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Supporting Information

Chow et al. 10.1073/pnas.1307125110

SI Materials and Methods

Fly Cultures. This study used 114 lines from the *Drosophila* Genetic Reference Panel (DGRP) (ref. 1; Dataset S1). Only males were assessed for endoplasmic reticulum (ER) stress response in this study. All flies were collected as virgins under CO_2 anesthesia and aged 3 d in vials of 20–30 male flies. All flies were maintained on standard agar–dextrose–yeast medium and housed at 24 °C on a 12-h light/dark cycle.

Survival Analysis. To measure survival under constant ER stress, 3-d-old male flies were fed a diet with or without the ER stress-inducing drug tunicamycin (TM; Sigma). The feeding protocol was similar to that used in a previous study (2). The diet was composed of 1.3% low-melting agarose (1.3 g /100 ml H_2O), 1% sucrose (1 g/ 100 ml H_2O), and 12 μM TM (drug) or no TM (control). Vials contained 2 mL of food (drug or control). For each DGRP line, 100 flies were fed the TM diet and 100 flies were fed the control diet. For ease of monitoring survival and to prevent overcrowding, flies were housed in groups of 20 per vial; thus, we monitored five TM vials and five control vials for each DGRP line. Survival was measured by counting the number of dead flies every 2–3 h until all of the flies in the drug condition were dead. Survival was measured in two blocks of 77 lines. Both blocks were initiated at the same time in the 12:12 light cycle.

Survival analysis was performed in R (Version 2.8.1; R Development Core Team) by using the Survival package (3). The Cox proportional hazards test (4) was used to calculate a hazard ratio (HR) for each line. The HR is the ratio of the death rate of the drug group compared with the control group, within a line. Thus, there is a single HR value for each DGRP line, and the HR takes into account all 100 flies exposed to TM and all 100 flies exposed to control conditions. Kaplan–Meier survival plots were also generated where indicated.

Agilent Microarray Analysis. Expression was measured at two time points: 8 h (early time point) and 20 h (late time point) of drug exposure. Because increased *Xbp1* splicing is detectable between 4 and 5 h (Fig. S1), 8 h was chosen as the early time point to ensure that all lines had initiated the ER stress response. To avoid measuring the death process, 20 h was chosen as the late time point because the most susceptible lines began to die at ~24 h. Twenty DGRP lines were randomly chosen for the 8-h analysis, and 8 lines (a random subset of the 20 lines used in the early time point) were chosen for the 20-h analysis. At both time points, gene expression was measured for the drug and control conditions. Twenty flies per line per treatment were collected for RNA and flash frozen on dry ice. Gene expression was measured on Agilent 4 × 44K *Drosophila* Gene Expression Microarrays (catalog no. G2519F-021791). Total RNA was isolated with a standard phenol/chloroform extraction protocol and stored at −80 °C. Samples were prepared for hybridization following the Ambion amino allyl messageAmp II aRNA Kit and hybridized overnight at 60 °C. Microarrays were scanned on an Axon 4000B scanner, and images were analyzed with GenePix Pro-6.0 software. To reduce processing variability, matched drug and control samples were prepared in parallel and hybridized on the same Agilent chip.

All analysis of microarray data was conducted in the R Bioconductor Linear Models for Microarray Data (LIMMA) library (5). After background correction, quantile normalization was performed across arrays. Gene expression was calculated as a $\log_2$ fold change between the matched control and drug samples for each line and time point. Transcripts that showed >1.5-fold up-regulation in response to ER stress in >25% (at 8 h) or >50% (at 20 h) of the lines assayed were designated as ER stress-responsive genes. Because of the lower sample number at 20 h, we used a higher cutoff. This higher cutoff ensured that we included both the common and most variable probes in the analysis and excluded single line differences.

Gene Ontology (GO) and pathway analysis was performed by using the FlyMine tools (www.flymine.org) (6). False discovery rate (FDR) was calculated with the Benjamini–Hochberg method (7). Cluster analysis was performed by using the Modulated Modularity Clustering online tool developed for the DGRP (http://mmc.gnets.ncsu.edu/) (8, 9). Correlations reported are Pearson correlations.

Association Study. To associate TM-induced ER stress survival time with genome-wide SNPs, we used a web tool developed for the DGRP that applies a simple linear model to test the null hypothesis that the means of the genotypes at each SNP, tested one at a time, were homogeneous (http://dgrp.gnets.ncsu.edu/) (2). The phenotype used in the association was the HR for each DGRP line. Because the HR calculation is a comparison of the death rate in the drug-treated and control groups, when the number of deaths in the control group is very small, the precision of the HR to estimate the true rate is relatively low. In our case, lines were excluded where <2% of control flies died during the experiment, resulting in a total of 89 lines included in the association analysis. Multiple testing correction was performed by calculating the FDR with the Benjamini–Hochberg method (7).

Functional Testing. To test association candidates for a potential role in ER stress, *P*-element insertion lines for 25 candidate genes were tested (Dataset S5; Bloomington *Drosophila* Stock Center, Indiana University). In most cases, the *P* element was inserted into the coding sequence, inserted in an intron, or inserted within 500 bp upstream of the gene. To standardize genetic background, all *P*-element insertion lines that were not isogenic with the laboratory strain w^{1118} were back-crossed to the w^{1118} background for more than five generations. Because many of the *P* elements were homozygous lethal, we assessed effects of each *P* element as a heterozygote, comparing them to control w^{1118} flies. To generate flies for testing, each *P*-element line was crossed to w^{1118}. Survival analysis was performed as described above, in which each line was subjected to drug and control conditions and compared with the survival rate observed for the w^{1118} line. Control-treated survival curves (all *P* elements and w^{1118}) were not statistically different from each other; thus, they were eliminated from the analysis. To test whether the survival curve of each *P*-element line was different from the survival curve of w^{1118}, we calculated the HR and significance for each drug-treated *P*-element survival curve relative to the drug-treated w^{1118} survival curve (3).

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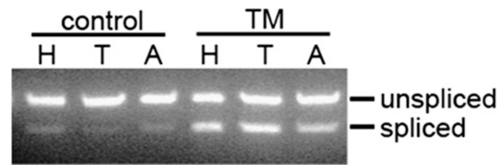

Fig. S1. ER stress is evident in flies exposed to TM for as few as 4 h. Increase in *Xbp1* splicing (lower band) indicates the presence of ER stress. H, head; T, thorax; A, abdomen.

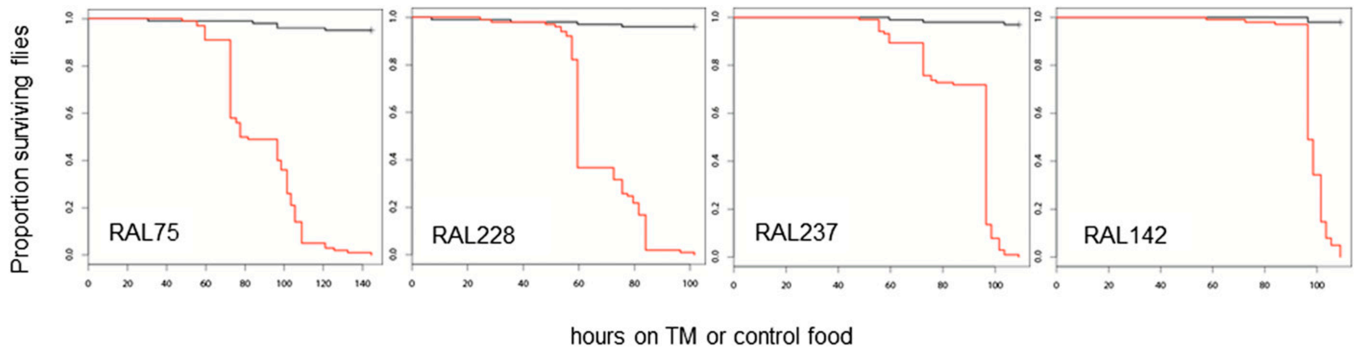

Fig. S2. Sample Kaplan–Meier Survival curves for four DGRP lines. Black line, control food; red line, TM food.

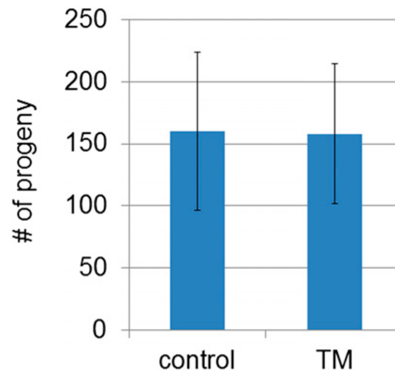

Fig. S3. Fertility of male flies is not affected by exposure to a diet supplemented by TM. Male flies from a single DGRP line were exposed for 20 h to control (n = 38) and TM-supplemented (n = 40) food and were singly mated to females from a standard laboratory strain. Females were allowed to lay eggs on standard laboratory medium for 4 d.

Fig. S4. (*A*) Examples of known ER stress genes with correlated expression in the largest module (module 43). (*B*) Examples of unknown and known ER stress genes with correlated expression in the largest module. Expression values are $\log_2$ fold change.

Fig. S5. Minor allele frequency is negatively correlated with the effect size of the SNP.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)
Dataset S4 (XLSX)
Dataset S5 (XLSX)