# Time- and stimulus-dependent characteristics of innate immune cells in organ cultured human corneal tissue

### Authors

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### Figure Legend

**Supplemental figure 1 - Bulk RNA-seq of human corneal tissue.** A Heatmap of the top 25 expressed protein-coding genes in three corneal specimens; genes are ordered according to mean expression in all three samples. TPM: transcripts per million (A). To verify if there was evidence of the presence of monocytes in the corneal samples, RNA-seq data were analyzed with respect to the expression of hundreds of macrophage and monocyte specific markers (from xCell main signature), respectively (B). A higher percentage of macrophage specific markers (55.3 %) was found to be expressed in corneal samples when compared to monocyte markers (36.3 %, p<0.001, chi-squared test), indicating a predominance of macrophages when compared to monocytes in corneal tissue. All marker genes with >10 TPM were defined as expressed.

**Supplemental Figure 2 -** **Examples of FMO control without CD45 staining (A) and after CD45 staining (B).** Two separate, fully stained control samples are shown in green and blue; the FMO controls are plotted in red. The dot plot on the lower rows in A and B are gated according to the gating strategy in figure 2. Without CD45 staining, the positive population can be distinguished from the negative population, but the gates are relatively inaccurate (A). By using CD45 as a marker for leukocytes, the positive population, with the exception of CD86, can be more clearly distinguished from the negative population.

**Supplemental Figure 3 - Macrophages in corneal tissue and culture medium (n=5 per group).** To allow cells to emigrate from the corneal tissue, the culture period of corneas in tissue medium was extended to a maximum of 45 days. Subsequently, the culture supernatants were analyzed by FC. The culture medium has far fewer viable cells than the actual corneal tissue (A). The macrophages present in the tissue culture medium showed expression of cell markers similar to macrophages in the cornea (B).

**Supplemental Figure 4 – Expression of M1 and M2 markers within CD45+ cells.** The percentage of CD11b+/CD14+/CD68+ cells as well as CD282+, CD284+, HLA DR+, CD163+, and CD206+ cells within CD45+ cells is visualized. In human corneal samples, up to two thirds of the CD45+ cells are also positive for CD11b/CD14/CD68. In unstimulated samples, there is approximately a 2:1 (M1 to M2) ratio among macrophages.