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## Supplemental information

# Anti-angiogenic collagen IV-derived peptide target engagement with $\alpha_v\beta_3$ and $\alpha_5\beta_1$ in ocular neovascularization models

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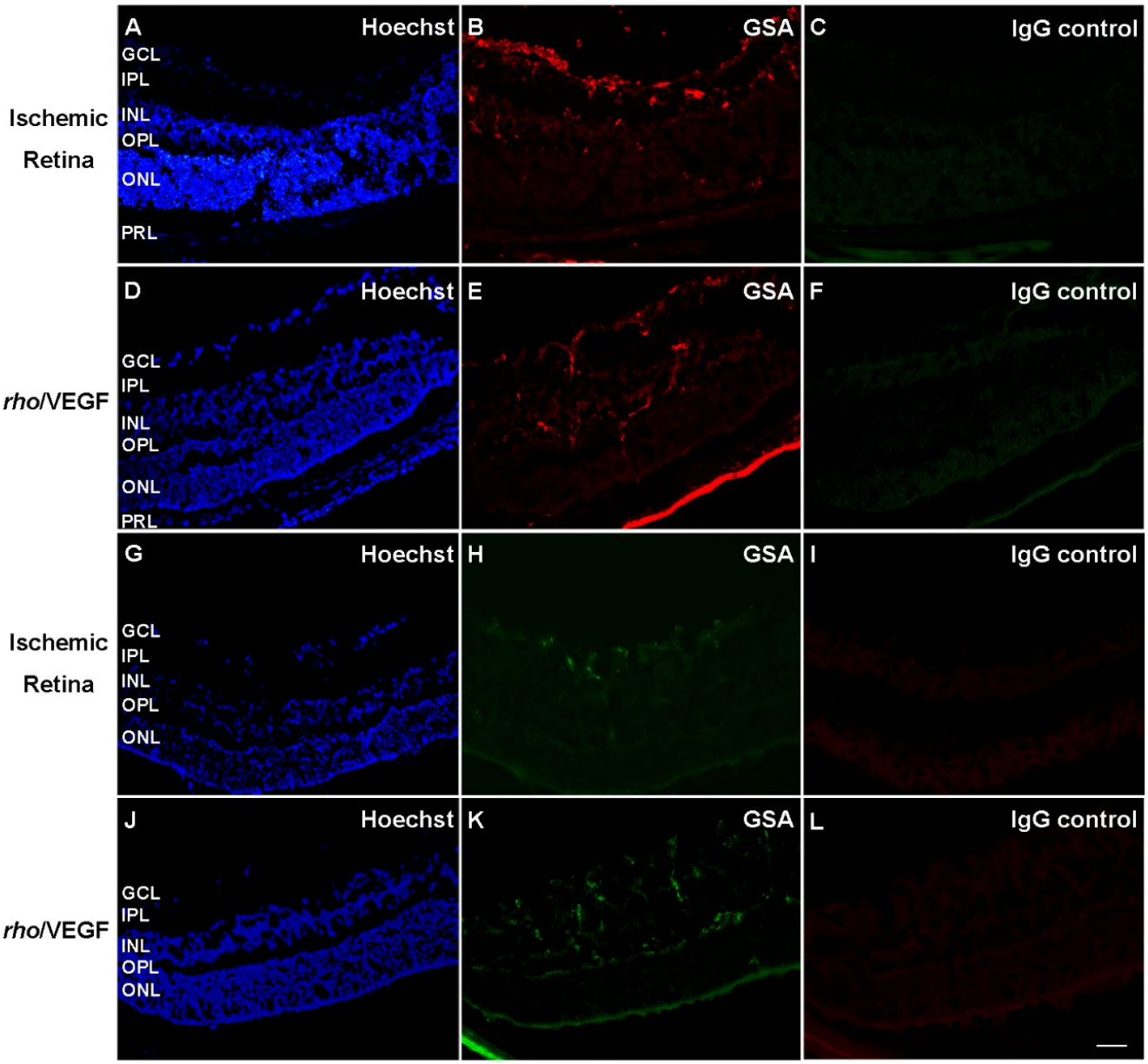
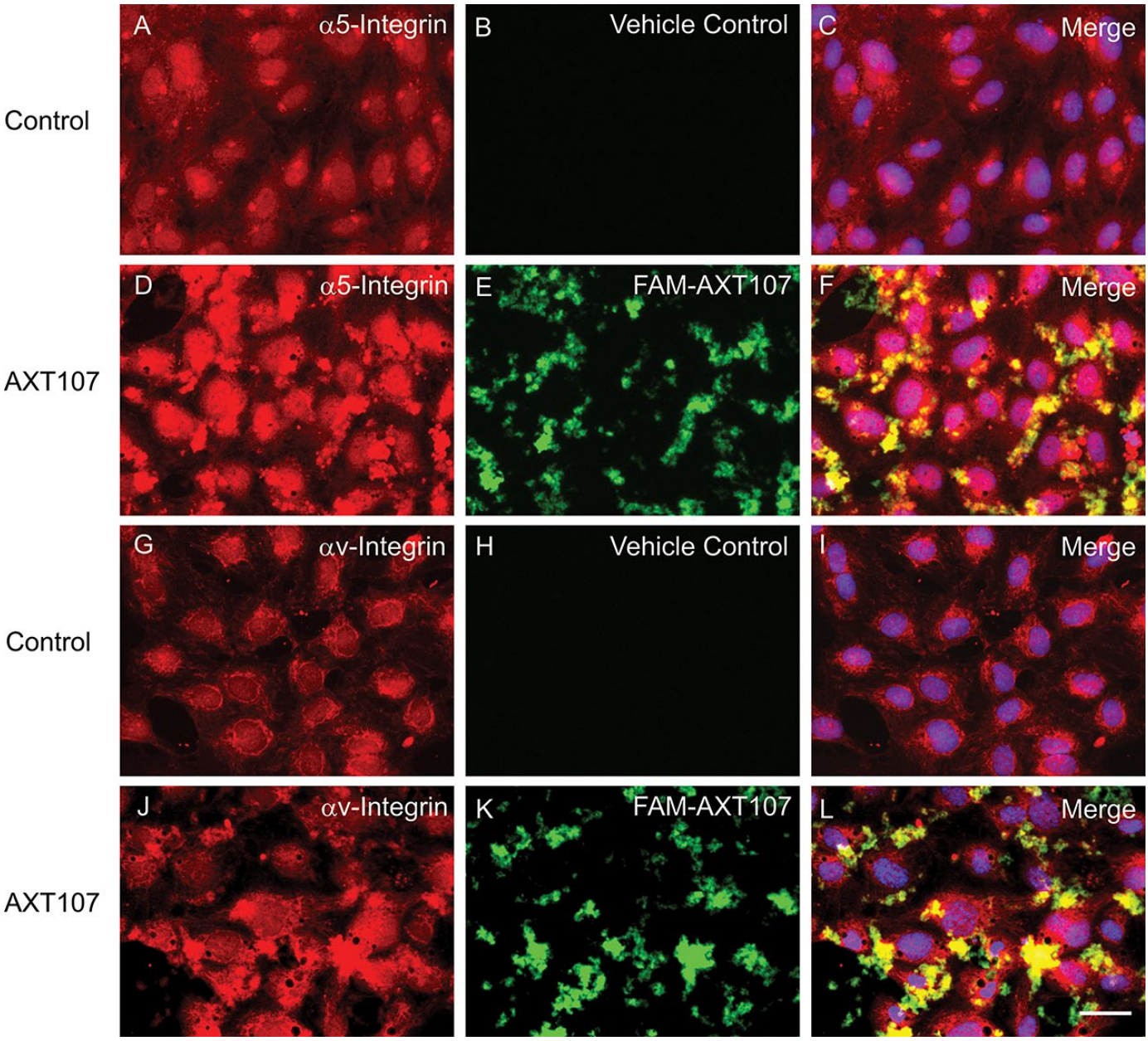
**Figure S1. Absence of nonspecific staining from secondary antibodies. Related to Figures 1-3.**



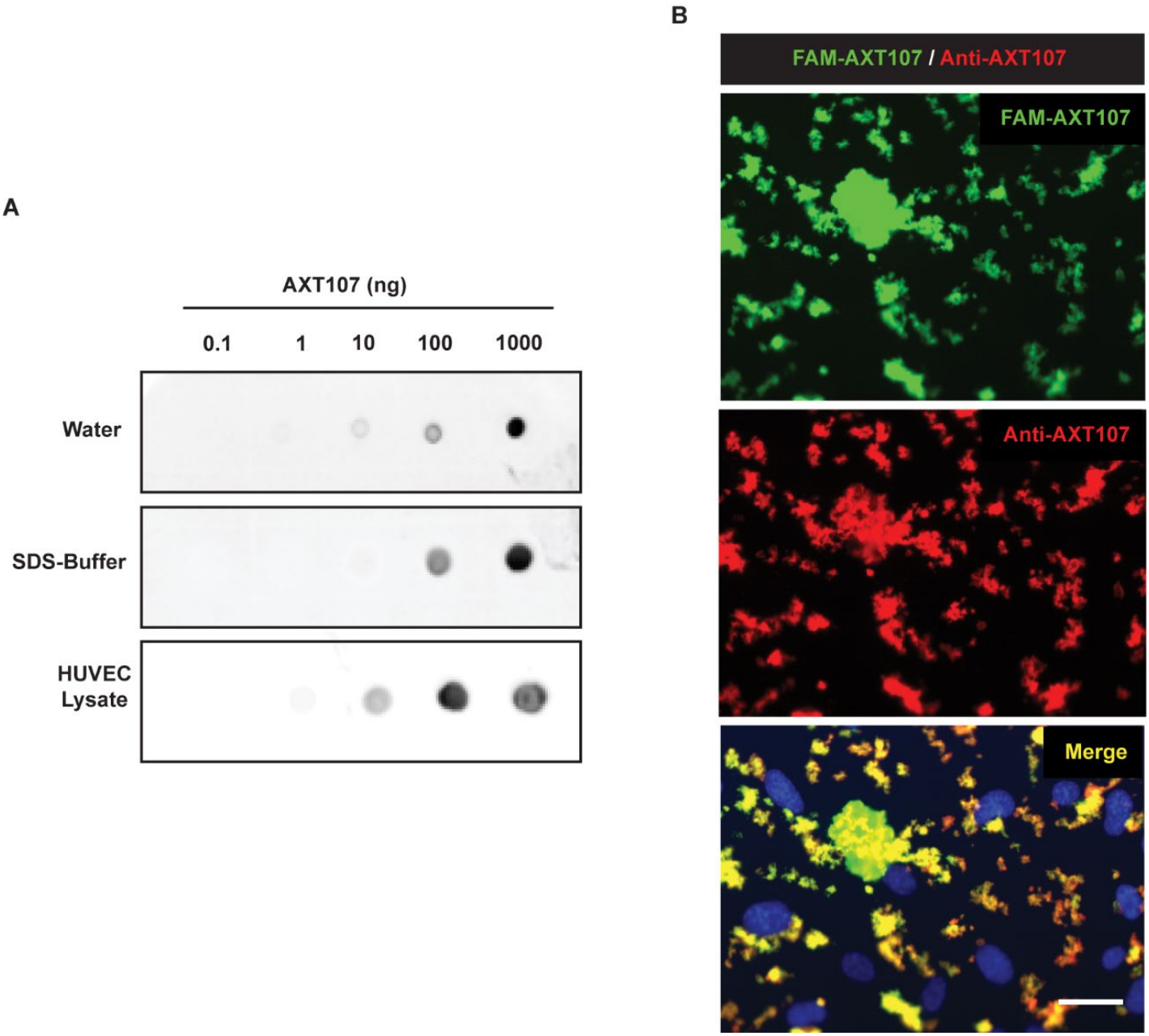

Ocular frozen sections from P17 C57BL/6 mice with oxygen-induced ischemic retinopathy (n=10, OIR, A-C and G-I) or P21 *rho*/VEGF transgenic mice (n=10, D-F and J-L) were incubated with Alexa Fluor 594-labeled Griffonia Simplicifolia Agglutinin lectin (GSA) and incubated with FITC-conjugated anti-rabbit IgG secondary antibody (A-F) or incubated with FITC-Griffonia Simplicifolia Agglutinin lectin (GSA) and then incubated with Alexa Fluor 594-conjugated anti-rat IgG secondary antibody (G-L) in the absence of primary antibody. There was no nonspecific staining with either secondary antibody (C, F, I, L). A minimum of 20 ocular sections were assessed per group. The study was conducted in duplicates and qualitatively similar results were found in the replicates. Scale bar = 100 μm.

**Figure S2. AXT107 co-localizes with $\alpha_5$ and $\alpha_v$ integrins in cultured human umbilical vein endothelial cell (HUVEC) monolayers. Related to Figure 4.**



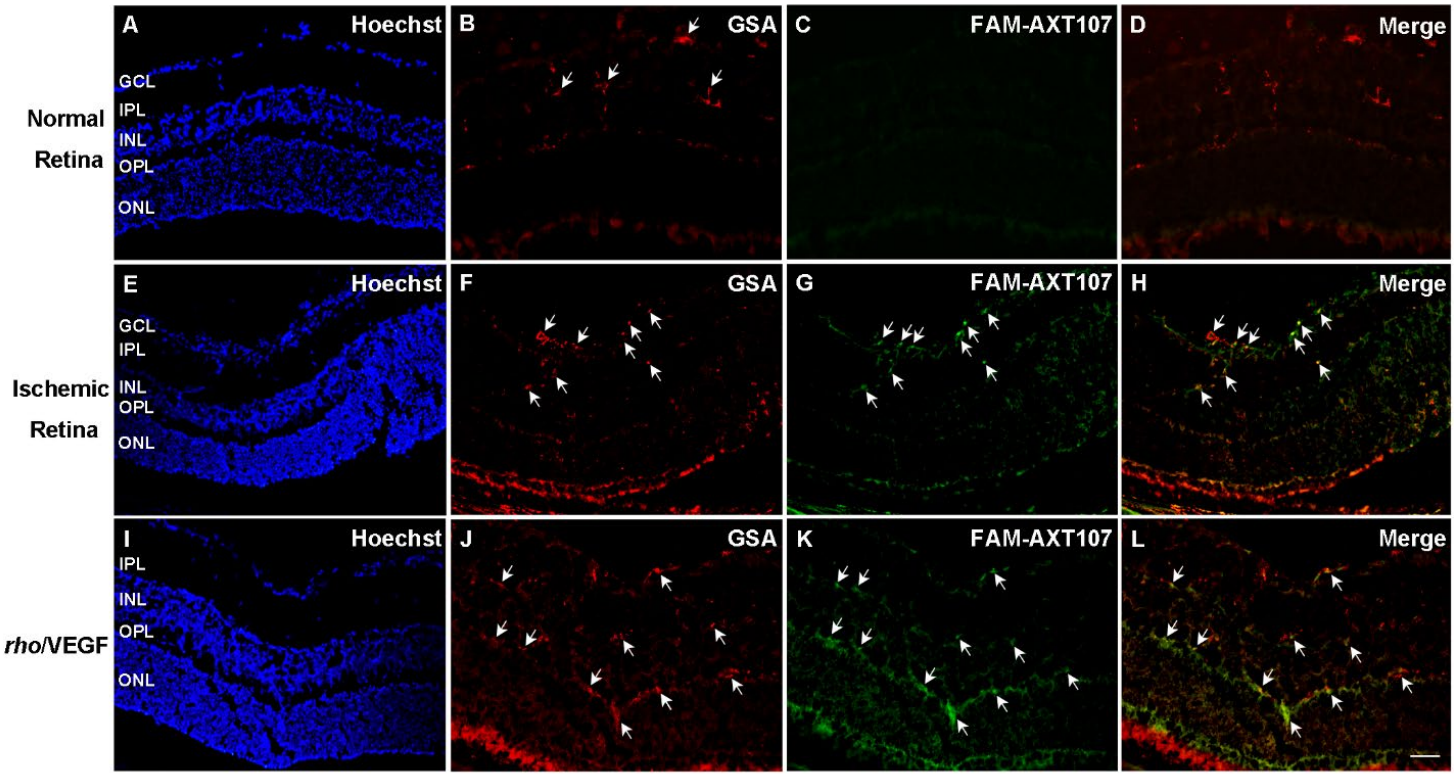

HUVEC monolayers to which no AXT107 was added (A-C) or to which 25 μM AXT107 containing 25% FAM-labeled AXT107 was added (D-F) were immunohistochemically stained with anti-$\alpha_5$ antibody. FAM-AXT107 co-localized with $\alpha_5$ integrin (F). HUVEC monolayers to which no AXT107 was added (G-I) or to which 25 μM AXT107 containing 25% FAM-labeled AXT107 was added (J-L) were immunohistochemically stained with anti-$\alpha_v$ antibody. FAM-AXT107 co-localized with $\alpha_v$ integrin (L). No green channel signal was detected in control groups (B, H). Nuclear staining (blue) is included in merged images. Scale bar = 25 μm.

**Figure S3. Anti-AXT107 antibody specifically stains AXT107. Related to Figures 5-8.**



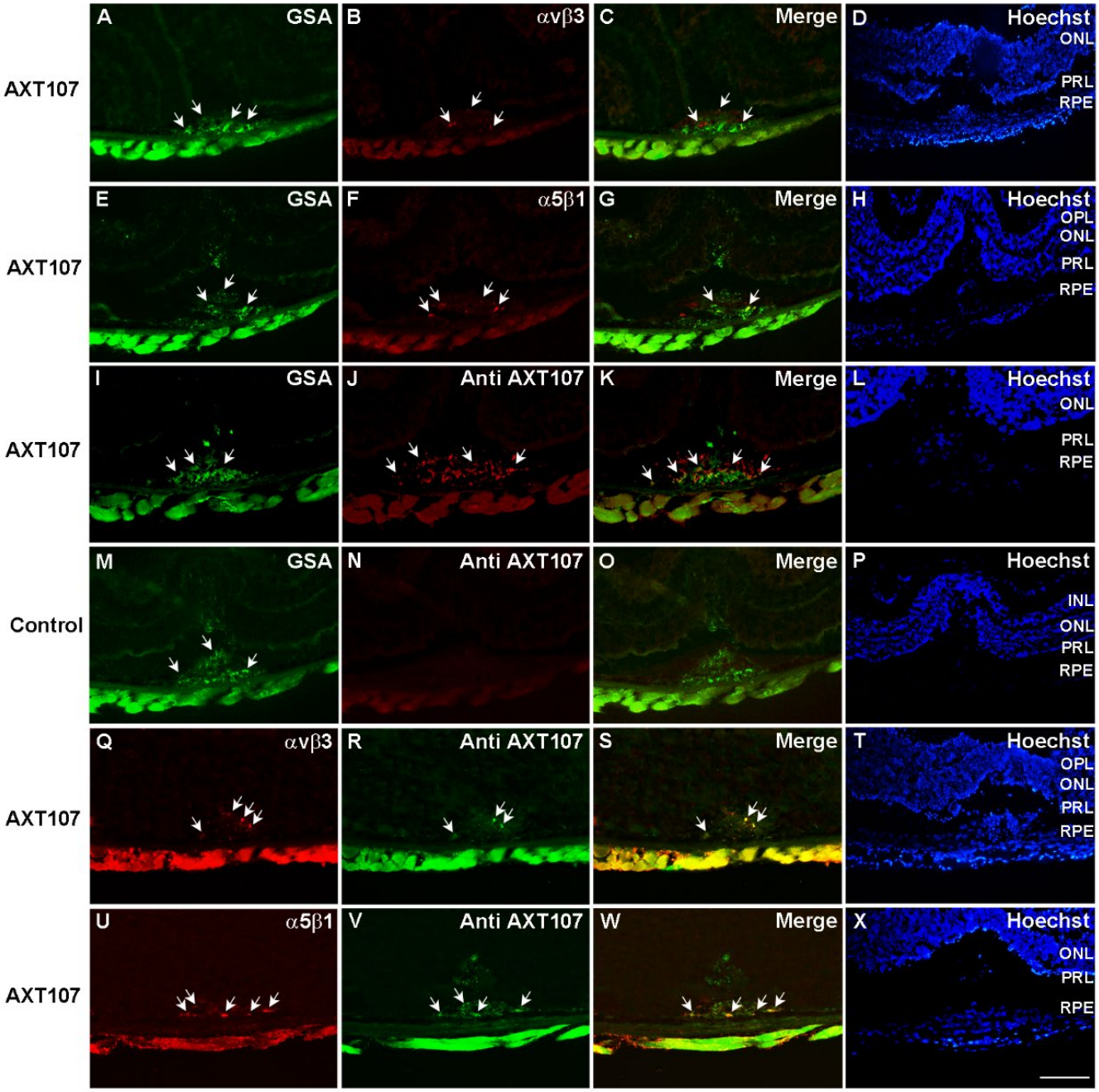

Dot blots of AXT107 in water, SDS buffer, or HUVEC lysates showed AXT107 concentration-dependent staining with anti-AXT107 (A). Addition of FAM-labeled AXT107 to HUVEC monolayers followed by immunohistochemical staining with anti-AXT107 antibody showed co-localization of FAM-labeled AXT107 (green) with antibody-stained AXT107 (red). Nuclear staining (blue) was included in the merged image (B). Scale bar = 25 μm.

**Figure S4. FAM-AXT107 localizes to blood vessels in diseased retinas but not in normal retinas. Related to Figure 6.**



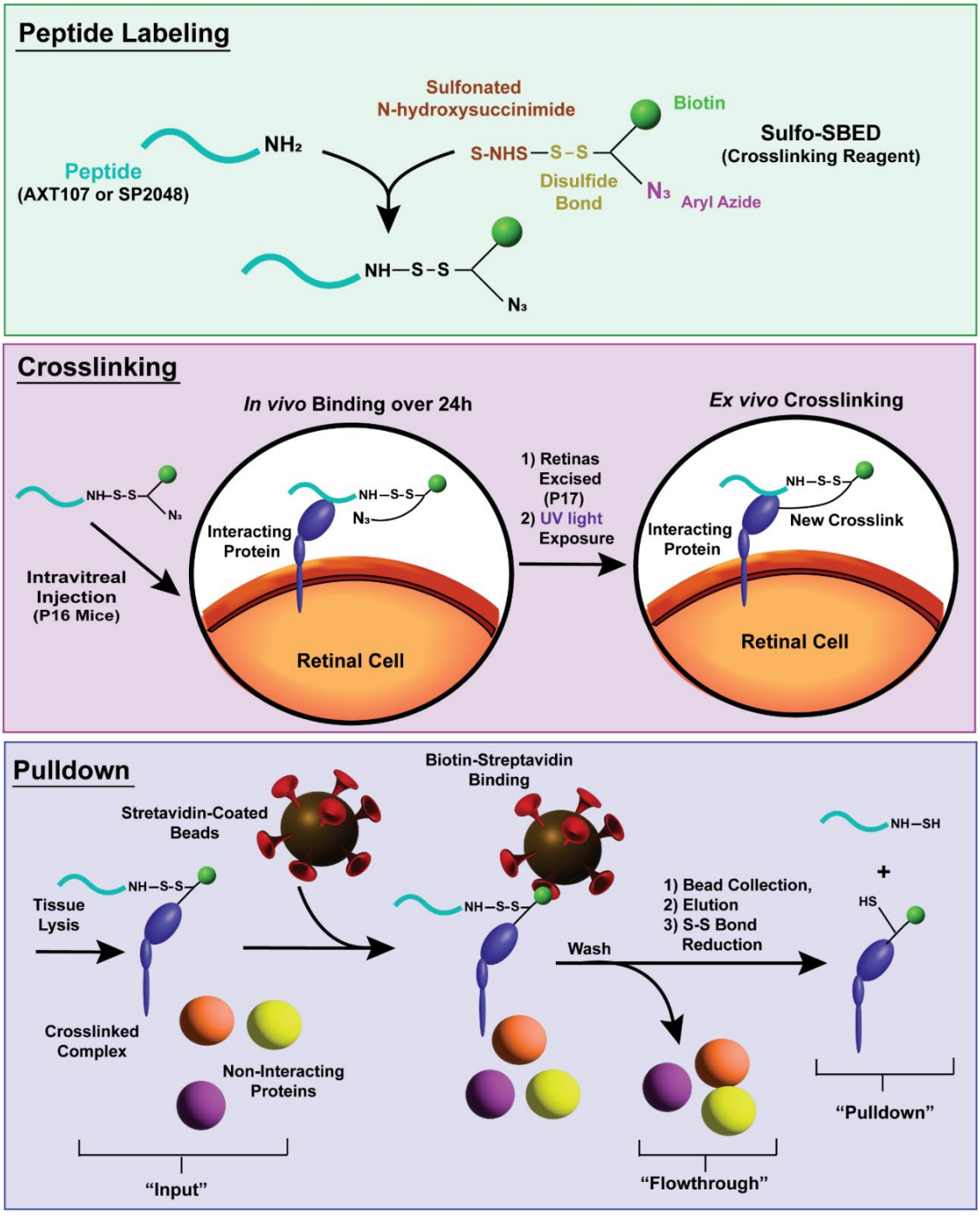

Ocular frozen sections from P21 normal mice (n=10) showed no fluorescence in the retina or in the GSA-stained normal retinal vessels, one day after injection of FAM-AXT107. P17 mice with oxygen-induced ischemic retinopathy (n=10, OIR), or P21 *rho*/VEGF transgenic mice (n=10) that had been given an intravitreous injection of 1 µg of FAM-AXT107 3 days before, were incubated with Alexa Fluor 594-labeled Griffonia Simplicifolia Agglutinin lectin (GSA). There was no detectable FAM-AXT107 in normal retina (A-D), but in retinas from OIR mice (E-H) or *rho*/VEGF mice (I-L) there was co-localization of FAM-AXT107 with GSA-stained neovascularization and pre-existent retinal vessels. A minimum of 20 ocular sections were assessed per group. The study was conducted in duplicates and qualitatively similar results were found in the replicates. Scale bar = 100 µm.

**Figure S5. After intraocular injection of AXT107, integrins αvβ3 and α5β1 are expressed in cells within type 2 choroidal neovascularization (NV) and AXT107 co-localizes within choroidal NV and integrins in serial ocular sections. Related to Figure 8.**



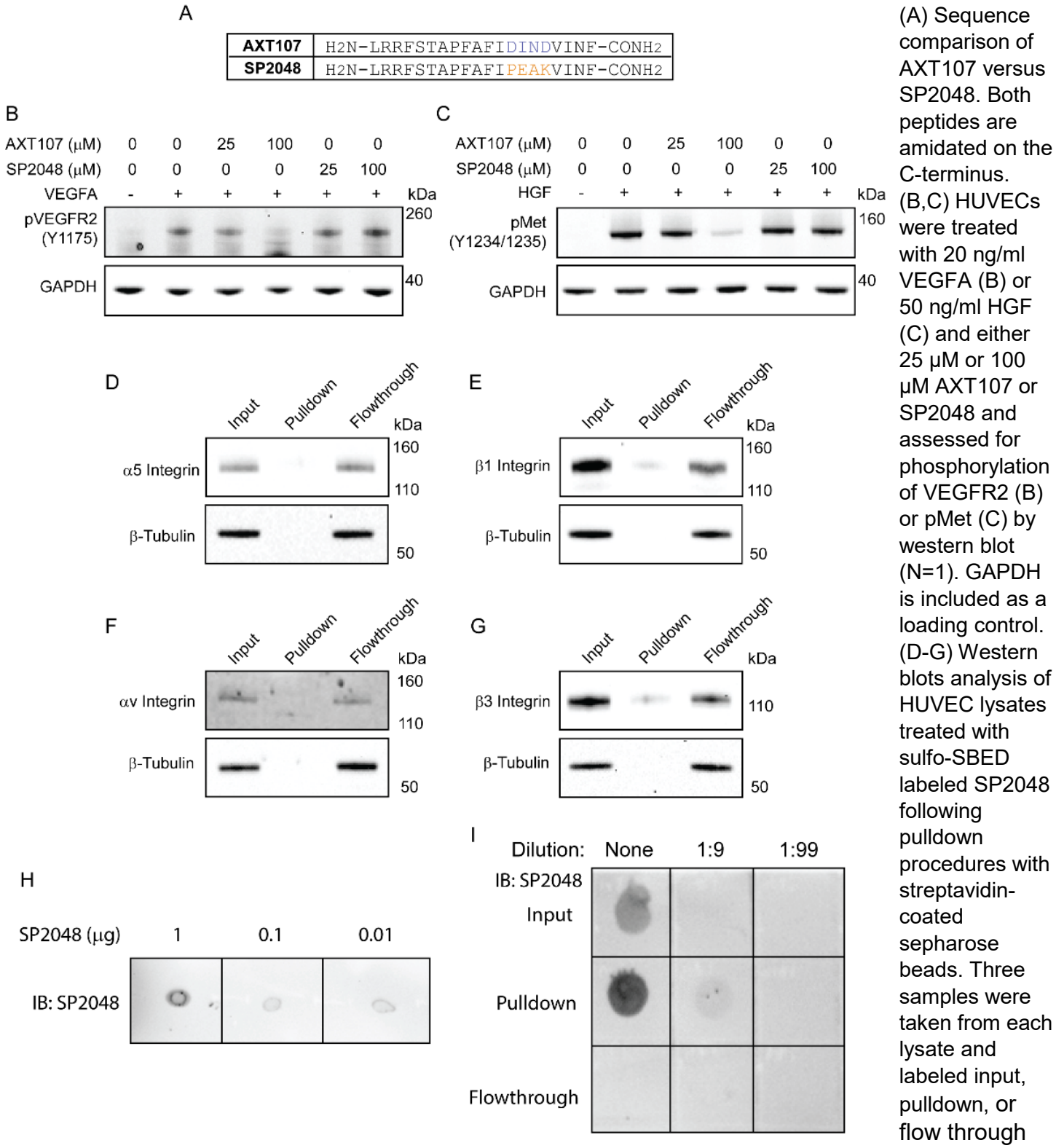

Seven days after laser-induced rupture of Bruch's membrane, and 1 day after intravitreous injection of 1 µg of AXT107 or PBS in 10 C57BL/6 mice, ocular frozen sections were incubated with FITC-Griffonia Simplicifolia Agglutinin lectin (GSA) and immunostained for αvβ3, α5β1, or AXT107. There was staining for αvβ3 (A-D), α5β1 (E-H), and localization of AXT107 (I-L) within choroidal NV (arrows) in the subretinal space above the retinal pigmented epithelium. There was no staining for AXT107 in the PBS-injected controls (M-P). Ocular sections stained for AXT107 and αvβ3 (Q-T) or for AXT107 and α5β1 (U-X), showed colocalization of AXT107 with each of the integrins. A minimum of 20 ocular sections were assessed per group. The study was conducted in duplicates and qualitatively similar results were found in the replicates. Scale bar = 100 µm.

**Figure S6. Summary diagram of sulfo-SBED crosslinking and pulldown experiments. Related to Figure 9.**




Peptide Labeling – Sulfo-SBED labeled peptide (sulfo-SBED peptide) was formed through room temperature incubation of the sulfo-SBED reagent with either AXT107 or SP2048. Primary amines from the the peptides react with the sulfonated N-hydroxysuccimide of the sulfo-SBED resulting in an N-terminal linkage of the sulfo-SBED and free biotin and aryl azide groups. Crosslinking – 1 µg of the sulfo-SBED peptide was injected intravitreally into P16 mice and binding allowed to occur over 24h. The retinas were then excised and pooled by treatment sets. Crosslinking was initiated by activating the aryl azide through UV light exposure, allowing it to covalently attach to nearby proteins. Pulldown – After crosslinking, the retinas were lysed and a sample saved as input, which contains all protein in the retina, regardless of interactions with the peptide. The remaining lysate was incubated with strepavidin-coated beads for 2h, after which the beads were pelleted by centrifugation and subsequently washed several times while the initial supernatant was saved as flow through, representing proteins that did not bind the beads (i.e. no biotin). The washed beads were then eluted by denaturation in SDS-loading dye and the peptide separate from interaction partners through reduction of the disulfide bond. All samples were then resolved by SDS-PAGE and analyzed by western blot.

**Figure S7. SP2048 does not crosslink with integrins. Related to Figure 9.**




(A) Sequence comparison of AXT107 versus SP2048. Both peptides are amidated on the C-terminus. (B,C) HUVECs were treated with 20 ng/ml VEGFA (B) or 50 ng/ml HGF (C) and either 25 μM or 100 μM AXT107 or SP2048 and assessed for phosphorylation of VEGFR2 (B) or pMet (C) by western blot (N=1). GAPDH is included as a loading control. (D-G) Western blots analysis of HUVEC lysates treated with sulfo-SBED labeled SP2048 following pulldown procedures with streptavidin-coated sepharose beads. Three samples were taken from each lysate and labeled input, pulldown, or flow through for the lysates before pulldown, components that associated with the beads, and components that did not associate with the beads respectively. Samples were each run four times and stained separately for integrins $\alpha_5$ (D), $\beta_1$ (E), $\alpha_v$ (F), and $\beta_3$ (G) with β-tubulin as a loading control (N=3). (H,I) Dot-blot analyses of 1 μg, 0.1 μg, and 0.01 μg SP2048 diluted in water (H) or samples used in panel C above (I) and stained using an anti-AXT107 antibody (N=3).