Supplemental Methods

Evaluation of the retinal vascularization and vascular remodeling.

Eyes of WT and periostin KO mice (3 mice each) at P5 were obtained, and the flat mounted retinas were stained with isolectin B4. We calculated the percentage of the retina that was vascularized relative to the total area in retinal flat mount. Additionally, we counted the number of vascular sprouts in 4 areas (360 x 270 μm) selected randomly from anterior region of retinal vascularization for each retina. The sum of the number of sprouts in the 4 areas/retina was calculated. The results of 3 samples were averaged and compared between WT and periostin KO mice.

The retinal vascular networks consisted of 3 layers. The superficial capillaries in the nerve fiber layer began to sprout from P7. The deep plexus in the outer plexiform layer forms at P12, followed by the intermediate plexus in the inner plexiform layer between P12 and P15. To evaluate the retinal vascular remodeling, eyes of WT and periostin KO mice were obtained at P6, P12, and P17, and paraffin sections were prepared. The sections were immunostained with isolectin B4 and Hoechst 33258 to detect the 3 layers of the retinal vascular networks. Retinal flat mounts from WT and periostin KO mice at P17 were also stained with isolectin B4 for the evaluation of retinal vascular networks.
Supplemental Figure.

Retinal physiological angiogenesis is normal in periostin KO mice.

A. Isolectin B4 staining of retinal flat mounts of WT (left) and periostin KO (right) mice at P5.

B. Quantification of the vascularized retinal area.

C. Growth fronts of retinal vascularization. Vascular sprouts are highlighted by dots.

D. Quantification of the number of vascular sprouts.

E. Retinal cross sections of P6, 12, and 17 mice were stained with isolectin B4 (green) and Hoechst 33258 (blue).

F. Three layer of retinal vascular network in WT and periostin KO mouse at P17. GCL: ganglion cell layer, INL: inner nuclear layer, ONL: outer nuclear layer, NFL: nerve fiber layer, IPL: inner plexiform layer, OPL: outer plexiform layer.

Scale bar: 40 μm.