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Single-cell transcriptional landscapes of *Aedes aegypti* midgut and fat body after a bloodmeal

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22 **SUMMARY**

23

24 *Aedes aegypti* mosquitoes are vectors for numerous arboviruses that have an increasingly
25 substantial global health burden. Following a bloodmeal, mosquitoes experience significant
26 physiological changes, primarily orchestrated by the midgut and fat body tissues. These
27 changes begin with digestion and culminate in egg production. However, our understanding of
28 those key processes at the cellular and molecular level remains limited. We have created a
29 comprehensive cell atlas of the mosquito midgut and fat body by employing single-cell RNA
30 sequencing and metabolomics techniques. This atlas unveils the dynamic cellular composition
31 and metabolic adaptations that occur following a bloodmeal. Our analyses reveal highly
32 diverse cell populations, specialized in digestion, metabolism, immunity, and reproduction.
33 While the midgut primarily comprises enterocytes, enteroendocrine and intestinal stem cells,
34 the fat body consists not only of trophocytes and oenocytes, but also harbors a substantial
35 hemocyte population and a newly found fat body-yolk cell population. The fat body exhibits a
36 complex cellular and metabolomic profile and exerts a central role in coordinating immune and
37 metabolic processes. Additionally, an insect-specific virus, PCLV (Phasi Charoen-Like Virus)
38 was detected in single cells, mainly in the midgut a week after the bloodmeal. These findings
39 highlight the complexity of the mosquito's abdominal tissues, and pave the way towards the
40 development of exquisitely refined vector control strategies consisting of genetically targeting
41 specific cell populations and metabolic pathways necessary for egg development after a
42 bloodmeal.

43 INTRODUCTION

44

45 Blood-feeding female *Aedes aegypti* mosquitoes transmit numerous arboviruses
46 (arthropod-borne viruses) including dengue, Zika, chikungunya and yellow fever viruses.
47 These viruses pose a substantial global health risk that is set to be exacerbated by the
48 changing climate and increased urbanization^{1,2}. Developing effective control strategies against
49 insect vectors entails a deep understanding of their physiology at the cellular and molecular
50 level. Recent advances in insect genetic manipulation now enable more precise studies and
51 targeting of these vectors, elevating our capacity to devise effective control strategies^{3,4}.

52 When a mosquito bites a vertebrate host to take a bloodmeal, physiological and
53 metabolic changes unfold within the mosquito, orchestrated by two key organs: the midgut and
54 the fat body^{5,6}. The midgut digests the bloodmeal, supplying essential nutrients for egg
55 development^{5,7}. During this process, the midgut epithelium also undergoes considerable
56 remodeling, including an initial expansion phase followed by a cell-death program to maintain
57 functional digestion capabilities⁸. Upon receiving nutrients from the midgut, the fat body, acting
58 as the metabolic and reproductive hub, engages in vitellogenesis, the production of yolk protein
59 essential for egg development⁹. Operating between 24 and 72 hours after the bloodmeal¹⁰, this
60 synchronized process is crucial for egg production and species survival. Beyond these primary
61 roles, the midgut and fat body are also pivotal immune organs, serving as the first line of
62 defense against ingested pathogens^{11,12}. The midgut epithelium locally produces antimicrobial
63 peptides (AMPs) and reactive oxygen species (ROS)¹³ while the fat body orchestrates
64 systemic humoral and cellular immune responses, involving secreted immune factors (AMPs,
65 cytokines) and activation of hemocytes, the circulating immune cells¹⁴.

66 While the mosquito midgut has been well studied on its own¹⁵⁻¹⁷, limited data
67 considering its spatial proximity with the fat body and potential inter-organ complementarity is
68 available. In addition, the fat body organ has never been studied at single-cell resolution and
69 its two main cell types have been identified using morphological criteria^{12,18}. In this study, we
70 exploit the power of single-cell RNA sequencing (scRNA-seq) to characterize midgut and fat
71 body cell populations. We generated single-cell atlases at two critical stages: two days post
72 bloodmeal, marking the completion of digestion and tapering of vitellogenesis, and seven days
73 post bloodmeal, with the return of tissue homeostasis. Analysis of these datasets reveal the
74 cellular landscape and transcriptional signatures associated with mosquito midgut and fat body
75 organs in a comprehensive and integrated manner, demonstrating their complementarity and
76 unveiling specialized roles for very diverse, and sometimes newly described cell populations.
77 We validate key findings by metabolomic profiling, confirming distinct physiological and
78 metabolic states and roles of each organ. Additionally, we detected the presence of the insect-
79 specific virus PCLV (Phasi Charoen-like Virus) in midgut and abdominal fat body tissues at the

80 single-cell level, demonstrating the power of single-cell transcriptomics for characterizing
81 mosquito viromes at the tissular and cellular levels. Finally, we release our transcriptomes via
82 the interactive *Aedes cell Atlas* (<https://aedes-cell-atlas.pasteur.cloud>), providing a community
83 platform for gene exploration and analysis in *Ae. aegypti*.

84

85 RESULTS

86

87 Mapping the cellular landscape of mosquito midgut and abdominal fat body following a 88 bloodmeal

89

90 To examine how a bloodmeal shapes cell populations and gene expression in the
91 mosquito midgut and abdominal fat body, we performed single-cell RNA sequencing
92 (scRNAseq) on female *Ae. aegypti*. We collected midgut and fat body tissues from a field-
93 derived colony originating from Kumasi (Ghana) at 2- and 7-days post bloodmeal (dpbm)^{19,20}.
94 For each timepoint, we collected 7 midguts and 7 fat bodies, with each pair originating from
95 one individual. Specifically, after the midgut was collected, the abdomen of each mosquito was
96 dissected to isolate what we refer to as the 'fat body', which includes the abdominal fat body
97 and associated tissues (e.g. muscles, epidermis), while excluding all other visible organs (cf.
98 Methods section; **Fig. 1A**). The tissues were then dissociated into single cells using an
99 enzymatic digestion at 4°C^{21,22}. Single-cell transcriptomes were generated using the 10X
100 Genomics® technology (cf. Methods section; **Fig. 1A**). After quality control (cf. Methods and
101 **Fig. S1A, C**), we retained 7,512 and 6,935 cells from the midgut at 2 and 7 dpbm, respectively,
102 and 13,400 and 14,326 cells from the fat body at 2 and 7 dpbm, respectively (**Fig. S1B, D**,
103 **Table S1**). To compare expression profiles across time within each organ, we integrated
104 datasets from both timepoints (2 and 7 dpbm) using Seurat²³. Next, we identified distinct
105 cellular clusters and markers (**Fig. 1B-C, Fig. S1B, D**) and carried out a comprehensive
106 annotation by manual curation of the literature available in *Drosophila*, *Anopheles*, and *Ae.*
107 *aegypti*, detailed below.

108 In midguts, we identified 13 cell clusters, encompassing well-known populations such
109 as enterocytes (EC), enteroendocrine cells (EE), intestinal stem cells/enteroblasts (ISC/EB),
110 cardia cells (CA), visceral muscle cells (VM), and hemocytes (HC) (**Fig. 1B**). These main
111 populations were identified by known marker genes, including *nubbin* and serine proteases for
112 enterocytes, *orcokinin* and *prospero* for enteroendocrine cells, *escargot* for intestinal stem
113 cells/enteroblasts, *gambicin* for cardia cells, *titin* for visceral muscle cells, and *NimB2/SPARC*
114 for hemocytes. Our data aligns with established findings from the *Drosophila* midgut single-
115 cell atlas²⁴ and previously published single-nuclei and single-cell transcriptomes of *Ae. aegypti*
116 midgut cells¹⁵⁻¹⁷, validating the robustness of our current scRNAseq approach (**Fig. S2, Table**

117 **S2)**. Additionally, it reveals cellular changes at two critical time points post bloodmeal. The
118 overall cellular composition remained largely stable between 2 and 7 dpbm, with enterocytes
119 representing over 70% of all cells, followed by visceral muscle cells (~7%) and enteroendocrine
120 cells (~4-6%) (**Table S2**). We observed a significant increase in intestinal stem
121 cells/enteroblasts at 7 dpbm (3,6% to 11,2%), consistent with a bloodmeal-induced epithelium
122 regeneration²⁵.

123 In fat bodies, we identified 16 cell clusters, revealing a complex cellular ecosystem (**Fig.**
124 **1C**). The cellular composition of the fat body was subject to greater temporal changes,
125 demonstrating a high degree of reshuffling after a bloodmeal (**Fig. S1D, Table S3**).
126 Trophocytes (TP), the primary storage sites for blood-derived nutrients, are characterized by
127 the expression of vitellogenic and biosynthetic genes. They constitute the majority of cells at 2
128 dpbm (59,8% of all cells). However, their proportion significantly declines by 7 dpbm (to 39,3%
129 of all cells), likely reflecting the completion of vitellogenesis and major yolk protein production.
130 This shift in trophocyte abundance over time suggests a return towards a homeostatic state
131 after an intense metabolic activity linked to egg development²⁶. Beyond trophocytes, the fat
132 body harbors a diverse range of cell types (**Fig. 1C, Table S3**). First, we detect a population
133 that we named as 'Fat body-Yolk Cells' (FYCs; 18% of all cells) expressing a transcriptional
134 profile associated with vitellogenesis and oogenesis, suggesting a link with the physically
135 adjacent ovaries. We also observe a significant increase in *NimB2/SPARC*-positive hemocytes
136 at 7 dpbm (18,9% of all cells at day 7, compared to 9% at day 2), likely reflecting heightened
137 immune activity within the fat body post bloodmeal²⁷. These are likely sessile hemocytes that
138 associate with tissues in the abdominal cavity^{28,29}, and are composed of granulocytes (HC-G)
139 and oenocytoids (HC-O). Tracheoblast-like cells (TB; ~6%) express genes involved in the
140 regeneration and development of the tracheal system. Muscle cells (MU; ~3-5%) likely
141 contribute to structural support within the fat body and mosquito heart, while oenocytes (OE;
142 ~1-3%) contribute to lipid synthesis. Epidermal cells (EP; ~2-7%) and neuroendocrine cells
143 (NE; ~1-3%) might be involved in barrier function and regulation of various hormonal and
144 physiological processes.

145 Collectively, we have determined the cellular landscape of midgut and fat body tissues
146 in *Aedes aegypti*, 2 and 7 days after a bloodmeal and propose a sketch of both organs based
147 on our cellular atlases (**Figure 1D**). Our single-cell atlases are accessible and explorable on a
148 dedicated website (<https://aedes-cell-atlas.pasteur.cloud>).

149

150 **Cell population dynamics in the midgut: from bloodmeal digestion to the return to**
151 **homeostasis**

152

153 The midgut is key to blood digestion and provides the first line of defense against
154 ingested pathogens³⁰. While its cellular composition is increasingly understood, the cell
155 population dynamics following a bloodmeal and over extended periods remain largely
156 unexplored. After identifying the main cell types (**Fig.1**), we analyzed each cell cluster
157 separately and provided a set of highly enriched and sometimes specific new marker genes
158 (**Fig. 2A-B**).

159 **Enterocytes (EC)**. We delineated six distinct enterocyte clusters (EC and EC-like1-5) that are
160 specialized in proteolysis and metabolism (**Fig. 2B**). The cluster we call EC stands out for its
161 enriched expression of *nubbin*, a well-established marker gene for differentiated enterocytes
162 in *Drosophila* and mosquitoes^{15,24} (**Fig. 2A & 2C**). In addition, EC displays enhanced
163 transcriptional and signal transduction activity, with expression of *Pde9*, *doublesex (dsx)*, and
164 *yorkie (yki)*, previously linked to *Ae. aegypti* enterocytes¹⁵ (**Fig. 2D**). Clusters EC-like1-5 likely
165 represent varying functional states of enterocytes and highly express the juvenile hormone-
166 regulated serine protease *JHA15*, previously associated with midgut epithelial cells (**Fig. 2D**)
167 ^{24,31,32}. EC-like1 cells express high levels of digestive enzymes (serine proteases,
168 carboxypeptidases, and lipases) at 2 dpbm, indicating robust activity during bloodmeal
169 breakdown^{15,24}. EC-like2 and EC-like5 cells express increasing levels of oxidative
170 phosphorylation genes over time, reflecting a transition from digestion at 2 dpbm towards
171 midgut homeostasis by 7 dpbm (**Fig. 2A, D**). Furthermore, the increased abundance of EC-
172 like2 cells at 7 dpbm supports a shift towards midgut stabilization (**Fig. 2D, Table S4**). EC-
173 like3 and EC-like4 cells share digestive markers with EC-like1 at 2 dpbm but exhibit a
174 significant decrease in abundance by 7 dpbm, similar to EC-like1, suggesting these three
175 clusters represent sequential stages or functional states of enterocyte-like cells (**Fig. 2D, Table**
176 **S4**).

177 **Cardia cells (CA)**. Present mainly at 7 dpbm, cardia cells are characterized by immune and
178 metabolic genes (**Fig. 2A, B, E**). Likely originating from the stomodeal valve near the
179 proventriculus³³, they express genes encoding antimicrobial peptides such as *gambicin*
180 (*GAM1*), sugar transporters (*Tret1*, *SWEET*), detoxification and insecticide resistance genes
181 (*TPX4*, *GSTE4*), and cytochrome P450 genes, potentially contributing to food processing at
182 the juncture with the anterior gut (**Fig. 2E**). Of note, the variable presence of cardia cells may
183 originate from the dissection method, which involves cutting near the proventriculus, potentially
184 impacting the consistent collection of these cells.

185 **Intestinal stem cells/enteroblasts (ISC/EB)**. This cluster is identified using established stem
186 cell markers *fibulin 1* and *C-type lysozyme A (LYSC11)*¹⁵ (**Fig. 2A**). This population also
187 express the *Drosophila* ISC markers *escargot (esg)* and *Delta*, along with a lower level of the
188 EB marker *Klumpfuss (klu)*^{34,35} (**Fig. 2F**). Subclustering allowed us to fully distinguish stem
189 cells from enteroblasts (**Fig. S3A**). ISC display high *esg* and *Delta* expression, while EB display

190 low *esg* and *Delta* but high *nubbin* expression, indicating a transition stage towards enterocytes
191 prominently at 7 dpbm. Both groups display low *prospero* expression, suggesting limited
192 progression towards enteroendocrine cells.

193 **Enteroendocrine cells (EE).** Similar to *Drosophila*, EE populations express a set of gut
194 hormones³⁶ (**Fig. 2A, G**). The EE-Orco cluster, expressing the *orcokinin* neuropeptide gene
195 linked to egg development, contrasts with the EE-NPF cluster, which expresses *neuropeptide*
196 *F (npf)* and *Islet Antigen-2 (IA-2)* genes involved in regulating lipid synthesis and insulin
197 metabolism^{37–39}. The transcription factor *prospero*, crucial for EE differentiation from ISC, is
198 primarily enriched in the EE-NPF cluster⁴⁰. Furthermore, genes associated with hormone
199 synthesis and secretion are differentially expressed between EE-Orco and EE-NPF,
200 suggesting distinct functional roles (**Fig. 2G**).

201 **Visceral muscle cells (VM).** Two visceral muscle cell populations (VM-1 and VM-2) are
202 identified based on their expression of structural and regulatory muscle proteins (**Fig. 2A-H**).
203 While both VM express high levels of *sallimus (sls)* and *titin* genes, VM-2 displays a broader
204 repertoire of muscle genes (*myofilin*, *tropomyosin*, *mlc-2*, *troponin*, *actin*), suggesting a more
205 specialized contractile function (**Fig. 2H**).

206 **Hemocytes (HC).** Lastly, a HC population is primarily identified at 2 dpbm, expressing
207 established hemocyte marker genes such as *Nimrod B2*, *SPARC*, and granulocyte marker
208 *LRIM16*⁴¹ (**Fig. 2A-I**).

210 **From metabolism to reproduction and defense: the complex cellular landscape of the** 211 **fat body**

212
213 Our study establishes the first comprehensive single-cell atlas of mosquito fat body
214 cells, revealing a remarkably diverse cellular landscape (**Fig. 1C, 3A**). As expected, two major
215 cell types previously described in mosquito fat bodies based on their distinct morphology –
216 trophocytes (TP) and oenocytes (OE) – are readily identified through robust expression of
217 metabolism-related genes^{5,18,42} (**Fig. 3A,B**).

218 **Trophocytes (TP).** Four trophocyte clusters (TP1-TP4) are identified, differentiated by the
219 presence of unique vitellogenic markers and distinct gene expression profiles (**Fig. 3A, C**).
220 TP1 displays a pronounced shift in cellular abundance from 47% at 2 dpbm to 12% at 7 dpbm,
221 suggesting a dynamic remodeling in response to nutrient status changes (**Fig. 3C, Table S5**).
222 TP1 and TP2, representing the majority of trophocytes, show significant expression of
223 vitellogenin genes (*Vg*, *vit-A*, *Vcp*) at 2 dpbm, aligning with the peak of proteolytic activity in
224 the midgut associated with yolk protein synthesis⁴³ (**Fig. 3B, C**). Beyond vitellogenesis, TP2
225 cluster, particularly prominent at 7 dpbm, also engages in trehalose biosynthesis, as evidenced
226 by strong expression of *Tps1* and *Tpp* genes. This highlights trophocyte's crucial role in

227 maintaining energy homeostasis and synthesizing the primary circulatory sugar in insect
228 hemolymph^{44,45}. TP2 and TP3 also display a diverse genetic repertoire, encompassing
229 metabolic genes along with a suite of immune modulatory markers (*Vago-like* genes, LRIMs,
230 SERPINS, CLIPs, and lectins). Notably, both clusters expressed the abdomen-specific
231 antimicrobial peptide *holotricin* (*GRRP*)³⁰ (**Fig. 3C**). These data highlight the dual role of fat
232 body cells in metabolism and defense, as observed in *Anopheles* mosquitoes⁴⁶. Although TP4
233 shares the vitellogenin membrane receptor *15a-1* with TP1, it shows a unique signature
234 enriched for lysosomal function (*LManII*), and metabolism/antioxidant defense (*GILT*, *Alp4*)
235 (**Fig. 3C**). TP3-4 may represent subsets of TP1-2, transitioning towards broader functions
236 beyond yolk production, as indicated by their increasing proportion at 7 dpbm.

237 **Oenocytes** (OE). Representing only 1-3% of the fat body cell population at both 2 and 7 dpbm
238 (**Fig. 3A, D; Table S5**), oenocytes stand out for their robust metabolic and fatty acid synthesis
239 machinery⁴⁷⁻⁴⁹. Specialized in producing cuticular hydrocarbons and pheromones in
240 *Drosophila*^{50,51}, they express high levels of genes encoding fatty acid synthases (*AaFAS2*,
241 *AaFAS3*, *AaFAS5*), elongase, reductase (*FarO*), desaturase (*SCD*), and P450 enzymes
242 (CYP4G family) (**Fig. 3D**). Notably, oenocytes also exhibit activity in lipid degradation (*Mfe2*,
243 *Mcad*), transport (*Galz*), and the TCA cycle (*Men*, *ACS*, *Idh*), highlighting their multifaceted role
244 in mosquito energy homeostasis. Intriguingly, *AaFAS1* is broadly detected in all fat body cells
245 and overexpressed in the TP2 subpopulation, suggesting distinct functions for AaFAS family
246 members. This is particularly relevant considering *AaFAS1*'s recent identification as a major
247 fatty acid synthase and potential dengue virus proviral factor⁵². Moreover, our findings align
248 with reports of *AaFAS3* and *FarO* as prominent markers of abdominal tissue, significantly
249 contributing to bulk transcriptomic datasets³⁰. Additionally, a minor OE-like cluster is detected
250 at 7 dpbm and subclustering revealed a specific gene set associated with this cell population
251 (**Fig. S3B**). Despite their low abundance, oenocytes appear to strongly contribute to the
252 mosquito's overall metabolism.

253 **Hemocytes-Granulocytes/Oenocytoids** (HC-G/O). Contrasting with the midgut, the fat body
254 display a diverse landscape of hemocytes, with their abundance doubling at 7 days post-
255 bloodmeal (**Fig. 3E, Table S3**). We identify 2 hemocyte clusters using established universal
256 markers⁴¹ (*NimB2*, *SPARC*, *Cg25C/Col4a1*), likely delineating circulating and sessile
257 populations within the fat body^{28,53} (**Fig 3A, E**). The granulocyte cluster HC-G, express markers
258 of mature granulocytes⁴¹ (*LRIM16*, *LRIM15*). In addition, HC-G displays a wide repertoire of
259 stress and immune response genes^{30,54} (*GPXH2*, *CLIPD1*, *FREP*), highlighting their potential
260 contribution to local immunity within the fat body. Further subclustering reveals additional
261 heterogeneity within HC-G, ranging from antimicrobial granulocytes, prohemocytes to
262 unidentified granulocytes that differ from circulating hemocytes typically observed in *Ae.*
263 *aegypti*⁴⁶ (**Fig. S3C**). Particularly, a subcluster highly abundant at 7 dpbm (Gra-Cut), exhibit a

264 distinct profile with lower granulocyte marker expression and a specific increase in cuticle
265 protein (*CPLCA3*, *CPF3*, *Ccp84A*) and trypsin (*Sb*) genes (**Fig. S3C**). This suggests a potential
266 specialization of granulocytes towards abdominal/cuticle tissues, particularly at later stages
267 post bloodmeal²⁸. Finally, a distinct population (HC-O) express several PPO gene family, a
268 hallmark of oenocytoids with roles in melanization and immune signaling^{41,46,55} (**Fig. 3E, S3C**).
269 These findings suggest a diverse and dynamic hemocyte population associated with or
270 circulating near the fat body after a bloodmeal, likely contributing to local or systemic immune
271 responses induced by the bloodmeal⁵⁶.

272 **Fat body-yolk Cells (FYC)**. We uncovered an undescribed cell population within the fat body
273 of female *Ae. aegypti* following a bloodmeal and named them fat body-yolk cells (FYC) due to
274 their expression of genes essential to fat body and ovary function (**Fig. 3A, B**). FYC uniquely
275 express a suite of genes critical for reproductive processes such as *Nasrat*, crucial for eggshell
276 formation (**Fig. 3A**). Mosquitoes in our experiment received a bloodmeal, without oviposition
277 sites (cf. Methods section). By 7 dpbm, we observed ovaries filled with mature eggs, indicative
278 of an oviposition readiness typically beginning by 3 dpbm⁵. At 2 and 7 dpbm, the FYC
279 population remains stable (**Fig. 3F, Table S3**), suggesting a role in assuring reproductive
280 readiness⁵. Clustering suggests that FYC1, FYC2, and FYC4 likely represent different states
281 of the same cell population, based on shared expression of ecdysone receptor genes (*EcR*,
282 *USP*, *E75*, *Hnf4*) crucial for oogenesis regulation^{10,57–59}, and structural proteins (*Nasrat*, *closca*,
283 *polehole*, *torso*) necessary for eggshell formation and embryo protection^{60–62}. Additionally,
284 FYC1-2-4 express genes required for eggshell integrity (*Pxt*, *HPX8B*, *yellow*)⁶⁰, and genes
285 associated with the LDL receptor family, suggesting a role in ovary lipid transport^{63,64}. The
286 enrichment of histone protein genes (*H2A*, *H2B*, *H3*) and histone methyltransferase genes
287 (*eggless*, *ash1*, *PR-Set7*) hint at potential epigenetic remodeling activity^{65,66}. In addition, FYC
288 highly express autophagy genes (*Tor*, *Atg1*, *Atg2*, *APG3*), suggesting resource reallocation
289 during late vitellogenesis and egg maturation^{26,67}. Moreover, FYC2 and FYC4 exhibit higher
290 transcriptional activity and express stem cell markers (*klu*, *Delta*), suggesting a role in fat body
291 differentiation and regeneration. Interestingly, FYC highly express piRNA pathway (PIWI
292 family) genes that are crucial for transposon silencing and genomic defense in ovarian
293 development^{30,65,68}. FYC3 was particularly enriched in *PIWI2*, hinting to its role in germline
294 protection. Overall, the newly identified FYC population sheds light on *Ae. aegypti* adaptive
295 mechanisms that possibly maintain reproductive readiness and cellular integrity despite
296 environmental restrictions on egg laying⁶⁹. This resilience underscores the sophisticated
297 biological orchestration ensuring the mosquito's reproductive potential and life cycle continuity.

298
299 Four less abundant populations are identified in association with the fat body (**Fig. S4**).
300 **Tracheoblasts (TB)**. This cluster displays markers of growth and cell regeneration (*nell1*, *br*,

301 *rgn*, *goe*), as well as signaling and trachea cell marker (*sprouty*, *Egfr*)^{70–72} (**Fig. 3A, S4A**). In
302 addition, these cells express specific metabolic genes (*Gcat*, *Tdh*). Their constant presence at
303 2 and 7 days post bloodmeal suggests a role in tissue maintenance and regeneration. **Muscle**
304 **cells** (MU). A distinct muscle cell cluster remains stable in proportion between 2 and 7 dpbm,
305 expressing genes characteristic of muscle structure and regulation (**Fig 3A, S4B**). These
306 genes encompassed a variety of muscle fiber types and regulatory pathways shared with
307 cardiac and other muscle cells found in the adult *Ae. aegypti* heart^{73–75}. **Epidermal cells** (EP).
308 A distinct cluster is enriched for epidermal cell markers, with cell abundance increasing at 7
309 dpbm (**Fig. 3A, S4C**). Epidermal cells express significant levels of juvenile hormone binding
310 protein (*JHbp14*) and genes associated with cuticle formation (*Lcp65Ad*, *Cpr49Ab*, *Cpr49Ae*,
311 *dumpy*) crucial for epidermal-cuticle attachment⁷⁶. Cuticular proteins, associated to the most
312 abundant EP subcluster (**Fig. S3D**), are known to be either synthesized by epidermal cells or
313 transported from the hemolymph^{77,78}. In addition, epidermal cells display markers for adhesion
314 (*SCR5*, *Tsp*, *CTLGA3*). Interestingly, the tyrosine amino acid pathway appears specifically
315 expressed (*Hpd*, *hgo*, *Tat*), which is essential in insect cuticle pigmentation and homeostasis.
316 Lastly, epidermal cells distinctly exhibit expression of acetylcholinesterase (*Ace*) and odorant
317 binding proteins (*OBP11*, *OBP10*), suggesting their involvement in neuronal signaling and
318 chemosensation. **Neuroendocrine cells** (NE). A NE cell cluster, prominent at 7 dpbm (**Fig**
319 **3A, S4D**), is identified based on markers shared with *Drosophila* brain neuropeptides and
320 midgut EE cells (**Fig. 2G**). Neuroendocrine cells notably express amyloid precursor protein
321 (*App*) and nervous system antigen (*nrv*), key components in insect neuronal signaling^{80,81}.
322 Enriched expression of neuro-specific genes, including *futsch*, a GABA receptor (*Rdl*), and
323 acetylcholinesterase (*Ace*), further emphasizes the broad spectrum of neuronal functions
324 manifested within these cells, underpinning complex neuroendocrine regulation in the fat body.
325 Interestingly, the genes *Ace* and *Rdl*, coding for proteins targeted by insecticides in
326 mosquitoes⁸², highlight the potential role of these cells in environmental adaptation and
327 underscore their significance as precise targets in insecticide-based control strategies.

328 The abdominal fat body environment of *Ae. aegypti*, traditionally viewed as a metabolic
329 center, is highly complex. Our study unveils distinct trophocyte subpopulations, oenocytes, and
330 a diverse hemocyte population, suggesting multifaceted roles spanning metabolism, immunity
331 and reproduction. Furthermore, the identification of fat body-yolk cells and the presence of
332 epidermal, tracheoblast, muscular, and neuroendocrine cells highlight the fat body as a
333 microenvironment orchestrating diverse physiological processes.

334

335 **Specific midgut and fat body cells mutually contribute to immune and metabolic**
336 **pathways.**

337

338 To examine the respective roles of midgut and abdominal fat body tissues in *Ae. aegypti*
339 following a bloodmeal, we focused on key genes implicated in immune response and
340 metabolism at 2 and 7 dpbm (**Fig. 4**). We computed the proportion of cells expressing specific
341 gene sets per cluster to assess gene expression. The use of control reference genes, including
342 the digestive enzyme *Chymotrypsin-1* (CHYM1) and the vitellogenic protein *Vitellogenin A* (vit-
343 A), confirmed a steady physiological status for each condition. Moreover, the consistent
344 expression of the housekeeping gene 60S ribosomal protein L14 (*RpL14*) across all clusters
345 confirmed overall data quality, with small variations only observed in the midgut at 2dpbm.
346 Both midgut and fat body organs are pivotal in nutrient metabolism and coordination of immune
347 defenses^{83–85}. Given these established roles, we decided to analyse expression of canonical
348 immune pathways traditionally associated with host defense, specifically TOLL, IMD, JAK-
349 STAT, antimicrobial peptides and RNA interference.

350 **TOLL/IMD** pathway components, including *REL1A*, *cactus*, and *REL2*, are broadly
351 expressed across cell clusters, and particularly enriched in EC, CA, VM, and FYC clusters.
352 IMD signaling is specifically upregulated in FYC at 7 dpbm. Peptidoglycan recognition proteins
353 (PGRP), key activators of TOLL/IMD signaling, exhibit tissue-specific expression patterns⁸⁶.
354 *PGRP-LB* is predominant in midgut cells (EC, CA) and OE, while *PGRP-LD* is enriched in the
355 fat body (TP, FYC). *PGRPS* is specifically expressed in midgut cells, with increased expression
356 at 7 dpbm. The **JAK-STAT** pathway is predominantly activated in the fat body. Cytokine-like
357 *Vago-like* genes (*VLG-2*, *VLG-1*) are significantly expressed in TP2-3, with an increase at 7
358 dpbm, and to a lesser extent in FYC and EP clusters⁸⁷. Core components of this pathway,
359 including *DOME*, *HOP*, and *STAT*, are broadly expressed in FYC. *STAT* is the only member
360 of this pathway highly expressed in midgut cells, particularly by EC. Cell clusters with high
361 *STAT* expression in both tissues also express the *SOCS* regulator at a lower level.

362 **Antimicrobial peptides (AMPs)** genes are differentially expressed across tissues. *Gambicin*
363 (*GAM1*) is predominantly expressed in midgut CA cluster at 2 dpbm, expanding to the other
364 midgut clusters at 7 dpbm. *Holotricin* (*GRRP*) is extensively expressed across all fat body
365 clusters, particularly in TP2-3. Other antimicrobial peptides, such as *defensin* (*Def*) and
366 lysozymes, display distinct patterns in the midgut (CA and ISC/EB) and the fat body (TP2 and
367 OE). **RNA interference (RNAi)** pathway components are extensively expressed in the fat
368 body, with FYC exhibiting particular activity for key genes such as *Dcr-1*, *Dcr-2*, *logq*, and
369 PIWIs. This highlights the crucial role of FYC in preserving the mosquito's germline integrity.

370

371 The midgut and fat body tissues in mosquitoes are highly metabolically active,
372 particularly following a bloodmeal^{43,83,88}. This is demonstrated by a high proportion of cells in
373 both tissues expressing a broad array of metabolic genes, exceeding those involved in immune
374 pathways, observed not only at the end of digestion but also at 7 dpbm (**Fig. 4**).

375 **Carbohydrate metabolism** including glycolysis and the pentose phosphate pathway
376 (*Hex-A*, *GAPDH*, *Tkt*), is active in both tissues, with specific cell groups showing elevated
377 activity: EC, ISC/EB, CA in the midgut, TP2-3, OE and FYC in the fat body. Starch metabolism,
378 particularly trehalose synthesis (*Tps1*, *Tpp*), is predominantly a fat body function, with a
379 dynamic expression pattern. Glycogen metabolism displays distinct profiles, with glycogen
380 catabolism (*GlyP*) highly active in the fat body at 2 dpbm trough TP and FYC clusters and
381 minimal biosynthesis (*GlyS*) in both tissues. **Energy metabolism**, encompassing the TCA
382 cycle and oxidative phosphorylation, is robust in both tissues, with overlapping cell-type
383 expression. Similar to glycolysis, midgut (EC, CA) and fat body clusters (TP, OE, FYC) show
384 distinct expression patterns for specific genes within these pathways. Notably, oxidative
385 phosphorylation became more widespread at 7 dpbm in both tissues.

386 **Lipid metabolism** exhibits diverse patterns across the midgut and the fat body. Fatty
387 acid biosynthesis, a core lipid metabolic process, shows tissue-specific profiles. While *AaFAS1*
388 is ubiquitously expressed in the fat body and sporadically by few midgut clusters, *AaFAS2*
389 demonstrates a specific expression pattern led by oenocytes. Interestingly, a fatty acid
390 desaturase (*Fad2*), is consistently expressed in both tissues. Genes involved in fatty acid
391 elongation (*elongase*), reduction (*FarO*, *FAR*), and beta-oxidation, exhibit diverse expression
392 patterns in midgut (EC-like2, CA, ISC/EB, EE-NPF), and fat body clusters (TP2, OE, and FYC).
393 Glycerolipid and sphingolipid metabolism are expressed relatively low in both tissues.
394 However, a glycerol kinase (*Gk1*), involved in triglycerides and phospholipids synthesis, is
395 predominantly active in TP and FYC cells. Apolipoproteins such as *Glaz* and *apoLp-III*, crucial
396 for lipid transport, display cell-specific expression in both tissues, highlighting vital biological
397 communication. *Glaz*, initially expressed in OE cluster, expands to FYC over time. Meanwhile,
398 *apoLp-III* demonstrates dynamic expression across specific midgut (EC-like, CA, ISC/EB) and
399 fat body clusters (TPs, OE, FYC). Lipoprotein receptors (*LRP1*, *arrow/yolkless*), mainly
400 expressed in the fat body, likely facilitate lipid uptake, particularly in FYCs.

401 Beyond lipid metabolism, midgut and fat body cell clusters exhibit diverse metabolic
402 functions. **Amino acid** metabolism is especially prominent in the midgut, with pathways
403 focusing on arginine, glutamine, and glutamate (*Argk1*, *Gs1*, *Gs*, *Gdh*) enriched in EC-like,
404 cardia, and VM2 cells. Similarly, the fat body contributes to amino acid metabolism, primarily
405 in OE and FYCs. Notably, a tyrosine/dopamine synthetic gene (*ple*) is exclusive to the fat body,
406 predominantly in FYC. **Purine and pyrimidine** metabolisms show similar patterns between
407 both tissues. The midgut, especially EC-like2, CA and ISC/EB cells, exhibits high expression
408 of key purine genes (*Adk2*, *awd*, *AdSS*), while in the fat body elevated expression is notable
409 in TP2 and FYCs. Notably, intensive expression of nucleoside triphosphate regulatory gene
410 (*awd*) is observed across all fat body clusters. **Vitamin** metabolism exhibits diverse, cell-type
411 specific expression profiles. Nicotinate (*Naprt*), pantothenate (*fbl*), and riboflavin (*Acph-1*)

412 pathways, show varying levels of expression in both tissues, with specific enrichment in certain
413 cell types (EC, EC-like, CA, TP, OE and FYCs). Gene related to thiamine (*Alp4*, *Alp9*) are
414 mainly represented in diverse midgut clusters, while vitamin B6 (*Pdxk*) displays high
415 expression levels in cardia cells, TP2 and oenocytoids. Additionally, folate metabolism (*Pcd*)
416 is actively expressed in the fat body. Cytochrome **P450** metabolism, involved in xenobiotic
417 detoxification and associated with insecticide resistance⁸⁹, showcases distinct tissue-specific
418 and cell-type-specific expression patterns. Notably, two CYP4G family genes are prominently
419 expressed in fat body clusters, primarily OE. Meanwhile, the expression of *CYP9J26* and
420 *CYP6AH1* are enriched in midgut CA cluster, highlighting a clear tissue and cell tropism of
421 P450 enzyme activity. **Insect hormone biosynthesis** genes show high levels of expression
422 with distinct tissue specificity, particularly for two 4-nitrophenyl phosphatase genes, which
423 serve as precursors for juvenile hormone synthesis. Notably, no expression of genes directly
424 associated with ecdysone hormone synthesis are observed. However, ecdysone receptor
425 genes, such as *EcR* and *E75*, are broadly expressed at low levels in midgut EC and EC-like
426 cells, as well as in FYCs of the fat body, as previously illustrated (**Fig. 3F**).

427 Our findings portray the distinct and key roles played by the midgut and fat body,
428 highlighting their unique contributions to immune and metabolic functions after a bloodmeal.
429 The midgut, with enterocytes, cardia cells, and ISC/EB cells, mounts a focused immune
430 response while actively participating in metabolic processes. Conversely, the fat body and
431 associated cells exhibit a broader role in immunity and metabolism, with trophocytes,
432 oenocytes and fat body-yolk cells emerging as key players. Consistent with recent research
433 linking metabolic processes to immune cell functions, our findings suggest that metabolic
434 activity and immune responses are intertwined after blood ingestion in mosquitoes⁹⁰.

435

436 **A metabolomic analysis confirms the transcriptional landscape of midgut and fat body** 437 **tissues post bloodmeal.**

438

439 To further validate our findings, we carried out an extensive metabolomic analysis to
440 characterize physiological states and find metabolic specializations in the midgut and fat body
441 of blood-fed *Ae. aegypti* mosquitoes. In parallel with the scRNA-seq experiment, abdominal
442 tissues dedicated to metabolite extraction were dissected from mosquitoes belonging to the
443 same cohort at 2 and 7 dpbm. Ultra-high-performance liquid chromatography–high-resolution
444 mass spectrometry (UHPLC–HRMS) analysis of pooled midgut and pooled fat bodies (n=5)
445 generated a comprehensive metabolic profile encompassing 974 metabolites, subsequently
446 analyzed by Principal Component Analysis (PCA), (**Fig. 5A**). PCA validates clear tissue-
447 specific and temporal metabolic distinctions, with samples clustering separately based on
448 tissue type and days post bloodmeal (**Fig. 5B**). Differential metabolite abundance analysis

449 reveals distinct metabolic profiles between midgut and fat body tissues (**Fig. 5C**). At 2- and 7-
450 dpbm, 155 and 149 unique metabolites, respectively, differentiate the two tissues, highlighting
451 significant physiological divergence. Moreover, the midgut exhibits a more pronounced time-
452 dependent metabolic shift, with 222 differentially abundant metabolites between 2 and 7 dpbm,
453 compared to 63 in the fat body.

454 Pathway analysis reveals divergent metabolic pathway enrichment between tissues
455 across time (**Fig. 5D**). The fat body exhibits consistent enrichment in vitamins, including
456 sustained abundance in vitamin B6 (pyridoxal) and early abundance of riboflavin and
457 pantothenate. This enrichment highlights a potential fat body role, notably for FYC (**Fig. 4**), in
458 storing vitamins for subsequent utilization in metabolic processes as enzyme cofactors.
459 Additionally, a gene associated with vitamin B6 metabolism (*Pdxk*), shows expression across
460 tissues, including notable activity in EC4 and cardia cells within the midgut, as well as TP2 and
461 HC-O in the fat body. This pattern highlights the fat body's central role in supplying essential
462 vitamins to dependent tissues and cells. Nucleotide metabolites are enriched in the fat body
463 with an abundance shift observed from pyrimidine metabolism at 2 dpbm to purine metabolism
464 at 7 dpbm, accompanied by a significant increase in amino acid metabolism. While systemic
465 *awd* gene expression potentially supports nucleotide synthesis in the fat body at both time
466 points, the high levels of amino acid metabolites observed at 7 dpbm in this tissue may result
467 from increased expression in amino acid pathway genes in the midgut (**Fig.4**). In contrast, the
468 midgut shows enrichment in porphyrin metabolism early on, followed by ether lipid metabolism
469 at 7 dpbm.

470 We then identify notable differences in metabolite content between tissues, with the fat
471 body displaying a higher abundance across most classes compared to the midgut (**Fig. 5E**).
472 However, xanthurenate, a metabolite derived from the tryptophan/kynurenine pathway and
473 known for its antioxidant and heme-chelating properties in mosquito midgut after a
474 bloodmeal⁸⁸, is highly abundant in the midgut at 2 dpbm and subsequently shifted to the fat
475 body by 7 dpbm. In addition, the presence of heme exclusively in the midgut at 2 dpbm aligns
476 with the breakdown of blood cells during the early stages of digestion (**Fig. 5F**). The fat body's
477 role in vitamin storage is further underscored by the high levels of vitamin detected, with B6
478 being particularly abundant (**Fig. 5E-F**).

479 Additionally, carbohydrate-related compounds, notably sucrose, are enriched in the fat
480 body (**Fig. 5E-F**). This finding aligns with the expression of trehalose biosynthesis genes in fat
481 body clusters (**Fig. 4**), given that trehalose synthesis requires sucrose as a substrate. Amino
482 acids and nucleobase metabolites are slightly more abundant in the fat body (**Fig. 5E**). For
483 instance, L-Glutamine, pivotal in metabolic and neuronal pathways, illustrates similar
484 abundance in both tissues (**Fig. 5F**). This observation confirms that amino acid metabolism is
485 as actively engaged in the midgut as it is in the fat body (**Fig. 4**). Levels of carboxylic acid and

486 fatty acid show no significant differences in abundance between the midgut and fat body (**Fig.**
487 **5E**), suggesting that both tissues maintain comparable levels of these metabolites. Palmitic
488 acid, a prevalent saturated fatty acid, is present at similar levels across tissues (**Fig. 5F**),
489 despite fatty acid synthases being predominantly expressed in the fat body (**Fig. 4**), pointing
490 to lipid exchanges between compartments. As for membrane lipids, the midgut is enriched in
491 ceramide and phosphatidic acid (PA), a precursor for multiple lipid classes. (**Fig. 5E**). Notably,
492 phospholipids with specific head groups characteristic of cell membranes (PC, PE, PI, PS),
493 are significantly less abundant at 2 dpbm in the midgut, exemplified by phospholipid
494 PE(18:1/16:1), (**Fig. 5F**). It suggests that bloodmeal digestion may have a profound impact on
495 phospholipid remodeling within the midgut, a process known to intensify when the blood
496 contains dengue virus⁹¹.

497 This comprehensive metabolomic analysis validates the distinct physiological states
498 and specialized metabolic functions observed by scRNAseq on the midgut and the fat body of
499 blood-fed *Ae. aegypti*, illustrating the specific metabolic pathways operational following a
500 bloodmeal.

501

502 **Expression of insect-specific viruses**

503

504 *Ae. aegypti* mosquitoes host a variety of microbial and viral communities, including
505 several insect-specific viruses (ISVs)⁹². In our study, we aimed to detect the presence of well-
506 described ISVs such as Phasi Charoen-like virus (PCLV), Humaita-Tubiaca virus (HTV),
507 and Cell-fusing agent virus (CFAV) in midgut and fat body cells. We quantitatively assessed
508 the percentage of cells harboring viral RNA (**Fig. 6A**).

509 At 2 dpbm, PCLV transcripts are detectable in less than 2% of cells in both tissues. By
510 7 dpbm, however, 14% of midgut cells and 8% of fat body cells contain detectable levels of
511 PCLV RNA, indicating a significant shift in infection, particularly in the midgut. Conversely,
512 CFAV presence is consistently low, detected in less than 0.4% of cells across both time points
513 and tissues, while HTV was absent in all samples (**Fig. 6B**). These data highlight insect-
514 specific viral replication dynamics within mosquitoes, with an increase in PCLV viral replication
515 following a bloodmeal, as previously reported in arbovirus-infected mosquitoes⁹³.

516 Specifically, PCLV expression is slightly enriched in midgut EC-like2 cells and
517 predominantly in fat body oenocytes (OE, OE-like) by 7 dpbm (**Fig. 6B-C**), and in a lesser
518 extent in FYC4, MU and EP clusters. To explore how PCLV infection influences cellular
519 function, we analyze gene expression differences between PCLV-positive and PCLV-negative
520 cells at 7 dpbm (**Fig. 6D**). PCLV-infected midgut cells are enriched in genes associated with
521 amino acid synthesis, oxidative respiration, and stress response (e.g., *serine-pyruvate*
522 *aminotransferase*, *GST*, *NADH dehydrogenase*), indicative of EC-like2 activity. In the fat body,

523 PCLV-positive cells upregulate lipid biosynthesis (*SCD*, *elongase*) and cytochrome P450
524 pathways (*CYP4G15*), which are characteristic of oenocytes. We then delve into the distinct
525 transcriptional responses in the most infected cell cluster of each tissue (**Fig. 6E**). EC-like2
526 PCLV-positive cells display increased expression of genes associated in proteolysis
527 modulation (*trypsin-eta*, *Cys*), ATP synthesis (*Mpcp2*), cellular homeostasis (*CREG*) and
528 hormone synthesis (*Antdh*), supporting enhanced metabolic processes and energy production
529 for viral replication. Meanwhile, PCLV-positive oenocytes (pooled Oe and Oe-like clusters),
530 display a reduction in ribosomal protein expression (*RpS21*) and mRNA translation (*pAbp*)
531 coupled with a mild upregulation of immune-associated serine proteases (*CLIPC3*) and
532 mitochondrial metabolism (*MPPA*). These changes may constitute counter-defense
533 mechanisms against viral invasion.

534 These results highlight the complex and specific responses of mosquito cells to
535 endogenous virus infection, with a particular focus on PCLV in the midgut at 7 dpbm. The
536 presence of PCLV prompts highly infected cells to display subtle transcriptional changes that
537 indicate an active response to infection.

538

539 **DISCUSSION**

540

541 Leveraging the power of single-cell transcriptomics and untargeted metabolomics led us to
542 comprehensively characterize the tissular and cellular responses of the midgut and abdominal
543 fat body of *Ae. aegypti* mosquitoes to a bloodmeal. Our cell atlases, including the first fat body
544 cell atlas in arthropods, are available for exploration and analysis on an interactive online
545 platform (<https://aedes-cell-atlas.pasteur.cloud>).

546 Our study reports 14,447 transcriptomes of individual mosquito midgut cells. We
547 identify new marker genes for several cell populations, including enterocytes, enteroendocrine
548 cells, intestinal stem cells/enteroblasts, cardia, visceral muscle cells, and associated
549 hemocytes. While previous *Ae. aegypti* mosquito single-cell midgut studies focused on the
550 effects of food (blood or sugar) or Zika virus infection, our analysis reveals the temporal
551 transcriptional shifts in midgut cell populations at 2 and 7 days after the bloodmeal intake and
552 digestion^{15–17}. We performed a comparative analysis (**Fig. S2**) of all published data revealing
553 an overall consistency in midgut cell type proportions and markers with other *Aedes* cell
554 atlases and the reference *Drosophila* gut atlas^{15–17,24}. Importantly, both single-nucleus RNA-
555 Seq and scRNA-Seq yielded highly concordant results on midgut cells, suggesting that these
556 approaches may be considered equivalent for future investigations¹⁵. Furthermore, marker
557 genes identified in our study to associate with midgut cell clusters such as enterocytes (ECs),
558 enteroendocrine cells (EE) and cardia cells (CA) aligned well with previously described bulk
559 transcriptomic signatures of *Ae. aegypti* gut regions³⁰. Together, the range of physiological

560 states and the use of different *Ae. aegypti* strains (laboratory and field-collected) enrich our
561 understanding of mosquito midgut cell composition while reinforcing the consistency of marker
562 genes, cell types, and their proportions. However, accurately comparing cell numbers across
563 conditions in single-cell studies is challenging, highlighting the need for improved validation
564 methods in differential abundance analysis⁹⁴.

565 Our study also provides the first fat body cell atlas published for arthropods,
566 encompassing 27,726 individual transcriptomes. Our analysis uncovers a surprising level of
567 cellular heterogeneity within the fat body, identifying well-known populations such as
568 trophocytes and oenocytes, alongside neuroendocrine cells, muscle cells, tracheoblast-like
569 cells, and epidermal cells^{18,42,74,95–98}. We confirm the anticipated roles of trophocytes and
570 oenocytes in vitellogenesis and metabolism, with trophocytes involved in hemolymph sugar
571 synthesis and oenocytes in lipid biosynthesis. Additionally, our results support the proposed
572 role of trophocytes in modulating antimicrobial humoral immunity, a critical fat body function
573 observed as early as two days after the bloodmeal, likely influenced by the gut
574 microbiome^{43,46,99}. These observations also illustrate the tight links between metabolism and
575 immunity, and their interplay with blood digestion mechanism.

576 Importantly, our analysis reveals previously unknown cell population in the fat body,
577 that we called fat body-yolk cells. The gene expression profiles of fat body-yolk cells were
578 reminiscent with those of ovarian cells^{30,60,100,101}, that were however specifically removed during
579 organ dissection. The significant and consistent abundance of fat body-yolk cells (18% of all
580 cells at 2 and 7 dpbm) also discredits the hypothesis that fat body-yolk cells are simply residual
581 ovarian cells. Rather, it suggests that fat body-yolk cells are inherent components of the
582 abdominal fat body and play a critical role, possibly in close association with ovarian functions,
583 in egg development. Specific fat body-yolk cells subpopulations (FYC2-3-4) express stem cell
584 markers and showed enrichment of piRNA genes, similar to a germarium-like profile, which is
585 essential for initiating egg chamber development^{101,102}. Given the lack of oviposition sites
586 during our experiment and the consistent presence of these cells over time, fat body-yolk cells
587 might be instrumental in preserving egg integrity within mosquito females carrying mature
588 eggs⁶⁹. This finding challenges our current view of the fat body's role in mosquito fertility.

589 The discovery and annotation of dozens of marker genes for mosquito cell population
590 opens possibility for genetic tool development, such a tissue- and cell-specific drivers. Widely
591 available in the model organism *D. melanogaster*, specific drivers combined with expression
592 of fluorophores, apoptotic genes, or short-hairpin RNAs enable in-depth functional studies and
593 elucidation of fundamental biological processes¹⁰³. In mosquitoes, they might also lead to the
594 development of the next generation of vector control tools, targeting only subsets of cells that
595 are critical to pathogen transmission, and enable pathogen blockage with exquisite specificity,
596 at each key step of the mosquito infectious cycle.

597 Our study constitutes a foundation for short- to long-term future investigations, aiming
598 to validate the presence of distinct cell populations, such as fat body-yolk cells, using RNA *in*
599 *situ* hybridization techniques or spatial transcriptomics. Thus, cell populations and states could
600 be placed in the context of tissular architecture and a wider range of cellular interactions^{104,105}.
601 Such work could also provide a comprehensive view of the three-dimensional organization of
602 mosquito abdominal tissues, shedding light on potential inter-organ interactions and metabolic
603 gradients^{106,107}. Recent advances in single-cell metabolomics also hold great promise for
604 complementing single-cell transcriptomics data sets through integrated multi-omics
605 approaches¹⁰⁸.

606 Hitherto, only one study has described the impact of an arbovirus infection, namely Zika
607 virus, on mosquitoes at the single-cell level, and only on midguts 4 days post virus exposure.
608 Building a comprehensive dataset, recapitulating the trajectory of a virus through a mosquito's
609 body, organ by organ, at the single-cell level would provide critical insights into the cellular
610 mechanisms of pathogen susceptibility and transmission¹⁷. It could also shed light on the
611 differential susceptibility and vectorial capacity of certain mosquito strains over others,
612 informing spread of arboviruses and epidemiology. Moreover, in the context of the release of
613 lab-modified mosquitoes carrying *Wolbachia*, an intracellular bacteria with virus-blocking
614 properties in several regions of the globe, elucidating the fundamental processes underlying
615 virus blockage at the single-cell level could provide insights into the heterogeneous efficiency
616 rates observed on the field¹⁰⁹.

617 Finally, enhancing our understanding of mosquito physiology at the cellular level,
618 particularly in the fat body, is important for unraveling the mechanisms of insecticide
619 resistance. Our study reveals that *Ae. aegypti* oenocytes robustly express key cytochrome
620 P450 genes, essential for metabolic resistance to insecticides, aligning with observations in
621 *Anopheles* and *Drosophila* that underscore the universal detoxification role of these cells¹¹⁰.
622 Elucidating tissue-specific expression pattern of key insecticide resistance gene could lead to
623 better identification and characterization of insecticide-resistant mosquito strains and
624 development of counter strategies. Also, intriguing links between insecticide resistance and
625 vector competence could be explored at the cellular and molecular level¹¹¹.

626 In sum, the study of arthropod vectors and their interactions with symbionts,
627 microbiomes and human pathogens at a single-cell resolution will hopefully generate deeper
628 fundamental understanding of those complex interactions and pave the way towards the
629 development of innovative disease control strategies.

630 **METHODS**

631

632 **Mosquito rearing**

633 Experiments were conducted with *Ae. aegypti* mosquitoes derived from a wild-type colony
634 originating from Kumasi, Ghana, predominantly comprised of *Aedes aegypti formosus*, with a
635 minor component of admixed ancestry from *Aedes aegypti aegypti*^{19,20}. Mosquitoes were
636 reared under controlled conditions (28°C, 12-hour light/12-hour dark cycle and 70% relative
637 humidity). The mosquito colony was maintained by feeding female mosquitoes on rabbit blood
638 (BCL), to ensure their survival, laying and egg collection. Prior to performing the experiments,
639 their eggs were hatched synchronously in a SpeedVac vacuum device (Thermo Fisher
640 Scientific) for 45 minutes. Their larvae were reared in plastic trays containing 1.5 L of tap water
641 and supplemented with Tetramin (Tetra) fish food at a density of 200 larvae per tray. After
642 emergence, adults were kept in BugDorm-1 insect cages (BugDorm) with permanent access
643 to 10% sucrose solution.

644

645 **Mosquito bloodmeal**

646 Five- to 7-day-old female mosquitoes were deprived of 10% sucrose solution 18-24 hours
647 before exposure to an artificial bloodmeal consisting of a 2:1 mix of washed rabbit erythrocytes
648 (BCL) supplemented with 10 mM adenosine triphosphate (Sigma) and Leibovitz's L-15
649 medium (Gibco) supplemented with supplemented with 0.1% penicillin/streptomycin
650 (pen/strep; Gibco ThermoFisher Scientific), 2% tryptose phosphate broth (TBP; Gibco Thermo
651 Fischer Scientific), 1× non-essential amino acids (NEAA; Life Technologies) and 10% fetal
652 bovine serum (FBS; Life Technologies). Mosquitoes were exposed to the bloodmeal for 15
653 min through a desalted pig-intestine membrane using an artificial feeder (Hemotek Ltd) set at
654 37°C. Fully engorged females were placed into small containers and maintained for 7 days at
655 28°C, 70% relative humidity and under a 12-hour light-dark cycle with permanent access to
656 10% sucrose. To meet biosafety requirements, containers did not provide space for
657 oviposition, effectively preventing egg-laying.

658

659 **Dissection of midgut and fat body**

660 Midgut dissection consisted of isolating the digestive track from the mosquito's body by
661 gripping the second to last abdominal segment using a forceps and pulling it out. Only the
662 midgut, devoid of the crop, foregut, hindgut and Malpighian tubules was kept for analysis. Fat
663 body dissection consisted of collecting the dorsal and ventral abdomen to which the fat body
664 lobes are attached. Crop, Malpighian tubules, hindgut and ovaries were removed if needed.

665

666

667 **Single-cell dissociation of midgut and fat body**

668 A cold dissociation method was used for the mosquito midgut and fat body, adapting protocols
669 originally developed for dissociating *Anopheles sp.* midgut into single-cell suspensions suitable
670 for scRNAseq²². Mosquitoes were dissected on ice and pools of 7 organs collected in a drop
671 of ice-cold SF900 III media (Sigma-Aldrich). Each organ was sectioned into 3 smaller pieces
672 using spring scissors (Fine science tools). Sectioned organs were transferred immediately to
673 a chambered coverglass well (Thermo scientific) containing 500ul of dissociation media
674 (SF900 III media supplemented with 10mg/ml Bacillus licheniformis protease (Sigma-Aldrich)
675 and 25 unit/ml DNaseI (Sigma)) for 15 minutes in the dark on ice. Tissues were then gently
676 triturated using 15 back-and-forth pipetting motions. After 5 minutes, 100uL of supernatant
677 containing dissociated cells was collected and transferred to a collection tube containing 45
678 mL of SF900 III media on ice. The dissociation well was supplemented with 100uL of fresh
679 dissociation media and incubated for 10 min in the dark on ice. Tissues were triturated again,
680 and 100uL of supernatant transferred to the collection tube. Three to four more dissociation
681 rounds were performed until only single cells remained. The collection tube was then spun at
682 300g for 15 min at 4°C. The pellet was resuspended gently in 900 ul ice-cold SF900 II media
683 and filtered through Flowmi cell strainers (Sigma) with a 40 um porosity for midguts and 70 um
684 porosity for fat bodies. The resulting cell suspension was spun at 300 g for 5 min at 4°C and
685 resuspended in 30 ul of PBS 1x-BSA 0,04%. Cell concentration was determined using a Kova
686 slide, and final concentration adjusted to 700-1000 cells per microliter.

687

688 **Single-cell gene expression assay and sequencing**

689 Single-cell RNA sequencing was performed using the Chromium Next GEM Single Cell 5' Kit
690 v2 dual index (10x Genomics) according to the manufacturer's protocol. Following a protocol
691 developed in the context of a pilot study, a primer specific to the dengue virus envelope
692 sequence was added at a concentration of 1uM, (Sequence 5'-3':
693 AAGCAGTGGTATCAACGCAGAGTACGAACCTGTTGATTCAACAGC). The targeted
694 recovery rate was 10,000 cells per sample. Sample quality control was performed at Omics
695 core facility at Institut Pasteur using Bioanalyzer DNA1000 high sensitivity (Agilent) and Qubit
696 4 Fluorometer (Thermo Fisher Scientific) assays. Libraries were sequenced by Macrogen on
697 a Novaseq 6000 platform (Illumina).

698

699 **Reference generation and sample processing with STARsolo.**

700 As a reference genome for *Aedes aegypti*, we use the AaegL5 genome from Vectorbase (NCBI
701 GenBank assembly id: GCA_002204515.1
702 https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_002204515.2/). The fastas and the gtf's
703 from *Aedes aegypti* were merged in one fasta file and one gtf respectively. The merged fasta

704 and gtf were used by STAR to generate the index files with the command¹¹². The index files
705 were generated with the command STAR --runMode genomeGenerate using the gtf file as
706 sjdbGTFfile and the fasta file as genomeFastaFiles. All sequencing data were processed and
707 mapped to the *Ae. aegypti* with STAR (version 2.7.9a) using the following parameters: --
708 soloType CB_UMI_Simple --soloBarcodeMate 1 --clip5pNbases 42 0 --soloCBstart 1 --
709 soloCBlen 16 --soloUMIstart 17 --soloUMIlen 10 --outFilterMultimapNmax 50 --
710 outFilterScoreMinOverLread 0.4 --outFilterMatchNminOverLread 0.4 --outBAMcompression
711 10 --soloMultiMappers EM --soloStrand Forward.

712

713 **Single-cell quality control and cell filtering**

714 STARsolo raw output files were individually read into RStudio (R version 4.3.2 from 2023-10-
715 31) and processed using Seurat (Seurat v5.0.1) and SingleCellExperiment (version 1.24.0).
716 Samples were converted to Seurat objects and SingleCellExperiment objects. A first filter was
717 applied by using the DropletUtils::barcodeRanks function (DropletUtils version 1.22.0): the
718 knee point of the barcoderank was used as a cutoff to remove empty and /or bad quality
719 droplets with a total UMI number under this value (**Table S6**). After this first filter, we obtained
720 for samples Midgut-2dpbm, Midgut-7dpbm, Fatbody-2dpbm and Fatbody-7dpbm respectively
721 33,832, 49,826, 46,158 and 48,205 cells that is at least twice as much as the number of cells
722 targeted (20000 cells). Samples were converted to Seurat objects. Mitochondrial gene
723 percentage was calculated for each cell. Features were log normalized, variable features were
724 identified, the top 2,000 variable features were scaled, a principal component (PC) analysis
725 dimensionality reduction was run. Nearest neighbors were computed, and appropriate
726 clustering granularity was determined with Clustree (v0.5.1). Uniform Manifold Approximation
727 and Projection (UMAP) dimensional reduction was performed, and clusters were visualized
728 with UMAP reduction. In each sample, a second filter for cells was applied by removing cells
729 contained in clusters 0 and 1, as these clusters have, in all samples, few to no significant
730 marker genes and/or mitochondrial genes as genes markers (**Table S6**).

731 We also applied the following criteria to exclude poor quality cells and potential doublets or
732 multiplets^{15,24}, which can display aberrantly high gene counts and do not represent true single
733 cells:

- 734 - Cells were retained if they had more than 100 detected genes.
- 735 - For midgut samples, cells with fewer than 2,400 detected genes were kept, based on the
736 99th percentile distribution.
- 737 - For fat body samples, cells with fewer than 5,300 detected genes were kept, following the
738 same percentile-based exclusion.

739 - Mitochondrial genes were filtered with the following criteria: cells with more than 5%
740 mitochondrial genes were excluded.

741 These metrics ensured a cell complexity greater than 0.75, defined as the $\log_{10}(\text{genes}$
742 $\text{detected})$ divided by the $\log_{10}(\text{UMI counts per cell})$, and no cells retained for further analysis
743 contained fewer than 400 total reads.

744

745 **Seurat workflow**

746 Raw counts for cells passing all our filters were loaded into a Seurat object. For midgut and fat
747 body, both samples at day 2 and day 7 were merged (Seurat::merge). Samples from the same
748 tissue were integrated, following the integration tutorial proposed by Seurat authors
749 (https://satijalab.org/seurat/articles/seurat5_integration). Features were log normalized, the
750 top 2000 variable features were identified. All variable features were scaled and a principal
751 component (PC) analysis dimensionality reduction was run. We integrated both samples (day
752 2 and day 7) together for midgut and fat body tissues using the CCAIntegration method from
753 Seurat. After integration, nearest neighbors were computed, and appropriate clustering
754 granularity was determined with Clustree (res 0.5 and 0.3 for midgut and fat body respectively).
755 Uniform Manifold Approximation and Projection (UMAP) dimensional reduction was performed
756 (parameters: n.neighbors=100, min.dist = 0.3), and clusters were visualized with UMAP
757 reduction.

758

759 **Metabolite extraction**

760 Mosquitoes were dissected in PBS 1X, and tissues collected in pools of 5 in tubes containing
761 500 μL of ice-cold methanol:water (80:20) and ~20 1-mm glass beads (BioSpec). Samples were
762 homogenized for 30 sec at 6,000 revolutions per minute (rpm) in a Precellys 24 grinder (Bertin
763 Technologies), and sonicated in an ultrasonic bath (J.R. Selecta) for 15 min at 4 °C.
764 Homogenates were centrifuged at 10,000 rpm for 1 min at 4 °C and 400 μL of supernatant
765 collected. Remaining pellets were resuspended in 500 μL of methanol:water (80:20) solution.
766 After another round of sonication and centrifugation, 400 μL of supernatant was collected.
767 Combined supernatants were vacuum-dried using Speed-Vac for 4 hours (Thermo Fisher
768 Scientific) and stored at -20 °C. Four to six biological replicates consisting of pools of 5 tissues
769 were prepared per condition.

770

771 **Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Metabolic** 772 **Profiling.**

773 Dry extracts were resuspended at 2 mg/mL in an 80:20 methanol:water solution. Ultra-high-
774 performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS)
775 analyses were performed on a Q Exactive Plus quadrupole (Orbitrap) mass spectrometer,

776 equipped with a heated electrospray probe (HESI II) coupled to a U-HPLC Vanquish H
777 (Thermo Fisher Scientific, Hemel Hempstead, U.K.). Hilic separation mode was done using a
778 Poroshell 120 Hilic-Z (2.7 μ m, 150 \times 2.1 mm, Agilent, Santa Clara, USA) equipped with a guard
779 column. The mobile phase A (MPA) was water with 0.1% formic acid (FA) and 10 mM
780 ammonium formate, and the mobile phase B (MPB) was acetonitrile with 0.1% FA and 10 mM
781 ammonium formate. The solvent gradient was 10% MPA (0 – 0.5 min), 10% MPA to 50% MPA
782 (0.5 - 9 min), 50% MPA (9 - 11 min), 10% MPA (11.1 - 20 min). The flow rate was 0.3 mL/min,
783 the column temperature was set to 40 °C, autosampler temperature was set to 5 °C, and
784 injection volume fixed to 1 μ L. Mass detection was performed in positive and negative
785 ionization (PI and NI) mode at resolution 35 000 power [full width at half-maximum (fwhm) at
786 400 m/z] for MS1 and 17 500 for MS2 with an automatic gain control (AGC) target of 1×10^6
787 for full scan MS1 and 1×10^5 for MS2. Ionization spray voltages were set to 3.5 kV for PI and
788 2,6 kV for NI, and the capillary temperature was kept at 256 °C. The mass scanning range was
789 m/z 100–1500. Each full MS scan was followed by data-dependent acquisition of MS/MS
790 spectra for the four most intense ions using stepped normalized collision energy of 20, 40, and
791 60 eV.

792

793 **Metabolomic data processing**

794 UHPLC-HRMS raw data were processed with MS-DIAL version 5.1¹¹³ for mass signal
795 extraction between 100 and 1500 Da from 0.5 to 12 min. Respectively MS1 and MS2 tolerance
796 were set to 0.01 and 0.05 Da in centroid mode. The optimized detection threshold was set to
797 1×10^5 concerning MS1 and 10 for MS2. Peaks were aligned on a quality control (QC)
798 reference file with a retention time tolerance of 0.1 min and a mass tolerance of 0.015 Da. All
799 MS-CleanR filters were applied with RSD tolerance of 50% and blank suppression ratio of
800 0.8¹¹⁴. Peak annotation was performed with in-house database (level 1) and FragHub database
801 (level 2) and finally using from MS-FINDER internal DB (level 3)^{115,116}.

802

803 **Insect-specific virus detection**

804 To get the ISV count, we performed a second run of read alignment using STAR (solo mode),
805 counting reads on both strands (--soloStrand Unstranded) on a reference genome containing:
806 - AaegL5 genome (NCBI GenBank assembly id: GCA_002204515.1 -
807 https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_002204515.2/)
808 - CFAV (Cell fusing agent virus) strain Galveston, complete genome
809 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000862225.3/ GenBank assembly
810 id : GCA_000862225.1)

811 - HTV (Humaita-Tubiacanga virus) strain Ab-AAF complete genome (two segments)
812 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_031535205.1/ - GenBank assembly
813 id GCA_031535205.1)

814 - PCLV (Phasi Charoen-like virus) isolate Rio, complete genome (three segments)
815 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_002814835.1/ - GenBank assembly
816 id : GCA_002814835.1)

817 ISV counts were added as metadata for fat body and midgut cells. These counts were not
818 considered for normalization, scaling, dimensional reduction or clustering steps.

819

820 **Statistical analysis**

821 Statistical analyses were performed in GraphPad Prism version 10.2.2. Difference in cell
822 clusters proportions and ISV-infected cell proportions were measured by chi-squared test.
823 Difference in metabolite intensity were measured by 2-way ANOVA and multiple comparison
824 test. Metabolomic dataset was analyzed using MetaboAnalyst 6.0 (www.metaboanalyst.org)¹¹⁷
825 and data were normalized by log transformation and auto scaling, for principal component
826 analysis (PCA). Venn diagram were produced using InteractiVenn¹¹⁸, based on significant
827 metabolites (fold changes >2, DFR adjusted p-value <0,05). Pathway analysis was based on
828 well-annotated HMDB compounds for global test enrichment on *Aedes aegypti* (yellow fever
829 mosquito) (KEGG).

830

831 **Data availability**

832 The scRNAseq data discussed in this publication have been deposited in NCBI's Gene
833 Expression Omnibus and are accessible through GEO Series accession number available
834 upon request. The metabolomic data discussed in this publication have been deposited in
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836

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855 **AUTHOR CONTRIBUTIONS**

856 Conceptualization: T.V., H.L.M., E.C., S.P., V.H., J.A., M.L., G.M. S.H.M.
857 Investigation: T.V., H.L.M., E.C, S.H.M.
858 Data curation: T.V, H.L.M.
859 Formal analysis: T.V., H.L.M., S.H.M.
860 Visualization: T.V., H.L.M., S.H.M.
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862 Writing – review and editing: T.V., H.L.M., E.C., S.P., J.A., M.L., G.M. S.H.M.
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864 **Declaration of interests**

865 The authors declare no competing interests.

866

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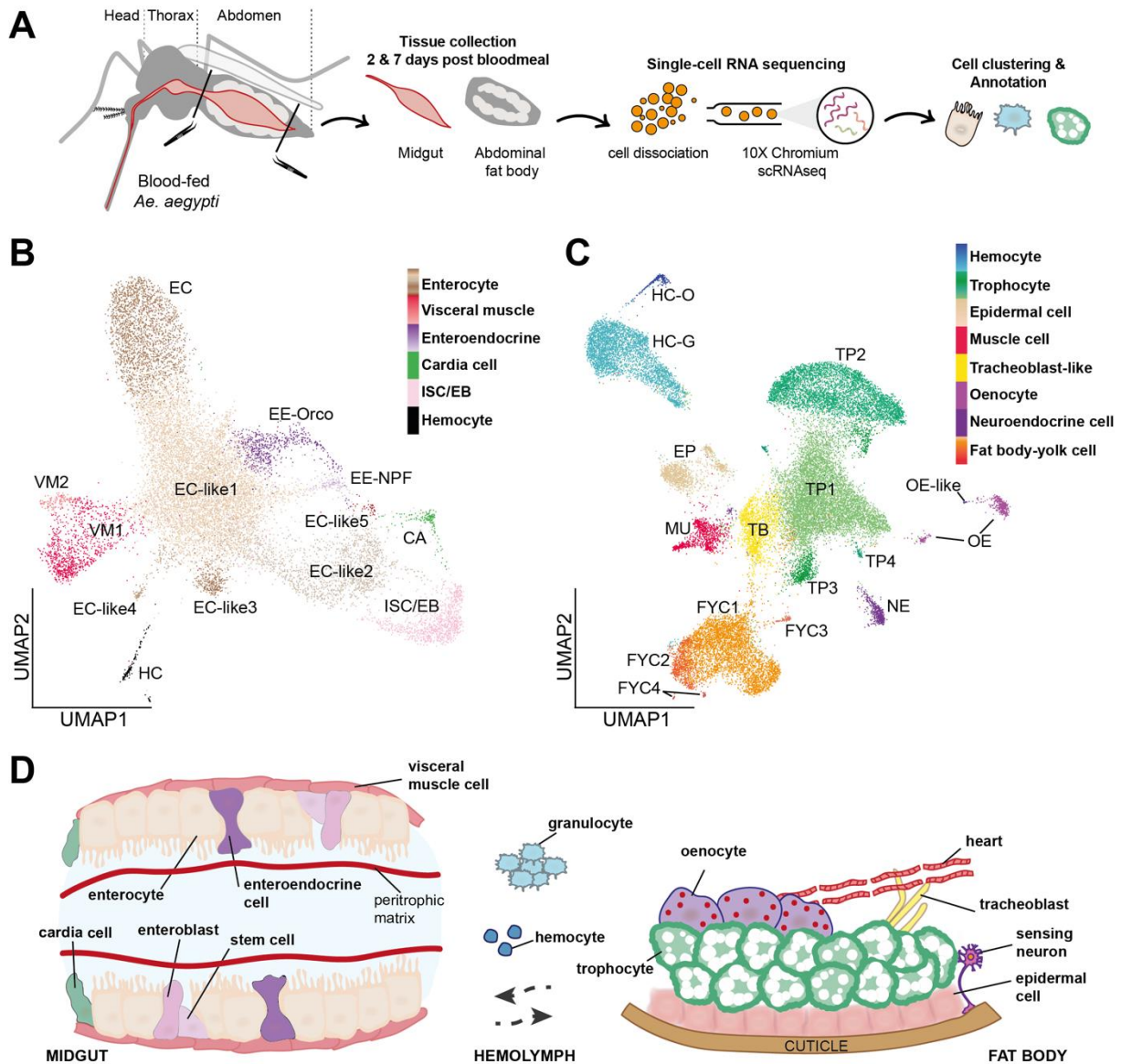
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1278 **FIGURES AND LEGENDS**

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1282 **Figure 1. Cellular landscape of *Ae. aegypti* abdominal tissues after a bloodmeal.**

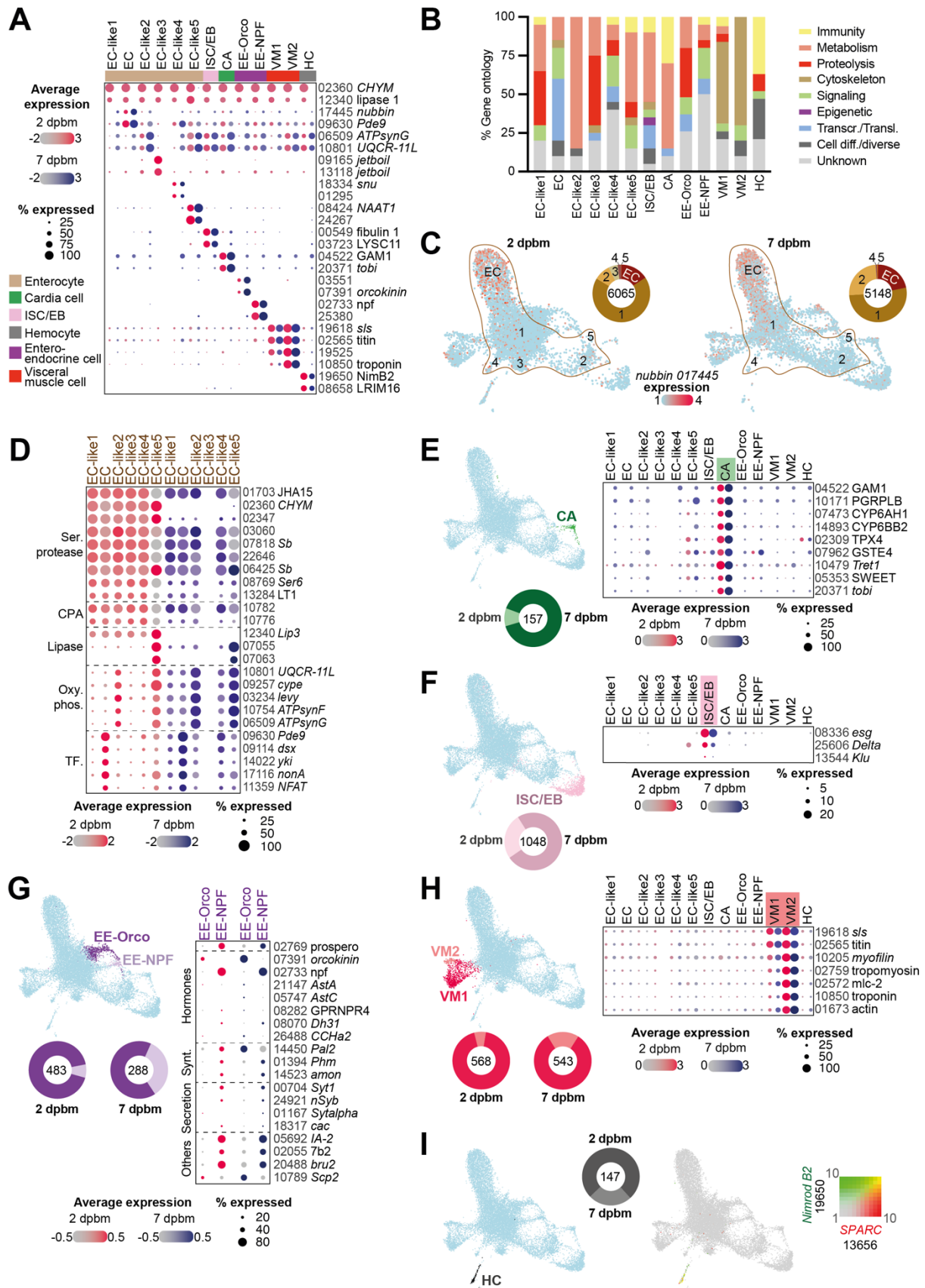
1283 (A) Schematic representation of the experimental workflow. Female *Ae. aegypti* mosquitoes
 1284 were fed a bloodmeal, and pools of 7 midgut and abdominal fat body tissues were collected at
 1285 2 and 7 days post bloodmeal (dpbm), for subsequent cell isolation and single-cell RNA
 1286 sequencing (scRNAseq).

1287 (B and C) Integrated UMAP (Uniform Manifold Approximation and Projection) visualization of
 1288 scRNAseq data from the midgut (B) and fat body (C) at 2 dpbm and 7 dpbm. Each dot
 1289 represents a single cell, colored according to its assigned cell cluster. EC: enterocytes;
 1290 ISC/EB: intestinal stem cells/enteroblasts; EE: enteroendocrine cells; VM: visceral muscle
 1291 cells; HC: hemocytes; CA: cardia cells; TP: trophocytes; FYC: fat body-yolk cell; HC-G:
 1292 hemocytes-granulocytes; HC-O: hemocytes-oenocytoids; TB: tracheoblast-like; EP: epidermal
 1293 cells; MU: muscle cells; NE: neuroendocrine cell; OE: oenocytes.

1294 (D) Schematic representation of cell types that compose mosquito midgut and fat body
 1295 associated tissues.

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Figure 2. Dynamic midgut cell composition after a bloodmeal.

(A) Expression of top 2 marker genes for each midgut cell cluster, based on integrated samples collected at 2 and 7 dpbm (Supplementary file 2).

1302 **(B)** Gene ontology of top 20 gene markers for each midgut cell cluster. The gene ontology
1303 categories were manually curated based on *Aedes aegypti* GO terms, GO terms of *Drosophila*
1304 or *Anopheles* orthologs, and annotations of immune-related and digestive-related genes from
1305 Hixson *et al*^{β0} (Supplementary file 4). Transcr: transcription; Transl: translation; Cell diff: cell
1306 differentiation.

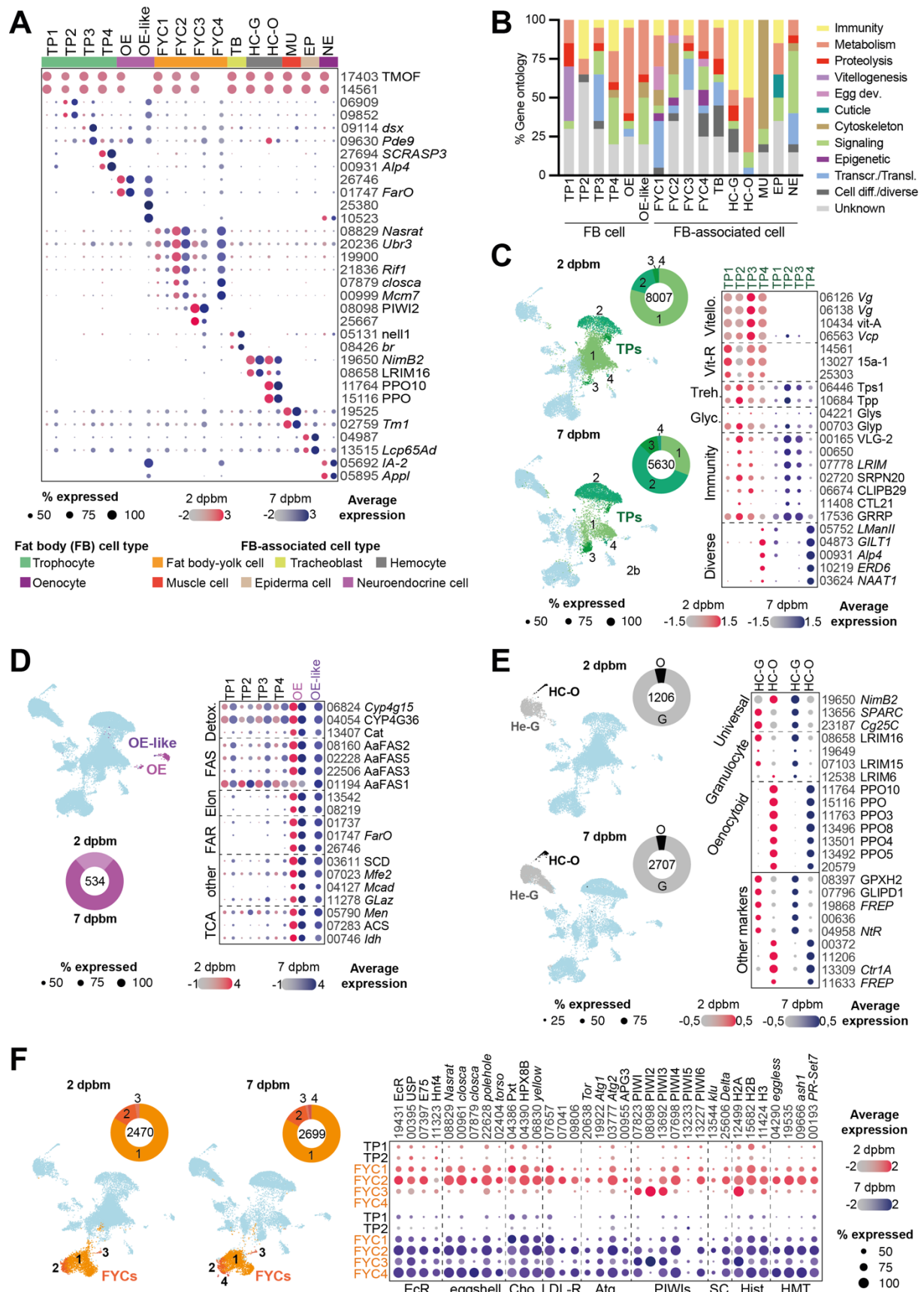
1307 **(C)** Expression of the *nubbin* (AAEL017445) gene marker in enterocytes (EC) and EC-like
1308 clusters at 2 and 7 dpbm, displayed by feature plot. The EC clusters are outlined by a brown
1309 line and EC proportion are shown by part of whole plot with total number of cells.

1310 **(D)** Dot plot showing the expression patterns of EC and EC-like gene group markers within
1311 each cluster at 2 and 7 dpbm. Ser: serine; CPA: carboxypeptidase; Oxy. Phos: oxidative
1312 phosphorylation; TF: transcription factor.

1313 **(E-H)** Specific cell clusters visualized on UMAP associated with their cell proportions by part
1314 of whole plot and gene markers representative of **(E)** cardia cells (CA), **(F)** intestinal stem cells
1315 and enteroblasts (ISC/EB), **(G)** enteroendocrine cells (EE) and **(H)** visceral muscle cells (VM).

1316 **(I)** Hemocyte cluster (HC) visualized by UMAP and co-expression of *Nimrod B2* (AAEL019650)
1317 and SPARC (AAEL013656) as universal hemocyte gene markers.

1318 Dot color shows relative average expression intensity for each gene at 2 dpbm (red) and 7
1319 dpbm (blue). Dot size reflects the percentage of cells expressing corresponding genes in each
1320 cell cluster. Each gene is identified by the AAEL0 truncated Vectorbase IDs and their
1321 respective abbreviation if available, not italicized. *Drosophila* or *Anopheles* ortholog
1322 abbreviations are indicated in italic.

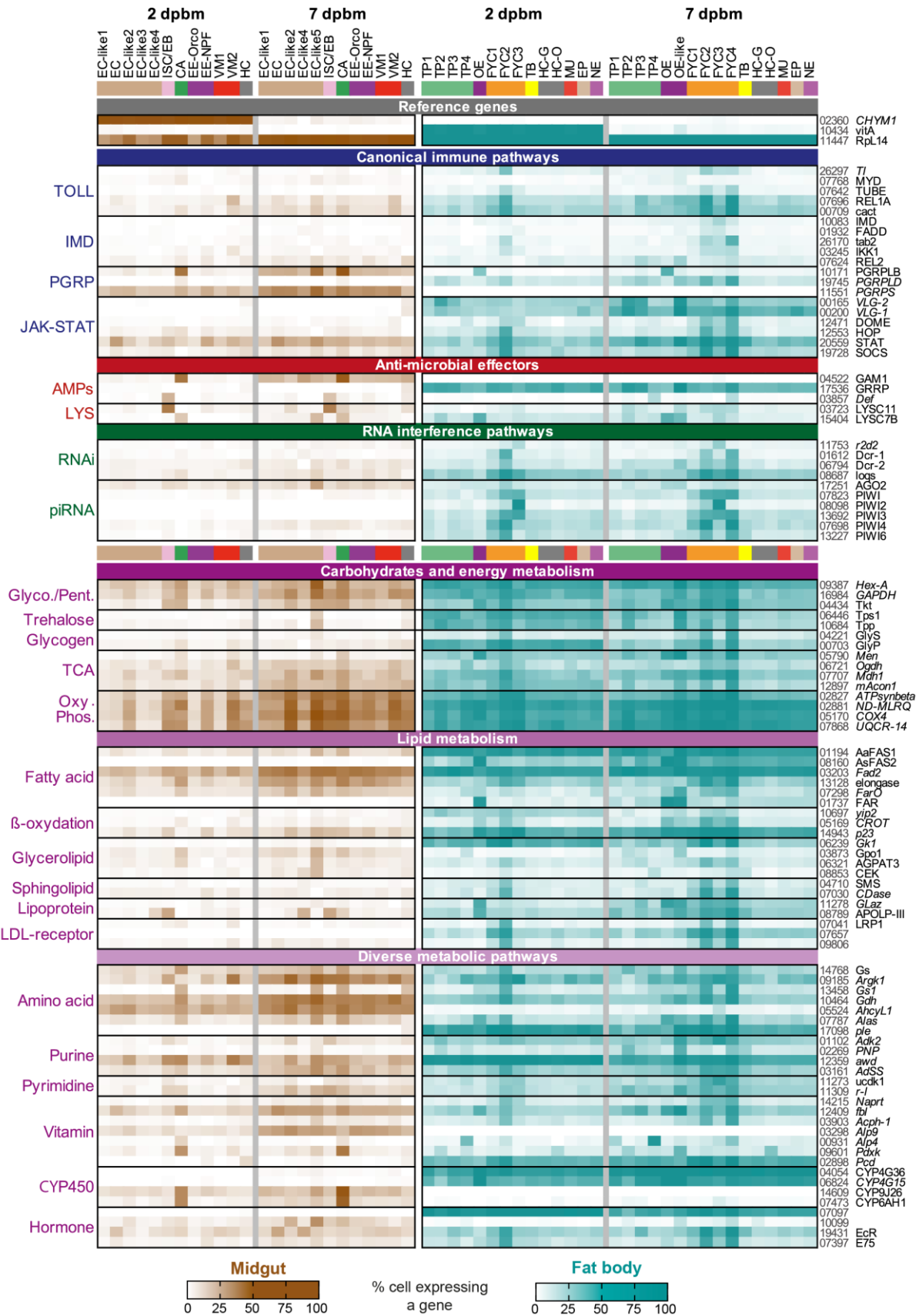


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1327 **(B)** Gene ontology of top 20 gene markers for each cell cluster. The gene ontology categories
1328 were manually curated based on *Aedes aegypti* GO terms, GO terms of *Drosophila* or
1329 *Anopheles* orthologs, and annotations of immune-related and digestive-related genes from
1330 Hixson *et al* (**Supplementary file 4**). Egg dev: egg development; Transcr: transcription;
1331 Transl: translation; Cell diff: cell differentiation.

1332 **(C-F)** Specific cell clusters visualized on UMAP associated with their cell proportions by part
1333 of whole plot and gene markers representative of **(C)** trophocytes, **(D)** oenocytes **(E)**
1334 hemocytes and **(F)** fat body-yolk cell.

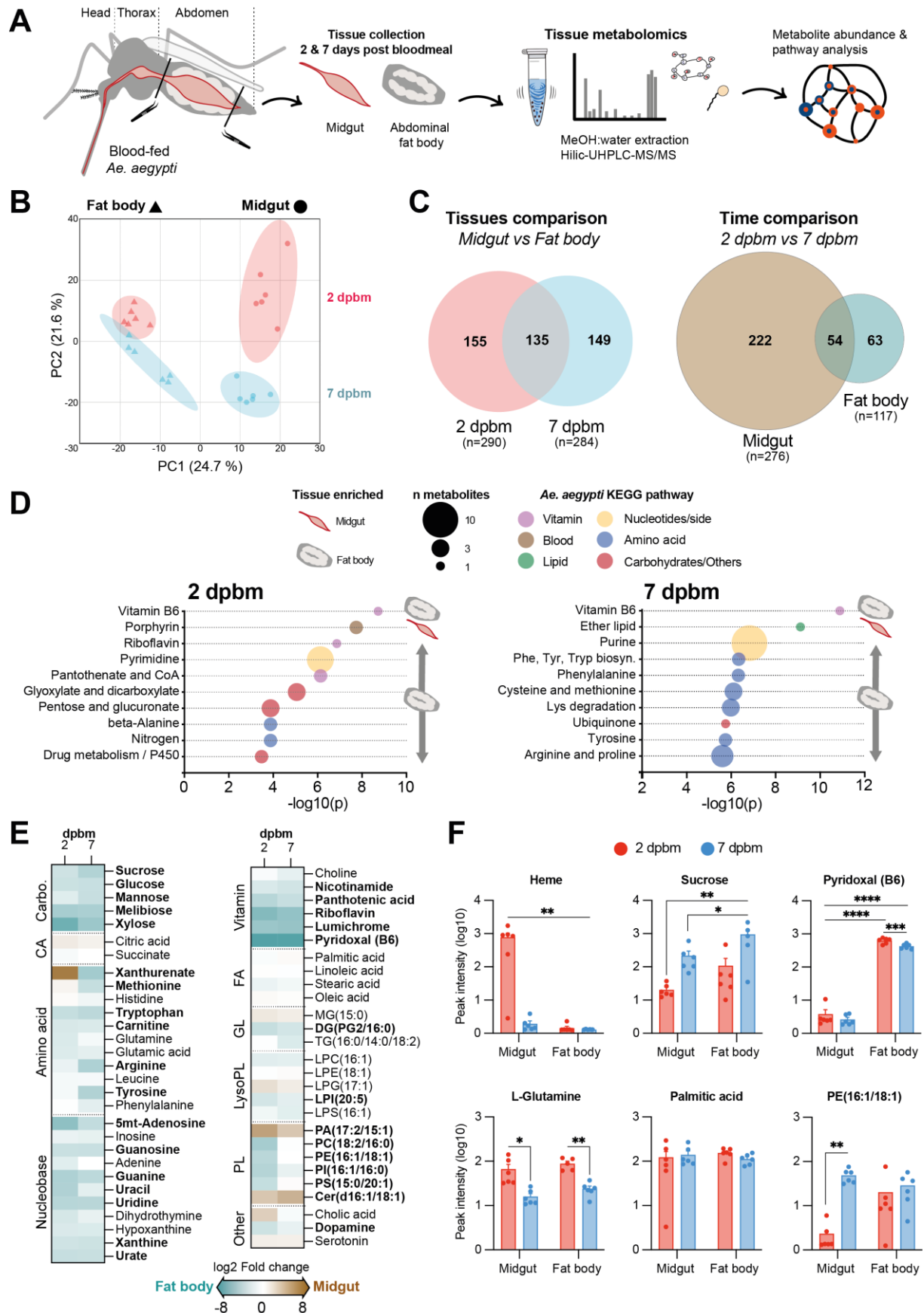
1335 Dot color shows relative average expression intensity for each gene at 2 dpbm (red) and 7
1336 dpbm (blue). Dot size reflects the percentage of cells expressing corresponding genes in each
1337 cell cluster. Each gene is identified by the AAEL0 truncated Vectorbase IDs and their
1338 respective abbreviation if available, not italicized. *Drosophila* or *Anopheles* ortholog
1339 abbreviations are indicated in italic. Vittelo.: vitellogenin genes; Vit-R: vitellogenin membrane
1340 receptor; Treh: trehalose metabolic genes; Glyc: glycogen metabolic genes; Detox:
1341 detoxification genes; FAS: fatty acid synthase; Elon: elongase; FAR: Fatty acyl-CoA reductase;
1342 TCA: tricarboxylic acid cycle genes; EcR: nuclear ecdysone receptor; Cho: chorion; LDL-R:
1343 low-density lipoprotein receptor; Atg: autophagy; SC: stem cell gene markers; Histone: histone
1344 protein genes; HMT: histone methyltransferase.



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Figure 4. Immune and metabolic pathways are expressed mainly by fat body cells and disparately in midgut cells. The heatmap displays the proportion of cells expressing specific genes within each cluster of midgut and fat body cells. Genes were chosen for their role in key immune and metabolic pathways (left column), with a criterion of at least 15% expression in a

1350 minimum of one cluster across the tissues (**Supplementary file 5**). The heatmap features the
1351 expression of Chymotrypsin-1 (*CHYM*) and Vitellogenin A (*vitA*) to monitor physiological
1352 changes post-bloodmeal, and 60S ribosomal protein L14 (*RpL14*) as a housekeeping gene.
1353 Each gene is identified by the AAEL0 truncated Vectorbase IDs and their respective
1354 abbreviation if available, not italicized (right column), with *Drosophila* or *Anopheles* ortholog
1355 abbreviations in italics. PGRP: Peptidoglycan Recognition Protein; LYS: lysozyme; Glyco:
1356 glycolysis; Pent: pentose phosphate pathway; TCA: tricarboxylic acid cycle; Oxy. Phos:
1357 oxidative phosphorylation; CYP450: cytochrome P450.



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Figure 5. Tissue metabolomics highlight the physiological differences and the metabolic expertise of the fat body.

1361 (A) Graphical overview of the midgut and fat body collection on blood-fed mosquitoes for
1362 subsequent metabolites extraction using methanol: water (80:20) and metabolomics by
1363 UHPLC-MS/MS. Mosquitoes used for both metabolomic analysis and scRNAseq were from
1364 the same batch and experiments were conducted concurrently within the same facility.
1365 (B) Principal Component Analysis (PCA) of metabolomic dataset from dissected midgut and
1366 fat body tissues (**Supplementary file 6**). Each dot represents a pool of 5 tissues and pairs of
1367 midgut and fat body originated from the same individual. Samples were normalized by the total
1368 ion chromatogram. The dataset was \log_{10} transformed and scaled by autoscaling.
1369 (C) Venn diagram of significantly regulated metabolites between midgut and fat body or
1370 between 2 and 7 days post-bloodmeal (dpbm). Significant metabolites were selected with a p-
1371 value FDR adjusted <0.05 and at least $|2|$ fold change difference.
1372 (D) Pathway analysis of the 10 most different metabolic pathways between midgut and fat body
1373 at 2 and 7 dpbm. Pathway analysis used HMDB annotated metabolites and global test
1374 enrichment method, based on *Aedes aegypti* KEGG pathway library. Significant pathways
1375 were selected with $-\log_{10}(p) >1,5$ and p-value FDR adjusted <0.05 .
1376 (E) Heatmap showing the differential abundance of selected metabolites between the midgut
1377 and the fat body at 2 and 7 dpbm. Significantly regulated metabolites in at least one time point
1378 are shown in bold and correspond to a fold change $>|2|$ and p-value adjusted < 0.05 , between
1379 midgut and fat body tissues. Metabolites are grouped by chemical classes. Carbo:
1380 carbohydrates; CA: carboxylic acids; FA: fatty acids; GL: glycerolipids; LysoPL:
1381 lysophospholipids, PL: phospholipids.
1382 (F) Selected metabolite intensity repartition in midgut and fat body at 2 and 7 dpbm. Significant
1383 differences were based on a two-way ANOVA Tukey's multiple comparison p-value <0.05 .
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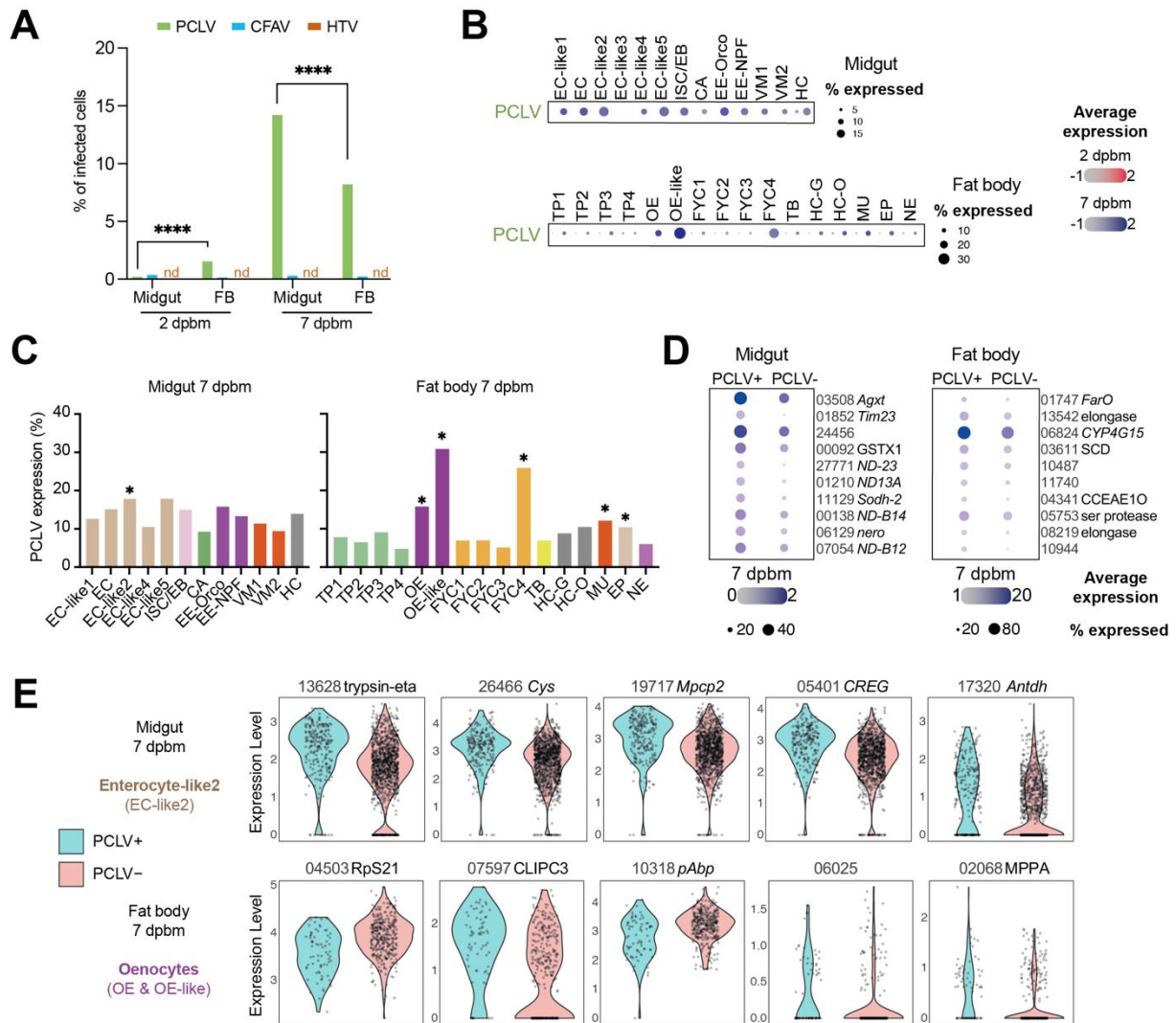


Figure 6. Insect-specific virus PCLV is highly expressed in midgut and fat body cells at later stage post-blood meal.

(A) Proportion of cells expressing insect-specific viruses PCLV, CFAV and HTV in the midgut and the fat body, at 2 and 7 dpbm. nd: not detected.

(B) Dot plot showing ISV expression of PCLV for midgut (top) and fat body (bottom) clusters. Dot color shows relative average expression intensity for ISVs at 2 dpbm (red) and 7 dpbm (blue). Dot size reflects the percentage of cells expressing PCLV in each cell cluster.

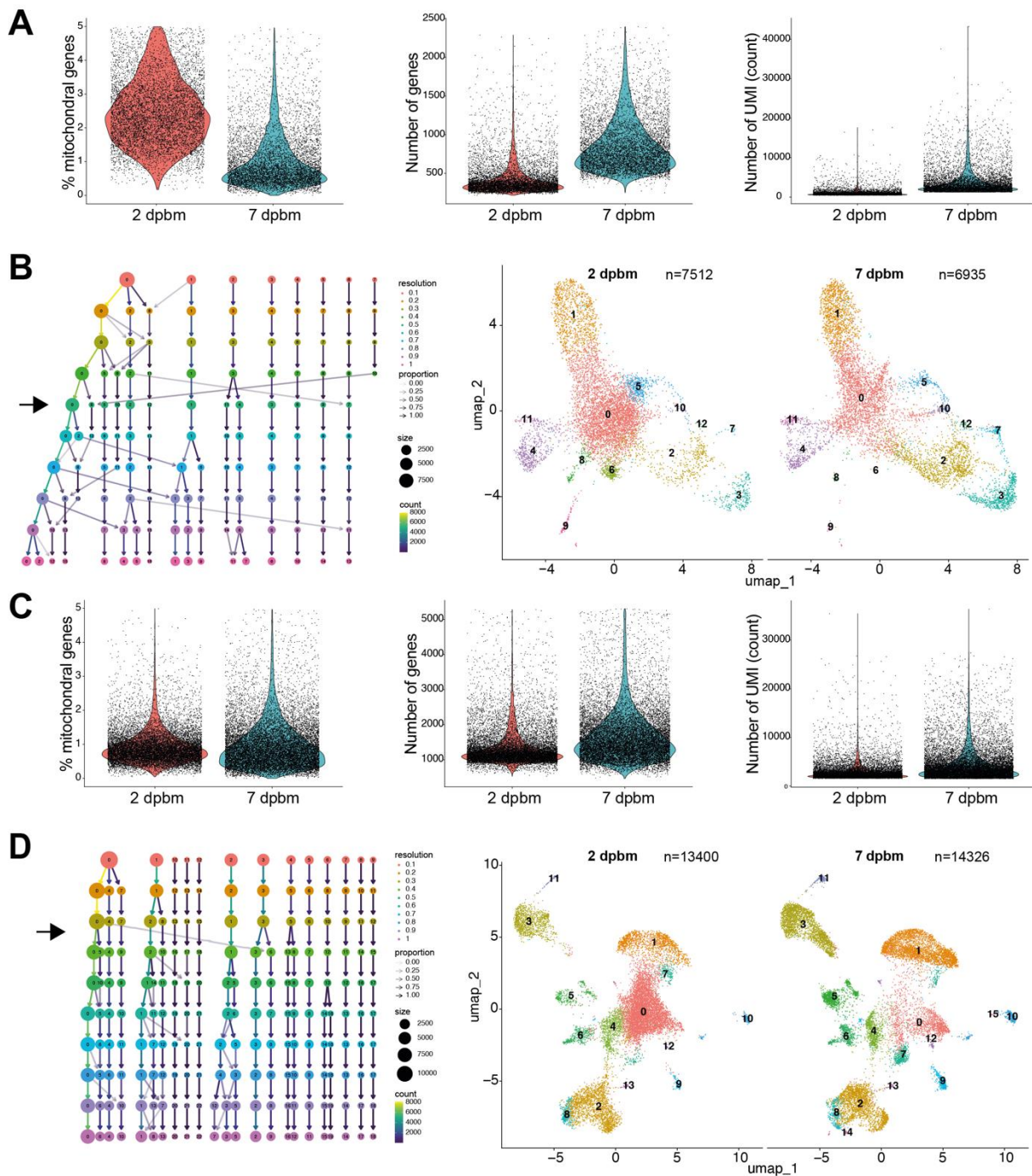
(C) Proportion of cells expressing PCLV in each midgut and fat body clusters at 7 dpbm. Higher proportions of PCLV-positive cells were determined to be statistically significant by the chi-square test with Bonferroni correction, * = p < 0.05.

(D) Dot plot showing top 10 markers differentially expressed between PCLV-positive and PCLV-negative cells within midgut and fat body tissues. Dot color shows relative average gene expression intensity at 7 dpbm. Dot size reflects the percentage of cells expressing corresponding genes in each cell cluster.

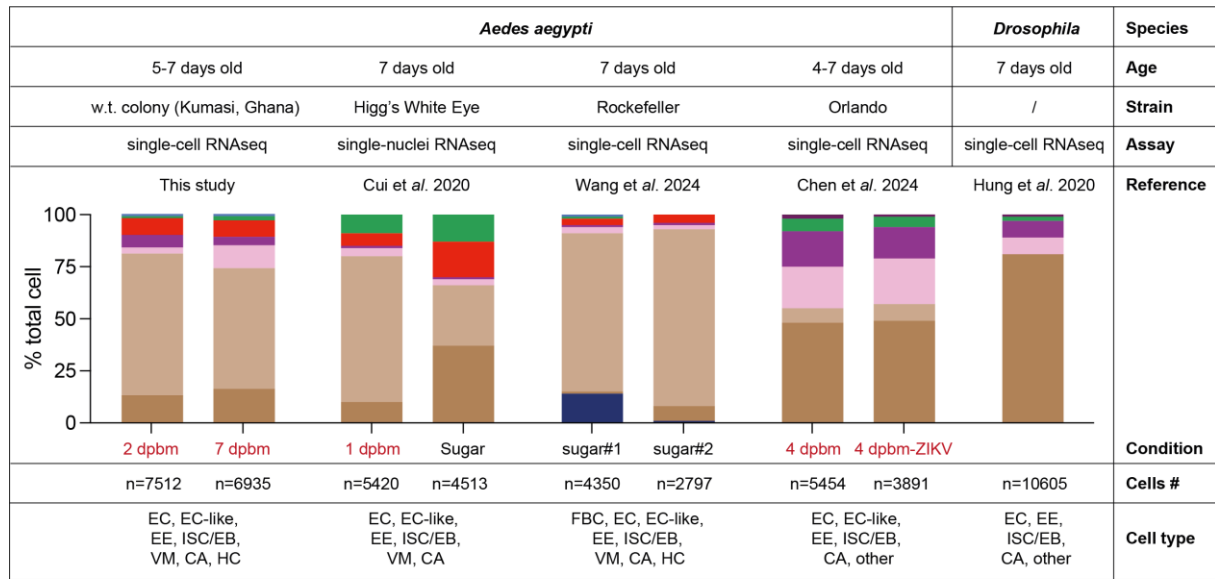
(E) Violin plot showing top 5 gene markers differentially expressed between PCLV-positive and PCLV-negative cells within enterocyte-like2 (EC-like2) and merged oenocytes (OE and OE-like) clusters, at 7 dpbm.

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1404 SUPPLEMENTARY FIGURES AND LEGENDS

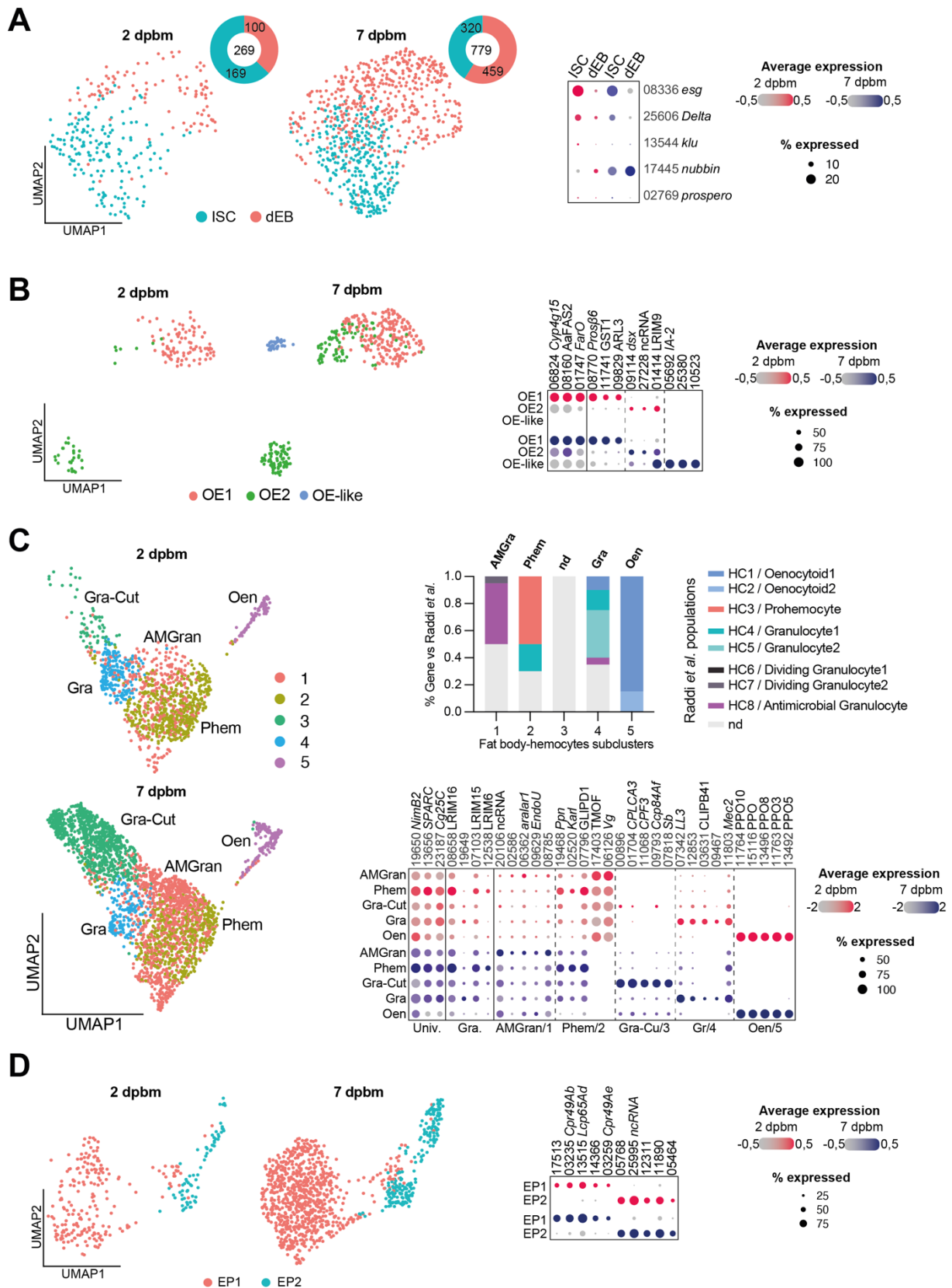


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1406 **Figure S1. Quality control and clustering analysis of midgut and fat body cell samples.**
1407 (A) Quality control metrics for midgut cells with mitochondrial gene proportion, gene count, and
1408 UMI count per cell at 2 and 7 dpbm.
1409 (B) Clustree visualization and UMAP analysis of midgut cell clustering across integrated 2 and
1410 7 dpbm time-points, with chosen Seurat resolution of 0.5.
1411 (C) Quality control metrics for fat body cells with mitochondrial gene proportion, gene count,
1412 and UMI count per cell at 2 and 7 dpbm.
1413 (D) Clustree visualization and UMAP analysis of fat body cell clustering across integrated 2
1414 and 7 dpbm time-points, with chosen Seurat resolution of 0.3.



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Figure S2. Comparison of female *Ae. aegypti* midgut cell composition with reported *Ae. aegypti* and *Drosophila* midgut cell studies. FBC: fat body cells; EC: enterocytes; ISC/EB: intestinal stem cells/enteroblasts; EE: enteroendocrine cells; CA: cardia cells; VM: visceral muscle cells; HC: hemocytes; w.t.: wild-type; dpbm: day post-blood meal.



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Figure S3. Sub-clustering improves characterization of differentiated cells within midgut and fat body populations.

(A) UMAP visualization of intestinal stem cell/enteroblast (ISC/EB) group-specific clustering within the midgut at 2 and 7 dpbm using 0.2 resolution, separating ISC and differentiated EB (dEB) with their proportions at both time point by part of whole plot. Canonical stem cell gene

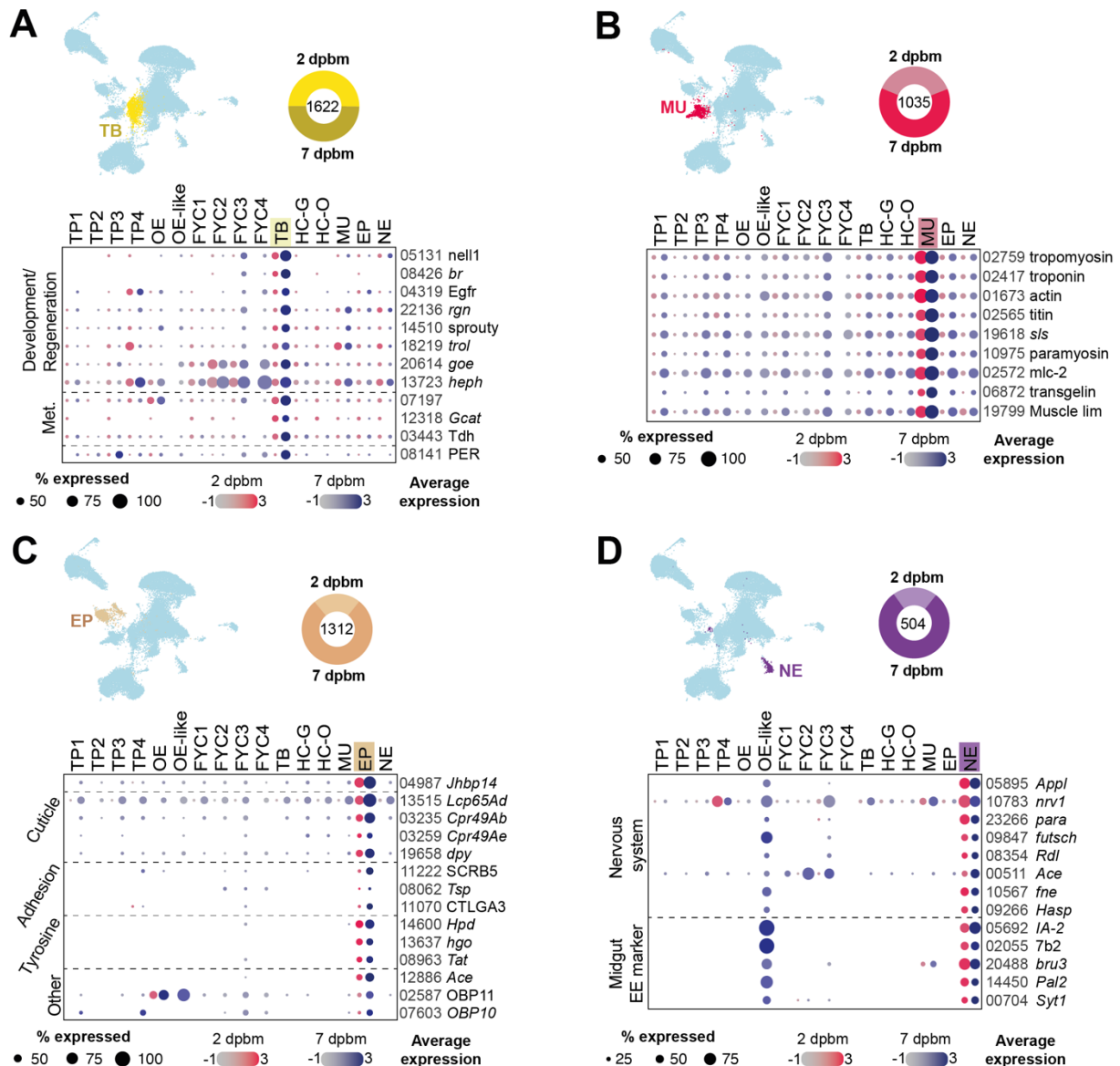
1426 markers (*esg*, *delta*, *klu*) and markers for differentiating EB (*nubbin*, *prospero*) are displayed
1427 in a dot plot.

1428 **(B)** UMAP visualization of oenocyte (Oe and Oe-like) group-specific clustering within the fat
1429 body at 2 and 7 dpbm using 0.1 resolution. The dot plot displays gene expression for highly
1430 specific marker presented in Fig. 3D and the top 3 gene markers from each oenocyte sub-
1431 cluster.

1432 **(C)** UMAP visualization of hemocyte group-specific clustering within the fat body at 2 and 7
1433 dpbm using 0.3 resolution. Clusters are annotated based on the expression of genes matching
1434 those reported in hemocyte subpopulations by Raddi *et al*⁴⁶. The bar plot illustrates the
1435 proportion of gene expression within each sub-cluster that corresponds to expression profiles
1436 in Raddi's hemocyte populations. The dot plot displays gene expression for universal (Univ.)
1437 and granulocyte (Gra.) markers, along with the top 5 gene markers from each hemocyte sub-
1438 cluster.

1439 **(D)** UMAP visualization of epidermal cell (EP) group-specific clustering within the fat body at 2
1440 and 7 dpbm using 0.2 resolution. The dot plot displays gene expression of the top 5 gene
1441 markers from each epidermal cell sub-cluster.

1442 Dot color shows relative average expression intensity for each gene at 2 dpbm (red) and 7
1443 dpbm (blue). Dot size reflects the percentage of cells expressing corresponding genes in each
1444 cell cluster. Each gene is identified by the AAEL0 truncated Vectorbase IDs and their
1445 respective abbreviation if available, not italicized. *Drosophila* or *Anopheles* ortholog
1446 abbreviations are indicated in italic.



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Figure S4. Heterogeneous and specialized cell populations in the fat body-associated tissues. Specific cell clusters visualized on UMAP associated with their cell proportions by part of whole plot and gene markers expression representative of (A) tracheoblast-like cells, (B) muscle cell (C), epidermal cell and (D) neuroendocrine cells. Dot color shows relative average expression intensity for each gene at 2 dpbm (red) and 7 dpbm (blue). Dot size reflects the percentage of cells expressing corresponding genes in each cell cluster. Each gene is identified by the AAEL0 truncated Vectorbase IDs and their respective abbreviation if available, not italicized. *Drosophila* or *Anopheles* ortholog abbreviations are indicated in italic. Met: metabolism; TB: tracheoblast; MU: muscle cells; EP: epidermal cells; NE: neuroendocrine cells.

1458 **SUPPLEMENTARY TABLES**

1459 **Table S1. Number of cells collected in midgut and fat body samples.**

Samples	Day of collection	N cells total	N genes detected >1	genome coverage*	Median UMI/cell	Median Gene/cell
Midgut	2	7512	11358	57%	759	360
	7	6935	11690	59%	2707	776
Fat body	2	13400	14170	72%	2320	1184,5
	7	14326	15162	77%	3373,5	1506

1460 * (Aaeg L5.3: 14,718 protein coding genes, 4704 ncRNA genes, and 382 pseudogenes from
1461 Vectorbase)¹¹⁹

1462 **Table S2. Proportion of cells grouped into cell types in the midgut.**
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Cell group	2 dpbm	7dpbm	2 dpbm	7dpbm	Chi-squared test
EC-like	5106	4025	68,0%	58,0%	0.0008
EC	959	1123	12,8%	16,2%	<0.0001
ISC/EB	269	779	3,6%	11,2%	<0.0001
VM	568	543	7,6%	7,8%	0.95
EE	483	288	6,4%	4,2%	<0.0001
CA	16	141	0,2%	2,0%	0.563
HC	111	36	1,5%	0,5%	0.763
Total	7512	6935			

1464 **Table S3. Proportion of cells grouped into cell types in the fat body.**
1465

Cell group	2 dpbm	7dpbm	2 dpbm	7dpbm	Chi-squared test
TP	8007	5630	59,8%	39,3%	0.003
FYC	2470	2699	18,4%	18,8%	0.87
HC	1206	2707	9,0%	18,9%	<0.0001
TB	814	808	6,1%	5,6%	0.59
EP	286	1026	2,1%	7,2%	<0.0001
MU	392	643	2,9%	4,5%	<0.0001
NE	103	401	0,8%	2,8%	<0.0001
OE	122	412	0,9%	2,9%	<0.0001
Total	13400	14326			

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1470 **Table S4. Proportion of midgut cells by cluster.**

Cell cluster	cluster	2 dpbm	7 dpbm	2 dpbm	7 dpbm
EC-like1	0	4112	2688	54,7%	38,8%
EC	1	959	1123	12,8%	16,2%
EC-like2	2	558	1270	7,4%	18,3%
ISC/EB	3	269	779	3,6%	11,2%
VM1	4	531	447	7,1%	6,4%
EE-Orco	5	447	190	6,0%	2,7%
EC-like3	6	312	1	4,2%	0,0%
CA	7	16	141	0,2%	2,0%
EC-like4	8	115	38	1,5%	0,5%
HC	9	111	36	1,5%	0,5%
EE-NPF	10	36	98	0,5%	1,4%
VM2	11	37	96	0,5%	1,4%
EC-like5	12	9	28	0,1%	0,4%
total		7512	6935		

1471 **Table S5. Proportion of fat body cells by cluster.**

Cell cluster	cluster	2 dpbm	7 dpbm	2 dpbm	7 dpbm
TP1	0	6350	1737	47,4%	12,1%
TP2	1	1354	3161	10,1%	22,1%
FYC1	2	2058	2247	15,4%	15,7%
HC-G	3	1129	2554	8,4%	17,8%
TB	4	814	808	6,1%	5,6%
EP	5	286	1026	2,1%	7,2%
MU	6	392	643	2,9%	4,5%
TP3	7	275	606	2,1%	4,2%
FYC2	8	350	359	2,6%	2,5%
NE	9	103	401	0,8%	2,8%
OE	10	122	373	0,9%	2,6%
HC-O	11	77	153	0,6%	1,1%
TP4	12	28	126	0,2%	0,9%
FYC3	13	61	39	0,5%	0,3%
FYC4	14	1	54	0,0%	0,4%
OE-like	15	0	39	0,0%	0,3%
Total		13400	14326		

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1482 **Table S6. Single-cell quality control process and cell filtering**

	Midgut- 2dpbm	Midgut- 7dpbm	Fatbody- 2dpbm	Fatbody- 7dpbm
Inflection	109	129	202	125
knee	484	1,248	1,597	1,528
Cells after knee cutoff	33,832	49,826	46,158	48,205
Resolution for clustering (filtering step)	0.4	0.35	0.3	0.3
Nb cells after removing cluster 0+1	8,010	6,991	13,474	14,744
Final cell number (filter percent.mt + min/max number of genes)	7,512	6,935	13,400	14,326

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1484 **SUPPLEMENTAL INFORMATION**

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1486 Supplementary file 1: List of genes and orthologs used in this study.

1487 Supplementary file 2: Significant markers of midgut cell clusters identified by FindAllMarkers.

1488 Supplementary file 3: Significant markers of fat body cell clusters identified by FindAllMarkers.

1489 Supplementary file 4: Top 20 most significantly expressed genes by cluster for ontology
1490 visualization.

1491 Supplementary file 5: Proportion of cell cluster expressing selected immune and metabolic
1492 genes.

1493 Supplementary file 6: Metabolites detected from midgut and fat body tissues at 2 and 7 dpbm.