

SUPPLEMENTARY INFORMATION:

***In Vivo* Imaging of Retinal Hypoxia using HYPOX-4-dependent Fluorescence in a Mouse Model of Laser-induced Retinal Vein Occlusion (RVO)**

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Running Title: Characterization of Retinal Hypoxia in a Mouse Model of RVO

Key Words: retinal hypoxia, ischemia, retinal vein occlusion, *in vivo* imaging, HYPOX-4

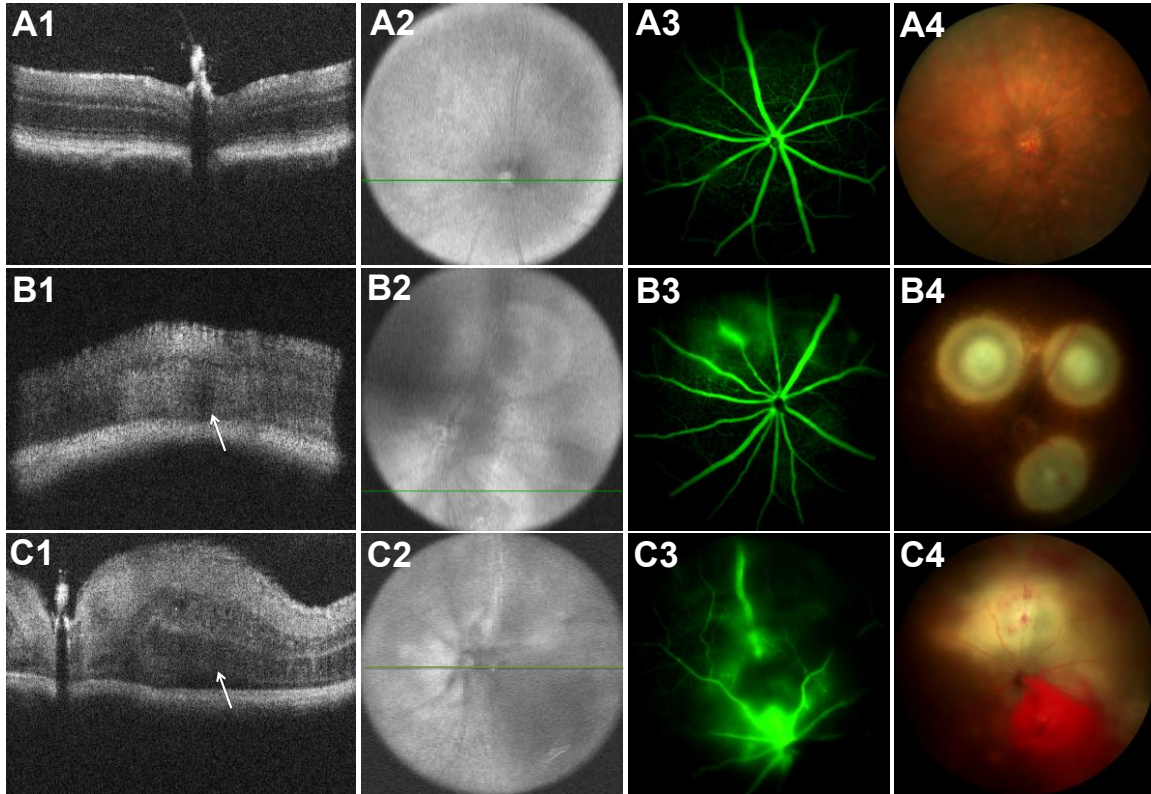


Figure S1: The green lines in the fundus images A2, B2 and C2 represent the location of the b-scans in the corresponding en face images in A1, B1 and C1 respectively. Changes in retinal thickness and vascular tortuosity post-PRVT in mouse RVO are indicated as follows: a control retina appears normal in the b-scan (A1), en face OCT image (A2), fluorescein angiogram (A3) and fundus image (A4) respectively. Minimal retinal edema (white arrow) was observed after laser treatment in sham control retina (B1). Areas of severe edema (C1) (white arrow) with increased vascular tortuosity (C3) was observed in a RVO retina.

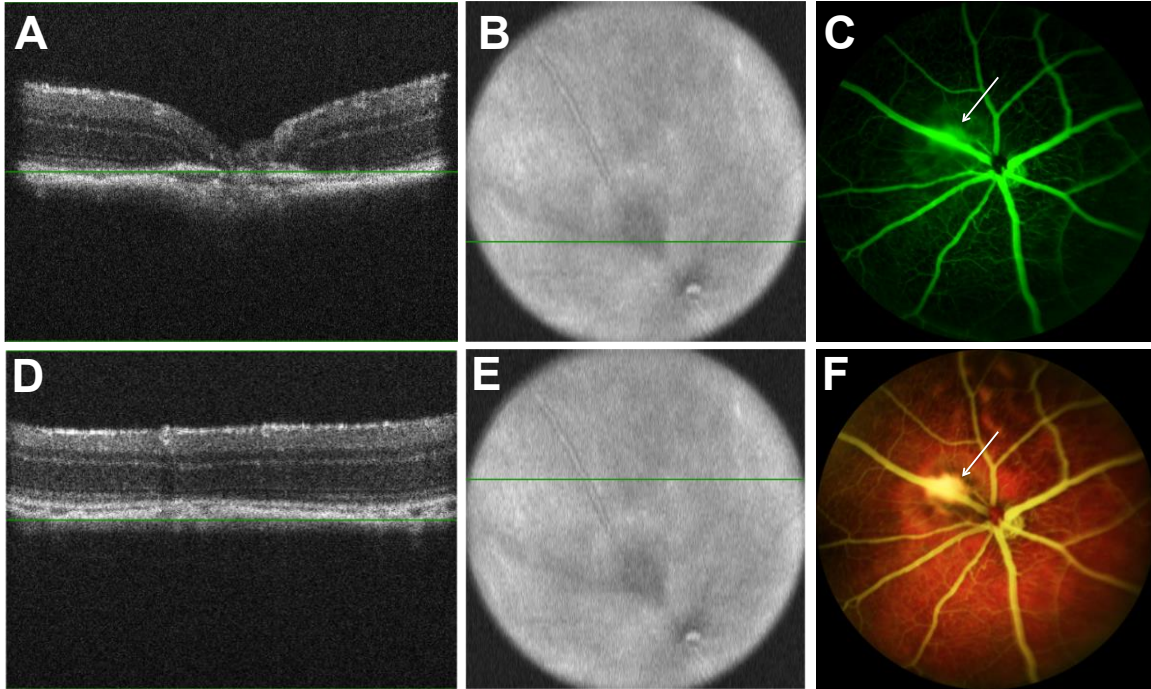


Figure S2: Recovery from retinal edema and vascular recannulation 8 days post-PRVT. Representative b-scan images of retinal thickness, at the location of the occluded vein (A) and at the retinal mid-periphery (D). The green line in the fundus image in B and E represent the location of the b-scan in A and D respectively. (A) Retinal edema resolved at day 8. The fluorescein angiogram (C) and fundus image (F) of the same eye at 8 days post-PRVT indicating recannulation of the occluded vein. White arrow shows the position of the vein occlusion.

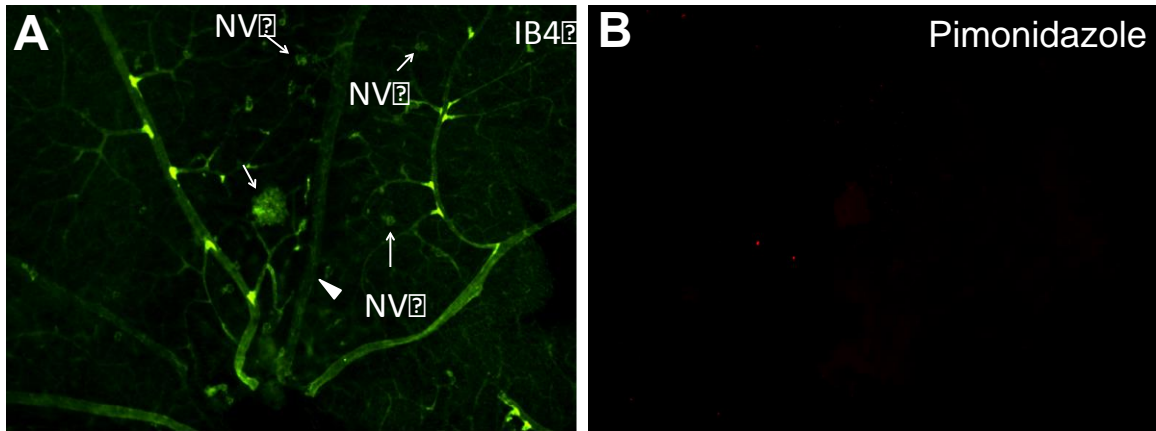


Figure S3: *Ex vivo* analysis of the RVO retina at 10 days post-PRVT. (A) IB4 immunostaining at 10 days post-PRVT showing neovascularization (arrows) originating from the dependent capillaries of the occluded vein (arrowhead); (B) the level of hypoxia was minimal at this time, as determined by pimonidazole-adduct immunostaining technique.

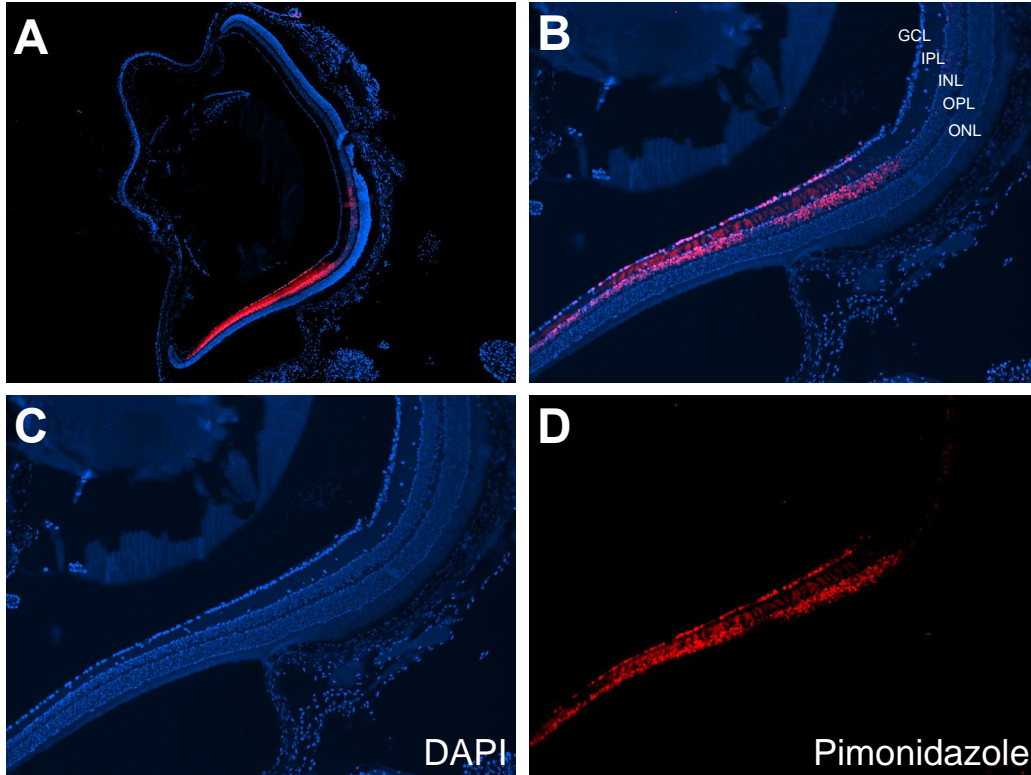


Figure S4: RVO mice were treated with pimonidazole and the spatial distribution of hypoxia was determined in retinal cross-sections. (A) Pimonidazole-adduct immunostaining confirmed retinal hypoxia (red) in the peripheral retina. Nuclei were stained with DAPI (blue). (B to D) Hypoxia was observed in the ganglion cell layer, inner plexiform and inner nuclear layers. Abbreviations: GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer.

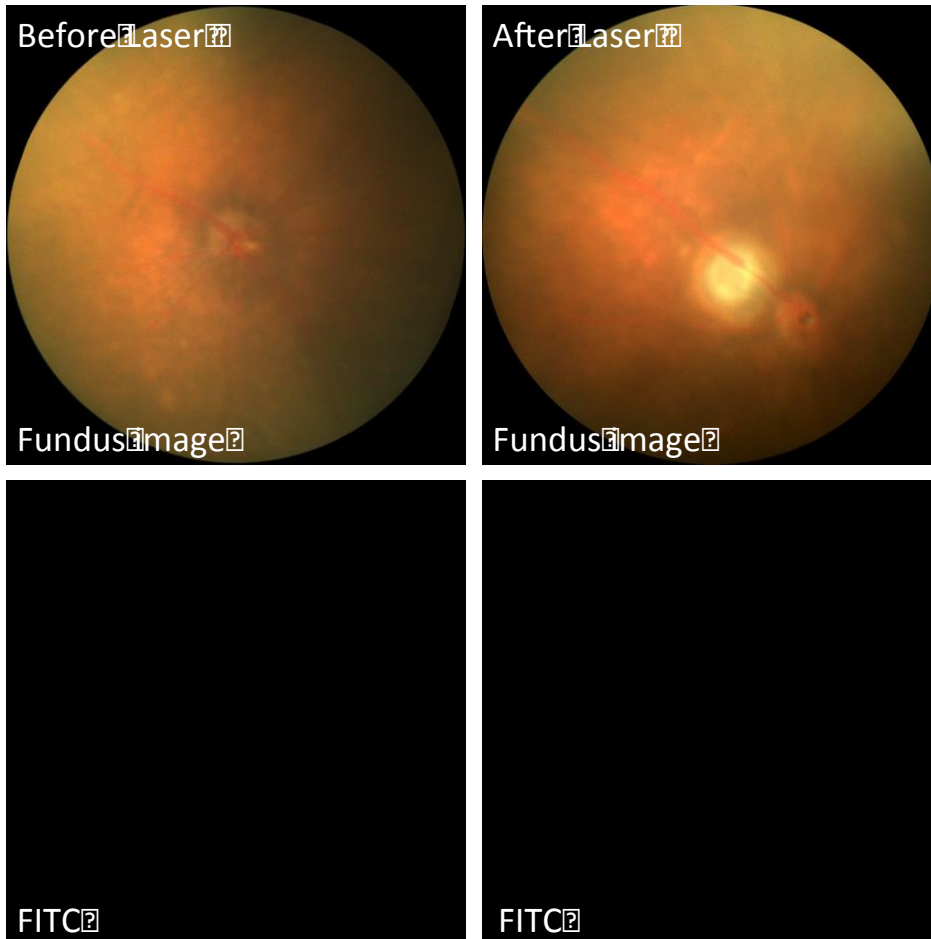


Figure S5: Change in the background auto-fluorescence in RVO mice (n=5) was monitored using Micron IV imaging system using same image acquisition parameters used for HYPOX-4 *in vivo* imaging. There was no change in auto-fluorescence observed in these RVO mice.

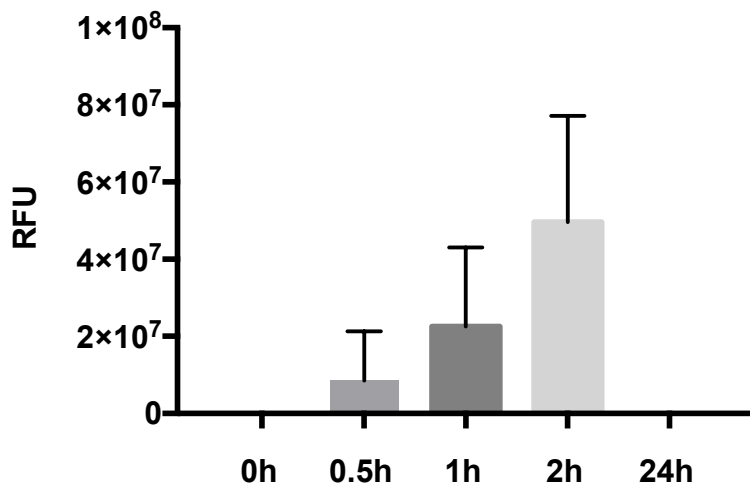


Figure S6: *In vivo* clearance of HYPOX-4 from ocular tissues was assessed by fluorescence measurement using Micron IV imaging system. HYPOX-4 was injected intraperitoneally at a dose of 60 mg/kg in healthy adult C57BL/6 mouse (n=3) and fluorescence images were captured *in vivo*. Relative fluorescence intensities were calculated using ImageJ software. Clearance profile of HYPOX-4 was similar in all the injected animals and complete clearance of HYPOX-4 from ocular tissues was observed after 24 hours.