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SUPPLEMENTARY MATERIAL

corresponding to:

In silico identification of the genes for sperm-egg interaction in the internal fertilization of the newt *Cynops pyrrhogaster*

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A

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                               20                    40                    60                    80
                                |                     |                     |                     |
comp59911   TGTAAAAAAG GCATTTATAT TGAAAAATAC AACTTCAAAC AGTACAGACC TCAGCAATGT ACAAAGAAAT ATTCCTGAAT
protamine 1
                              100                   120                   140                   160
                                |                     |                     |                     |
comp59911   TGTGTTAAAC GTTAACAGAT AATTACAGGT CACCATGGAC AGTTTTAGCC AGTCCCTAGT ACTTTTAGGC AGACTGCCTC
protamine 1
                              180                   200                   220                   240
                                |                     |                     |                     |
comp59911   TTGAAACATG GATTGCCTTC CAAAGGGTCA TCTTTTCACG GTAAAAAAGA TTTTAACAAA GACGTCAATG AACTAGACAA
protamine 1
                              260                   280                   300                   320
                                |                     |                     |                     |
comp59911   AAGTTAATTT CATTTTTTAG GTGGAAAGGA GTGTGTTTGT CCACCAATTC CTCGGTAAAT AAAACTATAT CTGTAGTGCT
protamine 1
                              340                   360                   380                   400
                                |                     |                     |                     |
comp59911   AGCATGTACT CCCCCATTTG AAACTGTGGA ATTTATTTCA CTGAATTGAC AATAATCAGT AGTAAATTCC CAGGAAGCTC
protamine 1
                              420                   440                   460                   480
                                |                     |                     |                     |
comp59911   TTCTCCCTCA TTAGAACATT TTGTATACCC CCCTCCCCCA CACACTTCTA GCAAACTGTG ATAAATCCTT GTCTTAAAAA
protamine 1
                              500                   520                   540                   560
                                |                     |                     |                     |
comp59911   AAAAATGAGA GCTTGGTTTG GCAACAAACC AATGGAAAGC ATCTTGACCT TGAGAAGGGT GGAAACAATT TCAACTGCAC
protamine 1
                              580                   600                   620                   640
                                |                     |                     |                     |
comp59911   TAGAATAATA AACTAAGTTT GTCAACATAG AACATACATG GACAGTCAGT GCTTGAAATG GGAAAATAGA AGTCCAGGTA
protamine 1
                              660                   680                   700                   720
                                |                     |                     |                     |
comp59911   CTCTATAGCA GAGTACCTGC TTGTTTCTGA GAAGTGCCGG TACTCTCCAA TTAAAAGTAT TACTTCGCTC TTGAAAAGTG
protamine 1
                              740                   760                   780                   800
                                |                     |                     |                     |
comp59911   CCGGTACTCT CCTCAAAATA AAAAAGTGCC GGCCCATTTA AAGCACTAAT GGGAGCTGTA CAAGATTGTG AATGAGGACT
protamine 1
                              820                   840                   860                   880
                                |                     |                     |                     |
comp59911   CTACAGCAAA AGAAGGAAAA TGTACCTGCA CACAGCCTGC AGGTGGACTG ATGGTCAGAA AGGCAATGCA CGGGTAGGCC
protamine 1
                              900                   920                   940                   960
                                |                     |                     |                     |
comp59911   GAGAGGCCAG AGGCTTCAAG TGACACAGCA GATACTTGAG TCAAATGACA GGTGGCAACA TAGTCAGGTG ATGTCACAAT
protamine 1
                              980                 1,000                 1,020                 1,040
                                |                     |                     |                     |
comp59911   CAGCACTGCT ATATATGTGA GGTTGAAACT GCCCCACAGC ATAGGGCACC TGCTCCTGGA CCTGAAAGGA TAAGAGCAAG
protamine 1                                                       ........ ........-. ......G...
                            1,060                 1,080                 1,100                 1,120
                                |                     |                     |                     |
comp59911   ACATGGCTTA TGGAAGAGCG AGGAGCAGGG GCAGAAGTGT ACGCAGAAGG CGAAGGGGAA GGTCACCCGG CAGGCGCAGA
protamine 1 .......... .......... .......... .......... .......... .......... .......... ..........
                            1,140                 1,160                 1,180                 1,200
                                |                     |                     |                     |
comp59911   AGAGGTAGAA GGAGCGACAA CGATGCTCCT CGTCGTCGTC GTCGCAGAAG ACAATAGA-C TCCCATGTTG ACACTTCCTG
protamine 1 .......... .......... .......... .......... .......... ........G. .......... ..........
                            1,220                 1,240                 1,260                 1,280
                                |                     |                     |                     |
comp59911   TTGCTGTGCT GCCTGGGTCC TCGAACTTCG TCTTTGATCT TCCATAGTGA TTATGATAAA ACACAAGCAG ATCCAGAAAA
protamine 1 .......... .......... .......... ......-... .........- .......... .......... ..........
                            1,300                 1,320                 1,340
                                |                     |                     |
comp59911   CATCTACAGC ATTGATTGAC AGCTACAGCA GGAAAAGTAG TAGCAAAATA AACTAAATTG CAAAAT
protamine 1 .......... .......... ....G...G. .......... .......... .......... ......
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B

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                               20                    40                    60                    80
                                |                     |                     |                     |
comp43001   GAAGTTACCT GCACCTAAGA CTGAAAGCAC AATAACAAGC TATGGCAAGA GGAAGGAGCC GGAGCAGAAG TGTACGGAGA
protamine 2                       .......... .......... .......... .....A.... .......... ..........
                              100                   120                   140                   160
                                |                     |                     |                     |
comp43001   CGGAGACGAG GAGGGTCTCC GCGCAGGCGC AGGAGAGCCG GCAGGAGGAG CCAAAGAGCA GGAGCAGGAG GTTTACGCCG
protamine 2 .......... .......... .......... .......... .......... .......... .......... ..........
                              180                   200                   220                   240
                                |                     |                     |                     |
comp43001   TCGCCGCCAC AGACGGGCAG ACCAGGAGTA GGTTCCGTGT CAACTCTTCC TGTTGCGTGC TGTCTGGGTC CCATCGCTCC
protamine 2 .......... .......... .......... .......... .......... .......... .......... ..........
                              260                   280                   300                   320
                                |                     |                     |                     |
comp43001   CTGCGGCCAG TCT-GAACTC TCCAACGGTG ATATTACAAG CACAAACTTT TTAATGAGAT CCAGTATAAA CAGCAACGGC
protamine 2 .......... ...T...... .......... .......... .......... .......... .........C AGCA......
                              340                   360                   380                   400
                                |                     |                     |                     |
comp43001   ATTAA-TCAC CAGCT-ACGG CAGGAAAAAA ATGACTAAAT AAACGTCCTG GCAAAACTTT AAAAAAAAAA AAAAAAAAAA
protamine 2 .....A.... .....T.... .......... .......... .......... ..........

comp43001   A
protamine 2
```

Fig. S1. Alignment of contig base sequences including base sequences of *protamine* genes. (A) *Comp59911 including whole* protamine 1 *gene sequence (dots) in 3'-region.* **(B)** *Comp43001 including whole* protamine 2 *gene (dots).*

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                             20                    40
                              |                     |
comp68248 LCEEAGMVVA VQRDLFGTGK LVQAADLKLG TALCRYTSLG ADNTVTFQIA
comp38201 Q.L..D...T .......... ...PG..R.. A.F.S.K.VN .N......S.
comp64853 Q.Q..A...T .......... .I.....R.. ....K....N .N..I.....
comp62734 N.NDS.I.L. MK.NPL...A .L-PQY.A.. --W.G.SIAD MGGY.I.KYR
comp60094                            .R.. ..F.NH...N .....I..R.
comp46343
                  60                    80                   100
                   |                     |                     |
comp68248 LQDCGNSLQV TADSLIYTSI LSYNPTRSDG SRVITRTNPT SVVIQCMYPR
comp38201 ......T... .PTL...S.V .T...SP.-. .A..I.S..V ..LL..L...
comp64853 .H....T... .P...V.S.. .T....P.II .T........ ..LVR.I...
comp62734 .NS..F.RLT SGMTVE.SAD .I.S.PAGTS N.WY--ST.F AER.N.----
comp60094 PH.....R.. .Q.AV..S.. .T.T..P.-. .H......QV ..LL..V...
comp46343
                            120                   140
                              |                     |
comp68248 SGNVSSNAIQ PTWIPFSST- IYSEANLLFS LKLMNDDWSA VRTSTFFHLG
comp38201 N......P.R .........- VSV.SS.V.. .R..S.N... E..T.I.Q..
comp64853 N........H ...V.....- ..A.ES.Y.. .T......IS A.NN.T.Q..
comp62734 --TLFNP.PP ..RSKSAV.H LSGSGV.N.R AR.L.A.F.. PSRTSTYS..
comp60094 HA....K..A ...T.LRT.- VSV.EA.S.. .R..I...TS A.S..T.Q..
comp46343                                     ...L.Y G.RYQT.QW.
                 160                   180                   200
                   |                     |                     |
comp68248 DVFNIEASVE SGNHAPLRVF VDSCVATLTP D-RKLTPSYD LIKSNGCLVD
comp38201 ...H.....T T.S.MEM..L .......... .-KTSS.R.. I.A.......
comp64853 .........Q T.S.VD.... ........N. N-MSSS.R.. ..G.....L.
comp62734 SNI...L.LQ T.F.Q.MQIY .EE.T.S.S. .LENA.VK.S VVNNH...T.
comp60094 ...HL....N A.SQF...I. ......SP.T .-PDSRLQ.. IVS.Y.....
comp46343 FPI.L..R.D TASGEA.KIY ..E.YGAP.A .LKTSGKT.A IMHNH.....
                            220                   240
                              |                     |
comp68248 GKLSDSSSAF RTPRLQQDKL QFMIDSFIFI GDSRSSIYIT CNLKAVPTIR
comp38201 ...P...... IS..S.PN.. ..TV...... ..A..L.... ......DASV
comp64853 ...P...... IS..D.L... K.TV.A.R.. A.T..Q.... .......A.Q
comp62734 ...G..K--. V.-.PSPSRI RLSLQV.R.A VE-NTDV.LH FKVLVWDPSV
comp60094 ....-..... VS..V.P.I. R.TV.A.R.. .TTN.M.F.. .S.R...ADS
comp46343 ..FGN..FWH .S----NNSI I.T.QA.MLR EGTEEQV..H .S.SVWDLEK
                 260
                   |
comp68248 DPDGMNKACS YN
comp38201 V.SAE..... .S
comp64853 ..SPTS.... ..
comp62734 LTGLTR.... ..
comp60094 A.SP.T....
comp46343 PMGLTR.S.. .
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Fig. S2. Alignment of amino acid sequences of ZP domains in ZPC homologues identified in RNAseq data from ovary. *Comp38201 is the homologue of ZPC in* C. pyrrhogaster *(accession: KM085177) reported in Makabe-Kobayashi* et al. *(2003).*

TABLE S1

PRIMER SEQUENCES USED IN RT-PCR

molecule	forward primer	reverse primer
α1 S (CaV1.1)	TTGATGTTGCAGTTGTTGGCGTT	AGGAGATGATGAAGTCACAGTAGGG
α1 C (CaV1.2)	TCCAGGATACCGCCACACACA	ACCATCGCCTCTGAGCACTAC
α1 D (CaV1.3)	TTTGCGTACCGTCCTAGTCCTTC	ACCTCTCACACCACCTTTTCATC
α1 B (CaV2.2)	GACTTTGGCGTCATCTTGGGAAG	TTCCAGTCTTCACCAGTGAGGAT
α1 H (CaV3.2)	CTCGTCTCTCCTATCCTCGTCAT	CGAGCAACACCAACCCACACAA
α1 I (CaV3.3)	AACTTCTCGTCTCTCCTATCCTC	CGCGCATTCCTTCTACAACTTCA
CRISP 2	ATGCAAAAAGTGGAATGGAG	CTGAGGAGCGTAATGGCAAA
CRISP-like	CCCTTTTCTTTGGTTTCAACTG	ATTTTTGGTATTCCCCGGTG
protamine 1	ATGGCTTATGGAAGAGCGAGGAG	CGACGACGACGAGGAGCATCGTT
ER-alpha	GAAAGAGGACGCAGATCAGAAGC	CACCAGCATGTCGAAGATCTCCA
EF1	AGGTATTGGCACAGTCCCAGTGG	TCAAGCTTCTTGCCAGATCGTCTG

TABLE S2

MULTIPLE CONTIG SEQUENCES ANNOTATED TO EACH α1 SUBUNIT OF VDCC

	length (bp)	accession	species	E-value	bit score	identity (%)
α1 S (CaV1.1)						
comp387802	443	XP_004323285.1	*Tursiops truncatus*	9.12E-84	265	96.2
		CAA81218.1	*Homo sapiens*	1.60E-82	264.2	94.7
		XP_002809678.1	*Pongo abelii*	2.62E-82	266.9	95.4
comp29074	583	XP_003212878.1	*Meleagris gallopavo*	5.78E-66	230.3	81.8
		XP_419259.3	*Gallus gallus*	2.21E-62	220.3	81.8
		ADU17253.1	*Acipenser ruthenus*	7.65E-62	218.8	78.7
comp29074	565	XP_419259.3	*Gallus gallus*	2.24E-09	65.1	35.3
		XP_003448283.1	*Oreochromis niloticus*	1.42E-08	62.8	40.6
		NP_001033694.1	*Felis catus*	1.89E-08	62.4	44.0
α1 C (CaV1.2)						
comp648010	320	BAD92253.1	*Homo sapiens*	5.08E-24	105.1	71.7
		EAW88906.1	*Homo sapiens*	9.07E-24	105.1	71.7
		XP_003313479.1	*Pan troglodytes*	1.51E-23	104.8	71.7
comp672552	248	CAR62397.1	*Capra hircus*	2.84E-50	164.9	96.3
		XP_416388.3	*Gallus gallus*	4.32E-46	168.3	98.8
		XP_003220884.1	*Anolis carolinensis*	4.39E-46	168.3	98.8
α1 D (CaV1.3)						
comp158103	761	XP_003210118.1	*Meleagris gallopavo*	4.19E-126	405.2	84.5
		NP_990365.1	*Gallus gallus*	4.95E-126	404.8	84.5
		EMC88796.1	*Columba livia*	1.43E-125	403.7	84.5
comp227364	497	NP_058994.1	*Rattus norvegicus*	5.94E-28	119.8	55.8
		EHH16293.1	*Macaca mulatta*	2.43E-27	118.2	55.2
		XP_003894337.1	*Papio anubis*	2.72E-27	117.9	55.2
comp433395	318	AAA40895.1	*Rattus norvegicus*	1.51E-39	142.9	82.5
		XP_001173076.3	*Pan troglodytes*	2.31E-39	150.2	82.5
		XP_002713437.1	*Oryctolagus cuniculus*	3.11E-39	149.8	82.5
comp54305	540	NP_058994.1	*Rattus norvegicus*	0.843408	38.9	32.3
		BAA07283.1	*Rattus norvegicus*	0.871595	38.9	32.3
		AAK72959.1|AF370009_1	*Rattus norvegicus*	1.59626	38.1	34.5
comp12217	208	EMC88796.1	*Columba livia*	1.21E-12	71.2	72.1
		XP_002192685.2	*Taeniopygia guttata*	1.44E-12	71.2	72.1
		NP_990365.1	*Gallus gallus*	3.60E-12	70.1	70.6
α1 B (CaV2.2)						
comp161304	981	DAA24110.1	*Bos taurus*	0	543.5	0.8957
		DAA24127.1	*Bos taurus*	3.35E-180	543.1	0.8957
		EAW88415.1	*Homo sapiens*	3.77E-179	533.9	0.8804
α1 H (CaV3.2)						
comp541465	212	XP_003210681.1	*Meleagris gallopavo*	5.09E-24	104.4	0.7465
		XP_414830.3	*Gallus gallus*	1.96E-23	102.8	0.7324
		EMP35527.1	*Chelonia mydas*	6.74E-21	94.4	0.75
comp423987	214	XP_002932520.1	*Xenopus (Silurana) tropicalis*	3.43E-24	104.8	0.7746
		XP_414830.3	*Gallus gallus*	5.98E-23	101.3	0.7761
		XP_003210681.1	*Meleagris gallopavo*	2.22E-22	99.8	0.7612
comp55396	5503	XP_002932520.1	*Xenopus (Silurana) tropicalis*	0	1508	0.7332
		XP_414830.3	*Gallus gallus*	0	1358.6	0.6691
		XP_002187949.2	*Taeniopygia guttata*	0	1320.5	0.6811
comp30821	287	XP_001374015.2	*Monodelphis domestica*	7.98E-31	125.2	0.5682
		AAG59899.1|AF334706_1	*Mus musculus*	1.25E-27	107.1	0.5545
		XP_002932520.1	*Xenopus (Silurana) tropicalis*	8.23E-27	113.6	0.6117
comp40193	565	ABL63743.1	*Rattus norvegicus*	1.58E-82	258.8	0.9231
		XP_004021144.1	*Ovis aries*	6.29E-82	274.6	0.9309
		XP_004270522.1	*Orcinus orca*	8.12E-82	276.2	0.9309
α1 I (CaV3.3)						
comp42249	1288	XP_002933803.1	*Xenopus (Silurana) tropicalis*	8.72E-70	251.5	0.602
		XP_425474.2	*Gallus gallus*	2.08E-64	235.7	0.4966
		XP_003221064.1	*Anolis carolinensis*	2.32E-54	206.5	0.5209
comp43042	276	XP_003905624.1	*Papio anubis*	5.46541	33.9	0.3134
comp44384	1125	XP_003221064.1	*Anolis carolinensis*	8.98E-156	493.8	0.754
		XP_003202350.1	*Meleagris gallopavo*	3.14E-155	492.3	0.7507
		XP_425474.2	*Gallus gallus*	3.86E-155	492.3	0.7507