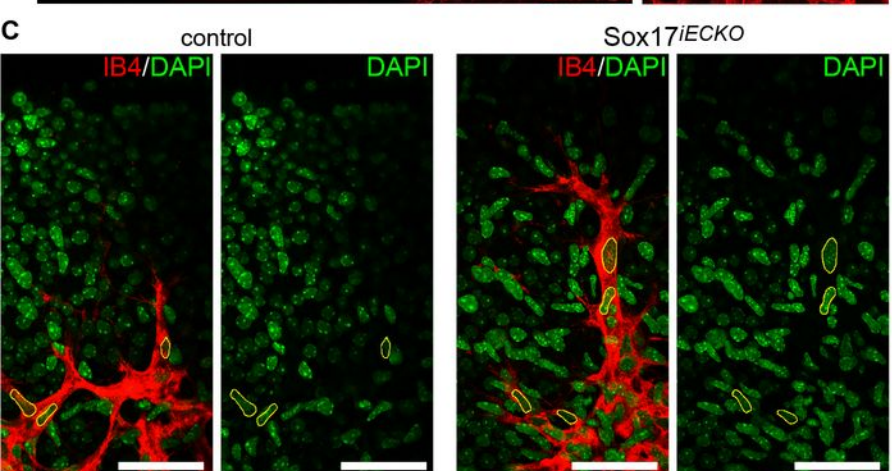
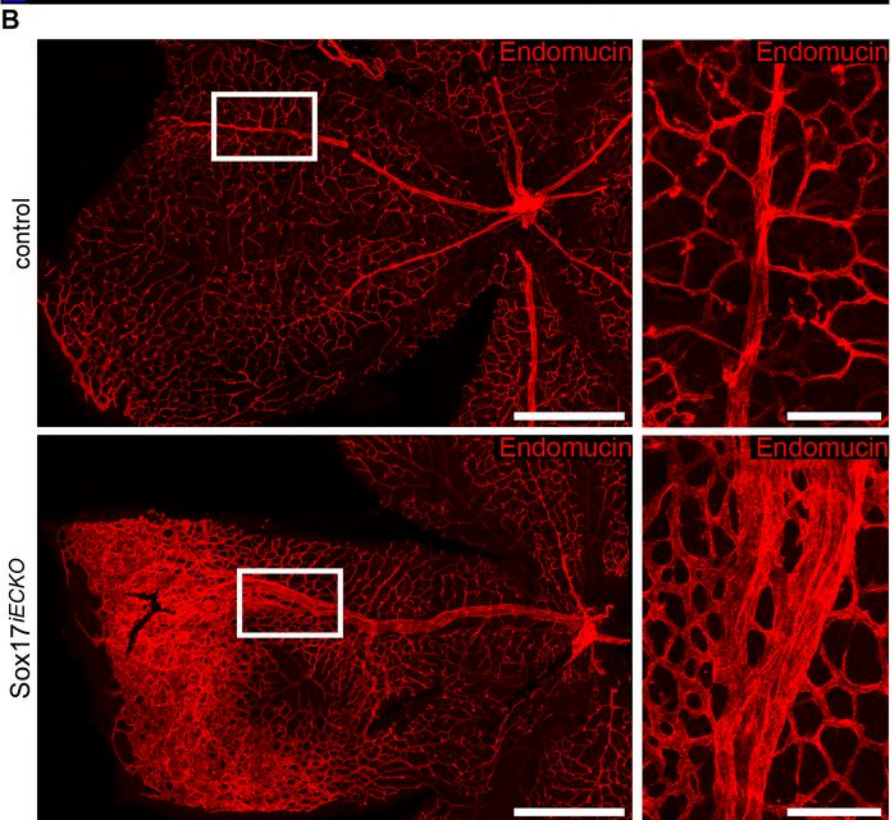
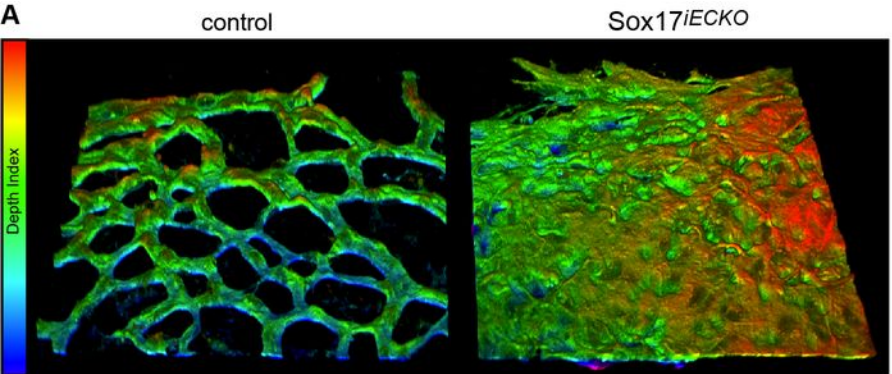


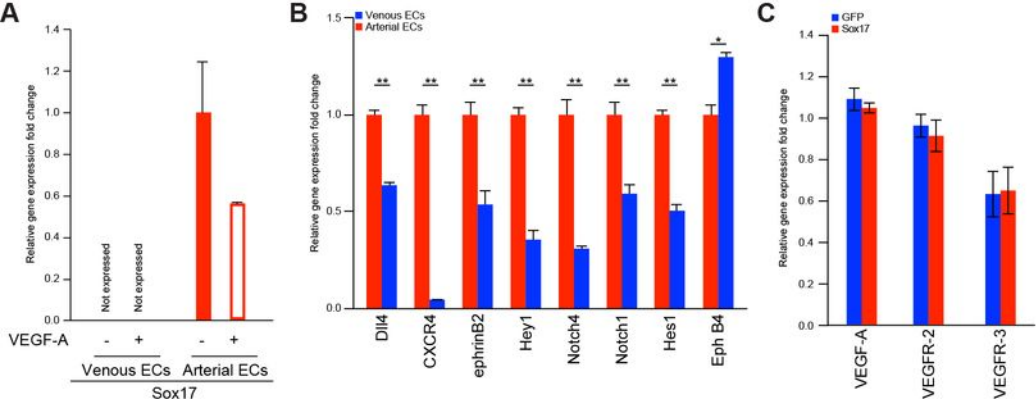
Supplementary Figure S1. Sox17 is required for arterio-venous specification

(A) Whole mount staining of E10.5 ephrin-B2/ β -gal reporter embryo. The images show co-localization of Sox17 (red) and the arterial marker ephrin/ β -gal (green) in the intersomitic vessels. (B) Quantification of nuclear fluorescence intensity of Sox17 staining in arteries (red), veins (blue) and capillaries (green) of developing retina. Data are represented as mean fluorescence value \pm SD, $n = 40$. (C, D) Sox17 expression in arteries and veins. Levels of Sox17 expression in human endothelial cells from veins (HUVECs) and arteries (HAECs) (C) and freshly isolated endothelial cells from mouse mesenteric arteries and veins (D). qRT-PCR analysis show that arteries present significantly higher levels of Sox17. Data are mean \pm SD, $n = 4$ (E) Altered vascular morphology in Sox17^{ECKO} embryos. Whole mount view of E9.5 control and Sox17^{ECKO} embryo and yolk sac (left panels) and whole mount staining with PECAM-1 is shown in yolk sac (right panels). (F) Images show E10.5 control and Sox17^{ECKO} embryos (left panels) and whole mount staining with PECAM-1 is shown in embryos head and intersomitic vessels (right panels). (G) Isolectin B4 (green) and Sox17 (red) staining of retinal vasculature in control (left) and Sox17^{ECKO} (right) pups showing absence of Sox17 staining in Sox17^{ECKO} arteries. Scale bar: 200 μ m in E and F; 100 μ m in A and G. * $p < 0.01$, two-tailed t-test assuming unequal variances.



Supplementary Figure S2. Vascular morphology of the retinal vasculature in controls and Sox17^{iECKO} new born mice.

(A) Depth coded stacks of retinal angiogenic front of control (left) and Sox17^{iECKO} (right). (B) Confocal images of whole mount control (upper panel) and Sox17^{iECKO} (lower panel) retinas stained with endomucin antibody. The lumen of veins was frequently enlarged (compare bottom and upper panels) and vascular lacunae could be observed at the venous front. (C) Single confocal plane from Fig. 2E showing an example of the analysis of vascular sprouts stained with Isolectin B4 (red) and DAPI (green) in control and Sox17^{iECKO} retinas. Tip cells nuclei are surrounded in yellow. Scale bar: 500µm in left panels of B, 100µm in right panels of B and in C.



Supplementary Figure S3. Sox17 expression in cultured endothelial cells.

(A-C) qRT-PCR analysis of cultured endothelial cells derived from control mice and FACS-sorted for expression of venous or arterial markers (CD45-/PECAM-1+/CXCR4- for veins and CXCR4+ for arteries). (A) Activation of venous and arterial endothelial cells by VEGF-A (80ng/ml, for 8 hr) did not increase Sox 17 expression, n=3. (B) Several arterial markers and members of the Notch signaling pathway are downregulated in venous endothelial cells while the venous marker EphB4 is increased, n=4. (C) qRT-PCR analysis of *VEGF-A*, *VEGFR-2* and *VEGFR-3* from cultured GFP or Sox17 infected venous endothelial cells did not show any significant difference in expression of these genes, n=4. Data are presented as mean \pm SD. * $p < 0.05$; ** $p < 0.01$, two-tailed t-test assuming unequal variances.

Supplementary Table S1– List of TaqMan probes used in qRT-PCR

<i>Gene Symbol</i>	<i>NCBI Gene Ref</i>	<i>Assay ID Target</i>
<i>Jag1</i>	<i>NM 013822</i>	<i>Mm00496902 ml</i>
<i>Notch4</i>	<i>NM 010929</i>	<i>Mm00440525 ml</i>
<i>Notch1</i>	<i>NM 008714</i>	<i>Mm00435245 ml</i>
<i>Dll4</i>	<i>NM 019454</i>	<i>Mm00444619 ml</i>
<i>Hey1</i>	<i>NM 010423</i>	<i>Mm00468865 ml</i>
<i>Hey2</i>	<i>NM 013904</i>	<i>Mm00469280 ml</i>
<i>Hes1</i>	<i>NM 008235</i>	<i>Mm00468601 ml</i>
<i>Efnb2</i>	<i>NM 010111</i>	<i>Mm00438670 ml</i>
<i>Ephb4</i>	<i>NM 010144</i>	<i>Mm00438750 ml</i>
<i>COUP-TFII</i>	<i>NM 183261.3</i>	<i>Mm00772789 ml</i>
<i>Sox17</i>	<i>NM 011441</i>	<i>Mm00488363 ml</i>
<i>Sox7</i>	<i>NM 011446</i>	<i>Mm00776876 ml</i>
<i>Sox18</i>	<i>NM 009236</i>	<i>Mm00656049 gh</i>
<i>Flt4</i>	<i>NM 008029.3</i>	<i>Mm00433337 ml</i>
<i>VEGF-A</i>	<i>NM NM 009505.4</i>	<i>Mm00437304 ml</i>
<i>VEGFR2/KDR</i>	<i>NM 010612.2</i>	<i>Mm00440099 ml</i>
<i>CXCR4</i>	<i>NM 009911.3</i>	<i>Mm01292123 ml</i>
<i>Dll1</i>	<i>NM 007865</i>	<i>Mm00432841 ml</i>

Supplementary Table S2– List of primers used in ChIP

Dll-4 (Region A)	5'-CCAGTAATCCTGCCCACTTG-3'	5'-TCGGCTTTTCCTCATACCTC-3'
Dll-4 (Region B)	5'-TCTCTCCTAGCCCTGGGATT-3'	5'-GTCAGAGTGGATTCCCCAGA-3'
Dll-4 (Region C)	5'-CGCTCACAACTCATGTTTC-3'	5'-GTGGTAGGGGGAAGTGGAAC-3'
Dll-4 (Region D)	5'-ACGAGCCAGGAGGGAGTG-3'	5'-GGTAGGCGTGTACCTCAAG-3'
Dll-4 (Region E)	5'-GTGCTGGGACTGTAGCCACT-3'	5'-CCGCTACTGAAACCTGCTTG -3'
Notch1	5'-GTGGGCTCTGAGGACAAGAG-3'	5'-TGTGTGTGTTGGAGGGTTTC-3'
Dll1	5'-GGGAGTCTTTCAAACATGTGC-3'	5'-GTGCGCTCAGCTGTATGGTA-3'
Notch4	5'-TCAACTAGGGCCTGCTATGG-3'	5'-CCCCACCCATTTTGTTAAT-3'