Natural variation in starvation sensitivity maps to a point mutation in phospholipase IPLA2-VIA in *Drosophila melanogaster*

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Abstract

Resistance to starvation is a classic complex trait, where genetic and environmental variables can greatly modify an animal's ability to survive without nutrients. In this study, we investigate the genetic basis of starvation resistance using complementary quantitative and classical genetic mapping in *Drosophila melanogaster*. Using the Drosophila Genetics Reference Panel (DGRP) as a starting point, we queried the genetic basis of starvation sensitivity in one of the most sensitive DGRP lines. We localize a major effect locus modifying starvation resistance to the phospholipase iPLA2-VIA. This finding is consistent with the work of others which demonstrate the importance of lipid regulation in starvation stress. Furthermore, we demonstrate that iPLA2-VIA plays a role in the maintenance of sugar reserves post-starvation, which highlights a key dynamic between lipid remodeling, sugar metabolism and resistance to starvation stress.

Introduction

Coping with periods of low nutrient availability is essential to the survival of any animal. At the simplest level, several metabolic processes need to integrate nutritional signals to successfully cope with periods of starvation. An animal has to be able to convert the energy obtained from its diet into a usable form. Energy that is not needed immediately has to be converted into a form amenable for efficient storage, and that stored energy has to be accessible for utilization during periods of starvation. For these reasons, the genetics of starvation resistance is an attractive trait for understanding the integrative aspects of animal physiology (Brown et al. 2019). Furthermore, a better understanding of the genetic basis of nutrient catabolism and storage will also shed a light on genes relevant to metabolic diseases and fitness traits in nature (Hardy et al. 2018).

Starvation resistance is a classic complex (or quantitative) trait (Harbison et al. 2004; Mackay et al. 2009). Complex traits refer to continuously distributed phenotypes across individuals within a population. These traits are believed to result from complex integration of genetic and environmental factors. While many contend that these phenotypes are primarily due to a multitude of relatively common variants with small to modest effect sizes(Lango Allen et al. 2010), others suggest that complex traits result from a smaller number of low frequency, but highly penetrant variants (Frazer et al. 2009). What remains clear is that effectively linking genetic variants to phenotypes is a central challenge of modern biology. A forward-genetic approach to establishing these associations would be to interrogate the role(s) of genes on phenotypes by assessing the effect of introducing mutations in specific genotypes. At a population level, genome wide association studies (GWAS) have contributed significantly to our understanding of the genetic basis of

complex traits (Mackay et al. 2012). However, the rapidly declining cost of next generation sequencing technologies has made multiple tools available to associate genetic markers with factors underlying complex traits. A variety of methods have been used to study starvation resistance in *Drosophila melanogaster.*

The Drosophila Genetics Reference Panel (DGRP), is a collection of 205 fully sequenced inbred *Drosophila* lines which has been used as a resource to study many complex traits. Starvation resistance shows large variation across individual lines of the DGRP consistent with a typical quantitative trait. Genome wide association analyses have identified hundreds of statistically significant associations between nucleotide variants and starvation resistance (Mackay et al. 2012). Studies in the DGRP have revealed that for starvation resistance, the effect size of variants has an inverse relationship with minor allele frequency (MAF) (Mackay et al. 2012). Interestingly, rarer variants appear to have a large degree of pleiotropy, with different variants having opposing effects on various complex traits (Mackay and Huang 2018). This is noteworthy as rare variants (Minor Allele Frequency (MAF) <0.05) are often ignored in individual variant-based association tests. One can therefore imagine a case where a variant present in only a handful of lines, with a large effect size, would not be identified since the rare variant would not be included in the study. As GWAS sample sizes have exceeded 10^5 in some cases, the definition of rare alleles and their effect sizes has changed (Smith, et al. 2022)

The use of outbred advanced intercross populations (AIPs) is a useful tool to explore the possibility that nucleotide variants have different effects on phenotype in different genetic

80 backgrounds and at different frequencies in mapping populations. A small set of parental
81 lines can be used to generate many genetically distinct individuals through recombination
82 over multiple generations. Variants that are rare in the source population will then be
83 present at appreciable levels in the recombinant individuals, allowing one to look for
84 associations in this generated population.
85 Whole-genome sequencing of pools of individuals (Pool-seq) allows for efficient estimates
86 of allele frequency differences across populations. This method can be particularly useful
87 when combined with extreme QTL mapping (Pool 2016; Mackay and Huang 2018). Since
88 causal alleles are expected to be enriched in the individuals at the tails of a given
89 phenotypic distribution, sequencing pools of individuals from these tails can be used to
90 identify alleles whose frequency differ significantly from each tail as candidate causal
91 alleles.
92
93 In the current study, we sought to use a combination of quantitative genetic and classical
94 genetic mapping to examine the genetic basis of starvation. Combining pool-seq of AIPs
95 with more classical approaches offers the best of both worlds, rapid and cost- effective
96 identification of genetic differences across populations, and finer resolution mapping to
97 distinguish between effect loci and linked mutations. Here, we present a study where
98 existing variation in the starvation resistance of two DGRP lines was used to identify the
99 genetic basis of starvation sensitivity in a specific sensitive line (DGRP-765). We created
100 replicate advanced intercross populations derived from the two DGRP lines. Pool-seq and
101 extreme QTL analyses revealed a polygenic basis for the difference between starvation
102 sensitive and starvation resistant flies, with a major factor on chromosome 3L and other

103 factors on 3R, 2L, but not X and 2R. Using classical mapping approaches and gene-
104 specific deficiencies, we were able localize the major effect locus to a single gene, the
105 phospholipase iPLA2-VIA. The correspondence of these approaches contributes to our
106 understanding of the genetic architecture of starvation resistance and identifies a novel
107 mutation in iPLA2-VIA that uncovers an association between starvation sensitivity, lipid
108 metabolism and carbohydrate metabolism.

109 Materials and Methods

110 *Fly stocks*

111 The fly lines selected for this study were based on our previously published work
112 demonstrating epistatic interactions between mitochondrial haplotypes and nuclear
113 genotypes from the DGRP that modify development time (Mossman et al. 2016). Briefly,
114 a panel of 72 mitochondria-nuclear combinations (using the notation: mitochondrial DNA
115 haplotype; nuclear DNA haplotype) was created through introgression 6 mitochondrial
116 haplotypes (3 *Drosophila melanogaster* (*Dmel*) and 3 *Drosophila simulans* (*Dsim*)), into
117 12 different DGRP lines via balancer chromosome substitution. We screened 12 of
118 these 72 lines (2 mtDNAs from *Dmel* and 2 mtDNAs from *Dsim* on each of three DGRP
119 nuclear backgrounds: 765, 315 and 820) of these lines to identify mito-nuclear
120 interactions which modify starvation resistance. The 765 nuclear background was
121 selected because it was the nuclear background most sensitive to starvation resistance
122 included in our panel. The 315 nuclear background was selected because the *Dsim*
123 mtDNAs were distinct from the *Dmel* mtDNAs in development time, demonstrating
124 species-specific mtDNA effects. The 820 nuclear background was selected because it
125 was the line most resistant to starvation included in our panel and demonstrated mtDNA

126 haplotype-specific effects on development time (Mossman et al 2016). During our
127 screen, we found one unique combination, *sil;765* which appeared to significantly
128 increase resistance to starvation and proceeded to validate whether the increase in
129 starvation resistance was due to the introgression of the *sil* mitochondrial haplotype or
130 residual heterozygosity from the creation of the lines.

Stock Name	**Mitochondrial haplotype**	**Nuclear haplotype**	**Phenotype**
Zim53;765	Zim53	DGRP-765	Starvation sensitive
line_765-hybrid (*sil;765*)	sil	DGRP-765/Unknown	Starvation resistant
Zim53;315	Zim53	DGRP-315	Starvation resistant
DGRP-765	Not altered	DGRP-765	Starvation sensitive
iPLA2-VIA[Delta174](Lin et al. 2018)	Not altered	Dmel\P{EPgy2}iPLA2-VIA^{EY05103}	Starvation sensitive

131 **Table of main stocks used in this study.** A table of all stocks used in this study can
132 be found in supplemental file 1.

133 ***Starvation Stress***

134 Survival during starvation stress was measured by placing approximately 125 female flies
135 per genotype in each of three replicate demography cages. Flies were collected and
136 sorted over CO_2 and allowed to recover in quart-sized plastic demography cages affixed
137 with 25x95 mm vials of high sugar food (15% sucrose, 5% Yeast, 2% agar) for 7 days
138 unless otherwise indicated. After 7 days, any dead flies were removed, and the food

139 supplied to each demography cage is replaced with 2% Difco Bacto-agar prepared with
140 deionized H_20. The number of dead flies was recorded every 12 hours and removed from
141 each demography cage until all flies were dead. The survival data from each cage were
142 modeled using a Kaplan-Meir survival model implemented in R (Therneau and Grambsch
143 2000). Significant differences in survivals were determined using a log rank test.
144

145 ***Whole genome sequencing of sensitive and resistant pools from Advanced***
146 ***Intercross Populations***

147 *Population Cages.* Five replicate advanced intercross populations (AIPs) were
148 established between the sensitive (*Zim53;765*) and resistant (*Zim53;315*) lines, as
149 follows: 25 *Zim53;765* males were crossed to 25 *Zim53;315* virgin females, in addition to
150 the reciprocal cross, to yield a total number of 50 male and 50 female parents of each line
151 as the founders of each replicate cage. These parents were introduced into Plexiglas
152 cages (20 x 20 x 20cm) and allowed to lay eggs in four 125mL culture bottles with 30mL
153 of Drosophila medium in each bottle. The four bottles in each cage were left with their
154 tops open for 3 days, and then each bottle was capped to allow flies to develop. Prior to
155 eclosion, the caps were removed from each bottle, and all flies were allowed to emerge
156 into the cage and mate at random. Four new culture bottles were placed into each
157 replicate cage, and the adults were allowed to lay eggs for 48 hours. After egg laying,
158 adults were cleared from each bottle, plugs were inserted, and cultures were allowed to
159 develop for 7 days following the termination of egg laying, after which the plugs were
160 removed from all bottles to allow eclosion of the next generation into each cage.
161 Population sizes exceeded 1000 individuals after the first generation. All five cages were

162 maintained at large population sizes (>2000 adults) for 10 generations to allow
163 recombination to take place between the sensitive (765) and resistant (315) DGRP
164 nuclear genotypes.

165 *Starvation Stress.* The female flies from each generation-10 population cage were
166 collected over CO_2 and moved to demography cages with a density of approximately 150
167 flies per cage. The flies were allowed to recover for 7 days with access to high sugar food
168 and then subjected to starvation conditions by replacing the food vials with a 2% agar and
169 water mix. Every six hours from the start of starvation, all the dead flies were collected,
170 flash frozen and stored at -80C.

171 *Pool-sequencing.* Pools of approximately 50 dead flies were made by combining flies from
172 the two earliest time points where deaths were observed (24 hour and 30 hour) and the
173 last two time points (96 hour and 102 hour). DNA from each replicate pool was isolated
174 using the DNeasy DNA purification kit from Qiagen (Cat No 69506). Each sample was
175 RNase treated prior to DNA elution using a 10-minute RNase A (Cat No 19101) treatment.
176 Libraries were prepared and sequenced using the DNBseq platform by the Beijing
177 Genomics Institute (BGI).

178 *Estimation of genetic differentiation using Popoolation2.* Raw reads were mapped to the
179 dm6 build of the *Drosophila* genome using *bwa* version 0.7.15 (Li and Durbin 2009).
180 Variants were called using *samtools* version 1.9 (Li et al. 2009). Allele frequency
181 differences and estimates of population subdivision (F_{ST}) were determined using
182 *Popoolation2* (Kofler et al. 2011), and the significance of differences in allele frequencies
183 between the early-dying and late-dying sample across the five replicate populations was
184 determined using the Cochran-Mantel-Haenszel test. P-values were corrected using the

185 Bonferroni method and differences with FDR-corrected p-values < 0.05 were considered
186 to be significant. Linkage disequilibrium was estimated from paired end reads, following
187 the procedure described in (Feder et al. 2012).
188
189 ***Forward genetic mapping of starvation sensitivity***
190 To generate *Zim53;765* selectively heterozygous for different autosomes, 20 *Zim53;765*
191 females were first crossed to 20 males from an in-house double balanced stock (*wg[Sp-*
192 *1]/CyO; Dr[1]/TM3, Sb[1]*). Twenty male progeny with both the 2nd chromosome *CyO* and
193 3rd chromosome *Dr* markers present were collected and sorted over CO_2 and crossed to
194 20 *Zim53;765* virgin females in eight replicate 20-female x 20-male crosses. Progeny from
195 this second cross were segregated into four groups based on the presence of the *CyO*
196 and *D*r markers. The four groups were, none (reconstituted wild-type *Zim53;765*),
197 chromosome 2 heterozygotes (*CyO/765*), chromosome 3 heterozygotes (*Dr/765*) and
198 heterozygotes for both chromosome 2 and 3 (*CyO/765;Dr/765*). Flies from each group
199 were moved to 3 replicate demography cages with approximately 125 flies each. The
200 survival of each group under starvation stress was assayed as described earlier.
201 ***Chromosome 3 recombination***
202 The chromosome segregation experiments indicated that the majority of the difference in
203 starvation resistance between the lines mapped to chromosome 3. We attempted to
204 generate *Zim53;765* chromosome 3 recombinants by crossing *Zim53;765* virgin females
205 to 20 males from a multiply marked recessive stock (BSC_576). The marked stock
206 contains the *ru, h Diap, st, cu, sr, e* and *ca* markers. The progeny from this cross however
207 failed to eclose. As an alternative, we crossed the *sil;765* virgin females to *BSC_576*

208 males in an effort to allow recombination of the 765 alleles that is present in the *sil;765*
209 stock. Twenty F_1 virgin females were collected and crossed to 20 males from a similarly
210 multiply marked recessive stock with identical recessive markers in addition to a dominant
211 marker present on one copy of chromosome 3 (BSC_1711) in three replicate 20-female
212 x 20-male crosses. F_1 males from this second cross were collected and segregated based
213 on the recessive and dominant markers present. Five classes were initially designated
214 based on the visible markers present, class i*: ru, h*; class ii: *ru, h, Diap, st*; class iii: *ru, h,*
215 *Diap, st, cu*; class iv: *ru, h, Diap, st, cu, sr*; and class v: *ru, h, Diap, st, cu, sr, e*. Twenty
216 males from each group were crossed to 20 *Zim53;765* virgin females and allowed to mate
217 for 48 hours in three replicate 20-female x 20-male crosses. The males were removed
218 and similarly mated to *sil;765* virgin females. Females from each cross were collected
219 over CO_2. Sufficient flies for a starvation stress survival assay were only available from
220 the class i, class ii and class v crosses, so only these were considered. The survival of
221 these females under starvation stress was measured as described above.

222 ***Chromosome 3L Deficiency Screen***

223 The recombination mapping implicated chromosome 3L as harboring the major starvation
224 factor, so deficiency mapping was pursued. Twenty males from each deficiency line
225 (Supplemental Table 1) were crossed to 20 *Zim53;765* virgin females in eight replicate
226 20-female x 20-male crosses. F_1 females were collected and sorted over CO_2. Females
227 were separated based on the presence of the respective balancer marker (flies
228 heterozygous for the deficiency and the 765 chromosome do not have the balancer
229 present) and moved to 3 replicate demography cages with approximately 125 flies each,

230 for each genotype. The flies were allowed to recover for 7 days on high sugar food and
231 survival under starvation stress measured as described above.

232 ***iPLA2-VIA Complementation***

233 The chromosome 3L deficiency mapping, in conjunction with the available DGRP
234 sequences, identified the iPLA2-VIA as a candidate gene, and deficiencies within that
235 gene were obtained for further complementation mapping. Twenty
236 *Zim53;765/BSC_25204* flies were crossed to twenty *BSC_80133/ iPLA2-VIA[Delta174]*
237 and independently to *BSC_80134/iPLA2-VIA[Delta192]* in reciprocal. Each cross was
238 done in replicates of 4. As a control, males from each *iPLA2-VIA* deficiency stock were
239 also crossed to *Zim53;315* females. F_1 females were collected and sorted over CO_2 and
240 moved to 3 replicate demography cages with approximately 125 flies each. The flies were
241 allowed to recover for 7 days on high sugar food and survival under starvation stress
242 measured as described above.

243 ***Glucose Assay***

244 The free glucose levels were measured for *Zim53;765*, *Zim53;315*, *iPLA2-VIA[Delta174]*,
245 *Zim53;315* crossed to *iPLA2-VIA[Delta174]* and *Zim53;765* crossed to iPLA2-
246 VIA[Delta174] as described in (Tennessen et al. 2014). 120 female flies from each
247 genotype were collected and sorted over CO_2 and allowed to recover in demography
248 cages affixed with 25x95 mm vials of high sugar food (15% sucrose, 5% Yeast, 2% agar)
249 for 5 days. Three replicates were done for each genotype. After 5 days, any dead flies
250 are removed. Half of the remaining flies were removed from the demography cage,
251 immediately flash frozen in liquid N_2 and stored at -80°C. The food supplied to each
252 demography cage was replaced with 2% Difco Bacto-agar prepared with deionized H_20.

253 After 12 hours, dead flies were discarded and the remaining flies were removed from the
254 demography cage, immediately flash frozen in liquid N_2 and stored at -80°C. Six frozen
255 flies from each replicate were transferred to a 1.5uL micro-centrifuge tube and
256 homogenized in 120uL of Phosphate Buffered Saline (PBS) using a steel bead and
257 Qiagen TissueLyser II set to 30Hz for 2 minutes. A 10uL aliquot was removed to measure
258 protein content using the Pierce™ BCA Protein Assay Kit (23225). The remaining
259 homogenate was heat treated for 10 minutes at 70°C. The homogenate was diluted 1:4
260 with PBS. The free glucose was measured with the Glucose Oxidase (GO) kit (MAK097-
261 1KT) by following the protocol for free glucose measurement laid out in (Tennessen et al.
262 2014).

263 **Data Availability**

264 Drosophila strains, code and raw data are available at the Rand Lab GitHub site for this
265 publication (https://github.com/DavidRandLab/Williams-et-al.-Starvation-Genetics-2021).
266 The authors affirm that all analyzed data necessary for confirming the conclusions of the
267 article are present within the article, figures, tables and GitHub site. Raw sequences for
268 the pool-seq mapping are available at BioProject accession number: PRJNA1130456.

269 Results

270 Selection of Genotypes for this study

271 Based on our interest in the genetics of starvation resistance, we designed a screen to
272 examine the effect of gene x gene and gene x environment interactions on starvation
273 resistance. We created a panel of unique mito-nuclear introgression lines based on
274 introgression of six different mitochondrial DNA haplotypes into several individual fly
275 lines from the DGRP as previously described (Mossman et al. 2016). Using these
276 introgression lines, we examined how different mito-nuclear introgression strains

277 responded to starvation stress. *mito;765* lines were of particular interest because the
278 765 nuclear background represents one of the least resistant lines in terms of starvation
279 resistance in the DGRP, and it appeared that introgression of the *sil* mitochondrial DNA
280 haplotype significantly improved resistance to starvation in the 765 nuclear background.
281 To test whether the rescue of starvation resistance mapped to a cytoplasmic or nuclear
282 factor, we performed reciprocal crosses to examine if maternal inheritance of mtDNA
283 was the source of the rescue of starvation sensitivity. We observed that when we
284 crossed *Zim53;765* to this *sil;765*, the direction of the cross (*Zim53;765 female* crossed
285 *sil;765* males vs *sil;765* females to *Zim53;765 males*) made no difference to starvation
286 resistance of its progeny. This implied that the *sil* mtDNA haplotype was not the source
287 of the rescue, as would be expected if the trait was being inherited maternally
288 (Supplemental figure 1a). This prompted us to analyze the reads from an RNA-
289 sequencing data set from each fly line. We compared the genotype calls for each SNP
290 present in *Zim53;765* reads (restricted to the coding regions) and the genotype calls at
291 the same locations in *sil;765*. We observed that at the vast majority of these sites, the
292 *sil;765* was heterozygous for the 765 allele and an alternative allele (in most cases the
293 reference allele) (Supplemental figure 1b and 1c). We did a similar comparison of
294 *sil;765* to an alternate DGRP line (*Zim53;315*) and did not see a similar overlap
295 between the *sil;765* allele and the *Zim53;315* allele (Supplemental figure 1b and 1c).
296 Based on these results we concluded that the *sil;765* was largely heterozygous for 765.
297 This suggested to us that starvation sensitivity in *Zim53;765,* and the other 4 *mito;765*
298 combinations, were homozygous recessive and the improvement in resistance to
299 starvation seen in *sil;765* was due to its nuclear heterozygosity. Indeed, we observed

300 that the offspring from a *Zim53;765* to *Zim53;315* cross were significantly more resistant
301 to starvation than *Zim53;765* (data not shown). For the rest of the study we refer to the
302 *sil;765* as *line_765-hybrid* to represent the true nature of its nuclear genome.

303 **Sites on chromosome 2R and 3 have significant differences in allele frequency**
304 **between early and late dying flies.**
305
306 To understand the genetic basis of 765 starvation sensitivity, we sought to identify genetic
307 differences characteristic of starvation sensitive flies. We created an advanced intercross
308 population in which *Zim53;765* was allowed to recombine with *sil;315*. We set up five
309 independent populations where *Zim53;765* flies were crossed to *sil;315* in reciprocal and
310 the populations maintained for 10 generations at population sizes >1000 adults. Following
311 this, females from each population were subjected to starvation stress and dead flies were
312 collected every six hours until all flies were dead. Flies that died during the first three and
313 last two six-hour intervals were pooled and sequenced (24 - 36 hours vs. 96 and 102
314 hours, hereafter 'early' and 'late' dying pools). Consistent differences in allele frequencies
315 across the early and late dying populations were determined using the Cochran-Mantel-
316 Haenszel test implemented in Popoolation2 (Kofler et al. 2011). There was an enrichment
317 in significantly different variants across chromosome 3 and a localized region of
318 chromosome 2R (Figure 1). These results suggest that *Zim53;765* and as a consequence
319 DGRP-765, starvation sensitivity is based on variants present on chromosome 3 and
320 chromosome 2.

321 **Chromosome 3 heterozygosity partially suppresses *Zim53;765* starvation**
322 **sensitivity**
323
324 Based on the suppression of starvation sensitivity seen in *Zim53;765* heterozygotes, we
325 reasoned that starvation sensitivity in *Zim53;765* was a homozygous recessive trait and

326 that we could localize the effect locus/loci to chromosomes by using chromosome
327 segregation tests and observing which chromosome segregants masked the trait. We
328 selectively made *Zim53;765* heterozygous for individual autosomes. Using chromosome
329 segregation tests, we generated the four 2-chromosome segregant populations of flies as
330 follows: heterozygous for chromosome 2, chromosome 3, chromosome 2 and 3 in
331 combination or neither. We observed that flies heterozygous for chromosome 3 had an
332 80% increase in mean survival relative to the non-heterozygotes (Figure 2a). Whereas
333 flies heterozygous for chromosomes 2 and 3 had a 140% increase in mean survival
334 (Figure 2a). Taken together, these results suggest that the main effect locus/loci
335 controlling *Zim53;765* starvation sensitivity are present on chromosome 3 with a
336 secondary locus/loci present on chromosome 2. Importantly, these findings are consistent
337 with the extreme QTL analysis described earlier for the Advanced Intercross Populations.

338 **Heterozygosity of chromosome 3L region partially suppresses Zim53;765**
339 **starvation sensitivity**
340

341 To localize the main effect loci on chromosome 3 we decided to conduct a deficiency
342 screen along chromosome 3. As there are over 200 deficiencies spanning chromosome
343 3, we first sought to narrow the region of interest further. We generated a series of
344 *Zim53;765* recombinants which were heterozygous for overlapping regions of
345 chromosome 3. Each recombinant was generated by crossing *line_765-hybrid* virgin
346 females to a multiply marked recessive stock, then crossing the F_1 virgin females to males
347 from a similarly marked recessive stock with one dominant marker added to distinguish
348 progeny. Three sets of recombinants (classes i, ii, v) were selected based on the
349 presence of visible recessive markers and sufficient flies for starvation analyses (Figure

350 2b). Recombinant class i is likely heterozygous for the region of 3L from the telomere up
351 to at least the regions marked by *h,* with recombination breakpoints existing anywhere
352 between h and the *cu* gene. Recombinant class ii is heterozygous for most of 3L and up
353 to *cu* gene on 3R, with breakpoints existing anywhere between the *cu* gene and the *ca*
354 gene. Recombinant class v is likely heterozygous for most of chromosome 3 with
355 breakpoints existing from the *ca* gene up to the telomere of 3R. It is important to note that
356 because these recombinants were generated from *line_765-hybrid*, the pool of
357 recombinants will have both the *Zim53;765* allele and the alternate (unknown) allele
358 present. Males from each recombinant class were initially crossed to *Zim53;765* females,
359 and then the same males crossed to *line_765-hybrid* as a control. The starvation
360 sensitivity of the female offspring of the *Zim53;765* and recombinant males was compared
361 to the female offspring of the *line_765-hybrid* and recombinant male controls. While we
362 cannot discern genotype at the individual fly level, 765 chromosome 3 alleles will be
363 enriched in the progeny from the *Zim53;765* vs recombinant cross, while the alternative
364 will be true for the *line_765-hybrid* vs recombinant cross. We expected that flies
365 homozygous for the causal *Zim53;765* allele would die earlier during starvation stress,
366 thus revealing the region of *Zim53;765* chromosome 3 responsible for its starvation
367 sensitivity. Although the heterogeneity of respective offspring populations will mask this
368 effect, the enrichment of the 765 allele in the *Zim53;765* vs recombinant cross population
369 and the alternate allele in the *line_765-hybrid* vs recombinant cross population should
370 result in appreciable differences in deaths early on during starvation stress. Indeed, we
371 observed a significant difference between the starvation sensitivity of class i offspring, but
372 not in the case of the cross ii offspring; class v offspring showed a small but significant

373 difference (see Figure 2C). These results indicate that heterozygosity of a region between
374 the *h* marker of recombinant i and the *cu* marker of recombinant ii is likely the largest-
375 effect factor required to suppress *Zim53;765* starvation sensitivity.

376 **Hemizygosity of 3L: 9812381 – 9899255 prevents partial suppression of *Zim53;765***
377 **starvation sensitivity**
378
379 We employed a deficiency screen across the region between recombinant class i and
380 recombinant class ii markers. The objective of this screen was to identify the region(s) of
381 heterozygosity required for the suppression of *Zim53;765* starvation sensitivity.
382 *Zim53;765* females were crossed to a series of flies deficient for various parts of
383 chromosome 3L. The deficient region of each line is maintained over a balancer
384 chromosome so the resulting offspring can be positive for the balancer, indicating
385 complete heterozygosity of chromosome 3 or only partially heterozygous for chromosome
386 3 and hemizygous for the deficient region. Significant differences in the starvation
387 resistance of the two classes of offspring indicates that heterozygosity of the missing
388 deficiency region is required for the suppression of starvation sensitivity. We initially
389 screened 10 deficiency lines and identified three where the starvation sensitivity was not
390 suppressed (failed to complement) in the partial chromosome 3L hemizygote (Figure 3a).
391 All three lines had deficient regions between 3L:9812381 – 3L:9899255 (Figure 3b),
392 indicating that heterozygosity of parts of the region is required for the suppression of
393 *Zim53;765* starvation sensitivity.

394 **Mutation in iPLA2-VIA is a candidate cause of *Zim53;765* starvation sensitivity**

395 Next, we sought to identify *Zim53;765* variants in the chromosome 3L region required for
396 suppression of *Zim53;765* starvation sensitivity which could underlie its sensitivity to

397 starvation. We utilized the available genomic sequencing data of each DGRP line to
398 identify 765 variants (Mackay et al. 2012). As the causal variant is deleterious, we focused
399 our initial analysis on low frequency variants (present in less than 10 % in the DGRP
400 population). We identified 83 variants which fit our criteria (Figure 4a), of these only 3
401 were predicted to lead to non-synonymous changes (Figure 4b and Table 1). A C-to-A
402 mutation, predicted to result in a G-to-V amino acid residue change in the conserved
403 GXGXXG motif of the phospholipase iPLA2-VIA protein, appeared as the most likely
404 candidate. Mutations in iPLA2-VIA have previously been shown to cause starvation
405 sensitivity and reduced lifespan in *Drosophila* (Kinghorn et al. 2015; Kinghorn and
406 Castillo-Quan 2016; Lin et al. 2018). In addition, PolyPhen-2 predicted the mutation as
407 probably damaging, with a score of 0.999 out of 1 (Adzhubei et al. 2010). Based on this
408 analysis, we decided to determine if *Zim53;765* has a functional copy of the iPLA2-VIA
409 gene.

410 ***iPLA2-VIA[Del174]* fail to complement *Zim53;765*/ DGRP-765 starvation sensitivity**
411 **and glucose homeostasis**
412 To determine if *Zim53;765* and as a consequence DGRP-765, results from a defective
413 copy of iPLA2-VIA, we sought to determine if the 765 copy of iPLA2-VIA gene could
414 complement an iPLA2-VIA null. We crossed *Zim53;765* virgin females to two previously
415 characterized iPLA2-VIA null lines (*iPLA2-VIA[Del174]*, *iPLA2-VIA[Del192],* caused by 2
416 different deletions generated by imprecise excision of a mobile element) (Lin et al. 2018).
417 To rule out the potential effects of mtDNA haplotype on starvation resistance, we also
418 utilized the DGRP-765 strain obtained from the Bloomington Stock center. We found that
419 progeny from DGRP-765 crossed *to iPLA2-VIA[Del174]* or *iPLA2-VIA[Del192]* were also
420 starvation sensitive (Figure 5). In contrast, crossing *Zim53;765*, *iPLA2-VIA[Del174]* or

421 *iPLA2-VIA[Del192]* to *Zim53;315* resulted in the suppression of starvation sensitivity
422 (Figure 5). Ablation or pharmaceutical inhibition of calcium independent phospholipases
423 have been shown to reduce insulin secretion in response to a glucose challenge (Song
424 et al. 2005; Ali et al. 2013). This raised the question of whether *Zim53;765* showed signs
425 of impaired glucose metabolism. We observed that following 12 hours of starvation,
426 *Zim53;765* significantly depletes its available free glucose in contrast to *Zim53;315*
427 suggesting improper regulation of glucose during acute starvation (Figure 6).
428 Interestingly, this phenotype was not observed in iPLA2-VIA[Del174] (Figure 6). However,
429 we found that progeny from *Zim53;765* crossed to iPLA2-VIA[Del174] also significantly
430 deplete their available free glucose following 12 hours of starvation (Figure 6). Taken
431 together, these results implicate iPLA2-VIA in *Zim53;765* starvation sensitivity, with a
432 parallel effect on glucose homeostasis. In addition, its effects extend beyond a simple
433 loss of function as evidenced by the *Zim53;765* specific phenotypes observed.

434 # Discussion

435 **Polygenic basis of *Zim53;765* starvation sensitivity**

436 Our advanced intercross population (AIP) design used a line at the extreme end of the
437 starvation resistance spectrum (*Zim53;765*, most sensitive) and another line more central
438 in the distribution. Though a compelling case could be made for the use a line at the
439 opposite end of the distribution (most resistant). We focused our effort on attempting to
440 identify why *Zim53;765* was starvation sensitive and reasoned that intercrossing
441 *Zim53;765* with *Zim53;315* would result in populations with the starvation sensitivity
442 allele(s) and the more common allele. We sequenced pools of flies from the extreme ends
443 of the AIP's starvation resistance distribution and found that there were several variants

444 with significant differences in allele frequencies. It is likely that many of these different
445 variants are in linkage disequilibrium since the populations recombined over a period of
446 only 10 generations. Nevertheless, the clustering of significant variants on chromosome
447 2 and 3 are suggestive of a polygenic basis for *Zim53;765* starvation sensitivity. This is
448 supported by the results of the chromosome segregation analysis. There we found
449 chromosome 3 heterozygosity was necessary to suppress *Zim53;765* starvation
450 sensitivity. In addition, heterozygosity of chromosome 2 and 3 together further improved
451 starvation resistance. Notably, chromosome 2 heterozygosity in isolation was not
452 sufficient to even partially suppress *Zim53;765* starvation sensitivity. This indicates that
453 the chromosome 2 loci/locus acts as a modifier that genetically interacts with the
454 chromosome 3 locus/loci. This may provide a potential explanation for why the vast
455 majority of significant variants identified were localized to chromosome 3L, while there
456 were clusters with fewer significant variants on chromosome 2L and 3R.

457 **Narrowing the main effect locus**

458 We attempted to narrow the target region, by generating recombinants between
459 *Zim53;765* and an unaffected genotype. We designed these crosses to be done with
460 multiple marked recessive stocks, as this would allow us to have a general idea of the
461 regions of recombination. We however failed to do this directly as crossing *Zim53;765*
462 females to males from each of the multiple marked recessive stocks failed to yield an
463 appreciable number of progeny. While not ideal, we decided to use the *line_765-hybrid*
464 to generate recombinants for a few reasons. Reciprocally crossing *line_765-hybrid* to
465 *Zim53;765* revealed that starvation resistances was not maternally inherited, suggesting
466 that mitochondrial DNA was not responsible for the phenotypic differences between the

467 two. *Zim53;765* and *line_765-hybrid* were generated using the same method described
468 in the materials and methods section, so a priori we knew that *line_765-hybrid* was a
469 hybridized version of 765 nuclear chromosomes, a fact confirmed by comparing the
470 alleles of *Zim53;765* variants to *line_765-hybrid*'s. The preponderance of the evidence
471 from the deficiency screen supported heterozygosity of the region of 3L 9812381 –
472 9899255 being required for suppression of *Zim53;765* starvation sensitivity. Four of the
473 six lines deficient for the region showed the expected result where suppression of
474 starvation sensitivity was masked in the hemizygous progeny. However, when *Zim53;765*
475 was crossed with Df(3L)BSC282, there was only a modest difference in the starvation
476 resistance of the heterozygous and homozygous progeny (Supplemental figure 2b). In
477 the case of *Zim53;765* crossed with Df(3L)BSC283, there was no statistical difference in
478 the starvation resistance of the heterozygous and homozygous progeny. It is possible
479 that the predicted deficient region of Df(3L)BSC282 and Df(3L)BSC283 are not
480 completely deleted.

481 **Complementation points to Zim53;765 iPLA2-VIA**

482 Of all the variants in the 3L 9812381 – 9899255 region, the nonsynonymous mutation
483 present in the calcium-independent phospholipase iPLA2-VIA immediately stood out, due
484 to the location of the mutation in the functional domain of the enzyme, and the phenotypes
485 previously associated with iPLA2-VIA loss of function. Mutations in the human homolog
486 PLA2G6 gene are characteristic of multiple autosomal recessive disease (Guo et al.
487 2018). Several studies have independently characterized starvation sensitivity in iPLA2-
488 VIA mutants (Kinghorn et al. 2015; Iliadi et al. 2018; Lin et al. 2018; Mori et al. 2019). In
489 each case, targeted mutations were introduced in iPLA2-VIA to generate null models,

490 with the goal of better understanding its role in neurodegeneration. These studies
491 identified aberrant lipid metabolism, lipid peroxidation and mitochondrial dysfunction as
492 potential causes of stress sensitivity and neurodegeneration. We should not necessarily
493 expect point mutations in iPLA2-VIA to universally have the same effect as these loss of
494 function models, as (Engel et al. 2010) mutations in PLA2G6 have varying effects on
495 catalytic activity. However, we can expect that if mutation(s) in iPLA2-VIA do in fact
496 underlie *Zim53;765* starvation resistance, an iPLA2-VIA null should fail to complement
497 *Zim53;765* starvation sensitivity. We showed that two separate nulls generated by (Lin et
498 al. 2018) do in fact fail to complement *Zim53;765* starvation sensitivity, indicating iPLA2-
499 VIA underlies *Zim53;765* starvation sensitivity.
500
501 Some phospholipases are have been associated with modulation of insulin secretion in
502 response to glucose by releasing arachidonic acid (AA), and exogenous AA
503 supplementation is sufficient to promote insulin release from rat islet cells (Metz 1988a).
504 This insulin secretion is believed to promote phospholipid hydrolysis and accumulation of
505 phospholipid-derived intermediates (Metz 1988b). This turnover of phospholipids could
506 promote insulin release, creating an interesting dynamic between glucose and lipid
507 metabolism, with phospholipases at the center. The premature depletion of glucose and
508 glycogen in *Zim53;765* early during starvations could be one consequence of its mutated
509 iPLA2-VIA. Consistent with this, we found that iPLA2-VIA[Del174] fail to complement the
510 premature glucose and glycogen depletion phenotypes associated with *Zim53;765*
511 (Figure 6). What is especially interesting is that the iPLA2-VIA null themselves do not
512 display this phenotype. This raises the possibility that affected pathways which ultimately

513 result in starvation sensitivity differ between the deletion mutation of iPLA2-VIA[Del174]
514 and the point mutation unique to *Zim53;765*. It will be an interesting target of a future
515 study to characterize the additional molecular bases underlying the starvation sensitivity
516 of *Zim53;765,* as this could potentially highlight novel roles for iPLA2-VIA and asses the
517 effects of the *Zim53;765* specific mutations have on the function of iPLA2-VIA.
518

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523 resistance, which provided the impetus for this work.
524

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528 acknowledges support of COBRE award P20GM109035 and MIRA R35GM139607
529

530 Conflicts of Interest

531 None declared.
532
533

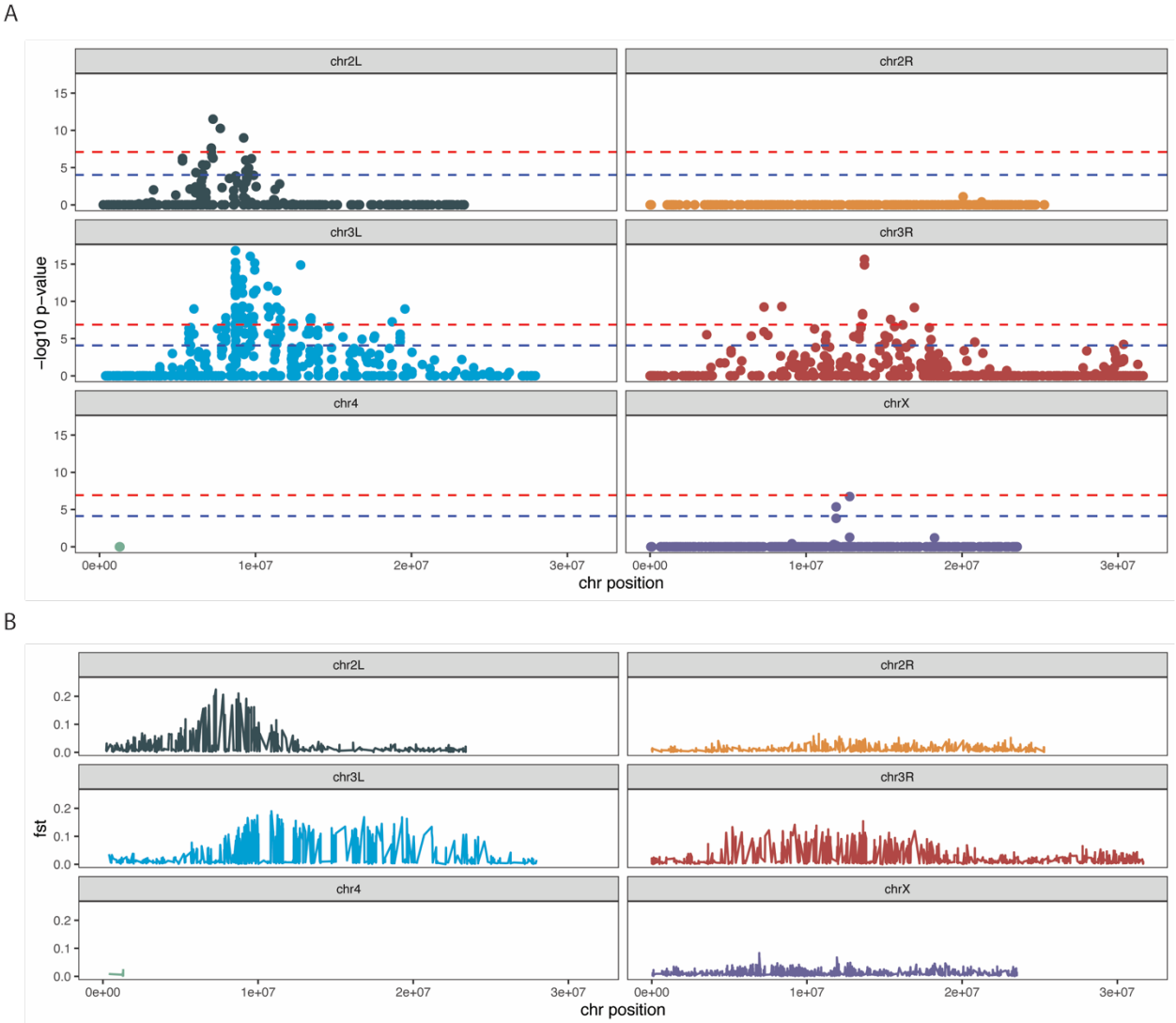


Figure 1. Significant allelic frequency differences between the tails of replicate advanced intercross populations subjected to starvation stress.
An advanced intercross population was created by crossing *Zim53;765* and *sil;315* in reciprocal. The hybrids were allowed to recombine freely for 10 generations before being subjected to starvation stress. Dead flies were collected every 6 hours and dead flies from the first 3 timepoints were combined and subjected to sequencing analysis. The same was done with the last two timepoints. In total 5 replicates AIP populations were generated and sequenced. (A) Consistent differences in allele frequencies across replicates were determined using the Cochran-Mantel-Haenszel (CMH) test and Popoolation2. The y-axis is the negative log-transformed, Bonferroni corrected p-value of each SNP (represented by each circle). The x-axis is the position of each SNP along the indicated chromosome. Blue and red lines highlight corrected p-values above $1x10^{-5}$ and $1x10^{-6}$ respectively. (B) F_{ST} between pools for each site averaged across replicate populations. The location of elevated FST values is generally concordant with significant CMH test statistics.

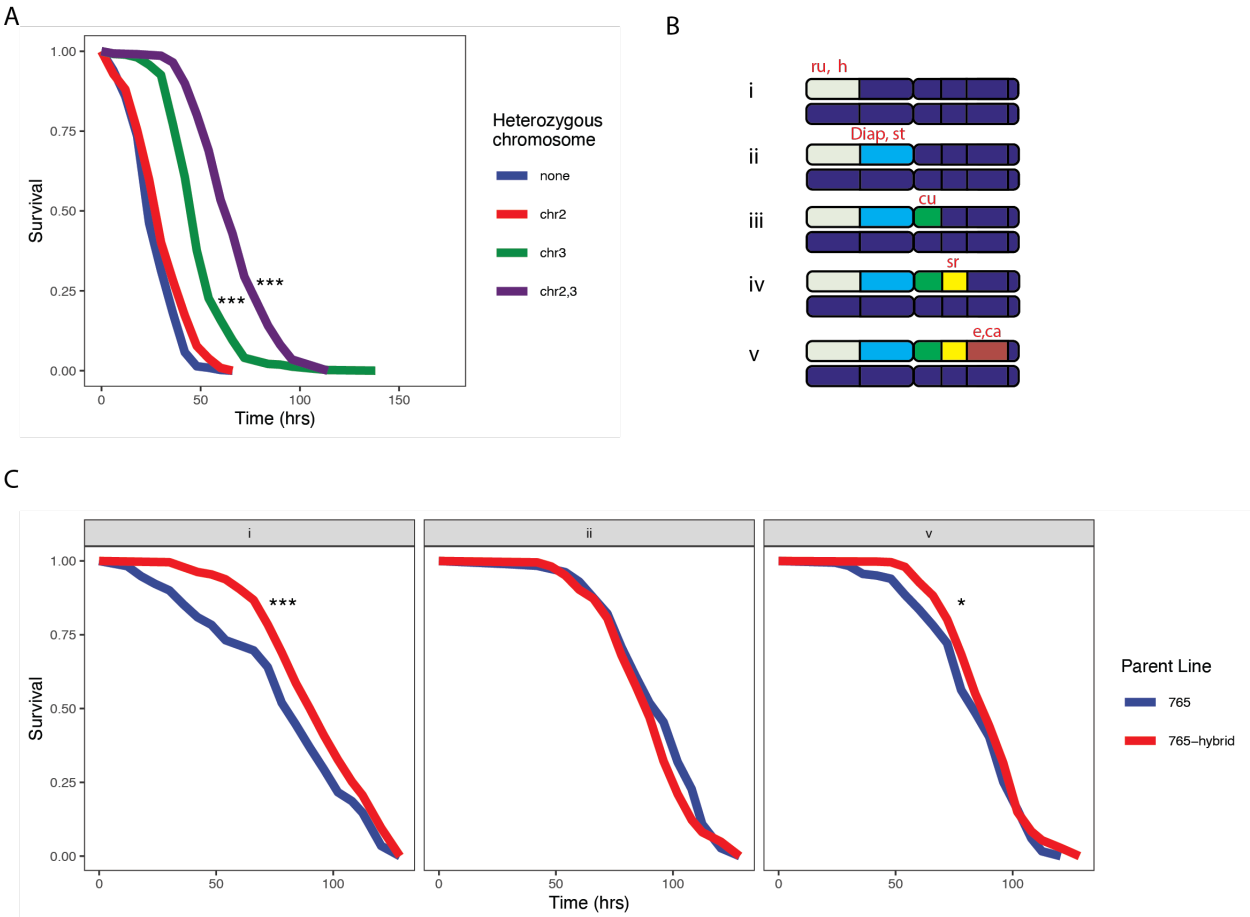


Figure 2. Heterozygosity of chromosome 3 partially suppresses *Zim53;765* starvation sensitivity

(A) Survival of female *Zim53;765* flies heterozygous for no chromosome, chromosome 2, chromosome 3 or chromosome 2 and 3 following starvation stress. Differences in survival relative to flies with no marker (none) were determined using a log-rank test (implemented in the R survival package) and included in the supplemental relevant statistics file.(B) Cartoon representation of recombination breakpoints for each recombinant class generated through meiotic recombination of the chromosome 3 mapping line. (C) Survival of female offspring produced by crossing each recombinant class to *Zim53;765* homozygotes or heterozygotes. Differences in survival were determined using a log-rank test (implemented in the R survival package) and included in the supplemental relevant statistics file.

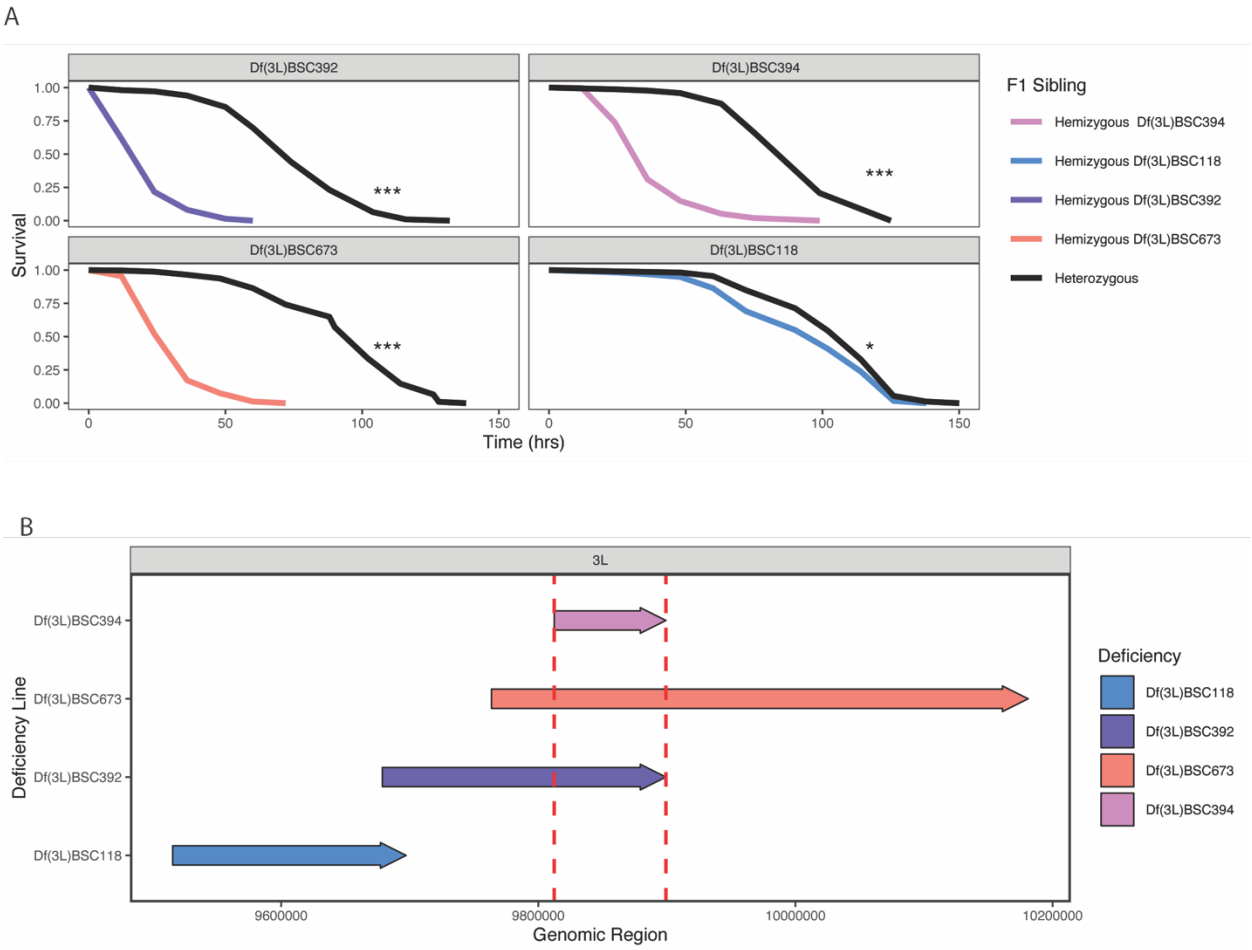


Figure 3. Hemizygosity of 3L: 9812381 – 9899255 prevents partial suppression of *Zim53;765* starvation sensitivity

(A) Survival of offspring from the cross between *Zim53;765* and the respective deficient male following starvation stress. Heterozygous refers to progeny which inherited the balancer chromosome, hemizygous refers to progeny which inherited the deficient chromosome. Line numbers correspond to Bloomington Stock Center names. Differences in survival was determined using a log-rank test (implemented in the R survival package) and included in the supplemental relevant statistics file. (B) Linear representation of hemizygous regions of respective deficiency lines. Highlighted region is common to the three deficiency regions which failed to suppress *Zim53;765* starvation sensitivity.

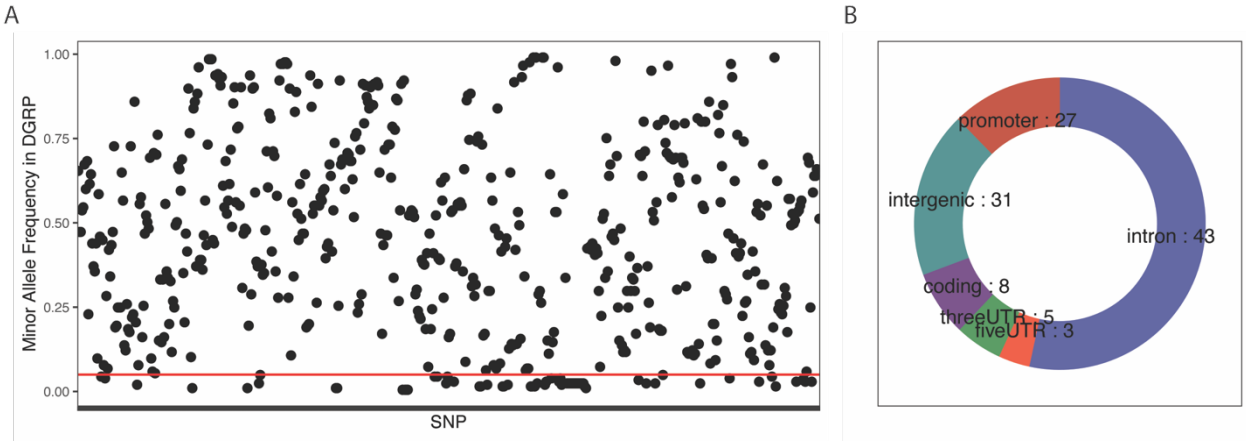
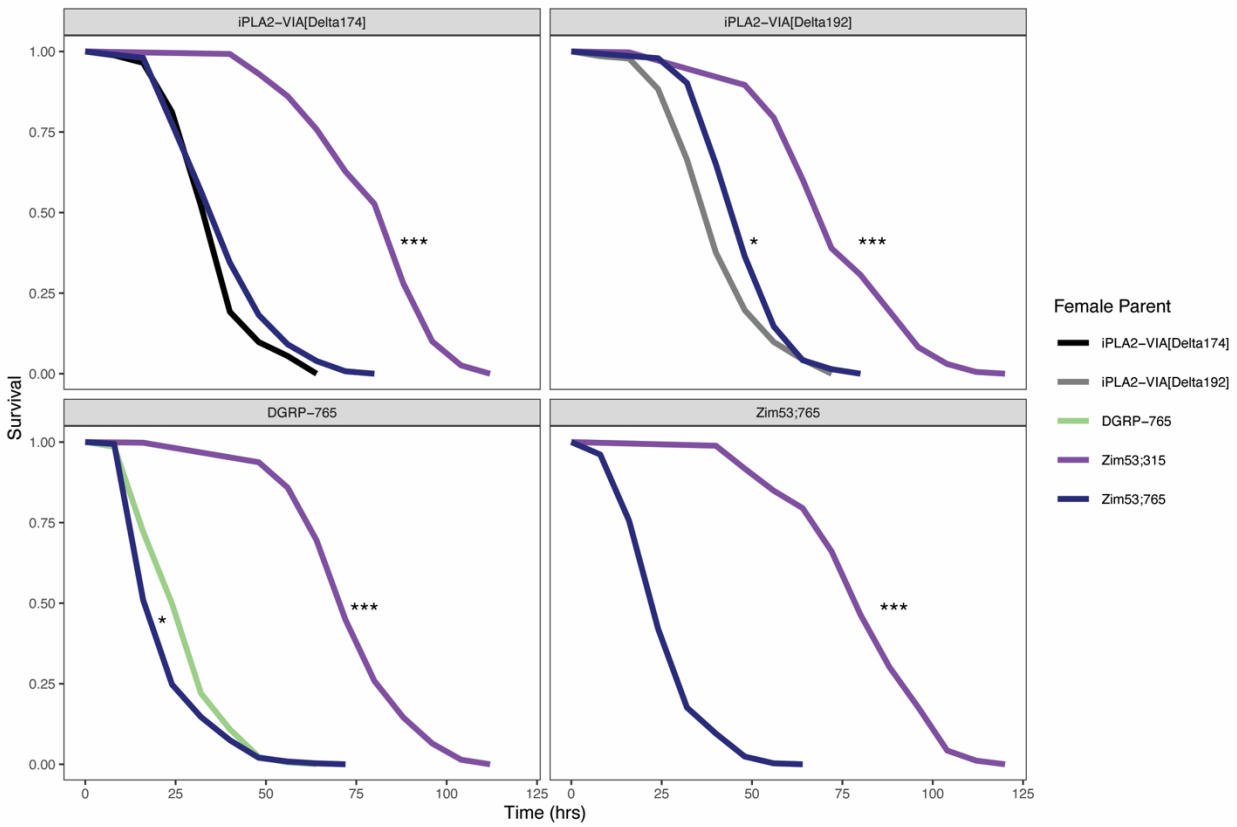


Figure 4. Frequency of 765 variants among the Drosophila Genetics Reference Panel

(A) Allele frequency of 765 variants in the 3L candidate region among the lines of the Drosophila Genetics Reference Panel. Variants found below the red line are present in less than ten percent of the populations. (B) Analysis of the types of low frequency (minor allele frequency less that 0.1 in the Drosophila Genetics Reference Panel) variants in 3L candidate region present in 765.



588
589 **Figure 5. *Zim53;765* iPLA2-VIA fails to complement iPLA2-VIA null lines**
590 Survival of female progeny from indicated crosses following starvation stress. Panels
591 are divided by male parent and colors segregate progeny from each type of female
592 parent. Differences in survival were determined using a log-rank test (implemented in
593 the R survival package) and included in the supplemental relevant statistics file.
594

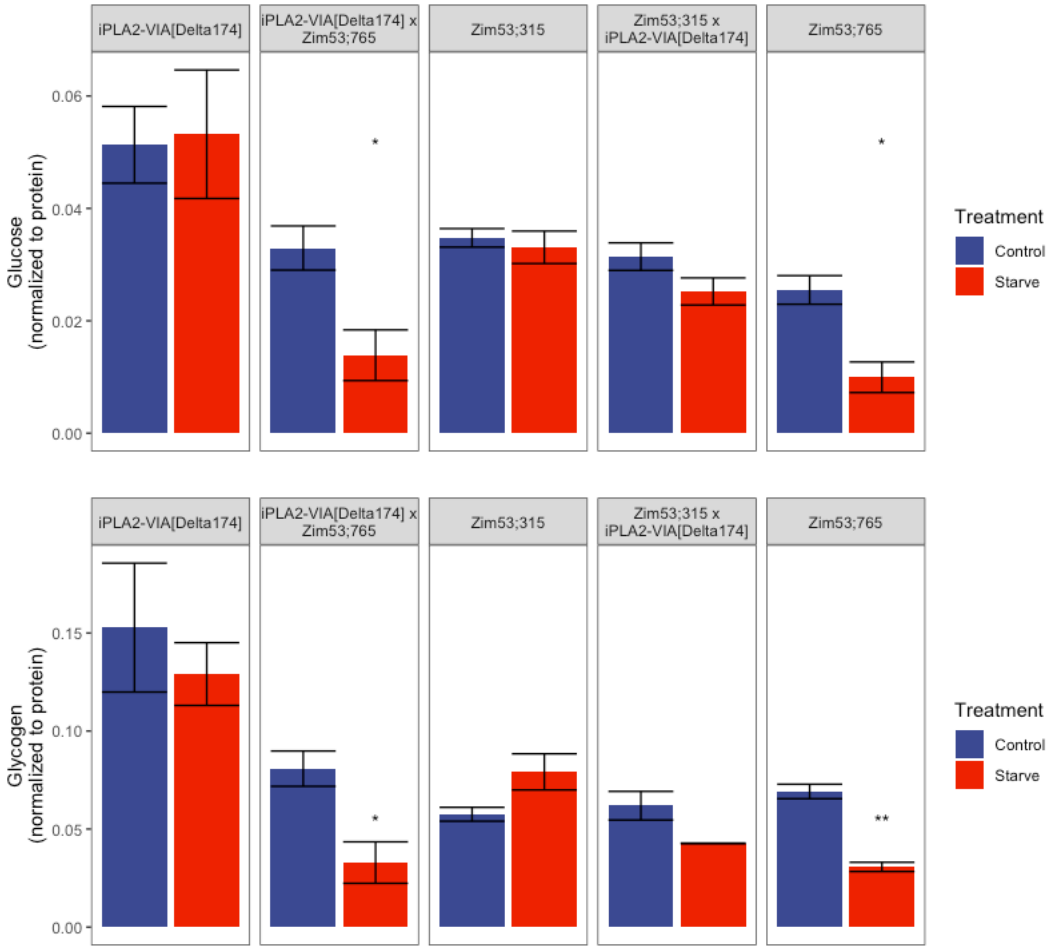
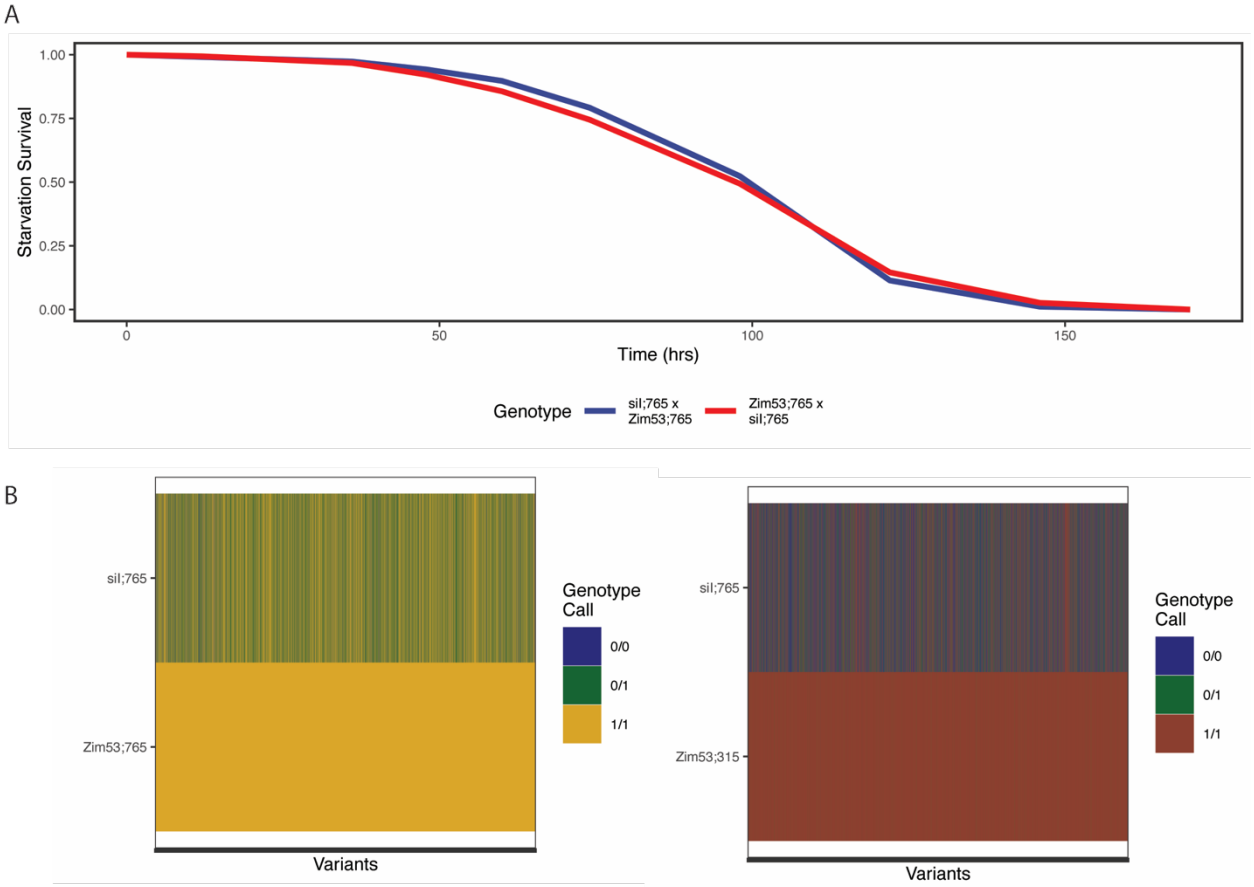


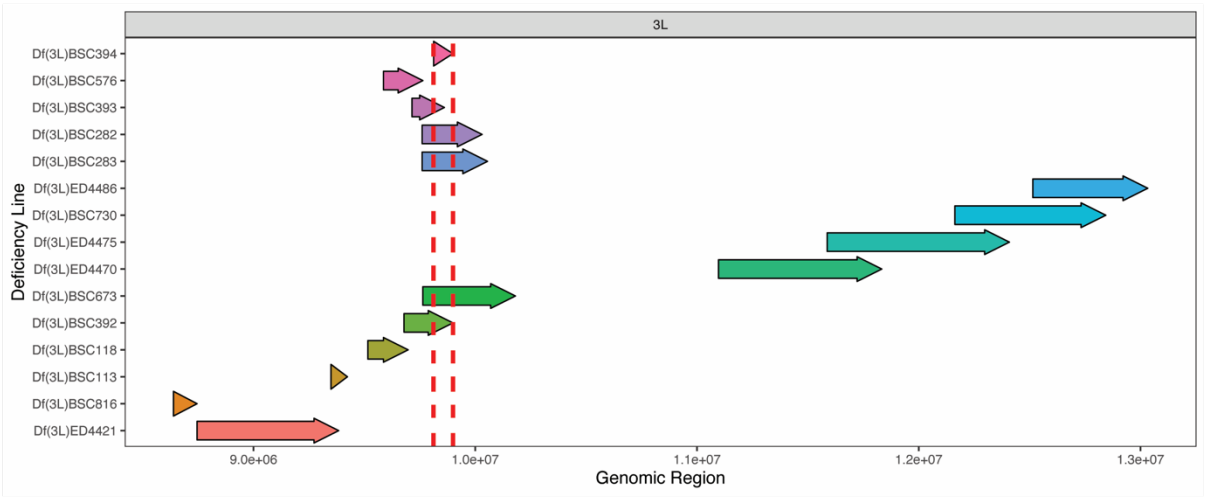
Figure 6. iPLA2-VIA[Delta174] fails to complement glucose and glycogen depletion phenotype of *Zim53;765*

The free glucose and total glycogen levels were measured for *Zim53;765*, *Zim53;315*, iPLA2-VIA[Delta174], *Zim53;315* crossed to iPLA2-VIA[Delta174] and *Zim53;765* crossed to iPLA2-VIA[Delta174]. Values in the un-starved (control) was compared to the values in the starved samples (12 hours) for 3 replicates of 6 flies using a student's t-test. Significant differences are indicated by * <0.05, ** <0.01.



Supplemental Figure 1. *sil;765* is a hybrid of DGRP-765
(A) Survival of female offspring of *Zim53;765,* and *sil;765* crossed in reciprocal. There is no statistical difference in survival based on a log-rank test for significance. (B and C) Genotype of *sil;765* at homozygous variant sites in RNA sequencing reads from (C) *Zim53;765* and (D) *Zim53;315* Green indicates that *sil;765* was heterozygous for both the variant and the reference allele, blue indicates that *sil;765* was homozygous for the reference allele.

A



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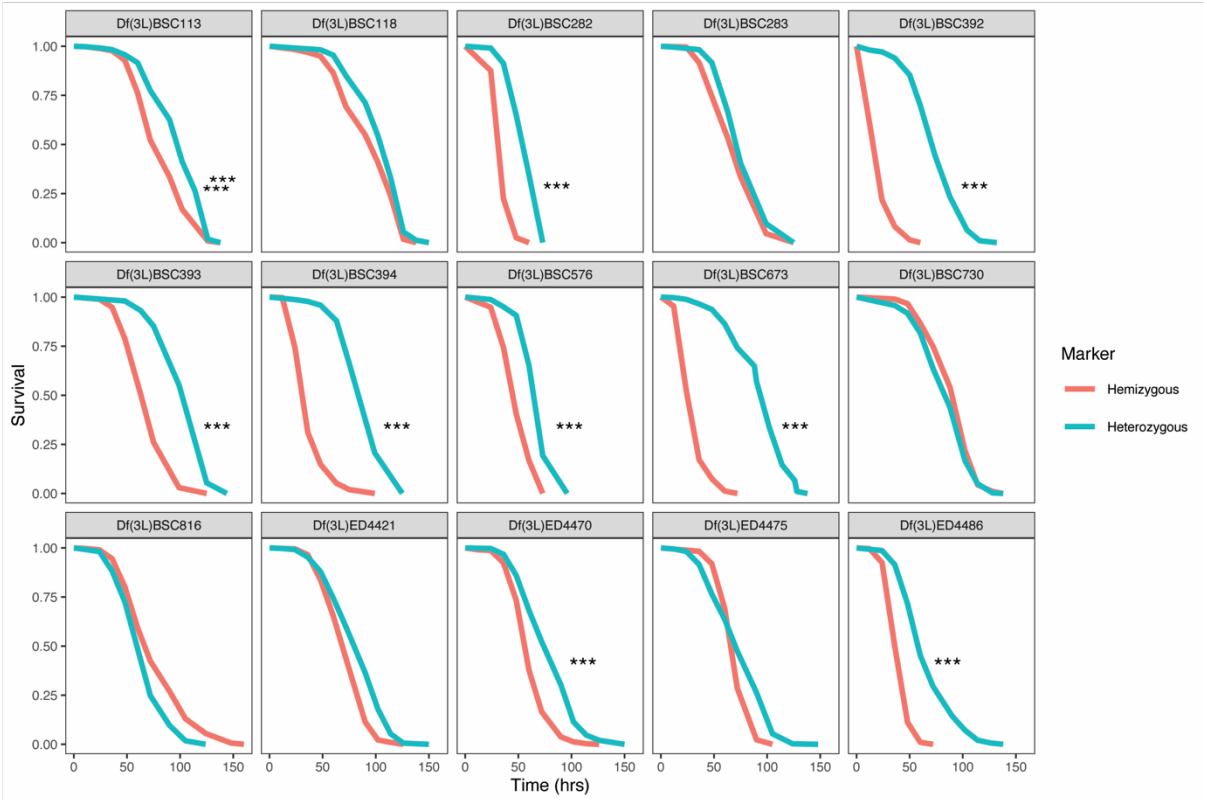

Supplemental Figure 2

(A) Linear representation of deficient region of respective deficient lines in region identified in meiotic recombination mapping. (B) Survival of offspring from the cross between Zim53;765 and the respective deficient male following starvation stress. Differences in survival were determined using a log-rank test (implemented in the R survival package) and included in the supplemental relevant statistics file. Heterozygous refers to progeny which inherited balancer chromosome, hemizygous refers to progeny

626 which inherited deficient chromosome. Line numbers correspond to Bloomington Stock
627 Center names.

References

Adzhubei, I. A., S. Schmidt, L. Peshkin, V. E. Ramensky, A. Gerasimova *et al.*, 2010 A method and server for predicting damaging missense mutations. Nat Methods 7**:** 248-249.

Ali, T., G. Kokotos, V. Magrioti, R. N. Bone, J. A. Mobley *et al.*, 2013 Characterization of FKGK18 as inhibitor of group VIA Ca2+-independent phospholipase A2 (iPLA2beta): candidate drug for preventing beta-cell apoptosis and diabetes. PLoS One 8**:** e71748.

Brown, E. B., M. E. Slocumb, M. Szuperak, A. Kerbs, A. G. Gibbs *et al.*, 2019 Starvation resistance is associated with developmentally specified changes in sleep, feeding and metabolic rate. J Exp Biol 222.

Engel, L. A., Z. Jing, D. E. O'Brien, M. Sun and P. T. Kotzbauer, 2010 Catalytic function of PLA2G6 is impaired by mutations associated with infantile neuroaxonal dystrophy but not dystonia-parkinsonism. PLoS One 5**:** e12897.

Feder, A. F., D. A. Petrov and A. O. Bergland, 2012 LDx: estimation of linkage disequilibrium from high-throughput pooled resequencing data. PLoS One 7**:** e48588.

Frazer, K. A., S. S. Murray, N. J. Schork and E. J. Topol, 2009 Human genetic variation and its contribution to complex traits. Nat Rev Genet 10**:** 241-251.

Guo, Y. P., B. S. Tang and J. F. Guo, 2018 PLA2G6-Associated Neurodegeneration (PLAN): Review of Clinical Phenotypes and Genotypes. Front Neurol 9**:** 1100.

Harbison, S. T., A. H. Yamamoto, J. J. Fanara, K. K. Norga and T. F. C. Mackay, 2004 Quantitative Trait Loci Affecting Starvation Resistance in Drosophila melanogaster. Genetics 166**:** 1807-1823.

Hardy, C. M., M. K. Burke, L. J. Everett, M. V. Han, K. M. Lantz *et al.*, 2018 Genome-Wide Analysis of Starvation-Selected Drosophila melanogaster-A Genetic Model of Obesity. Mol Biol Evol 35**:** 50-65.

Iliadi, K. G., O. B. Gluscencova, N. Iliadi and G. L. Boulianne, 2018 Mutations in the Drosophila homolog of human PLA2G6 give rise to age-dependent loss of psychomotor activity and neurodegeneration. Sci Rep 8**:** 2939.

Kinghorn, K. J., and J. I. Castillo-Quan, 2016 Mitochondrial dysfunction and defects in lipid homeostasis as therapeutic targets in neurodegeneration with brain iron accumulation. Rare Dis 4**:** e1128616.

Kinghorn, K. J., J. I. Castillo-Quan, F. Bartolome, P. R. Angelova, L. Li *et al.*, 2015 Loss of PLA2G6 leads to elevated mitochondrial lipid peroxidation and mitochondrial dysfunction. Brain 138**:** 1801-1816.

Kofler, R., R. V. Pandey and C. Schlotterer, 2011 PoPoolation2: identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). Bioinformatics 27**:** 3435-3436.

Lango Allen, H., K. Estrada, G. Lettre, S. I. Berndt, M. N. Weedon *et al.*, 2010 Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature 467**:** 832-838.

673 Li, H., and R. Durbin, 2009 Fast and accurate short read alignment with Burrows-Wheeler
674 transform. Bioinformatics 25**:** 1754-1760.
675 Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan *et al.*, 2009 The Sequence
676 Alignment/Map format and SAMtools. Bioinformatics 25**:** 2078-2079.
677 Lin, G., P. T. Lee, K. Chen, D. Mao, K. L. Tan *et al.*, 2018 Phospholipase PLA2G6, a
678 Parkinsonism-Associated Gene, Affects Vps26 and Vps35, Retromer Function,
679 and Ceramide Levels, Similar to alpha-Synuclein Gain. Cell Metab 28**:** 605-618
680 e606.
681 Mackay, T. F., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles *et al.*, 2012 The
682 Drosophila melanogaster Genetic Reference Panel. Nature 482**:** 173-178.
683 Mackay, T. F., E. A. Stone and J. F. Ayroles, 2009 The genetics of quantitative traits:
684 challenges and prospects. Nat Rev Genet 10**:** 565-577.
685 Mackay, T. F. C., and W. Huang, 2018 Charting the genotype-phenotype map: lessons
686 from the Drosophila melanogaster Genetic Reference Panel. Wiley Interdiscip Rev
687 Dev Biol 7.
688 Metz, S. A., 1988a Exogenous arachidonic acid promotes insulin release from intact or
689 permeabilized rat islets by dual mechanisms. Putative activation of Ca2+
690 mobilization and protein kinase C. Diabetes 37**:** 1453-1469.
691 Metz, S. A., 1988b Membrane Phospholipid Turnover as an Intermediary Step in Insulin-
692 Secretion - Putative Roles of Phospholipases in Cell Signaling. American Journal
693 of Medicine 85**:** 9-21.
694 Mori, A., T. Hatano, T. Inoshita, K. Shiba-Fukushima, T. Koinuma *et al.*, 2019 Parkinson's
695 disease-associated iPLA2-VIA/PLA2G6 regulates neuronal functions and alpha-
696 synuclein stability through membrane remodeling. Proc Natl Acad Sci U S A 116**:**
697 20689-20699.
698 Mossman, J. A., L. M. Biancani, C. T. Zhu and D. M. Rand, 2016 Mitonuclear Epistasis
699 for Development Time and Its Modification by Diet in Drosophila. Genetics 203**:**
700 463-484.
701 Pool, J. E., 2016 Genetic Mapping by Bulk Segregant Analysis in Drosophila:
702 Experimental Design and Simulation-Based Inference. Genetics 204**:** 1295-1306.
703 Smith, S. P., S. Shahamatdar, W. Cheng, S. Zhang, et al. , 2022 Enrichment analyses
704 identify shared associations for 25 quantitative traits in over 600,000 individuals
705 from seven diverse ancestries. Am J Hum Genet. 2022 May 5;109(5):871-884.
706 Song, K., X. Zhang, C. Zhao, N. T. Ang and Z. A. Ma, 2005 Inhibition of Ca2+-independent
707 phospholipase A2 results in insufficient insulin secretion and impaired glucose
708 tolerance. Mol Endocrinol 19**:** 504-515.
709 Tennessen, J. M., W. E. Barry, J. Cox and C. S. Thummel, 2014 Methods for studying
710 metabolism in Drosophila. Methods 68**:** 105-115.
711 Therneau, T. M., and P. M. Grambsch, 2000 *Modeling survival data : extending the Cox*
712 *model*. Springer, New York.
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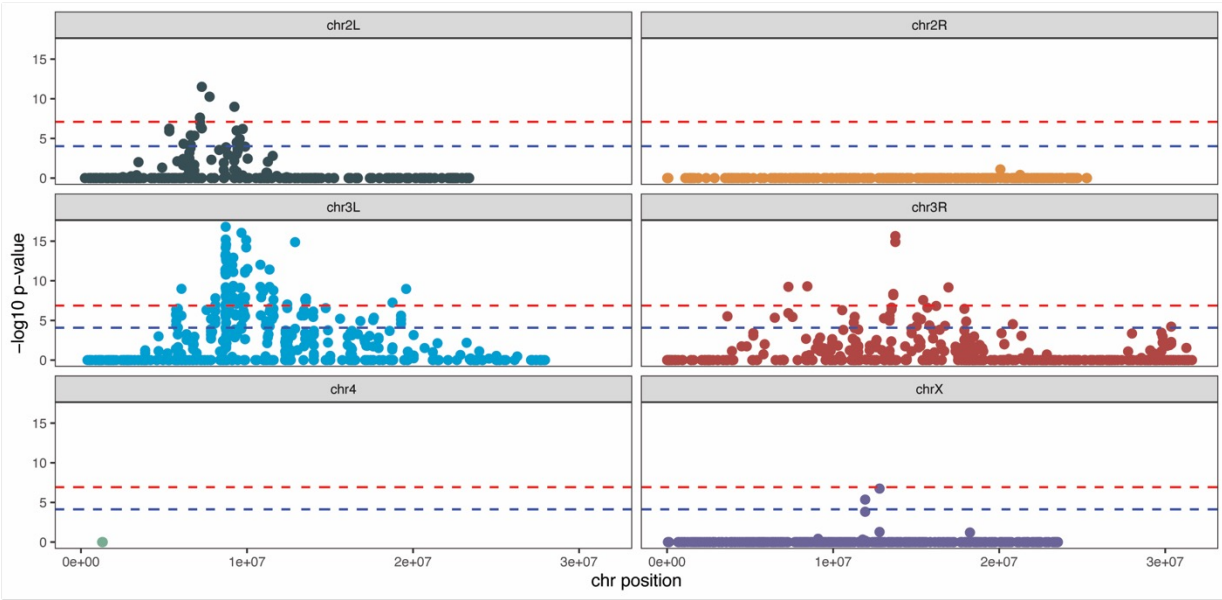
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chr position

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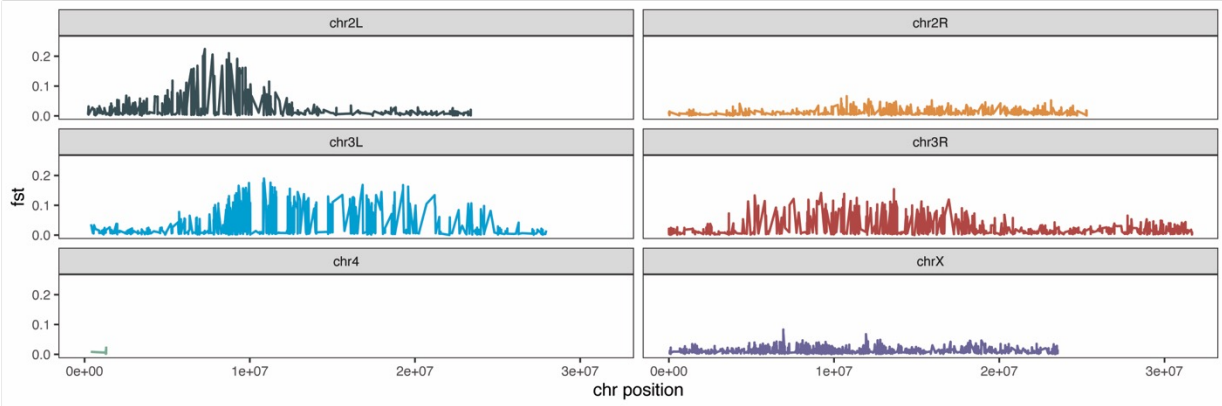
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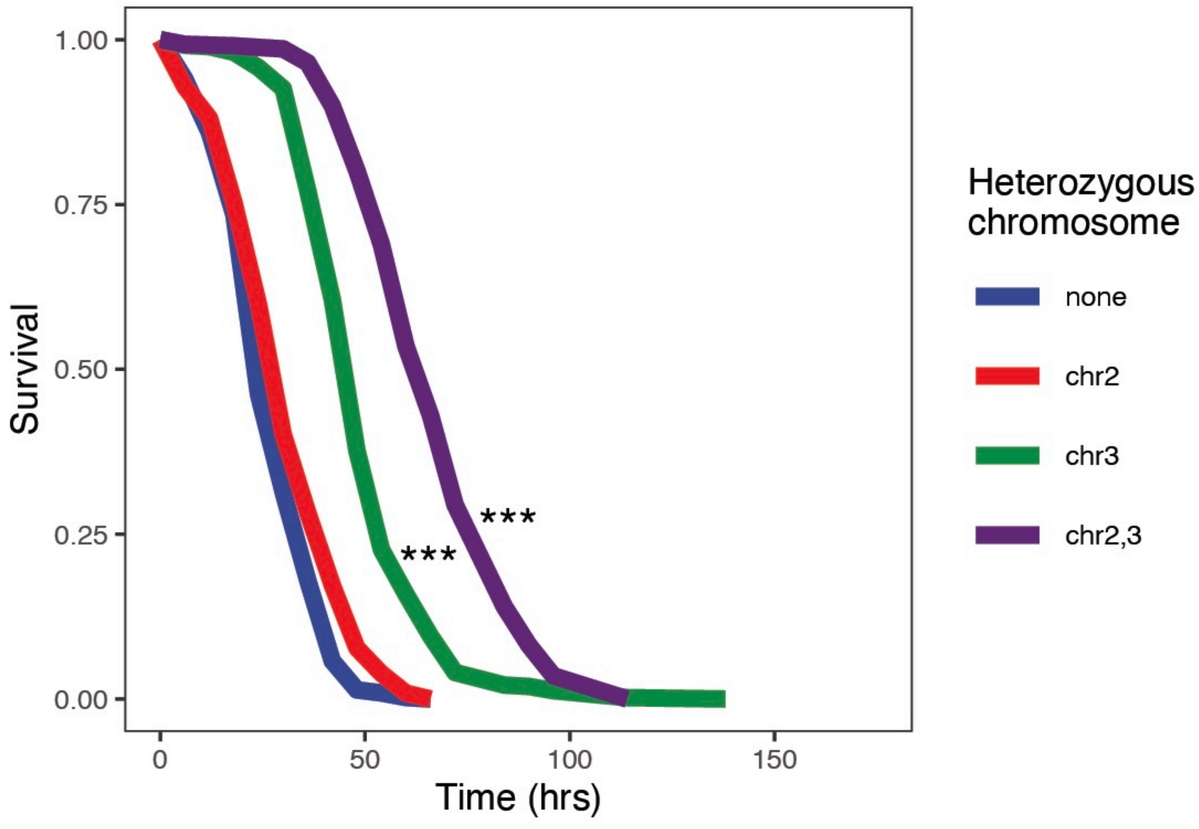
1.00
0.75
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Survival
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Time (hrs)
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chr3
chr2,3

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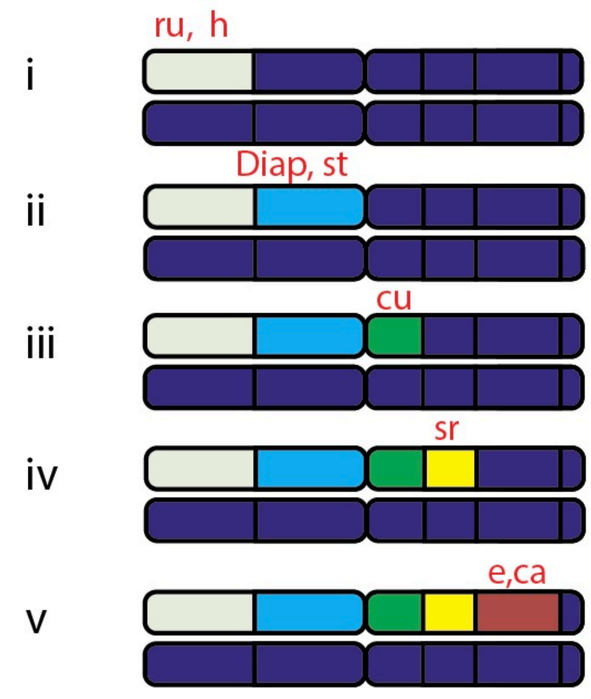
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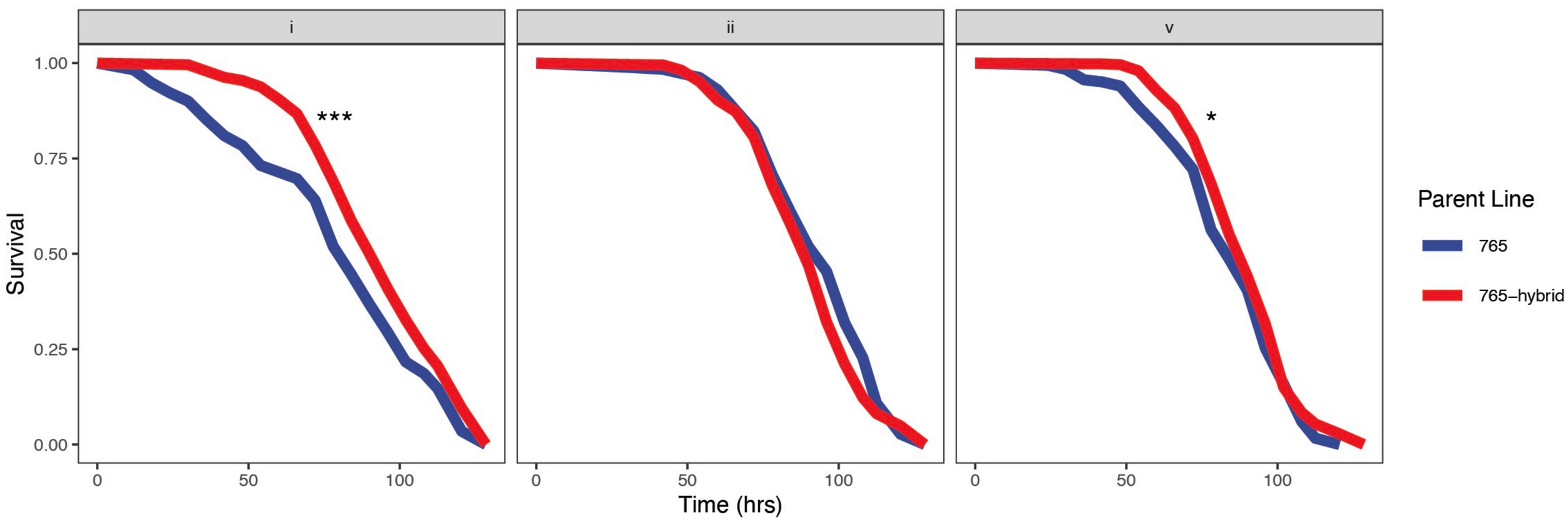
i
ii
v
1.00
0.75
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Survival
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Time (hrs)
Parent Line
765
765–hybrid

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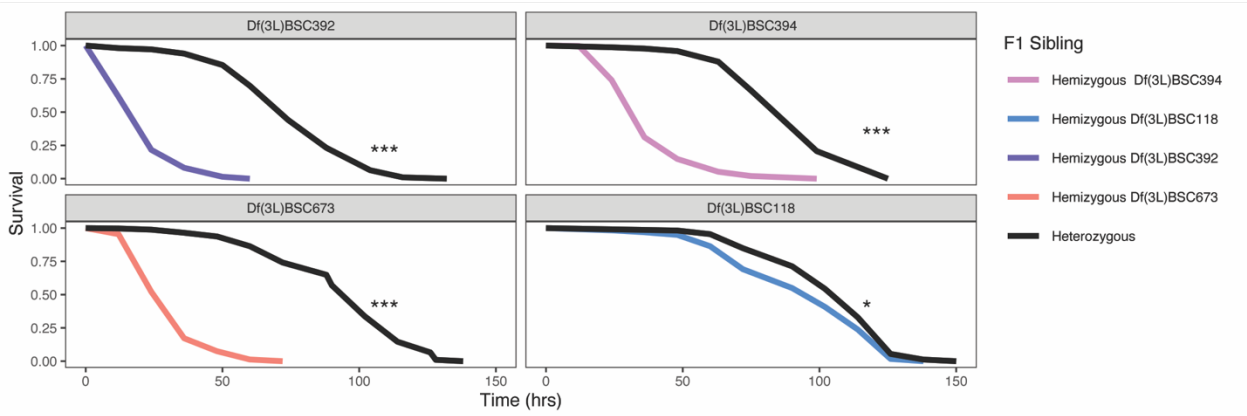
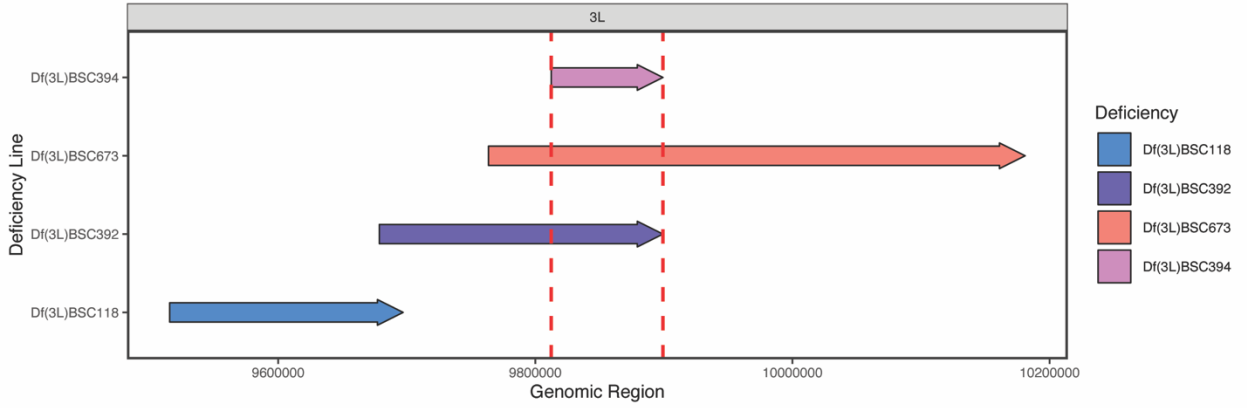

Df(3L)BSC392
Df(3L)BSC394
Df(3L)BSC673
Df(3L)BSC118
Survival
1.00
0.75
0.50
0.25
0.00
***
*
0
50
100
150
Time (hrs)
F1 Sibling
Hemizygous Df(3L)BSC394
Hemizygous Df(3L)BSC118
Hemizygous Df(3L)BSC392
Hemizygous Df(3L)BSC673
Heterozygous

B


3L
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Df(3L)BSC673
Df(3L)BSC392
Df(3L)BSC118
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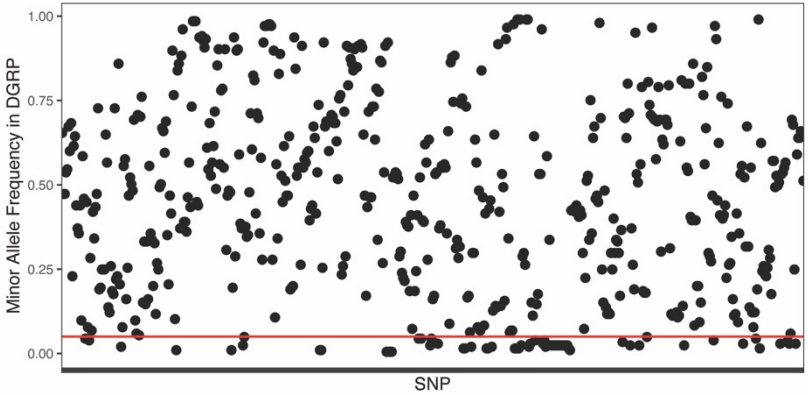
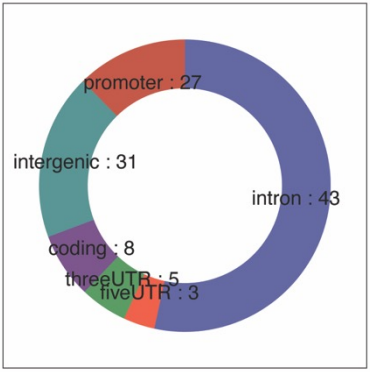
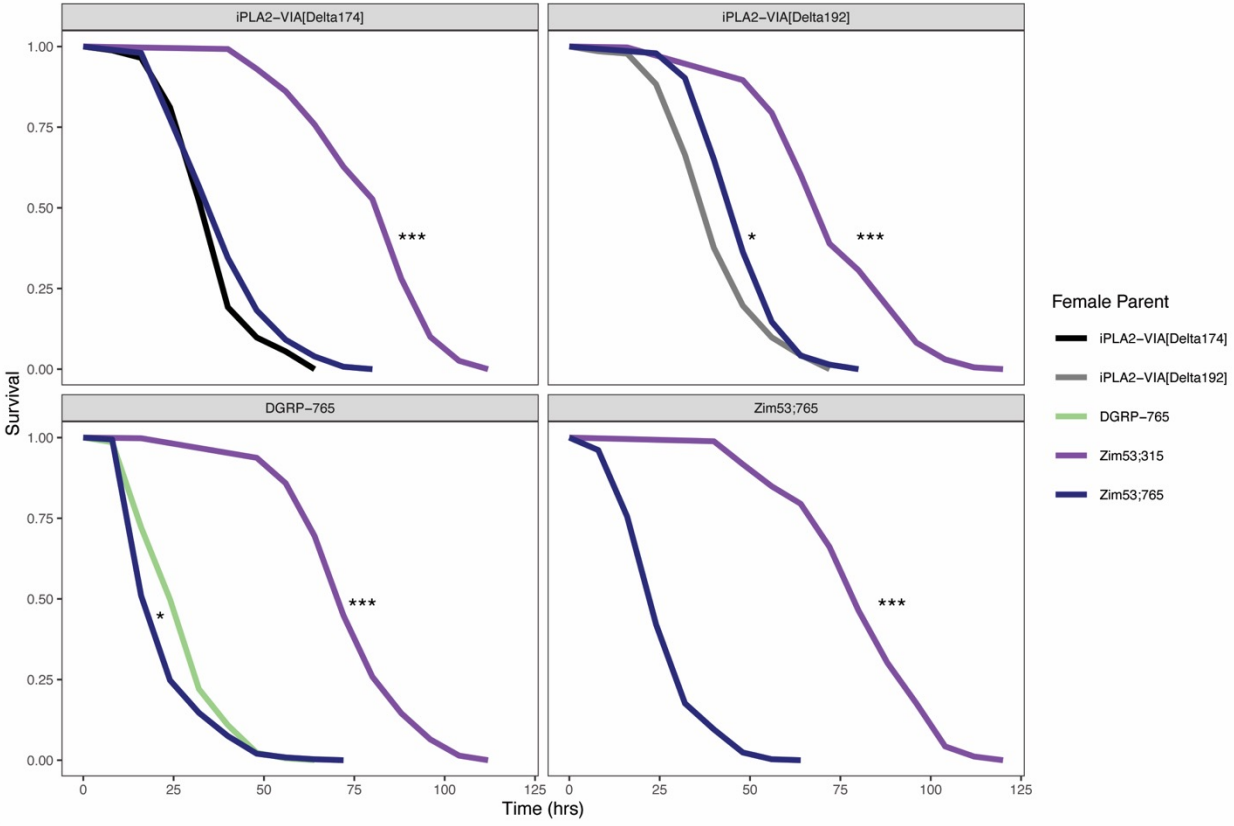
9800000
10000000
10200000
Genomic Region
Deficiency
Df(3L)BSC118
Df(3L)BSC392
Df(3L)BSC673
Df(3L)BSC394

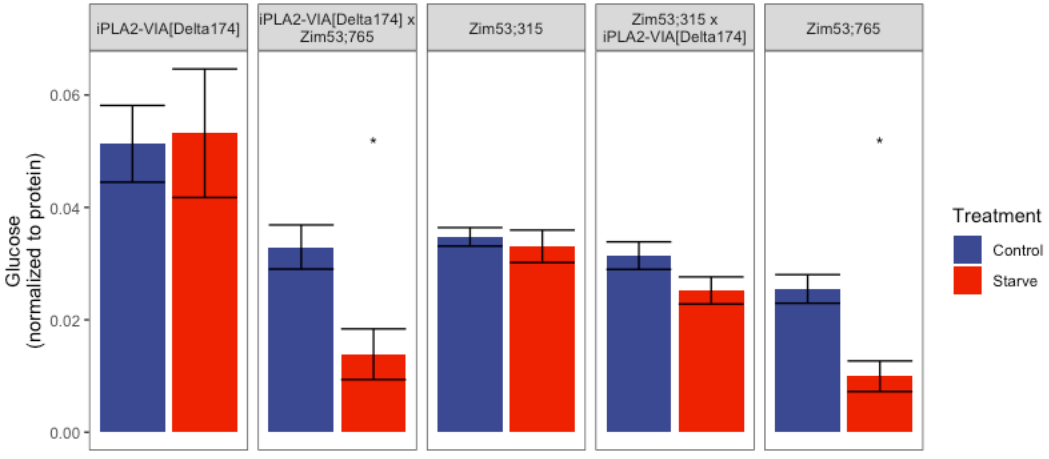
A

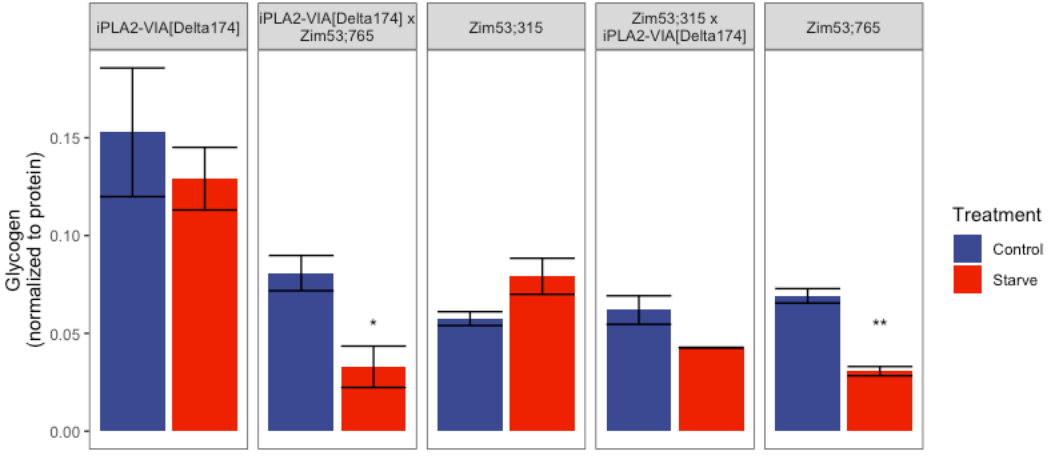

1.00
0.75
0.50
0.25
0.00
Minor Allele Frequency in DGRP
SNP

B


promoter : 27
intergenic : 31
intron : 43
coding : 8
threeUTR : 5
fiveUTR : 3


iPLA2−VIA[Delta174]
iPLA2−VIA[Delta192]
DGRP−765
Zim53;765
Survival
1.00
0.75
0.50
0.25
0.00
***
*
0
25
50
75
100
125
Time (hrs)
Female Parent
iPLA2−VIA[Delta174]
iPLA2−VIA[Delta192]
DGRP−765
Zim53;315
Zim53;765


iPLA2-VIA[Delta174]
iPLA2-VIA[Delta174] x Zim53;765
Zim53;315
Zim53;315 x iPLA2-VIA[Delta174]
Zim53;765
Glucose (normalized to protein)
0.06
0.04
0.02
0.00
*
*
Treatment
Control
Starve


iPLA2-VIA[Delta174]
iPLA2-VIA[Delta174] x Zim53;765
Zim53;315
Zim53;315 x iPLA2-VIA[Delta174]
Zim53;765
Glycogen (normalized to protein)
0.15
0.10
0.05
0.00
*
**
Treatment
Control
Starve

Chromosome	start	end	REF	ALT	GENEID	CONSEQUENCE	REFCODON
chr3L	9824166	9824166	C	T	FBgn0264489	nonsynonymous	CCA
chr3L	9833935	9833935	G	A	FBgn0004390	synonymous	AGC
chr3L	9854881	9854881	C	A	FBgn0036053	nonsynonymous	GGC
chr3L	9880027	9880027	A	G	FBgn0011836	synonymous	TTT
chr3L	9880564	9880564	G	T	FBgn0011836	synonymous	ATC
chr3L	9881643	9881643	C	A	FBgn0011836	nonsynonymous	GAG
chr3L	9884329	9884329	A	T	FBgn0025866	synonymous	ATA
chr3L	9884605	9884605	A	G	FBgn0025866	synonymous	GGA

VARCODON	REFAA	VARAA	SYMBOL
TCA	P	S	CG43897
AGT	S	S	RasGAP1
GTC	G	V	iPLA2-VIA
TTC	F	F	Taf2
ATA	I	I	Taf2
GAT	E	D	Taf2
ATT	I	I	CalpB
GGG	G	G	CalpB