Figure S1

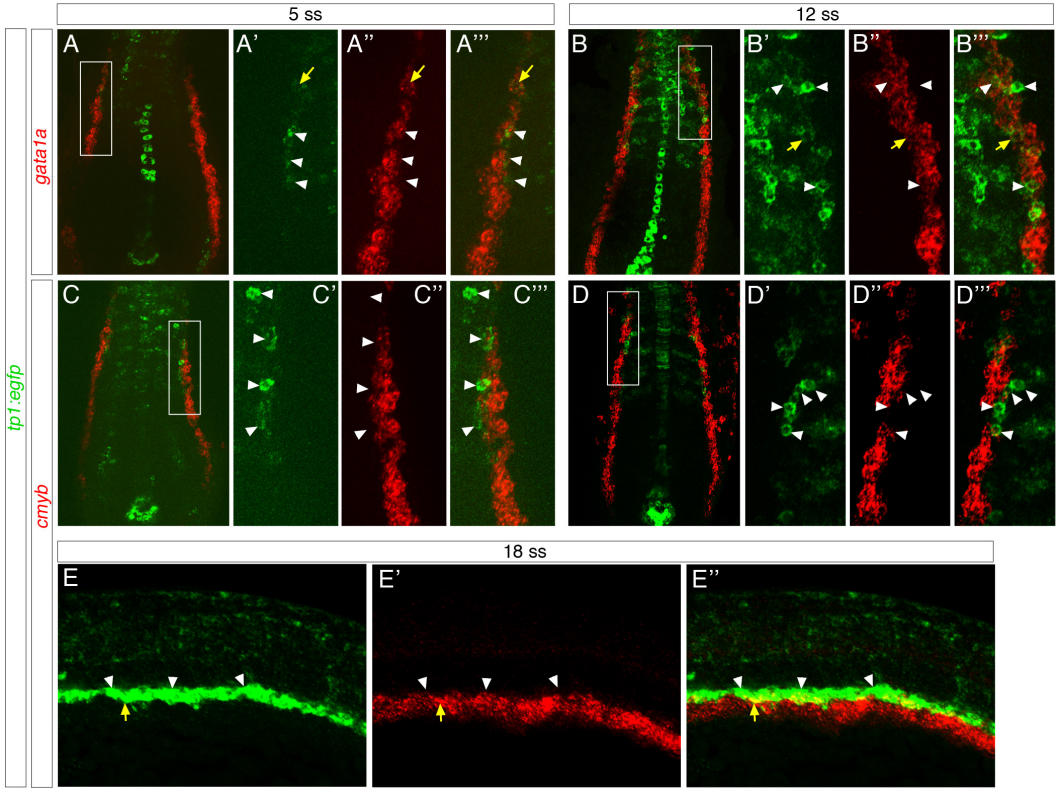


Fig S2

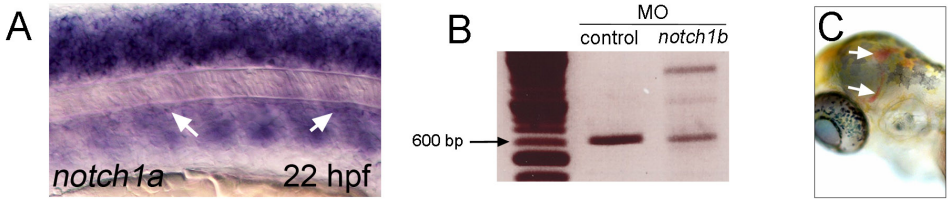


Figure S3

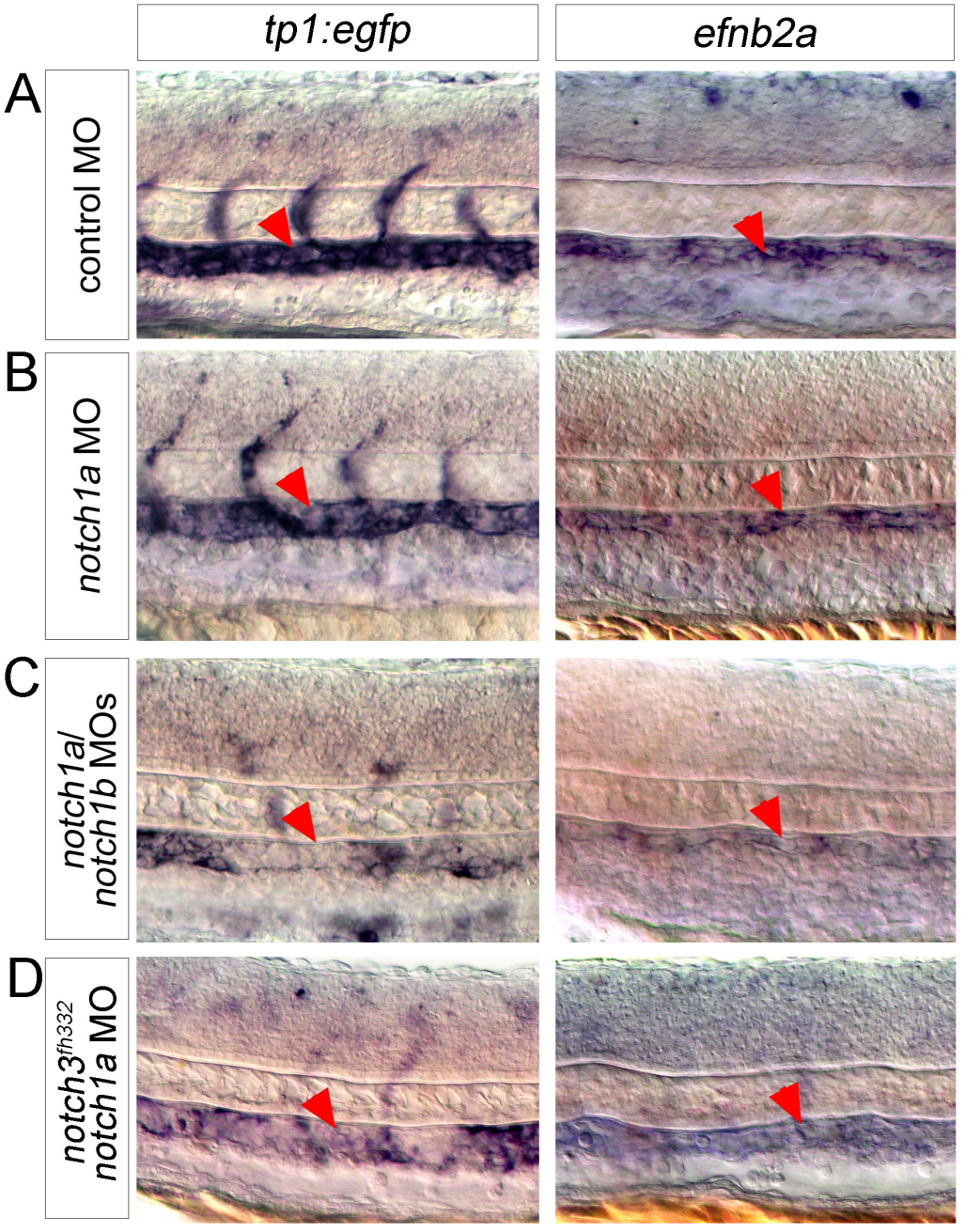

tp1:egfp
efnb2a
A
control MO
B
notch1a MO
C
notch1a/
notch1b MOs
D
notch3fh332
notch1a MO

Fig S4

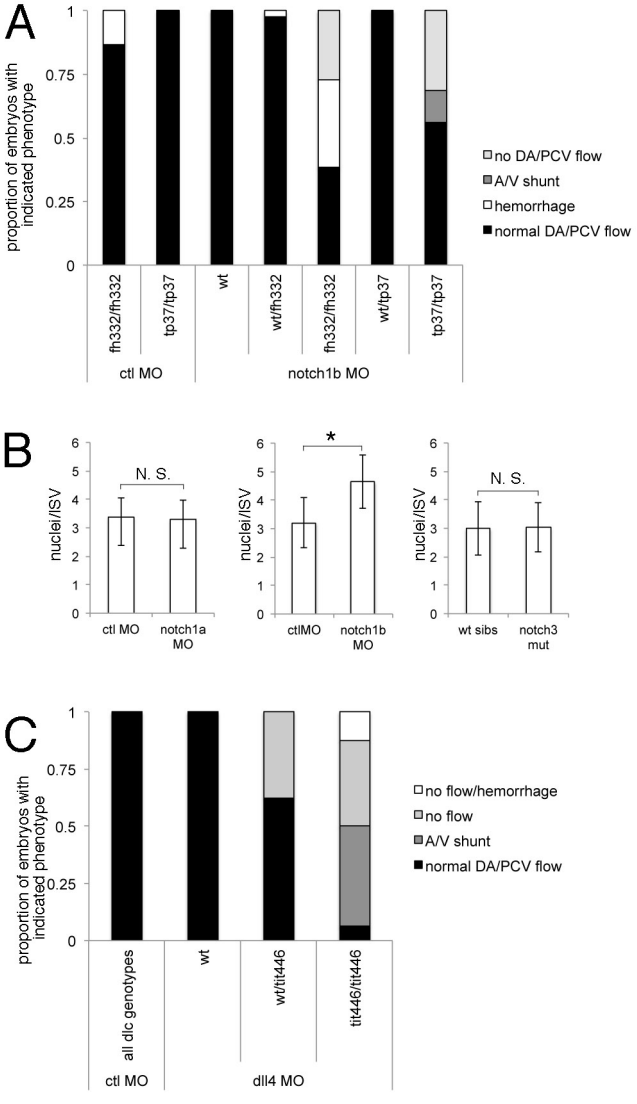
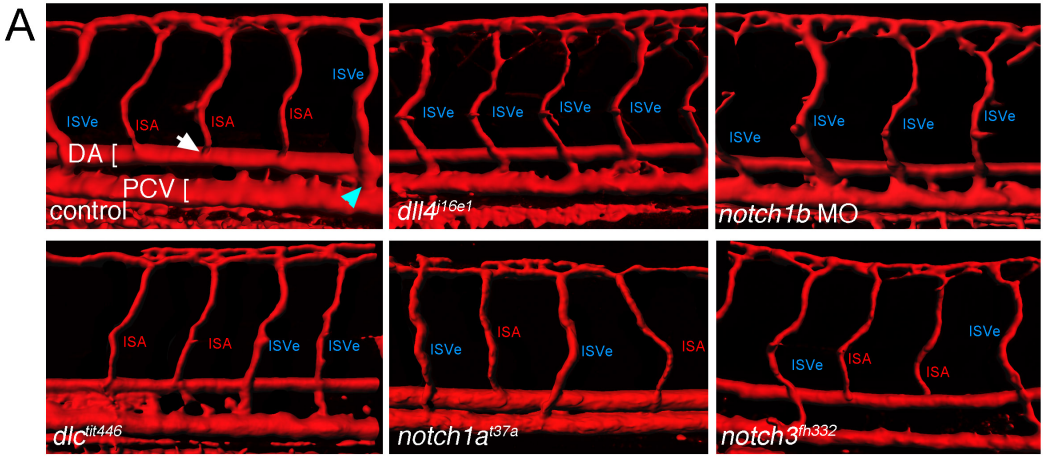
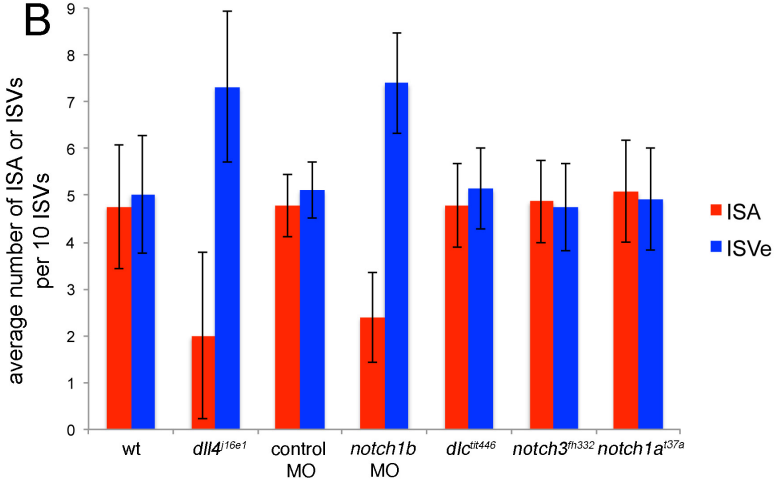

A
proportion of embryos with indicated phenotype
1
0.75
0.5
0.25
0
fh332/fh332
tp37/tp37
wt
wt/fh332
fh332/fh332
wt/tp37
tp37/tp37
ctl MO
notch1b MO
no DA/PCV flow
A/V shunt
hemorrhage
normal DA/PCV flow
B
nuclei/ISV
N. S.
ctl MO
notch1a MO
*
ctlMO
notch1b MO
N. S.
wt sibs
notch3 mut
C
proportion of embryos with indicated phenotype
all dlc genotypes
wt
wt/tit446
tit446/tit446
ctl MO
dll4 MO
no flow/hemorrhage
no flow
A/V shunt
normal DA/PCV flow

Fig S5


A
ISVe
ISVe
ISA
ISA
ISA
DA [
PCV [
control
ISVe
ISVe
ISVe
ISVe
dll4i16e1
ISVe
ISVe
ISVe
ISVe
notch1b MO
ISA
ISA
ISVe
ISVe
dlctit446
ISA
ISVe
ISVe
ISA
notch1at37a
ISVe
ISA
ISA
ISVe
ISA
notch3fh332


B
average number of ISA or ISVs per 10 ISVs
0
1
2
3
4
5
6
7
8
9
wt
dll4i16e1
control MO
notch1b MO
dlctit446
notch3fh332
notch1at37a
ISA
ISVe

Supplementary Information

Supplementary Figure Legends

Supplementary Figure S1. Notch activation in *gata1a* and *cmyb* expressing cells during somitogenesis. (A-E) Confocal images of embryos at indicated stages subjected to whole mount double fluorescent in situ hybridization with riboprobes against *egfp* (green) and (A-B) *gata1a* (red) or (C-D) *cmyb* (red). (A-D) Dorsal views of flat mounted embryos; anterior is up. (E) Lateral view, dorsal is up, anterior to the left. (A-E) Yellow arrows denote cells co-expressing *gata1a* or *cmyb* and egfp. White arrowheads denote cells expression only *egfp*.

Supplementary Figure S2. (A) DIC image of wild type embryo at 22 hours post fertilization (hpf) following whole mount in situ hybridization with a riboprobe against *notch1a*. White arrows indicate low level expression in presumptive endothelial cells in the dorsal aorta. (B) RT-PCR of a *notch1b* fragment spanning exon 8. The expected fragment size with normal splicing is approximately 600 bp, which is indicated on the marker lane. (C) Dorsal-lateral view of head region in a *notch3*^{fh332} mutant embryo injected with 2.5 ng *notch1b* MO. White arrows denote red blood cells that have leaked out of the cranial vessels indicative of hemorrhage.

Supplementary Figure S3. Multiple Notch receptors contribute to artery differentiation. (A-D) *Tg(tp1:egfp)*^{um14} embryos subjected to whole mount in situ hybridization with riboprobes against *egfp* (left panels) or *efnb2a* (right panels). Wild type embryos injected with (A) 2.5 ng control MO, (B) 1 ng *notch1a* MO or (C) 1 ng *notch1a* and 2.5 ng *notch1b* MO. (D) *notch3*^{fh332} mutant embryo injected with 1 ng *notch1a* MO. Red arrowheads denote dorsal aorta.

Supplementary Figure S4. Quantification of circulatory defects in Notch receptor- and ligand-deficient embryos. (A) Graph of proportion of embryos displaying indicated circulatory phenotype. Genotypes (fh332=*notch3*^{fh332}; tp37=*notch1a*^{tp37}) and injected Morpholino (MO) are indicated on the X-axis. Data for *notch1b* MO-injections are from 4 clutches of *notch3*^{fh332} embryos (total: 42 +/+, 82 +/*fh332*, and 52 *fh332*/*fh332*) and 3 clutches of *notch1*^{tp37} mutant embryos (total: 108 +/+ or +/tp37, 64 *tp37*/*tp37*). (B) Number of endothelial cells per intersomitic vessel (ISV) in embryos injected with indicated MO, or of indicated genotype. For MO injections, the number of endothelial cells in 6 ISVs per embryo were counted in embryos from two separate clutches (total sprouts counted – control (v. *notch1a*) MO=90, *notch1a* MO=84; control (v. *notch1b*) MO=84, *notch1b* MO=108). For *notch3*^{fh332}, embryos from 3 separate clutches were used (wt sibs: 156 ISVs, *notch3*^{fh332} mutant sibs: 168). N. S. – difference not statistically significant. *- p-value <0.001 (C) Graph of proportion of embryos displaying indicated circulatory phenotype. *dlc*^{tit446} genotype and injected Morpholino (MO) are indicated on the X-axis. Data for *dll4* MO-injections are from 3 clutches of *dlc*^{tit446} embryos (total: 34 +/+, 48 +/*tit446*, and 32 *tit446*/*tit446*).

Supplementary Figure S5. Quantification of intersomitic artery (ISA) and vein (ISVe) connections. (A) Surface renderings of trunk blood vessel 2-photon micrographs

following angiography with QDots. Dorsal aorta (DA) and posterior cardinal vein (PCV) denoted by brackets. Connection of an intersomitic vessel to the DA (white arrow; defined as an ISA) and PCV (light blue arrowhead; defined as an ISVe) are indicated in the first panel. (B) Graph showing average number of ISA and ISV connections per 10 vessels in embryos of indicated genotype, or injected with indicated Morpholino. Intersomitic vessels were quantified as arterial or venous based on circulation at 72 hpf in at least 10 embryos for each genotype or Morpholino injection. For Morpholino injections, embryos were injected with either 2.5 ng control or 2.5 ng *notch1b* Morpholino.

Supplementary Movie Legends

Movie 1. Normal circulation in trunk vessels in a wild type embryo at 72 hours post fertilization following injection with 2.5 ng control MO. DA – dorsal aorta, PCV – posterior cardinal vein, ISA – intersomitic artery, ISVe – intersomitic vein.

Movie 2. Normal circulation in trunk vessels in a *notch3*^{fh332} mutant embryo at 72 hpf following injection with 2.5 ng of control MO. DA – dorsal aorta, PCV – posterior cardinal vein, ISA – intersomitic artery, ISVe – intersomitic vein.

Movie 3. Normal circulation in dorsal aorta (DA) and posterior cardinal vein (PCV) in a wild type embryo at 72 hours post fertilization following injection with 2.5 ng *notch1b* MO. ISVe – intersomitic vein.

Movie 4. Normal circulation in dorsal aorta (DA) and posterior cardinal vein (PCV) in a *notch1a*^{tp37} mutant embryo at 72 hours post fertilization following injection with 2.5 ng control MO.

Movie 5. Loss of trunk circulation in *notch3*^{fh332} mutant embryo at 72 hpf following injection with 2.5 ng of *notch1b* MO. Red arrows denote poorly formed ventral wall of the dorsal aorta; blue arrow indicates possible gap between dorsal aorta (DA) and posterior cardinal vein (PCV).

Movie 6. Loss of circulation in *notch3*^{fh332} mutant embryo at 72 hpf following injection with 2.5 ng of *notch1b* MO. Red arrows denote well-formed ventral wall of the dorsal aorta. A single blood cell can be seen to traverse the length of the dorsal aorta in this clip.

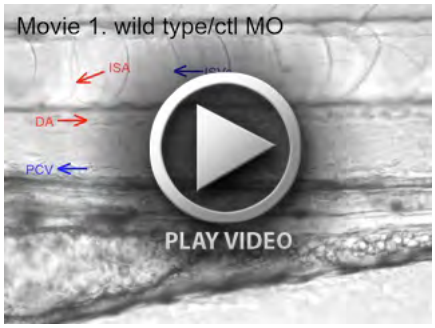
Movie 7. Arteriovenous shunt (denoted by red arrow) between the dorsal aorta (DA) and posterior cardinal vein (PCV) in a *notch1a*^{tp37} mutant embryo at 72 hpf following injection with 2.5 ng of *notch1b* MO.

Movie 8. Normal circulation in dorsal aorta (DA) and posterior cardinal vein (PCV) in a *dlc*^{tit446} mutant embryo at 72 hours post fertilization following injection with 2.5 ng control MO.

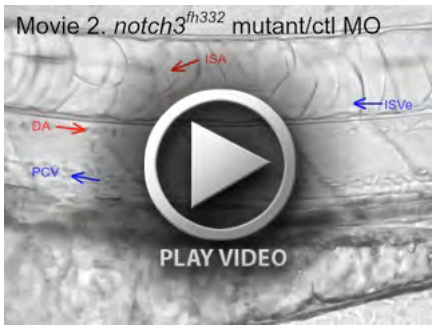
Movie 9. Normal circulation in dorsal aorta (DA) and posterior cardinal vein (PCV) in a wild type embryo at 72 hours post fertilization following injection with 10 ng *dll4* MO.

Movie 10. Loss of dorsal aorta (DA) and posterior cardinal vein (PCV) circulation in *dlc*^{tit446} mutant embryo at 72 hpf following injection with 10 ng *dll4* MO. Red arrows denote poorly formed ventral wall of the dorsal aorta.

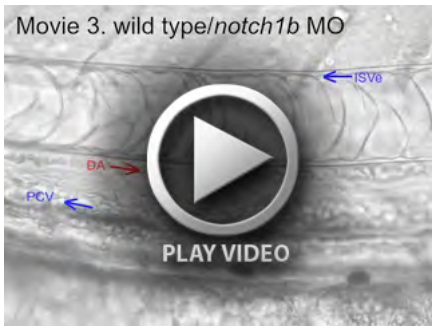
Movie 11. Arteriovenous shunt (denoted by red arrow) between the dorsal aorta (DA) and posterior cardinal vein (PCV) in a *dlc*^{tit446} mutant embryo at 72 hpf following injection with 10 ng of *dll4* MO.



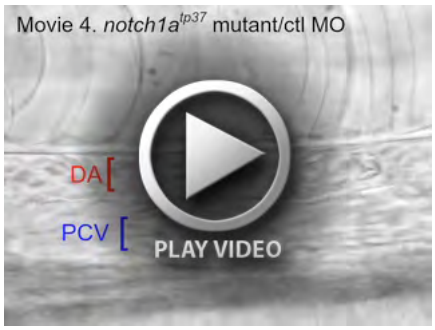
Movie 1.



Movie 2.



Movie 3.



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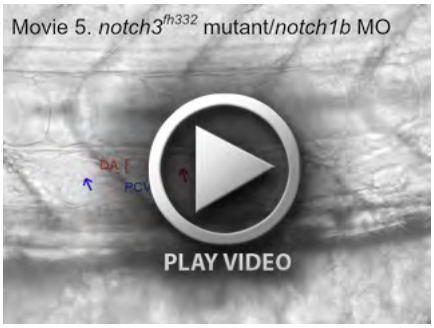
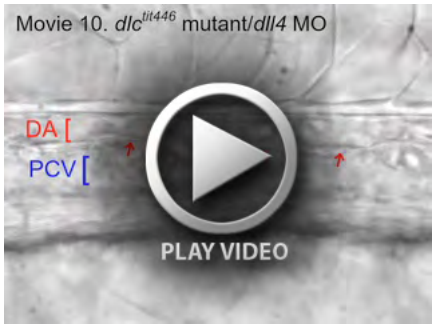


Movie 5.

Movie 6.

Movie 7.

Movie 8.



Movie 9.

Movie 10.

Movie 11.