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### *Identification and temporal expression of putative circadian clock transcripts in the amphipod crustacean Talitrus saltator*

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*Published in:*

PeerJ

*DOI:*

[10.7717/peerj.2555](https://doi.org/10.7717/peerj.2555)

*Publication date:*

2016

*Citation for published version (APA):*

O'Grady, J. F., Hoelters, L. S., Swain, M., & Wilcockson, D. (2016). Identification and temporal expression of putative circadian clock transcripts in the amphipod crustacean *Talitrus saltator*. *PeerJ*, 2016(10), Article e2555. <https://doi.org/10.7717/peerj.2555>

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Danpl-CRY2 -----MSVAETLPLRARSPTAQKSSQPAGVPKEKHTV  
 Tal-CRY2 MSPNKIASPGRSIIHRTILGDNNLTFSESGSSSGLAFTAEEKKNTSKVVS-----KHVV  
 Ep-CRY2 -----MKMTG--TNGSGKAKP-----KNVV  
 Bomte-CRY1 -----MTGSRSEINPDVTVRGE--KHTV  
 : \* : \*

Danpl-CRY2 HWFRKGLRLHDNPALREGLVDATTFRCVFIIDPWFASSSNVGINWRFLQCLELDLKNL  
 Tal-CRY2 HWFRGLRLHDNPALRDAIVNCETFRCIYILDWPWFAGSSNVGVNWRFLQCLELDVNSL  
 Ep-CRY2 HWFRKGLRIHDNPALRLGLRNCTTFRCIFILDWPWFAGSSNVGVNRWRFLQSLLEDLDRSL  
 Bomte-CRY1 HWFRKGLRLHDNPALREGLTGATTFRCVFLDPWFAGSTNVGINWRFLQCLELDLCSL  
 \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 RKLNSRLFVVRGQPADALPKLFREWGTTALTFEEDPEPYGRVDRDHNIMTKREVGVIQVTS  
 Tal-CRY2 RNLNSRLFVVRGQPANALPQLFKEWNTTVLSFEEDPEPFGRARDASIIIGIAQEMGIEVIV  
 Ep-CRY2 RTFNSRLFVIRGQPAKALPELFKEWDTTILTFEEDPEPFGRARDANVVAMAKEMGIKVIIV  
 Bomte-CRY1 RKLNSRLFVIRGQPADALPKLFKEWGTNTLTFEEDPEPFGRVDRDHNISALCKELGISVVO  
 \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 RVSHTLYKLDDIIEKNGGKAPLTYHQFQALIASMPPPPSAEPTISLETNRAVTPISDNH  
 Tal-CRY2 RTSHTLYELDKIIEKNGGKAPLTYKTFQNILAMMDPPPPVAPTEASDLKHAYTPLQHDH  
 Ep-CRY2 RTSHTLYKLDRIIETNGGKAPLTYKSFQNILSHIGPPSKPDSPVNREEINGGITPVSDDH  
 Bomte-CRY1 KVSHTLYKLDEIIEKNGGKAPLTYHQFQNVVASMDPPEPSVSTVTSACIGSAYTPLKEDH  
 : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 DERFGVPTLEELGFDTEGLKPPWIWIGGENEALLRRLERHLERKAWVASFGRPKMTPESLLS  
 Tal-CRY2 DDKYGVPNLEHLGFETEHLPPAVWKGGETEALSRLKHHLERKAWVASFGRPKMTPQSLFA  
 Ep-CRY2 DDKFGVPSLEDLGFDTDHLNPNVWKGGETEALTRLEHHLERKAWVASFGRPKMTPQSLYA  
 Bomte-CRY1 DDHYGVPNLEELGFDTEGLLPPVWKGGESEALARLERHLERKAWVASFGRPKMTPQSLLE  
 \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 SQTGLSPYLRFGCLSRLFFYYQLSELYKRIKQERPPLSLHGQILWREFFYCAATRNPFD  
 Tal-CRY2 CPTGLSPYLRFGCLSARKFYTELNVLYTKIKKVPAPVSLHGHLWREFFYTAATNPKFD  
 Ep-CRY2 SPTGLSPYLRFGCLSPRFYVKNLNFQIKKLPPLSIHGQLLWREFFYCAATNPKFD  
 Bomte-CRY1 SQTGLSPYLRFGCLSRLFFYYQLTDLKIKKAVPPLSLHGQLLWREFFYCAATKPNFD  
 . \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 RMEGNPICVQIPWEKNQEALKKWANGQTGF PWIDAIMIQLRNDGWIHHLARHAVACFLTR  
 Tal-CRY2 HMKGNPICVQIPWDKNPEALAKWAHGQTGF PWIDAIMTQLRTEGWIHNVARHAVACFLTR  
 Ep-CRY2 HMEGNPICVQIPWDKNPEALAKWANGQTGF PWIDAIMTQLRQEGWVHNVARHAVACFLTR  
 Bomte-CRY1 RMQGNPICVQIPWDKNVEALAKWANGQTGF PWIDAIMTQLREEGWIHHLARHAVACFLTR  
 : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 GDLWISWEEGMKVFDELLDADWSVNAGMWMWLSCSSFFQFFHCYCPVRFRKTDPNGD  
 Tal-CRY2 GNLWVSWEEMKVFDELLDADWSVNAGSWMWLSCSSFFQFFHCYCPVRYGRKADPNGD  
 Ep-CRY2 GDLWVSWEEMKVFDEPLLDADWAVNAGSWMWLSCSSFFQFFHCYCPVRYGRKADPNGD  
 Bomte-CRY1 GDLWISWEEGMKVFDELLDADWSVNAGMWMWLSCSSFFQFFHCYCPVRFRKADPNGD  
 \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 FIRKYIPVLKNMPTRYIHEPWCPEEIQKSIRCIIGKDYPMPIVDHTKASEINLERIKQV  
 Tal-CRY2 FIRTYPVLKNFPTRYIHEPWPAPESVQRNARCIIGQHYPLPMVDHGTQSQNNIERMKQV  
 Ep-CRY2 YIRTYLPLKNFPSRYIHEPWTAPGVRQAACIIGIDYPMVMVDHKSQSOHNIERMKQV  
 Bomte-CRY1 YIRRYLPILKNFPTRYIHEPWNAPLSVQRAACIIGKDYSLPMVNHSSSRINIERMKQV  
 : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Danpl-CRY2 YAQLAKFKPQGALIPQMLQRPNVLQSSPSPTSIIANINQSNYLCSSSDVPTPTNQTNTQ  
 Tal-CRY2 YQQLAHYR---ANISTRPCGDSKLRCKYPLH---STA-----  
 Ep-CRY2 YQQLTHYRGTAAPPFSKSMKRNHNIEFPPP---RFEFEKFPKDKREVKTAVD-----  
 Bomte-CRY1 YQQLNKYRGNASLKG---ETVGLLNLALPPPS--VKEKEEEEKRTKQSP--PPPENQSK--  
 \* \* \* : : : . : . : . : .

Danpl-CRY2 FKEDAVFLKPTVNNIKSNVDKQQQFKQVIVQEDKHSENQRHSVGNKYIVNEINKNINDI  
 Tal-CRY2 -----  
 Ep-CRY2 -----  
 Bomte-CRY1 -----MEALAKTTQHQQQHHQH-----

Danpl-CRY2	PVKQNNYDFKALTLNLNKFSNEPLTFLNQTTPNKNESFGQDVNNVIDVYSTSKPKFYFTDN
Tal-CRY2	-----
Ep-CRY2	-----
Bomte-CRY1	-----
Danpl-CRY2	GVITHNENAQTFKRDSYSDNYNKESTGSRVGEVHSNNPQTDKISSEKKN
Tal-CRY2	-----
Ep-CRY2	-----
Bomte-CRY1	-----

**Figure S1. Putative *Talitrus saltator* CRYPTOCHROME 2 (CRY2) protein**

Alignment of *Danaus plexippus* CRY2 (Danpl-CRY2; Accession No. ABA62409) with the *T. saltator* CRY2 (Tal-CRY2) deduced from homology cloning, together with the top two tblastn species homologue sequences *Eurydice pulchra* CRY2 (Ep-CRY2; Accession No. KC885970) and *Bombus terrestris* cryptochrome-1-like (Bomte-CRY1; Accession No. XM\_012312885). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains of one DNA photolyase domain and one FAD binding 7 domain are highlighted in yellow and green respectively.

Drome-PER -----MEGGESTESTHNTKVSDS-----  
Tal-PER MVLGDHRTSNGSSCTQEAMEVLPKLEEAGNQSLPSGVPTDQHVSNSPPESQEDTAAASA  
Ep-PER -----MDTGTPHEDVPSE-----  
Peram-PER -----MEETATHNTKISDS-----

. : \* :

Drome-PER -----AYSNSCSNSQSQRS-GSSKSRLSGSHSSGSSGYGK-PSTQASSSD  
Tal-PER IESDPEGRRQQPQNGQDPTNPCSQADQGYGSTESSFNG-QSRKSSSYSGGSLTKSRNSG  
Ep-PER -----DHTLEEGDSKNPSCQQESAYGSLESSSNG-QSQKS----FGGSGSKSLNSG  
Peram-PER -----AYSNS-SNSQSQRSSGSSKSRHS--NSSGSSGYCGHSSSIQSSNE

. . . \* : . \*\* : \* . : \* \* . : . . .

Drome-PER -----MIKRNKDKSRKKKKNKGAGQGAGQAQT LISASTSLEGRDEEKPRPSGTGCVEQQ  
Tal-PER SSQSSGFGEQHKKDAHTASITSSDKESATRAPHIDTQHSNDGNTTEELTDNLA AISCKSKS  
Ep-PER SSHSSGFGDQNDFKG-----IHLHEAKHIALKKKKTGKGGEKVAEIPFQTASEAE  
Peram-PER PFPQPSVTKRNKDKKHKKLLKSSVTTAATATVTSVVTTVSEYTEHENGTSHMSLGVSGT

. . : . . \* . . .

Drome-PER ICRELQDQQHGEDHSEFQAIEQLQEEEEEDQSGSESEADRVEGV-----  
Tal-PER SSVGVDKLHDGAHYGSSTPREKRTKEQKLRDFKRKKVEESHYQTYDSFPRPESYSFAAA  
Ep-PER LSS-----KGNETEKEKETSLEESPAAKEEAIIE-----  
Peram-PER VVPLSGTVAEETEITEAGSEGSVISSHAGVALGAAGVVPATTPG-----

. . . .

Drome-PER -----  
Tal-PER AARASDLPDKHVENFTAPLISLQTKDEFREPLRNFSGPSCSKNDYAIGSKATCLQSASM  
Ep-PER -----  
Peram-PER -----

Drome-PER -----AKSEAAQSFPIPSPLSVTIVPPSMGGCGGVGHAA  
Tal-PER PSASGVKRSLDSSVPGAQQSFFSQCAASTSYTVSSSCRVKNEITDQPTLIYTQALNYIR  
Ep-PER -----KESRYIHPRNSFFEERHGSQTSLVYQTALNYIR  
Peram-PER -----PENEAHQMASLTQTLNSIKMKMKMK---DLSTDI

. . . : . .

Drome-PER GLDSGLAKFDKTWEAGPGKLESMTGVGAAAAGTGQRGERVKEDSFCCVISMHDGIVLYTT  
Tal-PER RIKEGFTNKGQVFQSP--REHLSSNQSEAIADFIKSGSNQRGFTMVLSIRDGTIIRVS  
Ep-PER KIKERSAEQGVFPFASPY--LDQLSSNQ-EDI TEFFKSFISS-RGFTVAISIQDGSVLQVS  
Peram-PER PEETEGHSFSLPMVAEE-KE EHIRNSFDAEPPAHNEGE-----FCVVVSMQDGVVVFTT

. . . : : . . \* . : \* : \* : . .

Drome-PER PSITDVLGYPRDMWLGRSFIDFVHLKDRATFASQITTGIP IAESRGS---VPKDAKSTFC  
Tal-PER TNISEVLGFPEDMVVGHSFIDFVYPRDSVHFSSKIVSGMSLFTKNSSTRAESLMP--PFY  
Ep-PER PTITDILGFPKDMLLGQSFIDFVYPKDSINFSSKI INGLNIPFRN-SAIKDTHTGT--TF  
Peram-PER PSITDVVGFPKDMWLGRSFIDFVHPRDRTA FANHITSGVITPLSNSNPKGGSHPGKNSFY

. . : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-PER VMLRRYRGLKSGGFGVIGRPVSYEPFRLGLTFREAP-----EEARPDMVMSNGTN-M  
Tal-PER CRVRENKCYQASAFDMRGK-NSYKPKITLKFNDTLT----PLEEEVMAPISPFHLPTSD  
Ep-PER CRLRMYGHLKNTGYGIRNKTKLYKPKCIFKSQDAAR----FTDSKSVAHSS-----  
Peram-PER CCLRRYRGLKSTGYGVTEKEVSYLPFQLNMTFRELLPHSNPLEVEGNTSPESVPGGCNSM

\* : : . . : : \* \* : : . . .

Drome-PER LLVICATPIKSSYKVPDEILSQKSPKFAIRHTATGIISHVDSAAVSALGYLPQDLIGRSI  
Tal-PER VLLAEVIPVPSFYQVPDEVIN--GGNFVIRHSASCNFSEYDPAIPFLGHLPQDLTGNSI  
Ep-PER LIIAEIPIESAYKETDEATA--MGSFIRHSASCNFSEYDPEAIPYLGHLPQDLTGNSI  
Peram-PER FLVITAKLIC SAYKHAGETCA--SPKFVTRHLATCKLNVVDPECMPYLGYPHEMLGNSV

. : : \* \* : \* \* . \* \*\* \* : . . \* . . . \*\* : \* : \* : \* : \*

Drome-PER MDFYHHEDLSVMKETYETVMKKGQTAGASFC SKPYRFLIQNGCYVLETEWTSFVNPWSR  
Tal-PER FDCYHPEDLPLLLTIYREIREE---GKPFRESYRFRTFNGSWVLETEWLCFVNPWTR  
Ep-PER FDCYHPEDMSKLNIVYIVKHQ---GKPYRSEPYRFRTFNNSWVTLITWELCFVNPWTK  
Peram-PER LDFYHPEDLPFLKEVYQIVMQEN---GAPFR SKPYRFRSHNGGYILLETEWSSFVNPWSK

\* \* \* \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-PER KLEFVVGHHRVFGGPKQCNVFEA-APTCKLKISEEAQSRNTRIKEDIVKRLAETVSR---  
Tal-PER KIDSIIQGHKVIKGP RDISIYMEQCEGSLSTYSEEVCI IARKARREI IDLLSRPVAASLA  
Ep-PER RIESIIQHRILKVPKDINLFMDPDKALSPLPEDILLEAQAQKEI LDLLMKPVTS---  
Peram-PER KLEFVVGQHRVLKGFENADVFMAPVEDDTLQISEEVLEKSKI IQEEIRSLSEMVKN---  
: : : : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-PER -----PSDTVKQEVSRRCQALASFMETLMDEVSRADLKLELPHENEL  
Tal-PER EVLRHETRHPTVSPMQHENCVGIKVPASKRRRTLAEEMMSCLVNDIHAQGMKNLTSAAKIS  
Ep-PER -----YQDPCKEPMVKKRRRTLADIMSPFVEELDNIKKNSGDYHQTN  
Peram-PER -----NGHLEKQQMSKRCRDLATFMESLMDDITKPDCLKLELPQE-EH  
\* : \* : \* \* : \* : : : : \*

Drome-PER TVSER-----DSVMLGEISPHHDYDYSKSSSTE  
Tal-PER TLPTSGISTSTSTAVTSSAAINNSMAVQTSAPARNAVEFPSASA AVTQAAKGESLLSSSE  
Ep-PER LSMSCGVGRKSVGVSIANEGHMGHGTPLHKNS-LRHCVQSGVMSIITPGQ--ESLNSSIE  
Peram-PER SFSEH-----DSVMLGEISPHHDYDYSKSSSTE  
. : . : : \* \* \*

Drome-PER TPPSYNQLNYNENLLRFFNSKPVTAPAELDPPKTEPPEPRGTCVSGASGPMSPVHEGSGG  
Tal-PER SPSTYSRLNYSATMLRFFQSNPRTASTD-----ASAESKMESSIRGSVDEAKSSSP  
Ep-PER TPSSYQLNYSATLQRFRRSNPKTVSSD-----ESGDSKMEISHPESIGFSTSASA  
Peram-PER TPPSYNQLNYNDNIQRFESKPKTTLS D-----ESGESKIEANRSLMSTDEEGKSG  
: \* : \* : \* \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-PER SGSSGNFTTASNIHMSVNTNTSIAGTGGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT  
Tal-PER KQOYCKFNSVPSGVNKSL-----YMSGYSGSGSDTIYLTRK  
Ep-PER NRKSQS QS QS QS QS Q T Q S Q -----SGSGSGSDNFNFNESNS  
Peram-PER PAADSSLGSSNRKCCSPVN-----GSGSGSGSGHSGSGS-----  
. : . : . : \* \* . .

Drome-PER TGTGTGTGNGTNSGTGTGTASSSKGGSAI PPVTLTESELNKHNDMEKFM LKKHRES -  
Tal-PER YGSKDVTDSGSGESASGPSSASGGAGSANYRHVPLTEEVLSRHNQEMQMLFMERQKKNV  
Ep-PER FRKSN TKCKFAKNEESG---EGQSTDQPYMLPLTEEILSRHNQHMQKFMECQRKNER  
Peram-PER ---AGIGGSAESRRDTSATNTSHG---SYKPPLLTEALLCRHNEDEKMKMVQKHREQR-  
: . . . . \* \* \* : \* : \* \* : \* : : : :

Drome-PER -----GRTEGKSKKSANDTLKMLEYSGPGHGIKRGSSHSWEGEANKPK  
Tal-PER AITTPSRQRERLSRQKLQVKKASRKPPSKCALKRPN SGSRGDNRAHKFPFIEKTSAKQ  
Ep-PER ILNSNK-----YDFKTRTFKHKTKKRERSYAMKRSCTPKVEGN-AHKTHINSSAANS  
Peram-PER -----NKGSERESK-LKKCVHDKLLQEQCHGVKRS GSHSWEGEVYK--  
: \* . : . . .

Drome-PER QQLTLGTDAIKGAAGSAGGAVGTGGVSGGAGVAGGGSGTGVAGTPEGRATTTSGTGT  
Tal-PER SEQTGPSHLPGPLHTTHGNKLTINSFHSGSGSRADHVTRRTDLAVPVI PATCSSEQPQAP  
Ep-PER NKTLHISAMNTAKSS IAPGNEGSKREKSFSS---PMVSRQVPNI PDGRTMKEAGPSFQ  
Peram-PER -----ASKHPhVEGKELNPGSGGGGGVTSGLSQC LGVAAT--GKQYSGVGSVPP  
\* . . . .

Drome-PER GGAGGGGGGAGAAAAGASSVGSSTPGPSSYPTCTQININLWPPFSVGITPPVHSTHTAMA  
Tal-PER YFFPGHPLTPSNGFQNTSLPSNPSTSLSQEPQLPSNPHYPMT P M M M G P H Q T H F M T P Q  
Ep-PER RVLPNYI I HANHTSS PVKSTEP IVGHSTLP PCPVSSAGTFP SAPL M P P Q T T F L P S Q P  
Peram-PER IFQGG-----TNVNLWPPFSVTVTP-----  
. : . : : \*

Drome-PER -QSSFS SAGLFPTFYI PASLTPTS PTRSPRMHKH PHKGGTDMPTTSQQAAAAAAQA---  
Tal-PER LALPLHYMGAYPGMYI PHPSFNQAMYGAGFLMMSNMMMPHFFIQST SADPLHVSPTNLN  
Ep-PER --LPIQYVTGLPGVIYQP--IAPPLFSAPPVMLPNLVFQHTLVQPPMGHMVQVPILTD---  
Peram-PER -----LQPTPCSTHG F A T N-----NIPTP-----  
: . : : : .

Drome-PER -----MPLQYMAGVMYPHPSLFYTHPAAAAATAMMY  
Tal-PER TPSRQADNQSYIAQVAPVERS QSLSQQRHTDHHHSHSHHQQAQLQQPLPHPKSPHG VVH  
Ep-PER -----ERRYFTESEHETTETTHQATKSLQTDKQVVT  
Peram-PER -----PHMASMI PVY  
: :

```

Drome-PER      QPMFPFGMANALQIPERPLGSQSAYN-----KSVYTTT
Tal-PER        LKRSDSRATSVKVEPGSVRGSVASASGQLRCSMSHRPESLRSETDEPGHAEQGLVYVPE
Ep-PER         LR-RPDSQATSVKAEPGSVRGSNASASCKVNAAESIRSYVDDLSELLQNI PNSKQDRSHTP
Peram-PER      IEKRAPNFKNN-----

Drome-PER      PASMTKKVPGAFHVSVTFPAQVQRPSSQSASVKTEPGSSAAVSDPCKKEV-----
Tal-PER        VASHASHFSRSTSVLGEAEFSIASPDKRPSGMEDQHAE--NSSDTVMSDS-----
Ep-PER         NYSQVSPFQSSTSVIAEPESVRSPEKQDRLVEDTETKKNYSSDMILSMDSYSGPDDKSD
Peram-PER      -----

Drome-PER      -----PDSSPIPSVMGDYNSDPPCSSSNPANNKKYTDSNGNSDDMDGSSFSFYSSFI
Tal-PER        ----SPAQDSMPMLSEFSVDTSESIGVIQPPCDDRVLHDFAWLDHIEVTPQLLFYQLH
Ep-PER         EDSPSPDMGTSKDLEEDNERRDGSKMSMKRKDGTRPVLCEPPWIEDVKVTPPELLFRYQLK
Peram-PER      -----

Drome-PER      KTTDGSESPDTEKDPKHKRKLKSMSTSESKIMEHPEEDQTQHGDG-----
Tal-PER        TKELVDVLKNDMAALKTLNQPALVEDQLSSLYQELEIDGELQLDEGITSSSGEMLDVS
Ep-PER         TSELVDVLRQDMDLLKNTKQVKMNMWYKACWIYKFNRTYYTQRNRAHICV-----
Peram-PER      -----

Drome-PER      -----
Tal-PER        TKTSSEAGAARGAKKARSSRYFDKQAILHEVEAAIPPPDLSISHISPYSVARRLPNKHKS
Ep-PER         -----
Peram-PER      -----

Drome-PER      -
Tal-PER        S
Ep-PER         -
Peram-PER      -

```

**Figure S2. Putative *Talitrus saltator* PERIOD (PER) protein**

Alignment of *Drosophila melanogaster* PER (Drome-PER; Accession No. AAF45804) with the *T. saltator* PER (Tal-PER) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Eurydice pulchra* PER (Ep-PER; Accession No. KC885967) and *Periplaneta americana* (Peram-PER; Accession No. U12772). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains of two PAS domains, one PAC domain and one Period C domain are highlighted in yellow, green and blue respectively. The region of Ep-PER that corresponds to the PAC domain is shown in green text, though no PAC domain *per se* was identified in this protein using SMART. Drome-PER DBT/CK1ε binding site is highlighted in pink. Dark red text indicates Drome-PER CLK/CYC inhibitory domain (CCID). Pink text indicates regions in Tal-PER, Ep-PER and Peram-PER corresponding to sequence within both the overlapping Drome-PER DBT/CK1ε binding site and CCID regions.

Drome-TIM MSRRVRLHNNHIWNNQNFDKVKSVMDDLATPQLYSAFSSLGCL**EGD**--**TYVVNPNALAIL**  
 Tal-TIM -----  
 Lotgi -----MVMHVELQATCSALGYL**EGK**--**RYIKEPDCLETV**  
 Trica-TIM -----MSSLLSAELAATCSALGYD**ATSNKY**Y**ADSNTLETV**

Drome-TIM **EEINYKLT**YEDQTLRTF**FRAIGFGQNV**RS**DLIPLLEN**-**AKDDAVLES**VIRILVNLTVPVE  
 Tal-TIM -----RVLQTDWLPLLREHSDSALLDLVLRLLVNLTPAL  
 Lotgi **KDLIRFLK**REDDTC-**DIRRQLGHAG**IVQNDLLQ**LKIKYKDD**AVLFD**TVLRLLVNL**T**QPAY**  
 Trica-TIM **KDLIRYLRR**DD**ESH**-**DIRRQLGEIKV**LQNDLV**PLLKSYWE**ETDLFDVLRLLVNLTPAL  
 : . : . \* . : . : . : . : . : . : . : . : . : . : . : . : . : . : .

Drome-TIM **CLFSVDVM**YRTDVGRHTIFELNK**LLYSKEAF**TEARSTK**SVVEYMKHILES**D-**PKLSPHK**  
 Tal-TIM **LVFHQE**IPE-**DKAGREMYLRLVS**QQQ**GFKEAFT**DAGVWASVAGVLG**SRLQQG**-**SERDDDA**  
 Lotgi **LCFNNQ**IPE-**DKTLRNYLEI**ESHLYN**YKEAFV**DEELFAVLTEKIGDIL**KL**DWEHRQ**EED**  
 Trica-TIM **MLWNEEL**PT-**DKITRNHYLQ**IEDHLK**SYKQ**TFADE**TFWAVL**STRLSK**I**LET**SYAERG**DEN  
 : : : . \* . : . : . : . : . : . : . : . : . : . : . : . : .

Drome-TIM **CDQINNC**LLLLRNILH**IPETHAHC**VMP**MMQSM**PHG**ISMQNT**ILWNL**FIQ**SIDK**LLLY**LMT  
 Tal-TIM **NLI**EMCLVLLRN**V**LAV**PGHQ**-----**DT**TRTSDDADL**HDQV**L**WSL**HLAGI**PD**LLLY**ST**  
 Lotgi **KLT**LERMLILLRN**V**LH**IPDPS**-----**MEKRT**DD**ASL**HDQ**VIW**F**HVS**G**MED**MLLY**IAS**  
 Trica-TIM **SLI**IERILILIR**N**ILY**VPDPN**-----**TEK**RPD**N**AS**I**HDQ**V**L**WAL**HQ**S**G**MLD**IIL**F**IT**S**  
 : : \* : \* : \* : \* : . . : : : : \* : . : : : \* : : : .

Drome-TIM **CPQRAF**WGVT**M**VQL**I**AL**IYK**D**QH**SGD**SSP**MLTSD**PTS**D**SSD**NG**S**NGR**GM**GG**GM**REG**TAA**  
 Tal-TIM **STDES**DL**S**LHTLE**I**IS**L**MLRQ**Q**DPQ**N**LAGS**AL**HRS**AEE**QR**KD**-----  
 Lotgi **ADDER**QL**CM**HILE**I**IS**L**MF**R**E**Q**KPE**V**LAS**AG**V**K**SK**SE**KE**AE**-----  
 Trica-TIM **STNEK**AY**M**HTLE**I**IS**F**ML**R**E**Q**K**A**E**L**ARA**A**L**Q**R**S**E**T**E**K**M**R**D-----  
 . : . : : : : : : : \* . : . : . : . : .

Drome-TIM **TLQEV**SR**KG**Q**EY**Q**N**AMAR**V**PA**D**K**P**D**G**SE**E**AS**D**M**T**G**N**D**S**E**Q**P**G**S**P**E**Q**S**Q**P**A**G**E**S**M**D**D**G**D**Y**E**  
 Tal-TIM -----  
 Lotgi -----  
 Trica-TIM -----

Drome-TIM **DQRHR**QLNEHG**E**DEDE**VE**EEEE**YLQ**LG**P**ASE**PL**NLT**Q**Q**P**AD**K**V**N**NT**T**N**P**T**S**SA**P**Q**G**CL  
 Tal-TIM -----**AEAL**V**Q**V**R**Q**A**E**K**ARR**Q**Q**V**R**K**H**Y**NAR**H**S**R**F  
 Lotgi -----**ERE**LEM**I**RE**Q**ERAK**R**RA**E**FL**K**Q**S**-**SR**H**S**R**F**  
 Trica-TIM -----**EA**ELL**A**I**R**H**R**E**T**N**Q**Q**K**I**K**LY**NG**AR**H**S**R**F  
 : . : . : . : . : .

Drome-TIM **GNEP**FK**PP**PL**P**VRA**S**T**S**A**H**A**Q**M**Q**K**F**NESS**Y**ASH**V**SA**V**KL**G**Q**K**SP**H**AG**Q**L**Q**LT**K**G**K**CC**P**Q  
 Tal-TIM **GG**-----  
 Lotgi **GG**-----  
 Trica-TIM **GG**-----  
 \* .

Drome-TIM **KREC**PS**Q**SELSD**CG**Y**TQ**VEN**Q**ES**I**ST**SS**N**DD**D**G**P**Q**G**P**Q**H**Q**K**PP**C**NT**K**PR**N**K**P**RT**I**MS  
 Tal-TIM -----**TY**V**V**Q**N**MS**I**SD**R**D**V**IA**H**K**P**V**A**D**V**NS**F**N**F**D**Q**N**K**R**G**K**I**P**K**NR**A**P  
 Lotgi -----**TY**V**V**K**N**L**K**S**I**S**E**R**E**M**I**Y**H**K**A**G**D**VE**A**IT**L**N**D**K**R**R**T**K**I**AK**N**R**Q**P  
 Trica-TIM -----**TY**V**L**K**S**M**K**S**I**S**D**N**E**L**I**Y**H**K**P**L**N**K**L**E**A**L**N**F**D**AD**K**R**K**P**K**T**P**K**N**R**M**P  
 : . : \* \* \* . . . : . : . : . : .

Drome-TIM **PMD**KK**L**RR**K**LV**K**RS**K**SSL**I**N**M**K**L**V**Q**HT**P**T**D**DD**I**S**N**LL**K**E**F**T**V**D**F**LL**K**G**Y**S**Y**L**V**E**L**H  
 Tal-TIM **LP**DS**A**V**T**RR**S**T**L**A**I**R-----**L**F**L**Q**E**F**C**V**E**F**L**NG**A**Y**N**N**I**M**S**I**V**K  
 Lotgi **IK**N**T**E**V**C**R**R**S**T**L**S**I**R-----**L**C**L**K**E**F**C**V**Q**F**L**E**N**C**Y**N**P**I**M**F**A**V**K**  
 Trica-TIM **V**Q**S**ST**F**ERR**S**A**F**S**I**R-----**L**F**L**K**E**F**C**V**E**F**L**NG**A**Y**N**T**L**M**Y**H**V**K  
 . . \* . : \* \* : \* : \* \* \* . : . : .

Drome-TIM **MQL**LS**N**AK**V**P**I**D**T**SH**F**W**L**V**T**Y**FL**K**F**AA**Q**L**E**L**D**M**E**H**I**D**T**I**L**T**Y**D**V**L**S**Y**L**T**Y**E**G**V**S**L**C**E**Q**L  
 Tal-TIM **D**N**L**N**R**AR**V**Q**E**H**D**ES**Y**L**W**AM**K**F**F**M**E**F**N**R**H**E**F**K**V**E**L**V**T**E**T**L**S**I**Q**S**V**H**Y**V**Q**T**N**I**E**T**Y**H**E**M**M**  
 Lotgi **D**N**L**V**R**E**K**T**Q**D**H**D**E**T**Y**L**W**S**V**R**F**F**M**E**F**C**R**F**H**S**K**R**V**E**L**V**S**E**T**M**S**T**T**T**F**H**I**Y**T**N**L**L**N**Y**E**M**M**  
 Trica-TIM **D**N**L**V**R**ARA**Q**A**H**D**E**S**Y**L**W**AL**R**F**F**M**E**F**N**R**C**Y**K**F**E**V**K**L**V**S**E**T**M**A**V**Q**T**F**R**Y**V**Q**L**T**E**K**Y**F**D**M**I**  
 : \* \* : : : \* : : \* : \* . : . : . : . : . : .

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Drome-TIM      ELNARQEGSDLKPYLRRMHLVVTAIREFLQAIDTYNKVTHLNEDDKAHLRQLQLQISEMS
Tal-TIM        TT----EKKKIPLWSRRRHNGLRAYQEIIMMSLSAMDKSP--DQ-----
Lotgi          IT----EKKEAKVWGRRAHLALKAYQELMLTLDSDMRSG--NPQVMESKVIKGNLFYMM
Trica-TIM      QS----DKKNAVLWSRRLHLALLAYRELFLTLCAMDKSP--DETVRDSARVIKSNIFYIV
                : ..      : * * *   : *  :*:: :: : :: :

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**Figure S3. Putative *Talitrus saltator* TIMELESS (TIM) protein**

Alignment of *Drosophila melanogaster* TIM (Drome-TIM; Accession no. AAC46920) with the *T. saltator* TIM (Tal-TIM) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Lottia gigantea* hypothetical protein partial (Lotgi; Accession No. XM\_009048115) and *Tribolium castaneum* TIM (Trica-TIM; Accession No. XM\_008202829). 3' sequence removed from all sequences except Tal-TIM. '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure the SMART identified Timeless domain is highlighted in yellow.



```

Drome-CLK      -----GMEKVDANAYELVKFVGYFR-----NDTNTSTGSSSEV
Tal-CLK        -----QQHQQQQPQQHQ
Macro-CLK     -----SVSSCSRSMFGGNAG-----GGVTGSSNAGSGL
Ep-CLK        PEEYRERYTIAIHIQRGGMTPTAVPVYERIHILTGYFERYKCPSEDGVLDFSCSEAEDSSM
                . .

Drome-CLK      SNGSNGQPAVLPRIFQQNPNAEVDKLVFVGTGRVQNPQLIREMSIIDPTSNEFTSKHSM
Tal-CLK        QPQHPPQRPQEQQQQLQRNDQPEQNQPVFVGICRLEQPQLIREMRLLERTNTEFVSRHSL
Macro-CLK     CQSS-----LVQTNPSQEP-TKLVFVAIGRLERPQLVREMMIIEPSKTEFTSRHSL
Ep-CLK        SVGGTSRSSNPSPFTHSQHHSPEPTKIVFVAIARLEHPQLLRELIVLEPTKSEFTSRHSL
                . . . . .
                .: ***. *:::***:***: ::: :..**.*:***:

Drome-CLK      EWKFLFLDHRAPPIIGYMPFEVLGTSGYDYYHFDDLDSIVACHEELRQTGEGKSCYYRFL
Tal-CLK        EWKFLFLDQRASAIIGYLPFEVLGTSGYDYYHVDDLERVSTCHQFLIRTGKSSSCYYRFL
Macro-CLK     EWKFLFLDHRAPTIIGYLPFEVLGTSGYDYYHVEDLDKVASCHEQLMKTGKGTSCYYRFL
Ep-CLK        EWKFLFLDHRAPTIIGYLPFEVLGTSGYDYYHVDDLKLVSECHEMLMKKKGKISCFYRFL
                *****:*. .****:*****:*****:***: : **:* .:* * **:*

Drome-CLK      TKGQQWIWLQTDYVVSYHQFNPKPDYVCTHKVVSYAEVLKDSRKEGQKSGNSNSITNNG
Tal-CLK        TKGHQVWVWLQSHYYISYHQWNSKPEFVCTNTVVSYDDIKAEKLNSSSTNYNDSSTNLQTS
Macro-CLK     TKGQQWIWLQTYIITYHQWNSKPEFVCTNTVVSYS DVKAEKLVKEQMPNGLSELEINQS
Ep-CLK        TKGQQWIWLRSHYCITYHQWNSKPEFVCTNTVVSYGNIKGNQONHSEEREKSDMEFES
                ***:***:***:.* :*:***:***:***:***:***: : : . . . .

Drome-CLK      SSKVIASTGTSSKSA-----
Tal-CLK        SLTEDKTVSSSCPSQNCTFAEKIDPETKCKSEQNQRFFQKIQSFEEQCMNSSSRAEQSSS
Macro-CLK     ES----SMGLSGAGP-----
Ep-CLK        HSQP-TSEGGGSFAP-----
                : . . .

```

**Figure S4. Putative *Talitrus saltator* CLOCK (CLK) protein**

Alignment of *Drosophila melanogaster* CLK (Drome-CLK; Accession No. AAC62234) with the *T. saltator* CLK (Tal-CLK) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Macrobrachium rosenbergii* CLK (Macro-CLK; Accession No. AY842303) and *Eurydice pulchra* CLK (Ep-CLK; Accession No. KC885976). 3' sequence removed from all sequences. 5' sequence removed from all sequences except Tal-CLK to aid alignment. '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains consisting of one PAS domain and one PAC domain are highlighted in green and blue respectively.

Drome-CYC -----MEVQEFCE-----MEEIEDENYDEEKSA RTS-DENRKQN  
 Tal-BMAL1 -----SVASLSSDGVN-----IKKKLPTLGESHNDELDLDCSKNNRSSAEWNKRQN  
 Pacle-BMAL1A MFGMSNFYDYPKYRSECNSLASFSSDGGSKKRRGSFIDSNDDDADSIKIPRTAGDWNKRQN  
 Ep-BMAL1 MFGVVGFDPYPEYRSDSASGASYPSEGASKKRRGSTAESTEDDKSSKMPTSTDWNKRQN  
 . . .: . : : \* \* \* : : \* : : \*

Drome-CYC HSEIEKRRRDKMNTYINELSSMIPCFAMQRKLDKLTVLRMAVQHLRGIRGSGSLHPFNGL  
 Tal-BMAL1 HSEIEKRRRDKMNTYISELSRMIPQC--QSRKLDKLSVLRMAVQHIKMLRG--SINSYTE  
 Pacle-BMAL1A HSEIEKRRRDKMNTYIMELSSIIPVC--TSRKLKLDKLTVLRMAVQHMMLRG--SLNSYTE  
 Ep-BMAL1 HSEIEKRRRDKMNTYIMELSSIIPVG--TSRKLKLDKLTVLRMAVQHMMLRG--SLNSYTE  
 \*\*\*\*\* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-CYC SDYRPSFLSDQELKMIILQAS-EGFLFVVGCDRGRILYVSDSVSSVLNSTQADLLGQSWF  
 Tal-BMAL1 GQYKPSFVSDQEQVQLLQQQCTEGFLFVVGCDRGKILFVSESVSHILQYSQCELLGLSWF  
 Pacle-BMAL1A GHYKPAFLSDDLKKNLILQAA-DGFLFVVGCDRGRILYVSESVYQTLHYTQCELLGTSWF  
 Ep-BMAL1 GHYKPSFLSDDLKKNLILQAA-DGFLFVVGCDRGRILYVSESVSQTLYTHSELLGTSWF  
 . : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-CYC DVLHPKDIGKVKEQLSSLEQCPRERLIDAKTMLPVKT-----DVPQSLCRL  
 Tal-BMAL1 DILHPKDLTKVKEQLSCGDISRRERLVDAKTLLPVHHPNSSSSSSCGNYPSPQLDTRL  
 Pacle-BMAL1A DILHPKDLTKVKEQLSCSDISRRERLVDAKTLLPVKT-----DVPQGLTKL  
 Ep-BMAL1 DILHPKDLAKVKEQLSSADINRRDRLVDAKTLLPVKT-----DVPQGLSKL  
 \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-CYC CPGARRSFFCRMKLRTASNQIKEESDTSSSSRSTKRKSRLLTGHKYRVIQCTGYLKS  
 Tal-BMAL1 CPGSRRAFYARIRRPNVH---KAPADDASD-----DASAMTGDKRYMSIHFTGYLKS  
 Pacle-BMAL1A CPGSRRAFFCRMRCKTSP--VLKEEADSSTG--CPRKSKSQSSDKKYSVIHFTGYLKS  
 Ep-BMAL1 CPGFRRSFFCRMRCSAH--VIKEEADTTG--CHKKNKPKHCSDKKYSVIHFTGYLKS  
 \* \* \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-CYC TPIKD-EDQDADSDEQTTNLSCLVAIGRIPPNVRNSTVPLAS--LDNHPNIRHVLFISRHS  
 Tal-BMAL1 HGAFRPAGVGDE-DHDSGDAACLVAIGRLHRPLSCASVFLK-----FVAKLS  
 Pacle-BMAL1A APTKDPLEEDSGSDSDSCNLSCLVAVGRVHQPLLASSVLDTSLGSAAPTOPIEFISKHT  
 Ep-BMAL1 APTKEPVEEETEVDSDSCNLSCLVAVGRVHQPLLSSVPKDTRLSRFLPTKPIEFISKHN  
 \* : : : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-CYC GEGKFLFIDQRATLVIGFLPQEIIGTSTFYEYFHNEAALMESHKVMQVPEKVTQVYR  
 Tal-BMAL1 PEAKYNYVDQRMSVVLGWLQEVLGASVFEIHSPSDHCRLAHAHRALLSKTTVVESELQHR  
 Pacle-BMAL1A NDGKFVVIDQRASLLGLWLPQELLGSSMYEYFHQDDIAFLAETHKTTLQNSESCNTQVYR  
 Ep-BMAL1 SEGKFVVIDQKASLLGLWLPQELLGTSMYEYHQQDDIPILAEAHRLLLQANEPTQVYR  
 : : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \*

Drome-CYC FRCKDNSYIQLQSEWRAFKNPWTSEIDYIIAKNSVFL-----  
 Tal-BMAL1 CRHKDGRWRLEGSWKLFINPWTNELEYIVASNTVFSDDKL---SSTDDSSQRNSLQY  
 Pacle-BMAL1A FRTKEGSFVRLQSNWWTFKNPWTKEIYIISKNVVTSEAEELVESTMANESVS-QSYN  
 Ep-BMAL1 FRTKDGSFMNFKSMWKTFKNPWTKDVEYIISNNFLILSEGE-VESTMVSDCASSQSF  
 \* \* : . : : : \* \* \* \* : : \* : \* : \* : \*

Drome-CYC -----  
 Tal-BMAL1 GDSVPPDP-----SLSPSLPSTPLGATLQQNGGSAVPDAPAS-----TCGS  
 Pacle-BMAL1A SEYLNSPDPNHNQDTPIGGAGSNNSRLIGGGIQAGKIGRGIADVLNDQWKNSTCK  
 Ep-BMAL1 SEFINSPG-----GGSSSVSSSRMMGNVQANKIGRkiaEILDPHRRPESPAS

Drome-CYC -----  
 Tal-BMAL1 DVTDSYTVIQGQAVGSSSLASIQMARAVSGDSDDTASQCRASGAGEMVSDQVSTEGKDA  
 Pacle-BMAL1A SPVSPFEGILGSGASDRSFAALLRSDMTAHRNTVKNNAVLSSNTSTCSSEDTSRSTQLSN  
 Ep-BMAL1 TPISPLEAAILG-ANSDRSFSSLLKPEPTDSSR--MMN--ICGSSTDTSDSDSTSKQQA

Drome-CYC -----  
 Tal-BMAL1 SSGDGNPLQLN--LDVPARLPFLHHYN-----ARSESEASGVGEATSDDSD  
 Pacle-BMAL1A NHHRLPSMHNNNLINSSEGGFSFHNNSHRQPVS---NEGDLMDVVSGRDLETDTGSDSD  
 Ep-BMAL1 SHKPLLPPQ-----PKPENTLNHTNNHLQQVAQVNNNTDLMDVVSGRDVESEVTSDDSD

Drome-CYC	-----
Tal-BMAL1	EAAMAIIMSLLLEADAGLGGPAD-----
Pacle-BMAL1A	EAAMAVIMSLLLEADAGLGGPVDFSHLPWLP
Ep-BMAL1	EAAMAVIMSLLLEADAGLGEVPDFSHLPWLP

**Figure S5. Putative *Talitrus saltator* BMAL1 protein**

Alignment of *Drosophila melanogaster* CYC (Drome-CYC; Accession No. AAF49107) with the *T. saltator* BMAL1 (Tal-BMAL1) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Pacifastacus leniusculus* BMAL1A (Pacle-BMAL1A; Accession No. JQ670886) and *Eurydice pulchra* BMAL1 (Ep-BMAL1; Accession No. KC885968). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains of bHLH domain, PAS domains and PAC domain are highlighted in yellow, green and blue respectively. Red text indicates sequence with high similarity to C-terminal transactivation domain of *Mus musculus* BMAL1.

Drome-CKII- $\alpha$  -MTLP SAARVYTDVNAHKPDEYWDYENYVVVDWGNQDDYQLVVRKLGRGKYSEVF EAINIT T  
Tal-CKII- $\alpha$  MMPFRSRARVYADVNTLRPQDYWDYESHLEI EWGQQDDYQLVVRKLGRGKYSEVF EAININN  
Harsa-CKII- $\alpha$  -MALPSRARVYADVNSHKPREYWDY ES YVVDWGQQDDYQLVVRKLGRGKYSEVF EAINVTN  
Oruab-CKII- $\alpha$  -MALPSRGRVYADVNSHKPRDYWDY ES YVVDWGQQDDYQLVVRKLGRGKYSEVF EAINVTN  
\*.: \* .\*\*\*:\*\*\*: :\* :\*\*\*\*\*.::::\*:\*\*\*\*\*:.....

Drome-CKII- $\alpha$  TEKCVVKILKPVKKKKIKREIKILENLRGGTNIITLLAVVKDPVSRTPALIFEHVNNITDF  
Tal-CKII- $\alpha$  NEKCVVKILKPVKKKKIKREIKILENLRGGTNIITLQAVVKDPVSRTPALVFEHVNNITDF  
Harsa-CKII- $\alpha$  NEKCVVKILKPVKKKKIKREIKILENLRGGTNIITLQAVVKDPVSRTPALIFEHVNNITDF  
Oruab-CKII- $\alpha$  NEKCVVKILKPVKKKKIKREIKILENLRGGTNIITLQAVVKDPVSRTPALIFEHVNNITDF  
.\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*

Drome-CKII- $\alpha$  KQLYQTLTDYEIRYYLFE LLKALDYCHSMGIMHRDVKPHNVMIDHENRKLRLIDWGLAEF  
Tal-CKII- $\alpha$  KQLYQTLNDYDIRYYLYELLKALDYCHSMGIMHRDVKPHNVMIDHENRKLRLIDWGLAEF  
Harsa-CKII- $\alpha$  KQLYQTLNDYDIRYYLYELLKALDYCHSMGIMHRDVKPHNVMIDHENRKLRLIDWGLAEF  
Oruab-CKII- $\alpha$  KQLYQTLTDYDIRYYLYELLKALDYCHSLGIMHRDVKPHNVMIDHENRKLRLIDWGLAEF  
\*\*\*\*\*.\*\*\*:\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:.....

Drome-CKII- $\alpha$  YHPGQ EYNVRVASRYFKGPPELLVDYQMYDYSLDMWSLGCLASMI FRKEPFFHGHNDYDQ  
Tal-CKII- $\alpha$  YHPGQ EYNVRVASRYFKGPPELLIDYQMYDYSLDMWSLGCLASMI FRKEPFFHGHNDYDQ  
Harsa-CKII- $\alpha$  YHPGQ EYNVRVASRYFKGPPELLVDYQMYDYSLDMWSLGCLASMI FRKEPFFHGHNDYDQ  
Oruab-CKII- $\alpha$  YHPGQ EYNVRVASRYFKGPPELLVDYQMYDYSLDMWSLGCLASMI FRKEPFFHGHNDYDQ  
\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*

Drome-CKII- $\alpha$  LVRIAKVLGTEELYAYLDKYNIDLDPFRFDILQRHSRKRWERFVHSDNQHLV SPEALDFL  
Tal-CKII- $\alpha$  LVRIAKVLGTEELFEYVEKYHVELDPRFNDILGRHSRKRWERFVHSDNQHLV SPEALDFL  
Harsa-CKII- $\alpha$  LVRIAKVLGTEELFEYLDKYHI ELDPFRFNDILGRHSRKRWERFVHSDNQHLV SPEALDFL  
Oruab-CKII- $\alpha$  LVRIAKVLGTEELFEYLEKYHI ELDPFRFNDILGRHSRKRWERFVHSDNQHLV SPEALDFL  
\*\*\*\*\*:\*\*\*:\*\*\*:\*\*\*\*:\*\*\* \*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*

Drome-CKII- $\alpha$  DKLLRYDHVDRLTAREAMAHPYF LPIVN--GQMNPNQO-----  
Tal-CKII- $\alpha$  DKLLRYDHQERLTAHEAMEHPYF APIVKDQGIMIGSPFPQPPPAVISGIQDE-----  
Harsa-CKII- $\alpha$  DKLLRYDHYERLTAHEAMEHPYF YPIVKEQGRLTMVSSSPTPMTGSVPVGDHYRSSWRNN  
Oruab-CKII- $\alpha$  DKLLRYDHYERLTAHEAMEHPYF YPIVKEQGRLTIVSSSPTPMPGSLPLDE-----  
\*\*\*\*\*:\*\*\*\*\*:\*\*\* \*\*\*\*\* \*\*\*: \*:

Drome-CKII- $\beta$  ----MSSSEEV SWVTFWCGLRGNEFFCEVDEDIQDKFNLTGLNEQVPHYRQALDMIL  
Tal-CKII- $\beta$  ----MSSSEEV SWIAWFCGLRGNEFFCEVDEDIQDKFNLTGLNEQVPHYRQALDMIL  
Attce-CKII- $\beta$  MTTE TKMSSEEV SWISWFCGLRGNEFFCEVDEDIQDKFNLTGLNEQVPHYRQALDMIL  
Oruab-CKII- $\beta$  ----MSSSEEV SWISWFCGLRGNEFFCEVDEDIQDKFNLTGLNEQVPHYRQALDMIL  
\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*

Drome-CKII- $\beta$  DLEPEDELEDNPLQSDMTEQA AEMLYGLIHARYILTNRGIAQMIEKYQAGDFGH CPRVYC  
Tal-CKII- $\beta$  DLEF--DEEEDLPHQGD LVEQA AEMLYGLIHARYILTNRGIAQMIEKYQAGDFGH CPRVYC  
Attce-CKII- $\beta$  DLEPDDDLDDNPNQSD LIEQA AEMLYGLIHARYILTNRGIAQMIEKYQAGDFGH CPRVYC  
Oruab-CKII- $\beta$  DLEPDDDI EDNPNQSD LIEQA AEMLYGLIHARYILTNRGIAQMIEKYQAGDFGH CPRVYC  
\*\*\*\* \* : : \* \* . \* . \* : \*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*

Drome-CKII- $\beta$  ESQPMLPLGLSDIPGEAMVKTYCPKCIDVYTPKSSRHHTDGAYFGTGFPHMLFMVHPEY  
Tal-CKII- $\beta$  ENQPMLPIGLSDVPGEAMVKLYCPNCCDVYTPKSSRYNHIDGSYGTGFPHMLFMVHPEY  
Attce-CKII- $\beta$  ESQPMLPLGLSDVPGEAMVKSYPKCM DVYTPKSSRHHTDGAYFGTGFPHMLFMVHPEY  
Oruab-CKII- $\beta$  ESQPMLPLGLSDVPGEAMVKSYPKCM DVYTPKSSRHHTDGAYFGTGFPHMLFMVHPEY  
\* . \*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\* \*\*\*\*\*: \* \*\*\*\*\*: \* : \* \* . \*\*\*\*\*:\*\*\*\*\*

Drome-CKII- $\beta$  RPKRPTNQFVPRLYGFK IHSLAYQIQLQAAANFKMPLRAKN-----  
Tal-CKII- $\beta$  RPKRPGTQFVPRLYGFK IHPMAYQLQQAAANFNAPMPVFNNGKR  
Attce-CKII- $\beta$  RPKRAANQFVPRLYGFK IHPVAYQIQQASASTFKAPLRALNYNNGKR  
Oruab-CKII- $\beta$  RPKRATNQFVPRLYGFK IHPLAYQIQQASASTFKAPLRALTVNYNNGKR  
\*\*\*\*. .\*\*\*\*\*:\*\*\*\*\*:\*\*\*: \* \* : \* . \* : \* : \* . \*

Figure S6. Putative *Talitrus saltator* CASEIN KINASE II  $\alpha$  and  $\beta$  (CKII- $\alpha$  and CKII- $\beta$ ) proteins

Alignment of *Drosophila melanogaster* CKII- $\alpha$  and CKII- $\beta$  (Drome-CKII- $\alpha$  and Drome-CKII- $\beta$ ; Accession No.s AAN11415 and AAF48093 respectively) with the *T. saltator* CKII- $\alpha$  and CKII- $\beta$  (Tal-CKII- $\alpha$  and Tal-CKII- $\beta$ ) deduced from the Trinity *de novo* transcriptome assembly, together with the top two Tal-

CKII- $\alpha$  tblastn species homologue sequences *Harpegnathos saltator* CKII- $\alpha$  (Harsa-CKII- $\alpha$ ; Accession No. XM\_011140473) and *Orussus abietinus* CKII- $\alpha$  (Oruab-CKII- $\alpha$ ; Accession No. XM\_01242321) and the top two Tal-CKII- $\beta$  tblastn species homologue sequences *Atta cephalotes* CKII- $\beta$  (Attce-CKII- $\beta$ ; Accession No. XM\_012208415) and *Orussus abietinus* CKII- $\beta$  (Oruab-CKII- $\beta$ ; Accession No. XM\_012432316). 3' sequence removed from Harsa-CKII- $\alpha$ . '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains of serine/threonine protein kinase catalytic domain and casein kinase regulatory subunit domain are highlighted in yellow and green respectively.

```

Drome-CWO      RQDPLSHRIIEKRRRDRMNSCLADLSRLIPPQYQRKGRGRIEKTEIIEMAIRHLKHLQSE
Tal-CWO        ---PMSHRIIEKRRRDRMNNCLADLSRLIPAIYLKKGRGRIEKTEIIEMAIKHLKHLQAH
Metoc          KRDPMSHRIIEKRRRDRMNNCLADLSRLIPAIYLKKGRGRVEKTEIIEMAIKHLKHLQSH
Ixosc          ARDPMSHRIIEKRRRDRMNNCLADLSRLIPAVYLKKGRGRVEKTEIIEMAIKHLRHLQAH
                *.*****.*****.* :*****:*****:***:***:.

Drome-CWO      CQQKE SDYRSGYDCMKEAAKFLYDVHMQDFCHRLLGRLQEHIDEMFTDCYKSTRSCHM
Tal-CWO        SCKDPATCEVAHR--IETDHRHQYRLGFHECMS-ECVRFVVEIEGMYAGDDLICIRLMNHL
Metoc          ACNDPATCEVAQR--IDTDHRHQYRLGFQECMS-ECVRFVLDIEGRYANDEFICIRLMTHL
Ixosc          SCKDPTTCEVAQR--VDSDHRLQYRLGFQECLS-ETARFLVDLDGSC TADDTCFRLVAHL
                . . . : . . : * : ::: * : .:: * :

Drome-CWO      PDNVSASSGSPHQAYHPPLCHLRDMLATSASDVEHSQDHDNDVKDLSFRNHLNQLQRSQQA
Tal-CWO        QKHFDKVGHGFCYQIPG--GSGPTQ-----NNSSSPNP-----I
Metoc          KKHLDKIQGHNICYQVPGAVGSGPGQSTRTSPVASSSTASNGNSSSSSSGATTPATQIIQ
Ixosc          QKHFDKVSAGASCYPAGLVLSDEEAT-----M
                .:.. *

Drome-CWO      AAAAAVAAA VAVANGSSPASNAGVDSKVPLTNGGGTGGAPPAADNVPSNSTGSGSAAAC
Tal-CWO        QVNTQSASISIQIRLPFKGM-----
Metoc          QTPQQVPAIPIQIHPSSGVVNVMGSPAATTRLSSDDVRSLESEPSSESAFTRRAGGT FAP
Ixosc          EPPERQPKQEPTAALPDGSCS-----SSSSCS-----

```

**Figure S7. Putative *Talitrus saltator* CLOCKWORK ORANGE (CWO) protein**

Alignment of *Drosophila melanogaster* CWO (Drome-CWO; Accession no. AAF54527) with the *T. saltator* CWO (Tal-CWO) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Metaseiulus occidentalis* uncharacterized (Metoc; Accession No. XM\_003744642) and *Ixodes scapularis* conserved hypothetical protein (Ixosc; Accession No. XM\_002400882). 5' and 3' sequence removed from all sequences except Tal-CWO. '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains of bHLH domain and Orange of the Hairy/E(SPL) family domain are highlighted in yellow and green respectively.

```

Drome-DCO      -MELRVGNKYRLGRKIGSGSFGDIYLGTTINTGEEVAIKLECIKTKHPQLHIESKFYKTM
Tal-DBT        -MELRVGNKYRLGRKIGSGSFGDIYLGTTINTGEEVAIKLECIKTKHPQLHIESKFYKMM
Ep-CKI-ε      MMELRVGNKYRLGRKIGSGSFGDIYLGTTINTGEEVAIKLECIKTKHPQLHIESKFYKMM
Cioin-CKI      -MELRVGNKYRLGRKIGSGSFGDIYLGTTINTGEEVAIKLECVKTKHPQLHIESKFYKMM
                *****.*.*.*****.:***** *

Drome-DCO      QGGIGIPRIIWCGSEGDNVMMVME LLGPSLEDLNFNFCRRFSLKTVLLADQMISRIDIYI
Tal-DBT        AGGVGIPAIKWCGSEGDNVMMVME LLGPSLEDLNFNFCRTRKFSKTVLLADQLITRIEYI
Ep-CKI-ε      AGGVGIPSIKWCGSEGDNVMMVME LLGPSLEDLNFNFCRKFSLKTVLLADQLITRIEYI
Cioin-CKI      QGGVGIP TVKWC GAEGDYNVLMVME LLGPSLEDLNFNFCRKFSLKTVLLADQLISRIFI
                **.* ** : **.******:*****.*:* *****:*.**:**

Drome-DCO      HSRDFIHRDIKPDNFMGLGKKNLVYIIDFGLAKKFRDARSLKHIPYRENKNTGTARY
Tal-DBT        HSKNFIHRDIKPDNFMGLGKKNLVYIIDFGLAKKYRDPRTHQHIPYRENKNTGTARY
Ep-CKI-ε      HSKNFIHRDIKPDNFMGLGKKNLVYIIDFGLAKKYRDSRTHLHIPYRENKNTGTARY
Cioin-CKI      HSKNFIHRDIKPDNFMGLGKKNLVYIIDFGLAKKYRDGRTHQHIPYRENKNTGTARY
                **.:*****:*** * : *****

Drome-DCO      ASINHLGIEQSRRDDLES LGYVLMYFNLGALPWQGLKAANKRQKYERISEKKLSTSIIVV
Tal-DBT        ASVNHLGIEQSRRDDLES LGYILMYFN RGS LPWQGLKAATKRQKYERISEKKMQTRIED
Ep-CKI-ε      ASVNHLGIEQSRRDDLES LGYVLMYFN RGS LPWQGLKAATKRQKYERISEKKMQTPIEE
Cioin-CKI      ASINHLGIEQSRRDDLES LGYILMYFNLG LTPWQGLKAATKRQKYERISEKKMSTPIEL
                **.******:***** *:*****.******:.* *

Drome-DCO      LCKGFPSEFVNYLNFRCRQMHFDQRPDYCHLRKLEFRNLFHRLGFTYDYVFDWNLLKFGGPR
Tal-DBT        LCKGFPAEFATYLNVCRTLRFEEKPDYSHLRQLFRQLFHRQGFTYDYVFDWNLLKFVSEH
Ep-CKI-ε      LCKSFPNEFATYLNFCRSLRFEEKPDYSYLRQLFRQLFHRQGFTYDYVFDWNMLKFGGPR
Cioin-CKI      LCKGHPSEFATYLN YCRSLRFDDKPDYSYLRQLFRNLFHRLGFTYDYVFDWNMLKFGNRS
                ***..* **..** ** :*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*.

Drome-DCO      NPQAIQQAQDGADG-----
Tal-DBT        PSSKQHQQQQQ-----
Ep-CKI-ε      NQEEG-DRRDRGSNRPQGT-----
Cioin-CKI      STDDGKDHRTSASRGLVGS SATRQASTQKKL VVRQSSTMSKAKSKVSSWLTSSVVLHPKK
                . : :

```

**Figure S8. Putative *Talitrus saltator* DOUBLTETIME (DBT) protein**

Alignment of *Drosophila melanogaster* DCO (Drome-DCO; Accession No. AAF57110) with the *T. saltator* DBT (Tal-DBT) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Eurydice pulchra* CKI-ε (Ep-CKI-ε; Accession No. KC885972) and *Ciona intestinalis* CKI (Cioin-CKI; Accession No. XM\_002125796). 5' and 3' sequence removed from all sequences except Tal-DBT. '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure a SMART identified serine/threonine protein kinase catalytic domain is highlighted in yellow.

```

Drome-PDP1-ε -----
Tal-PDP1-ε MDTLYSGKNCASLSQMADSTFPSFDGGTTEGAFRASPSCASKDRDGTYPGAVTLLGSNL
Attce-TEF -----
Brafl -----

Drome-PDP1-ε -----MDPQSNAAAAAQLLXPVAMLPLLQRCSCCKWQPG
Tal-PDP1-ε WNKTIPTYDPLKVALEFMDLEEFLENENSIPVDDSTRSSNVLPENS DVEASHNCG-RVTTT
Attce-TEF -----MSDKDRSSPTLVENS LKSLLD-----QP-
Brafl -----

Drome-PDP1-ε IEWECQWNGNAVVAATGNGNGGNPQGNNNNNNNGNSGNSNNNSNNNVSSVQHVANAVAAA
Tal-PDP1-ε VADSCSFASLTITAGASSASSSPLRLNLQTHPGYSPASLHDSNSSTYFPPQSELNFMNSD
Attce-TEF -----SIFPLIGNVNG-----
Brafl -----

Drome-PDP1-ε ----VIANEHHNHLNSLKARFQPASSGKSTSNSKEIIC---PDDKYKE---EGDIW-NVE
Tal-PDP1-ε SKLLLDSDDEAPDSSHMPGGRRSPSPENLTSLSLRLRSMNADDKTVMRSVNSTISSTSE
Attce-TEF -----KSLSNGKEILCKDGP EKKDA---DGELWGNVE
Brafl -----

Drome-PDP1-ε AQTAF LG-----PNLWDKTLPYD---AD-----LKVT-QYADLD-----
Tal-PDP1-ε VSNASSQPF GKASEFPNFDDYSVDFHGV TADQSSGGGENP SMLKIDQTLRDNSEPHQK
Attce-TEF AQAALFG-----PNLWDKTLPYD---AD-----LKVLNHYVDLD-----
Brafl -----

Drome-PDP1-ε -----EFLSENNIP-DGLPGTHLGHSSG--LG--HRSDSLGHAAGLSLGLG
Tal-PDP1-ε IDESCASEITSGSWESLRASSQRCDTTSASTVETTGQERGR-RKNQGRGSAK PQSTGVR
Attce-TEF -----EFLSENGIPVDGVAGGGQGTMQGSQLHKINNT EAAAGHQGPAGLHLE
Brafl -----SPPHISME
:

Drome-PDP1-ε HITTKRERSPSPSD-----CISPDTLNPPSPAESTFSFAS-SGRDFDPRTRAFS
Tal-PDP1-ε HRRRRGITSYAEIDDDMISELCDFTTDKPEQVDDDRITPSQHNPNSKTGEKFNPRTRKFS
Attce-TEF PVT-KRERSPSPSE-----CCSPDTLNPPSPADSTLSMAS-SGRDFDPRTRAFS
Brafl EVI-----PEVEYDVSPTDVALASIP-GKEEFNPRKRAFS
* : : . . : * * * *

Drome-PDP1-ε DEELKPQPMIKKSRKQFVPEDE LKDDKYWARRRKNNI AAKRSRDARRQKENQIAMR RARYLE
Tal-PDP1-ε EDELKPQPMVKKSRKQYVPPDLKDDKYWARRQKNNVAAKRSRDARRVKENQIALRASFL E
Attce-TEF DEELKPQPMIKKSRKQFVPPDLKDDKYWARRRKNNMAAKRSRDARRMKENQIALRAGFL E
Brafl EEELRPQPMIKKSRKIFVPED LKDDKYWERRKNNVAAKRSRDARRIKENQVALRASFL E
: * * : * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Drome-PDP1-ε KENATLHQEVEQLKQENMDLRARLSK FQDV---
Tal-PDP1-ε EMHSLRKLKQLDEANDTISDLRKRLSK YEAV---
Attce-TEF KENMGLRQELDRLKNENMLLRDKLSK YTDV---
Brafl KENATLKEELLKAKEENVILSKLLK YEQQNRV
: : * * * * : : * * * :

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**Figure S9. Putative *Talitrus saltator* Par Domain Protein 1ε (PDP1ε) protein**  
Alignment of *Drosophila melanogaster* PDP1ε (Drome-PDP1-ε; Accession No. AAF04509) with the *T. saltator* PDP1ε (Tal-PDP1-ε) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Atta cephalotes* TEF (Attce-TEF; Accession No. XM\_012203776) and *Branchiostoma floridae* hypothetical protein (Brafl; Accession No. XM\_002601463). '\*' indicates identical amino acid residues in



the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure a SMART identified basic region leucin zipper domain is highlighted in green.

```

Drome-PP1      -----MSDIMNIDSIIISR-----LLEVRGARPGK-----NVQLSE
Tal-PP1       MSSNANTTTPKLS TKERVVKSVPFPPSHKLTCAEVFDPKTKPKFEWLNHFIEGR IEE
Acrec-PPα2    -----MAETDKNIDNIIAR-----LLEVRGARPGK-----NVQLTE
Volem-PPα2    -----MAETDKNIDNIIAR-----LLEVRGARPGK-----NVQLTE
                :   :. . . :   :                               ** . . . **           : : : *

Drome-PP1      SEIRSLCLKSREIFLSQPIILLELEAPLKICGDIHGQYYDLLRRLFYGGFPPESNYLFLGD
Tal-PP1       AAALRIINEGAALLRQEKTMIDIEAPVTVCEDIHGQYYDLLRRLFYGGFPPESNYLFLGD
Acrec-PPα2    VEIRGLCLKSREIFLSQPIILLELEAPLKICGDIHGQYYDLLRRLFYGGFPPESNYLFLGD
Volem-PPα2    VEIRGLCLKSREIFLSQPIILLELEAPLKICGDIHGQYYDLLRRLFYGGFPPESNYLFLGD
                :   :. . . :   :   : : : * : : : * : : : * : : : * : : : * : : : *

Drome-PP1      YVDRGKQSLETICLLLAYKIKYAENFFLLRGNHECASINRIYGFYDECKRRYTIKLWKTF
Tal-PP1       YVDRGKQSLETICLLLAYKIKYPENFFLLRGNHECASINRIYGFYDECKRRYNIKLWKTF
Acrec-PPα2    YVDRGKQSLETICLLLAYKIKYPENFFLLRGNHECASINRIYGFYDECKRRYNIKLWKTF
Volem-PPα2    YVDRGKQSLETICLLLAYKIKYPENFFLLRGNHECASINRIYGFYDECKRRYNIKLWKTF
                ***** . ***** . *****

Drome-PP1      TDCFNCLPVAAIIVDEKIFCCHGGLSPDLQSMEQIRRIMRPTDVPDQGLLCDLLWSDPKD
Tal-PP1       TDCFNCLPVAAIIVDEKIFCCHGGLSPDLQSMEQIRRIMRPTDVPDQGLLCDLLWSDPKD
Acrec-PPα2    TDCFNCLPVAAIIVDEKIFCCHGGLSPDLQSMEQIRRIMRPTDVPDQGLLCDLLWSDPKD
Volem-PPα2    TDCFNCLPVAAIIVDEKIFCCHGGLSPDLQSMEQIRRIMRPTDVPDQGLLCDLLWSDPKD
                ***** . *****

Drome-PP1      TMGWGENDRGVSFTFGAEVVGKFLQKHEFDLICRAHQVVEDGYEFFAKRQLVTLFSAPNY
Tal-PP1       TMGWGENDRGVSFTFGAEVVAKFLHKHDFDLICRAHQVVEDGYEFFAKRQLVTLFSAPNY
Acrec-PPα2    TMGWGENDRGVSFTFGAEVVAKFLHKHDFDLICRAHQVVEDGYEFFAKRQLVTLFSAPNY
Volem-PPα2    TMGWGENDRGVSFTFGAEVVAKFLHKHDFDLICRAHQVVEDGYEFFAKRQLVTLFSAPNY
                ***** . * : : * : : * : : * : : * : : * : : *

Drome-PP1      CGEFDNAGAMMSVDDTLMCSFQILKPADKRRFVYPNFGSSGRPLTPPRGANNKNKKK-
Tal-PP1       CGEFDNAGAMMSVDETLMCSFQILKPADKKKFSYVSLN-SGRPVTPPRGAANQKPKKK
Acrec-PPα2    CGEFDNAGAMMSVDETLMCSFQILKPADKRKFTYGGLN-AGRPVTTPPRGANNKNKKK-
Volem-PPα2    CGEFDNAGAMMSVDETLMCSFQILKPADKRKFTYGGLN-AGRPVTTPPRGANNKNKKK-
                ***** : ***** : * * . . . : * : : * : : * : : *

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**Figure S10. Putative *Talitrus saltator* Protein phosphatase 1 (PP1) protein.**

Alignment of *Drosophila melanogaster* PP1 (Drome-PP1; Accession No. CAA39820) with the *T. saltator* PP1 (Tal-PP1) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Acromyrmex echinatio*r PPα2 (Acrec-PPα2; Accession No. XM\_011065955) and *Vollenhovia emeryi* PPα2 (Volem-PPα2; Accession No. XM\_012019400). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure a SMART identified protein phosphatase 2Ac catalytic domains of the serine/threonine phosphatase family is highlighted in yellow.

Drome-MTS	MEDKATTKDLQWIEQLNECNQ	LTETQVRTLCDKAKEILSKESNVQEVKCPVTVCGDVHG
Tal-MTS	MEEKTQIKELDQWIEQLMECKQ	LGENVKTLCEKAKEVLAKESNVQEVKSPVTVCGDVHG
Pedhu-PP-V	MEEKTSLKELDQWIEQLNECKQ	LTESQVKTLCDKAKEILAKESNVQEVKCPVTVCGDVHG
Nasvi-PPIIA	MEEKASLKELDQWIEQLNDCKQ	LTESQVKTLCDKAKEILAKESNVQEVKSPVTVCGDVHG
	**:*:	*:***** :*:** *:**:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:
Drome-MTS	QFHDLMELFRIGGKSPDTNYLFMGDYVDRGYYSVETVLLVALKVRYRERITILRGNHES	
Tal-MTS	QFHDLMELFKIGGRSPDTNYLFMGDYVDRGYYSVETVLLVTLKVRFRERITILRGNHES	
Pedhu-PP-V	QFHDLMELFRIGGKSPDTNYLFMGDYVDRGYYSVETVLLVALKVRYRERITILRGNHES	
Nasvi-PPIIA	QFHDLMELFRIGGKSPDTNYLFMGDYVDRGYYSVETVLLVALKVRYRERITILRGNHES	
	*****:	***:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Drome-MTS	RQITQVYGFYDECLRKYGNANVWKYFTDLFDYLPLTALVDGQIFCLHGGLSPSIDSLDHI	
Tal-MTS	RQITQVYGFYDECLRKYGNANVWKYFTDLFDYLPLTALVDNQIFCLHGGLSPSIDTLDHI	
Pedhu-PP-V	RQITQVYGFYDECLRKYGNANVWKFFTDLFDYLPLTALVDGQIFCLHGGLSPSIDTLDHI	
Nasvi-PPIIA	RQITQVYGFYDECLRKYGNANVWKFFTDLFDYLPLTALVDGQIFCLHGGLSPSIDTLDHI	
	*****:	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Drome-MTS	RALDRLQEVPHGPMCDLLWSDPDDRGGWGISPRGAGYTFGQDISETFNNTNGLTLVSRA	
Tal-MTS	RALDRLQEVPHGPMCDLLWSDPDDRGGWGISPRGAGYTFGQDISETFNHSNGLTLVSRA	
Pedhu-PP-V	RALDRLQEVPHGPMCDLLWSDPDDRGGWGISPRGAGYTFGQDISETFNHSNGLTLVSRA	
Nasvi-PPIIA	RALDRLQEVPHGPMCDLLWSDPDDRGGWGISPRGAGYTFGQDISETFNHSNGLTLVSRA	
	*****:	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Drome-MTS	HQLVMEGYNWCHDRNVVTIFSA	PNYCYRCGNQAALMELDDSLKFSFLQFDPAPRRGEPHV
Tal-MTS	HQLVMEGYNWCHERNVVTIFSA	PNYCYRCGNQAAIMELDDSLKYSFLQFDPAPRRGEPHV
Pedhu-PP-V	HQLVMEGYNWCHDRNVVTIFSA	PNYCYRCGNQAAIMELDDALKYSFLQFDPAPRRGEPHV
Nasvi-PPIIA	HQLVMEGYNWCHDRNVVTIFSA	PNYCYRCGNQAAIMELDDALKYSFLQFDPAPRRGEPHV
	*****:	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Drome-MTS	TRRTPDYFL	
Tal-MTS	TRRTPDYFL	
Pedhu-PP-V	TRRTPDYFL	
Nasvi-PPIIA	TRRTPDYFL	
	*****	

### Figure S11. Putative *Talitrus saltator* MICROTUBULE STAR (MTS) protein

Alignment of *Drosophila melanogaster* MTS (Drome-MTS; Accession no. AAF52567) with the *T. saltator* MTS (Tal-MTS) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Pediculus humanus corporis* PP-V (Pedhu-PP-V; Accession No. XM\_002426681) and *Nasonia vitripennis* PPIIA (Nasvi-PPIIA; Accession No. XM\_001602456). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified protein phosphatase 2Ac catalytic domains of the serine/threonine phosphatase family are highlighted in green.

```

Drome-WBT      MSSG--TFVDRIDPFAKRSLLKKKQSSSRYRNSQDVELQQLPPLKADCSSELEELF
Tal-WBT       MSAGSGNFVDRIDPFAKRSLLKKPKRSQSSSRYRTANDVDLQPLPLLK-DVPGGEQEDLF
Trica-PPIIA   MSTT--AFVDRIDPFAKRSLLKKTKKSQSSSRYRNSQDVELQPLPLLK-DVPASEQEELF
Bommo-PPIIA   MSSG--TFVDRIDPFAKRSLLKKTKKSQSSSRYRNTQDVELQALPLLK-DVPSSEQEELF
**:*          ***** *:* ***** .:.*: ** * * * .. **:*

Drome-WBT      IRKLRQCCVSDFDMPVTDLKGKEIKRAALNDLSTYITHGRGVLTEPVYPEIIRMISCNL
Tal-WBT       LRKLRQCCVGFDFLDPVADLKGKETKRSTLNELVDYITAGRGVLTEPVYPEIAMIACNL
Trica-PPIIA   IRKLRQCCVDFDMPVADLKGKEVRRCTLNELVDYITGGRGVLTEPVYPEIIMKISANL
Bommo-PPIIA   IRKLRQCCVAFDFDMPVADLKGKEIKRATLNELVEYIHGGRGVLTEPVYPEIIMKISANL
:*:***** **:* **:* ***** *:* **:* * * ***** **:* **

Drome-WBT      FRTLPPSENPDFDPEEDDPTLEASWPHLQLVYEVFLRFLESQDFQATIGKRVIDQKFVLQ
Tal-WBT       FRTLPPSDNPDFDPEEDDPTLEASWPHLQLVYEFFLRFLESDFQPAIGKKVIDQKFVLQ
Trica-PPIIA   FRTLPPSENPDFDPEEDDPTLEASWPHLQLVYEFFLRFLESDFQPAIGKRVIDQKFVLQ
Bommo-PPIIA   FRTLPPSENPDFDPEEDDPTLEASWPHLQLVYEFFLRFLESTDFQPTIGKKVIDQKFVLQ
*****:* ***** .***** **:* **:* *****

Drome-WBT      LLELFDSEDPREDFLKTVLHRIYGKFLGLRAFIRKQINNIFLRFIYETEHFNGVGELLE
Tal-WBT       LLELFDSEDPREDFLKTVLHRIYGKFLGLRAFIRKQINNIFLRFIYETEHFNGVGELLE
Trica-PPIIA   LLELFDSEDPREDFLKTVLHRIYGKFLGLRAFIRKQINNIFLRFVYETEHFNGVGELLE
Bommo-PPIIA   LLDLFDSEDPREDFLKTVLHRIYGKFLGLRAFIRKQINNIFLRFVYETEHFNGVGELLE
*:* ***** **:* ***** **:* *****

Drome-WBT      ILGSIINGFALPLKAEHKQFLVKVLLPLHKVKCLSLYHAQLAYCIVQFLEKDPFLTEPVV
Tal-WBT       ILGSIINGFALPLKAEHKQFLIKVLIPLHKAKCLSLYHAQLAYCVVQFLEKDPFLTEPVI
Trica-PPIIA   ILGSIINGFALPLKAEHKQFLVKVLIPLHKVKCLSLYHAQLAYCVVQFLEKDPFLTEPVV
Bommo-PPIIA   ILGSIINGFALPLKSEHKQFLVKVLLPLHKVKCLSLYHAQLAYCVVQFLEKDATLTEPVV
*****:* ***** **:* ***** **:* *****

Drome-WBT      RGLLKFWPKTCSQKEVMFLGEIEEILDVIDPPQFVKIQEPLFRQIAKCVSSPHFQVAERA
Tal-WBT       KGLLKIWPKTCSQKEVMFLSEIEEILDVIEPNQFGKVQEPLFKQISKCVSSPHFQVAERA
Trica-PPIIA   RGLLKYWPKTCSQKEVMFLGEIEEILDVIEPSQFTRIQEPLFRQIAKCVSSPHFQVAERA
Bommo-PPIIA   RGLLKFWPKTCSQKEVMFLGEIEEILDVIEPAQFAKIQEPLFRQIAKCVSSPHFQVAERA
:*:*** ***** **:* ***** **:* *****

Drome-WBT      LYLWNNNEYAMSLIEENNAVIMPIMPALYRISKEHWNQTIVALVYNVLKTFMEMNSKLF
Tal-WBT       LYFWNNEYIMSLIEENSNVILPIMPALYRISKEHWNQTIVALVYNVLKTFMEMNSKLF
Trica-PPIIA   LYFWNNEYIMSLMEENNHVIMPIMPALYRISKEHWNQTIVALVYNVLKTFMEMNSKLF
Bommo-PPIIA   LYFWNNEYIMSLMEENNHVIMPIMPALYRISKEHWNQTIVALVYNVLKTFMEMNSKLF
*:* ***** **:* ***** **:* *****

Drome-WBT      ELTSSYKAERQKEKKRERDREELWKKLHELESNRSSGRTAGGSATTSNSAASAASTSIQ
Tal-WBT       ELTASYKSERQREKKRDREEREELWKKLQALELNRTQ-----PKE-----
Trica-PPIIA   ELTVSYKAERQKEKKREKERDELWKKLAELELNHNKNLPAQNNSPPSKK-----
Bommo-PPIIA   ELTASYKAERQKEKKREKERDELWKKLGELEISHKR---QQHAAPLAK-----
*** **:* ***** **:* ***** **:* *****

Drome-WBT      PSSAGLNSHQQSSNSGSSGSLSSGGAGGDNNPATTNAKIKQDKADN
Tal-WBT       -----
Trica-PPIIA   -----
Bommo-PPIIA   -----

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**Figure S12. Putative *Talitrus saltator* WIDERBORST (WBT) protein**

Alignment of *Drosophila melanogaster* WBT (Drome-WBT; Accession No. AAF56720) with the *T. saltator* WBT (Tal-WBT) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Tribolium castaneum* PPIIA (Trica-PPIIA; Accession No. XM\_008197190) and *Bombyx mori* PPIIA (Bommo-PPIIA; Accession No. XM\_004924466). '\*' indicates identical amino acid residues in the two

proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure a SMART identified B56 domain is highlighted in yellow.

```

Drome-TWS      MGRWGRQSPVLEPPDPQMOTTPPPPTLPRTTFMRQSSITKIGNMLNTAININGAKKPASN
Tal-TWS        -----MAGN
Scypa-PPIIA    -----MAGN
Megro-PPIIA    -----MAGN
* . *

Drome-TWS      G-EASWCFSQIKGALDDDDVTADII SCVEFNHDGELLATGDKGGRVVFQ RDPASKAANP
Tal-TWS        GSDTQWCFSQVKGTLDDDEITDADVISCVFESHGDLLATGDKGGRVVFQ RDP LSKGCSP
Scypa-PPIIA    G-DIQWCF SQVKGTLDDDVSEADI I SCVEFNHDGDLLATGDKGGRVVFQ RDPSSKNCHP
Megro-PPIIA    G-DIQWCF SQV KGTLEDDVTEADI I SCVEFNHDGDLLATGDKGGRVVFQ RDPISKNSIP
* : .*****:*.*:*.:::*.:*****.***:*****:***** ** . *

Drome-TWS      RRGEYNVYSTFQSHEPEFDYLSLEIEEKINKIRWLQOKNPVHFLLSNDKTVKLWVSE
Tal-TWS        TRGEYNVYSTFQSHEPEFDYLSLEIEEKINKIRWLKRKNPAHFLLSNDKTIKLWVSE
Scypa-PPIIA    RRGEYNVYSTFQSHEPEFDYLSLEIEEKINKIRWLKRKNPAHFLLSNDKTIKLWVSE
Megro-PPIIA    RRGEYNVYSTFQSHEPEFDYLSLEIEEKINKIRWLKRKNPAHFLLSNDKTIKLWVSE
*****:*****:*****:*****:*****:*****:*****:*****

Drome-TWS      RDKSFGGYNTKEENGLIRDPQNVLTALRVPSVKQIPLLV EASPRRTFANAHTYHINSISVN
Tal-TWS        RDKRAEGYNLRDDSGQIRDSSSITSLRVPTLQKQELMVEASPRRIFANAHTYHINSISIN
Scypa-PPIIA    RDKRAEGYNLRDESGQIRDPTSLTALRVVLPKPMELMVEASPRRIFANAHTYHINSISIN
Megro-PPIIA    RDKRVEGYNTKEENGTRDPACITSLRVPTIKPMELMVEASPRRIFANAHTYHINSISVN
*** ** :*: * ** . :*:***** :* : * :***** *****:***

Drome-TWS      SDQETFLSADDLRINLWHELVNQSYNIVDI KPTNMEELTEVITAAEFHPTECNVYVYSS
Tal-TWS        SDQETFLSADDLRINLWHELVTDQSFNIVDI KPSNMEELTEVITAAEFHPRDCNAFYVYSS
Scypa-PPIIA    SDQETFLSADDLRINLWHEVTDQSFNIVDI KPTNMEELTEVITAAEFHPDCNVYVYSS
Megro-PPIIA    SDQETFLSADDLRINLWHEITDQSFNIVDI KPTNMEELTEVITAAEFHPAECNVLYVYSS
*****:*****:*****:*****:*****:*****:*****:*****

Drome-TWS      SKGTIRLCDMRSAAALCDRHSKQFEEPE NPTNRSFFSEI ISSISDVKLSNSGRYMI SRDYL
Tal-TWS        SKGTIRLCDMRAAALCDSHAKMFEEAE DPSNRSFFSEI ISSISDVKFSNSGSLMISRDYL
Scypa-PPIIA    SKGTIRLCDMRQAALCDSHSKLFEEPE DPTNRSFFSEI ISSISDVKFSNSGRYMI SRDYL
Megro-PPIIA    SKGTIRLCDMRSAAALCDQHSKLFEEPE DPTNRSFFSEI ISSISDVKLSNSGRYMI SRDYL
***** ***** *:* ** .*:*****:***** *****

Drome-TWS      SIKVVDLHMETKPIETYPVHEYLRAKLCSLYE NDCIFDKFECCWNGKDSIMTGSYNNFF
Tal-TWS        TIKVVDLVRKENQPLETYSVHDYLRKLCSLYE NDCIFDKFECCWNGNDKHIMTGSYNNFF
Scypa-PPIIA    SVKVVDLHMETKPIETYPVHEYLRPKLCSLYE NDCIFDKFECCWNGNSAIMTGSYNNFF
Megro-PPIIA    SVKVVDLQMETKPIECYPVHEYLRSKLCSLYE NDCIFDKFECCWNGNSAIMTGSYNNFF
:*****: *.:*: * .*:*****:*****:*****:*****:*****

Drome-TWS      RVFD RNSKKDVTLEASRDI I KPKTVLKPRKVCTGG-KRKK DEISVDCLDFNKKILHTAWH
Tal-TWS        RMFD RENKKDVTLEAARE I AKPRTVLKPRKIGSGSKRKK DEINVDCLDFSKKILHTAWH
Scypa-PPIIA    RMFD RRTSKRDVTLEASRETA KPRYLKPRKVCTAG-KRKK DEISVDCLDFNKKILHTAWH
Megro-PPIIA    RVFD RRTKRDLTLEAARDI I AKPRTLKPRKVCTGG-KRKK DEISVDCLDFNKKILHTAWH
*:* ** .*:*****: * :* :*****: . * *****.*****.*****

Drome-TWS      PEENI IAVAATNNLFIFQDKF
Tal-TWS        PTENVI IAVAATNNLYIFQDKQ
Scypa-PPIIA    PHENI IAVAATNNLYIFQDKF
Megro-PPIIA    PSENVVAVAATNNLFLFQDKL
* **.:*****:*****

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**Figure S13. Putative *Talitrus saltator* TWINS (TWS) protein**

Alignment of *Drosophila melanogaster* (Drome-TWS; Accession no. AAF54498) with the *T. saltator* TWS (Tal-TWS) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Scylla paramamosain* PPIIA (Scypa-PPIIA; Accession No. JQ867383) and *Megachile rotundata* PPIIA (Megro-PPIIA; Accession No. XM\_012288516). '\*' indicates identical amino acid residues in the two proteins, '.' and ':'

indicate similar amino acid residues between the two proteins. In this figure seven SMART identified WD40 domains are highlighted in green.

```

Drome-SGG      MSGRPRTSSFAEGNK-QSPSLVLGGVKTCSRDSKITTVVATPGQGTDRVQEVSYTDTKV
Tal-SGG       MSGRPRTTSFAEGNKGPLAAVNFPGMKISSKDGKVTVVATAGLGSDRPQEVSYMDTKV
Athro-GSK-III MSGRPRTTSFAEGNK-PPTNPPLGGMKISSKDGSKVTVVASAGAGPDRPQEVGYTDTKV
Linhu-GSK-III MSGRPRTTSFAEGNK-PPANPLGGMKISSKDGSKVTVVATPGAGPDRPQEVAYTDTKV
*****:***** . :*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:

Drome-SGG      INGSFGVVVFQAKLCDTGEIQAIVKVLQDRRFKNRELQIMRKLHCNIVKLLYFFYSYSGE
Tal-SGG       INGSFGVVVFQAKLVESEIQAIVKVLQDKRFKNRELQIMRRLEHCNIVELKYFFYSCGD
Athro-GSK-III INGSFGVVVFQAKLCDTGEMVAIKKVLQDKRFKNRELQIMRRLEHCNIVKLLYFFYSYSGD
Linhu-GSK-III INGSFGVVVFQAKLCDSGEMVAIKKVLQDKRFKNRELQIMRRLEHCNIVKLLYFFYSYSGD
*****:***** :*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:

Drome-SGG      KRDEVFLNLVLEYIPETVYKVARQYAKTKQTIPINFIRLYMYQLFRSLAYIHSGLGICHRD
Tal-SGG       KKDEVFLNLVLEYVPEIYKVARHHGKQKQTIPIMSYIKLYMYQLFRSLAYMHALGVCHRD
Athro-GSK-III KKDEVFLNLVLEYIPETVYKVARHYSKSKQTIPIISFIKLYMYQLFRSLAYIHSGLGICHRD
Linhu-GSK-III KKDEVFLNLVLEYIPETVYKVARHYSKSKQTIPIISFIKLYMYQLFRSLAYIHSGLGICHRD
*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:

Drome-SGG      IKPQNLLLDPEAVLKLKCDFGSAKQLLHGEPNVSYICSRYYRAPELIFGAINYTTKIDVW
Tal-SGG       IKPQNLLVDPETGVLKLKCDFGSAKHLVLRGEPNVSYICSRYYRAPELIFGATDYTTNIDVW
Athro-GSK-III IKPQNLLLDPEAVLKLKCDFGSAKHLVKGEPNVSYICSRYYRAPELIFGAIDYTTKIDVW
Linhu-GSK-III IKPQNLLLDPEAVLKLKCDFGSAKHLVKGEPNVSYICSRYYRAPELIFGAIDYTTKIDVW
*****:*.*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:

Drome-SGG      SAGCVLAELLLGQPIFPDGSVDQLVEIIVKVLGTPTREQIREMNPNYTEFKFPQIKSHPW
Tal-SGG       SAGCVLSELLLAQPIFPDGSVDQLVEIIVKVLGTPTREQIREMNPNYTEFKFPQIKSHPW
Athro-GSK-III SAGCVLAELLLGQPIFPDGSVDQLVEIIVKVLGTPTREQIREMNPNYTEFKFPQIKSHPW
Linhu-GSK-III SAGCVLAELLLGQPIFPDGSVDQLVEIIVKVLGTPTRDQIREMNPNYTEFKFPQIKSHPW
*****:***** .*****:*****:*****:*****:*****

Drome-SGG      QKVFRIRTPTEAINLVSLLLEYTPSARITPLKACAHPPFDELREMGHNTLPNGRDMPPPLF
Tal-SGG       QKVFRQRTPEDAINLVSRLLEYTPSARISPLQACTHRFFDELREPN-TRLPNNRPLPPLF
Athro-GSK-III QKVFRARTPPEAMELVARLLEYTPSLRWTPLQACAHSFFNELREQG-TRLPNGRELPLPLF
Linhu-GSK-III QKVFRARTPPEAMDVARLLEYTPSLRMTPLQACAHSFFNELREQG-TRLPNGRELPLPLF
***** ***:*.*:*.*:*.*:*.*:*.*:*.*:*.*:*.*:

Drome-SGG      NFTEHELSIQPSLVPQLLPKHLQNASGPGGNRPSAGGAASIAASGSTSVSSTGSGASVEG
Tal-SGG       NFTELELKIQPELASKLIPSHYQS-----
Athro-GSK-III NFTEQELQIQPTLNSILIPKYM-----
Linhu-GSK-III NFTEHELRIQPVLNSMLIPKYMSTA-----
**** ** ** * . *:*.*:

Drome-SGG      SAQPQSQGTAAAAGSGSGGATAGTGGASAGGPGSGNNSSSGGASGAPSAVAAGGANAAVA
Tal-SGG       -----ESGSG-----GGAVGSEAPTGAAGGS-----
Athro-GSK-III -----TADNPG-----AQSEASNVTAGASGNSSDNSA
Linhu-GSK-III -----AATTTGTEGNPGSGGGGGQSDTTGATASAGNSNDNSV
...* . . .:*.*:

Drome-SGG      GGAGGGGGAGAATAAATATGAIGATNAGGANVTGSQNSALNSSGGSGNGEAAAGSGSG
Tal-SGG       -----VTSANTATAAGAE-----
Athro-GSK-III N-----ISTPTTQNTDPSQSNMA-----
Linhu-GSK-III TNP-----NTTANTSTNTQNTDPSQSNMA-----
: : . .

Drome-SGG      SSGSGGGGNGGDNDAGDSGAIASGGGAAETEAAASG
Tal-SGG       -----
Athro-GSK-III -----
Linhu-GSK-III -----

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**Figure S14. Putative *Talitrus saltator* SHAGGY (SGG) protein**

Alignment of *Drosophila melanogaster* SGG (Drome-SGG; Accession no. AAN09084) with the *T. saltator* SGG (Tal-SGG) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Athalia rosae* GSK-III



(Athro-GSK-III; Accession No. XM\_012400594) and *Linepithema humile* GSK-III (Linhu-GSK-III; Accession No. XM\_012372547). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure a SMART identified serine/threonine protein kinase catalytic domain is highlighted in yellow.

```

Drome-SLIMB      -----MMKMETDKIMDETNSNAQAFTTTMLYDPVR-----KKDS-----SPTYQTEREL
Tal-SLIMB       MDTSSPLLDLDDSHDDSKTNIYSAMVHSTTPTATHNNANNRRKESKSGSEYTSQRES
Athro-βTrCP    -----MTILYDPA-----KKEPPTGASAQYQERET
Linhu-βTrCP    -----MINMETDKIIDDTNA-SLQYPMTILYDPSC-----KKEPSTGVSTQYQEREI
                *           .           :..           *   *   :..*

Drome-SLIMB      CFQYFTQWSESGQVDFVEHLLSRMCHYQHGHINAYLKPMLQRDFITLLPIKGLDHI AENI
Tal-SLIMB       CLKMFEHWSEQDQLEFMEHLLSRMCHYQHGHINAYLKPMLQRDFISLLPKKGLDHVAEKI
Athro-βTrCP    CMKLFERWSEPEQVQFVEQLLARMCHYQHGHINAYLKPMLQRDFISLLPKKGLDHVAESI
Linhu-βTrCP    CMKFFERWTEPEQVHFVEQLLARMCHYQHGHINAYLKPMLQRDFISLLPKKGLDHVAESI
                *:: *   :..*   *::*   :..*   :..*   :..*   :..*   :..*   :..*   :..*   :..*

Drome-SLIMB      LSYLDAESLKSSELVCKEWRVISEGMLWKKLIERKVRTDSLWRGLAERRNWMQYLFKPR
Tal-SLIMB       LSYLDGKSLRDAELVCREWQRVIADGVLWKKLIERKVRTDPLWKGLSERRGWGQFLFKPR
Athro-βTrCP    LSYLDADSLCAAEMVCKGWYRVISEGMLWKKLIERKVRTDSLWRGLAERTGWIQYFFKPR
Linhu-βTrCP    LSYLDAKSLVSAELVCKEWHRVISEGMLWKKLIERKVRTDSVWRGLAERRGWIQYLFKPR
                ***** . **   :..*   :..*   *   :..*   :..*   :..*   :..*   :..*   :..*

Drome-SLIMB      PGQTQRPHSFHRELFPKIMNDIDSIENNWRTGRHMLRRINCRSENSKGVYCLQYDDGKIV
Tal-SLIMB       PGEQPHSHSYRKMYPKIIQNIKTIEANWRMGRHNLQKINCRSETSKGVYCLQYDDNKIV
Athro-βTrCP    PGETHPNHIFYRTLFPKIIITDIDNIEENNWARMGRHNLQRINCRSENSKGVYCLQYDDQKIV
Linhu-βTrCP    PGESHPNHNFYRSLYPKIVKDIESIENNWARMGRFNLQRINCRSENSKGVYCLQYDDQKIV
                ** :   *   :..*   :..*   :..*   :..*   *   *   *   :..*   :..*   :..*   :..*

Drome-SLIMB      SGLRDNTIKIWRDRTLQCVKTLMGHTGSVLCLQYDDKVIISGSSDSTVRVWDVNTGEMVN
Tal-SLIMB       SGLRDNTIKMWRDRTLQCYKVLGTGHTGSVLCLQYDERVVISGSSDSTVRVWDANTGEMTN
Athro-βTrCP    SGLRDNTIKIWRDRTLQCIKVLGTGHTGSVLCLQYDDKAIISGSSDSTVRVWDANTGEMLN
Linhu-βTrCP    SGLRDNTIKIWRDRTLQCIKVLGTGHTGSVLCLQYDDKAIISGSSDSTVRVWDANTGEMVN
                ***** :..*   *   *   *   *   *   *   *   *   *   *   *

Drome-SLIMB      TLIHHCEAVLHLRFNNGMMVTC SKDRSIAVWDMTSPSEITLRRVLVGHRAAVNVVDFDEK
Tal-SLIMB       TLIHHCEAVLHLRFNTGLLVTC SKDRSIAVWDMVSASEINLRRVLVGHRAAVNVVDFDEK
Athro-βTrCP    TLIHHCEAVLHLRFNNGMMVTC SKDRSIAVWDMTSQTEITLRRVLVGHRAAVNVVDFDEK
Linhu-βTrCP    TLIHHCEAVLHLRFNNGMMVTC SKDRSIAVWDMTSQTEIALRRVLVGHRAAVNVVDFDEK
                ***** :..*   :..*   :..*   :..*   :..*   :..*   :..*   :..*

Drome-SLIMB      YIVSASGDRTIKVWTS SCEFVRTLNHGKRGIAQLYRDLVSVSGSSDNTIRLWDIECGA
Tal-SLIMB       YIVSASGDRTIKVWGTSTCEFVRTLNHGKRGIAQLYRDLVSVSGSSDNTIRLWDIEYGA
Athro-βTrCP    YIVSASGDRTIKVWNTS TCEFVRTLIGHKRGIAQLYRDLVSVSGSSDNTIRLWDIECGA
Linhu-βTrCP    YIVSASGDRTIKVWNTS NCEFVRTLNHGKRGIAQLYRDLVSVSGSSDNTIRLWDIECGA
                ***** :..*   *   *   *   *   *   *   *   *   *   *

Drome-SLIMB      CLRVLEGHEELVRCIRFDTKRIVSGAYDGKIKVVDLVAALDPRAASNTLCLRTLVEHTGR
Tal-SLIMB       CLRILDGHEELVRCIRFDNKRIVSGAYDGKIKVVDLHAALDPRSPAGTLCRLTLVEHSGR
Athro-βTrCP    CLRVLEGHEELVRCIRFDNKRIVSGAYDGKIKVVDLVSALDPRAPASTLCLRTLVEHTGR
Linhu-βTrCP    CLRVLEGHEELVRCIRFDSKHIVSGAYDGKIKVVDLVAALDPRAVASTLCLRTLVEHTGR
                ** :..*   :..*   :..*   :..*   :..*   :..*   :..*   :..*

Drome-SLIMB      VFRLQFDEFQIVSSSHDDTILIWDFLNFTPNEN--KTGR-----
Tal-SLIMB       VFRLQFDEFQIVSSSHDDTILIWDFLNCSPDTPPLQGPLASSPASDTGVSAAGGGGGTA
Athro-βTrCP    VFRLQFDEFQIVSSSHDDTILIWDFLNFSPEGT PASGNASINTS-----
Linhu-βTrCP    VFRLQFDEFQIVSSSHDDTILIWDFLNYPSSGSVCSGRLPMDG-----
                ***** :..*   *   *

Drome-SLIMB      -----TPSPALMEH-----
Tal-SLIMB       TAGAAQPVVQPPGPVLF RGAEGPAPLPCPTPPLPSNDSMDRSIDEGQD
Athro-βTrCP    ---VANQSSTRSPSFFE-----
Linhu-βTrCP    ---APIQSDTRSPSFFE-----
                . . *   :..

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**Figure S15. Putative *Talitrus saltator* SUPERNUMERARY LIMBS (SLIMB) protein**

Alignment of *Drosophila melanogaster* SLIMB (Drome-SLIMB; Accession No. AAF55853) with the *T. saltator* SLIMB (Tal-SLIMB) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Athalia rosae*

$\beta$ TrCP (Athro- $\beta$ TrCP; Accession No. XM\_012405697) and *Linepithema humile*  $\beta$ TrCP (Linhu- $\beta$ TrCP; Accession No. XM\_012367510). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains of one D domain of beta-TrCP, one F box domain and seven WD40 domains are highlighted in yellow, green and blue respectively.

Drome-VRI MSIVCTLEQKVNFKAATTKNLLILKNNTDTNYINNYKQDNPSNNKFPRIQAQSNNSHL  
Tal-VRI -----MTAVMQN-----V  
Cluma-VRI -----  
Acypi-VRI -----MVADTANSYHHQOKTQQQP-----

Drome-VRI QHQQQIQKLAQLHHYSQQKLSGSDFPYGP RPPTGGKEEKLLLLLAPPGLYPEASVSTAM  
Tal-VRI LQREIMVAETVKSYPYP-----ALLPLA  
Cluma-VRI -----VMVTELHHQE---SG-----APINGG-----TIQRNI  
Acypi-VRI QQLNQHQHQQQQHHHQ-----QQQKQL  
: :

Drome-VRI PEVLSGTPTNSHNKANIAMNNVRLSNISPTLSMNGSSNEASNLHPLSMYGGISIPQSN  
Tal-VRI QQGLNYSCTTSSCGPSLLPQALGQPYPAQASPI MERNHVGRARNAPSLGANVSSTPSG--  
Cluma-VRI DSGLS-KSCHYSNDSVLMML---RSDSMSPTMMS-----EMGDEH---YSKDSSPNS--  
Acypi-VRI HQQQQQQLHQIHLQRQQQQ---QEQNSSGAMPQYCS DMEQSGGPGSMRYDDSVSPGP--  
. . . : : . . . : \*

Drome-VRI SGMSDSL GKYPVPGSGYGDGMM AQSPSQGGNGPQ---SALTAAQKELFSQRKQREFTPDNK  
Tal-VRI -----LGASGMIGAN-----SALLKDSIFAQRKQREFIPDSK  
Cluma-VRI -----PTPYDG-----SVKRKDI FSRKQREFIPDAK  
Acypi-VRI -----ESPGPEPYPPGFDLTAHLQHK EFFAQRKQREFIPDNK  
\* : . . . : \* \* \*

Drome-VRI KDES YWDRRRRNNEAAKRSREKRRYNDM VLEQRVIELTKENHVLKAQLDAIRDKNISGE  
Tal-VRI KDES YWDRRRRNNEAAKRSREKRRFNDM ILEQRVIELSKENHILRAQLTALENKFHVKGE  
Cluma-VRI KDDS YWDRRRRNNEAAKRSREKRRFNDM VLEQRVIELTKENHVLKAQLDAIKDKYNISGE  
Acypi-VRI KDES YWDRRRRNNEAAKRSREKRRFNDM ILEQRV VELS KENHVLKAQLSAIKDKFGISGE  
\*\* : \*\*\*\*\* : \*\* : \*\*\*\*\* : \* : \*\*\*\*\* : \* : \* : \* : \* : \* : \* : \*

Drome-VRI NLVS VEKILASLPTSEQVLSNTKRAKMSGSGSSSGSSPSGSGSGEGSPQGGHNGYPVGP  
Tal-VRI GLVNEEQVLSSMPQADQILSLTRRSNLSLLSMTSP-----TS  
Cluma-VRI NLVSVDQIMATLPTSEQVLSLTKR-----  
Acypi-VRI AVVS VEQVMASLPTNEQVLSITKRPKLSTSSSSSS-----VG-  
: \* . : : : : \* : \* : \* \* : \*

Drome-VRI PLSPLIYGPNGNARPEATVKS VHHIHHAGVAPPPHTLQQLVVPQSQTQHLYQPQPQHQ  
Tal-VRI LLSPTSMPTSPAP-----P  
Cluma-VRI -----P  
Acypi-VRI -----YSQNGSG-----P

Drome-VRI HQQQQISQPPQQQQQQEFPSPSAGSSSPVISDPHNRPPSTTIANLQVQLQALNRNVRPE  
Tal-VRI QQSSHSEDDHHFAVPQYSQH QHMEQSLPSPQSNARSQSPDY YQRDVP LQAQSSQ-----  
Cluma-VRI -----  
Acypi-VRI IPTSVIHQPVQSTTPKMNGSRTMAVHSPTAT-----

Drome-VRI DLDSL R KVVAAAGALYNAAAVVGAPPPPSAGLYVPAPSAYKDHLEAAAASHNVEAAVSS  
Tal-VRI -----AHSTYSSSESHLYES  
Cluma-VRI -----  
Acypi-VRI -----

Drome-VRI SAVDAVSSSSVSGSAASVNLNSRRACSPSYEHMLSSTTSSTLSSASSSGAVSGDDEQEHE  
Tal-VRI TALNLSRSSRSPNNMDCCYEQNRSLDYGGSSLPKLRHKNQ QHNTHSMSNNFNPNPCPM  
Cluma-VRI -----  
Acypi-VRI -----

Drome-VRI PAHMAPLQLQRSSPQQGSDANNCLPLKLRH KSHLGDKDAATA LLSLQHIKQEPNC SRAS  
Tal-VRI GRPLSTSP LQDQMPPTSPHHYQQISSPMMVNGQHS GTRSSSTSPSSPTSSHMLYPIKSEPI  
Cluma-VRI -----  
Acypi-VRI -----

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Drome-VRI      PPAWNDGGDNSSDERDSGISIASAEWTAQLQRKLLAPKEANVVVTS AERDQMLKSQLERLE
Tal-VRI       SREAGEESPGSSDDRDSGISLASSPPLSGAQSYPSSNRDSTEDMDCDSEQQLRVELQRLA
Cluma-VRI     -----
Acypi-VRI     -----

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Drome-VRI      SEVASIKMILAE-----
Tal-VRI       TEVRSLKSIISRNVDSNRQRDSPRR
Cluma-VRI     -----
Acypi-VRI     -----

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**Figure S16. Putative *Talitrus saltator* VRILLE (VRI) protein**

Alignment of *Drosophila melanogaster* VRI (Drome-VRI; Accession No. AAF52237) with the *T. saltator* VRI (Tal-VRI) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Clunio marinus* VRI (Cluma-VRI; Accession No. JQ011276) and *Acyrtosiphon pisum* VRI (Acypi-VRI; Accession No. FM998650). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure a SMART identified basic region leucin zipper domain is highlighted in yellow.

Drome-EBONY MGSLPQLSIVKGLQDFVPRALHRI FEEQQLR-HADKVALIYQPSTTGQGMAPSQSSYRQ  
Tal-EBONY -----MVSTLRGPTQCPPPTAHSVVEGFQWAALAHPHKAALVDP-----QRGTRMTYAQ  
Trica-EBONY MGSLPQLSILKGPTRPLVVSQLGRI FEKVAAGPAASNIALIFE--ENGQ---SRQLSYSQ  
Peram-EBONY MGSIPTLSILRGDPRPLDSEASLHRL LERAAGASAQQTALLFH-----NQSTSEFGA  
: \* : : \* : : : \* : : . : :

Drome-EBONY MNERANRAARLLVAETHGRFLQPNSDGDFIVAVCMQPSEGLVTTLLAIWKAGGAYLPIDP  
Tal-EBONY LRDEVNAIARVLEAHLR-DAPNHNRDGDVLVALCMSPPRLVATILAVMQVGAAYVALDP  
Trica-EBONY VDQITNKFARVISETIKTKNLKPNSDGDHIIAVNMHPSDHLILVLLSIWKS GCAYLPLDH  
Peram-EBONY LDAASRLARALVRRARNVVGARPNSDGDFLVAVCMESP SERLVVALLAVWKAGAAYLPLDP  
: . . \* \* : : . \* \* \* : : \* \* \* \* : : \* \* \* \* : : \* \* \* \* : \*

Drome-EBONY SFPANRIHHILLEAKP-TLVIRDDDDIDAGRFOGTPTLSTTELYAKSLQLAGSNLLSEEML  
Tal-EBONY NFPPSRVDHILQDCAPQLLVVEDGGIMPOSAPYTTTTLTSTIERQMRSQEGTNSPSVRV  
Trica-EBONY SFPASRIDHIIQESKP-VIVIYDE--DSKNFP--AKLSYEELLTASDGRSDKRLEDKERV  
Peram-EBONY AFPQARVAHILGEARP-VLVLAKE-DPELFS--DAALYEDLRREAADLSSEPLPDAETL  
\* \* \* : \* \* : : \* : : . : : . : : :

Drome-EBONY RGGNDHTAIVLYTSGSTGVPKGVRLPHESILNRLQWQWATFPYTANEAVSVFKALTTFVD  
Tal-EBONY CG-GEDVAVVLYTSGSTGVPKGVRLPHRALLNRLQWQWHTFPYEENE-VCVFKALTTFVD  
Trica-EBONY YQ-KDDLAIIVLYTSGSTGIPKGVRI PHKII LNRLQWQFKTFPFSETEKVCVFKALTTFVD  
Peram-EBONY PGCAPTALVLYTSGSTGVPKGVRLPHAVVLNRLRWQWRTFPYGPQERVCVFKALTTFVD  
\* : \* \* \* \* \* \* : \* \* \* \* \* \* : \* \* \* \* \* \* : \* \* \* \* \* \* : \* \* \* \* \* \* :

Drome-EBONY SIAELWGLMCLGAILVVPKAVTKDPQRLVALLERYKIRRLVLPVPTLLRSLLMYLKMEGG  
Tal-EBONY SLSEVLGPLLTGRTL VVAKSTVARVESLVEVLTQHRVRLVLPVPTLLRALLHCSRLEP-  
Trica-EBONY SVCEIWGPLISGISILIVPKSVTLDPKLIQKLDYKIERLVLVPSLLRSILMCLELK--  
Peram-EBONY SVGELWGPLLQERSVLVVPREVTKDPERLLRVLEDHQIERLVLVPSLLRSLLLALGLES-  
\* : \* : \* \* \* : : : : \* : . . : \* : \* : : : \* \* \* \* \* \* : \* \* \* \* \* \* :

Drome-EBONY GAAQKLLYNLQI WVCSGEP LSVSLASSFFDYFDEGVHRLYNFYGSTEVLGDVTFYFACESK  
Tal-EBONY --SHARLPHLKLWVCSGEPFLD LLENFFQVFTQ-EQGICNFYGSTEVMGDVTTFVHFKS  
Trica-EBONY -KNRTLKLNKLWVCSGETLTTSLAEFFRYFPENEYKLCNFYGSTEIMGDVTFYIIISGM  
Peram-EBONY -RNKGM LSR LKTWVCSGEP LSAQLAREFFTYFESGDHILCNFYGSTEVMGDVTYHVVRSA  
: \* . \* : \* \* \* \* \* : . . \* . \* \* : \* \* \* \* \* : \* \* \* \* \* : \* \* \* \* \* :

Drome-EBONY KQLSL---YDNVPIGIPLSNTVVYLLD--ADYRP-----  
Tal-EBONY EDVRLKVVSSVPIGEVVSNNCIVLGS DVEELRQRCAAAGGASAAPH SASSAGSANSVTN  
Trica-EBONY QQLKN---LLTVPIGAPVDNTIVYLLD--PELRP-----  
Peram-EBONY AELKD---STKVP IGRPLDNTAIYLLD--DNFRP-----  
: : . \* \* \* : : \* . : \* : \* : \*

Drome-EBONY -----VKNG-----  
Tal-EBONY GNGVTNGNGVANGNGVANGNGVTNGTIVTHRNGVANGNGVQNGNSVANGNGITNGSSITN  
Trica-EBONY -----VKTG-----  
Peram-EBONY -----VVSG-----  
\* . \*

Drome-EBONY -----EIGEIFASGLNLAAGYVNGRD  
Tal-EBONY GNGTANGNAVTDSTTSGKDCSSPPGVEGHGAPQGGRGGHQKGEMLVYGANLALGYVGVGG  
Trica-EBONY -----DIGELFVSGLNLASGYVNNRD  
Peram-EBONY -----EAGELYVSGLNLAQGYVKGRD  
: \* \* : . \* \* \* \* \* .

Drome-EBONY PERFLENPLAVEKKYARLYRTGDYGS LK-NGSIMYEGRTDSQVKIRGHRVDLS EVEKNVA  
Tal-EBONY ADKFFH--LLIDGKTEL VYRTGDFATLHPSG TLLYDGRTDGQIKIRGHRVDLCEVQRAVQ  
Trica-EBONY KEKFL ENQLAIDPIFSKLYRTGDFARLQ-NDVLLYEGRTDSQVKIRGHRVDLS EVEKAVS  
Peram-EBONY PERFLANPLTVDPEHSRLYRTGDFAREV-KGTLVYEGRTDSQVKIRGHRVDLAEVERAVA  
: : \* : \* : : \* \* \* \* : : . . : \* : \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* :

Drome-EBONY ELPLVDKAI V L CYHAGQVDQAILAFVKLRDDAPMVTE TQMEARLKD K LADYMT PQVVILE  
Tal-EBONY ATEGVTSCAVLCHAPGTVQQALVAFYCS-ETVEDDYEEERLEASLASRLLTYMRPKLVRVE  
Trica-EBONY GVEGVEKAVVLCYQPGEMNQALLAFVKS--SALMNENQIENILRSKLTYSYMPVQVILVE  
Peram-EBONY GLPGVDKCVVLCYKPGELEQALLAYVTTCKDSSMSQ-QLEAVLHKNLAAAYMLPQVAVVD  
\* . . \* \* \* : . \* : \* \* \* \* \* : : : \* \* . . \* \* \* \* \* : :

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Drome-EBONY      HVPLLVNGKVDRQALLKTYETANNNEGDSSIVLDFDYDQVPEDLKLTARDLFETVGGVIG
Tal-EBONY        HMPLLVNGKVDRQKLLGDYSAALHHN-----EGSVDVSGAPEGLQREAVALLTVVGRVLG
Trica-EBONY      SIPLLVNGKIDRQALLKSYENTNND--DSSVEIEIDYSGVKPAQMAAAKVLFDTVASVLN
Peram-EBONY      SIPLLVNGKIDRQALLRRYSEYSTSRNSESEGEPELDYTGVPANRLDAAKVLFFETVAEVLG
                  :***** ** * * * . . . * : . * * : . * * * : .
Drome-EBONY      RSTETLPPHSNFYELGGNSLNSIFTVTLREKGYNIGISEFIAAKNLGEIEKMAANHD
Tal-EBONY        GSKKKLSLSDNFFKIGGDSLNSVLVVTALGDLGYSGLTDFLDCDDLEAVVRKMGGLKG
Trica-EBONY      RSARSAISLDSNFYEIGGNSLNSIYTIIRLNEEGYQISIGDFLSAIDLGEVLERMTSSND
Peram-EBONY      GGTRSKVGLQANFYELGGNSLNSVFTVTKLRQLGYTIGITDFISSVNLQOVLERMQSND
                  . . . : **::**:*:*:* : : * * : ** : : : * : . : * : : : * . . .
Drome-EBONY      AVQLEESLNACPHLKMEAVPLRLEHRQEVIDIIVASFYNKADLEQWLKPGVLRDYSDI
Tal-EBONY        GSEGTVP---PSCSSNYTFHPLQEEMEQEVIELLAVSFSSKSDME--VATGAKKEDFYPL
Trica-EBONY      -----IHCSPPTYTSELLKNEHKSAVLDIITTSFYQKADLEQWLMPDIFESDYKEL
Peram-EBONY      TTATVS---EKRESKRYSAEMLQDQHKQSVNHMITESFYEKADLEQWLKPDIHREDYKDL
                  * : : . . * . . . * * . * : * * : . . . * : :
Drome-EBONY      LNDIWNVLVERDLSFVVYDTNTDRIIGTALNFDARNEPEVDIKSKLLIVFDFLEFCEGPI
Tal-EBONY        LHGIYGDVLLQRLSFVVQDA--GGRIVACSLNFDLQAEPPVDVDYPLTWVFDLQCESKV
Trica-EBONY      MDALWEPLVEKALSFAKAE--SGKIIGVGLNFDARDEPDVQITSKLTVIFEFLETVEGPV
Peram-EBONY      TDKLWEPLVQKNLSFIVKDT--QGVPVGVALNFDADHDEPAVEITSKLLIVDFLEFLEGPV
                  . : : : : * : . . . . * * : * * : * : * : * : * . :
Drome-EBONY      RDNYLPKGLNQILHSFMMGTAEKLNPRENIACMHFMEHEVLRVAREKQFAGIFTNTSPL
Tal-EBONY        R-NKLPQNVGEVVHSFMMSTLVDLSPKQNIELVKLMEEHNIRLARERQYKAVFTNTSAA
Trica-EBONY      RDKCLPGGKGIKILHSFMMGTHSSLSPKENVAVMQFMEDEVLKLATERHFEGIFTNTSPL
Peram-EBONY      RDNKLPQGKGQVLHSFMMATHPSLNYQQNVEVIQFMEAEVLTIAHSRGFAGIFTNTNPL
                  * : * * . . : : * * * * . * . * : * : : * * . : : * . : : * * * * . .
Drome-EBONY      TQQL-ADVYHYKTLNLFQVNEYVHSDGSRPFGDAPDEQRAIVHWKEVGK-----
Tal-EBONY        TRYVCDLLGYTVLAETPFCSYTAADGQQPFASIPPDHVAECTVFYV-----
Trica-EBONY      TQQLGTDVYHYQTLQVNRVYASDNTRPFGMAPDSQRAIVQWKPIKSDIVVDV
Peram-EBONY      TQQLGTDIYDYKVFNDYQVNLYEAPDGSKPFSEAPDSQRAVCSWRPI-----
                  * : : * : * . : : . * . * . * * . * : * : * :

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**Figure S17. Putative *Talitrus saltator* EBONY protein**

Alignment of *Drosophila melanogaster* EBONY (Drome-EBONY; Accession No. AAF55870) with the *T. saltator* EBONY (Tal-EBONY) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Tribolium castaneum* EBONY (Trica-EBONY; Accession No. XM\_008199683) and *Periplaneta americana* EBONY (Peram-EBONY; Accession No. AJ865468). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains consisting of one AMP binding domain, one AMP binding C domain and one PP binding domain are highlighted in yellow, green and blue respectively.

```

Ep-PDH          --MRFIILGVLFIAVASMILSNV-----
Tal-PDH-I      -MLSTTVAVWSAVVMLGCVAGGQD-----
Marja-PDH-II   -----MCRAAVLLFLMLAVAA-----
Dapma-PDH      MHQLSAKLSHFSIALLVLFVSLTMDVESAPAATSSINRPEAQLSIQEMEKFLEGLTRYLH
Eupcr-PDH-Lα   -----MRASVVFLGLFLAFIA-----
Eupcr-PDH-LβI  -----MRGLVMI CFMVLVVGVT-----
Eupcr-PDH-LβII -----
Tal-PDH-II     ----MASFKHQLLLLLLGCMLCLAL-----

Ep-PDH          -----MAQ-----SRDFSISEREIVASLAKQLLRVARMGYVPEGDLPR---
Tal-PDH-I      -----LGDLG-----SAPLTVPQRLAISDWAQSIARLADGGLDGPSGLSRRLE
Marja-PDH-II   -----VMVTEA-----QREPTASKCQAATELAIQILQAVKGAHTGVAAGPH--
Dapma-PDH      RQRLEQQRMHQPAKSLMSEEVTYDPDAIDRAILGEMAAPLDAERSSSVELANNSMSHAR
Eupcr-PDH-Lα   -----INDVV-----AQLSTEQDSIVVASLAAQILDVVAGSENDGDSGVQAV-
Eupcr-PDH-LβI  -----IAI-----VHAQEDQERQAVGNLALDILRVVGRAPSAMQ-----
Eupcr-PDH-LβII -----QEDQERQVVGELALGILRIVGQESSGPQ-----
Tal-PDH-II     -----VA-----AQPRRHDPDLYSDDDVAQAQLDGEESYGAVPDDLAAWLI-
                .       . *       :

Ep-PDH          -----KRNAELINSLLGVP-----RVMSDAGRR
Tal-PDH-I      LLQQQVELGNLGGWRGSDRLSYGSTRPHDKRNSELINSLGLP-----KFLRE----
Marja-PDH-II   -----KRNSELINSLGLP-----KFMIDAGRR
Dapma-PDH      PPTNNKWPWSLSNFERIEDQNVKQRPYGKRNSELINSLGLP-----RFMKVVG--
Eupcr-PDH-Lα   -----KRNSGTINSMLGLPRTYNLRRMMMHAGRK
Eupcr-PDH-LβI  -----KRNSELINSMLGLPQTLRAQKLMANMGR-
Eupcr-PDH-LβII -----KRNAETINTMLGLPQTLRAQKLMAKLGR-
Tal-PDH-II     -----RSRMP--KRNSELLNTLLGSKNI-----VALRAAGRR
                ***:   :*:***       :

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**Figure S18. Putative *Talitrus saltator* PIGMENT DISPERSING HORMONE (PDH) peptides**

Alignment of *Eurydice pulchra* PDH (Ep-PDH; Accession no. GQ380440) with the *T. saltator* PDH-I and PDH-II (Tal-PDH-I and Tal-PDH-II) deduced from the Trinity *de novo* transcriptome assembly, together with the top two Tal-PDH-I tblastn species homologue sequences *Marsupenaeus japonicus* PDH-II (Marja-PDH-II; Accession No. AB073368), *Daphnia magna* PDH (Dapma-PDH; Accession No. HQ843173) and the *Euphausia crystallorophias* sequences PDH-La, PDH-LβI, and PDH-LβII (Eupcr-PDH-La, Eupcr-PDH-LβI, and Eupcr-PDH-LβII respectively). '\*' indicates identical amino acid residues in the two peptides, '.' and ':' indicate similar amino acid residues. In this figure the SMART identified PDH domain is highlighted in yellow. The region of Tal-PDH-II that corresponds to the PDH regions of Drome-PDF and Tal-PDH-I is highlighted in blue, though no PDH domain *per se* was identified in this peptide using SMART.



Drome-RORA MASLLGSAPPAQILANQPIIVKIEPTQSFHIVDEGDTRVLSLPLSDADKLGASWIDLKDI  
Tal-RORA -----MDDSTLDLDTSGLLRVGGLLQSSLQDGGSGMGSSSNRDEMMGITHISPANS  
Drogr-RORA -----MYTQRMFDMWSSVTSKLEA-----HANNLG-----  
Droan-RORA -----MYTQRMFDMWSSVTSKLEA-----HANNLG-----  
: . . . : : \*

Drome-RORA AGLQAGGGATLLDVCFEQANEDGTIIATVQPDLENELEAELKAEGEPEDETEPEPPAPKR  
Tal-RORA SQFKQQQESQLSTTTPNILGFPGGNPSSNSGSTLLNISSIPNLTSPLSCASSNQNSER  
Drogr-RORA -----  
Droan-RORA -----

Drome-RORA LATTRPAQSRPQQQQQQQQQVKFLSDPPALARSSSFS--SLSSFSSISNISSVCKNMAS  
Tal-RORA ----SPLHQPHSSNNNRDCPAAFETNRSFLESCEDFTLDEISSDRSASTMSSLQNIPI  
Drogr-RORA ----QSNVQSPAAQNN-----  
Droan-RORA ----QSNVQSPAGQNN-----  
. . \* . : :

Drome-RORA NTSQEGSLKRTKERTPPMPPLTTHKPAATTTATSATSAAAATSATASATATSARENSR  
Tal-RORA SGKMGLLERKADRD-----LLNEILAGINEDRDRERDRDRDMADTTTPTSSTPTPTP  
Drogr-RORA -----  
Droan-RORA -----

Drome-RORA EHSSSSSGSNGAMTAQIEIIPCKVCGDKSSGVHYGVITCEGCKGFFRRSQSSVVNYQCPR  
Tal-RORA RIQSVADKKCNSIKAQIEIIPCKVCGDKSSGVHYGVITCEGCKGFFRRSQSSVVNYQCPR  
Drogr-RORA -----SSGSIKAQIEIIPCKVCGDKSSGVHYGVITCEGCKGFFRRSQSSVVNYQCPR  
Droan-RORA -----SSGSIKAQIEIIPCKVCGDKSSGVHYGVITCEGCKGFFRRSQSSVVNYQCPR  
. . : : .\*\*\*\*\*

Drome-RORA NKQCVDVDRVNRNRCQYCRLOKCLKLGMSRD AVKFGRMSKKQREKVEDEVRFHRAQMRAS  
Tal-RORA QKNCVVDVDRVNRNRCQYCRLOKCLQLGMSRD AVKFGRMSKKQREKVEDEVRYHKSQMRAS  
Drogr-RORA NKQCVDVDRVNRNRCQYCRLOKCLKLGMSRD AVKFGRMSKKQREKVEDEVRFHRAQMRAS  
Droan-RORA NKQCVDVDRVNRNRCQYCRLOKCLKLGMSRD AVKFGRMSKKQREKVEDEVRFHRAQMRAS  
: : .\*\*\*\*\*:\*\*\*\*\*:\*. :\*\*\*\* \*

Drome-RORA --DAAPDSSVYDTQTPSSSDQLHNNYN--SGGYSNNEVGYGSPYGYASVTPQQTMOYD  
Tal-RORA GPETSPDSSVYEPTSP--SQDMFSAQY----GFGSNELNFNHYSFSPRPGGASGSSLD  
Drogr-RORA --DAAPDSSVYDTQTPSSSDQLQHTNYNSYGGYSSNEVGYGSPYGYASVTPQQTMOYD  
Droan-RORA --DAAPDSSVYDTQTPSSSDQLHNNYNYSYGGYSNNEVGYGSPYGYASVTPQQTMOYD  
: : .\*\*\*\*\*: . \* \* : : \* \* : : . \* . : : . \* . : . \*

Drome-RORA ISADYVDSTTYEPRSTIIDPEFISHADGDINDVLIKTAEAHANTNTKLEAVHDMFRKQP  
Tal-RORA ISS--ADSTTYDPQSR-----GCLDIQAQTSLDS-GTDSHSSPLLSACRNER  
Drogr-RORA ISADYVDSTTYEPRSTMIDPEFISHADGDINDVLIKTAEAHANTNTKLESVNDMFRKQP  
Droan-RORA ISADYVDSTTYEPRSTIIDPEFISHADGDINDVLIKTAEAHNNTNTKLEAVHDMFRKQP  
\*\* : .\*\*\*\*\*:\*. \* : : \* : : : : : . \* . : . \*

Drome-RORA DVSRIIYYKNLQGEELWLDCAEKLTMQIONIEFAKLI PGFMRLSQDDQILLKTG--SE  
Tal-RORA Q-----W-----KNSEAVPASWDYRKSQGFRLPLD-----IRSGGCSS  
Drogr-RORA DLSRIIYYKNLQGEELWLDCAEKLTMQIONIEFAKLI PGFMRLSQDDQILLKTG--SE  
Droan-RORA DVSRIIYYKNLQGEELWLDCAEKLTMQIONIEFAKLI PGFMRLSQDDQILLKTG--SE  
: : \* \* : : : : \* \* . \* . \* : : \* \*

Drome-RORA ELAIVRMSRLLDLSQNAVLYG--DVMLPQEAFTSDSEEMRLVSRIFQTAKSIAELKLTET  
Tal-RORA NMQGAMKPDLMEGGGSLSHDMDILIKQEV----DGLDMNNLQSSHISGDSVETDMASPD  
Drogr-RORA ELAIVRMSRLLDLSQNAVLYG--DVMLPQEAFTSDSEEMRLVSRIFQTAKSIAELKLTET  
Droan-RORA ELAIVRMSRLLDLSQNAVLYG--DVMLPQEAFTSDSEEMRLVSRIFQTAKSIAELKLTET  
: : . \* : : . : : . \* : : \* . \* . : \* . : . : . \* : :

Drome-RORA ELALYQSLVLLWPERNGVRGNTETIQRLEFNLSMNAIRQELETNHAPLKGDVTVLDTLLNNI  
Tal-RORA HMTSVDSTTFLPCPSVSPQPNSQA---GLHLHHRPHMGFFGAADHGDFDLHHHQQQQ  
Drogr-RORA ELALYQSLVLLWPERNGVRGNTETIQRLEFNLSMNAIRQELETNHAPLKGDVTVLDTLLNNI  
Droan-RORA ELALYQSLVLLWPERNGVRGNTETIQRLEFNLSMNAIRQELETNHAPLKGDVTVLDTLLNNI  
: : \* \* : : \* . \* : : \* : . \* . : \* . \* : : \*

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Drome-RORA      PNF RD IS ILHME SL SKFKLQH PNVVFPALYKELFSIDSQQDLT
Tal-RORA        QQQQ-----EQQQQQQ
Drogr-RORA      PNF RD IS ILHME ALSKFKQQHPTVVFPALYKELFSIDSQQDLT
Droan-RORA      PNF R-----
: .

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#### **Additional file 24. Putative *Talitrus saltator* RORA protein**

Alignment of *Drosophila melanogaster* RORA (Drome-RORA; Accession No. NP\_788301) with the *T. saltator* RORA (Tal-RORA) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Drosophila grimshawi* RORA (Drogr-RORA; Accession No. XM\_001987136) and *Drosophila ananassae* RORA (Droan-RORA; Accession No. XM\_001959222). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains consisting of one C4 zinc finger domain and one HOLI ligand binding domain are highlighted in yellow and green respectively.

Drome-E75 MEAVQAAAAATSSGGSSGVPVGGSSGASKLKTEPIDFEMHLHEENERQQDIEREPPSS  
Tal-REVERB -MGSTSSDAGVPLISSTQDGDNRRTIGHSTNKLRQGREVFQNIIEFPYTLKTTDIVRATSSD  
Blage-E75 -----MTDTKDFRIPTSVI  
Apido-E75 -----MSADVARD-----

\*.

Drome-E75 NSNSNSNSLTPQRYTHVQVQVPPRQPTGLTTPGGTQKVILTTPRVEYVQQRATSSTGGGM  
Tal-REVERB DRKFDFAYSLADGSQAGIIASSTMHSKNG-----  
Blage-E75 A-----  
Apido-E75 -----

Drome-E75 KHVYSQQQGTAAASRSAPPETTALLTTTSGTPQIIITRTLPSNQHLRHRHSASPSALHHYQ  
Tal-REVERB KSIYPYPHEEKIKIKQEPSEYADLQSQQATSVLHMKGEFDSKVGHVKQEKKEDFARWWKN  
Blage-E75 -----  
Apido-E75 -----

Drome-E75 QQQPQRQQSPPPLHHQQQQQHVVRVIRDRGLYDEATVVVAARRHSVSPPLHHHSRSAP  
Tal-REVERB DDDEKPLKSEPQMKQRDEDMM-----PPPKG-----  
Blage-E75 -----PPPL-----  
Apido-E75 -----PSEM-----

\*.

Drome-E75 VSPVIARRGGAAAYMDQQYQQRQTPLAPP PPPPPPPPPPPPPPPQQQQQQYISTGVPPPTA  
Tal-REVERB -----PPSSSLGNIHRFSKPGKHHKIQDFRKNFPGAQY  
Blage-E75 -----  
Apido-E75 -----

Drome-E75 AARKFVVSTSTRHVNIASNHFQQQQQHQAQQHQQQHQHQHQHVIASVSSSSSSSA  
Tal-REVERB LPSYPNITDVSRLFSPVVSSEMWHAVSSYRMSLDQRAMYETEGAP-----  
Blage-E75 -----  
Apido-E75 -----

Drome-E75 IGGSSSSSHIFRTPVSSSSSSSNMHHQQQQQQSSSLGNSVMRPPP-----P  
Tal-REVERB -----SSSSVNELAKSSSDAQEPSSSGHLAPKSTKPEANIFTVKKNQ  
Blage-E75 -----VSSSTCXPPGTASSTMRPPP-----P  
Apido-E75 -----VDHTTTRNNGV-----P

. . \*

Drome-E75 P-PPKVKHASSSSGNSSSSNTNNSSSSSNGEEPSSSIPDLEFDGT TVLCRVCGDKASGF  
Tal-REVERB P-PANVENFYQTH--GTSGRKAAHTRRRDENIVSSSIDLEFDGT TLLCRVCGDRSSGF  
Blage-E75 P-PPKARS-----NGVAGGCDVKSERNHHEEPSSSIPDLEFDGT TVLCRVCGDKASGF  
Apido-E75 IYPIEVCE-----ATI AVNETQNPERRH-----LRVKEFDGT TVLCRVCGDKASGF

\* : . . . . . : \* \* \* \* \* . \* \* \* \* \* . \* \* \*

Drome-E75 HYGVHSCEGCKGFFRRSIQQKIQYRPCTKNQQCSILRINRNRQCQCRLKCCI AVGMSRDA  
Tal-REVERB HYGVHSCEGCKGFFRRSIQQKIQYRPCTKNQQCLVQKTNRNRQCQCRLKCCIEVGM SKDA  
Blage-E75 HYGVHSCEGCKGFFRRSIQQKIQYRPCTKNQQCSILRINRNRQCQCRLKCCI AVGMSRDA  
Apido-E75 HYGVHSCEGCKGFFRRSIQQKIQYRPCTKNQQCSILRINRNRQCQCRLKCCI AVGMSRDA  
\*\*\*\*\*:\*\*\*\*\* : . \*\*\*\*\* \* \* \* . \*

Drome-E75 VRFGRVPKREKARILAAMQQSTQNRGQQRALATELDDQPRLLAAVLAHLETCEFTKEKV  
Tal-REVERB VRFGRVPKNEKERIRQAMEKASSNIPQQDY-----NDDFLIQSITRSHLQSSAVANENM  
Blage-E75 VRFGRVPKREKARILAAMQQSSNSRSQEKAVAAELEDEQRLLSTVVRHLDTCDFTREKV  
Apido-E75 VRFGRVPKREKARILAAMQQSSHSRSQEKAVAAELEDEQRLLATVVQAHLDTCDFTRDKV  
\*\*\*\*\*.\* \* \* \* \* : : . \* : : \* : : . : \* \* \* . . : : :

Drome-E75 SAMRQRARDPCSYS-MPTLLACPLNPAPPE-LQSE----QEFS QRFVHVRGVIDFAGMIF  
Tal-REVERB REIIRTSEEHPQVANIDPISDCPLNP----YVGT----EDFQ QKFSPIVRDVVAFAKNIF  
Blage-E75 EPMLARARDQPSYTACPTLACPLNPNPQPLTGQQEELLQDFS KRFSPAIRGVVEFAKRIF  
Apido-E75 APILVRARETPNYTACPTLACPLNPNPQPLTGQQEELLQDFS KRFSPAIRGVVEFAKRIF  
: : : \* . : . \* \* \* \* . : \* \* \* \* : \* \* \* \* \* \* \* \*

Drome-E75 GFQLLTQDDKFTLLKAGLFDALFVRLICMFDSSINSIICLNGQVMRRDAIQNGANARFLV  
Tal-REVERB GFSDIKHDDKITLLRACVFEVLLIRFARLIDAKNRRMITISGYIINASIYANIKTSNDFE  
Blage-E75 GFALLPQDDQVTLKAGVFEVLLVRLACMFDAQTNSMICLNGQVVKREAIHNSNARFLM  
Apido-E75 GFSLLAQDDQVTLKAGVFEVLLVRLACMFDAQTNSMICLNGQVVKRESIHNSNARFLM  
\*\* : :\*\*\*:\*\*\*.\* :\*:\*:\*: :\*:\*. . :\* :.\* :.. . \* .. :.

Drome-E75 DSTFNFAERMNSMNLTDAEIGLFCIAIVLITPDRPGLRNLELEIKMYSRLKGCLQYIVAQN  
Tal-REVERB NTLFTFVDRINTLKLSDDEEMALFSAIVINPKRQGLHELKSVSSLHKRIVHCLQVIMQRE  
Blage-E75 DSMFDFAERLNSLRLSDAEVGLFCSVVVIAPDRPGLRNTELIERMQGKLKAALQMVSQN  
Apido-E75 DSMFDFAERVNSLRLSDAELGLFCSVVVIAADRPGLRNTELVERMHNKLRNALQTVLAQN  
: : \* \* .:\*\*\*:\*\*\*.\* \*\*:\*\*\*.: :\*: .\*. \*\*.: . :. : . : . \*\* :. :.

Drome-E75 RPDQPEFLAKLLETMPDLRRTLSTLHTEKLVVFR-TEHKELLRQQMWSMEDGNSSDGQQNK  
Tal-REVERB RPNDFSLCQDLLSTLNDLWILNGMHS-----KQTQAKQKGSNSDCDGRMDVSGP  
Blage-E75 HPGHANI CHELMKKIPDLRRTLNLHSEKLLAFKMTEQQQLQQQQQQHLLWGTSPPEESNS  
Apido-E75 HPQHPDILRELLKKIPDLRRTLNLHSEKLLAFKMTEQQQMQAQQQQHQ-----  
. \* .. : .\*:\*\*\*:\*\*\*.\* \*\*:\*\*\*.: :\*: .\*. \*\*.: . :. : . : . \*\* :. :.

Drome-E75 SPSG-----SWADAMDVEAAKSPGVSSTESADLDYGPSSSSQPGVSLPSPPPQQQPS  
Tal-REVERB QTEGFSSQYPCYSDYEVVRCGPMRYSNAS-----EYDEK-----RAPMEEQTS  
Blage-E75 KSPAGSSSSWSSSDVTMDEAVKSPGVS-----STESVCSGEVASLTEYQPNHHPVS  
Apido-E75 -----

Drome-E75 ALASSAPLLAATLSGG-CPLRNRRANSRNSGSDSGAAEMDIVGSHAHLTQNGLTITPIVRHQ  
Tal-REVERB QTSPTSPSSSTFCPDEEMRSPGSADSGCSTDSGCSIESGCSSDSG---CSMNIASN----  
Blage-E75 HQASSAPLLAATLAGGICPHRHRANSRNSG--SGDDMSGLPHHS---HGLTITAV----  
Apido-E75 -----

Drome-E75 QQQQQQQQIGILNNAHSRNLNGGHAMCQQQQQHPQLHHHLTAGAARYRKLDSPTDSGIES  
Tal-REVERB -----DHARSTLLSDEPR-----ETMSVPDETTER  
Blage-E75 -----NPPSRQQPLMPQHHRFQ-----RKLDSPSDSGIES  
Apido-E75 -----

Drome-E75 GNEKNECKAVSSGGSSS-----CSSPRSSVDDALDCSDAAANHNQVQHPQLSVVSVSPV  
Tal-REVERB FRKCYIKRKPN-----VEESRIPEK-----  
Blage-E75 GTEKLDKLTSGGGSTGSAPTVCSSPRSSLED-----  
Apido-E75 -----

Drome-E75 RSPQPSTSSHLKRQIVEDMPVLKRVLQAPPLYDTNSLMD-----EAYKPHKKFRALRHR  
Tal-REVERB GRPMTWHKPQKESEKLADSPVLRMCLEAPSTLKMDSFKDVYRGHSAHSHPHKKFRPTVDR  
Blage-E75 KDEEKHHNGSSGSHIDDMPVLKRVLQAPPLYDTNSLMD-----EAYKPHKKFRACRKN  
Apido-E75 -----

Drome-E75 EFETAADASSSTSGSNSLSAGSPRQSPVNSVATPPPSAASAAAGNPAQSOLHMLTRS  
Tal-REVERB -----SGHPHETLDYSHSSRPSTTPSPSYSSSPSSSISSPSRIS----SSPY  
Blage-E75 -----DSAEAEPMIVHVSPPPSHPVPPQHHSFQLHLHLTSNNHQSQ----SSTS  
Apido-E75 -----

Drome-E75 SPKASMASSHSVLAKSLMAEPRMTPEQMKRSDIIQNYLKRENSTAASSTTNGVGNRSPSS  
Tal-REVERB SSSSSSNPSSPANVSILAHRLAMPCKKFKPCTLVQSLVQSLQESK-----FGTGKALV  
Blage-E75 STSSSLSTHSTLAKSLMESPRMTAEQLKRTDIIHNYIMRADSPNPVTIEFPPSAPSPSA  
Apido-E75 -----

Drome-E75 SSTPPPSAVQNQRWGSSSVITTCQ-----QRQQSVSPHNSGSSSSSS-----  
Tal-REVERB SDTLHDCIMNDQSKIQRQHVLTASPS-----RYDCGRNSSLRSPSYMSPP-----  
Blage-E75 NSASTTSTSSYKMNNSGNLLVCANSTPSTGYHYIPQIQPQQQQQTVVGRWQSGPFGSNGA  
Apido-E75 -----QQQQQTQHVINVGW-----  
: . . .

```

Drome-E75          -----SSSSSSSSSS-----TSSNCSSSS
Tal-REVERB        -----THSMTSPPPH-----D--DVPPHS
Blage-E75         SVITTTTGRNPTPQQTYVLLQNSTNISPPVHHAEMQQSEFSRIYFHPGNAVSPHAAAASS
Apido-E75         -----

Drome-E75          ASSCQYFQSPHSTSNGTSA PASSSSSGSNSATP-----LLELQVDIADSAQPLNLSKKS
Tal-REVERB        YSSPIPVAHSSPSHTPTGLGASAMSGCNFSSS-----YICDNAEPLNLSKKT
Blage-E75         STSPSPPLPIPHRKTPPAVASCPPSPSSSGVTPIVISSPKMMELQVDIADSQQPLNLSKKS
Apido-E75         -----

Drome-E75          PTPPPSKLHALVAAANAVQRYPTLSADVTVTASNGGPPSAAASPAPSSSPASVGSPPNG
Tal-REVERB        PSPPPA-----
Blage-E75         PSPSPH-----PMASPA
Apido-E75         -----

Drome-E75          LSAAVHKVMLEA
Tal-REVERB        -----MET
Blage-E75         TTVTHKVVSLA
Apido-E75         -----

```

**Figure S20. Putative *Talitrus saltator* REVERB protein**

Alignment of *Drosophila melanogaster* E75 (Drome-E75; Accession No. NP\_730321) with the *T. saltator* (Tal-REVERB) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Blattella germanica* E75 (Blage-E75; Accession No. AM710419) and *Apis dorsata* E75 (Apido-E75; Accession No. XM\_006618394). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART identified domains consisting of one C4 zinc finger domain and one HOLI ligand binding domain are highlighted in yellow and green respectively.

Drome-SIR2 -----MMENYEEIRLGHIR--SKDLGNQVPDTTQFY--PPTKFDFGAEILASTSTEAEA  
 Tal-SIRT1 --EGLRVMSTVPAKRLRLDYSGEMLANEQKESFYQYIRTPDMLSTGG-RVSSSTSSSV  
 Micde-SIRT1 MASGSELPEYSSSVKRRKIE-----GQVYRVSEPLDLDFSESQPRQSSSSDL  
 Fopar-SIR2 MASGSELPEYSSQVKRRKIE-----SKVYGVSVHFNIDFPEVKVNRDTONIDI  
 : : . : : : : .

Drome-SIR2 EAEATATTEPATSEL-----AGKANGEIKTKTLAAREEQE  
 Tal-SIRT1 AMEGGLTCDSGFNELTPRLLLEEEPQSQLSSVSGSLSSHSPQPHGIKRSHASCIDEDDE  
 Micde-SIRT1 E---EPLSGDSGFTEL-----S---DGSKSMSTTP-----  
 Fopar-SIR2 E---EPLGGDSGFMDM-----S---DGAKSMSNTP-----  
 . :.. .: : \*

Drome-SIR2 IGA-----NLEHKTKNPT----KSMGEDEDEEEEEEDDEEEE---  
 Tal-SIRT1 ETAPPARRSPVSLSLGAQRSSMHCNSNSPSPSHFHNNHSSLDGGDDPDANDGDDN--S  
 Micde-SIRT1 -----DLMHLSSTPS-----RTDSTSDDTGCPLDTADEKDEVSS  
 Fopar-SIR2 -----ESTNLSSTPS-----RTDSTSDDTGCPLDTADEKDEVSS  
 . : :..\* : . \* : \* .:

Drome-SIR2 -EDDEEGITGTSNEDEDSSNSCVSEPDWK---LRWLQREFYTGRVPRQVIASIMPHF  
 Tal-SIRT1 VSSDSDRSVRLSNGDDAAAGGEGSDCGEGTPEPAHWWQQMRRGQNPRAVLSRVLGD  
 Micde-SIRT1 TVSNLSDLSGLSELSSEDS-----GHLWR-NASSWVQKQMITGANPRELLQHLL-MDP  
 Fopar-SIR2 TVSNLSDLSGLSELSSEET-----GQLWR-SASAWVQKQMTGTDPDLLQHLL-MDP  
 . : . : \* : : : \* : \* : \* \* : : .

Drome-SIR2 TGLAGDTDDSVLWDYLALHLLNEPKRRNKLASVNTFDDVISLVKKSQKI IVLT **GAGVSVSC**  
 Tal-SIRT1 SDIPEHVSDEMIWRLLLNVLTDGPRRERLRHLNLTDDAVRLMRTSRRI IVLT **GAGVSVSC**  
 Micde-SIRT1 TQIPEQVDDITLWKLVNIMSDPPRRHKLEHINTLADVVRLIKNSKKI IVLT **GAGVSVSC**  
 Fopar-SIR2 TQIPEQVDDITLWKLVNIMSDPPRRKLSYVNTLNDVVRLIKDSKKI IILT **GAGVSVSC**  
 : : . . \* : \* : : : : \* \* . \* : \* : \* . \* : \* : \* : \* : \*

Drome-SIR2 **GIPDFRSTNGIYARLAHDFPDLDPDQAMFDINIFYKRDRPRPFYKFAREIYPGEFQPSPCR**  
 Tal-SIRT1 **GIPDFRSRDIYARLAVDFPNLPDQAMFDINFFKRDRPRPFYKFAREIYPGQFTPSLCHR**  
 Micde-SIRT1 **GIPDFRSRDIYSRLAQDFPDLDPDQAMFDINIFYGQDRPRPFYKFAREIYPGQFKPSPCR**  
 Fopar-SIR2 **GIPDFRSRDIYSRLAQDFPNLPDQAMFDINFFAQDRPRPFYKFAREIYPGLFKPSPCR**  
 \* \* \* \* \* : \* \* \* \* \* \* \* : \* \* \* \* \* : \* \* \* \* \* \* \* \* \* \* \* \*

Drome-SIR2 **FIKMLETKGKLLRNYSQNIIDTLEQVAGIENVIECHGSFATASCTKCFKCNADALRADIF**  
 Tal-SIRT1 **FIRSMENHNKLLRNYSQNIIDTLEQVAGINNVIQCHGSFASATCQRCGFKVSAGAIAKDDIF**  
 Micde-SIRT1 **FIKMLDKNKLLRNYSQNIIDTLEQVAGIENVIECHGSFATASCTKCKYQVTAEDIREDF**  
 Fopar-SIR2 **FIKMLEKHKLLRNYSQNIIDTLEQVAGIENVIECHGSFATASCTKCFKQVTAEDIRTDIF**  
 \* . : : : : \* \* \* \* : \* \* \* \* : \* \* \* \* : \* \* \* \* : \* \* \* \* : \* \* \* \* \*

Drome-SIR2 **AQRI P V C P Q C Q P N K E Q**-----  
 Tal-SIRT1 **QQRIPMCPHCTPPTPLFCYGPDRDSFYGHGHDGTDTPPLQSSASLQASPARNESTSPQP**  
 Micde-SIRT1 **AQRIPLCPKCYTNSLP**-----  
 Fopar-SIR2 **SQRIPLCPKCSTNSLP**-----  
 \* \* \* \* \* : \* \* \* \* \* . . .

Drome-SIR2 ----- **SVDASVAVTEEEELRQLV** ----- **ENGIMKPD**  
 Tal-SIRT1 **GAAGSESPQOATGASHNLNLDSPAHNDDSNLETFNLATLCARSDSSNLMSQSPIMKPD**  
 Micde-SIRT1 ----- **SLSSTNTNDNYANFV** ----- **SQGIMKPD**  
 Fopar-SIR2 ----- **SLATTNTSQNYTDLV** ----- **SLGIMKPD**  
 . : : : : . \* \* \* \* \*

Drome-SIR2 **IVFFGEGLPDEYHTVMATDKDVCDLLIVIGSSLKVRP**VAHIPSSIPATVPQILINREQLH  
 Tal-SIRT1 **IVFFGEGLPDEFHDSISEDQNCDLLIVIGSSLKVRP**VAHIPNSIPHPVPQILINREPLD  
 Micde-SIRT1 **IVFFGEGLPDAFHDAIANDKDECDLLIVIGSSLKVRP**VALIPSSIPSVVPQILINRESLP  
 Fopar-SIR2 **IVFFGEGLPDAFHDSIAKDKDECDLLIVIGSSLKVRP**VALIPSSIPSHIPQILINRESLP  
 \* \* \* \* \* \* : \* : : \* : : \* \* \* \* \* \* \* \* \* \* \* \* : \* \* \* \* \* \*

Drome-SIR2 HLKFDVELLGSDSVIINQICHRLSDNDDCWRQLCCDESVLTESKELMPPEHSNHHLLHHHL  
 Tal-SIRT1 HLTFDIELLGDCDVIVNELCHRLGPS---WTVNCNSHVRLSEIRDL-PPKPETP-----  
 Micde-SIRT1 HLKFDIELLGDGDIINQLCHLIGEE---FSEICWKKDILEETPHLLPPRFITD-----  
 Fopar-SIR2 HLKFDVELLGDGDIINQLCHLMGEE---FLQLCWESEKLEETPHLLPPRFEDS-----  
 \* \* . \* \* : \* \* \* \* : \* \* \* \* : \* \* \* \* : \* \* \* \* : \* \* \* \* \*

```

Drome-SIR2      LHHRHCSSESERQSQLDTDTSIKSNSSADYILGSAGTCSDSGFESSTFSCGKRSTAAEA
Tal-SIRT1       -----SSSPASARLVAGDGDASVAGSGVAPVAAV-----ADAPHPKDEDDIEALRA
Micde-SIRT1     -----DSWERSQDSTINQELSQDSTEVLKLT-----YHHNVSTESQDSLIMNS
Fopar-SIR2     -----WERSTTS-----TQDSTEVIKQ-----HNLFNESQDSLINS
                : ... : . . :
Drome-SIR2      AAIERIKTDILVELNETTAL--SCDRLGLEGPQTTVESYRHLSDSSKDSGIEQCDNEAT
Tal-SIRT1       CWAPKIREVAARLPENCYLYNGGHRVVFKGAEVFDYDPPDVEGDDDSQNEQDDDDDDDED
Micde-SIRT1     NTPPKRLENTDICIISP----FHAGH---MEATEESFA-----LLGESPKRPLGDSLESS
Fopar-SIR2     NTPPKHLGSTEICISP----FDAGH---MEGNDGF-----SLLESPKRPLDESSVESS
                . . : . . : . * . : . :
Drome-SIR2      PSYV-----R----PSNLVQETKTVPAPSLTPIQ--QRGKRQTAERLQPGTFY
Tal-SIRT1       GELVDNDDSSPPLHE----ASNSLNPKLVSGDALSGIPSEKEFLPSFSLQNTKEPATGV
Micde-SIRT1     PKRINLEVRASPPSSGSSGSSGSSGISRSLDSDQDINYTTS--RYNRVIVSESTSENGHI
Fopar-SIR2     PKRLN-----SR----SCGSTPRSSDDGERF-----RFTRVVSFESTPENNGQV
                . : ... . . : : . :
Drome-SIR2      SHTNNSYVFPGAQVFD-----NDYSDDDDEEEERSHNRHSDLFG
Tal-SIRT1       SPPHSLDDVVEAVQAVEEA-----DQLLYSSCDTITHSDCTR--RTLSCDSSASS
Micde-SIRT1     ---YNLEECHVVPRIIDSTLKNISITLDDLTQSNNSNTPVDVDSSNDSEELLDSDKNG
Fopar-SIR2     ---FNLEECHVLPRIILDQ-----DSGVKGEERRDERE---GEDKYG
                . . . . : : : . . .
Drome-SIR2      NVGHNYKDDDE----DACD-LNAVPLSPLLPPSLEAHIVTD---IVNGSNEPLPNSSPG
Tal-SIRT1       SSCHLSTAATV-----TVEAPSVQEMDSSLGSEQEEDCVDSW-WM-----TLHASLAD
Micde-SIRT1     KSRHISIDSAIDSGLDSCNSVDSSDDKSTIEQGDSKRHNLQRHCWQPEVKESLASRLPE
Fopar-SIR2     KSRHASIDSAIDSGIGDSCNSVDSSEAKEI----EEKRQEIERR-WQGEIKESLANRLPE
                . * : : . . . : . * .
Drome-SIR2      QKRTACIEQQPTPAIETEIPPLKRRPSEENKQQTQIERSEESPPPGLAAV
Tal-SIRT1       TPSFSSDVAAPTLPSEDCSRPESQPQFNEPSESAPKLNKIESISRTSVAL-
Micde-SIRT1     NTYYQVSPGKIIFTGAEIYIEPDDYQLSSSSSRSPSMSTMIRPATEYNTT--
Fopar-SIR2     GSYQVSSGKIIFPGAEIYLEGDD---FGEPETRANEISSLTYTEDECRIT--
                * . . . . : . :

```

**Figure S21. Putative *Talitrus saltator* SIRT1 protein**

Alignment of *Drosophila melanogaster* SIR2 (Drome-SIR2; Accession No. NP\_477351) with the *T. saltator* (Tal-SIRT1) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Microplitis demolitor* SIRT1 (Micde-SIRT1; Accession No. XM\_008555996) and *Fopius arisanus* SIR2 (Fopar-SIR2; Accession No. XM\_011303457). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure one SMART identified SIR2 domain is highlighted in yellow.

```

Drome-SIRT2      -----MDKVRFFANTLHLG-GSSDAKEEVKVEK-----
Tal-SIRT2       -----KSFAMASASPKRDADAQDSPDPQPEKAVDDSGVFDASSEETTAGA
Brafl-HYPO      -----VEHLRRLFARTLGLSQERETEEGTARPQQ-----
Trica-SIRT2     MLKRCKSLLNSTSNLYKTVLKMSRDSSKDRAEAKPKQPF-----EAQSEEDNASGT
                . : .                ::

Drome-SIRT2     -----VIPDLSFDGFAEHWRVHGFRKIVTMV
Tal-SIRT2       SATDDDSGVFESLMSLIEQKLNFKKTDEEPQ---ILSSLDVDGIVDFMKSDKCHNVITLA
Brafl-HYPO      -----VLDEVTVEGIAKYITDGKCKNIIVLT
Trica-SIRT2     SMS-----IDSLRKYLAEKLGISDKDDEKEKIKILDVSVVDGIAVYIKKKCKNIITMA
                :: .: .:*:. .      .:.....

Drome-SIRT2     GAGISTSAGIPDFRSPGSGLYSNLKKYELPHPTAIFDLDYFEKNPAPFFALAKELYPGSF
Tal-SIRT2       GAGISTSAGIPDFRSPGSGLYNNLQKYNLAYPEAIFELGFFRENPPFFTLAKELYPGSF
Brafl-HYPO      GAGISTSAGIPDFRSPGTGLYDNLQKYNLNPNAIFELGFFKENPEPFALAKELYPGKF
Trica-SIRT2     GAGISTSAGIPDFRSPGSGLYDNLQKYNLPHQAI FELDFFHENPKPFFTLAKELYPGSF
                *****:***.***:*:*.* * *****:.* :** ***:*****.*

Drome-SIRT2     IPTPAHYFIRLLNDKGLLQRHYTQNIIDTLDRLTGLPEDKII EAHGSFHTNHCIK--CRKEY
Tal-SIRT2       NPTPCHFFIKLLEQKGLLRHYTQNIIDTLEHVAGISEEKLIEAHGSFRTAHCLG--CRKSY
Brafl-HYPO      KPTWCHYFIKLLSDKGLLRNFTQNIIDTLERVAGVSAGAMVEAHGTFYTAHCLGECRKEY
Trica-SIRT2     KPTISHYFIKLLIEKDVLLRHYTQNIIDTLERIAGIPEEKIVEAHGTFYTGCLA--CRKEY
                ** .*:*.** :*:* * :*****:.*:*. :*****:* * **: ***.

Drome-SIRT2     DMDWMKAEIFADRLPKCQ--KCQGVVKPDIVFFGENLPKRFYSSPEEDFQDCDLIIMGT
Tal-SIRT2       DQQWIKDEVFADRVPTCE--SCGSVVKPDIVFFGEGLPKDFFGAVRSDFRKCDLLIVMGT
Brafl-HYPO      TQEWVKEKVFENDEVPRCP--DCDGVVKPDIVFFGEAMPKFFPSVLADFPRCDLLIVMGT
Trica-SIRT2     QLEWMKERIFKDEVPECESKDCKGVVKPDIVFFGEALPKDFYSLIENDFKKCDLLIILGS
                :*:* :* * :* * . * ***** :* .*: ** *****:.*:

Drome-SIRT2     SLEVQPFASLVWRPGRPCIRLLINRDAV--GQASCVLFPNTRSLFLDKPNNTRDVAF
Tal-SIRT2       SLTVQPFASLIDNVSSTCPRLINRDAVGPAGAAATIQRMLMGHSGFQPDSPKNKRDAVAL
Brafl-HYPO      SLQVQPFASLVDRVPETCPRLINREKC--GQVDPIMRMLGFGGGMFDSENNYRDVAW
Trica-SIRT2     SLVVQPFASLVDRVLDTCPRLLINREKV--NNGSGIMAMFGFGAGFDFDGNKNNTRDVAW
                ** *****: . * *****: . : : .: * :* ****

Drome-SIRT2     LGDCDAGVMALAKALGWDQELQQLITSERKKLSGSQNSEELQQGKEKPSDPDKMTSGDR
Tal-SIRT2       LGDCDDGCLMLAEKLGWKEDLQKLIIEGKKKPDAT-----
Brafl-HYPO      LGDCDEGCKALAEELGWKEQLEELVHREHTRIEAQ-----
Trica-SIRT2     IGDCDEGCQLLADKLGWDELKLRQTEIEKIEKA-----EA
                :**** * ** .*** :*:*:* . .

Drome-SIRT2     DKKDASL
Tal-SIRT2       -----
Brafl-HYPO      -----
Trica-SIRT2     TPVKSSM

```

**Figure S22. Putative *Talitrus saltator* SIRT2 protein**

Alignment of *Drosophila melanogaster* SIRT2 (Drome-SIRT2; Accession No. NP\_650880) with the *T. saltator* SIRT2 (Tal-SIRT2) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Tribolium castaneum* SIRT2 (Trica-SIRT2; Accession No. XM\_963962) and *Branchiostoma floridae* hypothetical protein (Brafl-HYPO; Accession No. XM\_002596789). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure one SMART identified SIR2 domain is highlighted in yellow.



```

Drome-SIRT4 -----MRVQQLRFRSTSLRSSTARQ-----
Tal-SIRT4 MYILQKSCCTCHIFFIIKQKPIAATVTRNVLLQPPTKSHFKFLSNLRTDSTIKCFRSNRHQ
Brafl-HYPO -----MPVR--LQSNATTRVNTANF-----
Diasi-SIRT4 -----MTTCRIQRLFFKHFIVPPDVTSRSI-----
                        : . * .

Drome-SIRT4 -----EYVPHHKPVVEDDIKRLLEDFLLSKPNVLVLTGAGISTESGIPDYRSEGVG
Tal-SIRT4 FLLSRFCNNSAFVPKHDPDCSEELIEQLQAFVTRSNKIFVLTGAGISTESGIPDYRSEGVG
Brafl-HYPO -----HFVVPVSDPADLQDVEELQDFVSTSKRLFVITGAGISTESGIPDYRSEGVG
Diasi-SIRT4 -----SFIPKHKPVEESDINKLKQFIEKYNKILVVVTGAGISTESGIPDYRSEGVG
                        : :* . * . : : * : * : . : : * : *****

Drome-SIRT4 LYARSNHKPVQHMEFVKSSAVRKRYWARNFVGWPKFSATQPNAHHALARFEREERVQAV
Tal-SIRT4 LYATSSKRVPVQIKDFIAKAEVRQSYWARNYVGWPRFSAFEPNTHHTLAWEWQKGVVAHI
Brafl-HYPO LYARSDNRPVQYADFLKSGAIRQRYWARNYVGWPKFSSFPNISHE TL SGWESVGLHWL
Diasi-SIRT4 LYARSDKRPVQFQDFLKSRRVRIRYWARNFVGWPRFSSFPNANH YALKQMEDNEKLSYI
                        *** * : . : *** : * : . : * ***** : * * * . * * : * * . : :

Drome-SIRT4 VTQNVDRDLHTKAGSRNVVEVHGSGYVVKCLSCEYRIDRHEFQSILASLNPAF--KDAPDMI
Tal-SIRT4 VTQNVDALHQKAGSIKVTELHGSAHVQCMQCSHSLPRHALQPLLASLNPHLALPSSQLI
Brafl-HYPO VTQNVDSLHIKAGSRKVTTELHGSAARMCLSCPSVI PR TDMQTRIKHLNPVW--HAESQEM
Diasi-SIRT4 ITQNVDDLH YKAGNKKV IEMHGTAFRVMCLGCDYE I DRHKFQKILEDLNPD L--MIESQEM
                        : * * * * * * * * . : * * : * : . * * : * : * : * : * * * . : :

Drome-SIRT4 RPDGDVEI PLEYIENFRIP ECTQC GGD LKPEIVFFGDSVPRPRVDQIAGMVYNSDGLLVL
Tal-SIRT4 RPDGDVELTQEAVSRFVPPCPSCGGLLKPHVVFVFDNVPRARVEAVKEELRRC DALLAL
Brafl-HYPO APDADVFLAPEQIAGFRVPECEKCGGILKQPQIVFFGDNV PKPTVQFVHKMLEESDAMLVA
Diasi-SIRT4 RPDGDVEMSEETISKFHVPQCPCPHCHGDLKPDIVFFGDNI PRHRMEKIDHLVRSCDGLVVL
                        ** . * * : . * : * : * * * * * : * * * * . : : : : . * : * .

Drome-SIRT4 GSSL LVFSGYRVVLQTKDLKLPVGIVNIGETRADHLADIKISAKCGDVIPKLFDFRNSKS
Tal-SIRT4 GTSLQVYSAYRFILLASELGLPMAAVNIGTTRGDHLLQLRVPARCGLVLP LLHL-----
Brafl-HYPO GSSLQVYSAYRFVSAARDQKKPIAVLNIGPTRGDKLADLKVSARCGDVLPQIHL-----
Diasi-SIRT4 GSSLTVYSGYRILLEATDLHLPIAIVNIGPTRGDSLALQKVDKCSQVLASLRKDQS---
                        * : * * * : * : * : * : * * * * * * * * : : : * : * * : . :

Drome-SIRT4 VS
Tal-SIRT4 --
Brafl-HYPO --
Diasi-SIRT4 --

```

### Additional file 23. Putative *Talitrus saltator* SIRT4 protein

Alignment of *Drosophila melanogaster* SIRT4 (Drome-SIRT4; Accession No. NP\_572241) with the *T. saltator* SIRT4 (Tal-SIRT4) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Branchiostoma floridae* hypothetical protein (Brafl-HYPO; Accession No. XM\_002605838) and *Diaphorina sitri* SIRT4 (Diasi-SIRT4; Accession No. XM\_008482696). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure one SMART identified SIR2 domain is highlighted in yellow.

```

Drome-SIRT6 -----MSCNYADGLSAYDNKGILGAPESFDSDEVVAEKQCQELAELIKSGHVVL
Tal-SIRT6 FTTFYCFHRFAMSCNYAEGLSPLYDPKGQVGMPEFAGKEEVSRKVSELAALMKASRHTVF
Pappo-SIRT6 -----MSCNYAEGLSPYEHKGVVLTGPIPEKFEAIEKLNKCEMLARLITESKHIVV
Papxu-SIRT6 -----MSCNYAEGLSPYEHKGVVLTGPIPEKFEAIEKLNKCELLARLISESKHIVV
          *****:****.* ** :* ** * . * : * . ** *:. * * * .

Drome-SIRT6 HTGAGISTTSAGIPDFRFGPKGVWVTLLEKGEKPDFNVSFDEARPTKTHMAIALIESGVVQY
Tal-SIRT6 HTGAGISTTSAGIPDFRFGPKGVWVTLLEKGLRPEVNVSFDDARPTYTHMALVALEKAGLIHY
Pappo-SIRT6 HTGAGISTTSAGIPDFRFGPNGVWVTLLEKGRPSTNISFTDAKPTKTHMILKKLIESNKIQY
Papxu-SIRT6 HTGAGISTTSAGIPDFRFGPNGVWVTLLEKGRPSTNISFTDAKPTKTHMILKKLIDCNKIQY
          *****:*****:* .*. *: * :*. ** * : * . . . :.*

Drome-SIRT6 VISQNIIDGLHLKSGLDKRYLSELHGNIIYIEQCKKCRQFVSPSAVETVGQKSLQRACKSS
Tal-SIRT6 LVTQNIIDGLHLRSGFPRRKMALHGNMYLDKCSVCKREFVRCCTAVSTVGQKSLGVGCPGK
Pappo-SIRT6 IVSQNIIDGLHMKSGLSRKYLSELHGNNMFIDEC SLCKRQFIRSSPVETVGKKCSGVPCASG
Papxu-SIRT6 IVSQNIIDGLHLKSGLSRKYLSELHGNNMFIDEC SLCKRQFIRSSPVETVGKKCSGVPCASG
          :.:*****:.*: * . :*****:*****. * .*: * : .*. ** * : * .

Drome-SIRT6 MDSKGRSCRSGLYDNVLDWEHDLPENDLEMGVMHSTVADLNIALGTTLQIVPSGDLPLK
Tal-SIRT6 RDT-GRRCR-GRLHDNILDWEHDLPHLDYNLAEKHSVGSGLSVCLGTTLQIIPSGVLPPL
Pappo-SIRT6 YNG-GRPCR-GRLYDGVLDWEHSLPEDDLLMAEWHSS IADLSICLGTTLQIVPSGNLPLE
Papxu-SIRT6 HNG-GRPCR-GRLYDGVLDWEHSLPENDLLMAEWHSS IADLSICLGTTLQIIPSGNLPLE
          : * * * * * * . : * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Drome-SIRT6 NLKCGGKLVICNLQPTKHKDKKANLISSYVDVVLKVKLLGVEIPEYSEASDP-----
Tal-SIRT6 AKQSGGKLVICNLQPTKHKDISDIIINGYVDDVMRELLSLLNITCPEYSMEQDPVLI TRA
Pappo-SIRT6 TVKYGGKLVICNLQPTKHDNKSDLI INYYVDDILEKVMNLIKLDIPEYNEDTDL-----
Papxu-SIRT6 TVKYGGKLVICNLQPTKHDNKADLI INYYVDDILEKVMNINLNDI PQYNEDTDL-----
          : * * * :*****: :.:* . ** * : : : . : * : * * . *

Drome-SIRT6 --TKQSKP-----MEWTIPTS---NVNTFHRQYK-----KVKDSKIESKAKK
Tal-SIRT6 LKTKLACSSDAELIEWTIPDSWFDDKDLIEKVS RNKAIADQKNAGKYPRAKIDRRKRK
Pappo-SIRT6 --TKLAST---EIVEWTIKKK---DVLVLEKVFKA KC-----KGVKKKKILIKTKR
Papxu-SIRT6 --TKLAST---SIVEWTINKK---DVLVLEKVFKA KC-----KGVKKKKILIKNKR
          ** : . :**** . : : . . * . . ** . .

Drome-SIRT6 T-----KYT-----
Tal-SIRT6 SPIKPEVKKEETGESSQDEGLSARNERYRKRSLDAGQLGTNKR
Pappo-SIRT6 N-----STESNGIENGCKVIKLEVKQECDISEIK
Papxu-SIRT6 N-----STEINNGIENGCKVIKLEIKDECV-----
          .

```

### Additional file 29. Putative *Talitrus saltator* SIRT6 protein

Alignment of *Drosophila melanogaster* SIRT6 (Drome-SIRT6; Accession No. NP\_649990) with the *T. saltator* SIRT6 (Tal-SIRT6) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Papilio polytes* SIRT6 (Pappo-SIRT6; Accession No. XM\_013284914) and *Papilio xuthus* SIRT6 (Papxu-SIRT6; Accession No. XM\_013314262). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure one SMART identified SIR2 domain is highlighted in yellow.

Drome-SIRT7 MEKDLGEEKDQDQEONTEMEPKQEMDVAQS YITRAKMNPAKKDNEKR RRK DAMRRVSMIL  
 Tal-SIRT7 -----VLMDERKTTVNMMDEEGDGVVENAKRKAATNATLNL SKSSSKMKRVTWLL  
 Megro-SIRT7 -----MEEAASEKFLSRRRSAAALKA FKVKDERVATFKKVAAIL  
 Apido-SIRT7 -----MEETSNEKFLSRRRSATLKA FKVKDERIATFKKVAAIL  
 \* . . . . . : . . . . . : . . . . . : . . . . .

Drome-SIRT7 RKCDSMR--TTEDRQFLEKHPDMVKTTKRKRERVEIYKERVVEREDAPHVIEAKVEQLAN  
 Tal-SIRT7 RKRQEHKQLSNDEQKFLADNPKLVDQFNKRREKRQEYQSRHIEKEDSPETLREKCAELAQ  
 Megro-SIRT7 QKSETDR--TAEETGILVSCSDVVKVNLQEKRRHRVKARLEEVEDAPEILEEK CIRLAA  
 Apido-SIRT7 QKSETDR--TAE EIGILISCS DIVKEVNLQEKRRHRVKARLEEIEDAPELLEEKCMRLAA  
 . \* : . : : : \* . . . : \* . \* . : \* \* \* \* : \* \* \*

Drome-SIRT7 IISQAKHLVCYT GAGISTAALIPDYRGSQGIWTL LQKGDIGEHDLSSANPTYTHMALYE  
 Tal-SIRT7 ALHDAEFLVVYT GAGISTSANIPDYRGPNGIWTRLQKGEDIGSHDLTEAEPTVTHMALSA  
 Megro-SIRT7 AISRATSLAIYT GAGISTAASIPDYRGTVGVWTRLQKGDIGNHDL SQAEPTVTHMALYA  
 Apido-SIRT7 AISRATSLAVYT GAGISTAASIPDYRGTVGVWTRLQKGDIGNHDL SQAEPTLTHMALYA  
 : \* \* . \*

Drome-SIRT7 LHRRRLHHVVSQNC DGLHLRSLPRNSLSEIHGNMYEVCKNCRPN SVYWRQFDTTEMT  
 Tal-SIRT7 LQKTGRLQHIVSQNC DGLHLRSLPKTALSEVHG NMYIEVCRGCV PAREYVRTFDVTE RT  
 Megro-SIRT7 LYKARVLKHIVSQNC DGLHLRSGIPRTLLSEVHG NMYIEVCRTCKPYREYWR LFDVTEKT  
 Apido-SIRT7 LYKARVLKHIVSQNC DGLHLRSGIPRTLLSEVHG NMYIEVCRICKPYREYWR LFDVTEKT  
 \* . \* : \*

Drome-SIRT7 ARYCHKTHRLCHRCSEPLYDTIVHFG ERGNV K WPLNWAGATANAQRADVILCLGSSLKVL  
 Tal-SIRT7 STYKHATGRRCYRCGEPLVDSIVHFG ERGKLRWPLNWQACQAANAADTILCLGSSLKVL  
 Megro-SIRT7 ARYSHGTGRLCHRCNSVLHDSIVHFG ERGNLPWPINWNGATRAAKQADVILCLGSSLKVL  
 Apido-SIRT7 ARYSHGTGRLCHRCNSVLQDSIVHFG ERGNLPWPINWNGATRAAKQADVILCLGSSLKVL  
 : \*

Drome-SIRT7 KKYTWLWQMDRPARQAKICV VNLQWTPKDAIASIKINGKCDQVMAQLMHL LHPV P VYT  
 Tal-SIRT7 KKYTWLWQMDRPARRPRLY VVNLQWTPKDK EATMKINGKCD EVMKLVMDYMGIEIPRYN  
 Megro-SIRT7 KKY P W L W Q M D R P I H K R P S L Y I V N L Q W T P K D E N A V L K I N G K C D E V M K R V M V H L G L E I P Q Y D  
 Apido-SIRT7 KKY P W L W Q M D R P I H K R P S L Y I V N L Q W T P K D E N A V L K I N G K C D E V M K R I M T H L G L E I P Q Y N  
 \* \* \* . \*

Drome-SIRT7 KEKDPIFAHASLLMPEELHTLTQPLLN-----ADEEEAFTTTTEETQDSTISSESC  
 Tal-SIRT7 RSEDLIFDMATPLHPDELRTTTRPSPVPPIDLPIPIEESNNLEENVTERNKSPVSVETC  
 Megro-SIRT7 RTKDP IFFHAVRLRSNEQQTTSQPCLEEPANM---TLKEIDEITDNFHEDIASETKEEDC  
 Apido-SIRT7 RAKDP IFFHAVRLRNSEQHTTSQPCLEEPTNI---VHKELNQIDDNFHEDISSSTKEKEC  
 . : \*

Drome-SIRT7 SFNYS-----DLP I G K G P R I R T P I K N G R V K T N L E L R Q K F K T L N G Q D E E I K V E H V K T N  
 Tal-SIRT7 QNSRRFDRDDLGNVSVDERCSKVLNCA PVCNSSGDRISKYDGISASTMTNDIECGETI  
 Megro-SIRT7 ASPIK-----  
 Apido-SIRT7 ISSKR-----

Drome-SIRT7 GEVTEKDLITLESSIKIETEVKLEKLECSDTNFQQLK-----  
 Tal-SIRT7 IELPSVAQCKNGISLEK LKHFTREDNLNCCSSQTCCHTRSQYSDILQVNSKCSNYDSRLC  
 Megro-SIRT7 -----  
 Apido-SIRT7 -----

Drome-SIRT7 -----LLELLPKLEPLSLKEETEETPSNGFPELPK  
 Tal-SIRT7 ISTSDSVVRTLATSTASVVVTRVRSRPAVSWESVPAEVLSETCSSQSDNPGSEFVHESS  
 Megro-SIRT7 -----  
 Apido-SIRT7 -----

Drome-SIRT7 LVAIQKTHAECLSAVPTESRLKPLQLPPLVPIGAPLSTPFVPEKLVLPASQSSS-----  
 Tal-SIRT7 VSPSVEINTSSVTLSSSTSCSPSYSTVATPRMTTTLLEGEISG PGLAPSKCEHCTTGSCIH  
 Megro-SIRT7 -----ISQNPS-----QLVP-----  
 Apido-SIRT7 -----VTESMSAYSAPFFTALPFLSMGLPFP P M Y M C P Q L T P-----  
 : : : \* .

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Drome-SIRT7      ---IQIKSEGDGDSSTENDNEEEEESELAQM-----DLLRQNNDEELLRQLP-----
Tal-SIRT7        CEVLRLFSGMDGCELLSGDQCKCEVVMKKMFSCGAAPKLMKCDKCGFLRFFLLGTCRP
Megro-SIRT7      -----
Apido-SIRT7      -----LFYYP-----

Drome-SIRT7      -----TWYDAKYAYSGLHSILIPPPADLNIWNSQVVPNFA
Tal-SIRT7        ASRNGELNVNGKLNFGCTVSHLFCDCFEKQQLAVNGAKLPSSASGDASVAATAAAAATVT
Megro-SIRT7      -----
Apido-SIRT7      -----FVQVPNIT

Drome-SIRT7      --MNRSAAS-CFFCFDRYAELECQFYRRWNLS-----QRKHKKRARSGRFVVCECCPTS
Tal-SIRT7        SIIATAAESRSLVTLHVDASELCSQESSGEDSIVMLDCDDMEFKDQKFQDGDIDIKIDDEV
Megro-SIRT7      -----SVWRTRGHLL-----
Apido-SIRT7      SDVPKPKPT-CTFCMENEGSLTCLYYQRETDNSTLIGTESKQIKDTK-----

Drome-SIRT7      DDDDDYDENISLAHIAAAETAKRRQQLSTSFPRKLARTQAGWYGKGYKGRKRR-----
Tal-SIRT7        KDVKNFEIFNDACKERKEDIKISKPTFLTNHALSRLSSDHELKNDFRKNFELHSDSLKT
Megro-SIRT7      -----VFITNEIPIILV-----
Apido-SIRT7      -----TLVIHADPPIAAKNPGWFGKGYRKGMKKKR-----

Drome-SIRT7      -----
Tal-SIRT7        EDVKEEKLDVHSSCPVLNFDNTSCKLEKKDENCKLYDSLASSIVNMTSKSKSFCQED
Megro-SIRT7      -----
Apido-SIRT7      -----

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**Figure S25. Putative *Talitrus saltator* SIRT7 protein**

Alignment of *Drosophila melanogaster* SIRT7 (Drome-SIRT7; Accession No. NP\_651664) with the *T. saltator* SIRT7 (Tal-SIRT7) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Megachile rotundata* SIRT7 (Megro-SIRT7; Accession No. XM\_012287821) and *Apis dorsata* SIRT7 (Apido-SIRT7; Accession No.). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure one SMART identified SIR2 domain is highlighted in yellow.

```

Drome-JET          -----MCTLHPEEEAHLMMAMASGGQPTRTA
Tal-JET           HIILAHLPPIFSHIFWERISRKMKMINQNNINRLELVKVFSEDEALINKKLPRELLLRVL
Oruab-FBOX/LRR    MI-----HSGRTRLELTWVFHDDEAQINKKLPKELLLRIM
Harsa-FBOX/LRR    MI-----HSGRTRLELTWVFHDDEAQINKKLPKELLLRIL
                  :  .. ::* :      .      *

Drome-JET          SPRPLVTASIAA--PRSLFDVCWDDVLIPQVAVYLSLKDL-----FN
Tal-JET           SFLDVVSLCRCAQVSHYWHSLALDGANWQRI DLFNFQTDIEGVVVEQIGRRC GGFLKELS
Oruab-FBOX/LRR    SYLDVVSLCRCAQVSKAWNVLALDGSNWQRI DLFDFQRDVEGPVIENISRRC GGFLRQLS
Harsa-FBOX/LRR    SYLDVVSLCRCAQVSKAWNVLALDGSNWQRI DLFDFQRDVEGPVIENISRRC GGFLRQLS
                  *  :*: . .* ..  .: * .  .: : :  *:      :.

Drome-JET          LRCCSRTAQ--RFVEAALEKRQELHLSGNNTKNI-DVAFRVLARC CQRLEVLHLACCRW
Tal-JET           LRGCSVKDDPLITITSLCRNIERLNLT--NCKNITDLTCGALSRHCPRLRLLELESCSK
Oruab-FBOX/LRR    LRGCSIGNNSMRTLAQSCPNIEDLNLR--QCKKISDATCAALSSHCPKLQRLNLDSCPE
Harsa-FBOX/LRR    LRGCSIGNNSMRTLAQSCPNI EELNLS--QCKKISDATCAALSSHCPKLQRLNLDSCPE
                  ** * .  :      :      : : *:*  : *:* * : .*: * . * * * . *

Drome-JET          LTDELLLPL-----
Tal-JET           VTDQSLQLLADGCPHLVFLNIAWCSEITSAGVEAVAKGCPELEVFAKGVKELRDQALLO
Oruab-FBOX/LRR    VTDLSLKSLSDGCPPLLTHINLSWCELLTDNGVEALARGCPELRSFSLSKGCRQLTDRAVKC
Harsa-FBOX/LRR    ITDISLKSLSDGCPPLLTHINLSWCELLTDNGVEALARGCPELRSFSLSKGCRQLTDRAVKC
                  : ** * *

Drome-JET          LAN NKKRLWAVNLNECVNITALSLQPIIVE CKELRVLKLSKCQWLTTGAVDALTLHQSKL
Tal-JET           LASNCPKLRHVNLHSCVLTVDASVTVLAEKCPGLRYLCLSNCALSASDASLQTLALHNTKL
Oruab-FBOX/LRR    LARYCPNLEA INLHECRNITDDAVRELSERCPRLHYVCLSNCPNLTDASLCTLAQHCPLL
Harsa-FBOX/LRR    LARYCPNLEA INLHECRNITDDAVRELSEQCPRLHYVCLSNCPNLTDASLVTLAQHCPLL
                  ** . *  :*: .* : *  : :  : * * . : **:* * : .: : : * . *

Drome-JET          VEFDISYCGAIGERCLIIFFRKLNKLTVLSLANTPSVTDQVLIQIGNYCRELEHINVIGC
Tal-JET           ETLEVANCAQFTDNGFQALARSCLLERIDLEDCLLITDATLSHLAMGCPRLEKLSLSHC
Oruab-FBOX/LRR    SVLECVACTHFTDAGFQALARNCRLEKMDLEECVLITDATLIHLAMGCPRLEKLSLSHC
Harsa-FBOX/LRR    SVLECVGCTHFTDAGFQALAKNCRLEKMDLEECVLITDATLIHLAMGCPRLEKLSLSHC
                  : : * : : : : . . * :.* : : ** .* :. : * **::: : *

Drome-JET          AAISDYGVAH---TVHCLRRLTLIRRCPRVTELSLAPLRQR LRYIDRPQ-----
Tal-JET           ELITDEGIRHLGSA ACSTEQLSVLELDNCPLITDASLDHLL-ACHNLHRIELYDCQQITR
Oruab-FBOX/LRR    ELITDEGIRQLALS PCA SEHLAVLELDNCPLITDASLDHLLQACHNLQRIELYDCQLITR
Harsa-FBOX/LRR    ELITDEGIRQLALS PCA AEHLAVLELDNCPLITDASLDHLLQACHNLRIELYDCQLITR
                  *:* * : . * . . * . * : .** :* : ** * : * :

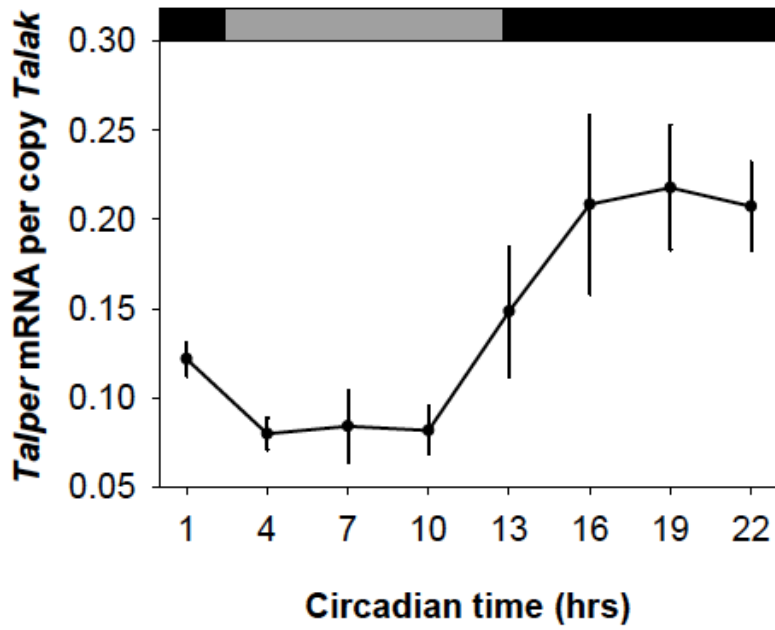
Drome-JET          -----PDVGLNAYNLNDFYPSD-----FLVY
Tal-JET           AGIRRLKAHL PNIKVQAYFAPQTPVPENTP-HRLHRCCTIL
Oruab-FBOX/LRR    AGIRRLRTHLPNIKVHAYFAPVTPPPSAGASTQRYCRCCVIL
Harsa-FBOX/LRR    AGIRRLRTHLPNIKVHAYFAPVTPPPSAGASRPYCRCCVIL
                  *:: :** *      :

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**Figure S26. Putative *Talitrus saltator* JETLAG protein**

Alignment of *Drosophila melanogaster* JETLAG (Drome-JET; Accession No. NP\_608880) with the *T. saltator* JETLAG (Tal-JET) deduced from the Trinity *de novo* transcriptome assembly, together with the top two tblastn species homologue sequences *Orussus abietinus* F-box/LRR repeat protein 20 (Oruab-FBOX/LRR; Accession No. XM\_012430170) and *Harpegnathos saltator* F-box/LRR repeat protein 20 (Harsa-FBOX/LRR; Accession No. XM\_011152298). '\*' indicates identical amino acid residues in the two proteins, '.' and ':' indicate similar amino acid residues between the two proteins. In this figure SMART

identified domains consisting of one F-box domain and multiple leucine-rich repeat domains are highlighted in yellow and green respectively.



**Figure S27. Expression profiling of *Talper* mRNA by Taqman PCR**

Timecourse samples taken from behaviourally rhythmic animals held in DD yield rhythmic changes in *Talper* abundance with peak mRNA levels in early night.

*Talper* expression is normalised to *Talak* and shown as mean expression  $\pm$  SEM (N=4). Black and grey bars illustrate time of expected day and night, respectively.

**Supplementary Table S1. Primer, probes and adapter sequences used for *Talitrus saltator* degenerate Taqman and RACE PCR.** T7 refers to the phage promoter sequence added at the 5' of the oligonucleotide; VIC® and NED® are probe fluors; MGB- Minor groove binding.

Method	Oligonucleotide name	Sequence
<i>Talitrus</i> Cry2 Degenerate PCR	Tal_Degen Cry2 F	GARGARYTIGGNTTYGAYAC
	Tal_Degen Cry2 F Nested	GARMGIAARGCNTGGGTNGC
	Tal_Degen Cry2 R	ATRWARTCICCRTTNGGRTC
	Tal_Degen Cry2 R Nested	ACYTTCATNCCYTCYTCCCA
<i>Talitrus</i> Clk Degenerate PCR	Tal_Degen Clk F	TGGAARTTYTNTTYTNGAYCA
	Tal_Degen Clk F Nested	AYCAYMGNGCNCNCNCANT
	Tal_Degen Clk R	CCADATCCAAYTGYTGNCCYTT
	Tal_Degen Clk R Nested	TGNCCYTTNGTNARRAANCKRTA
<i>Talitrus</i> Cry2 3' RACE PCR	Tal_Cry2 3' RACE F4	GAGTTTTTCTACACGGCAGCCACCAACA
	Tal_Cry2 3' RACE F5	ACGGCAGCCACCAACAACCCAAA
	Tal_Cry2 3' RACE F6	GACCACATGAAAGGCAATCCCATATG
	Adapter Oligo(dT)	GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTT
	Adapter Oligo(dT) primer	GACCACGCGTATCGATGTCGAC
<i>Talitrus</i> Clk 3' RACE PCR	Tal_Clk 3' RACE F2	GAGGGCCTCTGCAATCATCGGTTA
	Tal_Clk 3' RACE F3	ATGTGGACGACCTGGAACGGGTGTCTAC
<i>Talitrus</i> PDH 3' RACE PCR	Tal_PDH 3' RACE F1	TGGGGCTGCCAAAGTTTTTGAGGGAG
	Tal_PDH 3' RACE F2	GACAAGAGGAACTCGGAGCTCATCAACTC
<i>Talitrus</i> Cry2 5' RACE PCR	Tal_Cry2 5' RACE R8	CAGGCGAACAACGATTGTGGTGTCTAT
	Tal_Cry2 5' RACE R9	CCGAACGAGGGCAGCCAGGCTTT
	GeneRacer™ 5'	CGACTGGAGCAGGAGCACTGA
	GeneRacer™ 5' Nested	GGACTGACATGGACTGAAGGAGTA
	Adapter GeneRacer™ RNA Oligo	CGACUGGAGCAGGAGCACUGACAUGGACUGAAGGAGUAGAAA
<i>Talitrus</i> PDH 5' RACE PCR	Tal_PDH 5' RACE R1	GAGTTGATGAGCTCCGAGTTCCTCTTGTC
	Tal_PDH 5' RACE R2	CTCCCTCAAAAACCTTGGCAGCCCA
<i>Talitrus</i> Taqman PCR	TalPerTaqF1	GCCTCATGCCTCCATTCTACTG
	TalPerTaqR1	CGCAGAGGCTTGGTAGCATT
	TalPerTaqProbe	VIC®-CGAGTTCGCGAGAAC
	TalPerTaqStandF1	CTCGAGATTCGGTGCATTTT
	TalPerTaqStandR1T7	T7-GCCATCACCTCTTCTTCAA
	TalAKTaqF1	TCGAGGAACGGCTGTCCTT
	TalAKTaqR1	AGCAGCGTCAACCATTTTGTT
	TalAKqProbe	NED®-CCTGAGCCAAGACAG-MGB
	TalAKTaqStandF1	TATTTGGGGGCACAATTTGA
	TalAKTaqStandR1T7	T7-CCAAGCCAGTTTTCTTGTC



**Supplementary Table S2. tblastn analyses of all *Talitrus saltator* circadian proteins vs. all NCBI curated non-redundant sequences**

Query	Top NCBI non-redundant sequence Accession no.	Species	E-value	% amino acid identity/similarity
<i>Core clock proteins</i>				
Tal-CRY2	KC885970	<i>Eurydice pulchra</i>	0.0	78/89
Tal-CLK	AY842303	<i>Macrobrachium rosenbergii</i>	3e-64	76/90
Tal-PER	KC885967	<i>Eurydice pulchra</i>	3e-121	44/64
Tal-TIM	XM_009048115	<i>Lottia gigantea</i>	3e-108	49/68
Tal-BMAL1	JQ670886	<i>Pacifasticus leniusculus</i>	4e-139	43/55
<i>Clock associated proteins</i>				
Tal-PDH I	AB073368	<i>Marsupenaeus japonicus</i>	0.073	90/95
Tal-PDH II	-	-	-	-
Tal-CK2 $\alpha$	XM_011140473	<i>Harpegnathos saltator</i>	0.0	92/97
Tal-CK2 $\beta$	XM_012208415	<i>Atta cephalotes</i>	5e-124	82/90
Tal-CWO	XP_003744690	<i>Metaseiulus occidentalis</i>	8e-80	82/89
Tal-DBT	KC885972	<i>Eurydice pulchra</i>	0.0	95/97
Tal-PDP1 $\epsilon$	XM_012203776	<i>Atta cephalotes</i>	2e-35	70/85
Tal-PP1	XM_011065955	<i>Acromyrmex echinator</i>	0.0	79/85
Tal-MTS	XM_002426681	<i>Pediculus humanus corporis</i>	0.0	95/98
Tal-WBT	XM_008197190	<i>Tribolium castaneum</i>	0.0	88/94
Tal-TWS	JQ867383	<i>Scylla paramamosain</i>	0.0	86/92
Tal-SGG	XM_012400594	<i>Athalia rosae</i>	0.0	79/86
Tal-SLIMB	XM_012405697	<i>Athalia rosae</i>	0.0	82/90
Tal-VRI	JQ011276	<i>Clunio marinus</i>	6e-41	64/91
Tal-EBONY	XM_008199683	<i>Tribolium castaneum</i>	7e-85	39/56
Tal-RORA	XM_001987136	<i>Drosophila grimshawi</i>	3e-71	68/77
Tal-REVERB	AM710419	<i>Blattella germanica</i>	2e-102	45/64
Tal-SIRT1	XM_008555996	<i>Microplitis demolitor</i>	2e-81	69/84
Tal-SIRT2	XM_963962	<i>Tribolium castaneum</i>	5e-130	62/75
Tal-SIRT4	XM_002605838	<i>Branchiostoma floridae</i>	4e-110	57/69
Tal-SIRT6	XM_011552750	<i>Plutella xylostella</i>	3e-120	55/71
Tal-SIRT7	XM_012287821	<i>Megachile rotundata</i>	3e-144	56/71
Tal-JET	XM_012430170	<i>Orussus abietinus</i>	0.0	65/77

**Supplementary Table S3. blastp analyses of all *Talitrus saltator* circadian proteins vs. all NCBI curated non-redundant protein sequences**

Query	Top NCBI non-redundant sequence Accession no.	Species	E-value	% amino acid identity/similarity
<i>Core clock proteins</i>				
Tal-CRY2	AGV28717	<i>Eurydice pulchra</i>	0.0	78/89
Tal-CLK	AAX44045	<i>Macrobrachium rosenbergii</i>	2e-67	71/84
Tal-PER	AGV28714	<i>Eurydice pulchra</i>	0.0	37/53
Tal-TIM	KDR17447	<i>Zootermopsis nevadensis</i>	6e-139	52/72
Tal-BMAL1	AFV39705	<i>Pacifasticus leniusculus</i>	2e-154	43/57
<i>Clock associated proteins</i>				
Tal-PDH I	BAB91011	<i>Marsupenaeus japonicus</i>	0.041	90/95
Tal-PDH II	BAD13514	<i>Meimuna opalifera</i>	0.091	34/48
Tal-CK2 $\alpha$	1NA7_A	<i>Homo sapiens</i>	0.0	88/94
Tal-CK2 $\beta$	XP_012287730	<i>Orussus abietinus</i>	8e-144	86/94
Tal-CWO	XP_003744690	<i>Metaseiulus occidentalis</i>	2e-80	82/89
Tal-DBT	AGV28719	<i>Eurydice pulchra</i>	0.0	95/97
Tal-PDP1 $\epsilon$	EZA50108	<i>Cerapachys biroi</i>	7e-37	68/82
Tal-PP1	XP_011136198	<i>Harpegnathos saltator</i>	0.0	94/97
Tal-MTS	XP_002426726	<i>Pediculus humanus corporis</i>	0.0	95/98
Tal-WBT	XP_971164	<i>Tribolium castaneum</i>	0.0	88/94
Tal-TWS	AFK24473	<i>Scylla paramamosain</i>	0.0	87/93
Tal-SGG	XP_012256017	<i>Athalia rosae</i>	0.0	80/87
Tal-SLIMB	KDR19729	<i>Zootermopsis nevadensis</i>	0.0	84/92
Tal-VRI	KDR16467	<i>Zootermopsis nevadensis</i>	3e-46	50/64
Tal-EBONY	CAI26307	<i>Periplaneta americana</i>	1e-95	39/57
Tal-RORA	XP_011290218	<i>Musca domestica</i>	2e-75	70/80
Tal-REVERB	XP_011259848	<i>Camponotus floridanus</i>	1e-105	48/65
Tal-SIRT1	ABG78545	<i>Schistosoma mansoni</i>	1e-115	47/62
Tal-SIRT2	EFA06770	<i>Tribolium castaneum</i>	4e-141	60/73
Tal-SIRT4	XP_008480918	<i>Diaphorina citri</i>	8e-116	57/72
Tal-SIRT6	EFX74386	<i>Daphnia pulex</i>	2e-123	50/67
Tal-SIRT7	XP_012143211	<i>Megachile rotundata</i>	8e-147	56/71
Tal-JET	XP_008193983	<i>Tribolium castaneum</i>	0.0	63/78

Supplementary Table S4. blastp analyses of all *Talitrus saltator* circadian proteins vs. all FlyBase annotated protein sequences

Query	Top FlyBase annotated protein FlyBase no.	Species	E-value	% amino acid identity/similarity
<i>Core clock proteins</i>				
Tal-CRY2	-	-	-	-
Tal-CLK	FBpp0306710	<i>Drosophila melanogaster</i>	3e-56	56/77
Tal-PER	FBpp0304590	<i>Drosophila melanogaster</i>	4e-50	29/50
Tal-TIM	FBpp0082180	<i>Drosophila melanogaster</i>	4e-82	42/65
Tal-BMAL1	FBpp0074693	<i>Drosophila melanogaster</i>	1e-95	44/63
<i>Clock associated proteins</i>				
Tal-PDH I	FBpp0084396	<i>Drosophila melanogaster</i>	0.03	61/71
Tal-PDH II	-	-	-	-
Tal-CK2 $\alpha$	FBpp0070041	<i>Drosophila melanogaster</i>	7e-160	86/93
Tal-CK2 $\beta$	FBpp0300330	<i>Drosophila melanogaster</i>	7e-109	85/91
Tal-CWO	FBpp0081723	<i>Drosophila melanogaster</i>	4e-30	55/69
Tal-DBT	FBpp0306615	<i>Drosophila melanogaster</i>	3e-154	82/90
Tal-PDP1 $\epsilon$	FBpp0289727	<i>Drosophila melanogaster</i>	1e-34	57/74
Tal-PP1	FBpp0306442	<i>Drosophila melanogaster</i>	3e-153	84/91
Tal-MTS	FBpp0310063	<i>Drosophila melanogaster</i>	1e-174	92/97
Tal-WBT	FBpp0084575	<i>Drosophila melanogaster</i>	0.0	86/92
Tal-TWS	FBpp0081671	<i>Drosophila melanogaster</i>	0.0	80/91
Tal-SGG	FBpp0070450	<i>Drosophila melanogaster</i>	0.0	79/88
Tal-SLIMB	FBpp0303082	<i>Drosophila melanogaster</i>	0.0	78/88
Tal-VRI	FBpp0312171	<i>Drosophila melanogaster</i>	5e-41	58/77
Tal-EBONY	FBpp0083505	<i>Drosophila melanogaster</i>	1e-85	38/58
Tal-RORA	FBpp0297438	<i>Drosophila melanogaster</i>	4e-68	69/78
Tal-REVERB	FBpp0297726	<i>Drosophila melanogaster</i>	1e-92	45/65
Tal-SIRT1	FBpp0080015	<i>Drosophila melanogaster</i>	5e-80	60/78
Tal-SIRT2	FBpp0310647	<i>Drosophila melanogaster</i>	1e-100	58/72
Tal-SIRT4	FBpp0070817	<i>Drosophila melanogaster</i>	6e-84	53/70
Tal-SIRT6	FBpp0293897	<i>Drosophila melanogaster</i>	4e-99	56/72
Tal-SIRT7	FBpp0084733	<i>Drosophila melanogaster</i>	3e-115	56/71
Tal-JET	FBpp0111980	<i>Drosophila melanogaster</i>	9e-140	59/76

1 Supplementary File S1- Supplementary Methods (degenerate and RACE PCR and qPCR)

2

3 *Degenerate PCR*

4 Messenger RNA was enriched from total RNA using Dynabeads® mRNA DIRECT™ oligodT<sub>25</sub> magnetic  
5 beads (Life Technologies, UK) according to manufacturer's instructions. This was then reverse  
6 transcribed using AMV-RT kit (Promega, UK) in the following 20µl reaction mix: 1µl AMVRT, 4µl 5X  
7 AMVRT Buffer, 1µl random hexamer primers, 2µl dNTPs, 1µl RNase Inhibitor, 6µl DEPC treated water  
8 and 5µl of mRNA. The reaction was incubated at 37°C for 1 h and subsequently terminated by heating  
9 to 95°C for 5 min. In order to amplify *TalCry2*, a 10µl PCR reaction mix, including Amplitaq Gold® 2X PCR  
10 mix (Life Technologies, UK), 0.5µl 100µM each of Tal\_Degen Cry2 F primer (see Supplementary Table 1  
11 for all primer sequences) and Tal\_Degen Cry2 R primer, 1µl cDNA and 8µl DEPC-treated water was  
12 subject to the following thermal cycling conditions: 95°C 4 min activation followed by 40 cycles of 94°C  
13 30 sec, 42°C 45 sec, 72°C 45 sec with a final extension at 72°C for 7 min. One microliter of this product  
14 was then used in a second (nested) round of PCR using a mix of 10µl Bioline MyTaq™ Red 2X PCR mix  
15 (Bioline, UK), 0.5µl 10µM each Tal\_Degen Cry2 F Nested primer, 0.5µl Tal\_Degen Cry2 R Nested primer,  
16 and 8µl DEPC water. Cycling conditions were 95°C 4 min activation followed by 35 cycles 94°C 30 sec,  
17 46°C 45 sec, 72°C 45 sec with a final extension at 72°C for 7 min. PCR amplicons were resolved and  
18 visualised on a 1.5 % agarose gel. Bands were excised, purified and cloned for sequencing as detailed  
19 below.

20 PCR procedures used to amplify TalClk were identical to those for TalCry2 except that TalClk primers  
21 were used (Table S1), and annealing temperatures differed as follows. For the first round PCR an  
22 annealing temperature of 40°C was used whilst second round (nested) PCR was done with an annealing  
23 temperature of 45°C.

24

25 *5' and 3' Rapid amplification of cDNA ends (RACE) PCR*

26 RACE PCR was done using GeneRacer™ kit (Life Technologies, UK). mRNA extracted from 1 µg total RNA  
27 was reverse-transcribed with Superscript™ III reverse transcriptase (Life Technologies, UK) at 50°C for

1 50 minutes and using the GeneRacer 3' oligo (dT) adapter primer (see Table 1 for all adapter  
2 sequences). For 5' RACE, mRNA was dephosphorylated, decapped, ligated to a 5' RACE RNA oligo, and  
3 reverse-transcribed with Superscript III™ and using random primers, according to the manufacturer's  
4 instructions. After reverse transcription, templates were removed by incubation with RNase H (2 U, 15  
5 minutes, 37°C). The reagents were heated to 65°C for 5 min then transferred to ice for 1 min. The cDNA  
6 was subsequently used as a starting template for nested RACE PCR. For 3' RACE each of 3 PCR rounds  
7 was done in the following 20µl reactions: 10µl Bioline MyTaq™ Red 2X PCR Mix (Bioline, UK), 3' RACE  
8 Primer, 0.5µl Gene Specific Primer (1st round - Tal\_Cry2 3' RACE F4, 2nd round Tal\_Cry2 3' RACE F5,  
9 3rd round Tal\_Cry2 3' RACE F6), 8.5µl water and 0.5µl cDNA (each successive PCR round used the  
10 product from the preceding PCR round as template). For each round of PCR the cycling conditions were:  
11 95°C 4 min activation followed by 40 cycles of 94°C 30 sec, 58°C 45 sec, 72°C 45 sec with a final  
12 extension at 72°C for 7 min. Amplicons were resolved and visualised on a 1.5% agarose gel and bands  
13 excised, purified and cloned for sequencing as detailed below. The same procedure was used for TalClk  
14 and TalPDH-I however only two rounds of PCR were used along with the appropriate primers (Tal\_Clk  
15 3' RACE F2 for first round then Tal\_Clk 3' RACE F3 for second round for TalClk; Tal\_PDH 3' RACE F1 for  
16 first round then Tal\_PDH 3' RACE F2 for second round for TalPDH-I).

17 The 5' ends of TalCry2 and TalClk were amplified in the following PCRs: 10µl Amplitaq Gold PCR 2X Mix  
18 (Applied Biosystems, UK), 1.2µl GeneRacer™ 5' Primer, 0.4µl Gene Specific Primer (Tal\_Cry2 5' RACE  
19 R8), 8µl water and 0.4µl cDNA. Cycling conditions were; 95°C 5 min activation followed by 5 cycles 94°C  
20 30 sec, 72°C 2 min, 5 cycles 94°C 30 sec, 70°C 2 min, 25 cycles 94°C 30 sec, 65°C 30 sec, 72°C 2 min with  
21 a final extension at 72°C for 7 min. The cDNA product was used to seed a second round (nested) PCR as  
22 follows. Ten microliters Bioline MyTaq™ Red 2X PCR Mix (Bioline, UK), 0.5µl GeneRacer™ 5' Nested  
23 Primer, 0.5µl Gene Specific Primer (Tal\_Cry2 5' RACE R9), 8µl water and 1µl template from first round  
24 PCR. Cycling conditions were; 95°C 5 min activation followed by 40 cycles 94°C 30 sec, 65°C 30 sec,  
25 72°C 2 min with a final extension at 72°C for 10 min. Amplicons were resolved and visualised on a 1.5%  
26 agarose gel before bands were excised, purified and cloned into TOPO PCR4 vector, grown in TOP10' E.  
27 coli and prepared for sequencing as described elsewhere (see Wilcockson and Webster 2008). An

1 identical strategy was used for TalPDH-I using the appropriate primers (Tal\_PDH 5' RACE R1 for first  
2 round and Tal\_PDH 5' RACE R2 for the second round).

#### 3 4 *Quantitative PCR*

5 We validated FPKM values for *Talper* since this showed the most robust pattern of cycling (see results  
6 section).

7 Quantitative RT-PCR was done using Applied Biosystems TaqMan® MGB hydrolysis probes 5' labeled  
8 with VIC (*Talper*) or NED (*TalAK*) as described previously [1]. Sequences TaqMan primers and probes  
9 are given in Table 1. RNA was extracted and reverse transcribed as detailed (Methods). Standard  
10 curves in the range  $1 \times 10^{11}$  copies per  $\mu\text{L}$  for qPCR were made from cRNA produced by *in vitro*  
11 transcription using DNA templates amplified from brain cDNA and using T7 phage promoter-flanked  
12 PCR primers (Table 1). Quantitative RT-PCR (qRT-PCR) was done using Bioline Sensimix™ Probe  
13 mastermix containing the internal reference dye, ROX, according the manufacturers  
14 recommendations. Each 20  $\mu\text{L}$  reaction contained 0.5  $\mu\text{L}$  each primer (10  $\mu\text{M}$ ) and probe (2.5  $\mu\text{M}$ ) and  
15 1  $\mu\text{L}$  cDNA. *Talper* was measured in duplex reactions with the internal reference gene *arginine kinase*  
16 (*Talak*, Contig # 96331\_c1\_seq1). We investigated several candidate reference genes including  $\beta$ -*actin*  
17 and  $\beta$ -*tubulin* and found that only *Talak* provided reliable normalization in time series data.

18 Quantitative PCRs were run in triplicate on an Applied Biosystems StepOne Plus machine. For each  
19 assay, standards in serial ten-fold dilutions were run in the range  $10^9$ - $10^3$  copies per reaction. PCR  
20 efficiencies were in the range 90-100%. Data were expressed as copies *Talper* per copy of *Talak*.

21 Time-course qPCR data were analysed by one-way ANOVA.

- 22  
23
- 24 1. Sharp JH, Wilcockson DC, Webster SG: **Identification and expression of mRNAs encoding**  
25 **bursicon in the plesiomorphic central nervous system of *Homarus gammarus*.** *General*  
26 *and comparative endocrinology* 2010, **169**(1):65-74.
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