Supplementary Information

Patterning Mechanisms of the Sub-Intestinal Venous Plexus in Zebrafish

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Table S1. Small molecule inhibitor doses

Drug Name	Dose	Reference	Catalogue Number	Company
DMH1	25, 50 or 100 µM	(Hao et al., 2010)	D8946	Sigma
DMH4	25, 50 or 100 µM	(Hao et al., 2010)	D8696	Sigma
Dorsomorphin	50 µM	(Hao et al., 2010)	P5499	Sigma
SL327	30 µM	(Wiley et al., 2011)	S4069	Sigma
DAPT	100 µM	(Geling et al., 2002)	D5942	Sigma
LY-411575	25 µM	(Grotek et al., 2013)	SML0506	Sigma
Pdgfr Tyrosine Kinase Inhibitor V	0.25 µM	(Wiens et al., 2010)	521234	Calbiochem
Imatinib mesylate	50 µM	(Nishioka et al., 2008)	SC-202180	Santa Cruz Biotechnology
Cyclopamine	50 µM	(Lamont et al., 2010)	C988400	Toronto Research Chemicals

Table S2. *In situ* hybridization probes

Gene	Source
gata6	(Reiter et al., 1999)
***vegfr2* (*kdrl*)**	Addgene plasmid 22417
vegfaa	F-primer: 5'-GCTCTCCTCCATCTGTCTGC-3' R-primer: 5'-CCTTTGGCCTGCATTCACAC-3'
vegfab	F-primer: 5'-GCAAAACCGTGGTTCCAGAC-3' R-primer: 5'-GACGAGCTGAAACGACAACG-3'
plexinD1	F-primer: 5'-AAGATTGAGCCACTGTCGGG-3' R-primer: 5'-CACGCCAGAGACGGAGATAC-3'
***sema3aa* (*sema3a1*)**	(Yee et al., 1999)
***sema3ab* (*sema3a2*)**	(Torres-Vazquez et al., 2004)
***alk2* (*acvrl1l*)**	F-primer: 5'-CTGGCTGTGCGAATACTGAA-3' R-primer: 5'-GCCAGAAAAGACAGCAGACC-3'
bmp4	(Zeng and Childs, 2012)
***vegfr3* (*flt4*)**	F-primer: 5'-CAGTCCAAAACAGCCAGCAC-3' R-primer: 5'-TCGAGTGCCCTTCCCATAGA-3'
vegfC	F-primer: 5'-ATGCCATGCAGGAGCATTCA-3' R-primer: 5'-TGCGGTTGAGAGGTTGACTC-3'
mrc1	(Wong et al., 2009)
ephrinB2a	(Lawson et al., 2001)
gridlock	(Zhong et al., 2000)
etsrp	(Wong et al., 2009)

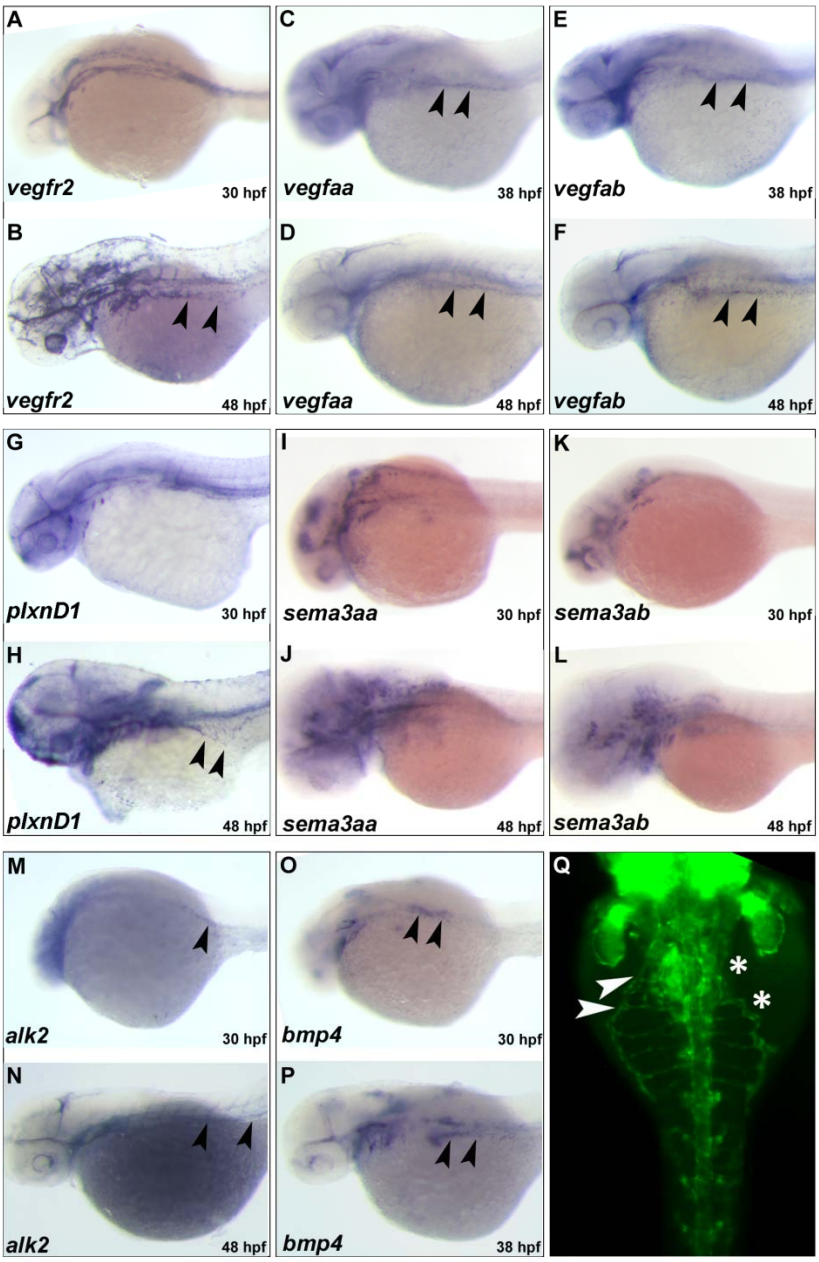

Fig. S1. Expression of growth factors and ligands during SIVP development and SIVP left-right asymmetry

(A-B) *vegfr2* is expressed in the SIVP (arrowheads). (C-F) *vegfaa* and *vegfab* are expressed in the pronephric ducts (arrowheads), close to the region where SIVP develops. (G-H) *plexinD1* is expressed in the SIVP (arrowheads). (I-L) *sema3aa* and *sema3ab* are expressed in the head but have weak expression in the trunk at both 30 and 48 hpf. (M-N) *alk2* is expressed in blood vessels including the axial vein (arrowheads) at 30 and 48 hpf. (O-P) *bmp4* is expressed in the gut (arrowheads). (Q) Dorsal view of a 2 dpf *Tg(fli:EGFP)*^{y1} embryo with head at the top showing left-right SIVP asymmetry. The left SIVP extends to a greater distance and also more anteriorly (white arrowheads) while the right SIVP is smaller and posterior only (white asterisks).

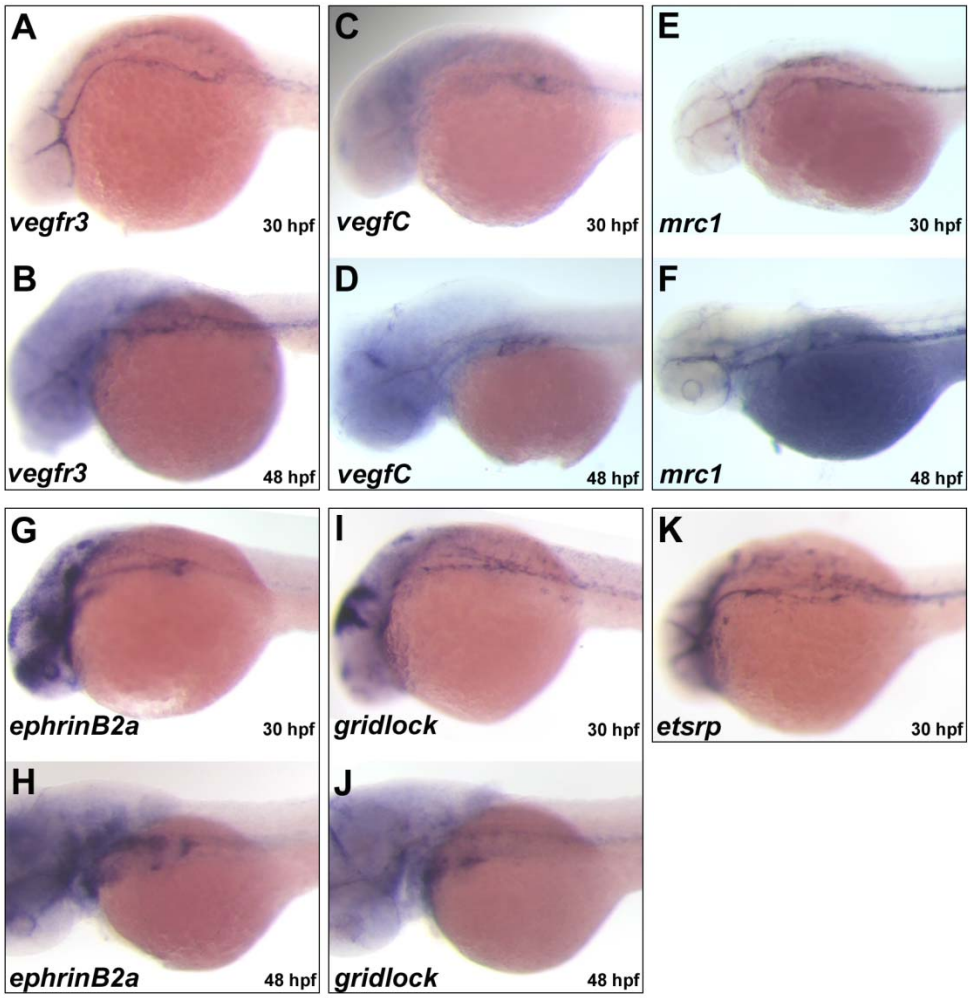

Fig. S2. Expression of venous and arterial markers in the SIVP region during the window of sprouting.
(A-B) *vegfr3* receptor. (C-D) *vegfC,* ligand for Vegfr3. (E-F) *mrc1*. (G-H) *ephrinB2a.* (I-J) *gridlock* (K) *etsrp*.

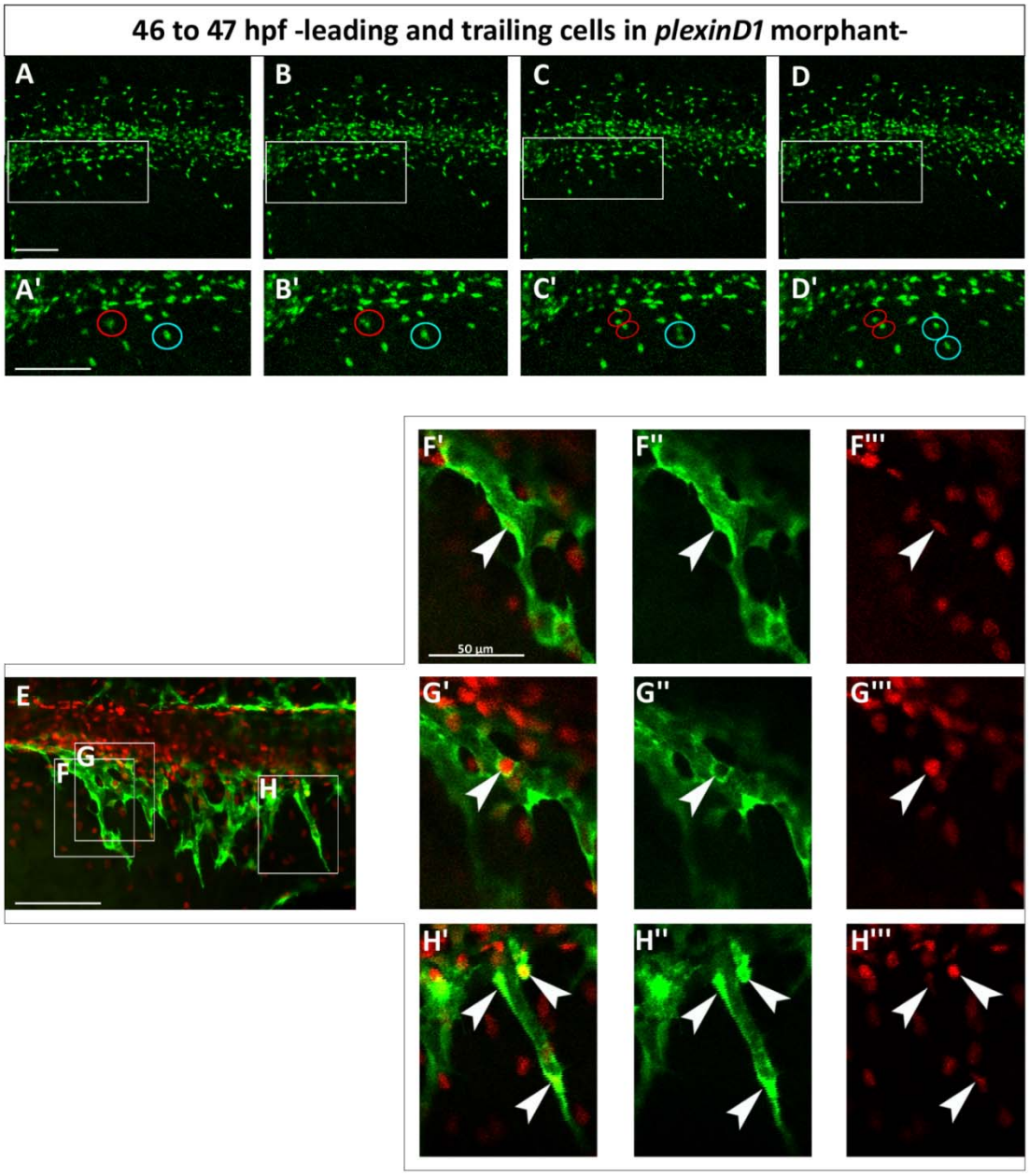

Fig. S3. Both leading and trailing cells proliferate during migration in *plexinD1* morphants
Confocal micrographs from a time-lapse of a *Tg(fli:EGFP)*^{y7} embryo injected with *plexinD1* morpholino. Enlargements are below each single original frame. (A'-D') Red circles indicate a dividing trailing cell and the blue circles point a dividing leading cell from about 46 to 47 hpf. (E-H) Analysis of proliferation using EdU staining in obd mutants. Red marks EdU staining and green marks endothelial cells in *Tg(fli:EGFP)*^{y1} wild type and *plexinD1* morphant embryos at 48 hpf. Boxes in E indicate the position of the enlargements. White arrowheads indicate the SIVP proliferating cells. Unspecified scale bars represent 100 µm.

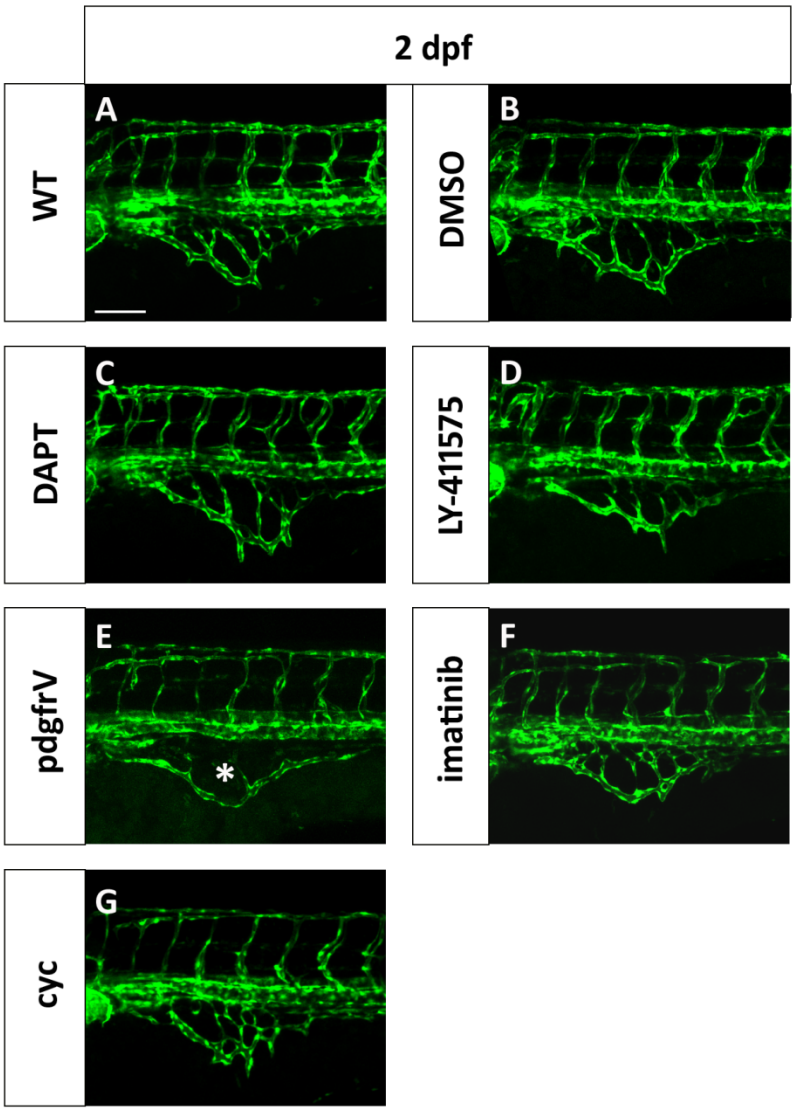

Fig. S4. Notch, Pdgf and Shh pathways do not play an essential role in SIVP development
(A-H) Phenotype of embryos treated with small molecule inhibitors from 24 hpf to 48 hpf. (A) Untreated control embryo (B) DMSO treated control embryos. (C) 100 µM DAPT treated embryos to examine the role of Notch. (D) 25 µM LY-411575, a γ-secretase inhibitor to examine the role of Notch. (E) 0.25 µM Pdgfr inhibitor V treated embryo, blocking Pdgfrα and β and Vegfr2, shows a similar phenotype (asterisk) to that seen with the VEGFR2 inhibitor DMH4. However a second Pdgfr inhibitor (I, 50 µM imatinib) causes no alteration in SIVP morphology. (G) 50 µM cyclopamine treated embryo, a hedgehog receptor antagonist. Scale bar represents 100 µm.

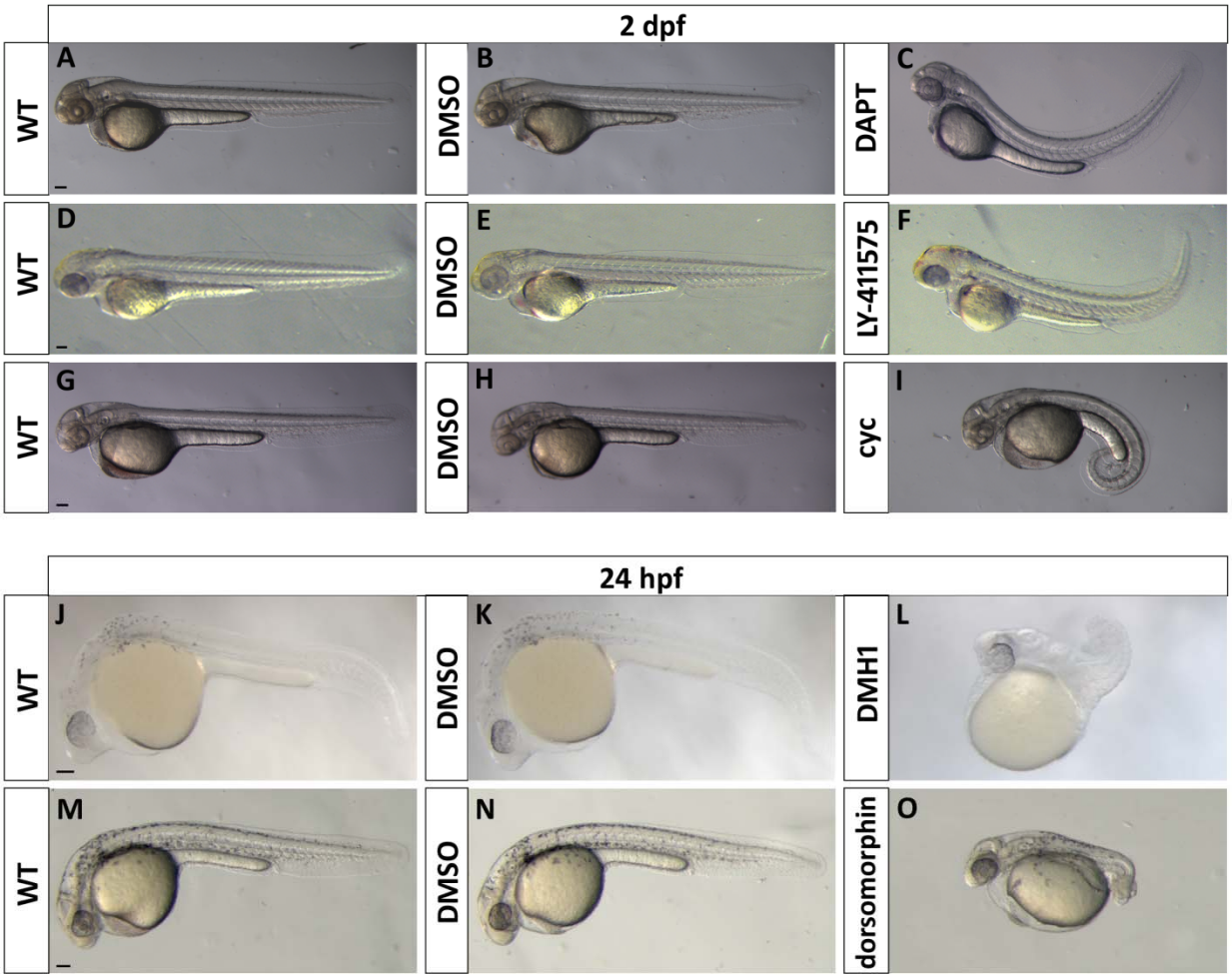

Fig. S5. Positive controls for drug treatments.
(A) Brightfield image of 2 dpf wild-type embryo. (B-C) 2 dpf embryos treated with DMSO and 100 µM DAPT from 24 hpf. Inhibition of Notch by DAPT causes a curved body axis. The same curved axis phenotype is obtained using 25 µM LY-411575 (D-F). (G) Brightfield image of 2 dpf wild-type embryo. (H-I) 2 dpf embryos treated with DMSO and 50 µM cyclopamine from 4 hpf. Cyclopamine treated embryos show a characteristic curly-down body axis. (J) Brightfield image of 24 hpf wild-type embryo. (K-L) 24 hpf embryos treated with DMSO and 1 µM DMH1 from 4 hpf. The dorsalizing effect of inhibition of Bmp signaling is observed (M-O). The same phenotype is obtained using 1 µM dorsomorphin. Scale bars represent 100 µm.

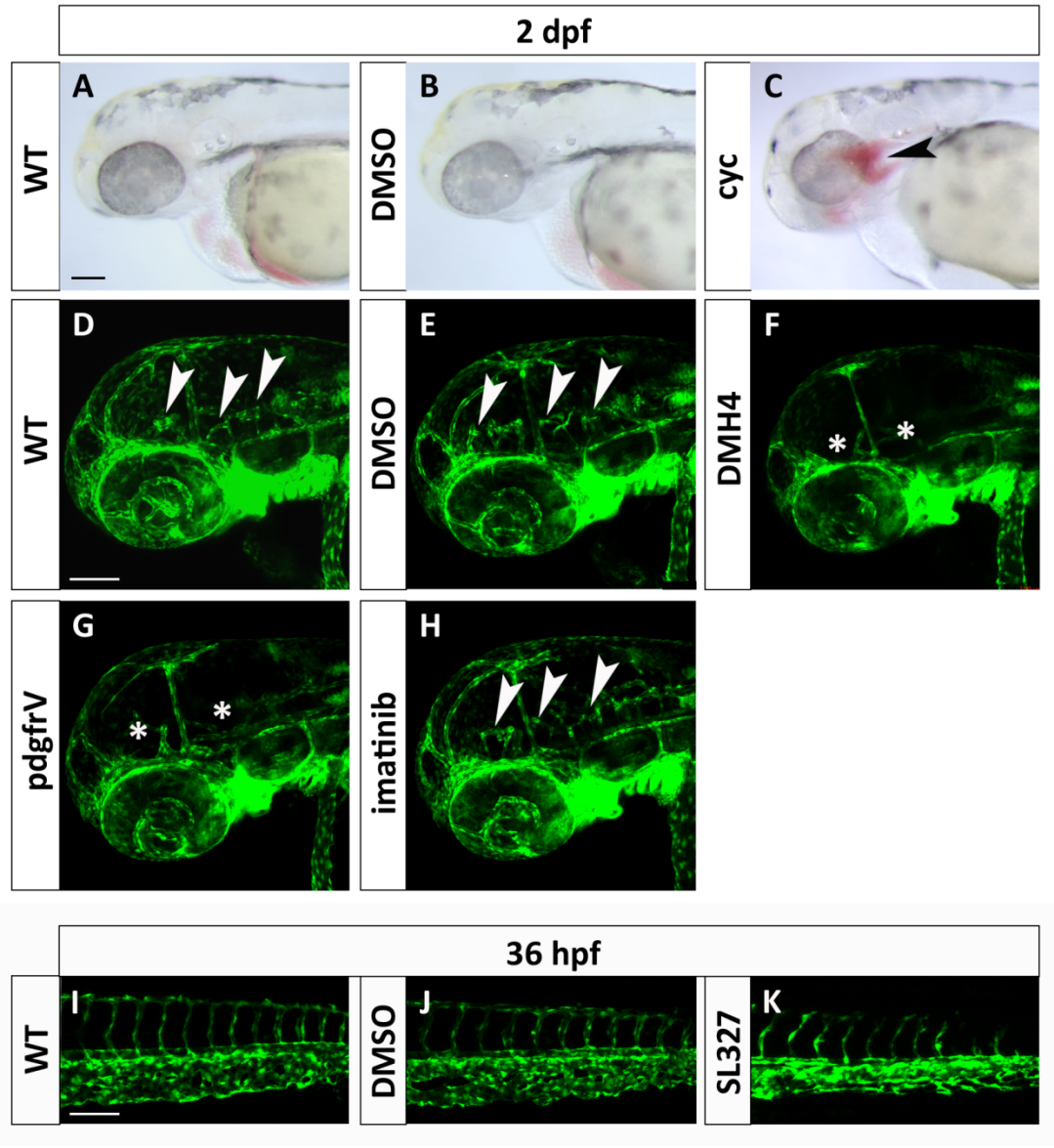

Fig. S6. Positive controls for cyclopamine, DMH4, Pdgfr inhibitor V, imatinib and SL327 drug treatments.

(A-C) Brightfield images of 2 dpf wild-type control and treated embryos from 24 hpf. (A-B) Wild-type and DMSO treated control embryos. (C) Embryo treated with 50 µM cyclopamine showing hemorrhage in the head indicated by the black arrowhead.

(D-H) Confocal images of 2 dpf *Tg(fli:EGFP)*^{y1} embryos untreated or treated with different inhibitors from 24 hpf. (D-E) Untreated and DMSO treated control embryos. White arrowheads mark the presence of CtAs in the head. (F-G) Embryos treated with 50 µM DMH4 and 0.25 µM Pdgfr inhibitor V show absence of CtAs (asterisks) but 50 µM imatinib treatment (H) does not affect CtA development (white arrowheads) suggesting that inhibition of arterial sprouts with Pdgfr inhibitor V is due to inhibition of Vegfr2 in addition to Pdgfr. (I-J) Untreated and DMSO treated control embryos from 24 to 36 hpf. (K) 60 µM SL327 inhibits Mek-1 and Mek-2 and causes alteration of caudal vein plexus development. Scale bars represent 100 µm.

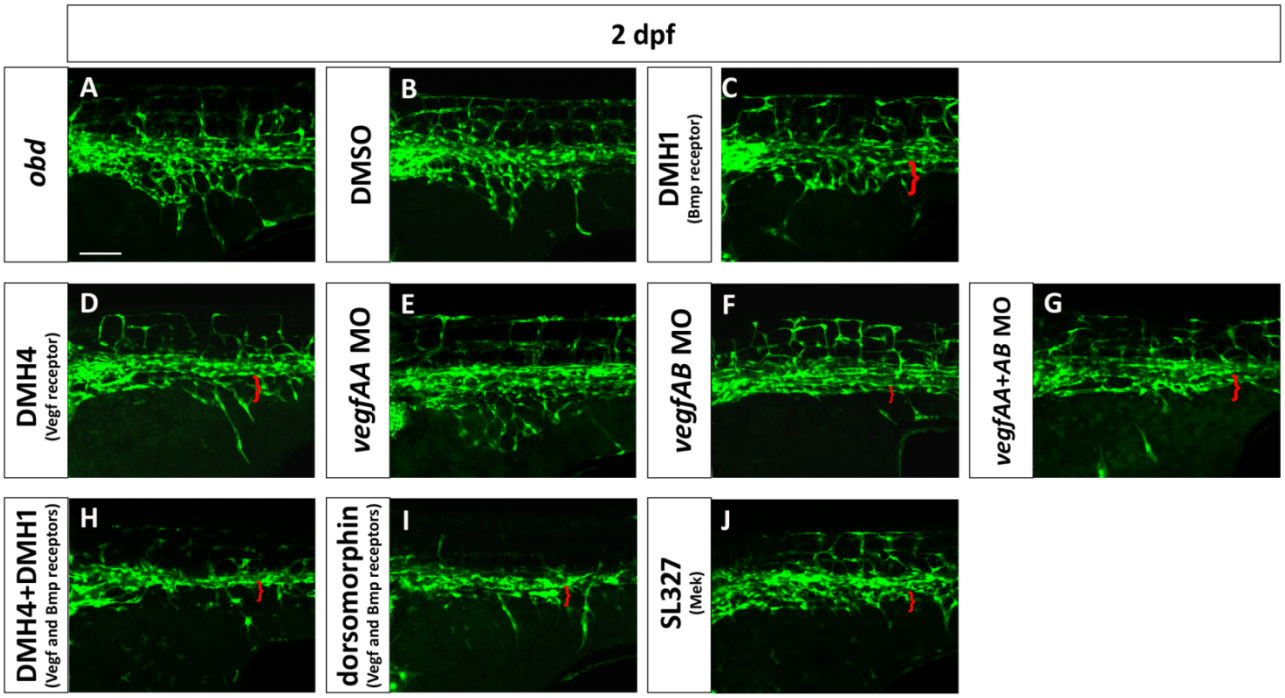

Fig. S7. Vegf and Bmp control *obd* SIVP development

(A-J) Phenotype of *obd* mutant embryos treated with small molecule inhibitors from 24 hpf to 48 hpf or morpholinos. (A) Untreated control *obd* mutant (B) DMSO vehicle treated *obd* mutant. (C) 100 µM DMH1 treated *obd* mutant. (D) 100 µM DMH4 treated *obd* mutant. (E) ~ 2 ng/e *vegfaa* morpholino treated *obd* mutant. (F) ~ 2 ng/e *vegfab* morpholino treated *obd* mutant. (G) *vegfaa* and *vegfab* treated *obd* mutant. (I) DMH4 and DMH1, 50 µM each-treated *obd* mutant. (J) 50 µM dorsomorphin treated *obd* mutant. (K) 30 µM SL327, a Mek-1/Mek-2 inhibitor-treated *obd* mutant. Red brackets indicate the reduced expansion of the SIVP. Scale bar represents 100 µm.

Movie 1
SIVP development in a *Tg(fli:EGFP)^{y1}* transgenic embryo from around 27 to 47 hpf. Anterior is to the left.

Movie 2
SIVP development in a *Tg(fli:EGFP)^{y1}* transgenic embryo from around 30 to 76 hpf. Anterior is to the left.

Movie 3
SIVP cell proliferation in a *Tg(fli:EGFP)^{y7}* transgenic embryo from around 32 to 75 hpf. Anterior is to the left.

Movie 4
SIVP development in a *obd^{fov01b}*; *Tg(fli:EGFP)^{y1}* transgenic embryo from around 30 to 76 hpf. Anterior is to the left.

Movie 5
SIVP cell proliferation in a *Tg(fli:EGFP)^{y7}* embryo injected with *plexinD1* morpholino and imaged from around 32 to 75 hpf. Anterior is to the left.

References

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