

Supplemental Figures

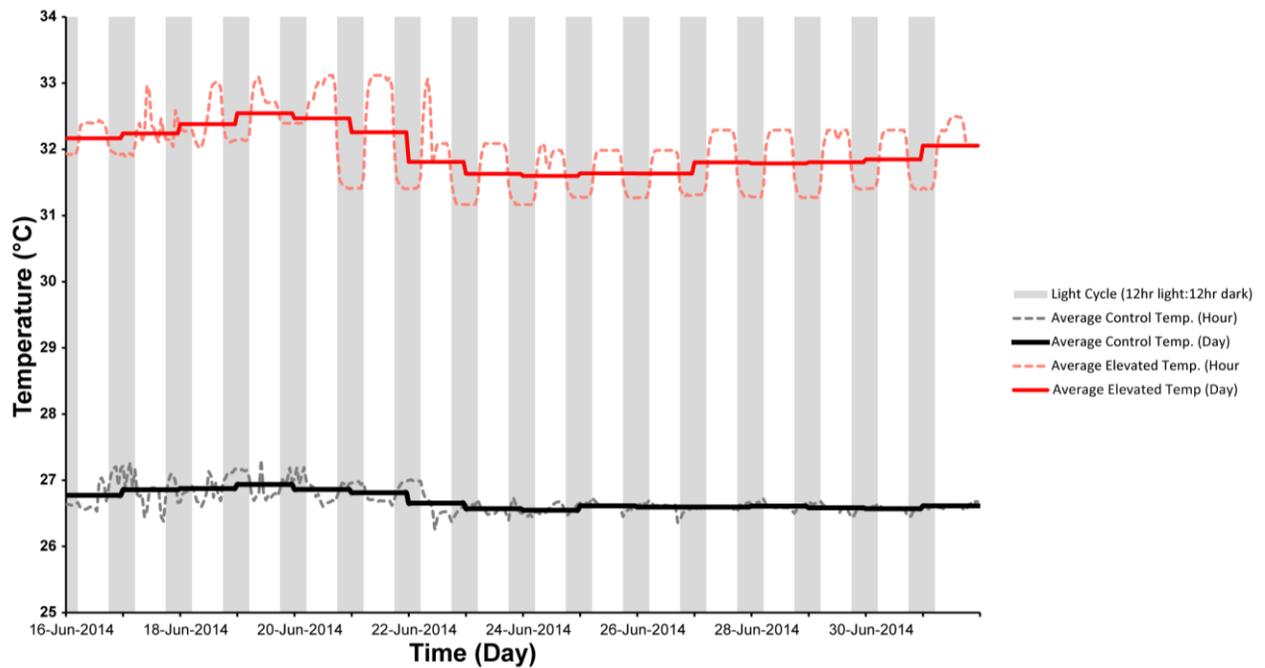


Figure S1. Experimental temperatures over 14 days. Temperature loggers submerged in water recorded temperature at 1 min intervals from each experimental chamber. Average temperatures by hour (dashed line) and by day (solid line) are displayed with the 12 h light: 12 h dark cycle indicated by shaded bars. Light cycle caused approximately 0.5 °C oscillation in temperature in the 32 °C treatment group, but not with control temperature. Control treatment = black; high temperature treatment = red.

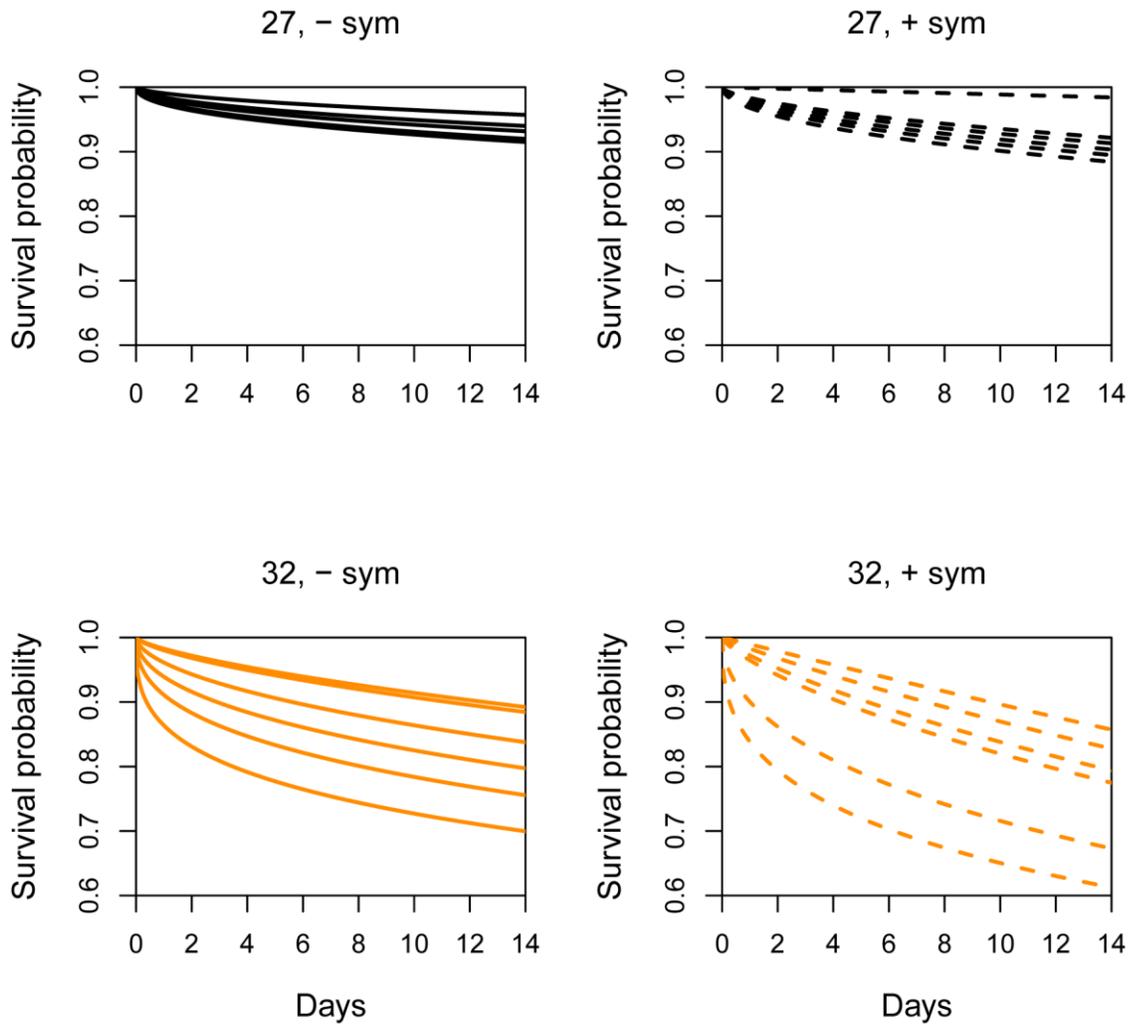


Figure S2. Survival probability for each replicate tube in a given treatment. Fitted survival probabilities under the Weibull proportional hazard model for each treatment group ($n= 300$ larvae), averaged across replicate tubes ($n= 6$ tubes per treatment group). The odds of survival decreased significantly with both temperature and symbiosis, but no interaction of these treatments was detected. Solid line = aposymbiotic (A), dashed line = symbiont colonized (S), black = 27 °C control temperature (C), orange = 32 °C high temperature (H).

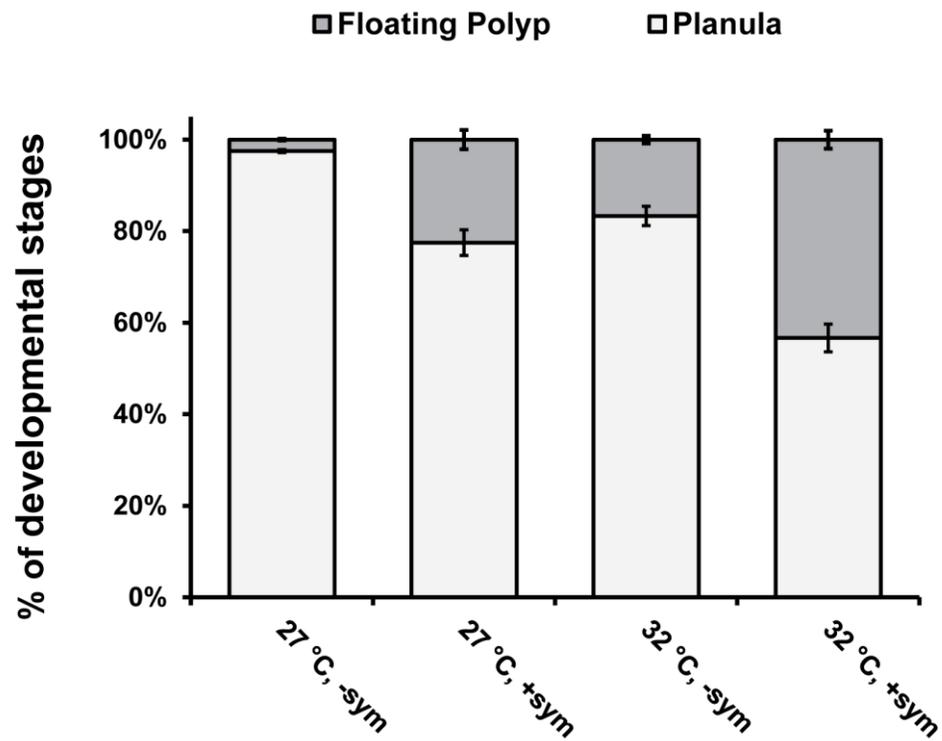


Figure S3. Percentage of metamorphosed floating polyps at 3 dpi in samples used for RNAseq. Twenty individuals were randomly collected from each replicate tube ($n= 6$ per group) and categorized into floating polyps (dark gray) or planulae (light gray). The odds of pre-mature development with temperature or colonization were tested using a logistic regression. The bars represent average for each developmental stage with standard error bars.

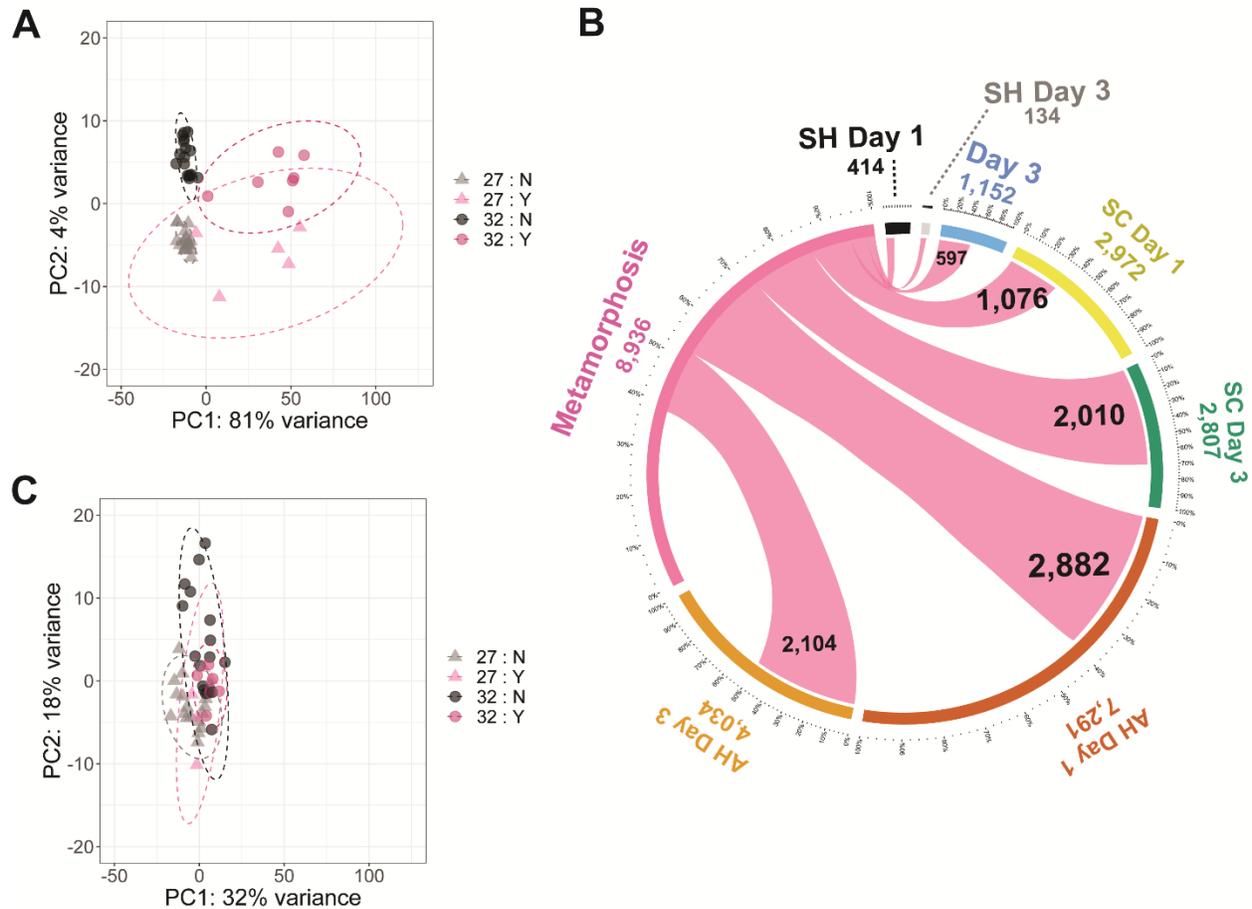


Figure S4. Identification and removal of metamorphosis differentially expressed genes.

(A) Principle component analysis of top 500 genes with highest variance of rlog transformed counts for each *A. digitifera* larval sample. Larval samples that did not undergo metamorphosis (N) formed tight clusters with their respective temperature treatments (gray triangle = 27 °C, black circle = 32 °C). The samples that had some level of metamorphosis (Y) ranging from 5 to 85% did not fall within the non-metamorphosed groups, but were also separated by temperature (light pink triangle = 27 °C, dark pink triangle = 32 °C). (B) 8,936 DEGs identified with metamorphosis were shared with the respective treatments. The colored segments outside the circle are the total number of DEGs for each category. The inner pink segments show the number of those DEGs that are shared with metamorphosis. (C) Principle component analysis of rlog transformed counts after the removal of the metamorphosis DEGs.

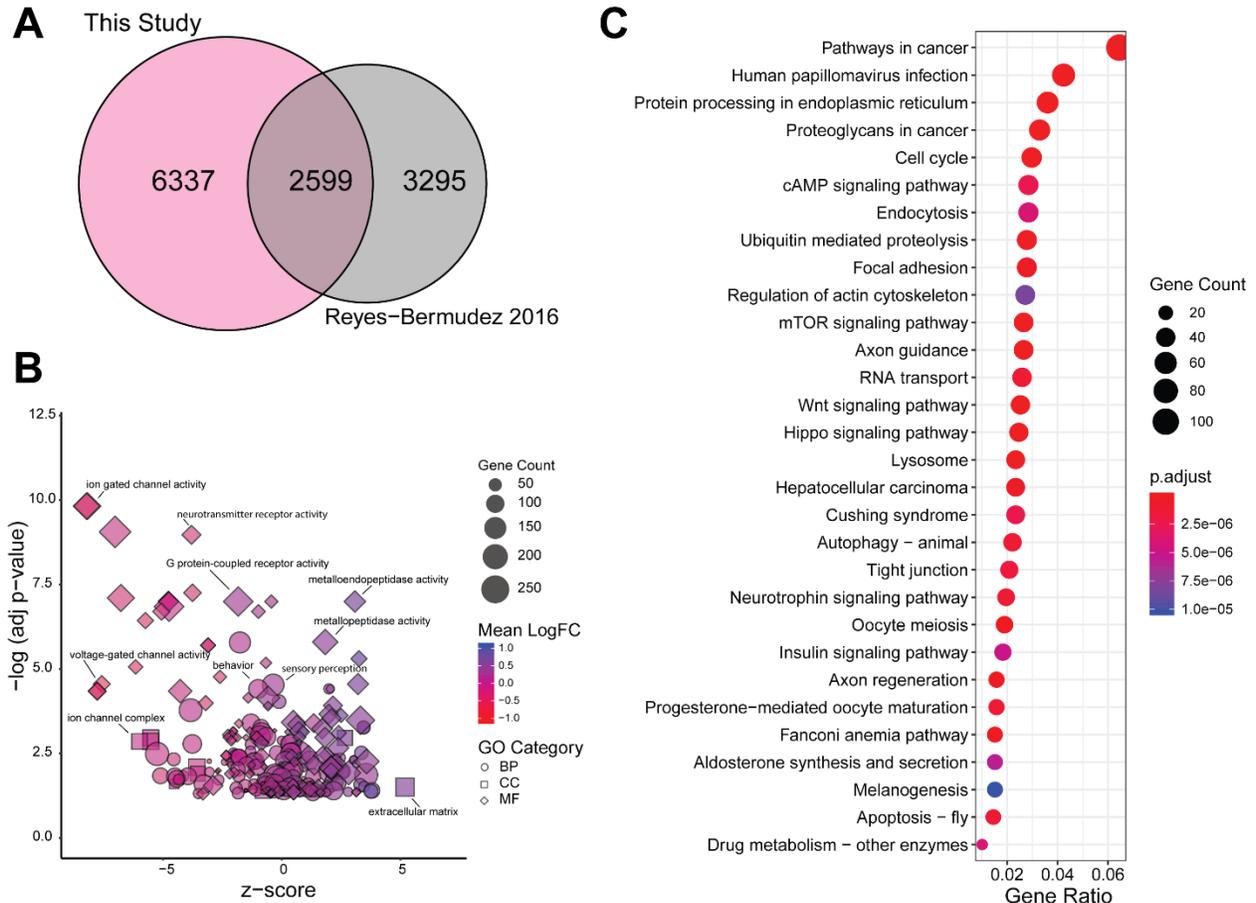


Figure S5. Enrichment of GO terms and KEGG pathways in differentially expressed genes with metamorphosis. (A) A Venn diagram shows overlap of 2,599 metamorphosis DEGs identified in this study with a previous study by Reyes-Bermudez et al. (2016) on the differences between planula larvae and adult *A. digitifera*. (B) Over-representation analysis identified 110, 159 and 16 GO terms from molecular function, biological process and cellular component categories, respectively. The adjusted p-values were \log_{10} transformed for each term and a z-score was calculated based on the number of genes upregulated vs. downregulated out of all genes assigned to a given GO term. The shape is based on the GO category (shapes: MF= diamond, BP= circle, CC=square), the size is based on the number of genes, and the color is based on the mean \log_2 fold-change calculated for all genes for a given GO term. (C) KEGG pathway enrichment analysis identified 187 pathways, of which the top 30 are shown. The size of the circles is based on gene counts and the color is based on the adjusted p-value.

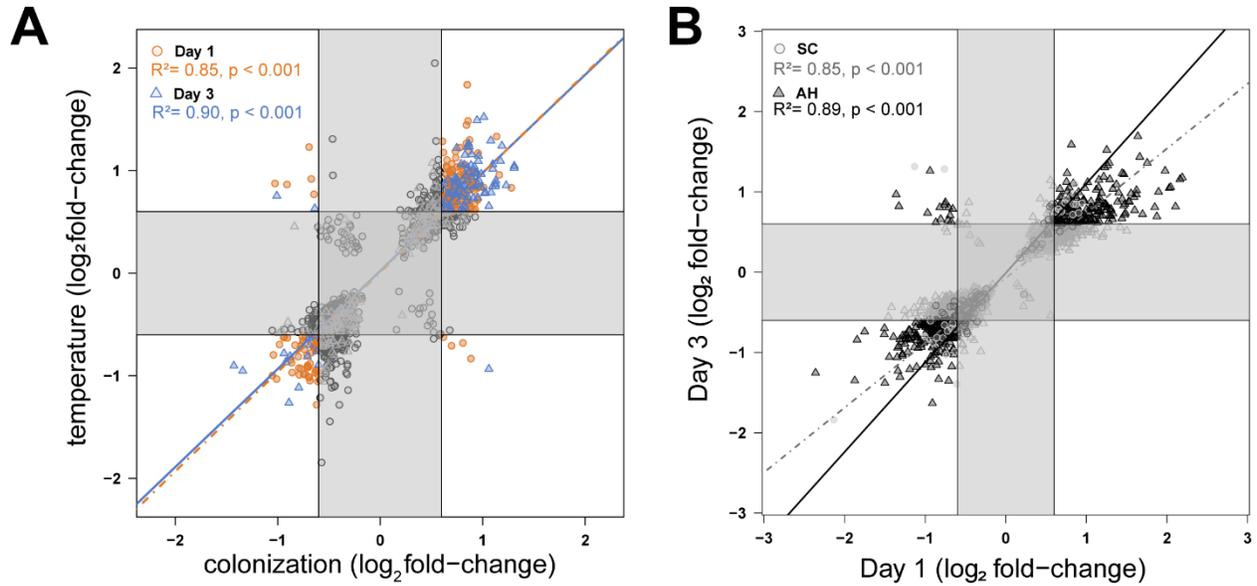


Figure S6. Correlation of gene expression from SC- and AH-treated larvae. (A) A positive correlation of DEGs shared between SC and AH treatment groups ($n = 942$ DEGs) on days 1 (orange circles) and 3 (blue triangles) (day 1 Pearson's correlation = 0.85, day 3 Pearson's correlation = 0.90). (B) Genes shared between days 1 and 3 by the SC (grey circles) or AH (black triangles) treatment groups were positively correlated (SC 1 and 3 dpi Pearson's correlation = 0.85, $p < 0.001$; AH 1 and 3 dpi Pearson's correlation = 0.890, $p < 0.001$). Genes within the gray boxes were differentially expressed but below the threshold of fold-change in this study, between 0.6 to -0.6.

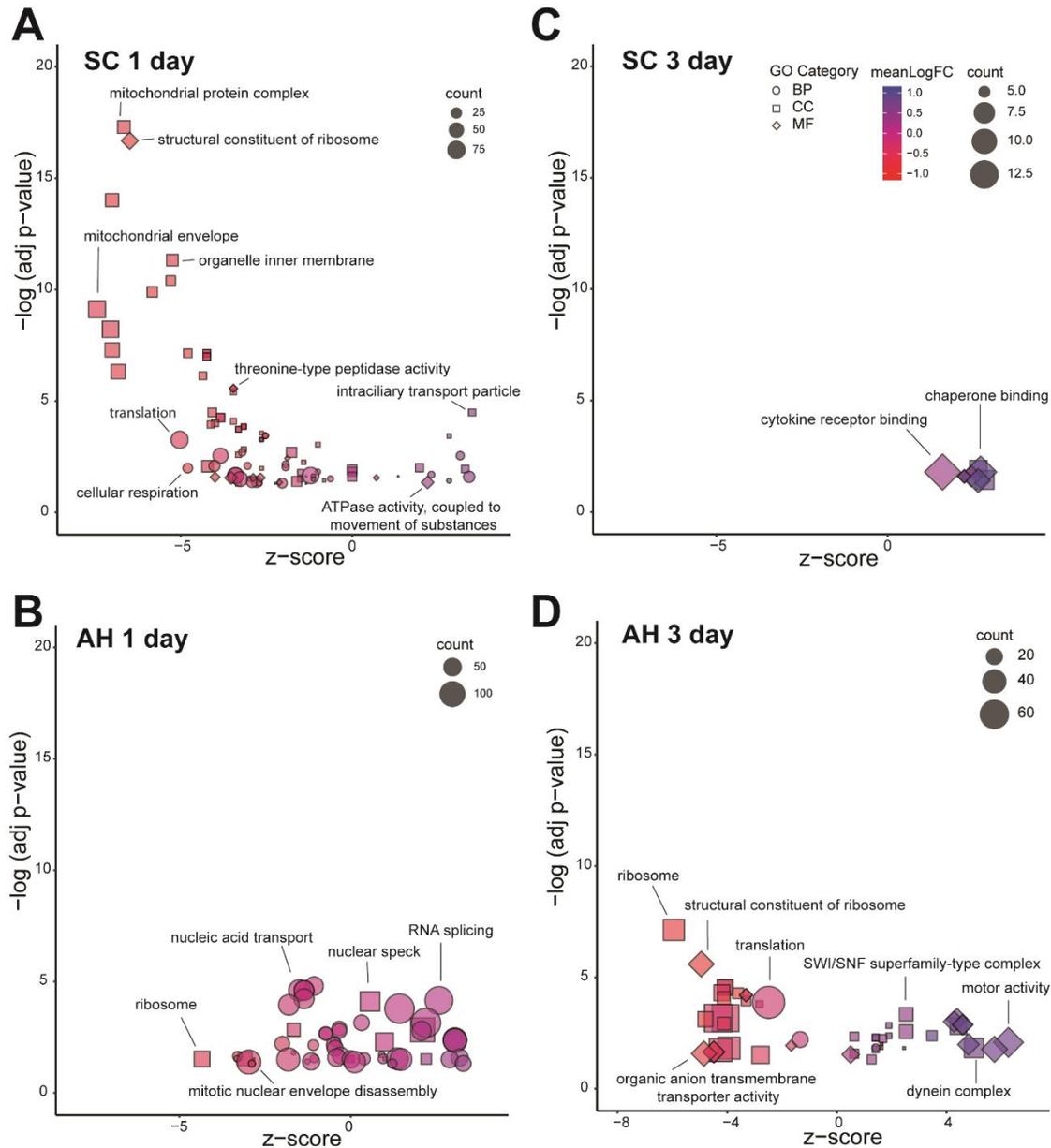


Figure S7. Enriched GO terms for each treatment by day in *A. digitifera* larval samples. GO term enrichment was performed on DEGs from day 1 SC (A) and AH (B) and day 3 SC (C) and AH (D) treatment groups with a hypergeometric model in clusterProfiler (Yu et al., 2012). The adjusted p-values were \log_{10} transformed for each term and a z-score was calculated based on the number of genes upregulated vs. downregulated out of all genes assigned to a given GO term. The shape is based on the GO category (shapes: MF= diamond, BP= circle, CC=square), the size is based on the number of genes, and the color is based on the mean \log_2 fold-change calculated for all genes for a given GO term.

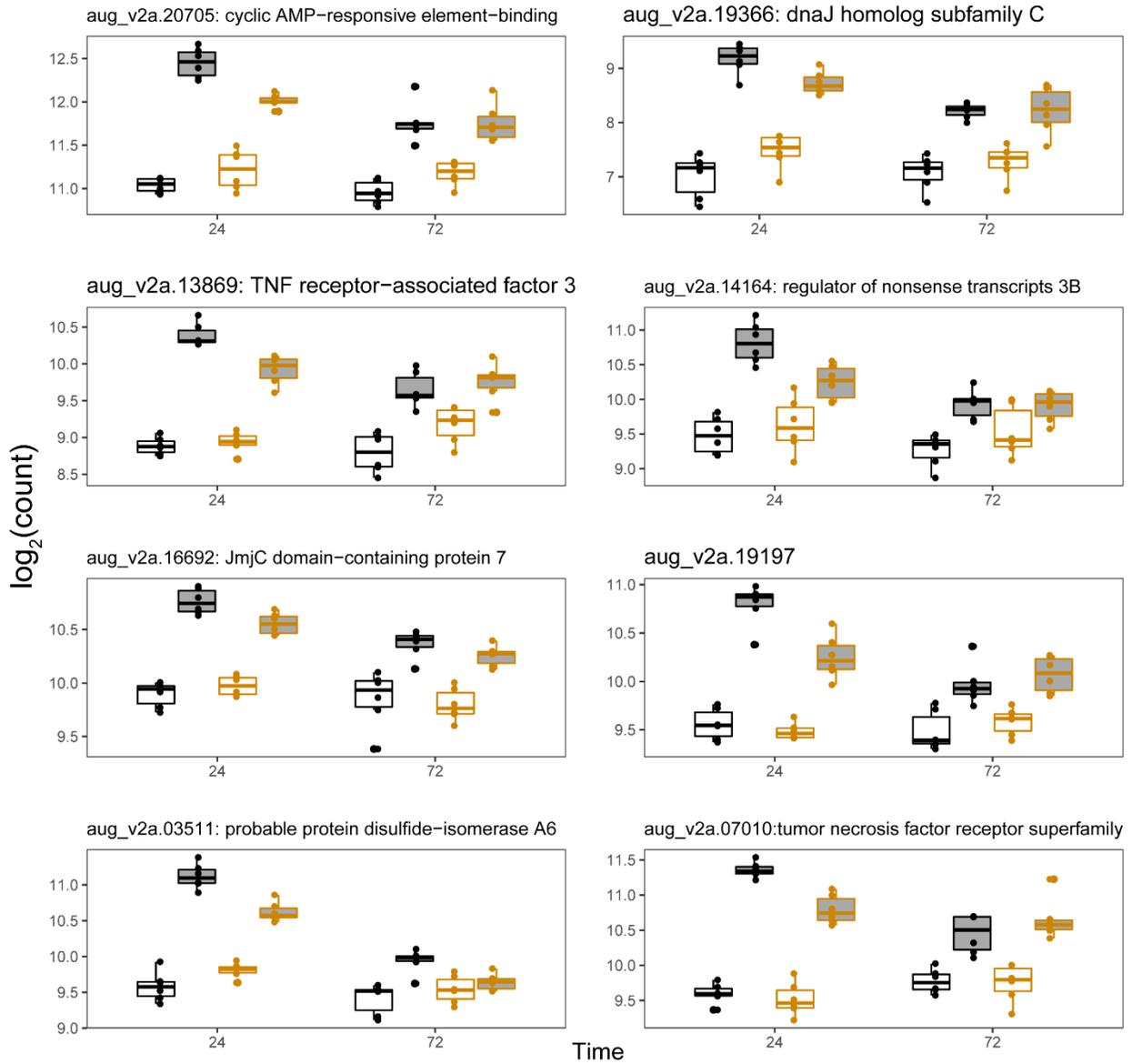


Figure S8. Normalized gene expression of the top eight hub genes in module M21. Black outline and white fill = AC, black outline and color fill = AH, orange outline and white fill = SC, orange outline and color fill = SH.

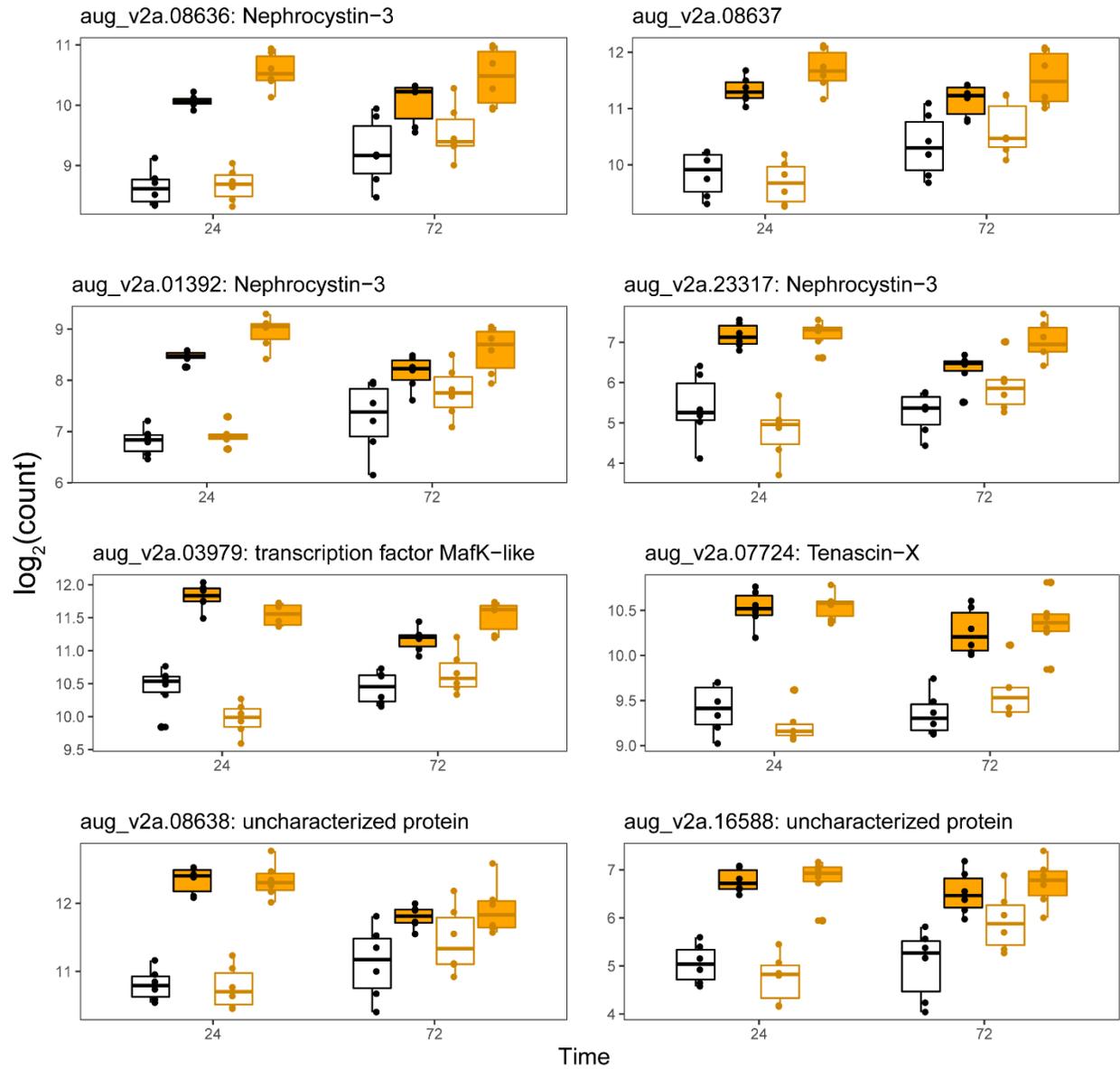


Figure S9. Normalized gene expression of the top eight hub genes in module M12. Black outline and white fill = AC, black outline and color fill = AH, orange outline and white fill = SC, orange outline and color fill = SH.

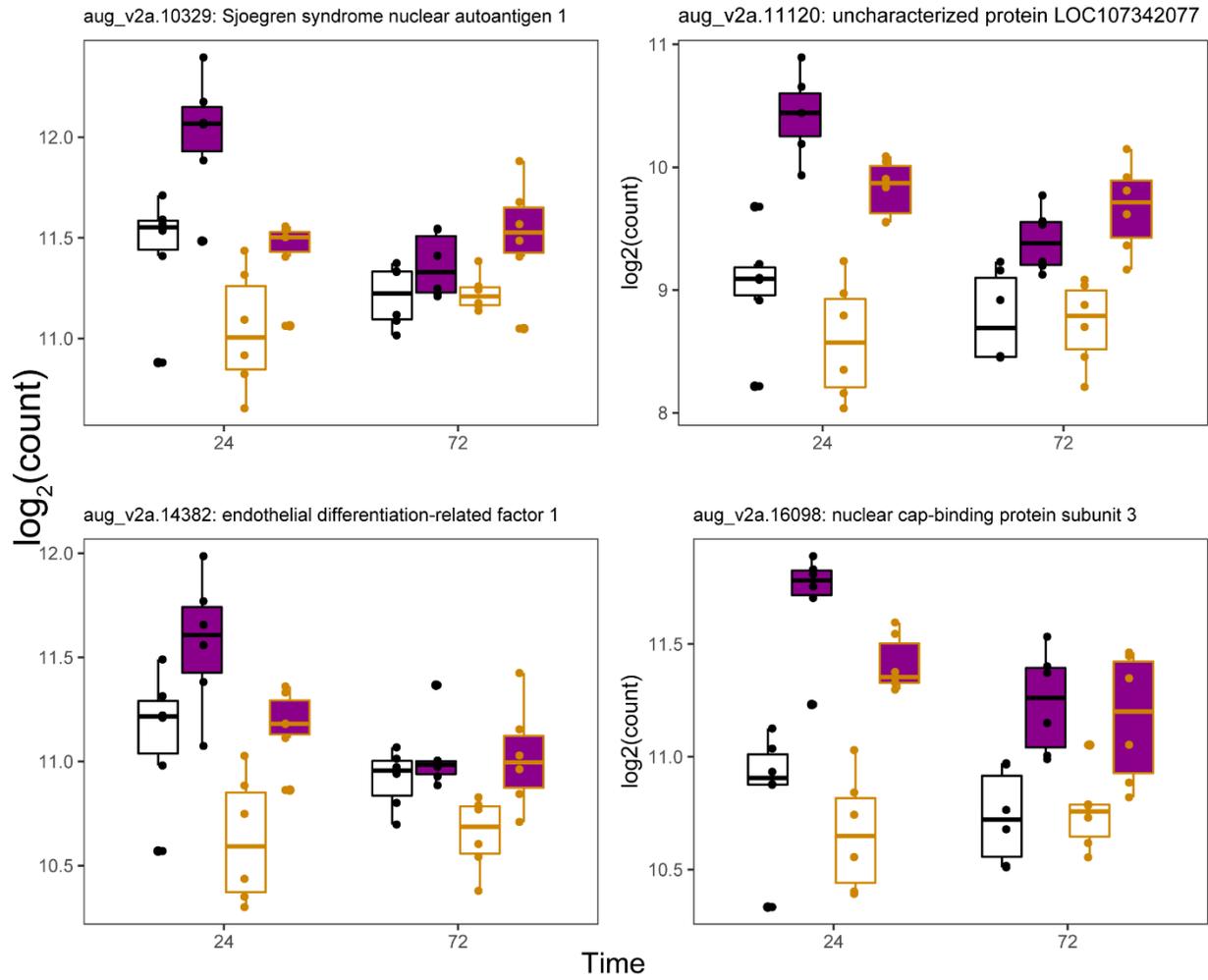


Figure S10. Normalized gene expression of the top four hub genes in module M24. Black outline and white fill = AC, black outline and color fill = AH, orange outline and white fill = SC, orange outline and color fill = SH.

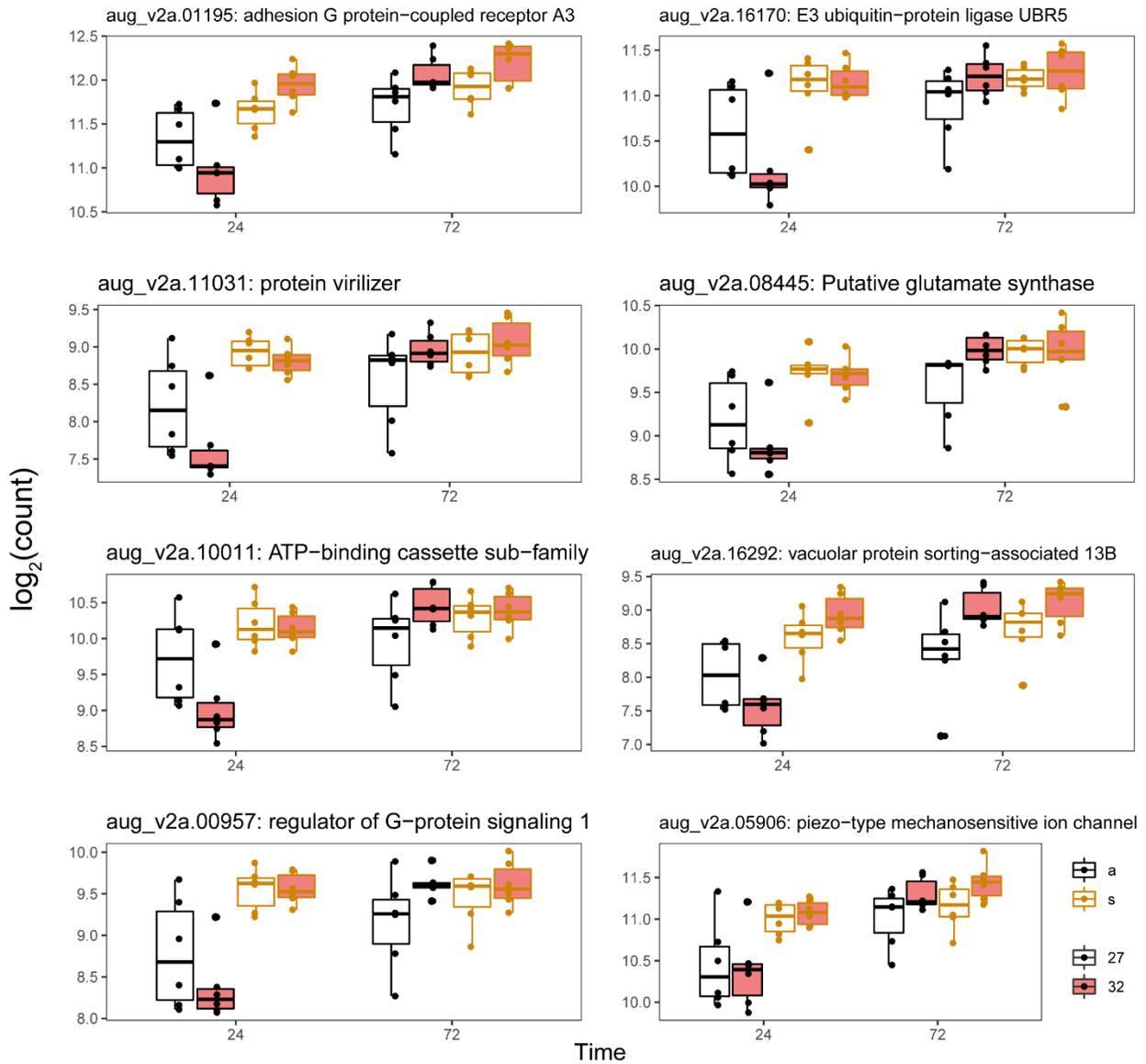


Figure S11. Normalized gene expression of the top eight hub genes in module M13. Black outline and white fill = AC, black outline and color fill = AH, orange outline and white fill = SC, orange outline and color fill = SH.

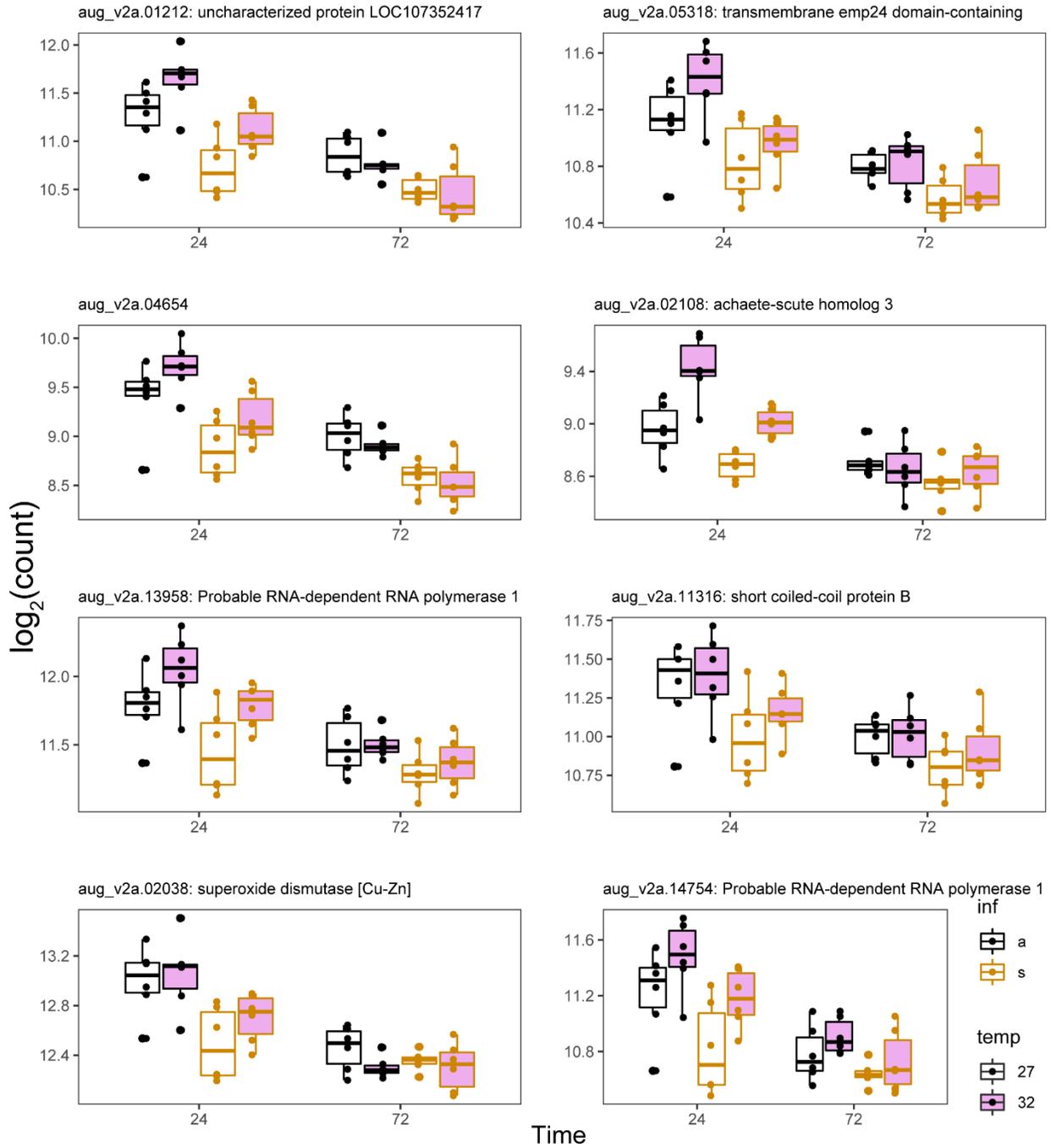


Figure S12. Normalized gene expression of the top eight hub genes in module M25. Black outline and white fill = AC, black outline and color fill = AH, orange outline and white fill = SC, orange outline and color fill = SH.

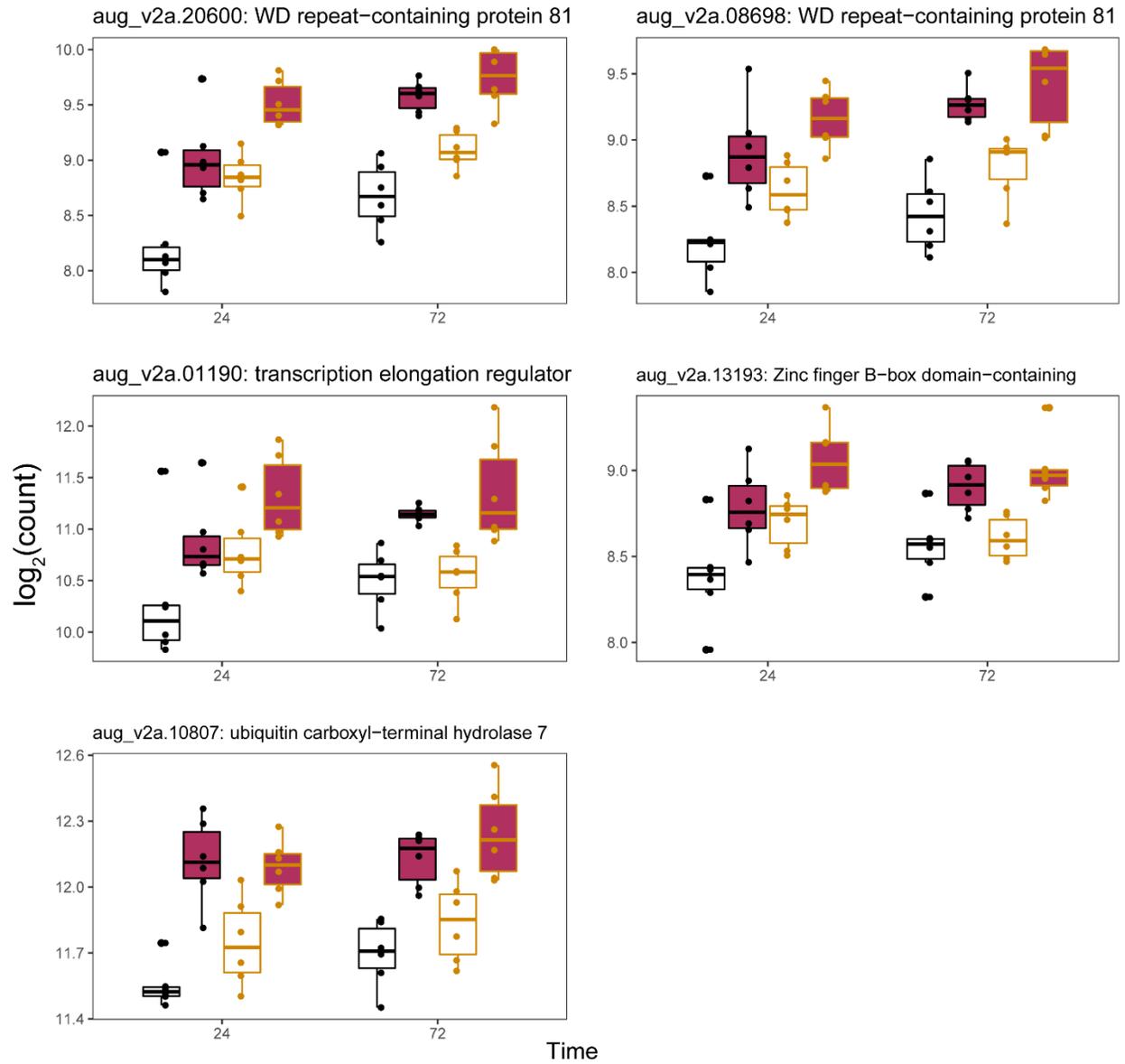


Figure S13. Normalized gene expression of the five hub genes in module M14. Black outline and white fill = AC, black outline and color fill = AH, orange outline and white fill = SC, orange outline and color fill = SH.

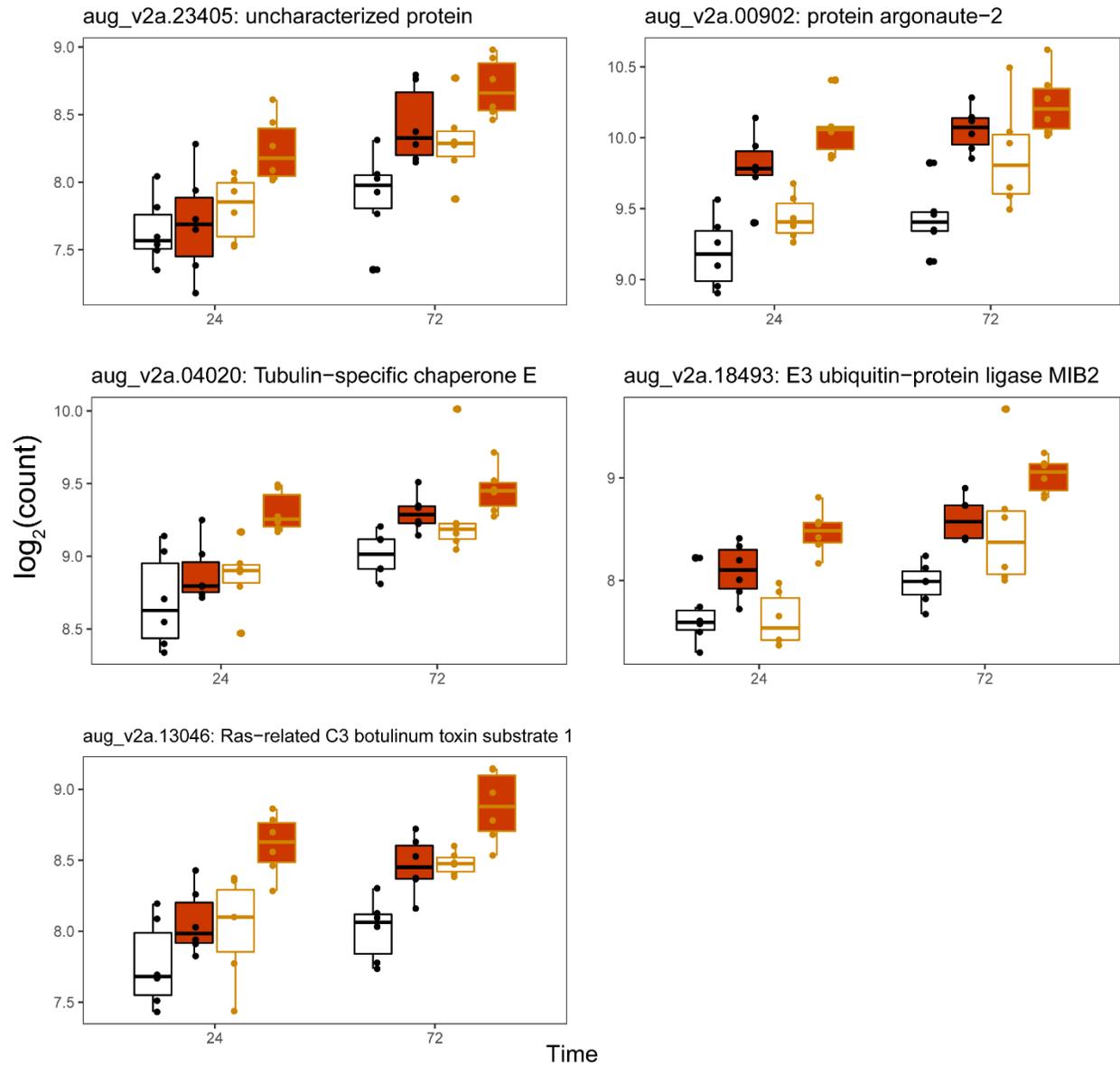


Figure S14. Normalized gene expression of the five hub genes in module M15. Black outline and white fill = AC, black outline and color fill = AH, orange outline and white fill = SC, orange outline and color fill = SH.

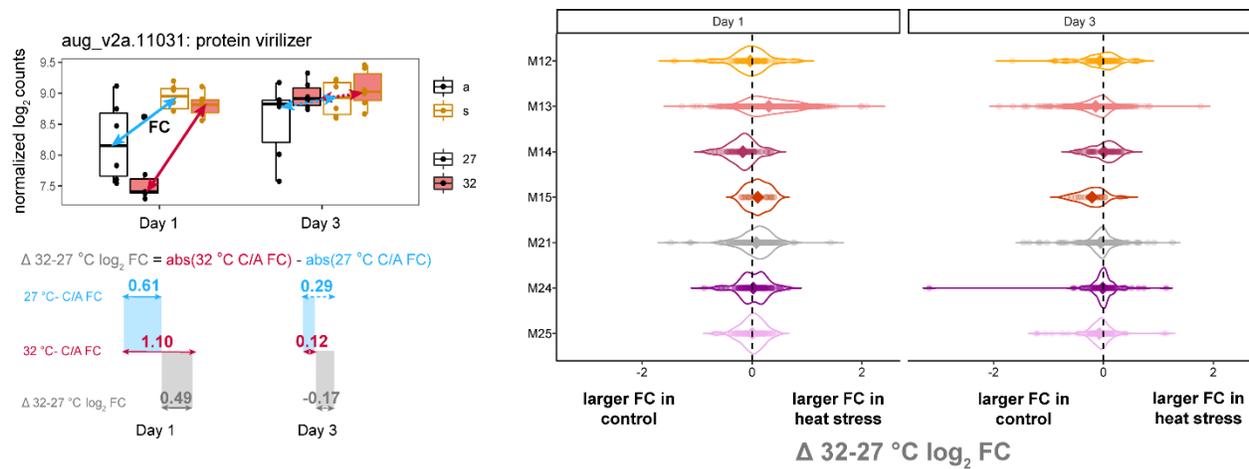


Figure S15. Comparison of colonization fold-change in the elevated and control temperature treatments. The difference in the absolute value of high temperature colonized larvae to not colonized larvae fold-change from the absolute value of the low temperature colonized larvae to not colonized larvae fold-change (Δ 32-27 °C log₂ expression) was calculated as exemplified for one of the hub genes in module M13 that was highly correlated with colonization. Distribution of Δ 32-27 °C log₂ expression for each significant gene (individual points) within each module is displayed by the violin plots with the mean difference represented by the diamond. Negative values indicate larger fold-changes in the control temperature treatment while positive values indicate larger fold-changes in the high temperature treatment.

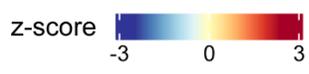
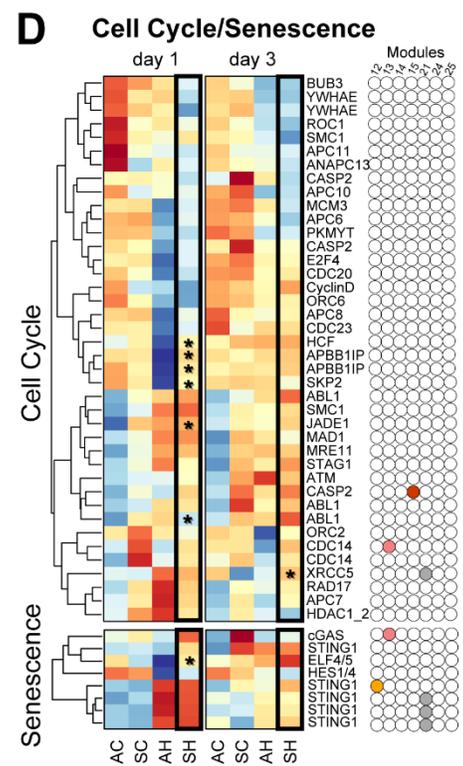
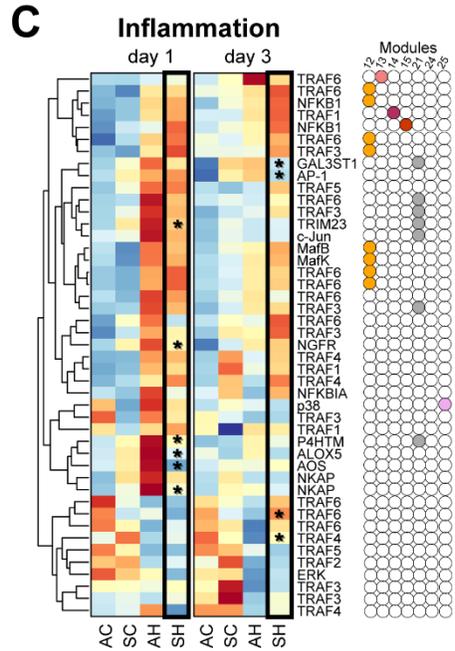
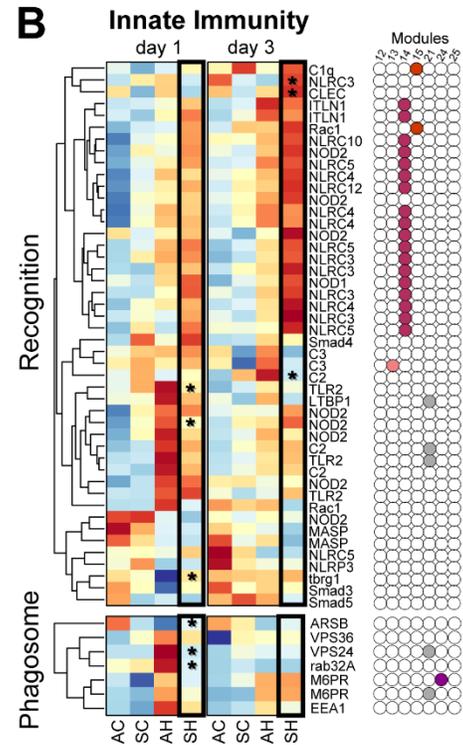
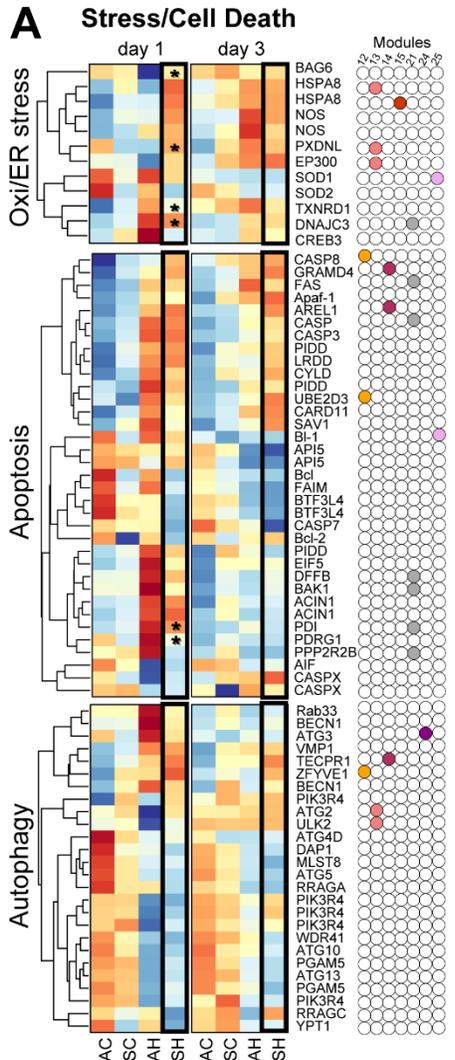


Figure S16. Heat maps of the specific pathways differentially expressed in colonization and temperature treatments. The average normalized, rlog counts of the transcripts (n=6 samples) involved in stress and apoptosis (A), immunity (B), inflammation (C), and cell cycle and cellular senescence (D) are presented. Expression was scaled row wise using a z-score transformation, ranging from -3 (blue) to 3 (red). Circles next to each heat map indicate the co-expression module membership of select modules referred to in the main text. Asterisk denote significant colonization-by-temperature interaction transcripts.