

**B**

1	MKKVLLTKAQHIQPVVASSSDVLVIKPVGSLRVISISAPHSASTYIQRKS	50
51	FQPVYEDIFKLLMKIPDKALTQRVIDAINESNNNDKVAISNHGKQCSCGV	100
101	QKVDSSTQTDWDTEETHMPGITNNSCEAKGNRHSMTSSSSEP IKPPVDP	150
151	TKVP <b>KKRGRKR</b> NTCVPQVVKRSAEMALKEREKQLTPV <b>ITKRKKRDVTQ</b>	200
201	AQNFESYQITNKTPVRRNSTLSDISFNSDELTRVENFISGNTEDRIIRIM	250
251	ANEFRKSHIMSEGLLP IHEEILHGDVYGVKRQIFVCCHAKMDINELLTR	300
301	DGEDCLELAL TNDTDTEIVSLILDARMMTDHLYENSNTALHLAVINHINI	350
351	<u>ESIRLLLRRIDLNSLLLTNDDGYTVLHLAVRNNQFLVVEAILDSIDEREL</u>	400
401	GETVYRRITLEAANPNEWDEKGFAYYDRACERLELNKTLNRAHKRDI	450
451	NASEARGGNPPLFYAVEGEQEHL CYFLLAHLADPDEENLSGHSPKSYHYE	500
501	YARTLRINLKVARVMEKVISILNT	524

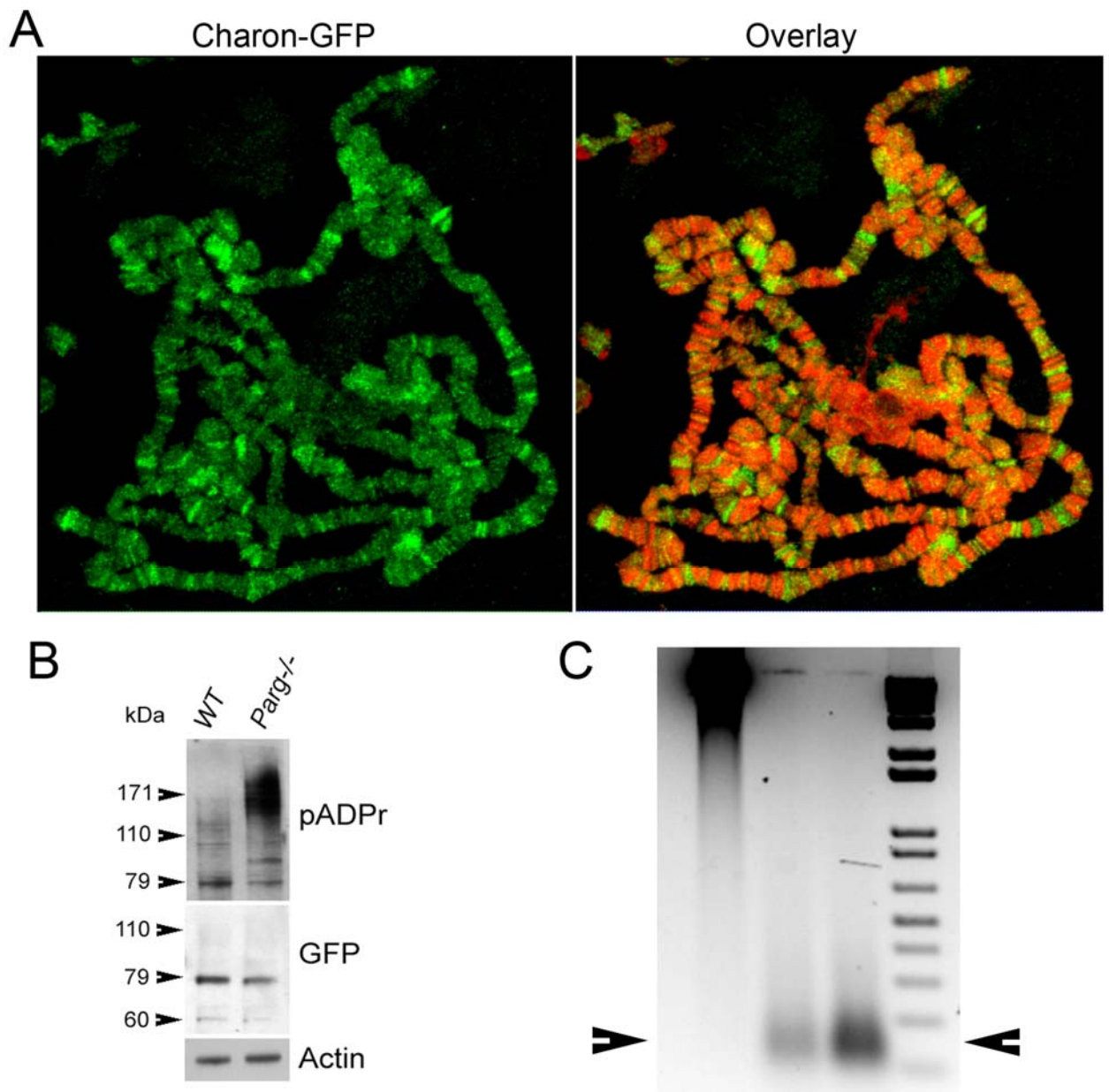
**Supplemental Figure S1. The characterization of *Charon* locus in *Drosophila melanogaster*.** **A.** Structure of *Charon* locus in *Drosophila* genome based on Flybase [26]. **B.** Sequence of *Drosophila* Charon protein. NLS sequences are shown in Red. Ankyrin repeats are shown in blue.

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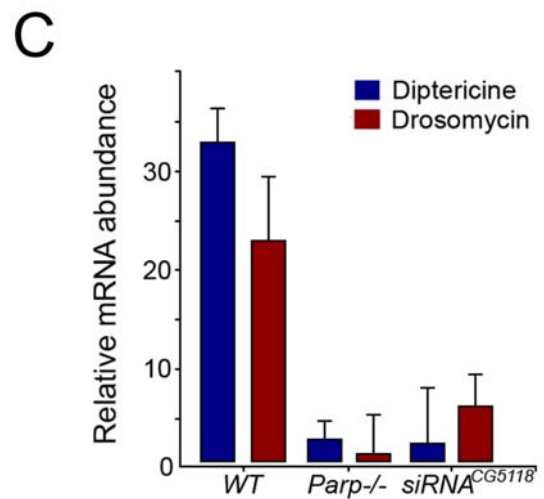
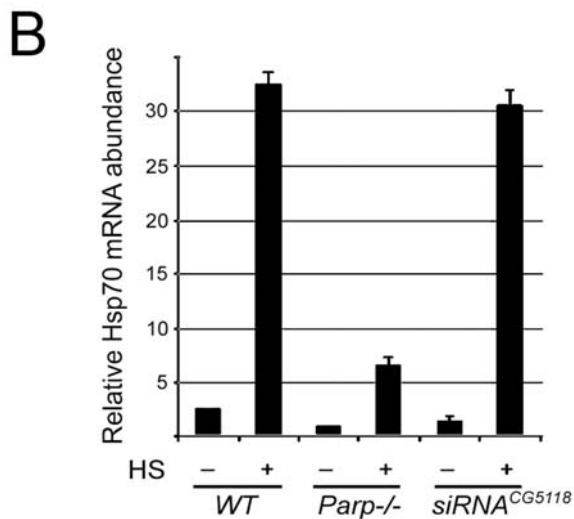
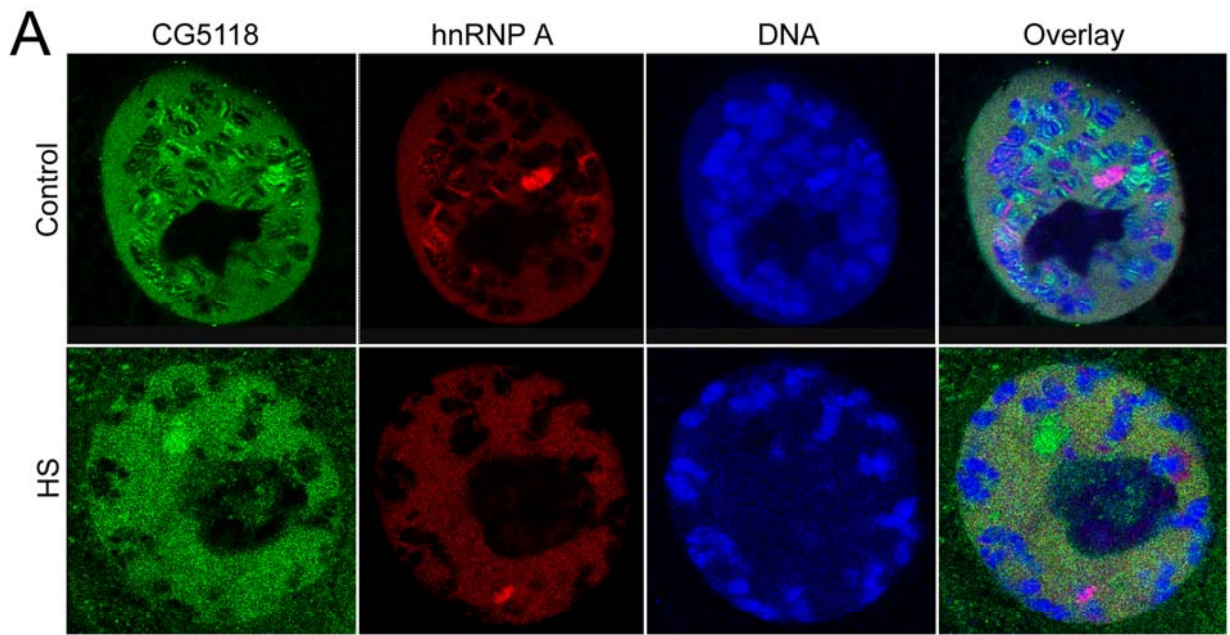
Ceratitis      -----MKKVILTKSSNREFATTMKVTPSPDNAKSKIDYSELFRVVLKIPEKDVQLRILDA
Bactrocera    -----MEKVIVRRSNRN--TAALKVISPDNAKSKSAYAELFRVVLQIPEKDVQDRILAA
CG5118        -----MKKVLLTKAQHIQPVVASSSD-----VLVIKPVGSLRVISIS
Lucilia       MITVRDFKQKNDTKTNKEDEVIKQKAAT-VDDSKNKFSVIHVVSATQQTVAMVPRFKCTI
Musca         MPNIKPTTEKAPGACKDISIVVGSPLPLSITTEMAKKSISRATVMVKNEMIGVPTSNAPV
:
Ceratitis     VNGRTSTGNIVHAICPNCKHTFDNAVLSGKEAGTQTDLFLEGANKSD----TTVVVPMQVVT
Bactrocera    VNGKASNAQIIHAMCPNCKKTFGNSNDPGKEIATQTDIAEIIISVSKNKATKIIITPPPPQT
CG5118        APHSASTYIQRKSFQPVYEDIFKLLMKIPDKALTQRFVIDAINESNN-----N
Lucilia       LKHVAPTKDKTPRPLVPLAPAVEGTGN-----GQVYQQRKRMLPEYKELFAALMKLPD
Musca         VVKNETVKVPTPNATVIVKREMKPPIQNDMEQKPSHIYQQRKYMLNEYKELFSVLMRFSD
.
Ceratitis     QTVSISSSNGQDFNIMSPVLQKAKVLSAKQNLSTIPQNLTVSKDTESQTPNMIEPQSA
Bactrocera    SQNRSHSSNGANSVEISSTISRRTVIFTTVPVDHAI AQQSSIINAIESIEPMEDQTAQVE
CG5118        DKVAISNHGKQCSCGVQKVDSSQTQDWDTEETHMPGITNNSCEAKGNRHSHMTSSSSEPI
Lucilia       KEMQNKLLAVINSHHSPREYRTIETQTDVPEIKDLLSKE-TEEISIRETANGSGNETPNS
Musca         KEISQKVVRCLN-----AKYANKEQTQDTPVEILETSSSK-ELVPPVNTNTTESTPTSPN-
.
Ceratitis     DATAVTSNLPMKRKRKRKVKLPQVVKRSHAQMALTHMQPR-----LKRKVKQKSPELYQ
Bactrocera    EP-----TNPVVKRKRKRKVKLPQVVKRSHAQMAHTQLQPK-----LKARKIDKSMDLGQ
CG5118        KPPVDPTKVPKRRGRKRNTCVPQVVKRSHAAEMALKEREKQLTPVITKRKRKRDVTAQNF
Lucilia       KDSNASEEMVKRKRKRKVKVSLPQANKESRAKQLAKMKNPK-----TSMNNNDAA
Musca         ---NSRPPTPKRKRKRKRKVAEPQANKESRVAQRSLLEKS-----VQRKPSSTQ
          *: *:*:*.. **.*.* . :
Ceratitis     KASDDGNDACRRDISISLSDSLIKDVLWLSF-DEHKSKEHDHIMRTMAEECLEADRR-TEG
Bactrocera    KPLNELIKRNRSDSMISLADILNDFMYSDDPELHKTKEEHIMRLMAAECSDADIR-IDG
CG5118        ESYQITNKTPVRRNSTLSDISFNSDELTRVENFISGNTEDRIIRIMANEFRKSHIMSEEG
Lucilia       KPSAPNGQQLNSPKRQRIDSFSISECSSDIVNIFDDHQTN-----VIMEFNKLNPEPFENG
Musca         PEIAQSNNDPCAKRRRSNSVCSNDSTLFNMITDMDLSRKDELYESFIKDCIIADVPLENG
.
Ceratitis     LLTIHRTIVQNDLYALRRQIFVWKKFKKQVQDLNLLTDEDENCLQLAIAQDCFPKI IDVL
Bactrocera    LLAIHRTIVVNDLLALRRQIFVWKKLQVDDLNTLLTDDDENCLQLAIVQDCFPKIEVL
CG5118        LLPHEEILHGDVYGVKRQIFVVCCHAK--MDINELLTRDGEDCLELALNTDTEIVSLI
Lucilia       LLPIQDGVVKNQTYKLSLHPIWQTYIG-TDLNDILTEDEDELLQLAVINMCDPSIISLL
Musca         LLPIYDCIVRDQDRTLKTQILIWKEYKR-VDLNELITDDEDELLQLAINNRRSPPIKLL
**.* : : : : * : *:* : * :*: *:* : : . *:*:*
Ceratitis     ISEGLNANEIDCHSNTCVHLAILNEIENESLRLLMRKIDPQLLLHLNDDGYTPLHMAVRA
Bactrocera    LNEGLNVNEIDDQSNTCVHLAVLNEIDDDSLILLFKKIDLNLLHLNDDGYTPLHMAVRA
CG5118        LDARMMTDHLYENSNTALHLAVINHINIESIRLLLRRIDLNSLLLNTDGYTVLHLAVRN
Lucilia       LEKGLNPNTFDAESNTIVHLAILNDITVKSEHLMNKIELKLLTLNDEGYTPLHLAIRQ
Musca         LTNGLRPNVSDCQGNIIHLVCLNDLVPDSMDHLMSHIDLKMLLELNDDGYTCLQLAVRQ
: : : . ..*.* :*:*:*:* . * : * : * : * : * : * : * : * : * : *
Ceratitis     NSYLRAEIMLNTLDERLLGKPIFNRNIRASLS-----EKEFGKYEEICKNLEKQCGNVT
Bactrocera    NGYLRAEIIILNEIDRRLGEPSPFRNRSKVLTS-----EAEFHKYEEACKKLEKNYSNTT
CG5118        NQFLVVEAILDSIDERELGETVYRRTLEAANPNE-WDEKGFAYYDRACERLELNKTLK
Lucilia       DRFLLAECMLNILDERLMKKVYKQRLDELETDKVLKKQFHNYEKICLRMALDDETN
Musca         DNFLLAECILNGVDKRLSGSIFYTRYNKIESDEKTMKLDYKDYKVCQDMIVEDNVA
: * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
Ceratitis     R----NAQPKLKKKVLLETGDRKSGNTALFFAVENKLEHFVFFLLAHLTDPRILNYCGQDA
Bactrocera    R----SAQPKLKKKFLMGDRKSGNTALFFAIENKLEHFIFFLAHLTDPRVMNFSGQDA
CG5118        N----RAH---KRDVINASEARGGNPPLFYAVEGEQEHLICYFLAHLADPDEENLSGHS
Lucilia       S---IIDNHDLKQKLLQAGDRRSGNTVLYFAIDNR-----
Musca         ANGNIIKNSDLKQKLLQVTDVRSSTALFFAIENQSEHLIYFLAHLSDPRTENLSGQDC
: * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
Ceratitis     KSYSEFGKMLQLSLRVDSAMENVVSLLR-
Bactrocera    KSYTEFGKMLQISLKVNDAMESVVTILG-
CG5118        KSYHYEYARTLRINLKVARVMEKVISILNT
Lucilia       -----YGEQELQ-----
Musca         KTFNEFGKSLKLSLDIDSAMERVIQLLS-
          * :

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**Supplemental Figure S2. Evolutionary conservation for Charon protein in *Insects*.**  
Multiple sequence alignments of Charon family members: Lucilia - *Lucilia cuprina* (KNC31104.1); CG5118 - *Drosophila melanogaster* (CG5118); Musca - *Musca domestica* (101889410); Ceratitis - *Ceratitidis capitata* (101454591); Bactrocera - *Bactrocera cucurbitae* (105210874).



**Supplemental Figure S3. Charon is a nuclear chromatin associated protein.** **A.** Charon protein labels ~ 400 loci in *Drosophila* chromatin: *Drosophila* larvae expressing Charon-GFP were cultured for 60hrs with *E.coli* bacteria; salivary glands were dissected from third instar larvae, squashed and stained with monoclonal anti-GFP (green) antibodies; DNA was detected using propidium iodide (red). **B.** Charon is not covalently modified with pADPr. Total nuclear protein extracts from animals expressing Charon-GFP wild type (WT) and *parg*<sup>27.1</sup> mutants were subjected to Western-blot analysis using anti-GFP, anti-pADPr antibodies. Proteins which covalently modified with pADPr demonstrate super-shift on Western-blot in *parg* mutants. Band corresponding to Charon-GFP does not demonstrate this. Anti-Actin antibody was used as a loading control. **C.** Quality control of MNase treated mononucleosomal chromatin for Co-IP and ChIP. Digested chromatin was checked on agarose gel after different time intervals of incubation with MNase.



**Supplemental Figure S4. Charon represents a third PARP-1 dependent pathway. A.** Charon protein is not required for PARP-1-dependent hnRNP-A1 pathway: Charon-GFP (green) is not colocalized with a key component of PARP-1 dependent RNA-fate-regulating pathway, hnRNP A1 (red) genomewide in steady state conditions (top). Upon heat shock hnRNP A1 remains bound to chromatin, while Charon relocates to soluble nucleoplasm. **B.** Charon is not required for PARP-1-dependent *hsp70* gene transcription. Quantitative RT-PCR experiment is shown. The amounts of PARP-1 dependent *hsp70* mRNA was detected using specific primers before (-) and after (+) 30 minutes heat shock in wild type (WT), *parp-1* mutants and Charon knockdowns. **C.** Expression of endogenous *Drosomycin* and *Diptericine* genes was detected after 20 hours infection in wild type (WT), *Parp-1*<sup>C03256</sup> mutant, and siRNA<sup>CG5118</sup>-expressing third instar larvae using quantitative RT-PCR.