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## Supplemental Information

### **Peptidoglycan-Sensing Receptors Trigger the Formation of Functional Amyloids of the Adaptor Protein Imd to Initiate *Drosophila* NF- $\kappa$ B Signaling**

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**A**

<i>D. melanogaster</i>	VATPTSPAGGATQGGIGSIALTNSDVTDFGDKHFY--EGPVTIQQFLIDNRDKW	- 241
<i>D. mojavensis</i>	LATPTAPA--ATQQIGSIALSNTDVTDFGDKHYY--EGPVTIQQFLIDNREKW	- 255
<i>M. domestica</i>	VLHPRQVAASSSIRPQVAISSSTDVTIGDKHFY--EGPVTIQQFLIDSRNKW	- 420
<i>G. morsitans morsitans</i>	QSLGTTSPSSVATAKIGSVAVSNSTDITDFGDKHFY--EGPVTIQQFLIDNRNKL	- 161
<i>A. aegypti</i>	SSSP---VSARGPNIGSIAVQNSSDITDFGNKTYI--KGQVVIKNIYHDKQN--	- 165
<i>C. quinquefasciatus</i>	VTAT---ALQERPNIIGSIAVQNSSDITDFGNKTYI--KGQVVIKNIYHDRKATN	- 177
<i>A. gambiae</i>	AIAPGVRPSPAASTIGAIAVHNSDITDFGNKTYI--KGQVVIKNIYQDRKN--	- 170
<i>T. castaneum</i>	-----PEVPSFGNISVMNSNDIHFGNKTFY--QGQVVIKQFLYANGKTT	- 82
<i>A. mellifera</i>	ADGNI VLPNADI SNFGNVRVKNSTNVHLGNKTFY--KGPVTIKQFVYTNPTSI	- 169
<i>B. terrestris</i>	VDGNV VLPNADV SNFGDVRVKNSSNVHLGNKTFY--KGPVTIKQFVYTNPTSI	- 181
<i>C. floridanus</i>	ANGDV VLPNADPSNFGDVCVKNSTNVHLGNKTFY--KGPVTIKQFVYTNPIPI	- 165
<i>D. plexippus</i>	NLPSNLVPNTGAPVYESVSVTNSENVQFGNNTYF--NGPVIKQIVQPKSGVD	- 98
<i>B. mori</i>	PMEN---LYSSLSFGNVTIKESNNTHEGNNTYF--NGPVIKQISQNGNGTV	- 95
<i>N. vitripennis</i>	EVNGVALPGSDAIQIGQLHATNTQNMHIGQRFVFIKSKGDVILIKSVNYTAPI SP	- 182

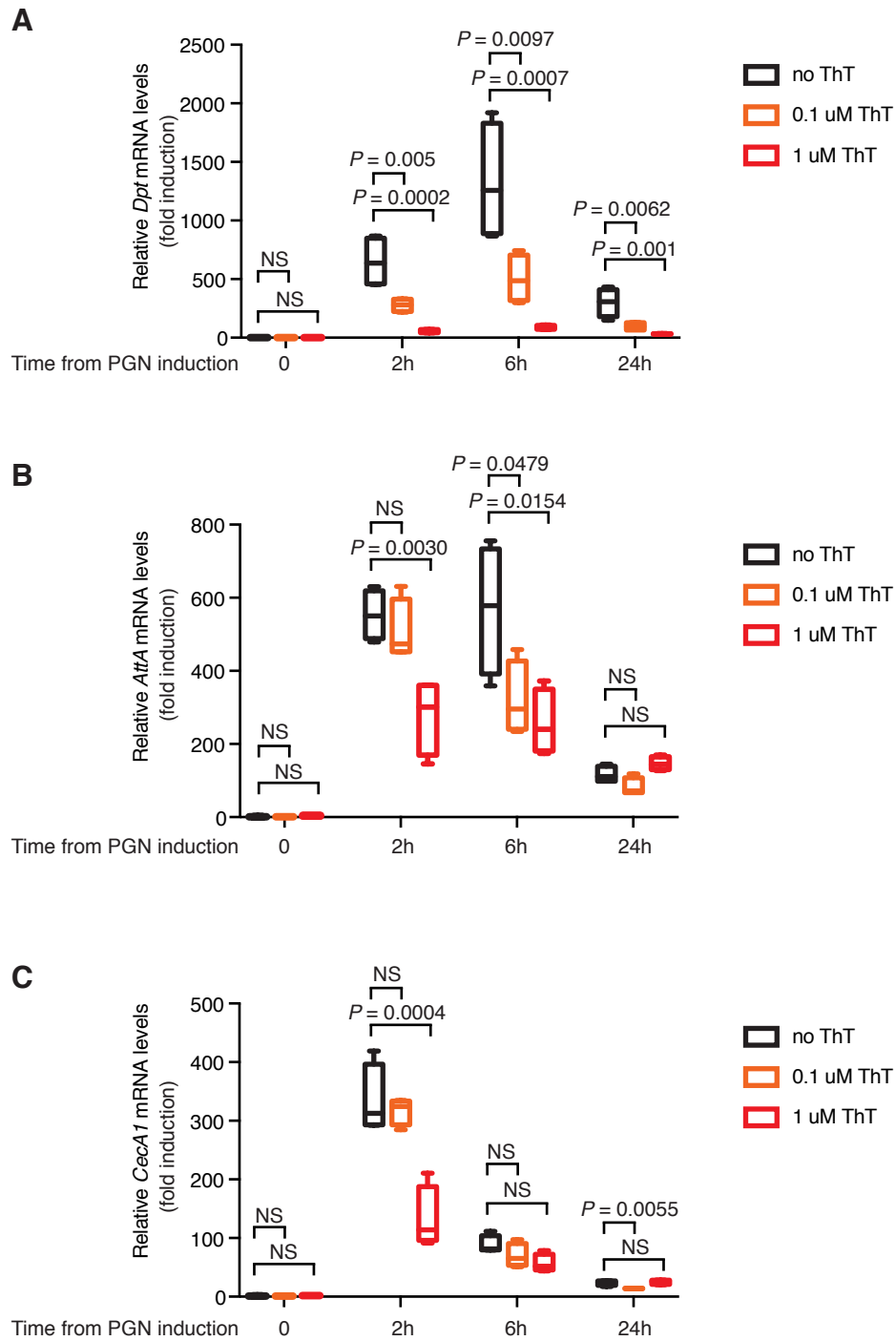
**B**

<i>D. melanogaster</i>	TIRDLMSINSIQTLGNVNI SNSTNVHIGNVTNINGNIQI IADGLTQNRDRR	- 129
<i>D. mojavensis</i>	TLRDLNSINSIQTVGNVNI SNSTNVHIGNVTNINGNIQI IAEHMKHPRNRR	- 142
<i>T. castaneum</i>	TIDNIL-SKGPNQ-YNNIQIYKSNVHIGDVTHINGPVIYNHLLPTINQNI VI	- 136
<i>A. aegypti</i>	SELPADWEGPSARQLAARQVENS NKVHIGNVTYVVGPIHI IHTGNNLGSIQQ	- 339
<i>C. quinquefasciatus</i>	EILQTVFKGNSTQTFGNIQIENS NKIHIGNVTYVVGPIHI IHTGNNLGSVQQ	- 147
<i>H. saltator</i>	ANGDV VLPNADPSNFGDVCVKNSTNVHLGNKTFYKGPVTIKQFVYTPASVQD	- 185
<i>M. domestica</i>	RQLSEIVSNMPQQNFGNFSISNSNVIMGHVIKINGNI IINKDGNES-----	- 130

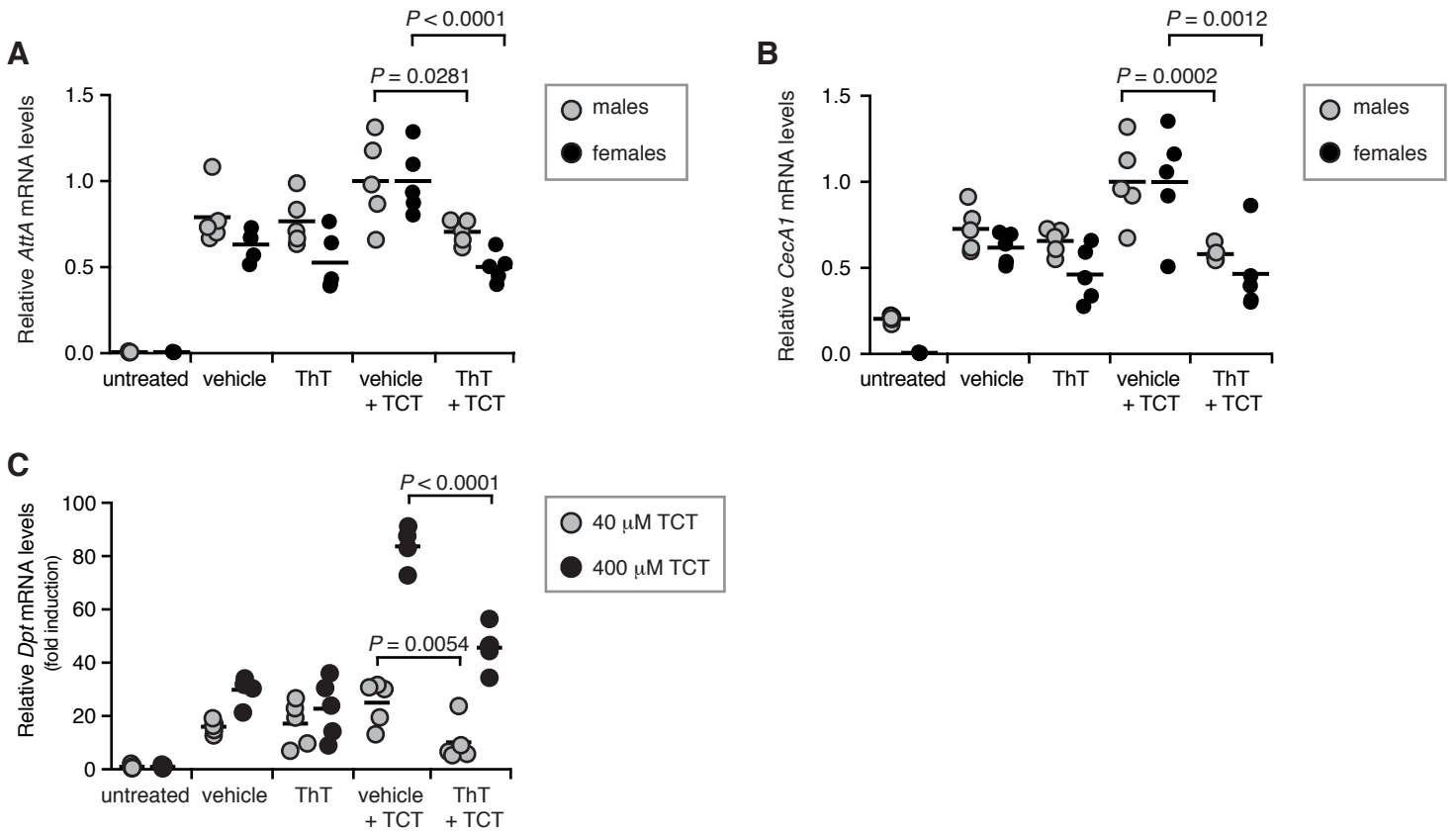
**C**

<i>D. melanogaster</i>	VQTNNTMNVHSAQQQVVMNFSNANLHFGSVYNFNQNL SACSSRKGSTSTAE E	- 145
<i>D. mojavensis</i>	TNTMNTMNVHSAQQQVVMNFSNASNLHFGSVYNFNQNL TGSSRKGSTSTTE D	- 151
<i>M. domestica</i>	QSQYNT LNVHNDNQRVLMQISNANSVHFGHVININSGKSEVCE SKREDTSCSH	- 98
<i>A. aegypti</i>	ALTAPQTSITNATGVQVYQIKNARNVHIGNSFTFNSASTEEDRTRPSTNVNGP	- 155
<i>A. gambiae</i>	TLTAPQTSISNTTGMQVFQIRNASNLHIGNSYTFNTAAVVDEGASTSGSLPGA	- 157
<i>C. quinquefasciatus</i>	NTLSGPQTTISAEGVHVHVIKNAKNEFQIGNSFTFNLATENGNNKPTN--SNGQ	- 143
<i>C. floridanus</i>	KTKSKHNKYSQGTNVINYNI VNSNGVKIGSRTSYICNVNQYSASNNASTAQPE	- 184
<i>B. terrestris</i>	KTKSKQRKHSQGTHVVNYNI INSNGVKIGSKTSYICNINQFAKNNSHASEETW	- 161
<i>N. vitripennis</i>	KKKPKQHKSSKGS HVINYNI INSSDVKIGSRTSYICNINQF SKNK--VEEDV	- 149
<i>A. mellifera</i>	NYSKPRTKKKSKPINTFYNI VNSENVKIGPTTVHTYNLNNFQNFDP SAFNRAK	- 141
<i>T. castaneum</i>	VEKKP-KKSGPSAFASVINISNANGIHVGSNYNVYLSNKSETQSQQQ-----	- 90

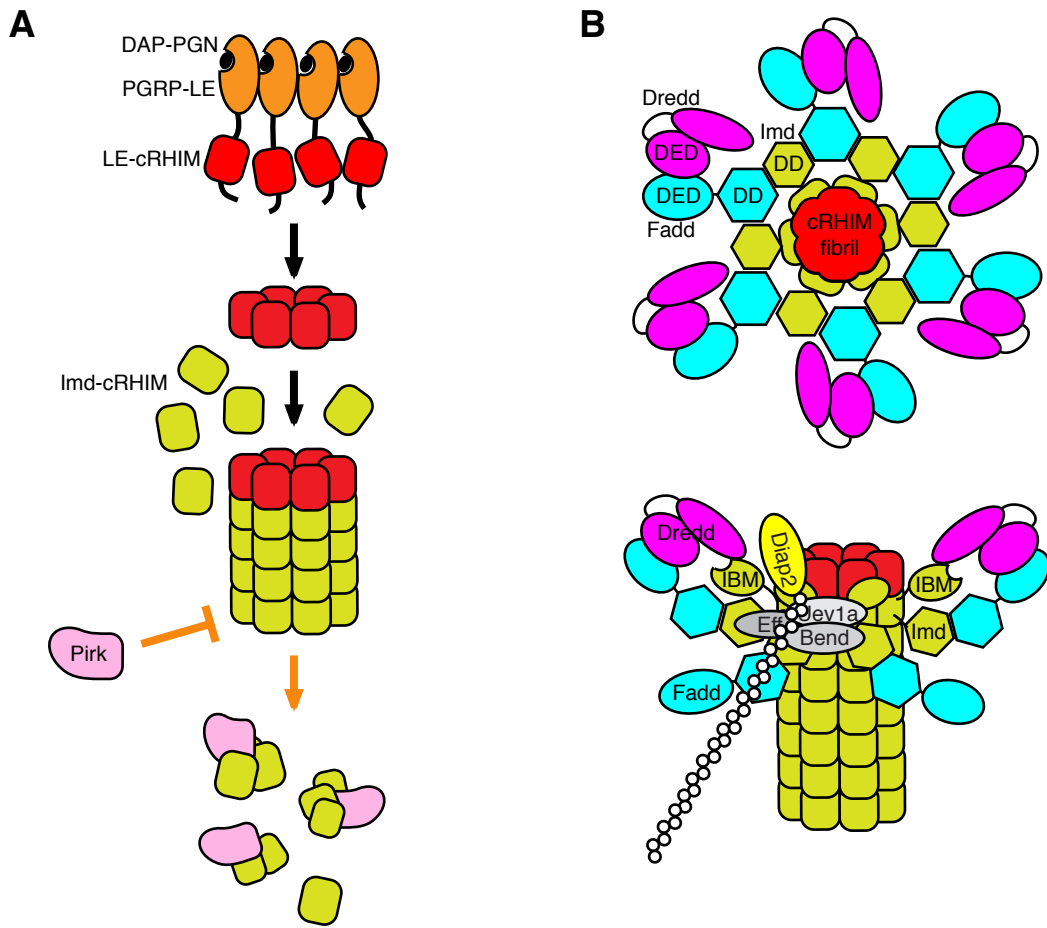
**Figure S1. Sequence variation in insect cRHIMs. Related to Figure 1.** Alignments of protein sequences homologous to *D. melanogaster* PGRP-LC (A), PGRP-LE (B), and Imd (C). The four core amino acids of cRHIM are boxed with red. Shading represents conservation according to Blosum 62 scoring matrix. Sequence IDs are the following: PGRP-LC and putative homologs in (A): *Drosophila melanogaster* (*D.mel*): NP\_729468, *Drosophila mojavensis* (*D.moj*): EDW18394.1, *Musca domestica* (*M.dom*): AFP63901.1, *Glossina morsitans morsitans* (*G.mor*): ABC25065.1, *Aedes aegypti* (*A.aeg*): XP\_001649251.1, *Culex quinquefasciatus* (*C.qui*): XP\_001848058.1, *Anopheles gambiae* (*A.gam*): EAL40488.3, *Tribolium castaneum* (*T.cas*): EFA01280.1, *Apis mellifera* (*A.mel*): XP\_006565566.1, *Bombus terrestris* (*B.ter*): XP\_003396511.1, *Camponotus floridanus* (*C.flor*): XP\_011263357.1, *Danaus plexippus* (*D.ple*): EHJ67829.1, *Bombyx mori* (*B.mori*): XP\_004929966.1, *Nasonia vitripennis* (*N.vit*): NP\_001164440.1. PGRP-LE and putative homologs in (B): *D.mel*: NP\_573078, *D.moj*: XP\_015016121.1, *T.cas*: EFA01375.1, *A.aeg*: XP\_001656352.1, *C.qui*: XP\_001842237.1, *Harpegnathos saltator*: XP\_011150487.1, *M.dom*: XP\_005187371.1. Imd and putative homologs in (C): *D.mel*: NP\_573394, *D.moj*: EDW08753.1, *M.dom*: XP\_005192241.1, *A.aeg*: XP\_001660624.1, *A.gam*: EAL40204.1, *C.qui*: XP\_001861391.1, *C.flor*: EFN61166.1, *B.ter*: XP\_003395628.1, *N.vit*: NP\_001135910.1, *A.mel*: NP\_001157189.1, *T.cas*: EFA11587.1



**Figure S2. ThT inhibits Imd pathway mediated induction of antimicrobial peptide genes in *Drosophila* S2\* cells. Related to Figure 5A.** Relative mRNA levels of *Diptericin* (A), *Attacin A* (B), and *Cecropin A1* (C) at different time points and ThT concentrations after PGN induction. N = 4 biological replicates. Boxes represent 95% confidence interval, line showing the mean. Box colors indicate different ThT concentrations: black = no ThT, orange = 0.1 μM ThT, red = 1 μM ThT. NS = not significant.



**Figure S3. ThT inhibits TCT-induced expression of antimicrobial peptide genes in flies. Related to Figure 5C-E.** Relative *Attacin A* (A), and *Cecropin A1* (B) mRNA levels in male (grey dots) and female (black dots) flies co-injected with 40  $\mu$ M TCT, and 1 mM ThT or vehicle (5% DMSO in PBS). (C) Comparison of *Diptericin* mRNA levels (fold induction) in female flies co-injected with 40  $\mu$ M TCT (grey dots) or 400  $\mu$ M TCT (black dots) in vehicle (5% DMSO in PBS), or 1 mM ThT. Each dot represents a biological replicate (N = 5), the line indicating the mean.



**Figure S4. cRHIM fibrils as a platform for Imd signaling. Related to Figure 6.** (A) Proposed model for cRHIM-mediated nucleus formation, subsequent amyloid polymerization of the adaptor protein Imd, and amyloid inhibition by Pirk. (B) Speculative model of the receptor-proximal signaling complex, where cRHIM motifs of PGRP-LC, PGRP-LE, and Imd form the amyloid core of the complex, from which the death domain (DD) and N-terminal domain of Imd branch out. The Imd death domain interacts with Fadd, which recruits the caspase-8 homolog Dredd to the complex. Activated Dredd cleaves the Imd N-terminus and reveals a binding site (IBM) for the ubiquitin E3 ligase (Diap2), which together with the E2 ligases Uev1a, Bendless (Bend), and Effete (Eff) links Imd with K63 polyubiquitin chains (only one depicted here for simplicity), and further activates Imd signaling.