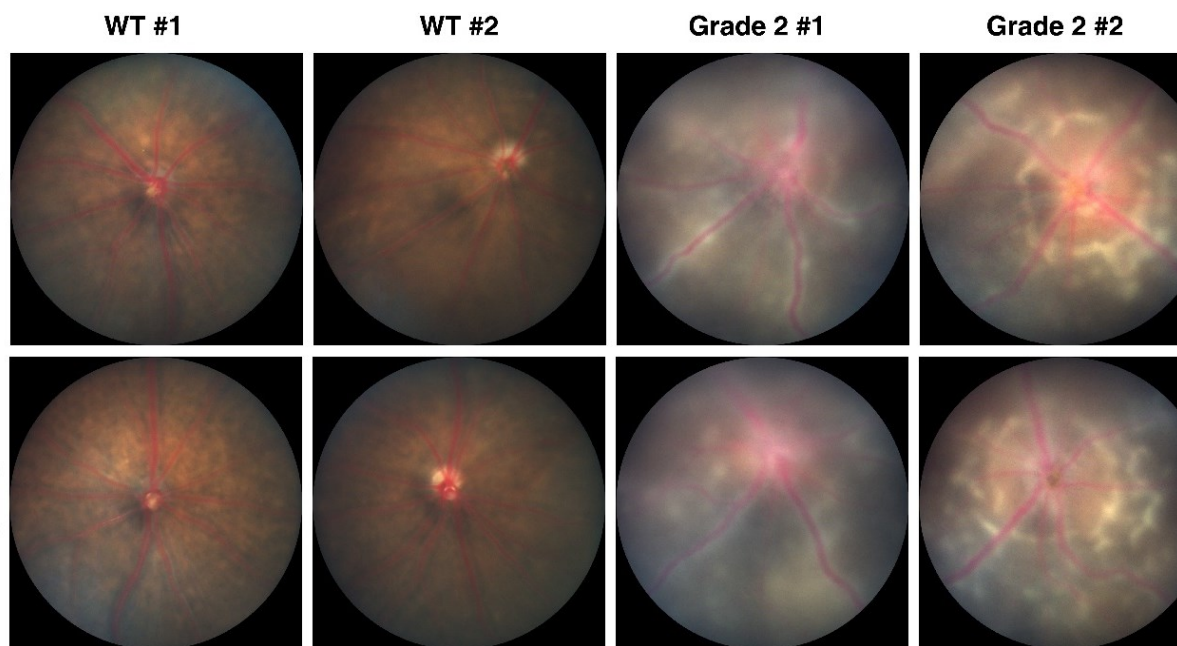
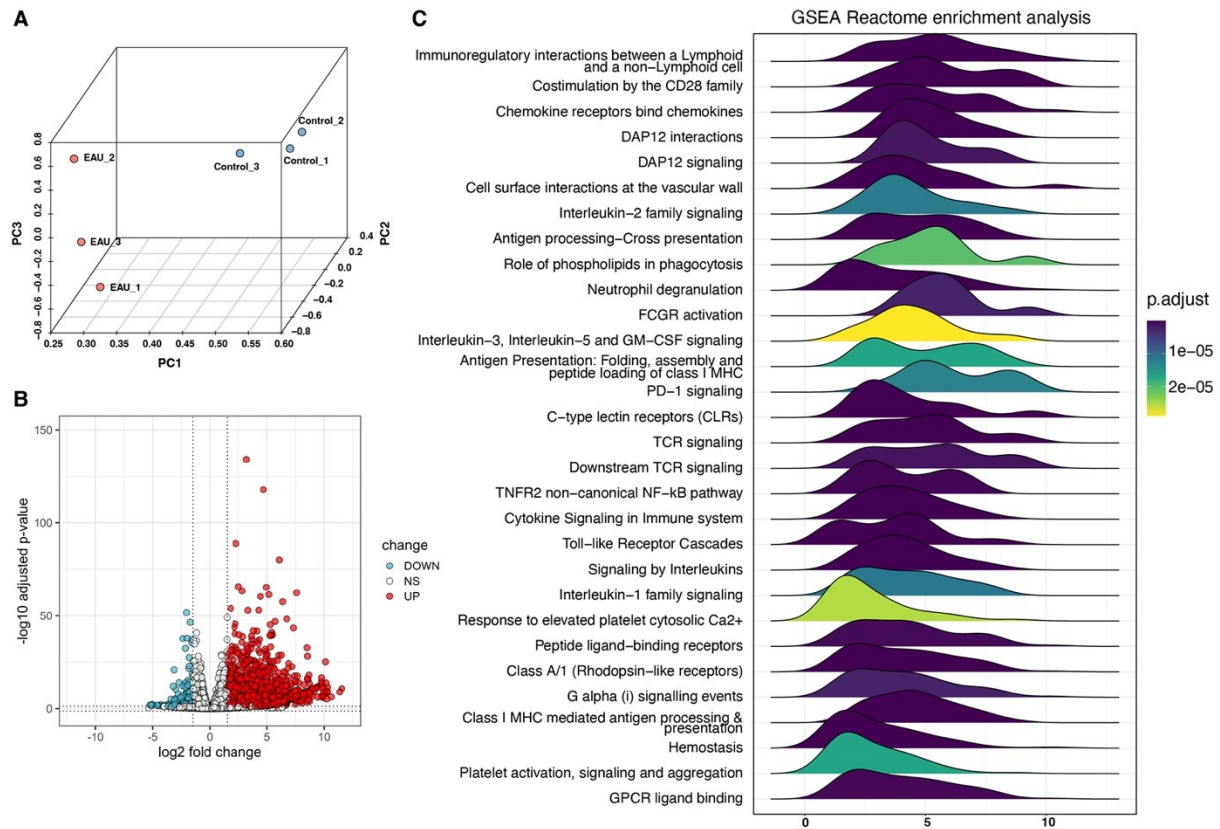


## **Supplementary Materials**

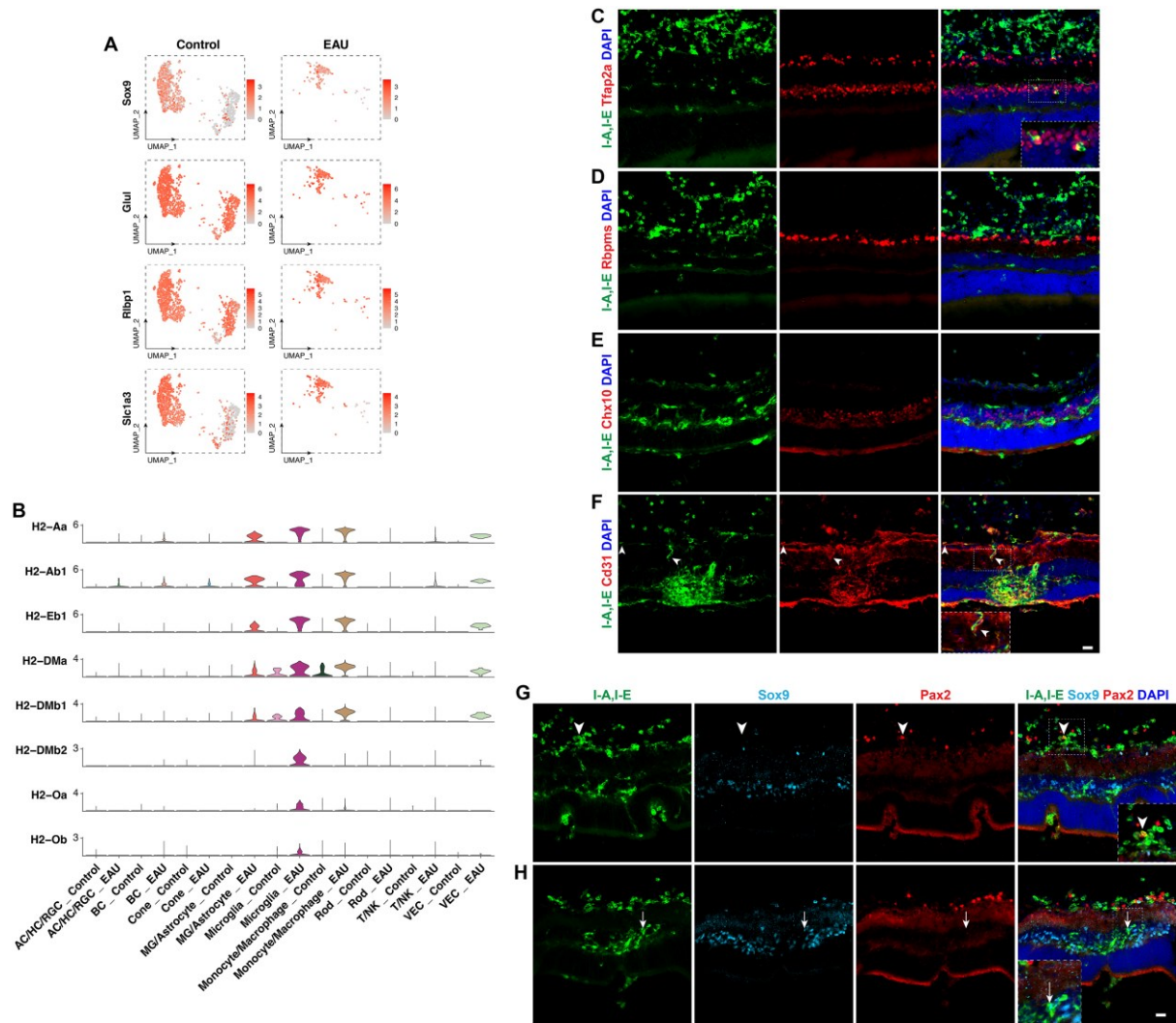
**Figures S1-S7 and Tables S1-S7**



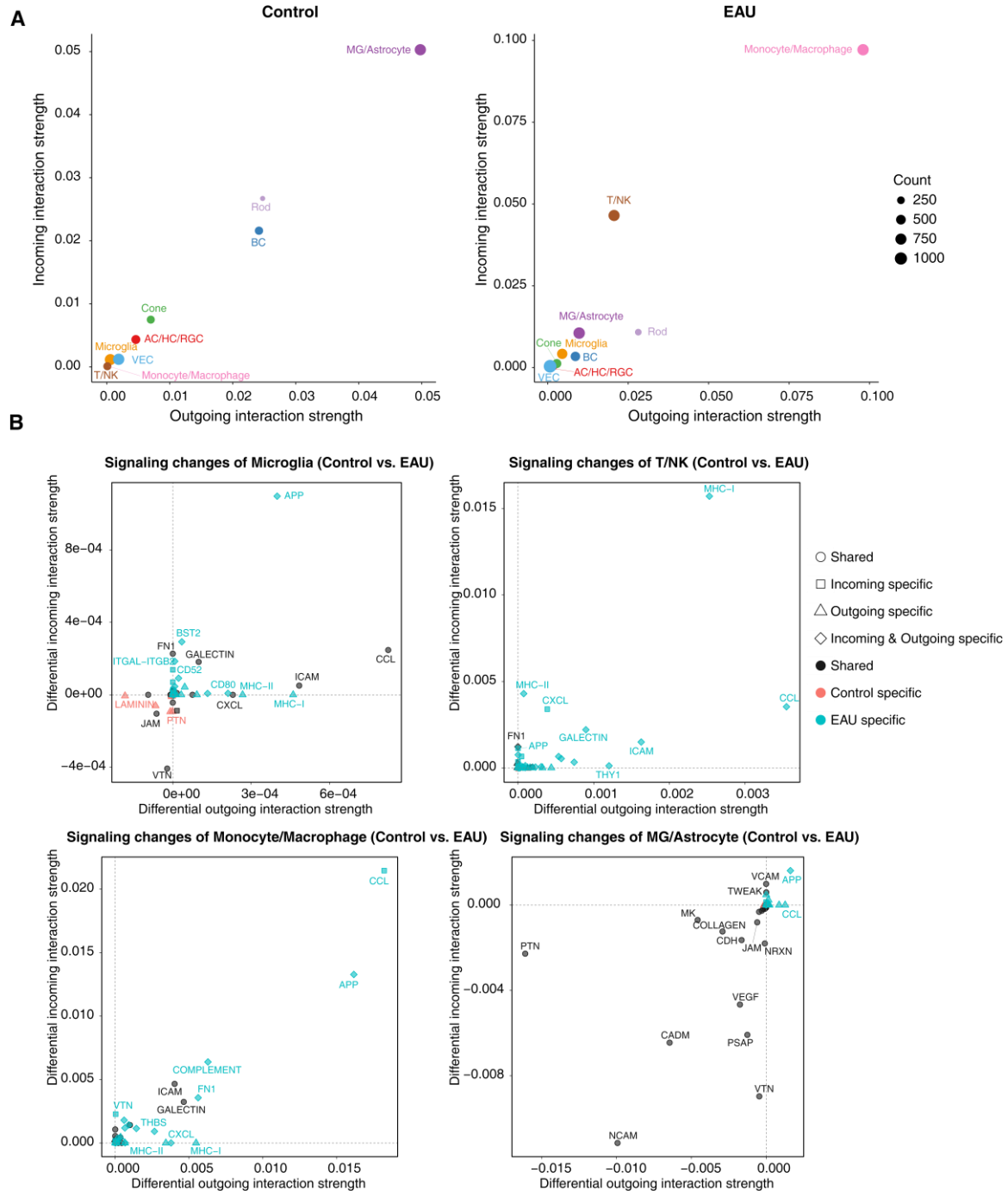
**Figure S1. Fundus images of retinas used for scRNA-seq analysis.** Two wild-type control mice and two grade 2 mice (14 d.p.i.) were used for scRNA-seq analysis.



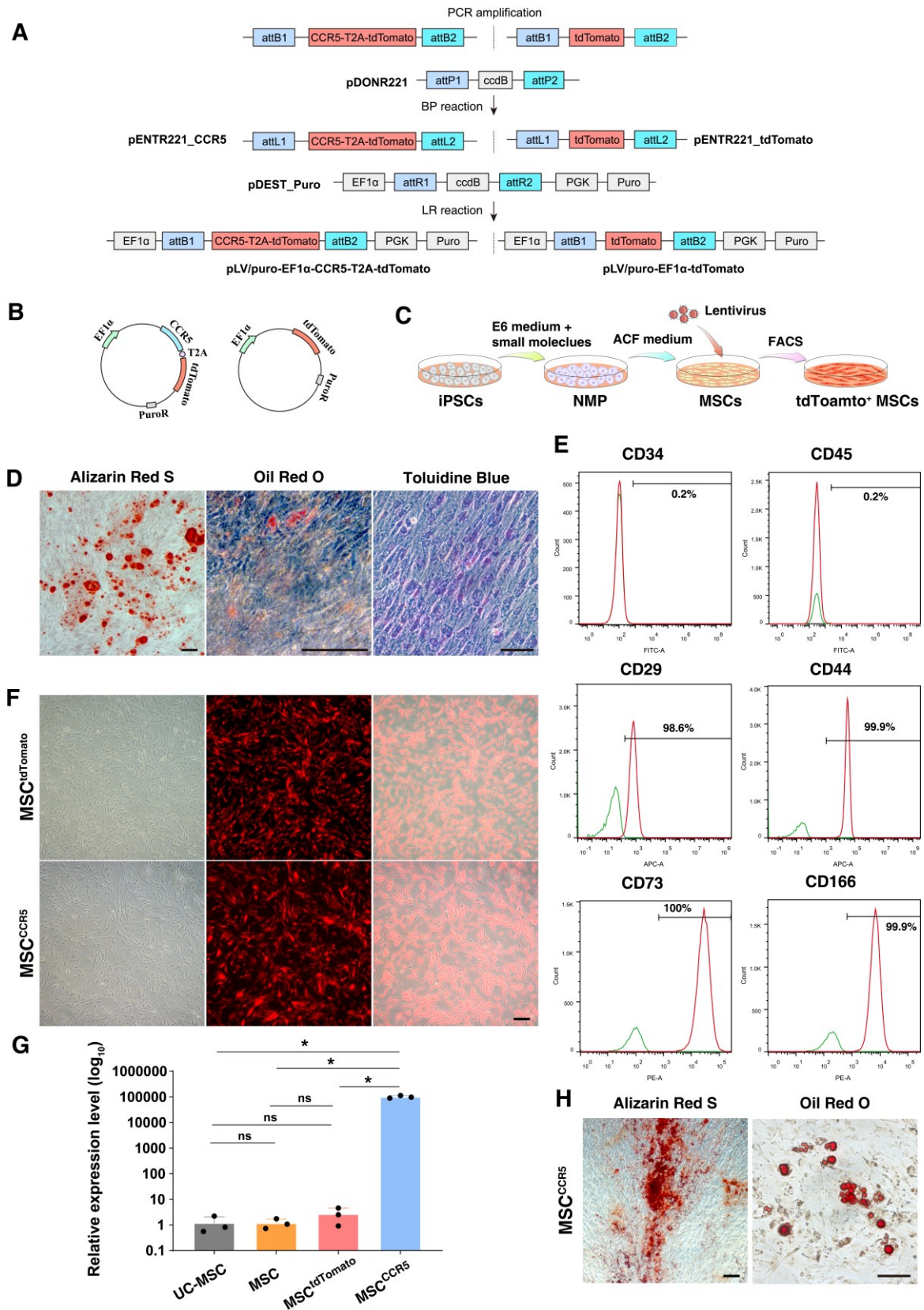
**Figure S2. RNA-seq analysis of control and EAU retinas.** (A) PCA (principal component analysis) plot showing that EAU retinal samples were well discriminated from the control retinal samples. (B) Volcano plot (significance vs fold change) of significantly downregulated (blue) and upregulated (red) genes (fold change  $\geq 1.5$  and  $p < 0.05$ ) between EAU and control retinas. (C) GSEA Reactome pathway enrichment analysis of all known genes.



**Figure S3. Expression of MHC II genes in control and EAU retinas.** (A) Feature plots comparing the expression of known Müller glia marker genes in control and EAU retinas. (B) Stacked violin plots showing expression patterns of MHC-II genes in different cell types. (C) Immunostaining of EAU retinal sections for MHC II proteins I-A/I-E (green) and Tfap2a (red) with DAPI counter-labeling (blue). (D) Immunostaining of EAU retinal sections for MHC II proteins I-A/I-E (green) and Rbpms (red) with DAPI counter-labeling (blue). (E) Immunostaining of EAU retinal sections for MHC II proteins I-A/I-E (green) and Chx10 (red) with DAPI counter-labeling (blue). (F) Immunostaining of EAU retinal sections for MHC II proteins I-A/I-E (green) and Cd31 (red) with DAPI counter-labeling (blue). Arrowheads indicate representative MHC II<sup>+</sup>Cd31<sup>+</sup> VECs. (G, H) Immunostaining of EAU retinal sections for MHC II proteins I-A/I-E (green), Sox9 (cyan), and Pax2 (red) with DAPI counter-labeling (blue). The arrowheads indicate a representative MHC II<sup>+</sup>Pax2<sup>+</sup>Sox9<sup>-</sup> astrocyte, and the arrows indicate a representative MHC II<sup>+</sup>Sox9<sup>+</sup>Pax2<sup>-</sup> Müller glia.

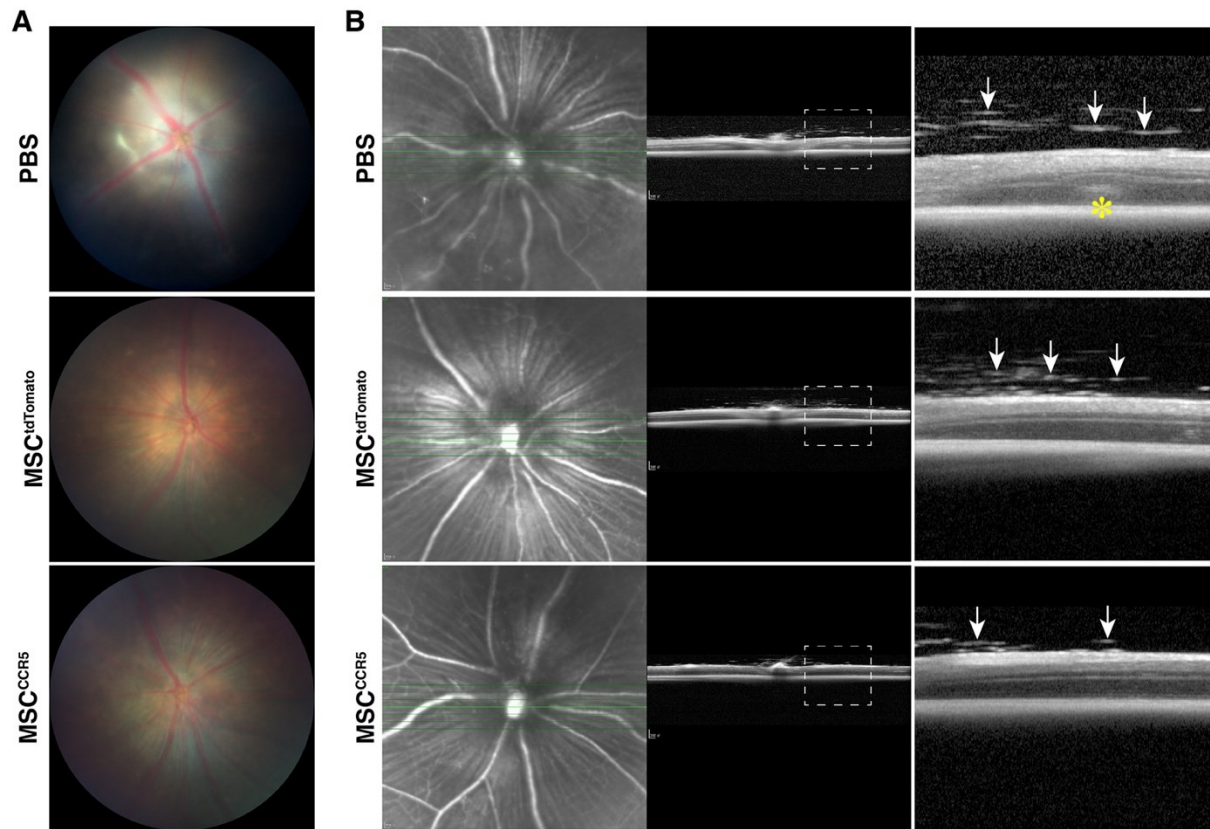


**Figure S4. Changes in strength of outgoing and incoming signal interactions in EAU retinas.** (A) Comparison of the strength of outgoing and incoming interactions in 2D space to identify cell populations that send or receive signals that change significantly between control and EAU retinas. (B) Visualization of differential outgoing and incoming signaling changes in **Microglia**, **T/NK**, **Monocyte/Macrophage**, and **MG/Astrocyte** cell clusters in EAU retinas compared to controls.

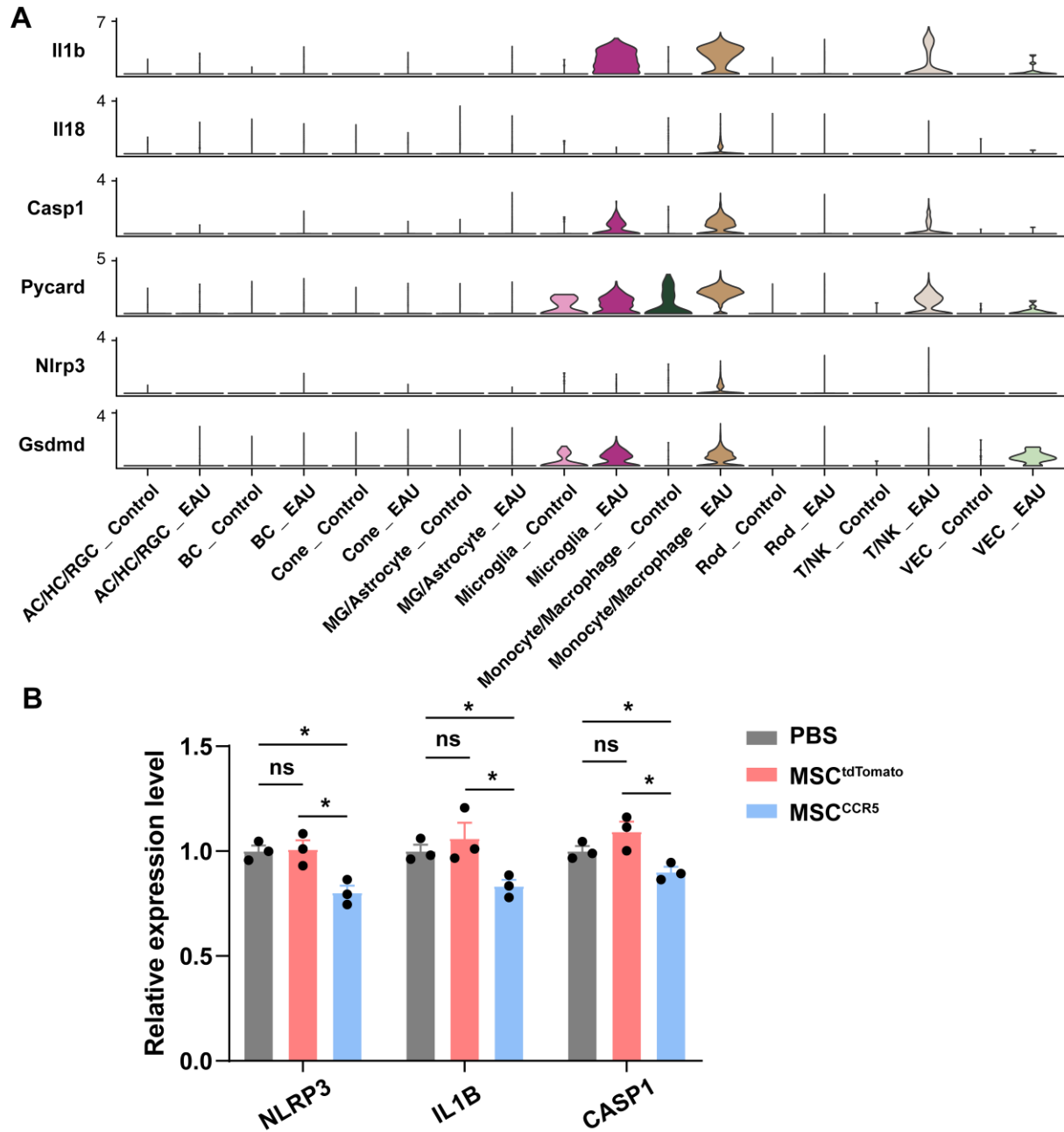


**Figure S5. MSC induction and modification.** (A) Schematic of the procedure for construction of the pLV/Puro-EF1 $\alpha$ -CCR5-T2A-tdTomato and pLV/Puro-EF1 $\alpha$ -tdTomato lentiviral expression plasmids. These plasmids were constructed using the gateway cloning

method. (B) Schematic diagrams of the pLV/Puro-EF1 $\alpha$ -CCR5-T2A-tdTomato and pLV/Puro-EF1 $\alpha$ -tdTomato plasmids. (C) Schematic diagram of the procedure for MSC induction from human iPSCs, lentiviral infection, and enrichment of tdTomato<sup>+</sup> MSCs. (D) Following differentiation, the osteogenic, adipogenic, and chondrogenic differentiation potential of hiPSC-derived MSCs were verified by staining with alizarin red S, oil red O and toluidine blue, respectively. (E) Flow cytometric analysis for detection of several typical MSC surface protein markers (CD29, CD44, CD73, and CD166) and hematopoietic markers (CD34 and CD45). (F) Normal morphologies of MSC<sup>tdTomato</sup> and MSC<sup>CCR5</sup> cells under bright field and fluorescence microscopy (red fluorescence resulted from expressed tdTomato). (G) qRT-PCR assay of CCR5 in UC-MSC, MSC, MSC<sup>tdTomato</sup> and MSC<sup>CCR5</sup>. Data are presented as mean  $\pm$  SD (n = 3 individual samples per group). \*p < 0.000001, ns, no significance. (H) The osteogenic and adipogenic differentiation potential of MSC<sup>CCR5</sup> cells were verified by staining with alizarin red S and oil red O, respectively. ACF: animal component-free; FACS: fluorescence-activated cell sorting; NMP: neuromesodermal progenitor; UC: umbilical cord. Scale bar: (D, F, H) 100  $\mu$ m.



**Figure S6. Transplantation of MSCs ameliorated EAU *in vivo*.** (A) Representative fundus photographs of the eyes from EAU mice at 14 d.p.i., transplanted with MSC<sup>tdTomato</sup> or MSC<sup>CCR5</sup> cells, or administered with PBS. (B) Representative fundus photographs (left) and OCT images (middle and right) of the eyes from EAU mice at 14 d.p.i., transplanted with MSC<sup>tdTomato</sup> or MSC<sup>CCR5</sup> cells, or administered with PBS. The right panels are higher magnification views of the corresponding outlined regions in the middle panels. The arrows point to inflammatory infiltrating cells and the asterisk indicates retinal fold and detachment in EAU eyes.



**Figure S7. The NLRP3 inflammasome was activated in EAU and suppressed by MSCs.**

(A) Stacked violin plots showing the expression patterns of Nlrp3 inflammasome genes in control and EAU retinas based on scRNA-seq analysis. (B) qRT-PCR analysis was performed for *NLRP3*, *IL1B* (IL-1 $\beta$ ) and *CASP1* (Caspase-1) expressed in PMA and LPS-treated THP-1 cells cultured in the absence (PBS) or presence of MSC<sup>tdTomato</sup> or MSC<sup>CCR5</sup> cells. Data are presented as mean  $\pm$  SEM ( $n = 3$  individual samples per group). \* $p < 0.05$ , ns, no significance.

**Table S1. Adapted clinical scoring criteria of EAU**

| <b>Clinical Grade</b> | <b>Criteria</b>                                                                                                                                 |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| 0                     | Normal retina                                                                                                                                   |
| 0.5                   | Few (1~2), very small and peripheral focal lesions; minimal vasculitis and vitritis                                                             |
| 1                     | Mild vasculitis; <5 small, peripheral and focal lesions; 1 linear lesion                                                                        |
| 2                     | Retinal edema; severe vasculitis (large size, thick wall, infiltrations); diffuse chorioretinal lesions and/or infiltrations; <5 linear lesions |
| 3                     | Papilledema; a pattern of linear lesions; large confluent chorioretinal lesions; subretinal neovascularization; subretinal hemorrhages          |
| 4                     | Large retinal detachment; retinal atrophy                                                                                                       |

**Table S2. Marker genes used for identifying major retinal cell types**

| <b>Retinal Cell Types</b> | <b>Marker genes</b>                                                                                            |
|---------------------------|----------------------------------------------------------------------------------------------------------------|
| Amacrine cell             | <i>Gad1, Slc6a9, Stx1b, Calb2, Chat, Th, Slc17a8, Ebf3, Tfap2a, Tfap2b</i>                                     |
| Astrocyte                 | <i>Pax2, Gfap, Vim, S100b</i>                                                                                  |
| Bipolar cell              | <i>Scgn, Grik1, Vsx1, Lhx4, Glra1, Fezf2, Zfhx4, Otx2, Vsx2, Trpm1, Grm6, Sebox, Prkca, Vstm2b</i>             |
| Cone                      | <i>Opn1sw, Opn1mw, Gnat2, Arr3, Pde6h</i>                                                                      |
| Horizontal cell           | <i>Lhx1, Calb1, Gja10, Onecut1, Lim1</i>                                                                       |
| Müller glia               | <i>Abca8a, Aldh1a1, Kncj10, Sox9, S100a10, Apoe, Slc1a3, Apoe, Dkk3, Gpr37, Rlbp1, Rax, Hes1, Notch1, Glul</i> |
| Retinal ganglion cell     | <i>Sncg, Pou4f1, Nrn1, Slc17a6, Pou4f2, Rbpms</i>                                                              |
| Rod                       | <i>Rho, Gnat1, Cnga1, Nrl, Nr2e3, Rcvrn</i>                                                                    |
| Vascular endothelial cell | <i>Cldn5, Cdh5, Pecam1, Vwf, Erg, Tek, Kdr, Flt1</i>                                                           |

**Table S3. Marker genes used for identifying major immune cell types**

| Immune Cell Types        | Marker genes                                                                      |
|--------------------------|-----------------------------------------------------------------------------------|
| Microglia                | <i>Tmem119, P2ry12, Crybb1, Rspo1, Serpine2, Astn1, Cx3cr1, Csf1r, Cd68, Aif1</i> |
| Monocytes/Macrophages    | <i>Ccl2, Ccr2, Ly6c2, Mrc1, Itgax, Cx3cr1, Csf1r, Aif1</i>                        |
| T cells                  | <i>Cd3d, Cd3e, Cd3g, Il2, Cd4, Cd8a, Cd8b1, Foxp3</i>                             |
| Cd8 T cells              | <i>Cd8a, Cd8b1, Cd3e</i>                                                          |
| NK cells                 | <i>Ncr1, Klra1, Klra6, Klrb1c, Klrb1a, Klrb1b, Klrb1f</i>                         |
| Th1 cells                | <i>Tbx21, Cxcr6, Ifng, Tnf, Stat4, Cd4</i>                                        |
| Th17 cells               | <i>Il17a, Il17f, Rorc, Stat3, Cd4</i>                                             |
| Treg cells               | <i>Foxp3, Il2ra, Il10, Tgfb1, Cd4</i>                                             |
| Undifferentiated T cells | <i>Cd3e, S1pr1, Ccr7, Il6ra, Lef1, Cd44, Cd69, Cd4</i>                            |

**Table S4. Gene-specific primer sequences used for qRT-PCR analysis**

| Gene                                 | Forward Primer               | Reverse Primer                |
|--------------------------------------|------------------------------|-------------------------------|
| <i>Ccl2</i>                          | CCCAAAGAAGCTGTAGTTTTGTCA     | GACCTTAGGGCAGATGCAGTT         |
| <i>Ccl3</i>                          | CCAAGTCTTCTCAGCGCCA          | CGGTTTCTCTTAGTCAGGAAAATGA     |
| <i>Ccl4</i>                          | CTCTCCTCTTGCTCGTGGC          | TGCCTCTTTTGGTCAGGAATACCA      |
| <i>Ccl5</i>                          | CATATGGCTCGGACACCACT         | TCGAGTGACAAACACGACTG          |
| <i>Ccl6</i>                          | ATCAAGCCGGGCATCATCTTT        | TTCCCAGATCTTGGGCCTTG          |
| <i>Ccl7</i>                          | TCCCTGGGAAGCTGTTATCTTCAA     | GCTATAGCCTCCTCGACCCA          |
| <i>Ccl8</i>                          | ATGGAAGCTGTGGTTTTCCAGA       | ACTTCTGGTCAAGGATCTCCA         |
| <i>Ccl9</i>                          | CAGGCCGGGCATCATCTTTA         | AGGTCCGTGGTTGTGAGTTT          |
| <i>Ccl12</i>                         | CGGGAAGCTGTGATCTTCAGGA       | TGAAGGTTCAAGGATGAAGGTTTG      |
| <i>Ccl17</i>                         | ACTTCAAAGGGGCCATTCTT         | TGGACAGTCAGAAACACGATGG        |
| <i>Ccl22</i>                         | CAAGCCTGGCGTTGTTTTGA         | TGGAGTAGCTTCTTCACCCAG         |
| <i>Ccl25</i>                         | GCAACCTACGTGCTGTGAGA         | GGCATCATCACCATCCTGGG          |
| <i>Ccl27a</i>                        | GGAGGAGATCTACTGGGGCT         | GGCAGAGGCAAGGCTTCTT           |
| <i>Gsdmd</i>                         | GCGATCTCATTCCGGTGGACAG       | TTCCCATCGACGACATCAGAGAC       |
| <i>Il1b (IL-1<math>\beta</math>)</i> | TGAAATGCCACCTTTTGACAG        | CCACAGCCACAATGAGTGATAC        |
| <i>Nlrp3</i>                         | GGTCCTCTTTACCATGTGCTTC       | AAGTCATGTGGCTGAAGCTGTA        |
| <i>Caspase1</i>                      | CAGGCAAGCCAAATCTTTATCACT     | GTGCCATCTTCTTTGTTCTGTTCTT     |
| <i>Gapdh</i>                         | AGGTCATCCCAGAGCTGAACG        | CACCCTGTTGCTGTAGCCGTAT        |
| <b>GAPDH</b>                         | <b>GGA</b> CTCATGACCACAGTCCA | <b>TC</b> AGCTCAGGGATGACCTTG  |
| <b>NLRP3</b>                         | <b>AAC</b> ATGCCCAAGGAGGAAGA | <b>GG</b> CTGTTACCAATCCATGA   |
| <b>IL1B</b>                          | <b>TG</b> CTCAAGTGTCTGAAGCAG | <b>TG</b> GTGGTCGGAGATTCGTAG  |
| <b>CASP1</b>                         | <b>GC</b> ACACGTCTTGCTCTCATT | <b>GC</b> CTCCAGCTCTGTAGTCAT  |
| <b>CCR5</b>                          | <b>CA</b> ATGTGAAGCAAATCGCAG | <b>GG</b> ATGAGGATGACCAGCATGT |

**Table S5. Antibodies used in flow cytometry**

| <b>Antibodies</b> | <b>Supplier</b> | <b>Cat. No.</b> | <b>Host</b> |
|-------------------|-----------------|-----------------|-------------|
| CD29-APC          | BD Biosciences  | 559883          | Mouse       |
| CD34-FITC         | BD Biosciences  | 555821          | Mouse       |
| CD44-APC          | BD Biosciences  | 559942          | Mouse       |
| CD45-FITC         | BD Biosciences  | 555482          | Mouse       |
| CD73-PE           | BD Biosciences  | 550257          | Mouse       |
| CD166-PE          | BD Biosciences  | 559263          | Mouse       |

**Table S6. Primary antibodies used in western blotting and immunostaining**

| <b>Primary antibodies</b> | <b>Supplier</b>          | <b>Cat. No.</b>    | <b>Host</b>   | <b>Dilution</b> |
|---------------------------|--------------------------|--------------------|---------------|-----------------|
| Nlrp3                     | Abcam                    | ab214185           | Rabbit        | 1:500           |
| Nlrp3                     | R&D system               | NBP2-12446         | Rabbit        | 1:500           |
| Il-1 $\beta$              | Abcam                    | ab234437           | Rabbit        | 1:500           |
| $\beta$ -Tubulin          | Abcam                    | ab179513           | Rabbit        | 1:1000          |
| Sox9                      | Millipore                | AB5535             | Rabbit        | 1:1000          |
| tdTomato                  | Sicgen                   | AB8181-200         | Goat          | 1:1000          |
| Calbindin                 | Swant                    | CB-38              | Rabbit        | 1:7000          |
| Chx10                     | Santa Cruz               | sc-21690           | Goat          | 1:1000          |
| Tfp2a                     | Abcam                    | ab108311           | Rabbit        | 1:1000          |
| RBPMS                     | Novus Biologicals        | NBP2-20112         | Rabbit        | 1:1000          |
| Cd45                      | ThermoFisher             | 14-0451-82         | Rat           | 1:1000          |
| Cd4-647                   | Biolegend                | 100533             | Rat           | 1:500           |
| I-A/I-E-488               | Biolegend                | 107615             | Rat           | 1:1000          |
| Cd8a                      | ThermoFisher             | 14-0081-82         | Rat           | 1:1000          |
| Cd13/32                   | Abcam                    | ab223200           | Rabbit        | 1:1000          |
| Foxp3                     | R&D system               | MAB8214-SP         | Rabbit        | 1:1000          |
| Cd45                      | R&D system               | AF114-SP           | Goat          | 1:1000          |
| Cd11b                     | Abcam                    | ab8878             | Rat           | 1:1000          |
| Iba1                      | Abcam                    | ab178847           | Rabbit        | 1:1000          |
| Glutamine Synthetase      | Millipore                | MAB302             | Mouse         | 1:1000          |
| Tbx21                     | Novus Biologicals        | NBP1-43299         | Mouse         | 1:1000          |
| Cd206                     | R&D System               | AF2535             | Goat          | 1:1000          |
| Rhodopsin                 | Millipore                | MAB5356            | Mouse         | 1:1000          |
| Arrestin                  | Millipore                | AB15282            | Rabbit        | 1:2000          |
| <b>F4/80</b>              | <b>Novus Biologicals</b> | <b>NB600-404SS</b> | <b>Rat</b>    | <b>1:400</b>    |
| <b>Ccl5</b>               | <b>Abcam</b>             | <b>ab9679</b>      | <b>Rabbit</b> | <b>1:500</b>    |
| <b>Tbx21-488</b>          | <b>Biolegend</b>         | <b>644830</b>      | <b>Mouse</b>  | <b>1:500</b>    |

**Table S7. Secondary antibodies used in Western blotting and immunostaining**

| <b>Secondary antibodies</b>      | <b>Supplier</b>           | <b>Cat. No.</b> | <b>Dilution</b> |
|----------------------------------|---------------------------|-----------------|-----------------|
| Goat anti-Rabbit HRP-linked IgG  | Abcam                     | ab6721          | 1:5000          |
| Horse anti-Mouse HRP-linked IgG  | Cell Signaling Technology | 7076            | 1:2000          |
| Donkey anti-Mouse Alexa 594 IgG  | Invitrogen                | A-21203         | 1:1000          |
| Donkey anti-Mouse Alexa 488 IgG  | Invitrogen                | A-21202         | 1:1000          |
| Donkey anti-Goat Alexa 594 IgG   | Invitrogen                | A-11058         | 1:1000          |
| Donkey anti-Rabbit Alexa 594 IgG | Invitrogen                | A-21207         | 1:1000          |
| Donkey anti-Rabbit Alexa 488 IgG | Invitrogen                | A-21206         | 1:1000          |
| Donkey anti-Rat Alexa 647 IgG    | Invitrogen                | A-78947         | 1:1000          |
| Donkey anti-Rat Alexa 594 IgG    | Invitrogen                | A-21209         | 1:1000          |
| Donkey anti-Rat Alexa 488 IgG    | Invitrogen                | A-21208         | 1:1000          |