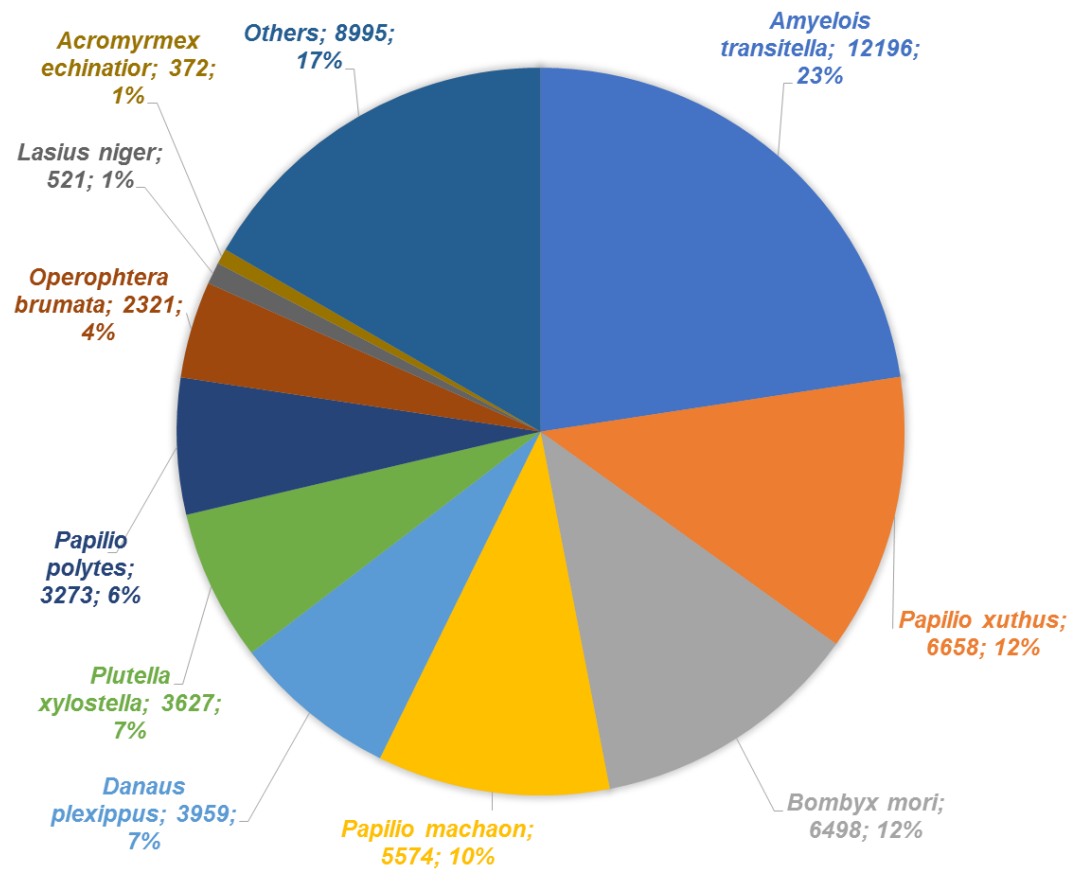
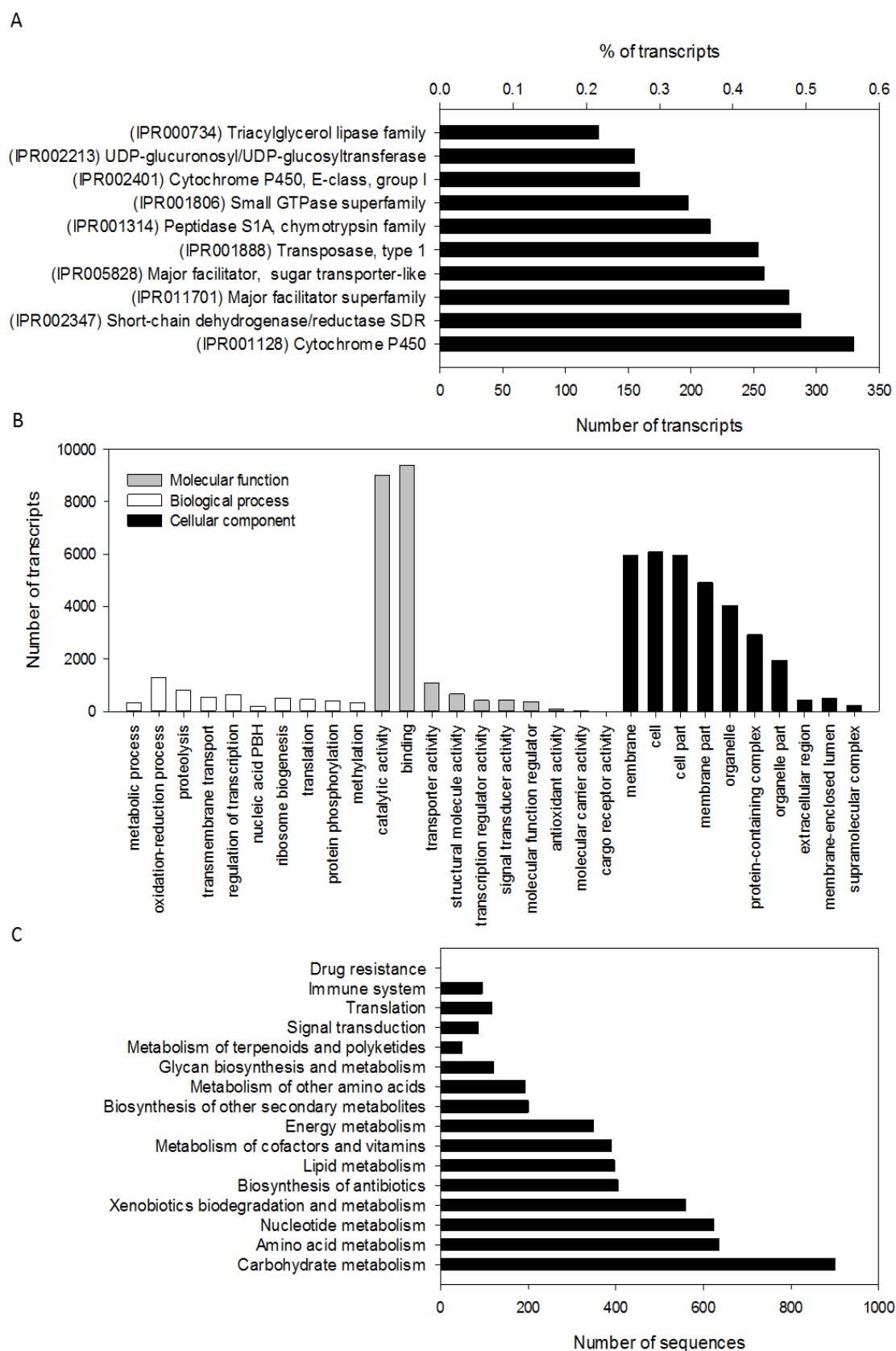
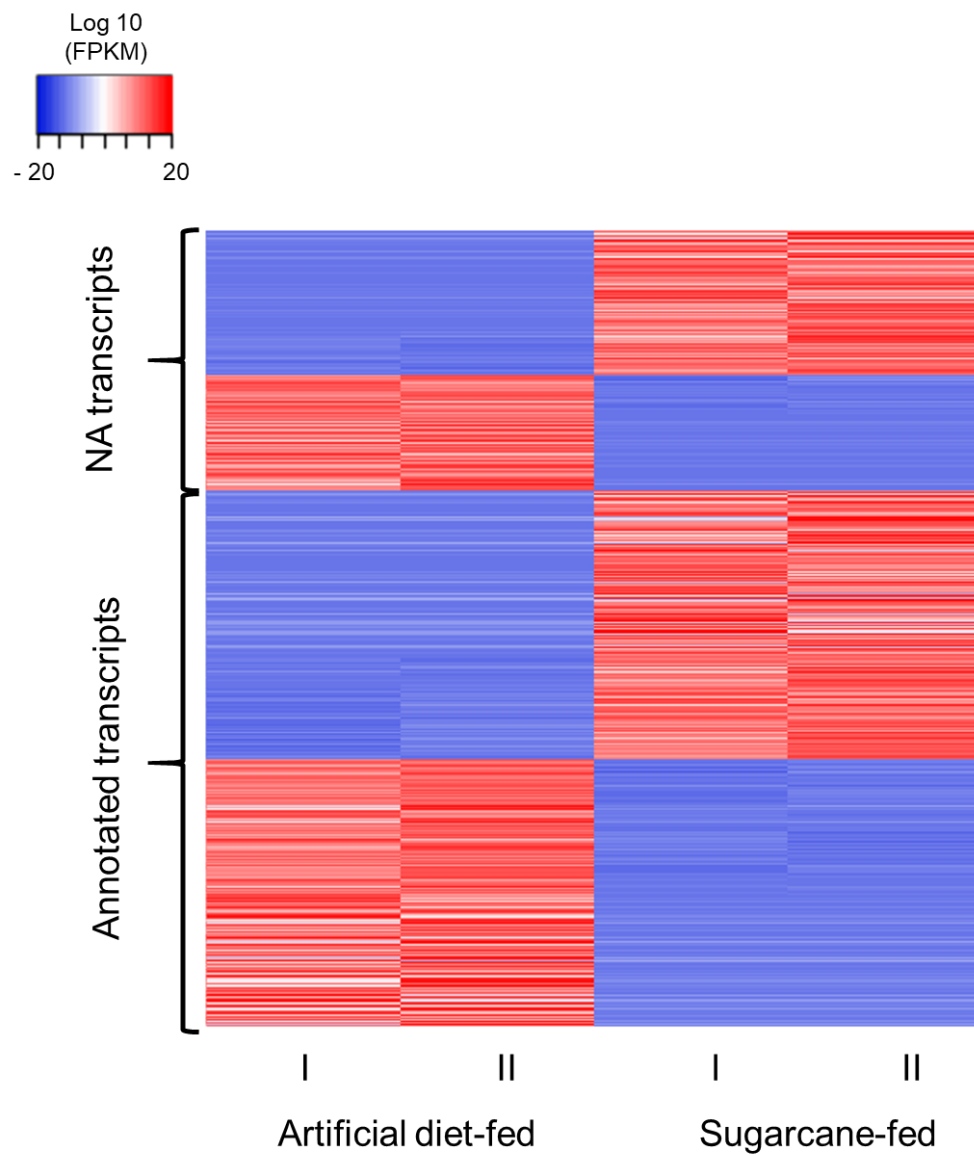




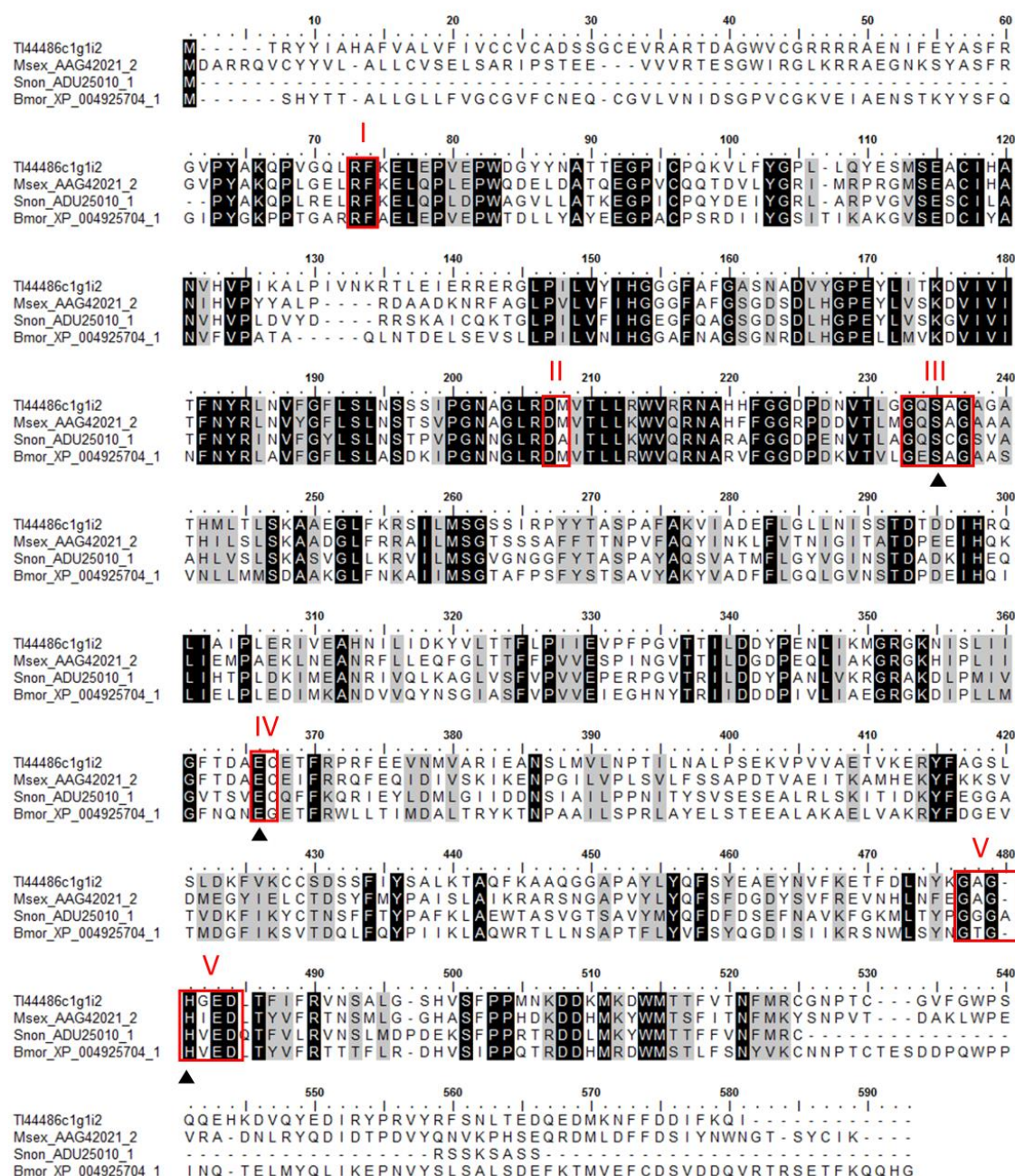
**Figure S1.** Distribution of top-hit species from BLAST analysis comparing the SGB transcriptome to the NR database of the NCBI. Legend shows species name; number of hits (match contigs); percent.



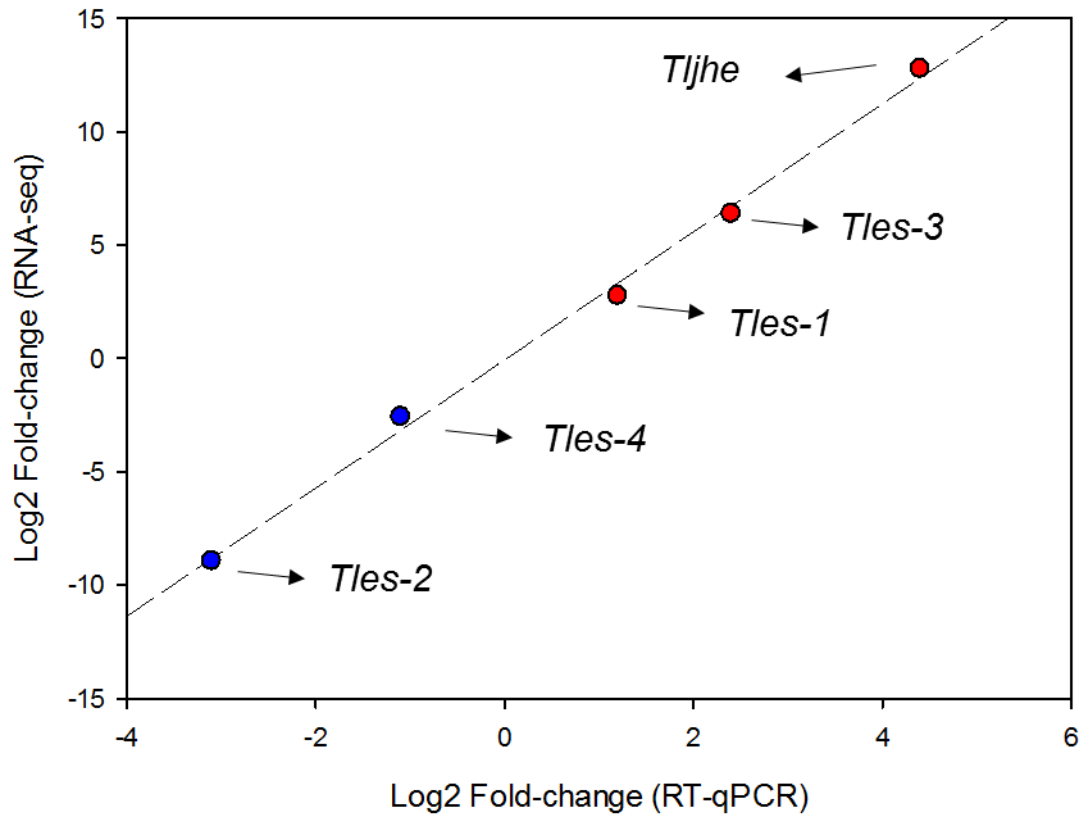
**Figure S2.** Annotation summary of SGB transcriptome. A) Top ten protein families (y-axis) annotated by using InterPro database. B) Gene ontology distribution of level 2 terms (x-axis). C) Distribution of metabolic pathways (y-axis) annotated with KEGG database.



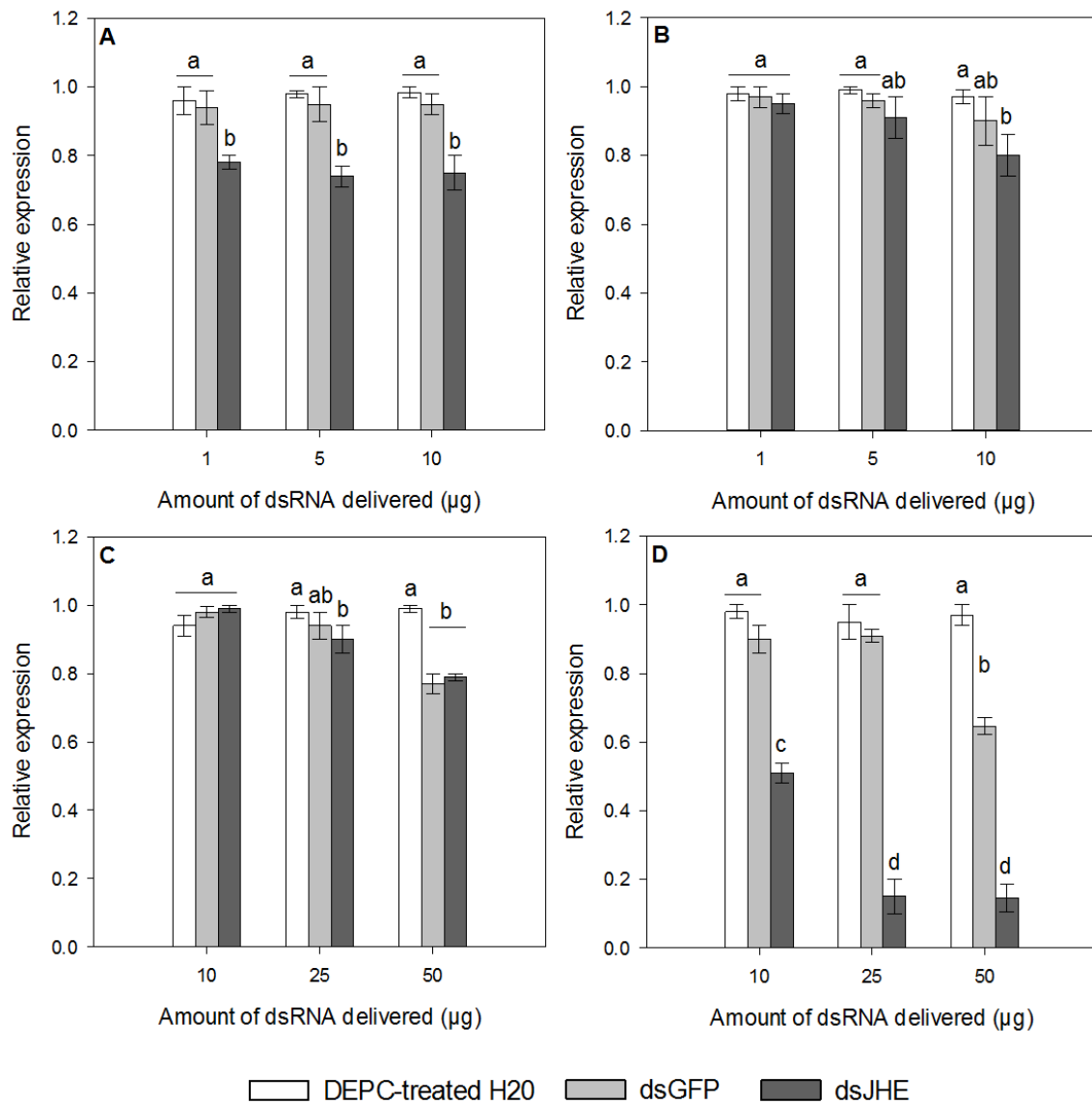
**Figure S3.** Heatmap showing distribution of differentially expressed genes of the SGB treated with different diet sources. Roman numerals represent different biological replicates. FPKM: fragments per kilo base per million of reads. NA: without hits in the protein NR database from the NCBI.



**Figure S4.** Amino acid sequence comparison between Tljhe (TI44486c1g1i2) and other insect JHEs. Sequences are aligned by using the MAFFT algorithm. The major catalytic motifs conserved in all insect JHEs are within red boxes and identified by roman numerals in red. The residues that form the catalytic triad are indicated by black triangles. Species abbreviations correspond to Msex: *Manduca sexta*. Bmor: *Bombyx mori*. Snon: *Sesamia nonagrioides*. Accession numbers (NCBI) are given right to the species abbreviation. Residues shaded in black and grey represent 100% and 80% conserved regions, respectively.



**Figure S5.** Correlation between expression values of DEGs encoding predicted esterases from *T. l. licus*, measured by RNA-seq and RT-qPCR. Circles represent transcripts overexpressed (red) and underexpressed (blue) in sugarcane-fed larvae compared with larvae fed on artificial diet. ***Tljhe***: juvenile hormone esterase. ***Tles-1***: esterase from contig Tlic2317c4g1i1. ***Tles-2***: esterase from contig Tlic44789c0g1i1. ***Tles-3***: esterase from contig Tlic38218c1g4i5. ***Tles-4***: esterase from contig Tlic48599c3g1i4.



**Figure S6. Expression of *TlJhe* as response to the delivery of dsJHE molecules.** (A) Expression in carcass after microinjection (B) Expression in carcass after oral delivery (C) Expression in gut after microinjection (D) Expression in gut after oral delivery. Treated larvae were all at L5 instar. Error bars were obtained from at least three independent biological replicates. Different lowercase letters represent statistical differences between treatments (Tukey-TSD  $p < 0.05$ ).

**Table S1.** Composition of artificial diet for rearing of *T. l. licus* larvae.

| COMPOUND               | AMOUNT     |
|------------------------|------------|
| Casein                 | 20 g       |
| Yeast extract          | 10 g       |
| Sugar                  | 60 g       |
| Ascorbic acid          | 10 g       |
| Vitamins solution      | 11 mL      |
| Wesson salts           | 7.5 g      |
| Cholesterol            | 300 mg     |
| Sodium benzoate        | 300 mg     |
| Ampicillin             | 80 mg      |
| Nystatin               | 800,000 UI |
| Choline chloride (50%) | 2 mL       |
| Distillated-water      | 800 mL     |
| Agar                   | 25.1 g     |

**Table S2.** List of primers used for RT-qPCR. Efficiency show was calculated using Real-time PCR Miner software. **Fw:** Forward primer. **Rv:** Reverse primer. ***β-act*:** β-actin. ***α-tub*:** α-tubulin. ***gapdh*:** Glyceraldehyde 3-phosphate dehydrogenase. ***ef-1α*:** Translation elongation factor 1 alpha. ***ubq*:** ubiquitin. ***prpp*:** Phosphoribosyl pyrophosphate synthetase. ***rps18*:** ribosomal subunit 18S. ***Tljhe*:** juvenile hormone esterase. ***Tles-1*:** esterase from contig Tlic2317c4g1i1. ***Tles-2*:** esterase from contig Tlic44789c0g1i1. ***Tles-3*:** esterase from contig Tlic38218c1g4i5. ***Tles-4*:** esterase from contig Tlic48599c3g1i4.

| Gene                 | Primer Sequence Forward (5'-3')                        | Amplicon (bp) | Mean Efficiency |
|----------------------|--------------------------------------------------------|---------------|-----------------|
| <b><i>β-act</i></b>  | Fw: ATGGTCGGTATGGGTCAGAA<br>Rv: ATGTCGTCCCAGTTGGTGAT   | 116           | 1.81            |
| <b><i>α-tub</i></b>  | Fw: ACTTGGTACTCGACCGCATC<br>Rv: ATGAGGAGGGAGGTGAAACC   | 115           | 1.88            |
| <b><i>gapdh</i></b>  | Fw: AAAGTAAAGGAGGCCGCTGT<br>Rv: CAGCAGCATACGAAGATGGA   | 116           | 1.91            |
| <b><i>ef-1α</i></b>  | Fw: AACATTGTCGTCATCGGACA<br>Rv: GGCCTCCTTCTCGAACTTCT   | 114           | 1.89            |
| <b><i>ubq</i></b>    | Fw: CAATGCAAGTTGTTTCGATTCA<br>Rv: GGTCTGCTGCTGGTAAACCT | 113           | 1.83            |
| <b><i>prpp</i></b>   | Fw: ACAGCTATCATGGTGGACGA<br>Rv: CACACCGTGGGTTGATATTG   | 115           | 1.93            |
| <b><i>rps18</i></b>  | Fw: ACGGTGAAAATCCAGTTTGG<br>Rv: GGACACGGATTCCCAGTAGA   | 118           | 1.92            |
| <b><i>Tljhe</i></b>  | Fw: TTGTTGAGTCGTCGCTTCC<br>Rv: TGAAGCCCACGATGAATGG     | 105           | 1.90            |
| <b><i>Tles-1</i></b> | Fw: AGGCTGAGATGCTTGAATGG<br>Rv: GCTGCGTGGTATGGATACTG   | 109           | 1.89            |
| <b><i>Tles-2</i></b> | Fw: ATCCATGGAGGTGGATTCTG<br>Rv: CCAAGTACTTCGAGTCTGTAG  | 118           | 1.88            |
| <b><i>Tles-3</i></b> | Fw: CAGCGAAACAACCAATATACC<br>Rv: CAATTCATCGGCATGACACAC | 114           | 1.89            |
| <b><i>Tles-4</i></b> | Fw: TGCTGGCCTCAAAGATGTAG<br>Rv: ACCACAGCTCTCACCAAATAC  | 106           | 1.89            |

**Table S3.** Summary of reads generated by Illumina sequencing and assembly statistics.

| <b>Library (Treatment)</b> | Number of sequenced reads (high-quality reads) |
|----------------------------|------------------------------------------------|
| Sugarcane-fed I            | 12,322,293 (11,817,513)                        |
| Sugarcane-fed II           | 26,067,335 (25,024,195)                        |
| Artificial diet-fed I      | 14,837,428 (14,244,726)                        |
| Artificial diet-fed II     | 20,991,014 (20,219,338)                        |
| Reference sample           | 57,057,629 (42,450,109)                        |
| <b>Assembly statistics</b> |                                                |
| Number of Unigenes         | 85,201                                         |
| Number of Contigs          | 121,538                                        |
| GC %                       | 36.71                                          |
| Completed BUSCOs           | 1,045 (98.0%)                                  |
| Single copy BUSCOs         | 596 (55.9%)                                    |
| Duplicated BUSCOs          | 449 (42.1%)                                    |
| <b>Contigs Statistics</b>  | Contigs                                        |
| Median (bp)                | 643                                            |
| Mean (bp)                  | 1,190.69                                       |
| Total assembled bases:     | 144,713,645                                    |
| Contig N50 (bp)            | 2,014                                          |

**Table S4.** List of significant GO terms obtained by enrichment analysis of the DEGs found in the transcriptome of the SGB. FDR: false discovery rate value. \*Active with either ribo- or deoxyribonucleic acids and producing 5'-phosphomonoesters. N: number of transcripts.

| <b>GO Category</b>        | <b>GO name</b>                | <b>GO Id</b> | <b>N Up</b> | <b>N Down</b> | <b>FDR</b> |
|---------------------------|-------------------------------|--------------|-------------|---------------|------------|
| <b>Biological Process</b> | transmembrane transport       | GO:0055085   | 67          | 0             | 0.00       |
|                           | establishment of localization | GO:0051234   | 91          | 0             | 0.00       |
|                           | transport                     | GO:0006810   | 91          | 0             | 0.00       |
|                           | localization                  | GO:0051179   | 91          | 0             | 0.01       |
|                           | DNA integration               | GO:0015074   | 30          | 0             | 0.02       |
|                           | transposition                 | GO:0032196   | 18          | 0             | 0.02       |
|                           | transposition, DNA-mediated   | GO:0006313   | 18          | 0             | 0.02       |
|                           | DNA recombination             | GO:0006310   | 19          | 0             | 0.03       |
|                           | amino sugar metabolic process | GO:0006040   | 2           | 12            | 0.00       |
|                           | aminoglycan metabolic process | GO:0006022   | 2           | 12            | 0.04       |
|                           | chitin metabolic process      | GO:0006030   | 2           | 12            | 0.00       |

|                           |                                                           |            |     |     |      |
|---------------------------|-----------------------------------------------------------|------------|-----|-----|------|
|                           | glucosamine-containing compound metabolic process         | GO:1901071 | 2   | 12  | 0.00 |
|                           | lipid metabolic process                                   | GO:0006629 | 7   | 23  | 0.00 |
|                           | metabolic process                                         | GO:0008152 | 243 | 289 | 0.00 |
|                           | oxidation-reduction process                               | GO:0055114 | 42  | 68  | 0.00 |
| <b>Cellular Component</b> | integral component of membrane                            | GO:0016021 | 92  | 0   | 0.00 |
|                           | intrinsic component of membrane                           | GO:0031224 | 92  | 0   | 0.00 |
|                           | membrane part                                             | GO:0044425 | 95  | 0   | 0.00 |
|                           | membrane                                                  | GO:0016020 | 119 | 0   | 0.02 |
|                           | mitochondrial outer membrane                              | GO:0005741 | 6   | 0   | 0.04 |
|                           | organelle outer membrane                                  | GO:0031968 | 6   | 0   | 0.04 |
|                           | outer membrane                                            | GO:0019867 | 6   | 0   | 0.04 |
|                           | collagen trimer                                           | GO:0005581 | 0   | 3   | 0.02 |
|                           | extracellular region                                      | GO:0005576 | 4   | 24  | 0.00 |
|                           | microbody                                                 | GO:0042579 | 1   | 6   | 0.01 |
|                           | peroxisome                                                | GO:0005777 | 1   | 6   | 0.01 |
| <b>Molecular Function</b> | transmembrane transporter activity                        | GO:0022857 | 56  | 0   | 0.00 |
|                           | hydrolase activity, acting on ester bonds                 | GO:0016788 | 31  | 0   | 0.02 |
|                           | active transmembrane transporter activity                 | GO:0022804 | 17  | 0   | 0.02 |
|                           | endoribonuclease activity                                 | GO:0004521 | 11  | 0   | 0.02 |
|                           | transporter activity                                      | GO:0005215 | 57  | 0   | 0.02 |
|                           | RNA-DNA hybrid ribonuclease activity                      | GO:0004523 | 9   | 0   | 0.02 |
|                           | hydrolase activity                                        | GO:0016787 | 132 | 0   | 0.02 |
|                           | endoribonuclease activity, producing 5'-phosphomonoesters | GO:0016891 | 10  | 0   | 0.02 |
|                           | endonuclease activity*                                    | GO:0016893 | 10  | 0   | 0.03 |
|                           | cofactor binding                                          | GO:0048037 | 43  | 48  | 0.04 |
|                           | neurotransmitter transporter activity                     | GO:0005326 | 6   | 0   | 0.04 |
|                           | neurotransmitter:sodium symporter activity                | GO:0005328 | 6   | 0   | 0.04 |
|                           | solute:sodium symporter activity                          | GO:0015370 | 6   | 0   | 0.04 |
|                           | secondary active transmembrane transporter activity       | GO:0015291 | 9   | 0   | 0.04 |
|                           | ribonuclease activity                                     | GO:0004540 | 11  | 0   | 0.04 |

|                                                                                                                      |            |     |     |      |
|----------------------------------------------------------------------------------------------------------------------|------------|-----|-----|------|
| symporter activity                                                                                                   | GO:0015293 | 8   | 0   | 0.05 |
| endonuclease activity                                                                                                | GO:0004519 | 11  | 0   | 0.05 |
| nuclease activity                                                                                                    | GO:0004518 | 14  | 0   | 0.05 |
| catalytic activity                                                                                                   | GO:0003824 | 255 | 271 | 0.00 |
| chitin binding                                                                                                       | GO:0008061 | 2   | 12  | 0.00 |
| cofactor binding                                                                                                     | GO:0048037 | 43  | 48  | 0.00 |
| endopeptidase activity                                                                                               | GO:0004175 | 26  | 37  | 0.01 |
| heme binding                                                                                                         | GO:0020037 | 20  | 33  | 0.00 |
| iron ion binding                                                                                                     | GO:0005506 | 18  | 33  | 0.00 |
| oxidoreductase activity                                                                                              | GO:0016491 | 45  | 68  | 0.00 |
| oxidoreductase activity,<br>acting on paired<br>donors, with<br>incorporation or<br>reduction of molecular<br>oxygen | GO:0016705 | 19  | 35  | 0.00 |
| peptidase activity                                                                                                   | GO:0008233 | 50  | 52  | 0.05 |
| peptidase activity,<br>acting on L-amino acid<br>peptides                                                            | GO:0070011 | 49  | 52  | 0.04 |
| protein-N-terminal<br>asparagine<br>amidohydrolase activity                                                          | GO:0008418 | 0   | 3   | 0.02 |
| serine-type<br>endopeptidase activity                                                                                | GO:0004252 | 22  | 26  | 0.05 |
| tetrapyrrole binding                                                                                                 | GO:0046906 | 20  | 33  | 0.00 |

**Table S5.** Stability (M) values of candidate reference genes evaluated with geNorm. Smaller M values correspond to more stable genes.

| Reference gene | M value |
|----------------|---------|
| <i>β-act</i>   | 1.00    |
| <i>α-tub</i>   | 0.33    |
| <i>gapdh</i>   | 0.19    |
| <i>ef-1α</i>   | 0.41    |
| <i>ubq</i>     | 0.22    |
| <i>prpp</i>    | 0.70    |
| <i>rps18</i>   | 0.20    |

**Table S6.** Significant hits from blastn analysis using as query the DNA sequence of *Tljhe* gene identified in the transcriptome of *Telchin licus licus*.

|                                            | Accession number | % identity | alignment length | mismatches | gap opens | evaluate |
|--------------------------------------------|------------------|------------|------------------|------------|-----------|----------|
| <i>Drosophila melanogaster</i><br>(7 hits) | CP023337.1       | 83.33      | 54               | 9          | 0         | 8.41E-06 |
|                                            | CP023331.1       | 83.33      | 54               | 9          | 0         | 8.41E-06 |
|                                            | AE014296.5       | 83.33      | 54               | 9          | 0         | 8.41E-06 |
|                                            | NM_141064.3      | 83.33      | 54               | 9          | 0         | 8.41E-06 |
|                                            | AC010049.7       | 83.33      | 54               | 9          | 0         | 8.41E-06 |
|                                            | AC010701.3       | 83.33      | 54               | 9          | 0         | 8.41E-06 |
|                                            | AY051599.1       | 83.33      | 54               | 9          | 0         | 8.41E-06 |
| <i>Apis mellifera</i><br>(4 hits)          | XM_026442009.1   | 78.18      | 55               | 12         | 0         | 0.002    |
|                                            | XM_016912970.2   | 78.18      | 55               | 12         | 0         | 0.002    |
|                                            | XM_026442008.1   | 78.18      | 55               | 12         | 0         | 0.002    |
|                                            | XM_026442007.1   | 78.18      | 55               | 12         | 0         | 0.002    |
| <i>Dendroctonus ponderosae</i><br>(7 hits) | XM_019913955.1   | 83.02      | 53               | 9          | 0         | 2.01E-06 |
|                                            | BT128413.1       | 83.02      | 53               | 9          | 0         | 2.01E-06 |
|                                            | XM_019900697.1   | 68.00      | 125              | 40         | 0         | 0.001    |
|                                            | XM_019900696.1   | 68.00      | 125              | 40         | 0         | 0.001    |
|                                            | XM_019898804.1   | 68.00      | 125              | 40         | 0         | 0.001    |
|                                            | BT126863.1       | 68.00      | 125              | 40         | 0         | 0.001    |
|                                            | XM_019913361.1   | 96.00      | 25               | 1          | 0         | 0.044    |
| <i>Bombyx mori</i><br>(11 hits)            | AK383719.1       | 68.98      | 361              | 108        | 2         | 3.25E-32 |
|                                            | AK383719.1       | 69.30      | 228              | 52         | 8         | 1.14E-12 |
|                                            | AK383719.1       | 68.35      | 158              | 46         | 2         | 3.71E-06 |
|                                            | AY489292.1       | 68.98      | 361              | 108        | 2         | 3.25E-32 |
|                                            | AY489292.1       | 68.86      | 228              | 53         | 8         | 4.83E-11 |
|                                            | AY489292.1       | 68.99      | 158              | 45         | 2         | 3.05E-07 |
|                                            | NM_001043562.1   | 68.98      | 361              | 108        | 2         | 3.25E-32 |
|                                            | NM_001043562.1   | 69.30      | 228              | 52         | 8         | 1.14E-12 |
|                                            | NM_001043562.1   | 68.35      | 158              | 46         | 2         | 3.71E-06 |
|                                            | XM_012689988.2   | 66.30      | 273              | 90         | 2         | 4.83E-11 |

|                |       |     |    |   |          |
|----------------|-------|-----|----|---|----------|
| XM_012689986.2 | 66.30 | 273 | 90 | 2 | 4.83E-11 |
|----------------|-------|-----|----|---|----------|

**Table S7.** Summary of GAM and linear regression models for analysis of weight loss in larvae feed on dsRNA-containing diets. Linear regression model is described as Weight Mean =  $b_0 + b_1 \cdot \text{Day after initial delivery of diet}$ . H<sub>2</sub>O: nuclease-free water.

| Treatment                        | GAM deviance (%) | Day Z | Regression Before day Z                                                   | Regression After day Z                                                    |
|----------------------------------|------------------|-------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| L5-treated with H <sub>2</sub> O | 97,0             | 18    | b <sub>0</sub> : 2.23 ± 0.404 (se)<br>b <sub>1</sub> : 0.06 ± 0.029 (se)  | b <sub>0</sub> : 5.88 ± 0.250 (se)<br>b <sub>1</sub> : -0.13 ± 0.010 (se) |
| L5-treated with dsGFP            | 98,5             | 18    | b <sub>0</sub> : 2.10 ± 0.349 (se)<br>b <sub>1</sub> : 0.07 ± 0.025 (se)  | b <sub>0</sub> : 5.67 ± 0.136 (se)<br>b <sub>1</sub> : -0.12 ± 0.005 (se) |
| L5-treated with dsJHE            | 99.5             | 10    | b <sub>0</sub> : 2.16 ± 0.082 (se)<br>b <sub>1</sub> : 0.05 ± 0.013 (se)  | b <sub>0</sub> : 3.32 ± 0.150 (se)<br>b <sub>1</sub> : -0.08 ± 0.007 (se) |
| L6-treated with H <sub>2</sub> O | 67.5             | x     | b <sub>0</sub> : 3.52 ± 0.211 (se)<br>b <sub>1</sub> : -0.10 ± 0.076 (se) |                                                                           |
| L6-treated with dsGFP            | 65.9             | x     | b <sub>0</sub> : 3.56 ± 0.412 (se)<br>b <sub>1</sub> : -0.11 ± 0.055 (se) |                                                                           |
| L6-treated with dsJHE            | 60.0             | x     | b <sub>0</sub> : 3.55 ± 0.321 (se)<br>b <sub>1</sub> : -0.12 ± 0.096 (se) |                                                                           |

**Table S8.** Predicted number of siRNAs obtained from mapping the dsJHE molecule to the sequences with significant hits in the BLAST analysis.

| Parameters             | Sequence (Accession number: GeneBank) | Non-suitable siRNAs | Suitable siRNAs |
|------------------------|---------------------------------------|---------------------|-----------------|
| <b>19-lenght siRNA</b> |                                       |                     |                 |
| <b>0 mismatches</b>    |                                       | 0                   | 0               |
|                        | <i>Tljhe</i> (Control)                | 285                 | 139             |
| <b>1 mismatches</b>    | AK383719.1                            | 3                   | 0               |
|                        | AY489292.1                            | 3                   | 0               |
|                        | NM_001043562.1                        | 3                   | 0               |
|                        | CP023337.1                            | 2                   | 0               |
|                        | AE014296.5                            | 2                   | 0               |
|                        | CP023331.1                            | 2                   | 0               |
|                        | <i>Tljhe</i> (Control)                | 285                 | 0               |
|                        |                                       |                     |                 |
| <b>2 mismatches</b>    | AE014296.5                            | 83                  | 0               |
|                        | CP023331.1                            | 60                  | 0               |
|                        | CP023337.1                            | 59                  | 0               |
|                        | AK383719.1                            | 10                  | 0               |
|                        | AY489292.1                            | 10                  | 0               |

|                        |                        |     |     |
|------------------------|------------------------|-----|-----|
|                        | NM_001043562.1         | 10  | 0   |
|                        | <i>Tljhe</i> (Control) | 285 | 0   |
| <b>20-lenght siRNA</b> |                        |     |     |
| <b>0 mismatches</b>    |                        | 0   | 0   |
|                        | <i>Tljhe</i> (Control) | 284 | 140 |
| <b>1 mismatches</b>    | AK383719.1             | 2   | 0   |
|                        | AY489292.1             | 2   | 0   |
|                        | NM_001043562.1         | 2   | 0   |
|                        | <i>Tljhe</i> (Control) | 284 | 0   |
| <b>2 mismatches</b>    | AE014296.5             | 32  | 0   |
|                        | CP023337.1             | 20  | 0   |
|                        | CP023331.1             | 20  | 0   |
|                        | AK383719.1             | 8   | 0   |
|                        | AY489292.1             | 8   | 0   |
|                        | NM_001043562.1         | 8   | 0   |
|                        | <i>Tljhe</i> (Control) | 284 | 0   |
| <b>21-lenght siRNA</b> |                        |     |     |
| <b>0 mismatches</b>    |                        | 0   | 0   |
|                        | <i>Tljhe</i> (Control) | 283 | 148 |
| <b>1 mismatches</b>    | AK383719.1             | 1   | 0   |
|                        | AY489292.1             | 1   | 0   |
|                        | NM_001043562.1         | 1   | 0   |
|                        | <i>Tljhe</i> (Control) | 283 | 0   |
| <b>2 mismatches</b>    | AE014296.5             | 11  | 0   |
|                        | AK383719.1             | 6   | 0   |
|                        | AY489292.1             | 6   | 0   |
|                        | NM_001043562.1         | 6   | 0   |
|                        | CP023337.1             | 4   | 0   |
|                        | CP023331.1             | 4   | 0   |
|                        | <i>Tljhe</i> (Control) | 283 | 0   |