Supplementary Information

Eph-ephrin signaling couples endothelial cell sorting and arterial specification

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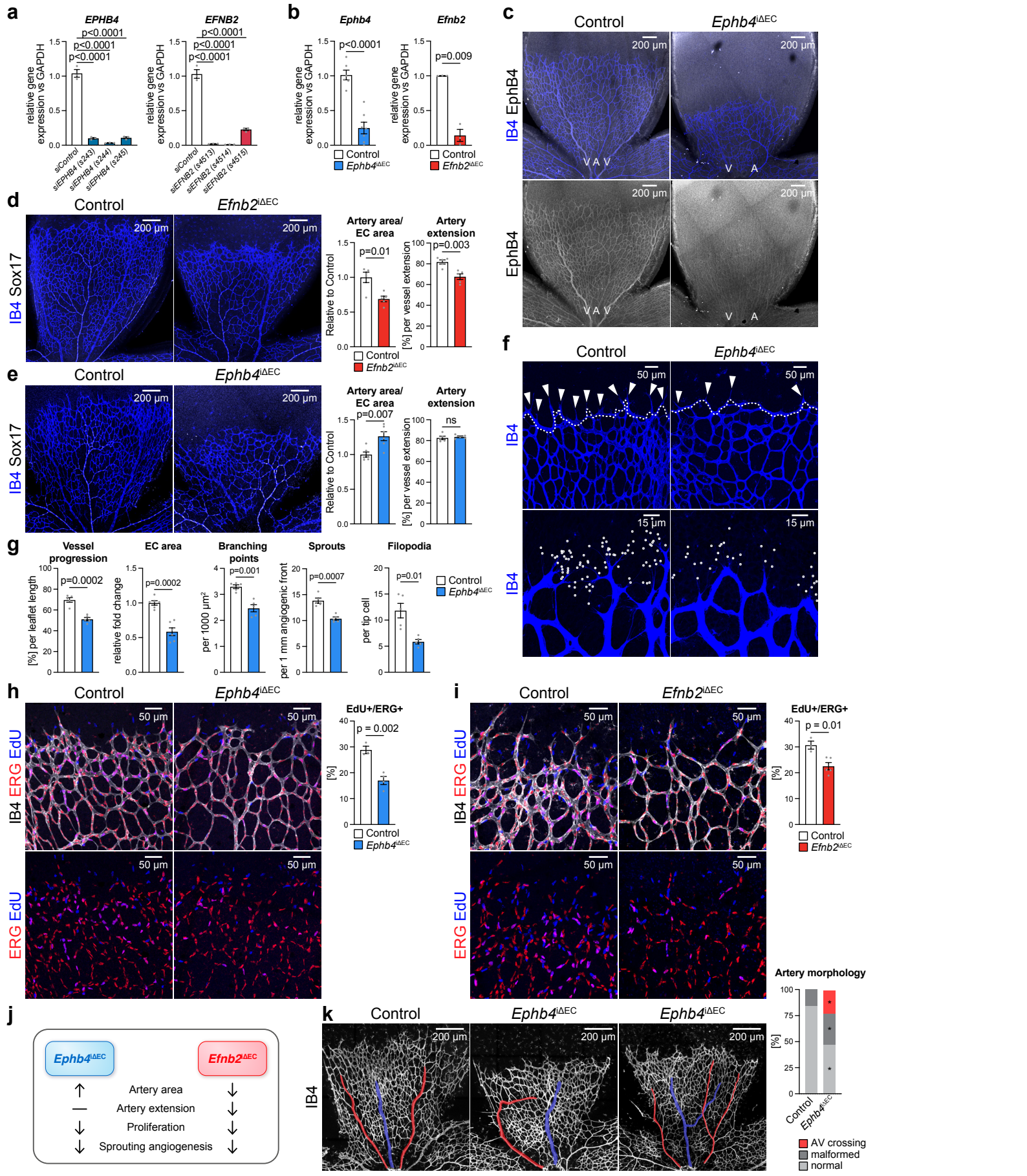
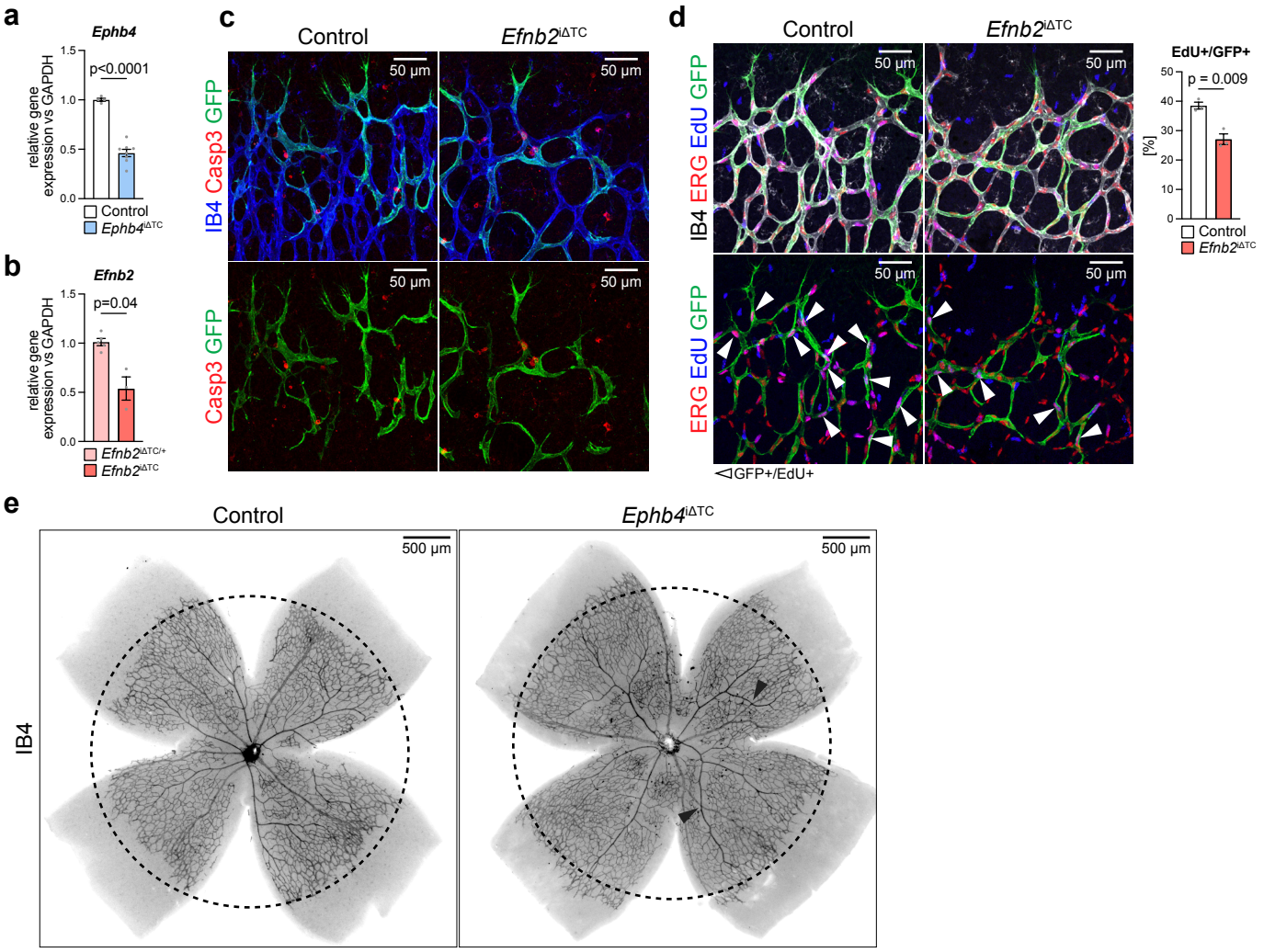
Supplementary Figure 1: Common and distinct roles of EphB4 and ephrin-B2 in the retinal angiogenesis.



Supplementary Figure 1: Common and distinct roles of EphB4 and ephrin-B2 in the retinal angiogenesis.

(**a**) Validation of *siEPHB4* and *siEFNB2* efficiency by RT-qPCR for 3 different siRNAs (n=3 experiments). (**b**) Validation of *Ephb4* and *Efnb2* gene deletion using *Cdh5CreERT2*. RT-qPCR of FACS sorted retinal ECs (n=6 control and 7 *Ephb4*$^{i\Delta EC}$ and n=3 control and 3 *Efnb2*$^{i\Delta EC}$ mice). (**c**) Confirmation of *Ephb4* inactivation. IB4 and EphB4 staining of control and *Ephb4*$^{i\Delta EC}$ retinas. (**d**, **e**) Confocal images of isolectin B4 (IB4) and Sox17 stained P6 retinal vessels (n=5 control and 5 *Efnb2*$^{i\Delta EC}$ mice (**d**) and n=6 control and 6 *Ephb4*$^{i\Delta EC}$ mice (**e**)). Quantitation of arterial area per total EC area and of artery extension defined by Sox17 arterial immunosignal. (**f**, **g**) EphB4 is required for sprouting angiogenesis. High magnification pictures of IB4 stained retinas (**f**). Graphs show quantitation of vessel progression (n=5 control and *Ephb4*$^{i\Delta EC}$), EC area (n=6 mice), branching points (n=5 mice), number of sprouts (n=5 mice) and filopodia per angiogenic front line (n=5 mice) (**g**). (**h**, **i**) Reduced proliferation of ECs in pan-endothelial *Ephb4* and *Efnb2* mutants. Graphs show quantitation of EdU+ per total ERG+ ECs (n=3 control and 4 *Ephb4*$^{i\Delta EC}$ and n=3 control and 5 *Efnb2*$^{i\Delta EC}$ mice). (**j**) Scheme summarizes vascular changes in mutant retinas. (**k**) Representative images of *Ephb4*$^{i\Delta EC}$ retina with malformed arteries and arteriovenous crossings compared to control. Graph shows quantitation of malformed arteries and normal vascular morphology (n= 19 control and 17 *Ephb4*$^{i\Delta EC}$). *P* values were calculated by one-way ANOVA (**a**), two-tailed unpaired *t*-test (**b**, **d**, **e**, **g**, **h**, **i**) and Chi-square test (**k**). *In vivo* experiments were performed with tamoxifen injections at P1-P3 following analysis at P6 (**c**, **d**, **e**, **f**, **h**, **i**, **k**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

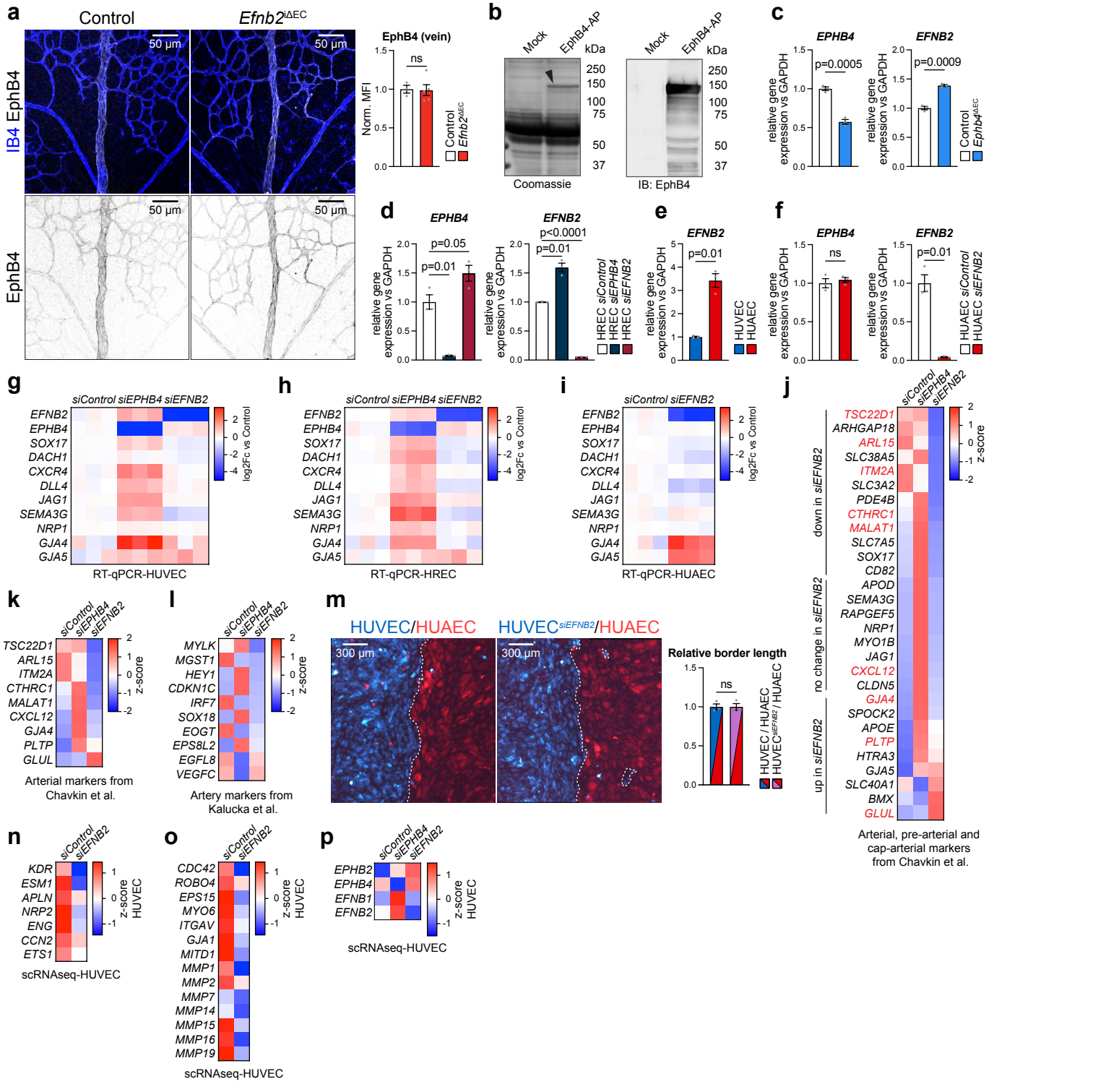
Supplementary Figure 2: Tip cell deletion of *Ephb4* and *Efnb2*.



Supplementary Figure 2: Tip cell deletion of *Ephb4* and *Efnb2*.

(**a**, **b**) Validation of *Ephb4* and *Efnb2* gene inactivation using *Esm1-CreERT2*. RT-qPCR of FACS sorted GFP+ retinal ECs (n=4 control and 8 *Ephb4*$^{i\Delta TC}$ and n=5 control (*Efnb2*$^{i\Delta TC/+}$) and 3 *Efnb2*$^{i\Delta TC}$ mice). (**c**) Confocal images of control and *Efnb2*$^{i\Delta TC}$ P6 retinas showing IB4+, GFP+ and Casp3+ cells. Note that there are no GFP+/Casp3+ ECs (n=3 mice). (**d**) EC proliferation (arrowheads) is reduced in *Efnb2* depleted tip cell progeny. Confocal images and quantitation of proliferating GFP+ ECs using IB4, ERG, EdU and GFP immunostaining (n=3 control and 3 *Efnb2*$^{i\Delta TC}$ mice). (**e**) IB4-stained overview pictures of control and *Ephb4*$^{i\Delta TC}$ mouse retinas. Arrowheads point to AV crossings. *P* values were calculated using two-tailed unpaired *t*-test (**a**, **b**, **d**). *In vivo* experiments were performed with tamoxifen injections at P1-P3 following analysis at P6 (**c**, **d**, **e**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

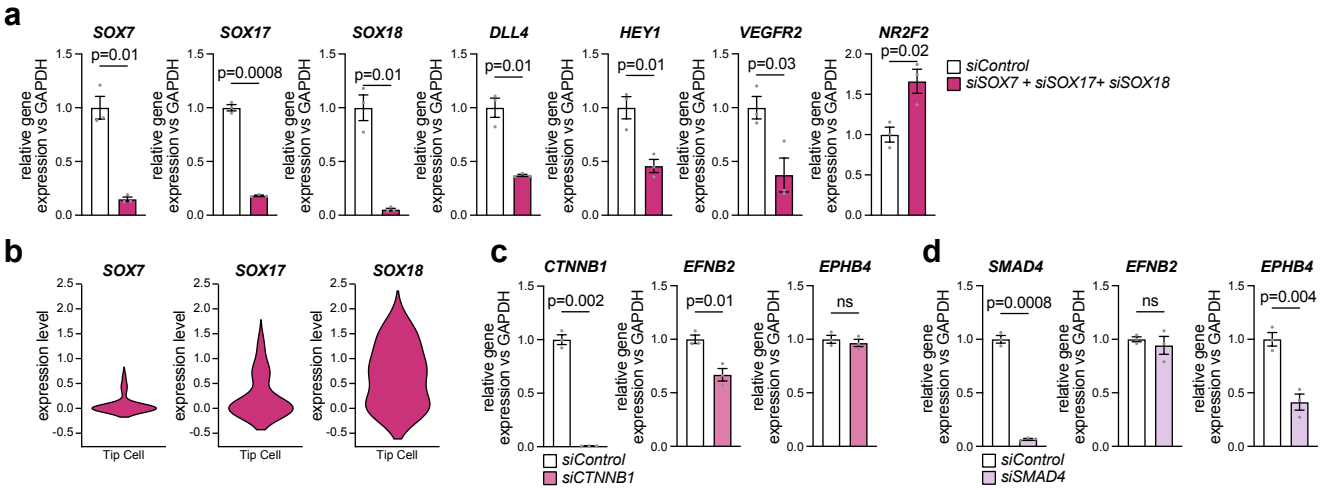
Supplementary Figure 3: Ephrin-B2 represses EphB4 expression but promotes VEGFR2 signaling.



Supplementary Figure 3: Ephrin-B2 represses EphB4 expression but promotes VEGFR2 signaling.

(**a**) EphB4 is unchanged in *Efnb2*$^{i\Delta EC}$ mature retinal vessels and central capillary plexus. EphB4 staining and immunosignal quantitation (n=3 control and 5 *Efnb2*$^{i\Delta EC}$ mice). (**b**) EphB4-AP protein expression. Coomassie blue and immunoblot detection of overexpressed EphB4-AP protein in HEK293 cell supernatant. (**c**) RT-qPCR of indicated genes in FACS-isolated P6 brain ECs (n=3 control and 3 *Ephb4*$^{i\Delta EC}$). (**d**, **e**, **f**) RT-qPCR of *EPHB4* and *EFNB2* in primary human retinal ECs (HRECs) upon *EPHB4* or *EFNB2* depletion (**d**), in HUVECs and HUAECs (**e**) and upon knockdown of *EFNB2* in HUAECs (**f**) (n=3 experiments). (**g**, **h, i**) RT-qPCR of indicated genes upon *EPHB4* and *EFNB2* knockdown in HUVECs (**g**) and HRECs (**h**) and upon knockdown of *EFNB2* in HUAECs (**i**) (n=3 experiments). (**j**, **k**, **l**) Comparative expression analysis of top arterial marker genes from P6 postnatal retina (Chavkin et al.[52]) and adult mouse brain (Kalucka et al.[53]) dataset, respectively, in *siControl*, *siEPHB4* and *siEFNB2* HUVEC scRNAseq own dataset. Z-score for tip cell-like main cluster was calculated in (**j**) for arterial (marked in red), pre-arterial and cap-arterial marker genes and in (**k)** only for arterial marker genes from Chavkin et al. retina dataset[52], while in (**l**) for arterial marker genes from Kalucka et al. brain dataset[53]. (**m**) Confrontation assay for heterotypic co-culture of HUVECs and HUAECs, as indicated. Final snapshots of CellTracker-labeled cells imaged for 48 h after removal of Ibidi insert. Quantitation graph for relative border length (n=3 experiments). (**n, o**) Decreased expression of genes related to angiogenesis (**n**) and cell migration (**o**) upon *EFNB2* KD in HUVECs compared to control. Genes selected from pseudobulk DGE analysis comparing *siControl* and *siEFNB2* in tip cell-like main cluster. (**p**) EphB and ephrin-B family genes are differentially affected by KD of EphB4 and ephrin-B2 in HUVECs. Results are based on pseudobulk DGE analysis comparing *siControl*, *siEPHB4* and *siEFNB2* in tip cell-like main cluster. *P* values were calculated using two-tailed unpaired *t*-test (**a**, **c-i, m**). *In vivo* experiments were performed with tamoxifen injections at P1-P3 following analysis at P6 (**a**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

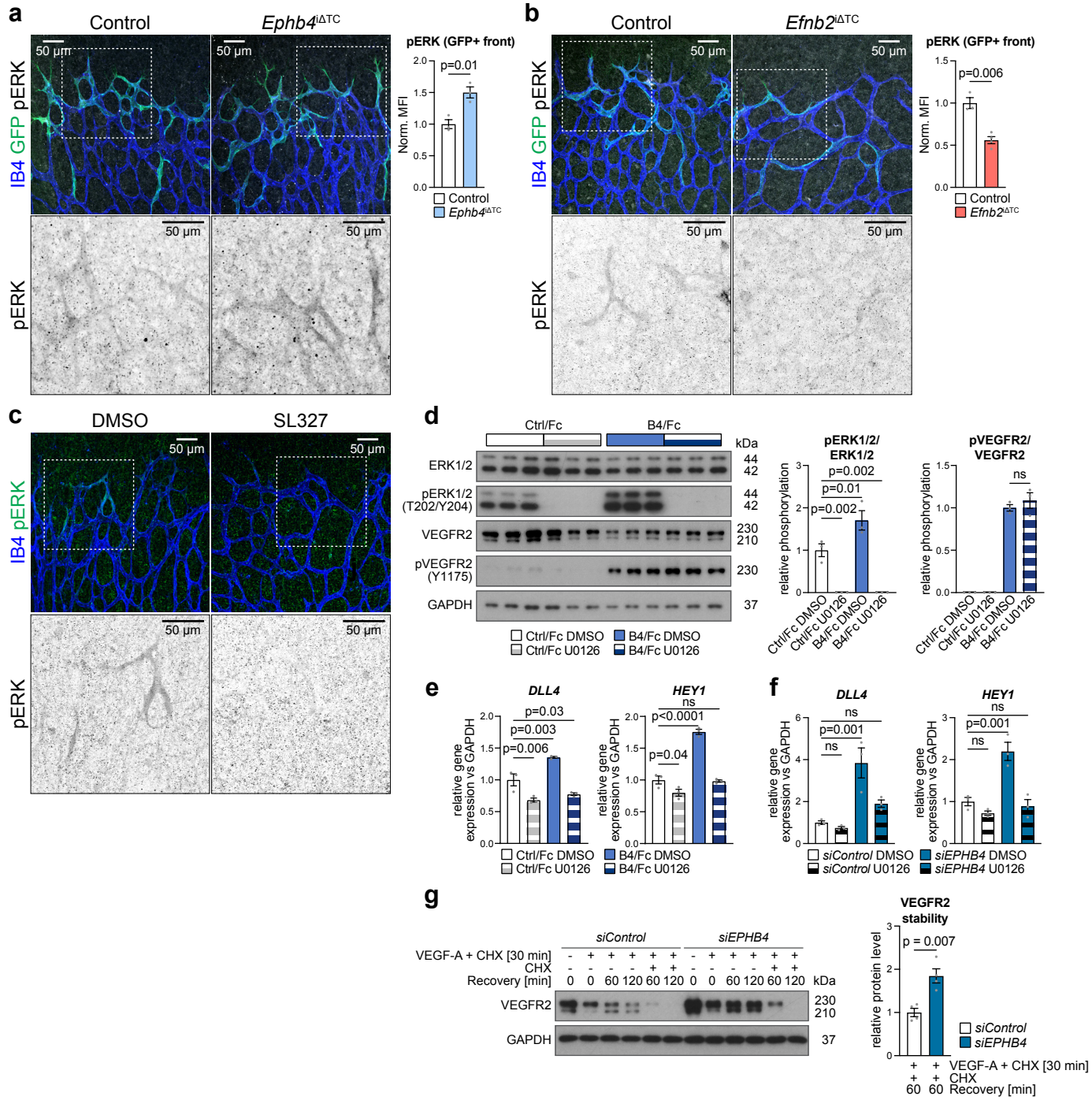
Supplementary Figure 4: Regulation of *EFNB2* and *EPHB4* expression in HUVECs.



Supplementary Figure 4: Regulation of *EFNB2* and *EPHB4* expression in HUVECs.

(**a**) SoxF factors promote arterial and repress venous gene expression. Validation by RT-qPCR of *SOX7* + *SOX17* + *SOX18* triple knockdown in HUVECs (n=3 experiments). (**b**) Violin plots of *SOX7*, *SOX17* and *SOX18* expression in P6 retinal tip cells. Data extracted from published scRNA-seq results[52]. (**c**, **d**) Not all transcription factors involved in vascular development and AV specification mediate reciprocal regulation of *EPHB4* and *EFNB2*. Graphs show RT-qPCR analysis of *siCTNNB1* vs *siControl* HUVECs (**c**) and *siSMAD4* vs *siControl* HUVECs (**d**) (n=3 experiments). *P* values were calculated using two-tailed unpaired *t*-test (**a**, **c**, **d**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

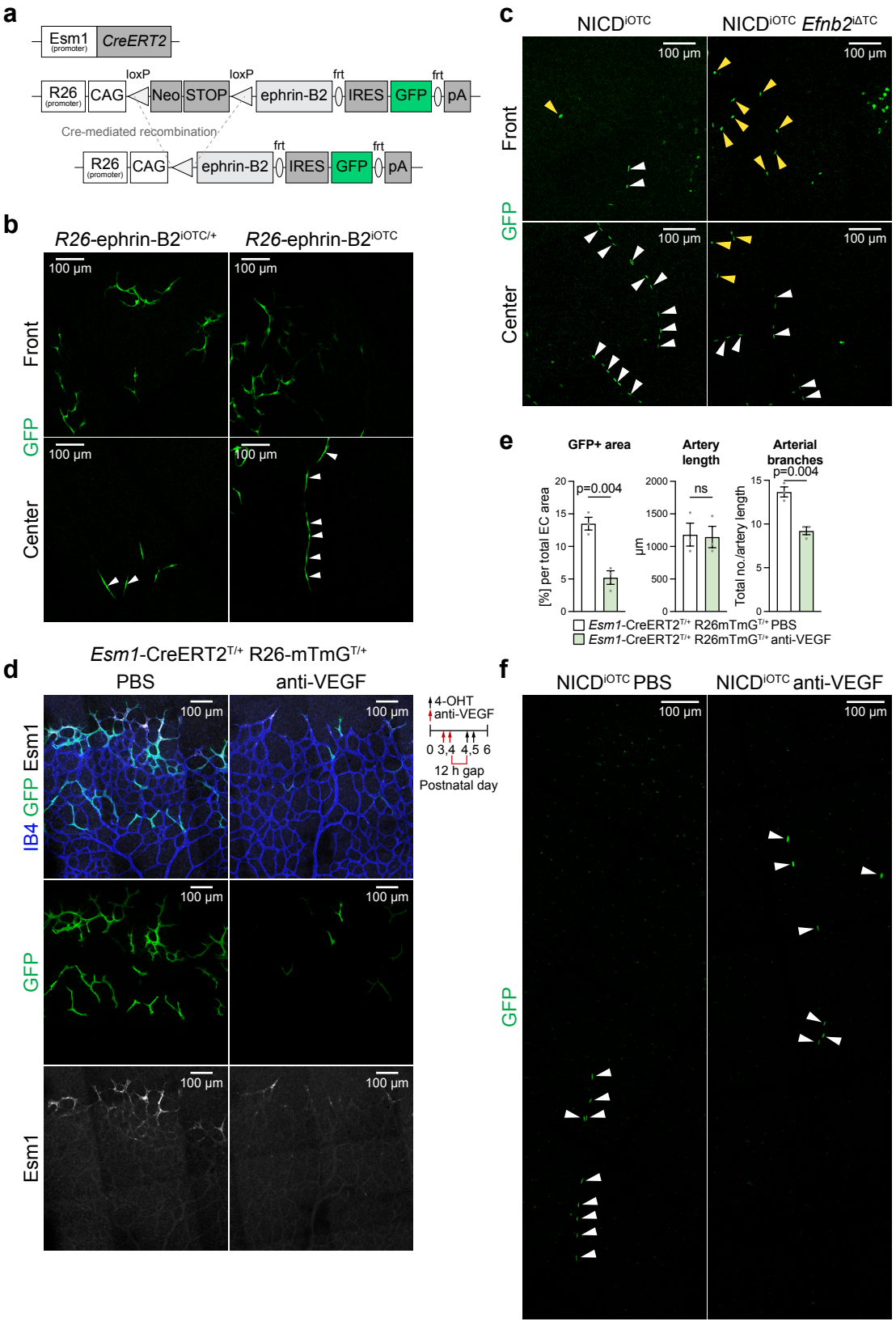
Supplementary Figure 5: ERK1/2 signaling promotes arterial gene expression.



Supplementary Figure 5: ERK1/2 signaling promotes arterial gene expression.

(**a**) Increased pERK1/2 (pERK) in *Ephb4*-deleted TC-derived progeny (n=3 control and 3 *Ephb4*$^{i\Delta TC}$). (**b**) Decreased pERK in *Efnb2*-deleted TC-derived progeny (n=3 control and 4 *Efnb2*$^{i\Delta TC}$). (**c**) Validation of pERK retinal immunosignal in mice treated with SL327 MEK inhibitor or vehicle. (**d**) VEGFR2 and ERK1/2 activation upon EphB4/Fc stimulation. Immunoblot for pVEGFR2 (Y1175) and pERK1/2 from stimulated HUVECs, treated with ERK inhibitor (U0126) or vehicle. Immunoblot quantitation of pVEGFR2 and pERK1/2 relative levels upon U0126 inhibitor or vehicle treatment (n=3 experiments). (**e**, **f**) ERK signaling is required downstream of ephrin-B2 stimulation (**e**) and *EPHB4* knockdown (**f**) to increase *DLL4* and *HEY1* expression. RT-qPCR for *HEY1* and *DLL4* (n=3 experiments). (**g**) Increased VEGFR2 stability in *EPHB4* KD cells. VEGFR2 immunoblotting of *siControl* and *siEPHB4* HUVECs pre-treated with VEGF-A and cycloheximide (CHX), washed and recovered in the absence or presence of CHX. Graph shows quantitation of VEGFR2 levels at 60 min of recovery (+ CHX) relative to 0 min (VEGF-A+CHX) (n=3 experiments). *P* values were calculated using two-tailed unpaired *t*-test (**a**, **b**, **g**) and one-way ANOVA (**d**, **e**, **f**). *In vivo* experiments were performed with 4-OHT injections at P4.5 following analysis at P6 (**a**, **b**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

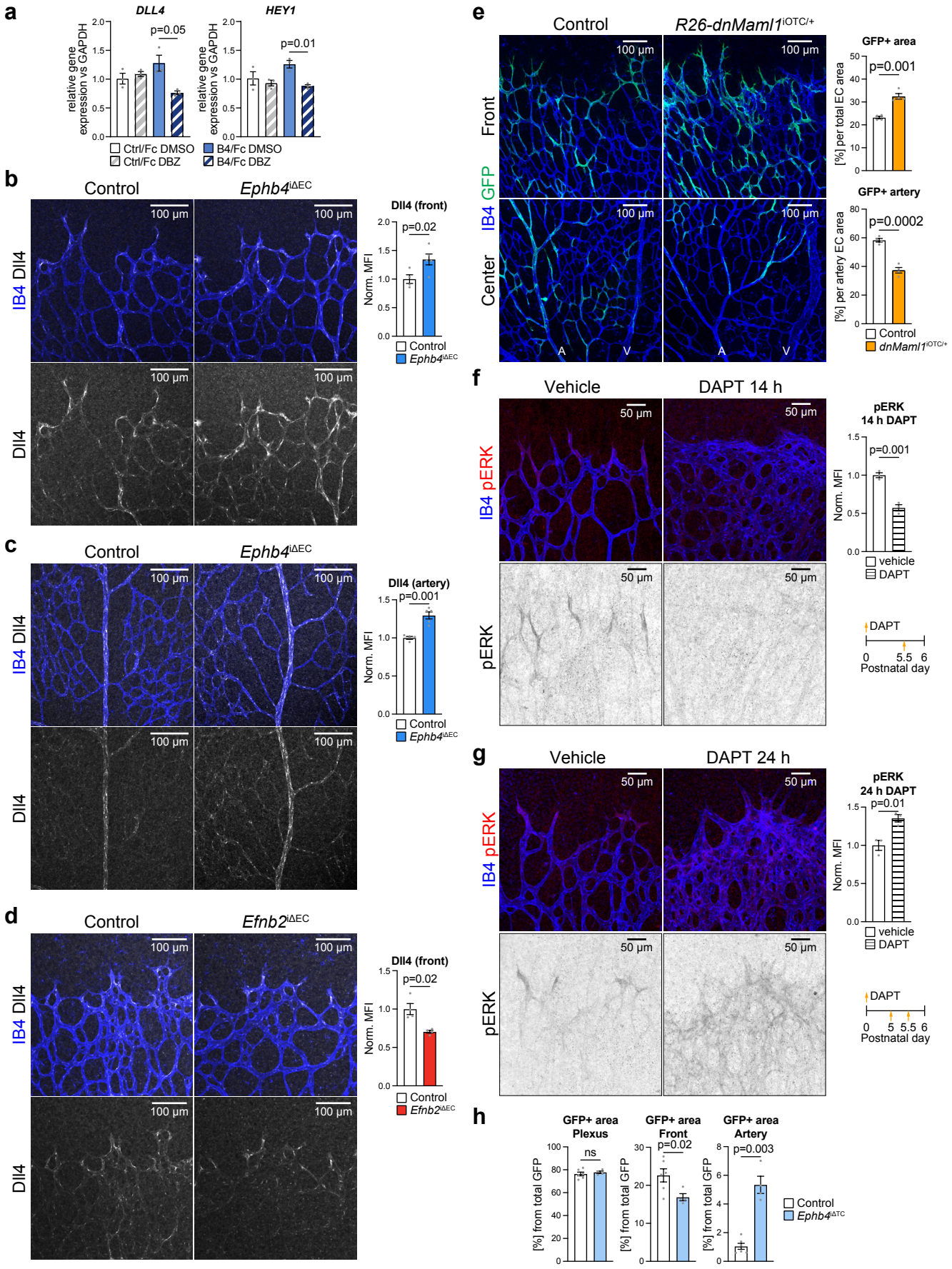
Supplementary Figure 6: Notch and VEGF-A control arterial progenitors in the retina.



Supplementary Figure 6: Notch and VEGF-A control arterial progenitors in the retina.

(**a**) Schematic depiction of the *Esm1-CreERT2* transgene and Cre-mediated ephrin-B2 overexpression. (**b**) Confocal images showing GFP+ ephrin-B2 overexpressing cells. (**c**) Confocal images of GFP+ (NICD+ cells) ECs in the $NICD^{iOTC}$ P6 retinal vasculature. (**d**) Induction of Esm1 expression in tip cells requires VEGF signaling. Confocal images of vehicle or anti-VEGF treated *Esm1-CreERT2 R26*-$mTmG^{+/T}$ retinas showing IB4+, Esm1+ and GFP+ (Esm1-derived) ECs. (**e**) Quantitation of GFP+ area per total EC area, artery length and arterial branches (n=3 vehicle and n=3 anti-VEGF). (**f**) Confocal images of vehicle or anti-VEGF treated $NICD^{iOTC}$ retinas showing GFP+ (NICD+) ECs. *P* values were calculated using two-tailed unpaired *t*-test (**c**). *In vivo* experiments were performed with tamoxifen injections at P1-P3 (**b**, **c**), 4-OHT injections at P4-P5 following analysis at P6 (**d**) or 4-OHT injections at P3-P4 followed by anti-VEGF injections at P5-P6 and analysis at P7 (**f**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

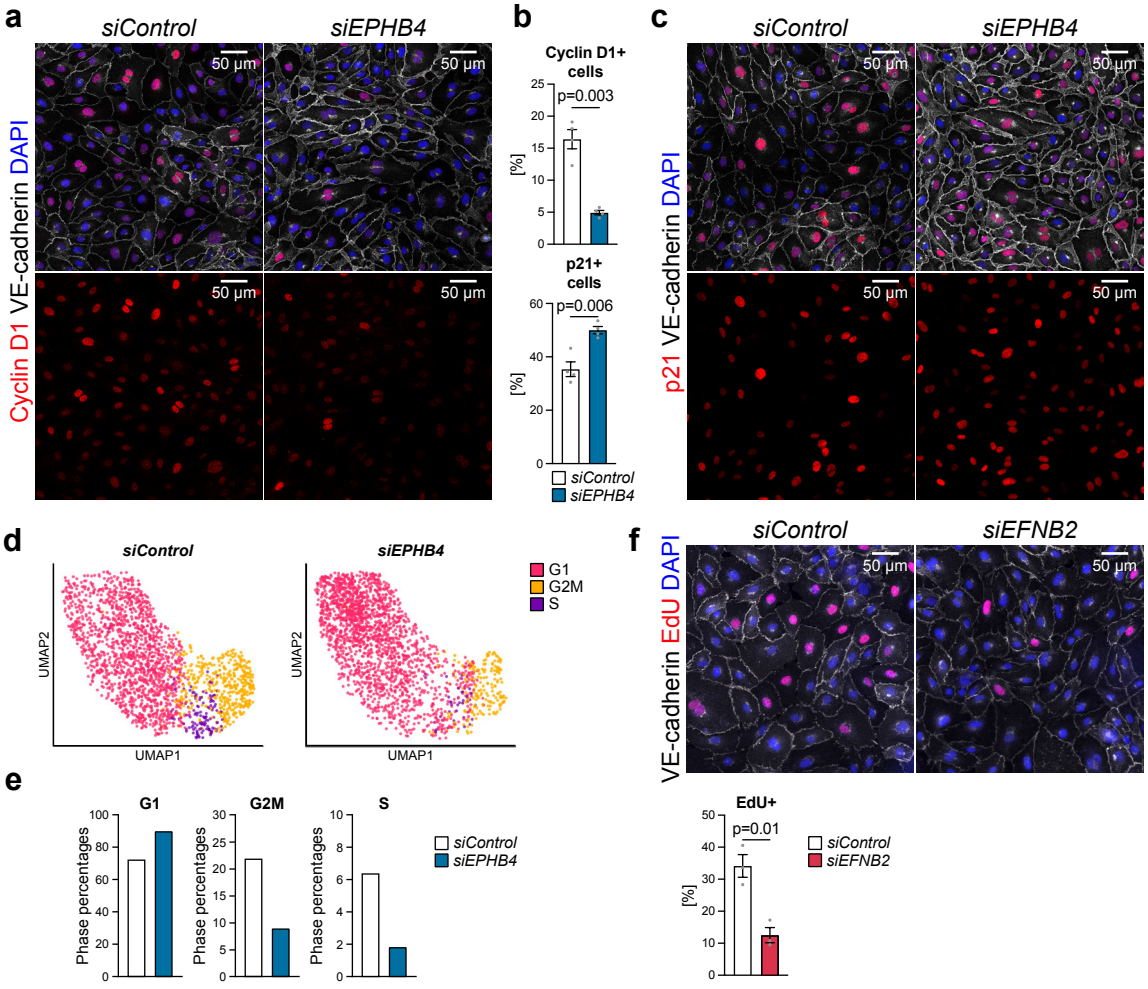
Supplementary Figure 7: Interactions between EphB4 and Notch during artery formation.



Supplementary Figure 7: Interactions between EphB4 and Notch during artery formation.

(**a**) Normalization of *DLL4* and *HEY1* expression upon DBZ treatment of EphB4/Fc stimulated cells (n=3 experiments). (**b**, **c**) Quantitation of Dll4 immunosignal in the P6 control (n=4 animals) and *Ephb4*$^{i\Delta EC}$ (n= 5) vascular front (**b**) and retinal arteries (**c**). (**d**) Decreased Dll4 capillary plexus expression upon EC-specific *Efnb2* inactivation. Quantitation of Dll4 MFI in the retinal vascular front area (n=4 control and 4 *Efnb2*$^{i\Delta EC}$). (**e**) dnMaml1 overexpression in tip cells impairs artery growth. Graphs show quantitation of total GFP+ area and GFP+ arterial area (n=4 control and 4 *R26*-dnMaml1$^{iOTC/+}$). (**f**) Acute Notch inhibition (DAPT) for 14 h reduces pERK1/2. Graph shows quantitation of pERK MFI in angiogenic front area (n=3 vehicle and n=3 DAPT injected in WT C57BL6 mice). (**g**) Robust Notch inhibition (DAPT) for 24 h increased pERK. Graph shows quantitation of pERK MFI in angiogenic front area (n=3 vehicle and n=3 DAPT injected in WT C57BL6 mice). (**h**) Graphs showing the distribution of GFP+ area in capillary plexus, front and artery per total GFP positive area (n=6 control and 4 *Ephb4*$^{i\Delta TC}$ mice). *P* values were calculated by one-way ANOVA (**a**) and two-tailed unpaired *t*-test (**b**, **c**, **d**, **e**, **f, g, h**). *In vivo* experiments were performed with tamoxifen injections at P1-P3 following analysis at P6 (**b**, **c**, **d**, **e**). DAPT or vehicle was administered once, 14 h prior to analysis (**f**) or twice, at 24 h and 14 h prior to analysis (**g**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

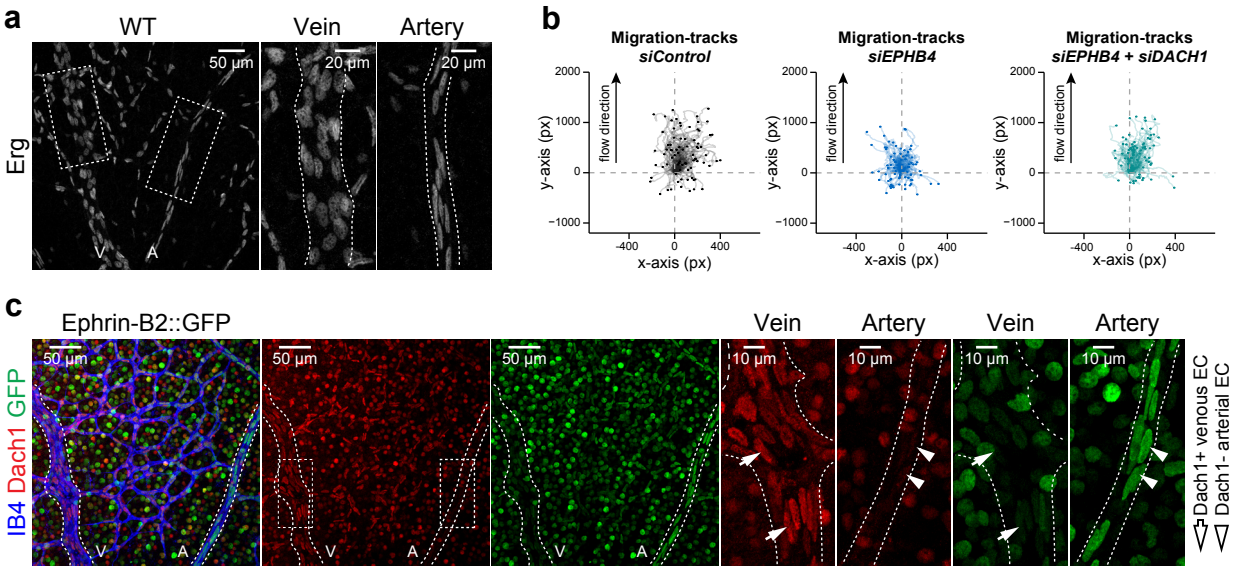
Supplementary Figure 8: EphB4 and ephrin-B2 in cell cycle regulation.



Supplementary Figure 8: EphB4 and ephrin-B2 in cell cycle regulation.

(**a**-**c**) Decreased Cyclin D1 and increased P21 protein expression in *siEPHB4* cells at 48 h of knockdown (n=3 experiments). (**d**, **e**) *siEPHB4* cells show impaired cell cycle progression, as indicated by 2D UMAP representation of cell cycle phase distribution relative to *siControl* cells (**d**). Graphs represent proportion of cells in G1, G2/M and S phases (**e**). (**f**) Reduced proliferation of HUVECs after *EFNB2* KD. Graphs shows quantitation of EdU+ per total cells. *P* values were calculated by two-tailed unpaired *t*-test (**b**, **f**). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

Supplementary Figure 9: EphB4, ephrin-B2 and Dach1 expression levels correlate with EC nuclear morphology and cell migration behavior under flow.



Supplementary Figure 9: EphB4, ephrin-B2 and Dach1 expression levels correlate with EC nuclear morphology and cell migration behavior under flow.

(**a**) Arterial ECs align better to flow than venous cells. Example of wild-type (WT) P6 retinal vasculature immunostained for ERG. Boxed areas show representative parts of artery (A) or vein (V). (**b**) Examples of quantified migration tracks for *siControl*, *siEPHB4* and *siEPHB4/ DACH1* cells (80 randomly selected tracks/experiment). (**c**) Dach1 is expressed in retinal veins but not in arteries. IB4, GFP and Dach1 staining of the P6 *Efnb2-H2B-GFP* knock-in reporter retinal vasculature. Source data are provided as a Source Data file.

Supplementary Table S1. **Summary of genetic mouse models used in this study**

Full name	Short name	Source/Reference
Rosa26-mT/mG	*R26-mTmG*	Muzumdar et al. 2007
Ephb4$^{lox/lox}$	/	Wang et al. 2015
Efnb2$^{lox/lox}$	/	Grunwald et al. 2004
Cdh5(PAC)-CreERT2	*Cdh5-CreERT2*	Wang et al. 2010
Esm1-CreERT2		Rocha et al. 2014
Gt(ROSA)$^{26Sortm1(Notch1)dam}$	*NICD*	Murtaugh et al. 2003
Efnb2-GFP		Davy and Soriano, 2007
C57BL/6J	WT	Janvier Labs
Rosa26-ephrin-B2-GFP	*R26-ephrin-B2-GFP*	this study
Rosa26-Dach1OE	*R26-Dach1*	Raftrey et al. 2021
Gt(ROSA)$^{26Sortm1(MAML1)Wsp}$	*R26-dnMaml1*	Tu et al. 2005

Control genotype	Mutant genotype	Tamoxifen treatment	Figure
Efnb2$^{lox/lox}$	*Efnb2*$^{lox/lox}$, *Cdh5(PAC)-CreERT2*$^{+/T}$	P1-P3 50 µg	2d, 3c, S1d, S1i, S3a, S7d
Ephb4$^{lox/lox}$	*Ephb4*$^{lox/lox}$, *Cdh5(PAC)-CreERT2*$^{+/T}$	P1-P3 50 µg	2f, 3a, 4c, 5d, S1c, S1e, S1f, S1h, S1k, S7b, S7c
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*Ephb4*$^{lox/lox}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P1-P3 50 µg	1c, 1g, 1i, 6b, 7a, S2e
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*Efnb2*$^{lox/lox}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P1-P3 50 µg	1d, 1h, 7b, S2c, S2d
NICD$^{lox/lox}$, *Esm1-CreERT2*$^{+/T}$	*Efnb2*$^{lox/lox}$, *NICD*$^{lox/lox}$, *Esm1-CreERT2*$^{+/T}$	P1-P3 50 µg	5b
R26-ephrin-B2-GFP$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*R26-ephrin-B2-GFP*$^{lox/lox}$, *Esm1-CreERT2*$^{+/T}$	P1-P3 50 µg	5a
Ephb4$^{lox/lox}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*Ephb4*$^{lox/lox}$, *R26-dnMaml1*$^{lox/+}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P1-P3 50 µg	6c
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*Ephb4*$^{lox/lox}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P4.5 50 µg 4OHT	6g, 9h, S5a
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*Ephb4*$^{lox/lox}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P4.5 50 µg 4OHT	6h
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*Efnb2*$^{lox/lox}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P4.5 50 µg 4OHT	S5b
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*R26-dnMaml1*$^{lox/+}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P1-P3 50 µg	S7e
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*R26-Dach1*$^{lox/+}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P4.5 50 µg 4OHT	9i
NICD$^{lox/lox}$, *Esm1-CreERT2*$^{+/T}$ vehicle	*NICD*$^{lox/lox}$, *Esm1-CreERT2*$^{+/T}$ anti-VEGF	P3, 4 50 µg 4OHT	5c, S6d
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	*R26-dnMaml1*$^{lox/+}$, *Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$	P4.5 50 µg 4OHT	6e
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$ vehicle	*Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$ DAPT	P4.5 50 µg 4OHT	6f
Efnb2-GFP		/	9c, S9c
Efnb2-GFP, *Esm1-CreERT2*$^{+/T}$, *Rosa26-RFP*$^{lox/+}$		P5 50 µg 4OHT	9e
Rosa26-mT/mG$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$ vehicle	*Rosa26-mT/mG*$^{lox/+}$, *Esm1-CreERT2*$^{+/T}$ anti-VEGF	P4, 5 50 µg 4OHT	S6b

Supplementary Table S2. Antibodies used for retina immunostaining

a

Primary antibodies	Species	Clone	Company	Catalog #	Dilution
α-Dach1	Rabbit	Polyclonal	Proteintech	10914-1-AP	1:200
α-DLL4	Goat	Polyclonal	R&D Systems	AF1389	1:50
α-EphB4	Goat	Polyclonal	R&D Systems	AF446	1:50
α-ERG	Rabbit	Monoclonal	Abcam	ab110639	1:200
α-GFP	Chicken	Polyclonal	Abcam	ab13970	1:1000
α-GFP-AF-488	Rabbit	Polyclonal	Invitrogen	A21311	1:500
α-IB4-biotinylated	Griffonia simplicifolia		Vector	B-1205	1:50
α-pERK	Rabbit	Monoclonal	Cell Signaling	4370	1:200
α-Sox17	Goat	Polyclonal	R&D Systems	AF1924	1:100
α-Casp3	Rabbit	Polyclonal	Cell Signaling	9661	1:100

b

Secondary antibodies	Species	Clone	Company	Catalog #	Dilution
α-chicken-AF-488	Donkey	Polyclonal	Jackson ImmunoResearch	703-545-155	1:500
α-goat-AF-647	Donkey	Polyclonal	Invitrogen	A21447	1:500
α-goat-AF-647	Donkey	Polyclonal	Thermo Scientific	A32849	1:500
α-rabbit-AF-488	Donkey	Polyclonal	Invitrogen	A21206	1:500
α-rabbit-AF-546	Donkey	Polyclonal	Invitrogen	A10040	1:500
α-rabbit-AF-594	Donkey	Polyclonal	Invitrogen	A21207	1:500
Streptavidine-AF-405			Invitrogen	S32351	1:100
Streptavidine-AF-546			Invitrogen	S11225	1:100

Supplementary Table S3. **Taqman probes used for qRT-PCR**

Name/Gene	Target Species	Company	Assay ID/Cat#
CXCR4 FAM	Human	Thermo Fisher Scientific	Hs00607978_s1
DACH1 FAM	Human	Thermo Fisher Scientific	Hs00974297_m1
DLL1 FAM	Human	Thermo Fisher Scientific	Hs01011330_m1
DLL4 FAM	Human	Thermo Fisher Scientific	Hs00184092_m1
EFNB2 FAM	Human	Thermo Fisher Scientific	Hs00187950_m1
EPHB4 FAM	Human	Thermo Fisher Scientific	Hs00174752_m1
HEY1 FAM	Human	Thermo Fisher Scientific	Hs01114113_m1
JAG1 FAM	Human	Thermo Fisher Scientific	Hs01070036_m1
NR2F2 FAM	Human	Thermo Fisher Scientific	Hs00819630_m1
SOX17 FAM	Human	Thermo Fisher Scientific	Hs00751752_s1
SOX18 FAM	Human	Thermo Fisher Scientific	Hs00746079_s1
SOX7 FAM	Human	Thermo Fisher Scientific	Hs00846731_s1
VEGFR2 FAM	Human	Thermo Fisher Scientific	Hs00911700_m1
GAPDH Endogenous Control VIC	Human	Thermo Fisher Scientific	4326317E
SEMA3G FAM	Human	Thermo Fisher Scientific	Hs00928870_g1
NRP1 FAM	Human	Thermo Fisher Scientific	Hs01546494_m1
GJA4 FAM	Human	Thermo Fisher Scientific	Hs01098016_m1
GJA5 FAM	Human	Thermo Fisher Scientific	Hs00979198_m1
Ephb4 FAM	Mouse	Thermo Fisher Scientific	Mm01201156_m1
Efnb2 FAM	Mouse	Thermo Fisher Scientific	Mm00438670_m1
Gapdh Endogenous Control VIC	Mouse	Thermo Fisher Scientific	4352339E

Supplementary Table S4. Antibodies used for western blotting

a

Primary antibodies	Species	Clone	Company	Catalog #	Dilution
α-Akt	Rabbit	Polyclonal	Cell Signaling	9272	1:1000
α-pAkt(S473)	Rabbit	Monoclonal	Cell Signaling	4060	1:500
α-bTAN20	Rat	Monoclonal	DSHB	bTAN 20	1:700
α-Dll4	Rabbit	Polyclonal	Cell Signaling	2589	1:1000
α-EphB4	Goat	Polyclonal	R&D Systems	AF446	1:2000
α-Ephrin-B2	Goat	Polyclonal	R&D Systems	AF496	1:2000
α-Epsin1	Rabbit	Monoclonal	Abcam	ab75879	1:1000
α-ERK1/2	Rabbit	Monoclonal	Cell Signaling	4695	1:1000
α-pERK1/2(T202/Y204)	Rabbit	Monoclonal	Cell Signaling	4370	1:1000
α-GAPDH	Rabbit	Monoclonal	Cell Signaling	2118	1:10000
α-Notch1	Rabbit	Monoclonal	Cell Signaling	4380	1:1000
α-PLCy	Rabbit	Monoclonal	Cell Signaling	5690	1:1000
α-pPLCy(Y783)	Rabbit	Polyclonal	Cell Signaling	2821	1:500
α-Sox17	Goat	Polyclonal	R&D Systems	AF1924	1:1000
α-Tubulin	Mouse	Polyclonal	Sigma	T5168	1:6000
α-VEGFR2	Rabbit	Monoclonal	Cell Signaling	2479	1:1000
α-pVEGFR2(Y1175)	Rabbit	Monoclonal	Cell Signaling	2478	1:500
α-pVEGFR2(Y951)	Rabbit	Monoclonal	Cell Signaling	4991	1:500

b

Secondary antibodies	Species	Clone	Company	Catalog #	Dilution
α-goat HRP-linked	Bovine	Polyclonal	Jackson Immuno Research	805-035-180	1:15000
α-mouse HRP-linked	Sheep		Amersham	NA931	1:40000
α-rabbit HRP-linked	Goat		Cell Signaling	7074	1:15000
α-rat-HRP-linked	Goat		Amersham	NA935	1:15000

Supplementary Table S5. Antibodies used for HUVEC immunostaining and FACS of murine ECs

a

Primary antibodies	Species	Clone	Company	Catalog #	Dilution
α-Dach1	rabbit	polyclonal	Proteintech	10914-1-AP	1:200
α-CyclinD1	rabbit	monoclonal	Cell Signaling	2978	1:50
α-GOLPH4	rabbit	polyclonal	Abcam	28049	1:500
α-p21	rabbit	monoclonal	Cell Signaling	2947	1:100
α-Phalloidin			Invitrogen	A22287	1:40
α-VE-Cadherin	rabbit	monoclonal	Cell Signaling	3195	1:100
α-VE-Cadherin	mouse	monoclonal	Santa Cruz	sc-9989	1:100
α-VEGFR2	goat	polyclonal	R&D Systems	AF644	1:100
DAPI			Sigma	D9542	1:500

b

Secondary antibodies	Species	Clone	Company	Catalog #	Dilution
α-goat-AF-488	donkey	polyclonal	Invitrogen	A11057	1:500
α-mouse-AF-488	donkey	polyclonal	Invitrogen	A21202	1:500
α-mouse-AF-546	donkey	polyclonal	Invitrogen	A10036	1:500
α-rabbit-AF-546	donkey	polyclonal	Invitrogen	A10040	1:500
α-rabbit-AF-647	donkey	polyclonal	Invitrogen	A31573	1:500

c

Conjugated primary antibodies	Species	Clone	Company	Catalog #	Dilution
α-CD45-BV421	rat	monoclonal	Biolegend	103134	1:100
α-Ter119-BV605	rat	monoclonal	Biolegend	116239	1:100
α-CD31-PerCP/ Cy5.5	rat	monoclonal	Biolegend	102420	1:50
α-CD326-PE/Cy7	rat	monoclonal	Biolegend	118216	1:50
α-CD140a-PE/Cy7	rat	monoclonal	eBioscience	25-1401-82	1:100
α-CD140b-APC	rat	monoclonal	eBioscience	17-1402-82	1:25
α-Podoplanin-eFluor660	hamster	monoclonal	eBioscience	50-5381	1:100
α-CD208-A647	rat	monoclonal	Dentrics	DDX0192A647-100	1:50