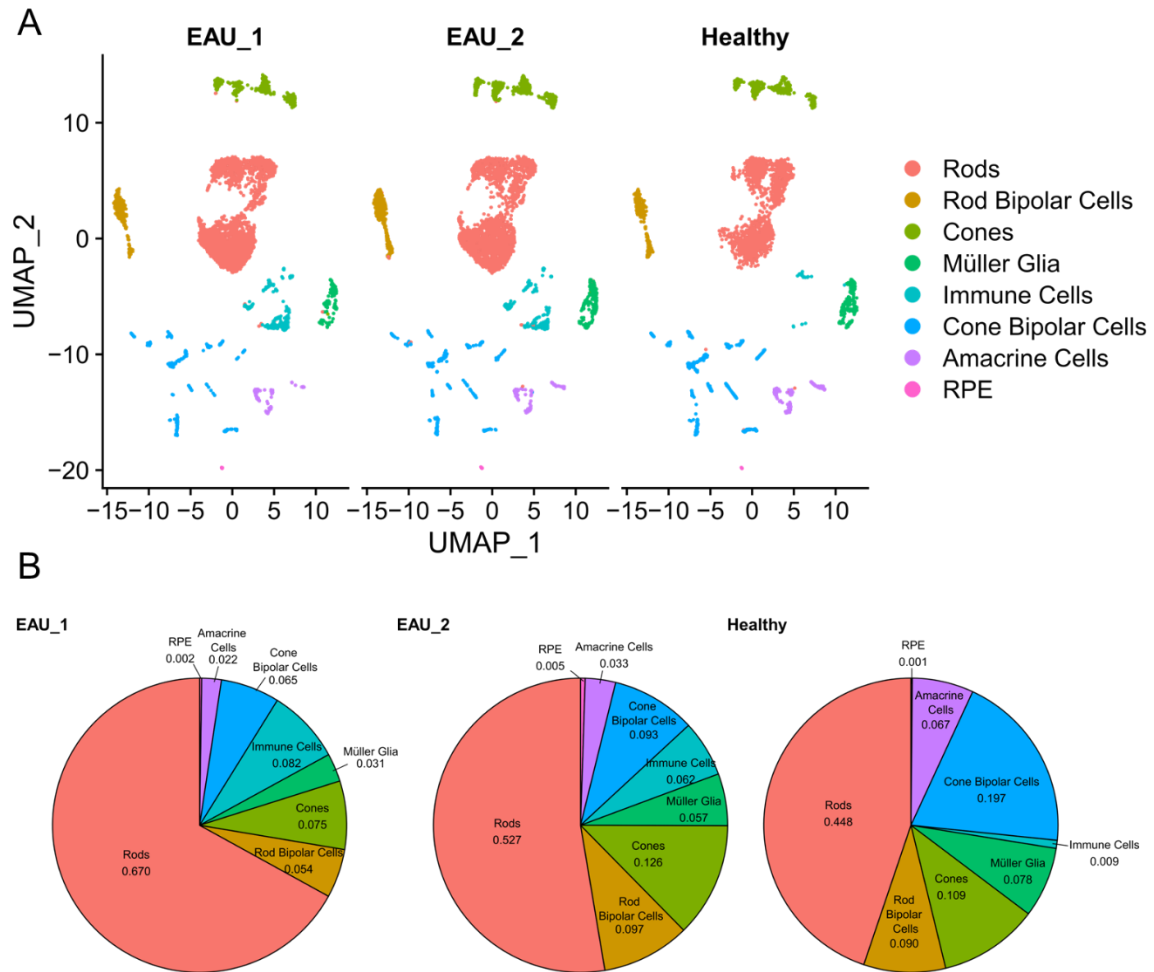


## **Supplementary Information**

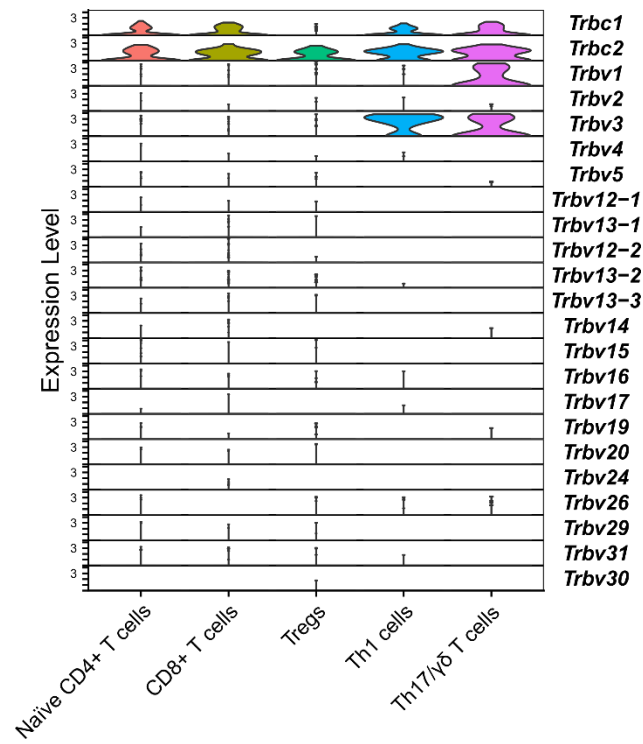
Single-cell transcriptomic analysis of retinal immune regulation and blood-retinal barrier function during experimental autoimmune uveitis

**Joel Quinn<sup>1</sup>, Ahmed Salman<sup>1</sup>, Christopher Paluch<sup>2,3,4</sup>, Matthew Jackson-Wood<sup>2</sup>, Michelle E McClements<sup>1</sup>, Jian Luo<sup>5</sup>, Simon J Davis<sup>3,4</sup>, Richard J Cornall<sup>6,7</sup>, Robert E MacLaren<sup>1,8</sup>, Calliope A Dendrou<sup>9,10</sup>, Kanmin Xue<sup>1,8,\*</sup>**

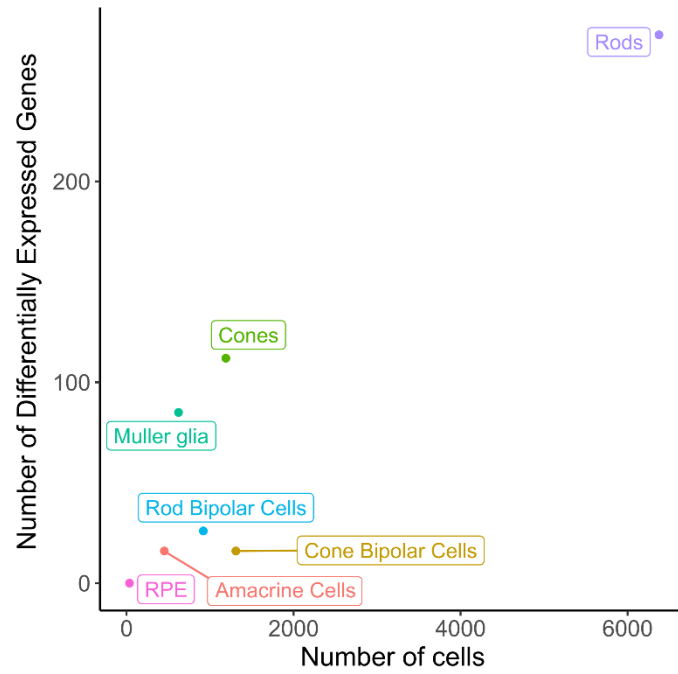
Kanmin Xue  
Email: [enquiries@eye.ox.ac.uk](mailto:enquiries@eye.ox.ac.uk)



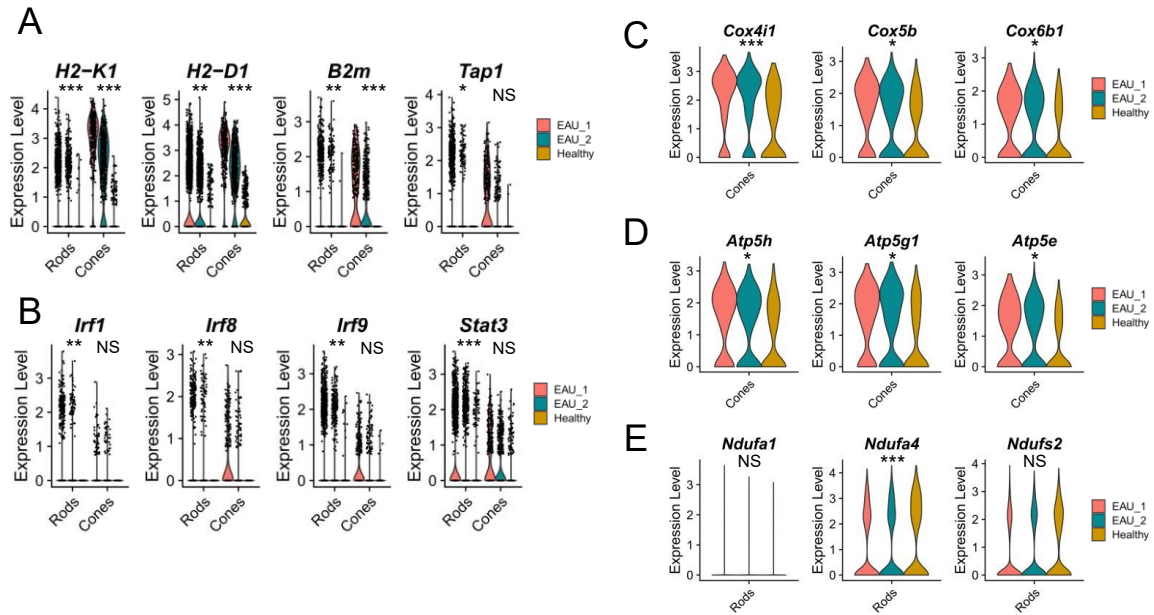
**Fig. S1. Distribution of cell types by sample. (A)** UMAPs split by sample showing presence of each annotated cell type in each sample. EAU samples contributed greatest to the immune cell cluster, as expected. **(B)** Proportions of each cell type by sample.



**Fig. S2. TCR-β gene expression among T cell subsets.** *Trbv3* was found to be a marker gene for the Th1 cell subset, and was also expressed by Th17/γδ T cells.



**Fig. S3. Relationship between number of differentially expressed genes vs number of cells in cluster.**



**Fig. S4. Leading edge genes for significantly enriched gene sets by GSEA.** Genes contributing to enrichment of inflammatory gene sets included (A) MHC-I antigen presentation pathway genes and (B) interferon regulatory factors. Enrichment of the Oxidative Phosphorylation gene set in cone photoreceptors was primarily due to upregulation of (C) cytochrome c oxidase genes and (D) ATP synthase genes during EAU. (E) Downregulation of Mitochondrial Complex I genes were the primary contributors to negative enrichment of the Oxidative Phosphorylation gene set in rod photoreceptors. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , DESeq2 Wald test.

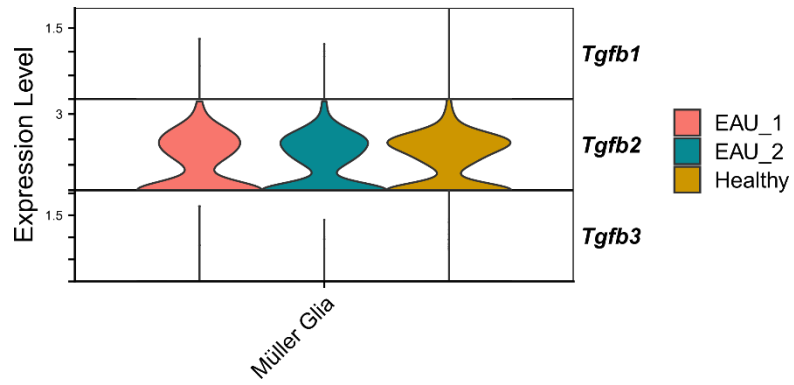


Fig. S5. TGF- $\beta$  isoform expression by Müller glia for each sample.

**Table S1. Summary of cell numbers for single-cell RNAseq by sample.**

| <b>Sample ID</b> | <b>Cell number</b> |
|------------------|--------------------|
| EAU_1            | 4029               |
| EAU_2            | 4108               |
| Healthy          | 3379               |
| <b>Total</b>     | <b>11516</b>       |

**Table S2. Summary of cell numbers by annotated cell type per sample.**

| <b>Cell type</b>   | <b>EAU_1</b> | <b>EAU_2</b> | <b>Healthy</b> | <b>Total</b> |
|--------------------|--------------|--------------|----------------|--------------|
| Rods               | 2699         | 2163         | 1514           | <b>6376</b>  |
| Rod Bipolar Cells  | 216          | 399          | 304            | <b>919</b>   |
| Cones              | 303          | 517          | 369            | <b>1189</b>  |
| Muller Glia        | 125          | 235          | 262            | <b>622</b>   |
| Immune Cells       | 329          | 255          | 30             | <b>614</b>   |
| Cone Bipolar Cells | 261          | 381          | 667            | <b>1309</b>  |
| Amacrine Cells     | 87           | 137          | 228            | <b>452</b>   |
| RPE                | 9            | 21           | 5              | <b>35</b>    |

**Table S3. Summary of immune cell numbers by annotated cell type per sample.**

| <b>Cell type</b>   | <b>EAU_1</b> | <b>EAU_2</b> | <b>Healthy</b> | <b>Total</b> |
|--------------------|--------------|--------------|----------------|--------------|
| CD8+ T cells       | 46           | 24           | 0              | <b>70</b>    |
| Microglia          | 46           | 47           | 22             | <b>115</b>   |
| Monocytes          | 44           | 20           | 1              | <b>65</b>    |
| Naive CD4+ T cells | 36           | 35           | 2              | <b>73</b>    |
| Neutrophils        | 27           | 6            | 1              | <b>34</b>    |
| NK cells           | 30           | 11           | 0              | <b>41</b>    |
| pDCs               | 12           | 16           | 0              | <b>28</b>    |
| Th1 Cells          | 22           | 20           | 0              | <b>42</b>    |
| Th17/gd T cells    | 9            | 20           | 0              | <b>29</b>    |
| Tregs              | 26           | 20           | 0              | <b>46</b>    |

**Table S4. Antibodies used in this study.**

| <b>Antibody</b>                           | <b>Clone</b> | <b>Supplier Product</b> | <b>Dilution</b> |
|-------------------------------------------|--------------|-------------------------|-----------------|
| Rat anti-mouse CD45                       | S18009D      | BioLegend #160302       | 1:200           |
| Rat anti-mouse I-A/I-E                    | M5/114.15.2  | BioLegend #107601       | 1:100           |
| Rabbit anti-mouse RPE65                   | EPR22579-44  | abcam #ab231782         | 1:200           |
| Rabbit anti-mouse glutamine synthetase    | Polyclonal   | abcam #ab228590         | 1:200           |
| Rat anti-mouse CD45 AlexaFluor 594        | S18009D      | BioLegend #160307       | 1:100           |
| Rat anti-mouse I-A/I-E AlexaFluor 647     | M5/114.15.2  | BioLegend #107617       | 1:100           |
| Rat anti-mouse CD4 AlexaFluor 488         | GK1.5        | BioLegend #100425       | 1:100           |
| Armenian hamster anti-mouse TCR g/d       | GL3          | BioLegend #118101       | 1:100           |
| Rat anti-mouse CD3 AlexaFluor 488         | 17A2         | BioLegend #100212       | 1:100           |
| Goat anti-rat IgG AlexaFluor 647          | Polyclonal   | Invitrogen #A21247      | 1:400           |
| Goat anti-rabbit AlexaFluor 568           | Polyclonal   | Invitrogen #A11036      | 1:400           |
| Goat anti-armenian hamster AlexaFluor 568 | Polyclonal   | Invitrogen #A78965      | 1:400           |
| Goat anti-rabbit IgG AlexaFluor 405       | Polyclonal   | Abcam #ab175652         | 1:400           |