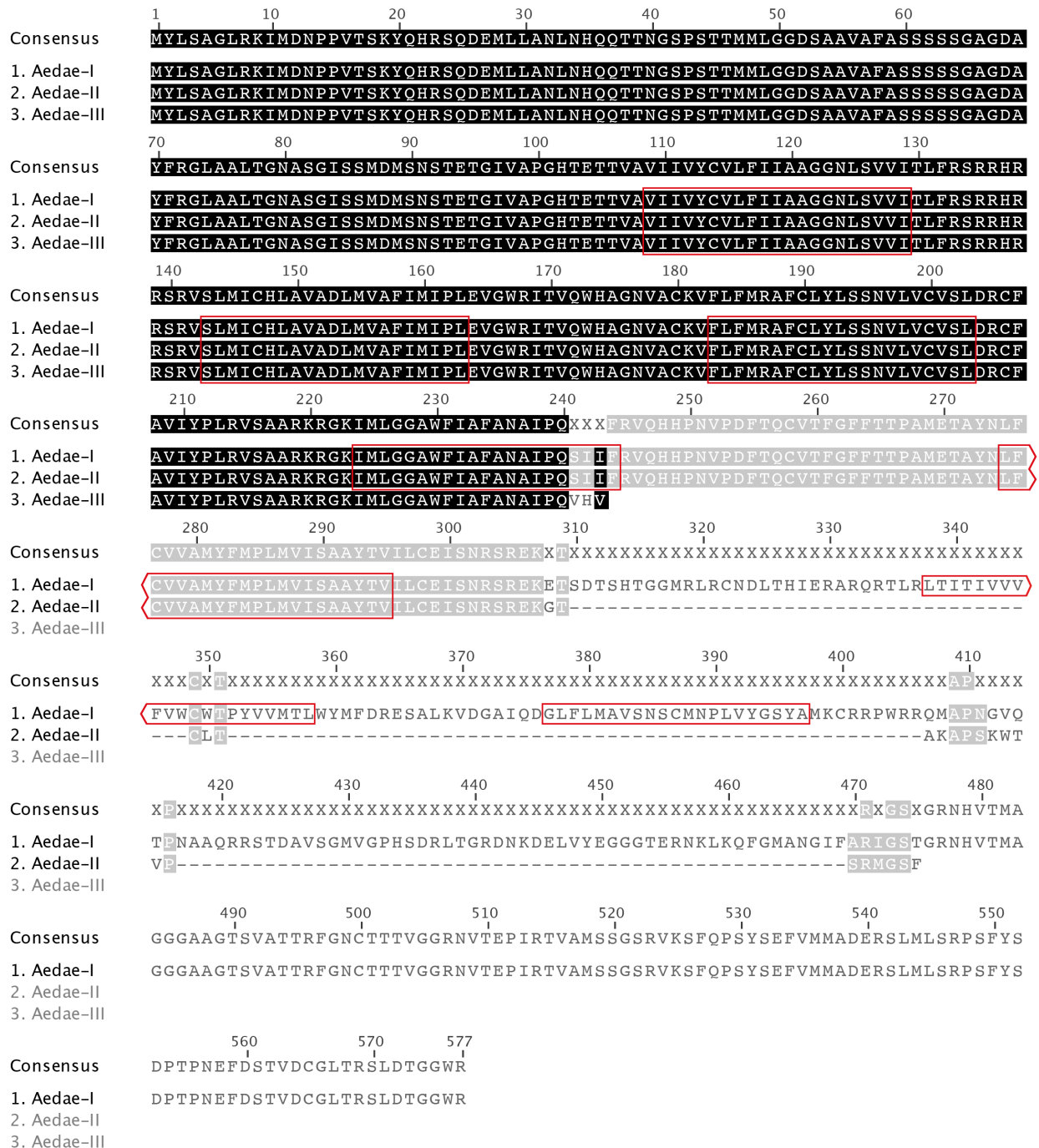


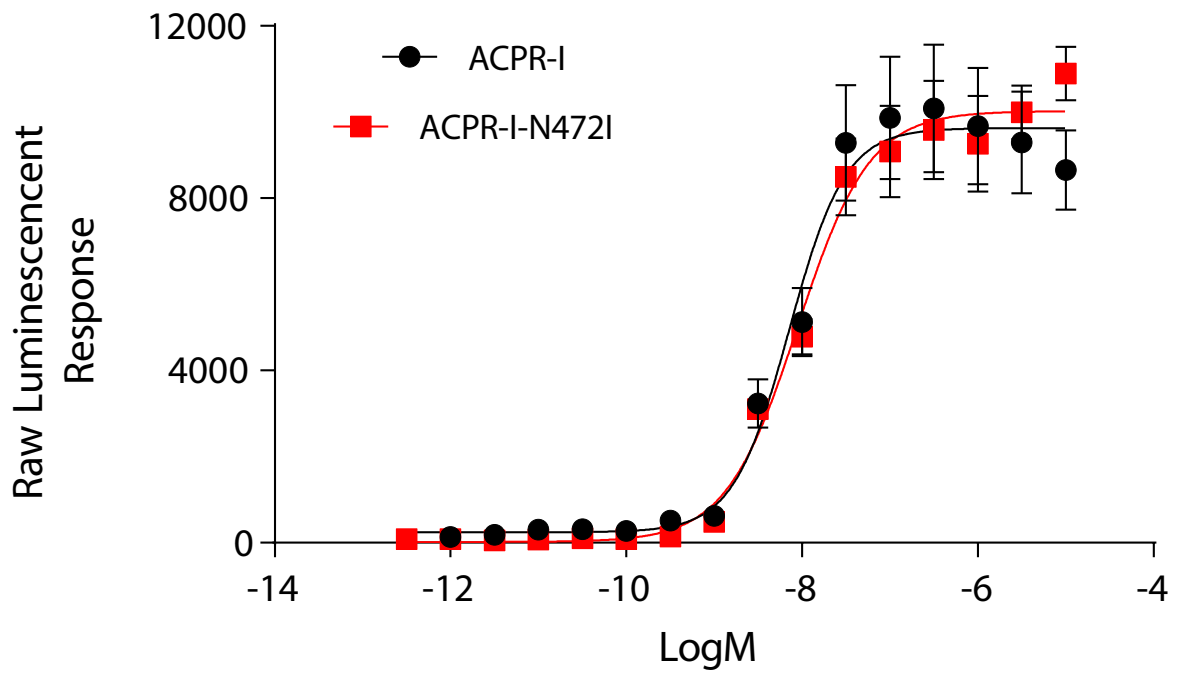
**Molecular identification, transcript expression, and functional deorphanization of  
the adipokinetic hormone/corazonin-related peptide receptor in the disease vector,  
*Aedes aegypti*.**

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SUPPLEMENTARY INFORMATION



**Figure S1. Sequence alignment of *Aedes aegypti* ACP receptor variants I, II, and III.** Aligned amino acid sequences of ACPRs from *A. aegypti*, transcript variant I, (GenBank: MF461644), transcript variant II, (GenBank: MF461645), transcript variant III, (GenBank: MF461646). Residues outlined in red indicate predicted transmembrane domains based on the *A. aegypti* sequences. Highlighting of residues indicates % identity with black denoting 100% sequence identity, dark grey denotes 80-100% identity, and light grey represents amino acid positions with 60-80% sequence identity.



**Figure S2. Functional heterologous receptor assay of CHO-K1 aequorin cells transiently expressing the *AedaeACPR-I* and *AedaeACPR-I-N472I*.** Dose dependent effect on the bioluminescence response (mean 0-15s) after the addition of between  $10^{-12}$  –  $10^{-5}$  M doses of *AedaeACP* to *AedaeACPR-I* and *AedaeACPR-I-N472I*. Luminescence is normalized to the BSA control. Data represent the mean  $\pm$  standard error (n=4).

**Table S1.** GenBank accession numbers and references for receptor sequences in Fig. 3.

Receptor	Putative Ligand(s)	Accession Number	Reference(s)
<i>Acyrtosiphon pisum</i> AKHR	AKH	XP_003245941	1
<i>Aedes aegypti</i> AKHR1	AKH	XP_001655248	2
<i>Aedes albopictus</i> AKHR	AKH	XP_019540948	
<i>Anopheles gambiae</i> AKHR	AKH	ABD60146	3
<i>Anopheles darlingi</i>	AKH	ETN67429	4
<i>Anopheles sinensis</i>	AKH	KFB53098	5
<i>Culex pipiens</i> AKHR	AKH	AAWU01041683	1
<i>Apis mellifera</i> AKHR	AKH	NP_001035354	6
<i>Bombyx mori</i> AKHR	AKH, ACP	NP_001037049	7-11
<i>Musca domestica</i> AKHR		XP_019895730	
<i>Manduca sexta</i> AKHR	AKH	ACE00761	12
<i>Drosophila melanogaster</i> AKHR	AKH	AAC61523	7,13
<i>Locusta migratoria</i> AKHR		ANW09575	
<i>Polyrhachis vicina</i> AKHR		ADK55068	
<i>Nasonia vitripennis</i> AKHR	AKH	NP_001161243	14
<i>Tribolium castaneum</i> AKHR	AKH	NP_001076809	14
<i>Periplaneta Americana</i> AKHR	AKH	ABB20590	14
<i>Rhodnius prolixus</i> AKHR	AKH	AIJ49751	15
<i>Gryllus bimaculatus</i> AKHR	AKH	ADZ17179	16
<i>Glossina morsitans</i> AKHR	AKH	AEH25943	
<i>Aedes aegypti</i> CRZR	CRZ	AAGE02020874	2
<i>Aedes albopictus</i> CRZR		XP_019554625	
<i>Anopheles gambiae</i> CRZR	CRZ	AAQ67361	17
<i>Apis mellifera</i> CRZR	CRZ	NP_001137393	6
<i>Culex pipiens</i> CRZR	CRZ	AAWU01031675	1
<i>Bombyx mori</i> CRZR	CRZ	NP_001127719	8,14,18
<i>Drosophila melanogaster</i> CRZR	CRZ	AAM21341	19
<i>Nasonia vitripennis</i> CRZR	CRZ	AAZX01006363	20
<i>Bactrocera dorsalis</i> CRZR	CRZ	AQX83392	21
<i>Rhodnius prolixus</i> CRZR-A	CRZ	AND99324	22
<i>Manduca sexta</i> CRZR	CRZ	AAR14318	23
<i>Musca domestica</i> CRZR	CRZ	NP_001295994	
<i>Anopheles gambiae</i> ACPR	ACP	ABX52399	14
<i>Anopheles darlingi</i> ACPR	ACP	ETN61096	24
<i>Aedes albopictus</i> ACPR	ACP	XP_019559258	
<i>Culex pipiens</i> ACPR	ACP	XP_001842868	1
<i>Tribolium castaneum</i> ACPR	ACP	ABX52400	14
<i>Nasonia vitripennis</i> ACPR	ACP	NP_001164571	14
<i>Bombyx mori</i> ACPR1	ACP, AKH	NP_001127726	14,23
<i>Bombyx mori</i> ACPR2	ACP, AKH	NP_001127745	14,23
<i>Rhodnius prolixus</i> ACPR-C	ACP, AKH	AKO62858	25

## References

1. Li, S. *et al.* Adipokinetic hormones and their G protein-coupled receptors emerged in Lophotrochozoa. *Sci Rep* 1–13 (2016). doi:10.1038/srep32789
2. Nene, V. *et al.* Genome Sequence of *Aedes aegypti*, a Major Arbovirus Vector. *Science* **316**, 1718–1723 (2007).
3. Kaufmann, C. & Brown, M. R. Adipokinetic hormones in the African malaria mosquito, *Anopheles gambiae*: Identification and expression of genes for two peptides and a putative receptor. *Insect Biochem Mol Biol* **36**, 466–481 (2006).
4. Mendes, N. D., Freitas, A. T., Vasconcelos, A. T. & Sagot, M.-F. Combination of measures distinguishes pre-miRNAs from other stem-loops in the genome of the newly sequenced *Anopheles darlingi*. *BMC Genomics* **11**, 529 (2010).
5. Zhou, D. *et al.* Genome sequence of *Anopheles sinensis* provides insight into genetics basis of mosquito competence for malaria parasites. *BMC Genomics* **15**, 42 (2014).
6. Elsik, C. G. *et al.* Finding the missing honey bee genes: lessons learned from a genome upgrade. *BMC Genomics* **15**, 86 (2014).
7. Staubli, F. *et al.* Molecular identification of the insect adipokinetic hormone receptors. *Proc Natl Acad Sci USA* **99**, 3446–3451 (2002).
8. Ou, J. *et al.* Transcriptomic analysis of developmental features of *Bombyx mori* wing disc during metamorphosis. *BMC Genomics* **15**, 820 (2014).
9. Suetsugu, Y. *et al.* Large scale full-length cDNA sequencing reveals a unique genomic landscape in a lepidopteran model insect, *Bombyx mori* G3 (*Bethesda*). **3**, 1481–92 (2013).
10. Huang, H. *et al.* Bombyx adipokinetic hormone receptor activates extracellular signal-regulated kinase 1 and 2 via G protein-dependent PKA and PKC but  $\beta$ -arrestin-independent pathways. *Biochemistry* **49**, 10862–10872 (2010).
11. Zhu, C. *et al.* Molecular and functional characterization of adipokinetic hormone receptor and its peptide ligands in *Bombyx mori*. *FEBS Lett* **583**, 1463–1468 (2009).
12. Ziegler, R., Isoe, J., Moore, W., Riehle, M. A. & Wells, M. A. The putative AKH receptor of the tobacco hornworm, *Manduca sexta*, and its expression. *J Insect Sci* **11**, 40 (2011).
13. Hauser, F., Søndergaard, L. & Grimmelikhuijzen, C. J. Molecular cloning, genomic organization and developmental regulation of a novel receptor from *Drosophila melanogaster* structurally related to gonadotropin-releasing hormone receptors for vertebrates. *Biochem Biophys Res Commun* **249**, 822–828 (1998).
14. Hansen, K. K. *et al.* Discovery of a novel insect neuropeptide signaling system closely related to the insect adipokinetic hormone and corazonin hormonal systems. *J Biol Chem* **285**, 10736–10747 (2010).
15. Zandawala, M., Hamoudi, Z., Lange, A. B. & Orchard, I. Adipokinetic hormone signalling system in the Chagas disease vector, *Rhodnius prolixus*. *Insect Mol Biol* **24**, 264–276 (2015).
16. Konuma, T., Morooka, N., Nagasawa, H. & Nagata, S. Knockdown of the adipokinetic hormone receptor increases feeding frequency in the two-spotted cricket *Gryllus bimaculatus*. *Endocrinology* **153**, 3111–3122 (2012).
17. Belmont, M., Cazzamali, G., Williamson, M., Hauser, F. & Grimmelikhuijzen, C. J. P. Identification of four evolutionarily related G protein-coupled receptors from the

- malaria mosquito *Anopheles gambiae*. *Biochem Biophys Res Commun* **344**, 160–165 (2006).
18. Yamanaka, N. *et al.* Neuropeptide receptor transcriptome reveals unidentified neuroendocrine pathways. *PLoS One* **3**, e3048 (2008).
  19. Cazzamali, G., Saxild, N. & Grimmelikhuijzen, C. Molecular cloning and functional expression of a *Drosophila* corazonin receptor. *Biochem Biophys Res Commun* **298**, 31–36 (2002).
  20. Werren, J. H. *et al.* Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science* **327**, 343–348 (2010).
  21. Hou, Q. L. *et al.* A role of corazonin receptor in larval-pupal transition and pupariation in the oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Front Physiol* **8**, 1–14 (2017).
  22. Hamoudi, Z., Lange, A. B. & Orchard, I. Identification and characterization of the corazonin receptor and possible physiological roles of the corazonin-signaling pathway in *Rhodnius prolixus*. *Front Neurosci* **10**, 1–12 (2016).
  23. Kim, Y.-J. *et al.* Corazonin receptor signaling in ecdysis initiation. *Proc Natl Acad Sci USA* **101**, 6704–6709 (2004).
  24. Marinotti, O. *et al.* The genome of *Anopheles darlingi*, the main neotropical malaria vector. *Nucleic Acids Res* **41**, 7387–7400 (2013).
  25. Zandawala, M., Haddad, A. S., Hamoudi, Z. & Orchard, I. Identification and characterization of the adipokinetic hormone/corazonin-related peptide signaling system in *Rhodnius prolixus*. *FEBS J* **282**, 3603–3617 (2015).