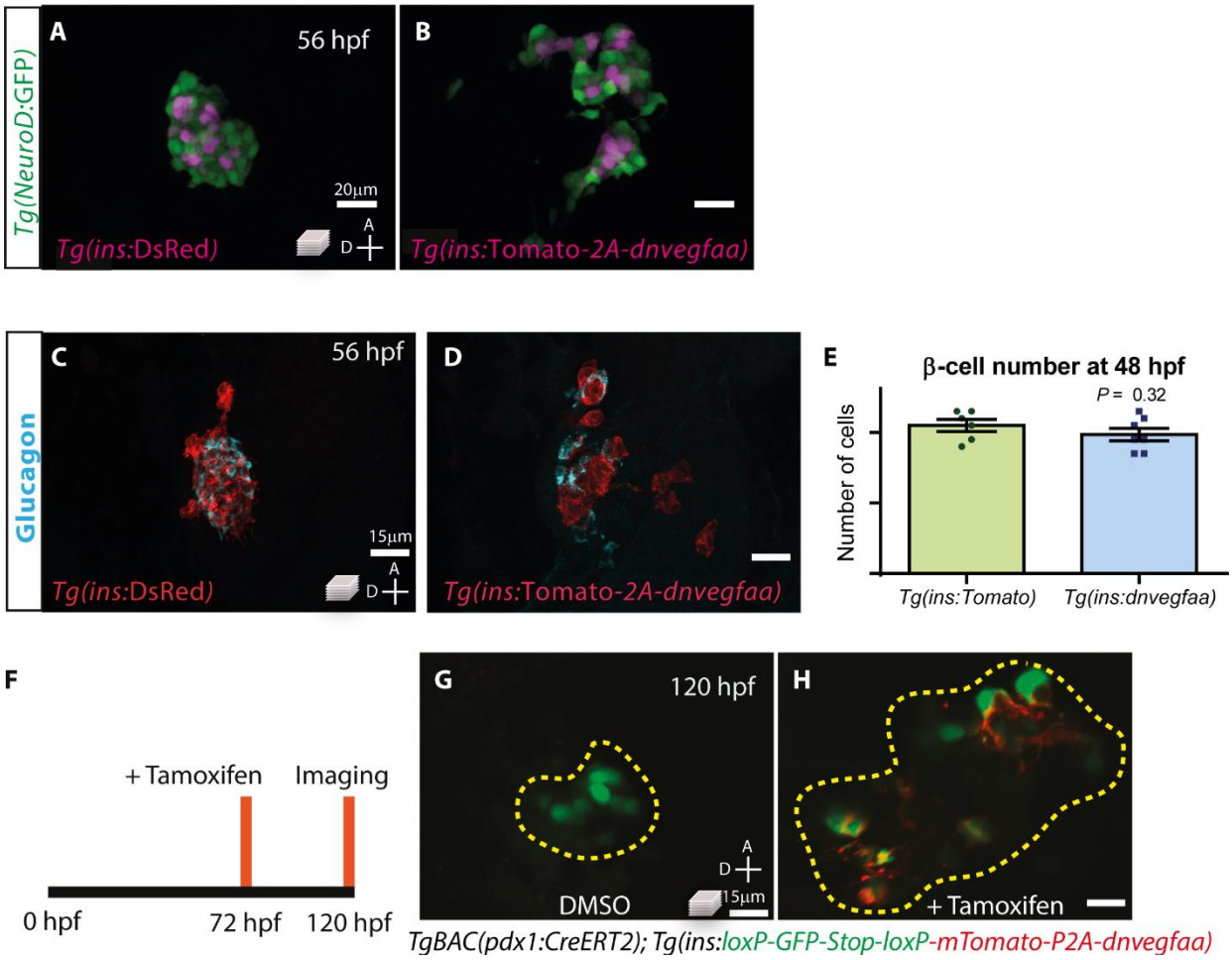


Figure S1: β-cell specific expression of dnVegfaa at embryonic or post-embryonic stages results in islet disruption. A-B) Pancreatic endocrine cells (green) in 56 hpf *Tg(NeuroD:GFP); Tg(ins:DsRed)* and *Tg(NeuroD:GFP); Tg(ins:dnvegfaa)* embryos. C-D) Confocal projection images of the pancreatic islet in 56 hpf *Tg(ins:DsRed)* and *Tg(ins:dnvegfaa)* animals showing Glucagon-positive α-cells (cyan). E) Number of β-cells in 48 hpf *Tg(ins:dnvegfaa)* and *Tg(ins:DsRed)* animals; mean ± SEM, n = 6-7 animals. *P* values from t-tests are presented. F) Induction of *dnVegfaa* expression in *TgBAC(pdx1:CreERT2); Tg(ins:loxP-GFP-STOP-loxP-mTom-P2A-dnVegfaa)* animals with 10 µM Tamoxifen treatment starting at 72 hpf and imaging at 120 hpf. G-H) β-cells in 120 hpf *TgBAC(pdx1:CreERT2); Tg(ins:loxP-GFP-STOP-loxP-mTom-P2A-dnvegfaa)* larvae treated with DMSO (vehicle) or Tamoxifen. Yellow dotted lines outline the spread of the β-cells in the primary islet. Maximum intensity projections are presented; A, anterior; D, dorsal.

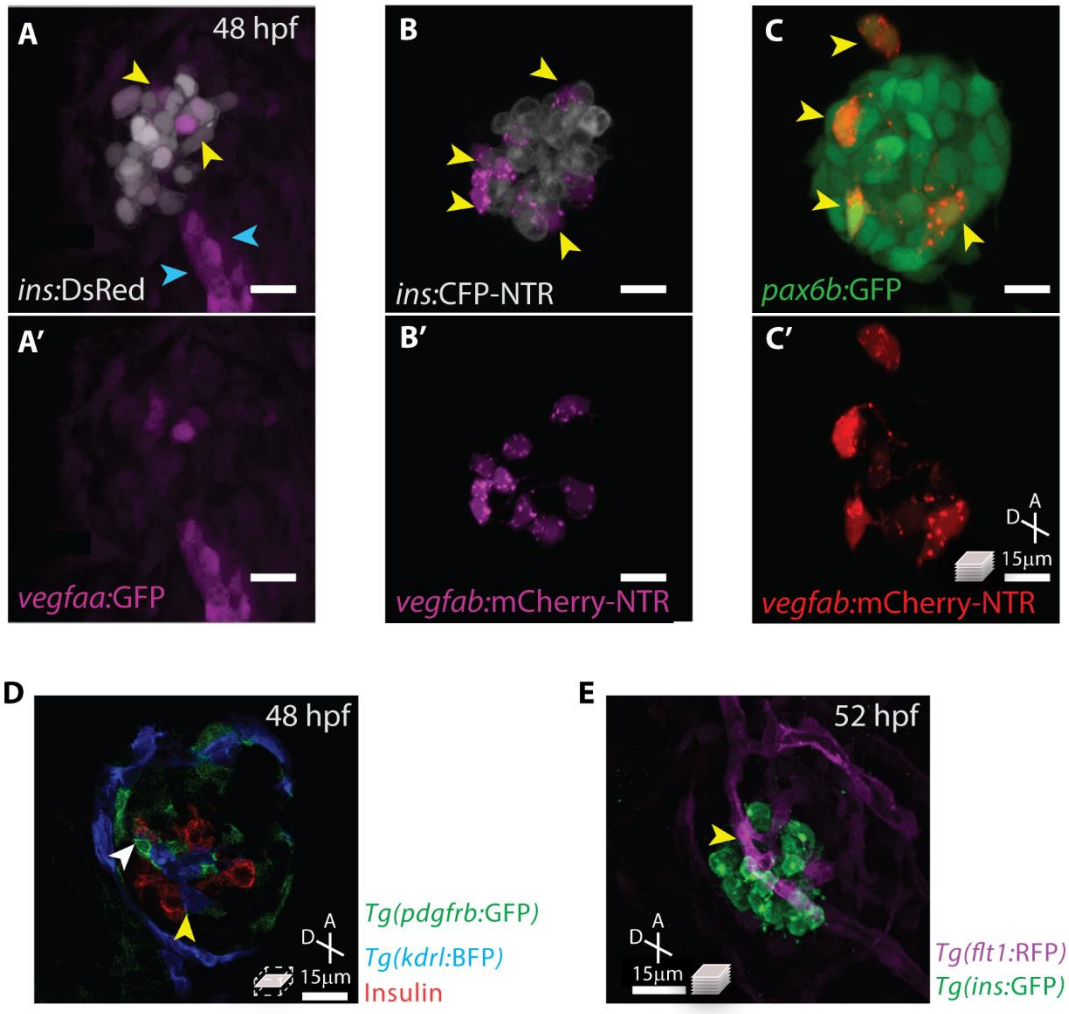


Figure S2: *vegfaa* and *vegfab* are expressed in and around the pancreatic endocrine cells. A) β-cells (white) in 48 hpf *TgBAC(vegfaa:GFP); Tg(ins:DsRed)* embryos. Yellow arrowheads point to *vegfaa* expressing β-cells. Blue arrowheads point to *vegfaa* expressing cells close to the islet that likely belong to non-endocrine lineages. B) β-cells (white) in 48 hpf *TgBAC(vegfab:gal4ff); Tg(UAS-E1b:Nfsb-mCherry); Tg(ins:CFP-NTR)* embryos. *TgBAC(vegfab:gal4ff);Tg(UAS-E1b:NfsB-mCherry)* is referred to as *Tg(vegfab:mCherry-NTR)*. Yellow arrowheads point to *vegfab* expressing cells around β-cells. C) Pancreatic endocrine cells (green) in 48 hpf *Tg(vegfab:mCherryNTR); Tg(pax6b:GFP)* embryos. Yellow arrowheads point to *vegfab* expressing endocrine cells. Maximum intensity projections are presented. D) Confocal plane of the primary pancreatic islet in a 48 hpf *Tg(kdrl:BFP); Tg(pdgfrb:GFP)* embryo showing β-cells (red). Yellow arrowhead points to a *kdrl* expressing endothelial cell in contact with a β-cell. White arrowhead points to a *pdgfrb* expressing cell in contact with a β-cell. E) Confocal projection image of the primary pancreatic islet in a 52 hpf *Tg(ins:GFP); Tg(flt1:RFP)* embryo showing β-cells (green). Yellow arrowhead points to a *flt1* expressing blood vessel in the islet. ; A, anterior; D, dorsal.

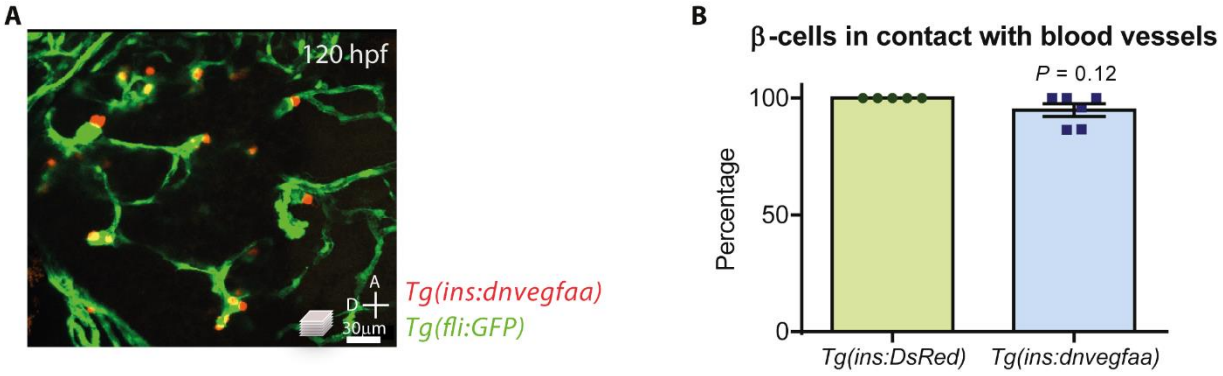
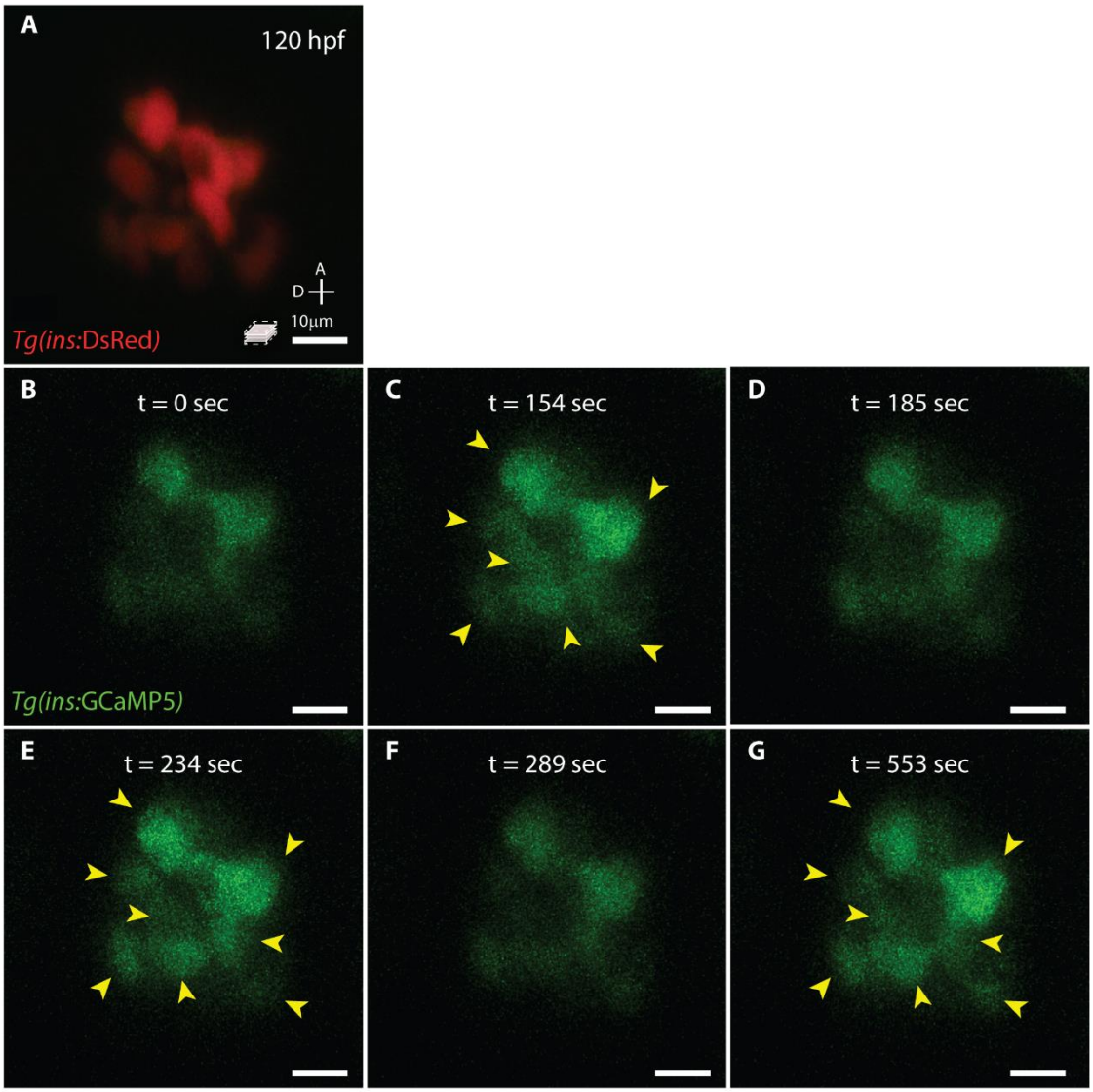
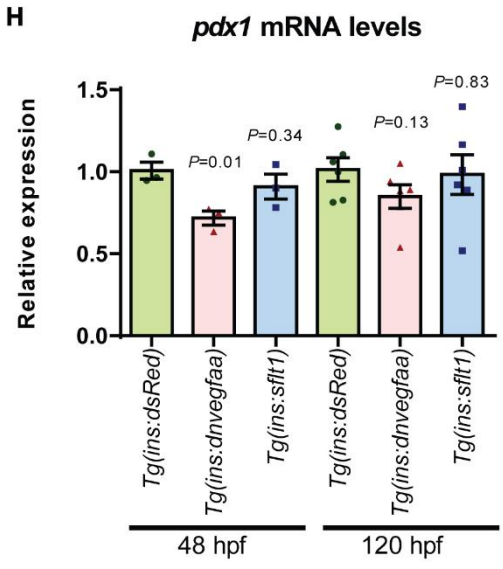


Figure S3: β-cells in *Tg(ins:dnvegfaa)* animals are in contact with blood vessels. A) Confocal projection image of the pancreatic islet in a 120 hpf *Tg(ins:dnvegfaa);Tg(fli:GFP)* animal showing β-cells (red). Maximum intensity projection is presented. B) Percentage of β-cells in contact with blood vessels in 120 hpf *Tg(ins:DsRed)* and *Tg(ins:dnvegfaa)* animals; mean ± SEM, n = 6-7 animals. *P* values from t-tests are presented.


A
120 hpf
A
D
10μm
Tg(ins:DsRed)
B
t = 0 sec
C
t = 154 sec
D
t = 185 sec
Tg(ins:GCaMP5)
E
t = 234 sec
F
t = 289 sec
G
t = 553 sec


H
pdx1 mRNA levels
Relative expression
1.5
1.0
0.5
0.0
P=0.01
P=0.34
P=0.13
P=0.83
Tg(ins:dsRed)
Tg(ins:dnvegfaa)
Tg(ins:sflt1)
Tg(ins:dsRed)
Tg(ins:dnvegfaa)
Tg(ins:sflt1)
48 hpf
120 hpf

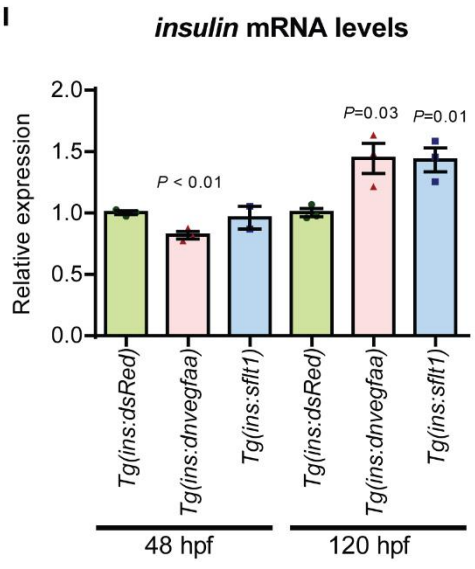
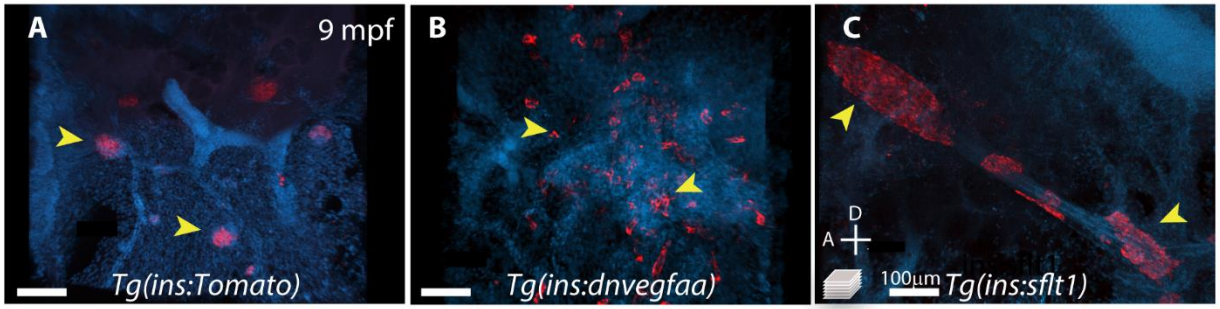
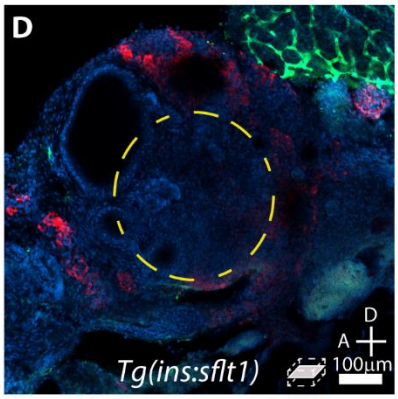

I
insulin mRNA levels
Relative expression
2.0
1.5
1.0
0.5
0.0
P < 0.01
P=0.03
P=0.01
Tg(ins:dsRed)
Tg(ins:dnvegfaa)
Tg(ins:sflt1)
Tg(ins:dsRed)
Tg(ins:dnvegfaa)
Tg(ins:sflt1)
48 hpf
120 hpf

Figure S4: Live imaging shows dynamics of calcium signaling in β-cells. A) Pancreatic β-cells (red) in 120 hpf *Tg(ins:DsRed); Tg(ins:GCaMP5)* animals. B-G) Calcium responsive fluorescence signal in 120 hpf *Tg(ins:DsRed); Tg(ins:GCaMP5)* animals, at the indicated time point, from live confocal imaging. Yellow arrowheads point to β-cells showing an observable increase in fluorescence signal intensity. Maximum intensity projections of planes in the central 30 μm region of the islet are shown. A, anterior; D, dorsal. H-I) *pdx1* (H) and *ins* (I) mRNA levels in 48 and 120 hpf animals; 3-6 replicates, 10 animals per replicate, mean ± SEM. *P* values from t-tests are presented.

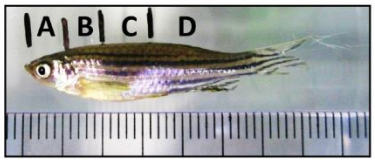


Insulin DNA

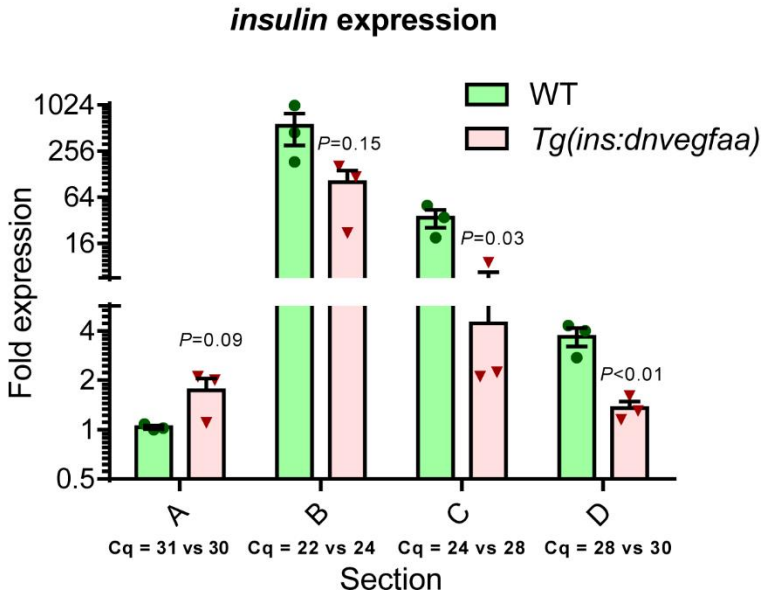


Insulin *Tg(fli:*GFP) DNA

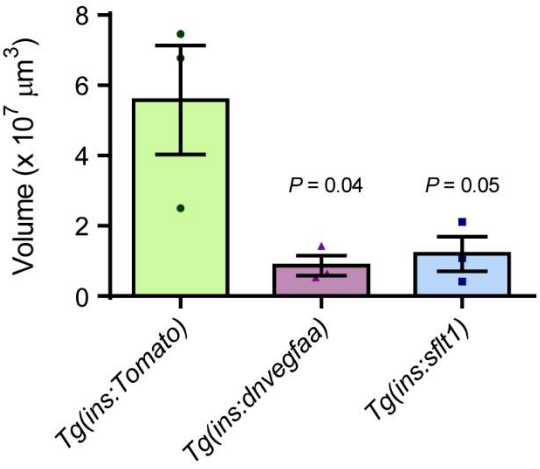
E



F



G



H

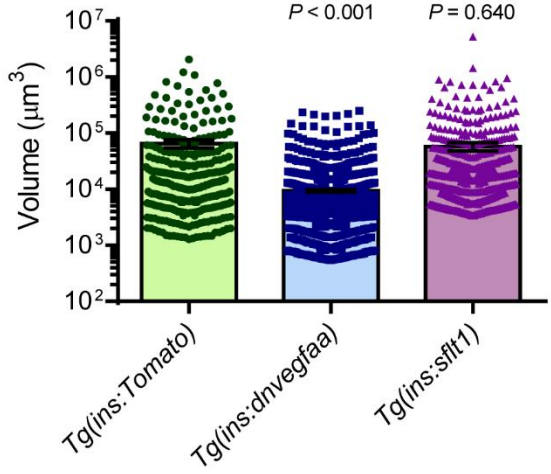
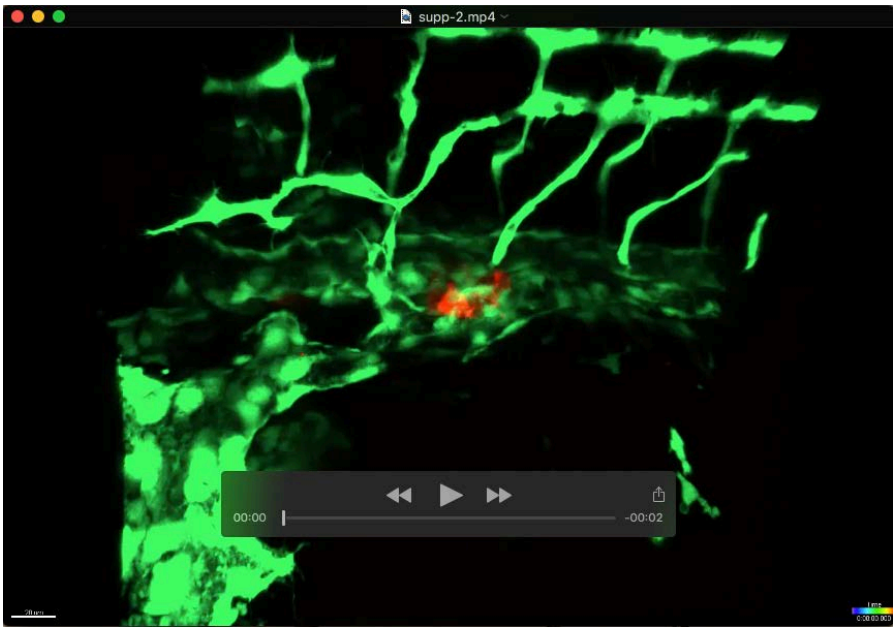


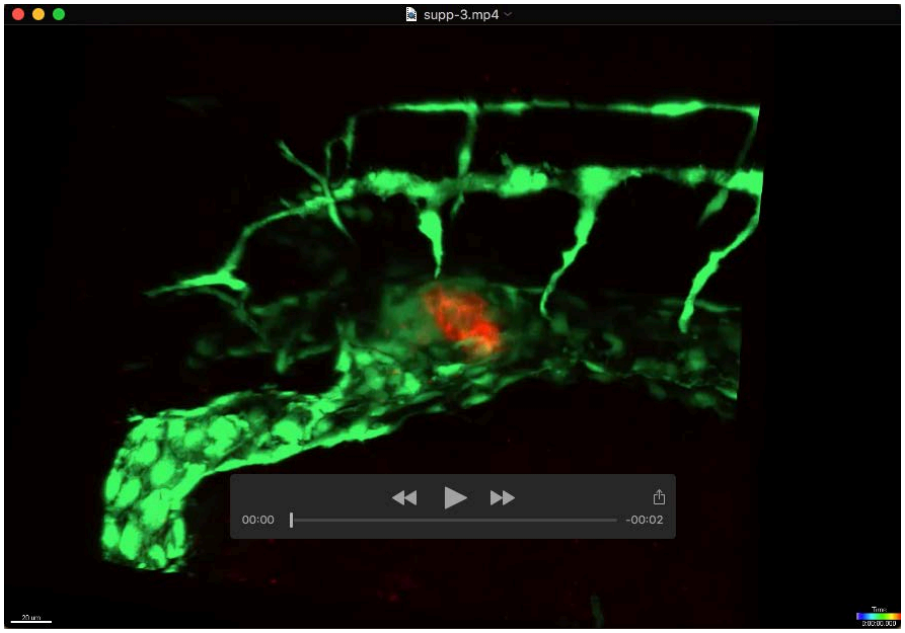
Figure S5: β-cell specific expression of dnVegfaa or sFlt1 causes defects in secondary islet formation. A-C) Wholemount immunostaining for β-cells (Insulin) along the pancreatic ducts of 9 mpf zebrafish after CLARITY-based tissue clearing. Yellow arrowheads point to β-cell clusters. D) Wholemount immunostaining for β-cells (Insulin) in the primary islet of a 9 mpf *Tg(ins:sflt1); Tg(fli:GFP)* zebrafish after CLARITY-based tissue clearing. Yellow dotted line outlines the nuclei in the core of the islet. E) Adult zebrafish divided up into four sections, as marked, to obtain RNA and quantify *insulin* expression in each section separately; section A - head, heart, eyes; section B - anterior gastrointestinal (GI) tract; section C - posterior GI tract; section D - lower trunk and tail regions. F) *insulin* mRNA levels (qPCR) from each of the four sections along with Ct values. G) Volume of Insulin antibody immunostaining (β-cells) in adult zebrafish pancreas, computed using surface construction in Imaris. H) Volume of Insulin antibody immunostaining (β-cells) in secondary islets, computed using surface construction in Imaris. Data points greater than 5.0×10^5 μm^3 in *Tg(ins:Tomato)* and *Tg(ins:sflt1)* animals correspond to the largest secondary islet clusters located close to the primary islet (Figure 4), mean ± SEM, n = 3 animals, 100 to 560 secondary islets per animal, *P* values from t-tests are presented.

	Ensembl Gene ID	Gene	Normalized Read Count (β-cells)	% of pancreatic cells with expression
Ligands	ENSDARG00000035350	*ins*	4324351	99.76
	ENSDARG00000103542	*vegfaa*	4101	6.64
	ENSDARG00000034700	*vegfab*	11588	13.72
	ENSDARG00000069640	*vegfc*	0	0.4
	ENSDARG00000092480	*vegfbb*	0	ND
	ENSDARG00000077588	*pdgfc*	147	0.56
	ENSDARG00000058424	*pdgfab*	420	1.61
	ENSDARG00000055505	*pdgfaa*	194	1.05
	ENSDARG00000077677	*pdgfd*	442	ND
	ENSDARG00000038139	*pdgfbb*	0	0.93
	ENSDARG00000005001	*grem2*	178	1.57
Receptors	ENSDARG00000019371	*flt1*	1	1.01
	ENSDARG00000017321	*kdr*	0	0.08
	ENSDARG00000105215	*kdrl*	16	1.97
	ENSDARG00000015717	*flt4*	1	0.64
	ENSDARG00000070494	*pdgfra*	2	0.68
	ENSDARG00000100897	*pdgfrb*	0	ND
	ENSDARG00000006456	*pdgfrl*	755	0.60
	ENSDARG00000027290	*nrp1b*	58	0.32
	ENSDARG00000071865	*nrp1a*	139	2.01
	ENSDARG00000096546	*nrp2a*	175	0.40
	ENSDARG00000038446	*nrp2b*	0	0.20

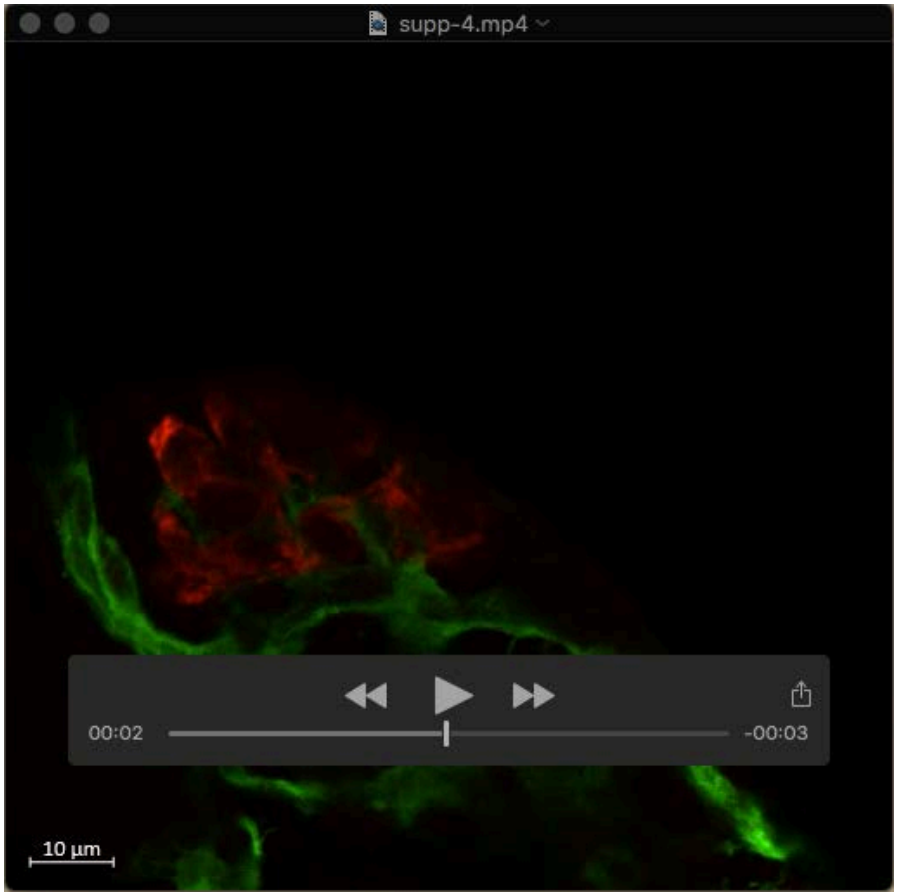
Table S1: Pancreatic β-cell expression of Vegf family ligand and receptor genes. Normalized read counts (Tarifeño-Saldivia et al., 2017) and percentage of pancreatic cells (Salem et al., 2019) expressing different Vegf family ligand and receptor genes; ND, not detected.



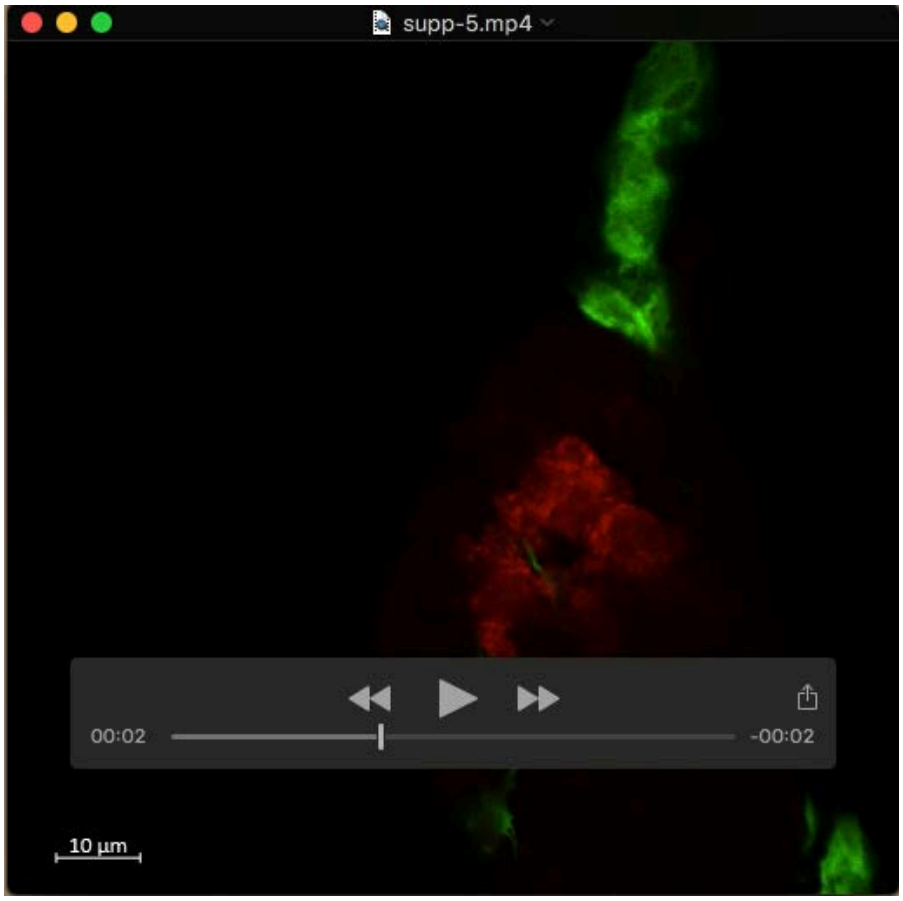
Movie 1: Time-lapse imaging shows collective migration of β-cells and their interaction with the vasculature in wild-type embryos. Confocal imaging of *Tg(ins:Tomato); Tg(fli:GFP)* zebrafish from 34 to 48 hpf at 60 min time intervals. Movie corresponds to Figure 1e.



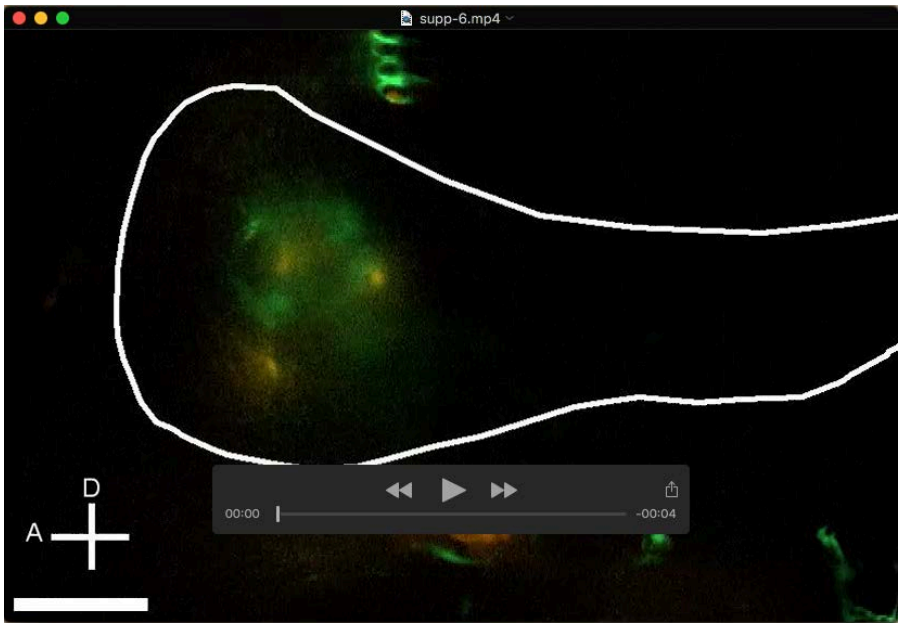
Movie 2: Time-lapse imaging shows dispersion of β-cells in *Tg(ins:dnvegfaa)* embryos. Confocal imaging of *Tg(ins:dnvegfaa); Tg(fli:GFP)* zebrafish from 34 to 48 hpf at 60 min time intervals. β-cells are marked in red. Movie corresponds to Figure 1f.



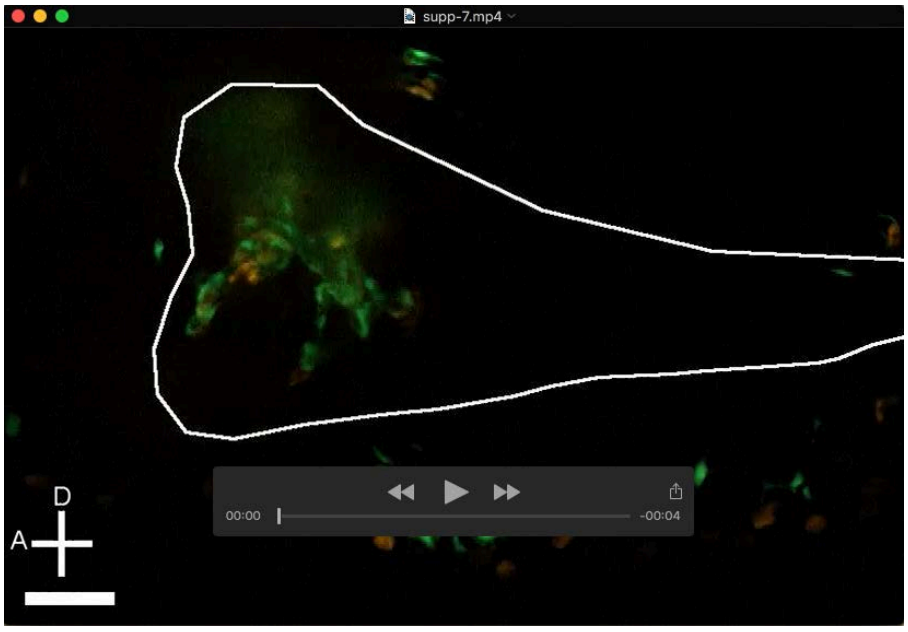
Movie 3: Wild-type pancreatic islets contain intra-islet vessels. Progressive planes in a Z-stack from confocal imaging of pancreatic islets in 48 hpf *Tg(ins:DsRed); Tg(fli:GFP)* zebrafish, with immunostaining for Insulin (red) and GFP. Movie corresponds to Figure 2c.



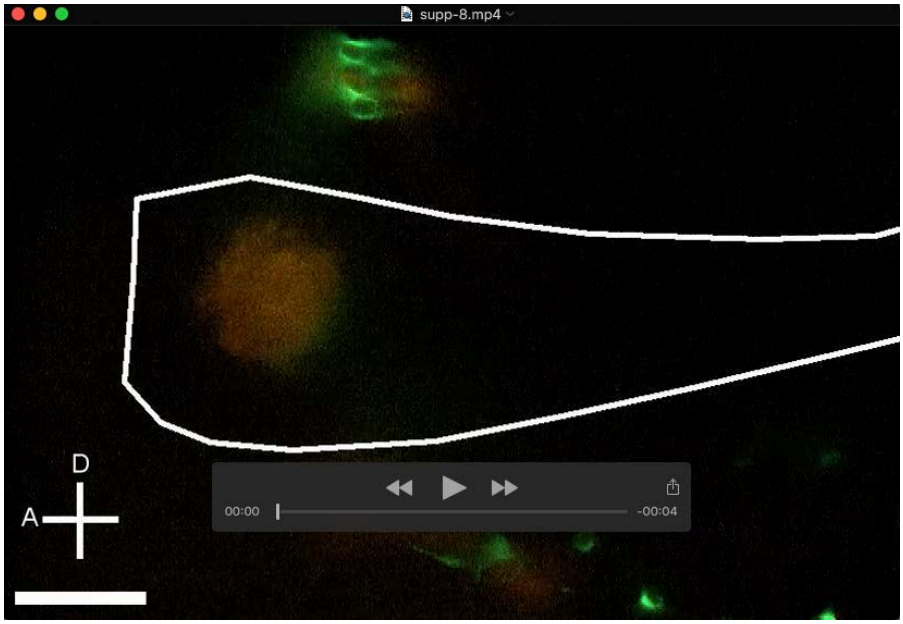
Movie 4: β-cell-specific sFlt1 expression results in markedly reduced intra-islet vasculature.
Progressive planes in a Z-stack from confocal imaging of pancreatic islets in 48 hpf *Tg(ins:sflt1); Tg(fli:GFP)* zebrafish, after immunostaining for Insulin (red) and GFP. Movie corresponds to Figure 2j.



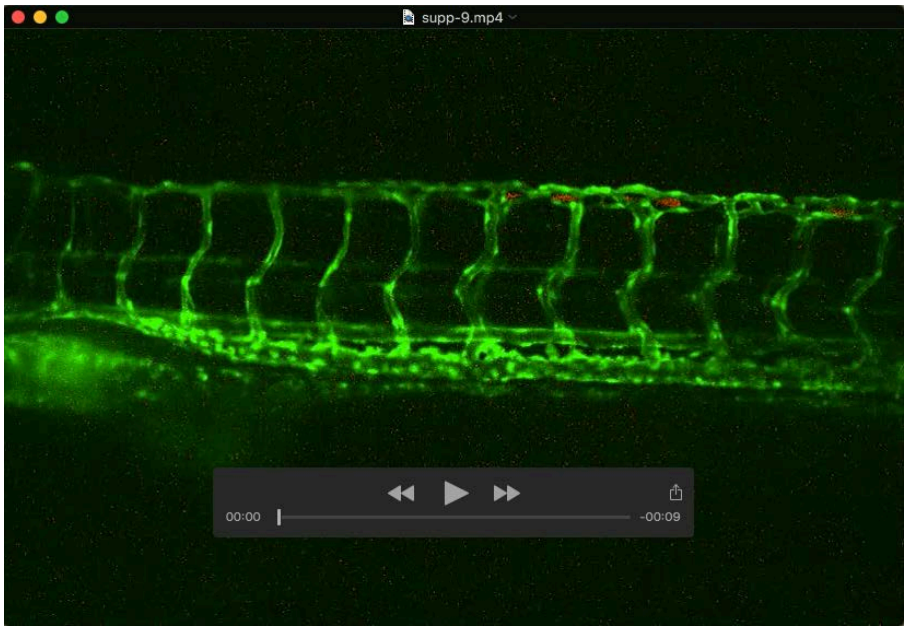
Movie 5: Live imaging shows circulating red blood cells in wild-type larval pancreas. Spinning disk confocal imaging of the pancreatic islet in 120 hpf *Tg(gata1:DsRed); Tg(fli:GFP)* zebrafish, showing circulating red blood cells (red). Pancreas is outlined in white. Scale bar, 40 μm.



Movie 6: Live imaging shows circulating red blood cells and disrupted vasculature in *Tg(ins:dnvegfaa)* pancreas. Spinning disk confocal imaging of the pancreatic islet in 120 hpf *Tg(ins:dnvegfaa); Tg(gata1:DsRed); Tg(fli:GFP)* zebrafish. Pancreas is outlined in white. Scale bar, 40 μm.



Movie 7: Live imaging shows lack of vasculature and circulation in *Tg(ins:sflt1)* pancreas. Spinning disk confocal imaging of the pancreatic islet in 120 hpf *Tg(ins:sflt1); Tg(gata1:DsRed); Tg(fli:GFP)* zebrafish. Faint red signal in the anterior region of the pancreas (outlined in white) is from β-cells. Scale bar, 40 μm.



Movie 8: Live imaging shows circulating β-cell in *Tg(ins:dnvegfaa)* larvae. Spinning disk confocal imaging of 72 hpf *Tg(ins:dnvegfaa); Tg(fli:GFP)* zebrafish. Arrowhead points to the β-cells circulating in the trunk region.