



**HAL**  
open science

## Novel cell- and stage-specific transcriptional signatures defining *Drosophila* neurons, glia and hemocytes

Rosy Sakr, Pierre B Cattenoz, Alexia Pavlidaki, Laura Ciapponi, Marta Marzullo, Hariharan Nivedita, Tina Mukherjee, Angela Giangrande

### ► To cite this version:

Rosy Sakr, Pierre B Cattenoz, Alexia Pavlidaki, Laura Ciapponi, Marta Marzullo, et al.. Novel cell- and stage-specific transcriptional signatures defining *Drosophila* neurons, glia and hemocytes. 2023. hal-04296340

**HAL Id: hal-04296340**

**<https://cnrs.hal.science/hal-04296340>**

Preprint submitted on 20 Nov 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Novel cell- and stage-specific transcriptional signatures defining *Drosophila* neurons, glia**  
2 **and hemocytes**

3 **Authors:**

4 Rosy Sakr<sup>1,2,3,4</sup>, Pierre B. Cattenoz<sup>1,2,3,4</sup>, Alexia Pavlidaki<sup>1,2,3,4</sup>, Laura Ciapponi<sup>5</sup>, Marta  
5 Marzullo<sup>5,6</sup>, Nivedita Hariharan<sup>7</sup>, Tina Mukherjee<sup>8</sup>, Angela Giangrande<sup>\*,1,2,3,4</sup>

6 **Affiliations:**

7 <sup>1</sup>Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France

8 <sup>2</sup>Centre National de la Recherche Scientifique, UMR7104, Illkirch, France

9 <sup>3</sup>Institut National de la Santé et de la Recherche Médicale, U1258, Illkirch, France

10 <sup>4</sup>Université de Strasbourg, Illkirch, France

11 <sup>5</sup>Department of Biology and Biotechnologies, Sapienza University of Rome, Rome 00185, Italy

12 <sup>6</sup>IBPM CNR c/o Department of Biology and Biotechnology, Sapienza University of Rome, 00185  
13 Rome, Italy

14 <sup>7</sup>The University of Trans-Disciplinary Health Sciences & Technology (TDU), Bengaluru,  
15 Karnataka 560064, India.

16 <sup>8</sup>Institute for Stem Cell Science and Regenerative Medicine (inStem), GKVK, Bellary Road,  
17 Bangalore 560065, India.

18 \*Corresponding author. Tel: +33 388653381; E-mail: [angela@igbmc.fr](mailto:angela@igbmc.fr)

19

20 **Abstract:**

21 Cell types can be now defined at unprecedented resolution using high throughput assays. We  
22 analyzed the transcriptional signatures of *Drosophila* neurons, glia and hemocytes, as examples of  
23 cell types that are related by position (glia/neurons) or function (glia/hemocytes) or that are  
24 unrelated (neurons/hemocytes). The most related cells display the highest similarity level (neurons  
25 and glia), the least related ones, the lowest (neurons and hemocytes), however, cells can show  
26 plastic features. Glia are much more similar to neurons than to hemocytes in the embryo, but are  
27 equally similar to the two cell types in the larva, when hemocytes acquire more immune functions.  
28 Larval glia and hemocytes display common as well as specific immune features, such as the glia-

29 specific NimA receptor, in agreement with the different environment faced by each cell types.  
30 Surprisingly, time represents a key identity parameter, as neurons, hemocytes and glia group more  
31 significantly by the stage than by the cell type and larval cells show upregulation of genes involved  
32 in chromatin organization and in DNA repair. This latter group of genes is linked to changes in  
33 gene expression levels and chromatin organization, revealing a function of these genes beyond  
34 DNA repair. Finally, the metabolic profiles reveal cell type-specific signatures and an overall shift  
35 from an embryonic, anabolic state to a larval, catabolic state.

36

37

## 38 **Introduction**

39 Traditionally, cell types have been defined by morphology, position, origin and function, relying  
40 on the expression of few proteins. While the combination of these parameters has produced  
41 valuable information, each of them on their own can lead to misleading conclusions. Cells in  
42 different organs can display similar functions and cells within the same organ can have different  
43 properties. Moreover, the *a priori* selection of proteins can introduce bias and neglect the wealth  
44 of molecular information intrinsic to each cell type. Finally, functional plasticity and dynamic  
45 states may conduct to a reductionist classification. To ensure a more unbiased and quantitative  
46 analysis, we used a high throughput approach and a data-driven computational method on neurons,  
47 hemocytes and glia. To account for plasticity, we analyzed them at two stages, embryo and larva.

48 *Drosophila* neurons and glia differentiate from common, ectodermal, precursors. Neurons sense,  
49 process and transmit information or secrete neuropeptides (Garces and Thor, 2006; Landgraf and  
50 Thor, 2006; Schmid et al., 1999). Glia are involved in nervous system development, function, and  
51 maintenance. They control cell proliferation (Ebens et al., 1993), axonal and synapse development  
52 (Brink et al., 2012; Ou et al., 2014), neuronal insulation and survival (Shepherd, 2000; Stork et al.,  
53 2008; Volkenhoff et al., 2015). They also establish the blood-brain barrier (BBB) (Abbott, 2005)  
54 and provide the immune function within the nervous system (Awasaki and Ito, 2004; Sonnenfeld  
55 and Jacobs, 1995a; Watts et al., 2004). The mesodermally derived hemocytes patrol the organism  
56 to ensure cellular immunity outside the nervous system (Lebestky et al., 2000; Lemaitre et al.,

57 1996; Tepass et al., 1994), hence sharing the function but not the origin with glial cells, while  
58 sharing neither the origin nor the function with neurons.

59 Transcriptome comparison highlights cell-specific features. Neurons and hemocytes show the least  
60 related transcriptional landscapes regardless of the stage. Glia and neurons stably share the highest  
61 similarity, in line with the fact that they constantly interact to ensure the homeostasis of the nervous  
62 system. Glia and hemocytes display significant similarity in the larva. This goes along with a  
63 significant change of the hemocyte transcriptional profile from the embryo to the larva, an open  
64 system that has to react to external stimuli as pathogens. Although larval glia and hemocytes share  
65 an immune function, the two cell types encounter distinct environments, pathogens for hemocytes,  
66 axon and dendrite debris for glia. Accordingly, the Eater and the Nimrod C1 (NimC1) scavenger  
67 receptors are specific to hemocytes, NimA is specific to glia.

68 Similarly, some metabolic features are common, others are specific with respect to tissue and stage.  
69 The key glycolytic gene *lactate dehydrogenase (ldh)* is upregulated in all the three cell types in  
70 the embryo, down-regulated in the larva. The expression of lipogenic genes, on the other hand, is  
71 upregulated in all three embryonic cell types, but changes discretely as the embryo transitions to  
72 the larval stage. Hemocytes dramatically shift to a lipolytic state while neurons maintain their  
73 lipogenic state. Larval glia maintain the expression of lipogenic genes but shows additional  
74 upregulation of lipolytic pathway genes. The observed common metabolic states may be a  
75 reflection of the overall developmental program, but the cell-specific states may highlight  
76 metabolic needs necessary to accommodate distinct functional roles.

77 Transcriptome comparison also highlights common stage-specific features. Larval cells express  
78 genes controlling chromatin organization as well as DNA repair at high levels. One of the main  
79 DNA repair genes is *rad50*, involved in DNA double strand break (DSB) repair. Its depletion  
80 triggers a genome-wide change in chromatin organization and mis-regulation of gene expression,  
81 pointing to a role beyond DSB. Finally, the three cell types display a glycolytic and lipogenic state  
82 in the embryo, a lipolytic and oxidative state in the larva, changes that are likely linked to external  
83 inputs such as feeding and respiration.

84 Altogether, our genome-wide analysis makes it possible to disentangle the relative importance of  
85 origin, position, function and time in the definition of cell types. The expression profile of a cell  
86 population is strongly affected by their origin in the embryo, while larval cells acquire additional

87 levels of specialization and become more affected by the function. Moreover, the three cell types  
88 display different degrees of plasticity, the migratory hemocytes exposed to the external challenges  
89 being the most plastic ones. This work paves the way for systematic identification of gene networks  
90 and expression programs, which will ultimately shed light on the biological mechanisms  
91 characterizing cell diversity in multicellular organisms.

92

93

## 94 **Results**

### 95 **Impact of origin, stage and function on the transcriptional landscapes**

96 To assess the impact of origin, function and position on the definition of a cell type, we compared  
97 the transcriptomes of neurons and glia, which share the origin; those of hemocytes and glia, which  
98 share the scavenging activity; and those of neurons and hemocytes, which share neither origin nor  
99 function. We performed the comparison in mature embryos (stage 16 or E16) and in wandering  
100 third instar larvae (L3), to understand whether expression profiles change from a small, closed and  
101 immobile system to a bigger, open system that is able to grow and to respond and adapt to the  
102 environment. Glia, hemocytes and neurons were purified from transgenic lines specific to each  
103 cell type: *repo-nRFP*, *srp(hemo)Gal4/+; UAS-RFP/+* and *elav-nRFP*, respectively. The  
104 hierarchical clustering of the transcriptomes shows a good correlation between the biological  
105 replicates, highlighting a low biological variability (**Figure 1-a**).

106 The developmental stage is the main factor of heterogeneity, more important than the cell type, as  
107 the highest correlation in the dendrogram is seen for samples of the same stage. Thus, each cell  
108 type is more similar to the two others at the same stage than to the same cell type at a different  
109 stage. The dendrogram also shows that glia and neurons group together, sharing the highest degree  
110 of similarity. We next performed pairwise comparisons of the number of differentially expressed  
111 genes to evaluate the commonalities and the differences between neurons, glia and hemocytes. We  
112 defined significantly upregulated genes based on transcripts that are enriched in one population  
113 compared to the other following three parameters: number of reads > 15, adjusted p-value <0.05  
114 and fold change >1.5 between the number of reads. Upregulated genes in each cell type were  
115 compared in the Venn diagram. The comparison of the cell-specific transcriptomes is shown in

116 **(Figure 1-b)** for the embryo, in **(Figure 1-c)** for the larva. The highest number of shared  
117 upregulated genes concerns E16 neurons and glia (in green) (31.3% of all genes included in the  
118 diagram), while only 5.7% of shared upregulated genes are found between E16 hemocytes and glia  
119 (in orange). Interestingly, the shared enriched transcripts between hemocytes and glia reach 18.3%  
120 by the larval stage, suggesting that glia and hemocytes acquire common molecular features by L3,  
121 despite their distinct origin. Finally, the Venn diagram and the dendrogram indicate that glia and  
122 neurons maintain a high degree of similarity in the larva (27.1%). Neurons and hemocytes remain  
123 the most unrelated cell populations, with 5.9% of shared enriched transcripts at E16, 3,8% at L3.

124 Interestingly, the number of expressed genes is overall lower in L3 compared to E16, and this is  
125 true for all cell types (transcripts with at least 15 reads, **Figure 1-d,e, top three lines**). By contrast,  
126 the number of upregulated genes in each cell type increases by L3 (**Figure 1-d,e, bottom three**  
127 **lines**). 10838 genes are expressed in E16 glia, 11281 in E16 neurons and 9811 in E16 hemocytes.  
128 By L3, 9187 genes are expressed in glia, 9312 in neurons and 7828 in hemocytes, which means  
129 that at least 1500 genes are only expressed at E16 in each cell type compared to L3 (**Figure 1-d,e**).  
130 This is not due to a low sequencing depth, since the same number of cells was used for each sample  
131 and there are no differences between the number of reads mapped for embryonic and larval cells  
132 (**Sup. Figure 1-a**). To further understand the molecular mechanisms underlying the stage  
133 differences, we analyzed the expression profiles of transcription factors, proteins that coordinately  
134 control downstream effector genes. In line with the increased number of cell-specific upregulated  
135 genes in the larva, the number of transcription factors commonly expressed in all three cell types  
136 decreases by L3 (**Sup. Table 1-a,b**). Thus, differentiated cells become more specialized over time.

137 In sum, our results show that the developmental stage has a stronger impact on the transcriptome  
138 than the cell type. In agreement with their respective origin and position, glia and neurons display  
139 the strongest similarity, hemocytes and neurons display the opposite behavior. Glia and hemocytes  
140 become more similar by L3. Finally, larval differentiated cells express fewer genes than the  
141 embryonic counterparts, but, proportionally, more genes that are upregulated in a cell-specific  
142 manner.

143

144 **Cell-specific signatures in the embryo define neurons and glia but not hemocytes**

145 The comparison of the transcriptomes indicates that almost half of the transcripts are common to  
146 neurons, glia and hemocytes (see **Figure 1-d,e**: number of genes expressed – number of genes  
147 upregulated in each cell type), calling for more refined parameters to define the three cell types.  
148 Since a significant fraction is enriched only in one cell type or shared by two cell populations, we  
149 predicted that a pairwise comparison associated with quantitative and Gene Ontology (GO)  
150 analyses would allow the disclosure of the signatures and functions that better define cell types.

151 In the embryo, 1649 (out of 11281) genes are upregulated in neurons compared to glia (in blue),  
152 992 in glia compared to neurons (out of 10838) (in yellow) (**Figure 2-a**). Thus, relatively few  
153 genes are upregulated in a specific cell type. The vast majority of the genes are non-differentially  
154 expressed, with number of reads > 15 and adjusted p-value of fold change > 0.05. On average,  
155 these genes are expressed at lower levels than those upregulated in a specific cell type (**Sup. Figure**  
156 **1-b,c**). Amongst the non-differentially expressed genes, the ones with fold change < 1.5 were  
157 considered as commonly expressed, mostly involved in biological processes found in all cells such  
158 as ncRNA processing, ribosome biogenesis and mRNA metabolic process (**Sup. Figure 1-d, Sup.**  
159 **Table 2-a**). The transcripts enriched in neurons are mostly involved in neuronal pathways, such  
160 as synaptic transmission, neurotransmitter secretion and neuron projection guidance (**Figure 2-b,**  
161 **Sup. Table 2-b**). Those enriched in glia are involved in the establishment of the BBB, axon  
162 ensheathment and the development of glial cells (**Figure 2-c, Sup. Table 2-c**).

163 The comparative analysis between E16 glia and hemocytes reveals a much higher number of  
164 transcripts specifically enriched in either cell type. This is in line with the Venn diagram in **Figure**  
165 **1-b**. 1945 transcripts are enriched in hemocytes (in red) and 2937 in glia (in yellow) (**Figure 2-d**).  
166 Interestingly, the hemocyte enriched transcripts only highlight one GO term related to immunity,  
167 ‘positive regulation of immune functions’, which comprises 39 genes (**Figure 2-e, Sup. Table 2-**  
168 **d**). Among them are 14 members of the Toll pathway and 6 of the Immune deficiency (Imd)  
169 pathway (**Figure 2-g**). The Imd pathway is one of the conserved NF- $\kappa$ B (nuclear factor kappa-  
170 light-chain-enhancer of activated B cells) immune signaling pathways in insects that regulates an  
171 antibacterial defense response. The remaining GO terms include “macroautophagy” and “Golgi  
172 vesicle transport”. In the case of glia, we find glia-specific functions such as septate junction



173 assembly (involved in BBB establishment), glial cell differentiation and neural functions such as  
174 motoneuron axon guidance (Abbott, 2005; Barres, 2008) (**Figure 2-f, Sup. Table 2-e**).

175 The comparison between E16 neurons and hemocytes also shows poor similarity, with 5261 genes  
176 non differentially expressed, 3608 upregulated in neurons, 1990 in hemocytes (**Figure 2-h**).

177 Altogether, these data reveal that only a minority of transcripts is cell-specific and that embryonic  
178 glia and neurons are closer to each other than to hemocytes. The genes that are specifically  
179 upregulated in E16 recapitulate the specific functions of neurons and glia, while few cell-specific  
180 terms are identified in hemocytes.

181

### 182 **Over development, hemocytes display higher plasticity compared to neurons and glia**

183 Cell functions adapt to changing environments during the life cycle. Typically, embryos live in  
184 autarchy, whereas larvae function as open systems, due to feeding, respiration and exposure to  
185 pathogens. Accordingly, although already functional in the embryo, we found that the  
186 differentiated cells become increasingly specialized by the larval stage.

187 A pairwise comparison reveals that 2500 genes are upregulated in L3 neurons compared to glia  
188 and 2279 genes are upregulated in L3 glia compared to neurons (**compare Figure 3-a and Figure**  
189 **2-a**). The genes upregulated in L3 neurons highlight neuronal functions and molecular cascades  
190 linked to signaling (neuropeptide signaling pathway, regulation of neurotransmitter levels and  
191 synapse organization, **Figure 3-b, Sup. Table 3-a**), compared to the GO terms linked to  
192 development found at E16 (**Figure 2-b**). L3 glia display the GO term septate junction assembly  
193 also found at E16, likely due to the fact that the BBB starts forming in the embryo and continues  
194 to expand as the CNS grows during the larval stages (reviewed in (Limmer et al., 2014). In  
195 addition, terms related to the differentiation of glia and gliogenesis were also found (**Figure 3-c,**  
196 **Sup. Table 3-b**), in line with the extensive glial proliferation ongoing in the larva (Pereanu et al.,  
197 2005).

198 We performed the pairwise comparison between L3 hemocytes and glia. Unlike their embryonic  
199 counterpart, L3 hemocytes present several terms related to immune functions such as  
200 phagocytosis, positive regulation of antimicrobial humoral response and positive regulation of



201 immune effector process (**compare Figure 2-e and Figure 3-e, Sup. Table 3-c**). The clear shift  
202 in the hemocyte expression profile indicates a major change in the function of these cells during  
203 development. The genes upregulated in glia (2941 genes in yellow) are involved in glial functions  
204 such as synapse organization and axon guidance (**Figure 3-f, Sup. Table 3-d**), a function that has  
205 already been investigated (reviewed in (Bittern et al., 2021)).

206 The MA plot comparing larval neurons and hemocytes shows that these cell types display the  
207 lowest level of similarity, comparable to that observed in the embryo (**Figure 3-g**).

208 The comparative analyses show that the divergence between neurons and glia increases over time:  
209 higher number of upregulated genes at L3 compared to E16 (Compare **Figure 3-a to Figure 2-a**),  
210 increased fold change of upregulated genes at L3 (**Sup. Figure 2-a**). By contrast, in the case of  
211 hemocytes and glia, the number of upregulated genes as well as their median fold change value  
212 are similar in E16 and L3 (compare **Figure 2-d and Figure 3-d, Sup. Figure 2-a**).

213 To shed light on the hemocyte / glia similarities at L3, we identified the genes that are upregulated  
214 in L3 glia and hemocytes but not in neurons (**Figure 1-c genes in orange**). A GO term analysis  
215 highlights genes involved in immune processes and in metabolism (**Sup. Table 4-a**). Typically, 4  
216 genes are involved in fatty acid oxidation including CPT2, Mtpalpha, Echs1, Mfe2, CG4860 and  
217 CG17544 (**Sup. Figure 2-b, Sup. Table 4-b**), indicating the significant reliance of L3 glia and  
218 hemocytes on lipid break-down. Moreover, the upregulated genes in the L3 hemocytes and glia  
219 show several GO terms related to immune functions such as positive regulation of antimicrobial  
220 humoral response and positive regulation of immune effector process (**Sup. Figure 2-b, Sup.**  
221 **Table 4-a,c**).

222 To better understand the nature of this evolving cell behavior, we compared each cell type at E16  
223 and L3. The MA plots show that neurons and glia evolve in a similar manner: a comparable number  
224 of genes is non-differentially expressed in neurons and in glia between E16 and L3; also, many  
225 more genes are upregulated in E16 glia and neurons, than in L3 glia and neurons (**Sup. Figure 2-**  
226 **c,d**). By comparison, the number of non-differentially expressed genes in E16 and L3 hemocytes  
227 is significantly lower than that detected in glia and neurons (**Sup. Figure 2-e**). This highlights the  
228 robust shift in the hemocytes' transcriptome during development compared to the relatively less  
229 dynamic transcriptomes of neurons and glia.

230 Finally, and in agreement with the gene expression profiles, neurons and glia share the highest  
231 number of transcription factors at E16 (**Sup. Figure 2-f, Sup. Table 1-c,d,e**), hemocytes and glia  
232 at L3 (**Sup. Figure 2-g, Sup. Table 1-f,g,h**), further highlighting the increased commonality  
233 between hemocytes and glia at L3.

234 In sum, cell-specific features and specialization increase over time, especially for hemocytes,  
235 which acquire a stronger immune potential and share more similarities with glia in the larva.

236

### 237 **Glia and hemocytes express specific scavenger receptors**

238 The vast majority of the hemocytes (95%) act as scavenger cells in homeostatic and challenged  
239 conditions. In the embryo, they are necessary for the elimination of apoptotic bodies and for the  
240 clearance of cellular debris. Prior to the formation of the BBB, they also contribute to this function  
241 within the developing CNS (Franc et al., 1996; Kurant et al., 2008; Manaka et al., 2004; Sonnenfeld  
242 and Jacobs, 1995b). Once glial cells form the BBB, however, hemocytes no longer have access to  
243 the CNS and by late embryogenesis the scavenging function within the nervous system is taken up  
244 by glia solely (Kurant et al., 2008). Thus, hemocytes and glia represent respectively the  
245 macrophages acting outside and inside the nervous system. The question then arises as to whether  
246 glia express the same immune pathways of hemocytes or represent a distinct class of macrophages.

247 A key molecular signature of macrophages is the expression of scavenger receptors (Kocks et al.,  
248 2005; Kurucz et al., 2007; Lebestky et al., 2000; Lemaitre et al., 1996; R met et al., 2002; Tepass  
249 et al., 1994), which we therefore used to compare hemocytes and glia. The MA plots comparing  
250 E16 (**Figure 4-a, Sup. Table 5-a**) or L3 hemocytes and glia (**Figure 4-b, Sup. Table 5-b**) allowed  
251 us to subdivide the receptors based on their relative levels of expression and fold change between  
252 the two cell types. Group 1 is specific to hemocytes, group 2 is expressed in both cell types, group  
253 3 is specific to glia (**Figure 4-a,b group 1 in red, group 2 in black, group 3 in yellow**).

254 Group 1 receptors are involved in the phagocytosis of Gram-positive and Gram-negative bacteria  
255 (Pearson et al., 1995; R met et al., 2001), matching the hemocyte-specific function of pathogen  
256 phagocytosis. They include the well-studied genes *eater*, *NimC1*, *NimC2* and *Scavenger receptor*  
257 *class C, type I (Sr-CI)*. *eater*, *NimC1* and *NimC2* contain multiple epidermal growth factor (EGF)  
258 like repeats called NIM repeats (Kurucz et al., 2007). SR-CI and SR-CIV belong to the *Drosophila*

259 SR-C class of scavenger receptors, SR-CI contains several known motifs, including two  
260 complement control protein domains, a somatomedin B domain and a Meprin, A-5 protein, and  
261 receptor protein-tyrosine phosphatase Mu domain (Rämet et al., 2001).

262 Group 2 receptors include *NimC4*, *draper (drpr)* and *croquemort (crq)*, which was believed to be  
263 only expressed in hemocytes (Franc et al., 1996) and belongs to the SR-B class of scavenger  
264 receptors homologous to the mammalian CD36 (Franc et al., 1999). Although *crq* is expressed at  
265 higher levels in hemocytes, it is still expressed in glia at relatively high levels and cannot be  
266 considered hemocyte-specific (**Sup. Table 5-a,b**). These receptors are involved in the  
267 phagocytosis of apoptotic bodies and debris, in line with the fact that *Drpr* and *NimC4* act in  
268 hemocytes and in glia to promote the clearance of apoptotic cells and axonal debris (Kurant et al.,  
269 2008; MacDonald et al., 2006; Manaka et al., 2004; Melcarne et al., 2019; Roddie et al., 2019).

270 Group 3 includes three genes: *bark beetle (bark)* encodes a transmembrane scavenger receptor-  
271 like protein important for the establishment of the BBB (Hildebrandt et al., 2015; Parker and Auld,  
272 2006; Stork et al., 2008). *Secreted Wg-interacting molecule (Swim)* contains a somatomedin B  
273 domain similar to Sr-CI, however it has not been investigated for its role in phagocytosis. *NimA*  
274 is a *Drpr*-like receptor containing one NIM domain followed by two EGF repeats and one Emilin  
275 domain (**Sup. Figure 3-a**, Callebaut et al., 2003). Its *C. elegans* orthologs are involved in the  
276 phagocytosis of apoptotic cells (Kurucz et al., 2007; Mangahas and Zhou, 2005).

277 The expression profile of the receptors evolves with time. The number of scavenger receptors  
278 specific to hemocytes and their expression levels increases in the larva (4 at E16, 8 at L3, shown  
279 in red, **Figure 4-a,b**). In parallel, the number of receptors non-differentially expressed between  
280 hemocytes and glia decreases in the larva (8 at E16, 5 at L3, shown in black). Moreover, within  
281 the same cell type, the expression levels of some receptors change significantly, *eater* being highly  
282 upregulated in L3, *NimC4* at E16.

283 To validate the expression of these genes, we performed quantitative reverse transcription PCR  
284 (RT-qPCR) assays on L3 glia and hemocytes. We tested and confirmed the purity of the sorted  
285 cell populations by quantifying the expression levels of *Hemolectin (Hml)* and *reverse polarity*  
286 (*repo*), as markers of hemocytes and glia, respectively (**Sup. Figure 3-b,c**). *NimC1* and *eater* are  
287 significantly upregulated in L3 hemocytes compared to glia (**Sup. Figure 3-d,e**), *drpr* shows

288 comparable expression levels (**Sup. Figure 3-f**); *crq* seems to be more expressed in hemocytes  
289 however the difference is not statistically significant (**Sup. Figure 3-g**), similarly to the  
290 transcriptome results. *NimA* is only found in glia (**Sup. Figure 3-h**). The finding that some  
291 receptors are expressed in glia and hemocytes, whereas others are cell-specific recapitulates their  
292 common function of phagocytosing apoptotic cells and debris and at the same time emphasizes the  
293 role of hemocytes in host defense against pathogens. In addition, the higher levels of the scavenger  
294 receptors in L3 than in E16 hemocytes prove an increased functional specificity of hemocytes in  
295 the larva.

296 Given the high specificity and levels of expression of *NimA*, we performed an *in situ* hybridization  
297 RNAscope assay on wild type (*WT*) L3 CNS and found very specific labeling at the position of  
298 the neuropile (**Sup. Figure 3-i,i'**). We complemented this analysis by labeling *NimA-Gal4; UAS-*  
299 *eGFP* E16, L3 and adults with Repo, a pan-glial marker and Nazgul, a NADP-retinol  
300 dehydrogenase specifically expressed in embryonic astrocyte and ensheathing glia, the two cell  
301 types that constitute the neuropile glia (Peco et al., 2016; Ryglewski et al., 2017) (**Figure 4-c-e'**).  
302 Nazgul and *NimA* nicely colocalize in the embryonic glial cells (**Figure 4-c,c'**). By contrast, *NimA*  
303 and Nazgul are found in larval glial cells that surround the neuropile and are in close proximity,  
304 suggesting that at that stage Nazgul is expressed in the astrocyte glia that invade the neuropile,  
305 while *NimA* is expressed in the ensheathing glia as well as in the wrapping glia that surround the  
306 peripheral nerves (**Figure 4-d,d'**). In the adult CNS, *NimA* is also found in glial cells surrounding  
307 the neuropile and, based on single-cell RNA seq data, *NimA* is expressed in adult ensheathing glia  
308 but not in astrocyte-like glia (**Figure 4-f,f'**) (Davie et al., 2018). Thus, *NimA* represents a novel  
309 glia-specific scavenger receptor expressed in a subset of glia cells throughout the life of the fly.

310 In sum, hemocytes have a strong commitment to immune functions in L3 and each phagocytic cell  
311 type expresses common as well as specific receptors, such as the glia-specific *NimA* gene and the  
312 hemocyte-specific *NimC1* and *eater* genes. This highlights a cell-specific phagocytic potential that  
313 differentiates glia from the macrophages outside the CNS.

314

### 315 **Stage-specific transcriptional signatures**

316 The transition from the embryo to the larva involves massive physiological changes that affect the  
317 organism as a whole. This likely explains why the transcriptomes group by stage more than cell  
318 types (**Figure 1-a**). To get more mechanistic insights on the changes associated with time, we  
319 assessed whether a stage-specific molecular signature is common to all cell types. We combined  
320 all cells deriving from E16 and compared their transcriptomes to those of cells deriving from L3.

321 The MA plot comparing E16 to L3 transcriptomes suggests that embryonic cells are overall closer  
322 to each other than larval cells (**Figure 5-a**), in line with the dendrogram in **Figure 1-a**. This does  
323 hint to a stage-specific signature common to all cells. The transcripts enriched at E16 compared to  
324 L3 are involved in cytoplasmic translation and cuticle development (**Figure 5-b, Sup. Table 6-a**).  
325 Genes included in the GO term cytoplasmic translation code for ribosomal proteins and translation  
326 machinery, such as translation initiation factors. Ribosomal protein transcripts and ribosomes are  
327 known to be maternally deposited and highly abundant in early embryos (Qin et al., 2007), but  
328 new ribosomes are built by the end of embryogenesis, in line with the transcriptomic data. The  
329 ‘cuticle development genes’, include 42 genes coding for Cuticular proteins, 21 genes coding for  
330 members of the Tweedle family and 7 genes coding for CPR cuticle protein family (**Figure 5-c,**  
331 **Sup. Table 6-b**). These proteins are predicted to be present in the extracellular matrix (ECM)  
332 (Cornman, 2009; Karouzou et al., 2007; Naba et al., 2016; Zuber et al., 2020), which implies a  
333 role of all three cell types in the deposition of ECM and cuticle at embryonic stage, a feature that  
334 has already been described for the hemocytes (Brown, 2011; Martinek et al., 2008) and is in  
335 accordance with the important role of the ECM in organogenesis.

336 Fewer genes are upregulated in L3 compared to E16 (**Figure 5-a**) and many of them belong GO  
337 terms related to tRNA aminoacylation, DNA repair and epigenetics (**Figure 5-d, Sup. Table 6-c**).  
338 The enrichment of tRNA aminoacylation related transcripts hints to increased translation, which  
339 could be due to the fact that, unlike embryos, larvae take up amino acids and nutrients through  
340 feeding and are thus able to aminoacylate tRNAs, as lack of amino acids and starvation has been  
341 shown to cause significant decreases in protein synthesis (Deliu et al., 2017).

342 We were puzzled by the enrichment of transcripts involved in DNA repair. Among them are *rad50*,  
343 *Nijmegen breakage syndrome (nbs)* and meiotic recombination 11 (*mre11*), members of the *MRN*

344 complex, as well as *Telomere fusion (Tefu)*, the ortholog of *Ataxia Telangiectasia Mutated (ATM)*  
345 **(Figure 5-e, Sup. Table 6-d)**. These genes have been implicated in DSB repair (Wang et al.,  
346 2014), in DNA replication restart after replication stress (Gatei et al., 2014) and in preventing  
347 telomere fusion as well as chromosome breakage in *Drosophila* (Ciapponi et al., 2004, 2006). To  
348 validate the stage-specific enrichment of these transcripts, we performed qPCR assays on RNA  
349 extracted from whole E16 and L3 animals: *rad50* **(Sup. Figure 4-a)** and *mre11* **(Sup. Figure 4-**  
350 **b)** are upregulated in L3 and *nbs* tends to be upregulated **(Sup. Figure 4-c)**. Based on the fact that  
351 double strand breaks trigger the phosphorylation of the histone H2AX, a variant of the H2A protein  
352 family (Redon et al., 2002), we hypothesized that the increase in DNA repair genes is due to an  
353 increase in DSBs. Therefore, we estimated the relative levels of DSB using the phosphorylated  
354 H2A.v (H2A.v-P) *Drosophila* marker, the equivalent of gamma-H2AX in mammals. When DNA  
355 damage forms double strand breaks, H2A.v is phosphorylated by kinases such as ATM and the  
356 phosphorylated protein, gamma-H2AX, is the first step in recruiting and localizing DNA repair  
357 proteins. Western blot assays on histone extracts show that the levels of H2A.v-P decrease in L3  
358 compared to E16 **(Sup. Figure 4-d,e)**, contrary to our initial hypothesis.

359 A recent study showed that the yeast equivalent of the MRN complex (MRX) is involved in  
360 controlling gene expression levels through modulating chromatin interaction (Forey et al., 2021).  
361 Moreover, MRN components interact with Heterochromatin Protein 1 (HP1), which is involved in  
362 gene repression through heterochromatin formation (James and Elgin, 1986; James et al., 1989),  
363 and they regulate HP1 expression and stability (Bosso et al., 2019). This, in addition to the finding  
364 of GO terms related to epigenetics “negative regulation of gene expression, epigenetic”,  
365 “chromatin remodeling” and “histone modification” in the genes upregulated in L3 compared to  
366 E16 **(Figure 5-f, Sup. Table 6-e)** led us to ask whether Rad50 might be involved in chromatin  
367 conformation and in the regulation of gene expression.

368 Chromatin states are defined by a highly complex epigenetic code comprised of histone post-  
369 translational modifications. Histone methylation is complex in that residues may be mono-, di-, or  
370 trimethylated, and these marks may activate or repress gene transcription. Di- and trimethylation  
371 of H3K4, for instance, strongly correlate with active gene transcription, whereas H3K9 and H3K27  
372 methylation are mostly associated to a closed heterochromatin state and therefore to transcription  
373 repression (Aranda et al., 2015; Conway et al., 2015; Peters et al., 2003; Rea et al., 2000; Santos-



374 Rosa et al., 2002). To determine whether Rad50 is required for chromatin conformation and  
375 repression of gene expression in L3 larvae, we labeled polytene chromosomes of wild-type or  
376 *rad50Δ5.1* mutant larvae with antibodies against H3K4me3, H3K9me2 or H3K9me3. The absence  
377 of Rad50 causes a significant increase of H3K4me3 and a decrease of H3K9me2/me3 epigenetic  
378 marks (**Figure 6-a,b**). Polytene chromosomes are characterized by the alternation of dense  
379 (heterochromatic) and less dense (euchromatic) bands, a reflection of the chromatin condensation  
380 state. Interestingly, we observed an alteration of the H3K4me3 band signal profile accompanied  
381 by almost complete absence of H3K9me3 in *rad50Δ5.1* polytene chromosomes compared to *WT*  
382 (**Figure 6-c,d**). While the repression marks H3K9me2/me3 show a clear decrease in *rad50Δ5.1*,  
383 the H3K27me3 signal increases significantly in *rad50Δ5.1* (**Figure 6-e,f**). Due to the link between  
384 H3K27me3 and DNA damage shown by studies highlighting an increase in H3K27me3 at DNA  
385 DSB sites (Basenko et al., 2015; O'Hagan et al., 2008, 2011) and the role of Rad50 in DNA  
386 damage, we asked whether the increase in H3K27me3 is simply due to increased DSBs.  
387 Comparing H3K27me3 and H2A.v-P distribution shows a clear lack of colocalization of the two  
388 marks thus highlighting that the increase in H3K27me3 is independent from the increase of DSBs  
389 (**Figure 6-e-g**).

390 The changes in the H3K4me3 and H3K27me3 profiles and the strong reduction of H3K9me3,  
391 observed in *rad50Δ5.1* polytene chromosomes, suggest that Rad50 depletion affects chromatin  
392 organization and hence gene expression regulation. These findings, together with the published  
393 data, underline a novel role of the DNA damage factors, in particular Rad50, in regulating  
394 chromatin states.

395 To understand whether Rad50 depletion and its effect on chromatin state also impinge on gene  
396 expression, we analyzed several transcripts that show high expression in E16 but low expression  
397 in L3 in all cell types in the *WT*. Assessing the expression of these genes in L3 *WT* compared to  
398 *rad50Δ5* showed significant upregulation of four genes upon Rad50 depletion including the  
399 *TweedleW* (*TwdlW*), *ftz transcription factor 1* (*ftz-ft1*), *Uncoordinated 76* (*Unc-76*) and the member  
400 of ATM *Mre11* (**Figure 6-h,k**), while *olf413* and *snail* (*sna*) show significant decrease of  
401 expression levels (**Figure 6-l,m**). Altogether, these and the published data highlight a novel role  
402 of the DNA damage factors, and specifically Rad50, in transcriptional regulation.



403 In sum, our data reveal that different developmental stages are associated with specific molecular  
404 signatures, regardless of the cell type, including chromatin organization.

405

#### 406 **Metabolic signatures of embryonic and larval neurons, glia and hemocytes**

407 A careful comparison of the embryonic and larval cells also revealed distinct metabolic states.  
408 Compared to their larval counterpart, E16 hemocytes express more glycolytic enzymes such as  
409 *lactate dehydrogenase (ldh)*, essential for the conversion of pyruvate to lactate (Cattenoz et.al.,  
410 2020, **Figure 7**). This implies the activation of a Warburg-like glycolytic state and the conversion  
411 of pyruvate to lactate. E16 hemocytes are also lipogenic as they highly express *de novo* the  
412 lipogenic enzyme, *Acetyl-CoA carboxylase (ACC)* and enzymes of the triacylglycerol biosynthetic  
413 pathway (TAG) that include *Fatty acid synthase (FAS)*, *Glycerol-3-phosphate acyltransferase*  
414 (*Gpat*), *1-Acylglycerol-3-phosphate O-acyltransferase (Agpat)*, *Lipin (Lpin)* and *Diacylglycerol*  
415 *O-acyltransferase (DGAT)*. By contrast, L3 hemocytes are more oxidative, they most likely  
416 internalize and metabolize lipids through the beta-oxidation pathway to generate acetyl CoA and  
417 drive the tricarboxylic acid (TCA) cycle. This is supported by the enrichment of transcripts  
418 encoding lipid-scavenging receptors and by the poor expression of genes involved in the TAG  
419 pathway (**Figure 7, Sup. Table 7**). Moreover, L3 hemocytes do not rely on glycolysis to drive the  
420 TCA cycle, as the genes involved in glycolysis, mainly *phosphofructokinase* and *pyruvate*  
421 *dehydrogenase (PDH)*, of which *PDH* controls the rate-limiting conversion of pyruvate to acetyl  
422 CoA and critically controls TCA activity, are not upregulated in those cells. Finally, the poor  
423 expression of *phosphoenolpyruvate carboxykinase (PEPCK)* and *fructose 1,6-bisphosphatase*  
424 (*FBPase*), as well as a significant enrichment of the *Glut1* sugar transporter transcripts suggest the  
425 absence of gluconeogenesis and the active uptake of glucose in the larva. Because the glycolytic  
426 genes downstream of *glucose 6, phosphate (G6P)* are poorly expressed while those of the pentose  
427 phosphate pathway (PPP) are highly expressed in the L3 hemocytes, the internalized glucose could  
428 be used to generate pentose sugars for ribonucleotide synthesis and redox homeostasis through the  
429 generation of NADPH. Hence, apart from the glycolytic pathway, E16 and L3 hemocytes display  
430 anabolic (gluconeogenic, lipogenic) and catabolic (oxidative, lipolytic) signatures, respectively.

431 Comparative analyses revealed interesting commonalities in a few metabolic pathways, in addition  
432 to metabolic changes that are cell-specific, which projects underlying differences in the metabolic  
433 states (**Figure 7**). The one feature observed across all cell types is a clear metabolic shift in the  
434 key glycolytic enzyme *ldh*, which is highly expressed in the embryo (**Figure 7, Sup. Table 7**).  
435 This implies a global activation of a Warburg like state specifically in the embryo.

436 The transcripts of the glycolytic pathway genes are enriched in E16 glia and neurons: *Glucokinase*  
437 (*Hexokinase-C*, *FBgn0001187*), *Aldolase* and *Glyceraldehyde 3-phosphate dehydrogenase*  
438 (*Gapdh*) show prominent expression in glia, while only *Glucokinase* and *pyruvate kinase* are  
439 expressed at high levels in the neurons. The transcripts coding for the *Glucose 6-phosphate*  
440 *dehydrogenase (G6PD)*, a rate-limiting enzyme of the PPP pathway, is also strongly enriched in  
441 E16 glia and neurons. This implies that these cells most likely use glucose for driving the PPP  
442 pathway and glycolysis. Neither E16 glia nor E16 neurons are gluconeogenic, as the *PEPCK* gene  
443 is poorly expressed at that stage. We therefore assessed the underlying source of glucose driving  
444 glycolysis and evaluated the capacity for glucose uptake via transporters as a predictable source.  
445 This revealed a strong enrichment of transcripts coding for sugar transporters like *sut1*, *sut3*, *sut4*  
446 and specifically glucose transporters *Glut1* and *Glut3*. L3 glia and neurons seem both  
447 gluconeogenic and glycolytic. Thus, E16 glia and neurons rely on extracellular sugar uptake,  
448 sustained glycolysis and PPP while L3 glia and neurons utilize *de novo* gluconeogenesis to fuel  
449 glycolysis.

450 The fate of glucose, post its breakdown to pyruvate via glycolysis, apart from driving lactate, can  
451 be diverted to the TCA cycle (oxidative). We did observe a significant upregulation the TCA  
452 enzymes in E16 glia compared to their larval counterpart. For instance, *Citrate synthase*,  
453 *Aconitase*, *Isocitrate dehydrogenase (IDH)*, *oxoglutarate dehydrogenase (Ogdh)*, *succinate*  
454 *dehydrogenase (SDH)* and *malate dehydrogenase (MDH)* are highly expressed in the embryo. By  
455 contrast, only a subset of TCA enzymes (*Aconitase*, *succinyl CoA synthetase (SCS)*, *Fumarate*  
456 *hydratase (FH)*) show prominent expression in L3 glia. Thus, glia are both glycolytic and oxidative  
457 in the embryo and transition to being primarily oxidative in the larva. By contrast, neurons undergo  
458 a clear metabolic shift from glycolytic in the embryo to oxidative in the larva, as L3 neurons  
459 display high expression of most TCA enzymes, including *PDH*.

460 Both E16 glia and neurons show significant enrichment of lipogenic transcripts. The key enzyme,  
461 *ACC*, and TAG biosynthetic enzymes (*FAS*, *Gpat*, *Agpat*, *Lpin*) are highly expressed. Interestingly,

462 the expression of enzymes involved in lipid break down, such as *Acyl-CoA oxidase (AOX)*, the  
463 first enzyme of the beta-oxidation system, and *Carnitine O-palmitoyltransferase (CPT1* and  
464 *CPT2)*, which transfers fatty acids into the mitochondrion for  $\beta$ -oxidation, is also high in E16  
465 neurons but not glia. Thus, L3 glia continues to show elevated expression of a subset of TAG  
466 biosynthetic enzymes (*FAS, Gpat, Agpat, Lpin*). Additionally, L3 glia show high expression of the  
467 fatty acid breakdown pathway. Taken together, these data suggest that glia are lipogenic both in  
468 embryo and larvae but activate lipolytic genes specifically in the larval stage. Neurons, on the other  
469 hand, sustain a lipogenic and lipolytic state in the embryo but in the larva, they are only lipogenic.

470

471

## 472 **Discussion**

473 The definition of cell types makes the object of intense investigation for the potential impact on  
474 medical science and in particular on regenerative medicine. We here identify the molecular  
475 signatures that characterize *Drosophila* neurons, glia and hemocytes, as representative examples  
476 of related and unrelated cell types. We show that the molecular identity of a cell type results from  
477 the combined action of intrinsic and extrinsic cues and we establish the impact of origin, position,  
478 metabolic state, function and time. We identify cell-specific as well as stage-specific signatures  
479 and we show that cells maintain their identity during development while keeping their ability to  
480 accommodate to the evolving needs of the organism.

481

### 482 **Cell types are defined by a relatively low number of specific transcripts**

483 Several technologies allow the genome-wide characterization of cells at the molecular level. Bulk  
484 transcriptomes provide an adapted tool to extrapolate the most significant and specific features  
485 that identify a cell type as a whole, due to the sequencing depth and to the average measurement  
486 of gene expression across a population of cells. Using this approach, we show that the majority of  
487 the genes are not differentially expressed between neurons, glia and hemocytes, both in the embryo  
488 and in the larva. Such high number of similar genes cannot be due to common origin/function  
489 because neurons and hemocytes share neither. This data nevertheless goes along with a study

490 showing that 48.8% of all Flybase genes are non-differentially expressed between all stages  
491 (Daines et al., 2011). No more than 2000 genes are upregulated in each cell type, hence, a relatively  
492 low number of genes is sufficient to define a cell type.

493 In average, the transcripts enriched in a specific cell type are expressed at high levels, suggesting  
494 that they are under the control of signaling pathway rather than being expressed constitutively and  
495 at low levels. Thus, cell types are defined by a combination of cell-specific transcript enrichment  
496 and high levels of expression. Of note, even amongst the common transcripts we find a similar  
497 distinction: the genes that have a house keeping function shared by all cell types are expressed at  
498 lower levels than those that are associated with more specific functions. For instance, the  
499 comparison of neurons and glia shows that the genes involved in DNA replication display a  
500 number of reads < 300 while those related to axons (which are common to neurons and glia but  
501 not to hemocytes) are expressed at higher levels (> 3000 reads).

502 In sum, cell types are defined by few genes expressed at high levels.

503

#### 504 **Transcriptional landscapes and the impact of time**

505 The establishment of a cell type involves the stable expression of specific morphological and  
506 functional features, even though cells may lose some traits or assume others over time. We hence  
507 expected each cell type to group together throughout development. Instead, the developmental  
508 stage represents the first factor of homogeneity, more than the cell type: embryonic hemocytes are  
509 closer to embryonic glia and neurons than to larval hemocytes. Thus, although differentiated cells  
510 accomplish the same function throughout the life of the organism, time affects their state.

511 Amongst the stage-specific genes, ECM related GO terms are upregulated in E16 compared to L3  
512 cells. In line with the fact that the ECM is a key player in morphogenesis and organogenesis,  
513 different types of embryonic cells likely express large quantities of ECM transcripts to prepare for  
514 the larval life and the immense growth happening during this stage. L3 cells show an enrichment  
515 of transcripts involved in DNA damage and repair. In particular, the three components of the MRN  
516 complex (Rad50, Mre-11 and NBS) and Tefu, that respond to DNA DSB and are involved in  
517 replication fork dynamics, telomere maintenance and even response to viral infection (Ciapponi et  
518 al., 2004; Syed and Tainer, 2018). The increased expression of these genes is seemingly not due

519 to DSB since there are more DBS markers in E16 than in L3. Our results suggest that Rad50  
520 functions beyond DNA repair and controls gene expression through the modulation of chromatin  
521 state, in line with recent finding showing that the yeast ortholog represses the loci near its binding  
522 site (Forey et al., 2021). Future studies will address whether the changes in both euchromatic and  
523 heterochromatic marks observed in the mutant larval salivary glands are due to late, indirect,  
524 effects or reflect a novel regulatory mechanism.

525 The RNAseq data suggest that cells are able to sense time, a process that at least in the case of  
526 neurons is disconnected from the cell cycle and from cell division, as these cells do not undergo  
527 mitosis. The upregulation of genes involved in histone modification and chromatin remodeling in  
528 L3 suggests a stage-specific epigenetic control of the transcriptional landscape.

529 Finally, the analysis of the transcription factors at the two stages suggests that the three cell types  
530 are endowed with distinct degrees of plasticity. The E16 and the L3 hemocytes share many fewer  
531 transcription factors (n=25, **Sup. Table 1-i,j in red**) than those that are common to the three  
532 embryonic cell types (n=129, **Sup. Table 1-a**). This behavior is also found in glia (n=17, **Sup.**  
533 **Table 7-k,l in yellow**) but not in neurons (n= 124, **Sup. Table 1-m,n in blue**), which share more  
534 than 50% of upregulated transcription factors at E16 and L3. The transcriptome of neurons as well  
535 as the transcription factors shared at E16 and L3 suggest that neurons are a relatively stable  
536 population compared to hemocytes. In the case of glia, though comparing their transcriptome at  
537 E16 and L3 shows similar behavior to neurons, the fact that they share few transcription factors at  
538 E16 and L3 suggests an intermediate level of stability. It is tempting to speculate that the neural-  
539 immune phenotype of glial cells and the strong reliance of hemocytes on the environment could  
540 account for the plastic behavior of these cell types. An increased number of glial and hemocytes  
541 subtypes in larval stages could also account for such behavior. Future single cell analyses on the  
542 different cell populations at different stages will help distinguishing between these possibilities.

543

#### 544 **Hemocytes and glia, macrophages outside and within the nervous system**

545 The origin and position of neurons and glia have a significant impact on their transcriptional  
546 landscapes, as shown by the large number of common molecular features. A substantial fraction  
547 of the shared GO terms concern processes related to axons, synapses, and neural processes. In  
548 contrast, neurons and hemocytes share many fewer transcripts (5,9% at E16, 3,8% at L3), with no

549 GO terms related to cell-specific processes (**Sup. Table 8-a,b**). The situation is different for the  
550 glia/hemocyte comparison. E16 glia and hemocytes share the same very low degree of similarity  
551 as that observed between neurons and hemocytes (5,7%). In addition, genes upregulated in E16  
552 hemocytes do not seem to be involved in hemocyte-specific functions. This is likely due to the fact  
553 that the major function of the E16 hemocytes is to secrete ECM components and to phagocytose  
554 apoptotic cells, both of which are also done by glia. By contrast, L3 hemocytes and glia share  
555 18,3% of enriched transcripts, some of which are related to immunity. The presence of hemocyte-  
556 specific GO in the larva indicates a real developmental switch (see also Cattenoz et al., 2020) and  
557 a strong specialization of the hemocytes, with more refined groups of genes being upregulated and  
558 a strong immune component.

559 Glia are not just macrophages that are also able to perform neural functions. Much like microglia,  
560 the vertebrate myeloid cells that invade the nervous system during development (Neumann et al.,  
561 2009; Wu et al., 2015) (DePaula-Silva et al., 2019; Hickman et al., 2013; Li and Barres, 2017;  
562 Ritzel et al., 2015), fly glia express very specific scavenging features compared to the immune  
563 cells located outside the nervous system. Typically, *NimA* is not expressed in hemocytes but only  
564 in a subset of glia. Pursuing the role of *NimA* will help deciphering the mode of action of glia in  
565 the immune response, which so far has relied only on the study of Draper and NimC4, the latter  
566 being only expressed in embryonic glia. *NimA* is likely not the only additional scavenger receptor  
567 possibly involved in the phagocytosis of debris and apoptotic bodies. A recent study identified the  
568 role of *NimB4* in apoptotic bodies phagocytosis in glia and in hemocytes in the larva (Petrigiani  
569 et al., 2021), even though *NimB4* expression in hemocytes is at least 70-fold higher than in glia (our  
570 data).

571 The RNAseq data highlight an intermediate neuro-immune phenotype of fly glia. We propose that  
572 simple organisms display relatively few cell types with multiple potentials whereas more complex  
573 organisms need a more refined division of labor accompanied by the appearance of dedicated cell  
574 types, such as the microglia in vertebrates. Interestingly, the comparative analysis of the  
575 transcription factor reveals the upregulation of the Nuclear factor of activated T-cells (NFAT)  
576 ortholog in L3 glia and hemocytes compared to neurons (**Sup. Table 1-g in gray**). While the  
577 *Drosophila* gene has not yet been explored in the context in immunity, NFAT is a member of a  
578 Calcium dependent protein family that plays an important role in the immune response in lymphoid  
579 and myeloid vertebrate lineages, including microglia (reviewed in Müller and Rao, 2010).



580 In sum, hemocytes become more specialized as the animal hatches. Fly glia are at the interface  
581 between the nervous and the immune systems: the neural origin shared with neurons is already  
582 apparent in the embryo, while the immune function shared with hemocytes becomes apparent in  
583 the larva. Given the importance of the immune resident cells of the CNS in development and  
584 homeostasis, fly glia represent an interesting tool to unravel the role of myeloid-like cells in  
585 diseases as severe and diverse as brain tumor, neurodegeneration and autoimmune diseases.

586

### 587 **Cell and stage-specific metabolic states**

588 Metabolism and cell identity are closely coupled so that cells can carry out their function and meet  
589 the changing needs during the life cycle. Our transcriptomic data reveal the following key  
590 observations: a) all cell types show high levels of *ldh* expression in the embryo, b) they appear  
591 predominantly lipogenic in the embryo and c) they switch from being glycolytic in the embryo to  
592 being oxidative in the larva. While these features are shared by the three cell types across  
593 development, there are stark differences in terms of glucose uptake and subsequent break down.  
594 E16 hemocytes rely on *de novo* glucose and lipid biosynthesis and sustain a glycolytic state, while  
595 their larval counterpart depend more on the uptake of sugars and lipids and their subsequent  
596 breakdown to generate intermediates that run the TCA to sustain an oxidative state. E16 glia  
597 internalize sugars, generate lipids and metabolize them via glycolytic and oxidative routes, while  
598 L3 glia rely on gluconeogenesis and TAG biosynthesis and their breakdown to drive TCA and  
599 maintain an oxidative state. E16 neurons mediate sugar uptake and *de novo* lipogenesis and sustain  
600 a glycolytic state, whereas L3 neurons are more oxidative, which could be driven by *de novo* sugar  
601 synthesis and its breakdown to sustain TCA. L3 neurons also show reliance on TAG biosynthesis,  
602 which is perhaps critical for generating lipids essential for synaptic vesicle release. We speculate  
603 that in E16 neurons lipid synthesis and catabolism may function as a potential energy source while  
604 in E16 glia, lipid synthesis may be relevant for functions like secretory vesicular based intercellular  
605 communication.

606 In sum, embryonic cells are capable of intracellular synthesis of sugars and lipids and are also  
607 involved in internalizing extracellular metabolites. Later on, neurons and glia indulge in active  
608 uptake of metabolites and also continue to drive intracellular synthesis of lipids and sugars. Thus,  
609 both extracellular and intra-cellular resources drive the TCA in these tissues. By contrast, L3



610 hemocytes appear more reliant on extracellular nutrients to drive their internal lipid oxidation and  
611 TCA catabolic pathways. Such reliance on extracellular metabolites as the predominant source  
612 makes hemocytes competent sensors of systemic metabolites and any fluctuation in their levels.

613 The analyses of the metabolic pathways revealed an unexpected aspect of stage-specificity: the  
614 utilization of distinct paralogs in embryos and larvae. The fatty acid metabolic enzyme, *Fatty acid*  
615 *synthase 1* (*FASN1*, *FBgn0027571*), *CG3523*, is expressed in larvae, while its paralog *CG3524*  
616 (*FASN2*, *FBgn0042627*), was detected in embryos. The enzyme of lipid breakdown pathway,  
617 *CG17544* (*FBgn0032775*) with predicted acyl-CoA oxidase activity, is expressed in larvae, while  
618 its paralog *CG9709*, *acyl-Coenzyme A oxidase at 57D distal* (*Acox57D-d*, *FBgn0034629*), in  
619 embryos. Additionally, we identified genes that are not referred to as paralogs in Flybase, but share  
620 functional similarity, showing a similar trend: *CG6650* (*FBgn0036402*), predicted to have ADP-  
621 glucokinase activity, is expressed in larvae, while the glycolytic enzyme *CG8094*, *Hexokinase C*  
622 (*Hex-C*, *FBgn0001187*), in embryos. For the TCA enzymes, *CG7024* (*FBgn0029722*), predicted  
623 to have pyruvate dehydrogenase activity, is expressed in larvae while *CG11876*, which is *Pyruvate*  
624 *dehydrogenase E1 beta subunit* (*Pdhb*, *FBgn0039635*), in embryos. The expression of *CG12233*,  
625 *Idh3a* (*FBgn0027291*), was detected in larvae, while *CG32026* (*FBgn0052026*), predicted with  
626 isocitrate dehydrogenase activity, was noticed in embryos. Finally, the expression of *Ogdh*  
627 (*Fbgn0010352*), *CG11661*, was seen in embryos, while *CG5214* (*FBgn0037891*), predicted to be  
628 part of the oxoglutarate dehydrogenase complex, in larvae. The enzymes with predicted acyl-CoA  
629 activity are tandemly duplicated, but the vast majority of the other genes are distantly located or  
630 reside on different chromosomes, calling for independent transcriptional regulation and/or stage-  
631 specific differences in their enzymatic activities. Future studies will establish the overall relevance  
632 of paralog switching on metabolic state transitions, but such temporal specificity provides an  
633 unexpected level of control that adds to the post transcriptional modifications of those enzymes.

634

635 The unbiased identification of common and specific transcriptional pathways allows a better  
636 understanding of the molecular processes associated to cell type identity. This genome-wide study  
637 has identified the transcriptional features of three types of related and unrelated cells, including  
638 their metabolic states and their transcriptional regulators. It has revealed novel cell-specific and  
639 stage-specific players including NimA and Rad50, respectively. It has highlighted time as a novel

640 parameter defining a differentiated cell, paving the way to understand the role of high-order  
641 chromatin organization in the process. Altogether, this comparative high throughput analysis  
642 allows a better understanding of the mechanisms defining cell type identity, a central issue in  
643 biology and medical science.

644

645

## 646 **Materials and methods**

### 647 **Fly strains and genetics**

648 Flies were raised on standard medium at 25°C. Fly strains used are: *srp(hemo)Gal4/+; UAS-*  
649 *RFP/+* obtained upon crossing *srp(hemo)Gal4* (gift from K. Brückner) (Brückner et al., 2004) and  
650 *UAS-RFP* flies (RRID:BDSC\_8547), *HmlA-RFP/+* (Makhijani et al., 2011), *srp(hemo)3xmcherry*  
651 (gift from D. Siekhaus, (Gyoergy et al., 2018), *repo-nRFP 43.1* on the 3rd chromosome (Laneve  
652 et al., 2013), *elav-nRFP 28.2* insertion on the 3rd chromosome *nSybGal4/+; UAS-RFP/+* obtained  
653 upon crossing *nSybGal4/+* (RRID:BDSC\_51635) with *UAS-RFP/+*, *rad50Δ5.1* (Ciapponi et al.,  
654 2004) and *Oregon-R* strain was used as the control (referred to as *WT* in text). *NimAGal4* null  
655 mutant was generated by CRISPR-mediated mutagenesis and performed by WellGenetics Inc.  
656 using modified methods from (Kondo and Ueda, 2013). In brief, the upstream gRNA sequence  
657 ACTGCTCCTCCTGCTTGCAA[TGG] and downstream gRNA sequence  
658 GTGCCCTTCTAACATATACC[AGG] were cloned into U6 promote plasmid(s). Cassette *Gal4-*  
659 *3xP3-RFP*, which contains ribosome binding sequence (RBS), Gal4, SV40 polyA terminator and  
660 a floxed 3xP3-RFP, and two homology arms were cloned into pUC57-Kan as donor template for  
661 repair. *NimA*-targeting gRNAs and hs-Cas9 were supplied in DNA plasmids, together with donor  
662 plasmid for microinjection into embryos of control strain *w[1118]*. F1 flies carrying selection  
663 marker of 3xP3-RFP were further validated by genomic PCR and sequencing. CRISPR generates  
664 a 4,884-bp deletion removing the entire *NimA* CDS that is replaced by the cassette *Gal4-3xP3-*  
665 *RFP. NimAGal4; UAS-eGFP* animals were obtained upon crossing *NimAGal4* with *UAS-eGFP*.

666 **FACS sorting of hemocytes, glia and neurons**

667 Embryonic cells were isolated as follows. Staged egg laying was carried out to produce E16  
668 embryos. *srp(hemo)Gal4/+; UAS-RFP/+*, *repo-nRFP*, *elav-nRFP* and *Oregon-R* strains were  
669 amplified, staged lays were done on yeast apple juice agar at 25°C. After a pre-lay period of 30  
670 minutes, the agar plates and yeast were replaced with fresh plates and flies were left to lay for 3  
671 hours at 25°C. Agar plates were removed and kept at 25°C, embryos were collected 11 hours and  
672 40 minutes AEL, when they had reached stage 16. Embryos were then isolated from the medium  
673 and washed on a 100 µm mesh. The collected embryos were transferred into a cold solution of  
674 phosphate-buffered saline (PBS) in a Dounce homogenizer on ice. The embryos were dissociated  
675 using the large clearance pestle then the small clearance pestle, then filtered (70 µm filter).

676 L3 neurons, glia and hemocytes were purified from *elav-nRFP*, *repo-nRFP* and *HmlΔ-RFP/+*  
677 larvae, respectively. Staged lays of 3 hours were carried out at 25°C and wandering larvae were  
678 collected 108–117 hours AEL. L3 hemocytes were isolated as mentioned in (Cattenoz et al., 2020).  
679 Larvae were bled in cold PBS containing PTU (Sigma-Aldrich P7629) to prevent hemocyte  
680 melanization (Lerner and Fitzpatrick, 1950), and filtered (70 µm filter). For neurons and glia  
681 isolation, larval brains were dissected in cold PBS on ice and transferred to tubes containing 0.5  
682 µg of collagenase IV (Gibco, Invitrogen) in 220 µL of PBS. Brains were incubated at 37°C on a  
683 Thermomixer with shaking at 500 rpm for 20 minutes. Then the brains were dissociated by  
684 pipetting up and down with 10-gauge needles and syringes and filtered through a 70 µm filter.

685 Cells were sorted using FACS Aria II (BD Biosciences) at 4°C in three independent biological  
686 replicates for each genotype. Live cells were first selected based on the forward scatter and side  
687 scatter and only single cells were sorted according to the RFP signal. *Oregon-R* cells were used as  
688 a negative control to set the gate and sort the RFP positive cells (**Sup. Figure 3-n**), which were  
689 collected directly in TRI reagent (MRC) for RNA extraction. Around 100 000 cells were sorted  
690 for each replicate. The purity of the sorted populations was assessed by carrying out a post-sort  
691 step. The FACS sorter was set up to produce cell pools displaying at least 80% of purity on the  
692 post-sort analysis.

## 693 **RNA extraction and sequencing**

694 Sorted cells were homogenized in TRI reagent (MRC) for 5 minutes at room temperature (RT) to  
695 ensure complete dissociation of nucleoprotein complexes. 0.2 mL of chloroform was added to each  
696 sample followed by centrifugation at 12,000 g for 15 minutes at 4°C. The upper aqueous phase  
697 containing the RNA was collected and transferred to a new tube. 0.5 mL of 2-propanol were added,  
698 and the samples were incubated for 10 minutes at RT. The samples were then centrifuged at 12,000  
699 g for 10 minutes to precipitate the RNA. The RNA pellet was then washed with 1 mL of 75%  
700 ethanol then precipitated again at 7,500 g for 5 minutes and air dried. 20 µL of RNase-free water  
701 was added to each sample before incubation at 55°C for 15 minutes. Single-end polyA+ RNA-Seq  
702 (mRNA-seq) libraries were prepared using the SMARTer (Takara) Low input RNA kit for  
703 Illumina sequencing. All samples were sequenced in 50-length Single-Read.

## 704 **Data analysis**

705 The data analysis was done using the GalaxEast platform, the Galaxy instance of east of France  
706 (<http://www.galaxeast.fr/>). First, FastQC (Babraham Bioinformatics) was used on the raw FastQ  
707 files to generate summary statistics and assess the quality of the data. The raw files were then  
708 converted to FastQ Sanger using FastQ Groomer for downstream analysis. Data were then mapped  
709 to the *Drosophila melanogaster* August 2014 Dm6 (BDGF release 6 + ISO1 MT/Dm6) reference  
710 genome using TopHat (Trapnell et al., 2009). The number of reads per annotated gene were  
711 counted using Htseq-Count (Anders et al., 2015) and the comparison and normalization of the data  
712 between the different cell types was done using Deseq2 (Anders and Huber, 2010). To compare  
713 E16 stage to L3 using Deseq2, we considered all cell types deriving from E16 as replicates and  
714 compared to the cell deriving from L3 that were also considered replicates. Gene ontology studies  
715 were done using the Panther classification system version 16 (<http://pantherdb.org/>) (Mi et al.,  
716 2010; Thomas et al., 2003) for the identification of biological processes, Bonferroni correction  
717 was used for all analyses. Genes with a number of reads > 15 were considered significantly  
718 expressed. For the study of upregulated genes, only genes with fold change >1.5, adjusted p-value  
719 <0.05 and expression level >15 were taken into account. Genes with number of reads > 15 and  
720 adjusted p-value of fold change > 0.05 were considered non-differentially expressed. Amongst  
721 these, genes with number of reads > 15 and fold change < 1.5 were considered commonly  
722 expressed. For the study of scavenger receptors only genes with expression level > 500, adjusted  
723 p-value < 0.05 and fold change > 10 were considered as specific to either glia or hemocytes. The

724 Venn diagrams were generated using Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/>)  
725 (Oliveros, J.C. (2007-2015)) and data representation was done using Microsoft Excel. The  
726 heatmaps representing the log<sub>10</sub> of the absolute expression levels in glia, neurons and hemocytes  
727 at E16 and L3 or representing the z-score were plotted in R (version 3.4.0) (R CoreTeam) using  
728 pheatmap (version 0.2) package (Kolde and Kolde, 2015).

### 729 **Quantitative PCR**

730 For the comparison between the expression levels of scavenger receptors in hemocytes and glia,  
731 cells were isolated from *srp(hemo)-3xmCherry* and *repo-nRFP* wandering L3, respectively. FACS  
732 sorting and RNA extraction were as described above. The extracted RNA was then treated with  
733 DNase I recombinant RNase free (Roche) and the reverse transcription was done using the Super-  
734 Script IV (Invitrogen) with random primers. The cycle program used for the reverse transcription  
735 is 65°C for 10 minutes, 55°C for 20 minutes, 80°C for 10 minutes. The qPCR was done using  
736 SYBR Green I Master (Roche). Actin5C (Act5C) and Ribosomal protein 49 (RP49) were used to  
737 normalize the data. The primers are listed in **Sup. Table 9**. The p-values and statistical test used  
738 are indicated in the figure legends.

739 For the quantitative PCR on larval and embryonic tissues, whole larvae and whole embryos were  
740 crushed in cold PBS using a Dounce homogenizer, extracts were filtered first through a cell strainer  
741 of 100 µm and RNA extraction, reverse transcription and qPCR were done as described.

### 742 **Histone extraction and western blot**

743 Histones from E16 and L3 *Oregon-R* were extracted as described in Abcam histone extraction  
744 protocol. Histone extracts were separated by 15% SDS-PAGE, transferred onto nitrocellulose  
745 membrane and probed with the primary antibodies mouse anti- H2A.v-P (1:5000) recognizing the  
746 *Drosophila* Histone 2A gamma variant, phosphorylated H2A.v-P (Developmental Studies  
747 Hybridoma Bank (DSHB AB\_2618077, Lake et al., 2013) and rabbit anti-H3 (1:5000) (Abcam  
748 ab1791) for normalization. Signal was detected with Pierce ECL western blotting substrate  
749 (Thermo Fisher Scientific, Waltham, MA) using HRP-conjugated secondary antibodies (1:10000,  
750 Jackson).

751 **Immunolabeling of central nervous system**

752 *NimAGal4; UAS-eGFP* embryos were dechorionated in bleach for 5 minutes and rinsed in water.  
753 They were then fixed in 50% heptane/50% paraformaldehyde (PFA) for 25 minutes on a shaker at  
754 RT and devitellinized in methanol/heptane for 1 minute. Embryos were then rinsed in methanol  
755 followed by PTX (0.3% Triton X-100 in PBS 1x) and incubated in blocking reagent (Roche) for 1  
756 hour at RT. They were incubated with primary antibodies mouse anti-Repo 1:20 (DSHB #8D12),  
757 chicken anti-GFP 1:500 (Abcam ab13970) and rabbit anti-Nazgul 1:100 (gift From B. Altenhein)  
758 in blocking reagent overnight (O/N) at 4°C, then washed in PTX 3 × 10 minutes at RT and  
759 incubated with secondary antibodies 1:400 (Cy3 anti-mouse and FITC anti-chicken (Jackson)) for  
760 1 hour at RT and washed 3x 10 minutes at PTX. The embryos were mounted in Vectashield  
761 (Vector #H-1000) and analyzed by confocal microscopy (Leica spinning disk). For L3 larvae and  
762 adults, CNSs from *NimAGal4 ; UAS-eGFP* were dissected in cold PBS 1x and transferred into  
763 wells containing 4% PFA and fixed for 2 hours at RT. They were then washed in PTX for 1 hour  
764 and incubated in blocking reagent for 1 hour at RT. CNSs were then incubated with primary  
765 antibodies mouse anti-Repo, chicken anti-GFP and rabbit anti-Nazgul O/N at 4°C, then washed in  
766 PTX 3x 10 minutes and incubated with secondary antibodies (Cy5 anti-mouse, Cy3 anti-rabbit  
767 (Jackson)) for 1 hour at RT. After washing in PTX, CNSs were mounted in Vectashield imaged  
768 using Leica spinning disk microscope and analyzed using Fiji (National Institute of Mental Health,  
769 Bethesda, Maryland, USA, (Schindelin et al., 2012).

770 **Immunolabeling of polytene chromosomes**

771 Salivary glands were dissected from L3 larvae in NaCl 0.7% / NP-40 0.1% and fixed for 2 minutes  
772 in 3.7% formaldehyde/ 0.7% NaCl/ 1% Triton-X. Before squashing, the glands were transferred  
773 in 2% formaldehyde/ 45% acetic acid and incubated for 2 minutes on a siliconized coverslip.  
774 During incubation, salivary glands were fragmented. A poly-L-lysine slide was inverted on top of  
775 the coverslip and salivary glands were squashed. The chromosome spreads were examined by  
776 phase microscopy and frozen in liquid nitrogen. The coverslip was flicked off with a razor blade  
777 and slides were incubated in cold PBS 2× for 15 min. The slides were transferred to a blocking  
778 solution (3% BSA, 2% NP-40, 0.2% Tween-20, 10% non-fat dry milk in PBS) and blocked for 1  
779 hour at RT. After blocking, slides were incubated with primary antibody in blocking solution  
780 overnight at 4°C in a humid chamber. Slides were then rinsed in PBS and washed 2x 15 minutes  
781 in PBS 1x /0.2% Tween/0.1% NP-40. After washing, slides were rinsed in PBS and incubated with



782 secondary antibody in blocking solution 2 hours at RT in a humid chamber. Slides were then rinsed  
783 in PBS and washed 2x 15 minutes in PBS1x /0.2% Tween/0.1% NP-40. Finally, slides were  
784 drained and mounted with Vectashield medium H-1200 with DAPI to stain DNA. The primary  
785 antibodies used for immunolabeling were: rabbit anti-H3K4me3 1:400 (Abcam ab8580); mouse  
786 anti-H3K9me2 1:20 (Abcam ab1220); rabbit anti-H3K9me3 1:100 (Abcam ab8898); rabbit anti-  
787 H3K27me3 1:400 (Cell Signaling C36B11); mouse anti-H2A.v-P 1:20 (DSHB AB\_2618077). The  
788 secondary antibodies were: FITC-conjugated goat anti-rabbit 1:50 (Jackson) and Rhodamine  
789 conjugated goat anti-mouse 1:50 (Jackson). Polytene chromosomes preparations were analyzed on  
790 a fluorescence microscope (Zeiss Apotome) and images acquisition performed using the Zen Pro  
791 software (Zeiss). Polytene chromosome fluorescence intensity and bands plot profile analysis were  
792 performed using Fiji software (National Institute of Mental Health, Bethesda, Maryland, USA).

### 793 ***In situ* hybridization**

794 We used a RNAscope® probe and reagent kit. Larval CNSs from *Oregon R* were dissected in cold  
795 PBS 1x and fixed in 4% PFA O/N at 4°C then washed 3x 10 minutes in PBST (PBS 1x/ 0.1%  
796 Tween-20) followed by one wash in PBS 1x. Samples were then incubated in RNAscope reagents  
797 as in protease III at 40°C for 10 minutes and washed in 3x 2 minutes in PBS 1x. The probe  
798 targeting *NimA* was then added in addition to the positive control probe and samples were  
799 incubated O/N at 40°C. The amplifiers and detection solution in the RNAscope reagent kit were  
800 added sequentially for hybridization signal amplification for the indicated time. CNSs were then  
801 mounted in Vectashield, imaged using Leica spinning disk microscope and analyzed using Fiji.

### 802 **Metabolic pathway analysis**

803 All data points considered for our analysis has a read count of more than ten in at least one of the  
804 samples from the tissue under consideration. Differential expression analysis between the L3 and  
805 E16 developmental stages was done for each tissue using DESeq2. Genes associated with  
806 metabolic pathways considered in this study, were retrieved from the KEGG database (Kanehisa  
807 and Goto, 2000, <http://www.genome.jp/>). The heatmaps representing the z-score of the metabolic  
808 genes in glia, neurons and hemocytes at E16 and L3 were plotted using R (version 3.4.0) (R  
809 CoreTeam). For genes with paralogs, the paralogs show log<sub>2</sub>FC(L3/E16) greater or lesser than  
810 zero, the paralog with the highest fold-change has been plotted. For genes whose paralogs have  
811 log<sub>2</sub>FC(L3/E16) that is either positive or negative, the paralogs with the highest and lowest fold-



812 change have been plotted. The details of all the genes (including all the paralogs) as well as genes  
813 included in the figures are present in **Sup. Table 7**.

814

815

## 816 **Acknowledgments**

817 We thank the Imaging Center of the IGBMC for technical assistance. The sequencing was  
818 performed by the GenomEast platform, a member of the “France Génomique” consortium (ANR-  
819 10-INBS-0009). We thank K. Bruckner, D. Siekhaus and B. Altenhein for providing fly stocks and  
820 antibodies. In addition, stocks obtained from the Bloomington *Drosophila* Stock Center (NIH  
821 P40OD018537) and antibodies obtained from the Developmental Studies Hybridoma Bank created  
822 by the NICHD of the NIH and maintained at The University of Iowa (Department of Biology,  
823 Iowa City, IA 52242) were used in this study. This work was supported by INSERM, CNRS, UDS,  
824 Ligue Régionale contre le Cancer, Hôpital de Strasbourg, ARC, CEFIPRA, USIAS, FRM and  
825 ANR grants, and by the CNRS/University LIA Calim. R. Sakr was supported by the French state  
826 fund through a doctoral contract from the University of Strasbourg and the Fondation pour la  
827 Recherche Médicale (FDT2020010107630). The IGBMC was also supported by a French state  
828 fund through the ANR labex. L. Ciapponi’s lab Sapienza University grant RM120172B7D32C04.  
829 T. Mukherjee’s lab is supported by the DST-Core Research Grant, DBT-IYBA 2017, CEFIPRA  
830 and USIAS fellowship. NH is a Graduate Student at inStem and supported by Council for  
831 Scientific and Industrial Research fellowship (CSIR).

832

833

## 834 **Author contribution**

835 Conceptualization, RS and AG ; Methodology, RS, AG, PBC, RS, AG, PBC, AP, MM, LC, TM  
836 and NH. Investigation, RS, AG, PBC, AP, MM, LC, TM and NH. Writing—Original Draft, RS  
837 and AG, Writing—Review and Editing, RS, AG and PBC; Funding Acquisition, AG, LC and TM;  
838 Resources, AG; Supervision, AG and PBC.

839

840 **Conflict of interest**

841 The authors declare that they have no conflict of interest.

842

843

844 **References**

845 Abbott, N.J. (2005). Dynamics of CNS barriers: evolution, differentiation, and modulation. *Cell. Mol. Neurobiol.* *25*, 5–23.

847 Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biol.* *11*.

849 Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* *31*, 166–169.

851 Aranda, S., Mas, G., and Di Croce, L. (2015). Regulation of gene transcription by Polycomb proteins. *Sci. Adv.* *1*, e1500737.

853 Awasaki, T., and Ito, K. (2004). Engulfing action of glial cells is required for programmed axon pruning during *Drosophila* metamorphosis. *Curr. Biol.* *14*, 668–677.

855 Barres, B.A. (2008). The mystery and magic of glia: a perspective on their roles in health and disease. *Neuron* *60*, 430–440.

857 Basenko, E.Y., Sasaki, T., Ji, L., Prybol, C.J., Burckhardt, R.M., Schmitz, R.J., and Lewis, Z.A. (2015). Genome-wide redistribution of H3K27me3 is linked to genotoxic stress and defective growth. *Proc. Natl. Acad. Sci. U. S. A.* *112*, E6339-48.

860 Bittern, J., Pogodalla, N., Ohm, H., Brüser, L., Kottmeier, R., Schirmeier, S., and Klämbt, C. (2021). Neuron-glia interaction in the *Drosophila* nervous system. *Dev. Neurobiol.* *81*, 438–452.

862 Bosso, G., Cipressa, F., Moroni, M.L., Pennisi, R., Albanesi, J., Brandi, V., Cugusi, S., Renda, F., Ciapponi, L., Polticelli, F., et al. (2019). NBS1 interacts with HP1 to ensure genome integrity. *Cell Death Dis.* *10*, 951.

865 Brink, D.L., Gilbert, M., Xie, X., Petley-Ragan, L., and Auld, V.J. (2012). Glial processes at the *Drosophila* larval neuromuscular junction match synaptic growth. *PLoS One* *7*, e37876.

867 Brown, N.H. (2011). Extracellular matrix in development: insights from mechanisms conserved between invertebrates and vertebrates. *Cold Spring Harb. Perspect. Biol.* *3*.

869 Brückner, K., Kockel, L., Duchek, P., Luque, C.M., Rørth, P., and Perrimon, N. (2004). The PDGF/VEGF receptor controls blood cell survival in *Drosophila*. *Dev. Cell* *7*, 73–84.

871 Callebaut, I., Mignotte, V., Souchet, M., and Mornon, J.P. (2003). EMI domains are widespread and reveal the probable orthologs of the *Caenorhabditis elegans* CED-1 protein. *Biochem. Biophys. Res. Commun.* *300*, 619–623.

874 Cattenoz, P.B., Sakr, R., Pavlidaki, A., Delaporte, C., Riba, A., Molina, N., Hariharan, N., Mukherjee, T., and Giangrande, A. (2020). Temporal specificity and heterogeneity of *Drosophila* immune cells. *EMBO J.* e104486.

877 Ciapponi, L., Cenci, G., Ducau, J., Flores, C., Johnson-Schlitz, D., Gorski, M.M., Engels, W.R., and Gatti,

- 878 M. (2004). The *Drosophila* Mre11/Rad50 complex is required to prevent both telomeric fusion and  
879 chromosome breakage. *Curr. Biol.* *14*, 1360–1366.
- 880 Ciapponi, L., Cenci, G., and Gatti, M. (2006). The *Drosophila* Nbs protein functions in multiple pathways  
881 for the maintenance of genome stability. *Genetics* *173*, 1447–1454.
- 882 Conway, E., Healy, E., and Bracken, A.P. (2015). PRC2 mediated H3K27 methylations in cellular identity  
883 and cancer. *Curr. Opin. Cell Biol.* *37*, 42–48.
- 884 Cornman, R.S. (2009). Molecular evolution of *Drosophila* cuticular protein genes. *PLoS One* *4*, e8345.
- 885 Daines, B., Wang, H., Wang, L., Li, Y., Han, Y., Emmert, D., Gelbart, W., Wang, X., Li, W., Gibbs, R., et  
886 al. (2011). The *Drosophila melanogaster* transcriptome by paired-end RNA sequencing. *Genome Res.* *21*,  
887 315–324.
- 888 Davie, K., Janssens, J., Koldere, D., De Waegeneer, M., Pech, U., Kreft, Ł., Aibar, S., Makhzami, S.,  
889 Christiaens, V., Bravo González-Blas, C., et al. (2018). A Single-Cell Transcriptome Atlas of the Aging  
890 *Drosophila* Brain. *Cell* *174*, 982–998.e20.
- 891 Deliu, L.P., Ghosh, A., and Grewal, S.S. (2017). Investigation of protein synthesis in *Drosophila* larvae  
892 using puromycin labelling. *Biol. Open* *6*, 1229–1234.
- 893 DePaula-Silva, A.B., Gorbea, C., Doty, D.J., Libbey, J.E., Sanchez, J.M.S., Hanak, T.J., Cazalla, D., and  
894 Fujinami, R.S. (2019). Differential transcriptional profiles identify microglial- and macrophage-specific  
895 gene markers expressed during virus-induced neuroinflammation. *J. Neuroinflammation* *16*, 1–20.
- 896 Ebens, A.J., Garren, H., Cheyette, B.N., and Zipursky, S.L. (1993). The *Drosophila* anachronism locus: a  
897 glycoprotein secreted by glia inhibits neuroblast proliferation. *Cell* *74*, 15–27.
- 898 Forey, R., Barthe, A., Tittel-Elmer, M., Wery, M., Barrault, M.-B., Ducrot, C., Seeber, A., Krietenstein, N.,  
899 Szachnowski, U., Skrzypczak, M., et al. (2021). A Role for the Mre11-Rad50-Xrs2 Complex in Gene  
900 Expression and Chromosome Organization. *Mol. Cell* *81*, 183–197.e6.
- 901 Franc, N.C., Dimarcq, J.L., Lagueux, M., Hoffmann, J., and Ezekowitz, R.A. (1996). Croquemort, a novel  
902 *Drosophila* hemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity* *4*, 431–443.
- 903 Franc, N.C., Heitzler, P., Ezekowitz, R.A., and White, K. (1999). Requirement for croquemort in  
904 phagocytosis of apoptotic cells in *Drosophila*. *Science* *284*, 1991–1994.
- 905 Garces, A., and Thor, S. (2006). Specification of *Drosophila* aCC motoneuron identity by a genetic cascade  
906 involving even-skipped, grain and *zfh1*. *Development* *133*, 1445–1455.
- 907 Gatei, M., Kijas, A.W., Biard, D., Dörk, T., and Lavin, M.F. (2014). RAD50 phosphorylation promotes  
908 ATR downstream signaling and DNA restart following replication stress. *Hum. Mol. Genet.* *23*, 4232–  
909 4248.
- 910 Gyoergy, A., Roblek, M., Ratheesh, A., Valoskova, K., Belyaeva, V., Wachner, S., Matsubayashi, Y.,  
911 Sánchez-Sánchez, B.J., Stramer, B., and Siekhaus, D.E. (2018). Tools Allowing Independent Visualization  
912 and Genetic Manipulation of *Drosophila melanogaster* Macrophages and Surrounding Tissues. *G3*  
913 (Bethesda). *8*, 845–857.
- 914 Hickman, S.E., Kingery, N.D., Ohsumi, T.K., Borowsky, M.L., Wang, L.C., Means, T.K., and El Khoury,  
915 J. (2013). The microglial sensome revealed by direct RNA sequencing. *Nat. Neurosci.* *2013* *1612* *16*, 1896–  
916 1905.
- 917 Hildebrandt, A., Pflanz, R., Behr, M., Tarp, T., Riedel, D., and Schuh, R. (2015). Bark beetle controls  
918 epithelial morphogenesis by septate junction maturation in *Drosophila*. *Dev. Biol.* *400*, 237–247.
- 919 James, T.C., and Elgin, S.C. (1986). Identification of a nonhistone chromosomal protein associated with  
920 heterochromatin in *Drosophila melanogaster* and its gene. *Mol. Cell. Biol.* *6*, 3862–3872.

- 921 James, T.C., Eissenberg, J.C., Craig, C., Dietrich, V., Hobson, A., and Elgin, S.C. (1989). Distribution  
922 patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of *Drosophila*. *Eur. J. Cell*  
923 *Biol.* *50*, 170–180.
- 924 Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.*  
925 *28*, 27–30.
- 926 Karouzou, M. V, Spyropoulos, Y., Iconomidou, V.A., Cornman, R.S., Hamodrakas, S.J., and Willis, J.H.  
927 (2007). *Drosophila* cuticular proteins with the R&R Consensus: annotation and classification with a new  
928 tool for discriminating RR-1 and RR-2 sequences. *Insect Biochem. Mol. Biol.* *37*, 754–760.
- 929 Kocks, C., Cho, J.H., Nehme, N., Ulvila, J., Pearson, A.M., Meister, M., Strom, C., Conto, S.L., Hetru, C.,  
930 Stuart, L.M., et al. (2005). Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens  
931 in *Drosophila*. *Cell* *123*, 335–346.
- 932 Kondo, S., and Ueda, R. (2013). Highly improved gene targeting by germline-specific Cas9 expression in  
933 *Drosophila*. *Genetics* *195*, 715–721.
- 934 Kurant, E., Axelrod, S., Leaman, D., and Gaul, U. (2008). Six-microns-under acts upstream of Draper in  
935 the glial phagocytosis of apoptotic neurons. *Cell* *133*, 498–509.
- 936 Kurucz, E., Márkus, R., Zsámboki, J., Folkl-Medzihradzsky, K., Darula, Z., Vilmos, P., Udvardy, A.,  
937 Krausz, I., Lukacsovich, T., Gateff, E., et al. (2007). Nimrod, a putative phagocytosis receptor with EGF  
938 repeats in *Drosophila* plasmatocytes. *Curr. Biol.* *17*, 649–654.
- 939 Lake, C.M., Holsclaw, J.K., Bellendir, S.P., Sekelsky, J., and Hawley, R.S. (2013). The development of a  
940 monoclonal antibody recognizing the *Drosophila melanogaster* phosphorylated histone H2A variant ( $\gamma$ -  
941 H2AV). *G3 (Bethesda)*. *3*, 1539–1543.
- 942 Landgraf, M., and Thor, S. (2006). Development of *Drosophila* motoneurons: specification and  
943 morphology. *Semin. Cell Dev. Biol.* *17*, 3–11.
- 944 Laneve, P., Delaporte, C., Trebuchet, G., Komonyi, O., Flici, H., Popkova, A., D’Agostino, G., Taglini, F.,  
945 Kerekes, I., and Giangrande, A. (2013). The Gcm/Glide molecular and cellular pathway: new actors and  
946 new lineages. *Dev. Biol.* *375*, 65–78.
- 947 Lebestky, T., Chang, T., Hartenstein, V., and Banerjee, U. (2000). Specification of *Drosophila*  
948 hematopoietic lineage by conserved transcription factors. *Science* *288*, 146–149.
- 949 Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., and Hoffmann, J.A. (1996). The dorsoventral  
950 regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults.  
951 *Cell* *86*, 973–983.
- 952 LERNER, A.B., and FITZPATRICK, T.B. (1950). Biochemistry of melanin formation. *Physiol. Rev.* *30*,  
953 91–126.
- 954 Li, Q., and Barres, B.A. (2017). Microglia and macrophages in brain homeostasis and disease. *Nat. Rev.*  
955 *Immunol.* *2017* *184* *18*, 225–242.
- 956 Limmer, S., Weiler, A., Volkenhoff, A., Babatz, F., and Klambt, C. (2014). The *Drosophila* blood-brain  
957 barrier: development and function of a glial endothelium. *Front. Neurosci.* *8*, 365.
- 958 MacDonald, J.M., Beach, M.G., Porgiglia, E., Sheehan, A.E., Watts, R.J., and Freeman, M.R. (2006). The  
959 *Drosophila* cell corpse engulfment receptor Draper mediates glial clearance of severed axons. *Neuron* *50*,  
960 869–881.
- 961 Makhijani, K., Alexander, B., Tanaka, T., Rulifson, E., and Brückner, K. (2011). The peripheral nervous  
962 system supports blood cell homing and survival in the *Drosophila* larva. *Development* *138*, 5379–5391.
- 963 Manaka, J., Kuraishi, T., Shiratsuchi, A., Nakai, Y., Higashida, H., Henson, P., and Nakanishi, Y. (2004).

- 964 Draper-mediated and phosphatidylserine-independent phagocytosis of apoptotic cells by *Drosophila*  
965 hemocytes/macrophages. *J. Biol. Chem.* *279*, 48466–48476.
- 966 Mangahas, P.M., and Zhou, Z. (2005). Clearance of apoptotic cells in *Caenorhabditis elegans*. *Semin. Cell*  
967 *Dev. Biol.* *16*, 295–306.
- 968 Martinek, N., Shahab, J., Saathoff, M., and Ringuette, M. (2008). Haemocyte-derived SPARC is required  
969 for collagen-IV-dependent stability of basal laminae in *Drosophila* embryos. *J. Cell Sci.* *121*, 1671–1680.
- 970 Melcarne, C., Lemaitre, B., and Kurant, E. (2019). Phagocytosis in *Drosophila*: From molecules and cellular  
971 machinery to physiology. *Insect Biochem. Mol. Biol.* *109*, 1–12.
- 972 Mi, H., Dong, Q., Muruganujan, A., Gaudet, P., Lewis, S., and Thomas, P.D. (2010). PANTHER version  
973 7: improved phylogenetic trees, orthologs and collaboration with the Gene Ontology Consortium. *Nucleic*  
974 *Acids Res.* *38*.
- 975 Naba, A., Clauser, K.R., Ding, H., Whittaker, C.A., Carr, S.A., and Hynes, R.O. (2016). The extracellular  
976 matrix: Tools and insights for the “omics” era. *Matrix Biol.* *49*, 10–24.
- 977 Neumann, H., Kotter, M.R., and Franklin, R.J.M. (2009). Debris clearance by microglia: an essential link  
978 between degeneration and regeneration. *Brain* *132*, 288–295.
- 979 O’Hagan, H.M., Mohammad, H.P., and Baylin, S.B. (2008). Double strand breaks can initiate gene  
980 silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. *PLoS*  
981 *Genet.* *4*, e1000155.
- 982 O’Hagan, H.M., Wang, W., Sen, S., Destefano Shields, C., Lee, S.S., Zhang, Y.W., Clements, E.G., Cai,  
983 Y., Van Neste, L., Easwaran, H., et al. (2011). Oxidative damage targets complexes containing DNA  
984 methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer Cell* *20*, 606–619.
- 985 Ou, J., He, Y., Xiao, X., Yu, T.-M., Chen, C., Gao, Z., and Ho, M.S. (2014). Glial cells in neuronal  
986 development: recent advances and insights from *Drosophila melanogaster*. *Neurosci. Bull.* *30*, 584–594.
- 987 Parker, R.J., and Auld, V.J. (2006). Roles of glia in the *Drosophila* nervous system. *Semin. Cell Dev. Biol.*  
988 *17*, 66–77.
- 989 Pearson, A., Lux, A., and Krieger, M. (1995). Expression cloning of dSR-CI, a class C macrophage-specific  
990 scavenger receptor from *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* *92*, 4056–4060.
- 991 Peco, E., Davla, S., Camp, D., Stacey, S.M., Landgraf, M., and van Meyel, D.J. (2016). *Drosophila*  
992 astrocytes cover specific territories of the CNS neuropil and are instructed to differentiate by Prospero, a  
993 key effector of Notch. *Development* *143*, 1170–1181.
- 994 Pereanu, W., Shy, D., and Hartenstein, V. (2005). Morphogenesis and proliferation of the larval brain glia  
995 in *Drosophila*. *Dev. Biol.* *283*, 191–203.
- 996 Peters, A.H.F.M., Kubicek, S., Mechtler, K., O’Sullivan, R.J., Derijck, A.A.H.A., Perez-Burgos, L.,  
997 Kohlmaier, A., Opravil, S., Tachibana, M., Shinkai, Y., et al. (2003). Partitioning and plasticity of  
998 repressive histone methylation states in mammalian chromatin. *Mol. Cell* *12*, 1577–1589.
- 999 Qin, X., Ahn, S., Speed, T.P., and Rubin, G.M. (2007). Global analyses of mRNA translational control  
1000 during early *Drosophila* embryogenesis. *Genome Biol.* *8*, 1–18.
- 1001 Rämét, M., Pearson, A., Manfrulli, P., Li, X., Koziel, H., Göbel, V., Chung, E., Krieger, M., and  
1002 Ezekowitz, R.A. (2001). *Drosophila* scavenger receptor CI is a pattern recognition receptor for bacteria.  
1003 *Immunity* *15*, 1027–1038.
- 1004 Rämét, M., Manfrulli, P., Pearson, A., Mathey-Prevot, B., and Ezekowitz, R.A.B. (2002). Functional  
1005 genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli*. *Nature* *416*, 644–  
1006 648.



- 1007 Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K.,  
1008 Ponting, C.P., Allis, C.D., et al. (2000). Regulation of chromatin structure by site-specific histone H3  
1009 methyltransferases. *Nature* 406, 593–599.
- 1010 Redon, C., Pilch, D., Rogakou, E., Sedelnikova, O., Newrock, K., and Bonner, W. (2002). Histone H2A  
1011 variants H2AX and H2AZ. *Curr. Opin. Genet. Dev.* 12, 162–169.
- 1012 Ritzel, R.M., Patel, A.R., Grenier, J.M., Crapser, J., Verma, R., Jellison, E.R., and McCullough, L.D.  
1013 (2015). Functional differences between microglia and monocytes after ischemic stroke. *J.*  
1014 *Neuroinflammation* 12, 1–12.
- 1015 Roddie, H.G., Armitage, E.L., Coates, J.A., Johnston, S.A., and Evans, I.R. (2019). Simu-dependent  
1016 clearance of dying cells regulates macrophage function and inflammation resolution. *PLoS Biol.* 17,  
1017 e2006741.
- 1018 Ryglewski, S., Duch, C., and Altenhein, B. (2017). Tyramine Actions on *Drosophila* Flight Behavior Are  
1019 Affected by a Glial Dehydrogenase/Reductase. *Front. Syst. Neurosci.* 11, 68.
- 1020 Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C.T., Schreiber, S.L.,  
1021 Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. *Nature* 419,  
1022 407–411.
- 1023 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden,  
1024 C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat.*  
1025 *Methods* 9, 676–682.
- 1026 Schmid, A., Chiba, A., and Doe, C.Q. (1999). Clonal analysis of *Drosophila* embryonic neuroblasts: neural  
1027 cell types, axon projections and muscle targets. *Development* 126, 4653–4689.
- 1028 Shepherd, D. (2000). Glial dependent survival of neurons in *Drosophila*. *Bioessays* 22, 407–409.
- 1029 Sonnenfeld, M.J., and Jacobs, J.R. (1995a). Macrophages and glia participate in the removal of apoptotic  
1030 neurons from the *Drosophila* embryonic nervous system. *J. Comp. Neurol.* 359, 644–652.
- 1031 Sonnenfeld, M.J., and Jacobs, J.R. (1995b). Macrophages and glia participate in the removal of apoptotic  
1032 neurons from the *Drosophila* embryonic nervous system. *J. Comp. Neurol.* 359, 644–652.
- 1033 Stork, T., Engelen, D., Krudewig, A., Silies, M., Bainton, R.J., and Klambt, C. (2008). Organization and  
1034 function of the blood-brain barrier in *Drosophila*. *J. Neurosci.* 28, 587–597.
- 1035 Syed, A., and Tainer, J.A. (2018). The MRE11-RAD50-NBS1 Complex Conducts the Orchestration of  
1036 Damage Signaling and Outcomes to Stress in DNA Replication and Repair. *Annu. Rev. Biochem.* 87, 263–  
1037 294.
- 1038 Tepass, U., Fessler, L.I., Aziz, A., and Hartenstein, V. (1994). Embryonic origin of hemocytes and their  
1039 relationship to cell death in *Drosophila*. *Development* 120, 1829–1837.
- 1040 Thomas, P.D., Campbell, M.J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R., Diemer, K., Muruganujan,  
1041 A., and Narechania, A. (2003). PANTHER: A Library of Protein Families and Subfamilies Indexed by  
1042 Function. *Genome Res.* 13, 2129–2141.
- 1043 Trapnell, C., Pachter, L., and Salzberg, S.L. (2009). TopHat: discovering splice junctions with RNA-Seq.  
1044 *Bioinformatics* 25, 1105–1111.
- 1045 Volkenhoff, A., Weiler, A., Letzel, M., Stehling, M., Klämbt, C., and Schirmeier, S. (2015). Glial  
1046 Glycolysis Is Essential for Neuronal Survival in *Drosophila*. *Cell Metab.* 22, 437–447.
- 1047 Wang, Q., Goldstein, M., Alexander, P., Wakeman, T.P., Sun, T., Feng, J., Lou, Z., Kastan, M.B., and  
1048 Wang, X.-F. (2014). Rad17 recruits the MRE11-RAD50-NBS1 complex to regulate the cellular response  
1049 to DNA double-strand breaks. *EMBO J.* 33, 862–877.

- 1050 Watts, R.J., Schuldiner, O., Perrino, J., Larsen, C., and Luo, L. (2004). Glia engulf degenerating axons  
1051 during developmental axon pruning. *Curr. Biol.* *14*, 678–684.
- 1052 Wu, Y., Dissing-Olesen, L., MacVicar, B.A., and Stevens, B. (2015). Microglia: Dynamic Mediators of  
1053 Synapse Development and Plasticity. *Trends Immunol.* *36*, 605–613.
- 1054 Zuber, R., Wang, Y., Gehring, N., Bartoszewski, S., and Moussian, B. (2020). Tweedle proteins form  
1055 extracellular two-dimensional structures defining body and cell shape in *Drosophila melanogaster*. *Open*  
1056 *Biol.* *10*, 200214.
- 1057
- 1058

## 1059 **Figure legends**

### 1060 **Figure 1: RNAseq analysis in embryonic and larval glia, neurons and hemocytes**

1061 (a) Dendrogram showing the hierarchical clustering based on the distance between samples. The  
1062 height indicates the distance. Euclidean distance, Ward criterion were used for this representation.

1063 (b-c) Venn diagrams showing the distribution of differentially expressed genes between neurons  
1064 (blue), glia (yellow) and hemocytes (red) at E16 (b) and L3 (c). Since only upregulated genes are  
1065 used for the analysis, the intersection between all three cell types is empty (white). The percentage  
1066 of genes in each category is indicated in the diagram. The percentage was calculated based on all  
1067 genes used for the analysis. (d,e) The total number of genes expressed (top three lines) and  
1068 upregulated compared the other two cell types (bottom three lines) at E16 (d) and L3 (e).

### 1069 **Figure 2: Pairwise transcriptome comparisons in the embryo**

1070 (a) Transcriptome comparison of E16 neurons and glia, MA plot. The x-axis reports the average  
1071 gene expression levels, the y-axis, the log<sub>2</sub> fold change between the genes in E16 neurons/E16  
1072 glia. Genes significantly upregulated in E16 neurons are shown in blue, genes significantly  
1073 upregulated in E16 glia are shown in yellow and non-differentially expressed genes are shown in  
1074 gray. (b,c) Gene Ontology (GO) term enrichment analysis in E16 neurons (blue) and E16 glia  
1075 (yellow). The fold enrichment for a subset of representative GO terms is displayed on the x-axis,  
1076 the number of genes within the GO term and the P-value of the GO term enrichment are indicated  
1077 in brackets. The complete results of the GO term analysis are shown in **Sup. Table 2-b,c**. (d)  
1078 Transcriptome comparison of E16 hemocytes and glia. MA plot as in (a), the y-axis reports the  
1079 log<sub>2</sub> fold change E16 hemocytes/E16 glia. Genes significantly upregulated in E16 hemocytes are  
1080 shown in red, genes significantly upregulated in E16 glia are shown in yellow and non-  
1081 differentially expressed genes are shown in gray. (e,f) GO term enrichment analysis in E16



1082 hemocytes (red) and E16 glia (yellow). The fold enrichment for a subset of representative GO  
1083 terms is displayed on the x-axis, the number of genes and the P-value of the GO term enrichment  
1084 are indicated in brackets. The complete results of the GO term analysis are shown in **Sup. Table**  
1085 **2-d,e.** (g) List of genes upregulated in E16 hemocytes compared to E16 glia and involved in the  
1086 immune deficiency and Toll pathways. (h) Transcriptome comparison of E16 neurons and  
1087 hemocytes. MA plot as in (a), the y-axis reports the log<sub>2</sub> fold change E16 neurons/E16 hemocytes.  
1088 Genes significantly upregulated in E16 neurons are shown in blue, genes significantly upregulated  
1089 in E16 hemocytes in red, and non-differentially expressed genes in gray.

### 1090 **Figure 3: Pairwise transcriptome comparisons in the larva**

1091 (a) Transcriptome comparison of L3 neurons and glia, MA plot. The x-axis reports the average  
1092 gene expression levels, the y-axis, the log<sub>2</sub> fold change L3 neurons/ L3 glia. Genes significantly  
1093 upregulated in L3 neurons are shown in blue, genes significantly upregulated in L3 glia are shown  
1094 in yellow and non-differentially expressed genes are shown in gray. (b,c) Gene Ontology (GO)  
1095 term enrichment analysis in L3 neurons (blue) and L3 glia (yellow). The fold enrichment for a  
1096 subset of representative GO terms are displayed on the x-axis, the number of genes and the P-value  
1097 of the GO term enrichment are indicated in brackets. The complete results of the GO term analysis  
1098 are shown in **Sup. Table 3-a,b.** (d) Transcriptome comparison of L3 hemocytes and glia. MA plot  
1099 as in (a), the y-axis reports the log<sub>2</sub> fold change L3 hemocytes/L3 glia. Genes significantly  
1100 upregulated in L3 hemocytes are shown in red, genes significantly upregulated in L3 glia are  
1101 shown in yellow and non-differentially expressed genes are shown in gray. (e,f) Gene Ontology  
1102 (GO) term enrichment analysis in L3 hemocytes (red) and L3 glia (yellow). The fold enrichment  
1103 for a subset of representative GO terms is displayed on the x-axis, the number of genes and the P-  
1104 value of the GO term enrichment are indicated in brackets. The complete results of the GO term  
1105 analysis are shown in **Sup. Table 3-c,d.** (g) Transcriptome comparison of L3 neurons and  
1106 hemocytes. MA plot as in (a), the y-axis reports the log<sub>2</sub> fold change L3 neurons/L3 hemocytes.  
1107 Genes significantly upregulated in L3 neurons are shown in blue, genes significantly upregulated  
1108 in L3 hemocytes are shown in red, and non-differentially expressed genes are shown in gray.

### 1109 **Figure 4: *NimA*, a novel glia-specific scavenger receptor**

1110 (a,b) MA plots as in Figure 2-c and Figure 3-c comparing the scavenger receptors expressed in  
1111 embryonic and larval hemocytes and glia. In black, scavenger receptors non-differentially

1112 expressed between E16 (a) or L3 hemocytes and glia (b). In red, scavenger receptors that are  
1113 upregulated in E16 (a) or L3 hemocytes (b) and in yellow, scavenger receptors that are upregulated  
1114 in E16 (a) or L3 glia (b). (c,e') Immunolabeling of *NimAGal4;UAS-eGFP* embryos (c,c'), L3  
1115 (d,d',d'') and adults (e) with anti GFP (green), anti-Repo (white), anti-Nazgul (red) and DAPI  
1116 (blue). (c) Full stack projection, scale bar = 100  $\mu\text{m}$ . Square indicates region in c'. (c') Single  
1117 section, scale bar = 50  $\mu\text{m}$ . (d) Full stack projection, scale bar = 100  $\mu\text{m}$ . Squares indicate regions  
1118 in d' and d''. (d'-d'') Single section, scale bar = 50  $\mu\text{m}$ . (e) Full stack projection, scale bar = 100  
1119  $\mu\text{m}$ .

### 1120 **Figure 5: Pairwise transcriptome comparison between E16 and L3 cells**

1121 (a) Transcriptome comparison of L3 vs. E16 cells, MA plot. The x-axis reports the average gene  
1122 expression levels, the y-axis, the log<sub>2</sub> fold change L3 cells/E16 cells. Genes significantly  
1123 upregulated in E16 are shown in green, genes significantly upregulated in L3 are shown in purple  
1124 and non-differentially expressed genes are shown in gray. (b,d) GO term enrichment analysis in  
1125 L3 (purple) and E16 (green). The fold enrichment for a subset of representative GO terms is  
1126 displayed on the x-axis, the number of genes and the P-value of the GO term enrichment are  
1127 indicated in brackets. The complete results of the GO term analysis are shown in **Sup. Table 6-**  
1128 **a,c**. (c,e,f) Heatmaps showing the log<sub>10</sub> gene expression of genes upregulated in E16 cells  
1129 compared to L3 cells and involved in Chitin-based cuticle development (c), as well as that of genes  
1130 upregulated in L3 cells compared to E16 cells and involved in DNA repair (e) and epigenetics (f).  
1131 Scale shows low expression (blue) to high expression (red). Values for heatmaps shown in **Sup.**  
1132 **Table 6-b,d,e**.

### 1133 **Figure 6: Impact of *rad50* on polytene chromosomes and on gene expression**

1134 (a) Representative polytene chromosomes immunolabeled with anti-H3K4me<sub>3</sub> (magenta) and  
1135 anti-H3K9me<sub>2</sub> (green), or with anti-H3K9me<sub>3</sub> (red), from *WT* or *rad50 $\Delta$ 5.1* homozygous mutants  
1136 L3. Chromosomes were counter-stained with DAPI (blue). Scale bar = 20  $\mu\text{m}$ . (b) Fluorescence  
1137 intensity quantification of polytene chromosomes, labeled as in (a), shows an increase of the  
1138 euchromatic marker H3K4me<sub>3</sub> and a decrease of the heterochromatic markers H3K9me<sub>2</sub>  
1139 H3K9m<sub>3</sub>, in *rad50 $\Delta$ 5.1* mutants compared to *WT*. Each dot represents a single polytene  
1140 chromosome (n  $\geq$  13). Arbitrary Unit (A.U.). Error bars represent SEM. P = p-value calculated  
1141 using Welch parametric t-test. (c) Representative intensity profiles of H3K4me<sub>3</sub> and DAPI signals

1142 over a segment at the tip of chromosome 2L, showing an alteration of the H3K4me3 signal in  
1143 *rad50Δ5.1* mutant compared to the *WT*; arrowheads denote regions of strong difference of  
1144 H3K4me3 in *rad50Δ5.1* compared to *WT*. y-axis: fluorescence intensity; x-axis: distance from the  
1145 tip of the chromosome ( $\mu\text{m}$ ). Scale bar = 10  $\mu\text{m}$ . (d) Representative intensity profiles of H3K9me3  
1146 and DAPI signals over a segment at the tip of chromosomes 3R showing absence of H3K9me3  
1147 signal in *rad50Δ5.1* compared to *WT*. y-axis: fluorescence intensity; x-axis: distance from the tip  
1148 of the chromosome ( $\mu\text{m}$ ). Scale bar = 10  $\mu\text{m}$ . (e) Representative polytene chromosomes  
1149 immunolabeled with anti-H3K27me3 (purple) and anti-H2A.v-P (green), from *WT* or *rad50Δ5.1*  
1150 homozygous larvae. Chromosomes were counter-stained with DAPI (blue). Scale bar = 20  $\mu\text{m}$ . (f)  
1151 Fluorescence intensity quantification showing increase of both H3k27me3 and H2A.v-P signals in  
1152 *rad50Δ5.1* mutants compared to *WT*. Each dot represents a single polytene chromosome ( $n \geq$   
1153 15). Error bars represent SEM.  $P =$  p-value calculated using Welch parametric t-test. (g)  
1154 Representative intensity profiles of H3K27me3 and H2A.v-P signals over a segment at the tip of  
1155 the 2R chromosome. Note that the increase of H2A.v-P peaks in the *rad50Δ5.1* mutant is not  
1156 directly correlated with that of H3K27me3 (arrowhead). y-axis: fluorescence intensity; x-axis:  
1157 distance from the tip of the chromosome ( $\mu\text{m}$ ). Scale bar = 10  $\mu\text{m}$ . (e-j) RT-qPCR assays on  
1158 *rad50Δ5.1* /+ and *rad50Δ5.1* larvae. The levels of *TwdlW* (h), *ftz-fl* (i), *mre11* (j), *Unc-76* (k),  
1159 *olf413* (l) and *sna* (m) transcripts were quantified and the relative expression levels were calculated  
1160 based on the expression of *Act5C* and *RP49*. List of primers shown in **Sup. Table 9**,  $n =$  number  
1161 of biological replicates,  $P =$  p-value calculated using Student's t-test and indicated on graphs, error  
1162 bars represent the standard deviation.

1163 **Figure 7: Comparison of the metabolic states in hemocytes, glia and neurons at E16 and L3**  
1164 (a) Schematic showing the main steps of sugar and lipid metabolic pathways. (b,c) Heatmaps  
1165 showing the z-score of genes involved in the different metabolic processes (b) and transporters (c)  
1166 in hemocytes, glia and neurons at E16 and L3. Scale shows low expression (blue) to high  
1167 expression (red). Values are shown in **Sup. Table 7**. (d) Recapitulative table highlighting the main  
1168 processes for sugar and lipid metabolism in hemocytes, neurons and glia from E16 and L3.

1169

1170

## 1171 **Supplementary figure legends**

### 1172 **Sup. Figure 1: Read counts and commonalities between embryonic neurons and glia**

1173 (a) Comparison of the total read count between the different samples. The y-axis represents the  
1174 total read count. The x axis-represents the different samples: E16 glia, neurons and hemocytes  
1175 (blue) and L3 glia, neurons and hemocytes (red). (b,c) Dot plots comparing the expression levels  
1176 of non-differentially genes versus differentially expressed genes. y-axis reports the log<sub>2</sub> fold  
1177 change E16 neurons/E16 glia. Non-differentially expressed genes are shown in gray (b). The x-  
1178 axis reports the average gene expression levels. Genes significantly upregulated in E16 neurons  
1179 are shown in blue, genes significantly upregulated in E16 glia are shown in yellow (c). (d) Gene  
1180 Ontology (GO) term enrichment analysis on genes commonly expressed between E16 glia and  
1181 neurons. The fold enrichment for a subset of representative GO terms is displayed on the x-axis,  
1182 the number of genes and the P-value of the GO term enrichment are indicated in brackets. The  
1183 complete results of the GO term analysis are shown in **Sup. Table 2-a**.

### 1184 **Sup. Figure 2: Cell type differences between E16 and L3**

1185 (a) Box plot showing the log<sub>2</sub> fold change (y-axis) between the different samples at E16 and L3  
1186 (x-axis). P = p-value calculated using Student's t-test and indicated on graphs, error bars represent  
1187 the standard deviation. (b) Heatmap showing the Log<sub>10</sub> expression levels (absolute levels) of  
1188 genes upregulated in L3 hemocytes and L3 glia but not in neurons and involved in metabolism.  
1189 Scale shows low expression (blue) to high expression (red). Log<sub>10</sub> expression values are shown  
1190 in **Sup. Table 4-b,c**. (c-e) Transcriptome comparison of the cell types at E16 vs. L3. The x-axis  
1191 reports the average gene expression levels, the y-axis, the log<sub>2</sub> fold change of L3 glia / E16 glia  
1192 (c), L3 neurons / E16 neurons (d) and L3 hemocytes / E16 hemocytes (e). Genes significantly  
1193 upregulated in L3 glia, L3 neurons and L3 hemocytes are shown in orange, dark blue and dark red  
1194 respectively. Genes significantly upregulated in E16 glia, E16 neurons and E16 hemocytes are  
1195 shown in yellow, light blue and light red, respectively. Non-differentially expressed genes are  
1196 shown in gray. (f,g) Venn diagrams showing the distribution of transcription factors between E16  
1197 neurons (blue), glia (yellow) and hemocytes (red) (f), and L3 neurons (blue), glia (yellow) and  
1198 hemocytes (red) (g). Transcription factors common to all three cell types are shown in white. The  
1199 number of transcription factors in each category is indicated in the diagram.

1200 **Sup. Figure 3: Commonalities and specificities between hemocytes and glia**

1201 (a) Graphical representation of NimA and Drpr. (b-h) Quantitative reverse transcription PCR (RT-  
1202 qPCR) assays on sorted L3 glia and hemocytes. To test the purity of the sorted populations, the  
1203 expression of *Hml* (b) and *repo* (c) were assessed in hemocytes and glia, respectively. The levels  
1204 of *NimC1* (d), *eater* (e), *drpr* (f), *crq* (g) and *NimA* (h) were quantified and relative expression  
1205 levels were calculated based on the expression of *Act5C* and *RP49*. List of primers shown in **Sup.**  
1206 **Table 9**, n = number of biological replicates, P = p-value calculated using Student's t-test and  
1207 indicated on graphs, error bars represent the standard deviation. NS = non-significant. (i) *In situ*  
1208 hybridization using an anti-*NimA* RNAscope probe on L3 CNS, full stack projection. Region in  
1209 white square in (i) is shown magnified in (i'). *NimA* is shown in red, DAPI in blue. (i) Scale bar =  
1210 100  $\mu$ m, (i') Scale bar = 50  $\mu$ m.

1211 **Sup. Figure 4: Specificities of larval cells**

1212 (a-c) RT-qPCR assays on cells from E16 and L3 *WT* animals. The levels of *rad50* (a), *mre11* (b)  
1213 and *nbs* (c) transcripts were quantified and the relative expression levels were calculated based on  
1214 the expression of *Act5C* and *RP49*. List of primers shown in **Sup. Table 9**, n = number of  
1215 biological replicates, p-value calculated using Student's t-test and indicated on graphs, error bars  
1216 represent the standard deviation. (d,e) Western blot quantification of  $\gamma$ H2AV. Histones extracted  
1217 from cells from E16 and L3 *WT* animals were blotted on nitrocellulose membranes and probed  
1218 with anti- H2A.v-P antibody (e). An antibody that recognizes all histones H3, anti-H3, was used  
1219 to confirm equal loading of lysates (d). The ladder on the left shows protein size in KDa. (f) Cells  
1220 were first selected based on the forward scatter (FSC-A) and the side scatter (SSC-A) to eliminate  
1221 dead cells and debris. Doublets were removed and only single cells were taken into account. The  
1222 gate for sorting Cy3/RFP positive cells was based on the negative control. Only Cy3/RFP positive  
1223 were sorted. A post sort step was performed at the end to test the purity of the sorted population.

1224 **Sup. Figure 5: Comparison of the metabolic pathways expressed in the three cell types at**  
1225 **embryonic and larval stages**

1226 (a,b) Heatmaps showing the Log10 expression levels (absolute levels) of genes involved in the  
1227 main metabolic processes (a) and of the main transporters (b) in hemocytes, glia and neurons of  
1228 E16 and L3. Scale shows low expression (blue) to high expression (red). Expression values are  
1229 shown in **Sup. Table 7**.

1230 **List of supplementary tables:**

1231 **Sup. Table 1: List of transcription factors and their expression levels in E16 and L3 glia,**  
1232 **neurons and hemocytes.**

1233 **Sup. Table 2: Gene Ontology terms found in commonly expressed genes or genes upregulated**  
1234 **in E16 glia, E16 neurons or E16 hemocytes.**

1235 **Sup. Table 3: Gene Ontology terms found in genes upregulated in L3 glia, L3 neurons or L3**  
1236 **hemocytes.**

1237 **Sup. Table 4: Gene Ontology terms and list of genes upregulated in L3 glia and hemocytes**  
1238 **compared to neurons.**

1239 **Sup. Table 5: List of scavenger receptors expressed in glia and hemocytes.**

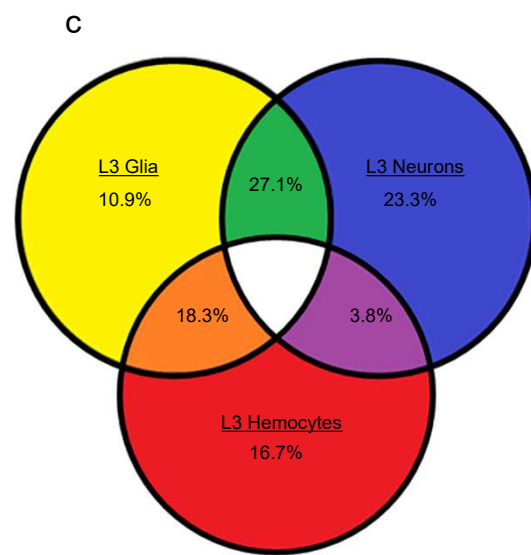
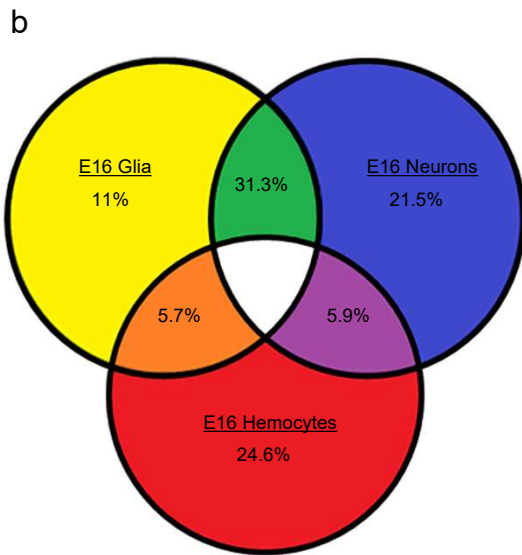
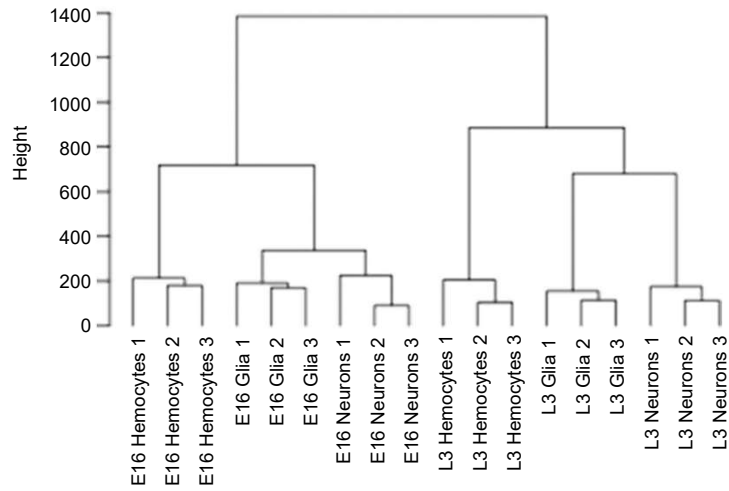
1240 **Sup. Table 6: Gene Ontology terms and lists of genes upregulated in E16 cells or L3 cells.**

1241 **Sup. Table 7: Lists of genes involved in different metabolism pathways.**

1242 **Sup. Table 8: Gene Ontology terms found in genes upregulated in E16 hemocytes or E16**  
1243 **neurons.**

1244 **Sup. Table 9: List of primers used in this study.**





**d**

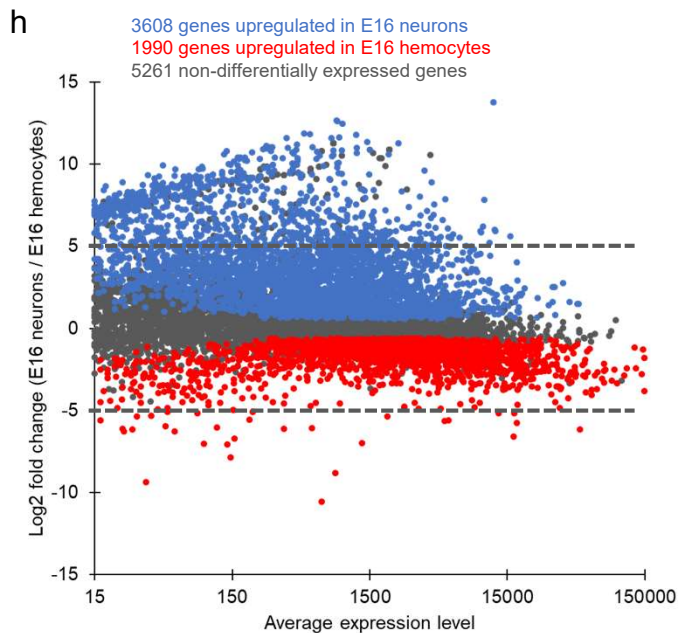
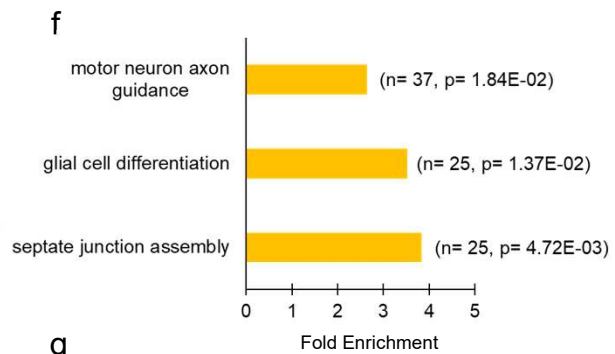
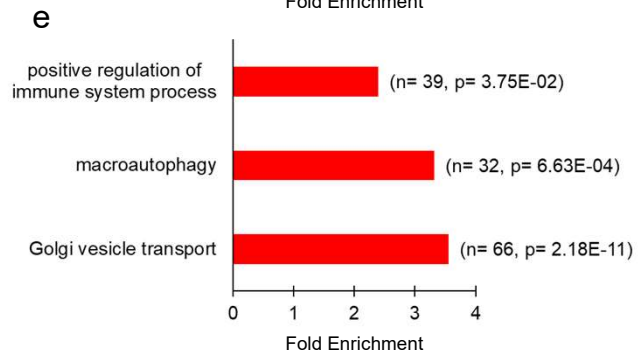
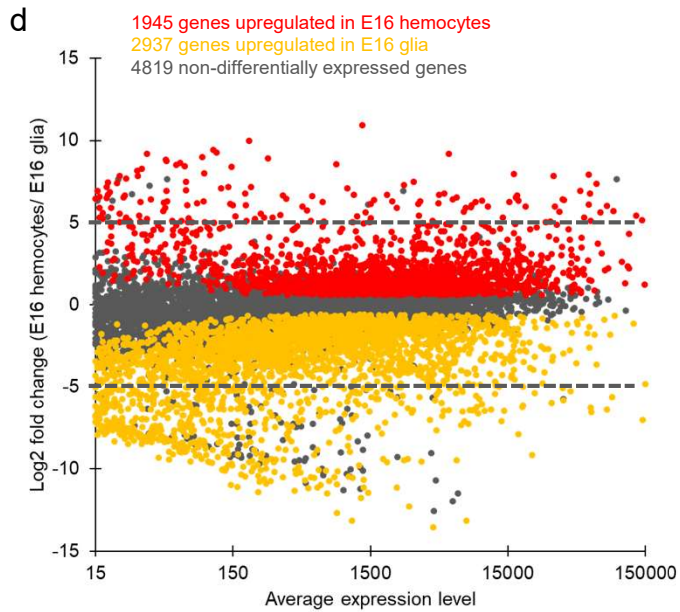
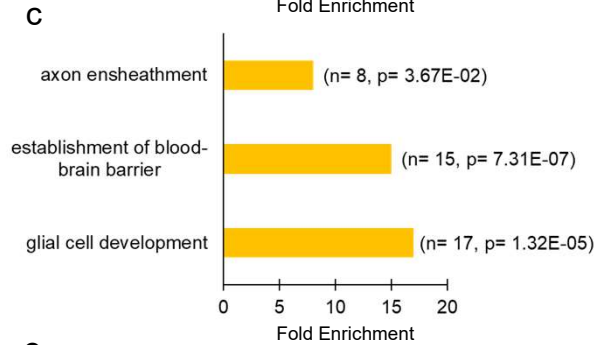
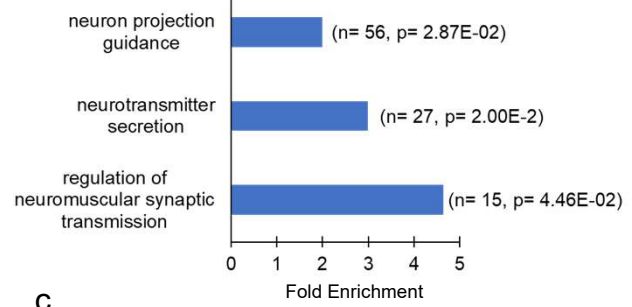
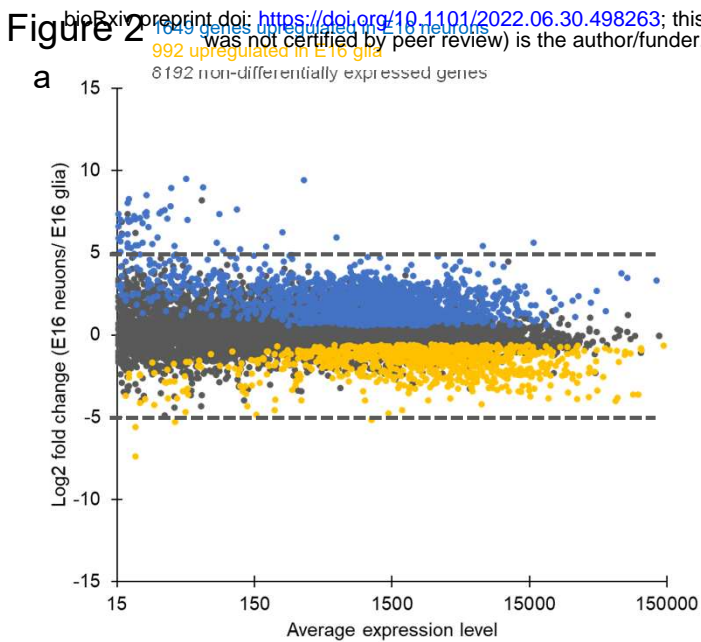
E16

# of genes expressed in E16 glia	10838
# of genes expressed in E16 neurons	11281
# of genes expressed in E16 hemocytes	9811
# of genes upregulated in E16 glia	3414
# of genes upregulated in E16 neurons	4177
# of genes upregulated in E16 hemocytes	2569

**e**

L3

# of genes expressed in L3 glia	9187
# of genes expressed in L3 neurons	9312
# of genes expressed in L3 hemocytes	7828
# of genes upregulated in L3 glia	4352
# of genes upregulated in L3 neurons	4191
# of genes upregulated in L3 hemocytes	2996

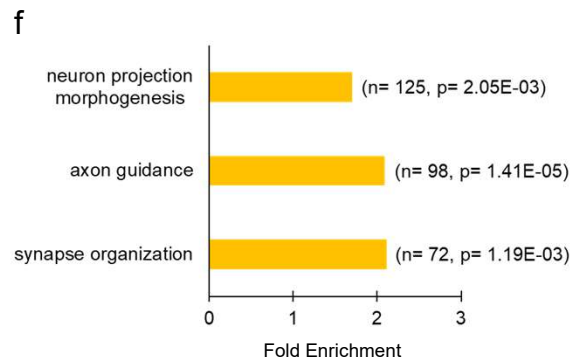
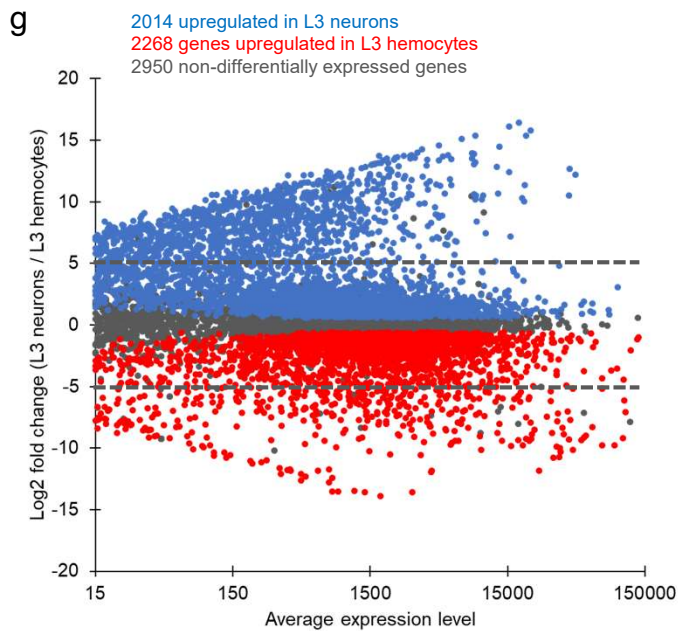
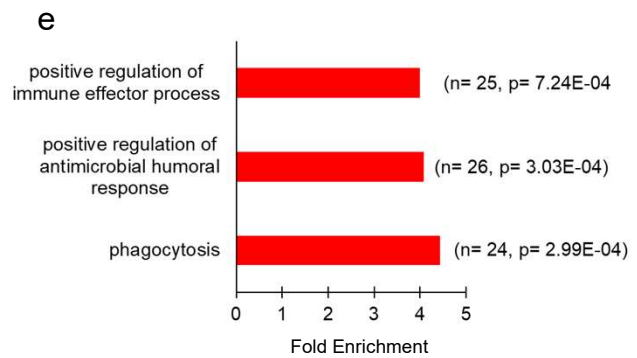
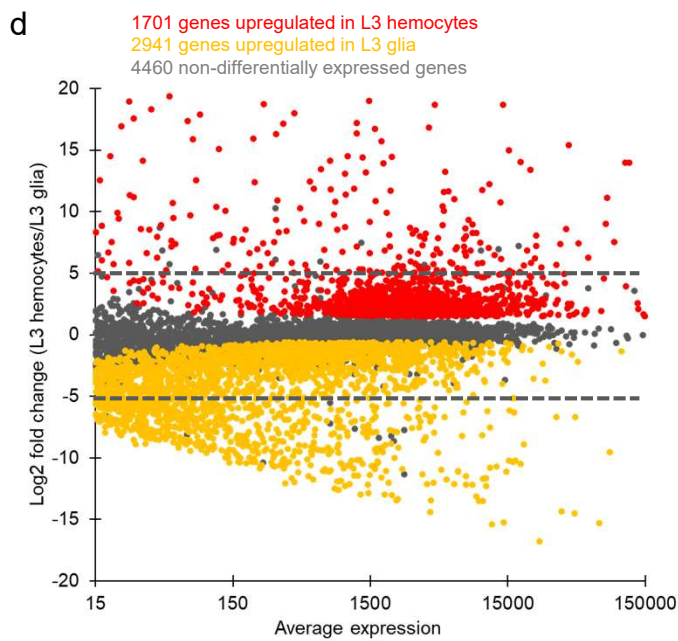
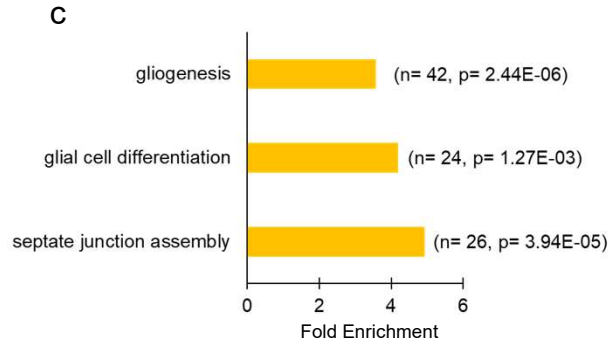
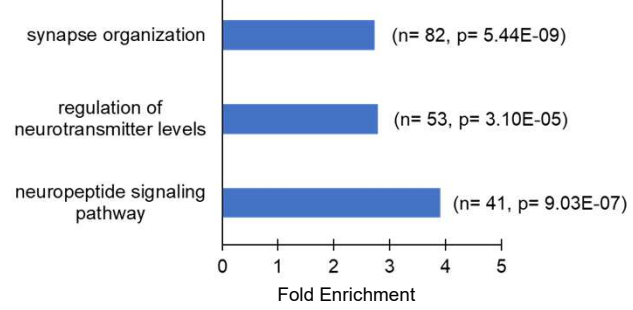
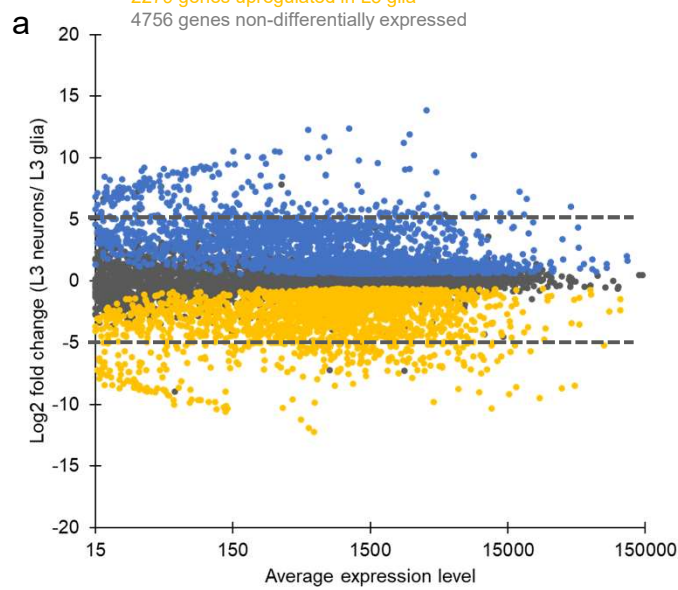


**g**

Immune Deficiency pathway		
FBgn0024222	I-kappaB kinase beta	IKKbeta
FBgn0013983	immune deficiency	imd
FBgn0041205	kenny	key
FBgn0035976	Peptidoglycan recognition protein LC	PGRP-LC
FBgn0031317	Charon	Charon
FBgn0010247	Poly-(ADP-ribose) polymerase	Parp

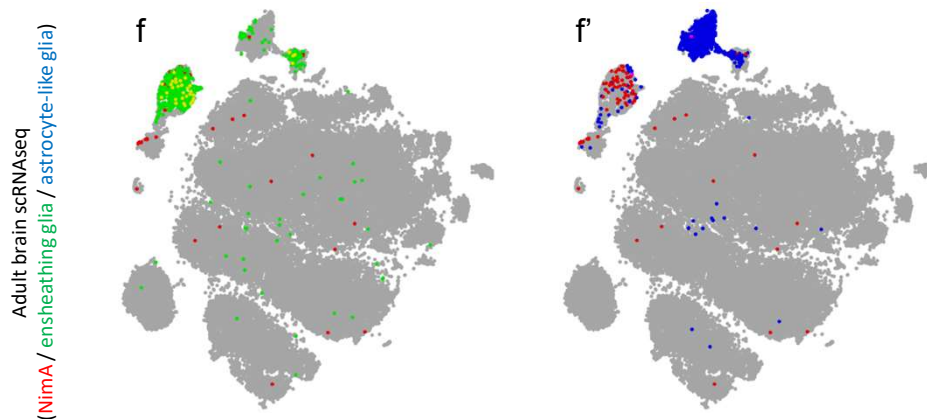
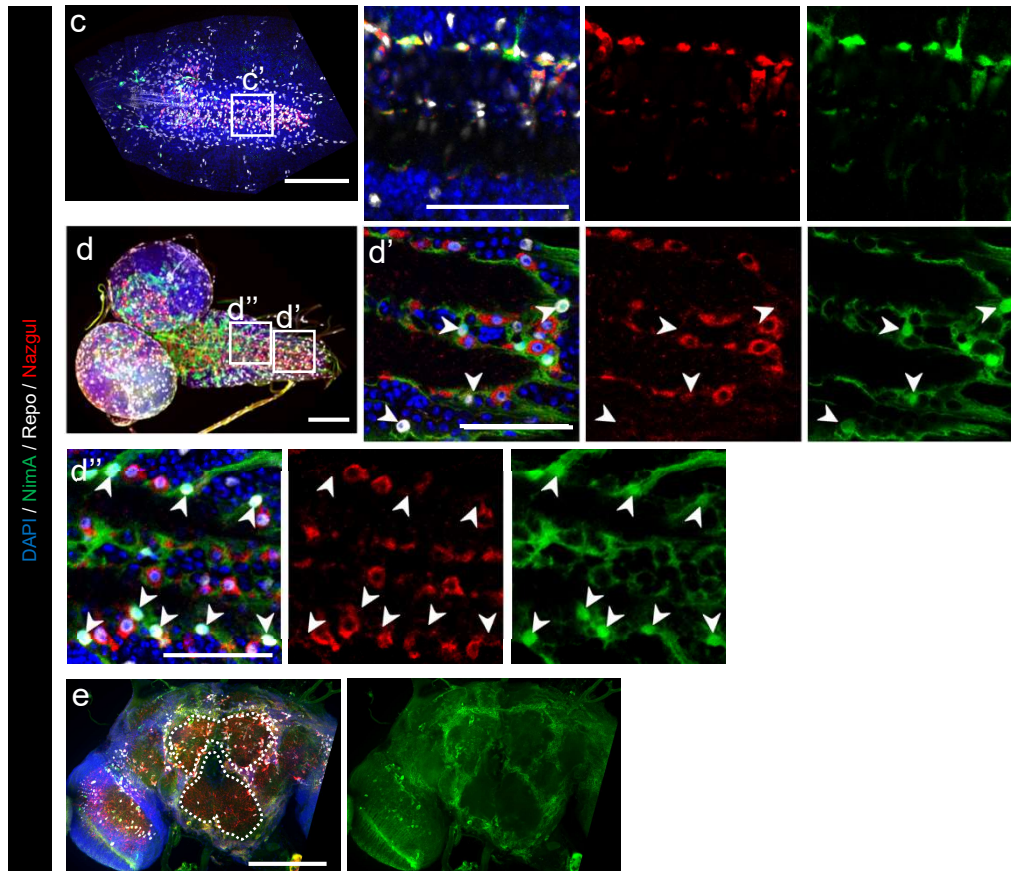
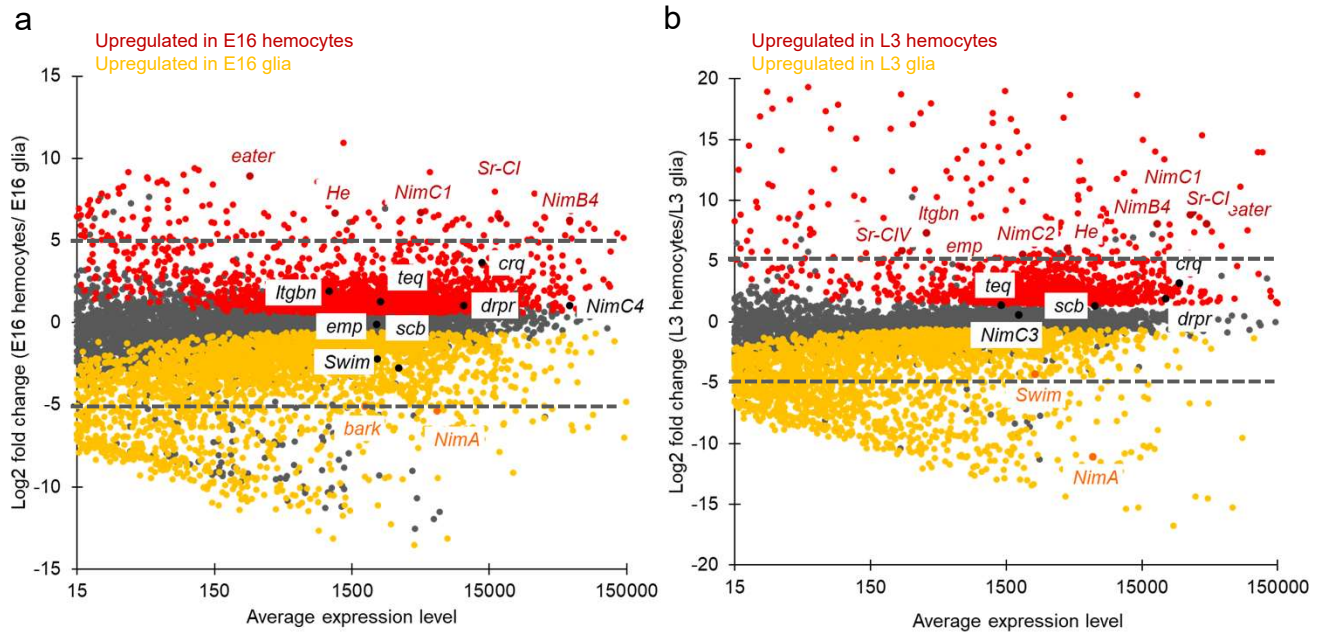
Toll pathway		
FBgn0011274	Dorsal-related immunity factor	Dif
FBgn0040323	Gram-negative bacteria binding protein 1	GNBP1
FBgn0030310	Peptidoglycan recognition protein SA	PGRP-SA
FBgn0010441	pelle	pll
FBgn0039102	Spatzle-Processing Enzyme	SPE
FBgn0003495	spatzle	spz
FBgn0262473	Toll	Tl
FBgn0034246	Dicer-2	Dcr-2
FBgn0265998	Darkener of apricot	Doa
FBgn0035207	HECT and RLD domain containing E3 ubiquitin ligase 4	Herc4
FBgn0010602	lesswright	lwr
FBgn0051217	modular serine protease	modSP
FBgn0030926	persephone	psh
FBgn0031676	senju	senju

# Figure 3

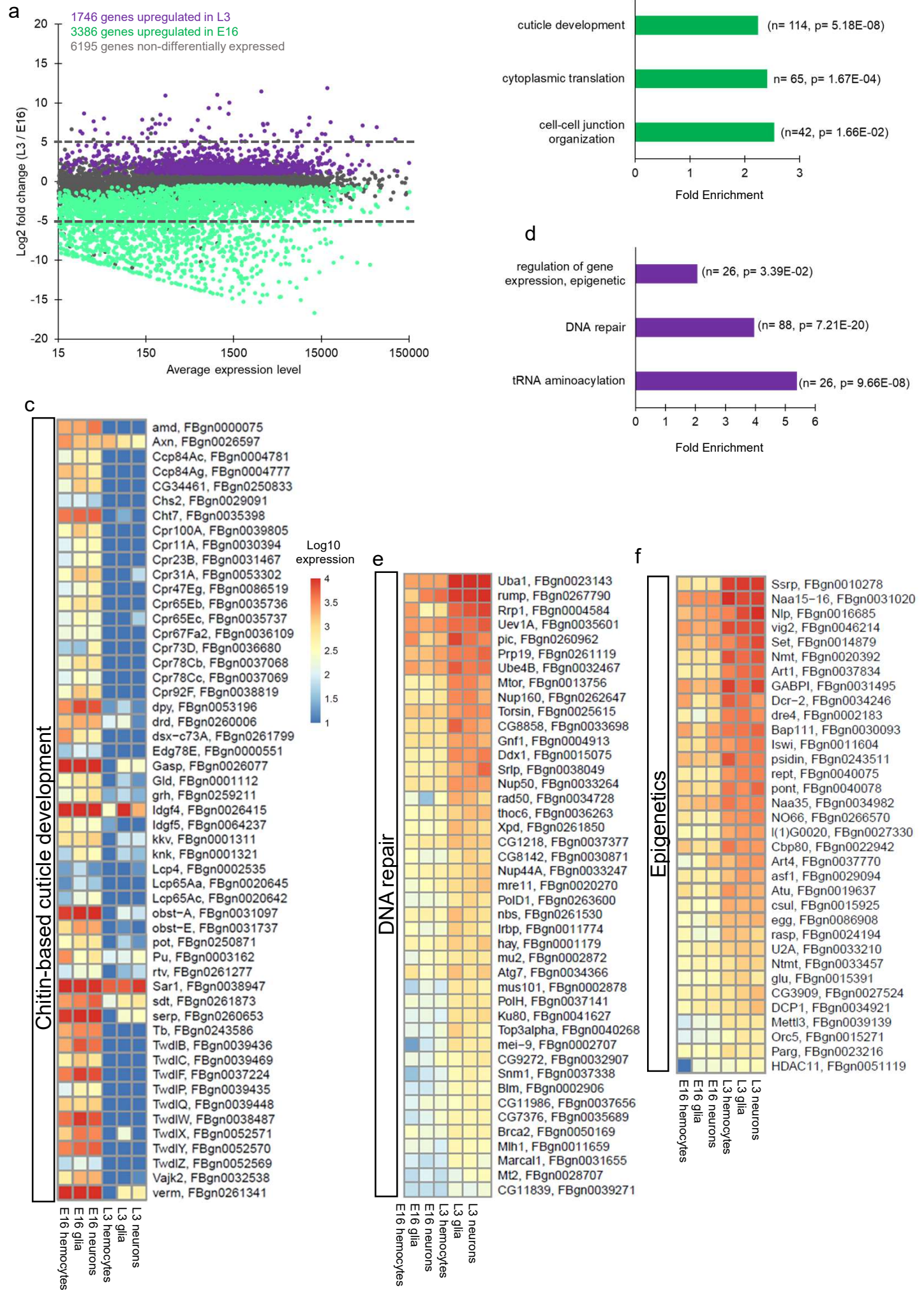




## Figure 4

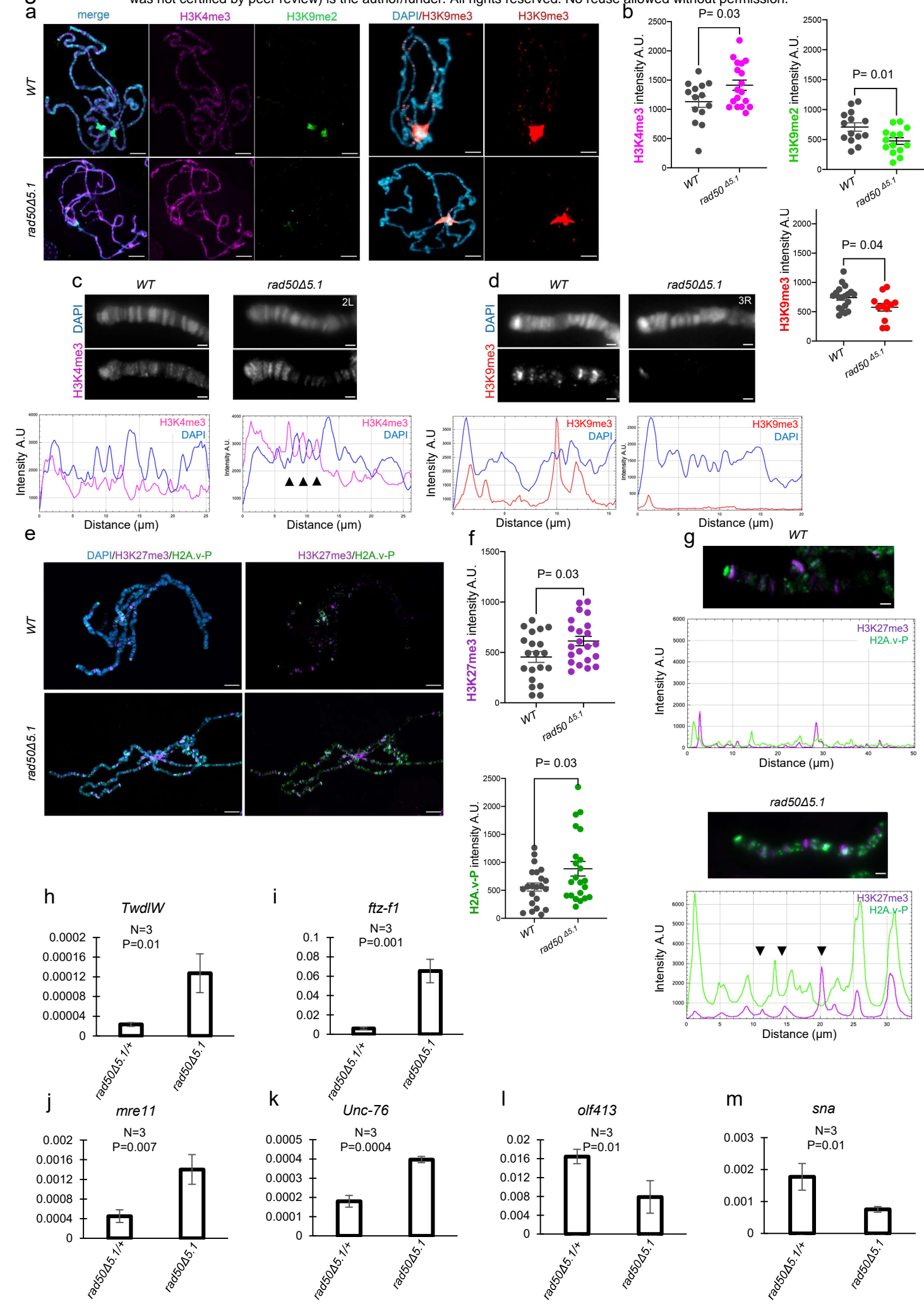


## Figure 5





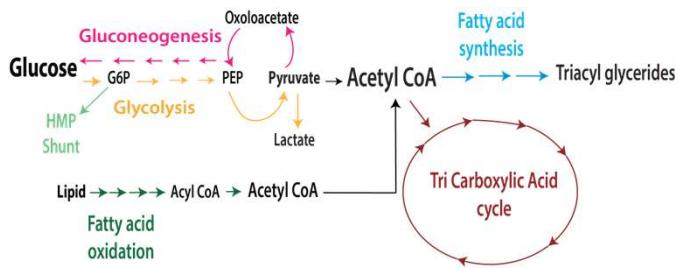
## Figure 6



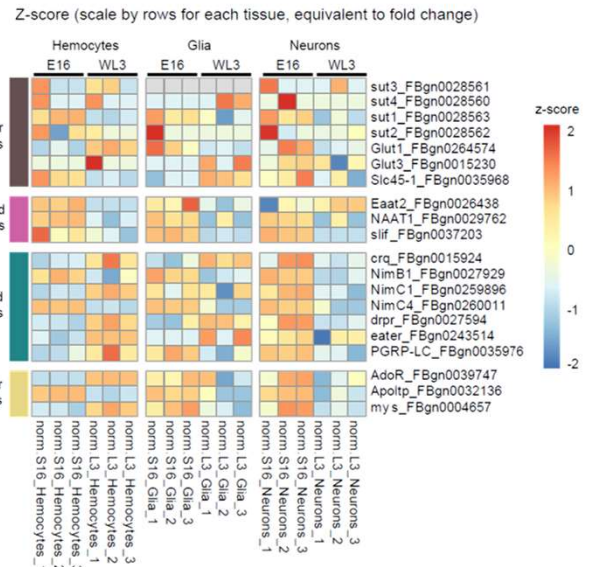


# Figure 7

a

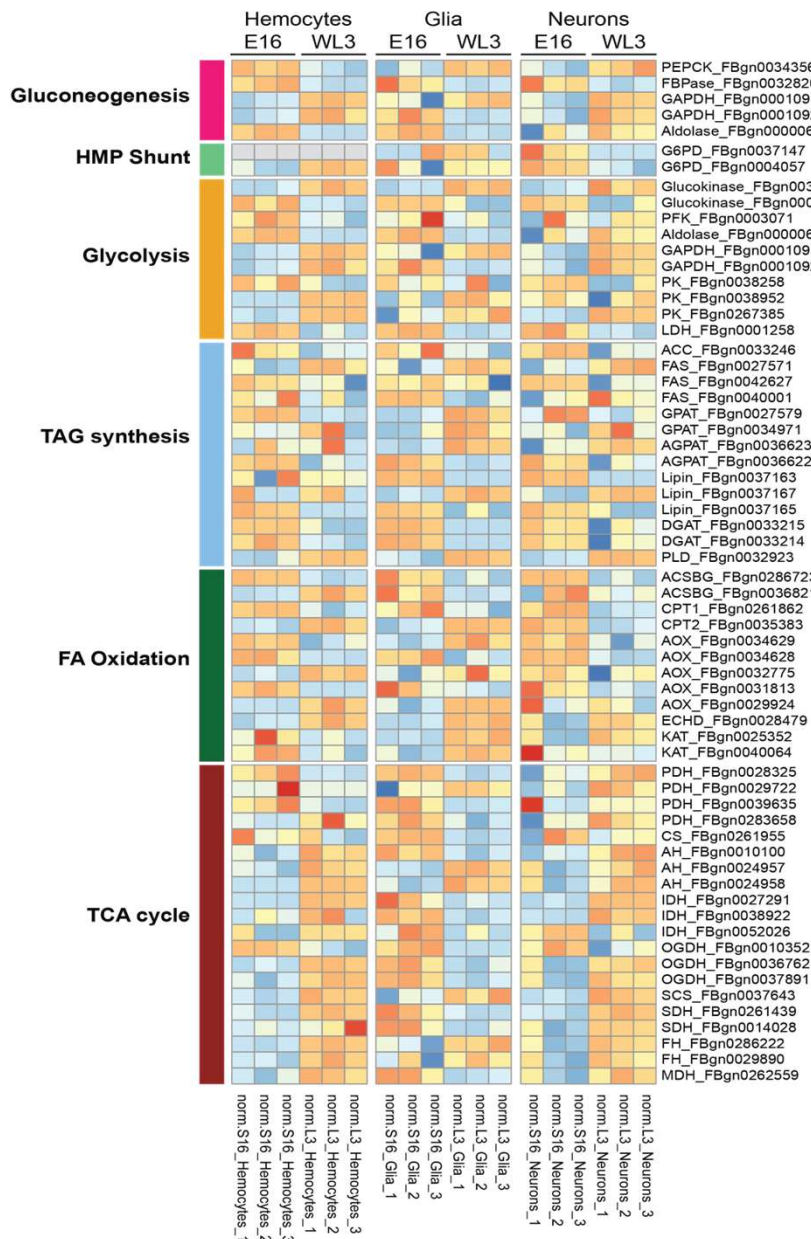


c



b

Z-score (scale by rows for each tissue, equivalent to fold change)



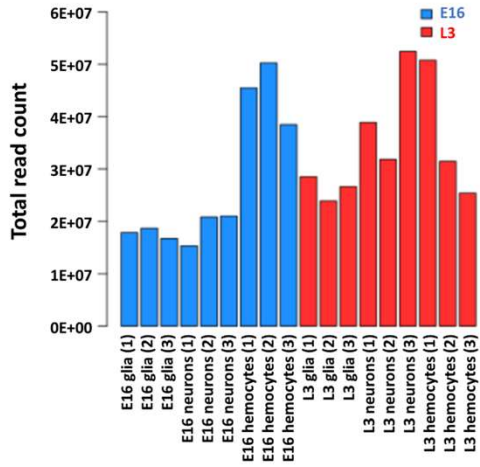
d

Sugar metabolism			
	Hemocytes	Neurons	Glia
Embryo	Gluconeogenic	Glycolytic	Glycolytic
	Glycolytic		Oxidative
Larva	Oxidative	Gluconeogenic	Gluconeogenic
		Oxidative	Oxidative

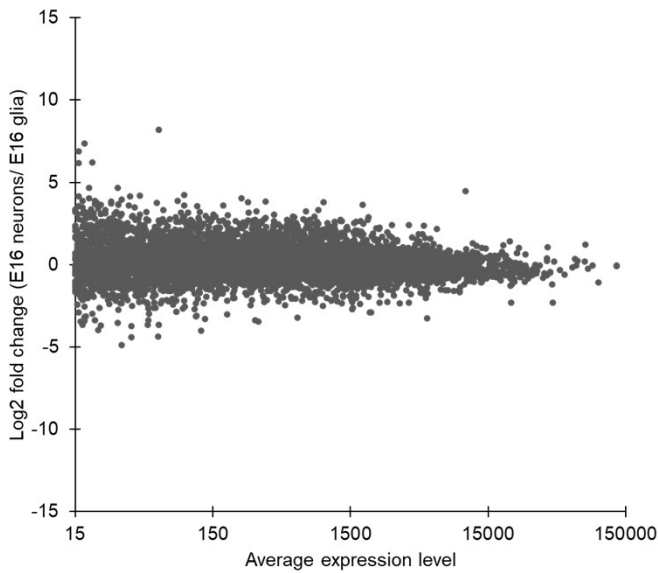
Lipid metabolism			
	Hemocytes	Neurons	Glia
Embryo	Lipogenic	Lipogenic	Lipogenic
		Lipolytic	
Larva	Lipolytic	Lipogenic	Lipogenic
		Lipolytic	Lipolytic



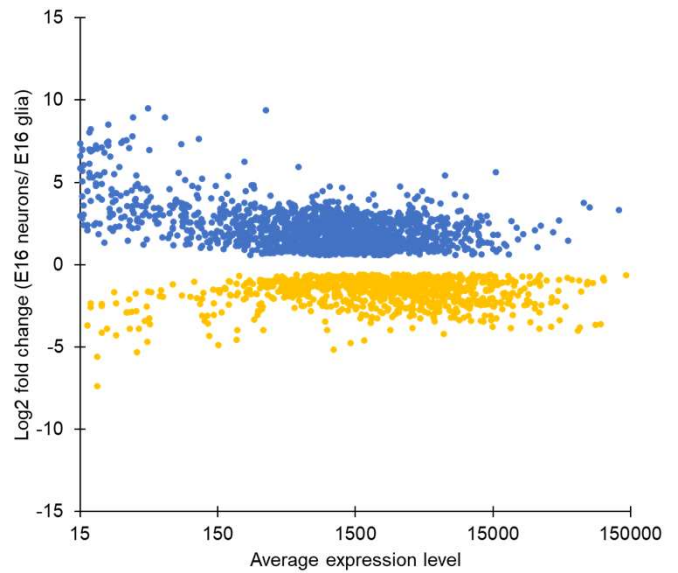
a



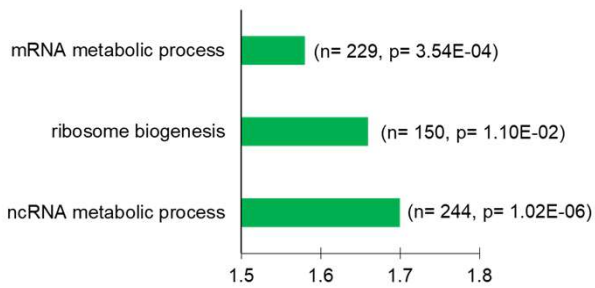
b

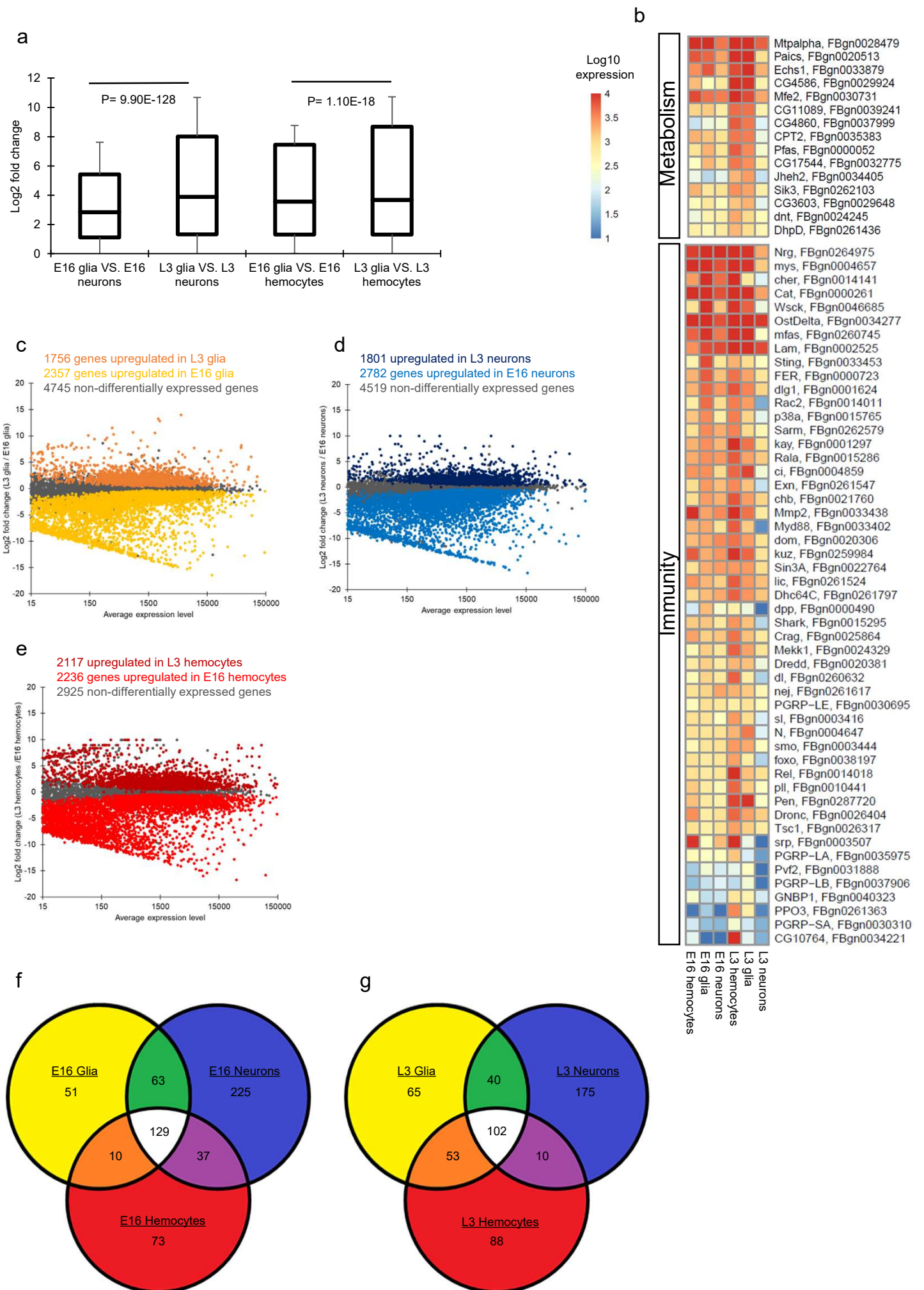


c



d







# Sup. Figure 3

