Supplementary Table 1. Primer sequence information. T7 promoter sequence is underlined.

Primer name	Sequence 5'-3'
intronVg1 F	TTTGCGAAACTACTCCTCCTCGCC
intronVg1 R	TTGTAGACGTATTCGCGGTTCGGT
intronVg2 F	TGGTGGAAGTTTGTTCTCATCGTT
intronVg2 R	ACCACCGTCCATCAACTTCTCGTA
intron2Vg1 F1	ACGAACGTCTGGGAACTTATGCCT
intron2Vg1 R1	ACTCGGTGTCAGCATTCTGGAAGT
intron2Vg2 F1	TACCTTGGCCACACCGAATCGTAA
intron2Vg2 R1	AGCAGGTTCCGGTTTCATAGTCGT
AgVg1intron1 F1	CAACTGCGAACAACCGAACCATGA
AgVg1intron1 R1	CACCAAATAGCCGTGCGTCACAAT
AaVgB intron1 F1	AGCTACTGGATCTTGGTGACAGGA
AaVgB intron1 R1	TGTAGCTGTAGCCCTGGTTGTTGT
AaVgC intron1 F2	CCAGTGATCCAGGGAATCATGTTAG
AaVgC intron1 R2	ATTACCAGGTAGGCACGGGTGAAA
AaVgA1intron1 F1	ACAGCTGCCGGATACTTGAAGACA
AaVgA1intron1 R1	TTGTAGCCAGCATCGTAGCCGTAA
rtAgVg F1	GCCAGAAGCTGTTTGTTTCGCAGA
rtAgVg R1	GGCAGCTTCATTACCACGCAGTTT
rtAgS7 F2	TGCACCTGGATAAGAACCAGCAGA
AgS7 R1	AGCTGCTGCAAACTTCGGCTATTC
T7 AgUPF1 F3	TAATACGACTCACTATAGGGCATTATAGTGGGCAACCCGA
T7 AgUPF1 R3	TAATACGACTCACTATAGGGGGGATTGAGCGTATTGATGA
T7AgUPF1 F5	TAATACGACTCACTATAGGGGTCGACGCTTACGACGGA

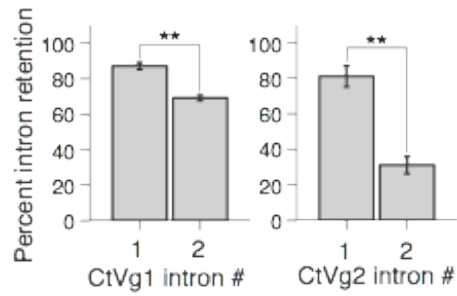
Supplementary table 2. Vitellogenin intron 1 and 2 lengths in base pairs (bp).

species/gene	intron 1 (bp)	intron 2 (bp)
CtVg1a	63	61
CtVg1b	64	59
CtVg2a	62	75
CtVg2b	62	75
AgVg1-3	99	74
AaVgA	70	57
AaVgB	82	59
AaVgC	67	68

Supplementary methods

The cycling parameters for end-point RT-PCR were 94 °C for 3 min, followed by 30 cycles of: 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, followed by a final extension of 72 °C for 3 minutes. The cycling parameters for quantitative PCR were 50ºC for 2 min, 95ºC for 15 min, followed by 40 cycles of 95ºC for 15 sec, 60ºC for 30 sec, 72ºC for 30 sec, followed by a melt curve ramping from 72ºC to 95ºC at the end of the 40 cycles. *AgVg* qPCR data was quantified relative to *AgS7* using Q-Gene (1).

1. Simon, P. (2003) Q-Gene: processing quantitative real-time RT-PCR data. *Bioinformatics (Oxford, England)*, **19**, 1439–1440.



Supplementary figure 1. Percent retention of the first and second intron of *CtVg* genes during combined non-reproductive stages (larvae and pupae) in *C. tarsalis*. Data were quantified from RT-PCR products using intron spanning primers, *Ct*Vg1intron1 and intron2 (left graph) and *Ct*Vg2intron1 and intron2 (right graph). The bar graphs represent the mean ±SEM. **$p<0.0001$, Student's t test.

Translation of first exon and retained intron and second exon (partial):

C. tarsalis

*Ct*Vg1a **Met** F A K L L L L A L G K Y **Stop** Q N H S **Stop** K N I W T N I N L S N A
*Ct*Vg1b **Met** F A K L L L L A L G K Y **Stop** Q N H S Y K K H L D **Stop** H Q P S K R
*Ct*Vg2a **Met** W W K F V L I V L G K L I V K I **Stop** P T Y K L N G **Stop** I Y L F Q
*Ct*Vg2b **Met** W W K F V L I V L G K L **Met** V K **Met** K ...(36 aa)...T R S **Stop** W T V

A. gambiae

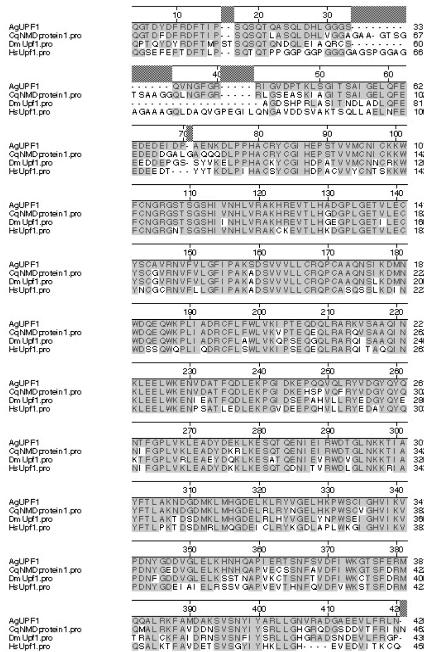
*Ag*Vg **Met** I A K L L L L T L G K C Y S F N R W L F H S K D F I S R A I **Stop** L I C

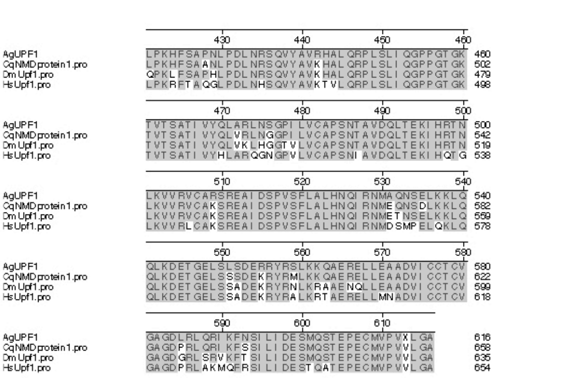
A. aegypti

*Aa*VgA **Met** L A K L L L L A L G K C S R E C S P Q T S N T I L F F P F D F H S G A H
C C L P I R E L V Q G L Q S W L **Stop** G L R C W
*Aa*VgB **Met** L T K I L L L A L G K C S **Stop** P A S T P S A I D Q I I L C N Q F F F I V H
*Aa*VgC **Met** L V K L F L L A L G K F Y L H D Q A S P V S Y R I T S L F I N S G D Q Y
C L S V L I P I R V P L W T S R **Stop** Q N

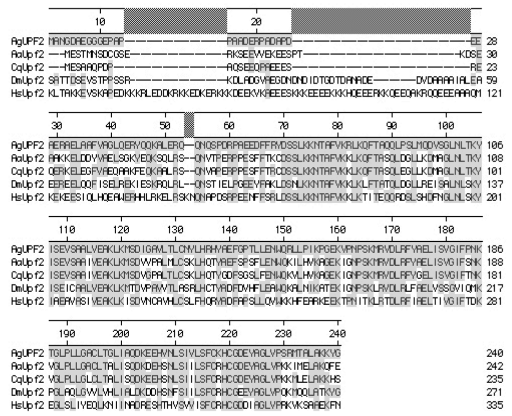
Supplementary figure 2. Translation of intron-retained vitellogenin transcripts from *C. tarsalis*, *A. gambiae* and *A. aegypti* reveals premature termination codons. The translation consists of the first exon (not underlined) followed by the retained first intron (underlined) and in some, partial sequences from the second exon (not underlined). “Stop” indicates a premature termination codon present due to the retention of the first intron in the sequence.

A

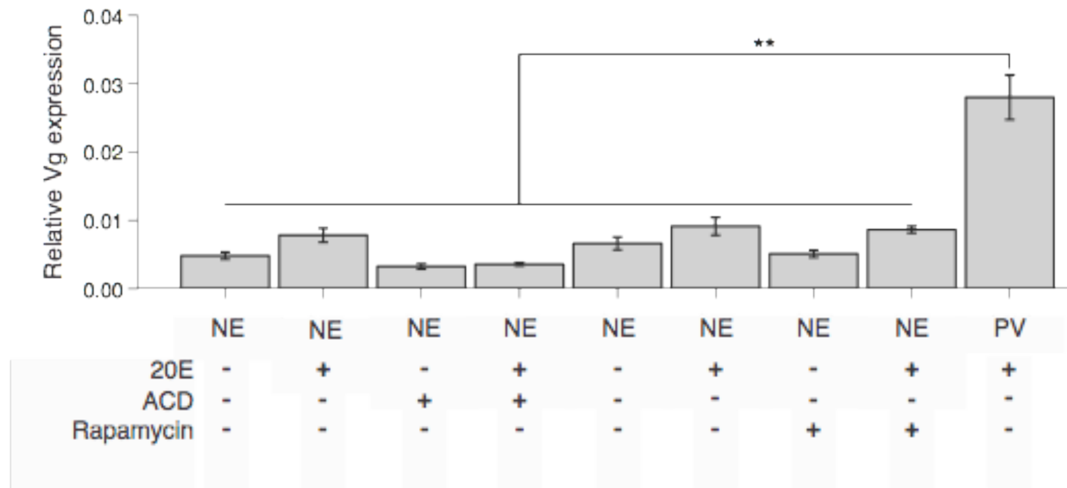




B



Supplementary figure 3. A. Partial amino acid alignment between a cloned *Anopheles gambiae* Upf1 (AgUpf1), predicted nonsense-mediated mRNA decay protein 1 from *Culex quinquefasciatus* (CqNMDprotein1, accession # XP_001862543), *Drosophila melanogaster* Upf1 (DmUpf1, accession # NP_572767) and *Homo sapiens* Upf1 (HsUpf1, accession # AAC51140) . Shaded residues match AgUPF1. **B.** Partial amino acid alignment between a cloned sequence of *Anopheles gambiae* Upf2 (AgUpf2), predicted Upf2 from *Aedes aegypti* and *Culex quinquefasciatus* (AaUpf2 and CqUpf2, accession # XP_001660932 and XP_001851054), *Drosophila melanogaster* Upf2 (DmUpf2, accession # NP_572434) and *Homo sapiens* Upf2 (HsUpf1, accession # EAW86331). Shaded residues match AgUPF2.



Supplementary figure 4. Expression of *AgVg* measured by quantitative PCR in newly emerged fat bodies treated with 20E, ACD and Rapamycin as compared to previtellogenic fat bodies treated with 20E. *AgVg* expression is shown relative to *AgS7*. The bar graphs represent the mean ±SEM. **p<0.0001. NE, newly emerged adult female; PV, previtellogenic adult female; 20E, 20-hydroxyecdysone; ACD, actinomycin D.

Supplementary figure 5. Mosquito fat body culture. The mosquito abdomen is dissected and cut on the ventral side down the anteroposterior axis. Abdomens are opened such that the cuticle is exposed to air and the fat body cells (which adhere to the wall of the abdomen) are exposed to the media. These are then referred to as “fat bodies”. Fat bodies are cultured 5 to a well of a 96-well plate as shown in the picture above. Arrow points to a single fat body.