

The genome and transcriptome of Japanese flounder provide insights into flatfish asymmetry

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Supplementary Note

1. Genome sequencing and assembly

We generated about 75.77 G of raw data for the Japanese flounder genome, and 52.6 G of effective data was used for assembly after filtering out low-quality reads and potential sequencing errors using SOAPdenovo³⁴ (<http://soap.genomics.org.cn>). (**Supplementary Fig. 1** and **Supplementary Table 1**). SOAPdenovo employs the *de Bruijn* graph algorithm to simplify the assembly and reduce computational complexity. First, SOAPdenovo constructs a *de Bruijn* graph by splitting the reads from short insert size libraries (170–800 bp) into 35-mers and then merges the 35-mers. The contigs were collated and exhibited unambiguous connections on the *de Bruijn* graphs. All reads were aligned onto the contigs to build scaffolds using the paired-end information. The paired-end information was used to link the contigs into scaffolds in a step-by-step fashion from short to long insert sizes. All high-quality reads were used to build the scaffolds. Some intra-scaffold gaps were filled by local assembly using the reads in a read-pair where one end uniquely aligned to a contig and the other end was located within a gap. We used a Kmer-based method to estimate genome size as described previously³⁵. The final total contig size and N50 were 534 Mb and 30.5 Kb, respectively. Total scaffold size and N50 was 546 Mb and 3.88 Mb, respectively (**Supplementary Table 2**). We further mapped the SSR and SNP markers to scaffolds, respectively, using BLASTN, and the consensus scaffolds, which were between the SSR and SNP maps, were linked onto chromosomes with a string of ‘N’s representing the gap between two adjacent scaffolds. Scaffolds with markers located on different chromosomes were filtered. Finally, 256 scaffolds with a length of 535 Mb (98% of scaffolds in length) were anchored onto 24 chromosomes (**Supplementary Table 3**).

We calculated that the Japanese flounder genome was 640 Mb using the depth-frequency distribution curve, 17-mer statistics, and the formula: $G = \text{kmer_num} / \text{kmer_depth}$ (**Supplementary Fig. 2**). We think that the 17-mer depth was

underestimated due to sequencing errors; thus, the size of the Japanese flounder genome should be slightly smaller than 640 Mb. The assembled contigs and scaffolds covered about 83.4% and 85.2% of the whole genome, respectively.

2. Whole genome alignment

The whole genomes of Japanese flounder and tongue sole, Japanese flounder and zebrafish, and tongue sole and zebrafish were aligned using their repeat masked genomes and LASTZ⁵¹. The results showed that the whole genome syntenic was 394 Mb, accounting for 86.2% and 84.3% of the tongue sole and Japanese flounder genomes, respectively (**Supplementary Fig. 3** and **Supplementary Table 4**).

3. Gene structural prediction and annotation

We first generated 20.5 Gb of transcriptome sequences to aid in the gene annotation (**Supplementary Table 5**). We used *de novo*, homology-based, and RNA-seq-based methods to predict the genes in the Japanese flounder genome. We predicted 24,402 and 28,024 genes using a *de novo* method, based on Augustus⁵² and Genscan⁵³, respectively. We also identified 18,825-23,908 homologous genes from eight species. We further downloaded 10,997 flounder EST sequences and obtained alignments for 7,145 genes. Glean⁵⁴ was run to combine the sequences and obtain a primary non redundant gene set that included 20,295 genes. Additionally, RNA-seq data were mapped to the genome to annotate 19,486 genes. Finally, homology-based, *de novo* derived, EST-mapped and transcribed gene sets were merged to form a comprehensive and non redundant gene set. We did some manual checking to obtain the final reference gene set containing 21,787 genes (**Supplementary Table 6**). Totals of 18,122, 15,359, 16,015, 19,778, and 20,714 genes were annotated from the reference gene set using the InterPro, GO, KEGG, Swissprot, and TrEMBL databases, respectively (**Supplementary Table 7**). A total of 688 genes were not annotated by known databases (**Supplementary Table 7**).

4. Evolution of gene families

We constructed gene families based on coding sequences and protein sequences from eight teleost species, including *Oreochromis niloticus*, *Cynoglossus semilaevis*, *Danio rerio*, *Gasterosteus aculeatus*, *Oryzias latipes*, *Takifugu rubripes*, *Tetraodon nigroviridis*, *Paralichthys olivaceus*, and human as an outgroup species. We used Treefam methodology to define a gene family as a group of genes that descended from a single gene in the last common ancestor of the species considered. First, BLASTP was used to compare all protein sequences with a database containing protein data sets for all species with an e-value $< 1 \times 10^{-7}$ and conjoined fragment alignments for each gene pair by Solar. Then, Hcluster_sg was used to cluster gene families. We finally obtained 13,940 and 14,074 gene families for Japanese flounder and tongue sole, respectively (**Supplementary Fig. 4** and **Supplementary Table 8**), and 3,793 single-copy gene families were defined using Treefam⁵⁵. These single-copy gene families were used to reconstruct the phylogeny with the 4d sites extracted from all single-copy gene families using PhyML (v3.0)⁴⁵ (**Supplementary Fig. 4**). We then selected the 291 conserved proteins (gap% < 5 and identity% > 0.65) to estimate species divergence time based on the calibration times using the MCMCTREE program in the PAML package⁵⁶. The results indicate that the two flatfish species were clustered in a sub-branch with tilapia, even though they diverged 72 million years ago, indicating that perciforms are their closest related sister group (**Fig. 1b** and **Supplementary Fig. 5**). This divergence time is longer than previously reported (52 Mya)⁵⁷, mainly owing to the fewer fish taxa with whole genomes and calibration points used in this study.

Japanese flounder and tongue sole are members of flatfish and show some unique characteristics, such as eye migration during metamorphosis. We abstracted 153 gene families clustered only in flatfish (**Supplementary Fig. 6**). The genes in such families were enriched in the GO terms of collagen (GO: 0005581, $P = 0.02309$), microtubule (GO: 0005874, $P = 0.03622$), regulation of appetite (GO: 0032098, $P = 0.01934$) and

protein polymerization (GO: 0051258, $P = 0.03254$), and seven KEGG pathways related to (i) cell proliferation and apoptosis, (ii) cytokines and immune responses, and (iii) carbohydrate metabolism (**Supplementary Tables 9 and 10**).

We identified 15 expanded gene families and 164 contracted gene families in flatfish ancestors. Among the 15 expanded gene families, 12 showed significant expansion (Fisher's exact test, $P < 0.05$) (**Supplementary Table 11**). The GO and KEGG analyses of the known genes in these families suggested they have important roles in flatfish metamorphosis and benthic adaptation (**Supplementary Tables 12 and 13**). The homeodomain-interacting protein kinase (Hipk) family is of particular interest, as it is a serine/threonine-protein kinase involved in p53-mediated cellular apoptosis and regulation of eye size, lens formation, and retinal lamination during late embryogenesis⁹. The *hipk* gene family has clearly expanded in flatfish ancestors (22 *hipk* genes in tongue sole, 21 in Japanese flounder, 5 in zebrafish, 8 in medaka, 5 in stickleback, 13 in tilapia, 6 in *Takifugu*, and 6 in *Tetraodon*; equal variance *t*-test, $P = 0.00847$). A phylogenetic analysis of all fish Hipk proteins showed two major clusters. One cluster (**Fig. 1c**, left side) comprised three subgroups of Hipk1, Hipk2, and Hipk3, which are distinct from each other. The numbers of genes encoding the proteins in all the species analyzed in these subgroups were very similar and mostly single gene copy. The second cluster (**Fig. 1c**, right side) was enriched in genes that were of the flatfish lineage-specific expansion. In particular, the Hipk2-like clade was expanded in tongue sole, and one Hipk1-like clade was expanded in tongue sole and Japanese flounder. We hypothesize that these expansion events contribute to flatfish-specific metamorphosis. Further functional analysis of the *hipk* gene during flatfish metamorphosis may help determine the role of this gene family in the origin of post-larval asymmetry.

5. Positively selected genes

Based on the phylogenetic tree using human and zebrafish as outgroups, we estimated the rate ratio (ω) of non-synonymous to synonymous nucleotide substitutions using CodeML (PAML package) to examine the selective constraints on candidate genes⁵⁶. After the high-quality alignments of related sequences were obtained as described above, we compared a series of evolutionary models in the likelihood framework using the species trees. A branch model was used to detect the average ω across the tree (ω_0), ω of the appointed branch to test (ω_2), and ω of all other branches (ω_1). We finally identified 106 positively selected genes arising in the flatfish ancestors (**Supplementary Tables 14-16**). A single KEGG pathway (cysteine and methionine metabolism; Fisher's exact test, $P = 2.55 \times 10^{-4}$) was enriched in this gene set and may could reflect the dietary changes that occur during metamorphosis (**Supplementary Table 15**). GO terms related to nucleotide metabolism (GO: 0017076, GO: 0032559, and GO: 0004019) and cell proliferation (GO: 0008283) were enriched (Fisher's exact test, $P < 0.05$) (**Supplementary Table 16**). Some other positively selected genes appeared to be involved in the multiple physiological and morphological changes accompanying flatfish metamorphosis, including axial patterning (*irx5* and *usp16*), neural patterning (*arid1a* and *igsf8*), musculoskeletal restructuring (*bmp1*, *ano6*, *coll10a1*, *pepd*, *nr1d2*, *mybpc1* and *myo1h*) (**Supplementary Table 14**). For example, *irx5* is involved in craniofacial neural patterning in the anterior/posterior and dorsal/ventral axes⁵⁸. Another axis patterning gene, *usp16*, regulates *hox* gene expression via histone H2A deubiquitination⁵⁹, and *arid1a* and *igsf8* play roles regulating neurite outgrowth and maintaining the neural network in the brain during metamorphosis, as massive restructuring of skeletal and body musculature occurs during this time⁶⁰. We also found some critical phototransduction pathway genes under positive selection in the tongue sole and Japanese flounder flatfish ancestors, such as *gucy2f*, which encodes an enzyme involved in cGMP resynthesis required for recovering the dark state after phototransduction, and *grk1*, that encodes a retina-specific kinase involved in the adaptation to changing light conditions via cone

opsin phosphorylation⁶¹. Besides, no positively selected gene was detected in tongue sole, whereas four genes (*grk6*, *grk7*, *pde6b* and *guca1b*) were under positive selection in only Japanese flounder. For examples, the *pde6b* participates in processes of transmission and amplification of the visual signal⁶². The *guca1b* encoding a protein that regulates the Ca²⁺ concentration during recovery of rod photoreceptors after light exposure⁶³. The divergence in genes under positive selection between the Japanese flounder and tongue sole we hypothesize (**Supplementary Tables 17 and 18**); i) reflects species-specific specializations, such as the sensory papillae, present in tongue sole⁸ and absent in Japanese flounder and also, ii) lineage specific subfunctionalization and the genetic redundancy widely reported in genomes of higher organisms⁶⁴

In order to detect which of the 106 genes identified in flatfish lineage are also under selection in other non-flatfish species, we ran the software condeml in the PAML package using the same conditions and identified the genes that are under positive selection in other species; (*D. rerio*, 0; *G. aculeatus*, 34; *Oryzias latipes*, 10; *Oreochromis niloticus*, 15; *Tetraodon nigroviridis*, 17; *Takifugu rubripes*, 9) (**Supplementary Table 19**). Of note is the failure in non-flatfish to detect positively selected genes involved in thyroid hormone signaling, retinoic acid signaling and phototransduction pathways among the non-flatfish with the exception of *notch2* in *O. niloticus* and *rdh14* in *T. nigroviridis*.

We checked the positively selected amino acids (AA) of the deduced proteins of the 106 genes in detail using a branch-site model. We found several deduced proteins had common positively selected AAs in flatfish. Importantly, most positively selected AAs were located in functional domains. For example, flatfish-specific mutations possibly resulting from positive selection in the CUB domain of Bmp1, the IG-like and immunoglobulin domains of Mybpc1, as well as the amino peptidase P and N-terminal domains of Pepd could affect the formation and metabolism of bone, muscle, and collagen. The extensively specific glycosyl hydrolase family 35

mutations in *Glb1* indicate that nutrition is important for initiating metamorphosis. In addition, specific mutations in the corresponding functional domains of *Irx5*, *Gucy2f* and *Plat*, which are involved in physiological changes and benthic adaptation, may affect flatfish metamorphosis (**Figure 2c** and **Supplementary Fig. 7**).

6. Common gene expression patterns during flatfish metamorphosis

We first obtained the differentially expressed genes (DEGs) from four developmental stages using the pairwise comparisons for tongue sole and Japanese flounder, respectively. A total of 6,496 and 8,117 DEGs were identified in tongue sole and Japanese flounder, respectively. The 2,307 orthologs of the DEGs for the two flatfish species were retrieved and enriched in KEGG pathways (Fisher's exact test, $P < 0.05$), that included immunity (map04610 and map04910), nutrition metabolism and biosynthesis (map00100, map04975 and map04974), tissue and organ morphogenesis (map04512 and map04380), visual adaptation (map04744), and TH action (map04919 and map04918) (**Supplementary Table 20**). The 2,307 orthologs of the DEGs across all four time points were grouped into two and three clusters for Japanese flounder and tongue sole, respectively, using the K-means clustering algorithm (**Supplementary Figs. 8 and 9**). In total, 624 Japanese flounder cluster A genes decreased in post-metamorphic larvae, and these genes were enriched in the steroid hormone biosynthesis pathway. The 1,574 Japanese flounder cluster B genes were highly expressed in post-metamorphic larvae and enriched in genes of calcium signaling and phototransduction pathways (**Supplementary Fig. 9** and **Supplementary Tables 21 and 22**). The 651 tongue sole cluster A genes were highly expressed during the pro-metamorphic stage and decreased at the metamorphic climax and were enriched in osteoclast differentiation and transforming growth factor- β signaling pathways. The 646 cluster B and the 990 cluster C genes were enriched in the phototransduction pathway (**Supplementary Fig. 9** and **Supplementary Tables 23-25**).

Gene expression levels at the pre-metamorphic stage were used as a reference, and expression levels at the pro-metamorphic, metamorphic climax, and post-metamorphic stages were labeled as up or down regulated relative to this group. We found 746 orthologs with the same labels in Japanese flounder and tongue sole. We sorted the gene expression levels of the four developmental stages (as levels 1, 2, 3, and 4) of Japanese flounder and tongue sole, respectively. Finally, 146 genes exhibiting a consistent expression pattern during metamorphosis were identified and were enriched in the phototransduction KEGG pathway (Fisher's exact test, $P = 6.84 \times 10^{-9}$) (**Supplementary Fig. 10** and **Supplementary Table 26**). We further divided the consistent gene set into subgroups. One subgroup of genes upregulated during pre-metamorphosis was enriched in the fat digestion and absorption ($P = 0.0016$), gastric acid secretion ($P = 0.01354$) ether lipid metabolism ($P = 0.01354$), and HTLV-I infection ($P = 0.0180$) KEGG pathways, indicating the requirement for appropriate larval nutrition before metamorphosis. A significant proportion of genes (9/49) acting in anterior-posterior patterning and neural development were detected during this stage, such as *hoxb6b*, *hoxa10*, *hoxc9*, *pax2a*, *atcay*, *wnt1*, *efna3*, *ecell* and *nfatc4*. Genes involved in neural development maintained high expression levels until the pro-metamorphic stage, particularly those for the brain, such as *eng2a*, *wnt1*, *atcay* and *kcnj10*, suggesting a critical role for neural remodeling during metamorphosis. The subgroup that was upregulated during the climax of metamorphosis contained 29 genes, many of which are involved in tissue remodeling (*arg2*, *syk*, *myf6* and *bhe40*), cell proliferation (*ccar1* and *rad21*), and development of photoreceptor cells (*olfm3* and *yieg*). The *crhr2* gene, the protein product of which controls corticotrophin release, was upregulated, which is similar to anurans where it stimulates thyrotropin during metamorphosis⁶⁵. The third subgroup was upregulated in the post-metamorphic stage and was enriched in phototransduction ($P = 2.6 \times 10^{-13}$), olfactory transduction ($P = 3.58 \times 10^{-5}$), and chemokine signaling pathway genes ($P = 0.0004$) (**Supplementary Table 27**). About one-quarter (20/75) of the genes were directly involved in visual adaptation. Other genes had functions related to neurodevelopment (*plp*, *pacsin1*, *avil*, *nefl* and *fabp7*), osmotic regulation (*scn4aa*,

best2, *clic6* and *kcna1*), and scale development (*st3gal4*, *stx11* and *nptn*), which accommodate benthic adaptation.

The essential role of THs in flatfish metamorphosis was reiterated by the detection of 21 differentially expressed orthologs of the thyroid hormone signaling pathway between different metamorphic stages of tongue sole and Japanese flounder (Supplementary Table 28).

7. Whole-mount immunohistochemistry and immunofluorescence

The negative control of larvae used for immunohistochemistry were treated with a secondary antibody but not the primary antibody. Whole-mount preparations were analyzed for tri-iodothyronine (T3) or thyroxine (T4) immunohistochemistry modified from the protocols described in⁴⁹. Briefly, larvae were incubated in MDH solution (methanol: DMSO: 30% H₂O₂, 4:1:1) for 5-8 h to inhibit endogenous peroxidase activity. The larvae were rinsed, frozen by submerging in methanol at -20 °C, and thawed at room temperature four times to expose T3 and T4 to the antibodies. The larvae were rinsed in different ratios of methanol and 0.5% Triton X-100 in phosphate-buffered saline with Tween 20 (PBST). Proteinase K (100 µg/mL in PBST) was used to digest the larvae at 37°C for 30-60 min (size dependent; smaller fish were more sensitive to Proteinase K). The larvae were rinsed three times with PBS for 20 min. The specimens were fixed in 4% PFA to maintain tissue integrity and rinsed three times in phosphate-buffered saline plus 1% Triton X-100 (PBSTX) for 20 min. Non-specific binding was blocked by incubating larvae with a blocking solution (Roche Applied Systems, Mannheim, Germany) for 10 h at room temperature. The larvae were incubated with a mouse monoclonal anti-T4 or anti-T3 antibody (Fitzgerald, North Acton, MA, USA) at room temperature for 10 h. After washing 12 times with PBST containing 0.5% Triton X-100 for 1 h, the larvae were incubated with a horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (Immunology Consultants Laboratory, Newberg, OR, USA) or fluorescein

isothiocyanate (FITC) conjugated goat anti-mouse secondary antibody (Fitzgerald) at room temperature for 3 h. After rinsing in PBSTX and PBS, the larvae were placed in 3-amino-9-ethyl carbazole (AEC; Amresco, Solon, OH, USA) solution until the appropriate staining density was attained. Staining was terminated by two PBS washes for 10 min each. The larvae were treated in a 1 M NaOH solution containing 3% H₂O₂ under bright light for 15 min to remove pigmentation. After washing in PBS, the specimens were stored in PBS for photography. Whole-mount specimens were observed and photographed under a dissecting microscope (Olympus, Tokyo, Japan) and documented using Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD, USA). After rinsing the specimens in blocking solution, PBSTX, and PBS, larval immunofluorescence was observed and photographed under a LSM710 NL0 laser scanning microscope (Carl Zeiss, Oberkochen, Germany).

8. Whole-mount *in situ* RNA hybridization

The larvae were rehydrated in a series of solutions with different ratios of methanol and PBS (80%, 60%, 40%, and 20%) for RNA *in situ* hybridization and then with 100% PBS for 20 min. The larvae were treated with 0.1 M PBS containing 100 mM glycine for 30 min to eliminate the PFA. Endogenous alkaline phosphatase activity was quenched with 0.2 M HCl for 30 min, followed by two 20 min washes in PBS. The larvae were treated in a 1 M NaOH solution containing 3% H₂O₂ under bright light for 15 min to remove pigmentation, followed by two rinses in PBS for 15 min each. The larvae were permeabilized by incubation in 0.3% Triton X-100 in PBS for 1 h, followed by two rinses in PBS for 20 min each. Proteinase K was used to digest the larvae for 60 min to further permeabilize the specimen, followed by refixation in 4% PFA and two rinses in PBS for 20 min each. The larvae were incubated for 4 h in prehybridization buffer (4× SSC with 50% formamide) and hybridized with 1 ng/μL labelled probe for 16 h. The hybridization buffer consisted of 50% formamide, 1% blocking reagent (Boehringer, Mannheim, Germany), 4×SSC, 10 mM DTT, 1 mg/mL yeast t-RNA, and the labelled probe. After hybridization, the larvae were washed

through a graded series of SSC (4×, 3.5×, 3×, and 2.5×) containing 50% formamide for 20 min at each concentration. The specimens were further washed in 2× SSC for 20 min, 0.2× SSC twice for 40 min, and finally in 50 mM PBS twice at room temperature for 20 min. The temperature for prehybridization, hybridization, and subsequent washes depended on the specific probe. Nonspecific immunoreactivity was blocked by incubating the larvae in 1% bovine serum albumin (BSA) and 0.4% Triton X-100 in PBS for 1 h at 37°C. The larval specimens were incubated for 4 h with anti-DIG-Fab-AP conjugate diluted 1:1000 (Boehringer) at 37°C. Excess anti-DIG-Fab-AP was removed by five rinses (20 min each) in PBS, and the signals were visualized after incubation in Fast Red Detection Solution (Boehringer) followed by an overnight wash in PBS and storage in 4% PFA at 4°C. Whole-mount specimens were observed and photographed under a dissecting microscope (Olympus) and documented using Image-Pro Plus 6.0 software.

9. Two- and three-color fluorescence *in situ* RNA hybridization

The retinoid x receptors (*rxrs*) and retinoic acid receptor (*rars*) RNA probes were prepared for two-color FISH by *in vitro* transcription from the T7 and SP6 promoters incorporating the Digoxigenin RNA Labeling Mix (Roche). Antisense RNA probes directed against digoxigenin were simultaneously hybridized overnight at 46 °C. After hybridization and washes in buffer, the larvae were developed for *rars* and *rxrs* using an anti-digoxigenin-Rhodamine antibody (Roche). At the same time, other RNA probes (*thrs*) were prepared by *in vitro* transcription from the T7 and SP6 promoters using the Biotin RNA Labeling Mix (Roche). Antisense RNA probes directed against biotin were simultaneously hybridized overnight at 46 °C. After hybridization and washes in buffer, the larvae were developed for *thrs* using anti-biotin-FITC (Sigma-Aldrich).

We labeled related mRNAs with digoxigenin, biotin, and fluorescence for three-color FISH. *rxra*, *rarb*, *rh1*, and *opn4* were labeled with DIG RNA Labeling Mix (Roche), and *sws2* and *kit* were labeled with Biotin RNA Labeling Mix (Roche). *sws2*, *kit*, and

dct were labeled with Fluorescence RNA Labeling Mix (Roche). The probes were mixed and hybridized overnight at 46 °C. After washing in buffer, the larvae were developed for the digoxigenin-labeled genes using an anti-digoxigenin-Rhodamine antibody (Roche). Larvae were developed for the biotin-labeled genes using DyLight TM405-IgG Fraction Monoclonal Mouse Anti-Biotin (Jackson Immuno Research Laboratories, West Grove, PA, USA). Larvae were developed for the fluorescence-labeled genes using anti-fluorescein (Roche). Whole mount *in situ* hybridization was carried out and processed using FISH methodology⁵⁰. The signal from antisense probe was photographed using an LSM 710 confocal microscope (Carl Zeiss, Jena, Germany), while no signal was detected from sense probe.

10. Whole-mount *in situ* cell proliferation assay

Larvae were treated with BrdU (Sigma-Aldrich) for 8 h, anesthetized in 0.1 µg/µL MS-222, fixed in 4% PFA overnight, and stored in methanol at 4°C. Larvae that were not labeled with BrdU were treated as negative controls. Whole-mount preparations were analyzed for BrdU immunostaining according to the techniques developed by Bao et al³⁰. Briefly, the larvae were rinsed in 0.1 M PBS after fixation, bleached with 3% H₂O₂ in 25 mM NaOH under strong light, and transferred to 2 M HCl at 37°C for 90 min. After neutralization with 0.1 M sodium borate for 20 min, the fish were rinsed three times with 0.5% Triton X-100 in PBST for 20 min. Proteinase K was used to digest the fish at 37°C for 30-60 min. Digestion was terminated by adding 100 mM glycine for 20 min. The larvae were rinsed twice with 0.5% Triton X-100 in PBST for 20 min each. Non-specific antisera binding was blocked using 5% goat serum, 1% BSA, and 0.5% Triton X-100 in PBST at 37°C for 90 min. The larvae were incubated with mouse monoclonal anti-BrdU antibody (Sigma-Aldrich) at 4°C overnight. After several washes in PBST containing 0.5% Triton X-100, the larvae were incubated with an HRP-conjugated goat anti-mouse secondary antibody (Immunology Consultants Laboratory) at 4°C overnight. After rinses in PBST and PBS, the larvae were placed in AEC solution until the appropriate staining density was attained. The

AEC stock solution was prepared by dissolving 10 mg AEC in 1 mL N, N-dimethylformamide (Amresco). Whole-mount specimens were observed and photographed under an SZX7 dissecting microscope (Olympus) and documented using Image-Pro Plus 6.0 software.

11. Detection of RA concentration by UPLC-MS/MS

The supernatant was analysed immediately by UPLC-MS/MS. The samples were protected from light and only manipulated and stored using glassware. The samples were separated using a Waters Acquity UPLC System with UPLC BEH C18 column (100mm × 2.1mm, 1.7µm) (Waters, Milford, MA). The mobile-phase consisted of (A) 0.1% formic acid in acetonitrile/methanol/water (40:30:30) and (B) 0.1% formic acid in acetonitrile/methanol/water (55:30:15). A linear gradient was generated at 400µl/min: 0-0.5 min, 0% B; 1.5 min, 30% B; 6.5-7.5 min, 100%B; 7.51-9.5 min, 0%B. The injection volume was 2µL. The column was maintained at 40°C and mass spectrometry was performed on a Triple Quad™5500 (AB Sciex, Framingham, MA) with ESI (electro spray ionization) sources in positive electrospray mode. Source temperature: 550°C, curtain gas (CUR): 30 psi, ion source gas 1 (GAS1): 50 psi, ion source gas 2 (GAS2): 50 psi, collision gas (CAD): 8 psi, ion spray voltage (IS): 5000V, entrance potential (EP): 7V, collision cell exit potential (CXP1): 10V. For quantification of samples, a stock solution of standard ATRA and 9CRA (Sigma-Aldrich, St. Louis, MO) was prepared in methanol and the working concentration was diluted to 1, 10, 50, 100, 200 or 1000 ng/mL with Acitretin 100 ng/mL. RAs were identified by comparison of their retention times (Rt) with those of the standards. We used the scheduled multiple reaction monitoring (S-MRM) mode for qualitative and quantitative analysis, with the following parameters (calibration curves of ATRA and 9-cis-RA were produced with $y=0.0283x+0.3155$ and $y=0.0083x+0.3308$).

12. Cross-talk between THs and RA during eye migration

Flatfish metamorphosis might involve cross-talk between THs and the RA system. Thus, we investigated the role of THs in detail by studying for the key components of the TH pathway as well as the RA signaling pathway in Japanese flounder. The asymmetric distribution or expression of *T4*, *T3*, *dio2*, *dio3*, *thraa*, and *thrb1* around climax of metamorphosis coincided with the pattern of proliferating cells around the eyes during metamorphosis (**Supplementary Figs. 11-18**). We also performed MMI treatment on pre-metamorphic larvae and found that MMI blocked flatfish metamorphosis inhibiting eye migration and reducing the asymmetric expression of *dio3*, *thraa* and *thrb1* (**Supplementary Figs. 19-21**). In order to see whether exogenous THs can rescue the MMI effect, larvae were rescued by administering T3 after MMI treatment. The results showed that MMI treatment for four days induced high mortality and dramatically slowed the progress of metamorphosis compared to control groups. No larvae of the MMI treatment group achieved climax of metamorphosis while 63% of control larvae reached metamorphic climax. After rescue by T3, about the 81% and 72% of larvae treated with MMI underwent metamorphosis and achieved the post-metamorphic stage as the control group (**Supplementary Table 29**). Therefore, exogenous THs rescue the effect caused by MMI treatment.

RAs are ligands of Rars nuclear receptors (ATRA is a ligand) and Rxrs (9-cis-RA is a ligand), which form heterodimers with Thrs during flatfish metamorphosis (**Supplementary Figs. 22 and 23**). We found enrichment of the RA signaling pathway in DEGs during metamorphosis (**Supplementary Table 30**). *thrs* and *rxrs* were asymmetrically expressed around the Japanese flounder blind eye during metamorphosis, whereas *rara*, but not the other *rars*, was also asymmetrically expressed (**Supplementary Fig. 24**). However, only the heterodimers (Rxra/Thra, Rxra/Thrb, Rara/Thra, and Rara/Thrb) exhibited a consistent left-right asymmetric distribution in epithelial tissue around the eyes during the pro-metamorphosis stage

(**Supplementary Fig. 24**). Other genes involved in RA metabolism, such as *cyp26*, *rbp2*, and *aldh1-3*, showed different degrees of asymmetric expression around metamorphic climax (**Supplementary Figs. 25 and 26**). Furthermore, treatment with MMI, an inhibitor of TH, significantly decreased expression of the *rars* and *rxrs*, indicating a strong interaction between TH and RA-dependent signaling (**Supplementary Fig. 27**).

We injected the 9-cis-RA, ATRA, 13-cis-RA and DMSO (control) into the suborbital area of the prospective blind side eye of pre-metamorphic Japanese flounder larvae, respectively. In 9-cis-RA treatment, 83 individuals survived: 40 pre-metamorphic larva (48%), 34 pro-metamorphic larva (41%), 5 at metamorphic climax (6%), and 4 post-metamorphic juveniles (5%). In ATRA treatment, 99 individuals survived: 24 pre-metamorphic larva (34%), 60 pro-metamorphic larva (52%), 12 at metamorphic climax (10%) and 3 post-metamorphic juveniles (3%). In 13-cis-RA treatment, 59 individuals survived: 25 at metamorphic climax (42%), and 34 post-metamorphic juveniles (58%). In the control group with DMSO treatment, 70 individuals survived: 24 at metamorphic climax (34%) and 44 post-metamorphic juveniles (63%). Only 5% and 3% of pre-metamorphic larvae which were injected with either 9-cis-RA or ATRA, respectively, in the suborbital area of the blind side eye, completed metamorphosis and developed into normal juvenile flatfish. This is significantly fewer larvae than those that completed metamorphosis following treatment with the non competitive Rxra ligand 13-cis-RA (58%) and the DMSO control (63%) ($P < 0.05$) (**Fig. 3b and 3c** and **Supplementary Fig. 28**). The right eye of larvae that failed to complete metamorphosis remained on the blind side, and such larvae were pigmented on both sides (**Fig. 3c** and **Supplementary Fig. 29**). Proliferation of cells in the suborbital area of the blind-side eye decreased significantly in 9-cis-RA- and ATRA-treated larvae compared with that in controls (**Fig. 3c**). *rara* expression, but not expression of the other *rars*, was upregulated after ATRA treatment, whereas expression of the two *thrs* was down-regulated (**Supplementary Figs. 30 and 31**).

13. Asymmetric pigmentation is controlled by RA

We firstly determined the concentration of RA in the pigmented (ocular) and the unpigmented (blind) side of juvenile fish skin. The concentration of ATRA and 9-cis-RA in the ocular side skin (0.0396 ng/mg and 0.1430 ng/mg, respectively) were significantly higher than that of the blind side skin (0.0036 ng/mg and 0.0172 ng/mg, respectively) (Fisher's exact test, $P < 0.05$) (**Supplementary Fig. 32**). We also conducted bioinformatics analysis to investigate the basis of asymmetry in the pigmentation pattern by comparing the transcriptomes obtained from the ocular and blind side skin of Japanese flounder and tongue sole (**Supplementary Figs. 33 and 34**). In particular, tyrosine metabolism (map00350, $P = 0.00012$) was the pigmentation-related KEGG pathway enriched for the up-regulated ocular side orthologs (**Supplementary Table 31**). We further detected components of a RA-induced pathway, which may be involved in pigmentation during flatfish metamorphosis including *aldh1*, *aldh2*, and *aldh3* gene expression. *aldh1* had a high expression before metamorphosis and decreased during pre-metamorphosis, and then reached a maximum at the climax stage. *aldh2* was also highly expressed before metamorphosis and then decreased to a relatively low level during metamorphosis. *aldh3* expression peaked post-metamorphosis (**Supplementary Fig. 35**). *aldh3* and *rxra*, but not the other *aldhs*, *rars*, or *rxrs*, were up-regulated on the ocular side compared to the blind side in post-metamorphic stages (**Figure 4a** and **Supplementary Figs. 35-38**). We injected 9-cis-RA, ATRA, or retinol into the dermal skin on the blind side of pre-metamorphic Japanese flounder to determine whether pigmentation would be affected (**Supplementary Fig. 39**). The 9-cis-RA and ATRA treatments increased the number of adult type chromatophores on the blind side of post-metamorphic fish, whereas retinol and DMSO treatments resulted in only a few adult type chromatophores (**Supplementary Figs. 40 and 41**). RA synthesis relies on opsins, which are expressed in the retina⁶⁶. We were struck by the ubiquitous expression of the genes of the phototransduction pathway (*rh1*, *rh2*, *lws*, *sws1* and *sws2*) in the skin during flatfish metamorphosis (**Fig. 4b** and **Supplementary Figs.**

42-46). To assess if this was flatfish specific we checked whether other teleost fishes express genes of the phototransduction pathway in skin. *In situ* hybridization revealed that opsins (*sws2* and *rh2*) are expressed both in the skin and eye of zebrafish (**Supplementary Fig. 47**). Using qPCR we also detected expression of *sws1* and *sws2* in the skin of other fish species including *Salmo salar*, *Scomberomorus niphonius*, *Cololabis saira* and *Hemirhamphus quoyi* (**Supplementary Fig. 48**), suggesting that the expression of opsins in the skin is a universal phenomenon in teleost species.

A series of experiments were performed to test our hypothesis that exposure to different wavelengths of light (**Supplementary Figs. 49 and 50**, and **Supplementary Table 32**) drives pigmentation and eye migration, possibly through release of opsin-bound retinol, which acts as a substrate for RA synthesis.

Supplementary Tables 1-33

Supplementary Table 1|Parameters of genome sequencing of Japanese flounder.

Pair-end libraries	Insert Size	Total Data(G)	Average Reads Length(bp)	Sequence coverage (X)	Physical coverage (X)
Solexa Reads	170bp	8.97	100	14.00	11.90
	500bp	19.45	150	30.36	50.59
	800bp	10.07	100	15.72	62.87
	2kb	17.05	44	26.61	604.66
	5kb	8.46	44	13.20	750.14
	10kb	7.34	44	11.46	1,302.54
	20kb	3.27	90	5.10	567.11
	40kb	1.16	49	1.81	737.47
Total		75.77		118.26	4087.29

Note: Assuming the Genome Size is 640 Mb.

Supplementary Table 2|Statistics of the assembly of the Japanese flounder genome.

	Contig		Scaffold	
	Size(bp)	Number	Size(bp)	Number
N90	8,364	17,917	973,391	147
N80	13,662	12,996	1,570,469	102
N70	18,837	9,683	2,470,950	75
N60	24,383	7,184	3,028,914	55
N50	30,544	5,224	3,880,601	40
Longest	275,324	-	13,318,689	-
Total Size	534,276,329	-	545,838,344	-
Total Number(>100bp)	-	38,618	-	7,200
Total Number(>2kb)	-	27,307	-	395

Supplementary Table 3|General statistics of anchored chromosomes in Japanese flounder.

Chr.	No. SSR	No. SNP	Contig		Scaffold	
			No.	Len.(bp)	No.	Len.(bp)
1	76	875	1,547	27,227,400	16	27,809,231
2	39	127	1,249	20,154,459	10	20,487,909
3	23	706	1,319	22,942,573	10	23,205,554
4	53	527	1,070	18,685,075	8	18,984,529
5	54	584	1,413	25,274,221	10	25,845,326
6	50	742	1,393	24,195,648	9	24,618,968
7	65	253	1,635	29,029,411	13	29,553,310
8	43	138	1,000	15,998,983	13	16,362,470
9	51	407	1,051	14,551,144	19	14,864,337
10	46	333	1,457	21,291,773	12	21,848,933
11	72	573	1,339	22,214,803	8	22,768,616
12	56	787	1,459	25,143,476	11	25,700,457
13	47	686	1,123	19,426,733	5	19,776,027
14	43	421	861	10,812,997	12	11,148,741
15	42	509	1,420	23,389,190	10	23,797,465
16	70	402	1,098	16,612,136	12	17,161,204
17	38	717	1,516	24,750,962	11	25,187,319
18	63	287	1,132	18,875,898	8	19,411,368
19	52	484	1,473	25,812,998	12	26,391,452
20	58	824	1,235	22,380,021	6	22,916,264
21	48	492	1,450	24,811,381	6	25,308,345
22	48	590	1,199	19,650,595	10	19,991,562
23	40	603	1,585	28,737,726	13	29,304,099
24	27	645	1,306	22,442,453	12	22,799,695
Total	1,204	12,712	31,330	524,412,056	256	535,243,181

Supplementary Table 4|Syntenic regions among different teleost species.

Species vs Species	Aligned Length (bp)	Target Genome Coverage Rate %	Query Genome Coverage Rate %
Pol vs Cse	393,780,132	86.22	84.32
Dre vs Pol	293,532,799	54.18	52.61
Dre vs Cse	175,101,522	38.28	32.39

Note: Pol, Japanese flounder; Cse, tongue sole; Dre, zebrafish

Supplementary Table 5|Transcriptome sequencing data statistics.

Species	Sample name	No. of read pairs	Read1 len. (bp)	Read2 len. (bp)	No. of base pairs (G)
Japanese flounder	Pre-	23,654,409	90	90	4.26
	Pro-	25,462,866	90	90	4.58
	climax	25,579,205	90	90	4.60
	Post-	26,202,463	90	90	4.72
	Sbs	6,646,111	90	90	1.20
	Sos	6,407,005	90	90	1.15
Tongue sole	Pre-	28,147,732	90	90	5.07
	Pro-	31,972,668	90	90	5.76
	climax	29,099,627	90	90	5.24
	Post-	27,043,571	90	90	4.87
	Sbs	32,131,226	90	90	5.78
	Sos	32,067,893	90	90	5.77

Note: Pre-, Pre-metamorphic larvae; Pro-, Pro-metamorphic larvae; climax, metamorphic climax; Post-, post-metamorphic juveniles; Sbs, the skins of blind side; Sos, the skins of ocular side.

Supplementary Table 6|General statistics of the predicted protein-coding genes in the Japanese flounder genome. Gene length includes the exon and intron regions but excludes UTRs.

Gene set		Number	Average gene length (bp)	Average CDS length (bp)	Average exon per gene	Average exon length (bp)	Average intron length (bp)
<i>De novo</i>	Augustus	24,402	10,865	1,484	8.6	173	1,234
	Genscan	28,024	14,006	1,594	9.1	175	1,532
Homolog	<i>Gasterosteus aculeatus</i>	23,908	8,229	1,334	8.09	165	972
	<i>Cynoglossus semilaevis</i>	19,233	10,567	1,606	9.6	166	1,037
	<i>Oreochromis niloticus</i>	18,825	10,938	1,689	10.2	165	999
	<i>Danio rerio</i>	23,501	8,324	1,370	7.92	173	1,005
	<i>Oryzias latipes</i>	23,819	7,738	1,293	7.75	167	955
	<i>Homo sapiens</i>	19,701	8,904	1,379	8.3	166	1,031
	<i>Takifugu rubripes</i>	20,816	9,417	1,473	8.94	165	1,001
	<i>Tetraodon nigroviridis</i>	20,563	8,952	1,437	8.82	163	961
EST		7,145	3,358	658	2.96	222	1,380
GLEAN		20,295	13,224	1,786	10.16	176	1,249
RNA-Seq		19,486	13,255	1,813	10.26	177	1,236
Final set		21,787	12,198	1,709	9.8	174	1,189

Supplementary Table 7|General statistics of the functional annotation.

		Number	Percent (%)
Total		21,787	100.00
Annotated	InterPro	18,122	83.18
	GO	15,359	70.50
	KEGG	16,015	73.51
	Swissprot	19,778	90.78
	TrEMBL	20,714	95.08
Unannotated		688	3.16

Supplementary Table 8|Statistics of gene families among 8 species. All other data was downloaded from Ensembl-60. Unclustered genes refer to specific genes of a given species; unique families refers to specific gene families of a given species. A total of 3,793 single copy gene families were detected among the 8 teleost species.

Species	Genes number	Genes in families	Unclustered genes	Family number	Average genes per family
<i>Paralichthys olivaceus</i>	21,787	20,332	1,455	13,940	1.46
<i>Cynoglossus semilaevis</i>	22,121	20,441	1,680	14,074	1.45
<i>Oreochromis niloticus</i>	21,437	21,226	211	13,149	1.61
<i>Oryzias latipes</i>	19,658	18,577	1,081	12,724	1.46
<i>Danio rerio</i>	24,007	22,962	1,045	13,623	1.69
<i>Takifugu rubripes</i>	18,508	18,251	257	12,449	1.47
<i>Tetraodon nigroviridis</i>	19,570	18,663	907	12,688	1.47
<i>Gasterosteus aculeatus</i>	20,756	19,733	1,023	13,313	1.48

Supplementary Table 9|GO enrichment of gene families in flatfish but absent from other teleosts.

GO_ID	GO_Term	GO_class	Gene NO.	<i>p</i> -value
GO:0005581	collagen	CC	3	0.02309
GO:0005874	microtubule	CC	3	0.03622
GO:0032098	regulation of appetite	BP	2	0.01934
GO:0043408	regulation of MAPK cascade	BP	2	0.01934
GO:0051258	protein polymerization	BP	3	0.03254

CC: cellular component; BP: biological process

Supplementary Table 10|KEGG enrichment of gene families in flatfish but absent from other teleosts.

Map ID	pathway	Gene no.	<i>p</i>-value	<i>q</i>-value
map04540	Gap junction	5	0.000168	0.013300686
map04650	Natural killer cell mediated cytotoxicity	4	0.000521	0.014580643
map04630	Jak-STAT signaling pathway	4	0.001628	0.034183385
map00053	Ascorbate and aldarate metabolism	2	0.002397	0.040271313
map04060	Cytokine-cytokine receptor interaction	4	0.004148	0.048301444
map04145	Phagosome	4	0.004377	0.048301444
map00040	Pentose and glucuronate interconversions	2	0.006118	0.048301444

Supplementary Table 11|Expanded gene families ($P<0.05$) in the flatfish ancestor.

family ID	Gene symbol	Protein annotation	Gene number	p- value
894	<i>hipk1,hipk2,hipk3</i> <i>hipk1*,hipk2*</i>	Homeodomain interacting protein kinase	37	0
2265	NA	NA	94	0
2595	<i>f2rl2, gpr4, gpr1</i>	Proteinase-activated receptor; G-protein coupled receptor	16	0
3935	NA	NA	9	0.0003
4142	<i>gabrg3,gabra4,gab</i> <i>rb4,gabrr2,gabrg1</i>	Gamma-aminobutyric acid receptor;	8	0.0003
5881	NA	NA	9	0.0003
8005	<i>cilp1,cilp2</i>	Cartilage intermediate layer protein	12	0.0001
8597	NA	NA	129	0
12771	<i>fndc7</i>	Fibronectin type III domain-containing protein 7	10	0.0001
15600	NA	NA	6	0.0008
15688	NA	NA	6	0.0019
15888	NA	NA	7	0.0003

NA: not anotated

Supplementary Table 12|GO enrichment of expanded gene families in the flatfish ancestor.

GO_ID	GO_Term	GO_class	Gene NO.	p-value
GO:0016021	integral to membrane	CC	23	4.73E-14
GO:0045202	synapse	CC	3	0.02342
GO:0004672	protein kinase activity	MF	31	5.63E-24
GO:0005230	extracellular ligand-gated ion channel activity	MF	8	4.75E-07
GO:0005216	ion channel activity	MF	8	0.01204
GO:0022838	substrate-specific channel activity	MF	8	0.01204
GO:0022803	passive transmembrane transporter activity	MF	8	0.0131
GO:0006278	RNA-dependent DNA replication	BP	11	2.43E-17
GO:0006468	protein phosphorylation	BP	31	1.11E-16
GO:0006260	DNA replication	BP	11	6.96E-11
GO:0043412	macromolecule modification	BP	31	1.09E-10
GO:0006793	phosphorus metabolic process	BP	31	4.81E-10
GO:0044267	cellular protein metabolic process	BP	31	4.86E-07
GO:0006259	DNA metabolic process	BP	11	6.37E-05
GO:0007186	G-protein coupled receptor signaling pathway	BP	16	0.00059
GO:0007166	cell surface receptor signaling pathway	BP	16	0.00627
GO:0034645	cellular macromolecule biosynthetic process	BP	11	0.047

CC: cellular component ; MF: molecular function; BP: biological process

Supplementary Table 13|KEGG enrichment of expanded gene families in the flatfish ancestor.

Map ID	Pathway	Gene NO.	<i>p</i> -value	<i>q</i> -value
map05033	Nicotine addiction	3	3.27E-06	1.63E-05
map04080	Neuroactive ligand-receptor interaction	4	9.25E-06	2.31E-05
map04727	GABAergic synapse	3	2.34E-05	3.47E-05
map05032	Morphine addiction	3	2.77E-05	3.47E-05
map04723	Retrograde endocannabinoid signaling	3	4.09E-05	4.09E-05

Supplementary Table 14|Positively selected genes in the ancestor of flatfish.

Gene name	w0	w1	w2	p-value
<i>pgd</i>	0.04202	0.03965	21.9028	0.001211
<i>ahcy-b</i>	0.03549	0.03235	31.0255	0.000123
<i>slc7a10</i>	0.08029	0.07729	0.75415	0.016539
<i>pepd</i>	0.10148	0.09768	0.55149	0.014876
<i>irx5</i>	0.07320	0.07014	0.64422	0.027727
<i>tgfbr3</i>	0.25158	0.24634	12.5435	0.046934
<i>tmem180</i>	0.12625	0.12173	0.97128	0.033512
<i>bms1</i>	0.08711	0.08525	0.48700	0.028268
<i>wee2</i>	0.13498	0.12898	33.8039	0.008471
<i>nr1d2</i>	0.08438	0.08100	0.44912	0.021679
<i>bmp1</i>	0.08126	0.07877	0.41976	0.012077
<i>pcyox1</i>	0.11359	0.10941	0.33871	0.024755
<i>abcc3</i>	0.10308	0.10021	1.35361	0.000544
<i>cse1l</i>	0.03365	0.03279	0.10414	0.042163
<i>tmem180</i>	0.08098	0.07762	1.23435	0.005636
<i>coll10a1</i>	0.14725	0.14240	0.87366	0.007336
<i>trappc3</i>	0.02382	0.02136	0.44459	0.010637
<i>ccdc28b</i>	0.03164	0.02363	0.41847	5.20E-06
<i>mthfd1</i>	0.05322	0.05170	0.17806	0.025707
<i>cct5</i>	0.03507	0.03334	1.72839	0.007833
<i>mtrr</i>	0.21469	0.20878	1.17313	0.037225
<i>kiaa0100</i>	0.09013	0.08846	0.30834	0.009239
<i>tsr1</i>	0.09563	0.09331	0.47258	0.044278
<i>ltn1</i>	0.15767	0.15473	0.58886	0.022782
<i>smg8</i>	0.05715	0.05556	0.43782	0.044086
<i>smg9</i>	0.06376	0.06129	0.21296	0.025272
<i>hspa8</i>	0.02692	0.02562	0.65637	0.016748
<i>pdp1</i>	0.03550	0.03415	0.25684	0.036512
<i>pcyt1a</i>	0.04709	0.04440	0.32549	0.016753
<i>acacb</i>	0.06653	0.06525	0.21186	0.009149
<i>myo1h</i>	0.07780	0.07579	0.37832	0.027896
<i>aass</i>	0.09038	0.08661	13.4328	0.000312
<i>adss</i>	0.05382	0.05159	0.21827	0.021306
<i>arid1a</i>	0.11043	0.10725	0.31337	0.00044
<i>uspl6</i>	0.20908	0.20349	3.68236	0.03639
<i>dopey2</i>	0.13345	0.13034	0.32665	0.001922
<i>tpp1</i>	0.11882	0.11537	1.84793	0.037581
<i>tmem260</i>	0.19121	0.18662	0.60689	0.049527
<i>adssl1c</i>	0.04568	0.04371	0.20340	0.042556

<i>ubp1</i>	0.08053	0.07757	0.21325	0.046337
<i>sms</i>	0.13939	0.13379	0.91151	0.010793
<i>rdh14</i>	0.09458	0.09029	3.18864	0.015194
<i>sesn1</i>	0.04137	0.03771	0.17875	0.000184
<i>fbxo25</i>	0.11975	0.11111	0.75731	0.002252
<i>acsl5</i>	0.10238	0.09929	0.39074	0.032376
<i>psmd11</i>	0.03323	0.03157	0.17324	0.033096
<i>chmp2a</i>	0.01629	0.01497	0.49912	0.030559
<i>pde12</i>	0.12949	0.12436	0.75150	0.012977
<i>ankrd52</i>	0.08756	0.08560	0.45119	0.013162
<i>acvr1</i>	0.07461	0.07162	0.22462	0.0331
<i>aifm3</i>	0.06645	0.06361	0.23470	0.007634
<i>aldh1l1</i>	0.06246	0.06062	0.30995	0.011923
<i>dnmt1</i>	0.06442	0.06296	1.44937	0.015492
<i>elavl3</i>	0.00174	0.00132	0.03445	0.045068
<i>dhx57</i>	0.12376	0.11962	1.20654	0.001491
<i>spg7</i>	0.03863	0.03745	0.16382	0.041299
<i>nckap1l</i>	0.12989	0.12657	0.30525	0.043246
<i>igsf8</i>	0.10241	0.09867	0.78681	0.028611
<i>gucy2f</i>	0.09609	0.09305	1.16636	0.014573
<i>EIF3L</i>	0.02163	0.02031	0.36049	0.005721
<i>polg</i>	0.09443	0.09084	14.0449	9.49E-05
<i>kars</i>	0.05513	0.05123	0.27775	0.00011
<i>ap1g1</i>	0.02468	0.02362	0.91618	0.008978
<i>lrrc8e</i>	0.10094	0.09637	0.41259	0.001794
<i>msh6</i>	0.09162	0.08834	0.29586	0.001383
<i>gmps</i>	0.03704	0.03589	0.43177	0.011066
<i>cap1</i>	0.10208	0.09861	1.15079	0.046037
<i>notch2</i>	0.06827	0.06552	0.65526	5.76E-07
<i>nr5a2</i>	0.16313	0.15662	88.8421	0.003539
<i>elfn1</i>	0.05731	0.05525	2.40093	0.015123
<i>ipo4</i>	0.13850	0.13546	0.35122	0.030331
<i>dpp3</i>	0.07506	0.07204	0.35879	0.004107
<i>uck1</i>	0.05348	0.05077	64.2040	0.048408
<i>slc35a5</i>	0.12101	0.11664	1.20794	0.047075
<i>drg2</i>	0.03043	0.02879	0.56408	0.013848
<i>aqp7</i>	0.14532	0.13847	1.19628	0.030299
<i>dnmt3b</i>	0.22566	0.22107	1.37619	0.027153
<i>eaf2</i>	0.07235	0.06821	0.46788	0.029152
<i>alcf</i>	0.06114	0.05873	0.33836	0.012868

<i>dap3</i>	0.11939	0.11474	0.55082	0.045142
<i>wsb1</i>	0.07970	0.07577	0.87830	0.001216
<i>syt10</i>	0.06715	0.06487	0.24202	0.029243
<i>chd4</i>	0.04907	0.04696	0.14320	7.08E-05
<i>rtcb</i>	0.01610	0.01485	16.7566	0.022511
<i>plat</i>	0.16728	0.16220	0.95602	0.040334
<i>ubr2</i>	0.09863	0.09648	0.30821	0.024888
<i>ttc37</i>	0.15147	0.14867	0.28463	0.031847
<i>inpp5a</i>	0.11693	0.11057	1.32929	0.000956
<i>abcb7</i>	0.07479	0.06484	1.14412	8.31E-13
<i>stag2</i>	0.03834	0.03727	0.16903	0.010295
<i>sqrcl</i>	0.08807	0.08409	2.41350	0.016087
<i>dbt</i>	0.09014	0.08585	5.25405	0.001624
<i>srpr</i>	0.09284	0.09031	0.55642	0.032708
<i>ocln</i>	0.13551	0.12969	1.12188	0.049204
<i>cyp4v2</i>	0.10191	0.09556	8.21253	0.001845
<i>ano6</i>	0.07988	0.07733	0.20658	0.03097
<i>serpine2</i>	0.12604	0.11743	0.76941	0.000727
<i>mybpc1</i>	0.07349	0.07100	0.59873	0.002066
<i>pcdh11x</i>	0.03758	0.03671	0.16827	0.037786
<i>lrfr2</i>	0.05919	0.05695	0.19664	0.019064
<i>pms1</i>	0.19723	0.19069	12.8651	0.002146
<i>glb1</i>	0.13217	0.12370	2.00650	8.47E-05
<i>pald1</i>	0.08557	0.08336	1.06476	0.024892
<i>pwp2</i>	0.04926	0.04761	1.13906	0.018868
<i>aldh3b1</i>	0.13080	0.12365	1.88219	0.029466
<i>grkl</i>	0.06172	0.05924	0.19752	0.034504

Supplementary Table 15|KEGG enrichment of positively selected genes in the flatfish ancestor.

Map ID	Pathway	Gene NO.	<i>p</i> -value	<i>q</i> -value
map00270	Cysteine and methionine metabolism	4	2.55E-04	2.09E-02

Supplementary Table 16|GO enrichment of positively selected genes in the flatfish ancestor.

GO ID	Description	Category	Sample size	p-value
GO:1901265	nucleoside phosphate binding	MF	26	0.00078
GO:0032553	ribonucleotide binding	MF	24	0.00252
	nuclear export signal receptor			
GO:0005049	activity	MF	2	0.00315
GO:0017076	purine nucleotide binding	MF	23	0.00734
GO:0004019	adenylosuccinate synthase activity	MF	2	0.00942
GO:1901363	heterocyclic compound binding	MF	37	0.018
	ligase activity, forming			
GO:0016879	carbon-nitrogen bonds	MF	6	0.03314
GO:0032559	adenyl ribonucleotide binding	MF	18	0.04404
GO:0044281	small molecule metabolic process	BP	11	0.01877
GO:0044710	single-organism metabolic process	BP	17	0.02957
GO:0008283	cell proliferation	BP	2	0.03727

Supplementary Table 17| Positively selected genes in the Japanese flounder.

Gene symbol	w0	w1	w2	P_value
<i>hnrnph1</i>	0.07419	0.06240	0.25617	6.69E-07
<i>gdi2</i>	0.08131	0.07353	0.22752	0.000211061
<i>snd1</i>	0.05057	0.04446	0.14627	4.32E-07
<i>kiaa0528</i>	0.06202	0.05141	0.22465	0
<i>pgd</i>	0.04202	0.03847	0.11707	0.001632467
<i>eif3b</i>	0.03842	0.03541	0.10511	0.000490131
<i>fkbp5</i>	0.15772	0.15039	0.27753	0.028913639
<i>apitd1</i>	0.13603	0.12198	0.71720	0.00823283
<i>zmynd10</i>	0.15698	0.14488	0.32733	0.002260372
<i>ppl</i>	0.08404	0.08179	0.12609	0.017626669
<i>pbx2</i>	0.03934	0.03653	0.08436	0.0271013
<i>tap1</i>	0.17694	0.16811	0.37564	0.001071894
<i>pdpr</i>	0.05078	0.04594	0.13772	2.45E-06
<i>igflr</i>	0.07075	0.06764	0.11577	0.002245395
<i>gpi</i>	0.06875	0.06526	0.11994	0.046328006
<i>sdr42e1</i>	0.13667	0.12969	0.21852	0.031114756
<i>sec11a</i>	0.03414	0.02124	0.15554	7.61E-07
<i>dhtkd1</i>	0.08404	0.08043	0.15342	0.003884411
<i>dtymk</i>	0.07824	0.06988	0.31789	0.002307289
<i>aqp12</i>	0.19259	0.17711	0.49934	0.004430528
<i>ddr2</i>	0.07272	0.06800	0.14624	0.000223116
<i>ubxn6</i>	0.10018	0.09431	0.19145	0.013248463
<i>dnaja3</i>	0.09246	0.08795	0.17890	0.025658352
<i>pdgfa</i>	0.14130	0.12260	0.28568	0.004412501
<i>tmem180</i>	0.12625	0.11954	0.22825	0.014078302
<i>aocx</i>	0.09061	0.08544	0.16296	0.004027856
<i>ndufa9</i>	0.09756	0.09200	0.17057	0.035191362
<i>agk</i>	0.12111	0.11536	0.19746	0.032005356
<i>lrrc10</i>	0.08619	0.08106	0.23896	0.027483692
<i>anxa2-a</i>	0.09483	0.08729	0.23263	0.006758444
<i>katnb1</i>	0.10568	0.09816	0.27900	0.000300234
<i>sod1</i>	0.09978	0.08674	0.29919	0.002179455
<i>rft1</i>	0.14317	0.12870	0.38419	1.08E-06
<i>creb1</i>	0.08527	0.07987	0.21457	0.018691375
<i>avil</i>	0.11811	0.11303	0.19561	0.009588953
<i>ist1</i>	0.07674	0.07222	0.15547	0.031094569
<i>kif18a</i>	0.13370	0.12888	0.20647	0.022613802
<i>litfl</i>	0.09377	0.08678	0.48765	0.021581612

<i>tubg1</i>	0.00597	0.00524	0.02296	0.027677437
<i>txndc9</i>	0.10751	0.09637	0.37957	0.001860259
<i>rps6ka</i>	0.02469	0.02130	0.08284	2.86E-06
<i>anxa4</i>	0.08563	0.08069	0.23198	0.027564005
<i>drg1</i>	0.02893	0.02507	0.13076	0.000179985
<i>sppl3</i>	0.02333	0.01078	0.29414	0
<i>ftcd</i>	0.11775	0.11069	0.24993	0.003091911
<i>lsg1</i>	0.11350	0.10896	0.18355	0.035103862
<i>aldh7a1</i>	0.03264	0.02999	0.07038	0.009447354
<i>actl6a</i>	0.02549	0.02166	0.09976	0.00012154
<i>lrpprc</i>	0.20679	0.19349	0.43279	1.07E-06
<i>abcg5</i>	0.08096	0.07681	0.13918	0.013950252
<i>hint1</i>	0.12742	0.11725	0.42548	0.041994347
<i>p4ha1</i>	0.10657	0.09743	0.21959	0.00020375
<i>ano5</i>	0.07810	0.07351	0.14105	0.001269579
<i>ccnd2</i>	0.23839	0.22374	0.42753	0.031128173
<i>col10a1</i>	0.14725	0.14000	0.30603	0.001937096
<i>prkcq</i>	0.04893	0.04538	0.15279	0.00099765
<i>rnpc3</i>	0.14973	0.14368	0.25065	0.038784767
<i>lrrc33</i>	0.20003	0.19126	0.32518	0.013195254
<i>bsdc1</i>	0.09723	0.08764	0.21818	0.000557607
<i>mocs3</i>	0.12889	0.12175	0.25951	0.01360818
<i>methfd1</i>	0.05322	0.05106	0.08535	0.040854545
<i>hsd3b7</i>	0.14063	0.12946	0.27645	0.002005014
<i>gfap</i>	0.09122	0.08467	0.21862	0.001608885
<i>asb16</i>	0.13506	0.12827	0.29497	0.017664497
<i>cacnb1</i>	0.03372	0.03025	0.08209	0.00088785
<i>aldh1l2</i>	0.04619	0.04413	0.07711	0.027389586
<i>EIF4G3</i>	0.15322	0.14982	0.20461	0.046707613
<i>dicer1</i>	0.06828	0.06625	0.09598	0.014307257
<i>sec24d</i>	0.09568	0.09240	0.15176	0.021468946
<i>pcdh17</i>	0.07216	0.06981	0.10606	0.036712959
<i>gba2</i>	0.08512	0.08196	0.14905	0.018535887
<i>slc17a5</i>	0.14995	0.14213	0.23263	0.029314303
<i>ints8</i>	0.06530	0.06220	0.11317	0.005718491
<i>pola1</i>	0.09439	0.09076	0.14347	0.003694459
<i>trpa1</i>	0.16404	0.15726	0.26589	0.003438005
<i>gpr12</i>	0.01745	0.01475	0.05208	0.006004592
<i>kcnj8</i>	0.02123	0.01998	0.09774	0.048359341
<i>mmaa</i>	0.08459	0.08029	0.21329	0.017871741

<i>sept6</i>	0.01306	0.01201	0.04774	0.02421468
<i>xpnpep2</i>	0.14365	0.13812	0.22829	0.024211831
<i>taf7</i>	0.03762	0.0277	0.19169	5.56E-11
<i>tex11</i>	0.19574	0.18909	0.30878	0.01530132
<i>cfl2</i>	0.09056	0.08193	0.22899	0.03716785
<i>tbc1b</i>	0.18397	0.17377	0.37524	0.037071238
<i>clptm1</i>	0.04372	0.03796	0.15442	3.78E-07
<i>rab4b</i>	0.01664	0.01422	0.08081	0.012146011
<i>tmem135</i>	0.10588	0.09859	0.21326	0.005544732
<i>ap2b1</i>	0.01195	0.01098	0.03297	0.006711493
<i>trip13</i>	0.13098	0.11959	0.27373	0.000816109
<i>ccdc12</i>	0.08369	0.06839	0.26788	0.00014327
<i>hspa8</i>	0.02692	0.02530	0.05275	0.048048578
<i>erp44</i>	0.06280	0.05783	0.14675	0.005140464
<i>galnt1</i>	0.03478	0.03285	0.07286	0.035821752
<i>itga6</i>	0.22817	0.21607	0.38395	0.000214023
<i>dnah11</i>	0.07790	0.07368	0.13906	1.56E-10
<i>gpx4</i>	0.12447	0.11222	0.29723	0.039418641
<i>cops2</i>	0.00926	0.00498	0.13398	7.77E-15
<i>gns</i>	0.09588	0.09064	0.19940	0.008088527
<i>syk</i>	0.09660	0.09155	0.20559	0.00226307
<i>slc27a6</i>	0.13189	0.12295	0.31519	0.000913149
<i>tubgcp4</i>	0.03664	0.03093	0.12758	1.31E-08
<i>acacb</i>	0.06653	0.06448	0.09999	0.003082787
<i>slc6a4</i>	0.04827	0.04031	0.15186	8.70E-08
<i>foxf1</i>	0.02704	0.02571	0.09860	0.045066426
<i>erbb2</i>	0.11438	0.11034	0.17469	0.007348164
<i>sult1c1</i>	0.10815	0.10029	0.21850	0.014162355
<i>mtol</i>	0.11982	0.11090	0.25630	0.000155249
<i>lrp2</i>	0.10524	0.10373	0.12414	0.046931516
<i>rbp4b</i>	0.12005	0.10422	0.46144	0.000674121
<i>ccar1</i>	0.08822	0.08458	0.15033	0.006781056
<i>rrp12</i>	0.10560	0.10195	0.16183	0.008467889
<i>zdhhc17</i>	0.03158	0.02916	0.06505	0.009018195
<i>ckmt1</i>	0.05264	0.04841	0.13696	0.002249023
<i>pdia3</i>	0.11592	0.11065	0.20144	0.017717646
<i>slc25a44</i>	0.09394	0.06680	0.55169	0
<i>aass</i>	0.09038	0.08589	0.17168	0.000821475
<i>smc1b</i>	0.19237	0.18587	0.27821	0.01252421
<i>dnajc8</i>	0.03695	0.03337	0.10673	0.007328043

<i>snx27</i>	0.04045	0.03667	0.09021	0.001303843
<i>aco1</i>	0.06726	0.06326	0.15072	0.000110088
<i>cd36</i>	0.17274	0.16430	0.32651	0.010332889
<i>far1</i>	0.04873	0.04074	0.16496	1.48E-08
<i>vezfl</i>	0.04438	0.03817	0.36914	1.64E-07
<i>stip1</i>	0.05643	0.05365	0.10902	0.030156546
<i>ruvbl2</i>	0.02437	0.02293	0.05821	0.043587855
<i>auts2</i>	0.13652	0.12359	0.27487	3.96E-07
<i>suv39h1</i>	0.07306	0.06851	0.25859	0.007342514
<i>ephb2</i>	0.04709	0.04555	0.07464	0.040663241
<i>cog6</i>	0.06666	0.06116	0.16046	7.99E-05
<i>rcc2</i>	0.04704	0.04458	0.13137	0.011401718
<i>zgpap</i>	0.13703	0.12930	0.33920	0.002993231
<i>ddost</i>	0.04347	0.03981	0.12973	0.0014374
<i>rpe</i>	0.03138	0.02804	0.07554	0.030679276
<i>dopey2</i>	0.13345	0.12953	0.19857	0.001528428
<i>myo16</i>	0.13109	0.12604	0.21625	0.000278507
<i>ivd</i>	0.06328	0.05717	0.16736	0.000152048
<i>six1</i>	0.01079	0.00877	0.05425	0.00545175
<i>rab38</i>	0.05566	0.05015	0.14778	0.011344287
<i>sat2</i>	0.13356	0.11662	0.43277	0.000661607
<i>liph</i>	0.22901	0.21696	0.38708	0.012708331
<i>fbxo25</i>	0.11975	0.10526	0.44315	5.35E-06
<i>elf2b2</i>	0.03850	0.03092	0.17819	1.39E-07
<i>elf2b4</i>	0.07702	0.07333	0.15387	0.033108729
<i>brcc3</i>	0.02166	0.01845	0.11775	9.05E-05
<i>ccnb1</i>	0.07817	0.07339	0.14162	0.044991876
<i>alad</i>	0.05307	0.04799	0.20868	0.00237661
<i>atp8b2</i>	0.08581	0.08154	0.15049	0.001257271
<i>gsn</i>	0.06601	0.06248	0.11989	0.010459924
<i>acs15</i>	0.10238	0.09852	0.14982	0.045589603
<i>pdhb</i>	0.05336	0.04916	0.10715	0.014156401
<i>ankrd52</i>	0.08756	0.08378	0.17828	0.005276736
<i>cs</i>	0.02878	0.02621	0.08078	0.006375609
<i>sptbn1</i>	0.05352	0.05185	0.07974	0.006001747
<i>atll</i>	0.03115	0.02784	0.07324	0.000966238
<i>cdkl1</i>	0.06231	0.04927	0.25234	6.45E-09
<i>slc8a3</i>	0.05141	0.04936	0.09703	0.017792004
<i>fgf12</i>	0.07988	0.05657	0.21208	1.78E-06
<i>zmynd8</i>	0.08593	0.08156	0.15044	0.003333641

<i>lrrcc1</i>	0.16201	0.15735	0.24292	0.031071535
<i>gps2</i>	0.06463	0.05210	0.30354	8.73E-09
<i>mls</i>	0.14390	0.13482	0.28936	0.001671302
<i>rhot2</i>	0.08390	0.08060	0.15191	0.030256097
<i>slc17a7</i>	0.05269	0.04492	0.16504	1.27E-07
<i>itfg3</i>	0.31563	0.28857	0.60856	2.94E-05
<i>cp062</i>	0.06391	0.06155	0.10452	0.021771978
<i>abpl</i>	0.11361	0.10946	0.17553	0.02462284
<i>uqcrb</i>	0.12384	0.09860	0.95233	0.000102842
<i>gmpr</i>	0.03669	0.03373	0.08738	0.014559122
<i>ahcyl2</i>	0.03597	0.03392	0.06742	0.045551207
<i>dhx57</i>	0.12376	0.11258	0.28728	3.39E-11
<i>arhgap21</i>	0.12247	0.11973	0.17519	0.034104209
<i>galns</i>	0.11350	0.10742	0.21951	0.014595729
<i>spg7</i>	0.03863	0.03601	0.08844	0.003151969
<i>spon2</i>	0.08772	0.08048	0.20531	0.005064673
<i>iars</i>	0.07079	0.06763	0.12087	0.001914102
<i>nckap1l</i>	0.12989	0.12260	0.23508	0.000158136
<i>tbb6</i>	0.03427	0.03000	0.10691	9.76E-05
<i>abtb1</i>	0.07597	0.07152	0.15849	0.015811614
<i>uckl1</i>	0.02968	0.02267	0.13085	1.67E-11
<i>smek1</i>	0.02147	0.01937	0.06470	0.00019767
<i>pdia6</i>	0.07289	0.06781	0.16090	0.007115758
<i>mus81</i>	0.10712	0.10255	0.16367	0.042571523
<i>gucy2f</i>	0.09609	0.09262	0.13790	0.023272413
<i>pdc</i>	0.09342	0.08151	0.42518	0.00021285
<i>efcab7</i>	0.14277	0.13647	0.24042	0.015302067
<i>slc12a5</i>	0.04651	0.04441	0.08163	0.018499174
<i>cyp24a1</i>	0.06636	0.06197	0.15347	0.008765455
<i>otud6b</i>	0.09927	0.09267	0.22239	0.021082955
<i>kat2b</i>	0.03318	0.03005	0.07961	0.000171039
<i>dad1</i>	0.02044	0.01551	0.07466	0.009376534
<i>prmt5</i>	0.04410	0.04184	0.09461	0.020393061
<i>mmp14</i>	0.08552	0.08205	0.15249	0.03160356
<i>mocos</i>	0.17828	0.17311	0.27812	0.048155006
<i>btafl</i>	0.06072	0.05832	0.10178	0.001150217
<i>etfdh</i>	0.04837	0.04494	0.12707	0.001357633
<i>pan2</i>	0.04408	0.04156	0.08369	0.000777793
<i>unc45a</i>	0.08289	0.07823	0.16008	0.001460575
<i>rfx4</i>	0.05342	0.05097	0.10764	0.018690392

<i>nr1h4</i>	0.11062	0.09759	0.26293	1.12E-05
<i>eeal</i>	0.10775	0.10383	0.17382	0.00337689
<i>polg</i>	0.09443	0.08789	0.22475	5.14E-07
<i>kars</i>	0.05513	0.05106	0.16007	0.00036828
<i>ap1g1</i>	0.02468	0.02145	0.11028	3.34E-06
<i>myole</i>	0.05079	0.04682	0.12143	5.58E-06
<i>rnaseh2a</i>	0.08032	0.07696	0.21011	0.042476441
<i>sptlc3</i>	0.08575	0.07903	0.17961	0.001028011
<i>msh6</i>	0.09162	0.08694	0.17102	8.27E-05
<i>folr2</i>	0.11607	0.10754	0.21024	0.042795549
<i>bbs4</i>	0.08297	0.07765	0.20975	0.00331153
<i>rbm47</i>	0.06799	0.06454	0.17409	0.006006592
<i>dyrk2</i>	0.04402	0.04127	0.08899	0.011650794
<i>clic4</i>	0.11620	0.10917	0.24836	0.033603522
<i>gpn3</i>	0.05850	0.05250	0.14184	0.004677141
<i>det1</i>	0.01889	0.01589	0.07464	1.29E-06
<i>lipe</i>	0.14471	0.13740	0.27107	0.007294657
<i>cap1</i>	0.10208	0.09734	0.17169	0.047413897
<i>sema3ab</i>	0.07303	0.05659	0.23741	1.38E-12
<i>gnl2</i>	0.09267	0.08848	0.15174	0.021671499
<i>notch2</i>	0.06827	0.05916	0.23213	0
<i>qtrt1</i>	0.07559	0.06998	0.15416	0.005140936
<i>mut</i>	0.03727	0.03407	0.09133	0.000271692
<i>psme1</i>	0.11379	0.10207	0.30104	0.00161685
<i>snx12</i>	0.07574	0.06889	0.21735	0.02963196
<i>ubtd2</i>	0.05751	0.04384	0.16940	1.52E-05
<i>tmod3</i>	0.06525	0.06054	0.19244	0.004374789
<i>uck1</i>	0.05348	0.04924	0.14079	0.021264129
<i>ltk</i>	0.09581	0.09120	0.21530	0.006259396
<i>kpna1</i>	0.03002	0.02795	0.06960	0.020975269
<i>plcd1</i>	0.12277	0.11551	0.21219	0.001074831
<i>myd88</i>	0.12285	0.11223	0.30435	0.002195775
<i>trim45</i>	0.15103	0.14416	0.22600	0.030111523
<i>psmb7</i>	0.10606	0.09900	0.19187	0.037369286
<i>sh3glb1</i>	0.06570	0.06201	0.11472	0.049892737
<i>lix1l</i>	0.05368	0.04517	0.15413	5.46E-05
<i>ptpn2</i>	0.09731	0.08857	0.19497	0.001566996
<i>slc2a6</i>	0.16476	0.15732	0.27217	0.04203645
<i>wdr74</i>	0.08788	0.08305	0.22809	0.035551234
<i>sand</i>	0.09545	0.09063	0.18780	0.015354721

<i>cxxc1</i>	0.05298	0.05042	0.11484	0.024876196
<i>uqcrc1</i>	0.08689	0.08272	0.16979	0.032563737
<i>gnrh2</i>	0.13816	0.12127	0.46169	0.035117106
<i>tubgcp3</i>	0.04821	0.04595	0.10166	0.005667818
<i>cnn2</i>	0.07476	0.06702	0.28624	0.000226939
<i>grk7</i>	0.07283	0.06962	0.14296	0.022790094
<i>cmpk1</i>	0.07263	0.06546	0.30108	0.001728077
<i>phr</i>	0.11308	0.10342	0.27961	7.07E-05
<i>ddr2</i>	0.09103	0.08729	0.13568	0.025844057
<i>scp2</i>	0.10320	0.09488	0.31873	0.000146187
<i>EIF5</i>	0.05414	0.04424	0.22713	4.56E-08
<i>tmem147</i>	0.03936	0.02121	0.31099	4.66E-15
<i>dnmt3b</i>	0.22566	0.21666	0.40453	0.000258204
<i>UCKL1</i>	0.04551	0.04266	0.11179	0.009588243
<i>CACNA1D</i>	0.06364	0.05858	0.13024	1.29E-07
<i>GUCA1B</i>	0.02284	0.01985	0.10805	0.014789737
<i>tdrd3</i>	0.13348	0.12896	0.21949	0.038262291
<i>SLITRK1</i>	0.04727	0.04499	0.07847	0.037144458
<i>SLC22A13</i>	0.10497	0.09966	0.19113	0.017407243
<i>FAM18B1</i>	0.16905	0.15394	0.42450	0.009111744
<i>capn7</i>	0.05035	0.04793	0.08360	0.022279532
<i>MRPL22</i>	0.11272	0.09754	0.28622	0.000668861
<i>grk6</i>	0.05401	0.04879	0.12718	6.70E-05
<i>dcaf13</i>	0.06577	0.05974	0.17628	0.001547663
<i>ibtk</i>	0.13054	0.12686	0.20720	0.014013737
<i>galnt6</i>	0.11087	0.10646	0.18692	0.021534068
<i>scn8a</i>	0.06070	0.05523	0.12409	6.26E-09
<i>asb8</i>	0.02871	0.02641	0.08005	0.033858244
<i>smc5</i>	0.12047	0.11437	0.20326	0.000935012
<i>poli</i>	0.21719	0.20873	0.38130	0.01534351
<i>jak2</i>	0.07003	0.06708	0.12446	0.005257046
<i>cpd</i>	0.13348	0.12963	0.19153	0.019133377
<i>sarm1</i>	0.08963	0.07903	0.27048	1.14E-09
<i>osbpl2</i>	0.06597	0.06047	0.13786	0.002780005
<i>dpyd</i>	0.08041	0.07421	0.17992	3.19E-06
<i>agl</i>	0.10314	0.09854	0.18572	9.39E-05
<i>MRPL37</i>	0.14614	0.13716	0.24197	0.012491256
<i>IFIH1</i>	0.21340	0.20368	0.35230	0.001554909
<i>tmem41aa</i>	0.09391	0.08686	0.20472	0.013298387
<i>pnkd</i>	0.10348	0.09552	0.28986	0.00122963

<i>acmsd</i>	0.09507	0.08459	0.29542	0.000296309
<i>lhx1a</i>	0.02108	0.01264	0.15449	3.35E-11
<i>ddr1</i>	0.06499	0.06076	0.11250	0.001705193
<i>gnl1</i>	0.11447	0.10917	0.23403	0.011041052
<i>tpi1b</i>	0.05003	0.04563	0.10268	0.040709802
<i>aicda</i>	0.11144	0.09290	0.37035	2.13E-05
<i>leng1</i>	0.09266	0.08529	0.43998	0.00289619
<i>slc25a40</i>	0.14872	0.14004	0.29441	0.00952579
<i>slc16a7</i>	0.05403	0.05057	0.10758	0.01398923
<i>utp20</i>	0.12725	0.12366	0.17572	0.00304423
<i>fntb</i>	0.07826	0.07382	0.19631	0.010134151
<i>pr1bp</i>	0.17711	0.17087	0.32535	0.035167993
<i>rtcb</i>	0.01610	0.01485	0.04034	0.018145657
<i>hoxc13a</i>	0.03998	0.03640	0.11727	0.022236966
<i>uba1</i>	0.07254	0.06988	0.12617	0.012021944
<i>c12orf41</i>	0.06967	0.06614	0.12869	0.023680229
<i>dhcr7</i>	0.06663	0.06200	0.15325	0.002859391
<i>llgl2</i>	0.07727	0.07484	0.11357	0.045088934
<i>kdm1a</i>	0.02187	0.02083	0.04596	0.034805533
<i>ikbkb</i>	0.09287	0.08823	0.16694	0.008693043
<i>mfi2</i>	0.20613	0.19660	0.33066	0.008021974
<i>ckap5</i>	0.07123	0.06932	0.09726	0.017628398
<i>dhx30</i>	0.15911	0.14472	0.34719	1.73E-08
<i>wnt3a</i>	0.03537	0.02929	0.10562	1.38E-05
<i>npc1</i>	0.10869	0.10518	0.16298	0.011172396
<i>zdhhc3</i>	0.05807	0.04607	0.18990	1.65E-07
<i>dapk1</i>	0.05948	0.05757	0.09081	0.0271819
<i>yipf3</i>	0.06817	0.06261	0.22045	0.003432118
<i>ubr2</i>	0.09863	0.09542	0.13292	0.014604473
<i>gys1</i>	0.03851	0.03551	0.09328	0.000656037
<i>atp2a1</i>	0.04570	0.04361	0.07675	0.01857622
<i>ncs1</i>	0.00502	0.00322	0.03518	0.015071008
<i>cds2</i>	0.03617	0.03382	0.07791	0.026949366
<i>upb1</i>	0.07743	0.07296	0.15295	0.032202763
<i>imp4</i>	0.04294	0.03864	0.15902	0.000927335
<i>atp5o</i>	0.14750	0.13591	0.41478	0.008370827
<i>abcb7</i>	0.07479	0.07103	0.13724	0.013180816
<i>znf711</i>	0.04444	0.04141	0.09946	0.009690616
<i>cetn2</i>	0.02051	0.01737	0.07031	0.012337283
<i>bnip1</i>	0.07886	0.06897	0.22729	0.000510627

<i>pdgfrl</i>	0.14328	0.13438	0.27154	0.011189303
<i>hdac3</i>	0.02042	0.01853	0.04950	0.015891969
<i>gne</i>	0.05936	0.05549	0.11021	0.004184244
<i>dnajb1</i>	0.06825	0.06347	0.16664	0.010514327
<i>nt5c2</i>	0.03273	0.02165	0.18430	0
<i>taf5</i>	0.06045	0.05687	0.10943	0.002974712
<i>dysfip1</i>	0.13719	0.12659	0.61534	0.029714878
<i>psmb3</i>	0.0198	0.01779	0.07876	0.039610242
<i>lrp2</i>	0.06920	0.06610	0.11247	1.04E-08
<i>slc15a1</i>	0.15756	0.14561	0.34960	3.64E-05
<i>sqrcl</i>	0.08807	0.07310	0.31991	1.16E-10
<i>galk2</i>	0.12592	0.11989	0.21864	0.024991891
<i>cyp2u1</i>	0.14400	0.13590	0.25905	0.007511771
<i>mogs</i>	0.08754	0.08024	0.17477	3.78E-05
<i>med23</i>	0.01928	0.01797	0.03703	0.004496246
<i>ehd3</i>	0.01811	0.01468	0.06480	5.04E-06
<i>pigh</i>	0.12491	0.11301	0.27579	0.014314071
<i>psme2</i>	0.10393	0.09043	0.26162	0.000265347
<i>ilvbl</i>	0.05646	0.05383	0.11095	0.023879516
<i>tsku</i>	0.14249	0.13085	0.48660	0.0012666
<i>ppwd1</i>	0.03312	0.03094	0.06296	0.023865727
<i>ch25hl2</i>	0.05780	0.05353	0.17521	0.014674837
<i>gfm2</i>	0.11378	0.10943	0.18422	0.032810423
<i>hexb</i>	0.11330	0.10743	0.20551	0.012058156
<i>slc28a2</i>	0.12826	0.12332	0.21556	0.040340447
<i>pex11a</i>	0.19047	0.17460	0.40372	0.004490379
<i>EIF3M</i>	0.04125	0.03760	0.12324	0.006208743
<i>rcn1</i>	0.05671	0.04921	0.13347	0.000298336
<i>slc43a1</i>	0.12802	0.11966	0.25870	0.001834205
<i>cyp4v2</i>	0.10191	0.09512	0.30056	0.001283514
<i>cpe</i>	0.04854	0.03615	0.16569	2.10E-10
<i>anxa5</i>	0.12108	0.11312	0.28149	0.012417564
<i>slmo1</i>	0.05016	0.04316	0.21686	0.003611646
<i>ighmbp2</i>	0.12450	0.12052	0.18174	0.034639049
<i>cyp11a1</i>	0.08564	0.08046	0.13443	0.031832705
<i>adipor2</i>	0.05435	0.03682	0.26795	1.31E-12
<i>pomgnt1</i>	0.03639	0.03468	0.07162	0.034710907
<i>tshz1</i>	0.05664	0.05289	0.12105	0.000830998
<i>brox</i>	0.05681	0.05255	0.25007	0.000901702
<i>actr6</i>	0.04035	0.03617	0.11872	0.000695469

<i>wwp2</i>	0.06542	0.05510	0.16384	2.51E-10
<i>xrn1</i>	0.10540	0.10247	0.15158	0.0109886
<i>farsb</i>	0.06340	0.05999	0.13395	0.010248476
<i>cdc5l</i>	0.06046	0.05805	0.10819	0.030643607
<i>arl2</i>	0.03545	0.03181	0.08815	0.034810832
<i>dnai2</i>	0.12116	0.11259	0.21479	0.000695126
<i>hiat1</i>	0.06848	0.06467	0.11770	0.037205765
<i>slc31a1</i>	0.06096	0.05611	0.27946	0.014352321
<i>prvu</i>	0.12329	0.10462	0.99390	0.000362039
<i>capn9</i>	0.07683	0.07251	0.11295	0.018682766
<i>taf5l</i>	0.05799	0.04865	0.17690	3.99E-09
<i>cyp51a1</i>	0.07065	0.06623	0.13444	0.006769748
<i>wdr43</i>	0.11214	0.10578	0.18806	0.010537366
<i>pms1</i>	0.19723	0.18969	0.32245	0.010346273
<i>adat3</i>	0.13073	0.12377	0.30300	0.029585138
<i>alkbh6</i>	0.09426	0.08676	0.27002	0.011659399
<i>ddx18</i>	0.05903	0.05314	0.11527	0.000328201
<i>appbp2</i>	0.01998	0.00901	0.19371	0
<i>thop1</i>	0.05489	0.05243	0.09423	0.02549412
<i>cpne1</i>	0.07562	0.06326	0.26674	6.88E-10
<i>gfpt2</i>	0.09094	0.08594	0.16340	0.004636315
<i>exoc8</i>	0.03096	0.02817	0.05677	0.003389553
<i>ssu72</i>	0.03501	0.02319	0.21063	1.85E-07
<i>myst1</i>	0.03131	0.02496	0.13111	1.98E-08
<i>pik3cg</i>	0.16494	0.16058	0.24235	0.035794964
<i>mgat4c</i>	0.18401	0.17361	0.37531	0.005639345
<i>glud1</i>	0.04356	0.03755	0.13920	6.92E-06
<i>irs2</i>	0.09403	0.09031	0.15056	0.010846479
<i>lig4</i>	0.06345	0.05904	0.13130	2.94E-05
<i>cnl66</i>	0.05444	0.04751	0.34638	8.06E-05
<i>hpx</i>	0.19736	0.17918	0.49522	0.000101301
<i>pde6b</i>	0.05673	0.04643	0.23981	2.58E-10
<i>lancl1</i>	0.09623	0.09092	0.16757	0.033131445

Supplementary Table 18|Positively selected genes in the tongue sole.

Gene symbol	w0	w1	w2	P_value
<i>gtf2h2</i>	0.08573	0.07680	0.13872	0.003293492
<i>poli</i>	0.21719	0.20745	0.29410	0.029723623
<i>upb1</i>	0.07743	0.07209	0.12584	0.021671524
<i>snx18</i>	0.04440	0.04147	0.06913	0.025652005
<i>slc20a1a</i>	0.11480	0.10820	0.15867	0.022661497
<i>hsd17b4</i>	0.13251	0.11822	0.22541	1.26E-05
<i>gtf2h3</i>	0.07360	0.06622	0.13116	0.016455053
<i>pole</i>	0.04348	0.04126	0.05919	0.001933989
<i>thoc5</i>	0.08520	0.07976	0.11981	0.014442971
<i>imp4</i>	0.04294	0.03709	0.09041	0.000939114
<i>slc7a4</i>	0.09716	0.08751	0.15777	0.000281882
<i>arvcf</i>	0.02900	0.02489	0.04780	0.000248389
<i>znf593</i>	0.04390	0.03532	0.12860	0.003019665
<i>khdrbs1</i>	0.06754	0.05866	0.13069	0.000808366
<i>mocs3</i>	0.12889	0.12121	0.17741	0.049306602
<i>id2</i>	0.06762	0.05635	0.54098	0.00628466
<i>slc8a3</i>	0.05141	0.04380	0.10075	2.00E-08
<i>six6</i>	0.02204	0.01786	0.06428	0.000630307
<i>vps39</i>	0.05059	0.04673	0.08478	0.001069975
<i>ppp2r3c</i>	0.04867	0.04452	0.07748	0.014655488
<i>nrd1</i>	0.07886	0.07090	0.15379	1.99E-06
<i>iah1</i>	0.12096	0.10752	0.22373	0.002965075
<i>plekhh1</i>	0.09440	0.08621	0.15296	6.90E-07
<i>rdh12</i>	0.08221	0.07479	0.14912	0.008981369
<i>fuca2</i>	0.11710	0.10904	0.17501	0.012373424
<i>mnat1</i>	0.10188	0.08950	0.19934	0.000824789
<i>mad2l1</i>	0.04636	0.04023	0.09634	0.009013813
<i>dicer1</i>	0.06828	0.06634	0.08435	0.046470259
<i>ttc5</i>	0.11666	0.10909	0.17775	0.016647918
<i>acadv1</i>	0.06752	0.06388	0.09796	0.029870217
<i>sptbn1</i>	0.05352	0.05181	0.06442	0.038061269
<i>lmbr1l</i>	0.06783	0.06049	0.11747	0.002383361
<i>hsd17b10</i>	0.05235	0.04538	0.10513	0.006708828
<i>clstn1</i>	0.03756	0.03161	0.07857	2.00E-07
<i>tshz2</i>	0.06750	0.06461	0.09573	0.023119632
<i>agmat</i>	0.06723	0.06319	0.11868	0.045732345
<i>dnajc1l</i>	0.05258	0.04164	0.13007	1.52E-08
<i>cacnal1f</i>	0.06364	0.05562	0.12586	2.10E-12

<i>pan2</i>	0.04408	0.04135	0.06018	0.010334712
<i>slc25a33</i>	0.07486	0.06823	0.11358	0.040179941
<i>aaas</i>	0.11564	0.09217	0.28665	9.40E-12
<i>kcna2</i>	0.04738	0.04212	0.07740	0.003385159
<i>trpc4ap</i>	0.03906	0.03635	0.05943	0.014743408
<i>gnl3</i>	0.16954	0.16011	0.23959	0.024154117
<i>thoc7</i>	0.03195	0.02711	0.06741	0.02157834
<i>glt8d1</i>	0.11850	0.11146	0.18113	0.038706288
<i>tardbp</i>	0.03546	0.02755	0.07460	0.000777097
<i>acad9</i>	0.08418	0.07962	0.12154	0.025637181
<i>rabif</i>	0.10385	0.08796	0.24749	0.00361894
<i>pacsin1</i>	0.09435	0.08964	0.14630	0.046789776
<i>rhobtb3</i>	0.04338	0.03824	0.07148	0.002595891
<i>ski</i>	0.11600	0.10823	0.16399	0.010743734
<i>urocl</i>	0.06510	0.05870	0.10717	0.000392248
<i>rft1</i>	0.14317	0.13228	0.23169	0.001687876
<i>ncl1</i>	0.11861	0.11098	0.18611	0.008913235
<i>aldh1l1</i>	0.06246	0.05715	0.10359	0.000193006
<i>uqcrc1</i>	0.08689	0.07298	0.19232	9.12E-07
<i>itpr1</i>	0.03321	0.02765	0.06593	0
<i>dnajc11</i>	0.08370	0.07474	0.14901	0.000351761
<i>smarcd1</i>	0.01592	0.01121	0.05528	6.73E-07
<i>rae1</i>	0.01633	0.01442	0.03224	0.041535214
<i>nprl2</i>	0.04515	0.04009	0.08441	0.004344016
<i>ece1</i>	0.08210	0.07719	0.12511	0.002960276
<i>ycp9</i>	0.10055	0.08849	0.18817	0.002116784
<i>slc17a5</i>	0.10607	0.09558	0.19492	0.000357079
<i>slc16a9</i>	0.12191	0.11349	0.19918	0.005998038
<i>map4k3</i>	0.04506	0.04082	0.08245	0.000527053
<i>lrpprc</i>	0.20679	0.18848	0.35530	5.34E-09
<i>rgr</i>	0.07419	0.06540	0.14392	0.002642732
<i>gba</i>	0.12897	0.10991	0.26740	3.29E-07
<i>fhl3</i>	0.04180	0.03333	0.10345	1.78E-05
<i>mdh2</i>	0.04152	0.03820	0.07437	0.037042862
<i>slc40a1</i>	0.07397	0.06367	0.13359	0.000194127
<i>gtpbp10</i>	0.15725	0.13995	0.31182	9.83E-05
<i>wdr41</i>	0.12825	0.11877	0.19750	0.007047236
<i>gtf3c4</i>	0.15088	0.14352	0.20500	0.01711091
<i>ak1</i>	0.04147	0.03583	0.08392	0.036746117
<i>ppwd1</i>	0.03312	0.03085	0.05914	0.02277659

<i>cryaa</i>	0.08217	0.07130	0.14477	0.01535132
<i>irs2</i>	0.09403	0.08873	0.13052	0.00657828
<i>ggt3p</i>	0.18891	0.17957	0.24845	0.039962312
<i>ipo5</i>	0.03781	0.03554	0.05212	0.01539897
<i>musk</i>	0.09447	0.08438	0.17616	3.37E-07
<i>vldlr</i>	0.04545	0.04262	0.06707	0.013380106
<i>dbh</i>	0.12749	0.10789	0.27828	3.84E-09
<i>grial</i>	0.05394	0.05112	0.07098	0.045850403
<i>mls</i>	0.14390	0.13686	0.19021	0.049116882
<i>atp5o</i>	0.14750	0.12073	0.56170	5.75E-07
<i>brap</i>	0.04617	0.04123	0.08593	0.000986701
<i>rpl26</i>	0.04859	0.01853	0.28814	5.59E-08
<i>zdhhc15</i>	0.08766	0.07060	0.20391	1.27E-05
<i>elf3l</i>	0.02163	0.01939	0.04097	0.010789318
<i>slitrk2</i>	0.06306	0.05621	0.10523	4.32E-05
<i>ibtk</i>	0.13054	0.12496	0.16931	0.01036063
<i>wnt6</i>	0.03035	0.02416	0.13284	3.30E-10
<i>ehhadh</i>	0.14770	0.13691	0.22801	0.000418734
<i>tmem177</i>	0.13819	0.12674	0.22747	0.006217003
<i>tsn</i>	0.04016	0.03449	0.08285	0.008265382
<i>fastkd1</i>	0.19249	0.16722	0.40901	1.63E-12
<i>spopla</i>	0.02496	0.02197	0.04503	0.049701226
<i>wdfy2</i>	0.06488	0.06053	0.10782	0.027759079
<i>dusp19</i>	0.07438	0.06579	0.12978	0.023524671
<i>glb1</i>	0.12581	0.11377	0.21582	5.75E-05
<i>hat1</i>	0.06415	0.05812	0.11528	0.006703061
<i>igf2bp2</i>	0.09879	0.08882	0.22108	0.000115088
<i>galnt13</i>	0.02788	0.02294	0.05741	5.31E-05
<i>tbc1d17</i>	0.08331	0.07657	0.12998	0.003121812
<i>ndufb10</i>	0.14686	0.12256	0.33348	0.000448115
<i>dnmt1</i>	0.06442	0.06065	0.08905	0.002142195
<i>rnf151</i>	0.06108	0.04625	0.14983	0.000279142
<i>smcr7l</i>	0.05222	0.04167	0.12901	6.64E-08
<i>tmem184a</i>	0.06611	0.05972	0.10451	0.008461727
<i>arl5b</i>	0.07398	0.06319	0.15640	0.005835814
<i>cox6a</i>	0.17391	0.15528	0.50587	0.019666095
<i>ndufa6</i>	0.08729	0.06677	0.29511	1.47E-05
<i>fam18b1</i>	0.16905	0.15230	0.35936	0.005248609
<i>tyh3</i>	0.11363	0.10609	0.16888	0.02389932
<i>kank2</i>	0.10189	0.09287	0.15967	9.59E-05

<i>ttyh2</i>	0.14002	0.13097	0.20363	0.012849059
<i>slc25a17</i>	0.10697	0.09851	0.17061	0.035182417
<i>dnaja3</i>	0.09246	0.08551	0.14758	0.011500372
<i>prkaca</i>	0.02073	0.01614	0.05160	0.003256764
<i>angpt1</i>	0.07074	0.06018	0.13646	8.58E-05
<i>cpsfl</i>	0.05026	0.04632	0.08067	4.32E-05
<i>clic4</i>	0.07634	0.06437	0.18742	0.000245691
<i>mrpl24</i>	0.07792	0.06423	0.20427	2.37E-05
<i>fabp3</i>	0.15206	0.14011	0.31279	0.047203222
<i>cap1</i>	0.10208	0.09058	0.21343	2.91E-05
<i>slc7a1</i>	0.10393	0.09657	0.17152	0.00188222
<i>wdr44</i>	0.07912	0.06896	0.14979	1.18E-07
<i>cwc15-a</i>	0.03517	0.02942	0.07525	9.40E-06
<i>tbc1d23</i>	0.05288	0.04353	0.11386	3.50E-09
<i>lass1</i>	0.07471	0.06801	0.11602	0.03357721
<i>rx1</i>	0.07994	0.06757	0.16558	0.000102822
<i>selrc1</i>	0.07703	0.06652	0.17158	0.000626065
<i>creb3l3a</i>	0.18021	0.17049	0.26730	0.028061876
<i>ano8</i>	0.05753	0.05198	0.09765	0.000173164
<i>polr2e</i>	0.00553	0.00396	0.02066	0.021490952
<i>copb2</i>	0.02339	0.02039	0.04731	0.000265476
<i>fam78b</i>	0.02026	0.01303	0.08648	4.40E-07
<i>abcb8</i>	0.09118	0.08563	0.13176	0.012271199
<i>tmem53</i>	0.09039	0.07883	0.18664	0.001588257
<i>bag1</i>	0.06486	0.05686	0.12501	0.015606024
<i>scp2</i>	0.10320	0.09372	0.16017	0.002828168
<i>mapre2</i>	0.07773	0.06291	0.20217	6.12E-06
<i>dhx30</i>	0.15911	0.14859	0.23766	0.000192297
<i>sntg1</i>	0.04160	0.03811	0.07304	0.019842866
<i>ptchd1</i>	0.04798	0.04363	0.06604	0.011225445
<i>armc3</i>	0.32491	0.31387	0.40945	0.044990596
<i>lmbr1</i>	0.05205	0.04215	0.10519	2.46E-05
<i>fry</i>	0.09920	0.09423	0.13201	3.02E-05
<i>slc6a4</i>	0.04444	0.03739	0.08868	1.32E-05
<i>vmn2r1</i>	0.09523	0.08527	0.17577	5.21E-07
<i>fibin</i>	0.04748	0.04093	0.10982	0.012520278
<i>ttc17</i>	0.08119	0.07385	0.14094	7.98E-06
<i>pros1</i>	0.13922	0.13341	0.18318	0.042783048
<i>ndufa9</i>	0.09756	0.07209	0.33989	2.89E-15
<i>ckap5</i>	0.07123	0.06849	0.09025	0.005959169

<i>rlbp1</i>	0.03418	0.03122	0.06298	0.042922597
<i>pdp1</i>	0.12326	0.11352	0.19606	0.002532809
<i>prep</i>	0.04010	0.03755	0.06066	0.031740617
<i>nr2e1</i>	0.02140	0.01897	0.04062	0.022328577
<i>fbxo25</i>	0.11975	0.10972	0.20503	0.004144093
<i>gnpat</i>	0.14114	0.13263	0.19810	0.008769616
<i>pla2g7</i>	0.12348	0.10899	0.26505	2.10E-05
<i>brox</i>	0.05681	0.05139	0.09772	0.006080183
<i>bmp2</i>	0.08434	0.07765	0.14238	0.020691251
<i>fdft1</i>	0.05273	0.04385	0.11237	3.92E-05
<i>slc35b2</i>	0.08344	0.07343	0.17176	4.82E-05
<i>mrpl19</i>	0.10785	0.09877	0.20441	0.009498186
<i>parp1</i>	0.07449	0.07144	0.09618	0.041566768
<i>cb044</i>	0.07396	0.06763	0.12119	0.000743359
<i>rhot2</i>	0.08390	0.07767	0.12377	0.008771157
<i>abca5</i>	0.09083	0.08690	0.11692	0.004106229
<i>mto1</i>	0.11982	0.11188	0.17571	0.005435035
<i>dock4</i>	0.02655	0.02540	0.03437	0.045691382
<i>tbc1d24</i>	0.08053	0.06866	0.16136	6.92E-07
<i>sult1c1</i>	0.10815	0.10057	0.16399	0.048813303
<i>csrp2</i>	0.06024	0.04962	0.12696	0.001855838
<i>samm50b</i>	0.06242	0.05626	0.09852	0.006232823
<i>ndufb7</i>	0.05349	0.04178	0.16420	0.000669822
<i>f8a1</i>	0.04136	0.03553	0.11769	0.001104891
<i>lgsn</i>	0.10305	0.08952	0.18752	1.23E-06
<i>cacng5</i>	0.04473	0.03828	0.09533	0.002309456
<i>wdr83</i>	0.05503	0.05033	0.09490	0.026247655
<i>lias</i>	0.06712	0.06153	0.12014	0.010348238
<i>capn2</i>	0.05347	0.04773	0.09127	0.000123597
<i>rhot1a</i>	0.06478	0.05825	0.10832	0.001117782
<i>p4hb</i>	0.08721	0.07524	0.23156	4.27E-07
<i>smox</i>	0.09685	0.08588	0.16791	0.000505401
<i>cyp4v3</i>	0.10191	0.09545	0.15028	0.013834191
<i>npepps</i>	0.04719	0.04423	0.06864	0.022692002
<i>EIF4A3</i>	0.01212	0.01051	0.02464	0.033410612
<i>ampd3</i>	0.08742	0.08265	0.11889	0.021711298
<i>gatad2b</i>	0.04770	0.04366	0.07143	0.035240173
<i>vipar</i>	0.12355	0.11614	0.17263	0.040672117
<i>lhx1a</i>	0.07513	0.06371	0.14620	0.000103201
<i>acac</i>	0.04336	0.04008	0.06216	0.000107116

<i>dgat2</i>	0.09425	0.08244	0.17186	0.000879943
<i>tcf21</i>	0.03668	0.03090	0.06562	0.039255184
<i>dhcr24</i>	0.06659	0.06083	0.10973	0.003417239
<i>prvu</i>	0.12329	0.10180	0.36698	0.001529583
<i>cox7c</i>	0.04845	0.03674	0.17209	0.031311105
<i>abpl</i>	0.11361	0.10568	0.16558	0.001673177
<i>foxl2</i>	0.02550	0.02019	0.05955	0.00055897
<i>xpot</i>	0.04827	0.04555	0.06764	0.019533479
<i>tim22</i>	0.11390	0.09092	0.50448	2.76E-08
<i>wdr36</i>	0.11801	0.11228	0.15495	0.030939507
<i>ndufb4</i>	0.15491	0.13446	0.31459	0.013070091
<i>pola1</i>	0.09439	0.09062	0.12628	0.008623414
<i>cat</i>	0.09819	0.09274	0.13670	0.034944766
<i>dpagtl</i>	0.06684	0.06112	0.09852	0.032098844
<i>galns</i>	0.11350	0.10578	0.15856	0.018930893
<i>spg7</i>	0.03863	0.03598	0.05919	0.027562023
<i>mltk</i>	0.13459	0.12868	0.18374	0.031297204
<i>gcsf</i>	0.16953	0.15071	0.32720	0.007582096
<i>tpst2</i>	0.07541	0.06406	0.17319	6.55E-07
<i>vps36</i>	0.04913	0.04321	0.10060	0.0044066
<i>zw10</i>	0.11229	0.10101	0.19584	3.32E-05
<i>hddc3</i>	0.08850	0.08092	0.19615	0.022378428
<i>etnk1</i>	0.06144	0.05669	0.10258	0.035469033
<i>aph1b</i>	0.05349	0.04577	0.11489	0.003499164
<i>cd031</i>	0.04627	0.04010	0.08615	0.00019243
<i>dhx29</i>	0.09198	0.08843	0.11498	0.03374077
<i>nav3</i>	0.07367	0.06753	0.10876	2.53E-07
<i>ss18</i>	0.03959	0.03554	0.07355	0.045757049
<i>cfl2</i>	0.09056	0.07449	0.19503	0.004472282
<i>vwf</i>	0.17757	0.17203	0.20834	0.006364622
<i>kiaa1033</i>	0.03957	0.03619	0.06288	0.001125248
<i>esg1</i>	0.03237	0.02750	0.07546	1.21E-05
<i>lap3</i>	0.11604	0.10818	0.17484	0.020028691
<i>galnt4</i>	0.08404	0.07588	0.15491	0.000155943
<i>mgnl1</i>	0.07984	0.07144	0.13303	0.000609337
<i>tyh3</i>	0.06119	0.05560	0.09470	0.013934379
<i>rab4a</i>	0.01958	0.01235	0.05655	0.000170777
<i>ndufs7</i>	0.10034	0.08510	0.29276	0.000349323
<i>ctdSpl2a</i>	0.10878	0.08721	0.26449	3.04E-09
<i>kctd3</i>	0.06414	0.05883	0.09210	0.007229818

<i>suv39h1</i>	0.07306	0.06267	0.13931	9.32E-05
<i>ephb2</i>	0.04709	0.04451	0.06498	0.019625156
<i>ppfibp1</i>	0.10307	0.09796	0.13666	0.015124355
<i>asb8</i>	0.02871	0.02321	0.07697	0.000635929
<i>tm9sf3</i>	0.01867	0.01414	0.05631	8.36E-06
<i>capzb</i>	0.04414	0.03035	0.15043	1.53E-07
<i>aco1</i>	0.06726	0.06421	0.09499	0.019277099
<i>aadac</i>	0.12055	0.10985	0.18557	0.004564126
<i>prph2</i>	0.10898	0.10017	0.17730	0.025259636

Supplementary Table 19 | Positively selected genes shared by the flatfish lineage and other teleost species.

Species	No. of selection genes
<i>Danio rerio</i>	0
<i>Gasterosteus aculeatus</i>	34
<i>Oryzias latipes</i>	10
<i>Oreochromis niloticus</i>	15
<i>Tetraodon nigroviridis</i>	17
<i>Takifugu rubripes</i>	9

Supplementary Table 20|KEGG enrichment of the 2,307 orthologs from DEGs.

Pathway ID	Pathway	Gene NO.	p-value	q-value
map04610	Complement and coagulation cascades	41	0	0
map04744	Phototransduction	14	1.07E-09	2.75E-08
map04152	AMPK signaling pathway	29	1.80E-06	2.97E-05
map04670	Leukocyte transendothelial migration	27	2.59E-06	3.99E-05
map04360	Axon guidance	30	3.40E-06	4.91E-05
map05161	Hepatitis B	35	1.01E-05	0.000137
map05020	Prion diseases	14	2.08E-05	0.000253
map04910	Insulin signaling pathway	28	2.29E-05	0.000264
map04922	Glucagon signaling pathway	20	2.60E-05	0.000286
map04510	Focal adhesion	39	0.000132	0.00105
map05210	Colorectal cancer	18	0.000131	0.00105
map05200	Pathways in cancer	71	0.00021	0.001616
map04976	Bile secretion	20	0.000278	0.002072
map04380	Osteoclast differentiation	29	0.00029	0.002091
map04725	Cholinergic synapse	26	0.000332	0.002321
map04022	cGMP-PKG signaling pathway	32	0.000559	0.003624
map04512	ECM-receptor interaction	19	0.000565	0.003624
map04974	Protein digestion and absorption	18	0.000603	0.003763
map04914	Progesterone-mediated oocyte maturatin	19	0.000682	0.003996
map04978	Mineral absorption	12	0.000709	0.003996
map00100	Steroid biosynthesis	12	0.000709	0.003996
map04520	Adherens junction	19	0.00083	0.004458
map04920	Adipocytokine signaling pathway	17	0.000894	0.004692
map04151	PI3K-Akt signaling pathway	54	0.001176	0.005905
map04068	FoxO signaling pathway	27	0.001614	0.00761
map04261	Adrenergic signaling in cardiomyocytes	25	0.00165	0.007623
map04930	Type II diabetes mellitus	13	0.001779	0.008058
map04972	Pancreatic secretion	19	0.001947	0.008647
map04024	cAMP signaling pathway	36	0.002119	0.009235
map05410	Hypertrophic cardiomyopathy HCM	21	0.002395	0.010243
map04921	Oxytocin signaling pathway	28	0.002719	0.010675
map04724	Glutamatergic synapse	23	0.002724	0.010675
map05414	Dilated cardiomyopathy	23	0.002567	0.010675
map04950	Maturity onset diabetes of the young	10	0.002773	0.010675
map04975	Fat digestion and absorption	10	0.002773	0.010675
map04710	Circadian rhythm	9	0.002742	0.010675
map04918	Thyroid hormone synthesis	15	0.002962	0.011217
map04611	Platelet activation	23	0.003576	0.012906
map03320	PPAR signaling pathway	17	0.003683	0.013088
map05220	Chronic myeloid leukemia	17	0.004056	0.014195
map05322	Systemic lupus erythematosus	14	0.005084	0.017267

map05212	Pancreatic cancer	16	0.005373	0.017984
map04010	MAPK signaling pathway	42	0.007536	0.024175
map05031	Amphetamine addiction	13	0.00871	0.027557
map05223	Non-small cell lung cancer	13	0.010345	0.031437
map04919	Thyroid hormone signaling pathway	21	0.011521	0.033682
map05205	Proteoglycans in cancer	35	0.013564	0.038206
map04917	Prolactin signaling pathway	16	0.014168	0.038955
map04014	Ras signaling pathway	36	0.014608	0.039693
map05146	Amoebiasis	20	0.015198	0.040815
map04066	HIF-1 signaling pathway	18	0.015834	0.042035
map05166	HTLV-I infection	43	0.017726	0.046059
map04728	Dopaminergic synapse	20	0.017948	0.046059
map04923	Regulation of lipolysis in adipocytes	13	0.017922	0.046059

Supplementary Table 21|KEGG enrichment of the orthologs in cluster A of the Japanese flounder.

Map	ID	Pathway	Gene NO.	p-value	q-value
map04512		ECM-receptor interaction	13	3.73E-07	3.15E-05
map04974		Protein digestion and absorption	11	6.59E-06	0.000417
map04670		Leukocyte transendothelial migration	12	3.09E-05	0.001303
map05410		Hypertrophic cardiomyopathy HCM	12	3.09E-05	0.001303
map01110		Biosynthesis of secondary metabolites	17	0.000109	0.003066
map05414		Dilated cardiomyopathy	12	0.0001	0.003066
map00100		Steroid biosynthesis	7	0.000148	0.003407
map01130		Biosynthesis of antibiotics	11	0.000183	0.003856
map04510		Focal adhesion	16	0.000221	0.004296
map04151		PI3K-Akt signaling pathway	22	0.000289	0.005218
map04110		Cell cycle	13	0.000353	0.005957
map04115		p53 signaling pathway	9	0.000535	0.008467
map04978		Mineral absorption	6	0.001067	0.015882
map05161		Hepatitis B	12	0.001663	0.023369
map04914		Progesterone-mediated oocyte maturation	8	0.002356	0.031368
map05200		Pathways in cancer	24	0.003119	0.037571

Supplementary Table 22|KEGG enrichment of the orthologs in cluster B of the Japanese flounder.

Map ID	Pathway	Gene NO.	p-value	q-value
map04610	Complement and coagulation cascades	33	0	0
map04744	Phototransduction	14	1.00E-11	2.52E-10
map04910	Insulin signaling pathway	26	1.54E-07	3.10E-06
map04152	AMPK signaling pathway	24	6.85E-07	1.15E-05
map01200	Carbon metabolism	11	1.81E-06	2.80E-05
map04360	Axon guidance	24	2.29E-06	3.29E-05
map05161	Hepatitis B	27	1.89E-05	0.000221545
map04920	Adipocytokine signaling pathway	16	1.98E-05	0.000221545
map04930	Type II diabetes mellitus	13	3.90E-05	0.000413408
map04724	Glutamatergic synapse	21	5.90E-05	0.000594142
map04921	Oxytocin signaling pathway	25	8.01E-05	0.000768213
map04922	Glucagon signaling pathway	15	0.000136	0.001191936
map04068	FoxO signaling pathway	23	0.000153	0.001287367
map04710	Circadian rhythm	9	0.000161	0.001293731
map04520	Adherens junction	16	0.000227	0.001756104
map04010	MAPK signaling pathway	36	0.00024	0.001791974
map03320	PPAR signaling pathway	15	0.000335	0.002247368
map04725	Cholinergic synapse	20	0.000401	0.002385632
map04066	HIF-1 signaling pathway	17	0.000408	0.002385632
map04972	Pancreatic secretion	16	0.000415	0.002385632
map05220	Chronic myeloid leukemia	15	0.000412	0.002385632
map04919	Thyroid hormone signaling pathway	19	0.000596	0.003323816
map05212	Pancreatic cancer	14	0.000611	0.003323816
map05210	Colorectal cancer	13	0.000906	0.004676728
map04022	cGMP-PKG signaling pathway	24	0.00095	0.004782713
map05032	Morphine addiction	15	0.001072	0.005203779
map05223	Non-small cell lung cancer	12	0.001085	0.005203779
map04723	Retrograde endocannabinoid signaling	17	0.00114	0.005338338
map05200	Pathways in cancer	50	0.001227	0.005618392
map05231	Choline metabolism in cancer	14	0.001896	0.007957082
map04976	Bile secretion	14	0.001896	0.007957082
map04024	cAMP signaling pathway	27	0.002086	0.008401049
map04380	Osteoclast differentiation	20	0.002272	0.008647966
map05160	Hepatitis C	17	0.002276	0.008647966
map04261	Adrenergic signaling in cardiomyocytes	19	0.002351	0.008769307
map04370	VEGF signaling pathway	10	0.002988	0.01055681
map04740	Olfactory transduction	6	0.003505	0.01217101
map04923	Regulation of lipolysis in adipocytes	11	0.004414	0.01481623
map05221	Acute myeloid leukemia	11	0.004414	0.01481623
map04012	ErbB signaling pathway	14	0.005284	0.01744662

map04014	Ras signaling pathway	28	0.005791	0.01881247
map04611	Platelet activation	17	0.006292	0.02011589
map04917	Prolactin signaling pathway	13	0.00662	0.02083398
map04510	Focal adhesion	25	0.007622	0.02361613
map04912	GnRH signaling pathway	13	0.007863	0.0239951
map04726	Serotonergic synapse	16	0.009099	0.02694982
map04670	Leukocyte transendothelial migration	15	0.009545	0.02786045
map04727	GABAergic synapse	13	0.01069	0.0307565
map04666	Fc gamma R-mediated phagocytosis	12	0.011714	0.03322774
map04914	Progesterone-mediated oocyte maturation	12	0.015751	0.04345762

Supplementary Table 23|KEGG enrichment of the orthologs in cluster A of the tongue sole.

Map ID	Pathway	Gene NO.	<i>p</i>-value	<i>q</i>-value
map04744	Phototransduction	9	6.80E-09	1.67E-06
map00100	Steroid biosynthesis	8	2.35E-05	0.001156
map04360	Axon guidance	12	0.000212	0.008673
map04115	p53 signaling pathway	9	0.000726	0.025507

Supplementary Table 24|KEGG enrichment of the orthologs in cluster B of the tongue sole.

Map	ID	Pathway	Gene NO.	<i>p</i> -value	<i>q</i> -value
map04260		Cardiac muscle contraction	30	1.28E-14	3.75E-12
map05146		Amoebiasis	28	4.03E-08	5.90E-06
map04744		Phototransduction	15	1.57E-06	0.000153
map05414		Dilated cardiomyopathy	26	1.04E-05	0.000764
map05410		Hypertrophic cardiomyopathy (HCM)	24	3.32E-05	0.001945
map04940		Type I diabetes mellitus	11	5.42E-05	0.002648
map04974		Protein digestion and absorption	21	8.08E-05	0.003382
map05330		Allograft rejection	8	0.000159	0.005162
map00330		Arginine and proline metabolism	14	0.000195	0.005705
map05332		Graft-versus-host disease	7	0.000357	0.008721
map00910		Nitrogen metabolism	6	0.001729	0.035923
map00520		Amino sugar and nucleotide sugar metabolism	12	0.002335	0.038353
map05310		Asthma	5	0.002768	0.040544
map00260		Glycine, serine and threonine metabolism	10	0.003062	0.041547

Supplementary Table 25|KEGG enrichment of the orthologs in cluster C of the tongue sole.

Map	ID	Pathway	Gene NO.	<i>p</i> -value	<i>q</i> -value
map03010		Ribosome	45	4.45E-19	1.35E-16
map03030		DNA replication	19	7.58E-10	1.15E-07
map04110		Cell cycle	38	1.23E-09	1.25E-07
map00240		Pyrimidine metabolism	29	1.04E-06	5.97E-05
map04744		Phototransduction	15	2.44E-05	0.000790
map00100		Steroid biosynthesis	10	2.60E-05	0.000790
map04111		Cell cycle - yeast	20	5.46E-05	0.001383
map03430		Mismatch repair	10	6.51E-05	0.001521
map00190		Oxidative phosphorylation	26	0.000104	0.002259
map04115		p53 signaling pathway	19	0.000191	0.003639
map03440		Homologous recombination	10	0.000209	0.003753
map05012		Parkinson's disease	30	0.000224	0.003797
map03420		Nucleotide excision repair	13	0.000538	0.00796
map04113		Meiosis - yeast	16	0.000848	0.011214
map05322		Systemic lupus erythematosus	14	0.002292	0.024885
map05016		Huntington's disease	36	0.005604	0.04867

Supplementary Table 26|KEGG enrichment of 146 DEGs common to the Japanese flounder and tongue sole during metamorphosis.

Map ID	Pathway	Gene No.	<i>p</i> –value	<i>q</i>-value
map04744	Phototransduction	8	6.84E-09	1.14E-06

Supplementary 27|KEGG enrichment for sub-groups of genes from the 146 orthologs.

Sub-groups	Pathway ID	Pathway	Gene No.	p-value	q-value
Group1	map04975	Fat digestion and absorption	3	0.00163076	0.1337228
	map04971	Gastric acid secretion	3	0.0135423	0.4753862
	map00565	Ether lipid metabolism	2	0.01807253	0.4753862
	map05166	HTLV-I infection	4	0.02789811	0.4753862
Group2	map04930	Type II diabetes mellitus	2	0.00880572	0.2243144
	map04730	Long-term depression	2	0.01121572	0.2243144
	map04666	Fc gamma R-mediated phagocytosis	2	0.03740761	0.337148
Group3	map04744	Phototransduction	11	2.60E-13	2.65E-11
	map04740	Olfactory transduction	6	3.58E-05	1.82E-03
	map04062	Chemokine signaling pathway	7	0.000411	1.40E-02

Supplementary Table 28|Flatfish orthologs in the thyroid hormone signaling pathway enriched in the DEGs during metamorphosis.

Gene symbol	Protein annotation
<i>prkaca</i>	cAMP-dependent protein kinase catalytic subunit alpha
<i>mapk1</i>	Mitogen-activated protein kinase 1
<i>armc4</i>	Armadillo repeat-containing protein 4
<i>thrb</i>	Thyroid hormone receptor beta
<i>thra</i>	Thyroid hormone receptor alpha
<i>rxraa</i>	Retinoic acid receptor RXR-alpha-A
<i>rxrgb</i>	Retinoic acid receptor RXR-gamma-B
<i>ncoa3</i>	Nuclear receptor coactivator 3
<i>gata5-b</i>	GATA-binding factor 5-B
<i>hif1a</i>	Hypoxia-inducible factor 1-alpha
<i>megf10</i>	Multiple epidermal growth factor-like domains protein 10
<i>prkca</i>	Protein kinase C alpha type
<i>slc16a2</i>	Monocarboxylate transporter 8
<i>slc16a10</i>	Monocarboxylate transporter 10
<i>pik3ca</i>	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform
<i>pik3r2</i>	Phosphatidylinositol 3-kinase regulatory subunit beta
<i>bad</i>	Bcl2 antagonist of cell death
<i>foxo1</i>	Forkhead box protein O1
<i>slc2a1</i>	Solute carrier family 2, facilitated glucose transporter member 1
<i>dio2</i>	Type II iodothyronine deiodinase
<i>dio3</i>	Type III iodothyronine deiodinase

Supplementary Table 29|Rescue effect of T3 on metamorphosis of Japanese flounder treated with MMI.

No. of larvae at 20 DAH		No. of larvae at 24 DAH			No. of larvae at 30 DAH			
pre-metamorphosis	pre-	pro-	climax	Post-	Pre-	Pro-	climax	Post-
600 (group A)	100	27	0	0	0	0	16	42
800 (group B)	156	82	0	0	0	1	14	64
200 (control)	0	62	106	0	0	0	0	142

Supplementary Table 30|Flatfish orthologs in the retinoic acid signaling pathway enriched in the DEGs during metamorphosis.

Gene symbol	Protein annotation
<i>rxra</i>	Retinoic acid receptor RXR-alpha-A
<i>rxrg</i>	Retinoic acid receptor RXR-gamma-B
<i>rarg</i>	Retinoic acid receptor gamma-A
<i>aldh1l2</i>	Aldehyde dehydrogenase family 1 member L2
<i>rbp2</i>	Retinol-binding protein 2
<i>raet1</i>	Iodotyrosine dehalogenase 1
<i>rdh12</i>	Retinol dehydrogenase 12
<i>rbp3</i>	Retinol-binding protein 3
<i>lrat</i>	Lecithin retinol acyltransferase

Supplementary Table 31|KEGG enrichment of up-regulated genes in the pigmented ocular skin of Japanese flounder and tongue sole.

Pathway ID	Pathway	Gene No.	<i>p</i> -value	<i>q</i>-value
map00350	Tyrosine metabolism	3	0.0001201207	0.004324345

Supplementary Table 32| Rate of pigmental cell formation after irradiation of Japanese flounder with different wavelengths of light on the dorsal and ventral surface.

light	λ /nm	TM(°C)	Fish number at 16 DAH	Fish number at 50 DAH	Fish number at 50 DAH (PBS)	Ratio MP/total fish, 50 DAH
yellow	580-600	26	300	62	6	9.7%
green	480-560	24.5	300	71	10	14.1%
blue	450-480	22	300	19	14	73.7%
red	650-760	24.5	300	32	4	12.5%
violet	400-450	22	300	32	12	37.5%
white		24	300	42	26	61.9%

Note: DAH, days after hatching; PBS pigmentation of the blindside; MP malpigmentation

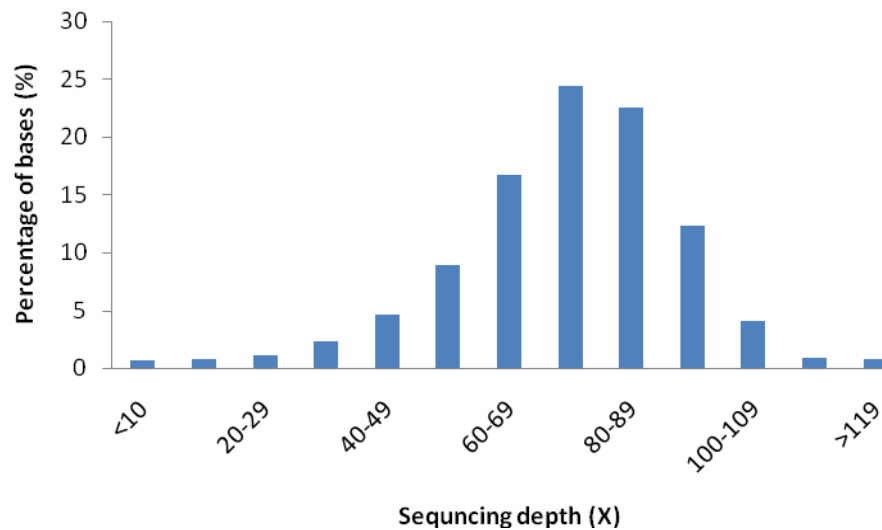
Supplementary Table 33|PCR primers, amplicon length, primer annealing temperature and the use of the amplicon in the study.

genes	Upper primer (5'-3')	Lower primer (5'-3')	PCR product length (bp)	Anneal temperature (°C)	Usage
<i>dio1</i>	CATCGCAGAGGCTCATTCAACAG	GACTTTCCCAGCCTGAAGCAC	214	58	Probe
<i>dio2</i>	GAAACTTGGCTGCGAGGCAC	TCGTTCGTTAGACACACCGTAAGC	478	59	Probe
<i>dio3</i>	CGTCTCCGACTCCAACAAGATG	TCATGAACGGGGGTCAGGAG	221	57	Probe
<i>aldh1</i>	CAGCGGGGTCTGCTCTGCCGG	TCGCCGGCTGGGCTGACAAAA	203	52	Probe
<i>aldh1</i>	ACAAAATGGGAGGGGAAGAC	AGTTCCACGGTATGATCTGTCC	121	60	qPCR
<i>aldh2</i>	GATGGTCATGCCGTGGATTTTGT	TAAGTACACCAAGATCTTCAT	382	52	Probe
<i>aldh2</i>	GCAGACGACCTCTCTCCGATG	CCTCTCTGCAACCCAGCTACTG	151	60	qPCR
<i>aldh3</i>	GGATCGCCAAGGAGGAAATATT	TCGGTGTACTCGGCCAAGGCATA	280	50	Probe
<i>aldh3</i>	CCATAGAGAGAGCAAACAGCTCA	TTATCCACACAGTGCCAGTCTC	114	60	qPCR
	C				
<i>rxra</i>	CCGTGACTGAGCGATGTG	GTTTCAACGAGGAGATGCC	276	52	Probe
<i>rxrb</i>	TTCGCAGACCTCACAAC	TGCTTACGATGACTTCCCTT	350	50	Probe
<i>rxrg</i>	GAGTGATCTGGTCCGCGATGGT	CGTGAAGGAGGAGGTGGTGCTT	145	55	Probe
<i>thraa</i>	CAACAAGCAGGATAGCAACTCGT	CTGGAACAGAGACGCTAAGAGCAG	125	60	Probe
	CAGAGG	ACATG			
<i>thrab</i>	CACATGGCTCAATGGCCCGAAAA	CAGCTACTCCTGCAAGTACGACTG	231	60	Probe
	GG	CTG			
<i>thrb1</i>	ATATGGCCACGAATGCACAGGGC	TTCCTTCAGGTGTATTGCCATGGA	126	60	Probe
	AAC	CGC			
<i>thrb2</i>	AGCAGAAGCGGAAATTCCTGGTC	TTCACCCCCGCTGCACTCTGGTC	97	60	Probe
	GAG				
<i>cyb26</i>	GTGCCAGATGTCGGAAGATTGAT	TCTTCACAAAGTCAGAAGAA	303	58	Probe
	TCA				

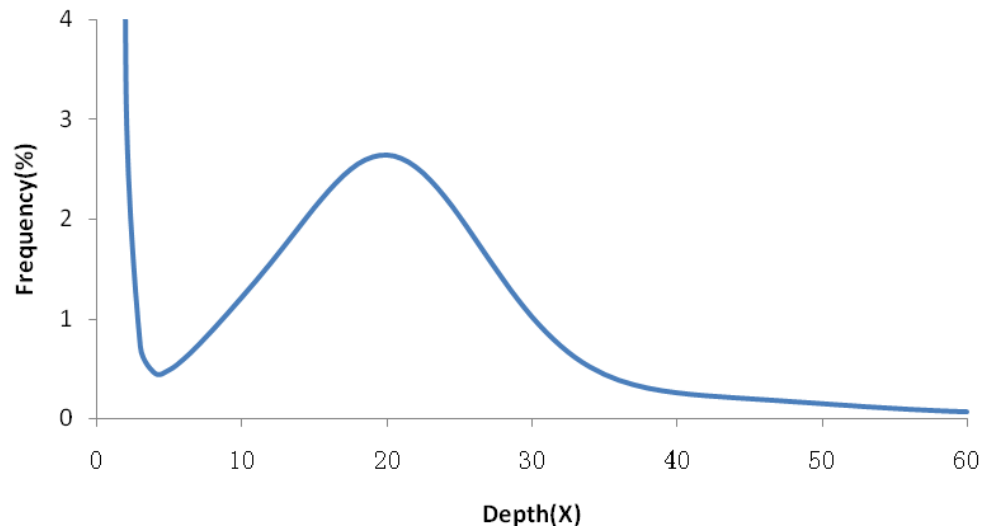
<i>rbp2</i>	ACATGGGCTACACATACACGCAC AGCAGT	GTTGTCGAGTCCCTTGGTGTACTCG	285	59	Probe
<i>rara</i>	CTGGTTGGCGAAGGCGAAAACC	GTCCCGACACGGAGCAGATGAT	404	55	Probe
<i>rarb</i>	AGAAACCTTCCCCTCCCTC	TGTCTCCGTGTCGTCCATC	391	56	Probe
<i>rarg</i>	GAGTGATCTGGTCCGCGATGGT	CGTGAAGGAGGAGGTGGTGCTT	278	55	Probe
<i>rh1</i>	ATGAACGGCACAGAGGGACCAT ATT	CATCGTTGTGGTGAATCCTCCAAA	285	52	Probe
<i>rh2</i>	CAGAACAAGAACTCAGGCAAC CTCT	CGAAGAAGTGGACGACAAACATGT AG	451	53	Probe
<i>lws</i>	CAAGAACTCCGTCACCCACTGA ACTGG	TGTACCTGCTCCAGCCAAGGATTG GGGGA	341	53	Probe
<i>sws1</i>	CGAACTTGAAGGAGCCGAAAGG TT	TCGTCCTGTTCGTGACCATGAAGTA TA	276	53	Probe
<i>sws2</i>	GCTGCGTATTACCTGGGTGTTT GCACTT	GGCATAGTAGCCAATCTCAG	406	53	Probe
<i>opn4</i>	GCCTCTTCCTCCTCATCTA	GTCCTCTGTGTCCTCAATC	267	52	Probe
<i>pax3</i>	CCACATCCGGCACAAGATTGTTG	TTTGTCCCGGATCTCCCATCT	250	54	Probe
<i>dct</i>	GGACGAGGTTGTGAAGGTTGTTG	TAATCGCCTGGTGA CTCTGTGT	242	52	Probe
<i>kit</i>	AGCAGTCTTCCTCCATCTTG	CCTCTGTCTGGTCCTCGTCA	492	54	Probe
<i>sws1-ss</i>	CTTATTAATTCTCGTGGTGNCMG TGHART	TTCGGTCTGCTATTGAACVCHTGC WAG	212	62	qPCR
<i>sws2-ss</i>	CGGCTTCCCTTANNGTYWCAAGA ACNG	GGCTTCCTAACACAACCKCWTCY YGCNT	161	63	qPCR
<i>sws1-hq</i>	CTGCGGGCAGTGMCTGTMTACN ACTCNYT	TTCTCGTGAAAAATTGMMTGATNA	277	60	qPCR
<i>sws1-sn</i>	GGTCTGGCACACCTTTAAACYWS GTT	GTCACGCATGGNKGCTAAWAACAC CTG	175	62	qPCR
<i>sws2-cs</i>	TGGACCGTGGTGCTHDCGNNAC	GAATTTACCAGCCCTGCTAVNNG SG	216	61	qPCR
<i>gapdh</i>	TGACTTCAACAGCGACACCCA	ACCCTGTTGCTGTAGCCAAA	121	60	qPCR
<i>β-actin</i>	GGAAATCGTGCGTGACATTAAG	CCTCTGGACAACGGAACCTCT	161	60	Probe

Note: *sws1-ss*, *sws1* *Salmo salar*; *sws2-ss*, *sws2* *Salmo salar*; *sws1-hq*, *sws1* *Hemirhamphus quoyi*; *sws1-sn*, *sws1* *Scomberomorus niphonius*; *sws2-cs*, *sws2* *Cololabis saira*.

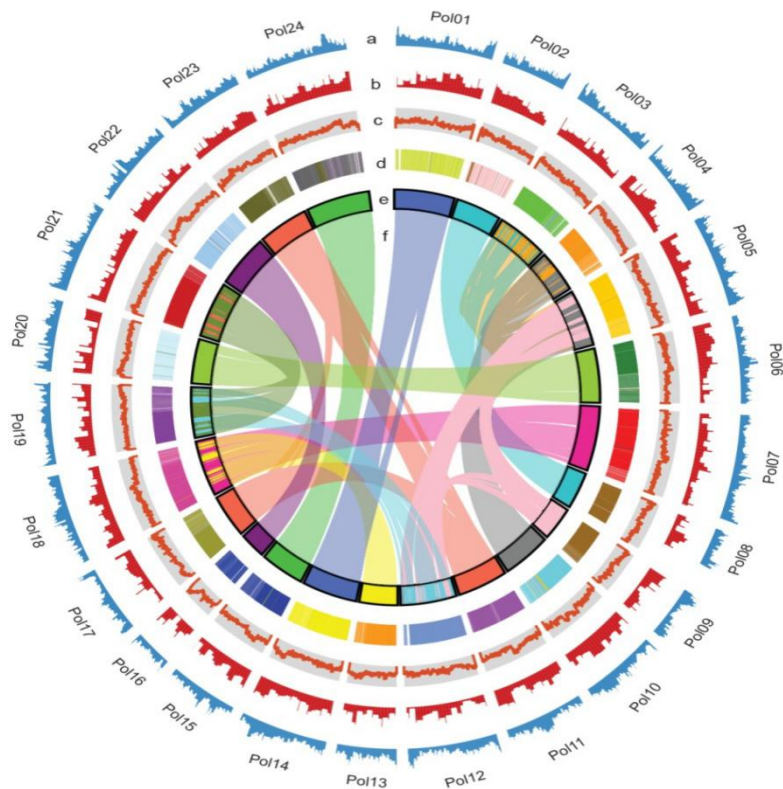
Supplementary Figures 1-50



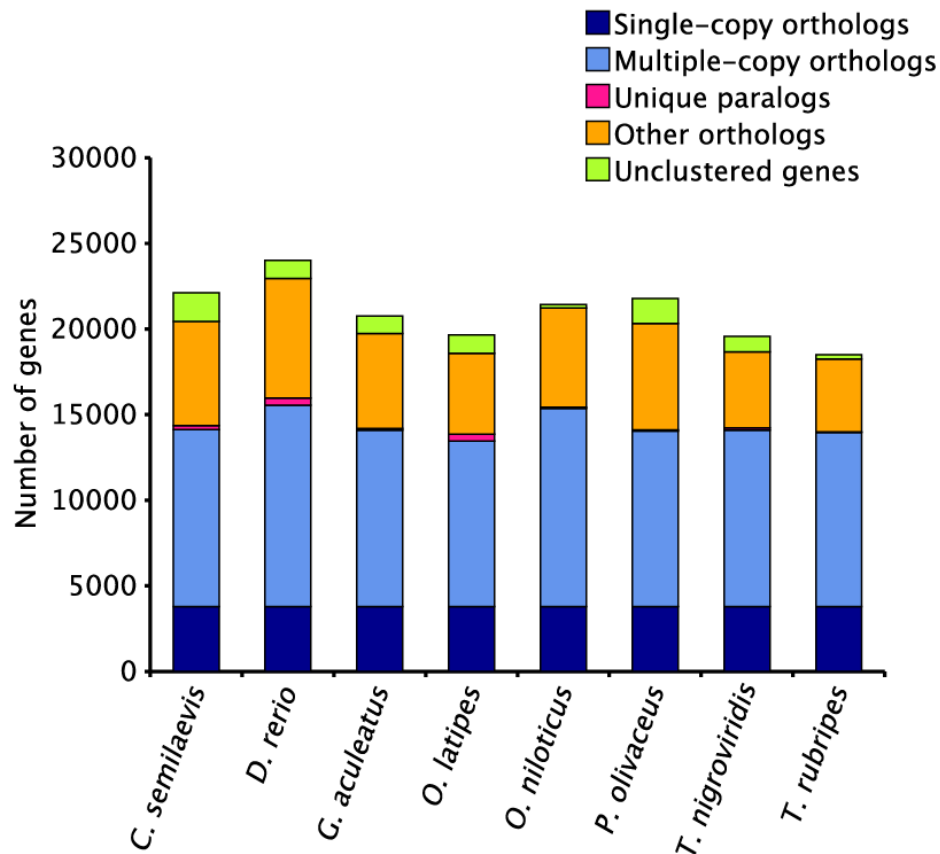
Supplementary Figure 1|Sequencing depth distribution of the Japanese flounder genome. To test for completeness of the assembly, the sequencing depth of each base was calculated from the alignment, the proportion followed the theoretical Poisson distribution.



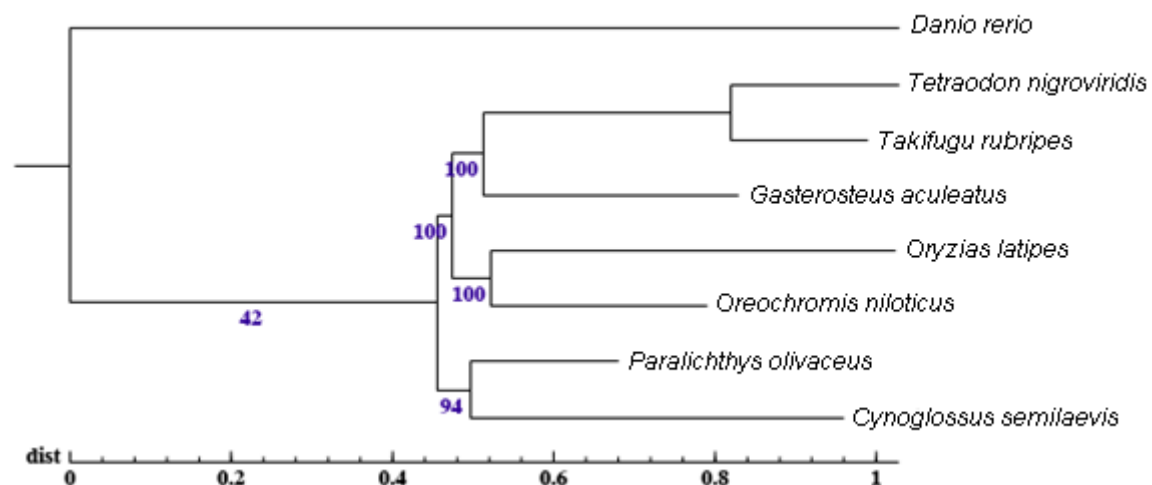
Supplementary Figure 2|Seventeen-mer estimation of genome size. The genome size, G , was defined as $G = K_num / K_depth$, where the K_num is the total number of k-mers, and K_depth is the most frequently occurring frequency. In the present study, K is 17, K_num is 12,814,861,831 and K_depth is 20. Thus, the Japanese flounder genome size is estimated to be 640 Mb.



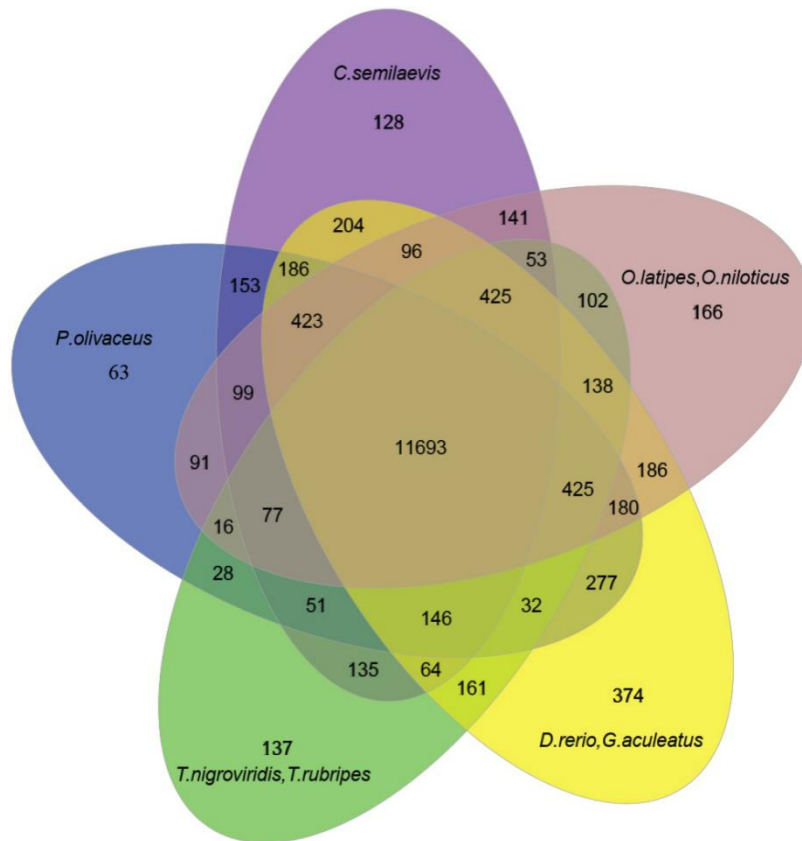
Supplementary Figure 3|The genomic landscape of the 24 Japanese flounder pseudo-chromosomes. The heatmap tracks (outer to inner circle) illustrate: repeat coverage (a, 14-594 per Mb, 100 kb windows), gene density (b, number per Mb, range 0-88), GC content (c, 37.69%-48.16%, 100 kb windows). d and e represent the Japanese flounder chromosomes painted by the corresponding tongue sole and ancestral chromosome respectively. f, central tracks connect the ortholog pairs from the teleost whole genome duplication in Japanese flounder.



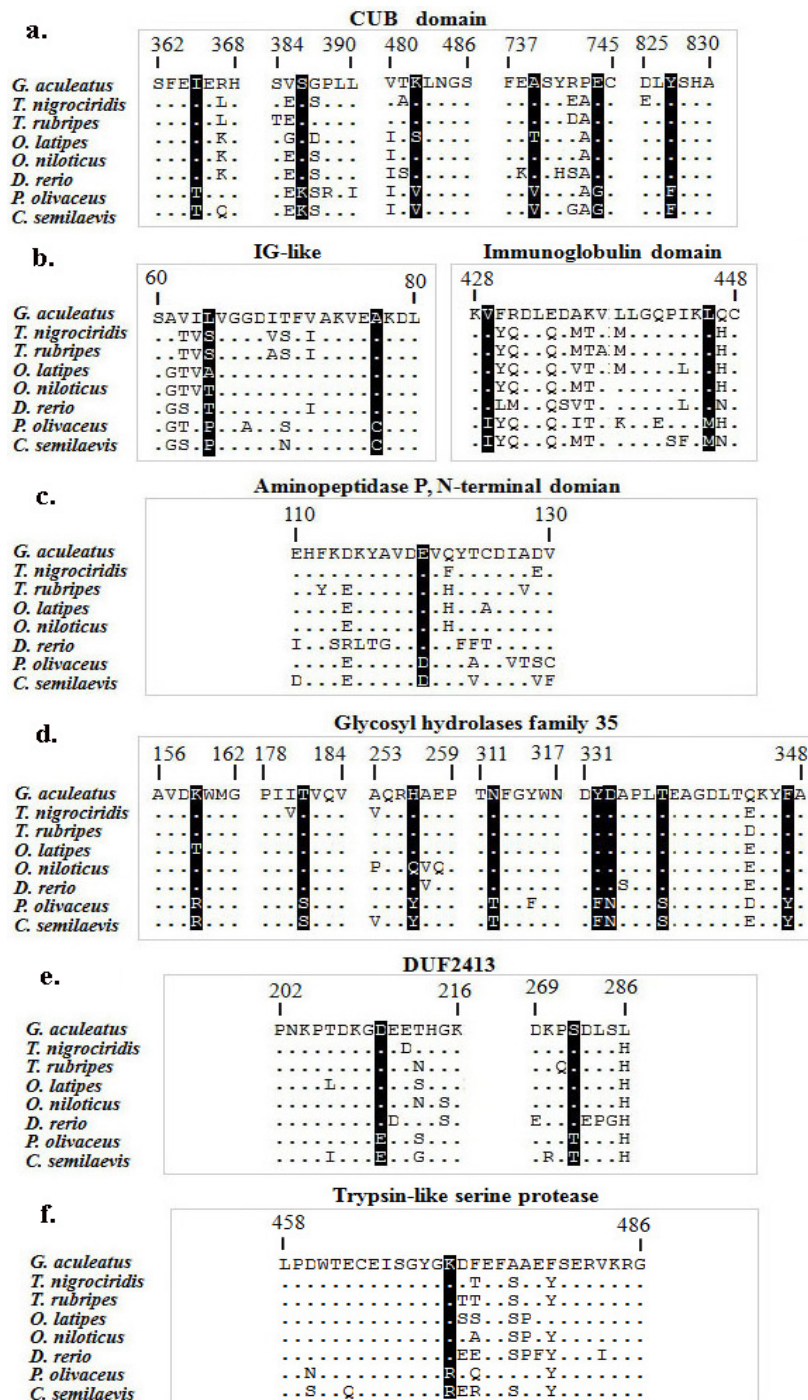
Supplementary Figure 4|Comparison of the number of homologue genes among *C. semilaevis*, *D. rerio*, *G. aculeatus*, *Oryzias latipes*, *Oreochromis niloticus*, *P. olivaceus*, *Tetraodon nigroviridis*, *Takifugu rubripes*. The results are based on the clustering of orthologs found in all species. If only one copy was present in a given species, the orthologs was defined as single copy and indicated in dark blue in the graph; if more than one gene copy was present in a given species, the orthologs was defined as multiple copy and indicated in the graph in light blue. Unique paralog refers to genes present in only one species with one or more copies; one of the copies was defined as a unique paralog and coloured deep pink. Orthologs in orange were identified in at least two species, but not all species. The remaining genes of each species were defined as unclustered and indicated in green. Based on the above definitions, the unique genes represent those specific for a single species.



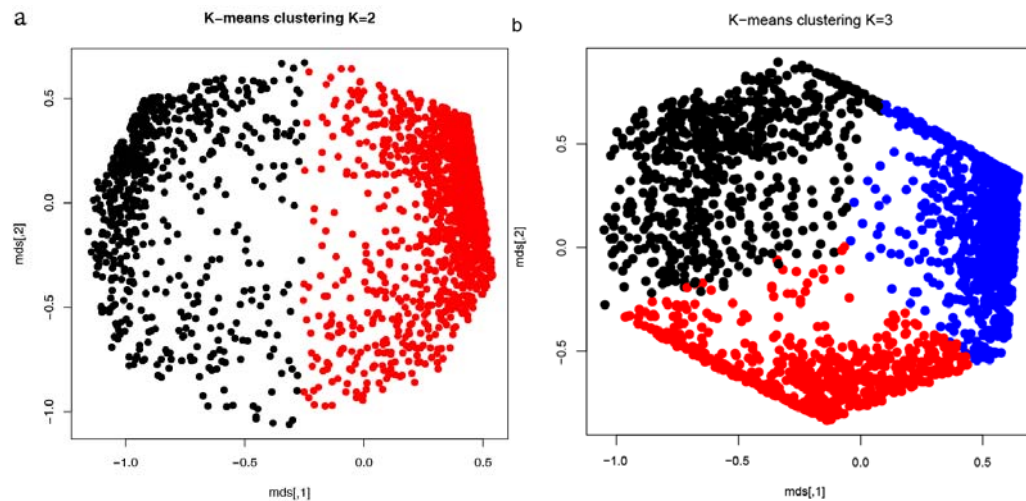
Supplementary Figure 5|Phylogenetic tree constructed with orthologous genes. The branch length represents the neutral divergence rate. The 3,793 single copy orthologous gene families from the eight teleost species were used for the analysis to construct the phylogenetic tree.



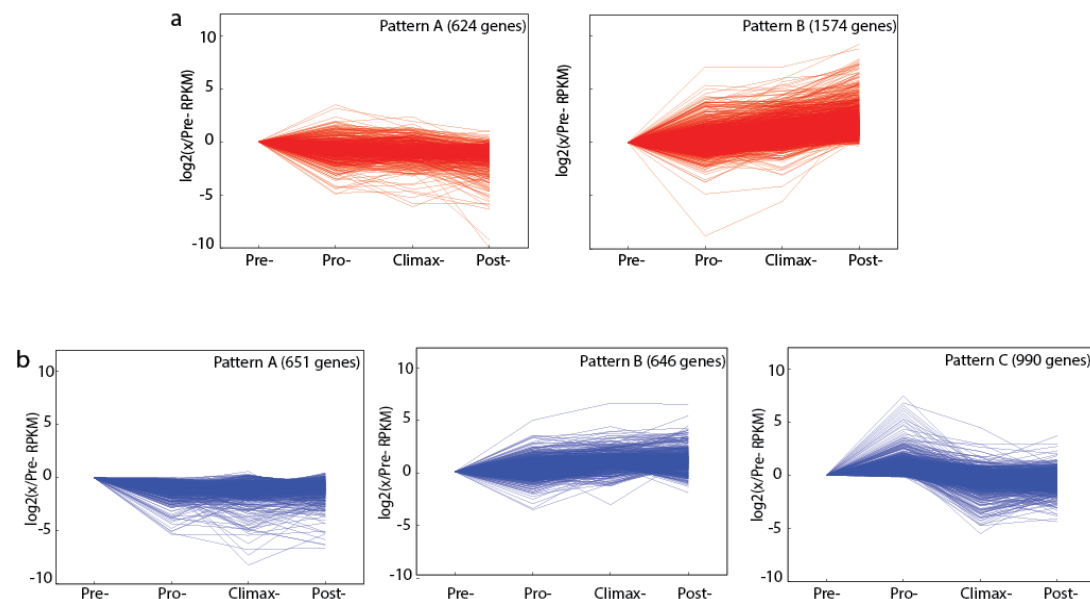
Supplementary Figure 6|Venn diagram of orthologous gene families. Eight teleost species (*Oreochromis niloticus*, *C. semilaevis*, *D. rerio*, *G. aculeatus*, *Oryzias latipes*, *Takifugu rubripes*, *Tetraodon nigroviridis* and *P. olivaceus*) were used to generate the Venn diagram based on the gene family cluster analysis. A total of 17,019 gene families were identified, of which 11,693 families are conserved in the eight teleost species. The species-specific gene families are those for which no orthologs were detected in the gene repertoires of the 8 genomes analysed. A total of 63 and 128 species-specific gene families were identified for the Japanese flounder and tongue sole, respectively. There were 153 families that were common for the tongue sole and flounder but different from the other 6 teleost species.



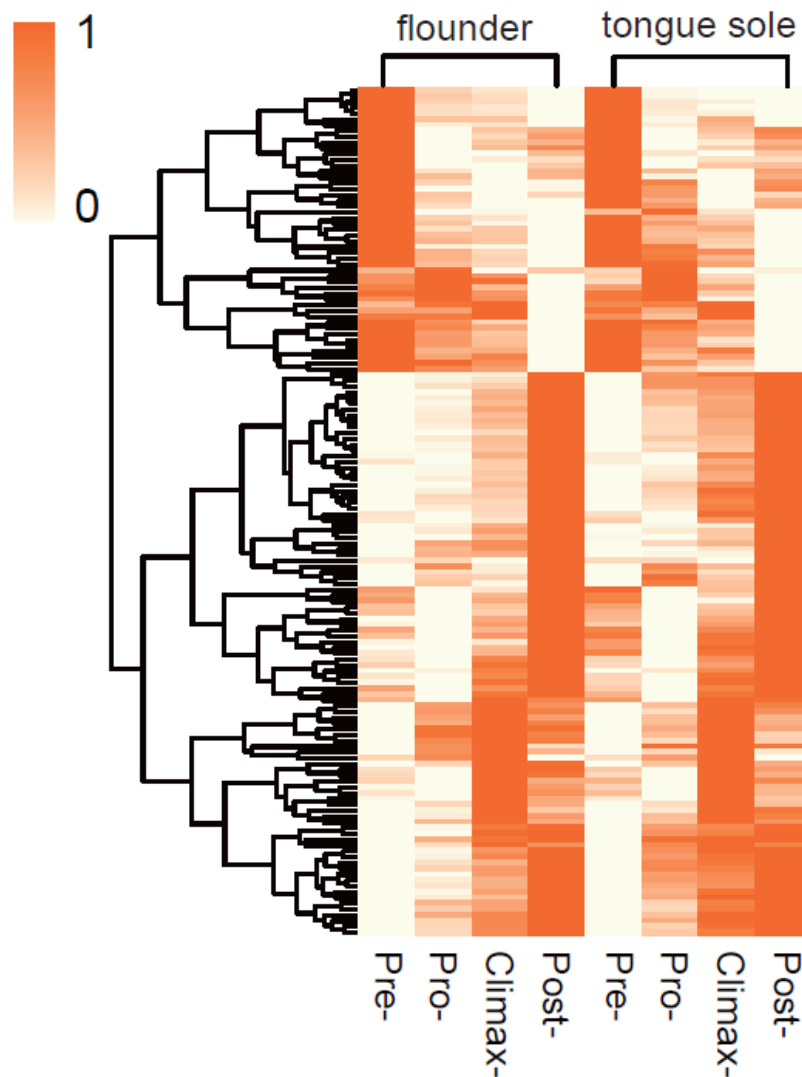
Supplementary Figure 7|Flatfish specific mutations of positively selected genes. a, specific mutations in the CUB domain of Bmp1; b, specific mutations in domains IG_like and immunoglobulin domains of Mybpc1; c, specific mutations in the aminopeptidase P N-terminal domain of Pepd; d, specific mutations in the glycosyl hydrolases family 35 domain of Glb1; e, specific mutations in the DUF2413 domain of Irx5; f, specific mutation in the trypsin-like serine protease domain of Plat. *G. aculeatus* (*Gasterosteus aculeatus*), *T. nigroviridis* (*Tetraodon nigroviridis*), *T. rubripes* (*Takifugu rubripes*), *O. latipes* (*Oryzias latipes*), *O. niloticus* (*Oreochromis niloticus*), *D. rerio* (*Danio rerio*), *P. olivaceus* (*Paralichthys olivaceus*), *C. semilaevis* (*Cynoglossus semilaevis*).



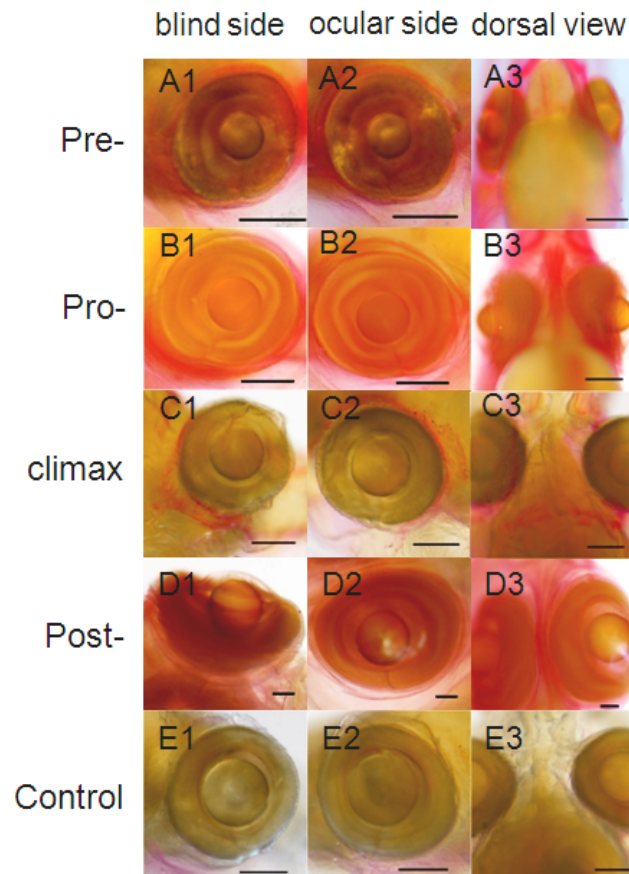
Supplementary Figure 8|K-means clustering analysis of the orthologs involved in the development of metamorphosis in flatfishes. a, Japanese flounder; **b**, tongue sole. To elucidate the developmental stage-specific gene expression profiles, we analyzed the expression patterns of differentially expressed orthologs (2,307). between Japanese flounder and tongue sole. We firstly calculated the number of clusters (k) based on the silhouette coefficient. After K-means clustering, the 2,307 differentially expressed orthologs of Japanese flounder were grouped into 2 patterns shown in black and red, and the orthologs in tongue sole were grouped into 3 patterns shown in black, red and blue.



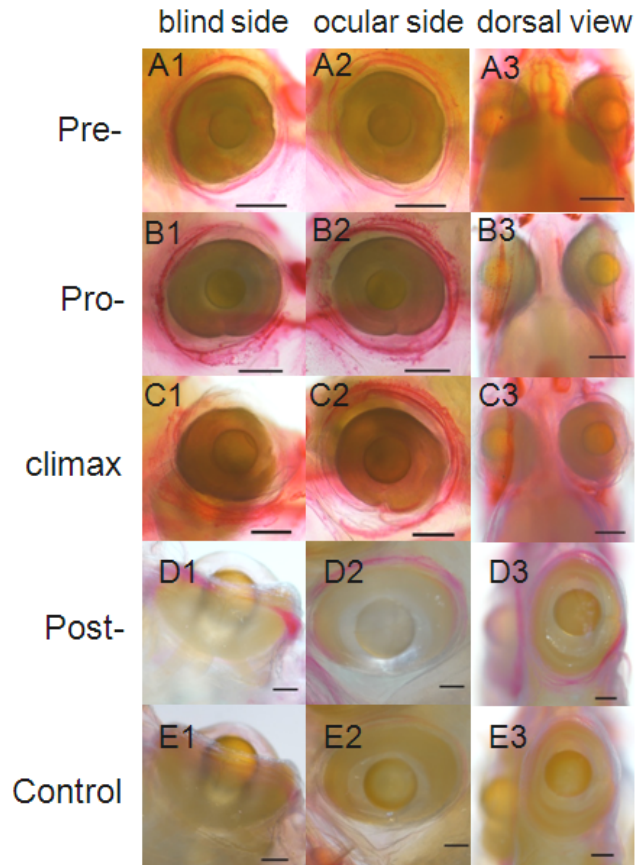
Supplementary Figure 9|Patterns of ortholog expression by K-means cluster analysis in the developing metamorphosis of flatfishes. Differentially expressed orthologs across all four metamorphosis time-points were grouped into two and three clusters for Japanese flounder (a) and tongue sole (b) respectively, using the K-means clustering algorithm. The y-axis gives the tag count (on a log scale) of differentially expressed genes. Each line represents a different gene. a, Pattern A- 624 genes in the Japanese flounder were decreased to lower levels in the post-metamorphic larval stage. Pattern B- 1,574 genes of Japanese flounder were highly expressed in the post-metamorphic larval stage; b, Three patterns (A, 651 genes; B, 646 genes; C, 990 genes) were enriched in genes of the phototransduction pathway. Pattern C- 990 genes in tongue sole were highly expressed in the pro-metamorphic stage and decreased at the metamorphic climax.



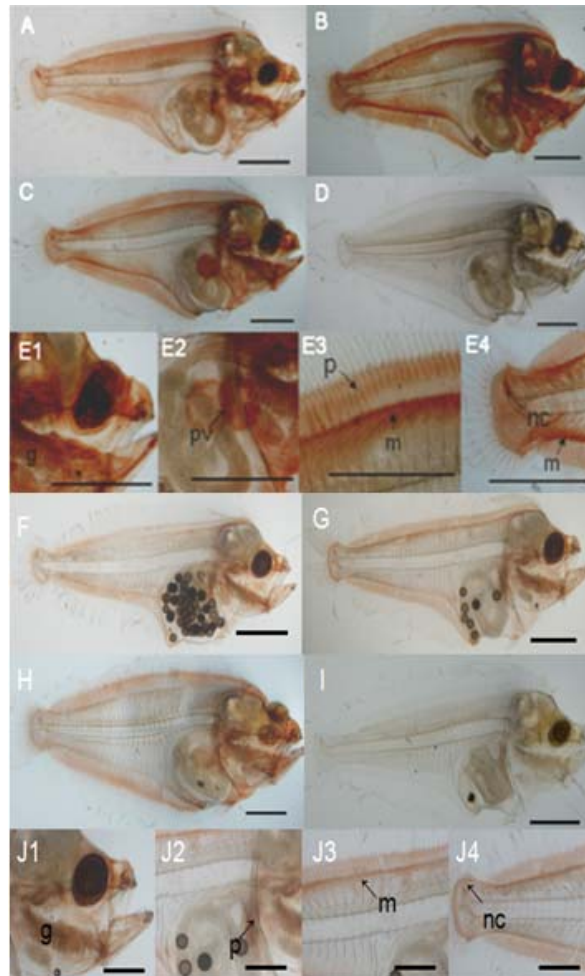
Supplementary Figure 10|Common expression pattern of 146 orthologs during flatfish metamorphosis. The 146 genes in Japanese flounder and tongue sole with a common expression pattern were selected by using the gene expression levels in the pre-metamorphic stage as a reference, and then determining the expression levels (up- or down-regulated) of differentially expressed genes in the pro-metamorphic, metamorphic climax, and post-metamorphic stages. We found 746 orthologs with the same labels in Japanese flounder and tongue sole, compared their expression levels in the different stages of metamorphosis and identified 146 orthologs exhibiting a consistent expression pattern during metamorphosis in both species.



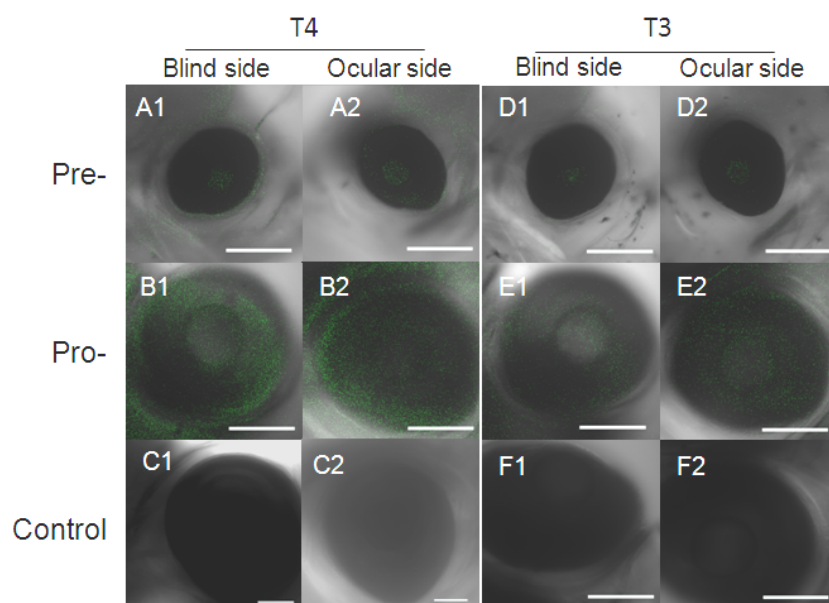
Supplementary Figure 11|Asymmetric expression of *dio2* in the skin around the eyes of Japanese flounder during metamorphosis. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, Metamorphic climax. D, post-metamorphic juvenile. E, Negative control - no signal was detected with the sense probe in larva at metamorphic climax. All scale bars, 200 μm .



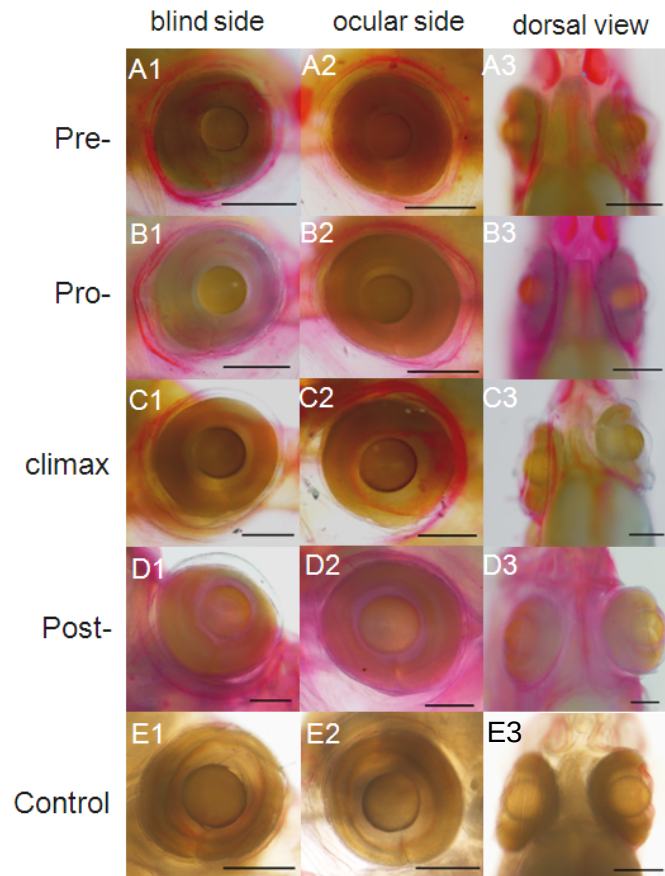
Supplementary Figure 12|Asymmetric expression of *dio3* in the skin around the eyes of Japanese flounder during metamorphosis. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, larva at metamorphic climax. D, post-metamorphic juvenile. E, Negative control - no signal was detected with the sense probe in post-metamorphic juvenile. All scale bars, 200 μ m.



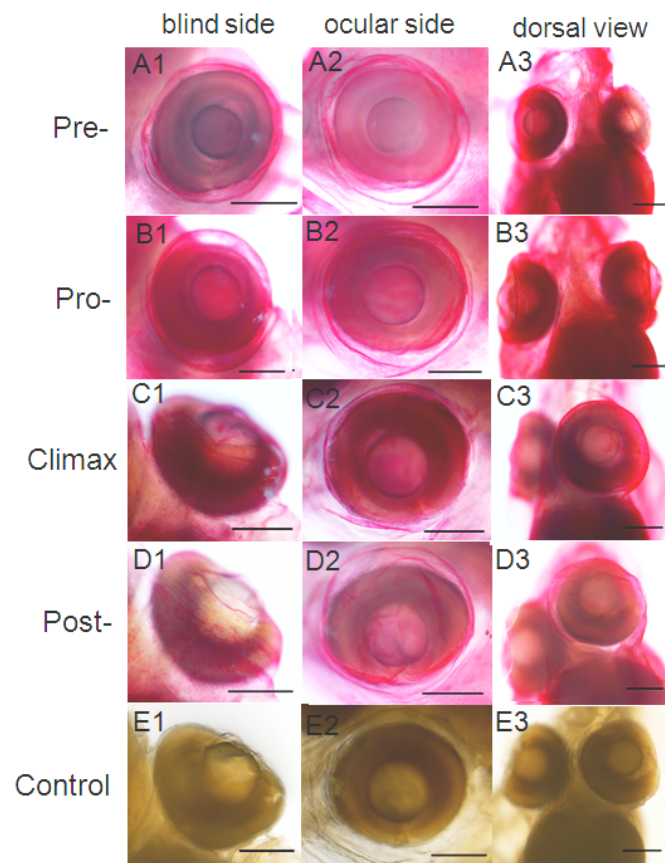
Supplementary Figure 13|Distribution of T4 and T3 in metamorphic Japanese flounder. T4 distribution pattern in pre-metamorphic larva (A), pro-metamorphic larva (B), and larva at metamorphic climax (C). D, Negative control (larva at metamorphic climax) in which T4 antisera was omitted. E, a higher magnification of pro-metamorphic larva (B), view of the head (E1), pectoral fin (E2), dorsal fin (E3) and caudal fin (E4). T3 distribution pattern in pre-metamorphic larva (F), pro-metamorphic larva (G), and larva at metamorphic climax (H). I, Negative control (larva at metamorphic climax) in which T3 antisera was omitted. J, a higher magnification of pro-metamorphic larva (G), (J1), pectoral fin (J2), dorsal fin (J3) and caudal fin (J4). Arrows indicate the specific signal. g, gill; pv, pectoral fin; p, pterygiophore; m, muscle; nc, notochord. All scale bars, 1 mm.



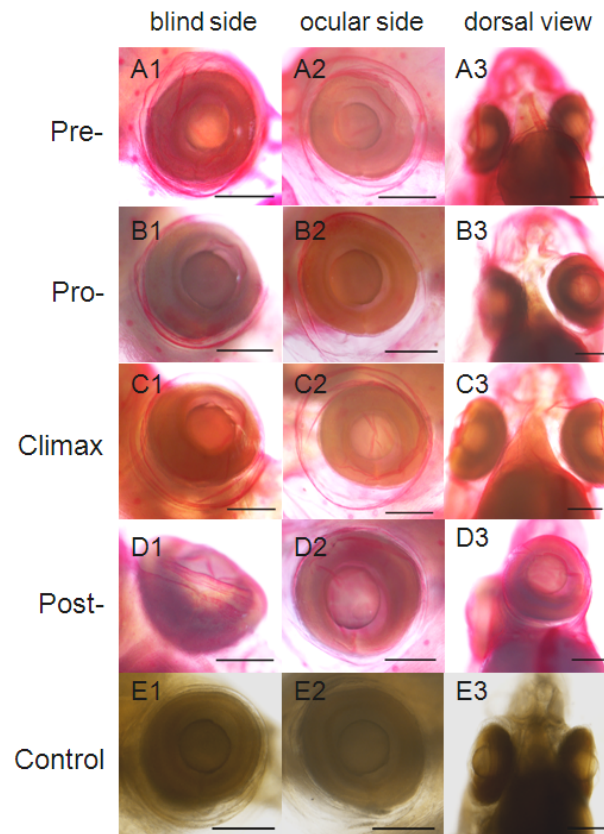
Supplementary Figure 14|Whole mount immunofluorescence of T4 and T3 in the skin around the eyes of metamorphic Japanese flounder. T4 distribution in pre-metamorphic larva (A) and pro-metamorphic larva (B) and negative control (C). T3 distribution in pre-metamorphic larva (D) and pro-metamorphic larva (E) and negative control (F). All scale bars, 200 μ m.



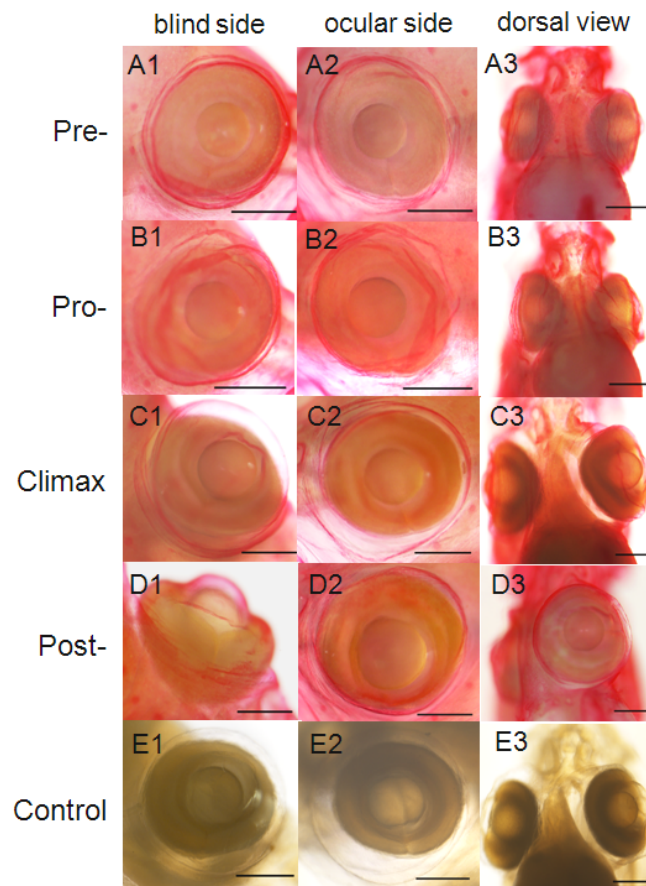
Supplementary Figure 15|Asymmetric expression of *thraa* in the skin around the eyes of Japanese flounder during metamorphosis. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, larva at metamorphic climax. D, post-metamorphic juvenile. E, Negative control - no signal was detected with the sense probe in post-metamorphic juveniles. All scale bars, 200 μ m.



Supplementary Figure 16|Symmetric expression of *thrab* in the skin around the eyes of Japanese flounder during metamorphosis. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, larva at metamorphic climax. D, post-metamorphic juvenile. E, Negative control - no signal was detected with the sense probe in post-metamorphic juveniles. All scale bars, 200 μ m.



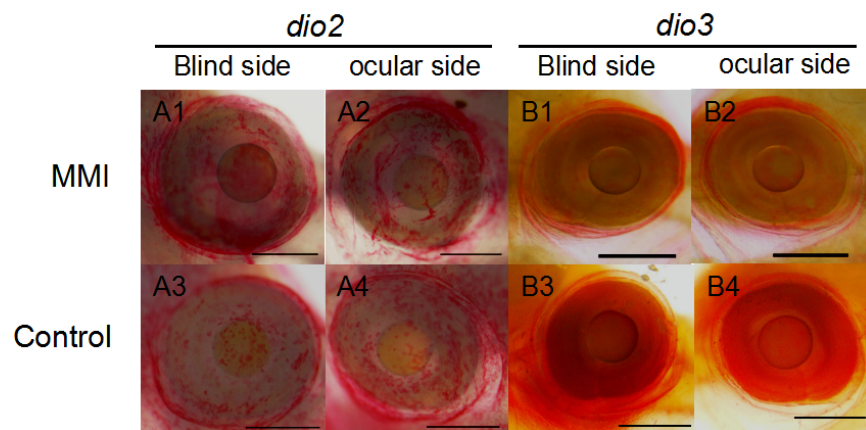
Supplementary Figure 17|Asymmetric expression of *thrb1* in the skin around the eyes of Japanese flounder during metamorphosis. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, larva at metamorphic climax. D, post-metamorphic juvenile. E, Negative control - no signal was detected with the sense probe in pro-metamorphic larva. All scale bars, 200 μm.



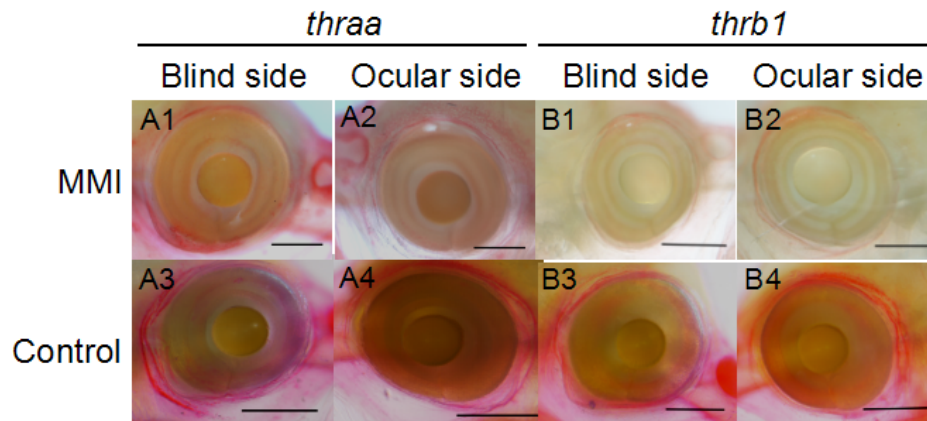
Supplementary Figure 18|Symmetric expression of *thrb2* in the skin around the eyes of Japanese flounder during metamorphosis. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, larva at metamorphic climax. D, post-metamorphic juvenile. E, Negative control - no signal was detected with the sense probe at metamorphic climax. All scale bars, 200 μ m.



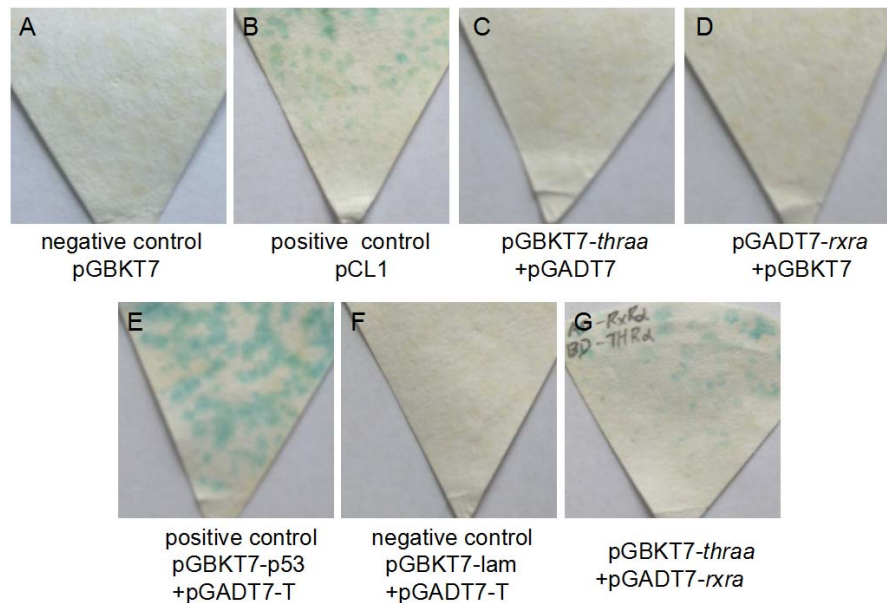
Supplementary Figure 19|Treatment with 2-Mercapto-1-methylimidazole (MMI) inhibited eye migration. A, Blind side of control; A1, increased magnification of a blind side view of the head; B, Ocular side of control; B1, increased magnification of an ocular side view of the head; C, Blind side of the fish after MMI treatment; C1, increased magnification of a blind side view of the head; D, Ocular side of the fish after MMI treatment; D1, increased magnification of an ocular side view of the head. Scale bars, 1 mm.



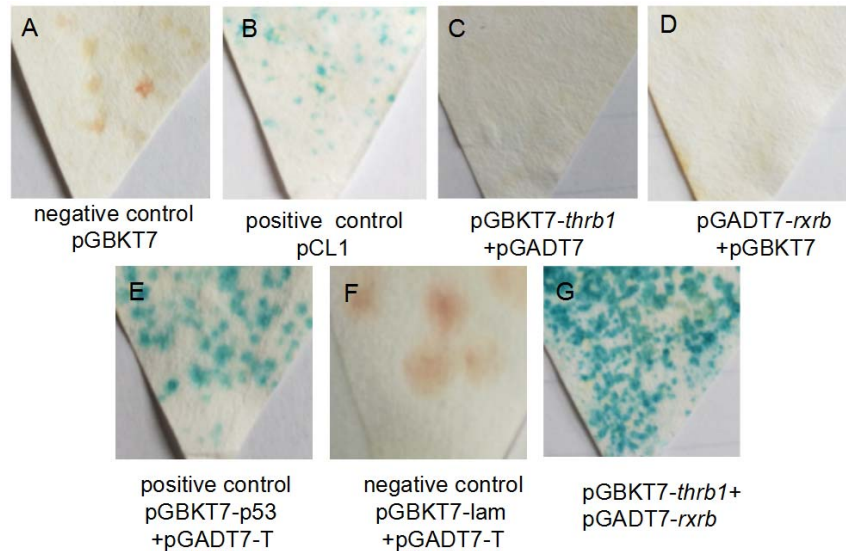
Supplementary Figure 20|Expression of *dio2* and *dio3* in the skin around the eyes of pro-metamorphic Japanese flounder after 4-days administration of MMI. Hybridization signals are red. A1-A4, *dio2* expression pattern. B1-B4, *dio3* expression pattern. Scale bars, 200 μ m.



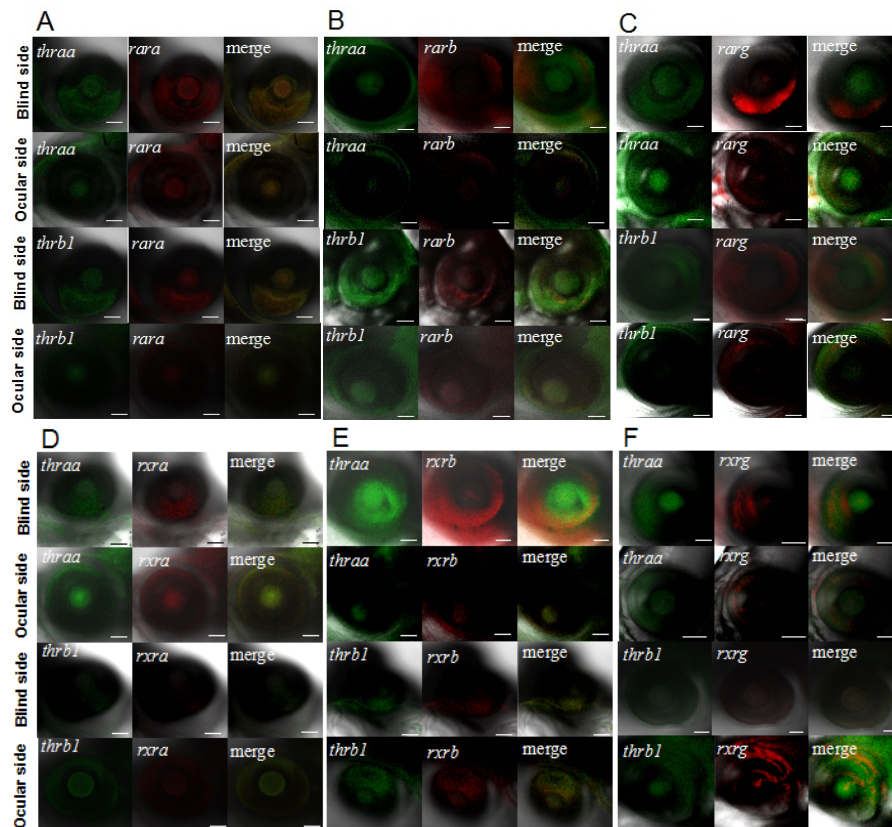
Supplementary Figure 21|Asymmetric expression of *thraa* and *thrb1* in the skin around the eyes of pro-metamorphic Japanese flounder was down-regulated after 4-days treatment with MMI. Hybridization signals are red. A1-A4, *thraa* expression pattern. B1-B4, *thrb1* expression pattern. Scale bars, 200 μ m. Note, the control figures (A3 and A4) are the same as those presented in B1 and B2 in Supplementary Figure 15 because the samples are all taken from the same experiment designed to compare normal development and development after MMI treatment.



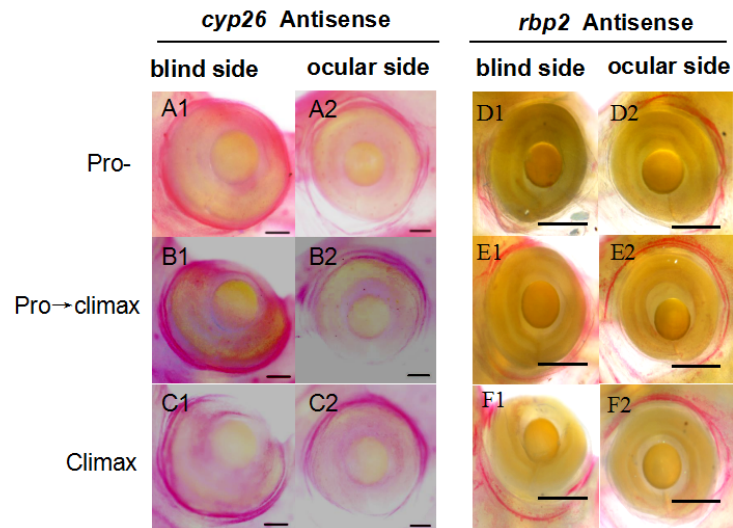
Supplementary Figure 22|Yeast two hybrid assay indicating interaction of Thraa with Rxra. Self-activation tests of the negative control pGBKT7 vectors (A), positive control pCL1 vectors (B), combinations of pGBKT7- *thraa* with pGADT7 (C), combinations of pGADT7- *rxra* with pGBKT7 (D). Colonies in (C) and (D) did not test positive in the X-gal assay. Positive control of pGBKT7-p53 and pGADT7-T (E), negative control of pGBKT7-Lam and pGADT7-T (F), experimental group of pGBKT7-*thraa* and pGADT7-*rxra* (G). Colonies in (E) and (G) tested positive in the X-gal assay, demonstrating that these fusion proteins can interact.



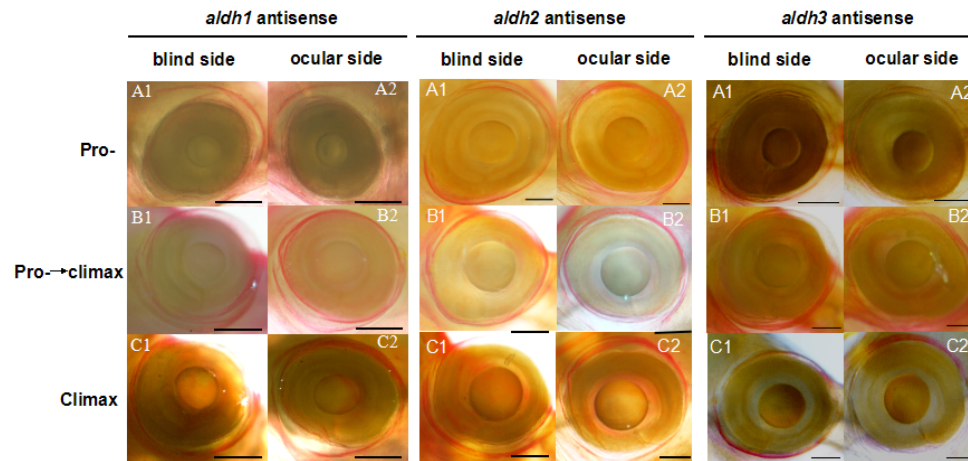
Supplementary Figure 23|Yeast two hybrid assay indicating interaction of Thr1 with Rxrb. Self-activation tests of the negative control pGBKT7 vectors (A), positive control pCL1 vectors (B), combinations of pGBKT7- *thrb1* with pGADT7 (C), combinations of pGADT7- *rxrb* with pGBKT7 (D). Colonies in (C) and (D) did not test positive in the X-gal assay. Positive control of pGBKT7-p53 and pGADT7-T (E), negative control of pGBKT7-Lam and pGADT7-T (F), experimental group of pGBKT7-*thrb1* and pGADT7-*rxrb* (G). Colonies in (E) and (G) tested positive in the X-gal assay, demonstrating that these fusion proteins can interact.



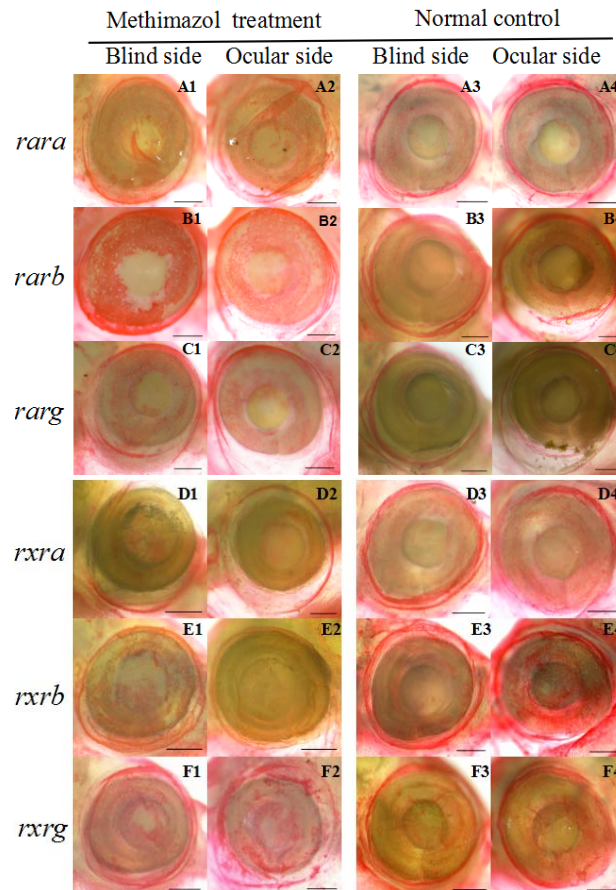
Supplementary Figure 24|Double fluorescence *in situ* hybridization (D-FISH) analysis for co-expression of *thraa*, *thrb1* and *rxr*, *rar* genes in Japanese flounder. The cRNA probes of *thraa* and *thrb1* genes were labeled with biotin and detected with anti-Biotin-FITC antibodies. *rxrs* (*rxra*, *rxrb* and *rxrg*) and *rar* (*rara*, *rarb* and *rarg*) cRNA probes were labeled with digoxigenin and detected with anti-Digoxigenin-Rhodamine antibodies. Panel A-F represents the blind side and ocular side views of the results obtained with the probes *thraa* and *thrb1* and *rxra*, *rxrb*, *rxrg*, and *rara*, *rarb*, *rarg*, respectively. Scale bars, 100 μ m.



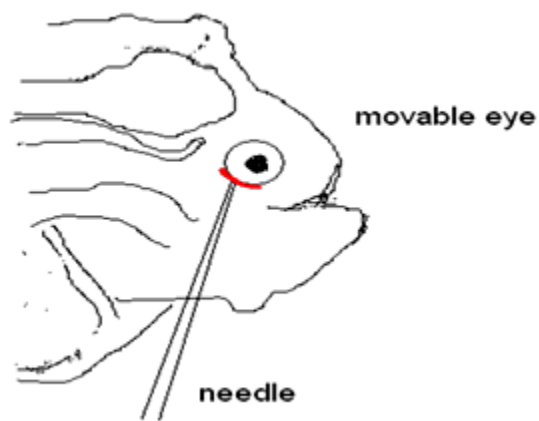
Supplementary Figure 25|The expression pattern of *cyp26* and *rbp2* in the skin around the eyes is similar to the distribution pattern of proliferating cells in the Japanese flounder. Hybridization signals are red. A and D, view in pro-metamorphic larva. B and E, view in pro-metamorphosis →climax. C and F, view in metamorphic climax. Scale bars, 100 μ m.



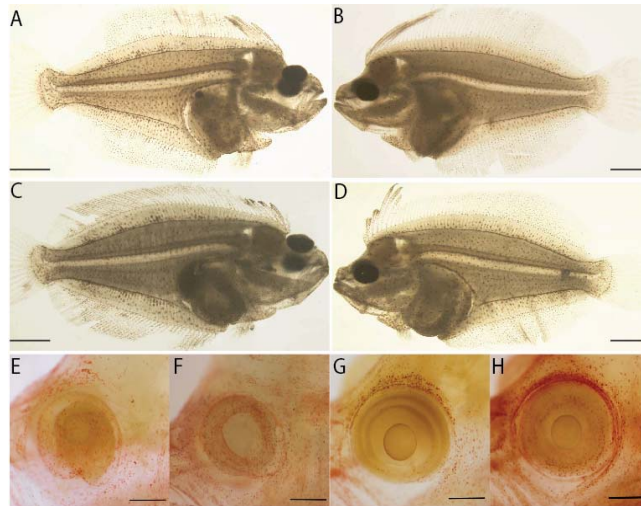
Supplementary Figure 26|Asymmetric expression pattern of *aldhs* genes around the eyes in several metamorphic stages of Japanese flounder. Hybridization signals are red. A1 and A2, blind side and ocular side views in pro-metamorphic larva; B1 and B2, blind side and ocular side views in pro-metamorphosis →climax; C1 and C2, blind side and ocular side views at metamorphic climax. Scale bars, 100 μ m.



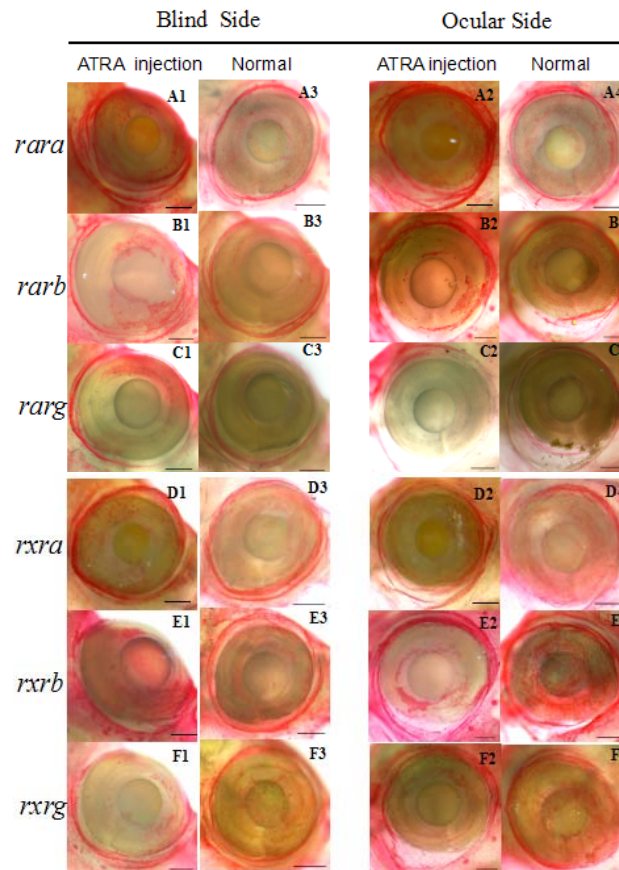
Supplementary Figure 27|The expression pattern of *rxrs* and *rars* in the skin around the eyes of pro-metamorphic Japanese flounder after 4-days treatment with MMI. Hybridization signals are red. A, *rara*, B, *rarb*, C, *rarg*, D, *rxra*, E, *rxrb* and F, *rxrg*. Scale bars, 100 μ m.



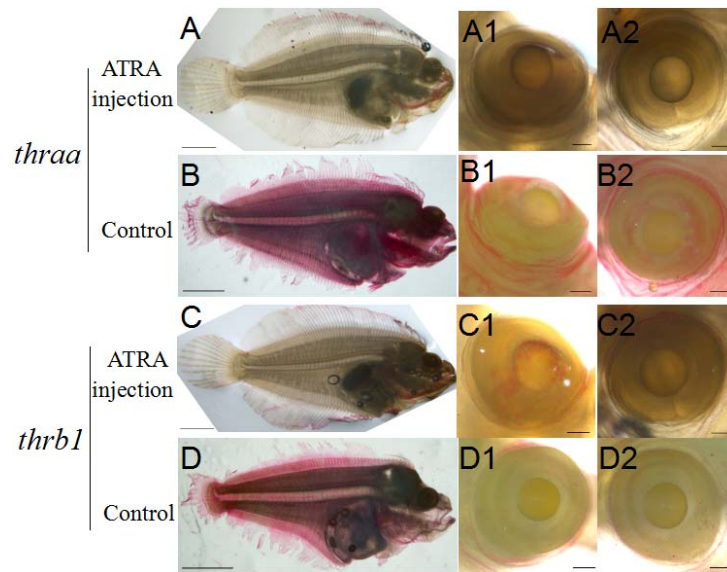
Supplementary Figure 28|The schematic view of retinoic acid-microinjection site (red) on the blind side of Japanese flounder larvae for inhibiting the eye migration.



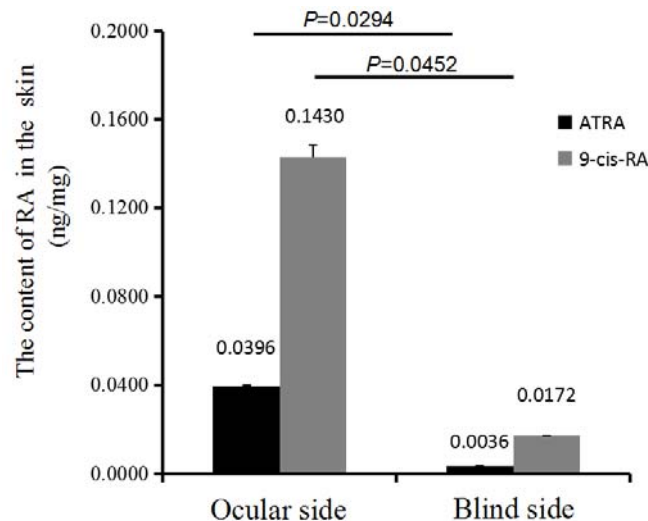
Supplementary Figure 29| Representative phenotypes caused by microinjection of RAs and in the lower panel cell proliferation in the suborbital area of the ocular side eye. A and C, eye-asymmetry individuals for 13-cis-RA (blind side) and control (blind side); B and D, eye-symmetry individuals for 9-cis-RA (ocular side) and ATRA (ocular side); Scale bar 1mm. E and G, eye-symmetry individuals for 9-cis-RA (ocular side) and ATRA (ocular side); F and H, the corresponding control (ocular side); Scale bar 200 μ m.



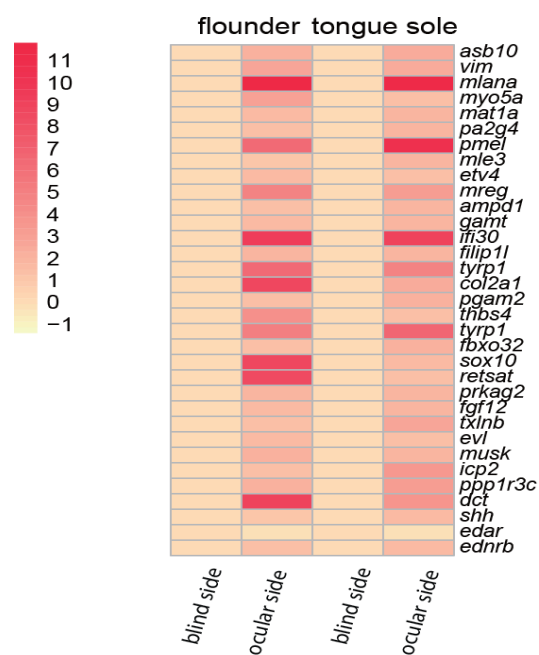
Supplementary Figure 30|Expression of *rars* and *rxrs* in the tissue around the eyes after microinjecting ATRA into the suborbital area on the blind side in Japanese flounder. Hybridization signals are red. Scale bars, 100 μ m. Note, the normal group presented in this figure is the same as the normal group in Supplementary Figure 27 because they came from a common experiment.



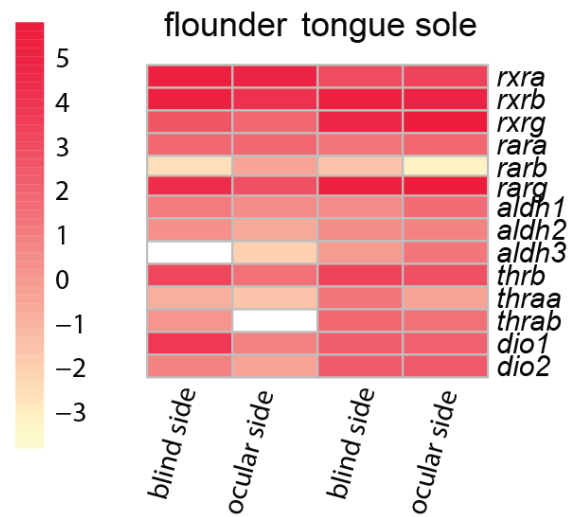
Supplementary Figure 31|The expression of *thr* genes in the tissue around the eyes after microinjecting ATRA into the suborbital area on the blind side of Japanese flounder. Hybridization signals are red. *thraa* expression in larva with ATRA treatment in a whole body view (A), blind side view (A1) and ocular side view (A2). *thraa* expression in normal larvae, a whole body view (B), blind side view (B1) and ocular side view (B2). *thrb1* expression in larva with ATRA treatment, a whole body view (C), blind side view (C1) and ocular side view (C2). *thrb1* expression in normal larva, a whole body view (D), blind side view (D1) and ocular side view (D2). Scale bars (A, B, C and D), 1 mm; Scale bars (A1, A2, B1, B2, C1, C2, D1, D2), 100 μ m.



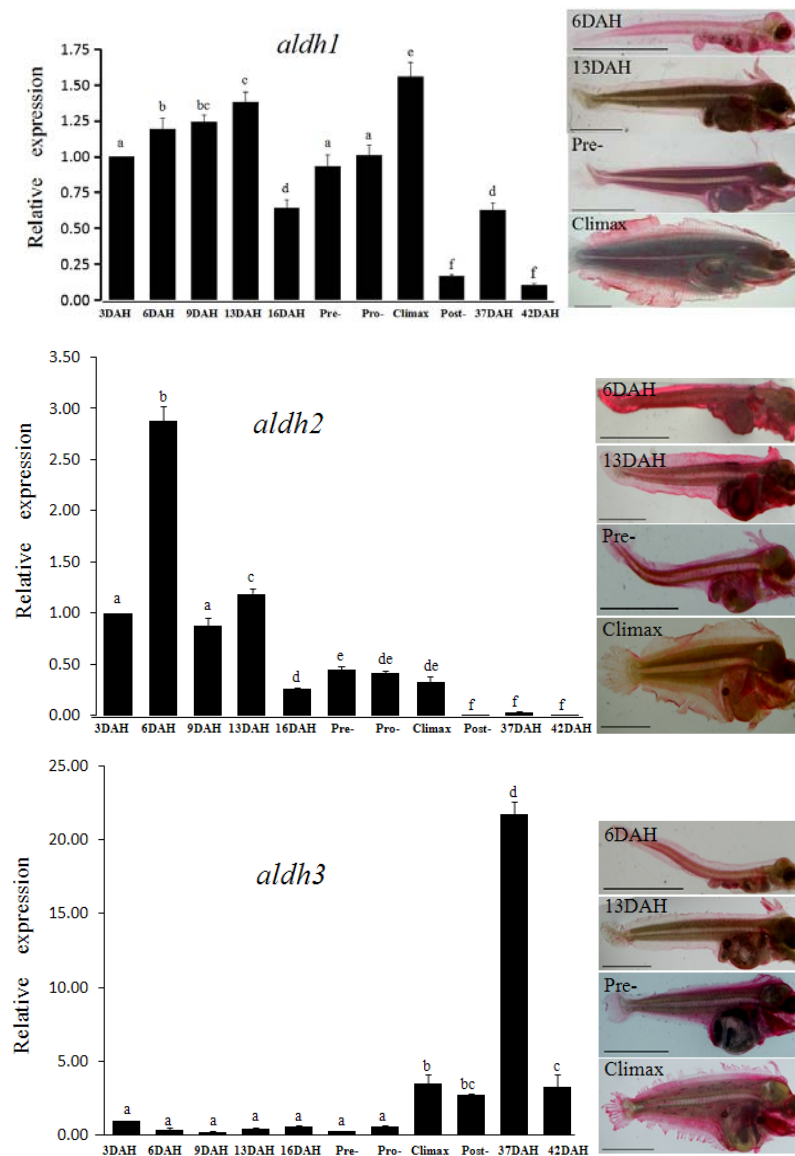
Supplementary Figure 32|The RA concentration in the skin of Japanese flounder post-metamorphic juvenile. RA concentration gradients were detected on the ocular and blind side skin from post-metamorphic juvenile Japanese flounder using UPLC-MS/MS in S-MRM mode with m/z 301.2/123.2. The RA isolates from skin samples had a peak with the same retention time as the standards ATRA and 9-cis-RA (5.58 and 5.43 min), which confirmed that RAs were isolated from the skin of Japanese flounder. For quantification of RAs using UPLC-MS/MS, calibration curves of ATRA and 9-cis-RA were produced with $y=0.0283x+0.3155$ and $y=0.0083x+0.3308$. The R^2 values (> 0.99) showed good linearity for the concentration range 1-1000 ng/mL. One-way ANOVA indicated that there were significant differences between ocular and blind side for RA concentration gradients. The numbers represent the means of concentration for each of three parallel experiments. Bars represent the standard deviation.



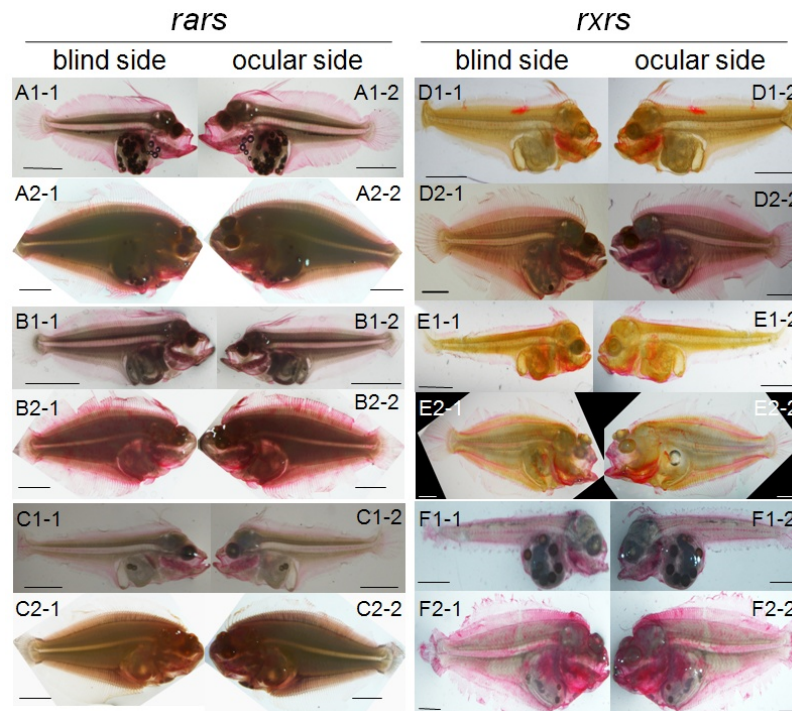
Supplementary Figure 33| Up-regulated pigment related orthologs in the ocular side skin of Japanese flounder and tongue sole.



Supplementary Figure 34| The expression of RA and TH signaling genes in the ocular and blind side skin of Japanese flounder and tongue sole.



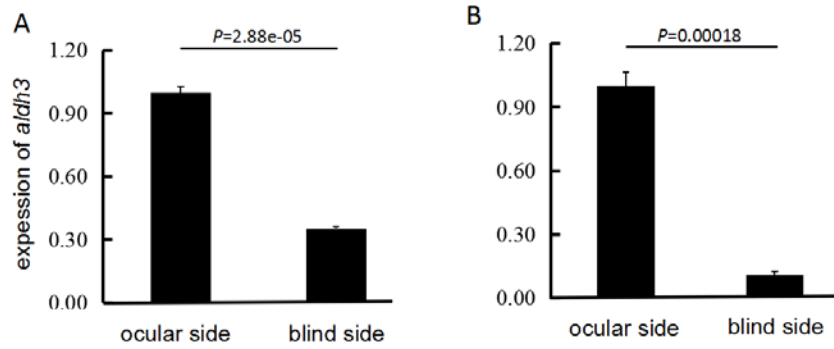
Supplementary Figure 35|Relative gene expression of *aldh1-3* in different developmental stages of the Japanese flounder. Hybridization signals are red. Graphs present the relative transcript abundance determined by qPCR of *aldh1-3* in early developmental stages and during metamorphosis. Scale bars, 1 mm. Bars represent the standard deviation.



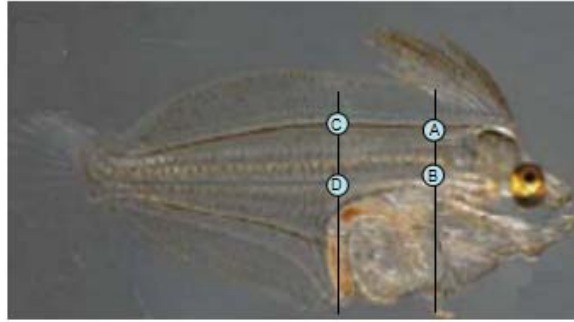
Supplementary Figure 36|The undifferentiated expression of *rar* and *rxr* genes on both sides of the body skin in pre-metamorphic larva and post-metamorphic juveniles in untreated Japanese flounder. Hybridization signals are red. Expression of the *rara* gene in pre-metamorphic larva (A1) and post-metamorphic juveniles (A2). Expression of the *rarb* gene in pre-metamorphic larva (B1) and post-metamorphic juveniles (B2). Expression of the *rarg* gene in pre-metamorphic larva (C1) and post-metamorphic juveniles (C2). Expression of the *rxra* gene in pre-metamorphic larva (D1) and post-metamorphic juveniles (D2). Expression of the *rxrb* gene in pre-metamorphic larva (E1) and post-metamorphic juveniles (E2). Expression of the *rxrg* gene in pre-metamorphic larva (F1) and post-metamorphic juveniles (F2). Scale bars, 100 μ m.



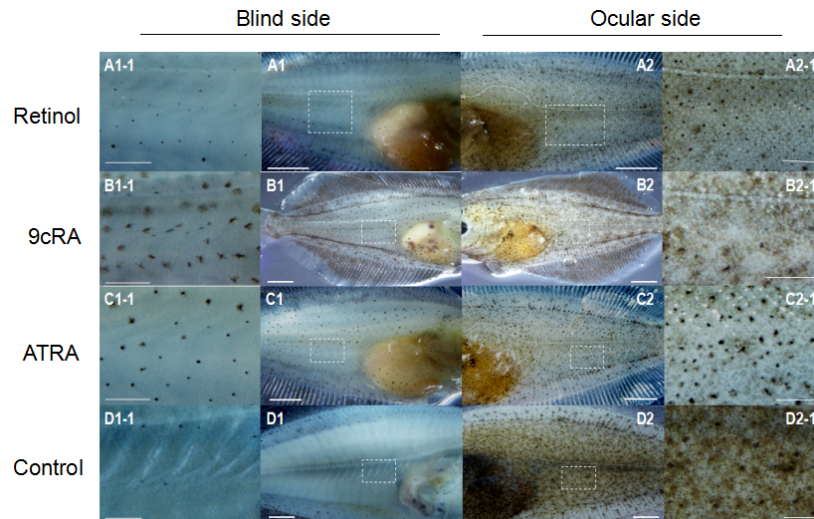
Supplementary Figure 37|Expression pattern of *aldhs* in the body skin during metamorphosis of the Japanese flounder. A. *aldh3* expressed in pre-metamorphic larva. A1, blind side view; A2, ocular side view; A3, increased magnification of the blind side view of the head; A4, increased magnification of the ocular side view of the head. B. *aldh3* expressed in larva at the post-metamorphic stage. B1, blind side view; B2, ocular side view; B3, increased magnification of the blind side view of the head; B4, increased magnification of the ocular side view of the head. *aldh1* (C) and *aldh2* (D) genes expressed in the skin on both sides. There were no significant differences in signal expression on the blind and ocular side skin in pre-metamorphic larva (C1, C2, D1, D2) and post-metamorphic juveniles (C3, C4, D3, D4). Hybridization signals are red. Scale bars, 100 μ m.



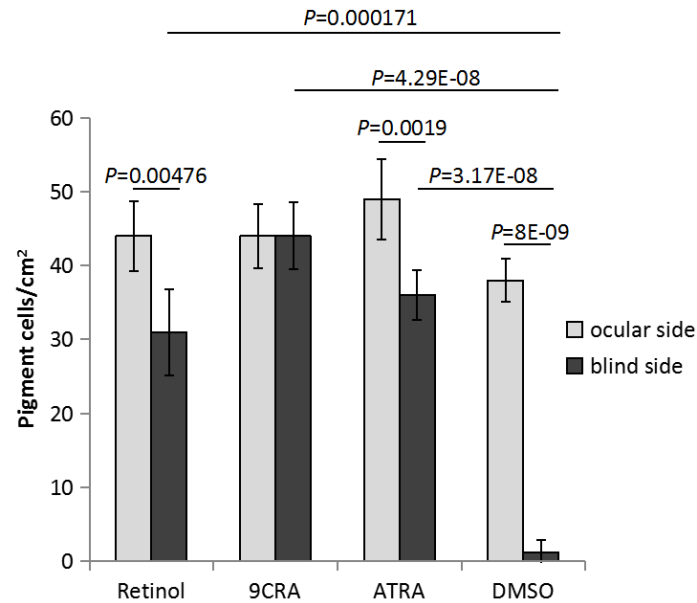
Supplementary Figure38|The expression of *ald3* in the skin of post-metamorphic juveniles (A) and adults (B) determined by qPCR analysis. One-way ANOVA indicated that there were significant differences between ocular and blind side for *ald3* expression. Bars represent the standard deviation.



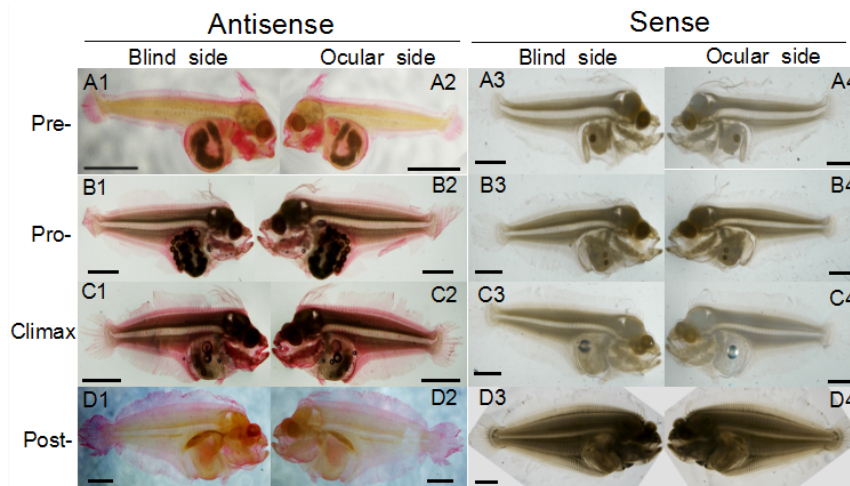
Supplementary Figure 39|The retinoic acid-microinjection site on the skin of blind side of Japanese flounder. A. intersection point of the upper terminal of the myotome and vertical line through the operculum; B. intersection point of the vertebrae and vertical line through operculum opening; C. intersection point of the uppermost point of the myotome and vertical line through the anus; D. intersection point of the vertebrae and vertical line through the anus.



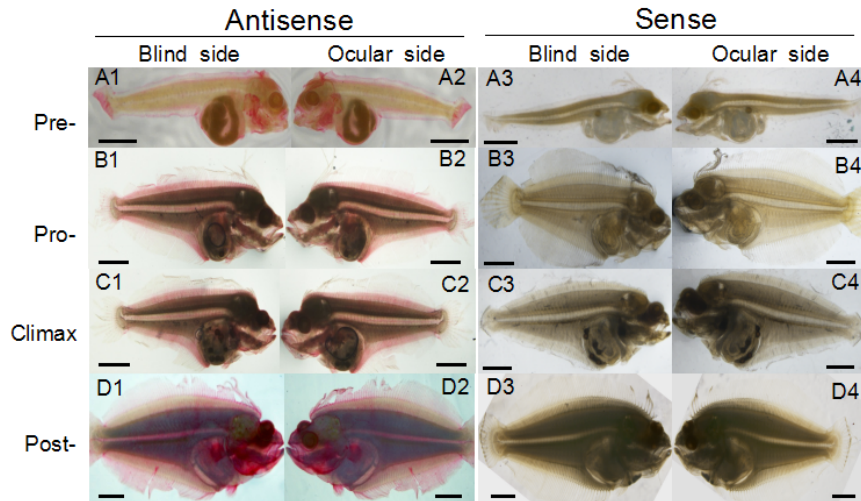
Supplementary Figure 40|Formation of pigment cells following microinjection of retinoic acid into the skin on the blind side of Japanese flounder. A1, distribution of adult chromatophores on the blind side after retinol injection; A1-1, increased magnification of the rectangular area in A1; A2, distribution of adult chromatophores in the ocular side; A2-1, increased magnification of the rectangular area in A2; B1, distribution of adult chromatophores on the blind side after 9cRA injection; B1-1, increased magnification of the rectangular area in B1; B2, distribution of adult chromatophores in the ocular side; B2-1, increased magnification of the rectangular area in B2; C1, distribution of adult chromatophores on the blind side after ATRA injection; C1-1, increased magnification of the rectangular area in C1; C2, distribution of adult chromatophores on the ocular side; C2-1, increased magnification of the rectangular area in C2; D1, distribution of adult chromatophores on the blind side after DMSO injection; D1-1, increased magnification of the rectangular area in D1; D2, distribution of adult chromatophores on the ocular side; D2-1, increased magnification of the rectangular area in D2. Scale bars for A1, A2, B1, B2, C1, C2, D1 and D2, 2 mm. Scale bars for A1-1, A2-1, B1-1, B2-1, C1-1, C2-1, D1-1 and D2-1, 0.5 mm.



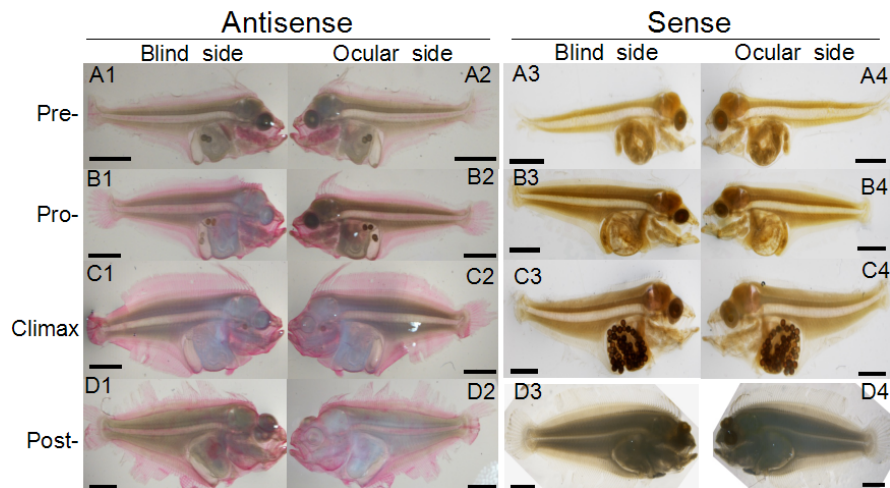
Supplementary Figure 41|Quantity of pigment cells after RA treatment. Retinol and RAs (9cRA and ATRA) were microinjected nine times into the skin on the blind side of Japanese flounder and the density of pigment cells (cells/cm²) on the blind and ocular sides were counted. Two samples *t*-test indicated that there were significant differences exist in pigment cell number between the blind and ocular sides in the different treatments and in the blind side relative to the control. Bars represent the standard deviation.



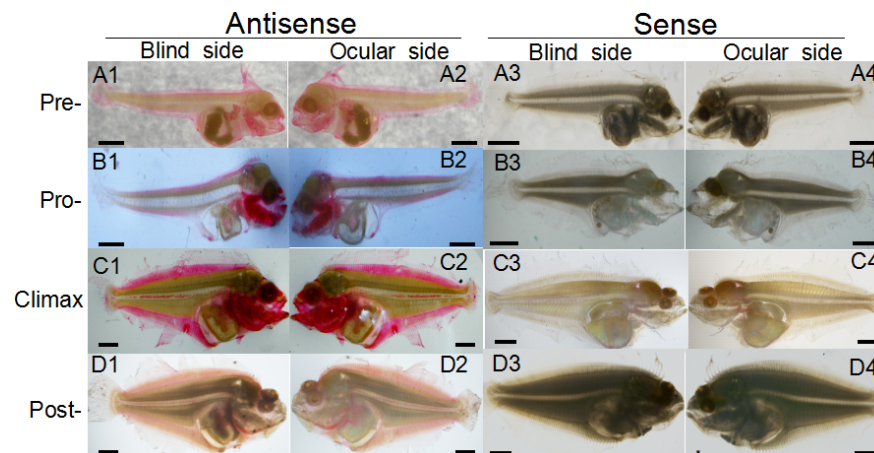
Supplementary Figure 42|Expression pattern of the rhodopsin (*rh1*) gene during metamorphosis of Japanese flounder. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, Metamorphic climax. D, post-metamorphic juvenile. Scale bars, 1 mm.



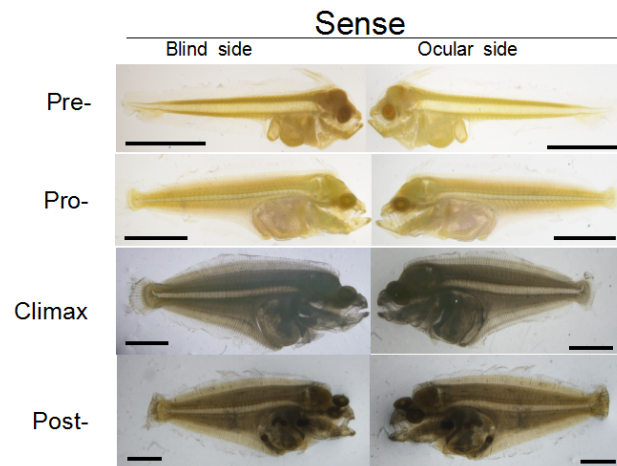
Supplementary Figure 43|Expression pattern of the *rh2* (*green-opsin*) gene during metamorphosis of Japanese flounder. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, Metamorphic climax. D, post-metamorphic juvenile. Scale bars, 1mm.



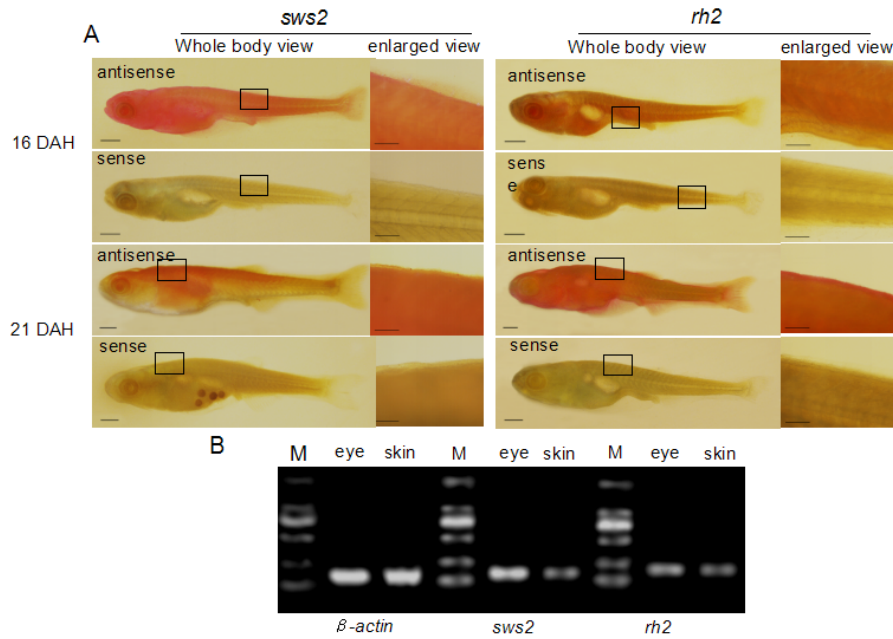
Supplementary Figure 44|Expression pattern of the *lws* (red) gene during metamorphosis of Japanese flounder. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, Metamorphic climax. D, post-metamorphic juvenile. Scale bars, 1mm.



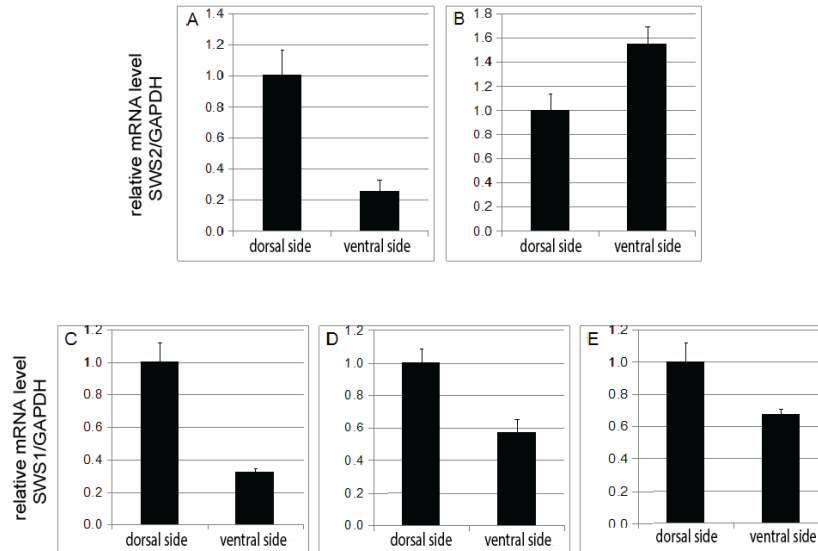
Supplementary Figure 45|Expression pattern of *sws1* (ultraviolet) gene during metamorphosis of Japanese flounder. Hybridization signals are red. A, pre-metamorphic larva. B, pro-metamorphic larva. C, Metamorphic climax. D, post-metamorphic juvenile. Scale bars, 1mm.



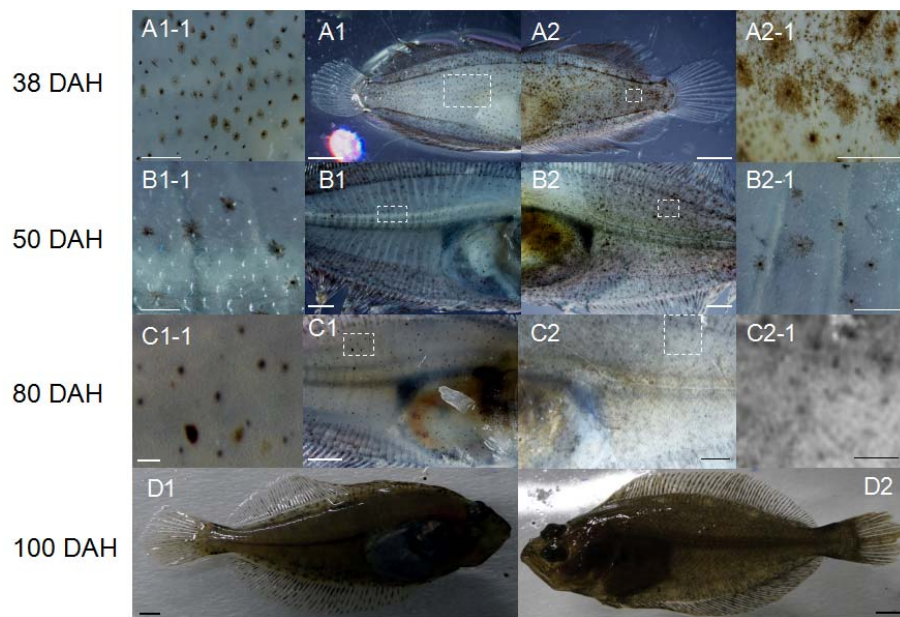
Supplementary Figure 46|Control of the *sws2* gene expression during metamorphosis of Japanese flounder. A, pre-metamorphic larva. B, pro-metamorphic larva. C, Metamorphic climax. D, post-metamorphic juvenile. Scale bars, 1mm.



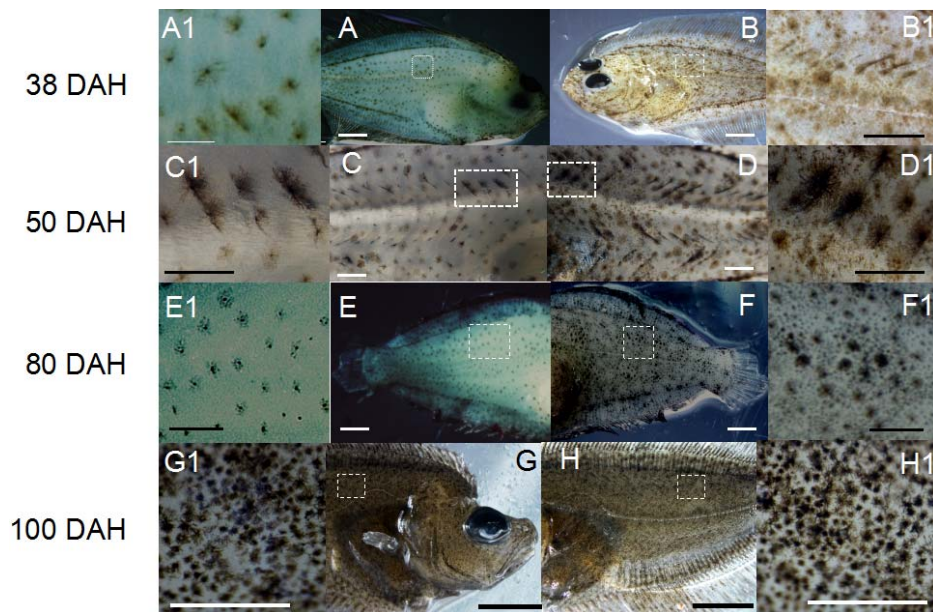
Supplementary Figure 47|Expression pattern of *sws2* and *rh2* in the skin and eye of zebrafish. Hybridization signals are red. A, *In situ* hybridization of *sws2* and *rh2* in the skin of zebrafish at 16 DAH and 21 DAH. B, RT-PCR analysis of *sws2* and *rh2* in the skin and eye of zebrafish. Black boxes represent the increased magnification for the corresponding figures. M, marker. Scale bars for the whole body view, 300 μ m, Scale bars for the enlarged view, 100 μ m.



Supplementary Figure 48|Expression of *sws1* and *sws2* in skin on the dorsal and ventral side of *Salmo salar*, *Scomberomorus niphonius*, *Cololabis saira* and *Hemirhamphus quoyi*. A, *sws2* expression in the dorsal and ventral skin of *Salmo salar*. B, *sws2* expression in the dorsal and ventral skin of *Cololabis saira*. C, *sws1* expression in the dorsal and ventral skin of *Salmo salar*. D, *sws1* expression in the dorsal and ventral skin of *Hemirhamphus quoyi*. E, *sws1* expression in the dorsal and ventral skin of *Scomberomorus niphonius*. Bars represent the standard deviation.



Supplementary Figure 49|The pigment cell distribution on ocular and blind skin of Japanese flounder after irradiation with white light. A, After exposure to the white light from the bottom of the tank for 38 days A1, Japanese flounder larvae blind side. A1-1, increased magnification of A1. A2, Japanese flounder larvae ocular side. A2-1, increased magnification of A2. B, After exposure to white light from the bottom of the tank for 50 days. B1, Japanese flounder larvae blind side. B1-1, increased magnification of B1. B2, Japanese flounder larvae ocular side. B2-1, enlarged view of B2. C, After exposure to white light from the bottom of the tank for 80 days. C1, Japanese flounder larvae blind side. C1-1, increased magnification of C1. C2, Japanese flounder larvae ocular side. C2-1, increased magnification of C2. D, After exposure to white light from the bottom of the tank for 100 days. D1, Japanese flounder larvae blind side. D2, Japanese flounder larvae ocular side. Scale bars for A1, A2, B1, B2, C1, C2, D1 and D2, 2 mm. Scale bars for A1-1, A2-1, B1-1, B2-1, C1-1 and C2-1, 0.5 mm.



Supplementary Figure 50|The pigment cell distribution on ocular and blind skin of Japanese flounder after irradiation with blue light. A, After exposure to blue light from the bottom of the tank for 38 days (Japanese flounder larvae blind side). A1, increased magnification of A (scale bar, 0.4 mm). B, After exposure to blue light from the bottom of the tank for 38 days (Japanese flounder larvae ocular side). B1, increased magnification of B (scale bar, 0.6 mm). C, After exposure to blue light from the bottom of the tank for 50 days (Japanese flounder larvae blind side). C1, increased magnification of C (scale bar, 1.8 mm). D, After exposure to blue light from the bottom of the tank for 50 days (Japanese flounder larvae ocular side). D1, increased magnification of D (scale bar, 1.4 mm). E, After exposure to blue light from the bottom of the tank for 80 days (Japanese flounder larvae blind side). E1, increased magnification of E (scale bar, 0.6 mm). F, After exposure to blue light from the bottom of the tank for 80 days (Japanese flounder larvae ocular side). F1, increased magnification of F (scale bar, 0.6 mm). G, After exposure to blue light from the bottom of the tank for 100 days (Japanese flounder larvae blind side). G1, increased magnification of G; H, After exposure to blue light from the bottom of the tank for 100 days (Japanese flounder larvae ocular side). H1, increased magnification of H. Scale bars, 1 mm.

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