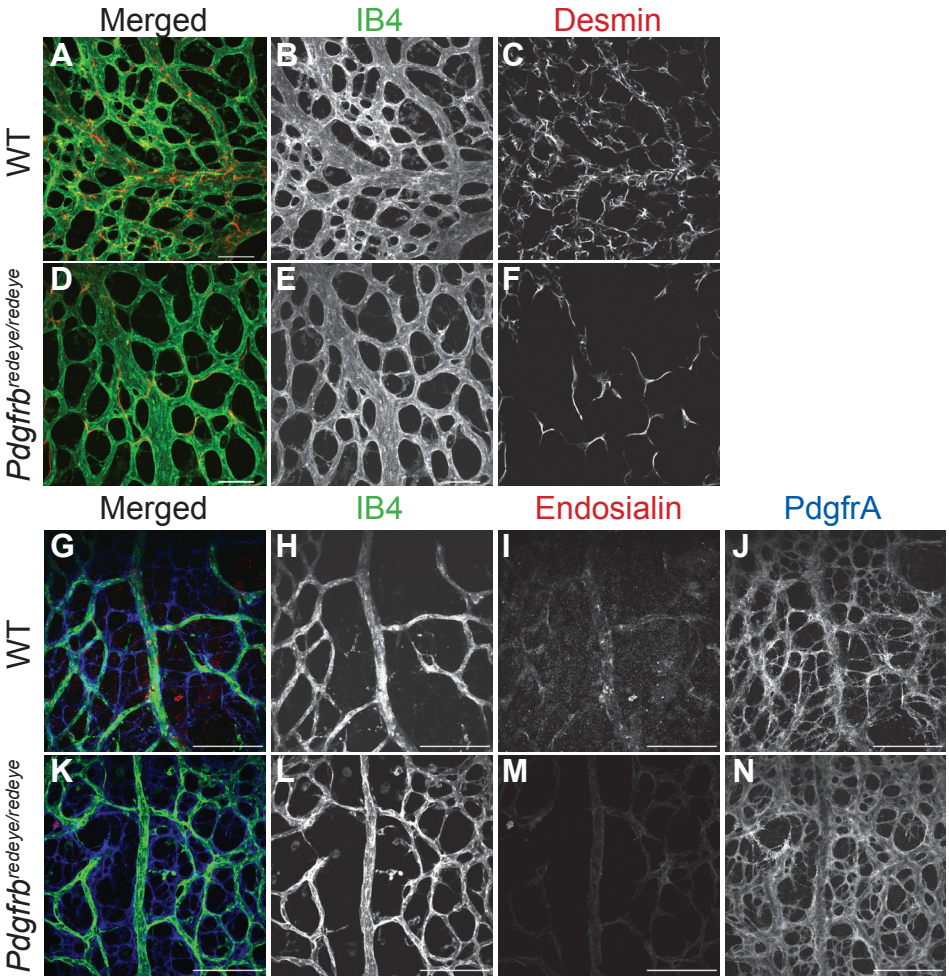
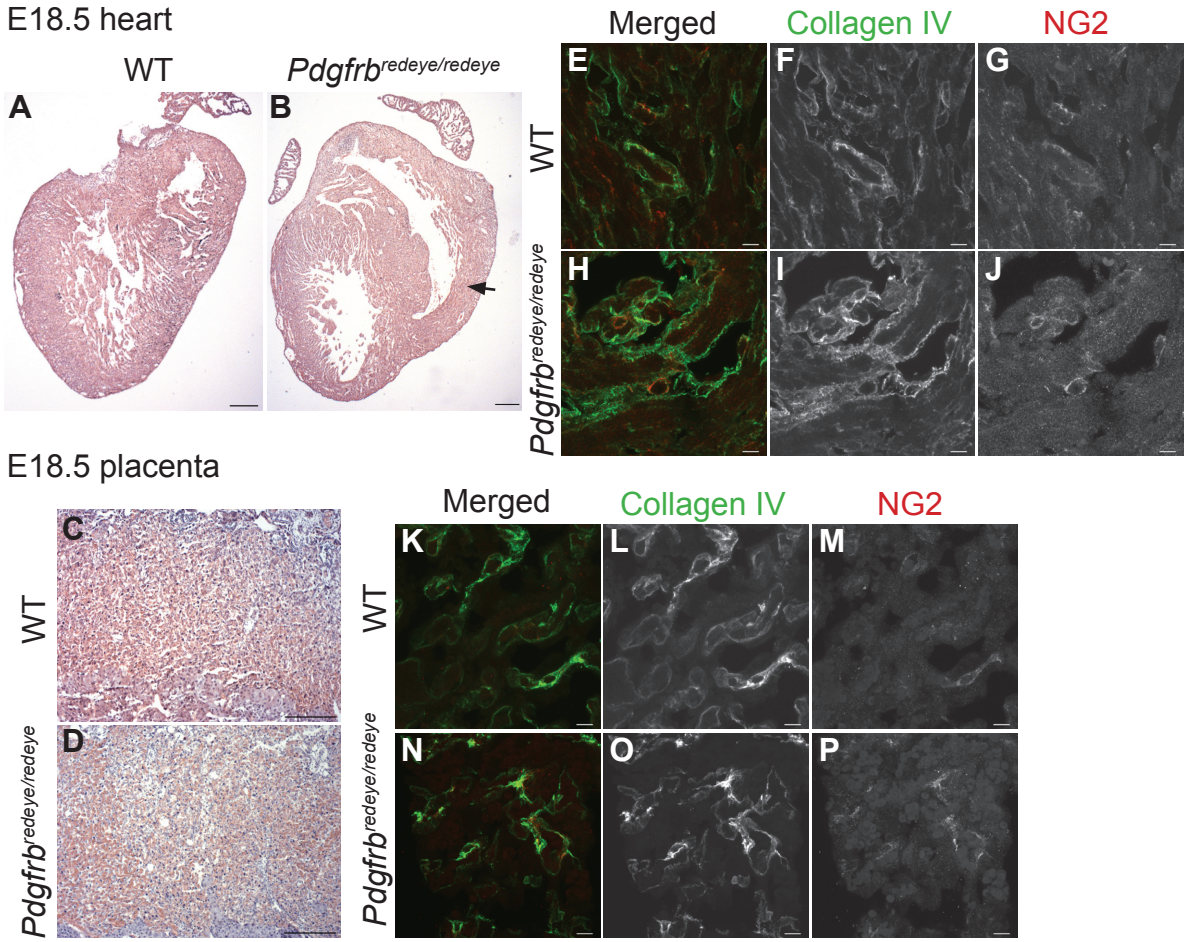


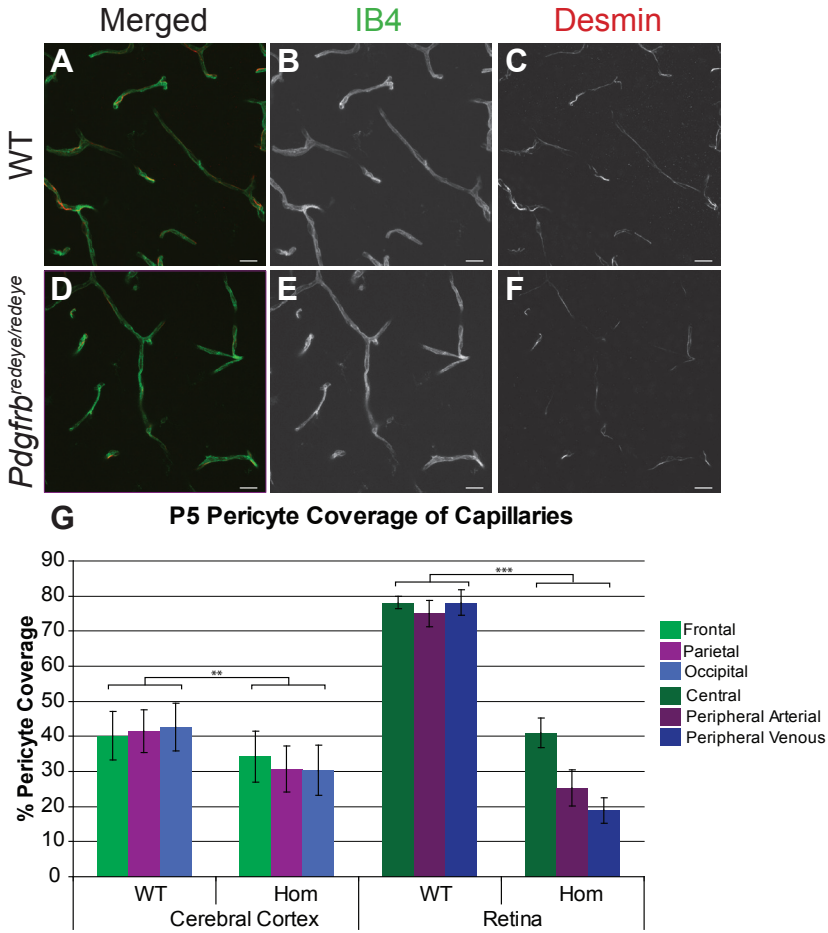
Supplementary Figure S1: Endosialin and Desmin expression are reduced in *Pdgfrb*$^{redeye/redeye}$ mutants but Pdgfrα expression is unchanged

Due to the lack of a specific pericyte marker, further markers were used to confirm the reduction in pericyte coverage in *Pdgfrb*$^{redeye/redeye}$ mutants. Representative images of P10 retinal vasculature show desmin, a marker for CNS pericytes, is expressed in WT retinae (A-C) but is reduced in *Pdgfrb*$^{redeye/redeye}$ mutants (D-F). Endosialin is a transmembrane glycoprotein expressed in immature pericytes as seen in WT (G-I) but not in *Pdgfrb*$^{redeye/redeye}$ mutants (K-M). Although Pdgfrα is expressed by astrocytes in the WT retina (J), it is able to bind PdgfB but is not upregulated as a consequence of reduced Pdgfrβ in the *Pdgfrb*$^{redeye/redeye}$ mutants (N). All scale bars: 100 μm.



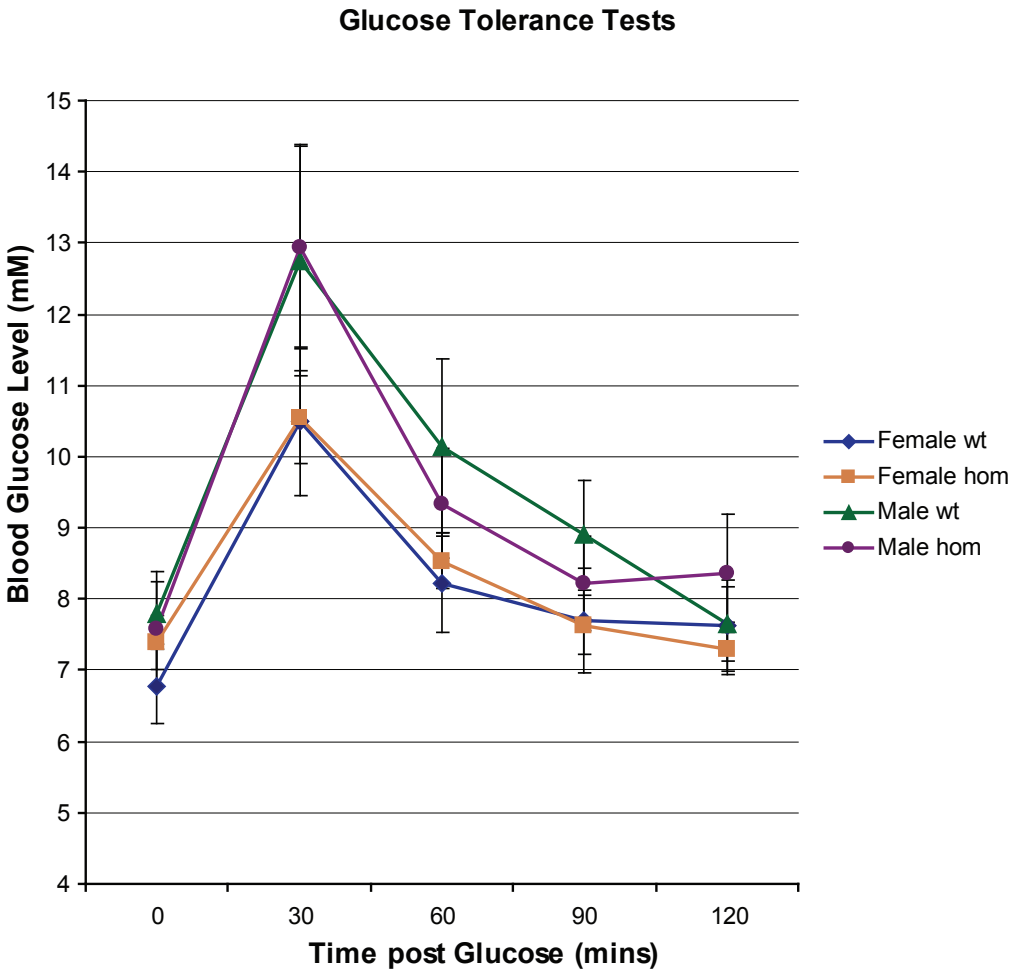
Supplementary Figure S2: Embryonic heart and placenta development are not impaired in *Pdgfrb*$^{redeye/redeye}$ mutants

To determine whether the *Pdgfrb*$^{redeye/redeye}$ mutants had a CNS-specific phenotype, we looked at embryonic E18.5 hearts and placentae which have been previously reported to be abnormal in endothelial knockouts of PdgfB. Haematoxylin and eosin staining shows that there are no gross defects in the heart (A, B), with the myocardium exhibiting normal thickness (arrow), and the histology of the placenta also revealed no gross pathology (C, D). Pericyte recruitment was analysed by staining cryosections of these organs with Collagen IV to label blood vessels and Proteoglycan NG2 to label pericytes. Pericytes were found at WT levels in both the heart (E-J) and placenta (K-P). Scale bars: 250 μm (A-D) or 10 μm (E-P).



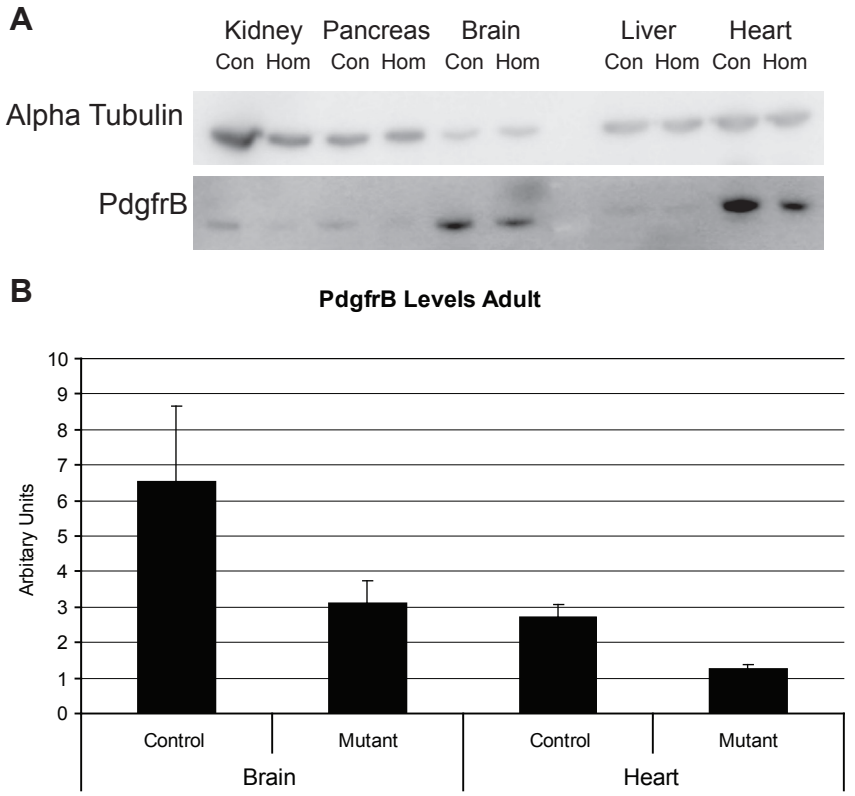
Supplementary Figure S3: Pericyte density is higher in retinal capillaries compared to cerebral capillaries

MetaMorph software was used for the quantification of pericyte coverage as determined by desmin (pericytes) and IB4 (vessels) staining in the cerebral cortex of WT (A-C) and *Pdgfrb*$^{redeye/redeye}$ P5 mice (D-F). This shows a statistically significant decrease in pericyte coverage in Homozygous animals (Hom) compared to WT (G). (SRH test $P < 0.005$, **Mann-Whitney U-test $P < 0.005$). To confirm that this lower coverage in wildtype cerebral cortex capillaries was not due to differences in staining from using desmin as a marker for pericytes rather than NG2, P5 retinae were also quantified using desmin. There is a statistically significant difference in pericyte coverage of retinal capillaries in P5 Homozygous animals (Hom) compared to WT (G) (SRH test $P < 0.001$, ***Mann-Whitney U-test $P < 0.001$).



Supplementary Figure S4: The *Pdgfrb*$^{redeye/redeye}$ mutants are not diabetic

In order to confirm that the retinopathy in the redeye mutants was not caused by diabetes we performed glucose tolerance tests in animals aged 8 weeks. Neither females nor males are intolerant to glucose and are able to clear excess glucose from their system. Error bars: standard deviation of the mean.



Supplementary Figure S5: Pdgfrβ expression in Adult Organs

In order to confirm that the retinopathy in the *redeye* mutants was not caused by diabetes we performed glucose tolerance tests in animals aged 8 weeks. Neither females nor males are intolerant to glucose and are able to clear excess glucose from their system. Error bars: standard deviation of the mean.

Antibody/ stain	Supplier	Dilution used
Flourescein-conjugated *Griffonia simplicifolia* Isolectin B4	Vector Laboratories, UK	1:500
Collagen IV	AbD Serotec, UK	1:500
ASMA	Abcam, UK	1:500
Proteoglycan NG2	Millipore UK Ltd, UK	1:200
Podocalyxin	R & D Systems, UK	1:100
Pdgfrβ	Abcam, UK	1:500
Desmin	Abcam, UK	1:500
Endosialin	MacFadyen et al., 2005	1:500
Claudin 5	Invitrogen, UK	1:500
Angiopoietin 1	Abcam, UK	1:200
ZO1	Invitrogen, UK	1:100
Brn3	Santa Cruz, Germany	1:200
Pdgfrα	BD Biosciences, UK	1:200
F4/80	AbD Serotec, UK	1:500
secondary Alexa-488	Molecular Probes, Invitrogen, UK	1:500
secondary Alexa-594	Molecular Probes, Invitrogen, UK	1:500
secondary Alexa-647	Molecular Probes, Invitrogen, UK	1:500

Supplementary Table S1: Antibodies and Stains used for immunofluorescence

Antibody	Supplier	Dilution used
Alpha tubulin	Abcam, UK	1:5000
Pdgfrβ	Source Bioscience, UK	1:10000
HRP-conjugated secondary antibodies	Fisher Scientific, UK	1:5000

Supplementary Table S2: Antibodies used for immunoblotting

Comparison	Figure	Primary Statistical Test	Post Test
Retinal pericyte coverage at P5 & P10	2K-L	SRH (Factors: genotype and region)	MWU Tests + Bonferroni Correction
No. of Avascular regions/ mm^2	5E	ANOVA (Factors: genotype and region)	TukeyHSD
No. of Branchpoints/ mm^2	5F	ANOVA (Factors: genotype and region)	TukeyHSD
RGC counts	6M	Kruskall-Wallis test	MWU Tests + Bonferroni Correction
Cerebral cortex and retinal pericyte coverage at P5 by Desmin staining	Supp. Fig. S3	SRH (Factors: genotype and region)	MWU Tests

Supplementary Table S3: Statistical Analysis Undertaken

The normality of the data analysed was assessed by Shapiro-Wilk test and by visual inspection and subsequently the appropriate parametric or non-parametric tests were performed.

SRH**:** two-way Scheirer-Ray-Hare (SRH) extension of the Kruskal-Wallis rank sum test, MWU Tests: pairwise Mann-Whitney U-tests. ANOVA: two-way ANalysis Of VAriance, TukeyHSD: pairwise Tukey's Honestly Significant Difference. Prior to analysis the avascular regions /mm^2 and branchpoints/ mm^2 data was root and probit transformed respectively.