



**SUPPLEMENTARY MATERIAL**

**corresponding to:**

**Latrophilin2 is involved in neural crest cell migration  
and placode patterning in *Xenopus laevis***

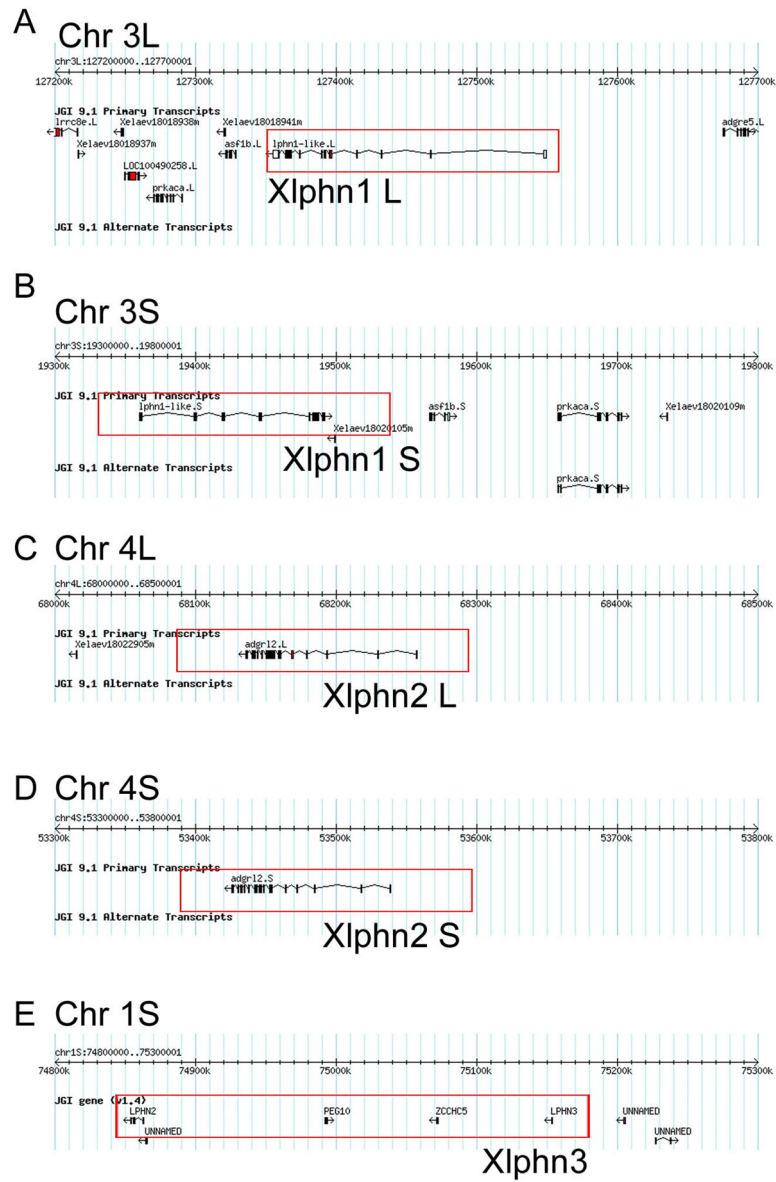
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and KOSUKE TANEGASHIMA\*

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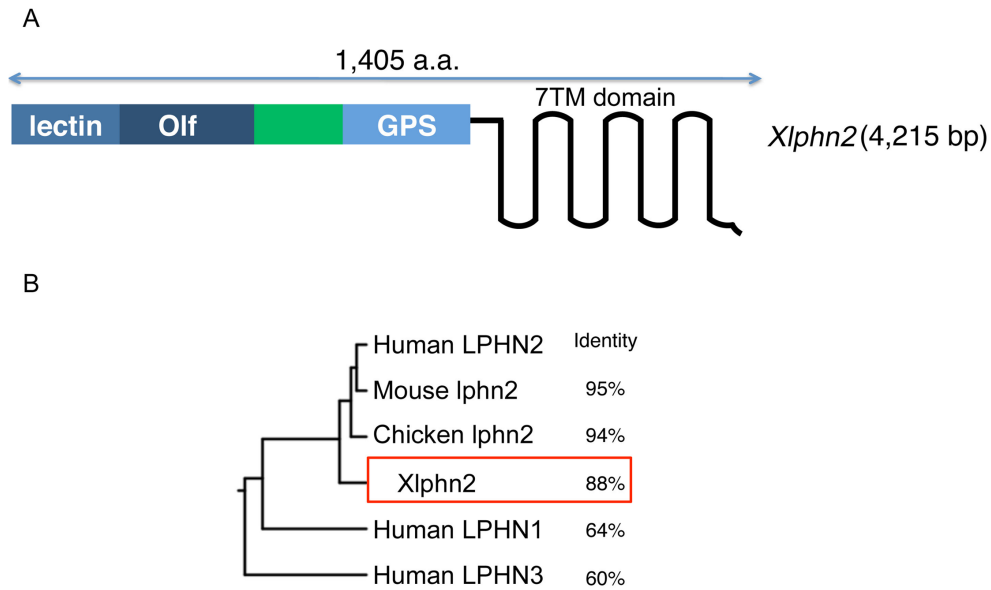
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#Note: These authors contributed equally to this study.

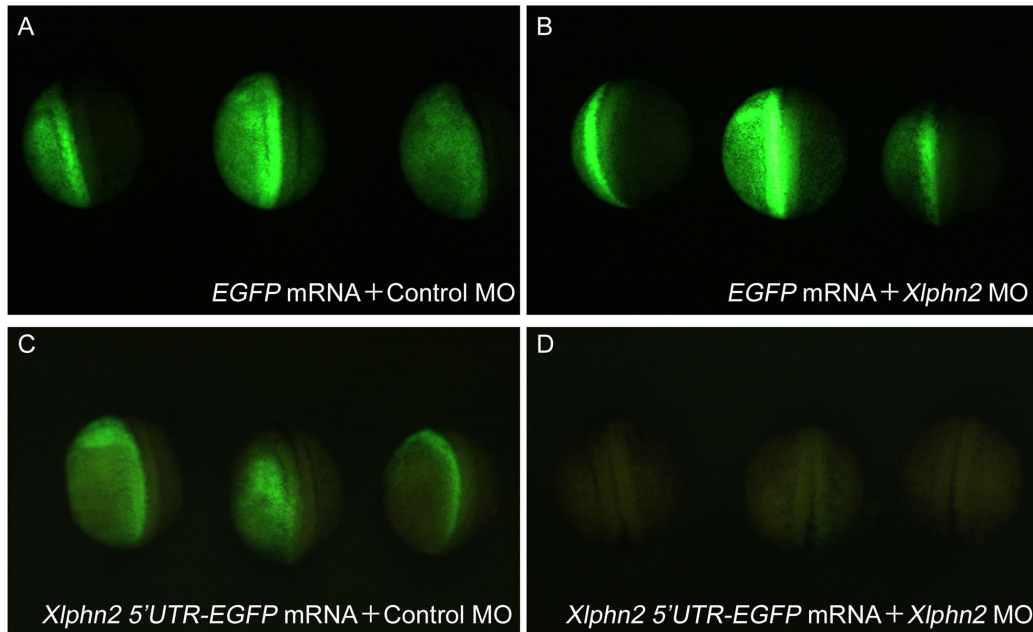
Full text for this paper is available at: <https://doi.org/10.1387/ijdb.180184kt>



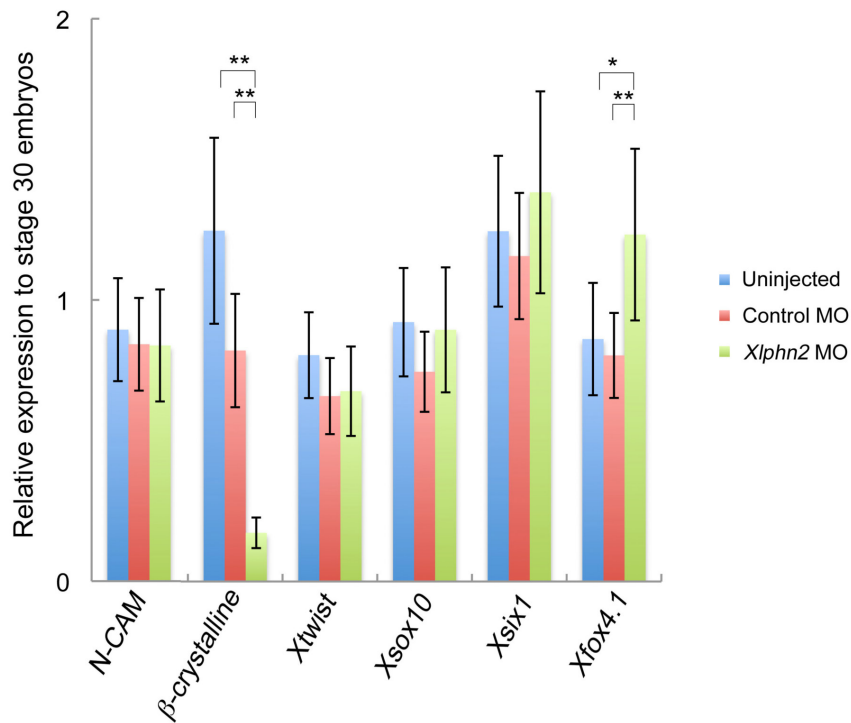
**Fig. S1. (A-E) Genomic structure of Latrophilin-related genes in *Xenopus laevis*.** Genomic region surrounding 500 kbp of *Xenopus latrophilin*-related genes is shown. Graphics were adapted and modified from *X.laevis* 9.1. on GB browser. Note that Xlphn3-like is not mapped completely on this region by JGI model because of low homology to human LPHN3.



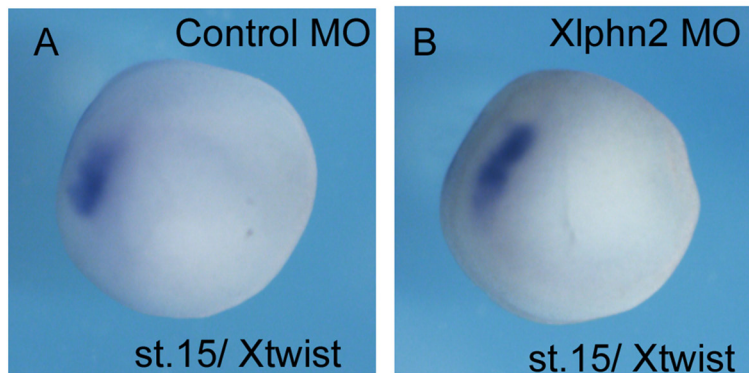
**Fig. S2. (A)** Analysis of the protein sequence coded by Xlphn2. Xlphn2 (4215 base pair long) protein contains a galactose binding lectin domain (lectin), an olfactomedin-like domain (olf), a G-protein-coupled receptor proteolytic site (GPS), and a seven transmembrane receptor domain (7TM domain). **(B)** Phylogenetic tree of Xlphn2 protein. Protein sequences of latrophilin-related gene products were analyzed by the Neighbor joining method. Percentages of identical amino acids (Identity) to the human LPHN2 protein are shown.



**Fig. S3. (A-D)** One hundred picograms of EGFP **(A,B)** or Xlphn2 5'UTR-EGFP **(C, D: containing Xlphn2 MO sequence upstream to ATG codon of EGFP mRNA)** was co-injected with 20 ng of control MO **(A,C)** or Xlphn2 MO **(B,D)** into one blastomere of 2-cell stage embryos. The injected embryos were cultured until stage 20 for microscopical observation of fluorescence.



**Fig. S4. qRT-PCR of stage 30 embryos.** Forty ng of control MO or Xlphn2 MO was injected into the animal pole of 2-cell stage embryos. The embryo was grown until stage 30 and total RNA was isolated from whole embryos to make cDNA for qRT-PCR. The expression level of each gene was measured using the relative quantification method with Elongation factor 1 $\alpha$  (EF1 $\alpha$ ) as a reference. cDNA of pooled embryos (stage 30) was used as a standard, and the expression levels of these embryos was set to 1. At least five individual embryos were used for quantification and statistical analysis. \*\*P<0.01 and \*P<0.05 were considered significant.



**Fig. S5. Xlphn2 is not required for induction of neural crest cells.** (A,B) Stage 15 embryos were stained with Xtwist as a probe. Lateral view is shown.

TABLE S1

## RT-PCR PRIMERS USED IN THIS STUDY

Gene names	Forward 5'-> 3'	Reverse 5'-> 3'	Product size
<i>ODC</i>	GTCAATGATGGAGTGTATGGATC	TCCATTCGGCTCTCCTGAGCAC	385
<i>EF1a</i>	TTGCCACACTGCTCACATTGCTTGC	ATCCTGCTGCCTTCTTTCCACTGC	251
<i>Xlphn1</i>	TGCTGTGTTAGAGGTCCAGG	TCTGCAGCACTTGGTTTGTC	294
<i>Xlphn2</i>	TAGGAGCAGATTTGGCTGGT	TGGCTACAGGCACATGTTGT	261
<i>Xlphn3</i>	GACTTACCTGGGCATTGGGA	GCTAAGCAAACATGCAAAACA	187
<i>N-CAM</i>	GCGGGTACCTTCTAATAGTCAC	GGCTTGGCTGTGGTTCTGAAGG	137
<i>β-crystallin</i>	CACTGACTTCAAGGGCAACA	TCTGGGGTTGATAGGCACTC	208
<i>Xtwist</i>	AGCAATGCCACTACAGCTCA	GAATGGATTTGGCGAACCTA	244
<i>Xsox10</i>	GGAGAAGGAGATGGGTCCTC	TTTCCTGCCTGAAGCTCTGT	168
<i>Xsix1</i>	TGGTATGCCATAACCCCTA	TAGCGACTTCCCTCCGTCTA	212
<i>Xfox4.1</i>	GGCACAATCTGTCCCTGAAT	GTCAGCATTGAAGGGCTCTC	237