Supplemental methods

FHV titration in hemolymph samples

Hemolymph (HL) from flies of desired ages was collected using a centrifugation method adapted from (TENNESSEN *et al.* 2014). The heads of ~ 80 FHV-injected female flies were removed using a razorblade under CO_2 anesthesia and the bodies were placed in a microcentrifuge tube filter (Corning Costar Spin-X Centrifuge Tube Filters) with pore diameters of 0.45µm. Tubes were spun at 9,000 rpm for five minutes at 4°C and immediately put onto ice. Each centrifugation yielded approximately 3µL of HL. After HL collection, DL-1 cells (Drosophila Genomics Resource Center (Indiana University, Bloomington, IN), Stock #9) were used to determine FHV titers in HL using the TCID50 method as previously described (ELEFTHERIANOS *et al.* 2011). The rabbit anti-FHV capsid protein antibody used in this assay at 1:1000 was a kind gift from Dr. Annette Schneemann (Scripps Research Institute, La Jolla, CA). A secondary goat anti-rabbit Alexa-488 antibody (Invitrogen) was used at 1:1000. Using epifluorescent microscope, the Reed-Muench method (REED AND MUENCH 1938) was used to calculate the TCID50.

Aligning RNA-Seq and estimating expression levels

For data presented in Figures 4, S8 and S9, we aligned mRNA sequences to the *D. melanogaster* reference genome sequence BDGP6.22 (downloaded from Ensembl July 2019) with HISAT2 v.2.1.0 (KIM *et al.* 2015). We used HTSeq v.0.11.1 (ANDERS *et al.* 2015) and the *D. melanogaster* BDGP6.22 gene set (downloaded from Ensembl July 2019) to quantify transcript abundances.

Analyzing differential expression

For data presented in Figures S8B and S9, transcript abundances were analyzed for differential expression with the R statistical computing/Bioconductor software package DESeq2 v.1.24.0 (LOVE *et al.* 2014). Briefly, DESeq2 assumes transcript abundances approximate a negative binomial distribution and identifies transcripts that are differentially expressed between groups. Here, pairwise comparisons were conducted among each of the different age, stage and treatment types to generate lists of differentially expressed genes (DEGs) using a false discovery rate (FDR) < 0.05. Transcript counts were transformed with the regularized logarithm function rlog to stabilize variances for PCA analyses and visualization.

Clustering genes with variable expression

For data presented in Figures 4 and S8, a heatmap was generated using the R statistical software/Bioconductor package pheatmap v.1.0.12 to visualize expression patterns among gene transcripts belonging to corresponding GO categories. The pheatmap hierarchical clustering function was used to group transcript expression into four modules.

References

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