Supplementary Data:

TABLE S1. **Oligonucleotide primers used for amplification of AMP cDNAs by real-time PCR**

TABLE S2. **Mass spectrometry identification of plasma proteins after injection of proHP6 and *M. luteus* (HP6 bands) or injection of activated HP8 (HP8 bands).**

FIGURE S1. **Alignment of the amino acid sequences of serine proteinase domains of the clip domain proteinases analyzed in Fig. 1.** The accession numbers for the protein sequences are given in Experimental Procedures.

FIGURE S2. **Analysis of HP6 and HP8 mRNA (A) and protein (B) levels in *M. sexta* larvae by RT-PCR and immunoblotting.** (A) RT-PCR analysis of HP6 and HP8 transcripts. Total RNA samples from hemocytes or fat body collected at 24 h after injection with water, *E. coli*, *M. luteus*, or curdlan were isolated for RT-PCR and analyzed by 1.0% agarose gel electrophoresis. *M. sexta* ribosomal protein S3 (rpS3) was used as an internal standard to normalize the templates. (B) Immunoblot analysis of HP6 and HP8 protein levels in plasma. Cell-free hemolymph samples (1 µl/lane) collected from *M. sexta* larvae at 24 h after injection of water, *E. coli*, *M. luteus*, or curdlan were subjected to 10% SDS-PAGE followed by immunoblot analysis using 1:2000 diluted antiserum against *M. sexta* HP6 (*left panel*) or HP8 (*right panel*) as the first antibody. Molecular masses and positions of the protein standards are marked on the *left*.

FIGURE S3. **Effect of recombinant HP6 and HP8 on plasma proPO activation in the absence of bacteria.** Samples of plasma (3 µl) were left untreated or mixed with combinations indicated of proHP6, proHP6$_I$, bovine Factor Xa, and proHP8. After incubation at room temperature for 10 min, PO activity was assayed using dopamine as a substrate, as described in Experimental Procedures. The bars represent mean ± S.D. (n=3). Bars labeled with different letters are significantly different (analysis of variance and Newman-Keuls test, $P < 0.05$).

TABLE S1 Specific primers used in real-time PCR for antimicrobial genes

Gene	ID	Forward primer	Reverse primer
moricin	AY232301	5'-TGCTTTCTTTAACCTTTGTCCTC-3'	5'-TATTCTAACACAGCCTATAATGCG-3'
cecropin	BI262670	5'-CCGTGTTTTATTCTTCGTCTTC-3'	5'-AATCCTTTGACCTGCACCC-3'
attacin	BI262533	5'-CGTGTCGAACTTCTTAAAGCC-3'	5'-CCTCTTCCACAACAACC-3'
lysozyme	S71028	5'-GTGTGCCTCGTGGAGAATG-3'	5'-ATGCCTTGGTGATGTCGTC-3'
gloverin	AM293324	5'-GCAAGTCGGCAACAATGG-3'	5'-ACCCTGTCCTGTCAGTTTG-3'
rpS3	U12708	5'-TGCGTTTCATCATGGAGTC-3'	5'-TCCTTGCCTGAGAAGTACG-3'

TABLE S2 Mass spectrometry identification of plasma proteins induced after injection of HP6 or HP8

Band	Protein	Accession #	Mascot Score	MSMS Peptides matched	% coverage by MSMS	Protein Mass(Da)
HP6-1	attacin-1	gi|67906420	332	4	22	24289
HP6-2	attacin-2	gi|29469969	197	2	14	22137
HP6-2	immune-induced protein 1(IId1)	gi|27733419	173	2	15	18195
HP6-3	lysozyme	gi|233964	221	5	33	14431
HP6-4	gloverin	gi|29469967	755	7	56	18433
HP6-5	cecropin B	Contig 2488*	105	2	65	3254
HP6-5	cecropin A	Contig 5774*	141	2	71	2875
HP8-1	attacin-1	gi|67906420	331	5	20	24289
HP8-2	gloverin	gi|29469967	229	4	29	18547
HP8-3	cecropin B	Contig 2488*	111	2	65	3254
HP8-3	cecropin A	Contig 5774*	98	2	71	2875

* *M. sexta* cecropin A and B sequences are partial sequences obtained from *M. sexta* ESTs available at http://entoplp.okstate.edu/profiles/7231contigs.pdf.

Fig. S1

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Manduca_HP8          RIVGGIQTEIDEHPWMVLLRYDK-----PSGWGFYCGGVLISSKYVLTAAHCVK-GSDLP-PNWKLSQVRLGEWNTSSQ- 72
Bombyx_BAEEase       RIFGGIQTEIDEHPWMALLRYDK-----PLGWGFYCGGVLIAPMYVLTAAHCVK-GSDLP-SSWQLSQVRLGEWNTSTE- 72
Tenebrio_SPE         RIYGGEKTDLDEFPWMALVEYEKP----GGSRGFYCGGVLISKRYVLTAAHCVK-GKDLP-KTWKLVSVRLGEYNTETD- 73
Drosophila_Easter    RIYGGMKTKIDEFPWMALIEYTKSQ---GKK-GHHCGGSLISTRYVITASHCVN-GKALP-TDWRLSGVRLGEWDTNTN- 73
Drosophila_SPE       RIFGGTNTTLWEFPWMVLLQYKKLF---SETYTFNCGGALLNSRYVLTAGHCLA-SRELDKSGAVLHSVRLGEWDTRTD- 75
Manduca_PAP1         RIYGGQITDLDEFPWMALLGYLTR----TGSTTYQCGGVLINQRYVLTAAHCTI-GAVER-EVGKLITVRLGEYDTQNS- 73
Holotrichia_PPAF1    KILNGDDTVPEEFPWTAMIGYKNS----SNFEQFACGGSLINNRYIVTAAHCVA-GRVLR-VVGALNKVRLGEWNTATD- 73
Holotrichia_PPAF3    KVLGGEDTDLGEYPWMALLQQTKT----SGAKSFGCGGSLISDRYVLTAAHCVV-SSSYT-VT----MVRLGEWDLRAT- 69
Manduca_PAP2         KILGGEATAIDQYPWLALIEYHKL-----AEIKLMCGGSLISAKYVLTAAHCVK-GPILE--KGTPKNVRLGEYNTTNNG 72
Bombyx_PPAE          KIVGGAPASIDSYPWLVVIEYVRL-----ERTMLLCGGALISGKYVLTAGHCVK-GGILD--VGTPKTVRLGEYNTTNPG 72
Manduca_PAP3         KIIGGNATDVDQYPWLTIIEYVKT-----GPIKLLCGGVLISGKYVLTAGHCLT-GPVLQ--IGTPTNVRLGEYNTKNDG 72
Drosophila_Grass     RVSNGYEVKLSSRPWMALLRYQQF-----GESRFLCGGAMISERYILTAAHCVH-GLQND-----LYEIRLGEHRISTE- 68
Limulus_PCE          RIIGGREAPIGAWPWMTAVYIKQG-----GIRSVQCGGALVTNRHVITASHCVVNSAGTDVMPADVFSVRLGEHNLYST- 74
Manduca_HP6          HILGGEEASLGEFPHMVALGFD----NGGGEYRFDCGGSLISNYYVLTAAHCIDTADREP-----PSVVRAGVVNIG-GP 70
Drosophila_PSh       HIVGGYPVDPGVYPHMAAIGYI----TFGTDFR--CGGSLIASRFVLTAAHCVNT-DANT-----PAFVRLGAVNIE-NP 67
Drosophila_Snake     LIVGGTPTRHGLFPHMAALGWTQGSGSKDQDIKWGCGGALVSELYVLTAAHCATS-GSKP-----PDMVRLGARQLN-ET 73
Drosophila_Spirit    SVVGGMPTRPREFPFMAALGWR---SNFDQRIYYRCGGALIANNFVLTAAHCADL-GGEP-----PSQVRLGGDNLT-LT 69
Manduca_HP21         LIIGGQNASRNEFPHMALLGYG----EEP-DVQWLCGGTLISENFILTAGHCISSRDIN------LTYVYLGA-LARSEV 68
Tenebrio_SAE         LIVGGTNATRKEFPHMAVIGFE----PQPGDIKWLCGGTVLSKHYILTAAHCLSHQEHGR-----ARYVRIG--VTDLED 69
                      : .*  .     *  . :              *** ::   .::**.**             :  *

Manduca_HP8          VDCVGD-------DCSQ-PVQDIRIEQIVAHESYDPEDNNQQNDIALLRLAQNVHLNDFVKPICLPTTEDLRDSNFDGLE 144
Bombyx_BAEEase       TDCVEG-------DCSG-PVQDIPVQQIIAHENYDPNDKDQQNDIALLRLSRNAQFNDFVSPICLPTSNELRQNEFESDY 144
Tenebrio_SPE         TDCINNGF--G-EDCAP-PPVNVQVEARIAHESYEPNNINQYHDIALLRLRREVKFSDYIKPICLPTTTEELSKSYLGQK 149
Drosophila_Easter    PDCEVDVR--GMKDCAP-PHLDVPVERTIPHPDYIPASKNQVNDIALLRLAQQVEYTDFVRPICLPLDVNLRSATFDGIT 150
Drosophila_SPE       PDCTTQMN--GQRICAP-KHIDIEVEKGIIHEMYAPNSVDQRNDIALVRLKRIVSYTDYVRPICLPTDG-LVQNNFVDYG 151
Manduca_PAP1         VDCVD-------DVCAD-PPQNIPIEVAYPHSGYSDNNKNRKDDIALVRLTRRAQYTYYVKPICLANNN---ERLATGND 142
Holotrichia_PPAF1    PDCYG-----AVRVCVPDKPIDLGIEETIQHPDYVDGSKDRYHDIALIRLNRQVEFTNYIRPVCLPQPN---EEVQVGQR 145
Holotrichia_PPAF3    QDCVGSG---SYQYCSP-PPQDIGIESITSHPNYEKSSRGVFNDIALIRLARPVNRNKYVQPICLPLPT---ERTPVGEN 142
Manduca_PAP2         PDCVPSDA--GSQDCTE-GMVLAPIEQTIPHPKYKPYSLNKQHDIALIRLRTFAPRTDFISPICLPKID-YAQSPPSAFS 148
Bombyx_PPAE          RDCVSVSA--GGTDCTD-PLVKIGIEKTIPHPDYQPYHFLRKHDIGLIRLQSIAPFTDFIRPICLPSTD-YTVNPPSKFA 148
Manduca_PAP3         ADCVTVEA--GGMDCTE-GAVIVPIEKTIPHPEYNPISRTRRNDIGLIRLKEMAPFTDFIRPICLPSLD-LTQAPPVNFT 148
Drosophila_Grass     EDCRQQ---GRKKKCAP-PVVNVGIEKHLIHEKYDAR--HIMHDIALLKLNRSVPFQKHIKPICLPITDELKEKAEQIST 144
Limulus_PCE          -----------DDDSNP-IDFAVTSVKHHEHFVLAT----YLNDIAILTLNDTVTFTDRIRPICLPYRK-LRYDDLAMRK 137
Manduca_HP6          AWDDET---------------DYRVAETILHPNYTRR--EKYHDVALLRLDRPVQFSSTLNAVCLFSSN---ENP--TSK 128
Drosophila_PSh       DHSYQ----------------DIVIRSVKIHPQYVG---NKYNDIAILELERDVVETDNIRPACLHTDA---TDPPSNSK 125
Drosophila_Snake     SATQQ----------------DIKILIIVLHPKYRSS--AYYHDIALLKLTRRVKFSEQVRPACLWQLP---ELQ--IPT 120
Drosophila_Spirit    EG--E----------------DISIRRVIIHPDYSAS--TAYNDIALLELETAAKP--ELKPTCIWTQK---EVT--NTL 122
Manduca_HP21         TDPSK----------------QYRIKKIHKHPEFAPP--VRYNDIALVELERNVPLDEWLKPACLHMGD---ETA--DDR 125
Tenebrio_SAE         TNHRQ----------------QLEVEELIPYPEYKSS--SHYHDIGLLRLKRSAKLDSFTVPACLYRKH---DIE--AEK 126
                                          :          .*:.:: *   .       . *:

Manduca_HP8          MEVAGWG------KTETRTESDVKLKVRVPVVSRRLCKSV---------YERVERLITDKQLCAGGVEG-KDSCRGDSGG 208
Bombyx_BAEEase       MEVAGWG------KTETRSESDVKLKVRVPIVNREECANV---------YSNVDRRVTNKQICAGGLAG-RDSCRGDSGG 208
Tenebrio_SPE         LFVAGWG------KTENRSESNIKLKVQVPVKQMSDCTAT---------YSSANVRLGSGQLCAGGESG-KDSCRGDSGG 213
Drosophila_Easter    MDVAGWG------KTEQLSASNLKLKAAVEGFRMDECQNV---------YSSQDILLEDTQMCAGGKEG-VDSCRGDSGG 214
Drosophila_SPE       MDVAGWG------LTENMQPSAIKLKITVNVWNLTSCQEK---------YSSFKVKLDDSQMCAGGQLG-VDTCGGDSGG 215
Manduca_PAP1         VFVAGWG------KTLSGKSSPIKLKLGMPIFDKSDCASK---------YRNLGAELTDKQICAGGVFA-KDTCRGDSGG 206
Holotrichia_PPAF1    LTVVGWG------RTETGQYSTIKQKLAVPVVHAEQCAKT---------FGAAGVRVRSSQLCAGGEKA-KDSCGGDSGG 209
Holotrichia_PPAF3    LLVAGWG------ATETKAQSDKKQKLKLPVTDLPACKTL---------YAKHNKIINDKMICAGGLKG-KDSCKGDSGG 206
Manduca_PAP2         LYVAGWGRYIQDVEAGIYRSSKIKLHVNVPFVDNERCLGGVRKL-----RNGENISLWKGQLCAGGVSG-KDSCKGDSGG 222
Bombyx_PPAE          LTVAGWGRYLQ-FDNGTVRSSKIKLHVTLPFVQRDVCEANQKPL-----RNGQRITLWKGQMCAGGEAG-KDSCKGDSGG 221
Manduca_PAP3         LYAAGWG-----AVSTSQPSSNVKLHVQLPFISYERCQPS-YAV-----QNRQ-IELWEKQVCAGGEAG-KDSCKGDSGG 215
Drosophila_Grass     YFVTGWG------TTENGSSSDVLLQANVPLQPRSACSQA----------YRRAVPLS--QLCVGGGDL-QDSCKGDSGG 205
Limulus_PCE          PFITGWG-----TTAFNGPSSAVLREVQLPIWEHEACRQA----------YEKDLNITNVYMCAGFADGGKDACQGDSGG 202
Manduca_HP6          LTITGWG----RTSNTRDIKSSKLLKADVVVVPSDKCGESYTN----WRKLPHGISQE--MMCAGDPKGVRDTCQGDSGG 198
Drosophila_PSh       FFVAGWG----VLNVTTRARSKILLRAGLELVPLDQCNISYAEQPGSIRLLKQGVIDS--LLCAIDQKLIADACKGDSGG 199
Drosophila_Snake     VVAAGWG----RTEFLG-AKSNALRQVDLDVVPQMTCKQIYRKERR----LPRGIIEG--QFCAGYLPGGRDTCQGDSGG 189
Drosophila_Spirit    VTAIGYG----QTSFAG-LSSAQLLKVPLKSVSNEECQHHYQKDQ-----LAQGVLGT--QMCAGDITGERDTCQGDSGG 190
Manduca_HP21         VWATGWG----LTEYKASSGANILQKVVLNKFSTFECILQYP-----PHRLMSQGFDVNSQMCYGDRSQSKDTCQGDSGG 196
Tenebrio_SAE         AIATGWG----HTTWGGS-GSNNLLKVTLDLFDHASCNRSYKNQI--SRRLKDGIIDDDIQVCAGSLDDEKDTCQGDSGG 199
                        *:*           :   .  :      *                     .*       *:* *****
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Fig. S1

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Manduca_HP8         ALMGQAPSA-NN----WLVVGVVSYGPSPCGTPGWPGVYTRVGAFMDWILSKLRP-------------------------- 258
Bombyx_BAEEase      ALMGQSPKA-NN----WYVFGVVSYGPSPCGTEGWPGVYTRVGSFMDWILSKLEQ-------------------------- 258
Tenebrio_SPE        PLMILSLDK-DKDI-HWYAAGVVSFGPSPCGMANWPGVYTKVSKYVDWIVGKLKP-------------------------- 266
Drosophila_Easter   PLIGLDTNK-VNTY--YFLAGVVSFGPTPCGLAGWPGVYTLVGKYVDWIQNTIES-------------------------- 266
Drosophila_SPE      PLMVPISTG-GRDV--FYIAGVTSYGTKPCGLKGWPGVYTRTGAFIDWIKQKLEP-------------------------- 267
Manduca_PAP1        PLMQRR-----PEG-IWEVVGIVSFG-NRCGLDGWPGVYSSVAGYSDWILSTLRSTNV----------------------- 257
Holotrichia_PPAF1   PLLAER-----ANQ-QFFLEGLVSFG-ATCGTEGWPGIYTKVGKYRDWIEGNIRP-------------------------- 257
Holotrichia_PPAF3   PLFGQTG---AGNA-QFYIEGIVSYG-AICGTEGFPAIYTRVSDHLDWIKQNVRV-------------------------- 256
Manduca_PAP2        PLMYDKE------R-KYEAVGVVSYGAEICGQQGIPGVYTNVHEYLPWIKATIKA-------------------------- 270
Bombyx_PPAE         PLMYEHS------K-KYEAVGIVSFGPEKCGQIDIPGVYTNVYEYLPWIQNTIEP-------------------------- 269
Manduca_PAP3        PLMYENG------Q-TYEVIGIVSFGPTPCGMQDIPGVYTKVHSYKDWIISNIKP-------------------------- 263
Drosophila_Grass    PLQAPAQYLGEYAP-KMVEFGIVSQGVVTCGQISLPGLYTNVGEYVQWITDTMASNGL----------------------- 260
Limulus_PCE         PMMLPVKTG------EFYLIGIVSFG-KKCALPGFPGVYTKVTEFLDWIAEHMV---------------------------- 249
Manduca_HP6         PL----QLMEKDG--LYRLVGVTSFGRGCGS--YVPGVYTRVSNYLGWIESIVWPN-------------------------- 246
Drosophila_PSh      PL--IHELNVEDG--MYTIMGVISSGFGCAT--VTPGLYTRVSSYLDFIEGIVWPDNRV----------------------- 252
Drosophila_Snake    PI---HALLPEYNC-VAFVVGITSFGKFCAAP-NAPGVYTRLYSYLDWIEKIAFKQH------------------------ 251
Drosophila_Spirit   P------LLMQDGL-LGYVVGITSLGQGCAS--GPPSVYTRVSSFVDWIEGIVWPAQQVTNAPQPNQMTSFSPEFDLRATI 262
Manduca_HP21        PLQIKH--KKINC--MWLIIGVTSFGKACGFI-GEPGIYTKVSHYIPWIESVVWP-------------------------- 246
Tenebrio_SAE        PLQIFHESKDIKC--MYDIIGVTSFG---------PGVYVRVSQYIGWIEDIVWPENS----------------------- 246
                    .                  *: * *         *.:*     .  :*
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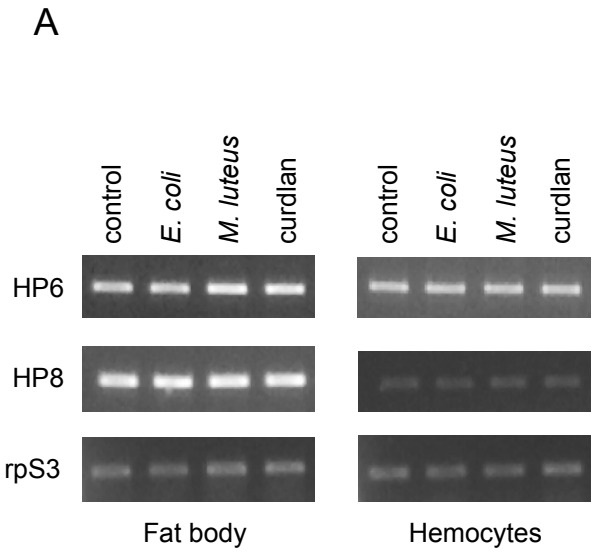
Fig. S2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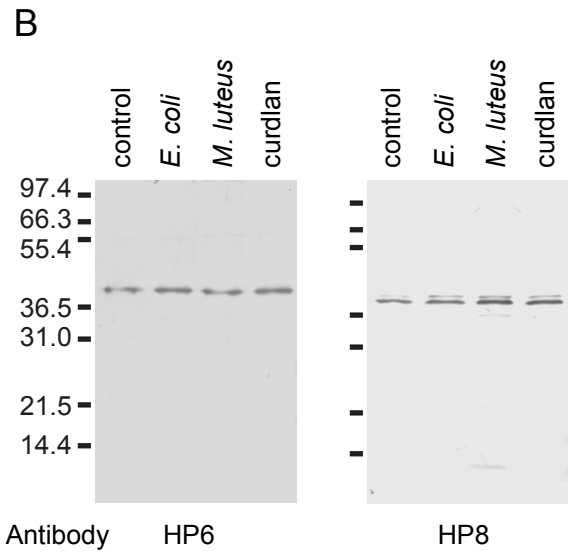
Fat body
Hemocytes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control
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M. luteus
curdlan
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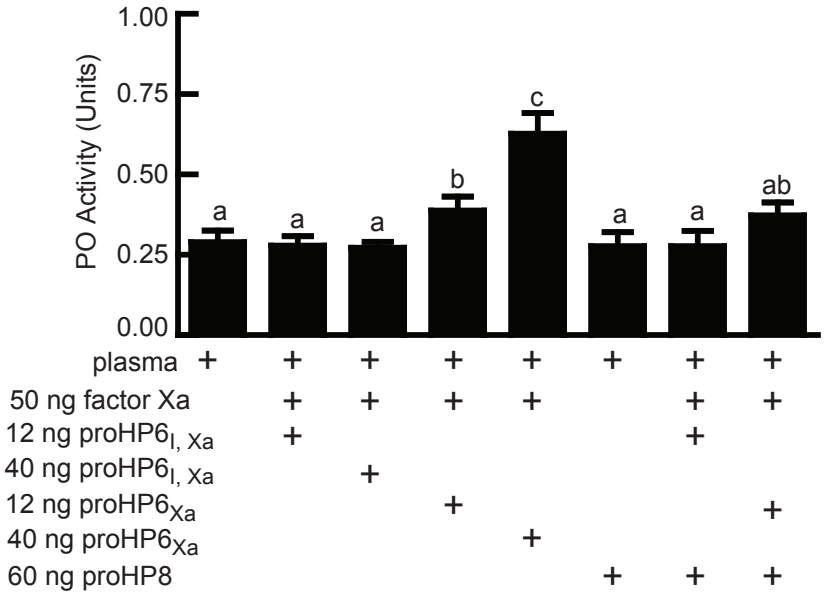
55.4
36.5
31.0
21.5
14.4
Antibody
HP6
HP8

Fig. S3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PO Activity (Units)
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a
a
b
c
a
a
ab
plasma + + + + + + + +
50 ng factor Xa + + + + + + +
12 ng proHP6I, Xa +
40 ng proHP6I, Xa +
12 ng proHP6Xa +
40 ng proHP6Xa +
60 ng proHP8 + + +