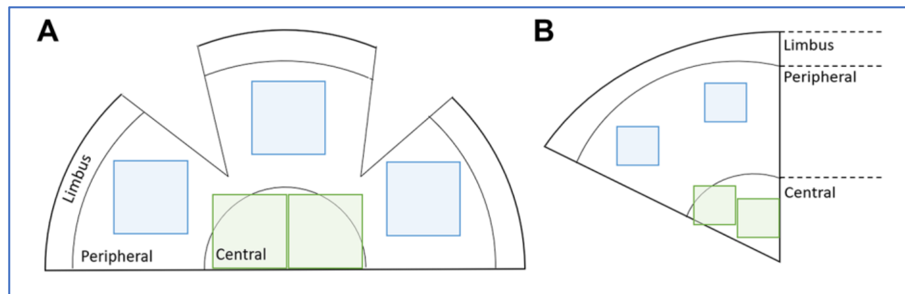
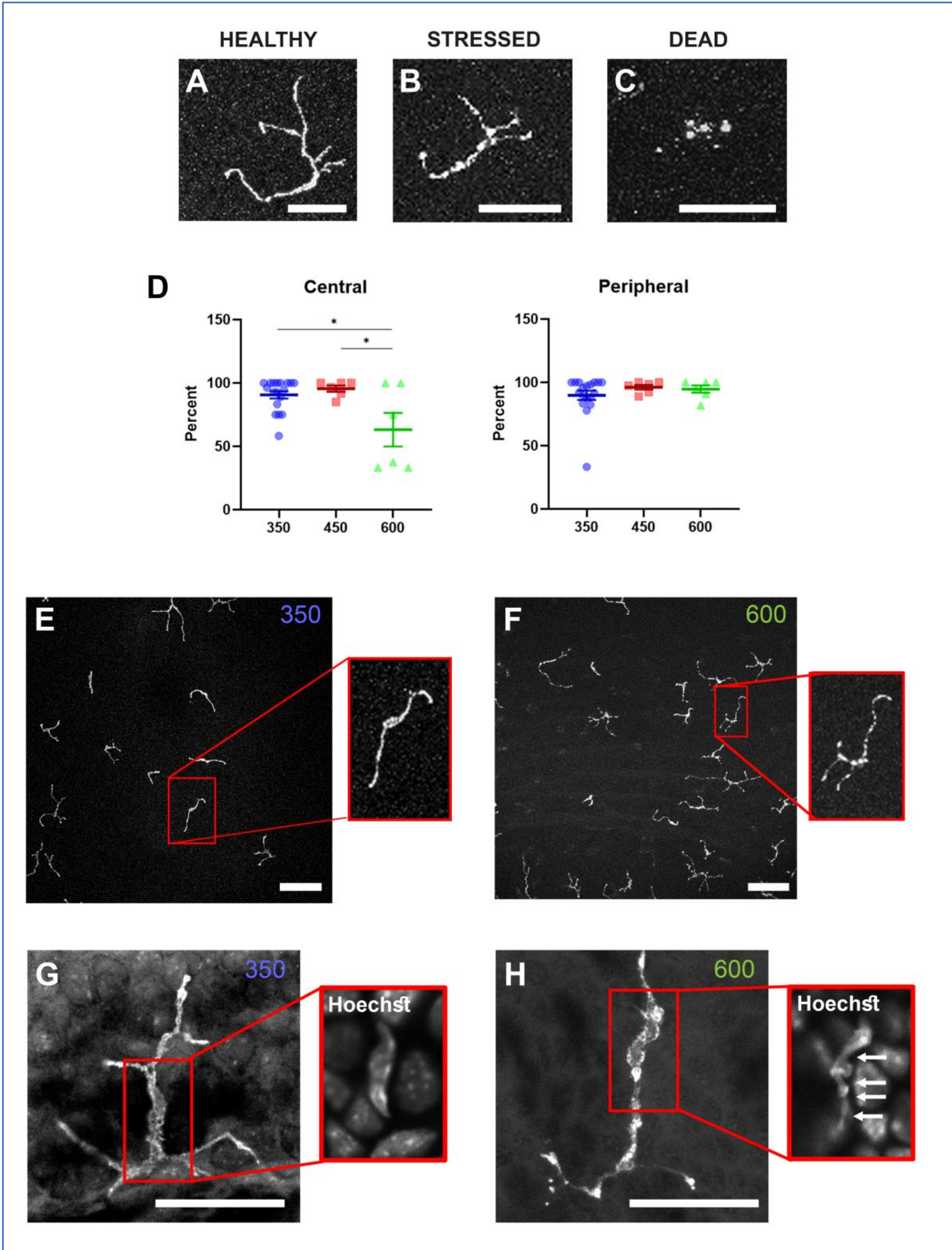
Supplementary material

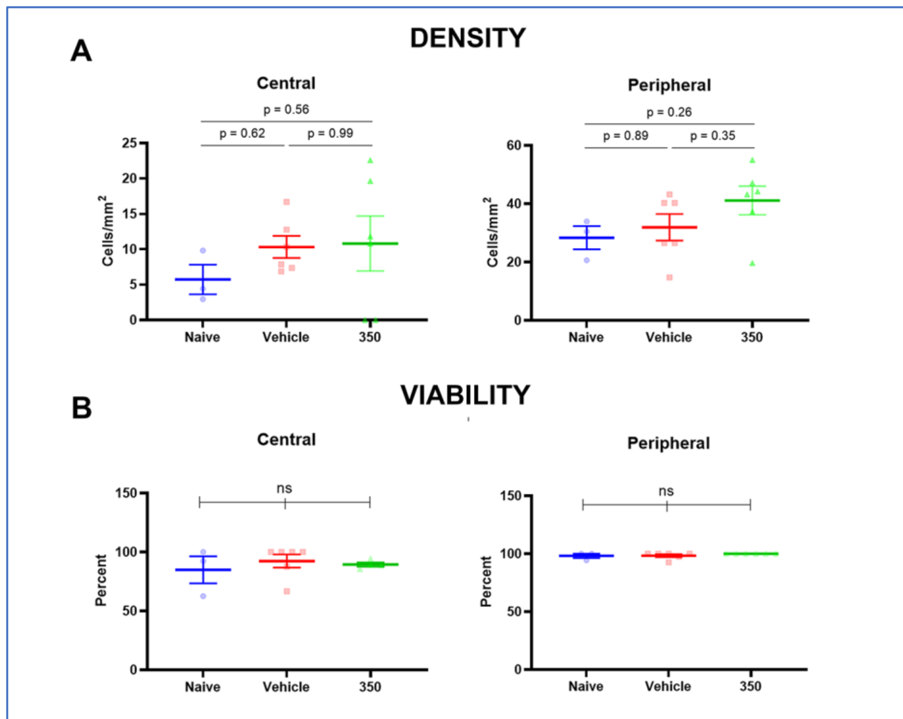


Supplementary Figure 1. Schematic of corneal image acquisition during immunofluorescence analyses. Morphological analyses were conducted in CD45-stained corneal halves (A) in which three 20x images were taken in the peripheral 2/3 of the cornea, excluding the limbus, and two 20x images were taken in the central 1/3 of the cornea. Immunophenotypic analysis was conducted in CD86- or CD68-stained corneal quadrants (B) in which two 40x images were taken from the peripheral 2/3 of the cornea (excluding the limbus) and two 40x images were taken from the central 1/3 of the cornea. Blue boxes represent images acquired from the "peripheral cornea" and green boxes represent images from the "central cornea".

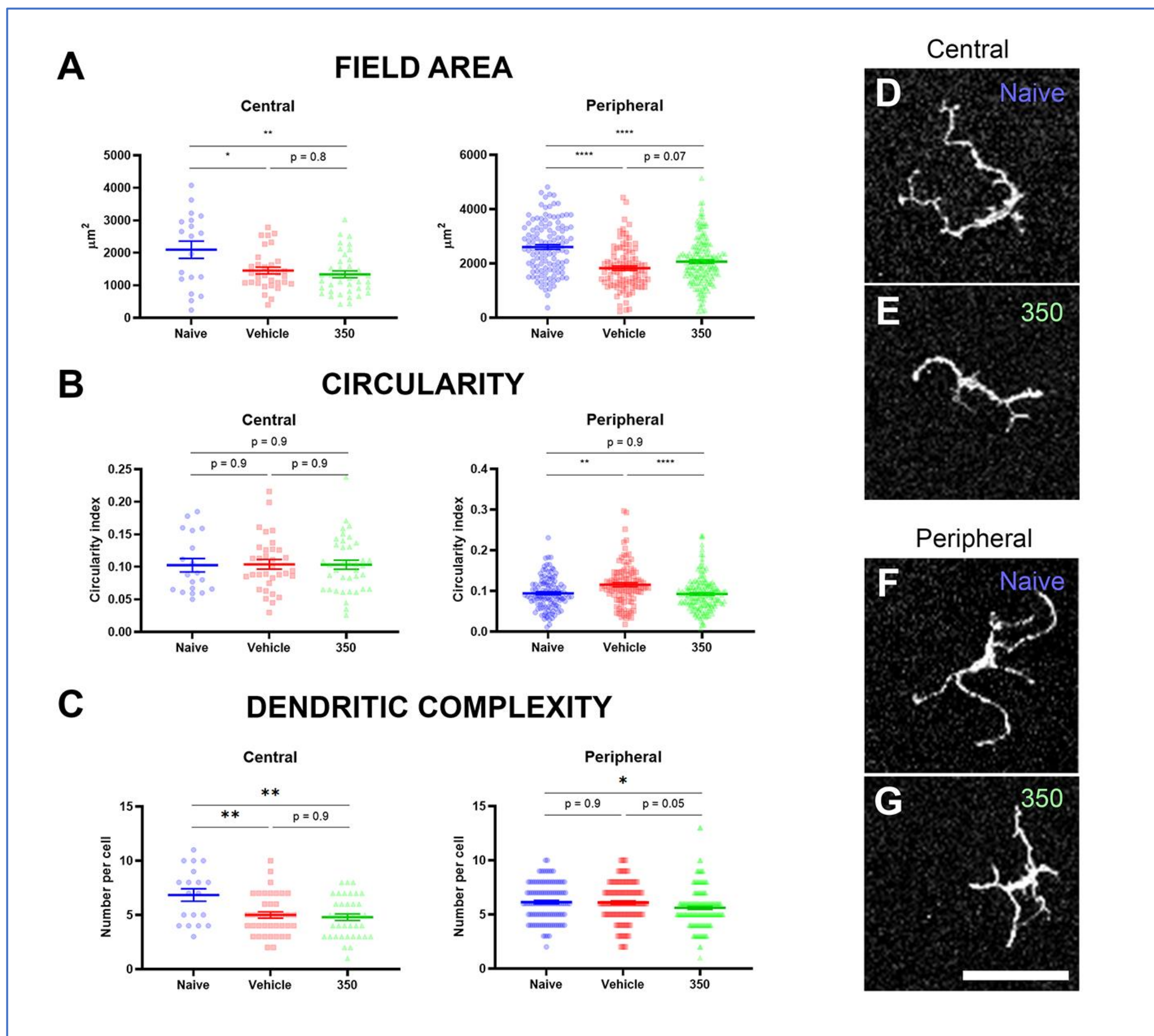


Supplementary Figure 2. Corneal dendritic cell (DC) viability. Membrane integrity was used as a marker for DC viability, as shown here in representative z-stack projections of murine CD45+ DCs following two hours of topical hyperosmolar stress (A-C). Smooth membranes are indicative of healthy DCs, while membrane blebbing (B) and membrane fragmentation (C) are indications of cellular stress and death. C57BL/6 mice that received topical application of 600 mOsm/L saline for two hours displayed a lower percentage of viable/non-fragmented DCs (D) in the central cornea and had membrane blebbing (F) that was not present in those that received 350 mOsm/L treatment (E). Nuclear morphology within

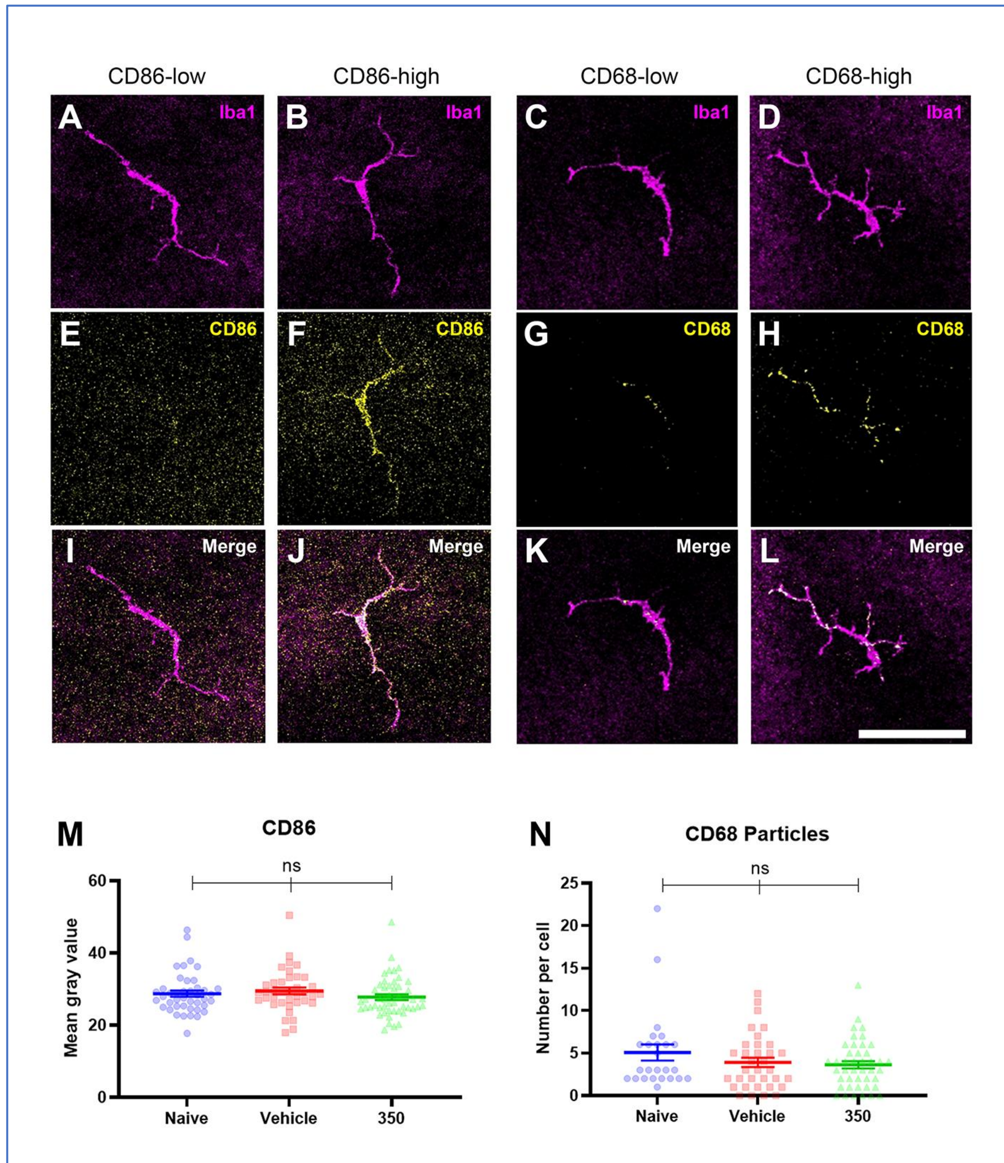
blebbed DCs, as visualised with Hoechst staining, also appeared fragmented at multiple points (white arrows) after 600 mOsm/L treatment (H) relative to nuclei from 350 mOsm/L-treated DCs (G). Each data point represents the average value taken from 3 images per cornea. N = 18, 6, 6 corneas in the 350, 450 and 600 mOsm/L groups, respectively. Data are presented as mean ± SEM. Asterisks denote statistical significance between groups (* $p < 0.05$). Scale bar is 50 µm for all images.



Supplementary Figure 3. CD45-labelled corneal dendritic cell density (A) and viability (B) are similar between naïve mice, and mice that received two-hour topical exposures of vehicle saline (290 mOsm/L) and 350 mOsm/L saline. Each data point represents the average value of 3 images per cornea. N = 3, 6, 6 corneas in the naïve, vehicle and 350 mOsm/L groups, respectively. Data are presented as mean ± SEM. ns denotes a comparison that was not statistically significant between groups ($p > 0.05$).



Supplementary Figure 4. Corneal dendritic cell morphology, including field area (A), circularity (B) and dendritic complexity (C), is different between naïve mice, and mice that received two-hour topical exposure to vehicle saline (280 mOsm/L) and 350 mOsm/L saline. In both the central and peripheral corneal epithelium, DCs treated with 350 mOsm/L saline had a lower field area and dendritic complexity compared to naïve mice, yet similar circularity. Representative confocal maximum z-stack projections (CD45-stained) display the visual appearance of these changes between the 350 mOsm/L (E, G) and naïve group (D, F) in the central (D-E) and peripheral cornea (F-G). Each data point represents a single DC. N (central) = 19, 33, 38 cells and N (peripheral) = 118, 102, 174 cells in the naive, vehicle and 350 mOsm/L groups, respectively. Data are presented as mean ± SEM. Asterisks denote statistical significance between groups (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). Scale bar for all images is 50 µm.



Supplementary Figure 5. Differing expression of CD86 and CD68 by Iba1+ epithelial DCs in naïve corneas. CD86-low (A,E, I), CD86-high (B, F, J), CD68-low (C, G, K) and CD68-high (D, H, L) DCs taken from the peripheral regions of naïve mice. Quantification of CD86 fluorescence intensity (M) and CD68 particles (N) in Iba1-labelled DCs is similar between naïve mice and mice that received topical exposure to vehicle saline (280 mOsm/L) or 350 mOsm/L saline for two hours. Data points represent individual DCs in both the central and peripheral cornea. N (CD86) = 42, 38, 49 cells and N (CD68) = 25, 34, 43 cells in the 350, 450 and 600 mOsm/L groups for CD86 and CD68 analysis, respectively. Data are presented as mean ± SEM; n.s denotes lack of a statistically significant difference between groups ($p > 0.05$). Scale bar for all images is 50 µm.