

1 **Title:** Muscle function and homeostasis require macrophage-derived cytokine inhibition of AKT
2 activity in *Drosophila*

3 **Short title:** Muscle Dome controls AKT and metabolism

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22

23 Abstract

24 *Unpaired* ligands are secreted signals that act via a GP130-like receptor, *domeless*, to activate JAK-
25 STAT signaling in *Drosophila*. Like many mammalian cytokines, *unpaired*s can be activated by
26 infection and other stresses and can promote insulin resistance in target tissues. However, the
27 importance of this effect in non-inflammatory physiology is unknown. Here, we identify a
28 requirement for *unpaired*-JAK signaling as a metabolic regulator in healthy adult *Drosophila* muscle.
29 Adult muscles show basal JAK-STAT signaling activity in the absence of any immune challenge.
30 Macrophages are the source of much of this tonic signal. Loss of the *dome* receptor on adult muscles
31 significantly reduces lifespan and causes local and systemic metabolic pathology. These pathologies
32 result from hyperactivation of AKT and consequent deregulation of metabolism. Thus, we identify a
33 cytokine signal from macrophages to muscle that controls AKT activity and metabolic homeostasis.

34 Introduction

35 JAK/STAT activating signals are critical regulators of many biological processes in animals. Originally
36 described mainly in immune contexts, it has increasingly become clear that JAK/STAT signaling is also
37 central to metabolic regulation in many tissues (Dodington et al., 2018; Villarino et al., 2017). One
38 common consequence of activation of JAK/STAT pathways in inflammatory contexts is insulin
39 resistance in target tissues, including muscle (Kim et al., 2013; Mashili et al., 2013). However, it is
40 difficult to describe a general metabolic interaction between JAK/STAT and insulin signaling in
41 mammals, due to different effects at different developmental stages, differences between acute and
42 chronic actions, and the large number of JAKs and STATs present in mammalian genomes (Dodington
43 et al., 2018; Mavalli et al., 2010; Nieto-Vazquez et al., 2008; Vijayakumar et al., 2013).

44 The fruit fly *Drosophila melanogaster* has a single, well-conserved JAK-STAT signaling pathway. The
45 *unpaired* (*upd*) genes *upd1-3* encode the three known ligands for this pathway; they signal by binding
46 to a single common GP130-like receptor, encoded by *domeless* (*dome*) (Agaisse et al., 2003; Brown et
47 al., 2001; Chen et al., 2002). Upon ligand binding, the single JAK tyrosine kinase in *Drosophila*,
48 encoded by *hopscotch* (*hop*), is activated; Hop then activates the single known STAT, STAT92E, which
49 functions as a homodimer (Binari and Perrimon, 1994; Chen et al., 2002; Hou et al., 1996; Yan et al.,
50 1996). This signaling pathway plays a wide variety of functions, including segmentation of the early
51 embryo, regulation of hematopoiesis, maintenance and differentiation of stem cells in the gut, and
52 immune modulation (Amoyel and Bach, 2012; Myllymaki and Ramet, 2014). Importantly, several
53 recent studies indicate roles for *upd* cytokines in metabolic regulation; for example, the *upds* are
54 important nutrient-responsive signals in the adult fly (Beshel et al., 2017; Rajan and Perrimon, 2012;
55 Woodcock et al., 2015; Zhao and Karpac, 2017).

56 Here, we identify a physiological requirement for Dome signaling in adult muscle. We observe that
57 adult muscles show significant JAK/STAT signaling activity in the absence of obvious immune
58 challenge and macrophages seem to be a source of this signal. Inactivation of *dome* on adult muscles
59 significantly reduces lifespan and causes muscular pathology and physiological dysfunction; these
60 result from remarkably strong AKT hyperactivation and consequent dysregulation of metabolism. We
61 thus describe a new role for JAK/STAT signaling in adult *Drosophila* muscle with critical importance in
62 healthy metabolic regulation.

63 Results

64 *dome* is required in adult muscle

65 To find physiological functions of JAK/STAT signaling in the adult fly, we identified tissues with basal
66 JAK/STAT pathway activity using a STAT-responsive GFP reporter (*10xSTAT92E-GFP*) (Bach et al.,

67 2007). The strongest reporter activity we observed was in legs and thorax. We examined flies also
68 carrying a muscle myosin heavy chain RFP reporter (*MHC-RFP*) and observed co-localization of GFP
69 and RFP expression in the muscles of the legs, thorax and body wall (Fig S1A). We observed strong,
70 somewhat heterogeneous reporter expression in all the muscles of the thorax and the legs, with
71 strong expression in various leg and jump muscles and apparently weaker expression throughout the
72 body wall muscles and indirect flight muscles (Fig 1A).

73 *dome* encodes the only known *Drosophila* STAT-activating receptor. To investigate the physiological
74 role of this signal, we expressed *dome^Δ*, a dominant-negative version of Dome lacking the
75 intracellular signaling domain, with a temperature-inducible muscle specific driver line (*w;tubulin-*
76 *Gal80^{ts};24B-Gal4*) (Fig S1B) (Brown et al., 2001). Controls (*24B-Gal80^{ts}/+*) and experimental flies (*24B-*
77 *Gal80^{ts}>dome^Δ*) were raised at 18° until eclosion to permit Dome activity during development. Flies
78 were then shifted to 29° to inhibit Dome activity and their lifespan was monitored. Flies with Dome
79 signaling inhibited in adult muscles were short-lived (Fig 1B, Fig S1C). This effect was also observed,
80 more weakly, in flies kept at 25° (Fig S1D). Upd-JAK-STAT signaling is important to maintain gut
81 integrity, and defects in gut integrity often precede death in *Drosophila*; however, our flies did not
82 exhibit loss of gut integrity (Fig S1E) (Jiang et al., 2009; Rera et al., 2012). To determine whether
83 Dome inhibition caused meaningful physiological dysfunction, we assayed climbing activity in *24B-*
84 *Gal80^{ts}/+* control flies and *24B-Gal80^{ts}>dome^Δ* flies. *24B-Gal80^{ts}>dome^Δ* flies showed significantly
85 impaired climbing compared to controls (Fig 1C). Adult muscle-specific expression of *dome^Δ* with a
86 second Gal4 line (*w;tub-Gal80^{ts};Mef2-Gal4*) gave a similar reduction in lifespan and decline in
87 climbing activity, confirming that the defect resulted from a requirement for Dome activity in muscle
88 (Fig S1F, G).

89 Impaired muscle function is sometimes accompanied by lipid accumulation (Baik et al., 2017).
90 Therefore, we stained thorax muscles with the neutral lipid dye LipidTox. In 14 day old flies, we
91 detected numerous small neutral lipid inclusions in several muscles, including the large jump muscle
92 (TTM), of *24B-Gal80^{ts}>dome^Δ* flies (Fig 1D).

93 **Muscle *dome* activity is required for normal systemic homeostasis**

94 Having observed lipid inclusions in adult muscles, we analysed the systemic metabolic state of *24B-*
95 *Gal80^{ts}>dome^Δ* flies. We observed significant reductions in total triglyceride, glycogen and free sugar
96 (glucose + trehalose) in these animals (Fig 1E, F). The reduction in free sugar was not detectable in
97 any dissected solid tissue, suggesting that it was due to a reduction in hemolymph sugar (Fig 1G).

98 Reduced hemolymph sugar could result from increased tissue glucose uptake. In this case, it should
99 be reflected in an increased metabolic stores or metabolic rate. Since metabolic stores were
100 decreased in our flies, we tested metabolic rate by measuring respiration. CO₂ production and O₂
101 consumption were both significantly increased in *24B-Gal80^{ts}>dome^Δ* flies, indicating an overall
102 increase in metabolic rate (Fig 1H).

103 ***dome* acts via *hop* to regulate AKT activity with little effect on other nutrient signaling pathways**

104 The observed metabolic changes imply differences in activity of nutrient-regulated signaling
105 pathways in *24B-Gal80^{ts}>dome^Δ* flies. Several signaling pathways respond to nutrients, or their
106 absence, to coordinate energy consumption and storage (Britton et al., 2002; Lizcano et al., 2003;
107 Ulgherait et al., 2014). Of these, insulin signaling via AKT is the primary driver of sugar uptake by
108 peripheral tissues.

109 We examined the activity of these signaling mechanisms in legs (a tissue source strongly enriched in
110 muscle) from *24B-Gal80^{ts}>dome^Δ* flies. We found an extremely strong increase in abundance of the

111 60-kDa form of total and activated (S505-phosphorylated) AKT (Fig 1I, J). This change was also seen in
112 legs from *Mef2-Gal80^{ts}>dome^Δ* flies, confirming that *dome* functions in muscles (Fig S1H, I). We also
113 saw this effect in flies carrying a different insertion of the *dome^Δ* transgene, under the control of a
114 third muscle-specific driver, *MHC-Gal4*, though the effect was weaker (Fig S1J). These *MHC-*
115 *Gal4>dome^Δ (II)* animals were also short-lived relative to controls (Fig S1K).

116 Elevated total AKT could result from increased transcript abundance or changes in protein
117 production or stability. We distinguished between these possibilities by assaying *Akt1* mRNA; *Akt1*
118 transcript levels were elevated in *24B-Gal80^{ts}>dome^Δ* muscle, but only by about 75%, suggesting that
119 the large effect on AKT protein abundance must be, at least in part, post-transcriptional (Fig S1L).
120 Similarly, AKT hyperactivation could be driven by insulin-like peptide overexpression; however, we
121 assayed the expression of *Ilp2-7* in whole flies and observed that none of these peptides were
122 significantly overexpressed (Fig S1M-R).

123 Unlike AKT, the amino-acid-responsive TORC1/S6K and the starvation-responsive AMPK pathway
124 showed no significant difference in activity in *24B-Gal80^{ts}>dome^Δ* flies (Fig 1K, L). However, flies with
125 AMPK knocked down in muscle did exhibit mild AKT hyperactivation (Fig S2A).

126 To identify signaling mediators acting between Dome and AKT, we first tested activity of the MAPK-
127 ERK pathway, which can act downstream of the JAK kinase Hop (Luo et al., 2002). We found an
128 insignificant reduction in ERK activity in *24B-Gal80^{ts}>dome^Δ* flies (Fig 1M). We then assayed survival
129 and AKT activity in flies with *hop* (JAK), *Dsor1* (MEK) and *rl* (ERK) knocked down in adult muscle. *rl*
130 and *Dsor1* knockdown gave mild or no effect on survival and pAKT (Fig S2B, C). In contrast, *hop*
131 knockdown phenocopied the milder *dome^Δ* transgene with regard to survival and pAKT (Fig S2D, E).

132 We further analysed the requirement for *hop* in muscle *dome* signaling by placing *24B-Gal80^{ts}>dome^Δ*
133 on a genetic background carrying the viable gain-of-function allele *hop^{Tum-1}*. Flies carrying *hop^{Tum-1}*
134 alone exhibited no change in lifespan, AKT phosphorylation, or muscle lipid deposition (Fig 2A-C).
135 However, *hop^{Tum-1}* completely rescued lifespan and pAKT levels in *24B-Gal80^{ts}>dome^Δ* flies (Fig 2D, E),
136 indicating that the physiological activity of muscle Dome is mediated via Hop and that this signal is
137 required, but not sufficient, to control muscle AKT activity.

138 **Increased AKT activity causes the effects of *dome* inhibition**

139 The phenotype of *24B-Gal80^{ts}>dome^Δ* flies is similar to that previously described in flies with loss of
140 function in *Pten* or *foxo* (Demontis and Perrimon, 2010; Mensah et al., 2015), suggesting that AKT
141 hyperactivation might cause the *dome* loss of function phenotype; however, to our knowledge, direct
142 activation of muscle AKT had not previously been analysed. We generated flies with inducible
143 expression of activated AKT (*myr-AKT*) in adult muscles (*w;tubulin-Gal80^{ts}/+;24B-Gal4/UAS-myr-AKT*
144 [*24B-Gal80^{ts}>myr-AKT*]) (Stocker et al., 2002). These animals phenocopied *24B-Gal80^{ts}>dome^Δ* flies
145 with regard to lifespan, climbing activity, metabolite levels, metabolic rate, and muscle lipid
146 deposition (Fig 3A-F).

147 We concluded that AKT hyperactivation could cause the pathologies seen in *24B-Gal80^{ts}>dome^Δ* flies.
148 We next tested whether reducing AKT activity could rescue *24B-Gal80^{ts}>dome^Δ* flies. We generated
149 flies carrying muscle-specific inducible dominant negative dome (*UAS-dome^Δ*) with dsRNA against
150 *Akt1* (*UAS-AKT-IR*). These flies showed significantly longer lifespan than *24B-Gal80^{ts}>dome^Δ* and *24B-*
151 *Gal80^{ts}>AKT-IR* flies, similar to all control genotypes analyzed (Fig 3G). Dome and AKT antagonism
152 synergised to control the mRNA level of *dome* itself, further suggesting strong mutual antagonism
153 between these pathways (Fig S3A).

154 AKT hyperactivation should reduce FOXO transcriptional activity. To test whether this loss of FOXO
155 activity caused some of the pathologies observed in *24B-Gal80^{ts}>dome^Δ* flies, we increased *foxo* gene
156 dosage by combining *24B-Gal80^{ts}>dome^Δ* with a transgene carrying a FOXO-GFP fusion protein under
157 the control of the endogenous *foxo* regulatory regions. These animals exhibited rescue of
158 physiological defects and lifespan compared to *24B-Gal80^{ts}>dome^Δ* flies (Fig 3H-J). They also
159 exhibited increased *dome* expression (Fig S3B). The effects of these manipulations on published *foxo*
160 target genes were mixed (Fig S3B); the strongest effect we observed was that Dome blockade
161 increased *upd2* expression, consistent with the observation that FOXO activity inhibits *upd2*
162 expression in muscle (none of the other genes tested have been shown to be FOXO targets in
163 muscle) (Zhao and Karpac, 2017). This may explain some of the systemic effects of Dome blockade.

164 The effect of the *foxo* transgene was stronger than expected from a 1.5-fold increase in *foxo*
165 expression, so we further explored the relationship between FOXO protein expression and AKT
166 phosphorylation. We found that *24B-Gal80^{ts}>dome^Δ* markedly increased FOXO-GFP abundance, so
167 that the increase in total FOXO was much greater than 1.5-fold (Fig S3C). This drove an apparent
168 feedback effect, restoring AKT in leg samples of *foxo^{GFP};24B-Gal80^{ts}>dome^Δ* flies to near-normal levels
169 (Fig S3D).

170 **Macrophages are a relevant source of *upd* signals**

171 Plasmatocytes—*Drosophila* macrophages—are a key source of *upd3* in flies on high fat diet and in
172 mycobacterial infection (Péan et al., 2017; Woodcock et al., 2015). Plasmatocytes also express *upd1-*
173 *3* in unchallenged flies (Chakrabarti et al., 2016). We thus tested their role in activation of muscle
174 Dome.

175 We found plasmatocytes close to STAT-GFP-positive leg muscle (Fig 4A, B). This, and the prior
176 published data, suggested that plasmatocytes might produce relevant levels of *dome*-activating
177 cytokines in steady state. We then overexpressed *upd3* in plasmatocytes and observed a potent
178 increase in muscle STAT-GFP activity (Fig 4C), confirming that plasmatocyte-derived *upd* signals were
179 able to activate muscle Dome.

180 To determine the physiological relevance of plasmatocyte-derived signals, we assayed STAT-GFP
181 activity in flies in which plasmatocytes had been depleted by expression of the pro-apoptotic gene
182 *reaper* (*rpr*) using a temperature-inducible plasmatocyte-specific driver line (*w;tub-Gal80^{ts};crq-Gal4*).
183 STAT-GFP fluorescence and GFP abundance were reduced in legs of plasmatocyte-depleted flies (*crq-*
184 *Gal80^{ts}>rpr*) compared to controls (*crq-Gal80^{ts}/+*) (Fig 4D, E). Activity was not eliminated, indicating
185 that plasmatocytes are not the only source of muscle STAT-activating signals.

186 We then examined the lifespan of flies in which we had depleted plasmatocytes in combination with
187 various *upd* mutations and knockdowns. Plasmatocyte depletion gave animals that were short-lived
188 (Fig 4F). (This effect was different from that we previously reported, possibly due to changes in fly
189 culture associated with an intervening laboratory move (Woodcock et al., 2015).) The lifespan of
190 these animals was further reduced by combining plasmatocyte depletion with null mutations in *upd2*
191 and *upd3*; plasmatocyte-replete *upd2 upd3* mutants exhibited near-normal lifespan (Fig 4F).
192 Similarly, plasmatocyte depletion drove muscle lipid accumulation, and *upd2 upd3* mutation
193 synergised with plasmatocyte depletion to further increase muscle lipid (Fig 4G). However, depleting
194 plasmatocytes in *upd2 upd3* mutants failed to recapitulate the effects of muscle Dome inhibition on
195 whole-animal triglyceride, free sugar, and glycogen levels (Fig S4A, B). This could be due to
196 antagonistic effects of other plasmatocyte-derived signals.

197 We attempted to pinpoint a specific Upd as the relevant physiological ligand by examining STAT-GFP
198 activity, first testing mutants in *upd2* and *upd3* because *upd1* mutation is lethal. However, these

199 mutants, including the *upd2 upd3* double-mutant, were apparently normal (Fig S4C). We then tested
200 plasmatocyte-specific knockdown of *upd1* and *upd3*; these animals were also essentially normal (Fig
201 S4D), and plasmatocyte *upd1* knockdown did not reduce lifespan (Fig 4H). However, plasmatocyte-
202 specific *upd1* knockdown gave significant compensating increases in expression of *upd2* and *upd3*
203 (Fig 4I). In keeping with this, combining plasmatocyte-specific *upd1* knockdown with mutations in
204 *upd2* and *upd3* reduced lifespan (Fig 4J) and also reduced STAT-GFP activity in these flies (Fig S4F).

205 Our results indicate that plasmatocytes are an important physiological source of the Upd signal
206 driving muscle Dome activity in healthy flies, and suggest that *upd1* may be the primary relevant
207 signal in healthy animals. However, plasmatocytes are not the only relevant source of signal, and Upd
208 mutual regulation prevents us from pinpointing a single responsible signal.

209 Discussion

210 Here we show that *upd-dome* signaling in muscle acts via AKT to regulate physiological homeostasis
211 in *Drosophila*. Loss of Dome activity in adult muscles shortens lifespan and promotes local and
212 systemic metabolic disruption. Dome specifically regulates the level and activity of AKT; AKT hyper-
213 activation mediates the observed pathology. Plasmatocytes are a primary source of the cytokine
214 signal. In healthy adult flies, insulin-like peptides are the primary physiological AKT agonists. The
215 effect we observe thus appears to be an example of a cytokine-Dome-JAK signal that impairs insulin
216 function to permit healthy physiology.

217 Our work fits into a recent body of literature demonstrating key physiological roles for JAK-STAT
218 activating signals in *Drosophila*. Upd1 acts locally in the brain to regulate feeding and energy storage
219 by altering the secretion of neuropeptide F (NPF) (Beshel et al., 2017). Upd2 is released by the fat
220 body in response to dietary triglyceride and sugar to regulate secretion of insulin-like peptides (Rajan
221 and Perrimon, 2012). More recently, muscle-derived Upd2, under control of FOXO, has been shown
222 to regulate production of the glucagon-like signal Akh (Zhao and Karpac, 2017). Indeed, we observe
223 that *upd2* is upregulated in flies with Dome signaling blocked in muscle, possibly explaining some of
224 the systemic metabolic effects we observe. Plasmatocyte-derived Upd3 in flies on a high fat diet can
225 activate the JAK/STAT pathway in various organs including muscles and can promote insulin
226 insensitivity (Woodcock et al., 2015). Our observation that Upd signaling is required to control AKT
227 accumulation and thus insulin pathway activity in healthy adult muscle may explain some of these
228 prior observations and reveals a new role for macrophage-derived cytokine signaling in healthy
229 metabolic regulation.

230 Several recent reports have examined roles of JAK/STAT signaling in *Drosophila* muscle. In larvae,
231 muscle JAK/STAT signaling can have an effect opposite to the one we report, with pathway loss of
232 function resulting in reduced AKT activity (Yang and Hultmark, 2017). It is unclear whether this
233 difference represents a difference in function between developmental stages (larva vs adult) or a
234 difference between acute and chronic consequences of pathway inactivation. Roles in specific muscle
235 populations have also been described: for example, JAK/STAT signaling in adult visceral muscle
236 regulates expression of Vein, an EGF-family ligand, to control intestinal stem cell proliferation
237 (Buchon et al., 2010; Jiang et al., 2011); the role of this system in other muscles may be analogous,
238 controlling expression of various signals to regulate systemic physiology.

239 The roles of mammalian JAK/STAT signaling in muscle physiology are more complex, but exhibit
240 several parallels with the fly. In mice, early muscle-specific deletion of Growth Hormone Receptor
241 (GHR) causes several symptoms including insulin resistance, while adult muscle-specific GHR deletion
242 causes entirely different effects, including increased metabolic rate and insulin sensitivity on a high-
243 fat diet (Mavalli et al., 2010; Vijayakumar et al., 2013; Vijayakumar et al., 2012). GHR signals via
244 STAT5; STAT5 deletion in adult skeletal muscle promotes muscle lipid accumulation on a high-fat diet

245 (Baik et al., 2017). Other STAT pathways can also play roles. For example, the JAK-STAT activating
 246 cytokine IL-6, which signals primarily via STAT3, increases skeletal muscle insulin sensitivity when
 247 given acutely but can drive insulin resistance when provided chronically (Nieto-Vazquez et al., 2008).
 248 STAT3 itself can promote muscle insulin resistance (Kim et al., 2013; Mashili et al., 2013). The
 249 relationship between these effects and those we have shown here, and the mechanisms regulating
 250 plasmatocyte Upd production during healthy physiology, remain to be determined.

251

252 **Materials and methods**

253 ***Drosophila melanogaster* stocks and culture**

254 All fly stocks were maintained on food containing 10% w/v Brewer's yeast, 8% fructose, 2% polenta
 255 and 0.8% agar supplemented with propionic acid and nipagin. Crosses for experiments were
 256 performed at 18° (for crosses with temperature inducible gene expression) or 25°. Flies were shifted
 257 to 29° after eclosion where relevant.

258 Male flies were used for all experiments.

259 The following original fly stocks were used for crosses:

Fly stocks	Description and Origin
<i>w¹¹¹⁸; tubulin-Gal80^{ts}/SM6a;24B-Gal4/TM6c, Sb¹</i>	Temperature sensitive muscle specific driver line; 24B-Gal4 a gift of Nazif Alic
<i>w¹¹¹⁸; tubulin-Gal80^{ts}/SM6a;Mef2-Gal4/TM6c, Sb¹</i>	Temperature sensitive muscle specific driver line; Mef2-Gal4 a gift of Michael Taylor
<i>w¹¹¹⁸; ;UAS-dome^A/TM6c, Sb¹</i>	Line for expression of a dominant-negative <i>dome</i> , gift of James Castelli-Gair Hombría
<i>w¹¹¹⁸;UAS-dome^A/CyO</i>	Line for expression of a dominant-negative <i>dome</i> , gift of James Castelli-Gair Hombría
<i>w¹¹¹⁸; ;UAS-myr-AKT/TM6c, Sb¹</i>	Line for over-expression of a constitutive active (myristoylated) AKT, gift of Ernst Hafen
<i>w;UAS-AMPKα-IR</i>	VDRC KK106200
<i>w;UAS-AMPKβ-IR</i>	VDRC KK104489
<i>w;UAS-rl-IR</i>	VDRC KK109108
<i>w;UAS-Dsor1-IR</i>	VDRC KK102276
<i>w¹¹¹⁸;foxo^{GFP}</i>	Expresses GFP-tagged <i>foxo</i> fusion protein (genomic rescue construct inserted at AttP40). Bloomington <i>Drosophila</i> Stock Center (BDSC) 38644
<i>w;UAS-AKT-IR</i>	VDRC KK103703
<i>w¹¹¹⁸;10xSTAT92E-GFP</i>	STAT-GFP reporter line (Bach et al., 2007). BDSC #26197
<i>w¹¹¹⁸;MHC-Gal4,MHC-RFP/SM6a</i>	Muscle specific driver line and muscle specific reporter line. Derived from BDSC #38464
<i>w upd2^Δ upd3^Δ; ;</i>	Gift of Bruno Lemaitre
<i>w¹¹¹⁸; ;crq-Gal4/ TM6c, Sb¹</i>	Plasmatocyte specific driver line, gift of Nathalie Franc
<i>w¹¹¹⁸; tub-Gal80^{ts};TM2/ TM6c, Sb¹</i>	Line for ubiquitous expression of <i>Gal80^{ts}</i> , BDSC #7108
<i>w¹¹¹⁸; ;UAS-rpr/ TM6c, Sb¹</i>	Line for over-expression of the pro-apoptotic protein rpr. Derived from BDSC #5824
<i>w¹¹¹⁸;UAS-CD8-mCherry</i>	Line for overexpression of a CD8-mCherry

	fusion protein. Derived from BDSC #27391
<i>w¹¹¹⁸;;srpHemo-3xmCherry/TM6c, Sb¹</i>	Plasmatocyte reporter line
<i>w;UAS-hop-IR</i>	VDRC GD40037
<i>w;UAS-upd1-IR/SM6a</i>	VDRC GD3282
<i>w;UAS-upd3-IR</i>	VDRC GD6811
<i>w¹¹¹⁸;;UAS-upd3/ TM6c, Sb¹</i>	Line for overexpression of upd3, gift of Bruce Edgar
<i>w¹¹¹⁸;UAS-2xeGFP/ SM6a</i>	Line for expression of bicistronic GFP, BDSC #6874
<i>w¹¹¹⁸ hop^{Tum-L}/FM7h</i>	Gain-of function mutant of <i>hop</i> ; derived by backcrossing from BDSC 8492 onto our control <i>w¹¹¹⁸</i> background

260

261 Genotype abbreviations were used for the different experimental flies in this study, in the following
262 table the complete genotypes are indicated:

Genotype abbreviation of flies used in the manuscript	Complete genotype of flies used in the manuscript
<i>10xSTAT92E-GFP/MHC-RFP</i>	<i>w¹¹¹⁸;10xSTAT92E-GFP/MHC-Gal4,MHC-RFP</i>
<i>24B-Gal80^{ts}/+</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/+;24B-Gal4/+</i>
<i>24B-Gal80^{ts}>dome^Δ</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/+;24B-Gal4/UAS-dome^Δ</i>
<i>24B-Gal80^{ts}>myr-AKT</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/+;24B-Gal4/UAS-myr-AKT</i>
<i>24B-Gal80^{ts}>AMPKα-IR</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/UAS-AMPKα-IR;24B-Gal4/+</i>
<i>24B-Gal80^{ts}>AMPKβ-IR</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/UAS-AMPKβ-IR;24B-Gal4/+</i>
<i>24B-Gal80^{ts}>rl-IR</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/UAS-rl-IR;24B-Gal4/+</i>
<i>24B-Gal80^{ts}>Dsor1-IR</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/UAS-Dsor1-IR;24B-Gal4/+</i>
<i>24B-Gal80>hop-IR</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/UAS-hop-IR;24B-Gal4/+</i>
<i>hop^{tum-L};24B-Gal80>dome^Δ</i>	<i>w¹¹¹⁸ hop^{tum-L};tub-Gal80^{ts}/+;24B-Gal4/UAS-dome^Δ</i>
<i>24B-Gal80^{ts}>AKT-IR</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/UAS-AKT-IR;24B-Gal4/+</i>
<i>24B-Gal80^{ts}>AKT-IR;dome^Δ</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/UAS-AKT-IR;24B-Gal4/UAS-dome^Δ</i>
<i>MHC-Gal4/+</i>	<i>w¹¹¹⁸;MHC-Gal4,Mhc-RFP/+;</i>
<i>MHC-Gal4>dome^Δ (II)</i>	<i>w¹¹¹⁸;MHC-Gal4,MHC-RFP/UAS-dome^Δ;</i>
<i>foxo^{GFP};24B-Gal80^{ts}/+</i>	<i>w¹¹¹⁸;foxo^{GFP};tub-Gal80^{ts}/+;24B-Gal4/+</i>
<i>foxo^{GFP};24B-Gal80^{ts}>dome^Δ</i>	<i>w¹¹¹⁸;foxo^{GFP};tub-Gal80^{ts}/+;24B-Gal4/UAS-dome^Δ</i>
<i>UAS-dome^Δ/+</i>	<i>w¹¹¹⁸;;UAS-dome^Δ/+</i>
<i>UAS-AKT-IR/+</i>	<i>w¹¹¹⁸;UAS-AKT-IR/+;</i>
<i>UAS-AKT-IR;dome^Δ/+</i>	<i>w¹¹¹⁸;UAS-AKT-IR/+; UAS-dome^Δ/+</i>
<i>Mef2-Gal80^{ts}/+</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/+;Mef2-Gal4/+</i>
<i>Mef2-Gal80^{ts}>dome^Δ</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/+;Mef2-Gal4/UAS-dome^Δ</i>
<i>srpHemo-3xmCherry</i>	<i>w¹¹¹⁸;; srpHemo-3xmCherry/+</i>
<i>crq-Gal4/+</i>	<i>w¹¹¹⁸;;crq-Gal4/+</i>
<i>crq-Gal80^{ts}>rpr</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/+;crq-Gal4/UAS-rpr or</i> <i>w¹¹¹⁸;tub-Gal80^{ts}/+;crq-Gal4,UAS-CD8-mCherry,10xSTAT92E-GFP/UAS-rpr</i>
<i>crq-Gal80^{ts}/+</i>	<i>w¹¹¹⁸;tub-Gal80^{ts}/+;crq-Gal4/+ or</i> <i>w¹¹¹⁸;tub-Gal80^{ts}/+;crq-Gal4,UAS-CD8-mCherry,10xSTAT92E-GFP/+</i>
<i>crq-Gal4/+</i>	<i>w¹¹¹⁸;;crq-Gal4,UAS-CD8-mCherry,10xSTAT92E-GFP/+</i>

<i>crq-Gal4>upd1-IR</i>	<i>w¹¹¹⁸;UAS-upd1-IR/+;crq-Gal4,UAS-CD8-mCherry,10xSTAT92E-GFP/+</i>
<i>crq-Gal4>upd3-IR</i>	<i>w¹¹¹⁸;UAS-upd3-IR/+;crq-Gal4,UAS-CD8-mCherry,10xSTAT92E-GFP/+</i>
<i>crq-Gal4>upd3</i>	<i>w¹¹¹⁸;crq-Gal4,UAS-CD8-mCherry,10xSTAT92E-GFP/UAS-upd3</i>
<i>upd2^Δ upd3^Δ;crq-Gal80^{ts}/+</i>	<i>w upd2^Δ upd3^Δ;tub-Gal80^{ts}/+;crq-Gal4/+</i>
<i>upd2^Δ upd3^Δ;crq-Gal80^{ts}>rpr</i>	<i>w upd2^Δ upd3^Δ;tub-Gal80^{ts}/+;crq-Gal4/UAS-rpr</i>
<i>upd2^Δ upd3^Δ;upd1-IR/+</i>	<i>w upd2^Δ upd3^Δ;UAS-upd1-IR/+</i>
<i>upd2^Δ upd3^Δ;crq-Gal4/+</i>	<i>w upd2^Δ upd3^Δ;crq-Gal4/+</i>
<i>upd2^Δ upd3^Δ;crq-Gal4>upd1-IR</i>	<i>w upd2^Δ upd3^Δ;UAS-upd1-IR/+;crq-Gal4/+</i>
<i>MHC^{YFP};srpHemo-3xmCherry</i>	<i>w¹¹¹⁸;MHC^{YFP}/+;srpHemo-3xmCherry/+</i>
<i>10xSTAT92E-GFP;srpHemo-3xmCherry</i>	<i>w¹¹¹⁸;10xSTAT92E-GFP/+;srpHemo-3xmCherry/+</i>

263

264 **Lifespan/Survival assays**

265 Male flies were collected after eclosion and groups of 20–40 age-matched flies per genotype were
 266 placed together in a vial with fly food (a cohort size of 20 is sufficient to detect a lifespan effect size
 267 of about 5% at p=0.05 with 90% confidence). All survival experiments were performed at 29°. Dead
 268 flies were counted daily. Vials were kept on their sides to minimize the possibility of death from flies
 269 becoming stuck to the food, and flies were moved to fresh food twice per week. Flies were
 270 transferred into new vials without CO₂ anaesthesia.

271 **Negative Geotaxis Assay/Climbing Assay**

272 Male flies were collected after eclosion and housed for 14 days in age-matched groups of around 20.
 273 The assay was performed in the morning, when flies were most active. Flies were transferred without
 274 CO₂ into a fresh empty vial without any food and closed with the open end of another empty vial.
 275 Flies were placed under a direct light source and allowed to adapt to the environment for 20 min.
 276 Negative geotaxis reflex was induced by tapping the flies to the bottom of the tube and allowing
 277 them to climb up for 8 seconds. After 8 seconds the vial was photographed. This test was repeated 3
 278 times per vial with 1 min breaks in between. The height each individual fly had climbed was
 279 measured in Image J and the average between all three runs per vial calculated.

280 **Staining of thorax samples**

281 For immunofluorescent staining of thorax muscles, we anaesthetized flies and removed the head,
 282 wings and abdomen from the thorax. Thorax samples were pre-fixed for 1 hour in 4% PFA rotating at
 283 room temperature. Thoraces were then halved sagittally with a razor blade and fixed for another 30
 284 minutes rotating at room temperature. Samples were washed with PBS + 0.1% Triton X-100, then
 285 blocked for 1 h in 3% bovine serum albumin (BSA) in PBS + 0.1% Triton X-100.

286 For Lipid-Tox staining, samples were washed with PBS and stained for 2 hours at room temperature
 287 with HCS Lipid Tox Deep Red (Thermo Fisher #H34477; 1:200). For Phalloidin labelling, the samples
 288 were washed in PBS after fixation and stained for 2 hours at room temperature with Alexa Fluor 488
 289 Phalloidin (Thermo Fisher #A12379, 1:20). Afterwards the samples were washed once with PBS and
 290 mounted in Fluoromount-G. All mounted samples were sealed with clear nail polish and stored at 4°
 291 until imaging.

292 **Confocal microscopy**

293 Imaging was performed in the Facility for Imaging by Light Microscopy (FILM) at Imperial College
 294 London and in the Institute of Neuropathology in Freiburg. A Leica SP5 and SP8 microscope (Leica)
 295 were used for imaging, using either the 10x/NA0.4 objective, or the 20x/NA0.5 objective. Images
 296 were acquired with a resolution of either 1024x1024 or 512x512, at a scan speed of 400Hz. Averages
 297 from 3-4 line scans were used, sequential scanning was employed where necessary and tile scanning
 298 was used in order to image whole flies. For imaging of whole live flies, the flies were anaesthetized
 299 with CO₂ and glued to a coverslip. Flies were kept on ice until imaging. For measuring mean
 300 fluorescence intensity, a z-stack of the muscle was performed and the stack was projected in an
 301 average intensity projection. Next the area of the muscle tissue analyzed was defined and the mean
 302 fluorescent intensity within this area was measured. Images were processed and analysed using
 303 Image J.

304 **RNA isolation and Reverse Transcription**

305 For RNA extraction three whole flies or three thoraces were used per sample. After anaesthetisation,
 306 the flies were smashed in 100µl TRIzol (Invitrogen), followed by a chloroform extraction and
 307 isopropanol precipitation. The RNA pellet was cleaned with 70% ethanol and finally solubilized in
 308 water. After DNase treatment, cDNA synthesis was carried out using the First Strand cDNA Synthesis
 309 Kit (Thermo Scientific) and priming with random hexamers (Thermo Scientific). cDNA samples were
 310 further diluted and stored at -20° until analysis.

311 **Quantitative Real-time PCR**

312 Quantitative Real-time PCR was performed with Sensimix SYBR Green no-ROX (Bioline) on a Corbett
 313 Rotor-Gene 6000 (Corbett). The cycling conditions used throughout the study were as follows: Hold
 314 95° for 10 min, then 45 cycles of 95° for 15s, 59° for 30s, 72° for 30s, followed by a melting curve. All
 315 calculated gene expression values were measured in arbitrary units (au) according to diluted cDNA
 316 standards run in each run and for each gene measured. All gene expression values are further
 317 normalized to the value of the loading control gene, Rpl1, prior to further analysis.

318 The following primer sequences have been used in this study:

Gene name	Forward	Reverse
<i>Akt1</i>	5'-ctttgagattactgacaga-3'	5'-ggatgtcacctgaggcttg-3'
<i>Ilp2</i>	5'-atccccgattccaccacaag-3'	5'-gcggttccgatatcgagttta-3'
<i>Ilp3</i>	5'-caacgcaatgaccaagagaa-3'	5'-tgagcatctgaaccgaact-3'
<i>Ilp4</i>	5'-gagcctgattagactgggactg-3'	5'-tggaccggctgcagtaac-3'
<i>Ilp5</i>	5'-gccttgatggacatgctga-3'	5'-agctatccaaatccgcca-3'
<i>Ilp6</i>	5'-ccctggcgtatgtattcc-3'	5'-cacaatcggttacgttctgc-3'
<i>Ilp7</i>	5'-cacaccgaggagggtctc-3'	5'-caatatagctggcgacca-3'
<i>dome</i>	5'-cggacttctggtactccatc-3'	5'-accttgatgaggccaggat-3'
<i>upd1</i>	5'-gcacactgatttcgatacgg-3'	5'-ctgccgtggtgctgtttt-3'
<i>upd2</i>	5'-cggaacatcacgatgagcgaat-3'	5'-tcggcaggaactgtactcg-3'
<i>upd3</i>	5'-actgggagaacacctgcaat-3'	5'-gcccgtttggttctgtagat-3'
<i>Pepck1</i>	5'-ggataaggtggacgtgaag-3'	5'-acctctcgaccagaact-3'
<i>Thor</i>	5'-caggaaggtgtcatctcgga-3'	5'-ggagtggaggtagagggtt-3'
<i>InR</i>	5'-gcaccattataaccggaacc-3'	5'-ttaattcatccatgacgtgagc-3'
<i>Rpl1</i>	5'-tccaccttgaagaagggcta-3'	5'-ttcggatctcctcagactt-3'

319

320 **Smurf Assay**

321 Smurf assays with blue-coloured fly food were performed to analyse gut integrity in different
322 genotypes. Normal fly food, as described above, was supplemented with 0.1% Brilliant Blue FCF
323 (Sigma Aldrich). Experimental flies were placed on the blue-coloured fly food at 9AM and kept on the
324 food for 2 h at 29°. After 2 h the distribution of the dye within the fly was analysed for each
325 individual. Flies without any blue dye were excluded, flies with a blue gut or crop were identified as
326 “non-smurf” and flies which turned completely blue or showed distribution of blue dye outside the
327 gut were classified as “smurf”.

328 **Western Blot**

329 Dissected legs or thoraces from three flies were used per sample and smashed in 75µl 2x Laemmli
330 loading buffer (100 mM Tris [pH 6.8], 20% glycerol, 4% SDS, 0.2 M DTT). Samples were stored at -80°
331 until analysis. 7.5µl of this lysate were loaded per lane. Blue pre-stained protein standard (11-
332 190kDa) (New England Biolabs) was used. Protein was transferred to nitrocellulose membrane (GE
333 Healthcare). Membrane was blocked in 5% milk in TBST (TBS + 0.1% Tween-20). The following
334 primary antibodies were used: anti-phospho(Ser505)-AKT (Cell Signal Technology (CST) 4054,
335 1:1,000), anti-AKT (CST 4691, 1:1,000), anti-phospho(Thr172)-AMPKα (CST 2535, 1:1,000), anti-
336 phospho(Thr389)-p70 S6 kinase (CST 9206, 1:1,000), anti-GFP (CST 2956, 1:1,000), anti-phospho-
337 p44/42 MAPK (Erk1/2) (CST 4370, 1:1,000) and anti-α-tubulin (clone 12G10, Developmental Studies
338 Hybridoma Bank, used as an unpurified supernatant at 1:3,000; used as a loading control for all
339 blots). Primary antibodies were diluted in TBST containing 5% BSA and incubated over night at 4°.
340 Secondary antibodies were HRP anti-rabbit IgG (CST 7074, 1:5,000) and HRP anti-mouse IgG (CST
341 7076, 1:5,000). Proteins were detected with Supersignal West Pico Chemiluminescent Substrate
342 (Thermo Scientific) or Supersignal West Femo Chemiluminescent Substrate (Thermo Scientific) using
343 a LAS-3000 Imager (Fujifilm). Bands were quantified by densitometry using Image J. Quantifications
344 reflect all experiments performed; representative blots from single experiments are shown.

345 **Thin Layer Chromatography (TLC) for Triglycerides**

346 Groups of 10 flies were used per sample. After CO₂ anaesthesia the flies were placed in 100µl of ice-
347 cold chloroform:methanol (3:1). Samples were centrifuged for 3 min at 13,000 rpm at 4°, and then
348 flies were smashed with pestles followed by another centrifugation step. A set of standards were
349 prepared using lard (Sainsbury’s) in chloroform:methanol (3:1) for quantification. Samples and
350 standards were loaded onto a silica gel glass plate (Millipore), and a solvent mix of hexane:ethyl
351 ether (4:1) was prepared as mobile phase. Once the solvent front reached the top of the plate, the
352 plate was dried and stained with an oxidising staining reagent containing ceric ammonium
353 heptamolybdate (CAM) (Sigma Aldrich). For visualization of the oxidised bands, plates were baked at
354 80° for 20 min. Baked plates were imaged with a scanner and triglyceride bands were quantified by
355 densitometry according to the measured standards using Image J.

356 **Measurement of Glucose, Trehalose and Glycogen**

357 5-7 day old male flies, kept at 29°, were used for the analysis. Flies were starved for 1 hr on 1% agar
358 supplemented with 2% phosphate buffered saline (PBS) at 29° before being manually smashed in
359 75µl TE + 0.1% Triton X-100 (Sigma Aldrich). 3 flies per sample were used. For measuring thorax,
360 head and abdomen samples, flies were first anaesthetized with CO₂. Afterwards, they were quickly
361 transferred to 1xPBS and the head was cut off. The guts were carefully removed from thorax and
362 abdomen and thorax were separated from each other. Afterwards the body parts were rinsed with
363 1xPBS before smashing them in 75µl TE + 0.1% Triton X-100. All samples were incubated at 75° for
364 20 min and stored at -80°. Samples were thawed prior to measurement and incubated at 65° for 5
365 min to inactivate fly enzymes. A total of 10µl per sample was loaded for different measurements into
366 flat-bottom 96-well tissue culture plates. Each fly sample was measured four times, first diluted in

367 water for calculation of background fly absorbance, second with glucose reagent (Sentinel
368 Diagnostics) for the measurement of free glucose, third with glucose reagent plus trehalase (Sigma
369 Aldrich) for trehalose measurement, and fourth with glucose reagent plus amyloglucosidase (Sigma
370 Aldrich) for glycogen measurement. Plates were then incubated at 37° for 1 h before reading with a
371 microplate reader (biochrom) at 492 nm. Quantities of glucose, trehalose and glycogen were
372 calculated according to measured standards.

373 **Respirometry**

374 Respiration in flies was measured using a stop-flow gas-exchange system (Q-Box RP1LP Low Range
375 Respirometer, Qubit Systems, Ontario, Canada, K7M 3L5). Ten flies from each genotype were put
376 into an airtight glass tube and supplied with our standard fly food via a modified pipette tip. Each
377 tube was provided with CO₂-free air while the 'spent' air was concurrently flushed through the
378 system and analysed for its CO₂ and O₂ content. All vials with flies were normalized to a control vial
379 with food but no flies inside. In this way, evolved CO₂ per chamber and consumed O₂ per chamber
380 were measured for each tube every ~ 44 min (the time required to go through each of the vials in
381 sequence)

382 **Statistical analysis and handling of data**

383 For real-time quantitative PCR, TLCs, MFI quantification, western blot quantifications and
384 colorimetric measurements for glucose, trehalose and glycogen levels an unpaired t-test was used to
385 calculate statistical significance for all experiments. Respirometer data was analysed with a Mann-
386 Whitney test. Lifespan/ Survival assays, where analysed with the Log-Rank and Wilcoxon test. Stars
387 indicate statistical significance as followed: * p<0.05, ** p<0.01 and *** p<0.001. All statistical tests
388 were performed with Excel or GraphPad Prism software.

389 All replicates are biological. No outliers were omitted, and all replicates are included in quantitations
390 (including in cases where a single representative experiment is shown). Flies were allocated into
391 experimental groups according to their genotypes. Masking was not used. For survival experiments,
392 typically, the 50% of flies that eclosed first from a given cross were used for an experiment. For
393 smaller-scale experiments, flies were selected randomly from those of a given age and genotype.

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407 **Author Contributions**

408 MSD and KK designed the project and wrote the manuscript. KK, FH, JS, CMV, PU, AG, DES and JD
409 constructed reagents, performed the experiments and analysed the experimental data.

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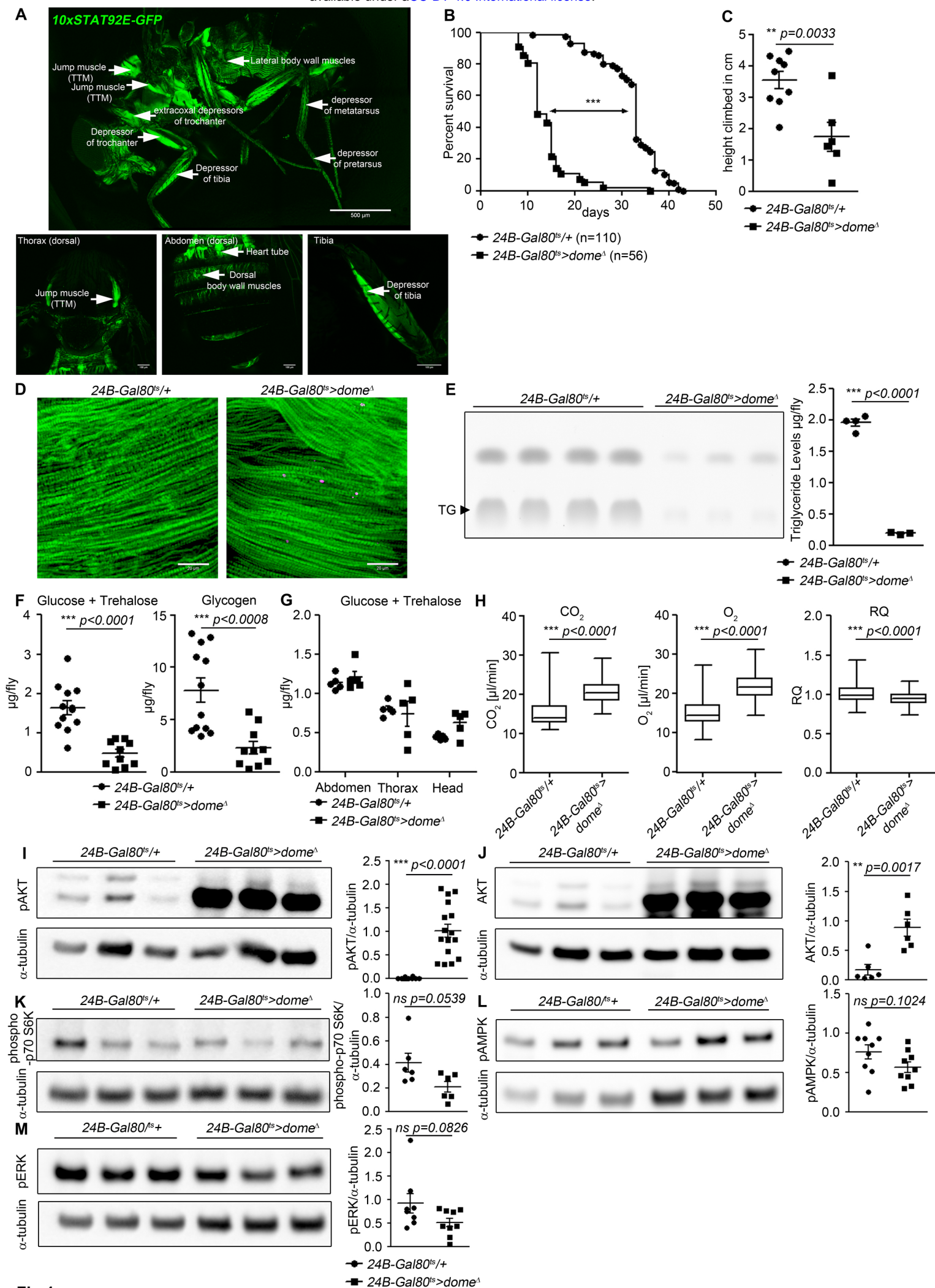


Fig 1

505 **Figure legends**

506 **Figure 1. Dome inhibition in adult muscle reduces lifespan, disrupts homeostasis, and causes AKT**
507 **hyperactivation.**

508 A) STAT activity in different muscles in 10xSTAT92E-GFP reporter fly. One fly out of 5 shown. Upper
509 panel: lateral view, Scale bar=500 μ m. Lower panels: dorsal thorax (left); dorsal abdomen (middle);
510 tibia (right), Scale bar=100 μ m

511 (B) Lifespan of *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>dome^Δ* at 29°, pooled from three independent
512 experiments. Log-Rank test: $\chi^2 = 166$, *** p<0.0001; Wilcoxon test: $\chi^2 = 157.7$, *** p<0.0001.

513 (C) Negative geotaxis assay of 14-day-old *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>dome^Δ* flies. Points
514 represent mean height climbed in individual vials (~20 flies/vial), pooled from three independent
515 experiments.

516 (D) Muscle (Phalloidin) and neutral lipid (LipidTox) of thorax samples from 14-day-old *24B-Gal80^{ts}/+*
517 and *24B-Gal80^{ts}>dome^Δ* flies. One representative fly per genotype is shown of six analysed. Scale
518 bar=50 μ m.

519 (E) Thin layer chromatography (TLC) of triglycerides in 7-day-old *24B-Gal80^{ts}/+* and *24B-*
520 *Gal80^{ts}>dome^Δ* flies, n=3-4 per genotype. One experiment of two is shown.

521 (F) Glucose and trehalose (left) and glycogen (right) in 7-day-old *24B-Gal80^{ts}/+* and *24B-*
522 *Gal80^{ts}>dome^Δ* flies, pooled from two independent experiments.

523 (G) Glucose and trehalose content of dissected abdomen, thorax, and head of 7-day-old *24B-*
524 *Gal80^{ts}/+* and *24B-Gal80^{ts}>dome^Δ* flies.

525 (H) CO₂ produced, O₂ consumed, and RQ of 7-day-old *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>dome^Δ* flies. Box
526 plots show data from one representative experiment of three, with data collected from a 24 h
527 measurement pooled from 3-4 tubes per genotype with 10 flies/tube. P values from Mann-Whitney
528 test.

529 (I-M) Western blots of leg protein from 14-day-old *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>dome^Δ* flies.

530 (I) Phospho-AKT (S505). One experiment of four is shown.

531 (J) Total AKT. One experiment of two is shown.

532 (K) Phospho-p70 S6K (T398). One experiment of two is shown.

533 (L) Phospho-AMPK α (T173). One experiment of three is shown.

534 (M) Phospho-ERK (T202/Y204). One experiment of three is shown. P values in C, E, F, I-M from
535 unpaired T-test.

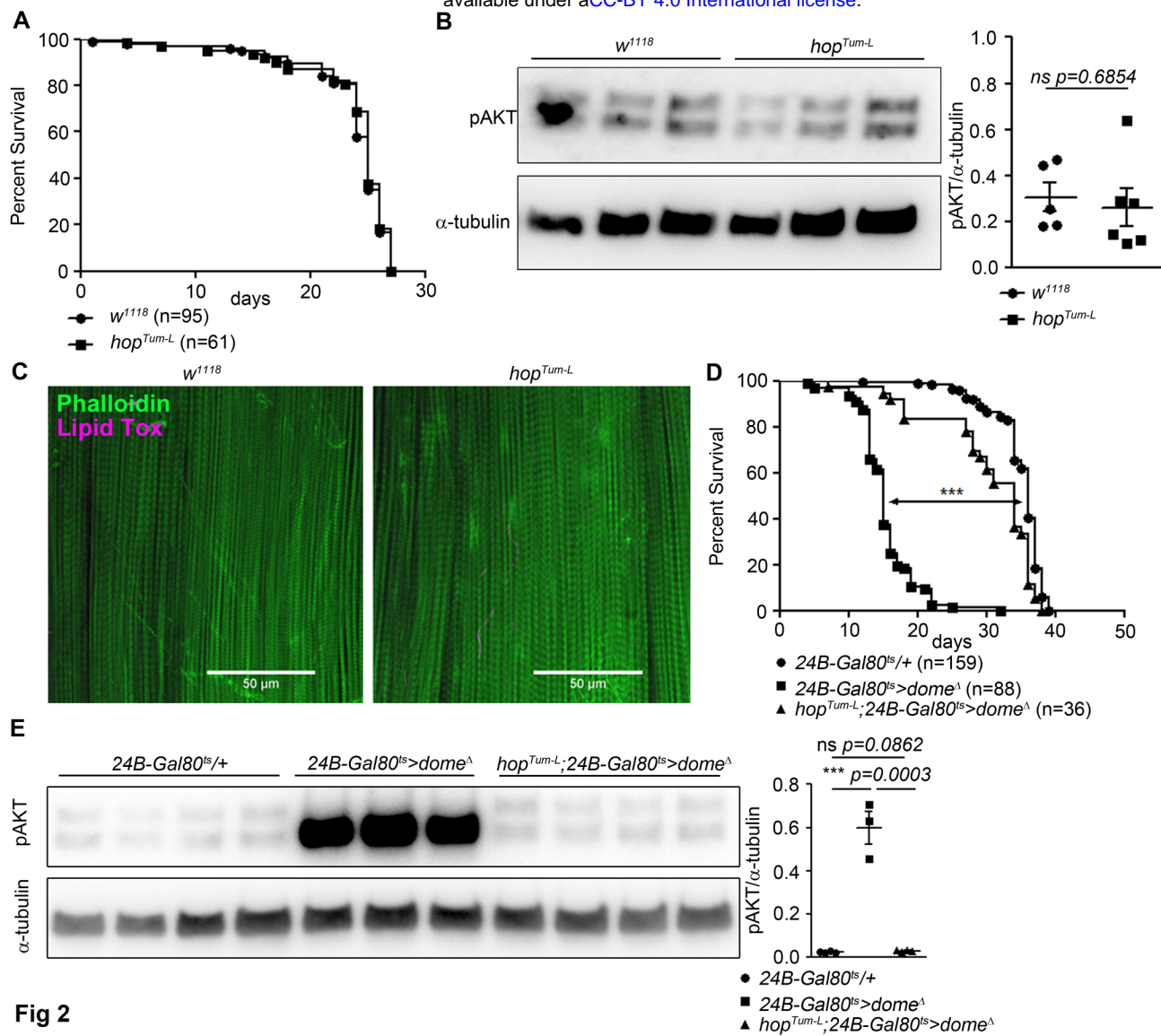


Fig 2

536 **Figure 2. Hop is required, but not sufficient, for Dome to control AKT.**

537 (A) Lifespan of w^{1118} and hop^{Tum-L} flies at 29°, pooled from two independent experiments. Log-Rank
538 test: $\chi^2 = 0.3223$, ns $p = 0.5702$; Wilcoxon test: $\chi^2 = 0.4756$, ns $p = 0.4906$.

539 (B) Phospho-AKT in leg samples from 14-day-old w^{1118} and hop^{Tum-L} flies. One experiment of two is
540 shown.

541 (C) Actin (Phalloidin) and neutral lipid (LipidTox) in flight muscle from 14-day-old w^{1118} and hop^{Tum-L}
542 flies. One representative fly shown of six analysed per genotype. Scale bar = 50 μm .

543 (D) Lifespan of $24B-Gal80^{ts}/+$, $24B-Gal80^{ts}>dome^{\Delta}$, and $hop^{Tum-L};24B-Gal80^{ts}>dome^{\Delta}$ flies at 29°,
544 pooled from four independent experiments. Log-Rank test ($24B-Gal80^{ts}/+$ vs. $24B-Gal80^{ts}>dome^{\Delta}$): χ^2
545 = 319.4, *** $p < 0.0001$; Wilcoxon test ($24B-Gal80^{ts}/+$ vs. $24B-Gal80^{ts}>dome^{\Delta}$): $\chi^2 = 280.2$, *** $p < 0.0001$.
546 Log-Rank test ($24B-Gal80^{ts}/+$ vs. $hop^{Tum-L} 24B-Gal80^{ts}>dome^{\Delta}$): $\chi^2 = 18.87$, *** $p < 0.0001$; Wilcoxon test
547 ($24B-Gal80^{ts}/+$ vs. $hop^{Tum-L} 24B-Gal80^{ts}>dome^{\Delta}$): $\chi^2 = 20.83$, *** $p < 0.0001$.

548 (E) Phospho-AKT in leg samples from 14-day-old $24B-Gal80^{ts}/+$, $24B-Gal80^{ts}>dome^{\Delta}$ and $hop^{Tum-L};24B-$
549 $Gal80^{ts}>dome^{\Delta}$ flies. P values in B, E from unpaired T-test.

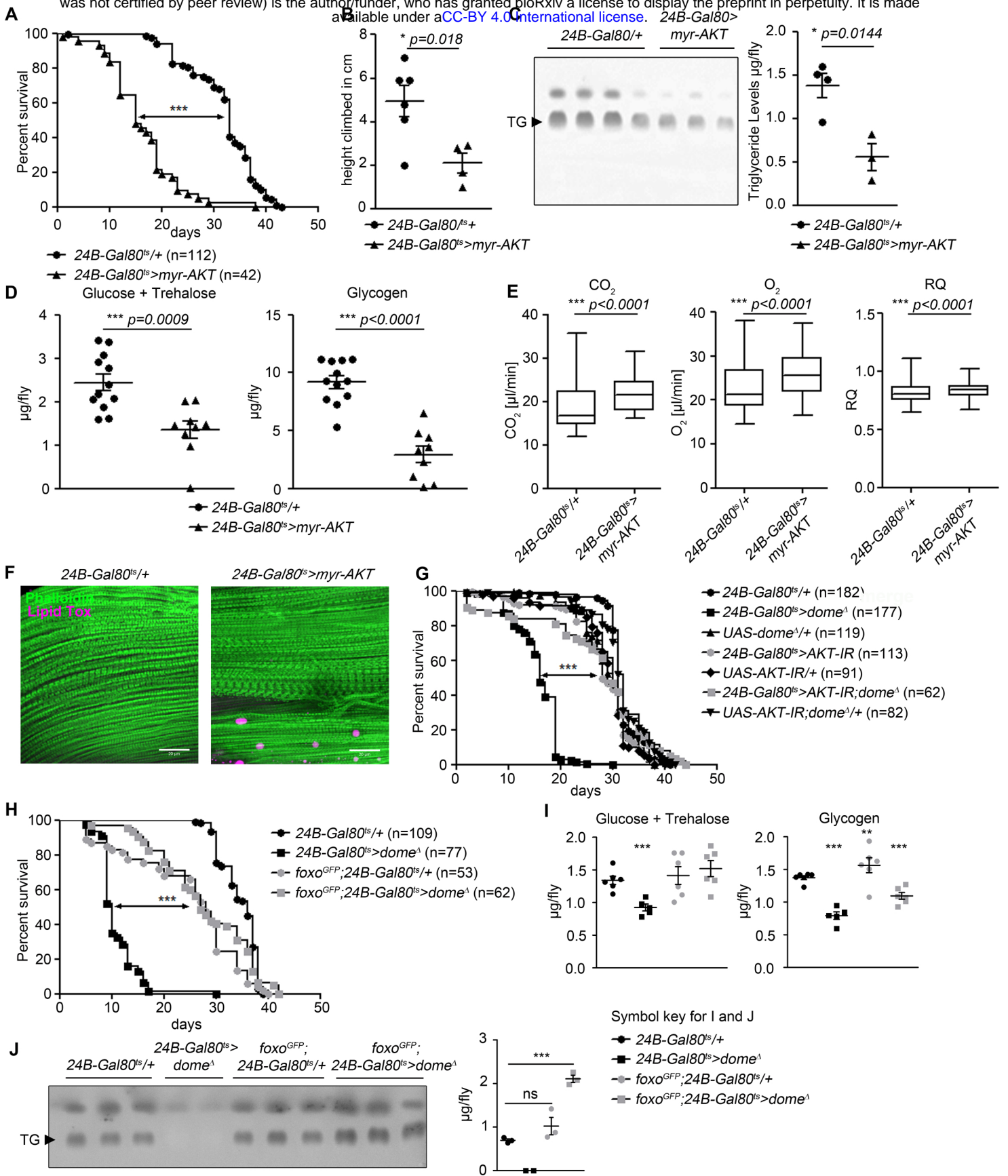


Fig 3

550 **Figure 3. AKT hyperactivation causes pathology in *24B-Gal80^{ts}>dome^Δ* flies.**

551 (A) Lifespan of *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>myr-AKT* at 29°, pooled data from three independent
552 experiments. Log-Rank test: $\chi^2 = 115.5$, *** $p < 0.0001$; Wilcoxon test: $\chi^2 = 123.6$, *** $p < 0.0001$.

553 (B) Negative geotaxis assay of 14-day-old *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>myr-AKT* flies. Points
554 represent mean height climbed in individual vials (~20 flies/vial), pooled from two independent
555 experiments.

556 (C) TLC of triglycerides in 7-day-old *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>myr-AKT* flies, n=3-4 per genotype.
557 One experiment of two is shown.

558 (D) Glucose and trehalose (left panel) and glycogen (right panel) in 7-day-old *24B-Gal80^{ts}/+* (n=12)
559 and *24B-Gal80^{ts}>myr-AKT* (n=9) flies, pooled from two independent experiments.

560 (E) CO₂ produced, O₂ consumed, and RQ of 7-day-old *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>myr-AKT* flies.
561 Box plots show data from one representative experiment of three, with data points collected from a
562 24 h measurement pooled from 3-4 tubes per genotype with 10 flies/tube. P values from Mann-
563 Whitney test.

564 (F) Phalloidin and LipidTox staining of thorax samples from 14-day-old *24B-Gal80^{ts}/+* and *24B-*
565 *Gal80^{ts}>myr-AKT* flies. One representative fly per genotype is shown of 3 analysed per group in 2
566 independent experiments. Scale bar=50μm.

567 (G) Lifespan of *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>dome^Δ*, *UAS-dome^Δ/+*, *24B-Gal80^{ts}>AKT-IR*, *UAS-AKT-IR/+*,
568 *24B-Gal80^{ts}>AKT-IR;dome^Δ* and *UAS-AKT-IR;dome^Δ/+* flies at 29°. One from four independent
569 experiments shown. Log-Rank test (*24B-Gal80^{ts}>dome^Δ* vs. *24B-Gal80^{ts}>AKT-IR;dome^Δ*): $\chi^2 = 101.0$,
570 *** $p < 0.0001$; Wilcoxon test (*24B-Gal80^{ts}>dome^Δ* vs. *24B-Gal80^{ts}>AKT-IR;dome^Δ*): $\chi^2 = 59.87$, ***
571 $p < 0.0001$.

572 (H) Lifespan of *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>dome^Δ*, *foxo-GFP;24B-Gal80^{ts}/+*, and *foxo-GFP;24B-*
573 *Gal80^{ts}>dome^Δ* flies at 29°, pooled from three independent experiments. Log-Rank test (*24B-*
574 *Gal80^{ts}>dome^Δ* vs. *foxo-GFP;24B-Gal80^{ts}>dome^Δ*): $\chi^2 = 114.0$, *** $p < 0.0001$; Wilcoxon test (*24B-*
575 *Gal80^{ts}>dome^Δ* vs. *foxo-GFP;24B-Gal80^{ts}>dome^Δ*): $\chi^2 = 93.59$, *** $p < 0.0001$. *24B-Gal80^{ts}/+* and *24B-*
576 *Gal80^{ts}>dome^Δ* controls in G and H are the same because a single survival experiment was split into
577 two graphs.

578 (I) Glucose + trehalose and glycogen in 7-day-old *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>dome^Δ*, *foxo-GFP;24B-*
579 *Gal80/+*, and *foxo-GFP; 24B-Gal80^{ts}>dome^Δ* flies.

580 (J) TLC of triglycerides in 7-day-old *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>dome^Δ*, *foxo-GFP;24B-Gal80^{ts}/+*, and
581 *foxo-GFP;24B-Gal80^{ts}>dome^Δ* flies. P values in B-D, I, J from unpaired T-test.

582

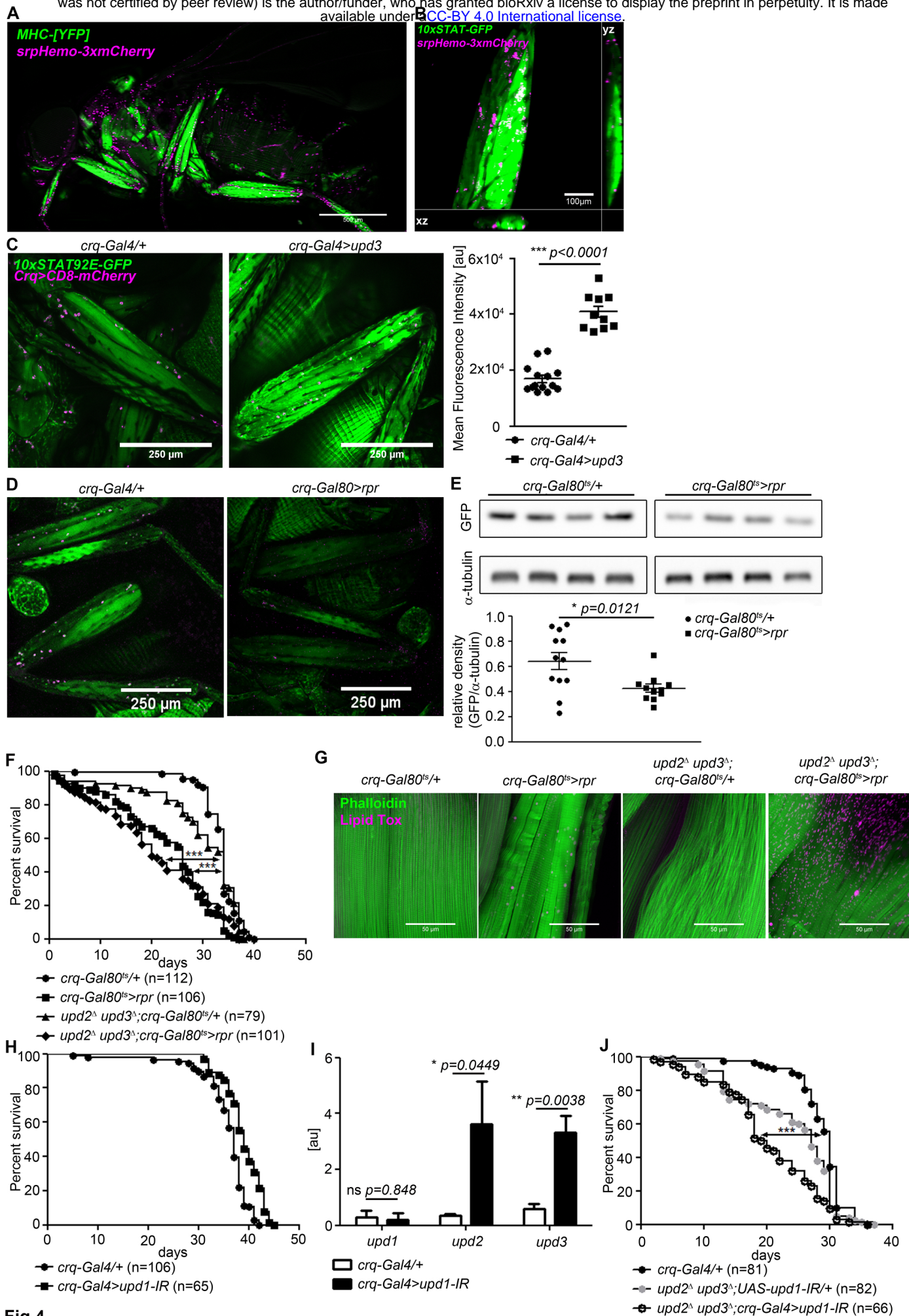


Fig 4

583 **Figure 4. Plasmatocytes promote muscle Dome activity.**

584 (A) Muscle (*MHC^{YFP}*) and plasmatocytes (*srpHemo-3xmCherry*) in 7-day-old flies. Plasmatocytes are
585 found in close proximity to adult muscles. One representative fly of 5 is shown. Scale bar=500 μ m.

586 (B) Legs and plasmatocytes in 7-day-old *10xSTAT92E-GFP;srpHemo-3xmCherry* flies. Muscle with high
587 JAK-STAT activity (green) is surrounded by plasmatocytes (magenta). One representative fly of 5 is
588 shown. Scale bar=100 μ m.

589 (C) STAT activity and plasmatocytes in legs from control (*10xSTAT92E-GFP;crq-Gal4>CD8-mCherry/+*)
590 and *upd3*-overexpressing (*10xSTAT92E-GFP;crq-4>CD8mCherry/UAS-upd3*) flies. One representative
591 fly of 10-14 is shown. Scale bar=100 μ m. Graph shows mean fluorescence intensity (MFI).

592 (D) STAT activity and plasmatocytes in legs from control (*10xSTAT92E-GFP;crq-Gal80^{ts}>CD8-*
593 *mCherry/+*) and plasmatocyte-depleted (*10xSTAT92E-GFP;crq-Gal80^{ts}>CD8mCherry/rpr*) flies. One
594 representative fly of six is shown. Scale bar=250 μ m.

595 (E) Western blot analysis of STAT-driven GFP in legs from 7-day-old control (*10xSTAT92E-GFP;crq-*
596 *Gal80^{ts}>CD8-mCherry/+*) and plasmatocyte-depleted (*10xSTAT92E-GFP;crq-Gal80^{ts}>CD8-mCherry/rpr*
597 flies). One representative experiment of three is shown. Graph shows STAT-GFP/ α -tubulin for control
598 (*crq-Gal80^{ts}/+*) and plasmatocyte-depleted (*crq-Gal80^{ts}>rpr*) leg samples.

599 (F) Lifespan of *crq-Gal80^{ts}/+*, *crq-Gal80^{ts}>rpr*, *upd2^Δ upd3^Δ;crq-Gal80^{ts}/+*, and *upd2^Δ upd3^Δ;crq-*
600 *Gal80^{ts}>rpr* flies at 29°; pooled data from three independent experiments shown. Log-Rank test (*crq-*
601 *Gal80^{ts}/+* vs. *crq-Gal80^{ts}>rpr*): $\chi^2 = 101.7$, *** $p < 0.0001$; Wilcoxon test (*crq-Gal80^{ts}/+* vs. *crq-*
602 *Gal80^{ts}>rpr*): $\chi^2 = 107.8$, *** $p < 0.0001$; Log-Rank test (*crq-Gal80^{ts}/+* vs. *upd2^Δ upd3^Δ;crq-Gal80^{ts}>rpr*):
603 $\chi^2 = 60.03$, *** $p < 0.0001$; Wilcoxon test (*crq-Gal80^{ts}/+* vs. *upd2^Δ upd3^Δ;crq-Gal80^{ts}>rpr*): $\chi^2 = 80.97$,
604 *** $p < 0.0001$.

605 (G) Actin (Phalloidin) and neutral lipid (LipidTox) in thorax samples from 14-day-old *crq-Gal80^{ts}/+*,
606 *crq-Gal80^{ts}>rpr*, *upd2^Δ upd3^Δ;crq-Gal80^{ts}/+*, and *upd2^Δ upd3^Δ;crq-Gal80^{ts}>rpr* flies. One
607 representative fly per genotype shown of 6 analysed per group. Scale bar=50 μ m.

608 (H) Lifespan of *crq-Gal4/+* and *crq-Gal4>upd1-IR* flies at 29°. Log-Rank test: $\chi^2 = 31.36$, *** $p < 0.0001$;
609 Wilcoxon test: $\chi^2 = 22.17$, *** $p = 0.0001$.

610 (I) Expression by qRT-PCR of *upd1*, *upd2* and *upd3* in thorax samples of *crq-Gal4/+* and *crq-*
611 *Gal4>upd1-IR* flies, data from four independent samples of each genotype.

612 (J) Lifespan of *crq-Gal4/+*, *upd2^Δ upd3^Δ;UAS-upd1-IR/+*, and *upd2^Δ upd3^Δ;crq-Gal4>upd1-IR* flies at
613 29°. Pooled data from three independent experiments shown. Log-Rank test (*crq-Gal4/+* vs. *upd2^Δ*
614 *upd3^Δ;crq-Gal4>upd1-IR*): $\chi^2 = 41.12$, *** $p < 0.0001$; Wilcoxon test (*crq-Gal4/+* vs. *upd2^Δ upd3^Δ;crq-*
615 *Gal4>upd1-IR*): $\chi^2 = 54.47$, *** $p < 0.0001$ Log-Rank test (*crq-Gal4/+* vs. *upd2^Δ upd3^Δ;UAS-upd1-IR/+*):
616 $\chi^2 = 14.46$, *** $p < 0.0001$; Wilcoxon test (*crq-Gal4/+* vs. *upd2^Δ upd3^Δ;UAS-upd1-IR/+*): $\chi^2 = 19.99$, ***
617 $p < 0.0001$. P values in C, E, H from unpaired T-test.

618

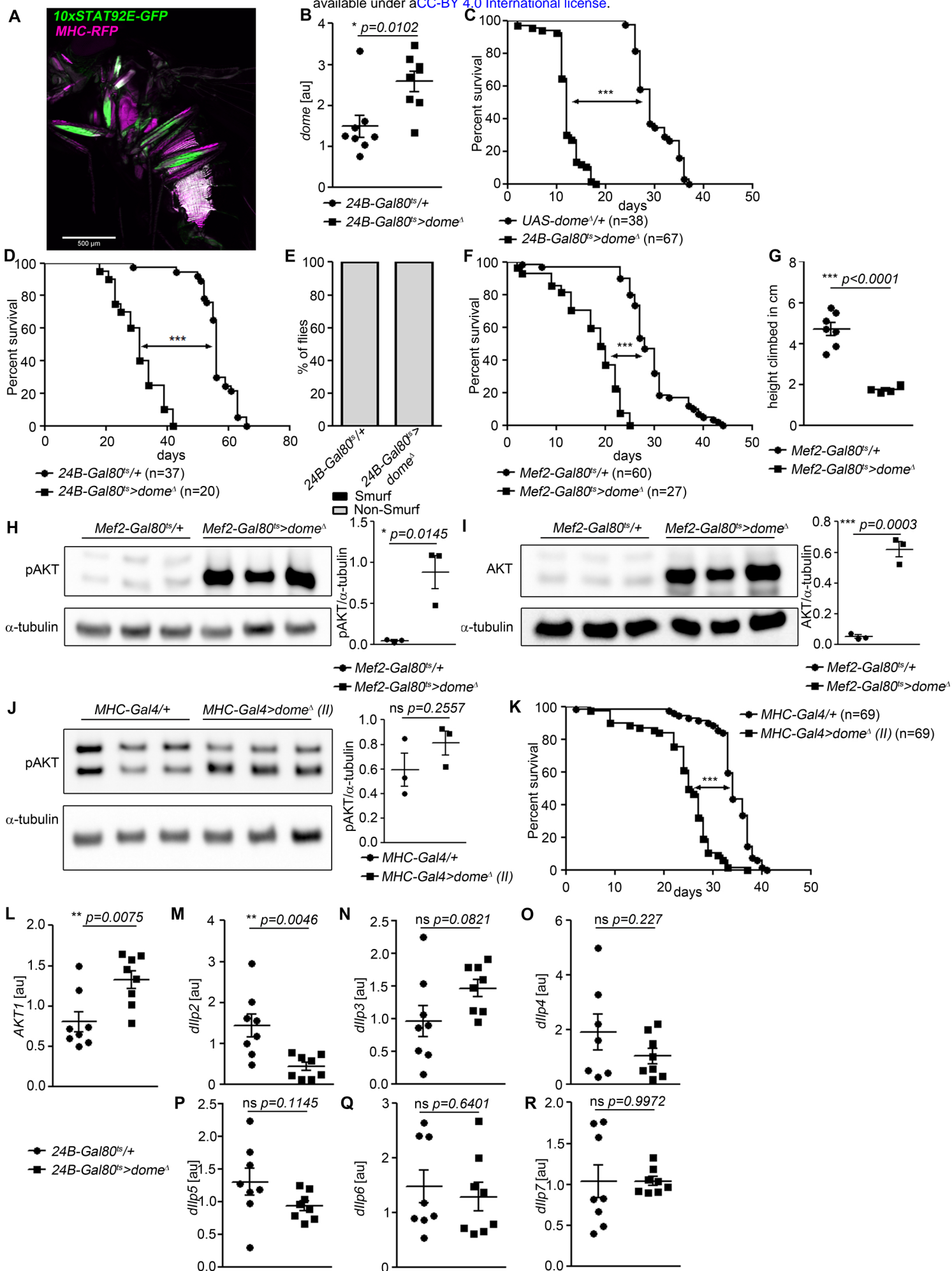


Fig S1

620 **Supplemental Information**

621 Figures S1-S4.

622

623 **Figure S1. Further characterisation of the requirement for *dome* in adult muscle.**

624 (A) STAT activity (*10xSTAT92E-GFP*) and muscle (*MHC-RFP*) colocalize in adult flies. One fly of 6
625 shown. Scale bar=500 μ m.

626 (B) *dome* expression by qRT-PCR in thorax samples from 14-day-old *24B-Gal80^{ts}/+* and *24B-*
627 *Gal80^{ts}>dome^Δ* flies.

628 (C) Lifespan of *UAS-dome^Δ/+* and *24B-Gal80^{ts}>dome^Δ* at 29°; pooled data from two independent
629 experiments shown. Log-Rank test: $\chi^2 = 100.8$, *** $p < 0.0001$; Wilcoxon test: $\chi^2 = 76.2$, *** $p < 0.0001$.

630 (D) Lifespan of *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>dome^Δ* at 25°; pooled data from two independent
631 experiments shown. Log-Rank test: $\chi^2 = 61.83$, *** $p < 0.0001$; Wilcoxon test: $\chi^2 = 55.18$, *** $p < 0.0001$.

632 (E) Smurf assay of 14-day-old *24B-Gal80^{ts}/+* (n=49) and *24B-Gal80^{ts}-dome^Δ* flies (n=18). Data pooled
633 from two independent experiments.

634 (F) Lifespan of *Mef2-Gal80^{ts}/+* and *Mef2-Gal80^{ts}>dome^Δ* flies at 29°, pooled from three independent
635 experiments. Log-Rank test: $\chi^2 = 86.96$, *** $p < 0.0001$; Wilcoxon test: $\chi^2 = 78.61$, *** $p < 0.0001$.

636 (G) Negative geotaxis assay of 14-day-old *Mef2-Gal80^{ts}/+* and *Mef2-Gal80^{ts}>dome^Δ* flies. Points
637 represent mean climbing height of individual vials analysed (~20 flies/vial), pooled from three
638 independent experiments.

639 (H, I) Western blots of protein from legs of 14-day-old *Mef2-Gal80^{ts}/+* and *Mef2-Gal80^{ts}>dome^Δ* flies.
640 One of three independent experiments is shown.

641 (H) Phospho-AKT.

642 (I) Total AKT.

643 (J) Western blots of Phospho-AKT in leg samples from 14-day-old *MHC-Gal4/+* and *MHC-Gal4>dome^Δ*
644 (II) flies. One of two independent experiments is shown.

645 (K) Lifespan of *MHC-Gal4/+* and *MHC-Gal4>dome^Δ* (II) flies at 29°, pooled from two independent
646 experiments. Log-Rank test: $\chi^2 = 82.9$, *** $p < 0.0001$; Wilcoxon test: $\chi^2 = 58.91$, *** $p < 0.0001$.

647 (L-R) Expression by qRT-PCR of *Akt1* and insulin-like peptides in whole fly samples from 14-day-old
648 *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}-dome^Δ* flies. All transcript levels are normalized to *Rpl1* and shown in
649 arbitrary units [au]. P values in B, G, H-J, L-R from unpaired T-test.

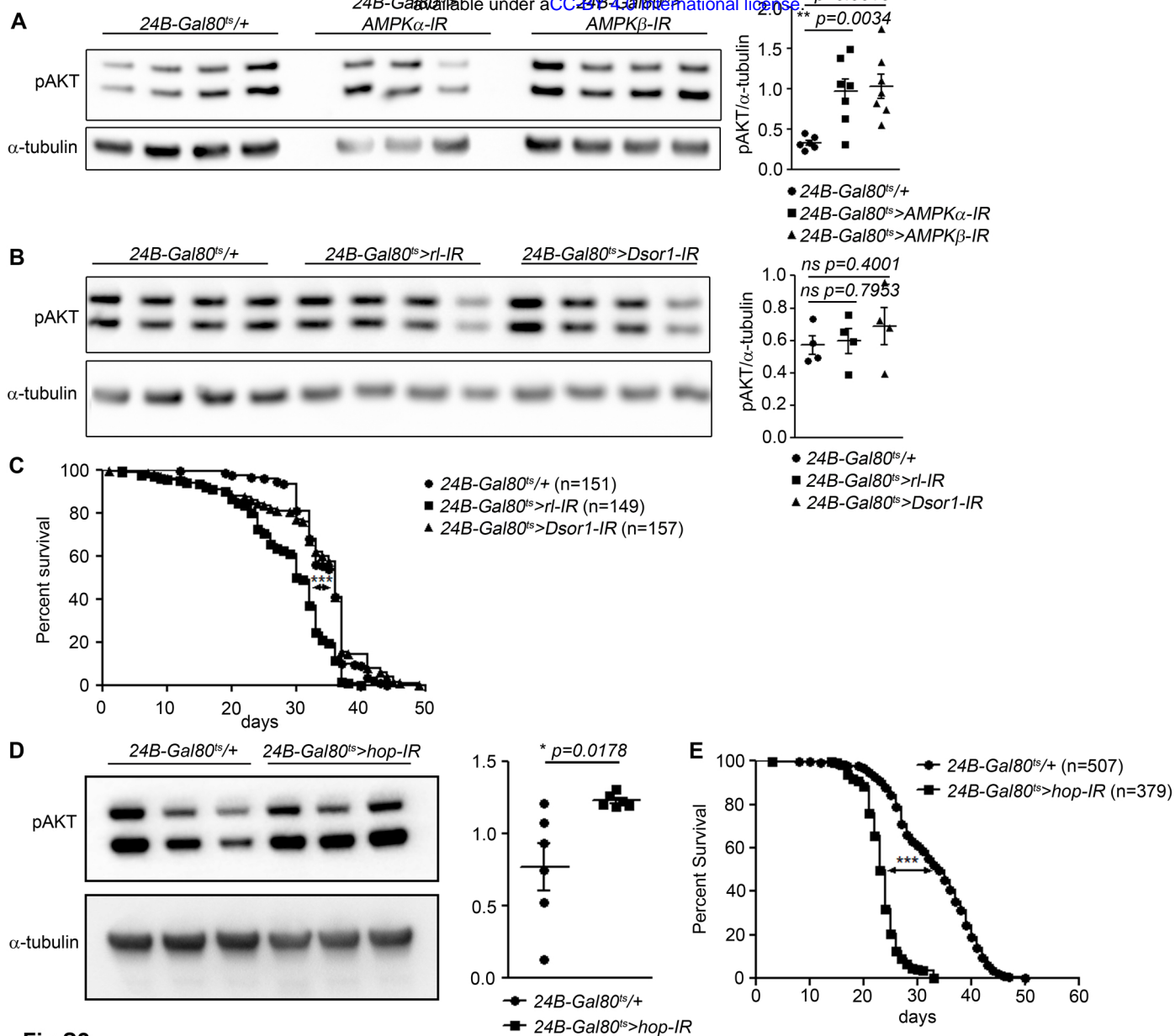


Fig S2

650 **Figure S2. Interactions of *dome* with AMPK, MAPK, and FOXO signaling in adult muscle.**

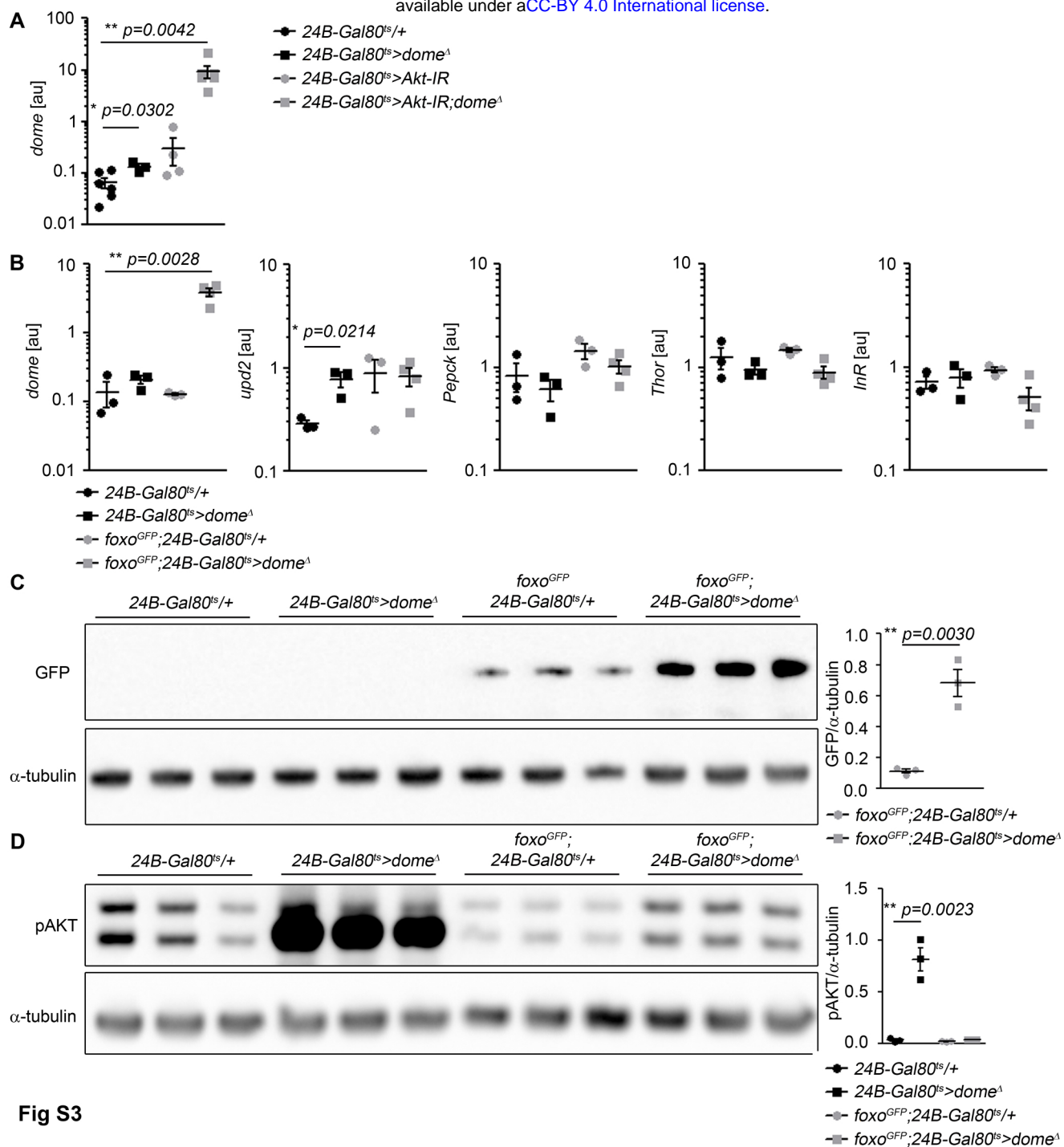
651 (A) Phospho-AKT in leg samples from 14-day-old *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>AMPK α -IR*, and *24B-*
652 *Gal80^{ts}>AMPK β -IR* flies. One of three independent experiments is shown.

653 (B) Phospho-AKT in leg samples from 14-day-old *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>rl-IR*, and *24B-*
654 *Gal80^{ts}>Dsor1-IR* flies. One of three independent experiments is shown.

655 (C) Lifespan of *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>rl-IR*, and *24B-Gal80^{ts}>Dsor1-IR* flies at 29°, pooled from
656 four independent experiments. Log-Rank test (*24B-Gal80^{ts}/+* vs. *24B-Gal80^{ts}>rl-IR*): $\chi^2 = 60.29$, ***
657 $p < 0.0001$; Wilcoxon test (*24B-Gal80^{ts}/+* vs. *24B-Gal80^{ts}>rl-IR*): $\chi^2 = 58.32$, *** $p < 0.0001$; Log-Rank test
658 (*24B-Gal80^{ts}/+* vs. *24B-Gal80^{ts}>Dsor1-IR*): $\chi^2 = 1.186$, ns $p = 0.2760$; Wilcoxon test (*24B-Gal80^{ts}/+* vs.
659 *24B-Gal80^{ts}>Dsor1-IR*): $\chi^2 = 0.0033$, ns $p = 0.9538$.

660 (D) Phospho-AKT in leg samples from 14-day-old *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>hop-IR* flies. One of
661 two independent experiments is shown.

662 (E) Lifespan of *24B-Gal80^{ts}/+* and *24B-Gal80^{ts}>hop-IR* flies at 29°, pooled from four independent
663 experiments. Log-Rank test (*24B-Gal80^{ts}/+* vs. *24B-Gal80^{ts}>hop-IR*): $\chi^2 = 546.4$, *** $p < 0.0001$;
664 Wilcoxon test (*24B-Gal80^{ts}/+* vs. *24B-Gal80^{ts}>hop-IR*): $\chi^2 = 458.1$, *** $p < 0.0001$. P values in A, C, E
665 from unpaired T-test.



666 **Figure S3. Mutual regulation by AKT, Foxo, and Dome.**

667 (A) *dome* expression by qRT-PCR in whole fly samples from 14-day-old *24B-Gal80^{ts}/+*, *24B-*
668 *Gal80^{ts}>dome^Δ*, *24B-Gal80^{ts}>Akt-IR*, and *24B-Gal80^{ts}>Akt-IR;dome^Δ* flies.

669 (B) Expression by qRT-PCR of *dome*, *upd2*, *Pepck*, *Thor* and *InR* in whole fly samples from 14-day-old
670 *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>dome^Δ*, *foxo-GFP;24B-Gal80^{ts}/+*, and *foxo-GFP;24B-Gal80^{ts}>dome^Δ* flies.

671 (C) Western blot for GFP to detect the Foxo-GFP fusion protein in leg samples from 14-day-old *24B-*
672 *Gal80^{ts}/+*, *24B-Gal80^{ts}>dome^Δ*, *foxo-GFP;24B-Gal80^{ts}/+*, and *foxo-GFP;24B-Gal80^{ts}>dome^Δ* flies.

673 (D) Western blot for Phospho-AKT in leg samples from 14-day-old *24B-Gal80^{ts}/+*, *24B-Gal80^{ts}>dome^Δ*,
674 *foxo-GFP;24B-Gal80^{ts}/+*, and *foxo-GFP;24B-Gal80^{ts}>dome^Δ* flies. One of two independent experiments
675 is shown. P values in A-D from unpaired T-test.

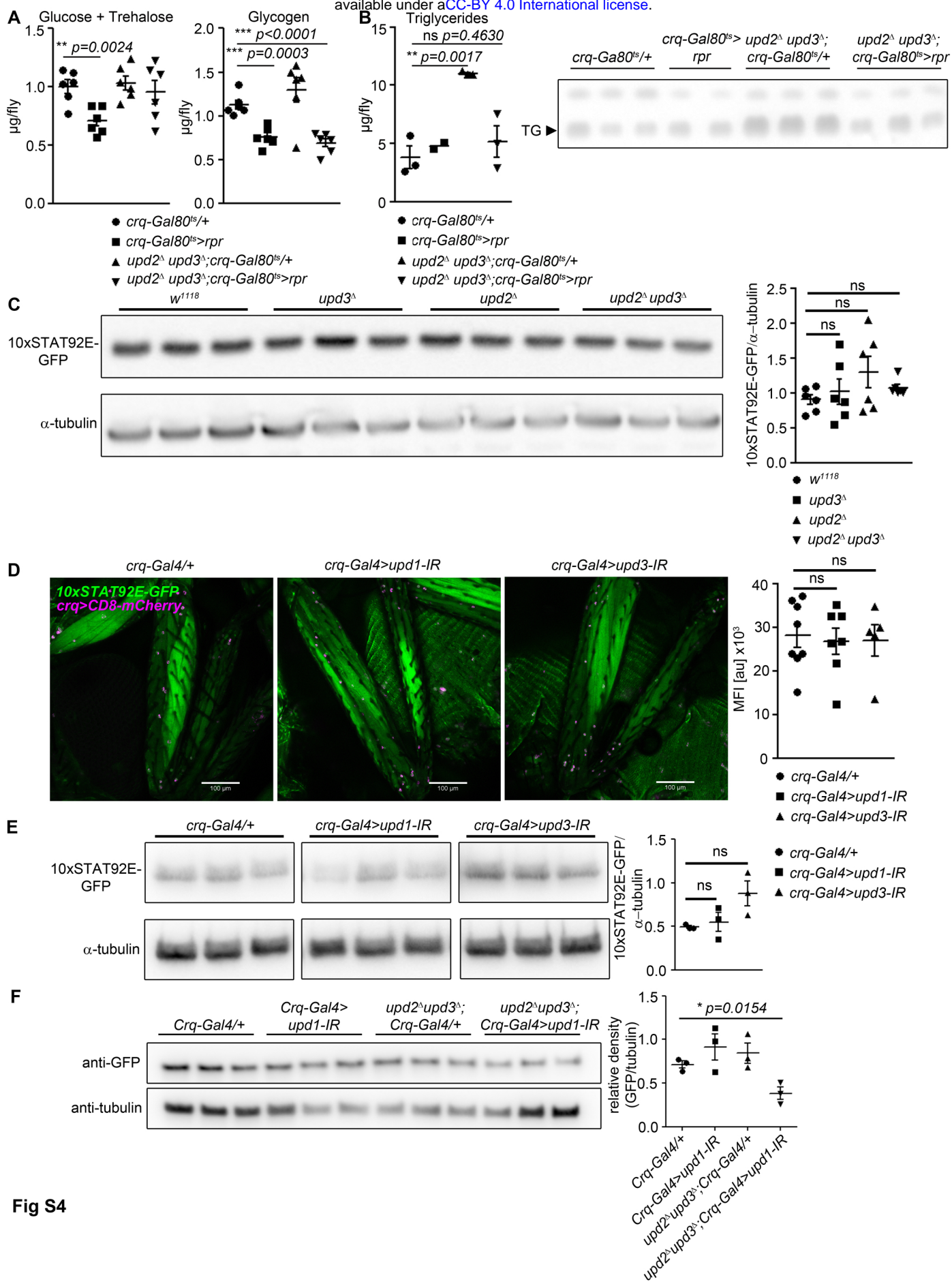


Fig S4

676 **Figure S4. Further characterisation of requirements for specific Upds.**

677 (A) Glucose + trehalose and glycogen in 7-day-old *crq-Gal80^{ts}/+*, *crq-Gal80^{ts}>rpr*, *upd2^Δ upd3^Δ;crq-*
678 *Gal80^{ts}/+*, and *upd2^Δ upd3^Δ; crq-Gal80^{ts}>rpr* flies.

679 (B) TLC of triglyceride in 7-day-old *crq-Gal80^{ts}/+*, *crq-Gal80^{ts}>rpr*, *upd2^Δ upd3^Δ;crq-Gal80^{ts}/+*, and
680 *upd2^Δ upd3^Δ;crq-Gal80^{ts}>rpr* flies, n=2-3 samples per genotype.

681 (C) Western blot analysis of STAT-driven GFP in legs from 7-day-old *w¹¹¹⁸*, *upd3^Δ*, *upd2^Δ*, and *upd2^Δ*
682 *upd3^Δ* flies. One representative experiment of two is shown.

683 (D) STAT activity and plasmatocytes in legs from 7-day-old control (*crq-Gal4/+*), *upd1* knockdown
684 (*crq-Gal4>upd1-IR*), and *upd3* knockdown (*crq-Gal4>upd3-IR*) flies. One representative fly is shown of
685 5-7 imaged for each genotype. Scale bar=100μm. Mean fluorescence intensity (MFI) is shown for all
686 flies imaged.

687 (E) Western blot analysis of STAT-driven GFP in legs from 7-day-old control (*crq-Gal4/+*), *upd1*
688 knockdown (*crq-Gal4>upd1-IR*), and *upd3* knockdown (*crq-Gal4>upd3-IR*) flies. One of two
689 independent experiments is shown.

690 (F) Western blot analysis of STAT-driven GFP in thorax from 7-day-old *crq-Gal4/+*, *crq-Gal4>upd1*,
691 *upd2^Δupd3^Δ;crq-Gal4/+* and *upd2^Δupd3^Δ;crq-Gal4>upd1-IR* flies. P values in A-F from unpaired T-test.

692