Supplementary Material

Molecular cloning, characterisation and expression analysis of the vitellogenin genes *vtgAo1* and *vtgC* during ovarian development in Chinese hook snout carp *Opsariichthys bidens*

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Table S1. Information on O. bidens used in this study.

Table S2. The Genebank accession numbers in multiple sequence alignments and NJ phylogenetic tree.

Fig. S1. Full-length Ob-vtgAol cDNA and deduced amino acid sequence.

Fig. S2. Full-length *Ob-vtgC* cDNA and deduced amino acid sequence.

Fig. S3. Multiple sequence alignment of *Ob*-VtgAo1.

Fig. S4. Multiple sequence alignment of *Ob*-VtgC.

^{*}These authors contributed equally to this study.

| Month | Sampling date | Number | Average body | Average | Ovary stage |
|-------|---------------|--------|------------------|--------------|-------------|
| age | | | length/cm | body | |
| | | | | height/g | |
| 6 | 2010.01 | 15 | 8 70+0 65 | 05⊥10 | п ш |
| 0 | 2019.01 | 15 | 8.79±0.05 | 0.J±1.0 | 11~111 |
| 7 | 2019.02 | 12 | $9.46{\pm}0.68$ | 12.6±3.5 | II~III |
| _ | | | | | |
| 8 | 2019.03 | 14 | 9.87±0.86 | 14.6±3.0 | II~III |
| 9 | 2019.04 | 16 | 9.55±0.96 | 15.4±3.2 | II~III |
| | | | | | |
| 10 | 2019.05 | 12 | 10.52 ± 0.72 | 22.6±5.3 | III~IV |
| 11 | 2019.06 | 15 | 12 44+0 90 | 28 8+7 1 | III. IV |
| 11 | 2019.00 | 15 | 12.44±0.90 | 20.0±7.1 | 111/~1 V |
| 12 | 2019.07 | 12 | 13.59±1.16 | 31.1±5.2 | IV~V |
| | | | | | |

Table S1. Information on O. bidens used in this study

| Species | Vtg type | Accession number (NCBI) | | | |
|--------------------------|----------|-------------------------|--|--|--|
| Labeo catla | VtgAol | ABP04034.2 | | | |
| Danio rerio | VtgAol | NP_001038362.3 | | | |
| Danio rerio | VtgAo2 | NP_001038378.1 | | | |
| Danio rerio | VtgC | NP_571340.1 | | | |
| Ctenopharyngodon idella | VtgAol | APB93352.1 | | | |
| Ctenopharyngodon idella | VtgAo2 | APB93353.1 | | | |
| Ctenopharyngodon idella | VtgC | APB93355.1 | | | |
| Cyprinus carpio | VtgAol | BAF73406.1 | | | |
| Cyprinus carpio | VtgAo2 | BAD51933.1 | | | |
| Gambusia affinis | VtgAa | BAD93697.1 | | | |
| Gambusia affinis | VtgAb | BAD93698.1 | | | |
| Gambusia affinis | VtgC | BAD93699.1 | | | |
| Morone americana | VtgAa | AAZ17415.1 | | | |
| Morone americana | VtgAb | AAZ17416.1 | | | |
| Morone americana | VtgC | AAZ17417.1 | | | |
| Mugil cephalus | VtgAa | BAF64835.1 | | | |
| Mugil cephalus | VtgAb | BAF64836.1 | | | |
| Mugil cephalus | VtgC | BAF64837.1 | | | |
| Thunnus thynnus | VtgAa | ACX32463.1 | | | |
| Thunnus thynnus | VtgAb | ADD63987.1 | | | |
| Thunnus thynnus | VtgC | ADD63988.1 | | | |
| Melanogrammus aeglefinus | VtgAa | AAK15158.1 | | | |
| Melanogrammus aeglefinus | VtgAb | AAK15157.1 | | | |
| Oryzias latipes | VtgAa | BAB79696.1 | | | |
| Oryzias latipes | VtgAb | BAB79591.1 | | | |
| Larimichthys crocea | VtgAa | AKK31325.1 | | | |
| Larimichthys crocea | VtgAb | AKK31327.1 | | | |
| Larimichthys crocea | VtgC | AKK31326.1 | | | |
| Gobiocypris rarus | VtgC | ATX74745.1 | | | |
| Cirrhinus molitorella | VtgAo1 | ADB77954.1 | | | |
| Tanichthys albonubes | VtgAo1 | ABN13867.1 | | | |
| Conger myriaster | VtgAe | BAD93275.1 | | | |
| Anguilla japonica | VtgAel | AAV48826.1 | | | |
| Anguilla japonica | VtgAe2 | AAR82899.1 | | | |
| Anguilla japonica | VtgAe3 | AAR82898.1 | | | |

Table S2. The Genebank accession numbers in multiple sequence alignmentsand NJ phylogenetic tree

| Oncorhynchus clarkii | VtgAs | AGQ04606.1 | | |
|------------------------|-------|----------------|--|--|
| Oncorhynchus mykiss | VtgAs | CAA63421.1 | | |
| Salmo salar | VtgAs | XP_014024135.1 | | |
| Crassostrea gigas | Vtg | BAC22716.1 | | |
| Haliotis discus hannai | Vtg | BAF98238.1 | | |
| Scapharca broughtonii | Vtg | AYE92811.1 | | |



Fig. S1. Full-length *Ob-vtgAo1* cDNA and deduced amino acid sequence. Blue letters above nucleotide sequence represent the corresponding amino acid sequence. Start (ATG) and stop (TAA) codons are indicated with grey boxes. The first 15 amino acids (underlined in blue) form the signal peptide sequence. Yellow highlights indicate the predicted Vtg receptor-binding region, and green highlights indicate the polyserine site of the Pv domain. Black double underlines indicate polyadenylation signal and poly-A sites.

070 F I E V G V H A D G I K E L L A G K I P 2041 ATTTTAGAGGTTGGTGCCACGCAGATGGAATAAAAGAGTTGTTAGCAGGAAAAACCC 121 GGGACTGGTACGAGTGGGACGGGAATTGCCAGACCTGGTCGAGTCCGCAGTGAAGCTGAG 2161 GAAGCTACCGAAAGACAGACCTCTACTGACCACATATGTACGCATGTTTGGACAGGAAGC 56 C T F E I T G E S P Q T F V L Q I S N V 181 GTGCACTTTTGAAATCACTGGTGAATCGCCACAAACTTTTGTACTTCAGATCTCAAATGT 736 F L M D V N G D F I Q S I T K S F S P T 2221 CTTTTTAATGGATGTAAACGGAGATTTCATTCAGAGTATCACAAAGTCATTTAGCCCTAC 241 AGACTTTGAAGACTTCAGTGGCATACCTGGGAAAAGTGACTTTAGCTCTTCCCAAAAGCT 2281 TCCAGGGAAGGAGAGTAAAGTTTGGGCAAAGATTCAAGATGTTCAAAAAGGAACTTCCTG 96 T K R L S A E F R Q P I V F E F S K G Q 301 CACTAAGCGTCTATCTGCCGAGTTCAGACAACCAATCGTTTTCGAATTCTCCAAAGGACA 776 H W T K P H L V Y E A R F M Q P T C L G 2341 GCACTGGACCAAGCCGCACCTTGTATATGAAGCTCGATTCATGCAGCCAACTTGTTTGGG 116 I T D I R T V P G V S N S V V N I V R G 361 AATTACTGACATTCGGACTGTACCTGGGGGTCTCAAATTCAGTTGTAAATATTGTGAGGGG 796 L P V E I S K Y F S I V N A V T M K V K 2401 TCTCCCAGTTGAAATCAGCAAATATTTCTCAATAGTAAATGCTGTTACAATGAAAGTTAA 816 A E I S P P P K E H L R E L L S S V I S 2461 GGCTGAAATTAGTCCTCCTCCCAAGGAACATCTGCGTGAACTGCTAAGCTCAGTCATTTC 136 V L G F L Q V T V K T T Q S F Y E L V E 421 GGTCCTTGGATTTCTACAAGTCACAGTCAAAACCACACAAGTTTTTATGAACTGGTGGA 481 GTTAGGAATTCATGGTGTATGTCAGAGCAGTTACACTGTTGAAGAAGACTCCAAAGCCAA 2521 CCTGCAAACAGATGGCTTTGCTGGAGTGACAAAGGATCATTTTGTCTTCCATGGAATCAA 2581 CACAGATCTTTTCCAGTGTGGTGTTGAGATGAGAGACTAAAAAGGTAACTGGCCTGCCATG 876 E F G L K I N I K E Q K Y Q M N L T P S 2641 GGAATTCGGCCTGAAAATAAACATAAAAGAGCAAAAGTATCAAATGAACTTGACTCCGAG 216 S T V K H N Y T V K S T E N G G L I T K 661 TTCCACAGTGAAACACCAACTACACAGTGAAGTCCACGGGAAAACGGCGGTCTAATCACTAA 896 K T D T E L F S V N S N V F A V L R N I 2701 CAAAACAGACACAGAGTTATTTTCAGTCAATTCTAATGTGTTCGCTGTTTTGAGAAATAT 236 A F A Q E R Q Y F S P F N V K G G N S R 721 AGCGTTTGCTCAGGAGCGTCAATATTTTCTCTCCGTTTAACGTAAAGGGAGGAACTCTCG 916 E D P S L S K I T P M M P E S E D S Q Q 2761 TGAGGATCCATCCTTATCCAAAATAACTCCCATGATGCCTGAATCAGAGGATTCCCAGCA 256 L L A L R D I E L L K V S E I E K K I V 781 ATTGTTGGCATTACGGGACATTGAGCTTCTCAAAGTTTCAGAAATAGAGAAAAAAATAGT 2821 GGGTTTGCCATTGGCCAGAATAATTTTACCCACTTCAAGGGATGAACAGAGTGAGAACTC N 841 GATCGGAAAGGTACAAAGTAGAGGCAACCTAATGTACAAGACAGAGAAGGACCTCCAACC 2881 TGAGGTGAAATTCAGGCAATGTGCTGAGGCCAAGATATATGGAACTGCGATCTGCATTGA 2941 GGCAGAAGCCAAACGATCACATCACCTACATGAATATCCCCTGTATTATTTCCTGGGACT 3001 TRESIREEPARSARPIERIQ 3001 TACCCGCTTTTCATATAAACTAGAACCAGCAAAGAGTGCAAAAACCCATTGAAAAGATTCA 961 GCGCTTGGCTCAGGCTAACATATATCATGTGAACAGTGCAAACAGCACAGATATTCTGGA 6 L I Q L L R V A T L E N L K Q I W K Q V 1 TCTAATTCAGTTGCTCCGTGTGGCAACGCTTGAAAATCTAAAGCAGATATGGAAGCAGGT 1016 I E V T A G I K H P Q A F S E M M D L S 3061 GATTGAGGTCACTGCGGGCATAAAACACCCCTCAAGCATTCAGTGAAATGATGGATCTCAG 356 S G N D E H R R W F L D L V V E V T D E 1081 CTCAGGAAATGATGAGCACAGGAGGTGGTTTCTGGACTTGGTGTGGGAGGTAACAGATGA 1036 R R V F K D T R D E I T T C R E H N L S 3121 TCGCAGAGTATTCAAGGACACAAGAGACGAGATCACCACCTGTCGTGAACACAATCTATC 376 R I L K F L E T R F K A G D I T V N E A 1141 AAGGATCCTCAAATTCCTTGAGACTAGATTCAAAGCAGGAGACATTACAGTGAATGAGGC 1056 S S F L A N Q D L G T T P D P V V T V K 3181 CAGTTCATTCCTTGCAAATCAGGACCTTGGTACTACTCCAGACCCTGTAGTCACAGTAAA 1076 A. L. S. L. S. P. P. G. K. S. L. G. Y. E. A. V. A. F. Y. L. 3241 AGCACTTAGTCTAAGTCCACCAGGAAAATCTCTGGGGCTATGAAGCTGTAGCCTTTTATCT 1096 P T A Q R D N V E M I V S E V G E E A N 3301 GCCAACAGCACAAAGAGACAATGTTGAAATGATAGTCTCCGAAGTTGGTGAAGAAGCAAA 416 A Q V F L T I P F S K S Q P L L W N T V 1261 GGCTCAGGTGTTTCTGACGATCCCTTTCAGTAAATCCCAACCTCTCTGTGGAACACTGT 1321 CGTTTTGGCATATGGATCTCTTCTATACAGATACTGTGTGTATACTGATCCCTGCCCTGT 456 T V V Q P L L D M A A S S L S K N S E V 1381 CACTGTGGTGCAGCCATTACTGGATATGGCTGCAAGTAGCCTAAGTAAAAACTCTGAAGT 3421 CAGATGGGGTGCAGAGTGTCAGACATATGATGTATCTATGAAGGTGTCCTCAGCATGCCA 476 D M V L A L K A L G N A A H P S S I K T 1441 GGACATGGTCCTTGCACTAAAGGCTTTGGGAAATGCAGCTCATCCACCTCCAGCATCAAGAC 3481 GCCAGAATCTAAAACTTCCTTATACACCAAGATTAAATGGGGAGCTCTGCCCCCAGTGTT 496 L L K F L P G Y S A G S E K L P I R V Q 1501 TCTCCTAAAGTTCCTTCCAGGCTACTCAGGCTGGATCTGAAAAACTTCCAATCAGAGTACA 1176 T I G Q T I Q E Y I P G L S Y T M G F Y 3541 CACAATTGGTCAAACAATTCAAGAGTACATTCCTGGCTTATCTTATACTATGGGTTTCTA 3601 CCAGAAATATGAGAAAAATCCAGAACGTCAAGCAGCTGTCACTGTTGTAGCATCCTCAAC F 1621 TATTGTCTTGAACCTCTTGTAAAGAAGACTCTTCCTGCTGAAATCCGCATGTTGGCCTG 3661 AGAGACCTTTGACATGAAAGTGAAAATTCCAGAGCGAACTATCGACAAAAAGGCAATTCC 1681 CATGGTGCTTCTAGACACCAAGCCATCCATGGTTTTGATCTCAGCAATAACTAATGTCCT 3721 3781 3841 3901 3961 4021 D L E E N D L Q V A S F S Y S L L K G I A L TTTAGAAGAGAATGATCTCCAGGTTGCCAGGTTGCCAGCTTCTCCTCTGCTCAAAGGCATTGC 596 K S R T P D N Q H L S T A R N I A M K I 1801 CAAGTCCCGCACCCCTGATAATCAACATCTATCCACTGCCCGTAATATTGCCATGAAGAT 4081 636 F H D D F L F G T S T D V Y M L Q N E S 1921 GTTCCATGATGACTTTTATTTGGGACATCCACTGATGTTTATATGCTGCAAAATGAAAG 4141 4201 AAACTTTGTGTGGAATGCTCAACTGTGTACCTGCAAATATATGGTGGCAGTCTCTTTGC 656 L I P S K L M L K G K I H F I G R I L Q 1981 TCTCATACCCTCCAAACTTATGCTAAAGGGAAAAATTCATTTCATTGGCAGAATATTGCA 4381 GCAAAAAAAAAA

Fig. S2. Full-length *Ob-vtgC* cDNA and deduced amino acid sequence. Gray boxes indicate start (ATG) and stop (TGA) codons. The predicted Vtg receptor-binding region is highlighted in yellow. The first 15 amino acids (blue underline) form the signal peptide sequence. Black double underlines show the polyadenylation signal and poly-A sites.

| | 1 Signal peptide |
|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (1) | MRAVVLALTVALVASQQINLVPEFAKDNTYEYKYEAQLLEGLPQEGLAHAGMKVSCKVHIRRITDNTFMMKLIDPQLQEVAGINPQSSVVPAPKITSALA |
| (1) | MRAVVLALIVALVASOTILVPE FALDKTYVK PEALLIGULPOEGLARAGIVNSKULSAVIENTELMKLIDDFILLEVAGUNEKDEVVPATKLTAALVAS |
| (1) | MRAVVIALTVALVASCOTNIN PETPORTYVYN YEALLLOGLEHEGLARAGTKYNSKVH.SAVTENTFLERIMDPLTYEYAGTWPKDPFVPATKLTSALA |
| (1) | MRAVVLALTVALVACDOFNLVPEFÄHDKTYVYKYEALLLGGLEOEGLARAGIKVSSKVLISATTENTYLMKLMDFILYEYAGTWEKDEFVPATKLISALA |
| (1) | MRAVVLALALAVVASCYVVFAPD PASSKTYVYKYFAVI MAGLPEGLASAGLKVLSKVLISNIAENTVILKLADPELESYSCVLPKDPPTPANNIKEALA |
| (1) | |
| (1) | MRVVVLALTTALVACCLHNLAPEFDAGKTYVYKYEALLLGGLPEEGLANAGLKVSSKVLISAAAONIYMLKIVE PELECYSGIWPKDELVPATKLTSALA |
| (1) | <mark>MRVVVLAITTALVA</mark> GHPQNFAP <mark>D</mark> FA <mark>AGKTYVYKYEALLLIGGLFEEGLARAGLKTSSKVLIS</mark> NAAQNIF <mark>MIK</mark> LA <mark>K</mark> FEL <mark>YEYSGINFKDFVTPATKLTAALA</mark> |
| (1) | MRAVVLALTLALVAGOQINLVPEFAAGKTYVYKYEALLLGGLPEEGLARAGLKVSSKVLISAVTENTFLLKLMDPLLFEYAGIWPKDPFVPATKLTSALA |
| | Signal peptide Lipovitelini heavy chain 200 200 |
| (101) | POLOT PIKFEYANGYI GKVFA PAGVSPTVI NLHRGTLNI LOLNI KKTONI YELOFAGAG GVCRTOVVI SEDPKTKH TTVTKSKDI SEC ERIMKDVI G |
| (101) | <mark>ağlığı pikfeyangyygkveapagvsptylnlergilne liçinfektionayeli deagargycrthyyi nedtkanhı ivtkskdirege erimkdyglay</mark> |
| (101) | AQLQI PIKFEYANG WGKVFAPAG VSPTVINLERGIINI LQINLKKTQNIYELQPAGAQ GVCRTHYVISEDPKANHIIVTKSKDISHCCFRIMKDVGLAY |
| (101) (101) | AQUQUPIKESIANG VOKVEAPAG VSPTVALEKGILNE QULNEKTON TEL QARAGO SVCKTETVI SODPKANELTVIKSKULE OLE KIMKOVGAN |
| (101) | SOLAT PIKFEYAN CV GKVYS PEGISTTVIN FROUN LOLNIKKTONVYELOFAGAC SVCSTIVAITEDEKAD FILLIKKTRUNC OF KIVKDEGLAN |
| (101) | PELNI PIKFEY <mark>SNGVYGKVFAPEGVS</mark> DIVLNF <mark>Y</mark> RGFLNI LOLNI KKTHNYYDLOBAGTOGYCKTLYSYNRDYKADRILLTKTRDM <mark>VHCHERISREI</mark> GLAY |
| (101) | POFAL PIKEEVINSVYCKTEAPEGVEPVV. NTHRGIVNVLQLNIKKTHKVPDLQEVGTCGVCKTIYSISEDARNINILLTKTRDINNCCEFLIKEMGLAY |
| (101) | AQLMI PIKESYANG VO SKMA PISO BYNY INVEGILNY I CENTRALINY VEDSAGAO GVOKTLYA I TEDDKAPILLITKTRDINEO OKITLYA I TEDDKAPILLITKTRDINEO OKITLYA I TEDDKAPILITKTRDINEO OKITLYA I TEDDKAPILITKI I TED |
| (101) | AOLOIPIKFEYANGVYGKVFAPEGVSPTVLNLHRGILNILOLNIKKTONVYELOBAGAOGVCRTLYVISED KAEHILVTKSKDLNHCOERIMKDVGLAY |
| (101) | 201 300 |
| (201) | TEKCAKCIERTKSLIETASYNYIMKPGATGVQITEATVELYYQFSPESEIHGAAQMEAKQTLAFVEIKKTPVAPIKGDYLARGSLQYEFATEILQTPIQ |
| (201) | TERCAR CTERVARCE LETTARYN YLWYRAAR GYL I AEATYFE WHO'FS PLIN E'I HGA LIMEAK CSLAFWEI EKTEWY PYKADY LARGS LOYTFATE LLOTPIO |
| (201) | TERCAR CITER IN SUBJECT IN THIS PARTY IN THE PARTY IS A DATA OF THE THE ADDRESS THE ADDRESS TO THE THE ADDRESS TO THE THE ADDRESS TO THE ADDR |
| (201) | TERCAE CTERVESLIETATYNY IMEPADNGAL IAEATVEEYYQES PENEIRGAAMMEAK CILAFYE IEKTPYVPIKAD YN PROSLOYEFATEI LOTPIQ |
| (201) | TEKCIKCOKEAKNIRGAAAYNYVLKEVPTGVLILOATVNELIQESPENEINGAAOMNIKCSLVFILEIQKTPLAPVNAQYLHRGSIKYEFABELLOTPITI |
| (201) | TERCOR CORDENING STORY TERP VP8GTMILEAD VNCI TO S PVSSFWGA ACTETE CTLVTLE TO SPTA VSAEVHINGSLKYEFSKE FELSPLUT |
| (201) | TOK CAR OF DEMANDED TITE TO THE FUNCTION TO THE STORY OF |
| (201) | TQSCPKCQQQB <mark>KNLRG</mark> ATGYNYTLKFVANGTLILEAAVNELIQPSPFTEMNGAAQMQTKCSLVPLEVQBAPTVPIQAEYLHRGSIKYEF <mark>STELLQTPIQ</mark> |
| (201) | ${\tt TERCAECQERSKNLRGTAAYNYILKPVAAGVLILEATVNELIQFSPFNEIHGAAQMETKQSLAFLEIQKTPIVPIKADYLHRGSLQYEFATELLQTPIQIAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA$ |
| (301) | |
| (301) | IKIS <mark>N</mark> APVQIVEVLKHIVVNNVAMVHDCAPIKEVQIJCILRVSTLENIEAIWDOFKDKPAYRWILDALPAVGTPVTVKIVKEKFLAGRITTEFTQAI |
| (301) | IKISDAPAOIIRVIKHLVANNVAMVHDOAPLKFVOLVOLLRAATLENIEAIWAOFKDKPVYRRWLLDALPAVGTPVIVKFIKEKFLAGELITPEFIOAIN |
| (301) | <mark>M</mark> KISDAPAQI <mark>IE</mark> VIKH <mark>IVANNVAMVHEDAPIKEVQIIQLIRVS</mark> TLENIEAI <mark>MA</mark> QEKDKEAY <mark>RRWLIDAIPEVGT</mark> EVI IK FIK <mark>EK</mark> FIAGELT <mark>I</mark> PEFIQAI <mark>N</mark> |
| (301) | KRISDAPAQIVEVIKILVSINKOMVHDDAPFKFVQIVQLUPVASEKIEKINSOFKDKPVYRKVLIDALPAVGTPVIIKFIKEKFLAGEFNTFEFIQTIV |
| (301) | IN THE CALL THE CALL AND THE ATTIC BELLOW IN SUM AND ACCOUNT OF A CONSTRUCT OF A CONS |
| (301) | VKISNAKAQTAE VMNKLATINVENIHENAPMKELELVQI LELARYED LEMYWNOVKKMSPHEHWELDTI PAACTPAAFETKEKFMAEDINTAEAVQA L |
| (301) | IKINNVQAQIYEILNHIVTNVERVHEDAPIKELEIIQLLRAARFEDIEMIWRQHRIKETRQMILDSIPAIGTEVAIREIKEKELDDITVASVAQALI |
| (301) | IRITNA QAQIABILAH LITINVEK VHEDARLKELELIQI. LAARREDI EMIWSQYEN KEANROWILDAI EVIGTPAALBEIKEKELAEQUTVA BITAQALI |
| (301) | IKISMA AQIVEVLMHLVINNVE VHEDAFEKELQLIQLIKVA LEDIEAIWSQENDKP IKKWLEDAIPAVGTPVIEKEIKEKELAGELTVPEEIQALV 401 |
| (401) | VALOMV PANLOT TOLTAS LAMERKIAT PALREVVMLCHGSVIAKHOVEVPTCPAELLR PIHELIAEAISKNDIPE ITLAL KVLGNAGHPASLKPIMKII |
| (401) | VAL <mark>OMVTA</mark> DLETIQLTASLA <mark>LHE</mark> KIATI PULREV <mark>VMLGYGSMIAKHC</mark> VAVPTCPAELLRPIYETAAEAIAKNNIPEITLALKVMGNAGHPASLKPIMKT |
| (401) | VALOWVTADLDTIQLTASLAMHEKIATTPALREVVMLGYGSMIAKHCVAVPTCPAELLRPIHETAAFATSKNDIPEITLALKVLGNAGHPASLKPIMKTL |
| (401) | VALON VALOUTIOTIASIAMHEKIA MEALKAVALAKVA VALA VOMIA VOMIAHOVA VETSABULA PIHALAKA TSKNOLKEITAL KVI INA HPASA PIMAL |
| (401) | ASTHWY TACCHAIT IVESLIDNNL VEN ALROTVHLOVOTWAR CSEKIAC SELTREI HEKIARAV EEN LEDI ILLKVI SNAGHPHSLKTITKI |
| (401) | AAVHWYKANPE <mark>SIKLFETL</mark> TEDNKIDANPULREIVFLGYCIMISKYCESSDYCFVEYIKFIQKRLSEAVSKGETEDIILYLKYLGNARHPSSIKSITKI |
| (401) | AAVHWYTADPEVIKLFESLLANDKYEKNPLLREVVELGYGTMVYKYCNKTAPCPAELVKPIQERITNAVAKNEEDKIVLYVKVLGNAGHPSSEKSLIKI |
| (401) | AS THAN TANTKATKU VEALATINSKU VESPVIRETULL OTTMISKOVENU AVCELLEPI ODLARAV KOS (ODTILLOKVICNA GATSLEPITUL |
| (401) | ALONYTAL TYLE INTERIA KIN VERVER I PAREVNER SAN AND AND AND AND AND AND AND AND AND A |
| (401) | 501 600 |
| (501) | P ilksaaaampiryovdaila lrniakkep <mark>klvopvaloiledralhpelknya</mark> sivlfea <mark>ap</mark> lval <mark>a</mark> ssaaylktetnmhvasesys <mark>yi</mark> ksutrinah |
| (501) | PGLETTA ATA OPERVOVDA LA LENIA KKEPKLVO OVALOLIV DEAL HEDVENTVACIV LEESKESVALVSSLA GALKTETIMH VISEATSHIIKSLTRITTA DGLETTA ATA OPURVATI A LA DITAVENU VODIA LA DITAVENU DALI UDVENTVATI VERSKESVALVSSLA GALKTETIMH VISEATSHIIKSLTR |
| (501) | FOLK TA SSL PLEVO VDALLA EN TAKKEF KLVO PVALO V DRALH P ZVENVACI V LFESK PSVALVSSLA GALKTETT MHVV SFA V H KSLT R TAK |
| (501) | PGLETAANALPIRVOVDAILALENIAKKEPKLVOPVALQLVLDEALHPEVEMVACIVLFEAEPSVALVSSLAGALEIEPNMHVASFAYSHIKSLTRITAE |
| (501) | PLEGEAAAALPTRVHVDAILAMENIAKKEPELIODVVQLEMOKGLQPELEMIACIVLEETKPRIGLVTALANVLKTEENLQVASETYSEMKSLTRSTA |
| (501) | PIHSTAASSEPTTVHILAIMA ENIAKKEPRIVOELALOITVIDKALDPELEMISOTVERTINSMALISTEANAVKSEENLOVASTTVSHIKSISSAN |
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Fig. S3. Multiple sequence alignment of *Ob-VtgAo1*. Black arrow indicates the cathepsin D hydrolysis site, the black line separates VtgAo1 signal peptide from the three yolk protein domains, red box is the conserved motif RGILN. Red arrow indicates the cathepsin D hydrolysis site, the red line separates the five yolk protein domains of the VtgAa signal peptide, and the red box is the conserved motif CGXC (X represents any amino acid). The predicted Vtg receptor-binding region is indicated with grey shadow.

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| Gobiocypris rarus VigC Danio rerio VigC Ctenopharyngodon idella VigC Gambusia affinis VigC | (101) (101) (101) (101) (101) | ABFROPIVFEFSROOT AEFSOPIVFEFSROOT AEFSOPIVFEFSROOT SELAOPFLFEYDNGEIO | DIRTVPGVSNTVV DIRTAPGVSNTVV DIRTAPGVSNSVV DIRTAPGVSNSVV DIRTAPGVSNSTTVV | /NIVRGILCFLQ /NIVRGILCFLQ /NIVRGILCFLQ /NIVRGILCFLQ /NI <mark>I</mark> RGILSFFQ | VTVKTTQSFTE VTVKTTQSFYE VTVKTTQSFYE VTVKTTQSFYE VTVKNTQ <mark>TT</mark> YE | SLVELGINGVC SLEELGINGVC SLVELGINGVC SLVELGINGVC | 28511 VEEDSAA 288 yivd edsaa 288yivdedsaa 288yiveedsaa Had <mark>y</mark> a <mark>iee</mark> kegi | KELIVTQMVDIT KELIVTRIVDIT KELIVTQMVDIT KELIVTQMVDIT | NCOOPAANISGNA NCOOPASIYRGMA NCOOPASIYRGMA NCOOPAALYSGMA NCWER <mark>AAMY</mark> SGMA |
| Morone Americana VtgC Thunnus thynnus VtgC Mugil cephalus VtgC Consensus | (101) (101) (101) (101) | AQLIKPFMENYTCGEV DQLVKPFMEDYASGEV AQLAKPFMFEYASGEV AELSQPIMFEYSKGQI1 201 | DIHA <mark>SAEISDTVV</mark> DIHASAEISDTVV DIRAAAEISDTVV DIRTAPGVSNTVV | /NIVRGILGFFQ /NIVRGILGFFQ /NIVRGILGFFQ NIVRGILGFLQ | VTVKTTQRIYE VTVKTTQRIYE F <mark>TVKTTQ</mark> RIYE VTVKTTQSIYE | LEEVGINGKO LEEASINGKO LEELGINGKO LEELGINGVOO | OSNYATEENMET OSNYA <mark>I</mark> EONKET OSNYATEEN <mark>T</mark> ET OSSYTVEEDS T | KDMTITQVVDVS KDMTITQVVDVG QDMTITQVVDVS KDLIVTQVVDVT | G <mark>CREKAAIYRGMA NCREKAAIYRGMA NCREK<mark>AAIYRGMA</mark> NCQQPAAIYRGMA 30</mark> |
| Opsariichthys bidens VtgC Gobiocypris rarus VtgC Danio rerio VtgC Ctenopharyngodon idella VtgC | (201) (201) (201) (201) | APEDKLSRORGESVVS ALEDKLSKORGESVVS APEDKLSKORGESVVS APEDKLSKORGENVVS | VKHN <mark>YTVKST</mark> ENG VKH <mark>T</mark> YTVKSTADG VKHTYTVKSTADG | GLITKAFAQER GLITKAFTQER GQITKAFAQER GLITKAFAQER | QYFSPFNVKGG QYFSPFNVKGG QYFSPFNVKGG | NS <mark>RLLALRDI</mark> NSOMFALRDI N <mark>FRMLALRDI</mark> | ELLKVSEIE <mark>K</mark> KI ELLKVSDTVDKI ELLKVSDTTDK <mark>N</mark> | VI <mark>G</mark> KVQS <mark>RG</mark> NLM VTGQVQSRGNLM VTGQVQSRGNLM VTGQVQSRGNLM | YKTEKDLOPIPV YKTEKDLKPIPV YKTNKDLKPIPV |
| Gambusia affinis VtgC Morone Americana VtgC Thumus thynnus VtgC Murail cenhalus VtgC | (201) (201) (201) (201) (201) | AEWDRLAKERGENVIS AVLDXVSKORGESVIS AVLDXVSKORGESIS AVLDXVSKORGESIS | MKYNYTIKPTGO VRYVYTVKATEC VRYVYTVKPTAEG | GLITRAQGFER GLITRAHGLER GLITRAHGLOO GLITRAHGLOO | 2HFSAFNVKGG 2HFSSFNVKGG 2HFSPFNVKGG 2HFSPFNVKGG | SFKMEATKDL SFKMQAMKEI SFKMQAMKEM | VLLSMNRTARGF VLLGVSDTARAV VLLGVSDTARAV | TYGPLEKKGKII IFGPMENKONLV TFGPMESKONII | YSFEDVDINIPT YKFVNAEANVPIN YKFVNAEANVPIN YKFVNAEANVPIN |
| Consensus | (201) | AVEDKLSKQRGESVIS1 | VKHVYTVKSTADO | GLITKAFGQER | QHFSPFNVKGO | SFKM ALKDI | VLLKVSDTARKI | VFGPVQSKGNLM | YKTEKDL PIPVV |
| Opsariichthys bidens VtgC Gobiocypris rarus VtgC Danio rerio VtgC | (301) (301) (301) | IN <mark>INCPLPKILDLIK</mark> RI ID <mark>INDPAPKILDLIK</mark> RI IN <mark>INDPVPKILDLIK</mark> RI | AQ <mark>AN IYH</mark> VNSANS AL <mark>AN IYHV</mark> DSA <mark>SS</mark> AQ <mark>AN</mark> IYHVDSETS | TDILDLIQLLR TDILDLIQLLR T <mark>EIL</mark> DLIQLMR | VATL <mark>ENL</mark> KQIM VATLENLEQLM VTTL <mark>DNLE</mark> HLM | VKQVSGNDEH <mark>R</mark> VKQVSGNDEHR VKQVSGNDEHRI | RWFLDL <mark>V</mark> VEVTE RWFLDLVVGVTE RWFLD <mark>LV</mark> VEVTE | ERILKFLETRFK ERILKFLEIRFK ERILK <mark>FLEAR</mark> YK | AG <mark>DITVNEAGQAI</mark> VGDITANEAGQAI AG <mark>DITANEA</mark> GQAI |
| Ctenopharyngodon idella VtgC Gambusia affinis VtgC Morone Americana VtgC Thunnus thynnus VtgC | (301) (301) (301) (301) | INLNNPAPKILDLIKRI OKLONPGPKATELIKRI ONLEDPIPKAVELIKQI ONLEDPVPKAIELIKHI | AQANIYHVDSA <mark>SS</mark> . <mark>S</mark> EANSGTINSATI .AEANKYQIDSATI .AQANSYRIDSATI | TDILDIQLLR EDSIKLYHLLR EDTIKLYQLLR EDTIKLYQLLR | VETLENLEQLW VTP <mark>YEELENM</mark> W VMP <mark>YEELEAVW</mark> VTP <mark>YEGLEIM</mark> W | KQVSGNDEHR KQLAVNPKYR KQLLGNEEHR ROFAENEEHR | RWFLD <mark>LV</mark> VEVTE RWFLD <mark>SIVEI</mark> AE RWFLD <mark>MIVEV</mark> SE RWFLD <mark>MIVEVT</mark> E | ERILKFLETRFK I <mark>KV</mark> LNF <mark>M</mark> EARFK ARILKFLEVRFQ ARILKFLEMRFQ | V <mark>CDITANEAGOAI</mark> AN <mark>DWI</mark> HF <mark>EALO</mark> T SCDISVIEAGEII ACDVSASEALEA |
| Mugil cephalus VtgC Consensus | (301) (301) | <mark>QNL</mark> DN <mark>PVPK</mark> A <mark>VEL</mark> FKQI QNLNDPVPKILDLIKRI 401 | AQAN <mark>RYQ<mark>I</mark>DKATI AQANIYHIDSATS</mark> | EDT <mark>IKLYQLLR</mark> TDIIKLYQLLR | VMP <mark>YE</mark> G <mark>l</mark> VV <mark>I</mark> W / TyenleqlW | IE <mark>QF<mark>AGNEEHR</mark> IKQVSGNDEHRI</mark> | RWFLD <mark>MIVEI</mark> GE RWFLDLIVEVID | A <mark>RILKFLETRFR ERILKFLETRFK</mark> | AG <mark>DVSA</mark> D <mark>EA</mark> LETI AGDITANEAGQAL 50 |
| Opsaruchthys bidens VtgC Gobiocypris rarus VtgC Danio rerio VtgC Ctenopharyngodon idella VtgC Gambusia affinis VtgC | (401) (401) (401) (401) (401) | VAFNHLPAKPYSVALA VAFNHLSAEPYSVALA VAFNHLSAEPYSVALA VAFNHLSAEPYSVALA TAFHHLQPTPENVGMAR | WFLTIPFSKSOPI EFLTIPFSKSOPI EFLTIPFSKSHPI EFLTIPFSKSOPI | LWNTVVLAYGS LWNTVVLAYGS LWNTVVLAYGS LWNTVVLAYGS LWNTVVLAYGS | LLYRYCVYTDE LU <mark>YRYC</mark> VYTDE L <mark>YRYCVYTDE LVNKYCVYTDE</mark> | CPUTUUQPEL CPUTUUQPEL CPUTUUQPEL CPUTUUQPEL CSUDUIQPE | DMAASSLSKNSE DLAVSSLNKNSE NMAASSLSKNSE DMAASSLSKNSE MATEALRNGNK | VDMVLALKALGN EDMVLALKALGN EDMVLALK <mark>S</mark> LGN EDMVLTLKALGN E <mark>EMIT</mark> ALKAMGN | AAHPSSIKTILKE A <mark>AHPSSIKTILKE</mark> A <mark>AHLSSFKTILKE</mark> A <mark>AHPSSTKTILKE</mark> A <mark>GHPKSM</mark> KTIMRE |
| Morone Americana VtgC Thunnus thynnus VtgC Mugil cephalus VtgC Consensus | (401) (401) (401) (401) | LAINHLQAIPELVEMAR LAFNHLQAIPELVEMAR V <mark>S</mark> INHLHAVPELVEMAR VAFNHL A PVSVALAÇ 501 | WFLTMPFSKS <mark>N</mark> IY MFLNMSFSKSKIY MFLTMPFSKSNTY MFLTIPFSKSNPL | (LWHTVALTYGS: (LWNTVVL <mark>S</mark> YGS: (LWHTVVL <mark>S</mark> YGS) LWNTVVLAYGSI | LVYKHCAYYTF LVYKHCAYYTF LVYKNCAYYTF LVYKYCVYYDP | CPINSVQPLL CPVTAVQPLL CPVTAVQPLL CPVTVVQPLLI | DMAMESLRNGNE DMAMEGLRNNNE DMATE <mark>SLRN</mark> NE DMA SSLRNNSE | ADMV <mark>IALKALGN</mark> ADMV <mark>IALR</mark> ALGN EDMTIALKALGN EDMVLALKALGN | A <mark>CHP</mark> GSIKTIMRE A <mark>CHP</mark> GSIKTIMRE A <mark>CHP</mark> GSIKTIIRE AAHPSSIKTILKE 60 |
| Opsariichthys bidens VtgC Gobiocypris rarus VtgC Danio rerio VtgC Ctanopharmaadan idalla VtaC | (501) (501) (501) | PGYSAGSEKLPIRVOG PGYSTCAEKLPTRVOG PGYSNGAEKLSTRVOG | AVQAFRLLA <mark>G</mark> RNF AVQAFRLLAYRDF AVQAFRLLASRAS | HNVQDIVLNLF HSVQDIVLDLF HSVQDIVLNLF | VK <mark>KTLPAEIRM</mark> VOKTLPAEIRM VOKHLPAEIRM | ILACMVLLDTK MACMVLLETK ILACTVLLETK | PSMVLISAITN PS <mark>IALVS</mark> VVTEV PSTALISVV <mark>S</mark> EV | LLEENDLOVASF LLEETDLOVASF LLEEADLOVASF | S <mark>YSLL</mark> KGIAKSRI SYSLLKGIAKSRI S <mark>YSLLKGFAKSRI</mark> |
| Gambusia affinis VtgC Morone Americana VtgC Thunnus thynnus VtgC Maril | (501) (501) (501) (501) | PGVSATPVDLPLRVQG PGVSATPVDLPLRVQSZ PGVAATPVDLPPRVLSZ PGVAATPVDLSPRVLSZ | AVOAFRELAGEDF A <mark>I</mark> QAMRLVANEDF AVO <mark>SMRLIA</mark> EDF CCCAV <mark>Y</mark> ETHECQEF | HIVODIVLNLF HNVREAALAVF HSVODITMSLF HSVOENHHESV | UCKNLPAEIR LHKHVASEVRM LOKNLPTEIRM LOKDLPTEIRM | HACMULLETK HAFKILFATK LAFMILFDTK LAFMILFDTK | PSTALISVITEN PSMALVSTVTAH PTLALVSVVTAH PSMALVSTMTAH | LLEEIDLOVASE ILQEEKDLOVASE ILQEEKDLHVVSE ILQEEKDVHVVSE | SISLLKGIARSHI AYTYLHGLARSRI AYSYLRSFARSSI AYSYLRSFAKSSI |
| Consensus | (501) | PGYSAGPVKLP RVQS/ | AVQAFRLLAARDP | HSVQDIVL LF1 | LQK LPAEIRM | ILACMILLDTKI | PSMALVSVVT V | LQEEKDLQVASF: | VICLOSICS SYSYLKGIAKSRI |
| Opsariichthys bidens VtgC Gobiocypris rarus VtgC Danio rerio VtgC | (601) (601) (601) | DNOHLSTARNIAMKIL DNONLSTACNIAMKIL DNOHLSIACNIAMKIL | 'RK <mark>LG</mark> HLS <mark>YRYS</mark> KS 'RKLGHLS <mark>YRYSKS</mark> 'RK <mark>LG</mark> HLS <mark>YRYS</mark> KN | MHF <mark>DWFHD</mark> DFL MHFDWFHDDFL LHF <mark>DWF</mark> HDDFL | F <mark>GTST</mark> DV <mark>YML</mark> (FGTSTDVYML(FGTSA <mark>DVYML</mark> (|)NES-LIPSKL))NES-LIPSKL))NES-PI <mark>PTK</mark> L) | MLK <mark>GKIHFIGRI</mark> MLNGKIHFIGRI MLK <mark>GK</mark> FHF <mark>IGR</mark> I | LQF <mark>IEWG</mark> VHAD <mark>G</mark> LQFIELGVHTDG LQFLEF <mark>GI</mark> RADG | I <mark>KELLA</mark> GKIPQL IKDLFAGK <mark>V</mark> PQLI IKDLFAGKIPELI |
| Ctenopharyngodon idella VtgC Gambusia affinis VtgC Morone Americana VtgC Thunnus thynnus VtoC | (601) (601) (601) | DNOHLSTACNIAMKIL NNOYRSIASSLAVKIL ENHFLSIACNYAVKVL DNHFLSTACNYAVKVL | RKLGHLSYRYSKS APRFGRLCYYYSKT APKFGRLSYHYSKA | MHEDWFHDDFL RKHADWFDDRWL MRMDWFNDDFL MRMDWFNDDFL | FGTSTDVYMLC IGMTSEFFMLF IGTAAEVFMLF IGTATEVFMLF | ONES-LVPSKLI NASNAIPSEI SAINIFPTEI SAINIFPTEI | MLK <mark>GKIHFIGRI</mark> SSGKLHIIGRI MMKGKFFFIGRI | LQFLELGVHADG IEILELGVRADG LQLLELGIRAEG | IKELFAGKI POL IKELIGAGI POFY IKELFCTSI POFT |
| Mugil cephalus VtgC | (601) | DNYF <mark>LSTACNVAVKIL</mark> A DNOHLSTACNIAMKIL | AP <mark>KF<mark>GRVSY</mark>Y<mark>YS</mark>Q4 BKLGRLSYBYSKA</mark> | RRM <mark>DWF</mark> N <mark>DDFL</mark> MHEDWFHDDEL | I <mark>GTAAEVEMLE</mark> FGTSTDVEMLE | RSAN <mark>NI</mark> F <mark>PT</mark> EF | MT <mark>KGKFY</mark> FIGRN | LOLLEFGTRAEG | IKDLFGASTPGF |



Opsariichthys bidens VtgC (1086) Gobiocypris rarus VtgC (1082) Danio rerio VtgC (1086) Ctenopharyngodon idella VtgC (1089) Gambusla affinis VtgC (1077) Morone Americana VtgC (1100) Thunnus thymnus VtgC (1100) Mugil cephalus VtgC (1097) Consensus (1101)

Opsariichthys bidens VtgC Gobiocypris raras VtgC Danio rerto VtgC Ctenopharyngodon idella VtgC Gamhusia affinis VtgC Morone Americana VtgC Thumus thynnus VtgC Mugil cephalus VtgC Consensus

Fig. S4. Multiple sequence alignment of *Ob-VtgC*. Arrow indicates the cathepsin D hydrolysis site, and the straight line separates the *Ob-VtgC* signal peptide from the yolk protein domain. The predicted Vtg receptor-binding region is indicated with grey shadow.

ANL AHF ANV

HSS

ES PORVE PERITORNA EPERANE PERITYKKA EDDINKE PERITYKKA ESTORE KEPENYYKKA SINKE KPENYYKA SINKE KPENYYKA SINKE KPENYYKA SINKE KPENYYKA

DDI DNI

FYQKYEKN FYQKKEEN FCQKYEKN FSEKNERN FSEQHERN FYQQHERN FYQQNERN

NAVM DAAI DTAV

> 'SYIMO ISYFMO 'AFLHO 'AFLLO IAFLLO

(1185)

(1185) (1182) (1186) (1189) (1174) (1199) (1199) (1196) (1201) EVGEEA

AV SA SA SA

VIVVAPSPE VIVVASSPE VIVVASSPE ATVIAAWTD /FTL /FTT (FTT

N<mark>WG</mark>T NWGA

ACQPE ACQPE AYTN<mark>G</mark>

STACQPGSKPSL 1277

TSPIELVGTEAVNIJT PSPIELVGTEAVNIJT PSPIELLGIEAANITMST------

PFLWNSQWFYFNMTCSAEY------PVPIPPASTLFPODTRNTTIDSFGRA FFLIPPASTOFFRDTRNTTIDSFGHA PFLIPPANEOFFNNTTNTTDSFGQA P PI F E N M