Supplementary Methods and Materials

RNA-seq

Before mapping, low quality (Q<20) reads and adaptor sequences were removed using Trimmomatic v0.32 (http://www.usadellab.org/cms/?page=trimmomatic). Clean data were mapped against the *X. laevis* v7.1 reference (downloaded from Xenbase) using TopHat v2.0.09 (ccb.jhu.edu/software/tophat). Only unique mapped reads were used in the subsequent analyses. BEDtools v2.16.2 was used to calculate the gene counts, and the Bioconductor edgeR package v3.8.5 was used to normalize the gene counts using the RPKM method. Then, the normalized gene counts were used to evaluate differential expression with the Bioconductor DEGseq package v1.20.0. The differentially expressed genes between two conditional samples were determined using the MARS (MA-plot-based method with random sampling model) method, with a fold change cutoff=1.0 and a p value cutoff=0.001 (signature called by DEGseq). Gene Ontology analysis of differentially expressed genes was performed using DAVID (Huang da et al., 2009). RNA-seq data can be found in Supplementary Table 4 (output scores) and Table 5 (depth of sequencing data).

Information for antibodies (see also supplementary Table S5 for additional information)

For Co-IPs: Anti-Flag-tag mAb-Magnetic Agarose (MBL #M185-10 10 µl/200 ug total protein), anti-HA (Roche #11867423001 0.5 µg/200 ug total protein), anti-Myc (Santa Cruz #sc-40 0.5 µg/200 ug/total protein).

For western blots: anti-Flag antibody (Sigma #F1804, 1:5000), anti-HA antibody (Roche #11867423001, 1:1000), anti-Myc-tag antibody (Santa Cruz #sc-40, 1:1000).

For chromatin immunoprecipitation, 1 µg relevant antibody (anti-H3K9ac, -H3K27ac, -H3K4me3, -CTD S5P) was used for embryonic lysate prepared from 50 embryos.

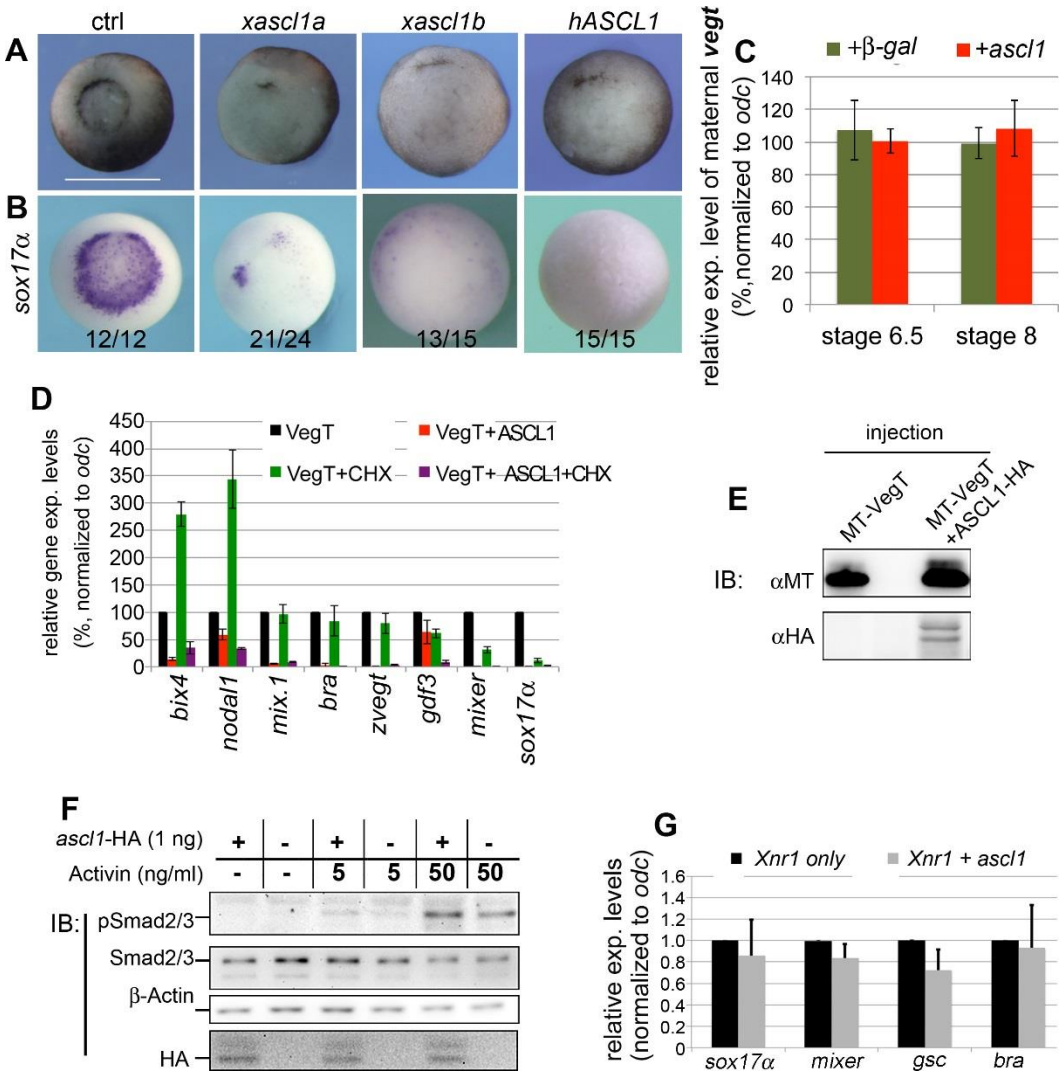

Fig. S1. (related to Fig. 1) Ectopic Ascl1 inhibits mesendoderm induction by VegT but not by Nodal/Activin

(A) Representative control and *ascl1* mRNA (500 pg)-injected embryos at stage 11 viewed from the vegetal pole. (B) Embryos at stage 10.5 *in situ* hybridized with *sox17α* and viewed from the vegetal pole. (C) *β-gal* or *ascl1* mRNA (500 pg) was injected into the vegetal pole at two-cell stage and the resultant embryos were collected at stages 6.5 and 8 for detecting maternal VegT mRNA by RT-qPCR. Values are mean ± s.d. (D) qPCR analysis for animal cap explants injected with indicated mRNAs and cultured in the presence of CHX (100 mM) from stage 8-10.5. (E) mRNA encoding Myc-tagged VegT (MT-VegT) was injected with or without Ascl1-HA at the two-cell stage, and the resultant embryos at stage 8 were analyzed for the expression of MT-VegT protein by western blotting. (F) Western blotting using anti-phospho-Smad2/3 (pSmad2/3) antibodies. Animal caps dissected from control or *ascl1*-injected (1 ng) blastulae were treated with 5-50 ng/ml Activin protein for 2 h followed by western blotting. (G) Animal caps isolated from blastulae injected with *Xnr1* (100 pg) alone or together with *ascl1* (500 pg) were analyzed by qPCR for mesendoderm gene expression. Values are mean ± s.d.

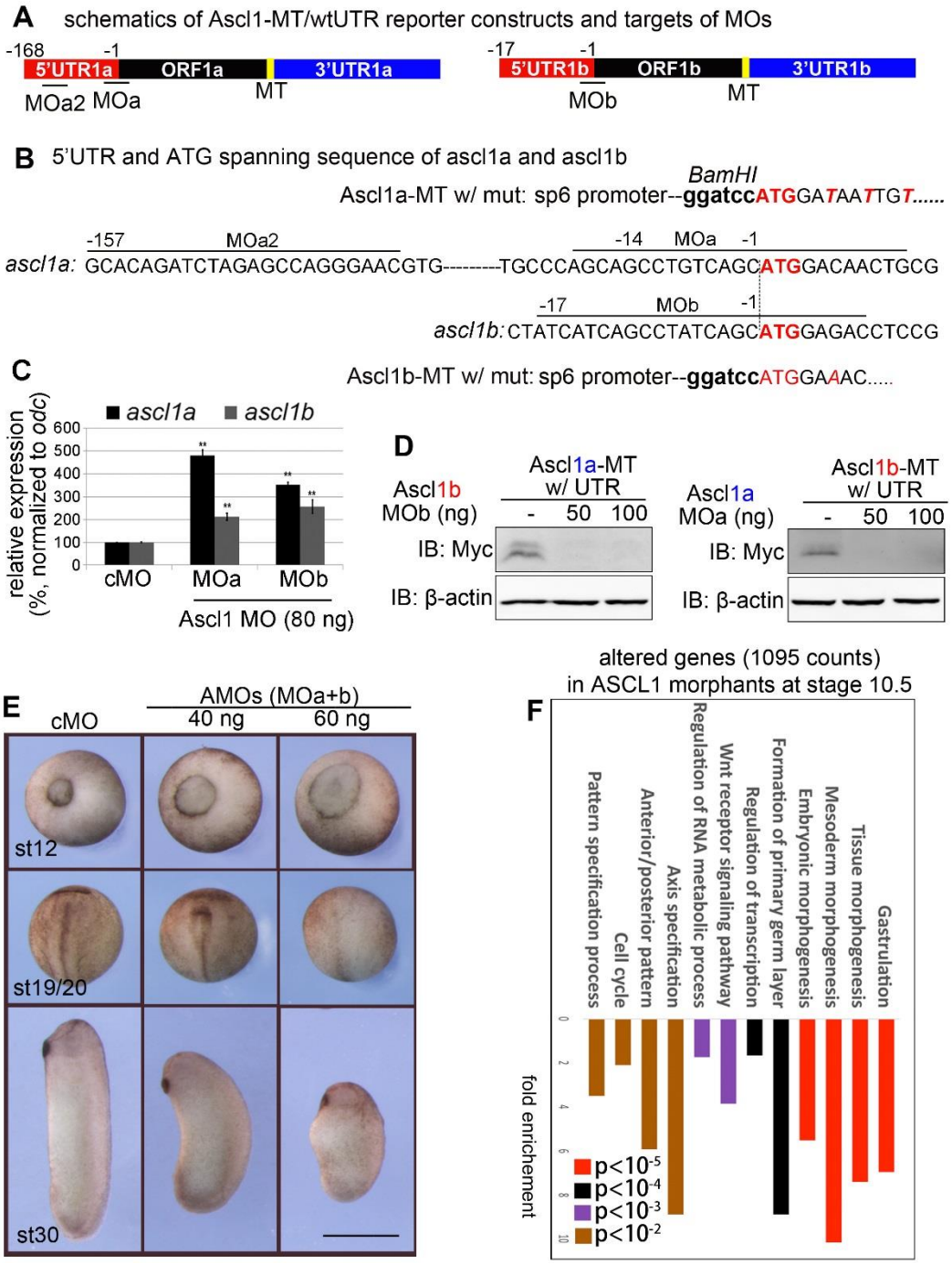

Fig. S2. (related to Fig.2) Ascl1 morpholinos and GO analysis for genes altered in Ascl1 morphants

(A) Schematic of the reporter constructs used to assess the translation blocking effects of the Ascl1 MOs: MOa, MOa2, and MOb. A Myc-tag (MT) is inserted in the C-terminus of Ascl1 polypetide. (B) cDNA sequences flanking the start codon (red characters) of two pseudoalleles of Ascl1. The MO-

recognizing sequences are indicated. (C) qPCR analysis of *ascl1a* and *1b* expression in Ascl1 morphants at stage 10.5. Values are mean ± s.d. ***P*<0.01. (D) Western blot analysis of MOa (MOb) inhibition of the translation of reporter Ascl1b (1a). (E) Representative control and Ascl1 morphants at different development stages. Incidence of blastopore closure delay: 97.8% (n=184) in Ascl1 morphants. All control morphants (cMO) closed blastopores by the end of gastrulation (n=168). The incidence body axis shortening with anteriorization of gut was near 100% (152/155) in Ascl1 morphants. The data were combined from 4 independent experiments. (F) Results from GO analysis for the genes (1095 counts) altered by Ascl1 depletion in embryos at stage 10.5.

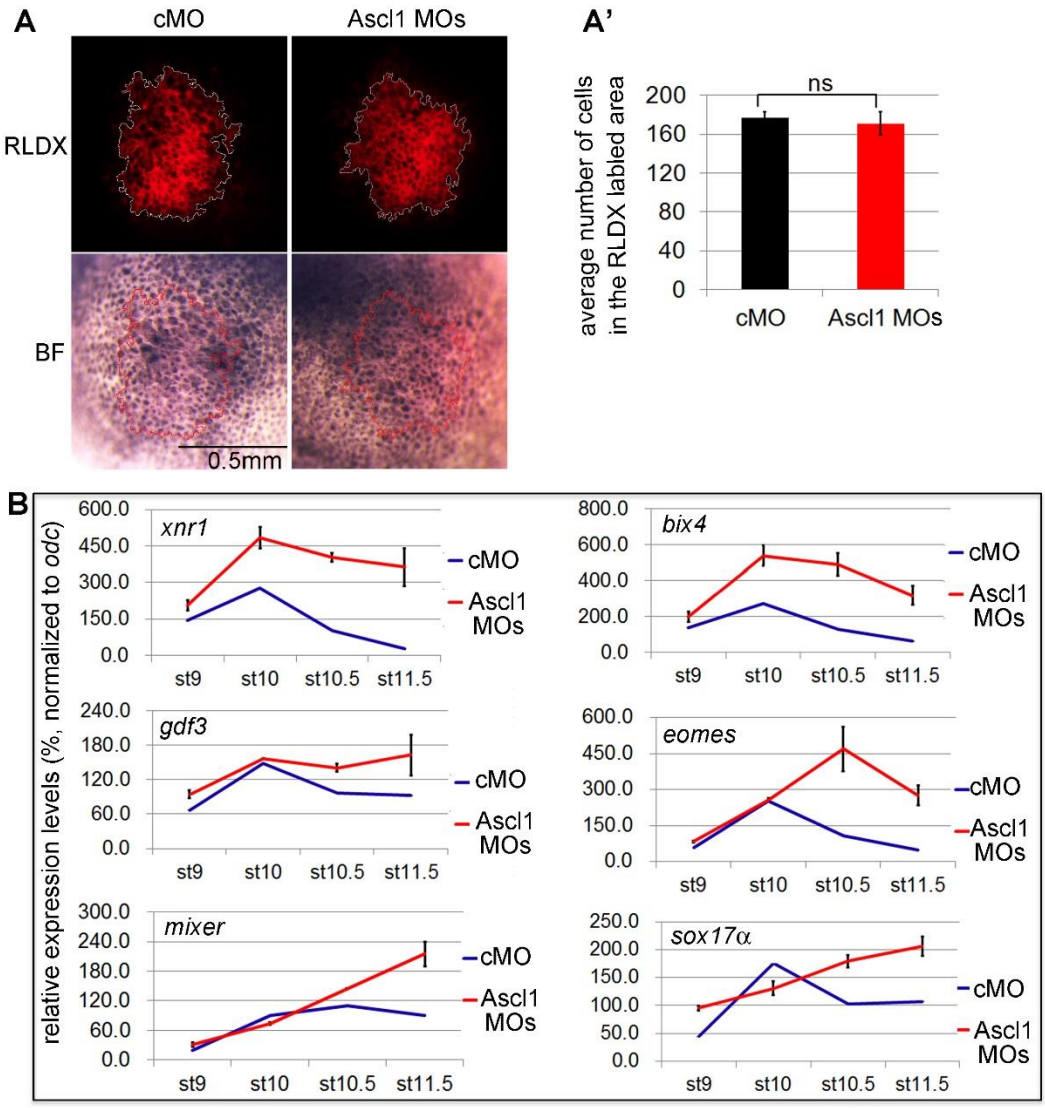

Fig. S3. (related to Fig. 2) Effects of Ascl1 depletion on cell division and gene expression

(A) 80 ng cMO or Ascl1 MOs injected at one-cell stage, RLDX injected into one animal-pole cell at 32-cs. Rhodamine channel (upper panels) or bright field (BR, lower panels) photographs taken at stage 10.5. **(A')** Comparison of cell numbers in the RLDX-labeled clones from 10 control or Ascl1 morphants at stage 10.5. n.s.: no significance by Student's *t*-test. **(B)** Gene expression in control and Ascl1 morphants during stage 9 to stage 11.3 analyzed through the semi-qPCR.

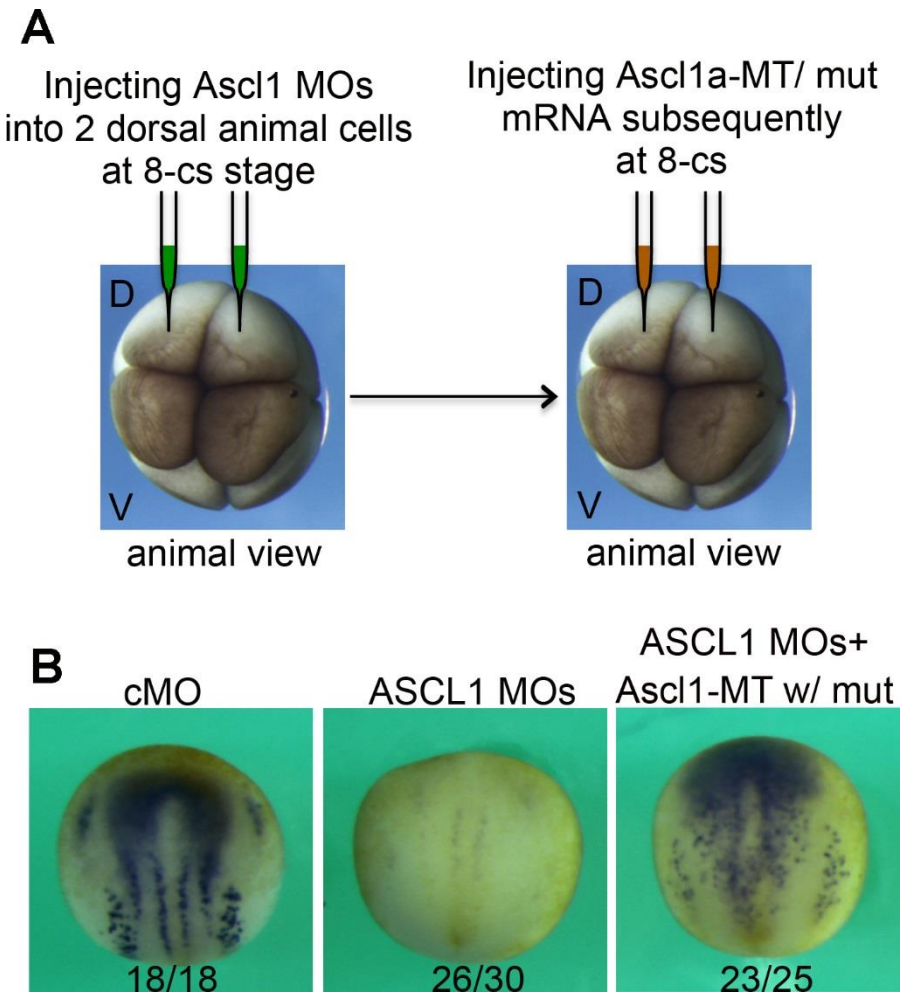

Fig. S4. (related to Fig. 4) Ascl1 is required for neuronal gene expression in early *Xenopus* embryos

(A). A schematic display of how Ascl1 MOs and Ascl1-MT/mut mRNA were sequentially injected into 2 dorsal animal cells at 8-cs. D: dorsal; V: ventral. (B) Representative embryos at stage 14/15 in situ hybridized with *tubb2b*, dorsal view, anterior to the up. Ascl1 MOs (MOa and MOb, 20 ng each) was injected into two dorsal animal cells at 8-cell stage. For the rescue attempt, synthetic Ascl1-MT/mut mRNA mRNA (30 pg) was injected into the same cells after injection of Ascl1 MOs.

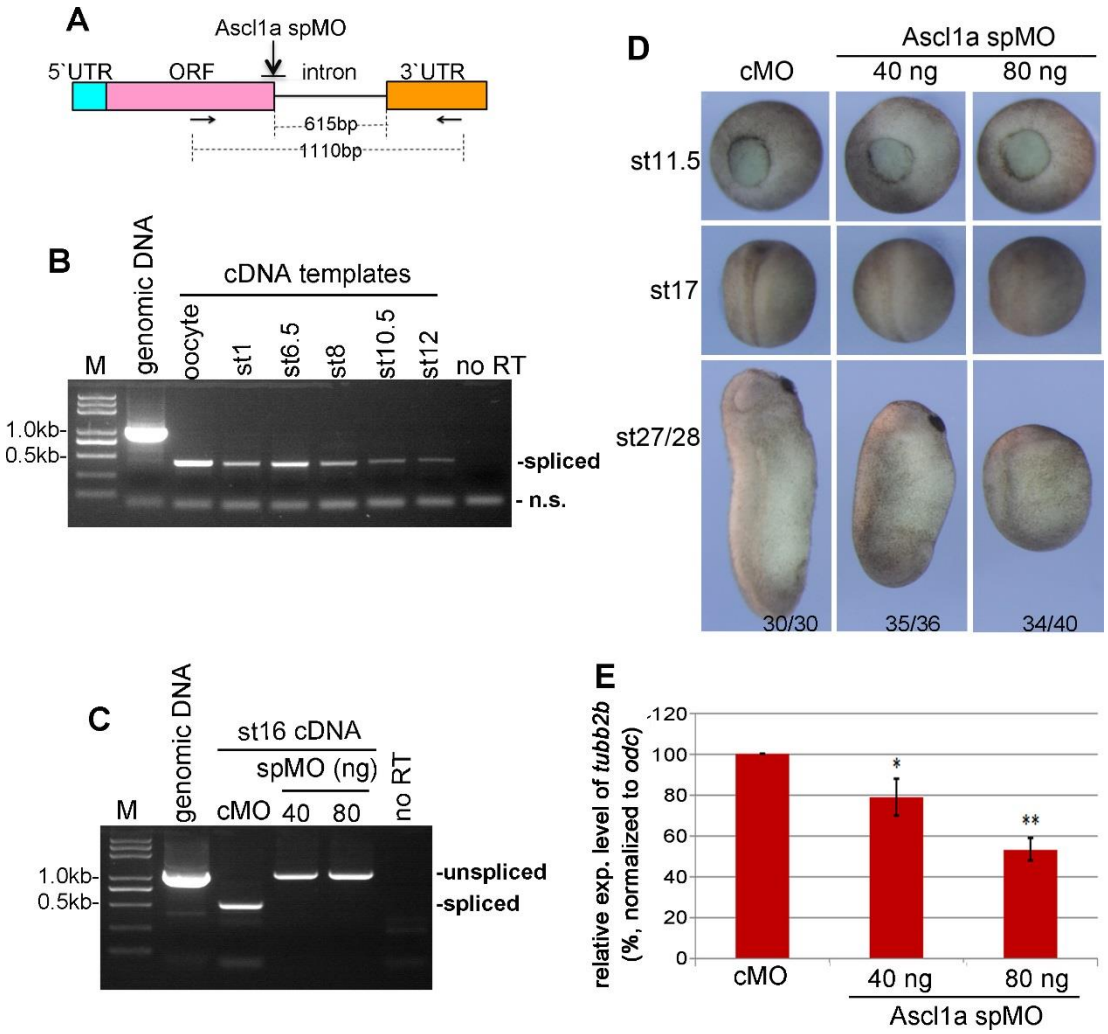

Fig. S5. (related to Fig. 4) Depleting the zygotic Ascl1a using a splice blocking MO (spMO)

(A). A schematic presentation of *ascl1a* gene containing a single intron of 615 bp long in the 3'UTR. The Ascl1a splice blocking MO (spMO) recognizes the donor sites. The unspliced mRNA is predicted to yield a PCR product of 1100 bp long. **(B)**. PCR verification of the primers designed for assessing the effects of Ascl1a spMO using genomic DNA template or cDNA templates made from oocytes and embryos at different developmental stages. **(C)**. RT-PCR results showing that injection Ascl1a spMO markedly increased the unspliced mRNA in stage 16 embryos and therefore the PCR products of 1100bp long. **(D)**. cMO and Ascl1a spMO injected embryos at different stages of development. **(E)**. qPCR detection of *tubb2b* expression in Ascl1a morphants at stage 18. Values are mean ± s.d. **P*<0.05.

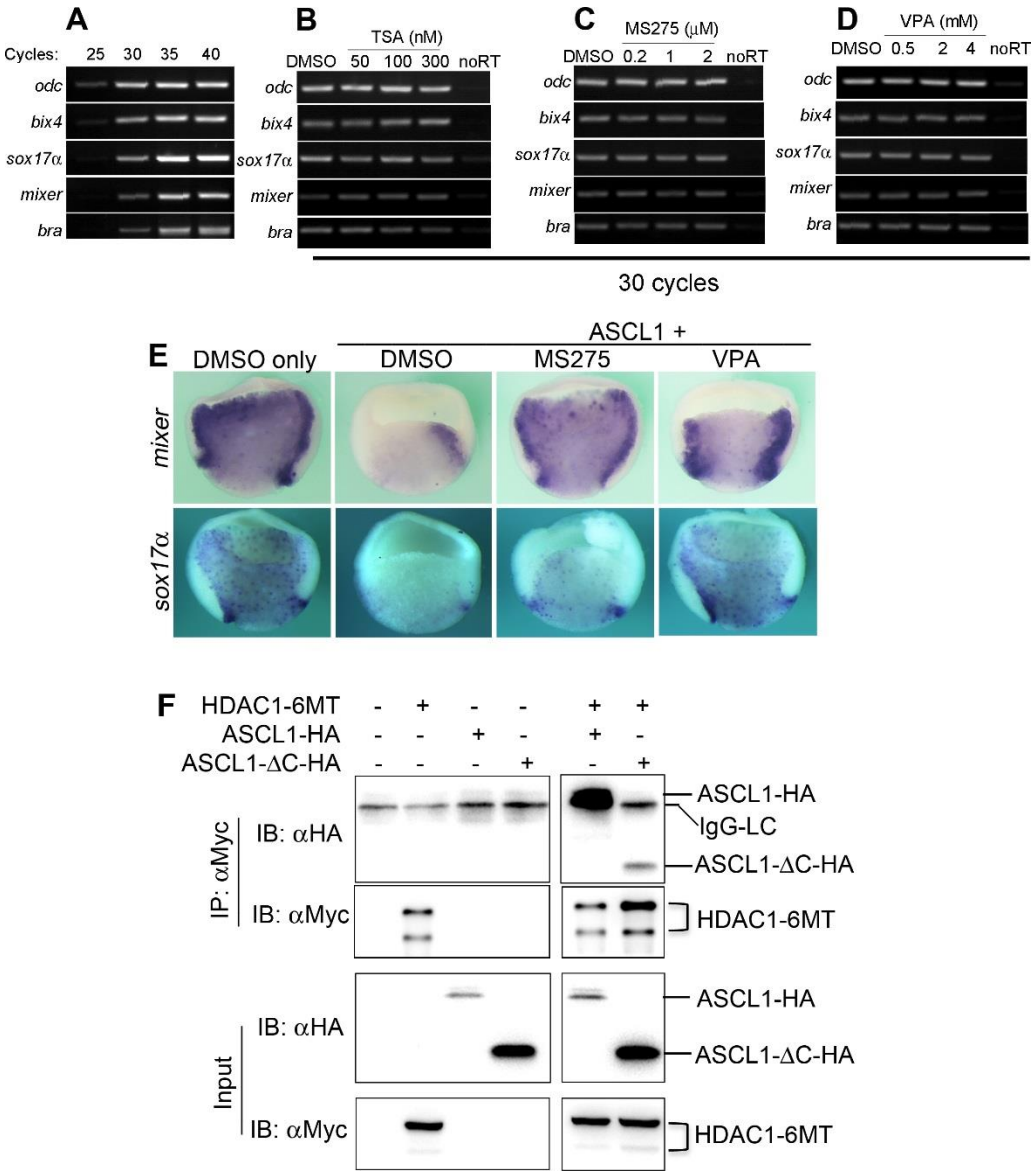

Fig. S6. (related to Fig. 6) Ascl1 interacts with HDAC1

(A). Optimization of the PCR cycle number for mesendoderm genes. (B-D) RT-PCR results showing the effects of HDACis at the indicated doses on mesendoderm gene expression. (E) Bisection view of embryos showing that the expression of *mixer* and *sox17α* was inhibited by Ascl1 overexpression and rescued by the application of MS-275 (1 μM) or VP (2 mM). (F) HDAC1-6MT and HA-ASCL1 mutants were used to confirm the association between Ascl1 and HDAC1 as detected under experimental conditions and shown in Fig. 6D.

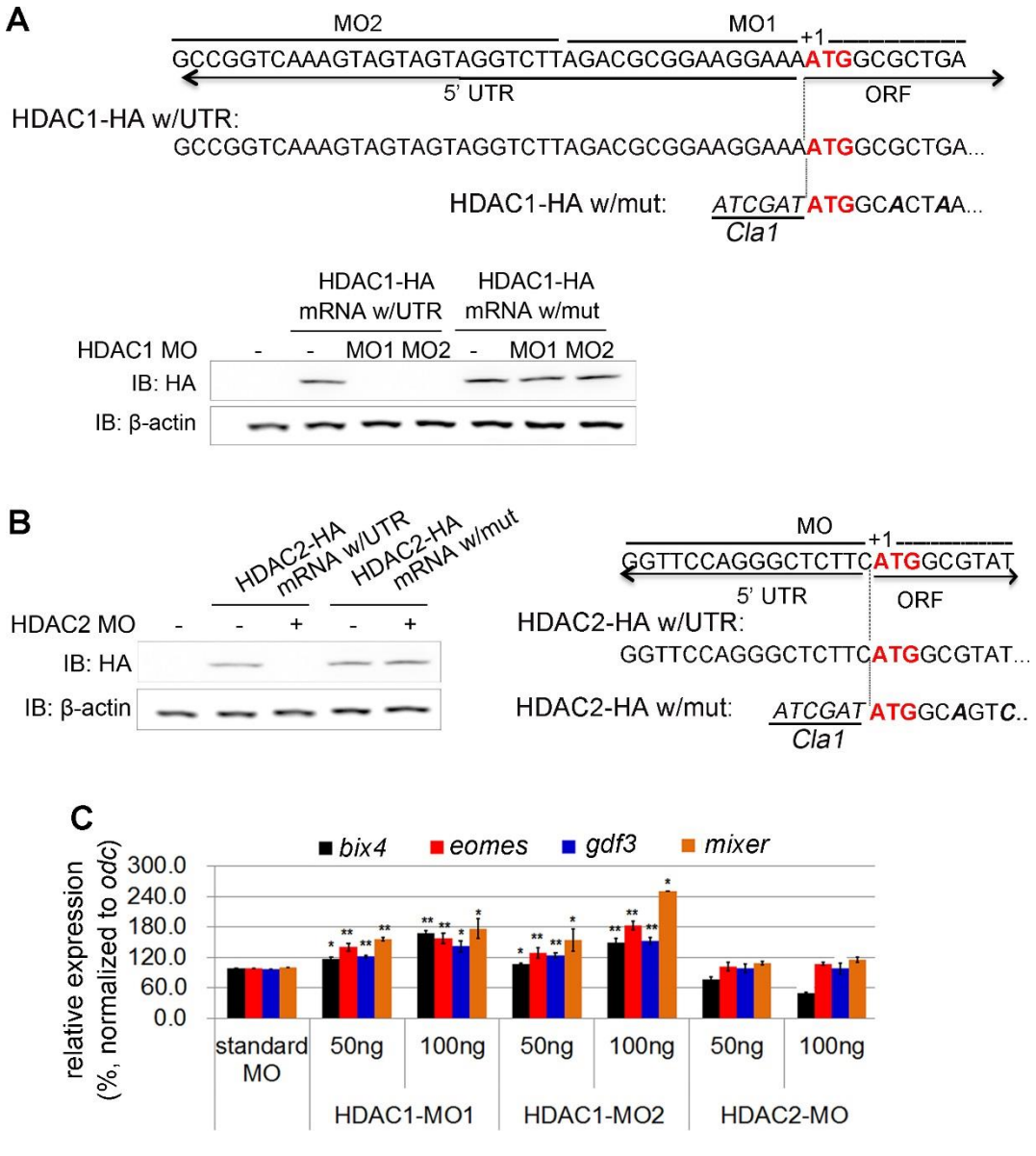

Fig. S7. (related to Fig. 6) Depleting HDAC1 using two non-overlapping MOs

(A). Sequence information for the 5'UTR proximal to the ATG and the sequences recognized by two non-overlapping HDAC1 MOs (HDAC1 MO1 and MO2), and the western blots showing HDAC1 MO1 and MO2 blocked the translation of the reporter mRNA with wild type 5'UTR, but not the reporter with a mutated 5'UTR. (B) Information related to HDAC2 MO and its translation-blocking efficacy assessed through a reporter assay. (C) qPCR detection of gene expression of HDAC1 or HDAC2 morphants at stage 10.5. Values are mean ± s.d. * indicates $p<0.05$; ** indicates $p<0.01$ by Student's *t*-test.

Supplementary Table S1: Asc1a gene sequence

Gene	NCBI ID
Ascl1a (Xenopus laevis achaete-scute family bHLH transcription factor 1)	**NM_001085778.1**

CAGCATTGAGCGCACAGAGCTAGAGCCAGGGAACGTGCGGGGCCGCTCCTGT
CACTTTGCAACAGCAGCATCATCCTCCTCCTCACCCGCACAGCCAGAGCAACA
CGCGAACGGCACGAGCCCCACTGCCTCCTCAGCTCACCTCCTCCTGCCCAGCA
GCCTGTCAGCATGGACAACTGCGTCGCTGCCAAGATCATGGACAGCAACTTAT
CCAGCCAGCAGCAGCATTTCCTACAGCCCCATTGCTTCTTCCCGCAGAACGTG
CAGCAGCTGAGCCCGGCGGAGGAGCAACAAGCGTCCAAAGCCAAGCCCATCA
AGAGGCAACGCTCGGCCTCCCCGGAGCTGATGAGGTGCAAGAGGCGGCTGAA
CTTCAATGGCTTCGGCTACAGCCTCCCGCAGCAGCAACCGGCCGCCGTGGCC
CGGAGGAACGAGAGGGAAAGGAACCGGGTCAAGCTGGTCAATCTCGGCTTTG
CCACCCTCAGGGAGCACGTCCCCAACGGTGCGGCCAACAAGAAGATGAGCAA
AGTGGAGACGCTGCGATCGGCAGTCGAGTACATCAGGGCGCTGCAACAGCTG
CTGGACGAACATGACGCTGTCAGCGCCGCTTTCCAGTCCGGGGTTCTTTCCCC
CACCATCTCCCCCAACTATTCCCACGATATGAACTCTATGGCGGGCTCCCCCGT
CTCCTCGTACTCCTCAGATGAAGGCTCCTATGATCCGCTGAGCCCGGAGGAGC
AAGAACTTCTCGACTTCACCACTTGGTTCTGAGATGCGGAGTAGCAGGTGAGTT
GCCTCCATCCCTGCTTCTTGTCCATCCTCCGGACCTGCTGCTGGACCACTCTGG
GAACCTCCCCCCCCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCCTACTTTCCTAAGCACTAGGTGCCCTTCTGTCATTGCTTTCCATTTCCA
GAGACCTGTCAGCCTCCACTTGCCAGGCATAGTGGCAGTTGTAGCAAGTGGCA
GTTGTAGTAACAACAACAGTAGGAGGGCTTGCAAGGGTGGCTGTCCCTGAATTA
GAATGGATCTGTTGCTTATGGGACTTTTACAACTTCCTTAGCAGTTGAGTTCCATT
CTCCAAGCTTTAAAATGGGACACATTTGGGATTTGAAGACCATTCTGAGTAGTTC
TGTAGCCATCTCTAGTTCTTGTGATAGCCTTTTGCACAATCATACTATTCCTTTGCT
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AATTTCTGCAGATGGTAATACAGGGAAAATAGTGTCTGTGGTGGGGATTGCCTAA
AACATATGTGATGTCAACAAGTCCCTTCTGTATACTGATTGTTTGTATTGTTCTTTC
AGGCTGTTTGCCTCCCTGCTTGGATAACAAAGTGAAGCACACAGGAGCAGTTG
GTCCCTCCAAGAACACTGAAGCACTACATTTGCACTCCAGTACGATTCACTGGA
TGGATAGAGACATAATGCCGTGGGGCTCGTGGGTGGTGAAGCACACAGACACC
ATCCCACTCACGCATGGAAGAGACGTGGTCCGTATGAACAACCCGGGGCTACC
CCATATAAAACCAAGCCCTTATTGACTATACCCTCCTTCTTTTATCGATGGAGGC
CGTTCCCGGATCAGAATTTGTGGCGTGTTTATCCAAACTATGCTGTTATTCAAAG
AAACCCCCCTCCCCTTCAGACTCTACCCCCTTTTTTTTTTTTGTACCACTTTCATC
CTTAAATGCTTCCAATCTTTTGTGAAATTTTTTTTTATTATAAGAAATATTTCTATC
TATCCCAACTGTTTTGGGATATATTAAGCTATTTTTGTACATAAGAGAGAAATTTA
TAGAAGTTTTGTACAAATGGTATGAAATATGTATATCTTGATACATTATTATGTAA
TGCTTATTACCTCTGCATATTTAGACTTGTAGTCTATAACCTTGCAACTGCAATTT
TGTATGTGGTTTTGTAAAGGACACTCCTTTTAAGGTGGGGTTGGAATTCCTACA
GATGTACTTTAGCACCAACGTGTCTTACTTTATAGAAACTTTGTTAATGCATTAAT
TGTGTTATTAAACAATGTTCACAGTGAACAAAGTTTATGCAGCTATTGTCCAAAC

GCAAAATAGTGGTCCACTGTATCTATGCTGCTTTCGGAACTCTATATACCACTG CCTTTTCTTACTGTTTTTAATACAAACTTACAAATGTCCTGCATAACTATGTTTTTT TACACAATAGTTTCATAATAAAACATTTTTATATAG (The intronic sequence highlighted with backgreen)

Supplementary Table S2: Asc1b gene sequence

Gene	**NCBI ID**
Ascl1b (Xenopus laevis MGC83023 protein)	**NM_001092525.1**
GTGCGGGGCCGCTCCTGTCACTTTGCAACAGCAGCAACTCCCCGTCACCCGCA CAGTCAGAGCAACACGCGAACGGCACGAGCCCCACTGGCACCCCAGCAGCAG CCGCCTATCATCAGCCTATCAGCATGGAGACCTCCGCTGCCAACCTGTCCAGCC AACAGCATTTCCTGCAGCCTCCTTGTTTCTTCCCGCACAACGTGCGGCTGAGCC CGGCGGAGGAGCAAGAAGCGGTCAAGCCGAAGCCCATCAAGAGGCAGCGCTC GAGTTCCCCGGAGCTGATGAGGTGCAAGAGGCGACTGAACTTCAATGGCTTCG GCTACAGCCTCCCGCAGCAGCAACCGGCCGCCGTGGCCCGGAGGAACGAGAG GGAGAGGAACCGAGTGAAGCTGGTCAATCTCGGCTTTGCCACCCTGAGAGAGC ACGTCCCCAACGGTGCGGCCAACAAGAAGATGAGCAAAGTGGAGACGCTGCGA TCGGCCGTCGAGTACATCAGGGCGCTGCAACAGCTGCTGGACGAACATGACGC GGTCAGCGCCGCTTTCCAGTCCGGCGTCTTTTCCCCCAACATCTCCCCTAACTA TTCTCATGGTATGAACTCTATGGCGGGGTCTCCCGTCTCCTCGTACTCCTCAGAT GAAGGCTCCTATGATCCGCTCAGCCCTGAGGAGCAAGAGCTGCTCGACTTCAC CACTTGGTTCTGAGACACTGAGAAGCAGGCTGTTTGCCTCCCTGCTTGGATAAC AAAGTGAAGCGCACAGGAGCAGTCGGTCCCTCCAAGAACACTGAAGCACTACA TTTGCACTCCAGTAGGATTCACTGGACGGATAGAGACATAATGCAGTGGGGGTC GTGGGTGGTGCAGCACACATACACCATCCCAGCTCACACGCGGAAGAGACGTG GTCCATATGAACAACCCGGGGCTACCCCGAATAAAACGCAAGCCCTTATTGACT GTATGCTCCTTTTTTTATCTATGGAGACGGTTCCCGGATCAGAATTTGTCTATCCA AACAATGCTGATATTCAAAGAAACCCCCTCCCCTTCAAGACTCTACCCCCTATTTT TTTGTACCACTTTCACCTTCAAATGCTTCCAAGCTTTTGTGAATTTTTTTATTATAA GAATATATTTCTATCTATCCCAACTGTTTTGGGATATATTAAGCTATTTTTGTACATA AGAGAGAGAGATTTATAGAAGTTTTGTACAAATGGTATAAAATGTGTATATCTTGAT ACATTATTATGTAATGCTTATTACCTCTGCATATTTAGACTTGTAGTCTATAACCTTG CAACTGCCATTTTGTATGTGGTTTTGTAAAGGACACTCCTTTTAAGGTGGGGTTG GAATTCCCACAGATGTACTTTAGCACCAACGTGTCTTACTTTATAGAAACTTTGTT AATGTATTAATTGTGTTATTAAACAATTGTTCACAGTGAACAAAGTTTATGCAGCTA TTGTCCAAACCTAAAATAGTGGTCCACTGTATCTATGCTGCCTTTCAGAACACTAT ATACCACTGCCTTTTCTTACTGTTTTTAATACAAACTTACAAATGTCCTGCATAACT GTTTTTTTACACAATAGTTTCATAATAAAACATTTTTTATAGAAAAAACAAAAAAAAA AAAAAA

Tables S3 and S4: RNA-seq data (S3: output scores; S4: the depth of RNA-seq data).

Click here to Download Table S3 and S4

Supplementary Table S5: antibodies used in this study

Antibody	**Supplier**	**Cat.#**
Anti-HA	Roche	11867423001
Anti-Myc	Santa Cruz	sc-40
Anti-Flag	MBL	M185-3L
	MBL	M185-10
Anti-H3K9ac	millipore	06-942
Anti-H3K27ac	millipore	07-360
Anti-H3K4me3	millipore	07-473
Anti-pol II S5P	Active motif	39233
Anti-phospho-smad2 (Ser465/467)	Cell signaling	3101
Anti-smad2	BD Transduction Laboratories	610843
Anti-phospho-smad1 (ser463/465)	Cell signaling	9511
Anti-smad1	Cell signaling	9743
Anti-phospho-Erk1/2 (Thr-202/Tyr204)	Cell signaling	9101
Anti-Erk1/2 (p44/42 MAPK)	Cell signaling	9102

Supplementary Table S6: oligos and primers used in this study

Antisense morpholino oligonucleotides		
Items	**Sequences**	**Notes/Reference**
Ascl1a MOa	5'CAGTTGTCCATGCTGACAGGCTGCT 3'	Gene Tools/ New
Ascl1a Moa2	5'GTTCCCTGGCTCTAGCTCTGTGC3'	Gene Tools/ New
Ascl1a 5mm cMO	5' CACTTCTCCATCCTCACACGCT 3'	Gene Tools/ New
Ascl1a spMO	5'GATGGAGGCAACTCACCTGCTACTC 3'	Gene Tools/ New
Ascl1b MOb	5'GTCTCCATGCTGATAGGCTGATGAT 3'	Gene Tools/ New
Ascl1b 5mm cMO	5'GTATCCATACTAATAGGCTAATAAT 3'	Gene Tools/ New
HDAC1 MO1	5' TCAGCGCCATTTTCCTTCCGCGTCT 3'	Gene Tools/ New
HDAC1 MO2	5' AAGACCTACTACTACTTTGACCGGC 3'	Gene Tools/ New
HDAC2 MO	5' ATACGCCATGAAGAGCCCTGGAACC 3'	Gene Tools/ New
VegT MO	5'CCCGACAGCAGTTTCTCATTCCAGC 3'	Gene Tools /Heasman et al., 2002
RT-PCR primers		
Items	**Sequences**	**Notes/Reference**
ascl1-f	5'GAGCTGATGAGGTGCAAGAG3'	New
ascl1-r	5'TTTGCTCATCTTCTTGTTGG 3'	
bix4-f	5' CTGCGCCTGAACAATAGAAA 3'	New
bix4-r	5' CAGGTAGCTAAAATCTTCAC 3'	
bra-f	5' TTCTGAAGGTGAGCATGTCG 3'	Sun et al.,1999
bra-r	5'GTTTGACTTTGCTAAAAGAGACAGG 3'	
chrd-f	5'AACTGCCAGGACTGGATGGT 3'	XMMR
chrd-r	5' GGCAGGATTTAGAGTTGCTTC 3'	
eomes-f	5' CCTCCACAGAGGTGGTTTGT 3'	New
eomes-r	5' GCTGCATCTGGGTAGTAGGC 3'	
gata4-f	5' AGTGCTACTGCTGCTACCTC 3'	Xathos and Janet,2001
gata4-r	5' ACTGTAGGAGACCTCTCTGC 3'	
gata5-f	5' ACCTTCAGAGCTGCGACACT 3'	Xathos and Janet,2001
gata5-r	5' CAGTGTATTGCCATACTGGTC 3'	
gdf3-f	5' TGGCAGAGTTGTGGCTATCA 3'	Xathos and Janet,2001
gdf3-r	5' CTATGGCTGCTATGGTTCCTT 3'	
gsc-f	5' TTCACCGATGAACAACTGGA 3'	New
gsc-r	5' TTCCACTTTTGGGCATTTTC 3'	
mix.1-f	5' GCAGATGCCAGTTCAGCCAATG 3'	Xathos and

mix.1-r	5’ TTTGTCCATAGGTTCCGCCCTG 3’	Janet,2001
mixer-f	5’ CACCAGCCCAGCACTTAACC 3’	Henry and Melton,1998
mixer-r	5’ CAATGTCACATCAACTGAAG 3’	
nodal 1-f	5’ AGAGGAATGTGGGTGCAGTT 3’	New
nodal 1-r	5’ CAACAAAGCCAAGGCATAAC 3’	
nodal 2-f	5’ TTACTGTATGAAGACGGAGAAGTTG 3’	New
nodal 2-r	5’ ATGCGACATGCCACAAAAC 3’	
odc-f	5’ GCCATTGTGAAGACTCTCTCCATTC 3’	Heasman et al., 2000
odc-r	5’ TTC GGG TGA TTC CTT GCC AC 3’	
pou5f3.3-f	5’ TGACGGAAATTGCCAAAGAAC 3’	New
pou5f3.3-r	5’ CCTTGGACATTCTGAATTTGCTC 3’	
sox17a-f	5’ GCAAGATGCTTGGCAAGTCG 3’	Xathos and Janet,2001
sox17a-r	5’ GCTGAAGTTCTCTAGACACA 3’	
vegt-f	5’ CAAGTAAATGTGAGAAACCGTG 3’	Zhang et al., 1998
vegt-r	5’ CAAATACACACACATTTCCCGA 3’	
ChIP-qPCR primers		
Items	**Sequences**	**Notes/Reference**
For VegT and Ascl1		
bix4-f	5’ GCCTCCAGCCTACTTCTACT 3’	New -148~-14 from TSS
bix4-r	5’ CCCTCCTTCTGGGGCTTTAT 3’	
sox17a-f	5’ GCCAATAGACACCTTTCTAG 3`	New -9801~-9538 from TSS
sox17a-r	5’ GAGAATGGGACTGTGTTAAC 3’	
mixer-f	5’ TGGTTCCATCCATGCGTTTC 3’	New -112 ~ +38 from TSS
mixer-r	5’ TCCCAAATCTTGTGGCTCCC 3’	
gsc-f	5’ CAGAGGACAATGGCATTAATCA 3’	New -239 ~ -55 from TSS
gsc-r	5’ AAGCACAGCAGCTCCACTCT 3’	
ef1α-f	5’ GTCTCGGCCCCTAAATATGA 3’	Blythe,2009
ef1α-r	5’ CAGCTCCCAGCTCTTTTGTC 3’	
For histone marks and Pol II S5P		
bix4-f	5’ TGGGAAGAGGCAGATTTGGA 3’	New +29 ~ +133 from TSS
bix4-r	5’ AGATGGTCACTGGAGGAGAA 3’	
mixer-f	5’ TGGTATAAGTAGGGACTCAGGT 3’	New -46 ~ +113 from TSS
mixer-r	5’ GAGAAGCAAGATGGGTAGAA 3’	
gsc-f	5’ CCCACCAAACCCTGTCCAAG 3’	New +102 ~ +190 from TSS
gsc-r	5’ ATGCTGAACATGCCAGAAGG 3’	
gdf3-f	5’ CAGAAGTTGCTGTAGGGAGA 3’	New -531 ~ -370 from TSS
gdf3-r	5’ GTAATAGTGCCAAGAGTGTC 3’	
bmp4-f	5’ CTGGGCAAGGGTGATTGTAT 3’	New

bmp5-r	5’ GGCGCTCAATAAATGAAGGT 3’	-605 ~ -432 from TSS
ef1α-f	5’ GTCTCGGCCCCTAAATATGA 3’	Blythe,2009
ef1α-r	5’ CAGCTCCCAGCTCTTTTGTC 3’	

Table S7: cDNA constructs used in this study.

cDNA cloning information, restriction enzymes for linearization and/or RNA polymerase for RNA probe labeling/in vitro transcription used in this study.

Click here to Download Table S7