Supplementary information

Purinergic dysregulation causes hypertensive glaucoma-like optic neuropathy

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Supplementary Figures

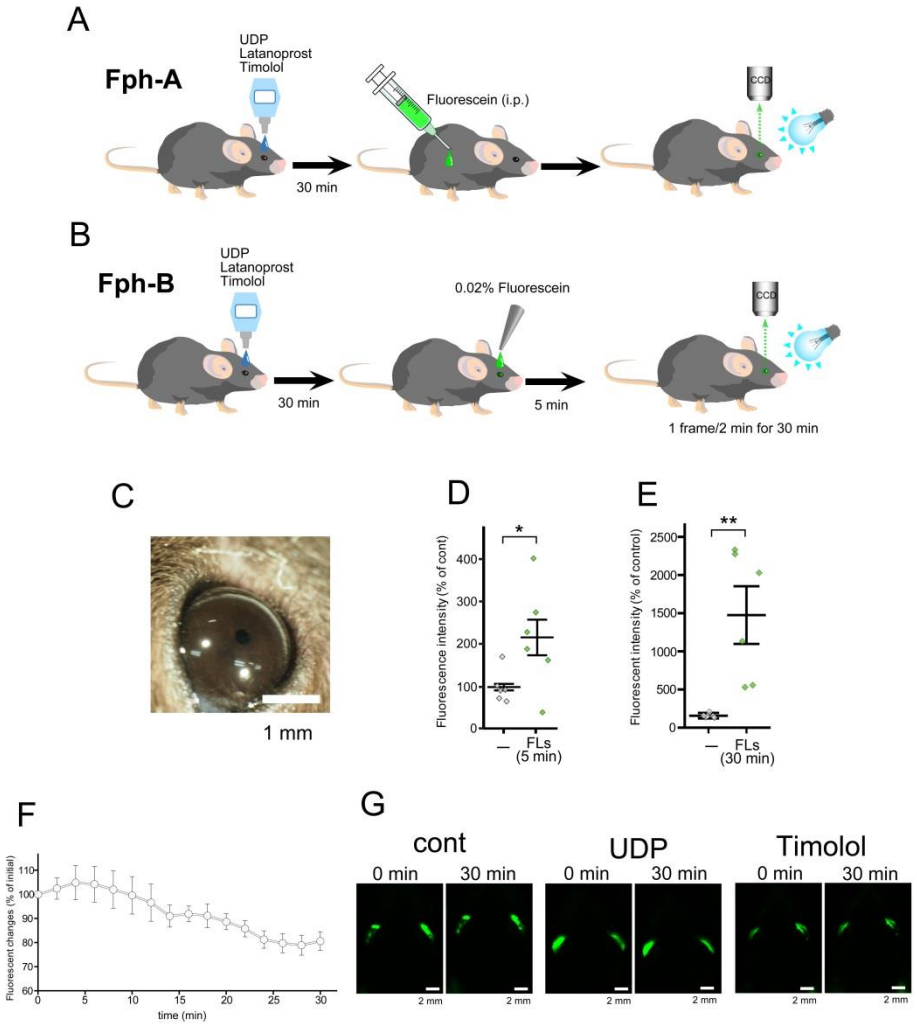

Figure S1. Aqueous humor dynamics assays.

(**A**) Fph-A procedures. Eye drops were administrated for 30 mins followed by fluorescein injection (i.p.). The fluorescence levels in the anterior chamber were monitored every 2 mins. (**B**) Fph-B procedures. Eye drops were administrated for 30 min followed by topical administration of fluorescein for 5 mins. After washing eyes with 500 μl of saline five times, fluorescence images of the anterior chamber were observed. (**C**) Pupil dilations were not induced for the assays to observe fluorescence limited to the anterior chamber. (**D, E**) Fluorescence levels of isolated aqueous humor at (**D**) 5 and (**E**) 30 mins after i.p. injection of fluorescein. Fluorescence levels in isolated aqueous humor showed a significant increase (n = 5, $*p < 0.05$ and $**p < 0.01$, unpaired *t*-test). (**F**) Fluorescence changes in control mice. During the first 4–6 minutes, the fluorescence level was slightly increased followed by a gradual reduction. (**G**) Representative fluorescence images at 0 and 30 mins. Data are expressed as the mean ± SEM.

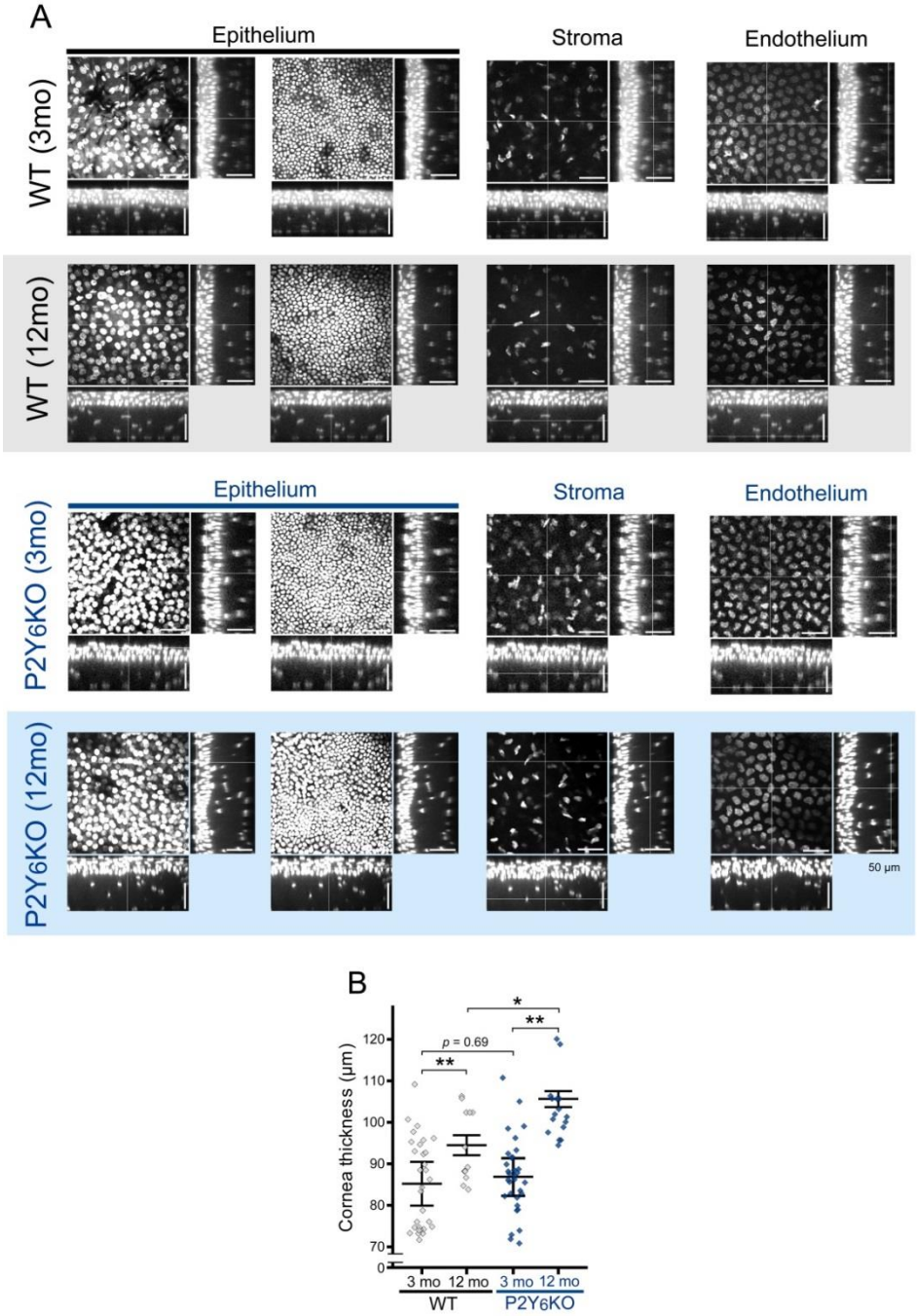

Figure S2. Comparison of central cornea thickness between WT and P2Y$_6$KO mice.

(**A**) Layer structure of the cornea. No significant structural differences were observed in the epithelial, stromal and endothelial cell layers between WT and P2Y$_6$KO mice. (**B**) Central cornea thickness (CCT). No significant differences in CCT at 3 months of age were observed. At 12 months, CCTs in both WT and P2Y$_6$KO mice were increased compared with 3-month-old groups, and P2Y$_6$KO had a thicker CCT than that of WT mice (n = 20, *$p < 0.05$, **$p < 0.01$, one-way ANOVA followed by Fisher's LSD test). Values are the mean ± SEM. Scale bars: 50 μm.

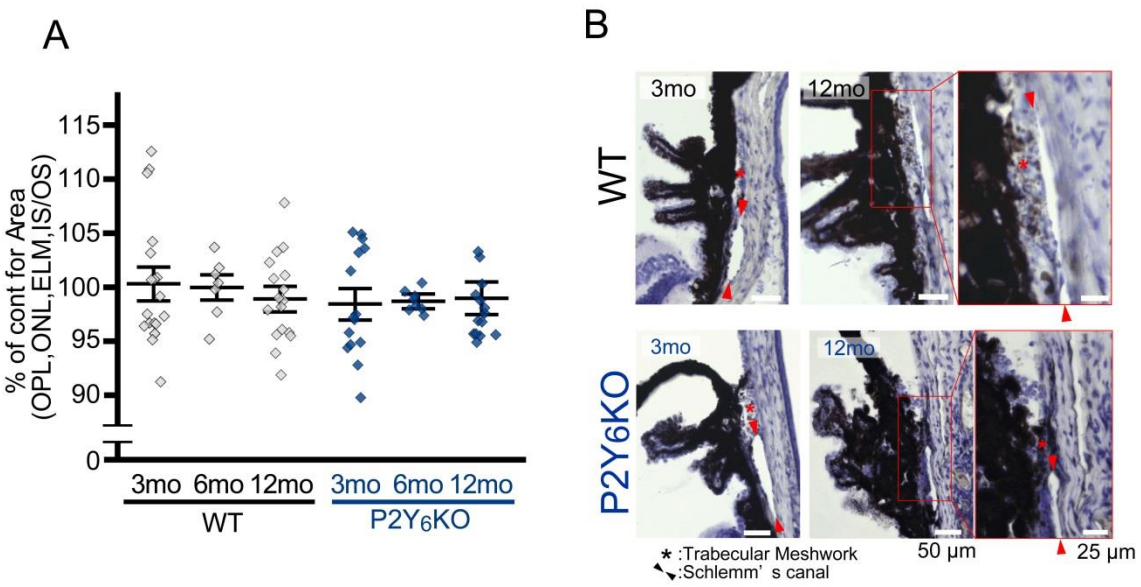

Figure S3. Histological differences in WT and P2Y$_6$KO mice.

(**A**) No changes in area size of the outer retina (OPL, ONL, ELM, IS/OS) were observed by OCT analysis (n = 10–20, one-way ANOVA followed by Fisher's LSD test). (**B**) No significant abnormalities were observed in anterior chamber structures in P2Y$_6$KO mice. Values are the mean ± SEM. Scale bars, 25 μm in (B, insert) and 50 μm in (B).

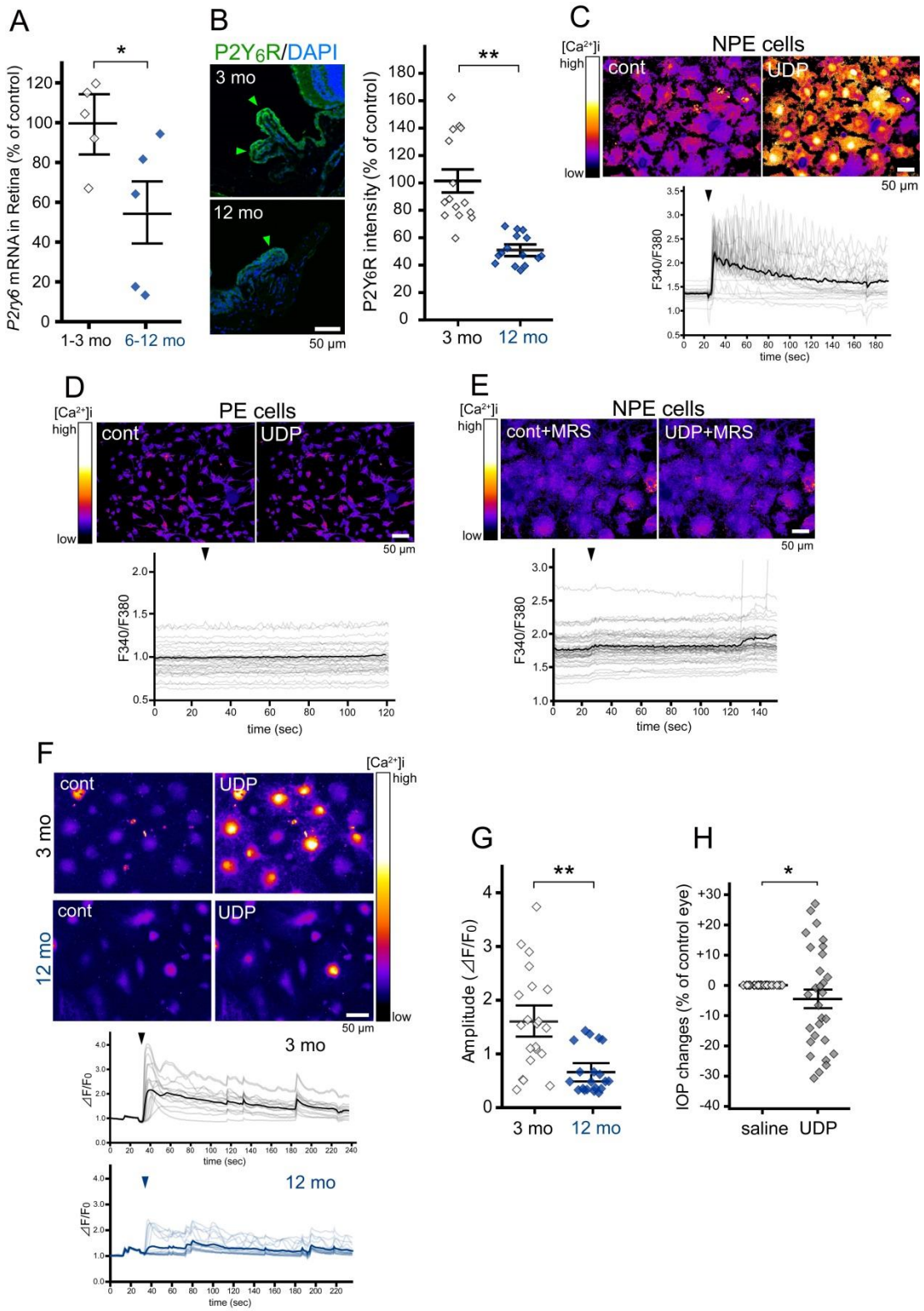

Figure S4. Expression level of $P2Y_6$ receptors in the eye changes with aging.

(A) The level of *P2ry6* mRNA in the retina was significantly reduced with age (n = 10, **p* = 0.05, unpaired *t*-test). Retinal *P2ry6* mRNA levels in WT mice at 6–12 months were

significantly lower compared with mice aged 1–3 months. **(B)** Immunoreactivity for $P2Y_6R$ in NPE cells of the ciliary body was decreased with age. Fluorescence intensities for $P2Y_6R$ signals in NPE cells of 3-month-old mice were significantly lower compared with those of 12-month-old mice (n = 10, $^{**}p < 0.01$, unpaired *t*-test). **(C)** Nonpigmented epithelial (NPE) cells showed clear Ca^{2+} responses evoked by UDP (100 μM, arrow). **(D)** Pigmented epithelial (PE) cells did not respond to UDP. **(E)** UDP-evoked responses in NPE cells were clearly blocked by MRS2578 (30 μM). Ca^{2+} imaging in (**B–D**) were performed in epithelial cells from 3-month-old WT mice. **(F** and **G)** Age-associated reduction in UDP-evoked Ca^{2+} responses in NPE cells. NPE cells from 12-month-old WT mice showed smaller amplitudes of Ca^{2+} transients evoked by UDP (100 μM, arrow) compared with NPE cells from 3-month-old mice (n = 20, $^{**}p < 0.01$, unpaired *t*-test). **(H)** IOP-lowering effect of UDP on WT mice aged 12 months. Instillation of UDP (500 μM, 5 μl/eye for 1.5 h) on 12-month old WT mice showed a 50% reduction in efficacy compared with 3-month-old WT mice (4.6±3.1 and 10.1±2.8% in 3- and 12-month-old WT mice, respectively). Scale bars, 50 μm in (B)-(F).