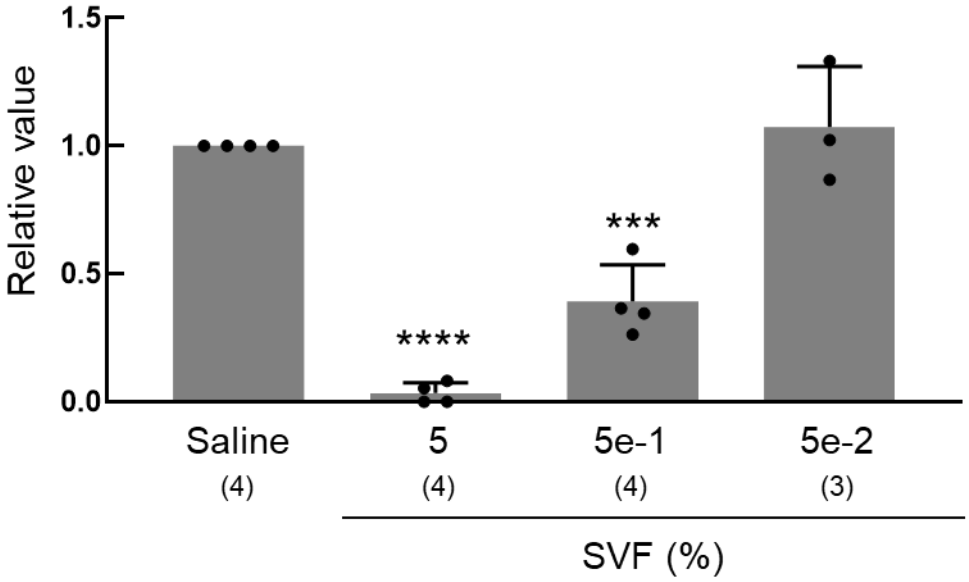

Fig. S1. Effect of *Corydoras* SVF on the motility of other species' sperm.

Medaka (*Oryzias latipes*) sperm was diluted with saline or SVF. Sperm motility was normalized to the number of motile spermatozoa in the saline control condition (control). Independent experiments were performed using spermatozoa collected from different male medaka. 50% SVF/DW was diluted to 0.1-fold (5%), 0.01-fold (5e-1%), and 0.001-fold (5e-2%). Unpaired two-tailed Student's *t*-test was conducted, ***$P < 0.001$, ****$P < 0.0001$ vs. Saline.

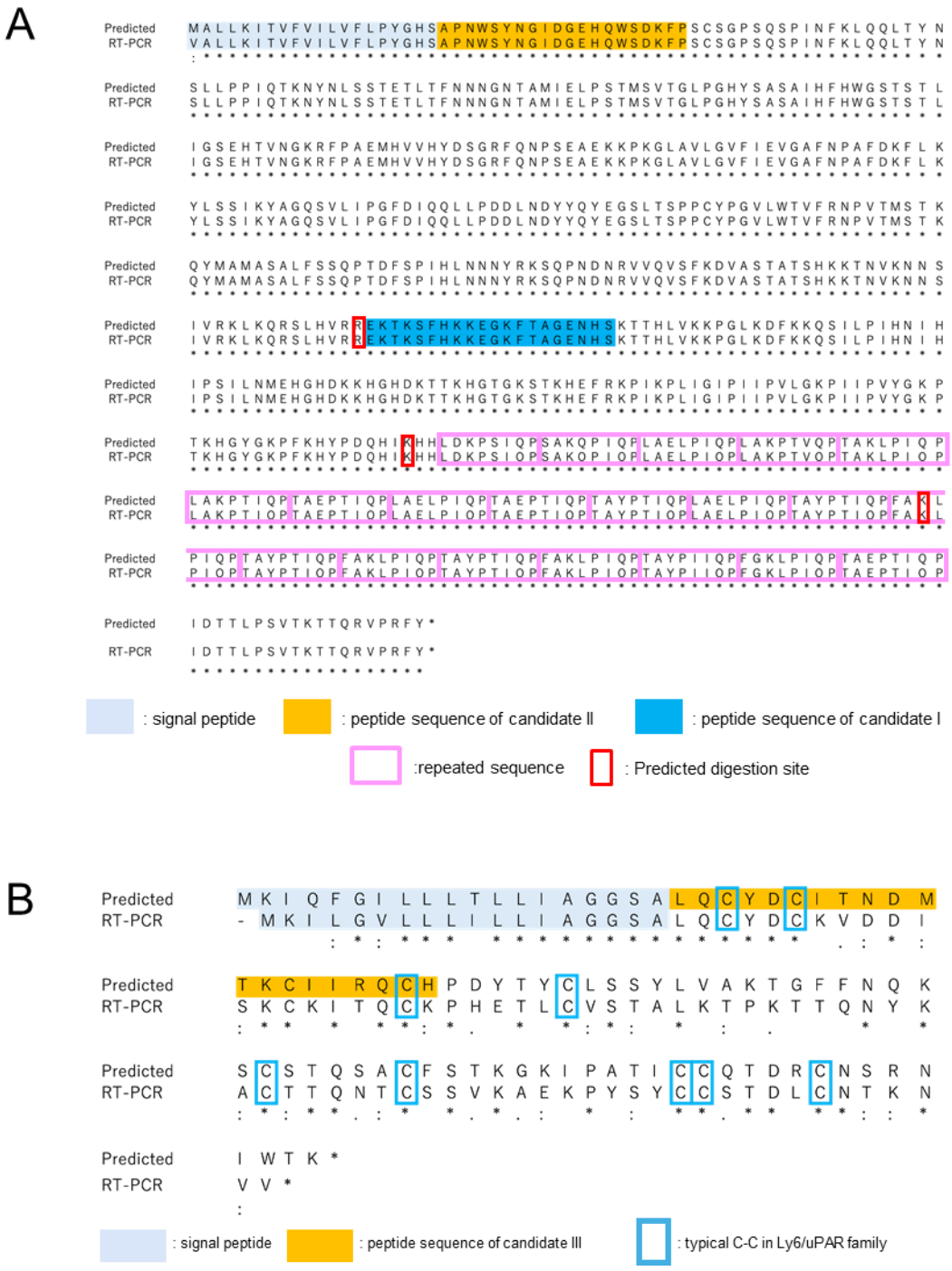

Fig. S2. Amino acid alignments of candidate II and III proteins.

(A) The top alignment was predicted by transcriptome analysis, and the bottom alignment was predicted from the results of DNA sequencing using RT-PCR. The gray marker indicates the signal peptide, orange marker indicates the matching part of the transcriptome result and

the peptide sequence of the candidate II band, and blue marker indicates the matching part of the transcriptome result and the peptide sequence of the candidate I band. Red plates indicate the predicted trypsin digestion sites. The pink plates indicate the repeated sequences. (B) The top alignment was predicted by transcriptome analysis, and the bottom alignment was predicted from the results of DNA sequencing using RT-PCR. The gray marker indicates the signal peptide and the orange marker indicates the matching part of the transcriptome result and the peptide sequence of candidate band III. Blue plates indicate the typical disulfide binding sites of Ly6/uPAR family proteins.

A

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   1 MCSRMKLLSGIPAVLLLLHI
  21 AAAQKLANDTIYLVTLNSQP
  41 VGGSTETLCVHVNPLRPIFS
  61 LLVNLKFGSTRQTLLTERFI
  81 NMEYYQCKQFQVPEVRAETE
 101 ASVTVLISGPKTSFNKTSMI
 121 LIKPGSEMVIMQTDKPIYKP
 141 GQTVKFRIASLYPSFLTYNQ
 161 MFPTIELQDPNGNRIGQWLN
 181 SSTGNGLIDLLYPTNPESPL
 201 GFYVITAWNKNNDVFTQTFE
 221 LKDYVLPKFEVTVKLPDVIT
 241 IVDASATLNICAKYTYGKPV
 261 SGSVKATVCRNKYPWLREET
 281 TDKDICIKFTQTTDATGCIS
 301 RVLDLTKFSLTKTNYEDMIQ
 321 VTCDVEEFATGIIISGSSAV
 341 YVTSELIRLTFENSASVFKV
 361 GMNFDGVVKAEDQNSKPLIN
 381 RLLYLKITYGDNAVSERTLT
 401 TDINGLAKFSLDTQMWGNSS
 421 VTLQARYYKSEKQPPYDPNV
 441 RLPIIPQAYLWLQSFISNSN
 461 SFLTLVPSADPFSCEQVATV
 481 TAKYLIHSSTLRASQQSLPI
 501 FYMVMSKGRVVQQGRLIIYV
 521 NARREENRGTVTLTLNSMKT
 541 LPPVAQVLLYAILPSGEAIA
 561 DSMNFPIENCLPNKVSLSFS
 581 SAQELPAGKTKLTLKAQPGS
 601 LCSVRAIDQSLLLLQPEKEL
 621 NAEAVFSLLPVQVLSGYPYN
 641 IDDERTYCVDTPPVDPLPVP
 661 ILLPRVRRSKFFFPYGSQSD
 681 VYGVFKNMAMKILTNADIKK
 701 PMSCYYGPFWDVRREPGVVF
 721 NFAVAKESSVSGASAPEQPV
 741 TTVRKFFPATWIWDLVPVGD
 761 SGMMAVDETLPDTITKWQAG
 781 AFCTSSVGFGVAPKVELTAF
 801 QPFFVSLTLPSSVIRKEMFT
 821 LKATVFNYLQGCMAVNVDLA
 841 PSPLFVARPCKGCIYSSCLC
 861 ADQSYTFSWIITANVVGEAS
 881 INVTAAAVQSSTLCGRNDIT
 901 VPQKGRIDTVINTLLVQAEG
 921 TKQTTSYNELICSSGGAVEV
 941 PVSLSLPELYVEGSVTAWVS
 961 VLGDIMGRALNNLASLLQMP
 981 YGCGEQNMLLFAPNIYILRY
1001 LESSGQLTEAIRSKAETFLI
1021 GGYQRELTYKHDDGSYSAFG
1041 MSDKSGNTWLTAFVMKAFAG
1061 AKRYIFIDDLYINQARIWLG
1081 QQQQENGCFASVGQLFHTDM
1101 KGGVDDEVTLTAYIMSAMLE
1121 LGLNLTDPVVGKALKCIRSA
1141 TPQLTSTYALALLSYTFTLA
1161 GDQSSRSSVISKLNSIAIIS
1181 DGTRHWSRQNTGTVDSLEVE
1201 MTSYVLLTLLTGPTLPGYDI
1221 SYTSSIVRWLAKQQNAFGGF
1241 ASTQDTVVALEALAKYSAAT
1261 YRPSGSMAVRVTSPLGKTKD
1281 FTVNQSNRLLYQESALQEVQ
1301 GNYKVKATGSGCVYVQFTLH
1321 YNIPPPADDSSFSIKASTKG
1341 NCSIPIPTVQVTVTVRFNGN
1361 RKKTNMMVIDLKPLSGFSLV
1381 SDSVLKVTESSDGNVKRVDQ
1401 KDGHVIVYLDYLIQGADRTY
1421 TLVIQQDVAVQNLQPAVVKV
1441 YDYYETVAEAVTEYTSPC
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B

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   1 MALLKITVFVILVFLPYGHS
  21 APNWSYNGIDGEHQWSDKFP
  41 SCSGPSQSPINFKLQQLTYN
  61 SLLPPIQTKNYNLSSTETLT
  81 FNNNGNTAMIELPSTMSVTG
 101 LPGHYSASAIHFHWGSTSTL
 121 IGSEHTVNGKRFPAEMHVVH
 141 YDSGRFQNPSEAEKKPKGLA
 161 VLGVFIEVGAFNPAFDKFLK
 181 YLSSIKYAGQSVLIPGFDIQ
 201 QLLPDDLNDYYQYEGSLTSP
 221 PCYPGVLWTVFRNPVTMSTK
 241 QYMAMASALFSSQPTDFSPI
 261 HLNNNYRKSQPNDNRVVQVS
 281 FKDVASTATSHKKTNVKNNS
 301 IVRKLKQRSLHVRREKTKSF
 321 HKKEGKFTAGENHSKTTHLV
 341 KKPGLKDFKKQSILPIHNIH
 361 IPSILNMEHGHDKKHGHDKT
 381 TKHGTGKSTKHEFRKPIKPL
 401 IGIPIIPVLGKPIIPVYGKP
 421 TKHGYGKPFKHYPDQHIKHH
 441 LDKPSIQPSAKQPIQPLAEL
 461 PIQPLAKPTVQPTAKLPIQP
 481 LAKPTIQPTAEPTIQPLAEL
 501 PIQPTAEPTIQPTAYPTIQP
 521 LAELPIQPTAYPTIQPFAKL
 541 PIQPTAYPTIQPFAKLPIQP
 561 TAYPTIQPFAKLPIQPTAYP
 581 IIQPFGKLPIQPTAEPTIQP
 601 IDTTLPSVTKTTQRVPRFY
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Fig. S3. The amino acid sequences of candidate I and II that matched with the transcriptome data from LC-MS/MS.

(A) The amino acid sequence was predicted as A2M from the transcriptome results, and the parts of red sequence were matched with the LC-MS/MS result of candidate I. (B) The amino acid sequence was predicted as CA12 from the transcriptome results, and the parts of the red sequence were matched with the LC-MS/MS result of candidate II.

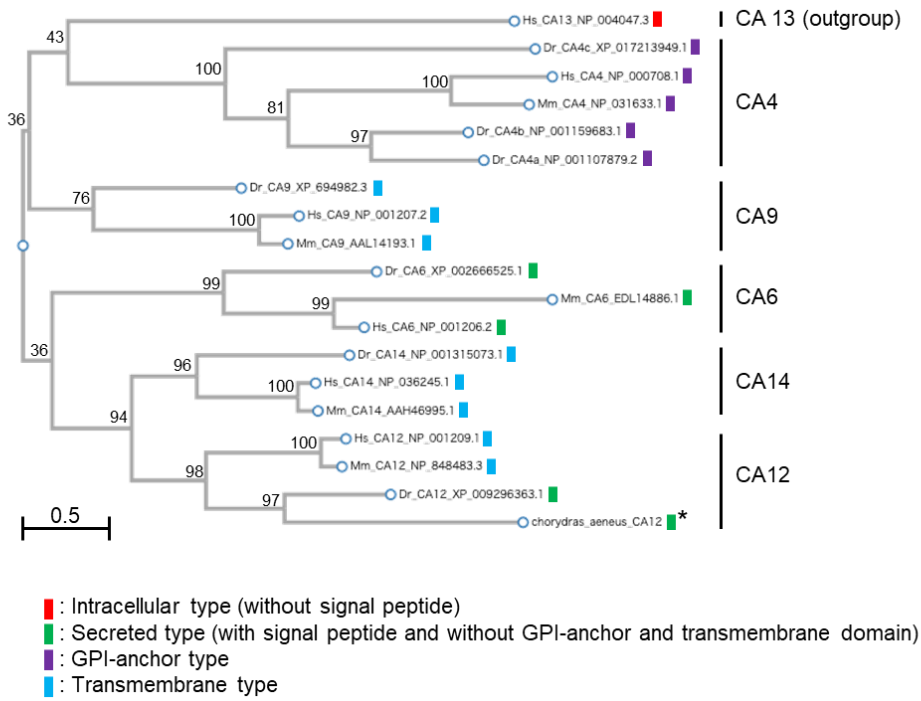

Fig. S4. The maximum-likelihood tree of carbonic anhydrase

Maximum likelihood tree showing the relationship between 19 samples of extracellular-type CA proteins (CA4, 6, 9, 12, and 14) of human, mouse, and zebrafish plus one outgroup intracellular-type CA protein (human CA13). Numerical values represent the bootstrap values (out of 100). Scale shows substitutions per site. Asterisk represents *ca*CA12 sequences obtained in this study.

Table S1. Settings for sperm motility analysis and PCR primers used in this study.

Table S1A. Parameters for SMAS analysis of *C. aeneus* sperm.

Data processing	min.	max.
Binarization	150	255
Deletion of particle out of edge	0	0
Deletion of particles (area [pixel])	50	250
Deletion of particles (roundness)	0.9	1.5
Deletion of particles (minimum center of gravity distance/ average center of gravity distance)	0	1
Deletion of particles (Oblateness)	1	2

Table S1B. Parameters for SMAS analysis of medaka sperm.

Data processing	min.	max.
Binarization	150	255
Deletion of particle out of edge	0	0
Deletion of particles (area [pixel])	10	39
Deletion of particles (roundness)	0.9	1.5
Deletion of particles (minimum center of gravity distance/ average center of gravity distance)	0	1
Deletion of particles (Oblateness)	1	2

Table S1C. Primers for PCR.

Primer ID	Sequence (5′→3′)
GeneRacer™ 5′Primer	CGACTGGAGCACGAGGACACTGA
Reverse GSP-1	AAGTTTATTGGAGACTGGGATGGT
Forward GSP	CGAGGACACTGACATGGACTG
GeneRacerTM 3′ Primer	CGTTACGTAGCGTATCGTTGACAGC
GeneRacerTM 5′ Nested Primer	GGACACTGACATGGACTGAAGGAGTA
Reverse Nested GSP	TCTGCTCCGAACTGGTCCTA
Forward Nested GSP	CACCAGTGGTCGGACAAGTT
GeneRacerTM 3' Nested Primer	CGTTACGTAGCGTATCGTTGACAGC
T7 promotor Primer	TAATACGACTCACTATAGGG
T3 promotor Primer	ATTAACCCTCACTAAAGGGA
forward Primer for cloning candidate I	ATGAAGCTCCTCAGTGGGATTC
Reverse Primer for cloning candidate I	TTAACATGGGGAGGTGTATTCA
forward Primer for cloning candidate II	ATGGCACTCCTCAAAATAACAG
Reverse Primer for cloning candidate II	ATAAAATCGAGGGACTCGCTG
forward Primer for cloning candidate III	GCATCCACCCACTTCTTCCT
Reverse Primer for cloning candidate III	TTGAACAGTCAGATGCTAATGCA
Forward Primer for subcloning caCA12-33	CCCGAATTCTGGTCGGACAAGTTCCCAT
Reverse Primer for subcloning caCA12-33	GGGCTCGAGGGTAGCAGGGAGGTGAGGTA
Forward Primer for subcloning caCA12-10	CCCCCATGGAGAAGACCAAAAGCTTCCAC
Reverse Primer for subcloning caCA12-10	CTCGAGATAAAATCGAGGGACTCGCTG
pGEX_5_Primer	GGGCTGGCAAGCCACGTTTGGTG
pGEX_3_Primer	CCGGGAGCTGCATGTGTCAGAGG
Forward Primer for subcloning caCA12-FL	CCCGAATTCGCTCCGAACTGGTCCTATAA
Reverse Primer for subcloning caCA12-FL	GGGCTCGAGATAAAATCGAGGGACTCGCTG
T7 terminator Primer	ATGCTAGTTATTGCTCAGCGG