Supplementary Materials and Methods

Genotyping of Sema3A-cECKO mice

Sema3A loxP PCR was performed with extracted DNA according to the following protocol: One incubation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 sec, an annealing step at 60°C for 30 sec, and an elongation step at 72° C for 2 min, followed by a last amplification step at 72°C for 5 min with the forward primer 5'- ACAACGCTTGCCTCGGGAGGTAAA -3' and the reverse primer 5'- ATGGTTCTGATAG-GTGAGGCATGG -3'. For the VECad-Cre transgene, the following protocol was used: One incubation at 94°C for 2 min, 39 cycles of denaturation at 94°C for 20 sec, an annealing step at 56°C for 20 sec and an elongation step at 70° C for 30 sec, followed by a last amplification step at 70°C for 5 min. The following primers were used: Forward primer 5'- GCAGGCAGCTCACAAAGGAACAAT -3', the reverse primer for the VECad gene 5'- TGTCCTTGCTGAGTGACAGTGGAA -3' and the reverse primer for Cre transgene 5'- ATCACTCGTTGCATCGACCGGTAA -3.

Quantification of *Sema3A* mRNA levels in sorted endothelial cells

We sorted 150,000 CD45- (BD Biosciences, FITC conjugated mouse-anti-CD45.1 antibody, 553775, 1:200; BD Biosciences, FITC conjugated mouse-anti-CD45.2, 553772, 1:200) and CD31+ (BD Biosciences, APC conjugated rat-anti-CD31, 551262, 1:200) ECs from livers of P8 Sema3A-WT and Sema3A-cECKO pups into 500 µL of RA1 buffer and isolated the RNA according to the instructions of the Nucleospin RNA XS kit (Macherey-Nagel). The RNA was transcribed to cDNA using the High Capacity Reverse Transcription kit (Applied Biosystems). The PCR reactions were performed using FastStart SYBR green master mix (Roche) with a 7900HT Fast Real-Time PCR System (Applied Biosystems). The following primer sets were used: *Sema3A* Exon1 - Set 1 (middle) F: 5'- ACGTTAGTGTTGCCATGAGGT-3', R: 5'- AGGCAATCCCAGTGAACCAG-3'; *Sema3A* Exon1 - Set 2 (end) F: 5'- GCGGAGCGGAGATAAAAGGA-3', R: 5'- CCTCATGGCAACACTAACGTC-3'; house keeping gene Rplp0 F: 5'- AGATTCGGGATATGCTGTTGGC-3', R: 5'- TCGGGTCCTAGACCAGTGTTC-3'.

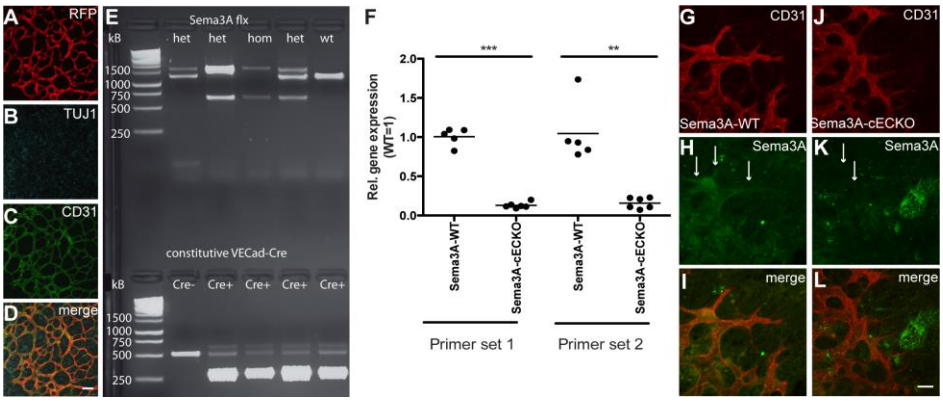

Fig. S1. **Confirmation of endothelial-cell specific Sema3A KO**. (A-D) Representative confocal images of a retinal whole mount of P7 constitutive VECad-Cre RFP pup stained for CD31 (green) and TUJ1 (cyan). (E) PCR analyses show amplicons for *Sema3A loxP* (1600 bp), *Sema3A wt* (1200 bp), and Cre-cut *Sema3A loxP* (650 bp). The Cre amplicon was present at 300 bp and the WT VECad amplicon at 550 bp. The Cre-cut amplicon was only present in Cre+ heterozygous or homozygous *Sema3A loxP* mice. (F) Quantification of relative *Sema3A* expression levels in Sema3A-cECKO pups compared to the Sema3A-WT littermates. A strong decrease in relative expression levels was detected with two different primer sets for *Sema3A* in Sema3A-cECKO compared to Sema3A-WT P8 pups: Primer set one: Sema3A-WT: 1.01±0.11, n=5, Sema3A-cECKO: 0.13±0.04, n=6, *t*-test, $p=1.97x10^{-5}$; Primer set two: Sema3A-WT: 1.05±0.39, n=5, Sema3A-cECKO: 0.16±0.07, n=6, *t*-test, $p=6.43x10^{-3}$. (G-L) High-magnification confocal images of retinal whole mounts of P7 Sema3A-WT and Sema3A-cECKO pups stained for CD31 (red) and Sema3A (green, arrows). Reduced Sema3A staining was visible in tip cell regions of Sema3A-cECKO compared to Sema3A-WT retinas. Scale bars: (A-D)=50 µm, (G-L)=20 µm. **=p<0.01, ***=p<0.001.

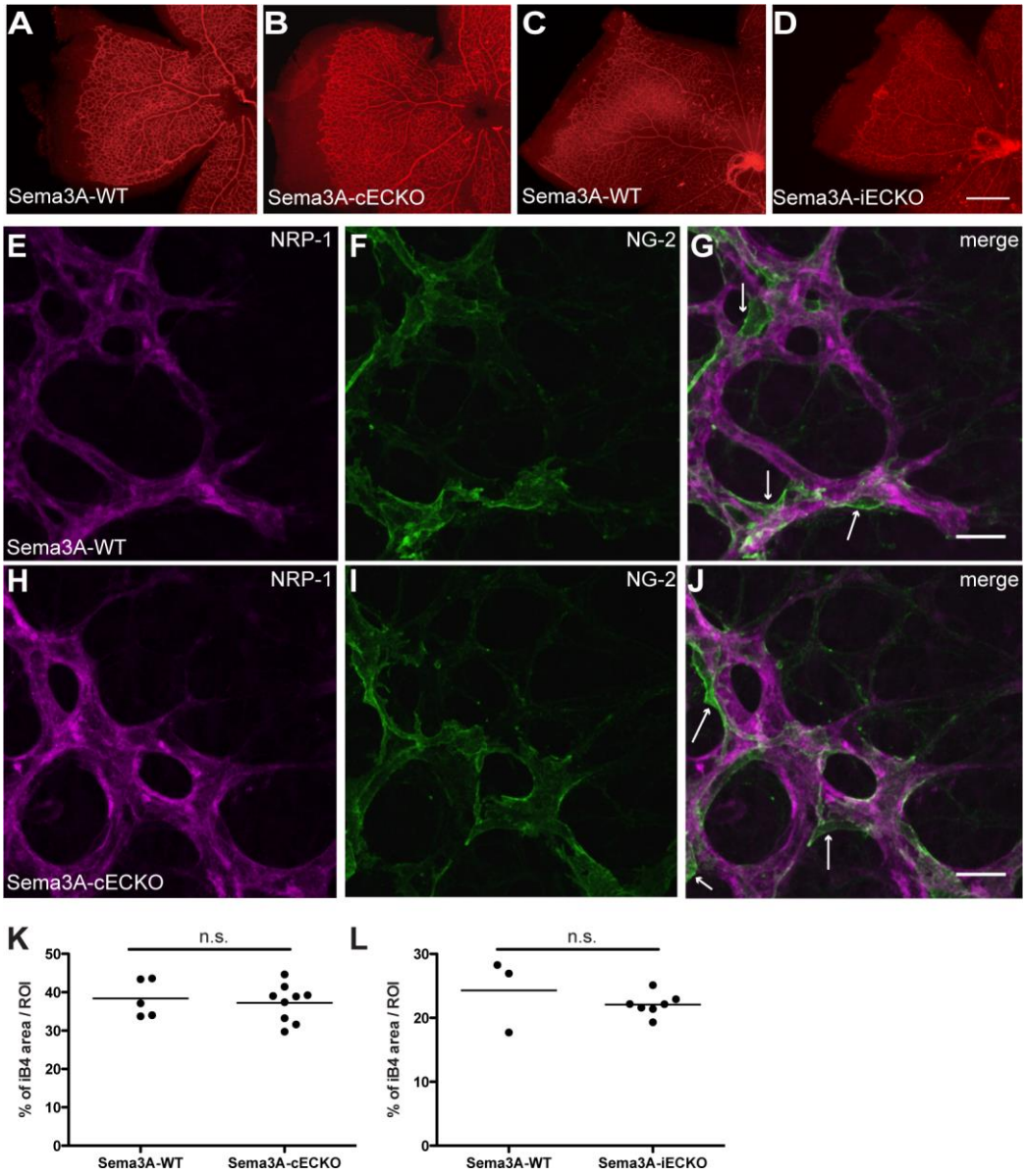

Fig. S2. **Endothelial cell-specific Sema3A KO does not alter BV density or pericyte coverage during development**. (A-D) IB4-stained P7 retinal whole mounts of Sema3A-WT, Sema3A-cECKO and Sema3A-iECKO pups. (E-J) High-magnification confocal images of retinal tip cells stained for Nrp-1 (magenta) and NG-2 (green) showed no overlap (arrows) and no gross alteration of pericyte coverage in P7 Sema3A-WT (A-C) or Sema3A-cECKO (D-F) pups. (K-L) Quantification of the IB4+ area per region of interest (%) in retinas of Sema3A-WT, Sema3A-cECKO and Sema3A-iECKO pups. No significant differences detectable. Scale bars: (A-D)=500 µm, (E-K)=20 µm.

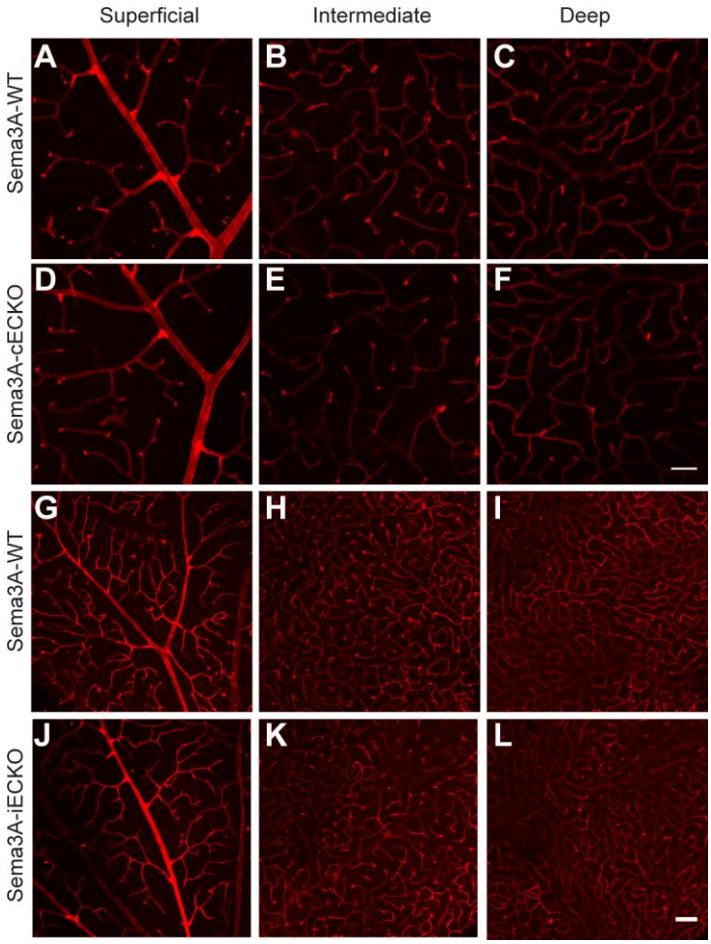

Fig. S3. **Endothelial cell-specific Sema3A KO does not change blood vessel density at later developmental stages**. (A-F) Confocal images of iB4 stained retinal whole mounts of 12 weeks old Sema3A-WT and Sema3A-cECKO mice. (G-L) Confocal images of iB4 stained retinal whole mounts of 3 weeks old control and Sema3A-iECKO mice. Scale bars: 50 µm.