

Pleiotropic fitness effects of the *Tre1-Gr5a* region in *Drosophila melanogaster*

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The abundance of transposable elements and DNA repeat sequences in mammalian genomes raises the question of whether such insertions represent passive evolutionary baggage or may influence the expression of complex traits. We addressed this question in *Drosophila melanogaster*, in which the effects of single transposable elements on complex traits can be assessed in genetically identical individuals reared in controlled environments¹. Here we demonstrate that single *P*-element insertions in the intergenic region between the gustatory receptor 5a (*Gr5a*, also known as *Tre*)²⁻⁴ and trapped in endoderm 1 (*Tre1*)⁵, which encodes an orphan receptor, exert complex pleiotropic effects on fitness traits, including selective nutrient intake, life span, and resistance to starvation and heat stress. Mutations in this region interact epistatically with downstream components of the insulin signaling pathway. Transposon-induced sex-specific and sex-antagonistic effects further accentuate the complex influences that intergenic transposable elements can contribute to quantitative trait phenotypes.

In a landmark paper in 1975 (ref. 6), it was noted that minor differences in protein sequences are unlikely to account for the profound morphological, physiological and behavioral differences among close relatives like chimpanzees and humans. Since then, their argument that the primary forces driving evolution act on regulatory elements rather than on coding regions has received ample experimental support^{7,8}. This notion is underscored by the recent completion of the draft sequence of the chimpanzee genome, which shows about 99% overall amino acid sequence identity between humans and their closest relative⁹.

The most notable difference between the chimpanzee and human genomes is the number and diversity of transposable element insertions. The human genome contains 7,082 *Alu* elements, threefold more than the chimpanzee genome. In contrast, the chimpanzee genome has acquired two new families of retroviral elements⁹. Given that only 2% of the human genome encodes genes, whereas the rest consists of transposons, various nucleotide repeats and what is

often referred to as 'junk DNA', these observations raise a profound question: to what extent do transposable element insertions influence the manifestation of complex traits? It is not possible to address this question in humans, because the vast number of short interspersed nuclear elements (SINEs), long interspersed nuclear elements (LINEs) and microsatellite insertions render it virtually impossible to resolve phenotypic effects of single insertions. *D. melanogaster* is an excellent system for assessing phenotypic effects of transposable elements, as many genetically identical individuals with single transposable element insertions can readily be generated and reared under controlled environmental conditions¹. We identified four co-isogenic lines with *P*-element insertions on the X chromosome in the intergenic region between *Tre1* and *Gr5a*¹⁰. *Gr5a* encodes a gustatory receptor for trehalose, the principal metabolic sugar in the hemolymph of insects²⁻⁴. *Tre1* has a role in transepithelial cell migration of *D. melanogaster* germ cells⁵. The *P*-element insertions in two of the lines are in the same genetic background and location, but oriented in opposite directions. In the other two lines, the *P*-element insertions are in the same location and orientation, but in different co-isogenic backgrounds.

We assessed the effects of these *P*-element insertions on taste preference, as *Gr5a* is a trehalose receptor²⁻⁴. Because ablation of gustatory or olfactory neurons results in alteration of life span in *Caenorhabditis elegans*¹¹, and because we have shown previously that one of the insert lines is associated with resistance to starvation stress¹², we also investigated the effects of the insertions on life span as well as starvation and heat-stress resistance. As the insulin-insulin growth factor (IGF) signaling pathway contributes a common mechanism that affects longevity in both *C. elegans*¹³⁻¹⁶ and *D. melanogaster*^{17,18}, we examined epistatic interactions between the *P*-element insertions and mutations in components of the insulin-IGF signaling pathway as a possible mechanistic explanation of the observed pleiotropy.

We found that the four *P*-element insertions are associated with opposite, and often sex-specific, effects on life span, stress resistance and taste perception. The effects on life span and stress resistance are in most instances positively correlated. *P*-elements in lines BG02514 and BG02257 are in the same co-isogenic background but are inserted

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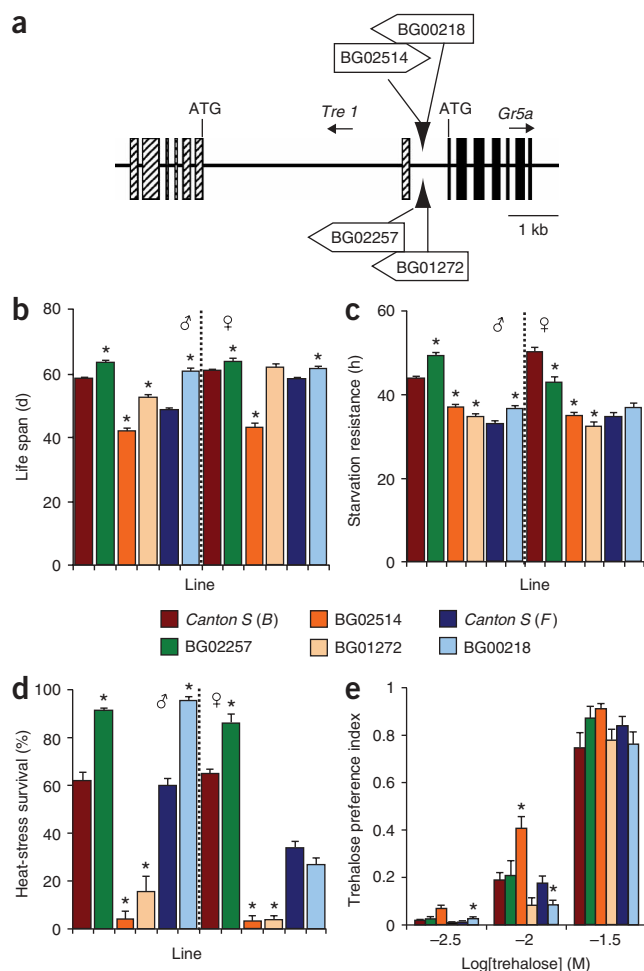


Figure 1 *P*-element insertion sites and pleiotropic effects of insertions in the *Tre1-Gr5a* region. **(a)** Locations of *P*[*GT1*] insertion sites in the *Tre1* and *Gr5a* region for four independent *P*-element insertion lines (BG02257, BG01272, BG02514 and BG00218). The horizontal line represents genomic DNA on the X chromosome at cytological position 5B. Exons of the *Tre1* (CG3171) and *Gr5a* (CG15779) genes are represented by hatched and black boxes, respectively, and their orientations are indicated by arrows. The two *P*[*GT1*] insertion sites are indicated with arrowheads. The insertion sites of *P*-elements in BG02514 and BG02257 are identical in location as are the insertion sites for BG01272 and BG00218. *P*-element orientations are indicated by rectangles with conjoined arrowheads. BG02257, BG01272 and BG02514 are in the Canton S (B) background, and BG00218 is in the Canton S (F) background. **(b–e)** Indicated phenotypes of the *P*-element insertion lines BG02257, BG02514 and BG01272 compared to their wild-type control, Canton S (B), and BG00218 compared to its control, Canton S (F), in males and females, respectively. As no significant differences were observed between males and females for trehalose preference, data for both sexes were pooled in **e**. Error bars represent s.e.m. The asterisks represent significant differences among means, as assessed by post-hoc Tukey tests at $P < 0.05$.

reduces the expression of *Tre1* without apparent effect on *Gr5a* (Fig. 2a). As expected from a previous report², trehalose preference was dramatically reduced in $\Delta 22$, but not significantly altered in $\Delta 157$ (Fig. 2b). In contrast, both *Tre1* and *Gr5a* influence starvation and heat-stress resistance, and life span. Whereas both deletion lines show a reduction in starvation-stress resistance in both sexes (Fig. 2c), their effects on heat-stress resistance and life span show sexual dimorphism. Heat-stress resistance is reduced in females only in $\Delta 157$, whereas it is reduced in both sexes in $\Delta 22$ (Fig. 2d). Similarly, life span is reduced in both sexes in $\Delta 22$, but in the $\Delta 157$ line life span is only reduced in males (Fig. 2e). These observations indicate that complex effects emanating from the entire *Tre1-Gr5a* region influence these fitness traits.

To assess genome-wide epistatic effects of these *P*-element insertions, we targeted the insulin–insulin growth factor (IGF) signaling pathway, because it has been implicated as a mechanism regulating longevity in *C. elegans*^{13–16}, *D. melanogaster*^{17,18} and mice^{19,20}. We identified *P*-element insertions in three genes of the insulin signaling pathway (*PDK1*, *tribbles* (*trbl*), *foxo*; Fig. 3a) in the same co-isogenic Canton S background as the *P*-element inserts in the *Tre1-Gr5a* region. We were thus able to directly evaluate whether *P*-element insertions in the *Tre1-Gr5a* region enhanced or suppressed the effects of *P*-element insertions in genes of the insulin/IGF signaling pathway on starvation-stress resistance and life span.

We observed epistatic interactions for starvation stress resistance with *foxo* and *trbl* (Fig. 3b). For *foxo*, we assessed epistatic interactions with two independent *P*-element insertion lines. One of these enhanced effects of the *P*-elements in the *Tre1-Gr5a* region in BG02514, but suppressed the effects of the BG02257 insertion for females. Epistatic interactions for life span were even more pronounced with both alleles of *foxo* suppressing the effects of the *P*-element insertions in the *Tre1-Gr5a* region. Additionally, two independent lines with *P*-element insertions in the same location in *PDK1* enhanced the effects of the *P*-elements in the *Tre1-Gr5a* region on life span (Fig. 3c). These epistatic effects point at the insulin signaling pathway as one possible mechanistic link for the observed modulation of life span and starvation-stress resistance arising from the *Tre1-Gr5a* region.

We predicted that the pleiotropic effects of the *P*-elements in BG02514 and BG02257 would be accompanied by alterations in expression of transregulated genes, and that opposite pleiotropic effects of inserts would be reflected in different transcriptional profiles.

in opposite orientations (Fig. 1a). Longevity, resistance to starvation and heat stress are reduced in both sexes in BG02514, and increased in both sexes in BG02257 (Fig. 1b–d). But the effect of the *P*-element in BG02257 on starvation stress is sexually antagonistic, with increased stress resistance in males and decreased stress resistance in females, as observed previously¹² (Fig. 1c). Thus, different orientations of the *P*-element in the same genetic background result in different phenotypic effects.

We next examined two *P*-element inserts (in lines BG01272 and BG00218) in the same location and orientation, but in different genetic backgrounds (Fig. 1a). The insert in BG01272 is associated with reduced resistance to starvation and heat stress in males and females and reduced longevity in males. In contrast, the insert in BG00218 is associated with increased resistance to heat and starvation stress in males only, and with enhanced longevity in both sexes (Fig. 1b–d).

We also tested whether the *P*-elements had pleiotropic effects on trehalose sensitivity. At intermediate concentrations of trehalose, we observed increased preference for trehalose for BG02514, and reduced trehalose preference for BG00218 for both sexes (Fig. 1e). These inserts differ in their location, orientation and genetic background.

We generated imprecise *P*-element excision alleles to determine whether *Tre1*, *Gr5a* or both were associated with the pleiotropic phenotypes. In one line, designated $\Delta 22$, a ~ 2.0 -kb deletion abolishes expression of the *Gr5a* gene and reduces expression of *Tre1*; in a second imprecise excision line, designated $\Delta 157$, a ~ 1.3 -kb deletion

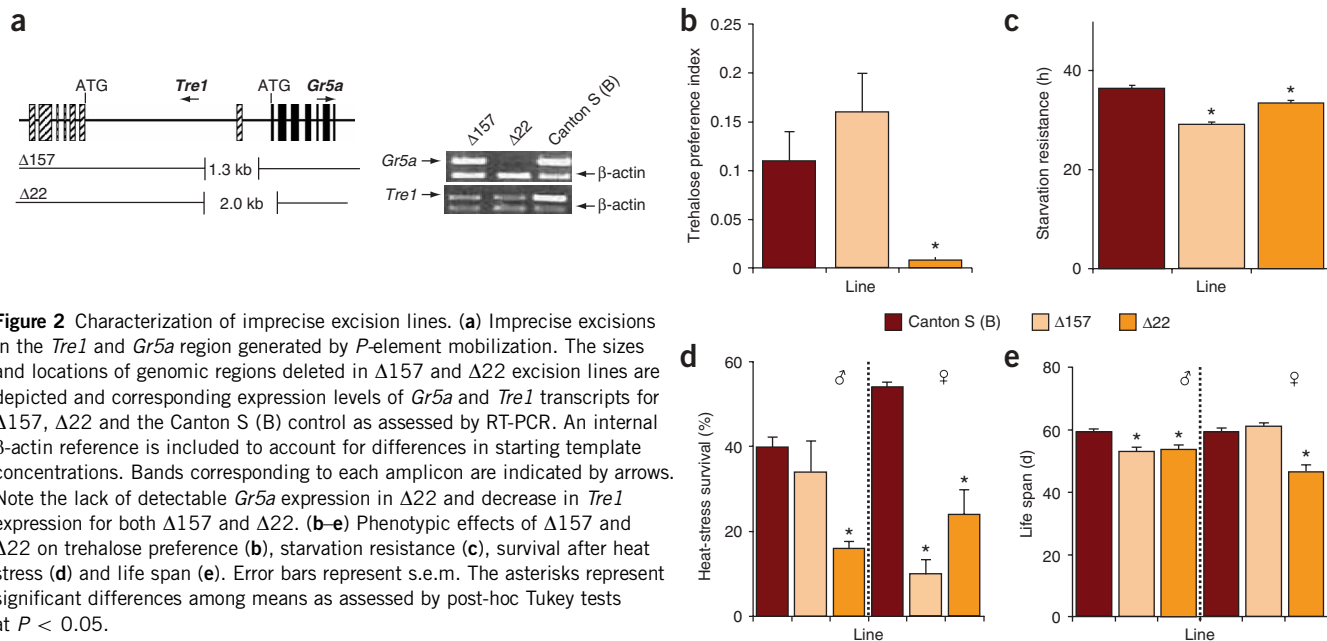


Figure 2 Characterization of imprecise excision lines. **(a)** Imprecise excisions in the *Tre1* and *Gr5a* region generated by *P*-element mobilization. The sizes and locations of genomic regions deleted in $\Delta 157$ and $\Delta 22$ excision lines are depicted and corresponding expression levels of *Gr5a* and *Tre1* transcripts for $\Delta 157$, $\Delta 22$ and the Canton S (B) control as assessed by RT-PCR. An internal β -actin reference is included to account for differences in starting template concentrations. Bands corresponding to each amplicon are indicated by arrows. Note the lack of detectable *Gr5a* expression in $\Delta 22$ and decrease in *Tre1* expression for both $\Delta 157$ and $\Delta 22$. **(b–e)** Phenotypic effects of $\Delta 157$ and $\Delta 22$ on trehalose preference **(b)**, starvation resistance **(c)**, survival after heat stress **(d)** and life span **(e)**. Error bars represent s.e.m. The asterisks represent significant differences among means as assessed by post-hoc Tukey tests at $P < 0.05$.

We tested these predictions by analyzing whole-genome transcription profiles of BG02514, BG02257 and the co-isogenic control strain.

We observed 14 probe sets for which BG02514 and/or BG02257 exhibited altered expression relative to their co-isogenic control, at a significance threshold of $P < 0.0002$ ($Q < 0.1$, **Table 1**; probe sets at $P < 0.001$ are shown in **Supplementary Table 1** online). We focused our analysis on transcription in heads, as *Gr5a* is expressed in the proboscis and taste information is processed via the subesophageal ganglion. Additional up- or downregulated transcripts might be observed in other tissues. The transcriptional profiles of BG02514 and BG02257 overlap, but are distinct. In BG02514, ten transcripts were upregulated and four were downregulated, whereas two transcripts were upregulated and three were downregulated in BG02257 (**Table 1**). The two upregulated transcripts in BG02257 were also upregulated in BG02514. Of the three downregulated transcripts in BG02257, two were also downregulated in BG02514. Seven probe sets were upregulated in BG02514 only and two transcripts, *Subc* and *CG4330*, which encode gene products associated with intermediary metabolism, were downregulated. Additionally, gene products associated with proteolysis were upregulated in BG02514. Five probe sets represented transcripts of unknown function.

Our results demonstrate that *P*-element insertions in intergenic regions have complex effects on quantitative trait phenotypes, which depend on the insertion site and orientation of the *P*-element and the host genetic background. Life span and stress resistance are life history traits that evolve in response to natural selection. It is likely that at least a substantial subset of the many transposable element insertions in presumably regulatory intergenic regions of mammalian genomes may also contribute to the evolutionary forces that drive the morphological, physiological and behavioral

divergence, in line with previous hypotheses⁶. Another implication is that polymorphisms in intergenic and other noncoding regions may contribute to standing variation in quantitative traits, including susceptibility to complex diseases in humans. Indeed, associations between noncoding variants and complex traits are common in *D. melanogaster*¹. It should, however, be noted that genes shown to contribute to the manifestation of quantitative traits via mutational effects in co-isogenic lines generated in the laboratory may not necessarily contribute to phenotypic variation in natural populations.

If complex traits in *D. melanogaster* are governed by epistatic networks of pleiotropic genes, it is not surprising that networks that determine life history traits overlap with networks that contribute to behavior, such as the link we found between gustatory perception and trehalose intake with starvation-stress resistance and life span in *D. melanogaster*. Our observations are in line with those from studies on *C. elegans*, which showed that ablation of chemosensory neurons either increases or decreases life span, dependent on which gustatory

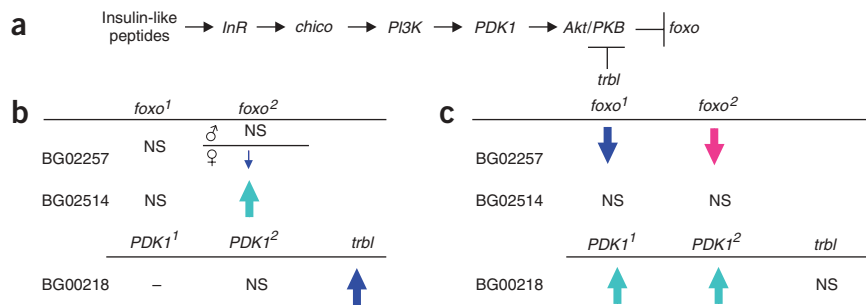


Figure 3 Epistatic interactions with the insulin signaling pathway. **(a)** *D. melanogaster* insulin signaling pathway^{26,27}. **(b–c)** Epistatic interactions between *Tre1-Gr5a* mutants (rows) and insulin signaling pathway mutants (columns) for starvation resistance **(b)** and life span **(c)**. Arrow colors reflect levels of significance, with pink, $P < 0.001$; dark blue, $P < 0.01$; and light blue, $P < 0.05$. Significant interactions on the phenotype are indicated by arrows and their upward or downward directions show enhancer and suppressor effects, respectively, on the original *P*-element-induced mutant phenotype. Sex-specific effects are denoted by male and female symbols. NS, not significant. Hyphen indicates no data was collected. See Methods for explanation of *foxo1*, *foxo2*, *PDK1* and *PDK2*.

Table 1 Loci with altered transcriptional regulation in *P*-element insertion lines

Gene ID	<i>F</i> statistic	<i>P</i> value	<i>Q</i> value	Gene description	Molecular function	Biological process	Canton S (B)	BG02257	BG02514
153070	1,310.94	1.19×10^{-8}	7.87×10^{-5}	Tequila	Chitin binding; ATP binding; serine-type endopeptidase activity	ATP synthesis-coupled proton transport; chitin metabolism; proteolysis and peptidolysis	A	<u>B</u>	<u>C</u>
141811	146.62	8.06×10^{-6}	0.022	Puromycin-sensitive aminopeptidase	Cytosol alanyl aminopeptidase activity; zinc binding; sugar porter activity	Phosphoenolpyruvate-dependent sugar phosphotransferase system; proteolysis and peptidolysis	B	B	<u>A</u>
142413	135.48	1.02×10^{-5}	0.022	<i>CG11314</i>	Unknown	Mesoderm development	B	<u>C</u>	<u>A</u>
145468	108.43	1.95×10^{-5}	0.032	<i>CG32523</i>	Serine-type peptidase activity; peptidase activity; chymotrypsin activity	Protein metabolism and peptidolysis	B	<u>B</u>	<u>A</u>
142911	77.84	5.11×10^{-5}	0.056	<i>Suchb</i>	Succinate-CoA ligase (GDP-forming) activity	Tricarboxylic acid cycle	A	<u>C</u>	<u>B</u>
141298	66.49	8.05×10^{-5}	0.076	<i>CG9186</i>	Unknown	Unknown	A	A	<u>B</u>
147983	59.27	1.12×10^{-4}	0.088	<i>CG12090</i>	Unknown	Unknown	B	B	<u>A</u>
151555	55.40	1.36×10^{-4}	0.088	<i>CG1090</i>	Alpha-type channel activity; calcium, potassium:sodium antiporter activity	Cation transport	B	B	<u>A</u>
150680	54.75	1.40×10^{-4}	0.088	<i>CG5484</i>	Unknown	Unknown	B	<u>A</u>	<u>A</u>
153453	50.89	1.73×10^{-4}	0.088	<i>CG4330</i>	High-affinity inorganic phosphate:sodium symporter activity	Carbohydrate metabolism; carbohydrate transport; cation transport	B	<u>B</u>	<u>A</u>
144908	50.40	1.77×10^{-4}	0.088	<i>CG10362</i>	Diacylglycerol binding; protein binding	Intracellular signaling cascade	A	A	<u>B</u>
148564	49.24	1.89×10^{-4}	0.088	<i>CG7607</i>	Unknown	Unknown	B	B	<u>A</u>
150404	48.42	1.99×10^{-4}	0.088	<i>CG17244</i>	Unknown	Unknown	B	B	<u>A</u>
141527	47.94	2.04×10^{-4}	0.088	Tequila	Chitin binding; ATP binding; serine-type endopeptidase activity	ATP synthesis coupled proton transport; chitin metabolism; proteolysis and peptidolysis	C	<u>B</u>	<u>A</u>

Expression levels in BG02257 or BG02514 were compared to the Canton S (B) control. Tukey tests were performed for each probe set to identify those lines in which expression was upregulated or downregulated relative to Canton S (B) (indicated by boldface and underlining). For each probe set, lines designated by the same letter are not statistically significantly different from one another. The relative level of expression of a probe set among lines is listed with the highest expression designated A and lowest C; expression levels that are significantly different from the control are indicated. All probe sets with altered regulation of expression at $P < 0.001$ are available in **Supplementary Table 1**. Entries above are statistically significant after correcting for a false positive discovery rate of $Q < 0.10$ (ref. 25). Probe sets in bold font correspond to genes that are represented independently multiple times on the microarray and that show altered regulation multiple times. Molecular and biological processes are listed in accordance with their gene ontology designations.

or olfactory neurons are affected¹¹. Neuronal ablation in *daf-2* and *daf-16* mutants implicated modulation of the insulin-IGF signaling pathway and subsequent activation of the FOXO transcription factor *daf-16*, as a possible link between gustatory perception and life span. The insulin-IGF signaling pathway has also been implicated in *D. melanogaster* longevity^{17,18,21}, and we note that the effects of mutations in the *Trel-Gr5a* intergenic region on starvation-stress resistance and longevity are modulated by epistatic interaction with mutations in genes involved in insulin signaling.

The complexity of the allelic effects we observed, including sex-specific or even sex-antagonistic effects depending on the location and orientation of the *P*-element and the genetic background, are at variance with the traditional expectation that all alleles of a particular gene have similar phenotypic effects. Our results show that the pleiotropic and epistatic effects on quantitative traits as well as transcriptional co-regulation are specific to each allele. Furthermore,

the pervasive epistasis we observed implies that effects of mutations should be examined in multiple genetic backgrounds to appreciate the full range of phenotypic effects, and that mapping quantitative trait loci affecting naturally occurring variation via their main effects will seriously underestimate the underlying complexity attributable to interactions. Our observations of the profound effects of intergenic *P*-element insertions in the *Trel-Gr5a* region on fitness traits in *D. melanogaster* raise the possibility that polymorphisms in previously considered 'junk DNA' in the human genome may substantially modulate the genetic architectures that drive expression and evolution of complex traits.

METHODS

***D. melanogaster* stocks.** We obtained four stocks (BG02514, BG02257, BG01272 and BG00218) with single *P*[*GTL*] element insertion²² at cytological position 5A from the Berkeley *Drosophila* Gene Disruption Project¹⁰. The



P-elements in BG02514 and BG02257 are inserted 747 bp upstream of *Gr5a*, in the same isogenic background, Canton S (B), but in opposite orientations. The *P*-elements in BG01272 and BG00218 are located 739 bp upstream of *Gr5a*, in the same orientation, but in different genetic backgrounds (Canton S (B) and (F), respectively). We generated revertant alleles by crossing BG01272 females to *w; Cy/Sp; SbA2-3/TM6,Tb* males. We crossed male offspring of genotype *w; Cy/BG01272; SbA2-3/B* to *w; Cy/Sp; B* females, and then again crossed individual male offspring in which the *P*-element was *Cy/B^P*; *B* to *w; Cy/Sp; B* females. We mated male and female *w; Cy/B^P*; *B* *inter se* to generate homozygous *P*-element excision lines in the isogenic Canton S (B) background. We analyzed excision of the construct by PCR using primers flanking the *P*-element insertion site, and then cloning and sequencing the PCR-amplified fragment.

We obtained lines with *P*-element insertions adjacent or within genes that encode components of the insulin signaling pathway from the Berkeley *Drosophila* Gene Disruption Project¹⁰. The lines each have a single *P*[*GT1*] element inserted adjacent to or within *foxo* (BG01018 (designated *foxo¹*) and BG01573 (designated *foxo²*), *PDK1* (BG00601 (designated *PDK¹*) and BG00872 (designated *PDK²*)) and *trbl* (BG00964), and are co-isogenic (BG01018, BG01573 are in the Canton S (B) background, whereas the others are in the Canton S (F) background). We reared all flies on agar-yeast-molasses medium at 25 °C and 70% humidity under a 12 h light-dark cycle. All phenotypes were measured contemporaneously with controls. Phenotypic values were stable over time, reflecting the integrity of the isogenic backgrounds of the lines.

Trehalose preference. We conducted two-choice taste-preference assays essentially as described previously^{2,3,23}. We removed flies, 5–7 days post-eclosion, from their food source 21 h before the assay and placed in vials containing 1.5% agarose to prevent desiccation. We conducted the assays in the dark at 25 °C. Each assay consisted of a 60-well plate containing 2 mM sucrose paired with variable concentrations of sucrose or trehalose. We observed no difference in sucrose preference among the lines (data not shown). We added 0.125 mg/ml FD&C Blue 1 or 0.5 mg/ml Acid Red 40 to either the sucrose reference or the test solution, and switched the dyes between replicates to control for differences in dye preference. We allowed the flies to feed for 2 h and then froze them at –20 °C. We calculated the preference index as $(N^R + 0.5 N^P)/(N^R + N^B + N^P)$, where N^R , N^B , and N^P refer to the number of flies with red, blue or purple abdomens, respectively. One replicate assay consisted of a single sex group of 50 flies. For each treatment, we conducted 6–10 replicate assays for each genotype and sex. Statistically significant deviations from wild-type taste preference were evaluated by three way factorial ANOVA according to the model $y = \mu + L + T + S + L \times T + L \times S + T \times S + L \times T \times S + E$, where line (*L*), treatment (*T*), concentration of sugar and sex (*S*) are fixed main effects, and *E* indicates error. Taste preferences for BG02514, BG02257 and Canton S (*B*) were determined for 1 mM, 2 mM and 3 mM sucrose and 3 mM, 10 mM or 30 mM trehalose. We measured taste preferences for imprecise excision lines at 10 mM trehalose.

Starvation-stress resistance. We placed ten same-sex virgin flies in a vial containing 10 ml of 1.5% agarose, and placed it at 25 °C under a 12 h light-dark cycle. We recorded the number of dead flies every 8 h. We scored a total of 10–15 replicates of 10 flies for each line and sex. Comparisons between lines and sexes were made by ANOVA, and post-hoc Tukey tests were performed to assess significant differences among means. As appropriate, the models used were $y = \mu + L + \text{Rep}(L) + E$ or $y = \mu + L + S + L \times S + \text{Rep}(L \times S) + E$, where line (*L*) and sex (*S*) are the fixed main effects and replicates (Rep) are treated as a random effect, and *E* indicates error.

Heat-stress resistance. We assayed four replicate vials for each line and sex. Each replicate vial contained 25 same-sex flies (5–7 d old), which were transferred without anesthesia into empty vials and placed in a water bath at 38 °C (±0.5 °C) for a set amount of time determined for each genetic background (Canton S (B) or Canton S (F)) and sex. We determined the assay time points for each genetic background and sex combination by regressing the percentage of flies that had survived after seven different heat-exposure times (every 20 min from 60 to 180 min). For lines in the Canton S (B) background, we heat-stressed females for 145 min and males for 116 min,

whereas we heat-stressed females and males of the Canton S (F) background line for 132 and 85 min, respectively. After heat stress, we immediately placed the flies in vials containing 5 ml of standard cornmeal-agar-molasses medium and returned them to an incubator at 25 °C and 60% humidity for 24 h. After 24 h we recorded the percentage of surviving flies per vial for each line and sex. Comparisons between lines and sexes were made by ANOVA according to the model $y = \mu + L + E$ where line (*L*) is the fixed main effect, and *E* indicates error.

Longevity. Every other day we transferred five virgin flies of a single sex to fresh food vials and recorded the number of dead flies. We scored at least 40 replicates of 5 flies each for each line. We made comparisons between lines by ANOVA, and performed post-hoc Tukey tests to assess significant differences among means. As appropriate, the models used were $y = \mu + L + \text{Rep}(L) + E$, or $y = \mu + L + D + \text{Rep}(L \times D) + E$, where line (*L*) and date (*D*) are the fixed main effects with replicates (Rep) treated as a random effect, and *E* indicating error.

Epistasis. We generated double-mutant heterozygous flies by crossing BG02257 or BG02514 virgin females to males from BG01018 and BG01573, and virgin females from BG00218 to males from BG00601, BG00872 and BG00964. We also generated appropriate controls by crossing either Canton S (B) or Canton S (F) to the mutant lines as well as to BG02257, BG02514 and BG00218, as appropriate. We assayed at least 5 replicates of either 10 flies per sex and genotype for starvation resistance, or 5 flies per sex and genotype for lifespan, as described above. We determine quantitative trait phenotypes for males and females of each genotype, as described below. We determined epistatic interactions by ANOVA according to the model $y = \mu + L1 + L2 + S + L1 \times L2 + S \times L1 + S \times L2 + L1 \times L2 \times S + \text{Rep}(L1 \times L2 \times S) + E$, where locus 1 (*L1*), locus 2 (*L2*), and sex (*S*) are fixed main effects and replicates (Rep) are a random effect and *E* indicates error variance.

Transcriptional profiling. We reared BG02514, BG02257 and Canton S (B) lines simultaneously at 25 °C under a 12 h light-dark cycle and 70% humidity. At 5–7 days after eclosion, we removed the heads of two replicate groups of 100 males and 100 females for each line. We isolated total RNA from each replicate and hybridized biotinylated cRNA probes to high-density oligonucleotide microarrays (Affymetrix, Inc.) and visualized the probes with a streptavidin-phycoerythrin conjugate, as described in the Affymetrix GeneChip Expression Analysis Technical Manual (2000), using internal references for quantification. The quantitative estimate of expression of each probe set is the signal (Sig) metric, as described in the Affymetrix Microarray Suite, Version 5.0. We analyzed Sig values by two-way ANOVA according to the model $y = \mu + L + S + L \times S + E$, where *L* is the effect of line, *S* is the effect of sex and *E* the error variance²⁴. To correct for multiple testing, we estimated the false discovery rate *Q* value²⁵. Unlike the *P* value, which is the number of false positives expected when truly nothing is significant, the false-discovery rate *Q* value controls the proportion of false positives among all terms declared significant²⁵. We used the 10% false-discovery rate criterion (*Q* < 0.10) for significance of any of the terms in the ANOVA model. This corresponded to *P* < 0.0002.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

All authors contributed to the experimental design and statistical analysis of data. S.M.R., R.R.H.A. and T.E.C.M. wrote the manuscript. S.M.R. measured starvation resistance and trehalose preference and performed molecular genetic analyses, M.M.M. measured life span, E.D.O. and T.J.M. measured heat stress, and A.Y. maintained *D. melanogaster* lines and constructed revertant lines.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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