

SUPPLEMENTARY INFORMATION

Novel pituitary actions of TAC4 gene products in teleost

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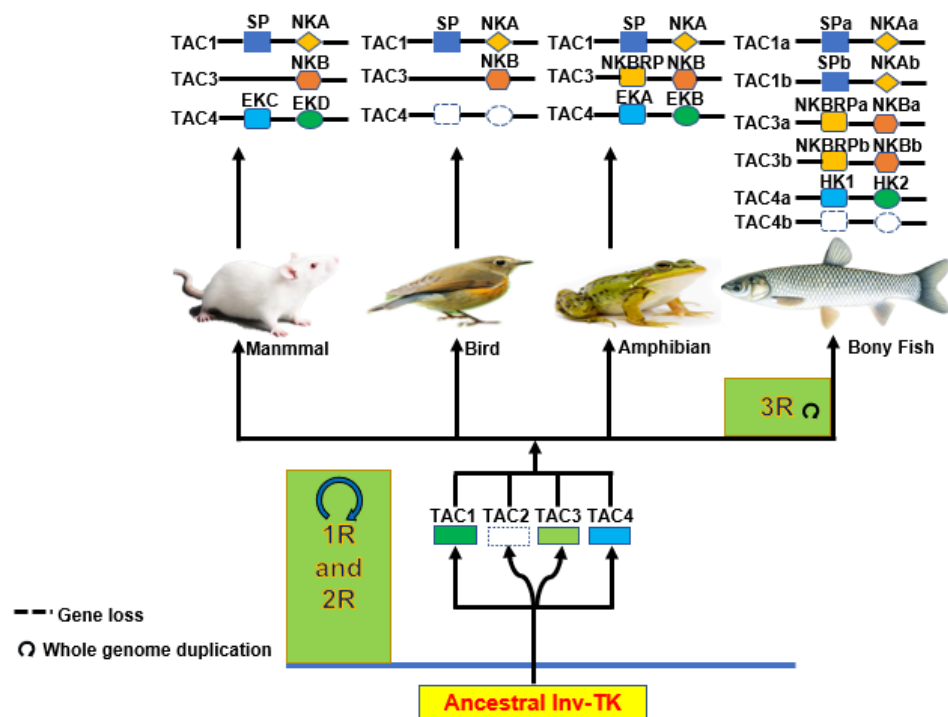
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Supplementary Table S1. Primer sequences used for quantitative real-time PCR of TAC4 and NKRs in grass carp

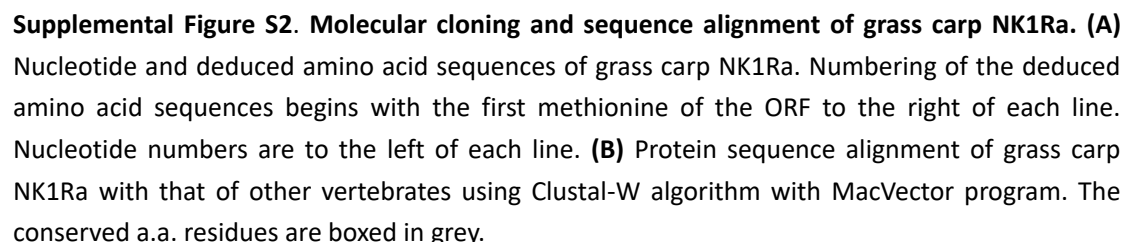
Gene	Forward primer	Reverse primer	Product size (bp)	Annealing T _m (°C)
TAC4	CAAATGCTGTTGTCTGCTT	TATGGTTCAACTACATTCCG	270 bp	52°C
NK1Ra	GGAATGGATTGCTCATCACTT	TAACGGTGTGTAATGCGGAC	280 bp	58°C
NK1Rb	CGTGTCGTGTGCTACATCAACT	TCATTTTCACCACCTTCCGT	220 bp	53°C
NK2R	AGATGATGATAGTGGTGGTGAC	GCAGTAGAGATGGGGTTGTA	177 bp	52°C
NK3Ra1	ATCGTAGTGGTGACCTTTGCC	CTCCATTCTGTGAGAGCGTGTA	315 bp	60°C
NK3Ra2	CCGCCTTCAACACGCTCATCAA	GGAAAGCCAAAATCACCGCCAA	246 bp	52°C
NK3Rb	GCCAAGAGAAAGGTTGTGAAGA	GTGTACATGCTGCTCTGGCG	330 bp	56°C
β-actin	CTGGTATCGTGATGGACTCT	AGCTCATAGCTCTTCTCCAG	280 bp	56°C

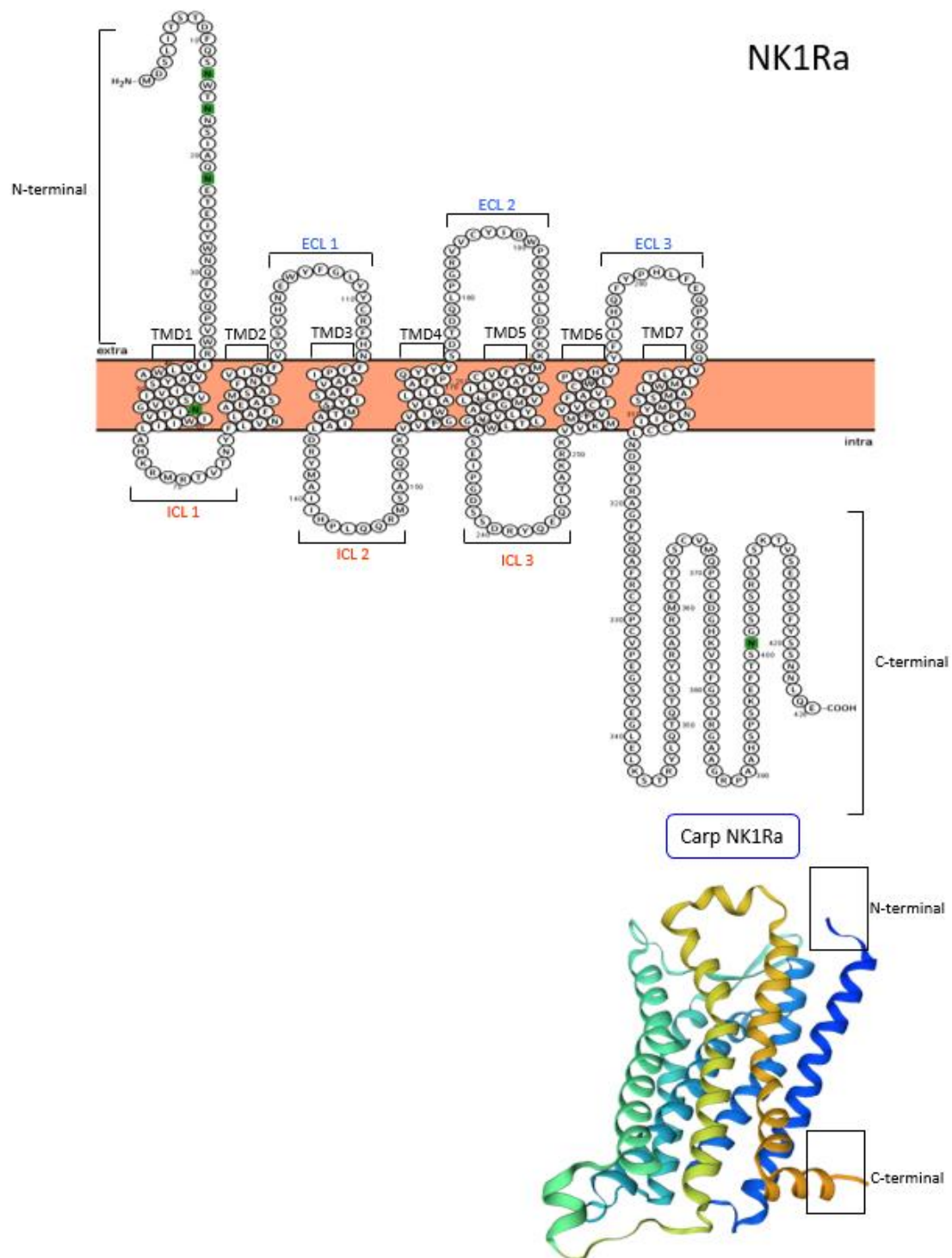
Supplementary Table S2. Primer sequences and PCR conditions for real-time PCR for selected gene targets in grass carp

Gene	Forward primer	Reverse primer	Product size (bp)	Annealing T _m (°C)
UTS1	CTGACCACCCACATCCCCCT	AGTTCGCGCTGTCCCTTTG	250 bp	59°C
CART2	CGCTGCTGCTCTGCTTGT	GCGGACAATCGCACATCT	280 bp	58°C
NMB1	AGCCTTGACTTGACTGAACTAA	CGGAACTTTAGGGCGGAT	220 bp	53°C
PRL	CTCAGCACCTCTCTCACCAATGA	GCGGAAGCAGGACAACAGAAAATG	400bp	60°C
SL α	ACCCACTGTACTTCAATCTCC	CGTCGTAACGATCAAGAGTAG	283 bp	58°C
β -actin	CTGGTATCGTGATGGACTCT	AGCTCATAGCTCTTCTCCAG	280 bp	56°C



Supplemental Figure S1. The speculative evolutionary process of several rounds of genome replication of tachykinin family. Peptides and genes are displayed by colored labels. The path of evolution is marked by arrows.

[illegible]



Supplemental Figure S3. Sequence analysis of grass carp NK1Ra. (A) Snake diagram of grass carp NK1Ra with Protter program. The seven transmembrane domains, three intracellular domains and three extracellular domains are labeled as TMD 1-7, ICL1-3 and ECL1-3, respectively. Sequence identities of transmembrane domains and extracellular domains, intracellular domains, N-termini and C-termini between grass carp and other vertebrates were showed in the Table. **(B)** 3-D protein model of grass carp NK1Ra using SWISS-MODEL program. The location of N-terminal and C-terminal were highlighted, respectively. The amino acids with hydrophobic side chains are colored blue, while those with hydrophilic side chains are colored red.

NK1Rb

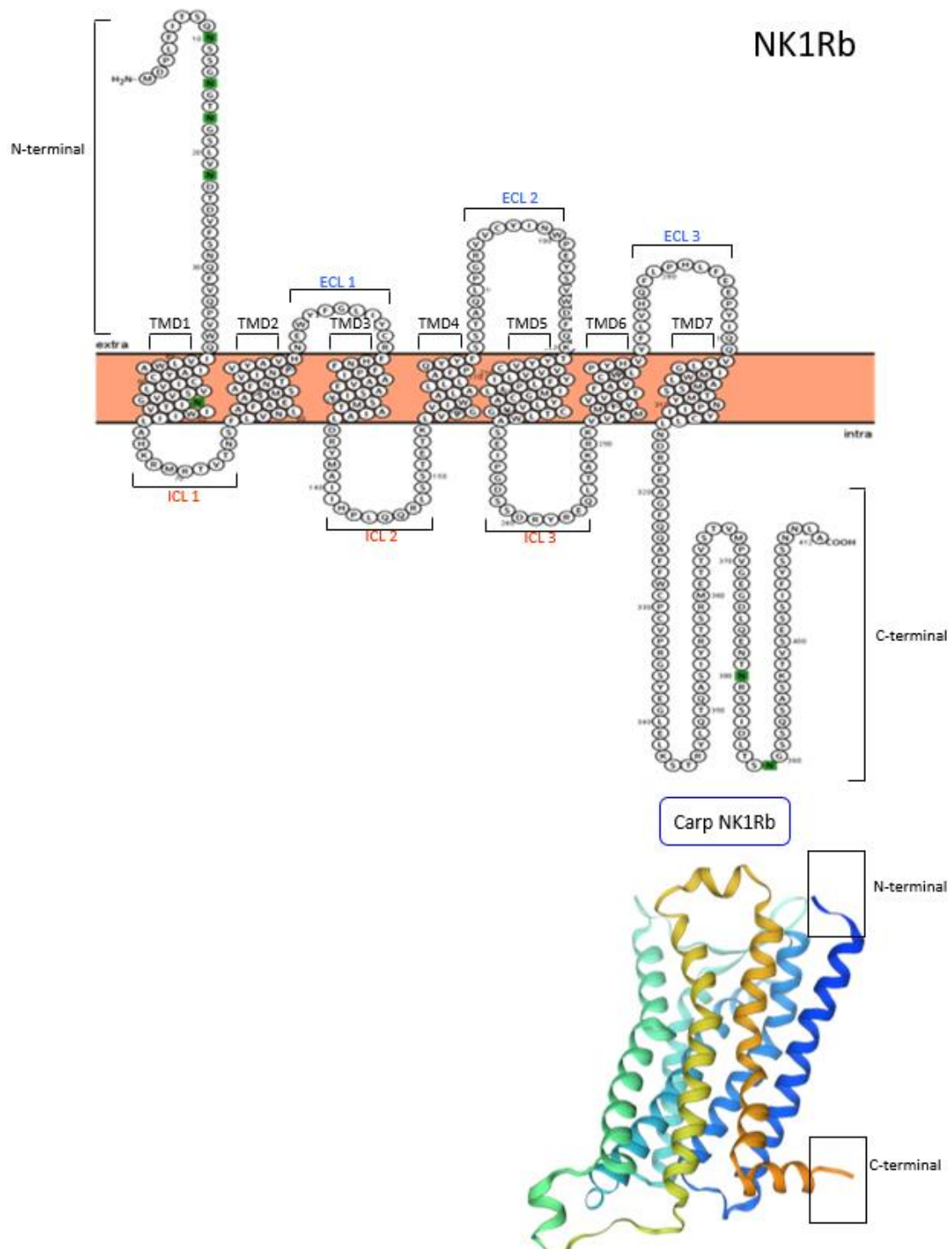
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   WAIAYCCIVLVSVVGNITVIWIILAHKRMRTVTYNSFLVNL 80
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   AVFASISYMTAIALDRYMAIIHPLQQRLSSTRTKLVVGV 160
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   WALALLLAFPPQYFSSSTAQLPGRVVCYINWPYYSVWDFQK 200
601 ACGTATTACGCTCTGGTGGTGTGCTTATTACTTTTCACTTGTCTCATCGGTTGTGCTTACCTGGTGGTAGGATGCACTCTCTGGGCCAGTGAGATACTGGAGACTCTTTCAGAC
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721 CGCTATCGAGACAGCTAACTGCCAAACGGAAGGTGGTGAATGATGATCGTTGTGTGTGCACCTTCGCGCTCTGCTGGCTGCCCTACCATGTCTACTTCTCTGGTGCACCACTTCTCT
   RYREQLTAKRKVVVMMIVVCTFAVCWLPYHVYFLVHQFL 280
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   FQQQAFFWCPCVPRGSEGLELKLSTRYLQTQASIRYTRM 360
1081 GAGACCACAGTATCCACAGTCATGCGGTTGGGAGGGGGACCTTCAGGAGAACACAAATGAAGTTCTATTGATCTCACTTCCAATGGTCTTCACAAAGCGCTCAAGAGCTGTATCA
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1239 GAGTCTCCATCTTCTATTCTAGTAACAACCTTGCAAA
   ESSIFYSSNNLA* 413

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<i>Ctenopharyngodon idella</i>	MDPLPITISQNSSG-NGTNGSLVNDIDVFSNQFVQPVVQIVLWAIAYCCIV
<i>Danio rerio</i>	MDPLYITSPNSSADNGSAGSPVNDTEVFGNQFVQPVVQIVLWAIAYCTIV
<i>Oryzias latipes</i>	MDPLG-----NGSDGTG-ACVNCSS--NQFVQPAWVQVGLWGAAYCGLIV
<i>Chelmon rostratus</i>	MDPLYDAF-DFNGTINWIN-APVNCSDGF-NQFVQPVVQIITLWAVAYCSIV
<i>Ctenopharyngodon idella</i>	LVSVVGNITVIWIILAHKRMRTVTNSFLVNLAFAEASMSAFNTVINVFVYA
<i>Danio rerio</i>	LVSVVGNITVIWIILAHKRMRTVTNYFLVNLAFAEASMSAFNTVINFLYA
<i>Oryzias latipes</i>	AVSVLGNLAIVWIILAHKRMRTVTNYFLVNLAFAEAAAMSAFNTVINFTYA
<i>Chelmon rostratus</i>	AVSVVGNMVIWIILAHKRMRTVTNYFLVNLAFAEACMSAFNTVINFLYA
<i>Ctenopharyngodon idella</i>	VHNEWYFGLIYCRFHNFFPIAAVFAISYMTAIALDRYMAIIHPLQQRLS
<i>Danio rerio</i>	VHNEWYFGLVYCRFHNFFPIAAVFAISYMTAIALDRYMAIIHPLQQRLS
<i>Oryzias latipes</i>	VHNEWYFGLAVYCRFHNFFPIAAIFASISYMTAIALDRYMAIIHPLKQRLS
<i>Chelmon rostratus</i>	VHNEWYFGLVYCRFHNFFPIAAIFASISYMTAIALDRYMAIIHPLKQRLE
<i>Ctenopharyngodon idella</i>	STETKLVVGIWALALLLAFPPQYFSSSTAQLPGRVVCYINWPYYSVWDFQ
<i>Danio rerio</i>	SAETKLVVCVIWALALLLAFPPQYFSSSTAQLPGRVVCYINWPYYSVWDFQ
<i>Oryzias latipes</i>	STETRVIGLIIWVLLALLLAFPPQYFSSSTTLPLGRITVCYIDWPEYATVDFR
<i>Chelmon rostratus</i>	STETRVVIGVIWMLALLLAFPPQYYSSTAPLPLGRITVCYIDWPEYITVDFE
<i>Ctenopharyngodon idella</i>	KTYVVCVVLIIYFLPLLIMGCAYLVVGCITLWASEIPGDSSEHYRQQLIAK
<i>Danio rerio</i>	KTYVVCVVLIIYFLPLLVMGCAYLVVGCFLWASEIPGDSSEHYRQQLIAK
<i>Oryzias latipes</i>	KIYVVSVALLIYFLPLCIMGWAYTIVAVTLWASKIPGDSSEHYRQQLIAK
<i>Chelmon rostratus</i>	KIYHVCVTLLIIYFLPLCIMGWAYITVGIITLWASEIPGDSSEHYKEQLIAK
<i>Ctenopharyngodon idella</i>	RKVVKMMIVVVCCTFAVCWLPYHVYFLVHQFLPHLFEEPIYIQQVYLCIMWL
<i>Danio rerio</i>	RKVVKMMIVVVCCTFAVCWLPYHVYFLVHQFLPHLFEEPIYIQQVYLCIMWL
<i>Oryzias latipes</i>	RKVVKMMIVVVCCTFAVCWLPYHVYFLVHQFFPELFEQRYIQQVYLAIMWL
<i>Chelmon rostratus</i>	RKVVKMMIVVVCCTFAVCWLPYHLYVFLVHQFFPELFEQRYIQQVYLAIMWL
<i>Ctenopharyngodon idella</i>	AMSSSTMYNPIIYCLLNDRFRAFGQQAFSWCPCVPRGSEGLELKLSTRYLQ
<i>Danio rerio</i>	AMSSSTMYNPIIYCLLNDRFRAFGQQAFSWCPCVPRGSEGLELKLSTRYLQ
<i>Oryzias latipes</i>	AMSSSTMYNPIIYCLNSRFRAFGQQVF-CCCSSSAAKEEELKSPRCCLQ
<i>Chelmon rostratus</i>	AMSSSTMYNPIIYCLNSRFRAFGQQVF-CCCAPIVAAKEEELKSRRYLH
<i>Ctenopharyngodon idella</i>	TQASIRASRMETTVSTVMPVGEGLQENINRSSIDLTSNGSSQS--ASK
<i>Danio rerio</i>	TQASIRASRMETTVSTVMPVGDGELPENPNRSSVDLTSNGSSRS--ASK
<i>Oryzias latipes</i>	TQVGPRTAFPPASAAQAGGE-----RPDETSTALSSC-----
<i>Chelmon rostratus</i>	TQVSIYRASRAETVSTAVHPA-----APEPKRAELPSCGPPGQPRAGV
<i>Ctenopharyngodon idella</i>	TVSESSI FYSSNNLA--
<i>Danio rerio</i>	TVSESSI FYSSNNVA--
<i>Oryzias latipes</i>	-----
<i>Chelmon rostratus</i>	TSNGSDTFTRSPDSPAQ

Supplemental Figure S4. Molecular cloning and sequence alignment of grass carp NK1Rb. (A) Nucleotide and deduced amino acid sequences of grass carp NK1Rb. Numbering of the deduced amino acid sequences begins with the first methionine of the ORF to the right of each line. Nucleotide numbers are to the left of each line. **(B)** Protein sequence alignment of grass carp NK1Ra with that of other vertebrates using Clustal-W algorithm with MacVector program. The conserved a.a. residues are boxed in grey.



Supplemental Figure S5. Sequence analysis of grass carp NK1Rb. (A) Snake diagram of grass carp NK1Rb with Protter program. The seven transmembrane domains, three intracellular domains and three extracellular domains are labeled as TMD 1-7, ICL1-3 and ECL1-3, respectively. Sequence identities of transmembrane domains and extracellular domains, intracellular domains, N-termini and C-termini between grass carp and other vertebrates were showed in the Table. **(B)** 3-D protein model of grass carp NK1Rb using SWISS-MODEL program. The location of N-terminal and C-terminal were highlighted, respectively. The amino acids with hydrophobic side chains are colored blue, while those with hydrophilic side chains are colored red.

NK2R

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1  ATGGATATCATCTTTGGACCCCTTTCAACATCTATTCTTTACGACGATGAAGACGGAAACGAAACCTCCACCAATCTGTTGGAGCAGCGGACTGGCAGGTGGCATTATGGGCGATTGCA
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121 TACTCTTTGATCGTAATTGTGTCCATGTCAGTGTATTGTTGATAATCTGGCAGATAAAGCATGAGGACAGTGACCAACTACTTCAATTGTAACCTGGCTTTTCTGAT
   YSLIVIVSIICNVTVIWIILAHKRMRTVTNIFYIVNLAFS 80
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   ASMATFNTVFNFFVYALHNDWYFGLGYCKFQNFPPITAMFS 120
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   FSLAFPQCYYASTKFYFPRITVCMVEWVDPDDYGGCKHQLSYQI 200
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721 CAGATGCAAGGCGAAAGGTTGTGAAGATGATAGTGGTGGTGAACCTTTGCAATATGTTGGCTGCCCTACCATCTACTTCACTTCTGGGAGCTTCAACAGGAGCATATAC
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961 GCATTCCAGTGGTGTCCCTCATCAAGATTCTGAGGAGGACACATGGAACCTGCAGCATGAGGACCTTTACATGAGAGCGAGTTATGCAACGAGACCCGGGTGGTGGTCCGA
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1131 AATCATGCCAACGAAACGAGACACCATCCAACTAATCAAGCCTGA
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Ctenopharyngodon idella MDITLDPLSLSTSLYDDEDEGNETSINLFEQPD
Danio rerio MDITLDPLSSSLLYYDEDEGNETSINLFEQPD
Takifugu rubripes MNKDKSMDAVSLIQHVERDMEATSFPCSVTADWLEEDGNETIWNQFQQPD
Oryzias latipes -----

Ctenopharyngodon idella WQVALWAIAYSLIVIVSIICNVTVIWIILAHKRMRTVTNIFYIVNLAFSDA
Danio rerio WQVALWAIAYSLIVIVSIICNVTVIWIILAHKRMRTVTNIFYIVNLAFSDA
Takifugu rubripes WQVALWAIAYSLIIVLSITGNITVIWIILAHKRMRTVTNIFYIVNLAFSDV
Oryzias latipes -----MRTVTNIFYIVNLAFSDV

Ctenopharyngodon idella SMATFNTVFNFFVYALHNDWYFGLGYCKFQNFPPITAMFSSSIYSMAAIAVD
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Takifugu rubripes SMAFNTLNFNFVYALHNDWYFGLGYCRFQNFPPITAMFSSSIYSMAAIAVD
Oryzias latipes SMATFNTLNFNFVYALHNDWYFGLGYCRFQNFPPITAMFSSSIYSMAAISVD

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Danio rerio RYMAIHPKRLKRLSSITTKVLIGVIVTVAFLAFPPQCYYASTKFYFPRITV
Takifugu rubripes RYMAIHPKRLKRLSSITTKVVIALLIWWVAVCLAFPPQCYYSVTRFYFPRITV
Oryzias latipes RYMAIHPKRLKRLSSITTKVVIALLIWWVAFSLAFPPQCYYSVTRFYFPRITV

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Danio rerio CMVEWVDPDDYGGCKHQLSYQIAVILIIYLLPLLVLMLVTYSLVGQRLWVGSEIP
Takifugu rubripes CMVDWPCDYGGCKHQLSYQIAVILIIYLLPLLVLMLVTYSIVGRSLWGGHIP
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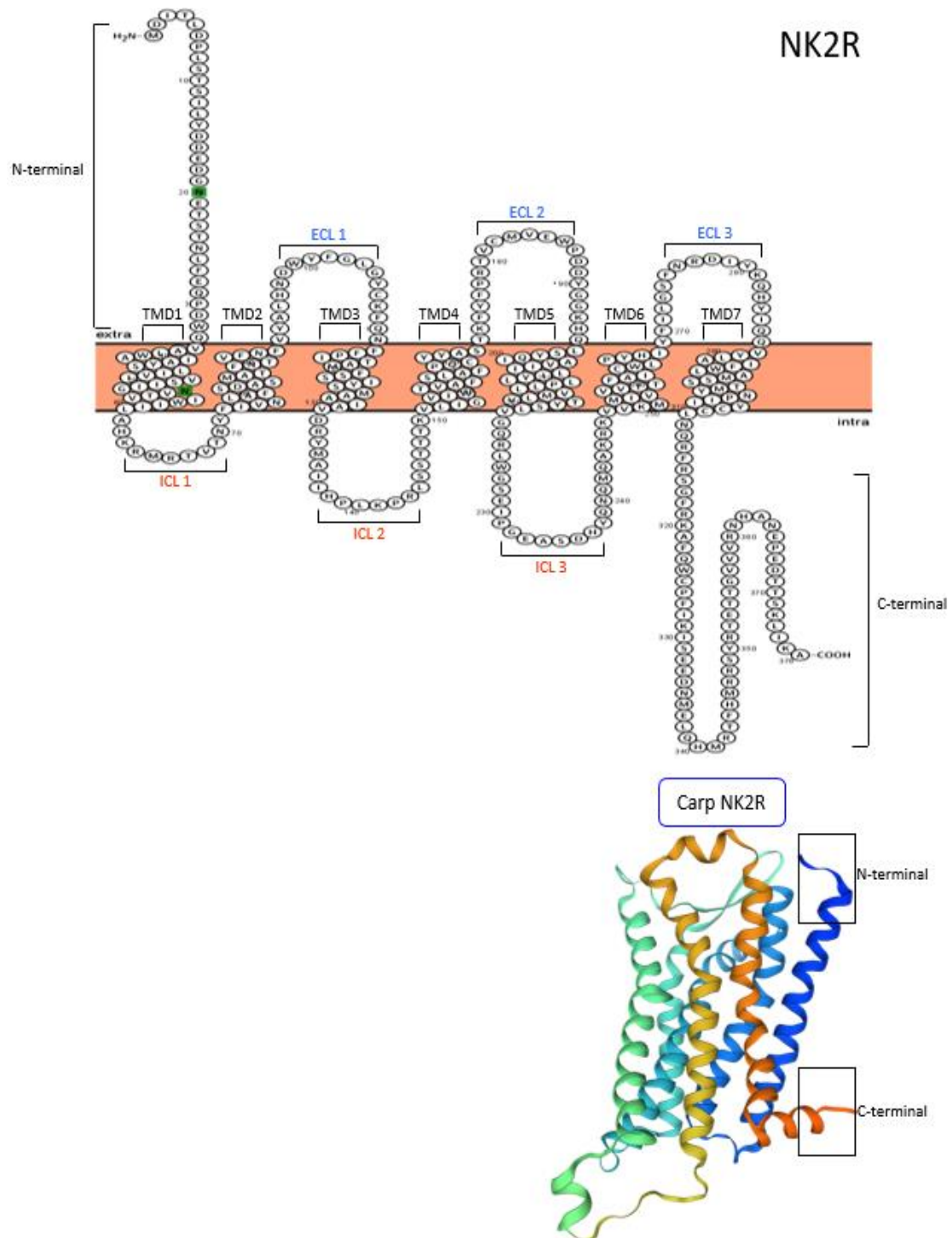
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Takifugu rubripes GEATDHYHNQITAKRKVVKMMIVVVTFALCWLPHYHYFILGSFNNDIYK
Oryzias latipes GEATDHYHSLQITSKRKVVKMMIVVVTFALCWLPHYHYFILGFNNDIYK

Ctenopharyngodon idella QHYIQQVYLAIFWLAMSSTMYNPIIYCCLNQRFRSGFRKAFQWCPFIKIS
Danio rerio QHYIQQVYLAIFWLAMSSTMYNPIIYCCLNQRFRSGFRKAFQWCPFIKIS
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Oryzias latipes QHYIQQVYLAIFWLAMSSTMYNPIIYCCLNQRFRAGFRHAFWCPFIKVS

Ctenopharyngodon idella EEDNMELQHMRTF--HMRRSYRTETTCVVVRNHANEPEDT-----
Danio rerio EEDNMELQHMRTF--HMRRSYRTETTSVVVRNHANEPEDT-----
Takifugu rubripes EEDKMEHQHTHTFRVIMTRSHRRESAQIKINHTCDANPPINMERDVK---
Oryzias latipes EEDKMEHQHTHTFRVIMTRSHRSDTHPRASIKTNIEKDSGLQRNKKQKCS

Ctenopharyngodon idella --TSKLIIKA-----
Danio rerio --TSKLIIKA-----
Takifugu rubripes AHGAHKVKKRQDGMVSSSIRLVQYTH
Oryzias latipes TQDAQLVKKLSDKRPAHDKLAEHRL

Supplemental Figure S6. Molecular cloning and sequence alignment of grass carp NK2R. (A) Nucleotide and deduced amino acid sequences of grass carp NK2R. Numbering of the deduced amino acid sequences begins with the first methionine of the ORF to the right of each line. Nucleotide numbers are to the left of each line. **(B)** Protein sequence alignment of grass carp NK1Ra with that of other vertebrates using Clustal-W algorithm with MacVector program. The conserved a.a. residues are boxed in grey.



Supplemental Figure S7. Sequence analysis of grass carp NK2R. (A) Snake diagram of grass carp NK2R with Protter program. The seven transmembrane domains, three intracellular domains and three extracellular domains are labeled as TMD 1-7, ICL1-3 and ECL1-3, respectively. Sequence identities of transmembrane domains and extracellular domains, intracellular domains, N-termini and C-termini between grass carp and other vertebrates were showed in the Table. **(B)** 3-D protein model of grass carp NK2R using SWISS-MODEL program. The location of N-terminal and C-terminal were highlighted, respectively. The amino acids with hydrophobic side chains are colored blue, while those with hydrophilic side chains are colored red.

NK3Ra1

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1  ATGGCACCGCCACAAAACGGATCTAACCTAACGGGGAACCTTTACAAACAGTTGTCGAGCCACCGTGGGCGGTGGGCTCTGGTCTGTGGCGGTACAGCTCCATCTAGOGATCGGGTG
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   F G N L I V M W I I L A H K R M R T V I N Y F L L N L A F S D A S M A A P N T L 80
241 A T T A A T T T T G T T A T G C A C G C A C G G T G A T T G G T A T T T G G A G A A G O C T A C T G C A A A T T T C A C A A C T T T T T C C C G G T C A C C T C C G T G T T T G C G A G C A T T A C T C A T G A G C G C A A T A G C A
   I N F V Y A T H C D W Y F G E A Y C K F H N F F P V T S V F A S I Y S M S A I A 120
361 G T O G A C A G G T A C A T G G C A T C A T C A T C O C C T G A A A C C A C G A C T C T C A G O C A C G C A C T A A G G T A T C A T T G T G T A T T T G G T G C T G G C T G T G G T T T T G G C C T T T C A C T G T G T T C
   V D R Y M A I I H P L K P R L S A T A T K V I I V C I W V L A V V L A F P L C F 160
481 T T T T C A A C C A T C A A A A A T T A C C A A G C G T A C C C T C T G C T A G C T G C C T G G C G A G A C C T T C A G A G G A C C C T T C A T G T A T C A T A T C A T G T G G C A A T G C T G G T G T A T G T T C T G C C T C T G
   F S T I K K L P K R T L C Y V A W P R P S E D P F M Y H I I V A M L V Y V L P L 200
601 G T G G T C A T G G G C A T T A A C T A C A C T A T T G T G G G T T G A C A C T T T G G G G A G G A G A T T C C T G G T G A C T C A T C A G A C A C T A T C A G G G T C A A C T G C G A G C A A A A A A A A G G T G G T G A A A T G
   V V M G I N Y T I V G L T L W C G E I P G D S S D N Y Q C Q L R A K R K V V K M 240
721 A T G A T C A T G T A G T G G T G A C C T T T G C C A T C T G C T G C C T G C C G T A C C A T G T A T T T C T G C C T A C G G A T T G A A C A A G C A A C T G G T A G A T G G A A G T T C A T T C A G C A G A T C T A T T T T C A
   M I I V V V T F A I C W L P Y H V Y F L A T G L N K Q L V R W K F I Q Q I Y L S 280
841 G T C A T G G C T T G C C A T G A G C T C C A C C A T G T A C A A C C C A T T A T T A C T G C C T G C C T G A C A G C A G G T T T G G G C G G C T T T A A C G G G T T T C C G C T G G T G C C C Q T T T G T G A G G G T G C T
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961 G A C T A C G A C G A G C T T G A G C T G C G G G C C A T G C G G C A T A A A G T A G C G C G T C A G A C A G C A T G T A C A C G C T C T C A C G A A T G G A G A C C A C G T T G C G C T G T G T G A C C C C T C A G A G C C A A C C
   D Y D E L E L R A M R H K V A R Q S S M Y T L S R M E T T V V A V C D P S E P T A H P G R K S L H N 360
1194 G O C C A C C A G G C G G A A G A G C C T T C A C A A C C A C G C A A A C C G T G C T C A A C C O G G C A A G A G C A A A G T A A C G T A T A T G C A A A G A C C O G A A G G A G G A A T T C T G T T G A
   A H P C R K S L H N H Q H N G C S N P A K S K E V T Y M Q S D P K E E F C 398

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Ctenopharyngodon idella -----MAPPQNGSNLIGNFT-----NQFVQPP
Danio rerio -----MDTITLDPLSSSLLVYDEEDGNETSINLFEQPD
Takifugu rubripes MNKDKSMDAVSLIQHVERDMEATISFPGSVIADWLEEDGNETITWNQFQQPD
Oryzias latipes -----

Ctenopharyngodon idella WRVALWSVAYSSTILAIIVFNGNLIWMWILAHKRMRTVINYFLLNLAFSDA
Danio rerio WQVALWAIAYTLIVILSFIGNVTVIWIILAHKRMRTVINYFIVNLAFSDA
Takifugu rubripes WQVALWAIAYSLLILVSLITGNITVIWIILAHRRMRTVINYFIVNLAFSDV
Oryzias latipes -----MRTVINYFIVNLAFSDV

Ctenopharyngodon idella SMAIFNTLINFVYATHGDWYFGEAYCKFHNFPPVTSVFASISYMSAIAVD
Danio rerio SMATFNTVFNFVYALHNDWYFGLGYCKFQNFPPITAMFSSSIYSMAAIAVD
Takifugu rubripes SMAIFNTLNFNFFVYALHNDWYFGLGYCRFQNFPPITAMFSSSIYSMAAIAVD
Oryzias latipes SMATFNTLNFNFFVYALHNDWYFGLGYCRFQNFPPITAMFSSSIYSMAAIAVD

Ctenopharyngodon idella RYMAIIHPLKPRLSATATKVIIVCIWVLAIVLAFPLCFFSTIKKLPKRTL
Danio rerio RYMAIIHPLKPRLSSTITKLLIGVIWIVAFSLAFPCYYASTKFYFPRIV
Takifugu rubripes RYMAIIHPLKPRFSSTITKVVIALLIWWVAVCLAFAPQCYYSVTRFYFPRIV
Oryzias latipes RYMAIIHPLKPRLSSTITKVVIALLIWWFAFSLAFPCYYSVTRFYFPRIV

Ctenopharyngodon idella CYVAVWPRPS--EDPFMYHIIVAMLVYVLPVVMGINVTIIVGLTLWGGEIF
Danio rerio CMVFWPDDYGGCKHQLSYQIAVITLIYLLPLLVMVLTYSVLVGRSLWGCSEIP
Takifugu rubripes CMVDWPCDYGGCKHQLSYQVALILLIYLLPLLVMVLTYSIVGRSLWGGHIP
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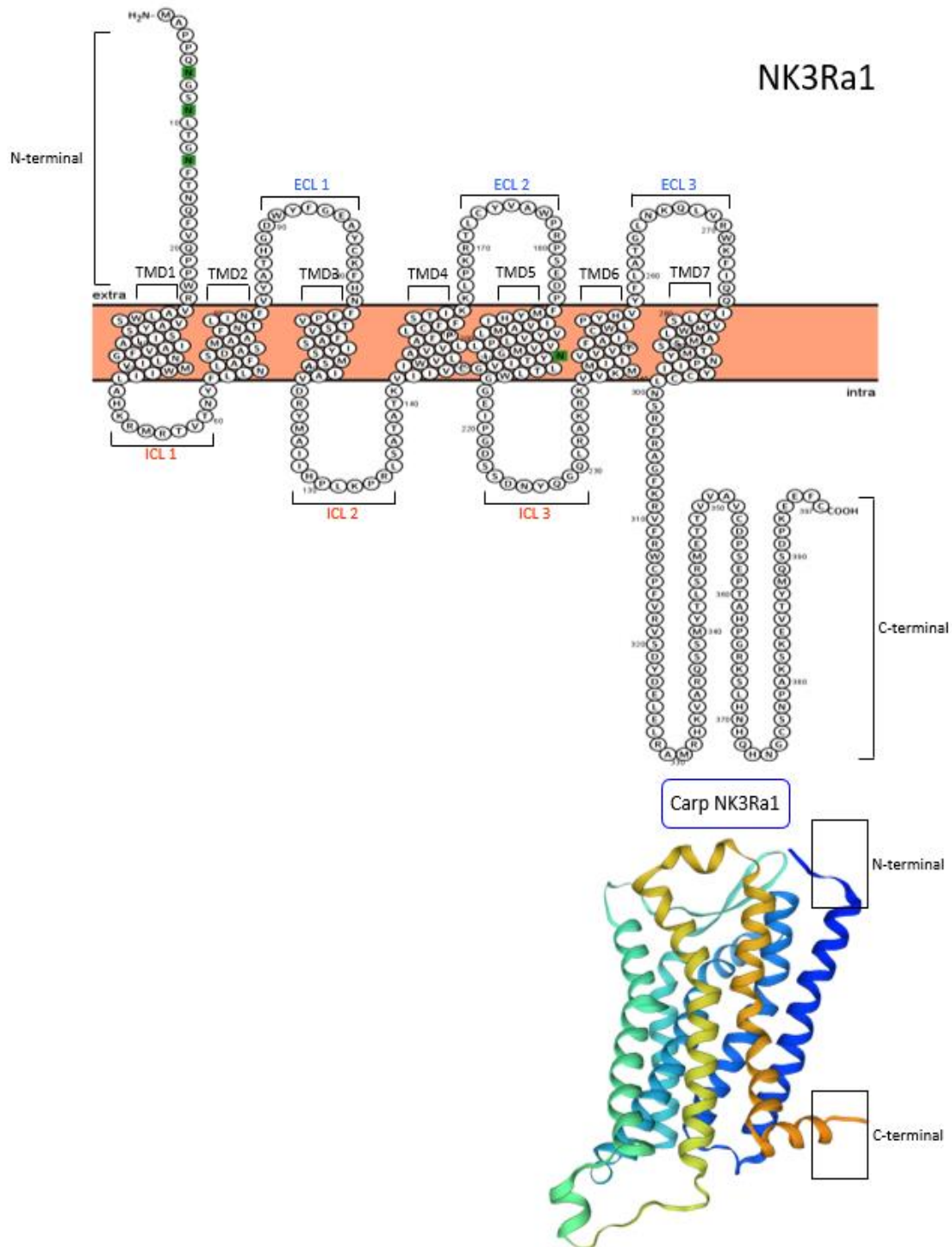
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Takifugu rubripes GEATDHYHNQITAKRRKVVVKMMIIVVVTIFALCWLPHYVYFILGSFNIDYK
Oryzias latipes GEATDHYHSQITISKRKVVVKMMVVVVVTFALCWLPHYVYFILGTFNIDYK

Ctenopharyngodon idella WKFIQQIYLSVMWLAMSSSTMYNPIIYCCLNSRFRAGFRKRVFRWCPFVRVS
Danio rerio QHYIQQVYLAIFWLAMSSSTMYNPIIYCCLNQRFRRSGFRKAFQWCPFVKIS
Takifugu rubripes QHYIQQVYLAIFWLAMSSSTMYNPIIYCCLNQRFRRAGFRHAFWCPFIKVS
Oryzias latipes QHYIQQVYLAIFWLAMSSSTMYNPIIYCCLNQRFRRAGFRHAFWCPFIKVS

Ctenopharyngodon idella DYDELELRAMRHKVARQS SMYTL SRMETTVVAVCDPSEPTAHPGRKSLHN
Danio rerio EEDNMEL---QHMRITF--HMRSSYRTETISVVVRNHANEPEDT-----
Takifugu rubripes EEDKMEL---QHTHTFRVTMTRSHRRESAQTKINHTCDANPPTNMERDVK
Oryzias latipes EEDKMEL---QHTNIFRVITMTRSHRSDTHPRASIKTNNIEKDSGLQRNKQ

Ctenopharyngodon idella HQHNGCSNPAKSKKEVTYMQSDPKIEFC-
Danio rerio -----TSKLIA-----
Takifugu rubripes ---AHGAHKVKRODGMVSSSIIRLVQYTH
Oryzias latipes KCSTQDAQLVKKLSDKRPAHDKLAERL

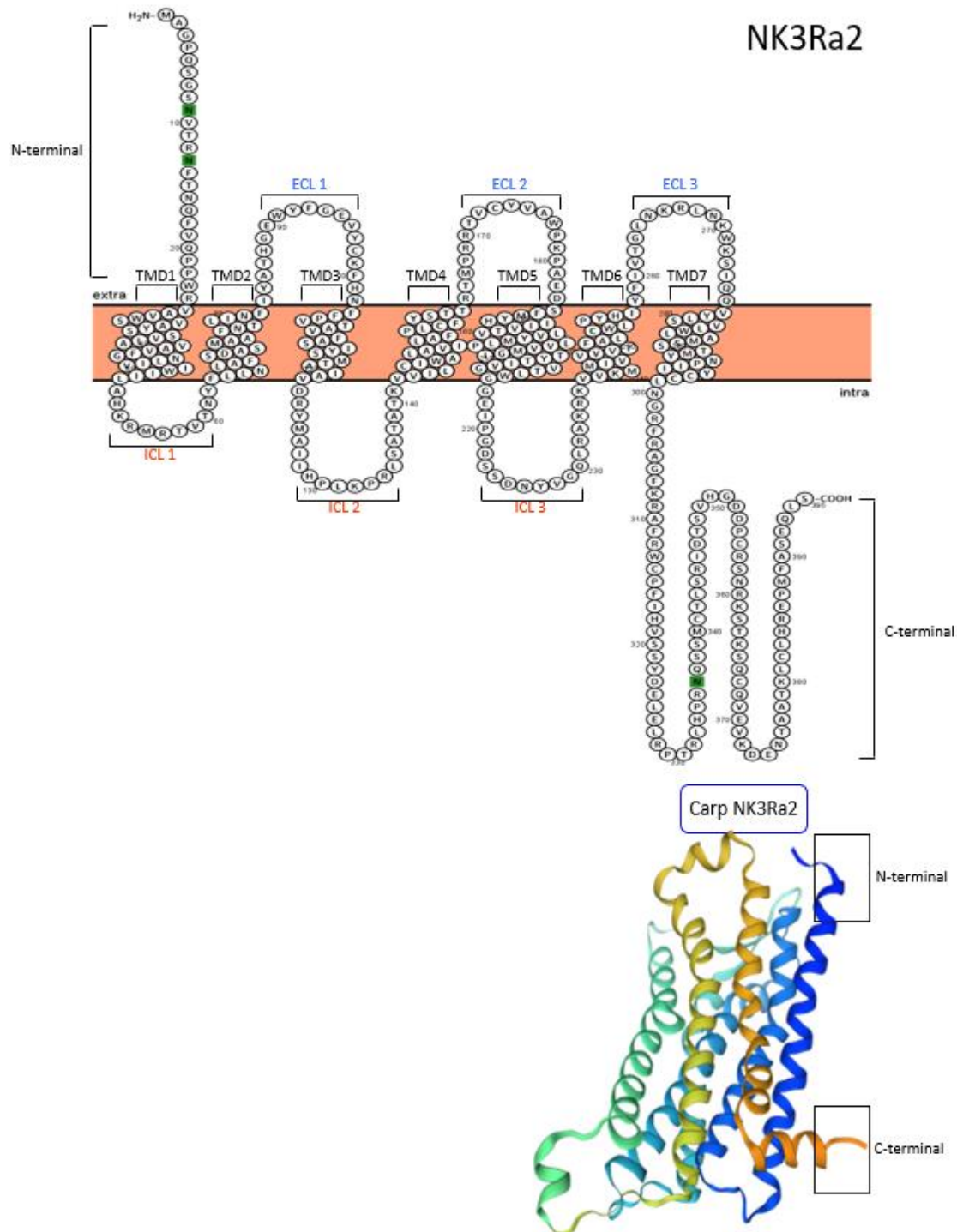
Supplemental Figure S8. Molecular cloning and sequence alignment of grass carp NK3Ra1. (A) Nucleotide and deduced amino acid sequences of grass carp NK3Ra1. Numbering of the deduced amino acid sequences begins with the first methionine of the ORF to the right of each line. Nucleotide numbers are to the left of each line. **(B)** Protein sequence alignment of grass carp NK3Ra1 with that of other vertebrates using Clustal-W algorithm with MacVector program. The conserved a.a. residues are boxed in grey.



Supplemental Figure S9. Sequence analysis of grass carp NK3Ra1. (A) Snake diagram of grass carp NK3Ra1 with Protter program. The seven transmembrane domains, three intracellular domains and three extracellular domains are labeled as TMD 1-7, ICL1-3 and ECL1-3, respectively. Sequence identities of transmembrane domains and extracellular domains, intracellular domains, N-termini and C-termini between grass carp and other vertebrates were showed in the Table. **(B)** 3-D protein model of grass carp NK3Ra1 using SWISS-MODEL program. The location of N-terminal and C-terminal were highlighted, respectively. The amino acids with hydrophobic side chains are colored blue, while those with hydrophilic side chains are colored red.

1	ATGGCTGGTCTCAGAGTGGCTCAAAAGTAACGGTAATTTCACGAATCAGTTCTGTCGACGCCGCGTGGCGGTGTCGGCGTCTGGTGGTTCGGGTACAGCTCCGTGCTCGCGGTTGCCGTG M A G P Q S G S N T T R N F I N Q F V Q P P V R V A V S V A Y S S V L A V A V	40
121	TTCGGAAACCTCATCGTATTATGGATAAATTTGGCTCATAAACGGATGCGCAACCGTTACCAATTATTTCTGCTCAACCTGGCGTCTCCGAGCGCTCGATGGCGGCTTCAACACGCTC F G N L I V I V I L L A H K R M R T V T N Y F L N L A F S D A S M A A F N T L	80
241	ATCAACTTCATTATCGCGACGACGCGAGGATGGTACTTTGGAGAGGTCTACTGCAAGTTCACAGACTCTCTTCCCGTGACCGCGCGTGTATGCCACGATTACTCCATGACACGGCATGCA I N F I Y A T A H G E W Y F G E Y C K F N H P P V T A V F A S I Y S M T A I A	120
361	GTGCACAGGTACATGGCCATTATTCACCTCTTGAAGCCCTGTGTCGACGCCAGCTACTAAAGTGGTGAATCTGTGTAATTTGGCGCTTGGCGGTGAATTTGGGCTTCCGCGTGTGTTTC V D R Y M A I I H P L K P R L S A T A K V V I L C I V A L A V I L A F P L C F	160
481	TACTCTACCCAGAGAACTATGCTTCGCAACGCTCTGCTACGTTTGCTGGCGGAAACCTCGTGAGGATTCAITCATATCATAGTAACGGTGTGCTGATGCTACATGCTGCCCTTA Y S T R T R I M P R R T V G C Y V A W P K P A E D S F M Y H I I V T V L V Y M L P L	200
601	GTGGTGATGGGTATCACTACACATATAGTCGGGCTGACACTTTGGGAGGAGAGATTCCTGGAGATTATCGGCAATATGTTGGACAACTGGTGCTAAAAGGAAGGGTGTGAAGATG V V M G C I T Y T I V G V T L W C G E I R P C D S S N D Y V G A L R A K R K V V K M	240
721	ATGATTGTGGTGGTGTACTTTGGCCCTCTGCTGGTGGTCCATATTTTCATGTGACGGGCTAAACACAGCGCTTGAACAAGTGGGAAGTCCATCCAGCAGGCTATCTGTCT M I V V V V T F A L C W L P Y H I Y F I V T G L N K R L N K W K S I Q V Y L S	280
841	GTGTTGGTGGTGGCGATCCACATGTACACACCCCATTAATTTACTGCTGTCTGAATGGCAGGTTTAGGGCGCGGTTCAAAAGGGCCCTTCAGGTGGTGTCCCTTCATCATGTATCA V L W L A M S T T M Y N P I I Y C C L N G R F R A G C F K R A F W C P F I H V S	320
961	AGCTACGATGAGCTAGAGCTCCGCTCCACCGTCTCCATCCAGCTAAACAGCAGCATGTGTCACCGCTGTCGGGATCGACACAGCGGTTCACGGGATGACCCCTTCGCGCAGCACCGA S Y D E L E L R G T P T R L L H P R N Q G S S M C T L S R I D T S V H G D D P C R S N R	360
1188	AAGAGCAOCCAAAGTCCCAAGTGCAGGTGCAAGAGCAAGCAACCGCAGCTACTAAACTCTGTCTTCCACAGAGGCCAATGTTTGGCAGGAGCAGCTCAGCTGA K S T K S O C O V E K D E N T A A T K L C L H R R P M F A S E O L S *	396

Supplemental Figure S10. Molecular cloning and sequence alignment of grass carp NK3Ra2. (A) Nucleotide and deduced amino acid sequences of grass carp NK3Ra2. Numbering of the deduced amino acid sequences begins with the first methionine of the ORF to the right of each line. Nucleotide numbers are to the left of each line. **(B)** Protein sequence alignment of grass carp NK1Ra with that of other vertebrates using Clustal-W algorithm with MacVector program. The conserved a.a. residues are boxed in grey.



Supplemental Figure S11. Sequence analysis of grass carp NK3Ra2. (A) Snake diagram of grass carp NK3Ra2 with Protter program. The seven transmembrane domains, three intracellular domains and three extracellular domains are labeled as TMD 1-7, ICL1-3 and ECL1-3, respectively. Sequence identities of transmembrane domains and extracellular domains, intracellular domains, N-termini and C-termini between grass carp and other vertebrates were showed in the Table. **(B)** 3-D protein model of grass carp NK3Ra2 using SWISS-MODEL program. The location of N-terminal and C-terminal were highlighted, respectively. The amino acids with hydrophobic side chains are colored blue, while those with hydrophilic side chains are colored red.

NK3Rb

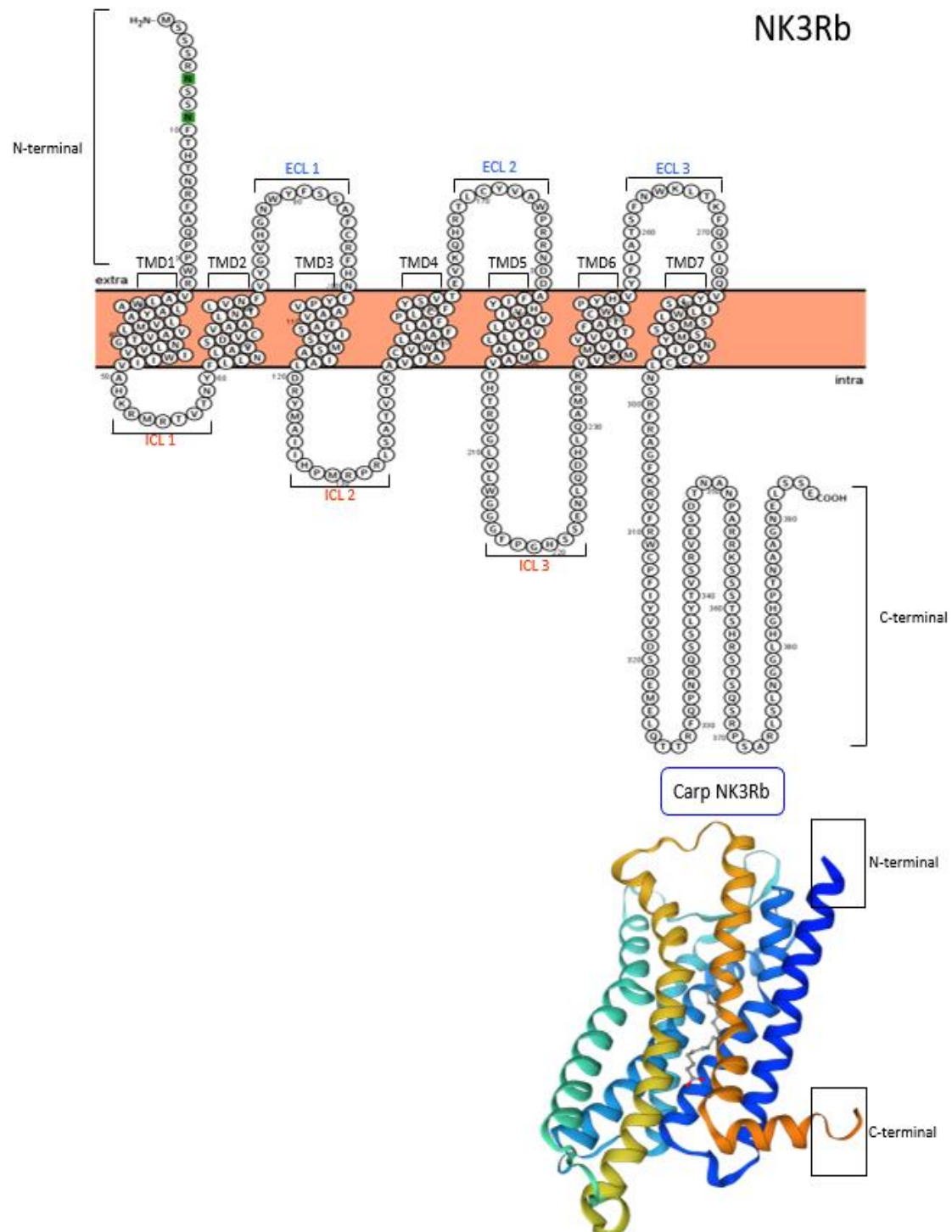
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121 AAOCGTGGTGCATCTGGATCATOGTTGGCACAAGAGATGAGAACCCTCAACCACTCTTCTGCTCAACCTGGGGTGTCTGACGTGTGGTGGCGCGCTCAACAGCTGGTGAAC
  N L V V I V I I V A H K R M R I V V I N Y F L L N L A V S D V C V A A L N T L V N 80
241 TTGCTGACGGGTGACAGGAACTGGTACTTCAGCAGCGCTTCTGCGCTTCCACAACTCTCTACCCGGTGGCGGGTGTTCGGAGCATCTACTCATGAGCGCATGCGCTTAGAC
  F V Y G V H C N W Y F S S A F C R F H N F Y P V A A V F A S I Y S M S A I A L D 120
361 AGGTACATGGGATCATCCACCGATGAGGCGCGCTCTCAGGAGCGGTCAACAAAGCTGTGATGCGATGTGTGGATTCTGGCGCATTTCTGGCTTTCCACTCTGCTTTTACTOC
  R Y M A I I H P M R P R L S A T V T K A V I A C V W I L A A F L A F P L C F Y S 160
481 GTAAACGGAAGTGAAGCAGCAGGAGCTCTGCTATGTGGCTGGCGCGGGCAACGACGAGCGCTTCATATATCACTGATGTGGCGGTGCTGGTATATCTCTGCTCTGGCCTG
  V T E V K Q H R T L C Y V A W P R R N D D A F I Y H V I V A V L V Y L L P L A L 200
601 ATGGCGTCAACACACCGGTTGGGCTGTGCTGTGGGCGGTGGATTCCCGGACACTCTCGGAAAACCTACAGGATCATCTGAGGOCATGAGAAGGTTGTGAAGATGATGGT
  N A V T H T R V G L V L W G C G F P G H S S E N L Q D H L Q A M R R V V K M M V 240
721 ATCTGGTAGTAACATTTGCCATCTGCTGGCTCCCATATCATGTGATTTCATCGGACGAGCTTCAACTGGAAGCTGACAAAATCCAGTGGATCCAGCAGGTGTATCTGCTGATCTG
  I V V V T F A I C W L P Y H V Y F I A T S F N W K L T K F Q S I Q Q V Y L S I L 280
841 TGGCTCTCCATGAGCTCTCTCATGTACAACCCATCATCTGCTGCTCAACAGCAGGTTCGCGCGCGCTTCAAGCGGTGTTTCGCTGGTCTCTCATTTATGTGCTGAGCTCT
  V L S M S S S M Y N P I I Y C C L N S R F R A G F K R V P R W C P P I Y V S D S 320
961 GATGAGATGGAATCCAGACCCAGCTTCCAGCCAAACCCAGAGAGCGCTGTACAGGCTCTCAGGTGTGGAGTCCGACCAACGCCAACCCCGCGCAGCAGCAAGAGCTCAGACCC
  D E M E L Q T T R F Q P N R Q S S L Y T V S R V E S D T N A N P A R R K S S S T 360
1188 AGCCACCGCAGCAGCAGCAATCAAGACCTCTGCGCGGTGTCACTTAATGGTGGTCTCCATGGACACCTACGAACGCCCGCGGTATGAATCACTCAGCTCAGAGTAG
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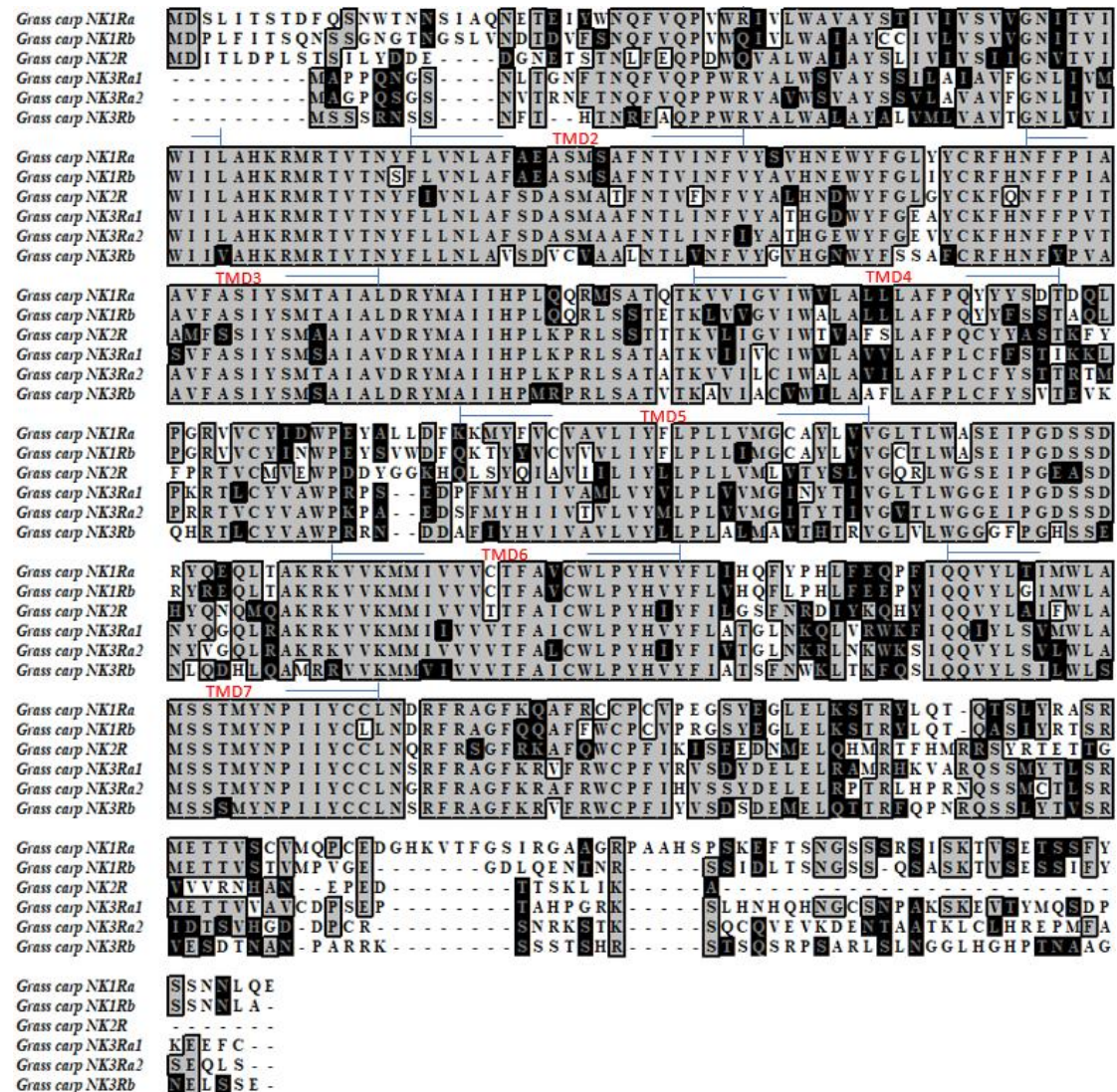
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<i>Danio rerio</i>	MAQSQNGSNLTCTNFTNQFVQPPWRVALWSVAYSSTLATAVFGNLIIVMWI
<i>Oryzias latipes</i>	MGAPNNGSNITISNFTNQFVQPPWRVALWSVAYSLLVLAFAVFGNVIWI
<i>Oreochromis niloticus</i>	MAASYNNGSNITITNHTNQFVQPPWRVALWSVAYSFVLAVAVFGNLIWI
<i>Ctenopharyngodon idella</i>	VAHKRMRTVINYFLNLAVSDVCVAAALNTLVNFVYGVHGNWYFSSAFCRF
<i>Danio rerio</i>	LAHKRMRTVINYFLNLAFSDASMAAFNTLINPVYATHGDNWYFGEAYCKF
<i>Oryzias latipes</i>	LAHKRMRTVINYFLNLAFSDASMAAFNTLINFIYAAHGEWYFGEAYCKF
<i>Oreochromis niloticus</i>	LAHKRMRTVINYFLNLAFSDASMAAFNTSINFIYAAHGEWYFGEAYCKF
<i>Ctenopharyngodon idella</i>	HNFFPVVAAVFASIYSMSAIALDRYMAIIHPMRPRLSATVTKAVIACVWL
<i>Danio rerio</i>	HNFFPVTSVFASIYSMSAIAVDRYMAIIHPLKPRLSATATKVJIVCIWVL
<i>Oryzias latipes</i>	HNFFPVTSVFASIYSMTAIAVDRYMAIIHPLKPRLSAKATTGIIVCIWSL
<i>Oreochromis niloticus</i>	HNFFPVTSVFASIYSMTAIAVDRYMAIIHPLKPRLSAKVTTGVIIVCIWSL
<i>Ctenopharyngodon idella</i>	AAFLAFPLCFYSVTEVK- -QHRTL CYVAWPRRNDDAFIYHVIVAVLVYL
<i>Danio rerio</i>	AVVLAFLPLCFSTIKKL- -PKRTL CYVAWPRPSEDPFMYHIIVAMLVYV
<i>Oryzias latipes</i>	AIVLAFLPLCFYSTITQVR- -PRRTICYVAWPRMADDSFMYHIIVAVLIVYV
<i>Oreochromis niloticus</i>	AIVLAFLPLCFYSTITQRTIPORTICYVAWPRNMKDDSFMYHIIVTVLVYV
<i>Ctenopharyngodon idella</i>	LPLALMAVTHTRVGLVLWGCGFPGHSSENLQDHLQAMRRVVKMMIIVVVT
<i>Danio rerio</i>	LPLVVMGINTYITVGLTLWGGEIPGDSNDNYGQLRAKRKVVVKMMIIVVVT
<i>Oryzias latipes</i>	LPLVVMGITITVGLTLWGGEIPGDTSDNYHGQLQAKRKVVVKMMIIVVVT
<i>Oreochromis niloticus</i>	LPLVVMGITITVGLTLWGGEIPGDSNDNYHGQLRAKRKVVVKMMIIVVVT
<i>Ctenopharyngodon idella</i>	FALCWLPHYHYFIIATSFNWKLTNFKQSITQQVYLSILWLSMSSSMYNPIIYC
<i>Danio rerio</i>	FAFCWLPHYHYFLVTGLNKQLARWKFIIQQIYLSIMWLAMSSSTMYNPIIYC
<i>Oryzias latipes</i>	FALCWLPHYHYFIVTGINKRLSKWKYIQQVYLSIMWLAMSSSTMYNPIIYC
<i>Oreochromis niloticus</i>	FALCWLPHYHYFIATGLNKLRLSKWKYIQQVYLSIMWLAMSSSTMYNPIIYC
<i>Ctenopharyngodon idella</i>	CLNSRFRAGFKRVFRWCPPIIVVSDSDMELEQTTRFQPNRQSSSLYTVSRVE
<i>Danio rerio</i>	CLNSRFRAGFKRVFRWCPPIVQVSDYDELELRAMRHVARQSSSMYITSRME
<i>Oryzias latipes</i>	CLNSRFRAGFKRAFRWCPPIKVSSYDELELRSTRLHPTQRSSSMYITLRMD
<i>Oreochromis niloticus</i>	CLNSRFRAGFKQAFRWCPPIKVSSYDELELRSTRLTQTRQSSSMYITLRMD
<i>Ctenopharyngodon idella</i>	SDIN- -ANPAR- - - - -RKSSSTISHRSISQSRP SARLSLNGGLHG
<i>Danio rerio</i>	TTVVITVCDPSE- - - - -PNTQFGRKS- - -LNHHHHNGCSNPAKS
<i>Oryzias latipes</i>	ITIMVVYDPAEAGDGCAGCGGTGRKHSLPARKRSYITSRQTDITISNNSGK
<i>Oreochromis niloticus</i>	NTIMVVYDPAEAGDGSAGGGTGRKHSLPGRKRSYVTSRHTETIAACSDTAVK
<i>Ctenopharyngodon idella</i>	HPNTAAAGNELSSE- -
<i>Danio rerio</i>	KETITMQSDPKIEEFS
<i>Oryzias latipes</i>	TQNGVAPSAEPEEFS
<i>Oreochromis niloticus</i>	TQNGSAPSAQPEEFP

Supplemental Figure S12. Molecular cloning and sequence alignment of grass carp NK3Rb. (A) Nucleotide and deduced amino acid sequences of grass carp NK3Rb. Numbering of the deduced amino acid sequences begins with the first methionine of the ORF to the right of each line. Nucleotide numbers are to the left of each line. **(B)** Protein sequence alignment of grass carp NK1Ra with that of other vertebrates using Clustal-W algorithm with MacVector program. The conserved a.a. residues are boxed in grey.



Supplemental Figure S13. Sequence analysis of grass carp NK3Rb. (A) Snake diagram of grass carp NK3Rb with Protter program. The seven transmembrane domains, three intracellular domains and three extracellular domains are labeled as TMD 1-7, ICL1-3 and ECL1-3, respectively. Sequence identities of transmembrane domains and extracellular domains, intracellular domains, N-termini and C-termini between grass carp and other vertebrates were showed in the Table. **(B)** 3-D protein model of grass carp NK3Rb using SWISS-MODEL program. The location of N-terminal and C-terminal were highlighted, respectively. The amino acids with hydrophobic side chains are colored blue, while those with hydrophilic side chains are colored red.



Supplemental Figure S14. Sequence alignment of grass carp NK1Ra, NK1Rb, NK2R, NK3Ra1, NK3Ra2 and NK3Rb. Protein sequence alignment of grass carp NK1Ra, NK1Rb, NK2R, NK3Ra1, NK3Ra2 and NK3Rb using Clustal-W algorithm with MacVector program. The lower conserved a.a. residues are boxed in grey where higher are in black. The seven transmembrane domains are labeled as TMD1-7, respectively.