

**Supplementary Figure 1. Pathologic changes after corneal alkali injury (CAI).**

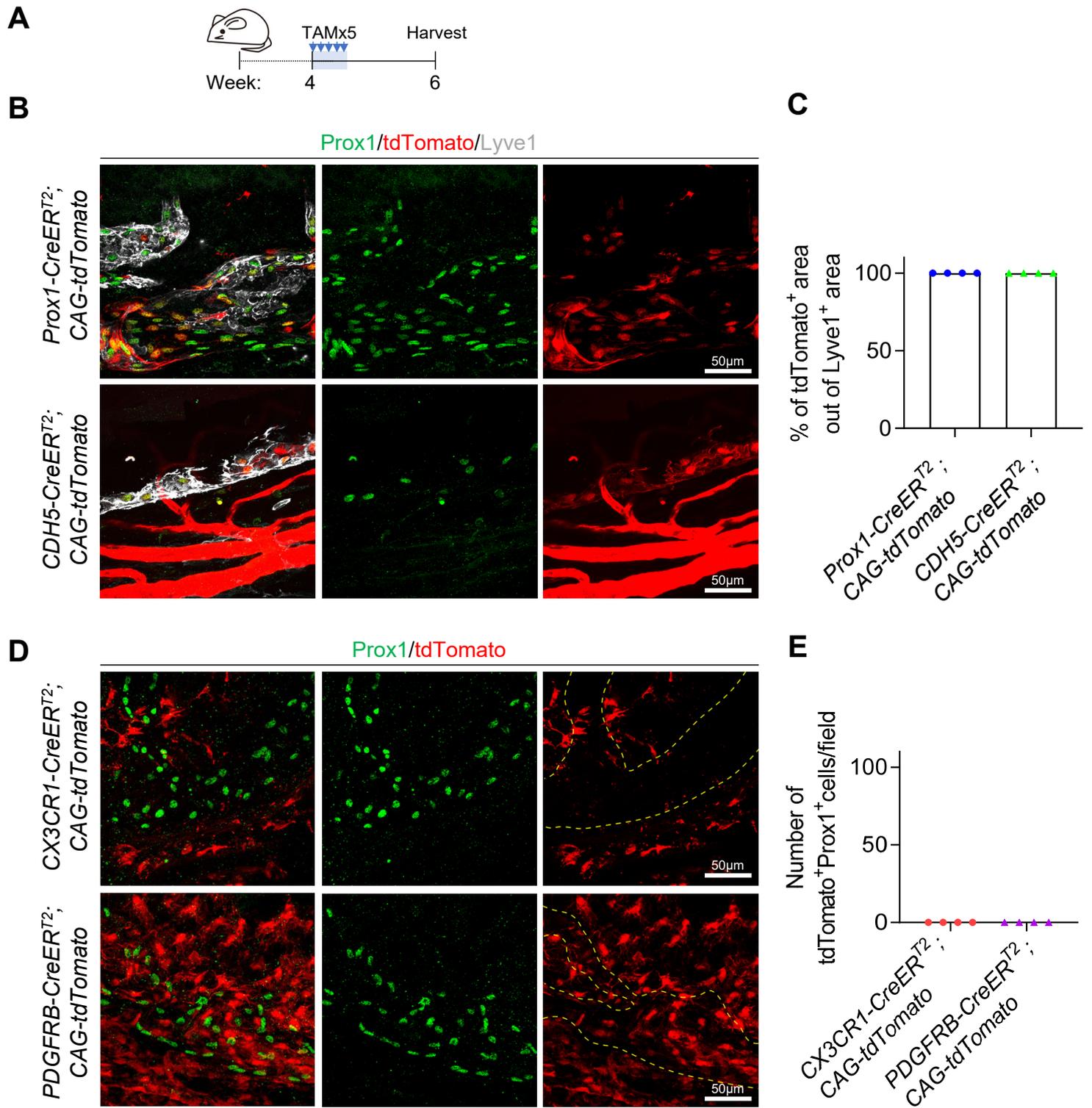
**A.** Slit lamp images and histological analyses of wildtype mouse eyes with CAI or sham treatment. The CAI or sham treatment was done by placing a 2-mm round filter paper infiltrated with 0.5  $\mu\text{l}$  of 1 M sodium hydroxide solution (CAI) or PBS (sham) on the cornea for 60 s. Note evident dilation and collagen deposition of the cornea and iris tissues in CAI-treated mouse group, as revealed by H&E and Masson staining of the cross-sectioned anterior segment. Scale bars: 1 mm and 100  $\mu\text{m}$ .

**B.** Immunostaining for Lyve1 and CD31 of CAI-treated wildtype mouse iris tissue whole-mounts. Note prominent neovascularization in the injured iridial tissue regions, regardless of the presence or absence of lymphatic vessels (LVs). Scale bar: 100  $\mu$ m.

**C.** Representative images for Lyve1 staining of cornea and iris tissue whole-mounts from wildtype mice that received CAI treatment at week 4, followed by analysis at week 10. Scale bars: 1 mm and 100  $\mu$ m.

**D.** Quantifications of the diameter, branches and terminal endpoints of corneal/limbal and iridial lymphatics in **C**. Data are mean $\pm$ SEM. Each dot represents one mouse. n=10 mice per group. \*p<0.05, \*\*\*p<0.001. Mann-Whitney *U* test.

**E.** Sexual ratio of mice with iridial lymphangiogenesis at day 28 after CAI. n=10 mice (5 male and 5 female) per group.



**Supplementary Figure 2. Verification of lymphatic lineage tracing mice.**

**A.** Experimental timeline of tamoxifen (TAM) administration for lineage tracing in the indicated tdTomato-reporter mouse lines (see **Figure 2**) in **B-E**. Arrows indicate 5XTAM (80 mg/kg) intraperitoneal (ip) injections in week 4, followed by analysis at week 6.

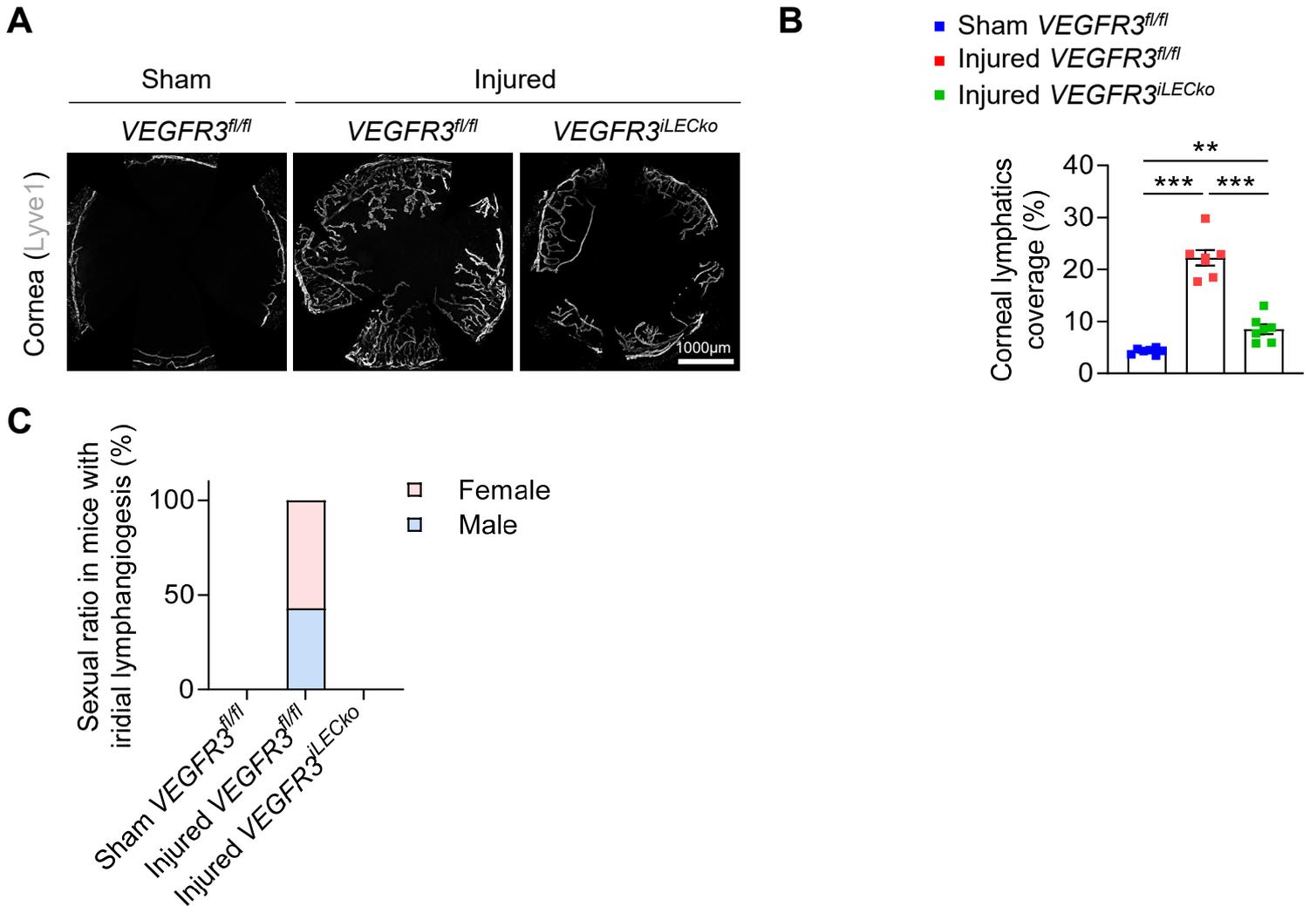
**B.** Representative tdTomato, Prox1 and Lyve1 immunostaining images of the cornea tissue whole-mounts from *Prox1-CreER<sup>T2</sup>;**CAG-tdTomato* and *CDH5-CreER<sup>T2</sup>;**CAG-tdTomato*. All mice received 5XTAM ip injections in week 4 and were analyzed at week 6. Note overlapping of tdTomato-labeled area

with Prox1/Lyve1-labeled lymphatic areas in *Prox1-CreER<sup>T2</sup>;CAG-tdTomato*, *CDH5-CreER<sup>T2</sup>;CAG-tdTomato* mouse lines, confirming faithful labeling of LECs via *Prox1-CreER<sup>T2</sup>* and *CDH5-CreER<sup>T2</sup>*-driven recombination expression of tdTomato in existing lymphatics. Scale bar: 50  $\mu$ m.

**C.** Quantification of the ratio of tdTomato-labeled area out of Prox1/Lyve1-labeled area in **B**. Data are mean $\pm$ SEM. n=4 mice per group. Each dot represents one mouse.

**D.** Representative tdTomato and Prox1 immunostaining images of the cornea tissue whole-mounts from *PDGFRB-CreER<sup>T2</sup>;CAG-tdTomato* and *CX3CR1-CreER<sup>T2</sup>;CAG-tdTomato* mice. All mice received 5XTAM ip injections in week 4 and were analyzed at week 6. Note no double labeling tdTomato and Prox1 in *PDGFRB-CreER<sup>T2</sup>;CAG-tdTomato* and *CX3CR1-CreER<sup>T2</sup>;CAG-tdTomato* mouse lines. Scale bar: 50  $\mu$ m.

**E.** Quantification of number of tdTomato and Prox1 double positive cells in **D**. Data are mean $\pm$ SEM. n=4 mice per group. Each dot represents one mouse.

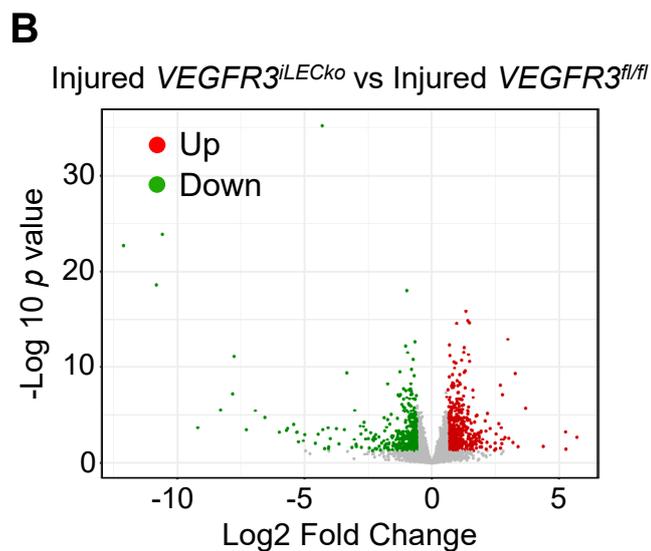
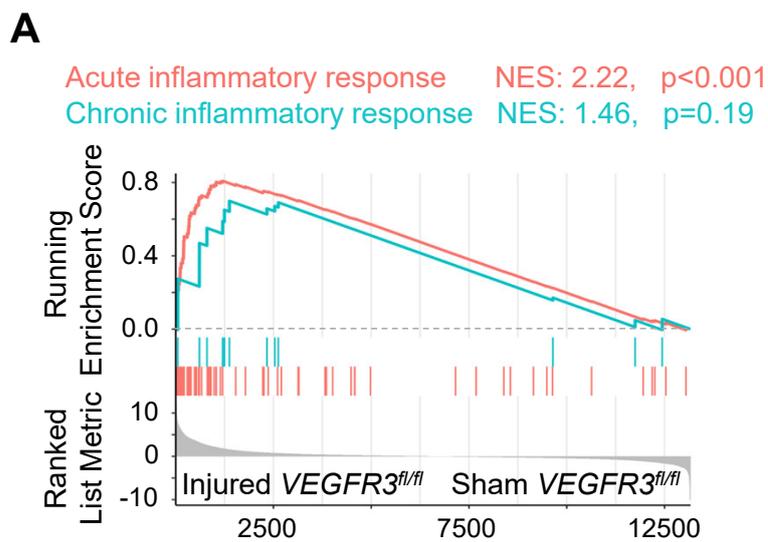


**Supplementary Figure 3. Inhibition of CAI-induced corneal lymphangiogenesis in *VEGFR3<sup>iLECKo</sup>* mice.**

**A.** Representative Lyve1 staining images of the cornea tissue whole-mounts from *VEGFR3<sup>fl/fl</sup>* and *VEGFR3<sup>iLECKo</sup>* mice with CAI or sham treatment. Note that CAI induces drastic lymphangiogenesis in *VEGFR3<sup>fl/fl</sup>* mice that is greatly inhibited in *VEGFR3<sup>iLECKo</sup>* mice. Scale bar: 1000 µm.

**B.** Quantification of corneal lymphatic coverage in mice in **A**. Data are mean±SEM. n=7 mice per group. Each dot represents one mouse. \*\* p<0.01. \*\*\* p<0.001. Welch's one-way ANOVA followed by the Dunnett T3 post hoc test.

**C.** Sexual ratio of mice shown in **Figure 3B-C**. n=7 mice (3 male+4 female) per group.



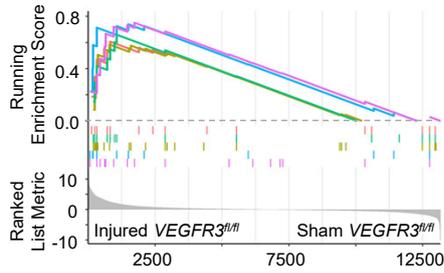
**Supplementary Figure 4. RNA-seq analysis of inflammatory pathways in the iris following CAI and *VEGFR3* depletion.**

**A.** GSEA showing expression pattern of acute and chronic inflammatory pathways following CAI. Adj.p value  $< 0.05$  (after Benjamini-Hochberg's multiple testing corrections) is considered statistically significant. NES: normalized enrichment score.

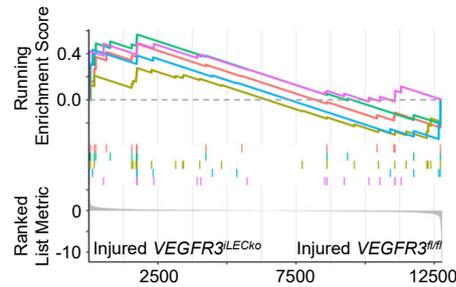
**B.** Volcano plot showing 259 upregulated differentially expressed genes (DEGs) and 222 downregulated DEGs in the Injured *VEGFR3<sup>iLECKo</sup>* vs. Injured *VEGFR3<sup>fl/fl</sup>* mouse iris tissues.

**A**

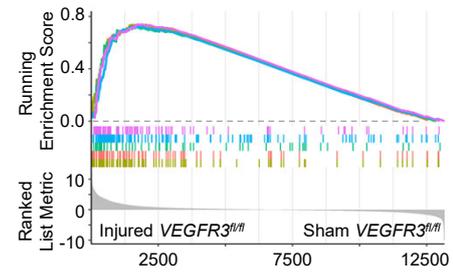
|                          |           |             |
|--------------------------|-----------|-------------|
| Th2 cell differentiation | NES: 1.64 | adj.p=0.065 |
| Th2 cytokine production  | NES: 1.51 | adj.p=0.23  |
| IL-4 production          | NES: 1.37 | adj.p=0.27  |
| IL-5 production          | NES: 1.40 | adj.p=0.26  |
| IL-13 production         | NES: 1.30 | adj.p=0.39  |



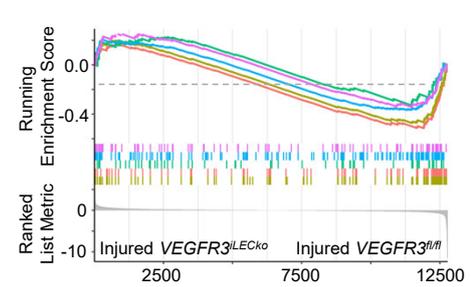
|                          |            |            |
|--------------------------|------------|------------|
| Th2 cell differentiation | NES: 1.29  | adj.p=0.45 |
| Th2 cytokine production  | NES: 1.04  | adj.p=0.65 |
| IL-4 production          | NES: -0.92 | adj.p=0.77 |
| IL-5 production          | NES: 1.44  | adj.p=0.33 |
| IL-13 production         | NES: 1.25  | adj.p=0.47 |

**B**

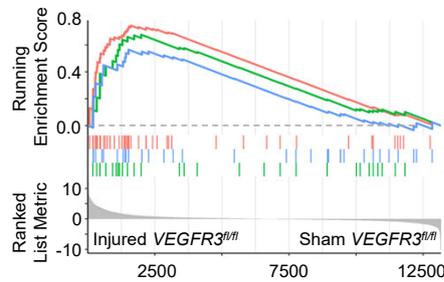
|                         |           |             |
|-------------------------|-----------|-------------|
| IL-1 production         | NES: 2.09 | adj.p<0.001 |
| IL-1 $\beta$ production | NES: 2.05 | adj.p<0.001 |
| IL-2 production         | NES: 1.90 | adj.p<0.001 |
| IL-6 production         | NES: 2.11 | adj.p<0.001 |
| IFN $\gamma$ production | NES: 2.07 | adj.p<0.001 |



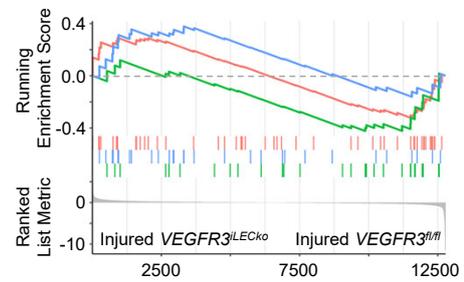
|                         |            |            |
|-------------------------|------------|------------|
| IL-1 production         | NES: -1.66 | adj.p=0.08 |
| IL-1 $\beta$ production | NES: -1.73 | adj.p=0.06 |
| IL-2 production         | NES: -1.02 | adj.p=0.69 |
| IL-6 production         | NES: -1.36 | adj.p=0.26 |
| IFN $\gamma$ production | NES: -1.17 | adj.p=0.52 |

**C**

|                                   |           |         |
|-----------------------------------|-----------|---------|
| Regulatory T cell differentiation | NES: 1.63 | p<0.05  |
| IL-10 production                  | NES: 1.94 | p<0.001 |
| TGF $\beta$ production            | NES: 1.38 | p=0.23  |



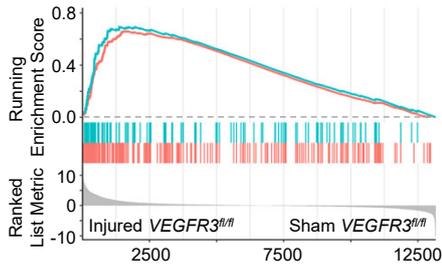
|                                   |            |        |
|-----------------------------------|------------|--------|
| Regulatory T cell differentiation | NES: -1.20 | p=0.49 |
| IL-10 production                  | NES: -0.98 | p=0.73 |
| TGF $\beta$ production            | NES: 1.12  | p=0.61 |



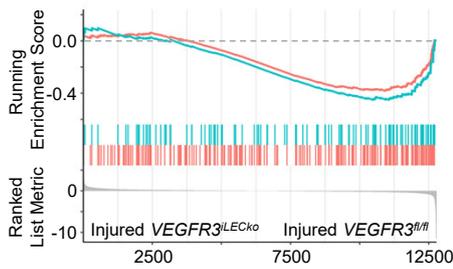
**Supplementary Figure 5.** GSEA showing expression profile of gene sets responsible for Th2-related pathway (**A**), proinflammatory cytokine production (**B**), regulatory T cell-related pathway (**C**) and in the indicated mouse iris tissues with or without CAI treatment. Adj.p value<0.05 (after Benjamini-Hochberg's multiple testing corrections) is considered statistically significant. NES: normalized enrichment score.

**A**

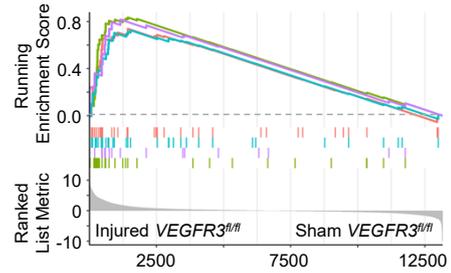
B cell activation NES: 1.96  $p < 0.001$   
 Immunoglobulin production NES: 1.99  $p < 0.001$



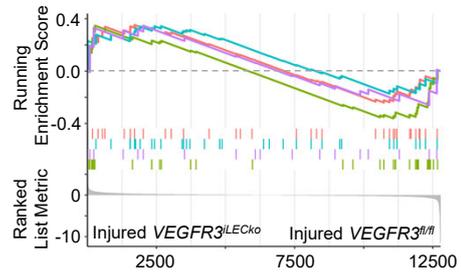
B cell activation NES: -1.48  $p = 0.13$   
 Immunoglobulin production NES: -1.61  $p = 0.13$

**B**

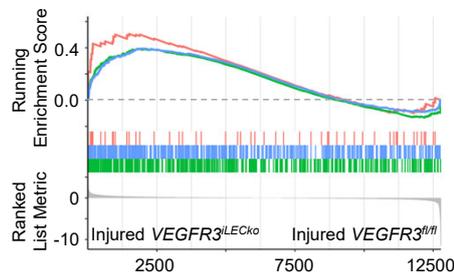
Neutrophil activation NES: 2.04  $p < 0.001$   
 TLR2 signaling pathway NES: 1.80  $p < 0.01$   
 Th17 immune response NES: 1.78  $p < 0.01$   
 IL-17 production NES: 1.83  $p < 0.001$



Neutrophil activation NES: -1.6  $p = 0.63$   
 TLR2 signaling pathway NES: 0.91  $p = 0.81$   
 Th17 immune response NES: 1.04  $p = 0.67$   
 IL-17 production NES: 1.08  $p = 0.63$

**C**

Collagen biosynthetic process NES: 1.70  $\text{adj.}p < 0.05$   
 Epithelium morphogenesis NES: 1.77  $\text{adj.}p < 0.001$   
 Epithelial cell differentiation NES: 1.76  $\text{adj.}p < 0.001$



**Supplementary Figure 6.** GSEA showing expression profile of gene sets responsible for B-cell and Neutrophil-Th17-related pathways (**A-B**) and collagen synthesis and epithelium morphogenesis/differentiation (**C**) in the indicated mouse iris tissues with or without CAI treatment. Adj.p value  $< 0.05$  (after Benjamini-Hochberg's multiple testing corrections) is considered statistically significant. NES: normalized enrichment score.