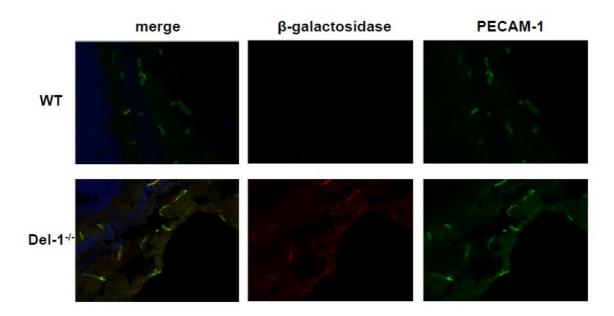
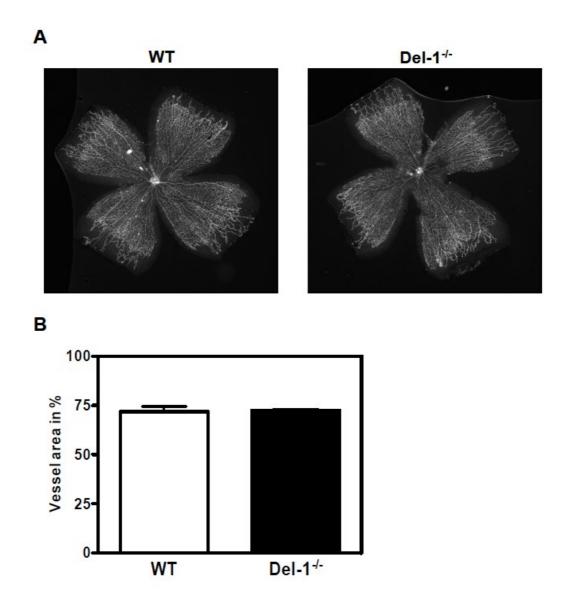
Supplementary Figures to Klotzsche – von Ameln, Cremer, et al. "Endogenous developmental endothelial locus-1 limits ischaemia-related angiogenesis by blocking inflammation" (https://doi.org/10.1160/TH16-05-0354)

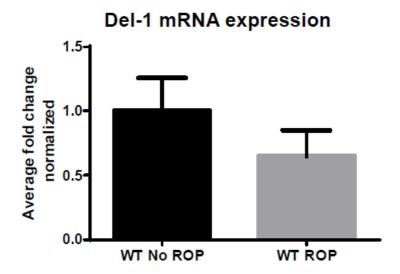


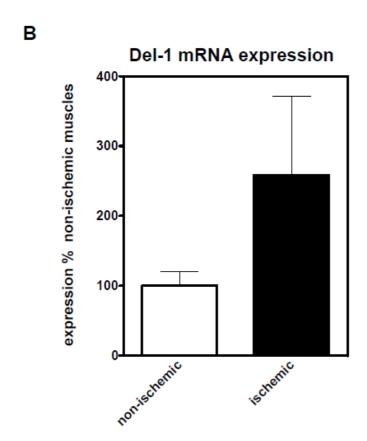
Suppl. Figure 1: Expression of Del-1 in the mouse retina. Representative images of Del-1 expression in the retina, which was assessed by β -galactosidase staining (red) in Del-1-LacZ-knock in mice. The green fluorescence indicates PECAM-1+ vessels. Retinal sections from WT mice were used as control and stained negative for β -galactosidase. Images were collected by confocal microscopy (40X objective).



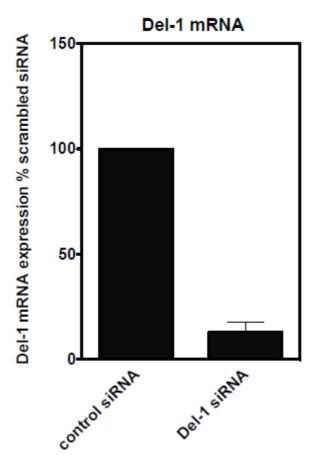
Suppl. Figure 2: Del-1–deficiency does not affect physiological developmental angiogenesis of the retina. A and B) Del-1 deficiency does not affect normal physiological vascular development of the retina (P6). A) Representative images of retinal wholemounts stained with Lectin; B) quantification of vessel area in % of total retinal area in WT and Del-1-/- mice (n=4-5 per group).

Α

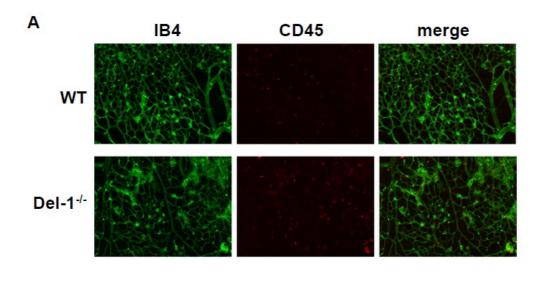


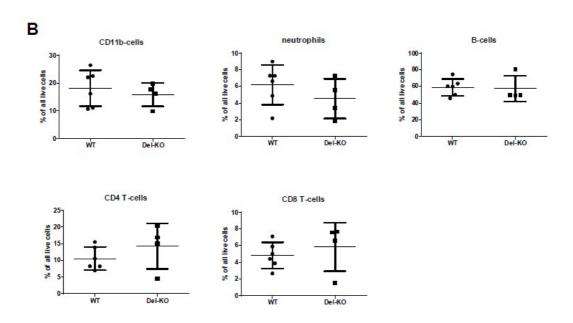


Suppl. Figure 3: Regulation of Del-1 expression in the ischemic mouse retina and hind limb. A) Expression of Del-1 mRNA in retinae from WT mice on P17 in the ROP model compared to Del-1 mRNA expression in retinae from WT control mice (kept at normal room air) was assessed by qPCR. B) Expression of Del-1 mRNA in ischemic and non-ischemic muscles from WT mice 4 days after the induction of hind limb ischemia was assessed by qPCR.



Suppl. Figure 4: siRNA-mediated Del-1 knockdown. Expression of the Del-1 mRNA 48 h after transfection with control siRNA or Del-1-siRNA in HUVEC was assessed by qPCR.





Suppl. Figure 5: Del-1-deficiency increases leukocyte infiltration of the retinas in the ROP model and no effect of Del-1-deficiency on peripheral blood leukocyte numbers. A) Representative images of infiltration of ischemic retinas on postnatal day 15 of the ROP model with CD45+ leukocytes (red fluorescence) in WT and Del-1-/- mice are depicted. Green fluorescence indicates lectin staining (IB4) of vessels. B) Flow cytometry analysis for different leukocyte subpopulations was performed in peripheral blood from WT and Del-1-/- mice on P17 in the ROP-model (n=4-6 mice per group). C) Flow cytometry analysis for different leukocyte subpopulations was performed in peripheral blood from WT and Del-1-/- mice 4 days after induction of hind limb ischemia (n=4-5 mice per group).