Supplementary Materials and Methods

Generation of transgenic fish

To generate *uas:mespba* fish, we amplified the full-length *mespba* fragment by PCR using ggaattcGACATGCAAACCTCAAGCAAG and ggctcgagTCATCTCCAGTAAGTCTGAGG, and cloned it into the EcoRI and XhoI site of pT2AUASMCS (a gift from Dr. Koichi Kawakami). For the generation of *tbx6:ggff* driver fish, pBS-hygroR-tol2R and pBS-tol2L-ampR cassettes were generated. The fragments of ampR and hygroR genes were amplified by PCR using GGAATTCTAGGGATAACAGGGTAATAACTTGGTCTGACAGTTACC, GGCTCGAGCACTTTTCGGGGAAATGTGC, GGAATTCTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACG ACTCACTATAGGAGGGCCATCATGAAAAAGCCTGAACTCAC and CTCGAGTAGGGATAACAGGGTAATCTATTCCTTTGCCCTCGGAC, and cloned into the EcoRI/XhoI and KpnI/ApaI sites of pBS-SK+ vector, respectively (pBS-ampR and pBS-hygroR). The *tol2L* and *tol2R* fragments were cut out from pT2AUASMCS and cloned into the NotI and EcoRI/KpnI site of pBS-ampR and pBS-hygroR, respectively. The homology arms were attached, flanking the ends of *hygroR-tol2R* and tol2L-ampR, by PCR using following primers; for *hygroR-tol2R* cassette, CCTCTGTGTGAAATGTGTCTGCAGTAGAACTCCAGTCGTTCTTGACAATTAA TCATCGGCATAGT and GTGAGCTGGGTCTTACTTCTCTGCTTGGCTGTTTTATTTTATCTGGCCTGTGT TTCAGACAC and for *tol2L-ampR* cassette,

Development • Supplementary information

TTAAAAGTCTTTTCCCACTTGCCCCTTAGTTTGATTTCCAGGATCCAGATCGA TCTGCGAAG and CTCAGTGTATAAGTCAGTGCCGTACGGATCGGTGGACGACCACTTTTCGGG GAAATGTGCG. The purified DNA fragments were introduced into the sw102 strain containing BAC (CH211-136M16) to introduce the *hygroR-tol2R* and *tol2L-ampR* cassettes into sites 14.5kbp upstream and 9.2kbp down stream of the Tbx transcription initiation site, respectively, by homologous recombination. Then the *ggff-pA-kanR* fragment was amplified by PCR using TTTAATATTCGATAAAGACAAACGTGAAGAAAGAGCAGACCCGGTCGCCAC CATGGTGAG and CTCTGTAATAGCAGTCGCTCAATCTCTGAGGTCCCAGAGCTGCGTGATCTGA TCCTTCAACTC. The fragment of *ggff-pA-kan* was introduced into the *tbx6* transcriptional initiation site of BAC (CH211-136M16) containing hygroR-tol2R and tol2L-ampR. The pT2A-hsf-ggff (a gift from Dr. Kawakami) was used as a template. Twenty-five picrograms of plasmid or BAC DNA was injected into the 1-cell- stage eggs of TL2 along with 50 pg of synthesized Tol2 transposase mRNA to obtain the transgenic fish. We used the following respective primers to check the genotypes of these transgenic fish; for *uas-mespba*, AGCGGAGACTCTAGAGGGTA and GGTTCTTCAGCCTCAATCTC and for *tbx6-ggff*, GTCTGAAGAACAACTGGGAG and TTCCGATGATGATGTCGCAC.

Genotyping of mutant and transgenic fish

To genotype the mutant generated by TALEN, we amplified the fragments of DNA

Development • Supplementary information

around the mutation site by PCR with primers used for the T7 endonuclease assay. To distinguish wild-type and mutant allele, we digested DNA fragments with BspHI, Hinp1I, PstI, HindIII, BccI, and HaeIII, for *mespba*^{kt1004}, *mespbb*^{kt1006}, *mespab*^{kt1002}, *mespab*^{kt1030}, *ripply1*^{kt1032} and *ripply2*^{kt1034}, respectively. Genotypes of *mespbb*^{kt1009} and *mespab*^{kt1017} were assessed by direct sequencing of PCR products. For genotyping *tbx6;ggff* and *uas;mespba*, the transgenes were detected by PCR by using the following primers: ; GTCTGAAGAACAACTGGGAG and TTCCGATGATGATGTCGCAC for *tbx6;ggff* and AGCGGAGACTCTAGAGGGTA and GGTTCTTCAGCCTCAATCTC for *uas;mespba*.

Development • Supplementary information

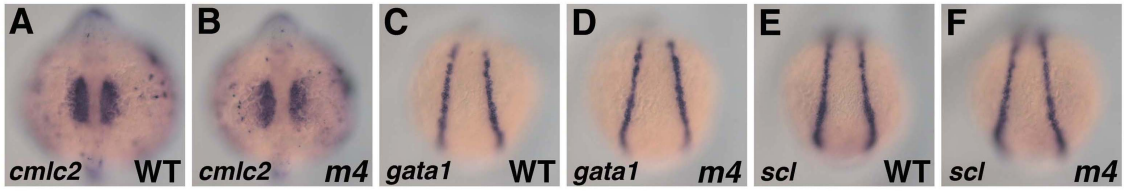
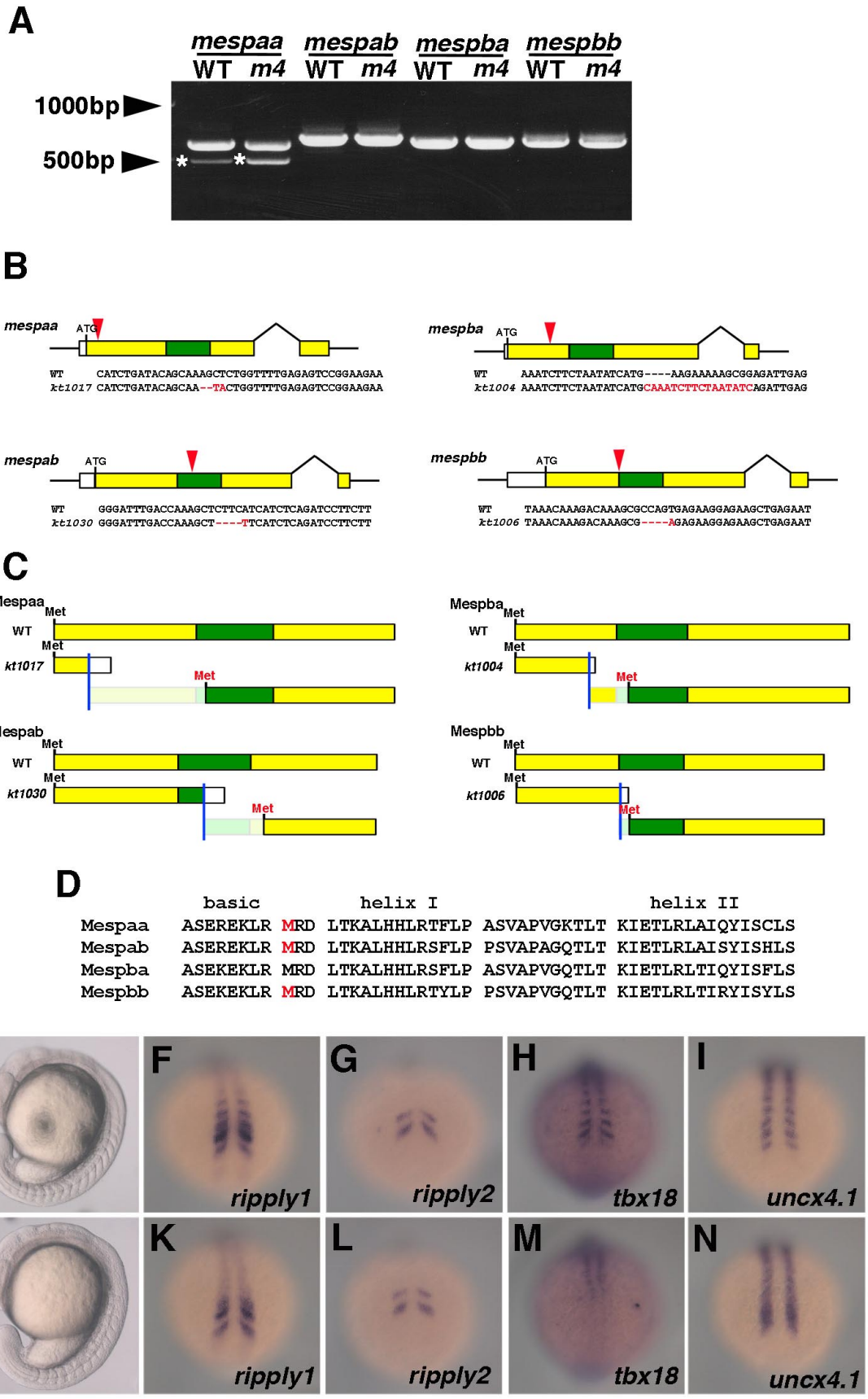


Fig. S1 Normal development of cardiac mesoderm and haematopoietic cells in the *mesp* quadruple mutant.

(A, B) Expression of *cmlc2* was not affected by mutations of the 4 mesp genes (100%; n=15). Embryos were fixed at the 17-somite stage. (C, D) Expression of *gata1* was not affected by mutations of the 4 *mesp* genes (100%; n=17). Embryos were fixed at the 11-somite stage. (E, F) Expression of *scl* was not affected by mutations of the 4 *mesp* genes (100%; n=15). Embryos were fixed at the11-somite stage.

Development • Supplementary information



Development • Supplementary information

Fig. S2 Complete lack of Mesp function in the *mesp* quadruple mutant

(A) RT-PCR products of *mesp* genes from the wild type and the *mesp* quadruple mutant embryo. Electrophoresis of *mesps* cDNA amplified by RT-PCR. The total RNA was isolated from wild type or *mesp* quadruple mutant embryos at the 8-somite stage. After reaction with reverse transcriptase, cDNA fragment containing the whole sequence of the coding region of th 4 *mesps* was amplified by PCR. All fragments were confirmed by direct sequencing and any unexpected splicing variant was not detected. Asterisks indicate non-specifically amplified PCR products, checked by direct sequencing.

(B-F) Generation of *mespab*^{kt1030} *and mespbb*^{kt1006} allele.

(B) Schematic diagrams showing mutations generated in the 4 *mesp* genes. Colored boxes indicate the coding regions; especially, green boxes show the basic helix-loop-helix domain. Red arrowheads indicate the approximate position of each mutation. Sequences around the mutation sites are also shown. Red characters in these sequences indicate mutated sequence. (C) Schematic diagrams of predicted protein structures produced from *mesp* mutant alleles shown in (B). Colored boxes indicate regions where amino acids in the same frame as the original could be translated and green boxes indicate the basic helix-loop-helix domain. White boxes indicate regions where different frames could be translated by frame-shift mutations. Blue lines indicate positions of the mutations. Possible coding frames containing the basic helix-loop-helix domain are also displayed. “Met” colored with red indicate presumptive positions of the translational initiation site. (D) Amino acids sequences of the basic helix-loop-helix domain of the 4 Mesp proteins. Red-colored M correspond to the red-colored Met shown in (C). Note that the presumptive translation products from mutant alleles of

Development • Supplementary information

mespaa, *mespab*, and *maspbb* could not contain the whole basic helix-loop-helix domain. (E,-N) Wild type (E, F, G,H,I) and another *mesp* quadruple mutant carrying distinct alleles from those mainly examined in this study (J,K,L,M,N). At the 16-somite stage, the morphology of *mespaa*$^{kt1017/kt1017}$; *mespba*$^{kt1004/kt1004}$; *mespab*$^{kt1030/kt1030}$; *mespbb*$^{kt1006/kt1006}$ was obviously identical to that of *mespaa*$^{kt1017/kt1017}$; *mespba*$^{kt1004/kt1004}$; *mespab*$^{kt1002/kt1002}$; *mespbb*$^{kt1009/kt1009}$, which were mainly examined in this study (E,F; See Figure 2). The expression *ripply1*, *ripply2*, *tbx18* and *uncx4.1* of *mespaa*$^{kt1017/kt1017}$; *mespba*$^{kt1004/kt1004}$; *mespab*$^{kt1030/kt1030}$; *mespbb*$^{kt1006/kt1006}$ was also identical those of *mespaa*$^{kt1017/kt1017}$; *mespba*$^{kt1004/kt1004}$; *mespab*$^{kt1002/kt1002}$; *mespbb*$^{kt1009/kt1009}$ at the 11-somite stage (F-I, K-N; See Figure 3 and 5)

Development • Supplementary information

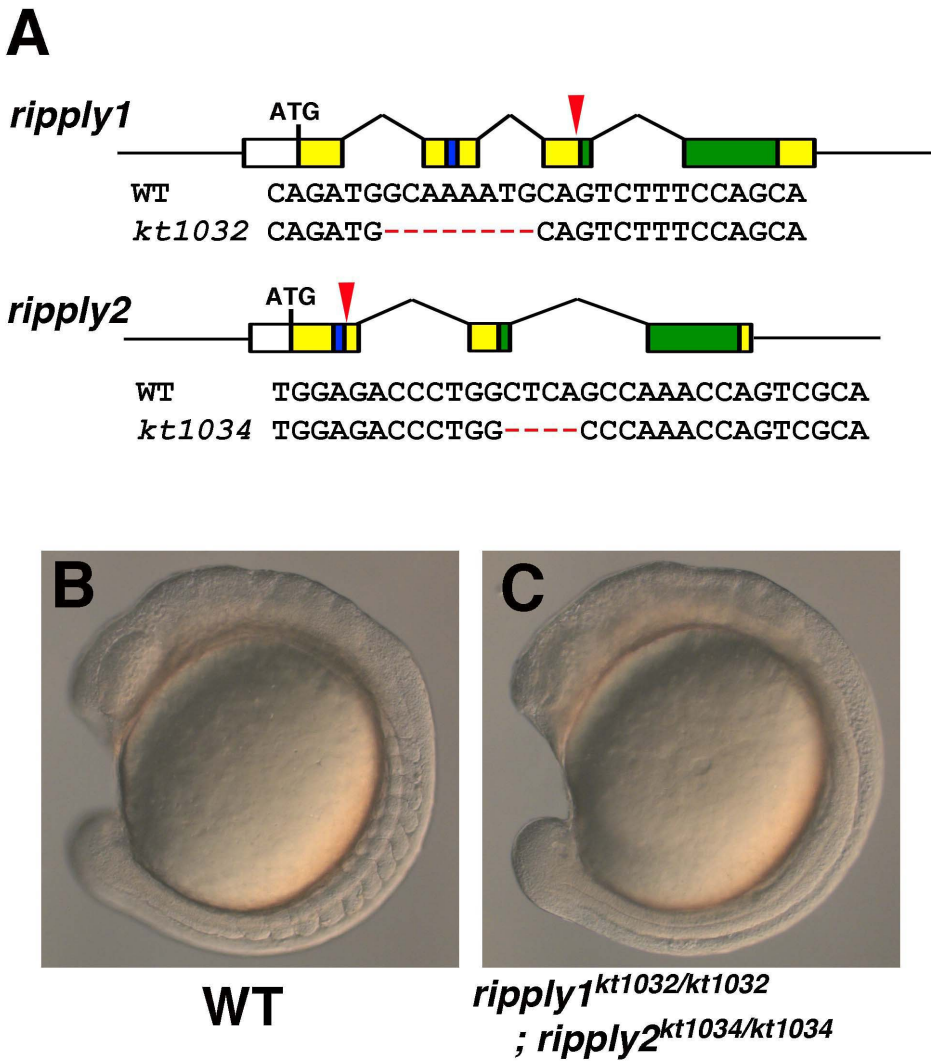


Fig. S3 Generation of *ripply1* and *ripply2* double mutant using TALENs

(A) Schematic diagram showing mutations of *ripply1* and *ripply2*. Colored boxes indicate the coding regions of Ripply1 and Ripply2 proteins; green boxes indicate the Ripply-homology domain, which is required for physical interaction with Tbx6, and blue boxes indicate the WRPW motif, which is essential for the interaction with Groucho. Red arrowheads indicate approximate positions of the mutations. The DNA sequences around the mutation sites are given below the schematic diagrams. Red bars indicate the mutated sequence. (B, C) The morphologies of *rippley1;ripply2* double mutant embryo at the 13-somites stage.

Development • Supplementary information

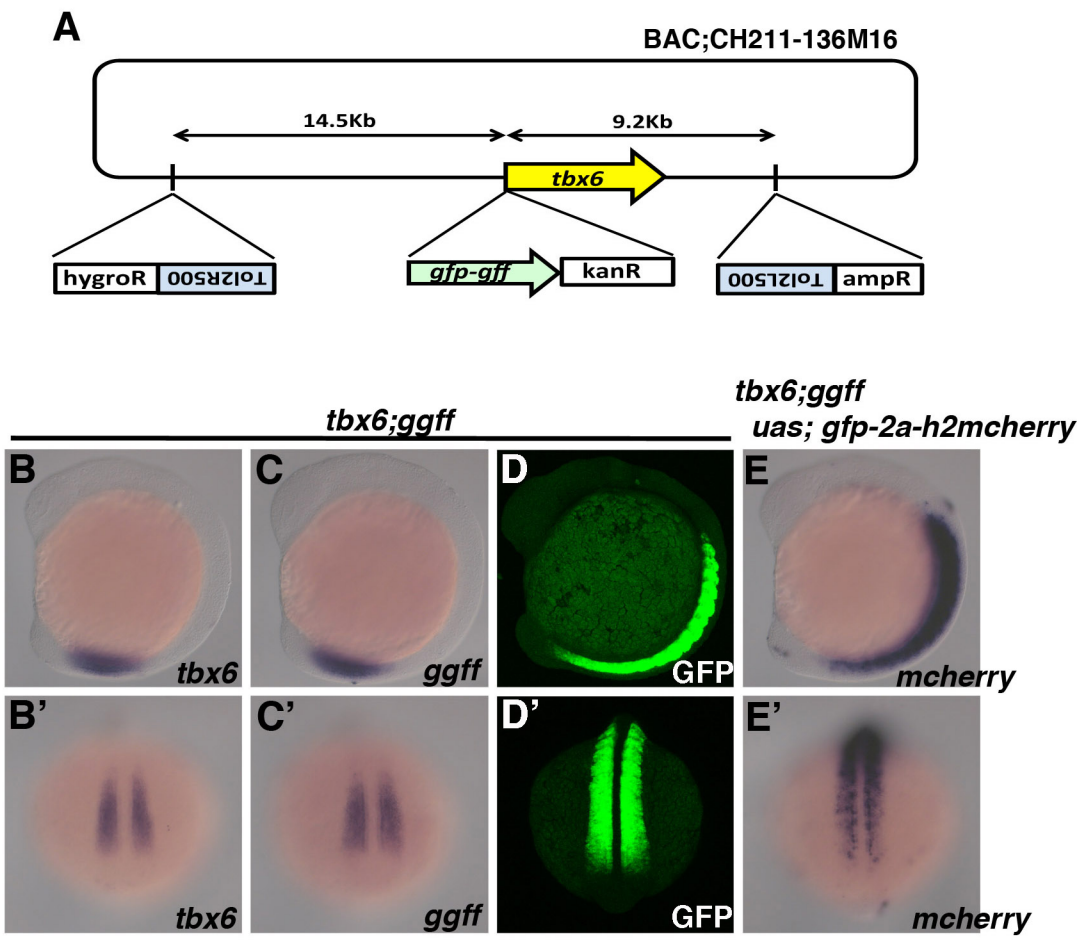


Fig. S4 Generation of *tbx6:ggff* transgenic fish

(A) Schematic diagram showing construction of the transgene. *tol2* and *ggff-pA-kan* cassette were introduced into CH211-136M16 by using homologous recombination in E.coli. (B,C) The expression pattern of *ggff* mRNA was almost identical to that of endogenous *tbx6* mRNA at the 11-somite stage. (D) The expression of GFP-GFF fusion protein (GGFF) was assessed by immunostaining using anti-GFP antibody. The expression GGFF was detected in the anterior PSM and mature somite region at the 11-somite stage. (E) *mcherry* expression in tbx6:ggff/uas:gfp-2a-h2a-mcherry double transgenic embryo at the 11-somite stage. Lateral and dorsal views of embryos are shown in B-E and B'-E', respectively.

Development • Supplementary information

Table S1. The list of the module of TALEN used for mutagenesis

	pCS2+TAL3-DD	pCS2+TAL3-RR
***mespaa*^{kt1017}**	HD NG NG HD HD NN NN NI HD NG HD NG HD NI NI	NI NI HD HD NI NN HD NI NG HD NG NN NI NG NI HD
***mespba*^{kt1004}**	NI NN NG HD NI NI NI NG HD NG NG HD NG NI NI NG NI	HD NG NG HD NI NN HD HD NG HD NI NI NG HD NG HD HD
***mespab*^{kt1002}**	HD HD NI NG HD NN NI NG NG HD HD NN NN NI NG NN HD NG NG NG	HD HD NI NN NG NG NN NG HD NG NG HD HD NI NN HD
***mespab*^{kt1030}**	HD HD NN NI NI NG NN NI NN NN NN NI NG NG NG NN NI HD HD	NN NN NI NN NN NG NI NI NN NI NI NN NN NI NG HD NG NN NI
***mespbb*^{kt1006}** ***mespbb*^{kt1009}**	HD HD NG NI NN NG NI NI NI HD NI NI NI NN NI HD NI	HD HD HD NG HD NI NG NG HD NG HD NI NN HD NG NG HD NG HD HD
***ripply1*^{kt1032}**	NN HD HD HD NN NN NG NI NI HD NI HD NG NI HD NI NI HD	NN NI HD NI NN NN NN NG NN HD NG NN NN NI NI NI NN NI HD
***ripply2*^{kt1034}**	HD NG NN NI NI NI NG NN NN NI HD NN HD NN NI NI NG HD NI	NN HD NN NI HD NG NN NN NG NG NG NN NN HD NG NN NI NN HD

Development • Supplementary information

Table S2. The list of the primers used for T7-endonuclease assay

	Forward primer	Reverse primer
***mespaa*^{kt1017}**	GCCTCCACGTTTTCTCTTCAGC	cCAGGAAACTTCGATTTGGGAC
***mespba*^{kt1004}**	AACCGATGGAGCAGTTCCAG	GTTTGTCCTACCGGAGCTAC
***mespab*^{kt1002}**	GACCATGGAGTTTAACCTTCCTCC	GGAGTTTCTCTCGTTCGCTTGCTG
***mespab*^{kt1030}**	GCTGGAAGACAACTGGAAGG	TGTAGCTAATGGCAAGACGG
***mespbb*^{kt1006}** ***mespbb*^{kt1009}**	ACTCCTGGAGCTCAGACTCC	GGTAGGTACGTCCTGAGGTG
***ripply1*^{kt1032}**	CATAAACACCGGACAGGAAGC	CAAACCAATTGCTCAAGCCAGAG
***ripply2*^{kt1034}**	CTCTTTCCACGGACACTATGG	GAAGATGGAGAGCTTGTGCTG

Development • Supplementary information

Table S3. The list of the primers used for cDNA cloning for mRNA probe synthesis.

	Forward primer	Reverse primer
cxcl12[7]	ggaattcTGATCGTAGTAGTCGCTCTG	ggctcgagTAACACGACAAACACGGAGC
smyhc	ggaattcACAACACACAGGACAACCC	ggctcgagCGAATCGGGAGGAGTTGTCA
s100t	ggaattcAACTCCGAGAATGCCTCCAC	cccggtaccGGGTTTGCGCCTCATGGAAC
pax7	ggaattcAGGAACAGTTCCTCGAATG	ggctcgagATGTCAGGGTAGTGTGTC

Development • Supplementary information