Supplementary Material

John E. Chesebro et al. doi: 10.1242/bio.20123699

A. *Periplaneta americana caudal*

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1        CGCGGCCGCGTCGACGCGCCGAGTTGTAGCGTGCGGGAGGTGTGCGAGAC     50
51       AGGATACGAACCCGTGTGCGCGAGCATGGAACCCGCGACTCTGTGACTTG    100
101      GGAACATGTGTCCGTGCTGACGAGATGTTGAGGAACAGGATGTGTAGTGT    150
151      CGTCTAGTGTCGGAGTGTGCTGTGCTCGGCTTCCAACAAGGTGGCATTCA    200
201      GACGAAAAAAGTGGAGGAGGAACTGGGTGCAAAGACAATGGTTTCATACT    250
1                                              M  V  S  Y        4
251      ACAACCCCTTAGCGATGTACCGGCACCAGCAGCAGGCCTCGGCTCCGGGG    300
5        Y  N  P  L  A  M  Y  R  H  Q  Q  Q  A  S  A  P  G      21
301      GGACCTCCGCAGTTCCACCACCCAACCAGCCCCGCCGCCACTTGGTACGG    350
22        G  P  P  Q  F  H  H  P  T  S  P  A  A  T  W  Y  G     38
351      GCCCCCCGGGAGTTACCAGCCCTCGCATCACCACCAGGTGCCTCCACCCC    400
39         P  P  G  S  Y  Q  P  S  H  H  H  Q  V  P  P  P       54
401      CGCTGCAACAGTACCCAAATTGCGTGCAGGACGATCAGCAGGGCACCGCG    450
55       P  L  Q  Q  Y  P  N  C  V  Q  D  D  Q  Q  G  T  A      71
451      GGGGCATGGCACCATCACCACCACCACATGTTTCAGCCGGAGTGGACCAG    500
72        G  A  W  H  H  H  H  H  H  M  F  Q  P  E  W  T  S     88
501      CGCGGGGGCCCCGGATTTTGGGAGTGCACCCGGAATGTCGCAGGGCCATC    550
89         A  G  A  P  D  F  G  S  A  P  G  M  S  Q  G  H      104
551      AGCCCTCTTCGGCGGGGCTCGAGGACCCCCAGTTACCGTCCCCGCCTATA    600
105      Q  P  S  S  A  G  L  E  D  P  Q  L  P  S  P  P  I     121
601      ACGGTGTCTAGTAGCGAACTGTCGAGTCCCGGCGCCGTTGGGGGTTCCGT    650
122       T  V  S  S  S  E  L  S  S  P  G  A  V  G  G  S  V    138
651      GACGCCCCCCCAGCACGCTGGGCGCCCCATTCCCGTCCGGAGCCCCTACG    700
139        T  P  P  Q  H  A  G  R  P  I  P  V  R  S  P  Y      154
701      AATGGATGAAGAAGCCGTCCTACCAGAGCCAGCCGAATCCAGTTGGCCCC    750
155      E  W  M  K  K  P  S  Y  Q  S  Q  P  N  P  V  G  P     171
751      AATCCCCCGCTTCTAGACCATACACGTGCCGGAATGCAGGAACTGTTGAG    800
172       N  P  P  L  L  D  H  T  R  A  G  M  Q  E  L  L  S    188
801      CAAAACGCGGACGAAgGACAAGTACCGAGTGGTATATAGCGATCACCAGC    850
189        K  T  R  T  K  D  K  Y  R  V  V  Y  S  D  H  Q      204
851      GACTAGARCTGGAGAAGGAGTTCCACTACAGCCGGTACATCACCATTAGG    900
205      R  L  E  L  E  K  E  F  H  Y  S  R  Y  I  T  I  R     221
901      CGTAAGGCGGAACTCGCTGCCAACCTGGGACTCTCAGAACGACAGGTGAA    950
222       R  K  A  E  L  A  A  N  L  G  L  S  E  R  Q  V  K    238
951      GATCTGGTTCCAGAACCGTCGCGCCAAGGAGCGCAAGCAGGTCAAGAAGC   1000
239        I  W  F  Q  N  R  R  A  K  E  R  K  Q  V  K  K      254
1001     GGGAGGAGCTGCTGCACAAGGGGAAGCTGGAGGCGGTGAGCGCGGCGCAC   1050
255      R  E  E  L  L  H  K  E  K  L  E  A  V  S  A  A  H     271
1051     CACCAGCTGCAGCACGCGCACCAGCAGCCGCAGCAGGCCGCGCAGGACCT   1100
272       H  Q  L  Q  H  A  H  Q  Q  P  Q  Q  A  A  Q  D  L    288
1101     GCTTCTGTGACGTCGCAGCGCTGCTACTGCTCATCAGCATTCAGACAATA   1150
289        L  L  *                                             290
1151     AACGTGCGAGTCGCGCCATCGTACATGACAACACGGGTTGTTGTCGGTAT   1200
1201     GGATCTGGCACCACGAGTGCTGCAGTGAACTCGGACACGGAACAAACTTC   1250
1251     TACAGGTTGCACAATATCCATGGAATCAGACTTTTTCAATTGACACAAAT   1300
1301     CTGACGACCTCATTCTTTACTGTTATTTATTTTGTCTGTCTTATTTAGCA   1350
1351     TTGAACATACCGCAGTCTGCTAAGTTATCGTAGCCGCCGTCTAAATTTTC   1400
1401     TACTTTGTCCAAAAAAAAAA                                 1420
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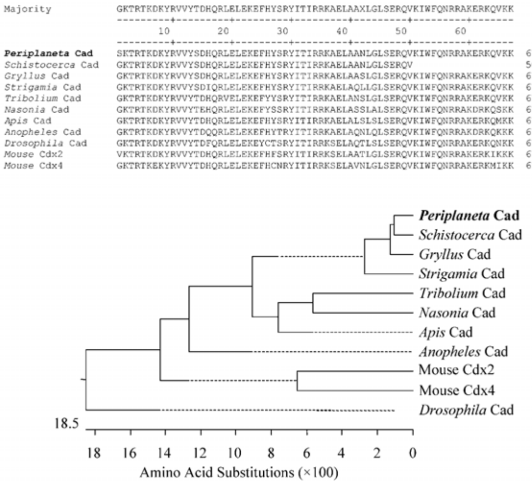
B. Alignment and phylogenetic analysis of *Pa*-Cad protein



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Majority            GKTRTKDKYRVVYTDHQRLELEKEFHYSRYITIRRKAELAAXLGLSERQVKIWFQNRRAKERKQVKK
                    ---------+---------+---------+---------+---------+---------+-------
                            10        20        30        40        50        60
                    ---------+---------+---------+---------+---------+---------+-------
Periplaneta Cad     SKTRTKDKYRVVYSDHQRLELEKEFHYSRYITIRRKAELAANLGLSERQVKIWFQNRRAKERKQVKK   67
Schistocerca Cad    GKTRTKDKYRVVYSDHQRLELEKEFHYSRYITIRRKAELAANLGLSERQV                    50
Gryllus Cad         GKTRTKDKYRVVYSDHQRLELEKEFHYSRYITIRRKAELAASLGLSERQVKIWFQNRRAKERKQVKK   67
Strigamia Cad       GKTRTKDKYRVVYSDIQRLELEKEFHYSRYITIRRKAELAQLLGLSERQVKIWFQNRRAKERKQVKK   67
Tribolium Cad       GKTRTKDKYRVVYTDHQRVELEKEFYYSRYITIRRKAELANSLGLSERQVKIWFQNRRAKERKQVKK   67
Nasonia Cad         GKTRTKDKYRVVYTEHQRLELEKEFYSSRYITIRRKAELASSLALSERQVKIWFQNRRAKDRKQSKK   67
Apis Cad            GKTRTKDKYRVVYTDHQRLELEKEFHYSRYITIRRKAELALSLSLSERQVKIWFQNRRAKERKQMKK   67
Anopheles Cad       GKTRTKDKYRVVYTDQQRLELEKEFHYTRYITIRRKAELAQNLQLSERQVKIWFQNRRAKDRKQKKK   67
Drosophila Cad      GKTRTKDKYRVVYTDFQRLELEKEYCTSRYITIRRKSELAQTLSLSERQVKIWFQNRRAKERKQNKK   67
Mouse Cdx2          VKTRTKDKYRVVYTDHQRLELEKEFHFSRYITIRRKSELAATLGLSERQVKIWFQNRRAKERKIKKK   67
Mouse Cdx4          GKTRTKEKYRVVYTDHQRLELEKEFHCNRYITIRRKSELAVNLGLSERQVKIWFQNRRAKERKMIKK   67
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C. List of primer sequences used in degenerate PCR, 5′RACE and 3′RACE for *Pa-cad*

Pa-cad degenerate forward: 5′ GAGCTSGAGAAGGARTT 3′

Pa-cad degenerate reverse: 5′ RCAGCACCARTGRAABGTRCA 3′

Pa-cad specific 5′RACE reverse (outer): 5′ ATCTTCACCTGTCGTTCTGAGAGTC 3′

Pa-cad specific 5′RACE reverse (inner): 5′ CTTACGCTTGATGGTGATGTACC 3′

Pa-cad specific 3′RACE forward (outer): 5′ ACTACAGCCGGTACAT CACCATCA 3′

Pa-cad specific 3′RACE forward (inner): 5′ CTCTCAGAACGACAGGTGAAGATC 3′

Fig. S1. Nucleotide and protein sequences for *Periplaneta caudal* and phylogenetic analysis. (**A**) The 1420 bp nucleotide sequence of *Pa-cad* codes for a 290 amino acid protein. The conserved homeodomain is underlined. (**B**) Phylogenetic analysis of the homeodomain protein sequence with other known *cad* sequences shows that *Pa-cad* is the *Periplaneta* orthologue and aligns with closely related insect species. (**C**) List of degenerate and specific primer sequences used to isolate and sequence the *Periplaneta caudal*.

A. *Periplaneta americana Wnt1*

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1     GAAGGCCATTGAAATTGTTTAGAAAATGTACTGTAATGTTAGAATGTCTG    50
51    CGACTAACATACTTCAATATTTTTCCGTGTGAGTGCTTGTTTAAAGTAGA   100
101   TTTTTATGTACAATTAACTACATTACGTTGGGTGATATTTACTTTACCGT   150
151   ATAATTTATGTTGAAGTGAATTTGTACAAGAATGGAAACAAATGTAACGT   200
201   ACAATTATAAGTGATAAAAATATAACCAATGAAACAACGACTTAAGGGAA   250
251   AATATTGTACTGGAATTAAGGTAAAGAGAAAATAAAATATTTTTGGTCAG   300
301   CGATAGCTACAGAATGTGAAATAATACAACATAAAAGTCAGAAATTCCGA   350
351   CGCATCAAAATATATACAAGAAATCTAACAAGAAGAGAATCAAGGGCAAT   400
401   CAAATGAGAGAACAAATTTGCTGACTAGAATAACAAACAATAGGCCTATA   450
451   GAATGTGAAGATGACGATTACAGGAAATGTGAAACAATTTAAGAAAAATT   500
501   GTTCTAAAAATACAAGACGTAAGTTCTTATCACTTACGAATAAAAATAGA   550
551   AATTAAAACAGACATCGTTGTACGTCACATCGACATCGAATATTCGAGGG   600
601   AAGACTTCAGAATACTGGTTGTATAATACTATCCGAAGATCGCTGAGGAT   650
651   TAGCACAAAACTAGACATTGCGGTACAAAACTAAATGCAAGATATTGTGC   700
1                                        M  Q  D  I  V       5
701   GCTCAGGAATACCTGTGTGTTTGTTCCAGGGGATTGCGAAAGCCGGCGAG   750
6     R  S  G  I  P  V  C  L  F  Q  G  I  A  K  A  G  E     22
751   CCCAACAACTTGCTTCCGCAGACGCCGGGCGCGCTCTACATGGACCCGGC   800
23     P  N  N  L  L  P  Q  T  P  G  A  L  Y  M  D  P  A    39
801   CGTGCACGCCATTCTGCGGCGGAAGCAGAGACGTCTAGTTCGGGAGAACC   850
40      V  H  A  I  L  R  R  K  Q  R  R  L  V  R  E  N      55
851   CGGGAGTTCTTGTGGCGGTAGCCAAAGGTGCTAACCAGGCCATCGTGGAA   900
56    P  G  V  L  V  A  V  A  K  G  A  N  Q  A  I  V  E     72
901   TGCCAGTTCCAGTTTCGAAACAGGAGGTGGAACTGCTCGACAAGAAATTT   950
73     C  Q  F  Q  F  R  N  R  R  W  N  C  S  T  R  N  F    89
951   TCTACGAGGCAAAAACCTCTTCGGAAAAATTGTTGACAGAGGTTGTCGGG  1000
90      L  R  G  K  N  L  F  G  K  I  V  D  R  G  C  R     105
1001  AGACGGCGTTCATATACGCGATCACAAGTGCGGGCGTGACACACGCTATC  1050
106   E  T  A  F  I  Y  A  I  T  S  A  G  V  T  H  A  I    122
1051  GCGCGGGCGCGCAGCGAGGGCAGCATCGAGTCGTGCACGTGTGATTACAG  1100
123    A  R  A  R  S  E  G  S  I  E  S  C  T  C  D  Y  S   139
1101  CCACCAGGCGCGGGCGCCGCAGGTGACGTCCGTGCCCGGCCTGCGCGACT  1150
140     H  Q  A  R  A  P  Q  V  T  S  V  P  G  L  R  D     155
1151  GGGAGTGGGGCGGCTGCTCCGACAACATCGGCTACGGCTTCAAGTTCTCC  1200
156   W  E  W  G  G  C  S  D  N  I  G  Y  G  F  K  F  S    172
1201  CGCGAATTCGTCGATACCGGCGAGCGGGGgCGCAACCTCCGCGAGAAGAT  1250
173    R  E  F  V  D  T  G  E  R  G  R  N  L  R  E  K  M   189
1251  GAATCTCCACAACAATGAGGCCGGCAGAGCGCACGTTTCCTCGGAGATGC  1300
190     N  L  H  N  N  E  A  G  R  A  H  V  S  S  E  M     205
1301  GTCAAGAATGTAAGTGCCACGGCATGTCTGGCTCCTGCACGGTCAAGACC  1350
206   R  Q  E  C  K  C  H  G  M  S  G  S  C  T  V  K  T    222
1351  TGCTGGATGCGGCTGCCCAGCTTCCGAGTCGTAGGCGACAACCTCAAGGA  1400
223    C  W  M  R  L  P  S  F  R  V  V  G  D  N  L  K  D   239
1401  CCGCTTCGACGGCGCCTCCAGAGTGATGGTGAGTAACGCGGGCAGCCTGC  1450
240     R  F  D  G  A  S  R  V  M  V  S  N  A  G  S  L     255
1451  GCGGCCAGGGTGGTAGCGGCGGCAGCGGCGTCGGTGGTAAGAAGAACAGA  1500
256   R  G  Q  G  G  S  G  G  S  G  V  G  G  K  K  N  R    272
1501  TACAACTTCCAACTGAAACCCTACAACCCGGACCACAAGCCGCCCGGCAC  1550
273    Y  N  F  Q  L  K  P  Y  N  P  D  H  K  P  P  G  T   289
1551  CAAAGACCTGGTCTACTTGGAGCCTTCCCCAGGGTTCTGCGAGCGCAACC  1600
290     K  D  L  V  Y  L  E  P  S  P  G  F  C  E  R  N     305
1601  CGAGACTCGGTATCCAAGGCACGCACGGACGTCAGTGCAACGATACGTCG  1650
306   P  R  L  G  I  Q  G  T  H  G  R  Q  C  N  D  T  S    322
1651  ATAGGCGTGGATGGTTGCGACCTCATGTGTTGTGGGCGAGGATATAGAAC  1700
323    I  G  V  D  G  C  D  L  M  C  C  G  R  G  Y  R  T   339
1701  TCATGAGGTGTCCGTGGTGCAGAGGTGTGCGTGCATGTTCCACTGGTGCT  1750
340     H  E  V  S  V  V  Q  R  C  A  C  M  F  H  W  C     355
1751  GCGAAGTCAAGTGCAACCTCTGTCGGACAAAGAAAACCATTCACACGTGT  1800
356   C  E  V  K  C  N  L  C  R  T  K  K  T  I  H  T  C    372
1801  CTGTGAGTGGTGAAAAAGAAACAATTCACCCATACTTGTGAGTGCTGCAA  1850
373    L  *                                                373
1851  AGAAAACCATCCACACGTGTCTGTGAGTGGTGAAAAAGAAACAATTCACC  1900
1901  CATACTAGTGAGTGCTGCGAAGAAAACCATCCACACGTGTCTGTGAGTGG  1950
1951  TGAAAAAGAAACCATTCACATTTGCTTGTGAGTGCTGCAAAGAAAAACTT  2000
2001  CCACACATATCTGTATGATCAGCATAGTGAAACTCGTTAATTGTCTGCGA  2050
2051  GTGCTTCAAAATCATTCATACATATCGATAAGTATTGGACAAAGAGAAAC  2100
2101  ATCACACTCGTTGTGATCGTTTCACAATGAAAACCATCCAGATGTGTTGT  2150
2151  GGGTGGTAGAAGAGTAACGTCACACTTGTATATGTTGCACATGTATGAGC  2200
2201  GCTGGACAAAGAGAACCACCCAAATGTGTTTACGTTGAGTACGTACTGCA  2250
2251  CAAACAAATACATTCACACGTCTTTATGAGCATACTGCAGAGATACGTAT  2300
2301  CTGTGGGTTACGCAAAAATAAAGTCACAACGCGTTTATGGTGAGTGTTAT  2350
2351  GAAAAAAAAAAAAA                                      2364
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B. Alignment and phylogenetic analysis of *Pa*-Wnt1 protein

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Majority              DNIGXGFKFSREFVDTGERGR--------DLREKMNLHNNEAGRAHVSSEMRQECKCHGMSGSCTVKTCWMRL
                      ---------+---------+---------+---------+---------+---------+---------+---
                              10        20        30        40        50        60        70
                      ---------+---------+---------+---------+---------+---------+---------+---
Periplaneta Wnt1      DNIGYGFKFSREFVDTGERGR--------NLREKMNLHNNEAGRAHVSSEMRQECKCHGMSGSCTVKTCWMRL
Cryptotermes Wnt1     DNIGYGFKFSREFVDRASADQ--------PARED-DVHNNEAGRAHVSS----EC
Gryllus Wnt1          DNIEYGFKFSRDFVDTGERGR--------TLREKMNLHNNEAGRLHVREEMRKECKCHGMSGSCTVKTCWMRL
Schistocerca Wg       -NIDYGFKFSREFVDTGERGR--------SLREKMNLHNNEAGRAHVVSEMRRECKCHGMSGSCTIRTCWMRL
Achaeranea Wnt1       DNIEFGSKFTKQFVGAAERGK--------DLRFTMNLHNNEAGRTHVAAGMRRQCKCHGMSGSCTVQTCWMQL
Apis Wnt              DNIGYGFKFSREFVDTGERGR--------NLREKMNLHNNEAGRAHVSSEMRQECKCHGMSGSCTVKTCWMRL
Bombyx Wnt1           DNIGFGFRFSREFVDTGERGK--------TLREKMNLHNNEAGRRHVQTEMKQECKCHGMSGSCTVKTCWMRL
Cupiennus Wnt1        DNIDFGAKFSRQFVDASERGK--------DLRYIMNLHNNEAGRAHVIGGMRRQCKCHGMSGSCTVQTCWMQL
Drosophila Wg         DNIGFGFKFSREFVDTGERGR--------NLREKMNLHNNEAGRAHVQAEMRQECKCHGMSGSCTVKTCWMRL
Mouse Wg-related      DNIDFGRLFGREFVDSGEKGR--------DLRFLMNLHNNEAGRTTVFSEMRQECKCHGMSGSCTVRTCWMRL
Nasonia Wg            DNIGYGFRFSREFVDTGERGR--------NLREKMNLHNNEAGRTHVSAEMRQECKCHGMSGSCTVKTCWMRL
Tribolium Wg          DNIGFGFTVSREFVDAGERGK--------TIREKMNLHNNEAGRWHVKDQMRQECKCHGMSGSCTIKTCWMRL
Cupiennius Wnt5       ----------------------------------MNLHNNEAGRRAVIRKIKVTCKCHGVSGSCSLVTCWQQL
Tribolium Wnt5        DNLEYGYKFTQNFVDVREKERKFKRGSKEQGRNLMNLHNNEAGRRAVIKKSKVTCKCHGVSGSCSLITCWQQL
Apis Wnt5             DNLEYGYKFTQAFVDVKERERSFKRGSREQGRSLMNLHNNEAGRRAVIKRSKVTCKCHGVSGSCSLITCWQQL
Danio Wnt5            DNVNYGYRFAREFVDAREREKNYPRGSVEHARTLMNLQNNEAGRMAVYNLANVACKCHGVSGSCSLKTCWLQL
Glomeris Wnt8         ENVHFGIDVTKDFLEARESGK--------DARALVNLHNNEAGRVAVSKTMRRLCKCHGVSGSCSIQTCWMQL
Tribolium Wnt8        DDSSFGEELVLKLLEDNEESS--------DAQAFINRHNNRIGREIIREKMLKTCKCHGVSGSCSFQTCWMQM
Parasteatoda Wnt8     DNVKIGNKMAKHYMDSKEHGR--------DIQAMINLHNNRVGRMMVKRNMRRMCKCHGVSGSCEMKTCWMRV
Danio Wnt8            DNVNFGDRIAKLFVDALENGH--------DSRAAVNLHNNEAGRLAVKATLKRTCKCHGLSGSCSIQTCWMQL
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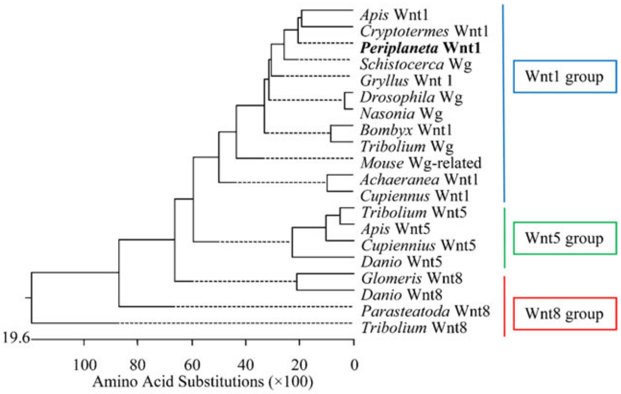


C. List of primer sequences used in degenerate PCR, 5′RACE and 3′RACE for *Pa-Wnt1*

*Pa-Wnt1*degenerate forward 1 (outer): 5′ CGNCCGVTGGAACTGYTCNAC 3′

*Pa-Wnt1*degenerate reverse 1 (outer): 5′ RCAGCACCARTGRAABGTRCA 3′

*Pa-Wnt1*degenerate forward 2 (inner): 5′ TGGGGYGGHTGCTSBGAYAAAT 3′

*Pa-Wnt1*degenerate reverse 2 (inner): 5′ GCCNCKKCCGSAGCACATVAG 3′

*Pa-Wnt1*specific 5′RACE reverse 1 (outer): 5′ TGGAGATTCATCTTCTCGCGGAGGT 3′

*Pa-Wnt1*specific 5′RACE reverse 1 (inner): 5′ CTTGTCGAGCAGTTCCACCTCCTG 3′

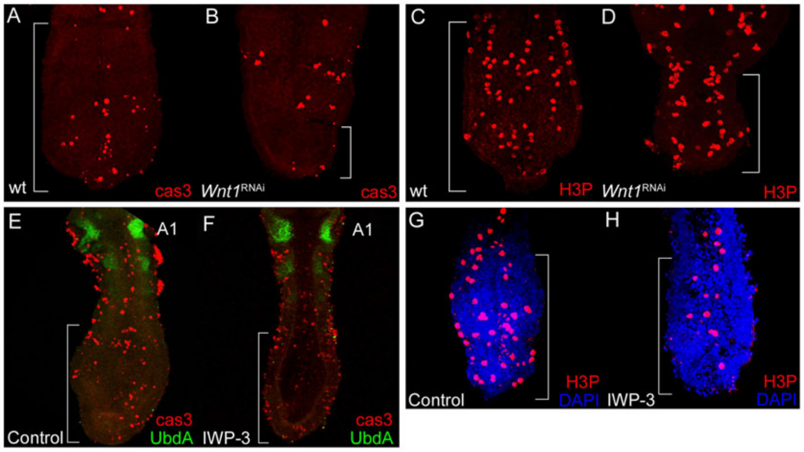
*Pa-Wnt1*specific 5′RACE reverse 2 (outer): 5′ GAATTCGCGGGAGAACTTGAAGCC 3′

*Pa-Wnt1*specific 5′RACE reverse 2 (inner): 5′ AACTAGACGTCTCTGCTTCCGCCG 3′

*Pa-Wnt1*specific 3′RACE forward 1 (outer): 5′ TTCTGCGAGCGCAACCCGAGA 3′

*Pa-Wnt1*specific 3′RACE forward 1 (inner): 5′GTCAGTGCAACGATACGTCGATAGGCT 3′

Fig. S2. Nucleotide and protein sequences for *Periplaneta Wnt1* and phylogenetic analysis. (**A**) The 2360 bp nucleotide sequence of *Periplaneta Wnt1* codes for a 373 amino acid protein. (**B**) Phylogenetic analysis of a conserved 108 aa region of the *Pa-Wnt1* protein sequence with other known sequences shows that *Pa-Wnt1* is the *Periplaneta* orthologue and aligns with closely related insect species. (**C**) List of degenerate and specific primer sequences used to isolate and sequence the *Periplaneta Wnt1*.



I

	cas3	H3P
wt	13 (n=4)	62 (n=3)
Pa-Wnt1^{RNAi}	8 (n=2)	44 (n=4)

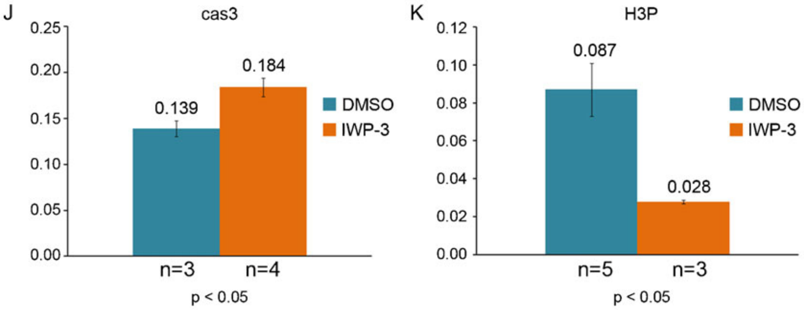


Fig. S3. *Wnt1* regulates growth but not cell death in the growth zone. In both wild type (**A**) and *Pa-Wnt1*^{RNAi} (**B**) embryos, apoptosis (as detected by α-cleaved caspase 3; cas3) is minimal and scattered throughout the GZ. (**C**) In wild type, numerous cells undergoing mitosis are seen (as detected by α-phosphorylated Histone 3; H3P). (**D**) Cell proliferation is lower in the small GZ of *Pa-Wnt1*^{RNAi} embryos. (**E**,**F**) The number of dying cells is increased in DMSO control (E) and IWP-3 (F) cultured embryos compared to A and B, yet similar to each other. This increase in cell death is likely due to the cytotoxic nature of DMSO and not to a role of Wnt signalling in apoptosis. Additionally, expression of the Hox genes Ubx and AbdA (as detected by mouse monoclonal antibody FP6.87; α-UbdA), remain unaffected in DMSO control (E) and IWP-3 (F) treated culture embryos, indicating that IWP-3 only affects Wnt signalling and not other patterning genes. In culture, the number of mitotic cells is reduced in control embryos (**G**), compared to wild type (C), and are much lower in IWP-3 treated embryos (**H**). Staining with the vital dye DAPI showed similar amounts of living cells in control and IWP-3 treated embryos, indicating a healthy GZ. (**I**) Table representing the average number of cells counted undergoing apoptosis (cas3) or mitosis (H3P) in the various control and Wnt depleted backgrounds. Apoptotic index (**J**) and mitotic index (**K**) comparisons between DMSO control and IWP-3 cultured embryos. All embryos are stage 9 and the growth zone is marked with a bracket. A1: first abdominal segment.

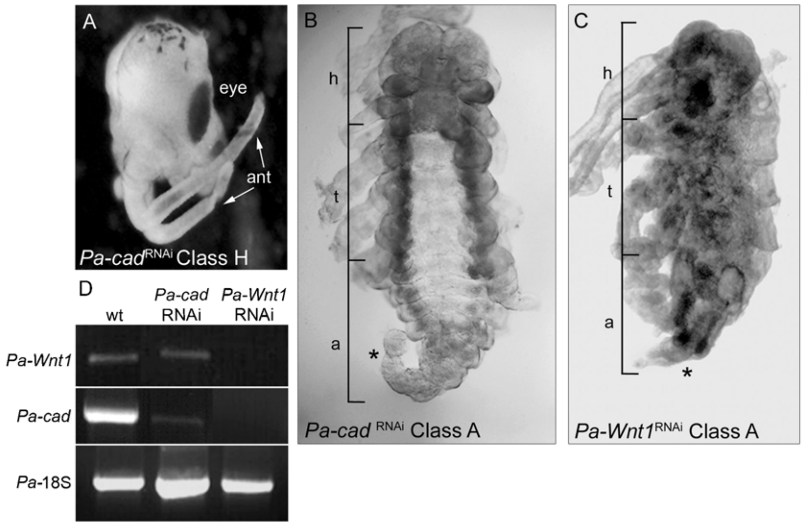


Fig. S4. Quantification of *Pa-cad*^{RNAi} and *Pa-Wnt1*^{RNAi} phenotypes. (**A**) Class 'H' *Pa-cad*^{RNAi} embryo in which only the anterior-most head segments, eyes and antennae, develop properly. (**B**) In Class 'A' *Pa-cad*^{RNAi} embryos the head and thorax develop properly, while the abdomen appears normal in the anterior, but tapers into a point at the posterior (*). (**C**) Similarly, Class 'A' *Pa-Wnt1*^{RNAi} phenotypes have a normally developed head and thorax with a truncated and tapered abdomen (*). (**D**) RT-PCR analysis of stage 9 Class 'T' *Pa-cad*^{RNAi} and *Pa-Wnt1*^{RNAi} embryos. *Pa-Wnt1* expression remains at wild type levels in *Pa-cad*^{RNAi} but is abolished in *Pa-Wnt1*^{RNAi}. Only trace amounts of *Pa-cad* expression can be detected in *Pa-cad*^{RNAi} and is absent in *Pa-Wnt1*^{RNAi}. *Periplaneta* 18S ribosomal subunit was used as a positive control in each case. These data suggest that the different phenotypic RNAi classes correlate with the degree of hypomorphy (RNA loss) caused by the RNAi treatment on a single, continuous function required for posterior segment development throughout embryogenesis. In the case of *Pa-cad* if enough expression is present during germ band elongation, further segments will develop, if not, posterior segment development will be arrested and the animals will display only the head and some thoracic segments formed at blastoderm. Thus, Class 'H' *Pa-cad*^{RNAi} embryos display a total or very strong loss of *Pa-cad* RNA whereas in Class 'A' *Pa-cad* can remain at near wild type levels (not shown). The same holds true for *Pa-cad* expression in *Pa-Wnt1*^{RNAi} embryos. ant: antennae; h: head; t: thorax; a: abdomen.

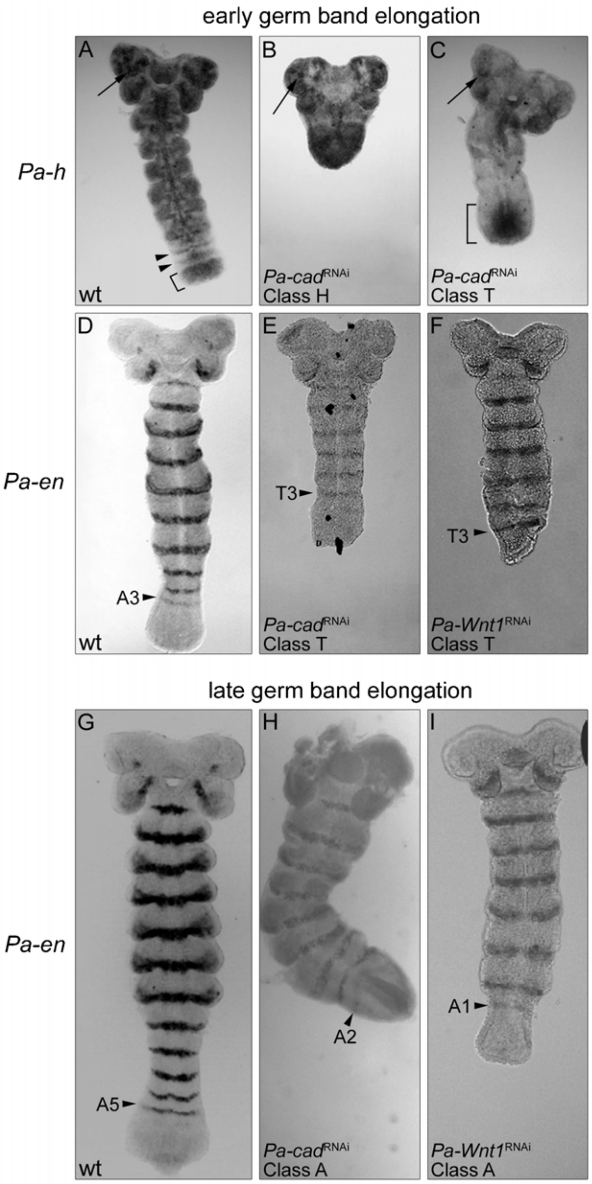


Fig. S5. Expression of segmental markers in *Pa-cad*^{RNAi} and *Pa-Wnt1*^{RNAi} phenotypic classes. (**A**) *Pa-hairy* (*Pa-h*) expression in wild type embryo (stage 7) showing a broad domain at the posterior GZ and in transient segmental stripes (A1 and A2) in the anterior GZ. Expression of *Pa-h* is also observed in the head, CNS midline and somites. (**B**) In a class 'H' *Pa-cad*^{RNAi} embryo *Pa-h* expression is in the head and in an expanded domain at the posterior but no segmental stripes are observed. (**C**) Similarly, in class 'T' *Pa-cad*^{RNAi} embryo, *Pa-h* segmental stripes are absent and *Pa-h* posterior GZ domain expanded. (**D**) Segmental expression of the *Pa-en* in a wild type embryo (stage 8) reaching the A3 segment. (**E**) *Pa-en* expression in a class 'T' *Pa-cad*^{RNAi} embryo shows that segment formation proceeds until the thoracic segments. (**F**) Likewise, in a class 'T' *Pa-Wnt1*^{RNAi} embryo *Pa-en* segmental stripes are observed up to T3 segment. (**G**) Expression pattern of *Pa-en* in a stage 9 wild type embryo showing expression up to the A5 segment. (**H**) Expression of *Pa-en* in a class 'A' *Pa-cad*^{RNAi} embryo with a reduced GZ shows that only two abdominal segments have been formed. (**I**) Similarly, in a class 'A' *Pa-Wnt1*^{RNAi} embryo the A1 *Pa-en* segmental stripe has been laid down. Note that the GZ is reduced in size.

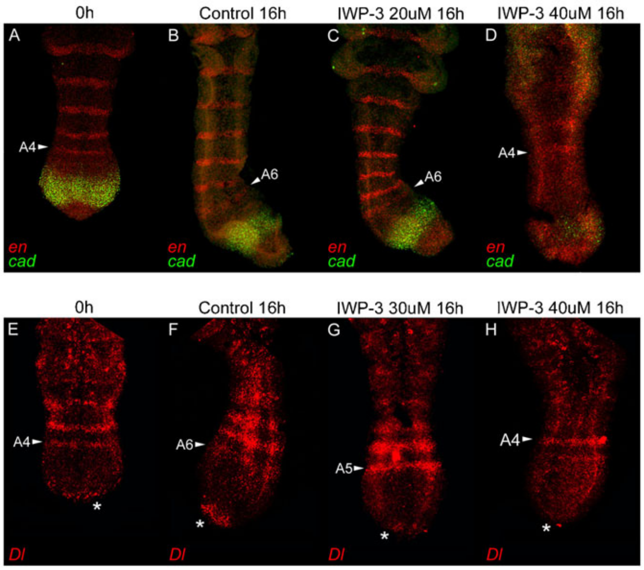


Fig. S6. IWP-3 dosage effects on *Pa-en*, *Pa-cad* (A–D) and *Pa-Dl* expression patterns (E–H). (A) *Pa-cad* (green) and *Pa-en* (red) patterns of expression in a stage 9 wild-type embryo prior culture. (B) A 16-hour DMSO control culture embryo labelled as in A. *Pa-en* is expressed segmentally up to the A6 segment and *Pa-cad* is expressed in the mid-GZ. (C) *Pa-en* and *Pa-cad* expressions have not been affected in a 16-hour 20 µM IWP3 culture embryo. (D) In a 16-hour 40 µM IWP3 culture embryo posterior *Pa-cad* expression is highly reduced and no newly *Pa-en* segmental stripes have been added.
(E) Pattern of expression of *Pa-Dl* in the posterior tip (*) and segmental stripes reaching the A3 segment in the anterior GZ in a stage 8 wild type embryo before culture. (F) In a 16-hour DMSO control culture embryo *Pa-Dl* is strongly expressed in the posterior tip (*) and in segmental stripes reaching the A6 segment. (G) *Pa-Dl* expression in a 16-hour culture embryo with 30 µM IWP3. *Pa-Dl* posterior tip (*) domain is reduced and A5 *Pa-Dl* segmental stripe has been just laid down. (H) A 16-hour 40 µM IWP3 culture embryo showing complete absence of *Pa-Dl* at the posterior tip and *Pa-Dl* segmental expression reaching the A4 segment.

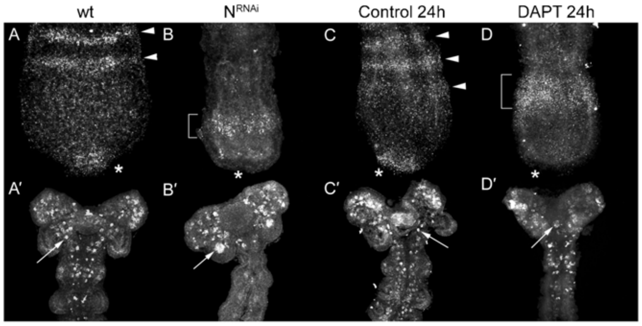


Fig. S7. Notch signalling regulates *Pa-Dl* in the growth zone. (**A**,**A′**) Wild-type *Pa-Dl* expression in the posterior tip (*, A) and segmental stripes in the anterior GZ (arrowheads, A), as well as in isolated neuroblasts in the developing head (arrow, A′) and ventral trunk. (**B**,**B′**) In *N*^{RNAi} embryos, *Pa-Dl* expression reveals a loss of expression in the posterior tip (*, B) and a single broad band of expression in the anterior GZ (bracket, B) resulting from a failure to form segmental stripes, along with a neurologic phenotype revealed by clusters of neuroblast cells in the head and trunk (arrow, B′). (**C**,**C′**) Similar to wild type, DMSO control cultured embryos express *Pa-Dl* in the posterior tip (*, C), in several stripes in the anterior GZ (arrowheads, C), and in the developing neuroblasts (arrow, C′). (**D**,**D′**) *Pa-Dl* expression in DAPT treated embryos is similar to that in *N*^{RNAi} embryos; a broad band, but no stripes, of expression in the anterior GZ (bracket, D) and no expression in the posterior tip (*, D) with large clusters of neuroblasts in the head and trunk (arrows, D′). These results confirm that Notch signalling regulates the stripes and cyclic expression of *Delta* in *Periplaneta*.