

**Characterization of Four orphan receptors (GPR3, GPR6, GPR12 and GPR12L) in Chickens and Ducks and
Regulation of *GPR12* Expression in Ovarian Granulosa Cells by Progesterone**

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Supplementary materials

Table S1. Primers used in this study

Gene	Primer name	Primer sequences (5'-3')
<i>Primers for cloning the 5'-UTR</i>		
<i>cGPR6</i>	raL1	ATGAGGCCGAGCCCGGCCAGCAGGTC
	raL2	ACACACAGCATCACGTCCCAGGGGTT
<i>cGPR12</i>	raL1	AGTGATGGCCAGCAAGCTGCCAACGG
	raL2	CCTATGAGCAGGAACATGGGCGCGCG
<i>cGPR12L</i>	raL1	CAGCAGGTCGGCCAGCGCCAGGCTGC
	raL2	CCCGTGGCGCACAGAGCGATGTCCC
<i>Primer for cloning the 3'-UTR</i>		
<i>cGPR6</i>	raU1	GCAGACATGCCCATCAGATAGCTCTC
	raU2	TTACGCCACGCTTCTACCTGCTACCT
<i>cGPR12</i>	raU1	CGTGATGCGCCATGCCCATCAGATTGC
	raU2	GCTGCTTGCTGGATGCCTTTCACGCT
<i>cGPR12L</i>	raU1	CCTCCACCCGCAAAGGGCTCTCCACG
	raU2	CGCCGTGTACACGTACTCCCTGGCGC
<i>^aPrimer for cloning the coding regions</i>		
<i>cGPR6</i>	U1	AGTCCAGTGTGGTGgaattcGCGGCGATGGAGCCAACGGC
<i>cGPR6</i>	L1	TGGATATCTGCAgaattcTCAGACATCACTAGGGGA
<i>cGPR12</i>	U1	AGTCCAGTGTGGTGgaattcGTTAAATGAATGAAGAGCC
<i>cGPR12</i>	L1	GCTGGATATCTGCAgaattcTCAGACATCGCTGGGCGATC
<i>cGPR12L</i>	U1	AGTCCAGTGTGGTGgaattcCTTTCGATGCTGCACGGCCC
<i>cGPR12L</i>	L1	GCTGGATATCTGCAgaattcTCACACGTCGCTGGATGTCC
<i>dGPR3</i>	U1	CCAGTGTGGTGgaattcGGCAGCATGATGGAGGACG
<i>dGPR3</i>	L1	TGCAgaattcGTCACCTCAGACGTCGCTGGGCGGACCT
<i>dGPR6</i>	U1	CAGTGTGGTGgaattcGAGGAGATGGAGCCAGAGCC
<i>dGPR6</i>	L1	ATCTGCgaattcGTCACCTCAGACATCGCTAGGGGA
<i>dGPR12</i>	U1	TCCAGTGTGGTGgaattcGTTAAATGAATGAAGATCT
<i>dGPR12</i>	L1	TCTGCgaattcGTCACCTCAGACATCACTGGGCGAT
<i>dGPR12L</i>	U1	CAGTGTGGTGgaattcCCAGCGATGCTGCACGGCCCC
<i>dGPR12L</i>	L1	TATCTGCAgaattcGTCACCTCACACGTCGCTGGACGT
<i>hGPR3</i>	U1	AGTCCAGTGTGGTGgaattcGGTACCATGATGTGGGGTGC
<i>hGPR3</i>	L1	ATATCTGCAgaattcGTCACCTAGACCATCACTGGGGGAGC
<i>hGPR6</i>	U1	CAGTGTGGTGgaattcGCCGCGATGAACGCGAGCGC
<i>hGPR6</i>	L1	ATCTGCAgaattcGTCACCTCAGACCTCACTGGGGGA
<i>hGPR12</i>	U1	AGTCCAGTGTGGTGgaattcGTTAAATGAATGAAGACCT
<i>hGPR12</i>	L1	ATATCTGCAgaattcGTCACCTACACATCACTGGGCGAGC
<i>pGPR3</i>	U1	GgaattcCGGTACCATGATGTGGGGTG
<i>pGPR3</i>	L1	GgaattcCTAGACGTCACCTGGGAGAA

<i>pGPR6</i>	U1	GgaattcCGCCGCGATGAACGCGAGCG
<i>pGPR6</i>	L1	GgaattcCTCAGACCTCACTGGGGGACC
<i>pGPR12</i>	U1	GgaattcCATGAATGAAGACCTGAAGGTC
<i>pGPR12</i>	L1	GgaattcCCTACACATCACTGGGCGAC
<i>zGPR3</i>	U1	AGTCCAGTGTGGTGgaattcTTCCTAATGGACCAGAACAG
<i>zGPR3</i>	L1	ATATCTGCAGaattcGTCACCTCAGACGTCGCTGGGGGTGT
<i>zGPR6</i>	U1	AGTCCAGTGTGGTGgaattcTCAGCGATGAACGAGAGCGA
<i>zGPR6</i>	L1	ATATCTGCAGaattcGTCACCTAGACCTCACTGGGCGATC
<i>zGPR12</i>	U1	AGTCCAGTGTGGTGgaattcGGCAGAATGAGTGAAGAGGT
<i>zGPR12</i>	L1	ATATCTGCAGaattcGTCACCTCAGACGTCGCTGGGGGTCC
<i>zGPR12La</i>	U1	TCCAGTGTGGTGgaattcTGAGCTATGATTCACTCTCT
<i>zGPR12La</i>	L1	TGGATATCTGCAGaattcCTATACATCGCTCGATGT
<i>zGPR12Lb</i>	U1	TCCAGTGTGGTGgaattcTGAGCTATGATTCACTCTCT
<i>zGPR12Lb</i>	L1	TGGATATCTGCAGaattcCTATACATCGCTCGATGT
<i>Primers for RT-PCR and quantitative RT-PCR assays</i>		
<i>cGPR12</i>	U1	CAGGACCGCGTAGAAGCCAG
<i>cGPR12</i>	L1	TGGCTTCGGAGCGCAGCAG
<i>BMP15</i>	U1	GGCCGCAGTCTGAGCACCAA
<i>BMP15</i>	L1	GGCAGCAGGCAGCAGCTCCA
<i>GDF9</i>	U1	CCGTGTTCTGAGTGCCAGCAC
<i>GDF9</i>	L1	CACAGACAAATGGCACGTGC
<i>Primers for amplifying cGPR12 promoter regions</i>		
<i>cGPR12</i> (-3050/+277)	U1	ATCTGCGATCTAAGTAAGCTCCACCAAGGTACAAGC
<i>cGPR12</i> (-1962/+277)	U1	ATCTGCGATCTAAGTAAGCTTCTCTGACAGAGGATG
<i>cGPR12</i>	L1	AAGCTTGGCATTCCGGTACTCAGCGCCCCGAGAGAGCAGC

Table S2. Lists of the GPCR sequences, their GenBank accession numbers and species used in amino acid sequence alignment.

	Chicken	Human	Pig	Giant panda	Duck	Zebrafish	Spotted gars
GPR3	-	NP_005272.1	MW310580	XP_034503372.1	MW310577	MW310585	XP_015204111.1
GPR6	MW310573	NP_001273028.1	MW310579	XP_002925693.2	MW310576	MW310584	XP_006626355.1
GPR12	MW310572	NP_005279.1	MW310578	XP_002924260.1	MW310575	MW310583	XP_015196671.1
GPR12L	MW310571	-	-	-	MW310574	MW310581 MW310582	XP_015206592.1

Table S3. Lists of genes and their GenBank accession numbers used to generate the phylogenetic tree in this study.

Gene names	Species	GenBank accession numbers
GPR3	Human	NP_005272.1
	Duck	MW310577
	Spotted gar	XP_015204111.1
	panda	XP_034503372.1
	pig	MW310580
	Zebrafish	MW310585
	Mouse	NP_032180.1
	Turtle	XP_006115571.1
	Finch	XP_030146224.1
GPR6	Human	NP_001273028.1
	Chicken	MW310573
	Duck	MW310576
	Spotted gar	XP_006626355.1
	Zebrafish	MW310584
	panda	XP_002925693.2
	pig	MW310579
	Human	NP_005279.1
	<i>Xenopus</i>	XP_017949566.1
	Turtle	XP_006123311.1
	Finch	XP_030123592.2
GPR12	Human	NP_005279.1
	Chicken	MW310572
	Duck	MW310575
	Spotted gar	XP_015196671.1
	Zebrafish	MW310583
	panda	XP_002924260.1
	pig	MW310578
	Mouse	NP_001010941.1
	Finch	XP_030130857.1
GPR12L	Chicken	MW310571
	Duck	MW310574
	Spotted gar	XP_015206592.1
	Zebrafish (GPR12La)	MW310581
	Zebrafish (GPR12Lb)	MW310582
	Japanese quail	XP_015716158.1
	Lizard	XP_028571577.1
MC4R	Human	NP_005903.2
	Mouse	NP_058673.2
	Duck	XP_005016300.1
	Chicken	NP_001026685.1

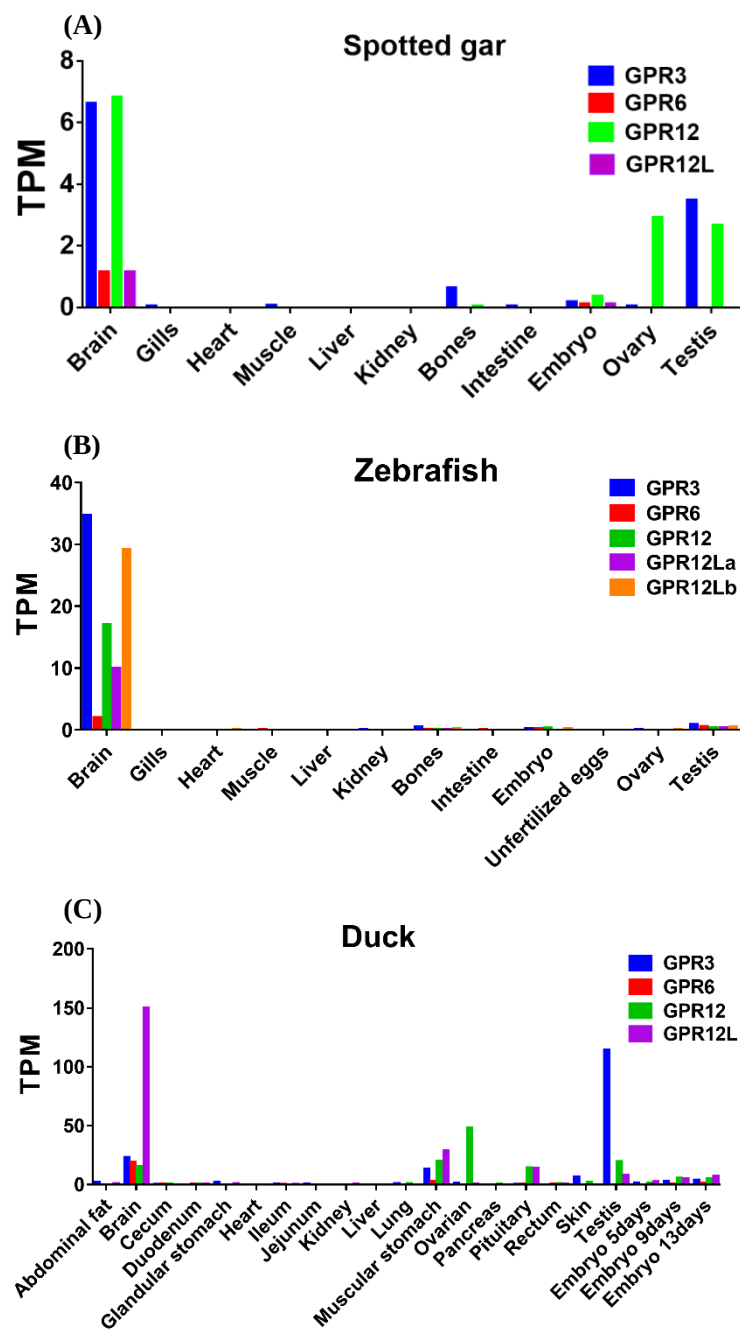
	<i>Xenopus</i>	XP_004915370.1
	Turtle	XP_006114320.1
	Zebrafish	NP_775385.1
	Finch	XP_030121249.1
	Spotted gar	XP_015210118.1
CNR1	Human	NP_001153698.1
	Mouse	NP_001341949.1
	Duck	XP_038033028.1
	Chicken	NP_001033741.1
	Turtle	XP_006114320.1
	Zebrafish	NP_775385.1
	Finch	XP_030121249.1
	Spotted gar	XP_015210118.1
LPAR1	Human	NP_001338326.1
	Mouse	NP_001277415.1
	Chicken	NP_001108554.1
	Duck	XP_027302018.1
	<i>Xenopus</i>	NP_001096313.1
	Turtle	XP_006118052.1
	Zebrafish	NP_001004502.2
	Finch	XP_002189340.1
	Spotted gar	XP_006626630.1
GHRHR	Human	NP_000814.2
	Pig	NP_999200.1
D1A	Human	NP_000785.1
	Mouse	NP_001278730.1
D1B	Human	NP_000789.1
	Rat	NP_036900.1

Table S4. Amino acid sequence identity of GPR3, GPR6, GPR12, GPR12L among vertebrate species including chickens, humans, ducks, zebrafish, and spotted gars.

		GPR3						GPR6						GPR12						GPR12L							
		human	pig	giant panda	duck	zebrafish	spotted gar	chicken	human	pig	giant panda	duck	zebrafish	spotted gar	chicken	human	pig	giant panda	duck	zebrafish	spotted gar	chicken	duck	zebrafish	zebrafish (zebra)	zebrafish (zebra)	spotted gar
GPR3	human	100.00%																									
	pig	95.10%	100.00%																								
	giant panda	96.60%	94.50%	100.00%																							
	duck	65.70%	66.30%	65.40%	100.00%																						
	zebrafish	45.20%	44.90%	45.50%	49.60%	100.00%																					
	spotted gar	63.90%	64.50%	65.10%	74.00%	50.90%	100.00%																				
GPR6	chicken	55.90%	56.20%	57.40%	61.40%	45.90%	67.40%	100.00%																			
	human	52.10%	52.10%	52.40%	56.30%	41.80%	57.70%	67.10%	100.00%																		
	pig	50.50%	50.20%	50.80%	54.60%	42.70%	56.00%	67.00%	93.90%	100.00%																	
	giant panda	51.60%	51.60%	51.90%	55.20%	43.30%	57.10%	67.90%	94.40%	94.50%	100.00%																
	duck	56.50%	56.80%	57.70%	61.70%	45.80%	66.50%	96.30%	67.10%	66.70%	67.40%	100.00%															
	zebrafish	53.40%	53.10%	53.70%	59.10%	44.50%	65.40%	74.80%	61.60%	60.70%	62.90%	74.20%	100.00%														
GPR12	spotted gar	54.00%	53.70%	54.30%	59.20%	43.20%	64.90%	79.80%	62.10%	61.80%	63.20%	79.50%	81.60%	100.00%													
	chicken	55.50%	56.10%	55.80%	62.10%	48.00%	65.30%	61.70%	51.10%	51.00%	51.60%	62.00%	59.50%	57.90%	100.00%												
	human	54.10%	54.70%	54.70%	62.10%	48.00%	65.30%	62.60%	52.20%	51.60%	53.00%	62.00%	60.40%	58.20%	88.30%	100.00%											
	pig	53.80%	53.80%	55.00%	61.20%	48.30%	65.00%	62.60%	52.20%	51.90%	53.30%	61.70%	60.70%	59.10%	87.10%	95.80%	100.00%										
	giant panda	53.80%	54.40%	55.00%	62.10%	48.00%	65.60%	62.30%	51.90%	51.60%	52.70%	61.70%	60.70%	58.50%	88.90%	97.90%	97.30%	100.00%									
	duck	55.50%	55.80%	56.40%	61.80%	47.70%	65.60%	61.40%	51.10%	51.00%	51.60%	61.40%	59.80%	57.90%	94.90%	91.60%	90.40%	92.20%	100.00%								
GPR12L	zebrafish	54.80%	55.30%	54.50%	60.40%	45.90%	62.70%	59.80%	51.60%	51.30%	52.10%	59.80%	55.80%	56.80%	74.40%	73.30%	71.80%	73.00%	74.10%	100.00%							
	spotted gar	53.80%	54.10%	54.70%	59.70%	47.90%	64.40%	60.50%	51.30%	50.80%	51.90%	60.50%	59.80%	58.10%	85.70%	85.10%	85.10%	86.90%	86.60%	78.00%	100.00%						
	chicken	45.10%	45.10%	46.00%	48.20%	43.30%	50.20%	48.40%	42.70%	42.20%	42.40%	47.30%	47.80%	45.70%	54.00%	53.50%	53.50%	53.70%	54.00%	50.90%	52.60%	100.00%					
	duck	43.70%	43.70%	44.20%	46.90%	40.80%	47.20%	46.10%	42.00%	41.10%	41.80%	45.00%	45.40%	44.00%	51.70%	51.20%	51.20%	51.20%	51.40%	49.20%	50.60%	84.00%	100.00%				
	zebrafish (zebra)	47.30%	47.00%	48.20%	51.10%	42.80%	52.90%	51.10%	41.60%	41.10%	42.20%	50.80%	49.40%	47.40%	54.00%	54.00%	54.00%	54.30%	54.30%	50.20%	53.10%	54.50%	52.50%	100.00%			
	zebrafish (zebra)	47.90%	47.90%	49.40%	51.60%	44.10%	56.90%	53.30%	43.70%	43.40%	44.20%	52.20%	52.00%	51.40%	59.50%	58.40%	57.80%	58.40%	58.70%	54.90%	56.90%	61.60%	58.40%	66.50%	100.00%		
GPR12L	spotted gar	48.30%	48.60%	48.90%	53.80%	44.10%	56.50%	54.10%	43.10%	42.90%	43.40%	53.50%	53.60%	53.70%	61.20%	60.30%	59.10%	60.30%	60.30%	55.60%	58.60%	63.90%	60.80%	66.70%	85.40%	100.00%	

-3050 TCCACCAAGGTACAAGCTTCAACATTTAAATCATCATATACATATGACATAGGAGTAGACACAGTTCCTGTCAGAGAAGTTGGTCATCTCAACTATTATAACTG
 -2945 AACACACCTTTCAGAAACATTCTCAGAAATATTTCCCATGCTTTTAAAGACTCAGGATGCCATTATCTCATTTTGAATACCTAGGATTATCAACGCGCCTTGAAT
 -2840 CATTTTCAGCTTTGAGATATTACTCTGCCAGCGAACAGCAACCAATACAGCTGGAAGCCAGCCCTCCACCTATGCATGTGTATATATGATTCATAGTAC
 -2735 AAACTTTTGCCATAAGAACACACACCTTTTAGTATTTTATTATGTATGTCTTGTCTTGGCTTAAAAAGCCTGAGAAGTGAGCACAGAACCAATGCCCTCTGT
 -2630 GGTCCCTATGATAGGACTAGAGGAATGGCTTCAAGCTGCAGCAGGGGAGGTTGAGGCTGGACATTAGGAAATACTACTCTGAAAGAGTGGTCAGGCACCTGGAATG
 -2525 GGCTGCCCGGAGAGGTGTGGAGTCACCGACCTGGTGGTGTTCAGGAACATTTGGACATTGTGTTGAGGGACATGGTTAGTGAGAACCATTTGGTGATGGGTGG
 -2420 GTGGTTGGACTCGGTGATCCTGTAGGTGTTTCCACCTTGGTGAATCTGTGATTCTATATTTTGTGGTGGCCTGAAGAAGCTCACTGCATATCACGTTTCATCTC
 -2315 AAGCTTTATTACAAATTAGTAATCAGAGACTGGCATGAAAGCACTTTTGTAGATGTAACAAAAAATAGATGAAAGATGTACAGAAGCATAAACAGTGTGCACTG
 -2210 TACCAGTTCTAGAAATATCCAGCCATGACAAATAAAAAAGGGGGGAGGGGGGAGCAAAATAGTTATTAAATGTACACATGCAGAAGAAGTAAATCACTGT
 -2105 GCTTTGTTAAAGGAAGCAGGAAAGAGAAACAGTGTTCGGGAAAAAAGCAAGCAGATATACATGCAGGGAACATGTCCCAAGTCAGGTACTTTTGAA
 -2000 GATTCCAAACACTGAATCAATTATCAGATAAAATAGAGTCTCTGCAGAGGATGCTCATATTAGACATGGCTATTGAGAGCAGTTGGCAACCAAGTGAGAAGG
 -1895 AATGGCTTTCAACACCTAAGGCCATTGTGTTCTTTACCTGAGGAGAAATAAAGTGTGAGTTATGAGAAAATTTCCAGCTATCATAGCCTATAAAGAAATAAA
 -1790 AATCAACTGCTACTAATTTGGGTACATTTTATTTCTGGCTTTTGCCCATGTAGAACTTTTATTTCTAAAGACTTCTTCTTAAAGGTATTAAAGGGAATCA
 -1685 TTTGTGCTGGTGTACTGTTCCAGTGATTAATTATCTTCTGATCGAATGTGCTTGATTTTGAATCTGAACCTTCTTAACCTTTGGCTTCCAGGCATTATACCTTT
 -1580 TCTGTAGAGGCATATAAAATCCTGGTGAAGAAATGCTCTAGTCCAGTCATCTCTCAGTTTCTTCTAGAGAACTGAAAAGAGTACTTGTATAAATCCTTCACT
 -1475 ATGAGGTAATTTCTCAGGTCTTCAATGCCATATCTGTAACCTTTCTCTGAATTTCTCTTCTTCTTCTCAGAACATTAAAAAATAAATATGTATCTAGCT
 -1370 TTCAAATACTAATCTCATCAGTGCCCTGTCCAGAGGTAACATAGAATCATAGGGCTTGGAAGGAAACTCTGGAGATCATCCAGTCAACCTCTGCTAAAGCAGGT
 -1265 TCCCTGCTAACACAGGTGGAGCAGGAAAGCACACAGGCAGGTCTTGAATATTGCCAGAGAGGAGGCTCCACCACCTCTCTGGGTACCTGTTCCAGTGCTCTGT
 -1160 CACCCCTCAAAGTAGAGAAGTTCTTTTGCATGTTGAATGTAACCTTTTGCATCCAGTTTGTGCTCATCATCCCTTGTCTGTCTACTGTATACCACTGACAGGAG
 -1055 CCTGGCTTCAACCAATTTGACTCTCACACCTCAGGTATTTATGAACAACGAAGAGATCCCTCTGTCTCTTTTCTCCAGACTCAACAGCTCCAACCTCTCTCAGCC
 -950 TTTTCCATAAGGAAGGTGCTCCAGGCCCGAGTCGTCTCGTGGTCTCAGCCCACTCTCCAATAGTTCCCTGTCTTCTTGAAGTGAAGGCCGAGAACTA
 -845 GACACAGCACTCCAGATGTGGCTCATTTGGGCCGAGTAGAGGGGAGGATCACTCTCCGACCTCTTGGCCACACTCTTTGTAATGTACCCAGGATCCCACT
 -740 GGCATTTCTGACTACAGGGGCACACTGTGGCTCATGGCCAACTGCTGCCAACGGAACACCCAGGTCTTCTCTAAAGAGCTCCTTGTGAGCAGGTGAGCCCC
 -635 GAGCCTGTACTGGTGATGTGGTTATTTCTCTCCATTTGCAGAACTCTACACTTGCCTTTGTGCCCTTTTCTCTCTACCCAACTCTCCAGTATGTGAGGTCC
 -530 TGCTGAATGGCAGCACAGCTTTCTAGCTTCGTATCATCAGCAAACTGCTGTGGTGAACCTACTCTTTTGTCCAGCTCACTTTTGAATATGTTGAACAAGACC
 -425 AGATCTGGTACTGACCTCAGGGAACACCACTAGTTATAGCTCTCCAACCTAGACTGTGTGCTGCTGACCACAACCTCTGAGCTCTGCCACTCAGTCAGTTCTCA
 CAAT
 -320 GTCC**ACTCTACTGCTTACCCACACCCCC**AAACATCACATCCTTGTTCCTGTTTCGGGCTCTCCCTATATACAGAGCCCTTTTGTGTACAGCCCTGCACAAGGAG
 CAAT
 -215 CTCTCGTATATAGCCGACCGGG**ACTGA**ACT**CCCAACCCGCTCCCTCAG**CGGCGCCCTCCCGCTCGCAGCAGGAGGCATCTGCGCAGGCGCGGAGCACGAGGGG
 CAP E2F
 -110 AACCCTCCGTTCCCGCGCAGAGTCCACGTGGCCGCGCGCGCCCTACCCCTCTCTCTCTCTCTCTCT**TCACCCGA**ACCG**CTGCGCGG**CGAGGGGAAGGGGAGGGG
 +1
 -5 GGGG**AG**CGCGCGCACCTGCGCTGCGCCCT**GGCCCCCTCACGGC**GAGCGGTGGAGGACGCGCCCTCGGAGGAGACCTGCGAGCCTCGTCTCCCGTCTCCGGG
 SUF AP2 SP1
 +101 AGAG**CCAGGTGT**GTGCGTCCGGGGGGGGGCTGCCGGGGGTTGCGGT**GGCCCGCGGGAG**TTTGTGAGGACCTCGGAGGACCTCGCGCC**CCCCTCCCC**ACCTT
 AP2 SP1
 +206 CCT**CCGGCGGGGC**TTCTTACAGGGGTCGGGGAAG**ACCGCGCGCCCT**CACCCGTACGCTCACCGC

Supplemental Fig. S1 Analysis of the promoter region of cGPR12. The nucleotide sequences of cGPR12 promoter region. The predicted binding sites for transcriptional factors were shaded. The transcription start site ‘G’ identified by 5’-RACE was boxed and designated as ‘+1’.



Supplemental Fig. S2 RNA-seq data analysis showing the tissue expression of *GPR3*, *GPR6*, *GPR12*, *GPR12La* (*GPR185a*), and *GPR12Lb* (*GPR185b*) in spotted gar (A), zebrafish (B), and duck (C) tissues.