INTRODUCTION

Social insects (ants, bees, wasps, and termites) are nearly ubiquitously distributed and ecologically very successful due to their advanced sociality including division of labor between castes and cooperation between individuals in a colony (Bourke & Franks, 1995). Importantly, they form colonies that show reproductive division of labor between the reproductive queen(s) and the sterile workforce, which help the queen reproduce. Social insect societies are under particular threat of pathogens and disease, because individuals in the colony usually are closely related offspring of the mother queen(s) and because the high number of individuals facilitates pathogen transmission (Schmid-Hempel, 1998). Each individual is protected against disease by its own hygiene...
The physiological immune defenses in insects comprise cellular and humoral responses, the former including phagocytosis of small microorganisms and encapsulation of larger parasites and the latter composed of several signaling pathways that culminate in the production of antimicrobial peptides and other effector molecules (Ferrandon, Imler, Hetru, & Hoffmann, 2007). The core genes encoding for these immune system components are retained across several insect orders (Viljakainen, 2015). Moreover, the immune responses are interconnected with stress responses, which in insects have an immune-enhancing effect via stress hormones releasing energy for both stress and immune responses (Adamo, 2017). This interconnection may be particularly relevant for our study where we test the effect of worker presence or absence in the context of infection, as it is known that social isolation may induce stress and interfere with disease defense abilities in insects (Boulay, Quagebeur, Godzinska, & Lenoir, 1999; Kohlmeier, Holländer, & Meunier, 2016; Koto, Mersch, Hollis, & Keller, 2015).

While previous work has focused mostly on worker–worker interactions, we here test how ant queens, the most important individuals of the colony, modulate their individual immune defenses after bacterial infection when they have access to social immunity or not (presence or absence of workers). We control for the fact of social isolation by also determining gene expression patterns of uninfected queens when alone or in the presence of their workers. We used queens of the Argentine ant Linepithema humile (Figure 1) that we injected with a combination of gram+ and gram− bacteria or sterile saline solution and then kept in either isolation or with workers. After injection, changes in gene expression patterns were analyzed at four time points using RNA-seq: shortly after injection (2 hr), during the development of the immune response (4 and 8 hr post-injection), and when the immune response was expected to be fully activated (12 hr post-injection) (Erler, Popp, & Lattorff, 2011). We hypothesized that both the infection state and the social environment will affect gene expression of the queens.
and tested for an interaction between the two, in particular if the response to bacterial infection differed between the two rearing conditions.

2 MATERIAL AND METHODS

2.1 Samples

Workers and queens of the Argentine ant L. humile were collected from the European main supercolony in Castell d’Aro, Spain, in April 2011 and kept in artificial nests in climate chambers (Sanyo) set to 27°C for 14 hr of light and 21°C for 10 hr of dark. The ants were fed with honey and cockroaches three times per week. Approximately 3 weeks prior to the experiments, small sub-colonies originating from two stock colonies and each consisting of a single queen and 10 workers were placed into petri dishes (diameter 9 cm) with a plastered ground and fed with 10% sugar water.

2.2 Bacteria used for infections

We used the gram-positive bacterium Staphylococcus aureus and the gram-negative bacterium Serratia marcescens in combination for infecting the queens with the aim to induce gene expression of both Toll and Imd innate immune signaling pathways, since in Drosophila, gram-positive bacteria are known to induce mainly the Toll pathway and gram-negative bacteria the Imd pathway (Ferrandon et al., 2007). The bacteria were grown overnight in LB medium (Merck). The bacterial suspension was centrifuged, and the pellet was washed three times in sterile saline solution (hereafter called Ringer) prepared following the protocol described in Aubert & Richard (2008). The pellet from the final wash was suspended in Ringer. For the injections, bacterial suspensions were diluted, bacterial cells counted using Neubauer counting chamber, and S. marcescens and S. aureus dilutions mixed to get a solution representing both bacterial species in equal quantity.

2.3 Injections and social environment

Linepithema humile queens were studied for effects on genomewide expression patterns at four time points (2, 4, 8, and 12 hr) after bacterial versus control injections in the presence or absence of five workers in a full factorial design. All the injections were made at the same time of day (in the morning) within a time window of 3 hr. Sample information is summarized in Table 1. The queens were randomly assigned for either bacterial or Ringer injection and were first transferred to small petri dishes on ice to cold-immobilize them for injection. Microinjections were performed using Picoliter Injector PLI-100 Plus (Harvard Apparatus) set at 10 psi for 1 s using spiked glass needles with inner diameter of 11.9 μm (Biomedical Instruments), resulting in an injection volume of about 65 nl. This volume was injected twice between the second and third tergite of the abdomen, containing approx. 1,300 bacterial cells (50:50 mix of S. marcescens and S. aureus). The controls were injected twice with 65 nl of sterile Ringer. After injection, the queens were transferred back to their original petri dish nests and kept together with five workers (social environment, the queens referred to as “social queens” hereafter) or reared alone by removing the workers (the queens referred to as “isolated queens” hereafter) at constant room temperature (22°C) and with 10% sugar water ad libitum. Each treatment at each time point was repeated three times.

2.4 RNA extractions and sequencing

At 2, 4, 8, or 12 hr post-injection (hpi), the ants were frozen in liquid nitrogen and kept in −80°C freezer until RNA extraction. The whole-body samples were disrupted and homogenized in TissueLyser II (Qiagen) using stainless steel beads (5 mm diameter). Total RNA was extracted using RNeasy Micro Kit (Qiagen) following the protocol provided with the kit and including DNA removal using RNase-free DNase I. RNA was quantified using Agilent 2100 Bioanalyzer, and the samples were sent to BGI Tech Solutions (Hong Kong) for library preparation (Illumina TruSeq RNA Sample Prep Kit) and mRNA sequencing (100 bp paired-end reads) with Illumina HiSeq2000.

2.5 Bioinformatic analyses

The filtering of raw sequence data was performed by BGI and included adapter removal, removal of reads with more than 10% of undetermined bases, and removal of reads with more than 50% of low quality bases (Q < 10). Quality controlled clean data obtained from BGI were used for further analyses. The clean reads were mapped...
to the *L. humile* reference genome (GCF_000217595.1) using STAR v.2.4.1b (Dobin & Gingeras, 2015). The mapped reads were counted for all exons defined in the NCBI *L. humile* Annotation Release 100 (GCF_000217595.1_Lhum_UMD_V04_genomic.gff) and counts per exons were summarized for genes using HTSeq 0.9.1 (Anders, Pyl, & Huber, 2015).

The count data of the samples were visualized by principal component analysis (PCA) in R version 3.4.1 (R Core Team, 2015). Analysis of differential gene expression in each contrast at each time point was carried out using DESeq2 version 1.16.1 (Love, Huber, & Anders, 2014) in R. In DESeq2, the count data for each gene in a sample are modeled with a negative binomial distribution where the mean and dispersion are estimated from the data. The mean is the read count of a gene normalized by a size factor based on the median of the ratios of observed counts (the read count of a gene in a given sample divided by the geometric mean of the read counts of that gene across all samples), thus allowing comparison of samples with variable sequencing depth. The dispersion estimate is obtained by first estimating dispersion for each gene using maximum likelihood, then fitting a curve to the maximum likelihood estimates (MLEs), and finally, shrinking the per gene dispersion estimates toward the expected dispersion values represented by the curve using empirical Bayes’ approach. Differential expression of a given gene between two conditions of interest is analyzed by using empirical Bayes shrinkage by fitting generalized linear model (GLM) to obtain MLEs for log2 fold change (LCF) between the conditions, then fitting normal distribution ($\mu = 0$) to the MLEs of all genes, and repeating the GLM fit for the given gene using this distribution as a prior. The maximum of the a posteriori distribution is the final estimate of the LFC, and the curvature of the distribution at its maximum is the standard error of the LFC. The significance of the LFCs is tested by Wald test, and the obtained p values are corrected for multiple testing by the method of Benjamini and Hochberg (1995). In this study, we used a false discovery rate (FDR) <10%.

Insects, including *L. humile*, are known to harbor RNA viruses (Gruber et al., 2017; Shi et al., 2016) which may have an effect on host gene expression (Doublet et al., 2017; Gerth & Hurst, 2017). We studied whether viruses are present and potentially have an effect on the gene expression patterns by assembling all the reads that could not be mapped to the *L. humile* genome by using default settings in Trinity v2.5.1, and by doing database searches with the obtained contigs against National Center for Biotechnology Information (NCBI Resource Coordinators, 2017) RefSeq virus databases “viral.1.protein.faa” and “viral.2.protein.faa” (accessed 7 January 2018) using BLASTX 2.6.0+ with an e-value threshold of $10^{-4}$. Contigs that matched insect viruses and that had a query coverage of at least 400 amino acids were used in the following steps. The unmapped reads from each sample were mapped against the selected blast-annotated virus contigs using default settings in BWA-MEM v.0.7.17 (Li & Durbin, 2009), and the mapped reads were counted using samtools v1.4 (Li et al., 2009) and normalized with the sum of genome-mapped and virus-mapped reads per sample. The resulting viral load per sample was ordered by magnitude, divided into three equal sized bins, and classified as low (normalized read count range $4.27 \times 10^{-3} - 4.45 \times 10^{-3}$, $n = 14$), medium (normalized read count range $4.64 \times 10^{-3} - 1.38 \times 10^{-3}$, $n = 16$), or high (normalized read count range $2.16 \times 10^{-3} - 0.18$, $n = 14$). The virus load classification was incorporated as a factor in the analysis of differential gene expression.

A list of manually annotated immune genes, hereafter called “the core immune genes”, of the *L. humile* genome including key genes for the main signaling pathways Toll, Imd, JAK-STAT, and JNK and additionally, genes involved in pathogen recognition, modulation of immune response, melanization, RNA interference, and clearance of microbes (antimicrobial peptides and phagocytosis; Viljakainen, 2015), was retrieved from Smith et al. (2011), Table S16. Thirteen C-type lectins, nine scavenger receptors, two Toll-like proteins, and transferrin were added to the list, which now totaled 121 immune genes. These immune genes were searched against the list of differentially expressed genes (DEGs). Predicted function for all the other DEGs outside the core immune genes was searched using PaperBLAST (Price & Arkin, 2017) and NCBI databases (NCBI Resource Coordinators, 2017).

A BED formatted file was parsed from the NCBI *L. humile* Annotation Release 100. The parsed BED file included the coding sequence (CDS) coordinates for genes, exon coordinates for non-coding RNAs, and exon coordinates for some other genes without CDS annotation, excluding 210 pseudogenes and 46 tRNA genes, and merging overlapping CDS and exon regions. Based on the genomic coordinates in the BED file, the CDS or exon sequences were retrieved from the genome and concatenated for each gene in FASTA format. This resulted in total of 12,696 gene sequences. Gene Ontology (GO) annotation was carried out for the 12,696 genes using Blast2GO version 3.3.5 (Conesa et al., 2005). First, BLASTX search was done with an E-value of $10^{-25}$ against all ant sequences in the NCBI non-redundant database restricting the number of BLAST hits to 20. In addition, InterProScan annotation was run for the sequences (Jones et al., 2014). For the resulting hits, GO mapping and annotation were performed and InterProScan GOs were merged to annotation. The first step resulted in 9,406 sequences with GO annotations. Next, a second round of BLASTX search was done with the sequences that remained without hits in the first step with an E-value of $10^{-25}$ against all arthropod sequences in the NCBI non-redundant database restricting the number of BLAST hits to 100. Again, mapping and annotation were run for the resulting hits and InterProScan GOs were merged to the annotations. After the second step, 9,702 genes had GO annotations. GO term enrichment analysis was performed for all comparisons and time points to find significantly over- and under-represented GO terms in the test set (DEGs, $N > 10$) with respect to the 9,702 genes with annotations as a reference set by using FatigO package and a FDR <5% (Al-Shahrour, Díaz-Uriarte, & Dopazo, 2004) implemented in Blast2GO. Only the results with significant enrichment are presented in the Results.
| Sample | Clean reads | Mapped reads | Mapping rate (%) | Reads mapped to genes | Gene mapping rate (%) |
|--------|-------------|--------------|------------------|-----------------------|-----------------------|
| B1     | 24,597,756  | 23,968,026   | 97               | 19,918,942            | 81                    |
| B14    | 26,624,416  | 25,858,946   | 97               | 21,931,220            | 82                    |
| B17    | 23,999,196  | 22,651,278   | 94               | 17,802,808            | 74                    |
| B18    | 23,322,168  | 22,207,576   | 95               | 18,369,706            | 79                    |
| B2     | 23,555,686  | 22,880,536   | 97               | 19,149,848            | 81                    |
| B21    | 34,493,492  | 33,386,034   | 97               | 28,086,624            | 81                    |
| B22    | 24,617,926  | 23,694,502   | 96               | 19,953,830            | 81                    |
| B26    | 29,655,470  | 28,194,828   | 95               | 24,070,260            | 81                    |
| B29    | 30,939,654  | 29,544,114   | 95               | 24,867,174            | 80                    |
| B30    | 36,209,766  | 24,433,280   | 67               | 20,189,926            | 56                    |
| B33    | 45,625,028  | 28,638,828   | 63               | 23,836,070            | 52                    |
| B37    | 44,147,540  | 43,202,868   | 98               | 36,453,038            | 83                    |
| B38    | 27,820,672  | 24,703,250   | 89               | 21,374,226            | 77                    |
| B41    | 29,808,498  | 27,583,454   | 93               | 23,167,504            | 78                    |
| B42    | 42,889,884  | 41,943,030   | 98               | 35,365,472            | 82                    |
| B45    | 36,409,122  | 35,492,646   | 97               | 29,598,516            | 81                    |
| B46    | 35,596,746  | 34,842,904   | 98               | 29,326,366            | 82                    |
| B49    | 32,376,284  | 31,729,918   | 98               | 27,374,740            | 85                    |
| B5     | 23,006,502  | 22,439,386   | 98               | 19,046,868            | 83                    |
| B50    | 27,917,926  | 26,788,868   | 97               | 22,619,380            | 82                    |
| B53    | 40,673,650  | 39,796,846   | 98               | 34,006,420            | 84                    |
| B55    | 35,647,646  | 34,806,466   | 98               | 29,083,614            | 82                    |
| B6     | 29,826,016  | 23,599,508   | 97               | 19,401,850            | 65                    |
| B9     | 23,367,018  | 22,771,162   | 97               | 19,052,478            | 82                    |
| C11    | 27,657,994  | 26,788,868   | 97               | 22,619,380            | 82                    |
| C12    | 26,274,850  | 25,191,632   | 96               | 21,050,254            | 80                    |
| C15    | 26,534,292  | 24,556,092   | 93               | 20,509,984            | 77                    |
| C16    | 22,399,506  | 21,708,568   | 97               | 17,984,018            | 80                    |
| C20    | 26,934,682  | 26,215,608   | 97               | 21,817,700            | 81                    |
| C24    | 29,162,756  | 27,998,298   | 96               | 23,807,766            | 82                    |
| C27    | 22,016,854  | 21,160,562   | 96               | 17,886,560            | 81                    |
| C28    | 20,395,710  | 19,525,038   | 96               | 16,514,588            | 81                    |
| C3     | 33,093,148  | 32,073,232   | 97               | 26,728,794            | 81                    |
| C11    | 40,544,886  | 37,664,904   | 93               | 32,121,400            | 79                    |
| C35    | 40,764,376  | 39,594,362   | 97               | 33,079,892            | 81                    |
| C39    | 38,215,078  | 37,412,872   | 98               | 30,819,540            | 81                    |
| C4     | 26,290,796  | 25,462,524   | 97               | 21,168,056            | 81                    |
| C40    | 28,242,682  | 27,614,780   | 98               | 23,711,616            | 84                    |
| C43    | 35,737,580  | 34,957,488   | 98               | 29,299,620            | 82                    |
| C47    | 44,515,826  | 43,220,512   | 97               | 36,959,312            | 83                    |
| C48    | 34,970,550  | 34,112,530   | 98               | 28,931,718            | 83                    |
| C51    | 32,295,292  | 31,631,658   | 98               | 26,822,394            | 83                    |
| C52    | 39,323,204  | 38,495,180   | 98               | 32,743,424            | 83                    |
| C54    | 44,103,734  | 27,761,612   | 63               | 23,261,200            | 53                    |

(Continues)
3 | RESULTS

In this study, 48 transcriptomes of *L. humile* queens were sequenced (Table 1). The mean number of clean reads per sample was 32 million of which on average 93% were mapped to the *L. humile* genome and 79% mapped to the 12,952 NCBI annotated genes including protein-coding, non-coding, and pseudogenes (Table 2). The genome-mapping rate was markedly lower (about 65%) for four samples: B30, B33, B50, and C54 (Tables 1 and 2), which were excluded from further analyses. The removal affected the following data points: bacteria-injected social queens at 2 and 12 hpi, bacteria-injected isolated queens at 4 hpi, and Ringer-injected social queens at 4 hpi, which all involved two instead of three biological replicates. In the PCA, the first PC explaining 37% of the variation in gene expression did not separate the samples according to the treatments, but the second PC explaining 9% of variance roughly separated the samples according to the type of injection (Figure 2).

### 3.1 Viral load of queens

Eight RNA viruses were identified in the Trinity-assembled contigs of reads that could not be mapped to the *L. humile* genome (Viljakainen, Holmberg, Abril, & Jurvansuu, 2018). We found that all our samples contained RNA viruses at different loads. The type of injection (bacteria or Ringer) did not affect the virus loads per sample (two-tailed t test: \( t = -1.36, df = 22.49, p = 0.19 \)), neither did the rearing condition (two-tailed t test: \( t = 0.05, df = 41.55, p = 0.95 \)), suggesting an a priori viral load of the ants, which ranged from low to high levels. Importantly, we found that the gene expression profiles of the ants were affected by viral load (Figure 3), so that we controlled for viral load in the analysis of differential gene expression.

### 3.2 Effect of social context

We first investigated the effect of social isolation per se by analyzing differentially expressed genes in Ringer-injected queens that were either
reared alone or with their workers (Figure 3, Table 3, and Appendix: Table A1). The total number of DEGs across all time points was 134 with 82 up-regulated and 52 down-regulated genes. GO enrichment analysis showed enrichment of biological processes "single-organism metabolic process," "carbohydrate phosphorylation," and "cellular glucose homeostasis" in the up-regulated genes at 12 hpi (Appendix: Table A2). We hence found that social context affected queen energy metabolism, but had no effect on immune gene expression.

### 3.3 Effect of bacterial infection depending on social context

We found that the effect of bacterial infection depended strongly on the social context the queens were reared at, even if the overall number of DEGs across all time points induced by the bacterial injection was similar across the social contexts, with 292 and 295 regulated genes in the presence and absence of workers, respectively (Figure 3, Table 3, Appendix: Tables A3 and A4). Some of the genes were regulated at several time points, and taking this into account, the number of unique DEGs was 272 in the socially reared and 268 in the isolated queens with 110 of the genes shared between the social contexts. Despite these similar numbers, the direction of regulation differed greatly: In the presence of workers, queens typically showed gene up-regulation as compared to their Ringer control (65% of DEGs up-regulated, $\chi^2 = 25.33, df = 1, p = 4.83 \times 10^{-7}$), whereas queens reared alone showed mostly down-regulation (63% of DEGs down-regulated; $\chi^2 = 21.16, df = 1, p = 4.23 \times 10^{-6}$), and only 22 of the up-regulated genes and 27 of the down-regulated genes were shared between the social treatments. Contrary to this overall difference in up-regulation versus down-regulation, both social and isolated queens showed a consistent up-regulation of core immune genes after bacterial injection (64% up-regulated out of 14 regulated core immune genes in social queens and 65% out of 23 in isolated queens; Table 4).

The majority of the DEGs were not directly related to immune response, the core immune genes representing only 5% and 9% of the regulated genes in social and isolated queens, respectively. To get insight on the affected biological processes and molecular functions, GO enrichment analysis was carried out for treatment contrasts with
| Locus          | Annotation                                              | Social | Isolated |
|---------------|---------------------------------------------------------|--------|----------|
|               | T2 | T4 | T8 | T12 | T2 | T4 | T8 | T12 |
| **Toll**      | LOC105673881          | BGGBP, recognition of microbes | 1.18 |        |        |        |        |        |
| LOC105673362 | Serine protease, activation of the pathway | 2.74 | 1.45 | 1.55 |        |        |        |        |
| LOC105675725 | Serine protease, activation of the pathway | 1.04 |        |        |        |        |        |        |
| LOC105671866 | Serine protease, activation of the pathway |        |        | 1.31 | 1.70 |        |        |        |
| LOC105673363 | Serine protease, activation of the pathway |        |        |        |        |        |        | 1.37  |
| LOC105678817 | Protein toll-like | 1.69 | -0.94 |        |        |        |        |        |
| LOC105678912 | Protein toll-like |        |        |        |        | -0.94 |        |        |
| LOC105678648 | Protein toll-like |        |        |        |        |        | 1.50  |        |
| LOC105678784 | Protein toll-like |        |        |        |        |        | 1.11  |        |
| LOC105678482 | Cactus-1a, Toll signaling |        |        | 2.78 | 1.40 |        |        |        |
| LOC105678483 | Cactus-1b, Toll signaling |        |        |        |        | 0.73  |        |        |
| LOC105676758 | Ninjurin, up-regulated by the Toll pathway |        |        | 2.82 | 1.41 |        |        |        |
| LOC105668729 | Relish, Imd signaling |        |        | 0.54 | 0.78 |        |        |        |
| LOC105675773 | PGRP-S2a, negative regulator of Imd pathway | -1.20 |        | -3.24 |        |        |        |        |
| LOC105678813 | Pirk, negative regulator of Imd pathway |        |        | 3.45 |        |        |        |        |
| **AMPS**      | LOC105670591          | Hymenoptaecin | 2.09 |        |        |        |        |        |
| LOC105675436 | Ixodidin | -1.69 |        |        |        |        |        |        |
| LOC105675717 | Defensin | -1.80 |        |        |        |        |        |        |
| **Melanization** | LOC105676303          | Ebony | 1.81 |        |        |        |        |        |
| LOC105667585 | Laccase | 1.48 | 1.66 |        |        |        |        |        |
| LOC105668871 | Prophenoloxidase (PPO) | -2.36 |        | -1.42 |        |        |        |        |
| LOC105674352 | Dopa decarboxylase | 1.78 | 3.03 |        | -0.77 | -1.57 |        |        |
| LOC105675407 | Inhibit beta E chain, limits infection induced melanization |        |        |        |        |        |        |        |
| LOC105677585 | Serine protease, activation of PPO |        |        |        |        |        |        | 1.06  |
| LOC105671136 | Serine protease, activation of PPO |        |        |        |        |        |        | 0.53  |
| LOC105678075 | Pale, involved in melanization |        |        |        |        |        |        | 1.40  |
| LOC105673744 | Serpin, inhibition of PPO activation |        |        |        |        |        |        | 0.91  |
| **Phagocytosis** | LOC105678691          | SCR-B-like, phagocytosis | -2.28 |        |        |        |        |        |
| LOC105677178 | TepII, phagocytosis |        |        | 1.89 | 0.73 | 1.02 |        |        |
| LOC105675281 | Nim-C, mediates phagocytosis |        |        |        |        | -1.67 |        |        |
| LOC105671541 | Croquemort, phagocytosis |        |        |        |        |        | -0.68 |        |
at least 10 DEGs. The up-regulated genes of social queens at 4 hpi showed enrichment of proteolysis and serine-type endopeptidase inhibitor activity (Appendix: Table A2). In the isolated queens, down-regulated genes at 2 hpi showed enrichment of proteolysis, molybdopterin cofactor biosynthetic process, and serine protease inhibitor activity (Appendix: Table A2). The genes categorized as being involved in proteolysis and having serine protease inhibitor activity were largely the same genes as in the social queens. Oxidation-reduction process was enriched in the down-regulated genes of isolated queens at 12 hpi including gene encoding for phenoloxidase (LOC105668871) and several cytochrome P450 protein-coding genes.

### 3.4 | Activation of Toll signaling pathway

The expression of immune genes indicated activation of the signaling pathway Toll (Table 4). In social queens, five Toll pathway genes were up-regulated. These included beta-1,3-glucan-binding protein (BGBP, LOC105673881), based on BLASTP search similar to Drosophila microbial recognition protein GNPB1 (GenBank Accession number NP_524142, 34% identity, 92% coverage) involved in recognition of gram-positive bacteria (Pilli-Floury et al., 2004). Up-regulated were also two genes encoding serine proteases: limulus clotting factor C-like (LOC105673362) similar to Drosophila modular serine protease, modSP (NP_536776, 27% identity, 97% coverage), and serine protease snake-like (LOC105675725) similar to Drosophila snake (NP_524338, 39% identity, 56% coverage). Both modSP and snake are involved in activation of the Toll receptor by transmitting microbial recognition signals from pattern-recognition proteins GNPB1 and PGRP-SA to Spätzle, which functions just upstream of Toll (Buchon et al., 2009). Two genes encoding Toll receptors were up-regulated, LOC105678817 and LOC105678648, both similar to Drosophila Toll (NP_524518, 29% identity, 70% coverage and 30% identity, 48% coverage, respectively).

In the isolated queens, seven Toll pathway genes were up-regulated and two down-regulated (Table 4). Three genes encoding for serine proteases, all involved in the activation of the Toll pathway in a similar way described above for modSP and snake, were up-regulated: two limulus clotting factor C-like (LOC105673362 and LOC105673363) similar to Drosophila modSP (NP_536776, 27% identity, 97% coverage and 31% identity, 94% coverage, respectively) and additionally, serine protease gd-like (LOC105671866) similar to gastrulation-defective in Drosophila (NP_001303552, 29% identity, 84% coverage) that presumably activates serine protease snake (Rose et al., 2003). One gene encoding Toll-like protein (LOC105678784) similar to Drosophila Toll (NP_524518, 36% identity, 90% coverage), which is a transmembrane receptor, was up-regulated, and two Toll-like protein-coding genes were down-regulated (LOC105678817 and LOC105678912). Two genes downstream of Toll receptor were up-regulated, cactus-1a (LOC105678482) and cactus-1b (LOC105678483), both similar to Drosophila cactus (NP_476943, 43% identity, 50% coverage and 38% identity, 76% coverage, respectively) which is an inhibitor of NF-κB transcription factor Dorsal that positively regulates the transcription of antimicrobial peptides (AMPs; Ferrandon et al., 2007). Notably, our analysis only revealed two Toll pathway genes overlapping between the social and isolated queens, limulus clotting factor C-like (LOC105673362) and protein Toll-like (LOC105678817), of which the latter was significantly up-regulated in the social queens while down-regulated in the isolated queens.

### 3.5 | Activation of Imd signaling pathway

Genes along the Imd pathway were not as widely represented among the DEGs as the Toll pathway genes (Table 4). Down-regulated in both social and isolated queens was a gene encoding for peptidoglycan-recognition protein SC2 (PGRP-SC2, LOC105675773), which is a negative regulator of the Imd pathway (Bischoff et al., 2006). In addition, isolated queens showed up-regulation of a gene encoding for the NF-xB-like transcription factor Relish (LOC105668729) (Ferrandon et al., 2007) and for uncharacterized protein (LOC105678813) similar to Drosophila poor Imd response upon knock-in (NP_001286686, 39% identity, 24% coverage), which, again, is a negative regulator of the Imd pathway (Kleino et al., 2008).

### 3.6 | Expression of antimicrobial peptides

The Toll and Imd signaling cascades culminate in the expression of AMPs (Ferrandon et al., 2007), which are immune effectors attacking fungal and bacterial pathogens in the host. In social queens, one AMP encoding gene, hymenoptaecin (LOC105670591) (Casteels, Ampe, Jacobs, & Tempst, 1993), was up-regulated at 12 hpi, and two AMP genes were down-regulated at 4 hpi: defensin-2 (LOC105675717) and chymotrypsin inhibitor-like (LOC105675436) similar (41% identity, 71% coverage) to ixodidin (P83516), which has been characterized in the Cattle tick Rhipicephalus microplus (Fogaça et al., 2006).

### 3.7 | Melanization

Melanization—an active mechanism to encapsulate pathogens within the host—was induced in both social and isolated queens indicated by a number of up-regulated genes (Table 4), for example, venom protease-like (LOC105677585) similar to venom serine protease (BSU2W0, 50% identity, 96% coverage) from Bombus ignitus, which is shown to activate the phenoloxidase cascade (Choo et al., 2010). Once activated, phenoloxidase catalyzes the production of quinones, which are polymerized to microbial melanin (De Gregorio et al., 2002). Up-regulated were also N-(5-aminomethylpentanoyl)-l-cysteinyl-l-valine synthase (LOC105676303) similar to ebony from Drosophila (NP_524431, 47% identity, 98% coverage) and aromatic-l-α-amino acid decarboxylase (LOC105674352), similar to Drosophila dopa decarboxylase (NP_724164, 74% identity, 90% coverage), both involved in melanization (Riedel, Vorkel, & Eaton, 2011).

### 3.8 | Phagocytosis

Three genes involved in phagocytosis were down-regulated (Table 4). These involved a gene encoding for scavenger receptor...
class B member 1-like (LOC105678691) and protein croquetom-like (LOC105671541), both similar to Drosophila croquetom (Q27367, 28% identity, 75% coverage and 37% identity, 90% coverage, respectively) required in the uptake of bacteria by phagocytes (Guillou, Troha, Wang, Franc, & Buchon, 2016), and fibrillin-1 (LOC105675281) similar to Drosophila eater (NP_651533, 37% identity, 49% coverage) that is also a phagocytic receptor promoting engulfment of bacteria (Kocks et al., 2005). Up-regulated was a gene encoding for alpha-2-macroglobulin-like protein 1 (LOC105677178) similar to Drosophila TepII (NP_723300, 34% identity, 37% coverage) that recognizes surface structures of bacteria leading to phagocytosis (Shokal, Kopydlowski, & Eleftherianos, 2017). Here, only the gene encoding for scavenger receptor class B member 1-like (LOC105678691) was regulated in both social and isolated queens.

3.9 | Interaction effect of social isolation and bacterial injection

An interaction analysis of social isolation and bacterial injection at all the four time points showed regulation of 20 genes across all time points in the bacteria-injected isolated queens (Tables 3 and 5). These included down-regulation at 4 hpi of the Toll receptor activator-gene spätzle (LOC105678357), up-regulation at 12 hpi of the Imd pathway signaling gene imd (LOC105672003) and down-regulation, also at 12 hpi, of hemocyte protein–glutamine gamma-glutamyl transferase-like (LOC105670674) similar to transglutaminase (NP_609174, 37% identity, 90% coverage) in Drosophila that inhibits the Imd pathway transcription factor Relish (Maki, Shibata, & Kawabata, 2017).

Hence, in both presence and absence of workers, all important immune defense pathways were triggered in the queens, with the highest number in the Toll pathway.

### 4 | DISCUSSION

In this study, we tested how the presence or absence of workers affects ant queen immune response after bacterial infection. When testing for the effect of our experimentally induced bacterial infections, we found that existing viral load had an effect on differential gene expression analyses and that virus presence and load should be taken into account in these types of analysis, as previously reported (Gerth & Hurst, 2017). To further control for the effect of social worker presence or absence per se in the absence of an infection, we first analyzed differential gene expression between Ringer-injected

| Time point | Locus            | Description                                      | LFC  | p-Value       | Adjusted p-value |
|------------|------------------|--------------------------------------------------|------|---------------|-----------------|
| T2         | LOC105672931     | Uncharacterized protein LOC105672931             | 1.41 | 5.31E−06      | 6.69E−02        |
| T4         | LOC105678357     | Protein spätzle                                  | −1.40| 2.61E−10      | 3.28E−06        |
|            | LOC105668988     | Uncharacterized LOC105668988                     | −7.82| 5.81E−08      | 3.64E−04        |
|            | LOC105667488     | Uncharacterized LOC105667488                     | −2.50| 1.71E−05      | 6.36E−02        |
|            | LOC105668757     | Non-coding RNA                                   | −1.74| 2.03E−05      | 3.63E−02        |
|            | LOC105668733     | Lambda-crystallin homolog                        | −0.55| 3.13E−05      | 6.55E−02        |
|            | LOC105678842     | Acyl-CoA Delta(11) desaturase-like                | −1.71| 3.13E−05      | 6.55E−02        |
|            | LOC105676303     | N-(5-amino-5-carboxypentanoyl)-\(\gamma\)-cysteinyl-\(\delta\)-valine synthase | −1.40| 5.04E−05      | 9.03E−02        |
| T8         | LOC105675386     | Sodium-coupled monocarboxylate transporter 1     | −0.65| 3.45E−06      | 4.35E−02        |
| T12        | LOC105677280     | Uncharacterized LOC105677280                    | 2.01 | 8.53E−11      | 9.68E−07        |
|            | LOC105674672     | Cholesterol desaturase daf-36                    | 1.49 | 6.29E−08      | 3.57E−04        |
|            | LOC105675029     | Uncharacterized LOC105675029                    | −0.83| 1.20E−06      | 4.54E−03        |
|            | LOC105677088     | Cytochrome P450 9e2-like                         | 0.99 | 2.24E−06      | 6.36E−03        |
|            | LOC105670674     | Hemocyte protein–glutamine gamma-glutamyl transferase-like | −0.59| 9.36E−06      | 1.77E−02        |
|            | LOC105672003     | Uncharacterized (imd)                            | 0.81 | 9.08E−06      | 1.77E−02        |
|            | LOC105673930     | MLX-interacting protein                          | 0.47 | 2.47E−05      | 4.01E−02        |
|            | LOC105669664     | Uncharacterized LOC105669664                    | 1.17 | 8.56E−05      | 9.87E−02        |
|            | LOC105671661     | Inositol polyphosphate multikinase               | 0.47 | 9.57E−05      | 9.87E−02        |
|            | LOC105675400     | Uncharacterized LOC105675400                    | 0.50 | 8.01E−05      | 9.87E−02        |
|            | LOC105676429     | Protein embryonic gonad-like                     | 0.73 | 9.19E−05      | 9.87E−02        |

LFC: log₂ fold change.
queens reared in the social environment or alone. Despite our sample size being large enough to detect significant effects of rearing on queen metabolism, we could not detect any general effect on queen immune gene expression.

We then tested whether worker presence or absence interfered with the queens’ individual immune response to bacterial infection over the course of infection. Overall, we found that injection of bacteria, over the four time points studied, affected the expression of similar numbers of genes in both social and isolated queens but interestingly, the social queens up-regulated the majority of the genes, whereas in the isolated queens, down-regulation was prevailing. This general down-regulation might be a consequence of social isolation, which has been shown to affect life-history traits by reducing longevity in workers of the ant *Camponotus fellah* (Boulay et al., 1999; Koto et al., 2015), yet did not compromise the innate immune response of bumblebees after pathogen challenge (Richter, Helbíng, Erler, & Lattorff, 2012). In the group-living earwig *Forficula auricularia*, rearing individuals alone also lead to a transiently increased susceptibility after pathogen exposure shortly after isolation, yet an indistinguishable survival of individuals living isolated or in groups for longer periods (Kohlsmeier et al., 2016), hence the effects of social isolation may be plastic, both varying over time and across species.

The isolated queens regulated slightly, but not significantly, higher number of the core immune genes than social queens (23 vs. 14, $\chi^2 = 3.38$, $df = 1$, $p = 0.07$) and the immune gene expression in the isolated queens did not show the overall pattern of down-regulation observed in all of their DEGs: 65% of immune genes were up-regulated as opposed to 37% of all DEGs ($\chi^2 = 8.11$, $df = 1$, $p = 0.004$). Therefore, even though gene expression in the isolated queens showed a trend of down-regulation, the activation of cellular and humoral immune cascades was comparable to the social queens.

An interesting observation was two enriched GO terms, serine protease inhibitor activity and proteolysis, both categories including approximately 20 genes, which were up-regulated at 4 hpi in the bacteria-injected social queens and down-regulated at 2 hpi in the bacteria-injected isolated queens. All except one of the genes in the serine protease inhibitor-category were long non-coding RNAs (IncRNA) which Blast2Go annotation found to contain a protease inhibitor domain suggesting they might regulate serine protease inhibitors (serpins). In insects, serpins are known to be involved in the regulation of immune signaling cascades, phagocytosis, and digestion (Gubb, Sanz-Parra, Barcena, Troxler, & Füllaondo, 2010), and the expression of serpin-related IncRNAs could be involved in the fine-tuning of various arms of immune response. It is striking that the gene expression patterns of these genes showed opposite directions in the two rearing conditions, highlighting the strong effect of social environment on the general response to bacterial infection.

As a conclusion, this study shows that ant queens were equally able to activate innate immune signaling cascades after bacterial injection when kept together with workers or when reared alone. This reveals that pathogen-injected queens raise an induced immune response even in the presence of rearing workers, yet that worker presence interferes with which exact set of genes is regulated. Hence, we could show that individual queen responses are not compromised, but modulated by their social context.

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**AUTHOR CONTRIBUTIONS**

LV and SC designed the research, LV performed the experimental work, LV carried out the bioinformatics analyses of RNA-seq data, LV and JJ carried out the virus-related bioinformatics analyses, LV and IH performed the statistical analyses, TB and SE helped LV to interpret the results related to differentially expressed genes, and LV and SC wrote the manuscript with contributions from all authors.

**DATA ACCESSIBILITY**

The short read sequence data are deposited at NCBI, BioProject PRJNA279338.

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### TABLE A1  Differentially expressed genes in Ringer-injected isolated queens with false discovery rate <10%. T2, T4, T8, and T12 indicate the post-injection time points

| Time point | Locus            | Description                              | LFC  | p-Value    | Adjusted p-value |
|------------|------------------|------------------------------------------|------|------------|------------------|
| T2         | LOC105680010     | Uncharacterized LOC105680010 (LOC105680010) | 7.62 | 5.41E-06   | 2.25E-02         |
|            | LOC105671203     | Uncharacterized LOC105671203 (LOC105671203) | 4.28 | 9.72E-13   | 1.21E-08         |
|            | LOC105675063     | Alkaline phosphatase 4-like (LOC105675063) | 3.35 | 1.55E-07   | 9.66E-04         |
|            | LOC105670077     | DNA repair protein RAD50-like (LOC105670077) | -20.67 | 1.56E-05 | 4.87E-02         |
| T4         | LOC105668186     | Uncharacterized LOC105668186 (LOC105668186) | 7.47 | 3.34E-07   | 4.14E-03         |
| T8         | LOC105668186     | Uncharacterized LOC105668186 (LOC105668186) | 8.89 | 4.74E-04   | 9.11E-02         |
|            | LOC105670097     | Spidrin-1 (LOC105670097)                  | 6.86 | 1.91E-06   | 1.34E-03         |
|            | LOC105678335     | Gephyrin-like (LOC105678335)              | 2.94 | 7.95E-05   | 3.06E-02         |
|            | LOC105677347     | Uncharacterized LOC105677347 (LOC105677347) | 2.34 | 2.36E-11   | 7.87E-08         |
|            | LOC105677332     | Uncharacterized LOC105677332 (LOC105677332) | 2.17 | 1.20E-07   | 1.99E-04         |
|            | LOC105678672     | Uncharacterized LOC105678672              | 1.82 | 2.49E-05   | 1.13E-02         |
|            | LOC105671207     | A disintegrin and metalloproteinase with thrombospondin motifs 7-like (LOC105671207) | 1.66 | 1.08E-04   | 4.00E-02         |
|            | LOC105677333     | Uncharacterized LOC105677333 (LOC105677333) | 1.65 | 2.00E-06   | 1.34E-03         |
|            | LOC105679161     | A disintegrin and metalloproteinase with thrombospondin motifs 17-like (LOC105679161) | 1.61 | 2.17E-04   | 6.99E-02         |
|            | LOC105678664     | Uncharacterized LOC105678664              | 1.57 | 2.57E-06   | 1.61E-03         |
|            | LOC105674354     | Tyrosine aminotransferase (LOC105674354)  | 1.47 | 3.40E-04   | 7.94E-02         |
|            | LOC105667964     | THAP domain-containing protein 4-like (LOC105667964) | 1.27 | 5.27E-04   | 9.77E-02         |
|            | LOC105677348     | Uncharacterized LOC105677348 (LOC105677348) | 1.17 | 9.58E-07   | 7.80E-04         |
|            | LOC105673216     | Putative fatty acyl-CoA reductase CG5065 (LOC105673216) | 1.15 | 3.47E-04   | 7.94E-02         |
|            | LOC105679050     | Transcription termination factor 2-like (LOC105679050) | 1.12 | 2.35E-05   | 1.12E-02         |
|            | LOC105671872     | Vitellogenin-2-like (LOC105671872)        | 0.98 | 1.88E-07   | 2.35E-04         |
|            | LOC105675620     | Aminopeptidase N-like (LOC105675620)      | 0.96 | 4.00E-04   | 8.24E-02         |
|            | LOC105670430     | Aminopeptidase N-like (LOC105670430)      | 0.90 | 3.45E-04   | 7.94E-02         |
|            | LOC105667719     | Uncharacterized protein PPB0145c-like (LOC105667719) | 0.87 | 1.13E-05   | 5.97E-03         |
|            | LOC105668487     | Uncharacterized LOC105668487 (LOC105668487) | 0.85 | 2.60E-04   | 7.42E-02         |
|            | LOC105678648     | Protein toll-like (LOC105678648)          | 0.84 | 2.11E-05   | 1.06E-02         |
|            | LOC105672608     | Facilitated trehalose transporter Tret1-like (LOC105672608) | 0.84 | 4.20E-04   | 8.24E-02         |

(Continues)
| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|------------|-------|-------------|-----|---------|------------------|
| LOC105669158 | Sorbitol dehydrogenase-like (LOC105669158) | 0.73 | 2.90E-04 | 7.60E-02 |
| LOC105674664 | Cytochrome P450 6a2-like (LOC105674664) | 0.71 | 2.59E-04 | 7.42E-02 |
| LOC105670239 | Uncharacterized LOC105670239 (LOC105670239) | 0.67 | 2.52E-04 | 7.42E-02 |
| LOC105671841 | Pleckstrin homology domain-containing family M member 2 (LOC105671841) | 0.66 | 2.85E-04 | 7.60E-02 |
| LOC105676405 | Fatty acid synthase (LOC105676405) | -0.64 | 1.67E-04 | 5.58E-02 |
| LOC105678111 | Uncharacterized LOC105678111 (LOC105678111) | -0.64 | 1.41E-04 | 4.87E-02 |
| LOC105675000 | Cartilage oligomeric matrix protein (LOC105675000) | -0.66 | 2.23E-04 | 6.99E-02 |
| LOC105676540 | Uncharacterized LOC105676540 (LOC105676540) | -0.78 | 4.14E-04 | 8.24E-02 |
| LOC105674426 | Beta-1 | -0.79 | 3.93E-04 | 7.60E-02 |
| LOC105674352 | Aromatic-α-amino acid decarboxylase (LOC105674352) | -0.95 | 4.96E-04 | 9.37E-02 |
| LOC105677051 | Protein Malvolio (LOC105677051) | -0.95 | 4.00E-04 | 8.24E-02 |
| LOC105670059 | Probable serine/threonine-protein kinase kinX (LOC105670059) | -0.96 | 5.60E-05 | 2.44E-02 |
| LOC105672810 | Protein yellow (LOC105672810) | -1.02 | 7.95E-05 | 3.06E-02 |
| LOC105678067 | Facilitated trehalose transporter Tret1-like (LOC105678067) | -1.05 | 7.31E-05 | 3.05E-02 |
| LOC105677852 | Uncharacterized LOC105677852 (LOC105677852) | -1.05 | 3.56E-06 | 2.10E-03 |
| LOC105676715 | Alpha-tocopherol transfer protein-like (LOC105676715) | -1.08 | 3.51E-04 | 7.94E-02 |
| LOC105674532 | ETS homologous factor-like (LOC105674532) | -1.10 | 3.64E-08 | 9.11E-05 |
| LOC105677500 | Glucosylceramidase-like (LOC105677500) | -1.15 | 2.75E-04 | 7.60E-02 |
| LOC105671381 | Uncharacterized LOC105671381 (LOC105671381) | -1.16 | 8.44E-06 | 4.69E-03 |
| LOC105668572 | Uncharacterized LOC105668572 (LOC105668572) | -1.18 | 2.42E-07 | 2.69E-04 |
| LOC105673352 | Sodium-coupled neutral amino acid transporter 9-like (LOC105673352) | -1.26 | 3.04E-04 | 7.60E-02 |
| LOC105667677 | Zinc carboxypeptidase-like (LOC105667677) | -1.27 | 1.52E-07 | 2.17E-04 |
| LOC105676197 | 5-aminolevulate synthase | -1.34 | 5.32E-08 | 1.06E-04 |
| LOC105670776 | Endocuticle structural glycoprotein SgAbd-4-like (LOC105670776) | -1.34 | 1.35E-04 | 4.82E-02 |
| LOC105670441 | Uncharacterized LOC105670441 (LOC105670441) | -1.43 | 7.43E-07 | 7.44E-04 |
| LOC105670071 | Glucosylceramidase-like (LOC105670071) | -1.45 | 4.18E-04 | 8.24E-02 |
| LOC105679194 | Muscle segmentation homeobox-like (LOC105679194) | -1.82 | 2.81E-12 | 1.40E-08 |
| LOC105668025 | Transmembrane protease serine 9-like (LOC105668025) | -2.01 | 3.57E-04 | 7.94E-02 |
| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|------------|-------|-------------|-----|---------|-----------------|
| LOC105676839 | LOC105679457 | l-lactate dehydrogenase-like (LOC105676839) | -2.06 | 1.01E-06 | 7.80E-04 |
| LOC105679189 | LOC105670077 | DNA repair protein RAD50-like (LOC105670077) | -7.90 | 1.99E-12 | 1.40E-08 |
| LOC105668565 | LOC105669318 | Kynurenine/alpha-aminoadipate aminotransferase | 4.72 | 9.56E-05 | 2.29E-02 |
| LOC105671889 | LOC105672897 | Synaptobrevin homolog YKT6-like (LOC105671889) | 3.73 | 2.30E-05 | 7.35E-03 |
| LOC105672083 | LOC105674428 | Uncharacterized LOC105668565 (LOC105668565) | 6.55 | 1.98E-04 | 3.56E-02 |
| LOC105672083 | LOC105671469 | d-arabinitol dehydrogenase 1-like (LOC105671469) | 2.22 | 1.99E-04 | 3.56E-02 |
| LOC105672083 | LOC105672698 | Patatin-like phospholipase domain-containing protein 3 (LOC105672698) | 2.36 | 6.73E-06 | 3.08E-03 |
| LOC105672083 | LOC10567310 | Uncharacterized LOC105670688 (LOC105670688) | 2.53 | 1.17E-05 | 4.57E-03 |
| LOC105672083 | LOC105673823 | Glucose dehydrogenase [FAD 2.16 | 4.39E-06 | 2.31E-03 |
| LOC105672083 | LOC105673839 | Succinate dehydrogenase [ubiquinone iron-sulfur subunit 2.16 | 1.30E-04 | 2.67E-02 |
| LOC105672083 | LOC105669349 | Uncharacterized LOC105669349 (LOC105669349) | 2.15 | 1.24E-04 | 2.60E-02 |
| LOC105672083 | LOC105669805 | Facilitated trehalose transporter Tret1-2 homolog (LOC105669805) | 2.08 | 5.43E-04 | 8.40E-02 |
| LOC105672083 | LOC105667792 | Synaptotagmin-6 (LOC105667792) | 2.00 | 1.08E-04 | 2.33E-02 |
| LOC105669349 | LOC105676997 | Patatin-like phospholipase domain-containing protein 3 (LOC105676997) | 1.97 | 3.37E-09 | 3.22E-06 |
| LOC105669349 | LOC105667804 | Uncharacterized family 31 glucosidase KIAA1161 (LOC105667804) | 1.92 | 5.44E-07 | 3.81E-04 |
| LOC105669349 | LOC105674420 | 3'(2') | 1.89 | 2.22E-05 | 7.30E-03 |
| LOC105669349 | LOC105670888 | Hexokinase type 2-like (LOC105670888) | 1.88 | 2.23E-10 | 2.61E-07 |

(Continues)
| Time point | Locus | Description | LFC | p-value | Adjusted p-value |
|------------|-------|-------------|-----|---------|-----------------|
| LOC105678328 | T-lymphocyte activation antigen CD86-like (LOC105678328) | 1.85 | 6.15E−05 | 1.59E−02 |
| LOC105674418 | Beta-1 | 1.78 | 8.76E−07 | 5.43E−04 |
| LOC105668703 | Membrane metalloendopeptidase-like 1 (LOC105668703) | 1.71 | 1.61E−05 | 5.70E−03 |
| LOC105668747 | C-1-tetrahydrofolate synthase | 1.70 | 2.05E−04 | 3.59E−02 |
| LOC105674681 | Cytochrome P450 6j1-like (LOC105674681) | 1.63 | 1.68E−04 | 3.21E−02 |
| LOC105677243 | Uncharacterized LOC105677243 (LOC105677243) | 1.53 | 1.67E−04 | 3.21E−02 |
| LOC105676329 | Insulin-like growth factor-binding protein complex acid labile subunit (LOC105676329) | 1.53 | 5.78E−06 | 2.77E−03 |
| LOC105674747 | Cytosolic purine 5’-nucleotidase (LOC105674747) | 1.51 | 2.64E−06 | 1.46E−03 |
| LOC105680118 | Cysteine proteinase 1-like (LOC105680118) | 1.49 | 5.94E−04 | 8.74E−02 |
| LOC105675420 | Protein yellow-like (LOC105675420) | 1.48 | 5.08E−05 | 1.37E−02 |
| LOC105669199 | 6-phosphofructo-2-kinase/fructose-2 | 1.43 | 1.35E−05 | 5.09E−03 |
| LOC105674352 | Aromatic-l-amino acid decarboxylase (LOC105674352) | 1.37 | 1.03E−05 | 4.32E−03 |
| LOC105667680 | Probable hydroxycacid-oxoacid transhydrogenase | 1.37 | 1.59E−04 | 3.16E−02 |
| LOC105657573 | Serine protease snake-like (LOC105657573) | 1.36 | 1.62E−05 | 5.70E−03 |
| LOC105668704 | Organic cation transporter protein (LOC105668704) | 1.36 | 4.04E−05 | 1.15E−02 |
| LOC105674248 | Branched-chain-amino acid aminotransferase | 1.35 | 2.78E−05 | 8.59E−03 |
| LOC105680007 | Protein toll (LOC105680007) | 1.35 | 2.01E−06 | 1.18E−03 |
| LOC105668437 | CCAAT/enhancer-binding protein (LOC105668437) | 1.33 | 1.81E−04 | 3.35E−02 |
| LOC105675016 | Peroxidase-like (LOC105675016) | 1.27 | 7.08E−04 | 9.94E−02 |
| LOC105677240 | General odorant-binding protein 56d-like (LOC105677240) | 1.18 | 1.06E−04 | 2.33E−02 |
| LOC105677283 | Alpha-aminoadipic semialdehyde synthase | 1.14 | 4.72E−04 | 7.52E−02 |
| LOC105671238 | Exosome component 10 (LOC105671238) | 1.14 | 6.13E−04 | 8.85E−02 |
| LOC105675407 | Inhibin beta E chain (LOC105675407) | 1.10 | 3.55E−04 | 5.92E−02 |
| LOC105667297 | Protein LTV1 homolog (LOC105667297) | 1.08 | 4.42E−04 | 7.27E−02 |
| LOC105671942 | Hexokinase-2-like (LOC105671942) | 1.08 | 8.18E−05 | 2.05E−02 |
| LOC105677187 | Serine protease easter-like (LOC105677187) | 1.05 | 1.09E−04 | 2.33E−02 |
| LOC105676715 | Alpha-tocopherol transfer protein-like (LOC105676715) | −1.38 | 3.07E−05 | 9.22E−03 |
| LOC105675717 | Defensing-2 (LOC105675717) | −1.39 | 4.68E−04 | 7.52E−02 |
| LOC105679818 | Cytosolic 10-formyltetrahydrofolate dehydrogenase (LOC105679818) | −1.47 | 5.42E−04 | 8.40E−02 |
| LOC105672088 | Lipid storage droplets surface-binding protein 2-like (LOC105672088) | −1.65 | 5.61E−04 | 8.55E−02 |

(Continues)
### TABLE A1 (Continued)

| Time point | Locus | Description | LFC  | p-Value | Adjusted p-value |
|------------|-------|-------------|------|---------|------------------|
| LOC105677041 | Cytochrome P450 4C1-like (LOC105677041) | -1.65 | 6.99E-04 | 9.94E-02 |
| LOC105671746 | Probable cytochrome P450 304a1 (LOC105671746) | -1.67 | 6.10E-08 | 5.35E-05 |
| LOC105667919 | Protein crumbs-like (LOC105667919) | -1.72 | 5.98E-04 | 8.74E-02 |
| LOC105677480 | Uncharacterized LOC105677480 (LOC105677480) | -1.73 | 5.26E-06 | 2.64E-03 |
| LOC105668144 | 1-acyl-sn-glycerol-3-phosphate acyltransferase alpha-like (LOC105668144) | -1.82 | 8.58E-06 | 3.76E-03 |
| LOC105677809 | Uncharacterized LOC105677809 (LOC105677809) | -1.86 | 1.81E-05 | 6.14E-03 |
| LOC105674506 | Annullin (LOC105674506) | -1.92 | 1.09E-05 | 4.43E-03 |
| LOC105675090 | Uncharacterized LOC105675090 (LOC105675090) | -1.94 | 4.21E-05 | 1.17E-02 |
| LOC105679194 | Muscle segmentation homeobox-like (LOC105679194) | -1.98 | 3.61E-05 | 1.05E-02 |
| LOC105679722 | Uncharacterized LOC105679722 (LOC105679722) | -2.06 | 2.27E-04 | 3.92E-02 |
| LOC105670118 | Uncharacterized methyltransferase-like protein SPBC21C3.07c (LOC105670118) | -2.07 | 5.80E-04 | 8.72E-02 |
| LOC105667917 | Protein crumbs-like (LOC105667917) | -2.45 | 1.06E-04 | 2.33E-02 |
| LOC105670857 | Leucine-rich repeat-containing protein egg-6 (LOC105670857) | -2.49 | 6.19E-05 | 1.59E-02 |
| LOC105671854 | Uncharacterized LOC105671854 (LOC105671854) | -3.69 | 3.19E-09 | 3.22E-06 |
| LOC105672186 | Uncharacterized LOC105672186 (LOC105672186) | -6.09 | 3.40E-04 | 5.78E-02 |
| LOC105668152 | Uncharacterized LOC105668152 (LOC105668152) | -6.68 | 1.66E-12 | 2.19E-09 |
| LOC105675449 | Uncharacterized LOC105675449 (LOC105675449) | -8.83 | 2.13E-13 | 3.74E-10 |
| LOC105670525 | Chondroadherin-like (LOC105670525) | -9.31 | 8.47E-30 | 8.92E-26 |
| LOC105668043 | Uncharacterized LOC105668043 (LOC105668043) | -10.32 | 3.15E-18 | 1.66E-14 |

### TABLE A2

Results of gene ontology enrichment analysis

| GO_name | GO_Category | FDR            | p-Value          |
|---------|-------------|----------------|-----------------|
| **Serine-type endopeptidase inhibitor activity** | MOLECULAR_FUNCTION | 1.13E-33 | 2.00E-37 |
| **Endopeptidase inhibitor activity** | MOLECULAR_FUNCTION | 6.65E-33 | 3.51E-36 |
| **Endopeptidase regulator activity** | MOLECULAR_FUNCTION | 6.65E-33 | 3.51E-36 |
| **Peptidase inhibitor activity** | MOLECULAR_FUNCTION | 8.42E-32 | 7.41E-35 |
| **Peptidase regulator activity** | MOLECULAR_FUNCTION | 8.42E-32 | 7.41E-35 |
| **Enzyme inhibitor activity** | MOLECULAR_FUNCTION | 8.24E-30 | 8.70E-33 |
| **Enzyme regulator activity** | MOLECULAR_FUNCTION | 1.89E-20 | 2.33E-23 |
| **Molecular function regulator** | MOLECULAR_FUNCTION | 4.10E-17 | 5.77E-20 |
| **Metallopeptidase activity** | MOLECULAR_FUNCTION | 1.07E-10 | 1.69E-13 |

*(Continues)*
| GO_name                                                                 | GO_Category                  | FDR     | p-Value  |
|------------------------------------------------------------------------|------------------------------|---------|----------|
| Peptidase activity, acting on l-amino acid peptides                     | MOLECULAR_FUNCTION          | 1.80E−06| 3.16E−09 |
| Peptidase activity                                                     | MOLECULAR_FUNCTION          | 3.77E−06| 7.30E−09 |
| Proteolysis                                                            | BIOLOGICAL_PROCESS          | 1.21E−04| 2.54E−07 |
| Metalloendopeptidase activity                                          | MOLECULAR_FUNCTION          | 0.0088  | 2.01E−05 |
| Transition metal ion binding                                           | MOLECULAR_FUNCTION          | 0.0466  | 1.15E−04 |
| **Enrichment of down-regulated genes in bacteria-injected isolated queens at 2 hpi** |                 |         |          |
| Serine-type endopeptidase inhibitor activity                           | MOLECULAR_FUNCTION          | 9.33E−24| 1.64E−27 |
| Endopeptidase inhibitor activity                                       | MOLECULAR_FUNCTION          | 2.11E−23| 1.12E−26 |
| Endopeptidase regulator activity                                       | MOLECULAR_FUNCTION          | 2.11E−23| 1.12E−26 |
| Peptidase inhibitor activity                                           | MOLECULAR_FUNCTION          | 9.97E−23| 8.77E−26 |
| Peptidase regulator activity                                           | MOLECULAR_FUNCTION          | 9.97E−23| 8.77E−26 |
| Enzyme inhibitor activity                                              | MOLECULAR_FUNCTION          | 2.17E−21| 2.29E−24 |
| Enzyme regulator activity                                              | MOLECULAR_FUNCTION          | 8.09E−15| 9.96E−18 |
| Metallopeptidase activity                                              | MOLECULAR_FUNCTION          | 5.74E−13| 8.08E−16 |
| Molecular function regulator                                           | MOLECULAR_FUNCTION          | 1.73E−12| 2.74E−15 |
| Peptidase activity, acting on l-amino acid peptides                    | MOLECULAR_FUNCTION          | 2.26E−07| 3.98E−10 |
| Peptidase activity                                                     | MOLECULAR_FUNCTION          | 4.47E−07| 8.65E−10 |
| Proteolysis                                                            | BIOLOGICAL_PROCESS          | 2.95E−06| 6.23E−09 |
| Metalloendopeptidase activity                                          | MOLECULAR_FUNCTION          | 3.96E−05| 9.05E−08 |
| Zinc ion binding                                                       | MOLECULAR_FUNCTION          | 5.47E−04| 1.35E−06 |
| Hydrolase activity                                                     | MOLECULAR_FUNCTION          | 0.0016  | 4.33E−06 |
| Transition metal ion binding                                           | MOLECULAR_FUNCTION          | 0.0032  | 8.95E−06 |
| Molybdopterin cofactor metabolic process                               | BIOLOGICAL_PROCESS          | 0.0403  | 1.35E−04 |
| Prosthetic group metabolic process                                     | BIOLOGICAL_PROCESS          | 0.0403  | 1.35E−04 |
| Molybdopterin cofactor biosynthetic process                            | BIOLOGICAL_PROCESS          | 0.0403  | 1.35E−04 |
| Endopeptidase activity                                                | MOLECULAR_FUNCTION          | 0.0479  | 1.77E−04 |
| Exopeptidase activity                                                 | MOLECULAR_FUNCTION          | 0.0479  | 1.77E−04 |
| **Enrichment of down-regulated genes in bacteria-injected isolated queens at 12 hpi** |                 |         |          |
| Oxidoreductase activity                                               | MOLECULAR_FUNCTION          | 0.0216  | 7.60E−06 |
| Oxidation-reduction process                                           | BIOLOGICAL_PROCESS          | 0.0216  | 7.49E−06 |
| **Enrichment of up-regulated genes in Ringer-injected isolated queens at 12 hpi** |                 |         |          |
| Single-organism metabolic process                                      | BIOLOGICAL_PROCESS          | 0.0024  | 4.28E−07 |
| Carbohydrate kinase activity                                           | MOLECULAR_FUNCTION          | 0.0120  | 6.36E−06 |
| Carbohydrate phosphorylation                                          | BIOLOGICAL_PROCESS          | 0.0120  | 4.47E−06 |
| Cellular glucose homeostasis                                          | BIOLOGICAL_PROCESS          | 0.0310  | 4.37E−05 |
| Glucose binding                                                       | MOLECULAR_FUNCTION          | 0.0310  | 4.37E−05 |
| Carbohydrate homeostasis                                              | BIOLOGICAL_PROCESS          | 0.0310  | 4.37E−05 |
| Hexokinase activity                                                   | MOLECULAR_FUNCTION          | 0.0310  | 4.37E−05 |
| Glucose homeostasis                                                   | BIOLOGICAL_PROCESS          | 0.0310  | 4.37E−05 |
**TABLE A3** Differentially expressed genes in bacteria-injected social queens with false discovery rate <10%. T2, T4, T8, and T12 indicate the post-injection time points

| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|------------|-------|-------------|-----|---------|------------------|
| T2         | LOC105671203 | Uncharacterized LOC105671203 (LOC105671203) | 3.69 | 7.03E−13 | 3.11E−09 |
|            | LOC105668751 | Uncharacterized LOC105668751 (LOC105668751) (Naickin-2) | 3.06 | 3.29E−08 | 7.27E−05 |
|            | LOC105670685 | Glycine-rich RNA-binding protein 1-like (LOC105670685) | 2.45 | 1.41E−04 | 9.84E−02 |
|            | LOC105677480 | Uncharacterized LOC105677480 (LOC105677480) | 2.29 | 1.74E−06 | 2.56E−03 |
|            | LOC105677479 | Uncharacterized LOC105677479 (LOC105677479) | 2.24 | 1.45E−04 | 9.84E−02 |
|            | LOC105675449 | Uncharacterized LOC105675449 (LOC105675449) | 1.79 | 3.19E−05 | 3.52E−02 |
|            | LOC105676587 | Trypsin-like (LOC105676587) | 1.73 | 9.33E−05 | 9.17E−02 |
|            | LOC105674885 | Purine nucleoside phosphorylase-like (LOC105674885) | −1.99 | 1.41E−04 | 9.84E−02 |
|            | LOC105678691 | Scavenger receptor class B member 1-like (LOC105678691) | −2.28 | 1.65E−05 | 2.08E−02 |
|            | LOC105668207 | Chymotrypsin-1-like (LOC105668207) | −4.01 | 9.23E−14 | 8.16E−10 |
|            | LOC105677784 | Uncharacterized LOC105677784 (LOC105677784) | −4.12 | 5.07E−10 | 1.49E−06 |
|            | LOC105668184 | Uncharacterized LOC105668184 (LOC105668184) | −22.70 | 1.35E−04 | 9.84E−02 |
|            | LOC105670097 | Spidroin-1 (LOC105670097) | −26.14 | 7.82E−07 | 1.38E−03 |
| T4         | LOC105670077 | DNA repair protein RAD50-like (LOC105670077) | 9.96 | 2.39E−06 | 4.18E−04 |
|            | LOC105670097 | Spidroin-1 (LOC105670097) | 9.00 | 6.39E−39 | 3.58E−35 |
|            | LOC105679457 | Uncharacterized LOC105679457 (LOC105679457) | 8.37 | 1.61E−04 | 1.54E−02 |
|            | LOC105679565 | A disintegrin and metalloproteinase with thrombospondin motifs 4-like (LOC105679565) | 7.51 | 1.67E−03 | 9.52E−02 |
|            | LOC105679203 | Cytochrome P450 4g15-like (LOC105679203) | 7.21 | 6.19E−11 | 4.34E−08 |
|            | LOC105671203 | Uncharacterized LOC105671203 (LOC105671203) | 6.80 | 1.90E−72 | 2.13E−68 |
|            | LOC105677347 | Uncharacterized LOC105677347 (LOC105677347) | 6.21 | 2.74E−05 | 3.41E−03 |
|            | LOC105668553 | A disintegrin and metalloproteinase with thrombospondin motifs 2-like (LOC105668553) | 5.68 | 7.90E−04 | 5.11E−02 |
|            | LOC105677333 | Uncharacterized LOC105677333 (LOC105677333) | 5.43 | 5.89E−07 | 1.32E−04 |
|            | LOC105676228 | Glutamyl aminopeptidase-like (LOC105676228) | 5.37 | 3.28E−05 | 3.89E−03 |
|            | LOC105677348 | Uncharacterized LOC105677348 (LOC105677348) | 5.28 | 1.98E−18 | 3.70E−15 |
|            | LOC105677332 | Uncharacterized LOC105677332 (LOC105677332) | 5.23 | 7.15E−12 | 6.16E−09 |

(Continues)
| Time point | Locus | Description | LFC  | p-Value | Adjusted p-value |
|------------|-------|-------------|------|---------|------------------|
| LOC105671207 | A disintegrin and metalloproteinase with thrombospondin motifs 7-like (LOC105671207) | 5.20 | 6.21E−05 | 6.82E−03 |
| LOC105670873 | Uncharacterized LOC105670873 (LOC105670873) | 4.97 | 2.43E−04 | 2.01E−02 |
| LOC105674384 | Uncharacterized LOC105674384 (LOC105674384) | 4.95 | 2.04E−04 | 1.82E−02 |
| LOC105676577 | Glutamyl aminopeptidase-like (LOC105676577) | 4.81 | 2.18E−04 | 1.88E−02 |
| LOC105674526 | Uncharacterized LOC105674526 (LOC105674526) | 4.80 | 5.82E−07 | 1.32E−04 |
| LOC105676006 | Uncharacterized LOC105676006 (LOC105676006) | 4.62 | 9.75E−10 | 5.20E−07 |
| LOC105678717 | Uncharacterized LOC105678717 (LOC105678717) | 4.60 | 1.09E−03 | 6.68E−02 |
| LOC105676008 | Uncharacterized LOC105676008 (LOC105676008) | 4.47 | 2.62E−05 | 3.34E−03 |
| LOC105670188 | Luciferin 4-monooxygenase-like (LOC105670188) | 4.37 | 3.59E−09 | 1.68E−06 |
| LOC105677172 | Uncharacterized LOC105677172 (LOC105677172) | 4.28 | 5.57E−04 | 3.81E−02 |
| LOC105674010 | Phosphotriesterase-related protein-like (LOC105674010) | 4.14 | 1.41E−04 | 1.37E−02 |
| LOC105673254 | Thyrotropin-releasing hormone-degrading ectoenzyme-like (LOC105673254) | 4.11 | 2.68E−04 | 2.19E−02 |
| LOC105672921 | Zinc finger protein 468-like (LOC105672921) | 4.10 | 1.10E−03 | 6.71E−02 |
| LOC105672283 | Uncharacterized threonine-rich GPI-anchored glycoprotein PJ4664.02-like (LOC105672283) | 4.04 | 6.91E−06 | 1.08E−03 |
| LOC105679148 | Uncharacterized LOC105679148 (LOC105679148) | 3.98 | 6.36E−07 | 1.40E−04 |
| LOC105678936 | Uncharacterized LOC105678936 (LOC105678936) | 3.92 | 1.05E−09 | 5.37E−07 |
| LOC105671306 | A disintegrin and metalloproteinase with thrombospondin motifs 17-like (LOC105671306) | 3.83 | 4.76E−04 | 3.37E−02 |
| LOC105674631 | Uncharacterized LOC105674631 (LOC105674631) | 3.82 | 3.84E−04 | 2.89E−02 |
| LOC105679175 | Uncharacterized LOC105679175 (LOC105679175) | 3.73 | 4.82E−06 | 7.60E−04 |
| LOC105671771 | Uncharacterized LOC105671771 (LOC105671771) | 3.57 | 5.05E−05 | 5.66E−03 |
| LOC105679161 | A disintegrin and metalloproteinase with thrombospondin motifs 17-like (LOC105679161) | 3.52 | 7.78E−04 | 5.08E−02 |
| LOC105674893 | Uncharacterized LOC105674893 (LOC105674893) | 3.48 | 5.07E−04 | 3.55E−02 |
| LOC105670869 | Uncharacterized LOC105670869 (LOC105670869) | 3.29 | 1.47E−10 | 9.16E−08 |

(Continues)
| Time point | Locus               | Description                                                                 | LFC  | p-Value    | Adjusted p-value |
|------------|---------------------|------------------------------------------------------------------------------|------|------------|------------------|
| LOC105670598 | LOC105670598 | A disintegrin and metalloproteinase with thrombospondin motifs 12-like (LOC105670598) | 3.28 | 2.82E-04  | 2.26E-02          |
| LOC105673458 | LOC105673458 | Thyrotropin-releasing hormone-degrading ectoenzyme-like (LOC105673458) | 3.18 | 2.90E-06  | 4.84E-04          |
| LOC105668847 | LOC105668847 | Uncharacterized LOC105668847 (LOC105668847) | 3.14 | 1.58E-03  | 9.20E-02          |
| LOC105674525 | LOC105674525 | Thyrotropin-releasing hormone-degrading ectoenzyme-like (LOC105674525) | 3.12 | 3.41E-06  | 5.62E-04          |
| LOC105679050 | LOC105679050 | Transcription termination factor 2-like (LOC105679050) | 3.10 | 9.41E-09  | 3.63E-06          |
| LOC105668490 | LOC105668490 | Uncharacterized LOC105668490 (LOC105668490) | 2.85 | 9.94E-05  | 1.03E-02          |
| LOC105670243 | LOC105670243 | Uncharacterized LOC105670243 (LOC105670243) | 2.81 | 2.94E-07  | 7.38E-05          |
| LOC105678344 | LOC105678344 | Gephyrin-like (LOC105678344) | 2.77 | 3.07E-05  | 3.70E-03          |
| LOC105674779 | LOC105674779 | Fatty acid synthase-like (LOC105674779) | 2.72 | 1.61E-14  | 2.00E-11          |
| LOC105671607 | LOC105671607 | Uncharacterized LOC105671607 (LOC105671607) | 2.69 | 1.24E-03  | 7.41E-02          |
| LOC105675620 | LOC105675620 | Aminopeptidase N-like (LOC105675620) | 2.64 | 1.15E-07  | 3.22E-05          |
| LOC105672800 | LOC105672800 | Uncharacterized LOC105672800 (LOC105672800) | 2.64 | 3.30E-05  | 3.89E-03          |
| LOC105679886 | LOC105679886 | Uncharacterized LOC105679886 (LOC105679886) | 2.64 | 5.65E-08  | 1.71E-05          |
| LOC105674397 | LOC105674397 | Uncharacterized LOC105674397 (LOC105674397) | 2.60 | 4.40E-08  | 1.37E-05          |
| LOC105670868 | LOC105670868 | Uncharacterized LOC105670868 (LOC105670868) | 2.56 | 2.31E-08  | 8.08E-06          |
| LOC105669605 | LOC105669605 | A disintegrin and metalloproteinase with thrombospondin motifs 20-like (LOC105669605) | 2.51 | 1.21E-03  | 7.34E-02          |
| LOC105670022 | LOC105670022 | Chymotrypsin-2-like (LOC105670022) | 2.47 | 3.77E-12  | 3.84E-09          |
| LOC105679174 | LOC105679174 | Uncharacterized LOC105679174 (LOC105679174) | 2.41 | 2.83E-04  | 2.26E-02          |
| LOC105670219 | LOC105670219 | Uncharacterized LOC105670219 (LOC105670219) | 2.38 | 4.34E-04  | 3.10E-02          |
| LOC105674848 | LOC105674848 | RCC1 and BTB domain-containing protein 1-like (LOC105674848) | 2.35 | 3.58E-04  | 2.78E-02          |
| LOC105670871 | LOC105670871 | Uncharacterized LOC105670871 (LOC105670871) | 2.34 | 3.47E-05  | 4.04E-03          |
| LOC105670872 | LOC105670872 | Uncharacterized LOC105670872 (LOC105670872) | 2.31 | 1.42E-06  | 2.65E-04          |
| LOC105668795 | LOC105668795 | Flavin-containing monooxygenase FMO GS-OX-like 3 (LOC105668795) | 2.30 | 7.32E-09  | 3.04E-06          |
| LOC105667907 | LOC105667907 | Thyroid receptor-interacting protein 11-like (LOC105667907) | 2.29 | 6.90E-07  | 1.43E-04          |
| LOC105668248 | LOC105668248 | Uncharacterized LOC105668248 (LOC105668248) | 2.28 | 1.80E-05  | 2.41E-03          |

(Continues)
| Time point | Locus                     | Description                     | LFC  | p-Value   | Adjusted p-value |
|------------|---------------------------|---------------------------------|------|-----------|------------------|
| LOC105670430 | Aminopeptidase N-like (LOC105670430) | 2.27                            | 2.06E−05 | 2.68E−03 |
| LOC105673464 | Venom carboxylesterase-6-like (pseudo) | 2.27                            | 4.42E−08 | 1.37E−05 |
| LOC105678987 | Uncharacterized LOC105678987 (LOC105678987) | 2.24                            | 2.97E−07 | 7.38E−05 |
| LOC105670899 | Uncharacterized LOC105670899 (LOC105670899) | 2.23                            | 6.89E−07 | 1.43E−04 |
| LOC105679177 | Uncharacterized LOC105679177 (LOC105679177) | 2.21                            | 2.08E−04 | 1.83E−02 |
| LOC105674677 | Uncharacterized LOC105674677 (LOC105674677) | 2.20                            | 1.81E−07 | 4.83E−05 |
| LOC105674740 | Zinc carboxypeptidase-like (LOC105674740) | 2.16                            | 7.34E−06 | 1.11E−03 |
| LOC105674403 | Uncharacterized LOC105674403 (LOC105674403) | 2.13                            | 1.16E−03 | 7.09E−02 |
| LOC105671625 | Uncharacterized LOC105671625 (LOC105671625) | 2.10                            | 1.12E−04 | 1.12E−02 |
| LOC105678984 | Uncharacterized LOC105678984 (LOC105678984) | 2.08                            | 8.18E−07 | 1.64E−04 |
| LOC105676005 | Uncharacterized LOC105676005 (LOC105676005) | 1.99                            | 2.92E−04 | 2.31E−02 |
| LOC105668487 | Uncharacterized LOC105668487 (LOC105668487) | 1.97                            | 2.73E−05 | 3.41E−03 |
| LOC105674716 | Zinc carboxypeptidase A 1-like (LOC105674716) | 1.92                            | 1.45E−05 | 2.03E−03 |
| LOC105672402 | Fatty acid synthase-like (LOC105672402) | 1.91                            | 7.20E−06 | 1.11E−03 |
| LOC105674892 | Uncharacterized LOC105674892 (LOC105674892) | 1.91                            | 3.83E−04 | 2.89E−02 |
| LOC105670241 | Uncharacterized LOC105670241 (LOC105670241) | 1.90                            | 7.66E−06 | 1.13E−03 |
| LOC105676597 | Uncharacterized LOC105676597 (LOC105676597) | 1.87                            | 1.43E−08 | 5.17E−06 |
| LOC105678934 | Uncharacterized LOC105678934 (LOC105678934) | 1.86                            | 2.95E−05 | 3.59E−03 |
| LOC105671177 | L-xylulose reductase-like (LOC105671177) | 1.85                            | 1.63E−04 | 1.55E−02 |
| LOC105671446 | A disintegrin and metalloproteinase with thrombospondin motifs 7-like (LOC105671446) | 1.85                            | 5.42E−04 | 3.75E−02 |
| LOC105670897 | Uncharacterized LOC105670897 (LOC105670897) | 1.85                            | 9.23E−08 | 2.72E−05 |
| LOC105674728 | Muscle M-line assembly protein unc-89-like (LOC105674728) | 1.84                            | 4.24E−04 | 3.08E−02 |
| LOC105678217 | Uncharacterized LOC105678217 (LOC105678217) | 1.84                            | 1.42E−07 | 3.88E−05 |
| LOC105668988 | Uncharacterized LOC105668988 (LOC105668988) | 1.83                            | 5.66E−10 | 3.17E−07 |
| LOC105679014 | Uncharacterized LOC105679014 (LOC105679014) | 1.83                            | 1.73E−04 | 1.60E−02 |

(Continues)
| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|-----------|--------|-------------|-----|---------|------------------|
| LOC105671241 | Aminopeptidase N-like (LOC105671241) | 1.83 | 4.86E-05 | 5.50E-03 |
| LOC105670900 | Uncharacterized LOC105670900 (LOC105670900) | 1.82 | 1.23E-04 | 1.22E-02 |
| LOC105678353 | Aminopeptidase N-like (LOC105678353) | 1.81 | 8.06E-04 | 5.19E-02 |
| LOC105676303 | N-(5-amino-5-carboxypentanoyl)-L-cysteinyl-L-valine synthase (LOC105676303) | 1.81 | 6.33E-09 | 2.73E-06 |
| LOC105670239 | Uncharacterized LOC105670239 (LOC105670239) | 1.79 | 2.90E-06 | 4.84E-04 |
| LOC105674406 | Uncharacterized LOC105674406 (LOC105674406) | 1.78 | 2.50E-04 | 2.06E-02 |
| LOC105678342 | Gephyrin-like (LOC105678342) | 1.77 | 1.53E-05 | 2.12E-03 |
| LOC105679156 | Uncharacterized LOC105679156 (LOC105679156) | 1.76 | 1.78E-04 | 1.63E-02 |
| LOC105668244 | Uncharacterized LOC105668244 (LOC105668244) | 1.75 | 9.96E-07 | 1.92E-04 |
| LOC105672123 | Uncharacterized LOC105672123 (LOC105672123) | 1.74 | 1.01E-03 | 6.24E-02 |
| LOC105667777 | Zinc carboxypeptidase-like (LOC105667777) | 1.72 | 2.13E-10 | 1.26E-07 |
| LOC105676007 | Uncharacterized LOC105676007 (LOC105676007) | 1.70 | 5.98E-04 | 4.06E-02 |
| LOC105678817 | Protein toll-like (LOC105678817) | 1.69 | 1.02E-04 | 1.05E-02 |
| LOC105670238 | Uncharacterized LOC105670238 (LOC105670238) | 1.67 | 7.51E-06 | 1.12E-03 |
| LOC105677480 | Uncharacterized LOC105677480 (LOC105677480) | 1.66 | 9.46E-07 | 1.86E-04 |
| LOC105677501 | Venom acid phosphatase Acph-1-like (LOC105677501) | 1.66 | 1.03E-04 | 1.05E-02 |
| LOC105670214 | Tissue factor pathway inhibitor-like (LOC105670214) | 1.60 | 8.25E-05 | 8.80E-03 |
| LOC105673951 | Chymotrypsin-1-like (LOC105673951) | 1.59 | 6.92E-07 | 1.43E-04 |
| LOC105668779 | Troponin C | 1.55 | 8.95E-06 | 1.30E-03 |
| LOC105675453 | Serine racemase-like (LOC105675453) | 1.55 | 6.50E-04 | 4.39E-02 |
| LOC105676003 | Uncharacterized LOC105676003 (LOC105676003) | 1.53 | 1.96E-04 | 1.78E-02 |
| LOC105676004 | Uncharacterized LOC105676004 (LOC105676004) | 1.52 | 8.36E-04 | 5.32E-02 |
| LOC105676346 | Uncharacterized LOC105676346 (LOC105676346) | 1.52 | 2.24E-06 | 3.99E-04 |
| LOC105678648 | Protein toll-like (LOC105678648) | 1.50 | 5.55E-04 | 3.81E-02 |
| LOC105667368 | 46 kDa FK506-binding nuclear protein-like (LOC105667368) | 1.49 | 4.87E-04 | 3.43E-02 |
| LOC105667585 | Laccase-1-like (LOC105667585) | 1.48 | 9.34E-05 | 9.87E-03 |
| LOC105670240 | Uncharacterized LOC105670240 (LOC105670240) | 1.47 | 1.27E-04 | 1.25E-02 |
| LOC105668195 | Uncharacterized LOC105668195 (LOC105668195) | 1.45 | 3.80E-07 | 8.88E-05 |
| Time point | Locus                                      | Description                                      | LFC  | p-Value     | Adjusted p-value |
|-----------|--------------------------------------------|--------------------------------------------------|------|-------------|------------------|
| LOC105671544 | Isthmin-like (LOC105671544)               | 1.44                                             | 2.33E−04 | 1.95E−02   |
| LOC105679722 | Uncharacterized LOC105679722 (LOC105679722) | 1.43                                             | 2.16E−04 | 1.88E−02   |
| LOC105670898 | Uncharacterized LOC105670898 (LOC105670898) | 1.43                                             | 2.15E−04 | 1.88E−02   |
| LOC105674374 | Uncharacterized LOC105674374 (LOC105674374) | 1.42                                             | 4.32E−04 | 3.10E−02   |
| LOC105675133 | Mitochondrial uncoupling protein 2-like (LOC105675133) | 1.42                                             | 2.67E−07  | 6.96E−05   |
| LOC105672982 | Chromosome-associated kinesin KIF4 (LOC105672982) | 1.39                                             | 7.11E−05  | 7.73E−03   |
| LOC105677706 | Bipolar kinesin KRP-130-like (LOC105677706) | 1.37                                             | 6.99E−04  | 4.69E−02   |
| LOC105677471 | Facilitated trehalose transporter Tret1-like (LOC105677471) | 1.33                                             | 3.92E−05  | 4.53E−03   |
| LOC105670134 | Uncharacterized LOC105670134 (LOC105670134) | 1.32                                             | 1.59E−03  | 9.20E−02   |
| LOC105677684 | E3 ubiquitin-protein ligase TRIM71 (LOC105677684) | 1.28                                             | 1.59E−05  | 2.17E−03   |
| LOC105672089 | Lipid storage droplets surface-binding protein 2-like (LOC105672089) | 1.28                                             | 2.24E−06  | 3.99E−04   |
| LOC105671386 | Organic cation transporter 1-like (LOC105671386) | 1.25                                             | 9.68E−04  | 6.03E−02   |
| LOC105675373 | Cyclin-dependent kinase 4 (LOC105675373) | 1.24                                             | 2.52E−05  | 3.25E−03   |
| LOC105669176 | Organic cation transporter protein-like (LOC105669176) | 1.22                                             | 1.69E−04  | 1.59E−02   |
| LOC105671746 | Probable cytochrome P450 304a1 (LOC105671746) | 1.22                                             | 2.24E−04  | 1.90E−02   |
| LOC105671768 | Uncharacterized LOC105671768 (LOC105671768) | 1.15                                             | 5.39E−04  | 3.75E−02   |
| LOC105668043 | Uncharacterized LOC105668043 (LOC105668043) | 1.13                                             | 1.31E−03  | 7.71E−02   |
| LOC105668144 | 1-acyl-sn-glycerol-3-phosphate acyltransferase alpha-like (LOC105668144) | 1.09                                             | 7.80E−04  | 5.08E−02   |
| LOC105675016 | Peroxidase-like (LOC105675016) | 1.01                                             | 3.90E−04  | 2.91E−02   |
| LOC105677622 | Uncharacterized LOC105677622 (LOC105677622) | 0.99                                             | 1.63E−03  | 9.34E−02   |
| LOC105676083 | Baculoviral IAP repeat-containing protein 7-A-like (LOC105676083) | 0.97                                             | 7.21E−04  | 4.81E−02   |
| LOC105668364 | Mesencephalic astrocyte-derived neurotrophic factor homolog (LOC105668364) | 0.91                                             | 1.74E−03  | 9.83E−02   |
| LOC105668947 | Endoplasmin (LOC105668947) | 0.83                                             | 1.69E−03  | 9.60E−02   |
| LOC105669096 | Spondin-1 (LOC105669096) | −0.90                                            | 1.22E−03  | 7.34E−02   |
| LOC105668946 | Uncharacterized LOC105668946 (LOC105668946) | −0.96                                            | 7.27E−04  | 4.82E−02   |
| LOC105673575 | Venom metalloproteinase 3-like (LOC105673575) | −0.98                                            | 3.16E−04  | 2.47E−02   |

(Continues)
| Time point | Locus                  | Description                                             | LFC  | p-Value     | Adjusted p-value |
|------------|------------------------|---------------------------------------------------------|------|-------------|------------------|
| LOC105670254 | Glycine-rich cell wall structural protein-like (LOC105670254) | -1.00 | 2.02E-04   | 1.81E-02     |
| LOC105672527 | Venom allergen 3-like (LOC105672527) | -1.00 | 2.70E-04   | 2.19E-02     |
| LOC105670714 | Alpha-amylase A (LOC105670714) | -1.01 | 3.79E-04   | 2.88E-02     |
| LOC105672704 | Receptor-type tyrosine-protein phosphatase epsilon-like (LOC105672704) | -1.04 | 8.56E-04   | 5.42E-02     |
| LOC105675546 | Uncharacterized LOC105675546 (LOC105675546) | -1.05 | 8.23E-04   | 5.27E-02     |
| LOC105676883 | Serine hydroxymethyltransferase | -1.08 | 4.27E-04   | 3.08E-02     |
| LOC10567184 | Troponin C | -1.09 | 2.32E-04   | 1.95E-02     |
| LOC105678432 | Acetyl-CoA carboxylase (LOC105678432) | -1.11 | 9.56E-04   | 6.02E-02     |
| LOC105671942 | Hexokinase-2-like (LOC105671942) | -1.12 | 9.69E-04   | 6.03E-02     |
| LOC105672608 | Facilitated trehalose transporter Tret1-like (LOC105672608) | -1.14 | 7.47E-04   | 4.92E-02     |
| LOC105678761 | Serine protease inhibitor 3/4-like (LOC105678761) | -1.17 | 4.04E-04   | 2.98E-02     |
| LOC105675773 | Peptidoglycan-recognition protein SC2-like (LOC105675773) | -1.20 | 9.78E-06   | 1.40E-03     |
| LOC105669805 | Facilitated trehalose transporter Tret1-2 homolog (LOC105669805) | -1.22 | 4.34E-05   | 4.97E-03     |
| LOC105674040 | Apolliporphins (LOC105674040) | -1.23 | 4.34E-06   | 7.05E-04     |
| LOC105671989 | Uncharacterized LOC105671989 (LOC105671989) | -1.28 | 4.03E-04   | 2.98E-02     |
| LOC105677003 | Aminopeptidase N-like (LOC105677003) | -1.28 | 1.09E-04   | 1.10E-02     |
| LOC105668704 | Organic cation transporter protein (LOC105668704) | -1.30 | 1.95E-05   | 2.57E-03     |
| LOC105670974 | Uncharacterized LOC105670974 (LOC105670974) | -1.30 | 1.87E-06   | 3.43E-04     |
| LOC105674681 | Cytochrome P450 6j1-like (LOC105674681) | -1.31 | 1.57E-04   | 1.51E-02     |
| LOC105677248 | Protein phosphatase 1 regulatory subunit 3C-B (LOC105677248) | -1.34 | 7.82E-05   | 8.42E-03     |
| LOC105672092 | Uncharacterized LOC105672092 (LOC105672092) | -1.35 | 9.67E-05   | 1.01E-02     |
| LOC105675916 | Major royal jelly protein 5-like (LOC105675916) | -1.37 | 1.39E-06   | 2.63E-04     |
| LOC105672956 | Ribose-phosphate pyrophosphokinase 1 (LOC105672956) | -1.39 | 3.22E-07   | 7.68E-05     |
| LOC105676485 | Uncharacterized LOC105676485 (LOC105676485) | -1.40 | 2.24E-04   | 1.90E-02     |
| LOC105672335 | Uncharacterized LOC105672335 (LOC105672335) | -1.41 | 3.70E-04   | 2.85E-02     |
| LOC105667549 | Vascular endothelial growth factor receptor 1-like (LOC105667549) | -1.42 | 5.42E-05   | 6.01E-03     |
| LOC105676142 | Vascular endothelial growth factor receptor kdr-like (LOC105676142) | -1.45 | 1.59E-03   | 9.20E-02     |

(Continues)
| Time point | Locus | Description                      | LFC  | p-Value      | Adjusted p-value |
|------------|-------|----------------------------------|------|--------------|------------------|
| LOC105669099 | Vascular endothelial growth factor B-like (LOC105669099) | −1.45 | 4.09E−04 | 2.99E−02 |
| LOC105674346 | Neural-cadherin (LOC105674346) | −1.49 | 3.92E−08 | 1.29E−05 |
| LOC105671703 | Facilitated trehalose transporter Tret1-like (LOC105671703) | −1.52 | 1.39E−03 | 8.15E−02 |
| LOC105672628 | Esterase FE4-like (LOC105672628) | −1.53 | 5.18E−09 | 2.32E−06 |
| LOC105670266 | Sodium/potassium/calcium exchanger 3-like (LOC105670266) | −1.54 | 1.72E−04 | 1.60E−02 |
| LOC105679180 | Solute carrier family 2 | −1.59 | 3.18E−07 | 7.68E−05 |
| LOC105679484 | d-arabinitol dehydrogenase 1-like (LOC105679484) | −1.66 | 1.69E−09 | 8.22E−07 |
| LOC105675436 | Chymotrypsin inhibitor-like (LOC105675436) | −1.69 | 1.42E−10 | 9.16E−08 |
| LOC105669509 | Arylsulfatase B-like (LOC105669509) | −1.71 | 4.52E−06 | 7.23E−04 |
| LOC105669158 | Sorbitol dehydrogenase-like (LOC105669158) | −1.71 | 8.01E−09 | 3.21E−06 |
| LOC105675717 | Defensing-2 (LOC105675717) | −1.80 | 1.05E−08 | 3.93E−06 |
| LOC105679484 | Sorbitol dehydrogenase-like (LOC105679484) | −1.80 | 3.71E−04 | 2.85E−02 |
| LOC105679620 | Uncharacterized LOC105679620 (LOC105679620) | −1.88 | 1.68E−05 | 2.26E−03 |
| LOC105679945 | Cytochrome P450 4C1-like (LOC105679945) | −1.95 | 1.30E−03 | 7.71E−02 |
| LOC105679847 | Probable plantanol-CoA dioxygenase (LOC105679847) | −2.03 | 2.39E−11 | 1.92E−08 |
| LOC105667920 | Phosphoenolpyruvate carboxykinase (GTP)-like (LOC105667920) | −2.08 | 1.30E−05 | 1.84E−03 |
| LOC105675714 | Uncharacterized LOC105675714 (LOC105675714) | −2.10 | 2.85E−04 | 2.27E−02 |
| LOC105672537 | Alpha-glucosidase-like (LOC105672537) | −2.15 | 4.98E−15 | 6.98E−12 |
| LOC105672219 | Leucine-rich repeat-containing protein 4-like (LOC105672219) | −2.17 | 3.72E−11 | 2.78E−08 |
| LOC105680018 | Uncharacterized LOC105680018 (LOC105680018) | −2.20 | 2.77E−06 | 4.78E−04 |
| LOC105675643 | Prismalin-14-like (LOC105675643) | −2.29 | 2.95E−05 | 3.59E−03 |
| LOC105678258 | Facilitated trehalose transporter Tret1-like (LOC105678258) | −2.35 | 1.24E−03 | 7.41E−02 |
| LOC105668871 | Phenoloxidase 2-like (LOC105668871) | −2.36 | 8.57E−17 | 1.37E−13 |
| LOC105676062 | Protein henna (LOC105676062) | −2.37 | 3.44E−08 | 1.17E−05 |
| LOC105679763 | Uncharacterized oxidoreductase C26H5.09c-like (LOC105679763) | −2.40 | 4.11E−13 | 4.60E−10 |
| LOC105671889 | Synaptobrevin homolog YKT6-like (LOC105671889) | −2.44 | 7.03E−07 | 1.43E−04 |
| LOC105667386 | Hexamerin-like (LOC105667386) | −2.63 | 4.83E−12 | 4.51E−09 |
| LOC105675433 | Dynein beta chain, ciliary (LOC105675433) | −2.97 | 1.07E−07 | 3.08E−05 |
| LOC105674885 | Purine nucleoside phosphorylase-like (LOC105674885) | −3.03 | 5.32E−23 | 1.19E−19 |

(Continues)
| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|------------|-------|-------------|-----|---------|------------------|
| T8         | LOC105670021 | Chymotrypsin-2-like (LOC105670021) | 1.78 | 8.18E-05 | 2.33E-02 |
| T8         | LOC105667585 | Laccase-1-like (LOC105667585) | 1.66 | 2.68E-12 | 5.00E-09 |
| T8         | LOC105671850 | Protein-S-isoprenylcysteine O-methyltransferase (LOC105671850) | 1.56 | 2.02E-05 | 7.47E-03 |
| LOC105669469 | Cytochrome b5-like (LOC105669469) | | 1.44 | 5.07E-06 | 2.33E-03 |
| LOC105671337 | Putative fatty acyl-CoA reductase CG5065 (LOC105671337) | | 1.39 | 2.23E-06 | 1.21E-03 |
| LOC105672439 | Alpha-sarcoglycan (LOC105672439) | | 1.37 | 3.77E-05 | 1.29E-02 |
| LOC105671866 | Serine protease gd-like (LOC105671866) | | 1.29 | 2.23E-07 | 1.48E-04 |
| LOC105673874 | 2-oxoisovalerate dehydrogenase subunit beta | | 1.26 | 3.26E-04 | 6.90E-02 |
| LOC105673821 | Vascular endothelial growth factor A-like (LOC105673821) | | 1.26 | 4.54E-05 | 1.43E-02 |
| LOC105669027 | Putative nuclease HARBI1 (pseudo) | | 1.19 | 3.03E-04 | 6.71E-02 |
| LOC105673881 | Beta-1,3-glucan-binding protein-like (LOC105673881) | | 1.18 | 1.43E-05 | 5.70E-03 |
| LOC105674055 | Uncharacterized protein DDB_ G0290685-like (LOC105674055) | | 1.10 | 2.83E-10 | 3.76E-07 |
| LOC105677650 | Speckle targeted PIP5K1A-regulated poly(A) polymerase-like (LOC105677650) | | 1.10 | 2.68E-04 | 6.04E-02 |
| LOC105675725 | Serine protease snake-like (LOC105675725) | | 1.04 | 1.68E-04 | 4.19E-02 |
| LOC105667656 | Uncharacterized LOC105667656 (LOC105667656) | | 1.01 | 2.12E-05 | 7.47E-03 |
| LOC105676251 | Uncharacterized LOC105676251 (LOC105676251) | | 0.99 | 2.00E-04 | 4.89E-02 |
| LOC105680007 | Protein toll (LOC105680007) | | 0.98 | 3.45E-04 | 7.12E-02 |
| LOC105672359 | Thiamine transporter 2-like (LOC105672359) | | 0.97 | 3.29E-04 | 6.90E-02 |
| LOC105673986 | G-protein coupled receptor moody (LOC105673986) | | 0.96 | 5.07E-04 | 9.06E-02 |
| LOC105669664 | Uncharacterized LOC105669664 (LOC105669664) | | 0.92 | 3.78E-04 | 7.67E-02 |

(Continues)
| Time point | Locus                          | Description                                                                 | LFC  | p-Value       | Adjusted p-value |
|------------|-------------------------------|-----------------------------------------------------------------------------|------|---------------|------------------|
|            | LOC105674673                  | Putative histone-lysine N-methyltransferase 1 (LOC105674673)                | 0.89 | 5.02E−04      | 9.06E−02         |
|            | LOC105676303                  | N-(5-amino-5-carboxypentanoyl)-l-cysteinyl-d-valine synthase (LOC105676303) | 0.86 | 2.17E−04      | 5.18E−02         |
|            | LOC105673707                  | Kinesin-like protein unc-104 (LOC105673707)                                 | 0.84 | 1.10E−04      | 2.99E−02         |
|            | LOC105669176                  | Organic cation transporter protein-like (LOC105669176)                       | 0.84 | 5.60E−06      | 2.48E−03         |
|            | LOC105672117                  | ATP-binding cassette sub-family G member 4 (LOC105672117)                    | 0.83 | 1.35E−05      | 5.56E−03         |
|            | LOC105678395                  | Uncharacterized LOC105678395 (LOC105678395)                                | 0.82 | 4.07E−04      | 8.09E−02         |
|            | LOC105671203                  | Uncharacterized LOC105671203 (LOC105671203)                                | 0.76 | 1.31E−04      | 3.40E−02         |
|            | LOC105671406                  | Putative phosphatidate phosphatase (LOC105671406)                           | 0.72 | 4.17E−04      | 8.09E−02         |
|            | LOC105676136                  | Guanine nucleotide-binding protein G(i) subunit alpha (LOC105676136)        | 0.69 | 1.99E−05      | 7.47E−03         |
|            | LOC105671687                  | Plastin-2 (LOC105671687)                                                    | 0.66 | 5.64E−04      | 9.93E−02         |
|            | LOC105673509                  | Ankyrin repeat and BTB/POZ domain-containing protein BTBD11 (LOC105673509)  | 0.58 | 2.40E−04      | 5.63E−02         |
|            | LOC105679739                  | Uncharacterized LOC105679739 (LOC105679739)                                | −0.59| 2.11E−05      | 7.47E−03         |
|            | LOC105678332                  | Pancreatic triacylglycerol lipase-like (LOC105678332)                       | −0.63| 7.11E−05      | 2.16E−02         |
|            | LOC105677611                  | Uncharacterized LOC105677611 (LOC105677611)                                | −0.68| 4.30E−05      | 1.39E−02         |
|            | LOC105671942                  | Hexokinase-2-like (LOC105671942)                                            | −0.71| 4.58E−04      | 8.56E−02         |
|            | LOC105669509                  | Arylsulfatase B-like (LOC105669509)                                         | −0.74| 2.60E−04      | 5.98E−02         |
|            | LOC105667685                  | Solute carrier organic anion transporter family member 2A1 (LOC105667685)   | −0.74| 3.18E−04      | 6.90E−02         |
|            | LOC105678768                  | Lipoyltransferase 1                                                          | −0.81| 1.31E−04      | 3.40E−02         |
|            | LOC105674040                  | Apolipoporphins (LOC105674040)                                              | −0.85| 7.22E−05      | 2.16E−02         |
|            | LOC105676627                  | Uncharacterized LOC105676627 (LOC105676627)                                | −0.97| 4.82E−04      | 8.87E−02         |
|            | LOC105667549                  | Vascular endothelial growth factor receptor 1-like (LOC105667549)            | −1.00| 4.19E−04      | 8.09E−02         |
|            | LOC105674437                  | Aquaporin-like (LOC105674437)                                               | −1.01| 8.98E−07      | 5.37E−04         |
|            | LOC105672704                  | Receptor-type tyrosine-protein phosphatase epsilon-like (LOC105672704)      | −1.03| 9.68E−05      | 2.69E−02         |
|            | LOC105675201                  | Glycine N-methyltransferase (LOC105675201)                                  | −1.03| 2.68E−08      | 2.29E−05         |
|            | LOC105676399                  | Netrin receptor UNC5C-like (LOC105676399)                                   | −1.04| 2.23E−07      | 1.48E−04         |
|            | LOC105675916                  | Major royal jelly protein 5-like (LOC105675916)                             | −1.08| 8.13E−05      | 2.33E−02         |
|            | LOC105672628                  | Esterase FE4-like (LOC105672628)                                            | −1.13| 4.28E−08      | 3.42E−05         |

(Continues)
| Time point | Locus                      | Description                                      | LFC     | p-Value       | Adjusted p-value |
|------------|----------------------------|--------------------------------------------------|---------|---------------|------------------|
| LOC105669186 | Uncharacterized LOC105669186 (LOC105669186) | -1.14                                           | 4.00E−05 | 1.33E−02     |
| LOC105676405 | Fatty acid synthase (LOC105676405) | -1.18                                           | 2.45E−09 | 2.67E−06     |
| LOC105678833 | Acyl-CoA Delta(11) desaturase-like (LOC105678833) | -1.19                                           | 2.75E−12 | 5.00E−09     |
| LOC105678901 | High affinity nerve growth factor receptor-like (LOC105678901) | -1.25                                           | 3.89E−06 | 1.94E−03     |
| LOC105675090 | Uncharacterized LOC105675090 (LOC105675090) | -1.27                                           | 4.99E−06 | 2.33E−03     |
| LOC105677615 | Alpha-tocopherol transfer protein-like (LOC105677615) | -1.42                                           | 2.93E−12 | 5.00E−09     |
| LOC105671862 | Uncharacterized LOC105671862 (LOC105671862) | -1.53                                           | 1.52E−04 | 3.88E−02     |
| LOC105679194 | Muscle segmentation homeobox-like (LOC105679194) | -1.54                                           | 2.47E−06 | 1.29E−03     |
| LOC105680137 | Tryptophan 2,3-dioxygenase-like (LOC105680137) | -1.67                                           | 8.66E−13 | 2.59E−09     |
| LOC105676142 | Vascular endothelial growth factor receptor kdr-like (LOC105676142) | -1.69                                           | 2.22E−07 | 1.48E−04     |
| LOC105680118 | Cysteine proteinase 1-like (LOC105680118) | -2.03                                           | 7.00E−06 | 2.99E−03     |
| LOC105677462 | Peroxisomal hydratase-dehydrogenase-epimerase-like (LOC105677462) | -2.07                                           | 1.32E−40 | 1.58E−36     |
| LOC105680018 | Uncharacterized LOC105680018 (LOC105680018) | -2.09                                           | 1.08E−09 | 1.29E−06     |
| LOC105675840 | Uncharacterized protein K02A2.6-like (LOC105675840) | -3.23                                           | 1.08E−13 | 4.29E−10     |
| LOC105678364 | Uncharacterized LOC105678364 (LOC105678364) | -3.29                                           | 1.39E−06 | 7.92E−04     |
| LOC105669037 | Uncharacterized LOC105669037 (LOC105669037) | -4.30                                           | 6.30E−14 | 3.77E−10     |
| LOC105670525 | Chondroadherin-like (LOC105670525) | -5.15                                           | 1.14E−08 | 1.05E−05     |
| T12        | Uncharacterized LOC105669627 (LOC105669627) | 10.40                                           | 9.22E−06 | 1.27E−02     |
| LOC105667928 | Uncharacterized LOC105667928 (pseudo) | 5.40                                            | 3.43E−10 | 2.13E−06     |
| LOC105669318 | Kynurenine/alpha-aminoadipate aminotransferase | 2.96                                            | 1.84E−06 | 2.86E−03     |
| LOC105673362 | Limulus clotting factor C-like (LOC105673362) | 2.74                                            | 1.11E−07 | 3.45E−04     |
| LOC105676242 | Uncharacterized LOC105676242 (LOC105676242) | 2.37                                            | 1.68E−07 | 4.16E−04     |
| LOC105670707 | Uncharacterized transmembrane protein DDB_G0289901-like (LOC105670707) | 2.29                                            | 2.50E−07 | 5.16E−04     |
| LOC105676540 | Uncharacterized LOC105676540 (LOC105676540) | 2.16                                            | 8.06E−07 | 1.43E−03     |
| LOC105670591 | Uncharacterized LOC105670591 (LOC105670591) | 2.09                                            | 7.28E−05 | 6.94E−02     |

(Continues)
**TABLE A3** (Continued)

| Time point | Locus              | Description                                          | LFC  | p-Value     | Adjusted p-value |
|------------|--------------------|------------------------------------------------------|------|-------------|------------------|
|            | LOC105677207       | Gamma-glutamyl transpeptidase 1-like (LOC105677207)  | 2.04 | 5.11E-05    | 5.28E-02         |
|            | LOC105677194       | Glucose dehydrogenase                                | 1.82 | 2.74E-05    | 3.09E-02         |
|            | LOC105674352       | Aromatic-L-amino acid decarboxylase (LOC105674352)   | 1.78 | 1.43E-05    | 1.77E-02         |
|            | LOC105677462       | Peroxisomal hydratase-dehydrogenase-epimerase-like (LOC105677462) | -3.04 | 2.10E-14    | 2.61E-10         |
|            | LOC105671854       | Uncharacterized LOC105671854 (LOC105671854)         | -3.30 | 5.52E-08  | 2.28E-04         |

LFC: log₂ fold change.

**TABLE A4** Differentially expressed genes in bacteria-injected isolated queens with false discovery rate <10%. T2, T4, T8, and T12 indicate the post-injection time points

| Time point | Locus              | Description                                          | LFC  | p-Value     | Adjusted p-value |
|------------|--------------------|------------------------------------------------------|------|-------------|------------------|
|            | LOC105672684       | Protein hairy (LOC105672684)                         | 1.75 | 3.31E-06    | 1.93E-03         |
|            | LOC105672434       | Uncharacterized LOC105672434 (LOC105672434)         | 1.53 | 2.39E-11    | 5.13E-08         |
|            | LOC105670707       | Uncharacterized transmembrane protein DDB_G0289901-like (LOC105670707) | 1.48 | 2.33E-07    | 2.27E-04         |
|            | LOC105675132       | Uncharacterized LOC105675132 (LOC105675132)         | 1.45 | 3.35E-04    | 4.66E-02         |
|            | LOC105673362       | Limulus clotting factor C-like (LOC105673362)        | 1.45 | 4.68E-07    | 4.18E-04         |
|            | LOC105669805       | Facilitated trehalose transporter Tret1-2 homolog (LOC105669805) | 1.20 | 1.75E-05    | 6.19E-03         |
|            | LOC105679763       | Uncharacterized oxidoreductase C26H5.09c-like (LOC105679763) | 1.18 | 9.17E-04    | 8.86E-02         |
|            | LOC105676470       | MAP/microtubule affinity-regulating kinase 3-like (LOC105676470) | 1.10 | 9.12E-10    | 1.40E-06         |
|            | LOC105676540       | Uncharacterized LOC105676540 (LOC105676540)         | 1.06 | 1.79E-05    | 6.19E-03         |
|            | LOC105676242       | Uncharacterized LOC105676242 (LOC105676242)         | 0.96 | 9.84E-08    | 1.17E-04         |
|            | LOC105668293       | Chymotrypsin-1-like (LOC105668293)                   | 0.94 | 7.93E-06    | 3.81E-03         |
|            | LOC105672325       | Calcium-binding mitochondrial carrier protein ScaMC-2-like (LOC105672325) | 0.90 | 6.63E-04    | 7.04E-02         |
|            | LOC105671678       | Collagen alpha-2(I) chain-like (LOC105671678)       | 0.89 | 5.94E-05    | 1.35E-02         |
|            | LOC105679973       | Fatty acid binding protein 1-B.1-like (LOC105679973) | 0.82 | 1.46E-04    | 2.56E-02         |
|            | LOC105677611       | Uncharacterized LOC105677611 (LOC105677611)         | 0.76 | 1.73E-04    | 2.86E-02         |
|            | LOC105672337       | Protein fem-1 homolog B (LOC105672337)               | 0.67 | 4.27E-04    | 5.51E-02         |
|            | LOC105674292       | Nose resistant to fluoxetine protein 6-like (LOC105674292) | 0.58 | 5.93E-04    | 6.77E-02         |
|            | LOC105668729       | Nuclear factor NF-kappa-B p100 subunit (LOC105668729) (Relish) | 0.54 | 1.01E-03    | 9.71E-02         |
|            | LOC105677684       | E3 ubiquitin-protein ligase TRIM71 (LOC105677684)    | -0.72 | 1.01E-04    | 2.03E-02         |
|            | LOC105678648       | Protein toll-like (LOC105678648)                     | -0.72 | 3.01E-04    | 4.30E-02         |
|            | LOC10566719        | Uncharacterized protein PFB0145c-like (LOC10566719)   | -0.81 | 2.73E-04    | 4.01E-02         |
|            | LOC105671848       | Putative exonuclease GOR (LOC105671848)              | -0.82 | 1.11E-04    | 2.12E-02         |

(Continues)
| Time point | Locus               | Description                                             | LFC  | p-Value     | Adjusted p-value |
|------------|---------------------|---------------------------------------------------------|------|-------------|------------------|
| LOC105674378 | Uncharacterized LOC105674378 (LOC105674378) | -0.82 | 6.36E-04 | 6.89E-02 |
| LOC105676004 | Uncharacterized LOC105676004 (LOC105676004) | -0.82 | 6.60E-04 | 7.04E-02 |
| LOC105671329 | Insulin-degrading enzyme-like (LOC105671329) | -0.83 | 7.00E-04 | 7.13E-02 |
| LOC105672982 | Chromosome-associated kinesin KIF4 (LOC105672982) | -0.84 | 3.02E-05 | 8.75E-03 |
| LOC105670214 | Tissue factor pathway inhibitor-like (LOC105670214) | -0.87 | 9.09E-04 | 8.86E-02 |
| LOC105673464 | Venom carboxylesterase-6-like (pseudo) | -0.88 | 6.57E-05 | 1.47E-02 |
| LOC105676004 | Chromosome-associated kinesin KIF4 (LOC105676004) | -0.82 | 6.60E-04 | 7.04E-02 |
| LOC105671329 | Insulin-degrading enzyme-like (LOC105671329) | -0.83 | 7.00E-04 | 7.13E-02 |
| LOC105672982 | Chromosome-associated kinesin KIF4 (LOC105672982) | -0.84 | 3.02E-05 | 8.75E-03 |
| LOC105670214 | Tissue factor pathway inhibitor-like (LOC105670214) | -0.87 | 9.09E-04 | 8.86E-02 |
| LOC105673464 | Venom carboxylesterase-6-like (pseudo) | -0.88 | 6.57E-05 | 1.47E-02 |
| LOC105670430 | Aminopeptidase N-like (LOC105670430) | -0.91 | 5.43E-05 | 1.27E-02 |
| LOC105664842 | Espin (LOC105668482) | -0.92 | 4.39E-04 | 5.06E-02 |
| LOC105670871 | Bipolar kinesin KRP-130-like (LOC105677706) | -0.92 | 4.01E-04 | 5.24E-02 |
| LOC105678817 | Protein toll-like (LOC105678817) | -0.94 | 4.06E-05 | 1.04E-02 |
| LOC105674728 | Muscle M-line assembly protein unc-89-like (LOC105674728) | -0.94 | 1.14E-06 | 8.71E-04 |
| LOC105678912 | Protein toll-like (LOC105678912) | -0.94 | 8.07E-04 | 8.09E-02 |
| LOC105670239 | Uncharacterized LOC105670239 (LOC105670239) | -0.98 | 4.27E-05 | 1.05E-02 |
| LOC105678342 | Gephyrin-like (LOC105678342) | -0.99 | 3.59E-04 | 4.87E-02 |
| LOC105678985 | Uncharacterized LOC105678985 (LOC105678985) | -1.00 | 1.95E-05 | 6.35E-03 |
| LOC105670219 | Uncharacterized LOC105670219 (LOC105670219) | -1.00 | 7.05E-04 | 7.13E-02 |
| LOC105674716 | Zinc carboxypeptidase A 1-like (LOC105674716) | -1.01 | 9.03E-05 | 1.90E-02 |
| LOC105670899 | Uncharacterized LOC105670899 (LOC105670899) | -1.01 | 3.63E-05 | 9.80E-03 |
| LOC105678986 | Uncharacterized LOC105678986 (LOC105678986) | -1.01 | 3.51E-04 | 4.83E-02 |
| LOC105678984 | Uncharacterized LOC105678984 (LOC105678984) | -1.02 | 6.48E-06 | 3.31E-03 |
| LOC105667907 | Thyroid receptor-interacting protein 11-like (LOC105667907) | -1.02 | 1.05E-03 | 9.99E-02 |
| LOC105669733 | Venom carboxylesterase-6-like (LOC105669733) | -1.02 | 1.16E-04 | 2.14E-02 |
| LOC105673217 | Uncharacterized LOC105673217 (LOC105673217) | -1.02 | 5.44E-05 | 1.27E-02 |
| LOC105671625 | Uncharacterized LOC105671625 (LOC105671625) | -1.06 | 3.10E-05 | 8.75E-03 |
| LOC105665758 | Thyrotropin-releasing hormone-degrading ectoenzyme-like (LOC105665758) | -1.06 | 5.30E-04 | 6.25E-02 |
| LOC105678934 | Uncharacterized LOC105678934 (LOC105678934) | -1.06 | 5.13E-04 | 6.19E-02 |
| LOC105678987 | Uncharacterized LOC105678987 (LOC105678987) | -1.07 | 2.68E-04 | 3.99E-02 |
| LOC105675620 | Aminopeptidase N-like (LOC105675620) | -1.07 | 2.58E-04 | 3.96E-02 |
| LOC105674397 | Uncharacterized LOC105674397 (LOC105674397) | -1.08 | 2.38E-05 | 7.32E-03 |
| LOC105670244 | Uncharacterized LOC105670244 (LOC105670244) | -1.10 | 2.22E-04 | 3.56E-02 |
| LOC105670868 | Uncharacterized LOC105670868 (LOC105670868) | -1.12 | 3.42E-06 | 1.93E-03 |
| LOC105674484 | RCC1 and BTB domain-containing protein 1-like (LOC105674484) | -1.14 | 2.31E-04 | 3.59E-02 |
| LOC105676597 | Uncharacterized LOC105676597 (LOC105676597) | -1.15 | 1.00E-04 | 2.03E-02 |

(Continues)
| Time point | Locus | Description | LFC  | p-Value  | Adjusted p-value |
|------------|-------|-------------|------|----------|-----------------|
| LOC105674665 | T-box transcription factor TBX20-like (LOC105674665) | -1.17 | 6.35E-04 | 6.89E-02 |
| LOC105674406 | Uncharacterized LOC105674406 (LOC105674406) | -1.17 | 2.39E-05 | 7.32E-03 |
| LOC105679050 | Transcription termination factor 2-like (LOC105679050) | -1.19 | 2.05E-07 | 2.20E-04 |
| LOC105674677 | Uncharacterized LOC105674677 (LOC105674677) | -1.20 | 1.10E-06 | 8.71E-04 |
| LOC105679162 | Uncharacterized LOC105679162 (LOC105679162) | -1.21 | 1.39E-04 | 2.48E-02 |
| LOC105672421 | Uncharacterized LOC105672421 (LOC105672421) | -1.22 | 1.02E-04 | 2.03E-02 |
| LOC105679916 | Kinesin-like protein KIF12 (LOC105679916) | -1.24 | 8.97E-06 | 4.01E-03 |
| LOC105657300 | Aminopeptidase N-like (LOC105675300) | -1.25 | 5.16E-04 | 6.19E-02 |
| LOC105672131 | Uncharacterized LOC105672131 (LOC105672131) | -1.26 | 8.11E-05 | 1.74E-02 |
| LOC105678936 | Uncharacterized LOC105678936 (LOC105678936) | -1.26 | 2.99E-04 | 4.30E-02 |
| LOC105670915 | Probable salivary secreted peptide (LOC105670915) | -1.29 | 7.81E-11 | 1.40E-07 |
| LOC105676006 | Uncharacterized LOC105676006 (LOC105676006) | -1.30 | 1.23E-05 | 5.08E-03 |
| LOC105668490 | Uncharacterized LOC105668490 (LOC105668490) | -1.33 | 6.14E-04 | 6.82E-02 |
| LOC105678344 | Gephyrin-like (LOC105678344) | -1.33 | 1.92E-04 | 3.11E-02 |
| LOC105671608 | Uncharacterized LOC105671608 (LOC105671608) | -1.35 | 2.62E-04 | 3.96E-02 |
| LOC105678353 | Aminopeptidase N-like (LOC105678353) | -1.43 | 2.18E-06 | 1.56E-03 |
| LOC105677450 | Phosphotriesterase-related protein-like (LOC105677450) | -1.44 | 1.15E-04 | 2.14E-02 |
| LOC105670356 | Sodium-dependent nutrient amino acid transporter 1-like (LOC105670356) | -1.45 | 1.64E-04 | 2.79E-02 |
| LOC105679178 | Uncharacterized LOC105679178 (LOC105679178) | -1.45 | 5.80E-04 | 6.69E-02 |
| LOC105679873 | Uncharacterized LOC105679873 (LOC105679873) | -1.45 | 4.31E-05 | 1.05E-02 |
| LOC105668011 | Uncharacterized LOC105668011 (LOC105668011) | -1.51 | 2.29E-04 | 3.59E-02 |
| LOC105670875 | Uncharacterized LOC105670875 (LOC105670875) | -1.53 | 6.10E-04 | 6.82E-02 |
| LOC105676970 | A disintegrin and metalloproteinase with thrombospondin motifs 7-like (LOC105676970) | -1.53 | 8.34E-04 | 8.28E-02 |
| LOC105679161 | A disintegrin and metalloproteinase with thrombospondin motifs 17-like (LOC105679161) | -1.54 | 3.75E-05 | 9.80E-03 |
| LOC105674012 | Phosphotriesterase-related protein-like (LOC105674012) | -1.55 | 1.04E-04 | 2.03E-02 |
| LOC105676008 | Uncharacterized LOC105676008 (LOC105676008) | -1.55 | 1.57E-05 | 6.03E-03 |
| LOC105679027 | Uncharacterized LOC105679027 (LOC105679027) | -1.58 | 4.64E-04 | 5.85E-02 |
| LOC105674893 | Uncharacterized LOC105674893 (LOC105674893) | -1.59 | 3.67E-04 | 4.89E-02 |
| LOC105673254 | Thyrotropin-releasing hormone-degrading ectoenzyme-like (LOC105673254) | -1.60 | 3.12E-04 | 4.40E-02 |
| LOC105674631 | Uncharacterized LOC105674631 (LOC105674631) | -1.61 | 5.14E-04 | 6.19E-02 |
| LOC105671207 | A disintegrin and metalloproteinase with thrombospondin motifs 7-like (LOC105671207) | -1.63 | 2.47E-05 | 7.36E-03 |
| LOC105677676 | Aminopeptidase N-like (LOC105677676) | -1.67 | 3.69E-04 | 4.89E-02 |
| LOC105674526 | Uncharacterized LOC105674526 (LOC105674526) | -1.71 | 8.17E-06 | 3.81E-03 |
| LOC105677348 | Uncharacterized LOC105677348 (LOC105677348) | -1.73 | 2.80E-15 | 1.00E-11 |
| LOC105674527 | Uncharacterized LOC105674527 (LOC105674527) | -1.74 | 2.66E-06 | 1.78E-03 |
| LOC105676143 | A disintegrin and metalloproteinase with thrombospondin motifs 8-like (LOC105676143) | -1.78 | 8.79E-04 | 8.65E-02 |

(Continues)
| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|------------|-------|-------------|-----|---------|------------------|
| LOC105677480 | Uncharacterized LOC105677480 (LOC105677480) | -1.80 | 6.99E-04 | 7.13E-02 |
| LOC105678343 | Gephyrin-like (LOC105678343) | -1.86 | 5.09E-06 | 2.73E-03 |
| LOC105668553 | A disintegrin and metalloproteinase with thrombospondin motifs 2-like (LOC105668553) | -1.93 | 1.66E-04 | 2.79E-02 |
| LOC105673493 | Alpha-tocopherol transfer protein-like (LOC105673493) | -1.94 | 6.81E-04 | 7.08E-02 |
| LOC105679148 | Uncharacterized LOC105679148 (LOC105679148) | -1.99 | 1.59E-08 | 2.13E-05 |
| LOC105676228 | Glutamyl aminopeptidase-like (LOC105676228) | -2.02 | 3.17E-06 | 1.93E-03 |
| LOC105677332 | A disintegrin and metolloproteinase with thrombospondin motifs 2-like (LOC105677332) | -2.05 | 2.87E-17 | 1.54E-13 |
| LOC105675423 | Uncharacterized LOC105675423 (LOC105675423) | -2.08 | 1.51E-05 | 5.98E-03 |
| LOC105674732 | Zinc carboxypeptidase-like (LOC105674732) | -2.10 | 1.60E-04 | 2.76E-02 |
| LOC105674384 | Uncharacterized LOC105674384 (LOC105674384) | -2.11 | 1.91E-05 | 6.35E-03 |
| LOC105675063 | Alkaline phosphatase 4-like (LOC105675063) | -2.13 | 1.79E-05 | 6.19E-03 |
| LOC105679017 | Uncharacterized LOC105679017 (LOC105679017) | -2.21 | 9.59E-06 | 4.11E-03 |
| LOC105673333 | Uncharacterized LOC105673333 (LOC105673333) | -2.25 | 5.21E-14 | 1.40E-10 |
| LOC105668554 | A disintegrin and metalloproteinase with thrombospondin motifs 2-like (LOC105668554) | -2.45 | 5.20E-04 | 6.19E-02 |
| LOC105675162 | Uncharacterized LOC105675162 (LOC105675162) | -2.77 | 6.92E-05 | 1.51E-02 |
| LOC105679873 | Sequestosome-1 (LOC105679873) | 3.08 | 3.32E-14 | 4.73E-11 |
| LOC105674352 | Aromatic-L-amino acid decarboxylase (LOC105674352) | 3.03 | 3.71E-13 | 4.39E-10 |
| LOC105675162 | Uncharacterized LOC105675162 (LOC105675162) | 3.02 | 1.13E-07 | 5.03E-05 |
| LOC105667758 | Ninjurin-1 (LOC105667758) | 2.82 | 2.79E-07 | 1.10E-04 |
| LOC105678482 | NF-kappa-B inhibitor cactus-like (LOC105678482) | 2.78 | 3.58E-09 | 2.12E-06 |
| LOC105678050 | Uncharacterized LOC105678050 (LOC105678050) | 2.64 | 8.90E-06 | 2.48E-03 |
| LOC105675162 | Uncharacterized LOC105675162 (LOC105675162) | 2.33 | 4.34E-06 | 1.40E-03 |
| LOC105679973 | Fatty acid binding protein 1-B.1-like (LOC105679973) | 1.97 | 1.70E-05 | 4.33E-03 |
| LOC105674105 | Beta-hexosaminidase subunit beta-like (LOC105674105) | 1.92 | 6.17E-05 | 1.19E-02 |
| LOC105678395 | Uncharacterized LOC105678395 (LOC105678395) | 1.89 | 6.75E-06 | 2.09E-03 |
| LOC105677178 | Alpha-2-macroglobulin-like protein 1 (LOC105677178) | 1.89 | 2.59E-06 | 9.12E-04 |
| LOC105671337 | Putative fatty acyl-CoA reductase CG5065 (LOC105671337) | 1.86 | 2.04E-04 | 3.46E-02 |
| LOC105679392 | Vanin-like protein 1 (LOC105679392) | 1.83 | 8.62E-06 | 2.48E-03 |
| LOC105677051 | Protein Malvolio (LOC105677051) | 1.80 | 2.15E-04 | 3.56E-02 |
| LOC10566443 | Uncharacterized LOC10566443 (LOC10566443) | 1.79 | 3.88E-05 | 8.35E-03 |
| LOC105679998 | Heat shock 70 kDa protein cognate 4 (LOC105679998) | 1.42 | 3.74E-04 | 5.42E-02 |
| LOC105669805 | Facilitated trehalose transporter Tret1-2 homolog (LOC105669805) | -1.56 | 7.81E-04 | 9.86E-02 |

(Continues)
| Time point | Locus                  | Description                                      | LFC   | p-Value     | Adjusted p-value |
|------------|------------------------|--------------------------------------------------|-------|-------------|------------------|
| LOC105675281 | Fibrillin-1 (LOC105675281) | -1.67                                           | 4.31E-04 | 6.13E-02    |
| LOC105670714 | Alpha-amylase A (LOC105670714) | -1.78                                           | 4.73E-05 | 9.49E-03    |
| LOC105675053 | Cytochrome P450 6a2-like (LOC105675053) | -1.79                                           | 4.80E-04 | 6.57E-02    |
| LOC105667318 | Dentin sialophosphoprotein-like (LOC105667318) | -1.87                                           | 2.69E-05 | 6.21E-03    |
| LOC105676465 | Facilitated trehalose transporter Tret1 (LOC105676465) | -1.87                                           | 3.55E-04 | 5.26E-02    |
| LOC105676200 | Short-chain dehydrogenase/reductase family 16C member 6-like (LOC105676200) | -1.88                                           | 2.75E-04 | 4.45E-02    |
| LOC105676829 | Stearoyl-CoA desaturase 5-like (LOC105676829) | -1.90                                           | 6.18E-04 | 8.14E-02    |
| LOC105669660 | Nose resistant to fluoxetine protein 6-like (LOC105669660) | -1.97                                           | 7.40E-04 | 9.57E-02    |
| LOC105673315 | Pheromone-binding protein Gp-9-like (LOC105673315) | -2.04                                           | 2.93E-04 | 4.64E-02    |
| LOC105677462 | Peroxisomal hydratase-dehydrogenase-epimerase-like (LOC105677462) | -2.13                                           | 3.14E-04 | 4.85E-02    |
| LOC105679763 | Uncharacterized oxidoreductase C26H5.09c-like (LOC105679763) | -2.15                                           | 9.02E-05 | 1.67E-02    |
| LOC105672608 | Facilitated trehalose transporter Tret1-like (LOC105672608) | -2.26                                           | 1.92E-04 | 3.32E-02    |
| LOC105671746 | Probable cytochrome P450 304a1 (LOC105671746) | -2.31                                           | 6.96E-07 | 2.61E-04    |
| LOC105677950 | Elongation of very long chain fatty acids protein 4-like (LOC105677950) | -2.51                                           | 4.67E-04 | 6.52E-02    |
| LOC105670857 | Leucine-rich repeat-containing protein egg-6 (LOC105670857) | -2.53                                           | 1.57E-05 | 4.14E-03    |
| LOC105671912 | Vitellogenin-1-like (LOC105671912) | -2.73                                           | 5.67E-04 | 7.62E-02    |
| LOC105678691 | Scavenger receptor class B member 1-like (LOC105678691) | -2.75                                           | 5.51E-09 | 3.02E-06    |
| LOC105676041 | Uncharacterized LOC105676041 (LOC105676041) | -2.80                                           | 4.87E-11 | 3.85E-08    |
| LOC105674885 | Purine nucleoside phosphorylase-like (LOC105674885) | -2.83                                           | 9.06E-06 | 2.48E-03    |
| LOC105674435 | Serine protease inhibitor dipetalogastin-like (LOC105674435) | -2.84                                           | 2.69E-06 | 9.12E-04    |
| LOC105677195 | Glucose dehydrogenase [FAD | -2.85                                           | 2.71E-05 | 6.21E-03    |
| LOC105669773 | Regucalcin-like (LOC105669773) | -2.85                                           | 1.81E-08 | 8.60E-06    |
| LOC105668188 | Uncharacterized LOC105668188 (LOC105668188) | -2.86                                           | 1.41E-08 | 7.16E-06    |
| LOC105674040 | Apolipoporins (LOC105674040) | -2.88                                           | 1.39E-07 | 5.80E-05    |
| LOC105679486 | Acyl-CoA Delta(11) desaturase-like (LOC105679486) | -2.92                                           | 7.97E-04 | 9.86E-02    |
| LOC105669905 | Uncharacterized LOC105669905 (LOC105669905) | -2.95                                           | 9.17E-05 | 1.67E-02    |
| LOC105675057 | Uncharacterized LOC105675057 (LOC105675057) | -2.95                                           | 3.30E-04 | 4.99E-02    |
| LOC105672219 | Leucine-rich repeat-containing protein 4-like (LOC105672219) | -2.98                                           | 2.37E-05 | 5.82E-03    |
| LOC105670441 | Uncharacterized LOC105670441 (LOC105670441) | -3.07                                           | 1.75E-04 | 3.11E-02    |
| LOC105672550 | Uncharacterized LOC105672550 (LOC105672550) | -3.13                                           | 4.27E-12 | 4.34E-09    |
| LOC105670856 | Negative regulator of reactive oxygen species-like (LOC105670856) | -3.21                                           | 2.74E-09 | 1.77E-06    |
| LOC105675773 | Peptidoglycan-recognition protein SC2-like (LOC105675773) | -3.24                                           | 3.51E-15 | 6.23E-12    |

(Continues)
| Time point | Locus | Description | LFC  | p-Value | Adjusted p-value |
|------------|-------|-------------|------|---------|------------------|
| LOC105678491 | Uncharacterized LOC105678491 (LOC105678491) | -3.67 | 9.17E-12 | 8.16E-09 |
| LOC105671873 | Uncharacterized LOC105671873 (LOC105671873) | -3.69 | 1.17E-20 | 2.78E-17 |
| LOC105677373 | Probable WRKY transcription factor protein 1 (LOC105677373) | -4.04 | 2.11E-09 | 1.50E-06 |
| LOC105667550 | Uncharacterized LOC105667550 (LOC105667550) | -4.95 | 4.11E-05 | 8.60E-03 |
| LOC105669522 | Glucose dehydrogenase [FAD] | -5.08 | 2.89E-05 | 6.42E-03 |
| LOC105671872 | Vitellogenin-2-like (LOC105671872) | -5.08 | 2.66E-36 | 1.89E-24 |
| LOC105668189 | Uncharacterized LOC105668189 (LOC105668189) | -11.64 | 4.80E-05 | 9.49E-03 |
| LOC105669806 | Cytochrome P450 307a1-like (LOC105669806) | 1.36 | 1.15E-07 | 4.64E-04 |
| LOC105668184 | Uncharacterized LOC105668184 (LOC105668184) | 8.58 | 1.64E-08 | 8.01E-06 |
| LOC105668572 | Uncharacterized LOC105668572 (LOC105668572) | 0.91 | 4.88E-08 | 3.94E-04 |
| LOC105668988 | Uncharacterized LOC105668988 (LOC105668988) | 0.90 | 1.43E-04 | 9.96E-02 |
| LOC105677178 | Alpha-2-macroglobulin-like protein 1 (LOC105677178) (TepII) | 0.73 | 1.63E-04 | 9.96E-02 |
| LOC105674427 | Beta-1,3-glucan-binding protein-like (LOC105674427) | 1.02 | 7.86E-05 | 6.35E-02 |
| LOC105668572 | Uncharacterized LOC105668572 (LOC105668572) | 0.91 | 4.88E-08 | 3.94E-04 |
| LOC105668184 | Uncharacterized LOC105668184 (LOC105668184) | 0.90 | 1.43E-04 | 9.96E-02 |
| LOC105668572 | Uncharacterized LOC105668572 (LOC105668572) | 0.91 | 4.88E-08 | 3.94E-04 |
| LOC105671866 | Serine protease gd-like (LOC105671866) | 1.31 | 5.74E-06 | 9.27E-03 |
| LOC105671866 | Serine protease gd-like (LOC105671866) | 1.31 | 5.74E-06 | 9.27E-03 |
| LOC105672123 | Uncharacterized LOC105672123 (LOC105672123) | 1.63 | 3.02E-05 | 5.73E-03 |
| LOC105674930 | Facilitated trehalose transporter Tret1-like (LOC105674930) | 1.60 | 2.18E-07 | 8.67E-05 |

(Continues)
| Time point | Locus               | Description                                                                 | LFC | p-Value   | Adjusted p-value |
|------------|---------------------|------------------------------------------------------------------------------|-----|-----------|------------------|
| LOC105668988 | Uncharacterized LOC105668988 (LOC105668988) | 0.90                          | 1.43E-04 | 9.96E-02   |
| LOC105671778 | Alpha-2-macroglobulin-like protein 1 (LOC105671778) (TepII) | 0.73                          | 1.63E-04 | 9.96E-02   |
| LOC105675407 | Inhibin beta E chain (LOC105675407) | -0.77                         | 7.26E-05 | 6.35E-02   |
| LOC105677462 | Peroxisomal hydratase-dehydrogenase-epimerase-like (LOC105677462) | -0.89                         | 1.54E-04 | 9.96E-02   |
| LOC105672608 | Facilitated trehalose transporter Tret1-like (LOC105672608) | -0.93                         | 7.57E-06 | 1.02E-02   |
| LOC105676062 | Protein henna (LOC105676062) | -1.30                         | 4.41E-06 | 8.90E-03   |
| LOC105668757 | Uncharacterized LOC105668757 (LOC105668757) | -2.03                         | 4.17E-07 | 1.12E-03   |
| T12         | Uncharacterized LOC105668813 (LOC105668813) | 3.45                          | 6.53E-43 | 3.50E-39   |
| LOC105669245 | Ejaculatory bulb-specific protein 3-like (LOC105669245) | 2.92                          | 5.39E-06 | 1.41E-03   |
| LOC105675404 | Uncharacterized LOC105675404 (LOC105675404) | 2.86                          | 7.75E-29 | 2.08E-25   |
| LOC105676242 | Uncharacterized LOC105676242 (LOC105676242) | 2.63                          | 3.00E-23 | 6.44E-20   |
| LOC105670707 | Uncharacterized transmembrane protein DDB, G0289901-like (LOC105670707) | 2.17                          | 5.77E-45 | 6.19E-41   |
| LOC105679181 | Facilitated trehalose transporter Tret1-like (LOC105679181) | 1.87                          | 1.22E-09 | 6.87E-07   |
| LOC105672089 | Lipid storage droplets surface-binding protein 2-like (LOC105672089) | 1.87                          | 1.94E-09 | 1.04E-06   |
| LOC105671866 | Serine protease gd-like (LOC105671866) | 1.70                          | 4.82E-11 | 3.44E-08   |
| LOC105672123 | Uncharacterized LOC105672123 (LOC105672123) | 1.63                          | 3.02E-05 | 5.73E-03   |
| LOC105674930 | Facilitated trehalose transporter Tret1-like (LOC105674930) | 1.60                          | 2.18E-07 | 8.67E-05   |
| LOC105673362 | Limulus clotting factor C-like (LOC105673362) | 1.55                          | 6.18E-07 | 2.14E-04   |
| LOC105669469 | Cytochrome b5-like (LOC105669469) | 1.46                          | 6.81E-13 | 7.30E-10   |
| LOC105675162 | Uncharacterized LOC105675162 (LOC105675162) | 1.46                          | 1.35E-16 | 2.41E-13   |
| LOC105677051 | Protein Malvolio (LOC105677051) | 1.44                          | 3.83E-12 | 3.42E-09   |
| LOC105676758 | Ninjurin-1 (LOC105676758) | 1.41                          | 1.24E-07 | 5.10E-05   |
| LOC105678482 | NF-kappa-B inhibitor cactus-like (LOC105678482) | 1.40                          | 8.63E-16 | 1.32E-12   |
| LOC105678075 | Tyrosine 3-monooxygenase (LOC105678075) | 1.40                          | 1.51E-13 | 1.80E-10   |
| LOC105673363 | Limulus clotting factor C-like (LOC105673363) | 1.37                          | 3.02E-09 | 1.54E-06   |
| LOC105675429 | Cyclic GMP-AMP synthase (LOC105675429) | 1.29                          | 5.14E-08 | 2.29E-05   |
| LOC105677645 | Cytochrome P450 6A1-like (LOC105677645) | 1.24                          | 9.63E-05 | 1.54E-02   |
| LOC105677207 | Gamma-glutamyl transpeptidase 1-like (LOC105677207) | 1.21                          | 4.40E-12 | 3.63E-09   |
| LOC105679973 | Fatty acid binding protein 1-B.1-like (LOC105679973) | 1.21                          | 3.53E-06 | 1.02E-03   |
| LOC105676478 | Cysteine sulfenic acid decarboxylase (LOC105676478) | 1.19                          | 1.21E-06 | 3.92E-04   |
| LOC105671174 | Lipopolysaccharide-induced tumor necrosis factor-alpha factor homolog (LOC105671174) | 1.15                          | 2.95E-06 | 8.78E-04   |
| LOC105672439 | Alpha-sarcoglycan (LOC105672439) | 1.15                          | 6.89E-08 | 2.95E-05   |

(Continues)
### Table A4 (Continued)

| Time point | Locus | Description | LFC  | p-Value     | Adjusted p-value |
|------------|-------|-------------|------|-------------|------------------|
| LOC105673362 | Limulus clotting factor C-like (LOC105673362) | 1.55 | 6.18E−07 | 2.14E−04 |
| LOC105669469 | Cytochrome b5-like (LOC105669469) | 1.46 | 6.81E−13 | 7.30E−10 |
| LOC105675162 | Uncharacterized LOC105675162 (LOC105675162) | 1.46 | 1.35E−16 | 2.41E−13 |
| LOC105677051 | Protein Malvolio (LOC105677051) | 1.44 | 3.83E−12 | 3.42E−09 |
| LOC105676758 | Ninjurin-1 (LOC105676758) | 1.41 | 1.24E−07 | 5.10E−05 |
| LOC105678482 | NF-kappa-B inhibitor cactus-like (LOC105678482) | 1.40 | 8.63E−16 | 1.32E−12 |
| LOC105678075 | Tyrosine 3-monooxygenase (LOC105678075) | 1.37 | 1.24E−07 | 5.10E−05 |
| LOC105673363 | Limulus clotting factor C-like (LOC105673363) | 1.37 | 3.02E−09 | 1.54E−06 |
| LOC105675429 | Cyclic GMP-AMP synthase (LOC105675429) | 1.29 | 5.14E−08 | 2.29E−05 |
| LOC105677051 | Protein Malvolio (LOC105677051) | 1.44 | 3.83E−12 | 3.42E−09 |
| LOC105676758 | Ninjurin-1 (LOC105676758) | 1.41 | 1.24E−07 | 5.10E−05 |
| LOC105678482 | NF-kappa-B inhibitor cactus-like (LOC105678482) | 1.40 | 8.63E−16 | 1.32E−12 |
| LOC105678075 | Tyrosine 3-monooxygenase (LOC105678075) | 1.37 | 1.24E−07 | 5.10E−05 |
| LOC105673363 | Limulus clotting factor C-like (LOC105673363) | 1.37 | 3.02E−09 | 1.54E−06 |
| LOC105675429 | Cyclic GMP-AMP synthase (LOC105675429) | 1.29 | 5.14E−08 | 2.29E−05 |
| LOC105677051 | Protein Malvolio (LOC105677051) | 1.44 | 3.83E−12 | 3.42E−09 |
| LOC105676758 | Ninjurin-1 (LOC105676758) | 1.41 | 1.24E−07 | 5.10E−05 |
| LOC105678482 | NF-kappa-B inhibitor cactus-like (LOC105678482) | 1.40 | 8.63E−16 | 1.32E−12 |
| LOC105678075 | Tyrosine 3-monooxygenase (LOC105678075) | 1.37 | 1.24E−07 | 5.10E−05 |
| LOC105673363 | Limulus clotting factor C-like (LOC105673363) | 1.37 | 3.02E−09 | 1.54E−06 |
| LOC105675429 | Cyclic GMP-AMP synthase (LOC105675429) | 1.29 | 5.14E−08 | 2.29E−05 |
| LOC105677051 | Protein Malvolio (LOC105677051) | 1.44 | 3.83E−12 | 3.42E−09 |
| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|------------|-------|-------------|-----|---------|-----------------|
| LOC105668941 | Probable citrate synthase 2, mitochondrial (LOC105668941) | 0.86 | 3.49E−05 | 6.23E−03 |
| LOC105669588 | Apoptosis-inducing factor 3 (LOC105669588) | 0.85 | 2.78E−07 | 1.03E−04 |
| LOC105674532 | ETS homologous factor-like (LOC105674532) | 0.84 | 3.01E−04 | 4.05E−02 |
| LOC105678679 | AN1-type zinc finger protein 2A-like (LOC105678679) | 0.84 | 5.66E−04 | 6.38E−02 |
| LOC105677494 | Annexin B9 (LOC105677494) | 0.83 | 7.92E−06 | 1.97E−03 |
| LOC105670266 | Sodium/potassium/calcium exchanger 3-like (LOC105670266) | 0.81 | 9.60E−06 | 2.19E−03 |
| LOC105679962 | Thioredoxin reductase 1, mitochondrial (LOC105679962) | 0.81 | 5.39E−06 | 1.41E−03 |
| LOC105668729 | Nuclear factor NF-kappa-B p100 subunit (LOC105668729) (Relish) | 0.78 | 3.01E−04 | 4.05E−02 |
| LOC105678483 | NF-kappa-B inhibitor cactus-like (LOC105678483) | 0.73 | 4.25E−04 | 5.12E−02 |
| LOC105675225 | Kelch-like ECH-associated protein 1 (LOC105675225) | 0.71 | 1.28E−04 | 1.96E−02 |
| LOC105678890 | Sodium-independent sulfate anion transporter (LOC105678890) | 0.71 | 7.95E−04 | 8.44E−02 |
| LOC105673596 | Putative inorganic phosphate cotransporter (LOC105673596) | 0.62 | 4.03E−04 | 5.03E−02 |
| LOC105675851 | Sphingosine kinase 2 (LOC105675851) | 0.62 | 7.01E−04 | 7.66E−02 |
| LOC105677558 | Kinectin-like (LOC105677558) | 0.61 | 1.64E−04 | 2.40E−02 |
| LOC105668823 | Uncharacterized LOC105668823 (LOC105668823) | 0.61 | 9.88E−04 | 9.77E−02 |
| LOC105674043 | Uncharacterized LOC105674043 (LOC105674043) | 0.60 | 2.51E−04 | 3.54E−02 |
| LOC105671136 | Serine protease 52-like (LOC105671136) | 0.53 | 8.59E−04 | 8.85E−02 |
| LOC105678761 | Serine protease inhibitor 3/4-like (LOC105678761) | −0.51 | 9.14E−04 | 9.33E−02 |
| LOC105672958 | Cytochrome P450 9e2-like (LOC105672958) | −0.67 | 1.01E−03 | 9.87E−02 |
| LOC105668778 | Leucine-rich repeats and immunoglobulin-like domains protein 2 (LOC105668778) | −0.67 | 9.81E−04 | 9.77E−02 |
| LOC105671541 | Protein croquemort-like (LOC105671541) | −0.68 | 9.94E−04 | 9.77E−02 |
| LOC105676100 | Tubulin-specific chaperone cofactor E-like protein (LOC105676100) | −0.71 | 5.35E−04 | 6.10E−02 |
| LOC105667678 | Monocarboxylate transporter 12-B (LOC105667678) | −0.75 | 6.13E−04 | 6.85E−02 |
| LOC105672522 | Rho-related BTB domain-containing protein 1 (LOC105672522) | −0.76 | 6.84E−04 | 7.56E−02 |
| LOC105677283 | Alpha-aminoisadipic semialdehyde synthase (LOC105677283) | −0.77 | 1.62E−04 | 2.40E−02 |
| LOC105673034 | PI-PLC x domain-containing protein 1-like (LOC105673034) | −0.78 | 8.02E−04 | 8.44E−02 |
| LOC105677249 | Glycogen-binding subunit 76A (LOC105677249) | −0.79 | 9.57E−05 | 1.54E−02 |
| LOC105667709 | Uncharacterized LOC105667709 (LOC105667709) | −0.79 | 4.18E−04 | 5.12E−02 |
| LOC105680137 | Tryptophan 2 | −0.79 | 4.02E−05 | 7.07E−03 |
| LOC105674346 | Neural-cadherin (LOC105674346) | −0.83 | 3.04E−04 | 4.05E−02 |
| LOC105670852 | Steroid receptor seven-up, isoforms B/C (LOC105670852) | −0.84 | 1.99E−04 | 2.84E−02 |
| LOC105678750 | Uncharacterized LOC105678750 (LOC105678750) | −0.85 | 4.64E−04 | 5.41E−02 |
| LOC105669560 | Inositol oxygenase (LOC105669560) | −0.85 | 8.16E−06 | 1.99E−03 |

(Continues)
| Time point | Locus | Description | LFC | p-Value | Adjusted p-value |
|------------|-------|-------------|-----|---------|------------------|
| LOC105668188 | Uncharacterized LOC105668188 (LOC105668188) | -0.85 | 8.11E-04 | 8.44E-02 |
| LOC105669693 | Aryl hydrocarbon receptor nuclear translocator-like protein 1 (LOC105669693) | -0.87 | 1.70E-04 | 2.46E-02 |
| LOC105669509 | Arylsulfatase B-like (LOC105669509) | -0.92 | 4.77E-05 | 8.24E-03 |
| LOC105679739 | Uncharacterized LOC105679739 (LOC105679739) | -0.96 | 5.10E-08 | 2.29E-05 |
| LOC105672956 | Ribose-phosphate pyrophosphokinase 1 (LOC105672956) | -0.98 | 7.94E-04 | 8.44E-02 |
| LOC105680118 | Cysteine proteinase 1-like (LOC105680118) | -0.99 | 3.23E-04 | 4.12E-02 |
| LOC105677063 | Organic cation transporter protein (LOC105677063) | -1.01 | 1.51E-05 | 3.06E-03 |
| LOC105674681 | Cytochrome P450 6j1-like (LOC105674681) | -1.03 | 3.11E-04 | 4.05E-02 |
| LOC105672502 | Alpha-tocopherol transfer protein-like (LOC105672502) | -1.03 | 3.13E-04 | 4.05E-02 |
| LOC105671942 | Hexokinase-2-like (LOC105671942) | -1.04 | 1.48E-10 | 9.89E-08 |
| LOC105667318 | Dentin sialophosphoprotein-like (LOC105667318) | -1.04 | 3.05E-05 | 5.73E-03 |
| LOC105680018 | Uncharacterized LOC105680018 (LOC105680018) | -1.11 | 1.16E-05 | 2.43E-03 |
| LOC105667320 | Homeobox protein Nkx-2.2a-like (LOC105667320) | -1.16 | 9.78E-04 | 9.77E-02 |
| LOC105671469 | D-arabinitol dehydrogenase 1-like (LOC105671469) | -1.17 | 2.77E-04 | 3.86E-02 |
| LOC105671862 | Uncharacterized LOC105671862 (LOC105671862) | -1.17 | 3.35E-05 | 6.08E-03 |
| LOC105677265 | Tachykinin-like peptides receptor 99D (LOC105677265) | -1.25 | 6.72E-07 | 2.25E-04 |
| LOC105671615 | Zinc finger protein 76-like (LOC105671615) | -1.26 | 1.12E-05 | 2.43E-03 |
| LOC105670688 | Uncharacterized LOC105670688 (LOC105670688) | -1.32 | 4.53E-04 | 5.33E-02 |
| LOC105678416 | Pancreatic lipase-related protein 2-like (LOC105678416) | -1.36 | 1.73E-11 | 1.32E-08 |
| LOC105676634 | Carboxypeptidase B-like (LOC105676634) | -1.39 | 1.55E-06 | 4.88E-04 |
| LOC105668871 | Phenoloxidase 2-like (LOC105668871) | -1.42 | 4.69E-06 | 1.29E-03 |
| LOC105679484 | Sorbitol dehydrogenase-like (LOC105679484) | -1.45 | 1.09E-04 | 1.70E-02 |
| LOC105675407 | Inhibin beta E chain (LOC105675407) | -1.57 | 1.85E-15 | 2.47E-12 |
| LOC105670471 | Trypsin epsilon-like (LOC105670471) | -1.64 | 1.15E-05 | 2.43E-03 |
| LOC105678258 | Facilitated trehalose transporter Tret1-like (LOC105678258) | -1.87 | 6.36E-06 | 1.62E-03 |
| LOC105672897 | Cytochrome P450 4C1-like (pseudo) | -1.97 | 1.08E-09 | 6.45E-07 |
| LOC105672608 | Facilitated trehalose transporter Tret1-like (LOC105672608) | -2.18 | 1.63E-10 | 1.03E-07 |
| LOC105672083 | Cytochrome P450 4c21-like (LOC105672083) | -2.42 | 9.57E-06 | 2.19E-03 |
| LOC105677462 | Peroxisomal hydratase-dehydrogenase-epimerase-like (LOC105677462) | -2.45 | 2.17E-12 | 2.11E-09 |

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