Supplemental Data

Supplemental Figures

Figure S1. Postembryonic zebrafish coronary vessel development. (Related to Figure 1)

Four different transgenic lines were used in different combinations to characterize the development of coronary vessels in zebrafish from 7dpf to 140dpf; *fli1a:EGFP*; *myl7:DsRed2-NLS* (labeled blue: A, C, D, G, J, L, M, O and Q), *wt1b:EGFP*; *tcf21:DsRed* (labeled maroon: B and F), *fli1a:EGFP; kdrl:mCherry* (labeled pink: E, H, K, N, P, R, S, U, V, X, Y, Z, BB, CC, DD, FF), *fli1a:EGFP; tcf21:DsRed* (labeled orange: I, T, W, AA, EE).

Pre-vessel formation (7 to 40dpf). After hatching the heart is composed of three layers, the *fli1a:EGFP* endocardium (A), *cmlc:nRFP* positive myocardium (A) and the epicardium partially labeled with *wt1b:EGFP* and *tcf21:DsRed* (B). The heart increases in size dramatically through the first six weeks of life (A-L) during which time the myocardium expands and the *tcf21*-poisitive epicardium partially covers the surface of both the atrium (a) and ventricle (v) (B, F, I). The bulbus arteriosus (ba) strongly expresses *kdrl:mCherry* as does the endocardium (E, H, K), which is visible through the myocardium. There are no endothelial cells (*fli1a:EGFP*) on the surface of the myocardium (*cmlc:nRFP*, A, C, D, G, J and L).

Coronary endothelial cell emergence (42 to 63dpf). Following this expansion in the six weeks after fertilization, endothelial cells are found on the surface of the heart and the coronary vasculature begins to form (M-R). These endothelial cells

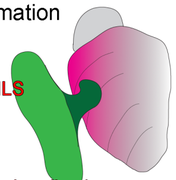
are found on the outside of the myocardium (*cmlc:nRFP*, M, O, Q) and express *kdrl:mCherry* (N, P, R). They are first observed at the juncture of the atrium and ventricle (M-O) and then connected from this point over the base of the ventricle (P-R).

Vessel formation and expansion (70 to 105dpf). The heart continues to grow into adulthood as it does so the vasculature continues to form over the surface of the ventricle, but not the atrium (S-Z). Endothelial cells remain connected to existing sprouts or vessels during this process (S-Z) and the heart becomes increasingly covered by *tcf21*-possitive epicardial cells as it proceeds (T and W).

Complete vascular network (105 to 140dpf). By 4 months post-fertilization the intricate network of vessels is formed, the pattern and density of vessels varies between adult zebrafish, but vessels are only observed on the ventricle of the heart (AA-FF).

A subset of endothelial cells of the coronary vasculature network express arterial markers *flt1:tdtomato* (GG) and *dll4:EGFP* (II), but none of the vasculature endothelial cells express venous marker *flt4:YFP* (GG and KK) nor lymphatic marker prox1:RFP (KK) at this stage. Expression of both *prox1a:RFP* and *flt4:YFP* is restricted to endothelium partially covering the BA (GG and KK, arrowheads). All four markers were found to be expressed in the adult fin of the same fish (*flt1:tdtomato and flt4:YFP*, HH; *dll4:EGFP*, JJ; *prox1a:RFP* and flt4:YFP, LL). Asterisk denotes artery. Scale bars, 50 μm.

Pre-vessel formation

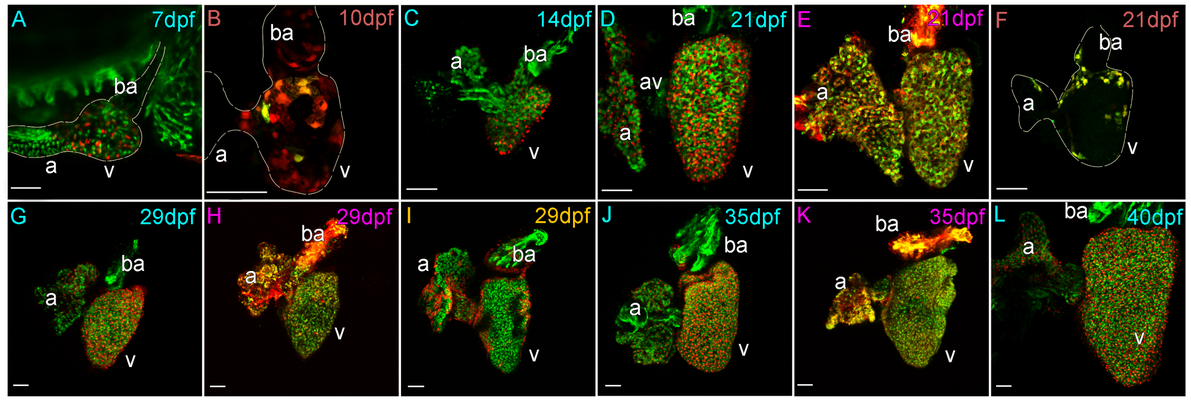


A, C, D, G, J, L: myl7:DsRed-NLS (myocardium), fli1a:EGFP (endothelium)

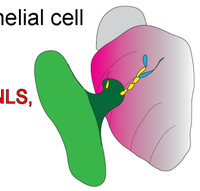
B, F: tcf21:DsRed (epicardium), wt1b:EGFP (epicardium)

E, H, K: kdrl:mCherry (vasculature), fli1a:EGFP

I: tcf21:DsRed, fli1a:EGFP

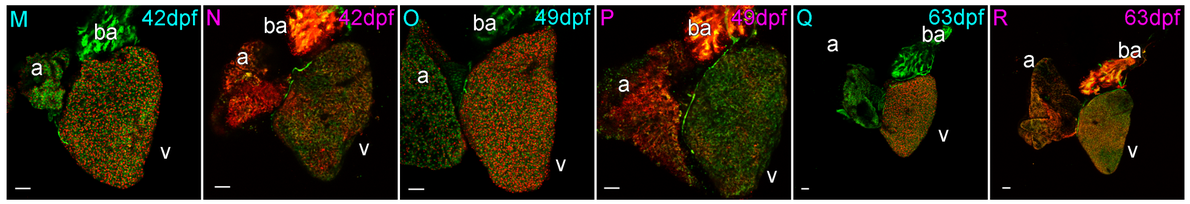


Coronary endothelial cell emergence

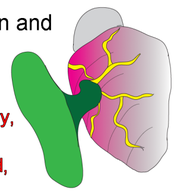


M, O, Q: myl7:DsRed-NLS, fli1a:EGFP

N, P, R: kdrl:mCherry, fli1a:EGFP

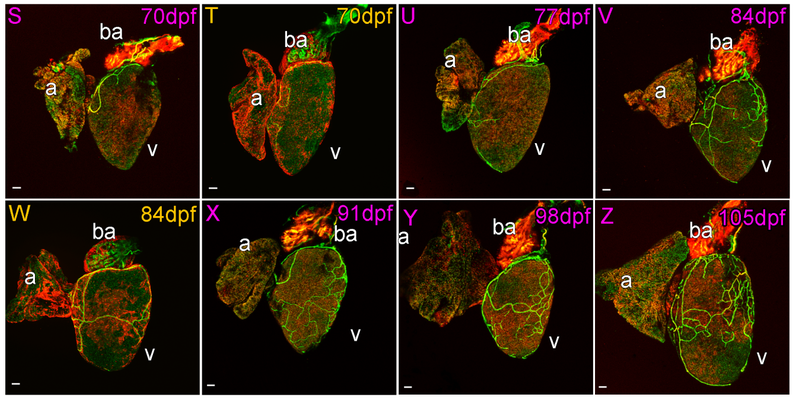


Vessel formation and expansion

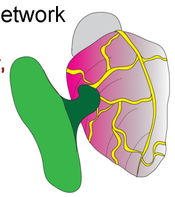


S, U, V, X, Y, Z: kdrl:mCherry, fli1a:EGFP

T, W: tcf21:DsRed, fli1a:EGFP

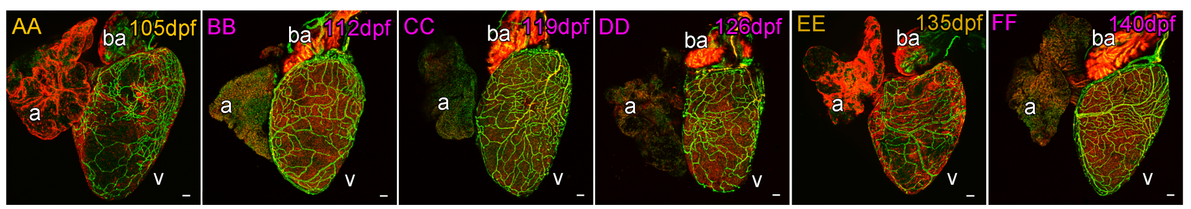


Complete vascular network



BB, CC, DD, FF: kdrl:mCherry, fli1a:EGFP

AA, EE: tcf21:DsRed, fli1a:EGFP



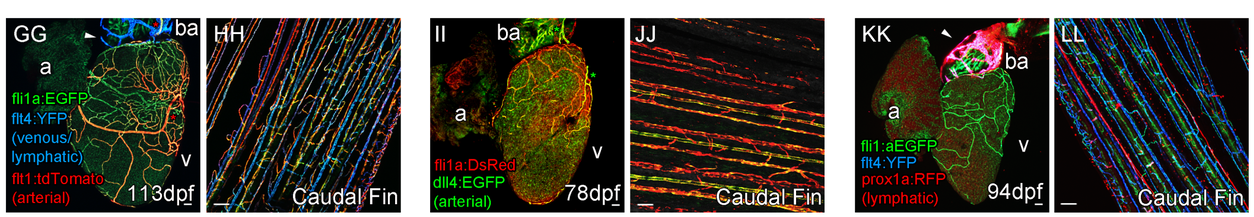


Figure S2. Coronary vasculature remains responsive to increases in heart size and output requirement. (Related to Figure 1)

Sporadic increases in the vessel density of large zebrafish (210 to 420dpf). Although formed and stable in many zebrafish the coronary vasculature remains capable of expanding in size and density with volumetric increases in heart size in older zebrafish (A-F). A proportion of zebrafish can continue to grow larger than their sibling tank mates and show increases in heart size and coronary vessel density (210dpf, A and B; 350dpf, C and D; 420dpf E and F; body length between 29 and 35mm labeled bottom left of each panel). The developmental timing, organization and extensiveness of the coronary vessel network are slightly variable, but highly correlative with fish size, age and the size of the heart ventricle (G-I). Graphs demonstrating the one-phase exponential correlation between zebrafish length and age (G, coefficient of determination (R^2)), linear correlation between heart ventricle size and age (H, Pearson correlation coefficient (r)) and the two-phase exponential correlation between heart ventricle size and the area covered by endothelial cells on the surface of the heart (I). Scale bars, 50 μm.

Adult sibling zebrafish are raised and maintained at a high density of 20 fish per 3 liter (*L*) until 8 mpf and then measured (8 mpf, preseparation) and divided into two groups: one maintained at high density (13/3L) and the other at low density (4/3L) (J). Three of these fish with an average length of 27mm were collected and imaged (8mpf collected, J). Four months later (12mpf), zebrafish from both

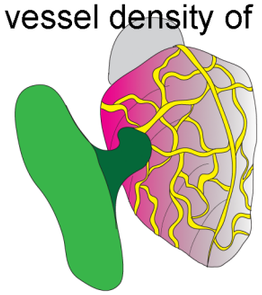
groups (4m 13/3L and 4/3L) were re-measured. Zebrafish maintained at low density are significantly larger (3mm on average) than those maintained at high density (J, 12mpf 4m 4/3L and 13/3L respectively; **p<0.01, t-test ±SD). Imaging of ventricle area (K) and vessel area (L) in 8 mpf (20/3L, collected), and 12 mpf hearts of fish maintained at high (4m 13/3L) and low (4m 4/3L) density reveals that the ventricle and corresponding coronary vessel area and density are significantly greater in the group of zebrafish maintained at low density (K and L, **p<0.01, t-test ±SD; M, collected preseparation; N, high density; O, low density). Scale bars, 50 μm.

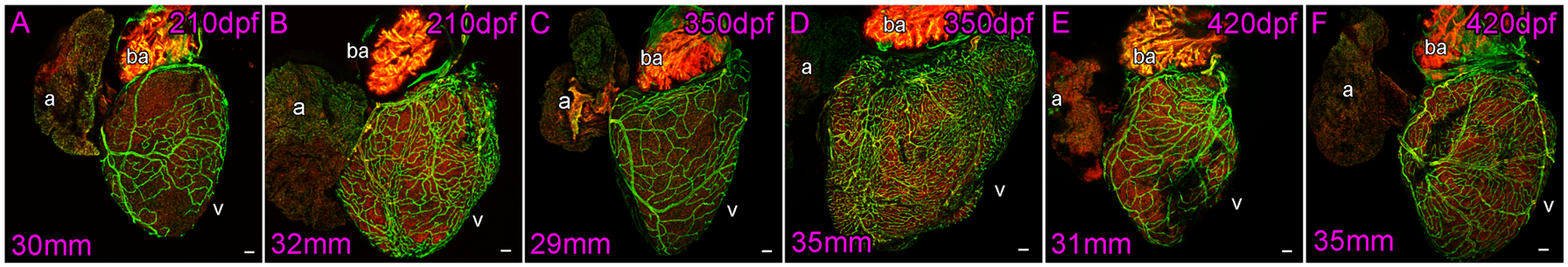
Sporadic increases in the vessel density of large zebrafish

A, C, E **Relatively small adult zebrafish**

B, D, F: **Relatively large adult zebrafish**

A, B, C, D, E, F: kdrl:mCherry (vasculature), fli1a:EGFP (endothlium)




A
210dpf
ba
a
v
30mm
B
210dpf
ba
a
v
32mm
C
350dpf
ba
a
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D
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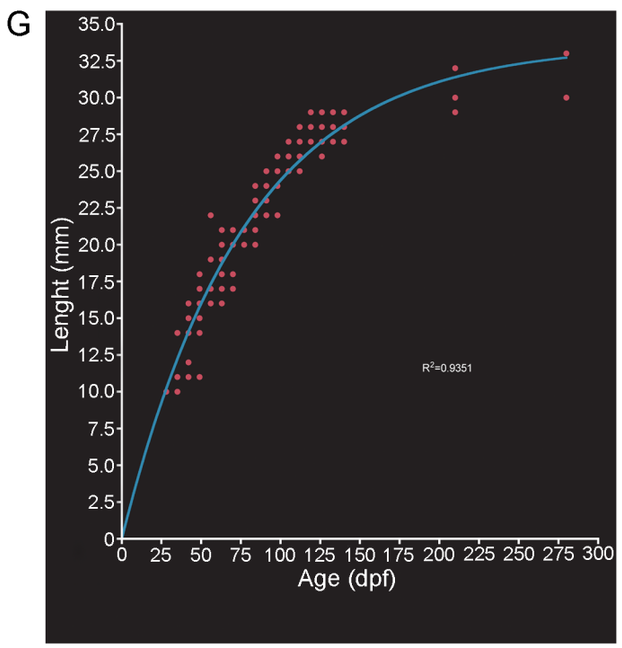
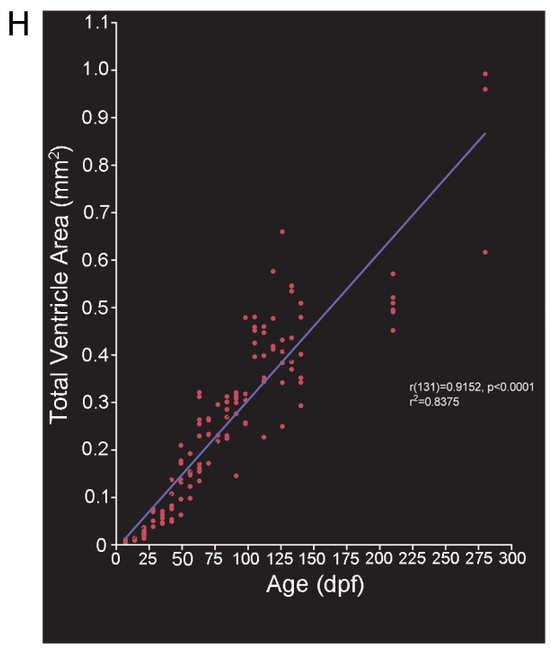
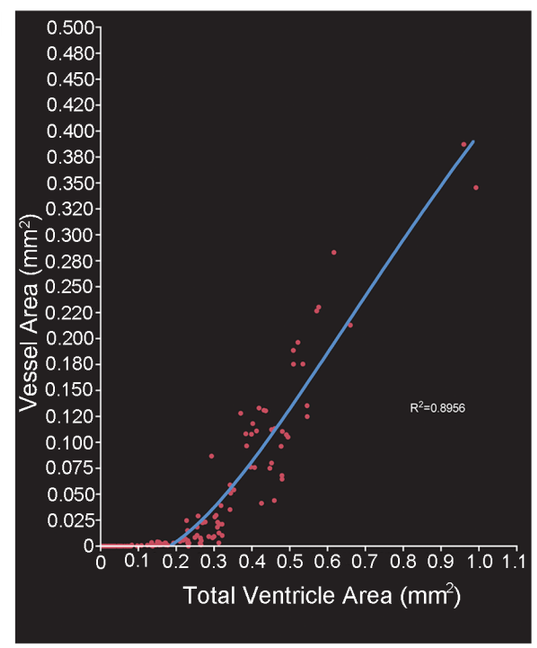
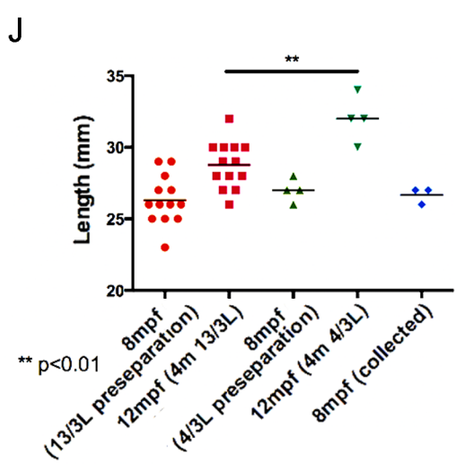
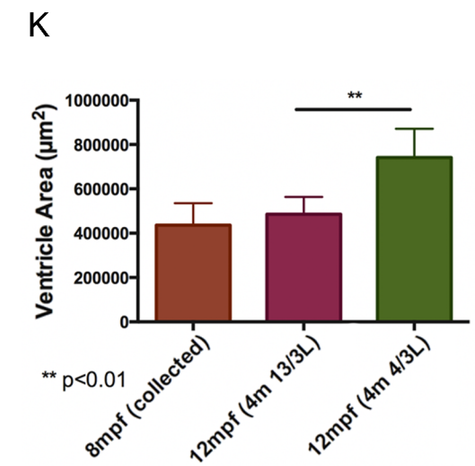
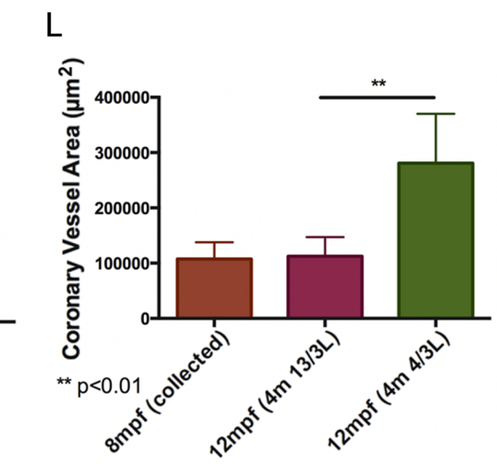












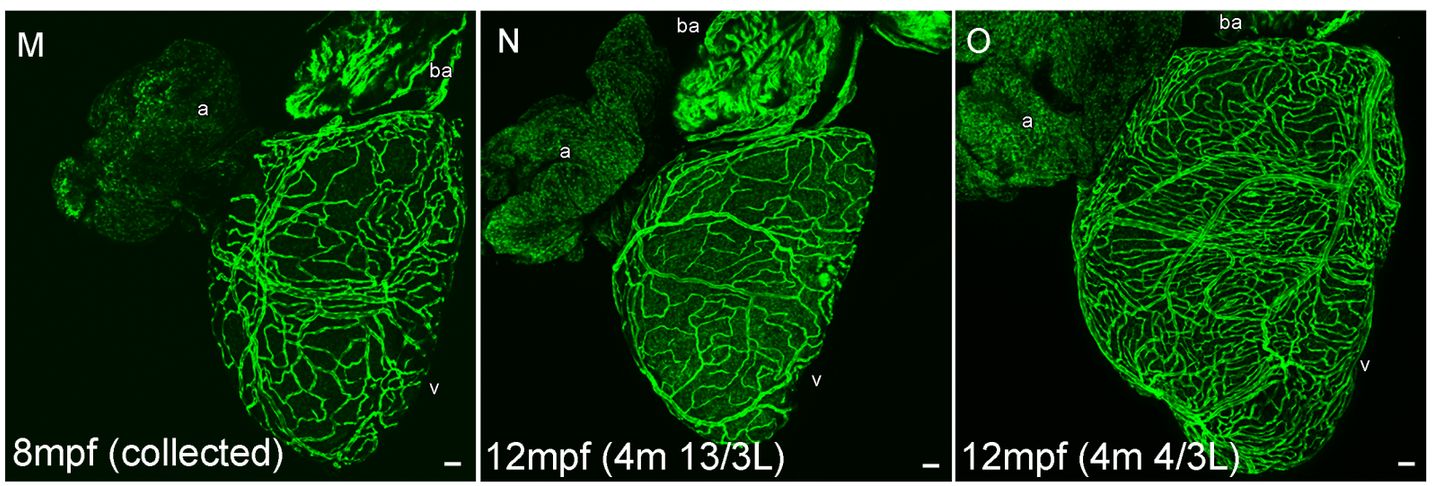
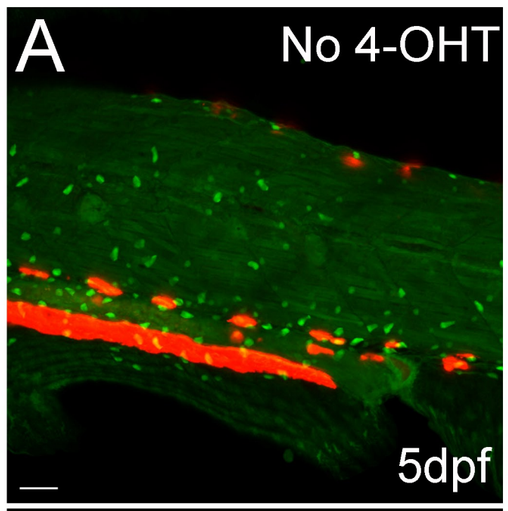


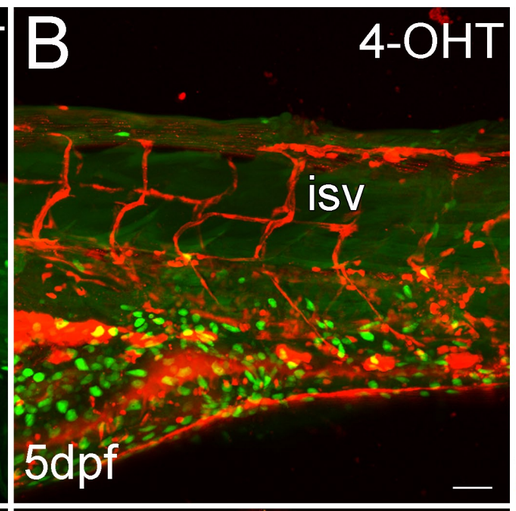
Figure S3. Cell lineage tracing of endocardial cells. (Related to Figure 3)

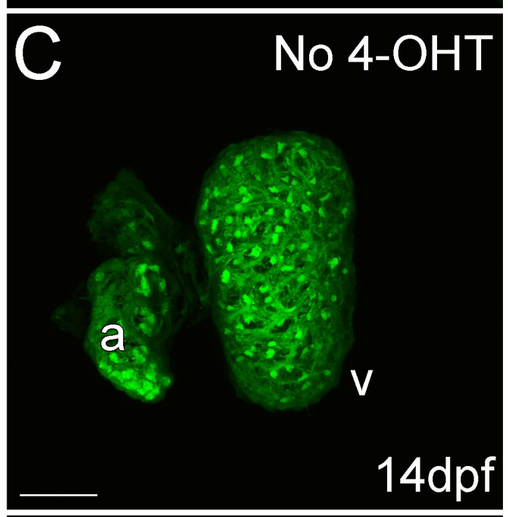
Cre activity is strictly dependent on 4OHT with no labeled endothelial cells observed in non-4OHT treated embryos (A, red autofluorescence from pigment cells), larval (C) or adult fish (E). Treatment of embryos for 5 days in 4-OHT results in clonal endothelial cell labeling in the embryo (B), larval (D) and adult (F) zebrafish. However, such labeling of the endothelial cells does not always result in the labeling of the later developing coronary vessels indicating that only a subset of endocardial cells contribute to the coronary vasculature (F). In addition, labeling of vessels in 4OHT treated hearts that lack any endocardial labeling has not been observed (n=6). Side view of ventricle at the AV boundary (atrium removed) (G and H, arrowhead, labeled endocardium; arrow, labeled surface vessel; G' and H' mCherry, G'' and H'' EGFP). Labeled endocardial cells in the AV boundary can be seen proximal to similarly labeled migrating endothelial cells on the ventricle surface. I, Quantification of triple transgenic zebrafish hearts (*fli1aep:ERt2CreERt2; fli1a:EGFP; ubb:LoxP-EGFP-LoxP-mCherry*) exposed to 4-OHT during embryogenesis and imaged at 95 to 155dpf, cumulative proportion of endocardium labeled in 1 to 7 patches (defined as a continuous group of cells). Individual hearts along x-axis, hearts with labeled coronary vessels or surface endothelial cells are marked with asterisk (I). To confirm the contribution from the AVC endocardium, recombination of *ubb:Zebrabow (ubb:Lox2272-LoxP-RFP-Lox2272-CFP-LoxP-YFP)* was induced in the embryonic endocardium by *kdrl:CreERt2* to produce a spectrum of colors which are plotted with respect to their hue and saturation (J). There was limited

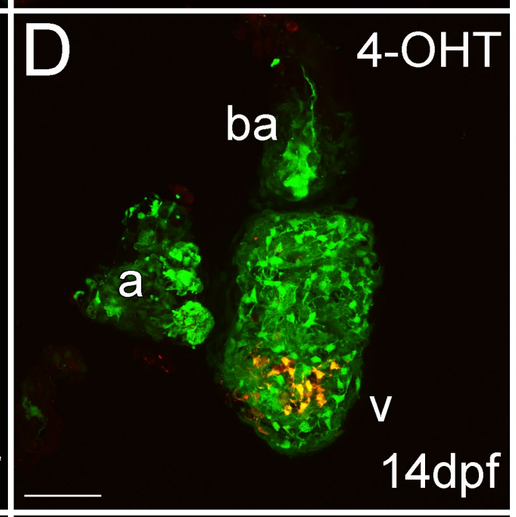
contribution to the spectrum from the red channel due the relatively weak expression of *ubb:Zebrabow* in endothelial cells and the imaging limitations due to the adjacent RFP-positive myocardium. Despite this, a range of distinguishable colors was detected with varying frequency (K). An average of 2.2 different colors where found in the regions surrounding the AVC and a maximum of 5 spectral labels were distinguishable within a single heart (L). An example of a color specific to the AVC endocardium that matches that of the vessel endothelium on the ventricle surface, there is also a direct connection between these cells (M; relative z position listed for AVC/vessel clone). Even without a full spectrum of color labeling the spectral matching of AVC endocardium with vessel endothelial cells is significantly higher than expected at random (N, Probability of randomly matching colors [P(Match)] is 24.4%, $p<0.0001$). Positional distribution of distinct spectral contributions shows that many are shared between different heart regions, but only those that label AVC endocardium match the labeling observed in vessel endothelial cells (O). The different spectral contributions are represented as a circle each of which is positioned with respect to their location within the endocardium of the heart (O). Colors that match those of the vessel endothelium on the ventricle surface are grouped together in the pink box; those that do not appear to match are below in the orange box (O). Unlike single-channel analysis, for multispectral analysis only cell spectral contribution is scored and not relative cell positioning (i.e. whether or not cells form a contiguous patch). Vessel density is defined as a ratio of this vessel area to total pixel area of a defined region. The positioning of the

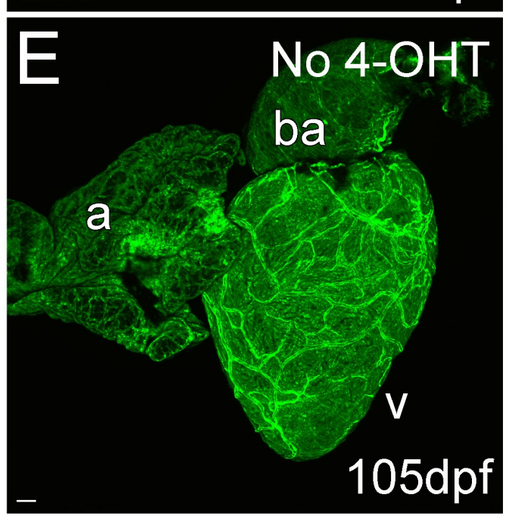
emerging sprouts from the AVC endocardium is consistent with the pattern of vascularization observed in older zebrafish hearts, which lack vessels on the surface of the atrium and sinus venous (sv, *fli1a:EGFP*) (P). Unlike the SV in other organisms studied, the zebrafish SV largely lacks cardiomyocytes (Q, *myl7:DsRed2-NLS,* red) and has no coronary sinus on its surface (Q, *fli1a:EGFP*, green; ventricle removed and atrium rotated to reveal SV). Scale bars, 50 μm. isv, intersegmental vessel; a, atrium; v, ventricle; av, AV canal; and sv, sinus venous.

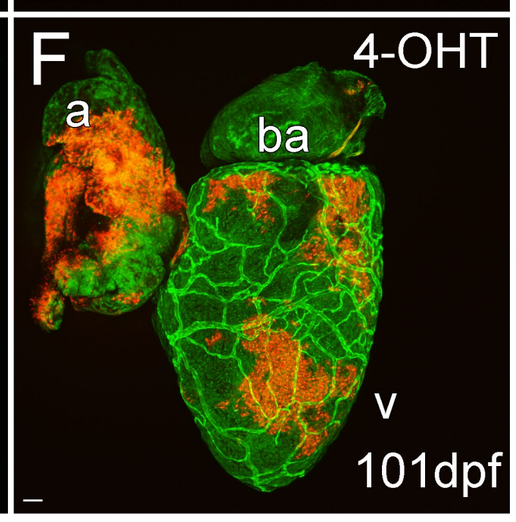


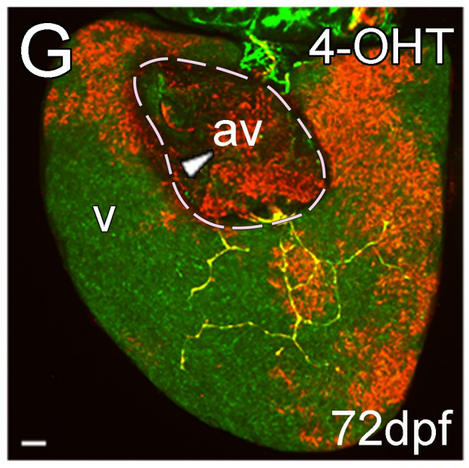


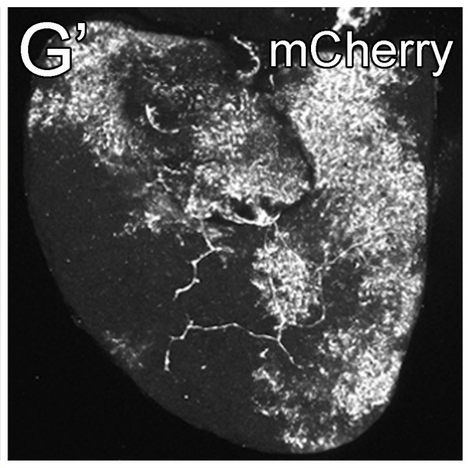


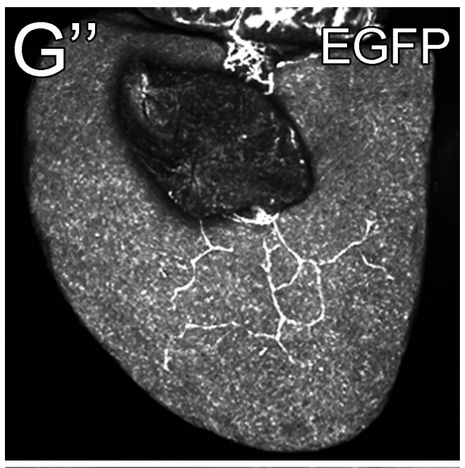


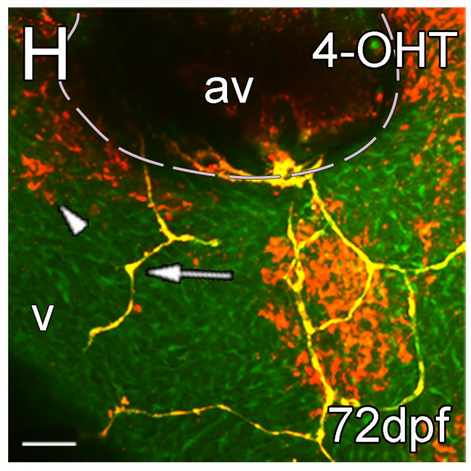


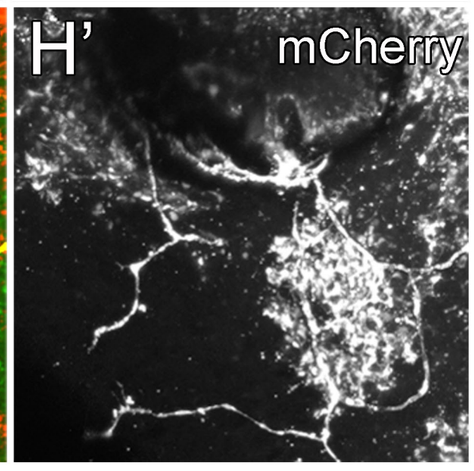


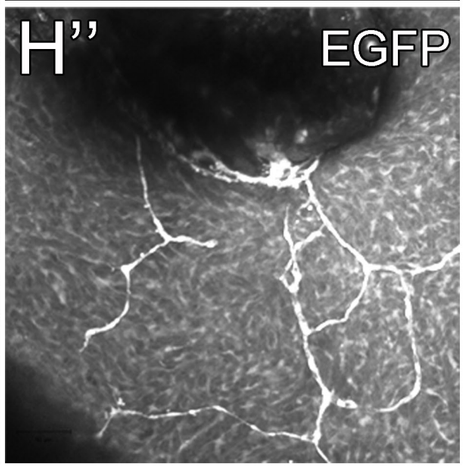


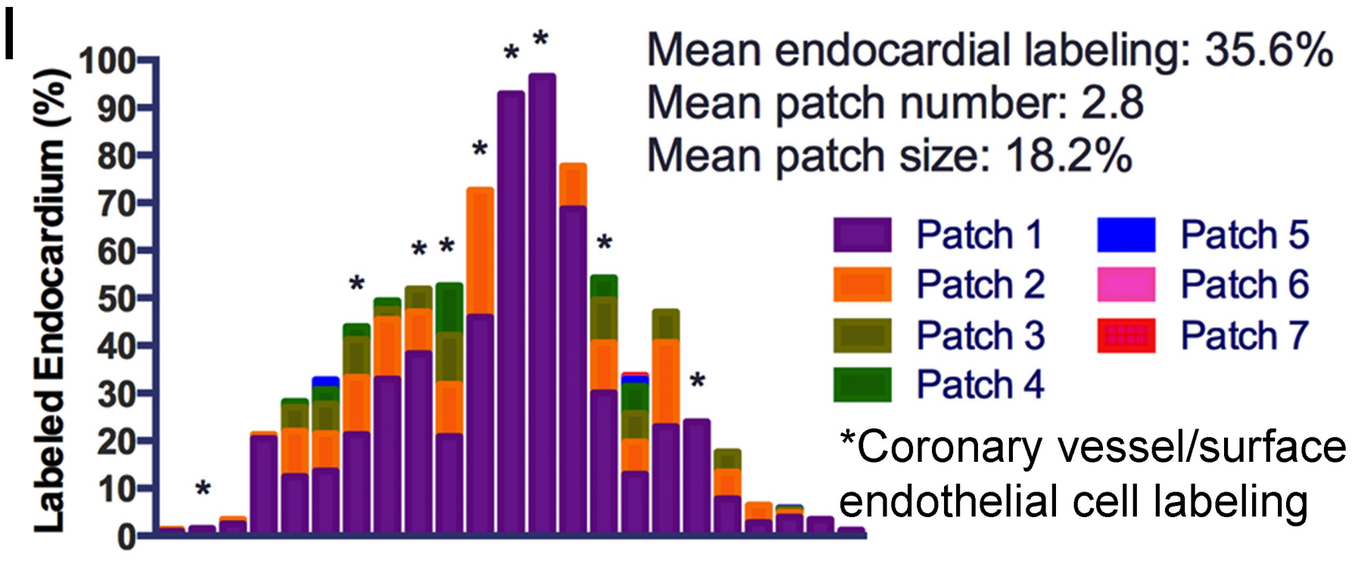


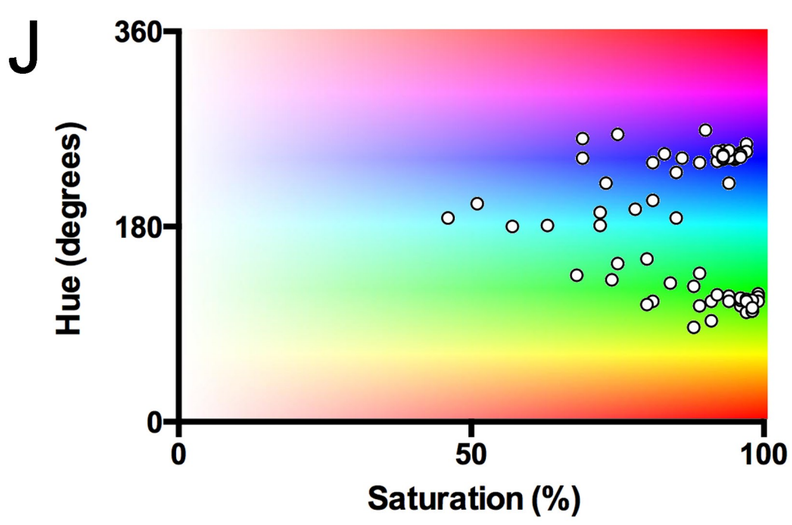






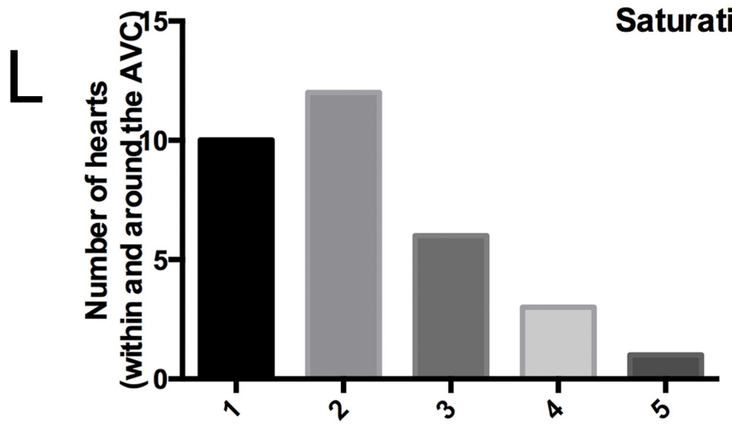


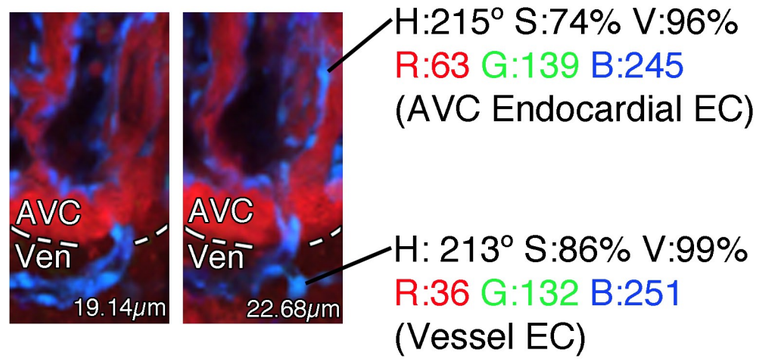


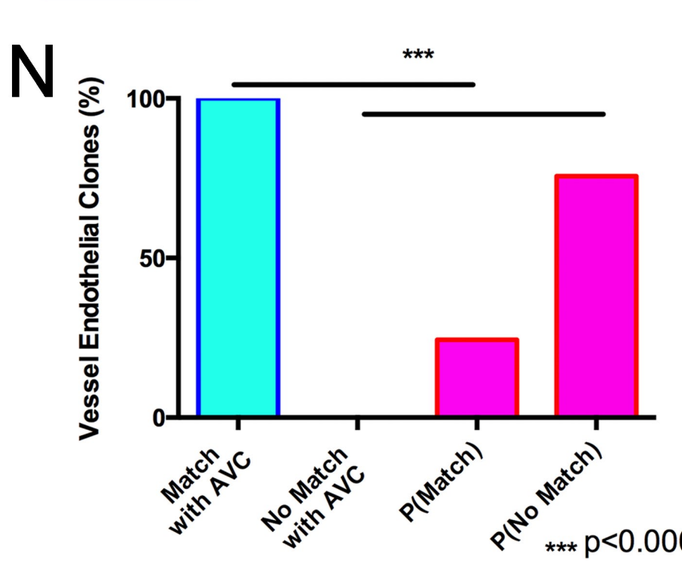


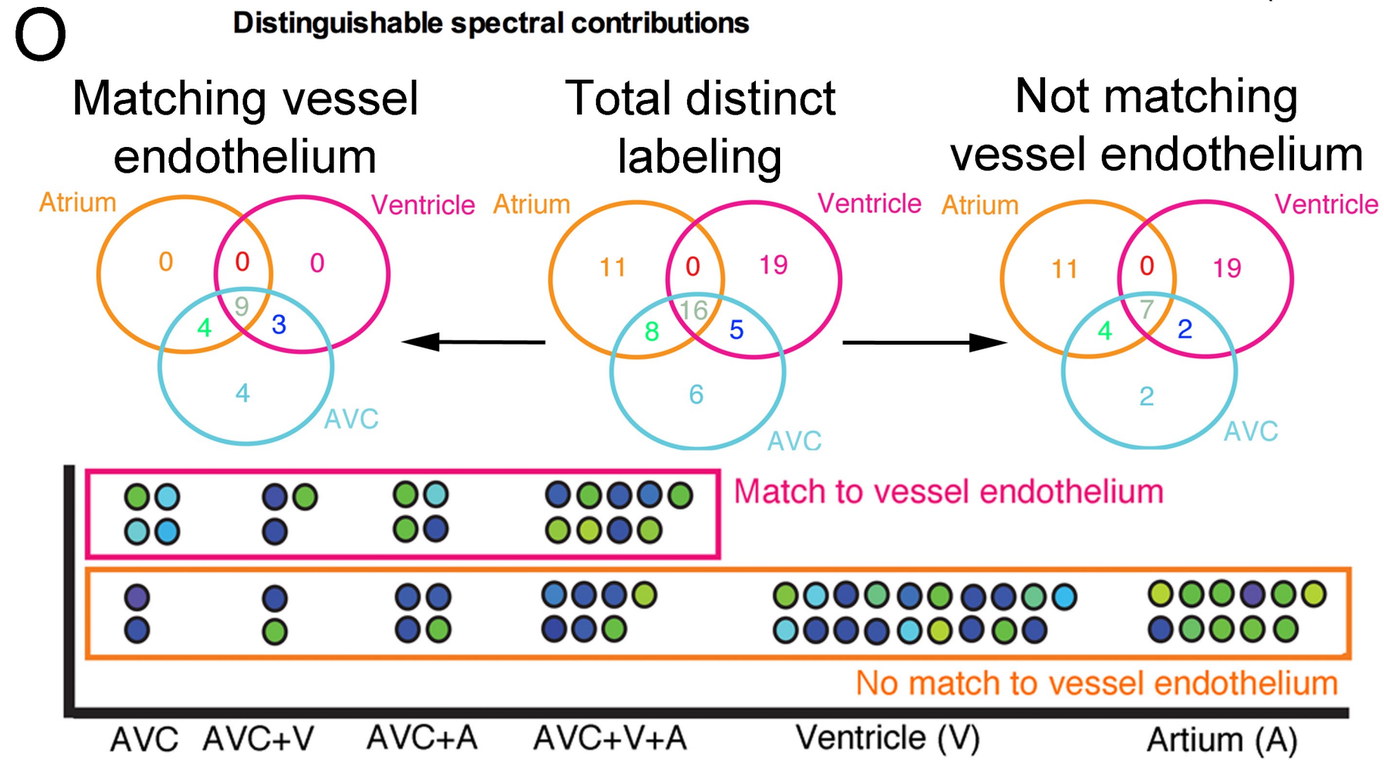
K

Hue (Degree, ±15)	Color Name	Color	Frequency	Probability
0/360	Red		0	0.000
30	Orange		0	0.000
60	Yellow		0	0.000
90	Chartreuse		5	0.071
120	Green		20	0.286
150	Spring Green		4	0.057
180	Cyan		6	0.086
210	Azure		5	0.071
240	Blue		26	0.371
270	Violet		4	0.057
300	Magenta		0	0.000
330	Rose		0	0.000









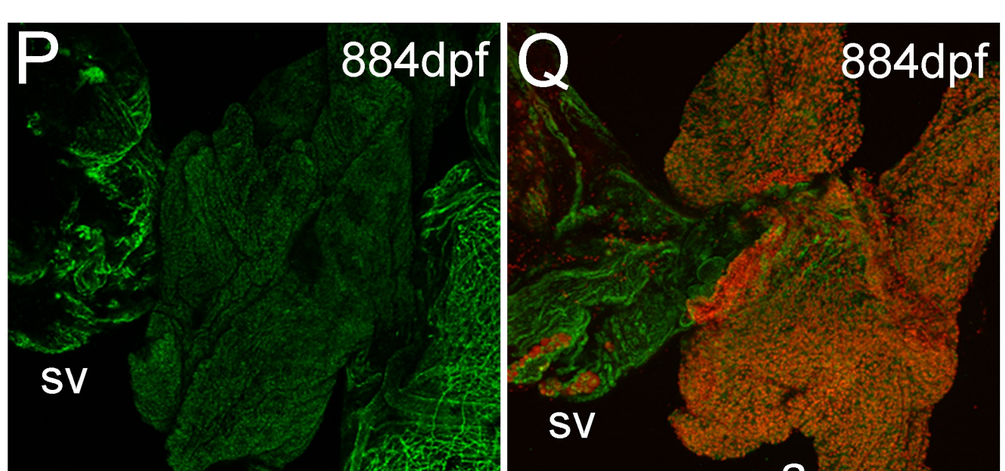


Figure S4. *cxcl12b:YFP* and *cxcr4a:YFP* expression during vessel formation. (Related to Figure 4)

Prior to the emergence of vessels, *cxcl12b:YFP* (pseudo colored blue in A, B, C, G, H, I; yellow in D) is detected in ventricular cardiomyocytes that also express *myl7:DsRed2-NLS* and are flanked by *fli1a:EGFP* positive endocardial cells (A-C). Expression of *cxcl12b:YFP* is stronger in the base than the apex (E, between 14dpf and 84dpf, n=36, measurements paired within hearts) and coronary vasculature is significantly more dense in the base of the ventricle (F, between 7 and 420dpf, n=153, means of 22 time points are paired), both $p<0.0001$ (Wilcoxon matched-pairs signed rank test). After the formation of coronary vessels expression is observed in mural cells (G-I). *tcf21:CreERt2, ubb:LoxP-EGFP-LoxP-mCherry, cxcl12b:YFP* embryos are treated with 4OHT to label the embryonic endocardium. *mCherry*-positive, epicardium (*tcf21*+) derived cells line the vessels and are *cxcl12b:YFP* positive (I, box enlarged to I', I'' mCherry, I''' YFP). Endothelial cells that migrate out over the ventricular cardiomyocytes express *cxcr4a:YFP* (J, K, L and M). *cxcr4a:YFP* expression becomes down regulated as vessels form (N-Q). *cxcr4a:YFP* expression is maintained in large vessels and this expression persists late into adulthood after the coronary vasculature has formed (P and Q). *cxcr4a:YFP* (peudocolored blue), *myl7:DsRed2-NLS* (red) and *fli1a:EGFP* (green) in J, K, N-Q. Colorimetric display is used to show *cxcr4a:YFP* intensity (L). Single *cxcr4a:YFP* transgenic fish (M; YFP, yellow; PMT, grey). Scale bars, 50 μm.

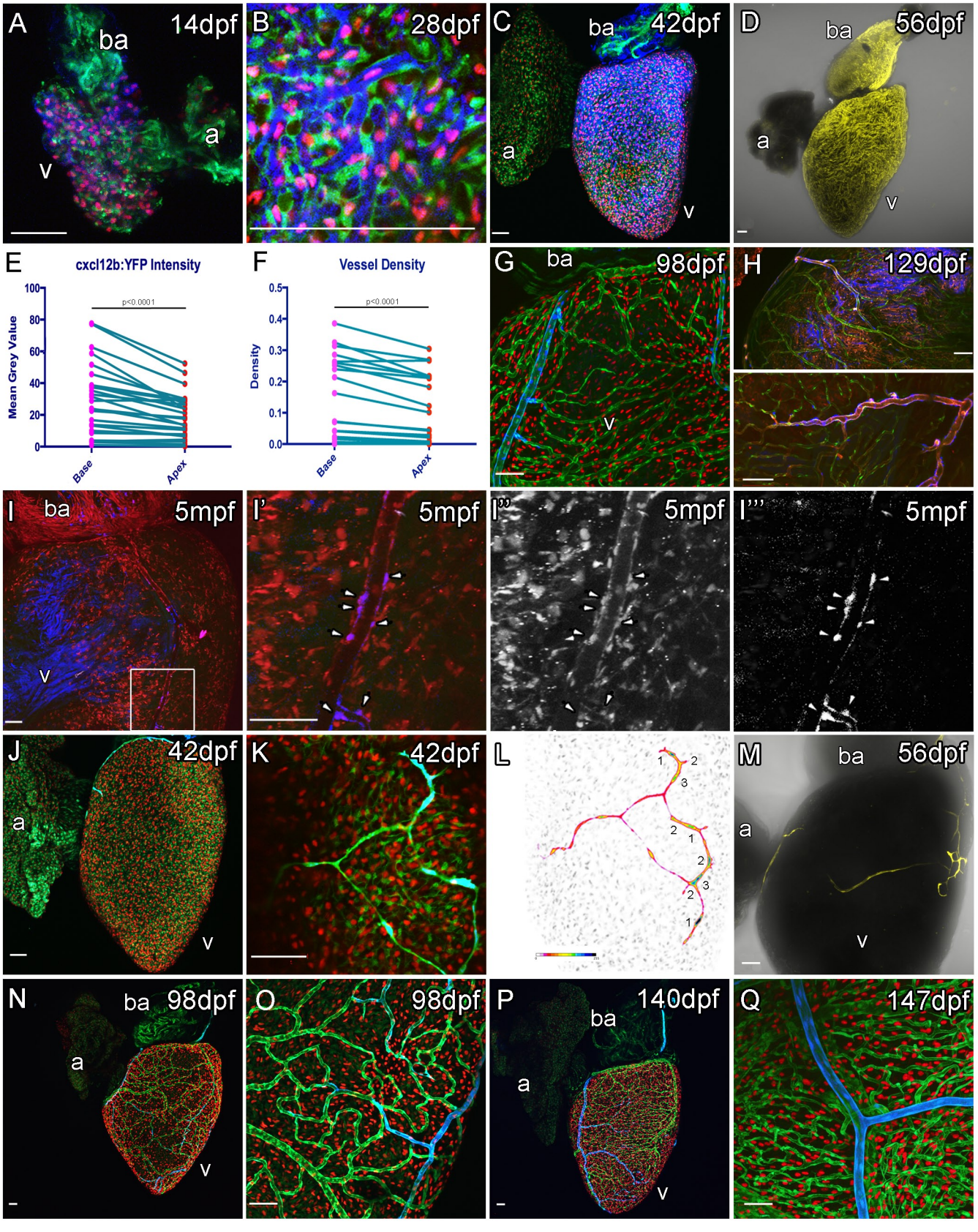

A
14dpf
ba
a
v
B
28dpf
C
42dpf
ba
a
v
D
56dpf
ba
a
v
E
cxcl12b:YFP Intensity
p<0.0001
Mean Grey Value
100
80
60
40
20
0
Base
Apex
F
Vessel Density
p<0.0001
Density
0.5
0.4
0.3
0.2
0.1
0.0
Base
Apex
G
ba
98dpf
v
H
129dpf
I
ba
5mpf
v
I'
5mpf
I''
5mpf
I'''
5mpf
J
42dpf
a
v
K
42dpf
L
1
2
3
2
1
2
2
3
2
1
M
ba
56dpf
a
v
N
ba
98dpf
a
v
O
98dpf
P
140dpf
ba
a
v
Q
147dpf

Figure S5. *cxcr4a* mutant zebrafish fail to develop functional coronary vasculature. (Related to Figure 5)

kdrl:grcfp allows visualization of the endocardium, bright atrial vessels and weaker expressing non-arterial vessels in 6mpf sibling adult zebrafish (A and B). This network of vessels is not detectable in the *cxcr4a* mutant zebrafish; instead unorganized endothelial cells are detectable on the surface of the heart (C and D). These cells fail to form vessels and remain as isolated cells into adulthood (E-H). Angiographs suggest that there is no major supply of blood to the surface of the heart in the mutant other than the systemic flow [I-K; Green, *fli1a:EGFP* (I'-K'); Red, retro-orbitally injected Rhodamine-Dextran (I''-K'')]. Scale bars, 50 μm.

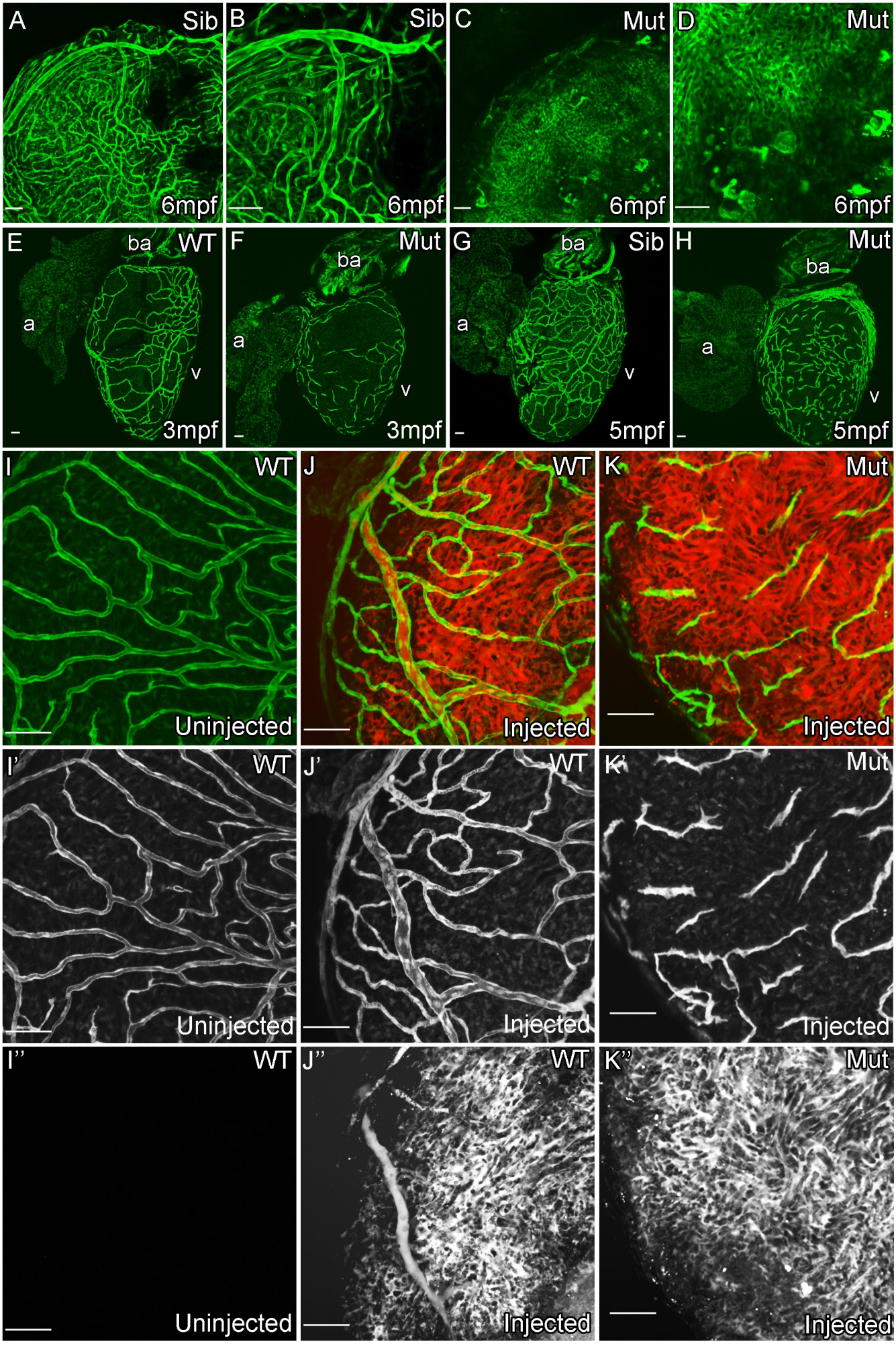

A
Sib
6mpf
B
Sib
6mpf
C
Mut
6mpf
D
Mut
6mpf
E
ba
WT
a
v
3mpf
F
Mut
ba
a
v
3mpf
G
ba
Sib
a
v
5mpf
H
Mut
ba
a
v
5mpf
I
WT
Uninjected
J
WT
Injected
K
Mut
Injected
I'
WT
Uninjected
J'
WT
Injected
K'
Mut
Injected
I''
WT
Uninjected
J''
WT
Injected
K''
Mut
Injected

Figure S6. Coronary vasculature develops normally *cxcr4b* mutants, but *cxcl12b* and *cxcl12a* act redundantly. (Related to Figure 5)

Hearts from zebrafish with genetic lesions in *cxcl12b* (B), *cxcr4b* (C) and *cxcl12a* (D) are phenotypically indistinguishable from those of non-mutant *fli1a:EGFP* transgenic zebrafish (WT, A). Cardiomyocyte expression of *cxcl12a:DsRed* is observed in *cxcl12b* mutant hearts, which could provide an alternative guidance source in the absence of Cxcl12b (46dpf, E and F; 68dpf, G and H). Cxcl12b and Cxcl12a appear to have general redundancy as loss of both genes prevents survival beyond one-month post fertilization under standard husbandry conditions (I). Double *cxcl12a/b* mutants maintained under optimized husbandry conditions can survive beyond one month (7 out of 136), but fail to develop coronary vasculature (K). The majority of zebrafish with a single copy of *cxcl12a* (and no *cxcl12b*) develop normal vasculature (L and M, 34/40), but some *cxcl12a +/-; cxcl12b -/-* do appear to have subtle abnormalities in vessel formation and positioning (N and O). Italicized age indicates zebrafish are raised using optimized husbandry, body length in parentheses. Scale bars, 50 μm.

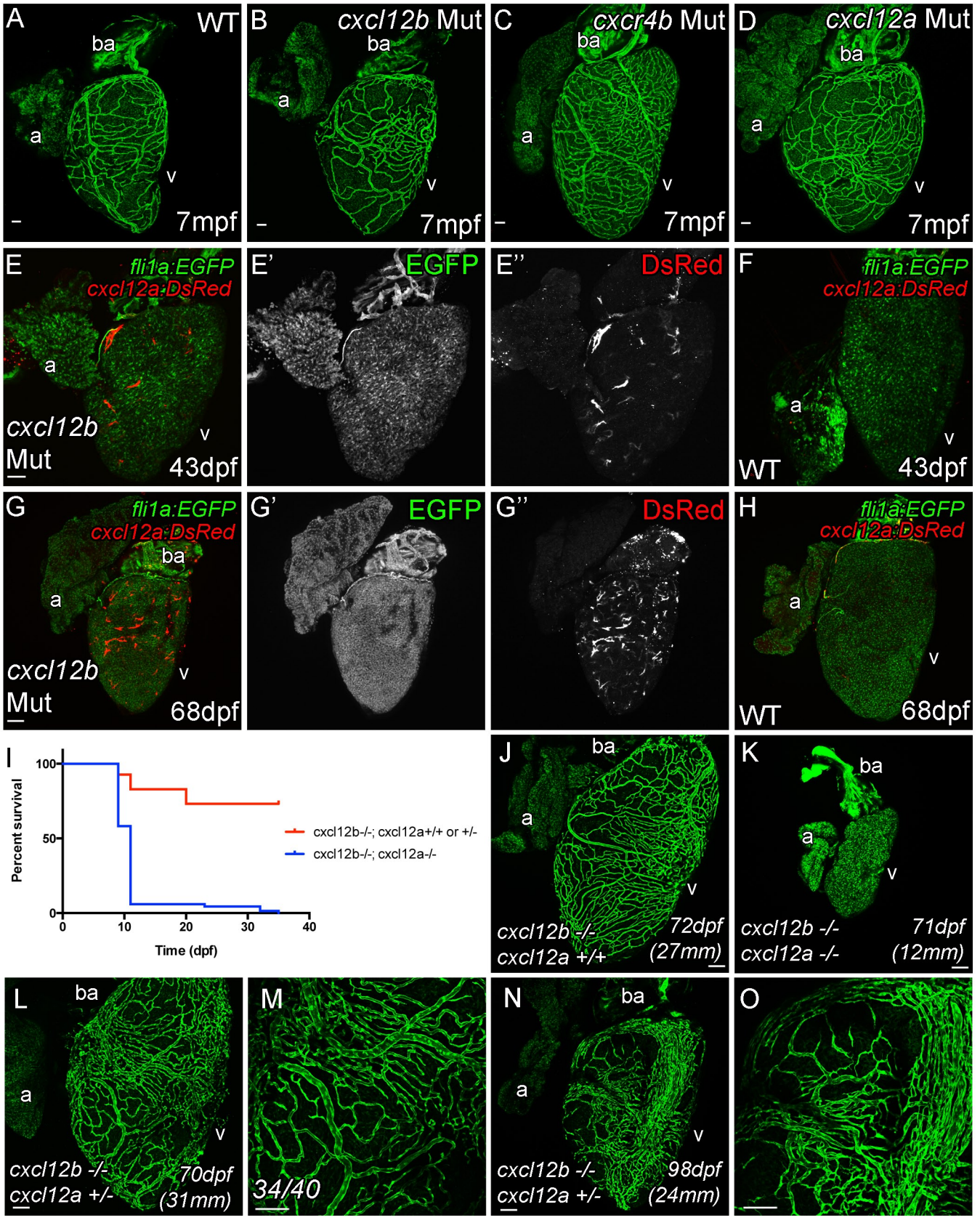
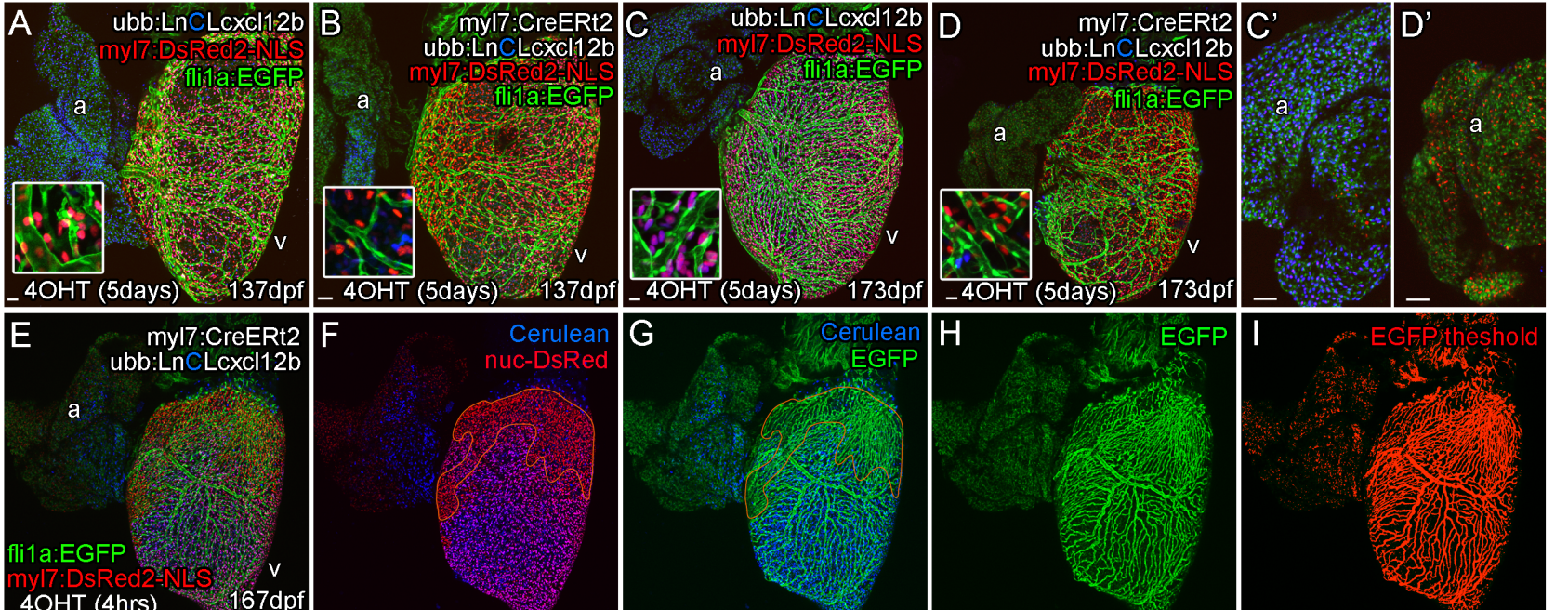

A
WT
ba
a
v
7mpf
B
cxcl12b Mut
C
cxcr4b Mut
D
cxcl12a Mut
E
fli1a:EGFP
cxcl12a:DsRed
cxcl12b
Mut
43dpf
E'
EGFP
E''
DsRed
F
WT
G
68dpf
G'
G''
H
I
Percent survival
100
50
0
0
10
20
30
40
Time (dpf)
cxcl12b-/-; cxcl12a+/+ or +/-
cxcl12b-/-; cxcl12a-/-
J
cxcl12b -/-
cxcl12a +/+
72dpf
(27mm)
K
cxcl12b -/-
cxcl12a -/-
71dpf
(12mm)
L
cxcl12a +/-
70dpf
(31mm)
M
34/40
N
98dpf
(24mm)
O

Figure S7. Cxcl12b directs coronary vessel formation.

(Related to Figure 6)

Uniform over-expression of *cxcl12b* resulted in a reduction in vessels and endothelial/vessel density in adult fish. However, this measurable reduction in endothelial cell density on the surface of the ventricle was not observed in older adult zebrafish over-expressing *cxcl12b* (137dpf A and B; 173dpf C and D). Ectopic expression of *cxcl12b* in atrial cardiomyocytes is not sufficient to direct vessel development on the surface of the atrium (C' and D', intensity levels adjusted to allow visibility of weaker atrial expression levels). Clonal overexpression of Cxcl12b in cardiomyocytes results in a significant increase in vessel density over the expressing region (E-I). Cxcl12b expression is indicated by the loss of H2B-Cerulean in *myl7:DsRed2-NLS*-positive cardiomyocytes (E-G, demarked by orange outline). Increase in density was measured using threshold level to define and estimate *fli1a:EGFP*-positive pixels that were part of the vascular network (H and I) and comparing this density between cxcl12b-expressing and non-expressing regions (demarcated by orange in this example, F and G) within an induced heart (paired t-test) and with siblings which lacked the *myl7:CreERt2* transgene (non-paired t-test). Scale bars, 50 μm.


A
ubb:LnCLcxcl12b
myl7:DsRed2-NLS
fli1a:EGFP
a
v
_4OHT (5days)
137dpf
B
myl7:CreERt2
ubb:LnCLcxcl12b
myl7:DsRed2-NLS
fli1a:EGFP
a
v
_4OHT (5days)
137dpf
C
ubb:LnCLcxcl12b
myl7:DsRed2-NLS
fli1a:EGFP
a
v
_4OHT (5days)
173dpf
D
myl7:CreERt2
ubb:LnCLcxcl12b
myl7:DsRed2-NLS
fli1a:EGFP
a
v
_4OHT (5days)
173dpf
C'
a
D'
a
E
myl7:CreERt2
ubb:LnCLcxcl12b
a
fli1a:EGFP
myl7:DsRed2-NLS
v
_4OHT (4hrs)
167dpf
F
Cerulean
nuc-DsRed
G
Cerulean
EGFP
H
EGFP
I
EGFP theshold

Supplementary Movies

Movie S1 (Movie 1.avi). Migration of endothelial cells over the surface of the ventricle between 77dpf and 79dpf. (Related to Figure 2)

Live imaging of an explanted wild type heart. A leading endothelial cell migrates across the surface of the ventricle from the arterial side of the heart. The cell then forms interconnections with endothelial cells descending from the junction between the ventricle and bulbus arteriosus. Grey box demarks field of view.

Movie S2 (Movie 2.mov). IMARIS analysis of clonal labeling of AV-canal endocardium and early angiogenic sprouts. Z-projection through AV-canal endocardial clone and contiguous angiogenic sprouts. (Related to Figure 3)

Recombination in a subset of endocardial cells is mediated by an endocardial expressed CreERt2 (*kdrl:CreERt2*, induction 36-52hpf). Unlabeled cells appear red (including the entire myocardium) labeled cells appear as a range of colors including green as observed in the AV-canal/atrium. Cells contiguous (i.e. pixels have a direct connection) with the forming surface vessel are highlighted in yellow showing that the angiogenic sprout on the surface of the ventricle has a direct connection to the labeled endocardium laying below the red-labeled myocardium. Finally this contiguous clone is isolated to show the morphology of the vessel-forming clone with angiogenic sprout attached at the lower end.

A small subset of cyan colored endocardial cells (within the yellow box) show a direct clonal relationship with the developing coronary vasculature (also cyan colored). Movement through the z-plane in the area demarked by the yellow box shows that these cells are located under the myocardium and are connected to the vessel forming endothelium.

Movie S3 (Movie 3.avi). Abnormal migration of *cxcr4a* mutant endothelial cells over the surface of the ventricle between 77dpf and 79dpf. (Related to Figure 5)

Live imaging of an explanted *cxcr4a* mutant heart. Cells tend to migrate as isolated cells rather than strings of cells as observed in wild-type hearts (Movie S1). Migrating cells also put out filopodial projections in multiple different directions, often changing directions as they move. Grey box demarks field of view.

Supplemental Experimental Procedures

Zebrafish strains

The *fli1a* endothelial enhancer-promoter (*fli1aep*) was used to drive expression of ERt2CreERt2 specifically in endothelial cells of the embryo (Das and Crump, 2012). Two ERt2 domains allowed tight temporal control of Cre activity such that there was no switching of the *ubb:LoxPEGFPLoxPmCherry* transgene in the absence of 4-hydroxytamoxifen (4OHT) and only limited activity with its addition

(Matsuda and Cepko, 2007). *Fli1aep:ERt2CreERt2* was constructed using the gateway system. 5' entry vector, p5E-fli1aep, contains 1.1kb of sequence directly upstream of the *fli1a* translation start site. This encompasses the 5'UTR, transcriptional start site and endothelial specific enhancer of *fli1a* (Das and Crump, 2012). The middle entry vector, pME-ERt2CreERt2, contains Cre flanked by two ERt2 sites within the middle entry gateway clone (Casanova et al., 2002). The two were combined with p3E-pA into pDEST injected at the one-cell stage from which the F1 generation were screened using *cry:CFP* transgene. Six lines where screened and individually characterized for Cre activity. The strongest specific expressing line was selected for lineage tracing studies.

cxcl12b:Citrine and *cxcr4a:Citrine* were constructed using BAC recombineering methods such that *citrine* was place into BAC CH73-291M8 and CH73-268G8 so as to be driven under the control of *cxcl12b* and *cxcr4a* regulator sequence respectively (Bussmann and Schulte-Merker, 2011).

Ubb:LoxPH2BCeruleanLoxPcxcl12b was constructed using the gateway system P5E-Ubb contains 3.5kb genomic sequence directly upstream of the *ubiquitin B* gene (Mosimann et al., 2011). An expression plasmid containing the nuclear localized fusion protein, H2B-Cerulean (pCS-H2BCerulean, kind gift from S. Megason, Harvard University), was modified to contain a second SV40 poly-adenylation signal down stream of the H2B-Cerulean ORF (pCS-H2BCeruleanpApA). This expression cassette was then further modified with the

addition of two flanking LoxP sites and cloned into the gateway middle entry vector to make pME-LoxPH2BCeruleanpApALoxP. The open reading frame of *cxcl12b* was cloned into gateway entry vector p3E from zebrafish cDNA. p3E-cxcl12b was then combined with p5E-Ubb, pME-LoxPH2BCeruleanpApALoxP and pDestTol2pA2 and injected with transpose mRNA to generate a transgenic line.

Husbandry and 4-OH tamoxifen induction

Fish were raised using standard husbandry conditions (Nüsslein-Volhard and Dahm, 2002), as the rate of fish growth is dependent on food amount and type, in addition to tank space, these were standardized for all developmental observations as far as possible. Fish where fed twice a day with brine shimp or dry food mix (Aquaneering Hatcheries Diet, Golden Pearls and Spirulina Flake), except for larvae (under 14dpf), which were fed fry powder (Argent Spirulina microfine powder and Hatchfry Encapsulation). Unless otherwise stated, thirty larval zebrafish were initially placed in a single 3L tank for the first month and then the density reduced after this to 10 to 15 fish per 3L tank, reaching sexual maturity in 3 months. Within a tank of sibling zebrafish size can vary considerably due to behavior and individual growth tendency. Therefore, only averaged sized fish where selected for analysis (unless otherwise stated). All fish where measured from the anterior tip of head to the caudal fin fork when analyzed (Figure S1 II). This standard husbandry was modified to raise *cxcl12a/b* double mutants and sibling control zebrafish. Embryos from an incross

of *cxcl12b-/-; cxcl12a+/-* zebrafish were analyzed for defects in lateral line migration by staining the neuromasts of live larvae at 5dpf with 4-Di-2-ASP (4-(4-(diethylamino)styryl)-*N*-methylpyridinium iodide, Invitrogen, 2μM in E3). Selected *cxcl12a/b* double mutant (those lacking neuromasts along the entire length of the trunk) and sibling larvae were then transferred into beakers (15 larvae per beaker containing approximately 150ml of system water, changed daily) and fed live rotifers until 14dpf, after which they were transferred into 3L tanks and fed as outlined above.

Embryonic endocardial endothelial cells were labeled in embryonic transgenic zebrafish by activating the ERt2CreERt2 with the addition of 4OHT (13μg/ml in E3; changed daily) during the first 5 days of development. Embryos were rinsed out of 4OHT on day 5 and raised in circulating system water in the normal way. Hearts from these treated fish and their untreated siblings were collected at 14, 28, 72 and 105dpf for confocal analysis. Multispectral clonal labeling of the endocardium was achieved by treating *kdrl:CreERt2; ubb:zebrabow* embryos with a pulse of 4OHT (5μg/ml in E3) between 32 and 56hpf. Tcf21-positive epicardial cells were labeled as described previously (Kikuchi et al., 2011).

Over expression of *cxcl12b* in cardiomyocytes was achieved by activation of *myl7:CreERt2* with 4OHT treatment (13μg/ml in E3; changed daily) during the first 5 days of development in fish which also had a single copy of the *ubb:LoxPH2BCeruleanLoxPcxcl12b* transgene. Cre activity resulted in the

removal of H2B-Cerulean and loss of Cerulean signal in cardiomyocytes (labeled with *myl7:DsRed-NLS*). Clonal patches of *cxcl12b* expression was accomplished with a single 0.5µg/ml 4OHT treatment between 52 and 56hpf after the majority of cardiomyocyte specification had taken place and in a similar manner to previous clonal labeling of cardiomyocytes during development (Gupta and Poss, 2012). In both *cxcl12b* over-expression experiments *myl7:CreERt2* and control non-*myl7:CreERt2* fish were treated and maintained together, only separated prior to imaging due to the potential effect of other external factors on vessel formation.

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