**Supplement**


**In-depth molecular profiling specifies human retinal microglia identity**

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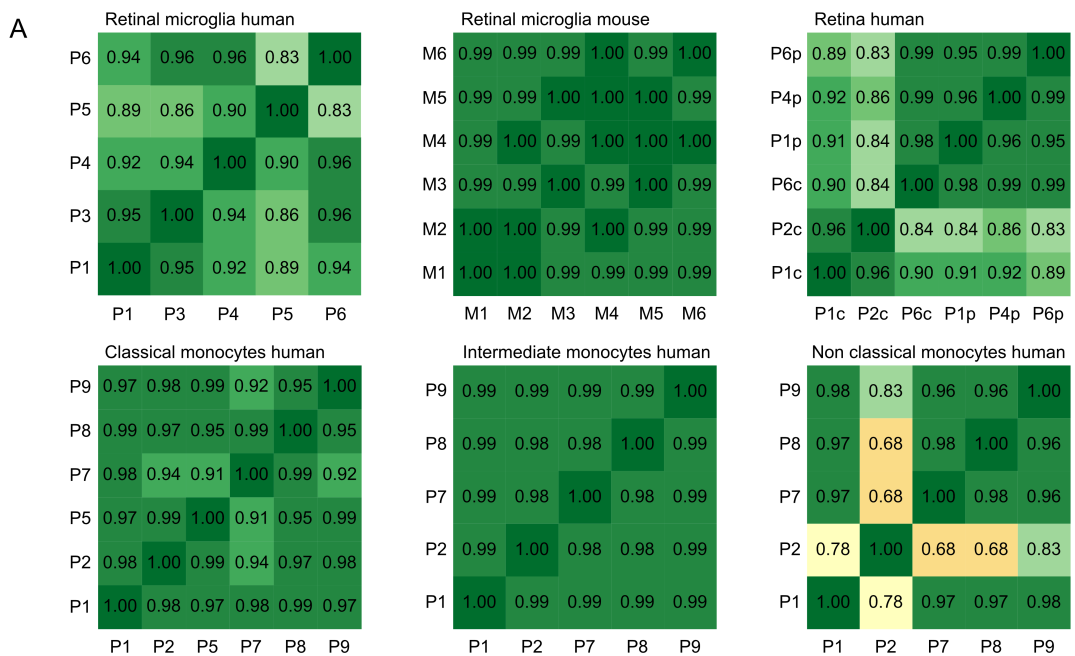
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**A**

Retinal microglia human

| | P1 | P3 | P4 | P5 | P6 |
|---|---|---|---|---|---|
| P6 | 0.94 | 0.96 | 0.96 | 0.83 | 1.00 |
| P5 | 0.89 | 0.86 | 0.90 | 1.00 | 0.83 |
| P4 | 0.92 | 0.94 | 1.00 | 0.90 | 0.96 |
| P3 | 0.95 | 1.00 | 0.94 | 0.86 | 0.96 |
| P1 | 1.00 | 0.95 | 0.92 | 0.89 | 0.94 |

Retinal microglia mouse

| | M1 | M2 | M3 | M4 | M5 | M6 |
|---|---|---|---|---|---|---|
| M6 | 0.99 | 0.99 | 0.99 | 1.00 | 0.99 | 1.00 |
| M5 | 0.99 | 0.99 | 1.00 | 1.00 | 1.00 | 0.99 |
| M4 | 0.99 | 1.00 | 0.99 | 1.00 | 1.00 | 1.00 |
| M3 | 0.99 | 0.99 | 1.00 | 0.99 | 1.00 | 0.99 |
| M2 | 1.00 | 1.00 | 0.99 | 0.99 | 0.99 | 0.99 |
| M1 | 1.00 | 1.00 | 0.99 | 0.99 | 0.99 | 0.99 |

Retina human

| | P1c | P2c | P6c | P1p | P4p | P6p |
|---|---|---|---|---|---|---|
| P6p | 0.89 | 0.83 | 0.99 | 0.95 | 0.99 | 1.00 |
| P4p | 0.92 | 0.86 | 0.99 | 0.96 | 1.00 | 0.99 |
| P1p | 0.91 | 0.84 | 0.98 | 1.00 | 0.96 | 0.95 |
| P6c | 0.90 | 0.84 | 1.00 | 0.98 | 0.99 | 0.99 |
| P2c | 0.96 | 1.00 | 0.84 | 0.84 | 0.86 | 0.83 |
| P1c | 1.00 | 0.96 | 0.90 | 0.91 | 0.92 | 0.89 |

Classical monocytes human

| | P1 | P2 | P5 | P7 | P8 | P9 |
|---|---|---|---|---|---|---|
| P9 | 0.97 | 0.98 | 0.99 | 0.92 | 0.95 | 1.00 |
| P8 | 0.99 | 0.97 | 0.95 | 0.99 | 1.00 | 0.95 |
| P7 | 0.98 | 0.94 | 0.91 | 1.00 | 0.99 | 0.92 |
| P5 | 0.97 | 0.99 | 1.00 | 0.91 | 0.95 | 0.99 |
| P2 | 0.98 | 1.00 | 0.99 | 0.94 | 0.97 | 0.98 |
| P1 | 1.00 | 0.98 | 0.97 | 0.98 | 0.99 | 0.97 |

Intermediate monocytes human

| | P1 | P2 | P7 | P8 | P9 |
|---|---|---|---|---|---|
| P9 | 0.99 | 0.99 | 0.99 | 0.99 | 1.00 |
| P8 | 0.99 | 0.98 | 0.98 | 1.00 | 0.99 |
| P7 | 0.99 | 0.98 | 1.00 | 0.98 | 0.99 |
| P2 | 0.99 | 1.00 | 0.98 | 0.98 | 0.99 |
| P1 | 1.00 | 0.99 | 0.99 | 0.99 | 0.99 |

Non classical monocytes human

| | P1 | P2 | P7 | P8 | P9 |
|---|---|---|---|---|---|
| P9 | 0.98 | 0.83 | 0.96 | 0.96 | 1.00 |
| P8 | 0.97 | 0.68 | 0.98 | 1.00 | 0.96 |
| P7 | 0.97 | 0.68 | 1.00 | 0.98 | 0.96 |
| P2 | 0.78 | 1.00 | 0.68 | 0.68 | 0.83 |
| P1 | 1.00 | 0.78 | 0.97 | 0.97 | 0.98 |

**B**

Celltype-specific marker

| Marker | group | R microglia mouse | Retinal microglia | Classical mono | Intermediate mono | Non classical mono | Retina |
|---|---|---|---|---|---|---|---|
| PTPRC (CD45) | leukocyte | 9719.1 | 12778.2 | 18444.8 | 28396.8 | 21603.5 | 267.4 |
| AIF1 | leukocyte | 7572.0 | 9096.1 | 8583.9 | 14507.0 | 345.6 | 23,4 |
| ITGAM (CD11b) | leukocyte | 4005.3 | 2243.8 | 4301.7 | 1013.5 | 3933.6 | 44.4 |
| ITGAL (CD11a) | leukocyte | 603.2 | 1513.2 | 3161.3 | 7702.2 | 8955.2 | 66.6 |
| TREM2 | brain MG | 5416.3 | 12455.5 | 7.0 | 6.2 | 7.8 | 213.7 |
| P2RY12 | brain MG | 72283.3 | 4873.5 | 54.0 | 122.1 | 0.2 | 2.4 |
| TMEM119 | brain MG | 57869.0 | 2370.3 | 8.3 | 27.2 | 5.2 | 255.9 |
| SALL1 | brain MG | 5418.5 | 91.7 | 0.3 | 0.3 | 0.5 | 96.8 |
| CD14 | classical mo | 11062.4 | 33708.6 | 41163.3 | 6151.1 | 9.2 | 806.1 |
| SELL (CD62L) | classical mo | 0.0 | 2540.4 | 31267.5 | 4484.1 | 7664.5 | 77.1 |
| CD36 | classical mo | 586.6 | 789.6 | 15888.8 | 3278.7 | 80.5 | 17.8 |
| CCR2 | classical mo | 28.6 | 1266.2 | 5164.2 | 641.0 | 25.2 | 30.1 |
| FCGR1A (CD64) | classical mo | 7657.6 | 3239.2 | 347.4 | 122.0 | 1.4 | 86.7 |
| CD74 | intermediate mo | 3314.9 | 454032.5 | 97470.9 | 100545.8 | 13961.4 | 6353.0 |
| HLA-DRB1 | intermediate mo | 0.0 | 41298.4 | 13372.6 | 16036.8 | 1323.2 | 162.4 |
| HLA-DRA | intermediate mo | 0.0 | 49018.0 | 11415.1 | 13266.1 | 982.6 | 46.3 |
| CD86 | intermediate mo | 6723.1 | 6375.9 | 4749.0 | 5752.9 | 218.2 | 47.6 |
| ITGAX (CD11c) | intermediate mo | 674.8 | 2144.5 | 2606.4 | 3550.5 | 1226.7 | 52.2 |
| FCGR3A (CD16) | non cl mo | 3234.7 | 42985.0 | 432.7 | 60515.6 | 25475.3 | 173.1 |
| CX3CR1 | non cl mo | 54163.6 | 33320.5 | 5355.6 | 15430.2 | 20083.9 | 168.0 |
| CXCR4 | non cl mo | 19.0 | 1348.2 | 616.7 | 345.9 | 2797.1 | 419.0 |
| NPVF | cones | 0.0 | 47.7 | 0.1 | 0.0 | 0.0 | 7366.7 |
| ARR3 | cones | 7.2 | 1.6 | 1.3 | 1.2 | 0.0 | 2286.5 |
| GNAT2 | cones | 34.3 | 9.4 | 1.9 | 2.8 | 5.3 | 39.4 |
| OPN1SW | cones | 85.8 | 0.0 | 0.3 | 0.4 | 0.0 | 10.9 |
| OPN1MW | cones | 7.1 | 0.0 | 0.0 | 0.0 | 0.0 | 2.8 |
| PDE6A | rods | 39.8 | 287.6 | 2.2 | 5.6 | 1.0 | 19173.2 |
| MAP1B | rods | 40.4 | 145.5 | 7.0 | 11.8 | 30.4 | 18399.6 |
| CNGA1 | rods | 48.0 | 85.5 | 0.5 | 0.0 | 0.0 | 15330.9 |
| NR2E3 | rods | 28.3 | 47.2 | 0.7 | 0.1 | 0.5 | 9557.1 |
| KCNB1 | rods | 26.4 | 64.8 | 0.3 | 0.6 | 0.2 | 3877.6 |
| STMN2 | RGCs | 7.8 | 5.3 | 0.0 | 0.2 | 0.0 | 2950.8 |
| NEFL | RGCs | 109.3 | 102.8 | 0.2 | 0.3 | 6.7 | 2634.8 |
| UCHL1 | RGCs | 25.0 | 10.1 | 0.0 | 0.7 | 1.7 | 1902.7 |
| SNCG | RGCs | 30.6 | 4.5 | 1.8 | 0.4 | 0.0 | 575.4 |
| SLC17A6 | RGCs | 8.3 | 0.4 | 0.4 | 0.2 | 0.1 | 131.0 |
| LRTM1 | BPs | 11.5 | 1.6 | 0.1 | 0.2 | 0.0 | 1997.5 |
| VSX2 | BPs | 18.8 | 5.1 | 0.0 | 0.1 | 0.3 | 1355.2 |
| TMEM215 | BPs | 0.8 | 0.3 | 0.3 | 0.1 | 0.6 | 726.3 |
| GRM6 | BPs | 0.1 | 0.1 | 0.5 | 0.2 | 0.2 | 585.3 |
| TRPM1 | BPs | 19.6 | 5.1 | 0.2 | 0.0 | 0.0 | 211.2 |
| CLDN5 | endothelial cells | 0.0 | 0.8 | 6.1 | 5.3 | 1.1 | 1036.6 |
| TM4SF1 | endothelial cells | 0.0 | 0.1 | 1.7 | 0.5 | 4.4 | 962.3 |
| CD34 | endothelial cells | 5230.8 | 14.7 | 1.3 | 0.4 | 1.3 | 273.5 |
| CDH5 | endothelial cells | 19.2 | 0.0 | 8.0 | 0.1 | 0.2 | 194.4 |
| ADAMTS9 | endothelial cells | 0.0 | 0.2 | 0.3 | 0.5 | 0.4 | 84.9 |

**Supplementary figure 1:** Quality and purity check. (A): Correlation plots visualizing Pearson correlation coefficients between any two patients (P) or mice (M) for all cell populations or tissue types (c = centre, p = periphery). (B): Expression of known celltype-specific marker genes in all 5 immune cell populations as well as in retinal tissue. Each column represents one group and each row one marker gene. Groups of marker genes for each celltype are visualized in the row annotation on the left. Numbers correspond to mean of normalized reads per group. Abbreviations: mo(no): monocyte, non cl: non classical, MG: microglia, R: retinal, RGC: retinal ganglion cell, BP: bipolar cell.
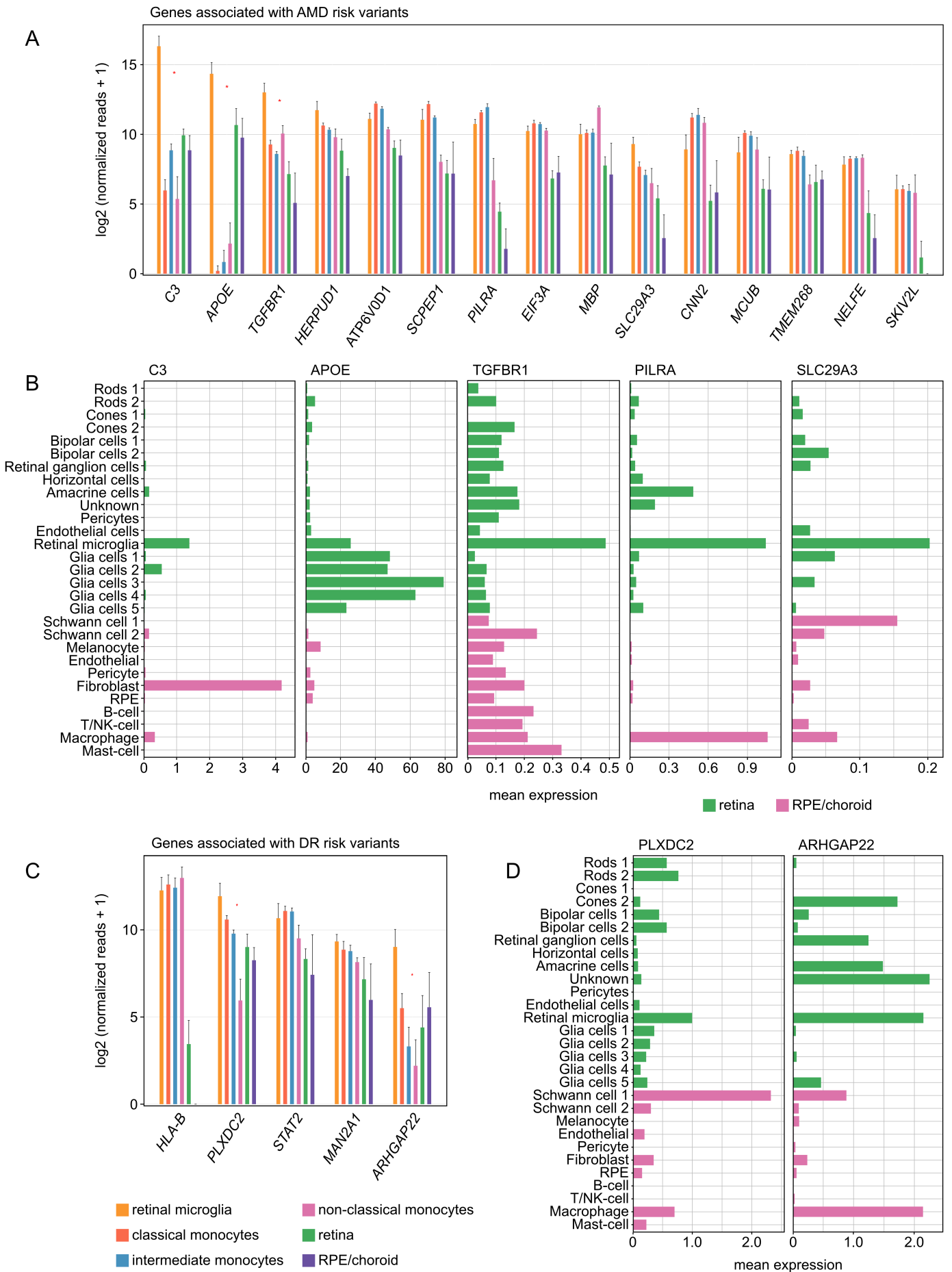
**Supplementary figure 2:** Bar graphs visualizing the expression levels of the 15 AMD (A) and 5 DR (C) risk genes identified to be overexpressed in retinal microglia when compared to retinal and RPE/choroid tissue (see Figure 1 F-G) in comparison to three sub-types of monocytes. Red asterisks indicate genes which were not only overexpressed in retinal microglia versus retinal and RPE/choroid tissue, but also when compared to all three subtypes of monocytes. (B+D) Expression of AMD (B) or DR (D) associated risk genes mainly expressed in retinal microglia compared to 28 other retinal and RPE/choroid cell types, as determined by reanalysis of published scRNA-Seq data of human retinal and RPE/choroid tissue.
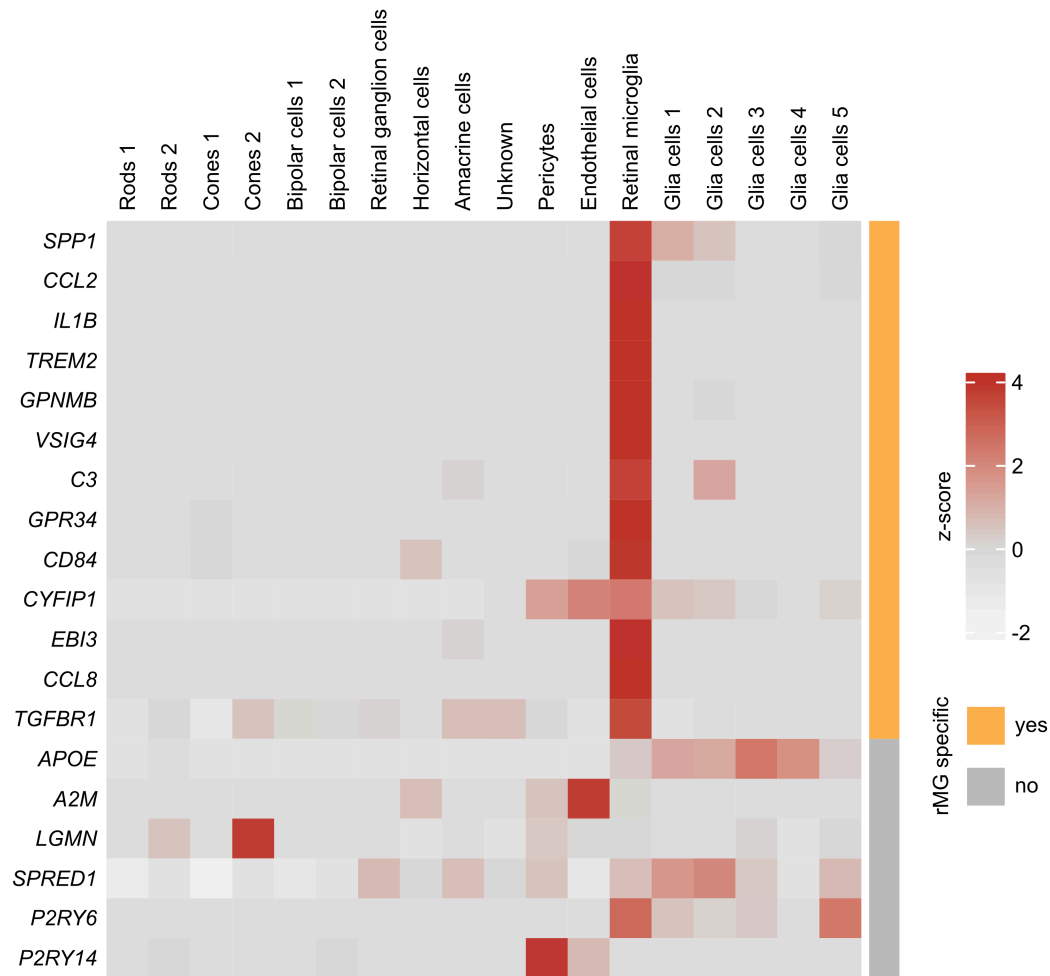
**Supplementary figure 3:** Heatmap visualizing expression of the 20 retinal microglia enriched genes identified in the network diagram in Figure 2E in published single cell RNA sequencing (scRNA-Seq) data of the human retina. 19 of these genes were detected by scRNA-Seq and 13 were specific for retinal microglia (yellow, see legend). The z-score represents a gene's expression in relation to its mean expression by standard deviation units.
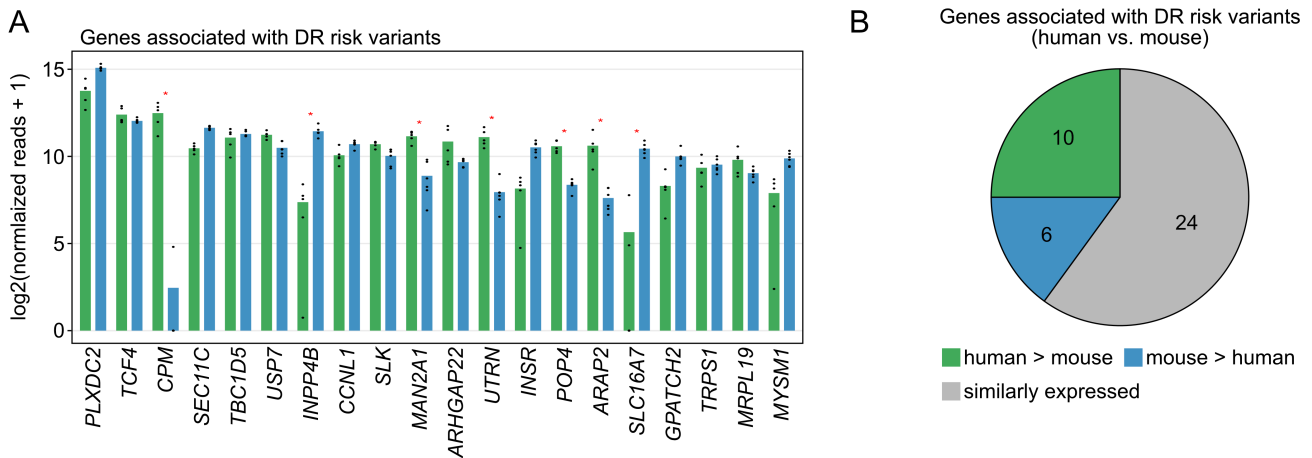


**Supplementary figure 4:** (A) Bar graphs visualizing the expression levels of the 20 highest expressed genes associated with DRP-risk variants in human and mouse retinal microglia. Red asterisks indicate differentially expressed genes (log2FC > 2, adjusted p < 0.05). (B) Pie chart illustrating relative expression of genes associated with DRP-risk variants in human and mouse retinal microglia.