

1 **Integrative single-cell and bulk RNA-seq analysis in human retina identified cell type-specific**  
2 **composition and gene expression changes for age-related macular degeneration**

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23 **Age-related macular degeneration (AMD) preferentially affects distinct cell types and**  
24 **topographic regions in retina. To characterize the impact of AMD on gene expression changes**  
25 **across retinal cell types and regions, we generated both single-cell RNA-seq (scRNA-seq) and**  
26 **bulk RNA-seq data from macular and peripheral retina in postmortem human donors with and**  
27 **without AMD. The scRNA-seq data revealed 11 major cell types with many previously reported**  
28 **AMD risk genes showing substantial cell type and region specificity. Cell type proportional**  
29 **changes with advancing AMD stage were significant for Müller glia, rods, astrocytes, microglia**  
30 **and endothelium.**

31  
32 AMD affects over 10 million Americans<sup>1</sup>, twice the number affected by Alzheimer's disease and equal  
33 to the total of all cancer patients combined<sup>2</sup>. While advances in retinal disease diagnostics have  
34 progressed rapidly, specific treatments for AMD directed at underlying genetic or metabolic defects have  
35 progressed slowly due to limited understanding of disease pathways and cell types involved in the  
36 initiation of AMD. The retina lines the inner surface of the eye and neurally connects to the brain via the

37 optic nerve (**Fig. 1a**). Photoreceptors and their support cells form a vertically organized, tightly integrated  
38 physiologic unit (**Fig. 1b**). AMD is a disease of this unit, with secondary effects including gliosis, cell  
39 death and synaptic circuitry corruption in inner retina<sup>3-5</sup>. Given the complexity of the retinal cell structure,  
40 there is an urgent need to identify cells contributing to the spectrum of AMD pathology.

41

42 Recent technologic breakthroughs in scRNA-seq make it possible to measure gene expression in single  
43 cells, resolve cell types, characterize the signature of gene expression across cells, and improve  
44 understanding of cellular function in health and disease<sup>6-9</sup>. We performed scRNA-seq on macula and  
45 peripheral retina from two postmortem normal eyes. In total, we obtained 36,959 macular and 55,426  
46 peripheral cells from the retina. Unsupervised deep learning based clustering identified 11 broadly  
47 defined cell types (**Fig. 1c**). Although some neuronal cell types, such as bipolar, amacrine, and ganglion  
48 cells can be further subdivided, we omitted further sub-clustering and maintained major cell types.

49

50 We assessed the cell-type specificity of 75 AMD GWAS<sup>6</sup> and transcriptome-wide association study  
51 (TWAS)<sup>10</sup> risk genes (**Fig. 1e, Methods and Supplementary Fig. 4**). Of 75 AMD risk genes, 23 showed  
52 cell type-specific expression either in macula or peripheral retina in the scRNA-seq data (**Methods,**  
53 **Supplementary Data 2**). For example, *MMP9* is specifically expressed in cones, and *PILRA* and *HLA-*  
54 *DQB1* are preferentially expressed in microglia. Further, 41 AMD risk genes have significant differential  
55 expression (DE) for retinal location (adjusted  $P < 0.05$ ) across 11 cell types.

56

57 To investigate the impact of AMD on cell types, we sequenced total RNA from macula and peripheral  
58 regions of 15 postmortem retinas that included normal, early and advanced AMD stages. Retinas were  
59 phenotyped by *ex vivo* fundus imaging and fellow-eye histology at The University of Alabama at  
60 Birmingham (UAB). Analysis identified 9,772 and 1,214 differentially expressed genes (DEGs) in macula  
61 and periphery for normal vs late AMD comparison (**Supplementary Data 3**). Interestingly, the DEG  
62 analysis between normal and early AMD found 169 DEGs in periphery and 21 genes in macula. We  
63 expected to see more DEGs in the macula than periphery and suspect that the larger sample size and  
64 higher sequencing depth in the peripheral retina samples increased the power. Among DEGs identified  
65 in macula from either comparison, 1202 (12.3%) are cell type specific, and 183 (14.6%) DEGs identified  
66 in periphery show cell type specificity (**Methods, Supplementary Fig. 5b and Supplementary Note 6**).  
67 Interestingly, we also found 17 DEGs for macula that may associate with AMD progression, as indicated  
68 by their increased fold change from early AMD to late AMD when compared to normal (**Supplementary**  
69 **Data 3**). Three of the AMD progression-associated DEGs show cell type-specificity, including *RAB41*  
70 (Cone), *ZMYND19* (Rod) and *COL4A3* (Müller glia).

71

72 It is known that AMD also has an impact on cell type composition of the retina, particularly in macula<sup>11,12</sup>.  
73 To characterize such changes in cell type composition, we estimated cell type proportions for each bulk  
74 RNA-seq sample by cell type deconvolution analysis using MuSiC in which the scRNA-seq data was  
75 used as a reference<sup>13</sup>. First, we considered a large bulk RNA-seq dataset generated by the EyeGEx  
76 study<sup>10</sup>, which includes 453 RNA-seq samples generated from peripheral retina in postmortem human  
77 donors. This dataset includes samples at different AMD stages based on the Minnesota Grading System  
78 (MGS) (MGS1: 105; MGS2: 175; MGS3: 112; MGS4: 61). For the normal eyes (MGS1), our  
79 deconvolution analysis revealed a noticeable proportion of rod photoreceptors (mean proportion=0.58)  
80 and Müller glia (mean proportion=0.14), but relatively small proportions ( $0.01 < \text{mean proportion} < 0.1$ ) of  
81 ganglion cells, cones, amacrine, bipolar, astrocytes and horizontal cells (**Fig 2a**). As AMD progresses  
82 (from MGS 2 to MGS 4) rods decrease. In contrast, the proportion of astrocytes increase, possibly  
83 reflecting an immune response<sup>14</sup> of the peripheral retina to AMD.

84  
85 The EyeGEx dataset is based is restricted to peripheral retina. Since AMD preferentially affects macula,  
86 we performed cell type deconvolution in our sample set which includes both macula and peripheral  
87 retina. In our peripheral retina samples, we found a decrease in rods and increase in astrocytes as AMD  
88 progresses, consistent with the EyeGEx data (**Fig 2b**). In macula, rods show a slightly decrease from  
89 normal to early AMD and a sharp decline from early to advanced AMD (**Fig. 2c**). Endothelium, astrocytes  
90 and microglia proportions increased in the macula when progressing from normal to advanced AMD.  
91 Rods are barely detectable in the macula of advanced AMD (**Fig. 2d and Supplementary Fig. 6**), in  
92 agreement with histological reports<sup>11,15</sup>.

93  
94 As bulk RNA-seq measures the average expression of genes (sum of cell type-specific gene expression  
95 weighted by cell type proportions), DEGs from bulk RNA-seq can result from changes in cell type-  
96 specific gene expression, as well as cell type composition. To determine if DE in the bulk RNA-seq  
97 samples were due to cell type-specific DE and not change in cell type composition, we developed a  
98 calibration-based method to detect cell type-specific DEGs (ctDEGs) by calibrating bulk level gene  
99 expression using cell type-specific marker genes from the scRNA-seq data (**Methods**). Applying this  
100 method to the EyeGEx peripheral retina data, we detected AMD associated ctDEGs for each of the 11  
101 major cell types. Across all cell types we identified 109 ctDEGs for MGS2 vs. MGS1, 201 ctDEGs for  
102 MGS4 vs. MGS1 (**Supplementary Data 4**), Fifty-one ctDEGs share the same cell type specificity for all  
103 comparisons. Only three ctDEGs were detected in astrocytes when comparing MGS3 vs. MGS1,  
104 possibly due to phenotype heterogeneity of the MGS3 samples. The largest set of cell type-specific  
105 DEGs were identified for microglia, 32 genes detected in MGS2 vs. MGS1 comparison, 82 genes  
106 detected for the MGS4 vs. MGS1 comparison (**Fig. 3a**), while 21 genes are in common and sharing the  
107 same directions of DE effect between two comparisons. Noticeably, for these 21 microglia-specific DEGs

108 shared by two comparisons, the degree of fold change is generally higher in MGS4 vs. MGS1 than in  
109 MGS2 vs. MGS1, especially for *FCGBP* and *HLA-DME* (**Fig. 3b and Supplementary Fig. 8**). The  
110 increased expression of these genes in MGS4 reflects microglia-specific AMD response with disease  
111 progression. We observed a similar tendency for astrocytes- and endothelium-specific DEGs  
112 (**Supplementary Fig. 8**). We further performed Gene Ontology (GO) enrichment analysis on cell type-  
113 specific DEGs identified between MGS4 vs. MGS1. The results revealed distinct functional enrichment  
114 for up- and down-regulated genes (**Supplementary Data 5**); for example, microglia-specific up-  
115 regulated genes are enriched for immune response (adjusted  $P = 5.34 \times 10^{-17}$ ), antigen processing and  
116 presentation of peptide antigen (adjusted  $P = 8.95 \times 10^{-14}$ ) and innate immune response (adjusted  $P =$   
117  $2.11 \times 10^{-12}$ ), while down-regulated genes are enriched for nuclear-transcribed mRNA catabolic process  
118 and nonsense-mediated decay (adjusted  $P = 5.27 \times 10^{-14}$ ), and establishment of protein localization to  
119 endoplasmic reticulum (adjusted  $P = 7.65 \times 10^{-13}$ ).

120

121 To investigate the cell type-specific impact of AMD on macular retina, we applied our calibration-based  
122 ctDEG detection method to the UAB data. Due to the limited sample size and moderate alterations in  
123 expression pattern in early AMD, 7 and 13 ctDEGs were identified in macula and periphery, respectively,  
124 when comparing normal and early AMD (**Supplementary Data 6**). In contrast, a larger number of  
125 ctDEGs were detected when comparing normal and late AMD, with 169 ctDEGs found in periphery and  
126 1,458 ctDEGs in macula (**Fig. 3c**). Among the 160 ctDEGs detected in periphery, a considerable number  
127 of them are microglia- ( $n=39$ ) and endothelium-specific ( $n=30$ ), which replicate the results in the EyeGEx  
128 periphery data. For macular retina, larger numbers (proportions of analyzed genes) of ctDEGs were  
129 identified for amacrine, bipolar, rod, cone and ganglion cells (**Fig. 3c**). This reflects the focus of AMD  
130 degeneration in the macula. Comparing DE of ctDEGs identified in the two regions we noticed a  
131 coordinated gene expression change between macula and periphery for immune related cell types  
132 (astrocytes and microglia) and photoreceptors (cones and rods) which indicate a greater impact on  
133 these cell types in the macula (**Supplementary Note 7 and Supplementary Fig 9**). Further, GO  
134 enrichment analysis on cell type-specific DEGs revealed that up- and down- regulated genes had  
135 distinct biological functions (**Supplementary data 7**). For example, microglia-specific up- and down-  
136 regulated DEGs between normal vs. late AMD identified in macula show a similar functional enrichment  
137 pattern as in the EyeGEx peripheral retina data (**Supplementary Data 5 and 7**). In particular, 41 rod  
138 specific up-regulated genes are enriched for negative regulation of cell death (adjusted  $P = 1.13 \times 10^{-2}$ )  
139 and negative regulation of apoptotic process (adjusted  $P = 3.56 \times 10^{-2}$ ), whereas the 85 down-regulated  
140 genes are enriched for visual perception (adjusted  $P = 2.25 \times 10^{-40}$ ), sensory perception of light stimulus  
141 (adjusted  $P = 3.65 \times 10^{-40}$ ), and detection of light stimulus (adjusted  $P = 2.11 \times 10^{-33}$ ) (**Fig. 3d**). These  
142 results reveal an impact on transcriptional profiles in different cell types that are common between  
143 macula and periphery, as well as transcriptional changes that are specific to each region.

144

145 In summary, we constructed a high-resolution human retina cell atlas with a particular focus on a  
146 comparison of regional differences. Our results linked GWAS genes for AMD with cell type-specific gene  
147 expression and enabled the use of GWAS data to inform the genetic architecture of AMD. We further  
148 leveraged scRNA-seq and bulk RNA-seq data, and our integrative analysis revealed both cell type-  
149 specific composition as well as gene expression changes associated with AMD progression. Our  
150 ongoing studies will aim to increase AMD sample size and add data from the RPE and choroid. Findings  
151 will overall provide novel insights into cell type-specific functions that can power precision therapeutic  
152 targeting of AMD.

153

154

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160

### 161 **Author contributions**

162 This study was conceived of and led by M.L. and D.S.. Y.L., R.Z., N.D., K.W., and Z.M. performed data  
163 analysis with input from M.L., D.S., and C.A.C.. C.S., S.L., and P.G. generated the human retina scRNA-  
164 seq data. N.P., Y.L. and M.L. created R Shiny apps for data visualization. Y.L. and M.L. wrote the paper  
165 with feedback from D.S., R.Z., C.A.C., K.W., Z.M., C.S., P.G..

166

167 **Figure Legends**

168

169 **Figure 1.** Summary of single-cell analysis from human retina. **(a)** Schematic cross-section of human  
170 eye (top) showing the retina lining the interior surface. The macula contains the fovea and is responsible  
171 for sharp vision. The periphery is responsible for detecting light and motion. Schematic of dissected  
172 tissue (bottom) shows retina adjoined to support tissues, flattened with relaxing cuts. Areas 8 mm in  
173 diameter were excised for RNA sequencing. **(b)** Layers of human retina and supporting tissues showing  
174 11 assayed cell types. Five neuronal classes are photoreceptors, bipolar cells, ganglion cells, horizontal  
175 and amacrine cells. Cone photoreceptors are sensitive to color and bright light. Rod photoreceptors are  
176 sensitive to low light. Ganglion cells transmit information to the brain. Horizontal cells and amacrine cells  
177 modulate signal from photoreceptors and bipolar cells, respectively. Müller glia span the retina and are  
178 involved in neurotransmission, fluid balance, and wound repair. Also depicted are microglia (with  
179 phagocytic and immune activity), astrocytes (regulation of metabolism and blood brain barrier,  
180 synaptogenesis, neurotransmission), vascular endothelium (vascular tone and blood flow; coagulation  
181 and fibrinolysis; immune response, inflammation and angiogenesis) and pericytes (integrity of  
182 endothelial cells, trans-regulation of vascular tone, stem cells). The retinal layers include: NFL, nerve  
183 fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer  
184 plexiform layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium; BrM, Bruch's membrane;  
185 ChC, choriocapillaris. The last three are shown for completeness and were not assayed. **(c)**  
186 Visualization of single-cell clusters using t-SNE. Cells are colored by cell types. **(d)** Visualization of  
187 single-cell clusters using t-SNE. Cells are colored by region. Cells from macular and peripheral retina  
188 were randomly mixed, suggesting the absence of batch effect. **(e)** Heatmap showing expression levels  
189 of AMD risk genes by cell type. Color in the heatmap represents expression intensity with red signifying  
190 higher expression in units of z-score. Left panel: AMD associated genes identified by loss- or gain-of-  
191 function mutations or by GWAS<sup>6</sup>. Right panel: target genes based on TWAS analysis listed<sup>10</sup>.

192

193 **Figure 2.** Cell type deconvolution analysis from bulk RNA-seq data. Cell type proportions for each bulk  
194 RNA-seq sample were estimated using MuSiC with the scRNA-seq data as reference. **(a)** Estimated  
195 cell type proportions for the EyeGEx peripheral retina bulk RNA-samples with four stages of AMD (MGS1:  
196 105; MGS2: 175; MGS3: 112; MGS4: 61). **(b)** Estimated cell type proportions for the UAB peripheral  
197 retina bulk RNA-seq samples (normal: 8; early AMD: 4; late AMD: 3). **(c)** Estimated cell type proportions  
198 for the UAB macular retina bulk RNA-seq samples (normal: 6; early AMD: 4; late AMD: 3). Note the  
199 similarity in **(a)** and **(b)** with respect to cell proportion increase in astrocytes and decrease in rods in  
200 peripheral retina as AMD progresses. Larger differences are noted in both cell types in macula along



201 with additional increases in Müller glia, microglia and vascular endothelium as AMD progresses. **(d)** Cell  
202 type proportion changes in the UAB macula retina samples for highlighted cell types.

203

204 **Figure 3.** Cell type-specific differential expression analysis. **(a)** Proportions of up- and down-regulated  
205 ctDEGs detected identified in the EyeGEx peripheral retina data. Colors show different test conditions  
206 used in the DE analysis; red: MGS2 vs. MGS1, green: MGS4 vs. MGS1. **(b)** Volcano plots and effect  
207 size comparison of microglia-specific DEGs identified using EyeGEx peripheral retina data. Left/Mid:  
208 Volcano plots of microglia-specific DEGs identified from two different tests. Significant ctDEGs are  
209 highlighted using red color and ctDEGs with large effect size are annotated. Right: effect size  
210 comparison of microglia specific DEGs. X-axis: effect size of ctDEGs identified between MGS2 vs.  
211 MGS1; y-axis: effect size of ctDEGs identified between MGS4 vs. MGS1. ctDEGs with increased effect  
212 size are annotated. **(c)** Proportions of up- and down-regulated ctDEGs between normal vs. late AMD  
213 identified in UAB retina data. Colors show different retina regions in the DE analysis; red: periphery,  
214 green: macula. **(d)** Representative GO terms of up- and down- regulated ctDEGs genes identified  
215 between normal vs. late AMD using UAB data. The complete table of GO analysis result can be found  
216 in **Supplementary Data 5 and 7.**

217

## 218 **Methods**

219

### 220 **Study subjects, scRNA-seq and bulk RNA-seq for the UAB data**

221 The scRNA-seq data were generated from macular and peripheral retina taken from two healthy adult  
222 donors using the 10X Genomics Chromium<sup>TM</sup> system. The bulk RNA-data were generated from 13  
223 macula samples (6 normal, 4 early AMD, and 3 late AMD) and 15 periphery samples (8 normal, 4 early  
224 AMD, and 3 late AMD) taken from the retina of 15 adult donors. All donor eyes were collected and  
225 characterized within 6 hours postmortem for presence of AMD and other pathology by author C.A.C.  
226 and a consulting medical retina specialist. Detailed sample preprocessing, donor characteristics,  
227 scRNA-seq and bulk RNA-seq data generation can be found in **Supplementary Note 1**.

228

### 229 **EyeGEx bulk RNA-seq data**

230 The Eye Genotype Expression (EyeGEx) study was designed to explore genetic landscape and post-  
231 GWAS interpretation of multifactorial ocular traits<sup>10</sup>. This study generated bulk RNA-seq data of 523  
232 peripheral retinal samples from postmortem human donors. We obtained the EyeGEx bulk RNA-seq  
233 data from the Gene Expression Omnibus (accession number GSE115828). This dataset includes gene  
234 expression measures for 523 samples and 58,051 genes. 453 of the samples with AMD phenotype  
235 information (MGS1: 105; MGS2: 175; MGS3: 112; MGS4: 61) were included in the analysis<sup>16</sup>. Genes  
236 that were expressed in less than 20% of the samples were eliminated, resulting in 14,709 genes in  
237 downstream analyses.

238

### 239 **scRNA-seq data clustering and cell type identification**

240 To identify cell types in the scRNA-seq data, we clustered cells into distinct cell types using DESC, a  
241 deep learning based clustering algorithm that is robust to batch effect<sup>17</sup>. To prepare the data for DESC  
242 clustering, the original gene count matrix obtained from CellRanger was normalized in which the UMI  
243 count for each gene in each cell was divided by the total number of UMIs in the cell. The normalized  
244 UMI count data were then multiplied by 10,000 and transformed to a natural log scale. We further  
245 standardize the log-transformed expression value for each gene by calculating a Z-score across cells  
246 within each batch. Lastly, 2,000 highly variable genes selected using *filter\_genes\_dispersion* function  
247 from the Scanpy package<sup>18</sup> were used as input for DESC clustering. In DESC analysis, we used a 2-  
248 layer autoencoder with 64 nodes on the first layer and 32 nodes on the second layer. The DESC  
249 clustering was performed using a grid of resolutions, and resolution = 0.4 was selected because it yields  
250 high maximum cluster assignment probability for most of the cells. DESC initially identified 18 cell  
251 clusters and 16 of them that contain more than 50 cells were kept for downstream analyses. We  
252 annotated these 16 cell clusters with cell type labels by examining expression patterns of known retina  
253 cell type markers (**Supplementary Data 8**). We further performed pairwise differential expression



254 analysis among cell clusters, and cell clusters with the same cell type annotation and very few  
255 differentially expressed genes were merged (**Supplementary Note 2 and Supplementary Fig. 2**). This  
256 procedure resulted in 11 major neuronal cell types, including cone photoreceptors, rod photoreceptors,  
257 bipolar cells, horizontal cells, amacrine cells, and ganglion cells; support cells (microglia, Müller glia,  
258 astrocytes), and vascular cells (endothelium, pericytes).

259

260 We are aware of that some of the cell types we identified, such as cone, rod and ganglion, are commonly  
261 called cell classes,<sup>19</sup> since each of them includes multiple (sub) types of cells with different expression  
262 patterns. However, to simplify the analysis of neural and non-neural cells, we use **cell type** to signify  
263 both **cell types** and **cell classes** in our data.

264

265

### 266 **t-SNE visualization for single-cell clustering**

267 To visualize cell type clusters from the scRNA-seq data, we generated a two-dimensional non-linear  
268 embedding of the cells using t-distributed Stochastic Neighbor Embedding (t-SNE)<sup>20</sup>. The low  
269 denominational representation of the original data from DESC were used as input. The algorithm was  
270 implemented using the *mTSNE* function from python package MulticoreTSNE<sup>21</sup>. We set perplexity = 50  
271 and learning rate = 500 and used the default values for all other parameters.

272

### 273 **Cell type- and region-specific expression of AMD risk genes**

274 We obtained AMD risk genes from previous studies, which include 51 AMD associated GWAS genes  
275 from Peng et al. 2019<sup>6</sup> and 26 target genes identified from TWAS analysis by Ratnapriya et al. 2019<sup>10</sup>.  
276 A gene that meets the following criteria was included for downstream analysis: 1) expressed in at least  
277 1% of the cells; 2) expressed in at least 10 cells for at least one cell type in the scRNA-seq data. In total,  
278 46 AMD associated genes and 22 TWAS target genes met these criteria. For these 68 genes, we tested  
279 whether they have significant high expression levels in a particular retina region and cell type(s). To test  
280 the region specificity, for each cell type, we tested whether these genes are differentially expressed  
281 between two retina regions. The analysis was conducted using the *FindAllMarkers* function in the R  
282 Seurat package<sup>22</sup> with the Wilcoxon test. Benjamini-Hochberg (BH)<sup>23</sup> adjusted p-value < 0.05 was used  
283 as threshold. To examine the cell type specificity, for each region, we compared AMD risk gene list to  
284 the identified cell type-specific genes and counted the overlap (**Supplementary Note 3 and**  
285 **Supplementary Data 1**).

286

287 To visualize cell type and region-specific expression, for each AMD risk gene, we calculated the mean  
288 expression across cells for each of the 11 major cell types for macular and peripheral retina separately.  
289 If a gene is expressed in less than 1% or 15 cells in a particular cell type, the mean expression of this

290 gene in this cell type will be set as 0. Genes that have 0 mean expression across all cell types will be  
291 removed from further analysis. To make cell type-wise mean expressions comparable across genes, we  
292 calculated z-score of cell type mean expressions for each gene, and visualized the z-scores using  
293 heatmap (**Fig. 1e**).

294

### 295 **DEG detection in bulk RNA-seq data**

296 Differential expression analysis for bulk RNA-seq data was performed using DESeq2 (v1.22.2)<sup>24</sup>. For  
297 the EyeGEx data, the filtered RSEM count matrix (14,709 genes by 453 samples) was used as input.  
298 Differential expression analysis was performed between normal vs. AMD samples defined by three  
299 different MGS levels. Also, sex was included as a covariate in the analysis. For the UAB data, we  
300 detected DEGs for macula and periphery separately. Genes that were expressed in less than 20% of  
301 the samples were eliminated, resulting in 19,313 genes in downstream analyses. The filtered read count  
302 matrices (19,313 genes by 13 samples for macula; 19,313 genes by 15 samples for periphery) were  
303 used as input. For each retina region, we detected DEGs between normal vs. early and normal vs. late  
304 AMD. All parameters for DESeq2 were set as default. We used BH adjusted p-value < 0.05 as  
305 significance threshold to correct for multiple testing. The significant DEGs are reported in  
306 **Supplementary Data 3**.

307

308 Further, we also examined whether these DEGs are cell type specific. For each retina region, we  
309 counted the overlap between identified DEGs and cell type-specific genes. Then we reported the  
310 proportions of cell type specific ones in AMD associated DEGs.

311

### 312 **Cell type deconvolution in bulk RNA-seq data**

313 We performed cell type deconvolution analysis for both the EyeGEx and UAB bulk RNA-seq data using  
314 the UAB scRNA-seq data as the reference. For the scRNA-seq data, we only kept genes that were  
315 expressed in at least 5% of cells and more than 10 cells in at least one cell type. Cell type deconvolution  
316 analysis was conducted using MuSiC<sup>13</sup> by setting  $\text{eps} = 0.0001$ ,  $\text{iter.max} = 1,000$  and default values for  
317 all other parameters. Also, we used the collection of 1,701 cell type-specific marker genes as reference  
318 genes in the deconvolution (**Supplementary Note 3 and Supplementary Data 1**).

319

### 320 **Detection of cell type-specific DEGs using calibrated gene expression**

321 Our analysis shows AMD may have specific impact on particular cell types. We are interested in  
322 detecting differential expression between normal and AMD eyes for different cell types separately.  
323 However, the bulk RNA-seq data with both normal and AMD subjects lack cell type level information. To  
324 bypass such limitation, we developed a procedure to detect cell type-specific DEGs using bulk RNA-  
325 seq data calibrated by cell type proportion, which can be obtained from scRNA-seq data.

326

327 Consider a scenario in which we aim to calculate fold change of gene expression between two conditions  
328 for a particular cell type. Let  $Y_{gi}$  denote the bulk RNA-seq expression for gene  $g$  in sample  $i$ .  $Y_{gi}$  is a  
329 weighted sum of cell type level gene expression,

330

$$Y_{gi} = \sum_{j=1}^C p_j^k X_{gij} \quad i \in S_k \quad (1)$$

331 where  $p_j^k$  is the proportion of cell type  $j$  ( $j = 1, 2, \dots, C$ ) under condition  $k$  ( $k = 1, 2$ ),  $X_{gij}$  is expression  
332 level of gene  $g$  in sample  $i$  for cell type  $j$ , and  $S_k$  is the set of samples under condition  $k$ . Here we  
333 assume that if gene  $g$  is cell type  $c$  specific, it is only expressed in that cell type so that

334

$$X_{gij} = 0 \quad \text{for all } j \neq c \quad (2)$$

336

337 Combine (1) and (2), then for genes that are cell type  $c$  specific, we have:

338

$$Y_{gi} = p_c^k X_{gic} \quad g \in G_c \quad (3)$$

340

341 where  $G_c$  is the set of genes that are cell type  $c$  specific. Let  $Z_g$  denote the fold change of gene  $g$   
342 between two conditions in cell type  $c$ . Then

343

$$Z_{gc} = \frac{(\sum_{i \in S_2} X_{gic})/n_2}{(\sum_{i \in S_1} X_{gic})/n_1} = \frac{p_c^1 (\sum_{i \in S_2} Y_{gi})/n_2}{p_c^2 (\sum_{i \in S_1} Y_{gi})/n_1} = \frac{(\sum_{i \in S_2} Y_{gi})/n_2}{(\sum_{i \in S_1} p'_c Y_{gi})/n_1} \quad g \in G_c \quad (4)$$

345

346 where  $p'_c = \frac{p_c^2}{p_c^1}$  is the proportion change of cell type  $c$  between the two conditions,  $n_k$  ( $k = 1, 2$ ) is the  
347 number of samples in condition  $k$ . Thus, for cell type  $c$  specific gene  $g$ , the cell type level fold change  
348  $Z_{gc}$  can be calculated using bulk level expression  $Y_{gi}$  calibrated by  $p'_c$ , which is the proportion change  
349 of cell type  $c$ .

350

351 For each of the 11 cell types, we aim to identify ctDEGs. Firstly, we calibrate bulk expression levels for  
352 identified cell type-specific markers (**Supplementary Note 3 and Supplementary Data 1**) according to  
353 (4), and then performed differential expression analysis for these genes using DESeq2<sup>24</sup>. All parameters  
354 in DESeq2 were set at default and genes with Benjamini-Hochberg (BH)<sup>23</sup> adjusted p-value < 0.05 was

355 declared to be significant. The detected cell type specific DEGs are reported in **Supplementary Data 4**  
356 **and 6**.

357

### 358 **Alternative way to calculate cell type proportion change**

359 Although we are able to estimate proportion change by averaging the between-condition difference of  
360 cell type proportion the from the deconvolution results, the proportion change obtained this way is  
361 subject to sample variation, prone to outliers, and it may result in larger number of false positives in the  
362 detected cell type specific DEGs. Therefore, we propose an alternative way to estimate cell type  
363 proportion change which can increase the robustness of cell type specific DEGs detection.

364

365 We assume that for a given cell type, only few cell type specific markers are differentially expressed  
366 between conditions for the cell type, and the average fold change across genes specific to the cell type  
367 is 1, that is,

368

$$369 \quad \frac{\sum_{g \in G_j} z_g}{m_j} = 1 \quad (5)$$

370

371 where  $m_j$  is the number of cell type specific genes for cell type  $j$ . Combine (4) and (5) we have:

372

$$373 \quad p'_j = \left( \sum_{g \in G_j} \frac{\sum_{i \in S_2} y_{gi}}{\sum_{i \in S_1} y_{gi}} \right) / m_j \quad (6)$$

374

375 Thus, we are able to calculate between-condition proportion change  $p'_j$  for cell type  $j$  directly using bulk  
376 level expression for cell type specific markers. Under our assumption, this method is a more direct way  
377 to estimate cell type proportion change between conditions. By avoiding the sample variation and  
378 complexity introduced in the deconvolution analysis, the calculation is more robust. The method was  
379 applied in the procedure of cell type specific DEGs detection (**Supplementary Fig.7**).

380

### 381 **GO enrichment analysis for ctDEGs**

382 We preformed GO-enrichment analysis using ToppGene Suite (<https://toppgene.cchmc.org/>)<sup>25</sup> for up-  
383 and down-regulated genes specific to each cell type. The analysis was performed only if there are at  
384 least 10 ctEGDs in the list. We used Bonferroni corrected P-value < 0.05 as the threshold for the  
385 significant GO-terms. The results are reported in **Supplementary data 5 and 7**. Representative GO  
386 terms for rod, bipolar, endothelium, astrocytes and microglia are shown in **Fig. 3d**.

387

388 **References**

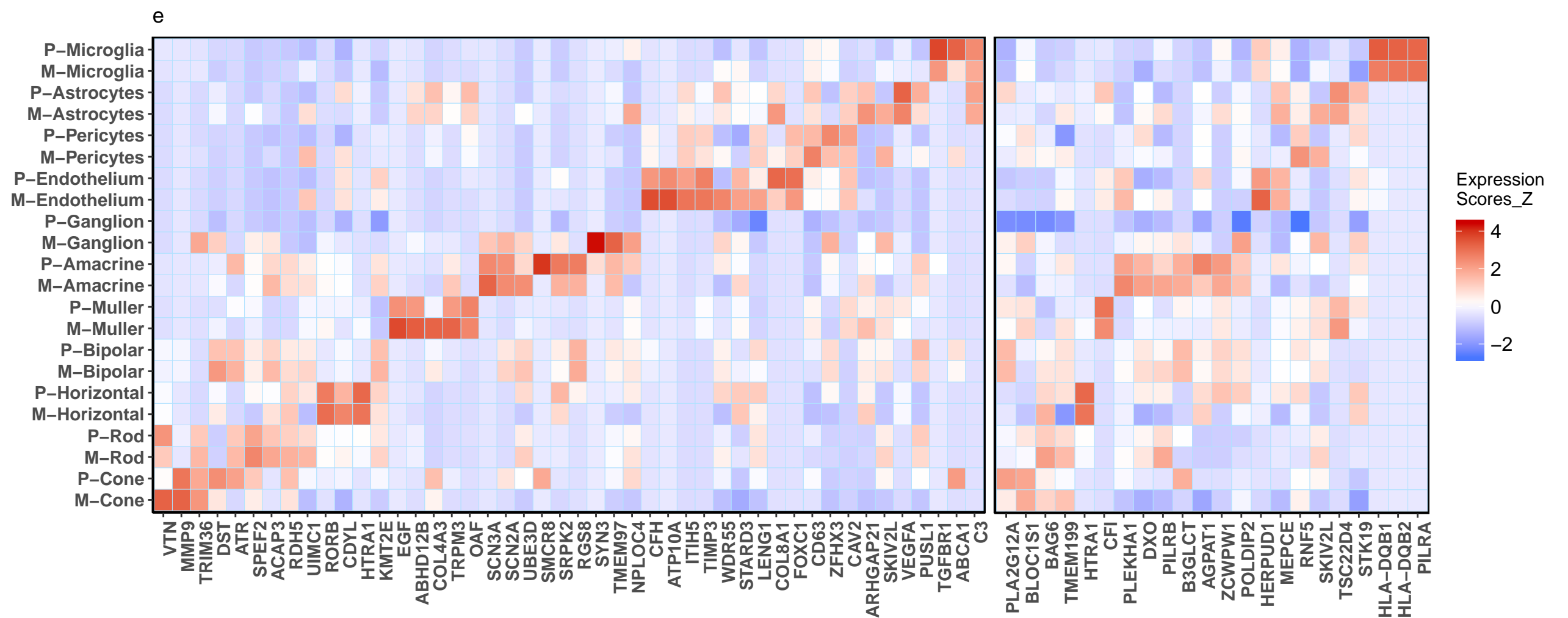
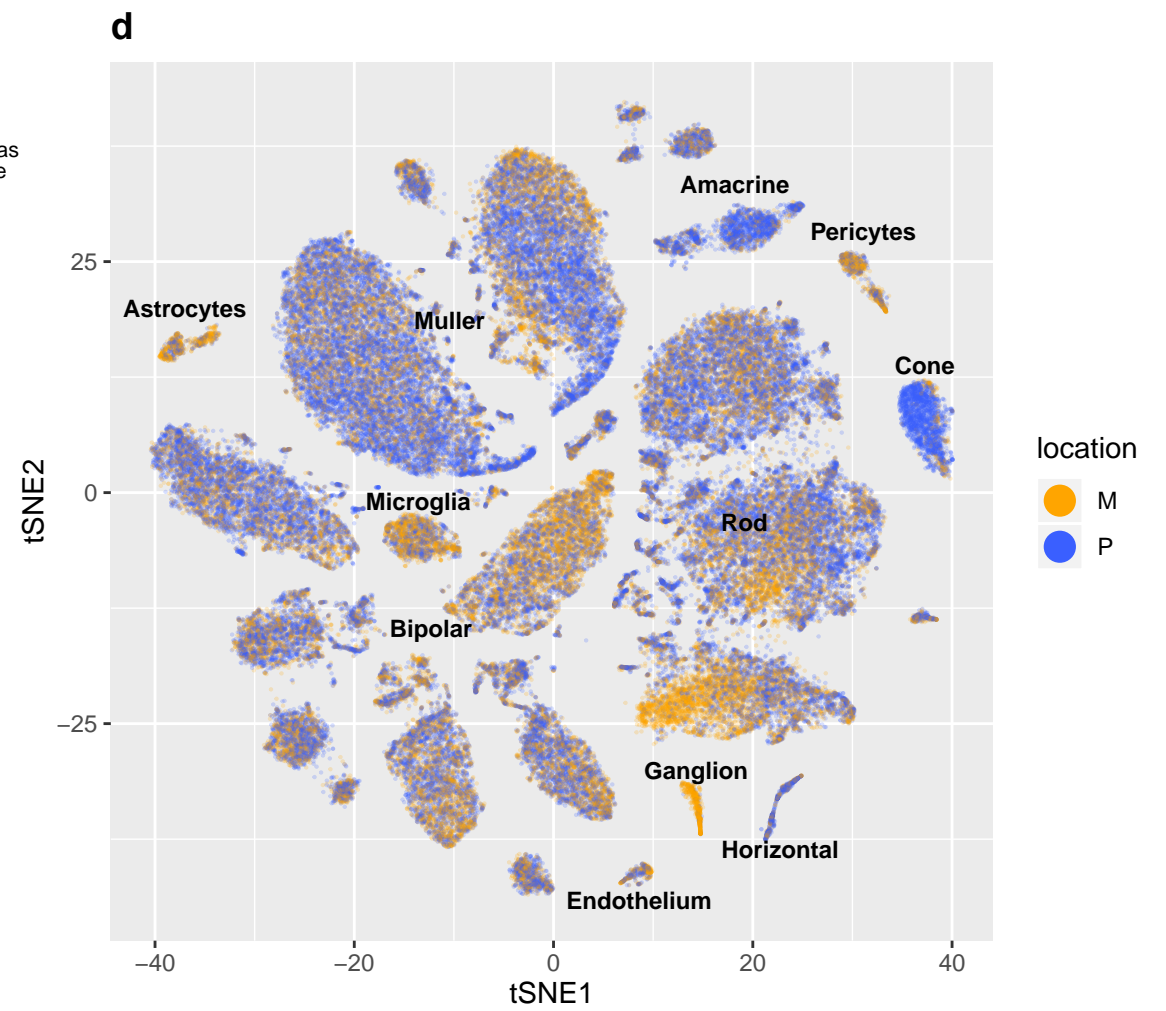
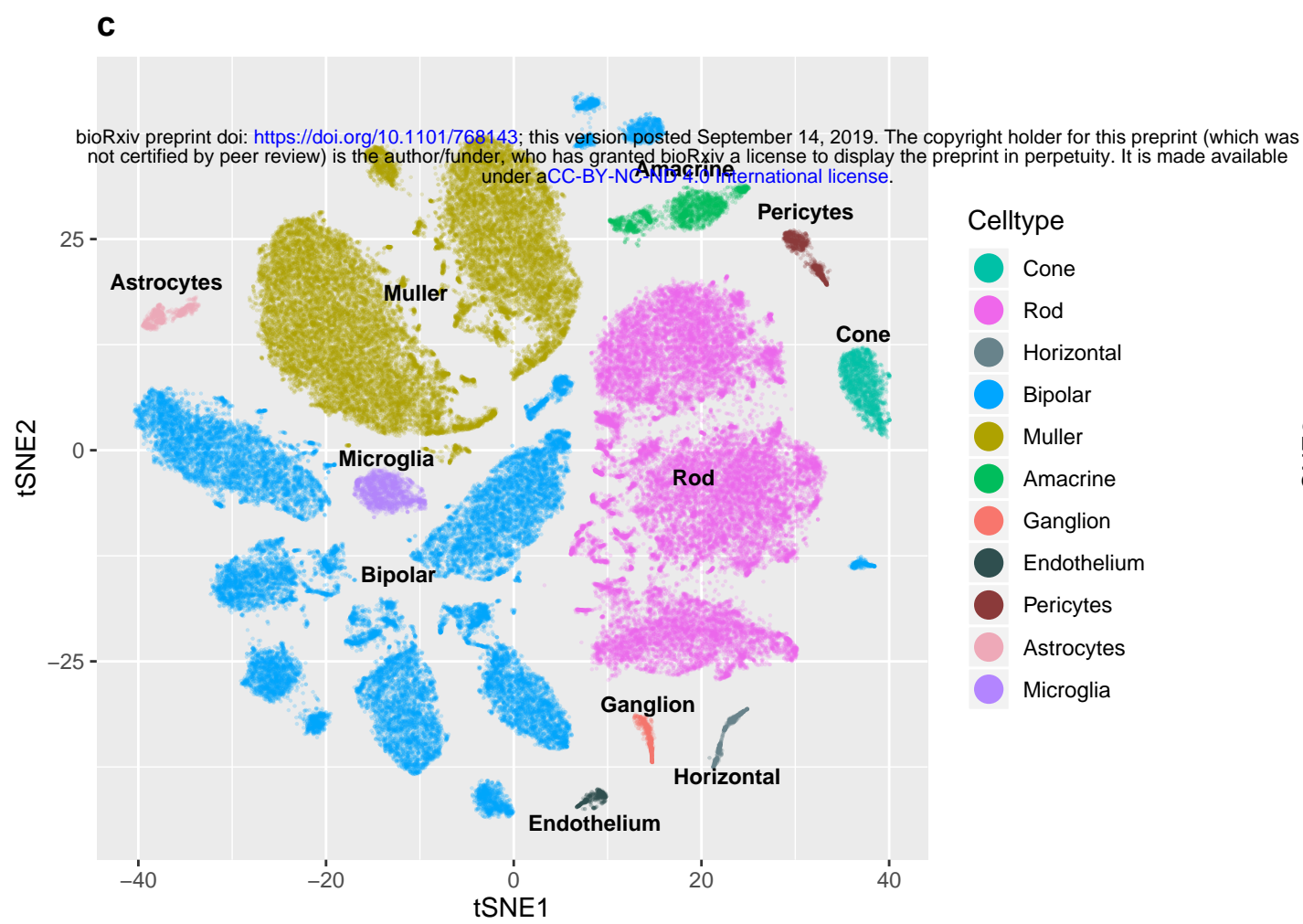
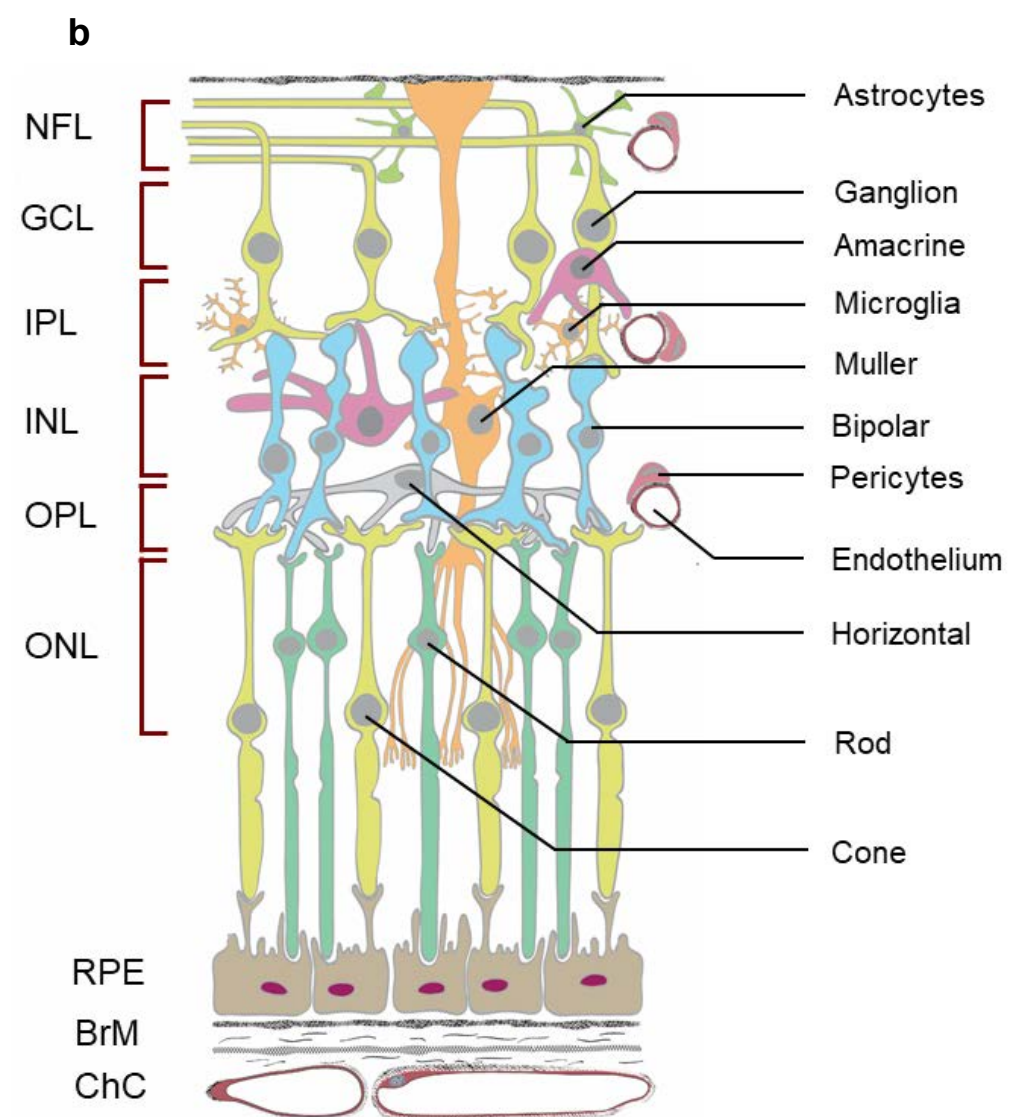
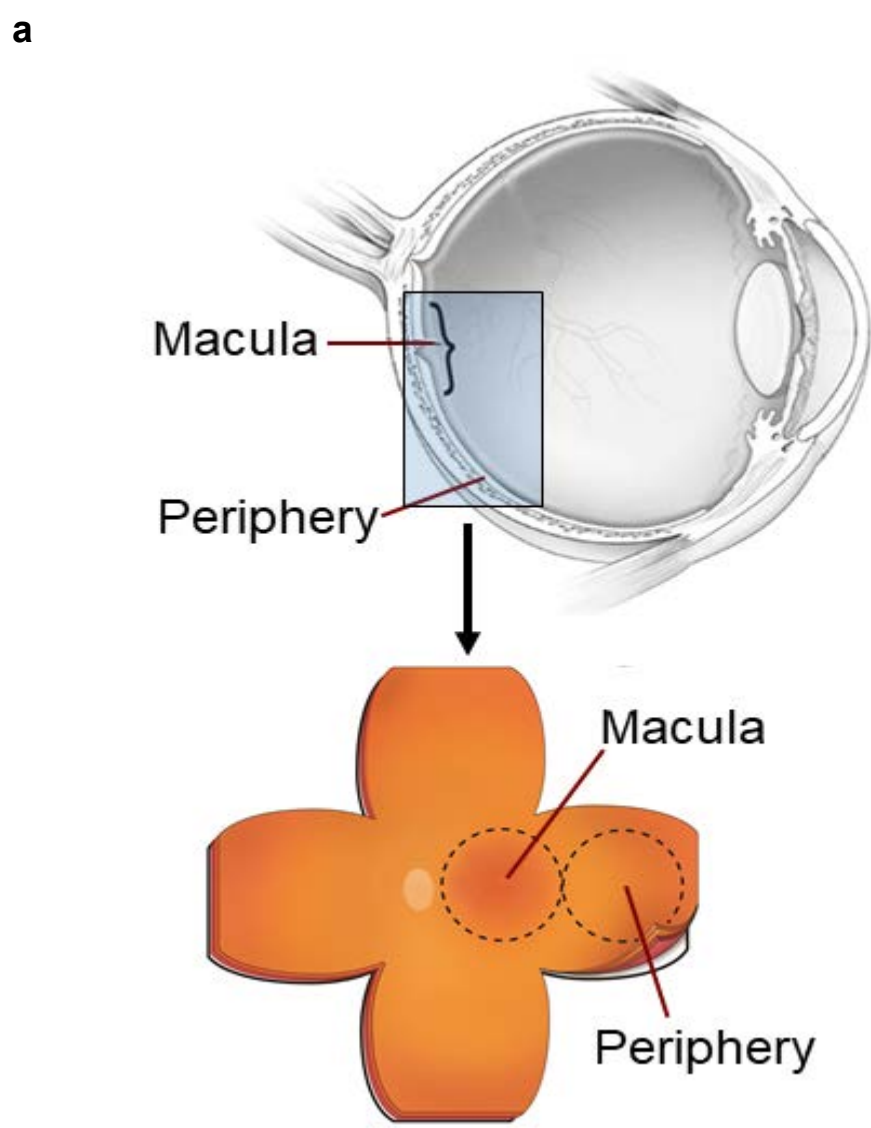
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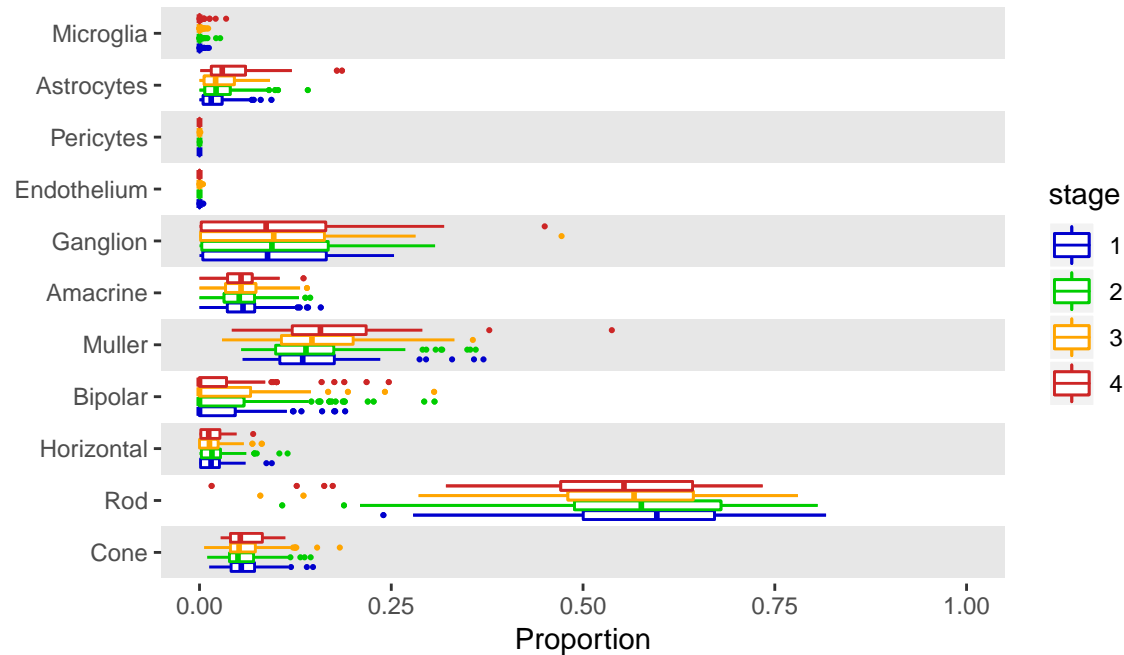
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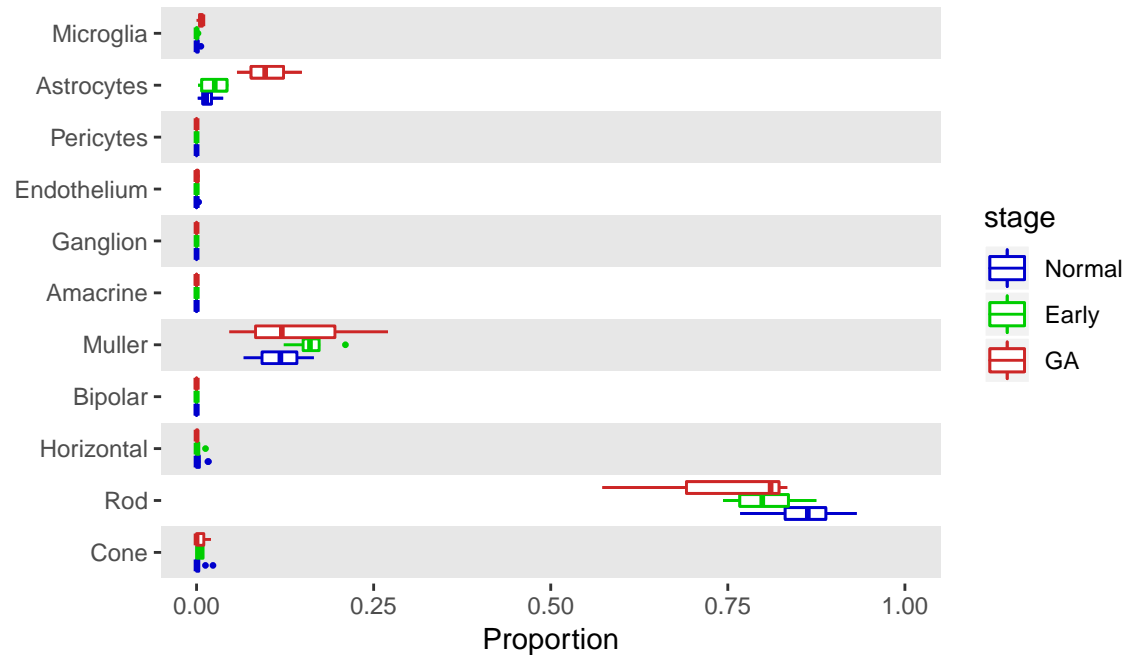




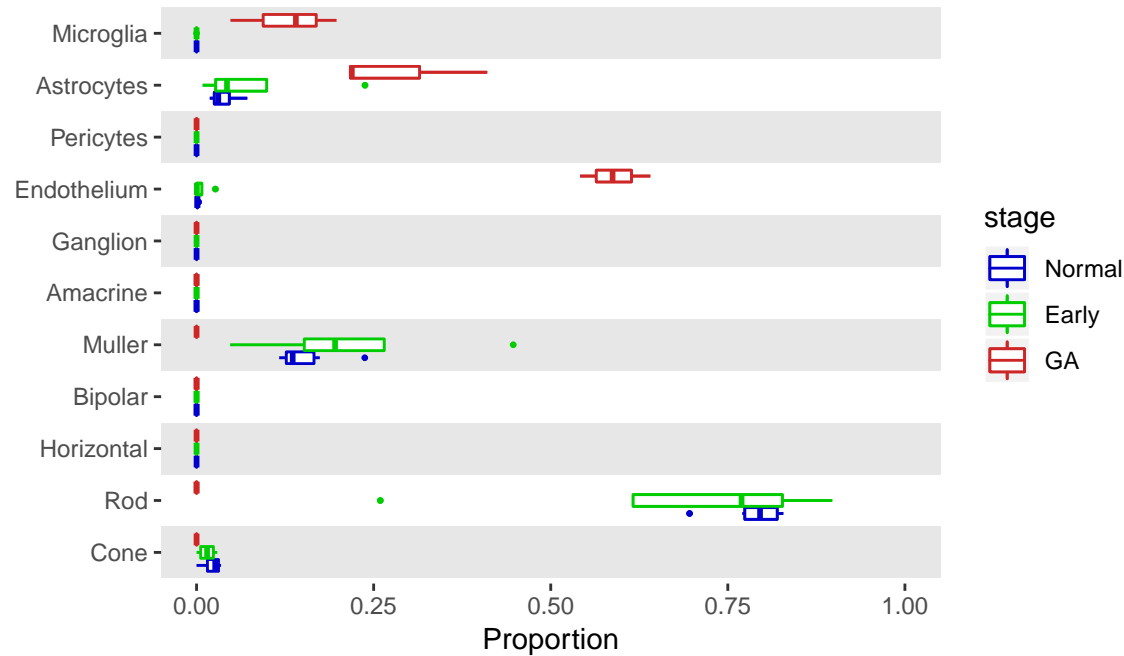
**a**  
EyeGEx periphery



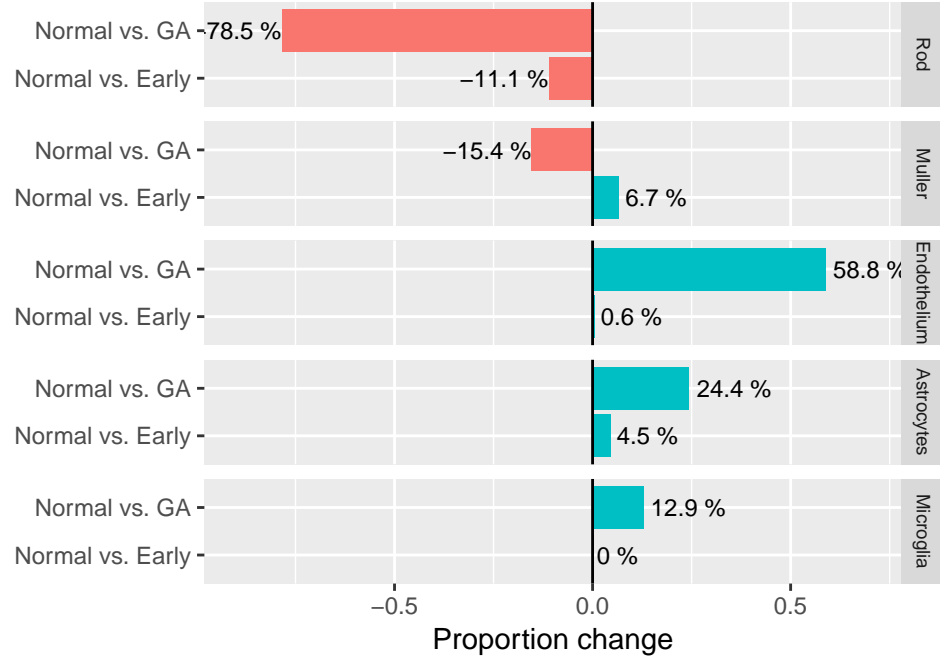
**b**  
UAB periphery

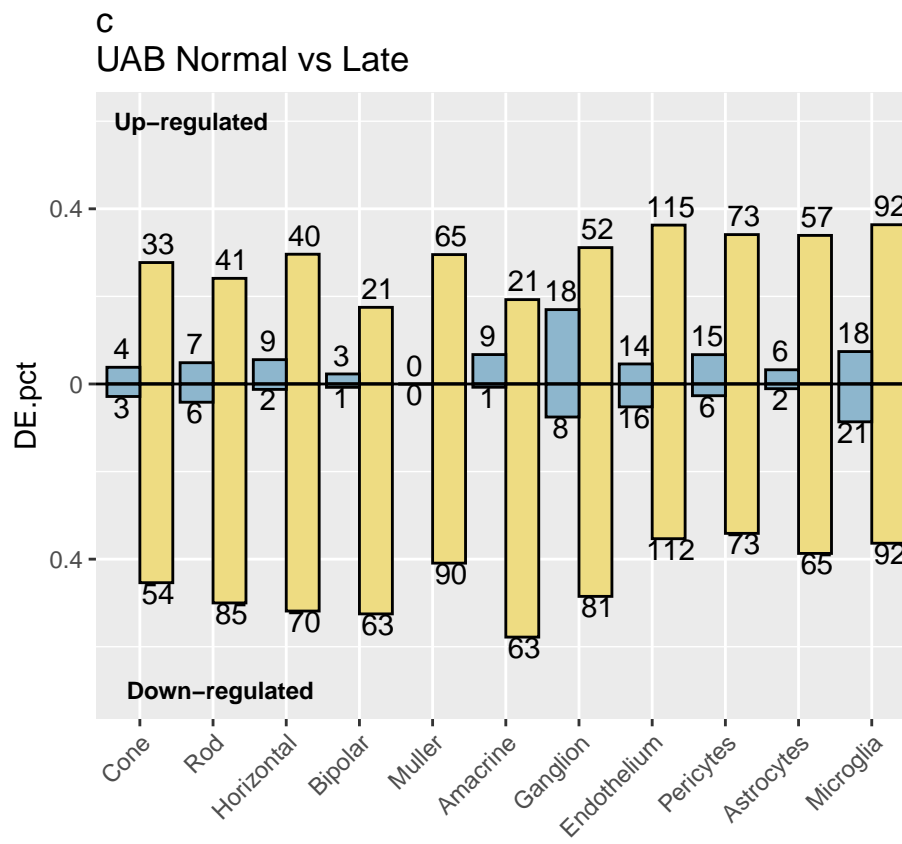
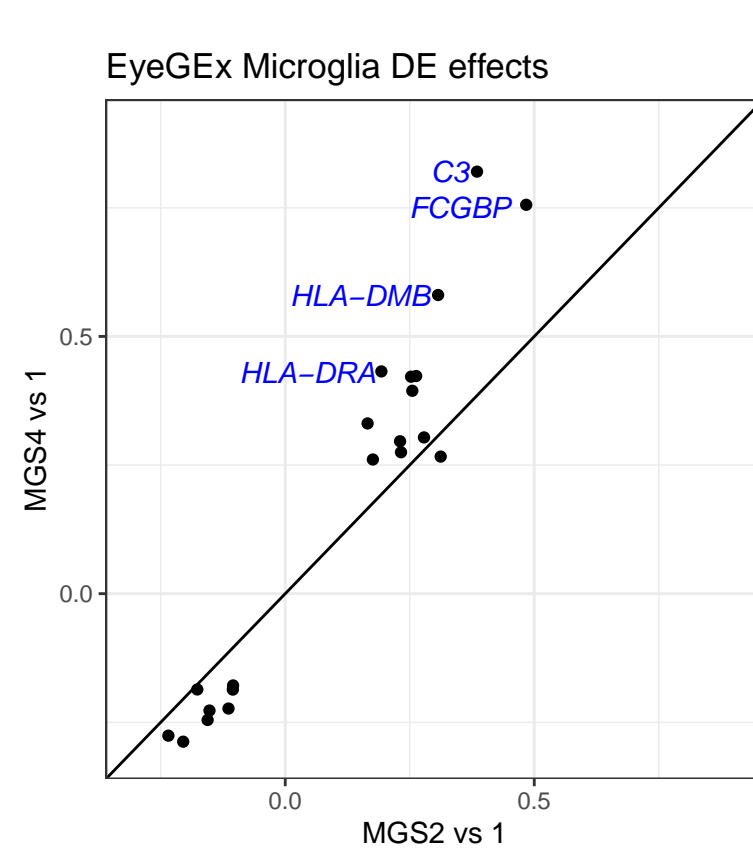
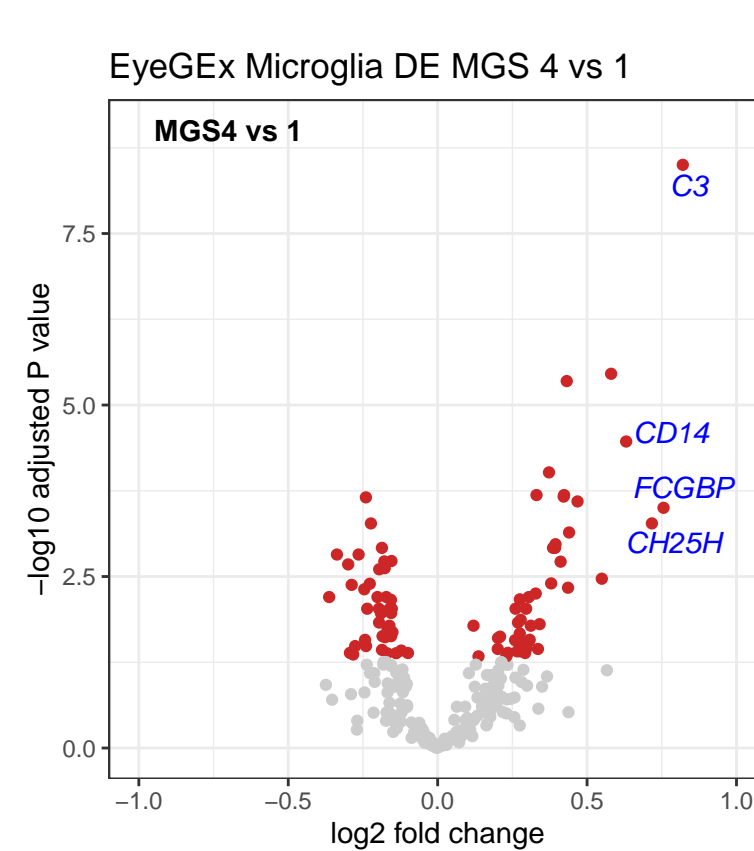
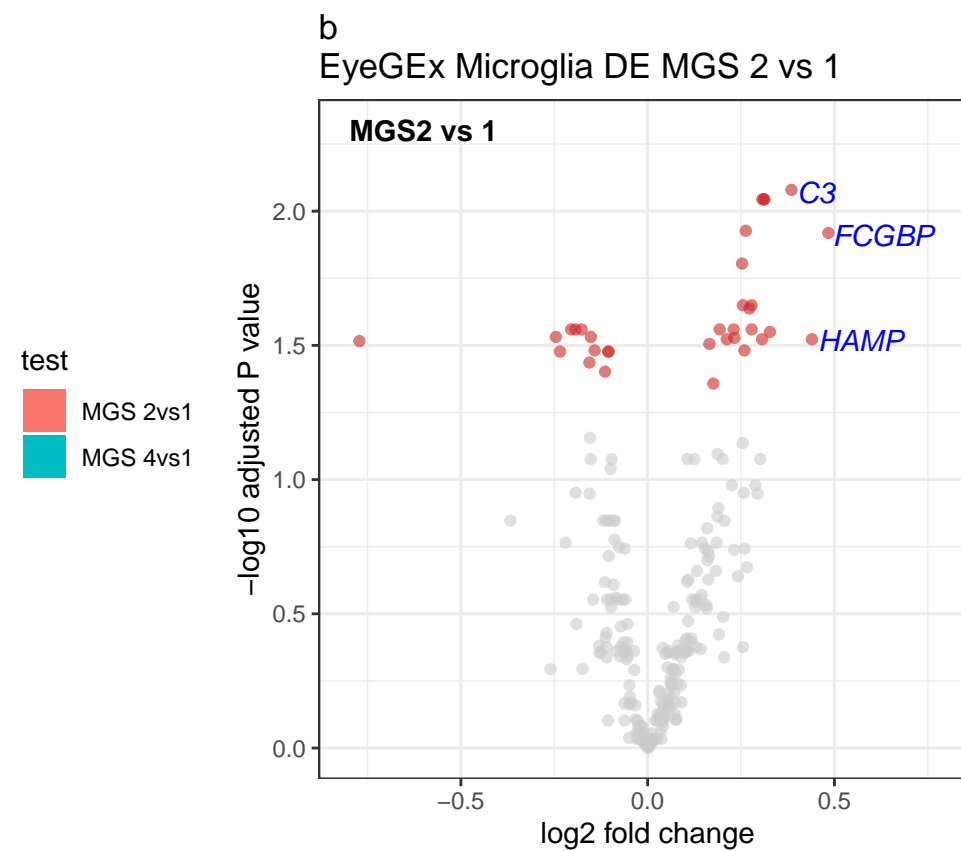
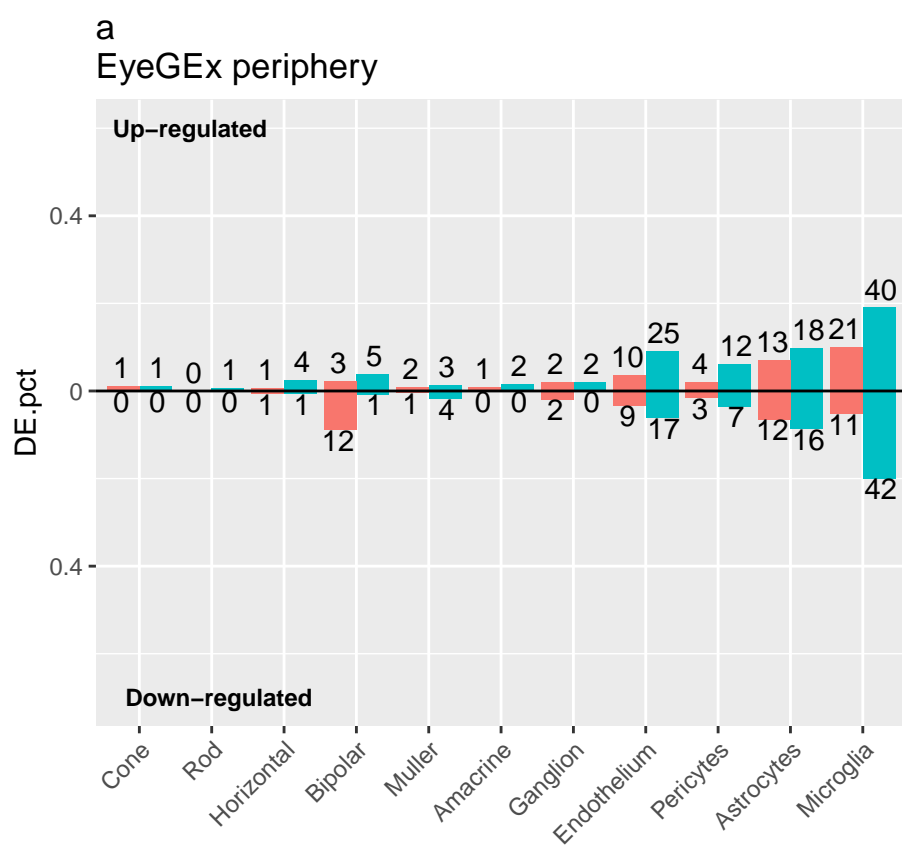


**c**  
UAB macula



**d**  
UAB proportion change





**d**

Cell type	DE direction	Representative GO term	Adjusted P value
Rod	Up	negative regulation of cell death	1.314e-04
Rod	Down	sensory perception of light stimulus	3.665e-40
Bipolar	Up	NA	NA
Bipolar	Down	chemical synaptic transmission	5.233e-09
Endothelium	Up	cell migration	1.223e-07
Endothelium	Down	protein localization to membrane	5.334e-07
Astrocytes	Up	type I interferon signaling pathway	1.005e-05
Astrocytes	Down	neurogenesis	3.495e-05
Microglia	Up	immune response	1.156e-27
Microglia	Down	SRP-dependent cotranslational protein targeting to membrane	7.015e-96