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Supplemental information

Drosophila parasitoids go to space: Unexpected effects of spaceflight on hosts and their parasitoids

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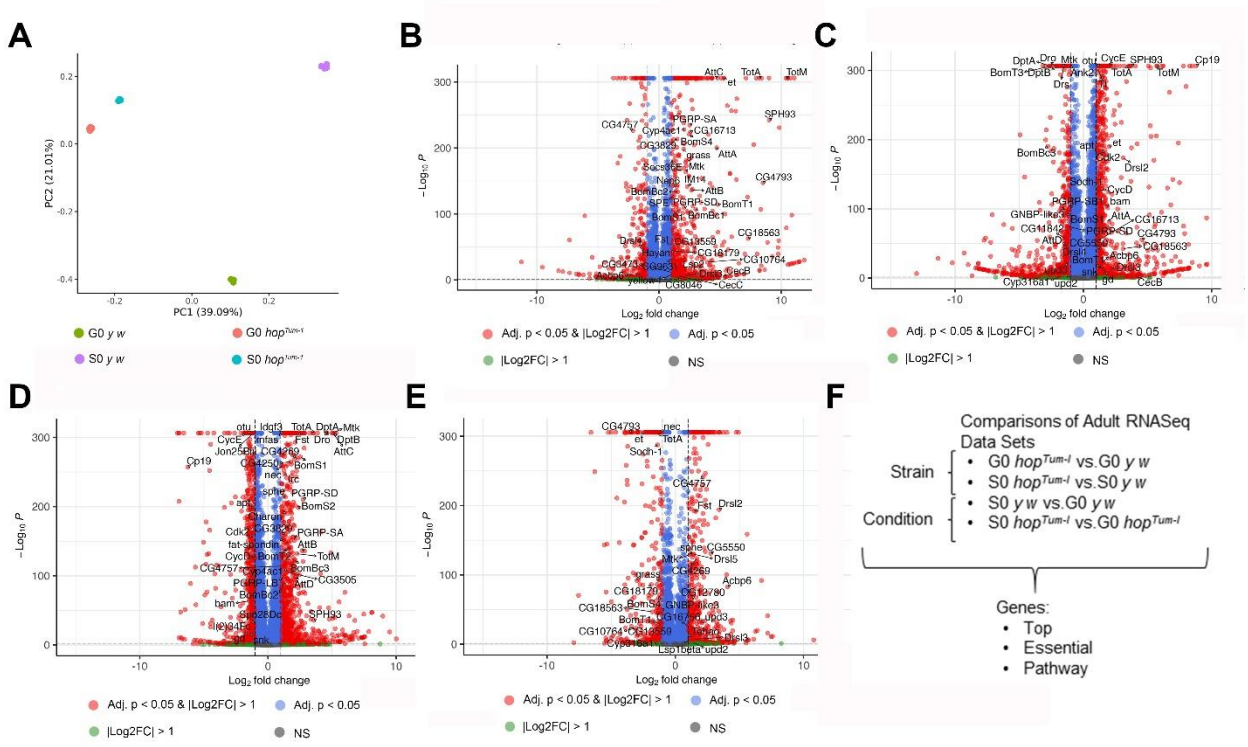

Figure S1. RNA-Seq analysis of adult fly samples, related to Figures 3-6.

(A) Principal component analysis for 16 samples with the log (2)-fold-transformed normalized gene-level counts data.

(B-E) Enhanced volcano plots comparing DEGs from adult fly samples. (B) G0 *hop*$^{Tum\text{-}l}$ vs. G0 *y w*; (C) S0 *hop*$^{Tum\text{-}l}$ vs. S0 *y w*; (D) S0 *y w* vs. G0 *y w*; (E) S0 *hop*$^{Tum\text{-}l}$ vs. G0 *hop*$^{Tum\text{-}l}$. Each dot represents the average value from four replicates for a single gene. Red colors indicate upregulated and downregulated genes with adjusted p < 0.05 and |log2FC| > 1 cutoff values; blue dots indicate genes with adjusted p < 0.05 and |log2FC| < 1. Multiple comparisons were accounted for by calculation of a Benjamini–Hochberg false discovery rate-adjusted p value (i.e., q value). Dashed horizontal lines mark a q value of 0.05 and dashed vertical lines indicate a log 2-FC of 1 and -1. Gene symbols correspond to differentially expressed immune genes.

(F) Genotypes and culture conditions of flies used in the bulk RNA-Seq study; (n = 4 replicates for each condition). Analysis of Top, Essential, and Immune Pathway DEGs was performed. S0 *y w* = space-flown adult flies (strain *y w*), G0 *y w* = ground control adult flies (strain *y w*), S0 $hop^{Tum\text{-}l}$ = space-flown adult flies (variant *y w* $hop^{Tum\text{-}l}$), G0 $hop^{Tum\text{-}l}$ = ground control adult flies (strain *y w* $hop^{Tum\text{-}l}$).

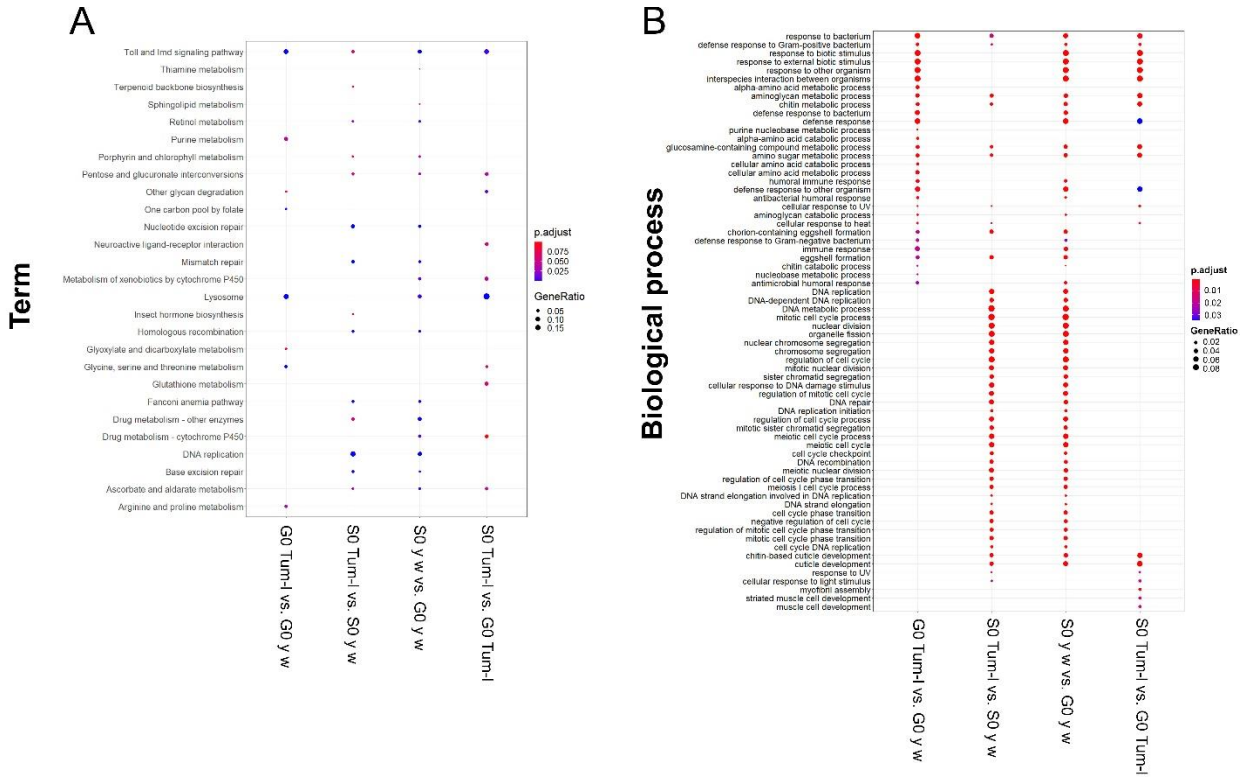

Figure S2. Enrichment analysis of differentially expressed genes in G0 and S0 adult flies, related to Figures 3-6.

(A) Enrichment analysis of KEGG pathways visualized by dot plot (adjusted p value < 0.1). The size of the dot is based on the gene count enriched in the pathway, while the color of the dot represents pathway significance. Experimental conditions analyzed are (a) G0 $hop^{Tum\text{-}l}$ versus G0 *y w* (G0 Tum-l vs. G0 y w); (b) S0 $hop^{Tum\text{-}l}$ versus S0 *y w* (S0 Tum-l vs. S0 y w); (c) S0 *y w* versus G0 *y w* (S0 y w vs. G0 y w); and (d) S0 $hop^{Tum\text{-}l}$ versus G0 $hop^{Tum\text{-}l}$ (S0 Tum-1 vs. G0 Tum-l).

(B) Gene ontology enrichment for biological process. Dot plot representing top 20 significantly enriched gene ontology terms (adjusted p value < 0.05) in biological processes for experimental conditions shown in panel A. Enrichment is represented as gene ratio, which indicates the number of genes differentially expressed in the experimental versus control condition, for each biological process indicated on the left.

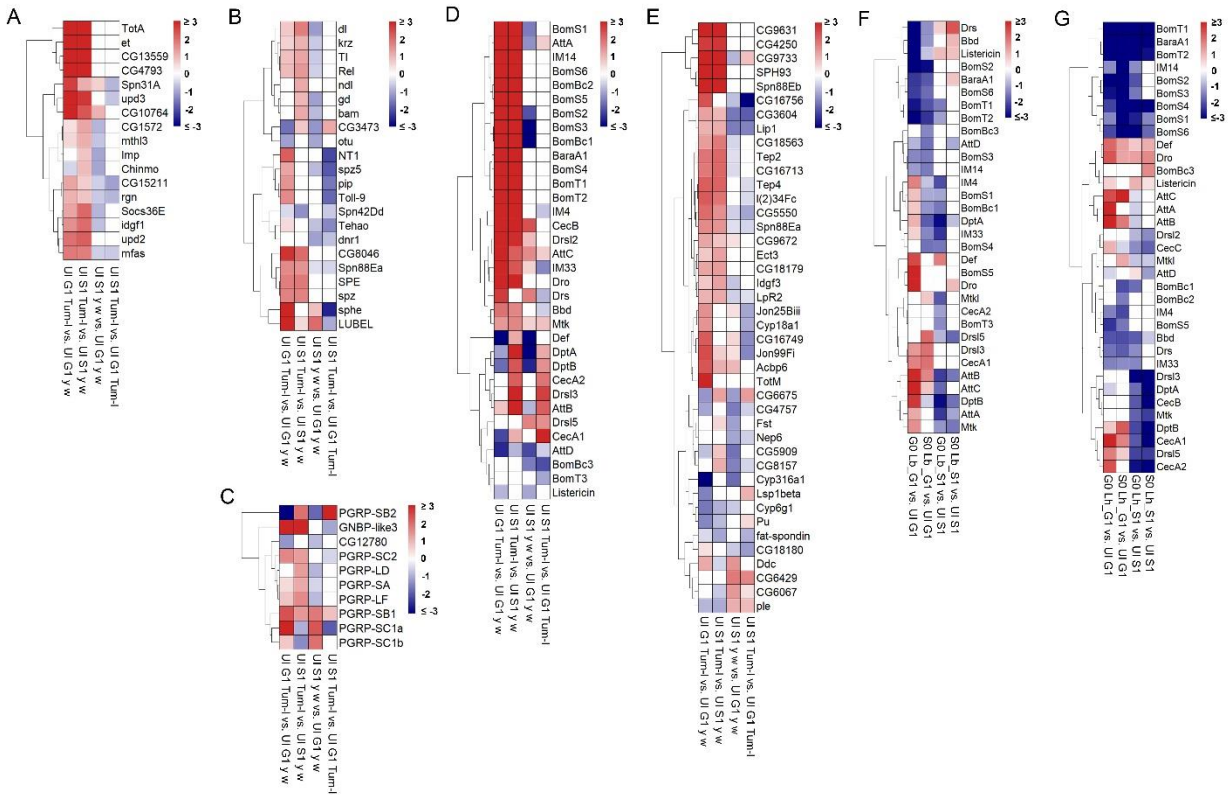

Figure S3. Differentially expressed immune signaling genes in naïve and wasp-infected G1 and S1 larvae, related to Figure 7.

(A) JAK-STAT pathway components and target genes in naïve S1 and G1 *y w* or *hop*$^{Tum\text{-}l}$ larvae are shown based on fold-induction. Genes were grouped based on their annotation in FlyBase [1]. Pathway genes that were significantly differentially expressed (adjusted $p < 0.05$ and $|\log2FC| > 1$) in at least one of the four comparisons are shown.

(B-E) Genes in the Toll/Imd pathway in naïve S1 and G1 *y w* or *hop*$^{Tum\text{-}l}$ larvae that are differentially expressed (adjusted $p < 0.05$ and $|\log2FC| > 1$) in at least one of the four comparisons are indicated. Core pathway components (B), Pattern Recognition Receptor genes (C), antimicrobial peptide genes (D), and other targets (E).

(F, G) Heat maps of AMP target immune genes comparing gene expression in G1 or S1 *hop*$^{Tum\text{-}l}$ hosts, parasitized with either G0/S0 *L. boulardi* (F), or G0/S0 *L. heterotoma* (G) relative to uninfected controls.

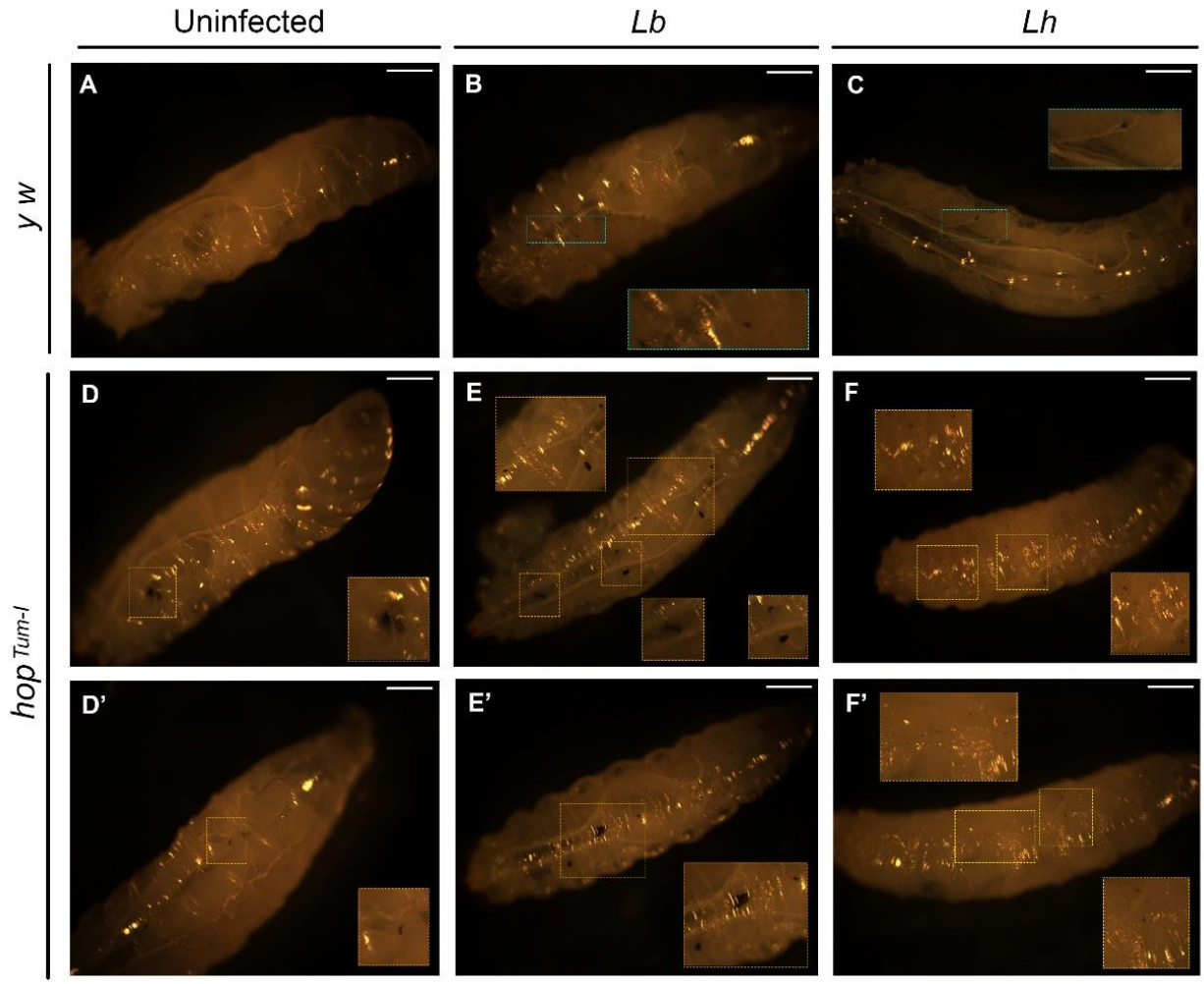

Figure S4. Immune reactions observed after wasp infection, related to Figure 7.

(A-C) Cellular encapsulation reactions in *Lb*- or *Lh*-infected G1 or S1 *y w* larvae. Fewer reactions are evident in *Lh*- versus *Lb*-infected *y w* larvae.

(D-F') Examples of tumors and encapsulation reactions in *Lb*- or *Lh*-infected G1 or S1 $hop^{Tum\text{-}l}$ hosts. These melanized structures have similar features of varying sizes as shown. Select areas with immune reactions are enlarged and shown as insets. Scale bars = 1 mm.

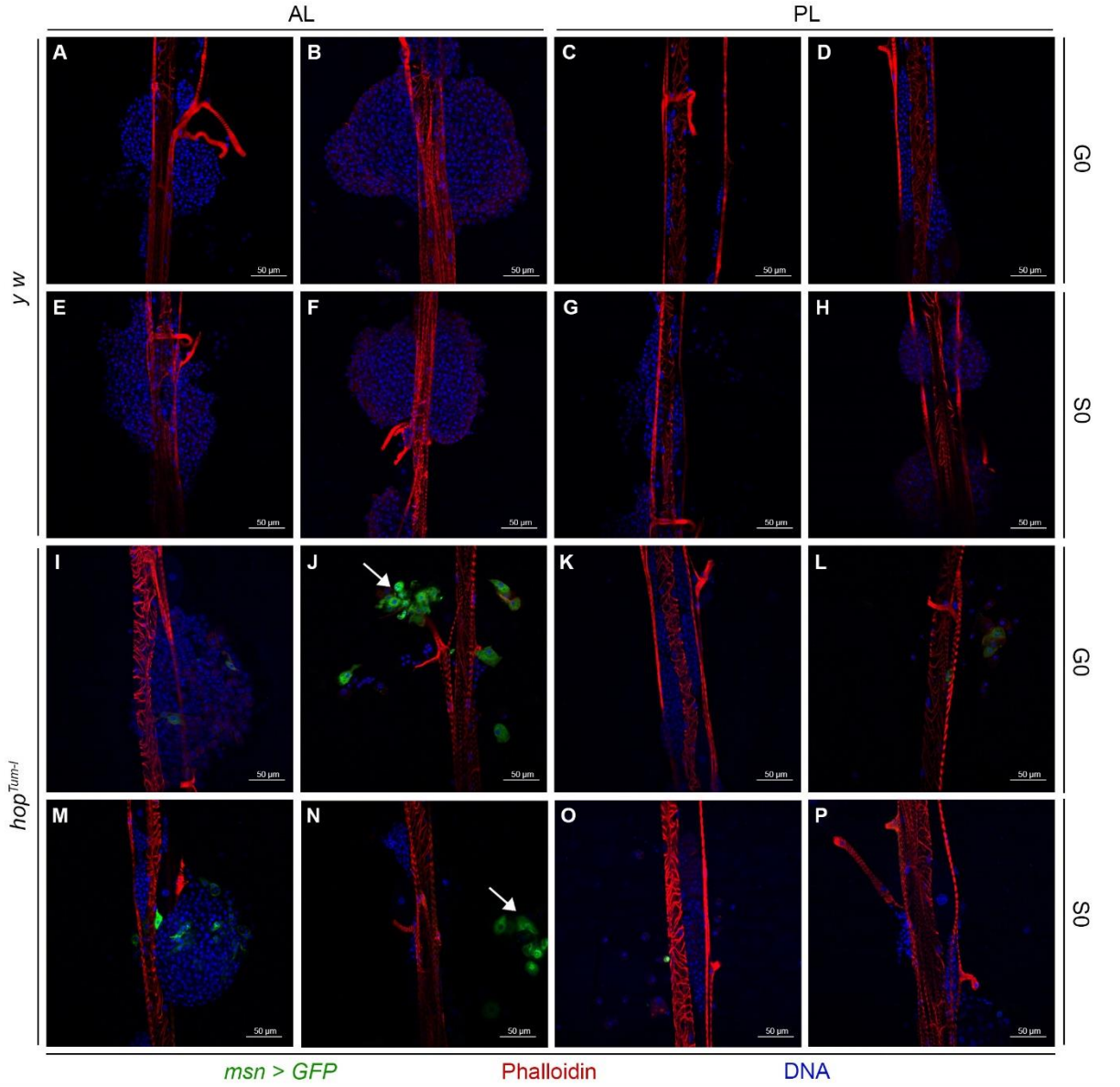

Figure S5. Lymph glands of naïve G0 and S0 hosts, related to Figure 7.

(A-H) Representative lymph glands from G0 (A-D) and S0 (E-H) *y w* larvae. Anterior (A, B, E, F) and posterior lobes (C, D, G, H) of *y w* lymph glands. n = 12 for each condition.

(I-P) Representative lymph glands from G0 (I-L) and S0 (M-P) $hop^{Tum\text{-}l}$ *msn > GFP* larvae. Anterior (I, J, M, N) and posterior (K, L, O, P) lobes. n = 12 for each condition.

Scale bars 50 μm. AL refers to anterior lobes, PL to posterior lobes. Arrows in panels J and N indicate GFP-positive lamellocyte aggregates.

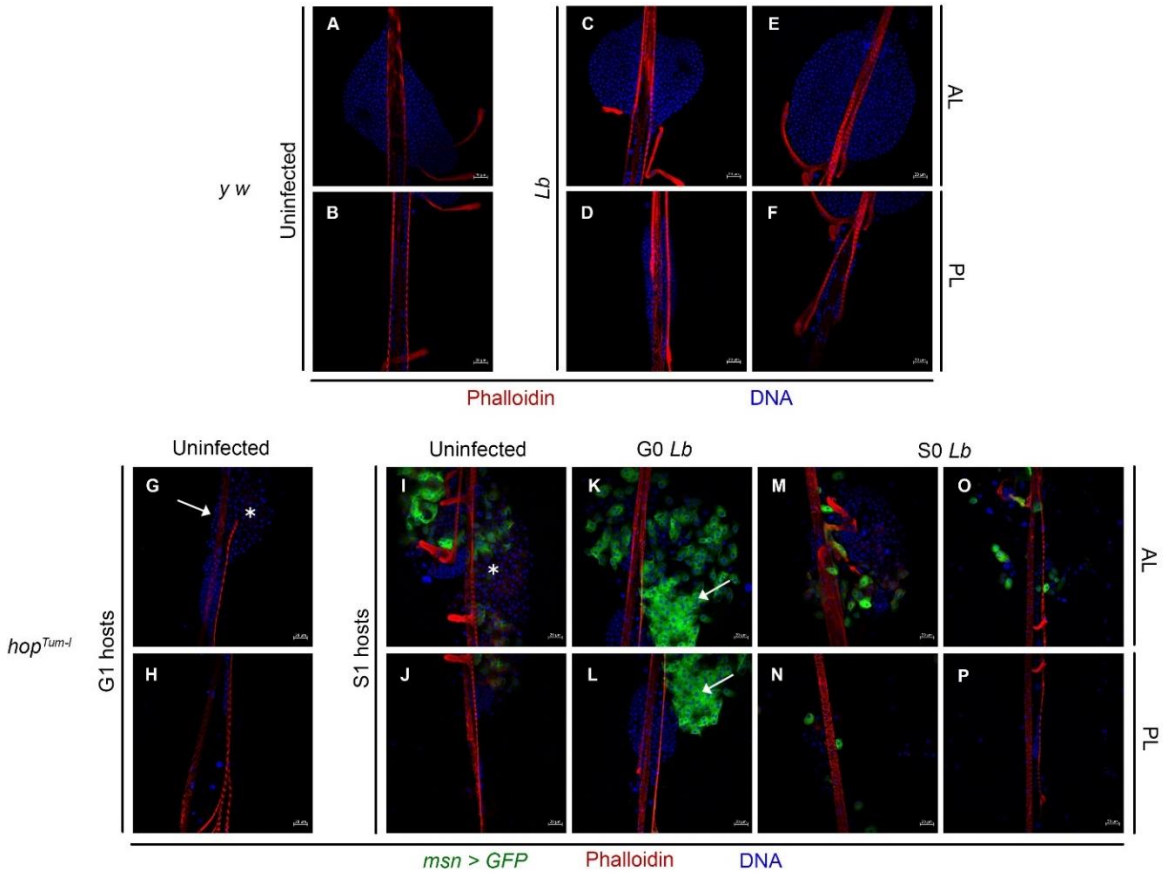

Figure S6. Lymph glands of naïve and *Lb*-parasitized G1 and S1 hosts, related to Figure 7.

(A, B) Lymph glands of naïve G1 *y w* larvae.

(C-F) Lymph glands of *Lb*-infected G1 (C, D) and *Lb*-infected S1 (E, F) *y w* hosts.

(G, H) Lymph glands of naïve G1 $hop^{Tum\text{-}l}$ hosts. Arrow in panel G shows absence of the left lobe and asterisk shows a reduced right lobe, likely due to release of differentiated hemocytes in the mutant background (compare with right lobe of panel I, as indicated by asterisk).

(I-P) Lymph glands of S1 $hop^{Tum\text{-}l}$ hosts. Naïve hosts (I, J). S1 hosts infected with G0 *Lb* (K, L) or S0 *Lb* (M-P). Arrow in panels K and L refer to tumor-containing, GFP-expressing lamellocytes. Anterior (AL) and posterior (PL) lobes are as shown. Scale bars 50 μm.

(n = 3 naïve *y w*, 8 G0 *Lb*-infected *y w*, and 3 S0 *Lb*-infected *y w* larvae).

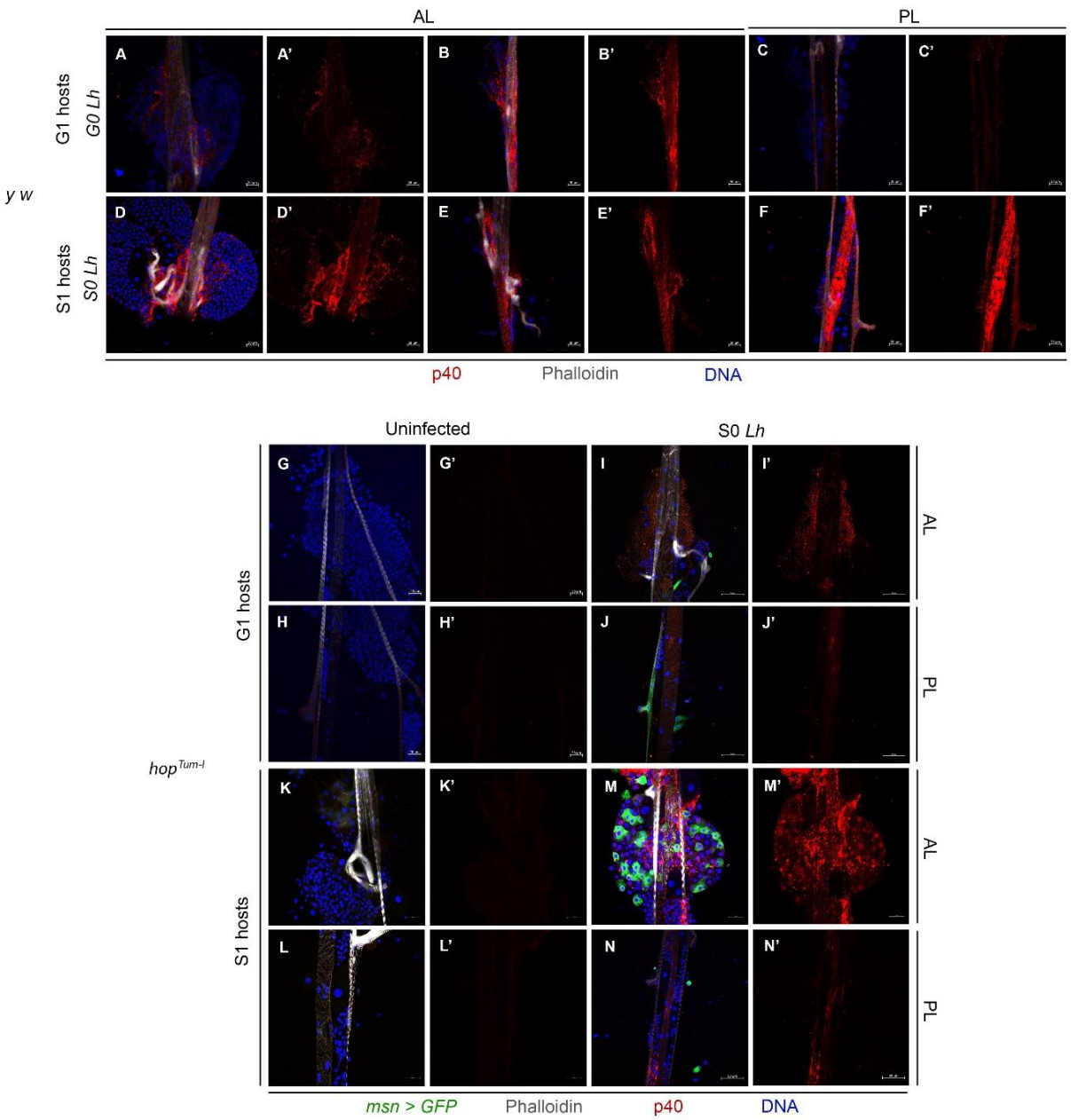

Figure S7. Lymph glands of naïve and *Lh*-infected G1 and S1 hosts, related to Figure 7.

(A-F') Lymph glands of *Lh*-infected G1 (A-C) and *Lh*-infected S1 (D-F) *y w* hosts.

(G-J') Lymph glands of naïve G1 $hop^{Tum\text{-}l}$ hosts (G, H), or infected by S0 *Lh* (I, J).

(K-N') Lymph glands of naïve S1 $hop^{Tum\text{-}l}$ hosts (K, L), or parasitized by S0 *Lh* (M, N). Primed panels show red-only channel to visualize EV distribution in the lymph glands.

Anterior (AL) and posterior (PL) lobes are as shown. Scale bars 50 μm.

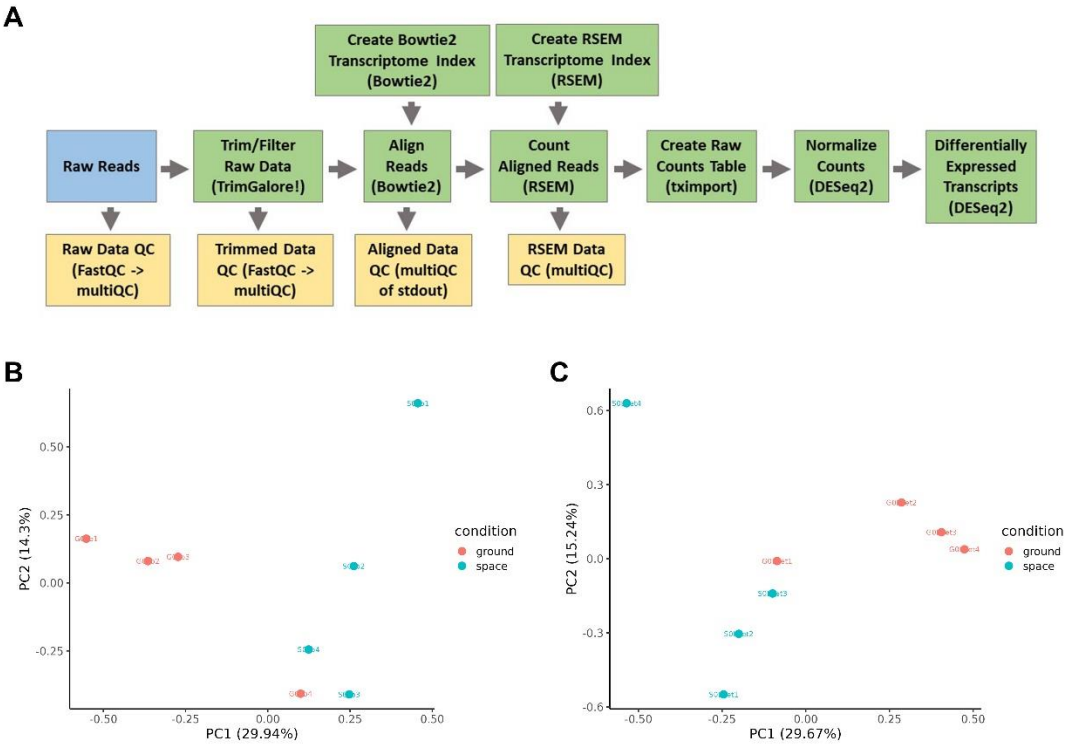

Figure S8. Evaluation of wasp parasite gene expression, related to Figure 8.

(**A**) Bioinformatics pipeline used for aligning RNA-Seq reads to available abdominal transcript sequences in the *Lb17* (GAJA00000000) and *Lh14* (GAJC00000000) transcriptomes [2]. Differentially expressed transcripts in G0 and S0 *Lb17* and G0 and S0 *Lh14* were then identified (see Methods for more details).

(**B, C**) Principal component analysis of normalized counts from RNA-Seq samples of *Lb17* (B), and *Lh14* (C), obtained from ground and spaceflight conditions, as indicated.

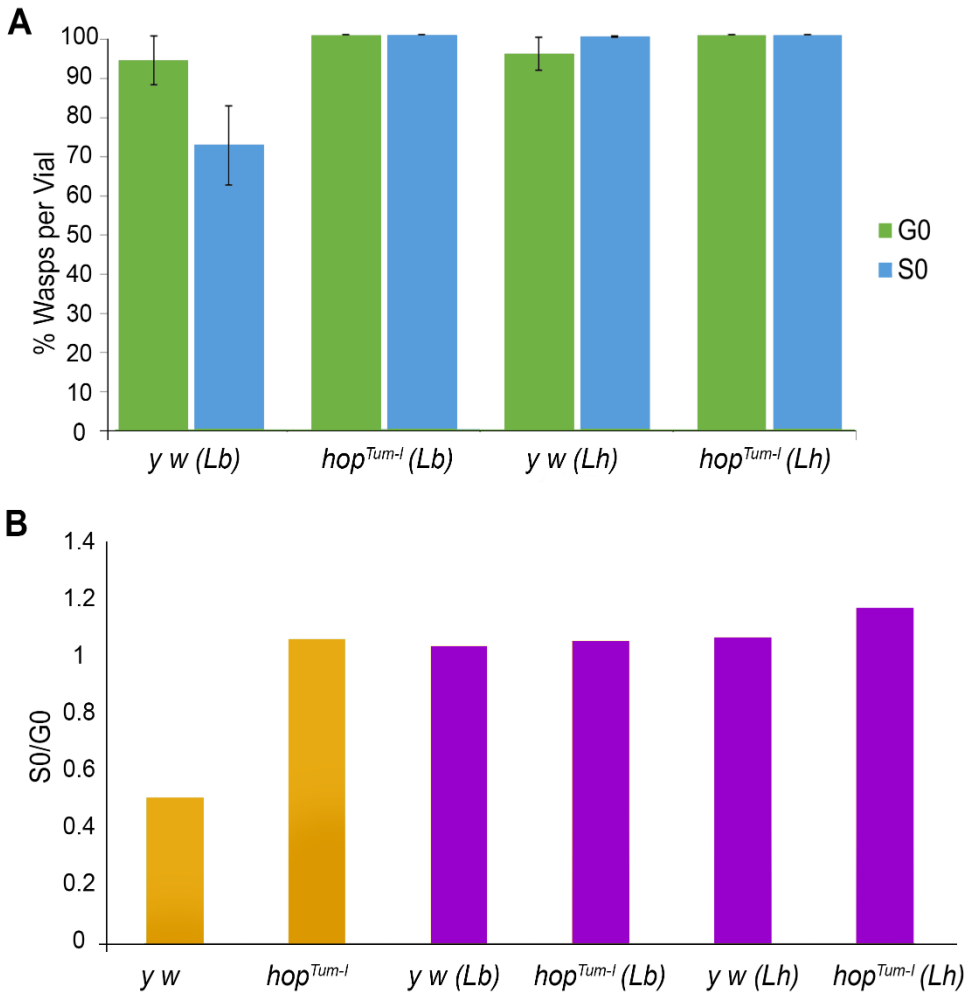

Figure S9. Survival of G0 and S0 adult flies and wasps, related to Figures 2 and 8.

(A) Percentage of wasps relative to total insects scored per vial. For raw data, see legend of Figures 2D and 2E. The p values for S0 versus G0 wasps are: *Lb17* = 0.08 and *Lh14* = 0.31 (Student t-test). No flies were observed in the *hop*$^{Tum\text{-}l}$ co-cultures. Error bars indicate standard error.

(B) Ratio of S0/G0 flies in fly-only cultures (orange) and wasps in co-cultures (purple). Insects were scored from all replicates.

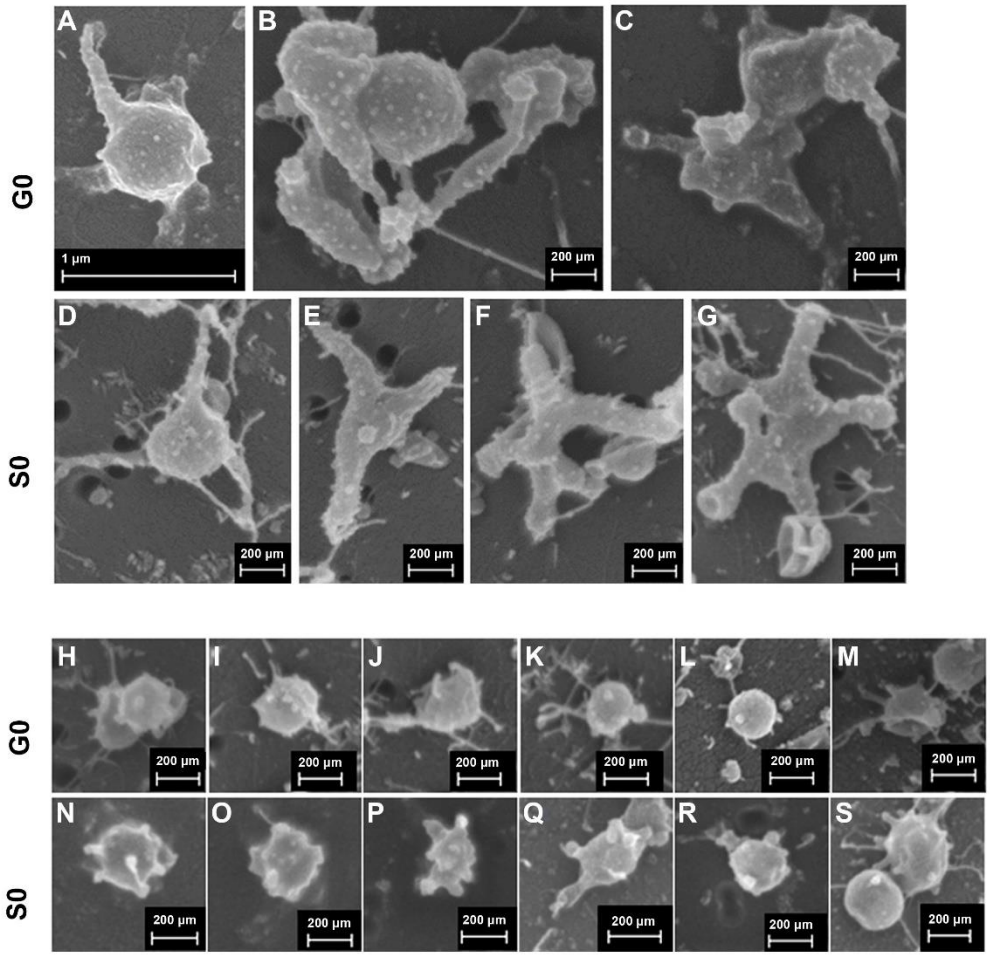

Figure S10. Extracellular vesicle-like particles from *L. boulardi* and *L. heterotoma*, related to Figures 2 and 8.

(A-S) Scanning electron micrographs of EVs from G0 *Lb* (A-C), S0 *Lb* (D-G), G0 *Lh* (H-M), and S0 *Lh* (N-S). Scale bars are as shown.

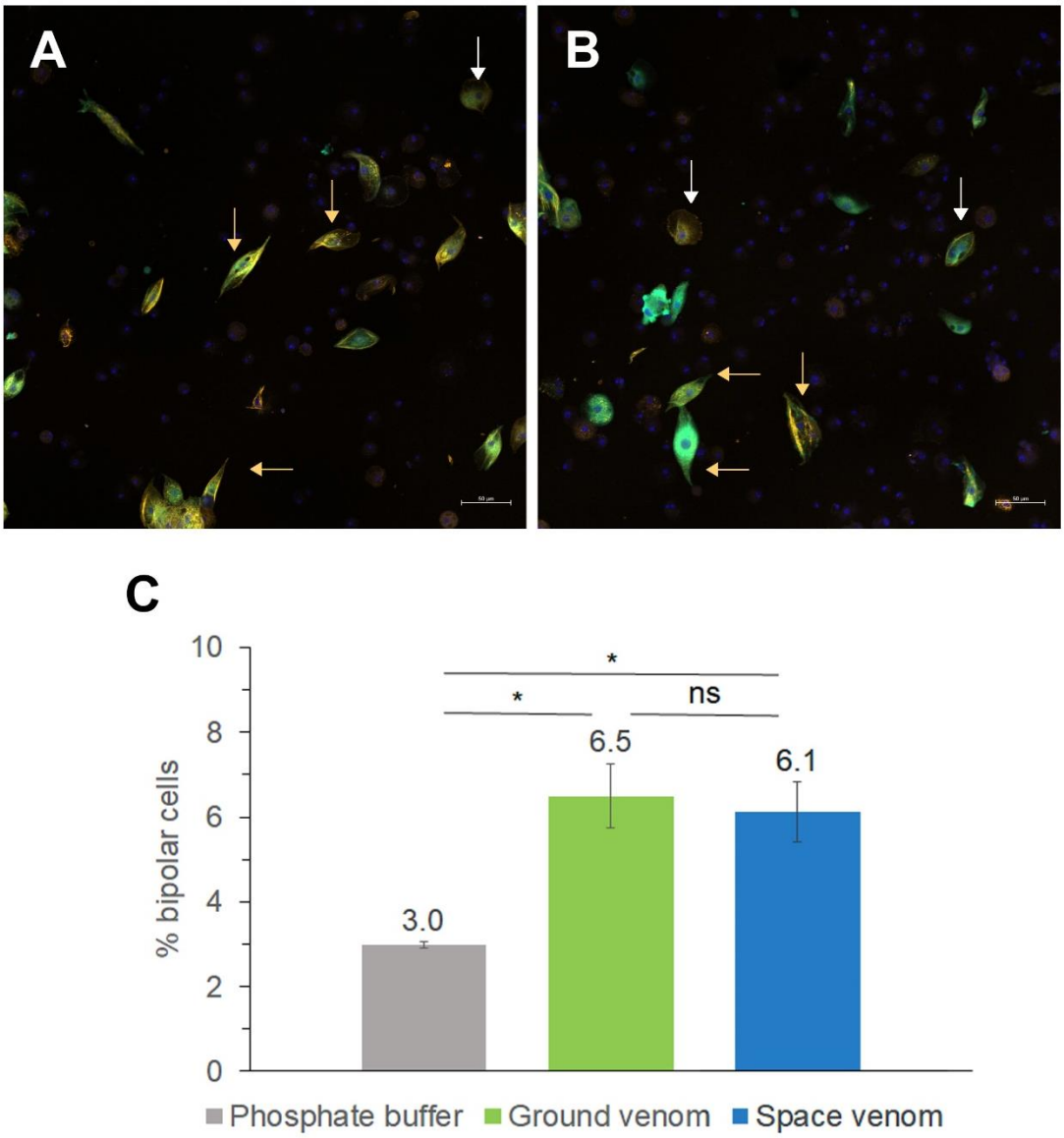

Figure S11. Assessing *L. heterotoma* venom activity *in vitro*, related to Figures 2 and 8.

(A-B) Confocal images of hemocytes from *hop*$^{Tum\text{-}l}$ *msn* > *GFP* larvae. Lamellocytes are GFP-positive; macrophages are GFP-negative. Normal *Lh* venom activity modifies a typical discoidal lamellocyte (white arrows) to assume a bipolar shape (yellow arrows). Hemocytes were incubated with venom from G0 (A) and S0 *Lh14* wasps (B), respectively. Scale bars 50 µm.

(C) Percent hemocytes with bipolar morphology after venom treatment (data are from 3-4 replicates). Differences between buffer control and G0/S0 venom activities are significant (indicated by *, Student t-test; $p = 0.02$ for both comparisons). G0/S0 venom effects on host lamellocytes are not significantly different (ns, $p = 0.73$). For phosphate buffer control, 129-347 lamellocytes were scored per replicate. The number of lamellocytes scored per replicate for G0 and S0 venom ranged from 290 to 721 and from 239 to 1,146, respectively. Error bars indicate standard error.

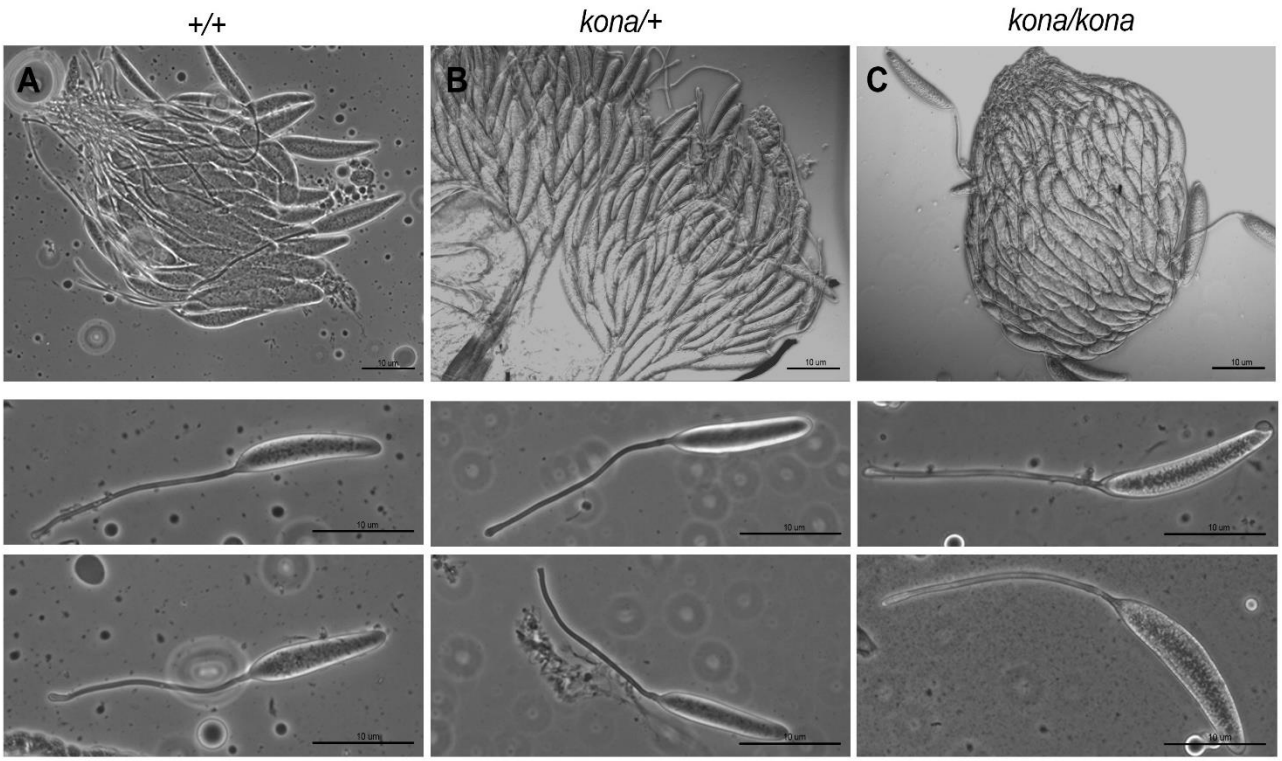

Figure S12. Ovaries from *L. heterotoma*, related to Figure 9.

(**A-C**) Ovaries and eggs from wild type (A), heterozygous (B), and homozygous *kona* mutant (C) wasps. Scale bars = 10 microns.

Table S1. Overall gene expression changes observed in naïve S0/G0 adult flies (top) and naïve S1/G1 larvae (bottom), related to Figures 3-5 and Figure S1.

The number and percent of expressed genes that are differentially expressed (DEGs, adjusted $p < 0.05$ and $|\log2FC| > 1$) and either up- or down-regulated are shown. A total of 15,628 genes were expressed in adult flies and 16,123 genes were expressed in larvae. A *Drosophila* gene was considered expressed if the sum of raw counts across all samples was > 10.

Adult Comparisons	Change	# Genes	% Genes	% DEGs
G *Tum-l* vs. G *y w*	up	1049	6.71	9.69
	down	466	2.98	
S *Tum-l* vs. S *y w*	up	1061	6.79	14.03
	down	1131	7.24	
S *y w* vs. G *y w*	up	1221	7.81	13.22
	down	845	5.41	
S *Tum-l* vs. G *Tum-l*	up	538	3.44	6.78
	down	521	3.33	

Larval Comparisons	Change	# Genes	% Genes	% DEGs
G *Tum-l* vs. G *y w*	up	1984	12.31	29.29
	down	2739	16.99	
S *Tum-l* vs. S *y w*	up	1092	6.77	10.06
	down	530	3.29	
S *y w* vs. G *y w*	up	609	3.78	5.07
	down	208	1.29	
S *Tum-l* vs. G *Tum-l*	up	2345	14.54	21.17
	down	1068	6.62	

References

1. Gramates, L.S., Agapite, J., Attrill, H., Calvi, B.R., Crosby, M.A., Dos Santos, G., Goodman, J.L., Goutte-Gattat, D., Jenkins, V.K., Kaufman, T., et al. (2022). Fly Base: a guided tour of highlighted features. Genetics *220*. 10.1093/genetics/iyac035.
2. Goecks, J., Mortimer, N.T., Mobley, J.A., Bowersock, G.J., Taylor, J., Schlenke, T.A. (2013). Integrative approach reveals composition of endoparasitoid wasp venoms. PLoS One *8*, e64125.