Supplemental Data

Overexpression of a *Drosophila* Homolog of Apolipoprotein D Leads to Increased Stress Resistance and Extended Lifespan

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Supplemental Experimental Procedures

Drosophila Strains

Da-Gal4, 24B-GAL4, GMR-GAL4, PO163-GAL4, Actin-GAL4, D42-GAL4, and *Elav-GAL4* were all obtained from the *Drosophila* stock center (Bloomington, IN). *DJ634* was isolated and characterized by L. Seroude in our laboratory. We thank Lily and Yuh-Nung Jan for providing us with *GAL4*$^{109(2)80}$. The *Drosophila* strain *white*^{1118} was used in all control crosses and as the background for generation of transgenic lines. Male flies were used throughout the study. We screened a publicly available collection of insertion lines containing enhancer-promoter transposable elements (Rorth collection of EP lines), out of which *EP(2)2383* was selected for this study.

Lifespans

All crosses were performed at 18ºC to minimize the effects of GAL4 during development. For each lifespan experiment (all at 29ºC), at least 100 2- to 4-day-old males were collected on a Peltier plate maintained at 4ºC to avoid possible negative effects of CO_2 anesthesia on lifespan. 20–30 flies were put in a single vial and transferred every 3–4 days to fresh standard food vials, and the number of dead flies recorded each time. Survival curves were analyzed with the Graphpad Prism 4 software, yielding p values for a logrank test.

Exposure to Hyperoxia

Adult males (2- to 4-day-old) in shell vials (20–30 flies per vial) containing standard food were maintained in a Plexiglas enclosure of 28 × 28 × 24 in at room temperature (22ºC–24ºC). Oxygen (100%) was passed through the box at a constant rate of 300 ml/min.

Starvation

For each lifespan experiment, at least 100 2- to 4-day-old males were collected as described above. 20–30 flies were put in a single vial and transferred every day to fresh vials, and the number of dead flies was recorded approximately every 2 hr. For dry starvation, the vials contained 5 g of silica gel (desiccant), while for wet starvation the vials contained 1% agar, providing water throughout the experiment, but no food source. Each experiment was repeated at least twice.

Generation of Transgenic Flies

An EST carrying the full *GLaz* cDNA (GH09946) was procured from the DGRC (Indiana) and partially digested with EcoRI and XhoI. The cDNA fragment was then subcloned into the expression vector pUASt, putting the *GLaz* cDNA 3′ of a series of five UAS repeats. The purified pUASt-*GLaz* vector was subsequently injected into *w*^{1118} embryos, and two single-insertion lines were selected. Both mapped to the second chromosome.

XGAL Staining

7-day-old adult males were cryosectioned, fixed for 20 min in 1% glutaraldehyde in PBS (pH 7.2), reacted with 0.1% X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and incubated at 37ºC for 4 hr. The reaction was stopped by rinsing twice with PBS before mounting in 70% glycerol/PBS.

Dry Weight Measurement

Male flies were raised on standard food for 7 days at room temperature, then desiccated overnight in a vacuum oven at 80ºC. They were weighed in three groups of 40, and the weights are reported as mg dry weight per fly. Each experiment was repeated at least twice.

Protein Content

40 7-day-old males were homogenized in a Potter apparatus, in a detergent buffer (10 mM Tris, 2 mM EDTA, 150 mM NaCl, 0.15% NP40, protease inhibitors). After a 10 min spin at 13,000 rpm, the supernatant was assayed with the BCA (Pierce, IL) assay according to manufacturer's instructions. Amounts are reported as mg protein per fly. Each experiment was done in triplicate and repeated at least twice.

Lipid Content

40 7-day-old males were desiccated and weighed as previously described. They were then submerged in 5 ml of ether for 24 hr to dissolve lipids. The ether was removed and the remaining tissue

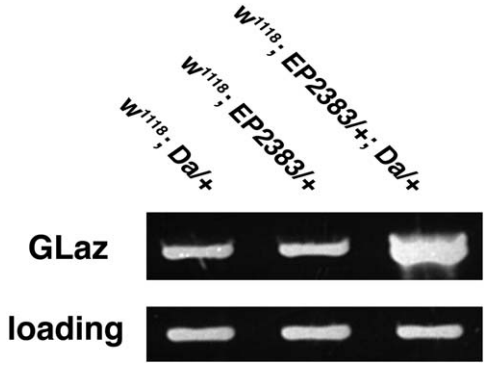

Figure S1. RT-PCR Showing a GAL4-Dependent Increase in *GLaz* Transcript with the EP Line

Marked increase in the amount of *GLaz* mRNA in *w*^{1118}; *EP(2)2383/+; da-GAL4/+* compared to the *w*^{1118}; *da-GAL4/+*, and *w*^{1118}; *EP(2)2383/+* controls, consistent with GAL4 activation of UAS in *EP(2)2383*, enhancing *Glaz* trancription. Amounts of total RNA reverse transcribed were normalized. A 361 bp rig/S15 RNA loading control was reverse transcribed and amplified to normalize the RT-PCR reaction, according to the Retroscript kit manufacturer's instructions.

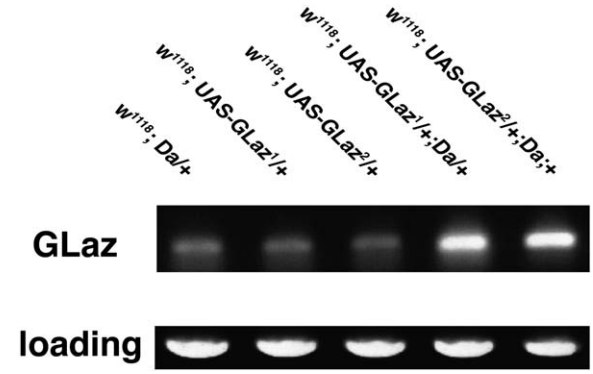

Figure S2. RT-PCR Showing a GAL4-Dependent Increase in *GLaz* Transcript with the cDNA Transgenic Lines

Transgenic cDNA insertion lines *UAS-GLaz*^{1} and *UAS-GLaz*^{2}. Both *w*^{1118}; *UAS-GLaz*^{1}*/+; da-GAL4/+* and *w*^{1118}; *UAS-GLaz*^{2}*/+; da-GAL4/+* displayed increases in the amount of *GLaz* transcript compared to controls. A 200 bp rp49 RNA fragment was reverse transcribed and amplified to normalize the RT-PCR reaction and to ensure that the RNA was loaded equally.

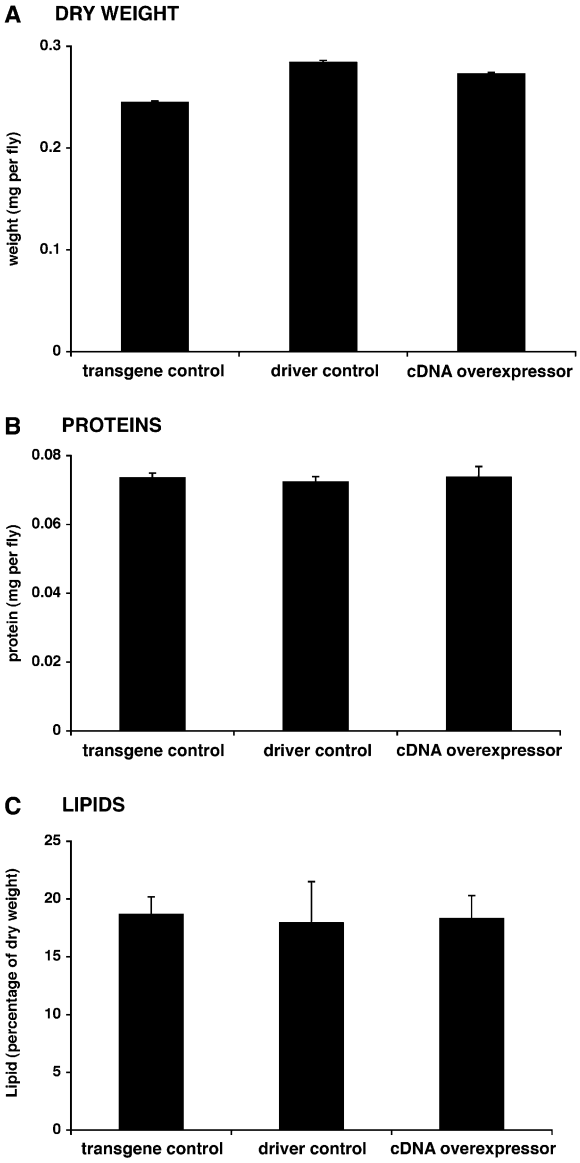

Figure S3. Overexpression of *Glaz* Does Not Alter Weight, Protein, or Lipid Content

Comparison between flies overexpressing Glaz (w^{1118}; $GAL4^{109(2)80}$/*UAS-GLaz2*) and controls (w^{1118}; $GAL4^{109(2)80}$/+ or w^{1118}; *UAS-GLaz2*/+) in terms of (A) dry weight (in mg/fly), (B) protein content (in mg/fly), and (C) lipid content (percentage of dry weight). Error bars represent SEM.

desiccated and weighed again. The weight difference corresponds to the lipid fraction, reported as percentage of dry weight. Each experiment was done in triplicate and repeated at least twice.

Behavioral Assays before and after Hyperoxia

All assays were performed at room temperature. Vertical locomotion performance was measured by putting 40 flies in a vial, tapping them to the bottom, and measuring the percentage of flies that reached the top third of the vial after 10 s. Horizontal locomotion performance was measured by putting 20 flies in a narrow tube covered in black tape and tapping them to one end, with a fiber-optic light at the other end. After 1 min, the number of flies having traveled more than 2 inches away from the origin was counted and expressed as a percentage of the total number. All assays were done in triplicate, and the experiment was repeated at least twice.

RT-PCR

Total RNA was extracted from 40 male flies of the various genotypes with Trizol reagent (Invitrogen, CA). RNA concentration was measured with a Nanodrop spectrophotometer, and sample concentrations were normalized. The Retroscript kit (Ambion, TX) was used according to the manufacturer's instruction, by means of the Oligo-dT primers provided in the kit. The cDNA was then amplified by PCR with two primers specific for *GLaz*, giving a single 600 bp amplicon (F: 5′-ATGATGAGTGGCCAGCCACTT-3′; R: 5′-GTCGCG GAGCCCCGTAA-3′).

Hypoxia Paradigm

At room temperature, 7-day-old adult males were placed in 100% N_2 for 30 min, then brought back to normoxia and allowed to recover for 20 min. All flies were awake by 20 min. 1 hr later, vertical locomotion performance was scored, as previously described.

Statistical Analyses

All survival curves were analyzed with the Prism4 software (Graphpad), and we report mean lifespan ±SEM. The p value given is the result of a logrank test on the Kaplan-Meier data. For all other assays (histogram charts), error bars represent SEM, and the p values are the result of the Student's t test.