


RESEARCH ARTICLE

Comparative RNA-Seq analyses of *Drosophila* plasmatocytes reveal gene specific signatures in response to clean injury and septic injury

Elodie Ramond *, Jan Paul Dudzic *, Bruno Lemaitre *

Global Health Institute, School of Life Science, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

 These authors contributed equally to this work.* elodie.ramond@inserm.fr (ER); jandudzic@uvic.ca (JPD); bruno.lemaitre@epfl.ch (BL) OPEN ACCESS

Citation: Ramond E, Dudzic JP, Lemaitre B (2020) Comparative RNA-Seq analyses of *Drosophila* plasmatocytes reveal gene specific signatures in response to clean injury and septic injury. PLoS ONE 15(6): e0235294. <https://doi.org/10.1371/journal.pone.0235294>

Editor: Andreas Bergmann, University of Massachusetts Medical School, UNITED STATES

Received: April 29, 2020

Accepted: June 11, 2020

Published: June 29, 2020

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0235294>

Copyright: © 2020 Ramond et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Data are available within the manuscript and its Supporting Information files. The raw data of the RNA-seq study are deposited at the NCBI sequence read

Abstract

Drosophila melanogaster's blood cells (hemocytes) play essential roles in wound healing and are involved in clearing microbial infections. Here, we report the transcriptional changes of larval plasmatocytes after clean injury or infection with the Gram-negative bacterium *Escherichia coli* or the Gram-positive bacterium *Staphylococcus aureus* compared to hemocytes recovered from unchallenged larvae via RNA-Sequencing. This study reveals 676 differentially expressed genes (DEGs) in hemocytes from clean injury samples compared to unchallenged samples, and 235 and 184 DEGs in *E. coli* and *S. aureus* samples respectively compared to clean injury samples. The clean injury samples showed enriched DEGs for immunity, clotting, cytoskeleton, cell migration, hemocyte differentiation, and indicated a metabolic reprogramming to aerobic glycolysis, a well-defined metabolic adaptation observed in mammalian macrophages. Microbial infections trigger significant transcription of immune genes, with significant differences between the *E. coli* and *S. aureus* samples suggesting that hemocytes have the ability to engage various programs upon infection. Collectively, our data bring new insights on *Drosophila* hemocyte function and open the route to post-genomic functional analysis of the cellular immune response.

Introduction

Drosophila blood cells, also called hemocytes, contribute to the cellular immune response by engulfing bacteria, combatting parasites and secreting antimicrobial and clotting factors. They also participate in regulating the immune response by secreting cytokines such as the JAK--STAT ligands Unpaired [1] or the Toll ligand Spätzle [2–4]. Hemocytes are also involved in wound healing notably through the engulfment of apoptotic cells and cellular debris, the stimulation of stem cell proliferation, and deposition of extracellular matrix [5–8]. Furthermore, hemocytes produce enzymes essential to the melanization reaction [9,10]. Recent evidence shows that *Drosophila* blood cells contribute not only to immunity and wound healing, but are also central to host metabolism [11–14]. That an excessive number of hemocytes can be detrimental to flies raised on a poor diet shows that hemocyte number must be tightly regulated

archive (SRA) with the accession number PRJNA638422.

Funding: ER, JPD and BL were supported by the SNF (Swiss National Science Foundations) sinergia grant CRSII5 186397.

Competing interests: The authors have declared that no competing interests exist.

[15]. Thus, there is a current effort to better characterize the role of hemocytes during the life cycle of flies.

Hematopoiesis occurs in several waves throughout the *Drosophila* life cycle. The first phase of hematopoiesis establishes a pool of hemocytes from the embryonic head mesoderm. These cells contribute to embryonic development by phagocytosing apoptotic cells, and through the deposition of extracellular matrix [8]. These embryonic derived hemocytes persist in larvae, where they are subjected to several rounds of division reaching about 6000 hemocytes at the end of the third instar larval stage [16]. Peripheral larval hemocytes are found either (i) in circulation in the hemolymph or (ii) in sessile patches [17–23]. Sessile hemocytes are attached to the internal surface of the larval body wall, forming patches, some of which are closely associated with secretory cells called oenocytes, as well as the endings of peripheral neurons [22,24]. Hemocytes are continuously exchanged between sessile patches and circulation [25,26]. The function of sessile hemocyte patches is not yet established but it has been proposed that they form a diffuse hematopoietic organ [22,27,28]. Larvae also possess a special hematopoietic organ, the lymph gland, that functions as a reservoir releasing hemocytes at the pupal stage or after parasitic infection. Both lymph gland and embryonic derived hemocyte populations contribute to the pool of adult hemocytes that will ultimately decline upon ageing. Whether active hematopoiesis occurs in adults is still debated [29,30].

Most studies on the cellular immune response focus on third instar larval hemocytes as both sessile and circulating hemocytes can easily be collected and FACS sorted. *Drosophila* larvae have two types of hemocytes in the unchallenged state: plasmatocytes, which are macrophage-like, and crystal cells, rounded hemocytes which contain crystals of prophenoloxidases, the zymogen form of phenoloxidases that catalyzes the melanization reaction against parasites or septic injury [9,17,31]. A third type of hemocytes, the lamellocytes, is restricted to the larval stage and originates either from progenitors in the lymph gland or in periphery by transdifferentiation of plasmatocytes or circulating progenitors [32–34]. These cells differentiate upon parasitoid wasp infestation and contribute to the encapsulation and melanization of larger parasites. At the larval stage, plasmatocytes represent the most abundant fraction of *Drosophila* blood cells (i.e. 90–95%) [35] and express several markers such as the clotting factor Hemolectin (Hml), or the phagocytic receptors Nimrod C1 (NimC1 or P1) or Eater [18]. The other 5–10% larval hemocytes are Lozenge (Lz) positive crystal cells [17]. Only rarely can lamellocytes be observed in the unchallenged larvae as these cells are induced upon wasp infestation or injury [32].

Until recently, there have been surprisingly few studies analyzing the hemocyte transcriptome, possibly due to difficulties in collect enough materials. The most comprehensive genome wide analysis was a characterization of whole larval hemocyte populations by Irving et al. in 2005, using an Affymetrix based oligonucleotide array [2]. Of the 13,000 genes (total number of genes > 17,500) represented in this microarray, they were able to identify 2500 with significantly enriched expression in hemocytes, notably genes encoding integrins, peptidoglycan recognition proteins (PGRPs), scavenger receptors, lectins, cell adhesion molecules and serine proteases. Interestingly, several single cell transcriptomic analyses have revealed the degree of heterogeneity of *Drosophila* hemocyte populations, but they did not characterize the full repertoire of genes expressed in hemocytes [36–39].

To better characterize the transcriptome of hemocytes, we have performed an RNAseq transcriptome analysis of FACS sorted Hml positive cells. The transcriptome of Hml positive (Hml+) plasmatocytes was determined in an unchallenged condition and 45 minutes following clean or septic injury with *Staphylococcus aureus* or *Escherichia coli*. Comparative transcriptomics allowed us to identify a set of genes specific to plasmatocytes in unchallenged or challenged condition, revealing the various contributions of these cells to host defense, wound healing and metabolism.

Results

Study design

We performed RNA sequencing of mRNA to analyze the global gene expression profile changes of *Drosophila* hemocytes from third instar larvae either unchallenged or collected 45 minutes after clean injury or septic injury with a needle dipped in concentrated bacterial pellets of *Staphylococcus aureus* or *Escherichia coli*. To isolate the plasmatocytes from other unwanted cells of the hemolymph preparation, we used the *Hml^Δ.Ds-Red.nls* fluorescent marker, which is specifically expressed in most plasmatocytes, and to a lesser extent in newly differentiated crystal cells [28,40,41]. We extracted hemolymph from wandering third instar L3 larvae by bleeding them onto a glass slide and subjected the collected hemolymph to fluorescence activated cell sorting (FACS) to isolate the *Hml+* hemocyte population. The collected hemocytes thus correspond to circulating hemocytes. Flow cytometer scatter-plot outputs were analyzed to delineate the hemocyte population based on the nucleic red-fluorescent signal, and total RNA extraction was performed on the isolated hemocytes (Fig 1). We collected approximately 20,000 to 40,000 larval *Hml*-positive plasmatocytes for each treatment. Three independent extractions were performed for each tested condition. As in Irving et al, 2005, we used unchallenged whole larvae as an external control to identify genes that were specifically enriched in plasmatocytes compared to the whole animal. RNA-Seq libraries were then constructed and sequenced using Illumina HiSeq, and we performed differential gene expression analysis between all sample groups. We obtained 62,320,223 reads from RNA samples extracted from whole larvae (L3), 42,284,187 reads from hemocytes of unchallenged larvae (UC), 42,519,937 reads from RNA extracted from hemocytes of clean-injured larvae (CI), 69,143,536 reads from RNA extracted from hemocytes of *E. coli* infected larvae (Ec) and 42,758,456 reads from RNA extracted from hemocytes of *S. aureus* infected larvae (Sa) (sum of triplicates). The total number of mapped reads per single library ranged from 5.30 to 23.17 million reads, with coverage ranging from 59.42% to 86.14% (Fig 2A). Genes with more than 5 reads per 1 million reads are listed in S1 File. One of our unchallenged hemocyte samples showed elevated immune gene expression. To account for a possible unrelated infection, we reduced unwanted variation from this sample as described in the methods.

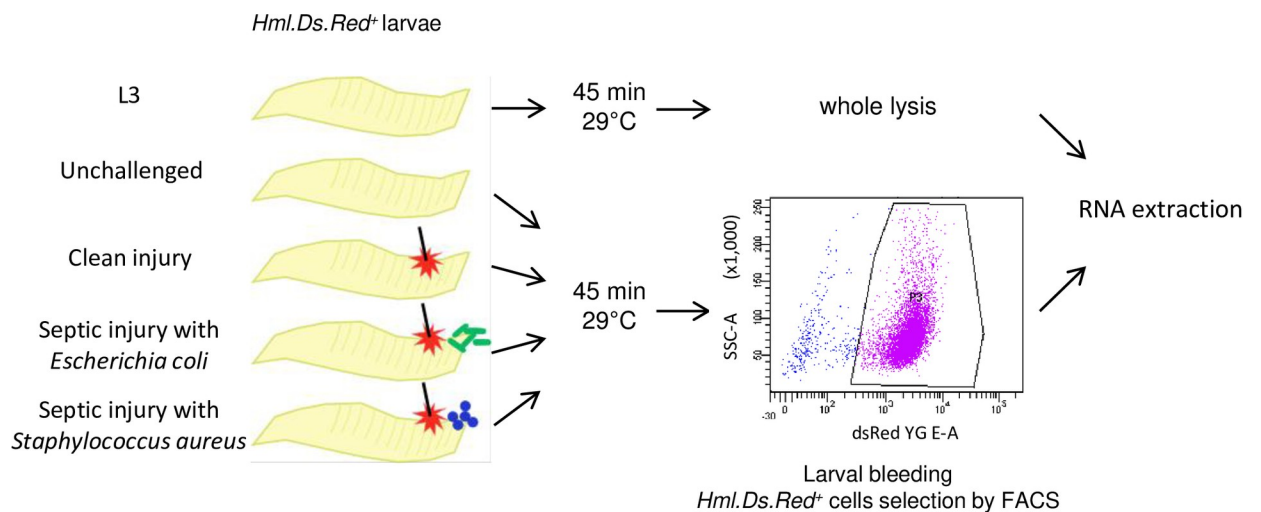


Fig 1. Experimental design of RNA sequencing experiment. Total RNA was extracted from whole larvae or hemocytes recovered from *Hml^Δ.Ds-red.nls* fluorescent larvae. Larvae were left unchallenged or challenged by a systemic infection with *Escherichia coli* or *Staphylococcus aureus* and incubated at 29°C for 45 minutes. Hemocytes were collected in PBS, on ice, and were immediately sorted FACS and processed for RNA extraction.

<https://doi.org/10.1371/journal.pone.0235294.g001>

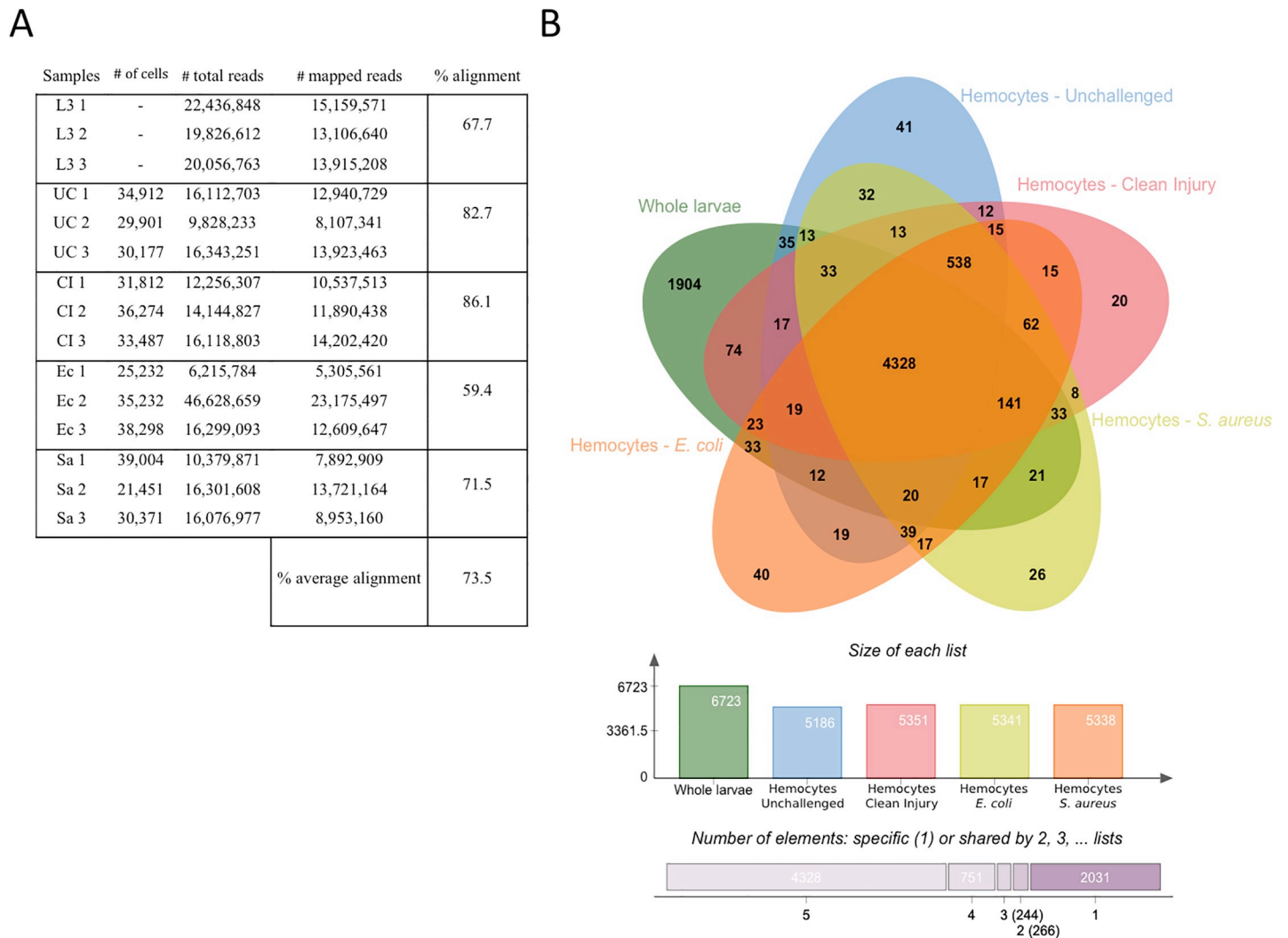


Fig 2. Transcriptome summaries from unchallenged whole larvae and hemocytes from unchallenged and infected larvae. (A) Transcriptome summary showing the number of reads for each triplicate in all experimental conditions with their corresponding number of mapped reads and the average percentage of alignment to the *D. melanogaster* genome. (B) Venn diagram representing the quantity of shared genes between all experimental treatments: Unchallenged wandering L3 larvae, hemocytes from unchallenged larvae, hemocytes from clean-pricked larvae (CI), hemocytes from larvae pricked with *Escherichia coli* (Ec), hemocytes from larvae pricked with *Staphylococcus aureus* (Sa).

<https://doi.org/10.1371/journal.pone.0235294.g002>

We then identified differentially expressed genes (DEGs) between all five samples by four pairwise comparisons: “unchallenged hemocytes” samples versus “unchallenged whole larva” samples, “unchallenged hemocytes” samples vs hemocytes from clean injured larva (CIH), CIH vs hemocytes from “*Escherichia coli* infected larvae” (EcH) and CIH vs hemocytes from “*Staphylococcus aureus* infected larvae” (SaH).

Identification of genes enriched in *Drosophila* larval plasmatocytes

Using our threshold that includes genes with at least 5 reads per 1 million reads, we found that 6,723 genes are expressed in L3 larvae, while unchallenged hemocytes express 5,186 genes. The number of genes expressed in hemocytes is roughly the same as observed by Irving et al. using Affymetrix arrays that identified around 5000 expressed genes in hemocytes [2]. To identify transcripts expressed in the unchallenged hemocyte population, we classified genes according

to their total number of reads (S1 File) and their degree of enrichment in Hml+ plasmatocytes compared to whole larvae (S2 File, for a selection see Table 1). We found that whole larvae and hemocytes shared expression of 4,477 genes, 2,246 genes were uniquely expressed in L3 larvae and 709 genes were uniquely expressed in hemocytes (Fig 2B). We confirmed the identity of the plasmatocyte population by the presence of reads for genes known to be specific for plasmatocytes (see below). We found 239 genes encoding transmembrane proteins in unchallenged hemocytes. Of those, 44 were enriched and 195 poorly expressed in hemocytes when compared to whole larvae (S3 File). This large set of transmembrane proteins likely contributes to the versatile functions of plasmatocytes. By secreting immune factors, the fat body plays a major role in the humoral response. Plasmatocytes are thought to play a similar role upon infection [42,43]. We therefore looked for genes encoding proteins with a secretion signal in plasmatocytes. We identified 329 such genes expressed in plasmatocytes. Among those, 70 were enriched and 259 were poorly expressed in plasmatocytes when compared to whole larvae (S4 File and selection in Table 1).

To better characterize gene repertoire of plasmatocytes, we will restrict our analysis to the 5393 genes that were differentially expressed in the whole larvae compared to plasmatocytes (S2 File). GO terms analysis (S1 Fig) identified many biological processes without clearly highlighting important classes of genes. Thus, we decided to analyze in depth the DEGs identified in our initial analysis.

We first focused our attention on genes known to play a role in *Drosophila* hematopoiesis. We found that genes encoding the transcription factors Serpent [44], U-shaped [45] and Yantar [46], which play a role in pro-hemocyte differentiation, were enriched in plasmatocytes with respective fold changes (FCs) of 7.0, 6.6 and 2.7 compared to whole larvae. We did not identify *glial cells missing* (*gcm*) in our screen, which is consistent with the fact that this gene encodes a transcription factor promoting plasmatocytes maturation only at the embryonic stage [36,47]. The three genes *dome*, *hedgehog* and *Antennapedia*, which positively regulate hematopoiesis in the lymph gland [48], were reduced in circulating plasmatocytes compared to whole larvae, with FCs of -5.3, -173.9 and -2903.3, respectively. Similarly, genes that promote pro-hemocyte maturation in the lymph gland, such as *jumu*, *pyramus*, *thisbe* and *heartless* were also down-regulated in hemocyte samples (with FCs of -2.2, -31.9, -1151.8 and -1655.5, respectively) [49,50]. The gene encoding the transcription factor *collier* (*knot*) that contributes to the lymph gland posterior signaling center [51] was not enriched in plasmatocytes. In contrast, the two genes encoding the transcription factors Pointed and Pannier, which promote hemocyte terminal differentiation [50,52] were enriched in circulating hemocytes with FCs of 3.5 and 15.6 respectively. Finally, genes implicated in crystal cell differentiation such as *Delta*, *serrate* and *notch* [48] were downregulated in plasmatocytes samples compared to the whole larvae samples, with respective FCs of -919.3, -9.9 and -4.5. In contrast, the expression of *lozenge* gene, which encodes the master regulator of crystal cell differentiation, was not affected. The expression of *lozenge* in Hml+ plasmatocytes possibly reflects the trans-differentiation of a subset of them into crystal cells [28]. These results confirmed that collected circulating peripheral plasmatocytes were mostly in the differentiated state. Consistent with this, the *Drosophila* hemocyte marker genes *hemese*, *peroxidase* and *hemolactin* had respectively 49.0, 15.5 and 6.7 fold higher expression in hemocytes compared to whole larvae samples.

As expected, plasmatocytes were strongly enriched in genes involved in phagocytosis. We found the *scavenger receptor class C, type I* (*ScrCI*), and the Nimrod receptors *Nimrod C1* [53] and *eater* [54] had respective FCs of 48.9, 26.5 and 15.3. We did not identify the *Integrin β subunit* to be differentially expressed in our screen. Two other Nimrod receptors, *draper* and *simu* (Six-Microns-Under, also named Nimrod C4) can bind phosphatidylserine on dying

Table 1. Selected DEGs of interest with Fold-changes of unchallenged hemocytes vs. whole larvae from S2 File.

CG	Full name	FC	CG	Full name	FC
Hemocyte marker			Stress response		
CG31770	<i>Hemese</i>	49.05	CG31359	<i>Heat-shock protein-70Bb</i>	55.07
CG3978	<i>pannier</i>	15.63	CG4183	<i>Heat-shock protein-26</i>	7.69
CG12002	<i>Peroxidasin</i>	15.51	CG11637	<i>Ninjurin B</i>	5.96
CG16707	<i>visgun</i>	13.62	CG4463	<i>Heat-shock protein-23</i>	4.43
CG3992	<i>serpent</i>	7.06	CG12101	<i>Heat-shock protein-60A</i>	4.36
CG7002	<i>Hemolectin</i>	6.76	CG4466	<i>Heat-shock protein-27</i>	4.27
CG2762	<i>u-shaped</i>	6.66	CG5436	<i>Heat-shock protein-68</i>	3.48
CG18426	<i>yantar</i>	2.73	CG4147	<i>Heat shock 70-kDa protein cognate 3</i>	3.3
Immune response			CG34246	<i>Heat shock protein cognate 20</i>	2.68
Antimicrobial defense			CG1242	<i>Heat shock protein 83</i>	2.59
CG4437	<i>PGRP-LF</i>	3.49	Oxidative stress response		
CG4432	<i>PGRP-LC</i>	3.23	Glutathione S transferase family		
CG5576	<i>Immune deficiency</i>	3.29	CG6776	<i>Glutathione S transferase O3</i>	10.43
CG8995	<i>PGRP-LE</i>	2.78	CG10045	<i>Glutathione S transferase D1</i>	8.55
CG11992	<i>Relish</i>	2.59	CG17523	<i>Glutathione S transferase E2</i>	8.51
CG32042	<i>PGRP-LA</i>	2.27	CG12242	<i>Glutathione S transferase D5</i>	5.22
CG6134	<i>spatzle</i>	10.5	CG4371	<i>Glutathione S transferase D7</i>	4.89
CG5974	<i>pelle</i>	5.31	CG4381	<i>Glutathione S transferase D3</i>	3.32
CG10520	<i>tube</i>	3.13	CG17530	<i>Glutathione S transferase E6</i>	3.12
CG7629	<i>Attacin-D</i>	7.8	CG11784	<i>Glutathione S transferase E13</i>	3.09
Melanization			CG5224	<i>Glutathione S transferase E11</i>	2.79
CG42640	<i>Prophenoloxidase 3</i>	25.59	CG10091	<i>Glutathione S transferase D9</i>	2.46
CG18550	<i>yellow-f</i>	13.55	CG5164	<i>Glutathione S transferase E1</i>	2.35
CG1102	<i>Melanization protease 1</i>	11.28	CG9362	<i>Glutathione S transferase Z1</i>	2.1
CG8193	<i>Prophenoloxidase 2</i>	9.1	CG30000	<i>Glutathione S transferase T1</i>	2
CG11331	<i>Serpin 27A</i>	7.93	Others genes		
CG7219	<i>Serpin 28Dc</i>	3.27	CG32495	<i>Glutathione synthetase 2</i>	20.18
Antiviral immunity			CG17753	<i>Copper chaperone for superoxide dismutase</i>	3.62
CG7138	<i>r2d2</i>	2.22	CG8905	<i>Superoxide dismutase 2 (Mn)</i>	3.57
Phagocytic receptors and markers			CG11793	<i>Superoxide dismutase 1</i>	2.8
CG4099	<i>Scavenger receptor</i>	48.99	CG1274	<i>thioredoxin peroxidase 2</i>	2.47
CG8942	<i>Nimrod C1</i>	26.55	CG1633	<i>thioredoxin peroxidase 1</i>	2.03
CG6124	<i>eater</i>	15.35	Metabolism		
CG2086	<i>draper</i>	3.19	Lipid metabolism / Peroxisome		
CG10106	<i>Tetraspanin 42Ee</i>	3.48	CG7291	<i>Niemann-Pick type C-2a</i>	7.59
CG4591	<i>Tetraspanin 86D</i>	4	CG3083	<i>Peroxin 19</i>	6.67
CG6120	<i>Tetraspanin 96F</i>	2.9	CG3639	<i>Peroxin 12</i>	6.06
CG10742	<i>Tetraspanin 3A</i>	3.2	CG7081	<i>Peroxin 2</i>	4.44
Opsonins			CG7864	<i>Peroxin 10</i>	2.94
CG33119	<i>Nimrod B1</i>	23.27	CG3947	<i>Peroxin 16</i>	2.41
CG33115	<i>Nimrod B4</i>	17.69	CG4289	<i>Peroxin 14</i>	2.2
CG16873	<i>Nimrod B5</i>	3.78	CG3415	<i>peroxisomal Multifunctional enzyme type 2</i>	2.27
CG2958	<i>lectin-24Db</i>	33.93	CG6783	<i>fatty acid binding protein</i>	9.73
CG7106	<i>lectin-28C</i>	24.47	CG4280	<i>croquemort</i>	4.74
CG18096	<i>Thioester-containing protein 1</i>	19.2	Adenosine metabolism		
CG5210	<i>Imaginal disc growth factor 6</i>	6.6	CG5992	<i>Adenosine deaminase-related growth factor A</i>	6.59

(Continued)

Table 1. (Continued)

CG4472	<i>Imaginal disc growth factor 1</i>	5.91	CG11994	<i>Adenosine deaminase</i>	5.59
Cellular encapsulation			Glucose metabolism		
CG14225	<i>eye transformer</i>	17.16	CG14816	<i>Phosphoglycerate mutase 5</i>	4.87
CG3715	<i>SHC-adaptor protein</i>	7.03	CG6453	<i>Glucosidase 2 beta subunit</i>	2.11
CG7830	<i>Oligosaccharide transferase gamma subunit</i>	2.68	CG7010	<i>Pyruvate dehydrogenase E1 alpha subunit</i>	2.06
			CG30410	<i>Ribose-5-phosphate isomerase</i>	2.04

<https://doi.org/10.1371/journal.pone.0235294.t001>

cells and promote apoptotic cell internalization, a process called efferocytosis [55,56]. In our screen, we found *draper* transcripts enriched in plasmatocytes samples with a FC of 3.1 whereas *simu* expression was unchanged. Hml+ plasmatocytes were also enriched in genes encoding opsonins such as *Tep1* (FC: 19.2) which has been shown to promote bacterial internalization [57,58]. In contrast, *Tep2* and *Tep3* were down-regulated in hemocytes compared to the whole larvae samples (FCs: -8.4 and -63.1). Furthermore, several genes encoding secreted components of the Nimrod family were enriched in plasmatocytes, most notably *Nimrod B1* and *Nimrod B4* and to a lesser extent *Nimrod B5* (FCs: 23.2, 17.6 and 3.7) as well as *hemese*. Genes encoding secreted Nimrod genes and *hemese* are clustered in the genome together with genes coding for phagocytic receptors such as *Nimrod C1*. Recently, *NimB5* has been shown to regulate plasmatocyte adhesion and proliferation [15]. The two plasmatocyte-enriched secreted Nimrod proteins, *NimB1* and *NimB4*, are promising candidate genes regulating important plasmatocyte functions, possibly phagocytosis [59]. *Drosophila* plasmatocytes were also enriched in several cytoskeleton proteins (see in S1 Table) such as SCAR, which has been shown contribute to phagocytosis and cell migration [60]. The two GTPases, *Rac1* and *Rac2*, which have been implicated in phagocytosis and cellular response were also enriched in plasmatocytes (FCs: 2.8 and 4.1) [61–63]. A recent study revealed an important role of peroxisomes in phagocytosis and immunity [64]. Consistent with this, several peroxins that encode components of peroxisomes (peroxins 19, 12, 2, 11, 10, 16, and 14) were strongly enriched in plasmatocytes. In addition, several genes encoding Tetraspanins were enriched in plasmatocytes. Tetraspanins are implicated in a wide range of functions in *Drosophila* such as protein stabilization at the plasma membrane and cell signaling regulation, and could contribute to phagocytosis or adhesion [65]. Specifically, we identified *late bloomer* and the *Tetraspanins 86D*, *42Ee*, *3A* and *96F* with respective FCs ranging from 2.8 to 3.9. Collectively, the enrichment of genes encoding phagocytic receptors, opsonins, and cytoskeletal proteins in plasmatocytes confirm their phagocytic ability.

The systemic antimicrobial response, which encompasses the production and release of many immune effectors into the hemolymph, is regulated by two NF- κ B pathways, namely *Imd* and *Toll* [66]. There is strong evidence that these two pathways are functional in hemocytes [67]. We found that plasmatocytes are enriched in several components of the *Imd* pathway, notably *Imd* and *Relish*. The genes encoding three transmembrane receptors of the Peptidoglycan recognition proteins (PGRP) family, *PGRP-LF*, *PGRP-LC*, and *PGRP-LA*, which are organized in a cluster in the *Drosophila* genome and contribute to *Imd* pathway activity, were also higher in plasmatocytes (FCs: 3.4, 3.2 and 2.2 respectively) [68,69]. The gene encoding the intracellular pattern recognition *PGRP-LE* that is involved in the sensing of monomeric peptidoglycan of Gram-negative bacteria and autophagy was also increased in plasmatocytes (FC: 2.7) [70–72]. We also confirmed that plasmatocytes have an increased expression of the gene *spatzle* (FC: 10.5), which encodes the ligand of the *Toll* pathway [66,73] as well as the genes encoding the adaptor *Tube* and the kinase *Pelle* (respective FCs: 3.1 and 5.3).

Crystal cells are the main hemocyte type involved in the melanization reaction, expressing both Prophenoloxidases 1 (PPO1) and 2 (PPO2) [9]. In contrast, Prophenoloxidase 3 (PPO3) is expressed in lamellocytes [10]. Surprisingly, we found that both *PPO2* and *PPO3* were enriched in circulating cells with fold changes of 9.1 and 25.5 compared to whole larvae. Their expression in plasmatocytes could reflect the transdifferentiation of Hml positive cells into crystal cells and lamellocytes or an unexpected contribution of plasmatocytes in the production of prophenoloxidase. Plasmatocytes had increased expression of genes encoding for several of the enzymes that have been linked to prophenoloxidase activity (e.g. *yellow f*, FC: 13.55; see [Table 1](#)). Complex serine protease cascades regulate important immune functions (i.e. melanization, Toll) in the hemolymph. We found that plasmatocytes have higher expression of the gene encoding the serine protease MP1 (FC: 11.2), which regulates melanization, and serpins such as *Serpin27A* (FC: 7.9) and *Serpin28Dc* (FC: 3.2), which negatively regulate melanization and Toll [74–77]. The immune function of lectins is poorly characterized in *Drosophila* but some of them have been implicated in immunity in other insects [2,78,79]. We found that two secreted lectins, lectin-24Db and lectin-28C were strongly enriched in plasmatocytes (FCs: 33.9 and 24.4).

Plasmatocytes also showed increased expression of many genes involved in the oxidative stress response, notably many glutathione S transferase genes and other detoxifying enzymes such as peroxidase (FC 15.51), superoxide dismutases 1 and 2 (FCs: 2.8 and 3.5) or thioredoxin peroxidase 1 and 2 (FC: 2 and 2.4). These genes may play a role in immune response activation [80]. In parallel, we observed an increase in gene transcripts involved in other cellular stress pathways, e.g. many heat shock proteins and Ninjurin B (FC: 5.9). We cannot exclude the possibility that this expression profile related to cellular stress also reflects a quick response of plasmatocytes to stresses imposed by procedures (i.e. temperature switch from 25°C to 29°C and FACS processing), despite maintaining samples on ice following extraction.

Plasmatocytes contribute to the production of the basal membrane in the embryo [81]. In agreement with this, larval plasmatocytes had increased expression of genes encoding components of the basal membrane, notably Laminins A, B1 and B2 (FCs: 5.7, 7.2 and 8.7) [8], Viking (collagen IV), and secreted enzymes that contribute to basal membrane formation (fat-spondin, Glutactin, Peroxidase, Matrix metalloproteinase 2 with FCs of 19.1, 18.6, 15.5 and 4.9). Consistent with previous studies that have suggested an important role of plasmatocytes in adenosine metabolism at the larval stage [82] we found that the two genes *Adenosine deaminase-related growth factor A* (*ADGF-A*) and *Adenosine deaminase* were upregulated (FCs: 6.59 and 5.59) while *Adenosine deaminase-related growth factor A2* and *D* were downregulated. Recent studies have also highlighted a significant contribution of plasmatocytes in lipid uptake and storage, complementary to the fat body [83]. Consistent with this, the gene *croquemort*, which encodes a lipid binding receptor of the CD36 family, is also enriched in plasmatocytes (FC: 4.7) [84]. The *fatty acid binding protein* (*fabp*) gene was enriched 9-fold in plasmatocytes. Finally, our transcriptome analysis shows that plasmatocytes express many transporters, the aquaporin *Prip* being one of the most enriched compared to other tissues (FC: 12.4).

The early plasmatocyte response to clean injury

We then explored the transcriptomic response of plasmatocytes to clean injury in further detail by comparing the transcriptome of hemocytes extracted from unchallenged larvae to hemocytes from larvae 45 minutes after clean injury. We choose this time point as it corresponds to the time necessary to fully internalize or phagocytose a particle [85]. We identified 664 DEGs after clean injury compared to hemocytes from unchallenged larvae. Among these genes, 358 were up-regulated and 306 were down-regulated in the clean injury samples using a two-fold

change criteria and $p < 0.05$ cut-off (S5 File, See selection in Table 2). We then proceeded to GO terms analysis by focusing on genes that are upregulated in hemocytes after clean injury compared to unchallenged hemocytes, and that have a P-value < 0.05 . We identified several GO-term groups significantly enriched upon clean injury. Genes with GO terms for molecular function assigned to cell metabolism, actin mobilization and cytoskeleton organization, anti-oxidant and stress responses were particularly affected (S2 Fig).

We found a significant increase in transcripts corresponding to *Glutathione-S-transferase* (GST) genes (D2, E1, D3, E3, E8, D7, D5, E6) which encode antioxidant enzymes that detoxify hydrogen peroxide and lipid peroxides [86,87]. Genes encoding three Cytochrome P450 enzymes (Cyp6a20, Cyp6a17 and Cyp6a23) and the ABC transporter Multidrug resistance protein 4 (Mrp4), which are involved in detoxification, were also enriched upon clean injury (FC: 10.5, 3.1, 2.8 and 2.8).

Other stress responsive genes such as *Frost*, *Hsp70*, *Hsp68*, *Ninjurin A*, the Hsp co-factor *starvin* and *DnaJ-like-1* were also more highly expressed in clean injury conditions. Consistent with this stress response, the JNK stress responsive pathway was activated as evidenced by an increase expression of *puckered* (*puc*, FC: 2.3).

Many other upregulated genes such as *Gadd45*, *kayak*, *Larval cuticle proteins 1, 3 and 4*, and *Matrix metalloproteinase 1* which play a key role in wound healing, extracellular matrix generation and cuticle repairing, were also more highly expressed in plasmatocytes [88,89].

Our study confirms that the JAK-SAT ligand gene *upd3* which orchestrates the systemic wound response [3,90] was upregulated in plasmatocytes upon clean injury (FC: 4.2). The gene encoding Wallenda, a MAP3K that regulates stress response by regulating the expression of the Materazzi lipid binding protein gene in Malpighian tubules [91], was also upregulated (FC: 2.6).

It is well established that clean injury, in the non-sterile condition used in this study, triggers a transient and weak antimicrobial response [92]. We found that genes encoding components of the immune responsive pathways Imd (*Relish*, *PGRP-LB*, *PGRP-LF*) and the Toll pathways (*cactus*) were upregulated (respective FCs: 4.5, 3.2, 2.4 and 2.1). A subset of antimicrobial peptide genes, notably *Cecropin B*, A2 and C (respective FCs: 13, 8.3 and 6.8) and *Attacin-B* (FC: 6.8), were upregulated by clean injury.

Interestingly, two genes whose mutations have been associated with refractoriness to virus C and Sigma, *pastrel* and *ref(2)P* respectively, were upregulated upon clean injury (FCs: 3.1 and 2.0) [93]. While the function of *ref(2)P* in autophagy is well established [94], the role of *pastrel* is poorly characterized. The induction of *pastrel* in plasmatocytes suggests that it could play an important function in activated plasmatocytes, as the result of a potential viral infection.

Genes such as *fondue* (FC: 4.2), *Larval-serum protein 1γ* (FC: 8.3) and *Hemolectin* (FC: 2.2) that are implicated in hemolymph clotting [95] were up-regulated in clean-injured samples. Of note, *dopa decarboxylase* and the GTP cyclohydrolase *punch*, genes which encode enzymes that regulate melanin formation, were enriched in clean injury samples confirming the contribution of plasmatocytes to the melanization process [96]. Collectively, our study shows that plasmatocytes contribute to wound healing by inducing genes involved in stress response, ROS detoxification and cytoskeletal remodeling. The induction of genes encoding components of the Toll and Imd immune signaling pathways may reinforce the reactivity of these immune cells.

Another major class of genes upregulated upon clean injury are those involved in the cytoskeleton (S1 Table). This includes genes involved in actin remodeling, microtubule formation and adhesion that likely reflect the change of shape observed in 'activated plasmatocytes' that are known to be more adhesive and display filopodia [97]. Among them, were the *Integrin*

Table 2. Selected DEGs of interest with Fold-changes of clean injury hemocytes vs. unchallenged hemocytes from S5 File.

CG	Full name	FC	CG	Full name	FC
Immune response			Gene regulation		
Antimicrobial defense			CG13194	<i>pyramus</i>	31.23
CG11992	<i>Relish</i>	4.51	CG10045	<i>Daughters against dpp</i>	4.82
CG43720	<i>sickie</i>	4.12	CG33542	<i>unpaired 3</i>	4.26
CG14704	<i>Peptidoglycan recognition protein LB</i>	3.21	CF13780	<i>PDGF- and VEGF-related factor 2</i>	3.87
CG4437	<i>Peptidoglycan recognition protein LF</i>	2.44	CG4371	<i>wallenda (MAP Kinase Kinase)</i>	2.60
CG1399	<i>Leucine-rich repeat</i>	2.62	CG32406	<i>PVR adaptor protein</i>	2.53
CG16712	<i>Immune induced molecule 33</i>	2.54	CG15154	<i>Suppressor of cytokine signaling at 36E</i>	2.52
CG5848	<i>cactus</i>	2.18	CG7850	<i>puckered</i>	2.30
CG1878	<i>Cecropin B</i>	13.04	Cytoskeleton organization		
CG1367	<i>Cecropin A2</i>	8.38	CG3259	<i>Intraflagellar transport 54</i>	14.41
CG1373	<i>Cecropin C</i>	6.85	CG11798	<i>charlatan</i>	7.49
CG18372	<i>Attacin-B</i>	6.86	CG4843	<i>Tropomyosin 2</i>	7.46
Clotting			CG5372	<i>Integrin alphaPS5 subunit</i>	7.19
CG15825	<i>fondue</i>	4.23	CG12008	<i>karst</i>	6.75
CG7002	<i>Hemolectin</i>	2.23	CG5695	<i>jaguar</i>	6.04
Phagocytosis			CG43976	<i>Rho guanine nucleotide exchange factor 3</i>	4.98
CG31962	<i>Scavenger receptor</i>	2.74	CG1212	<i>p130CAS</i>	4.85
Melanization			CG8865	<i>Ral guanine nucleotide dissociation stimulator-like</i>	4.15
CG10697	<i>Dopa decarboxylase</i>	15.09	CG10076	<i>spire</i>	4.08
CG9441	<i>Punch</i>	3.32	CG33103	<i>Papillin</i>	4.06
Repair			CG13503	<i>Verprolin 1</i>	3.60
CG2043	<i>Larval cuticle protein 3</i>	19.21	CG3937	<i>cheerio</i>	3.28
CG4859	<i>Matrix metalloproteinase 1</i>	9.77	CG11949	<i>coracle</i>	3.24
CG2044	<i>Larval cuticle protein 4</i>	3.84	CG14396	<i>Ret oncogene</i>	3.18
CG11086	<i>Growth arrest and DNA damage-inducible 45</i>	2.57	CG33558	<i>missing-in-metastasis</i>	3.06
CG33956	<i>kayak</i>	2.18	CG2184	<i>Myosin light chain 2</i>	3.02
Others			CG10119	<i>Lamin C</i>	2.73
CG8588	<i>pastrel</i>	3.10	CG18214	<i>trio</i>	2.68
CG6821	<i>Larval serum protein 1 gamma</i>	8.35	CG33694	<i>CENP-ana</i>	2.58
Stress response			CG5164	<i>cappuccino</i>	2.41
Heat shock proteins			CG9362	<i>kugelei</i>	2.40
CG5834	<i>Heat-shock protein-70Bbb</i>	16.44	CG10522	<i>sticky</i>	2.38
CG6489	<i>Heat-shock protein-70Bc</i>	15.67	CG18076	<i>short stop</i>	2.34
CG18743	<i>Heat-shock-protein-70Ab</i>	10.25	CG1560	<i>myospheroid</i>	2.12
CG31359	<i>Heat-shock protein-70Bb</i>	8.78	CG42274	<i>Rho GTPase activating protein at 18B</i>	2.11
CG5436	<i>Heat-shock protein-68</i>	6.28	CG1520	<i>WASp</i>	2.1
Cyp450			Vesicle trafficking		
CG10245	<i>Cyp6a20</i>	10.59	CG8024	<i>Rab32</i>	3.15
CG10241	<i>Cytochrome P450-6a17</i>	3.18	CG14001	<i>blue cheese</i>	2.34
CG10242	<i>Cyp6a23</i>	2.83	CG14296	<i>Endophilin A</i>	2.15
Others			Cell death effectors		
CG9434	<i>Frost</i>	9.19	CG1600	<i>Death resistor Adh domain containing target</i>	3.63
CG32130	<i>starvin</i>	8.80	CG33134	<i>Death executioner Bcl-2</i>	2.33
CG12703	<i>Peroxisomal Membrane Protein 70 kDa</i>	4.61	Metabolism		
CG14709	<i>Multidrug resistance protein 4</i>	2.85	Glucose metabolism		
CG10578	<i>DnaJ-like-1</i>	2.54	CG5932	<i>magro</i>	26.43

(Continued)

Table 2. (Continued)

CG	Full name	FC	CG	Full name	FC
CG6449	Ninjurin A	2.46	CG34360	Glucose transporter 4 enhancer factor	8.97
CG31216	Nicotinamide amidase	2.44	CG5889	Malic enzyme b	4.28
Oxidative stress response			CG4625	Dihydroxyacetone phosphate acyltransferase	4.24
Glutathione S transferase family			CG6906	Carbonic anhydrase	3.70
CG4181	Glutathione S transferase D2	10.36	CG8256	Glycerophosphate oxidase 1	2.69
CG5164	Glutathione S transferase E1	5.60	Lipid catabolism		
CG4381	Glutathione S transferase D3	4.19	CG3620	no receptor potential A	2.25
CG17524	Glutathione S transferase E3	4.19	CG11055	Hormone-sensitive lipase	2.22
CG17533	Glutathione S transferase E8	3.21	CG1882	pummelig	2.05
CG4371	Glutathione S transferase D7	3.20	Amino acid storage		
Others			CG6821	Larval serum protein 1 gamma	8.35
CG2259	Glutamate-cysteine ligase catalytic subunit	3.56	CG4178	Larval serum protein 1 beta	3.54
			CG2559	Larval serum protein 1 alpha	2.69

<https://doi.org/10.1371/journal.pone.0235294.t002>

alphaPS5 subunit gene (FC: 7.1) [98] and the integrins *charlatan* and *myospheroid* (FCs: 7.4 and 2.1) [99,100] which are induced upon lamellocyte differentiation. Interestingly, *Integrin β subunit*, a gene that encodes a transmembrane protein implicated in apoptotic corpses clearance in embryonic hemocytes [101], and *Scavenger receptor class C, type III (Sr-CIII)* were also induced (FCs: 3.9 and 2.7). Moreover, the gene encoding the FGF ligand *Pyramus* that has been shown to promote blood cell progenitors differentiation in the lymph gland [50] was the most up-regulated gene 45 minutes after clean injury. This suggests that FGF-R pathway activation in plasmatocytes by the ligand *Pyramus* could play a prominent role in promoting the differentiation of peripheral plasmatocytes upon injury, akin to the process observed in the lymph gland. On this line, Tattikota *et al.* observed an enrichment of transcripts encoding the FGF ligand *Branchless* and its receptor *Breathless* in crystal cells and lamellocytes subsets respectively, and revealed a role of the FGF pathway in the encapsulation of parasitoid wasps [37]. This also highlights the importance of the FGF pathway in cell specification. Two genes from the PVR (PDGF/VEGF-related factor) pathway that are implicated in hemocyte survival and migration [102–104], one encoding the PVR adaptor *PVRAP* and the other the PVR ligand *Pvf2*, were up-regulated upon clean injury (FCs: 2.5 and 3.8). Consistent with this, we also observed that clean injury triggers the down-regulation of apoptosis-associated genes such as *head involution defective (hid)*, FC: 15.7) [105] and *Deneddylase 1 (Den1)*, FC: 2.2) [106]. This suggests that wounding stimulates blood cell survival as well as blood cell pool expansion.

In mammals, macrophages undergo massive metabolic change upon activation [107,108]. Notably, lipid catabolism and glucose consumption are essential components of mammalian macrophage activation in order to fuel the cell as well as to produce inflammatory mediators [109]. We next investigated whether clean injury also induces a metabolic reprogramming in plasmatocytes. Interestingly, when analyzing the GO terms enrichment, we found an over-representation of “lipase activity” related genes (S2 Fig). Indeed, we observed the up-regulation of the *magro*, *alpha/beta hydrolase2 (Hydr2)*, the *phospholipase c at 21c* and *no receptor potential A (norpA)* genes with respective FCs of 26.4, 2.9, 2.6 and 2.2. Upregulation of *apolipoprotein (apoLpp)* and *ATP binding cassette subfamily A (ABCA)* genes (FCs: 2 and 4.2) indicate that both lipid catabolism and lipid uptake are induced upon clean injury in plasmatocytes, which may fuel the increased energy demand of the activated cells. In agreement with the concept of metabolic reprogramming, we noted the up-regulation of the *Glucose transporter 4 enhancer factor* gene (*Glut4EF*, FC 8.9) [110], a transcription factor regulating the *Glucose*

transporter 4 gene [111], also known as *solute carrier family 2 member 4*), and of the *Glycogen phosphorylase* (GlyP) gene coding for the enzyme catalyzing the rate-limiting step of glycogenolysis. The induction of these two genes suggests that upon injury plasmatocytes may increase their metabolic activity by increasing glucose provisioning. Genes of the mTOR signaling pathway, that is known to stimulate a glycolytic metabolism, were also upregulated: *thor*, *rictor* and *phosphoinositide-dependent kinase 1* (*pkd1*) and (FCs: 2.7, 2.5 and 2.0). Additionally, the *men* gene encoding malate dehydrogenase, which is known to sustain active glycolysis by replenishing the cytosolic NAD pool and by limiting tricarboxylic acid cycle (TCA) refueling [112], was also upregulated.

Plasmatocytes gene expression signature in response to bacterial infection

Finally, we explored the transcriptional response of blood cells upon septic injury with *Escherichia coli* (EcH samples, Table 3 and S6 File) and *Staphylococcus aureus* (SaH samples, Table 4 and S7 File) and compared it with the transcriptional profile of hemocytes from clean-injured larvae. We were interested to know if the presence of bacteria affects plasmatocyte response and whether hemocytes react in a different way to infection by Gram-negative versus Gram-positive bacteria (Fig 3). Studies have shown that *E. coli* is an efficient inducer of the Imd pathway and is sensitive to the action of antibacterial peptides [113]. In contrast, *S. aureus*, as a lysine-type bacterium, is a potent inducer of the Toll pathway [114] and is combatted by Toll mediated production of Bomanin [113], melanization [77], and phagocytosis [115]. Interestingly, we identified 104 and 92 uniquely expressed genes in hemocytes from larvae infected with *E. coli* (EcH) and *S. aureus* (SaH) respectively compared to hemocytes from clean injured larvae. In EcH samples, we identified 84 up-regulated genes and 151 down-regulated genes

Table 3. Selected DEGs of interest with Fold-changes of *E. coli* hemocytes vs. Clean injury hemocytes from S6 File.

CG	Full name	FC
Immune related genes—cellular immunity		
CG8175	<i>Metchnikowin</i>	7.31
CG10794	<i>Diptericin B</i>	4.97
CG9496	<i>Tetraspanin 29Fb</i>	4.53
CG1364	<i>Cecropin A1</i>	4.04
CG1367	<i>Cecropin A2</i>	3.33
CG32185	<i>Elevated during infection</i>	3.17
CG10146	<i>Attacin-A</i>	2.74
CG7629	<i>Attacin-D</i>	2.72
CG16844	<i>Bomanin Short 3</i>	2.67
CG1373	<i>Cecropin C</i>	2.52
CG14704	<i>Peptidoglycan recognition protein LB</i>	2.07
Cell migration / cytoskeleton reorganization		
CG31004	<i>mesh</i>	3.47
CG6976	<i>Myosin 28B1</i>	2.03
Stress response		
CG9434	<i>Frost</i>	2.99
CG3050	<i>Cyp6d5</i>	2.42
Glucose metabolism		
CG8693	<i>Maltase A4</i>	4.59
CG11909	<i>Target of brain insulin</i>	3.89

<https://doi.org/10.1371/journal.pone.0235294.t003>

Table 4. Selected DEGs of interest with Fold-changes of *S. aureus* hemocytes vs. Clean injury hemocytes from S7 File.

CG	Full name	FC
Immune related genes—cellular immunity		
CG32185	<i>elevated during infection</i>	3.96
CG8175	<i>Metchnikowin</i>	3.57
CG12143	<i>Tetraspanin42Ej</i>	2.37
CG15917	<i>Growth-blocking peptide 1</i>	2.29
CG10794	<i>Diptericin B</i>	2.19
CG16844	<i>Bomanin Short 3</i>	2.08
CG12840	<i>Tetraspanin 42El</i>	2.02
Cell migration / cytoskeleton reorganization		
CG32082	<i>Insulin receptor substrate 53 kDa</i>	2.71
Extracellular matrix components		
CG6281	<i>Tissue inhibitor of metalloproteases</i>	2.67
CG42768	<i>Muscle-specific protein 300 kDa</i>	2.55
Stress response—cell death		
CG4319	<i>reaper</i>	2.59
CG10391	<i>Cyp310a1</i>	2.43
CG8453	<i>Cyp6g1</i>	2.40
Oxidative stress response		
CG12242	<i>Glutathione S transferase D5</i>	2.02

<https://doi.org/10.1371/journal.pone.0235294.t004>

whereas in SaH samples, we identified 103 up-regulated genes and 81 down-regulated genes (see S6 and S7 Files). The two significantly enriched GO component categories upon infection with *E. coli* or *S. aureus* correspond to secreted components (GO term GO:0005615 and GO:0044421) and an “antibacterial humoral response” (GO:0019731) which is consistent with an increased expression of antimicrobial peptides (AMP) upon infection compared to clean injury (Fig 3, S6 and S7 Files, see selections in Tables 3 and 4).

Our RNAseq study reveals a small subset of genes that were induced upon both *E. coli* and *S. aureus*, notably the antimicrobial peptide coding gene *Metchnikowin* (*Mtk*, against Gram-positive and fungi, FC 7.3) and surprisingly, many genes annotated as 18S or 28S ribosomal RNA pseudogenes. Challenge with *E. coli* leads to specific induction of several Imd target genes, notably the antibacterial peptides *Diptericin B*, *Cecropins A1, A2 and C*, *Attacins A* and *D*, as well as *PGRP-LB*, a gene encoding a negative regulator of the Imd pathway that scavenges peptidoglycan [116]. Another immunity gene, *edin*, was also upregulated in EcH samples compared to clean injury samples (FC: 3.1). *Edin* has previously been described as upregulated in S2 cells upon *E. coli* infection and is needed for the increase in plasmatocyte numbers and for the release of sessile hemocytes into the hemolymph upon wasp infection [117]. Thus, the increase in *edin* could reflect the mobilization of sessile hemocytes into circulation. It is important to note the down-regulation of several heat-shock protein genes such as *Hsp27*, *Hsp70ab*, and *Hsp70Bc* in the EcH samples (FCs: -2.0, -3.0 and -3.0). Thus, septic injury with *E. coli* tends to orient the hemocyte towards an antibacterial response while clean injury directs a stress and repair response.

As in EcH samples, SaH samples also show an enrichment of GO processes associated with the immune response, such as the up-regulation of the *Metchnikowin* gene but also *Diptericin B* (FC: 3.57 and 2.19) and one Bomanin gene: *Bomanin Short 3* (*BomS3*, FC: 2.0). The specific induction of antibacterial peptide genes (*AttD*, *Cec*), known to be regulated by the Imd pathway in response to *E. coli* but not *S. aureus* indicates that the hemocytes can mount a

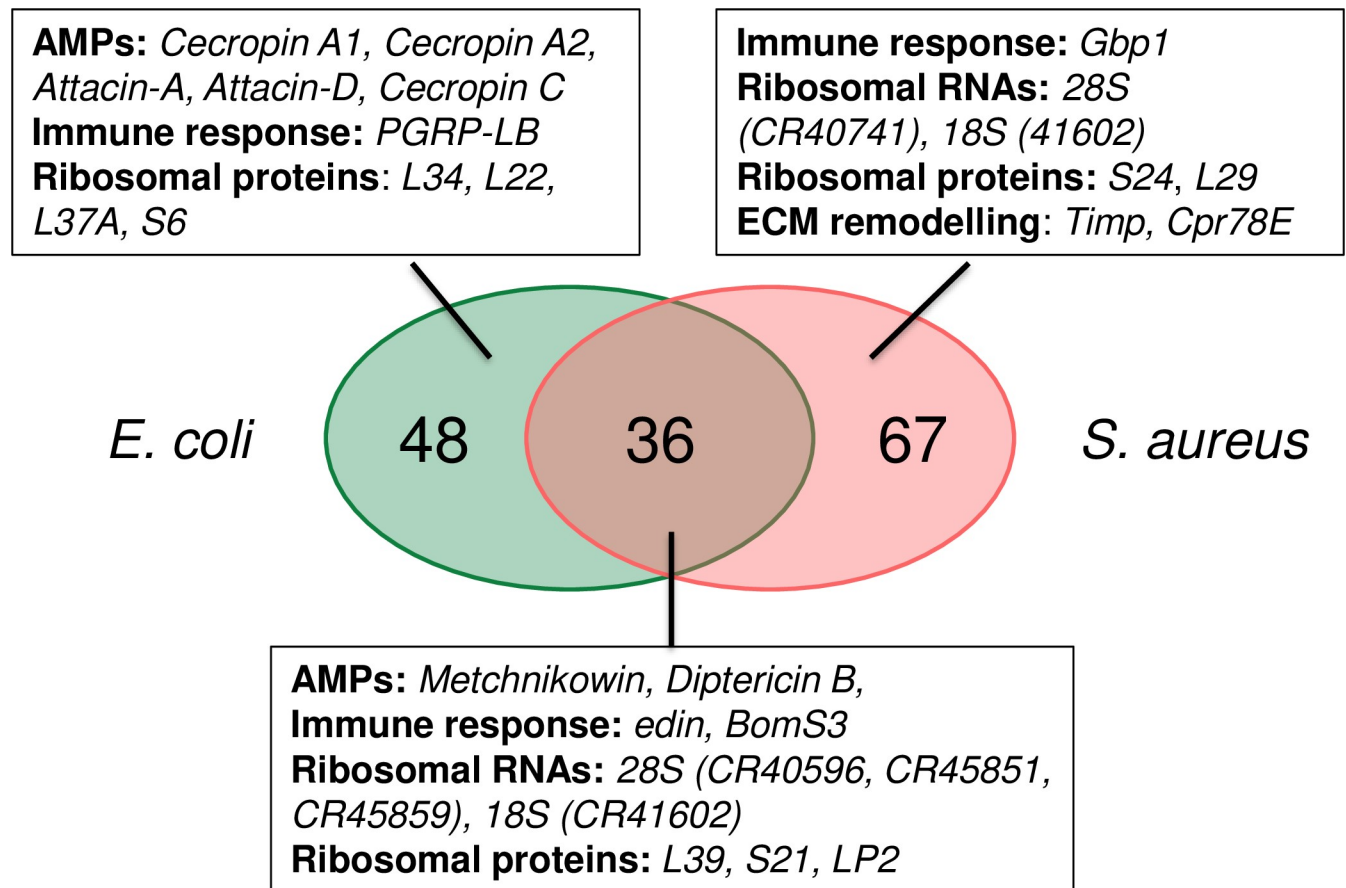


Fig 3. Genes induced in hemocytes upon bacterial infection. Venn diagram illustrating genes that were enriched in hemocytes from larvae pricked with *Escherichia coli* (*E. coli*) or *Staphylococcus aureus* (*S. aureus*) compared to clean-pricked larvae (CI).

<https://doi.org/10.1371/journal.pone.0235294.g003>

differentiated response to these two bacteria within 45 minutes. We also observed an increase in expression of *Growth-blocking peptide 1* (*Gbp1*). *Gbp1* was characterized as a cytokine that plays a role in IMD activation upon Gram-negative bacterial challenge in both the fat body and hemocytes [118]. Our study rather points to a specific induction of *Gbp1* in response to a Gram-positive bacterial challenge. Several genes encoding proteins that could function in phagocytosis such as *Biogenesis of lysosome-related organelles complex 1, subunit 2*, and *Tetra-spanin 42E1* were specifically enriched by two-fold in *S. aureus* versus clean injury. Several genes implicated in cell division were down-regulated in *S. aureus* samples compared to clean injury samples, such as *mitotic spindle* and *nuclear protein (mink, -2.4 fold change)* [119], *stathmin (stai, -3.8 fold change)* [120] and *cyclin B (cycB, -2.2 fold change)* [121] suggesting a cell cycle arrest in response to infection with this Gram-positive bacterium (S7 File) [122]. Surprisingly, the gene encoding the lipase *Magro* was expressed 14 times less upon systemic infection with *S. aureus* compared to clean injury or EcH. Thus, at the 45 minutes time point, a challenge by *S. aureus* tends to orient plasmatocytes towards a lower production of secreted factors and decreased lipid catabolism. We hypothesize that plasmatocytes contribute to host defense in different ways against *S. aureus* and *E. coli*. Phagocytosis of *S. aureus* may not be compatible with cell division. Decreased lipase activity may reflect a reduced energy demand of these plasmatocytes compared to energetically expensive AMP production in those infected with *E. coli*.

Discussion

The *Drosophila* immune response has been the focus of extensive genome-wide gene expression studies that open the route to successful post-genomic functional characterization of novel immune genes [123]. In contrast, transcriptome studies of hemocytes have been rather limited, or have used S2 or *mbn-2* hemocyte-derived cell lines that do not reflect an integrated model [2,124,125]. This was mostly due to the difficulties in collecting enough pure material, as hemocytes represent a tiny fraction of *Drosophila* larvae. Recently, FACS sorting of hemocytes, and the use of new hemocyte-specific markers has facilitated the extraction of plasmatocytes. Taking advantage of this, we performed a comprehensive RNAseq analysis of Hml+ plasmatocytes in absence of infection and 45 minutes following clean injury or septic injury with *E. coli* or *S. aureus*. We found that FACS purification did not affect the lineage characteristics of the sample allowing us to use these very pure populations to characterize transcriptomic variation in Hml+ positive plasmatocytes. As Hml+ cells are widely used to study hemocyte function such as adhesion, sessility, metabolism and phagocytosis, our dataset is an important contribution to the community. Our RNAseq study also complements a recent single cell analysis of *Drosophila* hemocytes that has revealed the various states of hemocyte differentiation [36,37,38,39]. It is important to note that our chosen experimental design may have affected the transcriptome of unchallenged hemocytes slightly. We incubated all larvae, previously reared at 25°C, for 45 minutes at 29°C after treatment to accelerate the transcriptional response, which might result in a small heat shock induction. Despite this limitation, comparisons between conditions revealed interesting patterns of expression that could be useful to further functional studies of hemocytes.

Our study provides the full repertoire of genes expressed in plasmatocytes and their expression levels, notably those encoding transmembrane proteins and secreted factors (S3 and S4 Files). Screening these genes in future studies will allow a better characterization of plasmatocyte functions in phagocytosis, migration, and sessility and a greater understanding of how these motile cells interact with other tissues. Consistent with a previous Affymetrix based study [2] and a recent single cell study [36], we found that plasmatocytes express a large repertoire of phagocytic receptors, opsonins and cytoskeleton proteins, reflecting their important function as phagocytes. Consistent with older studies that demonstrated that hemocytes produce antimicrobial peptides upon challenge [30,42,126], we confirmed that hemocytes express genes encoding several components of the Toll and Imd pathways, as well as some of their downstream target genes. They have the ability to induce a subset of antimicrobial peptides upon challenge. Use of AMP-reporter genes [42,126] and recent single cell analysis [36] has shown that only a fraction of plasmatocytes express antibacterial peptides upon immune challenge, indicating that some sub-populations of plasmatocytes are specialized for this task. One of the most surprising observations is the high expression of Attacin-D in plasmatocytes, as this antibacterial peptide is devoid of a signal peptide. The precise role of this AMP is unknown, and further studies should decipher whether it is secreted by an atypical mechanism or if it functions intracellularly. Our study confirms that plasmatocytes contribute to clotting and melanization, two important hemolymph immune functions [127]. Surprisingly, our RNAseq analysis detected an unexpected expression of PPO2 and PPO3 in Hml+ plasmatocytes, which were previously shown to be specific to crystal cells and lamellocytes. As single cell analysis studies have confirmed that PPO2 and PPO3 are indeed restricted to crystal cells and lamellocytes [36], the high expression of PPO2 and PPO3 in our sample can be explained by the presence of Hml+ plasmatocytes that are undergoing their transdifferentiation into crystal cells and lamellocytes [27,28,99]. The fat body, particularly at the larval stage, is the main organ producing hemolymphatic proteins. Our study confirms that like the fat body,

Hml+ plasmatocytes also express a large repertoire of secreted proteins, notably components of the extracellular matrix and opsonins. It is currently unclear how the synthesis of hemolymphatic protein is allocated between the fat body and plasmatocytes, or why plasmatocytes are involved in the secretion of molecules such as extracellular matrix components or AMPs. An interesting hypothesis is that plasmatocytes, by virtue of their migratory ability, function as local repairers that can locally supply and enrich specific factors. Indeed, a recent study indicated that a distinct pool of plasmatocytes, the "companion plasmatocytes" expressing collagen IV, are tightly associated with the developing ovaries from larval stages and onward [128]. Eliminating these companion plasmatocytes or specifically blocking their collagen IV expression during larval stages causes abnormal ovarian niches with excess stem cells in adults. This suggests that hemocytes could have short-range action consistent with the notion of local repairers. Deciphering specific roles for the hemocytes and fat body in the production of hemolymphatic proteins is an interesting prospect.

Despite extensive research on *Drosophila melanogaster* blood cell adaptation to wounding [33,129] only rare transcriptomic analyses has been performed, and have specifically analyzed their response to clean injury. One main study performed in 2008 tried to provide insight into specific hemocyte response to wounding. Stramer *et al.* compared transcriptional responses of wild-type embryos and *serpent* null embryos, which are devoid of hemocytes, 45 minutes following wounding. They identified a limited number of significantly affected genes, probably because of a dilution effect due to the limited number of hemocytes overall in embryos [88]. However, they identified the up-regulation of secreted phospholipase A2 that plays a role in the production of eicosanoids, key signaling molecules that limit inflammation [130]. In our study, we observed that the gene encoding the calcium-independent phospholipase A2 VIA was strongly induced upon wounding. This phospholipase has been shown in mammals to promote adhesion, clearance of debris and ROS production to act as a chemoattractant [131]. In contrast to the work done by Stramer *et al.*, we did not find any induction of *Drosomyacin* in the wounding condition, likely due to the short time point we used. Morphological studies have shown that hemocytes modify their shape, change their adhesive properties and start to transdifferentiate into plasmatocytes and lamellocytes upon clean injury. A recent single cell analysis has deciphered some of the changes that take place in hemocyte populations 24 hours after clean injury, reflecting a change in their differentiated states [37]. Our study reveals that major transcriptome changes have already begun 45 minutes post-challenge. These changes likely reflect the transformation of resting plasmatocytes into an activated form, and their differentiation into more specialized hemocyte sub-types. The observation that the FGF ligand Pyramus that mediates blood cell progenitors differentiation in the lymph gland [50] was the most up-regulated gene 45 minutes after clean injury suggests that FGF-R pathway activation could play a prominent role in promoting the differentiation of peripheral plasmatocytes upon injury. In this work, we also show that wounding reduces apoptotic processes in blood cells while promoting cell proliferation, consistent with a previous study showing that wounding stimulates *de-novo* peripheral blood cell proliferation [132]. In accordance with these data, we observed the up-regulation of *pvf2* [102] and the PVR adaptor [133] upon clean injury. Interestingly, the PVF pathway also plays a role in hemocyte migration, as Pvf2 acts as a chemoattractant [104]. It is possible that hemocytes in close contact to the wounding site stimulate nearby hemocytes to improve wound healing and accelerate repair processes. Our results also show that plasmatocytes, like mammalian macrophages, undergo major metabolic reprogramming following injury that likely fuels their transformation into an 'activated' plasmatocyte state that is more effective at producing secreted factors or engulfing bacteria. Future studies should better characterize how immune functions are coupled with metabolic reprogramming in hemocytes.

Finally, our study reveals that hemocytes can mount specific responses to different pathogens such as *E. coli* and *S. aureus*. The early time point we chose likely prevented us from fully capturing this differentiation. The polarization of T helper cells into sub-categories in response to different cytokine environments is well established. This concept has recently been extended to innate immune cells in mammals, notably to macrophages with their M1 pro-inflammatory and M2 pro repair subtypes [134]. It is tempting to speculate that *Drosophila* plasmatocytes can be polarized toward different functions such as enhanced production of antibacterial peptides or phagocytosis according to different inflammation and metabolic states. This would explain the existence of various plasmatocyte populations in different activity states [36,37,38,39]. In this vein, it would be interesting to further characterize the transcriptome of plasmatocytes in response to other challenges such as phagocytosis of apoptotic cells and yeast. Another interesting prospect is to decipher whether sessile and circulating plasmatocytes differ in their transcriptional activities. Collectively, our study and a recent single cell analysis underline the complexities of the cellular response and open the route to functional analysis.

Material and methods

Drosophila stocks and rearing

In this work, we used *w;Hml^A.DsRed.nls* line. Animals were reared on standard fly medium comprising 6% cornmeal, 6% yeast, 0.62% agar, and 0.1% fruit juice, supplemented with 10.6g/L moldex and 4.9ml/L propionic acid. Flies are maintained at 25°C on a 12 h light/ 12 h dark cycle. Both males and females were used for experiments.

Microorganism culture and infection experiments

The bacterial strains used and the respective optical density (O.D.) of the pellet at 600 nm were: *Staphylococcus aureus* (O.D. 0.5) and *Escherichia coli* (O.D. 0.5). L3 wandering larvae were pricked with a tungsten needle on the dorsal side, at the origin of the two trachea, corresponding to the A7 or A8 segments. Pricked larvae were placed into a small petri dish with fresh medium and incubated at 29°C for 45 minutes. We then dissected larvae on a glass slide in a 120 ul PBS droplet before cell sorting.

Cell sorting procedure

Cell sorting was performed on a BD FACS Aria II (Becton Dickinson, San Jose, CA, USA) fitted with a 100 µm nozzle and with pressure set at 20 PSI. The machine temperature was lowered at 4°C, samples were recovered in Eppendorf tubes kept on ice and immediately resuspended in TRIzol™ (#15596018, Thermo Fisher Scientific, Waltham, MA, USA). Hemocytes were selected and sorted based on DsRed fluorescence.

RNA extraction, sequencing and analysis

For whole larva RNA extraction, 20 animals were homogenized in tubes with glass beads and lysed with the use of a PRECELLYS™ homogenizer, with 0.5 mL of TRIzol™ reagent and 0.3 mL of chloroform. For recovery of hemocytes during FACS procedure, cells were directly resuspended in the same mix of TRIzol™-chloroform. RNA was extracted following the classical phenol-chloroform RNA extraction technique.

For all samples, RNA quality was assessed on a Fragment Analyzer (Agilent Technologies, Inc., Santa Clara, CA 95051, USA). RNA-seq libraries were prepared using 73–100 ng of total RNA and the Illumina TruSeq Stranded mRNA reagents (Illumina; San Diego, California, USA) according to the supplier's instructions. Cluster generation was performed with the

resulting libraries using the Illumina TruSeq SR Cluster Kit v4 reagents and sequenced on the Illumina HiSeq 2500 using TruSeq SBS Kit v4 reagents. Sequencing data were demultiplexed using the bcl2fastq Conversion Software (v. 2.20, Illumina; San Diego, California, USA).

The quality of the resulting reads was assessed with ShortRead (v. 1.28.0) [135]. Reads were then aligned to the reference genome (*Drosophila_melanogaster* BDGP6 dna.toplevel.fa) with TopHat (v2.1.0) and Bowtie (2.2.6.0). Mapping over exon-exon junctions was permitted by supplementing annotations (*Drosophila_melanogaster* BDGP6.87 GTF). The reads acquired in this way were used to create the lists of expressed genes (cutoff 5 counts per million [CPM] in the average of all triplicates) for each respective treatment. Unwanted variation from this data was removed by using RUVSeq (3.10) by estimating the factors of unwanted variation using residuals [136]. Differential expression analysis was performed with edgeR (3.26.4) [137].

Gene Ontology (GO) analysis was performed with Gorilla (online version <http://cbl-gorilla.cs.technion.ac.il/> - July and August 2019). Two unranked lists of genes were compared, where the background set of genes was all genes with expressed with minimum of 5 CPM reads from all three combined unchallenged hemocyte reads. The target set of genes was determined by the results of the differential expression analysis of the respective treatment with the following cutoffs: CPM > 5, P-value < 0.05, FC > +/-1.88. Fold changes are expressed as real values and Log2 based values.

Supporting information

S1 Fig. GO terms enriched in unchallenged hemocytes compared to whole larva.
(TIF)

S2 Fig. GO terms enriched in clean injury hemocytes compared to unchallenged hemocytes.
(TIF)

S1 Table. Selected DEGs of interest linked to extracellular matrix reorganization, extracted from the comparison of clean injury hemocytes vs. unchallenged hemocytes.
(DOCX)

S1 File. Raw reads numbers in all samples (L3, UC, CI, *E coli* and *S aureus*). Average of the number of mapped reads per million reads in the respective triplicate samples. GO terms were extracted from Flybase.
(XLSX)

S2 File. Differentially expressed genes in unchallenged hemocytes versus unchallenged whole larvae samples. Results of the differential gene expression analysis between unchallenged plasmatocytes and L3 whole larvae. Cut-off values are fold change ≥ 2 , logCPM > 2, P-value < 0.05.
(XLSX)

S3 File. Transmembrane proteins coding genes identified in S2 File. Differentially expressed genes between unchallenged hemocytes and L3 whole larvae which have the GO term 'integral component of plasma membrane' or 'cell surface'. Cut-off values are fold changes ≥ 2 , logCPM > 2, P-value < 0.05. Positive FC values indicate higher expression in plasmatocytes versus L3.
(XLSX)

S4 File. Secreted proteins coding genes identified in S2 File. Differentially expressed genes between L3 whole larvae and unchallenged hemocytes that have the GO term 'extracellular space' or 'extracellular region', which identifies putative secreted proteins regardless of the

presence of a signal peptide. Cut-off values are fold changes ≥ 2 , logCPM > 2 , P-value < 0.05 . Positive FC values indicate higher expression in plasmatocytes versus L3.

(XLSX)

S5 File. Differentially expressed genes in clean injury samples versus unchallenged samples. Results of the differential gene expression analysis between unchallenged hemocytes and hemocytes from animals with clean injury. Cut-off values are fold changes ≥ 2 , logCPM > 2.3 , P-value < 0.05 . Positive FC values indicate higher expression in clean injury samples versus unchallenged samples.

(XLSX)

S6 File. Differentially expressed genes in *E. coli* samples versus clean injury samples. Results of the differential gene expression analysis between hemocytes from animals infected with *E. coli* and hemocytes from animals with clean injury. Cut-off values are fold changes ≥ 2 , logCPM > 2.3 , P-value < 0.05 . Positive FC values indicate higher expression in *E. coli* samples versus clean injury samples.

(XLSX)

S7 File. Differentially expressed genes in *S. aureus* samples versus clean injury samples. Results of the differential gene expression analysis between hemocytes from animals infected with *S. aureus* and hemocytes from animals with clean injury. Cutoff values are fold changes ≥ 2 , logCPM > 2.3 , P-value < 0.05 . Positive FC values indicate higher expression in *S. aureus* samples versus clean injury samples.

(XLSX)

Acknowledgments

We thank Hannah Westlake and Samuel Rommelaere for useful comments on the manuscript. We also thank the EPFL Flow Cytometry Core Facility (FCCF) for help in cell sorting and especially Loïc Tauzin and André Mozes. We thank the Lausanne Genomic Technologies Facility (UNIL, Lausanne, Switzerland) for RNA sequencing and especially Johann Weber.

Author Contributions

Conceptualization: Elodie Ramond, Jan Paul Dudzic, Bruno Lemaitre.

Data curation: Jan Paul Dudzic, Bruno Lemaitre.

Formal analysis: Elodie Ramond, Jan Paul Dudzic, Bruno Lemaitre.

Funding acquisition: Bruno Lemaitre.

Investigation: Elodie Ramond, Jan Paul Dudzic, Bruno Lemaitre.

Methodology: Elodie Ramond, Jan Paul Dudzic, Bruno Lemaitre.

Project administration: Bruno Lemaitre.

Resources: Bruno Lemaitre.

Supervision: Bruno Lemaitre.

Validation: Bruno Lemaitre.

Visualization: Elodie Ramond, Jan Paul Dudzic, Bruno Lemaitre.

Writing – original draft: Elodie Ramond, Jan Paul Dudzic, Bruno Lemaitre.

Writing – review & editing: Elodie Ramond, Jan Paul Dudzic, Bruno Lemaitre.

References

1. Morin-Poulard I, Vincent A, Crozatier M. The Drosophila JAK-STAT pathway in blood cell formation and immunity. *JAKSTAT*. 2013; 2: e25700. <https://doi.org/10.4161/jkst.25700> PMID: 24069567
2. Irving P, Ubeda J-M, Doucet D, Troxler L, Lagueux M, Zachary D, et al. New insights into Drosophila larval haemocyte functions through genome-wide analysis. *Cell Microbiol*. John Wiley & Sons, Ltd (10.1111); 2005; 7: 335–350. <https://doi.org/10.1111/j.1462-5822.2004.00462.x> PMID: 15679837
3. Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N. Signaling role of hemocytes in Drosophila JAK/STAT-dependent response to septic injury. *Dev Cell*. 2003; 5: 441–450. [https://doi.org/10.1016/s1534-5807\(03\)00244-2](https://doi.org/10.1016/s1534-5807(03)00244-2) PMID: 12967563
4. Wang L, Kounatidis I, Ligoxygakis P. Drosophila as a model to study the role of blood cells in inflammation, innate immunity and cancer. *Front Cell Infect Microbiol*. Frontiers; 2014; 3: 113. <https://doi.org/10.3389/fcimb.2013.00113> PMID: 24409421
5. Evans IR, Wood W. Drosophila blood cell chemotaxis. *Curr Opin Cell Biol*. 2014; 30: 1–8. <https://doi.org/10.1016/j.ceb.2014.04.002> PMID: 24799191
6. Chakrabarti S, Dudzic JP, Li X, Collas EJ, Boquete J-P, Lemaitre B. Remote Control of Intestinal Stem Cell Activity by Haemocytes in Drosophila. Banerjee U, editor. *PLoS Genet*. 2016; 12: e1006089. <https://doi.org/10.1371/journal.pgen.1006089> PMID: 27231872
7. Arefin B, Kucerova L, Dobes P, Márkus R, Strnad H, Wang Z, et al. Genome-wide transcriptional analysis of Drosophila larvae infected by entomopathogenic nematodes shows involvement of complement, recognition and extracellular matrix proteins. *J Innate Immun*. Karger Publishers; 2014; 6: 192–204. <https://doi.org/10.1159/000353734> PMID: 23988573
8. Sánchez-Sánchez BJ, Urbano JM, Comber K, Dragu A, Wood W, Stramer B, et al. Drosophila Embryonic Hemocytes Produce Laminins to Strengthen Migratory Response. *Cell Rep*. 2017; 21: 1461–1470. <https://doi.org/10.1016/j.celrep.2017.10.047> PMID: 29117553
9. Binggeli O, Neyen C, Poidevin M, Lemaitre B. Prophenoloxidase activation is required for survival to microbial infections in Drosophila. Schneider DS, editor. *PLoS Pathog*. 2014; 10: e1004067. <https://doi.org/10.1371/journal.ppat.1004067> PMID: 24788090
10. Dudzic JP, Kondo S, Ueda R, Bergman CM, Lemaitre B. Drosophila innate immunity: regional and functional specialization of prophenoloxidases. *BMC Biol*. BioMed Central; 2015; 13: 81. <https://doi.org/10.1186/s12915-015-0193-6> PMID: 26437768
11. Dolezal T, Krejcová G, Bajgar A, Nedbalová P, Strasser P. Molecular regulations of metabolism during immune response in insects. *Insect Biochem Mol Biol*. 2019; 109: 31–42. <https://doi.org/10.1016/j.ibmb.2019.04.005> PMID: 30959109
12. Shin M, Cha N, Koranteng F, Cho B, Shim J. Subpopulation of Macrophage-Like Plasmatocytes Attenuates Systemic Growth via JAK/STAT in the Drosophila Fat Body. *Front Immunol*. Frontiers; 2020; 11: 63. <https://doi.org/10.3389/fimmu.2020.00063> PMID: 32082322
13. Green N, Walker J, Bontrager A, Zych M, Geisbrecht ER. A tissue communication network coordinating innate immune response during muscle stress. *J Cell Sci*. 2018; 131: jcs217943. <https://doi.org/10.1242/jcs.217943> PMID: 30478194
14. Mihajlovic Z, Tanasic D, Bajgar A, Perez-Gomez R, Steffal P, Krejci A. Lime is a new protein linking immunity and metabolism in Drosophila. *Dev Biol*. 2019; 452: 83–94. <https://doi.org/10.1016/j.ydbio.2019.05.005> PMID: 31085193
15. Ramond E, Petrigiani B, Dudzic JP, Boquete J-P, Poidevin M, Kondo S, et al. The adipokine NimrodB5 regulates peripheral hematopoiesis in Drosophila. *FEBS J*. 2020; 78: 1207. <https://doi.org/10.1111/febs.15237> PMID: 32009293
16. Holz A, Bossinger B, Strasser T, Janning W, Klapper R. The two origins of hemocytes in Drosophila. *Development*. The Company of Biologists Ltd; 2003; 130: 4955–4962. <https://doi.org/10.1242/dev.00702> PMID: 12930778
17. Lanot R, Zachary D, Holder F, Meister M. Postembryonic hematopoiesis in Drosophila. *Dev Biol*. 2001; 230: 243–257. <https://doi.org/10.1006/dbio.2000.0123> PMID: 11161576
18. Evans CJ, Hartenstein V, Banerjee U. Thicker than blood: conserved mechanisms in Drosophila and vertebrate hematopoiesis. *Dev Cell*. 2003; 5: 673–690. [https://doi.org/10.1016/s1534-5807\(03\)00335-6](https://doi.org/10.1016/s1534-5807(03)00335-6) PMID: 14602069
19. Jung S-H, Evans CJ, Uemura C, Banerjee U. The Drosophila lymph gland as a developmental model of hematopoiesis. *Development*. The Company of Biologists Ltd; 2005; 132: 2521–2533. <https://doi.org/10.1242/dev.01837> PMID: 15857916

20. Crozatier M, Meister M. *Drosophila* haematopoiesis. Cell Microbiol. Blackwell Publishing Ltd; 2007; 9: 1117–1126. <https://doi.org/10.1111/j.1462-5822.2007.00930.x> PMID: 17394559
21. Honti V, Csordás G, Márkus R, Kurucz É, Jankovics F, Andó I. Cell lineage tracing reveals the plasticity of the hemocyte lineages and of the hematopoietic compartments in *Drosophila melanogaster*. Mol Immunol. 2010; 47: 1997–2004. <https://doi.org/10.1016/j.molimm.2010.04.017> PMID: 20483458
22. Makhijani K, Alexander B, Tanaka T, Rulifson E, Brückner K. The peripheral nervous system supports blood cell homing and survival in the *Drosophila* larva. Development. Oxford University Press for The Company of Biologists Limited; 2011; 138: 5379–5391. <https://doi.org/10.1242/dev.067322> PMID: 22071105
23. Makhijani K, Brückner K. Of blood cells and the nervous system: hematopoiesis in the *Drosophila* larva. Fly (Austin). Taylor & Francis; 2012; 6: 254–260. <https://doi.org/10.4161/fly.22267> PMID: 23022764
24. Makki R, Cinnamon E, Gould AP. The development and functions of oenocytes. Annu Rev Entomol. Annual Reviews; 2014; 59: 405–425. <https://doi.org/10.1146/annurev-ento-011613-162056> PMID: 24397521
25. Babcock DT, Brock AR, Fish GS, Wang Y, Perrin L, Krasnow MA, et al. Circulating blood cells function as a surveillance system for damaged tissue in *Drosophila* larvae. Proc Natl Acad Sci USA. 2008; 105: 10017–10022. <https://doi.org/10.1073/pnas.07099511105> PMID: 18632567
26. Welman A, Serrels A, Brunton VG, Ditzel M, Frame MC. Two-color photoactivatable probe for selective tracking of proteins and cells. J Biol Chem. 2010; 285: 11607–11616. <https://doi.org/10.1074/jbc.M110.102392> PMID: 20139076
27. 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: 4805–4809. <https://doi.org/10.1073/pnas.0801766106> PMID: 19261847
28. Leitão AB, Sucena E. *Drosophila* sessile hemocyte clusters are true hematopoietic tissues that regulate larval blood cell differentiation. Elife. eLife Sciences Publications Limited; 2015; 4: 239. <https://doi.org/10.7554/eLife.06166> PMID: 25650737
29. Ghosh S, Singh A, Mandal S, Mandal L. Active hematopoietic hubs in *Drosophila* adults generate hemocytes and contribute to immune response. Dev Cell. 2015; 33: 478–488. <https://doi.org/10.1016/j.devcel.2015.03.014> PMID: 25959225
30. Sanchez Bosch P, Makhijani K, Herboso L, Gold KS, Baginsky R, Woodcock KJ, et al. Adult *Drosophila* Lack Hematopoiesis but Rely on a Blood Cell Reservoir at the Respiratory Epithelia to Relay Infection Signals to Surrounding Tissues. Dev Cell. 2019. <https://doi.org/10.1016/j.devcel.2019.10.017> PMID: 31735669
31. Rizki TM, Rizki RM, Grell EH. A mutant affecting the crystal cells in *Drosophila melanogaster*. Wilhelm Roux Arch Dev Biol. 2nd ed. Springer-Verlag; 1980; 188: 91–99. <https://doi.org/10.1007/BF00848799> PMID: 28304971
32. Rizki TM, Rizki RM. Lamellocyte differentiation in *Drosophila* larvae parasitized by *Leptopilina*. Dev Comp Immunol. 1992; 16: 103–110. [https://doi.org/10.1016/0145-305x\(92\)90011-z](https://doi.org/10.1016/0145-305x(92)90011-z) PMID: 1499832
33. Vlisidou I, Wood W. *Drosophila* blood cells and their role in immune responses. FEBS J. 2015; 282: 1368–1382. <https://doi.org/10.1111/febs.13235> PMID: 25688716
34. Anderl I, Vesala L, Ihalainen TO, Vanha-Aho L-M, Andó I, Rämetsä M, et al. Transdifferentiation and Proliferation in Two Distinct Hemocyte Lineages in *Drosophila melanogaster* Larvae after Wasp Infection. Schneider DS, editor. PLoS Pathog. 2016; 12: e1005746. <https://doi.org/10.1371/journal.ppat.1005746> PMID: 27414410
35. Tepass U, Fessler LI, Aziz A, Hartenstein V. Embryonic origin of hemocytes and their relationship to cell death in *Drosophila*. Development. 1994; 120: 1829–1837. PMID: 7924990
36. Cattenoz PB, Sakr R, Pavlidaki A, Delaporte C, Riba A, Molina N, et al. Temporal specificity and heterogeneity of *Drosophila* immune cells. EMBO J. 2020; 147: e104486. <https://doi.org/10.15252/embj.2020104486> PMID: 32162708
37. Tattikota SG, Cho B, Liu Y, Hu Y, Barrera V, Steinbaugh MJ, et al. A single-cell survey of *Drosophila* blood. Elife. eLife Sciences Publications Limited; 2020; 9: 597. <https://doi.org/10.7554/eLife.54818> PMID: 32396065
38. Fu Y, Huang X, Zhang P, van de Leemput J, Han Z. Single-cell RNA sequencing identifies novel cell types in *Drosophila* blood. Journal of Genetics and Genomics. 2020. <https://doi.org/10.1016/j.jgg.2020.02.004>
39. Cho B, Yoon S-H, Lee D, Koranteng F, Tattikota SG, Cha N, et al. Single-cell transcriptome maps of myeloid blood cell lineages in *Drosophila*. bioRxiv. Cold Spring Harbor Laboratory; 2020; 127: 2020.01.15.908350. <https://doi.org/10.1101/2020.01.15.908350>

40. Goto A, Kadowaki T, Kitagawa Y. Drosophila hemolymph gene is expressed in embryonic and larval hemocytes and its knock down causes bleeding defects. *Dev Biol.* 2003; 264: 582–591. <https://doi.org/10.1016/j.ydbio.2003.06.001> PMID: 14651939
41. Evans CJ, Liu T, Banerjee U. Drosophila hematopoiesis: Markers and methods for molecular genetic analysis. *Methods.* 2014; 68: 242–251. <https://doi.org/10.1016/j.ymeth.2014.02.038> PMID: 24613936
42. Samakovlis C, Asling B, Boman HG, Gateff E, Hultmark D. In vitro induction of cecropin genes—an immune response in a Drosophila blood cell line. *Biochem Biophys Res Commun.* 1992; 188: 1169–1175. [https://doi.org/10.1016/0006-291x\(92\)91354-s](https://doi.org/10.1016/0006-291x(92)91354-s) PMID: 1445351
43. Braun A, Lemaitre B, Lanot R, Zachary D, Meister M. Drosophila immunity: analysis of larval hemocytes by P-element-mediated enhancer trap. *Genetics.* Genetics Society of America; 1997; 147: 623–634. PMID: 9335599
44. Lebestky T, Chang T, Hartenstein V, Banerjee U. Specification of Drosophila hematopoietic lineage by conserved transcription factors. *Science.* 2000; 288: 146–149. <https://doi.org/10.1126/science.288.5463.146> PMID: 10753120
45. Sorrentino RP, Tokusumi T, Schulz RA. The Friend of GATA protein U-shaped functions as a hematopoietic tumor suppressor in Drosophila. *Dev Biol.* 2007; 311: 311–323. <https://doi.org/10.1016/j.ydbio.2007.08.011> PMID: 17936744
46. Sinenko SA, Kim EK, Wynn R, Manfrulli P, Andó I, Wharton KA, et al. Yantar, a conserved arginine-rich protein is involved in Drosophila hemocyte development. *Dev Biol.* 2004; 273: 48–62. <https://doi.org/10.1016/j.ydbio.2004.05.022> PMID: 15302597
47. Alfonso TB, Jones BW. gcm2 promotes glial cell differentiation and is required with glial cells missing for macrophage development in Drosophila. *Dev Biol.* 2002; 248: 369–383. <https://doi.org/10.1006/dbio.2002.0740> PMID: 12167411
48. Banerjee U, Girard JR, Goins LM, Spratford CM. Drosophila as a Genetic Model for Hematopoiesis. *Genetics.* Genetics; 2019; 211: 367–417. <https://doi.org/10.1534/genetics.118.300223> PMID: 30733377
49. Hao Y, Jin LH. Dual role for Jumu in the control of hematopoietic progenitors in the Drosophila lymph gland. *eLife.* eLife Sciences Publications Limited; 2017; 6: 97. <https://doi.org/10.7554/eLife.25094> PMID: 28350299
50. Dragojlovic-Munther M, Martinez-Agosto JA. Extracellular matrix-modulated Heartless signaling in Drosophila blood progenitors regulates their differentiation via a Ras/ETS/FOG pathway and target of rapamycin function. *Dev Biol.* 2013; 384: 313–330. <https://doi.org/10.1016/j.ydbio.2013.04.004> PMID: 23603494
51. Pennetier D, Oyallon J, Morin-Poulard I, Dejean S, Vincent A, Crozatier M. Size control of the Drosophila hematopoietic niche by bone morphogenetic protein signaling reveals parallels with mammals. *Proc Natl Acad Sci USA.* National Academy of Sciences; 2012; 109: 3389–3394. <https://doi.org/10.1073/pnas.1109407109> PMID: 22331866
52. Minakhina S, Tan W, Steward R. JAK/STAT and the GATA factor Pannier control hemocyte maturation and differentiation in Drosophila. *Dev Biol.* 2011; 352: 308–316. <https://doi.org/10.1016/j.ydbio.2011.01.035> PMID: 21295568
53. Kurucz É, Márkus R, Zsámboki J, Folkl-Medzihradzsky K, Darula Z, Vilmos P, et al. Nimrod, a putative phagocytosis receptor with EGF repeats in Drosophila plasmatocytes. *Curr Biol.* 2007; 17: 649–654. <https://doi.org/10.1016/j.cub.2007.02.041> PMID: 17363253
54. Bretscher AJ, Honti V, Binggeli O, Burri O, Poidevin M, Kurucz É, et al. The Nimrod transmembrane receptor Eater is required for hemocyte attachment to the sessile compartment in Drosophila melanogaster. *Biol Open.* The Company of Biologists Ltd; 2015; 4: 355–363. <https://doi.org/10.1242/bio.201410595> PMID: 25681394
55. Manaka J, Kuraishi T, Shiratsuchi A, Nakai Y, Higashida H, Henson P, et al. Draper-mediated and phosphatidylserine-independent phagocytosis of apoptotic cells by Drosophila hemocytes/macrophages. *J Biol Chem.* 2004; 279: 48466–48476. <https://doi.org/10.1074/jbc.M408597200> PMID: 15342648
56. Kurant E, Axelrod S, Leaman D, Gaul U. Six-microns-under acts upstream of Draper in the glial phagocytosis of apoptotic neurons. *Cell.* 2008; 133: 498–509. <https://doi.org/10.1016/j.cell.2008.02.052> PMID: 18455990
57. Blandin S, Levashina EA. Thioester-containing proteins and insect immunity. *Mol Immunol.* 2004; 40: 903–908. <https://doi.org/10.1016/j.molimm.2003.10.010> PMID: 14698229
58. Dostálová A, Rommelaere S, Poidevin M, Lemaitre B. Thioester-containing proteins regulate the Toll pathway and play a role in Drosophila defence against microbial pathogens and parasitoid wasps. *BMC Biol.* BioMed Central; 2017; 15: 79–16. <https://doi.org/10.1186/s12915-017-0408-0> PMID: 28874153

59. Somogyi K, Sipos B, Péntzes Z, Kurucz É, Zsámboki J, Hultmark D, et al. Evolution of genes and repeats in the Nimrod superfamily. *Mol Biol Evol.* 2008; 25: 2337–2347. <https://doi.org/10.1093/molbev/msn180> PMID: 18703524
60. Evans IR, Ghai PA, Urbančič V, Tan K-L, Wood W. SCAR/WAVE-mediated processing of engulfed apoptotic corpses is essential for effective macrophage migration in *Drosophila*. *Cell Death Differ.* Nature Publishing Group; 2013; 20: 709–720. <https://doi.org/10.1038/cdd.2012.166> PMID: 23328632
61. Williams MJ, Wiklund M-L, Wikman S, Hultmark D. Rac1 signalling in the *Drosophila* larval cellular immune response. *J Cell Sci.* The Company of Biologists Ltd; 2006; 119: 2015–2024. <https://doi.org/10.1242/jcs.02920> PMID: 16621891
62. Williams MJ, Andó I, Hultmark D. *Drosophila melanogaster* Rac2 is necessary for a proper cellular immune response. *Genes Cells.* John Wiley & Sons, Ltd; 2005; 10: 813–823. <https://doi.org/10.1111/j.1365-2443.2005.00883.x> PMID: 16098145
63. Avet-Rochex A, Perrin J, Bergeret E, Fauvarque M-O. Rac2 is a major actor of *Drosophila* resistance to *Pseudomonas aeruginosa* acting in phagocytic cells. *Genes Cells.* John Wiley & Sons, Ltd; 2007; 12: 1193–1204. <https://doi.org/10.1111/j.1365-2443.2007.01121.x> PMID: 17903178
64. Di Cara F, Sheshachalam A, Braverman NE, Rachubinski RA, Simmonds AJ. Peroxisome-Mediated Metabolism Is Required for Immune Response to Microbial Infection. *Immunity.* 2017; 47: 93–106.e7. <https://doi.org/10.1016/j.immuni.2017.06.016> PMID: 28723556
65. Charrin S, Jouannet S, Boucheix C, Rubinstein E. Tetraspanins at a glance. *J Cell Sci.* The Company of Biologists Ltd; 2014; 127: 3641–3648. <https://doi.org/10.1242/jcs.154906> PMID: 25128561
66. Lemaitre B, Hoffmann J. The host defense of *Drosophila melanogaster*. *Annu Rev Immunol.* Annual Reviews; 2007; 25: 697–743. <https://doi.org/10.1146/annurev.immunol.25.022106.141615> PMID: 17201680
67. Charroux B, Royet J. Elimination of plasmatocytes by targeted apoptosis reveals their role in multiple aspects of the *Drosophila* immune response. *Proc Natl Acad Sci USA.* 2009; 106: 9797–9802. <https://doi.org/10.1073/pnas.0903971106> PMID: 19482944
68. Gendrin M, Zaidman-Rémy A, Broderick NA, Paredes J, Poidevin M, Roussel A, et al. Functional analysis of PGRP-LA in *Drosophila* immunity. Leulier F, editor. *PLoS ONE.* 2013; 8: e69742. <https://doi.org/10.1371/journal.pone.0069742> PMID: 23922788
69. Myllymäki H, Valanne S, Rämetsä M. The *Drosophila* imd signaling pathway. *J Immunol.* 2014; 192: 3455–3462. <https://doi.org/10.4049/jimmunol.1303309> PMID: 24706930
70. Kurata S. Extracellular and intracellular pathogen recognition by *Drosophila* PGRP-LE and PGRP-LC. *Int Immunol.* 2010; 22: 143–148. <https://doi.org/10.1093/intimm/dxp128> PMID: 20089584
71. Neyen C, Poidevin M, Roussel A, Lemaitre B. Tissue- and ligand-specific sensing of gram-negative infection in *Drosophila* by PGRP-LC isoforms and PGRP-LE. *J Immunol.* American Association of Immunologists; 2012; 189: 1886–1897. <https://doi.org/10.4049/jimmunol.1201022> PMID: 22772451
72. Bosco-Drayon V, Poidevin M, Boneca IG, Narbonne-Reveau K, Royet J, Charroux B. Peptidoglycan sensing by the receptor PGRP-LE in the *Drosophila* gut induces immune responses to infectious bacteria and tolerance to microbiota. *Cell Host Microbe.* 2012; 12: 153–165. <https://doi.org/10.1016/j.chom.2012.06.002> PMID: 22901536
73. Shia AKH, Glittenberg M, Thompson G, Weber AN, Reichhart J-M, Ligoxygakis P. Toll-dependent antimicrobial responses in *Drosophila* larval fat body require Spätzle secreted by haemocytes. *J Cell Sci.* 2009; 122: 4505–4515. <https://doi.org/10.1242/jcs.049155> PMID: 19934223
74. De Gregorio E, Han S-J, Lee W-J, Baek M-J, Osaki T, Kawabata S-I, et al. An immune-responsive Serpin regulates the melanization cascade in *Drosophila*. *Dev Cell.* 2002; 3: 581–592. [https://doi.org/10.1016/s1534-5807\(02\)00267-8](https://doi.org/10.1016/s1534-5807(02)00267-8) PMID: 12408809
75. Scherfer C, Tang H, Kambris Z, Lhocine N, Hashimoto C, Lemaitre B. *Drosophila* Serpin-28D regulates hemolymph phenoloxidase activity and adult pigmentation. *Dev Biol.* 2008; 323: 189–196. <https://doi.org/10.1016/j.ydbio.2008.08.030> PMID: 18801354
76. Ligoxygakis P, Pelte N, Hoffmann JA, Reichhart J-M. Activation of *Drosophila* Toll during fungal infection by a blood serine protease. *Science.* American Association for the Advancement of Science; 2002; 297: 114–116. <https://doi.org/10.1126/science.1072391> PMID: 12098703
77. Dudzic JP, Hanson MA, Iatsenko I, Kondo S, Lemaitre B. More Than Black or White: Melanization and Toll Share Regulatory Serine Proteases in *Drosophila*. *Cell Rep.* 2019; 27: 1050–1061.e3. <https://doi.org/10.1016/j.celrep.2019.03.101> PMID: 31018123
78. Wilson R, Chen C, Ratcliffe NA. Innate immunity in insects: the role of multiple, endogenous serum lectins in the recognition of foreign invaders in the cockroach, *Blaberus discoidalis*. *J Immunol.* 1999; 162: 1590–1596. PMID: 9973417

79. Xia X, You M, Rao X-J, Yu X-Q. Insect C-type lectins in innate immunity. *Dev Comp Immunol*. 2018; 83: 70–79. <https://doi.org/10.1016/j.dci.2017.11.020> PMID: 29198776
80. Myers AL, Harris CM, Choe K-M, Brennan CA. Inflammatory production of reactive oxygen species by *Drosophila* hemocytes activates cellular immune defenses. *Biochem Biophys Res Commun*. 2018; 505: 726–732. <https://doi.org/10.1016/j.bbrc.2018.09.126> PMID: 30292413
81. Isabella AJ, Horne-Badovinac S. Building from the Ground up: Basement Membranes in *Drosophila* Development. *Curr Top Membr*. Elsevier; 2015; 76: 305–336. <https://doi.org/10.1016/bs.ctm.2015.07.001> PMID: 26610918
82. Dolezal T, Dolezelova E, Zurovec M, Bryant PJ. A role for adenosine deaminase in *Drosophila* larval development. Krasnow M, editor. *PLoS Biol*. Public Library of Science; 2005; 3: e201. <https://doi.org/10.1371/journal.pbio.0030201> PMID: 15907156
83. Woodcock KJ, Kierdorf K, Pouchelon CA, Vivancos V, Dionne MS, Geissmann F. Macrophage-derived upd3 cytokine causes impaired glucose homeostasis and reduced lifespan in *Drosophila* fed a lipid-rich diet. *Immunity*. 2015; 42: 133–144. <https://doi.org/10.1016/j.immuni.2014.12.023> PMID: 25601202
84. Franc NC, Dimarcq JL, Lagueux M, Hoffmann J, Ezekowitz RA. Croquemort, a novel *Drosophila* hemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity*. 1996; 4: 431–443. [https://doi.org/10.1016/s1074-7613\(00\)80410-0](https://doi.org/10.1016/s1074-7613(00)80410-0) PMID: 8630729
85. Melcarne C, Ramond E, Dudzic J, Bretscher AJ, Kurucz É, Andó I, et al. Two Nimrod receptors, NimC1 and Eater, synergistically contribute to bacterial phagocytosis in *Drosophila melanogaster*. *FEBS J*. John Wiley & Sons, Ltd (10.1111); 2019; 1248: 1116008. <https://doi.org/10.1111/febs.14857> PMID: 30993828
86. Singh SP, Coronella JA, Benes H, Cochrane BJ, Zimniak P. Catalytic function of *Drosophila melanogaster* glutathione S-transferase DmGSTS1-1 (GST-2) in conjugation of lipid peroxidation end products. *Eur J Biochem*. John Wiley & Sons, Ltd (10.1111); 2001; 268: 2912–2923. <https://doi.org/10.1046/j.1432-1327.2001.02179.x> PMID: 11358508
87. Saisawang C, Wongsantichon J, Ketterman AJ. A preliminary characterization of the cytosolic glutathione transferase proteome from *Drosophila melanogaster*. *Biochem J*. Portland Press Limited; 2012; 442: 181–190. <https://doi.org/10.1042/BJ20111747> PMID: 22082028
88. Stramer B, Winfield M, Shaw T, Millard TH, Woolner S, Martin P. Gene induction following wounding of wild-type versus macrophage-deficient *Drosophila* embryos. *EMBO Rep*. John Wiley & Sons, Ltd; 2008; 9: 465–471. <https://doi.org/10.1038/embor.2008.34> PMID: 18344972
89. Stevens LJ, Page-McCaw A. A secreted MMP is required for reepithelialization during wound healing. Gonzalez-Gaitan M, editor. *Mol Biol Cell*. 2012; 23: 1068–1079. <https://doi.org/10.1091/mbc.E11-09-0745> PMID: 22262460
90. Chakrabarti S, Dudzic JP, Li X, Collas EJ, Boquete J-P, Lemaitre B. Remote Control of Intestinal Stem Cell Activity by Haemocytes in *Drosophila*. Banerjee U, editor. *PLoS Genet*. 2016; 12: e1006089. <https://doi.org/10.1371/journal.pgen.1006089> PMID: 27231872
91. Li X, Rommelaere S, Kondo S, Lemaitre B. Renal Purge of Hemolymphatic Lipids Prevents the Accumulation of ROS-Induced Inflammatory Oxidized Lipids and Protects *Drosophila* from Tissue Damage. *Immunity*. 2020; 52: 374–387.e6. <https://doi.org/10.1016/j.immuni.2020.01.008> PMID: 32075729
92. Kenmoku H, Hori A, Kuraishi T, Kurata S. A novel mode of induction of the humoral innate immune response in *Drosophila* larvae. *Dis Model Mech*. The Company of Biologists Ltd; 2017; 10: 271–281. <https://doi.org/10.1242/dmm.027102> PMID: 28250052
93. Palmer WH, Varghese FS, van Rij RP. Natural Variation in Resistance to Virus Infection in Dipteran Insects. *Viruses*. Multidisciplinary Digital Publishing Institute; 2018; 10: 118. <https://doi.org/10.3390/v10030118> PMID: 29522475
94. DeVorkin L, Gorski SM. Monitoring autophagic flux using Ref(2)P, the *Drosophila* p62 ortholog. *Cold Spring Harb Protoc*. Cold Spring Harbor Laboratory Press; 2014; 2014: 959–966. <https://doi.org/10.1101/pdb.prot080333> PMID: 25183816
95. Theopold U, Krautz R, Dushay MS. The *Drosophila* clotting system and its messages for mammals. *Dev Comp Immunol*. 2014; 42: 42–46. <https://doi.org/10.1016/j.dci.2013.03.014> PMID: 23545286
96. De Gregorio E, Spellman PT, Rubin GM, Lemaitre B. Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. *Proc Natl Acad Sci USA*. 2001; 98: 12590–12595. <https://doi.org/10.1073/pnas.221458698> PMID: 11606746
97. Wood W, Faria C, Jacinto A. Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in *Drosophila melanogaster*. *J Cell Biol*. Rockefeller University Press; 2006; 173: 405–416. <https://doi.org/10.1083/jcb.200508161> PMID: 16651377

98. Kwon SY, Xiao H, Glover BP, Tjian R, Wu C, Badenhorst P. The nucleosome remodeling factor (NURF) regulates genes involved in *Drosophila* innate immunity. *Dev Biol*. 2008; 316: 538–547. <https://doi.org/10.1016/j.ydbio.2008.01.033> PMID: 18334252
99. Stofanko M, Kwon SY, Badenhorst P. Lineage tracing of lamellocytes demonstrates *Drosophila* macrophage plasticity. Schweisguth F, editor. *PLoS ONE*. Public Library of Science; 2010; 5: e14051. <https://doi.org/10.1371/journal.pone.0014051> PMID: 21124962
100. Márkus R, Kurucz É, Rus F, Andó I. Sterile wounding is a minimal and sufficient trigger for a cellular immune response in *Drosophila melanogaster*. *Immunol Lett*. 2005; 101: 108–111. <https://doi.org/10.1016/j.imlet.2005.03.021> PMID: 15964636
101. Nagaosa K, Okada R, Nonaka S, Takeuchi K, Fujita Y, Miyasaka T, et al. Integrin β v-mediated phagocytosis of apoptotic cells in *Drosophila* embryos. *J Biol Chem*. 2011; 286: 25770–25777. <https://doi.org/10.1074/jbc.M110.204503> PMID: 21592968
102. Munier A-I, Doucet D, Perrodou E, Zachary D, Meister M, Hoffmann JA, et al. PVF2, a PDGF/VEGF-like growth factor, induces hemocyte proliferation in *Drosophila* larvae. *EMBO Rep*. EMBO Press; 2002; 3: 1195–1200. <https://doi.org/10.1093/embo-reports/kvf242> PMID: 12446570
103. Brückner K, Kockel L, Duchek P, Luque CM, Rørth P, Perrimon N. The PDGF/VEGF receptor controls blood cell survival in *Drosophila*. *Dev Cell*. 2004; 7: 73–84. <https://doi.org/10.1016/j.devcel.2004.06.007> PMID: 15239955
104. Wood W, Faria C, Jacinto A. Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in *Drosophila melanogaster*. *J Cell Biol*. 2006; 173: 405–416. <https://doi.org/10.1083/jcb.200508161> PMID: 16651377
105. Steller H. Regulation of apoptosis in *Drosophila*. *Cell Death Differ*. Nature Publishing Group; 2008; 15: 1132–1138. <https://doi.org/10.1038/cdd.2008.50> PMID: 18437164
106. Broemer M, Tenev T, Rigbolt KT, Hempel S, Blagoev B, Silke J, et al. Systematic in vivo RNAi analysis identifies IAPs as NEDD8-E3 ligases. *Mol Cell*. 2010; 40: 810–822. <https://doi.org/10.1016/j.molcel.2010.11.011> PMID: 21145488
107. Owen KA, Pixley FJ, Thomas KS, Vicente-Manzanares M, Ray BJ, Horwitz AF, et al. Regulation of lamellipodial persistence, adhesion turnover, and motility in macrophages by focal adhesion kinase. *J Cell Biol*. 2007; 179: 1275–1287. <https://doi.org/10.1083/jcb.200708093> PMID: 18070912
108. Vergadi E, Ieronymaki E, Lyroni K, Vaporidi K, Tsatsanis C. Akt Signaling Pathway in Macrophage Activation and M1/M2 Polarization. *J Immunol*. American Association of Immunologists; 2017; 198: 1006–1014. <https://doi.org/10.4049/jimmunol.1601515> PMID: 28115590
109. Batista-Gonzalez A, Vidal R, Criollo A, Carreño LJ. New Insights on the Role of Lipid Metabolism in the Metabolic Reprogramming of Macrophages. *Front Immunol*. Frontiers; 2019; 10: 2993. <https://doi.org/10.3389/fimmu.2019.02993> PMID: 31998297
110. Crivat G, Lizunov VA, Li CR, Stenkula KG, Zimmerberg J, Cushman SW, et al. Insulin stimulates translocation of human GLUT4 to the membrane in fat bodies of transgenic *Drosophila melanogaster*. Bergmann A, editor. *PLoS ONE*. Public Library of Science; 2013; 8: e77953. <https://doi.org/10.1371/journal.pone.0077953> PMID: 24223128
111. Oshel KM, Knight JB, Cao KT, Thai MV, Olson AL. Identification of a 30-base pair regulatory element and novel DNA binding protein that regulates the human GLUT4 promoter in transgenic mice. *J Biol Chem*. 2000; 275: 23666–23673. <https://doi.org/10.1074/jbc.M001452200> PMID: 10825161
112. Hanse EA, Ruan C, Kachman M, Wang D, Lowman XH, Kelekar A. Cytosolic malate dehydrogenase activity helps support glycolysis in actively proliferating cells and cancer. *Oncogene*. Nature Publishing Group; 2017; 36: 3915–3924. <https://doi.org/10.1038/onc.2017.36> PMID: 28263970
113. Hanson MA, Dostálová A, Ceroni C, Poidevin M, Kondo S, Lemaitre B. Synergy and remarkable specificity of antimicrobial peptides in vivo using a systematic knockout approach. *Elife*. 2019; 8: 511. <https://doi.org/10.7554/eLife.44341> PMID: 30803481
114. Shiratsuchi A, Mori T, Sakurai K, Nagaosa K, Sekimizu K, Lee BL, et al. Independent recognition of *Staphylococcus aureus* by two receptors for phagocytosis in *Drosophila*. *J Biol Chem*. American Society for Biochemistry and Molecular Biology; 2012; 287: 21663–21672. <https://doi.org/10.1074/jbc.M111.333807> PMID: 22547074
115. Garg A, Wu LP. *Drosophila* Rab14 mediates phagocytosis in the immune response to *Staphylococcus aureus*. *Cell Microbiol*. John Wiley & Sons, Ltd; 2014; 16: 296–310. <https://doi.org/10.1111/cmi.12220> PMID: 24119134
116. Zaidman-Rémy A, Hervé M, Poidevin M, Pili-Floury S, Kim M-S, Blanot D, et al. The *Drosophila* amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity*. 2006; 24: 463–473. <https://doi.org/10.1016/j.immuni.2006.02.012> PMID: 16618604

117. Vanha-Aho L-M, Kleino A, Kaustio M, Ulvila J, Wilke B, Hultmark D, et al. Functional characterization of the infection-inducible peptide Edin in *Drosophila melanogaster*. Leulier F, editor. PLoS ONE. Public Library of Science; 2012; 7: e37153. <https://doi.org/10.1371/journal.pone.0037153> PMID: 22606343
118. Tsuzuki S, Ochiai M, Matsumoto H, Kurata S, Ohnishi A, Hayakawa Y. *Drosophila* growth-blocking peptide-like factor mediates acute immune reactions during infectious and non-infectious stress. Sci Rep. Nature Publishing Group; 2012; 2: 210–10. <https://doi.org/10.1038/srep00210> PMID: 22355724
119. Chen JWC, Barker AR, Wakefield JG. The Ran Pathway in *Drosophila melanogaster* Mitosis. Front Cell Dev Biol. Frontiers; 2015; 3: 74. <https://doi.org/10.3389/fcell.2015.00074> PMID: 26636083
120. Andersen SS. Spindle assembly and the art of regulating microtubule dynamics by MAPs and Stathmin/Op18. Trends Cell Biol. 2000; 10: 261–267. [https://doi.org/10.1016/s0962-8924\(00\)01786-4](https://doi.org/10.1016/s0962-8924(00)01786-4) PMID: 10856928
121. Ji J-Y, Crest J, Schubiger G. Genetic interactions between Cdk1-CyclinB and the Separase complex in *Drosophila*. Development. The Company of Biologists Ltd; 2005; 132: 1875–1884. <https://doi.org/10.1242/dev.01780> PMID: 15772129
122. Deplanche M, Filho RAE-A, Alekseeva L, Ladier E, Jardin J, Henry G, et al. Phenol-soluble modulin α induces G2/M phase transition delay in eukaryotic HeLa cells. FASEB J. 2015; 29: 1950–1959. <https://doi.org/10.1096/fj.14-260513> PMID: 25648996
123. Teixeira L. Whole-genome expression profile analysis of *Drosophila melanogaster* immune responses. Brief Funct Genomics. 2012; 11: 375–386. <https://doi.org/10.1093/bfpg/els043> PMID: 23023664
124. Johansson KC, Metzendorf C, Söderhäll K. Microarray analysis of immune challenged *Drosophila* hemocytes. Exp Cell Res. 2005; 305: 145–155. <https://doi.org/10.1016/j.yexcr.2004.12.018> PMID: 15777795
125. Troha K, Im JH, Revah J, Lazzaro BP, Buchon N. Comparative transcriptomics reveals CrebA as a novel regulator of infection tolerance in *D. melanogaster*. Schneider DS, editor. PLoS Pathog. Public Library of Science; 2018; 14: e1006847. <https://doi.org/10.1371/journal.ppat.1006847> PMID: 29394281
126. Reichhart JM, Meister M, Dimarcq JL, Zachary D, Hoffmann D, Ruiz C, et al. Insect immunity: developmental and inducible activity of the *Drosophila* dipterecin promoter. EMBO J. European Molecular Biology Organization; 1992; 11: 1469–1477. PMID: 1373375
127. Krautz R, Arefin B, Theopold U. Damage signals in the insect immune response. Front Plant Sci. Frontiers; 2014; 5: 342. <https://doi.org/10.3389/fpls.2014.00342> PMID: 25071815
128. Van De Bor V, Zimniak G, Papone L, Cerezo D, Malbouyres M, Juan T, et al. Companion Blood Cells Control Ovarian Stem Cell Niche Microenvironment and Homeostasis. Cell Rep. 2015; 13: 546–560. <https://doi.org/10.1016/j.celrep.2015.09.008> PMID: 26456819
129. Parsons B, Foley E. Cellular immune defenses of *Drosophila melanogaster*. Dev Comp Immunol. 2016; 58: 95–101. <https://doi.org/10.1016/j.dci.2015.12.019> PMID: 26748247
130. Ninnemann JL. Prostaglandins, Leukotrienes, and the Immune Response. 1988.
131. Ramanadham S, Ali T, Ashley JW, Bone RN, Hancock WD, Lei X. Calcium-independent phospholipases A2 and their roles in biological processes and diseases. J Lipid Res. American Society for Biochemistry and Molecular Biology; 2015; 56: 1643–1668. <https://doi.org/10.1194/jlr.R058701> PMID: 26023050
132. Pastor-Pareja JC, Wu M, Xu T. An innate immune response of blood cells to tumors and tissue damage in *Drosophila*. Dis Model Mech. 2008; 1: 144–54– discussion 153. <https://doi.org/10.1242/dmm.000950> PMID: 19048077
133. Tran TA, Kinch L, Peña-Llopis S, Kockel L, Grishin N, Jiang H, et al. Platelet-derived growth factor/vascular endothelial growth factor receptor inactivation by sunitinib results in Tsc1/Tsc2-dependent inhibition of TORC1. Mol Cell Biol. American Society for Microbiology Journals; 2013; 33: 3762–3779. <https://doi.org/10.1128/MCB.01570-12> PMID: 23878397
134. Kloc M, Ghobrial RM, Wosik J, Lewicka A, Lewicki S, Kubiak JZ. Macrophage functions in wound healing. J Tissue Eng Regen Med. 2019; 13: 99–109. <https://doi.org/10.1002/term.2772> PMID: 30445662
135. Morgan M, Anders S, Lawrence M, Aboyoun P, Pagès H, Gentleman R. ShortRead: a bioconductor package for input, quality assessment and exploration of high-throughput sequence data. Bioinformatics. 2009; 25: 2607–2608. <https://doi.org/10.1093/bioinformatics/btp450> PMID: 19654119
136. Risso D, Ngai J, Speed TP, Dudoit S. Normalization of RNA-seq data using factor analysis of control genes or samples. Nat Biotechnol. Nature Publishing Group; 2014; 32: 896–902. <https://doi.org/10.1038/nbt.2931> PMID: 25150836

137. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010; 26: 139–140. <https://doi.org/10.1093/bioinformatics/btp616> PMID: [19910308](https://pubmed.ncbi.nlm.nih.gov/19910308/)