Supplementary figures

Supplementary Figure 1

Sequences alignment of GNBP3 from 12 *Drosophila* species. The regions are delineated by backgrounds of different colors: green for the signal peptide, blue for the Glucan recognition domain, grey for the N-terminal domain, faint yellow for the Glucanase domain and dark yellow for the O-glycosyl hydrolase (E.C3.2.1) domain. The color code for the amino acids is the following: red for strict conservation, blue for conservative mutations and green for a percentage of conservation higher than 75. The catalytic residues (Glu (E) in bacterial glucanases) that are mutated into Gln (Q) and Tyr (Y) in the GNBPs are in frame.

Supplementary Figure 2

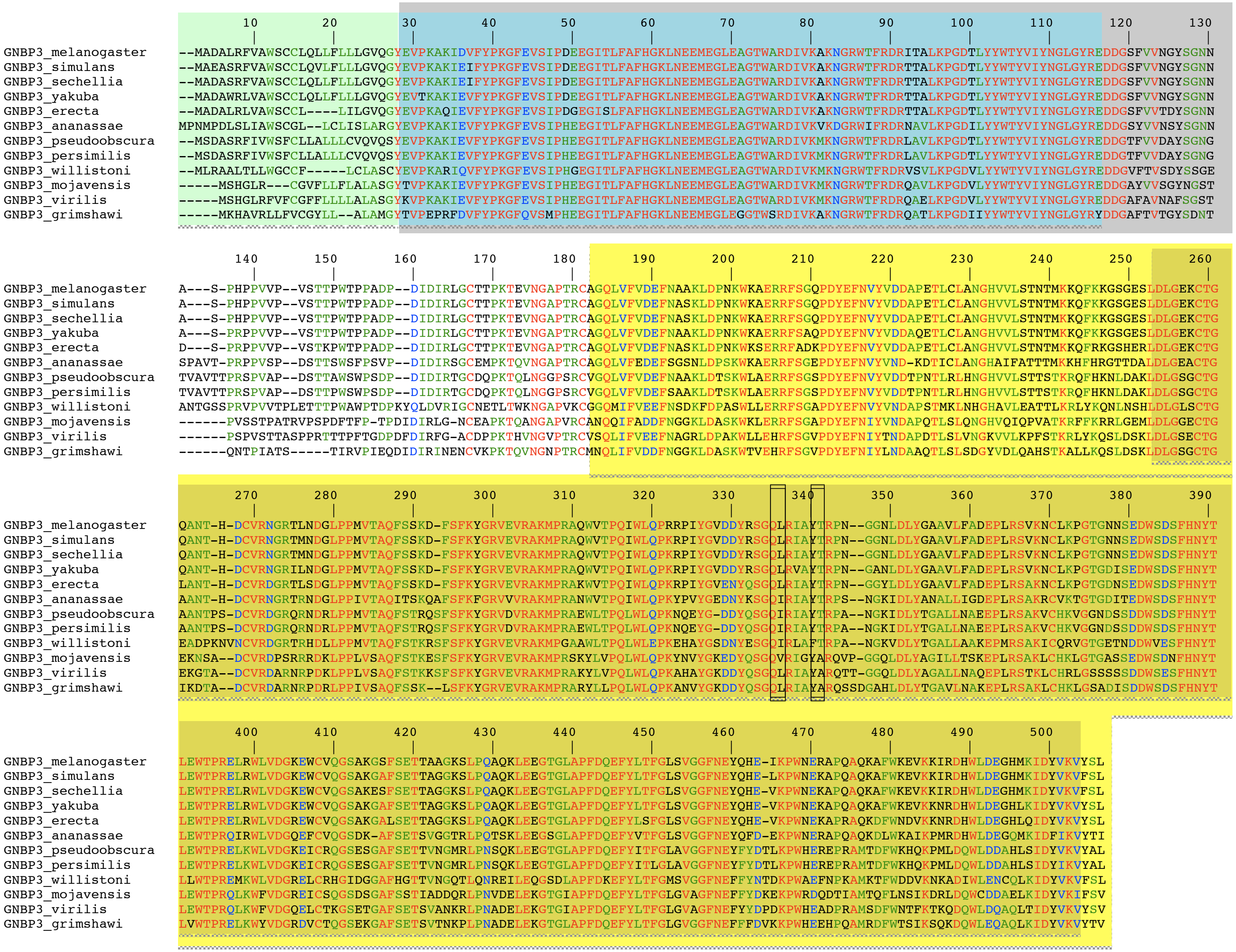
Sequence alignment of the N-terminal domain of GNBP1,2 and 3 of *Drosophila melanogaster* (GNBP1, 2 and 3), *Bombyx mori* and *Hyphantria cunea* GNBPs. The color code is the same as in supplementary Figure 1

Supplementary Figure 3

(A) Sequence modification in the short-loop mutant.

(B) CD spectra were recorded on a Jasco J-810 spectropolarimeter from 260 to 200 nm using a 1 mm jacketed quartz cell at 20°C, a scan rate of 20 nm.min^{-1}, a response time of 2ms, a band width of 1 nm, and a resolution of 1nm. The contribution of the buffer was subtracted and each spectrum represents the average of five scans. The spectra are presented without smoothing or further data processing. The three curves, corresponding to wild type, W77A and short-loop mutants, display the same minimum at 215 nm and cross the X axis at the same value (205 nm). A slight difference is observed for W77A mutant around 230 nm. This may be due to the removal of the tryptophane residue, which is in strong interaction with an histidine residue in the wild type and short-loop mutant.

Supplementary figure 1



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                               10        20        30        40        50        60        70        80        90       100       110       120       130
                                |         |         |         |         |         |         |         |         |         |         |         |         |
GNBP3_melanogaster    --MADALRFVAWSCCLQLLFLLLGVQGYEVPKAKIDVFYPKGFEVSIPDEEGITLFAFHGKLNEEMEGLEAGTWARDIVKAKNGRWTFRDRITALKPGDTLYYWTYVIYNGLGYREDDGSFVVNGYSGNN
GNBP3_simulans        --MAEASRFVAWSCCLQVLFLLLGVQGYEVPKAKIEIFYPKGFEVSIPDEEGITLFAFHGKLNEEMEGLEAGTWARDIVKAKNGRWTFRDRTTALKPGDTLYYWTYVIYNGLGYREDDGSFVVNGYSGNN
GNBP3_sechellia       --MADASRFVAWSCCLQLLFLLLGVQGYEVPKAKIEIFYPKGFEVSIPDEEGITLFAFHGKLNEEMEGLEAGTWARDIVKAKNGRWTFRDRTTALKPGDTLYYWTYVIYNGLGYREDDGSFVVNGYSGNN
GNBP3_yakuba          --MADAWRLVAWSCCLQLLFLLLGVQGYEVTKAKIEVFYPKGFEVSIPDEEGITLFAFHGKLNEEMEGLEAGTWARDIVKAKNGRWTFRDRTTALKPGDTLYYWTYVIYNGLGYREDDGSFVVNGYSGNN
GNBP3_erecta          --MADALRLVAWSCCL----LILGVQGYEVPKAQIEVFYPKGFEVSIPDGEGISLFAFHGKLNEEMEGLEAGTWARDIVKAKNGRWTFRDRTTALKPGDTLYYWTYVIYNGLGYREDDGSFVVTDYSGNN
GNBP3_ananassae       MPNMPDLSLIAWSCGL--LCLISLARGYEVPKAKIEVFYPKGFEVSIPHEEGITLFAFHGKLNEEMEGLEAGTWARDIVKVKDGRWIFRDRNAVLKPGDILYYWTYVIYNGLGYREDDGSYVVNSYSGNN
GNBP3_pseudoobscura   --MSDASRFIVWSFCLLALLLCVQVQSYEVPKAKIEVFYPKGFEVSIPHEEGITLFAFHGKLNEEMEGLEAGTWARDIVKMKNGRWTFRDRLAVLKPGDTLYYWTYVIYNGLGYREDDGTFVVDAYSGNG
GNBP3_persimilis      --MSDASRFIVWSFCLLALLLCVQVQSYEVPKAKIEVFYPKGFEVSIPHEEGITLFAFHGKLNEEMEGLEAGTWARDIVKMKNGRWTFRDRLAVLKPGDTLYYWTYVIYNGLGYREDDGTFVVDAYSGNG
GNBP3_willistoni      --MLRAALTLLWGCCF-----LCLASCYEVPKARIQVFYPKGFEVSIPHGEGITLFAFHGKLNEEMEGLEAGTWARDIVKMKNGRWTFRDRVSVLKPGDVLYYWTYVIYNGLGYREDDGVFTVSDYSSGE
GNBP3_mojavensis      -----MSHGLR---CGVFLLFLALASGYTVPKAKIEVFYPKGFEVSIPHEEGITLFAFHGKLNEEMEGLEAGTWARDIVKMKNGRWTFRDRQAVLKPGDVLYYWTYVIYNGLGYREDDGAYVVSGYNGST
GNBP3_virilis         -----MSHGLRFVFCGFFLLLLALASGYKVPKAKIEVFYPKGFEVSIPHEEGITLFAFHGKLNEEMEGLEAGTWARDIVKMKNGRWTFRDRQAELKPGDVLYYWTYVIYNGLGYREDDGAFAVNAFSGST
GNBP3_grimshawi       -----MKHAVRLLFVCGYLL--ALAMGYTVPEPRFDVFYPKGFQVSMPHEEGITLFAFHGKLNEEMEGLEGGTWSRDIVKAKNGRWTFRDRQATLKPGDIIYYWTYVIYNGLGYRYDDGAFTVTGYSDNT

                              140       150       160       170       180       190       200       210       220       230       240       250       260
                                |         |         |         |         |         |         |         |         |         |         |         |         |
GNBP3_melanogaster    A---S-PHPPVVP--VSTTPWTPPADP--DIDIRLGCTTPKTEVNGAPTRCAGQLVFVDEFNAAKLDPNKWKAERRFSGQPDYEFNVYVDDAPETLCLANGHVVLSTNTMKKQFKKGSGESLDLGEKCTG
GNBP3_simulans        A---S-PHPPVVP--VSTTPWTPPADP--DIDIRLGCTTPKTEVNGAPTRCAGQLVFVDEFNASKLDPNKWKAERRFSGQPDYEFNVYVDDAPETLCLANGHVVLSTNTMKKQFKKGSGESLDLGEKCTG
GNBP3_sechellia       A---S-PHPPVVP--VSTTPWTPPADP--DIDIRLGCTTPKTEVNGAPTRCAGQLVFVDEFNASKLDPNKWKAERRFSGQPDYEFNVYVDDAPETLCLANGHVVLSTNTMKKQFKKGSGESLDLGEKCTG
GNBP3_yakuba          A---S-PRPPVVP--VSTTPWTPPADP--DIDIRLGCTTPKTEVNGAPTRCAGQLVFVDEFNAAKLDPNKWKAERRFSAQPDYEFNVYVDDAQETLCLANGHVVLSTNTMKKQFKKGSGESLDLGEKCTG
GNBP3_erecta          D---S-PRPPVVP--VSTKPWTPPADP--DIDIRLGCTTPKTEVNGAPTRCAGQLVFVDEFNASKLDPNKWKSERRFADKPDYEFNVYVDDAPETLCLANGHVVLSTNTMKKQFRKGSHERLDLGEKCTG
GNBP3_ananassae       SPAVT-PRPPVSP--DSTTSWSFPSVP--DIDIRSGCEMPKTQVNGAPTRCAGQLVFEDEFSGSNLDPSKWKAERRFSGEPDYEFNVYVND-KDTICLANGHAIFATTTMKKHFHRGTTDALDLGEACTG
GNBP3_pseudoobscura   TVAVTTPRSPVAP--DSTTAWSWPSDP--DIDIRTGCDQPKTQLNGGPSRCVGQLVFVDEFNAAKLDTSKWLAERRFSGSPDYEFNVYVDDTPNTLRLHNGHVVLSTTSTKRQFHKNLDAKLDLGSGCTG
GNBP3_persimilis      TVAVTTPRSPVAP--DSTTPWSWPSDP--DIDIRTGCDQPKTQLNGGPSRCVGQLVFVDEFSAAKLDTSKWLAERRFSGSPDYEFNVYVDDTPNTLRLHNGHVVLSTTSTKRQFHKNLDAKLDLGSGCTG
GNBP3_willistoni      ANTGSSPRVPVVTPLETTTPWAWPTDPKYQLDVRIGCNETLTWKNGAPVKCGGQMIFVEEFNSDKFDPASWLLERRFSGAPDYEFNVYVNDAPSTMKLNHGHAVLEATTLKRLYKQNLNSHLDLGLSCTG
GNBP3_mojavensis      ------PVSSTPATRVPSPDFTFP-TPDIDIRLG-NCEAPKTQANGAPVRCANQQIFADDFNGGKLDASKWKLERRFSGAPDYEFNIYVNDAPQTLSLQNGHVQIQPVATKRFFKRRLGEMLDLGGECTG
GNBP3_virilis         ------PSPVSTTASPPRTTTPFTGDPDFDIRFG-ACDPPKTHVNGVPTRCVSQLIFVEEFNAGRLDPAKWLLEHRFSGVPDYEFNIYTNDAPDTLSLVNGKVVLKPFSTKRLYKQSLDSKLDLGSECTG
GNBP3_grimshawi       ------QNTPIATS-----TIRVPIEQDIDIRINENCVKPKTQVNGNPTRCMNQLIFVDDFNGGKLDASKWTVEHRFSGVPDYEFNIYLNDAAQTLSLSDGYVDLQAHSTKALLKQSLDSKLDLGSGCTG

                              270       280       290       300       310       320       330       340       350       360       370       380       390
                                |         |         |         |         |         |         |         |         |         |         |         |         |
GNBP3_melanogaster    QANT-H-DCVRNGRTLNDGLPPMVTAQFSSKD-FSFKYGRVEVRAKMPRAQWVTPQIWLQPRRPIYGVDDYRSGQLRIAYTRPN--GGNLDLYGAAVLFADEPLRSVKNCLKPGTGNNSEDWSDSFHNYT
GNBP3_simulans        QANT-H-DCVRNGRTMNDGLPPMVTAQFSSKD-FSFKYGRVEVRAKMPRAQWVTPQIWLQPKRPIYGVDDYRSGQLRIAYTRPN--GGNLDLYGAAVLFADEPLRSVKNCLKPGTGNNSEDWSDSFHNYT
GNBP3_sechellia       QANT-H-DCVRNGRTMNDGLPPMVTAQFSSKD-FSFKYGRVEVRAKMPRAQWVTPQIWLQPKRPIYGVDDYRSGQLRIAYTRPN--GGNLDLYGAAVLFADEPLRSVKNCLKPGTGNNSEDWSDSFHNYT
GNBP3_yakuba          QANT-H-DCVRNGRILNDGLPPMVTAQFSSKD-FSFKYGRVEVRAKMPRAQWVTPQIWLQPKRPIYGVDDYRSGQLRVAYTRPN--GANLDLYGAAVLFADEPLRSVKNCLKPGTGDISEDWSDSFHNYT
GNBP3_erecta          LANT-H-DCVRDGRTLSDGLPPMVTAQFSSKD-FSFKYGRVEVRAKMPRAKWVTPQIWLQPKRPIYGVENYQSGQLRIAYTRPN--GGYLDLYGAAVLFADEPLRSAKNCLKPGTGDNSEDWSDSFHNYT
GNBP3_ananassae       AANT-H-DCVRNGRTRNDGLPPIVTAQITSKQAFSFKFGRVVVRAKMPRANWVTPQIWLQPKYPVYGEDNYKSGQIRIAYTRPS--NGKIDLYANALLIGDEPLRSAKRCVKTGTGDITEDWSDSFHNYT
GNBP3_pseudoobscura   AANTPS-DCVRDGRQRNDRLPPMVTAQFSTRQSFSFKYGRVDVRAKMPRAEWLTPQLWLQPKNQEYG-DDYQSGQIRIAYTRPA--NGKIDLYTGALLNAEEPLRSAKVCHKVGGNDSSDDWSDSFHNYT
GNBP3_persimilis      AANTPS-DCVRDGRQRNDRLPPMVTAQFSTRQSFSFKYGRVDVRAKMPRAEWLTPQLWLQPKNQEYG-DDYQSGQIRIAYTRPA--NGKIDLYTGALLNAEEPLRSAKVCHKVGGNDSSDDWSDSFHNYT
GNBP3_willistoni      EADPKNVNCVRDGRTRHDLLPPMVTAQFSTKRSFSFKYGRVEVRAKMPGAAWLTPQLWLEPKEHAYGSDNYESGQIRLAFTRPA--NGKVDLYTGALLAAKEPMRSAKICQRVGTGETNDDWVESFHNYT
GNBP3_mojavensis      EKNSA--DCVRDPSRRRDKLPPLVSAQFSTKESFSFKYGRVEVRAKMPRSKYLVPQLWLQPKYNVYGKEDYQSGQVRIGYARQVP-GGQLDLYAGILLTSKEPLRSAKLCHKLGTGASSEDWSDNFHNYT
GNBP3_virilis         EKGTA--DCVRDARNRPDKLPPLVSAQFSTKKSFSFKYGRVEVRAKMPRAKYLVPQLWLQPKAHAYGKDDYQSGQLRIAYARQTT-GGQLDLYAGALLNAQEPLRSTKLCHRLGSSSSSDDWSESFHNYT
GNBP3_grimshawi       IKDTA--DCVRDARNRPDRLPPIVSAQFSSK--LSFKYGRVEVRAKMPRARYLLPQLWLQPKANVYGKDDYQSGQLRIAYARQSSDGAHLDLYTGAVLNAKEPLRSAKLCHKLGSADISDDWSDSFHNYT

                              400       410       420       430       440       450       460       470       480       490       500
                                |         |         |         |         |         |         |         |         |         |         |
GNBP3_melanogaster    LEWTPRELRWLVDGKEWCVQGSAKGSFSETTAAGKSLPQAQKLEEGTGLAPFDQEFYLTFGLSVGGFNEYQHE-IKPWNERAPQAQKAFWKEVKKIRDHWLDEGHMKIDYVKVYSL
GNBP3_simulans        LEWTPRELRWLVDGKEWCVQGSAKGAFSETTAGGKSLPQAQKLEEGTGLAPFDQEFYLTFGLSVGGFNEYQHE-LKPWNEKAPQAQKAFWKEVKKIRDHWLDEGHMKIDYVKVFSL
GNBP3_sechellia       LEWTPRELRWLVDGKEWCVQGSAKESFSETTAGGKSLPQAQKLEEGTGLAPFDQEFYLTFGLSVGGFNEYQHE-VKPWNEKAPQAQKAFWKEVKKIRDHWLDEGHMKIDYVKVFSL
GNBP3_yakuba          LEWTPRELRWLVDGKEWCVQGSAKGAFSETTAGGKSLPQAQKLEEGTGLAPFDQEFYLTFGLSVGGFNEYQHE-VKPWNEKAPQAQKAFWKEVKKNRDHWLDEGHLKIDYVKVYSL
GNBP3_erecta          LEWTPRELRWLVDGREWCVQGSAKGALSETTAGGKSLPQAQKLEEGTGLAPFDQEFYLSFGLSVGGFNEYQHE-VKPWNEKAPRAQKDFWNDVKKNRDHWLDEGHLQIDYVKVYSL
GNBP3_ananassae       LEWTPRQIRWLVDGQEFCVQGSDK-AFSETSVGGTRLPQTSKLEEGSGLAPFDQEFYVTFGLSVGGFNEYQFD-EKPWNERAPQAQKDLWKAIKPMRDHWLDEGQMKIDFIKVYTI
GNBP3_pseudoobscura   LEWTPRELKWLVDGKEICRQGSESGAFSETTVNGMRLPNSQKLEEGTGLAPFDQEFYITFGLAVGGFNEYFYDTLKPWHEREPRAMTDFWKHQKPMLDQWLDDAHLSIDYVKVYAL
GNBP3_persimilis      LEWTPRELKWLVDGKEICRQGSESGAFSETTVNGMRLPNSQKLEEGTGLAPFDQEFYITLGLAVGGFNEYFYDTLKPWHEREPRAMTDFWKHQKPMLDQWLDDAHLSIDYIKVYAL
GNBP3_willistoni      LLWTPREMKWLVDGRELCRHGIDGGAFHGTTVNGQTLQNREILEQGSDLAPFDKEFYLTFGMSVGGFNEFFYNTDKPWAEFNPKAMKTFWDDVKNKADIWLENCQLKIDYVKVFSL
GNBP3_mojavensis      LEWTPRQLKWFVDGREICSQGSDSGAFSSTIADDQRLPNVDELEKGTGIAPFDQEFYLTFGLGVAGFNEFFYDKEKPWRDQDTIAMTQFLNSIKDRLDQWCDDAELKIDYVKIFSV
GNBP3_virilis         LEWTPRQLKWFVDGQELCTKGSETGAFSETSVANKRLPNADELEKGTGIAPFDQEFYLTFGLGVAGFNEFYYDPDKPWHEADPRAMSDFWNTFKTKQDQWLDQAQLTIDYVKVYSV
GNBP3_grimshawi       LVWTPRELKWYVDGRDVCTQGSEKGAFSETSVTNKPLPNADELEKGTGLAPFDQEFYLTFGLGVGGFNEFFFDVKKPWHEEHPQAMRDFWTSIKSQKDQWLEQAQLKIDYVKVYTV
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Supplementary figure 2

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                                        10        20        30        40        50        60        70        80        90       100
                                         |         |         |         |         |         |         |         |         |         |
GNBP1_Drosophila_melanogaster   YKIPTPTVELLET-GFSVSIPDEEGVKVVAFNVNRNRNFTSFINEGQYNVRLTEPQNGRWTTNFSSVPLRSQDVLYLWTSVQHQKAVYQDLAQPLPVCNLGGEYR
GNBP2_Drosophila_melanogaster   FKVPSINFEMLKDEGFEVSIPDEPGIQRVFYMFQIDDTCPALMDY------ITEAVNGSWVSKQK-MSLQNNDKLQISMLVQFNEEIFEKSETRVIINTRLLTTK
GNBP3_Drosophila_melanogaster   YEVPKAKIDVFYPKGFEVSIPDEEGITLFAFHGKLNEEMEG-LEAGTWARDIVKAKNGRWTFRDRITALKPGDTLYYWTYVIYNGLGYREDDGSFVVNGYSGNN-
GNBP_Hyphantria                 -QVPQVTVQALKPRGFKASIPDSPSVSLFVFQGNINRAISK-SDIGTISGEILKAKDGRWTFEDPNVELKVGDVVNYYVVVVSNRGGYIKDNLSFTVSAL-----
GNBP_Bombyx_p50                 --MPDVKIQAFRPKGLRISVQDVPKMTLFAFQGNLNHKLDS-TSVGTLSAEVLDPVNGRWVYEEPDLKLKVKDVVYYNAVFSINKKIYEKTNQQFTVTEL-----
Consensus                        k!P    # l   GFe!SIPDepg!  va%    #       # g     itea NGrWt       L   D ly    Vq #   % k      !n
Prim.cons.                      YKVP5V222ALKPKGFEVSIPDEPG2TLFAFQGN2NR555S25D5GT4S4EI22A2NGRWTFEDP4V5LKVGDVLYYWTVVQ5NK52YEKDNQSFTVN5L3G332
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Supplementary Figure 3

A

NEEMEGLEAG

N---AGGEAG

B