Retinoic acid-driven Hox1 is required in the epidermis for forming the otic/atrial placodes during ascidian metamorphosis

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SUMMARY
Retinoic acid (RA)-mediated expression of the homeobox gene Hox1 is a hallmark of the chordate central nervous system (CNS). It has been suggested that the RA-Hox1 network also functions in the epidermal ectoderm of chordates. Here, we show that in the urochordate ascidian Ciona intestinalis, RA-Hox1 in the epidermal ectoderm is necessary for formation of the atrial siphon placode (ASP), a structure homologous to the vertebrate otic placode. Loss of Hox1 function resulted in loss of the ASP, which could be rescued by expressing Hox1 in the epidermis. As previous studies showed that RA directly upregulates Hox1 in the epidermis of Ciona larvae, we also examined the role of RA in ASP formation. We showed that abolishment of RA resulted in loss of the ASP, which could be rescued by forced expression of Hox1 in the epidermis. Our results suggest that RA-Hox1 in the epidermal ectoderm played a key role in the acquisition of the ASP-like structure during chordate evolution.

KEY WORDS: Hox1, Retinoic acid, Ascidian, Placode, Atrial siphon, Ciona intestinalis

INTRODUCTION
Hox1 plays a key role in anterior-posterior axis specification of the CNS in chordates (McGinnis and Krumlauf, 1992), and its expression is regulated by retinoic acid (RA) (Gavalas and Krumlauf, 2000). It has been suggested that this RA-Hox1 network functions in the general ectoderm of chordates (Holland, 2005), which gives rise to both the epidermis and the nervous system. Indeed, in extant cephalochordates and urochordates, RA-mediated Hox1 regulation is observed in the epidermis in addition to the CNS (Schubert et al., 2004; Ikuta et al., 2004; Kanda et al., 2009). In vertebrates, RA-Hox1 is necessary for formation of the otic placode (Hans and Westerfield, 2007; Makki and Capecchi, 2010), a chordate-specific structure in the cranial epidermal ectoderm (Shimeld and Holland, 2000). Because the RA-Hox1 network is crucial for specification of the CNS (Mark et al., 1993), which sends inductive signals to the overlying otic placode, the role of this network in otic placode development has generally been thought to be indirect. However, a recent study has indicated that mouse Hox1 is expressed in the otic epithelium (Makki and Capecchi, 2010). Thus, it is possible that the epidermal RA-Hox1 network contributed to the evolutionary innovation of the otic placode in higher chordates; however, the relationship between the epidermal RA-Hox1 network and the otic placode remains unclear.

Here, we report the epidermal functions of the RA-Hox1 cascade in the urochordate ascidian Ciona intestinalis. RA-Hox1-deficient animals do not form an atrial siphon placode (ASP), which is homologous to the vertebrate otic placode (Kourakis and Smith, 2007). Tissue-specific expression analysis of Hox1 showed that the RA-Hox1 cascade primarily functions in the epidermis to form the ASP. This study raises the possibility that RA-Hox1 in the epidermal ectoderm played a key role in the acquisition of the otic placode during chordate evolution.

MATERIALS AND METHODS
Transgenic lines
An enhancer detection line EJ[MiTSAdTPOG]124 was created using the jump-starter method (Sasakura et al., 2008). Tg[MiTSAdTPOG]8 (Awazu et al., 2007) was used as a transposon donor. The Minos insertion site of EJ[MiTSAdTPOG]124 was determined by thermal asymmetric interlaced (TAIL)-PCR (Liu et al., 1995). Two transgenic lines, Tg[MiTnigI]2 and Tg[MiTnigCiprgm]2, were used as muscle marker lines (Joly et al., 2007; Sasakura et al., 2008). Three transgenic lines, Tg[MiCepiI]3, Tg[MiCepiI]4 and Tg[MiCesA-CesA-CiEpiI]4, were used as marker lines for the ASP (Joly et al., 2007; Sasakura et al., 2009; Sasakura et al., 2010). The GFP fluorescent images were pseudocolored green.

Plasmids

**pRN3CiHox1**
The cDNA containing the full open reading frame (ORF) of Ci-Hox1 was amplified by RT-PCR with primers 5'–CGGATCCCATAGAATTCGTACATGAAATACC–3' and 5'–TTTCACGTGACTATATGTCATGTCC–3'. The amplified band was subcloned into the BglII and blunted EcoRI sites of pBS-RN3 (Lemaire et al., 1995) to create pRN3CiHox1.

**pSpeCFP-ter**
The ORF of eCFP was amplified by PCR with primers 5'–CTGAATCTTCTTGACGCTCGTCC–3' and 5'–ACCGGCGCATATGGTGACCAAGGGCGA–3'. The PCR fragment was inserted into the NotI and EcoRI sites of pBS-eGFP (Sasakura et al., 2003) to create pSpeCFP-ter.

**pSpeCFPCiHox1**
An EcoRI fragment of Ci-Hox1 cDNA was inserted into EcoRI site of pSPECFP-ter to create pSpeCFPCiHox1. A BamHI fragment of a cis element of Ci-CesA, Ci-ACK and Ci-β2TF were isolated from pCesA(–2080)-GFP

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(Sasakura et al., 2005), pSPCiAKRK (Hozumi et al., 2010) and pSPCiβ2TBK (Horie et al., 2011), respectively. These cis elements were subcloned into BamHI site of pSPeCFPCiHox1. A cis element of Ci-IF1 (Satou et al., 2001) was amplified from genomic DNA with primers 5'-TTTGGCGGCCGACATCTACAGCAAAGTTTCCAG-3’ and 5'-AAAAAGCCCACGCCATCTCTCTGAGTAGCAATGAC-3’, digested with NotI and subcloned into the NotI site of pSPeCFPCiHox1.

**Microinjection**

The sequence of the antisense morpholinol oligonucleotide (MO) for Ci-Hox1 is 5’-AACCTTACACCTTACGCTTTCG-3’. Ci-Hox1 mRNA was synthesized with Megascript T3 kit (Ambion), poly A tailing kit (Ambion) and cap structure analog (New England Biolabs) using pRN3CiHox1 as a template. The concentrations of MO, mRNA and lacZ, respectively. The sequence of the Raldh antisense MO is 5’-GTACTGTGATACGACTGAAGACAT-3’. Embryos were treated with U0126 at a concentration of 10 µM.

**Quantitative RT-PCR**

Total RNA was extracted from juveniles using the AGPC (acid guanidinium-phenol-chloroform) method (Chomczynski and Sacchi, 1987). Genomic DNA was digested with DNeasy (Takara Bio). Reverse transcription was performed with Superscript III reverse transcriptase (Invitrogen) and oligo(dT) primers. Quantitative reverse-transcription (RT)-PCR was carried out with SYBR Premix Ex Taq II (Takara Bio) and a Thermal Cycler Dice Real Time System TP800 (Takara Bio) following the manufacturer’s instructions. EF1α was used as a normalizer gene. The PCR primers for Ci-Hox1 were 5’-AAGCCAACTGTGTTACCACATG-3’ and 5’-ATGTGTTGGCGGATCTTGAAG-3’, and for EF1α they were 5’-CATGTACGGACAGCGAAACG-3’ and 5’-CAATGTGTTGGTAGCCATTCAA-3’.

**Whole-mount in situ hybridization**

Whole-mount in situ hybridization was carried out as described previously (Yoshida and Sasakura, 2012).

**RESULTS AND DISCUSSION**

**Loss of Ciona Hox1 function results in loss of the gill slit and body wall muscle, and disrupted atrial siphon muscle formation**

To investigate the role of the epidermal RA-Hox1 network in chordates, we examined the function of RA-Hox1 in urochordates, the closest evolutionary relatives of vertebrates (Delsuc et al., 2006). Previously, we used transposon-mediated enhancer trapping to create a green fluorescent protein (GFP)-enhancer trap reporter line, EJ[MITSAdBTOG]124, in the ascidian *Ciona intestinalis* (Sasakura et al., 2008; Ikuta et al., 2010). In these animals, GFP is expressed in the same pattern as endogenous *Ciona intestinalis* (Ci-) Hox1 (Fig.

![Fig. 1. GFP expression in EJ[MITSAdBTOG]124 enhancer detection line.](image)

**Fig. 1.** GFP expression in EJ[MITSAdBTOG]124 enhancer detection line. (A) Lateral view of a *Ciona intestinalis* larva (2 days post-fertilization) of EJ[MITSAdBTOG]124 enhancer detection line. Scale bar: 100 µm. (B) Lateral view of a EJ[MITSAdBTOG]124 juvenile. GFP is expressed in the posterior part of the endostyle (Es) and digestive tube, including the esophagus (Es0). Scale bar: 100 µm. (C) Ci-Hox1 expression at the juvenile stage. Ci-Hox1 is expressed in the posterior part of the esophagus and pancreatic part of the intestine including the esophagus. En, endoderm; Ep, epidermis; Nc, nerve cord.

**Fig. 2.** Ci-Hox1 is the affected gene in ngs mutants. (A) Insertion site of Minos in the EJ[MITSAdBTOG]124 transgenic line. Exons are indicated by boxes. Gray and white boxes correspond to the open reading frame and untranslated regions, respectively. (B, C) A wild-type heterozygous *Ciona intestinalis* juvenile (left) and an ngs mutant (right). Scale bar: 100 µm. (D) Genomic PCR of normal and ngs mutant juveniles. The scheme of the experiment is shown on the left. Bars represent chromosomes and PCR primers are shown by gray arrowheads. Transposon insertions are shown by black arrows. PCR bands were not amplified from the genome of homozygous animals, because long transposon insertion interfered with PCR amplification. Normal juveniles showed PCR bands, whereas ngs mutants showed no PCR bands. The lower column is the GAPDH loading control. (E) Quantitative RT-PCR of Ci-Hox1 transcripts. Experiments were performed in duplicate. In ngs mutants, the relative level of Ci-Hox1 mRNA was ~2-5% that of normal juveniles. (F, G) Juveniles injected with Ci-Hox1 antisense MO (left) or both Ci-Hox1 antisense MO and Ci-Hox1 mRNA (right). Gi, gill slit; BWM, body wall muscle.
1) (Ikuta et al., 2004) owing to a transposon insertion 192 bp upstream of the Ci-Hox1 transcriptional start site (Fig. 2A). To test whether the insertion disrupts function of the Ci-Hox1 promoter and generates a loss-of-function allele, we generated homozygous animals by crossing two heterozygous EJ[MiTSAdTPOG]124 animals (Fig. 2B-D). The homozygous animals showed notable phenotypes after metamorphosis, including loss of gill slits and body wall muscle (BWM) (Fig. 2B). The mutant was named no gill slit (ngs), after its gill-less phenotype. The ngs mutant phenotype showed the expected Mendelian frequency for a single recessive mutation (supplementary material Table S1), suggesting that a single gene underlies the observed phenotype.

To determine whether Ci-Hox1 is the gene for which function is abrogated in ngs mutants, we examined Ci-Hox1 expression levels in ngs mutant versus control animals by quantitative RT-PCR. ngs mutants showed a dramatic decrease in Ci-Hox1 expression (Fig. 2E). We also knocked down Ci-Hox1 function using an antisense MO that disrupts Ci-Hox1 splicing (supplementary material Fig. S1). Ci-Hox1-MO animals phenocopied ngs mutants (Fig. 2F) and could be rescued by co-injection of Ci-Hox1 mRNA (Fig. 2G). These results indicate that mutation of the Ci-Hox1 gene underlies the ngs phenotype, and that Ci-Hox1 is required for juvenile gill slit and BWM formation.

To determine whether Ci-Hox1 plays a role in the development of other muscle tissues, we knocked down Ci-Hox1 in transgenic lines expressing GFP in muscles (supplementary material Fig. S2A) (Joly et al., 2007; Sasakura et al., 2008). Formation of the atrial siphon muscle (ASM) was abnormal in these animals, whereas the oral siphon and heart muscles formed normally (supplementary material Fig. S2A,B, Table S2). Although GFP-positive muscle cells were present in the region of the presumptive ASM, they failed to form a ring-shaped, functional ASM muscle. Co-injection of Ci-Hox1 mRNA rescued the Ci-Hox1 MO phenotype (supplementary material Fig. S2C), demonstrating that Ci-Hox1 is required for proper formation of the ASM. Next, we examined whether the BWM and ASM are related to one another as previously suggested (Hirano and Nishida, 1997; Stolfi et al., 2010) by observing formation of the BWM by time-lapse imaging. We found that, indeed, the BWM formed as an extension of the ASM (supplementary material Movie 1). Thus, the absence of the BWM in Ci-Hox1-deficient juveniles is likely to be due to disruption of ASM formation, and a primary function of Ci-Hox1 is ASM formation. Because ASM and heart muscle originate from the same blastomeres (Hirano and Nishida, 1997; Stolfi et al., 2010), Ci-Hox1 should affect ASM formation after the two muscle cells separate.

**Ci-Hox1 is essential for formation of the epidermal structure homologous to the otic placode**

In ascidian juveniles, two atrial siphons are formed from the ASP, two thickenings of the larval epidermal ectoderm (Fig. 3A,B). The two atrial siphons then fuse at the midline to form one adult atrial siphon (Berrill, 1947). It has been suggested that the ascidian ASP is homologous to the vertebrate otic placode (Manni et al., 2004; Mazet and Shimeld, 2005; Mazet et al., 2005; Kourakis et al., 2010). In addition, a previous study showed that the ASP is also required for formation of the gill slit (Kourakis and Smith, 2007). To test whether ASP formation is affected in Ci-Hox1-deficient animals, we made use of epidermal GFP transgenic lines (Sasakura et al., 2010) in which the disc-like oral siphon primordium and the ASPs emit stronger GFP fluorescence than do the neighboring epidermal cells (Fig. 3C). In Ci-Hox1 knockdown animals, the ASP was lost, whereas formation of the oral siphon primordium was normal (Fig. 3D; supplementary material Table S3). This phenocopy could be rescued by co-injection of Ci-Hox1 mRNA (Fig. 3E; supplementary material Table S3), indicating that the Ci-Hox1 MO is specific. Taken together, our results demonstrate that Ci-Hox1 is essential for formation first of the ASP and then of the gill slit and ASM/BWM.

**Ci-Hox1 is expressed in several tissues at the larval stage, including the epidermal ectoderm, CNS and endoderm** (Ikuta et al., 2004). To determine which expression domain of Ci-Hox1 is

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**Fig. 3. Ci-Hox1 is essential for formation of the ASP.** (A) Lateral view of Ciona intestinalis larva with an ASP indicated by a dotted line. (B) Dorsal view of a juvenile. (C) An epidermal GFP transgenic line. (D) A larva injected with Ci-Hox1 antisense MO. No ASP was formed. (E) A larva simultaneously injected with Ci-Hox1 antisense MO and Ci-Hox1 mRNA. Two ASPs were formed. (F) A Ci-Hox1-MO-injected larva in which Ci-Hox1 is overexpressed in the epidermis. ASPs were formed. (G) A Ci-Hox1-MO-injected larva in which Ci-Hox1 was overexpressed in the CNS. ASPs were not formed. (H) A Ci-Hox1-MO-injected larva in which Ci-Hox1 was overexpressed in the mesenchyme. ASPs were not formed. (I) A Ci-Hox1-MO-injected larva in which Ci-Hox1 was overexpressed in the endoderm. ASPs were not formed. (J) A Raldh-MO-injected larva. ASPs were not formed. (K) A Raldh-MO-injected larva in which Ci-Hox1 was overexpressed in the epidermis. Two ASPs were formed. as, atrial siphon; osp, oral siphon primordium. Scale bars: 100 μm.
responsible for ASP formation, we generated tissue-specific Ci-
Hox1 expression constructs and tested their ability to rescue the Ci-
Hox1-MO ASP phenocopy. Strong rescue was observed when Ci-
Hox1 was expressed in the epidermal ectoderm (Fig. 3F; 
supplementary material Table S3). This result indicates that 
expression of Ci-Hox1 in the epidermal ectoderm is required 
for formation of the ASP. As two properly positioned ASPs were 
formed even though Ci-Hox1 was overexpressed throughout the 
embryo body, which was shown by rescue experiment of Ci-Hox1 
MO phenocopy with Ci-Hox1 mRNA, RA-driven Ci-Hox1 might 
render the epidermis competent to respond to the ASP-inducing 
signals. In contrast to the epidermis, expression of Ci-Hox1 in the 
CNS and mesenchyme failed to rescue the MO phenocopy (Fig. 
3G,H; supplementary material Table S3). A moderate rescue was 
observed when Ci-Hox1 was expressed in the endoderm (Fig. 3I; 
supplementary material Table S3), suggesting that Ci-Hox1 in the 
ednderm has a role in formation of the ASP.

Kourakis and Smith (Kourakis and Smith, 2007) previously 
suggested that FGF/MEK signaling after the early tailbud stage 
serves to induce the ASP. If Ci-Hox1 gives the epidermal ectoderm 
competence to respond to the inductive signal of the ASP, 
expression of Ci-Hox1 in the epidermis should be independent of 
the inductive signal. Ci-Hox1 expression is observed in the 
eddnem at the early tailbud stage, which is earlier than the 
induction of the ASP, suggesting that Ci-Hox1 expression is 
independent of the inductive signal. To address the independence 
between Ci-Hox1 in the epidermis and inductive signaling of ASP, 
we treated embryos with U0126 from the early tailbud stage, 
causiag loss of the ASP (supplementary material Table S4). 
Microinjection of Ci-Hox1 mRNA failed to overcome the effect 
(supplementary material Table S4), suggesting that inductive 
signaling of the ASP is not mediated by Ci-Hox1 in the epidermal 
ednderm.

Retinoic acid-driven epidermal expression of Hox1 is necessary for ASP formation

The expression of Ci-Hox1 in the epidermal ectoderm is directly 
upregulated by RA (Ishibashi et al., 2005; Kanda et al., 2009). 
To test the possibility that RA is involved in ASP formation, we 
disrupted RA synthesis by knocking down the gene encoding 
retinal dehydrogenase (Raldh), an RA synthesis enzyme 
(Niedereither et al., 1999), with an antisense MO. Raldh-MO 
larvae exhibited loss of the ASP (Fig. 3J; supplementary material 
Table S5), suggesting that RA is necessary for ASP formation. 
When Ci-Hox1 was overexpressed in the epidermis of Raldh-MO 
larvae, the phenotype was rescued and ASP formation was 
observed (Fig. 3K; supplementary material Table S5). These results 
indicate that RA functions in ASP formation via epidermal 
expression of Ci-Hox1.

Conclusions

We conclude that RA-driven Hox1 expression in the epidermal 
ednderm is essential for organizing the ASP/otic placode in the 
urochordate Ciona intestinalis (Fig. 4). In addition, this network 
functions directly in the ASP/otic placode to pattern it. Similarly, 
in amphioxus, RA functions in patterning of the epidermal sensory 
organ (Schubert et al., 2004), from which placodes are thought to 
originate (Holland and Holland, 2001). In vertebrates, otic placode 
formation depends on signals from a properly patterned CNS (Hans 
and Westerfield, 2007), and is therefore indirectly dependent on 
RA and Hox1. However, expression of Hox1 in the otic epithelium (Makki and Capeccchi, 2010) raises the possibility that 
the RA-Hox1 network might also function in the epidermal 
ednderm to form the otic placode in vertebrates. Furthermore, 
Hox1 expression in the epidermal ectoderm is observed in 
hemichordates (Aronowicz and Lowe, 2006). Thus, the role of RA-
Hox1 in specification of the epidermal sensory organ might have 
been inherited from the deuterostome ancestor of chordates and 
recruited for otic placode formation in the urochordate/vertebrate 
lineages. Because both the dorsal position of the CNS and the 
ednermally specialized placodes are hallmarks of chordates, the 
RA-Hox1 network appears to have played key roles in these 
evolutionary innovations crucial for acquiring the chordate body 
plan. Our study also suggests that Ci-Hox1 in the endoderm 
functions in ASP formation. In Ciona, the inductive signal for ASP 
formation is thought to be mediated by fibroblast growth factor 
(FGF) signaling (Kourakis and Smith, 2007). Although the source 
of the FGF signal has not been uncovered, the endoderm is a good 
candidate. Because Ci-Hox1 is probably the competence factor for 
ASP/otic placode formation, Ci-Hox1 might be upstream of the 
transcription factor genes expressed preferably in the placode, such 
as Fox1a, Pax2/5/8, eyes absent and Six1/2 (Mazet and Shimeld, 
2005). This issue also needs to be investigated for understanding 
the mechanisms underlying formation of the ASP/otic placode.

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Research Program [2009-801].

Competing interests statement

The authors declare no competing financial interests.


Table S1. Frequency of *ngs* mutants*

<table>
<thead>
<tr>
<th>Family ID</th>
<th>n</th>
<th>Number of normal juveniles</th>
<th>Percentage of <em>ngs</em> mutants</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>43 (72%)</td>
<td>16 (27%)</td>
<td>0.7&lt;P&lt;0.8</td>
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<tr>
<td>2</td>
<td>97</td>
<td>78 (80%)</td>
<td>19 (19%)</td>
<td>0.2&lt;P&lt;0.25</td>
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</tbody>
</table>

*Unhealthy juveniles whose phenotypes were difficult to judge were omitted from the count.*
Table S2. Effects of *Ci-Hox1* MO on the formation of juvenile muscle and gill slit

<table>
<thead>
<tr>
<th></th>
<th>Uninjected control</th>
<th><em>Ci-Hox1</em> MO</th>
<th><em>Ci-Hox1</em> MO + Ci-Hox1 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage oral siphon muscle formation (n)</td>
<td>100% (28)</td>
<td>95% (43)</td>
<td>100% (21)</td>
</tr>
<tr>
<td>Percentage atrial siphon muscle formation (n)</td>
<td>100% (44)</td>
<td>27% (48)</td>
<td>100% (25)</td>
</tr>
<tr>
<td>Percentage body wall muscle formation (n)</td>
<td>100% (44)</td>
<td>6% (48)</td>
<td>80% (25)</td>
</tr>
<tr>
<td>Percentage heart muscle formation (n)</td>
<td>100% (28)</td>
<td>97% (43)</td>
<td>100% (21)</td>
</tr>
<tr>
<td>Percentage gill slit formation (n)</td>
<td>100% (61)</td>
<td>17% (39)</td>
<td>86% (22)</td>
</tr>
</tbody>
</table>
Table S3. Effects of *Ci-Hox1* MO to the formation of atrial and oral siphon primordium

<table>
<thead>
<tr>
<th></th>
<th>Uninjected control</th>
<th><em>Ci-Hox1</em> MO</th>
<th><em>Ci-Hox1</em> MO + <em>Ci-Hox1</em> mRNA</th>
<th><em>Ci-Hox1</em> MO + <em>Ci-Hox1</em> in the epidermis</th>
<th><em>Ci-Hox1</em> MO + <em>Ci-Hox1</em> in the CNS</th>
<th><em>Ci-Hox1</em> MO + <em>Ci-Hox1</em> in the mesenchyme</th>
<th><em>Ci-Hox1</em> MO + <em>Ci-Hox1</em> in the endoderm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage atrial siphon primordium formation (<em>n</em>)</td>
<td>98% (97)</td>
<td>0% (28)</td>
<td>100% (23)</td>
<td>86% (53)</td>
<td>10% (20)</td>
<td>0% (20)</td>
<td>44% (29)</td>
</tr>
<tr>
<td>Percentage oral siphon primordium formation (<em>n</em>)</td>
<td>98% (90)</td>
<td>100% (28)</td>
<td>100% (23)</td>
<td>92% (13)</td>
<td>100% (20)</td>
<td>95% (20)</td>
<td>100% (29)</td>
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Table S4. The inductive signal of atrial siphon primordium is not mediated by *Ci-HoxI* expression

<table>
<thead>
<tr>
<th>Percentage atrial siphon primordium formation (n)</th>
<th>U0126 treatment*</th>
<th>U0126 + <em>Ci-HoxI</em> mRNA</th>
<th>DMSO treatment</th>
<th>DMSO + <em>Ci-HoxI</em> mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% (38)</td>
<td>0% (27)</td>
<td>100% (27)</td>
<td>100% (20)</td>
<td></td>
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</tbody>
</table>

*Embryos were treated with U0126 or DMSO from the early tailbud stage, and ASP formation was observed at the larval stage.*
Table S5. Effects of *Raldh* MO on the formation of atrial siphon primordium

<table>
<thead>
<tr>
<th>Percentage atrial siphon primordium formation (n)</th>
<th><em>Raldh</em> MO</th>
<th><em>Raldh</em> MO + <em>Ci-Hox1</em> in the epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4% (76)</td>
<td>50% (76)</td>
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