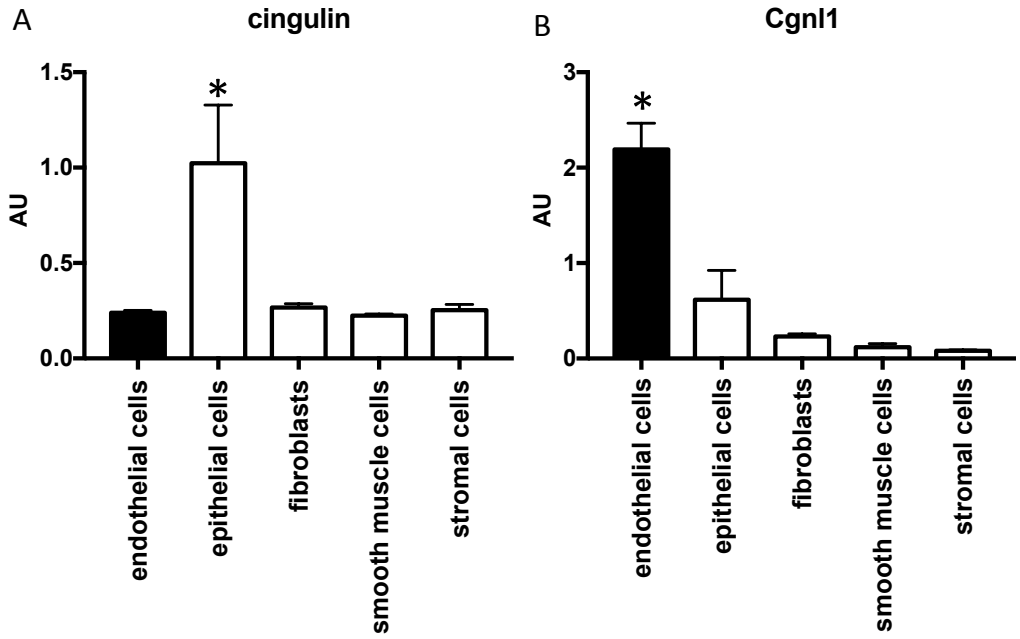
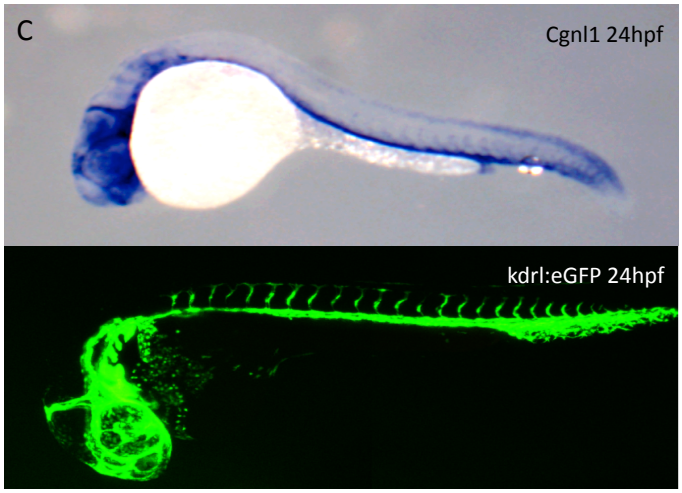
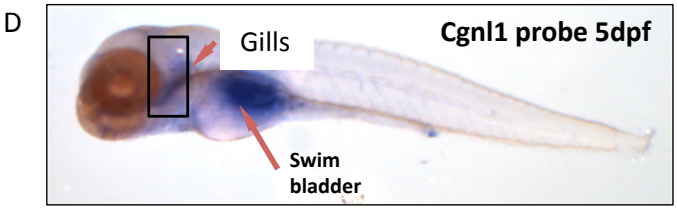
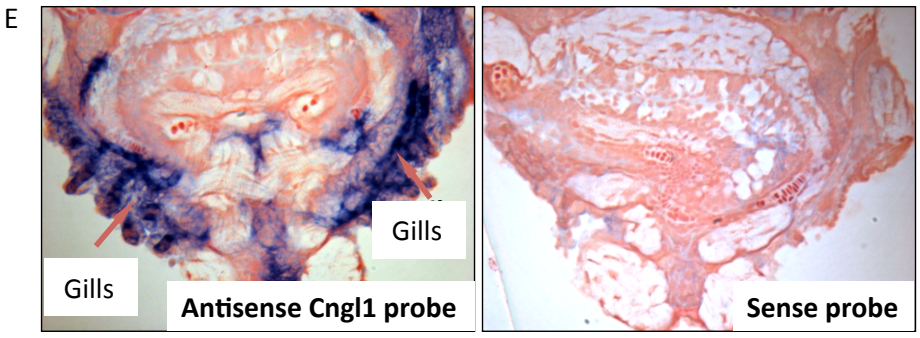
Supplemental data

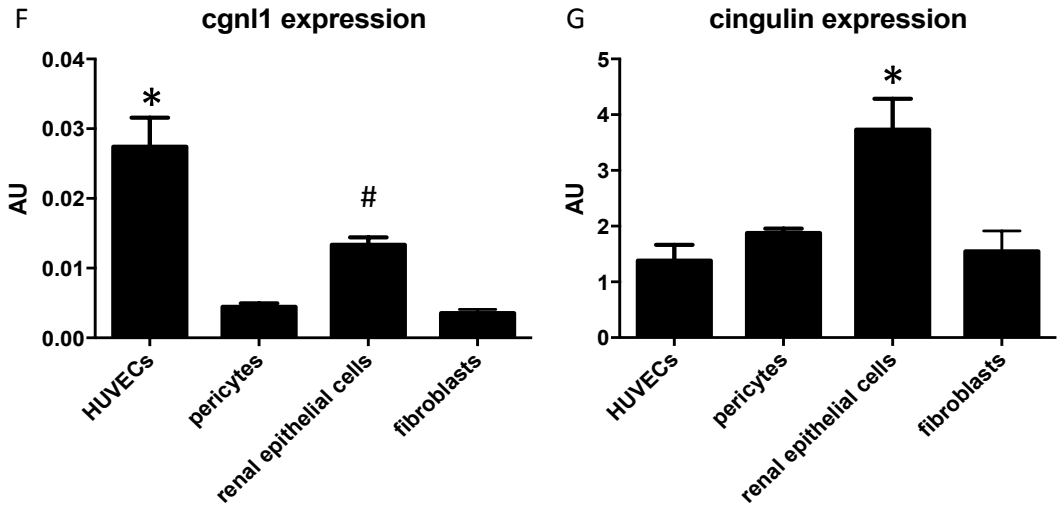
Results



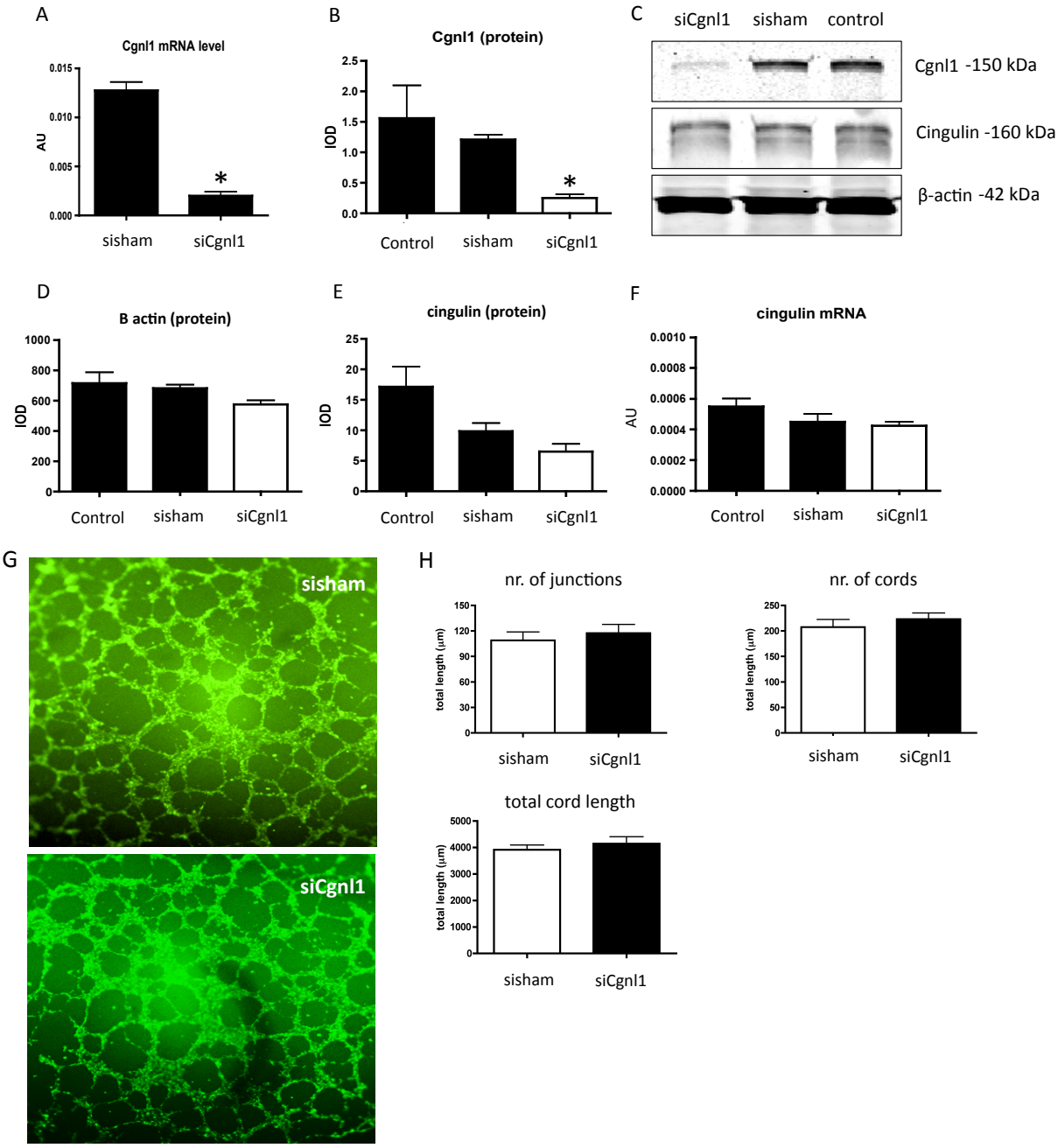








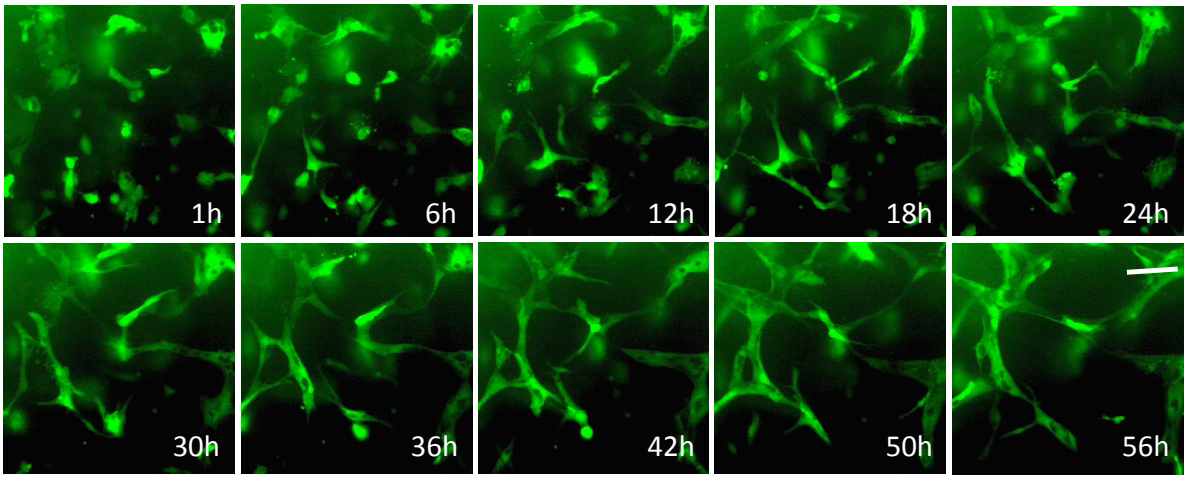
S.Figure 1: Data-mining of GEO-datasets show mRNA expression of different types of cultured human cells, including endothelial cells, epithelial cells, fibroblasts, smooth muscle cells and stromal cells, derived from different organs. Bar graphs indicate the expression levels of cingulin (A) and Cgnl1 (B). Shown are values of the mean ± SEM in arbitrary units (AU). *p<0.05 versus other cell types. (C) Upper image shows typical result of *in situ* hybridization (ISH) using an anti-sense probe specific for the zebrafish orthologue of Cgnl1 in developing zebrafish larvae at 24 hpf. The observed ISH pattern of the Cgnl1 anti-probe is very similar to the vascular GFP pattern (GFP expression driven by vascular kdrl promoter) observed in Tg(kdrl;eGFP)^{y1} zebrafish line at similar time point, shown in lower image. Lower and upper images do not display the same specimen. (D) Typical result of ISH using an anti-sense probe specific for the zebrafish orthologue of Cgnl1 in developing zebrafish larvae at 5 dpf. (E) Cross sections of the head region (gill area) showing the signal after ISH with anti-sense (Cngl1 detection) and sense (control) probe. Arrows point to Cngl1 ISH signal in gills. QPCR analysis of (F) Cgnl1 and (G) cingulin expression in human cells, including human venous endothelial cells (HUVECs compared to non-relevant cell types (pericytes, renal epithelial cells, and fibroblasts). Data obtained from 3 separate experiments, 2 samples per experiment. For F, *p<0.05 HUVECs versus pericytes, renal epithelial cells, and fibroblasts. $^{\#}$p<0.05 renal epithelial cells versus HUVECs, pericytes and fibroblasts. For G, *p<0.05 renal epithelial cells versus HUVECs, pericytes and fibroblasts. Values represent mean target/ house keeping gene ratio in AU ± SEM. One-way ANOVA.



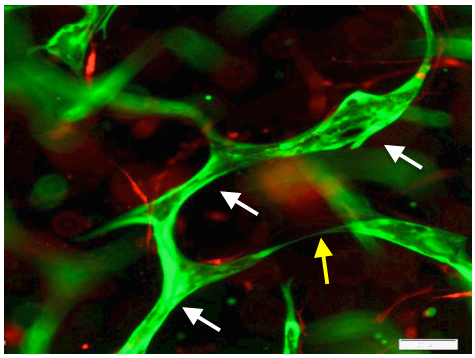
S.Figure 2: Validation of knockdown efficiency. (A) *In vitro* transfection of HUVECs Cgnl1-targeting siRNA followed by qPCR analysis at 2 days post-transfection demonstrates effect of Cgnl1 silencing on Cgnl1 expression compared to nontargeting scrambled siRNA (sisham) transfected HUVECs (~80% reduction) n=4. Student's t-test. (B) Western blot analysis of Cgnl1 protein signal in siCgnl1 as compared to sisham treated HUVECs or non-transfected cells. (C) A representative blot is shown of 3 separate experiments. (D) Quantified protein levels of ß actin loading control in the different groups. Effect of siRNA mediated knockdown of Cgnl1 on (E) cingulin protein (F) or mRNA levels. For protein quantification, values represent mean integrated optical

density (IOD) ± SEM corrected for β actin loading controls. For mRNA quantification, values represent mean target/ house keeping gene ratio in arbitrary units (AU) ± SEM. *p<0.05 siCgnl1 versus control and sisham. One-way ANOVA. (G) Representative result of a cord formation assay in standard 2D Matrigel following Cgnl1 silencing or sham siRNA transfection in HUVECs. HUVECs were visualized by Calcein-AM uptake. 4X magnification. (H) Quantitative analysis of the Matrigel assays shows effect of Cgnl1 silencing on the number of junctions, cords, and total cord length compared to sisham-treated HUVECs. Data obtained from 3 individual experiments with >8 wells analyzed per group. Values represent means ± SEM. Student's t-test.

A

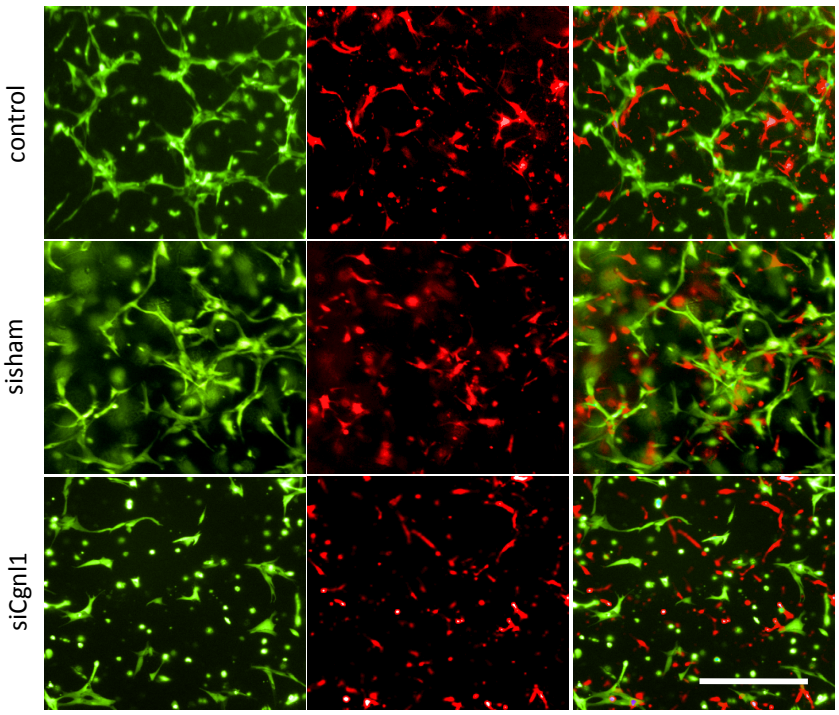


B

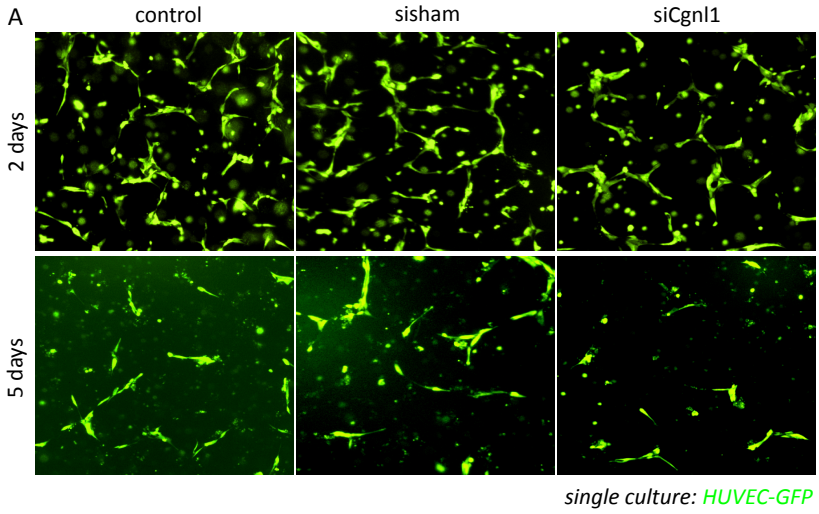


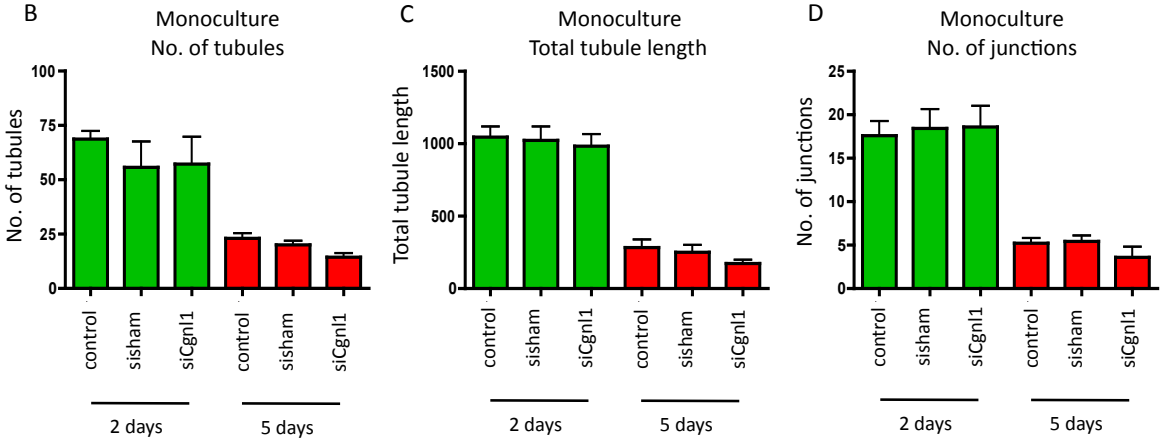
HUVEC-GFP = green
pericytes-RFP = red

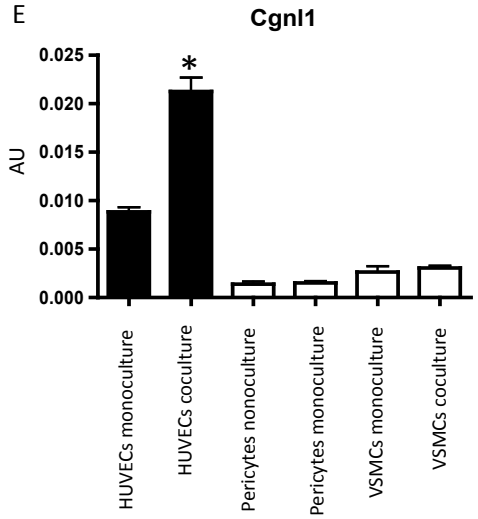
C

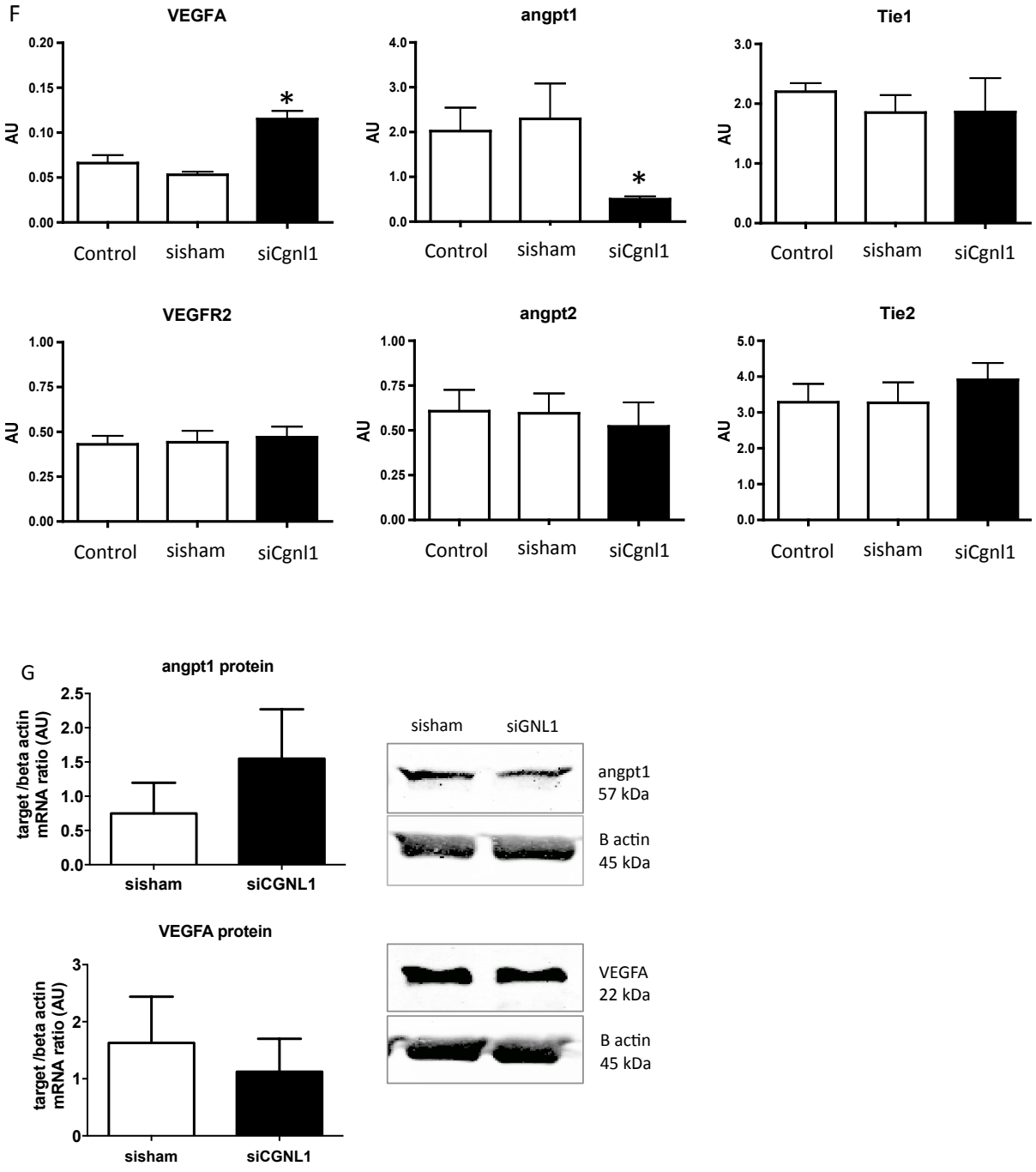


S.Figure 3: (A) Time series of a time lapse movie, showing angiogenesis in the 3D collagen coculture assay. Pericytes are present but not labelled. HUVECs are labelled with GFP marker. Indicated are hours post initiation of the assay. Scale bar represents 25 μm. (B) High magnification micrograph demonstrates lumenized vascular (GFP+) structures with pericyte (RFP+) coverage. Arrows indicate open (white) and closed (yellow) lumen areas. Scale bar represents 25 μm. (C) Representative results at day 2 in 3D collagen matrix coculture following Cgnl1 silencing or sham siRNA transfection in HUVEC-GFP (green). Pericytes are marked by RPF (red). Scale bar represents 100 μm.


A
control
sisham
siCgnl1
2 days
5 days
single culture: HUVEC-GFP

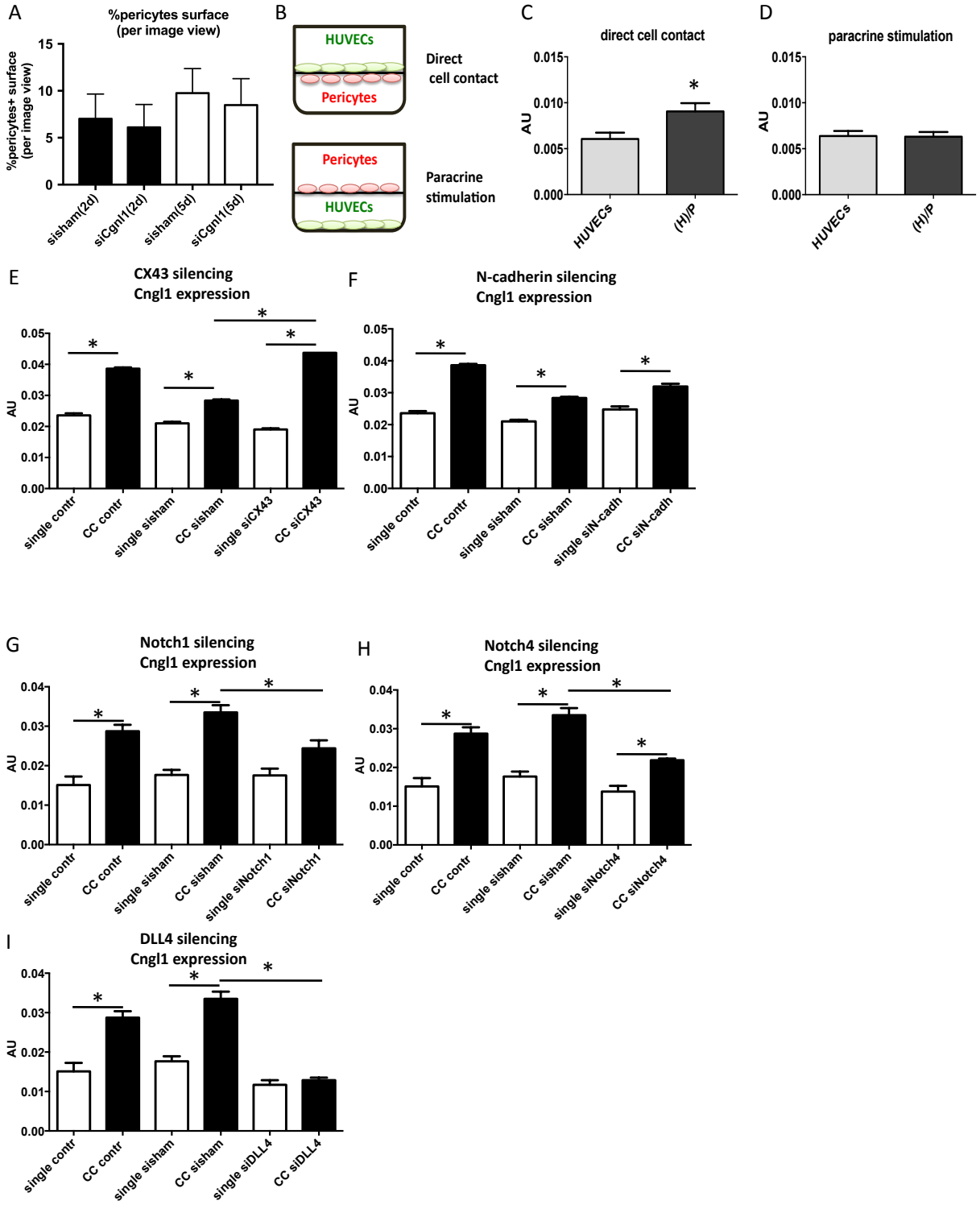

B
Monoculture
No. of tubules
No. of tubules
100
75
50
25
0
control
sisham
siCgnl1
control
sisham
siCgnl1
2 days
5 days
C
Monoculture
Total tubule length
Total tubule length
1500
1000
500
0
control
sisham
siCgnl1
control
sisham
siCgnl1
2 days
5 days
D
Monoculture
No. of junctions
No. of junctions
25
20
15
10
5
0
control
sisham
siCgnl1
control
sisham
siCgnl1
2 days
5 days


E
Cgnl1
AU
0.025
0.020
0.015
0.010
0.005
0.000
*
HUVECs monoculture
HUVECs coculture
Pericytes nonoculture
Pericytes monoculture
VSMCs monoculture
VSMCs coculture



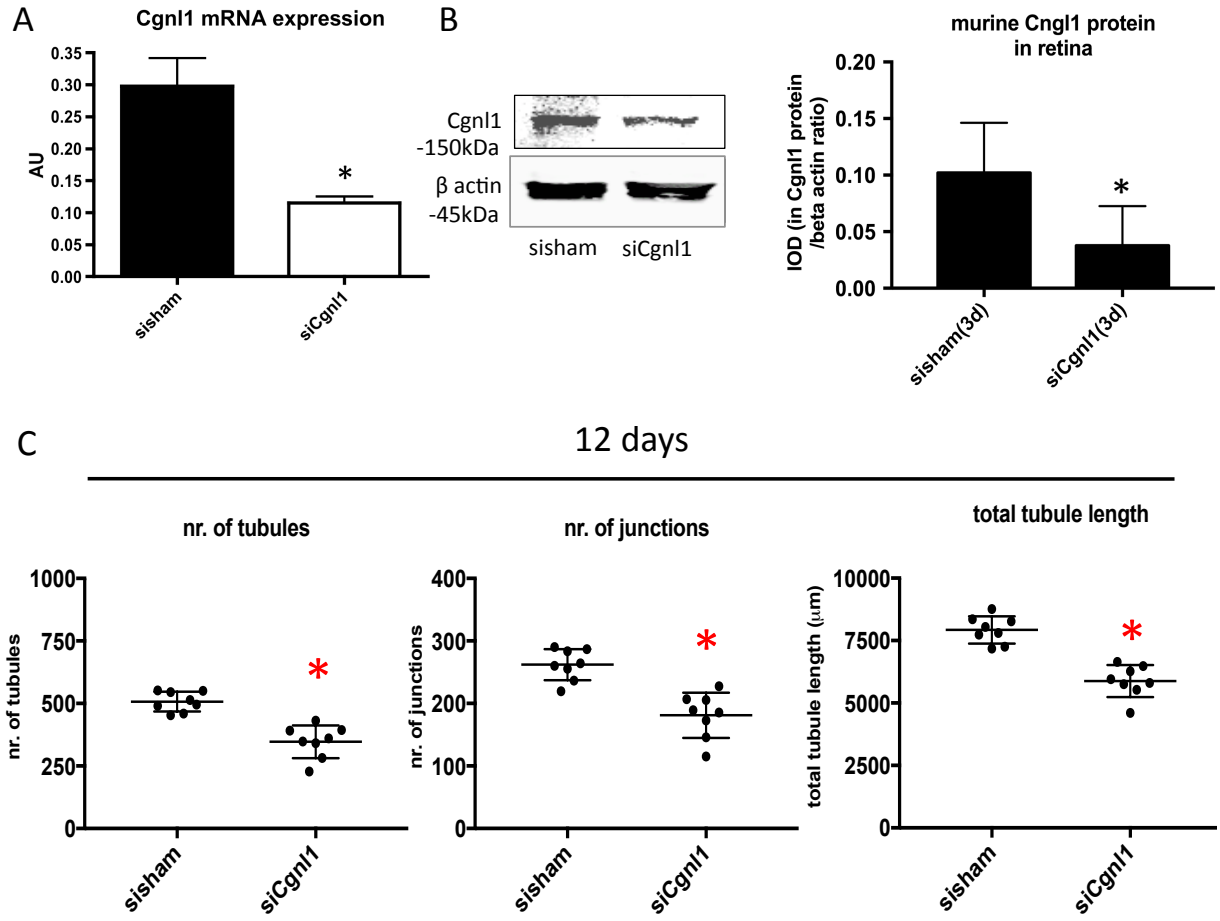
S.Figure 4: (A) Representative results at day 2 and 5 in 3D collagen assay without pericyte coculture, following Cgnl1 silencing or sham siRNA transfection in HUVEC-GFP. HUVECs are marked by GFP (green). 20X magnification. Quantitative analysis of assay results shows the effect of Cngnl1 silencing on the number of (B) tubules, (C) total tubule length, and number of (D) junctions compared to sisham-treated and non-transfected HUVEC-GFP in monoculture conditions. Data obtained from 3 individual experiments,

with >8 wells analyzed per group, per experiment. Values represent means target/ house keeping gene ratio in AU ± SEM. Red bars indicate data obtained from 5 days coculture, and green bars indicate data obtained from 2 days coculture. One-way ANOVA for comparisons within one time point. (E) QPCR analysis of Cngnl1 mRNA levels in HUVEC monocultures and HUVECs cocultured with mural cells (pericytes or VMSCs). Values represent means ± SEM. *p<0.05 HUVECs coculture versus other conditions. One-way ANOVA. (F) Cgnl1 silencing induced changes in endothelial expression profile of VEGFA, angpt1, Tie1, VEGFR2, angpt2, Tie2. Values represent mean target/ house keeping gene ratio in AU ± SEM. *p<0.05 versus control and sisham. Data obtained from 3 different experiments with 2 samples per experiment. One-way ANOVA. (G) Quantified western blot results for VEGFA and angpt1 protein levels. Shown are representative immuno blots. Values represent mean integrated optical density (IOD) ± SD corrected for β actin loading controls. (n=4). Student's t-test.

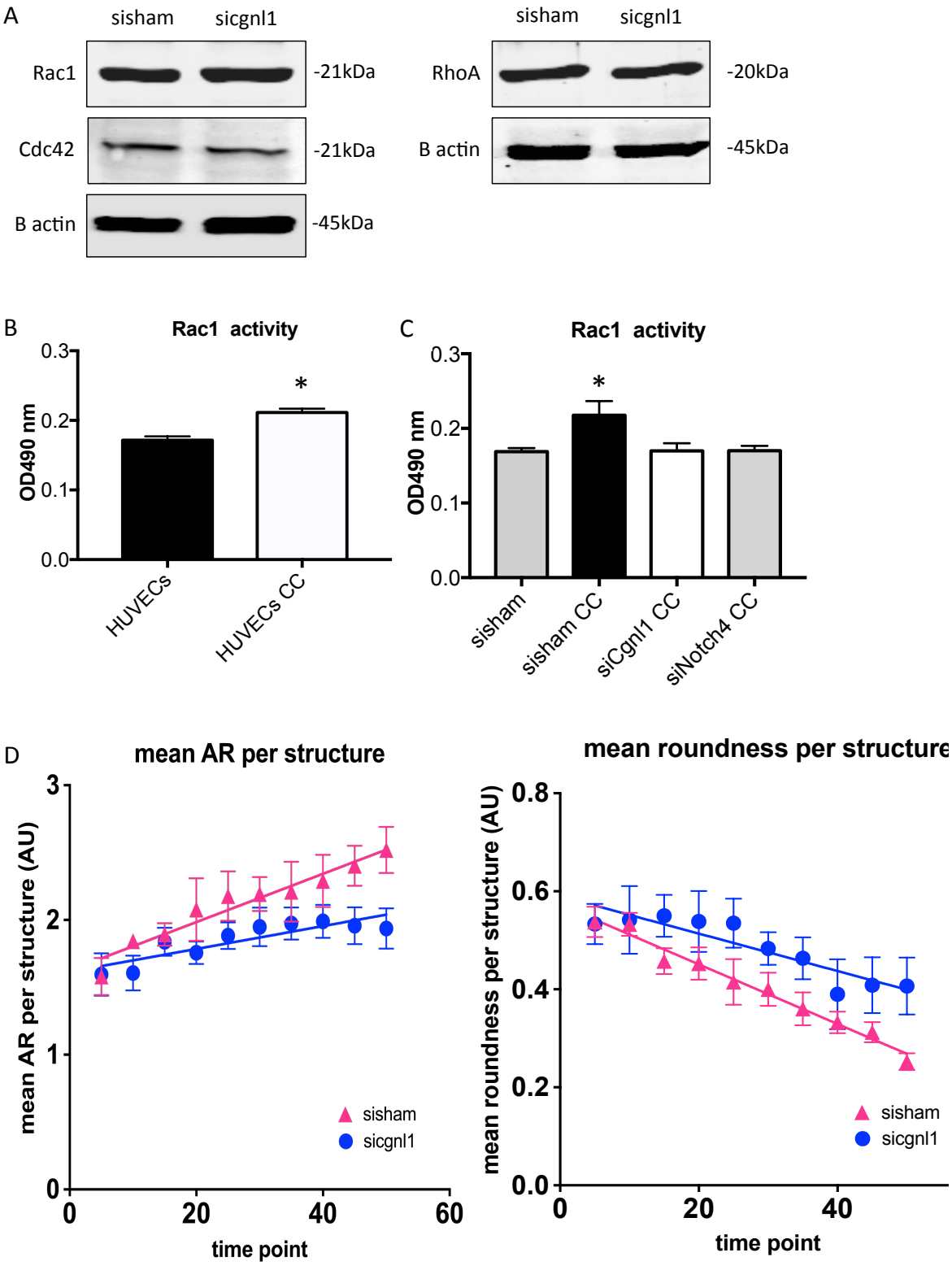


S.Figure 5: (A) Quantified results of %pericyte RFP surface per image view at day 2 (black bars) and day 5 (white bars) in the 3D collagen coculture assay, following Cgnl1 silencing or sham siRNA transfection in HUVEC-GFP. Values represent means ± SD (N>5). *p<0.05 siCgnl1 versus time-corresponding control and sisham. Student's t-test within 1 time point. (B) Diagrams showing the experimental setups of direct contact or paracrine stimulation of HUVECs by pericytes. (C) QPCR evaluation of Cngl1 mRNA

levels in HUVECs without (HUVECs) and with direct cell contact with pericytes ((H)/P). (D) QPCR evaluation of Cngl1 mRNA levels in HUVECs without (HUVECs) and with paracrine stimulation by pericytes ((H)/P). For C and D: n=3 *p<0.05 versus HUVECs without pericyte coculture. Values represent mean target/house keeping gene ratio in AU ± SEM. Student's t-test. (E) QPCR evaluation of Cngl1 mRNA levels in HUVECs without (single) and with direct contact stimulation by pericytes (CC) in HUVECs and pericytes untreated or treated with sisham or siCX43. (F) QPCR evaluation of Cngl1 mRNA levels in HUVECs without (single) and with direct contact stimulation by pericytes (CC) in HUVECs and pericytes untreated or treated with sisham or siN-cadherin. (G) QPCR evaluation of Cngl1 mRNA levels in HUVECs without (single) and with direct contact stimulation by pericytes (CC) in HUVECs and pericytes untreated or treated with sisham or siNotch1. (H) QPCR evaluation of Cngl1 mRNA levels in HUVECs without (single) and with direct contact stimulation by pericytes (CC) in HUVECs and pericytes untreated or treated with sisham or siNotch4. (I) QPCR evaluation of Cngl1 mRNA levels in HUVECs without (single) and with direct contact stimulation by pericytes (CC) in HUVECs and pericytes untreated or treated with sisham or siDLL4. For E-I: n>3 *p<0.05 Values represent mean target/house keeping gene ratio in AU ± SEM. One-way ANOVA.

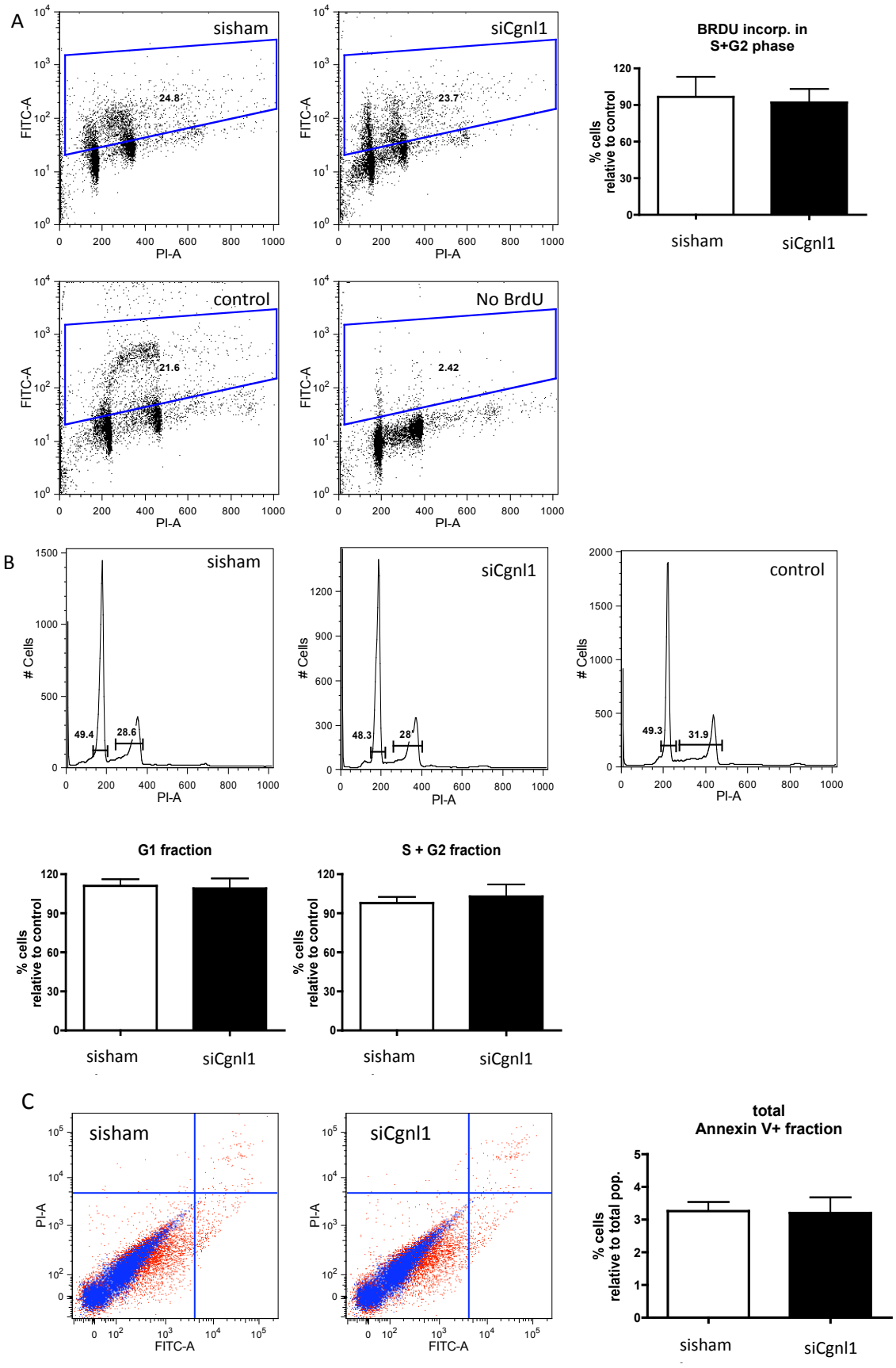


S.Figure 6: (A) QPCR validation of efficient knockdown in Accel siCgnl1 injected murine retina versus sisham treated samples. n=6 *p<0.05 versus sisham treated retinas. Values represent mean target/ house keeping gene ratio in AU ± SEM. Student's t-test. (B) Quantified western blot results for murine Cngl1 protein levels. Shown are representative immuno blots. Values represent mean integrated optical density (IOD) ± SD corrected for β actin loading controls. (n=6). Student's t-test. (C) Quantified results of retinal vascularization at day 12 after siCgnl1 injection at day 8 as compared to sisham-injected controls. Mean ± SD per group is indicated in scatter plots. *p<0.05 versus sisham-injected eyes. n=8 pups per group. Student's t-test.



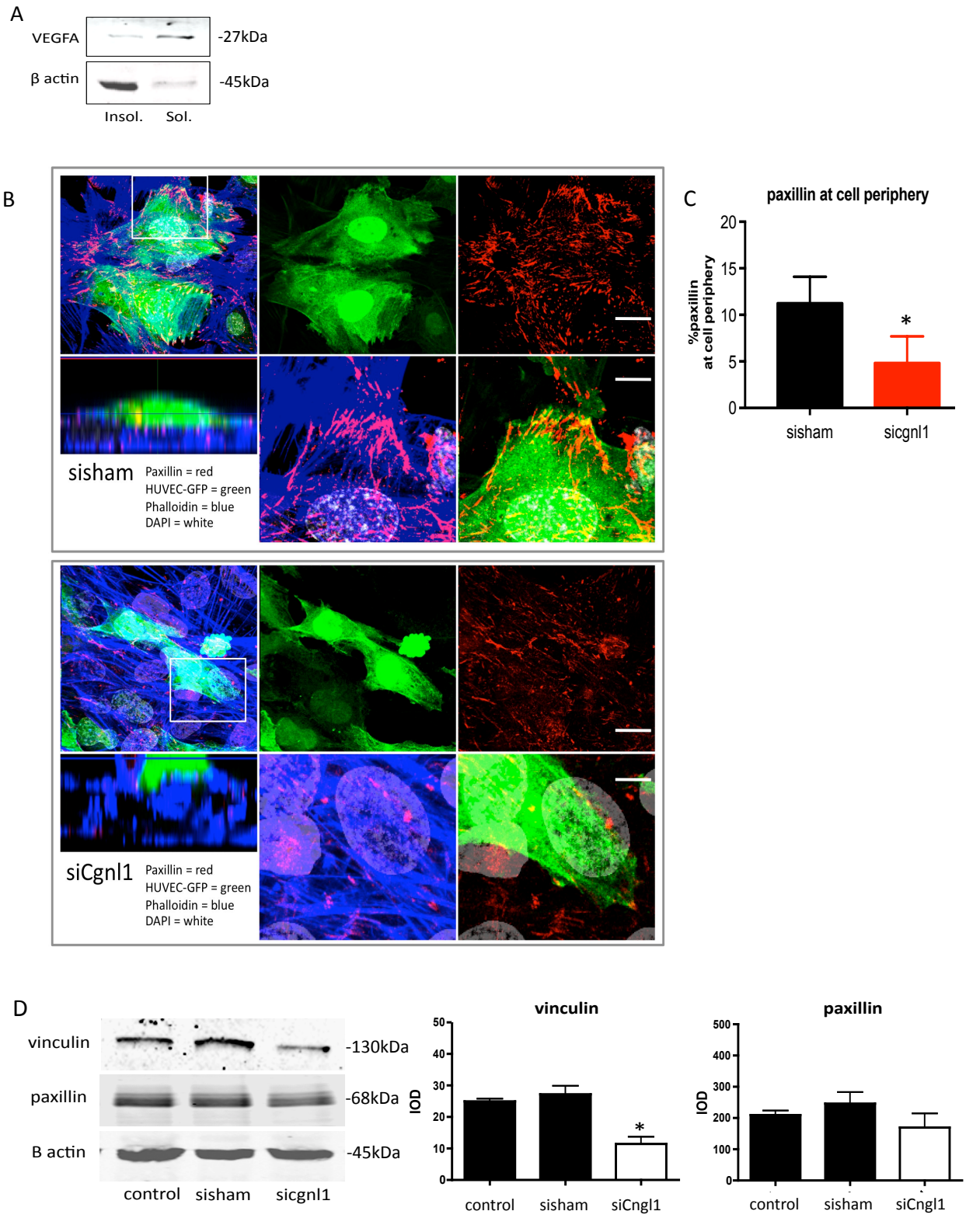
S.Figure 7: (A) Representative western blot for Rac1, Cdc42, RhoA, and β actin, of sisham and siCgnl1 treated HUVECs. (B) Chemo-luminescence measurement of the GTP-bound small G-proteins in cell lysates from HUVECs in single and pericyte coculture

conditions after 20 minutes of serum activation. Shown are the levels of GTP-Rac1. (C) Chemo-luminescence measurement in cell lysates from sisham, siCngl1, and siNotch4, transfected HUVECs cocultured with pericytes after 20 minutes of serum activation. Shown are the levels of GTP-Rac1. For B and C: Values represent means ± SEM. *P<0.05 other conditions, n=4. Student's t-test (B) and One-way ANOVA (C). (D) Serial images of time-lapse imaging of HUVECs GFP cells seeded in 3D collagen coculture with pericytes in siCgnl1 and sisham group. Different time points (T) are shown. 1 time point represents 1 hour post seeding. Quantification of aspect ratio (AR) and roundness per HUVEC-GFP+ structure (from T = 0 to T = 50 post seeding). Each symbol represents average ± SD of 5 time points. Each time point is composed of 5 individual measurements. p<0.0001 for AR and roundness, siCngl1 versus sisham group, linear regression analysis, overall comparison.



S.Figure 8: (A) Cell proliferation analysis was conducted by measuring BRDU

incorporation using flow cytometry. (A) Representative dot blot graphs are shown for sisham, siCgnl1 transfected, and non-transfected HUVECs. The Y-axes show the BRDU-FITC signal, the X-axes the PI signal. A negative control (HUVECs without BRDU incorportation) was used to define the gate settings. No difference was observed in BRDU signal was observed in the S+G2 phase cell population between the different groups (n=3, values represent means ± SEM, student's t-test). Data shown are measured 4 hours post activation. (B) Analysis of cell cycle distribution of sisham, siCgnl1 transfected, and non-transfected HUVECs. Representative histograms show the G1 and the S+G2 region in the different groups. Quantification of the percentages of cells in the G_1, and S+G_2 fractions show no effect of Cgnl1 silencing on cell cycle progression n=3, values represent means ± SEM, student's t-test, data shown are measured at 12 hours post activation. (C) Flow cytometry analysis of apoptosis in HUVECs transfected with sisham and siCgnl1. Dead cells are PI+, apoptotic cells are Annexin V+, and alive cells are PI-/Annexin V-. Quantification of the percentage of Annexin V+ cells show no difference between siCgnl1 or sisham treated HUVECs. n=3, values represent means ± SEM, student's t-test, data shown are measured at 4 hours post activation.


A
VEGFA
-27kDa
β actin
-45kDa
Insol.
Sol.
B
sisham
Paxillin = red
HUVEC-GFP = green
Phalloidin = blue
DAPI = white
siCgnl1
Paxillin = red
HUVEC-GFP = green
Phalloidin = blue
DAPI = white
C
paxillin at cell periphery
%paxillin at cell periphery
20
15
10
5
0
*
sisham
sicgnl1
D
vinculin
paxillin
B actin
-130kDa
-68kDa
-45kDa
control
sisham
sicgnl1
vinculin
IOD
50
40
30
20
10
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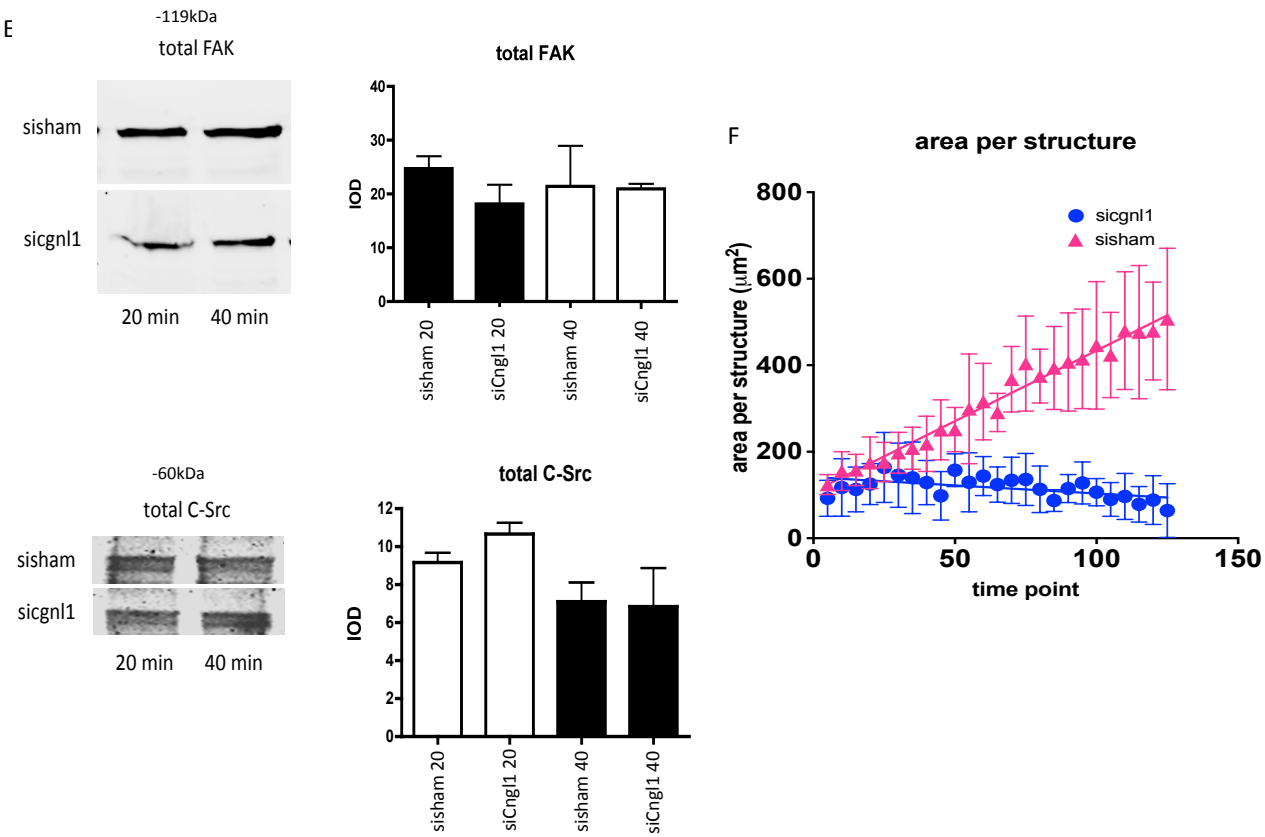
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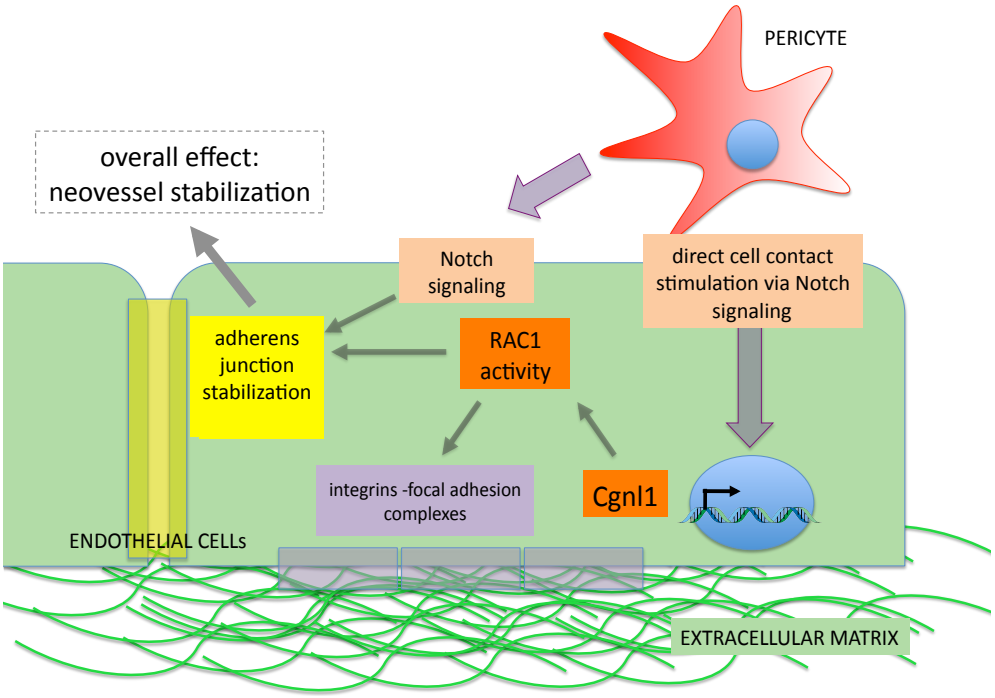
20 min
40 min
total C-Src
IOD
sisham 20
siCngl1 20
sisham 40
siCngl1 40
F
area per structure
area per structure (μm²)
time point
sicgnl1
sisham

S.Figure 9: (A) Representative western blot results of 2 different experiments for VEGFA and β actin detection in Triton-X insoluble fraction (the actin cytoskeleton associated compartment) versus the soluble fraction. (B) Representative images of intracellular staining of sisham (upper panel) and siCgnl1 (lower panel) treated HUVECs-GFP and human pericytes for paxillin (red signal), DAPI (white signal), and actin cytoskeleton (phalloidin blue signal). For both sisham and siCgnl1 panels: Upper row of images, scale bar represents 5 μm. Lower row of images; first image (left); Z stack showing HUVECs-GFP on top of phalloidin blue+ pericytes. 2nd and 3rd images; high magnification images of showing paxillin distribution HUVEC-GFP. Scale bar represents 2.5 μm. (C) Quantitative results of %paxillin distribution at cell borders of HUVECs-GFP per image view adjusted for cell numbers at 60 minutes post seeding on top of pericyte layer. Values represent means ± SD. *$p<0.05$ versus sisham. Data obtained from 4 different experiments with analysis of 12 different micrographs per group per experiment. Student's t-test. (D) Representative western blot results for vinculin, paxillin and β actin detection in siCngl1 versus sisham and non-treated control HUVECs. Graphs (right) show quantified results of immuno blotting of vinculin and paxillin. Values represent mean integrated optical density (IOD) ± SEM corrected for β actin loading controls. (n=3). One-way ANOVA. (E) Western blot analysis of total FAK and C-Src protein levels at 20 and 40 minutes after seeding of siCgnl1-treated compared to sisham-treated HUVECs. Graphs show quantified results. Values represent mean integrated optical density (IOD) ± SEM corrected for β actin loading controls. n=3. Student's t-test for comparison within corresponding time point. β actin protein level was assessed as a loading control and did not differ between the control, sisham and siCgnl1 samples (data not shown). (F) Quantification of area per HUVEC-GFP+ structure (from T = 0 to T = 125 post seeding). Each symbol represents average area per structure ± SD of 5 time points. Each time point is composed of 5 individual measurements. $p<0.0001$, siCngl1 versus sisham group, linear regression analysis, overall comparison.



S.Figure 10: A proposed working mechanism for Cngl1 in which pericyte induced upregulation of Cngl1 in endothelial cells via Notch signalling promotes the formation of strong Ve-cadherin adherens junctions via Rac1 activation. Cross cell type Notch signalling may also provide adherens junction stabilization via other unknown mechanisms. Simultaneously, Cngl1 mediated Rac1 activation stimulates assembly of integrins-focal adhesion complexes. Combined, formation of both strong adherens junctions and focal adhesions ensures stabilization and further elongation of neovascular tubules.

Supplemental material and methods

Small GTPase activation assay

For measuring small GTPase activity, HUVECs were serum starved in EBM-2/0.2% FCS overnight, followed by cell seeding in full supplemented EGM2 medium on gelatin/collagen coated surface for 20 and 40 minutes, before cells were harvested in NP40 buffer. proGTP-RhoA, Rac1, and cdc42 activation levels in the cell lysaters were measured using the G-lisa detection system (Tebu-Bio, Netherlands) following the manufacturer's protocol.

Flow cytometric analysis

BRDU incorporation assay: HUVECs were synchronized in the G_0/G_1 phase by serum deprivation in EGM-2/0.2% FCS for 12 hours, followed by incubation in EGM-2 with 10 μM BRDU for 4 hours at 37°C/5% CO2. Afterwards, cells were harvested, washed in PBS, fixed in 70% ethanol, treated with pepsin, and stained for BRDU incorporation using a direct FITC labeled mouse antibody directed against BRDU (Abcam, UK) and propidium-iodide (PI 1:300), followed by FACS analysis (FACScanto, BD Biosciences, The Netherlands) and subsequent data analysis by use of Flowjo®-software (Tree Star inc., US).

Cell cycle analysis: Cells were harvested at 0, 4 and 12 hours post activation, fixed in 70% ethanol/PBS for 15 min on ice, stained with propidium-iodide (PI 1:300), and analyzed by flow cytometry (FACScanto, BD Biosciences, Netherlands) with subsequent data analysis by use of Flowjo®-software (Tree Star inc., US).

Apoptosis analysis: Cells were harvested at 0, 4 and 12 hours post activation, stained for Annexin V and PI signals using an Annexin V apoptosis detection kit (BD Biosciences, The Netherlands), followed by analysis of the samples by flow cytometry (FACScanto, BD Biosciences, Netherlands) and subsequent data analysis by use of Flowjo®-software (Tree Star inc., US).

In vitro assays

2D matrigel assay: HUVECs were seeded at a density of 3×10^4 cells/ml in 200 µl EGM2 medium in a 96-well plate on serum-reduced Matrigel (BD Biosciences, The Netherlands) and incubated for 24 hours. Viable cells were visualized by Calcein-AM uptake according

to the manufacturer's protocol (BD Biosciences, The Netherlands) and fluorescence microscopy.

3D collagen monoculture and coculture assay: HUVECs (GFP labelled) were either suspended alone (monoculture in 3D collagen matrix) or with pericytes (RFP labelled) in 2,5 mg/ml collagen type 1 and assays were performed confirm the protocol developed by Stratman et al[1]. Live imaging was conducted using the Incucyte live cell analysis system (Essen Bioscience, The Netherlands).

Cell adhesion assay: HUVECs were harvested with accutase and seeded at density of 10000 cells per well of a 12 wells plate on gelatine coated glass slides. After 10, 20, 30, 60, or 120 minutes of incubation at 37°C/5% CO2 in EGM2 medium, the cells were washed with PBS and fixed with 4% PFA, followed by Rhodamine Phalloidin staining, and/or immunofluorescent staining of the FA components, paxillin, vinculin, and FAK using the protocol that was earlier described.

Quantification of the 2D matrigel, monoculture, and ccoculture assay, and time lapse imaging data: Angiosys analysis software (Angiosystems, UK) was used for 2D matrigel, monoculture and coculture assay to determine mean and total tubule length, and the number of tubules and junctions per field of view. FIJI (Image J) was used to analyze AR, roundness and GFP+ structure area data from time lapse imaging.

SiRNA mediated gene silencing

Targeted knockdown of genes was achieved by transfer of a mix of 4 specific siRNAs sequences directed against the target mRNA (Smartpool, Dharmacon, The Netherlands) in 50-60% sub-confluent HUVEC cultures, at 3 days prior to inclusion in experiments. As a control, cells were transfected with a mix of 4 scrambled non-targeting siRNAs (Dharmacon, The Netherlands). siRNA transfection efficiency of >80% of HUVECs was achieved at 72 hours, as validated by FITC-labelled siRNA (siglow, Dharmacon, The Netherlands, data not shown). Adequate overexpression or knockdown of the target genes was validated by qPCR and western blot analysis at 2 and 3 days post transfection respectively.

Quantitative PCR and western blot analysis

RNA was isolated using the RNAeasy kit (Qiagen, The Netherlands) and was checked for quality and quantity by capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies, The Netherlands), followed by reverse transcription into cDNA. qPCR

reactions were performed by real-time assessment of the sybergreen signal using the iCycler iQ Detection System (Bio-Rad, The Netherlands). qPCR analysis was performed for the murine transcripts of Cgnl1, and for the human transcripts of Cgnl1, cingulin, VEGFA, VEGFR2, angtp1, angtp2, Tie1, and Tie2. Target mRNA expression levels are reported relative to the housekeeping genes, hypoxanthine guanine phosphoribosyl transferase (Hprt1) in murine samples, and β actin in the human samples, as previously described[2]. For Western blot analysis, samples were lysed in NP40 buffer, and analysed on a 1.5% SDS-PAGE gel followed by western blotting using 1:1000 rabbit anti-FAK, rabbit anti-FAK phosphoY397, rabbit anti-ß actin (Abcam, UK), rabbit anti-CSrc, rabbit anti-CSrc phospho Y418 (Sigma, Netherlands), 1:500 mouse anti-vinculin, and mouse anti-paxillin (Abcam, UK), and 1:500 mouse anti-paracingulin/Cgnl1 (Invitrogen, Netherlands) and 1:500 rabbit anti-cingulin (Atlas Antibodies, Netherlands) for protein detection. Protein bands were visualized using the Li-Cor detection system (Westburg, The Netherlands), as previously described[3-5]. Tissue extracts were separated in Triton-X soluble and insoluble fractions following a modified protocol of Lampugnani et al. before Western blot analysis[6].

Primary cell culture condition and intracellular staining

Primary human umbilical vein endothelial cells (HUVECs, Lonza, Netherlands) were cultured on gelatin-coated plates at 37°C/5% CO2 in EGM2 medium (EBM2 medium supplemented with commercial bullet kit and 2% FCS) with penicillin/streptomycin (Lonza, The Netherlands). Only cell cultures of passages 3-6 were used throughout the experiments. For intracellular staining, HUVECs were grown on coverslips and fixed in 4% paraformaldehyde (Sigma, Netherlands) for 5 minutes, followed by membrane permeabilization in 0.2% tritonX/PBS for 10 minutes, incubation with 1:100 mouse anti-Cgnl1 (Invitrogen, Netherlands), and subsequent detection of the signal by using a 1:200 FITC labeled goat anti-mouse IgG antibody (Invitrogen, Netherlands). For dubbelstaining, the Cgnl1 staining protocol was followed by incubation with Rhodamine Phalloidin (Sigma, Netherlands) for visualization of the actin cytoskeleton. For the detection of FA components, fixed and permeabilized cells are incubated with 1:100 mouse anti-vinculin, or mouse anti-paxillin antibodies (Abcam, UK), followed by detection of the signal using a 1:200 dilution of a FITC labelled goat anti-mouse IgG antibody (Invitrogen, Netherlands) and subsequent Rhodamine Phalloidin staining (Sigma, Netherlands). For detection of adherens junction protein Ve-cadherin, fixed and permeabilized cells are incubated with

1:100 mouse anti-Ve-cadherin followed by detection using a 1:200 dilution of FITC labelled goat anti mouse IgG antibody (Invitrogen, Netherlands) followed by Rhodamine Phalloidin staining (Sigma, Netherlands). Coverslips were mounted using Vectashield/DAPI (Brunschwig, Netherlands) and stained cells were imaged by fluorescence microscopy (Carl Zeiss Inc., Netherlands). Quantification of Ve-cadherin at AJs was conducted by quantifying Ve-cadherin+ area per image view, presetting the dectection threshold on high to capture only the high fluorescent signal of Ve-cadherin accumulated at the AJs, excluding the weak Ve-cadherin signal in the cytoplasma. The obtained values were corrected for the number of cells per view. For 2D coculture staining, pericytes were seeded 24 hours prior adhesion of HUVECs-GFP on top of coverslips. Pericytes were visualized by phalloidin-blue staining, imaging of Ve-cadherin and paxillin distribution in HUVECs-GFP was monitored by confocal microscopy, using Z-stack analysis of Ve-cadherin and paxillin immuno signals in GFP+ cells.

References

1. Stratman AN, Malotte KM, Mahan RD, Davis MJ and Davis GE. Pericyte recruitment during vasculogenic tube assembly stimulates endothelial basement membrane matrix formation. *Blood*. 2009;114:5091-101.
2. Cheng C, Tempel D, Oostlander A, Helderman F, Gijsen F, Wentzel J, van Haperen R, Haitsma DB, Serruys PW, van der Steen AF, de Crom R and Krams R. Rapamycin modulates the eNOS vs. shear stress relationship. *Cardiovasc Res*. 2008;78:123-9.
3. Cheng C, Tempel D, Den Dekker WK, Haasdijk R, Chrifi I, Bos FL, Wagtmans K, van de Kamp EH, Blonden L, Biessen EA, Moll F, Pasterkamp G, Serruys PW, Schulte-Merker S and Duckers HJ. Ets2 determines the inflammatory state of endothelial cells in advanced atherosclerotic lesions. *Circ Res*. 109:382-95.
4. Cheng C, Noordeloos AM, Jeney V, Soares MP, Moll F, Pasterkamp G, Serruys PW and Duckers HJ. Heme oxygenase 1 determines atherosclerotic lesion progression into a vulnerable plaque. *Circulation*. 2009;119:3017-27.
5. Cheng C, Noorderloos M, van Deel ED, Tempel D, den Dekker W, Wagtmans K, Duncker DJ, Soares MP, Laman JD and Duckers HJ. Dendritic cell function in transplantation arteriosclerosis is regulated by heme oxygenase 1. *Circ Res*. 106:1656-66.
6. Khan TA, Bianchi C, Araujo E, Voisine P, Xu SH, Feng J, Li J and Sellke FW. Aprotinin preserves cellular junctions and reduces myocardial edema after regional ischemia and cardioplegic arrest. *Circulation*. 2005;112:I196-201.