SUPPLEMENTARY TABLE LEGENDS

Supplementary Table 1. Information about human donor eye tissue processed for snRNA-seq. Hu, human; OD, right eye; OS, left eye; MGH, Massachusetts General Hospital; COD, cause of death; DTP, death to processing time.

Supplementary Table 2. The number of high-quality single nucleus transcriptional profiles obtained for each donor, tissue and cluster.

Supplementary Table 3. Differentially expressed genes in each cluster compared with all other clusters.

Supplementary Table 4. The number, proportion and normalized proportion of single nucleus transcriptional profiles obtained for each cluster.

Supplementary Table 5. Differentially expressed genes between macular and peripheral RPEs. Highlighted genes (in blue) indicate differentially expressed genes that have been reported in previous literature.

Supplementary Table 6. A list of all glaucoma associated genes (grouped as “IOP only”, “POAG only”, “NTG”, “Early Onset”, and “POAG and IOP”) used to calculate scores in in Figs. 7 and S6A.

Supplementary Table 7. Antibodies used for histological studies.

Fig. S1

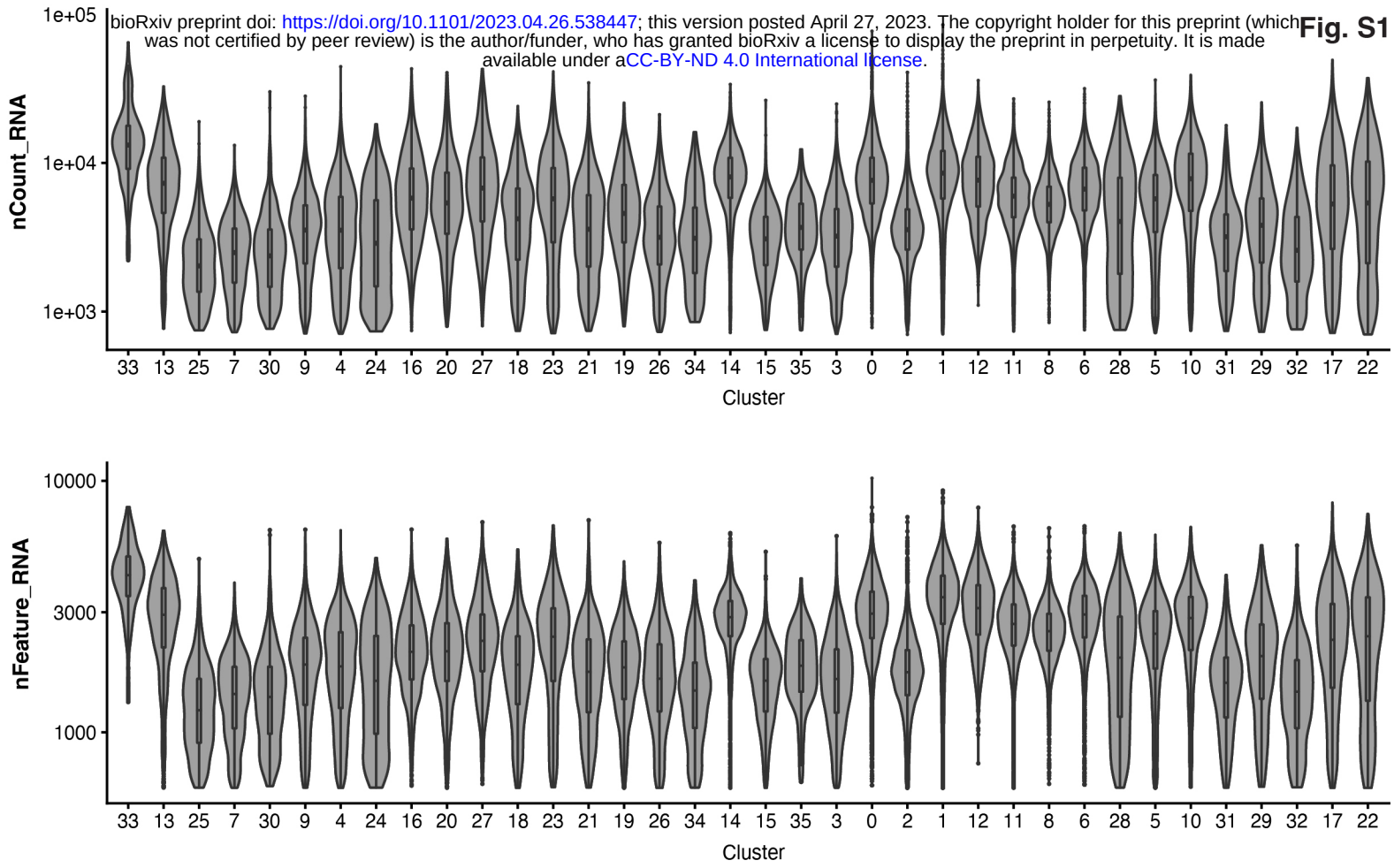


Fig. S1. Violin plot showing the number of transcripts and genes detected in each cluster.

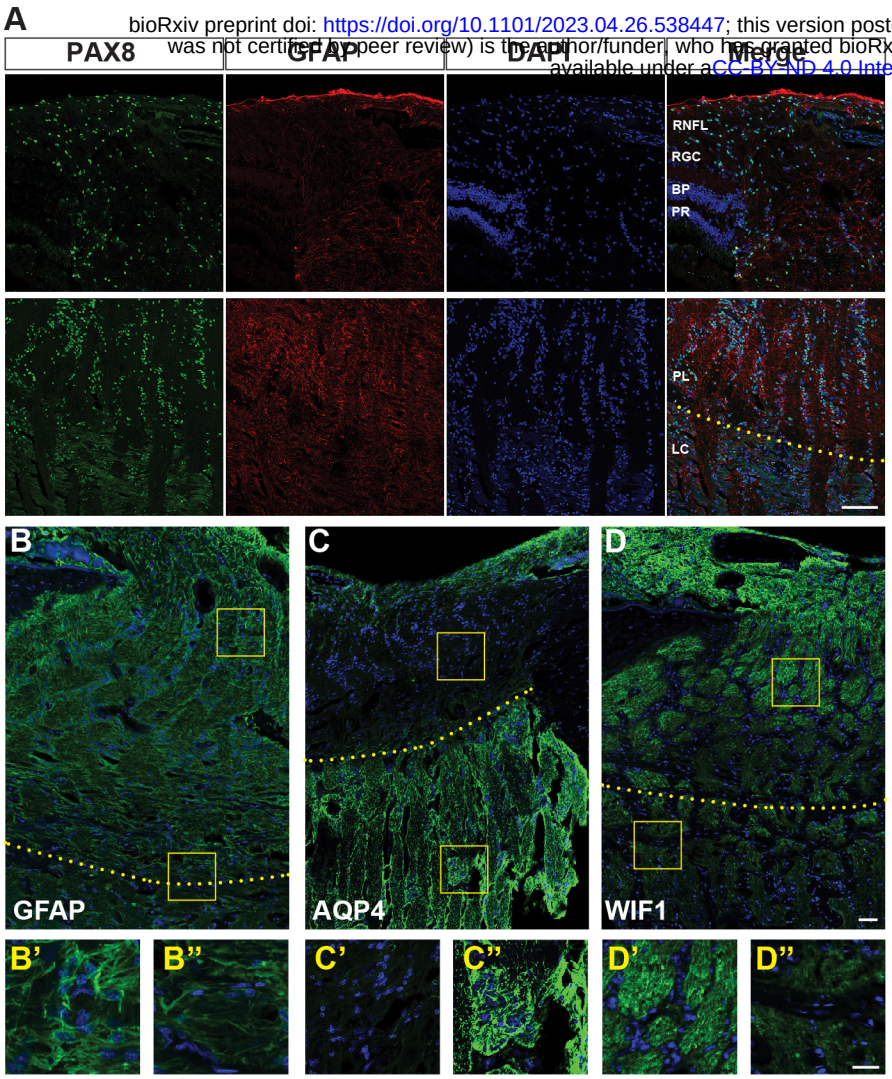
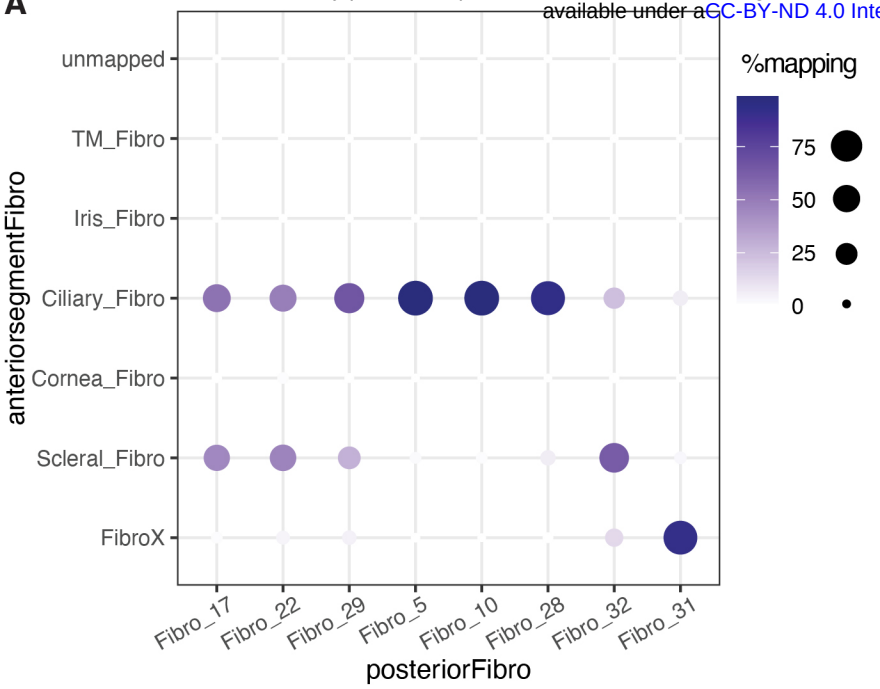
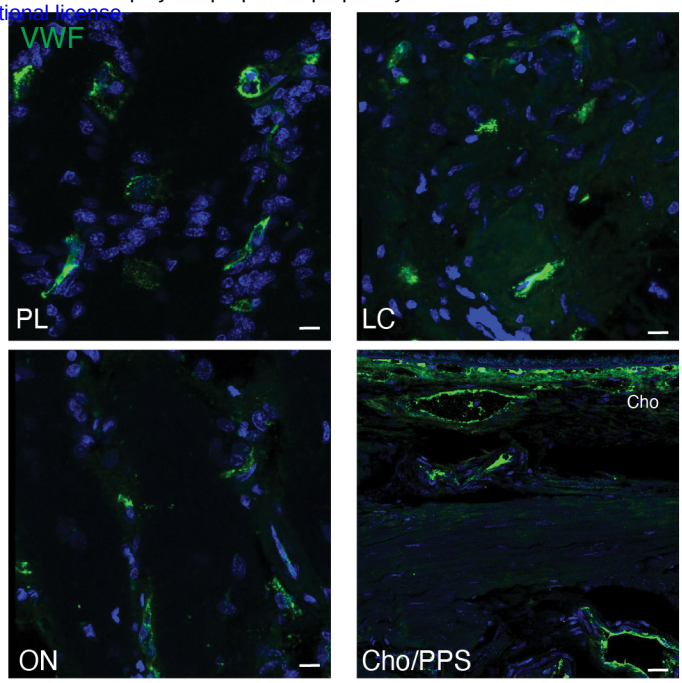


Fig. S2. (**A**) Immunostaining for PAX8 (as a novel pan marker of astrocytes) and GFAP showing distribution of PAX8+ astrocytes in two different regions of ONH tissue. (**B-D**) Immunostaining for GFAP(B), AQP4 (C), and WIF1 (D) highlights heterogeneity of astrocytes in ONH and ON tissues. Boxed areas in top panels are shown at higher magnification in lower panels. Yellow dotted line indicates the upper (in A) and lower (in B-D) border of LC. Bars show 100µm in B-D, and 50µm in magnified boxes B'-D''. RGC, Retinal Ganglion Cells; BP, Bipolars, PR, Photoreceptors; RNFL, Retinal Nerve Fiber Layer; LC, Lamina Cribrosa; PL, Prelaminar region.

A



B



C

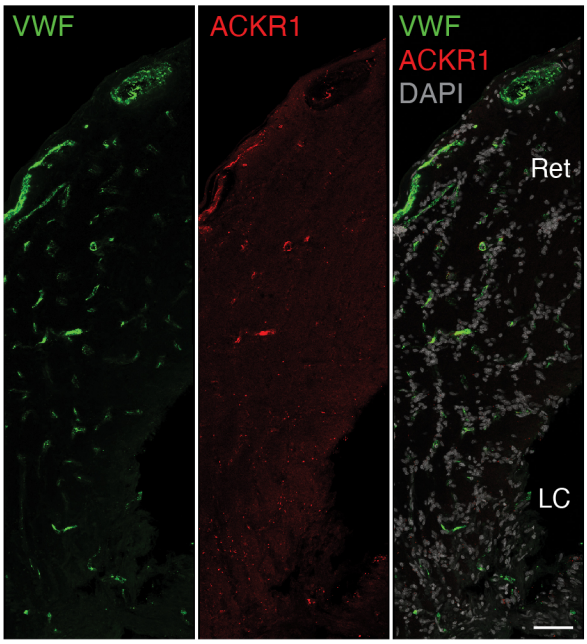
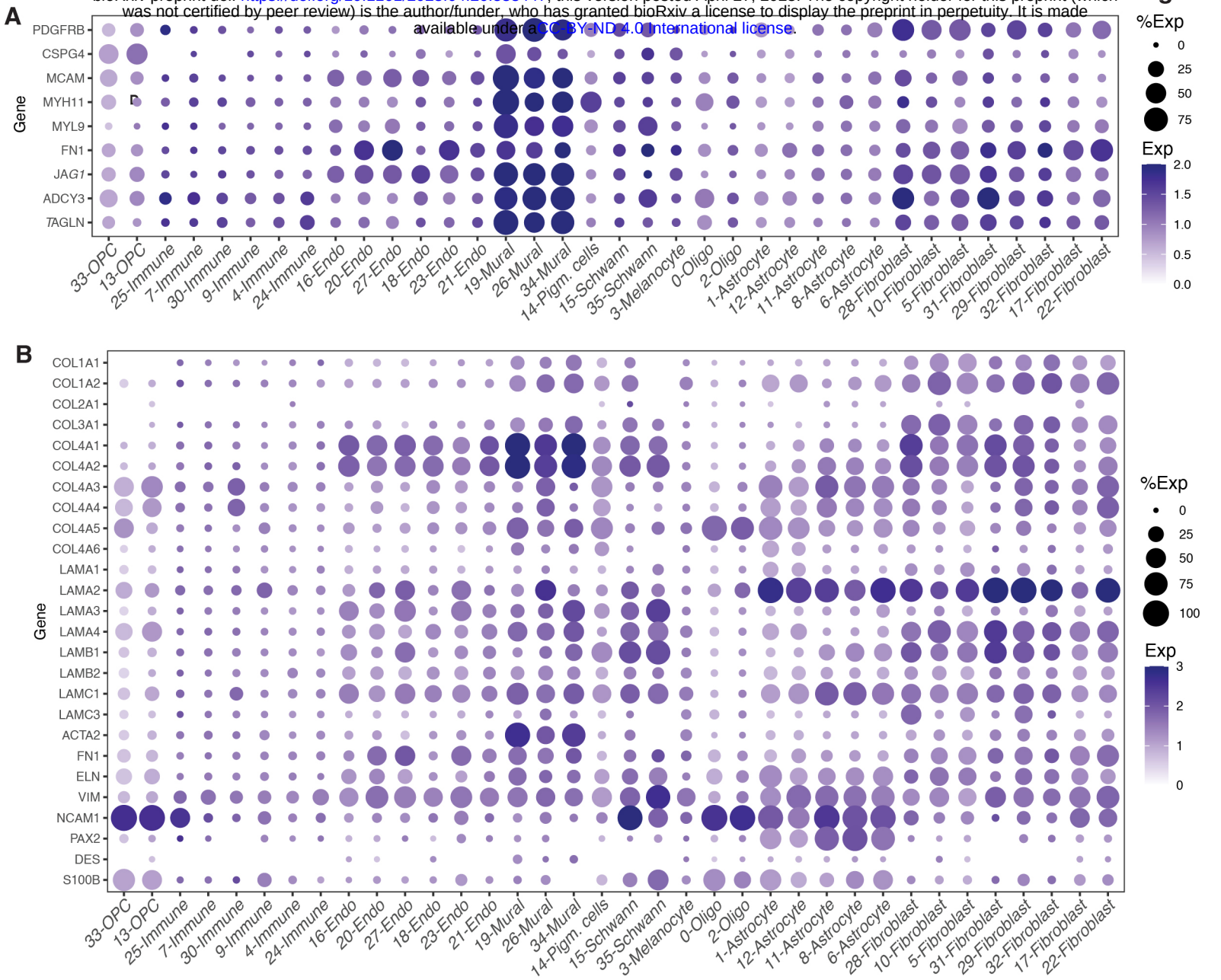
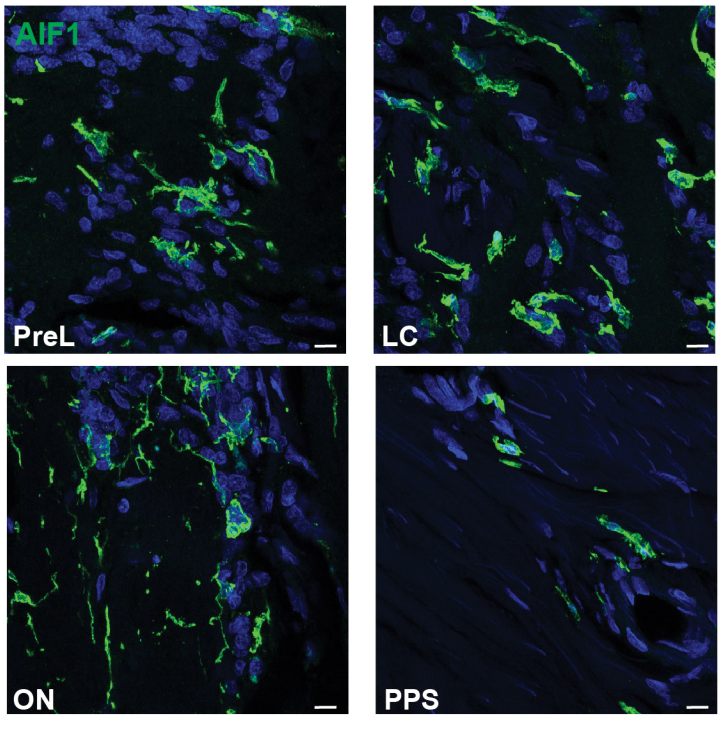


Fig. S3. (**A**) Confusion matrix showing transcriptional correspondence of fibroblasts in the current dataset to those in the anterior segment (van Zyl et al., 2022). (**B**) Immunostaining for VWF in prelaminar region of the ONH (PL), lamina cribosa (LC), optic nerve (ON), and peripapillary sclera (PPS). (**C**) Immunostaining for VWF (green) and ACKR1 (red) in human ONH. Bars show 10µm in B and 100µm in C.





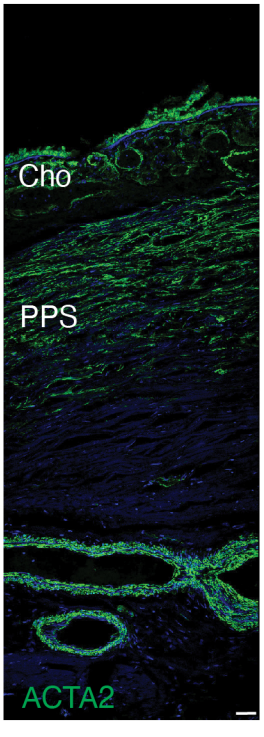


Fig. S4. (**A**) Dot plot showing expression of reported mural cell markers. (**B**) Dot plot showing expression of ECM genes and genes reported to be expressed by LC cells in all clusters. (**C**) Immunostaining for AIF1/IBA1 showing microglia and/or macrophages in ONH, ON, and scleral tissues. (**D**) Immunostaining for ACTA2 (green) in PPS. Bars show 10µm in C and 50µm in D.

Fig. S5

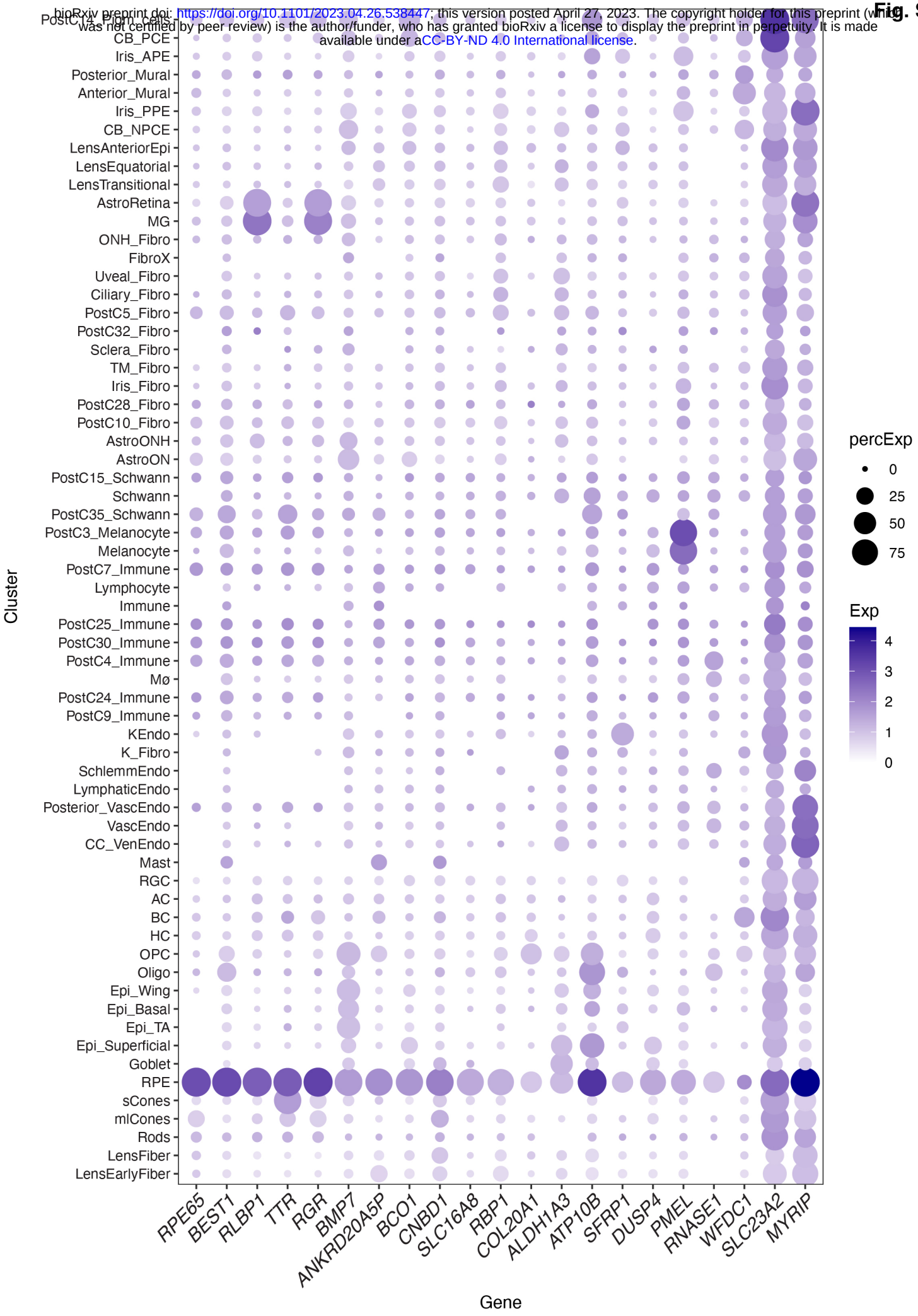


Fig. S5. Dot plot showing expression of known and novel marker genes for RPE.

Fig. S6

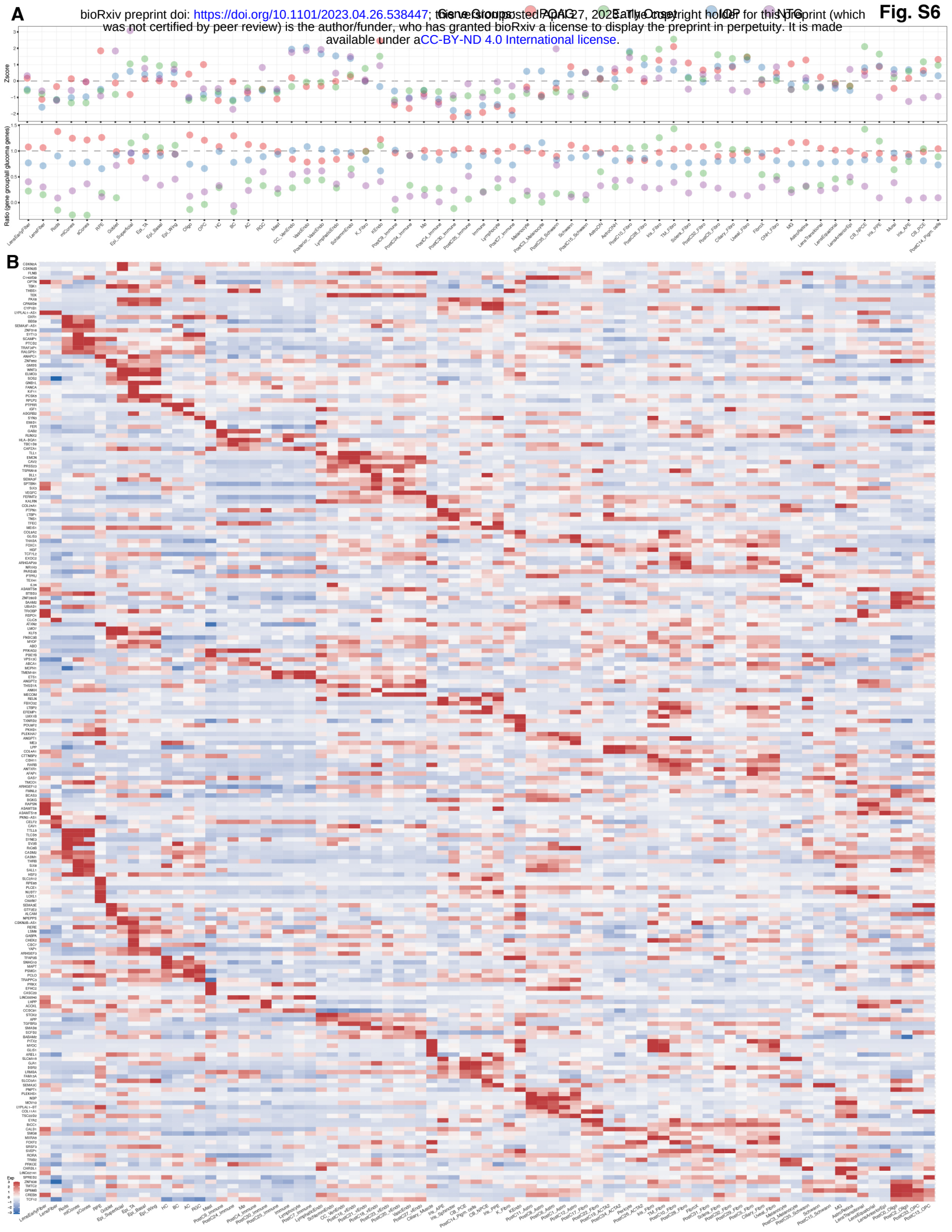


Fig. S6. (**A**) Bubble plot showing cell-type specific enrichment z-scores (top) and ratio score (bottom) of groups of genes that have been associated with POAG but not IOP, IOP but not POAG, normal tension glaucoma, or early onset/congenital glaucoma (**B**) Heat map showing expression of all genes associated with primary open angle glaucoma (POAG) and/or intraocular pressure (IOP). Genes are those identified through GWAS analysis (31) (30) and expression is mapped in cell types characterized in the current study and in the recent analysis of the anterior segment van Zyl et al., (9).