pcdh18a

Fig. S1. Expression of *pcdh18a* during the development of SCPs

(A-C) Whole-mount in situ hybridization for *pcdh18a* expression at 23, 26-27 and 36 hpf. sc, spinal cord; n, notochord. Scale bars, 50 μm. (A'-C') Magnified images of the areas indicated by dashed squares in (A-C), using VE-DIC microscopy. (A') In addition to the spinal cord (arrow), *pcdh18a* expression is first observed at the ventral side of the 5th somite from the tailbud (S_5), and continuously found in subsequent S_6 and older (more rostral) somites. It then persists in migrating SCPs (B', arrowheads), and in SCP derived stromal cells by 36 hpf (C', arrowheads).
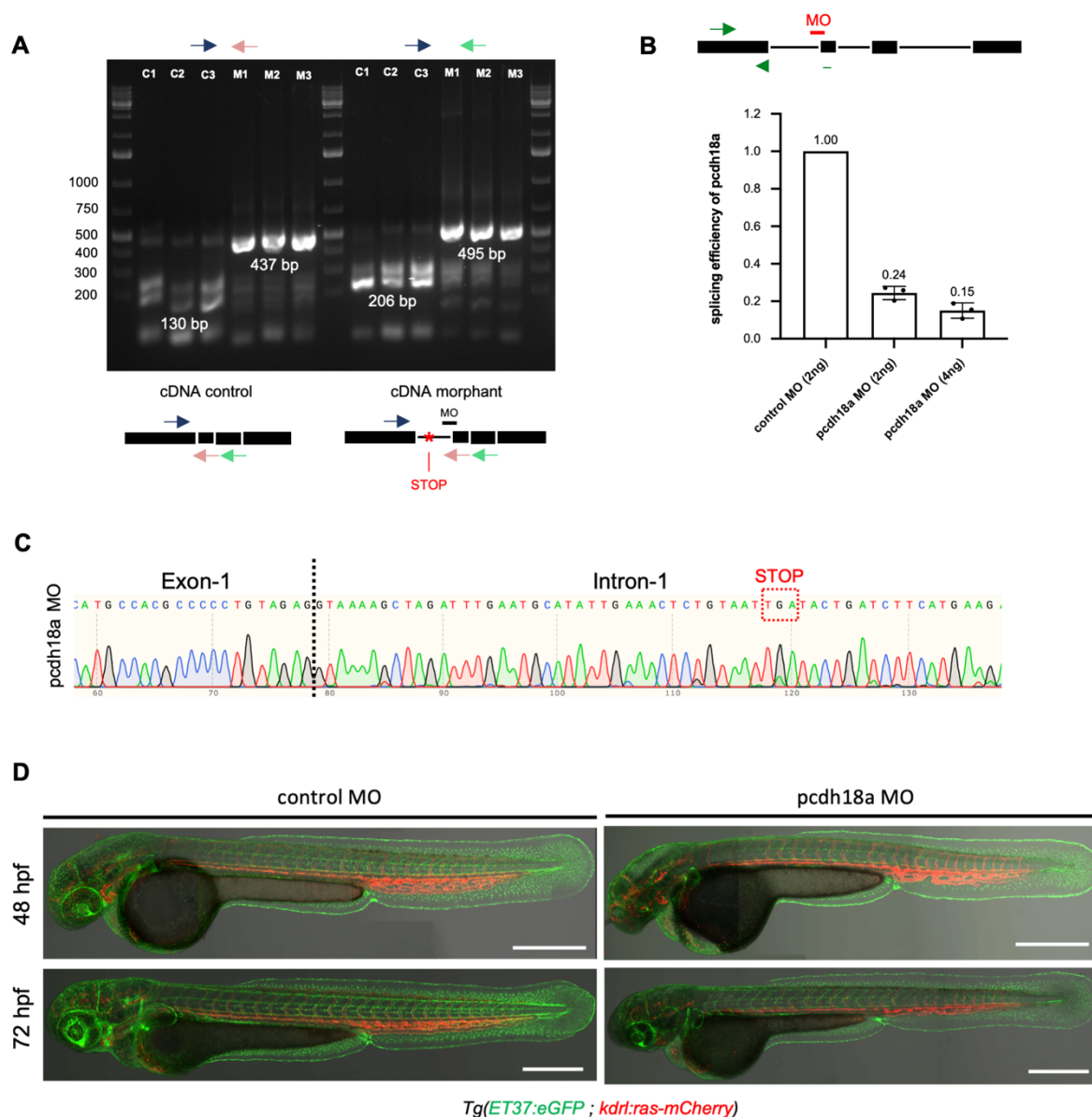
Fig. S2. A Pcdh18a i1-e2 MO efficiently induces intron 1 retention, resulting in truncation of most of the Pcdh18a cytoplasmic domain (A) Representative agarose gel showing pcdh18a mRNA products from the tail of control (C1-C3) and pcdh18a i1e2 morphant (M1-M3) embryos, as measured by RT-PCR using primers targeting exons 1 (blue arrows), 2 (pink arrows) and 3 (green arrows) indicated in diagrams of the zebrafish *pcdh18a* gene. Samples are biological replicates from the same experiment, each from 30-40 tails of 24 hpf embryos. Injection of the i1e2 MO led to reduction of the normal amplicon (130 bp, primer set exon 1 and 2 and 206 bp, primer set exon 1 and 3) and induced a corresponding increase in variants (437 and 495 bp) evidencing retentio of the first intron of *pcdh18a*. **(B)** RT-qPCR on embryos injected with 2 ng of control MO versus 2 or 4 ng of pcdh18a MO, using pcdh18a specific primers in the first exon and spanning the junction of the first and second exons. Reverse primer binding and qPCR amplification are possible only when the first intron is correctly spliced out. Samples correspond to biological triplicates of 30-40 tails from 23-24 hpf embryos. A 4 ng injection of pcdh18a i1e2 MO prevents intron-1 splicing of 85% of the pcdh18a RNA. **(C)** Extract of cDNA sequencing of bulk amplicon obtained from pcdh18a morphants shown in A. A vertical dotted line indicates the exon1/intron1 boundary. Retention of the first intron in the mRNA induces a premature STOP in the first intron. **(D)** Comparison of overall morphology of control and pcdh18a MO injected *Tg(ET37:eGFP; kdrl:ras-mCherry)* embryos at 48 hpf and larvae at 72 hpf. Fluorescence signals are superimposed on the bright field image. Scale bars, 500 μm.
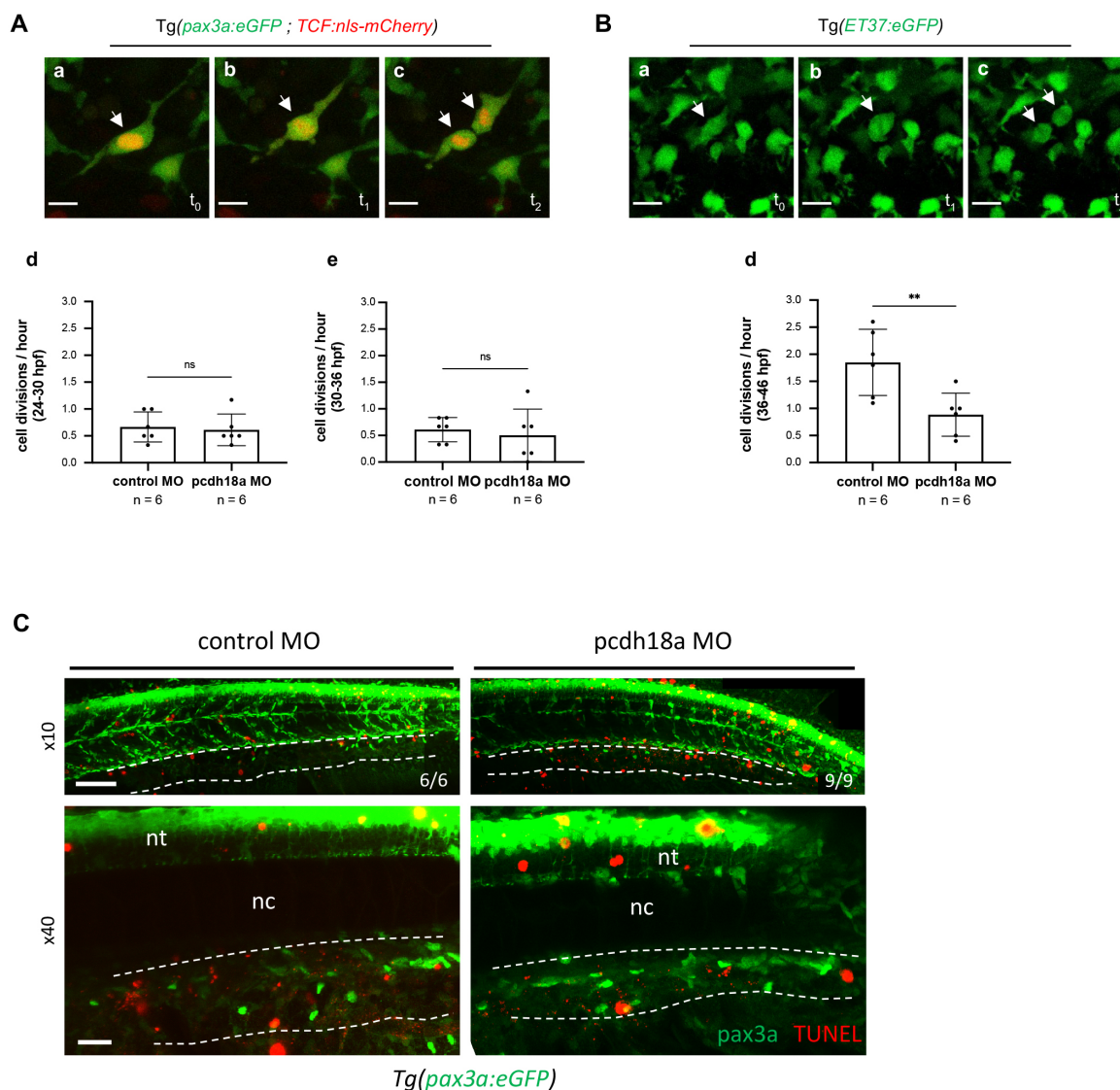
Fig. S3. Pcdh18a cytoplasmic truncation does not affect SCP division rate, nor causes their apoptosis.

(A) Counting of the number of SCP mitoses through time-lapse confocal imaging of Tg(*Pax3a:GFP*; *TCF:nls-mCherry*) during the period of SCP migration (24-36 hpf). **(a,b,c)** Confocal projections of control SCPs undergoing mitosis (arrows, t0: before, t1: during, t2: after mitosis). (d,e) Quantification of observed SCP mitoses in control and Pcdh18a-ΔCP106 morphant embryos, counted in an area spanning 5 caudal somites (d, from 24 to 30 hpf; e, from 30 to 36 hpf). Each dot represents the average number of observed mitoses per hour. n=6 for controls and morphants, from three independent experiments (mean±SD; ns, P>0.05; Student's t test). Scale bars, 10 μm. **(B)** Counting of SCP mitoses during the period of SCP settlement / maturation (36-46 hpf), through time-lapse confocal imaging of Tg(ET37:GFP) embryos. **(a,b,c)** Confocal projections of control SCPs undergoing mitosis (arrows, t0: before, t_1: during, t_2: after mitosis). **(d)** SCPs/SCs mitoses in control and morphant embryos were counted for 10 hours. Each dot represents the average number of mitoses per hour in a 5-somites wide area. n=6 for control and pcdh18a morphants from three independent experiments. (Mean±SD; **, P=0.0088; Student's *t* test). Scale bars, 10 μm. **(C)** TUNEL staining (red) at 48 hpf in the caudal region of Tg(*pax3a:eGFP*) control or pcdh18a MO injected embryos; eGFP+ cells are visualized by anti-GFP antibody. Dashed lines delimit the CHT. n=6 and n=9 for control and pcdh18a MO injected embryos respectively, from a single experiment. nc, notochord; nt, neural tube. Scale bar, 100 μm and 20 μm in the x10 and x40 panels, respectively.
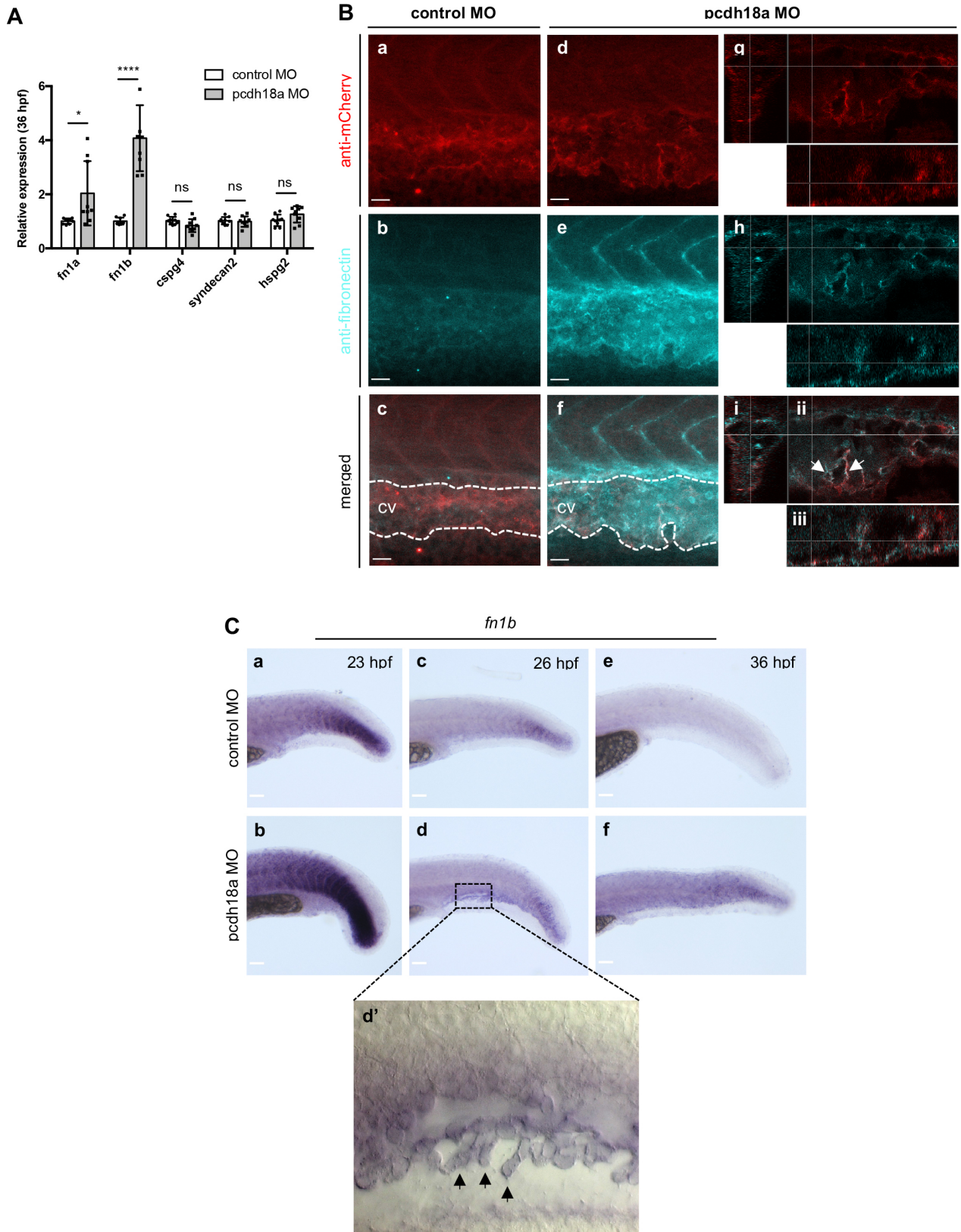
Fig. S4. Pcdh18a ICD truncation induces fibronectin expression in the CHT

(A) qPCR analysis for fibronectin 1a,1b (*fn1a* and *fn1b*), cspg4, syndecan2 and hspg2 in control and pcdh18a-ΔCP$_{106}$ morphant embryos at 36 hpf (n=9 for each, from 3 independent experiments) (mean±SD; *, P=0.0202; ****; P<0.0001; Student's t-test). **(B)** Immunostaining of Tg(*kdrl:ras-mCherry*) embryos for Fibronectin (cyan) and mCherry (red) in the caudal region at 36 hpf **(a-f)**. Confocal projections **(c,f)** are redisplayed in transverse **(i)**, sagittal **(ii)** and frontal **(iii)** sections using Imaris software. Dashed lines delineate the border of the caudal vein plexus (cvp). Arrows indicate cells double-labeled by anti-Fibronectin and anti-mCherry antibodies. Scale bars, 20 μm. **(C)** WISH for *fn1b* at 23, 26 and 36 hpf in control and pcdh18a-ΔCP$_{106}$ morphant embryos. Scale bars, 50 μm. **(d')** Magnified image showing *fn1b* expression at 26 hpf in the sprouting endothelial cells that will form the venous plexus (arrows).
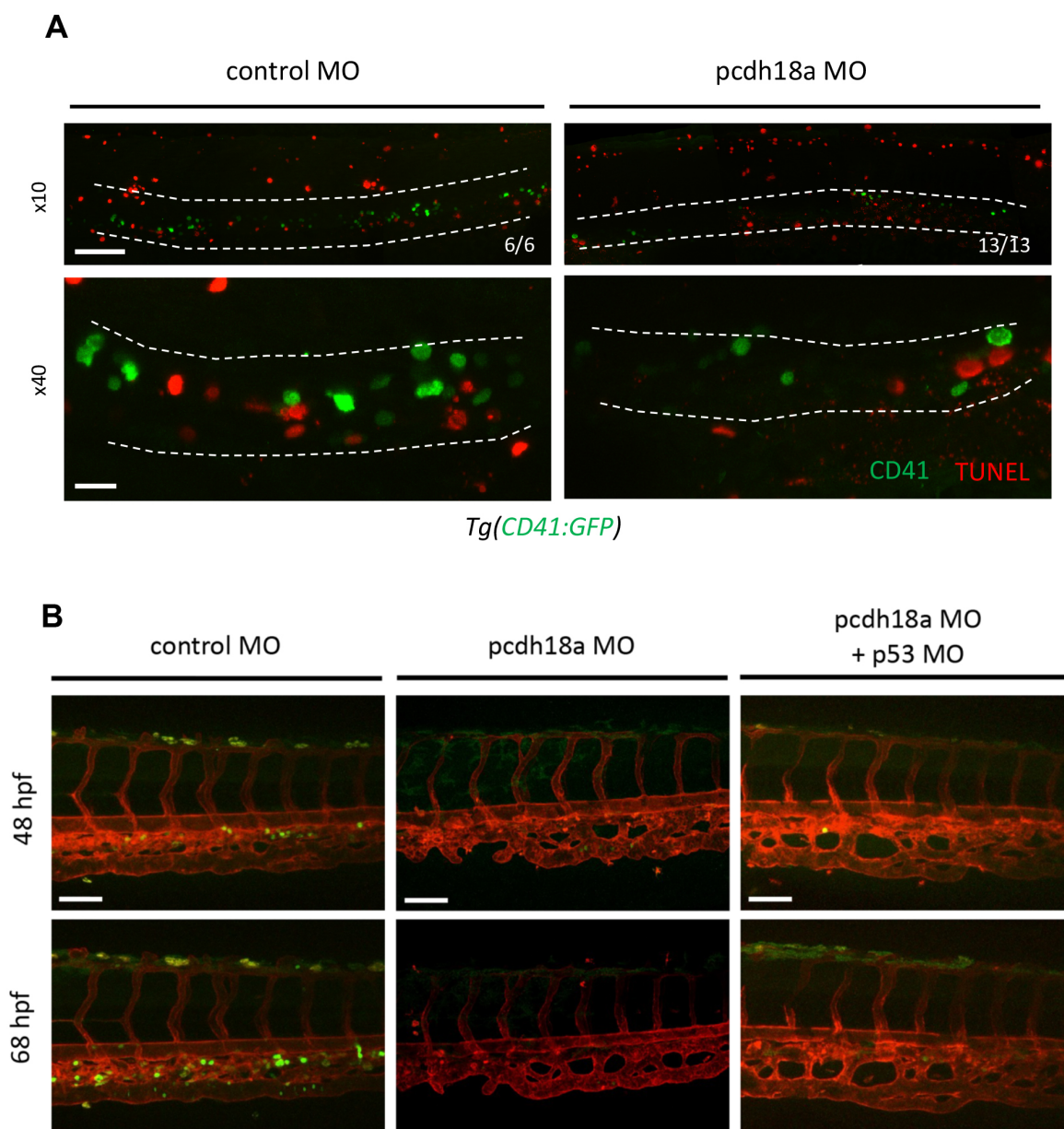
Fig. S5. Reduction of the number HSPCs in the CHT of pcdh18a morphants is not due to apoptosis nor to p53 pathway activation. (A) TUNEL staining (red) at 48 hpf of Tg(*CD41:GFP*) control or pcdh18a MO injected embryos. CD41:GFP+ cells are visualized by anti-GFP antibody. The area between the dashed lines indicates the CHT. n=6 and 13 for control and pcdh18a MO injected embryos respectively from single experiment. Scale bar, 100 and 20 µm in the x10 and x40 panels, respectively. **(B)** p53 MO does not rescue the pcdh18 morphant phenotype. Confocal maximum projections extracted at the beginning (48 hpf) and end (68 hpf) of time-lapse imaging sequences of control, pcdh18a MO, and pcdh18a MO +p53 MO (4ng each) injected Tg(*CD41:GFP; kdrl:ras-mCherry*) embryos. Scale bars, 100 µm.
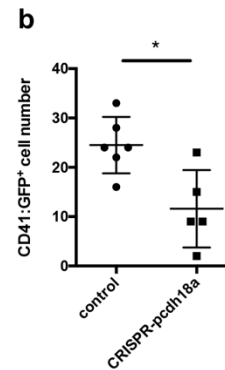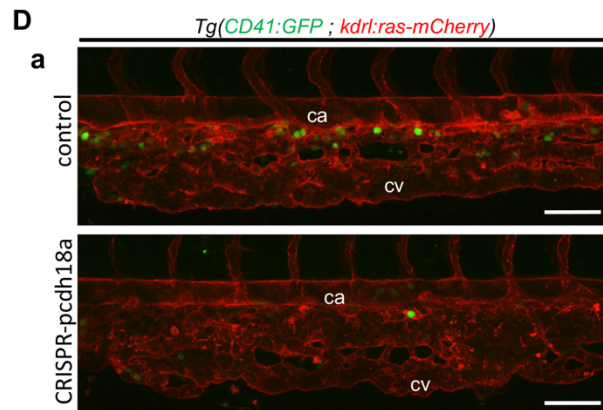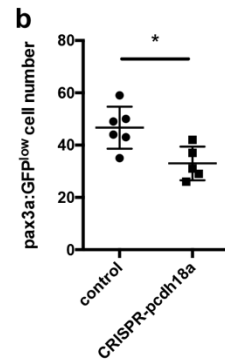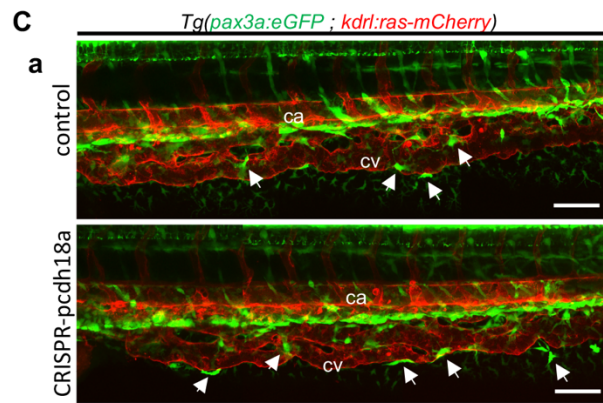
A

a pcdh18a

E1-F   E2-R

5'-UTR | Exon 1 | I1 | E2 | I2 | E3 | I3 | Exon 4 | 3'-UTR

(289)

pcdh18a-sgRNA

WT: GCCCCCTGTAGAGGTAAAAGCTAGATTTGAATGC--//--CTCCTCTCAATACTCCATCAGGGTCAGTATCA
P P V E

CRISPR-pcdh18a gDNA: GCCCCCTG---AGGTAAAAGCTAGATTTGAATGC--//--CTCCTCTCAATACTCCATCAGGGTCAGTATCA

CRISPR-pcdh18a cDNA: GCCCCCTGAGGTAAAAGCTAGATTTGAATGCATATTGAAACTCTGTAATTGATACTGATCTTCATG-----
P P E V K A R F E C I L K L C N *

b

Exon-1   Intron-1   STOP

CACGCCCCCTGAGGTAAAAGCTAGCTTTGAATGCATATTGAAACTCTGTAATGACTGATCTTCA

CRISPR-pcdh18a

c

B *Tg(pax3a:eGFP ; kdrl:ras-mCherry)*

a

control

CRISPR-pcdh18a

b

Abnormal CV
Large IVL
Normal

n=   20   33

Caudal plexus phenotype (percentage)

control: 20%, 80%
CRISPR-pcdh18a: 33%, 12%, 55%

C *Tg(pax3a:eGFP ; kdrl:ras-mCherry)*

a

control

CRISPR-pcdh18a

b

pax3a:GFP^low cell number

*

control   CRISPR-pcdh18a

D *Tg(CD41:GFP ; kdrl:ras-mCherry)*

a

control

CRISPR-pcdh18a
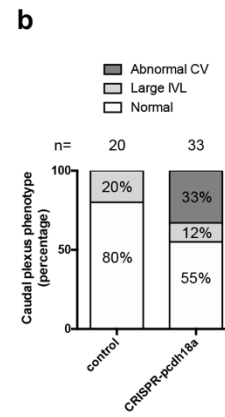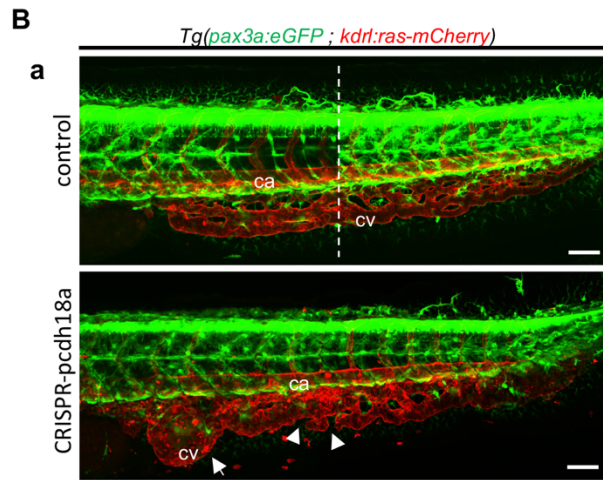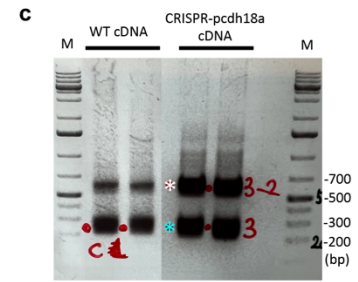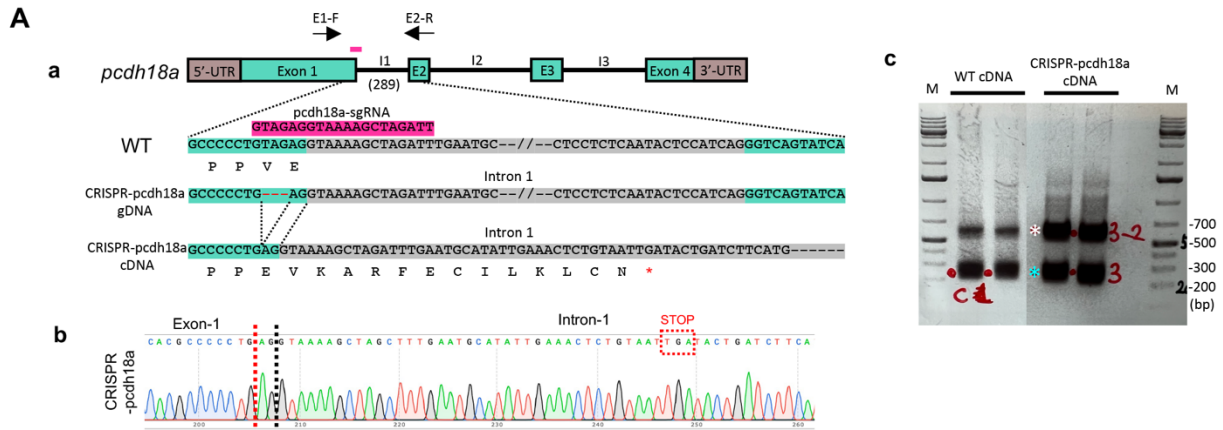

b

CD41:GFP+ cell number

*

control   CRISPR-pcdh18a

Fig. S6. pcdh18a-crispants leading to intron 1 retention in the mRNA phenocopy pcdh18a morphants. (A) a, The pcdh18a gene with coding sequences highlighted in green and intron sequences in grey. The indicated pcdh18a sgRNA led to a 3-nucleotide deletion, and thus to a single amino-acid deletion at the end of the exon 1, and to intron1 retention in the mRNA, so that 13 new amino acids are added, followed by a premature stop codon (red asterisk), just as in pcdh18a morphants. **b,** Extract of DNA sequencing of cloned amplicon obtained from pcdh18a-crispants shown in **a**. The black and red dotted lines indicate the border between exons and introns and the location of the 3-nucleotide deletion. **c,** Gel image of PCR products amplified with the primers shown in **a** using cDNA prepared from bulk uninjected control embryos and pcdh18a-crispants as templates. White and cyan asterisks indicate normal splicing products and fragments containing intron-1, respectively. **(B) a,** Confocal projections of uninjected control and pcdh18a-crispant *Tg(pax3a:eGFP; kdrl:ras-mCherry)* embryos at 48 hpf. The lower panel shows the characteristic phenotype frequently found in pcdh18a-crispants. Arrow and arrowheads indicate 'enlarged' and 'malformed' caudal vein (cv), respectively. **b,** Frequency histogram showing the incidence of abnormal CV plexus phenotype at 48 hpf. Morphology of the CV plexus in each of the uninjected control (n=20) and pcdh18a-crispant Tg(pax3a:eGFP; kdrl:ras-mCherry) (n=33) embryos was classified as 1) normal, 2) large inter-vascular loops (IVL), or 3) malformed CV plexus. **(C) a,** Uninjected control and pcdh18-crispant *Tg(pax3a:eGFP; kdrl:ras-mCherry)* at 48 hpf checked for the targeted genetic modification after live imaging. Arrows indicate pax3a:eGFP^{high} pigment cells. **b,** Quantification of pax3a:eGFP^{low} stromal cells in the CHT. Counting was done over 11 somite width in control (n=6) and genetically checked pcdh18a-crispant (n=5) embryos from a single experiment (mean±SD; *, P=0.0137, Student's t test). **(D) a,** Uninjected control and pcdh18-crispant *Tg(CD41:GFP; kdrl:ras-mCherry)* embryo checked for the targeted genetic modification after live imaging. **b,** Quantification of CD41:GFP^{+} cells in the CHT. Counting was done over 9 somite width in control (n=6) and genetically checked pcdh18a-crispant (n=5) embryos from a single experiment (mean±SD; *, P=0.0117, Student's t test). ca, caudal artery; cv, future definitive caudal vein. Scale bars, 100 µm.

## Table S1. Primers used for intermediate and Gibson cloning of *pcdh18a*

#	Primers	Sequences (5' – 3')
1	Int cloning _ pcdh18a FP1	GATACGCGCAACAGATTTCTCC
2	Int cloning _ pcdh18a RP1	TTCCCATTGGCCCCTTCATC
3	Int cloning _ pcdh18a FP2	CTGACTCGAACGACAACAATCC
4	Int cloning _ pcdh18a RP2	AACAACAGAGCAGGAGAGGTC
5	Int cloning _ pcdh18a FP3	ACGTGACTGTTGTGTTGACTG
6	Int cloning _ pcdh18a RP3	ACTCCTCCGTACACAATCTCATG
7	Int cloning _ pcdh18a FP4	CGAGGCCCAGCTTCAGAG
8	Int cloning _ pcdh18a RP4	CGTTCTCACGTGCGACTCAATC
9	Gib_hsp70_pcdh18a FP1	CTGGAATTCGGTACCCTCGAGGATATCGCCACCATGGAGACTAGCAAGGGTACAGTGCTC
10	Gib_hsp70_pcdh18a RP1	CTTCATCTGGGTCAGTGGCGTTCAG
11	Gib_hsp70_pcdh18a FP2	CTGAACGCCACTGACCCAGATGAAG
12	Gib_hsp70_pcdh18a RP2	CATTAGCACCAGCATCATGGTCCATCG
13	Gib_hsp70_pcdh18a FP3	CGATGGACCATGATGCTGGTGCTAATG
14	Gib_hsp70_pcdh18a RP3	CAGACTGAACTTATCCATGTCCTGGAGGG
15	Gib_hsp70_pcdh18a FP4	CCCTCCAGGACATGGATAAGTTCAGTCTG
16	Gib_hsp70_pcdh18a RP4	GGCTGATTATGATCGCGGCCGCGGATCCTTAGCTCTGGCGTACGTCCTGAAGC
17	Gib_UAS_pcdh18aDCD_FP1	GACGCGTGGATCCACCGGTCGCCACGCCACCATGGAGACTAGCAAGGGTACAGTG
18	Gib_UAS_pcdh18aDCD_RP2	GAGCCACGAATGTATCTACAGGAGCC
19	Gib_UAS_pcdh18aDCD_FP3	GGCTCCTGTAGATACATTCGTGGCTC
20	Gib_UAS_pcdh18aDCD_RP4	GTGAACAGCTCCTCGCCCTTGCTCACTGAGCAGCGAAGGGCAAAGGCC
21	Gib_UAS_pcdh18aDCD^AEAV_RP1	CTTGGAAGTTTGGACAAAACATCAGCGACA**G**CTTCT**GC**AAGTCTGGCTATCACAGTAC
22	Gib_UAS_pcdh18aDCD^AEAV_FP2	GGTGGGTACTGTGATAGCCAGACTT**GC**AGAAG**C**TGTCGCTGATGTTTTGTCCAAACTTC
23	Gib_UAS_pcdh18aDCD^AEAV_RP3	CCTGGACCAAAAATGAAATCTGACTTACA**G**CTTCA**GC**ATCAAAAGTACGTAGTGC
24	Gib_UAS_pcdh18aDCD^AEAV_FP4	CATTTATGCACTACGTACTTTTGAT**GC**TGAAG**C**TGTAAGTCAGATTTCATTTTTGGTC

Table S2. Primers and plasmids used to synthesize WISH probes

| From PCR	sense primer (5' – 3')	(+T7) antisense primer (5' – 3')
fn1b	GTTGGCGATGTGAGAACTGC	aagcttTAATACGACTCACTATAGGGCGGCAGTAAAACAAGGACGG
pcdh18a	TGGAAGAAATGGAGACGGCC	aagcttTAATACGACTCACTATAGGGCGCCAGGGTTTTGAAAGTGG
From plasmid		
myb	Plasmid obtained from L. Zon (Children Hospital Boston) _ T7 promoter _ GenbankID: NM_001309822.1	

Table S3. qPCR primers and efficiency measurement

gene	Sense primer (5' – 3')	Antisense primer (5' – 3')	Efficiency
ef1a	CAGCATTATCCAGTCCTTAAGTAGAGTGC	GCGTCATCAAGAGCGTTGAGAAG	95 %
fn1a	CCCCAGGAATCTCAGACTCAG	TGTTGATGTACCAGGCAGACG	91 %
fn1b	TGGGAGCGCATGTCTGATAC	CTCCCACTTTTCGCCAATGC	98 %
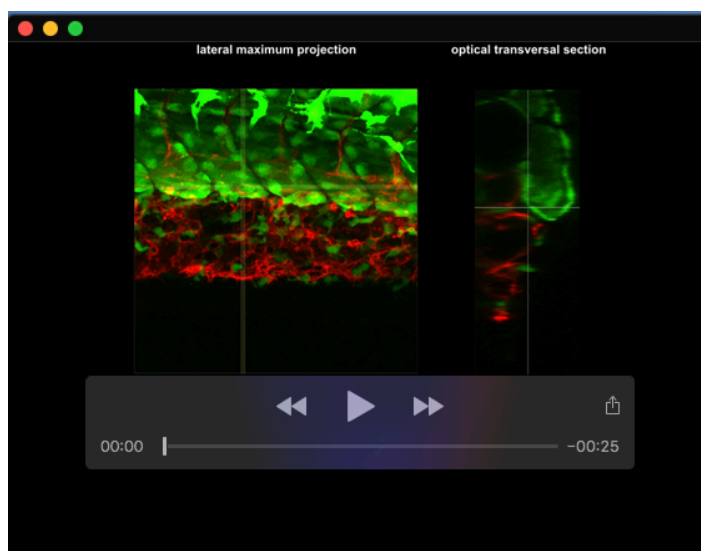
Movie 1 (related to Fig. 1). SCP migration on the primordial vein and caudal venous plexus formation

Maximum projection and orthogonal transverse section reconstituted by Imaris software from the confocal time-lapse imaging of a Tg(*Pax3a:GFP; kdrl:ras-mCherry*) embryo from 26 to 35 hpf. Horizontal lines at the beginning of the movie indicate the correspondence between the lateral and orthogonal view. Endothelial cells are mCherry+; the caudal artery is overlaid by the somites, while the forming venous plexus lies ventral to the somites. Migrating SCPs ar GFP+; GFP++ cells migrating from the top (dorsal side) into the future CHT (ventral side) after the SCPs are neural crest cells that will differentiate in pigment cells.
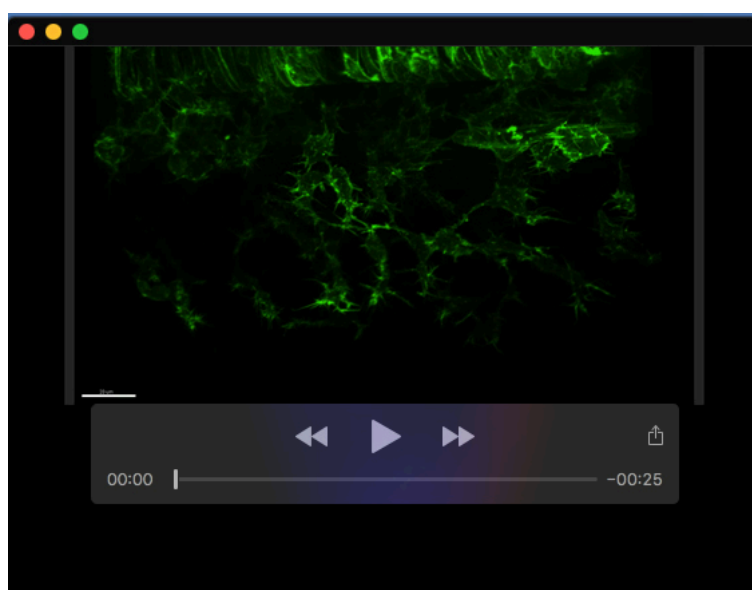

Movie 2 (related to Fig. 1). Visualization of SCP connections and filopodia during their migration

Live confocal spinning disk acquisition reconstructed in a 3D view with Imaris software of a Tg(*cspg4:Gal4; UAS:Lifeact-GFP*) embryo at 27 hpf.
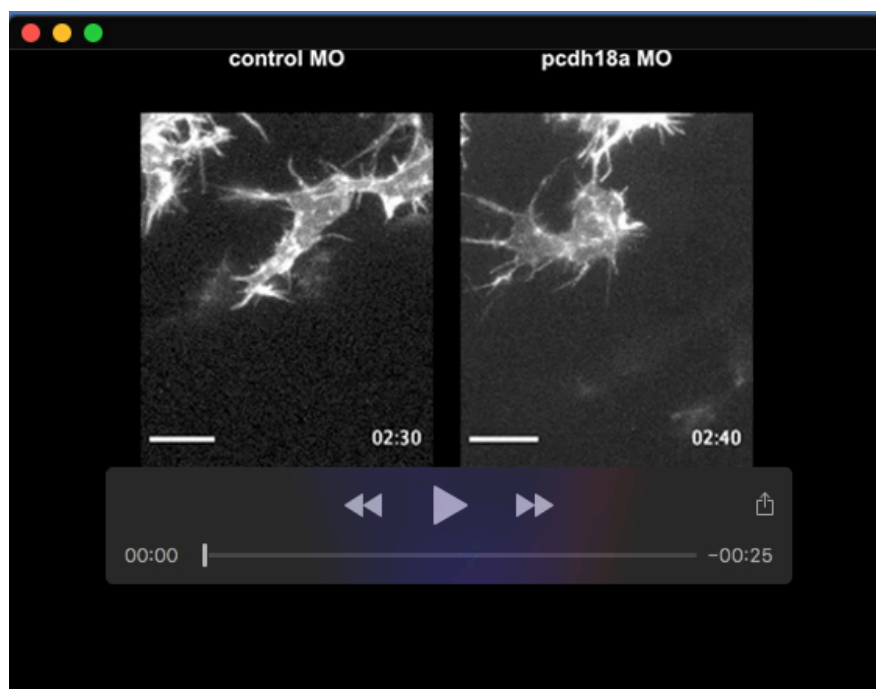
Movie 3 (related to Fig. 3). Actin remodeling and filopodia orientation during SCP leader cells migration in control or pcdh18-ΔCP$_{106}$ morphant embryos
Time-lapse confocal acquisitions of Tg(*cspg4:Gal4; UAS:Lifeact-GFP*) control or pcdh18a-ΔCP$_{106}$ morphant embryos from 26 to 27.5 hpf. Scale bars, 10 μm
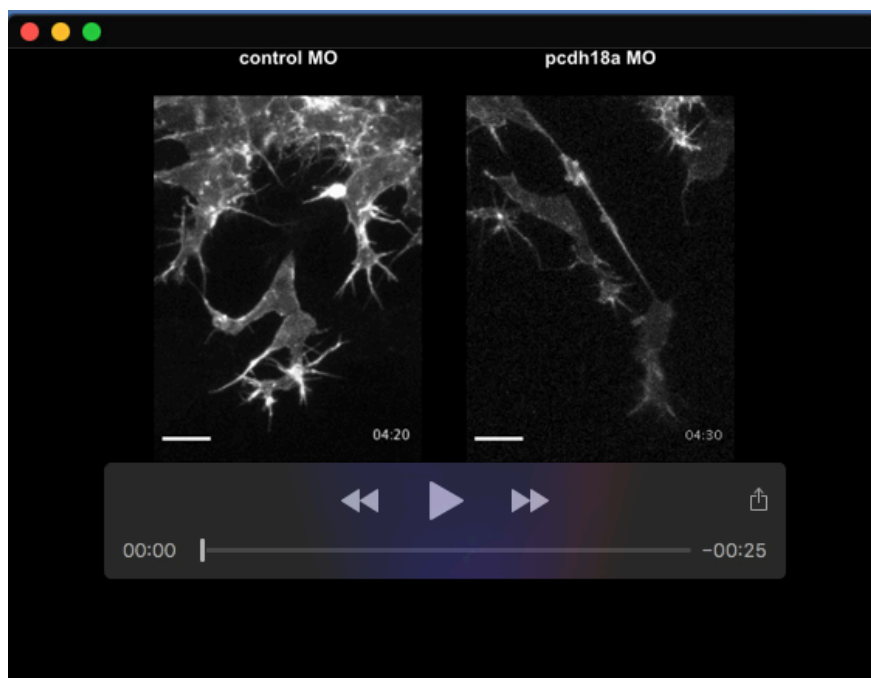
**Movie 4 (related to Fig. 3). Cellular connections between SCP leader and follower cells during their migration in control or pcdh18a-ΔCP$_{106}$ embryos**
Time-lapse confocal acquisitions of Tg(*cspg4:Gal4; UAS:Lifeact-GFP*) starting at 27 hpf, every 10 minutes, showing the long connection phenotype in morphants compared to control embryos. Scale bars, 10 μm.
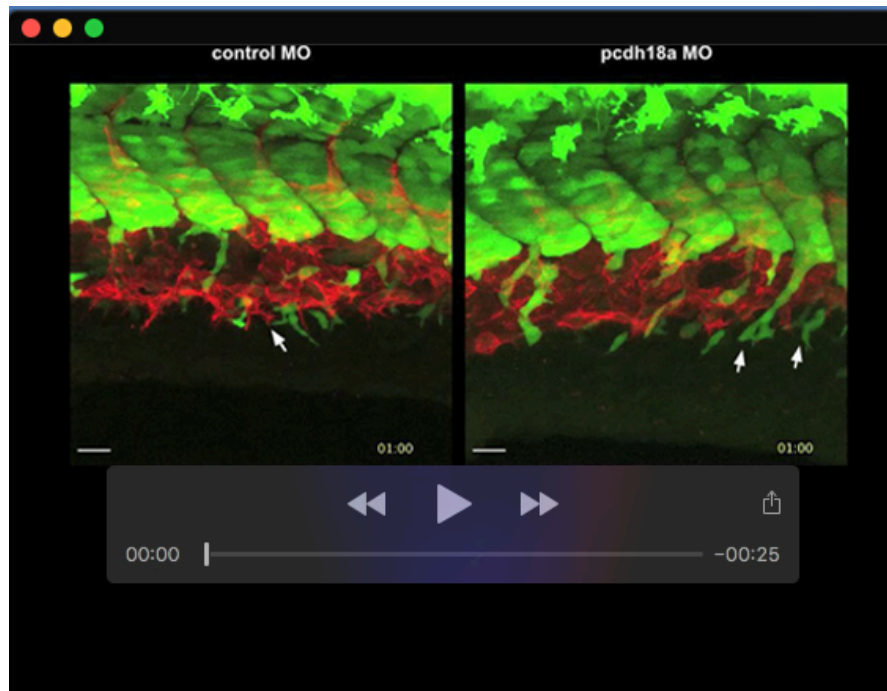
Movie 5 (related to Fig. 5). Stromal-endothelial cell interactions during caudal vein plexus formation in control or pcdh18a-ΔCP106 morphant embryos
Time-lapse confocal imaging of Tg(*pax3a:GFP; kdrl:ras-mCherry*) embryos from 26 to 36 hpf. Arrows indicate stromal−endothelial cell interactions in the control embryo, and solitary migration of stromal cells in the pcdh18a morphant. Scale bars, 20 μm.
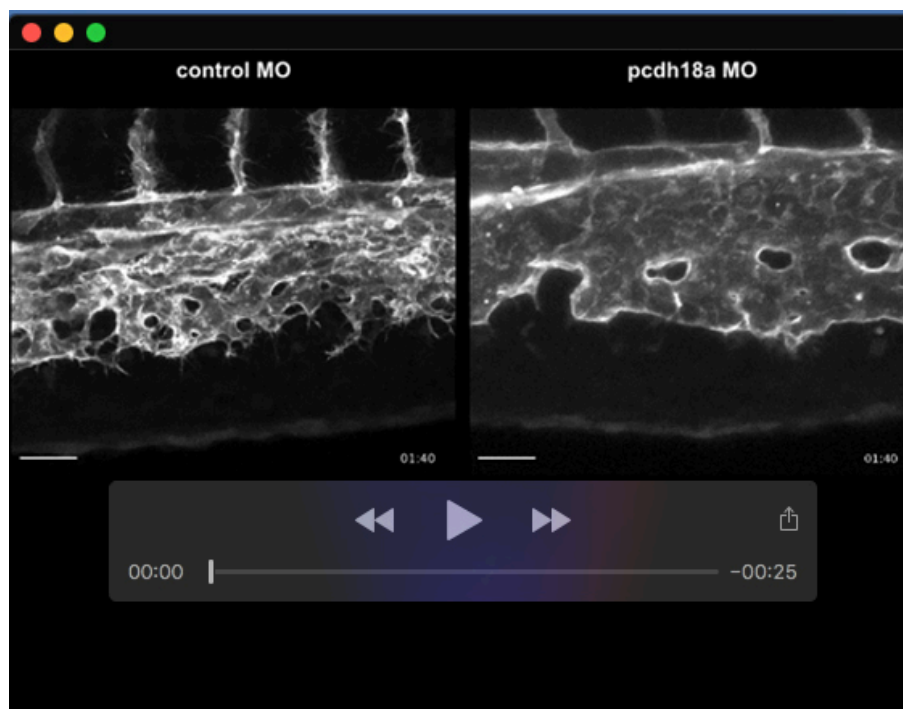
Movie 6 (related to Fig. 5). Endothelial cell projections and venous plexus formation in control or pcdh18a-ΔCP106 morphant embryos
Time-lapse confocal acquisitions of Tg(*kdrl:ras-mCherry*) embryos from 34 to 54 hpf. Scale bars, 30 μm.
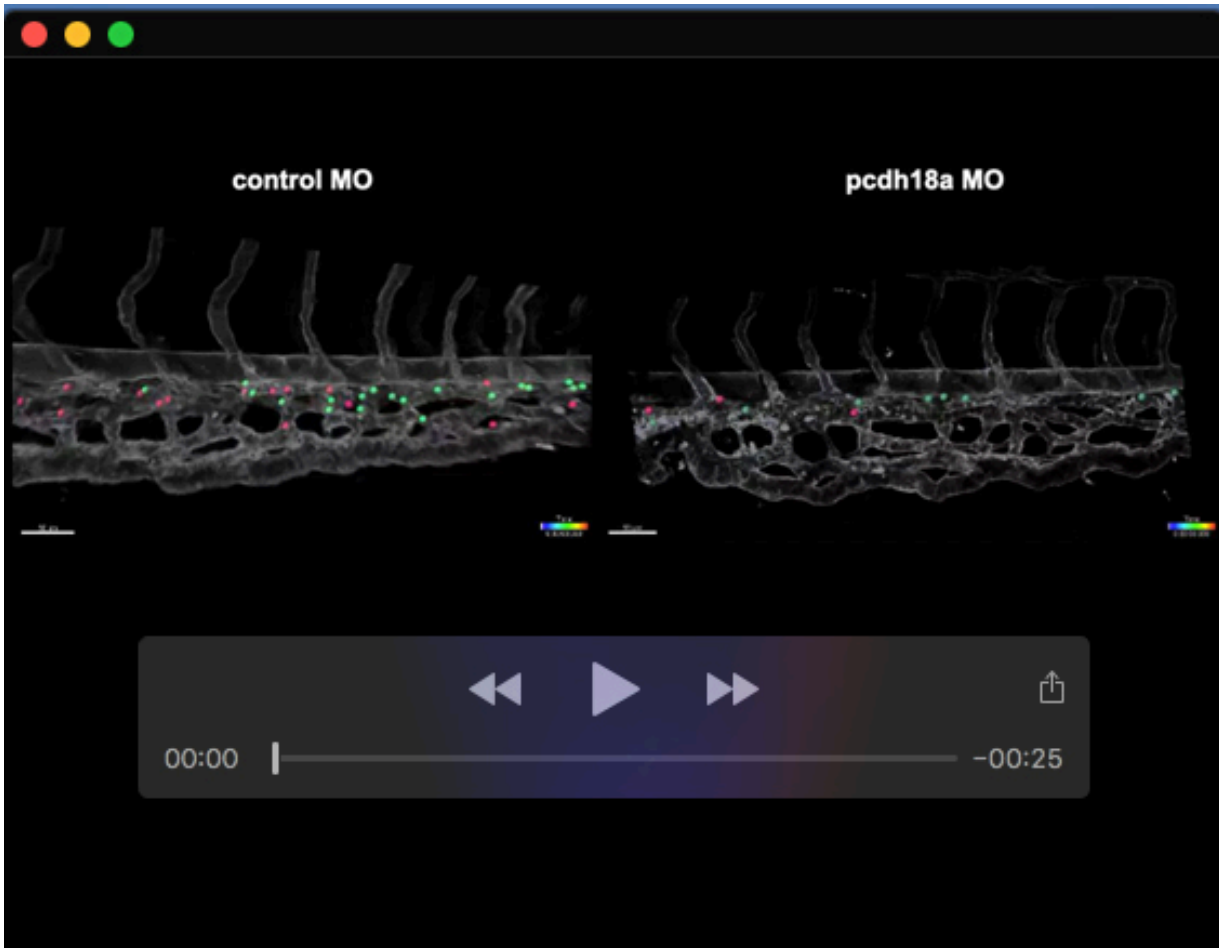
Movie 7 (related to Fig. 6). Tracking of CD41:GFP^{low} HSPCs and CD41:GFP^{high} pro-thrombocytes in the CHT of control and pcdh18a-ΔCP106 morphant larvae
Following time-lapse confocal imaging of Tg(*CD41:GFP; kdrl:ras-mCherry*) embryos for 20 hrs beginning at 48 hpf, GFP^{low} HSPCs and GFP^{high} pro-thrombocytes were tracked with Imaris software, and replaced in the resulting analysis by green and magenta spots, respectively. Scale bars, 50 μm.