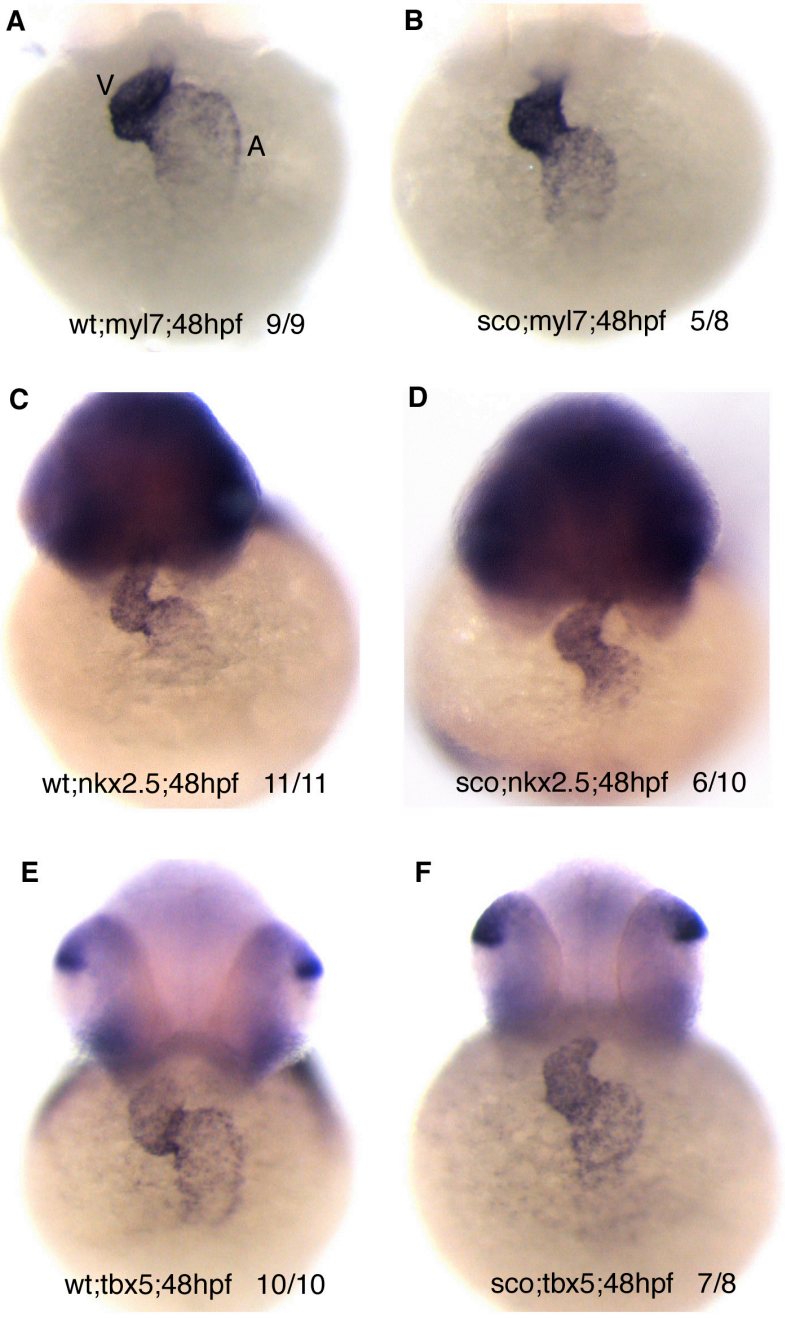


Fig. S1. Cardiac genes were not affected in *sco*^{te382} mutants. Wild-type (A, C, E) and *sco*^{te382} mutant (B, D, F) embryos at 48 hpf were subjected to whole-mount RNA in situ analyses with *myl7* (A, B); *nkx2.5* (C, D); or *tbx5* (E, F) probes. A, atrium; V, ventricle.

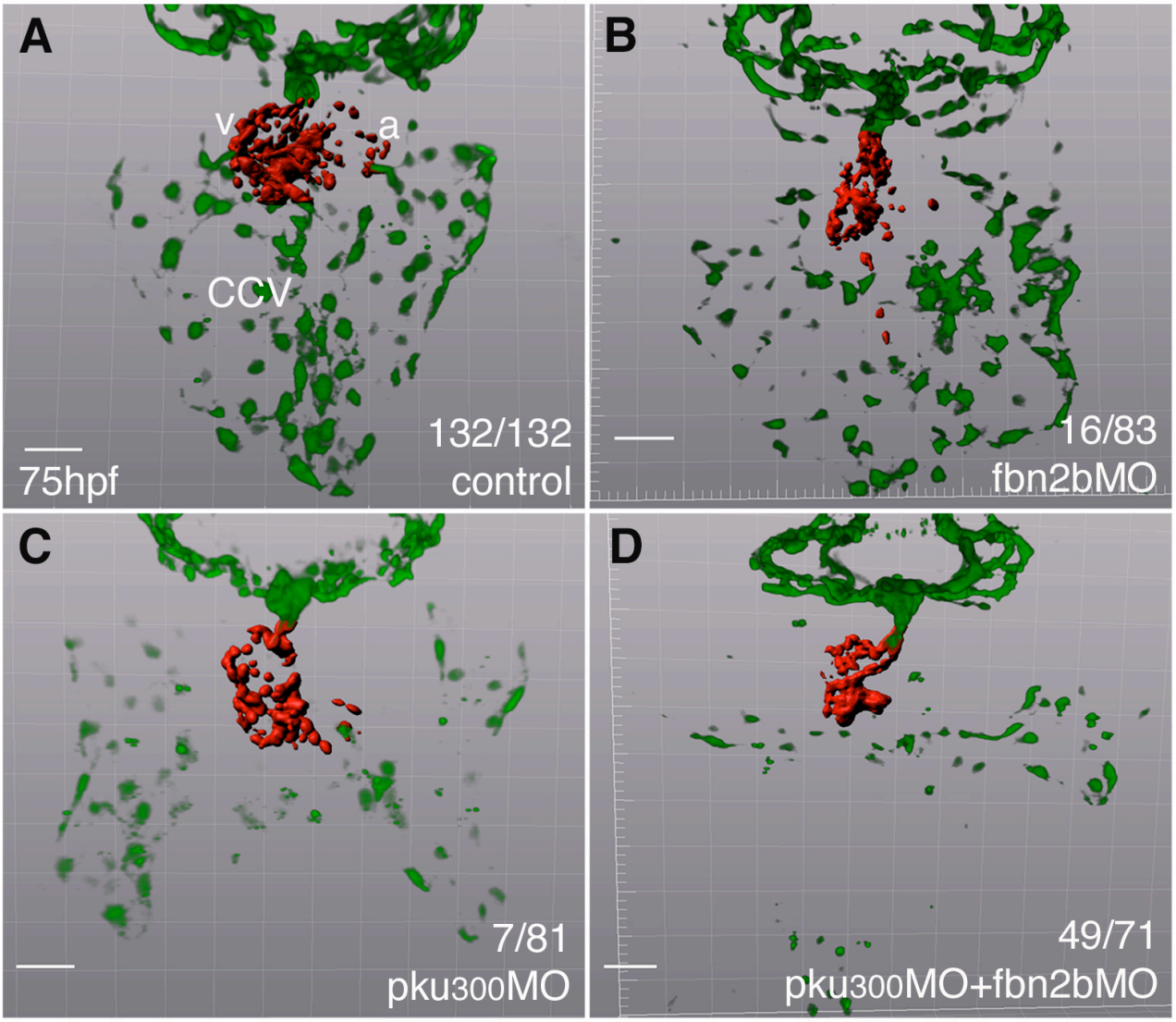


Fig. S2. Both *fbn2b* and *pku300* contribute to the formation of atrial endocardium and common cardinal vein (CCV). Compared with wild-type siblings (A), either fbn2bMO (0.4 ng) or pku300MO (4.8 ng) knockdown led to smaller percentages of morphants (16/83 by fbn2bMO; 7/81 by pku300MO) that had defects in the atrial endocardium or CCV (B, C), but simultaneous knockdown by low-dose of fbn2bMO and pku300MO resulted more morphants (49/71) that had defects in the atrial endocardium and CCV (D), suggesting a genetic interaction between *fbn2b* and *pku300* genes. Scale bar, 50 μm.

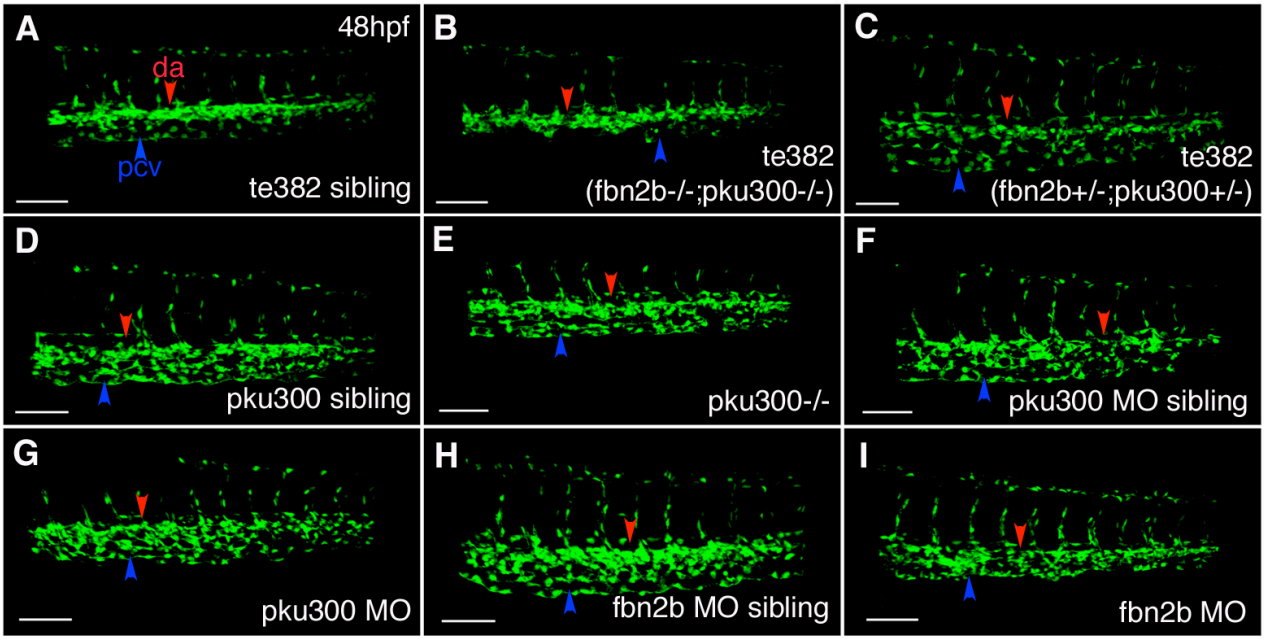


Fig. S3. Both *fbn2b* and *pku300* are required for the development of the caudal vein. (A-C) Compared with a wild-type sibling (A), a group II mutant (*fbn2b*$^{-/-}$;*pku300*$^{-/-}$) had severe defect in the caudal vein (B), while a group I mutant (*fbn2b*$^{+/-}$;*pku300*$^{+/-}$) had relatively normal caudal vein (C). (D-G) Either a *pku300*$^{-/-}$ mutant (E) or pku300MO morphant (G) had mild defect in the caudal vein as those of controls (D, F). (H-I) Compared with a fbn2bMO sibling (H), a fbn2bMO morphant had mild defect in the caudal vein (I). Scale bar, 100 μm.

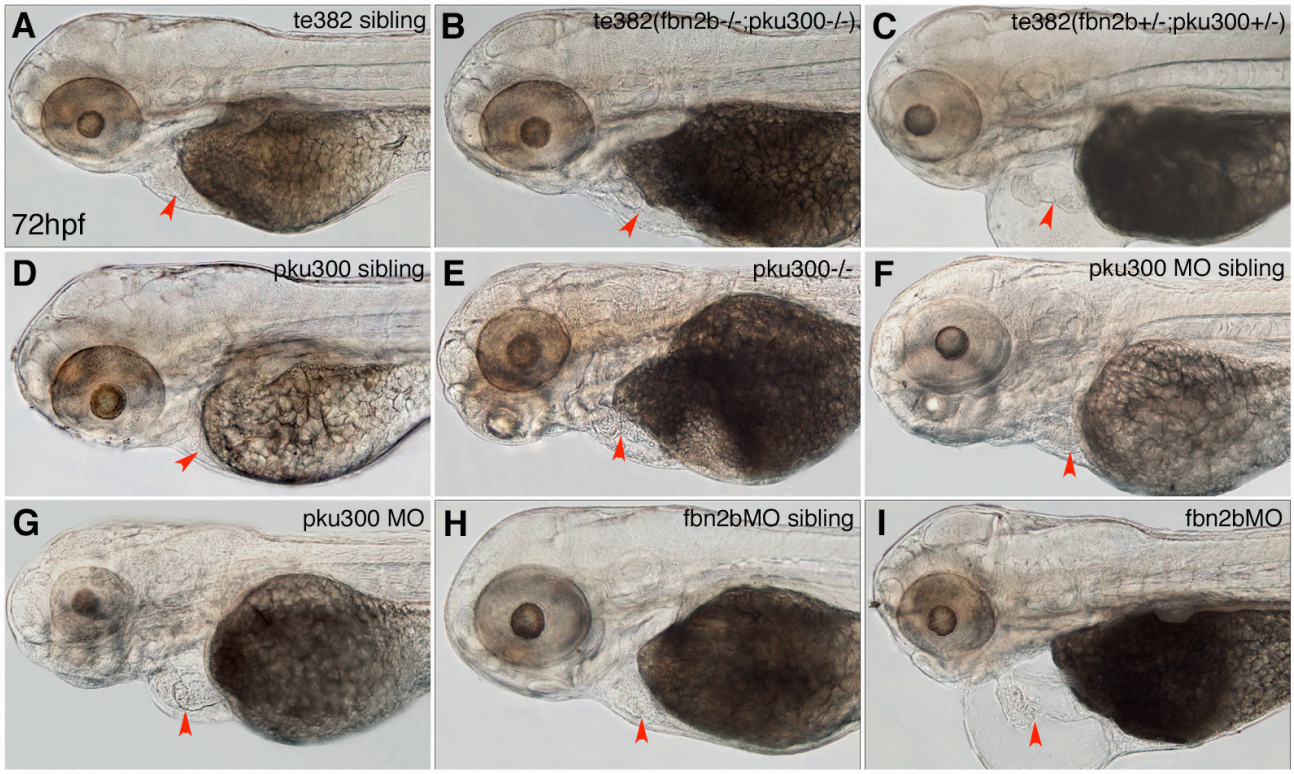


Fig. S4. Both *fbn2b* and *pku300* are required for heart development. (A-C) Compared with a wild-type sibling (A), either a group II mutant (*fbn2b*$^{-/-}$;*pku300*$^{-/-}$) (B), or a group I mutant (*fbn2b*$^{+/-}$;*pku300*$^{+/-}$) (C) had heart defects at 72 hpf. (D-G) Either a *pku300*$^{-/-}$ mutant (E) or pku300MO morphant (G) had heart defects, compared with their controls (D, F). (H-I) Compared with a fbn2bMO sibling (H), a fbn2bMO morphant had heart defects (I).

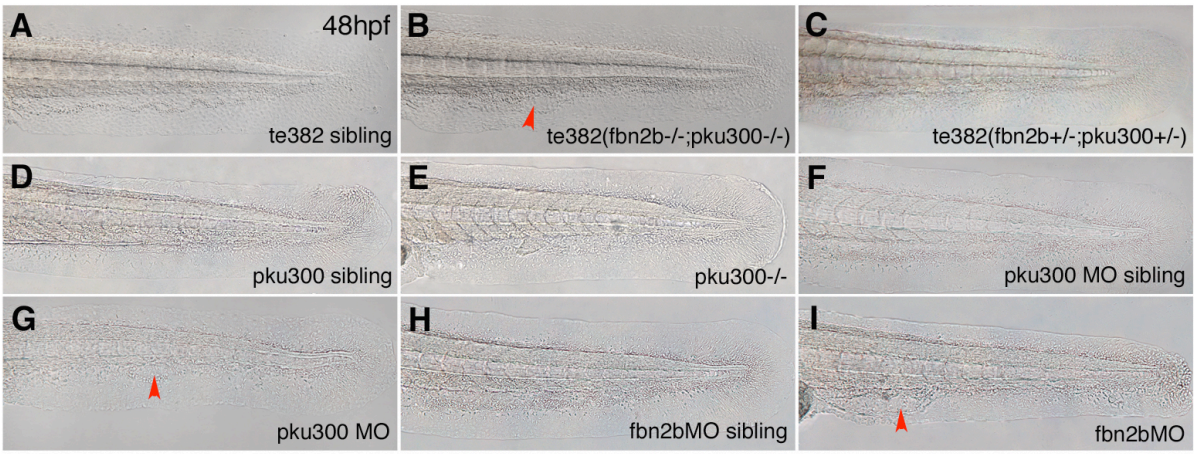


Fig. S5. Both *fbn2b* and *pku300* are required for the development of the ventral tail. (A-C) Compared with a wild-type sibling (A), a group II mutant (*fbn2b*$^{-/-}$;*pku300*$^{-/-}$) had severe defects in the ventral tail (B), while a group I mutant (*fbn2b*$^{+/-}$;*pku300*$^{+/-}$) had relatively normal ventral tail (C). (D-G) Either a *pku300*$^{-/-}$ mutant (E) or pku300MO morphant (G) had mild defects in the ventral tail, compared with those of controls (D, F). (H-I) Compared with a fbn2bMO sibling (H), a fbn2bMO morphant had defects in the ventral tail (I). Red arrowheads point to bubble-like deformed tissues.

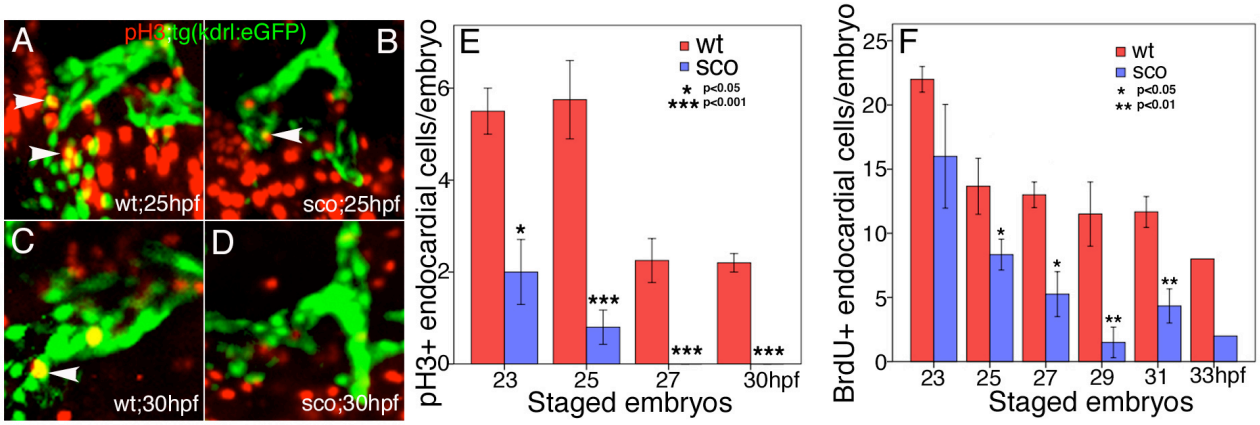


Fig. S6. Endocardial cell proliferation is defective in *sco*^{te382} mutants.

(A-E) wild-type (A, C) and *sco*^{te382} mutant (B, D) Tg(*kdrl:eGFP*) embryos at 23 hpf and 25 hpf (A, B), 27 hpf and 30 hpf (C, D) were subjected to immunostaining with anti-pH3 antibody. Images were photographed under Zeiss 510 confocal microscope. White arrowheads point to pH3-positive Tg(*kdrl:eGFP*) endocardial cells in the heart tube. (E) The pH3-positive endocardial cells were scored and statistically analyzed in wild-type and *sco*^{te382} mutant embryos from 23 to 30 hpf. (F) The BrdU-positive Tg(*kdrl:eGFP*) endocardial cells were scored and statistically analyzed in wild-type and *sco* mutant embryos from 23 to 33 hpf. Note that pH3- and BrdU-positive endocardial cells were gradually decreased in *sco*^{te382} mutants. (E-F) n=3-5; mean±SEM; student's t-test.

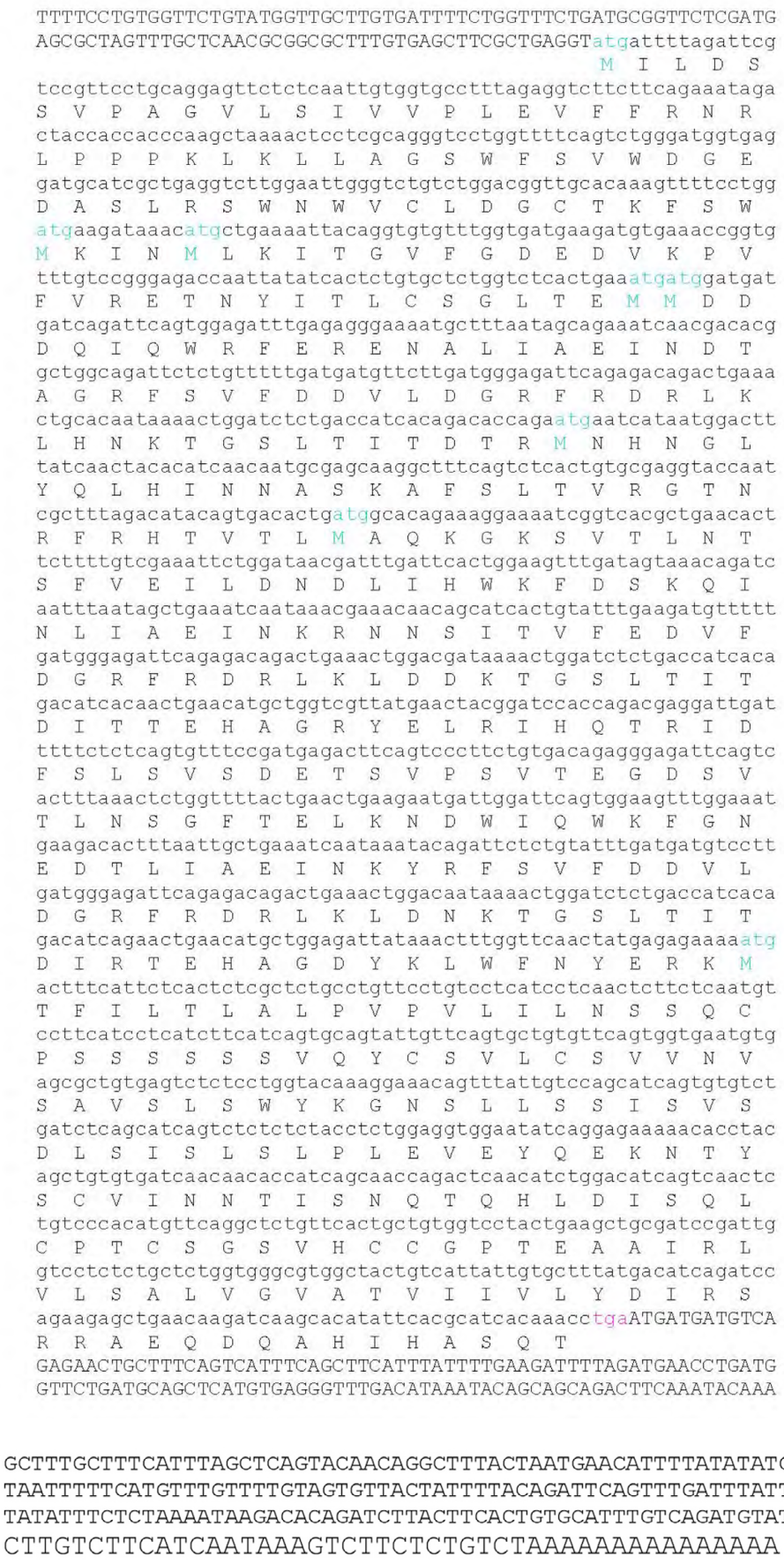
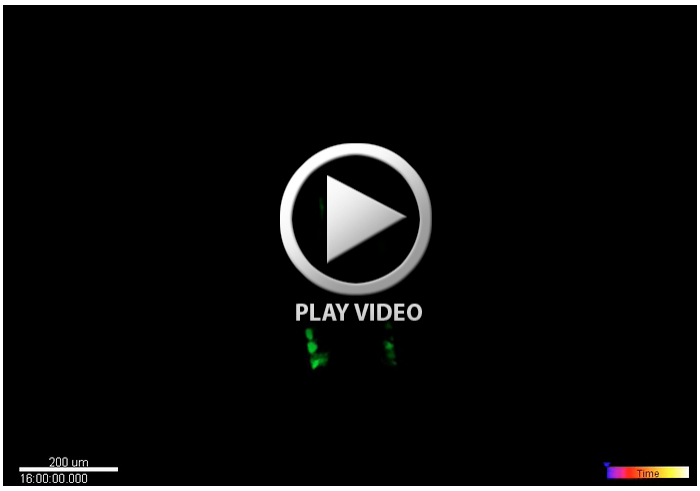
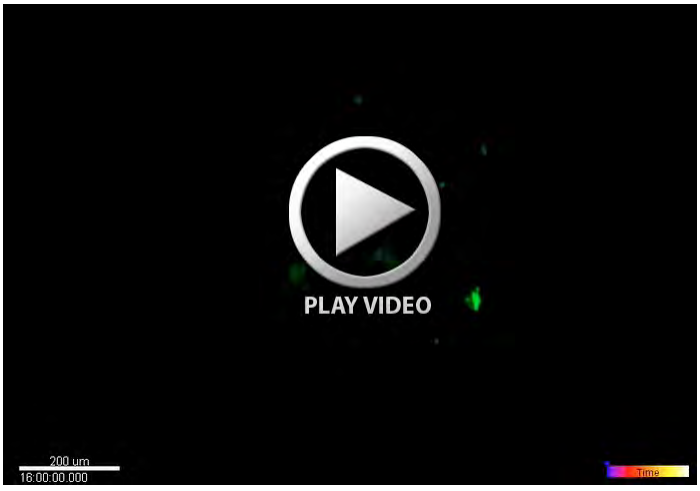


Fig. S7. Nucleotide and protein sequences of *pku 300.* The blue represents the codon encoding for methionine in the same reading frame and magenta for the stop codon.

Movie 1. A wild-type embryo at 48 hpf, noting that blood flowed from the atrium to ventricle; followed by a *sco*^{te382} mutant embryo at 48 hpf, noting blood flow from the atrium to ventricle, but leaked back from the ventricle to atrium.

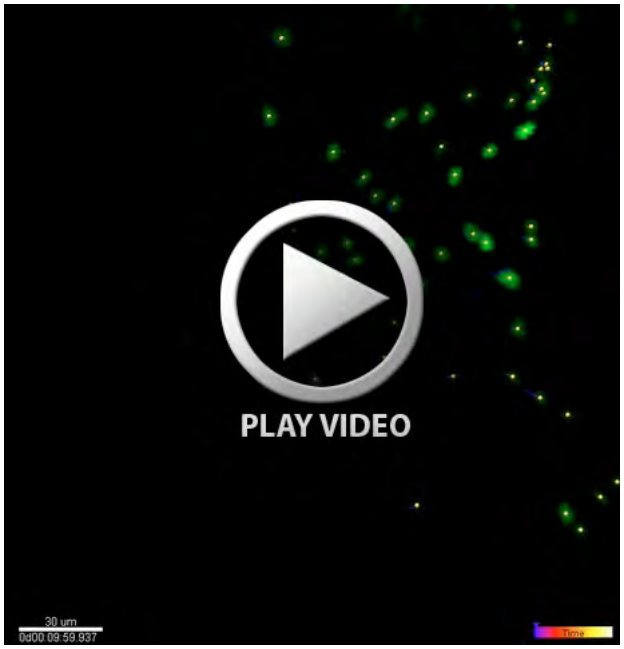


Movie 2. Live image showed that Tg(*kdrl:EGFP*) endocardial cells fused in the midline and then jogged toward the left in a wild-type embryo from 16 hpf to 22 hpf. Dorsal view, with the anterior on the bottom. Scale bar, 200 μm.

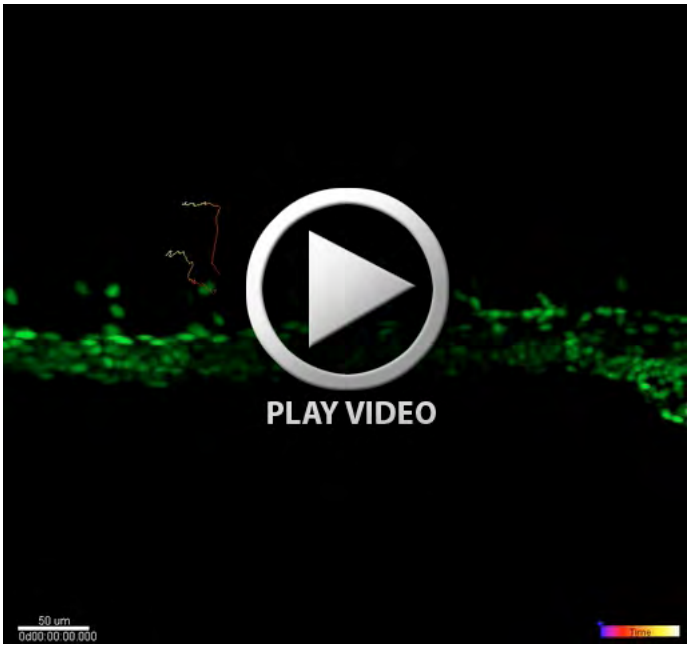


Movie 3. Live image showed that Tg(*kdrl:EGFP*) endocardial cells fused in the midline and then jogged toward the left in a *sco*^{te382} embryo from 16 hpf to 23 hpf. Dorsal view, with the anterior on the bottom. Scale bar, 200 μm.

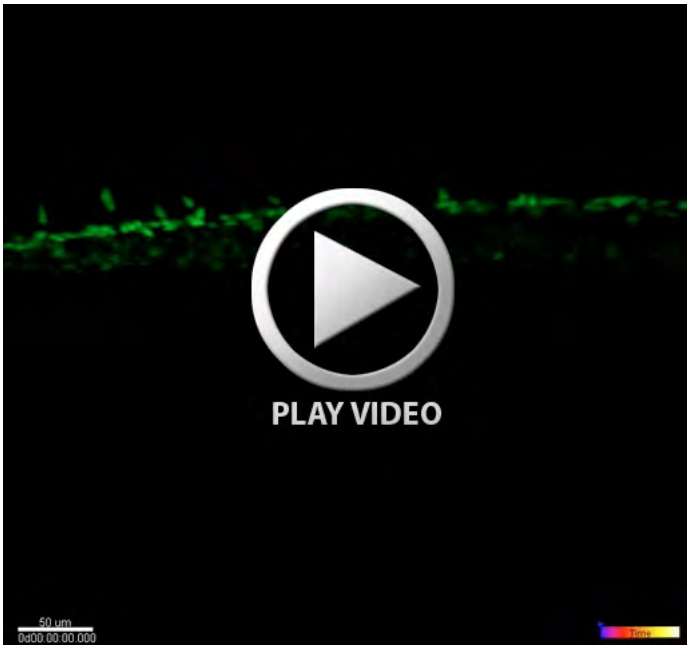
Movie 4. Live image showed 16 Tg(*fli1:nuEGFP*) endocardial cells underwent cell division in a wild-type embryo from 29 to 36 hpf. Ventral view, with the anterior on the top. Scale bar, 20 μm.



Movie 5. Live image showed no Tg(*fli1:nuEGFP*) endocardial cells underwent cell division in a *sco*^{te382} embryo from 29 to 36 hpf. Ventral view, with the anterior on the top. Scale bar, 30 μm.



Movie 6. Live image showed formation of intersegmental vessels in a wild-type Tg(*fli1:nuEGFP*) embryo from 23 to 33 hpf. Lateral view, with the anterior on the left. Scale bar, 50 μm.



Movie 7. Live image showed formation of intersegmental vessels in a *sco*^{te382} Tg(*fli1:nuEGFP*) embryo from 22 to 33 hpf. Note normal vascular endothelial cell division and migration. Lateral view, with the anterior on the left. Scale bar, 50 μm.

Table S1. Primer sequences for genetic markers during the chromosomal walking.

Marker	Forward Primer	Reverse Primer
1	AATCAGACCGGCTGAGAAGA	CGAAGCCTCGTGAGTGTGTA
2	CCCACATCCACGGAGACTAC	TGGCTCTCCAAGTGGTCTTT
3	TGGATGGCAGTGCTGATATG	GAAGAGGTTTGGAACCACCA
4	AATCAGACCGGCTGAGAAGA	AAACTCCCGCAAGTTCAGC
5	GTGTTGCTTGCCAGGCCTCT	AACAAAATGGGCAGGGCTTGA
6	TCACAATTGTTGTTGTGCAATGCT	CCTCATGCCATTTCCCCTTTC
7	GGGACACGTCCCACCCTAAA	GGAGTCTGTCAGGAACGCTGAA
8	CCGGTATGACTGTAGGGGTCCA	TGGACTGTAAAAGGCCAACTATTTAGA
9	TTCTCCGCTGGCCAAAAGTC	CATCGCCAGTGTTTCGTTCG

Table S2. Mutations of both *fbn2b* and *pku300* contributed to *sco*^{te382} mutant phenotypes.
Two groups of mutants were classified from heterozygous *sco*^{te382} crosses, one showing defects only in the heart (group I) and the other showing defects both in the heart and tail (group II). 12 embryos of each of wild-type siblings, group I mutants, and group II mutants were sequenced. Their corresponding phenotype (PT), genotype (GT), and embryo numbers (Em#) were shown. Note that phenotypical wild-type siblings had four types of genotypes, including 1) *fbn2b*$^{+/-}$ (G3935T) and *pku300*$^{+/-}$ (C946T); 2) *fbn2b*$^{+/-}$; 3) *pku300*$^{+/-}$; and 4) wild-type. Group I mutants were all both *fbn2b*$^{+/-}$ (G3935T) and *pku300*$^{+/-}$ (C946T). Group II mutants were all both *fbn2b*$^{-/-}$ (G3935T) and *pku300*$^{-/-}$ (C946T).

PT / GT / Em#	Wild-type siblings		Group I Abnormal heart		Group II Abnormal heart and tail	
	fbn2b	pku300	fbn2b	pku300	fbn2b	pku300
1	G/T	C/T	G/T	C/T	T	T
2	G/T	C/T	G/T	C/T	T	T
3	G/T	C/T	G/T	C/T	T	T
4	G	C	G/T	C/T	T	T
5	G	C	G/T	C/T	T	T
6	G/T	C/T	G/T	C/T	T	T
7	G/T	C	G/T	C/T	T	T
8	G/T	C/T	G/T	C/T	T	T
9	G	C/T	G/T	C/T	T	T
10	G	C	G/T	C/T	T	T
11	G/T	C	G/T	C/T	T	T
12	G	C/T	G/T	C/T	T	T