

Supporting Information

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A1

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>ILP1_BRAFL,Bf_V2_190,CDS=96160-96131,101650-102068,103191-103508
ATGAATCTATCCAGCGGTACGTGCTCGCCAGCCTGGCCGTGGTGTGCCTGCTCGTGAAGGAGACG
CAGGCGGAGTACCTGTGCGGTTCCACCCTCGCCGACGTGCTCTCCTTGTCTGTGGGAACAGAGGG
TACAACCTACAACCCAGGCGGTCCGTGAGCAAGAGAGCAATCGACTTCATCTCGGAACAACAAGCG
AAGGACTACATGGGCGCCATGCCTCACATCAGGCGGCGCCGCGGGCTGGTGGAGGAGTGTGCTAC
AACGTGTGCGACTACAGCCAACCTGGAGAGCTACTGCAACCCGTACACCACCGCTCCAGCCACCGCC
ACGCCCCTCAGAACCACCGAGCCGAGCCGAAGAAGCAGAAGACGACCCCTTGATGGCATGGTA
GGCGACCAAGCTCCTCTGGGATCTATCGAGAACATAGAAAACCTGGTCTATCATTACGACAGCGAC
GACATCACGATAGACGCGGCGAAAAAGGAGCCGAAGAACTTAAGGAAATCCTCGGGTCTTTCGAA
GATAAGAAGGCGAACCCCGTCTTTCCGTTTCATCAGACAATCCAAGAACATAAAACCAACAAGTTT
CCCGACAACCTCGCCACCAATACCCTACCGACCTGGTAGAAGAAGGACCAACCAACGAGATACCG
GAATCGCCATCACAGAAACCCACCCTGGAGCGTCTCGGATACAAGCACAAACCAACGACAAAGAAA
CAACCAACAGAAAACAACAACAACAACAGAGCCAGAGACAATAGAACCAAATCCTCCACTGTG
GAACCTCACACTGTACCAGAGTATATATCAAACAATACACACATAAACCGCTCATCACGTGCGG
CGAGGTACGCCAGAAGAATAGAATCCCCGGGACAGTTATCACTCAACTGA
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A2

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>ILP1_BRAFL
MNLSSVYVLASLAVVCLLVKETQAEYLCGSTLADVLSFVCGNRYNSQPRRSVSKRAIDFISEQQA
KDYMGAMPHIRRRRGLVEECCYNVCDYSQLESYCNPYTTAPATATPVRTTEPOPEEAEDDPLDGMV
GDQAPLGSNIENIENLVYHYSDDDITIDAAKKEPKLKEILGSFEDKKANPVFPFIROSKNIKPNKF
PDNFAHQYPTDLVEEGPTNEIPESPSQKPTLERLGYKHNQTDKKQPTENNNNNNRARDNRKTSSTV
EPHTVPEYISKQYTHKPLITLPRGTPRRIESPGQLSLN
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B1

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>ILP2_BRAFL.Bf_V2_243,CDS=576816-576969,583074-583348
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TCTGCGTACCTGTGCGGCTCGACGCTGTTTCGACGTGCTGAGCTGGGTGTGCGAGGGGCGCGGGAG
CCGGGGATTAGCAAGAACCAAGATGTTCCAACGTGGACAACGAAGCCAGGCTCCACCCCGATCA
CCGCAGTTTCCCGCGGAGTTCGCGAACTCATCGACGACTGTTGCTTCAATGTCTGCACCTTCGAC
ACTCTGGAGAGCTATTGCACTCCCTGGGCGGAAACACCCGAACCAACCTGAACGACGCCGAGGAC
GCCGCGGAAATTCGGATGGGGAGACGACGATCTCGGCGAAGTTCGGGAGTGACCACGTGACCAGA
GAACGAGACTCTCGCAATGGAGTCTTGTCATGA
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B2

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>ILP2_BRAFL
MSPSGVLLMTCLSLIGCAAPASSAYLCGSTLFDVLSWVCEGRGEPGISKNQDVPNVNDNEARLHPRS
PQFSRRVRELIDCCFNVCTFDTTLESYCTPWAETPEPNLNDAAEDAAEIPDGETTISAKFGSDHVTR
ERDSRNGVLS
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Fig. S1. Nucleotide and amino acid sequences of the amphioxus (*Branchiostoma floridae*) insulin-like peptides 1 and 2 (ILP1, ILP2). (A1) ILP1 CDS sequence was generated by joining segments 96160–96131, 101650–102068, and 103191–103508 of the genomic contig Bf.V2.190 (JGI), which was in turn determined by reference to the following EST clones: bfad002d22, bfad046n04, CAXG12536, and bflv006m14. (A2) Amino acid sequence translated from A1. (B1) ILP2 CDS sequence deduced by joining the regions 576816–576969 and 583074–583348 of the genomic contig Bf.V2.243 (JGI), which was determined by reference to the EST clones of the UniGene Bfl.6772 (NCBI). (B2) Amino acid sequence translated from B1.



Fig. S2. Alignment of the peptide sequences of the GSS and insulin/IGF/relaxin family. The abbreviations and the sequence sources (GenBank accession no.) are as follows: RLN3_HUMAN: relaxin 3 [*Homo sapiens*] (NP_543140), RLN3_MOUSE: relaxin 3 [*Mus musculus*] (NP_775276), INSL5_HUMAN: Insulin-like 5 [*Homo sapiens*] (NP_005469), INSL5_MOUSE: insulin-like 5 [*Mus musculus*] (NP_035961), RLN1_HUMAN: relaxin 1 [*Homo sapiens*] (NP_008842), RLN2_HUMAN: relaxin 2 [*Homo sapiens*] (NP_604390), RLN1_MOUSE: relaxin 1 [*Mus musculus*] (NP_035402), INSL4_HUMAN: insulin-like 4 [*Homo sapiens*] (NP_002186), INSL6_HUMAN: insulin-like 6 [*Homo sapiens*] (NP_009110), INSL6_MOUSE: insulin-like 6 [*Mus musculus*] (NP_038782), INSL3_HUMAN: insulin-like 3 [*Homo sapiens*] (NP_005534), INSL3_MOUSE: insulin-like 3 [*Mus musculus*] (NP_038592), IGF1A_HUMAN: insulin-like growth factor 1 [*Homo sapiens*] (NP_000609), IGF1_MOUSE: insulin-like growth factor 1 [*Mus musculus*] (NP_001104745), IGF2_HUMAN: insulin-like growth factor 2 [*Homo sapiens*] (NP_000603), IGF2_MOUSE: insulin-like growth factor 2 precursor [*Mus musculus*] (NP_034644), INS_HUMAN: insulin [*Homo sapiens*] (NP_000198), INS1_MOUSE: insulin I [*Mus musculus*] (NP_032412), INS2_MOUSE: insulin II [*Mus musculus*] (NP_032413), respectively. RLNL_BRAFL: relaxin-like peptide, predicted protein BRAFLDRAFT_100967 [*Branchiostoma floridae*] (EAA41967), ILP1_BRAFL: insulin-like peptide [*Branchiostoma floridae*] (see Fig. S1), ILP2_BRAFL: insulin-like peptide 2 [*Branchiostoma floridae*] (see Fig. S1). Asterisks indicate positions of amino acids that match completely; : high similarity; . low similarity.

NJ Trees

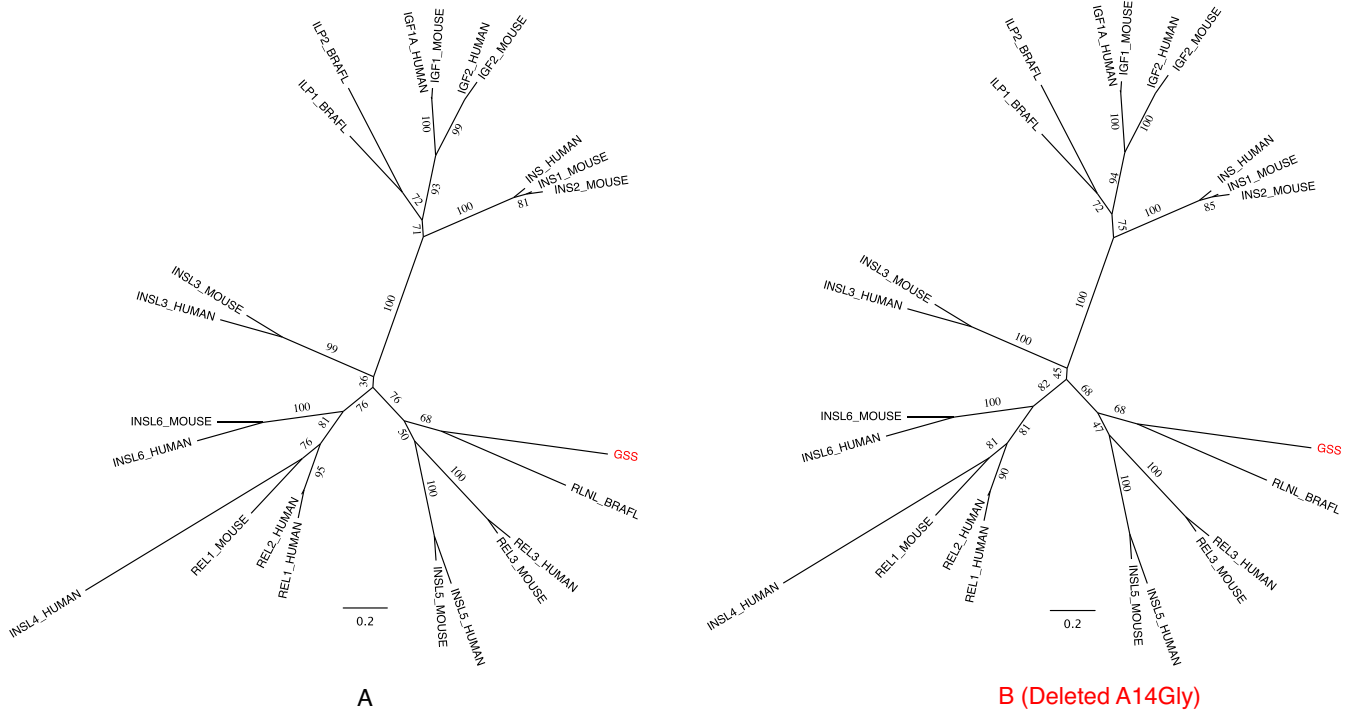
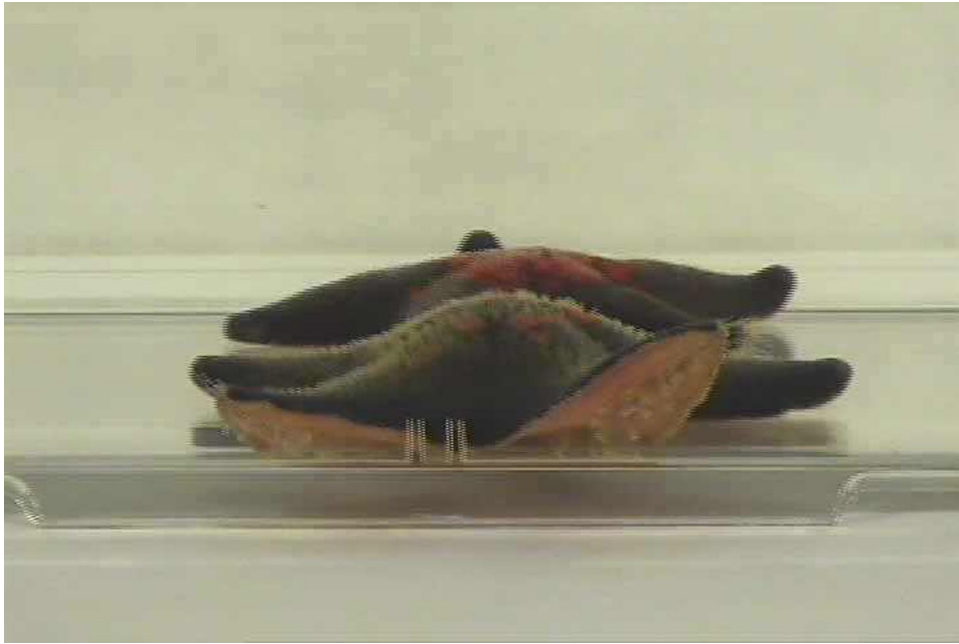


Fig. S3. Phylogenetic trees of insulin/IGF/relaxin superfamily. (A) The phylogenetic tree constructed from the alignment by using the neighbor-joining method. The number located beside each branch is the bootstrap score. GSS is indicated in red font. (B) The phylogenetic tree constructed after the removal of A14 residue (glycine) by using the neighbor-joining method. GSS is shown in red.



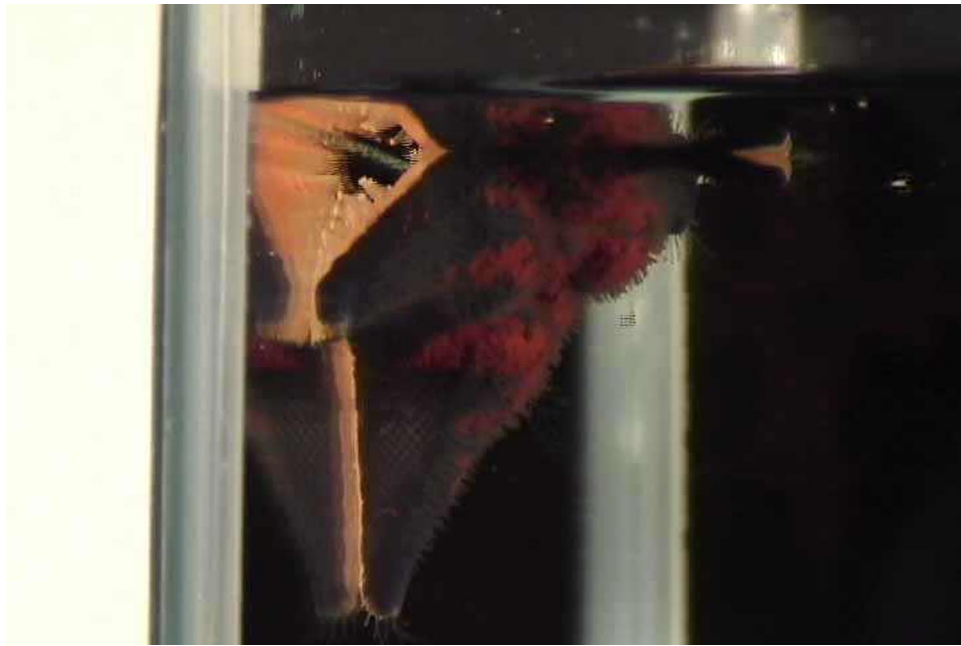
Movie S1. Gamete spawning induced by synthetic GSS. An ovarian fragment obtained from a mature female was incubated with 10 nM synthetic GSS as described in *Materials and Methods*. The serial photographs were taken every 4 seconds by using a DP70 digital camera system (Olympus) through a stereomicroscope (Leica). Time-lapse movie was constructed by 12 frames per second. The gametes were spawned after 20 min of incubation. The germinal vesicles had already been broken down in the spawned oocytes, demonstrating the completion of oocyte maturation.

[Movie S1 \(WMV\)](#)



Movie S2. Induction of spawning behavior in male starfish injected with synthetic GSS. Synthetic GSS at the concentration of ≈ 50 nM was injected into the body cavity of the mature male starfish. Five minutes after injection, the starfish moved around restlessly and climbed upward along the wall of the aquarium tank. Finally, the animal began to release sperm. On the other hand, the control starfish injected with seawater instead of GSS remained at the bottom without displaying any spawning behavior.

[Movie S2 \(WMV\)](#)



Movie S3. Induction of spawning behavior in female starfish injected with synthetic GSS. Synthetic GSS at the concentration of ≈ 50 nM was injected into the body cavity of the mature female starfish. After 5 min, the female starfish also climbed upward along the wall of the aquarium and began to release the eggs. In contrast, the control starfish remained at the bottom without showing any spawning behavior.

[Movie S3 \(WMV\)](#)