SUPPLEMENTAL MATERIAL

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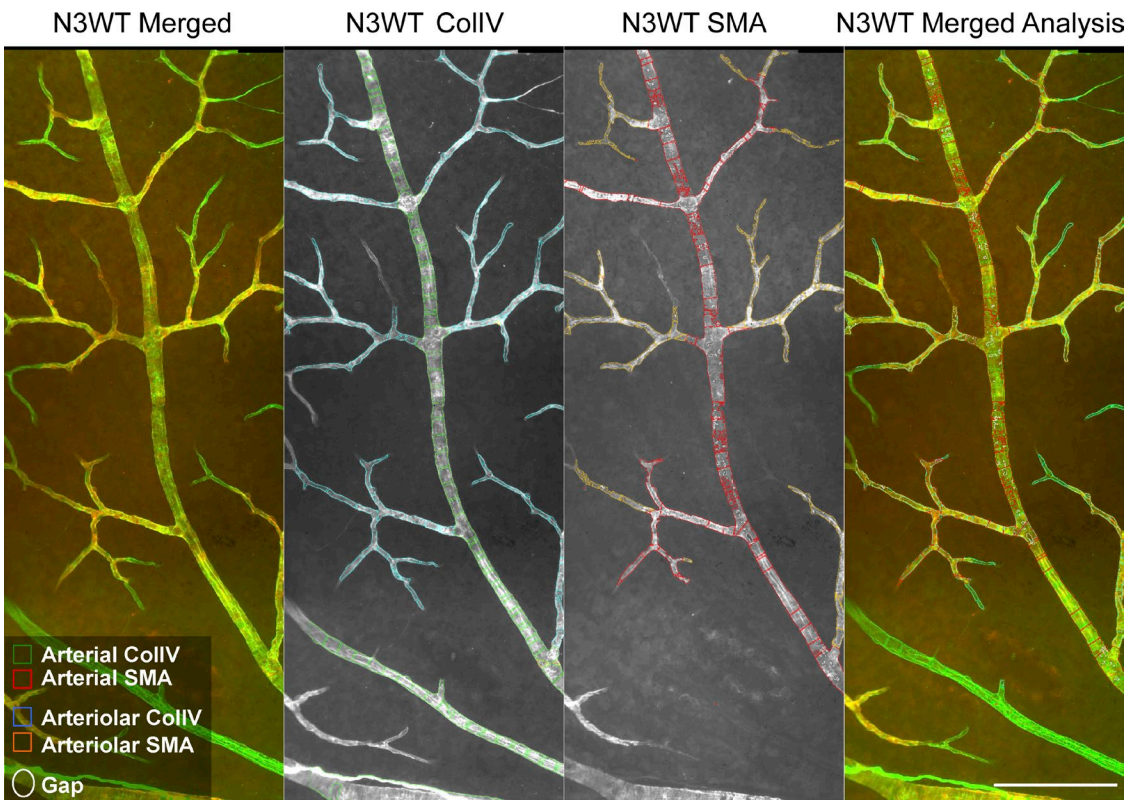

Figure S1. **Image processing of vessels via Fiji-based macro.** Images of retinal whole mounts stained with ColIV in green and SMA in red were processed. Seven images tracing a single vessel from optic nerve to periphery were stitched together using the Fiji MosaicJ macro. This was done for three vessels per retina/animal. The vascular analysis macro generates an outline of the vascularized area based on the ColIV silhouette, and is then cut into small rectangles, each of which is identified as part of the main vessel, shown as green rectangles, or as part of branching vessels, shown as blue rectangles. The squares are then superimposed onto the red, SMA binary image and determined to have, or not to have, SMA staining. Rectangles containing a value of 0, having no SMA staining are qualified as gaps. The SMA-positive areas are analyzed and qualified as the main vessel coverage, shown as red outlined areas, or as branching vessels, shown as orange outlined areas. The macro then saves parameters for each of the vessel types as an Excel spreadsheet. Bar, 200 µm. Images are representative of three mice analyzed. The results are representative of two independent experiments. Images tracing vessels were stitched together to generate the complete picture.

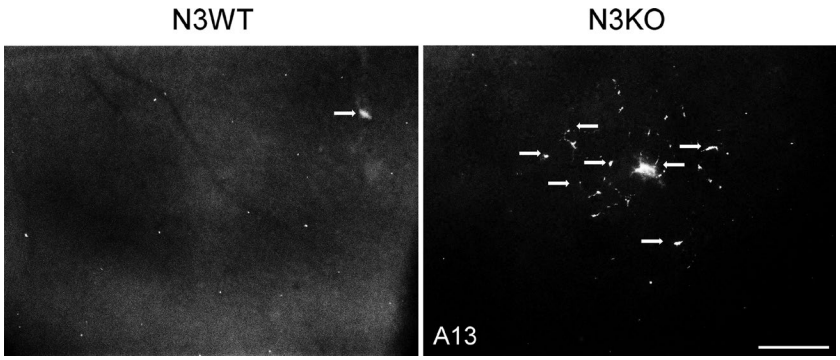

Figure S2. **Perivascular focal points of fluorescently labeled A13 antibody confirm penetration of blood–retinal barrier.** 25 µg of A13 antibody labeled with ReadiLink 647/674 were administered systemically through i.p. injection. Discrete areas of antibody penetration were observed 6 h after injection outside vessels after perfusion in N3KO mice (right), white arrows. As expected, antibody penetration was not detected outside vessels in N3WT with an intact blood–retinal barrier (left). Bar, 100 µm. Images are representative of three mice analyzed per genotype. The results are representative of two independent experiments.

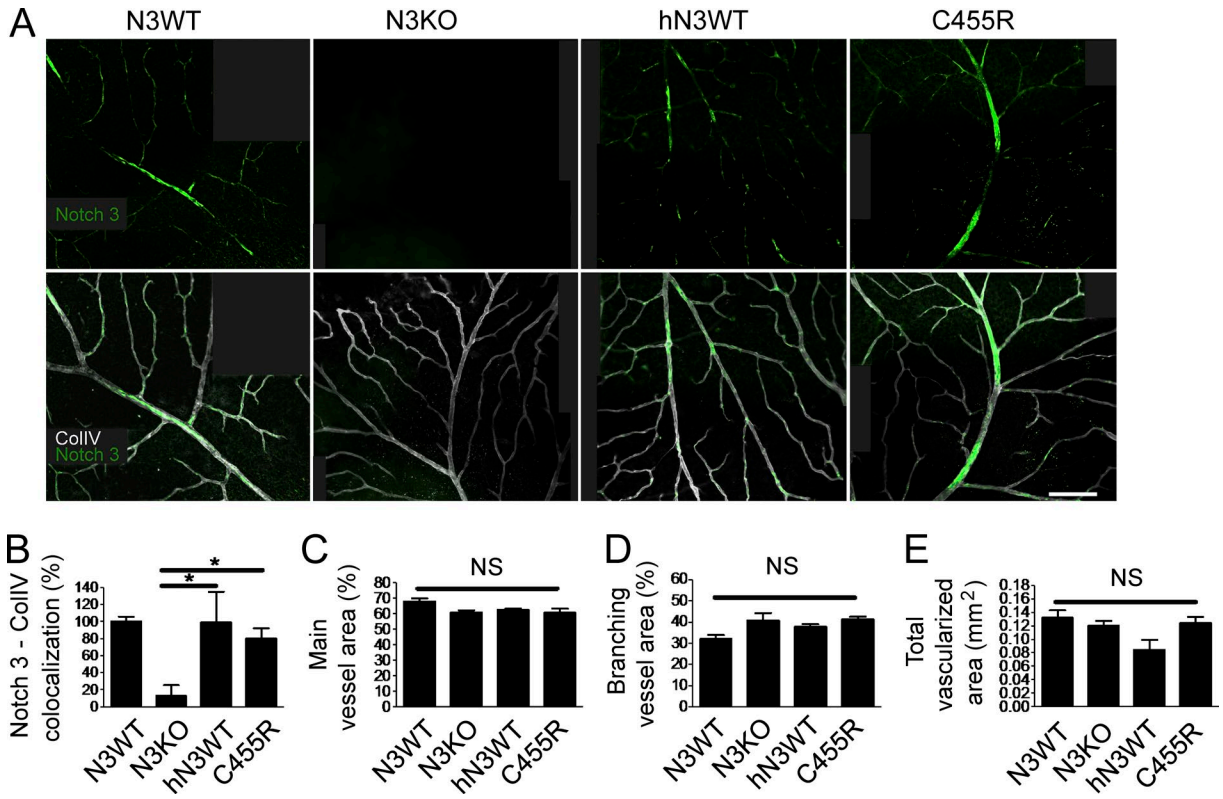

Figure S3. **Notch3 expression by SM22 promoter driven Cre-recombinase colocalizes with ColIV staining of vasculature.** (A) Specificity of Notch3 transgene driven by Cre-recombinase under an SM22 promoter was determined by immunofluorescence. Fixed retinas were stained for Notch3 (in green) and ColIV (in gray). Colocalization was determined as Notch3 and ColIV double-positive area as a percentage of the total vascularized area (ColIV). Three vessels/animal were imaged for quantification. Colocalization analysis was performed on N3WT, N3KO, hN3WT, and C455R mice. Images tracing vessels were stitched together to generate the complete picture (bar, 100 μm). (B) Percentage of colocalization was normalized to mean N3WT colocalization levels. N3KO mice lacked Notch3 staining compared with hN3WT and C455R; *, $P < 0.05$. No significant differences in normalized colocalization were observed between N3WT, hN3WT, and C455R lines. $n = 3$ for each genotype. (A and B) The results are representative of two independent experiments. Values in graphs are expressed as the means ± SEM. (C–E) Vascular density for arteries and arterioles shows no significant change. Main vessel area and branching vessel area (C and D) normalized to total retinal superficial plexus vascular density (E), measured across all strains using ColIV staining of retinal flat mounts, shows no significant differences. $n = 3$ for each genotype. The results are representative of two independent experiments. Values in graphs are expressed as the means ± SEM.