





**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<sup>te382</sup>* mutants.

(A-E) wild-type (A, C) and  $sco^{te^{382}}$  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  $sco^{te^{382}}$  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 and statistically analyzed in sco<sup>te382</sup> 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<sup>te382</sup>* mutants. (E-F) n=3-5; mean±SEM; student's t-test. tccgttcctgcaggagttctctcaattgtggtgcctttagaggtcttcttcagaaatagaS V P A G V L S I V V P L E V F F R N R  $\verb+ctaccacccaagctaaaactcctcgcagggtcctggttttcagtctgggatggtgag$ L P P P K L K L L A G S W F S V W D G E gatgcatcgctgaggtcttggaattgggtctgtctggacggttgcacaaagttttcctgg D A S L R S W N W V C L D G C T K F S W a tgaagataaaca tgctgaaaattacaggtgtgttttggtgatgaagatgtgaaaccggtg M KINMLKITGVFGDEDVKPV  ${\tt tttgtccgggagaccaattatatcactctgtgctctggtctcactgaa {\tt tggatgat}$ V R E T N Y I T L C S G L T E M M D D gatcagattcagtggagatttgagagggaaaatgctttaatagcagaaatcaacgacacg D Q I Q W R F E R E N A L I A E I N D T A G R F S V F D D V L D G R F R D R L K L H N K T G S L T I T D T R M N H N G L  ${\tt tatcaactacaactacaactacgagcaaggctttcagtctcactgtgcgaggtaccaat}$ Y Q L H I N N A S K A F S L T V R G T N cgctttagacatacagtgacactgatggcacagaaaggaaaatcggtcacgctgaacact R F R H T V T L M A Q K G K S V T L N T  ${\tt tcttttgtcgaaattctggataacgatttgattcactggaagtttgatagtaaacagatc}$ S F V E I L D N D L I H W K F D S K Q I aatttaatagctgaaatcaataaacgaaacaacagcatcactgtatttgaagatgttttt N L I A E I N K R N N S I T V F E D V F gatgggagattcagagacagactgaaactggacgataaaactggatctctgaccatcacaD G R F R D R L K L D D K T G S L T I T gacatcacaactgaacatgctggtcgttatgaactacggatccaccagacgaggattgat D I T T E H A G R Y E L R I H Q T R I D  ${\tt ttttctctcagtgtttccgatgagacttcagtcccttctgtgacagagggagattcagtc}$ F S L S V S D E T S V P S V T E G D S V  $a \verb+ctttaaactctggttttactgaactgaagaatgattggattcagtggaagtttggaaat$ L N S G F T E L K N D W I Q W K F G gaagacactttaattgctgaaatcaataaatacagattctctgtatttgatgatgtcctt EDTLIAEINKYRFSVFDDVL gatgggagattcagagacagactgaaactggacaataaaactggatctctgaccatcaca D G R F R D R L K L D N K T G S L T I T gacatcagaactgaacatgctggagattataaactttggttcaactatgagagaaaaatg DIRTEHAGDYKLWFNYERK M actttcattctcactctcgctctgcctgttcctgtcctcatcctcaactcttctcaatgt T F I L T L A L P V P V L I L N S S Q C  $\verb+cttcatcctcatcttcatcagtgcagtattgttcagtgctgtgttcagtggtgaatgtg$ P S S S S S V Q Y C S V L C S V V N V agcgctgtgagtctctcctggtacaaaggaaacagtttattgtccagcatcagtgtgtctS A V S L S W Y K G N S L L S S I S V S gatetcageatcagtetetetetetetggaggtggaatatcaggagaaaaacacetac D L S I S L S L P L E V E Y Q E K N T Y agetgtgtgtgatcaacaacaccatcagcaaccagactcaacatctggacatcagtcaactc S C V I N N T I S N Q T Q H L D I S Q L tgtcccacatgttcaggctctgttcactgctgtggtcctactgaagctgcgatccgattgC P T C S G S V H C C G P T E A A I R L  ${\tt gtcctctctgctctggtgggcgtggctactgtcattattgtgctttatgacatcagatcc}$ L S A L V G V A T V I I V L Y D I R S agaagagctgaacaagatcaagcacatattcacgcatcacaaacctgaATGATGATGTCA R R A E Q D Q A H I H A S Q T GAGAACTGCTTTCAGTCATTTCAGCTTCATTTATTTTGAAGATTTTAGATGAACCTGATG GTTCTGATGCAGCTCATGTGAGGGTTTGACATAAATACAGCAGCAGACTTCAAATACAAA

**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<sup>te382</sup>* 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  $\mu$ m.



**Movie 3.** Live image showed that Tg(kdrl:EGFP) endocardial cells fused in the midline and then jogged toward the left in a *sco<sup>te382</sup>* 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  $\mu$ m.



**Movie 5.** Live image showed no Tg(*fli1:nuEGFP*) endocardial cells underwent cell division in a *sco<sup>te382</sup>* embryo from 29 to 36 hpf. Ventral view, with the anterior on the top. Scale bar, 30  $\mu$ m.



**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  $\mu$ m.



**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  $\mu$ m.

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<sup>te382</sup>* mutant phenotypes.

Two groups of mutants were classified from heterozygous  $sco^{te^{382}}$  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	Wild-type siblings		Group I Abnormal heart		Group II Abnormal heart and tail	
Em#	fbn2b	pku300	fbn2b	pku300	fbn2b	pku300
1	G/T	C/T	G/T	C/ <mark>T</mark>	Т	Т
2	G/T	C/T	G/T	C/ <mark>T</mark>	Т	Τ
3	G/T	C/ <mark>T</mark>	G/T	C/ <mark>T</mark>	Т	Т
4	G	С	G/T	C/ <mark>T</mark>	Т	Т
5	G	С	G/T	C/ <mark>T</mark>	Т	Т
6	G/T	C/T	G/T	C/ <mark>T</mark>	Т	Ţ
7	G/T	С	G/T	C/ <mark>T</mark>	Т	Т
8	G/T	C/T	G/T	C/ <mark>T</mark>	Т	Т
9	G	C/T	G/T	C/T	Т	Т
10	G	С	G/T	C/T	Т	Ţ
11	G/T	С	G/T	C/T	Т	<sup>a</sup> T <sup>a</sup>
12	G	C/T	G/T	C/T	Т	Т