Figure S1

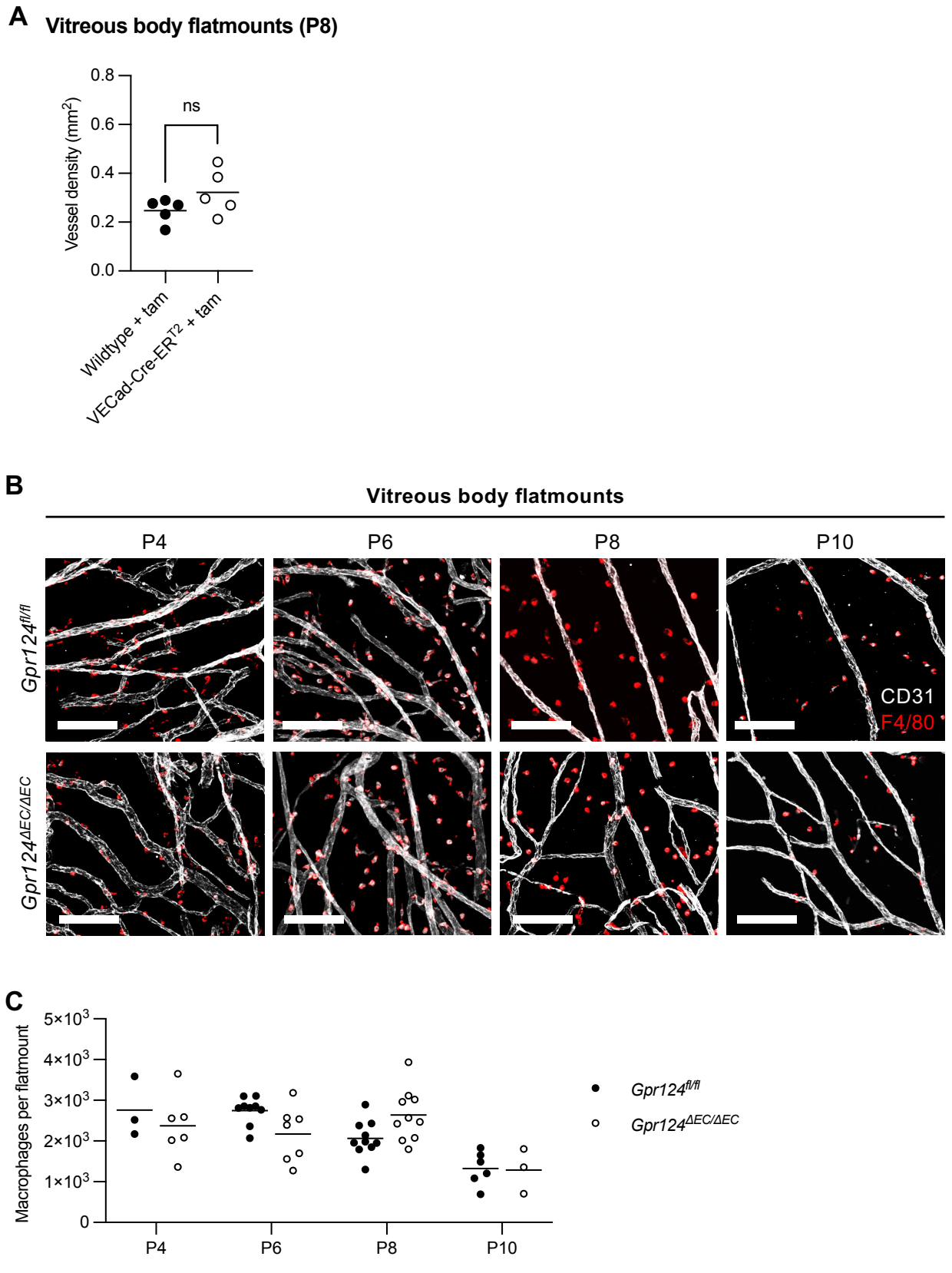

A
Vitreous body flatmounts (P8)
Vessel density (mm²)
0.8
0.6
0.4
0.2
0.0
ns
Wildtype + tam
VECad-Cre-ERT2 + tam
B
Vitreous body flatmounts
P4
P6
P8
P10
Gpr124fl/fl
Gpr124ΔEC/ΔEC
CD31
F4/80
C
Macrophages per flatmount
5×10³
4×10³
3×10³
2×10³
1×10³
0
P4
P6
P8
P10
Gpr124fl/fl
Gpr124ΔEC/ΔEC

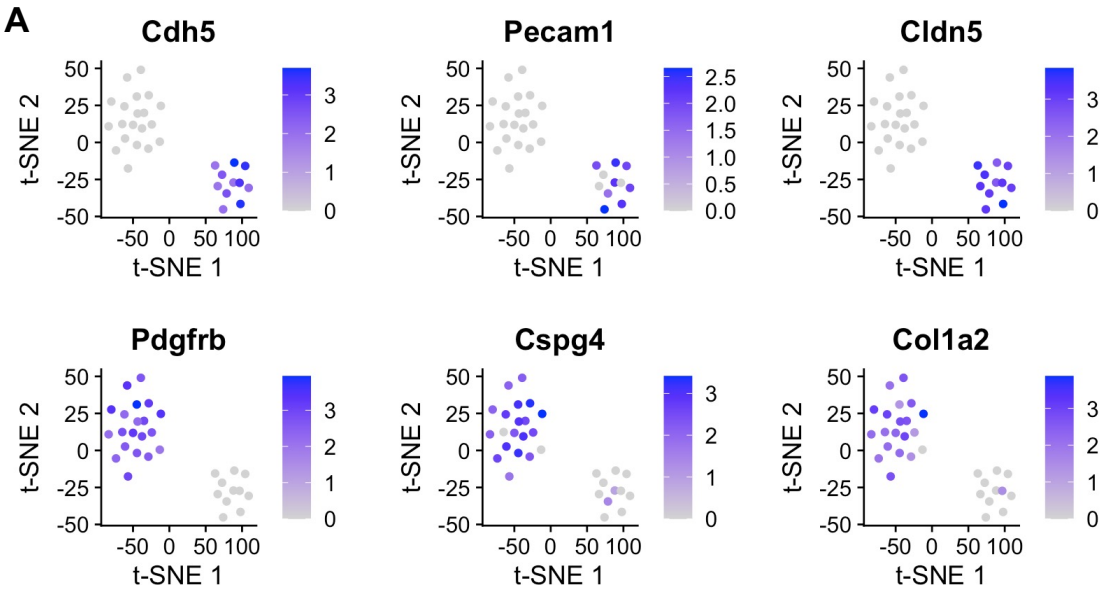
Figure S1. The VECad-Cre-ER^{T2} transgene does not affect hyaloid blood vessel regression, and endothelial GPR124 does not regulate hyaloid macrophage numbers. Related to Figure 1.

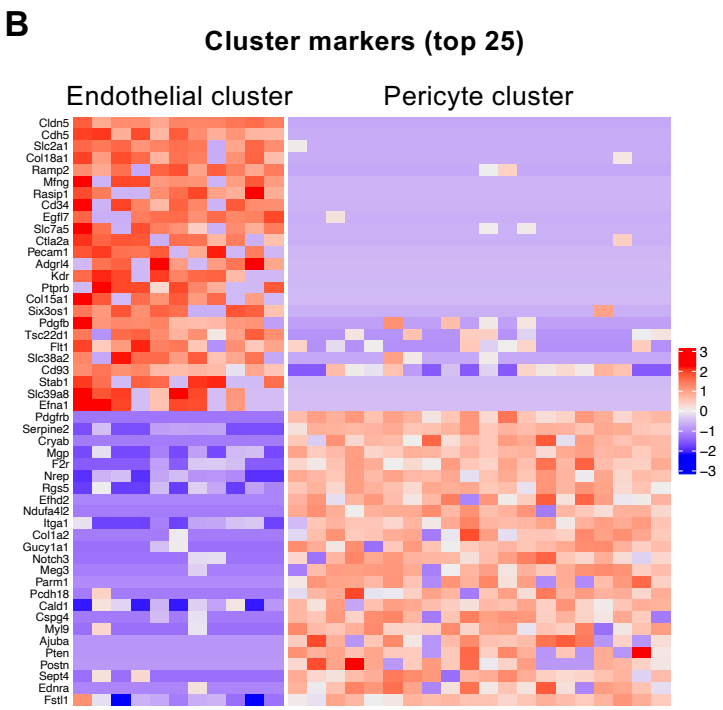
(A) Quantification of CD31 signal density (vessel density) in vitreous body flatmounts from P8 wildtype vs VECad-Cre-ER^{T2} transgenic mice treated with tamoxifen (P1-P4). Horizontal lines represent mean values from biological replicates (n ≥ 5). P, postnatal day; VECad-Cre-ER^{T2}, vascular endothelial cadherin promoter-driven Cre recombinase fused to tamoxifen-responsive estrogen receptor; tam, tamoxifen. ns, not significant.

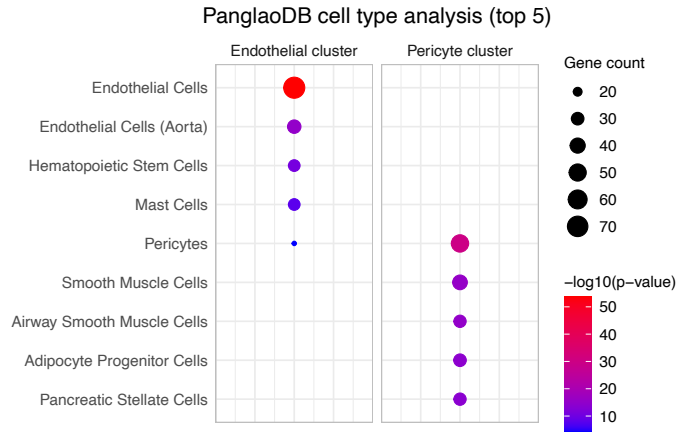
(B) Representative images of CD31 (white) and F4/80 (red) co-immunofluorescence staining of vitreous body flatmounts from *Gpr124$^{fl/fl}$* and *Gpr124$^{\Delta EC/\Delta EC}$* mice at indicated postnatal days. Scale bar: 50 µm.

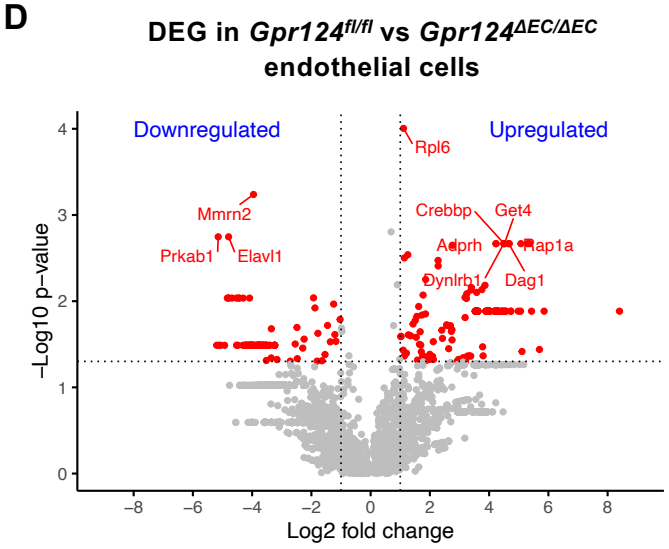
(C) Quantification of F4/80-positive macrophage number per flatmount in biological replicates (n ≥ 3) of (B). Horizontal lines represent mean values. No significant differences between genotypes were observed.

Figure S2


A
Cdh5
Pecam1
Cldn5
Pdgfrb
Cspg4
Col1a2
t-SNE 1
t-SNE 2


B
Cluster markers (top 25)
Endothelial cluster
Pericyte cluster


C
PanglaoDB cell type analysis (top 5)
Endothelial cluster
Pericyte cluster
Endothelial Cells
Endothelial Cells (Aorta)
Hematopoietic Stem Cells
Mast Cells
Pericytes
Smooth Muscle Cells
Airway Smooth Muscle Cells
Adipocyte Progenitor Cells
Pancreatic Stellate Cells
Gene count
−log10(p−value)


D
DEG in Gpr124fl/fl vs Gpr124ΔEC/ΔEC
endothelial cells
Downregulated
Upregulated
Rpl6
Mmrn2
Prkab1
Elavl1
Crebbp
Get4
Aplprh
Rap1a
Dynlrb1
Dag1
−Log10 p−value
Log2 fold change

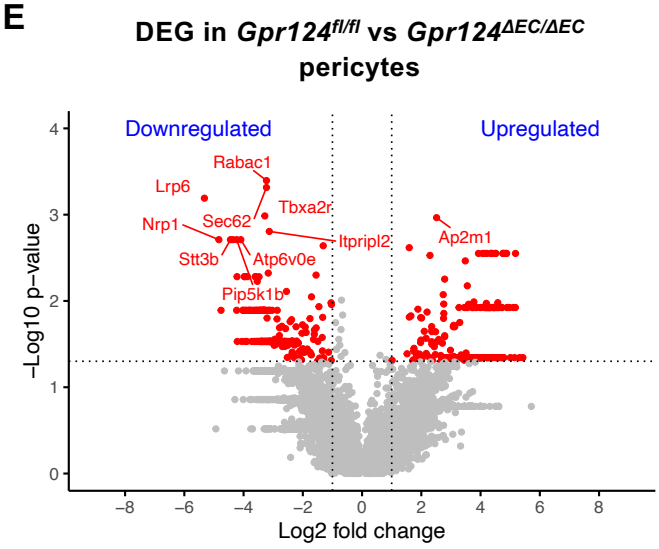

E
DEG in Gpr124fl/fl vs Gpr124ΔEC/ΔEC
pericytes
Downregulated
Upregulated
Rabac1
Lrp6
Tbxa2r
Nrp1
Sec62
Itpripl2
Ap2m1
Stt3b
Atp6v0e
Pip5k1b
−Log10 p−value
Log2 fold change

Figure S2. Single-cell RNA sequencing analysis of P6 hyaloid blood vessels. Related to Figure 5.

(A) t-SNE plots of sequenced cells displaying log-normalized mRNA expression (UMI counts) of indicated endothelial (top) and pericyte markers (bottom). t-SNE, t-distributed stochastic neighbor embedding; UMI, unique molecular identifier.

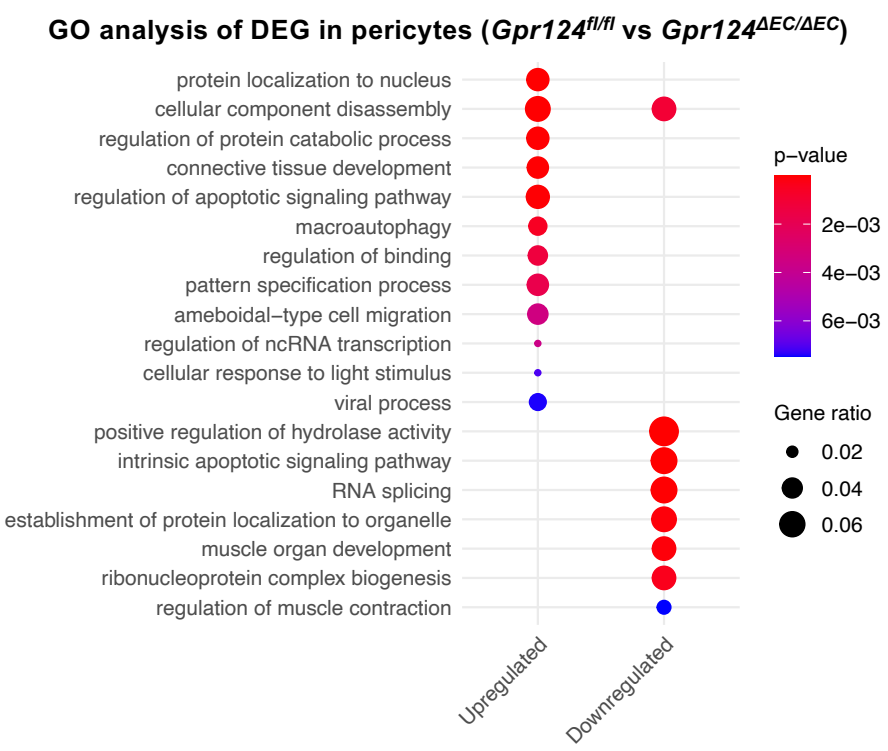
(B) Heatmap (scaled UMI counts) illustrating the top 25 differentially expressed genes (by p-value) between the endothelial and pericyte clusters. Red = upregulated, blue = downregulated or not detected.

(C) PanglaoDB cell type analysis using all markers from the indicated clusters. The top five identified cell types (by p-value) are shown. Gene count (circle size) indicates the number of matching genes.

(D-E) Volcano plots depicting differentially expressed genes (red dots) between P6 *Gpr124*$^{fl/fl}$ vs *Gpr124*$^{\Delta EC/\Delta EC}$ hyaloid endothelial cells **(D)** or pericytes **(E)**. The top ten differentially expressed genes (by p-value) are labeled. Differential expression criteria: absolute log2 fold change > 1 and $p < 0.05$. P, postnatal day; DEG, differentially expressed genes.

Figure S3

A


GO analysis of DEG in pericytes (Gpr124fl/fl vs Gpr124ΔEC/ΔEC)
protein localization to nucleus
cellular component disassembly
regulation of protein catabolic process
connective tissue development
regulation of apoptotic signaling pathway
macroautophagy
regulation of binding
pattern specification process
ameboidal−type cell migration
regulation of ncRNA transcription
cellular response to light stimulus
viral process
positive regulation of hydrolase activity
intrinsic apoptotic signaling pathway
RNA splicing
establishment of protein localization to organelle
muscle organ development
ribonucleoprotein complex biogenesis
regulation of muscle contraction
Upregulated
Downregulated
p−value
2e−03
4e−03
6e−03
Gene ratio
0.02
0.04
0.06

B

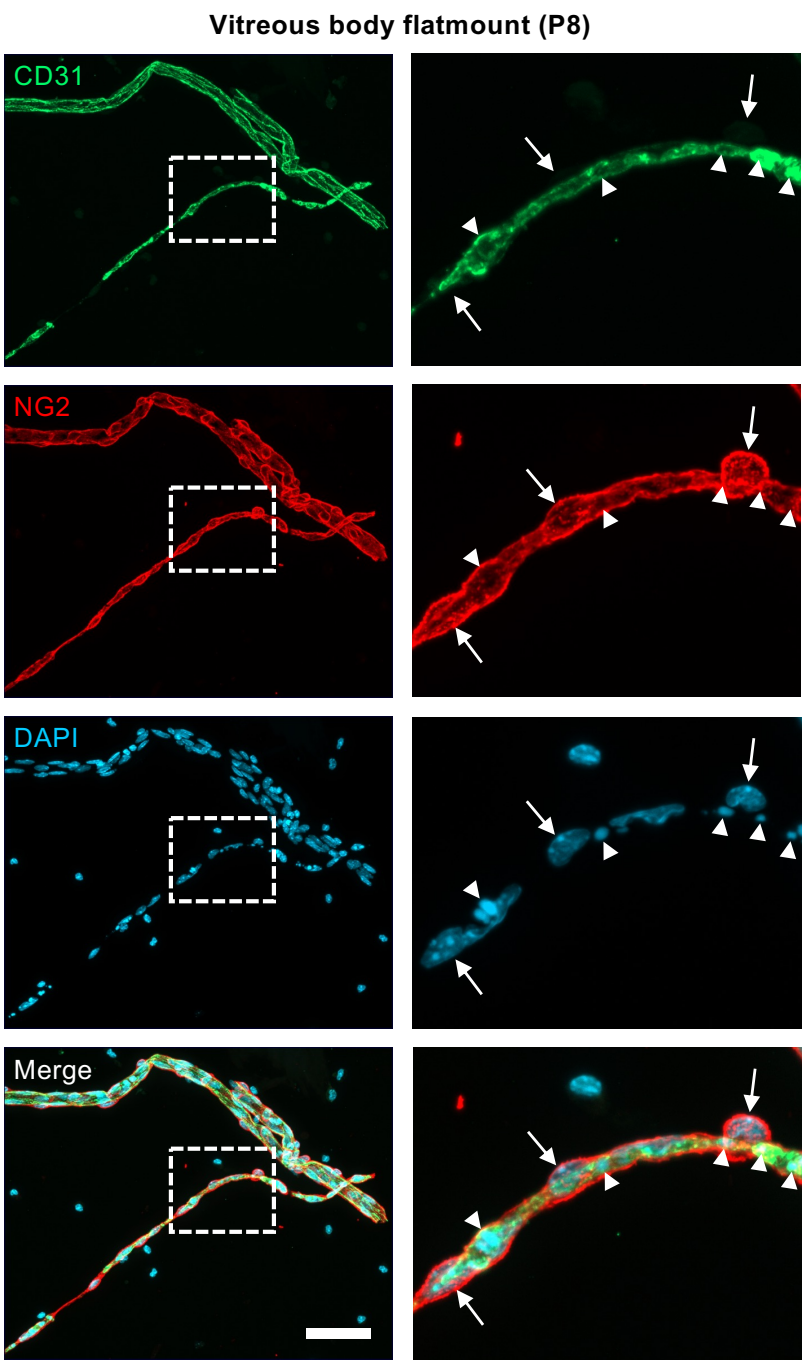

Vitreous body flatmount (P8)
CD31
NG2
DAPI
Merge

Figure S3. Analyses of hyaloid pericytes. Related to Figure 5.

(A) GO Biological Process analysis of differentially expressed genes between P6 *Gpr124*$^{fl/fl}$ vs *Gpr124*$^{\Delta EC/\Delta EC}$ hyaloid pericytes. Upregulated and downregulated genes were analyzed separately (columns). Redundant and semantically similar terms were consolidated into representative terms. Gene ratio = overlapping genes : input genes. P, postnatal day; GO gene ontology; DEG, differentially expressed genes.

(B) Representative images of CD31 (green), NG2 (red), and DAPI (blue) co-immunofluorescence staining of vitreous body flatmounts from P8 control mice (*Gpr124*$^{fl/fl}$). Dashed boxes are shown at higher magnification on the right. Arrows indicate pericyte nuclei. Arrowheads denote endothelial cell nuclei. Scale bar: 50 µm.