Supplementary Information for

Identification and characterization of genes involving the early step of Juvenile Hormone pathway in *Helicoverpa armigera*

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Supplementary Figures

Supplementary Figure 1： Diagram showing the JH biosynthetic pathway (modified from Bellés et al[11] and Noriega et al[10]). The mevalonate pathway is presented in the dashed box. Precursors are in bold and connected by arrows. Enzymes are in italics.

Supplementary Figure 2： Phylogenetic analysis of prenyltransferases from selected insects. The protein names and accession numbers used in this analysis are as follows: AgFpps: *Anopheles gambiae* (EAA04004); DaFpps: *Dendroctonus armandi* (ALF44684.1); TcFpps: *Tribolium castaneum* (NP_001164089.1); EgFpps: *Epicauta gorhami* (AHH34857.1); DmFpps: *Drosophila melanogaster* (NP_477380); DbFpps: *Drosophila busckii* (XP_017837938.1); HlFpps: *Habropoda laboriosa* (KOC62899.1); MpFpps1: *Myzus persicae* (ACA48701.1); MpFpps2: *Myzus persicae* (ABY19313.1); BmFpps1: *Bombyx mori* (BAF62113.1); BmFpps2: *Bombyx mori* (NP_001093301.1); BmFpps3: *Bombyx mori* (NP_001093302.1); MuFpps: *Mythimna unipuncta* (AAY33487.1); ObFpps: *Operophtera brumata* (KOB70829.1); CfFpps: *Choristoneura fumiferana* (AAY33486); PmFpps: *Papilio machaon* (KPJ18340.1); PxFpps: *Papilio xuthus* (KPJ05696.1).

Supplementary Figure 3： The expression levels among *HaFpps* genes in different tissues. He: heads; Ep: epidermis; Fb: fat body; Mg: midgut; Mt: malpighian tubules; Pm: peritrophic matrix. The bars represent the average (±SE) of biological repeats.

Supplementary Figure 4： Subcellular localization of HaFpps4-GFP in insect cells. The images were taken at 24 h post-transfection with the plasmid pHaFpps4-GFP. I, II and III represented three detected types.

Supplementary Figure 5： The full-length gels for each gene presented in Figure 2.

Supplementary Figure 6: The specificity of the qPCR primers

Supplementary Figure 7： Standard curve of JH-III pure chemical

Supplementary Figure 8： The chromatograph of JH-Ⅲ pure chemical (100 ng/μl) and sample (A standard, B sample)

Supplementary Tables

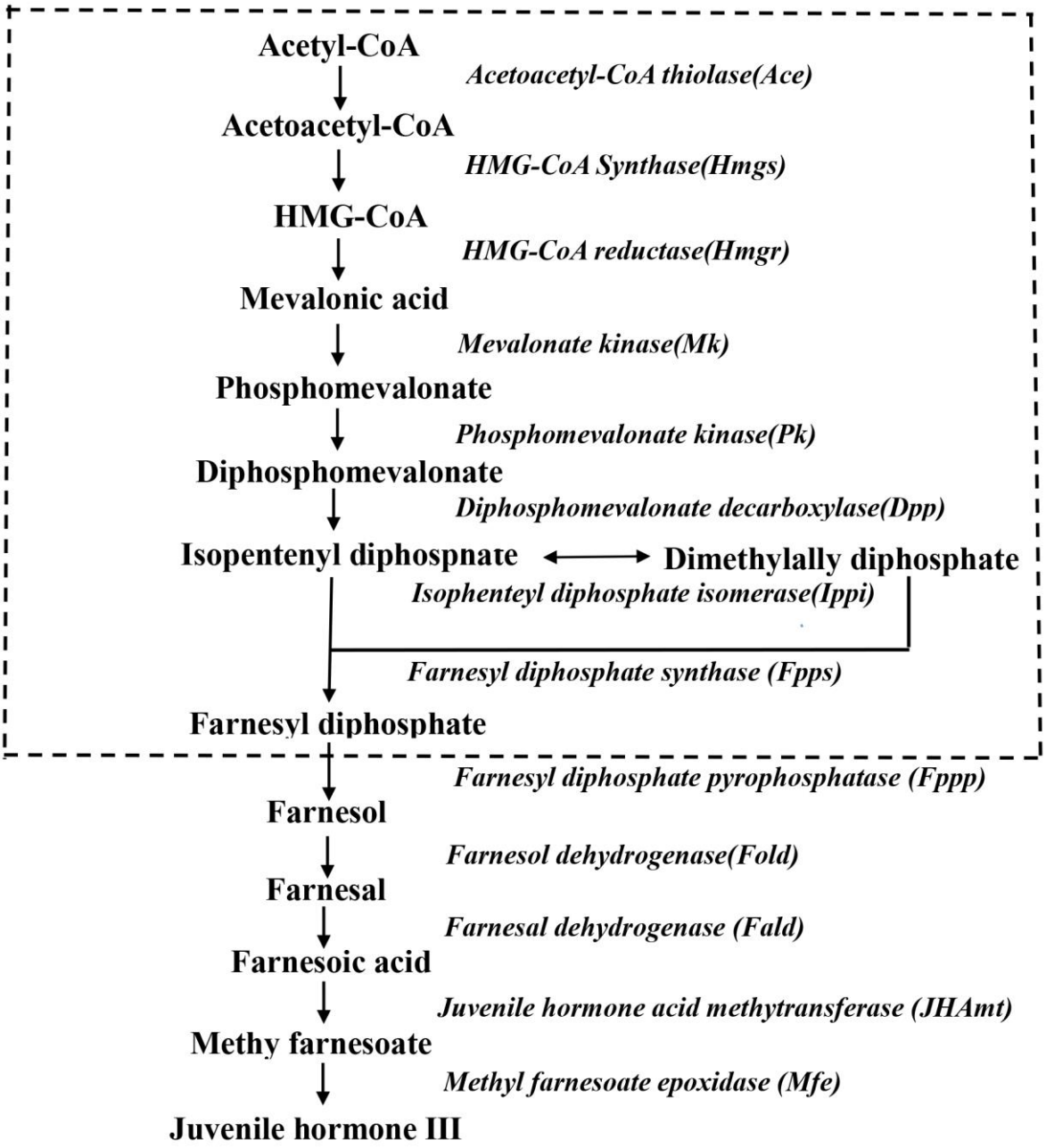
Supplementary Table 1： Enzyme-encoding genes in JH biosynthetic pathway in *H. armigera*

Supplementary Table 2： Primers used for the genomic sequence analysis.

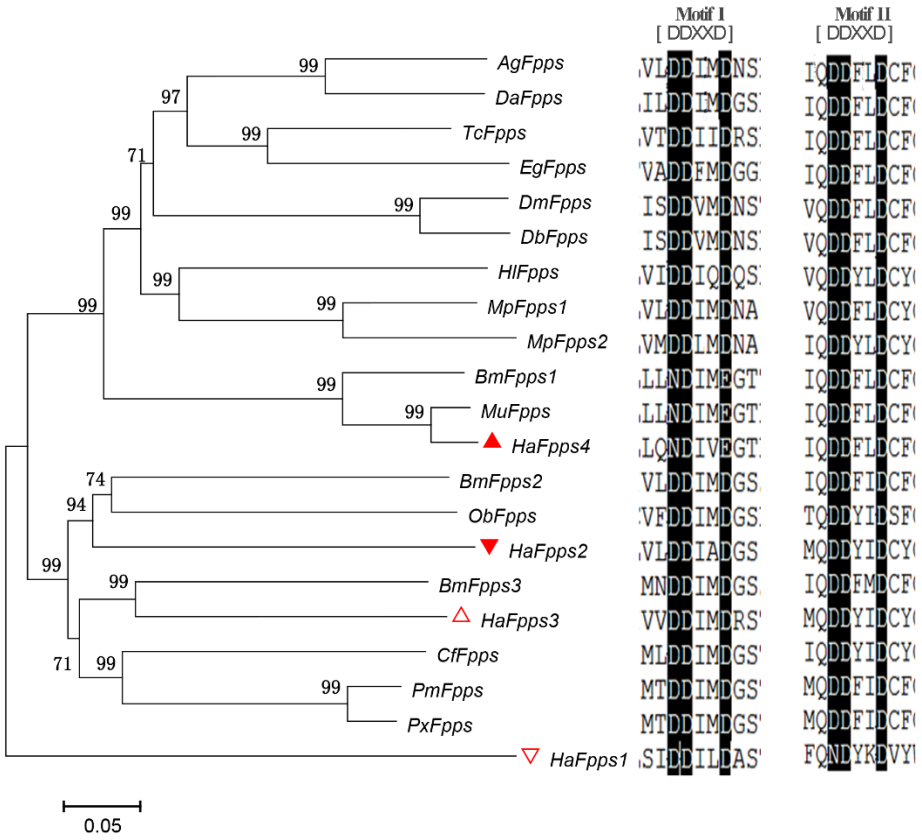
Supplementary sequences

Data set 1: Sequences of genes identified in our study

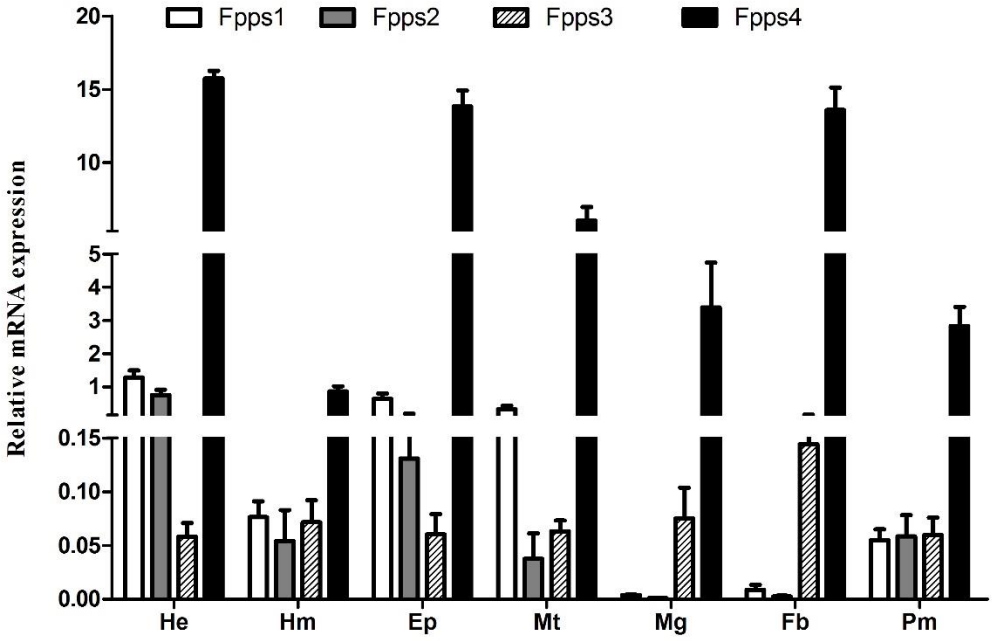
Supplementary Figure 1



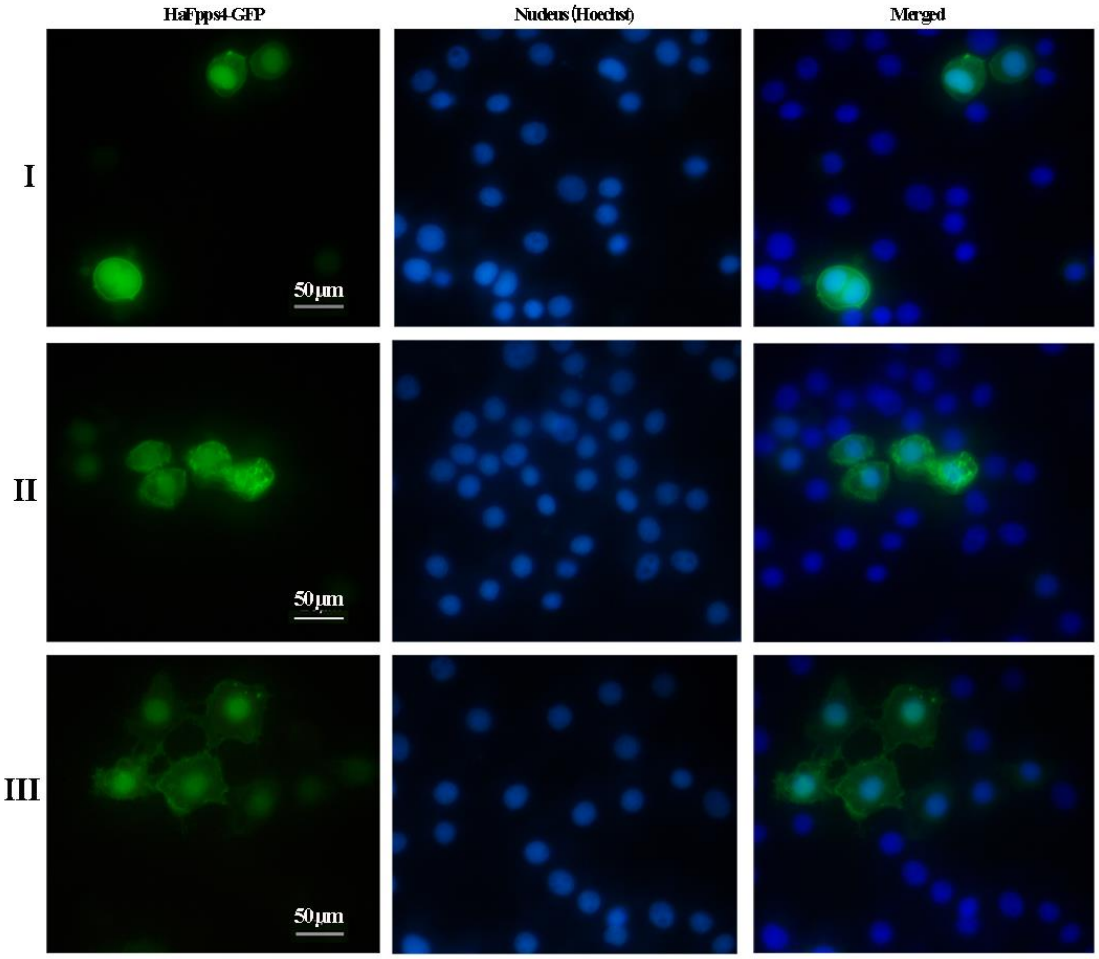
Supplementary Figure 2



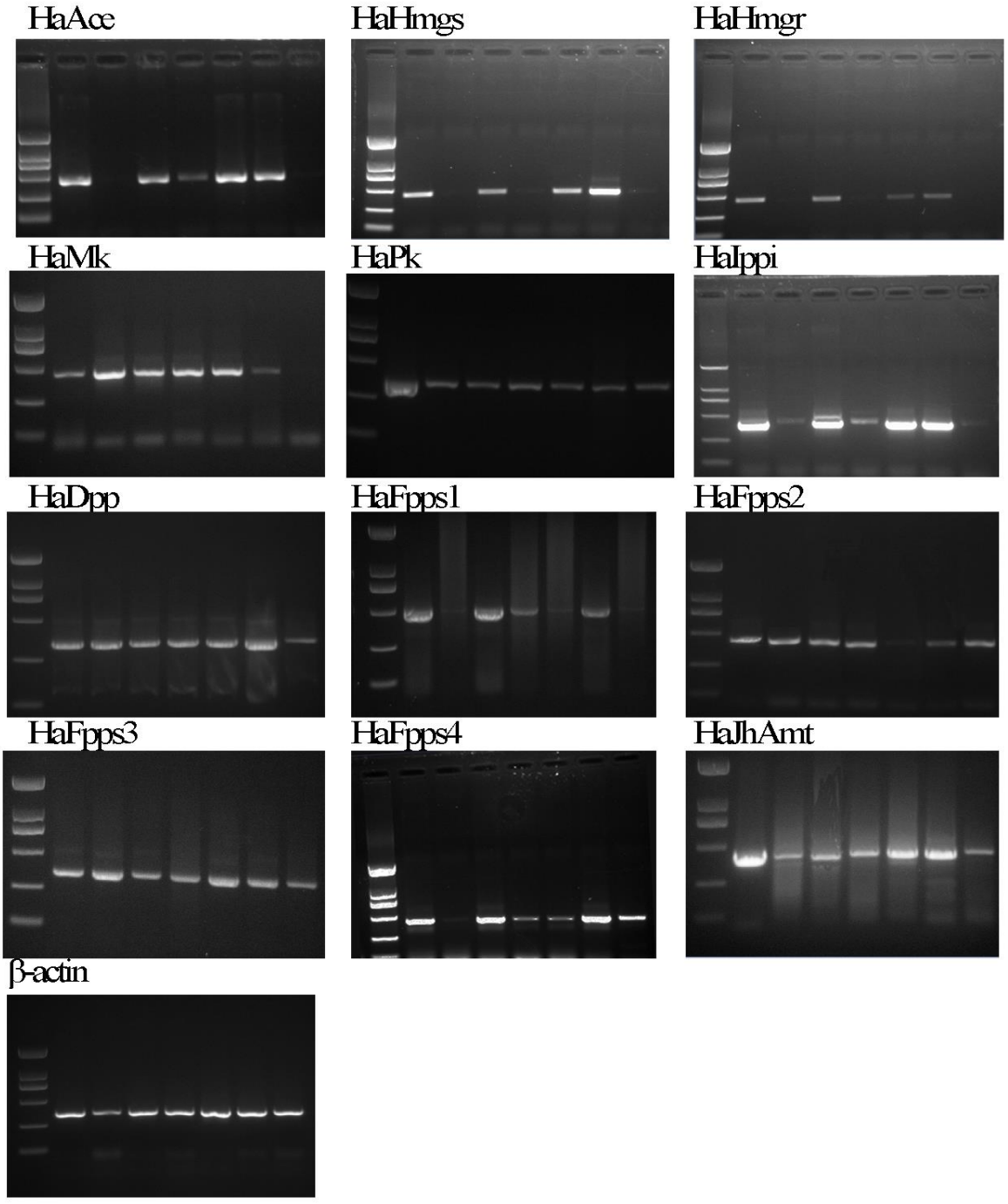
Supplementary Figure 3



Supplementary Figure 4

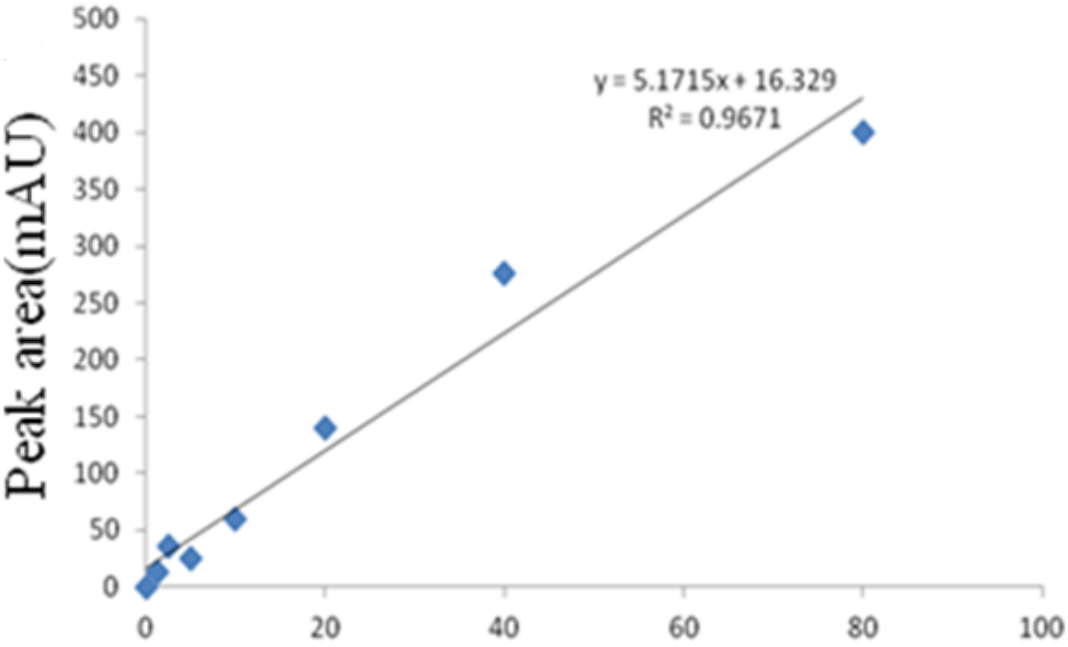


Supplementary Figure 5



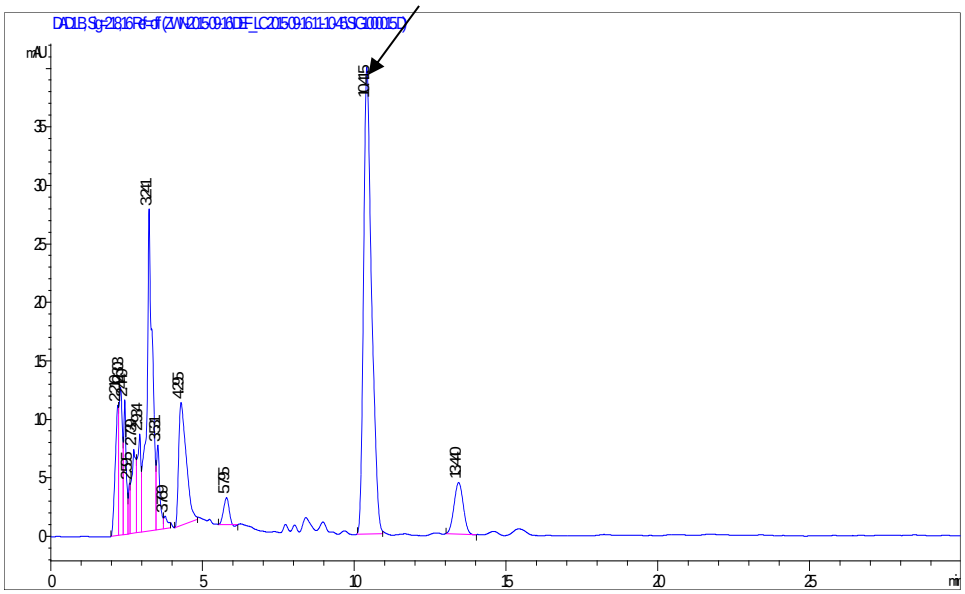
Supplementary Figure 6

Supplementary Figure 7

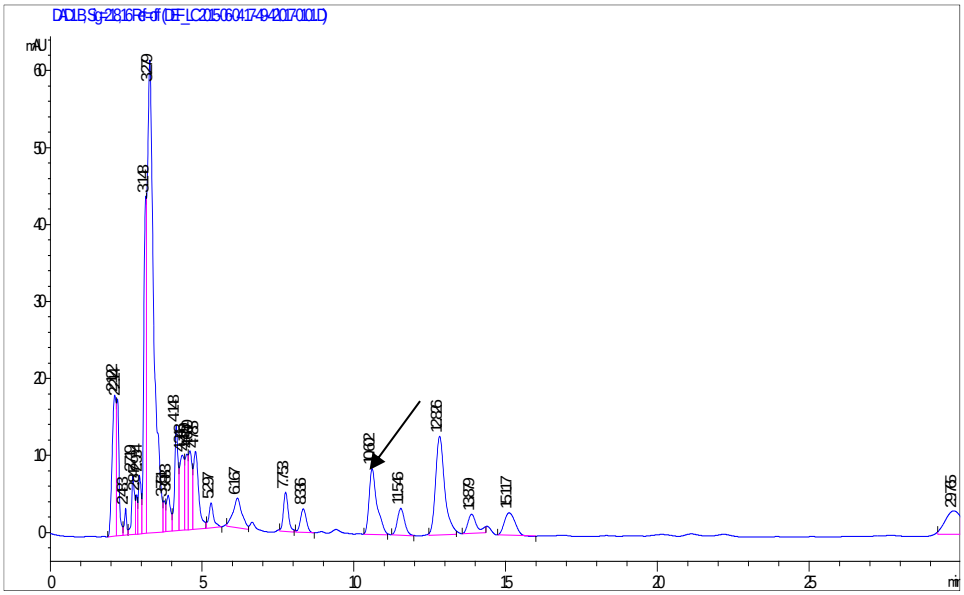


Supplementary Figure 8

A



B



Supplementary Table 1

Gene	Accession number	ORF(bp)	Peptide(aa)	Function	Identity	E-avlue
HaAce	KY71766	1272	423	catalyze 2 molecules of Acetyl-CoA to form acetoacetyl-CoA	81%	0.0
Hmgs	KY71767	1371	456	catalyze Acetoacetyl CoA+acetyl-CoA to form 3-hydroxy-3-methylglutaryl CoA, HMG-CoA	87%	0.0
HaMk	KY71768	1242	413	Phosphorylates mevalonate	83%	0.0
HaPk	KY71769	558	185	Phosphorylates phosphomevalonate	75%	$2e^{-87}$
HaDpp	KJ951986	1161	386	catalyze decarboxylation of diphosphomevalonate to form Isoamyl enol pyrophosphate	75%	0.0
HaIppi	KJ951988.1	759	252	isomerization of IPP to DMAPP	86%	$4e^{-166}$
HaFpps1	KY71770	1131	376	sequential condensation of IPP with DMAPP and then GPP to form FPP	39%	$3e^{-87}$
HaFpps2	KY71771	1164	387		51%	$1e^{-135}$
HaFpps3	KY71772	1155	384		57%	$3e^{-165}$
HaFpps4	KY71773	1284	427		94%	0.0

Supplementary Table 2

Name of Primers	Forward Sequences（5’-3’）	Reverse Sequences（5’-3’）
Ace-1	TTTTTTTTGAAAGGCAATAGA	TATTCTCAGCACAGTTACCCAT
Ace-2	TTTTTTTTGAAAGGCAATAGA	CCACCATTACAGACGGCA
Hmgr	ATGAAAGTCTGGGGAGCT	TAAATGTCTGCTGAGCTGCAAAC
Hmgs	ATGGGCGGAAGAGTTGA	TCAAACTTTCCTTGCATAA
Ippi-1	TCATTCTTTGTTGGGCTTTAC	GCAGCACTTCACCATCATCTC
Ippi-2	CAACTTCGTGGGAACTGCTA	TGAGACGATGAAGGTTGTCCC
Dpp	TCAAAGTAGTTTAGTAAATTATCGCTG	TTCTAAGAATATAAGATTTCAT
Mk-1	CGCAAAAGACTCTCACGGTT	TAATGATGTGTAGGAACTCGTCG
Mk-2	GTGGAGCCAACGATACGGAT	ATGCTGCCACCTTCACGA
Mk-3	TCGCTCATACAGCCACGC	TCAATCTCACACTTGCCACTG
Mk-4	TGAGATTGATAGTGAAGCGGA	CCGAGAGCAGACAGTGGTT
Mk-5	CCACTGTCTGCTCTCGG	GCTCACGATTCACTCACCCAT
Pk	TTGCTATTCAGCGGTAAGAGA	ATCATAAACCAGACAGCAACC
Fpps1-1	ATGGCCCTTTTGCTCCGTAA	GCAGTCATTAGTCGCCGAAGTA
Fpps1-2	TACTTCGGCGACTAATGACTGC	TTAATAATCTCTCAGTCGGCACG
Fpps2-1	TGCTGTTGGGAAAAGGATTAC	CACTATAAAGTCTCTGAAAGGT
Fpps2-2	ACCTTTCAGAGACTTTATAGTG	TCATGTTCTTACCGCCGATTA
Fpps2-3	TAATCGGCGGTAAGAACATGA	CTTTATTCGTTCTATGTGTGCC
Fpps3-1	AGAAAGTGAGCACGTAGCTAATATT	CTGGCAGAGCATCGTGAAA
Fpps3-2	AACTCCCTGAAGTGGGCAAT	AAGTCCAAGTGTTGCCCCAT
Fpps3-3	ATGGGGCAACACTTGGACTT	GTGACTGATTCGTCGCCGTA
Fpps3-4	ATGGGGCAACACTTGGACTT	GTAGGAGCGGTTTCAGAGGG
Fpps4-1	GCGAACAGACGCTACACCTA	GTAGCCATCGGCATTTCCT
Fpps4-2	ACGCAGGAAATGCCGATG	TGTAAGCCAGCACTGTCGC
Fpps4-3	ACGCAGGAAATGCCGATG	AGACCGACTTCTGGGTGCTT
Fpps4-4	CACAGTTCCACACTCACCAG	GAACAGGGTAAGGTCGGGCT
Fpps4-5	CACAGTTCCACACTCACCAG	CTGTAAGGCGACGACTGCGA

Data Set 1

>Helicoverpa armigera Acetyl-CoA thiolase

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>Helicoverpa armigera Hydroxymethylglutaryl-coenzyme A synthase

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>Helicoverpa armigera Mevalonate kinase

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>Helicoverpa armigera Phosphomevalonate kinase

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>Helicoverpa armigera Diphosphomevalonate decarboxylase

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>Helicoverpa armigera isopentenyl-diphosphate delta isomerase 1
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>Helicoverpa armigera Farnesyl pyrophosphate synthase 1
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>Helicoverpa armigera Farnesyl pyrophosphate synthase 2
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>Helicoverpa armigera Farnesyl pyrophosphate synthase 3

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>Helicoverpa armigera Farnesyl pyrophosphate synthase 4

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