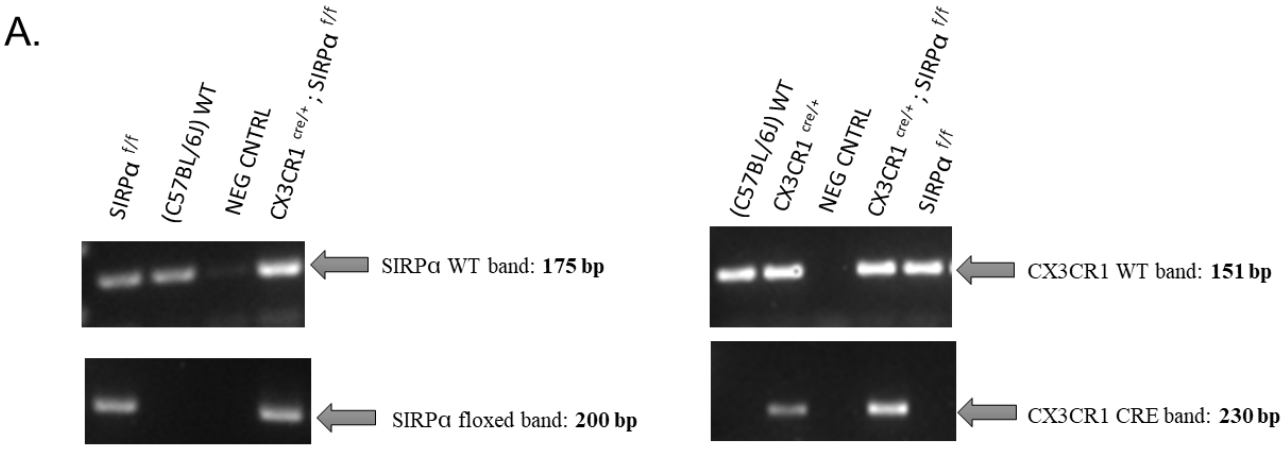
Supplementary figures

Disrupting the CD47/SIRPα Axis Protects Against Traumatic Optic Neuropathy

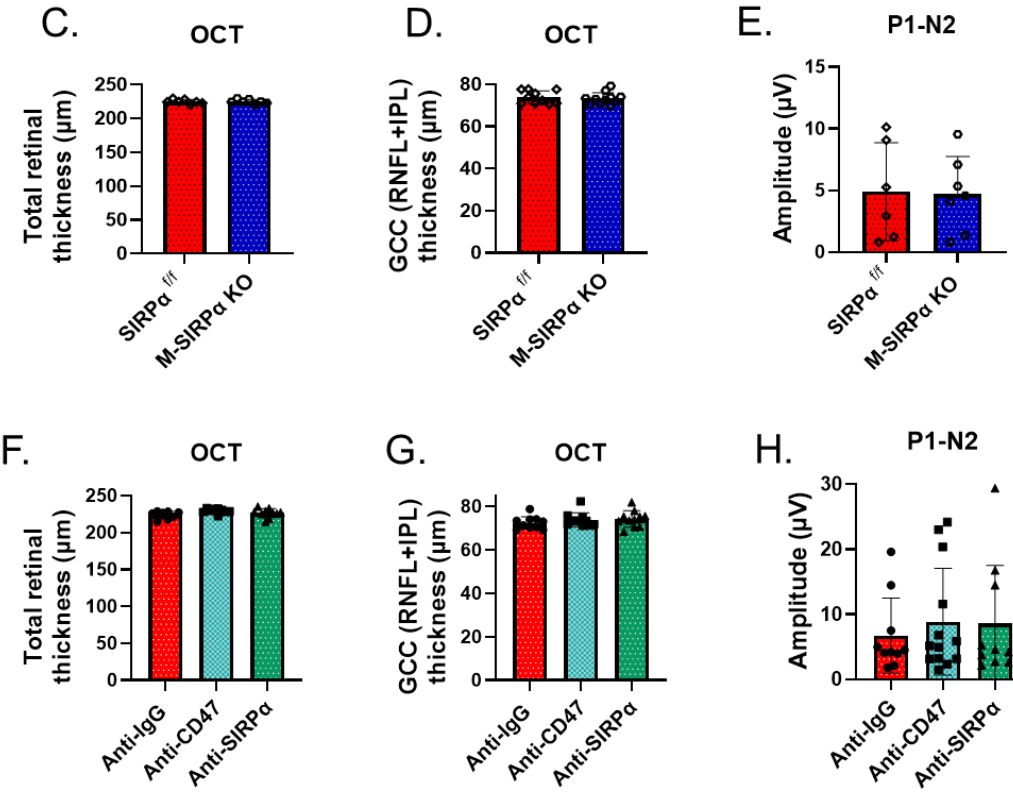
(Morris *et al.*)

Supplementary Figure 1.



B.

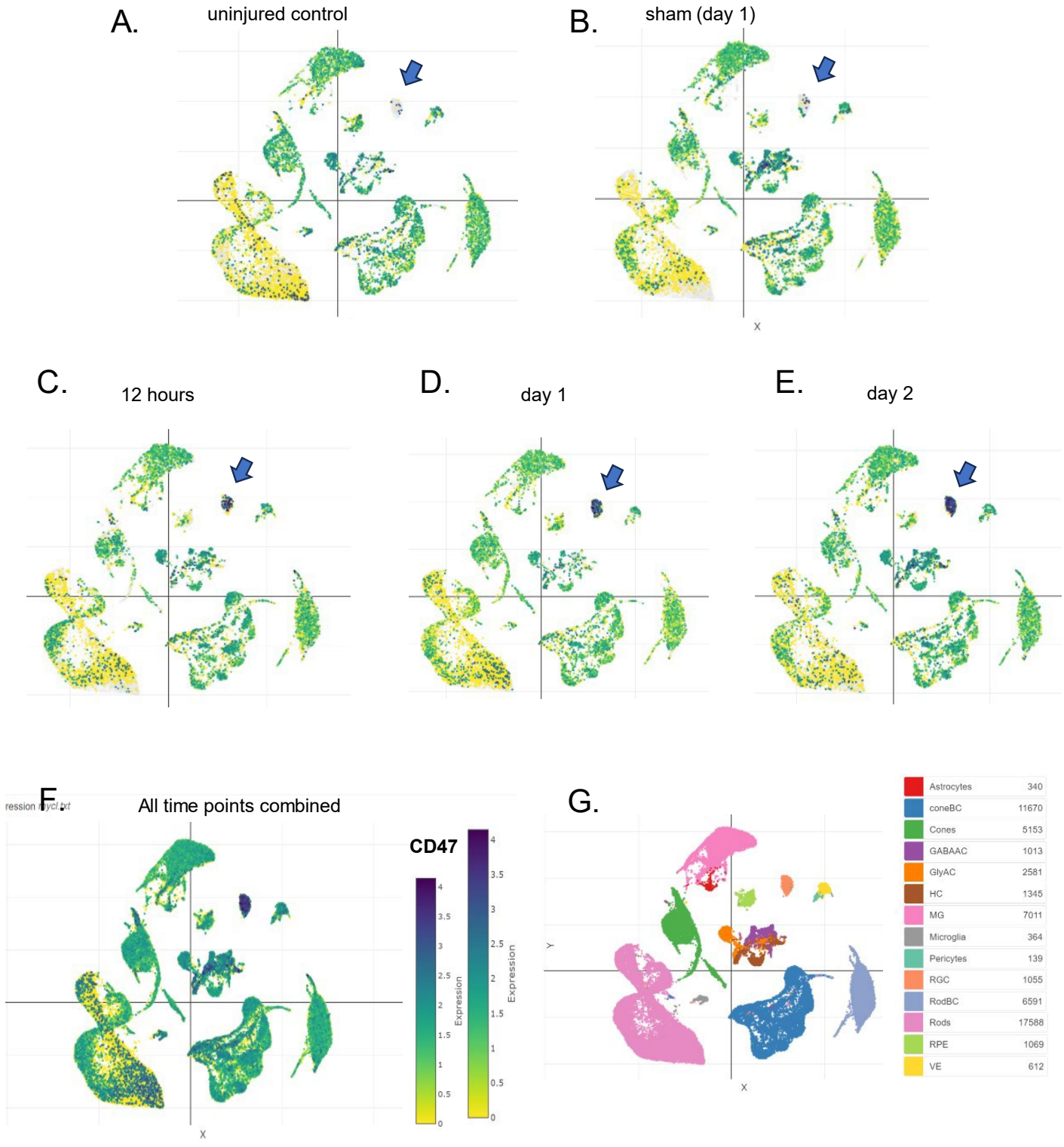
Genotyping Primer Sequences
SIRPa -FW genotyping (Sequence: TAG GGC CGC TGC TGC TCT GCC TGC TGC T)
SIRPa -RV genotyping (Sequence: TGG GCC TCA ACT CTG CAT CTG TAA)
SIRPa -F7 genotyping (Sequence: CCA GGA TCC GAA GTT CCT ATT CTC TAG)
Cx3cr1-Wild type Forward (Sequence: AGC TCA CGA CTG CCT TCT TC)
Cx3cr1-Common (Sequence: ACG CCC AGA CTA ATG GTG AC)
Cx3cr1-Mutant Forward (Sequence: GTT AAT GAC CTG CAG CCA AG)



Supplementary Figure 1. A. PCR genotyping shows the SIRPα WT band at 175 bp and floxed SIRPα band at 200 bp. CX3CR1 WT band at 151 bp and deletion of SIRPα with CX3CR1 Cre band at 230 bp. PCR results confirm successful generation of CX3CR1 cre/+; SIRPα $^{f/f}$ myeloid KO (M-SIRPα KO) mice. **B.** PCR Primer sequences used to genotype mice are listed. **C, D.** Optical coherence tomography (OCT) results show no

significant difference in total retina or ganglion cell complex (GCC) thickness between M-SIRPα KO and SIRPα $^{f/f}$ sham groups, N=10-11 per group. GCC was calculated as retinal nerve fiber layer (RNFL) + inner plexiform layer (IPL). **E.** Pattern electroretinography (PERG) results show no difference in P1-N2 peaks between M-SIRPα KO and SIRPα $^{f/f}$ sham groups, N=6-8 per group. **F, G.** OCT shows no significant difference in sham retinal thickness among the antibody-treated groups, N=10-11 per group. **H.** PERG results show no significant difference among the sham retinas of antibody-treated groups, N=10-13 per group.

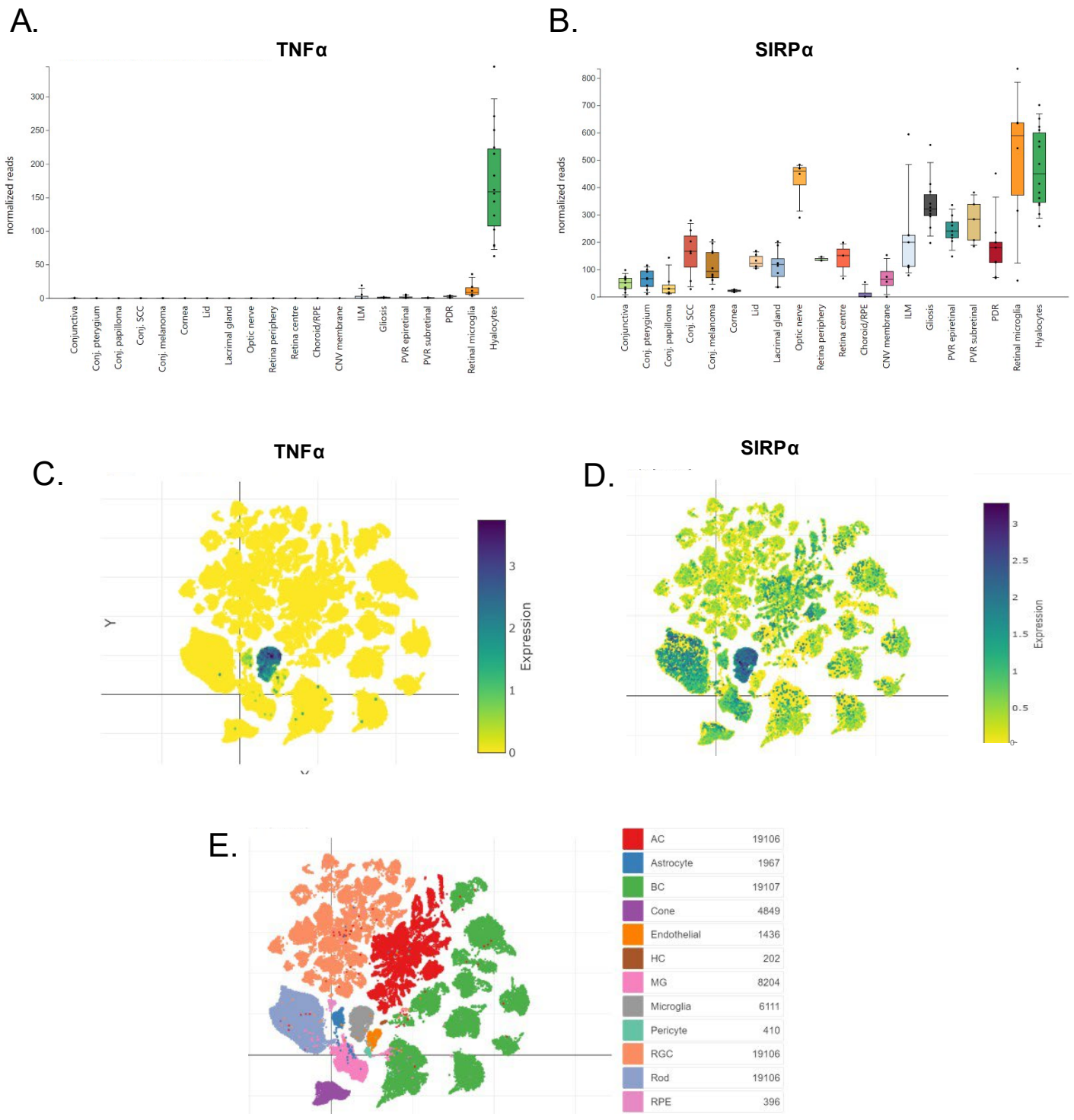
Supplementary Figure 2.



Supplementary Figure 2. Publicly available single-cell RNA-seq show CD47 mRNA upregulation in the retinal ganglion cells after ONC. A-F. Investigating publicly available single-cell RNA-seq of adult mouse whole retinal cell types show increased CD47 mRNA expression in the ganglion cell layer as early as 12 hours after ONC with further increases at days 1 and 2. Six t-distributed stochastic neighbor embedding (t-SNE) plots and a legend displaying the expression levels of CD47 across different cell types in an uninjured mouse retina, sham

retina, 12 hours, day 1, day 2 after ONC and a combined time point plot. The color gradient indicates the level of CD47 gene expression, with blue representing higher expression and yellow lower expression. **G.** Segmented and color-coded t-SNE plot representing different cell types with a corresponding legend listing these cell types and their respective counts. Figures were created with Single Cell Portal (Tarhan et al. BioRxiv, 2023, PMID: 37502904). Data were extracted from Qian et al. Nature Communications, 2024, PMID: 38467611. ConeBC: cone bipolar cells, GABAAC: GABAergic amacrine cells, GlyAC: glycinergic amacrine cells, HC: horizontal cells, MG: Müller glia, rodBC: rod bipolar cells, RGC: retinal ganglion cells, RPE: retinal pigment epithelial cells.

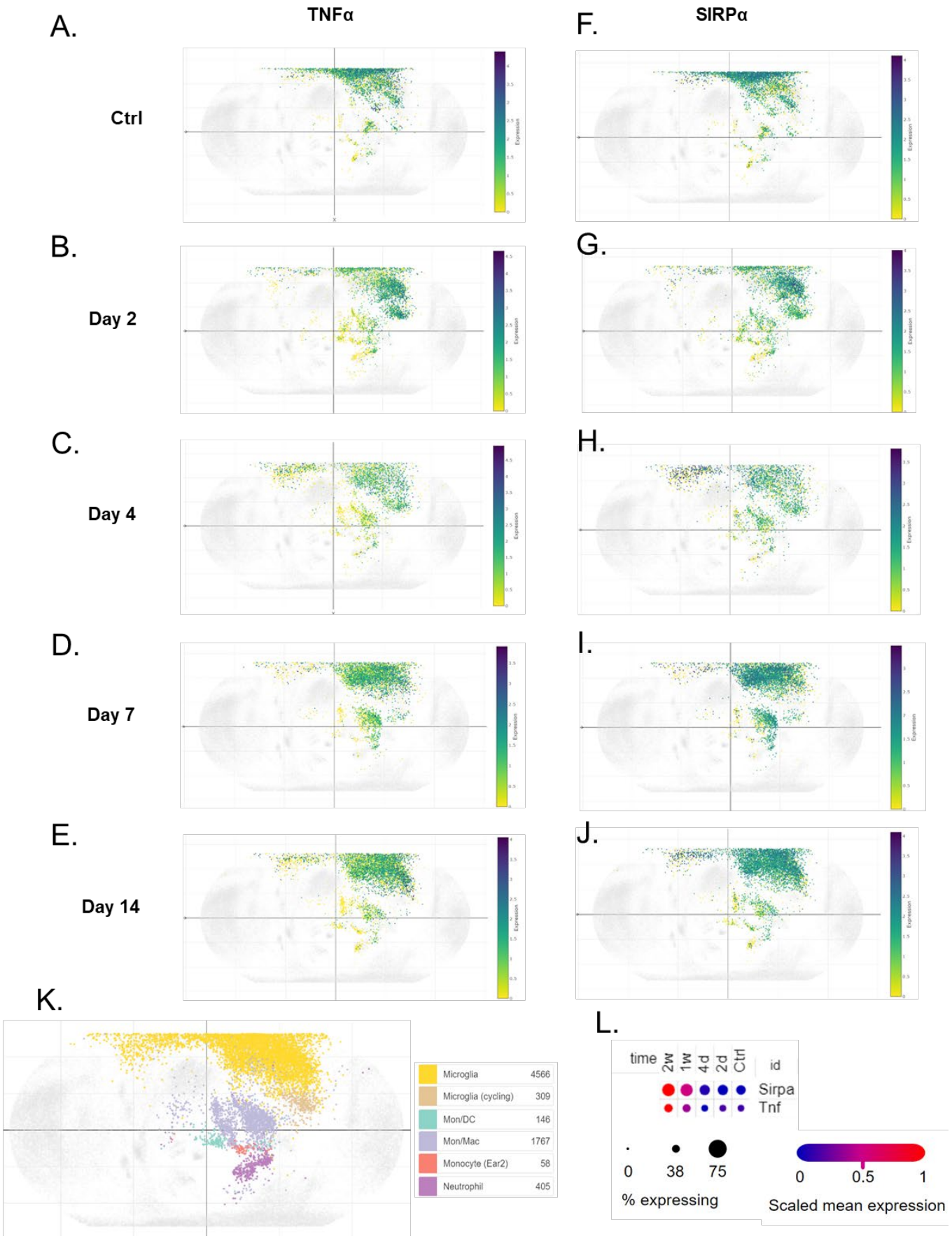
Supplementary Figure 3.



Supplementary Figure 3. Publicly available data show TNFα and SIRPα expression in retinal microglia. A, B. Graphs extracted from a searchable transcriptome database for human eye tissue (Wolf et al. Genetics 2022, PMID: 35124170) show high TNFα and SIRPα expression in retinal microglia and vitreous macrophages (hyalocytes). **C, D.** Publicly available single-cell RNA-seq of the uninjured adult mouse retina confirms TNFα and SIRPα expression in retinal microglia. Two t-SNE plots display the expression levels of TNFα and SIRPα, respectively, across different cell types. **E.** Segmented and color-coded t-SNE plot representing different cell

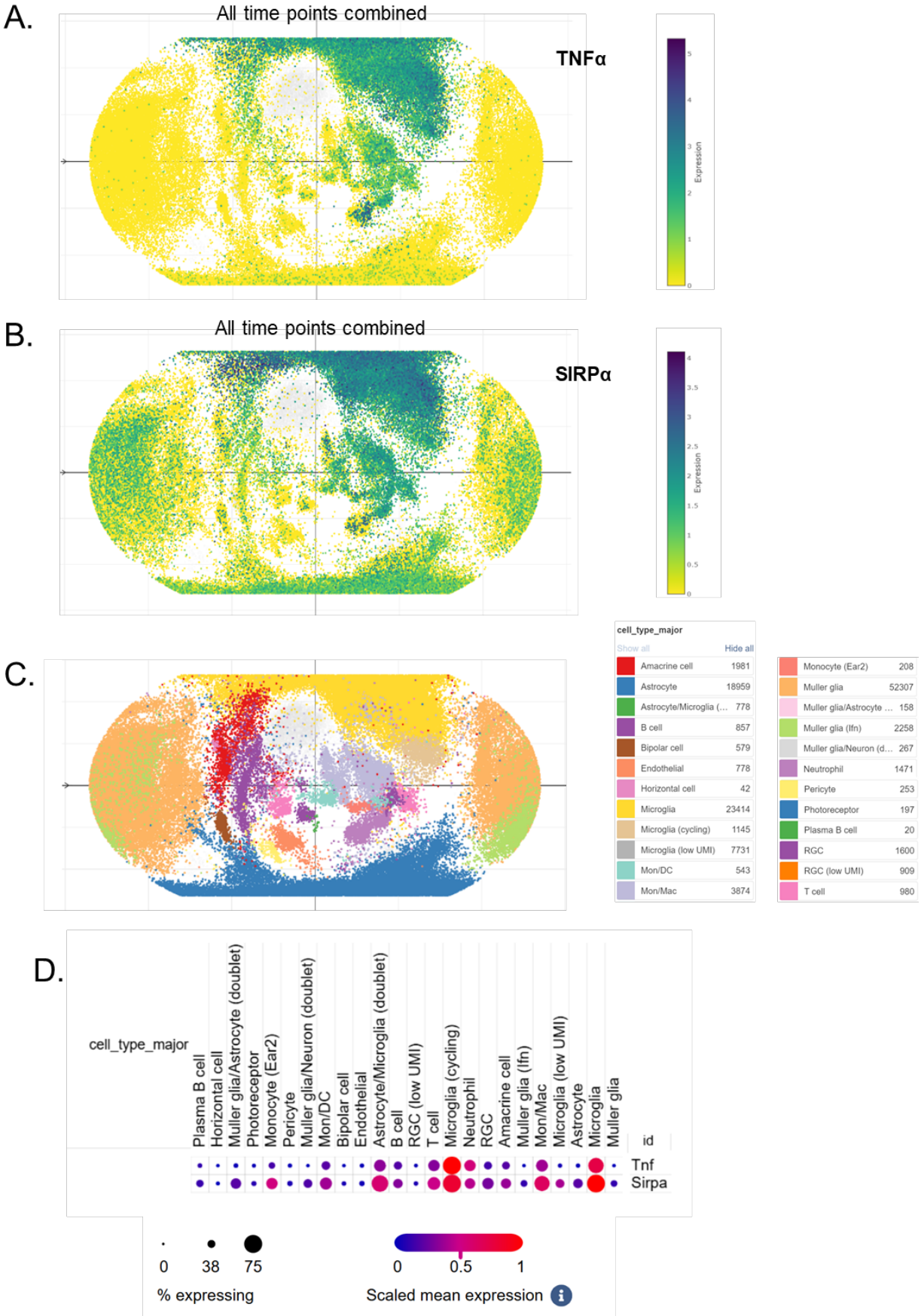
types, with a corresponding legend listing these cell types and their respective counts. The color gradients in the TNFα and SIRPα plots indicate the level of gene expression, with blue representing higher expression and yellow lower expression. Figures were created with Single Cell Portal (Tarhan et al. BioRxiv, 2023, PMID: 37502904). Data were extracted from Li et al. iScience, 2024, PMID: 38812536. AC: Amacrine cells, BC: bipolar cells, HC: horizontal cells, MG: Müller glia, RGC: retinal ganglion cells, RPE: retinal pigment epithelial cells.

Supplementary Figure 4.



Supplementary Figure 4. Temporal expression of TNFα and SIRPα mRNA in myeloid cells after ONC. A-J. Myeloid cell only t-SNE plots from publicly available single-cell RNA-seq data showing TNFα and SIRPα mRNA expression in microglia, macrophages (Mac), monocytes (Mon), dendritic cells (DC) and neutrophils at days 2, 4, 7, and 14 after ONC as compared to control (uninjured) retina. **K.** Key for single-cell RNA-seq data displaying myeloid cell types in the t-SNE plot. **L.** Dot plot showing temporal expression TNFα and SIRPα mRNA (on the y-axis) with a peak increase at 14 days after ONC. The X-axis shows control (Ctrl), days 2 and 4, as well as weeks 1 and 2. The size and color of the dots indicate the percentage of cells expressing a particular gene and the scaled mean expression level, respectively. The color gradient at the bottom shows the scale for mean expression, ranging from blue (low) to red (high). Figures were created with Single Cell Portal (Tarhan et al. BioRxiv, 2023, PMID: 37502904). Data were extracted from Benhar et al. Nature Immunology, 2023, PMID: 36807640.

Supplementary Figure 5.



Supplementary Figure 5. Cellular expression of TNFα and SIRPα mRNA after ONC. **A, B.** Two t-SNE plots of combined data from control and days 2, 4, 7, and 14 after ONC show TNFα and SIRPα mainly expressed in myeloid cells. **C.** Key for single-cell RNA-seq data displaying all cell types in the t-SNE plot. **D.** Dot plot showing expression of TNFα and SIRPα mRNA (on the y-axis) in different retinal cell types listed on the x-axis. The size and color of the dots indicate the percentage of cells expressing a particular gene and the scaled mean expression level, respectively. The color gradient at the bottom shows the scale for mean expression, ranging from blue (low) to red (high). Figures were created with Single Cell Portal (Tarhan et al. BioRxiv, 2023, PMID: 37502904). Data were extracted from Benhar et al. Nature Immunology, 2023, PMID: 36807640.