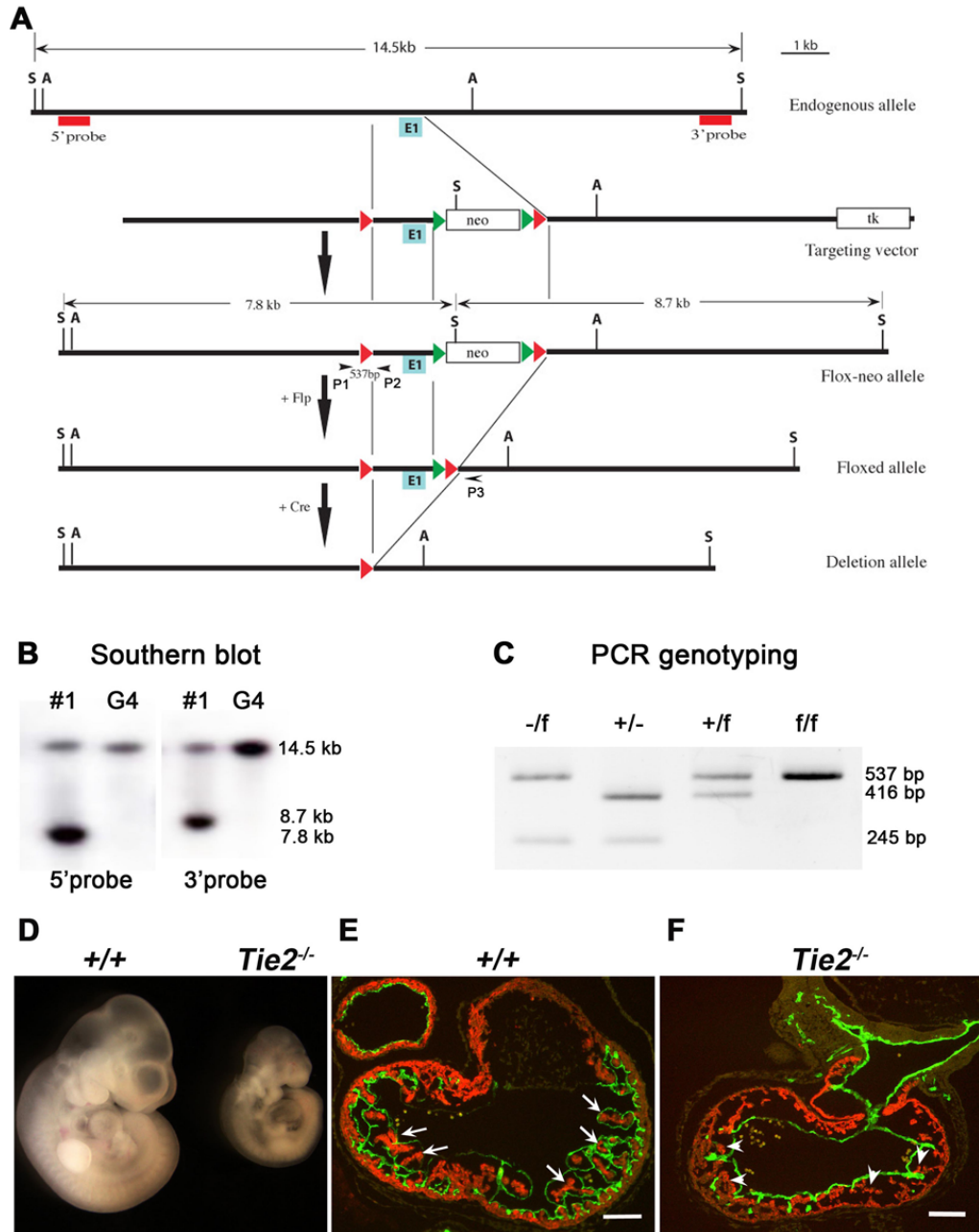
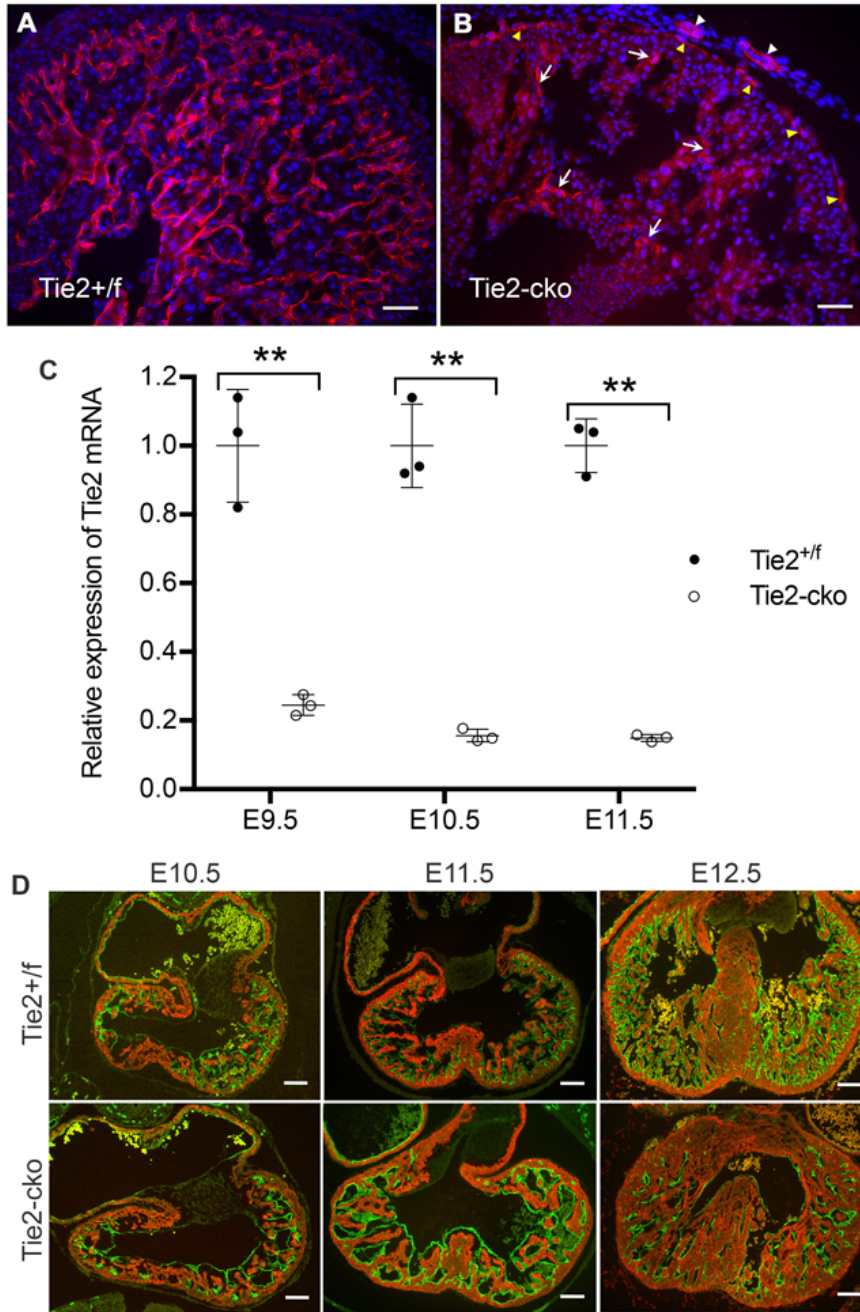


Supplemental Data



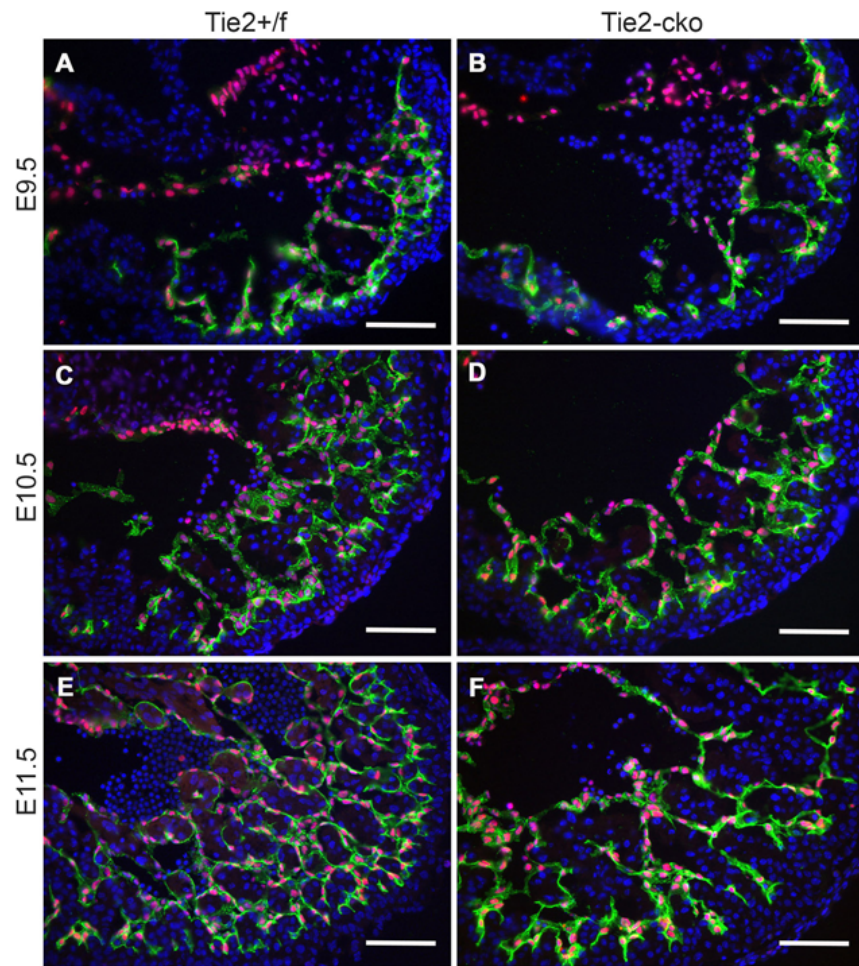
Supplemental Figure 1. Generation of mice with a floxed *Tie2* allele. (A) Schematic representation of the targeting strategy and deleting *Tie2* minimal promoter and exon 1 in mice. Top: *Tie2* genomic structure. The targeting vector was designed so that loxP sites would flank the 1267bp fragment containing *Tie2* minimal promoter and exon 1. Homologous recombination with the targeting vector generated the flox-neo allele; Flp deletion created the floxed allele; subsequent Cre deletion led to the Δ allele. Blue boxes, exon 1; red boxes, external probes for Southern blot analysis (diagnostic band lengths pre/post-recombination indicated); neo, Neomycin resistance cassette; tk, thymidine kinase cassette. Red and green arrowheads indicate LoxP and Frt sites,

respectively. A, *Afl*III; S, *Stu*I. Primers used for isolation of correctly targeted ES cells and for routine genotyping are indicated by small arrowheads. (B) Southern blot analysis of the parental (G4) and correctly targeted ES cell clones using the probes depicted in (A). *Stu*I-digested DNAs were hybridized to a 5' flanking probe (5'probe) or a 3' flanking probe (3'probe): A 14.5-kb band was observed for the wild-type allele, but a 7.8-kb band (5'probe) or an 8.7kb band (5'probe) for the mutant allele due to the presence of a *Stu*I site in the neo cassette. (C) Genotyping with 3 primers (P1, P2 and P3) to detect the four *Tie2* genotypes was carried out by PCR across the 5' *loxP* site, which produces a 416-bp fragment for the wild-type locus, 537-bp for the floxed allele, and 245-bp for the deletion allele. The position of the primers is indicated by small arrowheads in (A). (D) Gross images of *Tie2*^{+/+} (left) and *Tie2*^{-/-} (right) embryos at E10.5, showing growth retardation of mutant embryos. (E, F) Control and *Tie2*^{-/-} heart sections were stained with troponin T (myocardial marker, red) and endomucin (endocardial marker, green) antibodies. In comparison with the long trabeculae (arrow) in the control, only delaminated myocardial lamina (arrowhead) were present in *Tie2*^{-/-} ventricles at E10.5. Scale bars: 100 μm. For all studies in D-F, a total of more than 6 embryos per genotype collected from at least 3 independent litters was analyzed. A representative of >8 images was chosen for each panel.

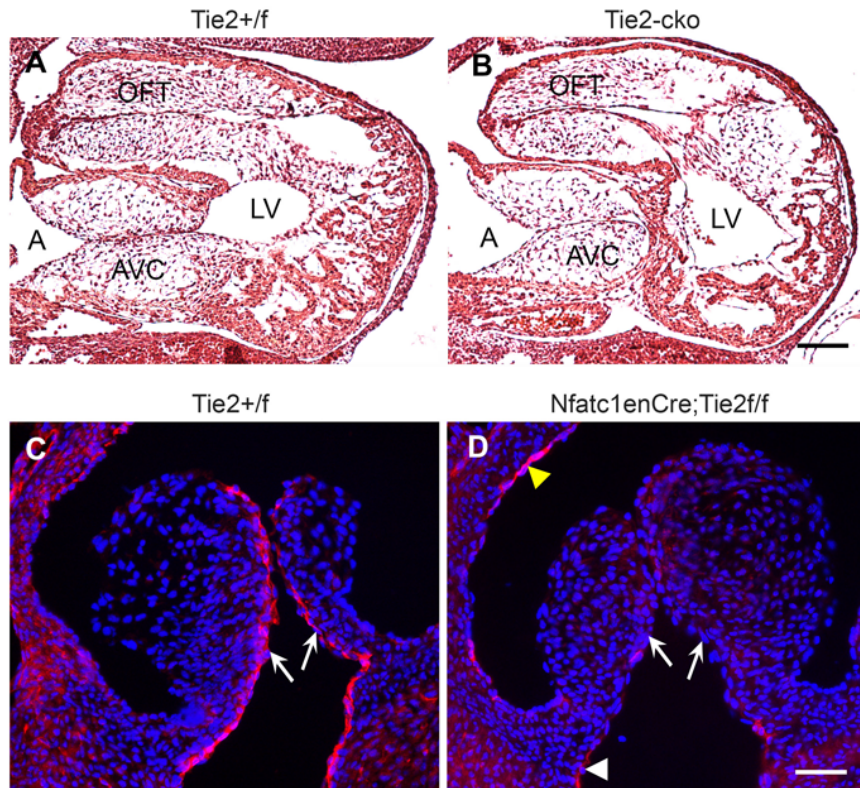


Supplemental Figure 2. Endocardial attenuation of Tie2 results in abnormal trabeculation.

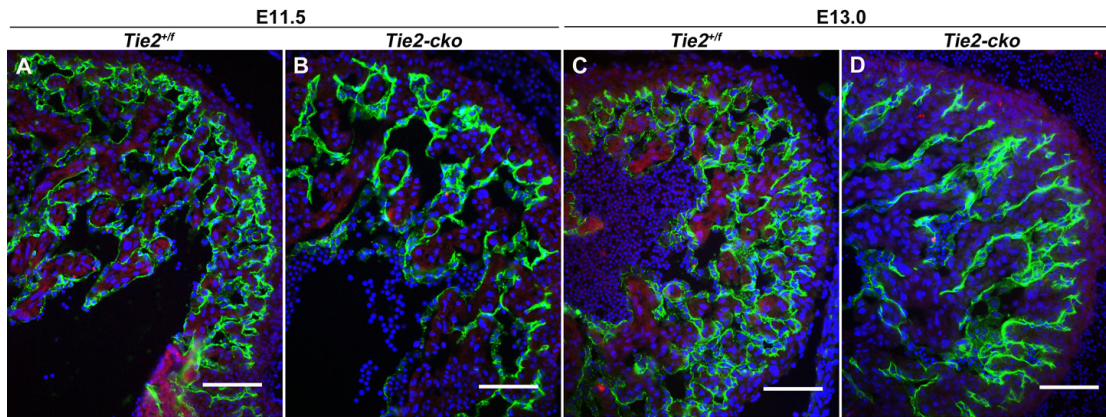
(A, B) Left ventricular sections of control (*Tie2*^{+/-}) (A) and *Tie2*^{cko} (B) embryos at E11.5 were stained with anti-Tie2 antibody, showing specific Tie2 deletion in endocardial cells without change of normal expression of Tie2 in epicardial (yellow arrowheads) or blood vessel endothelial cells (white arrowheads). Arrows indicate the very low level of the mosaic Tie2 expression in the mutant endocardium. Tie2, red. DAPI, blue. (C) The efficiency of Tie2 deletion in the whole heart at E9.5-E11.5 was examined by qPCR. N=3 per group, ***p* < 0.01, Student's *t* test. (D) Control and *Tie2*^{cko} heart sections at E10.5 to E12.5 were stained with troponin T (red) and endomucin (green) antibodies. Scale bars: 50 μm (A, B), 100 μm (D). A representative of >8 images was chosen for each panel.



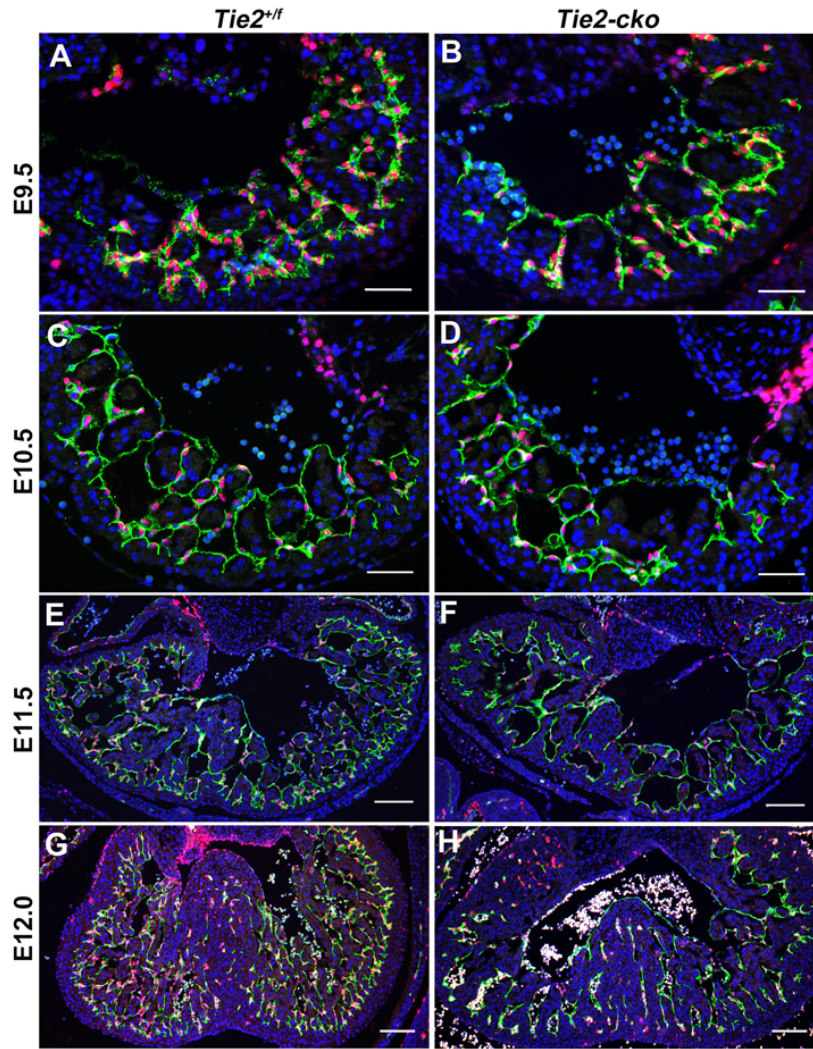
Supplemental Figure 3. Endocardial attenuation of Tie2 results in defective endocardium. Control (*Tie2*^{+/f}) and *Tie2-cko* left ventricle sections at E9.5-11.5 were dual immunostained with antibodies against endomucin (green)/Erg (red), showing simplified endocardial networks in mutant ventricles at all stages examined. Nuclei are counterstained with DAPI (blue). Scale bars: 100 μ m. For all studies, a total of more than 5 embryos per genotype collected from at least 3 independent litters was analyzed. A representative of >10 images was chosen for each panel.



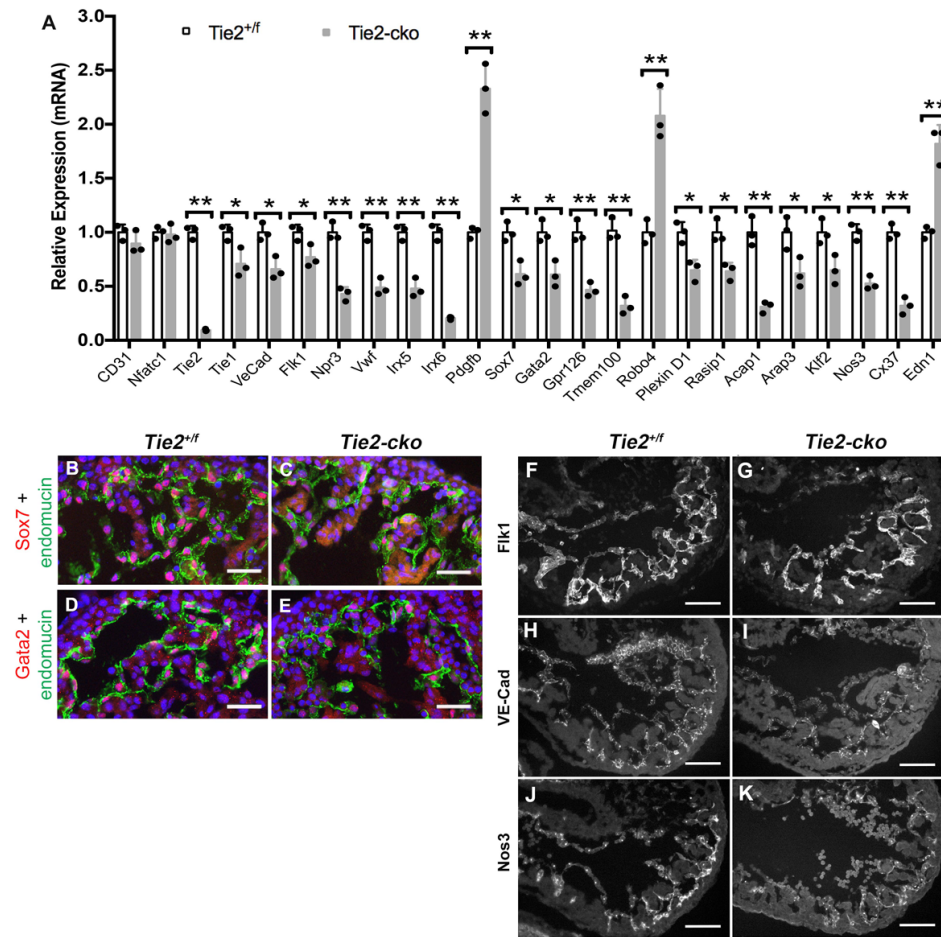
Supplemental Figure 4. Endocardial loss of Tie2 results in normal formation of endocardial cushions. (A and B) H&E stained heart sections of E11.5 *Tie2*^{+/f} (E) and *Tie2-cko* (F) embryos showing normal atrioventricular canal (AVC) and outflow tract (OFT). A, atrium; LV, left ventricle. (C and D) Representative images of immuno-staining of aortic valves (AoV) at P0 with anti-Tie2 antibody (red) showing intense Tie2 expression at the ventricular side (arrows) of *Tie2*^{+/f} AoV (C) and efficient specific Tie2 deletion in *Nfatc1*^{enCre};*Tie2*^{f/f} valvular endothelium (arrows) without change of normal expression of Tie2 in arterial endothelium (yellow arrowhead) or ventricular endocardium (white arrowhead). Nuclei are counterstained with DAPI (blue). Scale bars: A and B, 100 μ m; C and D, 50 μ m. For all studies, more than 5 embryos total per genotype collected from at least 3 independent litters were analyzed. A representative of >10 images was chosen for each panel.



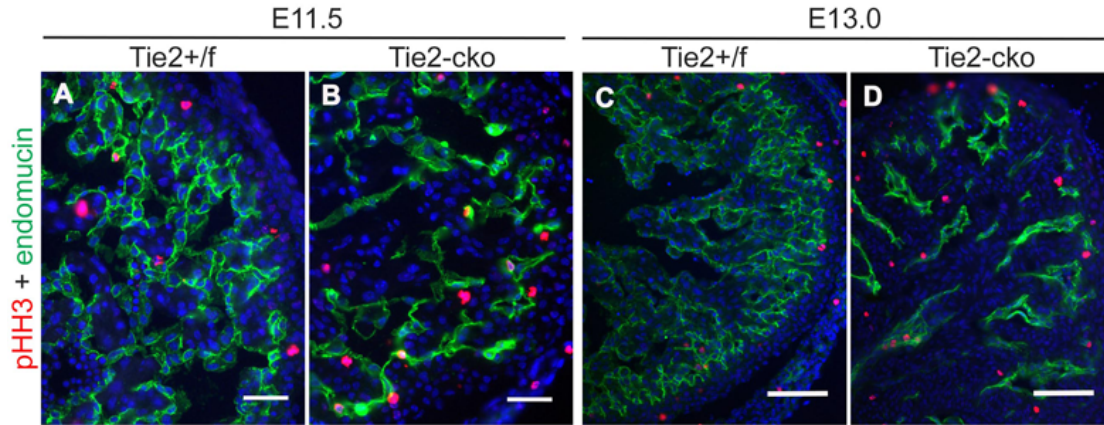
Supplemental Figure 5. Endocardial attenuation of Tie2 results in no alteration on apoptosis in trabecular CMs or ECs. (A-D) Dual immunostaining of control and *Tie2-cko* heart sections for active caspase 3 (red) and endomucin (green) at E11.5 and E13.0 detected no significant apoptosis in the control or mutant trabecular cardiomyocytes (CMs)/endocardial cells (ECs). DAPI, blue. Scale bars: 100 μ m. A total of more than 4 embryos per genotype collected from at least 3 independent litters was analyzed. A representative of >8 images was chosen for each panel.



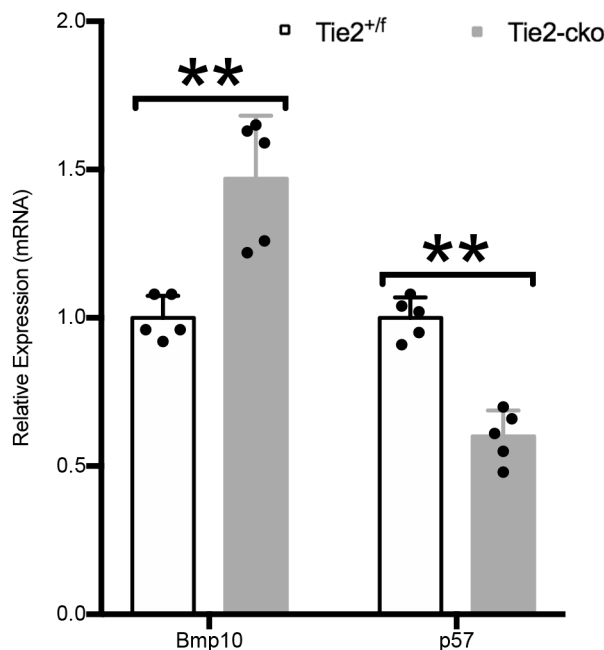
Supplemental Figure 6. Endocardial attenuation of Tie2 results in defective endocardium. (A-H) Control (*Tie2*^{+/*f*}) and *Tie2-cko* heart sections at E9.5, E10.5 (left ventricle), E11.5 and E12.0 (both ventricles) were dual immuno-stained with antibodies against endomucin (green)/N1ICD (red), showing reduced N1ICD+ endocardial cells in simplified endocardial networks of mutant ventricles. DAPI, blue. Scale bars: A-D, 50 μ m; E-H, 100 μ m. For all studies, a total of more than 5 embryos per genotype collected from at least 3 independent litters was analyzed. A representative of >8 images was chosen for each panel.



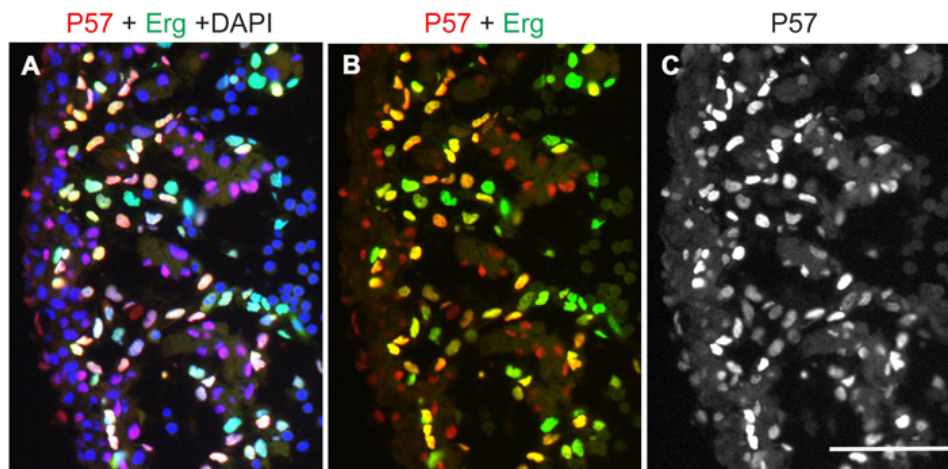
Supplemental Figure 7. Endocardial attenuation of Tie2 alters expression of endocardial genes. (A) qPCR analysis E11.5 *Tie2^{+/f}* and *Tie2-cko* hearts reveals that many endocardial genes were downregulated while a few (*Pdgfb*, *Robo4* and *Edn1*) were upregulated following endocardial loss of Tie2. N=3 per group, * $p < 0.05$; ** $p < 0.01$, Student's t test. (B-E) Dual immunostaining of control and *Tie2-cko* heart sections using antibodies for Sox7 (red)/endomucin (green) (B, C) or Gata2 (red)/endomucin (green) (D, E) at E10.5 showing significant reduced levels of Sox7 and Gata2 in the mutant endocardium. DAPI, Blue. (F-K) Immunostaining of control and *Tie2-cko* heart sections using antibodies for Flk1 (F, G), VE-Cad (H, I) or Nos3 (J, K) at E9.5 reveals reduced staining of Flk1, VE-Cad and Nos3 in the mutant endocardium. Scale bars: B- E, 50 μ m; F-K, 100 μ m. A representative of >8 images was chosen for each panel.



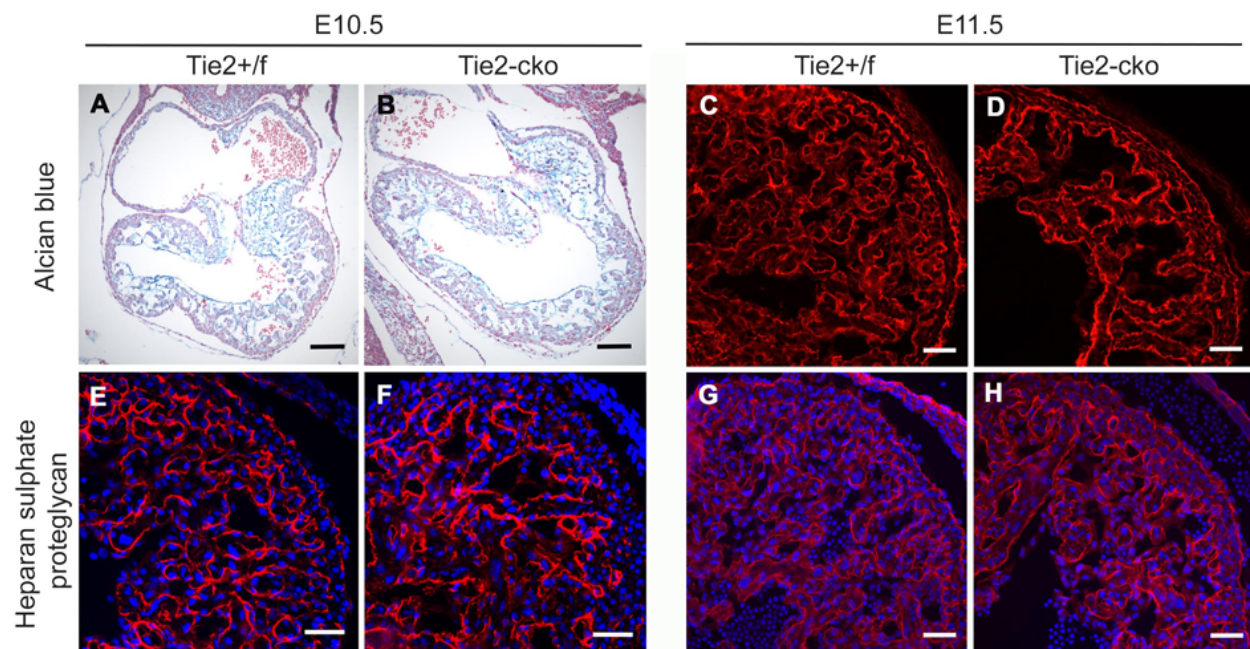
Supplemental Figure 8. Endocardial attenuation of Tie2 results in increased trabecular CM proliferation. (A-D) Dual immunostaining of control and *Tie2-cko* heart sections for pHH3 (red) and endomucin (green) at E11.5 and E13.0 showing significant increase in pHH3 staining in the mutant trabecular cardiomyocytes (CMs). DAPI, blue. Scale bars: 100 μ m. A total of more than 4 embryos per genotype collected from at least 3 independent litters was analyzed. A representative of >8 images was chosen for each panel.



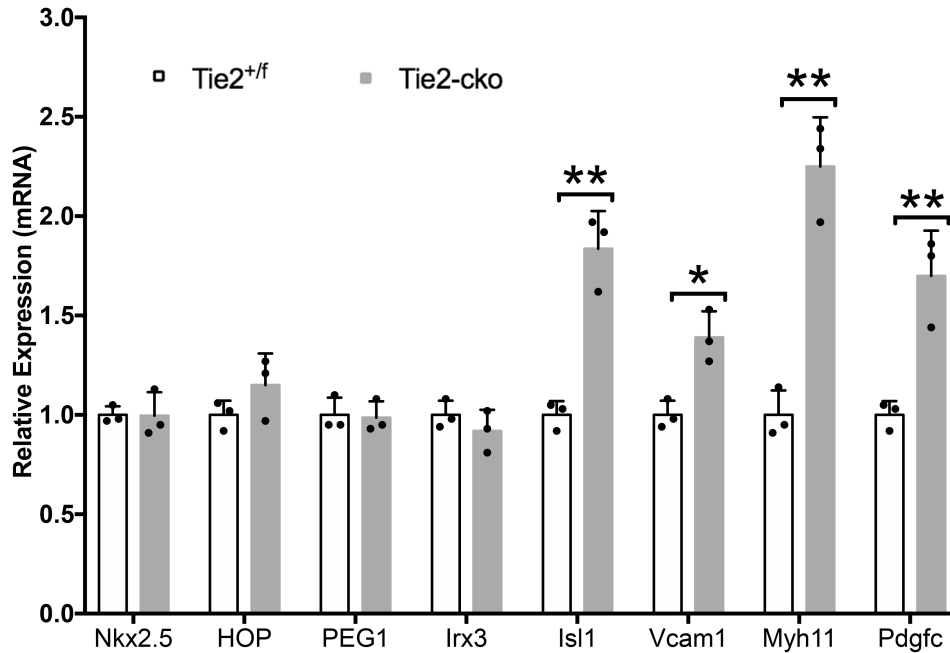
Supplemental Figure 9. Endocardial attenuation of Tie2 alters expression of *Bmp10* and *p57*. qPCR analysis E11.5 *Tie2^{+/f}* and *Tie2-cko* hearts detected increased expression of *Bmp10* (by 47%) and decreased expression of *p57* (by 40%) following endocardial loss of Tie2. N=5 per group, ** $p < 0.01$, Student's *t* test.



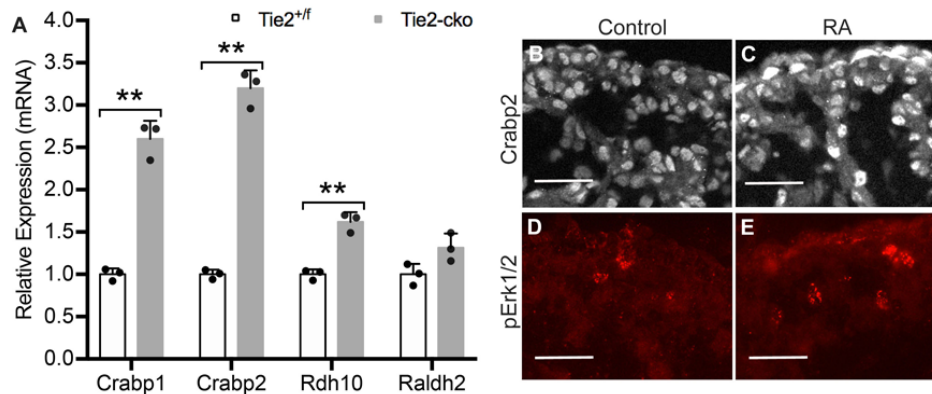
Supplemental Figure 10. Expression of p57 in endocardium is higher than that in myocardium of mouse embryos. (A-C) Dual immunostaining of wild-type heart sections for p57 (red) and Erg (green) at E10.5 showing that expression of p57 in endocardium is higher than that in myocardium of mouse embryos, which is a representative of >10 images chosen from 4 embryos. DAPI, blue. Scale bar: 100 μ m.



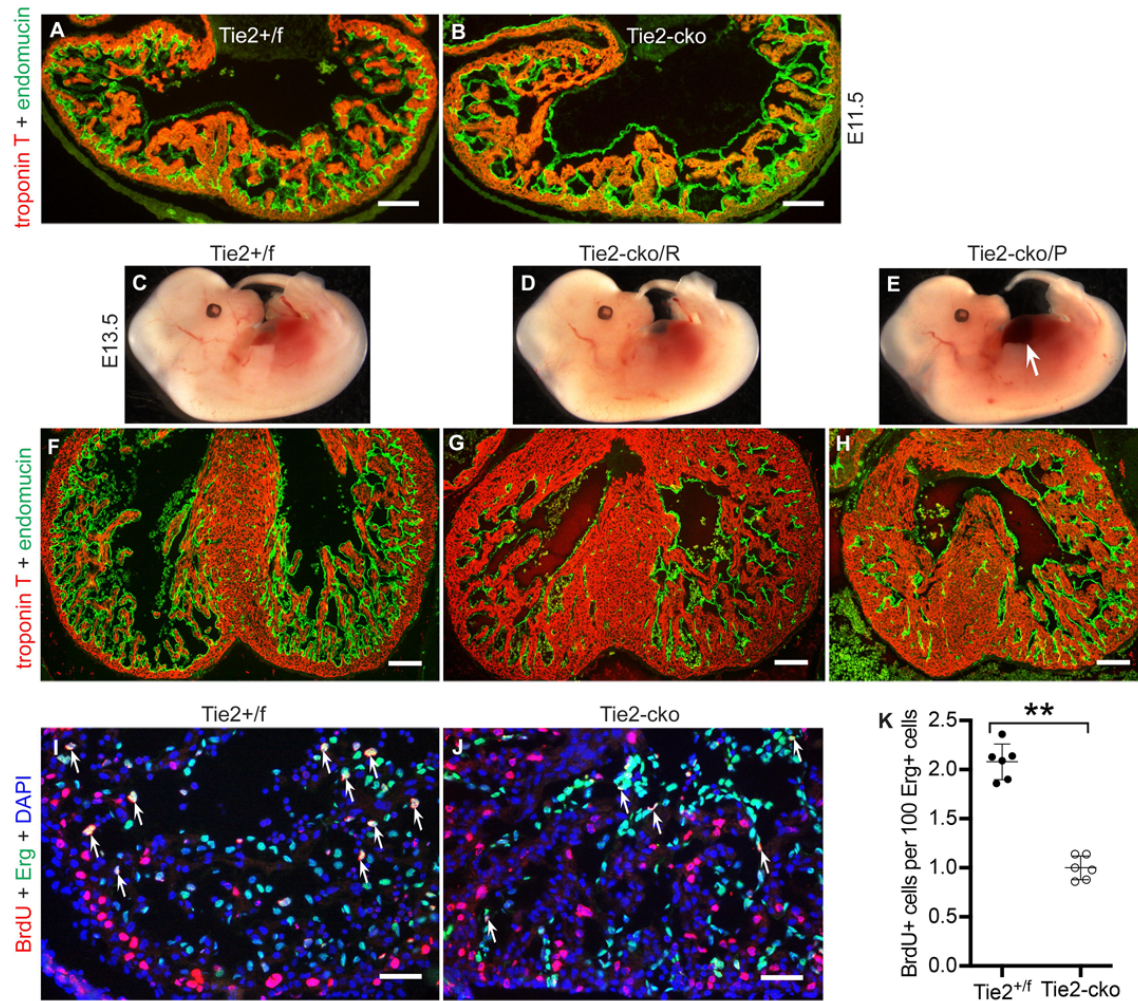
Supplemental Figure 11. Normal cardiac matrix/jelly in *Tie2-cko* embryonic ventricles. (A, B) Alcian blue staining of paraffin sections through ventricles of E10.5 control (*Tie2^{+/f}*) and *Tie2-cko* embryos. (C-H) Immunostaining of control and *Tie2-cko* heart frozen sections using antibodies for Fibronectin, Heparan sulphate proteoglycan and Laminin at E10.5 (E, F) and at E11.5 (C, D, G, H). Scale bars: A, B: 100 μ m; C-H: 50 μ m. A total of more than 4 embryos per genotype collected from at least 3 independent litters was analyzed. A representative of >8 images was chosen for each panel.



Supplemental Figure 12. Expression levels of a few genes associated with CM differentiation were changed in *Tie2-cko* embryos. qPCR analysis of genes associated with cardiomyocyte (CM) differentiation in E11.5 hearts reveals elevated levels of *Isl1*, *Vcam1*, *Myh11* and *Pdgfc* following endocardial loss of Tie2. N=3 per group, * $p < 0.05$; ** $p < 0.01$, Student's *t* test.



Supplemental Figure 13. RA-treated heart recapitulated the elevation of RA signaling and Erk1/2 hyperphosphorylation observed in *Tie2-cko* mutant myocardium. (A) qPCR analysis of control and Retinoic acid (RA)-treated wild-type heart extracts (treated at E8.0 and sampled at E10.5) reveals elevated RA signaling following RA treatment. N=3 per group, * $p < 0.05$; ** $p < 0.01$, Student's *t* test. (B and C) Immunostaining of control and RA-treated heart sections at E10.5 for Crabp2 (B and C) or p-Erk1/2 (red, D and E) showing elevated myocardial Crabp2 expression and Erk1/2 hyperphosphorylation following RA treatment. Scale bars: 50 μ m. A representative of >8 images was chosen for each panel.



Supplemental Figure 14. Myocardial phenotypes in *Tie2-cko* hearts were partially rescued by inhibiting in utero RA signaling with BMS493 treatment. (A and B) Heart ventricle sections of *Tie2*^{+f} (A) and *Tie2*^{-cko} (B) embryos at E11.5 treated with pan-retinoic acid (RA) receptor antagonist BMS493 were stained with troponin T (red) and endomucin (green) antibodies. (C-E) Gross images of in utero BMS493 treated *Tie2*^{+f} (C), *Tie2*^{-cko/R} (D, rescued group) and *Tie2*^{-cko/P} (E, poorly-rescued group) embryos at E13.5. Note that dorsal edema and pericardial effusions (arrow), common in the poorly-rescued group or untreated mutant embryos, were usually not obvious in the rescued group. (F-H) Heart sections of BMS493 treated *Tie2*^{+f} (F), *Tie2*^{-cko/R} (G) and *Tie2*^{-cko/P} (H) embryos at E13.5 were stained with troponin T and endomucin antibodies. (I and J) Dual immunostaining of BMS493 treated control (I) and *Tie2*^{-cko} (J) heart ventricle sections for BrdU (red) and Erg (green) at E11.5. The cells positive for both BrdU and Erg represent proliferating endocardial cells (ECs, arrows), which were decreased in the BMS493 treated mutant ventricles. (K) Quantification of BrdU/Erg-positive ECs as a percentage of total Erg-positive cells in endocardium indicated that BMS493 treated *Tie2*^{-cko} ventricles displayed lower proliferation rates of EECs at E11.5, similar to the untreated mutants. Scale bars: A, B, F-H, 100 μ m; I and J, 50 μ m. For the studies in A-H, a representative of >10 images was chosen from more than 5 embryos per genotype collected from at least 3 independent litters for each panel. For the studies in I-K, N=6 per group, ***p* < 0.01, Student's *t* test.

Supplemental Table 1. Sequences of qPCR primers

Gene	Forward primer	Reverse primer
18S	AGGAATCCCAGTAAGTGCG	GCCTCACTAAACCATCCAA
CD31	ATCCGCAAGGTFCGACCTAATCTCAT	ATACCCAACATGAACAAGGCAGCG
Nfatc1	CCAGCCTGCTTACAGTCCTC	TCCTCAGGCTCTTGCTTGAT
Tie1	TGCCAGTCTAGGGTATTGAAGTA	GGTCACACACACGGTGAACAA
Tie2	GAGGCCGAACATTCCAAGTA	GACAATTGTCACATGGCCAAAC
Notch1	GATGGCCTCAATGGGTACAAG	TCGTGTGTGTTGATGTCACAGT
Dll1	CAGGACCTTCTTTCGCGTATG	AAGGGGAATCGGATGGGGTT
Dll4	AGGTGCCACTTCGGTTACAC	GGGAGAGCAAATGGCTGATA
Jag1	GGGAGAGTGATACTTGATGGG	CTCATTGTGGCTTTTGTGGAG
Nrg1	AGCTGGCCTGTAATTCTTCCTGT	CCAATGGCCACATTGCCAATAGGT
ErbB2	CTTTGGTTACCCCCACTGCC	GGGGGAGCTGGTCGATGCTG
ErbB4	CATCTCAGCCGTTGCACCCT	TGCTGAGGAATATTTGGTCC
Bmp10	GGAAGGGGACAACCTGGAAT	TGCTCCTTGTCATGCTTTGG
Efnb2	CTAACCTCTCCTGCGCATT	AAACGTAGCCAACCGATGAC
EphB4	AGCGTCTGGACAAGATGAT	TGGTCCAAGAGTGGATGTGA
Hey2	CAGTGCCTTTGGAAACCATC	GAGGCTCTGGTGGAAATTGCT
Tbx20	TTACCGGGTGTGTTGCTTCCC	AGTGACAGGGCACTCACATG
Nkx2.5	AAGTGCTCTCCTGCCTTC C	CGTCTCGGCTTTGTCCAG
HOP	CGTAAGGTACAGCGACTTTC	TTATTTACGCCTGGGAGTGC
PEG1	GAGGTCTTGCCATCAAATAC	GAGTCCAGCTGCCTGATTC
Irx3	TCCTGCATCTCCACCTTCTC	CAGATCTGGGCTTCTGGAG
Isl1	TGTCAGGAGACTTGCCACTTT	GCCAAACGTTTATTAGTGAAATAGTC
Vcam1	AAGAGAACCAGGTGGAGGT	GGGGCAACGTTGACATAAAG
Myh11	GAGCAAACCTCAGGAGGAAAC	GTCCCGAGCGTCCATTTCTTC
Pdgfc	GCCAAAGAACGGGGACTCG	AGTGACAACCTCTCATGCCG
Tgfbr3	GGTGTGAAGTGTACCCGATCA	GTTTAGGATGTGAACCTCCCTTG
Crabp1	TTCCGCGGTACCTGGAAGATG	CCCCCTCAAGAAGTGTCTGTG
Crabp2	GGGTCTACGTCCGAGAGTGA	GTGGGAGGGAGGTTTGTGTC
Rdh10	CCTTTTGAAGCCGTCGTGTG	AGAACCGGCACCGTACAA
Raldh2	GCTCTCATGATATCCTCCGC	TCCCGTAAGCCAAACTCACC
Cyp1b1	ACGACGATGCGGAGTTCTTA	CGGGTTGGGAAATAGCTGC
Rbp4	CCACTGGATCATCGACCGG	GCCATTGGGGTCCAGAGAA
Fabp5	TGAAAGAGCTAGGAGTAGGACTG	CTCTCGGTTTGGACCGTATG
Stra6	CTGGTACATCGAGGAACCTCT	CCAGGAACGACAGTGAAGCC
Afp	ACCTTCTGTCTCAGTCATCT	CCTGACATCCAGGTAGATTCCA
p57	AGAGAAGTGCAGGAGAAAC	TCTGGCCGTTAGCCTCTAAAC
VE-Cad	ACATCTCATGCACCAGGGTACTA	AACTCACCTCCTTGTGGAATCCT
Flk1	TGCGGGCTCCTGACTACACTAC	TTCCCAAATGCTCCACCAACTCTG
Npr3	GTCTACAGCGACGACAAACTC	AGGTCCAAGTCTTTGGTCTCG
vWF	CTTCTGTACGCCTCAGCTATG	GCCGTTGTAATCCACACAAG
Irx5	TGGCTAAAGACCCGAAAATGT	GGGATACCGCACCAGAGTTA
Irx6	ACCCCGTTTCTCTTTACTGAGG	CCGCAGTGTGAGCTAAGGAC
Pdgfβ	AAGTGTGAGACAATAGTGACCCC	CATGGGTGTGCTTAAACTTTCCG
Sox7	ATGCTGGGAAAGTCAAGGAAAG	CGTGTCTGGTCCAGAGAGA
Gata2	GCCGGGAGTGTGCTCAACTG	AGGTGGTGGTGTGCTCTGA
Robo4	GGCAACGGTAGTGTCTTTGTG	ATGGCCTGCTCTAAAAGGAGG
Plxnd1	GCTGACTGTAGCCTATGGGGA	GCCATCTGGTGGATGTGAT
Rasip1	TCATGGAGCAGCTTACGGACT	GGGTGCGAGGAAAACTTTCA
Acap1	GTACTGCGTTTGGTTGAGGC	ACCCAATTCCTCCGATACTGAG
Arap3	GTTTGGCACTATGCCCTTGC	GCAGGGTCTGATGGCAGAG
Gpr126	CGCCATGTTTCATTTGGTCAT	ATCTCCTTCATCGCACAGTG
Tmem100	GACAAATGGAGAAAAACCCCAAGA	GGTAGCAGGAGAGTTCGGC
Klf2	CGCCTCGGGTTCATTTTC	AGCCTATCTTGCCGTCCTTT
Nos3	TGAAGATCTCTGCCCTCACTCATG	AGTCTCAGAGCCATACAGGGTT
Cx37(Gja4)	TCCCACATCCGATACTGGGT	CCCGCCGAGACAGGTAGAT
Edn1	GCACCGGAGCTGAGAAATGG	GTGGCAGAAGTAGACACTC

Supplemental Table 2. Sources of primary antibodies

Antibody	Supplier	Catalogue #	Application
rabbit anti-pHH3	Upstate	#06-570	IHC 1:100
rat anti-BrdU	abcam	ab6326	IHC 1:100
rabbit anti Cleaved Caspase-3 (Asp175)	Cell Signaling	#9664	IHC 1:400
mouse anti- α -Tublin	Sigma	T5168	WB 1:5000
rabbit anti-Erk1/2 (137F5)	Cell Signaling	#4695	WB 1:1000
Rabbit anti-Phospho-Erk1/2 (Thr202/Tyr204)	Cell Signaling	#9101	IHC 1:50 WB 1:1000
rabbit anti-Akt (pan) (C67E7)	Cell Signaling	#4691	WB 1:1000
rabbit anti-Phospho-Akt (Ser473)	Cell Signaling	#4060	WB 1:1000
mouse anti-Tnnt2 Antibody	Developmental Studies Hybridoma Bank	CT3	IHC 1:2
goat anti-mouse Tie2	R&D Systems	AF762	IHC 1:200
rat anti-mouse CD31	Pharmlngen,	557355	IHC 1:200
mouse anti-Nos3	BD Biosciences	610298	IHC 1:100
rat anti-CD34	eBioscience	14-0341-81	IHC 1:200
rat anti-CD54 (ICAM-1)	abcam	ab25375	IHC 1:150
rat anti-CD102 (ICAM-2)	eBioscience	14-1021	IHC 1:500
rabbit anti-Erg1	abcam	ab92513	IHC 1:100
rat anti-VE-Cadherin	PD Pharmlngen	555289	IHC 1:100
goat anti-Flk1	R&D Systems	AF644	IHC 1:200
rat anti-endomucin	abcam	ab106100	IHC 1:200
mouse anti-Crabp1	abcam	Ab2816	IHC 1:100
rabbit anti-Crabp2	Proteintech	10225-1	IHC 1:200
mouse anti-Fibronectin	Sigma	F6140	IHC 1:400
mouse anti-Laminin	Sigma-Aldrich	L8271	IHC 1:1000
rat anti-Heparan Sulphate Proteglycan	abcam	ab2501	IHC 1:5100
rabbit anti-Versican	Chemicon	AB1033	IHC 1:1500
Hyaluronic acid binding protein, biotinylated	Millipore	385911	IHC 1:200
rabbit anti-Cleaved Notch1	Cell Signaling	#4147	IHC 1:100
mouse anti-p57kip2	Thermo	MS-897-P1	IHC 1:100
rabbit anti-Gata2	ThermoFisher	PA1-100	IHC 1:1500
goat anti-Sox7	R&D Systems	AF2766	IHC 1:1500

WB: Western blot; IHC: Immunohistochemistry