Supplementary information.

Title: The juvenile hormone described in *Rhodnius prolixus* by Wigglesworth is juvenile hormone III skipped bisepoxide.

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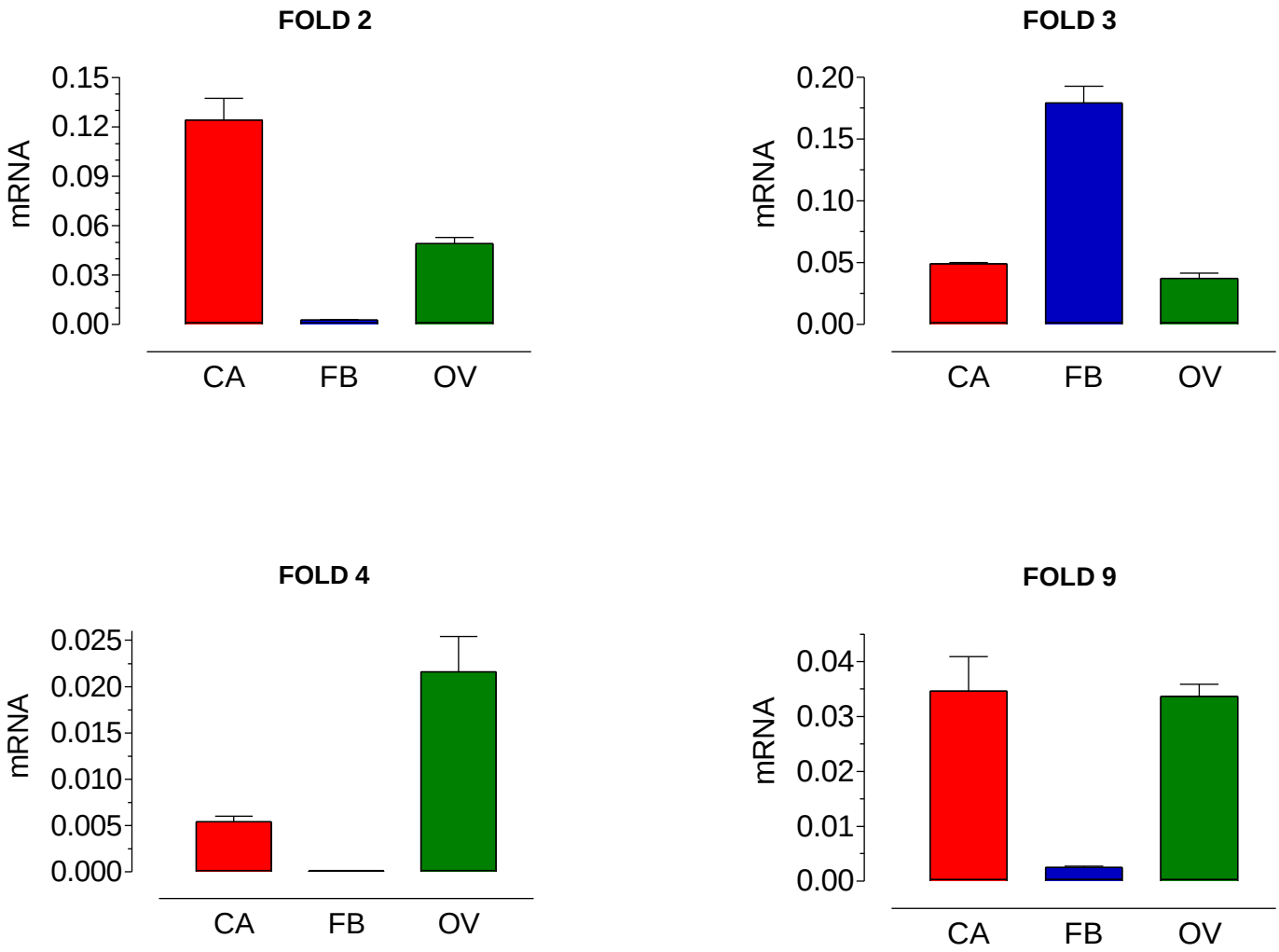
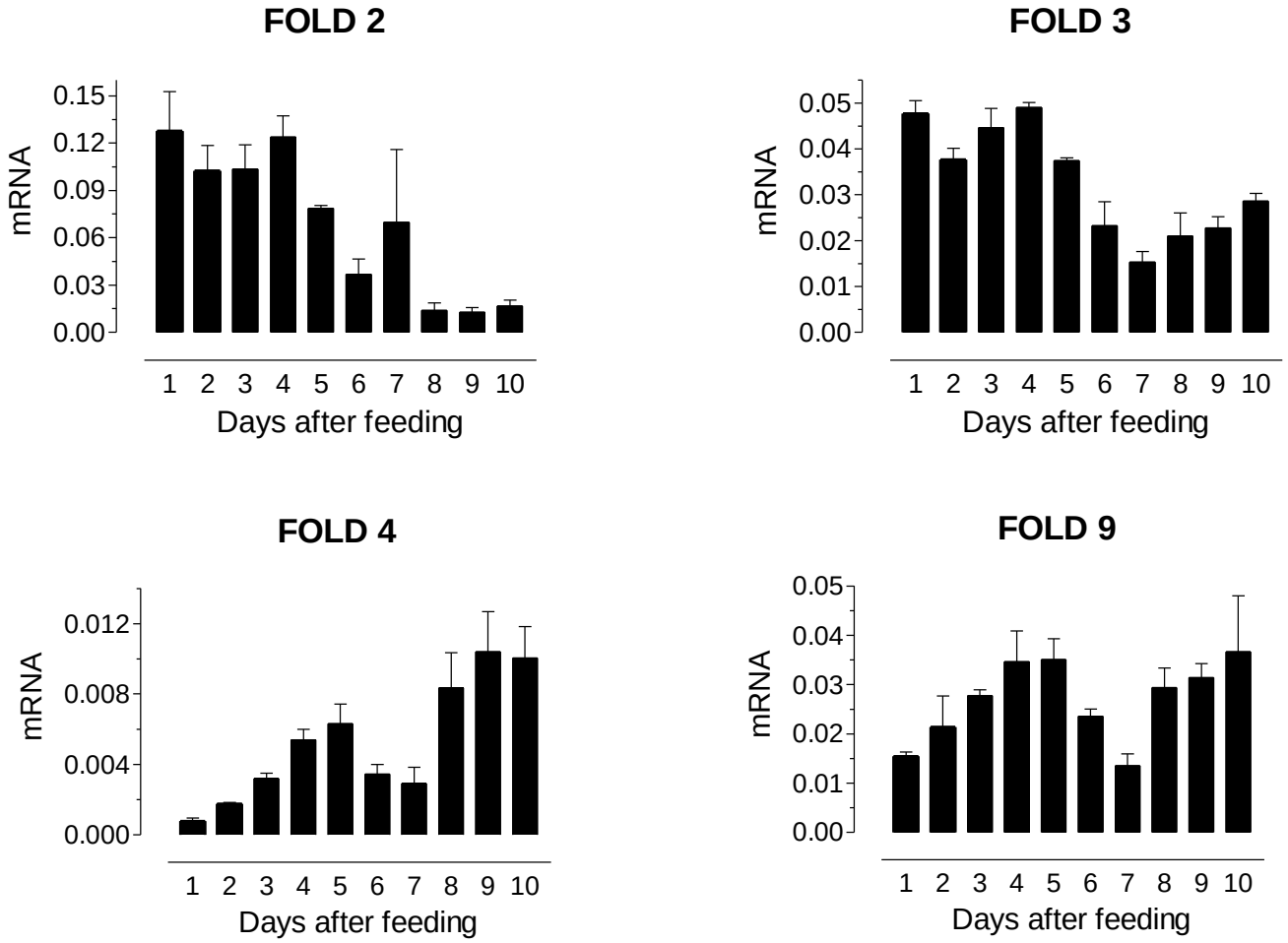
Supplemental Fig S1. Tissue specificity analysis of FOLD 2, 3, 4 and 9.

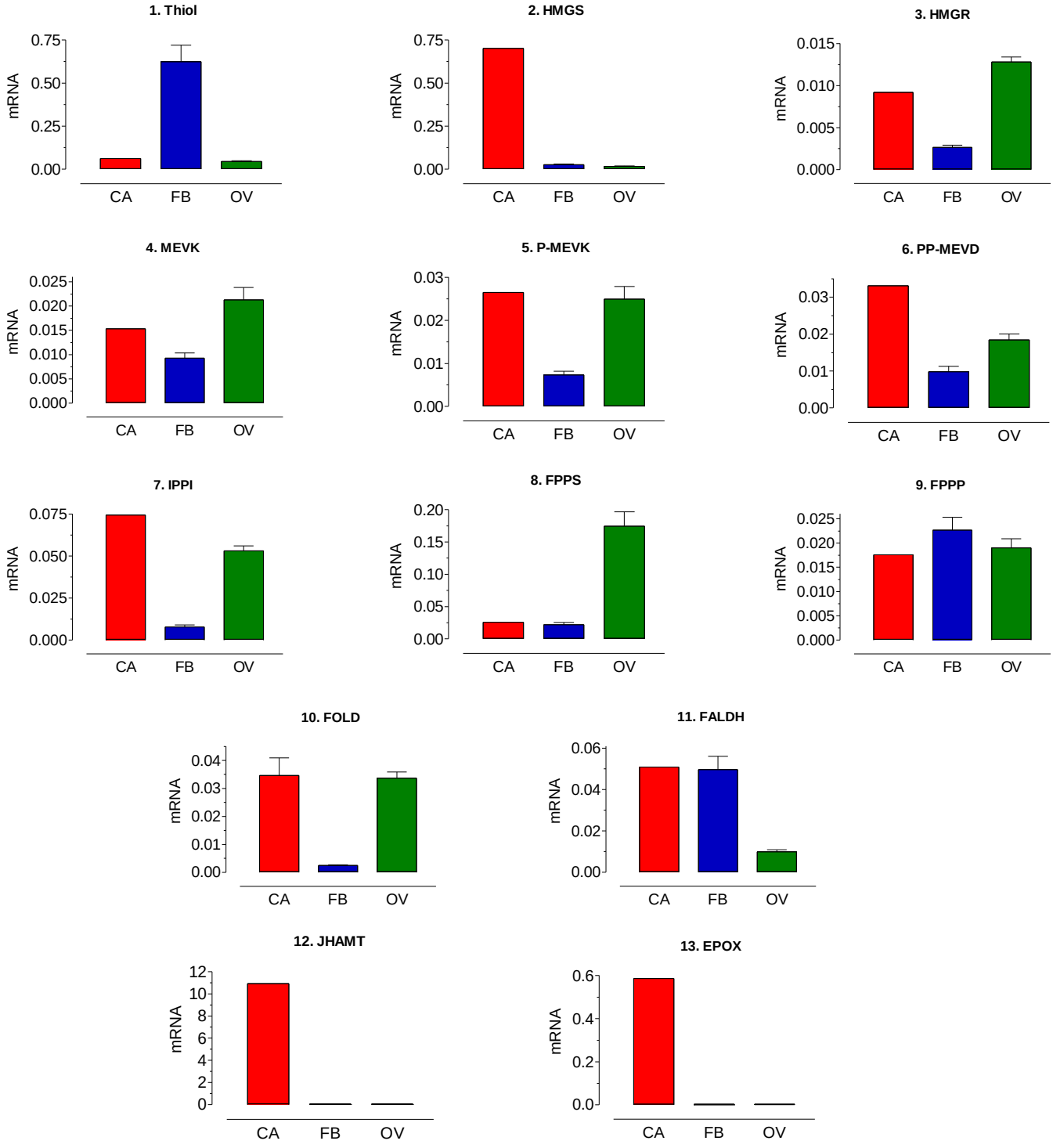
Groups of three pairs of ovaries, 3 fat bodies and 10 CA-CC were dissected in triplicate from females 8 days after a blood-meal. RNA extraction, reverse transcription and quantitative real-time PCR were performed as described in the Methods section. PCR reactions were run in triplicate. Transcript levels were normalized with rpL32 transcript levels in the same sample. Each RT-PCR data point is average of three independent biological replicates. Accession numbers for the different enzyme genes are included in Supplemental Table S4. CA: *corpora allata* complexes. FB: fat body. OV: ovaries



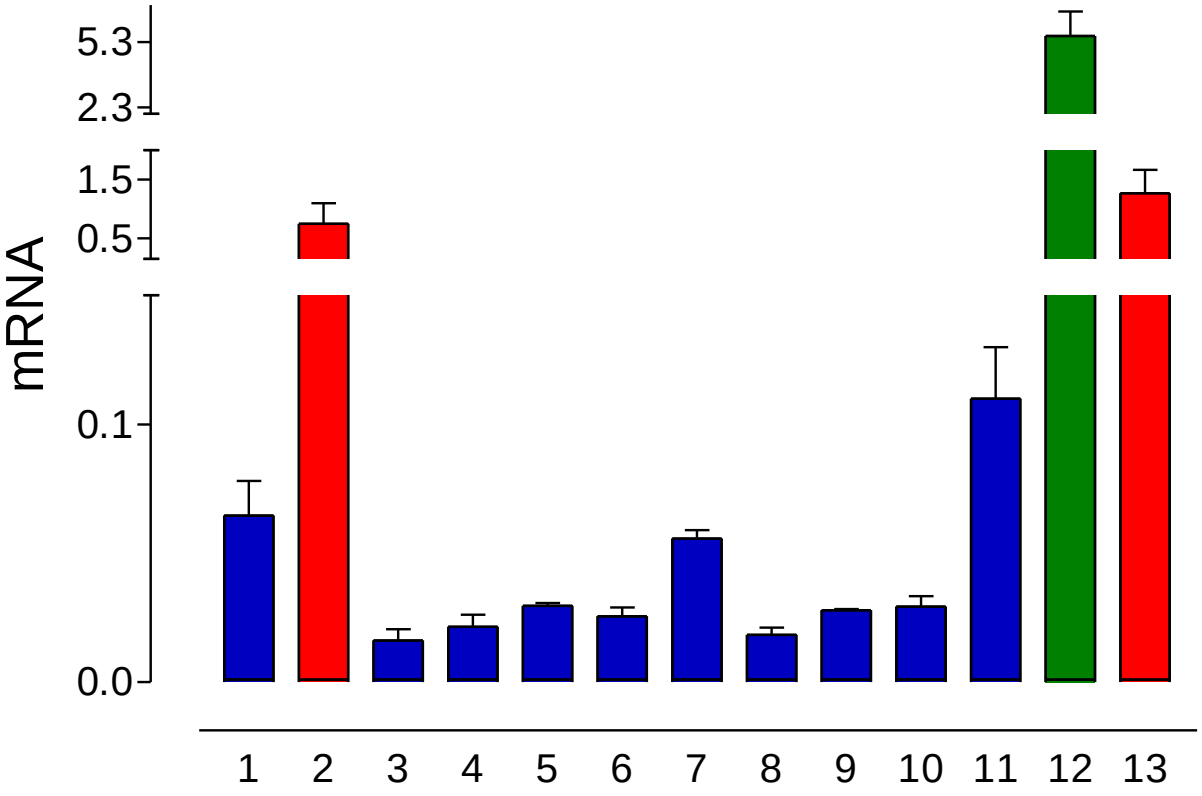
Supplemental Fig. S2. Developmental changes in the expression of FOLD enzymes in the CA. Expression of FOLD enzymes mRNAs in CA of 4th instar of *R. prolixus*. Nymphs were blood-fed and CAs were dissected at different days after blood feeding until molting to 5th instar (10 days later). Enzyme mRNA bars represent the number of transcripts detected by RT-qPCR, and normalized using the expression of the rpL32 gene. Each RT-PCR data point is average of three independent biological replicates of 15 CA complexes.



Supplemental Fig S3. **Tissue specificity of the mRNA for the JH biosynthetic enzymes.** Groups of three pairs of ovaries, 3 fat bodies and 10 CA-CC were dissected in triplicate from females 8 days after a blood-meal. RNA extraction, reverse transcription and quantitative real-time PCR were performed as described in the Methods section. PCR reactions were run in triplicate. Transcript levels were normalized with rpL32 transcript levels in the same sample. Each RT-PCR data point is average of three independent biological replicates. The primer sequences and accession numbers for the housekeeping gene 60S ribosomal protein L32 and for the different enzyme genes are included in Supplemental Table S3. CA: *corpora allata* complexes. FB: fat body. OV: ovaries.



Supplemental Fig. S4. Transcript levels for the JH biosynthetic enzymes in CA of 4th instar *R. prolixus*. Enzymes were grouped in three categories: Lower abundance (blue bars), moderate abundance (red bar) and higher abundance (green bar). Numbers for the enzyme refers to the position in the JH biosynthetic pathway (Fig. 3). Transcript levels were normalized with rpL32 transcript levels in the same sample. Each RT-PCR data point is the average of three independent biological replicates of mRNA from 10 CA-CC dissected from females 8 days after a blood-meal.



Supplemental Table S1. Parameters for multiple reaction monitoring (MRM) detection of the five most common JH homologs. Qualitative analysis tolerances were ±0.2 min from retention times and ±5% of relative abundances of secondary fragments. LC peak width at base was 0.15 min or lower for all compounds.

	RT	Precursor	Main MRM		2nd MRM		3rd MRM		4th MRM	
	(min)	(m/z)	(m/z)	CID (V)	(m/z)	CID (V)	(m/z)	CID (V)	(m/z)	CID (V)
JHB_3	8.60	283	233	10.0	251	7.0	-	-	-	-
$JHSB_3$	8.94	283	233	9.0	205	12.0	145	18.0	119	18.0
JH III*	9.40	267	235	5.0	147	10.0	-	-	-	-
JH II	9.64	281	249	5.0	231	6.0	-	-	-	-
JH I	9.82	295	263	5.0	161	12.0	-	-	-	-

* Detection of JH III-D3 internal standard was done on parent ion m/z 270 with same retention time, fragment ions and collision energies than JH III.

Supplemental Table S2. Ultra-high, resolution FT-ICR MS/MS derived parent and fragment ions of the JHSB3 standard. These four channels were utilized in the identification of JHSB3 in the LC-MS/MS experiments.

m/z	Formula	δ (ppm)
283.19039	$C_{17}H_{26}O_4^+$	0.01
233.15362	$C_{15}H_{21}O_2^+$	0.06
205.15860	$C_{14}H_{21}O^+$	-0.44
145.10110	$C_{11}H_{13}^+$	-0.53
119.08544	$C_9H_{11}+$	0.73

Supplemental Table S3: Accession # and primer sequences.

Gene	Accession #	Primer forward (5' → 3')	Primer reverse (5' → 3')
Acetyl-CoA-thiolase	RPRC007496	FW: CAAAGTTAATGTACACGGTGGTG	RV: CTCCAGACTTCAACGCTGTTA
HMG-CoA reductase	Supercontig RproC3:KQ034226 minus strand 878233-875494	FW: GGCATAGAAAGAAGATGACCAAAC	RV: GCACGAGTATCAAGACAACAATATG
HMG-CoA synthase	RPRC007884	FW: GCAACTGTTTGAAGAAAGTGGTA	RV: AAGCACTGGTACCTCCAAAG
Mevalonate Kinase	RPRC014277	FW: GAAAGATCAAGAGGAACGAGGAG	RV: CGCTTATGTGAGACACCTAATGAT
Phosphomevalonate Kinase	RPRC006212	FW: AAATCGTTTCTGACGAACAAGTG	RV: GCAATGACAACATCCCATTCAG
Diphosphomevalonate decarboxylase	RPRC012093	FW: CGTGGCCTTCCAGTTCAA	RV: GTATTTGAGGAGACCAGGTTCG
Isopentenyl diphosphate isomerase	RPRC003718	FW: CACCAATTACGCCTTGGTTTAG	RV: GTGGATATTCACGTGGTCTTGA
Farnesyl diphosphate synthase	RPRC014226	FW: CGCAGTAGTTGCAATGCATAAAG	RV: GCTTCTTGACAGCGGCTATT
Farnesyl diphosphate pyrophosphatase	RPRC004412	FW: GCTTGAATCCTAGAAGAGCGTTA	RV: ACCGGTAAGTACAAGCAATGTAT
Farnesol dehydrogenase	RPRC010547-RA	FW: AAACCGAGCGATGTTGT	RV: GTAGGTTGGATAACTAGTTCTGAT
Farnesal dehydrogenase	RPRC002910	FW: AGTACCTTACAGTCTAGTATTTGCC	RV: GATCTGTCTTCAGCACCGTT
Juvenil hormone acid methyltransferase	RPRC011659-RA	FW: GGACCAGGCGATGTTACTTT	RV: CCAAATCATCAGAAATATCGCTTCC
Methyl farneseoate epoxidase	RPRC000513	FW: CGGAGAATTGATTCATGATGATTGG	RV: GTAACGGCGGTGACAGTAAA
L32	RPRC014419	FW: ACATGCTTCCTACTGGTTTCA	RV: GACACACCATGCGCTATCT

Supplemental Table S4: Information on FOLD genes.

Sequence	Sequence		BLAST Score	Expressed in CA	Contig
Aedes_SDR1	RPRC010112-RA|1-720	RprSDR1	45	-	KQ034192
Aedes_SDR1	RPRC014554-RA|1-729	RprSDR2	41	+	KQ034087
Aedes_SDR1	RPRC011354-RA|1-726	RprSDR10	40	N/A	KQ034121
Aedes_SDR1	RPRC014549-RA|1-750	RprSDR3	42	+	KQ034087
Aedes_SDR1	RPRC014553-RA|1-723	RprSDR4	42	+	KQ034087
Aedes_SDR1	RPRC011377-RA|37-744	RprSDR5	36	-	KQ034121
Aedes_SDR1	RPRC011360-RA|1-708	RprSDR6	38	-	KQ034121
Aedes_SDR1	RPRC007083-RA|1-750	RprSDR7	35	-	KQ034115
Aedes_SDR1	RPRC011398-RA|34-561	RprSDR8	36	-	KQ034121
Aedes_SDR1	RPRC010547-RA|82-510	RprSDR9	43	+	KQ034899

In the Supplemental Table S4are included the 10 sequences with highest scores after BLAST with the FOLD from *Aedes aegypti* (Ae-SDR1), which is a CA farnesol dehydrogenase. Reference #16: Mayoral, J.G, Nouzova, M., Navare, A. & Noriega, F.G. NADP+-dependent farnesol dehydrogenase, a corpora allata enzyme involved in juvenile hormone synthesis *Proc Natl Acad Sci USA* **106**, 21091-21096 (2009).

The *Rhodnius* homologs are named RprSDRs.

Column 2 shows the accession numbers and names of the genes.

Column 3 shows the BLAST score (similarity).

Column 4 shows the expression of the genes in CA measured by qRT-PCR (see manuscript). RprSDR2, 3, 4 and 9 are expressed in CA.

Column 5 shows the contig location in the *R. prolixus* genome. RprSDR2, 3 and 4 are present in the same contig location. They are probably the product of gene duplication events. Based on the expression patterns, RprSDR4 or RprSDR9 are most likely the FOLD expressed in the CA that is part of the JH biosynthesis pathway.

RprSDR5, 6, 8 and 10 are present in the same contig location. RprSDR5, 6 and 8 are not expressed in the CA, so although we did not study the expression of RprSDR10 in CA (N/A: not analyzed), this gene is not expressed in CA.