Supplement figure 1: Fluorescence arises from NG2 DsRed expression. Using the same excitation intensity and camera gain settings in two NG2 hemizygous littermates, no excitation bleed through was observed through optimized DsRed filters (see methods). A carrier littermate shows strong DsRed fluorescence signal, whereas the non-carrier littermate (expressing no DsRed fluorescence) which showed negligible excitation bleed through and eliminated the potential that excited fluorophores were auto-fluorescent structures in the retina. Images are plotted on the same grayscale range for comparison.
Supplement figure 2: Spectral properties of emitted DsRed fluorescence from a single isolated capillary cell. LSM 510 run in spectral mode allowed confirmation of the DsRed fluorophore based on the known spectra of DsRed. The fluorescence band of the AOSLO was optimized to capture fluorescence at the fluorophore peak near 599 nm. To optimize DsRed capture, we selected 2× 579Δ34 nm band pass filter sets placed serially.
Supplement figure 3: Exciting with a 514 nm laser line, DsRed was confirmed to show strong fluorescence despite absorption off its maximal peak. Top, the fluorescence from a single pericyte was measured in lambda mode of the LSM 510 meta confocal scope. Excitation laser line was matched to the same wavelength as the AOSLO Argon source. Right, fluorescence intensity as a function of wavelength shows the emitted fluorescence is indicative of DsRed, and not endogenous autofluorophores in the retina. Based on the peak emission, with sufficient separation from the excitation source, the 579±34 nm band pass filter was selected to optimize collection efficiency.
Supplement figure 4: (Legend and static image. See Movie file included.)

A retinal flat mount was imaged at multiple locations at 10-20x magnification to create a multi-field of view composite image. At least 4 focus planes were imaged for each composite to ensure that each capillary was counted. Scale bar in figure 8. A zoom view is shown at right for real resolution at this magnification power. Capturing these planes focus ensured that each capillary pericyte was counted, regardless of vascular stratification in the inner retina.
Supplement figure 5:
Pericyte counts in retinal flat mount. Left: A composite image of four focal planes shows dense NG2 cellular labeling. Middle, each cell was counted manually and identified as being located on a vessel <= 7 μm (cyan) or >7 μm (white). This process was performed for each of 4 focal planes that imaged the flat mounted retina (right). Cells showed depth specific labeling which was a function of capillary stratification and orientation of the flat mount relative to the microscope objective. This process ensured each NG2 cell was counted regardless of depth of focus. Cells were not double counted in this paradigm by an exclusion criterion which rejected identified cells with identical X-Y coordinates in adjacent focal planes.
Supplement figure 5: [Legend and static image. See Movie file included.]

A simultaneous capture of vascular motion contrast (left) and DsRed fluorescence (right). The through-focus stack was captured by averaging frames as focus was stepped through different layers in the same retinal area. Numbers in lower right show relative defocus in diopters where more negative numbers represent superficial layers (retinal nerve fiber layer) and more positive going values represent deeper retinal layers (through outer plexiform layer).
Supplement figure 7: (Legend and static image. See Movie file included.)
Pericytes (red) and vascular perfusion (gray) information from figure 4 represented in 3D rendering. In vivo, NG2 cells show close apposition to the vascular endothelium. Field size is 5 degrees of visual angle.
Supplement figure 8: (Legend and static image. See Movie Gif file included.)

Left (figure 8a). An alternating view of NG2 fluorescence (red) and vessel blood motion contrast (cyan) reveals convergence of retinal arteries into the central retinal artery. At the end of the movie sequence, a single ring of NG2 fluorescence can be seen. This represents the deep scleral portion of the central retinal artery and the NG2 positive cells that surround it. Right (figure 8b), movie shows a 3D representation of the dense cluster of cells near the optic disc. Higher density is observed at the central retinal artery than on the adjacent central retinal vein. Field of view represents 5 degrees of visual angle.
Supplement figure 9: Entire retinal flat mount composite image with an overlay of a single HRA spectralis image of in vivo NG2 cell patterning. There is reproducibility in viewing vascular patterns in vivo and ex vivo. Areas undistorted by relief cuts show good match of large vascular structures. This colocalization allowed for accurate flat mount orientation relative to the known dorsal-temporal, inferior-superior axes captured with the wide field SLO.
Supplement figure 10: (Legend and static image. See Movie file included.)

Confocal images of NG2 positive cells in ex vivo flatmount reveal unique vascular morphologies. (Top left) NG2 cells showed a banded and incomplete morphology surrounding retinal arterioles. (Top right) Stellate cell morphology was observed on cells surrounding large venules. (Bottom left) On vessels less than 7 μm in diameter, single sparse capillaries were observed to have a single round soma, with long dendritic processes surrounding the capillary tube. Consistent with pericyte morphology, there is incomplete investment in the capillary endothelium, distinguishing these cells from their endothelial cell counterparts. (Bottom right) Capillary pericytes were often observed at capillary branch points often showing a "Y-shape" appearance. Image fields are 84μm across.
alpha-smooth muscle actin (FITC) (labeling vascular smooth muscle)

NG2, CSPG4 promoter (DsRed) (vascular mural cells)

Supplement figure 11: (Legend and static image. See Movie included.)
11a. Confocal stack of arteriole stained for labeled antibody against alpha-smooth muscle actin (FITC). 11b. 3D rendering of arteriole stained for labeled antibody against alpha-smooth muscle actin (FITC) showed similar banding structures to that seen in NG2 DsRed labeled cells on larger vessels (see 11c&d. 11c Confocal stack of arteriole labeled with NG2 DsRed. 11d. 3D rendering of arteriole transgenically expressed NG2 DsRed showed similar banding structures those seen in FITC labeled alpha-smooth muscle actin (11a&b). Image fields are 84um across.
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Supplement Table 1:
Cell counts on each of 5 retinas from three mice showing average cell density per retinal eccentricity in 100 μm radial bins. Cells in capillaries (Cap), were classified as soma on vessels <7 μm in diameter. Cells on arterioles or venules (A/V) were classified as soma on vessels >7 μm. Cell counts are reported as cells/mm² rounded to the nearest integer. An “x” represents data that did not meet our inclusion criterion >50% of retinal area for that expected retinal eccentricity based on a model eye (see Retinula and Hellet 1985). Regions that failed this criterion typically had microscopic tears or folded portions of retinal tissue. A “-” symbol represents that retina was intact, but no cells were counted in that annular region. Numbers reported are from eccentricities 0-2300 μm from the optic disc which encompasses >91% of the retinal area based on an expected average retinal area of 15.6 mm². Regions >2300 μm (remaining 9% of retinal area) generally did not meet the inclusion criteria of >50% of expected retinal area.