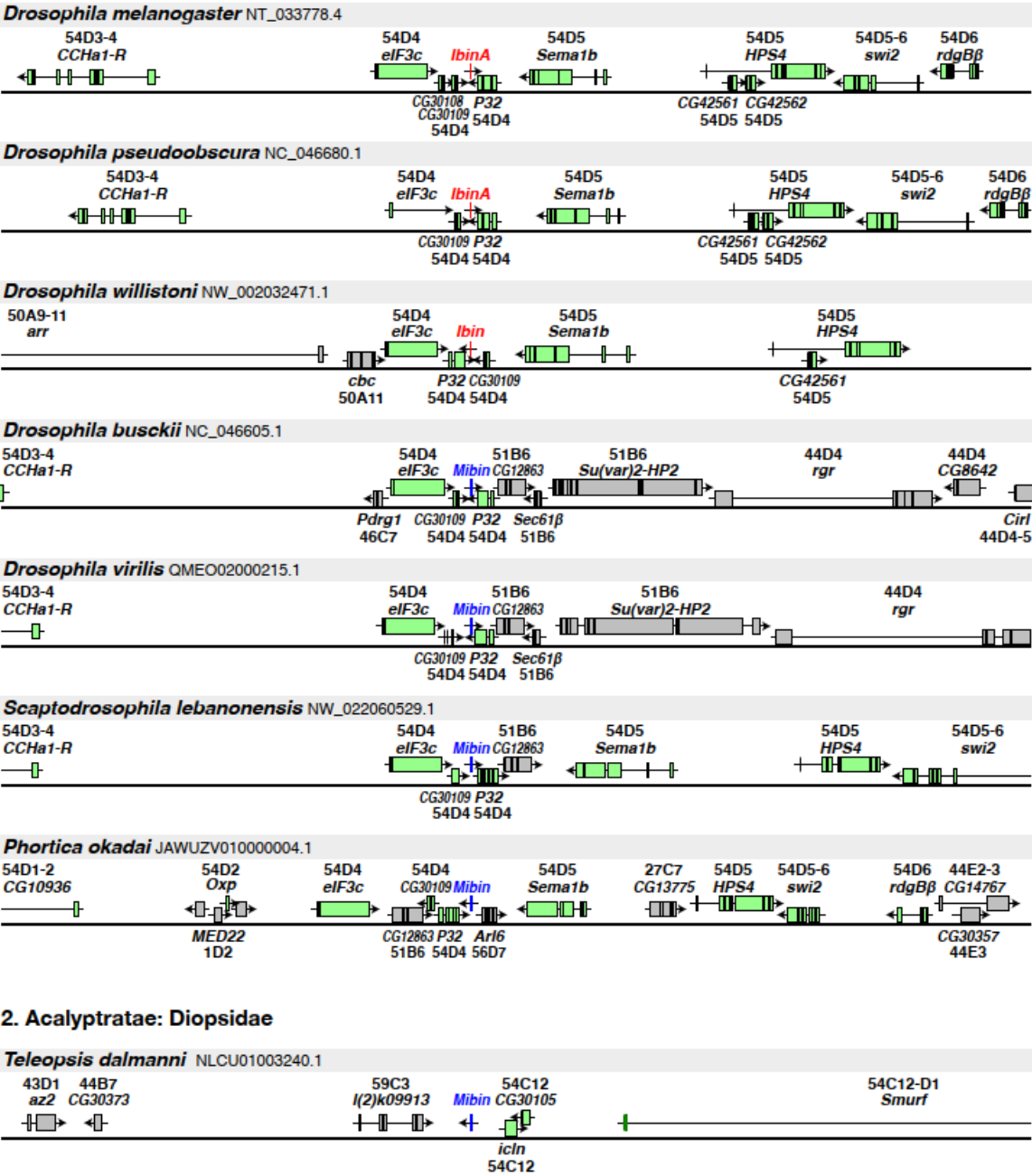


Ibin open reading frames

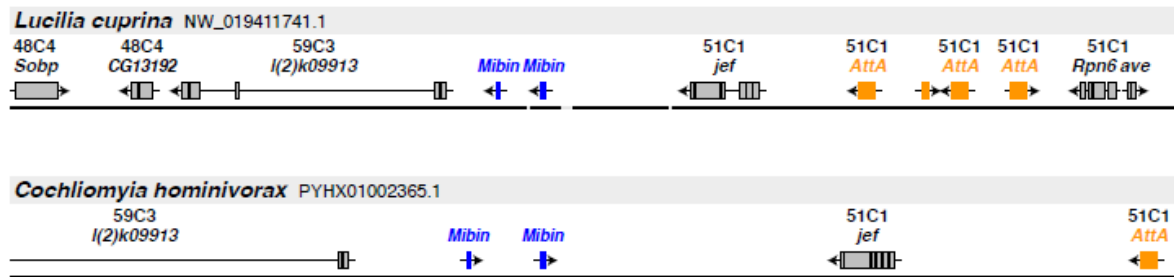


Chromosomal location of *Ibin* and *Mibin* genes in different species

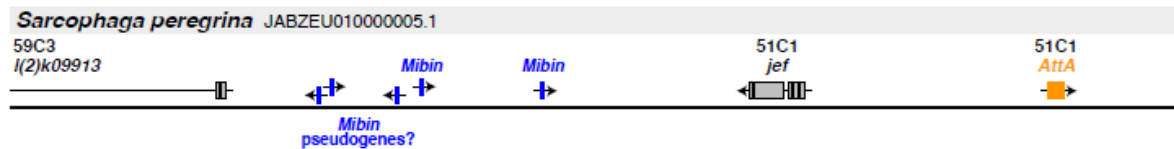
1. Acalypttratae: Drosophilidae



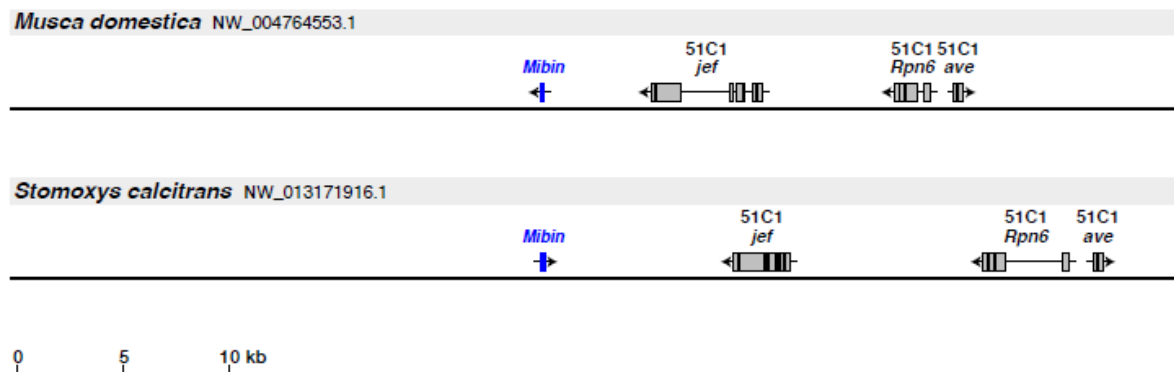
3. Calyptratae: Calliphoridae



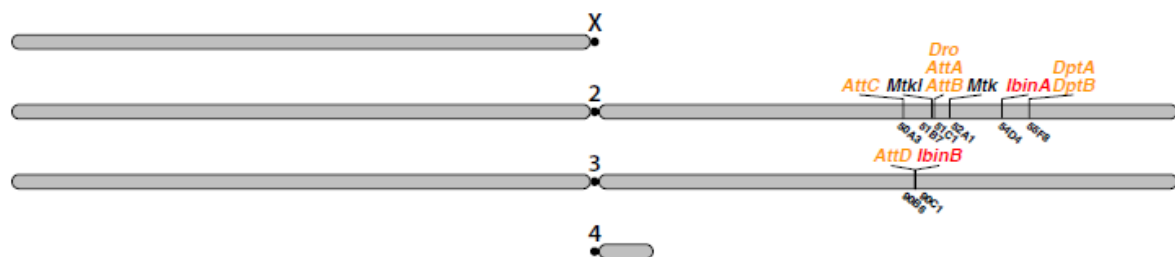
4. Calyptratae: Sarcophagidae



5. Calyptratae: Muscidae



6. Clustering of the *Ibin*, *Attacin*-related (*Att*, *Dro*, *Dpt*) and *Mtk* loci in the *D. melanogaster* genome

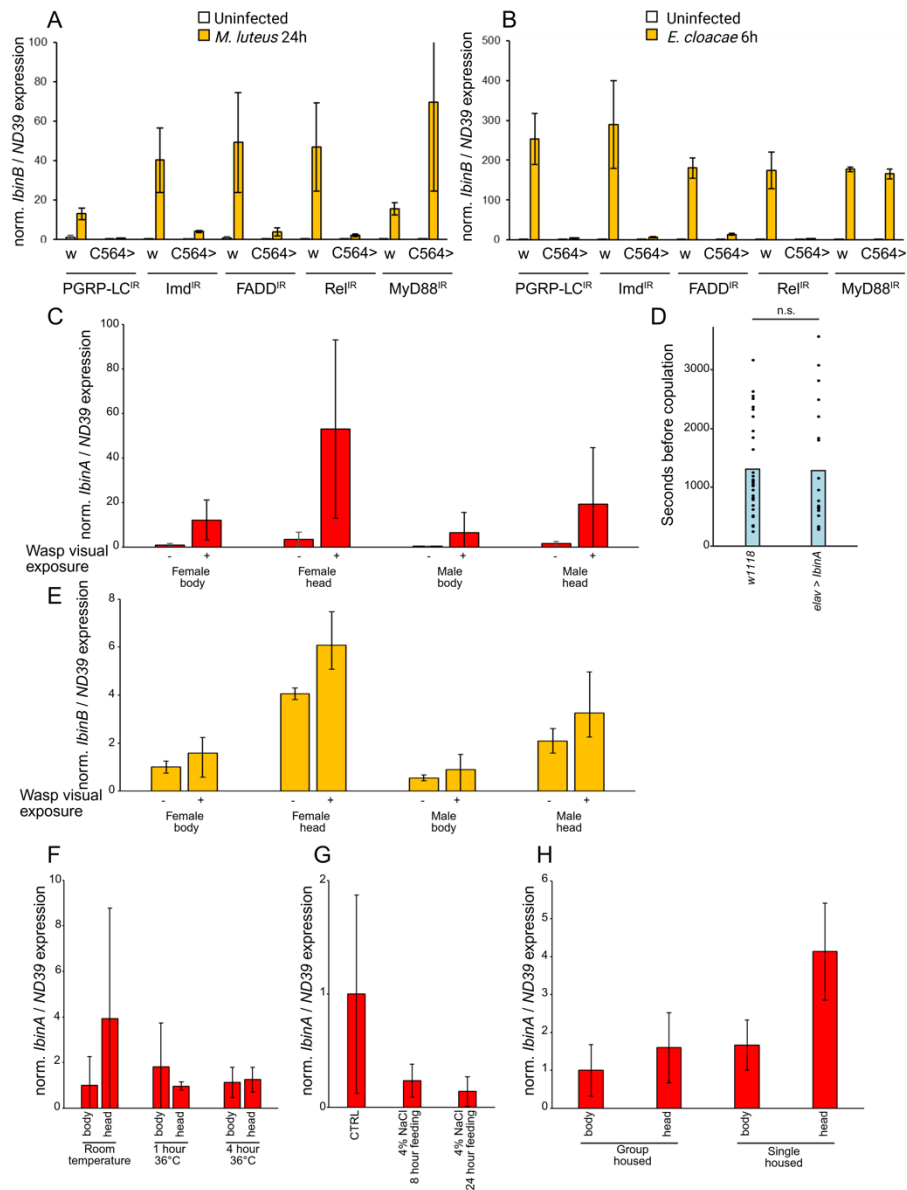


Sup. Fig. 2. Chromosomal location of *Ibin* and *Mibin* genes across species. Maps of genes around the *IbinA* and *Mibin* genes in selected acalyprate (page 1) and calyptrate (page 2) species. For each open reading frame, the name and chromosomal location of the orthologous *D. melanogaster* gene is indicated. Orthologs of genes in the near vicinity of *IbinA* in *D. melanogaster*, at 54C12-D6, are shown in green color.

Alignment of Mibin open reading frames across fly species

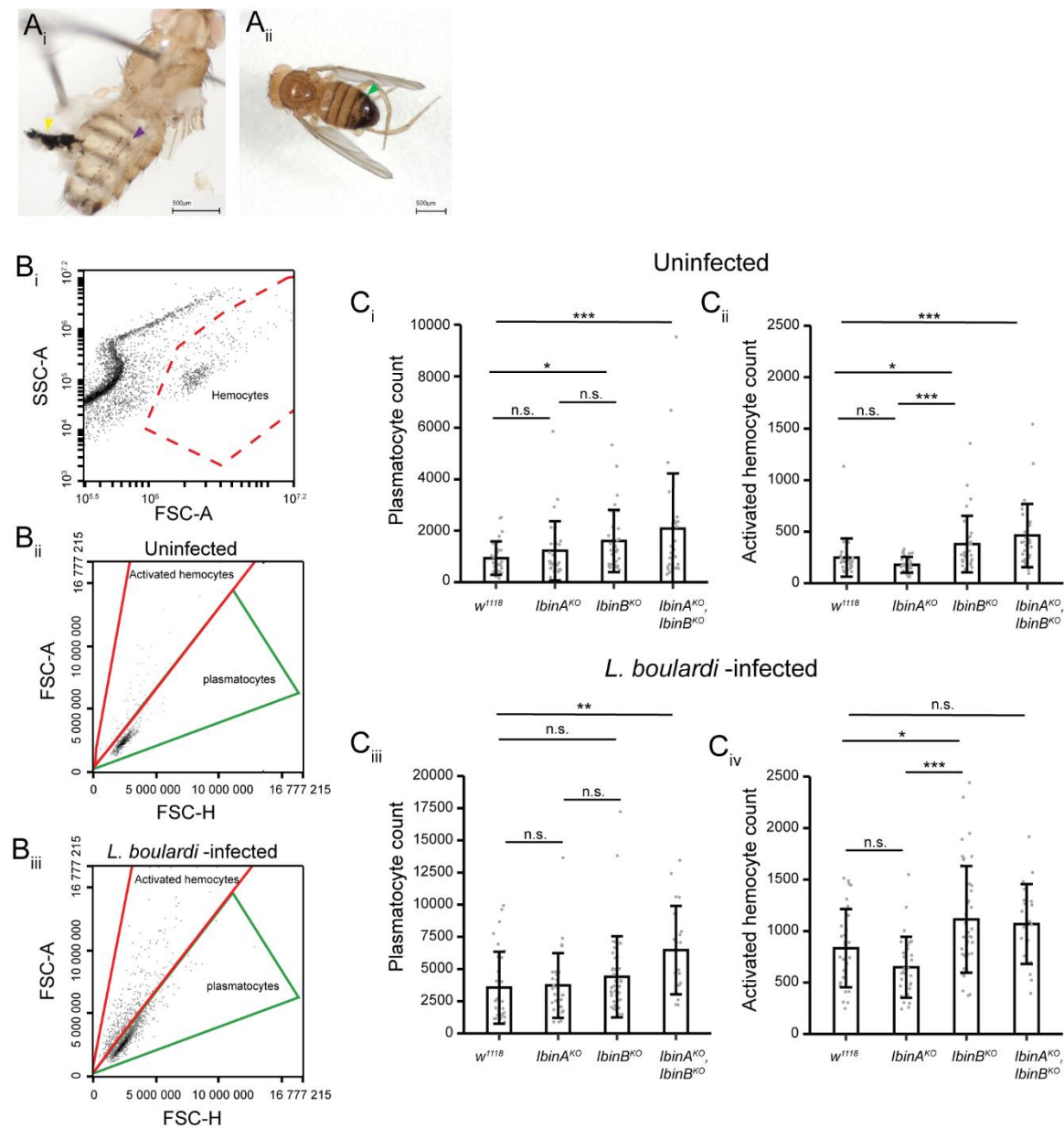


Sup. Fig. 3. Alignment of Mibin open reading frames across fly species



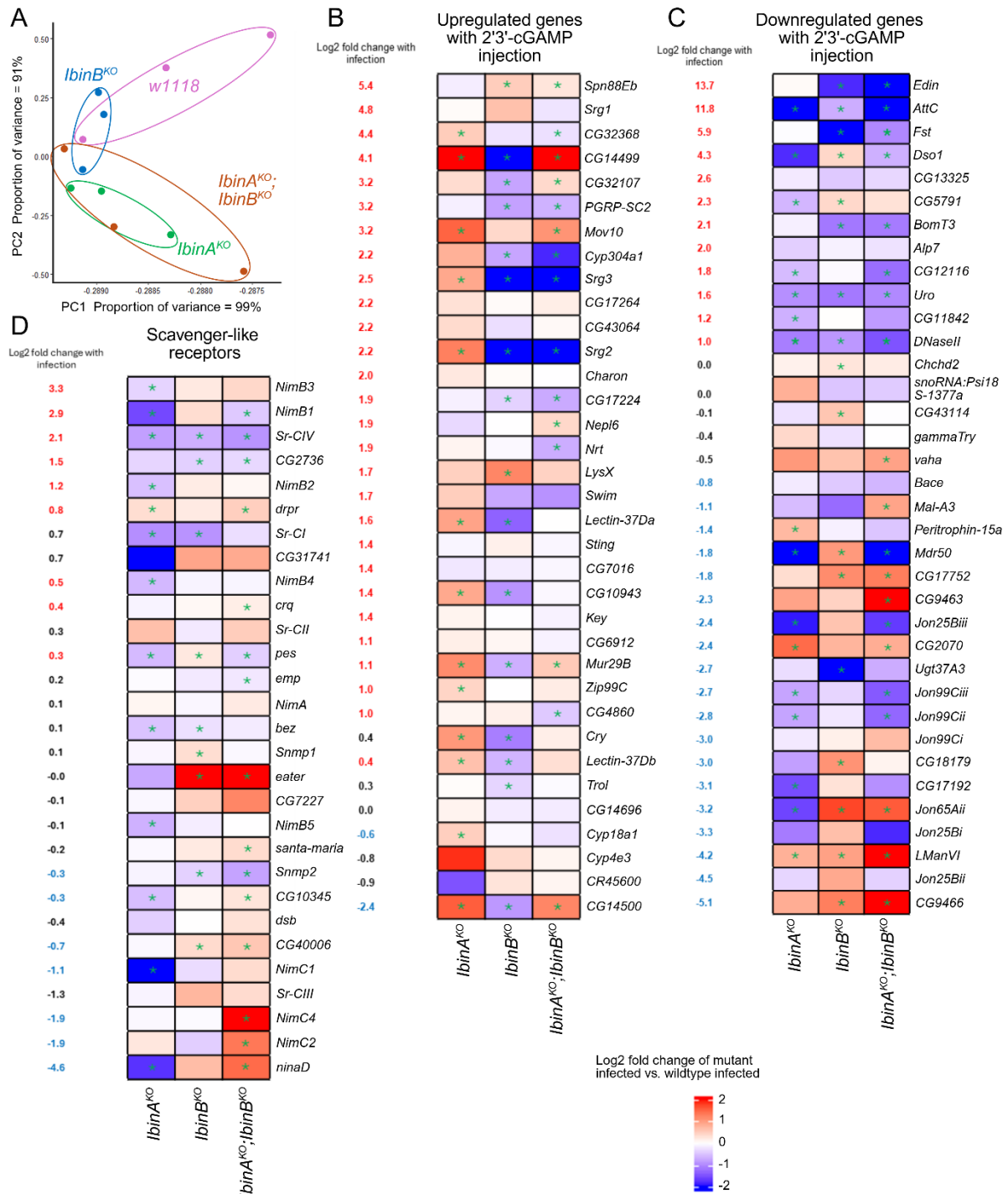
Sup. Fig. 4. *IbinB* expression is dependent on Imd components PGRP-LC, Imd, FADD and Rel, but not Toll pathway component MyD88, in the case of either *M. luteus* or *E. cloacae* infection. *IbinA* and *IbinB* do not show increased expression during physiological stress, but show higher expression during social isolation and exposure to parasitoid wasps. A) *IbinB* expression in female flies 24 hours post *M. luteus* infection with Imd and Toll pathway components knocked down. B) *IbinA* expression in female flies 24 hours post *M. luteus* infection with Imd and Toll pathway components knocked down. C) *IbinA* expression, determined by RT-qPCR, in heads and bodies of female and male flies visually exposed to *L. bouhardi* wasps in neighboring vials. D) Time to copulation of pairs of male and female *D. melanogaster* comparing flies overexpressing *IbinA* under control of a neuron-specific driver to wild type. E) *IbinB*

expression, determined by RT-qPCR, in heads and bodies of female and male flies visually exposed to *L. bouhardi* wasps in neighboring vials. **F)** *IbinA* expression in flies exposed to 36°C heat shock. **G)** *IbinA* expression following 4% NaCl feeding. **H)** *IbinA* expression in single housed (socially isolated) *D. melanogaster* vs. group housed flies. **C, E-H)** Canton-S flies were used in stress experiments.

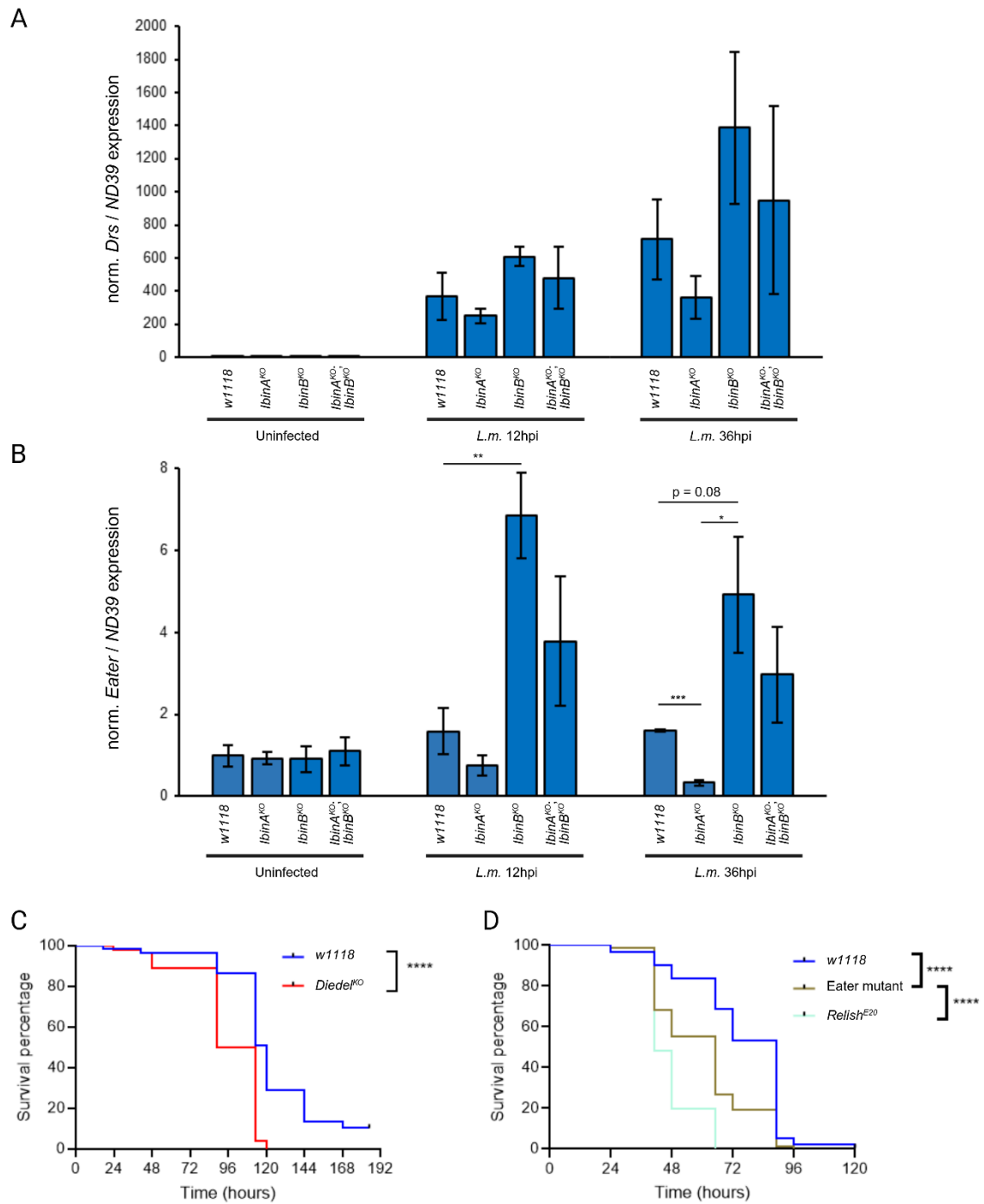


Sup. Fig. 5. Example images of *Ibin* mutant phenotype, flow cytometer gating strategy for hemocytes and hemocyte counts in *Ibin* mutant larvae. **Ai-Aii) Besides the melanized spots,**

lbin mutation also resulted in (Ai) brown coloration of cells on the dorsal side of the abdomen (purple arrowhead), putatively pericardial cells taking up melanized material and (Aii) a lack of melanization on the ventral striping pattern (green arrowhead). **Bi-Biii)** Gating strategy applied to hemocyte samples. **Bi)** Hemocytes form a population separated from cellular debris based on size and shape in the hemolymph on a forward scatter (FSC-A) vs. side scatter (SSC-A) area plot. Hemocytes in the gated area were used in subsequent analyses. **Bii-Biii)** hemocytes dissected from an uninfected (**Bii)** and *L. boulardi*-infected (**Biii)** larvae detected in the FSC-A vs. FSC-H plot. **Ci-Civ)** Quantification of hemocytes from uninfected (**Ci-Cii)** and wasp-infected (Ciii-Civ) larvae. n.s., not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



Sup. Fig. 7. Additional heatmaps of immune-relevant genes at 36hpi with *Listeria* in *IbinA^{KO}*, *IbinB^{KO}* and *IbinA^{KO};IbinB^{KO}* flies. **A) PCA plot of RNA sequencing data of uninfected samples. **B**) Heatmap of genes **B**) upregulated or **C**) downregulated when flies are injected with 2'3'-cGAMP (Hedelin et al 2024). **D**) Heatmap of scavenger receptors. **B-D**) Colors signify Log2 fold change of infected mutant flies compared to infected wildtype flies. Green asterisk signifies adjusted p value < 0.05. Log2 fold change with infection numerical values compares infected wildtype to uninfected wildtype. Red and blue numerical values signify adjusted p value < 0.05.**



Sup. Fig. 8. qPCR verification of *Eater* and *Drs* expression at 36hpi with *Listeria*. Survival of *Eater* and *Diedel* mutant flies. **A) *Drs* expression 12 and 36 hours post *L. monocytogenes* infection. **B)** *Eater* expression 12 and 36 hours post *L. monocytogenes* infection. **A-B)** For fold induction, w1118 uninfected sample was set to 1. **C)** Survival of *Diedel* mutant flies following *L. monocytogenes* infection. **D)** Survival of *Eater* mutant flies following *L. monocytogenes* infection. *Relish*^{E20} positive control. **A-D)** Statistically significant differences are marked with asterisks: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$, **** = $p < 0.0001$.**