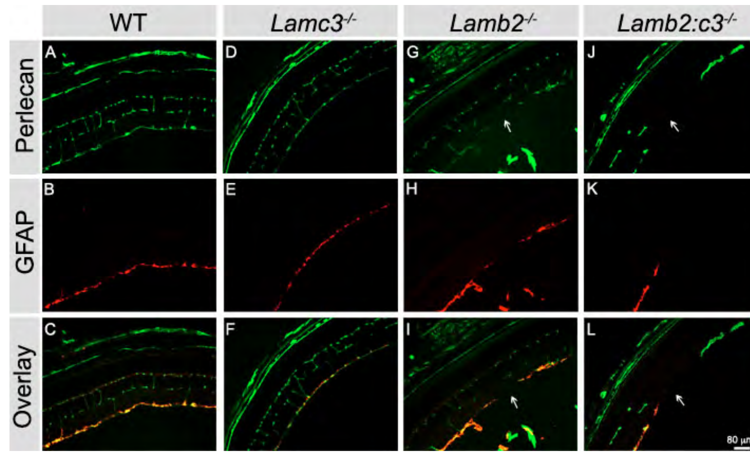

Fig. S1. Astrocytes are present only in retinal regions where the ILM is relatively intact. (**A-C**) Wild-type P15 retinal sections were reacted with antibodies to perlecan, a component of basement membranes (A), and GFAP, which is expressed by astrocytes (B). (C) Overlay of A and B. (**D-F**) *Lamc3*$^{-/-}$ P15 retinal sections were reacted with antibodies to perlecan (D) and GFAP (E). (F) Overlay of D and E. (**G-I**) *Lamb2*$^{-/-}$ P15 retinal sections were reacted with antibodies to perlecan (G) and GFAP (H). (I) Overlay of G and H. The ILM is disrupted in the *Lamb2*$^{-/-}$ retinal section (arrows). (**J-L**) *Lamb2:c3*$^{-/-}$ P15 retinal sections were reacted with antibodies to perlecan (J) and GFAP (K). (L) Overlay of J and K. The ILM is severely disrupted in the *Lamb2:c3*$^{-/-}$ retinal section (arrow).

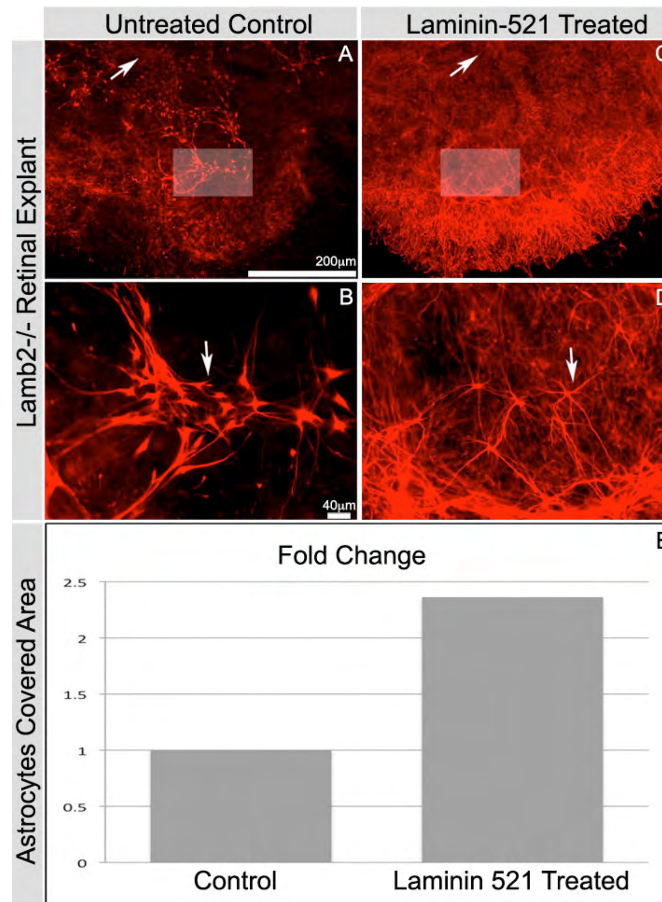

Fig. S2. Exogenous addition of laminin 521 restores astrocyte migration and patterning. (**A**,**B**) Untreated P1 *Lamb2*$^{-/-}$ retinal explants grown for 10 days in culture and analyzed for GFAP expression. Arrow indicates the head of the optic nerve. (B) A magnified region of A, indicated in by the shaded box in A. GFAP-positive astrocytes are clumped together (arrow in B). (**C**) Laminin 521-treated P1 *Lamb2*$^{-/-}$ retinal explant grown for 10 days in culture and analyzed for GFAP expression. Arrow indicates the head of the optic nerve. (**D**) A magnified region of C indicated by the shaded box in C. GFAP-positive astrocytes attain a stellate morphology with the addition of laminin 521 (arrow). (**E**) The difference in area covered by astrocytes between A and C was determined using Volocity software (v. 5.4.1) and the fold difference was recorded. This experiment was repeated using P0 and P3 *Lamb2*-null retinal cultures. Scale bar: 200 µm in A,C; 40 µm in B,D.

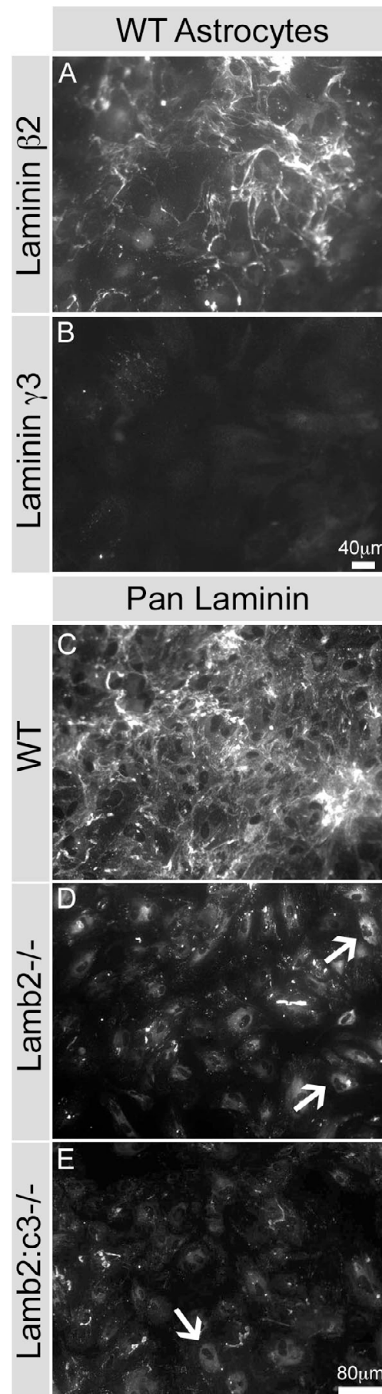

Fig. S3. Deletion of *Lamb2* and *Lamb2:c3* genes affects laminin secretion. (**A**) Wild-type retinal astrocytes were analyzed for laminin β2 chain expression after 6 days of culture. (**B**) Wild-type retinal astrocytes were analyzed for laminin γ3 chain expression after 6 days of culture. (**C**) Wild-type retinal astrocytes were analyzed for pan-laminin expression after 6 days of culture. (**D**) $Lamb2^{-/-}$ retinal astrocytes were analyzed for pan-laminin expression after 6 days in culture. Laminin immunoreactivity is mostly intracellular (arrows). (**E**) $\textit{Lamb2:c3}^{-/-}$ retinal astrocytes were analyzed for pan-laminin expression after 6 days in culture. Laminin immunoreactivity is mostly intracellular (arrows). Scale bar: 40 μm in A,B; 80 μm in C,E.

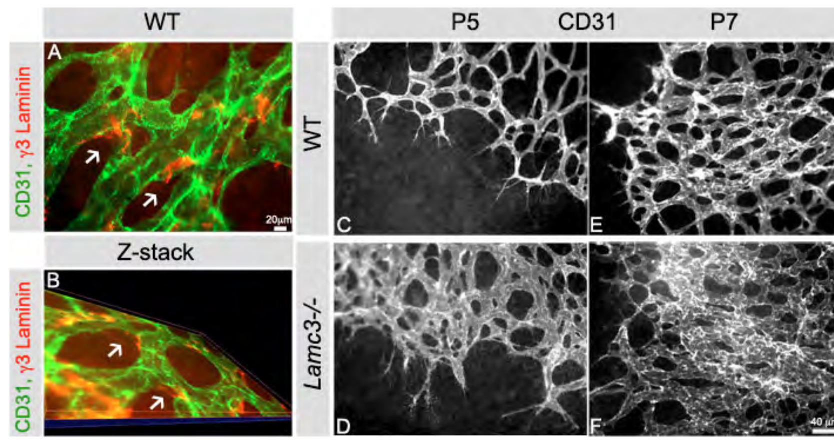

Fig. S4. Deletion of laminin γ3 chain affects vascular branching pattern during angiogenesis. (**A**) Wild-type P5 whole-mount retina was reacted with antibodies to laminin γ3 chain (red) and CD31 (green). The laminin γ3 chain is prominent at vascular branch points (arrows). This image was captured using fluorescent microscopy and de-convolved using Volocity. Scale bar: 20 µm. (**B**) Wild-type P5 whole-mount retina was reacted with antibodies to laminin γ3 chain (red) and CD31 (green). A *z*-stack was created from 0.5 µm steps using Volocity software (v. 5.4.1) and a three-dimensional image was created in Volocity and rotated to reveal the expression laminin γ3 chain around the vascular branch points (arrows). (**C**,**E**) Wild-type P5 and P7 whole-mount retinas were reacted with antibodies to CD31 to analyze the vascular branching pattern and tip cells at the vascular front. A regular branching array was observed in the wild-type retina. (**D**,**F**) *Lamc3*$^{-/-}$ P5 and P7 whole-mount retinas were reacted with antibodies to CD31 to analyze the vascular branching pattern and tip cells at the vascular front. The branching array is disrupted in the absence of the laminin γ3 chain. Scale bar: in F, 40 µm for C-F.

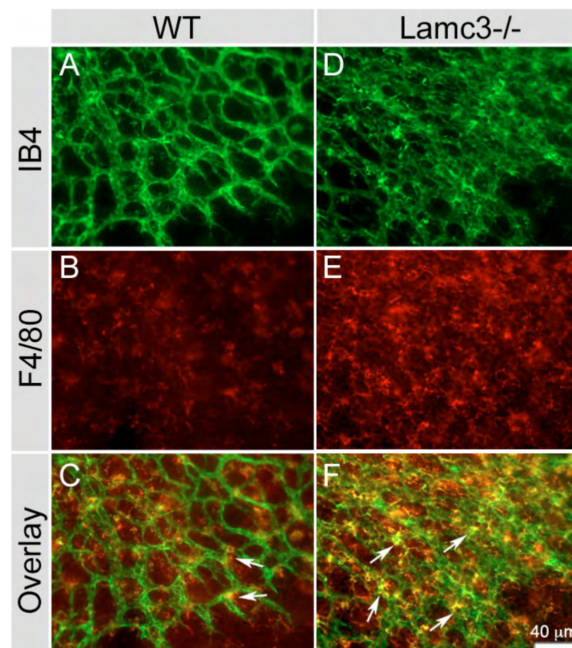

Fig. S5. Laminin γ3 chain regulates microglia-vasculature interactions. P3 whole-mount retinae from wild-type and *Lamc3*$^{-/-}$ were analyzed with isolection B4 (IB4, green) and F4/80 (a microglia-specific marker, red) to reveal blood vessel and microglia interactions. (**A**,**D**) Isolectin B4 demonstrates blood vessels as well as microglia in green. (**B**,**E**) F4/80 demonstrates only microglia in red. (**C**) Overlay of A and B demonstrates a few microglia at the vascular branch points (represented by arrows). (**F**) Overlay of D and E demonstrates more microglial associations with blood vessels at branch points (represented by arrows).