SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 1

List of primers used for cloning and for site directed mutagenesis.

Supplemental Table 2

List of primers used for quantitative RT-PCR.

Supplemental Figure 1

Early developmental expression of *nr2f2* in the zebrafish. (A-G) Whole mount *in situ* hybridization images of developing 10.5 hpf whole lateral (A), 16.5 hpf anterior dorsal (B), 16.5 hpf whole lateral (C), 18 hpf whole lateral (D), 19 hpf head dorsal (E), 19 hpf trunk lateral (F), 24 hpf trunk lateral (G), and 48 hpf trunk lateral (H) views of zebrafish embryos probed for *nr2f2*. At 3s (A) and 15s (B,C) nr2f2 is expressed in the midbrain and cerebellum (white arrowhead in A) and the spinal cord (white arrowhead in B). This expression persists throughout development (E), but at 18s (D) expression is first identified in the developing axial vessels (black arrowhead). At 20s (F), 24 hpf (G), and 48 hpf (H) this vascular expression is confined to the PCV. The images in panels G and H are the same as those in Fig. 1B and H, respectively. Scale bars, (A,C) 200 μm, (B,D-H) 100 μm.

Supplemental Figure 2

RT-PCR validation of morpholino efficacy. RT-PCR was used to verify strong reduction in properly spliced transcripts in animals injected with nr2f2 ATG (MO1) or splice (MO2) morpholinos. **(A)** Open boxes and black lines denote exons and introns of the early coding and non-coding region of nr2f2. MO1 and MO2 (in red) mark the target sites of the nr2f2 translation blocking and exon1 splice donor morpholino, respectively. P1, P2, and P3 indicate sites of primers. **(B)** Two- to four-cell embryos were injected with mismatch Ctl MO, nr2f2 MO1 (ATG MO) or nr2f2 MO2 (Splice MO) and cDNA was isolated from two pools of four embryos from each injection group. Fragments of nr2f2 sequences were amplified using indicated primers. In Ctl MO injected embryos the normal nr2f2 spliced P1-P3 RT-PCR product of approximately 550 bp was observed (lane 4) but not the P1-P2 splice donor site read-though PCR product (lane 3). In ATG MO1-injected embryos, little or no P1-P3 RT-PCR product is detected (lanes 5 and 6). In Splice MO-2 injected embryos, the normal nr2f2 spliced P1-P3 RT-PCR product is not observed (lanes 1 and 2), but a splice donor site read through product is observed instead (lanes 7 and 8). Translation of the read-through product is predicted to lead to a truncated polypeptide diverging from the normal product after amino acid 155 and terminating after an additional 21 amino acids (total predicted length 176 amino acids). **(C)** *B-actin* cDNA was also amplified from each sample as a loading control.

Supplemental Figure 3

Transgenes used for endothelial expression in zebrafish. (A) I-SceI vector for *kdrl* promoter-driven (endothelial) co-translational expression of wild type nr2f2 and cerulean

fluorescent protein ("kdrl-CF"). **(B)** I-SceI vector for *kdrl* promoter-driven (endothelial) co-translational expression of "activated" truncated nr2f2-VP16 fusion protein and cerulean fluorescent protein ("kdrl-VCt"). **(C)** I-SceI vector for *kdrl* promoter-driven (endothelial) co-translational expression of "repressive" engrailed- truncated nr2f2 fusion protein and cerulean fluorescent protein ("kdrl-ECt"). **(D)** Representative blue fluorescence image of cerulean expression in the trunk of a kdrl-CF transgene-injected animal, showing broad mosaic endothelial expression of the fluorescent protein. **(E)** Transgenes present in the *Tg(fli1a:GVEcRF)* and *Tg(UAS:N3ICDmyc)* (Lawson et al., 2001) transgenic lines that were crossed together to permit tebufenozide-inducible endothelial expression of "activated" Notch3 intracellular domain (ICD). **(F)** I-SceI vector for *kdrl* promoter-driven (endothelial) co-translational expression of the "Notch repressing" *suppressor of hairless (su[H])* DNA binding domain mutant *(su[H]DBM)* and mCherry fluorescent protein. In the constructs in A, B, C, and F the self-cleaving viral 2A peptide sequence (Provost et al., 2007; Szymczak et al., 2004) was used to co-translationally link other proteins to cerulean fluorescent protein, allowing stoichiometric production of both proteins.

Supplemental Figure 4

The *Tg(efnb2a:eGFP)* transgenic reporter is not responsive to nr2f2. (A) Transmitted light image of a 24 hpf *Tg(efnb2a:eGFP)* transgenic animal. **(B)** Green fluorescence confocal image of GFP expression in the animal in panel A, showing expression in muscle, neural, vascular, and other tissues. The red box shows approximate area magnified in panel C. **(C)** Higher magnification green fluorescence confocal image of GFP expression in the dorsal aorta (DA) but not in the posterior cardinal vein (PCV) in the animal in panel A. **(D)** Schematic diagram of a 1 dpf zebrafish (modified from (Kimmel et al., 1995)) with a red box showing the approximate position of images in B-F. **(E-I)** Green fluorescent confocal micrographs of 24 hpf *Tg(efnb2a:eGFP)* transgenic zebrafish. Image planes above and below the dorsal aorta and intersegmental vessels were not used in the reconstructed confocal images shown here, to eliminate obscuring somitic expression of the transgene and permit visiualization of the vessels. Animals in image panels E-I were either untreated (E), injected with *nr2f2* MO (F), injected with kdrl-CF (Full length nr2f2 under the control of the kdrl promoter; G), injected with kdrl-ECt (chimeric protein with nr2f2 lacking the transactivation domain fused to engrailed repressor domain; H), or injected with kdrl-VCt (chimeric protein with nr2f2 lacking the transactivation domain fused to VP16; I). Scale bar, 100 μm.

Supplemental Figure 5

The *Tp1:mCherry* and *efnb2a:eGFP* transgenic reporters are responsive to reduced Notch signaling. (A,B) Whole mount *in situ* hybridization of 24 hpf zebrafish probed for *dll4*. **(C,D)** Red fluorescent confocal micrographs of 24 hpf *Tg(Tp1bglob:hmgb1-mCherry)*^{h11} *("tp1:mCherry")* transgenic zebrafish. **(E,F)** Green fluorescent confocal micrographs of 36 hpf *Tg(efnb2a:egfp)* transgenic zebrafish. Animals were either treated with control DMSO carrier (A,C,E) or with 100μm Notch inhibitor DAPT (B,D,F). Animals in DMSO/DAPT panel pairs were imaged using identical conditions for comparison. Superficial image planes were removed for visualization of vessels in the reconstructions shown in panels E and F because of extensive fluorescence in muscle and epidermis in this transgenic line. **(G)** Quantitative RT-PCR measurement of *dll4* and *grl*

gene expression in excised trunks (see Materials and Methods) of 24 hpf animals. Values are all normalized to control gene expression levels, which are set equal to 1. Rostral is to the left and dorsal is up in all image panels. Scale bars, (A-D) 200 μm, (E,F) 50 μm.

Supplemental Table 1

List of primers used for cloning

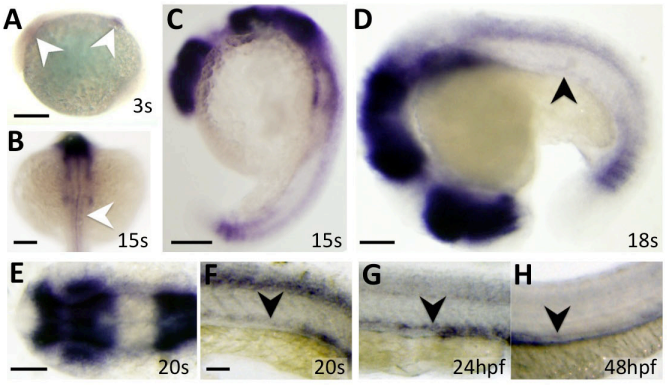
Clone name	Fwd primer (5'-3')	Reverse primer (5'-3')
nr2f2 full	caccatggcaatggtgtgg	ctactgaatcgacatataaggc
nr2f2 short	caccatggcaatggtgtgg	ctgcttttccgactggctc
nr2f2-Ct	caccatggcaatggtgtgg	aattggggttttacctac
sox7 (full)	caccatggcggctctgataagtgcgt	ttatgaaatgctgtagttgttgtagta
sox18 (full)	caccatgaatatatctgagtctagtt	ttatcctgtaatgcaggcgctg
XSu(H) DBM	caccatgcaacctggcattcc	ggacactactgctgcagtgga
GV-EcR F'	cacccggagcaagcttgatttagg	tggtttgtccaaactcatcaa

Supplemental Table 2

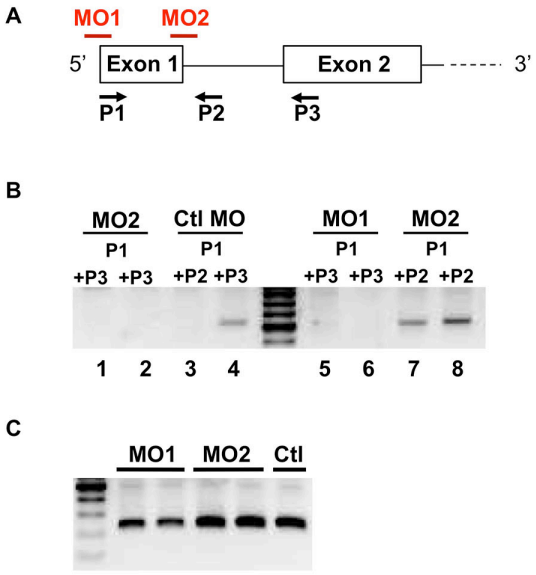
List of primers used for quantitative RT-PCR

Gene	Fwd primer (5'-3')	Reverse primer (5'-3')
ephrinB2a	acacgccaccacgttgtcactc	tgagccgttgttgttgccgc
grl	aagcagccgccatgaccacatc	tgcaggaacgcagcaggaatgg
fli1a	cgtcaagcgagagtatgacc	agttcatctgagacgcttcg
dll4	agtgtgacagcagcccacgc	tggggaatctgcgcaggtgag
sox7	ttgagtgctctgcgggaaacgc	tgcgctcatctttggcccac
sox18	tccttggacgctgtggaccaac	tcaaagcgctgctttcctcgc
stab1	tcaggcacaagaacgacgcc	atggttccgcaaacgccctg
dab2	agctccagccacagggcttttg	tgctgcaggagctgggtttgtg
lyve1	tgccatcacagccaaagaggc	gctcctcaacccaaccaaacctgc
ephB4a	agttcctgctcggatcagtggc	aggtccgcacactgttgttctcc
nr2f2	acacagtcaaccccgacgaacc	tttgtccccgcaaaccacgc
prox1a	tgtcatttgcgctcgcgctg	accgcaacccgaagacagtg
EF1a	ctggaggccagctcaaacat	atcaagaagagtagtaccgctagcat

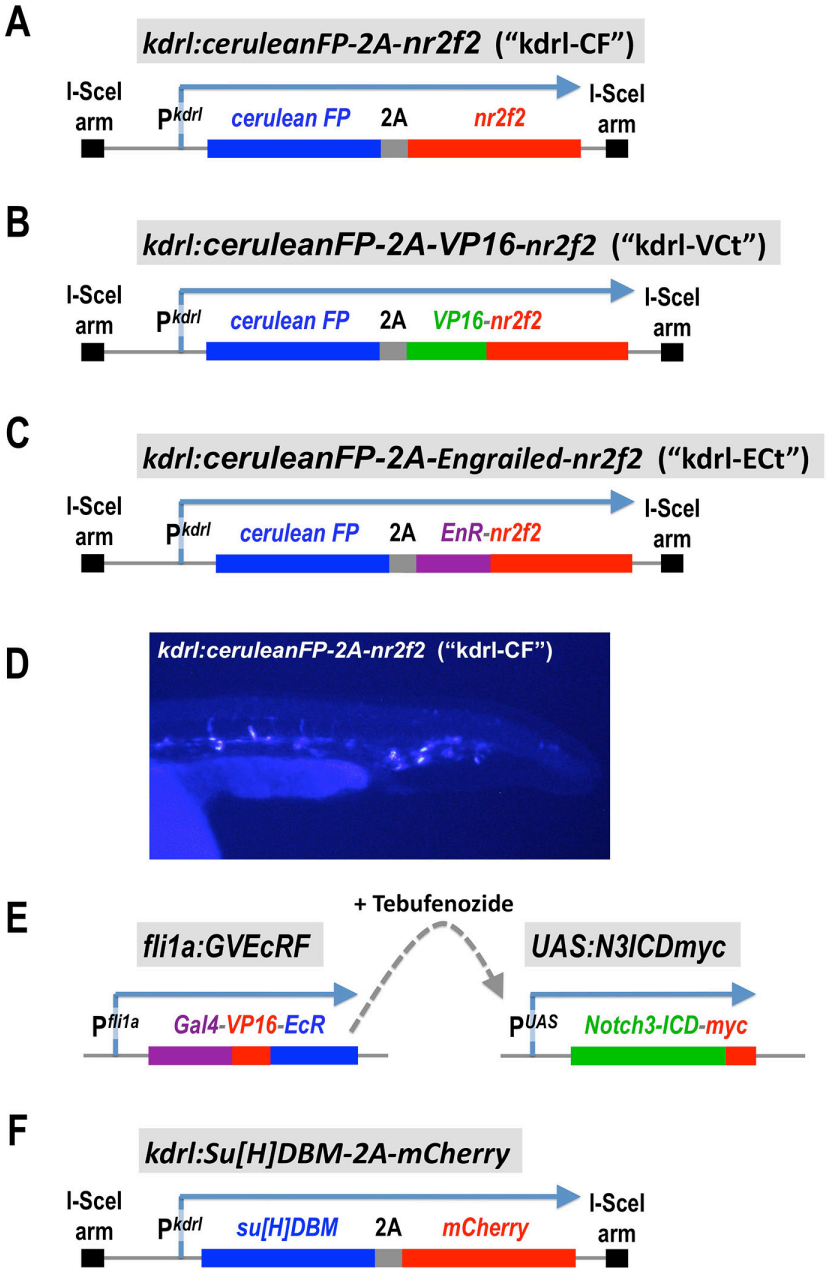
Swift et al., Supplemental Figure 1



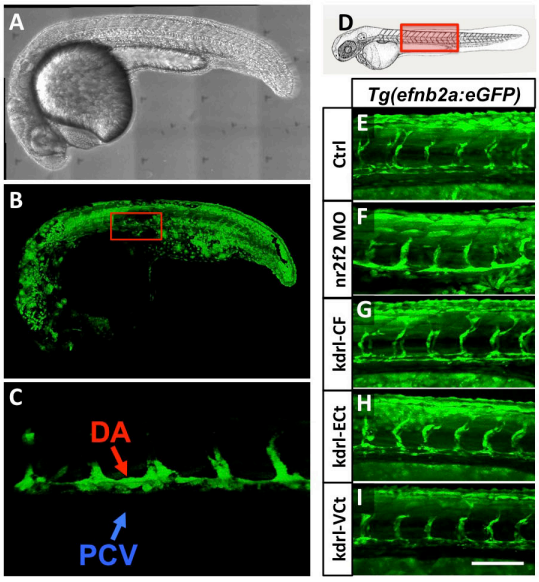
Swift et al., Supplemental Figure 2



Swift et al., Supplemental Figure 3



Swift et al., Supplemental Figure 4



Swift et al., Supplemental Figure 5