The **raspberry** Gene Is Involved in the Regulation of the Cellular Immune Response in *Drosophila melanogaster*

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**Abstract**

*Drosophila* is an extremely useful model organism for understanding how innate immune mechanisms defend against microbes and parasitoids. Large foreign objects trigger a potent cellular immune response in *Drosophila* larva. In the case of endoparasitoid wasp eggs, this response includes hemocyte proliferation, lamellocyte differentiation and eventual encapsulation of the egg. The encapsulation reaction involves the attachment and spreading of hemocytes around the egg, which requires cytoskeletal rearrangements, changes in adhesion properties and cell shape, as well as melanization of the capsule. Guanine nucleotide metabolism has an essential role in the regulation of pathways necessary for this encapsulation response. Here, we show that the *Drosophila* inosine 5'-monophosphate dehydrogenase (IMPDH), encoded by *raspberry* (*ras*), is centrally important for a proper cellular immune response against eggs from the parasitoid wasp *Leptopilina bouardi*. Notably, hemocyte attachment to the egg and subsequent melanization of the capsule are deficient in hypomorphic *ras* mutant larvae, which results in a compromised cellular immune response and increased survival of the parasitoid.

**Introduction**

Multicellular organisms have evolved diverse defense mechanisms against pathogenic microorganisms and parasites. Cellular encapsulation of invading parasites and endogenous tumors is a phylogenetically conserved mechanism of the innate immune response. Granuloma formation in vertebrates [1, 2] and capsule formation against endoparasitoid wasps in insects [3, 4] represent special forms of the cellular immune response. Furthermore, throughout the animal kingdom, these responses involve phylogenetically conserved signaling molecules and modules [5], as well as structures showing remarkable similarities, most likely as a result of convergent evolution of interactions between hosts and parasites [6]. In recent years, *Drosophila melanogaster* has revealed itself to be an excellent model for the investigation of granuloma formation and the encapsulation reaction.
The cellular immune response of *D. melanogaster* is confronted with a wide array of pathogenic microorganisms and parasites. Prokaryotes are ingested by phagocytic hemocytes (blood cells), the plasmatocytes [7], while large foreign objects, such as eggs from endoparasitoid wasps are encapsulated by hemocytes and destroyed within melanotic capsules [3]. The encapsulation response has a well defined order. First plasmatocytes attach and spread onto the wasp egg, after which they form septate junctions and enclose the egg in a capsule [8]. This is followed by the appearance of a specialized cell type, the lamellocyte, which differentiates in the sessile tissue, in the lymph gland and in the circulation [9, 10, 11]. These large flat cells bind to the egg, and eventually form a multilayered capsule. The final step in the encapsulation reaction is melanization of the capsule, which is accompanied by formation of potentially toxic quinones and free oxygen radicals [12, 13, 14]. This reaction is catalyzed by prophenoloxidase 3 (PPO3) expressed by lamellocytes [15].

To circumvent this cellular immune response, parasitoid wasps have evolved cytotoxic components or virus particles that are injected during oviposition [16]. In the case of successful encapsulation, reaction the fly survives, conversely, if the larva is immune deficient, or the wasp is able to inhibit the encapsulation response, the fly dies [17].

Guanine nucleotides have an evolutionary conserved role in the regulation of cell proliferation, differentiation, apoptosis, and are essential for cellular signaling and trafficking. Rho-family small GTPases (i.e. Rho, Rac1, Rac2 and Cdc42) are key regulators of the encapsulation process, operating in cytoskeletal rearrangements, lamellipodia and filopodia formation, cell shape changes and migration [18, 19, 20, 21, 22]. In the Rac2 mutant, due to defective filopodia formation, the abnormal spreading of plasmatocytes and lamellocytes results in an improper encapsulation reaction [20]. Furthermore, *Drosophila* small GTPases and other factors (i.e. the JNK homolog Basket, the dJNK kinase hemipterous, the TNF homolog Eiger), are involved in the release of prophenoloxidase from crystal cells [23].

The *Drosophila* raspberry gene encodes an inosine monophosphate dehydrogenase enzyme (IMPDH), which catalyzes the rate-limiting step of de novo synthesis of guanine nucleotides and thus regulates the GTP pool [24, 25]. It is a highly conserved essential enzyme found in all eukaryotes, as well as in most prokaryotes, and catalyzes the NAD+-dependent oxidation of inosine monophosphate (IMP) to xanthosine monophosphate (XMP). Recently, it was shown that IMPDH acts as a DNA binding transcriptional repressor attenuating the expression of the cell cycle-dependent transcription factor E2f, a key driver of cell proliferation. The catalytic activity of IMPDH is not required for sequence-specific DNA binding. As a nucleotide biosynthetic enzyme and transcription factor, IMPDH maintains the balance between metabolic state and cell proliferation [26]. Due to its crucial role, IMPDH is a major drug target for immunosuppressive, antiviral and anticancer therapy [24, 27, 28, 29].

In *Drosophila*, Raspberry is involved in Rho GTPase mediated cytokinesis [30], and in the phagocytosis of *Escherichia coli*, *Candida albicans* in S2 cells [31]. Overexpression of raspberry in hemocytes causes plasmatocyte accumulation along the dorsal vessel, possibly due to changes in their adherence or migratory properties [32]. Here, we report that the *Drosophila* raspberry gene is involved in regulating the encapsulation reaction by influencing hemocyte adhesion and melanization of the capsule, as inhibition of raspberry leads to encapsulation defects and higher survival rates of the wasp *Leptopilina boulardi*.

**Materials and Methods**

**Drosophila stocks**

The *Drosophila* stocks Oregon-R, w<sup>1118</sup>, ras<sup>y</sup>, y<sup>1</sup> sc<sup>-</sup> v<sup>1</sup>; P[TRIP.HMC03250] attP2, y<sup>1</sup> v<sup>1</sup>; P [TRIP.JF01446] attP2, Rac2<sup>Δ</sup> were obtained from the Bloomington Drosophila Stock Center.
(Bloomington, Indiana, USA). The following driver lines were used: Hemese-Gal4 UAS-GFPnls (He-Gal4) [33] and msnF9mo-Gal4 [34, 35]. The flies were kept on standard cornmeal-yeast diet at 25°C.

Antibodies

The P1a and P1b antibodies recognize the NimC1 molecule on plasmatocytes, the L1a, L1b and L1c antibodies [36] react with the Atilla molecule on lamellocytes [37, 38]. The antibodies were used as neat in the form of hybridoma tissue culture supernatants. The secondary antibody was goat anti-mouse CF-568 (Sigma-Aldrich), used at 1:1000 dilution.

Immune induction with parasitoid wasp

The cell-mediated immune response was induced by using the parasitoid wasp Leptopilina boulardi strain G486. 72 h old Drosophila larvae (n = 50) were exposed to 8 female wasps and incubated for 2h at 25°C. After wasp infestation, the wasps were removed, and larvae were kept at 25°C or at 29°C.

Eclosion of the D. melanogaster and L. boulardi adults after parasitization and statistics

Forty-eight hours after parasitization the Drosophila larvae were collected and washed in Drosophila Ringer’s solution (7.5 g NaCl, 0.35 g KCl, 0.21 g CaCl2, in 1000 ml dH2O, pH 7.0) and viewed for encapsulated and melanized wasp eggs or a small melanized black spot under a stereomicroscope. The parasitized larvae were transferred into vials containing standard fly food. The pupae were counted and then monitored for eclosing flies or wasps.

After 2 hours immune induction, the raspberry RNAi knockdown larvae were transferred to standard fly food and kept at 25°C or 29°C for 5 days respectively.

The experiments were repeated at least three times, summing up at least 100 flies/genotype. For the statistical analysis the Student’s t-test was used; the p-values of <0.05 were considered as significant.

Encapsulation assay

The larvae were dissected on 12-spot microscope slides (SM-11, Hendley Essex) 48 or 72h after parasitization. The number of the live, partially encapsulated and melanized or fully encapsulated and melanized wasp larvae in each individual Drosophila larva were counted.

Immunostaining of the encapsulated wasp eggs

Forty-eight and 72 hours after wasp infestation the larvae were dissected in multiwell glass plate, in Schneider’s medium containing 5% fetal bovine serum (FBS) and 0.003% 1-phenyl-2-thiourea (PTU) (Sigma-Aldrich), washed with PBS (137 mM NaCl, 2.7 mM KCl, 6.7 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2), fixed in PBS containing 2% paraformaldehyde and washed three times in PBS for 5 min. The samples were blocked with PBS containing 0.1% bovine serum albumin (PBS-BSA) for 20 min and incubated overnight with the corresponding primary antibody. After washing three times with PBS, the CF-568 secondary antibody was added (diluted in PBS-BSA) and incubated for 45 min. The nuclei were stained with DAPI (Sigma-Aldrich). The samples were washed three times in PBS for 5 min each, mounted in Flouromount G (SouthernBiotech) and analyzed with a Zeiss AxioScope 2 MOT fluorescence microscope or a Leica confocal LSM.
Hemocyte collection, counting and statistics

Third instar wandering larvae were bled into 30 μl Drosophila Ringer’s solution containing PTU by ripping the cuticle with two fine forceps. Hemocytes were counted from at least 8 larvae of each genotype in Bürker chamber. For the statistical analysis of total hemocyte number the Student’s t-test was used; the p-values of <0.05 were considered as significant.

Immunostaining of circulating hemocytes

Larvae were dissected on 12-spot microscope slides in Schneider’s media containing 5% FBS and PTU, at the indicated time points. Hemocytes were incubated on microscope slides to adhere for 1 hour in humid chambers in Schneider’s medium containing 5% FBS, then fixed in 2% paraformaldehyde containing PBS. The samples were washed three times in PBS for 5 min and blocked with PBS-BSA for 20 min, then incubated 1 hour with the primary antibody. After washing three times with PBS, the secondary anti-mouse CF-568 antibody (diluted in PBS-BSA) was added and incubated for 45 min. The nuclei were stained with DAPI. The samples were washed three times in PBS for 5 min each, then mounted in Fluoromount G and analyzed with Zeiss Axioscope 2 MOT fluorescence microscope.

Examination of pseudopod-like cytoplasmic extensions in hemocytes

Twenty-four hours after infestation with L. boulardi, six larvae were collected from each group and bled into Schneider’s media containing 5% FBS and PTU. Hemocytes were incubated on 12-spot microscope slides for 1h, fixed in PBS containing 2% paraformaldehyde and washed three times in PBS for 5 min. The samples were blocked with 0.1% BSA and 0.01% Triton X-100 containing PBS for 20 min. The actin cytoskeleton was stained with phalloidin conjugated Atto Rho6G (Sigma-Aldrich, 1:1000 final dilution in PBS-BSA) for 45 min, then washed three times in PBS for 5 min each. The nuclei were stained with DAPI. The samples were mounted in Fluoromount G (SouthernBiotech) and analyzed with a Zeiss Axioscope 2 MOT fluorescence microscope and the proportion of filopodia promoting hemocytes was established. Three independent biological samples were analyzed. The Student’s t-test was used to calculate the significant difference from the control samples. The p-values of <0.05 were considered significant.

Results

The ras^2^ allele affects the survival rate of the Drosophila larvae after infestation

We determined the rate of survival of the ras^2^ mutant after L. boulardi G486 parasitization in the hypomorphic, fully viable raspberry allele in D. melanogaster—as the ras null mutants are lethal [39, 40]. We found that the number of the wasps eclosed from the ras^2^ mutant was significantly higher (p<0.05) from the ras^2^ pupae (Fig 1A, red column) than from the Oregon-R control. The number of ras^2^ pupae from which neither flies nor wasps emerged was significantly lower compared to wild type controls (Fig 1A, green column). We observed that the number of hatching flies was relatively low both in the mutant and the control (Fig 1A, blue column). In the non-infested Oregon-R and ras^2^, there was no significant difference in the eclosion rate (Fig 1B).

We also investigated the survival of fly versus wasp after depletion the raspberry specific mRNA transcript in blood cells. We used the msnF9mo-Gal4 [34, 35] driver active in lamellocytes and lamellocyte precursors and the Hemese-Gal4 (He-Gal4) [32] driver, which is active in 80% of hemocytes including plasmatocytes and lamellocytes to silence ras by two independent
ras RNAi constructs (y¹ sc¹ v¹; P[TRIP.HMC03250] attP2 and y¹ v¹; P[TRIP.JF01446] attP2) at 25°C (S1 and S2 Figs) and 29°C. (Fig 1C and 1D, S3 Fig). We monitored the eclosion of flies or wasps and found that significantly more wasps hatched from the msnF9mo-Gal4>P[TRIP.JF01446] progeny compared to the parental lines (P<0.01) (Fig 1C). In the case of the other raspberry RNAi line (y¹ sc¹ v¹; P[TRIP.HMC03250] attP2)), the difference was also significant compared to the msnF9mo-Gal4/+ (p > 0.05) (Fig 1D). Similar results were obtained at 25°C using the msnF9mo-Gal4 driver (S1 Fig) or by using the He-Gal4 driver line at 25°C and 29°C (S2 and S3 Figs).

**raspberry** is involved in encapsulation and melanization reactions

We investigated the phenotype and number of capsules in *D. melanogaster* after parasitization with *L. boulardi* G486. We counted non-encapsulated living wasp larvae, partially encapsulated

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Fig 1. The eclosion of *D. melanogaster* versus *L. boulardi* G486 in ras² mutant and after RNAi silencing. (A) The eclosion of flies (blue) and wasps (brown) from the ras² mutant or from the Oregon-R after parasitization and from the (B) non-infested control. (C, D) Number of hatching flies from the *L. boulardi* G486 infested progenies (msnF9mo-Gal4>P[TRIP.JF01446] and msnF9mo-Gal4>P[TRIP.HMC03250] attP2)) and parental lines (P[TRIP.JF01446]/+ and msnF9mo-Gal4/+). The numbers in parenthesis indicate the number of the examined *D. melanogaster* pupae. The error bars indicate the standard error of the mean. *p<0.05, **p<0.01.

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and melanized or completely melanized wasp eggs (Fig 2A) in the hemocoel of the \( ras^2 \) and Oregon-R larvae.

Fig 2. The phenotype and proportion of living wasp larvae, partially or completely encapsulated and melanized wasp eggs in \( ras^2 \) mutant or after RNAi silencing. (A) The phenotypic categories of \( L. \text{boulardi} \) G486 larvae and capsules in \( D. \text{melanogaster} \) larvae after parasitization. Proportion of living wasp larvae (blue) and partially (red) or completely melanized (green) capsules in the \( ras^2 \) mutant or in the Oregon-R control (B, C) and from the parental lines \( \{\text{TRIP}.JF01446/+, \text{TRIP} \cdot \text{HMC03250}/+ \text{and msnF9mo/Gal4+/} \} \) and progenies \( \{\text{TRIP} \cdot \text{JF01446}/ \text{msnF9mo/Gal4} \} \) 72h after the \( L. \text{boulardi} \) G486 infestation. The numbers in parenthesis indicate the number of the examined \( Drosophila \) larvae. The error bars indicate the standard error of the mean. *\( p<0.05 \), **\( p<0.01 \), ***\( p<0.001 \).

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Seventy-two hours after oviposition, we counted the total number of parasitoids in the \textit{ras2} mutant and in the \textit{Oregon-R} larvae, and we found no significant difference in the number of parasitoids, which indicates that \textit{L. boulardi} G486 has no preference for depositing eggs into the \textit{ras2} or the \textit{Oregon-R} larvae. At the same time, we detected significantly higher number of living or partially encapsulated and melanized wasp larvae and significantly lower number of completely melanized eggs in the \textit{ras2} mutant compared to the \textit{Oregon-R} control after 48h (S4 Fig) or 72h following oviposition (Fig 2A). Similarly, using two different \textit{ras} RNAi constructs driven by the \textit{msnF9mo-Gal4} driver, we detected a significantly higher frequency of living wasp larvae in the progenies than in the parental lines 48h (S5 Fig) or 72h (Fig 2B and 2C) after oviposition. We obtained similar results using the hemocyte specific \textit{He-Gal4} driver to knockdown \textit{ras} expression (S6 and S7 Figs).

Incomplete lamellocyte adherence to the capsule in the \textit{ras2} mutant

We studied the morphological features of the capsules formed around the parasitoid egg by staining the capsule with the lamellocyte specific Atilla antibody. In \textit{Oregon-R}, the hemocytes formed a tight, continuous sheet around the parasite egg (Fig 3A) with strong melanization masking the nuclear staining, while in the \textit{ras2} mutant the lamellocytes formed a loose network around the egg (Fig 3B), accompanied with marginal melanization 72h after infestation (Fig 3C). We obtained similar results after RNAi silencing by driving the two different RNAi lines with the \textit{He-Gal4} driver line (Fig 3D and 3E), and with the lamellocyte specific \textit{msnF9mo-Gal4} driver (S8 Fig).

The number and morphological features of the circulating hemocytes in the \textit{ras2} mutant

Proliferation and differentiation are GTP-dependent processes in lymphocytes [41], and the IMPDH catalyzes the rate-limiting step of \textit{de novo} synthesis of guanine nucleotides [24]. Therefore we tested whether the observed defect in capsule formation in the \textit{ras2} mutant could
be the result of a decreased or abnormal hemocyte count in the hemolymph. Hemocyte counts of infested and non-infested Oregon-R and ras2 lines were determined, and the lamellocytes of the infested larvae were visualized. We found that the number of hemocytes (Fig 4A) was comparable and the number, as well as the morphological characteristics of circulating lamellocytes were the same (Fig 4B and 4C).

**raspberry** is involved in the formation of pseudopod-like cytoplasmic extensions in hemocytes

After immune induction, hemocytes undergo functional and morphological changes. As Rac2 has a role in these alterations [20], we tested ras2 mutants for morphological changes using Oregon-R and Rac2Δ as controls. In the ras2 and ras2; Rac2Δ larvae, hemocytes attached to the microscope slide were round with smooth margins and larger in size as compared to the Oregon-R, which had many pseudopod-like cytoplasmic extensions that show actin staining 24h after the immune induction (Fig 5). We found that the hemocytes of Oregon-R larvae had significantly more filopodia (7.2 ±2.9) compared to the ras2 (3.9±1.3) (p<0.01) and ras2; Rac2Δ (4.3±2.1) (p<0.01), but this difference was not significant compared to the Rac2Δ (5.6±4.0). In the ras2; Rac2Δ double mutant, the proportion of the extension forming plasmatocytes was lower compared to the Rac2Δ (Fig 5A), which suggests that ras is epistatic over Rac2. The length of the filopodia was similar in the Oregon-R, ras2, Rac2Δ and ras2; Rac2Δ (1–3 μm).

**Discussion**

The hemocytes of Drosophila melanogaster larvae form tightly compact melanized capsules around parasitoid wasp eggs. This cellular encapsulation reaction has long been studied in D. melanogaster and in other Drosophila species [3, 6], however not much is known about the regulation of this defense mechanism. Small GTPases Rac1, Rac2 and the Jun N-terminal kinase Basket were found to be required for the proper encapsulation reaction against the endoparasitoid wasp L. boulardi [20, 21]. Additionally, the Drosophila βPS-integrin Myospheroid is necessary for hemocytes to adhere to the cellular capsule surrounding L. boulardi eggs, and Rac1 is required for the topographic localization of Myospheroid in the cell membrane [42]. In a directed genetic screen, several genes were isolated that are related to encapsulation defects. The mammalian homologues of many of these genes are involved in wound healing, cellular adhesion and thrombosis [43].

In a screen [44] for genes involved in the regulation of cellular immune responses, we isolated a **raspberry** mutant (ras2) that shows significantly decreased chances for survival of the
Drosophila melanogaster host after parasitisation with the parasitoid wasp Leptopilina boulardi. The \textit{ras}^{2} mutant has a 5.0 kb insert in the \textit{raspberry} gene [39]. We localized the site of the insertion in the second intron (data not shown). We found significantly higher number of capsules with loosely attached lamellocytes and improper melanization compared to the tight, compact and melanized capsules of the \textit{Oregon-R} control. This shows that both the adhesion of lamellocytes and the melanization are affected by the mutation or, alternatively, improper lamellocyte attachment may inhibit melanization. To validate our results, we also used two independent \textit{ras} RNAi constructs. We found that the two RNAi lines showed different efficiency, which may be due to differences in their genetic backgrounds. Both \textit{ras} RNAi lines were able to mimic the mutant dark red eye color phenotype when driven with the \textit{lozenge-Gal4} driver (data not shown), which indicated that the RNAi lines were fully functional. We noticed similar phenotypes after knocking down the \textit{raspberry} transcript in hemocytes with the \textit{msnF9mo-Gal4} and the \textit{He-Gal4} drivers similarly to Bausek and Zeidler [45] after reduction of Ga73B levels in a \textit{HopT42} background. The lower Ga73B gene dose, compared to the wild type pretumors, prevented the formation of the tightly bound cell mass and resulted in the formation of loosely associated cell clumps, which were not able to develop and generate the melanized tumors visible in adults. These defects were not associated with a decrease in hemocyte number or abnormal lamellocyte morphological features. Ga73B is a GTP binding protein, the $\alpha$ subunit of the heterotrimeric G proteins and a novel JAK/STAT pathway gene involved in the JAK/STAT-mediated tumor formation. The JAK/STAT pathway is also involved in the proper encapsulation response [46, 47].
The regulation of the actin-based membrane protrusions, such as lamellipodia and filopodia, require coordinated events in cytoskeletal remodeling. Central to this process are the small GTPases [8, 20, 48]. Incomplete encapsulation reactions, similar to those we have observed in the \( ras^2 \) mutant, were described in the \( Rac2^\Delta \) mutant, in which plasmatocytes and lamellocytes adhered to the parasitoid egg fail to spread and there is a failure of melanization [20]. Rac2 is a member of the Ras small GTPase superfamily involved in the formation of filopodia and lamellipodia [49, 50]. Filopodia formation of hemocytes in the \( ras^2 \) and in the \( Rac2^\Delta \) mutant was defective, suggesting that similarly to the \( D. melanogaster \) Rac2 [20], myospheroid [42] and TM9SF4 [51], the Drosophila raspberry gene is also involved in the encapsulation reaction via regulation of filopodia or lamellocypodia formation. The lower proportion of filopodia forming plasmatocytes in the \( ras^2; Rac2^\Delta \) double mutant suggests that \( ras^2 \) has an epistatic effect on \( Rac2^\Delta \). Interestingly, it was shown that inhibition of the de novo GMP synthesis pathway has a strong effect on small GTPase function, including Rho GTPases such as Rac2 [52, 53]. Additional evidence for the involvement of de novo synthesis of guanine nucleotides in cellular processes was found in axon guidance too [54], where the Burgundy catalyzes the final reaction of the de novo GMP synthesis, while Raspberry catalyzes the first step of the same pathway both in neurons [54] and in hemocytes.

Possible explanation for the role of raspberry in the encapsulation reaction is that it encodes the rate-limiting enzyme for GTP synthesis and thus influences the function of enzymes requiring GTP. It is known that G proteins and small GTPases are involved in the regulation of processes related to the immune response. However, further studies must be conducted to elucidate the exact pathways affected in the case of decreased IMPDH level in the encapsulation reaction.

**Supporting Information**

**S1 Fig. The eclosion of \( D. melanogaster \) versus \( L. boulardi \) G486 from RNAi silenced pupae.** Two independent RNAi line were used (y\(^1\) sc\(^+\) v\(^1\); P[TRIP.HMC03250] attP2 and y\(^1\) v\(^1\); P[TRIP.JF01446] attP2) driven by msnF9mo-Gal4 driver line. The eclosion rate was monitored at 25°C. The numbers in parenthesis indicate the number of the examined \( D. melanogaster \) pupae. The error bars indicate the standard error of the mean. *\( p < 0.05 \), **\( p < 0.01 \).

(TIF)

**S2 Fig. The eclosion of \( D. melanogaster \) versus \( L. boulardi \) G486 from RNAi silenced pupae.** Two independent RNAi line were used (y\(^1\) sc\(^+\) v\(^1\); P[TRIP.HMC03250] attP2 and y\(^1\) v\(^1\); P[TRIP.JF01446] attP2) driven He-Gal4 driver line. The eclosion rate was monitored at 25°C. The numbers in parenthesis indicate the number of the examined \( D. melanogaster \) pupae. The error bars indicate the standard error of the mean. *\( p < 0.05 \).

(TIF)

**S3 Fig. The eclosion of \( D. melanogaster \) versus \( L. boulardi \) G486 from RNAi silenced pupae.** Two independent RNAi line were used (y\(^1\) sc\(^+\) v\(^1\); P[TRIP.HMC03250] attP2 and y\(^1\) v\(^1\); P[TRIP.JF01446] attP2) driven He-Gal4 driver line. The eclosion rate was monitored at 29°C. The numbers in parenthesis indicate the number of the examined \( D. melanogaster \) pupae. The error bars indicate the standard error of the mean. *\( p < 0.01 \).

(TIF)

**S4 Fig. The proportion of living, partially or completely encapsulated and melanized wasp larvae.** The encapsulation efficiency was examined at 48h following the wasp infestation. The numbers in parenthesis indicate the number of the examined \( D. melanogaster \) larvae. The error bars indicate the standard error of the mean. *\( p < 0.05 \).

(TIF)
S5 Fig. The proportion of living, partially or completely encapsulated and melanized wasp larvae in raspberry RNAi silenced Drosophila larvae. Two independent RNAi lines were used driven by msnF9mo-Gal4. The encapsulation efficiency was examined at 48h following the wasp infestation. The numbers in parenthesis indicate the number of the examined D. melanogaster larvae. The error bars indicate the standard error of the mean. **p<0.01, ***p<0.001. (TIF)

S6 Fig. The proportion of living, partially or completely encapsulated and melanized wasp larvae in raspberry RNAi silenced Drosophila larvae. Two independent RNAi lines were used driven by He-Gal4. The encapsulation efficiency was examined at 48h following the wasp infestation. The numbers in parenthesis indicate the number of the examined D. melanogaster larvae. The error bars indicate the standard error of the mean. *p<0.05, ***p<0.001. (TIF)

S7 Fig. The proportion of living, partially or completely encapsulated and melanized wasp larvae in raspberry RNAi silenced Drosophila larvae. Two independent RNAi lines were used driven by He-Gal4. The encapsulation efficiency was examined at 72h following the wasp infestation. The numbers in parenthesis indicate the number of the examined D. melanogaster larvae. The error bars indicate the standard error of the mean. **p<0.01, ***p<0.001. (TIF)

S8 Fig. The proportion of melanized wasp eggs with tight or loose capsule structure in msnF9mo-Gal4/UAS-rasRNAi larvae. The numbers in parenthesis indicate the number of the examined partially or completely encapsulated and melanized wasp eggs at 72h following the immune induction. **p<0.01. (TIF)

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Author Contributions

Conceived and designed the experiments: BK VH IA EK. Performed the experiments: BK G. Csordás VH G. Cinege IA EK. Analyzed the data: BK G. Csordás VH G. Cinege MJW IA EK. Contributed reagents/materials/analysis tools: BK G. Csordás VH IA EK. Wrote the paper: BK MJW IA EK.

References

1. Adams DO. The granulomatous inflammatory response. Am J Pathol. 1976; 84:164–192. PMID: 937513
2. Sarraf P, Sneller MC. Pathogenesis of Wegener’s granulomatosis: current concepts. Expert Rev Mol Med. 2005; 7:1–19.
3. Carton Y, Nappi AJ. Drosophila cellular immunity against parasitoids. Parasitol Today. 1997; 13:218–27. PMID: 15275074
4. Carton Y, Nappi AJ, Poirie M. Genetics of anti-parasite resistance in invertebrates. Dev Comp Immunol. 2005; 29:9–32. PMID: 15325520
5. Hoffmann JA. The immune response of Drosophila. Nature. 2003; 426:33–8. PMID: 14603309
6. Márkus R, Lerner Z, Honi V, Csordás G, Zsámboi J, Cinege G, et al. Multinucleated giant hemocytes are effector cells in cell-mediated immune responses of Drosophila. J Innate Immun. 2015; 7:340–53. doi: 10.1159/000369618 PMID: 25659047
7. Stuart LM, Ezekowitz RA. Phagocytosis: elegant complexity. Immunity, 2005; 22:539–50. PMID: 15894272
8. Williams MJ, Habayeb MS, Hultmark D. Reciprocal regulation of Rac1 and Rhox in Drosophila circulating immune surveillance cells. J Cell Sci. 2007; 120:502–11. PMID: 17227793
9. Márkus R, Laurinyecz B, Kurucz É, Honti V, Bajusz I, Sipos B, et al. Sessile hemocytes as a hematopoietic compartment in Drosophila melanogaster. Proc Natl Acad Sci USA. 2009; 106:4805–9. doi: 10.1073/pnas.0817661106 PMID: 19261847
10. Crozatier M, Meister M. Drosophila haematopoeisis. Cell Microbiol. 2007; 9:1117–26. PMID: 17394559
11. Stofanko M, Kwon SY, Badenhorst P. Lineage tracing of lamellocytes demonstrates 

9. doi:10.1371/journal.pone.0014051 PMID: 2124962
12. Russo J, Dupas S, Frey F, Carton Y, Brehelin M. Insect immunity: early events in the encapsulation process of parasitoid (Leptopilina boulardi) eggs in resistant and susceptible strains of Drosophila. Parasitology. 1996; 112:135–42. PMID: 8587797
13. Söderhäll K, Cerenius L. Role of prophenoloxidase-activating system in invertebrate immunity. Curr Opin Immunol. 1998; 10:23–8. PMID: 9523106
14. Nappi AJ, Christensen BM. Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. Insect Biochem Mol Biol. 2005; 35:443–59. PMID: 15804578
15. Dudzic JP, Kondo S, Ueda R, Bergman CM, Lemaitre B. Drosophila innate immunity: regional and functional specialization of prophenoloxidases. BMC Biol. 2015; 13.81. doi: 10.1186/s12915-015-0193-6 PMID: 26437768
16. Dubuffet A, Dupas S, Frey F, Drezen JM, Poirie M, Carton Y. Genetic interactions between the parasitoid wasp Leptopilina boulardi and its Drosophila hosts. Heredity. 2007; 98:21–7. PMID: 17039553
17. Fleury F, Ris N, Fouillet P, Carton Y, Bouletreau M. Genetic and ecological interactions in Drosophila-Parasitoids communities: a case study with Drosophila melanogaster, D. simulans and their Leptopilina species. Genetica. 2004; 120:181–94. PMID: 15086657
18. Burridge K, Wennerberg K. Rho and Rac take center stage. Cell. 2004; 116:167
19. Raftopoulou M, Hall A. Cell migration: Rho GTPases lead the way. Dev Biol. 2004; 265:23–32. PMID: 14697350
20. Williams MJ, Andó I, Hultmark D. Drosophila melanogaster Rac2 is necessary for a proper cellular immune response. Genes Cells. 2005; 10:813–23. PMID: 16098145
21. Williams MJ, Wiklund ML, Wikman S, Hultmark D. Reciprocal regulation of Rac1 and Rho1 in Drosophila larval cellular immune response. J Cell Sci. 2006; 119:2015–24. PMID: 16621891
22. Williams MJ. Drosophila hemopoiesis and cellular immunity. J. Immunol. 2007; 178:4711–6. PMID: 17404248
23. Bidla G, Dushay MS, Theopold U. Crystal cell rupture after injury in Drosophila requires the JNK pathway, small GTPases and the TNF homolog Eiger. J Cell Sci. 2007; 120:1209–15. PMID: 17356067
24. Hedstrom L. IMP dehydrogenase: structure, mechanism, and inhibition. Chem Rev. 2009; 109:2903–28. doi: 10.1021/cr900021w PMID: 19480389
25. Slee R, Bownes M. The raspberry locus encodes Drosophila inosine monophosphate dehydrogenase. Mol Gen Genet. 1995; 248:755–66. PMID: 7478679
26. Kozhevnikova EN, van der Knaap JA, Pindyurin AV, Ozgur Z, van Ijcken WF, Moshkin YM, Verrijzer CP. Metabolic enzyme IMPDH is also a transcription factor regulated by cellular state. Mol Cell. 2012; 47:133–9. doi: 10.1016/j.molcel.2012.04.030 PMID: 2268723
27. Ratcliffe AJ. Inosine 5′-monophosphate dehydrogenase inhibitors for the treatment of autoimmune diseases. Curr Opin Drug Discov Devel. 2006; 9:595–605. PMID: 17002220
28. Chen L, Pankiewicz K. Recent development of IMP dehydrogenase inhibitors for the treatment of cancer. Curr Opin Drug Discov Devel. 2007; 10:403–12. PMID: 17659481
29. Shu Q, Nair V. Inosine monophosphate dehydrogenase (IMPDH) as a target in drug discovery. Med Res Rev. 2008; 28:219–32. PMID: 17480004
30. Gregory SL, Shandala T, O'Keefe L, Jones L, Murray MJ, Saint R. A Drosophila overexpression screen for modifiers of Rho signalling in cytokinesis. Fly (Austin). 2007; 1:13–22.
31. Stroschein-Stevenson SL, Foley E, O'Farrell PH, Johnson AD. Identification of Drosophila gene products required for phagocytosis of Candida albicans. PLoS Biol. 2006; 4;e4. PMID: 16336044
32. Stofanko M, Kwon SY, Badenhorst P. A misexpression screen to identify regulators of Drosophila larval hemocyte development. Genetics. 2008; 180:253–67. doi: 10.1534/genetics.108.089094 PMID: 18757933
33. Zettervall CJ, Anderl I, Williams MJ, Palmer R, Kurucz É, Andó I, et al. A directed screen for genes involved in *Drosophila* blood cell activation. Proc Natl Acad Sci U S A. 2004; 101:14192–7. PMID: 15381778

34. Tokusumi T, Sorrentino RP, Russell M, Ferrarese R, Govind S, Schulz RA. Characterization of a lamellocyte transcriptional enhancer located within the misshapen gene of *Drosophila melanogaster*. PLoS One. 2009a; 4:e6429.

35. Tokusumi T, Shoue DA, Tokusumi Y, Stoller JR, Schulz RA. Characterization of a lamellocyte specific enhancer-reporter transgenes for the analysis of hematopoiesis in *Drosophila*. Genesis. 2009b; 47:771–4.

36. Kurucz É, Vácz B, Márkus R, Laurinyecz B, Vilmos P, Zsámboki J, et al. Definition of *Drosophila* hemocyte subsets by cell-type specific antigens. Acta Biol Hung. 2007a; 58:115–111.

37. Kurucz É, Márkus R, Zsámboki J, Folkli-Medzihradszky K, Darula Z, Vilmos P, et al. Nimrod, a putative phagocytosis receptor with EGF repeats in *Drosophila* plasmacocytes. Curr Biol. 2007b; 17:649–54.

38. Honti V, Kurucz É, Csordás G, Laurinyecz B, Márkus R, Andó I. In *Drosophila* melanogaster. Immunol Lett. 2009; 126:83–4. doi: 10.1016/j.imlet.2009.08.004 PMID: 19695290

39. Nash D, Hu S, Leonard NJ, Tong SY, Fillips D. The raspberry locus of *Drosophila melanogaster* includes an inosine monophosphate dehydrogenase like coding sequence. Genome. 1994; 37:333–44. PMID: 7911114

40. Irving P, Ubeda JM, Doucet D, Troxler L, Lagueux M, Zachary D, et al. New insights into *Drosophila* larval haemocyte functions through genome-wide analysis. Cell Microbiol. 2005; 7:335–350. PMID: 15679837

41. Gu JJ, Tolin AK, Jain J, Huang H, Santiago L, Mitchell BS. Targeted disruption of the inosine 5’-monophosphate dehydrogenase type I gene in mice. Mol Cell Biol. 2003; 23:6702–12. PMID: 12944494

42. Xavier MJ, Williams MJ. The Rho-family GTPase Rac1 regulates integrin localization in immunosurveillance cells. PLoS One. 2011; 6:e19504. doi: 10.1371/journal.pone.0019504 PMID: 21603603

43. Howell L, Sampson CJ, Xavier MJ, Bolukbasi E, Heck MM, Williams MJ. A directed miniscreen for pathway genes to larval hemocyte concentration and the egg encapsulation response in *Drosophila*. Genetics. 2006; 172:1633–44. doi: 10.1242/jcs.030163 PMID: 18796536

44. Nussbaum JM, Liu LJ, Hasan SA, Schaub M, McClendon A, Stainier DYR, Sakaguchi TF. Homeostatic generation of reactive oxygen species protects the zebrafish liver from steatosis. Hepatology. 2013; 58:1326–34. doi: 10.1002/hep.26551 PMID: 23744565

45. Wawrzyniak JA, Bianchi-Smiraglia A, Bshara W, Mannava S, Ackroyd J, Bagati A et al. A Purine Nucleotide Biosynthesis Enzyme Guanosine Monophosphate Cell Reports 2013; 5:493–507. doi: 10.1016/j.celrep.2013.09.015 PMID: 24139804

46. Long H, Cameron S, Yu L, Rao Y. De novo GMP synthesis is required for axon guidance in *Drosophila*. Genetics. 2006; 172:1633–42. PMID: 16322525