Functional state and age-related changes of oligodendrocytes and their precursors.

a, Heatmap showing the biological processes and pathways enriched in each oligodendrocyte precursor population. **b,** Heatmap showing the biological processes and pathways enriched in each oligodendrocyte subtype. **c**, Scatter plot showing cell proportions (Y-axis) of the oligodendrocyte precursor populations associated with age (X-axis). The pearson correlation coefficient (*R*) and *P.adj* from multivariable linear regression (*p*) were indicated. **d**, Box plot showing cell composition across three age ranges within oligodendrocyte populations. *: $P < 0.05$, **: $P<0.01$. **e**, Heatmap showing changes in gene expression (Y-axis) across age (X-axis). **f**, Bar plot showing biological processes enriched in age-related gene modules identified in Fig. 4e. **g**, UpSet plot showing the number of age-related differentially expressed genes (DEG) identified in each oligodendrocyte subtype. **h,** Heatmap showing the biological processes enriched among DEGs up-regulated with age. **i,** Heatmap showing the biological processes enriched among DEGs down-regulated with age.

Figure 5. Identification of astrocytes.

a, UMAP of astrocytes derived from snRNA-seq data, with cells colored by cell populations. **b,** Bar plot showing distribution of sample collection regions across astrocyte populations. **c**, Co-embedding of cells from snATAC-seq (left) and snRNA-seq (right) datasets for astrocytes, colored by subclass labels. **d,** Heatmap showing the identified regulons associated with region-specific astrocyte subclasses. **e.** Image Dim plot showing distribution of region-specific astrocyte subclasses in situ across optic nerve from Xenium data. **f,** Bar plot displaying cell proportional composition of astrocyte subclasses across optic nerve in situ derived from Xenium data. **g,** Validation of Astro_retina in situ (labeled by colocalization of *EBF1, NRP1, and SLC4A11* transcripts) with Xenium Explorer. **h,** Validation of Astro_ONH in situ (labeled by colocalization of *DSP, LRP2, and TMEM132B* transcripts) from Xenium data visualized with Xenium Explorer. **i,** Validation of Astro_ONHON in situ (dominated by *SLC4A11*

transcripts with lowly expressed *EFEMP1* transcripts) and Astro_ON in situ (dominated by *EFEMP1* transcripts) from Xenium data visualized with Xenium Explorer.

Figure 6. Functional state and age-related changes of Astrocytes

a, Heatmap showing the biological processes and pathways enriched in each astrocyte population. **b,** Dot plot showing the expression of genes associated with stemness across astrocyte populations. **c.** Heatmap showing the enrichment of gene modules associated with neuron stem cell states across astrocyte populations. **d**, Box plot showing cell proportions across three age ranges for each astrocyte population. *: $P < 0.05$, **: $P<0.01$. **e**, UpSet plot showing the number of age-related differentially expressed genes (DEG) identified in each astrocyte population. **f**, Heatmap showing the biological processed enriched among DEGs up-regulated with age. **g**, Heatmap showing the biological processed enriched among DEGs down-regulated with aging. **h,** Scatter plot showing cell-cell communication signaling changes in region-specific astrocyte subclasses in ages 20-40 compared with ages < 20 years. **i,** Scatter plot showing cell-cell communication signaling changes in region-specific astrocyte subclasses in ages >70 compared with ages 20-40 years.

Figure 7. Identification of other cell classes and cell niche

a, UMAP of fibroblasts derived from snRNA-seq data, with cells colored by cell types. **b,** Dot plot showing expression of marker genes across fibroblast types in the snRNA-seq data. Bar plot showing distribution of sample collection regions across astrocyte populations. **c**, Box plot showing cell proportions across three age groups for each fibroblast population. **d**, Heatmap showing the biological processed enriched among DEGs up-regulated with age. **e**, Heatmap showing the biological processed enriched among DEGs down-regulated with age. **f,** UMAP of microglia derived from snRNA-seq data, with cells colored by cell populations. **g,** Scatter plot showing cell proportion (Y-axis) of microglia associated with age (X-axis). **h,** UMAP of macrophages derived from snRNA-seq data, with cells colored by cell populations. **i,** Heatmap showing cell class composition for each identified spatial cell niche. **j.** Dim plot showing distribution of cell niches in situ across optic nerve from Xenium data. **k.** Bar plot displaying niche proportional composition across optic nerve in situ derived from Xenium data.

Figure 8. GWAS cell type enrichment and fine-mapping

a, Cell class enrichment of GWAS loci based on snRNA-seq gene expression with MAGMA. Celltyping. Rows represent enriched GWAS traits, and columns represent optic nerve cell classes and cell types. The dot size indicates –log10P. Significant results (FDR < 0.1) are

highlighted in purple, while non-significant results (FDR ≥ 0.1) are shown in grey. **b,** Cell class enrichment of GWAS loci based on snATAC-seq OCRs across major cell classes with LDSC. Rows represent enriched GWAS traits, and columns represent optic nerve cell classes. The dot size indicates $-\log_{10}$FDR. Significant results (FDR < 0.1) are highlighted in yellow, while non-significant results (FDR ≥ 0.1) are shown in grey. **c**, Categorization of fine-mapped GWAS variants according to their genomic locations. Categories include peak (namely, OCRs), linked CREs, DARs, promoter, exon, 5′ UTR and 3′ UTR. **d**, Dot plot showing the gene expression of prioritized GWAS genes. **e**, A fine-mapped POAG GWAS locus, rs7636574, in the *ARHGEF3* region.

Extended Figures

Extended Data Figure 1. The QC matrix of major cell classes in the optic nerve

a, Pie chart showing the proportions of cells derived from different data sources. **b,** Pie chart showing the ancestry distribution of donors. **c,** Pie chart showing the sex distribution of donors. **d**, Histogram showing the age distribution of donors. **e,** UMAP of the transcriptomic cell atlas derived from snRNA-seq data, including major cell classes from both the retina and the optic nerve. Cells are colored by major cell classes. **f,** UMAP of the transcriptomic cell atlas derived from snRNA-seq data, including major cell classes from the optic nerve. Cells are colored by sample tissue sources. **g,** UMAP of the transcriptomic cell atlas derived from snRNA-seq data, including major cell classes from the optic nerve. Cells are colored by sex. **h,** UMAP of the transcriptomic cell atlas derived from snRNA-seq data, including major cell classes from the optic nerve. Cells are colored by ancestry. **i,** UMAP of the transcriptomic cell atlas derived from snRNA-seq data, including major cell classes from the optic nerve. Cells are colored by data source. **j**, Bar plot showing the distribution of data source across major cell classes. summarizing the cell abundance of each major cell class.

Extended Data Figure 2. Benchmarking of the integration method

a, Benchmarking of the integration method in the cell class level. **b,** Benchmarking of the integration method in the cell type level.

Extended Data Figure 3. The sex- and ancestry- associated cell proportion distribution.

a, Box plot showing cell proportion distribution between sexes in optic nerve head samples. **b,** Box plot showing cell proportion distribution between sexes in optic nerve samples. **c,** Box plot showing cell proportion distribution across ancestries in optic nerve head samples. **d,** Box plot showing cell proportion distribution across ancestries in optic nerve samples. P-values

were calculated through multivariable linear regression controlling for potential confounders. *: $P < 0.05$. **: $P < 0.01$. ***: $P < 0.001$.

Extended Data Figure 4. Cell type annotation for Xenium data.
a, Heatmap showing expression of marker genes across cell classes in the Xenium data. **b**, Image Dim plot showing the spatial distribution of cells in the Xenium slide “ONH01”. **c**, Image Dim plot showing the spatial distribution of cells in the Xenium slide “ONH02”. **d**, Image Dim plot showing the spatial distribution of cells in the Xenium slide “PP_1”. **e**, Image Dim plot showing the spatial distribution of cells in the Xenium slide “PP_2”. **f**, Image Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_2”. **g**, Image Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_1”. **h**, Image Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_3”. **i**, Cell proportion distribution across Xenium slides.

Extended Data Figure 5. Annotation of oligodendrocyte populations in Xenium data
a, Heatmap showing expression of marker genes in Xenium data. **b**, Heatmap showing marker gene expression in Xenium data, scaled within each gene. **c**, Dot plot showing marker gene expression in snRNA-seq data. **d**, Dot plot showing marker gene expression in snRNA data, scaled within each gene. **e,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_1”. **f,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_2”. **g,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_3”. **h,** Dim plot showing the spatial distribution of cells in the Xenium slide “PP_1”. **i,** Dim plot showing the spatial distribution of cells in the Xenium slide “PP_2”. **j,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH01”. **k,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH02”.

Extended Data Figure 6. Annotation of astrocyte populations in Xenium data
a, Heatmap showing expression of marker genes in Xenium data. **b**, Heatmap showing marker gene expression in Xenium data, scaled within each gene. **c**, Dot plot showing marker gene expression in snRNA-seq data. **d**, Heatmap showing marker gene expression in snRNA data, scaled within each gene. **e,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_1”. **f,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_2”. **g,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH_3”. **h,** Dim plot showing the spatial distribution of cells in the Xenium slide “PP_1”. **i,** Dim plot showing the spatial distribution of cells in the Xenium slide “PP_2”. **j,** Dim plot showing the spatial

distribution of cells in the Xenium slide “ONH01”. **k,** Dim plot showing the spatial distribution of cells in the Xenium slide “ONH02”. **l**, Dot plot showing the expression of *PIEZO1* and *PIEZO2* across astrocyte populations.

Extended Data Figure 7. Ligand receptor expression in LAMININ-mediated signaling pathway

a, Dot plot showing gene expression of LAMININ ligand genes across cell classes. **b**, Dot plot showing expression of LAMININ receptor genes across astrocyte populations. **c**, Expression (Y-axis) of LAMININ ligand genes and mechanosensor genes associated with age (X-axis) in fibroblast populations. **d**, Expression (X-axis) of LAMININ receptor genes and mechanosensor genes with age (Y-axis) in astrocyte populations.

Extended Data Figure 8. Age-related cell proportion and gene expression change

a, Scatter plot showing cell proportion (Y axis) versus age (X axis). Each dot is a donor sample. Pearson correlation coefficients and P-values were indicated in the plots. **b**, UpSet plot showing the numbers of age-related differentially expressed genes across major cell classes. **c,** Heatmap showing the pathways enriched in genes up-regulated during aging. **d,** Heatmap showing pathways enriched in genes down-regulated during aging.

Extended Data Figure 9. Spatial cell niches across optic nerve

a, The 10 spatial cell niches in the Xenium slide “ONH_1”. **b**, The 10 spatial cell niches in the Xenium slide “ONH_2”. **c,** The 10 spatial cell niches in the Xenium slide “ONH_3”. **d**, The 10 spatial cell niches in the Xenium slide “PP_1”. **e,** The 10 spatial cell niches in the Xenium slide “PP_2”.

Extended Data Figure 10. Expression of reported GWAS genes

Dotplot showing expression of reported GWAS genes associated with glaucoma and IOP across optic nerve cell populations.

References:

1. Salazar, J. J. *et al.* Anatomy of the Human Optic Nerve: Structure and Function. in *Optic Nerve* (IntechOpen, 2018). doi:10.5772/intechopen.79827.

2. Monavarfeshani, A. *et al.* Transcriptomic analysis of the ocular posterior segment completes a cell atlas of the human eye. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2306153120 (2023).

3. Smith, A. M. & Czyz, C. N. Neuroanatomy, Cranial Nerve 2 (Optic). in *StatPearls [Internet]* (StatPearls Publishing, 2022).

4. Jb, S. & Y, C. The optic nerve. *Seminars in neurology* **29**, (2009).

5. London, A., Benhar, I. & Schwartz, M. The retina as a window to the brain—from eye research to CNS disorders. *Nat Rev Neurol* **9**, 44–53 (2013).

6.Fernández Blanco, L. *et al.* Immunopathology of the optic nerve in multiple sclerosis. *Clinical and Experimental Immunology* **209**, 236–246 (2022).

7. Tham, Y.-C. *et al.* Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040. *Ophthalmology* **121**, 2081–2090 (2014).

8. Jayaram, H., Kolko, M., Friedman, D. S. & Gazzard, G. Glaucoma: now and beyond. *The Lancet* **402**, 1788–1801 (2023).

9. Quaranta, L. *et al.* Glaucoma and neuroinflammation: An overview. *Survey of Ophthalmology* **66**, 693–713 (2021).

10. Biousse, V. & Newman, N. J. Diagnosis and clinical features of common optic neuropathies. *The Lancet Neurology* **15**, 1355–1367 (2016).

11. Hamel, A. R. *et al.* Integrating genetic regulation and single-cell expression with GWAS prioritizes causal genes and cell types for glaucoma. *Nat Commun* **15**, 396 (2024).

12. Van Zyl, T. *et al.* Cell atlas of the human ocular anterior segment: Tissue-specific and shared cell types. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2200914119 (2022).

13. Li, J. *et al.* Single-cell atlas of the transcriptome and chromatin accessibility in the human retina. *Nat Genet* **58**, 418–433 (2026).
14. Zuo, Z. *et al.* Single cell dual-omic atlas of the human developing retina. *Nat Commun* **15**, 6792 (2024).
15. Fang, R. *et al.* Conservation and divergence of cortical cell organization in human and mouse revealed by MERFISH. *Science (New York, N.Y.)* **377**, 56 (2022).
16. Sikkema, L. *et al.* An integrated cell atlas of the lung in health and disease. *Nat Med* **29**, 1563–1577 (2023).
17. Choi, J. *et al.* Spatial organization of the mouse retina at single cell resolution by MERFISH. *Nature Communications* **14**, 4929 (2023).
18. Coleman-Belin, J. *et al.* Aging Effects on Optic Nerve Neurodegeneration. *IJMS* **24**, 2573 (2023).
19. Jammal, A. A., Berchuck, S. I., Thompson, A. C., Costa, V. P. & Medeiros, F. A. The Effect of Age on Increasing Susceptibility to Retinal Nerve Fiber Layer Loss in Glaucoma. *Investigative Ophthalmology & Visual Science* **61**, 8 (2020).
20. Cc, S. *et al.* SoxD proteins influence multiple stages of oligodendrocyte development and modulate SoxE protein function. *Developmental cell* **11**, (2006).
21. T, B. *et al.* Transcription factors Sox5 and Sox6 exert direct and indirect influences on oligodendroglial migration in spinal cord and forebrain. *Glia* **64**, (2016).
22. Chelban, V. *et al.* Mutations in NKX6-2 Cause Progressive Spastic Ataxia and Hypomyelination. *American Journal of Human Genetics* **100**, 969 (2017).
23. Cai, J. *et al.* Co-localization of Nkx6.2 and Nkx2.2 Homeodomain Proteins in Differentiated Myelinating Oligodendrocytes. *Glia* **58**, 458 (2010).

24. Zhang, S. *et al.* Sox2 Is Essential for Oligodendroglial Proliferation and Differentiation during Postnatal Brain Myelination and CNS Remyelination. *The Journal of Neuroscience* **38**, 1802 (2018).

25. Birdsey, G. M. *et al.* The Endothelial Transcription Factor ERG Promotes Vascular Stability and Growth through Wnt/β-Catenin Signaling. *Developmental Cell* **32**, 82 (2015).

26. Shah, A. V. *et al.* The endothelial transcription factor ERG mediates Angiopoietin-1-dependent control of Notch signalling and vascular stability. *Nat Commun* **8**, 16002 (2017).

27. Ferdous, A. *et al.* Fli1 Promotes Vascular Morphogenesis by Regulating Endothelial Potential of Multipotent Myogenic Progenitors. *Circulation Research* **129**, 949–964 (2021).

28. Zhang, L., Ge, T. & Cui, J. FLI-1-driven regulation of endothelial cells in human diseases. *J Transl Med* **22**, 740 (2024).

29. Deng, H. *et al.* FOXO1 Integrates Endothelial Hemodynamic, Inflammatory, and Metabolic Pathways in Atherosclerosis. *Circ Res* **138**, e327592 (2026).

30. Wang, Y. *et al.* FoxC1 activates Notch3 signaling to promote the inflammatory phenotype of keloid fibroblasts and aggravates keloid. *Exp Cell Res* **444**, 114402 (2025).

31. Southard, K. M. *et al.* Comprehensive transcription factor perturbations recapitulate fibroblast transcriptional states. *Nat Genet* **57**, 2323–2334 (2025).

32. Sun, W. *et al.* SOX9 Is an Astrocyte-Specific Nuclear Marker in the Adult Brain Outside the Neurogenic Regions. *J Neurosci* **37**, 4493–4507 (2017).

33. Neyrinck, K. *et al.* SOX9-induced Generation of Functional Astrocytes Supporting Neuronal Maturation in an All-human System. *Stem Cell Rev Rep* **17**, 1855–1873 (2021).

34. Saeki, K., Pan, R., Lee, E., Kurotaki, D. & Ozato, K. IRF8 defines the epigenetic landscape in postnatal microglia, thereby directing their transcriptome programs. *Nat Immunol* **25**, 1928–1942 (2024).

35. Masuda, T. *et al.* IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. *Cell Rep* **1**, 334–340 (2012).

36. Jones, R. E., Andrews, R., Holmans, P., Hill, M. & Taylor, P. R. Modest changes in Spi1 dosage reveal the potential for altered microglial function as seen in Alzheimer's disease. *Sci Rep* **11**, 14935 (2021).

37. Sonn, I. *et al.* Single transcription factor efficiently leads human induced pluripotent stem cells to functional microglia. *Inflamm Regen* **42**, 20 (2022).

38. Liao, X. H. *et al.* VEGF-A Stimulates STAT3 Activity via Nitrosylation of Myocardin to Regulate the Expression of Vascular Smooth Muscle Cell Differentiation Markers. *Sci Rep* **7**, 2660 (2017).

39. Vanicek, L. R. *et al.* STAT3 expression is reduced in cardiac pericytes in HFpEF and its loss reduces cellular adhesion and induces pericyte senescence. *FEBS Lett* **599**, 1781–1794 (2025).

40. Simons, M. & Nave, K.-A. Oligodendrocytes: Myelination and Axonal Support. *Cold Spring Harb Perspect Biol* **8**, a020479 (2015).

41. Franklin, R. J. M. & Ffrench-Constant, C. Regenerating CNS myelin - from mechanisms to experimental medicines. *Nat Rev Neurosci* **18**, 753–769 (2017).

42. Rodríguez-Campuzano, A. G., Castelán, F., Hernández-Kelly, L. C., Felder-Schmittbuhl, M.-P. & Ortega, A. Yin Yang 1: Function, Mechanisms, and Glia. *Neurochem Res* **50**, 96 (2025).

43. Yu, Y. *et al.* Olig2 Targets Chromatin Remodelers to Enhancers to Initiate Oligodendrocyte Differentiation. *Cell* **152**, 248–261 (2013).

44. Choi, J. J., Svaren, J. & Wang, D. CoTF-reg reveals cooperative transcription factors in oligodendrocyte gene regulation using single-cell multi-omics. *Commun Biol* **8**, 181 (2025).

45. Li, W.-Y. *et al.* AAV-KLF7 Promotes Descending Propriospinal Neuron Axonal Plasticity after Spinal Cord Injury. *Neural Plasticity* **2017**, 1621629 (2017).

46. Kim, H.-J. *et al.* CREB5 promotes the proliferation and self-renewal ability of glioma stem cells. *Cell Death Discov.* **10**, 103 (2024).

47. Yu, T. *et al.* CREB5 Promotes the Proliferation of Neural Stem/Progenitor Cells in the Rat Subventricular Zone via the Regulation of NFIX Expression. *Cells* **14**, 1240 (2025).

48. Yuan, Y. *et al.* From Physiology to Pathology of Astrocytes: Highlighting Their Potential as Therapeutic Targets for CNS Injury. *Neurosci Bull* **41**, 131–154 (2025).

49. Zhang, Y.-M. *et al.* Astrocyte metabolism and signaling pathways in the CNS. *Front Neurosci* **17**, 1217451 (2023).

50. Verkhratsky, A. *et al.* Astrocytes in human central nervous system diseases: a frontier for new therapies. *Signal Transduct Target Ther* **8**, 396 (2023).

51. Wang, Y. *et al.* SOX2 is essential for astrocyte maturation and its deletion leads to hyperactive behavior in mice. *Cell Reports* **41**, 111842 (2022).

52. Kautzman, A. G. *et al.* Sox2 regulates astrocytic and vascular development in the retina. *Glia* **66**, 623–636 (2018).

53. Sardar, D., Cheng, Y.-T., Szewczyk, L. M., Deneen, B. & Molofsky, A. V. Mechanisms of astrocyte development. in *Patterning and Cell Type Specification in the Developing CNS and PNS* 807–827 (Elsevier, 2020). doi:10.1016/B978-0-12-814405-3.00032-1.

54. Laug, D. *et al.* Nuclear factor I-A regulates diverse reactive astrocyte responses after CNS injury. *J Clin Invest* **129**, 4408–4418 (2019).

55. Tchieu, J. *et al.* NFIA is a gliogenic switch enabling rapid derivation of functional human astrocytes from pluripotent stem cells. *Nat Biotechnol* **37**, 267–275 (2019).

56. Takouda, J., Katada, S., Imamura, T., Sanosaka, T. & Nakashima, K. SoxE group transcription factor Sox8 promotes astrocytic differentiation of neural stem/precursor cells downstream of Nfia. *Pharmacol Res Perspect* **9**, e00749 (2021).

57. Capilla-Gonzalez, V., Cebrian-Silla, A., Guerrero-Cazares, H., Garcia-Verdugo, J. M. & Quiñones-Hinojosa, A. Age-related changes in astrocytic and ependymal cells of the subventricular zone. *Glia* **62**, 790–803 (2014).

58. Doetsch, F., Caillé, I., Lim, D. A., García-Verdugo, J. M. & Alvarez-Buylla, A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703–716 (1999).

59. Balordi, F. & Fishell, G. Mosaic removal of hedgehog signaling in the adult SVZ reveals that the residual wild-type stem cells have a limited capacity for self-renewal. *J Neurosci* **27**, 14248–14259 (2007).

60. Balordi, F. & Fishell, G. Hedgehog signaling in the subventricular zone is required for both the maintenance of stem cells and the migration of newborn neurons. *J Neurosci* **27**, 5936–5947 (2007).

61. Magnusson, J. P. *et al.* Activation of a neural stem cell transcriptional program in parenchymal astrocytes. *eLife* **9**, e59733 (2020).

62. Kremer, L. P. M. *et al.* DNA methylation controls stemness of astrocytes in health and ischaemia. *Nature* **634**, 415–423 (2024).

63. Bernstein, S. L. *et al.* The optic nerve lamina region is a neural progenitor cell niche. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 19287–19298 (2020).

64. Colonna, M. & Butovsky, O. Microglia Function in the Central Nervous System During Health and Neurodegeneration. *Annu Rev Immunol* **35**, 441–468 (2017).

65. Yamasaki, R. Microglia/Macrophages in Autoimmune Demyelinating Encephalomyelitis (Multiple Sclerosis/Neuromyelitis Optica). *Int J Mol Sci* **26**, 3585 (2025).

66. Prinz, M., Masuda, T., Wheeler, M. A. & Quintana, F. J. Microglia and Central Nervous System-Associated Macrophages-From Origin to Disease Modulation. *Annu Rev Immunol* **39**, 251–277 (2021).

67. Sun, R. & Jiang, H. Border-associated macrophages in the central nervous system. *J Neuroinflammation* **21**, 67 (2024).

68. Gharahkhani, P. *et al.* Genome-wide meta-analysis identifies 127 open-angle glaucoma loci with consistent effect across ancestries. *Nat Commun* **12**, 1258 (2021).

69. Springelkamp, H. *et al.* New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics. *Hum Mol Genet* **26**, 438–453 (2017).

70. Khawaja, A. P. *et al.* Genome-wide analyses identify 68 new loci associated with intraocular pressure and improve risk prediction for primary open-angle glaucoma. *Nat Genet* **50**, 778–782 (2018).

71. Khor, C. C. *et al.* Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. *Nat Genet* **48**, 556–562 (2016).

72. Fritsche, L. G. *et al.* A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet* **48**, 134–143 (2016).

73. Zou, Y., Carbonetto, P., Wang, G. & Stephens, M. Fine-mapping from summary data with the “Sum of Single Effects” model. *PLoS Genet* **18**, e1010299 (2022).

74. Cullen, P. F., Gammerdinger, W. J., Ho Sui, S. J., Mazumder, A. G. & Sun, D. Transcriptional profiling of retinal astrocytes identifies a specific marker and points to functional specialization. *Glia* **72**, 1604–1628 (2024).

75. Zhu, Y. *et al.* Astrocytes in the Optic Nerve Are Heterogeneous in Their Reactivity to Glaucomatous Injury. *Cells* **12**, 2131 (2023).

76. Cullen, P. F. & Sun, D. Astrocytes of the eye and optic nerve: heterogeneous populations with unique functions mediate axonal resilience and vulnerability to glaucoma. *Front. Ophthalmol.* **3**, 1217137 (2023).

77. Mazumder, A. G., Julé, A. M. & Sun, D. Astrocytes of the optic nerve exhibit a region-specific and temporally distinct response to elevated intraocular pressure. *Mol Neurodegeneration* **18**, 68 (2023).

78. Mazumder, A. G., Julé, A. M., Cullen, P. F. & Sun, D. Astrocyte heterogeneity within white matter tracts and a unique subpopulation of optic nerve head astrocytes. *iScience* **25**, 105568 (2022).

79. Pandey, S. *et al.* Disease-associated oligodendrocyte responses across neurodegenerative diseases. *Cell Reports* **40**, 111189 (2022).

80. Jäkel, S. *et al.* Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature* **566**, 543–547 (2019).

81. Bernstein, S. L. *et al.* The optic nerve lamina region is a neural progenitor cell niche. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 19287–19298 (2020).

82. Zheng, G. X. Y. *et al.* Massively parallel digital transcriptional profiling of single cells. *Nat Commun* **8**, 14049 (2017).

83. Young, M. D. & Behjati, S. SoupX removes ambient RNA contamination from droplet-based single-cell RNA sequencing data. https://dx.doi.org/10.1093/gigascience/giaa151.

84. McGinnis, C. S., Murrow, L. M. & Gartner, Z. J. DoubletFinder: Doublet Detection in Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. *Cell Systems* **8**, 329-337.e4 (2019).

85. Stuart, T. *et al.* Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902.e21 (2019).

86. Virshup, I., Rybakov, S., Theis, F. J., Angerer, P. & Wolf, F. A. anndata: Access and store annotated datamatrices. *JOSS* **9**, 4371 (2024).

87. Wolf, F. A., Angerer, P. & Theis, F. J. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol* **19**, 15 (2018).

88. Gayoso, A. *et al.* A Python library for probabilistic analysis of single-cell omics data. *Nat Biotechnol* **40**, 163–166 (2022).

89. Liu, A. *et al.* Discovery of optimal cell type classification marker genes from single cell RNA sequencing data. *BMC Methods* **1**, 15 (2024).

90. Korsunsky, I. *et al.* Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat Methods* **16**, 1289–1296 (2019).

91. La Manno, G. *et al.* RNA velocity of single cells. *Nature* **560**, 494–498 (2018).

92. Bergen, V., Lange, M., Peidli, S., Wolf, F. A. & Theis, F. J. Generalizing RNA velocity to transient cell states through dynamical modeling. *Nat Biotechnol* **38**, 1408–1414 (2020).

93. Wolf, F. A. *et al.* PAGA: graph abstraction reconciles clustering with trajectory inference through a topology preserving map of single cells. *Genome Biol* **20**, 59 (2019).

94. Borcherding, N. *et al.* Mapping the immune environment in clear cell renal carcinoma by single-cell genomics. *Commun Biol* **4**, 122 (2021).

95. Barbie, D. A. *et al.* Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* **462**, 108–112 (2009).

96. DeTomaso, D. & Yosef, N. Hotspot identifies informative gene modules across modalities of single-cell genomics. *Cell Syst* **12**, 446-456.e9 (2021).

97. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).

98. Hoffman, G. E. & Schadt, E. E. variancePartition: interpreting drivers of variation in complex gene expression studies. *BMC Bioinformatics* **17**, 483 (2016).
99. Kuleshov, M. V. *et al.* Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research* **44**, W90 (2016).
100. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847–2849 (2016).
101. Jin, S., Plikus, M. V. & Nie, Q. CellChat for systematic analysis of cell–cell communication from single-cell transcriptomics. *Nat Protoc* **20**, 180–219 (2025).
102. Jin, S. *et al.* Inference and analysis of cell-cell communication using CellChat. *Nat Commun* **12**, 1088 (2021).
103. Satpathy, A. T. *et al.* Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nat Biotechnol* **37**, 925–936 (2019).
104. Granja, J. M. *et al.* ArchR is a scalable software package for integrative single-cell chromatin accessibility analysis. *Nat Genet* **53**, 403–411 (2021).
105. Cao, Z.-J. & Gao, G. Multi-omics single-cell data integration and regulatory inference with graph-linked embedding. *Nat Biotechnol* **40**, 1458–1466 (2022).
106. Zhang, Y. *et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**, R137 (2008).
107. Breiman, L. Random Forests. *Machine Learning* **45**, 5–32 (2001).
108. Pedregosa, F. *et al.* Scikit-learn: Machine Learning in Python. *MACHINE LEARNING IN PYTHON*.

109. Bravo González-Blas, C. *et al.* SCENIC+: single-cell multiomic inference of enhancers and gene regulatory networks. *Nat Methods* **20**, 1355–1367 (2023).

110. Mallet: MAchine Learning for LanguagE Toolkit. *Mallet* https://mimno.github.io/Mallet/.

111. Otsu, N. A Threshold Selection Method from Gray-Level Histograms. *IEEE Trans. Syst., Man, Cybern.* **9**, 62–66 (1979).

112. Janesick, A. *et al.* High resolution mapping of the tumor microenvironment using integrated single-cell, spatial and in situ analysis. *Nat Commun* **14**, 8353 (2023).

113. Hao, Y. *et al.* Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat Biotechnol* **42**, 293–304 (2024).

114. Cable, D. M. *et al.* Robust decomposition of cell type mixtures in spatial transcriptomics. *Nat Biotechnol* **40**, 517–526 (2022).

115. Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium *et al.* Genetic identification of brain cell types underlying schizophrenia. *Nat Genet* **50**, 825–833 (2018).

116. De Leeuw, C. A., Mooij, J. M., Heskes, T. & Posthuma, D. MAGMA: Generalized Gene-Set Analysis of GWAS Data. *PLoS Comput Biol* **11**, e1004219 (2015).

117. Murphy, A. E., Schilder, B. M. & Skene, N. G. MungeSumstats: a Bioconductor package for the standardization and quality control of many GWAS summary statistics. *Bioinformatics* **37**, 4593–4596 (2021).

118. Skene, N. G. & Grant, S. G. N. Identification of Vulnerable Cell Types in Major Brain Disorders Using Single Cell Transcriptomes and Expression Weighted Cell Type Enrichment. *Front. Neurosci.* **10**, (2016).

119. Finucane, H. K. *et al.* Heritability enrichment of specifically expressed genes identifies disease-relevant tissues and cell types. *Nat Genet* **50**, 621–629 (2018).

120. Berisa, T. & Pickrell, J. K. Approximately independent linkage disequilibrium blocks in human populations. *Bioinformatics* **32**, 283–285 (2016).

121. Wang, G., Sarkar, A., Carbonetto, P. & Stephens, M. A Simple New Approach to Variable Selection in Regression, with Application to Genetic Fine Mapping. *Journal of the Royal Statistical Society Series B: Statistical Methodology* **82**, 1273–1300 (2020).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryNote.pdf
- SupplementaryTable18.xlsx
- Fig7.pdf
- SupplementaryFigures.pdf
- ExtendedFig.3.pdf
- SupplementaryTable17.xlsx
- ExtendedFig.4.pdf
- SupplementaryTable16.xlsx
- ExtendedFig.6.pdf
- SupplementaryTable19.xlsx
- ExtendedFig.5.pdf
- SupplementaryTable14.xlsx
- ExtendedFig.1.pdf
- SupplementaryTable15.xlsx
- ExtendedFig.8.pdf
- SupplementaryTable111.xlsx
- Fig2.pdf
- SupplementaryTable20.xlsx
- ExtendedFig.9.pdf
- SupplementaryTable13.xlsx
- Fig5.pdf
- SupplementaryTable12.xlsx
- ExtendedFig.10.pdf
- ExtendedFig.7.pdf
- Fig4.pdf
- Fig3.pdf
- ExtendedFig.2.pdf
- Fig6.pdf
- Fig8.pdf
- Fig1.pdf