LARVAL HEMOLYMPH PROTEINS AND PHYSIOLOGICAL ROLE OF PROPHENOLOXIDASES IN *ANOPHELES GAMBIAE*

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Bachelor of Science in Biosciences

University of Science and Technology of China

Hefei, Anhui

2013

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2016

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PHYSIOLOGICAL ROLE OF PROPHENOLOXIDASE

IN *ANOPHELES GAMBIAE*

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Title of Study: LARVAL HEMOLYMPH PROTEINS AND PHYSIOLOGICAL ROLE OF PROPHENOLOXIDASES IN *ANOPHELES GAMBIAE*

Major Field: Entomology and Plant Pathology

Abstract: The African mosquito *Anopheles gambiae* is one of the major vectors for human malaria. Understanding its immune system may provide new means for disrupting the disease transmission. While the *Drosophila melanogaster* and *Manduca sexta* immune systems are well studied, most components of the mosquito system remain to be examined. Insect hemolymph contains important factors for humoral and cellular defense responses as well as immune signal transduction, including pattern recognition receptors, serine proteases, serpins, antimicrobial peptides. In the present study, we collected hemolymph samples from water- and *E. coli*-pricked *A. gambiae* larvae. The samples were separated on SDS-PAGE and subjected to LC-MS/MS analysis. The detected peptides were searched against *A. gambiae* proteins from VectorBase. We have identified a total of 1,756 proteins. Most of the abundant proteins contain putative signal peptides. Twenty-five most abundant proteins represent over half of the total protein amount, 109 proteins are up-regulated, 49 are down-regulated, and 235 are considered to be defense-related. After examining the protein distribution in the gel slices, we found that more abundant proteins tend to exist in more of the slices. We also obtained evidence for proteolysis, post-translational modification, serpin-protease complex formation, and high M_r immune complex formation based on the distribution data. In addition to the proteomic study, we generated monoclonal antibodies against prophenoloxidases PPO2 and PPO7 and found that PPO2 is presented in the adult hemolymph. Lastly, we tried to knockdown PPO gene expression in female adults by injecting double-stranded RNA and examined their survival following an *E. coli* challenge. No significant difference was observed between the test and control groups.

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CHAPTER I

INTRODUCTION

Vertebrates rely on their immune system to distinguish self from non-self and defend against invading pathogens. Immunity is divided into two types, innate and adaptive. Innate immunity is fast and can kill a broad spectrum of pathogens but lacks specificity. Adaptive immunity involves production of antibodies and T-cell receptors that recognize specific pathogens and development of immune cells that produce the specific proteins and, hence, is much slower. Only innate immune responses have been demonstrated to take place in most invertebrates including all insects.

In insects, cuticle lining of body surfaces, digestive tract and trachea acts as a physical barrier for exogenous parasites and comprises the first line of defense (Tzou et al., 2000). If microbes cross this line, they may encounter humoral and cellular responses in the hemolymph (Jiravanichpaisal et al., 2006). Cellular responses involve phagocytosis, encapsulation, and nodule formation of pathogens by hemocytes (Lavine and Strand, 2002), while humoral responses are initiated upon recognition of pathogen-associated molecular patterns (PAMPs) by pathogen recognition receptors (PRRs) (Janeway and Medzhitov, 2002). The recognition activates a serine protease cascade in hemolymph, which ultimately leads to the generation of active phenoloxidase (PO) (Ragan et al., 2009). PO catalyzes the melanotic encapsulation of invading parasites and mediates their clearance from the host. Apart from that, at least two signal transduction pathways exist in insects to mediate induced synthesis of antimicrobial peptides (AMPs). The Toll pathway, activated upon Gram-positive bacterial and fungal infection, employs transcription factors Dif and Dorsal for AMP

production (Leclerc and Reichhart, 2004; Pinheiro and Ellar, 2006). The IMD pathway, responsive to most Gram-negative bacterial infection, employs Relish to induce AMP production (Kurata, 2010; Stoven et al., 2003)

The genome sequence of *A. gambiae* was reported by Holt et al. (2002), and continuous efforts have been made to improve the gene annotation. Due to rapid development of proteomic methods, proteomic studies have been performed in *A. gambiae* as good complements to genomic and transcriptomic research. Dinglasan et al. (2009) found 12 peritrophins that contain chitin-binding domains in the peritrophic matrix proteome. They form the matrix and protect mosquito from pathogens in the midgut. A majority of the proteins identified are related to immunity in the hemolymph proteome, including PPO2, CLIPB4 and CLIPA6, TEP15, SRPN2 and SRPN15 (Paskewitz et al., 2005; Pinto et al., 2009). Others participate in iron or lipid metabolism, such as ferritin, apolipophorin III and MD2-related protein. In the proteome of saliva and salivary glands, D7-related proteins with pheromone/odorant binding domains were found to be highly abundant and gSGs were implicated in blood feeding (Francischetti et al., 2002; Kalume et al., 2005). Lefevre et al. (2007) found a wide range of molecules in the head proteome of *A. gambiae* which indicated an altered energy production in *Plasmodium*-infected mosquitoes. Phosphoglycerate mutase and tropomyosin in the head may be involved in behavioral manipulation. Lastly, two vitelline membrane proteins, seven chorion proteins and seven odorant binding proteins were identified in the eggshell proteome (Amenya et al., 2010). Enzymes involved in cross-linking and stabilizing the chorion, such as peroxidase, laccase 2, PPO9, thioredoxin, were also reported.

Here we employ a new proteomic method and focus on the larval hemolymph proteins of *A. gambiae*. The objectives of my research are: 1) obtain a complete profile of proteins present in *A. gambiae* larval hemolymph; 2) analyze the distribution patterns of proteins in polyacrylamide gel and explore possible implications in protein function; 3) find out the expression profile and physiological role of prophenoloxidases in *A. gambiae.*

CHAPTER II

REVIEW OF LITERATURE

Insect immune system

The first line of insect antimicrobial defense is the cuticle or exoskeleton, which is composed of proteins embedded in chitin and serves as a physical barrier against pathogens in the environment (Tzou et al., 2000; Feldhaar and Gross, 2008). Ingestion of food can introduce microbes to the digestive tract. Midgut cells in many insects produce a peritrophic membrane to protect the gut wall from abrasive food. It is permeable to digestive enzymes and nutrients, but not to microbes.

Some pathogens can bleach the barrier and enter hemocoel, where they will encounter the host cellular and humoral immune responses (Jiravanichpaisal et al., 2006). The cellular responses involve phagocytosis, nodule formation and encapsulation (Lavine and Strand, 2002). Phagocytosis is the engulfment of bacteria and fungi by plasmatocytes. When the size (or number) of microbes is too large to be engulfed, multicellular nodules are formed around the pathogens, which are often melanized subsequently. For parasites (*e.g*. nematodes) and parasitoids (*e.g*. wasp eggs), encapsulation by multi-layer hemocytes is employed to sequestrate them.

Humoral responses are initiated by binding of free pathogen recognition receptors (PRRs) to pathogen associated molecular patterns (PAMPs). PAMPs, like peptidoglycan, β-1,3-glucan, lipopolysaccharide (LPS), and lipoteichoic acid (LTA), are structural features of many bacteria and fungi, which can be used to distinguish non-self from self by the host. Correspondingly, insects use

PRRs, such as peptidoglycan recognition proteins (PGRPs), β-1,3-glucan recognition proteins (βGRPs), Gram-negative bacteria binding proteins (GNBPs), and C-type lectins (CTLs) to identify those PAMPs (Janeway and Medzhitov 2002). Binding of PRRs to PAMPs activates a serine protease cascade in the insect hemolymph, which ultimately leads to the activation of prophenoloxidase (PPO) (Ragan et al., 2009). Active PO is a key enzyme for melanization. The protease system is regulated by serine protease inhibitors of the serpin superfamily (Kanost et al., 2004).

Furthermore, recognition of the PAMPs by PRRs directly or indirectly activates two immune signal transduction pathways, which up-regulates the expression of distinct but overlapping sets of antimicrobial peptide (AMP) genes in *Drosophila*. One is the Toll pathway, which responds to Gram-positive bacteria and fungi (Leclerc and Reichhart, 2004). Upon PRR recognition and serine protease activation, pro-Spätzle is processed to form Spätzle, which binds and activates the Toll receptors on cell membrane. The receptor then interacts with intracellular Myd88/Tube/Pelle and leads to the phosphorylation of Cactus. Phosphorylated Cactus dissociates from transcription factors Dif and Dorsal, allowing them to translocate to nucleus and induce AMP expression (Belvin and Anderson, 1996; Pinheiro and Ellar, 2006). The other is the IMD pathway in which most Gram-negative bacteria are recognized by membrane-bound PGRP-LCs (Kurata, 2010). The intracellular domain of PGRP-LC forms complex with Imd/Dredd/Fadd and ultimately cleaves Relish, resulting in a DNA-binding N-terminal fragment (Hu and Yang, 2000; Naitza et al., 2002). This active Relish fragment translocates to nucleus and induce AMP expression (Hoffmann and Reichhart, 2002; Stoven et al., 2003).

Phenoloxidase

Phenoloxidase (PO) is an important component of the insect immune system. It is synthesized as a zymogen prophenoloxidase (PPO), which is activated by proteolytic cleavage *in vivo*. PPO is primarily synthesized in insect hemocytes (Ashida and Brey, 1997), but other locations are also reported, like hindgut epidermal cells in the silkworm *Bombyx mori* (Shao et al., 2013) and hind wing of red flour beetle *Tribolium castaneum* (Dittmer et al., 2012).

There are mainly three mechanisms for PPO activation (Lu et al., 2014). In *B. mori*, direct cleavage at Arg51-Phe52 by PPAE (PPO activating enzyme) is able to generate active PO (Yasuhara et al, 1995). In the tobacco hornworm *Manduca sexta*, PAP (PPO activating protease) cleavage at the same site (Arg51-Phe52 in MsPPO2) only generates a product with low PO activity. The cleavage has to occur in the presence of a high M_r complex of serine protease homolog-1 and -2 (SPH1 and SPH2) for the cleaved product to exhibit high PO activity (Jiang et al., 2003a, b; Yu et al., 2003; Gupta et al., 2005). In the beetle *Holotrichia diomphalia* and the fruit fly *Drosophila melanogaster*, PPO is first processed into a 76 kDa product with no activity, and then further cleaved to a 60 kDa high PO activity fragment (Lee et al., 1998; Kim et al., 2002; Lu et al., 2014a). In each model, the pathway is initiated via the binding of pathogen recognition receptors (PRRs, such as PGRPs, βGRPs) to pathogen associated molecular patterns (PAMPs, like peptidoglycans, β-1,3-glucan). Binding activates a downstream serine protease cascade, which finally leads to the activation of PPO. For example, the binding activates *M. sexta* hemolymph protease-14 (HP14), which cleaves proHP21 to form HP21. HP21 is responsible for the activation of proPAP2 and proPAP3 into PAP2 and PAP3 that cleave PPO. With the involvement of SPH1 and SPH2, PO is able to kill and melanize pathogens and parasites (Jiang, 2008).

The biological substrate of PO is generally thought to be tyrosine (Clark and Strand, 2013). Under PO catalysis, tyrosine will be converted to L-dopa. After several subsequent steps of chemical transformation, melanin will be produced at last. An important intermediate in the process

is 5,6-dihydroxyindole (DHI) which has been demonstrated to be potent antibiotic (Zhao et al., 2007; Charoensapsri et al., 2014).

Serpins in *Anopheles gambiae*

A total of 18 serpin genes have been identified in *A. gambiae*, which are SRPN1-14, 16-19 (Suwanchaichinda and Kanost, 2009). Only two serpins have alternative splicing isoforms, SRPN4 has three and SRPN10 has four. Most of the serpin genes form clusters: (SRPN1, 2, 3) and (SRPN7, 14, 18) on chromosome arm 2L, (SRPN11, 12, 17) on 2R, and (SRPN5, 6, 16) on 3R. Sixteen serpins were predicted to contain secretory signal peptides, indicating they are probably extracellular proteins. The exceptions are SRPN10 and 12. SRPN10 was reported to be intracellular and was translocated from nucleus to cytoplasm upon *Plasmodium* infection (Danielli et al., 2003 & 2005). Molecular mass of mature serpins in *A. gambiae* are generally 42−66 kDa. SRPN4A is unusually large (90.8 kDa). SRPN2 was demonstrated to inhibit CLIPB9 (An et al., 2011). SRPN1 and 6 were shown to inhibit *M. sexta* PAPs (Michel et al., 2006; An et al., 2012), but the exact substrate in mosquito was not yet illustrated. SRPN13 was found to be expressed predominately in eggs and young larvae, pointing to a role in early development (Suwanchaichinda and Kanost, 2009).

Quantitative proteomic analysis

Early proteomic studies employed two-dimensional gel electrophoresis to separate proteins from differently treated samples. Proteins of interest, such as differentially expressed gene products, were selected for mass spectrometry (MS) analysis. Although 2D gel is capable of distinguishing more than 1000 proteins, the number of proteins actually identified is far less. Also, it is impossible to resolve all proteins in one sample, and these reasons together prevent the method

from high-throughput applications (Schulze and Usadel, 2010). The next generation proteomic method takes advantage of stable isotopes to label proteins from different treatments or tissues. Isotopes can be chemically linked to proteins *in vitro* or metabolically incorporated into proteins *in vivo*. These proteins are then mixed and digested to produce differentially labeled peptides before LC-MS analysis. And peptides from different biological samples can be distinguished in MS due to the different masses of their isotopes (Schulze and Usadel, 2010). For example, in isotope coded affinity tag (ICAT), cysteine residues are covalently linked to the ICAT reagent, thus simplifying the system by focusing only on cysteines (Gygi et al., 1999). While stable isotope labeling approaches are the gold standard in protein detection, they have their limitations. First, they are time-consuming and relatively expensive in regarding to the labeling process and reagents needed. Also, comparisons can only be performed among 2−8 experiments due to technical constraints (Bantscheff et al., 2007). Recent years, label-free quantification (LFQ) methods are becoming more and more popular due to their ease of use (Asara et al., 2008). LFQ methods can be applied to all kinds of samples theoretically, and the number of experiments that can be compared is not limited. LFQ is based on spectral counting or the signal intensity of peptide precursor ions. The high-resolution power uncouples the quantification and identification progress, and thus provide higher dynamic range for quantification. Also, LFQ intensities are normalized across the whole experiment, which corrects for technical and biological variations in peptide peak intensities (Cox et al., 2014).

RNA interference

RNA interference, historically known as post-transcriptional gene silencing (PTGS), was first reported in plants (Fire et al., 1998). The best application of this technology is that exogenously introduced double-stranded RNAs (dsRNAs) can result in down-regulation of target gene expression. Upon introducing into cytoplasm, long dsRNAs will be cleaved by Dicer (Bernstein et

al., 2001) to produce 20-25 nucleotide dsRNA duplexes with 2-nucleotide 3' overhangs called small interfering RNAs (siRNAs) (Zamore et al., 2000; Vermeulen et al., 2005). siRNA is then unwound to passenger and guide strands, where only the guide strand is integrated into RNA inducing silencing complex (RISC) (Gregory et al., 2005). RISC is going to take advantage of the single-stranded guide RNA and mediate the cleavage of its complementary mRNA, which leads to PTGS (Ahlquist, 2002). The protein in RISC that mediates mRNA cleavage is called argonaute and it cleaves mRNA at the position corresponding to the middle of the guide RNA (Kupferschmidt, 2013). The RNAi effect can be amplified when siRNAs are taken as templates by RNA-dependent RNA polymerase (RdRP) to produce more siRNAs (Pak et al., 2007; Sijen et al., 2007). Thus, a few dsRNA molecules are able to mediate gene knockdown of whole cell or organism.

Endogenous RNAi can happen when microRNAs (miRNAs) encoded by RNA-coding genes are produced. miRNAs are transcribed firstly as pri-miRNAs with hairpin structures in the nucleus. Here, pri-miRNAs are processed by Drosha, a protein contains RNase III domain and dsRNA-binding domain, to generate pre-miRNAs with stem-loop structures (Lee et al., 2002 & 2003). The pre-miRNA product is then transported via exportin-5 to the cytoplasm (Yi et al., 2003; Bohnsack et al., 2004), where it is further digested by Dicer to generate ~21 nucleotide RNA duplexes (miRNAs) to block translation (Elbashir et al., 2001). Thus, the two pathways (siRNA and miRNA) converges in downstream cascades (Gregory et al., 2006).

Melanization and survival

Results varied a lot in the survival tests upon melanization disruption in *Drosophila melanogaster* (see Table below). This may be due to differences in immune challenge and gene manipulation methods. In general, RNAi experiments didn't elicit much change on survival, except that *B. bassiana* on Sp7 knockdown led to lower survival. Most challenges, including Gram-

positive bacteria, yeast and fungi, on PPO1/2 mutants had lower survival rates comparing to the control. For Sp7/MP2 mutants, nearly half had lower survival and the other half remained the same. Only the *S. pneumoniae* challenge in Sp7/MP2 mutants showed higher survival across all experiments. Also, the same challenge under the same mutant and RNAi conditions had consistent or different survival rates (*e.g*. *S. aureus* in Sp7/MP2 mutants). In *A. gambiae*, the role of melanization in survival is less studied. Schnitger and colleagues reported that the survival of CTL4 and CTLMA2 dsRNA-injected mosquitoes was reduced after Gram-negative bacterial infection, however the hemolymph PO activity of these mosquitoes was not altered (Schnitger et al., 2009). When PO activity and melanization were abolished by CLIPA8 knockdown, the survival of mosquitoes was not hampered after both *E. coli* and *S. aureus* infection (Schnitger et al., 2007).

Type	Challenge	Sp7/MP2 mutant	MP1-RNAi	Sp7-RNAi	PPO1/2 mutant
Wounding	Mild	Lower[3]			Same[5]
	Strong		Same[4]	Same[4]	
Gram-negative bacteria	*E. coli*	Same[1,3]			
	E. carotovora		Same[2]	Same[2]	Lower[5]
	S. typhimurium	Lower[3]			Same[5]
	E. cloacae				Same[5]
	B. cepacia	Same[3]			
	A. tumefaciens	Same[1]			
Gram-positive (Lys) bacteria	*E. faecalis*	Same[1,3]	Same[2]	Same[2]	Lower[5]
	S. aureus	Lower[3]+ Same[1]			Lower[5]
	S. saprophyticus				Lower[5]
	S. pneumoniae	Higher[3]			
Gram-positive (DAP) bacteria	*L. monocytogenes*	Lower[3]			Lower[5]
	B. subtilis				Lower[5]
Yeast	*C. ablicans*		Same[2]	Same[2]	Lower[5]
Fungi	*B. bassiana*	Same[3]	Same[2]	Lower[2]	Lower[5]
	M. anisopliae				Lower[5]
	A. fumigatus				Lower[5]

*Survival results of melanization disruption in *D. melanogaster* (modified from reference 5). 1. Leclerc et al., 2005; 2. Tang et al., 2006; 3. Ayres et al., 2008; 4. Nam et al., 2012; 5. Olivier et al., 2014.

CHAPTER III

METHODOLOGY

Mosquito rearing

A. gambiae G3 strain colony was maintained in an incubator where temperature, humidity and photoperiod were strictly controlled. Temperature was set to 27.5℃ and 80% relatively humidity was achieved by introducing a basin of water into the incubator. A 12h : 12h light-dark cycle with gradual sunset and sunrise transitions was programmed inside the incubator. To collect eggs, mosquito adults of 6 to 10-day-old were given a sheep blood meal (HemoStat Laboratories). Eggs were collected on wet filter papers and then transfer into distilled water to allow hatching. Larvae within first 2 days after hatching were feed on baker's yeast, and the following instars were fed with larvae food (ground fish food plus baker's yeast at a ratio of 2:1 (w/w)). Pupae were picked and concatenated in cups with water for molting. Newly emerged adults were maintained by 10% sucrose solution until a blood meal was taken.

Sample preparation for proteomic study

Appropriate number of fourth instar larvae were transferred to new cups with clean water before infection. For infection, they were first dried on a filter paper, and then pricked in the thorax

with a pulled tiny glass needle that was previously dipped into *E. coli* pellets or distilled water (control). Then the larvae were transferred back to the same cup, and a little bit of larval food was provided. An incubation period of 24 h was allowed before hemolymph extraction, so that the infection would be given enough time to elicit responses. For hemolymph extraction, larvae were first dried and laid down on paraffin film. Then protease inhibitor solution prepared using cOmplete ULTRA Tablets, Mini (Roche) containing 0.1% 1-phenyl-2-thiourea (PTU, phenoloxidase inhibitor) were added onto them (5 µl for 5 larvae), and they were torn slightly with forceps in the thorax in solution. So, bleeding hemolymph would mix with protease inhibitors and PTU immediately, as the abdomen of mosquito larvae were pressed with pipette tips. All samples (approximately 20 µl each tube) were centrifuged 5000 rpm (*c.a.* 2000×*g*) for 5 min to remove hemocytes and other contaminating tissues. Protein concentration of all samples were determined by a modified Bradford assay using BSA as a standard. A total of 40 µg total protein (volume adjusted to 20 µl by PBS) was taken from each of control and induced samples (4 biological replicates) and mixed with 4 µl 6×SDS sample buffer. After incubation at 95 ℃ for 5 min, eight samples were loaded onto 4−15% gradient polyacrylamide gel (Mini-Protein TGX Precast Gels, Bio-Rad) and electrophoresed for 40 min at 25 mA. The gel was stained by Coomassie blue for 20 min and destained for 1 h in 30% methanol and 10% acetic acid. Each lane was divided into 12 gel slices, resulting in 48 gel samples. Proteins in gel slices 1-5 (>80 kDa, 40 samples) were analyzed using a LTQ Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific) without technical replicate. The remaining gel slices (56 samples) were analyzed on an Orbitrap Fusion tribrid mass spectrometer, and at least one technical replicate was performed for each sample.

Proteomic data analysis

Software MaxQuant was used to process raw data and perform database searching. Data from Orbitrap and Fusion mass spectrometers were analyzed together. For analysis between biological

replicates, gel slices from the same biological replicate were designated as one experiment (8 experiments in total: CH1−4, IH1−4). For analysis across gel slices to look at protein distributions, gel slices were distinguished and designated as different experiments (96 experiments in total: CH(1−4)_(1−12), IH(1−4)_(1−12). Protein LFQ intensities in each gel slice (CH1−12, IH1−12) were represented by the average intensity in this slice across the biological replicates. Peptides were searched against *A. gambiae* protein database from VectorBase (*Anopheles gambiae* PEST PEPTIDES_AgamP4.2), with trypsin set as the digestion enzyme. Oxidation of methionine, acetylation of the protein N-terminus, iodoacetamide derivative of cysteine, pyro-Glu from glutamine and acrylamide adduct of cysteine were selected as variable modifications. No fixed modification was specified, and all peptides (with or without modifications) were used in searching. In protein search result, contaminants and proteins with one peptide count were not included in subsequent analysis. Here, we used the LFQ (label free quantification) intensity to calculate p value in Student's t-test. Proteins with LFQ intensity of zero in all 8 biological replicates were excluded. For IH/CH ratio, it was calculated by dividing IH group mean over CH group mean. Signal peptides were predicted by SignalP 4.0 (Petersen et al., 2011), and those without SignalP output were predicted using Phobius (Käll et al., 2007) and Signal-3L (Shen and Chou, 2007) again. Hypothetical proteins were searched against NCBI non-redundant protein sequences using BLASTP 2.3.0+ (Camacho et al., 2009), and annotated either by BLAST description annotator in BLAST2GO or manually. Those not identified by BLAST were subjected to domain prediction by InterProScan 5, and representative domains were taken as protein names. Remaining unknown ones not identified by any method were represented with VectorBase IDs.

PPO knockdown and mosquito survival

Female mosquito adults within 1-2 days after emergence were anesthetized on ice, and injected with 69 nl (1 ng/nl) dsRNA to the thorax, either targeting *A. gambiae* PPO proteins

(AgPPOs) or GFP as a negative control. AgPPO dsRNA is designed to target a conserved region of *A. gambiae* PPOs, and it is able to knockdown all 9 PPO mRNAs to different extent. MEGAscript RNAi kit (Ambion) was used for dsRNA synthesis and purification, and Nanojet II (Drummond) was utilized for micro-injection. Mosquitoes were allowed to recover for 4 days before microbial infection was performed. For bacterial challenge, *E. coli* strain BL21 was cultured overnight, pelleted, re-suspended in sterilized PBS to a concentration of OD_{600} = 0.4. Each female mosquito received 69 nl bacteria suspension. Mosquito survival was recorded for a consecutive period of seven days, with dead individuals counted and removed daily.

RNA analysis

Total RNA of five mosquito adults was isolated using TRIzol Reagent (Ambion) 1 and 12 days after dsRNA injection. Then first-strand cDNA was synthesized from total RNA using iScript Reverse transcription supermix for qRT-PCR (Bio-Rad) containing oligo-dT primers. The cDNA (400 ng) was used in a two-step qPCR protocol with iTaq Universal SYBR Green Supermix (Bio-Rad) and CFX Connect Real-Time System (Bio-Rad). The melting curves of PCR products were examined with non-pure amplification excluded from subsequent analysis. qRT-PCR primers (Table 1, Appendices) were generated to specifically amplify AgPPO1−9 transcripts respectively, taking actin mRNA as an internal reference.

Hemolymph PPO analysis

15−20 female adults were decapitated 4 days post injection of dsRNA, and their hemolymph samples were extracted into 10 μl ddH_2O containing 0.1% 1-phenyl-2-thiourea (PTU) and protease inhibitors (cOmplete ULTRA Tablets, Mini, Roche) using QIAshredder (QIAGEN). Hemolymph samples (3 μg total protein) were then subjected to western blot analysis. The hemolymph samples

were first separated by 6% SDS-PAGE and then transferred onto nitrocellulose membrane under 15 V constant voltage for 80 min. Membrane was then blocked with 3% BSA in Tris-buffered saline (137 mM NaCl, 2.7 mM KCl, 25 mM Tris-HCl, pH 7.4) for 20 min and incubated with 1:500 diluted polyclonal antiserum against *Aedes aegypti* PPO5 (AaPPO5) in TBS with 1% BSA overnight at room temperature. The recognition of AaPPO5 polyclonal antibody against AgPPOs was confirmed previously (Hu et al., unpublished data). After washing, the membrane was further incubated with alkaline phosphatase linked goat-anti-rabbit (GAR-AP) secondary antibody (1:1000 diluted in TBS containing 1% BSA) for 4−6 h. Development was performed in 0.1M Tris-HCl, pH 9.5 with 1% alkaline phosphatase (AP) color reagents A and B (Bio-Rad).

PPO monoclonal antibody generation

Antibodies are designed against peptide sequences (Table 2, Appendices) that are predicted to be on the surface of PPO proteins. The company (Ab-Mart, Shanghai, China) manually synthesized the peptides and conjugated them to BSA. Screening was performed using the conjugates across a well-established antibody library containing antibodies against all possible oligo-peptides. Positive hits were sent to us for verification and further examination on PPO proteins. 36 ng of each of the native PPO proteins (PPO1-9) were loaded onto nitrocellulose membranes. The membranes were blocked and cut into strips for probing with different primary antibodies (1:1000). Strips were further incubated in alkaline phosphatase linked goat-anti-mouse (GAM-AP) secondary antibody (1:1000) and developed in 0.1M Tris-HCl, pH 9.5 with 1% alkaline phosphatase (AP) color regent A and B (Bio-Rad).

CHAPTER IV

FINDINGS

Overview of proteomic results

There is no clear difference on band patterns between CH and IH lanes in polyacrylamide gel, suggestive of good repeatability and no major protein changes elicited by bacterial challenge (Fig. 1A). Each lane was cut into 12 gel slices in order to achieve comparable protein amounts and appropriate gel volume, as well as separating some intense bands from more diffused ones. By comparing with the protein marker, molecular mass ranges of the gel slices was estimated to be: 500−350, 350−250, 250−230, 230−140, 140−80, 80−70, 70−45, 45−30, 30−22, 22−20, 20−15, <15 kDa from top to bottom, corresponding to slices 1−12, respectively (Fig. 1A). Indeed, the number of proteins identified (LFQ intensity not zero) in each gel slice was consistent across the two groups. For CH, there are 583, 674, 544, 806, 765, 817, 1023, 1197, 1189, 781, 981, 958 proteins in slice 1−12; For IH, there are 687, 744, 698, 859, 742, 864, 1061, 1206, 1178, 890, 949, 907 proteins, confirming a good parallellism between experiments (Fig. 1B). Although slice 7−9 contain more proteins, identified protein numbers are comparable between gel slices, suggesting the gel cutting procedure was properly-designed. In order to further investigate the correlation between biological samples, we did a pairwise Pearson correlation analysis (Table 1). Correlation within groups are high: 0.897−0.978 (mean ± SD, 0.937 ± 0.029) for CH-CH, and 0.959-0.991

(0.976 ± 0.011) for IH-IH, suggesting good consistency among biological replicates. For comparison between CH and IH, the correlation is lower (0.908−0.954; mean ± SD: 0.941 ± 0.020), but not well separated from the intragroup correlations, demonstrating that no major protein change was induced by microbial challenge again.

Overall, we have identified 1,756 proteins after excluding those with only one peptide count. After determining their names by BLAST and InterProScan, we divided them into nine categories: immunity-related, metabolism, DNA/RNA & nucleus, ion binding, cytoskeleton/motor, ATP/NAD binding, sensory/cuticle, ribosomal protein and other, and each of them contains 235, 524, 105, 147, 73, 181, 52, 78, and 361 proteins (Fig. 2A), respectively. This includes a wide range of molecules, some of which were not expected to be presented in larval plasma. Among them, 602 proteins were predicted to contain signal peptides, and others were supposed to be intracellular. This may be due to the hemolymph collection procedure. In the procedure, we pressed the larval abdomen in order to extract more hemolymph, which may have led to contamination by gut content. Hemocyte rupture and incomplete removal of hemocytes by centrifugation can also result in the presence of intracellular proteins in MS results. However, the abundance of extracellular proteins are almost two thirds of the total protein abundance (69.7% in CH, 64.6% in IH), which is twice higher than the intracellular ones. That means the contamination is not severe, although there are quite a few number of intracellular proteins.

Most abundant proteins

We examined the distribution of proteins based on their abundance (Fig. 3A). There are 61 proteins identified in CH but not in IH (LFQ of IH is zero), and 68 proteins vice versa. Within total LFQ intensity ranges of $<1\times10^6$, 1×10^6 to 1×10^7, 1×10^7 to 1×10^8, 1×10^8 to 1×10^9, 1×10^9 to 1×10^{10}, and $>1\times10^{10}$, we identified 35, 339, 698, 453, 140, 23 proteins in CH, and 48, 340, 702, 444, 140,

21 proteins in IH. They are almost normally distributed. Then we calculated the total abundance of proteins within each LFQ range and represented them as percentage of the total protein abundance of CH or IH (Fig. 3B). Surprisingly, a few molecules represents more than half of the protein abundance in both groups. The 23 and 21 most abundant proteins in CH and IH account for 62.2% and 54.3% of the total protein amounts, respectively. Also, the 140 less abundant proteins in both groups account for 25.5% and 31.1% accordingly.

We closely examined the 23 and 21 (total 25) most abundant proteins (Table 2). Different isoforms of hexamerin compose a large proportion (27.8% for CH and 24.5% for IH in abundance) of the hemolymph proteins. They are also known as storage proteins in insects. Apolipophorin-III alone accounts for 7% of the total protein abundance. It associates with low density lipophorin (LDLp) and facilitates the transport of diacylglycerols in plasma. Also, vitellogenin, an egg yolk precursor, comprises 4−5% of the total protein amount. Actin is an intracellular protein and important component of cytoskeleton. It is a house-keeping gene and usually adopted as internal references. Its presence in the hemolymph probably results from incomplete removal of hemocytes or gut contamination. It is also the case with other intracellular metabolic enzymes, like creatine kinase, fructose-bisphosphate aldolase and glyceraldehyde 3-phosphate dehydrogenase. Gelsolin is a regulatory protein of the actin filament, and here we identified the extracellular form of this protein. Ferritin is a protein that stores and transports iron in the serum. Studies have also shown its role in immune and stress response (Larade and Storey, 2004; Ong et al., 2005). It is quite surprising to find PPO2 and PPO3 are among the most abundant proteins. They probably remain in inactive state in plasma prior to acute activation. TEP15 is a member of the TEP family, which belongs to the complement C3/α2 macroglobulin superfamily. One family member TEP1 was demonstrated to promote parasite melanization (Blandin et al., 2004) and bacteria phagocytosis (Levashina et al., 2001).

Moreover, we examined the presence of proteins in each gel slice. In the beginning, we divided the gel in a way that the intense bands could be separated (Fig. 1A). Here, we examined the contents of these three bands: slice 3 (250-230 kDa), slice 6 (80-70 kDa) and slice 10 (22-20 kDa). Different isoforms of hexamerin together account for 57% of CH and 47% of IH total protein amounts in slice 3. Similarly, hexamerin isoforms account for 53% of CH and 34% of IH in slice 6. It is much simpler in slice 10 – apolipophorin III alone represents most of the protein abundance (74% of CH and 67% of IH). Therefore, we conclude that the intense bands in slices 3, 6 and 10 are mainly hexamerins, hexamerins and apolipophorin III.

Up- and down-regulated proteins

A total of 158 proteins are significantly different (p <0.05) in abundance between CH and IH. 109 were up-regulated (IH/CH >1, minimum: 1.14) (Table 3) and 49 were down-regulated (IH/CH<1, maximum: 0.85) (Table 4) after *E. coli* challenge. They account for 9% of the total proteins identified. Surprisingly, only twenty are related to immunity. Their IH/CH ratios are close to 1.0 in most cases. 119 out of the 158 proteins ($p < 0.05$) have IH/CH ratio of 0.5−2, and 144 have IH/CH ratio of 0.3−3. One reason for this unusual phenomenon may be that, after pricking and placing the larvae back into aqueous environment, bacteria can diffuse into water through the wound site. On the other hand, due to wounding and exposure to food-containing water, the control larvae may be infected.

The 109 up-regulated proteins are devided into six groups: immunity-related, cytoskeleton/ motor, DNA/RNA & nucleus, metabolism, ATP/NAD binding, and other functions, with each comprised of 5, 10, 16, 37, 12 and 29 proteins (Table 3), respectively. Only 14 of them were predicted to be extracellular. The five immunity-related proteins are E3 SUMO-protein ligase RanBP2, LRR15, PGRP-LB, SRPN10B and TEP2, and their IH/CH ratios are 2, 1.34, 2.24, 1.93

and 1.51, respectively. Involvement of nucleus and DNA/RNA binding proteins may reflect regulations in transcription and translation in response to bacterial challenge. And indeed, quite a few of them are translation initiation factors. Also, microbial challenge can disturb the metabolism of the host, resulting in metabolic changes partly reflected by enzymes or other related proteins (*e.g.* ATP/NAD binding proteins) to resist invading pathogens. In the "other" group, some heat shock proteins and stress-induced proteins may be induced by injury or infection.

The 49 down-regulated proteins belong to three categories: immunity-related, metabolism and other, with 15, 19 and 15 proteins in each group, and signal peptides were predicted to be present in half (26) of them (Table 4). Interestingly, immunity-related proteins account for 31% of the down-regulated proteins. Indeed, 70% (158) of the 235 immunity-related proteins were found to have IH/CH ratio less than 1. In other words, there is a tendency for immunity-related proteins to be down-regulated in *A. gambiae* larvae after *E. coli* challenge. The 15 significantly down-regulated immune proteins were CLIPA7 homolog, CLIPB1, B8, B13, PPO1, PPO2, PPO3, βGBP, LRR1, TEP15, MDL2, lysozyme-4 (c-type), fibrinogen, coagulation factor X, and a chymotrypsin-like protease. Their IH/CH ratios are within 0.34−0.85.

Immunity-related proteins

A total of 235 immunity-related proteins were identified in our experiment, and 166 (71%) of them were predicted to be secretory (Table 5). Some proteins do not contain signal peptides but actually present in plasma, like PPOs, may result from cell rupture. These immunity-related proteins were further divided into 8 groups: AMP, PPO, TEP, PRR, SP, SPH, Serpin and other, with 9, 7, 8, 40, 63, 37, 15 and 56 proteins in each group (Fig. 2B), respectively. Unlike in *Manduca sexta* (Zhang et al., 2014), we identified only a small number of antimicrobial peptides (AMPs), including defensin, gambicin, transferrin and lysozyme. However, we detected seven

prophenoloxidaes (PPO1−4, 6−8) in the larval hemolymph. PPO2 and PPO3 are most abundant; PPO6 and PPO8 are moderate; PPO1, PPO4 and PPO7 are low. A few members of the thioester-containing proteins (TEPs), complement-like proteins in insects, were also identified, including TEP1, 2, 4, 6, 9, 12, 14, and 15. The function of TEP1 is well characterized while others remain unclear (Levashina et al., 2001; Blandin et al., 2004). Pathogen recognition receptors (PRRs) are important molecules which distinguish nonself from self in the host. Typical PRRs include βGBPs, CTLs, GNBPs, LRIMs, LRRs, and PGRPs, some of which were identified in this study. Serine proteases (SPs) and serine protease homologs (SPHs) comprise the largest groups, accounting for 43% of the immunity-related proteins. They are (chymo)trypsin-like proteins without or with clip-domains (CLIPs). CLIPs are major components of the hemolymph serine protease cascade which leads to the activation of PPOs or cytokines for signal transduction receptors on cell membrane (Cao et al., 2015), and 31 (including serine protease homologs) of them are found here. Many serpins are serine protease inhibitors that inhibit the activity of hemolymph proteases. There are 19 SRPNs in *A. gambiae*, and we identified 12: SRPN1−4, 7−12, 16, and 17. Other identified immune proteins include FBNs, fibrinogens, thioredoxins, thioredoxin peroxidases and so on.

Gel distribution of immune proteins

Theoretically, proteins should migrate to the position corresponding to their calculated molecular masses (M_r's) in polyacrylamide gel. However, multiple reasons can lead to irregular distribution patterns from calculated, such as post-translational modifications (*e.g.* glycosylation) (Table 6). We examined the gel distribution patterns of immunity-related proteins (Table 3, Appendices) and identified major discrepancies from theoretical M_r values. For some proteins, their abundances are very high and they are presented in nearly all 12 gel slices both in CH and IH (data not shown). To explain this, we examined the correlation between protein abundance and number of slices the protein presented. There is a trend for more abundant proteins to be found in more gel

slices (Fig. 4). Other than that, proteins over 160 kDa tend to migrate to lower positions with M_r less than 80 kDa, which may be due to proteolytic cleavage. Proteolytic cleavage can also account for the discrepancies of many serine proteases, since they exist as zymogens and need cleavage to become active (Table 6). Another possibility for the discrepancies of serine proteases is their complex formation with serpins, which results in SDS-stable 70-80 kDa molecules. In *M. sexta*, serpin-1E was known to form complex with HP1 and HP8 (Ragan et al., 2010). Serpin-3 through 6 were known to associate with PAPs and HP1, 6, 8, 21. In *A. gambiae*, however, it is not well-understood. Only SRPN2 was reported to form complex with CLIPB9 (An et al., 2011). SPRN1 and 6 were shown to inhibit *M. sexta* PAPs (Michel et al., 2006; An et al., 2012), but the substrate in mosquito was not illustrated. Nonetheless, it is likely that many serpins are able to form SDS-stable complexes with serine proteases in mosquito. In our results, quite a few CLIPs and serpins were found to form possible serpin-protease complexes in 70-80 kDa (slice 6), especially SRPN10D which exists 100% in slice 6 (Table 7). There is another type of discrepancy that cannot be explained with protein abundance, proteolysis or complex formation. Many proteins migrate to the first few gel slices (>140 kDa) which is far beyond their theoretical molecular mass. These include 4 trypsin-like proteins, 4 LRRs, 4 coagulation factor XI, 5 TEPs and 6 PPOs, and can be interpreted as components of high mass immune complexes (Table 8).

Examination of PPO monoclonal antibodies

To facilitate the detection of specific PPOs in tissues, we asked a company to develop and screen monoclonal antibodies against 29 unique and 3 common surface peptides of *A. gambiae* PPO2, 6, 7 and 8 (Table 2, Appendices). According to the company, 54 antibodies were generated against 27 peptides, 49 of which have detection limits below 25 ng against BSA-peptide conjugates. However, when all the antibodies were tested against native recombinant *A. gambiae* PPOs, only two (1P5 and 3P35) showed successful recognition at a sensitivity of 36 ng PPO2 and PPO7,

respectively (data not shown). Antibody 3P35 showed some cross-reactivity with BSA. After changing the blocking solution to 3% BSA in TBS and pre-incubating 3P35 with 1% BSA in TBS for 1h, the cross-reactivity was eliminated while the recognition on PPO7 remained unchanged. Antibody 1P5 reacted weakly with PPO7, and both antibodies were able to recognize denatured PPOs at a sensitivity of ~400 ng.

Mosquito hemolymph PPOs

According the proteomic results, the abundance of PPO2 and 3 are most abundant, PPO6 and 8 are moderate, PPO1, 4 and 7 are low. To confirm this using the monoclonal antibodies, we examined the expression profile of PPOs in hemolymph samples from mosquito larvae, pupae and adults (Fig. 5). In larva and pupa, not much signal was detected by 1P5 (against AgPPO2) and 3P35 (against AgPPO7) at the position of recombinant PPO, while there were proteins identified by the polyclonal antibody that recognizes all AgPPOs. In adult, however, one strong band was detected by 1P5, while no PPO7 was identified by 3P35. Although 1P5 cross-reacts with PPO7, since no PPO7 was detected, the band must be PPO2. There is also one band lower than PPO2/7 under AaPPO5 in adult, which may be other PPO isoforms (Fig. 5). The lack of PPO2 signal in larval hemolymph may be due to the low sensitivity of monoclonal antibodies comparing to mass spectrometry.

PPO knockdown and mosquito survival

There has being a long-existing debate about whether PPO is needed or not for mosquito immune response. But till present, direct evidence from PPO knockdown is not reported. In our proteomic result, PPO1−3 was shown to be down-regulated after *E. coli* challenge, indicating a role of PPOs in anti-bacterial response. Here, we tried to knockdown them by dsRNA injection and

examine the survival of mosquitoes upon bacterial challenging. Female mosquitoes (~40 per group) within 1-2 days of emergence were injected with either dsPPO or dsGFP (as negative control). The knockdown efficacy was confirmed by qRT-PCR and immunoblotting (Fig. 6), which demonstrated successful silencing at both mRNA and protein level. After four days of recovery, both groups were injected with *E. coli* of $OD_{600} = 0.4$ for the survival test. Survival was documented for the following seven days. The result didn't show significant difference between dsPPO and dsGFP groups (Fig. 7). One possible explanation is that the knockdown is not significant enough to elicit observable responses or not long-lasting enough to cover the documenting period. We have examined the PPO mRNA levels 12 day post dsRNA injection, which would be seven days post *E. coli* infection. The qRT-PCR data showed only 2 PPO genes still had significant knockdown (data not shown). Another possibility is that PPOs are not indispensable for mosquito immunity, which had been reported before (Schnitger et al., 2007).

CHAPTER V

CONCLUSION

In the present study, we identified a total of 1,756 proteins in the hemolymph of *A. gambiae* larvae. Although 69% of them are predicted to be intracellular, they only constitute a small portion (*c.a.* 33%) of the total protein amount. Among all these proteins, 109 (14 extracellular) were up-regulated and 49 (27 extracellular) down-regulated. IH/CH ratios for 1,577 proteins were not substantially changed (0.33−3) after the immune challenge. This may be related to the aquatic habitat of mosquito larvae, which may have affected the test. PPO1−3 and TEP15 were down-regulated. PPO2, PPO3 and TEP15 are among the most abundant proteins, including hexamerins, apolipophorin III, OBP9, ferritin and vitellogenin. We consider 235 proteins (70% extracellular) as defense-related. 100 (42%) of them are serine protease-related, suggesting these proteins play an important role in mediating immune responses in hemolymph. We examined the gel distribution patterns of all the proteins and found that abundant proteins tend to spread to more gel slices. Besides, while distributions of some proteins can be explained by post-translational modifications such as proteolysis, others suggest the existence of serpin-protease complexes and high M_r immune complexes. In addition to the proteomic study, we generated monoclonal antibodies against PPO2 and PPO7, and used them to examine their presence in mosquito hemolymph. Immunoblot analysis showed PPO2 (and not PPO7) is present in hemolymph of the adult mosquitos. We examined the role of PPOs in antibacterial defense in mosquito by knocking down PPO expression in adult females. *E .coli* infection of the adults did not cause any significant difference in survival between GFP and PPO dsRNA-treated groups.

Table 1. Pearson correlation between biological replicates

	CH1	CH2	CH3	CH4	IH1	IH2	IH3	IH4
CH1	1	0.915	0.948	0.897	0.965	0.98	0.965	0.918
CH2		1	0.978	0.956	0.943	0.924	0.921	0.908
CH3			1	0.929	0.938	0.954	0.935	0.918
CH4				1	0.949	0.936	0.942	0.954
IH1					1	0.977	0.984	0.959
IH2						1	0.991	0.967
IH3							1	0.978
IH4								1

Table 2. A list of 25 most abundant proteins

Protein IDs	Protein names	MW (kDa)	SP*	CH%	IH%	IH/CH	p-value
AGAP000651-PC	Actin	41.8		0.64	0.84	1.12	0.458
AGAP013365-PA	Apolipophorin-III	21.7	19	7.07	7.16	0.86	0.642
AGAP008054-PD	Chemosensory protein	14.6	17	1.68	1.23	0.63	0.126
AGAP005627-PD	Creatine kinase	39.8		1.09	1.37	1.07	0.643
AGAP002564-PE	Fructose-bisphosphate aldolase, class I	39.2		0.97	1.43	1.26	0.168
AGAP011369-PA	Gelsolin	42.7	20	0.78	0.49	0.54	0.053
AGAP009623-PA	Glyceraldehyde 3-phosphate dehydrogenase	35.5		0.77	0.72	0.8	0.49
AGAP001659-PA	Hexamerin	83.9	19	7.99	6.41	0.69	0.019
AGAP001657-PA	Hexamerin	84.2	18	9.1	6.36	0.6	0.039
AGAP005768-PA	Hexamerin	82.3	18	1.15	2.75	2.04	0.138
AGAP005766-PA	Hexamerin A	38.9	18	2.71	3.7	1.17	0.441
AGAP001345-PA	Hexamerin A	82.8	18	6.8	5.28	0.66	0.05
AGAP008060-PA	Imaginal disc growth factor	48.1	22	0.88	0.94	0.91	0.706
AGAP010657-PA	Larval serum protein 1 beta chain	23.5	18	1.26	1.68	1.14	0.351
AGAP008369-PA	Lipid transport protein vitellogenin	170.4	19	4.71	4.06	0.74	0.294
AGAP001826-PA	Lipophorin	371.3		2.5	1.1	0.38	0.036
AGAP007059-PA	LRR-7059	124		0.72	0.67	0.79	0.106
AGAP000278-PA	OBP9	15.7	17	2.98	1.83	0.52	0.051
AGAP006258-PA	PPO2	78.1		1.19	1.18	0.85	0.045
AGAP004975-PA	PPO3	78.6		2.1	1.96	0.8	0.024
AGAP013400-PA	Probable fatty acid-binding protein	14.7		0.67	1.34	1.72	0.021
AGAP012057-PA	RNA polymerase-associated protein RTF1	88.4		0.93	0.49	0.45	0.008
AGAP002464-PA	Ferritin G subunit	26.2	21	2.05	1.45	0.6	0.082
AGAP002465-PA	Ferritin heavy chain	24.6	26	1.13	0.8	0.61	0.032
AGAP008364-PA	TEP15	163.6	42	1.66	1.41	0.73	0.045

*SP indicates the predicted signal peptide cleavage site, and TMs indicates the predicted number of transmembrane domains. CH% and IH% indicate the abundance percentage that protein represents out of the total protein abundance of CH and IH.

Table 3. A list of 109 up-regulated proteins

Group	Protein IDs	Protein names	SP*	TMs	IH/CH	p-value
Immunity-related	AGAP002982-PA	E3 SUMO-protein ligase RanBP2			2	0.021
	AGAP003878-PA	LRR-15	19	1	1.34	0.013
	AGAP001212-PB	PGRPLB		1	2.24	0.04
	AGAP005246-PD	SRPN10B			1.93	0.044
	AGAP008366-PA	TEP2			1.51	0.021
Cytoskeleton/ motor	AGAP001306-PA	Actin related protein 2/3 complex, subunit 4			1.48	0.042
	AGAP002509-PA	Actin-interacting protein 1			1.4	0.018
	AGAP010175-PC	Adenylyl cyclase-associated protein 1			1.49	0.034
	AGAP012185-PA	Erythrocyte membrane protein band 4.1			1.8	0.008
	AGAP004335-PA	Filamin			1.37	0.017
	AGAP001315-PE	Microtubule-associated protein 7 family			2.1	0.022
	AGAP000749-PA	Muscular protein 20			1.34	0.043
	AGAP010895-PA	Spectrin beta			1.46	0.016
	AGAP001799-PA	Tropomyosin 1			1.52	0.008
	AGAP002130-PA	Tubulin-specific chaperone A			1.43	0.003
DNA/RNA & nucleus	AGAP002945-PA	Bifunctional glutamyl/prolyl-tRNA synthetase			2.61	<0.001
	AGAP006125-PA	Density-regulated protein			1.78	0.048
	AGAP001883-PA	ELAV-like 1			2.3	0.002
	AGAP002340-PA	Eukaryotic translation initiation factor 3A			1.45	0.039
	AGAP004725-PA	Eukaryotic translation initiation factor 3C			2.16	0.002
	AGAP002337-PA	Eukaryotic translation initiation factor 3D			1.33	0.033
	AGAP003486-PA	General transcriptional corepressor trfa			1.9	0.044
	AGAP005015-PA	Heterogeneous nuclear ribonucleoprotein K			1.75	0.029
	AGAP007299-PA	Importin-7			1.64	0.014
	AGAP012013-PA	Nuclear factor of activated T-cells 5			1.69	0.009
	AGAP002351-PA	Nuclear pore complex protein Nup98-Nup96			4.67	0.005
	AGAP002654-PB	Poly(A)-binding protein 1			1.87	0.04
	AGAP010553-PA	Poly(U)-binding-splicing factor PUF60			1.63	0.035
	AGAP002655-PA	RNA binding protein			2.88	0.045
	AGAP010640-PA	Translation initiation factor			1.68	0.003
	AGAP002502-PA	Translation initiation factor 4G			1.48	0.02
Metabolism	AGAP011350-PA	4-nitrophenyl phosphatase			1.41	0.029
	AGAP006227-PA	Alpha esterase			∞	0.026
	AGAP007809-PA	Aminopeptidase NPEPL1			1.26	0.036
	AGAP004236-PA	Beta-lactamase-like protein 2 homolog			∞	0.024
	AGAP001341-PA	Bleomycin hydrolase			1.43	0.045
	AGAP004940-PA	cAMP-dependent protein kinase regulator			1.41	0.028
	AGAP009405-PA	CPAP3-E	24		1.59	0.037
	AGAP005627-PE	Creatine kinase			10.27	0.027
	AGAP003124-PA	Dihydropyrimidinase			2.47	0.047
	AGAP001021-PB	Dihydropyrimidine dehydrogenase			1.32	0.025
	AGAP000513-PB	Dipeptidase E	23		1.81	0.032
	AGAP004394-PA	Dipeptidyl-peptidase III			1.38	0.029
	AGAP013400-PA	Fatty acid-binding protein			1.72	0.021
	AGAP004071-PB	Fimbrin			1.63	0.009
	AGAP006670-PA	Gamma-glutamyl hydrolase	33		1.91	0.049
	AGAP001512-PA	Glutamate-cysteine ligase catalytic subunit			1.4	0.027
	AGAP003077-PB	Glutamyl aminopeptidase		1	1.72	0.05
	AGAP004383-PA	GSTD10			3.07	0.01
	AGAP009191-PA	GSTE6			1.35	0.041
	AGAP003257-PA	GSTU2			2.03	0.035
	AGAP006353-PA	Histidine triad nucleotide binding protein 1			1.58	0.049
	AGAP004747-PA	Ion binding and proteolysis			1.58	0.049
	AGAP012008-PA	Na^+/H^+ exchange regulatory cofactor NHE-RF1			2.05	0.003
	AGAP007700-PA	N-acetylneuraminate lyase			1.33	0.04
	AGAP000500-PD	NADPH-ferrihemoprotein reductase			∞	0.001
	AGAP008305-PC	Phosphoglucomutase			1.14	0.03
	AGAP009172-PA	Prolyl oligopeptidase	15		1.56	0.006
	AGAP004758-PB	Proteasomal ubiquitin receptor adrm1 homolog			1.8	0.047
	AGAP006171-PA	Protein phosphatase			∞	<0.001
	AGAP005929-PA	Pyridoxine kinase			1.46	0.012

	AGAP004093-PA	Sterol carrier protein-2			2.25	0.036
	AGAP003052-PA	Tetratricopeptide repeat-containing protein alpha			1.62	0.029
	AGAP004870-PA	Tripeptidyl-peptidase II			1.46	0.025
	AGAP011872-PA	Ubiquitin-activating enzyme E1			1.61	0.023
	AGAP001056-PA	Ubiquitin-conjugating enzyme E2 L3			1.32	0.029
	AGAP009841-PA	UBX domain-containing protein 1			1.67	0.039
	AGAP009648-PA	Ureidoimidazoline decarboxylase			1.59	0.013
ATP/NAD binding	AGAP003405-PA	Adenylosuccinate synthase			1.28	0.038
	AGAP005981-PA	DnaJ homolog subfamily A			1.87	0.001
	AGAP000970-PA	DnaJ homolog subfamily C			1.26	0.036
	AGAP012010-PA	Fructose-2,6-bisphosphatase			1.23	0.038
	AGAP007699-PC	GTP-binding nuclear protein Ran			1.43	0.002
	AGAP011208-PA	Hexokinase			1.47	0.012
	AGAP001690-PA	Regulating synaptic exocytosis protein 2			∞	0.028
	AGAP003153-PD	V-type proton ATPase catalytic subunit A			1.9	0.006
	AGAP009486-PA	V-type proton transporting ATPase 54 kDa		2	1.97	0.026
	AGAP002884-PA	V-type proton transporting ATPase subunit B			1.76	0.018
	AGAP005845-PA	V-type proton transporting ATPase subunit C			1.41	0.013
	AGAP002401-PA	V-type proton transporting ATPase subunit E			1.41	0.019
Other	AGAP001467-PA	AGAP001467-PA			1.87	0.032
	AGAP013060-PA	AGAP013060-PA	20	1	2.71	0.048
	AGAP011762-PA	BAG domain-containing protein Samui			1.59	0.015
	AGAP010557-PA	B-cell receptor-associated protein 31		3	1.66	0.003
	AGAP005316-PA	Charged multivesicular body protein 4			1.71	0.008
	AGAP010251-PA	Coatomer protein complex alpha subunit			2.37	0.038
	AGAP004625-PB	Cortactin			1.32	0.027
	AGAP010900-PA	Cuticular protein 1 from fifty-one aa family	17		1.72	0.015
	AGAP005997-PA	Cuticular protein RR-1 family	17		1.46	0.021
	AGAP006103-PA	Farnesoic acid o-methyl transferase-like			1.81	0.009
	AGAP009738-PA	Glutaredoxin			1.66	0.015
	AGAP010331-PA	heat shock protein 110kDa			1.39	0.012
	AGAP013228-PA	Heat shock protein 67B2			1.43	0.001
	AGAP000941-PB	Heat shock protein beta-1 isoform x2			1.77	0.012
	AGAP000941-PA	Heat shock protein beta-1 isoform x2			1.76	0.007
	AGAP007310-PA	Klaroid		2	1.59	0.005
	AGAP005291-PA	Lupus la ribonucleoprotein			1.63	0.008
	AGAP003238-PC	N-myc downstream regulated protein			1.62	0.022
	AGAP005369-PA	NOLC1-like isoform x2			3.27	0.015
	AGAP008747-PA	Nsp1p			2.02	0.03
	AGAP008046-PA	PACSIN2			3.91	0.004
	AGAP004310-PA	Perq amino acid-rich protein 2			1.59	0.036
	AGAP012746-PA	Phyhd1 protein			1.58	0.029
	AGAP006946-PA	Prefoldin subunit 4			1.41	0.034
	AGAP003612-PA	Protein CDV3 homolog			1.35	0.013
	AGAP004520-PA	Ran-binding protein 3			1.83	0.007
	AGAP010188-PA	Stress-induced-phosphoprotein 1			1.3	0.037
	AGAP004273-PB	Synapse-associated protein			2.96	0.025
	AGAP000626-PA	Vesicle-associated membrane protein B		1	1.67	0.03

*SP indicates the predicted signal peptide cleavage site, and TMs indicates the predicted number of transmembrane domains.

Table 4. A list of 49 down-regulated proteins

Group	Protein IDs	Protein names	SP*	TMs	IH/CH	p-value
Immunity-related	AGAP009110-PA	GNBP	24		0.52	0.004
	AGAP005663-PA	Chymotrypsin-like protease	34	1	0.61	0.026
	AGAP011792-PA	CLIPA7 homolog	21		0.52	0.011
	AGAP003251-PA	CLIPB1	25		0.5	0.004
	AGAP004855-PA	CLIPB13	22		0.74	0.036
	AGAP003057-PA	CLIPB8	24	1	0.52	0.048
	AGAP005072-PA	Coagulation factor X	14		0.53	0.002
	AGAP006743-PA	Fibrinogen			0.79	0.042
	AGAP004832-PA	LRR-1		1	0.7	0.03
	AGAP007385-PA	Lysozyme 4 (c-type)	31	1	0.37	0.046
	AGAP002857-PB	MDL2	25	1	0.5	0.02
	AGAP002825-PA	PPO1			0.34	0.031
	AGAP006258-PA	PPO2			0.85	0.045
	AGAP004975-PA	PPO3			0.8	0.024
	AGAP008364-PA	TEP15	42		0.73	0.045
Metabolism	AGAP000558-PA	1,2-alpha-mannosidase			0.7	0.047
	AGAP003490-PA	Alanine-glyoxylate aminotransferase			0.64	0.032
	AGAP000862-PA	Alpha 1,3-glucosidase	22		0.78	0.03
	AGAP000679-PA	Aminoacylase			0.76	0.033
	AGAP008783-PA	Arginase			0.57	0.004
	AGAP000985-PA	ARP2 actin-related protein 2 homolog			0.81	0.017
	AGAP000756-PB	Carboxypeptidase M	22		0.75	0.026
	AGAP006726-PA	COEAE5G			0.67	0.02
	AGAP000162-PA	Cystathionine beta-synthase			0.71	0.001
	AGAP002465-PA	Ferritin heavy chain	26		0.61	0.032
	AGAP011107-PA	Glutaredoxin			0.74	0.021
	AGAP008798-PA	Guanine nucleotide exchange factor MSS4			0.16	0.011
	AGAP007237-PA	Heme peroxidase	37		0	0.024
	AGAP009033-PA	Heme peroxidase	18		0.56	0.015
	AGAP001826-PA	Lipophorin			0.38	0.036
	AGAP004654-PA	Phosphoadenylate 3'-nucleotidase			0.39	0.047
	AGAP008096-PA	Sphingomyelin phosphodiesterase	22	1	0.73	0.02
	AGAP000439-PA	Tetrahydrobiopterin dehydratase			0.63	0.02
	AGAP008064-PA	Uroporphyrinogen-III synthase			0.64	0.032
Other	AGAP010846-PA	AGAP010846-PA			0.29	0.036
	AGAP028095-PC	AGAP028095-PC	21		0.47	0.005
	AGAP004108-PB	Amalgam	13		0.59	0.02
	AGAP008052-PA	Chemosensory protein	17		0.61	0.039
	AGAP002822-PA	Condensin-2 complex subunit H2			0.16	0.003
	AGAP008013-PA	Filaggrin-2 isoform x1	18		0.63	0.013
	AGAP001768-PB	Gamma-interferon-inducible protein IP-30	32	1	0.49	0.038
	AGAP001657-PA	Hexamerin	18		0.6	0.039
	AGAP001659-PA	Hexamerin	19		0.69	0.019
	AGAP005471-PA	Muscle M-line assembly protein unc-89			0.7	0.009
	AGAP001127-PA	P37NB protein	24	1	0.42	0.033
	AGAP012057-PA	RNA polymerase-associated protein RTF1			0.45	0.008
	AGAP001989-PA	Secreted salivary gland protein	24	1	0.41	0.013
	AGAP007532-PA	Vinculin			0.7	0.033
	AGAP003095-PA	Yellow protein	21		0.71	0.003

*SP indicates the predicted signal peptide cleavage site, and TMs indicates the predicted number of transmembrane domains.

Table 5. A list of 235 immunity-related proteins

Group	Protein IDs	Protein names	MW (kDa)	SP*	TMs	IH/CH	p-value
AMP	AGAP004632-PA	Defensin	10		1	27.53	0.356
	AGAP007199-PA	Defensin	7	22		2.08	0.341
	AGAP008645-PA	Gambicin	8.8	20	1	2.67	0.089
	AGAP007347-PA	Lysozyme 1 (c-type)	15.3	20		0.76	0.64
	AGAP007345-PA	Lysozyme 3 (c-type)	16.6	18		1.12	0.817
	AGAP007385-PA	Lysozyme 4 (c-type)	17.4	31	1	0.37	0.046
	AGAP007344-PA	Lysozyme 8 (c-type)	16.5	18		13.93	0.356
	AGAP011119-PA	Lysozyme 3	18	21		0.98	0.916
	AGAP000376-PA	Transferrin precursor	69.2	18		1.61	0.055
PPO	AGAP002825-PA	PPO1	79.3			0.34	0.031
	AGAP006258-PA	PPO2	78.1			0.85	0.045
	AGAP004975-PA	PPO3	78.6			0.8	0.024
	AGAP004981-PA	PPO4	78.5			0.84	0.357
	AGAP004977-PA	PPO6	79			1.06	0.827
	AGAP004980-PA	PPO7	79.6			3.66	0.414
	AGAP004976-PA	PPO8	79.3			1.03	0.914
TEP	AGAP010815-PA	TEP1	152.1	21	1	0.4	0.071
	AGAP008654-PA	TEP12	96.3			0.32	0.127
	AGAP008368-PA	TEP14	139.4			0.17	0.091
	AGAP008364-PA	TEP15	163.6	42		0.73	0.045
	AGAP008366-PA	TEP2	154.6			1.51	0.021
	AGAP010812-PA	TEP4	149.4			1	0.993
	AGAP010814-PA	TEP6	151.3	26	1	0.12	0.108
	AGAP010830-PA	TEP9	151.5	21	1	0.31	0.537
PRR	AGAP007036-PA	APL1A	49.4	20		0	0.356
	AGAP007035-PA	APL1B	63.9	20		0.74	0.342
	AGAP007033-PA	APL1C	82.4	22	1	0.55	0.137
	AGAP004811-PA	CTL1	21.8	25	1	0.79	0.402
	AGAP004810-PA	CTL3	20.8	22	1	0.72	0.276
	AGAP005335-PA	CTL4	19.8	24	1	0.28	0.103
	AGAP003625-PA	CTL8	21.5	17		0.99	0.981
	AGAP006430-PB	CTLGA2	24.9	17		1.43	0.396
	AGAP010193-PA	CTLGA3	27	17		1.02	0.929
	AGAP007412-PA	CTLMA1	20.1	24	1	0.57	0.097
	AGAP007411-PA	CTLMA3	19.4	21		0.68	0.075
	AGAP002911-PA	CTLMA9	17.5	22		0.89	0.939
	AGAP010021-PA	Dumpy	172.4			4.71	0.414
	AGAP010024-PA	Dumpy	345.1			3.75	0.411
	AGAP003027-PA	Dumpy-like protein	43.6	31		1.63	0.31
	AGAP009106-PA	GNBP	32.1	26	1	0.53	0.064
	AGAP009110-PA	GNBP	42	24		0.52	0.004
	AGAP009146-PA	GNBP	33.5	31		0.33	0.084
	AGAP006761-PA	GNBPA1	55.7	17	1	0.67	0.176
	AGAP004455-PA	GNBPB1	44.1	24		0.75	0.338
	AGAP002798-PA	GNBPB2	43.7	19		1.07	0.829
	AGAP002799-PA	GNBPB3	43.1	18		146.34	0.356
	AGAP002796-PA	GNBPB4	46.7		1	1.63	0.232
	AGAP006327-PA	LRIM (Short)	39.6	28		0.71	0.095
	AGAP006348-PA	LRIM1	57.3	22		0.56	0.138
	AGAP005693-PA	LRIM17	48.8	21		0.63	0.081
	AGAP007039-PA	LRIM4	59.9	24		0.46	0.172
	AGAP006644-PA	LRR	77	25	1	0.5	0.359
	AGAP011503-PA	LRR	32	23		0.79	0.392
	AGAP005962-PA	LRR shoc-2	90.7	16		0.79	0.242
	AGAP004832-PA	LRR-1	117.8		1	0.7	0.03
	AGAP003878-PA	LRR-15	63.2	19	1	1.34	0.013
	AGAP007030-PA	LRR-7030	115.4	26	1	0	0.356
	AGAP007059-PA	LRR-7059	124			0.79	0.106
	AGAP007060-PA	LRR-7060	132.9	28	1	0.76	0.251
	AGAP009762-PA	Nimrod	141.6	23	1	0.76	0.497
	AGAP001212-PB	PGRPLB	23.3		1	2.24	0.04
	AGAP000536-PA	PGRPS1	22.4	26		0.58	0.078

	AGAP006343-PA	PGRPS2	20	20		0	0.356
	AGAP006342-PA	PGRPS3	20	20		0.98	0.942
SP	AGAP001198-PA	Chymotrypsin	29.4	18		0	0.078
	AGAP011608-PA	Chymotrypsin BI	36.4	22	2	1.38	0.417
	AGAP001365-PA	Chymotrypsin-c-like isoform x1	68.6	21	1	0.68	0.149
	AGAP005663-PA	Chymotrypsin-like protease	33.8	34	1	0.61	0.026
	AGAP005670-PA	Chymotrypsin-like protease	32.2	16		0.74	0.277
	AGAP005671-PA	Chymotrypsin-like protease	32.2	16		0.92	0.709
	AGAP005686-PA	Chymotrypsin-like protease	31.9	18		0.98	0.95
	AGAP007252-PA	Chymotrypsin-like protease	32.9	17		0.74	0.266
	AGAP009121-PA	Chymotrypsin-like protease	27.7	16		0.93	0.785
	AGAP005687-PA	Chymotrypsin-like protease	32.1	18		1.01	0.968
	AGAP006674-PA	Chymotrypsin-like protease	32.4	21		0.86	0.444
	AGAP006675-PA	Chymotrypsin-like protease	32.1	17		0.41	0.214
	AGAP003686-PA	CLIP	39.8			0.83	0.315
	AGAP003251-PA	CLIPB1	40.9	25		0.5	0.004
	AGAP009214-PA	CLIPB11	39.8	25	1	0.95	0.824
	AGAP004855-PA	CLIPB13	44.8	22		0.74	0.036
	AGAP009844-PA	CLIPB15	40.6	22		0.82	0.182
	AGAP003246-PA	CLIPB2	38.4	19		0.35	0.078
	AGAP003249-PA	CLIPB3	40.1	30		0.68	0.087
	AGAP003250-PA	CLIPB4	39.4	24		0.7	0.076
	AGAP004148-PA	CLIPB5	41.3	30		1.02	0.891
	AGAP003057-PA	CLIPB8	44.8	24	1	0.52	0.048
	AGAP013442-PB	CLIPB9	82.9	26	1	0.67	0.054
	AGAP008835-PA	CLIPC1	42.6	25		0.83	0.182
	AGAP000572-PA	CLIPC10	40.9	27	1	0.96	0.821
	AGAP004317-PA	CLIPC2	41.5	27		0.8	0.346
	AGAP004318-PA	CLIPC3	43.1	32	1	0.87	0.296
	AGAP000573-PB	CLIPC4	39.4	22		0.79	0.086
	AGAP000315-PA	CLIPC6	39.6	22	1	0.81	0.316
	AGAP004719-PA	CLIPC9	40.6		1	0.87	0.354
	AGAP002422-PA	CLIPD1	48.5	21	1	0.94	0.581
	AGAP002813-PA	CLIPD6	52.8	19		1.51	0.222
	AGAP001798-PA	Clotting factor C (limulus) homolog	72.6	18	1	1.31	0.464
	AGAP005072-PA	Coagulation factor X	96.4	14		0.53	0.002
	AGAP003960-PA	Coagulation factor XI	64.6	26	1	0.74	0.171
	AGAP012269-PA	Coagulation factor XI	72.3	20	1	1.69	0.139
	AGAP013252-PA	Coagulation factor XI	66.6	26		0.94	0.398
	AGAP001245-PA	Eupolytin	28.7	16		0.74	0.326
	AGAP001246-PA	Eupolytin	30.3	26	1	1.14	0.792
	AGAP001248-PA	Eupolytin	28.9	24		1.12	0.814
	AGAP001249-PA	Eupolytin	27.1	16		0.89	0.666
	AGAP006539-PA	Eupolytin	28.8	18	1	1.54	0.369
	AGAP011920-PA	Eupolytin	26.3	18		0.53	0.11
	AGAP012946-PA	Plasminogen	35.5	24		0.81	0.359
	AGAP013221-PA	Plasminogen	35	24		0.63	0.085
	AGAP006486-PA	Prss3	30.8	22	1	0.9	0.817
	AGAP004566-PA	Serine protease	35.7	20		0.95	0.837
	AGAP006673-PA	Serine protease	33.1	19		0.88	0.535
	AGAP002543-PA	Serine protease	29.8	23		0	0.142
	AGAP011917-PA	Serine protease	26.2	20	1	0.89	0.812
	AGAP001240-PA	Serine protease (thymus-specific)	55.8	16		1.69	0.219
	AGAP005914-PA	Serine protease (thymus-specific)	57		1	1.33	0.372
	AGAP012328-PA	Serine protease 14	36.5			0.77	0.636
	AGAP012614-PA	Serine protease 14	43.4			1.55	0.261
	AGAP013487-PA	Serine protease 14 homolog	34.2	29		1.44	0.331
	AGAP005625-PA	Serine Protease with SR-A	146.8		1	1.03	0.903
	AGAP010240-PA	Trypsin (late)	28.2	17		0.59	0.063
	AGAP006485-PA	Trypsin-alpha	30.5	19		0.91	0.858
	AGAP011427-PA	Trypsin-like protein	96.2			0.39	0.061
	AGAP012022-PA	Trypsin-like protein	97	18		1.41	0.146
	AGAP012504-PA	Trypsin-like protein	93.9			1.28	0.283
	AGAP027981-PA	Trypsin-like protein	98			1.91	0.09
	AGAP007043-PA	Urokinase-type plasminogen activator	59.9			0.68	0.166

SPH	AGAP005642-PA	Chymotrypsin-like protease	33	26	1	0.97	0.916
	AGAP011791-PA	CLIPA1 homolog	48.4	20		0.58	0.112
	AGAP011781-PA	CLIPA12 homolog	40.9	25		0.88	0.458
	AGAP011788-PA	CLIPA14 homolog	30.4	21		0.87	0.34
	AGAP011790-PB	CLIPA2 homolog	55.9	20		0.82	0.508
	AGAP011780-PA	CLIPA4 homolog	45.9	20		0.84	0.473
	AGAP011789-PA	CLIPA6 homolog	45.7	24		0.93	0.561
	AGAP011792-PA	CLIPA7 homolog	80.9	21		0.52	0.011
	AGAP010731-PA	CLIPA8 homolog	40.9	25		0.7	0.248
	AGAP013184-PA	CLIPB36 homolog	42.5		1	0.9	0.573
	AGAP002270-PA	CLIPB7 homolog	43.6	19		1.12	0.741
	AGAP003689-PA	CLIPC7 homolog	67	24	1	0.94	0.695
	AGAP008808-PA	Coagulation factor XI	67.5			1.02	0.784
	AGAP011919-PA	Eupolytin-like	28.1	20		0.55	0.401
	AGAP010730-PA	PPO activating factor homolog	28.2			0.69	0.072
	AGAP005707-PA	Serine collagenase 1 homolog	32.3	21		0.54	0.201
	AGAP005709-PA	Serine collagenase 1 homolog	28.7	17		0.88	0.864
	AGAP006676-PA	Serine collagenase 1 homolog	28.6	16		1.09	0.719
	AGAP004740-PA	Serine collagenase 1 homolog	27.8	19		0.71	0.489
	AGAP005703-PA	Serine collagenase 1 homolog	31.4	21		0	0.356
	AGAP005708-PA	Serine collagenase 1 homolog	29.6	23	1	0	0.165
	AGAP003248-PA	Serine protease 14 like	33.2	26	1	1.02	0.986
	AGAP004638-PA	Serine protease homolog	37.3	30	1	0.79	0.41
	AGAP009216-PA	Serine protease homolog	33.9	16		0	0.356
	AGAP013117-PA	Serine protease homolog	33.5	19	1	0.64	0.105
	AGAP000290-PA	Serine protease homolog	54	25	1	1.07	0.938
	AGAP003691-PA	Serine protease homolog	94.4		1	0.54	0.088
	AGAP011325-PA	Serine protease homolog	34.2			0.76	0.131
	AGAP001708-PA	Serine protease homolog gd-like	30.9	23		0.82	0.44
	AGAP001979-PA	Serine protease homolog with SR-A	226		1	2.21	0.422
	AGAP006677-PA	Trypsin (late) homolog	29.6	18	1	1.01	0.969
	AGAP009122-PA	Trypsin II-P29 like	29.2	21		1.62	0.084
	AGAP006487-PA	Trypsin-alpha like	30.4	19		0.97	0.945
	AGAP012505-PA	Trypsin-like protein	31.5			24.8	0.356
	AGAP008403-PA	Trypsin-like protein	99.3			0.87	0.593
	AGAP013164-PA	Trypsin-like protein	28.2	25		1.18	0.659
	AGAP003626-PA	Vitamin k-dependent protein c	34.5	23		0.71	0.215
Serpin	AGAP006909-PA	SRPN1	47.7	26		1.56	0.234
	AGAP005246-PD	SRPN10B	42.6			1.93	0.044
	AGAP005246-PE	SRPN10D	42.2			124.84	0.356
	AGAP001377-PA	SRPN11	57.1	16		1.04	0.905
	AGAP001375-PA	SRPN12	64.8	15		1.39	0.467
	AGAP009213-PA	SRPN16	61.1	28		0.78	0.314
	AGAP001376-PA	SRPN17	53.7	35	1	1.01	0.939
	AGAP006911-PA	SRPN2	46.5	21		0.79	0.199
	AGAP006910-PA	SRPN3	47.1	22		1.18	0.373
	AGAP009670-PA	SRPN4	68.9	25	1	0.86	0.596
	AGAP009670-PB	SRPN4	61.8	25	1	0.8	0.314
	AGAP007693-PA	SRPN7	44.3	25		0.95	0.792
	AGAP003194-PA	SRPN8	48.8	20		0.88	0.383
	AGAP003139-PA	SRPN9	50.4	28		1.26	0.365
	AGAP006813-PA	TIL domain-containing protein	13.4	22		1.5	0.377
Other	AGAP002585-PA	Cell wall cysteine-rich protein	175.6		1	2.35	0.34
	AGAP004631-PA	Coagulation factor deficiency 2 homolog	26.1	21		1.4	0.553
	AGAP003987-PA	Complement component 1 Q binding protein	29.6			0.48	0.209
	AGAP002878-PA	Cystatin-like protein	11			1.08	0.49
	AGAP011460-PA	Cysteine-rich protein (salivary)	11.2	20		0.39	0.11
	AGAP006253-PA	Cysteine-rich venom protein	9.5	20		1.14	0.761
	AGAP012970-PA	Cysteine-rich venom protein	8.8	18		0.64	0.45
	AGAP011832-PA	Death-associated protein 1	10.3			0.6	0.395
	AGAP008878-PA	Defense protein	17.7	21	1	0.93	0.799
	AGAP010884-PA	Down syndrome cell adhesion molecule A	214.7			0.47	0.645
	AGAP000025-PA	E3 SUMO-protein ligase 2	150.4			0	0.356
	AGAP002982-PA	E3 SUMO-protein ligase RanBP2	308.1			2	0.021
	AGAP010822-PA	Fasciclin	26.3			0.93	0.749

	AGAP010823-PA	Fasciclin isoform c	52.4	23	1	0	0.356
	AGAP011239-PA	FBN7	30.4			0.81	0.468
	AGAP009184-PA	FBN8	35.9	22		0.49	0.146
	AGAP010775-PA	FBN8	23.3			0	0.356
	AGAP011223-PA	FBN8	24.8			0.53	0.067
	AGAP011225-PA	FBN8	34.5	22		0.52	0.084
	AGAP009556-PA	FBN8	22.4	18		0.76	0.161
	AGAP004918-PA	Fibrinogen	35	19		1.03	0.835
	AGAP004996-PA	Fibrinogen	46.8	19		0.7	0.609
	AGAP006743-PA	Fibrinogen	37.4			0.79	0.042
	AGAP006790-PA	Fibrinogen	30.8			0.29	0.165
	AGAP011197-PA	Fibrinogen	32.3			0.98	0.941
	AGAP004917-PA	Fibrinogen-related protein 1	34.2	21		0.83	0.251
	AGAP006914-PA	Fibrinogen-related protein 1	31.3	18		0.84	0.343
	AGAP008797-PA	Immunoglobulin (CD79A) binding protein 1	42.2			14.42	0.356
	AGAP000032-PA	Integrin alpha-ps2 isoform x1	166.7	41	1	1.36	0.279
	AGAP008968-PA	Kazal domain-containing protein	6.5	18		0.88	0.757
	AGAP011482-PA	Kazal domain-containing protein	8.5	22	1	1.25	0.514
	AGAP007629-PB	Laminin gamma 1	179.6	28	1	1.01	0.965
	AGAP004993-PA	Laminin subunit alpha	412.1	23		0.76	0.207
	AGAP002857-PB	MDL2	18.1	25	1	0.5	0.02
	AGAP011319-PA	Pacifastin-related peptide	25.3	17		0.73	0.105
	AGAP008804-PB	Peroxin-19	33.2			0.36	0.25
	AGAP001325-PA	Peroxiredoxin 5, atypical 2-Cys peroxiredoxin	20.6			0.85	0.438
	AGAP004674-PA	Phenoloxidase inhibitor protein	36.3	21		0.92	0.727
	AGAP010477-PB	Phosducin-like 3	26.3			1.25	0.179
	AGAP005531-PA	Programmed cell death 6-interacting protein	94.1			1.26	0.091
	AGAP000378-PA	Programmed cell death protein 4	47.4			1.15	0.857
	AGAP005432-PA	Programmed cell death protein 5	14.8			1.23	0.642
	AGAP003476-PA	Protein BCP1	33.6			0.93	0.772
	AGAP004333-PA	Serine-type endopeptidase inhibitor	173.7			0.27	0.125
	AGAP003012-PA	SP71 isoform A	78.6	25	1	1.42	0.61
	AGAP000305-PA	SPARC	22.2			1.16	0.579
	AGAP011765-PA	Spondin-1	87	31		0.81	0.403
	AGAP003338-PA	Thioredoxin	15.5			0.32	0.384
	AGAP007201-PA	Thioredoxin	15.6			1.27	0.468
	AGAP009584-PA	Thioredoxin	12.1			0.64	0.119
	AGAP000396-PA	Thioredoxin peroxidase	26			0.82	0.433
	AGAP011054-PA	Thioredoxin peroxidase	22			1.09	0.209
	AGAP011824-PA	Thioredoxin peroxidase	25			0.95	0.703
	AGAP005462-PA	Thioredoxin-like protein 1	31.6			0.89	0.528
	AGAP001613-PA	Thioredoxin-related transmembrane protein 1	38.9	22	1	1.38	0.455
	AGAP003615-PA	Toll-interacting protein	30.4			1.42	0.516

*SP indicates the predicted signal peptide cleavage site, and TMs indicates the predicted number of transmembrane domains.

Table 6. Possible proteolysis and post-translational modifications

Proteins	MW (kDa)	RA*	Slice	1	2	3	4	5	6	7	8	9	10	11	12
			MW	500	350	250	230	140	80	70	45	30	22	20	15
AGAP004993-PA Laminin subunit alpha	412.1	0.9	CH	0	0	0	0	0	44	24	16	3	0	14	0
		0.7	IH	0	0	0	0	0	30	27	23	13	0	7	0
AGAP010024-PA Dumpy	345.1	1.2	CH	0	4	0	0	28	14	12	16	4	5	5	13
		10.8	IH	0	0	0	0	9	7	5	47	17	5	7	3
AGAP002982-PA E3 SUMO-protein ligase RanBP2	308.1	0.5	CH	0	0	0	0	0	0	0	60	40	0	0	0
		1.5	IH	0	0	0	0	0	0	8	54	21	10	7	0
AGAP001979-PA Serine protease homolog with SR-A	226	0.1	CH	0	0	0	0	0	0	0	32	23	46	0	0
		0.5	IH	0	0	0	0	0	0	0	35	6	46	13	0
AGAP007629-PB Laminin gamma 1	179.6	0.2	CH	0	0	0	0	0	0	47	21	32	0	0	0
		0.1	IH	0	0	0	0	0	47	0	53	0	0	0	0
AGAP002585-PA Cell wall cysteine-rich protein	175.6	0.1	CH	0	0	0	0	0	0	0	0	100	0	0	0
		0.2	IH	0	0	0	0	0	0	0	0	100	0	0	0
AGAP004333-PA Serine-type endopeptidase inhibitor	173.7	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	100	0	0	0	0
AGAP010021-PA Dumpy	172.4	0.8	CH	0	0	0	0	0	14	12	31	20	10	12	0
		6.8	IH	0	0	0	0	1	3	6	41	26	15	8	0
AGAP000032-PA Integrin alpha-ps2 isoform x1	166.7	0.8	CH	0	0	0	0	0	0	0	3	31	18	48	0
		1.5	IH	0	0	0	0	0	0	0	0	27	32	41	0
AGAP000025-PA E3 SUMO-protein ligase 2	150.4	0	CH	0	0	0	0	0	0	0	0	0	0	0	100
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP009762-PA Nimrod	141.6	40.2	CH	0	0	0	0	0	0	11	47	16	15	8	3
		66.2	IH	0	0	0	0	0	0	14	25	23	24	10	4
AGAP005072-PA Coagulation factor X	96.4	2.3	CH	0	0	0	13	11	17	15	40	2	3	0	0
		1.1	IH	0	0	0	27	0	12	5	53	3	0	0	0
AGAP011427-PA Trypsin-like protein	96.2	1.2	CH	0	0	0	0	0	0	0	39	19	0	42	0
		0.7	IH	0	0	0	0	0	27	0	32	41	0	0	0
AGAP004975-PA PPO3	78.6	0.5	CH	0	0	0	0	0	5	10	70	15	0	0	0
		1.2	IH	8	9	0	7	0	1	8	46	8	5	3	5
AGAP006644-PA LRR	77	0.2	CH	0	0	0	0	0	0	0	71	29	0	0	0
		0.2	IH	0	0	0	0	0	0	0	40	60	0	0	0
AGAP001798-PA Clotting factor C (limulus) homolog	72.6	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	0	0	0	100	0
	64.8	103.6	CH	0	0	0	1	1	1	2	7	16	31	37	4

AGAP001375-PA SRPN12		191.9	IH	1	0	0	1	0	0	1	5	17	28	42	4
AGAP002799-PA GNBPB3	43.1	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	100	0	0	0	0	0	0
AGAP005246-PE SRPN10D	42.2	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	100	0	0	0	0	0	0
AGAP010731-PA CLIPA8 homolog	40.9	18.6	CH	3	3	1	2	4	6	4	10	5	8	5	50
		15.5	IH	2	3	1	3	4	6	5	8	6	8	4	51
AGAP003686-PA CLIP	39.8	0.4	CH	0	0	0	0	0	0	0	34	7	0	0	59
		0.6	IH	0	0	0	0	0	0	0	5	0	0	0	95
AGAP012328-PA Serine protease 14	36.5	0.9	CH	0	0	0	0	0	0	0	10	0	0	0	90
		0.8	IH	0	0	0	0	0	0	0	7	0	0	0	93
AGAP004674-PA Phenoloxidase inhibitor protein	36.3	176.4	CH	0	0	0	0	1	0	0	2	5	7	49	34
		180.1	IH	0	0	0	0	0	0	0	4	5	7	54	28
AGAP005707-PA Serine collagenase 1 homolog	32.3	5.5	CH	3	2	0	3	4	0	1	3	8	57	17	3
		3.2	IH	7	4	1	4	0	0	0	5	13	59	7	0
AGAP006486-PA Prss3	30.8	0.1	CH	0	0	0	0	0	0	0	82	18	0	0	0
		0	IH	0	0	0	0	0	100	0	0	0	0	0	0
AGAP011920-PA Eupolytin	26.3	0.4	CH	0	0	0	0	0	0	5	0	19	0	69	7
		0.6	IH	0	0	0	0	0	9	21	0	20	0	49	0
AGAP011319-PA Pacifastin-related peptide	25.3	207.9	CH	0	0	0	0	0	0	0	1	4	19	63	14
		166.5	IH	0	0	0	0	0	0	0	1	4	15	58	21
AGAP004810-PA CTL3	20.8	23	CH	0	0	0	0	4	0	1	3	6	5	9	71
		28.7	IH	0	0	0	0	9	1	2	4	6	4	16	59
AGAP006343-PA PGRPS2	20	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP007344-PA Lysozyme 8 (c-type)	16.5	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP006813-PA TIL domain-containing protein	13.4	0.2	CH	0	0	0	0	0	0	0	0	100	0	0	0
		0.4	IH	0	0	0	0	0	0	0	0	100	0	0	0

*RA stands for relative abundance, it's represented as [protein abundance * 10000/total protein abundance of CH or IH]. Molecular weight under each gel slice indicates the upper limit of each slice. The values of each protein in each slice is the percentage of abundance out of the protein's total abundance in CH or IH, so 12 slices of each protein adds up to 100%. Red boxes indicate the calculated positions of the proteins.

Table 7. Possible serpin-protease complexes

Proteins	MW (kDa)	Relative abundance*	Slice	1	2	3	4	5	6	7	8	9	10	11	12
			MW	500	350	250	230	140	80	70	45	30	22	20	15
AGAP001376-PA SRPN17	53.7	3.6	CH	0	3	0	8	5	13	24	33	9	0	2	3
		5.1	IH	0	4	0	9	0	13	25	25	11	3	0	10
AGAP002813-PA CLIPD6	52.8	2.2	CH	0	4	7	8	12	18	14	16	4	0	5	12
		3.2	IH	3	7	13	8	13	7	15	16	3	4	2	9
AGAP003139-PA SRPN9	50.4	157.2	CH	3	3	4	5	11	14	28	29	2	0	1	1
		166.4	IH	2	2	3	4	13	17	44	11	2	1	1	1
AGAP003194-PA SRPN8	48.8	106.5	CH	7	10	6	18	13	10	28	3	1	0	1	1
		79.4	IH	9	13	8	22	12	8	20	3	1	2	1	1
AGAP006909-PA SRPN1	47.7	3.2	CH	0	0	0	14	10	20	28	29	0	0	0	0
		4	IH	4	8	0	11	5	15	40	17	0	0	0	0
AGAP006910-PA SRPN3	47.1	40.8	CH	5	4	4	7	7	10	18	20	19	2	2	3
		47.2	IH	5	4	4	8	6	11	26	11	18	2	3	3
AGAP006911-PA SRPN2	46.5	142	CH	3	3	4	8	7	19	25	24	2	0	1	3
		128.1	IH	3	4	4	10	4	22	34	11	3	2	1	2
AGAP003057-PA CLIPB8	44.8	88	CH	5	5	4	9	6	9	12	29	8	4	5	7
		72.3	IH	5	6	4	10	6	12	14	17	8	7	6	6
AGAP007693-PA SRPN7	44.3	23.2	CH	5	5	4	11	12	10	13	31	3	2	4	1
		26	IH	7	9	6	15	9	7	31	11	2	1	1	0
AGAP005246-PD SRPN10B	42.6	5	CH	8	7	6	10	7	10	15	8	5	0	19	4
		6.2	IH	7	6	5	9	8	10	8	6	6	3	27	3
AGAP008835-PA CLIPC1	42.6	29	CH	2	3	3	6	8	15	23	33	2	2	2	1
		68.5	IH	2	3	3	5	6	20	30	27	1	1	1	1
AGAP005246-PE SRPN10D	42.2	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	100	0	0	0	0	0	0
AGAP004317-PA CLIPC2	41.5	4.2	CH	4	3	1	7	9	9	11	19	7	7	5	19
		3.3	IH	4	2	0	13	11	14	0	16	8	5	5	22
AGAP004148-PA CLIPB5	41.3	9.3	CH	6	8	2	10	7	11	6	15	4	1	4	25
		11.2	IH	8	12	10	13	7	7	6	9	4	0	4	21
AGAP003251-PA CLIPB1	40.9	8.6	CH	6	5	6	9	14	13	21	25	0	0	0	0
		7.2	IH	10	9	8	15	12	9	19	17	0	2	0	0
AGAP004719-PA CLIPC9	40.6	1.8	CH	0	4	17	16	36	23	4	0	0	0	0	0
		2.1	IH	5	10	21	15	27	16	7	0	0	0	0	0
AGAP009214-PA CLIPB11	39.8	11.2	CH	7	7	9	6	12	15	16	6	9	6	4	4
		10.5	IH	9	8	10	8	9	17	17	7	9	7	0	1
AGAP003250-PA CLIPB4	39.4	12.1	CH	4	5	2	6	10	12	8	29	10	2	3	9
		11.8	IH	6	8	4	10	11	11	7	27	6	2	2	5
AGAP000573-PB CLIPC4	39.4	396.3	CH	7	6	7	10	9	15	10	13	4	7	5	8
		330.7	IH	7	7	7	11	8	13	11	10	4	8	5	10
AGAP003246-PA CLIPB2	38.4	1.5	CH	0	0	0	7	9	4	8	55	4	0	1	11
		0.4	IH	0	0	0	16	0	35	5	45	0	0	0	0

*Relative abundance is represented as [protein abundance * 10000/total protein abundance of CH or IH]. Molecular weight under each gel slice indicates the upper limit of each slice. The values of each protein in each slice is the percentage of abundance out of the protein's total abundance in CH or IH, so 12 slices of each protein adds up to 100%. Red boxes indicate the calculated positions of the proteins.

Table 8. Proteins of possible high M_r immune complexes

Proteins	MW (kDa)	Relative abundance	Slice MW	1 500	2 350	3 250	4 230	5 140	6 80	7 70	8 45	9 30	10 22	11 20	12 15
AGAP008366-PA TEP2	154.6	0.2	CH	58	34	0	0	8	0	0	0	0	0	0	0
		0.4	IH	57	32	8	2	0	0	0	0	0	0	0	0
AGAP010830-PA TEP9	151.5	0.2	CH	0	100	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP010814-PA TEP6	151.3	1.3	CH	30	0	45	0	0	0	11	9	5	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP008368-PA TEP14	139.4	0.2	CH	38	53	0	8	0	0	0	0	0	0	0	0
		0.1	IH	57	43	0	0	0	0	0	0	0	0	0	0
AGAP007060-PA LRR-7060	132.9	241.6	CH	17	30	22	22	6	2	1	0	0	0	0	0
		199.2	IH	20	36	23	16	4	1	1	0	0	0	0	0
AGAP007059-PA LRR-7059	124	387.9	CH	13	27	18	31	4	2	2	1	1	0	0	0
		383.5	IH	14	34	20	25	3	1	2	1	1	0	0	0
AGAP004832-PA LRR-1	117.8	49.8	CH	17	28	21	28	4	1	1	0	0	0	0	0
		38.8	IH	21	31	23	21	2	0	0	0	0	0	0	0
AGAP008403-PA Trypsin-like protein	99.3	6	CH	18	28	18	12	17	1	4	2	0	0	0	0
		4.8	IH	22	31	24	10	9	1	3	0	0	0	0	0
AGAP027981-PA Trypsin-like protein	98	1.1	CH	14	25	12	14	20	0	0	8	0	0	7	0
		2	IH	19	24	15	15	6	0	0	14	3	0	4	0
AGAP012022-PA Trypsin-like protein	97	0.3	CH	0	31	0	32	37	0	0	0	0	0	0	0
		0.4	IH	16	53	0	0	32	0	0	0	0	0	0	0
AGAP008654-PA TEP12	96.3	3	CH	29	33	15	9	5	2	4	1	1	0	0	0
		0.7	IH	36	41	14	5	0	0	4	0	0	0	0	0
AGAP003691-PA Serine protease homolog	94.4	35.4	CH	13	24	16	13	21	6	3	2	1	0	0	2
		16.1	IH	18	25	19	12	14	7	2	1	1	1	0	0
AGAP012504-PA Trypsin-like protein	93.9	2.9	CH	10	19	9	13	23	2	8	9	5	0	0	0
		4.3	IH	15	25	15	12	14	2	1	14	2	0	0	0
AGAP005962-PA LRR shoc-2	90.7	2.6	CH	11	23	22	22	21	0	0	0	0	0	0	0
		2.1	IH	19	31	24	21	6	0	0	0	0	0	0	0
AGAP004980-PA PPO7	79.6	0.1	CH	0	0	61	39	0	0	0	0	0	0	0	0
		1.2	IH	15	15	60	11	0	0	0	0	0	0	0	0
AGAP002825-PA PPO1	79.3	7	CH	6	12	15	16	8	20	10	5	4	4	0	0
		2	IH	0	28	23	13	0	24	10	2	0	0	0	0
AGAP004976-PA PPO8	79.3	28.8	CH	5	14	19	22	14	13	4	3	4	0	2	1
		35.3	IH	8	16	34	26	5	3	1	1	5	1	0	0
AGAP004977-PA PPO6	79	45.9	CH	11	11	25	12	10	16	5	3	2	0	1	4
		57.6	IH	12	13	31	11	9	9	4	3	2	2	1	5
AGAP003012-PA SP71 isoform A	78.6	1531.9	CH	7	9	21	10	8	24	6	4	3	3	3	2
		1367.7	IH	10	11	23	11	6	16	5	4	3	5	3	2
AGAP004981-PA PPO4	78.5	33.4	CH	7	9	19	10	9	19	8	5	4	4	5	3
		26.9	IH	11	14	28	15	7	9	5	3	2	4	2	1
AGAP006258-PA PPO2	78.1	907.4	CH	11	10	17	9	8	21	6	4	3	3	5	3
		826.7	IH	12	12	18	11	7	14	5	4	3	4	6	4
AGAP012269-PA Coagulation factor XI	72.3	0	CH	0	100	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	100	0	0	0	0	0	0	0	0	0	0
AGAP008808-PA Coagulation factor XI	67.5	0.3	CH	0	0	0	100	0	0	0	0	0	0	0	0
		1	IH	72	0	28	0	0	0	0	0	0	0	0	0
AGAP013252-PA Coagulation factor XI	66.6	6.8	CH	9	7	18	13	13	17	9	5	4	0	4	2
		8	IH	9	10	27	12	11	8	6	4	3	3	6	3
AGAP003960-PA Coagulation factor XI	64.6	21.8	CH	17	16	14	18	13	4	11	3	3	0	0	1
		21	IH	15	17	27	18	9	3	5	2	2	2	0	0
AGAP007035-PA APL1B	63.9	0.8	CH	0	0	0	46	14	9	30	0	0	0	0	0
		0.5	IH	0	0	0	85	0	0	15	0	0	0	0	0
AGAP006761-PA GNBPA1	55.7	0.2	CH	0	12	0	64	24	0	0	0	0	0	0	0
		0.1	IH	0	0	25	75	0	0	0	0	0	0	0	0
AGAP006743-PA Fibrinogen	37.4	0.9	CH	0	25	0	29	45	0	0	0	0	0	0	0
		0.6	IH	0	0	0	52	48	0	0	0	0	0	0	0

*Relative abundance is represented as [protein abundance * 10000/total protein abundance of CH or IH]. Molecular weight under each gel slice indicates the upper limit of each slice. The values of each protein in each slice is the percentage of abundance out of the protein's total abundance in CH or IH, so 12 slices of each protein adds up to 100%. Red boxes indicate the calculated positions of the proteins.

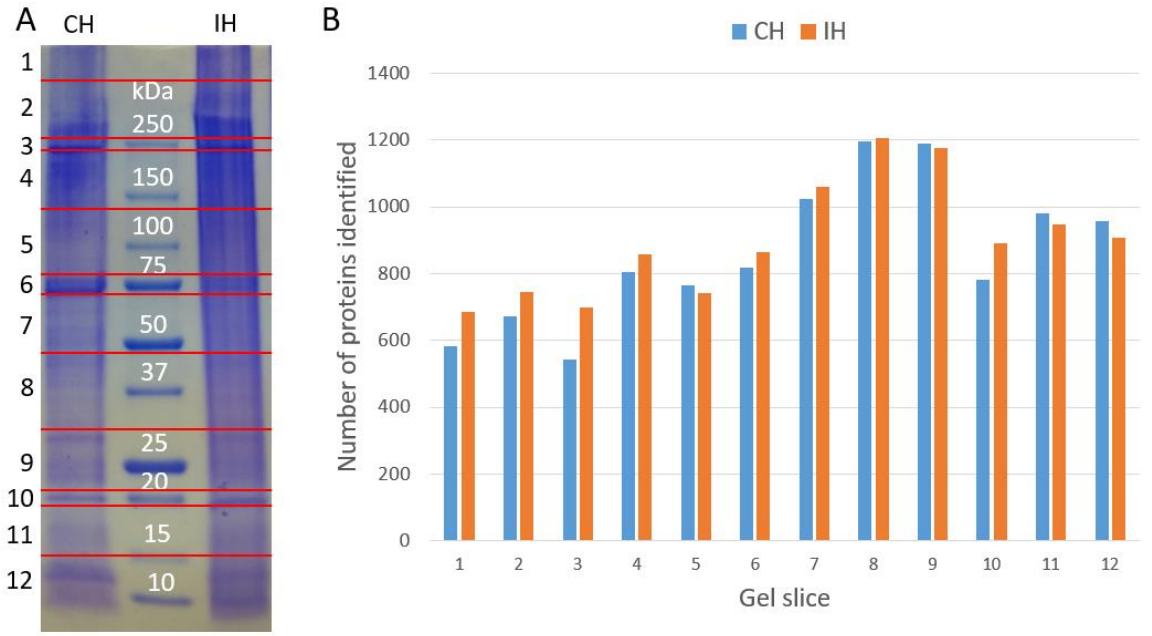

Figure 1. Gel cutting and proteins identified in each gel slice. (A) shows representative lanes of CH and IH. 40 μg total protein of each biological replicate was loaded to each lane and electrophoresed in 4-15% gradient gel. After staining and destaining, each lane was divided into 12 slices, with the molecular mass range of 500-350, 350-250, 250-230, 230-140, 140-80, 80-70, 70-45, 45-30, 30-22, 22-20, 20-15, 15-0kDa respectively. (B) shows the number of proteins identified (LFQ intensity not zero) in each gel slice of CH and IH. CH: 583, 674, 544, 806, 765, 817, 1023, 1197, 1189, 781, 981, 958 proteins from slice 1 to 12. IH: 687, 744, 698, 859, 742, 864, 1061, 1206, 1178, 890, 949, 907 correspondingly.

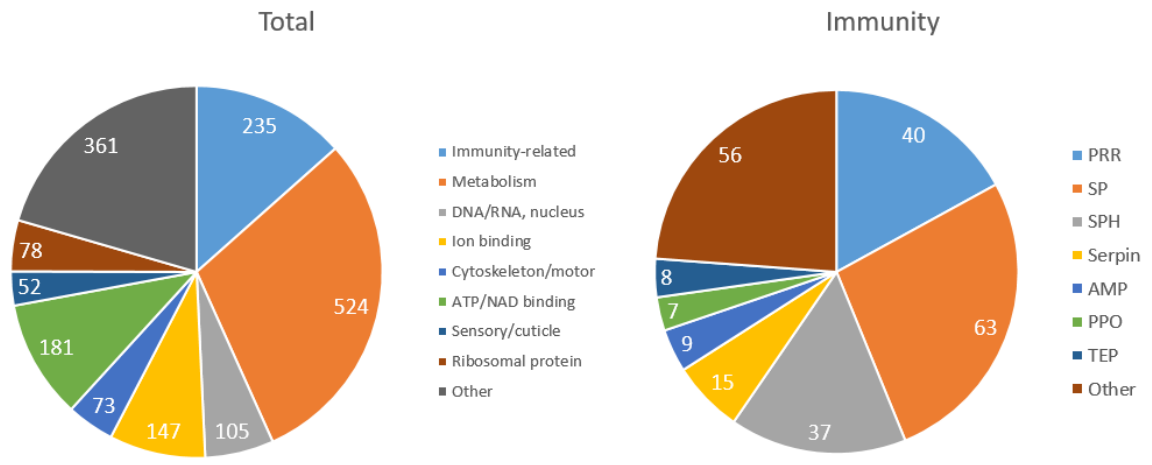

Figure 2. Composition of total and immunity-related proteins. The total 1756 proteins are grouped into 9 categories and their numbers are indicated in (A). The 235 immunity-related proteins are grouped into 6 categories and their numbers are indicated in (B).

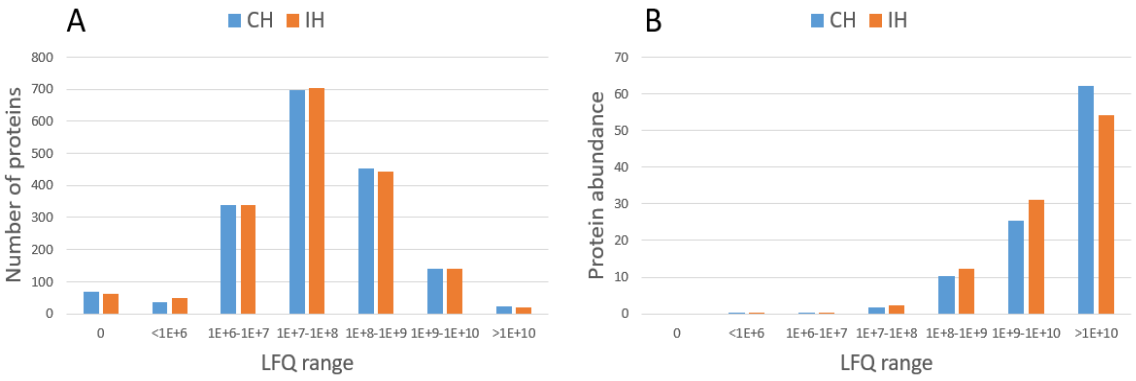

Figure 3. Distribution of protein number and abundance. (A) Distribution of protein numbers within each LFQ range. (B) shows the relative abundance of all proteins within each LFQ range. The total protein abundance of CH and IH are normalized to 100%.

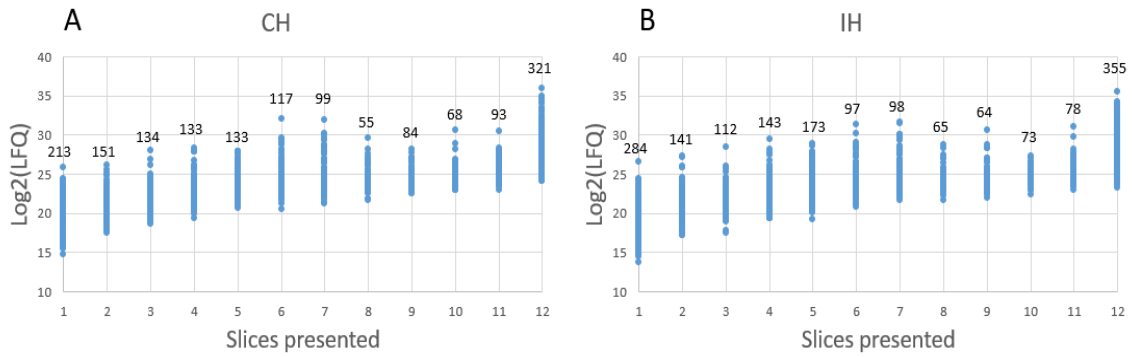

Figure 4. Correlation between protein abundance and slices presented. Correlation between protein abundance and the number of gel slices the protein is presented are indicated in (A) for CH and (B) for IH, respectively. Protein abundance is represented as the logarithm of LFQ intensity with base 2. The number of proteins within each group is indicated above each dot line.

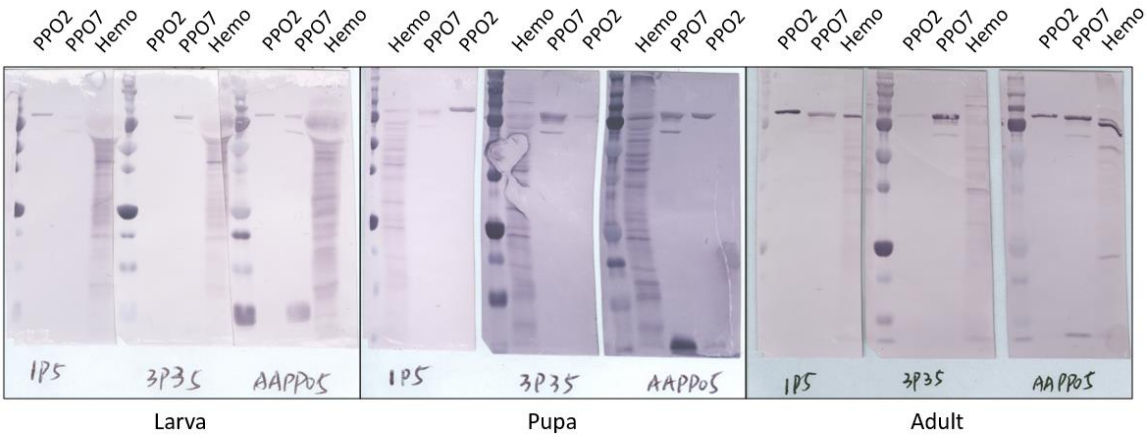

Figure 5. Western blot of hemolymph samples using monoclonal antibodies. Purified recombinant PPO2 and PPO7 (400 ng), and 7 µg total hemolymph protein of *A. gambiae* larva, pupa, adult were used. Membranes were blocked with 3% BSA in TBS, probed with the primary antibodies (1P5, 3P35, or AaPPO5 at 1ng/µl) and secondarily with GAM-AP (for 1P5 or 3P35) or GAR-AP (for AaPPO5) at 1:1000 dilution. Antibodies are all in 1% BSA containing TBS, and 3P35 antibody was always pre-mixed with BSA for 1 h to eliminate its cross-reactivity with BSA on the membrane.

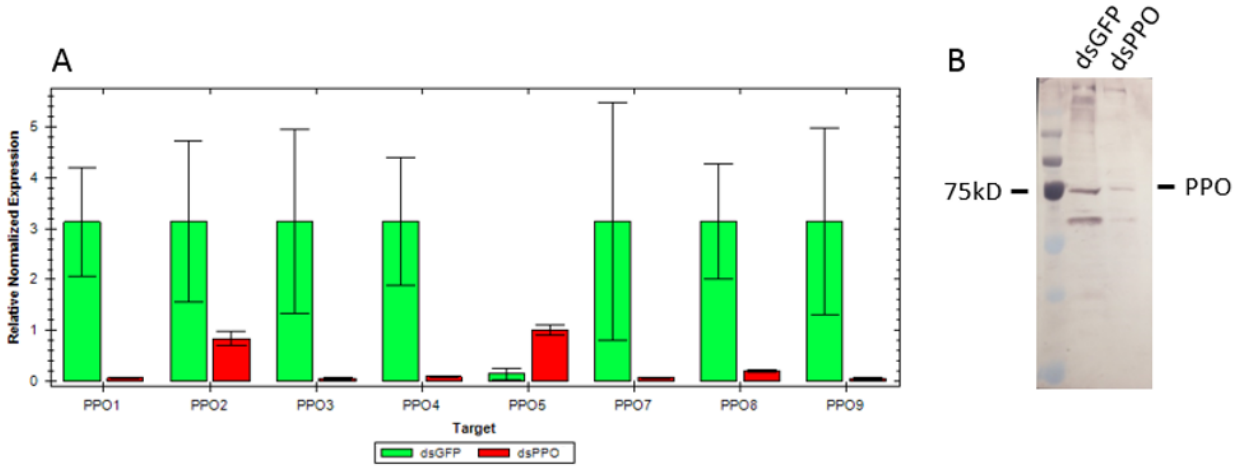

Figure 6. RNA knockdown efficiency at mRNA and protein level. (A) Total RNA was isolated from 5 female mosquitoes at 24 h after dsRNA injection. Then reverse transcription was performed to generate first strand cDNA, and mRNA level of each PPO gene was examined by qPCR using specific primers (Table 2, Appendices). PPO6 is omitted because of non-pure melting curves. (B) Hemolymph of 15 female mosquitoes was extracted 4 days post dsRNA injection. 3ug total protein was used for western blot analysis with anti- *Aedes aegypti* PPO5 primary antibody (1:1000) and GAR-AP secondary antibody (1:1000). PPO is around 80 kDa, and the lower band is probably PO since no PTU is added.

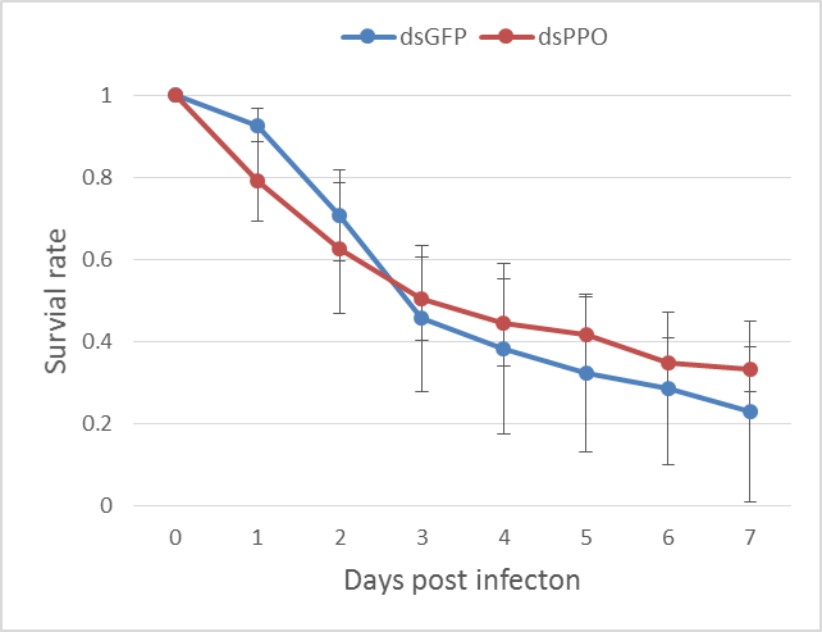

Figure 7. Mosquito survival upon *E. coli* infection after PPO knockdown. *A. gambiae* female adults within 1-2 days after emergence were injected with dsRNA targeting PPO or GFP (control). Then a 4-day interval was allowed for full recovery of mosquitoes before *E. coli* challenge was introduced. Survival rate was recorded for the following 7 days with dead individuals daily counted and removed. Brown dots represent dsPPO injected mosquitoes, and blue dots represent dsGFP. Result here is a summary of three independent replicates, each carried out with ~40 female mosquitoes per treatment group.

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APPENDICES

Table 1. Primers for qRT-PCR targeting PPO genes

Primers	Sequences
Actin-F*	5’- CACACCGTCCCAATCTATGAAGGTTATG -3’
Actin-R	5’- CTTCTCCTTGATGTCACGGACAATTTCAC -3’
AgPPO1-F	5’- AAGAACAAGCTGCCACCGTACA -3’
AgPPO1-R	5’- CAGATGGGTGAACCTTGCAAACAC -3’
AgPPO2-F	5’- ACAAAGATGCGCTGGCTCAGTT -3’
AgPPO2-R	5’- ACGGTGCGTTAGGGTCAAGTTC -3’
AgPPO3-F	5’- CACCGTTAACCTAAACCCTGGTACG -3’
AgPPO3-R	5’- CGTGCTTGGCTCGTTGATGTTAGA -3’
AgPPO4-F	5’- CGTCACACTTAATGCCGGTGCTAAT -3’
AgPPO4-R	5’- TTCGGCCAACCACAGTTACAGAAC -3’
AgPPO5-F	5’- GCCTACACAAGCTCGGAACTTTCA -3’
AgPPO5-R	5’- TCGAACTGTGATCGCTGCCAAA -3’
AgPPO6-F	5’- TCGTAAACAATCAAAGGGATCGGATCACAA -3’
AgPPO6-R	5’- GCTCACCACGGCGATCCTTATT -3’
AgPPO7-F	5’- GTGAATTTAACACCTGGCATTAACAACATC -3’
AgPPO7-R	5’- GCAGAAGCGGAAATTTGCATCAC -3’
AgPPO8-F	5’- TACGATGAGAATGCTGGGTGCGAT -3’
AgPPO8-R	5’- GTCACAGTCGCCAATCGCATGTTT -3’
AgPPO9-F	5’- CGTCAAGTTACATCCTGGCGATAA -3’
AgPPO9-R	5’- ACAGATCGAACGGTTGACCATC -3’

*F for forward primer and R for reverse primer.

Table 2. Peptides for PPO monoclonal antibody generation

Proteins	Epitope sequences	Proteins	Epitope sequences
PPO2	ELLTPYTAEQLGNPG	Common*	QSSVTIPYERTFRN
PPO2	TAEQLGNPGVTVNSV	Common	KDRRGELFYYMHQQL
PPO2	SVGVQLSRPNTPANV	Common	LLTFWQRSQVDLGTG
PPO2	VEVNNESGAVRKGTL	PPO6	SSEADTRIAVRATTL
PPO2	AIGTKSAPTDKDALA	PPO6	EGAVVNNQRDRITID
PPO2	TDFEQDSVAQELDPN	PPO6	NANQIGYAGVQIQSF
PPO2	TLADFVTPNSNMKTA	PPO6	MALSNINLPETEQFR
PPO2	NSNMKTATVQVKFNN	PPO6	SDFTRPNSNMTNIEV
PPO8	GPNSPASSQVSNDTG	PPO7	SAAAAAPAGTSADTP
PPO8	SNDTGVPPTVVTIKD	PPO7	DTPTMNRVSLNNIPD
PPO8	AGFAVSDDGVRVPLD	PPO7	VSLNNIPDPDIKFAE
PPO8	TMAELSNSNVTLEAL	PPO7	NPGVNLLSLETELDR
PPO8	ETQLDRAGGAVNSFV	PPO7	LETELDRRDSVKNTL
PPO8	LRINSTARSNRQDTV	PPO7	LQVAYSGTAKPATLR
PPO8	GNVEQANAGNAQSRF	PPO7	TFRNVANTNIGDANF
PPO8	FEDDNANVNYDENAG	PPO7	HEQDRVNPLFDERTD

*Common for consensus regions of PPO proteins.

Table 3. Gel distribution of immunity-related proteins

Proteins	MW (kDa)	RA*	Slice	1	2	3	4	5	6	7	8	9	10	11	12
			MW	500	350	250	230	140	80	70	45	30	22	20	15
AGAP004993-PA Laminin subunit alpha	412.1	0.9	CH	0	0	0	0	0	44	24	16	3	0	14	0
		0.7	IH	0	0	0	0	0	30	27	23	13	0	7	0
AGAP010024-PA Dumpy	345.1	1.2	CH	0	4	0	0	28	14	12	16	4	5	5	13
		10.8	IH	0	0	0	0	9	7	5	47	17	5	7	3
AGAP002982-PA E3 SUMO-protein ligase RanBP2	308.1	0.5	CH	0	0	0	0	0	0	0	60	40	0	0	0
		1.5	IH	0	0	0	0	0	0	8	54	21	10	7	0
AGAP001979-PA Serine protease homolog with SR-A	226	0.1	CH	0	0	0	0	0	0	0	32	23	46	0	0
		0.5	IH	0	0	0	0	0	0	0	35	6	46	13	0
AGAP010884-PA Down syndrome cell adhesion molecule A	214.7	0.7	CH	0	100	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP007629-PB Laminin gamma 1	179.6	0.2	CH	0	0	0	0	0	0	47	21	32	0	0	0
		0.1	IH	0	0	0	0	0	47	0	53	0	0	0	0
AGAP002585-PA Cell wall cysteine-rich protein	175.6	0.1	CH	0	0	0	0	0	0	0	0	100	0	0	0
		0.2	IH	0	0	0	0	0	0	0	0	100	0	0	0
AGAP004333-PA Serine-type endopeptidase inhibitor	173.7	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	100	0	0	0	0
AGAP010021-PA Dumpy	172.4	0.8	CH	0	0	0	0	0	14	12	31	20	10	12	0
		6.8	IH	0	0	0	0	1	3	6	41	26	15	8	0
AGAP000032-PA Integrin alpha-ps2 isoform x1	166.7	0.8	CH	0	0	0	0	0	0	0	3	31	18	48	0
		1.5	IH	0	0	0	0	0	0	0	0	27	32	41	0
	163.6	1128.1	CH	9	12	16	13	12	25	4	3	2	1	2	2

AGAP008364-PA TEP15		921.4	IH	12	15	20	13	8	14	5	3	2	3	2	3
AGAP008366-PA TEP2	154.6	0.2	CH	58	34	0	0	8	0	0	0	0	0	0	0
		0.4	IH	57	32	8	2	0	0	0	0	0	0	0	0
AGAP010815-PA TEP1	152.1	60.3	CH	18	16	20	16	16	6	2	1	2	0	1	2
		22.7	IH	21	18	30	15	8	2	2	1	2	0	0	2
AGAP010830-PA TEP9	151.5	0.2	CH	0	100	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP010814-PA TEP6	151.3	1.3	CH	30	0	45	0	0	0	11	9	5	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP000025-PA E3 SUMO-protein ligase 2	150.4	0	CH	0	0	0	0	0	0	0	0	0	0	0	100
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP010812-PA TEP4	149.4	16.3	CH	15	13	28	9	19	11	2	1	0	0	0	1
		16.7	IH	18	19	25	15	13	5	2	1	0	1	0	1
AGAP005625-PA Serine Protease with SR-A	146.8	8.5	CH	4	6	5	11	9	3	8	20	10	3	9	12
		13	IH	5	8	7	9	11	3	8	17	14	3	6	9
AGAP009762-PA Nimrod	141.6	40.2	CH	0	0	0	0	0	0	11	47	16	15	8	3
		66.2	IH	0	0	0	0	0	0	14	25	23	24	10	4
AGAP008368-PA TEP14	139.4	0.2	CH	38	53	0	8	0	0	0	0	0	0	0	0
		0.1	IH	57	43	0	0	0	0	0	0	0	0	0	0
AGAP007060-PA LRR-7060	132.9	241.6	CH	17	30	22	22	6	2	1	0	0	0	0	0
		199.2	IH	20	36	23	16	4	1	1	0	0	0	0	0
AGAP007059-PA LRR-7059	124	387.9	CH	13	27	18	31	4	2	2	1	1	0	0	0
		383.5	IH	14	34	20	25	3	1	2	1	1	0	0	0
AGAP004832-PA LRR-1	117.8	49.8	CH	17	28	21	28	4	1	1	0	0	0	0	0
		38.8	IH	21	31	23	21	2	0	0	0	0	0	0	0
AGAP007030-PA LRR-7030	115.4	0	CH	0	0	0	100	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP008403-PA Trypsin-like protein	99.3	6	CH	18	28	18	12	17	1	4	2	0	0	0	0
		4.8	IH	22	31	24	10	9	1	3	0	0	0	0	0
AGAP027981-PA Trypsin-like protein	98	1.1	CH	14	25	12	14	20	0	0	8	0	0	7	0
		2	IH	19	24	15	15	6	0	0	14	3	0	4	0
AGAP012022-PA Trypsin-like protein	97	0.3	CH	0	31	0	32	37	0	0	0	0	0	0	0
		0.4	IH	16	53	0	0	32	0	0	0	0	0	0	0
AGAP005072-PA Coagulation factor X	96.4	2.3	CH	0	0	0	13	11	17	15	40	2	3	0	0
		1.1	IH	0	0	0	27	0	12	5	53	3	0	0	0
AGAP008654-PA TEP12	96.3	3	CH	29	33	15	9	5	2	4	1	1	0	0	0
		0.7	IH	36	41	14	5	0	0	4	0	0	0	0	0
AGAP011427-PA Trypsin-like protein	96.2	1.2	CH	0	0	0	0	0	0	0	39	19	0	42	0
		0.7	IH	0	0	0	0	0	27	0	32	41	0	0	0
AGAP003691-PA Serine protease homolog	94.4	35.4	CH	13	24	16	13	21	6	3	2	1	0	0	2
		16.1	IH	18	25	19	12	14	7	2	1	1	1	0	0
AGAP005531-PA Programmed cell death 6-interacting protein	94.1	0.6	CH	0	7	0	32	61	0	0	0	0	0	0	0
		1.4	IH	8	26	28	15	23	0	0	0	0	0	0	0
AGAP012504-PA Trypsin-like protein	93.9	2.9	CH	10	19	9	13	23	2	8	9	5	0	0	0
		4.3	IH	15	25	15	12	14	2	1	14	2	0	0	0
AGAP005962-PA LRR shoc-2	90.7	2.6	CH	11	23	22	22	21	0	0	0	0	0	0	0
		2.1	IH	19	31	24	21	6	0	0	0	0	0	0	0
AGAP011765-PA Spondin-1	87	12.9	CH	10	11	7	6	10	6	7	9	7	10	9	7
		11.7	IH	10	9	7	6	4	3	5	13	13	15	10	5
AGAP013442-PB CLIPB9	82.9	31.3	CH	5	4	2	5	5	6	6	24	5	25	9	5
		25.9	IH	3	4	3	6	4	8	8	22	5	20	10	5
AGAP007033-PA APL1C	82.4	103.6	CH	7	9	14	9	15	13	3	3	5	8	8	6
		83.4	IH	8	11	13	10	10	5	2	3	8	16	10	3
AGAP011792-PA CLIPA7 homolog	80.9	99.4	CH	8	10	10	11	18	7	8	7	10	4	4	3
		75.4	IH	9	11	8	11	12	9	9	9	6	7	5	5

AGAP004980-PA PPO7	79.6	0.1	CH	0	0	61	39	0	0	0	0	0	0	0	0
		1.2	IH	15	15	60	11	0	0	0	0	0	0	0	0
AGAP002825-PA PPO1	79.3	7	CH	6	12	15	16	8	20	10	5	4	4	0	0
		2	IH	0	28	23	13	0	24	10	2	0	0	0	0
AGAP004976-PA PPO8	79.3	28.8	CH	5	14	19	22	14	13	4	3	4	0	2	1
		35.3	IH	8	16	34	26	5	3	1	1	5	1	0	0
AGAP004977-PA PPO6	79	45.9	CH	11	11	25	12	10	16	5	3	2	0	1	4
		57.6	IH	12	13	31	11	9	9	4	3	2	2	1	5
AGAP004975-PA PPO3	78.6	0.5	CH	0	0	0	0	0	5	10	70	15	0	0	0
		1.2	IH	8	9	0	7	0	1	8	46	8	5	3	5
AGAP003012-PA SP71 isoform A	78.6	1531.9	CH	7	9	21	10	8	24	6	4	3	3	3	2
		1367.7	IH	10	11	23	11	6	16	5	4	3	5	3	2
AGAP004981-PA PPO4	78.5	33.4	CH	7	9	19	10	9	19	8	5	4	4	5	3
		26.9	IH	11	14	28	15	7	9	5	3	2	4	2	1
AGAP006258-PA PPO2	78.1	907.4	CH	11	10	17	9	8	21	6	4	3	3	5	3
		826.7	IH	12	12	18	11	7	14	5	4	3	4	6	4
AGAP006644-PA LRR	77	0.2	CH	0	0	0	0	0	0	0	71	29	0	0	0
		0.2	IH	0	0	0	0	0	0	0	40	60	0	0	0
AGAP001798-PA Clotting factor C (limulus) homlog	72.6	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	0	0	0	100	0
AGAP012269-PA Coagulation factor XI	72.3	0	CH	0	100	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	100	0	0	0	0	0	0	0	0	0	0
AGAP000376-PA Transferrin precursor	69.2	278.7	CH	9	9	16	10	13	18	11	5	4	1	2	3
		546.5	IH	10	10	21	9	10	15	8	5	5	3	2	2
AGAP009670-PA SRPN4	68.9	22	CH	4	4	2	10	8	6	24	17	4	2	2	17
		24.6	IH	4	5	3	11	7	5	21	24	4	1	1	14
AGAP001365-PA Chymotrypsin-c-like isoform x1	68.6	1.2	CH	0	0	0	14	13	13	10	33	18	0	0	0
		0.7	IH	0	7	0	23	0	0	18	42	10	0	0	0
AGAP008808-PA Coagulation factor XI	67.5	0.3	CH	0	0	0	100	0	0	0	0	0	0	0	0
		1	IH	72	0	28	0	0	0	0	0	0	0	0	0
AGAP003689-PA CLIPC7 homolog	67	18	CH	7	11	10	12	7	15	16	16	2	0	2	2
		20.3	IH	11	13	13	11	5	11	12	18	2	1	0	2
AGAP013252-PA Coagulation factor XI	66.6	6.8	CH	9	7	18	13	13	17	9	5	4	0	4	2
		8	IH	9	10	27	12	11	8	6	4	3	3	6	3
AGAP001375-PA SRPN12	64.8	103.6	CH	0	0	0	1	1	1	2	7	16	31	37	4
		191.9	IH	1	0	0	1	0	0	1	5	17	28	42	4
AGAP003960-PA Coagulation factor XI	64.6	21.8	CH	17	16	14	18	13	4	11	3	3	0	0	1
		21	IH	15	17	27	18	9	3	5	2	2	2	0	0
AGAP007035-PA APL1B	63.9	0.8	CH	0	0	0	46	14	9	30	0	0	0	0	0
		0.5	IH	0	0	0	85	0	0	15	0	0	0	0	0
AGAP003878-PA LRR-15	63.2	3.5	CH	3	6	6	10	6	22	37	9	0	0	0	0
		6.5	IH	6	7	11	12	9	30	16	5	0	4	0	0
AGAP009670-PB SRPN4	61.8	111.1	CH	2	2	3	4	7	4	23	7	4	5	9	30
		133.8	IH	2	2	2	4	6	3	20	6	5	6	17	28
AGAP009213-PA SRPN16	61.1	109.7	CH	3	4	4	8	8	7	18	5	3	6	18	15
		125.4	IH	3	4	5	8	5	6	14	5	4	5	26	15
AGAP007043-PA Urokinase-type plasminogen activator	59.9	0.8	CH	0	6	18	18	21	17	20	0	0	0	0	0
		0.3	IH	20	18	23	18	0	0	20	0	0	0	0	0
AGAP007039-PA LRIM4	59.9	10.9	CH	12	11	14	16	15	8	10	7	6	0	1	0
		9.5	IH	12	11	21	12	17	6	7	10	4	2	0	0
AGAP006348-PA LRIM1	57.3	125.4	CH	7	7	6	11	16	8	25	5	4	2	5	3
		98.4	IH	7	7	9	13	19	12	12	4	6	3	5	2
AGAP001377-PA SRPN11	57.1	164.1	CH	3	4	4	7	8	7	38	15	6	3	2	2
		181.8	IH	3	3	4	6	5	9	44	13	6	3	2	2
AGAP005914-PA Serine protease (thymus-specific)	57	1.2	CH	0	7	0	31	14	15	34	0	0	0	0	0
		2.5	IH	8	17	15	26	14	0	20	0	0	0	0	0

AGAP011790-PB CLIPA2 homolog	55.9	62.7	CH	2	3	4	3	13	14	8	10	5	6	19	12
		71.6	IH	2	2	3	3	8	16	9	14	5	5	18	16
AGAP001240-PA Serine protease (thymus-specific)	55.8	1.1	CH	27	33	20	16	3	0	0	0	0	0	0	0
		2.1	IH	26	26	14	17	16	0	0	0	0	0	0	0
AGAP006761-PA GNBPA1	55.7	0.2	CH	0	12	0	64	24	0	0	0	0	0	0	0
		0.1	IH	0	0	25	75	0	0	0	0	0	0	0	0
AGAP000290-PA Serine protease homolog	54	0.1	CH	0	0	0	0	0	0	0	70	30	0	0	0
		0	IH	0	0	0	0	0	0	0	100	0	0	0	0
AGAP001376-PA SRPN17	53.7	3.6	CH	0	3	0	8	5	13	24	33	9	0	2	3
		5.1	IH	0	4	0	9	0	13	25	25	11	3	0	10
AGAP002813-PA CLIPD6	52.8	2.2	CH	0	4	7	8	12	18	14	16	4	0	5	12
		3.2	IH	3	7	13	8	13	7	15	16	3	4	2	9
AGAP010823-PA Fasciclin isoform c	52.4	0.1	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP003139-PA SRPN9	50.4	157.2	CH	3	3	4	5	11	14	28	29	2	0	1	1
		166.4	IH	2	2	3	4	13	17	44	11	2	1	1	1
AGAP007036-PA APL1A	49.4	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP005693-PA LRIM17	48.8	5.5	CH	7	6	7	11	16	14	31	5	2	0	0	2
		6	IH	9	9	9	14	22	15	19	2	0	2	0	0
AGAP003194-PA SRPN8	48.8	106.5	CH	7	10	6	18	13	10	28	3	1	0	1	1
		79.4	IH	9	13	8	22	12	8	20	3	1	2	1	1
AGAP002422-PA CLIPD1	48.5	3.2	CH	8	7	4	9	17	9	12	14	0	0	13	6
		2.9	IH	12	11	10	15	25	3	14	7	0	3	0	0
AGAP011791-PA CLIPA1 homolog	48.4	36.8	CH	6	6	7	10	11	8	21	8	3	2	11	8
		29.3	IH	6	5	8	11	12	10	13	8	3	3	13	9
AGAP006909-PA SRPN1	47.7	3.2	CH	0	0	0	14	10	20	28	29	0	0	0	0
		4	IH	4	8	0	11	5	15	40	17	0	0	0	0
AGAP000378-PA Programmed cell death protein 4	47.4	1.2	CH	0	7	4	11	22	6	12	28	11	0	0	0
		2	IH	7	5	11	10	21	7	8	17	8	5	0	0
AGAP006910-PA SRPN3	47.1	40.8	CH	5	4	4	7	7	10	18	20	19	2	2	3
		47.2	IH	5	4	4	8	6	11	26	11	18	2	3	3
AGAP004996-PA Fibrinogen	46.8	3.5	CH	5	3	0	5	9	6	21	9	3	3	4	31
		3.7	IH	7	4	2	6	8	2	17	6	5	6	7	30
AGAP002796-PA GNBPB4	46.7	0.8	CH	8	11	7	23	28	0	0	24	0	0	0	0
		2.1	IH	16	14	15	21	25	0	0	10	0	0	0	0
AGAP006911-PA SRPN2	46.5	142	CH	3	3	4	8	7	19	25	24	2	0	1	3
		128.1	IH	3	4	4	10	4	22	34	11	3	2	1	2
AGAP011780-PA CLIPA4 homolog	45.9	87.7	CH	5	4	4	6	7	6	19	10	9	15	7	7
		93.6	IH	6	5	4	8	8	9	16	12	8	12	7	7
AGAP011789-PA CLIPA6 homolog	45.7	315	CH	6	6	5	9	9	7	18	22	10	3	2	4
		377.6	IH	7	7	6	11	8	9	18	18	7	4	3	3
AGAP004855-PA CLIPB13	44.8	17.7	CH	11	9	8	16	16	10	18	6	5	0	0	1
		15.9	IH	12	12	11	20	16	6	10	5	3	3	0	2
AGAP003057-PA CLIPB8	44.8	88	CH	5	5	4	9	6	9	12	29	8	4	5	7
		72.3	IH	5	6	4	10	6	12	14	17	8	7	6	6
AGAP007693-PA SRPN7	44.3	23.2	CH	5	5	4	11	12	10	13	31	3	2	4	1
		26	IH	7	9	6	15	9	7	31	11	2	1	1	0
AGAP004455-PA GNBPB1	44.1	326.2	CH	7	7	5	13	13	9	14	17	3	3	7	2
		307.3	IH	9	9	6	15	13	9	18	6	4	4	5	3
AGAP002798-PA GNBPB2	43.7	13.2	CH	10	10	7	11	12	9	10	21	4	1	1	4
		18.4	IH	12	10	8	13	10	10	8	17	3	2	0	6
AGAP003027-PA Dumpy-like protein	43.6	4.4	CH	0	12	3	14	19	4	8	16	3	0	14	7
		7.1	IH	4	10	10	9	18	3	7	9	1	1	18	11
AGAP002270-PA CLIPB7 homolog	43.6	1.5	CH	0	0	0	0	0	0	0	14	21	31	34	0
		2.5	IH	0	0	0	0	0	0	0	16	25	25	30	4
AGAP012614-PA Serine protease 14	43.4	6.7	CH	0	0	3	6	3	9	10	53	12	1	1	3
		15.2	IH	1	1	1	7	4	7	5	50	15	5	3	1
AGAP002799-PA GNBPB3	43.1	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	100	0	0	0	0	0	0
AGAP004318-PA CLIPC3	43.1	6	CH	5	5	4	16	9	8	11	27	7	0	4	3
		6.7	IH	6	7	9	18	15	6	13	18	5	1	2	2

AGAP005246-PD SRPN10B	42.6	5	CH	8	7	6	10	7	10	15	8	5	0	19	4
		6.2	IH	7	6	5	9	8	10	8	6	6	3	27	3
AGAP008835-PA CLIPC1	42.6	29	CH	2	3	3	6	8	15	23	33	2	2	2	1
		68.5	IH	2	3	3	5	6	20	30	27	1	1	1	1
AGAP013184-PA CLIPB36 homolog	42.5	1.5	CH	0	0	0	0	0	9	24	25	12	0	19	10
		1.3	IH	0	0	0	0	0	0	24	32	6	0	38	0
AGAP008797-PA Immunoglobulin (CD79A) binding protein 1	42.2	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP005246-PE SRPN10D	42.2	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	100	0	0	0	0	0	0
AGAP009110-PA GNBP	42	27	CH	4	3	3	4	6	3	12	4	5	7	17	34
		19.9	IH	4	3	6	5	7	5	5	8	6	9	23	20
AGAP004317-PA CLIPC2	41.5	4.2	CH	4	3	1	7	9	9	11	19	7	7	5	19
		3.3	IH	4	2	0	13	11	14	0	16	8	5	5	22
AGAP004148-PA CLIPB5	41.3	9.3	CH	6	8	2	10	7	11	6	15	4	1	4	25
		11.2	IH	8	12	10	13	7	7	6	9	4	0	4	21
AGAP011781-PA CLIPA12 homolog	40.9	10	CH	11	9	7	13	13	9	13	12	12	0	0	0
		10.5	IH	12	10	8	14	14	9	12	12	7	2	0	0
AGAP010731-PA CLIPA8 homolog	40.9	18.6	CH	3	3	1	2	4	6	4	10	5	8	5	50
		15.5	IH	2	3	1	3	4	6	5	8	6	8	4	51
AGAP003251-PA CLIPB1	40.9	8.6	CH	6	5	6	9	14	13	21	25	0	0	0	0
		7.2	IH	10	9	8	15	12	9	19	17	0	2	0	0
AGAP000572-PA CLIPC10	40.9	2.2	CH	0	0	0	0	0	3	30	37	13	0	16	0
		2.7	IH	0	0	0	0	0	3	27	39	11	5	15	0
AGAP009844-PA CLIPB15	40.6	4.8	CH	3	5	4	13	8	12	10	29	3	0	1	12
		5.4	IH	8	8	4	16	11	9	9	16	1	0	1	16
AGAP004719-PA CLIPC9	40.6	1.8	CH	0	4	17	16	36	23	4	0	0	0	0	0
		2.1	IH	5	10	21	15	27	16	7	0	0	0	0	0
AGAP003249-PA CLIPB3	40.1	15.4	CH	6	4	5	7	7	8	7	32	8	5	6	6
		13.9	IH	4	4	4	7	8	11	8	28	6	7	6	6
AGAP009214-PA CLIPB11	39.8	11.2	CH	7	7	9	6	12	15	16	6	9	6	4	4
		10.5	IH	9	8	10	8	9	17	17	7	9	7	0	1
AGAP003686-PA CLIP	39.8	0.4	CH	0	0	0	0	0	0	0	34	7	0	0	59
		0.6	IH	0	0	0	0	0	0	0	5	0	0	0	95
AGAP006327-PA LRIM (Short)	39.6	4.6	CH	3	8	4	17	18	14	24	12	0	0	0	0
		4.3	IH	6	15	5	24	5	14	22	3	2	3	0	0
AGAP000315-PA CLIPC6	39.6	8.8	CH	0	0	0	0	0	7	15	28	10	9	14	16
		10	IH	0	0	0	0	0	1	15	20	13	14	19	18
AGAP003250-PA CLIPB4	39.4	12.1	CH	4	5	2	6	10	12	8	29	10	2	3	9
		11.8	IH	6	8	4	10	11	11	7	27	6	2	2	5
AGAP000573-PB CLIPC4	39.4	396.3	CH	7	6	7	10	9	15	10	13	4	7	5	8
		330.7	IH	7	7	7	11	8	13	11	10	4	8	5	10
AGAP001613-PA Thioredoxin-related transmembrane protein 1	38.9	0.3	CH	0	0	0	0	0	0	0	16	84	0	0	0
		0.8	IH	0	0	0	0	0	0	0	28	60	12	0	0
AGAP003246-PA CLIPB2	38.4	1.5	CH	0	0	0	7	9	4	8	55	4	0	1	11
		0.4	IH	0	0	0	16	0	35	5	45	0	0	0	0
AGAP006743-PA Fibrinogen	37.4	0.9	CH	0	25	0	29	45	0	0	0	0	0	0	0
		0.6	IH	0	0	0	52	48	0	0	0	0	0	0	0
AGAP004638-PA Serine protease homolog	37.3	19.4	CH	9	7	6	13	6	11	18	15	3	2	3	5
		17	IH	6	7	4	16	6	25	11	8	2	5	3	7
AGAP012328-PA Serine protease 14	36.5	0.9	CH	0	0	0	0	0	0	0	10	0	0	0	90
		0.8	IH	0	0	0	0	0	0	0	7	0	0	0	93
AGAP011608-PA Chymotrypsin BI	36.4	0.2	CH	0	0	0	0	0	0	23	23	54	0	0	0
		0.1	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP004674-PA Phenoloxidase inhibitor protein	36.3	176.4	CH	0	0	0	0	1	0	0	2	5	7	49	34
		180.1	IH	0	0	0	0	0	0	0	4	5	7	54	28
AGAP009184-PA FBN8	35.9	41.1	CH	10	8	9	15	13	7	10	20	2	1	3	2
		24.4	IH	11	8	10	18	17	9	6	15	1	2	1	1
	35.7	0.7	CH	0	0	0	0	0	6	19	56	20	0	0	0

AGAP004566-PA Serine protease		0.8	IH	0	0	0	0	0	11	19	55	16	0	0	0
AGAP012946-PA Plasminogen	35.5	18.1	CH	5	5	4	9	11	7	8	29	5	9	4	4
		21.8	IH	5	7	5	9	9	10	8	22	5	11	2	4
AGAP004918-PA Fibrinogen	35	7.8	CH	6	5	1	9	8	5	8	38	9	5	5	2
		5.3	IH	5	6	4	14	10	7	9	28	6	5	4	2
AGAP013221-PA Plasminogen	35	1.7	CH	2	7	6	16	19	18	7	23	2	0	0	0
		2.1	IH	14	16	6	27	14	5	7	12	0	0	0	0
AGAP011225-PA FBN8	34.5	175	CH	5	4	4	7	9	10	11	25	9	5	9	2
		175.4	IH	4	4	4	8	9	12	13	23	6	5	7	3
AGAP003626-PA Vitamin k-dependent protein c	34.5	4	CH	10	1	4	11	9	14	15	32	4	0	0	0
		2.2	IH	0	5	4	15	8	19	19	28	0	0	0	3
AGAP004917-PA Fibrinogen-related protein 1	34.2	4.4	CH	0	0	0	2	7	7	9	21	10	19	12	12
		4.3	IH	0	0	0	5	0	5	7	18	11	18	18	18
AGAP011325-PA Serine protease homolog	34.2	5.4	CH	0	3	0	9	5	8	12	53	4	0	3	3
		14	IH	5	4	1	10	7	9	11	40	3	1	2	7
AGAP013487-PA Serine protease 14 homlog	34.2	4.1	CH	2	5	0	10	13	13	15	32	9	0	1	1
		4.8	IH	5	6	4	11	14	12	14	25	7	1	0	0
AGAP009216-PA Serine protease homolog	33.9	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP005663-PA Chymotrypsin-like protease	33.8	38.4	CH	3	2	2	4	4	4	5	5	32	26	6	6
		29.4	IH	4	3	3	6	4	3	4	7	32	20	7	8
AGAP003476-PA Protein BCP1	33.6	1.2	CH	0	0	0	0	0	0	19	54	13	0	14	0
		2.1	IH	0	0	0	3	4	16	25	25	28	0	0	0
AGAP009146-PA GNBP	33.5	2.6	CH	6	8	0	13	9	9	14	36	2	0	4	0
		2.2	IH	7	12	4	19	11	11	14	21	0	0	0	0
AGAP013117-PA Serine protease homolog	33.5	10.3	CH	0	0	0	2	3	1	1	63	9	7	11	0
		3.9	IH	0	0	0	3	3	2	4	64	6	7	11	0
AGAP008804-PB Peroxin-19	33.2	0.2	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	100	0	0	0	0
AGAP003248-PA Serine protease 14 like	33.2	0.1	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	100	0	0	0	0
AGAP006673-PA Serine protease	33.1	0	CH	0	0	0	0	0	0	0	0	100	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP005642-PA Chymotrypsin-like protease	33	2.6	CH	12	2	0	6	7	4	10	13	12	12	20	2
		2.1	IH	9	6	0	8	4	12	23	13	10	12	3	0
AGAP007252-PA Chymotrypsin-like protease	32.9	2.8	CH	0	0	0	0	0	10	13	18	53	0	2	5
		2.6	IH	0	0	0	0	0	8	19	15	56	0	0	3
AGAP006674-PA Chymotrypsin-like protease	32.4	62.3	CH	4	3	3	7	5	6	9	10	36	5	4	8
		72.4	IH	5	3	3	7	5	7	11	11	33	5	3	6
AGAP011197-PA Fibrinogen	32.3	6.7	CH	7	8	4	13	12	5	11	22	15	1	0	1
		8.7	IH	8	8	7	12	12	7	11	15	16	3	0	1
AGAP005707-PA Serine collagenase 1 homolog	32.3	5.5	CH	3	2	0	3	4	0	1	3	8	57	17	3
		3.2	IH	7	4	1	4	0	0	0	5	13	59	7	0
AGAP005671-PA Chymotrypsin-like protease	32.2	186.4	CH	6	4	4	7	8	8	8	8	30	7	5	5
		251.7	IH	5	4	5	9	9	9	10	8	27	5	4	4
AGAP005670-PA Chymotrypsin-like protease	32.2	66.9	CH	5	3	4	7	6	7	8	8	32	11	5	4
		72.6	IH	6	5	4	8	7	9	9	8	28	9	4	3
AGAP009106-PA GNBP	32.1	0.5	CH	0	0	0	0	0	0	0	34	66	0	0	0
		0.2	IH	0	0	0	0	0	0	34	34	31	0	0	0
	32.1	0.6	CH	0	0	0	6	0	0	0	10	62	8	14	0

AGAP005687-PA Chymotrypsin-like protease		0.7	IH	0	0	0	5	0	0	27	40	17	11	0	0
AGAP006675-PA Chymotrypsin-like protease	32.1	23.6	CH	4	6	3	9	4	5	6	35	8	5	11	4
		16	IH	4	9	7	12	9	3	7	25	4	8	12	1
AGAP011503-PA LRR	32	1.9	CH	0	0	0	15	13	6	14	31	21	0	0	0
		1.4	IH	0	7	0	16	0	0	23	50	5	0	0	0
AGAP005686-PA Chymotrypsin-like protease	31.9	5.8	CH	6	5	4	11	11	7	11	13	28	1	2	2
		8.3	IH	7	6	6	14	10	9	8	13	22	2	2	0
AGAP005462-PA Thioredoxin-like protein 1	31.6	0.9	CH	0	0	0	0	0	0	19	56	17	0	0	8
		0.8	IH	0	0	0	0	0	16	30	47	7	0	0	0
AGAP012505-PA Trypsin-like protein	31.5	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	0	100	0	0	0	0
AGAP005703-PA Serine collagenase 1 homolog	31.4	0	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP006914-PA Fibrinogen-related protein 1	31.3	4.8	CH	9	12	0	16	13	11	13	13	12	0	0	0
		3.3	IH	9	14	11	30	8	8	15	4	0	0	0	0
AGAP001708-PA Serine protease homolog gd-like	30.9	5.9	CH	0	3	0	5	4	5	9	23	18	8	12	14
		5.8	IH	0	0	0	5	2	6	11	24	15	7	17	13
AGAP006790-PA Fibrinogen	30.8	2	CH	8	6	4	21	0	0	13	15	28	0	5	0
		2.6	IH	15	8	6	22	0	0	8	6	28	0	7	0
AGAP006486-PA Prss3	30.8	0.1	CH	0	0	0	0	0	0	0	82	18	0	0	0
		0	IH	0	0	0	0	0	100	0	0	0	0	0	0
AGAP006485-PA Trypsin-alpha	30.5	39.2	CH	5	5	4	9	9	7	11	38	7	1	3	3
		47.1	IH	6	6	4	14	9	7	14	26	7	2	3	2
AGAP011239-PA FBN7	30.4	150.1	CH	8	7	6	10	13	7	11	20	7	6	4	2
		188.8	IH	9	8	7	10	15	10	11	17	5	5	4	2
AGAP003615-PA Toll-interacting protein	30.4	0.1	CH	0	0	0	0	0	0	0	16	84	0	0	0
		0.3	IH	0	0	0	0	0	0	0	41	59	0	0	0
AGAP011788-PA CLIPA14 homolog	30.4	31.9	CH	6	5	4	12	8	6	10	11	26	4	2	8
		34.9	IH	7	7	5	14	8	8	13	8	19	5	2	5
AGAP006487-PA Trypsin-alpha like	30.4	4.6	CH	14	10	6	12	20	11	8	17	1	0	0	0
		4.9	IH	16	10	10	15	21	13	7	4	4	0	0	0
AGAP001246-PA Eupolytin	30.3	2	CH	0	0	0	11	0	8	9	13	55	2	2	0
		4.7	IH	0	3	2	6	4	6	8	18	35	6	8	4
AGAP002543-PA Serine protease	29.8	0.1	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP003987-PA Complement component 1 Q binding protein	29.6	3.6	CH	0	0	0	0	0	0	4	8	53	7	13	15
		2.8	IH	0	0	0	0	0	3	14	20	36	3	14	10
AGAP006677-PA Trypsin (late) homolog	29.6	0.4	CH	0	0	0	0	0	0	0	7	7	75	11	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP005708-PA Serine collagenase 1 homolog	29.6	1.6	CH	3	2	0	6	7	10	6	9	28	4	17	9
		2.2	IH	7	9	2	8	8	7	5	13	22	4	7	8
AGAP001198-PA Chymotrypsin	29.4	0.2	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP009122-PA Trypsin II-P29 like	29.2	2.7	CH	0	4	0	17	4	3	11	10	16	30	2	2
		3.6	IH	4	8	2	30	2	1	15	2	4	32	0	0
AGAP001248-PA Eupolytin	28.9	0.7	CH	0	0	0	14	14	0	0	12	48	0	6	6
		1.2	IH	11	0	0	15	6	0	6	16	28	6	0	12
AGAP006539-PA Eupolytin	28.8	2.6	CH	5	3	4	10	6	9	11	19	25	3	6	0
		4.1	IH	9	11	7	14	7	7	7	21	12	2	4	0
AGAP001245-PA Eupolytin	28.7	16.5	CH	0	2	0	8	0	3	7	12	55	0	0	14
		18.7	IH	2	0	0	10	0	0	3	10	56	0	0	19
	28.7	0	CH	0	0	0	0	0	0	0	100	0	0	0	0

AGAP005709-PA Serine collagenase 1 homolog		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP006676-PA Serine collagenase 1 homolog	28.6	32.7	CH	6	5	5	12	11	7	12	9	24	1	6	2
		37.6	IH	8	8	7	17	11	7	12	6	14	3	6	2
AGAP010240-PA Trypsin (late)	28.2	24.7	CH	7	10	8	11	17	9	7	13	16	0	1	1
		32.6	IH	8	12	9	17	12	5	15	11	9	1	0	1
AGAP010730-PA PPO activating factor homolog	28.2	11	CH	12	6	5	8	6	7	11	7	27	4	5	2
		8.6	IH	11	8	5	11	10	2	4	12	27	6	2	2
AGAP013164-PA Trypsin-like protein	28.2	52.6	CH	6	6	6	11	12	11	15	24	4	1	2	1
		51.8	IH	8	10	8	15	11	10	15	15	5	3	2	0
AGAP011919-PA Eupolytin-like	28.1	24.3	CH	4	2	2	3	4	3	3	4	40	4	10	21
		16.3	IH	6	3	2	6	4	2	6	4	37	6	7	17
AGAP004740-PA Serine collagenase 1 homolog	27.8	2.1	CH	0	0	0	9	0	10	15	17	39	0	1	9
		0.9	IH	0	0	0	39	0	6	28	0	26	0	0	0
AGAP009121-PA Chymotrypsin-like protease	27.7	3.4	CH	0	5	0	17	3	2	13	7	32	0	6	15
		3.1	IH	4	8	3	31	0	3	17	2	22	3	0	7
AGAP001249-PA Eupolytin	27.1	109.4	CH	4	4	3	10	3	8	8	9	25	15	7	4
		100.9	IH	5	5	4	15	2	7	14	6	18	14	7	3
AGAP010193-PA CTLGA3	27	12	CH	0	0	0	3	1	1	5	10	72	1	4	4
		17.4	IH	0	2	0	4	0	1	4	11	71	1	2	4
AGAP010822-PA Fasciclin	26.3	41.7	CH	5	4	4	6	5	5	8	8	34	6	4	10
		33.5	IH	6	5	3	9	5	7	9	8	26	8	3	10
AGAP010477-PB Phosducin-like 3	26.3	20.3	CH	5	5	4	7	10	11	12	21	6	3	7	9
		27	IH	6	5	4	8	7	11	15	14	6	4	12	8
AGAP011920-PA Eupolytin	26.3	0.4	CH	0	0	0	0	0	0	5	0	19	0	69	7
		0.6	IH	0	0	0	0	0	9	21	0	20	0	49	0
AGAP011917-PA Serine protease	26.2	0.8	CH	0	0	0	0	0	0	7	13	67	0	0	13
		0.7	IH	0	0	0	0	0	0	0	20	80	0	0	0
AGAP004631-PA Coagulation factor deficiency 2 homolog	26.1	0.1	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	100	0	0	0	0
AGAP000396-PA Thioredoxin peroxidase	26	2.1	CH	0	0	0	10	2	0	0	13	39	8	9	20
		2.3	IH	0	4	0	11	0	0	12	17	28	8	3	18
AGAP011319-PA Pacifastin-related peptide	25.3	207.9	CH	0	0	0	0	0	0	0	1	4	19	63	14
		166.5	IH	0	0	0	0	0	0	0	1	4	15	58	21
AGAP011824-PA Thioredoxin peroxidase	25	59.6	CH	4	3	3	5	5	5	7	9	40	5	5	10
		87.1	IH	3	3	3	5	4	7	12	13	32	4	5	9
AGAP006430-PB CTLGA2	24.9	1.6	CH	0	0	0	0	0	0	0	0	58	5	22	16
		2.2	IH	0	0	0	0	0	0	0	0	65	10	12	13
AGAP011223-PA FBN8	24.8	2.1	CH	3	6	0	18	16	13	17	22	5	0	0	0
		1.1	IH	0	0	0	23	22	10	20	21	3	0	0	0
AGAP010775-PA FBN8	23.3	0	CH	0	0	0	0	0	0	0	100	0	0	0	0
		0	IH	0	0	0	0	0	0	0	0	0	0	0	0
AGAP001212-PB PGRPLB	23.3	2.9	CH	0	0	0	0	0	0	1	1	37	55	7	0
		9.3	IH	0	0	0	0	0	0	0	2	26	69	1	2
AGAP009556-PA FBN8	22.4	18.3	CH	5	5	2	9	9	6	12	34	14	1	1	2
		16.4	IH	6	7	7	14	12	9	11	25	6	2	0	0
AGAP000536-PA PGRPS1	22.4	7.5	CH	0	0	0	0	0	1	1	2	5	75	15	1
		4.4	IH	0	0	0	0	0	2	7	3	11	53	20	3
AGAP000305-PA SPARC	22.2	1.5	CH	0	0	0	0	0	0	1	29	27	16	26	0
		2.7	IH	0	0	0	0	0	0	0	25	27	16	33	0
AGAP011054-PA Thioredoxin peroxidase	22	61.6	CH	4	4	3	6	6	5	8	11	24	13	6	9
		83.8	IH	4	5	4	8	6	7	12	10	19	11	5	9
AGAP004811-PA CTL1	21.8	4	CH	0	0	0	0	0	2	0	5	22	34	30	6
		1.5	IH	0	0	0	0	0	0	0	6	20	54	20	0
	21.5	123.1	CH	2	2	1	5	2	2	5	8	8	17	43	3

AGAP003625-PA CTL8		153.8	IH	3	3	2	5	4	4	10	8	11	14	29	8
AGAP004810-PA CTL3	20.8	23	CH	0	0	0	0	4	0	1	3	6	5	9	71
		28.7	IH	0	0	0	0	9	1	2	4	6	4	16	59
AGAP001325-PA Peroxiredoxin 5, atypical 2-Cys peroxiredoxin	20.6	60	CH	0	1	0	1	1	1	1	2	3	7	68	15
		66.3	IH	0	0	0	1	1	0	2	2	3	8	59	23
AGAP007412-PA CTLMA1	20.1	1.9	CH	0	0	0	0	0	0	0	20	7	46	27	0
		0.6	IH	0	0	0	0	0	0	0	31	15	25	29	0
AGAP006343-PA PGRPS2	20	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP006342-PA PGRPS3	20	36	CH	0	0	0	1	0	0	1	2	2	4	81	8
		49.8	IH	0	1	0	1	0	0	1	1	2	6	74	13
AGAP005335-PA CTL4	19.8	1.8	CH	0	0	0	0	0	0	0	0	6	0	76	19
		0.4	IH	0	0	0	0	0	0	0	0	0	0	100	0
AGAP007411-PA CTLMA3	19.4	12.8	CH	3	2	1	6	4	2	3	9	8	22	37	4
		8.9	IH	5	4	4	8	3	0	8	11	11	23	24	0
AGAP002857-PB MDL2	18.1	7.3	CH	1	0	0	3	5	2	2	5	5	7	62	8
		4	IH	0	0	0	0	3	0	6	7	7	6	50	20
AGAP011119-PA Lysozyme 3	18	11.5	CH	0	0	0	0	0	0	2	4	2	18	57	17
		12.4	IH	0	0	0	0	0	0	0	0	4	9	57	31
AGAP008878-PA Defense protein	17.7	59.2	CH	2	2	1	2	4	0	1	4	5	5	69	5
		70.1	IH	1	1	1	1	6	0	3	4	7	9	63	3
AGAP002911-PA CTLMA9	17.5	0.3	CH	0	0	0	0	0	0	0	0	0	0	0	100
		0.3	IH	0	0	0	0	0	0	0	0	0	0	0	100
AGAP007385-PA Lysozyme 4 (c-type)	17.4	1.1	CH	0	0	0	0	0	0	0	1	0	0	13	86
		0.5	IH	0	0	0	0	0	0	0	0	0	0	0	100
AGAP007345-PA Lysozyme 3 (c-type)	16.6	10.9	CH	0	0	0	0	3	2	0	3	6	5	23	58
		19.9	IH	0	0	0	1	6	0	2	7	8	7	29	39
AGAP007344-PA Lysozyme 8 (c-type)	16.5	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0	IH	0	0	0	0	0	0	100	0	0	0	0	0
AGAP007201-PA Thioredoxin	15.6	2.3	CH	0	0	0	0	0	0	0	0	0	0	0	100
		5.8	IH	0	0	0	0	0	0	7	3	4	4	0	82
AGAP003338-PA Thioredoxin	15.5	0.4	CH	0	0	0	0	0	0	0	0	0	0	0	100
		0.1	IH	0	0	0	0	0	0	0	0	0	0	100	0
AGAP007347-PA Lysozyme 1 (c-type)	15.3	1.9	CH	0	0	0	0	0	0	0	0	0	0	18	82
		1.8	IH	0	0	0	0	0	0	0	0	0	0	34	66
AGAP005432-PA Programmed cell death protein 5	14.8	1.3	CH	0	0	0	0	0	0	0	0	0	0	72	28
		1.9	IH	0	0	0	0	0	0	0	0	0	0	69	31
AGAP006813-PA TIL domain-containing protein	13.4	0.2	CH	0	0	0	0	0	0	0	0	100	0	0	0
		0.4	IH	0	0	0	0	0	0	0	0	100	0	0	0
AGAP009584-PA Thioredoxin	12.1	35.6	CH	4	3	2	2	7	0	0	4	6	7	8	57
		41	IH	4	3	3	3	11	0	1	10	9	8	12	36
AGAP011460-PA Cysteine-rich protein (salivary)	11.2	39.4	CH	0	0	0	0	0	0	0	0	0	1	6	93
		16	IH	0	0	0	0	0	0	0	0	1	0	10	89
AGAP002878-PA Cystatin-like protein	11	7.2	CH	0	0	0	3	7	0	0	4	6	5	43	33
		11.4	IH	0	0	0	1	5	0	0	6	8	8	46	26
AGAP011832-PA Death-associated protein 1	10.3	13.9	CH	0	0	0	0	0	0	0	0	0	3	87	10
		12	IH	0	0	0	0	0	0	0	0	0	2	90	7
AGAP004632-PA Defensin	10	0	CH	0	0	0	0	0	0	0	0	0	0	0	0
		0.1	IH	0	0	0	0	0	0	0	0	0	0	0	100
AGAP006253-PA Cysteine-rich venom protein	9.5	47	CH	0	0	0	0	0	0	0	0	0	0	1	99
		65.4	IH	0	0	0	0	0	0	0	0	0	0	2	98
AGAP008645-PA Gambicin	8.8	0.6	CH	0	0	0	0	0	0	0	0	0	0	0	100
		0.5	IH	0	0	0	0	0	0	0	0	0	0	0	100
	8.8	1.3	CH	0	0	0	0	0	0	0	0	0	0	0	100

AGAP012970-PA Cysteine-rich venom protein		4.3	IH	0	0	0	0	0	0	0	0	0	0	0	100
AGAP011482-PA Kazal domain-containing protein	8.5	3.3	CH	0	0	0	0	0	0	0	0	0	0	0	100
		5.1	IH	0	0	0	0	0	0	0	0	0	1	2	97
AGAP007199-PA Defensin	7	0.5	CH	0	0	0	0	0	0	0	0	0	0	0	100
		1.5	IH	0	0	0	0	0	0	0	0	0	0	0	100
AGAP008968-PA Kazal domain-containing protein	6.5	44.3	CH	0	0	0	0	0	0	0	0	1	2	1	95
		52	IH	0	0	0	0	0	0	0	0	0	1	1	97

*RA stands for relative abundance, it's represented as [protein abundance * 10000/total protein abundance of CH or IH]. Molecular weight under each gel slice indicates the upper limit of each slice. The values of each protein in each slice is the percentage of abundance out of the protein's total abundance in CH or IH, so 12 slices of each protein adds up to 100%. Red boxes indicate the calculated positions of the proteins.

Abbreviations:

PPO: Prophenoloxidase RNAi: RNA interference CTL: c-type lectin

PRR: pathogen recognition receptor PAMP: pathogen associated molecular pattern

LPS: lipopolysaccharide LTA: lipoteichoic acid IMD: immune deficiency

PGRP: peptidoglycan recognition protein βGRP: β-1, 3-glucan recognition protein

GNBP: Gram-negative bacteria binding protein AMP: antimicrobial peptide

PAP: PPO activating protease SPH: serine protease homolog CH: control hemolymph

HP: hemolymph protease DHI: 5, 6-dihydroxyindole MS: mass spectrometry

LFQ: label-free quantification PTU: 1-phenyl-2-thiourea IH: induced hemolymph

PTGS: post-transcriptional gene silencing RISC: RNA inducing silencing complex

qRT-PCR: quantitative reverse transcriptional PCR TBS: tris-buffered saline

GAR-AP: alkaline phosphatase linked goat-anti-rabbit secondary antibody

LDLp: low density lipophorin TEP: thioester-containing protein FBN: fibrillin

LRR: leucine-rich repeat SP: serine protease OBP: odorant-binding protein

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