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, Vancouver, BC, Canada, V6T 1Z3

§ 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. Circulating Akh 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 stimulates an intracellular signaling cascade that promotes fat breakdown (Braco et al., 2012; Gäde and Auerswald, 2003; Park et al., 2002; Patel et al., 2005; Staubli et al., 2002). 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 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 *Hormone sensitive lipase* (*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* support sex-biased effects on fat storage for many genetic loci (Nelson et al., 2016; Watanabe and Riddle, 2021). As evidence of sex-specific mechanisms underlying whole-body fat storage continues to mount, several reports have also identified male-female differences in phenotypes linked with fat metabolism. For example, sex differences have been reported in energy physiology, metabolic rate, food intake, food preference, circadian rhythm, sleep, immune response, starvation resistance, and lifespan (Andretic and Shaw, 2005; Austad and Fischer, 2016; Belmonte et al., 2020; Chandegra et al., 2017; Helfrich-Förster, 2000; Huber et al., 2004; Hudry et al., 2019; Millington et al., 2021; Park et al., 2018; Reddiex et al., 2013; Regan et al., 2016; Sieber and Spradling, 2015; Videlier et al., 2019; Wat et al., 2020). More work is therefore needed to understand the genetic and physiological mechanisms underlying the male-

female difference in fat storage, and to identify the impact of this sex-specific regulation on key life history traits. Further, it will be critical to elucidate how these mechanisms are linked with upstream factors that determine sex.

In *Drosophila*, sexual development is determined by the number of X chromosomes (Salz and Erickson, 2010). In females, the presence of two X chromosomes triggers the production of a functional splicing factor called Sex lethal (Sxl; FBgn0264270) (Bell et al., 1988; Bridges, 1921; Cline, 1978). Sxl's most well-known downstream target is *transformer* (*tra*; FBgn0003741), where Sxl-dependent splicing of *tra* pre-mRNA allows the production of a functional Tra protein (Belote et al., 1989; Boggs et al., 1987; Inoue et al., 1990; Sosnowski et al., 1989). In males, which have only one X chromosome, no functional Sxl or Tra proteins are made (Cline and Meyer, 1996; Salz and Erickson, 2010). Over several decades, a large body of evidence has accumulated showing that Sxl and Tra direct most aspects of female sexual identity, including effects on abdominal pigmentation, egg-laying, neural circuits, and behaviour (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; Cline, 1978; Cline and Meyer, 1996; Clough et al., 2014; Dauwalder, 2011; Demir and Dickson, 2005; Goodwin et al., 2000; Hall, 1994; Heinrichs et al., 1998; Hoshijima et al., 1991; Inoue et al., 1992; Ito et al., 1996; Nagoshi et al., 1988; Neville et al., 2014; 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

208 w^{1118} control females lacking ovaries (Figure 1B). Given that we reproduced this finding
209 in females carrying a distinct combination of *tra* mutant alleles (Figure 1C) (Hudry et al.,
210 2016), our findings suggest Tra regulates the sex difference in whole-body triglyceride
211 levels by promoting fat storage in females. While females have reduced fat breakdown
212 post-starvation compared with males (Wat et al., 2020), the magnitude of fat breakdown
213 post-starvation was not significantly different between $tra^{1}/Df(3L)st\text{-}j7$ mutants and sex-
214 matched w^{1118} controls (genotype:time interactions p=0.6298 [females], p=0.3853
215 [males]; Supplementary file 1) (Figure 1 - figure supplement 1B). Tra function is
216 therefore required to promote elevated fat storage in females, but does not regulate fat
217 breakdown post-starvation.
218 Given that males normally lack a functional Tra protein (Belote et al., 1989;
219 Boggs et al., 1987; Inoue et al., 1990; Sosnowski et al., 1989), we next asked whether
220 the absence of Tra in males explains their reduced whole-body triglyceride levels and
221 rapid triglyceride breakdown post-starvation (Wat et al., 2020). To test this, we
222 ubiquitously overexpressed Tra using *daughterless* (*da*)-*GAL4*, an established way to
223 feminize male flies (Ferveur et al., 1995; Rideout et al., 2015), and examined whole-
224 body fat metabolism. In 5-day-old *da-GAL4>UAS-*tra^{F} males, whole-body triglyceride
225 levels were significantly higher than in age-matched *da-GAL4>+* or *+>UAS-*tra^{F} control
226 males (Figure 1D). No increase in whole-body fat storage was observed in age-matched
227 *da-GAL4>UAS-*tra^{F} females compared with *da-GAL4>+* or *+>UAS-*tra^{F} control females
228 (Figure 1 - figure supplement 1C); therefore, the sex difference in fat storage was
229 reduced. Because high levels of Tra overexpression may influence viability (Siera and
230 Cline, 2008), we also measured fat storage in males carrying an allele of *tra* that directs
231 the production of physiological Tra levels ($tra^{F\ K\text{-}IN}$ allele) (Hudry et al., 2019). As in *da-*
232 *GAL4>UAS-*tra^{F} males, whole-body triglyceride levels were significantly higher in $tra^{F\ K\text{-}IN}$
233 males compared with w^{1118} control males (Figure 1E), indicating that the gain of a
234 functional Tra protein in males promotes elevated whole-body fat storage.
235 Importantly, the presence of rudimentary ovaries in $tra^{F\ K\text{-}IN}$ males did not explain
236 their increased fat storage, as whole-body fat storage was still higher in $tra^{F\ K\text{-}IN}$ males
237 lacking gonads compared with gonadless control males (Figure 1F). The elevated fat
238 storage in $tra^{F\ K\text{-}IN}$ males also cannot be attributed to ecdysone production by the

rudimentary ovaries, as no ecdysone target genes were upregulated (Figure 1 - figure supplement 1D) (Sieber and Spradling, 2015). Together, these data indicate that lack of Tra function contributes to the reduced whole-body triglyceride levels normally observed in males. In males, this role for Tra may also extend to regulation of fat breakdown, as triglyceride mobilization post-starvation was significantly reduced in *da-GAL4>UAS-tra*^{F} males compared with *da-GAL4*>+ or +>*UAS-tra*^{F} controls during a 24 hr starvation period (genotype:time $p<0.0001$ [males]; Supplementary file 1) (Figure 1 - figure supplement 1E), a finding we reproduced in *tra*$^{F\ K\text{-}IN}$ males (Figure 1 - figure supplement 1F). While this effect of Tra on fat breakdown in males does not perfectly align with our data from *tra* mutant females, we note a trend toward increased fat breakdown in *tra* mutant females that was not statistically significant (Figure 1 - figure supplement 1B). Taken together, these data support a clear role for Tra in regulating the sex difference in fat storage, and suggest that a role for Tra in regulating fat breakdown cannot be ruled out.

transformer **function in neurons regulates the sex difference in fat storage**

Tra function is required in many cell types, tissues, and organs to promote female sexual 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; Hall, 1994; Heinrichs et al., 1998; Hoshijima et al., 1991; Inoue et al., 1992; Ito et al., 1996; Nagoshi et al., 1988; Neville et al., 2014; 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). To determine the cell types and tissues in which Tra function is required to influence fat metabolism, we overexpressed Tra using a panel of GAL4 lines that drive expression in subsets of cells and/or tissues. To rapidly assess potential effects on fat metabolism, we measured starvation resistance, an established readout for changes to fat storage and breakdown (Beller et al., 2010; Bi et al., 2012; Choi et al., 2015; Grönke et al., 2003, 2005, 2007; Gutierrez et al., 2007).

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

To determine whether increased starvation resistance in *elav-GAL4>UAS-tra^F* males was due to altered fat metabolism, we measured whole-body triglyceride levels in males and females with neuronal Tra overexpression. We found that *elav-GAL4>UAS-tra^F* males (Figure 2D), but not females (Figure 2 - figure supplement 3B), showed a significant increase in whole-body fat storage compared with sex-matched *elav-GAL4>+* and *+>UAS-tra^F* controls. This suggests that the male-specific increase in starvation resistance (Figure 2C) was due to increased fat storage in *elav-GAL4>UAS-tra^F* males, which we confirm by showing that the rate of fat breakdown in *elav-GAL4>UAS-tra^F* males and females was not significantly different from sex-matched *elav-GAL4>+* and *+>UAS-tra^F* controls (Figure 2 - figure supplement 3C) (genotype:time interaction p=0.2789 [males], p=0.7058 [females]; Supplementary file 1). Neurons are therefore one cell type in which Tra function influences the sex difference in whole-body triglyceride storage.

To identify specific neurons that mediate Tra's effects on starvation resistance and whole-body fat storage, we overexpressed Tra in neurons known to affect fat metabolism and measured starvation resistance (Figure 2 - figure supplement 4A-E) (Al-Anzi and Zinn, 2018; Al-Anzi et al., 2009; Chung et al., 2017; Li et al., 2016; May et

al., 2020; Min et al., 2016; Mosher et al., 2015; Zhan et al., 2016). One group of neurons that significantly augmented starvation resistance upon Tra expression was the APCs (Figure 2E), a group of neuroendocrine cells in the corpora cardiaca that produce Akh and other peptide hormones such as Limostatin (Lst; FBgn0034140) (Alfa et al., 2015; Lee and Park, 2004). Flies with APC-specific Tra expression (*Akh-GAL4>UAS-tra^{F})* had significantly increased starvation resistance compared with sex-matched *Akh-GAL4>+* and *+>UAS-tra^{F}* controls (Figure 2E; Figure 2 - figure supplement 5A). To determine whether the starvation resistance phenotype indicated altered fat storage, we compared whole-body triglyceride levels in *Akh-GAL4>UAS-tra^{F}* males and females with sex-matched *Akh-GAL4>+* and *+>UAS-tra^{F}* controls. There was a significant increase in whole-body fat storage in males (Figure 2F) but not females (Figure 2 - figure supplement 5B) with APC-specific Tra expression. This indicates Tra function in the APCs promotes fat storage, revealing a previously unrecognized role for the APCs in regulating the sex difference in fat storage. Indeed, fat breakdown was unaffected in *Akh-GAL4>UAS-tra^{F}* males and females compared with sex-matched *Akh-GAL4>+* and *+>UAS-tra^{F}* controls (Figure 2 - figure supplement 5C) (genotype:time interaction p=0.1201 [males] and p=0.0596 [females]; Supplementary file 1).

Sex-specific regulation of Adipokinetic hormone leads to a male bias in pathway activity

Given that the sexual identity of the APCs impacts whole-body fat storage, we compared the regulation of *Akh*, APC activity, and Akh signaling between adult males and females. We first examined *Akh* and *AkhR* mRNA levels in both sexes using quantitative real-time polymerase chain reaction (qPCR). We found that mRNA levels of both *Akh* and *AkhR* were significantly higher in 5-day-old *w^{1118}* males than in females (Figure 3A,B). This male bias in *Akh* mRNA levels did not reflect an increased APC number in males, as we found no sex difference in the number of APCs (Figure 3C). Because Akh release from the APCs is regulated by APC activity (Kubrak et al., 2020; Oh et al., 2019), we next measured APC activity in males and females by driving APC-specific expression of calcium-responsive chimeric transcription factor *LexA-VP16-*

NFAT (*Akh-GAL4>UAS-LexA-VP16-NFAT* [called *UAS-CaLexA*]) (Masuyama et al., 2012). Sustained APC activity triggers nuclear import of LexA-VP16-NFAT, where it drives expression of a GFP reporter downstream of a LexA-responsive element (Masuyama et al., 2012). Monitoring GFP levels in the APCs therefore provides a straightforward way to monitor APC activity.

In 5-day-old *Akh-GAL4>UAS-CaLexA* males, GFP levels were significantly higher than in age- and genotype-matched females (Figure 3D-H). Because *GAL4* mRNA levels were not significantly different between males and females carrying the *Akh-GAL4* transgene (Figure 3 - figure supplement 1A), and the number of APCs did not differ between the sexes (Figure 3C), these findings indicate that the APCs are more active in males than in females. To determine whether the male bias in *Akh/AkhR* mRNA levels and APC activity affected Akh pathway activity, we next compared levels of an Akh pathway readout called phosphorylated Inositol-requiring enzyme 1 (Ire1; FBgn0261984) (p-Ire1) (Song et al., 2017) between 5-day-old male and female flies. We found that p-Ire1 levels were higher in w^{1118} males compared with genotype-matched females in three out of four biological replicates (Figure 3I-K; Figure 3 - figure supplement 1B), a finding that aligns with the sex difference in *Akh/AkhR* mRNA levels and APC activity. Taken together, our data reveal a previously unrecognized male bias in Akh pathway activity.

The Adipokinetic hormone pathway contributes to the sex difference in fat storage

As an initial step toward establishing whether the male bias in Akh pathway activity contributes to sex differences in fat metabolism, we used a published approach to ablate the APCs (*Akh-GAL4>UAS-reaper* (*rpr*)) (Lee and Park, 2004; White et al., 1996), and measured whole-body triglyceride levels in each sex. Because the sexual identity of the APCs affects fat storage and not fat breakdown (Figure 2F; Figure 2 - figure supplement 5C), we focused our analysis on measuring triglyceride storage rather than mobilization. Triglyceride levels were significantly higher in 5-day-old *Akh-GAL4>UAS-rpr* males than in *Akh-GAL4>+* and *+>UAS-rpr* control males (Figure 4A). In

363 contrast, triglyceride levels in 5-day-old *Akh-GAL4>UAS-rpr* females were not
364 significantly different from *Akh-GAL4>+* and *+>UAS-rpr* control females (Figure 4 -
365 figure supplement 1A). This suggests that the male bias in Akh pathway activity
366 normally contributes to the sex difference in fat storage by limiting triglyceride
367 accumulation in males. Importantly, we reproduced the male-biased effects on fat
368 storage in flies carrying mutant *Akh* and *AkhR* alleles (Akh^{A} and $AkhR^{1}$, respectively)
369 (Figure 4B,C; Figure 4 - figure supplement 1B,C), and show that APC-specific
370 knockdown of *Lst* had no effect on fat storage in either sex (Figure 4 - figure supplement
371 1D,E). These findings support a model in which it is Akh production by the APCs that
372 plays a role in regulating the male-female difference in fat storage. While Akh is a
373 known regulator of whole-body fat metabolism (Heier and Kühnlein, 2018; Heier et al.,
374 2021), our findings reveal a new role for Akh in regulating the sex difference in fat
375 storage. Notably, this Akh-mediated regulation of the male-female difference in fat
376 storage operates in a parallel pathway to the previously described sex-specific role of
377 triglyceride lipase *bmm* (Figure 4 - figure supplement 2A,B) (Wat et al., 2020).
378 Beyond the APC ablation or complete loss of Akh, we next wanted to test
379 whether the sex-specific Akh regulation we uncovered contributes to the male-female
380 difference in fat storage. To this end, we used a genetic approach to manipulate either
381 *Akh* mRNA levels or APC activity. To determine whether the male bias in *Akh* mRNA
382 levels contributes to the sex difference in fat storage, we measured whole-body
383 triglyceride levels in flies with APC-specific expression of *Akh-RNAi* (*Akh-GAL4>UAS-*
384 *Akh-RNAi*). Importantly, this manipulation effectively reduced *Akh* mRNA levels in both
385 sexes (Figure 4 - figure supplement 3A,B). In males, whole-body triglyceride levels were
386 significantly higher in *Akh-GAL4>UAS-Akh-RNAi* flies compared with *Akh-GAL4>+* and
387 *+>UAS-Akh-RNAi* control flies (Figure 4D). *Akh-GAL4>UAS-Akh-RNAi* female flies, in
388 contrast, showed no significant change in whole-body fat storage compared with *Akh-*
389 *GAL4>+* and *+>UAS-Akh-RNAi* control females (Figure 4 - figure supplement 3C). This
390 indicates a strongly male-biased effect on fat storage due to reduced *Akh* mRNA levels,
391 suggesting that the sex difference in *Akh* mRNA levels contributes to the male-female
392 difference in whole-body fat storage.

To determine whether the male bias in APC activity also influences the sex difference in fat storage, we silenced the APCs by APC-specific overexpression of an inwardly rectifying potassium channel Kir2.1 (Baines et al., 2001) and measured whole-body triglyceride levels. Whole-body fat storage in *Akh-GAL4>UAS-Kir2.1* adult males was significantly higher compared with *Akh-GAL4*>+ and +>*UAS-Kir2.1* control males (Figure 4E). In females, while we observed significantly elevated whole-body fat storage in *Akh-GAL4>UAS-Kir2.1* adults compared with *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls (Figure 4 - figure supplement 3D), the magnitude of this increase was larger in males (sex:genotype interaction p=0.0455; Supplementary file 1). Together, these data suggest that the male bias in APC activity contributes to the sex difference in fat storage by limiting triglyceride accumulation in males. Indeed, augmenting APC activity in females using a bacterial voltage-gated sodium channel (*UAS-NaChBac*) significantly reduced fat storage in females (Figure 4F; Figure 4 - figure supplement 3E). While Akh affects food-related behaviours in some contexts (Choi et al., 2015; Hentze et al., 2015; Huang et al., 2020), we observed no significant effects of altered APC activity on feeding behaviour in either sex (Figure 4 - figure supplement 4A-D). This suggests that the male-biased effect of APC manipulation on fat storage cannot be fully explained by effects on food intake. Thus, in addition to the contribution of elevated *Akh* mRNA levels in males to the sex difference in fat storage, we also identify a role for the male bias in APC activity in the sex-specific regulation of whole-body triglyceride levels.

transformer **regulates the sex difference in fat storage via the Adipokinetic hormone pathway**

Given that Tra function and the Akh pathway both contribute to the male-female difference in fat storage, we asked whether the presence of Tra affects the sex bias in Akh pathway activity. In 5-day-old $tra^{1}/Df(3L)st\text{-}j7$ females, levels of p-Ire1 were higher than in w^{1118} control females in three out of four biological replicates (Figure 5A-C; Figure 5 - figure supplement 1A). This suggests the presence of Tra in females normally represses Akh pathway activity. Indeed, loss of Tra significantly increased *Akh* mRNA levels in females (Figure 5D). Given Tra's effects on Akh pathway activity, we next

tested whether the change in Akh pathway function was significant for Tra's effects on whole-body triglyceride levels. We predicted that if increased Akh pathway activity caused the lower fat storage in *tra* mutant females, genetic manipulations that reduce Akh pathway activity should block this reduction in whole-body triglyceride levels. While all female genotypes lacking *tra* function had reduced fat storage compared with control females (Figure 5E), APC ablation in *tra* mutant females rescued this decrease in whole-body triglyceride levels (Figure 5E). Indeed, fat storage in *tra* mutant females lacking APCs was not significantly different from w^{1118} control females (p=0.9384; Supplementary file 1) (Figure 5E), indicating that the increased Akh pathway activity we observed in *tra* mutant females was one reason for their reduced fat storage. Given that APC activation in males expressing physiological levels of Tra similarly rescued the Tra-induced increase in whole-body triglyceride levels (Figure 5F), these findings suggest that the sex-specific regulation of Akh pathway activity represents one way *tra* influences the male-female difference in fat storage.

Loss of Adipokinetic hormone has opposite effects on reproductive success in each sex and mediates a fecundity-lifespan tradeoff in females

Our results suggest that adult females show lower Akh pathway activity and higher fat storage, whereas males maintain a higher level of Akh activity and lower fat storage. Because the correct regulation of fat storage in each sex influences reproduction (Buszczak et al., 2002; Grönke et al., 2005; Sieber and Spradling, 2015; Wat et al., 2020), we tested how loss of this critical regulator of the sex difference in fat storage impacted offspring production in each sex. In Akh^{A} mutant males, we found that the proportion of males copulating with a *Canton-S (CS)* virgin female was lower than in control w^{1118} males at each 10 min interval during a 60 min observation period (Figure 6A). When we counted viable offspring from these copulation events, we found that Akh^{A} mutant males had significantly fewer overall progeny than w^{1118} control males (Figure 6B). These results suggest that Akh function normally promotes reproductive success in males; however, it is important to note that Akh function is not absolutely

required for male fertility, as a prolonged 24 hr period of contact between Akh^{A} mutant males and *CS* females allowed the production of normal progeny numbers (Figure 6C).

In contrast to males, loss of Akh in females increased fecundity (Figure 6D). Specifically, Akh^{A} mutant females produced a significantly higher number of offspring compared with w^{1118} controls (Figure 6D). Thus, in females, a low level of Akh pathway activity promotes fecundity. Given that a change in one life history trait such as reproduction often affects traits such as longevity (Chapman et al., 1995; Flatt, 2011; Fowler and Partridge, 1989; Hansen et al., 2013), we also measured lifespan in females with reduced Akh pathway function. We found that lifespan was significantly shorter in *Akh-GAL4>UAS-Kir2.1* females compared with *Akh-GAL4>+* and *+>UAS-Kir2.1* control 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; Hall, 1994; Heinrichs et al., 1998; Hoshijima et al., 1991; Inoue et al., 1992; Ito et al., 1996; Nagoshi et al., 1988; Neville et al., 2014; 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), uncovering a role for *tra* in regulating fat storage significantly extends our understanding of how sex differences in metabolism arise. Given that sex differences exist in other aspects of metabolism (*e.g.* oxygen consumption) (Wat et al., 2020), this new insight suggests that more work will be needed to determine whether *tra* contributes to sexual dimorphism in additional metabolic traits. Indeed, one study showed that *tra* influences the sex difference in adaptation to hydrogen peroxide stress (Pomatto et al., 2017). Beyond metabolism, Tra also regulates multiple aspects of development and physiology such as intestinal stem cell proliferation (Ahmed et al., 2020; Hudry et al., 2016; Millington and Rideout, 2018), carbohydrate metabolism (Hudry et al., 2019), body size (Mathews et al., 2017; Rideout et al., 2015), phenotypic plasticity (Millington et al., 2021), and lifespan responses to dietary restriction (Regan et al., 2016). Because some, but not all, of these studies identify a cell type in which Tra function influences these diverse phenotypes, future studies will need to determine which cell types and tissues require Tra expression to establish a female metabolic and physiological state. Indeed, recent single-cell analyses reveal widespread gene expression differences in shared cell types between the sexes (Li et al., 2021).

Identifying neurons as the anatomical focus of Tra's effects on fat storage was another key finding from our study. While many sexually dimorphic neural circuits related to behaviour and reproduction have been identified (Anand et al., 2001; Auer and Benton, 2016; Baker et al., 2001; Billeter et al., 2006; Clyne and Miesenböck, 2008; Dauwalder, 2011; Demir and Dickson, 2005; Evans and Cline, 2007; Goodwin et al., 2000; Hall, 1994; Inoue et al., 1992; Ito et al., 1996; Kimura et al., 2019; Kvitsiani and Dickson, 2006; Neville et al., 2014; Nojima et al., 2014; Pavlou et al., 2016; von Philipsborn et al., 2014; Rezával et al., 2014, 2016; Rideout et al., 2007, 2010; Ryner et al., 1996; Sato et al., 2019; Shirangi et al., 2016; Wang et al., 2020), less is known about sex differences in neurons that regulate physiology and metabolism. Indeed, while many studies have identified neurons that regulate fat metabolism (Al-Anzi and Zinn, 2018; Al-Anzi et al., 2009; Chung et al., 2017; Li et al., 2016; May et al., 2020; Min et al., 2016; Mosher et al., 2015; Zhan et al., 2016), these studies were conducted in single- or mixed-sex populations. Because male-female differences in neuron number (Billeter et al., 2006; Castellanos et al., 2013; Demir and Dickson, 2005; Garner et al., 2018; Lee and Hall, 2001; Rideout et al., 2007, 2010; Robinett et al., 2010; Taylor and Truman, 1992), morphology (Cachero et al., 2010; Kimura et al., 2019), activity (Guo et al., 2016), and connectivity (Cachero et al., 2010; Nojima et al., 2021) have all been described across the brain and ventral nerve cord (Mellert et al., 2010, 2016), a detailed analysis of neuronal populations that influence metabolism will be needed in both sexes to understand how neurons contribute to the sex-specific regulation of metabolism and physiology. Indeed, while our identification of a role for APC sexual identity in regulating the male-female difference in fat storage represents a significant step forward in understanding how sex differences in neurons influence metabolic traits, more knowledge is needed of sexual dimorphism in this critical neuronal subset.

An obvious starting point for learning more about the sex-specific regulation of fat storage by the APCs is to examine how sexual identity influences known APC regulatory mechanisms. For example, there are physical connections between corazonin- and neuropeptide F-positive (CN) neurons and APCs in adult male flies (Oh et al., 2019), and between the APCs and a bursicon-α-responsive subset of DLgr2 neurons in females (Scopelliti et al., 2019). These connections inhibit APC activity: CN

neurons inhibit APC activity in response to high hemolymph sugar levels (Oh et al., 2019), whereas binding of bursicon-α to DLgr2 neurons inhibits APC activity in nutrient-rich conditions (Scopelliti et al., 2019). Future studies will therefore need to determine whether these physical connections exist in both sexes. Male-female differences in circulating factors that regulate the APCs may also exist. For example, gut-derived Allatostatin C (AstC; FBgn0032336) binds its receptor on the APCs to trigger Akh release; however, loss of AstC affects fat metabolism and starvation resistance in females but not in males (Kubrak et al., 2020). This suggests that sex differences in AstC production or release may exist. Another circulating factor that may influence the sex difference in fat storage is skeletal muscle-derived unpaired 2 (upd2; FBgn0030904), which regulates hemolymph Akh levels to maintain diurnal fat metabolism (Zhao and Karpac, 2017). Given that circulating peptides such as Allatostatin A (AstA; FBgn0015591), *Drosophila* insulin-like peptides (Dilps), and activin ligands also influence Akh pathway activity (Ahmad et al., 2020; Hentze et al., 2015; Post et al., 2019; Song et al., 2017), a systematic survey of circulating factors that modulate Akh production, release, and Akh pathway activity in each sex will be needed to fully understand the sex-specific regulation of fat storage.

In addition to fat metabolism, it will be important to extend our understanding of how sex-specific Akh regulation affects additional Akh-regulated phenotypes. For example, Akh has been linked with the regulation of lifespan (Bednářová et al., 2018; Liao et al., 2021), starvation resistance (Isabel et al., 2005; Kubrak et al., 2020; Mochanová et al., 2018), locomotion (Isabel et al., 2005; Lee and Park, 2004), immune responses (Adamo et al., 2008), cardiac function (Isabel et al., 2005; Noyes et al., 1995), oxidative stress responses (Gáliková et al., 2015), and fertility (Liao et al., 2021). Yet, most studies were performed in mixed- or single-sex populations. This suggests additional work is needed to determine how changes to Akh pathway function affect physiology, development, and life history in both sexes. Importantly, the lessons we learn may also extend to other species. Akh signalling is highly conserved across invertebrates (Gäde and Auerswald, 2003; Lorenz and Gäde, 2009; Staubli et al., 2002), and is functionally similar to the mammalian β-adrenergic and glucagon systems (Grönke et al., 2007; Lee and Park, 2004; Staubli et al., 2002). Because sex-specific

578 regulation of both glucagon and the β-adrenergic systems have been described in
579 mammalian models and in humans (Al-Gburi et al., 2017; Bell et al., 2001; Bilginoglu et
580 al., 2007; Brooks et al., 2015; Claustre et al., 1980; Dart et al., 2002; Davis et al., 2000;
581 Drake et al., 1998; Freedman et al., 1987; Hinojosa-Laborde et al., 1999; Hoeker et al.,
582 2014; Hogarth et al., 2007; Lafontan et al., 1997; Luzier et al., 1998; McIntosh et al.,
583 2011; Ng et al., 1993), detailed studies on sex-specific Akh regulation and function in
584 flies may provide vital clues into the mechanisms underlying male-female differences in
585 physiology and metabolism in other animals.
586
587 **MATERIALS AND METHODS**
588
589 ***Data availability.*** Details of all statistical tests and *p*-values are in Supplementary file 1.
590 All raw data generated in this study are in Supplementary file 2. All primer sequences
591 are in Supplementary file 3. Original image files for all images in this study are available
592 upon request.
593
594 ***Fly husbandry.*** Fly stocks were maintained at 25°C in a 12:12 light:dark cycle. All
595 larvae were reared at a density of 50 larvae per 10 ml of fly media (recipe in
596 Supplementary file 4). Males and females were separated either as early pupae by
597 gonad size, or late pupae by the presence of sex combs. Sex-transformed males and
598 females were distinguished by the presence (males) or absence (females) of $B^{S}Y$.
599 Single-sex groups of 20 pupae were transferred to damp filter paper within a food vial
600 until eclosion. Unless otherwise stated, all experiments used 5- to 7-day-old flies.
601
602 ***Fly strains.*** We obtained the following strains from the Bloomington *Drosophila* Stock
603 Center: *Canton-S* (#64349), w^{1118} (#3605), *UAS-nGFP* (#4775), *UAS-Akh-RNAi*
604 (#27031), *UAS-tra*^{F} (#4590), *tra*^{1} (#675), *Df(3L)st-j7* (#5416), *UAS-NaChBac* (#9468),
605 *UAS-Kir2.1* (#6595), *UAS-reaper* (#5823), *UAS-CaLexA* (#66542). We obtained *Akh*^{A},
606 *AkhR*^{rev}, *AkhR*^{1}, *bmm*^{1}, and *AkhR*^{1}*;bmm*^{1} as kind gifts from Dr. Ronald Kühnlein
607 (Gáliková et al., 2015; Grönke et al., 2005, 2007), *tra*^{KO} and *tra*$^{F\ K\text{-}IN}$ as kind gifts from
608 Dr. Irene Miguel-Aliaga (Hudry et al., 2016, 2019), and *Mex-GAL4* as a kind gift from Dr.

Claire Thomas (Phillips and Thomas, 2006). The following GAL4 lines were used for tissue-specific expression: *da-GAL4* (ubiquitous), *cg-GAL4* (fat body), *r4-GAL4* (fat body), *Lsp2-GAL4* (fat body), *Myo1A-GAL4* (enterocytes), *Mex-GAL4* (enterocytes), *dMef2-GAL4* (skeletal muscle), *repo-GAL4* (glia), *elav-GAL4* (neurons), *c587-GAL4* (somatic cells of the gonad), *tj-GAL4* (somatic cells of the gonad), *nos-GAL4* (germ cells of the gonad), *dimmed-GAL4* (peptidergic neurons), *TH-GAL4* (dopaminergic neurons), *Tdc2-GAL4* (octopaminergic neurons), *VT030559-GAL4* (mushroom body neurons), *dilp2-GAL4* (insulin-producing cells), *Akh-GAL4* (Akh-producing cells). All transgenic stocks were backcrossed into a w^{1118} background for a minimum of 5 generations.

Adult weight. To measure adult weight, groups of ten flies were weighed in 1.5 ml microcentrifuge tubes on an analytical balance (Mettler-Toledo, ME104).

RNA extraction, cDNA synthesis, and qPCR. One biological replicate consisted of five flies homogenized in 200 µl of Trizol. RNA was extracted following manufacturer's instructions, as previously described (Wat et al., 2020). cDNA was synthesized from RNA using the Quantitect Reverse Transcription Kit (Qiagen, 205311). qPCR was used to quantify relative mRNA transcript levels as previously described (Wat et al., 2020). See Supplementary file 3 for a full list of primers.

Whole-body triglyceride measurements. One biological replicate consisted of five flies homogenized in 200 µl of 0.1% Tween (AMresco, 0777-1L) in 1X PBS using 50 µl of glass beads (Sigma, 11079110) agitated at 8 m/s for 5 seconds (OMNI International Bead Ruptor 24). Assay was performed according to established protocols (Tennessen et al., 2014) as previously described (Wat et al., 2020).

Western blotting. One biological replicate consisted of ten flies homogenized in extraction buffer (females=200 µl, males=125 µl) containing 20 mM Hepes (pH 7.8), 450 mM NaCl, 25% glycerol, 50 mM NaF, 0.2 mM EDTA, 0.5% Triton X-100, 1 mM PMSF, 1 mM DTT, 1X cOmplete Protease Inhibitor Cocktail (Roche), and 1X PhosSTOP (Roche) using 50 µl of glass beads (Sigma, 11079110) agitated at 8 m/s for 5 seconds (OMNI

International Bead Ruptor 24). Samples were incubated on ice for 5 min before cellular debris was pelleted by centrifugation at 10,000 rpm for 5 min at 4°C and supernatant was removed (Thermo Scientific, Heraeus Pico 21 centrifuge). Centrifugation was repeated twice more to remove fat from the samples. Protein concentration of each sample was determined by a Bradford Assay (Bio-Rad, 550-0205); 20 µg of protein per sample was loaded onto a 12% SDS-PAGE gel. Immunoblotting was performed as previously described (Millington et al., 2021). Primary antibodies used were rabbit anti-p-Ire1 (1:1000; Abcam #48187) and mouse anti-actin (1:100; Santa Cruz #sc-8432). Secondary antibodies used were goat anti-rabbit (1:5000; Invitrogen #65-6120) and horse anti-mouse (1:2000; Cell Signaling #7076).

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

Capillary Feeder Assay. One biological replicate consisted of ten flies placed into a specialized 15 ml conical vial with access to 2 capillary tubes. Capillary tubes were filled with fly food media containing 5% sucrose, 5% yeast extract, 0.3% propionic acid, and 0.15% nipagin. Approximately 0.5 µl of mineral oil was added to the top of each capillary tube to prevent evaporation. All vials were placed into fitted holes in the lid of a large plastic container. A shallow layer of water was poured into the base of the container to

maintain high humidity throughout the experiment. The meniscus of the fly food media was marked before the start of the experiment and again after 24 hr. The distance between the marks is used to quantify the volume of fly food media that was consumed (1 mm=0.15 µl). The volume of fly food consumed was normalized to the weight of individual flies (protocol adapted from Stafford et al., 2012).

Male fertility. One singly-housed male was placed with a group of three virgin *Canton-S (CS*) females and allowed to interact for 60 min. At 10 min intervals during the 60 min observation period, we recorded whether a copulating male-female pair was present in the vial. After the 60 min observation period, the male was removed from the vial and the females were allowed to lay eggs for 72 hr (flies were transferred to new food every 24 hr). After 72 hr, the females were removed and progeny were allowed to develop. After 10 days, we counted the number of pupae in each vial. For the 24 hr mating assay, one singly-housed male was allowed to interact with three virgin *CS* females for 24 hr before the male was removed and females were allowed to lay eggs for 72 hr as described above.

***Female fecundity*.** One virgin female was placed with a group of three virgin *CS* males for 24 hr. The females were then transferred onto fresh food every 24 hr for 3 days and the number of pupae were counted as described above.

Starvation resistance. 5-day-old flies were transferred to vials containing 2 ml of starvation media (0.7% agar in 1X PBS). The number of deaths was recorded every 12 hr until no living flies remained in the vial.

Lifespan. Flies were transferred to new vials with 2 ml of fresh food every 2-3 days until no living flies remained in the vial. Deaths were recorded when the flies were transferred.

Statistics and data presentation. All figures and data were generated and analyzed using GraphPad Prism (v9.1.2). For experiments with 2 groups, a Student's *t*-test was

performed. For experiments with 3 or more groups, a one-way ANOVA with Tukey HSD post-hoc test was performed. For fat breakdown experiments, a two-way ANOVA was used to determine the interaction between genotype and time. Starvation resistance and lifespan statistics were performed using RStudio and a script for a Log-rank test with Bonferroni's correction for multiple comparisons. Note, the lowest *p*-value given by RStudio is 2.0×10^{-16}. The below packages and script were used:

```
library ("survminer")
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

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

Figure 2. *transformer* function in Akh-producing cells contributes to the sex difference in fat storage. (A) Starvation resistance was significantly reduced in *tra^{1}/Df(3L)st-j7* females compared with *w^{1118}* controls (*p*<2x10^{-16}; log-rank test,

762 Bonferroni's correction for multiple comparisons). *n*=344-502 animals. (B) Starvation
763 resistance was significantly enhanced in *da-GAL4>UAS-tra^{F}* males compared with *da-*
764 *GAL4>+* and *+>UAS-tra^{F}* controls ($p<2x10^{-16}$ and $p<2x10^{-16}$ respectively; log-rank test,
765 Bonferroni's correction for multiple comparisons). *n*=198-201 animals. (C) Starvation
766 resistance was significantly enhanced in *elav-GAL4>UAS-tra^{F}* males compared with
767 *elav-GAL4>+* and *+>UAS-tra^{F}* controls ($p<2x10^{-16}$ and $p<2x10^{-16}$ respectively; log-rank
768 test, Bonferroni's correction for multiple comparisons). *n*=248-279 animals. (D) Whole-
769 body triglyceride levels were significantly higher in *elav-GAL4>UAS-tra^{F}* males
770 compared with *elav-GAL4>+* and *+>UAS-tra^{F}* controls ($p=0.0001$ and $p=0.0006$
771 respectively; one-way ANOVA followed by Tukey's HSD). *n*=7-8 biological replicates.
772 (E) Starvation resistance was significantly enhanced in *Akh-GAL4>UAS-tra^{F}* males
773 compared with *Akh-GAL4>+* and *+>UAS-tra^{F}* controls ($p=3.1x10^{-9}$ and $p<2x10^{-16}$
774 respectively; log-rank test, Bonferroni's correction for multiple comparisons). *n*=280-364
775 animals. (F) Whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-*
776 *tra^{F}* males compared to *Akh-GAL4>+* and *+>UAS-tra^{F}* control males ($p<0.0001$ and
777 $p<0.0001$ respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological
778 replicates. Black circles indicate the presence of a transgene and open circles indicate
779 the lack of a transgene. *** indicates $p<0.001$, **** indicates $p<0.0001$; shaded areas
780 represent the 95% confidence interval; error bars represent SEM.

781 **Figure 3. Sex-specific regulation of Akh and the Akh signaling pathway.** (A) *Akh*
782 mRNA levels were significantly higher in *w^{1118}* males compared with genotype-matched
783 females ($p<0.0001$, Student's *t*-test). *n*=8 biological replicates. (B) *AkhR* mRNA levels
784 were significantly higher in *w^{1118}* males than in females ($p=0.0002$, Student's *t*-test). *n*=4
785 biological replicates. (C) Expression of *UAS-nGFP* in Akh-producing cells (APCs) (*Akh-*
786 *GAL4>UAS-nGFP*) revealed no significant difference in APC cell number between
787 males and females ($p=0.5166$; Student's *t*-test). *n*=22-23 animals. (D) GFP intensity
788 produced as a readout of calcium activity in the APCs (*Akh-GAL4>LexAop-CD8-*
789 *GFP;UAS-LexA-VP16-NFAT (UAS-CaLexA)*) was significantly higher in males
790 compared with females ($p=0.0176$; Student's *t*-test). *n*=11 biological replicates. (E-H)
791 Maximum Z-projections of images showing GFP produced as a readout for APC
792 calcium activity from both *Akh-GAL4>UAS-CaLexA* males and females. Yellow outline

represents APC location, scale bars=50 μm, *n*=11 biological replicates. (I-K) Whole-body p-Ire1 levels were higher in w^{1118} males compared with w^{1118} females in three biological replicates. * indicates *p*<0.05, *** indicates *p*<0.001, **** indicates *p*<0.0001, ns indicates not significant; error bars represent SEM.

Figure 4. Sex-specific regulation of Akh and APC activity influence the sex difference in fat storage. (A) Whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-reaper (rpr)* males compared with *Akh-GAL4*>+ and +>*UAS-rpr* controls (*p*=0.0002 and *p*=0.0215 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (B) Whole-body triglyceride levels were significantly higher in Akh^{A} males compared with w^{1118} controls (*p*<0.0001; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (C) Whole-body triglyceride levels were significantly higher in $AkhR^{1}$ males compared with $AkhR^{rev}$ controls (*p*<0.0001; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (D) Whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-Akh-RNAi* males compared with *Akh-GAL4*>+ and +>*UAS-Akh-RNAi* controls (*p*=0.0015 and *p*=0.0002 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (E) Whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-Kir2.1* males compared with *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls (*p*<0.0001 and *p*<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (F) Whole-body triglyceride levels were significantly lower in *Akh-GAL4>UAS-NaChBac* females compared with *Akh-GAL4*>+ and +>*UAS-NaChBac* controls (*p*<0.0001 and *p*<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. Black circles indicate the presence of a transgene and open circles indicate the lack of a transgene; * indicates *p*<0.05, ** indicates *p*<0.01, *** indicates *p*<0.001, **** indicates *p*<0.0001; error bars represent SEM.

Figure 5. *transformer* regulates the sex difference in fat storage via the Akh signalling pathway. (A-C) Whole-body p-Ire1 levels were higher in $tra^{1}/Df(3L)st\text{-}j7$ females compared with w^{1118} controls in three biological replicates. (D) Whole-body *Akh* mRNA levels were significantly higher in $tra^{1}/Df(3L)st\text{-}j7$ females compared with w^{1118} controls (*p*<0.0001; Student's *t*-test). *n*=8 biological replicates. (E) Whole-body

triglyceride levels were significantly lower in *tra^{KO}/Df(3L)st-j7* females carrying either *Akh-GAL4*>+ or +>*UAS-reaper* (*rpr*) transgenes compared with *w^{1118}* controls carrying a functional Tra protein (p<0.0001 and p<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). Whole-body triglyceride levels were not significantly different between *tra^{KO}/Df(3L)st-j7* females lacking APCs (*Akh-GAL4>UAS-rpr*) and *w^{1118}* controls (p=0.9384; one-way ANOVA followed by Tukey's HSD). n=8 biological replicates. (F) Whole-body triglyceride levels were significantly higher in *tra$^{F\ K\text{-}IN}$* males carrying either *Akh-GAL4*>+ or +>*UAS-NaChBac* transgenes compared with *w^{1118}* control males lacking Tra function (p<0.0001 and p<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). Whole-body triglyceride levels in *tra$^{F\ K\text{-}IN}$* males with APC activation (*Akh-GAL4>UAS-NaChBac*) were significantly lower than *tra$^{F\ K\text{-}IN}$* males carrying either the *Akh-GAL4*>+ or +>*UAS-NaChBac* transgenes alone (p<0.0001 and p<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). n=5 biological replicates. Black circles indicate the presence of a transgene or mutant allele and open circles indicate the lack of a transgene or mutant allele. **** indicates p<0.0001, ns indicates not significant; error bars represent SEM.

Figure 6. Sex-specific regulation of Akh signalling pathway promotes reproductive success in each sex. (A) At all observation points, a lower proportion of *Akh^{A}* males were successfully copulating with a wildtype *Canton-S* (*CS*) female compared with *w^{1118}* controls. n=31 males. (B) The number of pupae produced from a 60 min mating period was significantly lower in *Akh^{A}* males compared with *w^{1118}* controls (p=0.0003; Student's t-test). n=24-26 males. (C) The number of pupae produced from a 24 hr mating period was not significantly different between *Akh^{A}* males and *w^{1118}* control males (p=0.2501; Student's t-test). n=24-25 males. (D) The number of pupae produced from a 24 hr mating period was significantly higher in *Akh^{A}* females compared with *w^{1118}* controls (p=0.0006; Student's t-test). n=28-36 females. (E) Lifespan was significantly shorter in *Akh-GAL4>UAS-Kir2.1* females compared with *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls (p<2x10^{-16} and p=0.0015 respectively; log-rank test, Bonferroni's correction for multiple comparisons). n=160-198 females. (F) Lifespan of *Akh-GAL4>UAS-Kir2.1* males was intermediate between *Akh-GAL4*>+ and +>*UAS-Kir2.1* controls, indicating no overall effect of inhibiting APC neuronal activity on male lifespan

(p=0.00013 and p=7.0x10^{-6} respectively; log-rank test, Bonferroni's correction for multiple comparisons). n=196-200 males. ** indicates p<0.01, *** indicates p<0.001, **** indicates p<0.0001, ns indicates not significant; error bars represent SEM; shaded areas represent the 95% confidence interval.

SUPPLEMENTAL FIGURE LEGENDS

Figure 1 - figure supplement 1. Elucidating *transformer*'s effect on sex differences in fat metabolism. (A) Whole-body triglyceride levels were not significantly different between *tra^{1}/Df(3L)st-j7* males and *w^{1118}* controls (p=0.0685; Student's t-test). n=8 biological replicates. (B) The reduction in whole-body triglyceride levels post-starvation was not significantly different between *tra^{1}/Df(3L)st-j7* animals and sex-matched *w^{1118}* controls between 0-24 hr post-starvation (genotype:time p=0.6298 [female], p=0.3853 [male]; two-way ANOVA per sex). n=7-8 biological replicates. (C) Whole-body triglyceride levels in *da-GAL4>UAS-tra^{F} f*emales were intermediate between *da-GAL4*>+ and +>*UAS-tra^{F}* control females, indicating no overall effect of Tra overexpression in females (p=0.0160 and p=0.0002 respectively; one-way ANOVA followed by Tukey's HSD). n=7-8 biological replicates. (D) Whole-body mRNA levels of ecdysone responsive genes were not higher in *tra$^{F\ K\text{-}IN}$* males compared to *w^{1118}* control males (*Ecdysone receptor* (*EcR*)*: p*<0.0001; *Ecdysone-induced protein 75B (E75): p*=0.0072; *Ecdysone-induced protein 78C (E78): p*=0.0408; *broad (br): p*=0.0003; *ftz transcription factor 1* (*ftz-f1*)*: p*=0.002; Student's t-test for each gene). n=7-8 biological replicates. (E) The reduction in whole-body triglyceride levels between 0-24 hr post-starvation was significantly smaller in *da-GAL4>UAS-tra^{F}* males compared with *da-GAL4*>+ and +>*UAS-tra^{F}* control males (genotype:time p<0.0001; two-way ANOVA). The post-starvation reduction in triglyceride levels in *da-GAL4>UAS-tra^{F}* females was intermediate between both *da-GAL4*>+ and +>*UAS-tra^{F}* controls, suggesting no overall effect of Tra overexpression on female fat storage (genotype:time p=0.0223; two-way ANOVA per sex). n=7-8 biological replicates. (F) The reduction in whole-body triglyceride levels between 0-24 hr post-starvation was significantly lower in *tra$^{F\ K\text{-}IN}$* males, but not females, compared with sex-matched *w^{1118}* controls (genotype:time

885 *p*=0.0009 [male], *p*=0.9024 [female]; two-way ANOVA per sex). *n*=7-8 biological
886 replicates. F indicates female, M indicates male. Black circles indicate the presence of a
887 transgene and open circles indicate the lack of a transgene. * indicates *p*<0.05, **
888 indicates *p*<0.01, *** indicates *p*<0.001, **** indicates *p*<0.0001, ns indicates not
889 significant; error bars represent SEM except for graphs displaying fat breakdown where
890 error bars represent COE.
891
892 **Figure 2 - figure supplement 1. Effect of *transformer* gain in multiple cell types**
893 **and tissues on starvation resistance.** (A-F) Limited to no effects of Tra
894 overexpression on starvation resistance were observed for female fat body, muscle, and
895 gut (for individual *p*-values see Supplementary file 1; log-rank test with Bonferroni's
896 correction for multiple comparisons). In males, Tra overexpression in the fat body and
897 gut caused no extension of starvation resistance, with only a minor extension observed
898 upon Tra overexpression in muscle (for individual *p*-values see Supplementary file 1;
899 log-rank test with Bonferroni's correction for multiple comparisons). (A) *n*=397-413
900 females, *n*=295-431 males. (B) *n*=187-206 females, *n*=198-202 males. (C) *n*=293-402
901 females, *n*=330-452 males. (D) *n*=268-383 females, *n*=363-409 males. (E) *n*=226-374
902 females, *n*=250-362 males. (F) *n*=168-206 females, *n*=178-198 males. ** indicates
903 *p*<0.01, *** indicates *p*<0.001, **** indicates *p*<0.0001, ns indicates not significant;
904 shaded areas represent the 95% confidence interval.

905 **Figure 2 - figure supplement 2. Effect of *transformer* gain in additional cell types**
906 **and tissues on starvation resistance.** (A-D) Limited to no effects of Tra
907 overexpression were observed upon Tra expression in the female gonad and glia (for
908 individual *p*-values see Supplementary file 1; log-rank test with Bonferroni's correction
909 for multiple comparisons). Limited to no effects of Tra overexpression were observed
910 upon Tra expression in the male gonad and glia (for individual *p*-values see
911 Supplementary file 1; log-rank test with Bonferroni's correction for multiple
912 comparisons). (A) *n*=232-268 females, *n*=318-400 males. (B) *n*=234-349 females,
913 *n*=327-349 males. (C) *n*=374-442 females, *n*=293-364 males. (D) *n*=300-374 females,
914 *n*=329-355 males. * indicates *p*<0.05, ** indicates *p*<0.01, *** indicates *p*<0.001, ****

indicates $p<0.0001$, ns indicates not significant; shaded areas represent the 95% confidence interval.

Figure 2 - figure supplement 3. Gain of *transformer* function in neurons does not affect fat breakdown. (A) Starvation resistance in *elav-GAL4>UAS-tra^F* females was not significantly different compared with *elav-GAL4>+* and *+>UAS-tra^F* controls (p=0.3 and p=1, respectively; log-rank test with Bonferroni's correction for multiple comparisons). n=318-749 females. (B) Whole-body triglyceride levels were not significantly different between *elav-GAL4>UAS-tra^F* females and *elav-GAL4>+* and *+>UAS-tra^F* controls (p=0.3224 and p=0.7754 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological replicates. (C) The reduction in whole-body triglyceride levels post-starvation was not significantly different between *elav-GAL4>UAS-tra^F* flies and sex-matched *elav-GAL4>+* and *+>UAS-tra^F* controls (genotype:time p=0.2789 [male], p=0.7058 [female]; two-way ANOVA per sex). n=7-8 biological replicates. Black circles indicate the presence of a transgene and open circles indicate the lack of a transgene; ns indicates not significant; shaded areas represent the 95% confidence interval; error bars represent SEM except for graphs displaying fat breakdown where error bars represent COE.

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

Figure 2 - figure supplement 5. Gain of *transformer* function in Akh-producing cells does not affect fat breakdown. (A) Starvation resistance was significantly

extended in *Akh-GAL4>UAS-tra^{F}* females compared with *Akh-GAL4>+* and *+>UAS-tra^{F}* controls (p=0.00033 and p=9.4x10^{-11} respectively; log-rank test with Bonferroni's correction for multiple comparisons). n=168-219 females. (B) Whole-body triglyceride levels were not significantly different between *Akh-GAL4>UAS-tra^{F}* females and *Akh-GAL4>+* and *+>UAS-tra^{F}* controls (p=0.2195 and p=0.0731 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological replicates. (C) The reduction in whole-body triglyceride levels post-starvation was not significantly different between *Akh-GAL4>UAS-tra^{F}* animals and sex-matched *Akh-GAL4>+* and *+>UAS-tra^{F}* controls (genotype:time p=0.1201 [males], p=0.0596 [female]; two-way ANOVA per sex). n=8 biological replicates. Black circles indicate the presence of a transgene and open circles indicate the lack of a transgene; *** indicates p<0.001, **** indicates p<0.0001, ns indicates not significant; shaded areas represent the 95% confidence interval; error bars represent SEM except for graphs displaying fat breakdown where error bars represent COE.

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

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

975 significantly different from both *Akh-GAL4*>+ and +>*UAS-Lst-RNAi* controls (p=0.0357
976 and p=0.2364 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological
977 replicates. (E) Whole-body triglyceride levels in *Akh-GAL4>UAS-Lst-RNAi* females were
978 not significantly different from both *Akh-GAL4*>+ and +>*UAS-Lst-RNAi* controls
979 (p<0.0001 and p=0.6656 respectively; one-way ANOVA followed by Tukey's HSD). n=8
980 biological replicates. Black circles indicate the presence of a transgene and open circles
981 indicate the lack of a transgene. * indicates p<0.05, **** indicates p<0.0001, ns
982 indicates not significant; error bars represent SEM.

983 **Figure 4 - figure supplement 2. Akh and *brummer* operate in parallel pathways to**
984 **regulate the sex difference in fat storage.** (A) Whole-body triglyceride levels were
985 significantly higher in $AkhR^1$ and bmm^1 males, respectively, compared with w^{1118}
986 controls (p<0.0001 and p<0.0001; one-way ANOVA followed by Tukey's HSD). Whole-
987 body triglyceride levels were further increased in $AkhR^1; bmm^1$ males compared with
988 $AkhR^1$ males and bmm^1 males (p<0.0001 and p<0.0001 respectively; one-way ANOVA
989 followed by Tukey's HSD). n=7-8 biological replicates. (B) Whole-body triglyceride
990 levels were significantly higher in $AkhR^1$ females compared with w^{1118} controls; however
991 whole-body triglyceride levels were not significantly different between bmm^1 females
992 and w^{1118} control females (p=0.002 and p=0.4256 respectively; one-way ANOVA
993 followed by Tukey's HSD). Whole-body triglyceride levels were further increased in
994 $AkhR^1; bmm^1$ females compared to $AkhR^1$ females and bmm^1 females (p=0.0024 and
995 p<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). n=8 biological
996 replicates. Black circles indicate the presence of a mutant allele and open circles
997 indicate the lack of a mutant allele. ** indicates p<0.01, **** indicates p<0.0001, ns
998 indicates not significant; error bars represent SEM.

999 **Figure 4 - figure supplement 3. RNAi-mediated Akh knockdown effectively**
1000 **reduced Akh transcripts in both sexes.** (A) *Akh* mRNA levels in the head and anterior
1001 half of the thorax were significantly lower in *Akh-GAL4>UAS-Akh-RNAi* males compared
1002 with *Akh-GAL4*>+ controls (p=0.0008; Student's t-test). n=5-8 biological replicates. (B)
1003 *Akh* mRNA levels in the head and anterior half of the thorax were significantly lower in
1004 *Akh-GAL4>UAS-Akh-RNAi* females compared with *Akh-GAL4*>+ controls (p<0.0001;

Student's *t*-test). *n*=8 biological replicates. (C) Whole-body triglyceride levels were not significantly different between *Akh-GAL4>UAS-Akh-RNAi* females and both *Akh-GAL4>+* and *+>UAS-Akh-RNAi* controls (*p*=0.0136 and *p*=0.4845 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (D) Whole-body triglyceride levels were significantly higher in *Akh-GAL4>UAS-Kir2.1* females compared with *Akh-GAL4>+* and *+>UAS-Kir2.1* controls (*p*=0.0001 and *p*=0.0022 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (E) Whole-body triglyceride levels were significantly lower in *Akh-GAL4>UAS-NaChBac* males compared with *Akh-GAL4>+* and *+>UAS-NaChBac* controls (*p*<0.0001 and *p*<0.0001 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. Black circles indicate the presence of a transgene and open circles indicate the lack of a transgene. ** indicates *p*<0.01, *** indicates *p*<0.001, **** indicates *p*<0.0001, ns indicates not significant; error bars represent SEM.

Figure 4 - figure supplement 4. Activity of the Akh-producing cells does not regulate food consumption in either sex. (A) Food consumption was not significantly different between *Akh-GAL4>UAS-Kir2.1* females and *Akh-GAL4>+* and *+>UAS-Kir2.1* controls (*p*=0.6488 and *p*=0.0539 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. (B) Food consumption was not significantly different between *Akh-GAL4>UAS-Kir2.1* males and *Akh-GAL4>+* and *+>UAS-Kir2.1* controls (*p*=0.3623 and *p*=0.0638 respectively; one-way ANOVA followed by Tukey's HSD). *n*=7-8 biological replicates. (C) Food consumption was not significantly different between *Akh-GAL4>UAS-NaChBac* females and *Akh-GAL4>+* and *+>UAS-NaChBac* controls (*p*=0.9369 and *p*=0.9571 respectively; one-way ANOVA followed by Tukey's HSD). *n*=7-8 biological replicates. (D) Food consumption was not significantly different between *Akh-GAL4>UAS- NaChBac* males and both *Akh-GAL4>+* and *+>UAS-NaChBac* controls (*p*=0.0266 and *p*=0.8141 respectively; one-way ANOVA followed by Tukey's HSD). *n*=8 biological replicates. 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.

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.

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.

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

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

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

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

1074 Bell, C., Seals, D.R., Monroe, M.B., Day, D.S., Shapiro, L.F., Johnson, D.G., and Jones, P.P. (2001). Tonic
1075 sympathetic support of metabolic rate is attenuated with age, sedentary lifestyle, and female sex in
1076 healthy adults. J. Clin. Endocrinol. Metab. *86*, 4440–4444.

1077 Bell, L.R., Maine, E.M., Schedl, P., and Cline, T.W. (1988). *Sex-lethal*, a *Drosophila* sex determination
1078 switch gene, exhibits sex-specific RNA splicing and sequence similarity to RNA binding proteins. Cell *55*,
1079 1037–1046.

1080 Beller, M., Bulankina, A.V., Hsiao, H.-H., Urlaub, H., Jäckle, H., and Kühnlein, R.P. (2010). PERILIPIN-
1081 dependent control of lipid droplet structure and fat storage in *Drosophila*. Cell Metab. *12*, 521–532.

1082 Belmonte, R.L., Corbally, M.-K., Duneau, D.F., and Regan, J.C. (2020). Sexual Dimorphisms in Innate
1083 Immunity and Responses to Infection in *Drosophila melanogaster*. Front. Immunol. *10*, 3075.

1084 Belote, J.M., McKeown, M., Boggs, R.T., Ohkawa, R., and Sosnowski, B.A. (1989). Molecular genetics of
1085 transformer, a genetic switch controlling sexual differentiation in *Drosophila*. Dev. Genet. *10*, 143–154.

1086 Bharucha, K.N., Tarr, P., and Zipursky, S.L. (2008). A glucagon-like endocrine pathway in *Drosophila*
1087 modulates both lipid and carbohydrate homeostasis. J. Exp. Biol. *211*, 3103–3110.

1088 Bi, J., Xiang, Y., Chen, H., Liu, Z., Grönke, S., Kühnlein, R.P., and Huang, X. (2012). Opposite and
1089 redundant roles of the two *Drosophila* perilipins in lipid mobilization. J. Cell Sci. *125*, 3568–3577.

1090 Bilginoglu, A., Amber Cicek, F., Ugur, M., Gurdal, H., and Turan, B. (2007). The role of gender differences
1091 in beta-adrenergic receptor responsiveness of diabetic rat heart. Mol. Cell. Biochem. *305*, 63–69.

1092 Billeter, J.-C., Rideout, E.J., Dornan, A.J., and Goodwin, S.F. (2006). Control of male sexual behavior in
1093 *Drosophila* by the sex determination pathway. Curr. Biol. CB *16*, R766-776.

1094 Bjedov, I., Toivonen, J.M., Kerr, F., Slack, C., Jacobson, J., Foley, A., and Partridge, L. (2010). Mechanisms
1095 of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. Cell Metab. *11*, 35–46.

1096 Boggs, R.T., Gregor, P., Idriss, S., Belote, J.M., and McKeown, M. (1987). Regulation of sexual
1097 differentiation in *D. melanogaster* via alternative splicing of RNA from the transformer gene. Cell *50*,
1098 739–747.

1099 Braco, J.T., Gillespie, E.L., Alberto, G.E., Brenman, J.E., and Johnson, E.C. (2012). Energy-dependent
1100 modulation of glucagon-like signaling in *Drosophila* via the AMP-activated protein kinase. Genetics *192*,
1101 457–466.

1102 Bridges, C.B. (1921). TRIPLOID INTERSEXES IN *DROSOPHILA MELANOGASTER*. Science *54*, 252–254.

1103 Brooks, V.L., Shi, Z., Holwerda, S.W., and Fadel, P.J. (2015). Obesity-induced increases in sympathetic
1104 nerve activity: sex matters. Auton. Neurosci. Basic Clin. *187*, 18–26.

1105 Broughton, S.J., Piper, M.D.W., Ikeya, T., Bass, T.M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E.,
1106 Withers, D.J., Leevers, S.J., et al. (2005). Longer lifespan, altered metabolism, and stress resistance in
1107 *Drosophila* from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. U. S. A. *102*, 3105–
1108 3110.

1109 Brown, E.H., and King, R.C. (1961). Studies on the Expression of the *Transformer* Gene of *Drosophila*
1110 *Melanogaster*. Genetics *46*, 143–156.

1111 Burtis, K.C., and Baker, B.S. (1989). *Drosophila doublesex* gene controls somatic sexual differentiation by
1112 producing alternatively spliced mRNAs encoding related sex-specific polypeptides. Cell *56*, 997–1010.

1113 Buszczak, M., Lu, X., Segraves, W.A., Chang, T.Y., and Cooley, L. (2002). Mutations in the *midway* gene
1114 disrupt a *Drosophila* acyl coenzyme A: diacylglycerol acyltransferase. Genetics *160*, 1511–1518.

1115 Cachero, S., Ostrovsky, A.D., Yu, J.Y., Dickson, B.J., and Jefferis, G.S.X.E. (2010). Sexual dimorphism in the
1116 fly brain. Curr. Biol. CB *20*, 1589–1601.

1117 Camara, N., Whitworth, C., and Van Doren, M. (2008). The creation of sexual dimorphism in the
1118 *Drosophila* soma. Curr. Top. Dev. Biol. *83*, 65–107.

1119 Castellanos, M.C., Tang, J.C.Y., and Allan, D.W. (2013). Female-biased dimorphism underlies a female-
1120 specific role for post-embryonic Ilp7 neurons in *Drosophila* fertility. Dev. Camb. Engl. *140*, 3915–3926.

1121 Chandegra, B., Tang, J.L.Y., Chi, H., and Alic, N. (2017). Sexually dimorphic effects of dietary sugar on
1122 lifespan, feeding and starvation resistance in *Drosophila*. Aging *9*, 2521–2528.

1123 Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F., and Partridge, L. (1995). Cost of mating in *Drosophila*
1124 *melanogaster* females is mediated by male accessory gland products. Nature *373*, 241–244.

1125 Choi, S., Lim, D.-S., and Chung, J. (2015). Feeding and Fasting Signals Converge on the LKB1-SIK3 Pathway
1126 to Regulate Lipid Metabolism in *Drosophila*. PLoS Genet. *11*, e1005263.

1127 Christiansen, A.E., Keisman, E.L., Ahmad, S.M., and Baker, B.S. (2002). Sex comes in from the cold: the
1128 integration of sex and pattern. Trends Genet. TIG *18*, 510–516.

1129 Chung, B.Y., Ro, J., Hutter, S.A., Miller, K.M., Guduguntla, L.S., Kondo, S., and Pletcher, S.D. (2017).
1130 *Drosophila* Neuropeptide F Signaling Independently Regulates Feeding and Sleep-Wake Behavior. Cell
1131 Rep. *19*, 2441–2450.

1132 Claustre, J., Peyrin, L., Fitoussi, R., and Mornex, R. (1980). Sex differences in the adrenergic response to
1133 hypoglycemic stress in human. Psychopharmacology (Berl.) *67*, 147–153.

1134 Cline, T.W. (1978). Two closely linked mutations in *Drosophila melanogaster* that are lethal to opposite
1135 sexes and interact with daughterless. Genetics *90*, 683–698.

1136 Cline, T.W., and Meyer, B.J. (1996). Vive la différence: males vs females in flies vs worms. Annu. Rev.
1137 Genet. *30*, 637–702.

1138 Clough, E., Jimenez, E., Kim, Y.-A., Whitworth, C., Neville, M.C., Hempel, L.U., Pavlou, H.J., Chen, Z.-X.,
1139 Sturgill, D., Dale, R.K., et al. (2014). Sex- and tissue-specific functions of *Drosophila* doublesex
1140 transcription factor target genes. Dev. Cell *31*, 761–773.

1141 Clyne, J.D., and Miesenböck, G. (2008). Sex-specific control and tuning of the pattern generator for
1142 courtship song in *Drosophila*. Cell *133*, 354–363.

1143 Dart, A.M., Du, X.-J., and Kingwell, B.A. (2002). Gender, sex hormones and autonomic nervous control of
1144 the cardiovascular system. Cardiovasc. Res. *53*, 678–687.

1145 Dauwalder, B. (2011). The roles of fruitless and doublesex in the control of male courtship. Int. Rev.
1146 Neurobiol. *99*, 87–105.

1147 Davis, S.N., Shavers, C., and Costa, F. (2000). Differential gender responses to hypoglycemia are due to
1148 alterations in CNS drive and not glycemic thresholds. Am. J. Physiol. Endocrinol. Metab. *279*, E1054-
1149 1063.

1150 Demir, E., and Dickson, B.J. (2005). *fruitless* splicing specifies male courtship behavior in *Drosophila*. Cell
1151 *121*, 785–794.

1152 DiAngelo, J.R., and Birnbaum, M.J. (2009). Regulation of fat cell mass by insulin in *ii*. Mol. Cell. Biol. *29*,
1153 6341–6352.

1154 Drake, K., Gateva, E., Deutsch, J., and Cohen, W.R. (1998). Sex differences in the adrenal catecholamine
1155 response to hypoglycemia in rats. Metabolism. *47*, 121–124.

1156 Evans, D.S., and Cline, T.W. (2007). *Drosophila melanogaster* Male Somatic Cells Feminized Solely by
1157 TraF Can Collaborate With Female Germ Cells to Make Functional Eggs. Genetics *175*, 631–642.

1158 Ferveur, J.F., Stortkuhl, K.F., Stocker, R.F., and Greenspan, R.J. (1995). Genetic feminization of brain
1159 structures and changed sexual orientation in male *Drosophila*. Science *267*, 902–905.

1160 Flatt, T. (2011). Survival costs of reproduction in *Drosophila*. Exp. Gerontol. *46*, 369–375.

1161 Fowler, K., and Partridge, L. (1989). A cost of mating in female fruitflies. Nature *338*, 760–761.

1162 Francis, V.A., Zorzano, A., and Teleman, A.A. (2010). dDOR is an EcR coactivator that forms a feed-
1163 forward loop connecting insulin and ecdysone signaling. Curr. Biol. CB *20*, 1799–1808.

1164 Freedman, R.R., Sabharwal, S.C., and Desai, N. (1987). Sex differences in peripheral vascular adrenergic
1165 receptors. Circ. Res. *61*, 581–585.

1166 Gäde, G., and Auerswald, L. (2003). Mode of action of neuropeptides from the adipokinetic hormone
1167 family. Gen. Comp. Endocrinol. *132*, 10–20.

1168 Gáliková, M., and Klepsatel, P. (2018). Obesity and Aging in the *Drosophila* Model. Int. J. Mol. Sci. *19*,
1169 E1896.

1170 Gáliková, M., Diesner, M., Klepsatel, P., Hehlert, P., Xu, Y., Bickmeyer, I., Predel, R., and Kühnlein, R.P.
1171 (2015). Energy Homeostasis Control in *Drosophila Adipokinetic Hormone* Mutants. Genetics *201*, 665–
1172 683.

1173 Garner, S.R.C., Castellanos, M.C., Baillie, K.E., Lian, T., and Allan, D.W. (2018). *Drosophila* female-specific
1174 Ilp7 motoneurons are generated by Fruitless-dependent cell death in males and by a double-assurance
1175 survival role for Transformer in females. Dev. Camb. Engl. *145*, dev150821.

1176 Ghosh, A.C., and O'Connor, M.B. (2014). Systemic Activin signaling independently regulates sugar
1177 homeostasis, cellular metabolism, and pH balance in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.
1178 S. A. *111*, 5729–5734.

1179 Goodwin, S.F., Taylor, B.J., Villella, A., Foss, M., Ryner, L.C., Baker, B.S., and Hall, J.C. (2000). Aberrant
1180 splicing and altered spatial expression patterns in *fruitless* mutants of *Drosophila melanogaster*.
1181 Genetics *154*, 725–745.

1182 Grönke, S., Beller, M., Fellert, S., Ramakrishnan, H., Jäckle, H., and Kühnlein, R.P. (2003). Control of Fat
1183 Storage by a *Drosophila* PAT Domain Protein. Curr. Biol. *13*, 603–606.

1184 Grönke, S., Mildner, A., Fellert, S., Tennagels, N., Petry, S., Müller, G., Jäckle, H., and Kühnlein, R.P.
1185 (2005). Brummer lipase is an evolutionary conserved fat storage regulator in *Drosophila*. Cell Metab. *1*,
1186 323–330.

1187 Grönke, S., Müller, G., Hirsch, J., Fellert, S., Andreou, A., Haase, T., Jäckle, H., and Kühnlein, R.P. (2007).
1188 Dual lipolytic control of body fat storage and mobilization in *Drosophila*. PLoS Biol. *5*, e137.

1189 Grönke, S., Clarke, D.-F., Broughton, S., Andrews, T.D., and Partridge, L. (2010). Molecular Evolution and
1190 Functional Characterization of *Drosophila* Insulin-Like Peptides. PLOS Genet. *6*, e1000857.

1191 Guo, F., Yu, J., Jung, H.J., Abruzzi, K.C., Luo, W., Griffith, L.C., and Rosbash, M. (2016). Circadian Neuron
1192 Feedback Controls the *Drosophila* Sleep-Activity Profile. Nature *536*, 292–297.

1193 Gutierrez, E., Wiggins, D., Fielding, B., and Gould, A.P. (2007). Specialized hepatocyte-like cells regulate
1194 *Drosophila* lipid metabolism. Nature *445*, 275–280.

1195 Hall, J.C. (1994). The Mating of a Fly. Science *264*, 1702–1714.

1196 Hansen, M., Flatt, T., and Aguilaniu, H. (2013). Reproduction, Fat Metabolism, and Life Span: What Is the
1197 Connection? Cell Metab. *17*, 10–19.

1198 Heier, C., and Kühnlein, R.P. (2018). Triacylglycerol Metabolism in *Drosophila melanogaster*. Genetics
1199 *210*, 1163–1184.

1200 Heier, C., Klishch, S., Stilbytska, O., Semaniuk, U., and Lushchak, O. (2021). The *Drosophila* model to
1201 interrogate triacylglycerol biology. Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids *1866*, 158924.

1202 Heinrichs, V., Ryner, L.C., and Baker, B.S. (1998). Regulation of sex-specific selection of *fruitless* 5' splice
1203 sites by transformer and transformer-2. Mol. Cell. Biol. *18*, 450–458.

1204 Helfrich-Förster, C. (2000). Differential control of morning and evening components in the activity
1205 rhythm of *Drosophila melanogaster*--sex-specific differences suggest a different quality of activity. J.
1206 Biol. Rhythms *15*, 135–154.

1207 Hentze, J.L., Carlsson, M.A., Kondo, S., Nässel, D.R., and Rewitz, K.F. (2015). The Neuropeptide
1208 Allatostatin A Regulates Metabolism and Feeding Decisions in *Drosophila*. Sci. Rep. *5*, 11680.

1209 Hinojosa-Laborde, C., Chapa, I., Lange, D., and Haywood, J.R. (1999). Gender differences in sympathetic
1210 nervous system regulation. Clin. Exp. Pharmacol. Physiol. *26*, 122–126.

1211 Hoeker, G.S., Hood, A.R., Katra, R.P., Poelzing, S., and Pogwizd, S.M. (2014). Sex Differences in β-
1212 Adrenergic Responsiveness of Action Potentials and Intracellular Calcium Handling in Isolated Rabbit
1213 Hearts. PLoS ONE *9*, e111411.

1214 Hogarth, A.J., Mackintosh, A.F., and Mary, D.A.S.G. (2007). Gender-related differences in the
1215 sympathetic vasoconstrictor drive of normal subjects. Clin. Sci. Lond. Engl. 1979 *112*, 353–361.

1216 Hoshijima, K., Inoue, K., Higuchi, I., Sakamoto, H., and Shimura, Y. (1991). Control of *doublesex*
1217 alternative splicing by transformer and transformer-2 in *Drosophila*. Science *252*, 833–836.

1218 Huang, R., Song, T., Su, H., Lai, Z., Qin, W., Tian, Y., Dong, X., and Wang, L. (2020). High-fat diet enhances
1219 starvation-induced hyperactivity via sensitizing hunger-sensing neurons in *Drosophila*. ELife *9*, e53103.

1220 Huber, R., Hill, S.L., Holladay, C., Biesiadecki, M., Tononi, G., and Cirelli, C. (2004). Sleep homeostasis in
1221 *Drosophila melanogaster*. Sleep *27*, 628–639.

1222 Hudry, B., Khadayate, S., and Miguel-Aliaga, I. (2016). The sexual identity of adult intestinal stem cells
1223 controls organ size and plasticity. Nature *530*, 344–348.

1224 Hudry, B., de Goeij, E., Mineo, A., Gaspar, P., Hadjieconomou, D., Studd, C., Mokochinski, J.B., Kramer,
1225 H.B., Plaçais, P.-Y., Preat, T., et al. (2019). Sex Differences in Intestinal Carbohydrate Metabolism
1226 Promote Food Intake and Sperm Maturation. Cell *178*, 901-918.e16.

1227 Hughson, B.N., Shimell, M., and O'Connor, M.B. (2021). AKH Signaling in *D. melanogaster* Alters Larval
1228 Development in a Nutrient-Dependent Manner That Influences Adult Metabolism. Front. Physiol. *12*.

1229 Inoue, K., Hoshijima, K., Sakamoto, H., and Shimura, Y. (1990). Binding of the *Drosophila sex-lethal* gene
1230 product to the alternative splice site of *transformer* primary transcript. Nature *344*, 461–463.

1231 Inoue, K., Hoshijima, K., Higuchi, I., Sakamoto, H., and Shimura, Y. (1992). Binding of the *Drosophila*
1232 transformer and transformer-2 proteins to the regulatory elements of *doublesex* primary transcript for
1233 sex-specific RNA processing. Proc. Natl. Acad. Sci. U. S. A. *89*, 8092–8096.

1234 Isabel, G., Martin, J.-R., Chidami, S., Veenstra, J.A., and Rosay, P. (2005). AKH-producing neuroendocrine
1235 cell ablation decreases trehalose and induces behavioral changes in *Drosophila*. Am. J. Physiol. Regul.
1236 Integr. Comp. Physiol. *288*, R531-538.

1237 Ito, H., Fujitani, K., Usui, K., Shimizu-Nishikawa, K., Tanaka, S., and Yamamoto, D. (1996). Sexual
1238 orientation in *Drosophila* is altered by the satori mutation in the sex-determination gene *fruitless* that
1239 encodes a zinc finger protein with a BTB domain. Proc. Natl. Acad. Sci. U. S. A. *93*, 9687–9692.

1240 Jackson, A.S., Stanforth, P.R., Gagnon, J., Rankinen, T., Leon, A.S., Rao, D.C., Skinner, J.S., Bouchard, C.,
1241 and Wilmore, J.H. (2002). The effect of sex, age and race on estimating percentage body fat from body
1242 mass index: The Heritage Family Study. Int. J. Obes. *26*, 789–796.

1243 Johnson, A.A., and Stolzing, A. (2019). The role of lipid metabolism in aging, lifespan regulation, and age-
1244 related disease. Aging Cell *18*, e13048.

1245 Kamareddine, L., Robins, W.P., Berkey, C.D., Mekalanos, J.J., and Watnick, P.I. (2018). The *Drosophila*
1246 Immune Deficiency Pathway Modulates Enteroendocrine Function and Host Metabolism. Cell Metab.
1247 *28*, 449-462.e5.

1248 Kang, P., Chang, K., Liu, Y., Bouska, M., Birnbaum, A., Karashchuk, G., Thakore, R., Zheng, W., Post, S.,
1249 Brent, C.S., et al. (2017). *Drosophila* Kruppel homolog 1 represses lipolysis through interaction with
1250 dFOXO. Sci. Rep. *7*, 16369.

1251 Karastergiou, K., Smith, S.R., Greenberg, A.S., and Fried, S.K. (2012). Sex differences in human adipose
1252 tissues – the biology of pear shape. Biol. Sex Differ. *3*, 13.

1253 Kimura, K., Urushizaki, A., Sato, C., and Yamamoto, D. (2019). A novel sex difference in *Drosophila*
1254 contact chemosensory neurons unveiled using single cell labeling. J. Neurogenet. *33*, 116–124.

1255 Kubrak, O., Jensen, L., Ahrentløv, N., Koyama, T., Malita, A., Naseem, M.T., Lassen, M., Nagy, S., Texada,
1256 M.J., Halberg, K.A., et al. (2020). The gut hormone Allatostatin C regulates food intake and metabolic
1257 homeostasis under nutrient stress. BioRxiv 2020.12.05.412874.

1258 Kühnlein, R.P. (2012). Lipid droplet-based storage fat metabolism in *Drosophila*: Thematic Review Series:
1259 Lipid Droplet Synthesis and Metabolism: from Yeast to Man. J. Lipid Res. *53*, 1430–1436.

1260 Kvitsiani, D., and Dickson, B.J. (2006). Shared neural circuitry for female and male sexual behaviours in
1261 *Drosophila*. Curr. Biol. *16*, R355–R356.

1262 Lafontan, M., Barbe, P., Galitzky, J., Tavernier, G., Langin, D., Carpéné, C., Bousquet-Melou, A., and
1263 Berlan, M. (1997). Adrenergic regulation of adipocyte metabolism. Hum. Reprod. Oxf. Engl. *12 Suppl 1*,
1264 6–20.

1265 Lease, H.M., and Wolf, B.O. (2011). Lipid content of terrestrial arthropods in relation to body size,
1266 phylogeny, ontogeny and sex. Physiol. Entomol. *36*, 29–38.

1267 Lee, G., and Hall, J.C. (2001). Abnormalities of male-specific FRU protein and serotonin expression in the
1268 CNS of *fruitless* mutants in *Drosophila*. J. Neurosci. Off. J. Soc. Neurosci. *21*, 513–526.

1269 Lee, G., and Park, J.H. (2004). Hemolymph sugar homeostasis and starvation-induced hyperactivity
1270 affected by genetic manipulations of the *adipokinetic hormone*-encoding gene in *Drosophila*
1271 *melanogaster*. Genetics *167*, 311–323.

1272 Lee, B., Barretto, E.C., and Grewal, S.S. (2019). TORC1 modulation in adipose tissue is required for
1273 organismal adaptation to hypoxia in *Drosophila*. Nat. Commun. *10*, 1878.

1274 Lehmann, M. (2018). Endocrine and physiological regulation of neutral fat storage in *Drosophila*. Mol.
1275 Cell. Endocrinol. *461*, 165–177.

1276 Li, H., Janssens, J., Waegeneer, M.D., Kolluru, S.S., Davie, K., Gardeux, V., Saelens, W., David, F., Brbić,
1277 M., Leskovec, J., et al. (2021). Fly Cell Atlas: a single-cell transcriptomic atlas of the adult fruit fly. BioRxiv
1278 2021.07.04.451050.

1279 Li, Y., Hoffmann, J., Li, Y., Stephano, F., Bruchhaus, I., Fink, C., and Roeder, T. (2016). Octopamine
1280 controls starvation resistance, life span and metabolic traits in *Drosophila*. Sci. Rep. *6*, 35359.

1281 Liao, S., Amcoff, M., and Nässel, D.R. (2021). Impact of high-fat diet on lifespan, metabolism, fecundity
1282 and behavioral senescence in *Drosophila*. Insect Biochem. Mol. Biol. *133*, 103495.

1283 Lorenz, M.W., and Gäde, G. (2009). Hormonal regulation of energy metabolism in insects as a driving
1284 force for performance. Integr. Comp. Biol. *49*, 380–392.

1285 Luong, N., Davies, C.R., Wessells, R.J., Graham, S.M., King, M.T., Veech, R., Bodmer, R., and Oldham, S.M.
1286 (2006). Activated FOXO-mediated insulin resistance is blocked by reduction of TOR activity. Cell Metab.
1287 *4*, 133–142.

1288 Luzier, A.B., Nawarskas, J.J., Añonuevo, J., Wilson, M.F., and Kazierad, D.J. (1998). The effects of gender
1289 on adrenergic receptor responsiveness. J. Clin. Pharmacol. *38*, 618–624.

1290 Masuyama, K., Zhang, Y., Rao, Y., and Wang, J.W. (2012). Mapping neural circuits with activity-
1291 dependent nuclear import of a transcription factor. J. Neurogenet. *26*, 89–102.

1292 Mathews, K.W., Cavegn, M., and Zwicky, M. (2017). Sexual Dimorphism of Body Size Is Controlled by
1293 Dosage of the X-Chromosomal Gene *Myc* and by the Sex-Determining Gene *tra* in *Drosophila*. Genetics
1294 *205*, 1215–1228.

1295 May, C.E., Rosander, J., Gottfried, J., Dennis, E., and Dus, M. (2020). Dietary sugar inhibits satiation by
1296 decreasing the central processing of sweet taste. ELife *9*, e54530.

1297 McIntosh, V.J., Chandrasekera, P.C., and Lasley, R.D. (2011). Sex differences and the effects of
1298 ovariectomy on the β-adrenergic contractile response. Am. J. Physiol. - Heart Circ. Physiol. *301*, H1127–
1299 H1134.

1300 Mellert, D.J., Knapp, J.-M., Manoli, D.S., Meissner, G.W., and Baker, B.S. (2010). Midline crossing by
1301 gustatory receptor neuron axons is regulated by fruitless, doublesex and the Roundabout receptors.
1302 Dev. Camb. Engl. *137*, 323–332.

1303 Mellert, D.J., Williamson, W.R., Shirangi, T.R., Card, G.M., and Truman, J.W. (2016). Genetic and
1304 Environmental Control of Neurodevelopmental Robustness in *Drosophila*. PLoS ONE *11*, e0155957.

1305 Millington, J.W., and Rideout, E.J. (2018). Sex differences in *Drosophila* development and physiology.
1306 Curr. Opin. Physiol. *6*, 46–56.

1307 Millington, J.W., Brownrigg, G.P., Chao, C., Sun, Z., Basner-Collins, P.J., Wat, L.W., Hudry, B., Miguel-
1308 Aliaga, I., and Rideout, E.J. (2021). Female-biased upregulation of insulin pathway activity mediates the
1309 sex difference in *Drosophila* body size plasticity. ELife *10*, e58341.

1310 Min, S., Chae, H.-S., Jang, Y.-H., Choi, S., Lee, S., Jeong, Y.T., Jones, W.D., Moon, S.J., Kim, Y.-J., and
1311 Chung, J. (2016). Identification of a Peptidergic Pathway Critical to Satiety Responses in *Drosophila*. Curr.
1312 Biol. *26*, 814–820.

1313 Mochanová, M., Tomčala, A., Svobodová, Z., and Kodrík, D. (2018). Role of adipokinetic hormone during
1314 starvation in *Drosophila*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. *226*, 26–35.

1315 Mosher, J., Zhang, W., Blumhagen, R.Z., D'Alessandro, A., Nemkov, T., Hansen, K.C., Hesselberth, J.R.,
1316 and Reis, T. (2015). Coordination between *Drosophila* Arc1 and a specific population of brain neurons
1317 regulates organismal fat. Dev. Biol. *405*, 280–290.

1318 Murphy, D.J. (2001). The biogenesis and functions of lipid bodies in animals, plants and microorganisms.
1319 Prog. Lipid Res. *40*, 325–438.

1320 Nagoshi, R.N., McKeown, M., Burtis, K.C., Belote, J.M., and Baker, B.S. (1988). The control of alternative
1321 splicing at genes regulating sexual differentiation in *D. melanogaster*. Cell *53*, 229–236.

1322 Nelson, C.S., Beck, J.N., Wilson, K.A., Pilcher, E.R., Kapahi, P., and Brem, R.B. (2016). Cross-phenotype
1323 association tests uncover genes mediating nutrient response in *Drosophila*. BMC Genomics *17*, 867.

1324 Neville, M.C., Nojima, T., Ashley, E., Parker, D.J., Walker, J., Southall, T., Van de Sande, B., Marques, A.C.,
1325 Fischer, B., Brand, A.H., et al. (2014). Male-specific fruitless isoforms target neurodevelopmental genes
1326 to specify a sexually dimorphic nervous system. Curr. Biol. CB *24*, 229–241.

1327 Ng, A.V., Callister, R., Johnson, D.G., and Seals, D.R. (1993). Age and gender influence muscle
1328 sympathetic nerve activity at rest in healthy humans. Hypertens. Dallas Tex 1979 *21*, 498–503.

1329 Nojima, T., Neville, M.C., and Goodwin, S.F. (2014). Fruitless isoforms and target genes specify the
1330 sexually dimorphic nervous system underlying *Drosophila* reproductive behavior. Fly (Austin) *8*, 95–100.

1331 Nojima, T., Rings, A., Allen, A.M., Otto, N., Verschut, T.A., Billeter, J.-C., Neville, M.C., and Goodwin, S.F.
1332 (2021). A sex-specific switch between visual and olfactory inputs underlies adaptive sex differences in
1333 behavior. Curr. Biol. *31*, 1175-1191.e6.

1334 Noyes, B.E., Katz, F.N., and Schaffer, M.H. (1995). Identification and expression of the *Drosophila*
1335 *adipokinetic hormone* gene. Mol. Cell. Endocrinol. *109*, 133–141.

1336 Oh, Y., Lai, J.S.-Y., Mills, H.J., Erdjument-Bromage, H., Giammarinaro, B., Saadipour, K., Wang, J.G., Abu,
1337 F., Neubert, T.A., and Suh, G.S.B. (2019). A glucose-sensing neuron pair regulates insulin and glucagon in
1338 *Drosophila*. Nature *574*, 559–564.

1339 Park, A., Tran, T., and Atkinson, N.S. (2018). Monitoring food preference in *Drosophila* by
1340 oligonucleotide tagging. Proc. Natl. Acad. Sci. U. S. A. *115*, 9020–9025.

1341 Park, Y., Kim, Y.-J., and Adams, M.E. (2002). Identification of G protein-coupled receptors for *Drosophila*
1342 PRXamide peptides, CCAP, corazonin, and AKH supports a theory of ligand-receptor coevolution. Proc.
1343 Natl. Acad. Sci. *99*, 11423–11428.

1344 Patel, R.T., Soulages, J.L., Hariharasundaram, B., and Arrese, E.L. (2005). Activation of the Lipid Droplet
1345 Controls the Rate of Lipolysis of Triglycerides in the Insect Fat Body*. J. Biol. Chem. *280*, 22624–22631.

1346 Pavlou, H.J., Lin, A.C., Neville, M.C., Nojima, T., Diao, F., Chen, B.E., White, B.H., and Goodwin, S.F.
1347 (2016). Neural circuitry coordinating male copulation. ELife *5*, e20713.

1348 von Philipsborn, A.C., Jörchel, S., Tirian, L., Demir, E., Morita, T., Stern, D.L., and Dickson, B.J. (2014).
1349 Cellular and behavioral functions of fruitless isoforms in *Drosophila* courtship. Curr. Biol. CB *24*, 242–
1350 251.

1351 Phillips, M.D., and Thomas, G.H. (2006). Brush border spectrin is required for early endosome recycling
1352 in *Drosophila*. J. Cell Sci. *119*, 1361–1370.

1353 Pomatto, L.C.D., Carney, C., Shen, B., Wong, S., Halaszynski, K., Salomon, M.P., Davies, K.J.A., and Tower,
1354 J. (2017). The Mitochondrial Lon Protease Is Required for Age-Specific and Sex-Specific Adaptation to
1355 Oxidative Stress. Curr. Biol. CB *27*, 1–15.

1356 Post, S., Liao, S., Yamamoto, R., Veenstra, J.A., Nässel, D.R., and Tatar, M. (2019). *Drosophila* insulin-like
1357 peptide dilp1 increases lifespan and glucagon-like *Akh* expression epistatic to *dilp2*. Aging Cell *18*,
1358 e12863.

1359 Power, M.L., and Schulkin, J. (2008). Sex differences in fat storage, fat metabolism, and the health risks
1360 from obesity: possible evolutionary origins. Br. J. Nutr. *99*, 931–940.

1361 Predel, R., Wegener, C., Russell, W.K., Tichy, S.E., Russell, D.H., and Nachman, R.J. (2004). Peptidomics of
1362 CNS-associated neurohemal systems of adult *Drosophila melanogaster*: A mass spectrometric survey of
1363 peptides from individual flies. J. Comp. Neurol. *474*, 379–392.

1364 Rajan, A., and Perrimon, N. (2012). The *Drosophila* cytokine Unpaired 2 regulates physiological
1365 homeostasis by remotely controlling Insulin secretion. Cell *151*, 123–137.

1366 Recazens, E., Mouisel, E., and Langin, D. (2021). Hormone-sensitive lipase: sixty years later. Prog. Lipid
1367 Res. *82*, 101084.

1368 Reddiex, A.J., Gosden, T.P., Bonduriansky, R., and Chenoweth, S.F. (2013). Sex-specific fitness
1369 consequences of nutrient intake and the evolvability of diet preferences. Am. Nat. *182*, 91–102.

1370 Regan, J.C., Khericha, M., Dobson, A.J., Bolukbasi, E., Rattanavirotkul, N., and Partridge, L. (2016). Sex
1371 difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary
1372 restriction. ELife *5*, e10956.

1373 Rezával, C., Nojima, T., Neville, M.C., Lin, A.C., and Goodwin, S.F. (2014). Sexually dimorphic
1374 octopaminergic neurons modulate female postmating behaviors in *Drosophila*. Curr. Biol. CB *24*, 725–
1375 730.

1376 Rezával, C., Pattnaik, S., Pavlou, H.J., Nojima, T., Brüggemeier, B., D'Souza, L.A.D., Dweck, H.K.M., and
1377 Goodwin, S.F. (2016). Activation of Latent Courtship Circuitry in the Brain of *Drosophila* Females Induces
1378 Male-like Behaviors. Curr. Biol. CB *26*, 2508–2515.

1379 Rideout, E.J., Billeter, J.-C., and Goodwin, S.F. (2007). The sex-determination genes *fruitless* and
1380 *doublesex* specify a neural substrate required for courtship song. Curr. Biol. CB *17*, 1473–1478.

1381 Rideout, E.J., Dornan, A.J., Neville, M.C., Eadie, S., and Goodwin, S.F. (2010). Control of sexual
1382 differentiation and behavior by the *doublesex* gene in *Drosophila melanogaster*. Nat. Neurosci. *13*, 458–
1383 466.

1384 Rideout, E.J., Narsaiya, M.S., and Grewal, S.S. (2015). The Sex Determination Gene *transformer*
1385 Regulates Male-Female Differences in *Drosophila* Body Size. PLoS Genet. *11*, e1005683.

1386 Robinett, C.C., Vaughan, A.G., Knapp, J.-M., and Baker, B.S. (2010). Sex and the Single Cell. II. There Is a
1387 Time and Place for Sex. PLOS Biol. *8*, e1000365.

1388 Roth, S.W., Bitterman, M.D., Birnbaum, M.J., and Bland, M.L. (2018). Innate Immune Signaling in
1389 *Drosophila* Blocks Insulin Signaling by Uncoupling PI(3,4,5)P3 Production and Akt Activation. Cell Rep. *22*,
1390 2550–2556.

1391 Ryner, L.C., Goodwin, S.F., Castrillon, D.H., Anand, A., Villella, A., Baker, B.S., Hall, J.C., Taylor, B.J., and
1392 Wasserman, S.A. (1996). Control of male sexual behavior and sexual orientation in *Drosophila* by the
1393 *fruitless* gene. Cell *87*, 1079–1089.

1394 Salz, H.K., and Erickson, J.W. (2010). Sex determination in *Drosophila*: The view from the top. Fly (Austin)
1395 *4*, 60–70.

1396 Sato, K., Goto, J., and Yamamoto, D. (2019). Sex Mysteries of the Fly Courtship Master Regulator
1397 Fruitless. Front. Behav. Neurosci. *13*, 245.

1398 Sawala, A., and Gould, A.P. (2017). The sex of specific neurons controls female body growth in
1399 *Drosophila*. PLoS Biol. *15*, e2002252.

1400 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C.,
1401 Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat.
1402 Methods *9*, 676–682.

1403 Schreiber, R., Xie, H., and Schweiger, M. (2019). Of mice and men: The physiological role of adipose
1404 triglyceride lipase (ATGL). Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids *1864*, 880–899.

1405 Scopelliti, A., Bauer, C., Yu, Y., Zhang, T., Kruspig, B., Murphy, D.J., Vidal, M., Maddocks, O.D.K., and
1406 Cordero, J.B. (2019). A Neuronal Relay Mediates a Nutrient Responsive Gut/Fat Body Axis Regulating
1407 Energy Homeostasis in Adult *Drosophila*. Cell Metab. *29*, 269-284.e10.

1408 Shirangi, T.R., Wong, A.M., Truman, J.W., and Stern, D.L. (2016). Doublesex Regulates the Connectivity of
1409 a Neural Circuit Controlling *Drosophila* Male Courtship Song. Dev. Cell *37*, 533–544.

1410 Sieber, M.H., and Spradling, A.C. (2015). Steroid Signaling Establishes a Female Metabolic State and
1411 Regulates SREBP to Control Oocyte Lipid Accumulation. Curr. Biol. *25*, 993–1004.

1412 Siera, S.G., and Cline, T.W. (2008). Sexual back talk with evolutionary implications: stimulation of the
1413 *Drosophila* sex-determination gene *sex-lethal* by its target *transformer*. Genetics *180*, 1963–1981.

1414 Song, W., Veenstra, J.A., and Perrimon, N. (2014). Control of Lipid Metabolism by Tachykinin in
1415 *Drosophila*. Cell Rep. *9*, 40–47.

1416 Song, W., Cheng, D., Hong, S., Sappe, B., Hu, Y., Wei, N., Zhu, C., O'Connor, M.B., Pissios, P., and
1417 Perrimon, N. (2017). Midgut-Derived Activin Regulates Glucagon-like Action in the Fat Body and
1418 Glycemic Control. Cell Metab. *25*, 386–399.

1419 Sosnowski, B.A., Belote, J.M., and McKeown, M. (1989). Sex-specific alternative splicing of RNA from the
1420 *transformer* gene results from sequence-dependent splice site blockage. Cell *58*, 449–459.

1421 Stafford, J.W., Lynd, K.M., Jung, A.Y., and Gordon, M.D. (2012). Integration of taste and calorie sensing in
1422 *Drosophila*. J. Neurosci. Off. J. Soc. Neurosci. *32*, 14767–14774.

1423 Staubli, F., Jorgensen, T.J.D., Cazzamali, G., Williamson, M., Lenz, C., Sondergaard, L., Roepstorff, P., and
1424 Grimmelikhuijzen, C.J.P. (2002). Molecular identification of the insect adipokinetic hormone receptors.
1425 Proc. Natl. Acad. Sci. U. S. A. *99*, 3446–3451.

1426 Sturtevant, A.H. (1945). A Gene in *Drosophila Melanogaster* That Transforms Females into Males.
1427 Genetics *30*, 297–299.

1428 Suzawa, M., Muhammad, N.M., Joseph, B.S., and Bland, M.L. (2019). The Toll Signaling Pathway Targets
1429 the Insulin-like Peptide Dilp6 to Inhibit Growth in *Drosophila*. Cell Rep. *28*, 1439-1446.e5.

1430 Taylor, B.J., and Truman, J.W. (1992). Commitment of abdominal neuroblasts in *Drosophila* to a male or
1431 female fate is dependent on genes of the sex-determining hierarchy. Dev. Camb. Engl. *114*, 625–642.

1432 Teixeira, L., Rabouille, C., Rørth, P., Ephrussi, A., and Vanzo, N.F. (2003). *Drosophila* Perilipin/ADRP
1433 homologue Lsd2 regulates lipid metabolism. Mech. Dev. *120*, 1071–1081.

1434 Teleman, A.A., Chen, Y.-W., and Cohen, S.M. (2005). 4E-BP functions as a metabolic brake used under
1435 stress conditions but not during normal growth. Genes Dev. *19*, 1844–1848.

1436 Tennessen, J.M., Barry, W., Cox, J., and Thummel, C.S. (2014). Methods for studying metabolism in
1437 *Drosophila*. Methods San Diego Calif *68*, 105–115.

1438 Texada, M.J., Jørgensen, A.F., Christensen, C.F., Koyama, T., Malita, A., Smith, D.K., Marple, D.F.M.,
1439 Danielsen, E.T., Petersen, S.K., Hansen, J.L., et al. (2019). A fat-tissue sensor couples growth to oxygen
1440 availability by remotely controlling insulin secretion. Nat. Commun. *10*, 1955.

1441 Thiele, C., and Spandl, J. (2008). Cell biology of lipid droplets. Curr. Opin. Cell Biol. *20*, 378–385.

1442 Thurmond, J., Goodman, J.L., Strelets, V.B., Attrill, H., Gramates, L.S., Marygold, S.J., Matthews, B.B.,
1443 Millburn, G., Antonazzo, G., Trovisco, V., et al. (2019). FlyBase 2.0: the next generation. Nucleic Acids
1444 Res. *47*, D759–D765.

1445 Tian, Y., Bi, J., Shui, G., Liu, Z., Xiang, Y., Liu, Y., Wenk, M.R., Yang, H., and Huang, X. (2011). Tissue-
1446 autonomous function of *Drosophila* seipin in preventing ectopic lipid droplet formation. PLoS Genet. *7*,
1447 e1001364.

1448 Ugrankar, R., Liu, Y., Provaznik, J., Schmitt, S., and Lehmann, M. (2011). Lipin is a central regulator of
1449 adipose tissue development and function in *Drosophila melanogaster*. Mol. Cell. Biol. *31*, 1646–1656.

1450 Videlier, M., Rundle, H.D., and Careau, V. (2019). Sex-Specific Among-Individual Covariation in
1451 Locomotor Activity and Resting Metabolic Rate in *Drosophila melanogaster*. Am. Nat. *194*, E164–E176.

1452 Walther, T.C., and Farese, R.V. (2012). Lipid Droplets and Cellular Lipid Metabolism. Annu. Rev. Biochem.
1453 *81*, 687–714.

1454 Wang, F., Wang, K., Forknall, N., Patrick, C., Yang, T., Parekh, R., Bock, D., and Dickson, B.J. (2020).
1455 Neural circuitry linking mating and egg-laying in *Drosophila* females. Nature *579*, 101–105.

1456 Wang, H., Becuwe, M., Housden, B.E., Chitraju, C., Porras, A.J., Graham, M.M., Liu, X.N., Thiam, A.R.,
1457 Savage, D.B., Agarwal, A.K., et al. (2016). Seipin is required for converting nascent to mature lipid
1458 droplets. ELife *5*, e16582.

1459 Wat, L.W., Chao, C., Bartlett, R., Buchanan, J.L., Millington, J.W., Chih, H.J., Chowdhury, Z.S., Biswas, P.,
1460 Huang, V., Shin, L.J., et al. (2020). A role for triglyceride lipase *brummer* in the regulation of sex
1461 differences in *Drosophila* fat storage and breakdown. PLOS Biol. *18*, e3000595.

1462 Watanabe, L.P., and Riddle, N.C. (2021). GWAS reveal a role for the central nervous system in regulating
1463 weight and weight change in response to exercise. Sci. Rep. *11*, 5144.

1464 Wegener, C., Reinl, T., Jänsch, L., and Predel, R. (2006). Direct mass spectrometric peptide profiling and
1465 fragmentation of larval peptide hormone release sites in *Drosophila melanogaster* reveals tagma-
1466 specific peptide expression and differential processing. J. Neurochem. *96*, 1362–1374.

1467 White, K., Tahaoglu, E., and Steller, H. (1996). Cell killing by the *Drosophila* gene *reaper*. Science *271*,
1468 805–807.

1469 Womersley, J., and Durnin, J.V.G.A. (1977). A comparison of the skinfold method with extent of
1470 'overweight' and various weight-height relationships in the assessment of obesity. Br. J. Nutr. *38*, 271–
1471 284.

1472 Zhan, Y.P., Liu, L., and Zhu, Y. (2016). Taotie neurons regulate appetite in *Drosophila*. Nat. Commun. *7*,
1473 13633.

1474 Zhao, X., and Karpac, J. (2017). Muscle Directs Diurnal Energy Homeostasis through a Myokine-
1475 Dependent Hormone Module in *Drosophila*. Curr. Biol. CB *27*, 1941-1955.e6.

1476

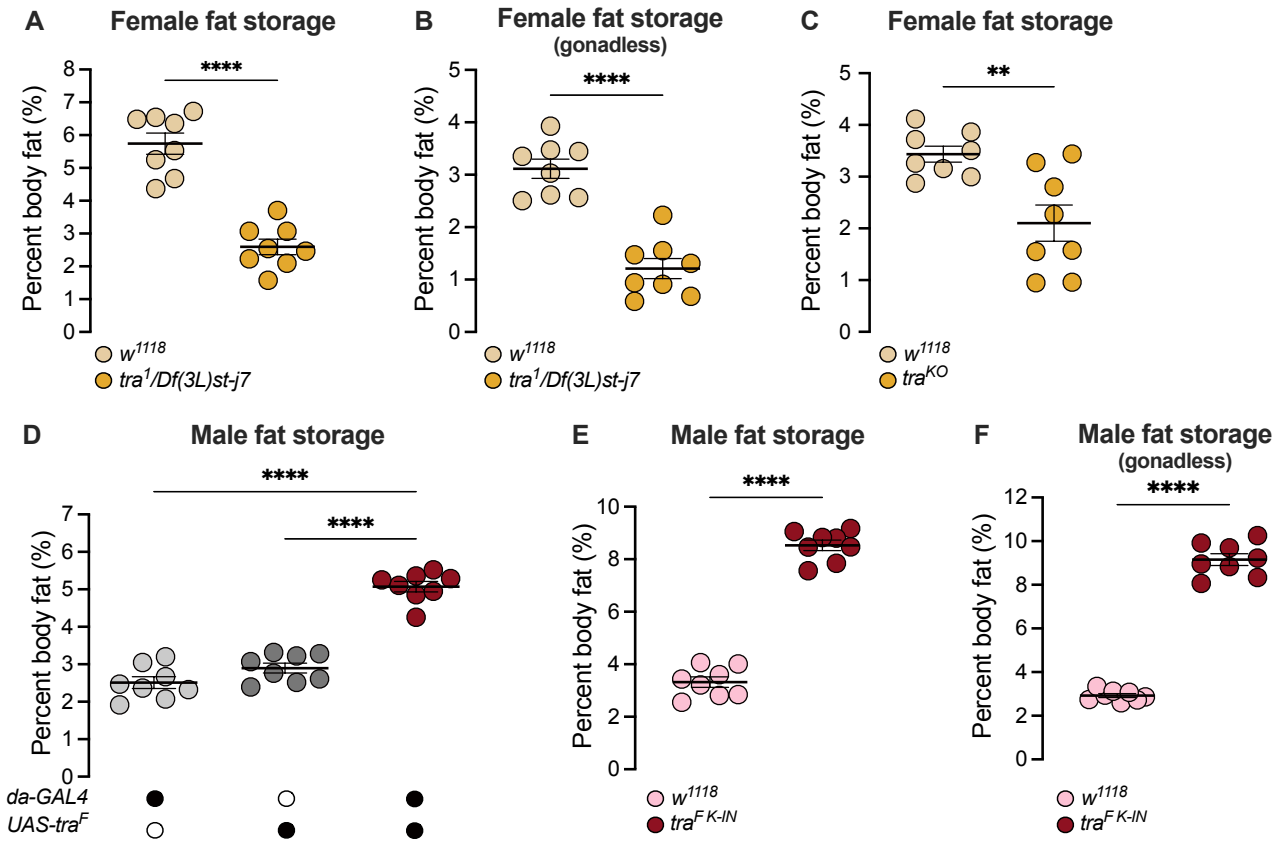
A
Female fat storage
Percent body fat (%)
****
w1118
tra1/Df(3L)st-j7
B
Female fat storage
(gonadless)
Percent body fat (%)
****
w1118
tra1/Df(3L)st-j7
C
Female fat storage
Percent body fat (%)
**
w1118
traKO
D
Male fat storage
Percent body fat (%)
****
****
da-GAL4
UAS-traF
E
Male fat storage
Percent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

FIGURE 1

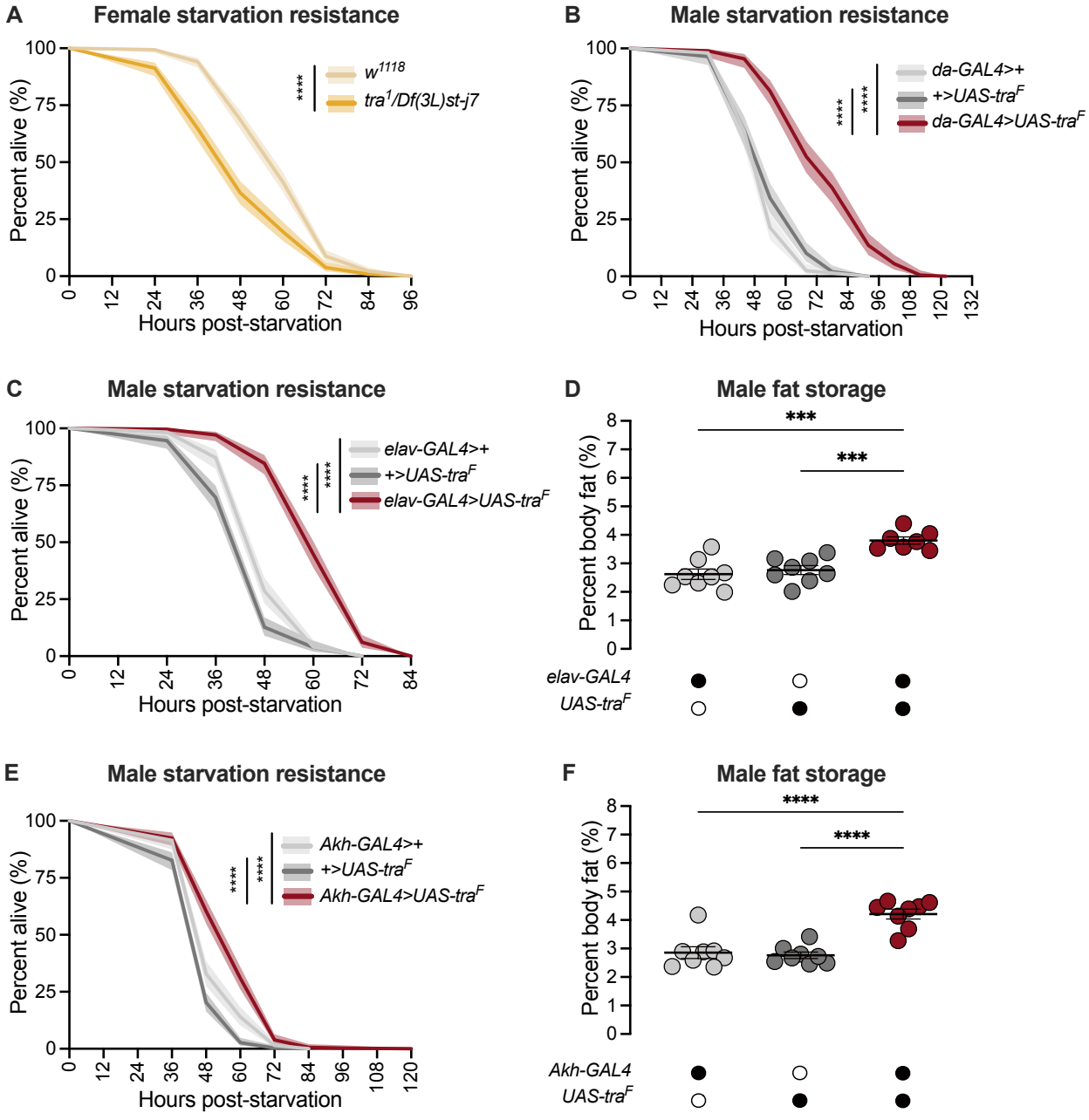
A
Female starvation resistance
Percent alive (%)
Hours post-starvation
****
w1118
tra1/Df(3L)st-j7
B
Male starvation resistance
Percent alive (%)
Hours post-starvation
****
****
da-GAL4>+
+>UAS-traF
da-GAL4>UAS-traF
C
Male starvation resistance
Percent alive (%)
Hours post-starvation
****
****
elav-GAL4>+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

FIGURE 2

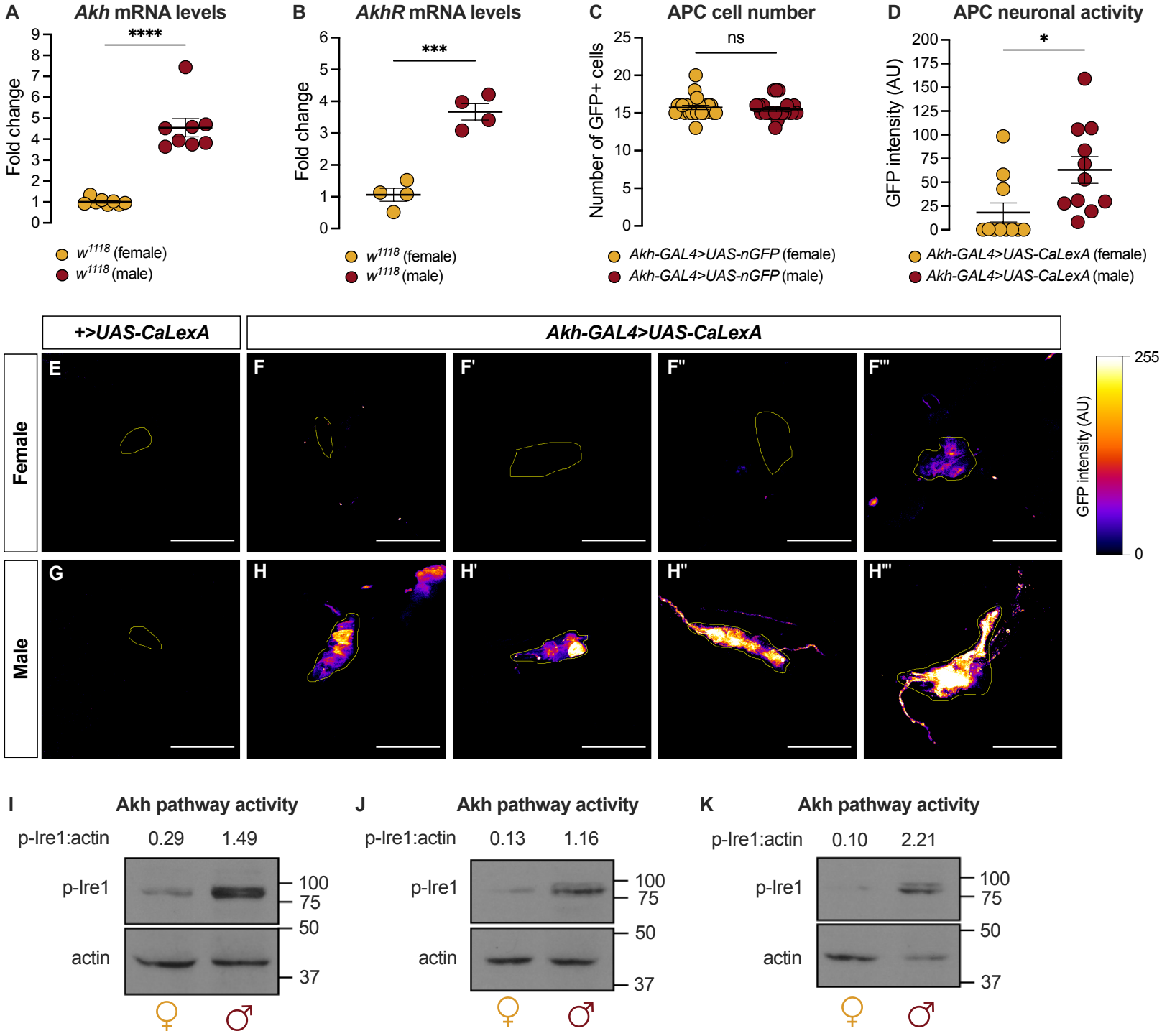
A
Akh mRNA levels
****
Fold change
w1118 (female)
w1118 (male)
B
AkhR mRNA levels
***
Fold change
w1118 (female)
w1118 (male)
C
APC cell number
ns
Number of GFP+ cells
Akh-GAL4>UAS-nGFP (female)
Akh-GAL4>UAS-nGFP (male)
D
APC neuronal activity
*
GFP intensity (AU)
Akh-GAL4>UAS-CaLexA (female)
Akh-GAL4>UAS-CaLexA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

FIGURE 3

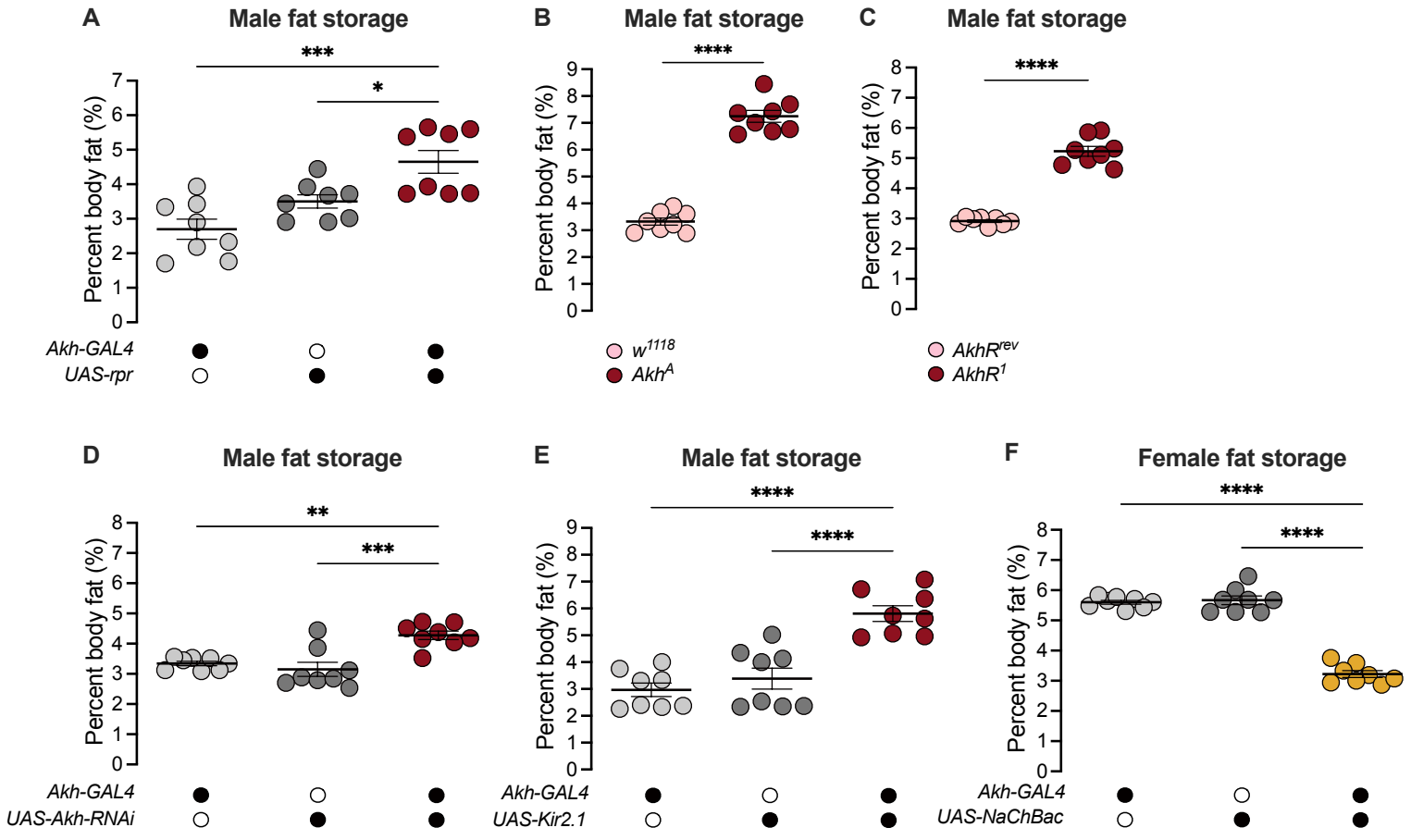
A
Male fat storage
***
*
Percent body fat (%)
Akh-GAL4
UAS-rpr
B
Male fat storage
****
Percent body fat (%)
w1118
AkhA
C
Male fat storage
****
Percent body fat (%)
AkhRrev
AkhR1
D
Male fat storage
**
***
Percent body fat (%)
Akh-GAL4
UAS-Akh-RNAi
E
Male fat storage
****
****
Percent body fat (%)
Akh-GAL4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

FIGURE 4

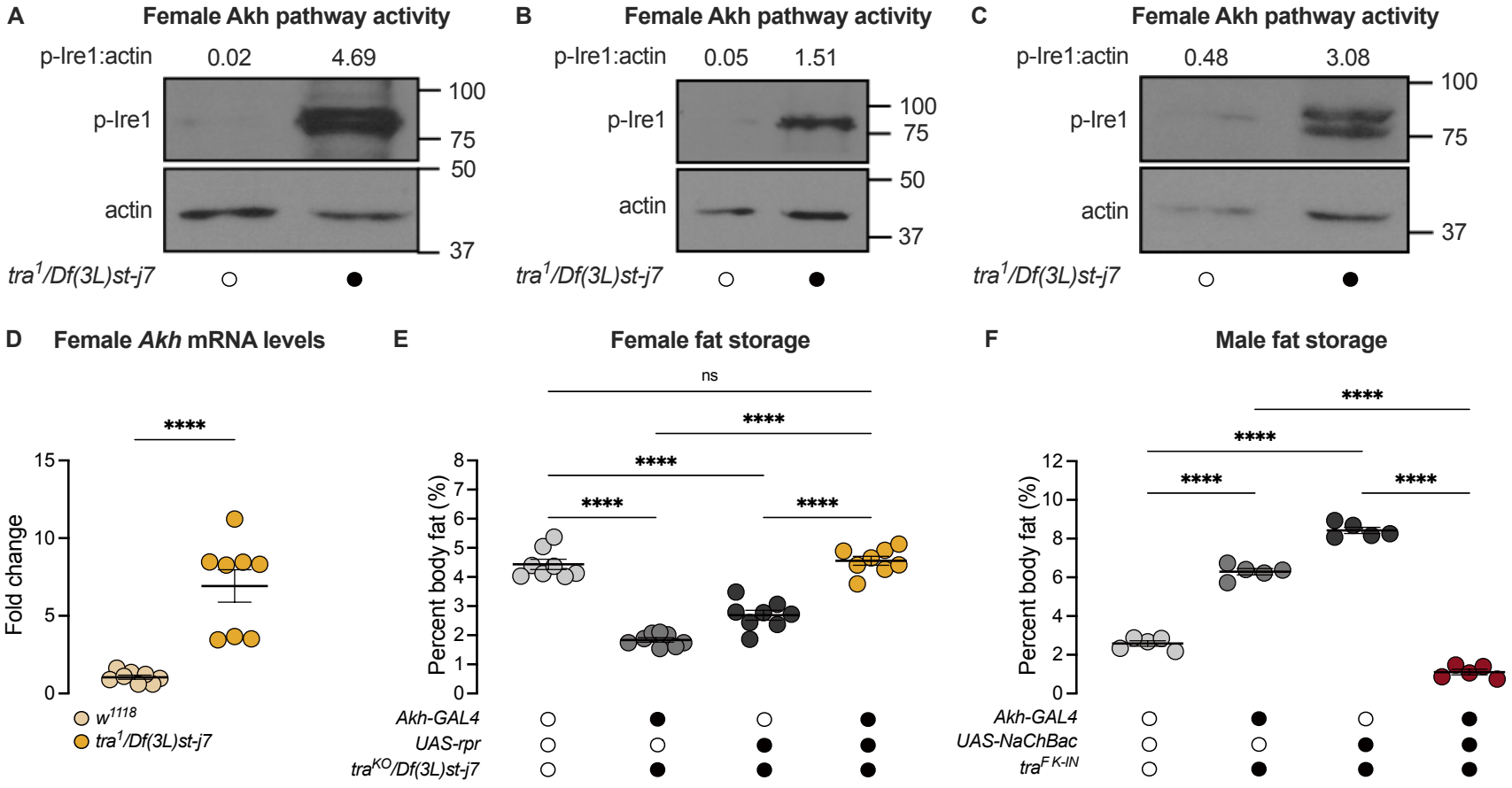
A
Female Akh pathway activity
p-Ire1:actin
0.02
4.69
p-Ire1
actin
100
75
50
37
tra1/Df(3L)st-j7
B
Female Akh pathway activity
p-Ire1:actin
0.05
1.51
C
Female Akh pathway activity
p-Ire1:actin
0.48
3.08
D
Female Akh mRNA levels
Fold change
****
w1118
tra1/Df(3L)st-j7
E
Female fat storage
Percent body 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

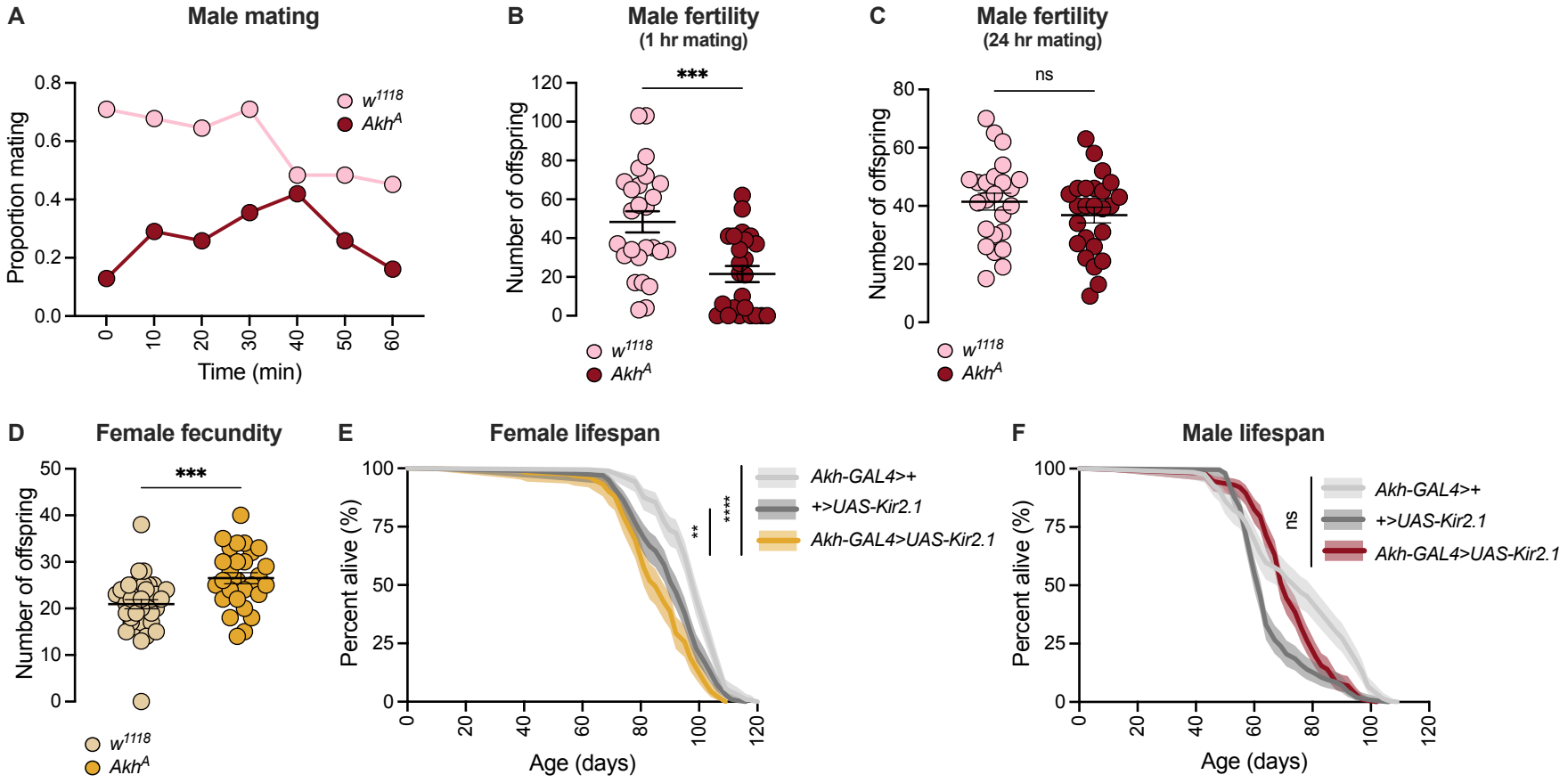
A
Male mating
Proportion mating
Time (min)
w^{1118}
Akh^{A}
B
Male fertility
(1 hr mating)
Number of offspring
***
C
Male fertility
(24 hr mating)
ns
D
Female fecundity
E
Female lifespan
Percent alive (%)
Age (days)
Akh-GAL4>+
+>UAS-Kir2.1
Akh-GAL4>UAS-Kir2.1
F
Male lifespan

FIGURE 6

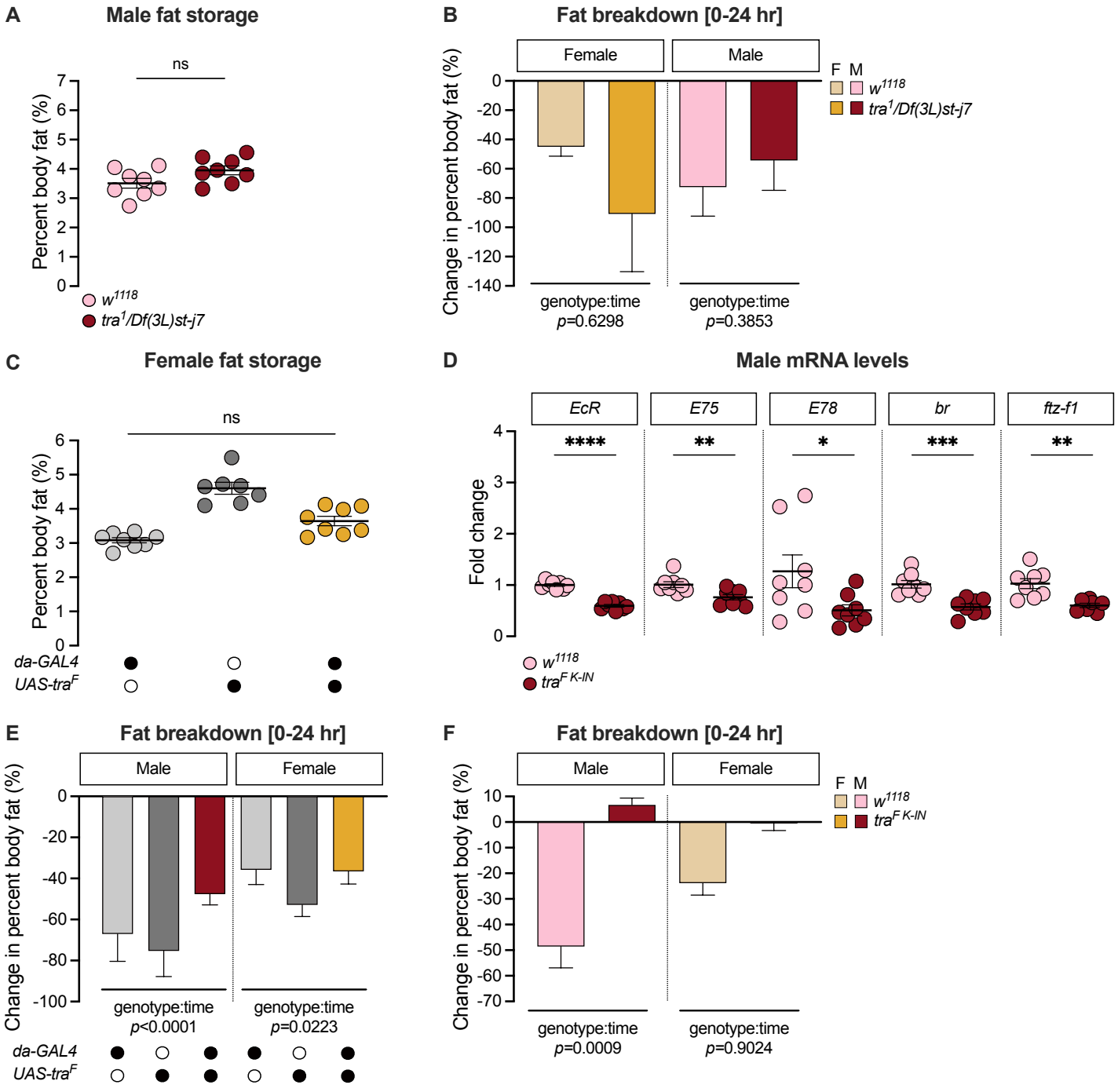

FIGURE 1 - FIGURE SUPPLEMENT 1

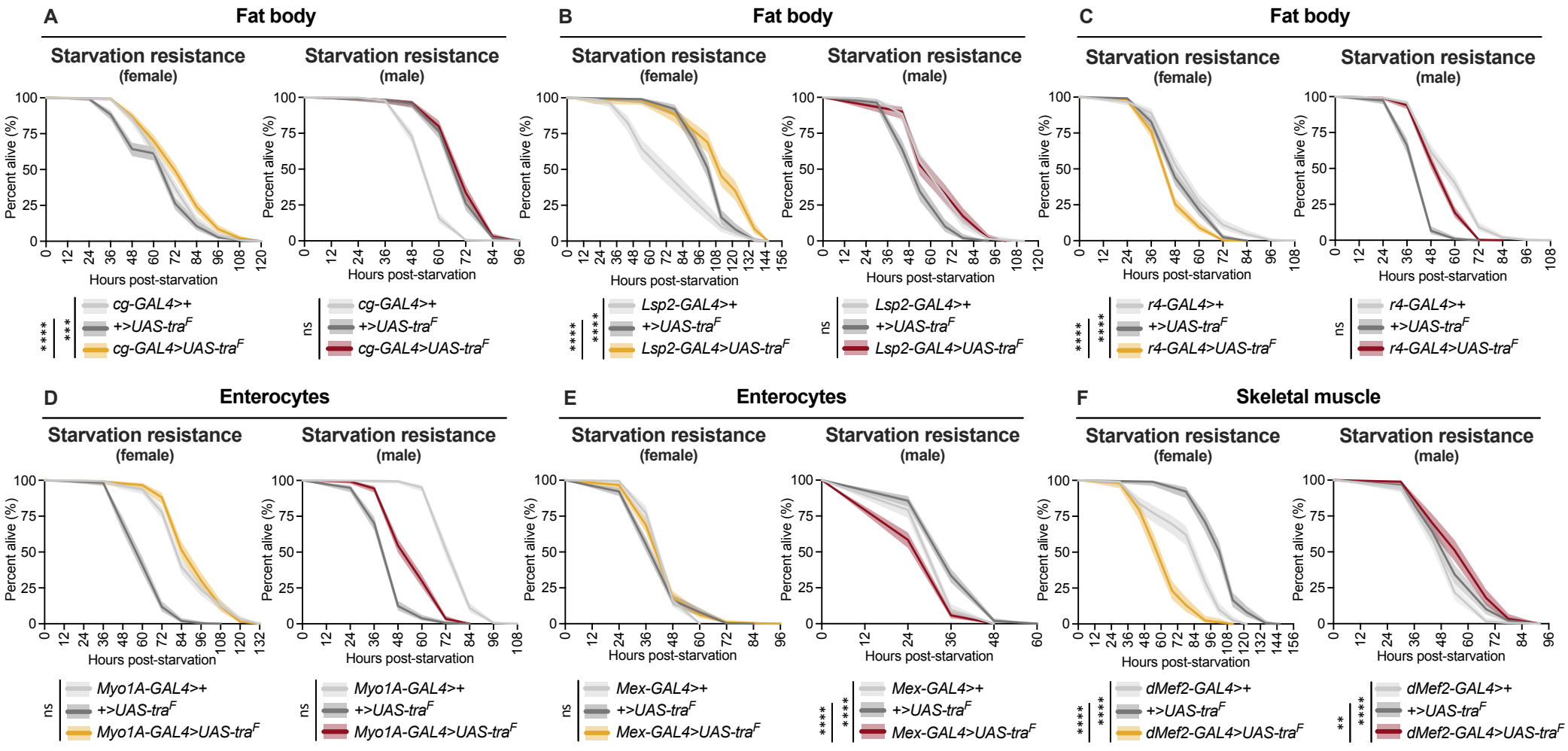

FIGURE 2 - FIGURE SUPPLEMENT 1

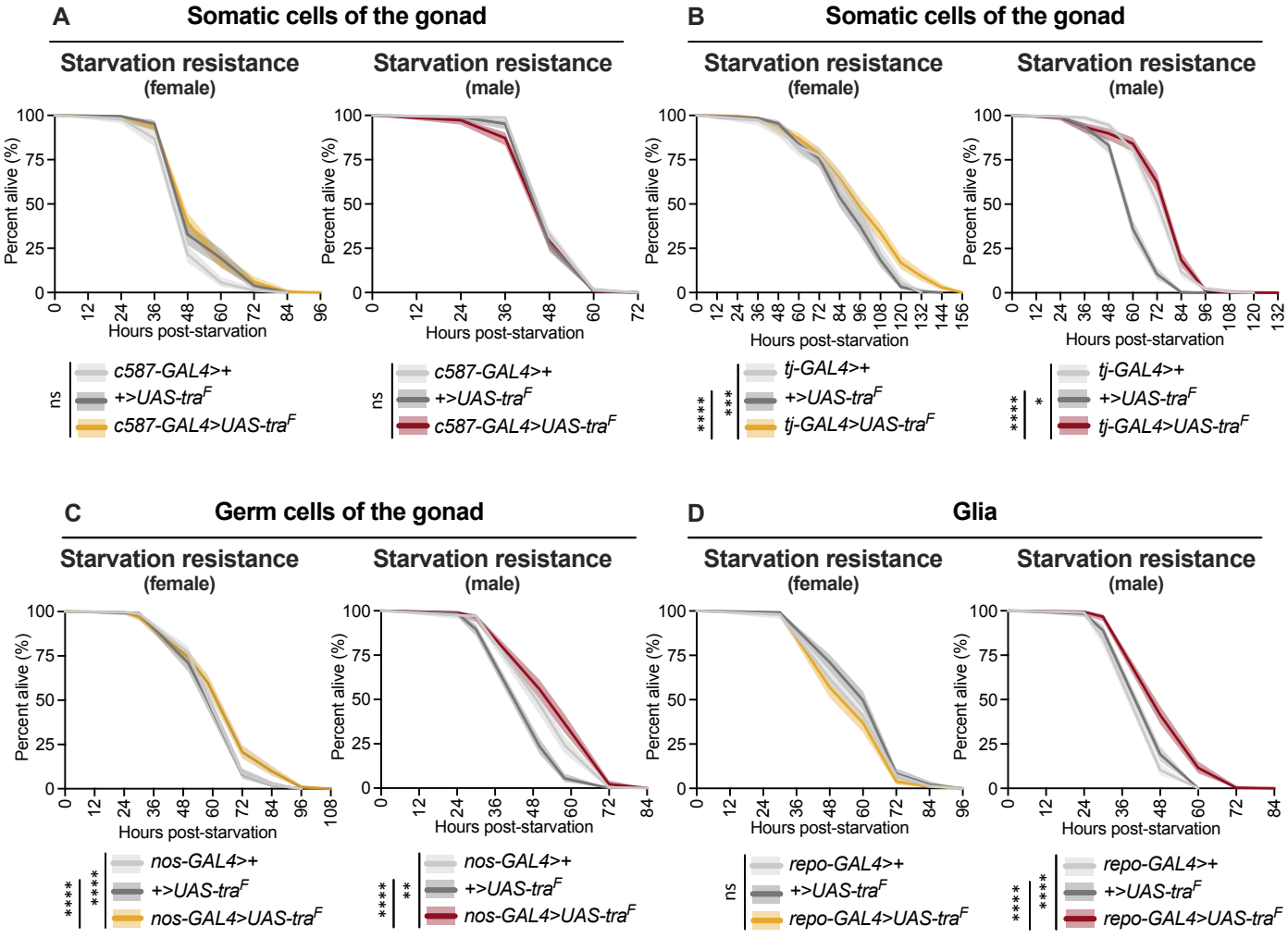

FIGURE 2 - FIGURE SUPPLEMENT 2

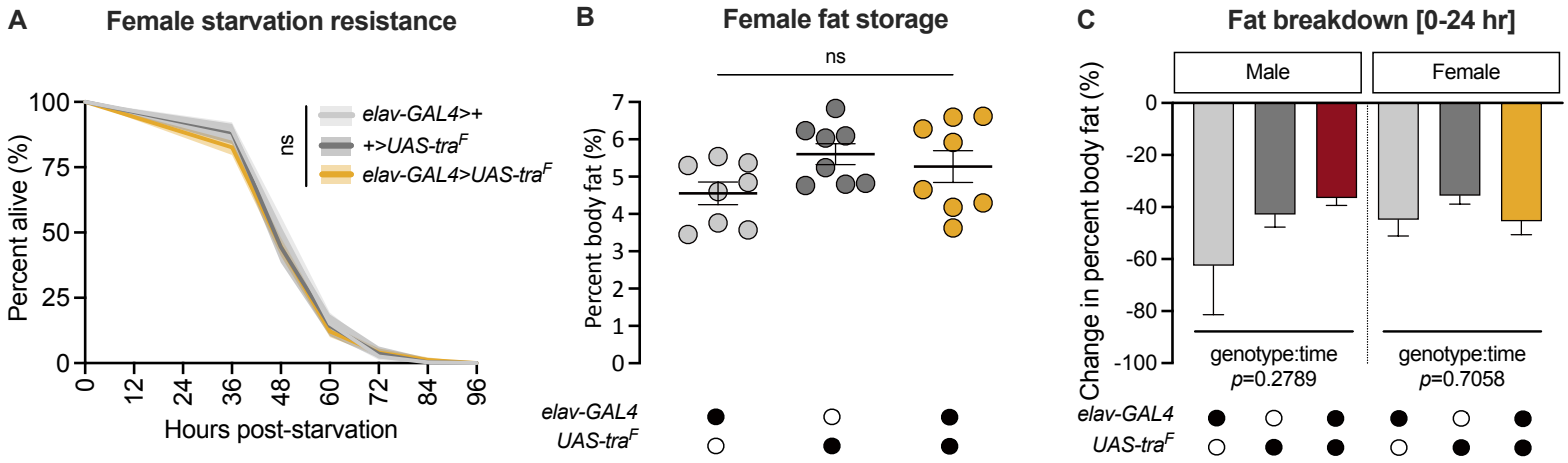

FIGURE 2 - FIGURE SUPPLEMENT 3

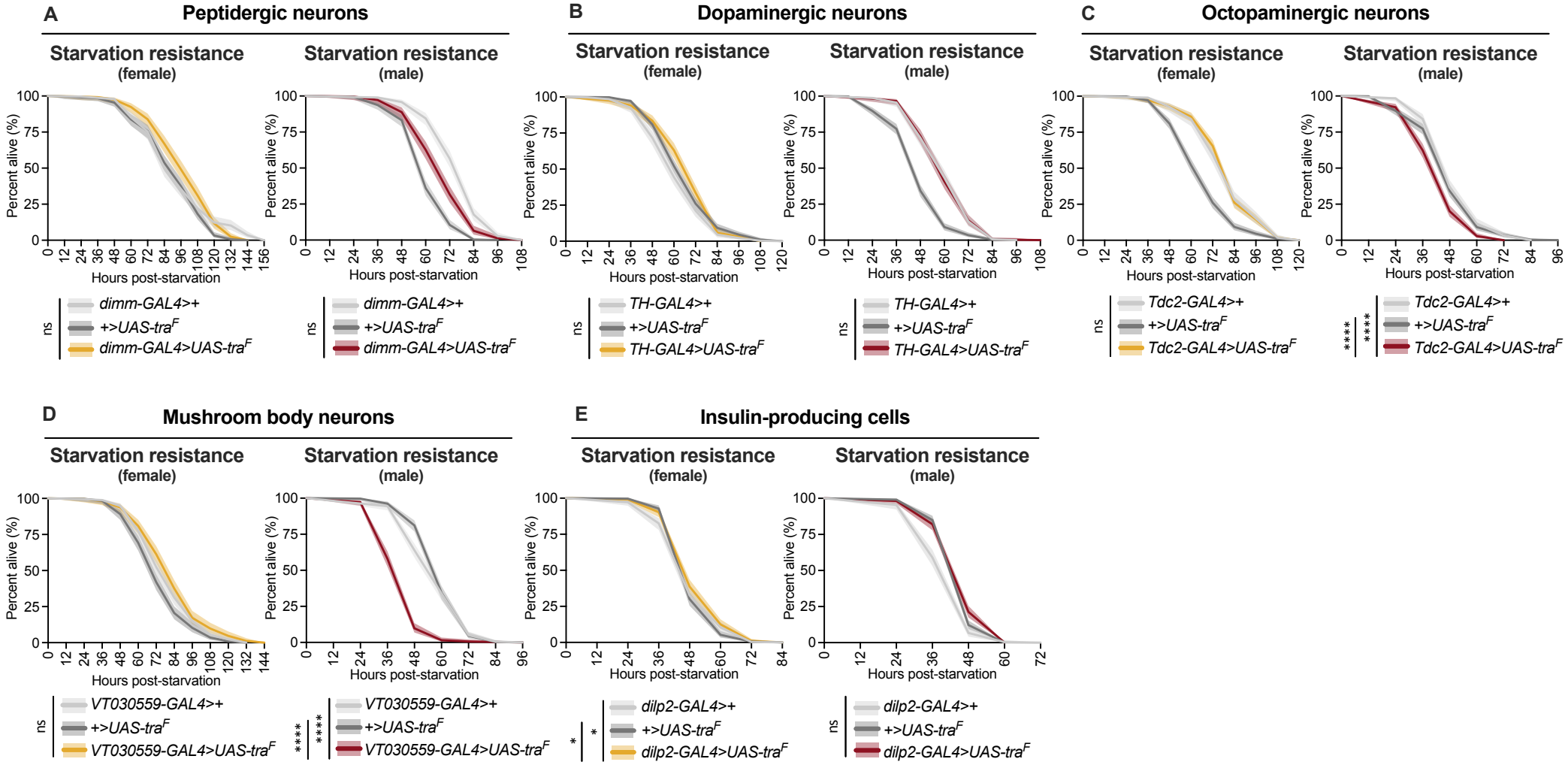

FIGURE 2 - FIGURE SUPPLEMENT 4

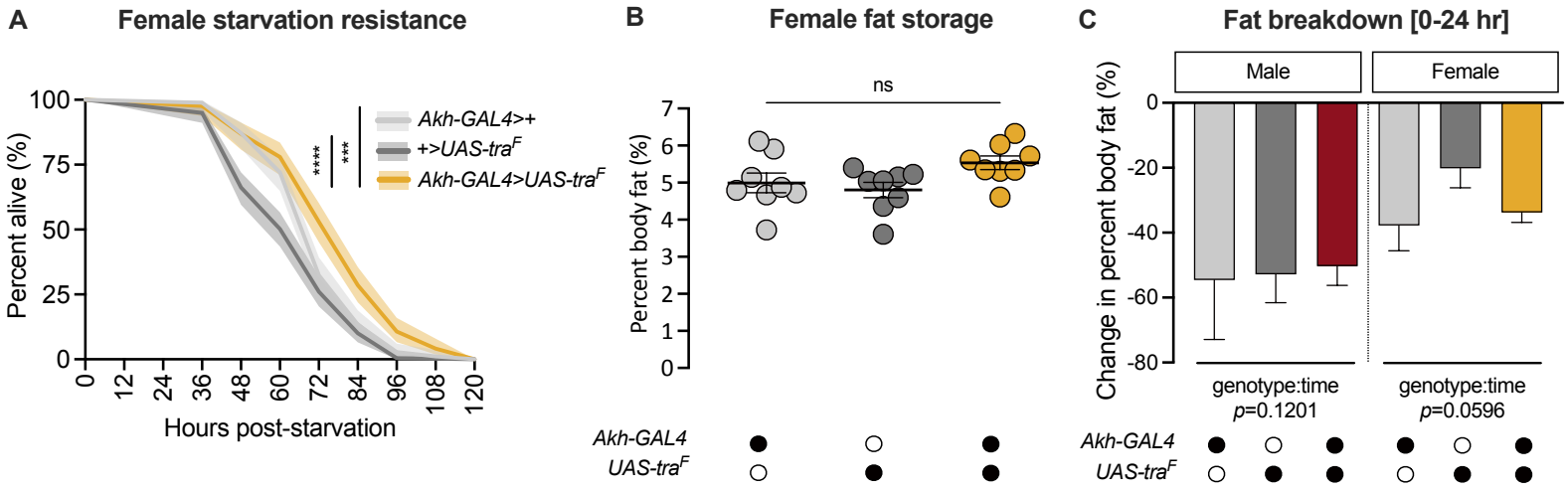

FIGURE 2 - FIGURE SUPPLEMENT 5

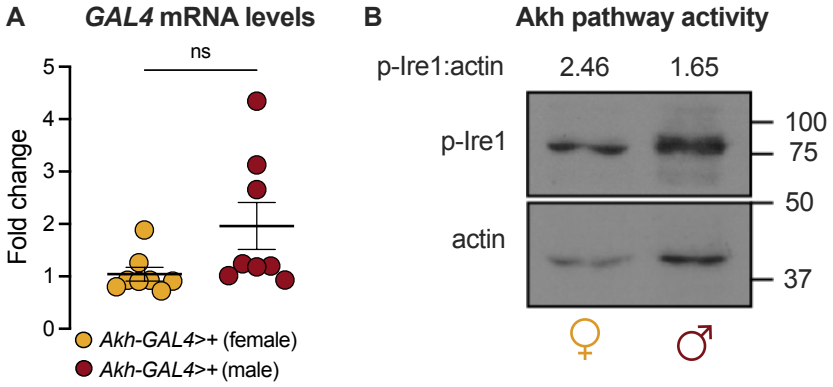

FIGURE 3 - FIGURE SUPPLEMENT 1

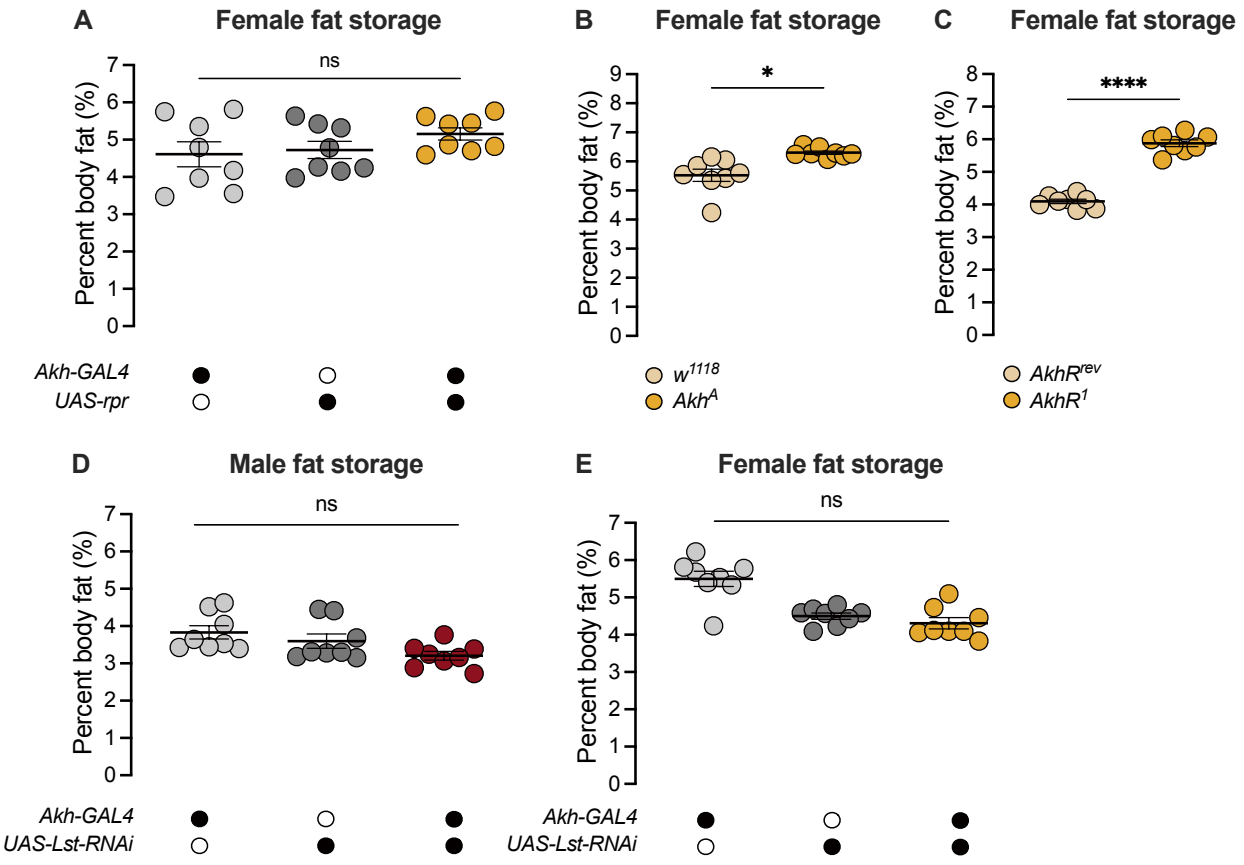

FIGURE 4 - FIGURE SUPPLEMENT 1

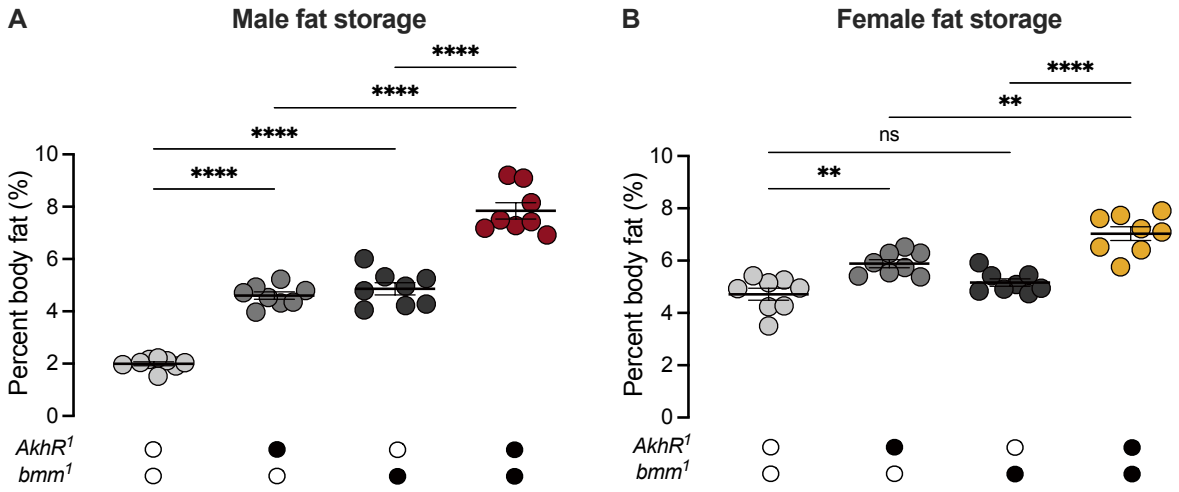

FIGURE 4 - FIGURE SUPPLEMENT 2

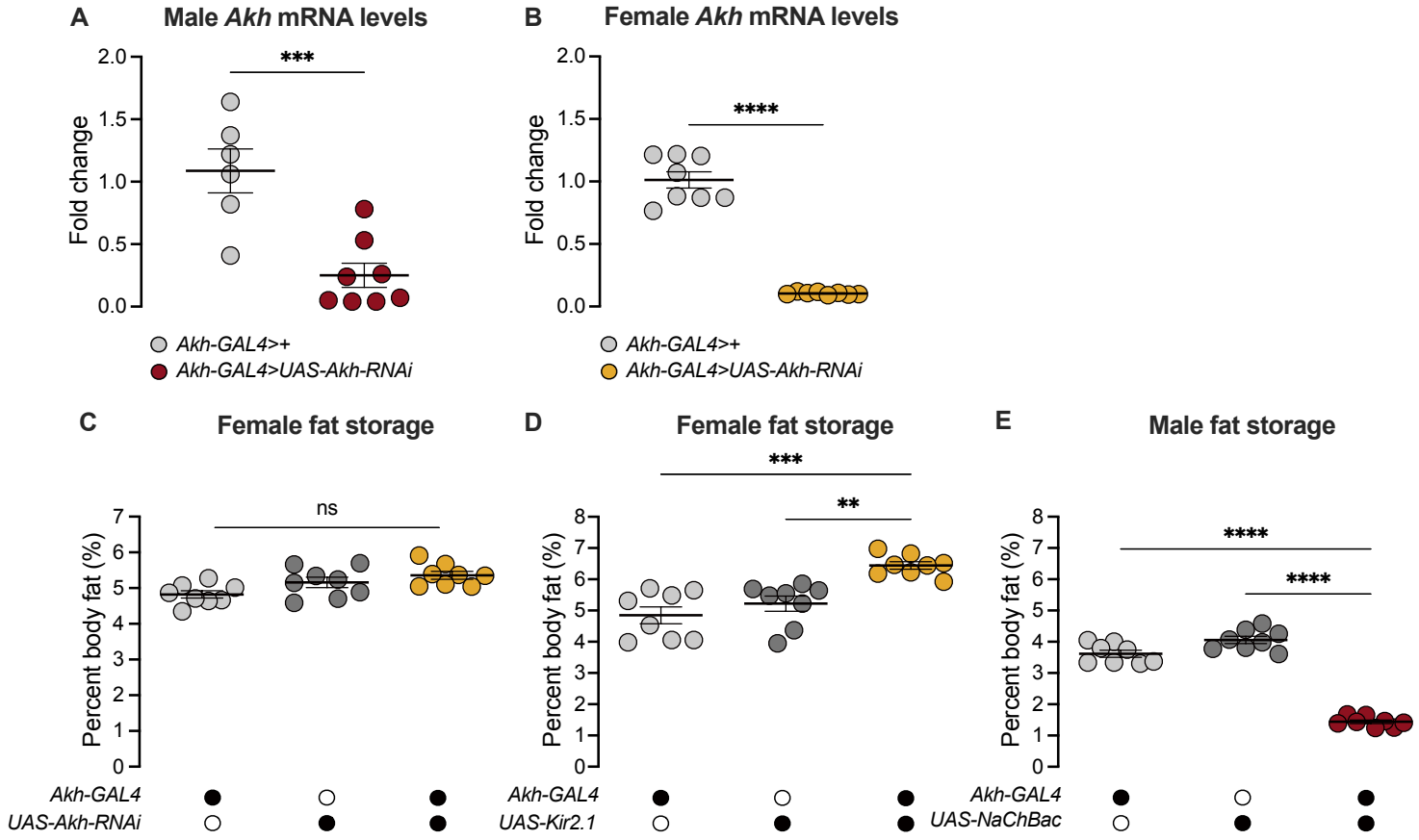

FIGURE 4 - FIGURE SUPPLEMENT 3

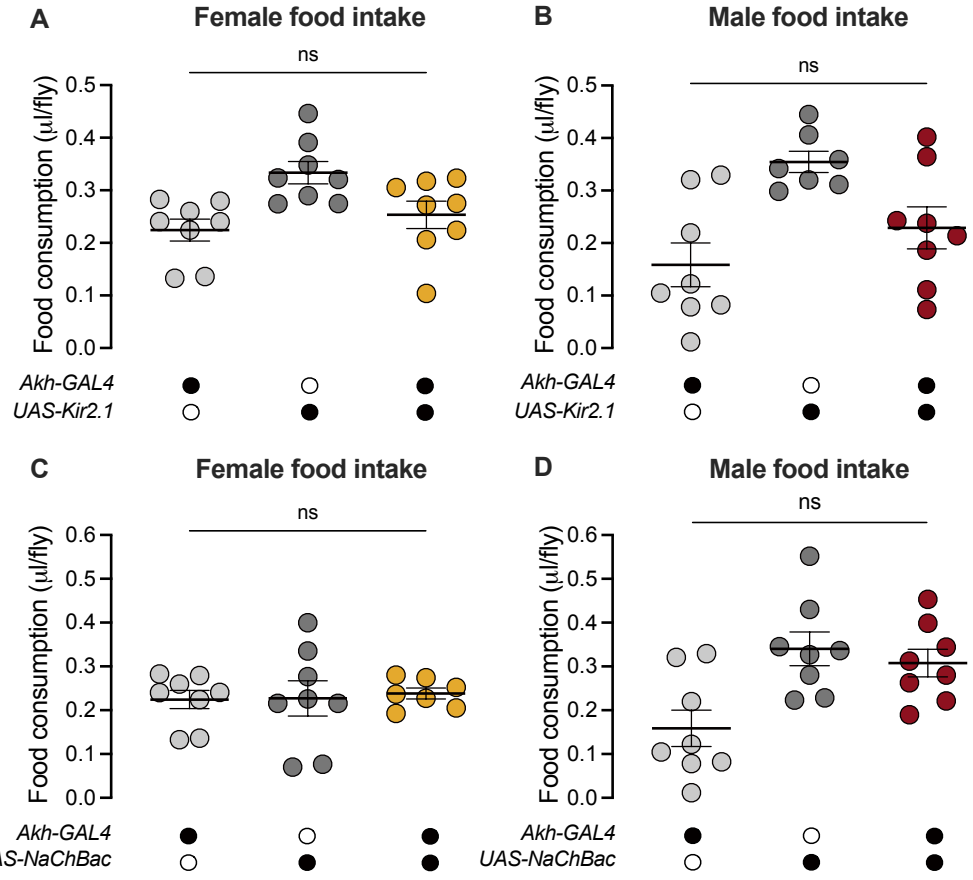

FIGURE 4 - FIGURE SUPPLEMENT 4

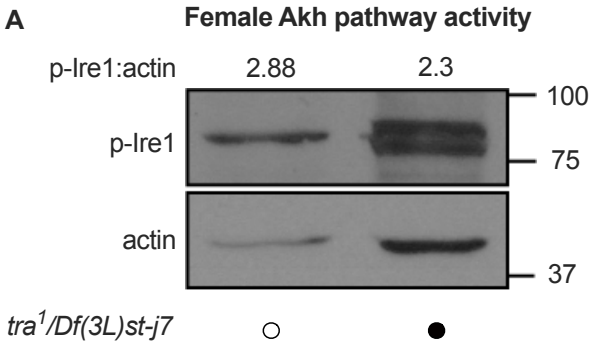

FIGURE 5 - FIGURE SUPPLEMENT 1