Figure S1

A

Individual Z-plane images through the three vascular plexi at 1 μm intervals

Orthogonal Projection
6 images

superficial

1 2 3 4 5 6

intermediate

7 8 9 10 11 12

deep

13 14 15 16 17 18

B

Corresponding Z-stacks processed through Angioutil

superficial

intermediate

deep

[Images of vascular plexi at different depths and angles]
Figure S1. Confocal images of the retinal vascular beds of an untreated 10.5 month old Ins2\textsuperscript{Akita} mouse. A. Sequential images (at 1 μm intervals) through the entire retinal vasculature illustrate distinct separation between the three vascular beds. Here, six sequential frames are orthogonally projected to create the resulting stacked image for analysis (although we show each layer here, only the deep vascular bed was analyzed in the current study). B. Angiotool software calculates vascular area (yellow lines), vessel lengths (red lines), and number of junctions (blue dots), as seen more clearly in the last panel inset. Scale bars, 150 μm.