Supporting Information for:

Pharmacological characterization of the stick insect *Carausius morosus* Allatostatin-C receptor with its endogenous agonist

Ali Işbilir[1,2#,3#], Burcin Duan Sahbaz[1], Gunes Tuncgenc[1], Moritz Bünemann[4], Martin J. Lohse[2,3,5], Necla Birgül-Iyison[1]*

[1] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bogazici University, Istanbul, Turkey.

[2] Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, 13125, Germany.

[3] Institute of Pharmacology and Toxicology, University of Würzburg, Würzburg 97078, Germany.

[4] Department of Pharmacology and Clinical Pharmacy, Philipps-University, 35043, Marburg, Germany.

[5] ISAR Bioscience Institute, Planegg/Munich, 82152, Germany

* Correspondence: birgul@boun.edu.tr

Current affiliation

TABLE OF CONTENTS

Figure S1: CmorAlstRC-mediated Gi activation is specific to CmorAST-C in HEK293 cells.

Figure S2: ΔN-term and ECL3 mutants of the CmorAlstRC do not have impaired expression and membrane localization.

Figure S3: Evolutionary relationship of *Carausius morosus* Gαi, arrestin and GRK transcripts with mammalian and insect species.

Table S1: Sequence identity of Allatostatin-C from Carausius morosus and other insect species.

Table S2: F test on the logEC50 and Emax values obtained from Figure 3C and Figure 3B.

Table S3: Sequence alignment of the conserved domains of C. morosus Gαi-, arrestin-, and GRK-like transcripts with multiple species.

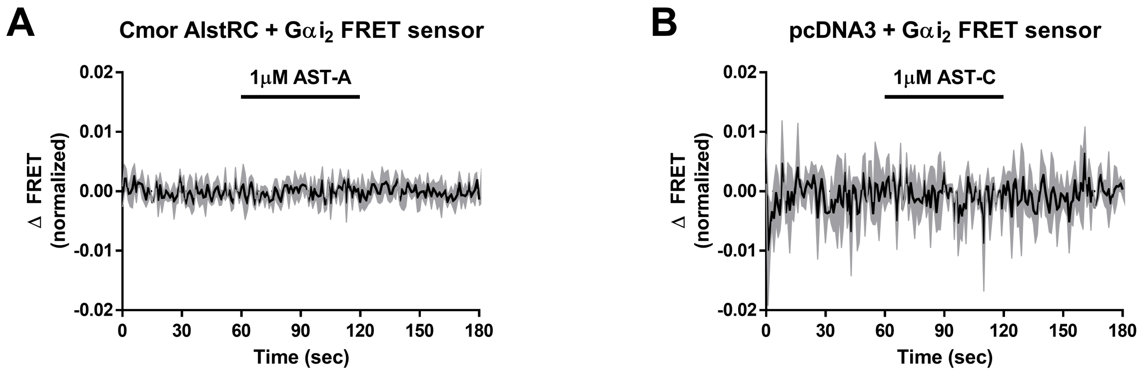

Figure S1: CmorAlstRC-mediated Gi activation is specific to CmorAST-C in HEK293 cells.

(**A)** Time lapse FRET microscopy measurements of Gαi2 activation in HEK293 cells expressing CmorAlstRC and Gαi2 FRET sensor, stimulated with AST-A. No FRET chang was observed within 60 seconds of superfusion. **(B)** Cells expressing Gαi2 sensor with pcDNA3 were sueprfused with CmorAST-C for 60 seconds. No FRET change was detected. Each FRET trace is generated by averaging 3 independent single cell measurements. Error bars represent SD.

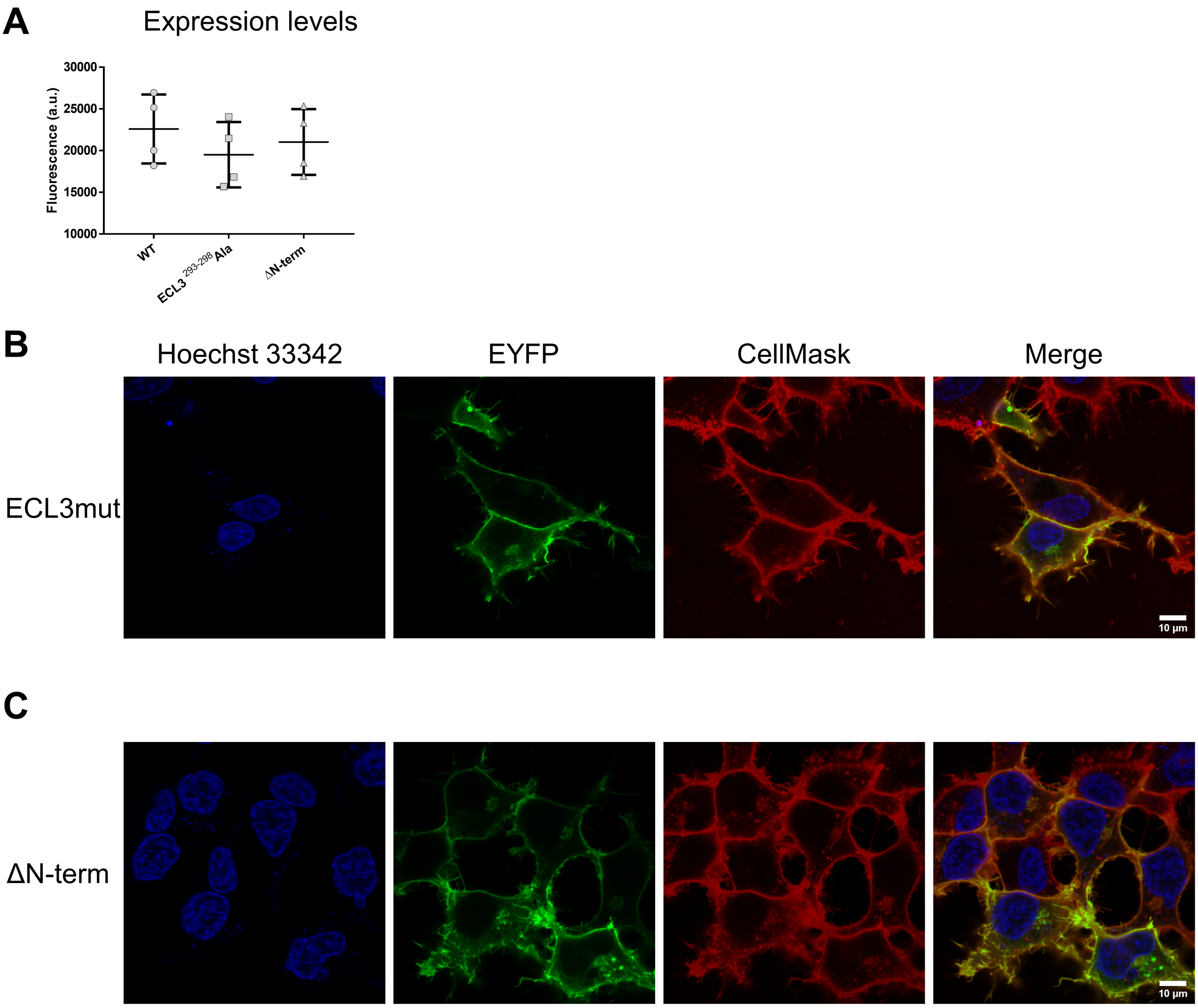

Figure S2: ΔN-term and ECL3 mutants of the CmorAlstRC do not have impaired expression and membrane localization.

(A) Expression levels of the C-terminally EYFP-tagged CmorAlstRC and its mutated forms. Bar graphs represent the mean and SD of 3 independent plate reader experiments. **(B)** Confocal microscopy images of the C-terminally EYFP-tagged ECL3 (B) and ΔN-term (C) mutants of the CmorAlstRC. Each image is representative of 3 independent experiments.

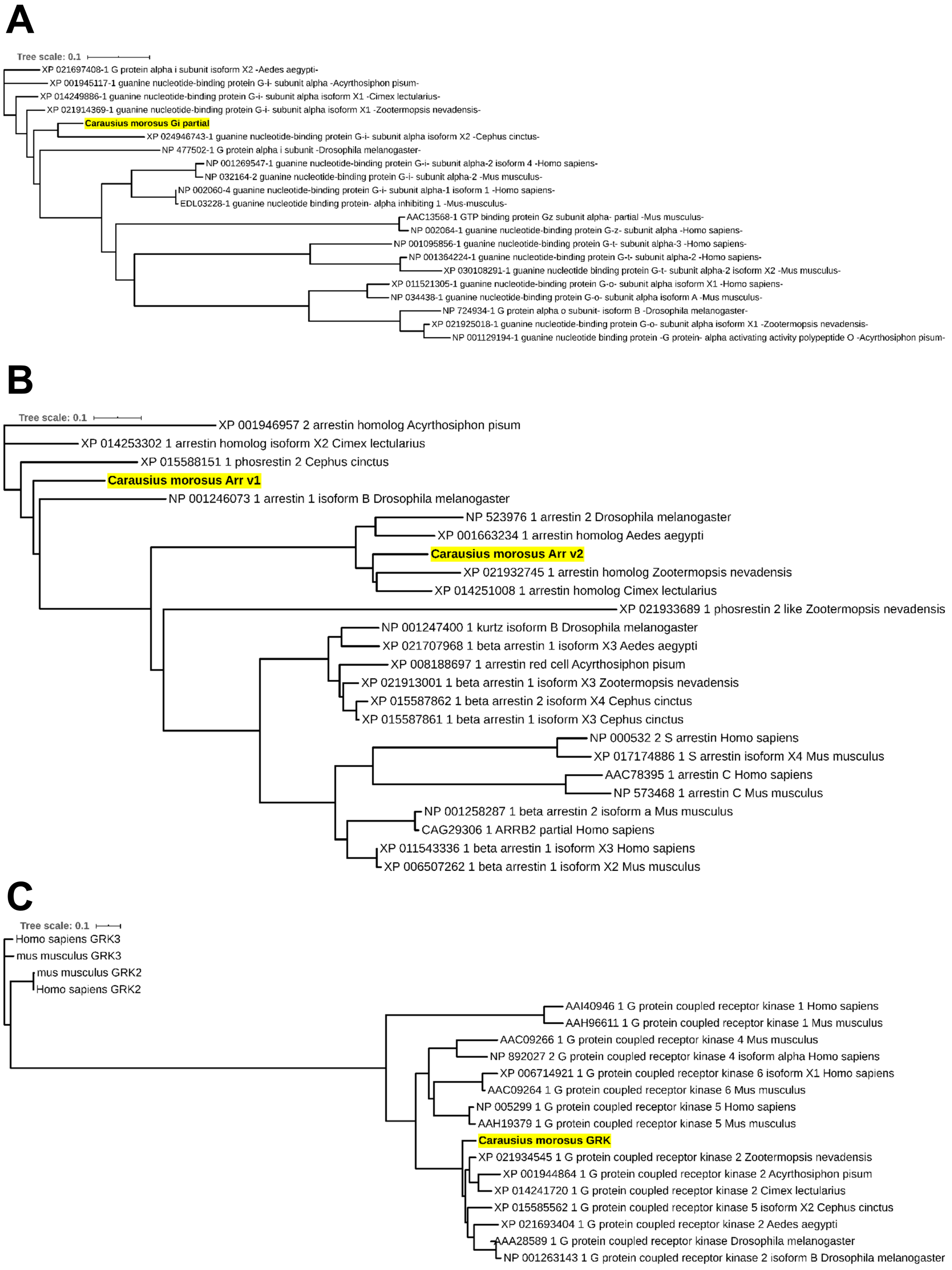

Figure S3: Evolutionary relationship of *Carausius morosus* Gαi, arrestin and GRK transcripts with mammalian and insect species.

Phylogenetic trees represent evolutionary distance of identified *Carausius morosus* Gαi (A), arrestin (B) and GRK (C) transcripts to the genomes of mammalian and insect species (*Carausius morosus, Homo sapiens, Mus musculus, Drosophila melanogaster, Acyrthosiphon pisum, Zootermopsis nevadensis, Aedes aegypti, Cimex lectularis* and *Cephus cinctus*).

TABLE S1

Sequence identity of Allatostatin-C from *Carausius morosus* and other insect species.

Description	**Max Score**	**Total Score**	**Query cover**	**E Value**	**Per. Ident.**	**Accession**
Allatostatin C preprohormone-like protein [*Daphnia magna*]	58.3	58.3	100%	1.00E-08	100	KZS21307.1
Allatostatin C [*Cryptotermes secundus*]	58.3	58.3	100%	1.00E-08	100	PNF40808.1
allatostatin C [*Acyrthosiphon pisum*]	58.3	58.3	100%	1.00E-08	100	XP_003244026.1
allatostatin C-like [*Myzus persicae*]	58.3	58.3	100%	1.00E-08	100	XP_022182906.1
allatostatin C-like [*Melanaphis sacchari*]	58.3	58.3	100%	1.00E-08	100	XP_025207556.1
allatostatin C *[Plautia stali*]	58.3	58.3	100%	1.00E-08	100	BAV78790.1
PREDICTED: allatostatin C-like [*Polistes canadensis*]	58.3	58.3	100%	1.00E-08	100	XP_014598613.1
allatostatin C1/prohormone 1 [*Cherax quadricarinatus*]	58.3	58.3	100%	1.00E-08	100	AWK57503.1
Allatostatin C Type 2 Precursor protein [*Hyalella azteca*]	58.3	58.3	100%	1.00E-08	100	KAA0201193.1
allatostatin C-like [*Pseudomyrmex gracilis*]	58.3	58.3	100%	1.00E-08	100	XP_020296756.1
allatostatin C [*Formica exsecta*]	58.3	58.3	100%	1.00E-08	100	XP_029677371.1
allatostatin C [*Odontomachus brunneus*]	58.3	58.3	100%	1.00E-08	100	XP_032688105.1
PREDICTED: allatostatin C-like [*Dinoponera quadriceps*]	58.3	58.3	100%	1.00E-08	100	XP_014471590.1
allatostatin C [*Camponotus floridanus*]	58.3	58.3	100%	1.00E-08	100	XP_011252628.1
allatostatin C [*Ooceraea biroi*]	58.3	58.3	100%	1.00E-08	100	XP_011338324.1
allatostatin C-like [*Pogonomyrmex barbatus*]	58.3	58.3	100%	1.00E-08	100	XP_011635356.1
allatostatin C [*Nylanderia fulva*]	58.3	58.3	100%	1.00E-08	100	XP_029159605.1
allatostatin C [*Harpegnathos saltator*]	58.3	58.3	100%	1.00E-08	100	XP_011138699.1
putative allatostatin C preprohormone [*Daphnia pulex*]	58.3	58.3	100%	1.00E-08	100	EFX85706.1
allatostatin C-like [*Daphnia magna*]	58.3	58.3	100%	1.00E-08	100	XP_032779583.1

TABLE S2

F test on the logEC50 and Emax values obtained from Figure 3C and Figure 3B.

Table S2A) logEC50 of Cmor AST-C

	WT vs ECL3mut	WT vs ΔN-ter	ECL3 vs ΔN-ter
Preferred model	LogIC50 different for each data set	LogIC50 different for each data set	LogIC50 different for each data set
p value	<0,0001	<0,0001	0.0019
F (DFn, DFd)	55,22 (1, 84)	32,46 (1, 84)	10,48 (1, 66)

Table S2B) Emax of Cmor AST-C

	WT vs ECL3mut	WT vs ΔN-ter	ECL3 vs ΔN-ter
Preferred model	Bottom same for all data sets	Bottom different for each data set	Bottom different for each data set
p value	0.5754	0.0004	0.0109
F (DFn, DFd)	0,3161 (1, 84)	13,82 (1, 84)	6,86 (1, 66)

Table S2C) logEC50 of Dmel AST-C

	WT vs ECL3mut	WT vs ΔN-ter	ECL3 vs ΔN-ter
Preferred model	LogIC50 different for each data set	LogIC50 same for all data sets	LogIC50 different for each data set
p value	<0,0001	0.4916	<0,0001
F (DFn, DFd)	34,57 (1, 66)	0,4784 (1, 66)	30,05 (1, 66)

Table S2D) Emax of Dmel AST-C

	WT vs ECL3mut	WT vs ΔN-ter	ECL3 vs ΔN-ter
Preferred model	Bottom same for all data sets	Bottom different for each data set	Bottom same for all data sets
p value	0.1406	0.0049	0.7579
F (DFn, DFd)	2,224 (1, 66)	8,496 (1, 66)	0,09579 (1, 66)

TABLE S3

Table S3A) Putative receptor binding site of *Carausius morosus* Gαi-like aligned with multiple species

```
                                                             ############# ####
query      86 .[1].PLTVC     FPEF.[ 4].TYEEAAAYIQLKFENLN.[5].KEIYTHFTCATDTSNIQFVFDAVTDVIIKNNL 153 C. morosus Gi
1AZT_A    304 .[3].KIEDY     FPEF.[17].RVTRAKYFIRDEFLRIS.[7].HYCYPHFTCAVDTENIRRVFNDCRDIIQRMHL 388 cattle
1ZCA_B    286 .[1].SIKKH     FPDF.[ 5].RLEDVQRYLVQCFDRKR.[4].KPLFHHFTTAIDTENIRFVFHAVKDTILQENL 353 house mouse
1CUL_C    290 .[3].KIEDY     FPEF.[17].RVTRAKYFIRDEFLRIS.[7].HYCYPHFTCAVDTENIRRVFNDCRDIIQRMHL 374 cattle
1U0H_C    304 .[3].KIEDY     FPEF.[17].RVTRAKYFIRDEFLRIS.[7].HYCYPHFTCAVDTENIRRVFNDCRDIIQRMHL 388 cattle
NP_506290 283 .[1].NITTA     FPDY.[ 4].NYEEAVSFIKQKFAELN.[5].KTIYMHETCATDTNQVQLVISSVIDTIIQKNL 350 nematode
CAG08423  307 .[1].HLATY     FPEF.[ 5].DPGAAQEFILKMYQEQN.[4].KTLYAHFTCATDTENIRFVFAAVKDTILRHNL 374 Tetraodon nigroviridis
AAS92627  289 .[3].KIEDY.[3].FAYY.[14].RVTRAKFFIRDEFLKIS.[7].HYCYPHFTCAVDTENIRRVFNDCRDIIQRMHL 373 zebrafish
AAQ24336  286 .[1].PINKH     FPDY.[ 5].DFDTAAKYFADRFRSIN.[4].REIYIHYTNATDTTLLKATMDSVQDMIIQKNL 353 Penicillium marneffei
AAS38582  289 .[3].KIEDY.[3].YAMY.[14].DVLRAKYFIRDEFLRIS.[7].HYCYPHFTCAVDTENIRRVFDDCRDIIQRMHL 373 purple urchin
```

Table S3B) beta - gamma complex interaction site (polypeptide binding site) of *Carausius morosus* Gαi-like aligned with multiple species

```
               # #   ### #  ## ####
query       1 FKMFDVGGQRSERKKWIHCFEGVTAIIFCVALsgydlvl--aedeemNRMIESMKLFDSICNskwfvetSIILFLNKKDL 78  C. morosus Gi
1AZT_A    219 FHMFDVGGQRDERRKWIQCFNDVTAIIFVVASssynmvi--rednqtNRLQEALNLFKSIWNnrwlrtiSVILFLNKQDL 296 cattle
1ZCA_B    201 FKMVDVGGQRSQRQKWFQCFDGITSILFMVSSseydqvl--medrrtNRLVESMNIFETIVNnklffnvSIILFLNKMDL 278 house mouse
1CUL_C    205 FHMFDVGGQRDERRKWIQCFNDVTAIIFVVASssynmvi--rednqtNRLQEALNLFKSIWNnrwlrtiSVILFLNKQDL 282 cattle
1U0H_C    219 FHMFDVGGQRDERRKWIQCFNDVTAIIFVVASssynmvi--rednqtNRLQEALNLFKSIWNnrwlrtiSVILFLNKQDL 296 cattle
NP_506290 198 FRVFDVGGQRSERRKWIHCFDNVESIIFITAIseydqvl--fedettNRMIESMQLFNSICNstwflstAMILFMNKKDL 275 nematode
CAG08423  220 FRMVDVGGQRSERRKWIHCFENVTSIIFLVALseydqvlaecdnevlNRMEESKALFKTIITypwfqrsSVILFLNKTDI 299 Tetraodon nigroviridis
AAS92627  204 FHMFDVGGQRDERRKWIQCFNDVTAIIFVVASssynmvi--rednntNRLREALALFRSIWNnrwlrtiSVILFLNKQDM 281 zebrafish
AAQ24336  201 FRMMDVGGQRSERKKWIHCFEGVQCLLFMVALsgydqsl--ledqnaNQMHEAMMLFESLANgewfkrkPIMLFLNKMDL 278 Penicillium marneffei
AAS38582  204 FHMFDVGGQRDERRKWIQCFNDVTAIIFVVACssynlvl--redpnqNRLRESLELFRSIWNnrwlrtiSVILFLNKQDL 281 purple urchin
```

Table S3C) Sequence alignment of *Carausius morosus* Gαi-like and *Homo sapiens* Gαi2 proteins

```
GFAX01054314_1_Carausius_morosus_Gi_partial      ------------------------------------------------------------ 0
NP_001269547.1_Homo_sapiens_Gi2_isoform_4        MAIVKAMGNLQIDFADPSRADDARQLFALSCTAEEQGVLPDDLSGVIRRLWADHGVQACF 60


GFAX01054314_1_Carausius_morosus_Gi_partial      -------------------------------------------------------FKMFD 5
NP_001269547.1_Homo_sapiens_Gi2_isoform_4        GRSREYQLNDSAAYYLNDLERIAQSDYIPTQQDVLRTRVKTTGIVETHFTFKDLHFKMFD 120
                                                                                                       *****

GFAX01054314_1_Carausius_morosus_Gi_partial      VGGQRSERKKWIHCFEGVTAIIFCVALSGYDLVLAEDEEMNRMIESMKLFDSICNSKWFV 65
NP_001269547.1_Homo_sapiens_Gi2_isoform_4        VGGQRSERKKWIHCFEGVTAIIFCVALSAYDLVLAEDEEMNRMHESMKLFDSICNNKWFT 180
                                                 ****************************.************** ***********.***.

GFAX01054314_1_Carausius_morosus_Gi_partial      ETSIILFLNKKDLFEEKISKSPLTVCFPEFTGSNTYEEAAAYIQLKFENLNKRKDQKEIY 125
NP_001269547.1_Homo_sapiens_Gi2_isoform_4        DTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEIY 240
                                                 :*****************::****:****:**:*.*:***:*** ***:****** ****
                                                                     ##############
GFAX01054314_1_Carausius_morosus_Gi_partial      THFTCATDTSNIQFVFDAVTDVIIKNNLKDCGLF  159
NP_001269547.1_Homo_sapiens_Gi2_isoform_4        THFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF  274
                                                 *********.*:**********************
```

Table S3D) Sequence alignment of *Carausius morosus* arrestin-like and *Homo sapiens* β-arrestin2 proteins

```
CAG29306_1_Homo_sapiens_ARRB2                ------MGEKPGTRVFKKSSPNCKLTVYLGKRDFVDHLDKVDPVDGVVLVDPDYLKDRKV      54
GFAX01134611_1_Carausius_morosus_ARR_v1      --------MGVNFKVFKKCSPNGKITVYLGKRDFVDHITGIEPIDGVVLLEEEYLRERKI      52
GDXB01033192_1_Carausius_morosus_ARR_v2      MFLLLLLAYVVAVKVFKKTTPNGKVTVYLGKRDFIDHLDNTDVIDGVVVVDNDYLRGRKV      60
                                                          :**** :** *:*********:**:   : :****::: :**: **:

CAG29306_1_Homo_sapiens_ARRB2                FVTLTCAFRYGREDLDVLGLSFRKDLFIATYQAFPPVPNPPRPPTRLQDRLLRKLGQHAH      114
GFAX01134611_1_Carausius_morosus_ARR_v1      FGQVVCGFRYGREEDEVMGLNFQKELFLVSEQVYPPPEKSEHNLTKLQERLLKKLGPNAF      112
GDXB01033192_1_Carausius_morosus_ARR_v2      FGELTTTYRYGREEDEVMGLKFSKEMPLCRDQIAPS-QETKQELTPIQERLVKKFGANAY      119
                                             *  :.  :*****: :*:**.* *:: :   *  *   :  :  * :*:**::*:* :*.

CAG29306_1_Homo_sapiens_ARRB2                PFFFTIPQNLPCSVTLQPGPEDTGKACGVDFEIRAFCAKSLEEKSHKRNSVRLVIRKVQF      174
GFAX01134611_1_Carausius_morosus_ARR_v1      PFTFVLPPNTPASVTLQPGPEDQGEPCGVQYYVKMFSGDSDTDRSHKRSTITLGFRKVQY      172
GDXB01033192_1_Carausius_morosus_ARR_v2      PFTFHFPGNSPSSVTLMPGEDDSGKPLGVEYSVKAYVGDHKDDKSHKRSSVSLAIKKLQY      179
                                             ** * :* * *.**** ** :* *:  **:: :: : ..   ::****.:: * ::*:*:

CAG29306_1_Homo_sapiens_ARRB2                APEKPG-PQPSAETTRHFLMSDRSLHLEASLDKELYYHGEPLNVNVHVTNNSTKTVKKIK      233
GFAX01134611_1_Carausius_morosus_ARR_v1      APSKPG-RQPCTMVRKDFLLSPGELELEATLDKQVYLHGEKIAVNISIRNNSNKMVKKIK      231
GDXB01033192_1_Carausius_morosus_ARR_v2      APPSRGHRLPSSLVSKGFTFSNGKLNLEVTLDKEIYYHGEKITANAIVTNNSRKAVRTIK      239
                                             ** . *   *.: . : * :*  .*.**.:***::* *** : .*  : *** * *:.**

CAG29306_1_Homo_sapiens_ARRB2                VSVRQYADICLFSTAQYKCPVAQLEQDD--QVSPSSTFCKVYTITPLLSDNREKRGLALD      291
GFAX01134611_1_Carausius_morosus_ARR_v1      AMVQQGVDVVLFQNGQYRTSIASLETQEGCPVQPGSTLQKVMYLVPTLESNKDRRGIALD      291
GDXB01033192_1_Carausius_morosus_ARR_v2      VFVVQHCEVT-MVNAQFSRHVASLETREGCPITPGASFTKVFYLVPLAASNKDRYGIALD      298
                                             . * *  ::  : ..*:   :*.**  :   : *.::: **  :.*   .*::: *:***

CAG29306_1_Homo_sapiens_ARRB2                GKLKHEDTNLASSTIVKEG-ANKEVLGILVSYRVKVKLVV-SRGGDVSVELPFVLMHPKP      349
GFAX01134611_1_Carausius_morosus_ARR_v1      GQLKHNSTNLASSTLLASP-DQRDAFGIVVSYALKVKLYLGALGGELTAELPLLLMHPKP      350
GDXB01033192_1_Carausius_morosus_ARR_v2      GYLKDDDVNLASSTLIAEGKCPAEAMGIVISYSLRVKLNCGTLGGDLVTDVPFKLMHPAL      358
                                             * **.:..******:: .     :.:**::** ::***   : **:: .::*: ****

CAG29306_1_Homo_sapiens_ARRB2                HDHIPLPRPQSAAPE---TDVPVDTNLIEFDTNY--ATDDDIVFEDFARLRLKGMKDDDY      404
GFAX01134611_1_Carausius_morosus_ARR_v1      TKGKVIHADSQADVEMFRQD-TV---DQEAEKC---------------------------      379
GDXB01033192_1_Carausius_morosus_ARR_v2      GTVERER------VQVLKKTKSVER-AKYENSCYATDDDDNIVFEDFARLRLNEPE----      407
                                                           :       *       :.

CAG29306_1_Homo_sapiens_ARRB2                DDQLC 409
GFAX01134611_1_Carausius_morosus_ARR_v1      ----- 379
GDXB01033192_1_Carausius_morosus_ARR_v2      ----- 407
```