

Supplementary Materials

Table S1. Primers used in this study

Table S2. GenBank accession numbers of different PLA₂ genes to construct phylogenetic tree of Figure S2A

Figure S1. Effects of different concentrations of aspirin ('ASP') on choriogenesis of *Ae. albopictus*. **(A)** Hemocoelic injection. ASP was injected into 5 days old females 10 min before blood feeding ('BF'). At 72 h post BF, ovaries were collected from treated females in PBS to count the number of chorionated oocytes. **(B)** Oral feeding. Different concentrations of ASP were prepared with a 10% sugar solution. The sugar-ASP solution (5 mL) was provided to an adult cage containing 10 females at 3 h before BF. After BF, females were allowed to continuously feed the sugar-ASP solution for 3 days and then assessed for the development of chorionated oocytes. Each treatment used 10 females. Different letters above standard error bars indicate significant differences among means at Type I error = 0.05 (LSD test).

Figure S2. Identification of six PLA₂ genes of *Ae. albopictus*. **(A)** Phylogenetic tree analysis of Aa-PLA₂s. The tree was generated by the Maximum likelihood tree method using software package MEGA6.0, where evolutionary distances were computed using the Poisson correction method. Amino acid sequences of different PLA₂s were retrieved from GenBank with accession numbers of Table S2. **(B)** Typical functional domains of six Aa-PLA₂s. Domains were predicted using Prosite (<https://prosite.expasy.org/>) and SMART protein (<http://smart.embl-heidelberg.de/>).

Figure S3. Prediction of peroxidases (POXs) associated with PG biosynthesis in *Ae. albopictus*. **(A)** Phylogenetic tree analysis of mosquito POXs. The tree was generated by the Maximum likelihood tree method using software package MEGA-X, where evolutionary distances were computed using the Poisson correction method. Amino acid sequences of different peroxidases were retrieved from GenBank with accession numbers indicated along with individual gene names in the tree. **(B)** Domain analysis of five Aa-POXs clustered with known COX-POXs.

Figure S4. Phylogenetic tree and domain analyses of Aa-Shade. **(A)** Phylogenetic analysis of Shade genes from different insects. The analysis was performed using MEGA-X. Bootstrapping values were obtained with 1,000 repetitions to support branching and clustering. Amino acid sequences of selected genes were retrieved from GenBank with accession numbers indicated along with individual gene names in the tree. **(B)** Domain analysis of Aa-Shade. 'SP' and 'HBD' stand for the signal peptide and heme-binding

domain, respectively. Domains were predicted using Prosite (<https://prosite.expasy.org/>) and SMART protein (<http://smart.embl-heidelberg.de/>).

Figure S5. RNAi of three *PLA₂* (*Aa-PLA₂-IIA*, *Aa-PLA₂-IIIB*, *Aa-PLA₂-VI*) and three *POX* (*Aa-POX19*, *Aa-POX20*, *Aa-POX15*) genes. RNAi was performed by injecting 100 ng of gene-specific dsRNA or control dsRNA ('dsCON') to 5 days old females at 24 h before blood-feeding (BF). Changes in mRNA levels were assessed at 72 h after injecting dsRNA or dsCON by RT-qPCR using *RpS6* as a reference gene to normalize target gene expression level. Each treatment was replicated three times with independent tissue preparations. Asterisk (*) above standard error bars indicates significant difference among means at Type I error = 0.05 (LSD test).

Figure S6. Molecular characteristics of *Ae. albopictus* PGE₂ receptor (*Aa-PGE₂R*: GenBank accession number: XP_029736412.1). (A) Domain analysis of *Aa-PGE₂R*. A cleavage site of signal peptide (23 residues) is denoted by scissor. Seven transmembrane domains are marked on phospholipid bilayer with locations. N-glycosylation sites are denoted by an orange circle and phosphorylation sites are marked by a blue triangle. Domains were predicted using Prosite (<https://prosite.expasy.org/>) and SMART protein (<http://smart.embl-heidelberg.de/>). (B) A phylogenetic analysis. The tree was generated by the Maximum likelihood tree method using software package MEGA6.0, where evolutionary distances were computed using the Poisson correction method. Amino acid sequences of different PGE₂R were retrieved from GenBank with accession numbers of KAB1277535.1 for *C. dromedarius* (DP2), NP_000944.1 for *H. sapiens* (DP2), NP_071577.1 for *R. norvegicus* (DP2), XP_021258611.1 for *N. meleagris* (DP2), AAI12966.1 for *H. sapiens* (FP), JAA36212.1 for *P. troglodytes* (FP), AAZ08074.1 for *B. bubalis* (FP), EDL82480.1 for *R. norvegicus* (FP), NP_032993.2 for *M. musculus* (IP), NP_001015622.2 for *B. Taurus* (IP), EDM08294.1 for *R. norvegicus* (IP), XP_005361096.1 for *M. ochrogaster* (IP), NP_001345441.1 for *M. musculus* (TXA), NP_001161391.1 for *B. Taurus* (TXA), JAA33130.1 for *P. troglodytes* (TXA), AAH74749.1 for *H. sapiens* (TXA), NP_000946.2 for *H. sapiens* (EP1), NP_001265404.1 for *R. norvegicus* (EP1), NP_038669.1 for *M. musculus* (EP1), NP_001179077.1 for *B. taurus* (EP1), NP_000947.2 for *H. sapiens* (EP2), NP_112350.1 for *R. norvegicus* (EP2), NP_032990.1 for *M. musculus* (EP2), NP_777013.1 for *B. taurus* (EP2), NP_942007.1 for *H. sapiens* (EP3), NP_036836.1 for *R. norvegicus* (EP3), NP_001346674.1 for *M. musculus* (EP3), NP_851375.1 for *B. taurus* (EP3), NP_000949.1 for *H. sapiens* (EP4), NP_114465.3 for *R. norvegicus* (EP4), NP_001129551.1 for *M. musculus* (EP4), and NP_777014.1 for *B. Taurus* (EP4).

Figure S7. Expression profile of *PGE₂R* in *Ae. albopictus*. (A) Expression pattern in different developmental stages from egg to male and female adults. (B) Expression in different body parts of *Ae. albopictus* females at 3 days after blood-feeding ('BF'): head ('HD'), thorax ('TH'), ovary ('OV'), and abdomen ('ABD'). (C) Relationship between the expression level of *Aa-PGE₂R* and the number of chorionated oocytes after BF. Expression of actin, a reference gene, was used to normalize gene expression levels in RT-qPCR. All measurements were independently replicated three times.

Figure S8. Molecular characterization of *Ae. albopictus* vitellogenin protein (*Aa-Vg1* and *Aa-Vg2*). **(A)** A phylogenetic tree analysis. The tree was generated by the Maximum likelihood tree method using software package MEGA6.0, where evolutionary distances were computed using the Poisson correction method. Amino acid sequences of different vitellogenin genes were retrieved from GenBank. **(B)** Domain analysis of *Aa-Vg1* and *Aa-Vg2*. Domains were predicted using Prosite (<https://prosite.expasy.org/>) and SMART protein (<http://smart.embl-heidelberg.de/>). **(C)** Expression pattern of *Aa-Vg1* and *Aa-Vg2* after blood-feeding (BF).

Table S1. Primers used in this study

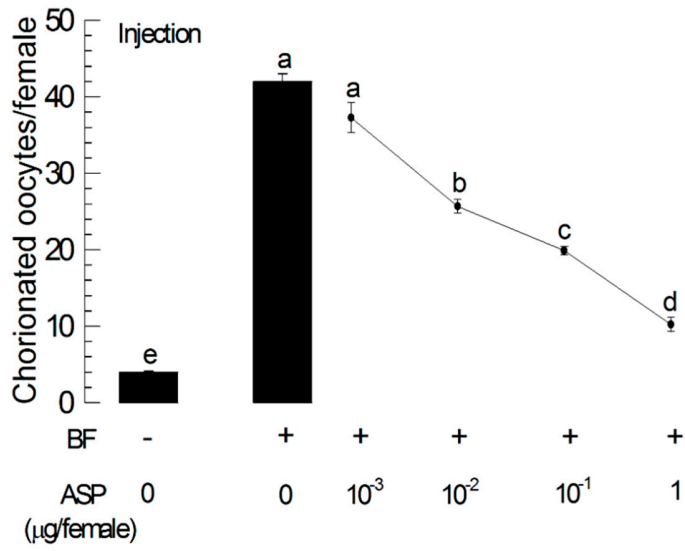
| Genes | Primer sequence (5' - 3') |
|--------------------------------|---|
| <i>Aa-PLA₂-IIIA</i> | CAAGTGTGACCGAGATTTTC |
| | GTCTCAAAAACAGCCTGTAAG |
| <i>Aa-PLA₂-IIIB</i> | CCTGAAAATGTCCGACTCC |
| | TTTCCTGTTGTACGGATG |
| <i>Aa-PLA₂-VI</i> | TCAAAACTGTACGGGAAGAG |
| | CACATCGATCGAGTTTTTCC |
| <i>Aa-PLA₂-VIII</i> | TTCACCGGACAAAGTGATAG |
| | ATCGAACAGCTCGAAAATCT |
| <i>Aa-PLA₂-XII</i> | ACATCCCACAATCGGATGGG |
| | TGGACATTTGAATATGCAAT |
| | TAATACGACTCACTATAGGGAGAACATCCCACAATCGGATGGG |
| | TAATACGACTCACTATAGGGAGATGGACATTTGAATATGCAAT |
| <i>Aa-PLA₂-XV</i> | CCGATATCAACAAACCGAAC |
| | TTCCGTTGTAGTTACCCTTG |
| <i>Aa-Shade</i> | AACAATATGACCCACCATCC |
| | GCTTGTAGACGTTTTGGTTT |
| | TAATACGACTCACTATAGGGAGAAACAATATGACCCACCATCC |
| | TAATACGACTCACTATAGGGAGAGCTTGTAGACGTTTTGGTTT |
| <i>Aa-POX4</i> | GTCCTTACAACGATTACCGA |
| | AAAATAGTACCGATCGCCTC |
| <i>Aa-POX9</i> | ATGCCGAAATCAAACACTTC |
| | TACAATGACTGTAGCTTGGC |
| | TAATACGACTCACTATAGGGAGAATGCCGAAATCAAACACTTC |
| | TAATACGACTCACTATAGGGAGATACAATGACTGTAGCTTGGC |
| <i>Aa-POX15</i> | CTTAATACTCCTCGACCGAC |
| | TCAAAGAGTGCTCAAAGTCT |
| | TAATACGACTCACTATAGGGAGACTTAATACTCCTCGACCGAC |
| | TAATACGACTCACTATAGGGAGATCAAAGAGTGCTCAAAGTCT |
| <i>Aa-POX19</i> | GACCTTACAACGACTACCTC |
| | TGCTGTAAAAGTACCGATCC |
| | TAATACGACTCACTATAGGGAGAGACCTTACAACGACTACCTC |
| | TAATACGACTCACTATAGGGAGATGCTGTAAAAGTACCGATCC |
| <i>Aa-POX20</i> | GTTTCTTCAGATTGCTCACC |
| | GGCTAGATCTGATCCGAAAG |
| | TAATACGACTCACTATAGGGAGAGTTTCTTCAGATTGCTCACC |
| | TAATACGACTCACTATAGGGAGAGGCTAGATCTGATCCGAAAG |
| <i>Aa-Vg1</i> | CCGTAGTCAAAATTCAAGC |
| | ACTCTTCAGCGATATTCCAC |
| <i>Aa-Vg2</i> | CAAGCCACAGTATCCTTCTT |
| | CTCGTACTCATCAAATCCGT |
| <i>Aa-PGE₂R</i> | CCAACATGCACCACTTCT |
| | GGAAGTGCCTTCGCATCT |
| | TAATACGACTCACTATAGGGAGACCAACATGCACCACTTCT |
| | TAATACGACTCACTATAGGGAGAGGAACTGCCTTCGCATCT |
| <i>RpS6</i> | ACAAGCTGCGTCACTTCTACGACA |
| | CTTGTCGTTTCCACCGCAATCTT |
| | CACGTTCACTCAGGATCTTC |

Table S2. GenBank accession numbers of different PLA₂ genes to construct phylogenetic tree of Fig. S2A

| Gene | Accession | Scientific name |
|----------------|----------------|-----------------------------------|
| <i>Ame-III</i> | XP 002915601.2 | <i>Ailuropoda melanoleuca</i> |
| <i>Ami-III</i> | XP 006032459.1 | <i>Alligator sinensis</i> |
| <i>Av-X</i> | XP 009862694.1 | <i>Apaloderma vittatum</i> |
| <i>Bt-XII</i> | NP 001091532.1 | <i>Bos taurus</i> |
| <i>Cf-V</i> | XP 006188390.1 | <i>Camelus ferus</i> |
| <i>Bt-III</i> | NP 001074379.2 | <i>Bos taurus</i> |
| <i>Cf-X</i> | XP 006179161.1 | <i>Camelus ferus</i> |
| <i>Cl-I</i> | NP 001003320.1 | <i>Canis lupus familiaris</i> |
| <i>Cl-III</i> | XP 025330373.1 | <i>Canis lupus dingo</i> |
| <i>Cp-I</i> | XP 003477984.1 | <i>Cavia porcellus</i> |
| <i>Cp-X</i> | XP 010001508.1 | <i>Chaetura pelagica</i> |
| <i>Cg-III</i> | EGW07116.1 | <i>Cricetulus griseus</i> |
| <i>Dr-III</i> | NP 001032489.1 | <i>Danio rerio</i> |
| <i>Egc-I</i> | XP 001489303.1 | <i>Equus caballus</i> |
| <i>Eh-X</i> | XP 010154523.1 | <i>Eurypyga helias</i> |
| <i>Fc-III</i> | XP 023097342.1 | <i>Felis catus</i> |
| <i>Fg-II</i> | XP 009572314.1 | <i>Fulmarus glacialis</i> |
| <i>Gg-I</i> | NP 001138961.1 | <i>Gallus gallus</i> |
| <i>Gg-X</i> | ADG46026.1 | <i>Gallus gallus</i> |
| <i>Gs-X</i> | XP 009813736.1 | <i>Gavia stellata</i> |
| <i>Hs-II</i> | NP 000291.1 | <i>Homo sapiens</i> |
| <i>Hs-III</i> | NP 073730.3 | <i>Homo sapiens</i> |
| <i>Hs-X</i> | NP 003552.1 | <i>Homo sapiens</i> |
| <i>Hs-V</i> | 5245948.1 | <i>Homo sapiens</i> |
| <i>Hs-XII</i> | NP 110448.2 | <i>Homo sapiens</i> |
| <i>Hs-III</i> | NP 056530.2 | <i>Homo sapiens</i> |
| <i>It-III</i> | XP 005339912.1 | <i>Ictidomys tridecemlineatus</i> |
| <i>Lc-XIIA</i> | XP 010754065.1 | <i>Larimichthys crocea</i> |
| <i>Ls-XIIB</i> | XP 021399547.1 | <i>Lonchura striata domestica</i> |
| <i>Mm-XIIA</i> | NP 001181806.1 | <i>Macaca mulatta</i> |
| <i>Mn-III</i> | XP 011748880.1 | <i>Macaca nemestrina</i> |
| <i>Lc-X</i> | XP 017689444.1 | <i>Lepidothrix coronata</i> |
| <i>Ma-III</i> | XP 005068966.1 | <i>Mesocricetus auratus</i> |
| <i>Md-III</i> | XP 008543925.1 | <i>Microplitis demolitor</i> |
| <i>Mm-X</i> | NP 036117.1 | <i>Macaca mulatta</i> |
| <i>Mm-IB</i> | NP 035237.1 | <i>Macaca mulatta</i> |
| <i>Mm-II</i> | NP 032894.2 | <i>Macaca mulatta</i> |
| <i>Mm-III</i> | NP 766379.2 | <i>Macaca mulatta</i> |
| <i>Mm-V</i> | P97391.2 | <i>Macaca mulatta</i> |
| <i>Mm-XII</i> | NP 075685.2 | <i>KAF1442899.1</i> |
| <i>Nn-X</i> | XP 009464949.1 | <i>Nipponia nippon</i> |
| <i>Oc-IB</i> | Q7M334.1 | <i>Oryctolagus cuniculus</i> |
| <i>Pp-III</i> | XP 003826977.1 | <i>Pan paniscus</i> |
| <i>Oa-IB</i> | XP 004017463.1 | <i>Ovis aries</i> |
| <i>Pp-V</i> | XP 034812955.1 | <i>Pan paniscus</i> |
| <i>Pl-X</i> | KFQ64262.1 | <i>Phaethon lepturus</i> |

| | | |
|-----------------|----------------|----------------------------------|
| Dp-X | XP 009897154.1 | <i>Dryobates pubescens</i> |
| Pa-III | XP 009215381.1 | <i>Papio anubis</i> |
| Pg-X | XP 010076871.1 | <i>Pterocles gutturalis</i> |
| Pa-X | XP 009323089.1 | <i>Pygoscelis adeliae</i> |
| Rn-X | NP 058872.1 | <i>Rattus norvegicus</i> |
| Rn-IB | NP 113773.1 | <i>Rattus norvegicus</i> |
| Rn-IIC | NP 062075.1 | <i>Rattus norvegicus</i> |
| Rn-IID | NP 001013446.1 | <i>Rattus norvegicus</i> |
| Rn-III | NP 001099485.1 | <i>Rattus norvegicus</i> |
| Rn-V | NP 058870.1 | <i>Rattus norvegicus</i> |
| Rn-XIIA | EDL82189.1 | <i>Rattus norvegicus</i> |
| Se-III | AZL90156.1 | <i>Spodoptera exigua</i> |
| Te-X | KFV16621.1 | <i>Tauraco erythrolophus</i> |
| XI-XIIA | NP 001085480. | <i>Xenopus laevis</i> |
| Aa-XII | XP 029724479.1 | <i>Aedes albopictus</i> |
| Dh-XII | XP 023164765.1 | <i>Drosophila hydei</i> |
| Hsa-I | 3ELO A | <i>Homo sapiens</i> |
| Pt-I | XP 001160305.1 | <i>Pan troglodytes</i> |
| Mm-I | NP 001343515.1 | <i>Mus musculus</i> |
| Rn-I | NP 113773.1 | <i>Rattus norvegicus</i> |
| Dre-I | NP 001107095.1 | <i>Danio rerio</i> |
| Hsa-IV | NP 077734.2 | <i>Homo sapiens</i> |
| Pt-IV | XP 001165694.1 | <i>Pan troglodytes</i> |
| Ggg-IV | XP 004028114.1 | <i>Gorilla gorilla gorilla</i> |
| Rn-IV | NP 598235.2 | <i>Rattus norvegicus</i> |
| Mm-IV | NP 032895.1 | <i>Mus musculus</i> |
| Hsa-VI | NP 001004426.1 | <i>Homo sapiens</i> |
| Ggg-VI | XP 030861468.1 | <i>Gorilla gorilla gorilla</i> |
| Poa-VI | XP 024095668.1 | <i>Pongo abelii</i> |
| Mmu-VI | XP 028683552.1 | <i>Macaca mulatta</i> |
| Pit-VI | XP 023077806.1 | <i>Ptilocolobus tephrosceles</i> |
| Hsa-VII | AAB04170.1 | <i>Homo sapiens</i> |
| Pp-VII | XP 034818104.1 | <i>Pan paniscus</i> |
| Ggg-VII | XP 004044188.1 | <i>Gorilla gorilla gorilla</i> |
| Pt-VII | XP 009449675.1 | <i>Pan troglodytes</i> |
| Aa-III A | XP 019933483.1 | <i>Aedes albopictus</i> |
| Aa-IIIB | XP 029713527.1 | <i>Aedes albopictus</i> |
| Aa-VIII | XP 029714924.1 | <i>Aedes albopictus</i> |
| Aa-XV | XP 029720738.1 | <i>Aedes albopictus</i> |
| Aa-VI | XP 029732314.1 | <i>Aedes albopictus</i> |
| Rn-XV | NP 001004277.1 | <i>Rattus norvegicus</i> |
| Hsa-VIII | NP 001242939.1 | <i>Homo sapiens</i> |
| Pp-VIII | XP 003811265.1 | <i>Pan paniscus</i> |
| Ggg-VIII | XP 030868609.1 | <i>Gorilla gorilla gorilla</i> |
| Pat-VIII | XP 009452256.1 | <i>Pan troglodytes</i> |
| Min-VIII | XP 016054620.1 | <i>Miniopterus natalensis</i> |
| Mm-XV | NP 598553.1 | <i>Mus musculus</i> |
| Hsa-XV | 4X90 A | <i>Homo sapiens</i> |
| Op-XV | XP 004584230.1 | <i>Ochotona princeps</i> |

A



B

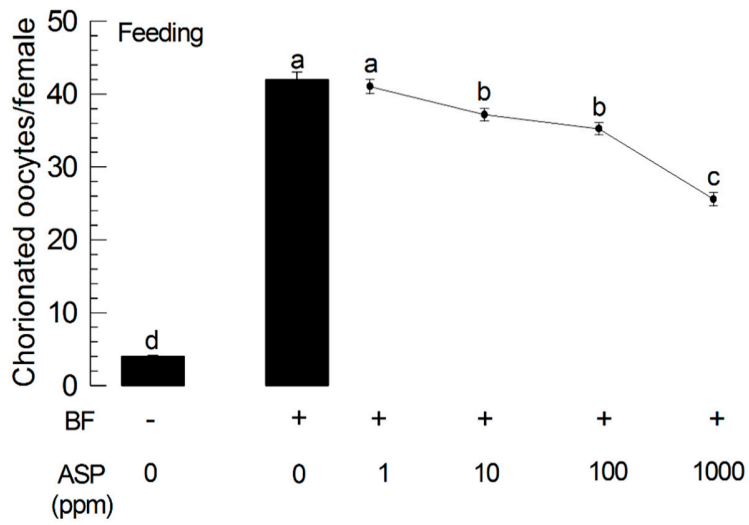
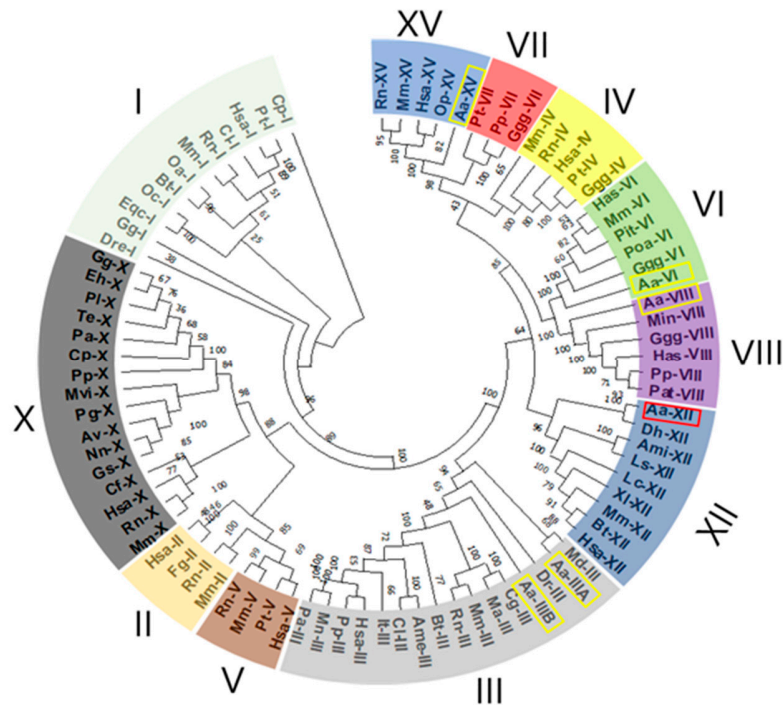


Figure S1

A



B

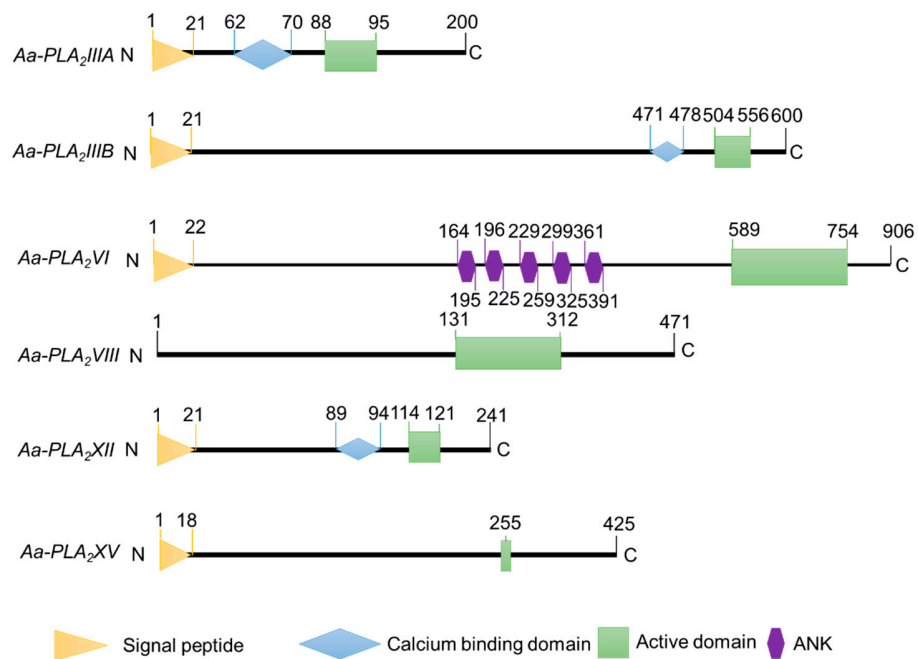
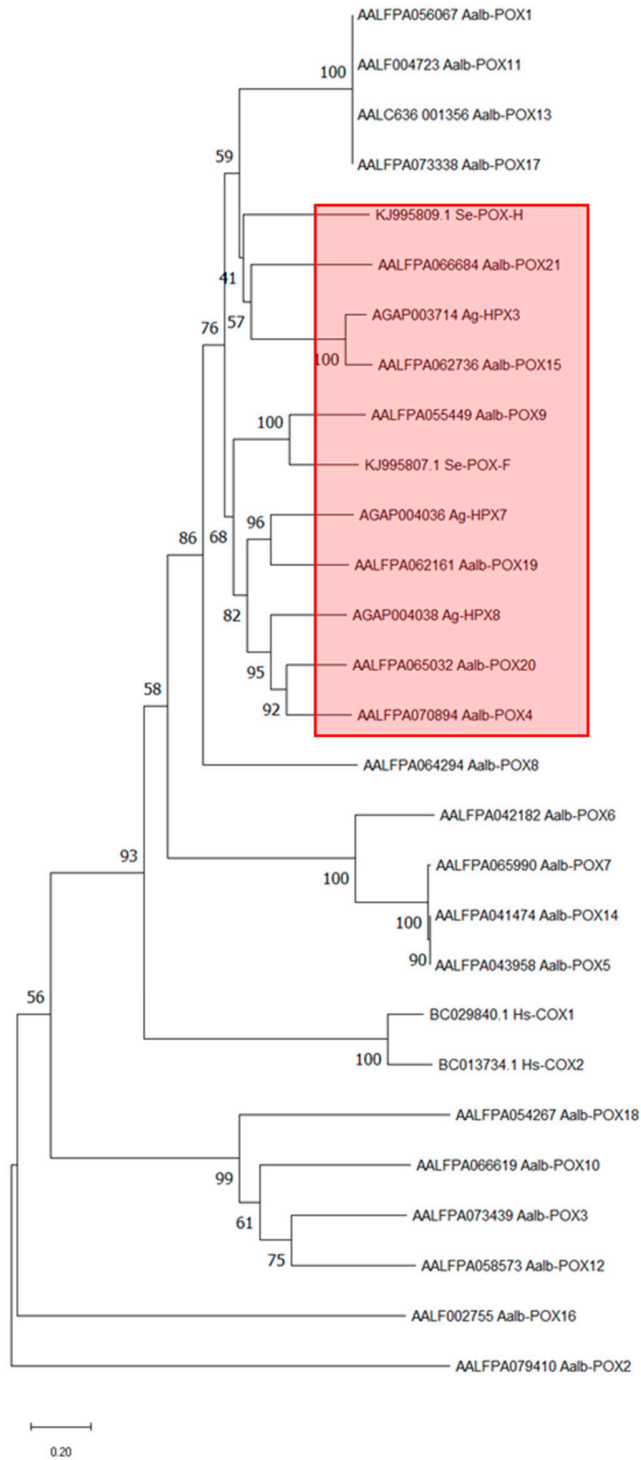


Figure S2

A



B

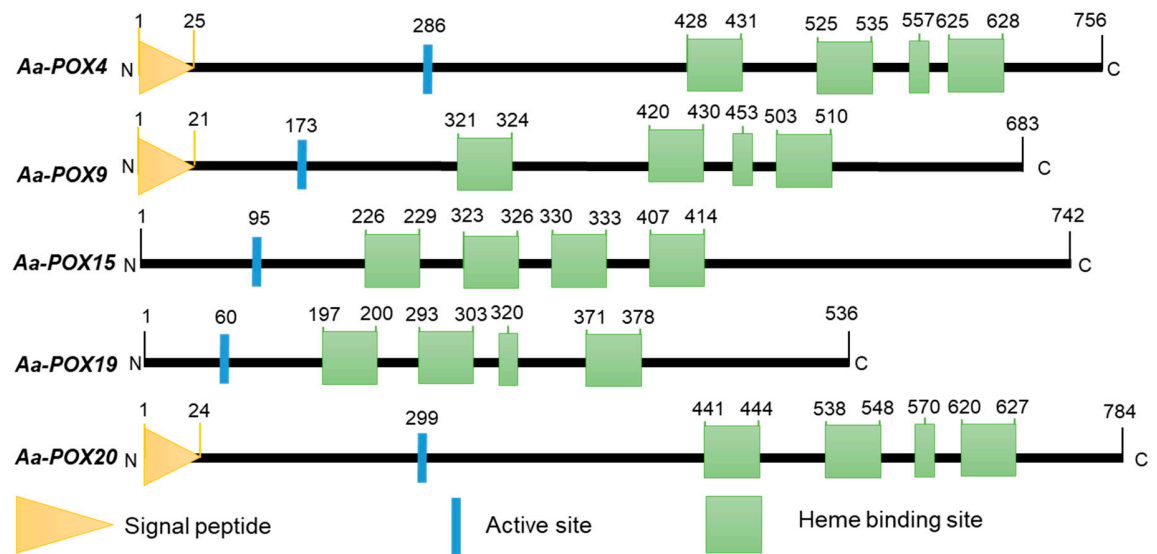
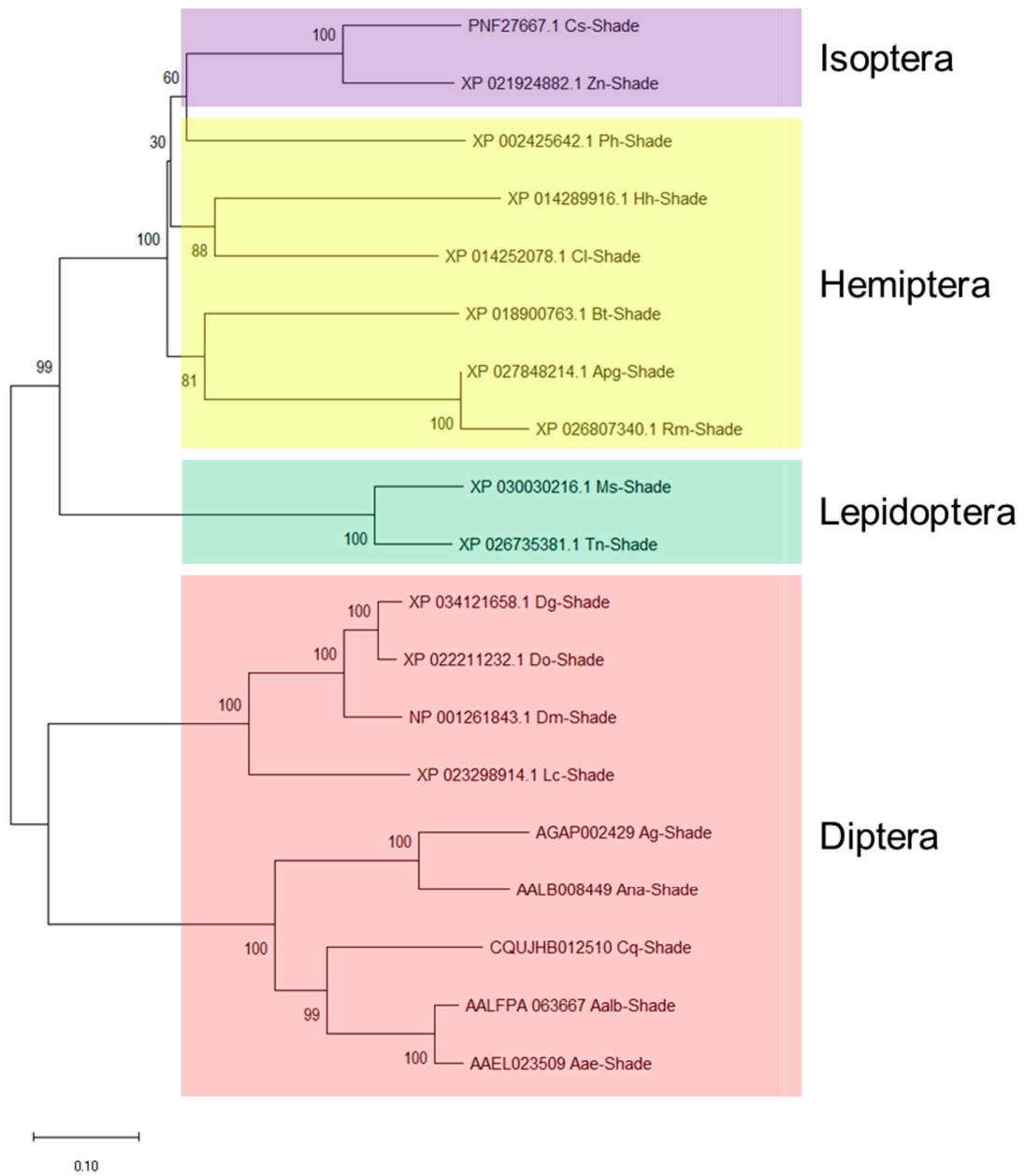


Figure S3

A



B

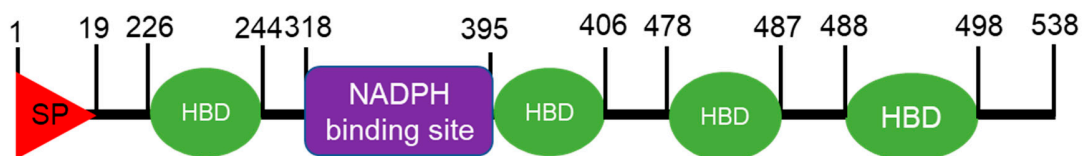


Figure S4

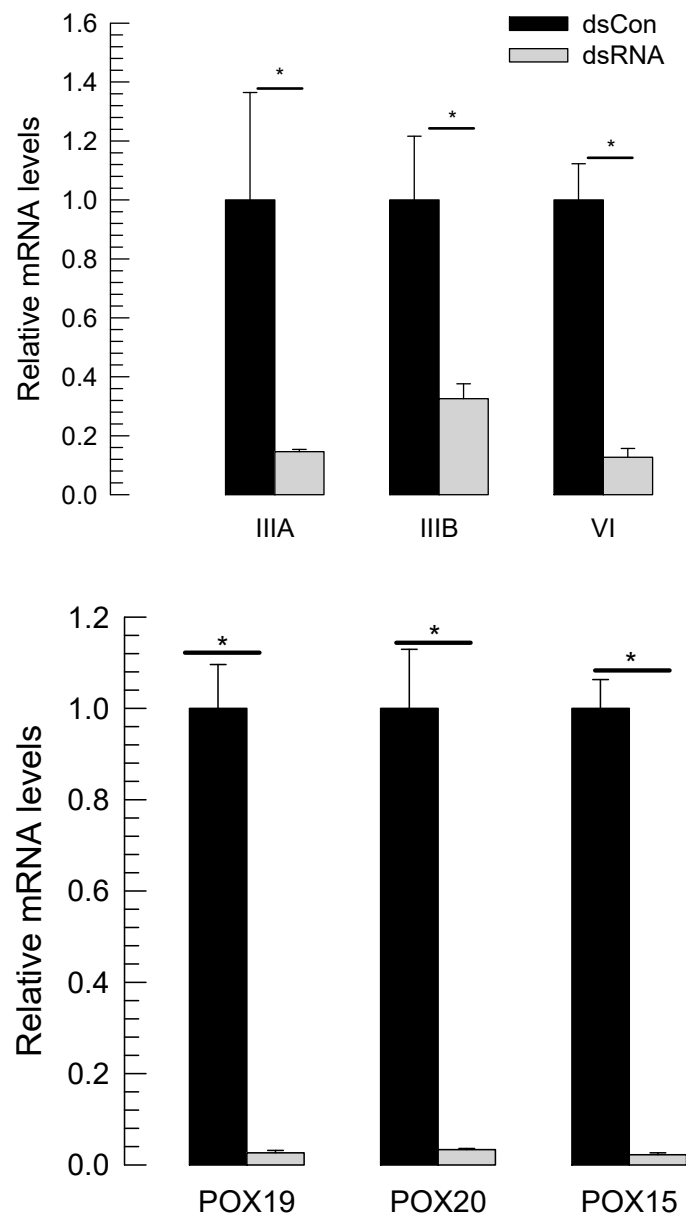
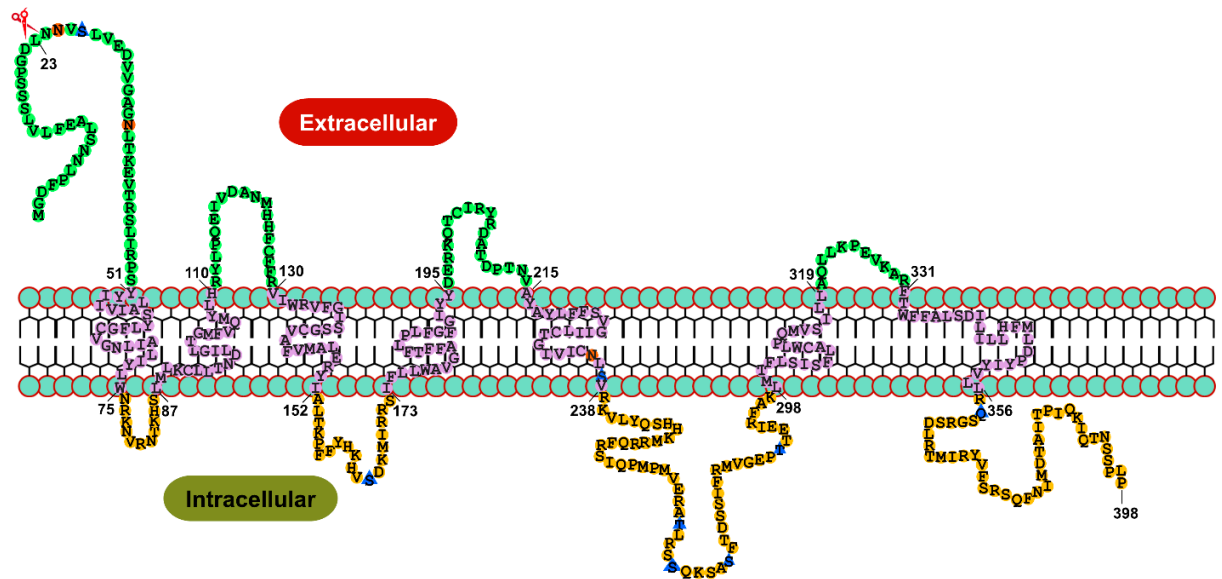


Figure S5

A



B

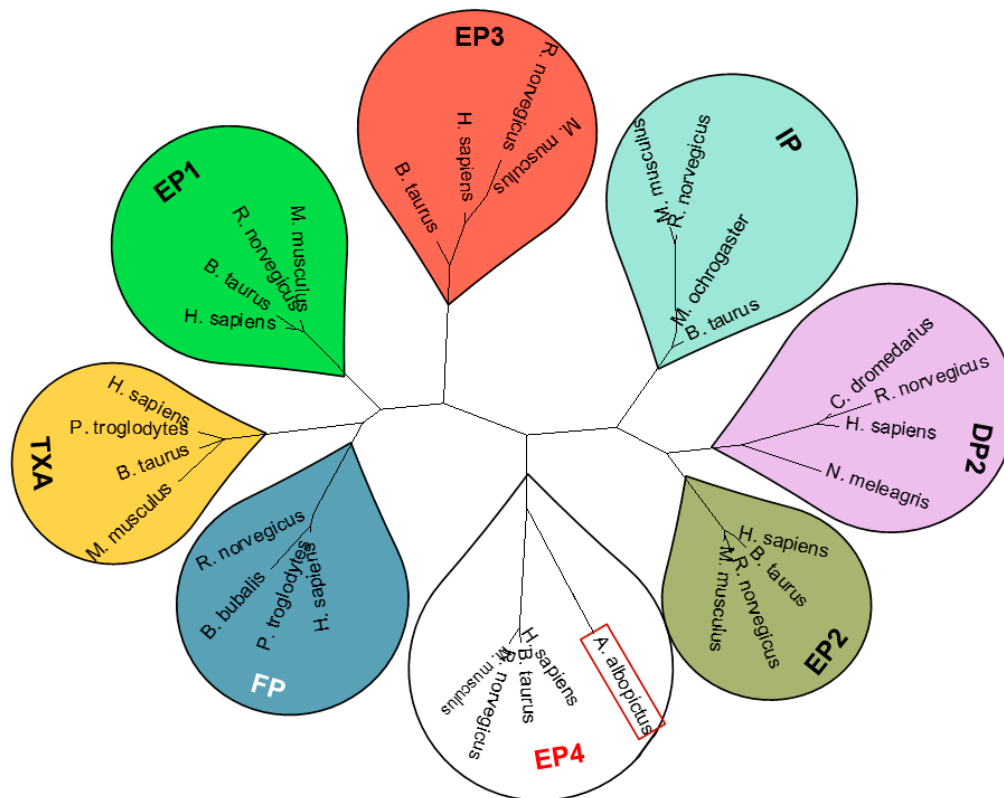


Figure S6

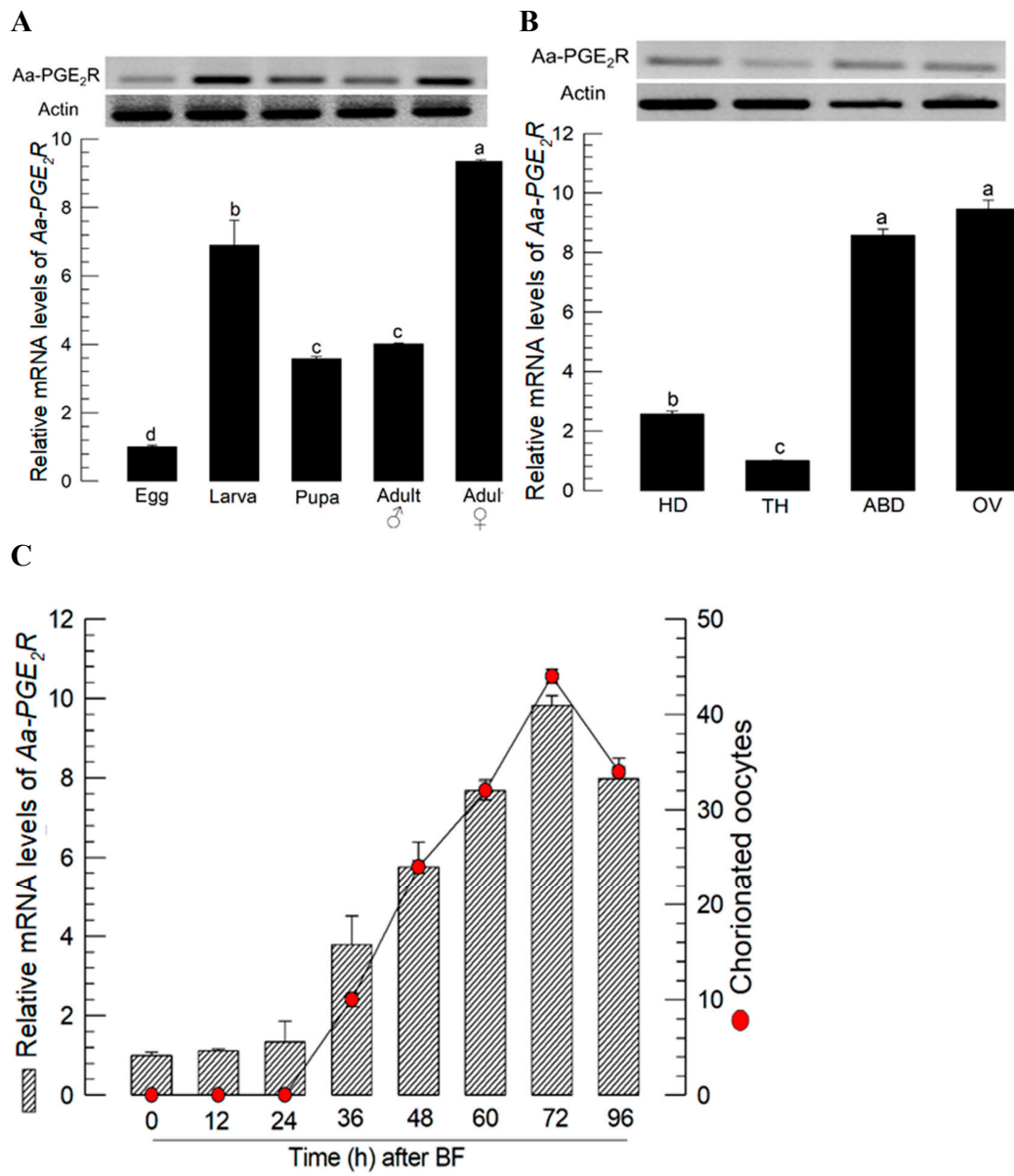
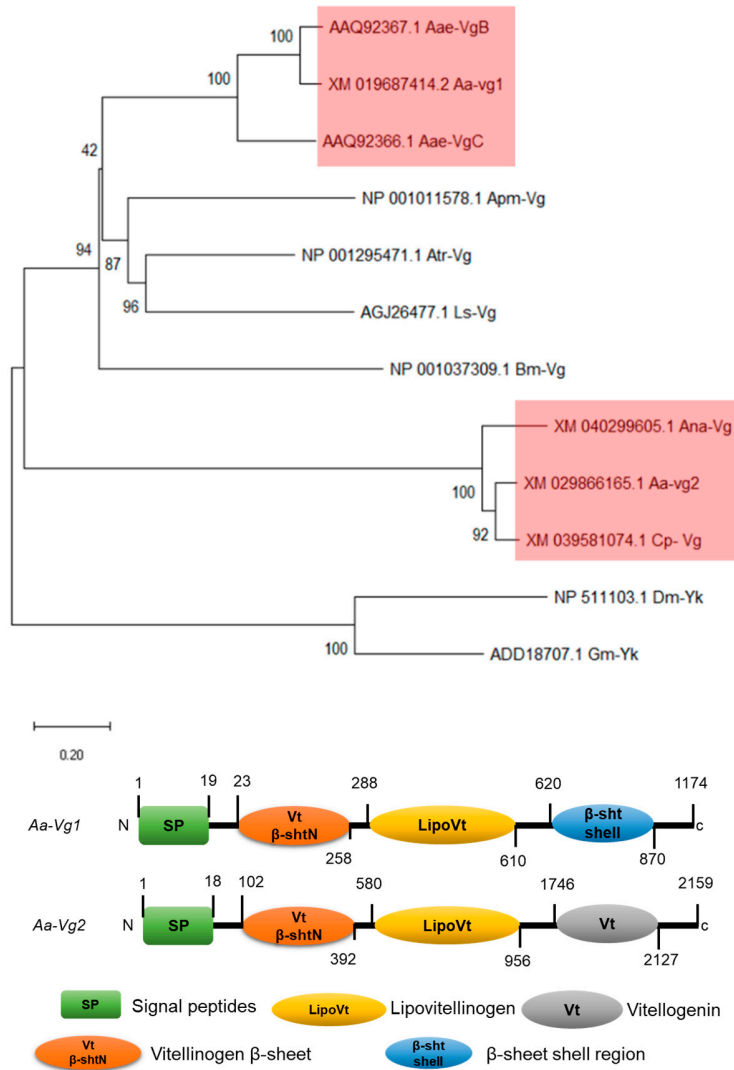


Figure S7

A



B

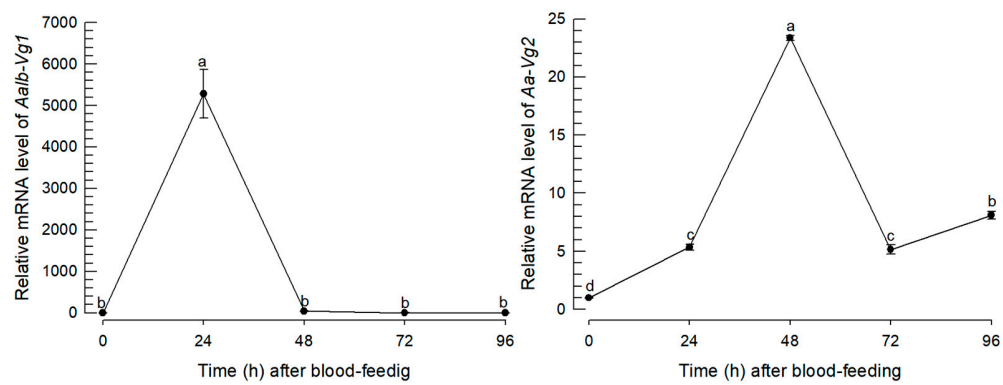


Figure S8