# Supplementary Online Content

Dmitriev PM, Wang H, Rosenblum JS, et al. Vascular changes in the retina and choroid of patients with *EPAS1* gain-of-function mutation syndrome. *JAMA Ophthalmol.* Published online December 26, 2019. doi:10.1001/jamaophthalmol.2019.5244



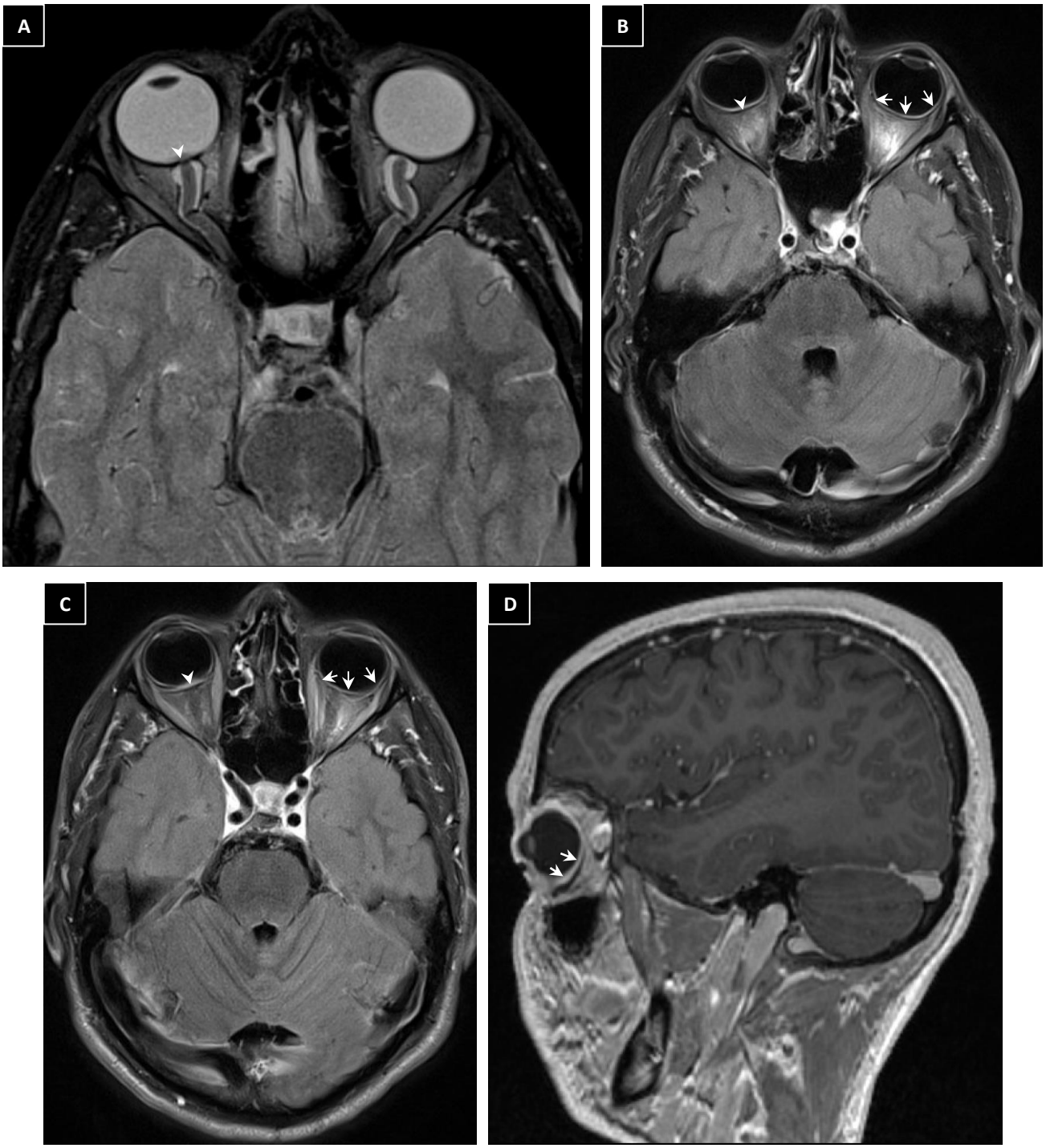
This supplementary material has been provided by the authors to give readers additional information about their work.

**eTable.** Ophthalmic Features of Three-Month-Old *Epas1*$^{A529V}$ Mutant Mice

| **Phenotype of Epas1$^{A529V}$ mutant mice** | **Mouse (n=7)** |
|---|---|
| Fibrosis overlying optic disc | 7 |
| Persistence of hyaloid vessels | 4 |
| Retinal vascular defects (vascular tortuosity, dilated retinal veins, abnormal patterning) | 7 |
| Retinal pigment epithelium (RPE) changes | 4 |
| Leakage from vessels on FA | 3 |

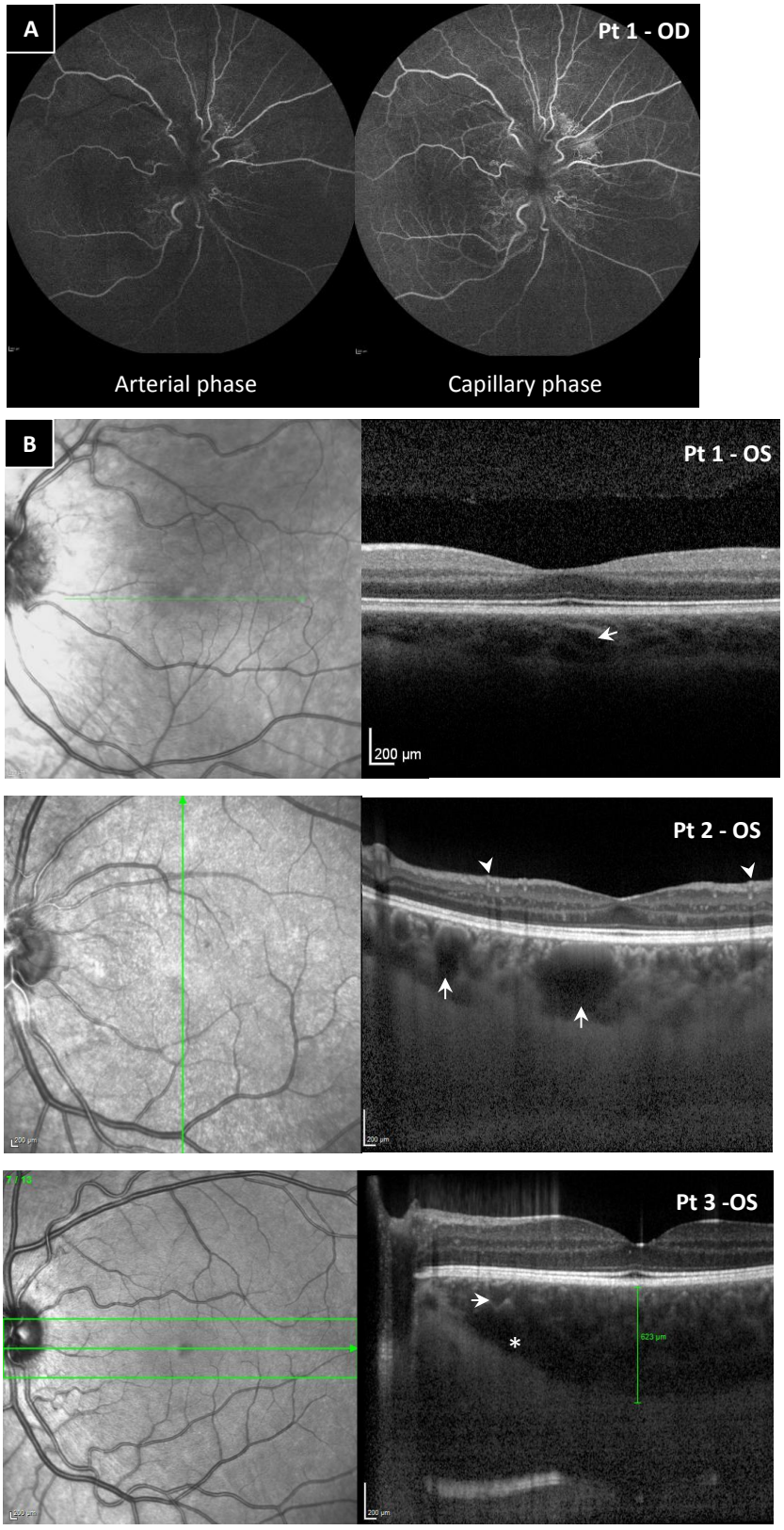
Summary of the fundoscopy and fluorescein angiography results of 7 three-month old Epas1$^{A529V}$ mutant mice. Control mice did not exhibit these findings.

**eFigure 1.** Patient Orbital and Intracranial MRI



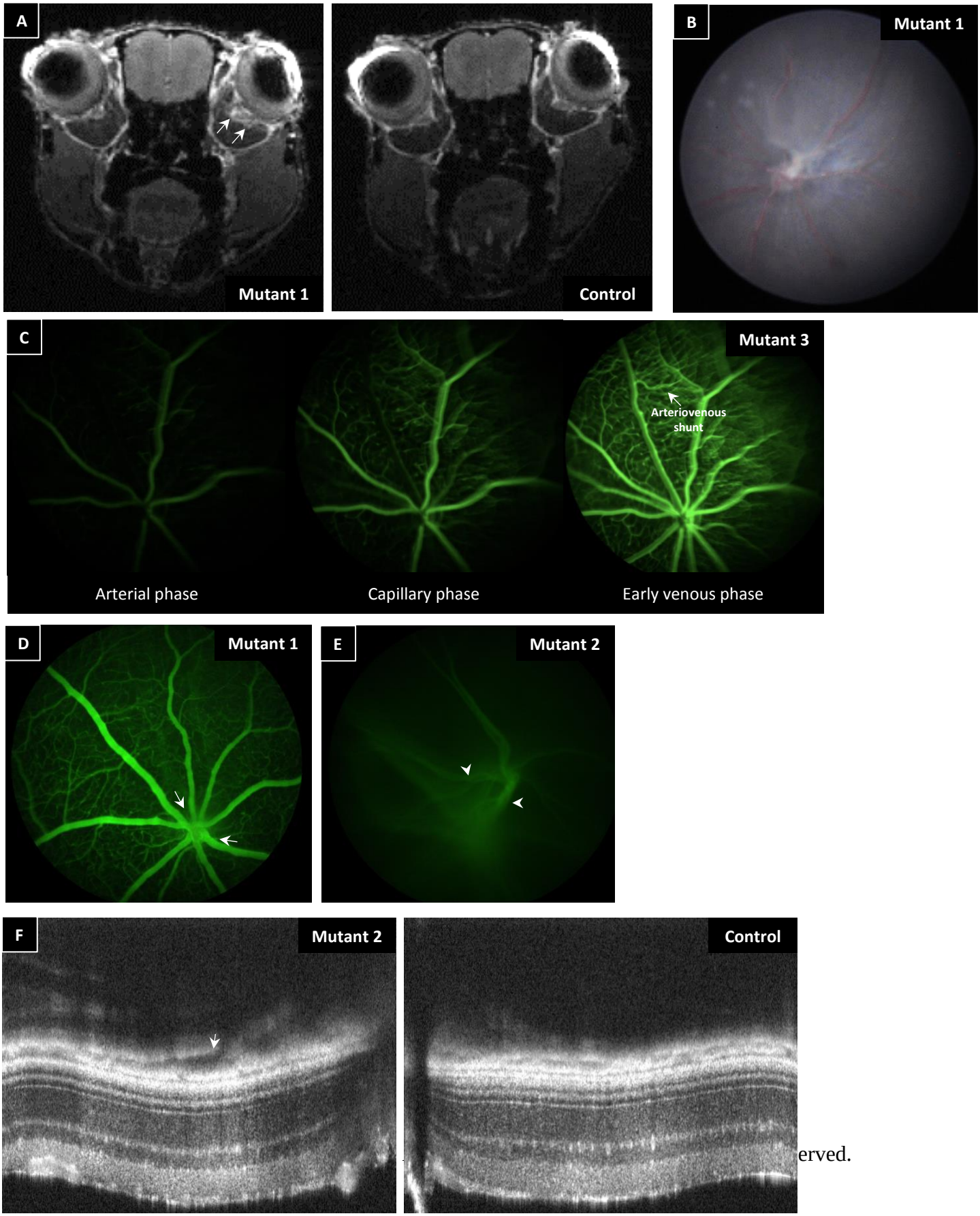
Representative images of all patients are shown. *Panel A*: T2-weighted Turbo spin echo (TSE) fat-saturated sequence of the orbit of Patient 1 at age 15 shows morning glory anomaly of the R eye (arrowhead). Tortuous optic nerve course and increased diameter of optic nerve sheath is seen bilaterally. *Panel B*: Axial T1-weighted post-contrast venous phase sequence of Patient 1 at age 17 shows evidence of a morning glory anomaly (arrowhead) in the right eye and contrast signal intensity in the posterior aspect of the globe bilaterally (arrows). *Panel C:* Axial T1-weighted FLAIR post-contrast venous phase sequence of Patient 1 at age 19 shows morning glory anomaly of the optic nerve head as it exits the back of the right eye (arrowhead). Signal intensity, consistent with contrast, is seen at the posterior aspect of the globe, extending laterally, consistent with the choroidal layer (arrows). *Panel D:* Sagittal T1-weighted post-contrast venous phase sequence of Patient 1 at age 17 demonstrates increased contrast accumulation and lateral extent in the posterior aspect of the globe in the right eye, off midline.

**eFigure 2.** Additional Patient Ocular Findings



*Panel A:* Fluorescein angiography (FA). Morning glory anomaly shown is specific to Patient 1. Early arterial phase FA demonstrates filling defect due to morning glory anomaly. Remaining arterial filling emphasizes vessel tortuosity. Early capillary filling phase shows increase in same regional leakage as seen in arterial phase. *Panel B*: EDI-OCT imaging of the left eyes of three patients with measurements of choroid thickness taken at the fovea showed thickening of the choroid layer, dilation of the choroidal vessels (arrows), and presence of interstitial fluid (*). Increased number of retinal vessels is also seen (arrowheads). Choroid measurements are as follows: Pt 1 OS 307 $\mu$m, Pt 2 OS 447 $\mu$m, and Pt 3 OS 623 $\mu$m. OD, right eye; OS, left eye.

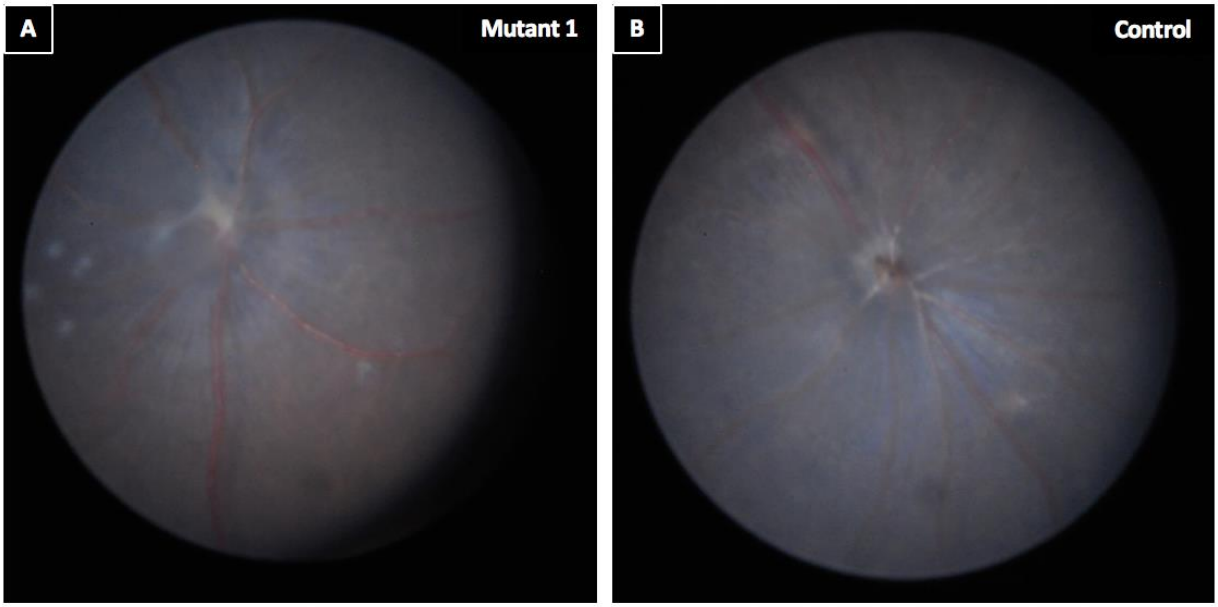
**eFigure 3.** Additional *Epas1*$^{A529V}$ Transgenic Mouse Model Ocular Findings




erved.

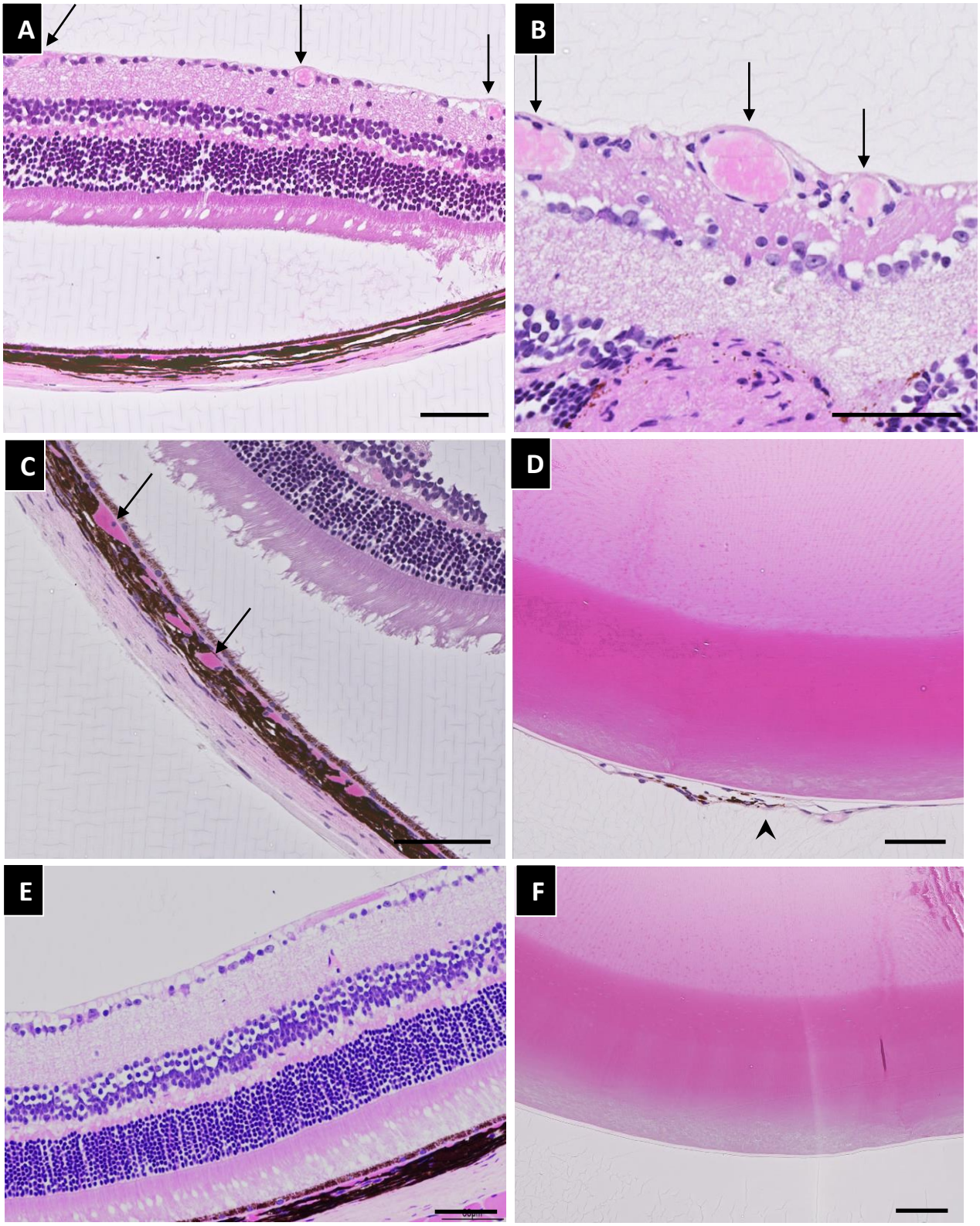
*Panel A:* MRI of mutant mouse brain and orbit demonstrated increased contrast accumulation in the posterior aspect of the eyes and dilation of the choroid vessels and vortex veins (arrows), bilaterally, when compared to littermate control. *Panel B:* Ophthalmoscopic image of mutant mouse eye showing dilated vessels, vessel tortuosity, fibrosis over the optic disc, and disorganized retinal vasculature. Ophthalmoscopic image of control mouse eye can be found in Figure 3. *Panel C*: FA images of representative mutant mouse at different time points since tail vein injection of fluorescein dye from a single mutant mouse showing evidence of an arteriovenous shunt (arrow) which is not present in the littermate control. From left to right: early arterial phase FA image, early capillary filling phase FA image, and early venous phase FA image. Video of shunt is provided in Supplementary Video 1. *Panel D*: FA image of a mutant mouse eye 5 minutes post-injection demonstrates leakage of dye from vessels surrounding the optic disc (arrows). *Panel E*: FA image of another mutant mouse eye 10 minutes post-injection shows persistence of hyaloid vessels (arrowheads), corresponding with the hazy opacity seen on mutant FA in Figure 3. *Panel F*: EDI-OCT imaging of a mutant mouse shows thickening of the choroid layer and presence of large vessels (arrow) when compared to a littermate control.

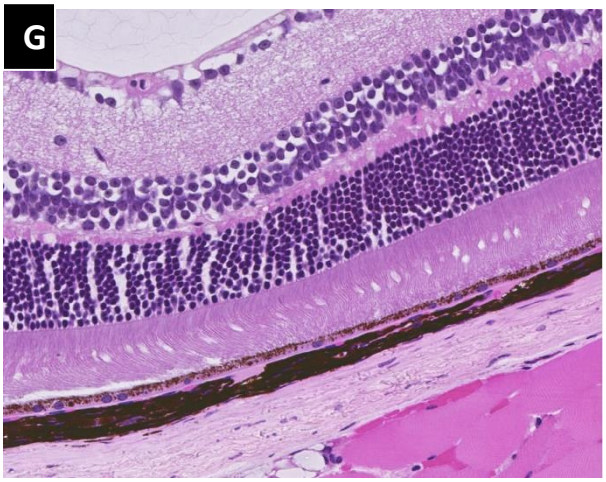
**eFigure 4.** Repeat Ophthalmoscopic Evaluation of *Epas1*$^{A529V}$ Mutant Mice




Representative images are shown. Three months after initial evaluation, ophthalmoscopy of the *Epas1*$^{A529V}$ mutant mice showed no progression of eye pathology in the mutant (A) when compared both to previous time point and to littermate control (B).

**eFigure 5.** Histologic Analysis of *Epas1*$^{A529V}$ Mutant Mice






Representative histologic images of a 6-month-old *Epas1*$^{A529V}$ mutant mouse showing dilated, congested vessels in the retina (A, B), and choroid (C) (arrows). A neovascular membrane and persistent hyaloid vasculature is also seen (D) (arrowhead). These findings were not seen in littermate control mice (E, F, G). Scale bars, 60 μm. C and G share the same scale bar.