TITLE

Sex determination gene *transformer* regulates the male-female difference in *Drosophila* fat storage via the adipokinetic hormone pathway

AUTHORS

Lianna W. Wat[1], Zahid S. Chowdhury[1], Jason W. Millington[1], Puja Biswas[1], Elizabeth J. Rideout[1§]

AFFILIATIONS

1 Department of Cellular and Physiological Sciences, Life Sciences Institute, The University of British Columbia

§ Corresponding author

RUNNING TITLE

Adipokinetic hormone pathway regulates the sex difference in fat storage

KEYWORDS

Drosophila, fat storage, triglyceride, adipokinetic hormone, sex determination, *transformer*, lifespan, fertility

CORRESPONDING AUTHOR DETAILS

Elizabeth J. Rideout, Life Sciences Center, 2350 Health Sciences Mall (RM3308), Vancouver, BC, Canada V6T 1Z3. Email: elizabeth.rideout@ubc.ca Phone: (604) 822-0623. Fax: (604) 822-2316.

ABSTRACT

Sex differences in whole-body fat storage exist in many species. For example, *Drosophila* females store more fat than males. Yet, the mechanisms underlying this sex difference in fat storage remain incompletely understood. Here, we identify a key role for sex determination gene *transformer* (*tra*) in regulating the male-female difference in fat storage. Normally, a functional Tra protein is present only in females, where it promotes female sexual development. We show that loss of Tra in females reduced whole-body fat storage, whereas gain of Tra in males augmented fat storage. Tra's role in promoting fat storage was largely due to its function in neurons, specifically the Adipokinetic hormone (Akh)-producing cells (APCs). Our analysis of Akh pathway regulation revealed a male bias in APC activity and Akh pathway function, where this sex-biased regulation influenced the sex difference in fat storage by limiting triglyceride accumulation in males. Importantly, Tra loss in females increased Akh pathway activity, and genetically manipulating the Akh pathway rescued Tra-dependent effects on fat storage. This identifies sex-specific regulation of Akh as one mechanism underlying the male-female difference in whole-body triglyceride levels, and provides important insight into the conserved mechanisms underlying sexual dimorphism in whole-body fat storage.

INTRODUCTION

In animals, stored fat provides a rich source of energy to sustain basal metabolic processes to survive periods of nutrient scarcity, and to support reproduction (Heier and Kühnlein, 2018; Heier et al., 2021; Walther and Farese, 2012). The main form of stored fat is triglyceride, which is deposited within specialized organelles called lipid droplets (Kühnlein, 2012; Murphy, 2001; Thiele and Spandl, 2008). Lipid droplets are found in many cell types throughout the body, but the main organ responsible for triglyceride storage is the adipose tissue (Murphy, 2001). The amount of triglyceride in the adipose tissue is regulated by many factors; however, one important factor that influences an individual's whole-body fat level is whether the animal is female or male (Karastergiou et al., 2012; Power and Schulkin, 2008; Sieber and Spradling, 2015; Wat et al., 2020). Typically, females store more fat than males. In mammals, females store approximately 10% more body fat than males (Jackson et al., 2002; Karastergiou et al., 2012; Womersley and Durnin, 1977). Female insects, on the other hand, can store up to four times more fat than males of the same species (Lease and Wolf, 2011) and break down fat more slowly than males when nutrients are scarce (Wat et al., 2020). These male-female differences in fat metabolism play a key role in supporting successful reproduction in each sex: females with reduced fat storage often show lower fecundity (Buszczak et al., 2002; Sieber and Spradling, 2015) whereas males with excess fat storage generally show decreased fertility (Grönke et al., 2005; Wat et al., 2020). Given that fat storage also influences diverse phenotypes such as immunity and lifespan (DiAngelo and Birnbaum, 2009; Gáliková and Klepsatel, 2018; Johnson and Stolzing, 2019; Kamareddine et al., 2018; Liao et al., 2021; Roth et al., 2018; Suzawa et al., 2019), the sex-specific regulation of fat storage has implications for several life history traits. Yet, the genetic and physiological mechanisms that link biological sex with fat storage remain incompletely understood in many animals.

Clues into potential mechanisms underlying the sex difference in fat storage have emerged from studies on the regulation of triglyceride metabolism in *Drosophila*. While many pathways impact whole-body triglyceride levels (Ballard et al., 2010; Bjedov et al., 2010; Broughton et al., 2005; Choi et al., 2015; DiAngelo and Birnbaum, 2009; Francis

et al., 2010; Ghosh and O'Connor, 2014; Grönke et al., 2010; Heier and Kühnlein, 2018; Heier et al., 2021; Hentze et al., 2015; Kamareddine et al., 2018; Kang et al., 2017; Kubrak et al., 2020; Lee et al., 2019; Lehmann, 2018; Luong et al., 2006; Rajan and Perrimon, 2012; Roth et al., 2018; Scopelliti et al., 2019; Sieber and Spradling, 2015; Song et al., 2014, 2017; Suzawa et al., 2019; Teleman et al., 2005; Texada et al., 2019), the Adipokinetic hormone (Akh; FBgn0004552) pathway plays a central role in regulating whole-body fat storage and breakdown (Heier and Kühnlein, 2018; Heier et al., 2021; Lehmann, 2018). Akh is synthesized as a preprohormone in the Akh-producing cells (APCs), and is subsequently cleaved by proprotein convertases to produce active Akh (Lee and Park, 2004; Noyes et al., 1995; Predel et al., 2004; Wegener et al., 2006). When the APCs are activated by stimuli such as peptide hormones or neurons that make physical connections with the APCs (Kubrak et al., 2020; Oh et al., 2019; Scopelliti et al., 2019; Zhao and Karpac, 2017), Akh is released into the hemolymph (Braco et al., 2012).

Circulating Akh then interacts with a G-protein coupled receptor called the Akh receptor (AkhR, FBgn0025595), where Akh binding to AkhR on target tissues such as the fat body increases intracellular cyclic adenosine monophosphate (cAMP) levels. High levels of cAMP activate protein kinase A (PKA; FBgg0000242) (Gäde and Auerswald, 2003; Park et al., 2002; Staubli et al., 2002a), which phosphorylates several downstream metabolic effectors to promote fat breakdown. For example, in insects, active PKA promotes fat breakdown via phosphorylation and activation of Lipid storage droplet-1 (Lsd-1; FBgn0039114) (Arrese et al., 2008; Bickel et al., 2009; Gäde and Auerswald, 2003; Patel et al., 2005). In mammals, fat breakdown is mediated by similar PKA-dependent phosphorylation of mammalian Lsd-1, and by PKA-dependent phosphorylation and recruitment of lipases, such as Hormone-sensitive lipase (Hsl), to lipid droplets to promote fat mobilization (Sztalryd and Brasaemle, 2017). Given that these genes are highly conserved in flies (Kühnlein, 2012), similar PKA-dependent mechanisms likely explain triglyceride mobilization from lipid droplets. Thus, high levels of Akh pathway activity limit fat storage whereas low levels of Akh signaling promote fat storage. While Akh-mediated triglyceride breakdown plays a vital role in releasing stored energy during times of nutrient scarcity to promote survival (Mochanová et al.,

2018), the Akh pathway limits fat storage even in contexts when nutrients are plentiful. Indeed, loss of *Akh* or *AkhR* augments fat storage in males under normal physiological conditions (Bharucha et al., 2008; Gáliková et al., 2015; Grönke et al., 2007), highlighting the critical role of this pathway in regulating whole-body triglyceride levels.

Additional clues into potential mechanisms underlying the sex difference in fat storage come from studies on metabolic genes. For example, flies carrying loss-of-function mutations in genes involved in triglyceride synthesis and storage, such as *midway* (*mdy*; FBgn0004797), *Lipin* (*Lpin;* FBgn0263593), *Lipid storage droplet-2* (*Lsd-2*; FBgn0030608), and *Seipin* (*Seipin*; FBgn0040336) show reduced whole-body triglyceride levels (Buszczak et al., 2002; Grönke et al., 2003; Teixeira et al., 2003; Tian et al., 2011; Ugrankar et al., 2011; Wang et al., 2016). Whole-body deficiency for genes that regulate triglyceride breakdown, on the other hand, generally have higher whole-body fat levels. This is best illustrated by elevated whole-body triglyceride levels found in flies lacking *brummer* (*bmm*; FBgn0036449) or *Hsl* (FBgn0034491), both of which encode lipases (Bi et al., 2012; Grönke et al., 2005). While these studies demonstrate the strength of *Drosophila* as a model in revealing conserved mechanisms that contribute to whole-body fat storage (Recazens et al., 2021; Schreiber et al., 2019; Walther and Farese, 2012), studies on *Drosophila* fat metabolism often use single- or mixed-sex groups of flies (Bednářová et al., 2018; Gáliková et al., 2015; Grönke et al., 2007; Hughson et al., 2021; Isabel et al., 2005; Lee and Park, 2004; Scopelliti et al., 2019). As a result, less is known about how these metabolic genes and pathways contribute to the sex difference in fat storage.

Recent studies have begun to fill this knowledge gap by studying fat metabolism in both sexes. In one study, higher circulating levels of steroid hormone ecdysone in mated females were found to promote increased whole-body fat storage (Sieber and Spradling, 2015). Another study showed that elevated levels of *bmm* mRNA in male flies restricted triglyceride storage to limit whole-body fat storage (Wat et al., 2020). Yet, neither ecdysone signaling nor *bmm* fully explain the male-female differences in whole-body fat metabolism (Sieber and Spradling, 2015; Wat et al., 2020), suggesting that additional metabolic genes and pathways must contribute to the sex difference in fat storage (Wat et al., 2020). Indeed, genome-wide association studies in *Drosophila*

146 support sex-biased effects on fat storage for many genetic loci (Nelson et al., 2016;
147 Watanabe and Riddle, 2021). As evidence of sex-specific mechanisms underlying
148 whole-body fat storage continues to mount, several reports have also identified male-
149 female differences in phenotypes linked with fat metabolism. For example, sex
150 differences have been reported in energy physiology, metabolic rate, food intake, food
151 preference, circadian rhythm, sleep, immune response, starvation resistance, and
152 lifespan (Andretic and Shaw, 2005; Austad and Fischer, 2016; Belmonte et al., 2020;
153 Chandegra et al., 2017; Helfrich-Förster, 2000; Huber et al., 2004; Hudry et al., 2019;
154 Millington et al., 2021; Park et al., 2018; Reddiex et al., 2013; Regan et al., 2016; Sieber
155 and Spradling, 2015; Videlier et al., 2019; Wat et al., 2020). More work is therefore
156 needed to understand the genetic and physiological mechanisms underlying the male-
157 female difference in fat storage, and to identify the impact of this sex-specific regulation
158 on key life history traits. Further, it will be critical to elucidate how these mechanisms
159 are linked with upstream factors that determine sex.
160 In *Drosophila*, sexual development is determined by the number of X
161 chromosomes (Salz and Erickson, 2010). In females, the presence of two X
162 chromosomes triggers the production of a functional splicing factor called Sex lethal
163 (Sxl; FBgn0264270) (Bell et al., 1988; Bridges, 1921; Cline, 1978). Sxl's most well-
164 known downstream target is *transformer* (*tra*; FBgn0003741), where Sxl-dependent
165 splicing of *tra* pre-mRNA allows the production of a functional Tra protein (Belote et al.,
166 1989; Boggs et al., 1987; Inoue et al., 1990; Sosnowski et al., 1989). In males, which
167 have only one X chromosome, no functional Sxl or Tra proteins are made (Cline and
168 Meyer, 1996; Salz and Erickson, 2010). Over several decades, a large body of evidence
169 has accumulated showing that Sxl and Tra direct most aspects of female sexual
170 identity, including effects on abdominal pigmentation, egg-laying, neural circuits, and
171 behaviour (Anand et al., 2001; Baker et al., 2001; Billeter et al., 2006; Brown and King,
172 1961; Burtis and Baker, 1989; Camara et al., 2008; Christiansen et al., 2002; Cline,
173 1978; Cline and Meyer, 1996; Clough et al., 2014; Dauwalder, 2011; Demir and
174 Dickson, 2005; Goodwin et al., 2000; Hall, 1994; Heinrichs et al., 1998; Hoshijima et al.,
175 1991; Inoue et al., 1992; Ito et al., 1996; Nagoshi et al., 1988; Neville et al., 2014;
176 Nojima et al., 2014; Pavlou et al., 2016; von Philipsborn et al., 2014; Pomatto et al.,

2017; Rezával et al., 2014, 2016; Rideout et al., 2007, 2010; Ryner et al., 1996; Sturtevant, 1945). More recently, studies have extended our knowledge of how Sxl and Tra regulate additional aspects of development and physiology such as body size and intestinal stem cell proliferation (Ahmed et al., 2020; Hudry et al., 2016; Millington and Rideout, 2018; Millington et al., 2021; Regan et al., 2016; Rideout et al., 2015; Sawala and Gould, 2017). Yet, the effects of sex determination genes on whole-body fat metabolism remain unknown, indicating a need for more knowledge of how factors that determine sexual identity influence this important aspect of physiology.

Here, we reveal a role for sex determination gene *tra* in regulating whole-body triglyceride storage. In females, Tra expression promotes a higher level of whole-body fat storage, whereas lack of a functional Tra protein in males leads to lower fat storage. Interestingly, neurons were the anatomical focus of *tra*'s effects on fat storage, where we show that ectopic Tra expression in male APCs was sufficient to augment whole-body triglyceride levels. Our analysis of Akh pathway regulation in both sexes revealed increased *Akh/AkhR* mRNA levels, APC activity, and Akh pathway activity in males. Our findings indicate that this overall male bias in the Akh pathway contributes to the sex difference in whole-body triglyceride levels by restricting fat storage in males. Importantly, we show that the presence of Tra influences Akh pathway activity, and that Akh lies genetically downstream of Tra in regulating whole-body fat storage. These results provide new insight into the mechanisms by which upstream determinants of sexual identity, such as *tra,* influence the sex difference in fat storage. Further, we identify a previously unrecognized sex-biased role for Akh in regulating whole-body triglyceride levels.

RESULTS

Sex determination gene *transformer* regulates the male-female difference in fat storage

Altered *Sxl* function in either sex causes significant lethality due to effects on the dosage compensation machinery (Cline, 1978; Cline and Meyer, 1996). We therefore

asked whether the presence of Tra in females, which promotes female sexual development, contributes to the elevated whole-body triglyceride levels observed in females (Sieber and Spradling, 2015; Wat et al., 2020). In 5-day-old virgin females lacking *tra* function (*tra*^{1}*/Df(3L)st-j7*), we found that whole-body triglyceride levels were significantly lower than in age-matched *w*^{1118} control females (Figure 1A). Because we observed no significant difference in fat storage between *tra*^{1}*/Df(3L)st-j7* mutant males and *w*^{1118} controls (Figure 1 - figure supplement 1A), the sex difference in whole-body triglyceride storage was reduced. Importantly, Tra's effect on whole-body triglyceride storage was not explained by the absence of ovaries in females lacking Tra function (Sieber and Spradling, 2015; Wat et al., 2020), as whole-body fat storage was significantly reduced in *tra*^{1}*/Df(3L)st-j7* mutant females without gonads compared with *w*^{1118} control females lacking ovaries (Figure 1B). Given that we reproduced this finding in females carrying a distinct combination of *tra* mutant alleles (Figure 1C) (Hudry et al., 2016), our findings suggest Tra regulates the sex difference in whole-body triglyceride levels by promoting fat storage in females. While females have reduced fat breakdown post-starvation compared with males (Wat et al., 2020), the magnitude of fat breakdown post-starvation was not significantly different between *tra*^{1}*/Df(3L)st-j7* mutants and sex-matched *w*^{1118} controls (genotype:time interactions p=0.6298 [females], p=0.3853 [males]; Supplementary file 1) (Figure 1 - figure supplement 1B). Tra function is therefore required to promote elevated fat storage in females, but does not regulate fat breakdown post-starvation.

Given that males normally lack a functional Tra protein (Belote et al., 1989; Boggs et al., 1987; Inoue et al., 1990; Sosnowski et al., 1989), we next asked whether the absence of Tra in males explains their reduced whole-body triglyceride levels and rapid triglyceride breakdown post-starvation (Wat et al., 2020). To test this, we ubiquitously overexpressed Tra using *daughterless* (*da*)-*GAL4*, an established way to feminize male flies (Ferveur et al., 1995; Rideout et al., 2015), and examined whole-body fat metabolism. In 5-day-old *da-GAL4>UAS-tra*^{F} males, whole-body triglyceride levels were significantly higher than in age-matched *da-GAL4*>+ or +>*UAS-tra*^{F} control males (Figure 1D). No increase in whole-body fat storage was observed in age-matched *da-GAL4>UAS-tra*^{F} females compared with *da-GAL4*>+ or +>*UAS-tra*^{F} control females

239 (Figure 1 - figure supplement 1C); therefore, the sex difference in fat storage was
240 reduced. Because high levels of Tra overexpression may influence viability (Siera and
241 Cline, 2008), we also measured fat storage in males carrying an allele of *tra* that directs
242 the production of physiological Tra levels (*tra*$^{F\ K\text{-}IN}$ allele) (Hudry et al., 2019). As in *da-*
243 *GAL4>UAS-tra*^{F} males, whole-body triglyceride levels were significantly higher in *tra*$^{F\ K\text{-}}$
244 ^{IN} males compared with *w*^{1118} control males (Figure 1E), indicating that the gain of a
245 functional Tra protein in males promotes elevated whole-body fat storage.
246 Importantly, the presence of rudimentary ovaries in *tra*$^{F\ K\text{-}IN}$ males did not explain
247 their increased fat storage, as whole-body fat storage was still higher in *tra*$^{F\ K\text{-}IN}$ males
248 lacking gonads compared with gonadless control males (Figure 1F). The elevated fat
249 storage in *tra*$^{F\ K\text{-}IN}$ males also cannot be attributed to ecdysone production by the
250 rudimentary ovaries, as no ecdysone target genes were upregulated (Figure 1 - figure
251 supplement 1D) (Sieber and Spradling, 2015). Together, these data indicate that lack of
252 Tra function contributes to the reduced whole-body triglyceride levels normally observed
253 in males. In males, this role for Tra may also extend to regulation of fat breakdown, as
254 triglyceride mobilization post-starvation was significantly reduced in *da-GAL4>UAS-tra*^{F}
255 males compared with *da-GAL4*>+ or +>*UAS-tra*^{F} controls during a 24 hr starvation
256 period (genotype:time $p<0.0001$ [males]; Supplementary file 1) (Figure 1 - figure
257 supplement 1E), a finding we reproduced in *tra*$^{F\ K\text{-}IN}$ males (Figure 1 - figure supplement
258 1F). While this effect of Tra on fat breakdown in males does not perfectly align with our
259 data from *tra* mutant females, we note a trend toward increased fat breakdown in *tra*
260 mutant females that was not statistically significant (Figure 1 - figure supplement 1B).
261 Taken together, these data support a clear role for Tra in regulating the sex difference in
262 fat storage, and suggest that a role for Tra in regulating fat breakdown cannot be ruled
263 out.
264
265 ***transformer* function in neurons regulates the sex difference in fat storage**
266
267 Tra function is required in many cell types, tissues, and organs to promote female
268 sexual development (Anand et al., 2001; Baker et al., 2001; Billeter et al., 2006; Brown
269 and King, 1961; Burtis and Baker, 1989; Camara et al., 2008; Christiansen et al., 2002;

270 Clough et al., 2014; Dauwalder, 2011; Demir and Dickson, 2005; Goodwin et al., 2000;
271 Hall, 1994; Heinrichs et al., 1998; Hoshijima et al., 1991; Inoue et al., 1992; Ito et al.,
272 1996; Nagoshi et al., 1988; Neville et al., 2014; Nojima et al., 2014; Pavlou et al., 2016;
273 von Philipsborn et al., 2014; Pomatto et al., 2017; Rezával et al., 2014, 2016; Rideout et
274 al., 2007, 2010; Ryner et al., 1996; Sturtevant, 1945). To determine the cell types and
275 tissues in which Tra function is required to influence fat metabolism, we overexpressed
276 Tra using a panel of GAL4 lines that drive expression in subsets of cells and/or tissues.
277 To rapidly assess potential effects on fat metabolism, we measured starvation
278 resistance, an established readout for changes to fat storage and breakdown (Beller et
279 al., 2010; Bi et al., 2012; Choi et al., 2015; Grönke et al., 2003, 2005, 2007; Gutierrez et
280 al., 2007).

281 Normally, adult females have elevated starvation resistance compared with age-
282 matched males due to higher fat storage and reduced fat breakdown (Wat et al., 2020).
283 Indeed, loss of *tra* reduced starvation resistance in females (Figure 2A) whereas gain of
284 Tra function enhanced starvation resistance in males (Figure 2B), in line with their
285 effects on fat metabolism (Figure 1A,D). From our survey of different GAL4 lines (Figure
286 2 - figure supplement 1A-F; Figure 2 - figure supplement 2A-D), we found that neurons
287 were the cell type in which gain of Tra most strongly extended male starvation
288 resistance (Figure 2C). Specifically, starvation resistance in males with Tra
289 overexpression in neurons (*elav-GAL4>UAS-tra*^{F}) was significantly extended compared
290 with *elav-GAL4*>+ and +>*UAS-tra*^{F} controls (Figure 2C), with no effect in females
291 (Figure 2 - figure supplement 3A). Because the increase in starvation resistance upon
292 neuron-specific Tra expression was similar in magnitude to the increase in survival
293 observed upon global Tra expression (Figure 2B,C), this finding suggests a key role for
294 neuronal Tra in regulating starvation resistance.

295 To determine whether increased starvation resistance in *elav-GAL4>UAS-tra*^{F}
296 males was due to altered fat metabolism, we measured whole-body triglyceride levels in
297 males and females with neuronal Tra overexpression. We found that *elav-GAL4>UAS-*
298 *tra*^{F} males (Figure 2D), but not females (Figure 2 - figure supplement 3B), showed a
299 significant increase in whole-body fat storage compared with sex-matched *elav-GAL4*>+
300 and +>*UAS-tra*^{F} controls. This suggests that the male-specific increase in starvation

301 resistance (Figure 2C) was due to increased fat storage in *elav-GAL4>UAS-tra^F* males,
302 which we confirm by showing that the rate of fat breakdown in *elav-GAL4>UAS-tra^F*
303 males and females was not significantly different from sex-matched *elav-GAL4*>+ and
304 +>*UAS-tra^F* controls (Figure 2 - figure supplement 3C) (genotype:time interaction
305 p=0.2789 [males], p=0.7058 [females]; Supplementary file 1). Neurons are therefore
306 one cell type in which Tra function influences the sex difference in whole-body
307 triglyceride storage.
308 To identify specific neurons that mediate Tra's effects on starvation resistance
309 and whole-body fat storage, we overexpressed Tra in neurons known to affect fat
310 metabolism and measured starvation resistance (Figure 2 - figure supplement 4A-E)
311 (Al-Anzi and Zinn, 2018; Al-Anzi et al., 2009; Chung et al., 2017; Li et al., 2016; May et
312 al., 2020; Min et al., 2016; Mosher et al., 2015; Zhan et al., 2016). One group of
313 neurons that significantly augmented starvation resistance upon Tra expression was the
314 APCs (Figure 2E), a group of neuroendocrine cells in the corpora cardiaca that produce
315 Akh and other peptide hormones such as Limostatin (Lst; FBgn0034140) (Alfa et al.,
316 2015; Lee and Park, 2004). Flies with APC-specific Tra expression (*Akh-GAL4>UAS-*
317 *tra^F)* had significantly increased starvation resistance compared with sex-matched *Akh-*
318 *GAL4*>+ and +>*UAS-tra^F* controls (Figure 2E; Figure 2 - figure supplement 5A). To
319 determine whether the starvation resistance phenotype indicated altered fat storage, we
320 compared whole-body triglyceride levels in *Akh-GAL4>UAS-tra^F* males and females
321 with sex-matched *Akh-GAL4*>+ and +>*UAS-tra^F* controls. There was a significant
322 increase in whole-body fat storage in males (Figure 2F) but not females (Figure 2 -
323 figure supplement 5B) with APC-specific Tra expression. This indicates Tra function in
324 the APCs promotes fat storage, revealing a previously unrecognized role for the APCs
325 in regulating the sex difference in fat storage. Indeed, fat breakdown was unaffected in
326 *Akh-GAL4>UAS-tra^F* males and females compared with sex-matched *Akh-GAL4*>+ and
327 +>*UAS-tra^F* controls (Figure 2 - figure supplement 5C) (genotype:time interaction
328 p=0.1201 [males] and p=0.0596 [females]; Supplementary file 1).
329
330 **Sex-specific regulation of Adipokinetic hormone leads to a male bias in pathway**
331 **activity**

332

333 Given that the sexual identity of the APCs impacts whole-body fat storage, we
334 compared the regulation of *Akh*, APC activity, and Akh signaling between adult males
335 and females. We first examined *Akh* and *AkhR* mRNA levels in both sexes using
336 quantitative real-time polymerase chain reaction (qPCR). We found that mRNA levels of
337 both *Akh* and *AkhR* were significantly higher in 5-day-old w^{1118} males than in females
338 (Figure 3A,B). This male bias in *Akh* mRNA levels did not reflect an increased APC
339 number in males, as we found no sex difference in the number of APCs (Figure 3C).
340 Because Akh release from the APCs is regulated by APC activity (Kubrak et al., 2020;
341 Oh et al., 2019), we next measured APC activity in males and females by driving APC-
342 specific expression of calcium-responsive chimeric transcription factor *LexA-VP16-*
343 *NFAT* (*Akh-GAL4>UAS-LexA-VP16-NFAT* [called *UAS-CaLexA*]) (Masuyama et al.,
344 2012). Sustained APC activity triggers nuclear import of LexA-VP16-NFAT, where it
345 drives expression of a GFP reporter downstream of a LexA-responsive element
346 (Masuyama et al., 2012). Monitoring GFP levels in the APCs therefore provides a
347 straightforward way to monitor APC activity.

348 In 5-day-old *Akh-GAL4>UAS-CaLexA* males, GFP levels were significantly
349 higher than in age- and genotype-matched females (Figure 3D-H). Because *GAL4*
350 mRNA levels were not significantly different between males and females carrying the
351 *Akh-GAL4* transgene (Figure 3 - figure supplement 1A), and the number of APCs did
352 not differ between the sexes (Figure 3C), these findings indicate that the APCs are
353 more active in males than in females. To determine whether the male bias in *Akh/AkhR*
354 mRNA levels and APC activity affected Akh pathway activity, we next compared levels
355 of phosphorylated Inositol-requiring enzyme-1 (Ire1; FBgn0261984) between males and
356 females. Because levels of phosphorylated Ire1 (p-Ire1) are higher in *Drosophila* cells
357 stimulated with Akh peptide, regulation that was dependent on the presence of AkhR,
358 high p-Ire1 levels indicate increased Akh pathway activity (Song et al., 2017). We found
359 that the ratio of p-Ire1 to loading control actin was higher in 5-day-old w^{1118} males
360 compared with age- and genotype-matched females in three out of four biological
361 replicates (Figure 3I-K; Figure 3 - figure supplement 1B), a finding that aligns with the

362 sex difference in *Akh/AkhR* mRNA levels and APC activity. Taken together, our data
363 suggests a previously unrecognized male bias in the Akh pathway.
364
365 **The Adipokinetic hormone pathway contributes to the sex difference in fat**
366 **storage**
367
368 Given that high Akh pathway activity limits fat storage via an established intracellular
369 signaling cascade that culminates in lipase recruitment and fat mobilization (Baumbach
370 et al., 2014; Grönke et al., 2007; Lee and Park, 2004; Mochanová et al., 2018), we
371 wanted to determine whether the male bias in Akh pathway activity influences the sex
372 difference in fat metabolism by restricting fat storage in males. We therefore used a
373 published approach to ablate the APCs (*Akh-GAL4>UAS-reaper* (*rpr*)) (Lee and Park,
374 2004; White et al., 1996), and measured whole-body triglyceride levels in each sex.
375 Because the sexual identity of the APCs affects fat storage and not fat breakdown
376 (Figure 2F; Figure 2 - figure supplement 5C), we focused our analysis on triglyceride
377 storage rather than mobilization. Triglyceride levels were significantly higher in 5-day-
378 old *Akh-GAL4>UAS-rpr* males than in *Akh-GAL4*>+ and +>*UAS-rpr* control males
379 (Figure 4A). In contrast, triglyceride levels in 5-day-old *Akh-GAL4>UAS-rpr* females
380 were not significantly different from *Akh-GAL4*>+ and +>*UAS-rpr* control females
381 (Figure 4 - figure supplement 1A). This suggests that the male bias in Akh pathway
382 activity normally contributes to the sex difference in fat storage by limiting triglyceride
383 accumulation in males via the established intracellular signaling cascade known to
384 regulate lipid droplet breakdown (Arrese et al., 2008; Heier and Kühnlein, 2018; Heier et
385 al., 2021; Kühnlein, 2012; Patel et al., 2005). Importantly, we reproduced the male-
386 biased effects on fat storage in flies carrying mutant *Akh* and *AkhR* alleles (Akh^{A} and
387 $AkhR^{1}$, respectively) (Figure 4B,C; Figure 4 - figure supplement 1B,C), and show that
388 APC-specific knockdown of *Lst* had no effect on fat storage in either sex (Figure 4 -
389 figure supplement 1D,E). These findings support a model in which it is Akh production
390 by the APCs that plays a role in regulating the male-female difference in fat storage.
391 While the mechanisms underlying the regulation of intracellular fat breakdown by Akh
392 have been well-documented (Bharucha et al., 2008; Gáliková et al., 2015; Grönke et al.,

393 2007; Heier and Kühnlein, 2018; Heier et al., 2021; Kubrak et al., 2020; Lee and Park,
394 2004; Lehmann, 2018; Scopelliti et al., 2019; Zhao and Karpac, 2017), our findings
395 reveal a new role for Akh in regulating the sex difference in fat storage. Notably, this
396 Akh-mediated regulation of the male-female difference in fat storage operates in a
397 parallel pathway to the previously described sex-specific role of triglyceride lipase *bmm*
398 (Figure 4 - figure supplement 2A,B) (Wat et al., 2020).

399 Beyond the APC ablation or complete loss of Akh, we next wanted to test
400 whether the sex-specific Akh regulation we uncovered contributes to the male-female
401 difference in fat storage. To this end, we used a genetic approach to manipulate *Akh*
402 mRNA levels or APC activity, and measured whole-body fat storage in both sexes. To
403 determine whether the male bias in *Akh* mRNA levels contributes to the sex difference
404 in fat storage, we measured whole-body triglyceride levels in flies with APC-specific
405 expression of *Akh-RNAi* (*Akh-GAL4>UAS-Akh-RNAi*). Importantly, this manipulation
406 effectively reduced *Akh* mRNA levels in both sexes (Figure 4 - figure supplement 3A,B).
407 In males, whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-*
408 *Akh-RNAi* flies compared with *Akh-GAL4>+* and *+>UAS-Akh-RNAi* control flies (Figure
409 4D). *Akh-GAL4>UAS-Akh-RNAi* female flies, in contrast, showed no significant change
410 in whole-body fat storage compared with *Akh-GAL4>+* and *+>UAS-Akh-RNAi* control
411 females (Figure 4 - figure supplement 3C). This indicates a strongly male-biased effect
412 on fat storage due to reduced *Akh* mRNA levels, suggesting that the sex difference in
413 *Akh* mRNA levels contributes to the male-female difference in whole-body fat storage.

414 To determine whether the male bias in APC activity also influences the sex
415 difference in fat storage, we silenced the APCs by APC-specific overexpression of an
416 inwardly rectifying potassium channel Kir2.1 (Baines et al., 2001) and measured whole-
417 body triglyceride levels. Whole-body fat storage in *Akh-GAL4>UAS-Kir2.1* adult males
418 was significantly higher compared with *Akh-GAL4>+* and *+>UAS-Kir2.1* control males
419 (Figure 4E). In females, while we observed significantly elevated whole-body fat storage
420 in *Akh-GAL4>UAS-Kir2.1* adults compared with *Akh-GAL4>+* and *+>UAS-Kir2.1*
421 controls (Figure 4 - figure supplement 3D), the magnitude of this increase was larger in
422 males (sex:genotype interaction p=0.0455; Supplementary file 1). Together, these data
423 suggest that the male bias in APC activity contributes to the sex difference in fat storage

424 by limiting triglyceride accumulation in males. Indeed, augmenting APC activity in
425 females using a bacterial voltage-gated sodium channel (*UAS-NaChBac*) significantly
426 reduced fat storage in females (Figure 4F; Figure 4 - figure supplement 3E). While Akh
427 affects food-related behaviours in some contexts (Choi et al., 2015; Hentze et al., 2015;
428 Huang et al., 2020), we observed no significant effects of altered APC activity on
429 feeding behaviour in either sex (Figure 4 - figure supplement 4A-D). This suggests that
430 the male-biased effect of APC manipulation on fat storage cannot be fully explained by
431 effects on food intake. Thus, in addition to the contribution of elevated *Akh* mRNA levels
432 in males to the sex difference in fat storage, we also identify a role for the male bias in
433 APC activity in the sex-specific regulation of whole-body triglyceride levels.
434
435 ***transformer* regulates the sex difference in fat storage via the Adipokinetic**
436 **hormone pathway**
437
438 Given that Tra function and the Akh pathway both contribute to the male-female
439 difference in fat storage, we asked whether the presence of Tra affects the sex bias in
440 Akh pathway activity. In 5-day-old $tra^{1}/Df(3L)st\text{-}j7$ females, levels of p-Ire1 were higher
441 than in w^{1118} control females in three out of four biological replicates (Figure 5A-C;
442 Figure 5 - figure supplement 1A). This suggests the presence of Tra in females normally
443 represses Akh pathway activity. Indeed, loss of Tra significantly increased *Akh* mRNA
444 levels in females (Figure 5D). Given Tra's effects on Akh pathway activity, we next
445 tested whether the change in Akh pathway function was significant for Tra's effects on
446 whole-body triglyceride levels. We predicted that if increased Akh pathway activity
447 caused the lower fat storage in *tra* mutant females, genetic manipulations that reduce
448 Akh pathway activity should block this reduction in whole-body triglyceride levels. While
449 all female genotypes lacking *tra* function had reduced fat storage compared with control
450 females (Figure 5E), APC ablation in *tra* mutant females rescued this decrease in
451 whole-body triglyceride levels (Figure 5E). Indeed, fat storage in *tra* mutant females
452 lacking APCs was not significantly different from w^{1118} control females (p=0.9384;
453 Supplementary file 1) (Figure 5E), indicating that the increased Akh pathway activity we
454 observed in *tra* mutant females was one reason for their reduced fat storage. Given that

455 APC activation in males expressing physiological levels of Tra similarly rescued the Tra-
456 induced increase in whole-body triglyceride levels (Figure 5F), these findings suggest
457 that the sex-specific regulation of Akh pathway activity represents one way *tra*
458 influences the male-female difference in fat storage.
459
460 **Loss of Adipokinetic hormone has opposite effects on reproductive success in**
461 **each sex and mediates a fecundity-lifespan tradeoff in females**
462
463 Our results suggest that adult females show lower Akh pathway activity and higher fat
464 storage, whereas males maintain a higher level of Akh activity and lower fat storage.
465 Because the correct regulation of fat storage in each sex influences reproduction
466 (Buszczak et al., 2002; Grönke et al., 2005; Sieber and Spradling, 2015; Wat et al.,
467 2020), we tested how loss of this critical regulator of the sex difference in fat storage
468 impacted offspring production in each sex. In Akh^{A} mutant males, we found that the
469 proportion of males copulating with a *Canton-S (CS)* virgin female was lower than in
470 control w^{1118} males at each 10 min interval during a 60 min observation period (Figure
471 6A). When we counted viable offspring from these copulation events, we found that
472 Akh^{A} mutant males had significantly fewer overall progeny than w^{1118} control males
473 (Figure 6B). These results suggest that Akh function normally promotes reproductive
474 success in males; however, it is important to note that Akh function is not absolutely
475 required for male fertility, as a prolonged 24 hr period of contact between Akh^{A} mutant
476 males and *CS* females allowed the production of normal progeny numbers (Figure 6C).
477 In contrast to males, loss of Akh in females increased fecundity (Figure 6D).
478 Specifically, Akh^{A} mutant females produced a significantly higher number of offspring
479 compared with w^{1118} controls (Figure 6D). Thus, in females, a low level of Akh pathway
480 activity promotes fecundity. Given that a change in one life history trait such as
481 reproduction often affects traits such as longevity (Chapman et al., 1995; Flatt, 2011;
482 Fowler and Partridge, 1989; Hansen et al., 2013), we also measured lifespan in females
483 with reduced Akh pathway function. We found that lifespan was significantly shorter in
484 *Akh-GAL4>UAS-Kir2.1* females compared with *Akh-GAL4>+* and *+>UAS-Kir2.1* control
485 females (Figure 6E). In contrast, male lifespan was not significantly different between

Akh-GAL4>UAS-Kir2.1 flies and *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls (Figure 6F). Our findings are in agreement with a previous study that demonstrated a female-specific reduction in lifespan in response to whole-body loss of *Akh* (Bednářová et al., 2018). This suggests that while low Akh activity in females promotes fertility, this benefit comes at the cost of a shorter lifespan.

DISCUSSION

In this study, we used the fruit fly *Drosophila melanogaster* to improve knowledge of the mechanisms underlying the male-female difference in whole-body triglyceride levels. We show that the presence of a functional Tra protein in females, which directs many aspects of female sexual development, promotes whole-body fat storage. Tra's ability to promote fat storage arises largely due to its function in neurons, where we identified the APCs as one neuronal population in which Tra function influences whole-body triglyceride levels. Our examination of *Akh*/*AkhR* mRNA levels and APC activity revealed several differences between the sexes, where these differences lead to higher Akh pathway activity in males than in females. Genetic manipulation of APCs and Akh pathway activity suggest a model in which the sex bias in Akh pathway activity contributes to the male-female difference in fat storage by limiting whole-body triglyceride storage in males. Importantly, we show that Tra function influences Akh pathway activity, and that Akh acts genetically downstream of Tra in regulating whole-body triglyceride levels. This reveals a previously unrecognized genetic and physiological mechanism that contributes to the sex difference in fat storage.

One key finding from our study was the identification of sex determination gene *tra* as an upstream regulator of the male-female difference in fat storage. In females, a functional Tra protein promotes fat storage, whereas lack of Tra in males leads to reduced fat storage. While an extensive body of literature has demonstrated important roles for *tra* in regulating neural circuits, behaviour, abdominal pigmentation, and gonad development (Anand et al., 2001; Baker et al., 2001; Billeter et al., 2006; Brown and King, 1961; Burtis and Baker, 1989; Camara et al., 2008; Christiansen et al., 2002; Clough et al., 2014; Dauwalder, 2011; Demir and Dickson, 2005; Goodwin et al., 2000;

517 Hall, 1994; Heinrichs et al., 1998; Hoshijima et al., 1991; Inoue et al., 1992; Ito et al.,
518 1996; Nagoshi et al., 1988; Neville et al., 2014; Nojima et al., 2014; Pavlou et al., 2016;
519 von Philipsborn et al., 2014; Pomatto et al., 2017; Rezával et al., 2014, 2016; Rideout et
520 al., 2007, 2010; Ryner et al., 1996; Sturtevant, 1945), uncovering a role for *tra* in
521 regulating fat storage significantly extends our understanding of how sex differences in
522 metabolism arise. Given that sex differences exist in other aspects of metabolism (*e.g*.
523 oxygen consumption) (Wat et al., 2020), this new insight suggests that more work will
524 be needed to determine whether *tra* contributes to sexual dimorphism in additional
525 metabolic traits. Indeed, one study showed that *tra* influences the sex difference in
526 adaptation to hydrogen peroxide stress (Pomatto et al., 2017). Beyond metabolism, Tra
527 also regulates multiple aspects of development and physiology such as intestinal stem
528 cell proliferation (Ahmed et al., 2020; Hudry et al., 2016; Millington and Rideout, 2018),
529 carbohydrate metabolism (Hudry et al., 2019), body size (Mathews et al., 2017; Rideout
530 et al., 2015), phenotypic plasticity (Millington et al., 2021), and lifespan responses to
531 dietary restriction (Regan et al., 2016). Because some, but not all, of these studies
532 identify a cell type in which Tra function influences these diverse phenotypes, future
533 studies will need to determine which cell types and tissues require Tra expression to
534 establish a female metabolic and physiological state. Indeed, recent single-cell analyses
535 reveal widespread gene expression differences in shared cell types between the sexes
536 (Li et al., 2021).
537 Identifying neurons as the anatomical focus of Tra's effects on fat storage was
538 another key finding from our study. While many sexually dimorphic neural circuits
539 related to behaviour and reproduction have been identified (Anand et al., 2001; Auer
540 and Benton, 2016; Baker et al., 2001; Billeter et al., 2006; Clyne and Miesenböck, 2008;
541 Dauwalder, 2011; Demir and Dickson, 2005; Evans and Cline, 2007; Goodwin et al.,
542 2000; Hall, 1994; Inoue et al., 1992; Ito et al., 1996; Kimura et al., 2019; Kvitsiani and
543 Dickson, 2006; Neville et al., 2014; Nojima et al., 2014; Pavlou et al., 2016; von
544 Philipsborn et al., 2014; Rezával et al., 2014, 2016; Rideout et al., 2007, 2010; Ryner et
545 al., 1996; Sato et al., 2019; Shirangi et al., 2016; Wang et al., 2020), less is known
546 about sex differences in neurons that regulate physiology and metabolism. Indeed,
547 while many studies have identified neurons that regulate fat metabolism (Al-Anzi and

548 Zinn, 2018; Al-Anzi et al., 2009; Chung et al., 2017; Li et al., 2016; May et al., 2020; Min
549 et al., 2016; Mosher et al., 2015; Zhan et al., 2016), these studies were conducted in
550 single- or mixed-sex populations. Because male-female differences in neuron number
551 (Billeter et al., 2006; Castellanos et al., 2013; Demir and Dickson, 2005; Garner et al.,
552 2018; Lee and Hall, 2001; Rideout et al., 2007, 2010; Robinett et al., 2010; Taylor and
553 Truman, 1992), morphology (Cachero et al., 2010; Kimura et al., 2019), activity (Guo et
554 al., 2016), and connectivity (Cachero et al., 2010; Nojima et al., 2021) have all been
555 described across the brain and ventral nerve cord (Mellert et al., 2010, 2016), a detailed
556 analysis of neuronal populations that influence metabolism will be needed in both sexes
557 to understand how neurons contribute to the sex-specific regulation of metabolism and
558 physiology. Indeed, while our identification of a role for APC sexual identity in regulating
559 the male-female difference in fat storage represents a significant step forward in
560 understanding how sex differences in neurons influence metabolic traits, more
561 knowledge is needed of sexual dimorphism in this critical neuronal subset.
562 An obvious starting point for learning more about the sex-specific regulation of fat
563 storage by the APCs is to examine how sexual identity influences known APC
564 regulatory mechanisms. For example, there are physical connections between
565 corazonin- and neuropeptide F (NPF; FBgn0027109)-positive (CN) neurons and APCs
566 in adult male flies (Oh et al., 2019), and between the APCs and a bursicon-α-responsive
567 subset of DLgr2 neurons in females (Scopelliti et al., 2019). These connections inhibit
568 APC activity: CN neurons inhibit APC activity in response to high hemolymph sugar
569 levels (Oh et al., 2019), whereas binding of bursicon-α to DLgr2 neurons inhibits APC
570 activity in nutrient-rich conditions (Scopelliti et al., 2019). Future studies will therefore
571 need to determine whether these physical connections exist in both sexes. Male-female
572 differences in circulating factors that regulate the APCs may also exist. For example,
573 gut-derived Allatostatin C (AstC; FBgn0032336) binds its receptor on the APCs to
574 trigger Akh release; however, loss of AstC affects fat metabolism and starvation
575 resistance in females but not in males (Kubrak et al., 2020). This suggests that sex
576 differences in AstC production or release may exist. Additionally, gut-derived NPF binds
577 to its receptor on APCs to inhibit Akh release (Yoshinari et al., 2021). Another
578 circulating factor that may influence the sex difference in fat storage is skeletal muscle-

579 derived unpaired 2 (upd2; FBgn0030904), which regulates hemolymph Akh levels to
580 maintain diurnal fat metabolism (Zhao and Karpac, 2017). Given that circulating
581 peptides such as Allatostatin A (AstA; FBgn0015591), *Drosophila* insulin-like peptides
582 (Dilps), and activin ligands also influence Akh pathway activity (Ahmad et al., 2020;
583 Hentze et al., 2015; Post et al., 2019; Song et al., 2017), a systematic survey of
584 circulating factors that modulate Akh production, release, and Akh pathway activity in
585 each sex will be needed to fully understand the sex-specific regulation of fat storage.
586 In addition to fat metabolism, it will be important to extend our understanding of
587 how sex-specific Akh regulation affects additional Akh-regulated phenotypes. For
588 example, Akh has been linked with the regulation of lifespan (Bednářová et al., 2018;
589 Liao et al., 2021), starvation resistance (Isabel et al., 2005; Kubrak et al., 2020;
590 Mochanová et al., 2018), locomotion (Isabel et al., 2005; Lee and Park, 2004), immune
591 responses (Adamo et al., 2008), cardiac function (Isabel et al., 2005; Noyes et al.,
592 1995), oxidative stress responses (Gáliková et al., 2015), and fertility (Liao et al., 2021).
593 Yet, most studies were performed in mixed- or single-sex populations. This suggests
594 additional work is needed to determine how changes to Akh pathway function affect
595 physiology, development, and life history in both sexes. Importantly, the lessons we
596 learn may also extend to other species. Akh signalling is highly conserved across
597 invertebrates (Gäde and Auerswald, 2003; Lorenz and Gäde, 2009; Staubli et al.,
598 2002b), and is functionally similar to the mammalian β-adrenergic and glucagon
599 systems (Grönke et al., 2007; Lee and Park, 2004; Staubli et al., 2002b). Because sex-
600 specific regulation of both glucagon and the β-adrenergic systems have been described
601 in mammalian models and in humans (Al-Gburi et al., 2017; Bell et al., 2001; Bilginoglu
602 et al., 2007; Brooks et al., 2015; Claustre et al., 1980; Dart et al., 2002; Davis et al.,
603 2000; Drake et al., 1998; Freedman et al., 1987; Hinojosa-Laborde et al., 1999; Hoeker
604 et al., 2014; Hogarth et al., 2007; Lafontan et al., 1997; Luzier et al., 1998; McIntosh et
605 al., 2011; Ng et al., 1993), detailed studies on sex-specific Akh regulation and function
606 in flies may provide vital clues into the mechanisms underlying male-female differences
607 in physiology and metabolism in other animals.
608
609 **MATERIALS AND METHODS**

610

611 ***Data availability.*** Details of all statistical tests and *p*-values are in Supplementary file 1.
612 All raw data generated in this study are in Supplementary file 2. All primer sequences
613 are in Supplementary file 3. Fly food media recipe is in Supplementary file 4. Original
614 image files for all images in this study are in their respective Source Data files.

615

616 ***Fly husbandry.*** Fly stocks were maintained at 25°C in a 12:12 light:dark cycle. All
617 larvae were reared at a density of 50 larvae per 10 ml of fly media (recipe in
618 Supplementary file 4). Males and females were separated either as early pupae by
619 gonad size, or late pupae by the presence of sex combs. Sex-transformed males and
620 females were distinguished by the presence (males) or absence (females) of B^SY.
621 Single-sex groups of 20 pupae were transferred to damp filter paper within a food vial
622 until eclosion. Unless otherwise stated, all experiments used 5- to 7-day-old flies.

623

624 ***Fly strains.*** We obtained the following strains from the Bloomington *Drosophila* Stock
625 Center: *Canton-S* (#64349), w^{1118} (#3605), *UAS-nGFP* (#4775), *UAS-Akh-RNAi*
626 (#27031), *UAS-tra*^{F} (#4590), *tra*^{1} (#675), *Df(3L)st-j7* (#5416), *UAS-NaChBac* (#9468),
627 *UAS-Kir2.1* (#6595), *UAS-reaper* (#5823), *UAS-CaLexA* (#66542). We obtained *Akh*^{A},
628 *AkhR*^{rev}, *AkhR*^{1}, *bmm*^{1}, and *AkhR*^{1}*;bmm*^{1} as kind gifts from Dr. Ronald Kühnlein
629 (Gáliková et al., 2015; Grönke et al., 2005, 2007), *tra*^{KO} and *tra*$^{F\ K\text{-}IN}$ as kind gifts from
630 Dr. Irene Miguel-Aliaga (Hudry et al., 2016, 2019), and *Mex-GAL4* as a kind gift from Dr.
631 Claire Thomas (Phillips and Thomas, 2006). The following GAL4 lines were used for
632 tissue-specific expression: *da-GAL4* (ubiquitous), *cg-GAL4* (fat body), *r4-GAL4* (fat
633 body), *Lsp2-GAL4* (fat body), *Myo1A-GAL4* (enterocytes), *Mex-GAL4* (enterocytes),
634 *dMef2-GAL4* (skeletal muscle), *repo-GAL4* (glia), *elav-GAL4* (neurons), *c587-GAL4*
635 (somatic cells of the gonad), *tj-GAL4* (somatic cells of the gonad), *nos-GAL4* (germ cells
636 of the gonad), *dimmed-GAL4* (peptidergic neurons), *TH-GAL4* (dopaminergic neurons),
637 *Tdc2-GAL4* (octopaminergic neurons), *VT030559-GAL4* (mushroom body neurons),
638 *dilp2-GAL4* (insulin-producing cells), *Akh-GAL4* (Akh-producing cells). All transgenic
639 stocks were backcrossed into a w^{1118} background for a minimum of 5 generations.

640

641 ***Adult weight.*** To measure adult weight, groups of ten flies were weighed in 1.5 ml
642 microcentrifuge tubes on an analytical balance (Mettler-Toledo, ME104).
643
644 ***RNA extraction, cDNA synthesis, and qPCR.*** One biological replicate consisted of
645 five flies homogenized in 200 µl of Trizol. RNA was extracted following manufacturer's
646 instructions, as previously described (Wat et al., 2020). cDNA was synthesized from
647 RNA using the Quantitect Reverse Transcription Kit (Qiagen, 205311). qPCR was used
648 to quantify relative mRNA transcript levels as previously described (Wat et al., 2020).
649 See Supplementary file 3 for a full list of primers.
650
651 ***Whole-body triglyceride measurements.*** One biological replicate consisted of five
652 flies homogenized in 200 µl of 0.1% Tween (AMresco, 0777-1L) in 1X PBS using 50 µl
653 of glass beads (Sigma, 11079110) agitated at 8 m/s for 5 seconds (OMNI International
654 Bead Ruptor 24). Assay was performed according to established protocols (Tennessen
655 et al., 2014) as previously described (Wat et al., 2020).
656
657 ***Western blotting.*** One biological replicate consisted of ten flies homogenized in
658 extraction buffer (females=200 µl, males=125 µl) containing 20 mM Hepes (pH 7.8), 450
659 mM NaCl, 25% glycerol, 50 mM NaF, 0.2 mM EDTA, 0.5% Triton X-100, 1 mM PMSF, 1
660 mM DTT, 1X cOmplete Protease Inhibitor Cocktail (Roche), and 1X PhosSTOP (Roche)
661 using 50 µl of glass beads (Sigma, 11079110) agitated at 8 m/s for 5 seconds (OMNI
662 International Bead Ruptor 24). Samples were incubated on ice for 5 min before cellular
663 debris was pelleted by centrifugation at 10,000 rpm for 5 min at 4°C and supernatant
664 was removed (Thermo Scientific, Heraeus Pico 21 centrifuge). Centrifugation was
665 repeated twice more to remove fat from the samples. Protein concentration of each
666 sample was determined by a Bradford Assay (Bio-Rad, 550-0205); 20 µg of protein per
667 sample was loaded onto a 12% SDS-PAGE gel. Immunoblotting was performed as
668 previously described (Millington et al., 2021). Primary antibodies used were rabbit anti-
669 p-Ire1 (1:1000; Abcam #48187) and mouse anti-actin (1:100; Santa Cruz #sc-8432).
670 Secondary antibodies used were goat anti-rabbit (1:5000; Invitrogen #65-6120) and
671 horse anti-mouse (1:2000; Cell Signaling #7076).

672

673 ***APC measurements.*** To isolate the APCs, individual flies were anesthetized on ice,
674 and the brain and foregut were removed in cold 1X PBS. Samples were fixed in 4%
675 paraformaldehyde for 30 min, followed by two 30 min washes in cold 1X PBS. Samples
676 were incubated with Hoechst (Sigma, 33342) at a concentration of 1:500 for 5 min and
677 mounted in SlowFade Diamond Antifade Mountant (ThermoFisher, S36967). Images
678 were captured using a Leica TCS SP5 Confocal Microscope and processed using Fiji
679 (ImageJ; Schindelin et al., 2012). To visualize APC neuronal activity (*Akh-GAL4>UAS-*
680 *CaLexA*), the mean GFP intensity of one APC cluster was quantified by measuring
681 average pixel intensity within the region of interest using Fiji (ImageJ; Schindelin et al.,
682 2012). To determine APC cell number (*Akh-GAL4>UAS-nGFP*), GFP punctae were
683 counted manually using Fiji (ImageJ; Schindelin et al., 2012). One biological replicate
684 consists of one cluster of APCs, where only one APC cluster was measured per
685 individual.

686

687 ***Capillary Feeder Assay.*** One biological replicate consisted of ten flies placed into a
688 specialized 15 ml conical vial with access to 2 capillary tubes. Capillary tubes were filled
689 with fly food media containing 5% sucrose, 5% yeast extract, 0.3% propionic acid, and
690 0.15% nipagin. Approximately 0.5 µl of mineral oil was added to the top of each capillary
691 tube to prevent evaporation. All vials were placed into fitted holes in the lid of a large
692 plastic container. A shallow layer of water was poured into the base of the container to
693 maintain high humidity throughout the experiment. The meniscus of the fly food media
694 was marked before the start of the experiment and again after 24 hr. The distance
695 between the marks is used to quantify the volume of fly food media that was consumed
696 (1 mm=0.15 µl). The volume of fly food consumed was normalized to the weight of
697 individual flies (protocol adapted from Stafford et al., 2012).

698

699 **Male fertility.** One singly-housed male was placed with a group of three virgin *Canton-*
700 *S (CS*) females and allowed to interact for 60 min. At 10 min intervals during the 60 min
701 observation period, we recorded whether a copulating male-female pair was present in
702 the vial. After the 60 min observation period, the male was removed from the vial and

703 the females were allowed to lay eggs for 72 hr (flies were transferred to new food every
704 24 hr). After 72 hr, the females were removed and progeny were allowed to develop.
705 After 10 days, we counted the number of pupae in each vial. For the 24 hr mating
706 assay, one singly-housed male was allowed to interact with three virgin *CS* females for
707 24 hr before the male was removed and females were allowed to lay eggs for 72 hr as
708 described above.
709
710 ***Female fecundity*.** One virgin female was placed with a group of three virgin *CS* males
711 for 24 hr. The females were then transferred onto fresh food every 24 hr for 3 days and
712 the number of pupae were counted as described above.
713
714 **Starvation resistance.** 5-day-old flies were transferred to vials containing 2 ml of
715 starvation media (0.7% agar in 1X PBS). The number of deaths was recorded every 12
716 hr until no living flies remained in the vial.
717
718 **Lifespan.** Flies were transferred to new vials with 2 ml of fresh food every 2-3 days until
719 no living flies remained in the vial. Deaths were recorded when the flies were
720 transferred.
721
722 ***Statistics and data presentation.*** All figures and data were generated and analyzed
723 using GraphPad Prism (v9.1.2). For experiments with 2 groups, a Student's *t*-test was
724 performed. For experiments with 3 or more groups, a one-way ANOVA with Tukey HSD
725 post-hoc test was performed. For fat breakdown experiments, a two-way ANOVA was
726 used to determine the interaction between genotype and time. Starvation resistance and
727 lifespan statistics were performed using RStudio and a script for a Log-rank test with
728 Bonferroni's correction for multiple comparisons. Note, the lowest *p*-value given by
729 RStudio is 2.0×10^{-16}. The below packages and script were used:
730
731 library ("survminer")
732 library ("survival")

```
data <- read.csv("xxx.csv")
survfit(Surv(time, event) ~ genotype, data)
pairwise_survdiff(Surv(time, event) ~ genotype, data, p.adjust.method = "bonferroni")
summary (data)
```


ACKNOWLEDGEMENTS

We would like to thank Dr. Ronald Kühnlein for *Akh*^{A}, *AkhR*^{rev}, *AkhR*^{1}, *bmm*^{1}, *and AkhR*^{1}*;bmm*^{1} fly strains. We would also like to thank Dr. Irene Miguel-Aliaga for the *tra*^{KO} and *tra*$^{F\ K\text{-}IN}$ strains, and Dr. Claire Thomas for *Mex-GAL4.* Stocks obtained from the Bloomington *Drosophila* Stock Center (NIH P40OD018537) were used in this study. We thank the TRiP at Harvard Medical School (NIH/NIGMS R01-GM084947) for providing transgenic RNAi fly stocks and/or plasmid vectors used in this study. We acknowledge critical resources and information provided by FlyBase (Thurmond et al., 2019); FlyBase is supported by a grant from the National Human Genome Research Institute at the U.S. National Institutes of Health (U41 HG000739) and by the British Medical Research Council (MR/N030117/1). Funding for this study was provided by grants to EJR from the Canadian Institutes for Health Research (CIHR, PJT-153072), CIHR Sex and Gender Science Chair Program (GS4-171365), Natural Sciences and Engineering Research Council of Canada (NSERC, RGPIN-2016-04249), Michael Smith Foundation for Health Research (16876), and the Canadian Foundation for Innovation (JELF-34879). LWW was supported by a British Columbia Graduate Scholarship Award and a 1-year CELL Fellowship from UBC. JWM and PB were each supported by a 4-year CELL Fellowship from UBC. We acknowledge that our research takes place on the traditional, ancestral, and unceded territory of the Musqueam people; a privilege for which we are grateful.

COMPETING INTERESTS STATEMENT

No competing interests declared.

MAIN FIGURE LEGENDS

763

764 **Figure 1. *transformer* regulates the sex difference in fat storage.** (A) Whole-body
765 triglyceride levels were significantly lower in *tra*^{1}*/Df(3L)st-j7* females compared with
766 *w*^{1118} controls (*p<0.0001*; Student's *t*-test). *n*=8 biological replicates. (B) Whole-body
767 triglyceride levels were significantly lower in *tra*^{1}*/Df(3L)st-j7* females with excised
768 gonads compared with *w*^{1118} controls lacking gonads (*p<0.0001*; Student's *t*-test). *n*=8
769 biological replicates. (C) Whole-body triglyceride levels were significantly lower in *tra*^{KO}
770 females compared with *w*^{1118} controls (*p*=0.0037; Student's *t*-test). *n*=8 biological
771 replicates. (D) Whole-body triglyceride levels were significantly higher in *da-*
772 *GAL4>UAS-tra*^{F} males compared with *da-GAL4*>+ and +>*UAS-tra*^{F} controls (*p*<0.0001
773 and *p*<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological
774 replicates. (E) Whole-body triglyceride levels were significantly higher in *tra*$^{F\ K\text{-}IN}$ males
775 compared with *w*^{1118} controls (*p*<0.0001, Student's *t*-test). *n*=8 biological replicates. (F)
776 Whole-body triglyceride levels were significantly higher in *tra*$^{F\ K\text{-}IN}$ males with excised
777 gonads compared with *w*^{1118} controls lacking gonads (*p*<0.0001; Student's *t*-test). *n*=8
778 biological replicates. Black circles indicate the presence of a transgene and open circles
779 indicate the lack of a transgene. ** indicates *p*<0.01, **** indicates *p*<0.0001; error bars
780 represent SEM.

781 **Figure 2. *transformer* function in Akh-producing cells contributes to the sex**
782 **difference in fat storage.** (A) Starvation resistance was significantly reduced in
783 *tra*^{1}*/Df(3L)st-j7* females compared with *w*^{1118} controls (*p*<2x10^{-16}; log-rank test,
784 Bonferroni's correction for multiple comparisons). *n*=344-502 animals. (B) Starvation
785 resistance was significantly enhanced in *da-GAL4>UAS-tra*^{F} males compared with *da-*
786 *GAL4*>+ and +>*UAS-tra*^{F} controls (*p*<2x10^{-16} and *p*<2x10^{-16} respectively; log-rank test,
787 Bonferroni's correction for multiple comparisons). *n*=198-201 animals. (C) Starvation
788 resistance was significantly enhanced in *elav-GAL4>UAS-tra*^{F} males compared with
789 *elav-GAL4*>+ and +>*UAS-tra*^{F} controls (*p*<2x10^{-16} and *p*<2x10^{-16} respectively; log-rank
790 test, Bonferroni's correction for multiple comparisons). *n*=248-279 animals. (D) Whole-
791 body triglyceride levels were significantly higher in *elav-GAL4>UAS-tra*^{F} males
792 compared with *elav-GAL4*>+ and +>*UAS-tra*^{F} controls (*p*=0.0001 and *p*=0.0006
793 respectively; one-way ANOVA followed by Tukey's HSD). *n*=7-8 biological replicates.

794 (E) Starvation resistance was significantly enhanced in *Akh-GAL4>UAS-tra^F* males
795 compared with *Akh-GAL4*>+ and +>*UAS-tra^F* controls (p=3.1x10^{-9} and p<2x10^{-16}
796 respectively; log-rank test, Bonferroni's correction for multiple comparisons). n=280-364
797 animals. (F) Whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-*
798 *tra^F* males compared to *Akh-GAL4*>+ and +>*UAS-tra^F* control males (p<0.0001 and
799 p<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological
800 replicates. Black circles indicate the presence of a transgene and open circles indicate
801 the lack of a transgene. *** indicates p<0.001, **** indicates p<0.0001; shaded areas
802 represent the 95% confidence interval; error bars represent SEM.

803 **Figure 3. Sex-specific regulation of Akh and the Akh signaling pathway.** (A) *Akh*
804 mRNA levels were significantly higher in *w^{1118}* males compared with genotype-matched
805 females (p<0.0001, Student's t-test). n=8 biological replicates. (B) *AkhR* mRNA levels
806 were significantly higher in *w^{1118}* males than in females (p=0.0002, Student's t-test). n=4
807 biological replicates. (C) Expression of *UAS-nGFP* in Akh-producing cells (APCs) (*Akh-*
808 *GAL4>UAS-nGFP*) revealed no significant difference in APC cell number between
809 males and females (p=0.1417; Student's t-test). n=8-12 animals. (D) GFP intensity
810 produced as a readout of calcium activity in the APCs (*Akh-GAL4>LexAop-CD8-*
811 *GFP;UAS-LexA-VP16-NFAT (UAS-CaLexA)*) was significantly higher in males
812 compared with females (p=0.0438; Student's t-test). n=6-8 biological replicates. (E-H)
813 Maximum Z-projections of representative images showing GFP produced as a readout
814 for APC calcium activity from both *Akh-GAL4>UAS-CaLexA* males and females. Scale
815 bars=50 µm, n=6-8 biological replicates. (I-K) Whole-body p-Ire1 levels were higher in
816 *w^{1118}* males compared with *w^{1118}* females in three biological replicates. * indicates
817 p<0.05, *** indicates p<0.001, **** indicates p<0.0001, ns indicates not significant; error
818 bars represent SEM. Original images for Figure 3C are found in Figure 3 – Source Data
819 1. Original images for Figure 3D-H are found in Figure 3 – Source Data 2. Original
820 images for Figure 3I-K are found in Figure 3 – Source Data 3.

821 **Figure 4. Sex-specific regulation of Akh and APC activity influence the sex**
822 **difference in fat storage.** (A) Whole-body triglyceride levels were significantly higher in
823 *Akh-GAL4>UAS-reaper (rpr)* males compared with *Akh-GAL4*>+ and +>*UAS-rpr*

824 controls (p=0.0002 and p=0.0215 respectively; one-way ANOVA followed by Tukey's
825 HSD). n=8 biological replicates. (B) Whole-body triglyceride levels were significantly
826 higher in *Akh*^{A} males compared with w^{1118} controls (p<0.0001; one-way ANOVA
827 followed by Tukey's HSD). n=8 biological replicates. (C) Whole-body triglyceride levels
828 were significantly higher in *AkhR*^{1} males compared with *AkhR*^{rev} controls (p<0.0001;
829 one-way ANOVA followed by Tukey's HSD). n=8 biological replicates. (D) Whole-body
830 triglyceride levels were significantly higher in *Akh-GAL4>UAS-Akh-RNAi* males
831 compared with *Akh-GAL4*>+ and +>*UAS-Akh-RNAi* controls (p=0.0015 and p=0.0002
832 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological replicates. (E)
833 Whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-Kir2.1* males
834 compared with *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls (p<0.0001 and p<0.0001
835 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological replicates. (F)
836 Whole-body triglyceride levels were significantly lower in *Akh-GAL4>UAS-NaChBac*
837 females compared with *Akh-GAL4*>+ and +>*UAS-NaChBac* controls (p<0.0001 and
838 p<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological
839 replicates. Due to independent experiments with a shared GAL4 control, *Akh-GAL4*>+
840 males are shared between Figure 4E and Figure 4 – figure supplement 3E. *Akh-*
841 *GAL4*>+ females are shared between Figure 4F and Figure 4 – figure supplement 3D.
842 Black circles indicate the presence of a transgene and open circles indicate the lack of a
843 transgene; * indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001, **** indicates
844 p<0.0001; error bars represent SEM.

845 **Figure 5. *transformer* regulates the sex difference in fat storage via the Akh**
846 **signalling pathway.** (A-C) Whole-body p-Ire1 levels were higher in *tra*^{1}*/Df(3L)st-j7*
847 females compared with w^{1118} controls in three biological replicates. (D) Whole-body *Akh*
848 mRNA levels were significantly higher in *tra*^{1}*/Df(3L)st-j7* females compared with w^{1118}
849 controls (p<0.0001; Student's t-test). n=8 biological replicates. (E) Whole-body
850 triglyceride levels were significantly lower in *tra*^{KO}*/Df(3L)st-j7* females carrying either
851 *Akh-GAL4*>+ or +>*UAS-reaper* (*rpr*) transgenes compared with w^{1118} controls carrying a
852 functional Tra protein (p<0.0001 and p<0.0001 respectively; one-way ANOVA followed
853 by Tukey's HSD). Whole-body triglyceride levels were not significantly different between
854 *tra*^{KO}*/Df(3L)st-j7* females lacking APCs (*Akh-GAL4>UAS-rpr*) and w^{1118} controls

855 (p=0.9384; one-way ANOVA followed by Tukey's HSD). n=8 biological replicates. (F)
856 Whole-body triglyceride levels were significantly higher in $tra^{F\ K\text{-}IN}$ males carrying either
857 *Akh-GAL4*>+ or +>*UAS-NaChBac* transgenes compared with w^{1118} control males
858 lacking Tra function (p<0.0001 and p<0.0001 respectively; one-way ANOVA followed by
859 Tukey's HSD). Whole-body triglyceride levels in $tra^{F\ K\text{-}IN}$ males with APC activation (*Akh-*
860 *GAL4>UAS-NaChBac*) were significantly lower than $tra^{F\ K\text{-}IN}$ males carrying either the
861 *Akh-GAL4*>+ or +>*UAS-NaChBac* transgenes alone (p<0.0001 and p<0.0001
862 respectively; one-way ANOVA followed by Tukey's HSD). n=5 biological replicates.
863 Black circles indicate the presence of a transgene or mutant allele and open circles
864 indicate the lack of a transgene or mutant allele. **** indicates p<0.0001, ns indicates
865 not significant; error bars represent SEM. Original images for Figure 5A-C are found in
866 Figure 5 – Source Data 1.

867 **Figure 6. Sex-specific regulation of Akh signalling pathway promotes**
868 **reproductive success in each sex.** (A) At all observation points, a lower proportion of
869 Akh^{A} males were successfully copulating with a wildtype *Canton-S* (*CS*) female
870 compared with w^{1118} controls. n=31 males. (B) The number of pupae produced from a
871 60 min mating period was significantly lower in Akh^{A} males compared with w^{1118} controls
872 (p=0.0003; Student's t-test). n=24-26 males. (C) The number of pupae produced from a
873 24 hr mating period was not significantly different between Akh^{A} males and w^{1118} control
874 males (p=0.2501; Student's t-test). n=24-25 males. (D) The number of pupae produced
875 from a 24 hr mating period was significantly higher in Akh^{A} females compared with w^{1118}
876 controls (p=0.0006; Student's t-test). n=28-36 females. (E) Lifespan was significantly
877 shorter in *Akh-GAL4>UAS-Kir2.1* females compared with *Akh-GAL4*>+ and +>*UAS-*
878 *Kir2.1* controls (p<2x10^{-16} and p=0.0015 respectively; log-rank test, Bonferroni's
879 correction for multiple comparisons). n=160-198 females. (F) Lifespan of *Akh-*
880 *GAL4>UAS-Kir2.1* males was intermediate between *Akh-GAL4*>+ and +>*UAS-Kir2.1*
881 controls, indicating no overall effect of inhibiting APC neuronal activity on male lifespan
882 (p=0.00013 and p=7.0x10^{-6} respectively; log-rank test, Bonferroni's correction for
883 multiple comparisons). n=196-200 males. ** indicates p<0.01, *** indicates p<0.001, ****
884 indicates p<0.0001, ns indicates not significant; error bars represent SEM; shaded
885 areas represent the 95% confidence interval.

886

887 **SUPPLEMENTAL FIGURE LEGENDS**

888

889 **Figure 1 - figure supplement 1. Elucidating *transformer*'s effect on sex differences**
890 **in fat metabolism.** (A) Whole-body triglyceride levels were not significantly different
891 between *tra*^{1}*/Df(3L)st-j7* males and *w*^{1118} controls (*p*=0.0685; Student's *t*-test). *n*=8
892 biological replicates. (B) The reduction in whole-body triglyceride levels post-starvation
893 was not significantly different between *tra*^{1}*/Df(3L)st-j7* animals and sex-matched *w*^{1118}
894 controls between 0-24 hr post-starvation (genotype:time *p*=0.6298 [female], *p*=0.3853
895 [male]; two-way ANOVA per sex). *n*=7-8 biological replicates. (C) Whole-body
896 triglyceride levels in *da-GAL4>UAS-tra*^{F} *f*emales were intermediate between *da-*
897 *GAL4*>+ and +>*UAS-tra*^{F} control females, indicating no overall effect of Tra
898 overexpression in females (*p*=0.0160 and *p*=0.0002 respectively; one-way ANOVA
899 followed by Tukey's HSD). *n*=7-8 biological replicates. (D) Whole-body mRNA levels of
900 ecdysone responsive genes were not higher in *tra*$^{F\ K\text{-}IN}$ males compared to *w*^{1118} control
901 males (*Ecdysone receptor* (*EcR*)*: p*<0.0001; *Ecdysone-induced protein 75B (E75):*
902 *p*=0.0072; *Ecdysone-induced protein 78C (E78): p*=0.0408; *broad (br): p*=0.0003; *ftz*
903 *transcription factor 1* (*ftz-f1*)*: p*=0.002; Student's *t*-test for each gene). *n*=7-8 biological
904 replicates. (E) The reduction in whole-body triglyceride levels between 0-24 hr post-
905 starvation was significantly smaller in *da-GAL4>UAS-tra*^{F} males compared with *da-*
906 *GAL4*>+ and +>*UAS-tra*^{F} control males (genotype:time *p*<0.0001; two-way ANOVA).
907 The post-starvation reduction in triglyceride levels in *da-GAL4>UAS-tra*^{F} females was
908 intermediate between both *da-GAL4*>+ and +>*UAS-tra*^{F} controls, suggesting no overall
909 effect of Tra overexpression on female fat storage (genotype:time *p*=0.0223; two-way
910 ANOVA per sex). *n*=7-8 biological replicates. (F) The reduction in whole-body
911 triglyceride levels between 0-24 hr post-starvation was significantly lower in *tra*$^{F\ K\text{-}IN}$
912 males, but not females, compared with sex-matched *w*^{1118} controls (genotype:time
913 *p*=0.0009 [male], *p*=0.9024 [female]; two-way ANOVA per sex). *n*=7-8 biological
914 replicates. F indicates female, M indicates male. Black circles indicate the presence of a
915 transgene and open circles indicate the lack of a transgene. * indicates *p*<0.05, **
916 indicates *p*<0.01, *** indicates *p*<0.001, **** indicates *p*<0.0001, ns indicates not

917 significant; error bars represent SEM except for graphs displaying fat breakdown where
918 error bars represent COE.
919
920 **Figure 2 - figure supplement 1. Effect of *transformer* gain in multiple cell types**
921 **and tissues on starvation resistance.** (A-F) Limited to no effects of Tra
922 overexpression on starvation resistance were observed for female fat body, muscle, and
923 gut (for individual *p*-values see Supplementary file 1; log-rank test with Bonferroni's
924 correction for multiple comparisons). In males, Tra overexpression in the fat body and
925 gut caused no extension of starvation resistance, with only a minor extension observed
926 upon Tra overexpression in muscle (for individual *p*-values see Supplementary file 1;
927 log-rank test with Bonferroni's correction for multiple comparisons). (A) n=397-413
928 females, n=295-431 males. (B) n=187-206 females, n=198-202 males. (C) n=293-402
929 females, n=330-452 males. (D) n=268-383 females, n=363-409 males. (E) n=226-374
930 females, n=250-362 males. (F) n=168-206 females, n=178-198 males. ** indicates
931 $p<0.01$, *** indicates $p<0.001$, **** indicates $p<0.0001$, ns indicates not significant;
932 shaded areas represent the 95% confidence interval.

933 **Figure 2 - figure supplement 2. Effect of *transformer* gain in additional cell types**
934 **and tissues on starvation resistance.** (A-D) Limited to no effects of Tra
935 overexpression were observed upon Tra expression in the female gonad and glia (for
936 individual *p*-values see Supplementary file 1; log-rank test with Bonferroni's correction
937 for multiple comparisons). Limited to no effects of Tra overexpression were observed
938 upon Tra expression in the male gonad and glia (for individual *p*-values see
939 Supplementary file 1; log-rank test with Bonferroni's correction for multiple
940 comparisons). (A) n=232-268 females, n=318-400 males. (B) n=234-349 females,
941 n=327-349 males. (C) n=374-442 females, n=293-364 males. (D) n=300-374 females,
942 n=329-355 males. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, ****
943 indicates $p<0.0001$, ns indicates not significant; shaded areas represent the 95%
944 confidence interval.

945 **Figure 2 - figure supplement 3. Gain of *transformer* function in neurons does not**
946 **affect fat breakdown.** (A) Starvation resistance in *elav-GAL4>UAS-tra*^{F} females was

947 not significantly different compared with *elav-GAL4*>+ and +>*UAS-tra*^{F} controls (*p*=0.3
948 and *p*=1, respectively; log-rank test with Bonferroni's correction for multiple
949 comparisons). *n*=318-749 females. (B) Whole-body triglyceride levels were not
950 significantly different between *elav-GAL4*>*UAS-tra*^{F} females and *elav-GAL4*>+ and
951 +>*UAS-tra*^{F} controls (*p*=0.3224 and *p*=0.7754 respectively; one-way ANOVA followed
952 by Tukey's HSD). *n*=8 biological replicates. (C) The reduction in whole-body triglyceride
953 levels post-starvation was not significantly different between *elav-GAL4*>*UAS-tra*^{F} flies
954 and sex-matched *elav-GAL4*>+ and +>*UAS-tra*^{F} controls (genotype:time *p*=0.2789
955 [male], *p*=0.7058 [female]; two-way ANOVA per sex). *n*=7-8 biological replicates. Black
956 circles indicate the presence of a transgene and open circles indicate the lack of a
957 transgene; ns indicates not significant; shaded areas represent the 95% confidence
958 interval; error bars represent SEM except for graphs displaying fat breakdown where
959 error bars represent COE.

960 **Figure 2 - figure supplement 4. Effect of *transformer* gain in multiple neuronal**
961 **subsets on starvation resistance.** (A-E) Limited to no effects of Tra overexpression in
962 several subsets of neurons on starvation resistance in females (for individual *p*-values
963 see Supplementary file 1; log-rank test with Bonferroni's correction for multiple
964 comparisons). In males, Tra overexpression in several subsets of neurons caused no
965 extension of starvation resistance (for individual *p*-values see Supplementary file 1; log-
966 rank test with Bonferroni's correction for multiple comparisons). (A) *n*=248-333 females,
967 *n*=249-333 males. (B) *n*=322-484 females, *n*=314-516 males. (C) *n*=282-478 females,
968 *n*=364-516 males. (D) *n*=256-343 females, *n*=326-392 males. (E) *n*=326-390 females,
969 *n*=285-466 males. * indicates *p*<0.05, **** indicates *p*<0.0001, ns indicates not
970 significant; shaded areas represent the 95% confidence interval.

971 **Figure 2 - figure supplement 5. Gain of *transformer* function in Akh-producing**
972 **cells does not affect fat breakdown.** (A) Starvation resistance was significantly
973 extended in *Akh-GAL4*>*UAS-tra*^{F} females compared with *Akh-GAL4*>+ and +>*UAS-tra*^{F}
974 controls (*p*=0.00033 and *p*=9.4x10^{-11} respectively; log-rank test with Bonferroni's
975 correction for multiple comparisons). *n*=168-219 females. (B) Whole-body triglyceride
976 levels were not significantly different between *Akh-GAL4*>*UAS-tra*^{F} females and *Akh-*

977 *GAL4*>+ and +>*UAS-tra*^{F} controls (*p*=0.2195 and *p*=0.0731 respectively; one-way
978 ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (C) The reduction in
979 whole-body triglyceride levels post-starvation was not significantly different between
980 *Akh-GAL4>UAS-tra*^{F} animals and sex-matched *Akh-GAL4*>+ and +>*UAS-tra*^{F} controls
981 (genotype:time *p*=0.1201 [males], *p*=0.0596 [female]; two-way ANOVA per sex). *n*=8
982 biological replicates. Black circles indicate the presence of a transgene and open circles
983 indicate the lack of a transgene; *** indicates *p*<0.001, **** indicates *p*<0.0001, ns
984 indicates not significant; shaded areas represent the 95% confidence interval; error bars
985 represent SEM except for graphs displaying fat breakdown where error bars represent
986 COE.

987 **Figure 3 - figure supplement 1. *Akh-GAL4* drives equivalent *GAL4* mRNA levels in**
988 **both sexes.** (A) Whole-body *GAL4* mRNA levels were not significantly different
989 between *Akh-GAL4*>+ females and males (*p*=0.0687; Student's *t*-test). *n*=8 biological
990 replicates. (B) Whole-body p-Ire1 levels were not higher in w^{1118} males compared with
991 w^{1118} females in one biological replicate. ns indicates not significant; error bars
992 represent SEM. Original image for Figure 3 – figure supplement 1B is found in Figure 3
993 – figure supplement 1 - Source Data 1.

994 **Figure 4 - figure supplement 1. APC-derived Limostatin does not regulate the sex**
995 **difference in fat storage.** (A) Whole-body triglyceride levels were not significantly
996 different between *Akh-GAL4>UAS-reaper (rpr)* females and *Akh-GAL4*>+ and +>*UAS-*
997 *rpr* controls (*p*=0.3024 and *p*=0.4673 respectively; one-way ANOVA followed by Tukey's
998 HSD). *n*=8 biological replicates. (B) Whole-body triglyceride levels were significantly
999 higher in *Akh*^{A} females compared with w^{1118} controls (*p*=0.0152; one-way ANOVA
1000 followed by Tukey's HSD). *n*=8 biological replicates. (C) Whole-body triglyceride levels
1001 were significantly higher in *AkhR*^{1} females compared with *AkhR*^{rev} control females
1002 (*p*<0.0001; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (D)
1003 Whole-body triglyceride levels in *Akh-GAL4>UAS-Limostatin (Lst)-RNAi* males were not
1004 significantly different from both *Akh-GAL4*>+ and +>*UAS-Lst-RNAi* controls (*p*=0.0357
1005 and *p*=0.2364 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological
1006 replicates. (E) Whole-body triglyceride levels in *Akh-GAL4>UAS-Lst-RNAi* females were

1007 not significantly different from both *Akh-GAL4*>+ and +>*UAS-Lst-RNAi* controls
1008 (p<0.0001 and p=0.6656 respectively; one-way ANOVA followed by Tukey's HSD). n=8
1009 biological replicates. Black circles indicate the presence of a transgene and open circles
1010 indicate the lack of a transgene. * indicates p<0.05, **** indicates p<0.0001, ns
1011 indicates not significant; error bars represent SEM.

1012 **Figure 4 - figure supplement 2. Akh and *brummer* operate in parallel pathways to**
1013 **regulate the sex difference in fat storage.** (A) Whole-body triglyceride levels were
1014 significantly higher in $AkhR^1$ and bmm^1 males, respectively, compared with w^{1118}
1015 controls (p<0.0001 and p<0.0001; one-way ANOVA followed by Tukey's HSD). Whole-
1016 body triglyceride levels were further increased in $AkhR^1; bmm^1$ males compared with
1017 $AkhR^1$ males and bmm^1 males (p<0.0001 and p<0.0001 respectively; one-way ANOVA
1018 followed by Tukey's HSD). n=7-8 biological replicates. (B) Whole-body triglyceride
1019 levels were significantly higher in $AkhR^1$ females compared with w^{1118} controls; however
1020 whole-body triglyceride levels were not significantly different between bmm^1 females
1021 and w^{1118} control females (p=0.002 and p=0.4256 respectively; one-way ANOVA
1022 followed by Tukey's HSD). Whole-body triglyceride levels were further increased in
1023 $AkhR^1; bmm^1$ females compared to $AkhR^1$ females and bmm^1 females (p=0.0024 and
1024 p<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological
1025 replicates. Black circles indicate the presence of a mutant allele and open circles
1026 indicate the lack of a mutant allele. ** indicates p<0.01, **** indicates p<0.0001, ns
1027 indicates not significant; error bars represent SEM.

1028 **Figure 4 - figure supplement 3. RNAi-mediated Akh knockdown effectively**
1029 **reduced Akh transcripts in both sexes.** (A) *Akh* mRNA levels in the head and anterior
1030 half of the thorax were significantly lower in *Akh-GAL4>UAS-Akh-RNAi* males compared
1031 with *Akh-GAL4*>+ controls (p=0.0008; Student's t-test). n=5-8 biological replicates. (B)
1032 *Akh* mRNA levels in the head and anterior half of the thorax were significantly lower in
1033 *Akh-GAL4>UAS-Akh-RNAi* females compared with *Akh-GAL4*>+ controls (p<0.0001;
1034 Student's t-test). n=8 biological replicates. (C) Whole-body triglyceride levels were not
1035 significantly different between *Akh-GAL4>UAS-Akh-RNAi* females and both *Akh-*
1036 *GAL4*>+ and +>*UAS-Akh-RNAi* controls (p=0.0136 and p=0.4845 respectively; one-way

1037 ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (D) Whole-body
1038 triglyceride levels were significantly higher in *Akh-GAL4>UAS-Kir2.1* females compared
1039 with *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls (*p*=0.0001 and *p*=0.0022 respectively;
1040 one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (E) Whole-body
1041 triglyceride levels were significantly lower in *Akh-GAL4>UAS-NaChBac* males
1042 compared with *Akh-GAL4*>+ and +>*UAS-NaChBac* controls (*p*<0.0001 and *p*<0.0001
1043 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. Due
1044 to independent experiments with a shared GAL4 control, *Akh-GAL4*>+ males are
1045 shared between Figure 4E and Figure 4 – figure supplement 3E. *Akh-GAL4*>+ females
1046 are shared between Figure 4F and Figure 4 – figure supplement 3D. Black circles
1047 indicate the presence of a transgene and open circles indicate the lack of a transgene.
1048 ** indicates *p*<0.01, *** indicates *p*<0.001, **** indicates *p*<0.0001, ns indicates not
1049 significant; error bars represent SEM.

1050 **Figure 4 - figure supplement 4. Activity of the Akh-producing cells does not**
1051 **regulate food consumption in either sex.** (A) Food consumption was not significantly
1052 different between *Akh-GAL4>UAS-Kir2.1* females and *Akh-GAL4*>+ and +>*UAS-Kir2.1*
1053 controls (*p*=0.6488 and *p*=0.0539 respectively; one-way ANOVA followed by Tukey's
1054 HSD). *n*=8 biological replicates. (B) Food consumption was not significantly different
1055 between *Akh-GAL4>UAS-Kir2.1* males and *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls
1056 (*p*=0.3623 and *p*=0.0638 respectively; one-way ANOVA followed by Tukey's HSD). *n*=7-
1057 8 biological replicates. (C) Food consumption was not significantly different between
1058 *Akh-GAL4>UAS-NaChBac* females and *Akh-GAL4*>+ and +>*UAS-NaChBac* controls
1059 (*p*=0.9369 and *p*=0.9571 respectively; one-way ANOVA followed by Tukey's HSD). *n*=7-
1060 8 biological replicates. (D) Food consumption was not significantly different between
1061 *Akh-GAL4>UAS- NaChBac* males and both *Akh-GAL4*>+ and +>*UAS-NaChBac*
1062 controls (*p*=0.0266 and *p*=0.8141 respectively; one-way ANOVA followed by Tukey's
1063 HSD). *n*=8 biological replicates. Due to independent experiments with a shared GAL4
1064 control, *Akh-GAL4*>+ females are shared between Figure 4 – figure supplement 4A and
1065 Figure 4 – figure supplement 4C. *Akh-GAL4*>+ males are shared between Figure 4 –
1066 figure supplement 4B and Figure 4 – figure supplement 4D. Black circles indicate the

presence of a transgene and open circles indicate the lack of a transgene. ns indicates not significant; error bars represent SEM.

Figure 5 - figure supplement 1. Whole-body p-Ire1 levels in *transformer* mutant flies. (A) Whole-body p-Ire1 levels were not higher in *tra*^{1}*/Df(3L)st-j7* females compared with *w*^{1118} control females in one biological replicate. Black circles indicate the presence of a mutant allele and open circles indicate the lack of a mutant allele. Original image for Figure 5 – figure supplement 1A is found in Figure 5 – figure supplement 1 – Source Data 1.

SOURCE DATA LEGENDS

Figure 3 – Source Data 1. Images used to quantify number of Akh-producing cells.
Figure 3 – Source Data 2. Images used to quantify neuronal activity of Akh-producing cells.
Figure 3 – Source Data 3. Original blots for p-Ire1 and actin in males and females.
Figure 3 – figure supplement 1 – Source Data 1. Original blots for p-Ire1 and actin in male vs. female.
Figure 5 – Source Data 1. Original blots for p-Ire1 and actin in females with whole-body loss of *transformer*.
Figure 5- figure supplement 1 – Source Data 1. Original blots for p-Ire1 and actin in females with whole body loss of *transformer.*

REFERENCES

Adamo, S.A., Roberts, J.L., Easy, R.H., and Ross, N.W. (2008). Competition between immune function and lipid transport for the protein apolipophorin III leads to stress-induced immunosuppression in crickets. J. Exp. Biol. *211*, 531–538.

Ahmad, M., He, L., and Perrimon, N. (2020). Regulation of insulin and adipokinetic hormone/glucagon production in flies. Wiley Interdiscip. Rev. Dev. Biol. *9*, e360.

Ahmed, S.M.H., Maldera, J.A., Krunic, D., Paiva-Silva, G.O., Pénalva, C., Teleman, A.A., and Edgar, B.A. (2020). Fitness trade-offs incurred by ovary-to-gut steroid signaling in *Drosophila*. Nature *584*, 415–419.

Al-Anzi, B., and Zinn, K. (2018). Identification and characterization of mushroom body neurons that regulate fat storage in *Drosophila*. Neural Develop. *13*, 18.

Al-Anzi, B., Sapin, V., Waters, C., Zinn, K., Wyman, R.J., and Benzer, S. (2009). Obesity-blocking neurons in *Drosophila*. Neuron *63*, 329–341.

Alfa, R.W., Park, S., Skelly, K.-R., Poffenberger, G., Jain, N., Gu, X., Kockel, L., Wang, J., Liu, Y., Powers, A.C., et al. (2015). Suppression of insulin production and secretion by a decretin hormone. Cell Metab. *21*, 323–334.

Al-Gburi, S., Deussen, A., Zatschler, B., Weber, S., Künzel, S., El-Armouche, A., Lorenz, K., Cybularz, M., Morawietz, H., and Kopaliani, I. (2017). Sex-difference in expression and function of beta-adrenoceptors in macrovessels: role of the endothelium. Basic Res. Cardiol. *112*, 29.

Anand, A., Villella, A., Ryner, L.C., Carlo, T., Goodwin, S.F., Song, H.J., Gailey, D.A., Morales, A., Hall, J.C., Baker, B.S., et al. (2001). Molecular genetic dissection of the sex-specific and vital functions of the *Drosophila melanogaster* sex determination gene *fruitless*. Genetics *158*, 1569–1595.

Andretic, R., and Shaw, P.J. (2005). Essentials of Sleep Recordings in *Drosophila*: Moving Beyond Sleep Time. In Methods in Enzymology, M.W. Young, ed. (Academic Press), pp. 759–772.

Arrese, E.L., Rivera, L., Masakazu, H., Mirza, S., Hartson, S.D., Weintraub, S., and Soulages, J.L. (2008). FUNCTION AND STRUCTURE OF LIPID STORAGE DROPLET PROTEIN 1 STUDIED IN LIPOPROTEIN COMPLEXES. Arch. Biochem. Biophys. *473*, 42–47.

Auer, T.O., and Benton, R. (2016). Sexual circuitry in *Drosophila*. Curr. Opin. Neurobiol. *38*, 18–26.

Austad, S.N., and Fischer, K.E. (2016). Sex Differences in Lifespan. Cell Metab. *23*, 1022–1033.

1122 Baines, R.A., Uhler, J.P., Thompson, A., Sweeney, S.T., and Bate, M. (2001). Altered
1123 Electrical Properties in *Drosophila* Neurons Developing without Synaptic Transmission.
1124 J. Neurosci. *21*, 1523–1531.

1125 Baker, B.S., Taylor, B.J., and Hall, J.C. (2001). Are complex behaviors specified by
1126 dedicated regulatory genes? Reasoning from *Drosophila*. Cell *105*, 13–24.

1127 Ballard, S.L., Jarolimova, J., and Wharton, K.A. (2010). Gbb/BMP signaling is required
1128 to maintain energy homeostasis in *Drosophila*. Dev. Biol. *337*, 375–385.

1129 Baumbach, J., Xu, Y., Hehlert, P., and Kühnlein, R.P. (2014). Gαq, Gγ1 and Plc21C
1130 Control *Drosophila* Body Fat Storage. J. Genet. Genomics *41*, 283–292.

1131 Bednářová, A., Tomčala, A., Mochanová, M., Kodrík, D., and Krishnan, N. (2018).
1132 Disruption of Adipokinetic Hormone Mediated Energy Homeostasis Has Subtle Effects
1133 on Physiology, Behavior and Lipid Status During Aging in *Drosophila*. Front. Physiol. *9*.

1134 Bell, C., Seals, D.R., Monroe, M.B., Day, D.S., Shapiro, L.F., Johnson, D.G., and Jones,
1135 P.P. (2001). Tonic sympathetic support of metabolic rate is attenuated with age,
1136 sedentary lifestyle, and female sex in healthy adults. J. Clin. Endocrinol. Metab. *86*,
1137 4440–4444.

1138 Bell, L.R., Maine, E.M., Schedl, P., and Cline, T.W. (1988). *Sex-lethal*, a *Drosophila* sex
1139 determination switch gene, exhibits sex-specific RNA splicing and sequence similarity to
1140 RNA binding proteins. Cell *55*, 1037–1046.

1141 Beller, M., Bulankina, A.V., Hsiao, H.-H., Urlaub, H., Jäckle, H., and Kühnlein, R.P.
1142 (2010). PERILIPIN-dependent control of lipid droplet structure and fat storage in
1143 *Drosophila*. Cell Metab. *12*, 521–532.

1144 Belmonte, R.L., Corbally, M.-K., Duneau, D.F., and Regan, J.C. (2020). Sexual
1145 Dimorphisms in Innate Immunity and Responses to Infection in *Drosophila*
1146 *melanogaster*. Front. Immunol. *10*, 3075.

1147 Belote, J.M., McKeown, M., Boggs, R.T., Ohkawa, R., and Sosnowski, B.A. (1989).
1148 Molecular genetics of *transformer*, a genetic switch controlling sexual differentiation in
1149 *Drosophila*. Dev. Genet. *10*, 143–154.

1150 Bharucha, K.N., Tarr, P., and Zipursky, S.L. (2008). A glucagon-like endocrine pathway
1151 in *Drosophila* modulates both lipid and carbohydrate homeostasis. J. Exp. Biol. *211*,
1152 3103–3110.

1153 Bi, J., Xiang, Y., Chen, H., Liu, Z., Grönke, S., Kühnlein, R.P., and Huang, X. (2012).
1154 Opposite and redundant roles of the two *Drosophila* perilipins in lipid mobilization. J.
1155 Cell Sci. *125*, 3568–3577.

1156 Bickel, P.E., Tansey, J.T., and Welte, M.A. (2009). PAT proteins, an ancient family of
1157 lipid droplet proteins that regulate cellular lipid stores. Biochim. Biophys. Acta *1791*,
1158 419–440.

1159 Bilginoglu, A., Amber Cicek, F., Ugur, M., Gurdal, H., and Turan, B. (2007). The role of
1160 gender differences in beta-adrenergic receptor responsiveness of diabetic rat heart.
1161 Mol. Cell. Biochem. *305*, 63–69.

1162 Billeter, J.-C., Rideout, E.J., Dornan, A.J., and Goodwin, S.F. (2006). Control of male
1163 sexual behavior in *Drosophila* by the sex determination pathway. Curr. Biol. CB *16*,
1164 R766-776.

1165 Bjedov, I., Toivonen, J.M., Kerr, F., Slack, C., Jacobson, J., Foley, A., and Partridge, L.
1166 (2010). Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila*
1167 *melanogaster*. Cell Metab. *11*, 35–46.

1168 Boggs, R.T., Gregor, P., Idriss, S., Belote, J.M., and McKeown, M. (1987). Regulation of
1169 sexual differentiation in *D. melanogaster* via alternative splicing of RNA from the
1170 *transformer* gene. Cell *50*, 739–747.

1171 Braco, J.T., Gillespie, E.L., Alberto, G.E., Brenman, J.E., and Johnson, E.C. (2012).
1172 Energy-dependent modulation of glucagon-like signaling in *Drosophila* via the AMP-
1173 activated protein kinase. Genetics *192*, 457–466.

1174 Bridges, C.B. (1921). TRIPLOID INTERSEXES IN *DROSOPHILA MELANOGASTER*.
1175 Science *54*, 252–254.

1176 Brooks, V.L., Shi, Z., Holwerda, S.W., and Fadel, P.J. (2015). Obesity-induced
1177 increases in sympathetic nerve activity: sex matters. Auton. Neurosci. Basic Clin. *187*,
1178 18–26.

1179 Broughton, S.J., Piper, M.D.W., Ikeya, T., Bass, T.M., Jacobson, J., Driege, Y.,
1180 Martinez, P., Hafen, E., Withers, D.J., Leevers, S.J., et al. (2005). Longer lifespan,
1181 altered metabolism, and stress resistance in *Drosophila* from ablation of cells making
1182 insulin-like ligands. Proc. Natl. Acad. Sci. U. S. A. *102*, 3105–3110.

1183 Brown, E.H., and King, R.C. (1961). Studies on the Expression of the *Transformer* Gene
1184 of *Drosophila Melanogaster*. Genetics *46*, 143–156.

1185 Burtis, K.C., and Baker, B.S. (1989). *Drosophila doublesex* gene controls somatic
1186 sexual differentiation by producing alternatively spliced mRNAs encoding related sex-
1187 specific polypeptides. Cell *56*, 997–1010.

1188 Buszczak, M., Lu, X., Segraves, W.A., Chang, T.Y., and Cooley, L. (2002). Mutations in
1189 the *midway* gene disrupt a *Drosophila* acyl coenzyme A: diacylglycerol acyltransferase.
1190 Genetics *160*, 1511–1518.

1191 Cachero, S., Ostrovsky, A.D., Yu, J.Y., Dickson, B.J., and Jefferis, G.S.X.E. (2010).
1192 Sexual dimorphism in the fly brain. Curr. Biol. CB *20*, 1589–1601.

1193 Camara, N., Whitworth, C., and Van Doren, M. (2008). The creation of sexual
1194 dimorphism in the *Drosophila* soma. Curr. Top. Dev. Biol. *83*, 65–107.

1195 Castellanos, M.C., Tang, J.C.Y., and Allan, D.W. (2013). Female-biased dimorphism
1196 underlies a female-specific role for post-embryonic Ilp7 neurons in *Drosophila* fertility.
1197 Dev. Camb. Engl. *140*, 3915–3926.

1198 Chandegra, B., Tang, J.L.Y., Chi, H., and Alic, N. (2017). Sexually dimorphic effects of
1199 dietary sugar on lifespan, feeding and starvation resistance in *Drosophila*. Aging *9*,
1200 2521–2528.

1201 Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F., and Partridge, L. (1995). Cost of
1202 mating in *Drosophila melanogaster* females is mediated by male accessory gland
1203 products. Nature *373*, 241–244.

1204 Choi, S., Lim, D.-S., and Chung, J. (2015). Feeding and Fasting Signals Converge on
1205 the LKB1-SIK3 Pathway to Regulate Lipid Metabolism in *Drosophila*. PLoS Genet. *11*,
1206 e1005263.

1207 Christiansen, A.E., Keisman, E.L., Ahmad, S.M., and Baker, B.S. (2002). Sex comes in
1208 from the cold: the integration of sex and pattern. Trends Genet. TIG *18*, 510–516.

1209 Chung, B.Y., Ro, J., Hutter, S.A., Miller, K.M., Guduguntla, L.S., Kondo, S., and
1210 Pletcher, S.D. (2017). *Drosophila* Neuropeptide F Signaling Independently Regulates
1211 Feeding and Sleep-Wake Behavior. Cell Rep. *19*, 2441–2450.

1212 Claustre, J., Peyrin, L., Fitoussi, R., and Mornex, R. (1980). Sex differences in the
1213 adrenergic response to hypoglycemic stress in human. Psychopharmacology (Berl.) *67*,
1214 147–153.

1215 Cline, T.W. (1978). Two closely linked mutations in *Drosophila melanogaster* that are
1216 lethal to opposite sexes and interact with *daughterless*. Genetics *90*, 683–698.

1217 Cline, T.W., and Meyer, B.J. (1996). Vive la différence: males vs females in flies vs
1218 worms. Annu. Rev. Genet. *30*, 637–702.

1219 Clough, E., Jimenez, E., Kim, Y.-A., Whitworth, C., Neville, M.C., Hempel, L.U., Pavlou,
1220 H.J., Chen, Z.-X., Sturgill, D., Dale, R.K., et al. (2014). Sex- and tissue-specific
1221 functions of *Drosophila* doublesex transcription factor target genes. Dev. Cell *31*, 761–
1222 773.

1223 Clyne, J.D., and Miesenböck, G. (2008). Sex-specific control and tuning of the pattern
1224 generator for courtship song in *Drosophila*. Cell *133*, 354–363.

1225 Dart, A.M., Du, X.-J., and Kingwell, B.A. (2002). Gender, sex hormones and autonomic
1226 nervous control of the cardiovascular system. Cardiovasc. Res. *53*, 678–687.

1227 Dauwalder, B. (2011). The roles of fruitless and doublesex in the control of male
1228 courtship. Int. Rev. Neurobiol. *99*, 87–105.

1229 Davis, S.N., Shavers, C., and Costa, F. (2000). Differential gender responses to
1230 hypoglycemia are due to alterations in CNS drive and not glycemic thresholds. Am. J.
1231 Physiol. Endocrinol. Metab. *279*, E1054-1063.

1232 Demir, E., and Dickson, B.J. (2005). *fruitless* splicing specifies male courtship behavior
1233 in *Drosophila*. Cell *121*, 785–794.

1234 DiAngelo, J.R., and Birnbaum, M.J. (2009). Regulation of fat cell mass by insulin in
1235 *Drosophila melanogaster.* Mol. Cell. Biol. *29*, 6341–6352.

1236 Drake, K., Gateva, E., Deutsch, J., and Cohen, W.R. (1998). Sex differences in the
1237 adrenal catecholamine response to hypoglycemia in rats. Metabolism. *47*, 121–124.

1238 Evans, D.S., and Cline, T.W. (2007). *Drosophila melanogaster* Male Somatic Cells
1239 Feminized Solely by TraF Can Collaborate With Female Germ Cells to Make Functional
1240 Eggs. Genetics *175*, 631–642.

1241 Ferveur, J.F., Stortkuhl, K.F., Stocker, R.F., and Greenspan, R.J. (1995). Genetic
1242 feminization of brain structures and changed sexual orientation in male *Drosophila*.
1243 Science *267*, 902–905.

1244 Flatt, T. (2011). Survival costs of reproduction in *Drosophila*. Exp. Gerontol. *46*, 369–
1245 375.

1246 Fowler, K., and Partridge, L. (1989). A cost of mating in female fruitflies. Nature *338*,
1247 760–761.

1248 Francis, V.A., Zorzano, A., and Teleman, A.A. (2010). dDOR is an EcR coactivator that
1249 forms a feed-forward loop connecting insulin and ecdysone signaling. Curr. Biol. CB *20*,
1250 1799–1808.

1251 Freedman, R.R., Sabharwal, S.C., and Desai, N. (1987). Sex differences in peripheral
1252 vascular adrenergic receptors. Circ. Res. *61*, 581–585.

1253 Gäde, G., and Auerswald, L. (2003). Mode of action of neuropeptides from the
1254 adipokinetic hormone family. Gen. Comp. Endocrinol. *132*, 10–20.

1255 Gáliková, M., and Klepsatel, P. (2018). Obesity and Aging in the *Drosophila* Model. Int.
1256 J. Mol. Sci. *19*, E1896.

1257 Gáliková, M., Diesner, M., Klepsatel, P., Hehlert, P., Xu, Y., Bickmeyer, I., Predel, R.,
1258 and Kühnlein, R.P. (2015). Energy Homeostasis Control in *Drosophila Adipokinetic*
1259 *Hormone Mutants*. Genetics *201*, 665–683.

1260 Garner, S.R.C., Castellanos, M.C., Baillie, K.E., Lian, T., and Allan, D.W. (2018).
1261 *Drosophila* female-specific Ilp7 motoneurons are generated by Fruitless-dependent cell
1262 death in males and by a double-assurance survival role for Transformer in females.
1263 Dev. Camb. Engl. *145*, dev150821.

1264 Ghosh, A.C., and O'Connor, M.B. (2014). Systemic Activin signaling independently
1265 regulates sugar homeostasis, cellular metabolism, and pH balance in *Drosophila*
1266 *melanogaster*. Proc. Natl. Acad. Sci. U. S. A. *111*, 5729–5734.

1267 Goodwin, S.F., Taylor, B.J., Villella, A., Foss, M., Ryner, L.C., Baker, B.S., and Hall,
1268 J.C. (2000). Aberrant splicing and altered spatial expression patterns in *fruitless*
1269 mutants of *Drosophila melanogaster.* Genetics *154*, 725–745.

1270 Grönke, S., Beller, M., Fellert, S., Ramakrishnan, H., Jäckle, H., and Kühnlein, R.P.
1271 (2003). Control of Fat Storage by a *Drosophila* PAT Domain Protein. Curr. Biol. *13*,
1272 603–606.

1273 Grönke, S., Mildner, A., Fellert, S., Tennagels, N., Petry, S., Müller, G., Jäckle, H., and
1274 Kühnlein, R.P. (2005). Brummer lipase is an evolutionary conserved fat storage
1275 regulator in *Drosophila*. Cell Metab. *1*, 323–330.

1276 Grönke, S., Müller, G., Hirsch, J., Fellert, S., Andreou, A., Haase, T., Jäckle, H., and
1277 Kühnlein, R.P. (2007). Dual lipolytic control of body fat storage and mobilization in
1278 *Drosophila*. PLoS Biol. *5*, e137.

1279 Grönke, S., Clarke, D.-F., Broughton, S., Andrews, T.D., and Partridge, L. (2010).
1280 Molecular Evolution and Functional Characterization of *Drosophila* Insulin-Like
1281 Peptides. PLOS Genet. *6*, e1000857.

1282 Guo, F., Yu, J., Jung, H.J., Abruzzi, K.C., Luo, W., Griffith, L.C., and Rosbash, M.
1283 (2016). Circadian Neuron Feedback Controls the *Drosophila* Sleep-Activity Profile.
1284 Nature *536*, 292–297.

1285 Gutierrez, E., Wiggins, D., Fielding, B., and Gould, A.P. (2007). Specialized hepatocyte-
1286 like cells regulate *Drosophila* lipid metabolism. Nature *445*, 275–280.

1287 Hall, J.C. (1994). The Mating of a Fly. Science *264*, 1702–1714.

1288 Hansen, M., Flatt, T., and Aguilaniu, H. (2013). Reproduction, Fat Metabolism, and Life
1289 Span: What Is the Connection? Cell Metab. *17*, 10–19.

1290 Heier, C., and Kühnlein, R.P. (2018). Triacylglycerol Metabolism in *Drosophila*
1291 *melanogaster*. Genetics *210*, 1163–1184.

1292 Heier, C., Klishch, S., Stilbytska, O., Semaniuk, U., and Lushchak, O. (2021). The
1293 *Drosophila* model to interrogate triacylglycerol biology. Biochim. Biophys. Acta BBA -
1294 Mol. Cell Biol. Lipids *1866*, 158924.

1295 Heinrichs, V., Ryner, L.C., and Baker, B.S. (1998). Regulation of sex-specific selection
1296 of *fruitless* 5' splice sites by transformer and transformer-2. Mol. Cell. Biol. *18*, 450–458.

1297 Helfrich-Förster, C. (2000). Differential control of morning and evening components in
1298 the activity rhythm of *Drosophila melanogaster*--sex-specific differences suggest a
1299 different quality of activity. J. Biol. Rhythms *15*, 135–154.

1300 Hentze, J.L., Carlsson, M.A., Kondo, S., Nässel, D.R., and Rewitz, K.F. (2015). The
1301 Neuropeptide Allatostatin A Regulates Metabolism and Feeding Decisions in
1302 *Drosophila*. Sci. Rep. *5*, 11680.

1303 Hinojosa-Laborde, C., Chapa, I., Lange, D., and Haywood, J.R. (1999). Gender
1304 differences in sympathetic nervous system regulation. Clin. Exp. Pharmacol. Physiol.
1305 *26*, 122–126.

1306 Hoeker, G.S., Hood, A.R., Katra, R.P., Poelzing, S., and Pogwizd, S.M. (2014). Sex
1307 Differences in β-Adrenergic Responsiveness of Action Potentials and Intracellular
1308 Calcium Handling in Isolated Rabbit Hearts. PLoS ONE *9*, e111411.

1309 Hogarth, A.J., Mackintosh, A.F., and Mary, D.A.S.G. (2007). Gender-related differences
1310 in the sympathetic vasoconstrictor drive of normal subjects. Clin. Sci. Lond. Engl. 1979
1311 *112*, 353–361.

1312 Hoshijima, K., Inoue, K., Higuchi, I., Sakamoto, H., and Shimura, Y. (1991). Control of
1313 *doublesex* alternative splicing by transformer and transformer-2 in *Drosophila*. Science
1314 *252*, 833–836.

1315 Huang, R., Song, T., Su, H., Lai, Z., Qin, W., Tian, Y., Dong, X., and Wang, L. (2020).
1316 High-fat diet enhances starvation-induced hyperactivity via sensitizing hunger-sensing
1317 neurons in *Drosophila*. ELife *9*, e53103.

1318 Huber, R., Hill, S.L., Holladay, C., Biesiadecki, M., Tononi, G., and Cirelli, C. (2004).
1319 Sleep homeostasis in *Drosophila melanogaster*. Sleep *27*, 628–639.

1320 Hudry, B., Khadayate, S., and Miguel-Aliaga, I. (2016). The sexual identity of adult
1321 intestinal stem cells controls organ size and plasticity. Nature *530*, 344–348.

1322 Hudry, B., de Goeij, E., Mineo, A., Gaspar, P., Hadjieconomou, D., Studd, C.,
1323 Mokochinski, J.B., Kramer, H.B., Plaçais, P.-Y., Preat, T., et al. (2019). Sex Differences
1324 in Intestinal Carbohydrate Metabolism Promote Food Intake and Sperm Maturation. Cell
1325 *178*, 901-918.e16.

1326 Hughson, B.N., Shimell, M., and O'Connor, M.B. (2021). AKH Signaling in *D.*
1327 *melanogaster* Alters Larval Development in a Nutrient-Dependent Manner That
1328 Influences Adult Metabolism. Front. Physiol. *12*.

1329 Inoue, K., Hoshijima, K., Sakamoto, H., and Shimura, Y. (1990). Binding of the
1330 *Drosophila sex-lethal* gene product to the alternative splice site of *transformer* primary
1331 transcript. Nature *344*, 461–463.

1332 Inoue, K., Hoshijima, K., Higuchi, I., Sakamoto, H., and Shimura, Y. (1992). Binding of
1333 the *Drosophila* transformer and transformer-2 proteins to the regulatory elements of
1334 *doublesex* primary transcript for sex-specific RNA processing. Proc. Natl. Acad. Sci. U.
1335 S. A. *89*, 8092–8096.

1336 Isabel, G., Martin, J.-R., Chidami, S., Veenstra, J.A., and Rosay, P. (2005). AKH-
1337 producing neuroendocrine cell ablation decreases trehalose and induces behavioral
1338 changes in *Drosophila*. Am. J. Physiol. Regul. Integr. Comp. Physiol. *288*, R531-538.

1339 Ito, H., Fujitani, K., Usui, K., Shimizu-Nishikawa, K., Tanaka, S., and Yamamoto, D.
1340 (1996). Sexual orientation in *Drosophila* is altered by the *satori* mutation in the sex-
1341 determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. Proc.
1342 Natl. Acad. Sci. U. S. A. *93*, 9687–9692.

1343 Jackson, A.S., Stanforth, P.R., Gagnon, J., Rankinen, T., Leon, A.S., Rao, D.C.,
1344 Skinner, J.S., Bouchard, C., and Wilmore, J.H. (2002). The effect of sex, age and race
1345 on estimating percentage body fat from body mass index: The Heritage Family Study.
1346 Int. J. Obes. *26*, 789–796.

1347 Johnson, A.A., and Stolzing, A. (2019). The role of lipid metabolism in aging, lifespan
1348 regulation, and age-related disease. Aging Cell *18*, e13048.

1349 Kamareddine, L., Robins, W.P., Berkey, C.D., Mekalanos, J.J., and Watnick, P.I.
1350 (2018). The *Drosophila* Immune Deficiency Pathway Modulates Enteroendocrine
1351 Function and Host Metabolism. Cell Metab. *28*, 449-462.e5.

1352 Kang, P., Chang, K., Liu, Y., Bouska, M., Birnbaum, A., Karashchuk, G., Thakore, R.,
1353 Zheng, W., Post, S., Brent, C.S., et al. (2017). *Drosophila* Kruppel homolog 1 represses
1354 lipolysis through interaction with dFOXO. Sci. Rep. *7*, 16369.

1355 Karastergiou, K., Smith, S.R., Greenberg, A.S., and Fried, S.K. (2012). Sex differences
1356 in human adipose tissues – the biology of pear shape. Biol. Sex Differ. *3*, 13.

1357 Kimura, K., Urushizaki, A., Sato, C., and Yamamoto, D. (2019). A novel sex difference
1358 in *Drosophila* contact chemosensory neurons unveiled using single cell labeling. J.
1359 Neurogenet. *33*, 116–124.

1360 Kubrak, O., Jensen, L., Ahrentløv, N., Koyama, T., Malita, A., Naseem, M.T., Lassen,
1361 M., Nagy, S., Texada, M.J., Halberg, K.A., et al. (2020). The gut hormone Allatostatin C

1362 regulates food intake and metabolic homeostasis under nutrient stress. BioRxiv
1363 2020.12.05.412874.

1364 Kühnlein, R.P. (2012). Lipid droplet-based storage fat metabolism in *Drosophila*:
1365 Thematic Review Series: Lipid Droplet Synthesis and Metabolism: from Yeast to Man. J.
1366 Lipid Res. *53*, 1430–1436.

1367 Kvitsiani, D., and Dickson, B.J. (2006). Shared neural circuitry for female and male
1368 sexual behaviours in *Drosophila*. Curr. Biol. *16*, R355–R356.

1369 Lafontan, M., Barbe, P., Galitzky, J., Tavernier, G., Langin, D., Carpéné, C., Bousquet-
1370 Melou, A., and Berlan, M. (1997). Adrenergic regulation of adipocyte metabolism. Hum.
1371 Reprod. Oxf. Engl. *12 Suppl 1*, 6–20.

1372 Lease, H.M., and Wolf, B.O. (2011). Lipid content of terrestrial arthropods in relation to
1373 body size, phylogeny, ontogeny and sex. Physiol. Entomol. *36*, 29–38.

1374 Lee, G., and Hall, J.C. (2001). Abnormalities of male-specific FRU protein and serotonin
1375 expression in the CNS of *fruitless* mutants in *Drosophila*. J. Neurosci. Off. J. Soc.
1376 Neurosci. *21*, 513–526.

1377 Lee, G., and Park, J.H. (2004). Hemolymph sugar homeostasis and starvation-induced
1378 hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding
1379 gene in *Drosophila melanogaster*. Genetics *167*, 311–323.

1380 Lee, B., Barretto, E.C., and Grewal, S.S. (2019). TORC1 modulation in adipose tissue is
1381 required for organismal adaptation to hypoxia in *Drosophila*. Nat. Commun. *10*, 1878.

1382 Lehmann, M. (2018). Endocrine and physiological regulation of neutral fat storage in
1383 *Drosophila*. Mol. Cell. Endocrinol. *461*, 165–177.

1384 Li, H., Janssens, J., Waegeneer, M.D., Kolluru, S.S., Davie, K., Gardeux, V., Saelens,
1385 W., David, F., Brbić, M., Leskovec, J., et al. (2021). Fly Cell Atlas: a single-cell
1386 transcriptomic atlas of the adult fruit fly. BioRxiv 2021.07.04.451050.

1387 Li, Y., Hoffmann, J., Li, Y., Stephano, F., Bruchhaus, I., Fink, C., and Roeder, T. (2016).
1388 Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*.
1389 Sci. Rep. *6*, 35359.

1390 Liao, S., Amcoff, M., and Nässel, D.R. (2021). Impact of high-fat diet on lifespan,
1391 metabolism, fecundity and behavioral senescence in *Drosophila*. Insect Biochem. Mol.
1392 Biol. *133*, 103495.

1393 Lorenz, M.W., and Gäde, G. (2009). Hormonal regulation of energy metabolism in
1394 insects as a driving force for performance. Integr. Comp. Biol. *49*, 380–392.

1395 Luong, N., Davies, C.R., Wessells, R.J., Graham, S.M., King, M.T., Veech, R., Bodmer,
1396 R., and Oldham, S.M. (2006). Activated FOXO-mediated insulin resistance is blocked
1397 by reduction of TOR activity. Cell Metab. *4*, 133–142.

1398 Luzier, A.B., Nawarskas, J.J., Añonuevo, J., Wilson, M.F., and Kazierad, D.J. (1998).
1399 The effects of gender on adrenergic receptor responsiveness. J. Clin. Pharmacol. *38*,
1400 618–624.

1401 Masuyama, K., Zhang, Y., Rao, Y., and Wang, J.W. (2012). Mapping neural circuits with
1402 activity-dependent nuclear import of a transcription factor. J. Neurogenet. *26*, 89–102.

1403 Mathews, K.W., Cavegn, M., and Zwicky, M. (2017). Sexual Dimorphism of Body Size Is
1404 Controlled by Dosage of the X-Chromosomal Gene Myc and by the Sex-Determining
1405 Gene *tra* in *Drosophila*. Genetics *205*, 1215–1228.

1406 May, C.E., Rosander, J., Gottfried, J., Dennis, E., and Dus, M. (2020). Dietary sugar
1407 inhibits satiation by decreasing the central processing of sweet taste. ELife *9*, e54530.

1408 McIntosh, V.J., Chandrasekera, P.C., and Lasley, R.D. (2011). Sex differences and the
1409 effects of ovariectomy on the β-adrenergic contractile response. Am. J. Physiol. - Heart
1410 Circ. Physiol. *301*, H1127–H1134.

1411 Mellert, D.J., Knapp, J.-M., Manoli, D.S., Meissner, G.W., and Baker, B.S. (2010).
1412 Midline crossing by gustatory receptor neuron axons is regulated by fruitless, doublesex
1413 and the Roundabout receptors. Dev. Camb. Engl. *137*, 323–332.

1414 Mellert, D.J., Williamson, W.R., Shirangi, T.R., Card, G.M., and Truman, J.W. (2016).
1415 Genetic and Environmental Control of Neurodevelopmental Robustness in *Drosophila*.
1416 PLoS ONE *11*, e0155957.

1417 Millington, J.W., and Rideout, E.J. (2018). Sex differences in *Drosophila* development
1418 and physiology. Curr. Opin. Physiol. *6*, 46–56.

1419 Millington, J.W., Brownrigg, G.P., Chao, C., Sun, Z., Basner-Collins, P.J., Wat, L.W.,
1420 Hudry, B., Miguel-Aliaga, I., and Rideout, E.J. (2021). Female-biased upregulation of
1421 insulin pathway activity mediates the sex difference in *Drosophila* body size plasticity.
1422 ELife *10*, e58341.

1423 Min, S., Chae, H.-S., Jang, Y.-H., Choi, S., Lee, S., Jeong, Y.T., Jones, W.D., Moon,
1424 S.J., Kim, Y.-J., and Chung, J. (2016). Identification of a Peptidergic Pathway Critical to
1425 Satiety Responses in *Drosophila*. Curr. Biol. *26*, 814–820.

1426 Mochanová, M., Tomčala, A., Svobodová, Z., and Kodrík, D. (2018). Role of
1427 adipokinetic hormone during starvation in *Drosophila*. Comp. Biochem. Physiol. B
1428 Biochem. Mol. Biol. *226*, 26–35.

1429 Mosher, J., Zhang, W., Blumhagen, R.Z., D'Alessandro, A., Nemkov, T., Hansen, K.C.,
1430 Hesselberth, J.R., and Reis, T. (2015). Coordination between Drosophila Arc1 and a
1431 specific population of brain neurons regulates organismal fat. Dev. Biol. *405*, 280–290.

1432 Murphy, D.J. (2001). The biogenesis and functions of lipid bodies in animals, plants and
1433 microorganisms. Prog. Lipid Res. *40*, 325–438.

1434 Nagoshi, R.N., McKeown, M., Burtis, K.C., Belote, J.M., and Baker, B.S. (1988). The
1435 control of alternative splicing at genes regulating sexual differentiation in *D.*
1436 *melanogaster*. Cell *53*, 229–236.

1437 Nelson, C.S., Beck, J.N., Wilson, K.A., Pilcher, E.R., Kapahi, P., and Brem, R.B. (2016).
1438 Cross-phenotype association tests uncover genes mediating nutrient response in
1439 *Drosophila*. BMC Genomics *17*, 867.

1440 Neville, M.C., Nojima, T., Ashley, E., Parker, D.J., Walker, J., Southall, T., Van de
1441 Sande, B., Marques, A.C., Fischer, B., Brand, A.H., et al. (2014). Male-specific fruitless
1442 isoforms target neurodevelopmental genes to specify a sexually dimorphic nervous
1443 system. Curr. Biol. CB *24*, 229–241.

1444 Ng, A.V., Callister, R., Johnson, D.G., and Seals, D.R. (1993). Age and gender
1445 influence muscle sympathetic nerve activity at rest in healthy humans. Hypertens.
1446 Dallas Tex 1979 *21*, 498–503.

1447 Nojima, T., Neville, M.C., and Goodwin, S.F. (2014). Fruitless isoforms and target genes
1448 specify the sexually dimorphic nervous system underlying *Drosophila* reproductive
1449 behavior. Fly (Austin) *8*, 95–100.

1450 Nojima, T., Rings, A., Allen, A.M., Otto, N., Verschut, T.A., Billeter, J.-C., Neville, M.C.,
1451 and Goodwin, S.F. (2021). A sex-specific switch between visual and olfactory inputs
1452 underlies adaptive sex differences in behavior. Curr. Biol. *31*, 1175-1191.e6.

1453 Noyes, B.E., Katz, F.N., and Schaffer, M.H. (1995). Identification and expression of the
1454 *Drosophila* adipokinetic hormone gene. Mol. Cell. Endocrinol. *109*, 133–141.

1455 Oh, Y., Lai, J.S.-Y., Mills, H.J., Erdjument-Bromage, H., Giammarinaro, B., Saadipour,
1456 K., Wang, J.G., Abu, F., Neubert, T.A., and Suh, G.S.B. (2019). A glucose-sensing
1457 neuron pair regulates insulin and glucagon in *Drosophila*. Nature *574*, 559–564.

1458 Park, A., Tran, T., and Atkinson, N.S. (2018). Monitoring food preference in *Drosophila*
1459 by oligonucleotide tagging. Proc. Natl. Acad. Sci. U. S. A. *115*, 9020–9025.

1460 Park, Y., Kim, Y.-J., and Adams, M.E. (2002). Identification of G protein-coupled
1461 receptors for *Drosophila* PRXamide peptides, CCAP, corazonin, and AKH supports a
1462 theory of ligand-receptor coevolution. Proc. Natl. Acad. Sci. *99*, 11423–11428.

1463 Patel, R.T., Soulages, J.L., Hariharasundaram, B., and Arrese, E.L. (2005). Activation of
1464 the Lipid Droplet Controls the Rate of Lipolysis of Triglycerides in the Insect Fat Body*.
1465 J. Biol. Chem. *280*, 22624–22631.

1466 Pavlou, H.J., Lin, A.C., Neville, M.C., Nojima, T., Diao, F., Chen, B.E., White, B.H., and
1467 Goodwin, S.F. (2016). Neural circuitry coordinating male copulation. ELife *5*, e20713.

1468 von Philipsborn, A.C., Jörchel, S., Tirian, L., Demir, E., Morita, T., Stern, D.L., and
1469 Dickson, B.J. (2014). Cellular and behavioral functions of fruitless isoforms in
1470 *Drosophila* courtship. Curr. Biol. CB *24*, 242–251.

1471 Phillips, M.D., and Thomas, G.H. (2006). Brush border spectrin is required for early
1472 endosome recycling in *Drosophila*. J. Cell Sci. *119*, 1361–1370.

1473 Pomatto, L.C.D., Carney, C., Shen, B., Wong, S., Halaszynski, K., Salomon, M.P.,
1474 Davies, K.J.A., and Tower, J. (2017). The Mitochondrial Lon Protease Is Required for
1475 Age-Specific and Sex-Specific Adaptation to Oxidative Stress. Curr. Biol. CB *27*, 1–15.

1476 Post, S., Liao, S., Yamamoto, R., Veenstra, J.A., Nässel, D.R., and Tatar, M. (2019).
1477 *Drosophila insulin-like peptide dilp1* increases lifespan and glucagon-like *Akh*
1478 expression epistatic to *dilp2*. Aging Cell *18*, e12863.

1479 Power, M.L., and Schulkin, J. (2008). Sex differences in fat storage, fat metabolism, and
1480 the health risks from obesity: possible evolutionary origins. Br. J. Nutr. *99*, 931–940.

1481 Predel, R., Wegener, C., Russell, W.K., Tichy, S.E., Russell, D.H., and Nachman, R.J.
1482 (2004). Peptidomics of CNS-associated neurohemal systems of adult *Drosophila*
1483 *melanogaster*: A mass spectrometric survey of peptides from individual flies. J. Comp.
1484 Neurol. *474*, 379–392.

1485 Rajan, A., and Perrimon, N. (2012). The *Drosophila* cytokine Unpaired 2 regulates
1486 physiological homeostasis by remotely controlling Insulin secretion. Cell *151*, 123–137.

1487 Recazens, E., Mouisel, E., and Langin, D. (2021). Hormone-sensitive lipase: sixty years
1488 later. Prog. Lipid Res. *82*, 101084.

1489 Reddiex, A.J., Gosden, T.P., Bonduriansky, R., and Chenoweth, S.F. (2013). Sex-
1490 specific fitness consequences of nutrient intake and the evolvability of diet preferences.
1491 Am. Nat. *182*, 91–102.

1492 Regan, J.C., Khericha, M., Dobson, A.J., Bolukbasi, E., Rattanavirotkul, N., and
1493 Partridge, L. (2016). Sex difference in pathology of the ageing gut mediates the greater
1494 response of female lifespan to dietary restriction. ELife *5*, e10956.

1495 Rezával, C., Nojima, T., Neville, M.C., Lin, A.C., and Goodwin, S.F. (2014). Sexually
1496 dimorphic octopaminergic neurons modulate female postmating behaviors in
1497 *Drosophila*. Curr. Biol. CB *24*, 725–730.

1498 Rezával, C., Pattnaik, S., Pavlou, H.J., Nojima, T., Brüggemeier, B., D'Souza, L.A.D.,
1499 Dweck, H.K.M., and Goodwin, S.F. (2016). Activation of Latent Courtship Circuitry in the
1500 Brain of *Drosophila* Females Induces Male-like Behaviors. Curr. Biol. CB *26*, 2508–
1501 2515.

1502 Rideout, E.J., Billeter, J.-C., and Goodwin, S.F. (2007). The sex-determination genes
1503 *fruitless* and *doublesex* specify a neural substrate required for courtship song. Curr.
1504 Biol. CB *17*, 1473–1478.

1505 Rideout, E.J., Dornan, A.J., Neville, M.C., Eadie, S., and Goodwin, S.F. (2010). Control
1506 of sexual differentiation and behavior by the *doublesex* gene in *Drosophila*
1507 *melanogaster*. Nat. Neurosci. *13*, 458–466.

1508 Rideout, E.J., Narsaiya, M.S., and Grewal, S.S. (2015). The Sex Determination Gene
1509 *transformer* Regulates Male-Female Differences in *Drosophila* Body Size. PLoS Genet.
1510 *11*, e1005683.

1511 Robinett, C.C., Vaughan, A.G., Knapp, J.-M., and Baker, B.S. (2010). Sex and the
1512 Single Cell. II. There Is a Time and Place for Sex. PLOS Biol. *8*, e1000365.

1513 Roth, S.W., Bitterman, M.D., Birnbaum, M.J., and Bland, M.L. (2018). Innate Immune
1514 Signaling in *Drosophila* Blocks Insulin Signaling by Uncoupling PI(3,4,5)P3 Production
1515 and Akt Activation. Cell Rep. *22*, 2550–2556.

1516 Ryner, L.C., Goodwin, S.F., Castrillon, D.H., Anand, A., Villella, A., Baker, B.S., Hall,
1517 J.C., Taylor, B.J., and Wasserman, S.A. (1996). Control of male sexual behavior and
1518 sexual orientation in *Drosophila* by the *fruitless* gene. Cell *87*, 1079–1089.

1519 Salz, H.K., and Erickson, J.W. (2010). Sex determination in *Drosophila*: The view from
1520 the top. Fly (Austin) *4*, 60–70.

1521 Sato, K., Goto, J., and Yamamoto, D. (2019). Sex Mysteries of the Fly Courtship Master
1522 Regulator Fruitless. Front. Behav. Neurosci. *13*, 245.

1523 Sawala, A., and Gould, A.P. (2017). The sex of specific neurons controls female body
1524 growth in *Drosophila*. PLoS Biol. *15*, e2002252.

1525 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T.,
1526 Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source
1527 platform for biological-image analysis. Nat. Methods *9*, 676–682.

1528 Schreiber, R., Xie, H., and Schweiger, M. (2019). Of mice and men: The physiological
1529 role of adipose triglyceride lipase (ATGL). Biochim. Biophys. Acta BBA - Mol. Cell Biol.
1530 Lipids *1864*, 880–899.

1531 Scopelliti, A., Bauer, C., Yu, Y., Zhang, T., Kruspig, B., Murphy, D.J., Vidal, M.,
1532 Maddocks, O.D.K., and Cordero, J.B. (2019). A Neuronal Relay Mediates a Nutrient

1533 Responsive Gut/Fat Body Axis Regulating Energy Homeostasis in Adult *Drosophila*.
1534 Cell Metab. *29*, 269-284.e10.

1535 Shirangi, T.R., Wong, A.M., Truman, J.W., and Stern, D.L. (2016). Doublesex Regulates
1536 the Connectivity of a Neural Circuit Controlling *Drosophila* Male Courtship Song. Dev.
1537 Cell *37*, 533–544.

1538 Sieber, M.H., and Spradling, A.C. (2015). Steroid Signaling Establishes a Female
1539 Metabolic State and Regulates SREBP to Control Oocyte Lipid Accumulation. Curr.
1540 Biol. *25*, 993–1004.

1541 Siera, S.G., and Cline, T.W. (2008). Sexual back talk with evolutionary implications:
1542 stimulation of the *Drosophila* sex-determination gene *sex-lethal* by its target
1543 transformer. Genetics *180*, 1963–1981.

1544 Song, W., Veenstra, J.A., and Perrimon, N. (2014). Control of Lipid Metabolism by
1545 Tachykinin in *Drosophila*. Cell Rep. *9*, 40–47.

1546 Song, W., Cheng, D., Hong, S., Sappe, B., Hu, Y., Wei, N., Zhu, C., O'Connor, M.B.,
1547 Pissios, P., and Perrimon, N. (2017). Midgut-Derived Activin Regulates Glucagon-like
1548 Action in the Fat Body and Glycemic Control. Cell Metab. *25*, 386–399.

1549 Sosnowski, B.A., Belote, J.M., and McKeown, M. (1989). Sex-specific alternative
1550 splicing of RNA from the transformer gene results from sequence-dependent splice site
1551 blockage. Cell *58*, 449–459.

1552 Stafford, J.W., Lynd, K.M., Jung, A.Y., and Gordon, M.D. (2012). Integration of taste
1553 and calorie sensing in *Drosophila*. J. Neurosci. Off. J. Soc. Neurosci. *32*, 14767–14774.

1554 Staubli, F., Jørgensen, T.J.D., Cazzamali, G., Williamson, M., Lenz, C., Søndergaard,
1555 L., Roepstorff, P., and Grimmelikhuijzen, C.J.P. (2002a). Molecular identification of the
1556 insect adipokinetic hormone receptors. Proc. Natl. Acad. Sci. U. S. A. *99*, 3446–3451.

1557 Staubli, F., Jorgensen, T.J.D., Cazzamali, G., Williamson, M., Lenz, C., Sondergaard,
1558 L., Roepstorff, P., and Grimmelikhuijzen, C.J.P. (2002b). Molecular identification of the
1559 insect adipokinetic hormone receptors. Proc. Natl. Acad. Sci. U. S. A. *99*, 3446–3451.

1560 Sturtevant, A.H. (1945). A Gene in Drosophila Melanogaster That Transforms Females
1561 into Males. Genetics *30*, 297–299.

1562 Suzawa, M., Muhammad, N.M., Joseph, B.S., and Bland, M.L. (2019). The Toll
1563 Signaling Pathway Targets the Insulin-like Peptide Dilp6 to Inhibit Growth in *Drosophila*.
1564 Cell Rep. *28*, 1439-1446.e5.

1565 Sztalryd, C., and Brasaemle, D.L. (2017). The perilipin family of lipid droplet proteins:
1566 Gatekeepers of intracellular lipolysis. Biochim. Biophys. Acta *1862*, 1221–1232.

1567 Taylor, B.J., and Truman, J.W. (1992). Commitment of abdominal neuroblasts in
1568 *Drosophila* to a male or female fate is dependent on genes of the sex-determining
1569 hierarchy. Dev. Camb. Engl. *114*, 625–642.

1570 Teixeira, L., Rabouille, C., Rørth, P., Ephrussi, A., and Vanzo, N.F. (2003). *Drosophila*
1571 Perilipin/ADRP homologue Lsd2 regulates lipid metabolism. Mech. Dev. *120*, 1071–
1572 1081.

1573 Teleman, A.A., Chen, Y.-W., and Cohen, S.M. (2005). 4E-BP functions as a metabolic
1574 brake used under stress conditions but not during normal growth. Genes Dev. *19*,
1575 1844–1848.

1576 Tennessen, J.M., Barry, W., Cox, J., and Thummel, C.S. (2014). Methods for studying
1577 metabolism in *Drosophila*. Methods San Diego Calif *68*, 105–115.

1578 Texada, M.J., Jørgensen, A.F., Christensen, C.F., Koyama, T., Malita, A., Smith, D.K.,
1579 Marple, D.F.M., Danielsen, E.T., Petersen, S.K., Hansen, J.L., et al. (2019). A fat-tissue
1580 sensor couples growth to oxygen availability by remotely controlling insulin secretion.
1581 Nat. Commun. *10*, 1955.

1582 Thiele, C., and Spandl, J. (2008). Cell biology of lipid droplets. Curr. Opin. Cell Biol. *20*,
1583 378–385.

1584 Thurmond, J., Goodman, J.L., Strelets, V.B., Attrill, H., Gramates, L.S., Marygold, S.J.,
1585 Matthews, B.B., Millburn, G., Antonazzo, G., Trovisco, V., et al. (2019). FlyBase 2.0: the
1586 next generation. Nucleic Acids Res. *47*, D759–D765.

1587 Tian, Y., Bi, J., Shui, G., Liu, Z., Xiang, Y., Liu, Y., Wenk, M.R., Yang, H., and Huang, X.
1588 (2011). Tissue-autonomous function of *Drosophila* seipin in preventing ectopic lipid
1589 droplet formation. PLoS Genet. *7*, e1001364.

1590 Ugrankar, R., Liu, Y., Provaznik, J., Schmitt, S., and Lehmann, M. (2011). Lipin is a
1591 central regulator of adipose tissue development and function in *Drosophila*
1592 *melanogaster*. Mol. Cell. Biol. *31*, 1646–1656.

1593 Videlier, M., Rundle, H.D., and Careau, V. (2019). Sex-Specific Among-Individual
1594 Covariation in Locomotor Activity and Resting Metabolic Rate in *Drosophila*
1595 melanogaster. Am. Nat. *194*, E164–E176.

1596 Walther, T.C., and Farese, R.V. (2012). Lipid Droplets and Cellular Lipid Metabolism.
1597 Annu. Rev. Biochem. *81*, 687–714.

1598 Wang, F., Wang, K., Forknall, N., Patrick, C., Yang, T., Parekh, R., Bock, D., and
1599 Dickson, B.J. (2020). Neural circuitry linking mating and egg-laying in *Drosophila*
1600 females. Nature *579*, 101–105.

1601 Wang, H., Becuwe, M., Housden, B.E., Chitraju, C., Porras, A.J., Graham, M.M., Liu,
1602 X.N., Thiam, A.R., Savage, D.B., Agarwal, A.K., et al. (2016). Seipin is required for
1603 converting nascent to mature lipid droplets. ELife *5*, e16582.

1604 Wat, L.W., Chao, C., Bartlett, R., Buchanan, J.L., Millington, J.W., Chih, H.J.,
1605 Chowdhury, Z.S., Biswas, P., Huang, V., Shin, L.J., et al. (2020). A role for triglyceride
1606 lipase *brummer* in the regulation of sex differences in *Drosophila* fat storage and
1607 breakdown. PLOS Biol. *18*, e3000595.

1608 Watanabe, L.P., and Riddle, N.C. (2021). GWAS reveal a role for the central nervous
1609 system in regulating weight and weight change in response to exercise. Sci. Rep. *11*,
1610 5144.

1611 Wegener, C., Reinl, T., Jänsch, L., and Predel, R. (2006). Direct mass spectrometric
1612 peptide profiling and fragmentation of larval peptide hormone release sites in *Drosophila*
1613 *melanogaster* reveals tagma-specific peptide expression and differential processing. J.
1614 Neurochem. *96*, 1362–1374.

1615 White, K., Tahaoglu, E., and Steller, H. (1996). Cell killing by the *Drosophila* gene
1616 *reaper*. Science *271*, 805–807.

1617 Womersley, J., and Durnin, J.V.G.A. (1977). A comparison of the skinfold method with
1618 extent of 'overweight' and various weight-height relationships in the assessment of
1619 obesity. Br. J. Nutr. *38*, 271–284.

1620 Yoshinari, Y., Kosakamoto, H., Kamiyama, T., Hoshino, R., Matsuoka, R., Kondo, S.,
1621 Tanimoto, H., Nakamura, A., Obata, F., and Niwa, R. (2021). The sugar-responsive
1622 enteroendocrine neuropeptide F regulates lipid metabolism through glucagon-like and
1623 insulin-like hormones in *Drosophila melanogaster*. Nat. Commun. *12*, 4818.

1624 Zhan, Y.P., Liu, L., and Zhu, Y. (2016). Taotie neurons regulate appetite in *Drosophila*.
1625 Nat. Commun. *7*, 13633.

1626 Zhao, X., and Karpac, J. (2017). Muscle Directs Diurnal Energy Homeostasis through a
1627 Myokine-Dependent Hormone Module in *Drosophila*. Curr. Biol. CB *27*, 1941-1955.e6.

1628

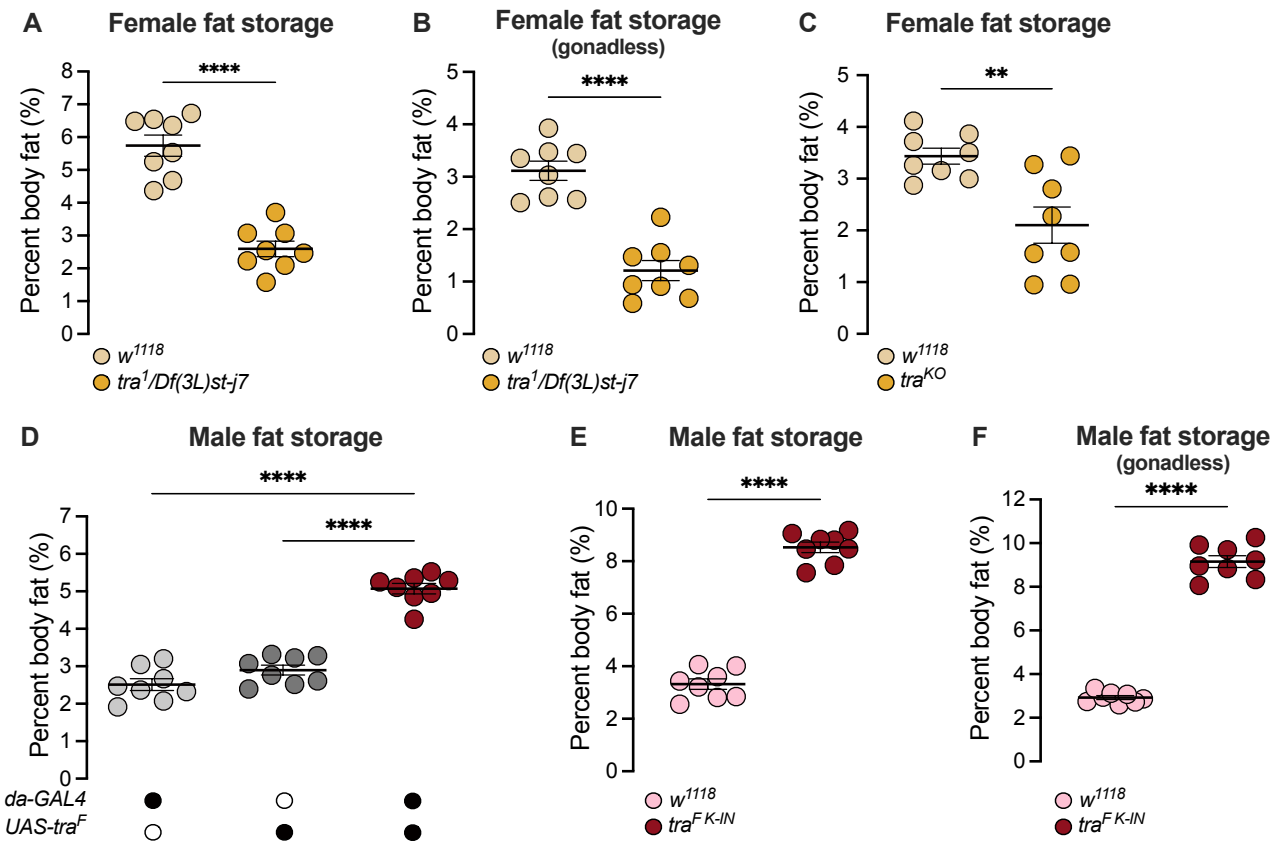
A
Female fat storage
Percent body fat (%)
****
w1118
tra1/Df(3L)st-j7
B
Female fat storage
(gonadless)
Percent body fat (%)
****
w1118
tra1/Df(3L)st-j7
C
Female fat storage
Percent body fat (%)
**
w1118
traKO
D
Male fat storage
Percent body fat (%)
****
****
da-GAL4
UAS-traF
E
Male fat storage
Percent body fat (%)
****
w1118
traF K-IN
F
Male fat storage
(gonadless)
Percent body fat (%)
****
w1118
traF K-IN

FIGURE 1

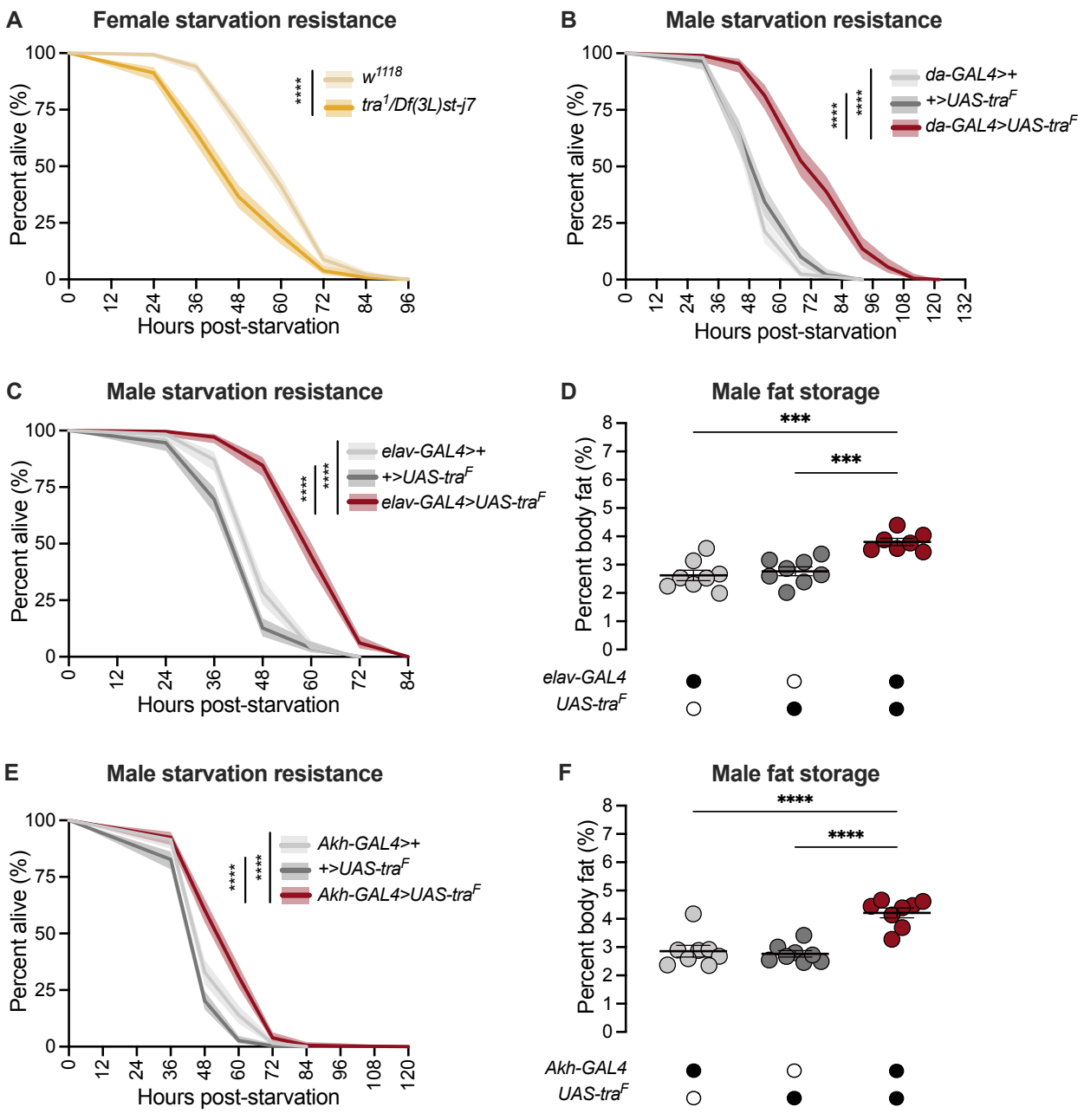
A
Female starvation resistance
Percent alive (%)
Hours post-starvation
w1118
tra1/Df(3L)st-j7
****
B
Male starvation resistance
da-GAL4>+
+>UAS-traF
da-GAL4>UAS-traF
C
Male starvation resistance
elav-GAL4>+
+>UAS-traF
elav-GAL4>UAS-traF
D
Male fat storage
Percent body fat (%)
***
elav-GAL4
UAS-traF
E
Male starvation resistance
Akh-GAL4>+
+>UAS-traF
Akh-GAL4>UAS-traF
F
Male fat storage
****
Akh-GAL4
UAS-traF

FIGURE 2

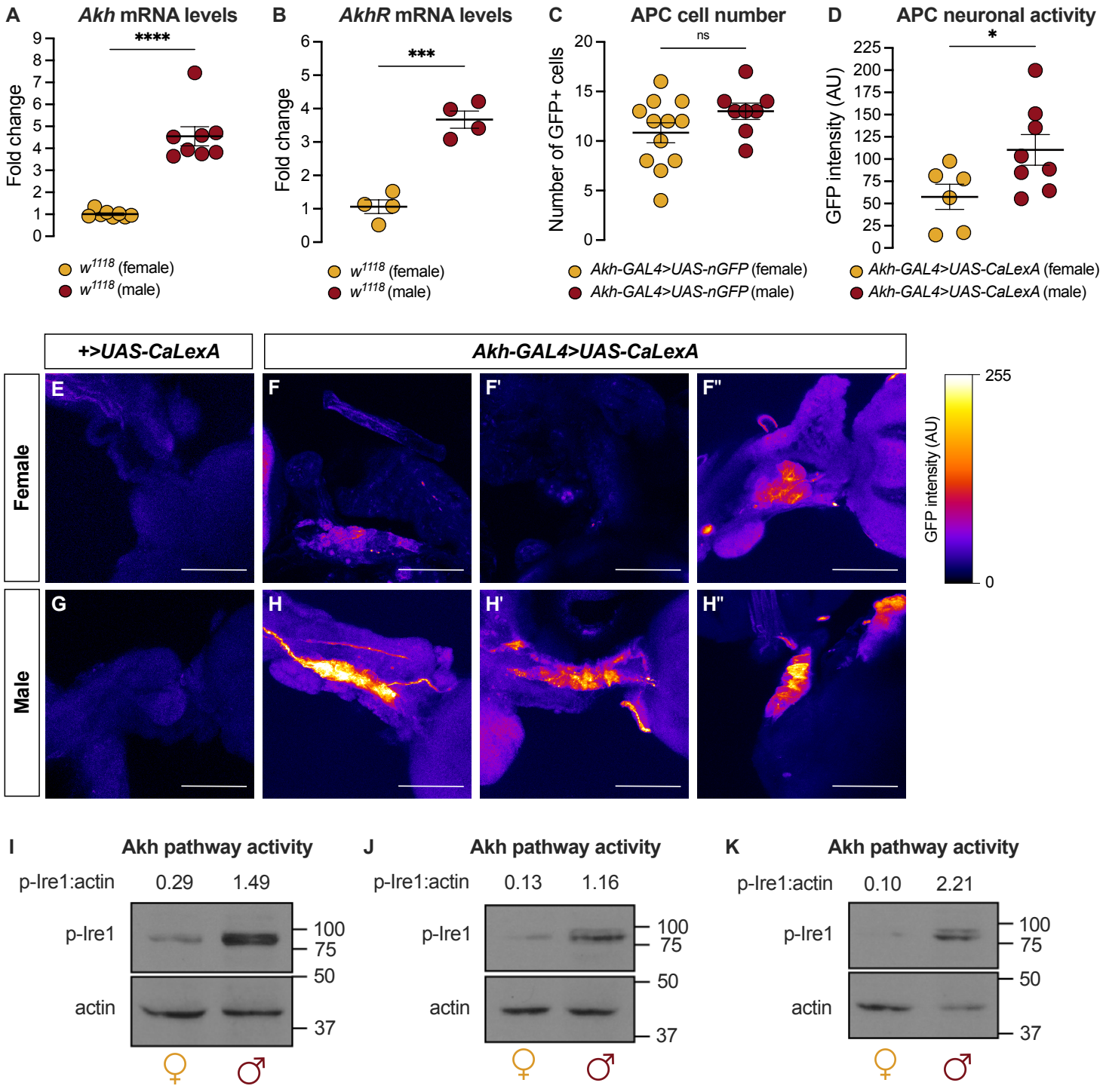
A
Akh mRNA levels
****
Fold change
w1118 (female)
w1118 (male)
B
AkhR mRNA levels
***
Fold change
w1118 (female)
w1118 (male)
C
APC cell number
ns
Number of GFP+ cells
Akh-GAL4>UAS-nGFP (female)
Akh-GAL4>UAS-nGFP (male)
D
APC neuronal activity
*
GFP intensity (AU)
Akh-GAL4>UAS-CaLexA (female)
Akh-GAL4>UAS-CaLexA (male)
+>UAS-CaLexA
Akh-GAL4>UAS-CaLexA
Female
Male
E
F
F'
F''
G
H
H'
H''
255
GFP intensity (AU)
0
I
Akh pathway activity
p-Ire1:actin
0.29
1.49
p-Ire1
actin
100
75
50
37
J
Akh pathway activity
p-Ire1:actin
0.13
1.16
p-Ire1
actin
100
75
50
37
K
Akh pathway activity
p-Ire1:actin
0.10
2.21
p-Ire1
actin
100
75
50
37

FIGURE 3

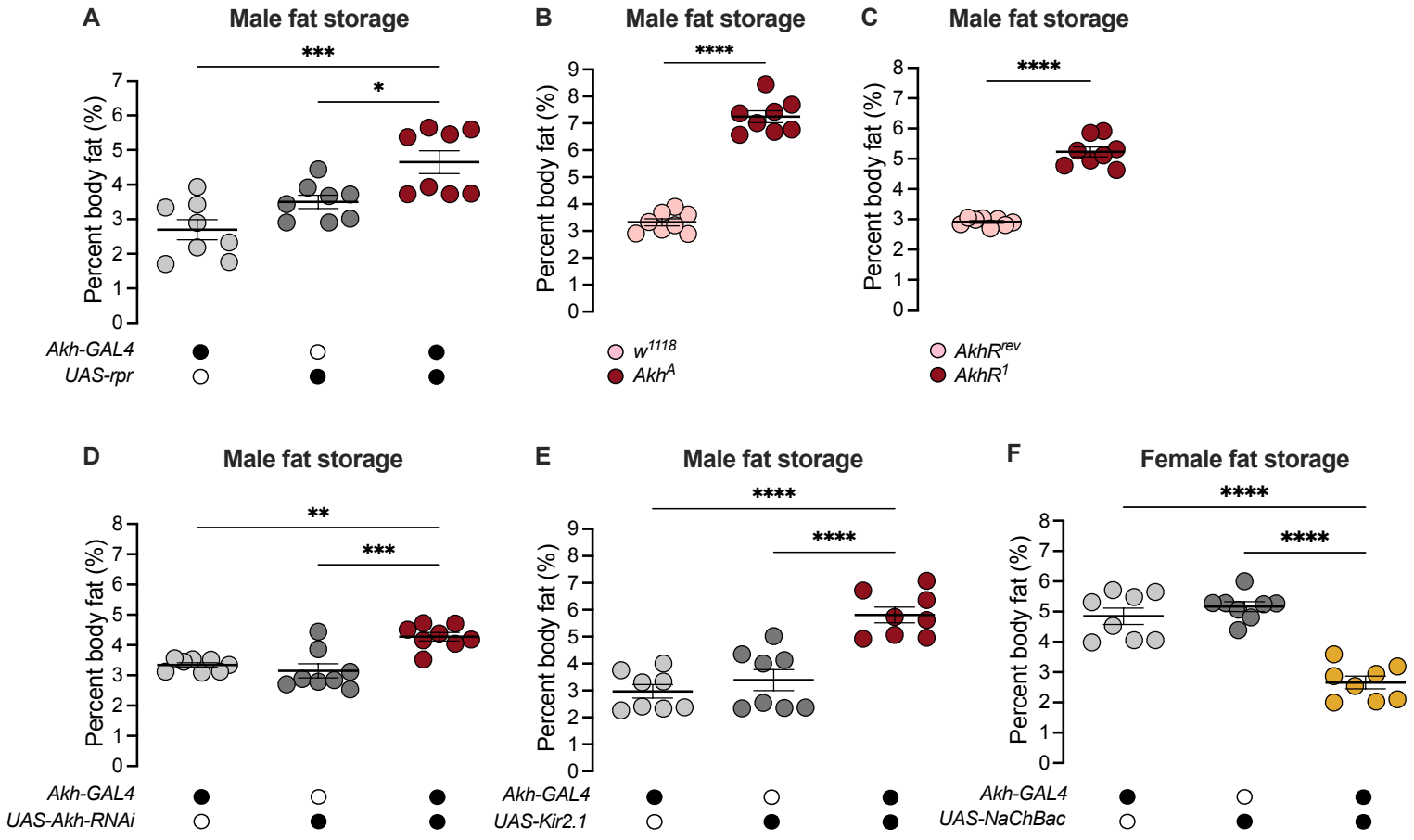
A
Male fat storage
***
*
Percent body fat (%)
Akh-GAL4
UAS-rpr
B
Male fat storage
****
Percent body fat (%)
w1118
AkhA
C
Male fat storage
****
Percent body fat (%)
AkhRrev
AkhR1
D
Male fat storage
**
***
Percent body fat (%)
Akh-GAL4
UAS-Akh-RNAi
E
Male fat storage
****
****
Percent body fat (%)
Akh-GAL4
UAS-Kir2.1
F
Female fat storage
****
****
Percent body fat (%)
Akh-GAL4
UAS-NaChBac

FIGURE 4

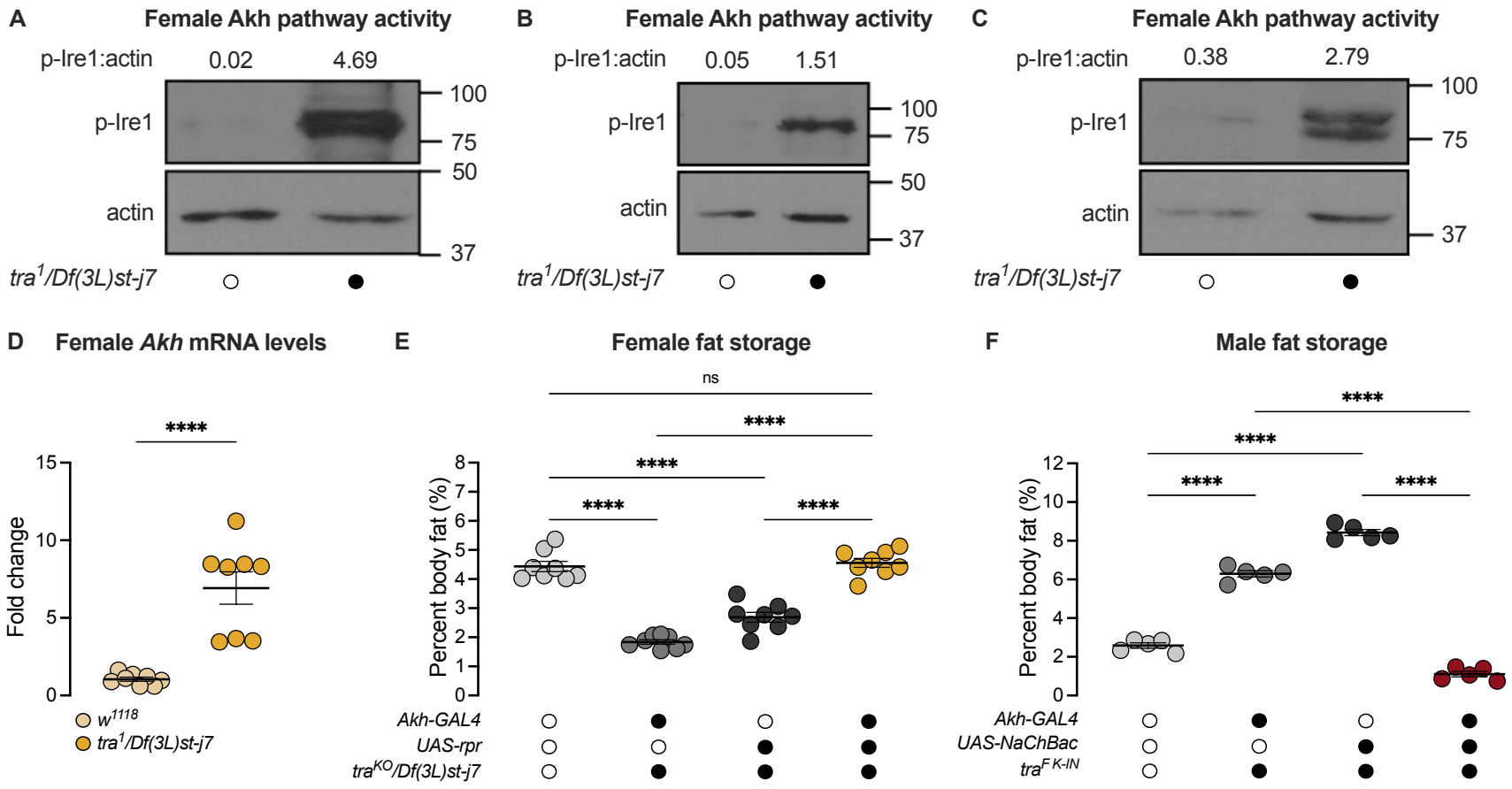
A
Female Akh pathway activity
p-Ire1:actin
0.02
4.69
p-Ire1
actin
100
75
50
37
tra1/Df(3L)st-j7
B
Female Akh pathway activity
p-Ire1:actin
0.05
1.51
p-Ire1
actin
100
75
50
37
tra1/Df(3L)st-j7
C
Female Akh pathway activity
p-Ire1:actin
0.38
2.79
p-Ire1
actin
100
75
37
tra1/Df(3L)st-j7
D
Female Akh mRNA levels
Fold change
****
w1118
tra1/Df(3L)st-j7
E
Female fat storage
Percent body fat (%)
ns
****
Akh-GAL4
UAS-rpr
traKO/Df(3L)st-j7
F
Male fat storage
Percent body fat (%)
****
Akh-GAL4
UAS-NaChBac
traF K-IN

FIGURE 5

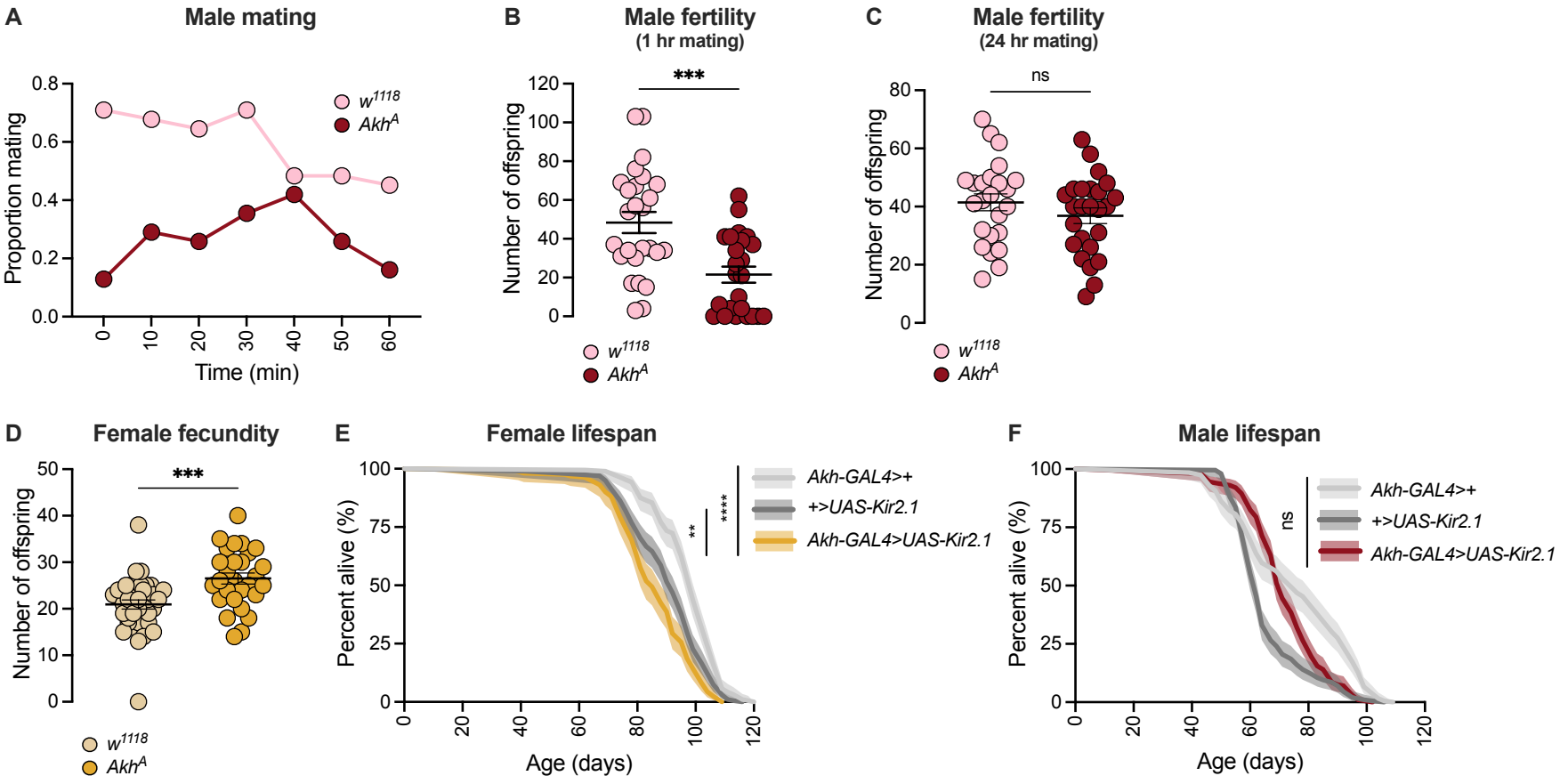
A
Male mating
Proportion mating
Time (min)
w^{1118}
Akh^A
B
Male fertility
(1 hr mating)
Number of offspring
***
C
Male fertility
(24 hr mating)
ns
D
Female fecundity
E
Female lifespan
Percent alive (%)
Age (days)
Akh-GAL4>+
+>UAS-Kir2.1
Akh-GAL4>UAS-Kir2.1
F
Male lifespan

FIGURE 6

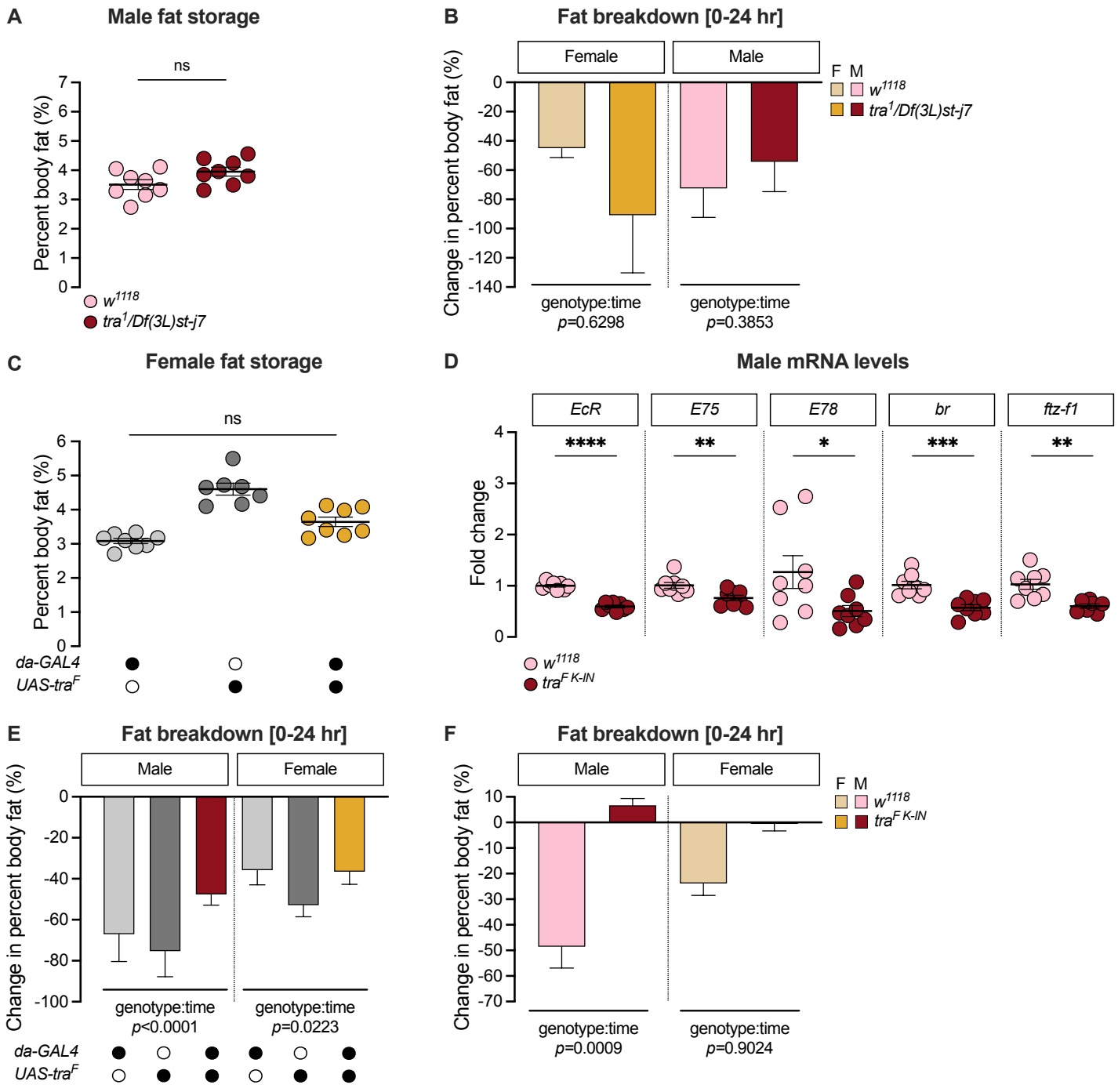

FIGURE 1 - FIGURE SUPPLEMENT 1

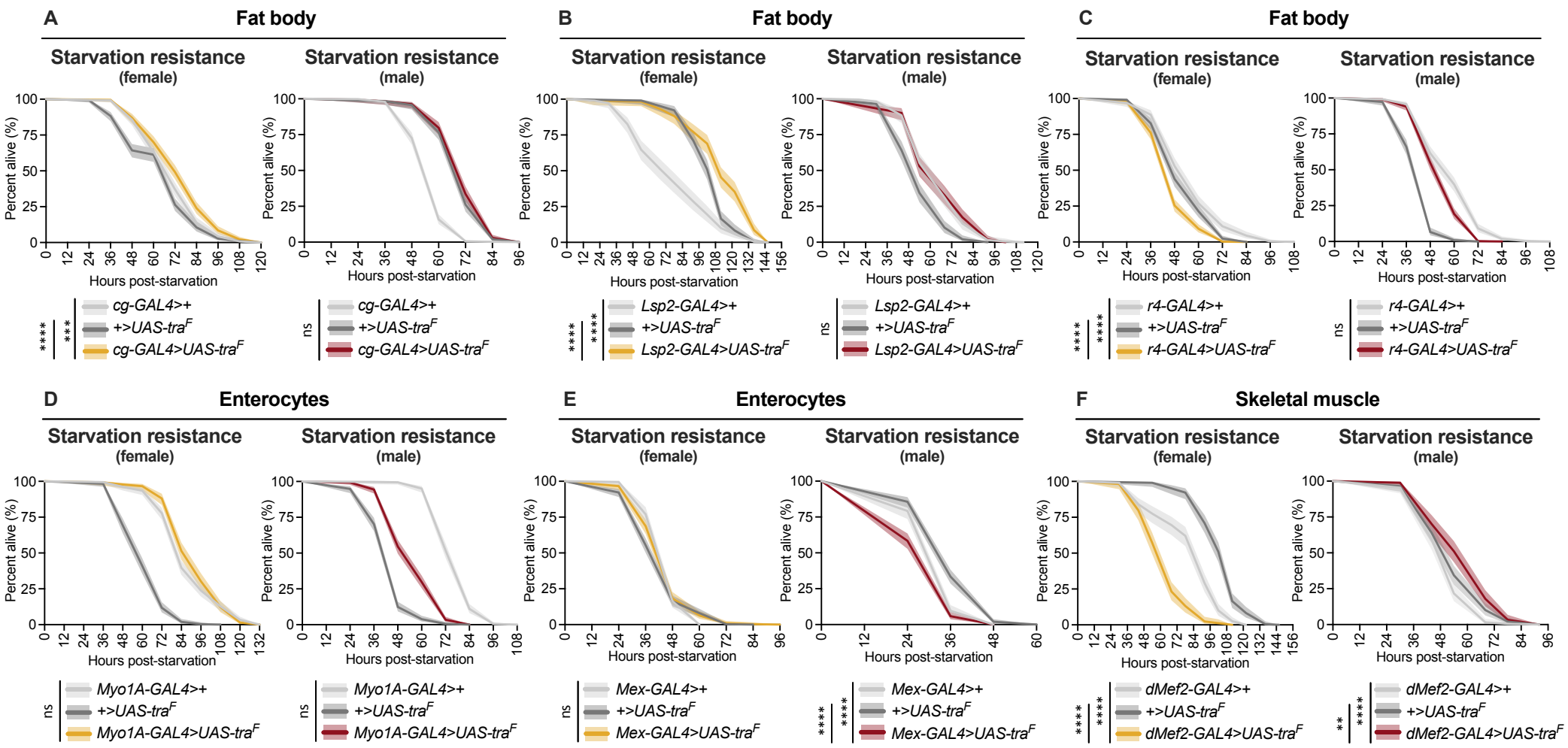

FIGURE 2 - FIGURE SUPPLEMENT 1

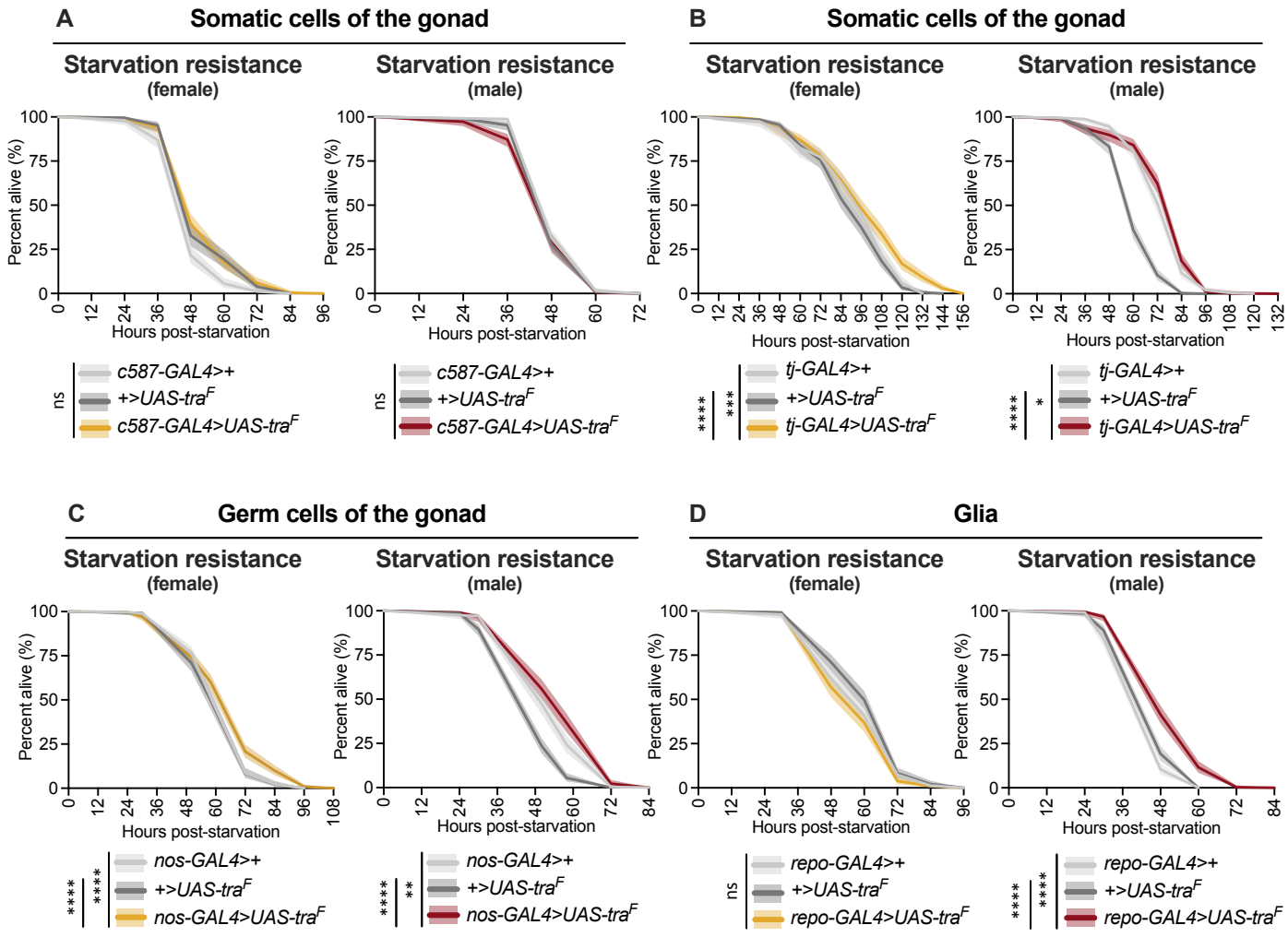

FIGURE 2 - FIGURE SUPPLEMENT 2

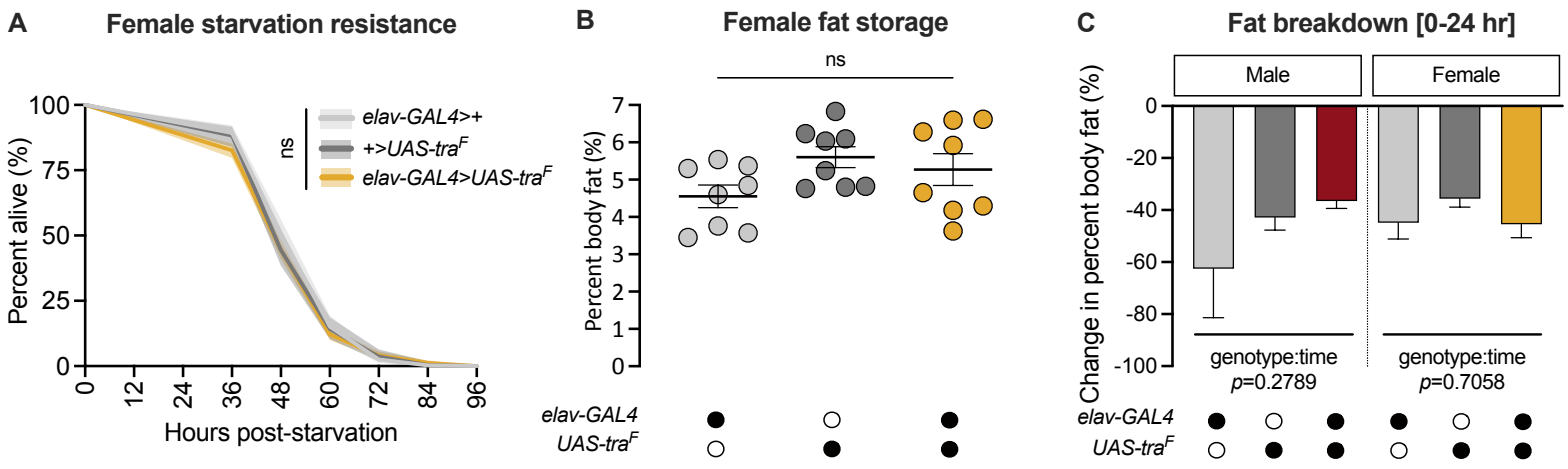

FIGURE 2 - FIGURE SUPPLEMENT 3

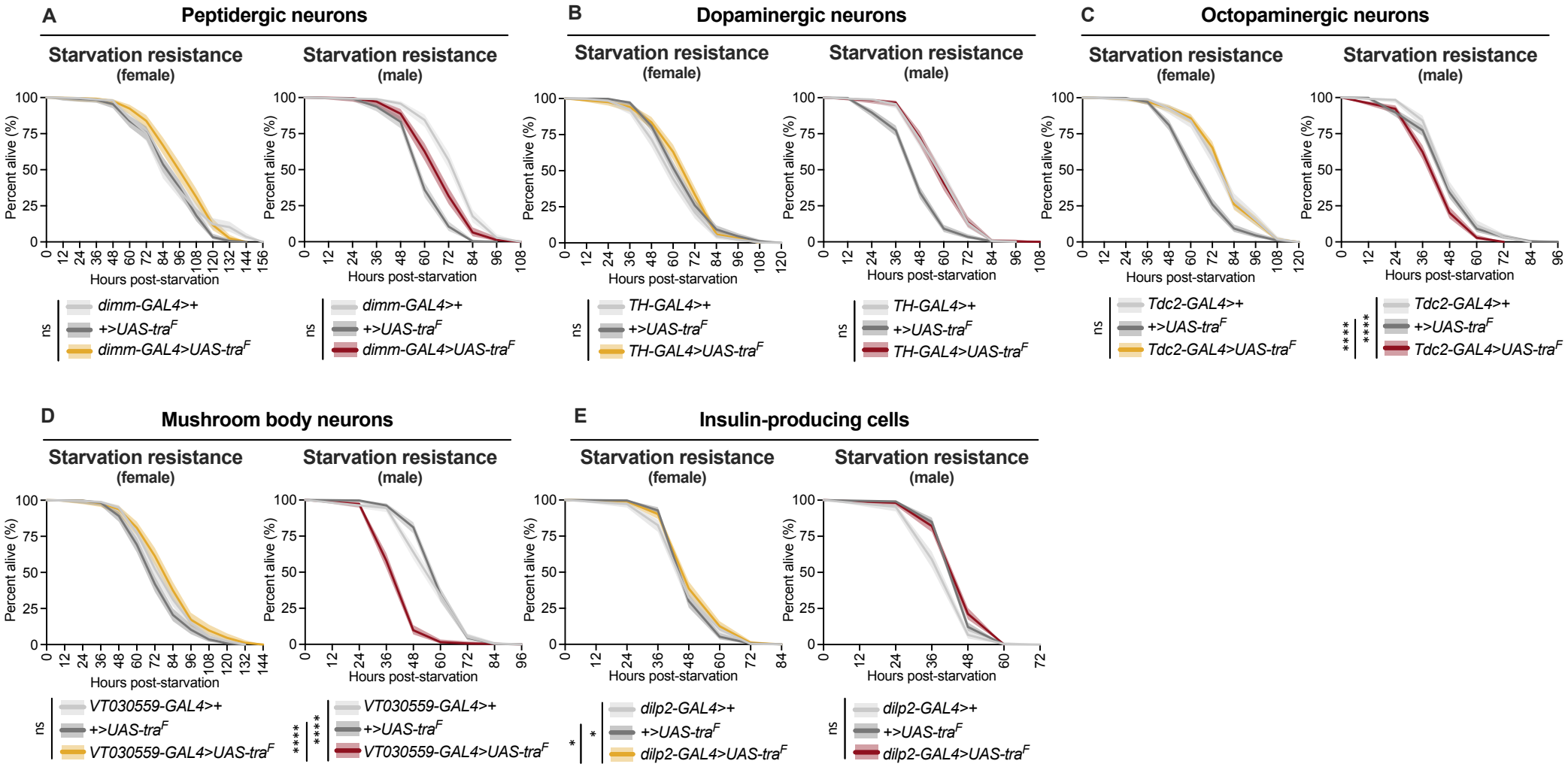

FIGURE 2 - FIGURE SUPPLEMENT 4

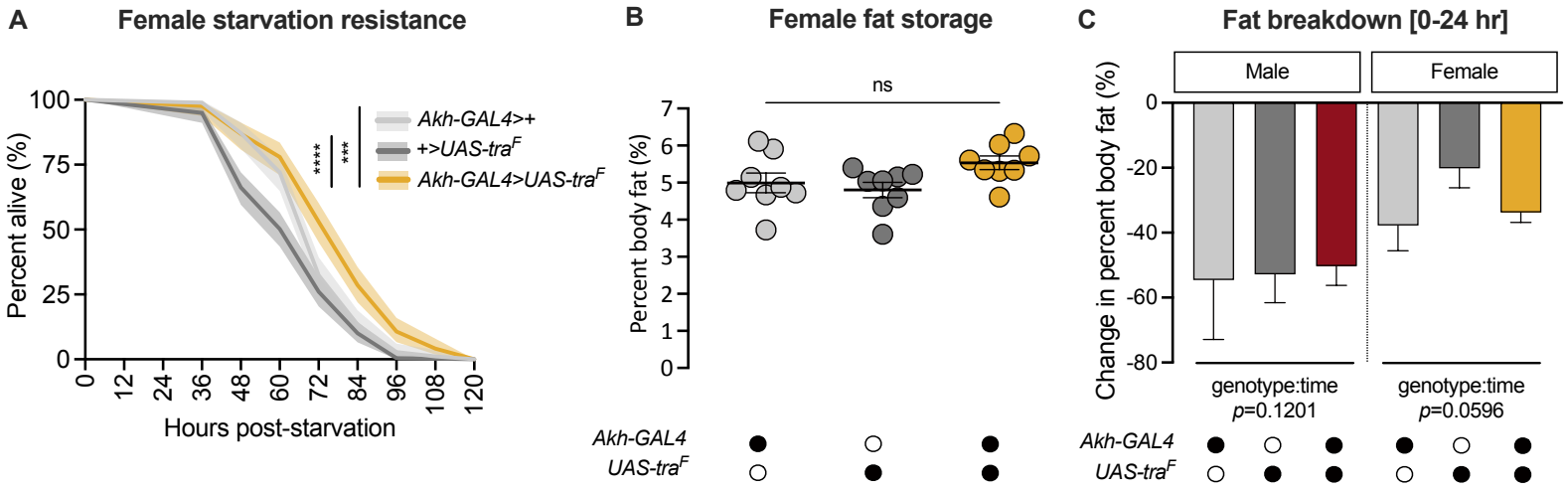

FIGURE 2 - FIGURE SUPPLEMENT 5

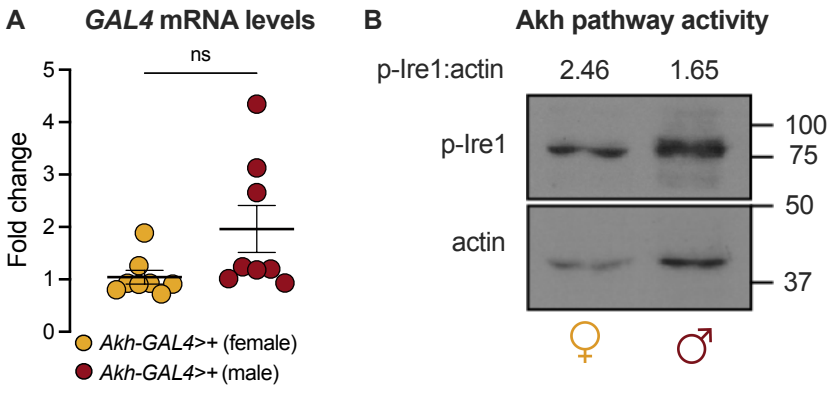

FIGURE 3 - FIGURE SUPPLEMENT 1

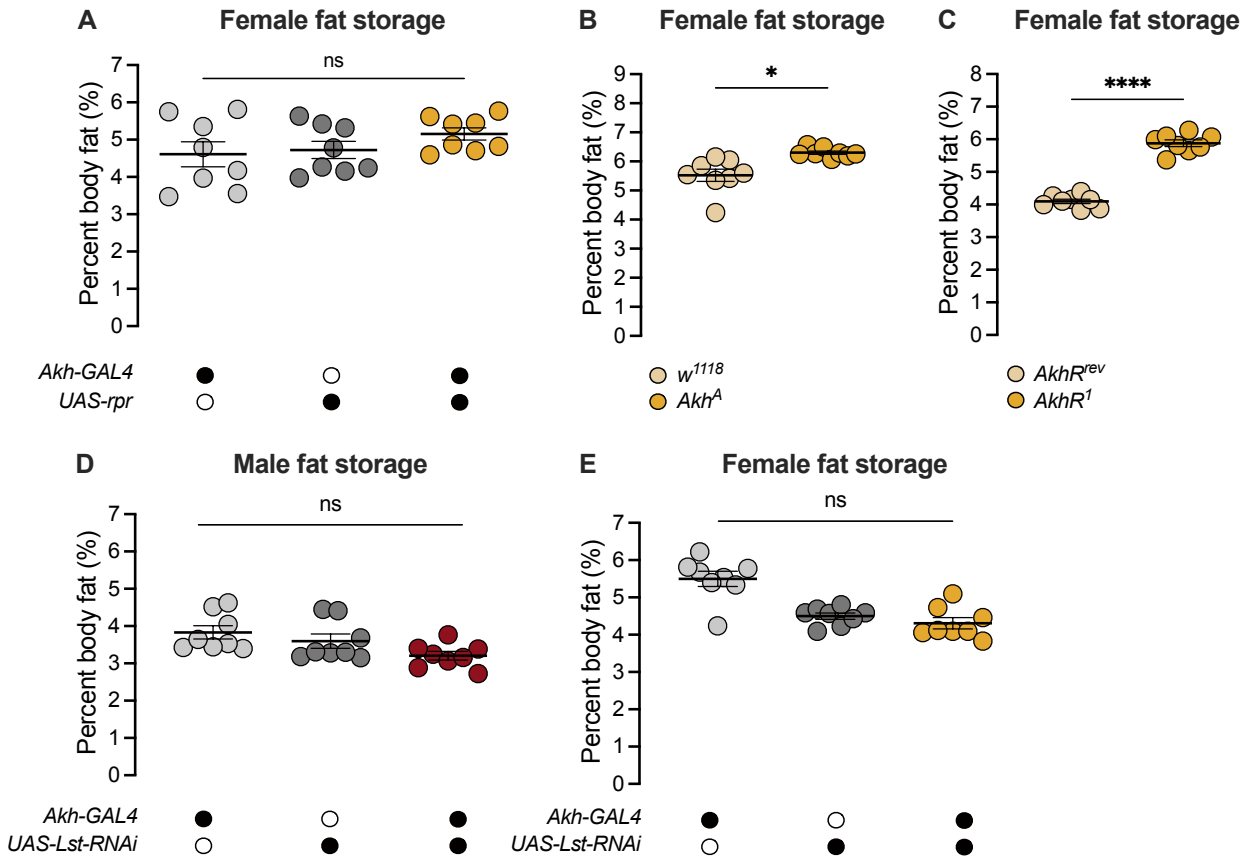

FIGURE 4 - FIGURE SUPPLEMENT 1

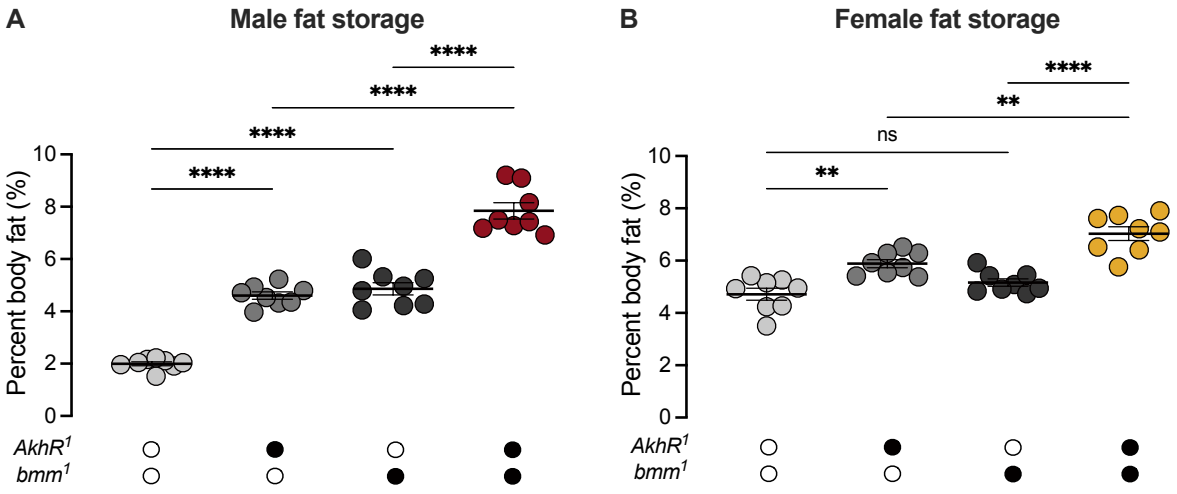

FIGURE 4 - FIGURE SUPPLEMENT 2

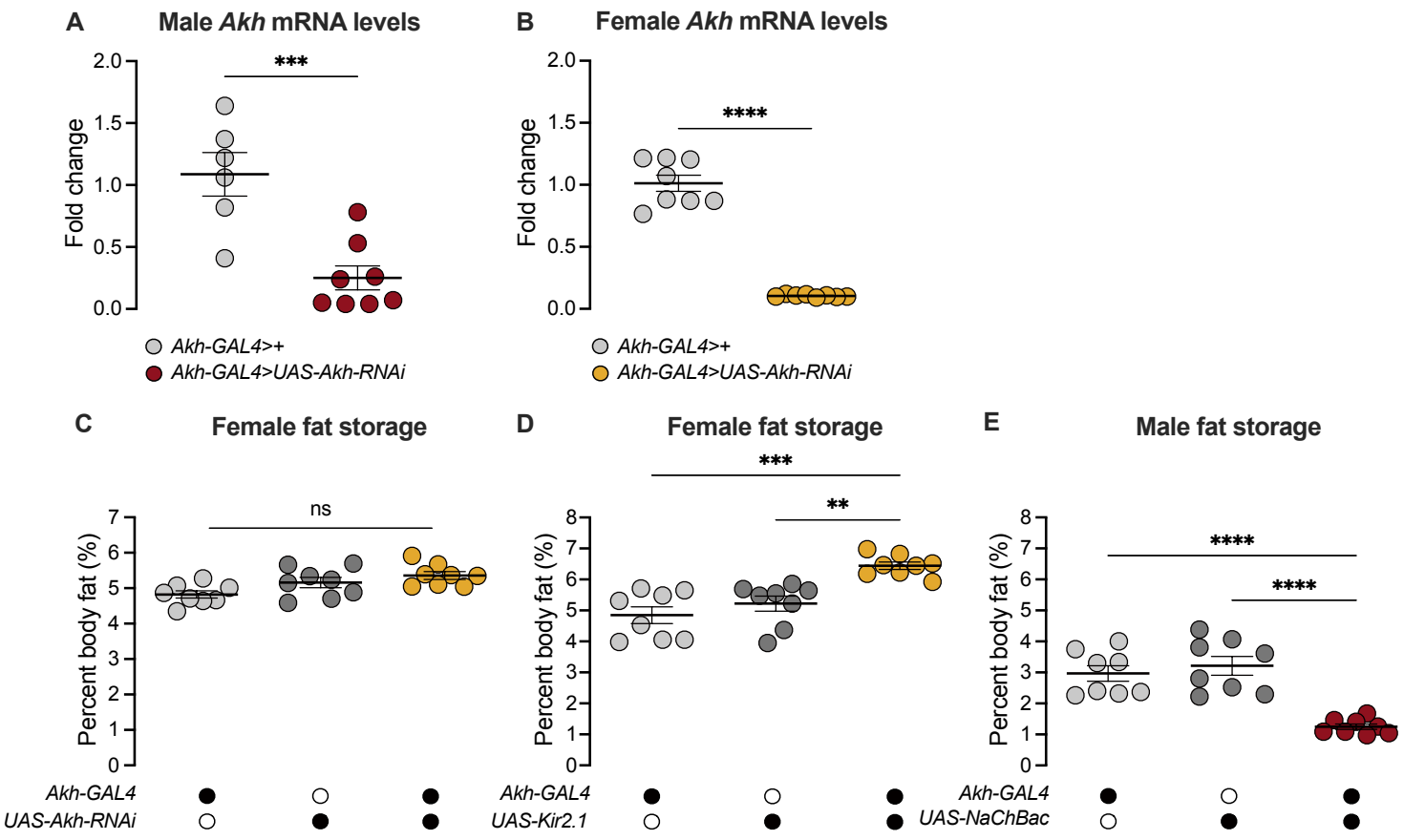

FIGURE 4 - FIGURE SUPPLEMENT 3

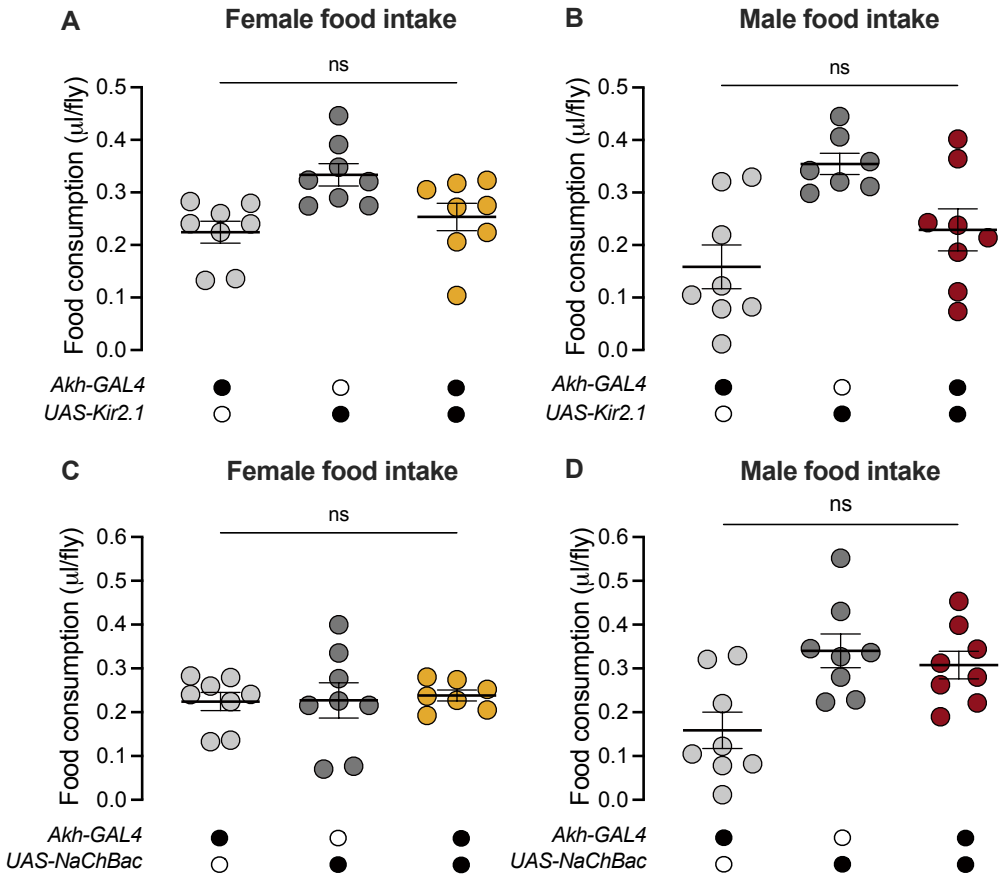

FIGURE 4 - FIGURE SUPPLEMENT 4

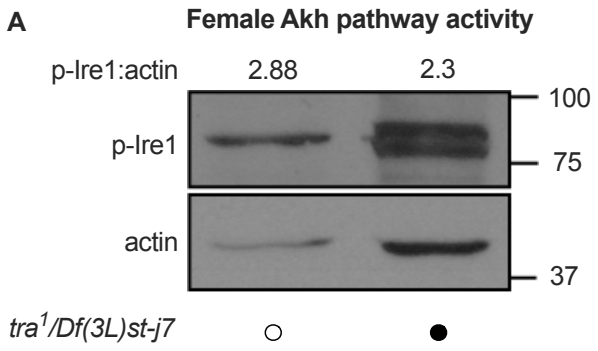

FIGURE 5 - FIGURE SUPPLEMENT 1