Supplemental Information

Drug uptake into corneal epithelial cells and tissue levels depend on among other drug efflux transporters. With particular relevance to dry-eye disease, cyclosporine is a potent inhibitor of P-glycoprotein 1 (Pg-P), which is highly expressed in HCE-T cells. In order to determine whether Xanthohumol exerts inhibitory effects on Pg-P, we performed an in vitro drug efflux transporter assay.

To this end, HCET-T cells seeded in 96-well plates were exposed to a dose-range of either Xanthohumol (10 nM – 10 µM) or to cyclosporine A (5 nM – 100 µM) for 30 min. Calcein-AM (2 µM) was added and fluorescence (excitation λ = 495 nm; emission λ = 525 nm) was measured in a Cytation 5 plate reader (BioTek Instruments, Inc.; Winooski, VT, USA) every 5 min for a 30 min period. The slope of the response was calculated and plotted over the drug concentration. Data were fitted using a Hill equation in Prism 9.0 (GraphPad, Inc., La Jolla, CA, USA).

Xanthohumol had no effect on P-gp drug transporter function. The slope did not change with an increasing concentration of Xanthohumol (Suppl. Fig. 1) suggesting the absence of an inhibitory effect on P-gp. In contrast, cyclosporine resulted in a dose-dependent increase in the slope that could be fitted with a Hill equation, confirming the inhibitory effect of cyclosporine on Pg-P (Suppl. Fig. 1).



**Supplemental Figure 1:** Xanthohumol had no effect on P-gp drug transporter function. Xanthohumol (filled circles) had no effect on the slope, while cyclosporine (open circles) showed a dose-dependent increase that was fitted using a Hill equation. Data were calculated from 8 technical replicates per concentration.

Given the potent effects of cyclosporine on Pg-P, care should be taken when co-administering ocular topical drugs together with cyclosporine, as this may results in increased concentrations in the corneal epithelial cells due to inihibition of Pg-P.