SI Appendix

Supplementary Methods

Data preprocessing

Quality control of raw sequencing data was conducted using FASTQC v0.10.1 (*1*). Barcodes and contaminating adapter sequences with >5bp overlap were removed using CUTADAPT v1.1 (*2*). Removal of low quality reads and extraction of orphaned reads were conducted using SICKLE (*3*). Overlapping pairs of sequencing reads were merged using FLASH v1.2.2 (*4*).

Transcriptome assembly and annotation

Preprocessed RNA-seq data were assembled using the ABYSS v1.3.5 assembler (*5*, *6*) and the tiled sub-read (kmer) length that optimized the N50 was estimated as 35 nucleotides. Coding sequences and Ensembl gene IDs for *Danio rerio* (Zv10) were downloaded from Ensembl. The BLASTSUITE v34 tools (*7*) were used to generate BLAST databases and conduct searches. Annotation of transcripts was completed in two steps. The translated largest open reading frame (ORF) from each *L. erinacea* transcript was used to query the *Danio rerio* coding sequences with tBLASTn (max e-value = 0.01) and hits from the reference database within 20 bp were merged. For each *L. erinacea* contig, the largest difference in log-2 e-values of the BLAST hits was chosen as the cutoff. For *L. erinacea* contigs of interest with greater than one BLAST hit, a reciprocal tBLASTn of the matching *D. rerio* genes was conducted in the *L. erinacea* transcriptome and the *D. rerio* gene with the lowest mean e-value was chosen as the annotation. For *L. erinacea* contigs without ORFs greater than 150 nucleotides or unannotated from the previous tBLASTn, a tBLASTx search was conducted in the *D. rerio* coding sequences. The annotations provided

with the assembled transcriptome fasta file include the Ensembl gene IDs, gene descriptions, and BLAST e-values.

Alignment and differential expression

We used BOWTIE2 v2.0.0 (*8*) for sequence alignment and SAMTOOLS v0.1.18 suite (*9*) for sorting, converting, removing duplicate, and indexing reads. The statistical environment R and Bioconductor packages were used for differential expression analysis. Biostrings v2.26.3 (*10*) was used for handling sequencing data. Transcripts less than 300 nucleotides in length were excluded. Transcripts that were unable to be annotated using the aforementioned methods were excluded such that 18,521 transcripts were included in the analyses. Log-2 RPKM (reads per kilobase of transcript per million mapped reads) values were computed for each transcript. Comparisons to identify differential expression were conducted essentially as in (*11*). However, the magnitude of differential expression (residuals) for individual transcript comparisons (linear regression) was modified to correct for heteroskedasticity. An apex was chosen at 1.02 times the maximum transcript score and the angular distance was used to weight individual transcript residuals.

Real time PCR for Hox genes

Total RNA was subtracted from the anterior or posterior pectoral fin by Trizol (Invitrogen,15596-026). Subsequently, cDNA was synthesized by SuperScript Ⅲ First-Strand Synthesis System (Invitrogen). Real time PCR was performed by ABI7300 with SYBR Green PCR master Mix (Invitrogen). The absolute quantification was performed with diluted series of each Hox cDNA vector as a copy number control. Primer

sequences for real time PCR are as the followings.

Hoxa1;

5’-GCCAAACCTTTGACTGGATG-3’ and

5’-TCGTATTGTGTTCGGCTGAC-3’

Hoxa2;

5’-ATCACCTGCCTGCTTCTTGT-3’ and

5’-GATCCCCCGCCTGTATTATC-3’

Hoxa3;

5’-GATGAAAGAATCACGCCAAAA-3’ and

5’-GGCTTTTCTCACCAGCAGAA-3’

Hoxa4;

5’-TCCGTGGATGAAGAAAATTCA-3’ and

5’-CGCACATCCGATTTCAAGT-3’

Hoxa5;

5’-TCAGATTTACCCCTGGATGC-3’ and

5’-CCGTCCTGGCTCTTTTACCT-3’

Hoxa6;

5’-CGTAAAGTTGAAATTAAGTGTCATAGG-3’ and

5’-CTTGGATGCAACGAATGAAT-3’

Hoxd1;

5’-GAACACCTTCGAGTGGATGAA-3’ and

5’-GCAGATGCGTGAGATGAAGA-3’

Hoxd4;

5’-ATCAAAACGGCACAGCAAC-3’ and

5’-CCCGTGTTAACGTGGATTTT-3’

Supplementary Figure1 | Cell proliferation in the pectoral and pelvic fins. A-D, Whole mount antibody staining of phosphorylated histoneH3(Green) and DAPI(Blue). **E-H,** Magnified pictures of anterior fin in **A-D**. **I,** Immunofluorescence of phosphorylated histone H3 and DAPI in the transverse section of the pectoral fin at stage 31. **J,** A magnified picture of **I**. **K,** Comparison of cell proliferation rate in each part of the pectoral and pelvic fin at stage 31. PecA; the anterior pectoral fin, PecC; the center pectoral fin, PecP; the posterior pectoral fin, PelA; the anterior pelvic fin, PelC; the center pelvic fin, PelP; the posterior pelvic fin. The anterior and posterior cell proliferation is higher than center at stage 31. *; t-test P<0.05, **;<P<0.01

Supplementary Figure2 | The volcano plots of RNA-seq at stage 29 and 31. A, The plot of stage 29. **B,** The plot of stage 31. The axis labels and the color coding for the plots are as in Figure2. **C**, Average differential expression scores versus average transcript expression values for stages 29 (N=3), 30 (N=3), and 31 (N=2). N is the number of paired anterior-posterior samples and higher differential expression scores indicate bias in anterior versus posterior fin.

Supplementary Figure3 | Phylogenetic analysis of *L.erinacea Wnt3*
Each number indicates the value of the bootstrap.

Supplementary Figure4 | Phylogenetic analysis of *L.erinacea Fgf7*

Supplementary Figure5 | Phylogenetic analysis of *L.erinacea Gli3*

Supplementary Figure6 | Phylogenetic analysis of *C.plagiosum Gli3*

Supplementary Figure7 | *Hoxa* and *Hoxd* expression levels by RNA-sequence analysis at stage 30. A, *Hoxd* expression levels at the anterior, center and posterior fin. **B,** *Hoxa* expression levels.

Supplementary Figure8 | Real time PCR analysis of *3'Hox* expression levels at stage 30. A. *Hoxa1*, **B.** *Hoxa2,* **C.** *Hoxa3,* **D.** *Hoxa4,* **E.** *Hoxa5,* **F.** *Hoxa6,* **G.** *Hoxd4,* **H.** *Hoxd8.* Pec-A; the anterior pectoral fin, Pec-C; the center pectoral fin, Pec-P; the posterior pectoral fin, Pel-A; the anterior pelvic fin, Pel-C; the center pelvic fin, Pel-P; the posterior pelvic fin. The vertical axis shows the copy number of each mRNA.

Supplementary Figure9 | A functional test of 3'HOX. A, The experimental scheme for testing *3'Hox* gene function. The top; wild type embryos, center; embryos treated by Cyclopamine from 24hpf to 36hpf followed by *Wnt3* ISH at 36hpf. The bottom; *Hoxa2b* expression vector was injected into samples at one cell stage in addition to the later Cyclopamine treatment. **B.** *Wnt3a* expression in wild type embryos at 36hpf. **C.** *Wnt3* expression in the embryos treated by Cyclopamine. **D.** *Hoxa2b* expression, driven by a distal fin enhancer (Island Ⅰ) at 36hpf. **E.** *Wnt3* expression in the embryos treated by Cyclopamine with Island Ⅰ- *Hoxa2b* injection.

Supplementary Figure10 | Expression patterns of took kit genes in paired fins. A, *Wnt3* expression in the anterior ectoderm of the skate pectoral fin at stage 30. **B,** *Gli3* expression in the posterior mesenchyme of the skate pectoral fin at stage 30. **C,** *Msx2* expression in the anterior mesenchyme of the skate pectoral fin at stage 30. **D,** *Bmp2* expression in the skate pectoral fin at stage 30. Dorsal view. **E,** *Bmp4* expression in the skate pectoral fin at stage 30. Ventral view. **F,**

Gli3 expression is enriched in the zebrafish posterior pectoral fin. **G,** *Gli3* expression in the zebrafish pelvic fin at 21dpf. The expression is enriched in the anterior fin.

Supplementary References

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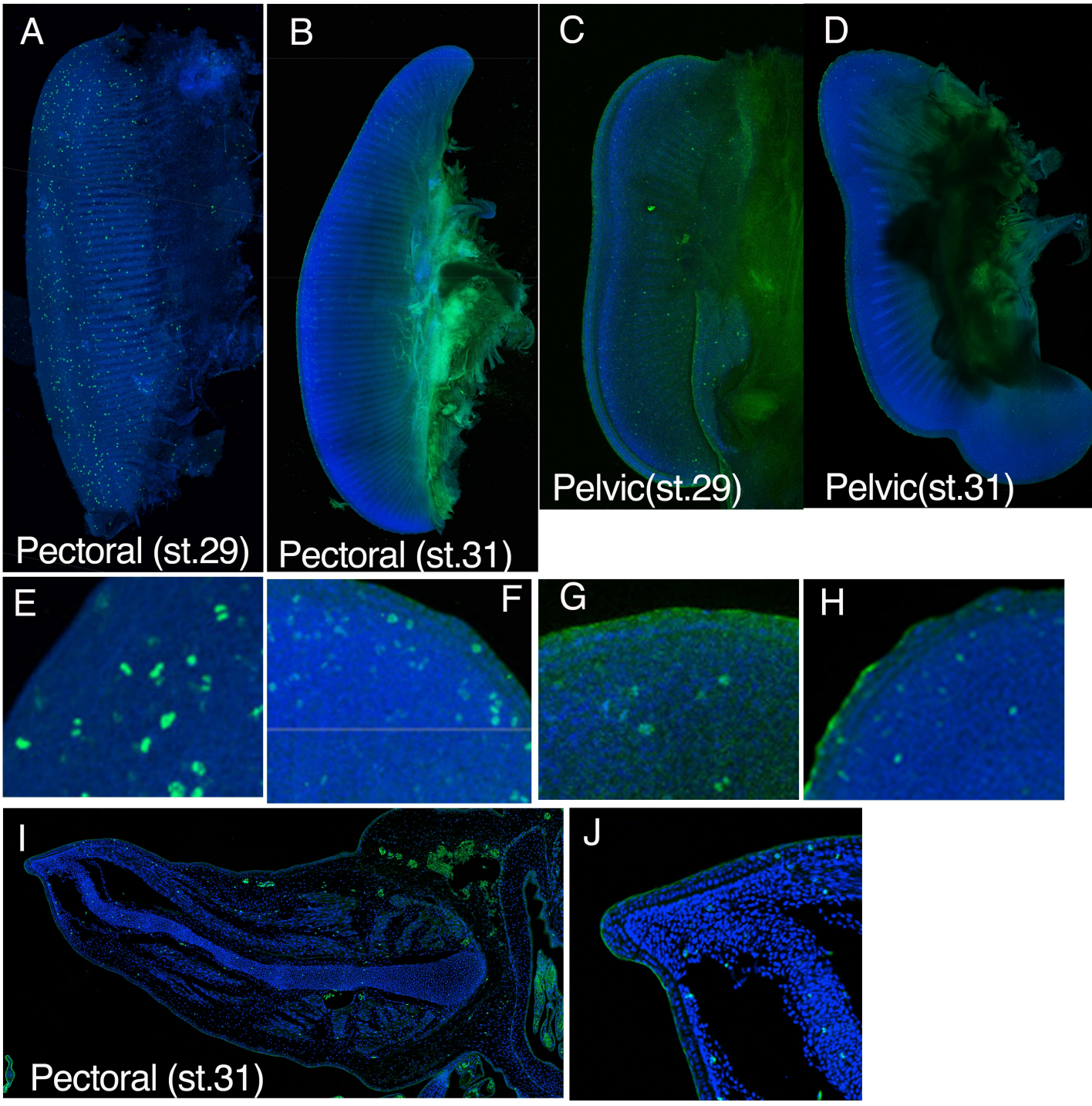
Supplementary Figure 1



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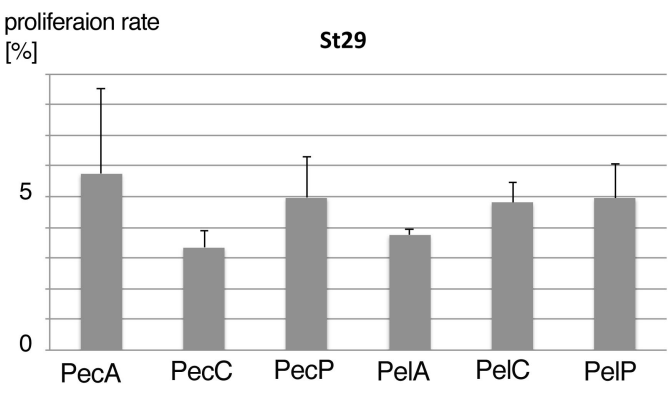

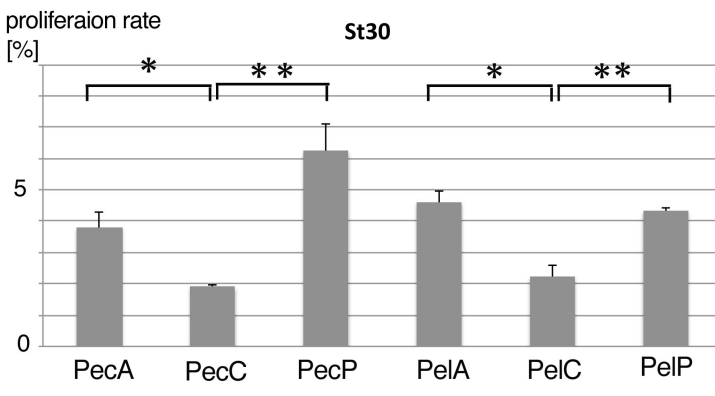

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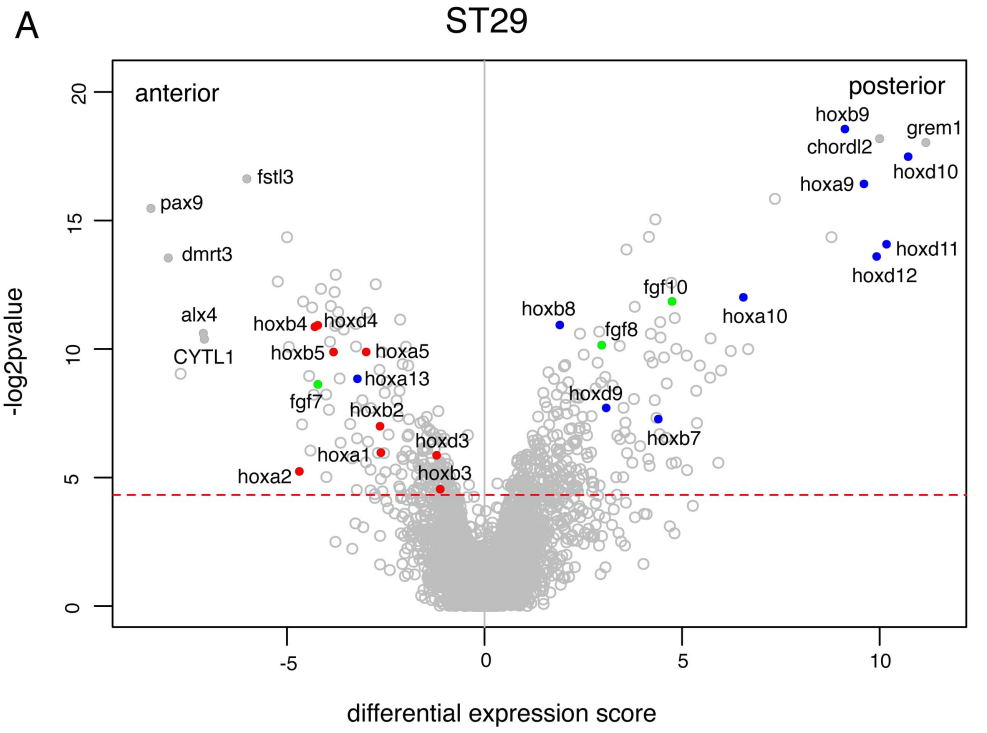

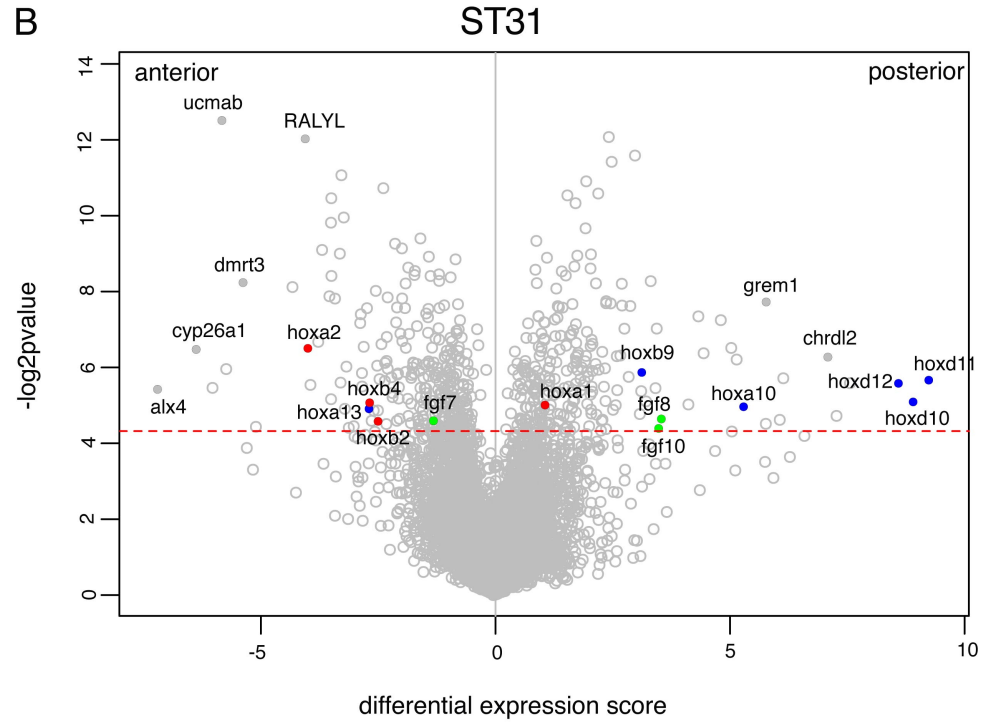

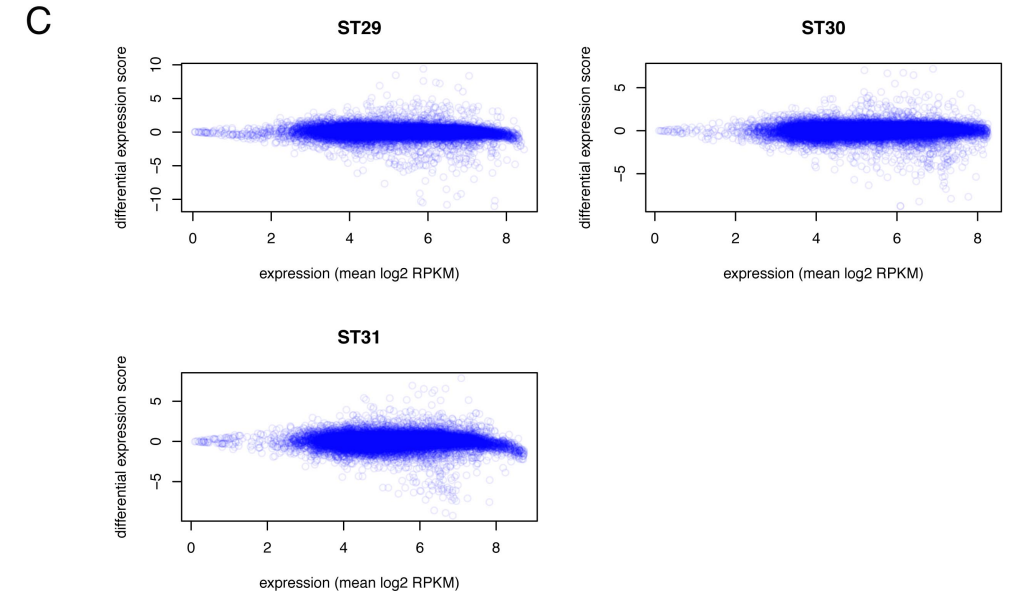

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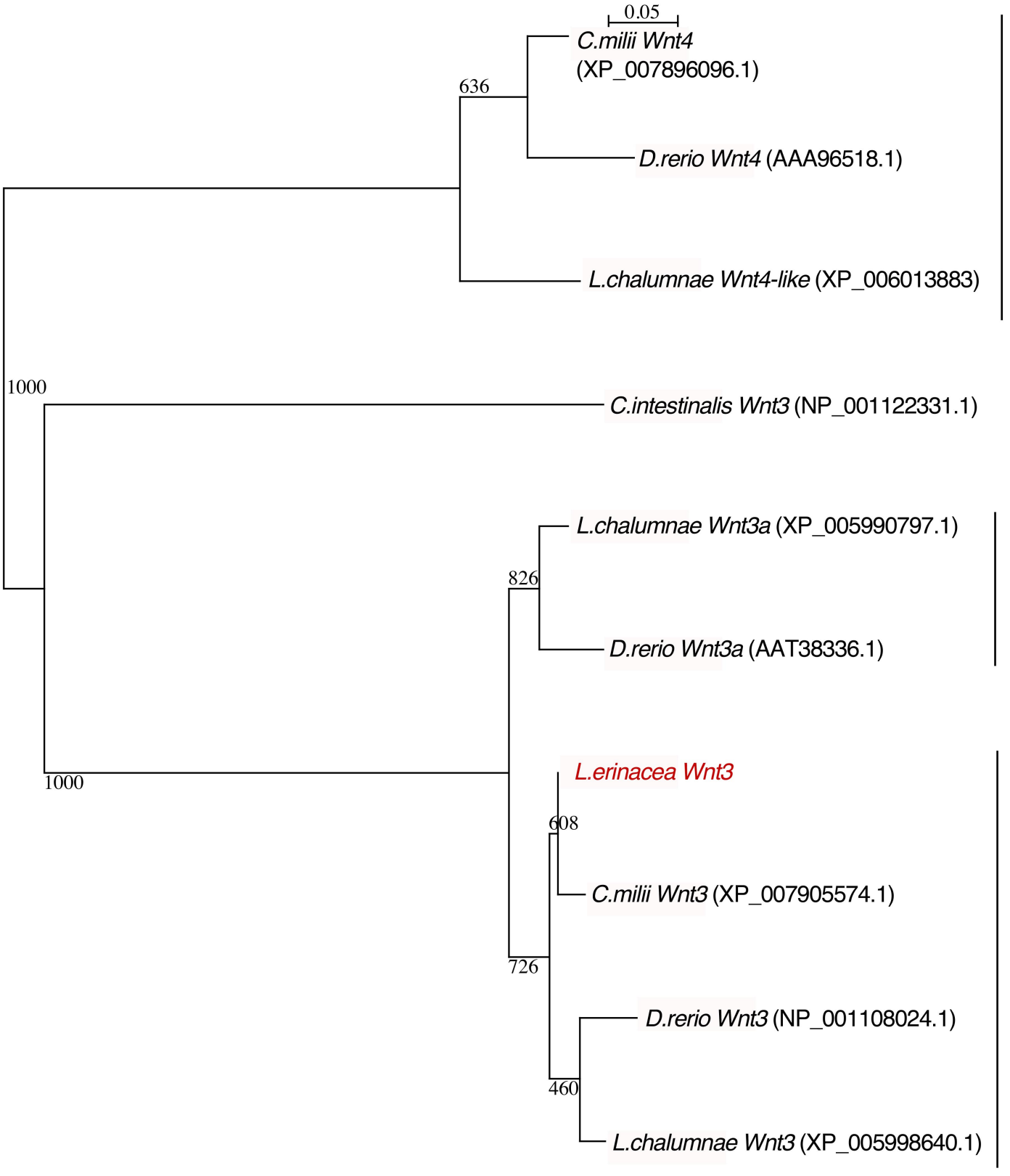

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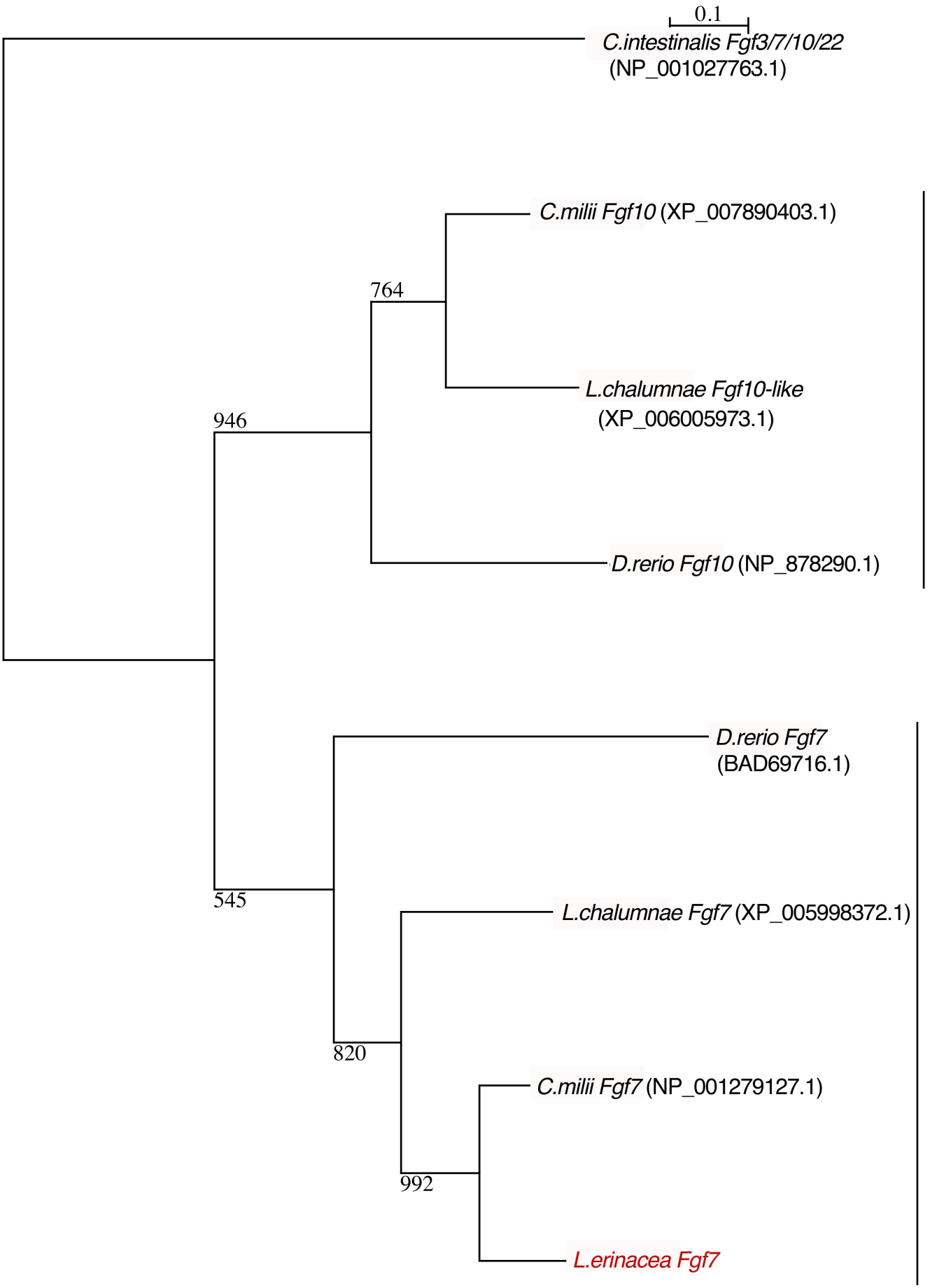

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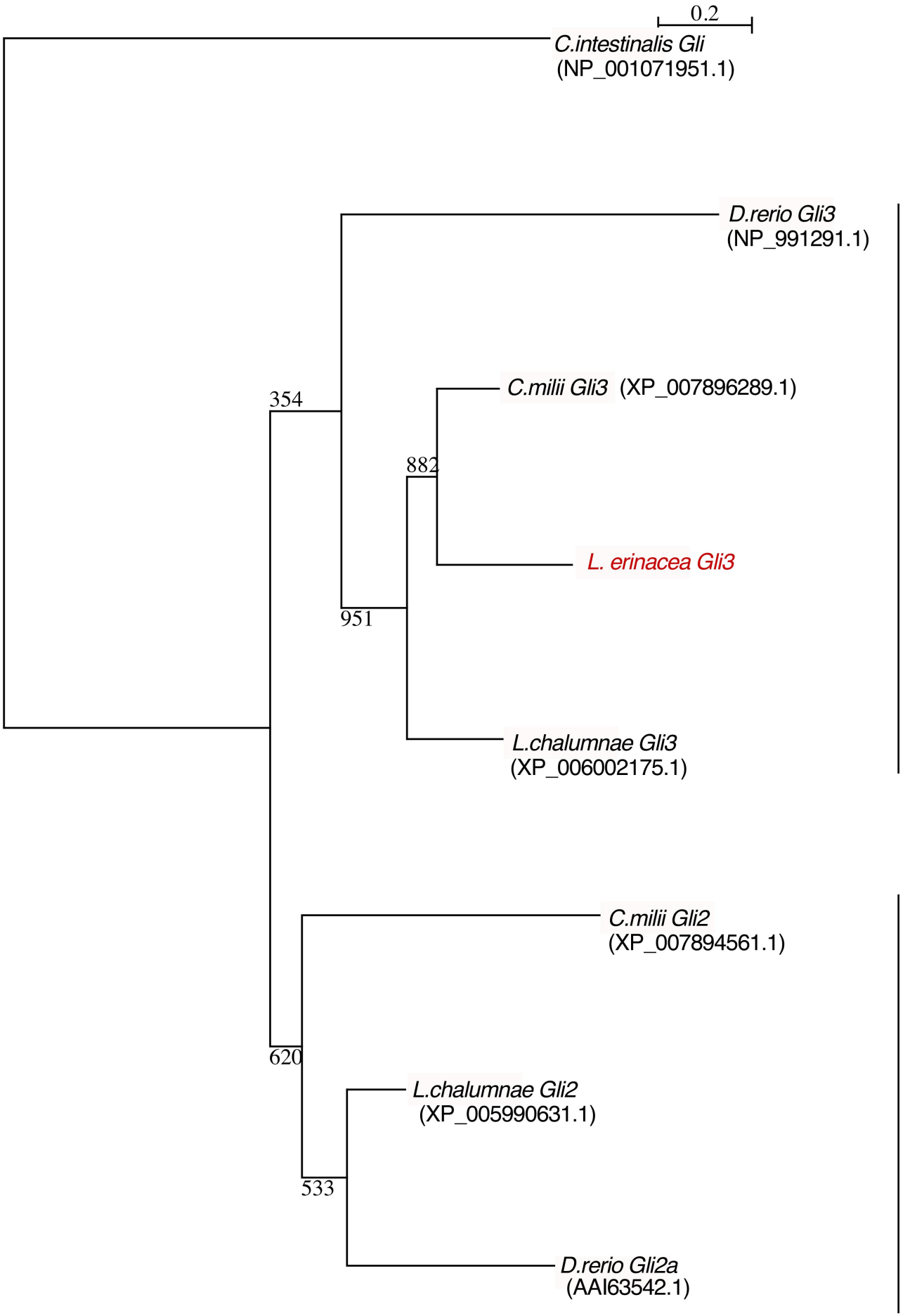

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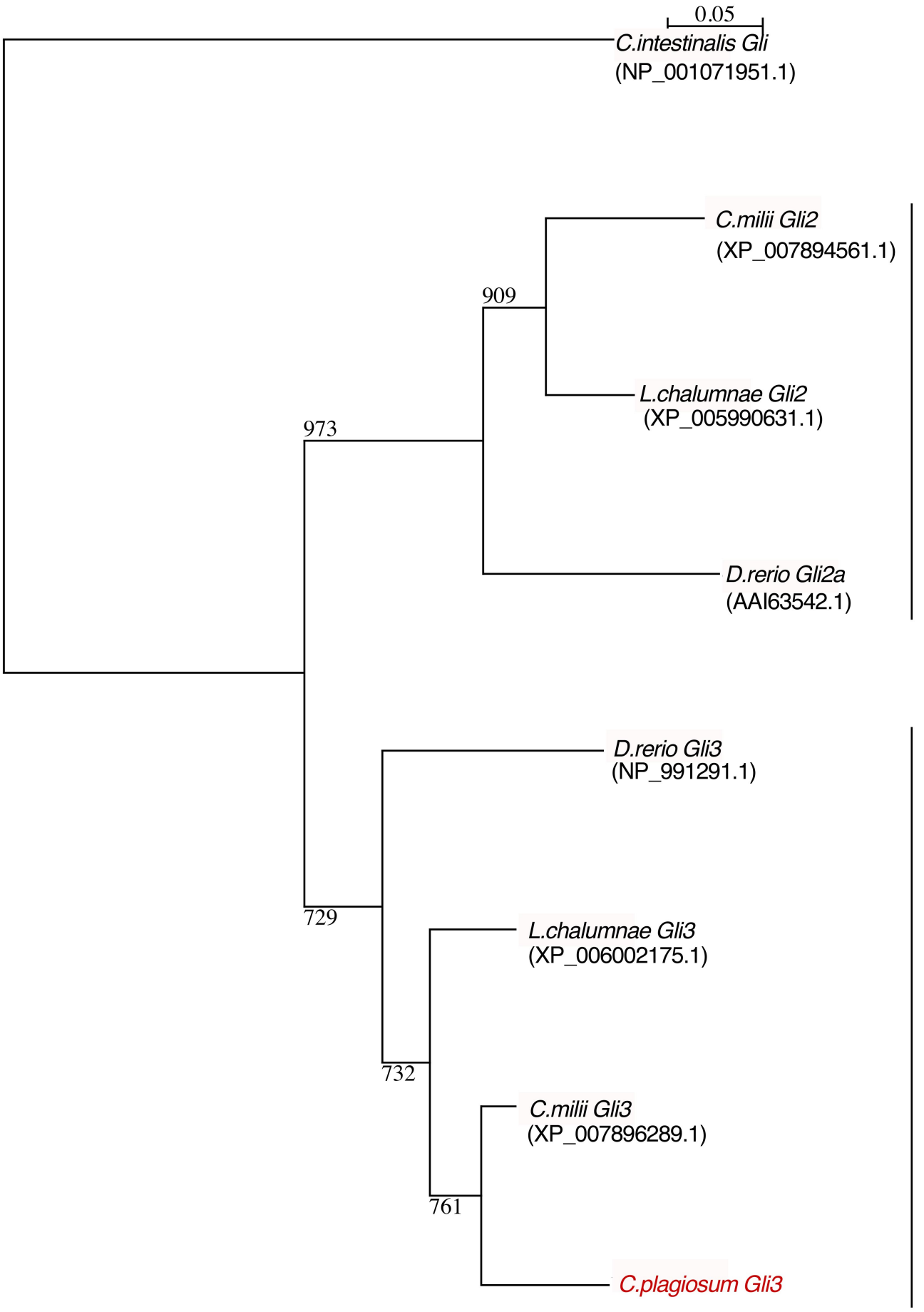

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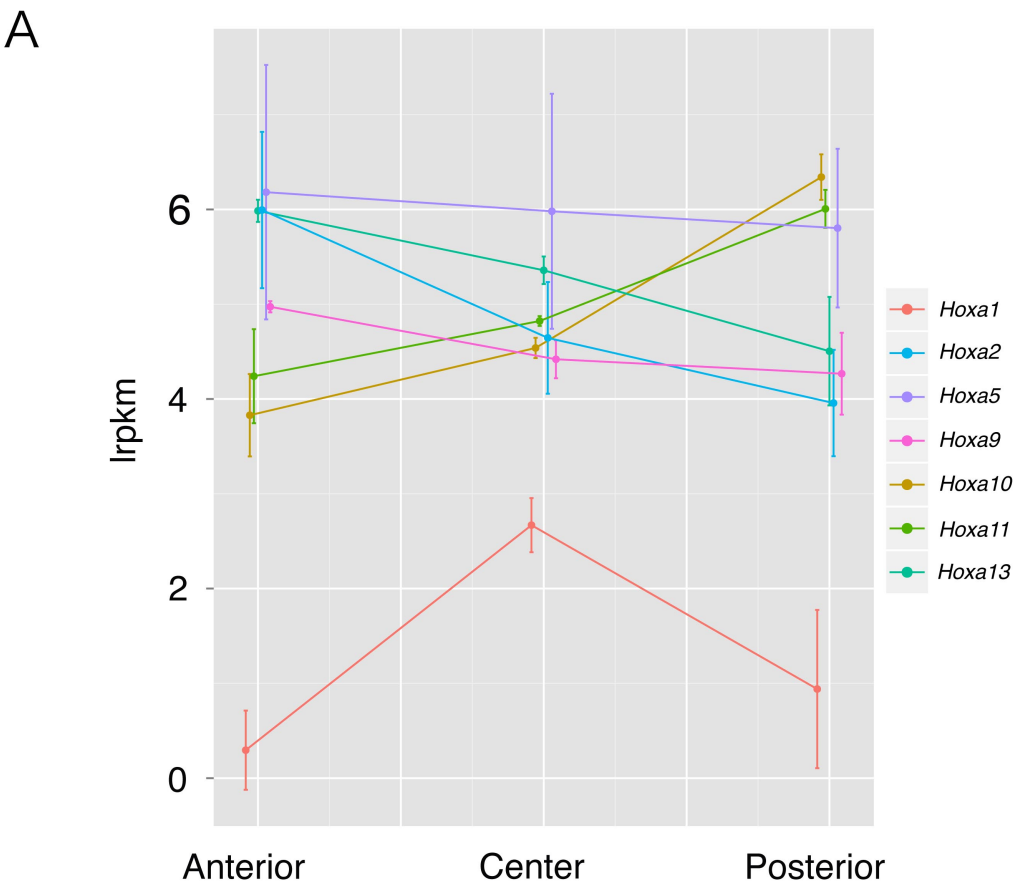

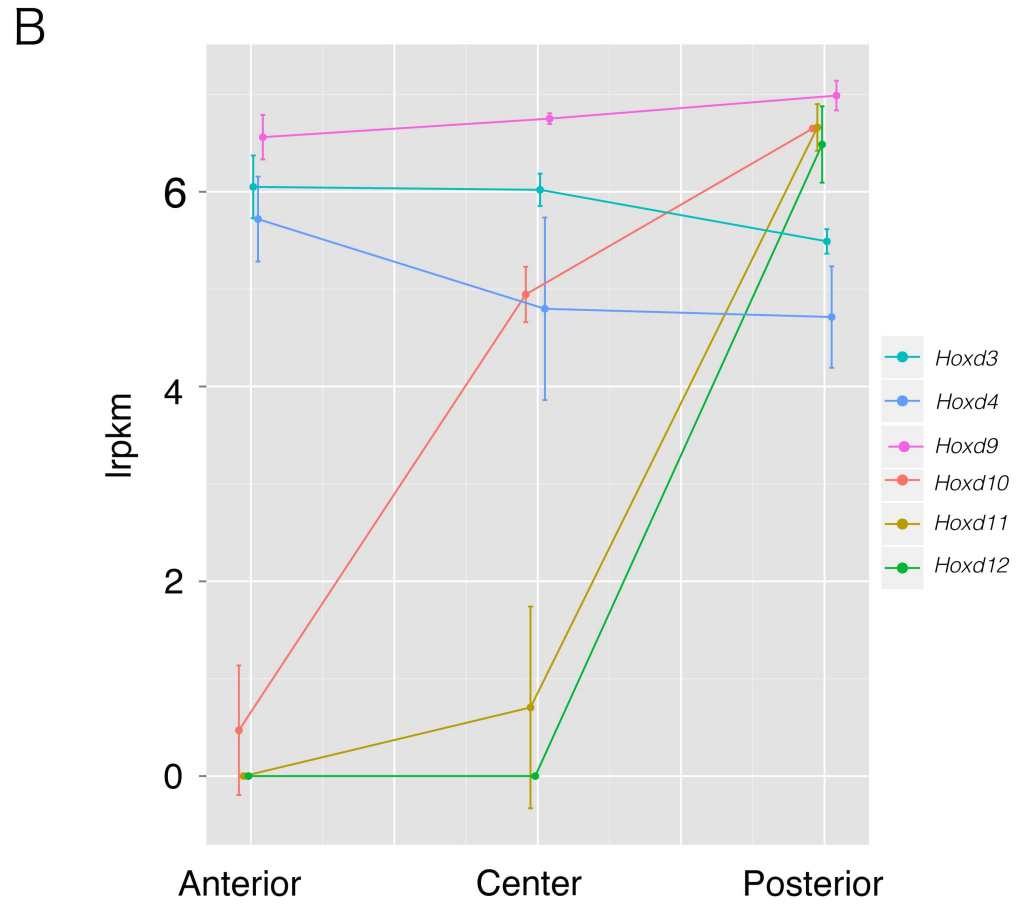

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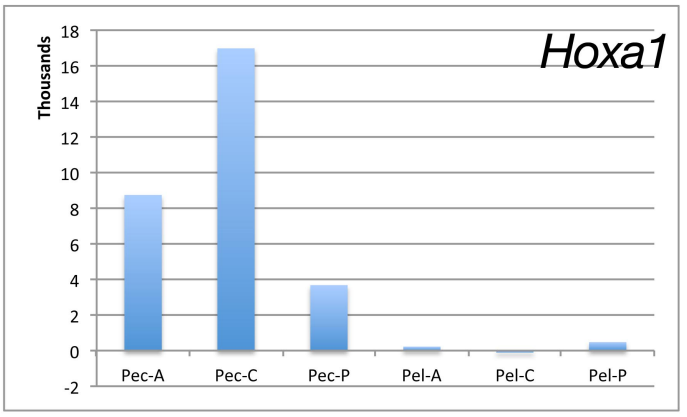

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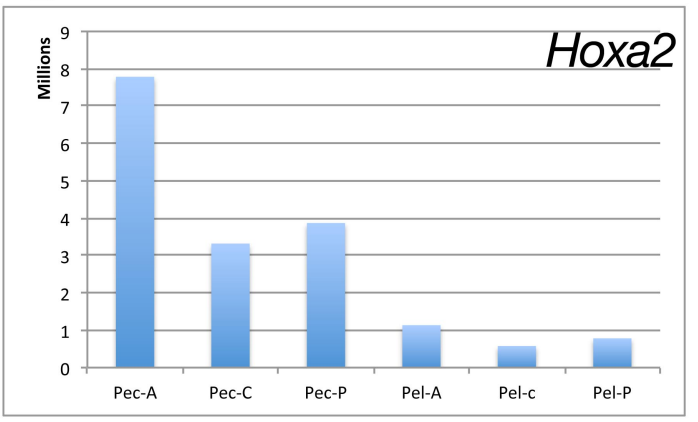

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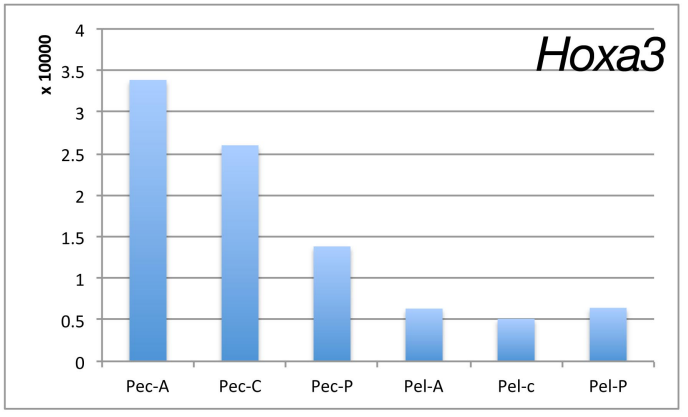

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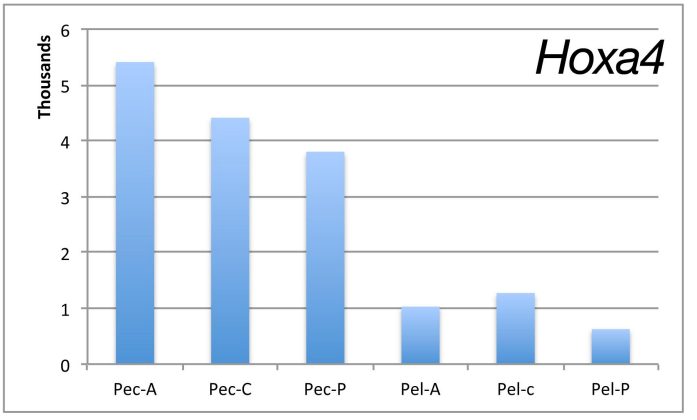

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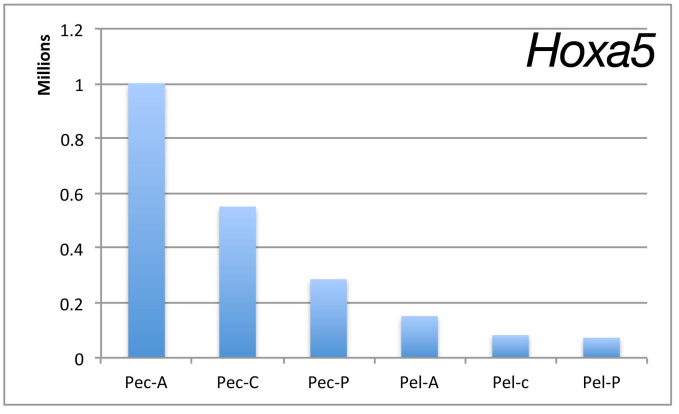

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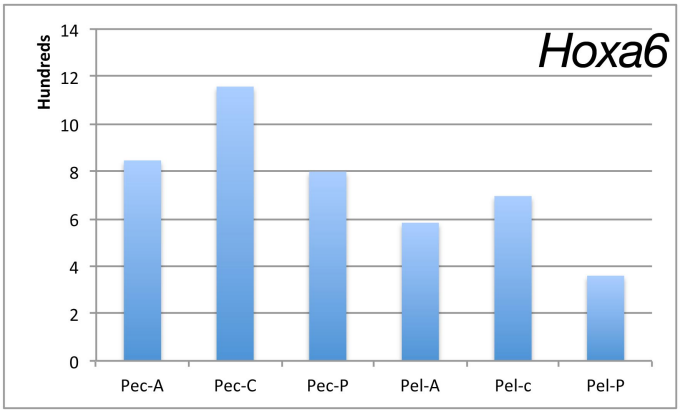

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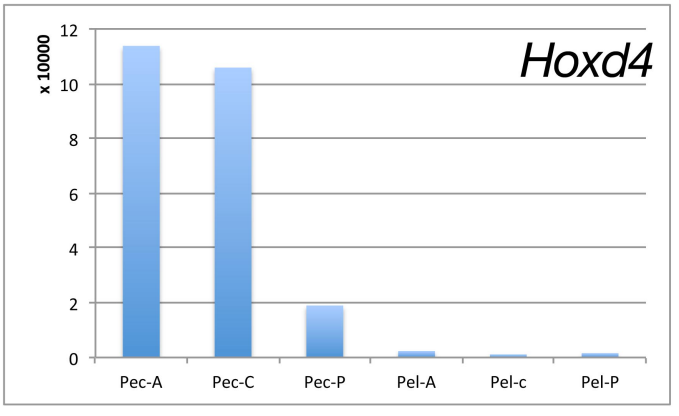

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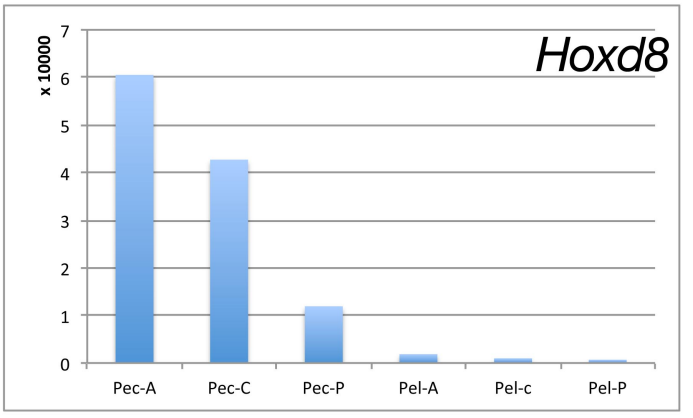

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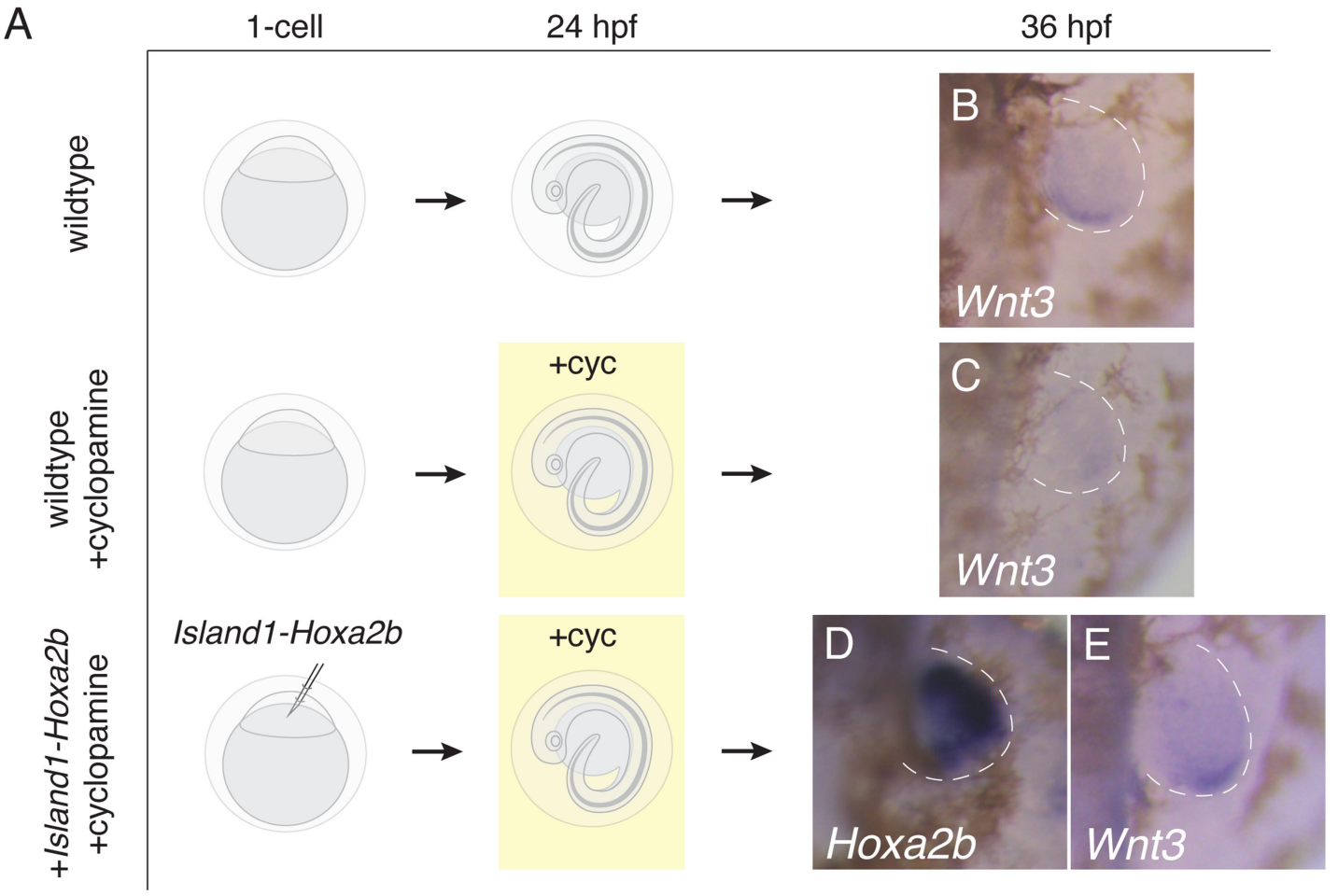

Supplementary Figure 10