Adipocyte-Derived Amino Acid Storage Proteins are Required for Germline Stem Cell Maintenance in Adult *Drosophila* Females

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Running title: Storage protein regulation of ovarian stem cells

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ABSTRACT

Tissue homeostasis is dependent on precise coordination between endocrine organs in response to changes in organism physiology. Secreted circulating factors from adipocytes (called adipokines) regulate the behavior of stem cell lineages in peripheral tissues in multiple organisms. In addition to their endocrine roles, *Drosophila* adipocytes store and secrete amino acid storage proteins throughout development. During the larval feeding period, adipocytes secrete storage proteins into the hemolymph, which are reabsorbed by the adipose tissue during metamorphosis to control adult organ size and fertility. Despite the known functions for storage proteins during the larval stages, their requirement during *Drosophila* adulthood and reproduction are uncharacterized. We discover that adipocyte-specific knockdown of the storage proteins *Larval serum protein 1* (*Lsp1*) $\alpha/\beta/\gamma$ and *Larval serum protein 2* (*Lsp2*) results in a decrease in GSC maintenance. We further reveal that decreased GSC number is due to downregulation of Target of Rapamycin (TOR) signaling in GSCs, suggesting compromised amino acid sensing directly in GSCs. We also find that the proteins that mediate storage protein adipocyte reabsorption, Fat body protein 1 (Fbp1) and Fat body protein 2 (Fbp2), are expressed in ovarian follicle cells. Intriguingly, Fbp1 nor Fbp2 appear to be required in follicle cells for GSC maintenance, suggesting undiscovered requirements for amino acid storage proteins in oogenesis. Our results highlight a novel role for *Drosophila* amino acid storage proteins during adulthood and in regulating tissue stem cell lineages.

KEYWORDS: Oogenesis, Fbp1, Fbp2, Lsp1, Lsp2

INTRODUCTION

Multicellular organisms rely on precise coordination of circulating factors between organs to regulate developmental processes and maintain tissue homeostasis during adulthood (Castillo-Armengol et al., 2019; Droujinine and Perrimon, 2016; Katagiri, 2023), and defects in

52 interorgan communication can result in tissue dysfunction and disease (Alvarez-Ochoa et al.,
53 2021). For example, fasting-induced intestinal secretion of the insulin peptide (INS-7)
54 coordinates the gut-to-brain axis to control fat mobilization in *C. elegans* (Liu et al., 2024).
55 Intermittent fasting in mammals activates adrenal glands to stimulate the release of free fatty
56 acids in the hair follicle stem cell (HFSC) niche, resulting in increased levels of reactive oxygen
57 species to induce HFSC death (Chen et al., 2025). Therefore, understanding the unique
58 mechanisms used by different organs to communicate their physiological state can provide
59 insight to how stem cell lineages and tissue homeostasis are maintained during adulthood.
60 The adult *Drosophila* ovary is an ideal model to understand interorgan control of stem
61 cell lineages due to the conservation of signaling pathways (Hales et al., 2015; Mohr, 2018) and
62 the unparalleled genetic toolkit that allows genetic manipulation distinct cell types (Roote and
63 Prokop, 2013). Each female contains two ovaries that are arranged in repeating units of 16-20
64 ovarioles with continuously developing egg chambers (**Figure 1A**). Continuous follicle
65 production is dependent on the germline stem cell (GSC) population located in the anteriorly
66 located germarium (**Figure 1B**). Each germarium houses two-to-three GSCs that divide
67 asymmetrically to produce a self-renewing GSC and differentiating stem cell progeny
68 (Drummond-Barbosa, 2019). Oogenesis is energetically intensive, and GSCs are sensitive to
69 nutrient changes (Drummond-Barbosa, 2019; Drummond-Barbosa and Spradling, 2001). For
70 example, a protein poor diet reduces GSC maintenance and results in death of differentiating
71 progeny (Drummond-Barbosa and Spradling, 2001; Hsu and Drummond-Barbosa, 2009).
72 Furthermore, brain-derived *Drosophila* insulin-like peptides directly regulate GSC division
73 (LaFever and Drummond-Barbosa, 2005) and GSCs directly sense amino acids to activate
74 Target of Rapamycin (TOR) signaling to influence GSC self-renewal and proliferation (LaFever
75 et al., 2010). These studies suggests that complex coordination between organism physiology,
76 organ homeostasis, and control of stem cell lineages is highly regulated in *Drosophila* females.

77 The adipose tissue (also known as the “fat body” in *Drosophila*) is a major site of fat
78 storage and carbohydrate metabolism and has significant endocrine functions to mediate
79 interorgan homeostasis through regulated secretion of adipokines (Morigny et al., 2021; Sakers
80 et al., 2022). The fat body is composed of adipocytes and hepatocyte-like oenocytes, making it
81 similar to the mammalian adipose and liver tissue (Arrese and Soulages, 2010; Chatterjee and
82 Perrimon, 2021; Li et al., 2019). In *Drosophila*, adipocyte-specific metabolic activity impacts
83 GSC maintenance. For example, loss of fatty acid beta oxidation, iron transport, and amino acid
84 transport in adipocytes have been shown to decrease GSC number (Matsuoka et al., 2017).
85 Furthermore, we previously demonstrated that signaling downstream of the nuclear receptor
86 Seven up (Svp) in adipocytes is required to maintain GSC number and prevent death of
87 differentiating stem cell progeny (Weaver and Drummond-Barbosa, 2019). However, the precise
88 signaling mechanisms from adipocytes to GSCs have not been fully elucidated.
89 In addition to lipids and fats, insect adipose tissue stores proteins in the form of amino
90 acid storage proteins (hereafter referred to as “storage proteins”) (Haunerland, 1996; Telfer and
91 Kunkel, 1991). Storage proteins are hexamerins composed of six identical or similar subunits
92 that range in molecular weight from 70 to 90 kDa each (Telfer and Kunkel, 1991). Storage
93 proteins are enriched for methionine or armomatic amino acids (phenylalanine, tryptophan, and
94 tyrosine) and show a high degree of sequence similarity to arthropod hemanocyins (Telfer and
95 Kunkel, 1991). In *Drosophila*, storage proteins have been extensively studied during the larval
96 stages and metamorphosis. The *Drosophila* genome contains four storage proteins, which
97 include the homohexamer, Larval serum protein 2 (Lsp2), and the heterohexamer composed of
98 Larval serum protein 1α (Lsp1α), Lsp1β, and Lsp1γ. During the larval feeding period Lsp1α/β/γ
99 and Lsp2 are synthesized by and secreted from the fat body (Burmester and Scheller, 1997;
100 Roberts et al., 1977). The proteins encoded by *fat body protein 1* (*Fbp1*) and *Fat body protein 2*
101 (*Fbp2*) are the predicted receptors for Lsps (Akam et al., 1978; Wolfe et al., 1977). Lsps are
102 reabsorbed by adipocytes during metamorphosis in a Fbp1-dependent manner, where they are

103 utilized to control adult organ size and fitness (Valzania et al., 2024). However, Fbp1 and Fbp2
104 lack transmembrane domains and are found in the hemolymph (Valzania et al., 2024),
105 suggesting an unidentified receptor may also influence storage protein reabsorption in
106 adipocytes.

107 Storage proteins have also been observed in adult *Drosophila*. Larval serum protein 2
108 (Lsp2) was previously shown to be synthesized in the adult *Drosophila* fat body (Benes et al.,
109 1990). While no clear role has been defined for storage proteins in adult *Drosophila*, protein
110 levels of Lsps, Fbp1, and Fbp2 in adult female adipocytes have been shown to be sensitive to
111 dietary changes (Matsuoka et al., 2017) and we previously reported that storage protein
112 transcript levels are positively regulated by Svp (Weaver and Drummond-Barbosa, 2020).
113 Furthermore, homozygous *Drosophila Lsp1* mutants are infertile, suggesting undiscovered
114 mechanisms for storage protein regulation of fertility in *Drosophila* (Roberts et al., 1991). These
115 studies suggest that storage proteins may have roles in adult *Drosophila* to regulate tissue
116 homeostasis and reproduction.

117 In this study we investigated the role of storage proteins in the regulation of the adult
118 female *Drosophila* GSC lineage. Using tissue-specific manipulations, we found that adipocyte-
119 derived storage proteins are required to regulate GSC maintenance. We found that adipocyte-
120 derived Lsp1 and Lsp2 are required for activation of TOR signaling in GSCs for their
121 maintenance. We also generated a suite of CRISPR knock-in fusion lines and demonstrate that
122 storage proteins are expressed during adulthood and are also produced in the adult female
123 ovarian follicle cells. We also present intriguing results that although Fbp1 is required for
124 storage protein uptake in larval adipocytes, knockdown of *Fbp1* nor *Fbp2* in adipocytes or
125 follicle cells influence GSC number. These results suggest a role for a yet-to-be identified
126 intermediary processor of adipocyte-derived Lsps in maintaining GSCs. Together, our results
127 highlight a previously unknown role for adipocyte-derived storage proteins during adulthood in
128 the regulation of oogenesis.

129

130 **MATERIALS AND METHODS**

131 ***Drosophila strains and culture***

132 *Drosophila* stocks were maintained on Bloomington *Drosophila* Stock Center (BDSC)
133 Cornmeal Food that consists of 15.9 g/L inactive yeast, 9.2 g/L soy flour, 67 g/L yellow
134 cornmeal, 5.3 g/L agar, 70.6 g/L light corn syrup, 0.059 M propionic acid at 22-25°C. Medium
135 was supplemented with active wet yeast paste for all experiments. Previously described *Gal4*
136 and *Gal80* transgenes were used, including *3.1Lsp2-Gal4* (Armstrong et al., 2014; Lazareva et
137 al., 2007) and *tubGal80*^{ts} (McGuire et al., 2003). *UAS-Luc*^{JF01355} (Matsuoka et al., 2017), *UAS-*
138 *Fbp2*^{GLC01665} (Ni et al., 2011) (Transgenic RNAi Project; fgr.hms.harvard.edu/), and *Oregon-R*
139 were obtained from the Bloomington *Drosophila* Stock Center (BDSC; bdsc.indiana.edu/). *UAS-*
140 *Fbp1*^{GD5143}, *UAS-Lsp1*$\alpha^{KK106904}$, *Lsp1*$\beta^{GD12786}$, *UAS-Lsp2*^{GD6088}, and *UAS-Lsp2*^{KK115182} were
141 obtained from the Vienna *Drosophila* RNAi Stock Center (VDRC;
142 https://shop.vbc.ac.at/vdrc_store/). Lines carrying multiple genetic elements were generated by
143 standard crosses. Balancer chromosomes and other genetic elements are described on Flybase
144 (www.flybase.org/).

145

146 ***Generation of CRISPR Knock-In GFP-tagged transgenes***

147 Briefly, gRNA donors were designed to knock in eGFP at the C-terminal end of each
148 protein. Transgenesis was performed using either *yw; nosCas9 attp40* or *yw; nosCas9attp2*.
149 CRISPR knock-in eGFP transgenic lines were designed, microinjected, and balanced by
150 Rainbow Transgenic Fly, Inc (Camarillo, CA). Positive eGFP transgenic lines were verified by
151 screening for eGFP fluorescence intensity.

152

153 ***Adipocyte-specific RNA interference (RNAi)***

154 Females containing the *UAS-hairpin* of interest (against *Luc*, *Fbp1*, *Fbp2*, *Lsp1*α, *Lsp1*β,
155 or *Lsp2*) in combination with *y w; tubGal80*^{ts}*/CyO; 3.1Lsp2-Gal4/TM3* were raised at 18°C to
156 block Gal4 activity (and therefore, transcript knockdown) during development. Zero-to-2-day-old
157 females (with *Oregon-R* males) were kept at 18°C for 2-3 days and subsequently switched to
158 29°C for RNAi induction. *UAS-Luc*^{JF01355} was used as an RNAi control. As additional controls,
159 females of similar genotypes but without *Gal4/Gal80*^{ts} (*UAS*-alone controls) were raised and
160 maintained under similar conditions for 5-15 days after switching to 29°C. For all conditions,
161 medium was supplemented with wet active yeast paste.
162
163 **Adult female tissue immunostaining and fluorescence microscopy**
164 Ovaries and other tissues were dissected in unsupplemented Grace's Insect Medium
165 (Gibco), fixed, and washed as described (Weaver and Drummond-Barbosa, 2019). Samples
166 were blocked for at least 3 hours at room temperature in 5% normal goat serum (NGS; MP
167 Biomedicals) plus 5% bovine serum albumin (BSA, Sigma Aldrich) in phosphate-buffered saline
168 (PBS; 10 mM NaH_2PO_4/$NaHPO_4$, 175 mM NaCl, pH 7.4) containing 0.1% Triton X-100 (PBST),
169 and incubated overnight at 4°C in primary antibodies diluted in blocking solution as follows:
170 mouse monoclonal anti-α-Spectrin (3A9) (DSHB; Developmental Studies Hybridoma Bank, 3
171 µg/ml); mouse monoclonal anti-Lamin C (LC28.26) (DSHB, 0.8 µg/ml); rat monoclonal anti-E-
172 Cadherin concentrate (DCAD2-c) (DSHB, 0.37 µg/ml); rabbit monoclonal anti-p4E-BP1
173 (Thr37/46) (Cell Signaling Technology, 1 µg/ml), and rabbit polyclonal anti-Vasa (Boster Bio, 1
174 µg/ml). Samples were washed in PBST and incubated for 2 hours at room temperature in 1:200
175 Alexa Fluor 488- or 568-conjugated goat species-specific secondary antibodies (Invitrogen) in
176 blocking solution. Stained samples were washed, mounted in Vectashield containing 1.5 µg/ml
177 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories), and imaged using a Nikon SP8
178 confocal microscope.

179 GSCs and cap cells were identified as described (Weaver and Drummond-Barbosa,
180 2019), and two-way ANOVA with interaction (GraphPad Prism) was used to calculate the
181 statistical significance of any differences among genotypes in the rate of cap cell or GSC loss
182 from at least three independent experiments, as described (Armstrong et al., 2014). Progression
183 through vitellogenesis was assessed using DAPI staining, as described (Weaver and
184 Drummond-Barbosa, 2019). Three independent experiments were performed and subjected to a
185 Student's *t* test for statistical analysis.
186 For Dad::nlsGFP quantification, the densitometric mean of individual GSC nuclei was
187 measured from optical sections containing the largest nuclear diameter (visualized by DAPI)
188 using FIJI (https://fiji.sc/) (Armstrong et al., 2014). For E-Cadherin quantification, the total
189 densitometric value from maximum intensity projections around the cap cells (identified using
190 LamC staining) was measured with FIJI (Weaver and Drummond-Barbosa, 2018).
191 Quantification of E-Cadherin levels by immunofluorescence is a well-established method to
192 determine whether adherence of GSCs to the niche is compromised (Hsu and Drummond-
193 Barbosa, 2011). p4E-BP1 levels were determined by taking Z-stacks through the whole GSC
194 and total stack intensity was projected. Densitometric data for Dad::nlsGFP, E-Cadherin, and
195 p4E-BP1 levels were subjected to the Mann-Whitney *U*-test.
196 For lipid droplet visualization, fixed and washed fat bodies attached to abdominal
197 carcasses were incubated with 2.5X Alexa Fluor 488-conjugated phalloidin (Molecular Probes)
198 in PBST for 20 min, rinsed, and washed three times for 15 min each in PBST. Abdominal
199 carcasses were incubated in 25 ng/ml Nile Red dye for 10 min. Samples were stored in
200 Vectashield plus DAPI (Vector Laboratories) and fat bodies were scraped off the abdominal
201 carcass and imaged using a Nikon SP8 confocal. The largest cell area of each adipocyte (based
202 on phalloidin staining) used to measure adipocyte area; whereas the longest width of each
203 nucleus per adipocyte (based on DAPI) determined nuclear diameter. Each measurement was

204 performed using FIJI. Three independent experiments were performed, and statistical analysis
205 was performed using a Student's *t* test.
206
207 **ApopTag Assays**
208 To detect dying germline cysts, the ApopTag Indirect *In Situ* Apoptosis Detection Kit (Millipore
209 Sigma) was used according to the manufacturer's instructions as previously described (Weaver
210 and Drummond-Barbosa, 2019). Briefly, fixed and teased ovaries were rinsed in equilibration
211 buffer twice for 5 minutes each at room temperature. Samples were incubated in 100 µl TdT
212 solution at 37ºC for 1 hour with mixing at 15-minute intervals. Ovaries were washed three times
213 in 1X PBS followed by incubation in anti-digoxigenin conjugate for 30 min at room temperature
214 protected from light. Samples were washed four times in 1X PBS and processed for
215 immunofluorescence as described above.
216
217 **RNA isolation**
218 Abdominal carcasses from 100 females of each genotype were dissected in Grace's
219 medium supplemented with 10% fetal bovine serum (FBS; Sigma) as previously described
220 (Weaver and Drummond-Barbosa, 2020). Fat body cells were dissociated from abdominal
221 carcasses with 500 µl dissociation buffer [0.2% Trypsin (Sigma) plus 2 mg/ml collagenase
222 (Sigma) in 1x PBS] per 50 carcasses for 30 minutes at room temperature. Samples were gently
223 agitated every 5 minutes to facilitate separation of cells from the cuticle. 500 µl of Grace's media
224 plus 10% FBS was added to stop the enzymatic reaction and supernatants were placed in new
225 tubes. Carcasses were rinsed with Grace's medium plus 10% FBS and the two supernatants
226 per genotype were combined. Dissociated cells were centrifuged at 3.3 rpm for 5 min at room
227 temperature. Supernatants were removed and cells were flash frozen in liquid nitrogen. Cells
228 were lysed in 250 µl lysis buffer from the RNAqueous-4PCR DNA-free RNA isolation for RT-
229 PCR kit (Ambion). RNA was extracted from all samples following the manufacturer's

230 instructions. Three independent experiments were performed for quantitative reverse-
231 transcriptase polymerase chain reaction (qRT-PCR) analysis.
232
233 **cDNA synthesis and quantitative reverse-transcriptase polymerase chain reaction (qRT-**
234 **PCR)**
235 cDNA was synthesized from 500 ng of total RNA described above for each sample using
236 Superscript II Reverse Transcriptase (Thermo Fisher Scientific) according to the manufacturer's
237 instructions. PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) was used for RT-
238 qPCR. The reactions for three independent biological replicates were performed in triplicate
239 using LightCycler 96 (Roche). Amplification fluorescence threshold was determined by
240 LightCycler 96 software, and ddCT were calculated using Microsoft Excel. Fold change of
241 transcript levels was calculated in Excel as described (Taylor et al., 2019). The primers used for
242 all PCR reactions are listed in Table S1. *Rp49* and *Act5C* transcript levels were used as
243 references.
244
245 **Fly Liquid-Food Interaction Counter (FLIC) assay**
246 The Fly Liquid-Food Interaction Counter (FLIC) system was used to determine
247 differences in feeding behavior as previously described (Fleck et al., 2024; Ro et al., 2014).
248 FLIC *Drosophila* Feeding Monitors (DFMs, Sable Systems International, models DFMV2 and
249 DFMV3) were used in the single choice configuration and each chamber was loaded with liquid
250 food solution [4% sucrose (m/v), 1.5% yeast extract (m/v)]. Females incubated at 29ºC for 10
251 days were switched from solid food to liquid food at 29ºC overnight (~8-12 hours) prior to
252 running each experiment. Flies were aspirated into the DFM chambers and feeding behavior
253 was measured for 24 hours. Each FLIC experiment contains pooled data from at least 30 flies
254 for each genotype. FLIC data were analyzed using previously described custom R code (Ro et
255 al., 2014), which is available at https://github.com/PletcherLab/FLIC_R_Code. Default

256 thresholds were used for analysis except for the following: minimum feeding threshold = 10,
257 tasting threshold = (0,10). Animals that did not participate (i.e., returned zero values), whose
258 DFM returned an unstable baseline signal, or who produced extreme outliers (i.e., exceeding
259 twice the mean of the population) were excluded from analysis. Data were subjected to a Mann-
260 Whitney *U*-test.
261
262 **RESULTS**
263 ***Adipocyte-derived amino acid storage proteins are required for GSC maintenance.***
264 We previously reported that Svp signaling in the female fat body is required for GSC
265 maintenance (Weaver and Drummond-Barbosa, 2019). We also had performed transcriptomics
266 on the adult female fat body to identify signaling components downstream of Svp. Analysis of
267 this data revealed that *Lsp1α/β* and *Lsp2* transcripts were downregulated in females where *svp*
268 was depleted from the fat body (**Fig. 1C**; Weaver and Drummond-Barbosa, 2020). Furthermore,
269 adipocyte-specific affinity labeling of secreted peptides identified storage proteins in adult
270 hemolymph (**Fig. 1C**; Droujinine et al., 2021), and a previous proteomics analysis determined
271 that storage proteins are sensitive to changes in diet in adult female adipocytes (**Fig. 1D**;
272 Matsuoka et al., 2017). We therefore decided to test the involvement of adipocyte-derived
273 storage proteins in regulating GSC maintenance. To assess whether storage proteins are
274 expressed in adult females, we utilized a CRISPR knock-in strategy to express enhanced GFP
275 (eGFP) at the C-terminal end of the endogenous protein (Lsp1γ and Lsp2) and analyzed protein
276 expression in female larval and adult adipocytes by immunofluorescence using *Oregon-R* as a
277 negative control (**Fig. 1E and F**). In larval female adipocytes, Lsp1γ::eGFP (representing the
278 heterohexameric complex of Lsp1α/β/γ) and Lsp2::eGFP are abundantly expressed (**Fig. 1E**)
279 and form granule-like structures in the cytosol. In contrast, storage protein expression in adult
280 female adipocytes is significantly decreased compared to larval female adipocytes (**Fig. 1F**),

281 showing small Lsp1γ::eGFP and Lsp2::eGFP puncta. Collectively, these results suggest that
282 although storage proteins are not as highly expressed in the adipocytes of adult females
283 compared to larvae, they are detectable by more sensitive methodologies and may have
284 functional roles during adulthood.
285
286 ***Adipocyte-derived amino acid storage proteins are required for GSC maintenance.***
287 We next asked whether adipocyte-secreted Lsp1α/β/γ and Lsp2 are required for GSC
288 maintenance or survival of early germline cysts [the specific ovarian phenotypes affected by *svp*
289 knockdown in adult female adipocytes (Weaver and Drummond-Barbosa, 2019)]. Individual
290 knockdown of *Lsp1α* or *Lsp1β* is sufficient to decrease the transcript levels of all components of
291 the Lsp1 complex (**Fig. S1A, B, and D**; Valzania et al., 2024). Therefore, we knocked down
292 *Lsp1α*, *Lsp1β*, or *Lsp2* (**Fig. S1C and E**) specifically in adult adipocytes using the previously
293 described *tubGal80^{ts}; 3.1Lsp2-Gal4* (*3.1Lsp2^{ts}*) driver (Armstrong et al., 2014) and assayed
294 GSC number over 15 days by staining ovaries with α-Spectrin to label GSCs and Laminin C to
295 label cap cell nuclear lamina (**Fig. 2A and B**). Knockdown of *Lsp1α/β* and *Lsp2* in adult female
296 adipocytes significantly increased the rate of GSC loss compared to *Luc* control (**Fig. 2C and D;**
297 **Fig. S1F and G**).
298 The loss of GSCs was not due to leaky expression of the *UAS* transgenes, as there was
299 no significant decrease in GSC number of *UAS-alone* controls over time compared to the *UAS-*
300 *Luc* RNAi hairpin (**Fig. S1H and I**). In addition, the decrease in GSC maintenance was not
301 dependent on the size of the niche (Hsu and Drummond-Barbosa, 2009), as cap cell numbers
302 were unaffected (**Fig. S1J and K**). Furthermore, GSC loss was not due to gross disruption of
303 adipose tissue as measured by adipocyte nuclear or cell size (**Fig. S2A-F**). We also tested
304 whether loss of Lsps in adipocytes altered feeding behavior and did not observe any change in
305 the number of feeding events or duration of feeding compared to *Luc* RNAi control (**Fig. S2G-I**).

306 In addition to GSC maintenance, knockdown of *svp* in adult female adipocytes
307 decreases early germline cyst survival (Weaver and Drummond-Barbosa, 2019). To assess
308 whether knockdown of *Lsp1α/β/γ* or *Lsp2* influences survival of early germline cysts, we labeled
309 germaria with ApopTag, which is a TUNEL-based assay to detect significant DNA fragmentation
310 that is indicative of apoptosis (Drummond-Barbosa and Spradling, 2001). Compared to *Luc*
311 control, knockdown of *Lsp1α/β/γ* or *Lsp2* in adipocytes did not significantly increase the
312 percentage of ApopTag positive germline cysts 10 days after transgene expression (**Fig. S3**).
313 Collectively, these results suggest that Lsp1α/β/γ and Lsp2 are required in adult female
314 adipocytes to specifically control GSC maintenance and other factors downstream of Svp in
315 adipocytes may be responsible for regulating germline cyst survival.
316
317 ***Storage protein reabsorption by Fbp1 or Fbp2 does not regulate GSC maintenance.***
318 Reabsorption of storage proteins in adipocytes during pupariation is dependent on Fbp1
319 (Valzania et al., 2024). Considering *Fbp1* and *Fbp2* transcripts are regulated by Svp and Fbp1
320 and Fbp2 protein levels in adult female fat are regulated by diet, we hypothesized that Fbp1-
321 mediated retrieval of storage proteins may promote GSC self-renewal. We assessed Fbp1 and
322 Fbp2 expression in adult female adipocytes using our eGFP CRISPR knock-in constructs.
323 Fbp1::eGFP is expressed in granules in female larvae adipocytes, however Fbp2::eGFP has a
324 more diffuse expression pattern (**Fig. 3A**). In contrast, Fbp1 and Fbp2 expression in adult
325 female adipocytes is negligible compared to the *Oregon-R* control (**Fig. 3B**). To determine
326 whether receptor-mediated adipocyte reabsorption of storage proteins is required for regulation
327 of GSC number, we knocked down *Fbp1* (as well as the other predicted storage protein
328 receptor, *Fbp2*) in adult female adipocytes (**Fig. S4A-C**) and analyzed GSC maintenance.
329 Relative to *Luc* control, knockdown of *Fbp1* or *Fbp2* did not significantly decrease GSC

330 maintenance over time (**Fig. 3C; Fig. S4D**). These results suggest that Fbp1 nor Fbp2 are not
331 required in adult female adipocytes for maintaining GSC number.
332
333 ***Fbp1 and Fbp2 are expressed in ovarian follicle cells but are not required for GSC***
334 ***maintenance.***
335 Because Fbp1 and Fbp2 are not required in adult female adipocytes for regulation of
336 GSC number, we hypothesized that Fbp1 or Fbp2 may be required in the ovarian germline or
337 somatic cell populations for storage protein absorption. We therefore assayed ovarian
338 expression of our CRISPR eGFP knock-in lines for expression in the ovary. Lsp1γ::eGFP was
339 not detected in either the germline or the soma of the adult ovary (**Fig. S5A**). Interestingly, we
340 did detect Lsp2::eGFP in the oocyte of egg chambers as early as stage 9 (**Fig. S5B**). This
341 suggests Lsp2 may be synthesized in oocytes or possibly transported to the oocyte from
342 another tissue (e.g., adipocytes) since *Lsp2* transcripts are not detected by single cell
343 sequencing of the ovary (Li et al., 2022; Rust et al., 2020). Relative to *Oregon-R* control (**Fig.**
344 **4A**), both Fbp1::eGFP (**Fig. 4B**) and Fbp2::eGFP (**Fig. 4C**) were detected in the adult ovary.
345 Fbp1::eGFP localized to ovarian follicle cells starting in stage 3 egg chambers and decreased
346 expression after stage 5. Fbp1::eGFP was also detected in the cytoplasm of nurse cells
347 beginning in stage 7 egg chambers. In contrast, Fbp2::eGFP exclusively localized to follicle
348 cells, beginning in the germarium and continuing through the remainder of oogenesis. To our
349 knowledge, this is the first reported localization of Lsp2, Fbp1, and Fbp2 in the adult *Drosophila*
350 ovary, suggesting functional roles in adult females.
351 To determine whether Fbp1 or Fbp2 are required in ovarian follicle cells for GSC
352 number, we designed a modified transgenic line of the commonly used follicle cell driver *tj-Gal4*
353 (Sahai-Hernandez and Nystul, 2013). In addition to follicle cell expression, *tj-Gal4* has ectopic
354 expression in the central nervous system, Malpighian tubules, and adipocytes (**Fig. S5C**;
355 Weaver et al., 2020). To alleviate expression in the brain, we combined the temperature

356 inducible *tj-Gal4 tubGal80^{ts}* driver with *nSyb-Gal80* (to prevent neuronal Gal4 expression
357 independent of temperature), which significantly decreased expression in the brain when
358 females were incubated at 29ºC (**Fig. S5C**). Note there is still significant expression in
359 adipocytes; however, because Fbp1 and Fbp2 are not required in adipocytes to regulate GSC
360 number, we reasoned any differences in GSC maintenance would be due primarily to their
361 requirement in follicle cells. However, knock down of *Fbp1* or *Fbp2* using *tj-Gal4 tubGal80^{ts};*
362 *nSyb-Gal80* did not significantly decrease the number of GSCs over 15 days relative to *Luc*
363 control. These results suggest that although Fbp1 and Fbp2 are expressed in the follicle cells of
364 the ovary, they are not required for GSC maintenance.
365
366 ***Adipocyte-derived amino acid storage proteins do not influence BMP signaling and E-***
367 ***Cadherin levels in GSCs.***
368 GSCs are maintained in the niche primarily through Bone Morphogenic Protein (BMP)
369 signaling from cap cells (Song and Xie, 2002) and by E-Cadherin adhesion to cap cells (Song et
370 al., 2004) to prevent premature differentiation. We asked whether loss of GSCs due to
371 knockdown of *Lsp1*α/β/γ and *Lsp2* in adult adipocytes was mediated by decreased BMP
372 signaling or a reduction in E-Cadherin levels at the niche. To test whether BMP signaling was
373 affected, we measured the nuclear intensity of the Dad::nlsGFP BMP signaling reporter (Ayyaz
374 et al., 2015) when *Lsp1*α/β or *Lsp2* were knocked down specifically in adult female adipocytes
375 after 10 days. Storage protein loss specifically in adult female adipocytes did not have a
376 significant decrease on Dad::nlsGFP levels relative to *Luc* control (**Fig. S6A-C**), suggesting that
377 adipocyte-derived amino acid storage proteins do not regulate BMP signaling. Furthermore,
378 adipocyte-specific loss of storage proteins did not affect E-Cadherin levels in the GSC-niche
379 interface, suggesting a different mechanism is required to maintain GSCs (**Fig. S6D-F**).
380

381 ***Adipocyte-derived storage proteins regulate GSC Target of Rapamycin signaling.***

382 mTOR is a conserved kinase that regulates cell growth and survival downstream of
383 amino acids (Gonzalez and Hall, 2017) and is required in adult female GSCs for stem cell
384 maintenance (LaFever et al., 2010). We hypothesized that adipocyte-derived storage proteins
385 may be transported to the ovary (or some other peripheral tissue) and are catabolized into
386 amino acids to promote TOR signaling. To test this hypothesis, we knocked down *Lsp1*α/β or
387 *Lsp2* specifically in adult female adipocytes using *3.1Lsp2-Gal4*^{ts} and assayed the levels of
388 phosphorylated 4E-BP1 (p4E-BP1), the eukaryotic translation initiation factor 4E binding protein
389 that is phosphorylated by mTOR, which serves as a direct read-out of TOR activity (**Fig. 5A;**
390 LaFever et al., 2010; Wei et al., 2019). In female GSCs, p4E-BP1 is present during M phase but
391 not G phase (LaFever et al., 2010), which limited the number of GSCs that could be analyzed.
392 Regardless, adipocyte-specific knockdown of amino acid storage proteins significantly
393 decreased p4E-BP1 levels (**Fig. 5B**). Collectively, our results suggest that adipocyte-derived
394 storage proteins act upstream of TOR in GSCs to control GSC maintenance.
395

396 **DISCUSSION**

397 Organism physiology is dependent on modulation of interorgan communication in
398 response to changes in macromolecules levels. Diet-derived amino acids and amino acid
399 sensing is required in multiple cell types to promote stem cell proliferation and self-renewal to
400 maintain tissue homeostasis. In insects, amino acid storage proteins are essential for
401 metamorphosis and organ growth, as well as reproduction. Our results herein show that
402 adipocyte-derived storage proteins are required in adult *Drosophila* females to regulate GSC
403 maintenance to facilitate egg production. Furthermore, our results highlight an uncharacterized
404 requirement for storage proteins in adulthood for female reproduction in *Drosophila*.
405
406

407 ***Storage proteins are expressed in adult insects.***

408 Although the requirement of storage proteins has been primarily studied for their roles
409 during larval feeding and metamorphosis, storage protein production has also been observed in
410 adult insects (Benes et al., 1990; Martins et al., 2008; Wheeler and Buck, 1995, 1996; Yan et
411 al., 2022). For example, transcripts of the honeybee storage protein *hex 70a* are more abundant
412 in adult female workers compared to males, and there is a significant increase in *hex 70a* fat
413 body transcripts in workers with active ovaries compared to those with inactive ovaries (Martins
414 et al., 2008). Furthermore, *Lsp2* transcripts are detected in the adult *Drosophila* fat body, and
415 the protein accumulates in the hemolymph, albeit at levels significantly decreased compared to
416 larvae (Benes et al., 1990). These results are consistent with our detection of storage proteins
417 (Lsp2::GFP, Fbp1::GFP, and Fbp2::GFP) in adipocytes and the ovary (**Figure 1E and F; Figure**
418 **4B and C; Figure S5B**), suggesting undescribed adult specific roles for storage proteins in
419 adult *Drosophila* females.

420 Consistent with our study focused on the roles of storage proteins in adult females, there
421 is evidence that that storage protein expression in adult insects is regulated in a sex-specific
422 manner. Synthesis of both *Lsp1* and *Lsp2* occurs in adult females after transplantation of 2nd-
423 instar larval fat bodies, whereas only Lsp1 synthesis can occur after transplantation in males
424 (Butterworth et al., 1979; Jowett and Postlethwait, 1981). In *Bombyx mori*, females accumulate
425 more storage protein compared to males (Tojo et al., 1980), and multiple insects utilize storage
426 proteins to support egg development during adulthood (Wheeler and Buck, 1995, 1996). It has
427 also been shown that females transcribe different hexamerin subunits compared to males
428 (Martins et al., 2010; Tojo et al., 1980; Zakharkin et al., 2001), suggesting a female-specific
429 systemic factor may regulate storage protein synthesis during adulthood. One possible
430 candidate is the steroid hormone ecdysone. In *Diptera* adults, ecdysone is primarily produced
431 by ovarian follicle cells, accumulating high levels in females but not males (Birnbaum et al.,
432 1984; Bownes et al., 1984; Ono et al., 2006). In *Drosophila* females, increased ecdysone levels

433 and ecdysone signaling in the nervous system promotes female feeding and the nutrient
434 storage required for oogenesis (Sieber and Spradling, 2015). Given that formation of storage
435 granules in larvae corresponds with an increase in ecdysone titers, female-specific activation of
436 ecdysone signaling may provide a feed-forward mechanism for continuous storage protein
437 production to support oogenesis and the developing egg.
438
439 ***Potential roles for storage proteins during oogenesis.***
440 The presence of elevated storage proteins in adult female insects also suggests specific
441 functions during oogenesis. Adult females produce large quantities of vitellogenin to supply
442 developing oocytes with a surplus of amino acids to support embryonic development (Berg et
443 al., 2024). To support developing oocytes, *Lepidoptera* adults rely on larval-derived protein
444 reserves to promote egg production, whereas autogenous insects (such as mosquitos) store
445 glycogen, fat, and protein in the larval fat body that is carried into adulthood (Wheeler and Buck,
446 1996). In contrast, the monarch butterfly produces two methionine-rich hexamerins specifically
447 during adulthood to support egg development (Pan and Telfer, 1996). Other insects have been
448 shown to synthesize and accumulate hexamerins, with storage protein accumulation greater in
449 females that is quickly depleted upon vitellogenesis. For example, virgin queen ants store large
450 quantities of storage proteins that are quickly depleted during the rearing of the first worker ants
451 (Wheeler and Buck, 1995). Although storage proteins have been considered in dispensable
452 during *Drosophila* adulthood in some studies, our results are consistent with another report
453 showing other adipokines that are primarily known for their roles in larvae also act as regulators
454 of adult oogenesis (Simmons et al., 2025). Furthermore, we found that Lsp2::eGFP is present in
455 the oocyte of developing egg chambers (**Figure S5B**) and both Fbp1::eGFP and Fbp2::eGFP
456 are present in ovarian follicle cells (**Figure 4B and C**), suggesting additional roles during
457 oogenesis or embryonic development undiscovered by our analysis.
458

459 ***Adipocyte-derived factors are required for oogenesis.***

460 Although present in other tissues, the primary site of hexamerin synthesis is in the insect
461 fat body (Burmester and Scheller, 1997; Haunerland, 1996; Telfer and Kunkel, 1991). The fat
462 body serves as a major endocrine organ in insects (Li et al., 2019) and fat body-specific
463 pathways indirectly influence oogenesis, including GSC maintenance (Armstrong and
464 Drummond-Barbosa, 2018; Armstrong et al., 2014; Matsuoka et al., 2017; Weaver and
465 Drummond-Barbosa, 2018, 2019). For example, adipocyte-secreted collagen IV is required to
466 maintain ovarian GSCs by promoting E-Cadherin mediated adhesion of GSCs to the niche
467 (Weaver and Drummond-Barbosa, 2018). In addition, multiple diet-dependent pathways that
468 regulate amino acid production are altered when adult *Drosophila* females are fed a protein-
469 poor diet (Matsuoka et al., 2017). Furthermore, amino acid sensing in adult female adipocytes
470 influences GSC number in a TOR-independent manner, whereas adipocyte-specific TOR
471 activity influences oogenesis (Armstrong et al., 2014). Considering storage protein synthesis in
472 larval adipocytes occurs downstream of TOR (Valzania et al., 2024), it was interesting we did
473 not observe defects in ovulation for when *Lsp1*$\alpha/\beta/\gamma$ or *Lsp2* were knocked down in adipocytes
474 compared to control (0% of ovaries has 2 or more stage 14 embryos per ovariole for all
475 genotypes). Therefore, our results suggest production and secretion of adipocyte-derived
476 storage proteins may occur in a TOR-independent manner.

477

478 ***Maintenance of GSCs requires activation of amino acid-dependent pathways.***

479 Stem cells and cancer stem cells rapidly proliferate and require increased amino acid
480 metabolism to maintain their self-proliferative and differentiation activities (Gong et al., 2025). In
481 *Drosophila*, larval neuroblasts require diet-derived amino acids to support growth and
482 proliferation (Chell and Brand, 2010). Loss of the amino acid transporter *slimfast* in
483 hematopoietic progenitors causes premature differentiation, resulting in decreased progenitor
484 cell number (Shim et al., 2012). In addition, dietary methionine is required for activation of S-

485 adenosylmethionine to regulate the division rate of intestinal stem cells (Obata et al., 2018).
486 Adult female GSCs directly sense amino acids and activate TOR signaling to control GSC
487 number (LaFever et al., 2010). Our results suggest that adipocyte-derived amino acid storage
488 proteins are required to promote TOR signaling in GSCs (**Figure 5**), suggesting that storage
489 proteins could be trafficked to the ovary where they are catabolized to their amino acid
490 precursors before transportation into GSCs. In larval adipocytes, Fbp1 is required for storage
491 protein reabsorption into the fat prior to metamorphosis (Valzania et al., 2024). However, we did
492 not observe Fbp1::eGFP localization in the GSC niche area suggesting that Fbp1 may not be
493 mediating trafficking of storage proteins into GSCs. It is possible that Fbp1 (or Fbp2) are
494 required in a separate tissue or cell type to catabolize Lsps to regulate GSC maintenance.
495 Single cell RNA sequencing analysis show that *Fbp2* transcripts are present in adult gut
496 enterocytes (Li et al., 2022). Therefore, future studies should systematically knock down *Fbp1*
497 and *Fbp2* to determine which cell type may be required for Lsp absorption to control GSC
498 number. Furthermore, multiple amino acid transporter transcripts are expressed in the ovary
499 (Wright and Armstrong, 2024), and single cell sequencing analysis suggests that multiple
500 transporters are expressed in female GSCs (Li et al., 2022). Future studies should also address
501 the germline requirement for transporters that traffic aromatic amino acids and their relationship
502 with adipocyte-derived storage proteins in regulating TOR signaling and GSC maintenance in
503 the adult female ovary.
504
505 **DATA AVAILABILITY**
506 *Drosophila* strains are available from the Weaver lab upon request. However, any strains
507 that are available from the Bloomington *Drosophila* Stock Center (BDSC) must be obtained
508 through the BDSC. All data generated for this study are included in the main text and figures or
509 in the supplemental file provided.
510

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523

524 **DECLARATION OF INTERESTS**

525 The authors declare no conflict of interests.

526

527 **AUTHOR CONTRIBUTIONS**

528 L.N.W. designed experiments, analyzed and interpreted data, and wrote the manuscript.
529 A.B.Z., M.G.A., R.C.E., and L.N.W. performed experiments.

530

531 **REFERENCES**

532 Akam, M.E., Roberts, D.B., Wolfe, J., 1978. Drosophila hemolymph proteins: purification,
533 characterization, and genetic mapping of larval serum protein 2 in D. melanogaster. Biochem
534 Genet 16, 101-119.

535

536 Alvarez-Ochoa, E., Froldi, F., Cheng, L.Y., 2021. Interorgan communication in development and
537 cancer. Wiley interdisciplinary reviews. Developmental biology 10, e394.
538
539 Armstrong, A.R., Drummond-Barbosa, D., 2018. Insulin signaling acts in adult adipocytes via
540 GSK-3beta and independently of FOXO to control Drosophila female germline stem cell
541 numbers. Developmental biology 440, 31-39.
542
543 Armstrong, A.R., Laws, K.M., Drummond-Barbosa, D., 2014. Adipocyte amino acid sensing
544 controls adult germline stem cell number via the amino acid response pathway and
545 independently of Target of Rapamycin signaling in Drosophila. Development 141, 4479-4488.
546
547 Arrese, E.L., Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. Annu
548 Rev Entomol 55, 207-225.
549
550 Ayyaz, A., Li, H., Jasper, H., 2015. Hemocytes control stem cell activity in the Drosophila
551 intestine. Nature Cell Biology 17, 736-748.
552 Benes, H., Edmondson, R.G., Fink, P., Kejzlarova-Lepesant, J., Lepesant, J.A., Miles, J.P.,
553 Spivey, D.W., 1990. Adult expression of the Drosophila Lsp-2 gene. Developmental biology 142,
554 138-146.
555
556 Berg, C., Sieber, M., Sun, J., 2024. Finishing the egg. Genetics 226.
557
558 Birnbaum, M.J., Kelly, T.J., Woods, C.W., Imberski, R.B., 1984. Hormonal regulation of ovarian
559 ecdysteroid production in the autogenous mosquito, Aedes atropalpus. Gen Comp Endocrinol
560 56, 9-18.
561

562 Bownes, M., Dübendorfer, A., Smith, T., 1984. Ecdysteroids in adult males and females of
563 Drosophila melanogaster. Journal of insect physiology 30, 823-830.
564 Burmester, T., Scheller, K., 1997. Conservation of hexamerin endocytosis in Diptera. Eur J
565 Biochem 244, 713-720.
566
567 Butterworth, F.M., Tysell, B., Waclawski, I., 1979. The effect of 20-hydroxyecdysone and protein
568 on granule formation in the in vitro cultured fat body of Drosophila. Journal of insect physiology
569 25, 855-860.
570
571 Castillo-Armengol, J., Fajas, L., Lopez-Mejia, I.C., 2019. Inter-organ communication: a
572 gatekeeper for metabolic health. EMBO reports 20.
573
574 Chatterjee, N., Perrimon, N., 2021. What fuels the fly: Energy metabolism in Drosophila and its
575 application to the study of obesity and diabetes. Sci Adv 7.
576
577 Chell, J.M., Brand, A.H., 2010. Nutrition-responsive glia control exit of neural stem cells from
578 quiescence. Cell 143, 1161-1173.
579
580 Chen, H., Liu, C., Cui, S., Xia, Y., Zhang, K., Cheng, H., Peng, J., Yu, X., Li, L., Yu, H., Zhang,
581 J., Zheng, J.S., Zhang, B., 2025. Intermittent fasting triggers interorgan communication to
582 suppress hair follicle regeneration. Cell 188, 157-174 e122.
583
584 Droujinine, I.A., Meyer, A.S., Wang, D., Udeshi, N.D., Hu, Y., Rocco, D., McMahon, J.A., Yang,
585 R., Guo, J., Mu, L., Carey, D.K., Svinkina, T., Zeng, R., Branon, T., Tabatabai, A., Bosch, J.A.,
586 Asara, J.M., Ting, A.Y., Carr, S.A., McMahon, A.P., Perrimon, N., 2021. Proteomics of protein
587 trafficking by in vivo tissue-specific labeling. Nat Commun 12, 2382.

588

589 Droujinine, I.A., Perrimon, N., 2016. Interorgan Communication Pathways in Physiology: Focus
590 on Drosophila. Annu Rev Genet 50, 539-570.
591

592 Drummond-Barbosa, D., 2019. Local and Physiological Control of Germline Stem Cell Lineages
593 in Drosophila melanogaster. Genetics 213, 9-26.
594

595 Drummond-Barbosa, D., Spradling, A.C., 2001. Stem cells and their progeny respond to
596 nutritional changes during Drosophila oogenesis. Developmental biology 231, 265-278.
597

598 Fleck, S.A., Biswas, P., DeWitt, E.D., Knuteson, R.L., Eisman, R.C., Nemkov, T., D'Alessandro,
599 A., Tennessen, J.M., Rideout, E., Weaver, L.N., 2024. Auxin exposure disrupts feeding behavior
600 and fatty acid metabolism in adult Drosophila. eLife 12.
601

602 Gong, Y., Wang, X., Chen, W., Tsai, H.I., Liu, Y., 2025. Cancer stem cells amino acid
603 metabolism: Roles, mechanisms, and intervention strategies. Cell Signal 134, 111903.
604

605 Gonzalez, A., Hall, M.N., 2017. Nutrient sensing and TOR signaling in yeast and mammals. The
606 EMBO journal 36, 397-408.
607

608 Hales, K.G., Korey, C.A., Larracuente, A.M., Roberts, D.M., 2015. Genetics on the Fly: A Primer
609 on the Drosophila Model System. Genetics 201, 815-842.
610

611 Haunerland, N.H., 1996. Insect storage proteins: gene families and receptors. Insect Biochem
612 Mol Biol 26, 755-765.
613

614 Hsu, H.J., Drummond-Barbosa, D., 2009. Insulin levels control female germline stem cell
615 maintenance via the niche in Drosophila. Proceedings of the National Academy of Sciences of
616 the United States of America 106, 1117-1121.
617
618 Hsu, H.J., Drummond-Barbosa, D., 2011. Insulin signals control the competence of the
619 Drosophila female germline stem cell niche to respond to Notch ligands. Developmental biology
620 350, 290-300.
621
622 Jowett, T., Postlethwait, J.H., 1981. Hormonal regulation of synthesis of yolk proteins and a
623 larval serum protein (LSP2) in Drosophila. Nature 292, 633-635.
624
625 Katagiri, H., 2023. Inter-organ communication involved in metabolic regulation at the whole-
626 body level. Inflamm Regen 43, 60.
627
628 LaFever, L., Drummond-Barbosa, D., 2005. Direct control of germline stem cell division and cyst
629 growth by neural insulin in Drosophila. Science 309, 1071-1073.
630
631 LaFever, L., Feoktistov, A., Hsu, H.J., Drummond-Barbosa, D., 2010. Specific roles of Target of
632 rapamycin in the control of stem cells and their progeny in the Drosophila ovary. Development
633 137, 2117-2126.
634
635 Lazareva, A.A., Roman, G., Mattox, W., Hardin, P.E., Dauwalder, B., 2007. A role for the adult
636 fat body in Drosophila male courtship behavior. PLoS genetics 3, e16.
637
638 Li, H., Janssens, J., De Waegeneer, M., Kolluru, S.S., Davie, K., Gardeux, V., Saelens, W.,
639 David, F.P.A., Brbic, M., Spanier, K., Leskovec, J., McLaughlin, C.N., Xie, Q., Jones, R.C.,

640 Brueckner, K., Shim, J., Tattikota, S.G., Schnorrer, F., Rust, K., Nystul, T.G., Carvalho-Santos,
641 Z., Ribeiro, C., Pal, S., Mahadevaraju, S., Przytycka, T.M., Allen, A.M., Goodwin, S.F., Berry,
642 C.W., Fuller, M.T., White-Cooper, H., Matunis, E.L., DiNardo, S., Galenza, A., O'Brien, L.E.,
643 Dow, J.A.T., sign, F.C.A.C.s., Jasper, H., Oliver, B., Perrimon, N., Deplancke, B., Quake, S.R.,
644 Luo, L., Aerts, S., Agarwal, D., Ahmed-Braimah, Y., Arbeitman, M., Ariss, M.M., Augsburger, J.,
645 Ayush, K., Baker, C.C., Banisch, T., Birker, K., Bodmer, R., Bolival, B., Brantley, S.E., Brill, J.A.,
646 Brown, N.C., Buehner, N.A., Cai, X.T., Cardoso-Figueiredo, R., Casares, F., Chang, A.,
647 Clandinin, T.R., Crasta, S., Desplan, C., Detweiler, A.M., Dhakan, D.B., Dona, E., Engert, S.,
648 Floc'hlay, S., George, N., Gonzalez-Segarra, A.J., Groves, A.K., Gumbin, S., Guo, Y., Harris,
649 D.E., Heifetz, Y., Holtz, S.L., Horns, F., Hudry, B., Hung, R.J., Jan, Y.N., Jaszczak, J.S.,
650 Jefferis, G., Karkanias, J., Karr, T.L., Katheder, N.S., Kezos, J., Kim, A.A., Kim, S.K., Kockel, L.,
651 Konstantinides, N., Kornberg, T.B., Krause, H.M., Labott, A.T., Laturney, M., Lehmann, R.,
652 Leinwand, S., Li, J., Li, J.S.S., Li, K., Li, K., Li, L., Li, T., Litovchenko, M., Liu, H.H., Liu, Y., Lu,
653 T.C., Manning, J., Mase, A., Matera-Vatnick, M., Matias, N.R., McDonough-Goldstein, C.E.,
654 McGeever, A., McLachlan, A.D., Moreno-Roman, P., Neff, N., Neville, M., Ngo, S., Nielsen, T.,
655 O'Brien, C.E., Osumi-Sutherland, D., Ozel, M.N., Papatheodorou, I., Petkovic, M., Pilgrim, C.,
656 Pisco, A.O., Reisenman, C., Sanders, E.N., Dos Santos, G., Scott, K., Sherlekar, A., Shiu, P.,
657 Sims, D., Sit, R.V., Slaidina, M., Smith, H.E., Sterne, G., Su, Y.H., Sutton, D., Tamayo, M., Tan,
658 M., Tastekin, I., Treiber, C., Vacek, D., Vogler, G., Waddell, S., Wang, W., Wilson, R.I., Wolfner,
659 M.F., Wong, Y.E., Xie, A., Xu, J., Yamamoto, S., Yan, J., Yao, Z., Yoda, K., Zhu, R., Zinzen,
660 R.P., 2022. Fly Cell Atlas: A single-nucleus transcriptomic atlas of the adult fruit fly. Science
661 375, eabk2432.
662
663 Li, S., Yu, X., Feng, Q., 2019. Fat Body Biology in the Last Decade. Annu Rev Entomol 64, 315-
664 333.
665

666 Liu, C.C., Khan, A., Seban, N., Littlejohn, N., Shah, A., Srinivasan, S., 2024. A homeostatic gut-
667 to-brain insulin antagonist restrains neuronally stimulated fat loss. Nat Commun 15, 6869.
668
669 Martins, J.R., Nunes, F.M., Cristino, A.S., Simoes, Z.L., Bitondi, M.M., 2010. The four
670 hexamerin genes in the honey bee: structure, molecular evolution and function deduced from
671 expression patterns in queens, workers and drones. BMC Mol Biol 11, 23.
672
673 Martins, J.R., Nunes, F.M., Simoes, Z.L., Bitondi, M.M., 2008. A honeybee storage protein
674 gene, hex 70a, expressed in developing gonads and nutritionally regulated in adult fat body.
675 Journal of insect physiology 54, 867-877.
676
677 Matsuoka, S., Armstrong, A.R., Sampson, L.L., Laws, K.M., Drummond-Barbosa, D., 2017.
678 Adipocyte Metabolic Pathways Regulated by Diet Control the Female Germline Stem Cell
679 Lineage in Drosophila melanogaster. Genetics 206, 953-971.
680
681 Mohr, S.E., 2018. First in fly: Drosophila research and biological discovery. Harvard University
682 Press, United States.
683
684 Morigny, P., Boucher, J., Arner, P., Langin, D., 2021. Lipid and glucose metabolism in white
685 adipocytes: pathways, dysfunction and therapeutics. Nat Rev Endocrinol 17, 276-295.
686
687 Ni, J.Q., Zhou, R., Czech, B., Liu, L.P., Holderbaum, L., Yang-Zhou, D., Shim, H.S., Tao, R.,
688 Handler, D., Karpowicz, P., Binari, R., Booker, M., Brennecke, J., Perkins, L.A., Hannon, G.J.,
689
690 Perrimon, N., 2011. A genome-scale shRNA resource for transgenic RNAi in Drosophila. Nature
691 methods 8, 405-407.

692

693 Obata, F., Tsuda-Sakurai, K., Yamazaki, T., Nishio, R., Nishimura, K., Kimura, M., Funakoshi,
694 M., Miura, M., 2018. Nutritional Control of Stem Cell Division through S-Adenosylmethionine in
695 Drosophila Intestine. Developmental cell 44, 741-751 e743.
696

697 Ono, H., Rewitz, K.F., Shinoda, T., Itoyama, K., Petryk, A., Rybczynski, R., Jarcho, M., Warren,
698 J.T., Marques, G., Shimell, M.J., Gilbert, L.I., O'Connor, M.B., 2006. Spook and Spookier code
699 for stage-specific components of the ecdysone biosynthetic pathway in Diptera. Developmental
700 biology 298, 555-570.
701

702 Pan, M.L., Telfer, W.H., 1996. Methionine-rich hexamerin and arylphorin as precursor reservoirs
703 for reproduction and metamorphosis in female luna moths. Archives of insect biochemistry and
704 physiology 33, 149-162.
705

706 Ro, J., Harvanek, Z.M., Pletcher, S.D., 2014. FLIC: high-throughput, continuous analysis of
707 feeding behaviors in Drosophila. PloS one 9, e101107.
708

709 Roberts, D.B., Turing, J.D., Loughlin, S.A.R., 1991. The advantages that accrue to Drosophila
710 melanogaster possessing larval serum protein 1. Journal of insect physiology 37, 391-400.
711

712 Roberts, D.B., Wolfe, J., Akam, M.E., 1977. The developmental profiles of two major
713 haemolymph proteins from Drosophila melanogaster. Journal of insect physiology 23, 871-878.
714

715 Roote, J., Prokop, A., 2013. How to design a genetic mating scheme: a basic training package
716 for Drosophila genetics. G3 (Bethesda) 3, 353-358.
717

718 Rust, K., Byrnes, L.E., Yu, K.S., Park, J.S., Sneddon, J.B., Tward, A.D., Nystul, T.G., 2020. A
719 single-cell atlas and lineage analysis of the adult Drosophila ovary. Nat Commun 11, 5628.
720
721 Sahai-Hernandez, P., Nystul, T.G., 2013. A dynamic population of stromal cells contributes to
722 the follicle stem cell niche in the Drosophila ovary. Development 140, 4490-4498.
723
724 Sakers, A., De Siqueira, M.K., Seale, P., Villanueva, C.J., 2022. Adipose-tissue plasticity in
725 health and disease. Cell 185, 419-446.
726
727 Shim, J., Mukherjee, T., Banerjee, U., 2012. Direct sensing of systemic and nutritional signals
728 by haematopoietic progenitors in Drosophila. Nat Cell Biol 14, 394-400.
729
730 Sieber, M.H., Spradling, A.C., 2015. Steroid Signaling Establishes a Female Metabolic State
731 and Regulates SREBP to Control Oocyte Lipid Accumulation. Current biology : CB 25, 993-
732 1004.
733
734 Simmons, C., Williams, I.H., Bradshaw, T.W., Armstrong, A.R., 2025. Adipocyte-Derived
735 CCHamide-1, Eiger, Growth-Blocking Peptide 3, and Unpaired 2 Regulate Drosophila
736 melanogaster Oogenesis. Biomolecules 15.
737
738 Song, X., Wong, M.D., Kawase, E., Xi, R., Ding, B.C., McCarthy, J.J., Xie, T., 2004. Bmp
739 signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of
740 marbles, in germline stem cells in the Drosophila ovary. Development 131, 1353-1364.
741

742 Song, X., Xie, T., 2002. DE-cadherin-mediated cell adhesion is essential for maintaining somatic
743 stem cells in the Drosophila ovary. Proceedings of the National Academy of Sciences of the
744 United States of America 99, 14813-14818.
745
746 Taylor, S.C., Nadeau, K., Abbasi, M., Lachance, C., Nguyen, M., Fenrich, J., 2019. The Ultimate
747 qPCR Experiment: Producing Publication Quality, Reproducible Data the First Time. Trends
748 Biotechnol 37, 761-774.
749
750 Telfer, W.H., Kunkel, J.G., 1991. The function and evolution of insect storage hexamers. Annu
751 Rev Entomol 36, 205-228.
752
753 Tojo, S., Nagata, M., Kobayashi, M., 1980. Storage proteins in the silkworm, Bombyx mori.
754 Insect Biochemistry 10, 289-303.
755
756 Valzania, L., Alami, A., Leopold, P., 2024. A temporal allocation of amino acid resources
757 ensures fitness and body allometry in Drosophila. Developmental cell 59, 2277-2286 e2276.
758
759 Weaver, L.N., Drummond-Barbosa, D., 2018. Maintenance of Proper Germline Stem Cell
760 Number Requires Adipocyte Collagen in Adult Drosophila Females. Genetics 209, 1155-1166.
761
762 Weaver, L.N., Drummond-Barbosa, D., 2019. The nuclear receptor seven up functions in
763 adipocytes and oenocytes to control distinct steps of Drosophila oogenesis. Developmental
764 biology 456, 179-189.
765

766 Weaver, L.N., Drummond-Barbosa, D., 2020. The Nuclear Receptor Seven Up Regulates
767 Genes Involved in Immunity and Xenobiotic Response in the Adult Drosophila Female Fat Body.
768 G3 (Bethesda) 10, 4625-4635.
769
770 Weaver, L.N., Ma, T., Drummond-Barbosa, D., 2020. Analysis of Gal4 Expression Patterns in
771 Adult Drosophila Females. G3 (Bethesda) 10, 4147-4158.
772
773 Wei, Y., Bettedi, L., Ting, C.Y., Kim, K., Zhang, Y., Cai, J., Lilly, M.A., 2019. The GATOR
774 complex regulates an essential response to meiotic double-stranded breaks in Drosophila. eLife
775 8.
776
777 Wheeler, D.E., Buck, N.A., 1995. Storage proteins in ants during development and colony
778 founding. Journal of insect physiology 41, 885-894.
779
780 Wheeler, D.E., Buck, N.A., 1996. A role for storage proteins in autogenous reproduction in
781 Aedes atropalpus. Journal of insect physiology 42, 961-966.
782
783 Wolfe, J., Akam, M.E., Roberts, D.B., 1977. Biochemical and immunological studies on larval
784 serum protein 1, the major haemolymph protein of Drosophila melanogaster third-instar larvae.
785 Eur J Biochem 79, 47-53.
786
787 Wright, Y., Armstrong, A.R., 2024. Differential amino acid transporter expression in adult
788 Drosophila melanogaster tissues. MicroPubl Biol 2024.
789

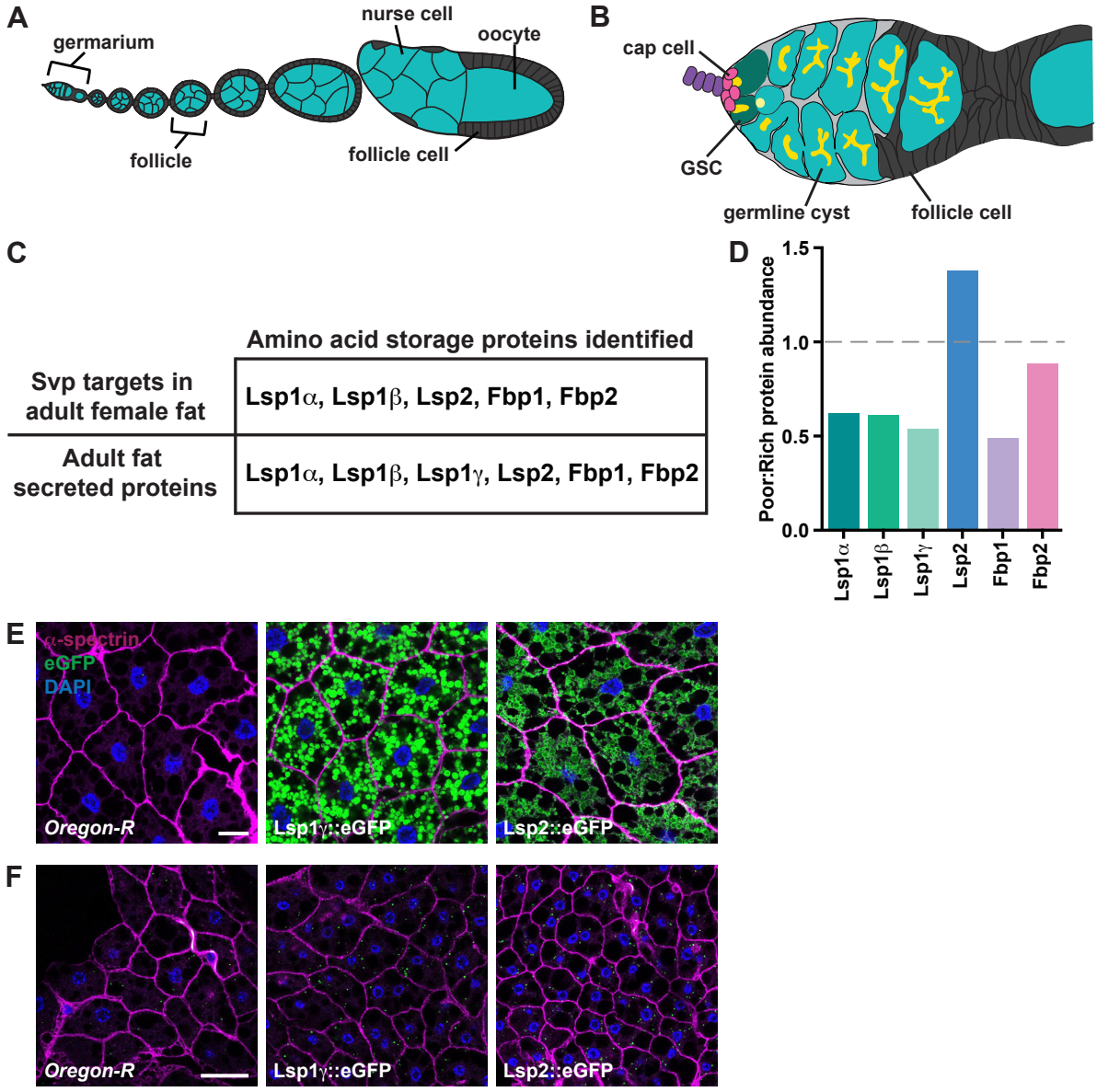
790 Yan, J., Xue, Z., Dong, H., Pang, J., Liu, H., Gong, J., Xia, Q., Hou, Y., 2022. Nutrition regulates
791 the expression of storage proteins in Bombyx mori via insulin-like/FoxO signaling pathway.
792 Insect Biochem Mol Biol 149, 103847.
793
794 Zakharkin, S.O., Headley, V.V., Kumar, N.K., Buck, N.A., Wheeler, D.E., Benes, H., 2001.
795 Female-specific expression of a hexamerin gene in larvae of an autogenous mosquito. Eur J
796 Biochem 268, 5713-5722.
797

798 **FIGURE LEGENDS**

799 **Figure 1. Amino acid storage proteins are present in larval and adult female fat tissue.**
800 **(A)** Cartoon schematic of the adult *Drosophila* ovariole showing the anterior germarium and
801 progressively older egg chambers. Each egg chamber consists of germ cells (teal; 15 nurse
802 cells and one oocyte) and somatic follicle cells (gray). **(B)** Germline stem cells (GSCs; dark teal)
803 reside in the germarium and are maintained by a somatic niche that is composed of cap cells
804 (pink). Germline cysts are supported by follicle cells (gray) that facilitate budding of the egg
805 chamber. GSCs are identified by the fusome (yellow), which changes morphology as the
806 differentiated cyst undergoes mitotic divisions with incomplete cytokinesis. **(C)** Amino acid
807 storage proteins identified by transcriptome analysis of Svp targets from (Weaver and
808 Drummond-Barbosa, 2020) and mass spectrometry analysis of adult hemolymph from
809 (Droujinine et al., 2021). **(D)** Abundance of amino acid storage proteins in adult females on a
810 rich versus protein poor diet identified in (Matsuoka et al., 2017). **(E,F)** Third-instar (E) or adult
811 (F) female adipocytes from *Oregon-R* control and amino acid storage eGFP::fusion CRISPR
812 lines. eGFP (green), amino acid storage protein; α-spectrin (magenta), cell outline; DAPI (blue),
813 nuclei. Scale bar = 25 μm.
814

815 **Figure 2. Adult adipocyte-specific knockdown of larval serum proteins decreases GSC**
816 **maintenance.**
817 **(A,B)** Germaria from *Luc* control (A) and adipocyte-specific *Lsp1*α knockdown (B) after 15 days
818 of transgene expression. Vasa (green), germ cells; α-spectrin (magenta), fusome; LamC
819 (magenta), cap cell nuclear lamina; DAPI (blue), nuclei. GSCs are outlined. Scale bar, 10 µm.
820 **(C,D)** Average number of GSCs over time in *Luc* control females and from adipocyte-specific
821 *Lsp1*α/β (C) or *Lsp2* (D) knockdown. Data shown as mean ± standard error of the mean (SEM)
822 from three independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001; two-way
823 ANOVA with interaction.
824
825 **Figure 3. Knockdown *Fbp1* or *Fbp2* in adult female adipocytes does not influence GSC**
826 **number.**
827 **(A,B)** Third-instar (A) or adult (B) female adipocytes from *Oregon-R* control and Fat body
828 protein eGFP::fusion CRISPR lines. eGFP (green), amino acid storage protein; α-spectrin
829 (magenta), cell outline; DAPI (blue), nuclei. Scale bar = 25 µm. Note that the *Oregon-R* control
830 is identical to Figure 1E and F as all of the fat samples were collected and analyzed at the same
831 time and under the same conditions. **(C)** Average number of GSCs over time for adipocyte-
832 specific *Fbp1* or *Fbp2* knockdown compared to *Luc* control. Data shown as mean ± SEM. n.s.,
833 no significant differences; two-way ANOVA with interaction.
834
835 **Figure 4. Fbp1 and Fbp2 are expressed in adult ovarian follicle cells, but are not required**
836 **for GSC maintenance.**
837 **(A-C)** Ovarioles from *Oregon-R* (A), *Fbp1::eGFP* (B), and *Fbp2::eGFP* (C) adult females. eGFP
838 (green), amino acid storage protein; α-spectrin (magenta), fusome; LamC (magenta), cap cell
839 nuclear lamina; DAPI (blue), nuclei. Scale bar = 100 µm. **(D,E)** Average number of GSCs over

840 15 days from follicle cell knockdown of *Fbp1* (D) and *Fbp2* (E). Data shown as mean ± SEM
841 from three independent experiments. n.s., no significant differences; two-way ANOVA with
842 interaction.
843
844 **Figure 5. Adipocyte-specific knockdown of storage proteins decreases TOR signaling in**
845 **GSCs.**
846 **(A)** Anterior portion of germaria from adipocyte-specific *Luc* control and *Lsp2* knockdown 10
847 days after transgene induction. Phosphorylated 4E-BP1 (p4E-BP1, green), a direct readout of
848 mTOR signaling (LaFever et al., 2010; Wei et al., 2019); α-spectrin (magenta), fusome; LamC
849 (magenta), cap cell nuclear lamina; DAPI (blue), nuclei. Scale bar = 2.5 μm. The dotted lines
850 outline GSCs. **(B)** Dot plot of p4E-BP1 intensity per GSC for each genotype. At least 20 GSCs
851 per genotype were analyzed from three independent experiments. Data shown as mean ± 95%
852 confidence interval for each genotype. *$P < 0.05$, ****$P < 0.0001$; Mann-Whitney *U*-test.



	Amino acid storage proteins identified
Svp targets in adult female fat	Lsp1α, Lsp1β, Lsp2, Fbp1, Fbp2
Adult fat secreted proteins	Lsp1α, Lsp1β, Lsp1γ, Lsp2, Fbp1, Fbp2

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Figure 1

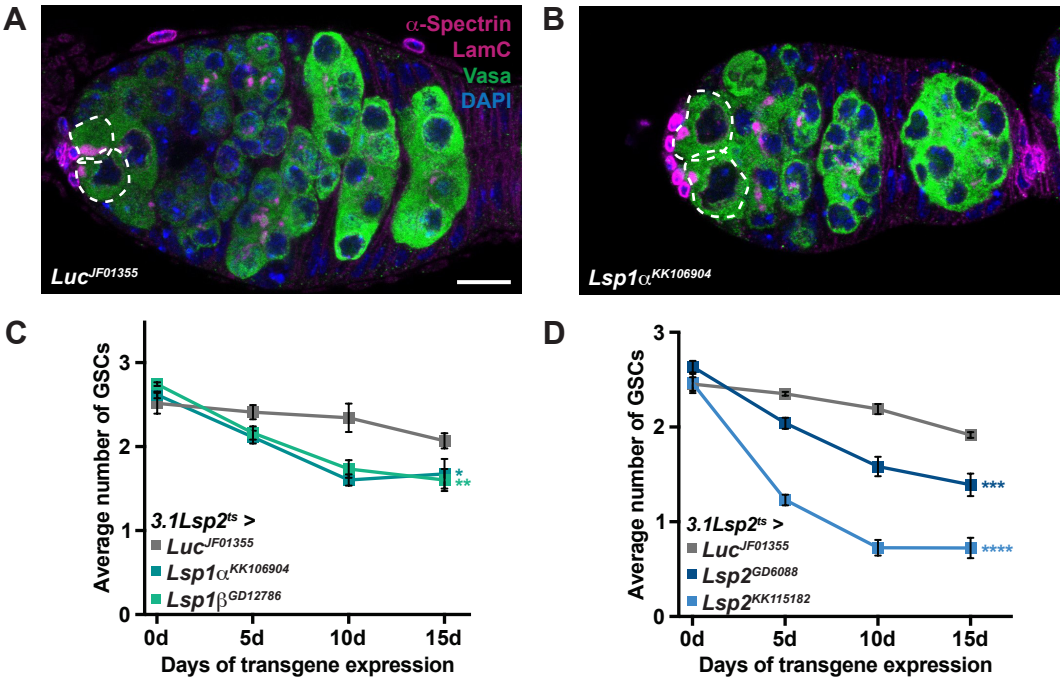

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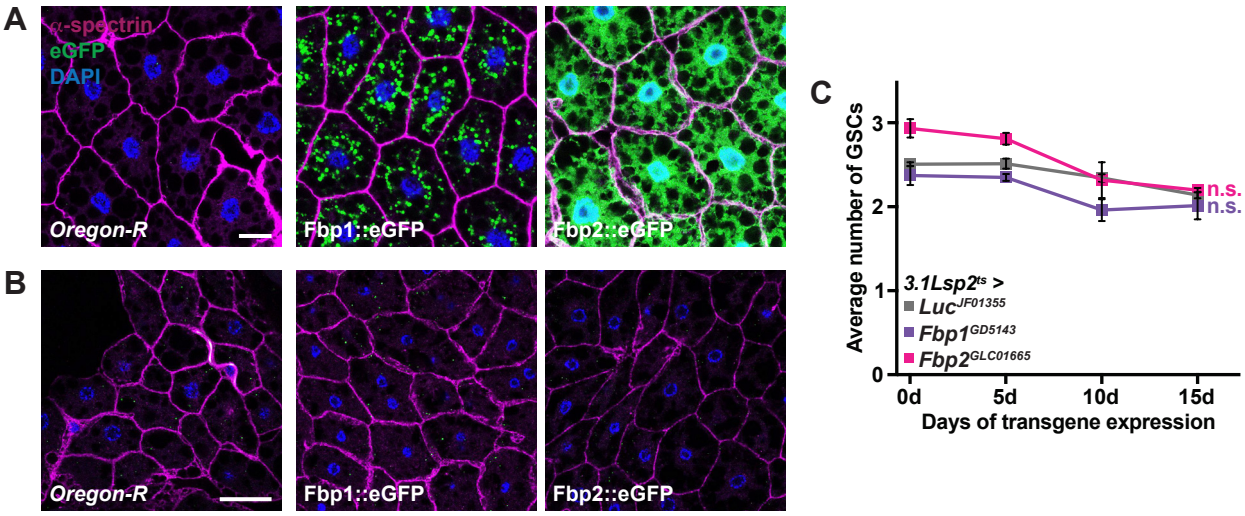
Figure 2



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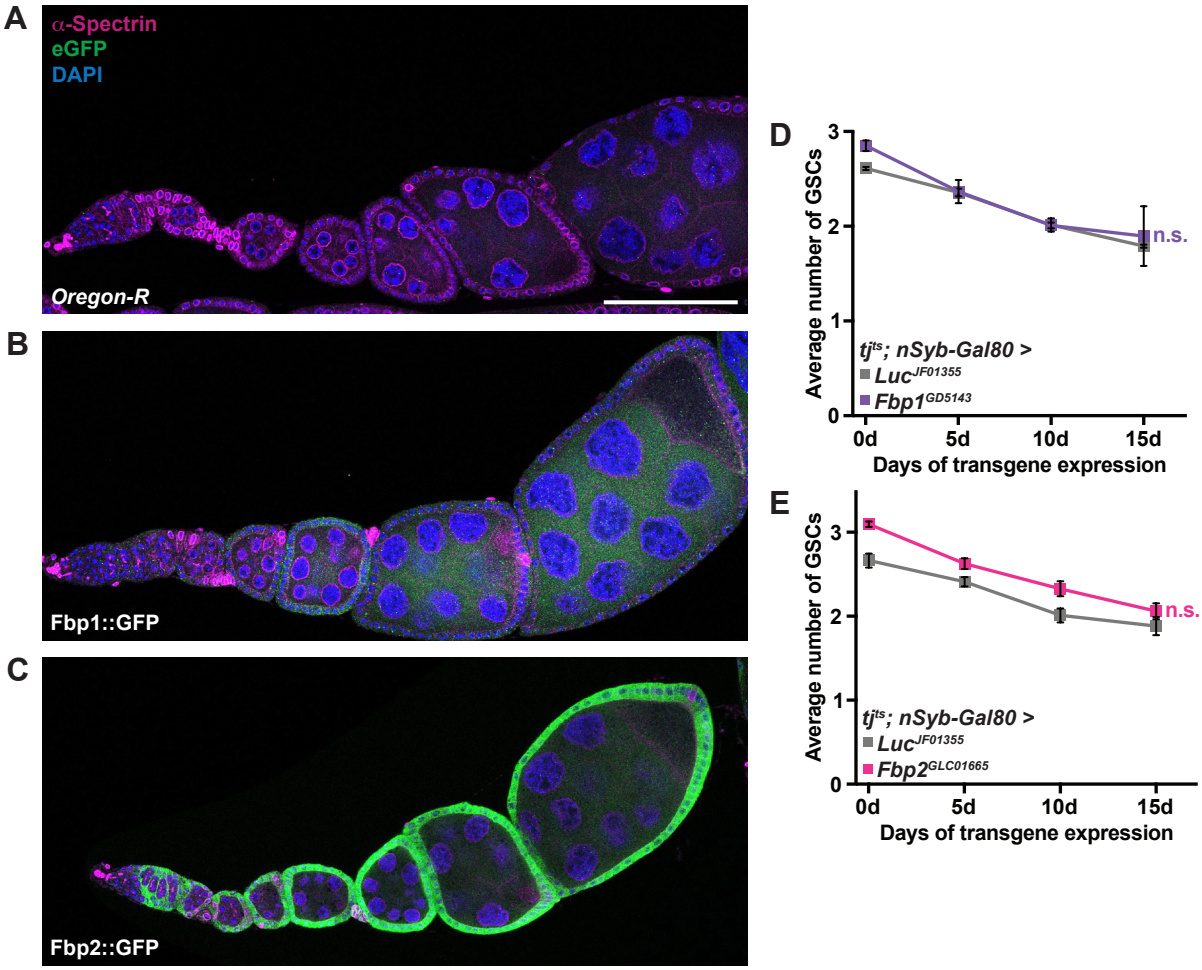

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Figure 4

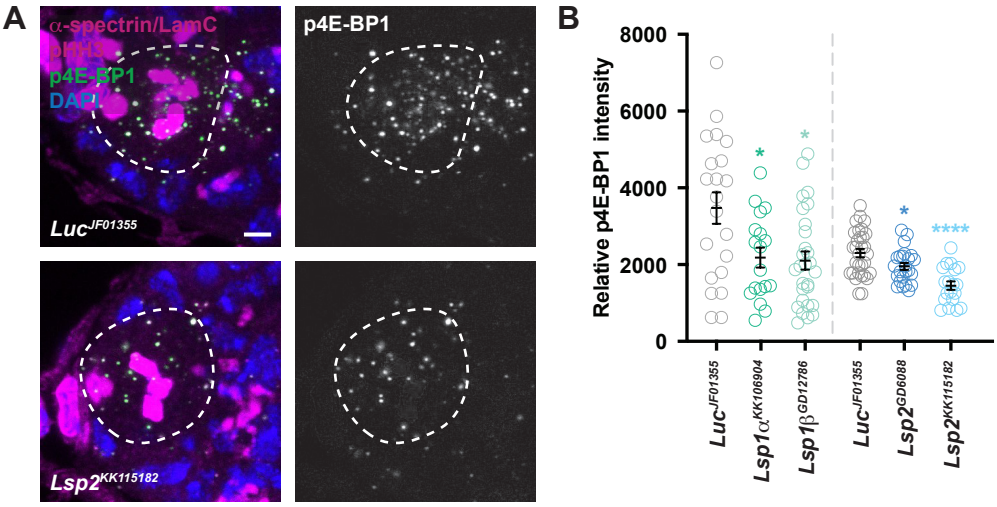

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Figure 5