Supporting Information for:

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

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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 change was observed within 60 seconds of superfusion. (B) Cells expressing Gαi2 sensor with pcDNA3 were superfused 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, Acrhythsion pismum, Zootermopsis nevadensis, Aedes aegypti, Cimex lectularis and Cephus cinctus*).
| 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_02096756.1 |
| 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 [Harpeggnathos saltator] | 58.3 | 58.3 | 100% | 1.00E-08 | 100 | XP_011386999.1 |
| putative allatostatin C proprehormone [Daphnia pulex] | 58.3 | 58.3 | 100% | 1.00E-08 | 100 | EFX5706.1 |
| 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

| Preferred model | WT vs ECL3mut | WT vs ΔN-ter | ECL3 vs ΔN-ter |
|-----------------|---------------|---------------|---------------|
| 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

| Preferred model | WT vs ECL3mut | WT vs ΔN-ter | ECL3 vs ΔN-ter |
|-----------------|---------------|---------------|---------------|
| 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

| Preferred model | WT vs ECL3mut | WT vs ΔN-ter | ECL3 vs ΔN-ter |
|-----------------|---------------|---------------|---------------|
| 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

| Preferred model | WT vs ECL3mut | WT vs ΔN-ter | ECL3 vs ΔN-ter |
|-----------------|---------------|---------------|---------------|
| 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 *Carassius morosus* Gai-like aligned with multiple species

| query | 86 | .[1]PLTVC | FPEF.[4]TYEEFAAYIQLKFECLN.|[5]|KHITHTCATAGIIPVFVDAVTDIIFKNL 153 | C. morosus Gi |
| 1AZT_A | 304 | .[3]KIEDY | FPEF.[17]NRTRAKYFIRDIEFLRIS.|[7]|HCTHIHTCAGTIIPVFVDNDRDIIIQRHML 388 | cattle |
| 12CA_B | 286 | .[1]SIKKH | FPDF.[5]KLEDVQYRLQCFDNKR.|[4]|KLEYPHTCAITIIPVFHVAKTDIILQENL 353 | house mouse |
| 1CUL_C | 290 | .[3]KIEDY | FPEF.[17]NRTRAKYFIRDIEFLRIS.|[7]|HCTHIHTCAGTIIPVFVDNDRDIIIQRHML 374 | cattle |
| 1U0H_C | 304 | .[3]KIEDY | FPEF.[17]NRTRAKYFIRDIEFLRIS.|[7]|HCTHIHTCAGTIIPVFVDNDRDIIIQRHML 388 | cattle |
| NP_506290 | 303 | .[1]NITPA | FPDY.[4]NYEAAVSKIKQFEALN.|[5]|KFLYMYHTCAITIIPVFVDNDRDIIIQRHML 350 | nematode |
| CAG08423 | 307 | .[1]HLATY | FPEF.[5]DPGAAQEFILKMYQEQN.|[4]|KFLYHYHTCAITIIPVFVAATKITLRLHNL 374 | *Tetraodon nigroviridis* |
| AAS92627 | 289 | .[3]KIEDY | .[3]FAYY.|[14]|VRTRAKYFIRDIEFLRIS.|[7]|HCTHIHTCAGTIIPVFVDNDRDIIIQRHML 373 | zebrafish |
| AAQ24336 | 286 | .[1]PINKH | FPDY.[5]DFDAAKKEAYADRIKSN.|[4]|KEIHYNTAHITIIPVFATMDNQDIIQKNL 353 | Penicillum marneffi |
| AAS38582 | 289 | .[3]KIEDY | .[3]YAMY.|[14]|DVLRAKYFIRDIEFLRIS.|[7]|HCTHIHTCAGTIIPVFVDNDRDIIIQRHML 373 | purple urchin |

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

| query | 1 | FIMVOCGGSKKKMNIKIEGVTAIIFCVALLSgydlvl|--|aeedefNRELIESMKLFSICNskwvetSIILFLNKDL 78 | C. morosus Gi |
| 1AZT_A | 219 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDL 296 | cattle |
| 12CA_B | 201 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQMDL 275 | house mouse |
| 1CUL_C | 205 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDL 282 | cattle |
| 1U0H_C | 219 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDL 296 | cattle |
| NP_506290 | 198 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDL 275 | nematode |
| CAG08423 | 220 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDL 296 | *Tetraodon nigroviridis* |
| AAS92627 | 204 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDM 281 | zebrafish |
| AAQ24336 | 201 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDM 278 | Penicillum marneffi |
| AAS38582 | 204 | FIMVOCGGSKKKMNIKIEGVTAIIFVVASSynvml|--|redngtNRLQALNLFKSNWTwrliiSVILFNKQDL 281 | purple urchin |
| GenBank Accession | Species                        | Alignment (Residues) |
|------------------|-------------------------------|----------------------|
| GFAX01054314_1   | Carausius morosus Gi-like     | 0                    |
| NP_001269547.1   | Homo sapiens Gi2 isoform_4    | 60                   |
| GFAX01054314_1   | Carausius morosus Gi-like     | 5                    |
| NP_001269547.1   | Homo sapiens Gi2 isoform_4    | 120                  |
| GFAX01054314_1   | Carausius morosus Gi-like     | 65                   |
| NP_001269547.1   | Homo sapiens Gi2 isoform_4    | 180                  |
| GFAX01054314_1   | Carausius morosus Gi-like     | 125                  |
| NP_001269547.1   | Homo sapiens Gi2 isoform_4    | 240                  |
| GFAX01054314_1   | Carausius morosus Gi-like     | 159                  |
| NP_001269547.1   | Homo sapiens Gi2 isoform_4    | 274                  |
Table S3D) Sequence alignment of *Carausius morosus* arrestin-like and *Homo sapiens* β-arrestin2 proteins

| Accession | Species          | Sequence Alignment |
|------------|------------------|--------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

**CAG29306_1_Homo_sapiens_ARRB2**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

**GFAX01134611_1_Carausius_morosus_ARR_v1**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

**GDB01033192_1_Carausius_morosus_ARR_v2**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

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**CAG29306_1_Homo_sapiens_ARRB2**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

**GFAX01134611_1_Carausius_morosus_ARR_v1**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

**GDB01033192_1_Carausius_morosus_ARR_v2**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

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**CAG29306_1_Homo_sapiens_ARRB2**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

**GFAX01134611_1_Carausius_morosus_ARR_v1**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

**GDB01033192_1_Carausius_morosus_ARR_v2**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

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**CAG29306_1_Homo_sapiens_ARRB2**

| **Accession** | **Species** | **Sequence Alignment** |
|---------------|-------------|-----------------------|
| CAG29306_1_Homo_sapiens_ARRB2 | CAG29306_1_Homo_sapiens_ARRB2 |
| GFAX01134611_1_Carausius_morosus_ARR_v1 | GFAX01134611_1_Carausius_morosus_ARR_v1 |
| GDB01033192_1_Carausius_morosus_ARR_v2 | GDB01033192_1_Carausius_morosus_ARR_v2 |

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