Supplementary File：Genome-wide identification, expression and response to estrogen of vitellogenin gene family in Sichuan bream (*Sinibrama taeniatus*)

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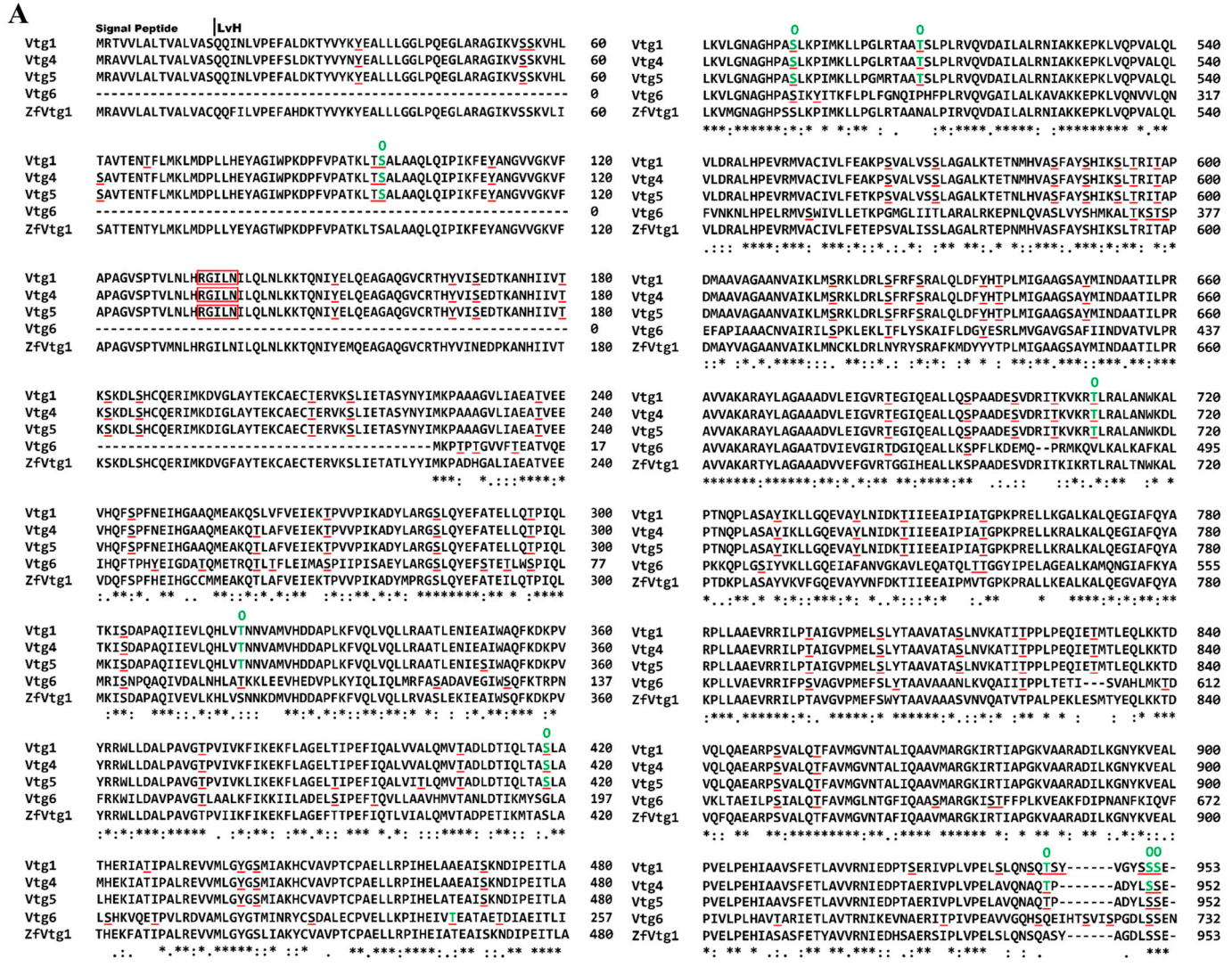
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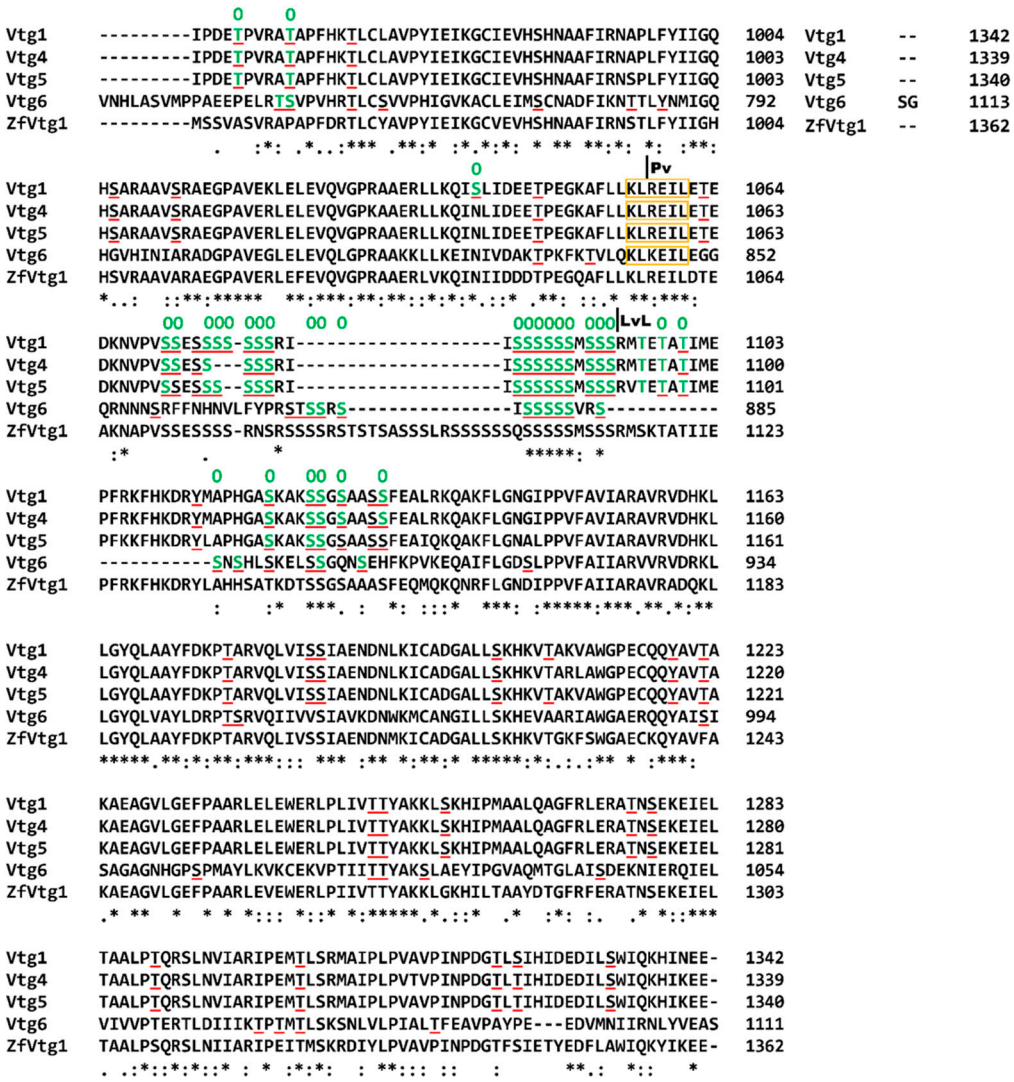
Vtg1
Vtg4
Vtg5
Vtg6
ZfVtg1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LvL
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Vtg5 -- 1340
Vtg6 SG 1113
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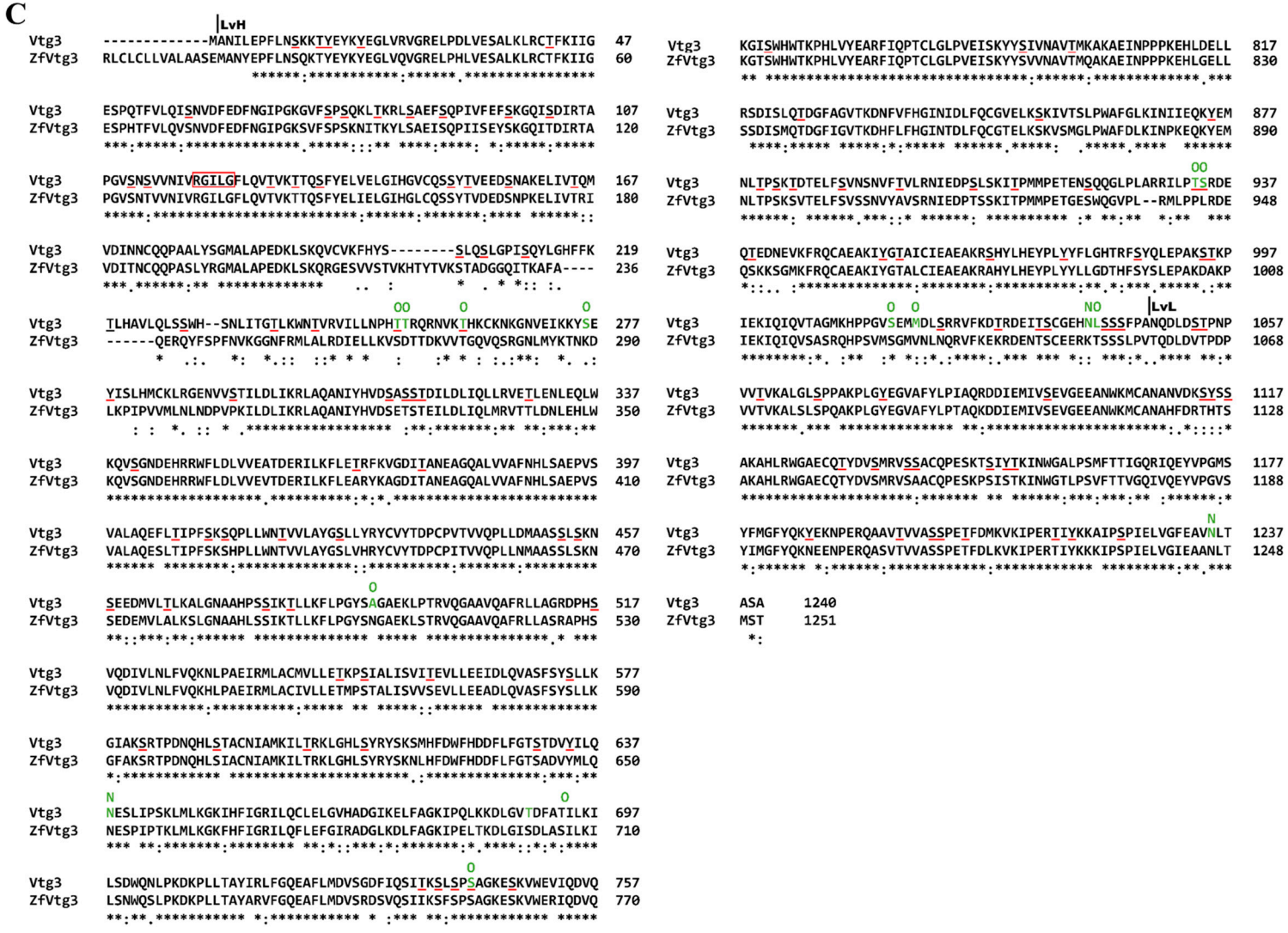
B

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OO O O
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N O
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ZfVtg3 YIMGFYQKNEENPERQASVTVVASSPETFDLKVKIPERTIYKKKIPSPIELVGIEAANLT 1248
Vtg3 ASA 1240
ZfVtg3 MST 1251

Supplementary Figure S1. Alignments of the deduced amino acid sequences of Sichuan bream and zebrafish (Zf) (A) type I, (B) type II and (C) type III Vtgs. Numbers on the right indicate amino acid residue positions. The expected primary cleavage sites for yolk protein (YP) are indicated above the row of sequence by vertical lines followed by the abbreviated name of the YP domains (LvH, Pv, LvL, β'-c, or Ct). Asterisks (*) under sequences indicate identical amino acids, semicolons (:) indicate residues with higher similarity, and periods (.) indicate residues with lower similarity. Dashes indicate gaps inserted for optimal alignment. The conserved RGILN/G, CGLC, QEY, and KLR/KEIL motifs are enclosed by red, black, blue, and orange boxes, respectively. The 14 conserved cysteine residues of the β'-c and Ct domains are highlighted in gray. Predicted O-glycosylation and N-glycosylation sites are indicated by the (O) and (N), respectively, shown above the sequences with the symbol for the relevant residues also set in green typefaces. Predicted phosphorylation sites are underlined in red.

Supplementary Table S1. Sequence consistency percentage (%) matrix of Vtgs

	St Vtg1	St Vtg4	St Vtg5	St Vtg6	Carp VtgAo1	Zf Vtg1	Croaker VtgAa	Bass VtgAa	St Vtg2	Carp VtgAo2	Zf Vtg2	Croaker VtgAb	Bass VtgAb	St Vtg3	Carp VtgC	Zf Vtg3	Croaker VtgC	Bass VtgC
StVtg1	100	97.7	97	42.2	89.3	82.8	41.6	44.2	73.2	71.1	65	42.8	43.4	23.4	25.5	24.9	26.2	25.5
StVtg4	97.7	100	98.1	42.4	89.9	82.4	41.5	44.1	72.9	71.1	64.7	43	43.4	23.3	25.5	24.7	26	25.4
StVtg5	97	98.1	100	42.8	89.8	82.8	41.7	44.3	73.2	71.1	64.8	43.2	43.6	23.1	25.5	24.4	26	25.4
StVtg6	42.2	42.4	42.8	100	42.1	41	28.8	30.2	34.5	35	33	30.1	29.6	18.6	19.3	18.7	18.9	17.8
CarpVtgAo1	89.3	89.9	89.8	42.1	100	82	41.8	44.1	70.5	72.6	64.5	43.7	44.1	22.7	25.2	24.3	26.1	25.9
ZfVtg1	82.8	82.4	82.8	41	82	100	40.1	42.7	65.3	65.8	65.7	42	42.3	22.3	24.7	23.9	25.5	25.4
CroakerVtgAa	41.6	41.5	41.7	28.8	41.8	40.1	100	76.4	51	51.8	50.4	56.4	56.5	17.5	18.9	18.7	20.8	20.4
BassVtgAa	44.2	44.1	44.3	30.2	44.1	42.7	76.4	100	53.9	55.2	52.4	58.9	59.2	18.3	19.9	19.3	21.2	20.8
StVtg2	73.2	72.9	73.2	34.5	70.5	65.3	51	53.9	100	87.8	80.5	51.9	52.6	19.6	21.1	21	21.5	21.1
CarpVtgAo2	71.1	71.1	71.1	35	72.6	65.8	51.8	55.2	87.8	100	80.1	54	54.6	19.7	21.3	20.9	21.8	21.4
ZfVtg2	65	64.7	64.8	33	64.5	65.7	50.4	52.4	80.5	80.1	100	50.6	51.4	18.9	20.5	19.8	21.2	20.6
CroakerVtgAb	42.8	43	43.2	30.1	43.7	42	56.4	58.9	51.9	54	50.6	100	81.4	18.8	20.3	19.7	22.2	21.8
BassVtgAb	43.4	43.4	43.6	29.6	44.1	42.3	56.5	59.2	52.6	54.6	51.4	81.4	100	18.8	20.6	20	22.3	21.9
StVtg3	23.4	23.3	23.1	18.6	22.7	22.3	17.5	18.3	19.6	19.7	18.9	18.8	18.8	100	80.1	76.4	45.1	45.6
CrucianVtgC	25.5	25.5	25.5	19.3	25.2	24.7	18.9	19.9	21.1	21.3	20.5	20.3	20.6	80.1	100	82.1	48.3	48.7
ZfVtg3	24.9	24.7	24.4	18.7	24.3	23.9	18.7	19.3	21	20.9	19.8	19.7	20	76.4	82.1	100	46.7	47.8
CroakerVtgC	26.2	26	26	18.9	26.1	25.5	20.8	21.2	21.5	21.8	21.2	22.2	22.3	45.1	48.3	46.7	100	80.4
BassVtgC	25.5	25.4	25.4	17.8	25.9	25.4	20.4	20.8	21.1	21.4	20.6	21.8	21.9	45.6	48.7	47.8	80.4	100

The comparison between types I, II, and III Vtgs are highlighted with yellow, green, and blue shadows, respectively. St: Sichuan bream (*Sinibrama taeniatus*), Carp: Common carp (*Cyprinus carpio*), Zf: Zebrafish (*Danio rerio*), Croaker: Large yellow croaker (*Larimichthys crocea*), Bass: Striped bass (*Morone saxatilis*).

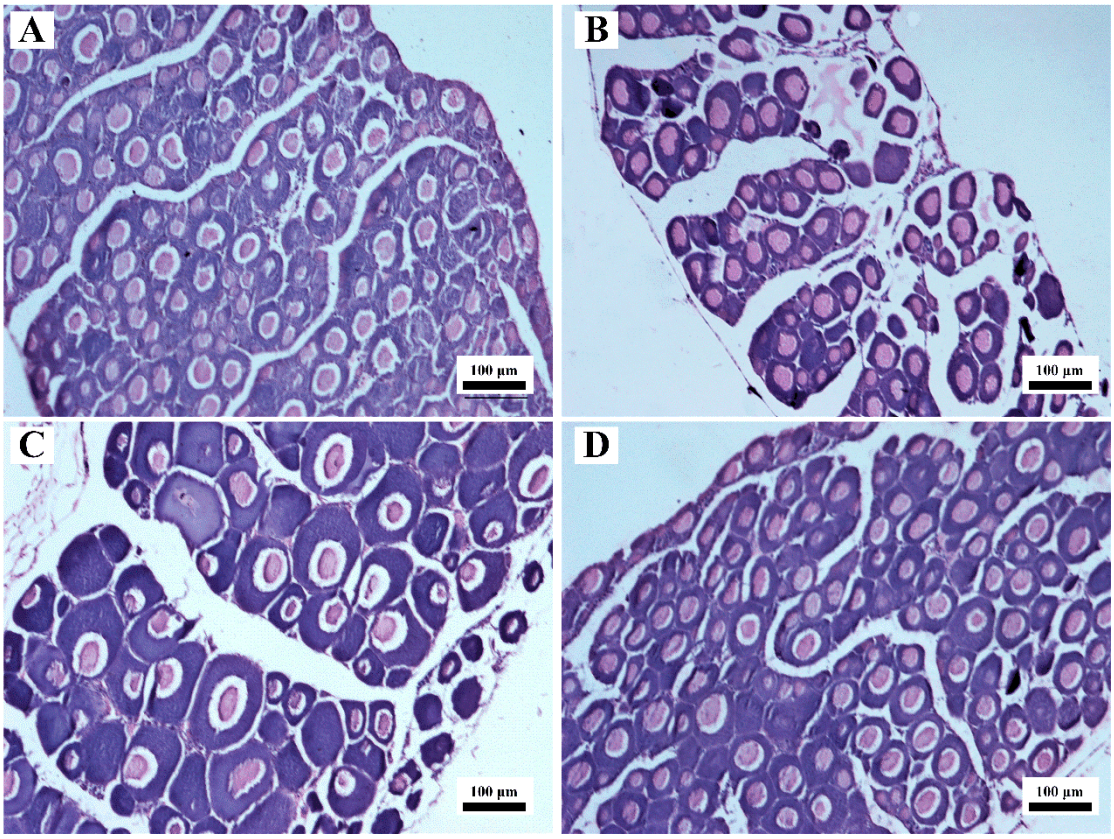
Supplementary Table S2 Primer sequences for qRT-PCR

Gene names	Upstream Primer (5'–3')	Downstream Primer (5'–3')
vtg1	5'CGCTGAAAATGACAACCTGAAG3'	5'TGGCATAGGTGGTGACGATTAG3'
vtg2	5'TGCTGGTCACCCTGCTAGTCT3'	5'TCTTTCTTGGCAATGTTCCTCA3'
vtg3	5'TCGGTGAATCGCCACAAAC3'	5'GCTGAACTCGGCAGATAGACG3'
vtg4	5'TGACAAGCCAACCGCAAGA3'	5'GGCATAGGTGGTGACGATTAGTG3'
vtg5	5'CCGAGTTCATTCAGGCTCTTGT3'	5'GCTGGGATTGTGGCGATTT3'
vtg6	5'CCTAGAAGCACCAGTAGCCGC3'	5'GGAAGTGAGTCCCCAAGGAATAT3'
β-actin	5' CGAGCTGTCTTCCCATCCA3'	5'TCACCAACGTAGCTGTCTTTCTG3'

Supplemental Table S3. Sequence information and accession numbers of vitellogenins for species shown in the neighbor-joining phylogenetic tree.

Species	Vtg type	cDNA full length (bp)	Amino acida residue (aa)	Accession number
Haliotis discus hannai	Vtg	7753	2391	AB360714
Ichthyomyzon unicuspis	VtgABCD	5976	1823	M88749
Cyprinus carpio	VtgAo1	4098	1353	AB331884
	VtgAo2	5000	1624	AB106873
Carassius auratus	VtgAo1	4241	1290	MT409425
	VtgC	4207	1203	MT409426
Danio rerio	Vtg1	4431	1363	NM_001044897
	Vtg2	5002	1631	NM_001044913
	Vtg3	3970	1253	NM_131265
	Vtg4	4232	1358	NM_001045294
	Vtg5	4225	1358	NM_001025189
	Vtg6	4244	1374	NM_001122610
	Vtg7	4212	1358	NM_001102671
	Vtg8	4489	1496	NC_007133
Rhodeus uyekii	VtgAo1	4275	1424	KM111547
Fundulus heteroclitus	VtgAa	5198	1704	U07055
	VtgAb	5166	1687	U70826
Oryzias latipes	VtgAa	5037	1678	AB064320
	VtgAb	5323	1725	AB074891
Sparus aurata	VtgAa	5143	1675	HG794235
	VtgAb	5252	1709	HG794236
Pagrus major	VtgAa	5239	1715	AB181838
	VtgAb	5348	1716	AB181839
	VtgC	4128	1272	AB181840
Morone americana	VtgAa	5752	1667	DQ020120
	VtgAb	5156	1682	DQ020121
	VtgC	4243	1275	DQ020122
Morone saxatilis	VtgAa	5216	1663	HQ846509
	VtgAb	5145	1682	HQ846510
	VtgC	4050	1275	HQ846511
Larimichthys crocea	VtgAa	5278	1717	KM362364
	VtgAb	5202	1695	KM405515
	VtgC	4236	1254	KM362365
Salmo salar	VtgAs	5127	1659	XM_014168660
Oncorhynchus clarkii	VtgAs	5003	1659	JX683452
	VtgC	3899	1281	JX683453
Anguilla japonica	VtgAe1	5395	1742	AY775788
	VtgAe2	5356	1734	AY423445
	VtgAe3	5423	1759	AY423444
Conger myriaster	VtgAe	5110	1663	AB185334
Gadus chalcogrammus	VtgAa	5195	1663	AB568267
	VtgAb	5098	1648	AB568268
	VtgC	4283	1268	AB568269
Melanogrammus aeglefinus	VtgAa	5189	1665	AF284035
	VtgAb	5103	1643	AF284034
Paralichthys olivaceus	VtgAa	5021	1658	XM_020081193

	VtgAb	5060	1663	XM_020081206
	VtgC	4287	1275	XM_020080687
Hippoglossus hippoglossus	VtgAa	4902	1633	EF582606
	VtgAb	4944	1647	EF582607
Cynoglossus semilaevis	VtgC	4410	1275	XM_008329605
	VtgAa	5190	1699	AB288932
Mugil cephalus	VtgAb	5216	1699	AB288933
	VtgC	4037	1272	AB288934



Supplementary Figure S2. Histological pictures of the ovaries of each group of experimental fish after treatment with SERMs. (A) Control group. (B) ICI-treated group. (C) PPT-treated group. (D) DPN-treated group.