

SUPPLEMENTAL FIGURE LEGENDS

Figure S1

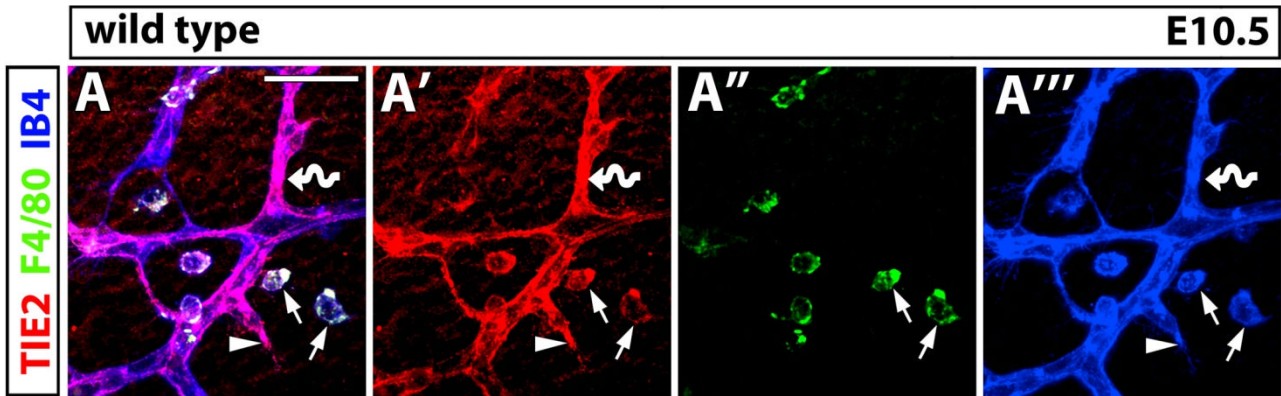


Fig. S1. *TIE2 is expressed by hindbrain tissue macrophages.* (A) An E10.5 wild type hindbrain was labelled for TIE2 (red), F4/80 (green) and IB4 (blue); single channels are shown in (A'-A'''). Arrowheads indicate examples of tip cells, curved arrows point to vessel segments composed of stalk cells; arrows indicate examples of tissue macrophages expressing NRP1. Scale bar: 50 μ m.

Figure S2

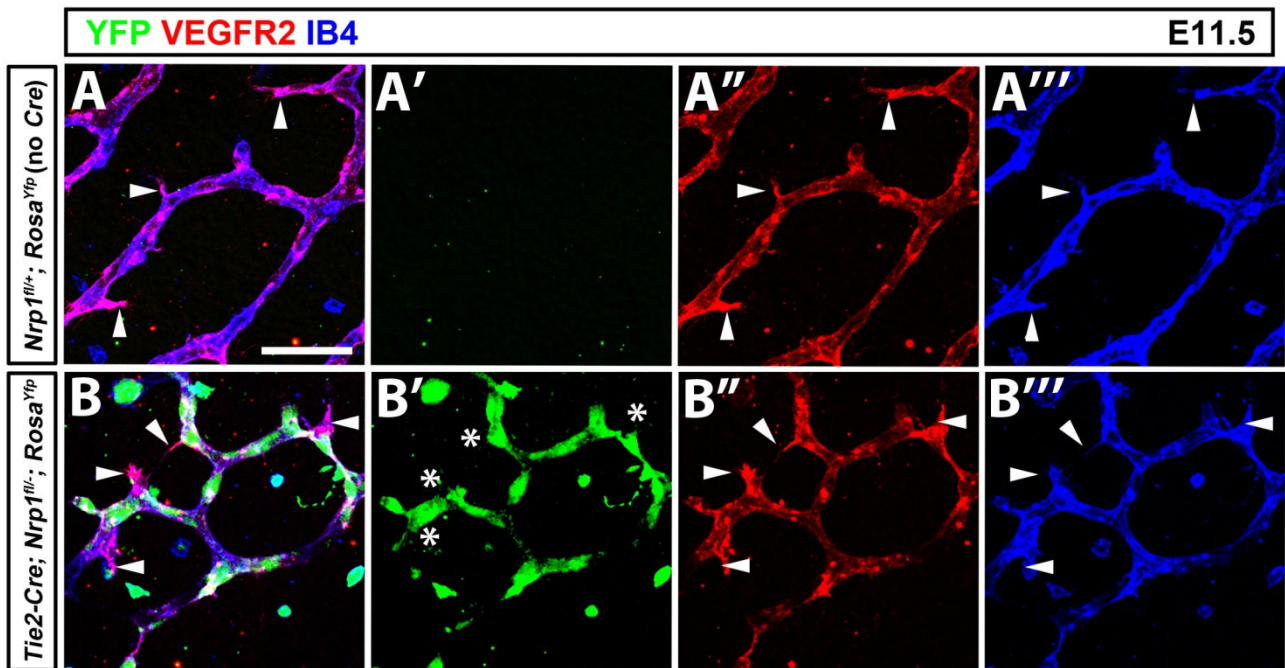


Fig. S2. *Tie2-Cre;Nrp1^{fl/-}* mutant tip cells retain *VEGFR2* expression. (A,B) VEGFR2 (red), YFP (green) and IB4 (blue) immunofluorescence staining of littermate E11.5 hindbrains of the indicated genotypes; single channels are shown in the adjacent panels (A'-A''' and B'-B''', respectively). Arrowheads indicate examples of tip cells, asterisks indicate examples of tip cells lacking expression of the YFP reporter. Scale bar: 50 μ m.

Figure S3

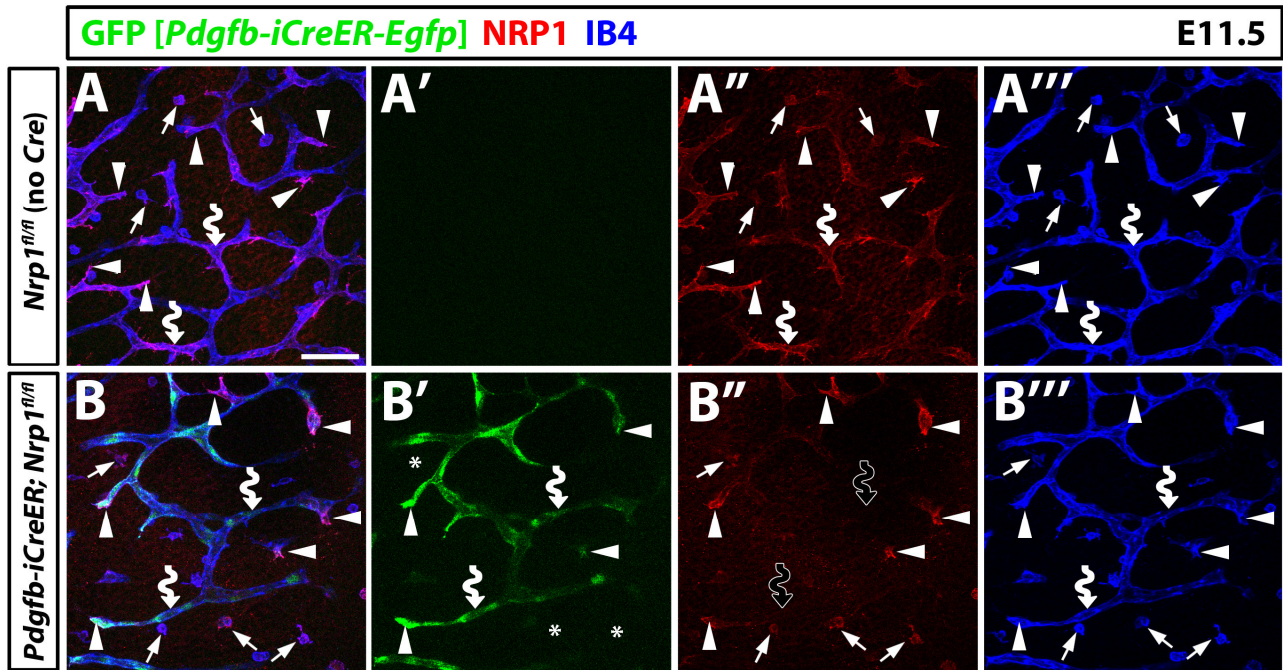


Fig. S3. Endothelial tip cells in *Pdgfb-iCreER-Egfp;Nrp1^{fl/fl}* mutant hindbrains retain NRP1 expression. (A,B) GFP (green), NRP1 (red) and IB4 (blue) immunofluorescence staining of tamoxifen-induced E11.5 littermate hindbrains of the indicated genotypes (*Pdgfb-iCreER-Egfp* was abbreviated to *Pdgfb-iCreER*); single channels are shown in the adjacent panels (A'-A''' and B'-B''', respectively). Arrowheads indicate examples of tip cells and curved arrows examples of stalk cells (note lack of NRP1 expression in stalks cells indicated with black curved arrows in B''). Arrows indicate examples of tissue macrophages expressing NRP1 and IB4, asterisks indicate lack of YFP expression in tissue macrophages. Scale bar: 50 μ m.

Figure S4

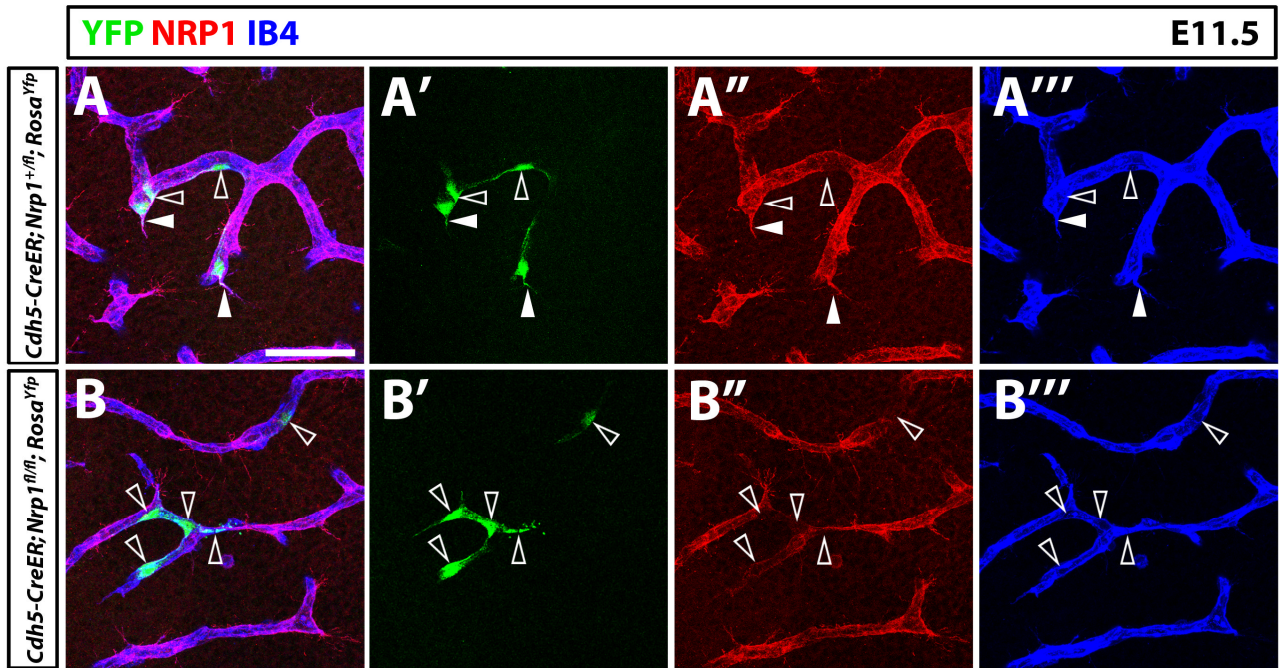


Fig. S4. *YFP-expressing endothelial cells in tamoxifen-induced *Cdh5-CreER* hindbrains.* (A,B) YFP (green), NRP1 (red) and IB4 (blue) immunofluorescence staining of tamoxifen-induced E11.5 littermate hindbrains of the indicated genotypes; single channels are shown in the adjacent panels (A'-A''' and B'-B''', respectively). Solid arrowheads indicate examples of YFP-positive tip cells, clear arrowheads examples of YFP-positive stalk cells. Note that YFP-positive cells in the control hindbrain (A', A'') express NRP1 and can assume both tip and stalk cell positions. In contrast, YFP-positive cells in the hindbrain with homozygously floxed *Nrp1* (B', B'') lack NRP1 and adopt only stalk cell positions. Scale bar: 50 μ m.