

Expanded View Figures

Figure EV1. Single-cell RNA-seq from all cells in the healthy murine eye.

- A t-SNE representation of individual cells from all eye compartments measured by scRNA-seq. Each dot represents an individual cell. Color code indicates the respective cell types.
- B Unbiased cluster analysis of cell subpopulations found in the retina, ciliary body, and cornea.
- C Stacked bar plot (Marimekko chart) representation of macrophage subsets to a given cluster.
- D Heatmap of the 30 most regulated genes per cluster (adjusted *P*-value < 0.05 based on the negative binomial distribution). The scale bar represents the color-coded z-scores. Genes presented in t-SNE plots in (E) are marked by asterisks.
- E t-SNE plot of typical genes for microglia subsets (*P2ry12*, *Tmem119*, *Hexb*), macrophage cluster (*Cd74*, *H2-Aa*, *Apoe*), Rods/Cone cluster (*Rho*, *Prph2*, *Pdc*), and epithelial cells cluster (*Emp1*, *Gsto1*, *Krt5*).

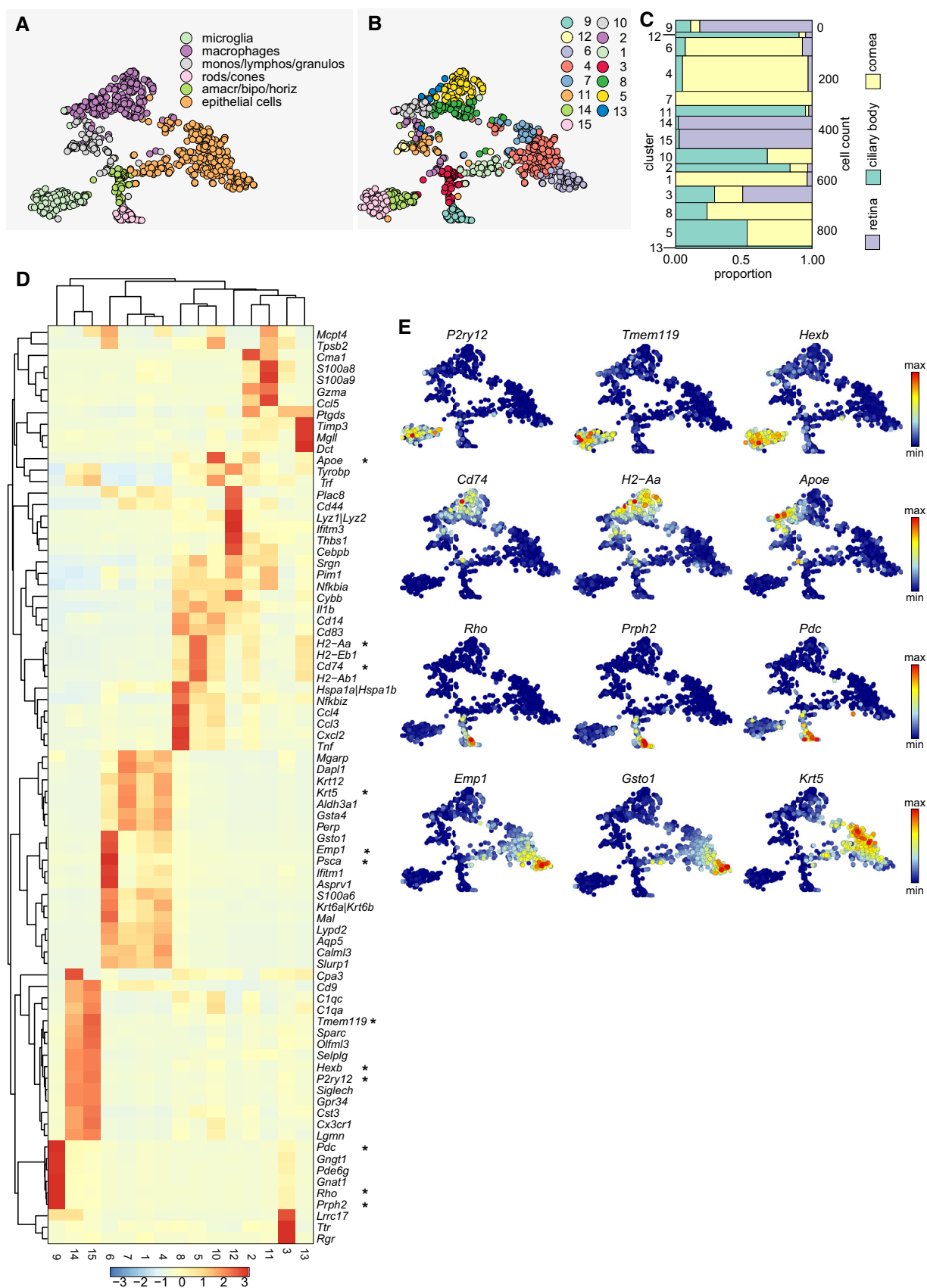


Figure EV1.

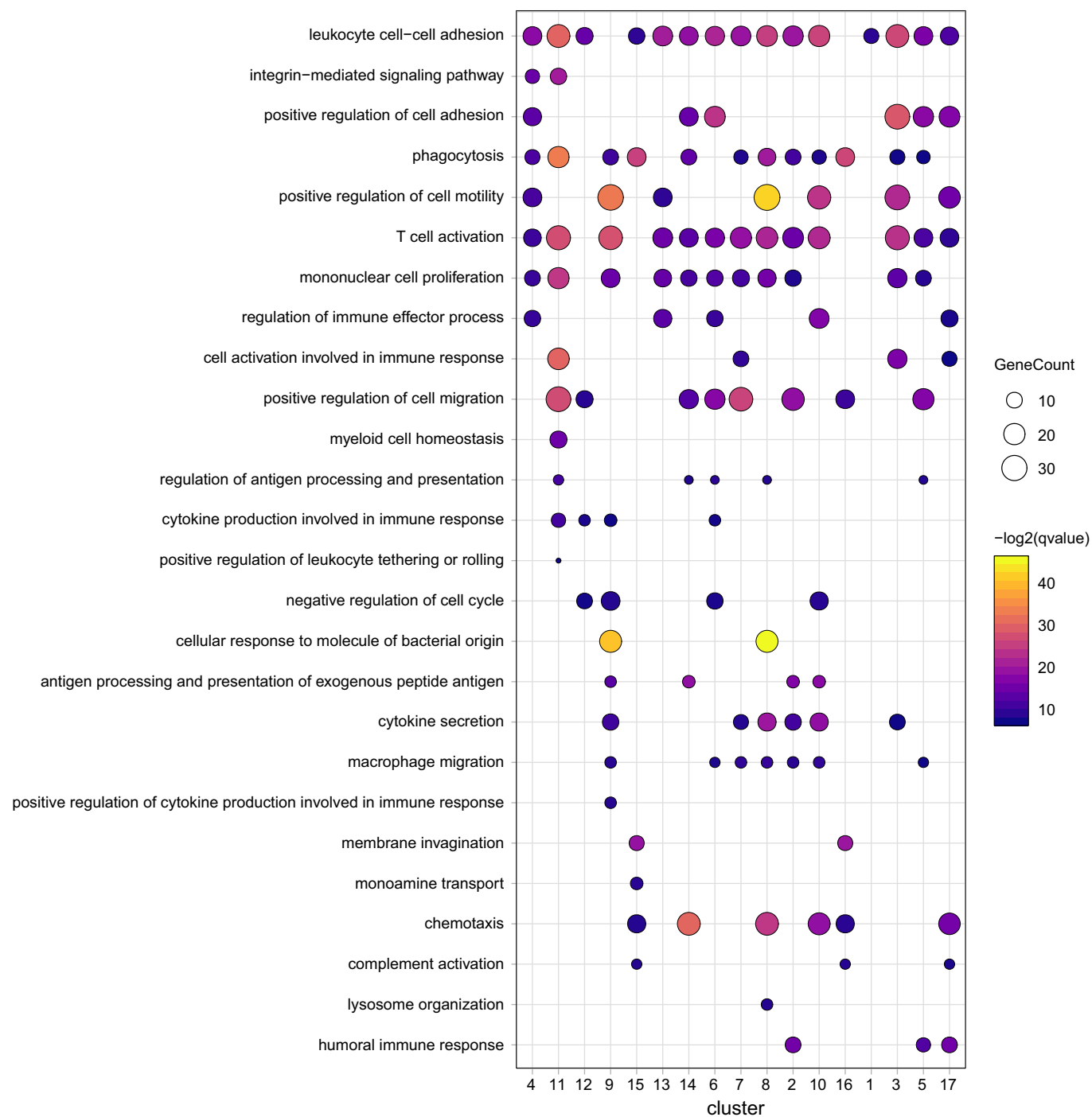


Figure EV2. Gene ontology enrichment (GO) analysis across all cell clusters of different eye compartments.

GO pathways were selected and depicted for all cell cluster identified in Fig 1 for the eye compartments retina, ciliary body, and cornea.

Figure EV3. Comparison of brain and eye macrophages by single-cell RNA-seq.

- A t-SNE representation of single cells based on the tissue of origin.
- B Unbiased cluster analysis of subpopulations of cells found in the steady-state adult brain and eye.
- C Stacked bar plot (Marimekko chart) representation of the proportional contribution of macrophage subsets from different tissues to a given cluster demonstrating a pure microglia from both brain and retina in clusters 0. Hypergeometric testing revealed significantly enriched microglia (cluster 0), and macrophage clusters in the ciliary body (3, 4, 5) are indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).
- D Heatmap of the most regulated genes per cluster (adjusted P -value < 0.05 based on the negative binomial distribution). Clusters are arranged from the left to the right. The scale bar represents the color-coded z-scores.
- E t-SNE expression and line plot of homeostatic gene signatures of microglia (*Tmem119*, *Hexb*, *Slc2a5*, *P2ry12*, *Siglech*, *Trem2*), macrophages (*Mrc1*, *Cd163*, *Lyve1*, *Siglec1*, *Stab1*, *Pf4*, *Ms4a7*, *Cbr2*, *Apoe*), boarder-associated macrophages (*Irf7*, *Crip1*, *Ccl6*, *Ccl9*, *Clec4b1*, *Ccr2*, *Vim*, *Lsp1*, *Lgals3*), monocytes (*Ly6c2*, *Ccr2*, *Anxa8*, *Plac8*, *Nr4a1*), dendritic cells (*Flt3*, *Zbtb46*, *Batf3*, *Clec9a*, *Itgae*), and antigen-presenting cells (APCs) (*Cd74*, *H2-Aa*, *H2-Eb1*, *H2-Ab1*, *Cd80*, *Cd86*, *Cd40*).



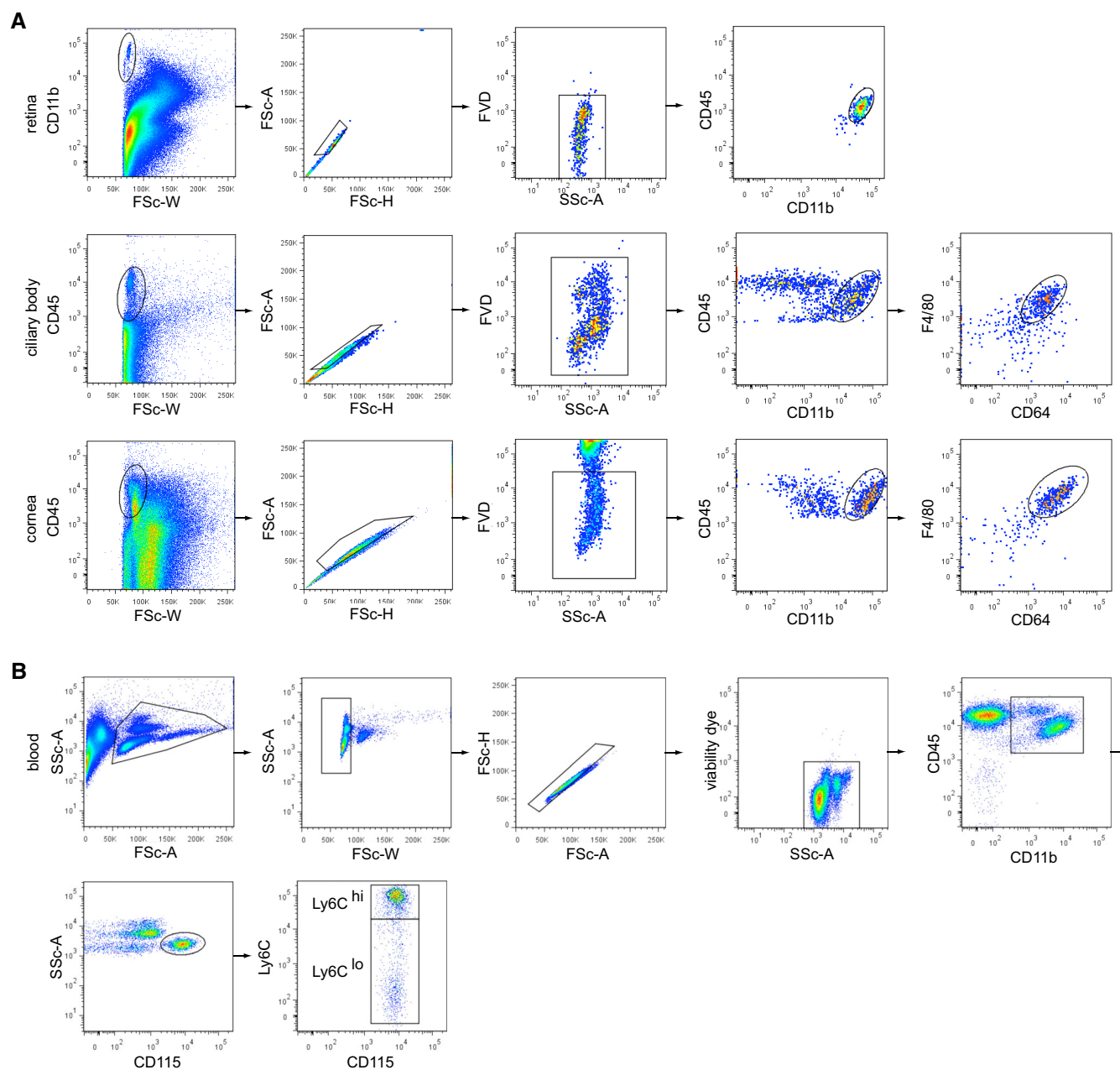


Figure EV4. Flow cytometric gating strategy for eye macrophages and monocytes.

A Gating strategy of eye macrophages. CD11b⁺ or CD45⁺ cells underwent doublet exclusion by FSc-W, -A, and -H gating and subsequent dead cell exclusion (fixable viability dye, FVD). Retinal microglia were specified as CD45^{lo}CD11b⁺, macrophages in the ciliary body and cornea as CD45^{lo}CD11b⁺CD64⁺F4/80⁺.

B Gating strategy of blood monocytes. Leukocytes underwent doublet exclusion by FSc-W, -A, and -H gating and subsequent dead cell exclusion (fixable viability dye, FVD). CD45⁺CD11b⁺ myeloid blood cells were further subdivided in CD45⁺CD11b⁺CD115⁺Ly6C^{hi} and CD45⁺CD11b⁺CD115⁺Ly6C^{lo} monocytes.

Figure EV5. Molecular survey of all retinal cells during neovascularization.

- A t-SNE representation of cells from control, CNV d3, and CNV d7 conditions.
- B t-SNE plot showing identity of individual cells. Color code indicates different cell types.
- C Unbiased cluster analysis of cell populations found in the retina upon CNV induction.
- D Heatmap of the 30 most regulated genes per cluster (adjusted *P*-value < 0.05 based on the negative binomial distribution). Scale bar represents the color-coded z-scores. Selected genes presented in t-SNE plots shown in (E) are marked by asterisks.
- E t-SNE presentations of genes characteristic for microglia subsets (*P2ry12*, *Tmem119*), dendritic cell cluster (*Cd74*, *H2-Aa*), Rods/Cone cluster (*Rho*, *Pdc*), and epithelial cells cluster (*Krt12*, *Gsto1*).

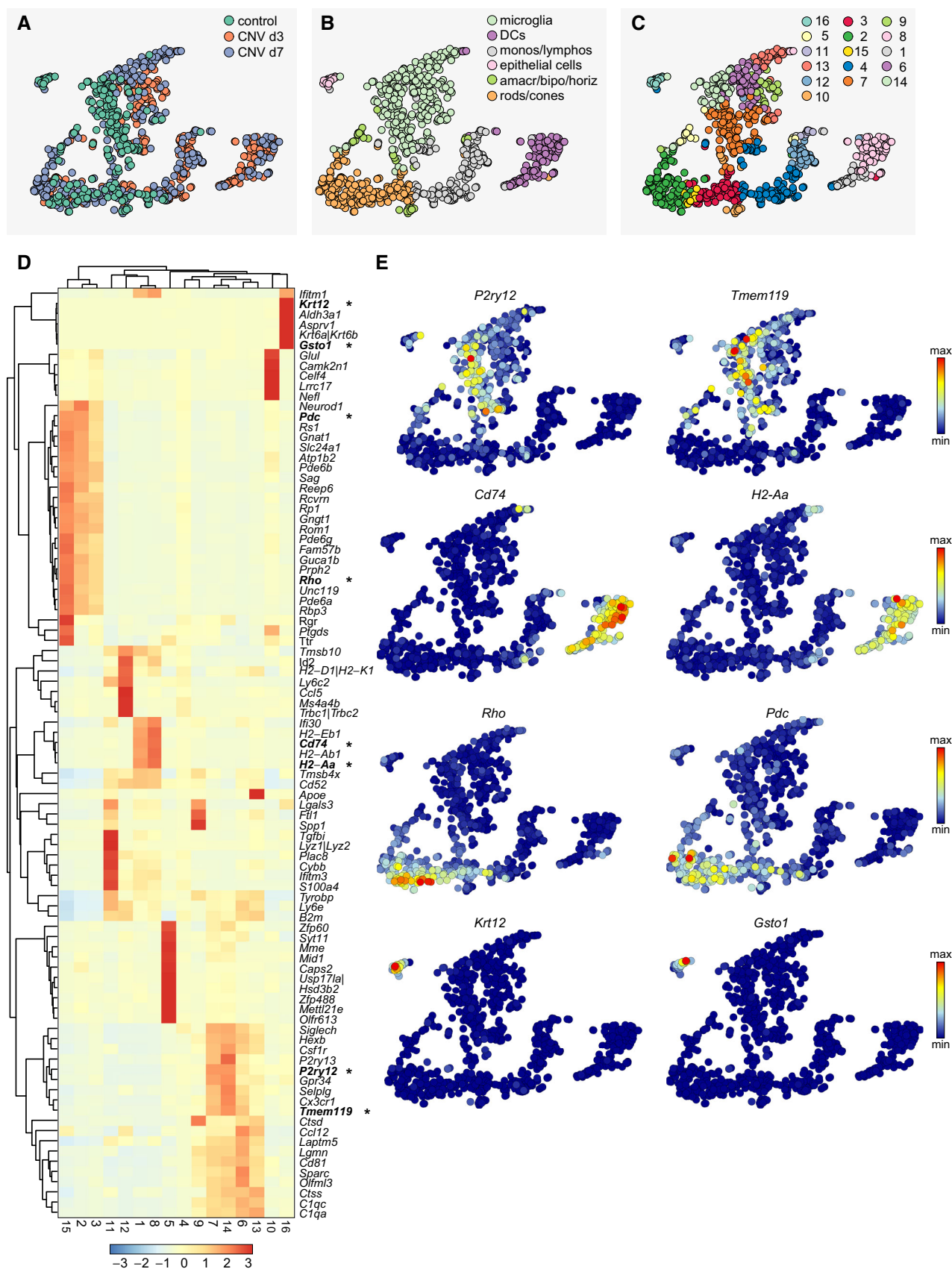


Figure EV5.

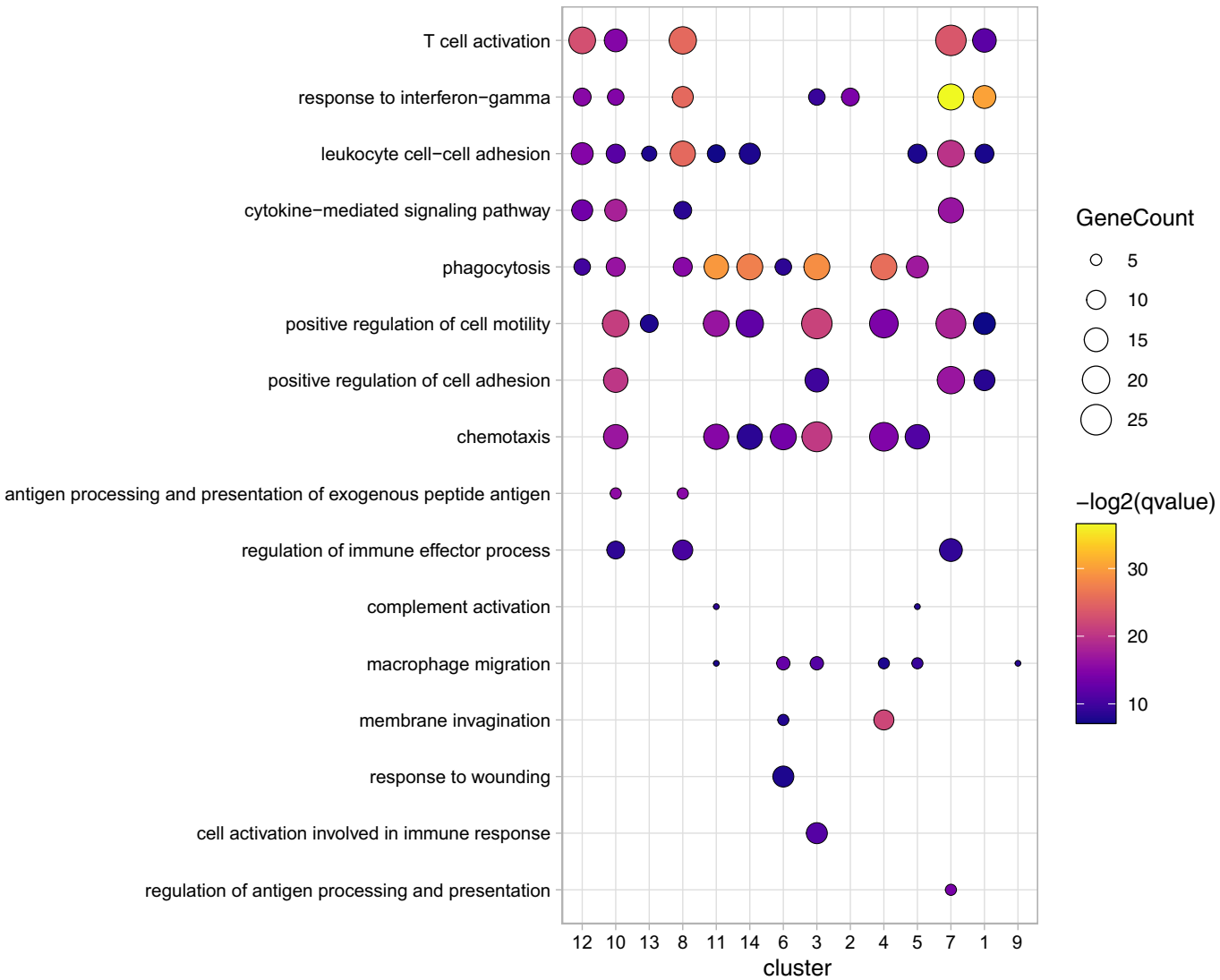


Figure EV6. Gene ontology enrichment (GO) analysis across all myeloid cell clusters during neovascularization.

GO pathways were selected and depicted for all cell cluster identified in Fig 7.