

Supplementary information

Tyrosine Hydroxylase is crucial for maintaining the pupae tanning and immune in *Anopheles sinensis*

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⁺Liang Qiao and Minghui Du contributed equally to this work.

Running title: *AsTH* for *Anopheles sinensis* pupae development

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Supplementary Figure legends

Figure S1. Alignment analysis of TH amino acid sequences among different insect species. Horizontal line indicates the Bioperin-H domain.

Figure S2. Detection of RNAi off-target effects. a. The pupa cuticle pigmentation pattern at 38th hour of pupation between *dsTH2* and *dsRed* group. Scale bar=500 μ m. b. The expression level of *AsTH* between *dsTH2* and *dsRed* individuals at 38th hour of pupation. *RPL49* was used as the internal control.

Figure S3. Precise region of cuticle frozen section. The red box region represents the observed sliced section region. Scale bar=100 μ m.

Figure S4. The expression pattern of *AsTH* gene expression in the egg (eggs were gathered from 1 hour to 1 day after born) stage, larval stage and adult stage, respectively. *RPL49* was used as the internal control.

Figure S5. Spatial expression pattern of *AsTH* in different tissues at 38th hour of pupation. IN represents the integument, FB represents the fat body, (HE+G) represents the mixture of the hemolymph and the gut. The "*" symbol represents treatment with bacteria. *RPL49* was used as the internal control.

Figure S6. The expression levels of 4 representative *Anopheles sinensis* pro-phenoloxidase (PPO) genes (The orthologous genes in *Anopheles gambiae* were expressed in the immune tissues) between *dsTH* and *dsRed* group at 38th hour of pupation. *RPL49* was used as the internal control.

Figure S1

An. gambiae : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
An. coluzzii : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
An. arabiensis : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
An. stephensi : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
An. dirus : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
An. funestus : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
An. sinensis : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
Cx. quinquefasciatus : **M**LAAAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
Ae. aegypti : -----**M**EAIVEEQO-----
Dr. melanogaster : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
Ma. sexta : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
Bm. mori : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
Da. plexippus : MAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
Tr. castaneum : AAVAAQAQKREMFIAKKSYS **EN**GYPSRRRSLVDDARFETIVVKTKQCTVLEARAKANEDEKTPQEVQQTVEDQNDDEEIRMVAVDELQPKQPEHVPFA
 a aaqknremfa kksys ElgypSrrrrslvddarfet vvkqtkq vl ear an

An. gambiae : DDEDKETDAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
An. coluzzii : DDEDKETDAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
An. arabiensis : DDEDKETDAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
An. stephensi : DDEDKETDAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
An. dirus : DDEDKETDAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
An. funestus : DDEDKETDAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
An. sinensis : DDEDKETDAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
Cx. quinquefasciatus : AODD-ETTAGLTFE **EV**VLV **NA**AS **SP**PAE **K**EWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
Ae. aegypti : **D**EID-**AG**LTFE **EV**VLV **NA**AS **SP**PAE **K**OWRAAVV **V**LR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
Dr. melanogaster : -----**Y**GLTFE **D**ITL **NA**AS **SP**PAE **A**QAQAAAL **L**MR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
Ma. sexta : DDNKADPDTYTFE **EV**VLV **NA**AS **SP**PAE **A**QAQAAAL **L**MR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
Bm. mori : DDDADPDTYTFE **EV**VLV **NA**AS **SP**PAE **A**QAQAAAL **L**MR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
Da. plexippus : NETGTDPDTYTFE **EV**VLV **NA**AS **SP**PAE **A**QAQAAAL **L**MR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
Tr. castaneum : **D**-----**S**GLTFE **EV**VLV **NA**AS **SP**PAE **A**QAQAAAL **L**MR **GM**SLGRILKAWAYE **ST**V **H**LESRSRSEGVQFDVILVKYDVA **BA**NLLQLIRSLRQI
 D gLTlEe66LqNaaS EspeAE 6 AA666r64dG6gSL R6Lk 6e G 6 H6E3R s gvqfD L6K6 M R nl6QLI4sLRQ3

An. gambiae : QSFQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
An. coluzzii : QSFQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
An. arabiensis : QSFQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
An. stephensi : QSFQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
An. dirus : QSFQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
An. funestus : QSFQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
An. sinensis : QSFQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
Cx. quinquefasciatus : AFSQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
Ae. aegypti : AFSQSVSLSENNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
Dr. melanogaster : GFSFNNMADNNVNYKAWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
Ma. sexta : TAFAGVNNHSDNNISSTKWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
Bm. mori : TAFAGVNNHSDNNISSTKWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
Da. plexippus : TAFAGVNNHSDNNISSTKWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
Tr. castaneum : SLELHCITGELNISA **K**AWPFF **H**AS **L**DCNHLMTKYE **P**DLDMNHGPFAD **Q**VYR **R**RRKEIA **IA**FAFYRGDP **Y** **I**Y **D**Y **T**ENK **WA**AVE **G**VKEL **M**VQ
 sf 66 e N6 K WFPF HAS LDCNHLMTK5EPdLDMNHGPFAD YR RRRK2IAe6AFaY4YGPdIP I Y3e En TW VF V Lm k

An. gambiae : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
An. coluzzii : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
An. arabiensis : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
An. stephensi : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
An. dirus : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
An. funestus : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
An. sinensis : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
Cx. quinquefasciatus : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
Ae. aegypti : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
Dr. melanogaster : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
Ma. sexta : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
Bm. mori : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
Da. plexippus : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
Tr. castaneum : HACSPYLAVR **R**KLDEK **I**VKER **P**OLQMS **D**L **R**KN **P**GET **L**RPAAGLL **T**ARDF **L**AS **L**AF **R**FOSTOYVRH **I**NSPYHTPEPD **I**HELLGHMPL **L**ADP **S**FA
 HAC BY a F kl2 e I fV R6PQLZ6eS FL44 TGF3LRPAAGLLTARDFLASLAFR6FQSTQYVRH N3P5HTPEPDcIHELLGH6PLLADP **S**FA

An. gambiae : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
An. coluzzii : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
An. arabiensis : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
An. stephensi : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
An. dirus : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
An. funestus : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
An. sinensis : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
Cx. quinquefasciatus : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
Ae. aegypti : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
Dr. melanogaster : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
Ma. sexta : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
Bm. mori : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
Da. plexippus : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
Tr. castaneum : QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE **D**EVKAY **G**AGLLSAY **G**ELLHA **I**SDK **D**RRR **F**EA **S**T **V**OPY **Q**DQY **Q**P **I**YVAES **F** **D**AK **K**FR
 QFSQIEGLASLGASD **E**IEKLS **T**VYWF **V**TEFGLCKE 6KAYGagLLS yGELLHA6SDKpE RpFePasTa6QYQDQYQPIY5VAE3FeDaK KFR

An. gambiae : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
An. coluzzii : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
An. arabiensis : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
An. stephensi : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
An. dirus : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
An. funestus : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
An. sinensis : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
Cx. quinquefasciatus : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
Ae. aegypti : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
Dr. melanogaster : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
Ma. sexta : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
Bm. mori : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
Da. plexippus : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
Tr. castaneum : RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC
 RVWSMSRPFVEFRNPH **T**DRVE **L**DSV **G**KL **T**LV **S**OLNTE **V**LHL **T**NA **I**KL **R** **P**FC K64 p5

Figure S2

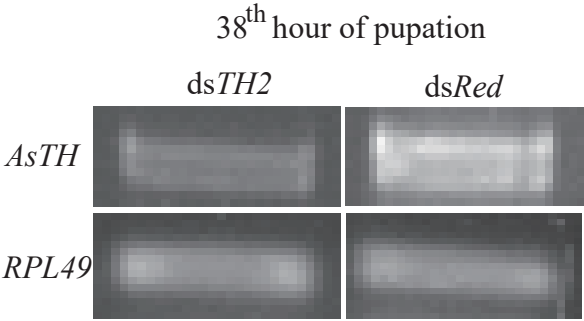


Figure S3

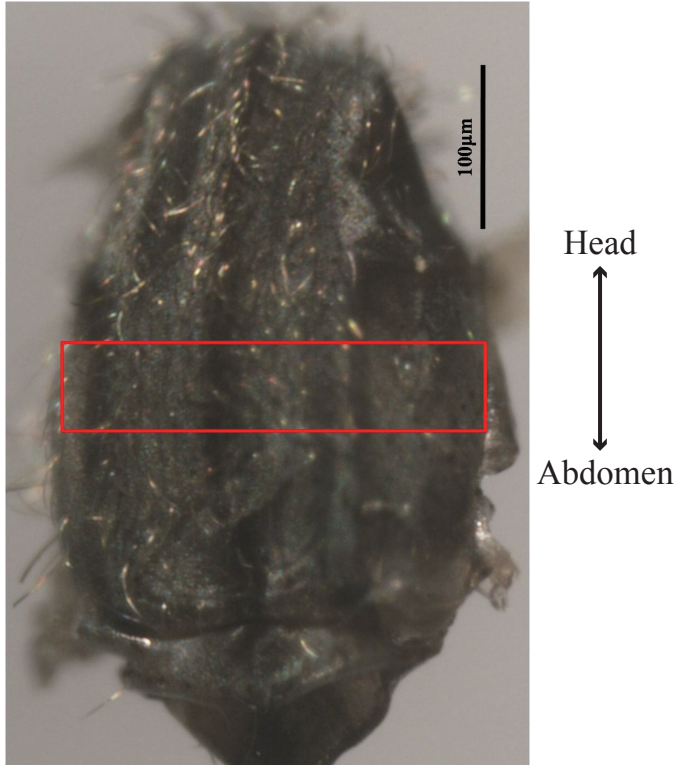


Figure S4

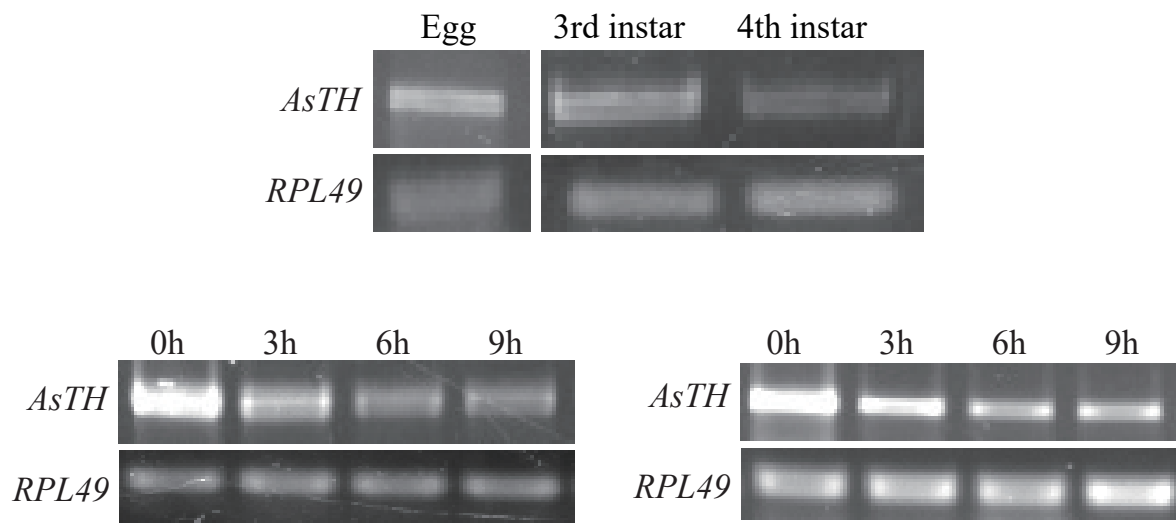


Figure S5

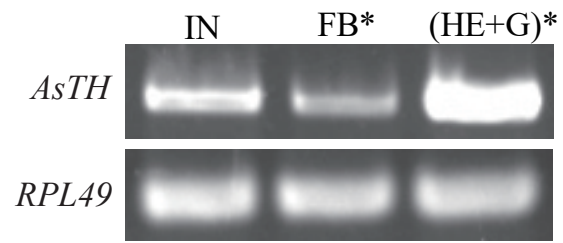


Figure S6

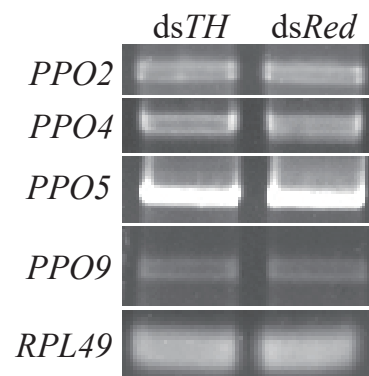


Table S1. Primers used in this study

Primer name	Sequence	
	F primer (5'---3')	R primer (5'---3')
Primers for <i>TH</i> cloning		
<i>AsTH</i> sequence 1	CCGATACCTGCCAAATCCAC	TGAGTCTGACGAGCCCTAACTCT
<i>AsTH</i> sequence 2	AAGACAAACTTCCGCAAGAGGT	CGTGTGGTAGGGCGAGTTG
<i>AsTH</i> sequence 3	CGGAAAGCGGTATGCATTCATA	TCATCCAGGACGGTTTGTITG
<i>AsTH</i> sequence 4	CCAGCTCAACACCGAACTGC	AGGGGAAGAGGGAGGATGAAC
3'RACE nested primer	CAGAGTTAGGGCTCGTCAGACTCAT	CGCTACGTAACGGCATGACAGTG
3'RACE primer	CTTATGAGGACGGACAGAGTTAGGG	GCTGTCAACGATACGCTACGTAACG
Primers used in RT-PCR		
<i>AsTH</i>	GCTGGCGTTCCGCATCTT	GCGATGGCATTGGTGAGGT
<i>RPL49</i>	GGAGCCGGTCGGTGATATGT	TTCCTTCTCGGTCGGCTTCG
<i>AsAttacin</i>	CGGGTTTTTCCTGATTTGTGC	GCGACCGAAGTGCTGCGTAT
<i>AsCecropin-A</i>	ATCTTCGTATTCGTCCTGCTGG	GCCTTCTTGCCGCCTTGA
<i>AsCecropin-B</i>	GCCTGTTCGTGGTGTGATG	AGCAGTGCCTTCTGAGCCG
<i>AsDefensin</i>	ATCGTGCTGGTTGTGGTGGTA	GCAGGTTGCCCGCTTGAA
<i>AsGambicin</i>	TTCTCCTCGCCGTCCTCCT	CCGTATCGTTGGCAGTCCG
<i>AsPPO 2</i>	CTGGACGAGGAGCGTGGTAT	AGTCACATGGTGCGTTAGGATC
<i>AsPPO 4</i>	GTGCGATGGCGGTGTTTG	TCGTGGATGTAGCCGAGAATG
<i>AsPPO 5</i>	GTGCCTTCGCCGACATCA	CATTGTATCGCAAACGACCCT
<i>AsPPO 9</i>	CGGGTGTCACGCTATGGGA	TCTTCACGGCATCGTCTTCG
Quantitative RT-PCR primers		
<i>AsDDC</i>	GGACAGGGTGGTGGTGTTATTCA	GATTGCCTTCTCCAGCGTTTC
<i>Aslaccase2</i>	GTTCCCCTAACGAGCACACATT	CTCCAAACCGTCTTCGCATC
<i>RPS7</i>	CGGAGAAGATGGCATGGGAGAT	ATAGTGAGCATAGGCCCGGTTA
Primers used for dsRNA template amplification		
<i>AsTH</i> sequence 1	TAATACGACTCACTATAGGGAGAAAGACAAACTTCCGCAAGAGGT	TAATACGACTCACTATAGGGAGACGTGTGGTAGGGCGAGTTG
<i>AsTH</i> sequence 2	TAATACGACTCACTATAGGGAGACCGATACTGCCAAATCCAC	TAATACGACTCACTATAGGGAGATGAGTCTGACGAGCCCTAACTCT
<i>Red</i>	TAATACGACTCACTATAGGGAGACTTCAAGGTGCGCATGGAG	TAATACGACTCACTATAGGGAGATGTGGATCTCGCCCTTCAG

Table S3. Statistic of RNAi (For Cuticle tanning, at 38th hour of pupation)

Treatment	Injected No.	No. of individuals with tanning impaired (alive)	No. of Death at 38 th hour of pupation
<i>dsTH</i>	58	32	6
<i>dsTH2</i>	42	18	5
dsRed (For <i>dsTH</i>)	49	0	5
dsRed (For <i>dsTH2</i>)	36	0	4