Supporting Information

Supplemental Material

Antibody information

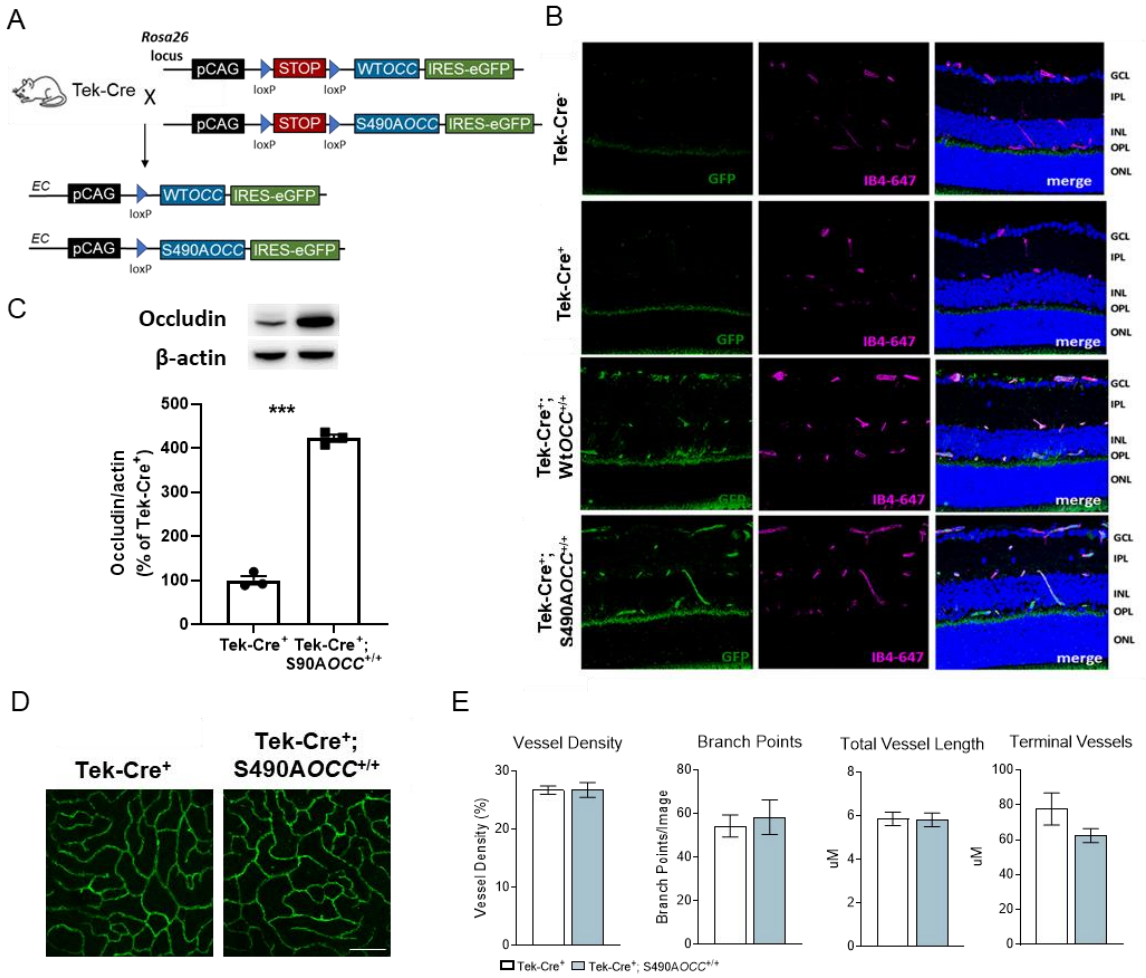
The following antibodies were used for western blotting: rabbit anti-occludin (1:1000, Cat. No. 406100, Thermo Fisher), rabbit anti-occludin pS490 (1:500, (1)), mouse anti p-44/42 MAPK (Erk1/2) (Thr202/Tyr204) (1:1000, Cat. No. 9106, Cell Signaling), rabbit anti-p44/42 MAPK (Erk1/2) (1:1000, Cat. No. 91022, Cell Signaling) and mouse anti-β-actin (1:10000, Cat. No. 3700, Cell Signaling)

The following antibodies were used for immunofluorescence: mouse anti-occludin-594 AlexaFluor conjugated (1:100, Cat. No. 331594, Thermo Fisher), rabbit anti-claudin-5 (1:100, Cat. No. 341600, Thermo Fisher), rat anti-ZO-1 (1:100, Cat. No. MABT11, Millipore), rat anti-CD45 (1:50, Cat. No. 550539, BD Pharmigen) and goat anti-Iba1 (1:100, Cat. No. NB100-1028, Novus Biologicals), anti-GFP-AF 488 (1:250, Cat. No. A21311, Thermo Fisher) and rabbit anti-RBPMS (1:100, Cat. No. 15187-1-AP, Proteintech)

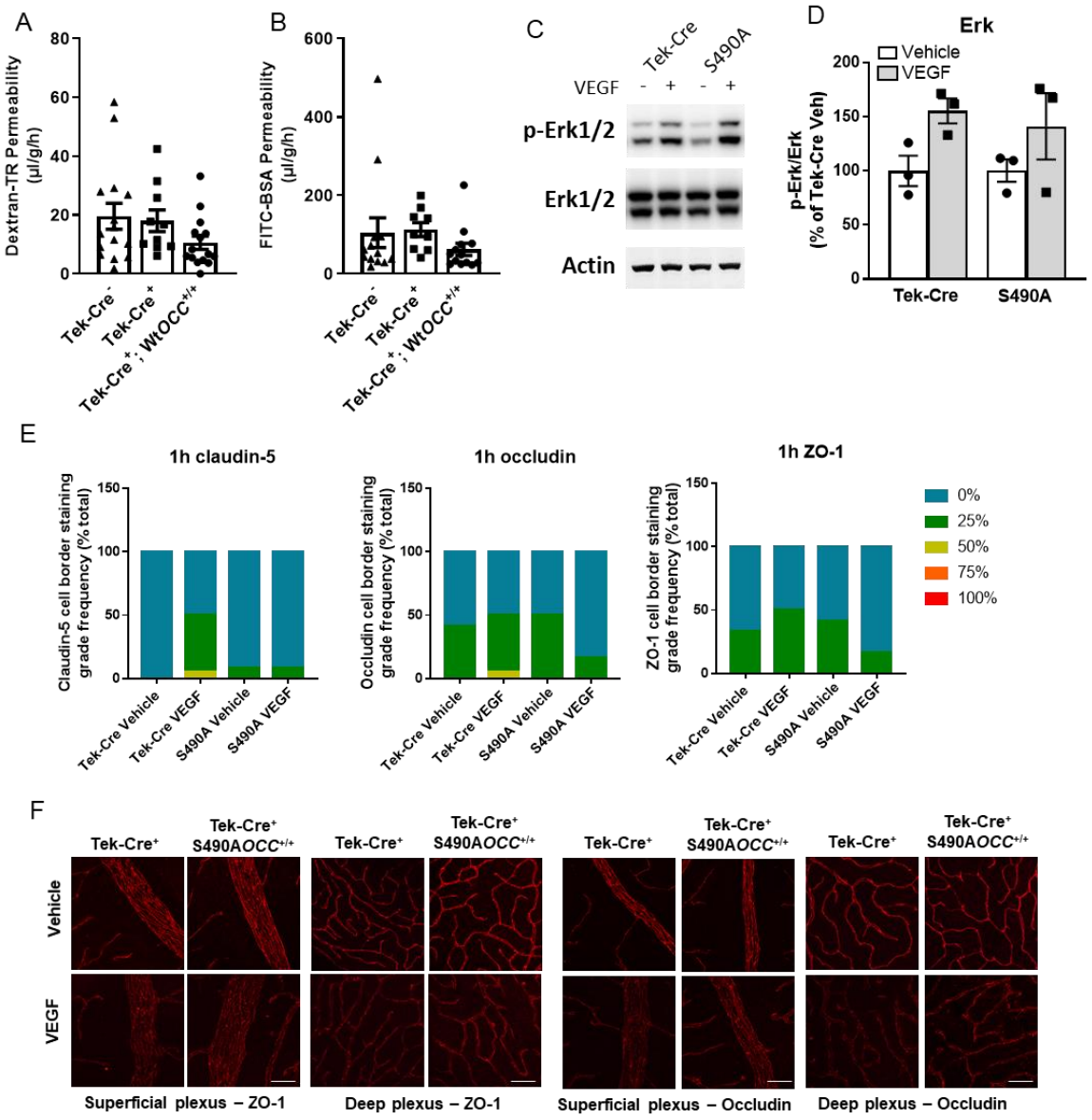
References

1. Sundstrom JM, Sundstrom CJ, Sundstrom SA, Fort PE, Rauscher RL, Gardner TW, Antonetti DA: Phosphorylation site mapping of endogenous proteins: a combined MS and bioinformatics approach. J Proteome Res 2009;8:798-807

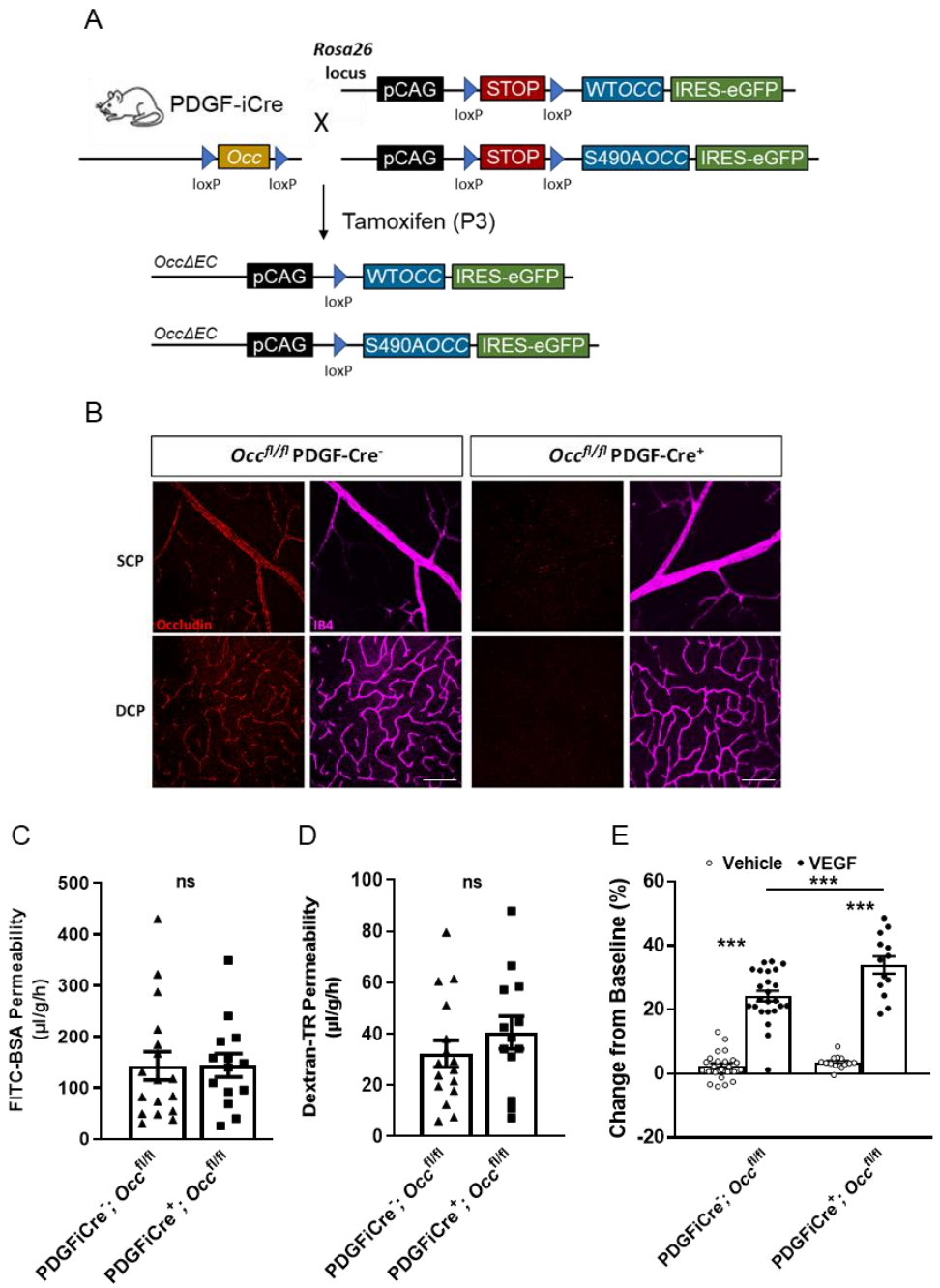
Supplemental Data



Supplementary figure 1: (A) Transgenic animals expressing either wild-type occludin (Wt*OCC*) or mutated Ser490 to Ala occludin (S490A*OCC*) followed by IRES-eGFP at the rosa26 site were crossed with Tek-Cre allowing for endothelial cell specific expression of Wt or S490A occludin. (B) Representative images of retinal cross-sections of Tek-Cre^{-}, Tek-Cre^{+}, Tek-Cre^{+}; Wt$OCC^{+/+}$ and Tek-Cre^{+}; S490A$OCC^{+/+}$ stained for GFP (green), IB4 (purple) and nuclei (blue). Legend: GCL — ganglion cell layer; IPL — inner plexiform layer; INL — inner nuclear layer; OPL — outer plexiform layer; ONL — outer nuclear layer. (C) Occludin protein levels from total retinal lysates from adult Tek-Cre^{+} and Tek-Cre^{+}; S490A$OCC^{+/+}$ mice. (D) Pictures from flat-mounted adult retinas from Tek-Cre^{+} and Tek-Cre^{+}; S490A$OCC^{+/+}$ were stained for the vessel marker IB4. Scale bar: 50µm. (E) Vessel architecture was quantified using an automated software and vessel density, branch points, total vessel length and terminal vessels were determined, n=4. Data are represented as mean ± SEM. ***P <0.001 Two-tailed student's t-test.

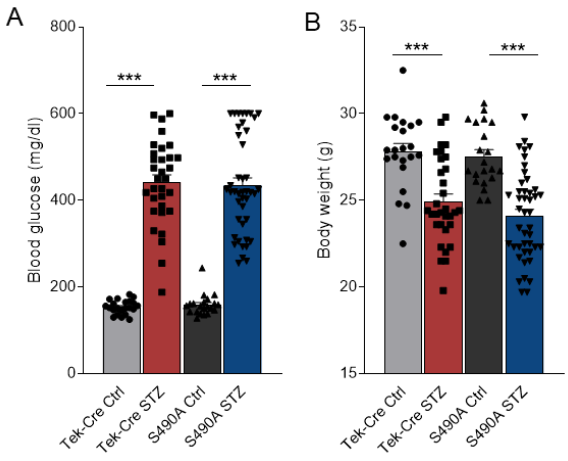


Supplementary figure 2: Tek-Cre^{-}, Tek-Cre^{+} and Tek-Cre^{+}; Wt*OCC*$^{+/+}$ mice were given an intravitreal injection of 200 ng VEGF in one eye and vehicle in the other and retinal vascular permeability to 70-kDa Dextran (A) and FITC-BSA (B) was determined after 36 h of VEGF intravitreal injection, by extracting the extravasated dye into the tissue. Data was normalized to the vehicle injected eye of each mouse. (C) Representative western blot for p-Erk1/2 and t-Erk1/2, 1h after VEGF injection (D) Quantification of the immunoreactive bands, normalized against actin protein levels. (E) Retinas were stained for tight junction protein and flat-mounted, 1h after VEGF injection, n=3. Masked scoring of claudin-5, occludin and ZO-1 border staining, ranking in 5 categories of loss, of at least 4 pictures per retina. (F) Representative images of occludin and ZO-1 staining in the superficial and deep capillary plexus in retina flat mounts, 36h after VEGF injection, n=3-4. Scale bar: 50µm.

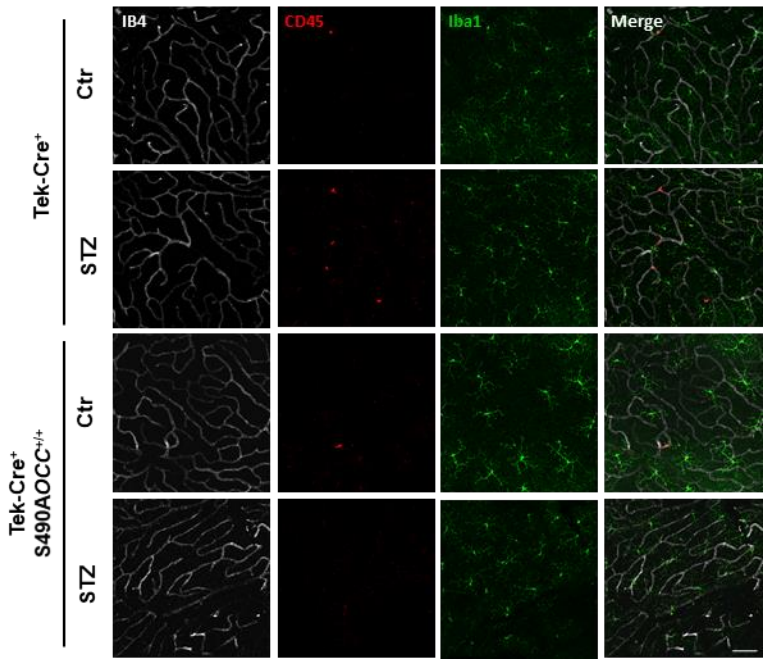


Supplementary figure 3: (A) Transgenic mice with floxed endogenous occludin and expressing either WT or S490A mutant occludin under the vascular-specific PDGFb promoter were generated. Cre was induced by tamoxifen injection at P3. (B) Whole-mount staining for occludin (red) and vessel marker IB4 (purple), n=2. Scale bar: 50µm. Legend: SCP – superficial capillary plexus; DCP – deep capillary plexus. PDGFiCre^{-}; *Occ*$^{fl/fl}$ and PDGFiCre^{+}; *Occ*$^{fl/fl}$ mice were given an intravitreal injection of 200 ng VEGF in one eye and vehicle in the other and retinal vascular permeability to FITC-BSA (C) and 70-kDa Dextran (D) was determined after 36 h of VEGF intravitreal injection, by extracting the extravasated dye into the tissue. Data was normalized to the vehicle injected eye of each

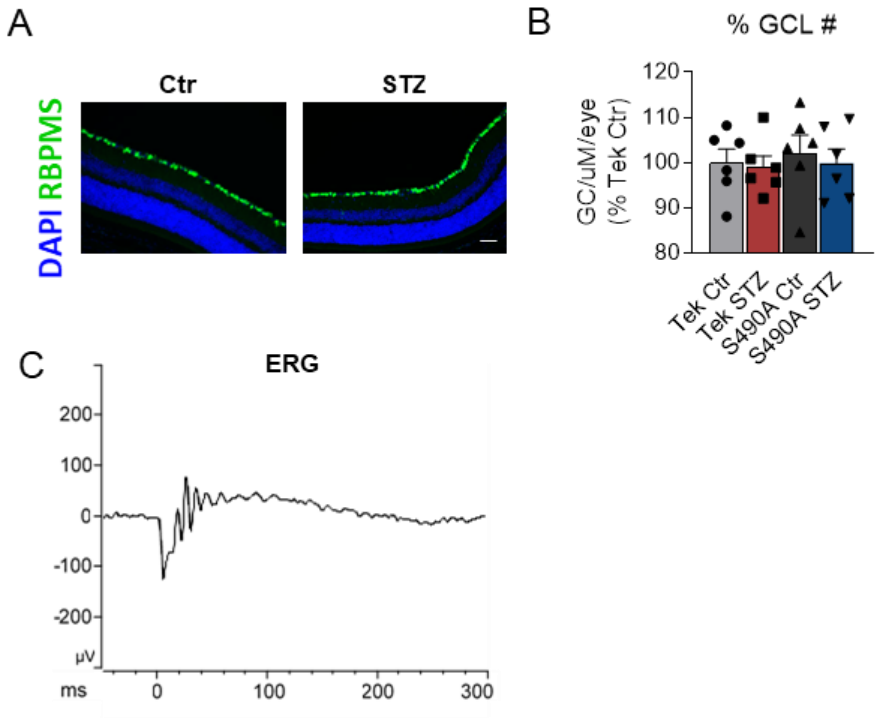
mouse. VEGF-induced retinal edema formation was determined by measuring retinal thickness by OCT and % change from baseline was calculated (E).



Supplementary figure 4: Diabetes was induced by STZ injection in Tek-Cre^{+} and Tek-Cre^{+}; S490A*OCC*$^{+/+}$ mice. After 4 months of diabetes induction blood glucose levels (A) and body weight (B) were determined. ***P <0.001 One-way ANOVA followed by Sidak's post-hoc test.



Supplementary figure 5: Representative confocal images from the deep capillary plexus of retinas stained with vessel marker IB4 (grey), leukocyte marker CD45 (red) and microglia/macrophage marker Iba1 (green). Scale bar: 50µm.



Supplementary figure 6: (A) After 4 months of diabetes induction retinal cross sections were stained for RBPMS (green) and Hoechst (blue). Scale bar: 50μm. (B) Quantification of the number of RBPMS-positive cells **P* <0.05, ***P* <0.01, ****P* <0.001 One-way ANOVA followed by Sidak's post-hoc test. (C) Representative ERG response elicited from 1.5 cd-s/m2 log flash intensity from a Tek^{+}-Cre control animal.