Supplementary Materials: Evolutionary Conservation of *pou5f3* Genomic Organization and Its Dynamic Distribution during Embryogenesis and in Adult Gonads in Japanese Flounder *Paralichthys olivaceus*

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Table S1. The Genbank accession numbers or Ensembl IDs of the POUV p	proteins analyzed in Figure 1C.
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Species	Genbank Accession No./ Ensembl IDs	Species	Genbank Accession No./ Ensembl IDs
Acipenser sinensis	AEK81554.1	Pan troglodytes	NP_001238970.1
Anolis carolinensis	ENSACAP00000017001	Ornithorhynchus anatinus	NP_001229656.1
Bos taurus	NP_777005.1	Oryzias latipes	NP_001098339.1
Carassius auratus	AET79963.1	Oreochromis niloticus	XP_003444455.1
Danio rerio	NP_571187.1	Pagrus major	BAH08689.1
Gadus morhua	ENSGMOP0000005053	Paralichthys olivaceus	KJ522774
Gasterosteus aculeatus	ENSGACP0000023125	Rattus norvegicus	NP_001009178.1
Homo sapiens	NP_002692.2	Takifugu rubripes	XP_003965650.1
Labeo rohita	ADC96616.1	Tetraodon nigroviridis	ENSTNIP0000016222
Latimeria chalumnae	XP_005994419.1	Xenopus tropicalis	ENSXETP0000008656
Mus musculus	NP_038661.2	Xiphophorus maculatus	XP_005799711.1

Table S2. The Ensembl IDs of the analyzed sequences in Figure 3B.

Species		Ensembl IDs
Human	Homo sapiens	ENST0000259915
Cow	Bos taurus	ENSBTAT0000028122
Mouse	Mus musculus	ENSMUST0000025271
Stickleback	Gasterosteus aculeatus	ENSGACT0000023169
Tilapia	Oreochromis niloticus	ENSONIT0000004857
Zebrafish	Danio rerio	ENSDART0000065817

${\tt gtgcccacgaaaacctgtggagccttggaggagagcagcatttgagcttgacggtggagcggcgtgaagggcaaggattcatggagtaggccggaggcgt$	100
${\tt gttggtggtaatgtggcaccaaaacgacagctgctaaggccttttgcaaaactctcccccaaacggactttacgcaagcaa$	200
gcgcgtcctccgctcgttcctctttgacttgtgcgtaaacttgaaacggatgagcacgtttaactttattcccattaactatttcttttaaaactttcta	300
$\verb+teteggacttgttetaataateteeceacagegtttggaag \verb+ATGTCTGAAAGATCTCAGAGTCCGGAGTGCCAAAGCAGGCCGTATGACTTCAGCC$	400
M S E R S Q S P E C Q S R P Y D F S	18
${\tt GCGCCGGCCATTGCGCTCAGGTTTTGGGTCAAGATGGTTTGGGCAACGCTGCGTCATTCCAGGCTCTCTCACGGCGTTTTGCCAGACCCGAGCCTCCTCTA$	500
R A G H C A Q V L G Q D G L G N A A S F Q L P H G V L P D P S L L Y	52
${\tt TAACAAAACGGCGTATGGTGGCATCACGTCGGCCTCTGCGCAGACTTTCTTCCCATTCCCACCCA$	600
N K T A Y G G I T S A S A Q T F F P F P P M A S D Y R G T D L Q A	85
gggagtttgggcaacccaaacactggtatcccttcgctgcgcccgagtacaccggccaggtacccggcgtaacagcagctacccagcctactaacacctga	700
GEFGQPKHWYPFAAPEYTGQVPGVTAATQPTNL	118
GTCCCCCTATGGCCGAGACCCCGGGAGCAAATCAAAATGCCCGAGATCAAAACCGAGAAGGACACCGGCGATGACTACTCAACGGAGATCAAGGGTCAACA	800
S	152
${\tt GTATCTGACACCGTCAGCCTCCGCCGCCATGGCTCATGGAGTCTTCTACCCCGCGGCCTGGAACCCGTCCTTCTGGCCTGGAATCGCCCACATCACCCCG$	900
Y L T P S A S A A M A H G V F Y H A A W N P S F W P G I A H I T P	185
ccccgtatcagtaatcaaaaatccttccacatccttccaccatcgtcgtcgtctatgtcccccttcacccccgagcaaccgggcttccaggaaacgcgtttttca	1000
PGISNQNPSTSSASSPSMSPSPPSNGLPGNAFF	218
GCGTGAACCCAACCCAAGCCGCCCCCGGGCCGCAGACGCCGGACCCGGCCTCCTCCACGCGGACGCGGTTCCTCCAGCGGCGGATGCAGCGACTCAGA	1100
S V N P T Q A A P G P Q T Q N P A S S T R S S G S S S G G C S D S E	252
${\tt GGAG} gt$ agattagaatcccttcaaccaaccatttaatccaacagttgtaatatgagccttcaagtcaaacacacatgtgttcaacttctgtaagttcta	1200
E	253
$catctatatggttttcattttcaacctgtctcaccag {\tt GAGAACCTCTCCACTGAGGAACTGGAGCAGTTTGCCAAGGAACTGAAACACAAACGCATCACTT}$	1300
ENLSTEELEQFAKELKHKRIT	274
${\tt TGGGTTTCACTCAAGCTGATGTTGGCCTTGCCCTGGGTAACCTCTATGgt aggttggtcccatgtggaaacgtcctattgggtcatattgcactccct$	1400
L G F T Q A D V G L A L G N L Y	290
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G	291
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K M F S Q T T I C R F E A L Q L S F K N M C K L K P L L Q R W L N	324
AGCCAGAGACCTCAGACATCCCCAGGATgtaagtctgcaacactgacatctgccagctagtttacaagacaacagatgatccttgagcctgatgggatc	1900
E A E T S D N P Q D	334
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atoacatgaaacattatttgaaccataacatccatacaaggcttaaaccataagggacacaaattgcttcggtacataggtagccctagttggggcttg	2100
	2200
	300
	2300
	2400
Ciosoci i okonomono ytaayaacaaaaytya ytaytu ytu tu t	2400
	2500
	123
CCCACTGARCATGGCCCCTTTCCCCATCCCCCATCCCCCAGTACCACCTACCCACGCACCCCCCTTTCAC	2600
	156
CGGCCCGATGTCCTGAAACAGGCCCTTCACCCCGGACTTATTGGTCACATGACTGGA TA acacgctccggttggcctagccaatcccgcagataacctt	2700
R Р D V I, К O A I, Н Р G I, I G Н М Т G *	475
cccaagettttetgtaaagggcaagteaaactggaggaggaggaggatgattagtggatgaagacggttcaagggaggaggaggatettttteetgttatte	2800
traaacta ca caaattu cattu to ca catocto ta accoura a concerca caa cattu tu da to a atto tu da ta adocta da aa t	2900
$\label{eq:constraint} t_{constraint} = t_{constraint} =$	3000
ccacttatactaaatctaatctutttctgggggtgggggggggg	3100
aattaggtaagtatttggtcaatattgtacaatggacagagtacggtattgtattgtattaaatggagaaaaatggagaaagggta	3200
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caccetatcaaetactaatatattttaaaaattgctttttaaaaataatatattaatatataatatataatattaaaa	3673
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${\tt gtctcqacaqattqtaaatqqattcattataatttattqaaatqttqqttaaqctqaqatqttttqactqtaaccacctqaqacctaaatqtaaaacqqa$	3873
cactttatttqaaaaattqtatatqcaaactaccaaattqttccttttcccaqtaaatctttqcccatqtcccaccaaa(aaaaaaaaaa	3973
aaaaaa)	3979

Figure S1. Nucleotide and protein sequences of Japanese flounder *pou5f3* gene (accession number: KJ522774). Coding sequences (CDS) are denoted in uppercase letters, whereas the UTRs or introns are denoted in lowercase letters. The deduced amino acids are indicated in one-letter code and exhibited below the coding regions. The GT/AG intron/exon boundaries are shown in italic and the start and termination codons are highlighted in bold. The POU-specific domain and POU homeodomain are shadowed with light and dark gray boxes, respectively. The red letters indicate the poly (A) tails of the two isoforms of *Popou5f3* transcripts.



Figure S2. Comparison of the full-length amino acid sequences of Japanese flounder Pou5f3 protein with other vertebrate orthologues. Identical and similar residues are highlighted in black and gray, respectively. POU specific and homeodomains are indicated with red and yellow frames, respectively. The linker regions between two domains are shown by green line. The alignment is generated with ClustalW and shaded with BOXSHADE 3.21. The hyphens (–) indicate gaps that were inserted to maximize sequence similarity.



Figure S3. The predicted three-dimensional model of *Po*Pou5f3 POU domain based on *M. musculus* Pou5f1 (PDB ID: 3L1P) by homology modelling method using SWISS-MODEL online server. The α -helices and loop regions are shown as red and green ribbons, respectively.