

Supplementary Table and Figures

Table S1 Primers used in this study

Name	Sequence 5'-3'	Target gene	Restriction site
AmphiCerF/R	F: <u>GGTACCATGAAGACGAGCGTGAGGAGC</u> R: <u>ACTAGTTCAGAAGTACTTATCCCCACATG</u>	<i>Cer</i>	KpnI/SpeI
AmphiNodalF/R	F: <u>GGTACCGCAGGCCGAGACCAACACCCGC</u> R: <u>ACTAGTCTACTGACAGCCGCATTCATCC</u>	<i>Nodal</i>	KpnI/SpeI
AmphiPitxF/R	F: <u>GGTACCACATATCTAAGGAGGACATCGTG</u> R: <u>ACTAGTTCCTTAGCAAACAATCCCATACGC</u>	<i>Ptix</i>	KpnI/SpeI
AmphiLeftyF/R	F: <u>CTCGAGTACGATGAAACCTGTTCTAGTT</u> R: <u>ACTAGTTTACTGTGTGCACGCACACTG</u>	<i>Lefty</i>	XhoI/SpeI
AmphiPtchF/R	F: ACGGTTGGACATATTCTGTTGC R: TGATACCATCCGCTCATTCTG	<i>Ptch</i>	NA
AmphiFoxE4F/R	F: <u>GAATTC</u> TGGGAGAAAAACACACACAAC R: <u>ACTAGTGGCATCTT</u> CACAAGAGACGA	<i>FoxE4</i>	EcoRI/SpeI
AmphiErrF/R	F: CCAGACTTCAGTGGACGATGAC R: GGGTCCCTATGTCCCTATGC	<i>Err</i>	NA
AmphiDkk1/2/4F/R	F: <u>GGTACCATGT</u> CGAACTCCATGCTGCAGCT R: <u>ACTAGTCTACTGCTGGC</u> ACGTGTACAGT	<i>Dkk1/2/4</i>	KpnI/SpeI
AmphiNkx2.1F/R	F: <u>GAATTC</u> ATGGAGTCCATAAGCCCTAAGC R: <u>ACTAGTTCACCACGCTCTGCCCTGCTGT</u>	<i>Nkx2.1</i>	EcoRI/SpeI
AmphiKroxF/R	F: <u>GGTACCACACACCGCTTCCCTGCTGA</u> R: <u>ACTAGTCGTA</u> AAATGAACCGTGAACCCA	<i>Krox</i>	KpnI/SpeI
AmphiHhF1/R1	F: <u>GAATTC</u> GAATTTAGCCGTTAATAGGGAG R: <u>ACTAGTACACACAGCCGAGTAGACACTT</u>	<i>Hh</i>	EcoRI/SpeI
AmphiHhF2/R2	F: GAATTTAGCCGTTAATAGGGAG R: GCGAGTAATCCGTCCGTTGA	<i>Hh</i>	NA
AmphiWnt3F/R	F: GGCATCCCATTGGAAGTACTC R: TCATTTGCACGTGTGCACCTG	<i>Wnt3</i>	NA
AmphiPax2/5/8	F: ATGGACAGGATGACCACGATG R: GTGAGAAGAGAAGAAGTTGCC	<i>Pax2/5/8</i>	NA
Amphi-m-actin	F: TCAGGGCGTGATGGTCCGGTAT R: GGTGGACAGGGAAGCCAAGAT	<i>m-actin</i>	NA
AmphiMRF2	F: ATGAACTACACAGAGCTGAGCA R: TTGAACAAGATTTTGGCACGGT	<i>MRF2</i>	NA
AmphiNetrin	F: TGTAACAGTGACCCATTCCG R: CACATGGCATGAAGGTTGA	<i>Netrin</i>	NA
AmphiBrachyury	F: AGACCAGCGTCAACAACGAGATG R: AACAACTGGAGCCCYATGAC	<i>Brachyury</i>	NA
AmphiZic	F: AGGCCTTTCGCATGGATTGT R: CTGCCTCTGCGTTCATTTGC	<i>Zic</i>	NA

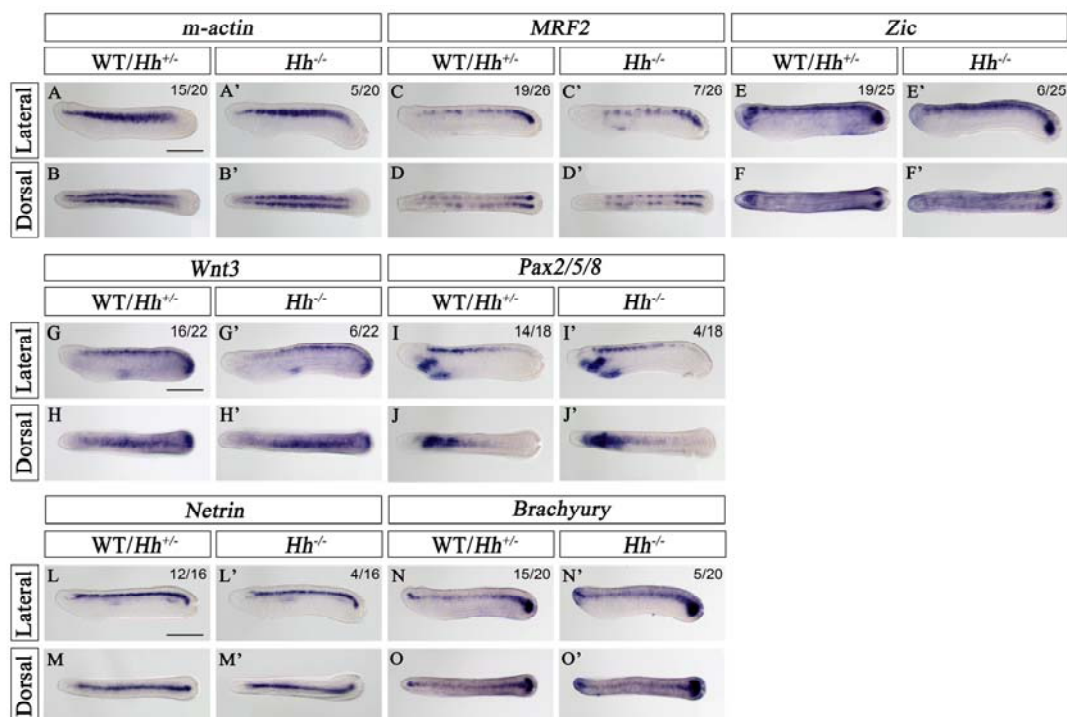


Figure S1. The expression pattern of middle line structure and somite marker genes in the embryos at late neurula stage.

(A-B') The expression of *m-actin* specific to somite in wild-type/heterozygote embryos, symmetric expression pattern of *m-actin* in *Hh*^{-/-} mutant. (C-D') The expression of *MRF2* limited to posterior somites in normal embryos, no difference were observed in *Hh*^{-/-} mutant. (E-F') The expression of *Zic* in the dorsal lateral somites, *Hh* knockout had no effects on *Zic* expression. (G-J') The expression of *Wnt3* and *Pax2/5/8* mark the neural tube in amphioxus, no differences are observable in *Hh*^{-/-} mutant. (L-O') *Netrin* and *Brachyury* expression in the neural tube and notochord in the embryos, *Hh* knockout did not effect on the *Netrin* and *Brachyury* expression. All embryos are positioned with anterior to the left. The scale bar is 200 μ m.

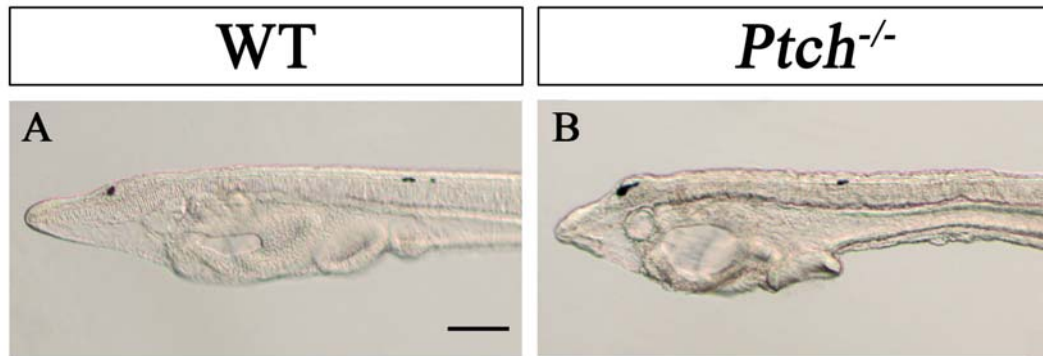


Figure S2. Phenotype resulted from *Ptch* knock out in amphioxus.

(A) The left lateral view of wild-type amphioxus showing a normal development of mouth and gill slits. (B) *Ptch* knockout did not effect on the left-right patterning but resulted in an enlarged pigment spot and mouth. The scale bar is 100 μ m.

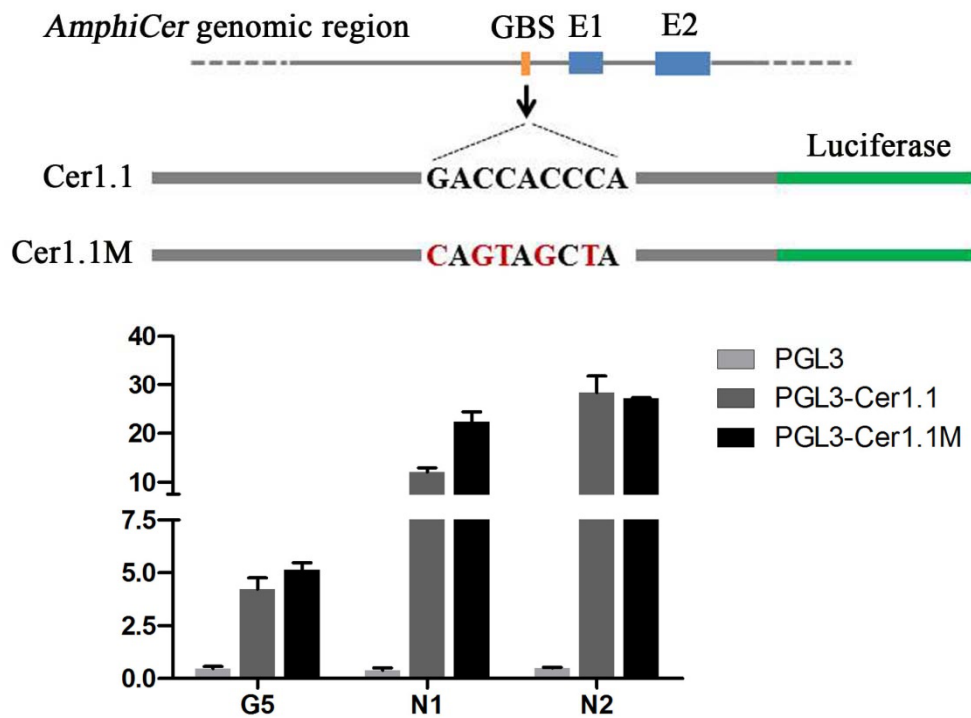


Figure S3. *Cer*-Luciferase reporter activity in amphioxus luciferase assay.

Luciferase reporter plasmids containing a deduced Gli-binding site or a mutant deduced Gli-binding site were injected into amphioxus eggs. Each egg were injected with reporter plasmid and reference plasmid pTK-Renilla luciferase (10:1). Luciferase values were obtained at G5, N1 and N2 stages respectively. Firefly and Renilla luciferase values were obtained by the standard protocol provided by the producer (Promega). **GBS**, Gli binding site; Point mutations are marked in red letters; **E**, exon; **G5**, late-gastrula; **N1**, early neurula; **N2**, mid-neurula.

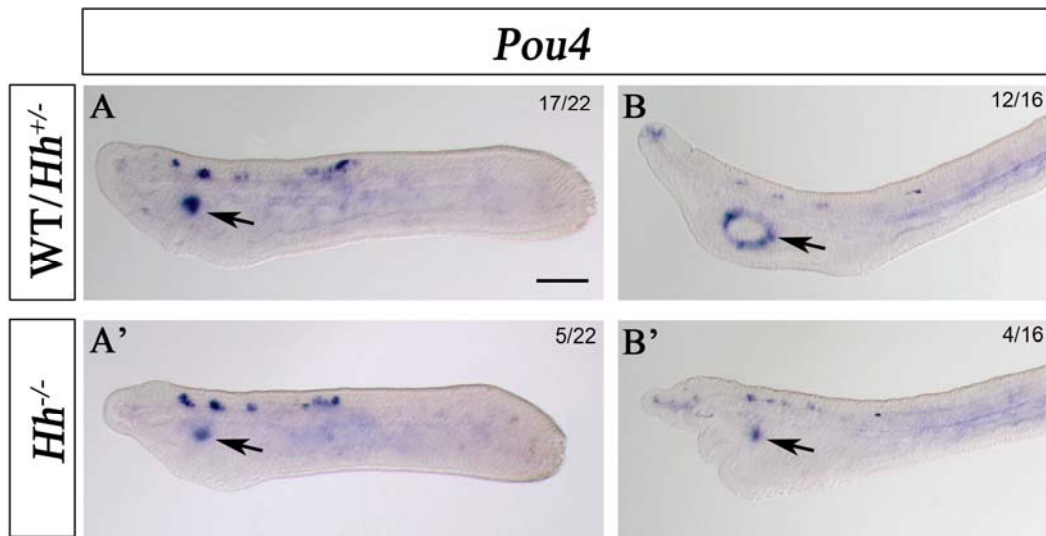


Figure S4. Oral mesovesicle and mouth opening shown with *Pou4* expression in amphioxus.

(A) Left lateral view showing *Pou4* expression in oral mesovesicle. (B) Mouth opening indicated by *pou4* expression. (A') *Pou4* expression at oral mesovesicle was reduced slightly in *Hh*^{-/-} embryos. (B') Disappeared mouth opening was disclosed by *Pou4* expression in *Hh*^{-/-} amphioxus. Arrows indicate mouths in the figures. The scale bar is 100 μ m.