1. The Genetic Architecture of Defense as Resistance to and Tolerance of Bacterial Infection in *Drosophila melanogaster*

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6. The Genetic Architecture of Infection Defense

Abstract

Defense against pathogenic infection can take two forms: resistance and tolerance. Resistance is the ability of the host to limit a pathogen burden, whereas tolerance is the ability to limit the negative consequences of infection at a given level of infection intensity. Evolutionarily, a tolerance strategy that is independent of resistance could allow the host to avoid mounting a costly immune response and, theoretically, to avoid a coevolutionary arms race between pathogen virulence and host resistance. Biomedically, understanding the mechanisms of tolerance and how they relate to resistance could potentially yield treatment strategies that focus on health improvement instead of pathogen elimination. In order to understand the impact of tolerance on host defense and identify genetic variants that determine host tolerance, we defined a novel measure of genetic variation in tolerance as the residual deviation from a binomial regression of fitness under infection against infection intensity. We then performed a genome-wide association study (GWAS) to map the genetic basis of variation in resistance to and

tolerance of infection by the bacterium *Providencia rettgeri*. We found a positive genetic correlation between resistance and tolerance and we demonstrated that the level of resistance is highly predictive of tolerance. We identified 30 loci that predict tolerance, many of which are in genes involved in the regulation of immunity and metabolism. We used RNAi to confirm that a subset of mapped genes have a role in defense, including putative wound repair genes *grainy head* and *debris buster*. Our results indicate that tolerance is not an independent strategy from resistance, but that defense arises from a collection of physiological processes intertwined with canonical immunity and resistance.

Introduction

To deal with infection, a host must control pathogen burden while maintaining health and fitness. The collection of these strategies is known as defense. Pathogen control strategies that either kill the pathogen or prevent it from proliferating are known as resistance, while processes that reduce the decline of health or fitness during infection are known as tolerance (Strauss & Agrawal 1999; Råberg *et al.* 2009). Host resistance mechanisms may apply selective pressure on the pathogen to overcome this defense, whereas tolerance strategies are predicted to have a neutral or positive impact on pathogen fitness and thereby potentially avoid a coevolutionary arms race (Boots & Bowers 1999; Roy & Kirchner 2000; Miller *et al.* 2006). Understanding the patterns of natural genetic variation in tolerance, as well as the underlying biological processes that promote tolerance, will provide a better understanding of the evolutionary trajectories of host-pathogen interactions and could yield novel sustainable therapeutic strategies. In this study, we performed a genome-wide association study (GWAS) using 172 inbred lines from the *Drosophila melanogaster* Genetic Reference Panel (DGRP) to quantify resistance and tolerance and to establish the genetic architecture of these traits.

To fully understand the contributions of resistance and tolerance to defense, the two strategies should be measured independently from one another. Resistance is measured as either immune system activity or pathogen burden after infection (Baucom & de Roode 2011). Tolerance can be estimated as either 'point tolerance' or 'slope tolerance'. Slope tolerance is measured as a reaction-norm or slope, where pathogen burden is plotted on the x-axis and health or fitness is plotted on the y-axis (Hochwender *et al.* 2000; Råberg *et al.* 2007, 2009). Point tolerance is defined as host health or fitness at a given pathogen burden(Ayres & Schneider

66 2008; Baucom & de Roode 2011). Slope tolerance estimates are conceptually pleasing because
67 experimental manipulation of pathogen burden could potentially allow tolerance to be estimated
68 independently of resistance, although the two traits may be linearly related across only a limited
69 range of infection severities (Louie *et al.* 2016). In this study, we define a new measure to
70 estimate genetic variation in tolerance as a point estimate of genotypic deviation in tolerance
71 across a population of *D. melanogaster*, as well as with a slope tolerance in a subset of
72 genotypes.

73 Tolerance and resistance are predicted to have divergent impacts on the evolution of host-
74 pathogen interactions. In theory, costly resistance alleles that negatively impact pathogen fitness
75 may be maintained as balanced polymorphisms, oscillating in a frequency-dependent manner
76 with pathogen prevalence or virulence alleles (e.g., Stahl et al. 1999). Alternatively, tolerance
77 alleles may drive an increase in pathogen prevalence, resulting in rapid fixation of these alleles
78 (Boots & Bowers 1999; Roy & Kirchner 2000; Miller *et al.* 2006). These models assume that
79 tolerance and resistance act independently. However, any genetic trade-off between resistance
80 and tolerance could constrain the evolutionary trajectories of defense alleles. Interest in tolerance
81 by both plant and animal biologists was piqued by empirical studies demonstrating a negative
82 relationship between resistance and tolerance in morning glory and in mouse (Fineblum &
83 Rausher 1995; Råberg *et al.* 2007). However, a meta-analysis of tolerance and resistance in
84 plants found that a negative relationship may not be a general phenomenon (Leimu & Koricheva
85 2006).

86 Although previous work has shown the genetic basis of natural variation in resistance to
87 bacterial pathogens in *Drosophila* (e.g., Lazzaro et al. 2004, 2006; Felix et al. 2012; Unckless et
88 al. 2015), little is known about the underlying genetic basis of variation in tolerance. Weinig et al

89 2003 measured resistance and tolerance to rabbit herbivory in *Arabidopsis*, and although several
90 resistance QTL were identified, no tolerance QTL were found despite having power to detect
91 alleles that explained greater than 5% of variance (Weinig *et al.* 2003). Additionally, a recent
92 study on tolerance of chronic HIV infection in humans did not identify any loci in genome-wide
93 association study after multiple test corrections (Regoes *et al.* 2014). This has led to the
94 hypothesis that the genetic architecture of tolerance is composed of many loci with individually
95 small effects (Weinig *et al.* 2003).

96 Functional studies using mutant and RNAi knock-down *Drosophila* have shown that
97 genes involved in protection of tissues and regulation of immunity and metabolic processes can
98 mechanistically determine tolerance of infection, but it is unknown whether these genes harbor
99 polymorphisms that contribute to natural variation in tolerance. Laboratory mutations in immune
100 genes involved in melanization (Ayres & Schneider 2008), phagocytic encapsulation (Shinzawa
101 *et al.* 2009), insulin signaling (Dionne *et al.* 2006), feeding behavior (Ayres & Schneider 2009),
102 and regulation of JAK-STAT (Merkling *et al.* 2015) alter tolerance in *D. melanogaster*. Based on
103 these studies, we hypothesized that polymorphisms in genes involved in regulation of immunity
104 and metabolic processes would predict tolerance of infection. For instance, regulatory genes may
105 influence tolerance by tightly regulating the immune response to avoid immunopathology while
106 simultaneously regulating resource allocation and tissue repair, thus allowing for adequate
107 pathogen control while maintaining and returning to homeostasis.

108 In this study we measured tolerance and resistance across a single population of inbred *D.*
109 *melanogaster* and used a genome-wide mapping approach to identify single-nucleotide
110 polymorphisms (SNPs) that predict variation in tolerance. We mapped polymorphisms in genes
111 involved in regulation of gene expression, metabolism, immunity, and other processes as

predictive of phenotypic variation in tolerance. We showed that tolerance and resistance are positively correlated and that tolerance estimates are dependent on host resistance across varying levels of infection severity. We found that resistance and tolerance are non-independent traits, and that they may be linked through shared biological processes. We have identified novel tolerance genes and confirmed their effects using RNAi.

Material and Methods

Drosophila and bacterial stocks

One hundred and seventy-two inbred lines from the *Drosophila* Genetic Reference Panel (DGRP) were phenotyped for the genome-wide association study. The DGRP is a set of fully sequenced, inbred lines collected from a single population in Raleigh, NC, USA (Mackay *et al.* 2012; Huang *et al.* 2014). A list of lines included in the GWAS for each trait can be found in Table S7. Six lines that spanned low, medium, or high levels of resistance and had phenotypically extreme (high or low) genotypic deviation tolerance were chosen for further investigation (RAL-801, RAL-26, RAL-882, RAL-359, RAL-138, and RAL-714; Figure S1). RNAi stocks from the Vienna Drosophila Resource Center KK (phiC31 generated) and GD (P-element generated) libraries (Dietzl *et al.* 2007) were used in the functional testing of candidate genes implicated in the GWAS (Table S8). All flies were maintained at 24°C on a 12:12 hour light:dark cycle on a rearing medium of 8.3% glucose, 8.3% Brewer's yeast, 1% agar, with 0.04% phosphoric acid and 0.004% propionic acid added to prevent microbial growth in the diet.

Bacterial Infection

Five to nine-day-old mated female flies were infected with the Gram-negative bacterium *Providencia rettgeri* (strain Dmel). This strain of *P. rettgeri* was isolated from the hemolymph of wild caught *D. melanogaster* and hence can be consider a natural pathogen (Juneja & Lazzaro

2009; Galac & Lazzaro 2011). Infection in the thorax of *D. melanogaster* results in a proliferation of the bacteria within the first 24 hours after inoculation, which corresponds to mortality of this acute infection within 72 hours after inoculation. The bacterial load decreases after 24 hours after infection and reaches a steady chronic infection (Howick & Lazzaro 2014). We have focused on the acute phase of infection in this study. For each day of infection, an overnight culture was started from a single bacterial colony and was grown overnight in liquid LB at 37°C with shaking. For the primary mapping experiment and the RNAi knockdown experiments, the overnight culture was diluted in LB to A_{600} of 1.0 +/- 0.1. Female flies were infected by pricking the thorax with a 0.15 mm dissecting pin dipped in the dilute overnight culture of *P. rettgeri* (Khalil *et al.* 2015). For experiments that measured defense across infection doses, flies were injected in the thorax with 23 nl of a dilute overnight culture at A_{600} of 0.1, 0.01, and 0.001 (Khalil *et al.* 2015). As a handling and treatment control, flies were sterilely wounded. When the pinprick method was used for infection, sterile wounding was performed by pricking with an aseptic needle. For the multi-dose experiment using the injector, the wounding control was injected with 23 nl of sterile PBS. In all experiments, 40 flies were infected for each infection treatment on each experimental day and housed in groups of 20. One group of 20 was arbitrarily assigned to the bacterial load assay while the other was used for the survival assay.

Survival and bacterial load assay

Approximately 20 hours after infection, groups of three flies were homogenized in 1 ml of PBS using a FastPrep-24 homogenizer (MP Biomedicals). Homogenates were diluted 1:100 or 1:1000 in PBS prior to plating. These diluted homogenates were then plated onto LB agar using a WASP 2 spiral plater (Microbiology International, Bethesda, MD, USA). Plates were incubated overnight at 37°C. Resulting colonies (colony forming units, CFU) were counted using the

158 ProtoCOL plate counting system (Microbiology International) to estimate the number of viable
159 bacteria per pool of flies.
160 In the primary mapping experiment, survival of infection was estimated for each DGRP
161 line at a single time point at two days after inoculation. The number of dead and living flies was
162 counted to estimate the proportion of flies surviving the infection. This time point corresponded
163 to the greatest mortality from the acute phase of *P. rettgeri* infection (Howick & Lazzaro 2014).
164 In subsequent experiments, including the multiple-dose experiment and the RNAi knockdown
165 experiments, survival was measured once a day for five days after inoculation, with the flies
166 transferred to fresh medium every 2-3 days. On the fifth day, the flies still alive were counted
167 and censored from the experiment. As a control, 20 females were wounded and survival was
168 monitored in the same fashion. Three independent biological replicates were performed for each
169 experiment.
170 *Genome-wide association study*
171 In total approximately 19,000 flies from 172 lines of the DGRP were infected for
172 phenotyping. Data was collected across three independent experimental blocks. For each block,
173 infections were performed over eight days, and within each day, 20 to 24 DGRP lines were
174 randomly assigned without replacement to be infected. For each DGRP line on each day, 40
175 female flies were infected and housed in groups of 20. One group of 20 was arbitrarily assigned
176 to the bacterial load assay while the other was used for the survival assay. Because of the high-
177 throughput nature of the experiment, no sterile-wound control was performed.
178 To estimate the genotypic deviation in tolerance, a mixed model was built using the
179 proportion of flies surviving the infection as the response variable and the bacterial load as a
180 covariate in the model to control for the level of resistance for each *Drosophila* genotype. For the

three traits mapped (bacterial load sustained, host survival of infection, and genotypic deviation in tolerance), the data was corrected for experimental variables (random factors: Block: k = 1-3; Day: l = 1-24) and whether the lines carried the endosymbiotic bacteria *Wolbachia pipientis* (fixed factor: Wolbachia: j = 1, 2) using the lme4 package in R (Bates et al. 2014; R Core Team 2014). The models for each trait are given below:

Bacterial Load:

$$\ln(CFU)_{jkl} = \mu + Wolbachia_j + Block_k + Day(Block_k)_l + \varepsilon_{jkl}$$

Host Survival:

$$Proportion\_Alive_{jkl} = \mu + Wolbachia_j + Block_k + Day(Block_k)_l + \varepsilon_{jkl}, \text{ Family=binomial}$$

Genotypic Deviation in Tolerance

$$Proportion\_Alive_{jkl} = \mu + Load + Wolbachia_j + Block_k + Day(Block_k)_l + \varepsilon_{jkl}, \text{ Family=binomial}$$

The residuals were extracted from each model and the predicted mean from each line was used for association testing. These values can be found in Table S7. Mapping was performed in PLINK version 1.9 (Purcell *et al.* 2007) using the publically available genome-sequences of the 172 DGRP lines we measured (Mackay *et al.* 2012; Huang *et al.* 2014). Approximately 2.5 million SNPs were used in the study with a minimum minor allele frequency of 0.05 required for inclusion in the study. A nominal p-value of $p < 10^{-5}$ was used as an initial significance threshold.

Gene Ontology analysis

To test for enrichment of functional groups of genes among our mapped hit, Gene Ontology was performed using GOWINDA, which accounts for an unequal probability of sampling genes as a consequence of gene length (Kofler & Schlötterer 2012). Analysis was performed using both the GOslim gene set (Adams *et al.* 2000) and the FuncAssociate2 gene set

(Berriz *et al.* 2009). The GOslim set contained fewer and broader terms than the FuncAssociate2 set. GOWINDA was run using version 5.46 of the *D. melanogaster* genome annotation with 100,000 simulations in gene mode that conservatively assumes complete linkage disequilibrium of all SNPs, where a "gene" was defined as all SNPs within 2000 bp of an annotated gene. The FuncAssociate2 set was run with a minimum gene set for each category of 10. SNPs that we identified as significantly associated with mapped phenotypes at $p < 10^{-5}$ were included in the analysis.

Functional testing of candidate genes

To unbiasedly test whether the genes bearing the mapped SNPs played a functional role in defense, we used RNAi to ubiquitously knock-down all candidate tolerance genes containing a SNP that mapped with $p < 10^{-5}$ within coding or intronic regions. To test the proportion of arbitrary genes that would alter defense when knocked-down, we randomly selected 10 control genes from the annotated list of all *Drosophila* genes and ubiquitously knocked-down these genes. To knock-down each gene, females carrying the RNAi construct (Table S8) were crossed to males from the driver line (Actin5C-Gal4/CyO), which ubiquitously expresses Gal4. A small number of genes were also tested using the c564-Gal4 driver line, which drives expression primarily in the fat body and hemocytes. A list of all genes tested can be found in Table S8. Bacterial load and survival of the knock-down genotypes were compared to the background genotype for that knock-down line crossed the driver line. Progeny were sorted 2-3 days prior to infection and kept in groups of 20 females and 5 males of the same genotype. Males were discarded at the time of infection.

Results

Defining tolerance as a genotypic deviation

Existing conceptual definitions of tolerance are not suitable for mapping genetic variation because they are either biologically unrealistic (e.g., assume a linear relationship between pathogen burden and fitness) or are experimentally untenable (e.g., require multiple measurements of each genotype across a range of infection doses). To overcome these limitations, we created a new measure of genetic variability in tolerance, which we call "genotypic deviation in tolerance". The premise of this approach is that the entire data set is used to predict a general, non-linear relationship between host fitness and sustained pathogen load after injection of a uniform initial dose. The degree to which individual genotypes depart from this relationship is the genotypic deviation in tolerance.

To estimate genotypic deviations in tolerance, we first determined the pathogen load sustained (Figure 1A) and proportion of flies surviving the infection (Figure 1B) for each genotype in the study. We then estimated a function describing the expected survival of *D. melanogaster* for *P. rettgeri* infection across the range of pathogen burdens experienced, incorporating the bacterial load and survival data from all genotypes measured. The vertical deviation of each measured genotype from that inferred relationship is our estimate of variation in tolerance. This definition allowed us to measure the departure of a given genotype from the overall population tolerance curve (Figure 1C).

We found significant genetic variation for survival, resistance and the genotypic deviation in tolerance ($p < 0.0001$). Bacterial loads ranged from a mean of 3.00×10^4 bacteria per pool of three flies in the most resistant line to 3.69×10^7 bacteria per three flies in the least resistant line (Figure 1A). Eight lines had no flies surviving the infection, whereas the line with

highest survival had 93% of flies surviving (Figure 1B). There was a strong negative relationship between bacterial load and proportion of hosts surviving the infection (r = -0.852, $p < 0.0001$, Figure 1C). There was a positive relationship between the tolerance deviation and the proportion of hosts surviving the infection (r = 0.772, $p < 0.0001$, Figure S2). There was a positive relationship between resistance and genotypic deviation in tolerance (r = 0.348, $p < 0.0001$: Figure 1D). This suggests that there is no tradeoff between resistance and tolerance, but instead genotypes that had high levels of resistance were also better able to deal with the relative consequences of the infection.

To understand whether our genotypic deviation in tolerance accurately represented tolerance across infection severity, we infected six DGRP lines that had high or low genotypic deviation in tolerance and experienced a range of bacterial loads (Figure S1A). We infected these six lines with three inoculation doses, introducing approximately $1x10^1$, $5x10^2$ or $1x10^4$ bacteria per fly—a 1000-fold range in inoculation dose (Figure S1B). Across these treatments, bacterial loads at 20 hours after inoculation ranged from 6.43 x 10^4 to 6.94 x 10^7 (Figure 2B) and survival of infection at 5 days post-inoculation ranged from 89% to 0% (Figure 2A). We found that resistance level was a stronger predictor of survival of infection than inoculation dose or genotype (bacterial load: $p < 2.2x10^{-16}$, dose: $p = 0.042$, genotype: $p = 3.03x10^{-7}$), and that bacterial load was determined much more strongly by genotype ($p < 2.2x10^{-16}$) than by initial infection dose ($p = 6.295x10^{-9}$). In other words, the lines sustained an approximate pathogen burden that was stereotypical for that genotype, regardless of initial infection dose, and the genetically fixed pathogen burden was in turn predictive of survival (Figure 2C). We note that this pattern renders tolerance estimates based on infection dose irrelevant and probably inaccurate (Figure 2D), a point we return to in Discussion.

The genetic architecture of resistance and tolerance of infection

To identify candidate genes that predict defense against bacterial infection, we performed a genome-wide association study on our three traits of interest (survival of infection, bacterial load, and genotypic deviation in tolerance) (Figure S3). We identified 63 SNPs in 49 genes that predicted survival of infection (Table S1), 25 SNPs in 20 genes that predicted pathogen load (Table S2), and 30 SNPs in 25 genes that predicted genotypic deviation in tolerance (Table 1). SNPs that explained variation in survival of infection are much more likely than random to fall in or around genes. We find that 24.36% of the SNPs mapped for survival fall within coding regions compared to only 9.71% of the of SNPs genome-wide (X_2 = 12.56, p = 3.94 x10$_{-4}$) and 25.64% lie within 5000 bp of an annotated gene relative to 14.98% of genome-wide SNPs (X_2 = 4.08, p = 0.04345). SNPs that explained variation in bacterial load were slightly enriched in coding regions (X_2 = 3.0373, p = 0.08137). There was no significant enrichment of any site class for SNPs that explained variation in tolerance relative to the rest of the genome.

Although only five SNPs overlapped between our mapped phenotypes (Table S3), there was a positive correlation between effect sizes of significant SNPs across the traits. This was seen most strongly in SNPs that predicted survival of infection. The effect sizes of the alleles that significantly predicted survival were positively correlated in magnitude and direction with the effect size of those alleles on both resistance and the genotypic deviation in tolerance (resistance: r = 0.993, p < 2.2x10$_{-16}$; tolerance deviation; r = 0.990, p < 2.2x10$_{-16}$; Figure 3). This was also seen to a slightly lesser degree for the effect sizes of the SNPs which significantly predicted resistance or the genotypic deviation in tolerance when compared to mapped effect sizes on the other traits. (resistance SNPs vs survival: r = 0.980, p < 2.2x10$_{-16}$, vs tolerance: r = 0.626, p =

8.2x10^{-4}; tolerance SNPs vs survival: $r = 0.966$, $p < 2.2\text{x}10^{-16}$, vs resistance: $r = 0.700$ $p = 3.32\text{x}10^{-5}$; Figure 3). This general positive correlation in effects implies that shared SNPs influence other defense traits even when the nominal significance threshold is not met, either because of pleiotropy or because the measured traits are inherently interdependent.

Genes that harbor tolerance SNPs are involved in regulation of gene expression

We performed gene ontology analysis to understand whether the genes we identified as harboring allelic variation for our mapped traits were enriched for specific biological processes. Genes that harbor SNPs that explained variation in survival were enriched for the GO categories "defense response" and "response to stress." We found enrichment for genes involved in "defense response," "protein kinase activity," and "structural molecule activity" in predicting bacterial load. In contrast to the survival and load enrichments, we found enrichment in genes involved in the "nucleus," "proteinaceous extracellular matrix," and "endoplasmic reticulum" (Table 2) among those that harbor tolerance SNPs. When the analysis was repeated using a more refined set of GO terms, these genes were enriched for "negative regulation of gene expression," "immune system processes," and "metabolic processes." In contrast, candidate resistance and survival genes were involved in the "antibacterial humoral response" and other categories (Table S4, Table S5, Table S6). Importantly, none of these terms survived FDR correction for multiple tests. Relaxing the *p*-value threshold of the GWAS to $p = 10^{-4}$ only slightly changed the GO results and did not provide further biological insight (data not shown). The lack of significance in GO enrichment is probably because GO analysis of GWAS results assumes an infinitesimal model of quantitative genetics, where many genes in each relevant GO category each make small but detectable contributions to overall phenotypic variation. This model is unlikely to hold in experimental practice because (a) trait variation is likely to be determined by a finite number of

genes, with very few causal genes representing each functional category, and (b) if the observed phenotypic variance were distributed among very many causal genes in few GO categories, the proportion of variance explained by each individual gene would become so small as to be undetectable in a study the size of ours so the GO analysis would still be underpowered.

Genetic variants that alter tolerance not transcriptionally induced under infection

To test whether any of the genes identified in the GWAS were induced under infection conditions, we compared each gene list with published transcriptomic data from *D. melanogaster* Canton-S mated females infected with *P. rettgeri* (Short & Lazzaro 2013). We found meaningful overlap between genes that contained SNPs that predicted survival and bacterial load and the 186 genes that had altered expression under infection conditions in the microarray study. We found that six of 49 genes that harbored SNPs that predicted survival of infection were induced under infection conditions (*Dpt*, *DptB*, *CG30098*, *TrpA1*, *IM23*, and *IM1*). Three of 20 genes that contained resistance SNPs had altered expression under infection. *Dpt* and *DptB* had increased expression and *dsb* was repressed after infection. Many of the genes that were modulated under infection are previously characterized immune genes. This is not surprising because, historically, the major humoral immune response pathways in *Drosophila* have been defined by genes that transcriptionally respond to infection (De Gregorio *et al.* 2002).We found that none of our 25 genes that contained SNPs predicting genotypic deviation in tolerance were transcriptionally altered by infection. The lack of transcriptional modulation of candidate tolerance genes suggest that tolerance is not determined by induced expression of effector molecules in the same manner as resistance, but may instead be determined by the state of the host at the time of infection.

Alternatively, mapped genetic variation in transcription factors could alter tolerance by regulating responsive genes that do not themselves harbor tolerance-altering polymorphisms.

Functional testing of candidate defense genes

Functional studies have characterized the main resistance pathways in *Drosophila* (Lemaitre & Hoffmann 2007); however, we are just beginning to understand how a host tolerates an infection. To confirm that the candidate genes mapped for variation in tolerance played a functional role in defense, we ubiquitously knocked-down expression of each gene using RNAi. Out of the 25 tolerance genes tested, 10 of the knocked-down genotypes produced viable offspring. Out of those 10 genes, 5 altered defense by either changing survival or load after infection relative to the control. To test the proportion of arbitrary genes that would alter defense when knocked-down, we randomly selected 10 genes from the annotated list of all *Drosophila* genes and measured bacterial load and host survival. Out of these 10 genes test, 5 produced viable offspring when ubiquitously knocked-down and only 1 (*Rbp9*) significantly altered survival of infection (Cox proportional hazard model: $p = 0.012$, Figure 4). Our mapped genes that gave defense phenotypes when disrupted with RNAi were *mspo*, *fhos*, *CG4174*, *gus*, and *beat-IIIc* (Figure 4, Figure S4). Knock-down of all five of these genes resulted in a change in tolerance: survival of infection or bacterial load was altered without the corresponding change in the other trait. Knock-down of *mspo* and *fhos* decreased survival of infection (*mspo*: $p = 0.022$, *fhos*: $p = 0.009$. Knock-down of *beat-IIIc* increased survival of infection ($p = 0.005$). Knock-down of *CG4174* and *gus* decreased bacterial load (increased resistance) (*CG4174*: $p = 0.003$, *gus*: $p = 0.015$). This demonstrates that the genes we have identified can alter tolerance through

perturbation of resistance levels without a corresponding change in survival or through altering survival of infection without a change in resistance.

We additionally selected a small number of mapped genes for further investigation because we hypothesized they might play a role in wound healing or defense based on previous studies (Mace *et al.* 2005b; Muratoglu *et al.* 2006; Gordon *et al.* 2008; Han *et al.* 2014). We knocked-down expression of *u-shaped* (*ush*), *grainyhead* (*grh*), *debris buster* (*dsb*), and *CG30098* using the c564 driver, which is expressed primarily in the fat body and hemocytes. *ush* and *grh* were tolerance GWAS hits, *dsb* was a resistance GWAS hit, and CG30098 was a survival of infection GWAS hit. Out of these four genes, three displayed tolerance phenotypes (*ush*, *grh* and *dsb*). Knock-down primarily in the fat body and hemocytes caused a major decrease in survival of the knock-down flies ($p < 0.001$), but no significant change in bacterial load ($p > 0.05$, Figure 5).

Discussion

We found that bacterial load was strongly predictive of survival of infection. This was seen across all the *D. melanogaster* lines tested and within the phenotypically extremes lines, where fitness under infection was much more strongly predicted by genetically-determined resistance level than by infection dose. Lines that fell at the phenotypic extremes stayed at those extreme levels of resistance regardless of dose, while those at intermediate levels of resistance spanned a greater range of bacterial loads based on infection dose (Figure 2C).The strong impact of resistance on the outcome of infection may have prevented our ability to fully separate resistance and tolerance. Methodologically, these results stress the importance of estimating slope tolerance from fitness plotted against pathogen burden after replication within the host

(Strauss & Agrawal 1999). If fitness is plotted against initial infection dose, inferred differences in tolerance may actually be the result of different levels of resistance (Figure 2D). Additionally, in systems where the pathogen replicates within the host, the use of multiple doses does not estimate or account for endogenous host resistance level. In such cases, only comparison of genotypes that have similar levels of resistance can prevent potential differences in tolerance from being confounded by infection severity.

The positive relationship between resistance and tolerance could be a result of distinct forms of defense acting at the extremes of infection severity and/or the boundedness of the survival data at 0% and 100%. The genetic correlations between the three traits measured were reflected in the association study where we found that the direction and magnitude of effect sizes of the mapped SNPs was positively correlated across traits (Figure 3). A positive correlation between traits could also be driven by differences in general vigor in the inbred lines used. However, we did not find any positive correlations between tolerance, bacterial load, or survival of infection and a study performed by Durham *et al.* (2014) that measured fecundity and life-span across the DGRP (Figure S5). Biologically, both the positive relationship between resistance and genotypic deviation in tolerance seen across the DGRP and the inability to separate the two traits by using multiple infection doses implies that we must consider the evolution of tolerance and resistance together. In systems like ours, tolerance will not evolve independently of resistance. These two traits are tangled.

Despite this non-independence between traits, we have identified candidate genes that alter tolerance and not resistance via our genome-wide association study. Importantly, none of the SNPs identified survived at a genome-wide significance level determined by permutation analysis (Figure S6). However, we expect a complex trait such as tolerance to be multi-allelic

and permutation analysis assumes a single large-effect allele determines the trait. The DGRP has been used to successfully identify large-effect alleles in traits with a simple genetic basis, such as resistance to viral infection (Magwire *et al.* 2012). However, the resource lacks power to detect small-effect alleles at genome-wide significance (Vaisnav *et al.* 2014). This relaxed *p*-value threshold allows for hypothesis generation, but requires follow-up studies to confirm the role of the loci in the mapped trait because of the high rate of false positives expected. Future work should be devoted to the development of resources for the identification of genetic architecture of complex traits such as the Drosophila synthetic reference panel (King *et al.* 2012a; b; Long *et al.* 2014).

Despite the limitations described above, we still have evaluated the set of genes carrying SNPs that predicted genotypic deviation in tolerance. These genes were nominally enriched for the GO term 'nucleus' and are involved in regulation of gene expression, metabolism or immunity included the transcription factors *grainyhead*, *pipsqueak*, *domino*, *Blimp-1*, and *C15*. *grainyhead* is involved in developmental processes and wounding healing via ERK signaling in embryo (Mace *et al.* 2005a; Kim & McGinnis 2011). *pipsqueak* is involved in embryonic patterning and regulation of chromatin silencing. *domino* is involved in regulation of hemocyte proliferation and defense (Braun *et al.* 1998; Evans *et al.* 2003). *Blimp-1* regulates development through response to ecdysone (Agawa *et al.* 2007). *C15* is involved in regulation of development including notch signaling (Campbell 2005). Based on this enrichment, we hypothesize that variation in tolerance may be determined by differential regulation of gene expression in essential biological processes, potentially at multiple stages of development and not just in response to infection. This is in contrast to survival and resistance, which are largely determined by variation in previously characterized immune and stress responses including the previously

431 characterized non-synonymous SNP in the antimicrobial peptide gene, *Diptericin* (Unckless *et*
432 *al.* 2015, 2016).

433 Using ubiquitous RNAi knock-down we were able to confirm the role of five mapped
434 tolerance genes in defense: *mspo*, *beat-IIIc*, *fhos*, *gus* and *CG4174*. Previous predictions,
435 expression data, or loss of function studies have shown *mspo*, *beat-IIIc*, and *fhos* may be
436 involved in immune processes or wound healing. *mspo* has been shown to be induced in cell
437 culture infected with *Escherichia coli* (Kleino *et al.* 2008), and beat-IIIc has an immunoglobin-
438 like fold which can be involved in immune function (Watson *et al.* 2005). *fhos* is involved in
439 wound healing (Lammel *et al.* 2014). *gus* and *CG4174* have not been implicated in canonical
440 immunity. *gus* is involved in developmental processes including axis specification, appendage
441 formation, and regulation of catabolic processes (Styhler *et al.* 2002; Kugler *et al.* 2010).
442 *CG4174* is predicted to be involved in oxidation-reduction processes including iron ion and
443 ascorbic acid binding (FlyBase Curators *et al.* 2004).

444 Using tissue specific knock-down in the fat body and hemocytes we were able to confirm
445 the role *grainy head, u-shaped* and *debris buster* in defense. *grainy head* is a transcription factor
446 that is involved in embryonic wound healing via epithelial repair (Mace *et al.* 2005a) and is
447 predicted to bind the promoters of characterized immune genes (Dobson et al. 2016). *debris*
448 *buster* is known to be involved in autophagy of dendritic debris by fusion of the phaogosome and
449 lysosome (Han *et al.* 2014). Autophagy plays an important role in resistance to some bacterial
450 infection and immunogenic tolerance to symbiotic organisms (Voronin *et al.* 2012; Moy &
451 Cherry 2013), both processes which we hypothesize may be associated with infection tolerance.
452 *u-shaped* is involved in lymph gland development and crystal cell differentiation as well as
453 regulation of antimicrobial peptide biosynthetic processes (Evans *et al.* 2003; Muratoglu *et al.*

2006; Valanne *et al.* 2010). These functional studies provide further support that the architecture of tolerance is composed of polymorphisms in the regulators of immune and stress responses. Future work can be done to identify the specific actions and timing of these elements in defense tolerance.

DATA ACCESSIBILITY

All data will be available on dryad.

ACKNOWLEDGMENTS

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Table 1 SNPs that predicted variation in tolerance to infection There were 30 SNPs that predicted variation in tolerance at a *p*-value of $< 10^{-5}$. At a nominal significant threshold several of these also appeared to predict survival of infection, but most did not alter bacterial load.

SNP	Gene	Site Class	Tolerance *p*-value	Survival *p*-value	Load *p*-value
X:21,581,423	none annotated	NA	7.81E-07	4.94E-04	2.00E-01
2R:10,563,254	psq	Intron	9.72E-07	1.14E-02	9.16E-01
2R:5,128,972	CR43256	Splice site acceptor	1.39E-06	1.21E-04	9.70E-02
X:12,140,511	Ten-a	Intron	1.85E-06	1.33E-03	1.78E-01
2R:5,128,902	CR43256	Intron	2.38E-06	2.35E-04	1.15E-01
2L:533,659	ush	Intron	2.50E-06	1.49E-05	2.19E-02
2R:17,840,479	grh	Synonymous	3.51E-06	3.59E-04	5.77E-02
2R:5,332,144	sxc	Downstream	3.72E-06	1.01E-05	3.32E-03
2L:16,628,423	CG42389	Intron	4.22E-06	6.90E-05	1.60E-02
2R:21,323,134	CG30394/dom	5' UTR/Upstream	4.88E-06	8.25E-05	3.56E-02
3R:21,510,537	C15	Intron	4.96E-06	9.43E-05	1.68E-02
2L:17,229,914	beat-IIIc	Intron	5.14E-06	1.69E-04	7.50E-02
3L:5,645,490	Blimp-1	Intron	5.32E-06	2.45E-05	5.09E-03
2R:17,988,140	dpr13	Intron	5.66E-06	2.44E-04	4.39E-02
2R:14,648,549	CG10139	Downstream	5.90E-06	3.47E-06	1.53E-03
3L:18,601,807	CG4174	Non-synonymous	5.91E-06	2.88E-03	5.30E-01
2R:15,517,660	Khc-73	3' UTR	6.15E-06	4.96E-06	1.92E-03
2L:16,578,056	CG42389	Intron	6.52E-06	1.12E-02	8.89E-01
2R:14,681,641	mspo	Intron	6.57E-06	1.15E-03	1.30E-01
2R:5,126,594	gus	Intron	6.58E-06	8.98E-04	2.25E-01
2L:1,766,281	none annotated	NA	8.31E-06	1.70E-04	4.38E-02
2L:8,896,128	CG42713	Downstream	8.49E-06	8.95E-04	4.16E-01
2L:8,896,128	CG34398	Upstream	8.49E-06	8.95E-04	4.16E-01
2L:15,772,825	CG31826	Intron	8.66E-06	8.57E-03	7.67E-01
2L:20,997,569	CG42238	Intron	8.98E-06	1.87E-03	2.61E-01
3R:7,586,053	CG34127	Intron	9.00E-06	8.93E-06	2.17E-03
2L:1,766,283	none annotated	NA	9.17E-06	3.60E-04	8.94E-02
2L:8,895,939	CG34398/CG42713	Upstream/Downstream	9.44E-06	5.36E-04	3.37E-01
3L:8,771,126	Fhos	Intron	9.54E-06	8.71E-04	1.42E-01

640 **Table 2** Nominally significant GOslim Gene Ontology terms from for survival of infection,
641 bacterial load, and tolerance.

Trait	GO Term	p-value
Survival	defense response	0.0027
	response to stress	0.0061
Bacterial Load	protein kinase activity	0.0058
	defense response	0.0125
	structural molecule activity	0.0410
	protein modification process	0.0482
Tolerance	proteinaceous extracellular matrix	0.0208
	endoplasmic reticulum	0.0344
	nucleus	0.0423

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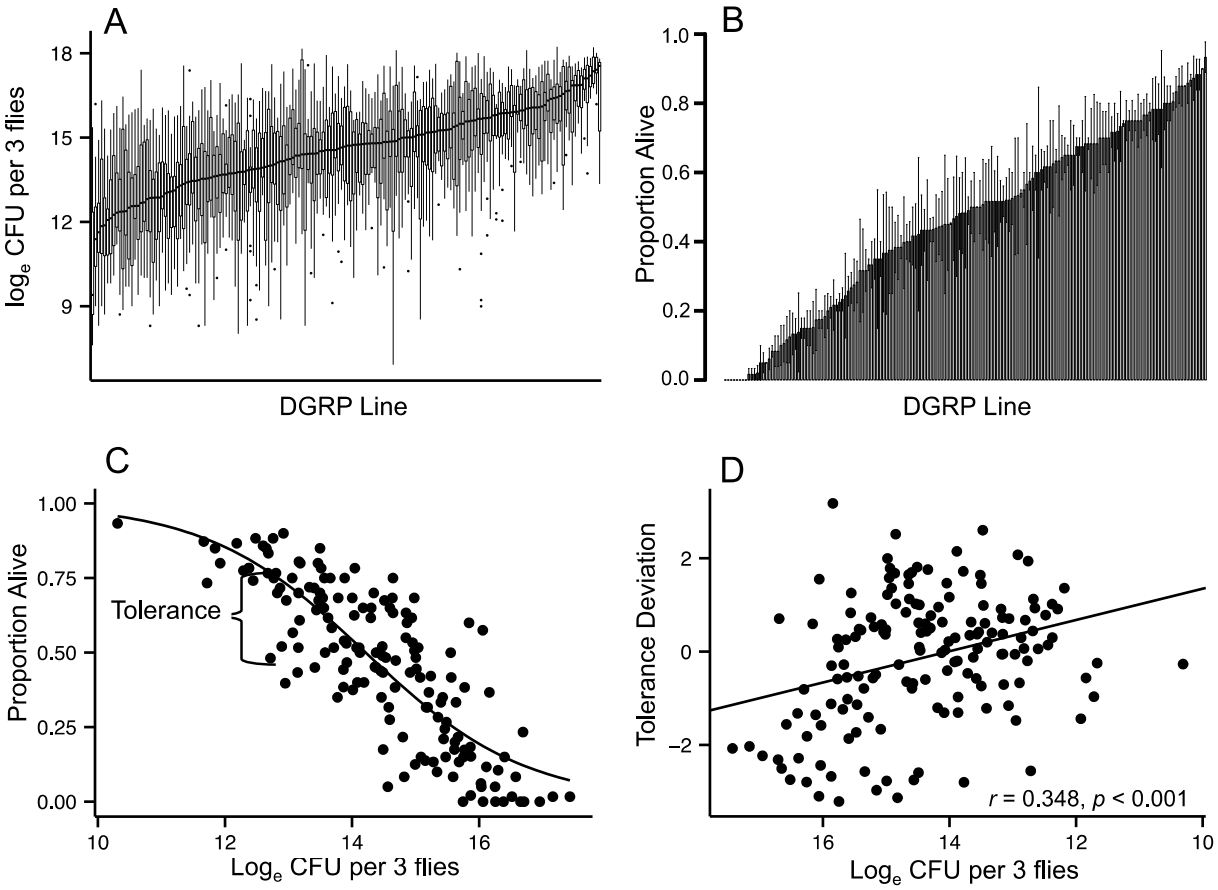


645
646
647 **Figure 1**

648 Distribution of bacterial load (A) and survival (B) across the DGRP; lines are sorted based on
649 phenotypic value for each trait. (C) The mean value of survival for each line plotted against the
650 mean bacterial load. The curve represents the fitted model with a binomial distribution;
651 representing the species tolerance across pathogen burdens. The vertical distance of each point
652 from the function is the genotypic deviation in tolerance. (D) Genotypic deviation in tolerance
653 plotted against mean bacterial load for each line. The bacterial load in A is represented as a box
654 and whiskers plot for each DGRP line where the box represents the first and third quantile and
655 the solid line represents the median. The error bars in B represent one standard error from the
656 mean.
657

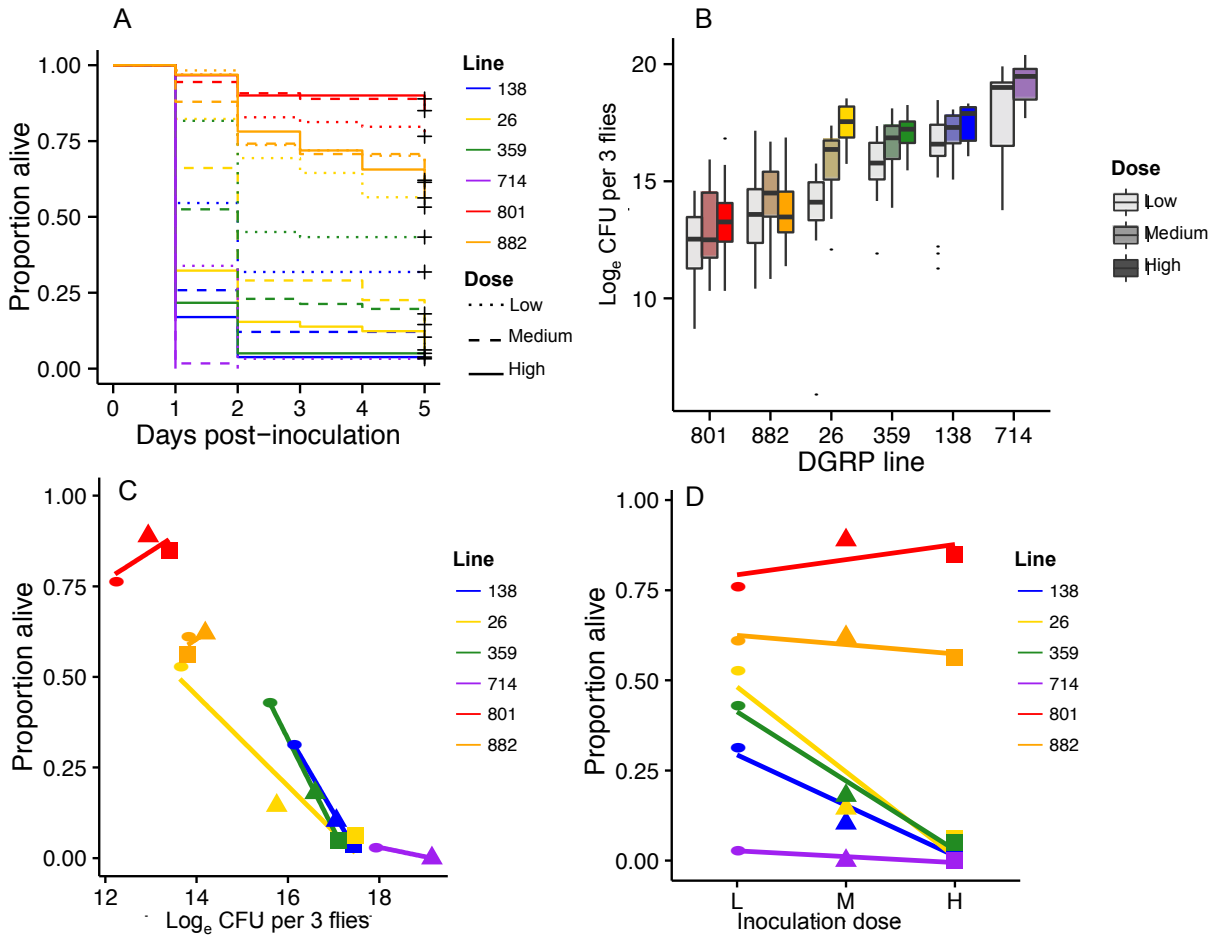


Figure 2

Genotype and resistance predict tolerance. Six DGRP lines were infected with three inoculation doses low (L), medium (M), and high (H), corresponding to approximately 10^1, $10^{2.5}$ or 10^4 bacteria per fly. (A) Survival was estimated daily for five days after inoculation for each line at each dose. Thickness of line dash corresponds to strength of dose, with solid lines representing the highest dose and dotted lines represented the lowest dose. (B) Bacterial load for each line at each dose measured 20 hours after inoculation. Shading of boxes represents the strength of the dose with the highest dose being the darkest box. No data was obtained for line 714 at the high dose because all flies were dead by 20 hours post-inoculation. (C) The proportion surviving five days after inoculation plotted against the mean bacterial load 20 hours after infection at low (circle), medium (triangle), and high (square) infection doses. (D) Proportion surviving the infection plotted against initial infection dose.

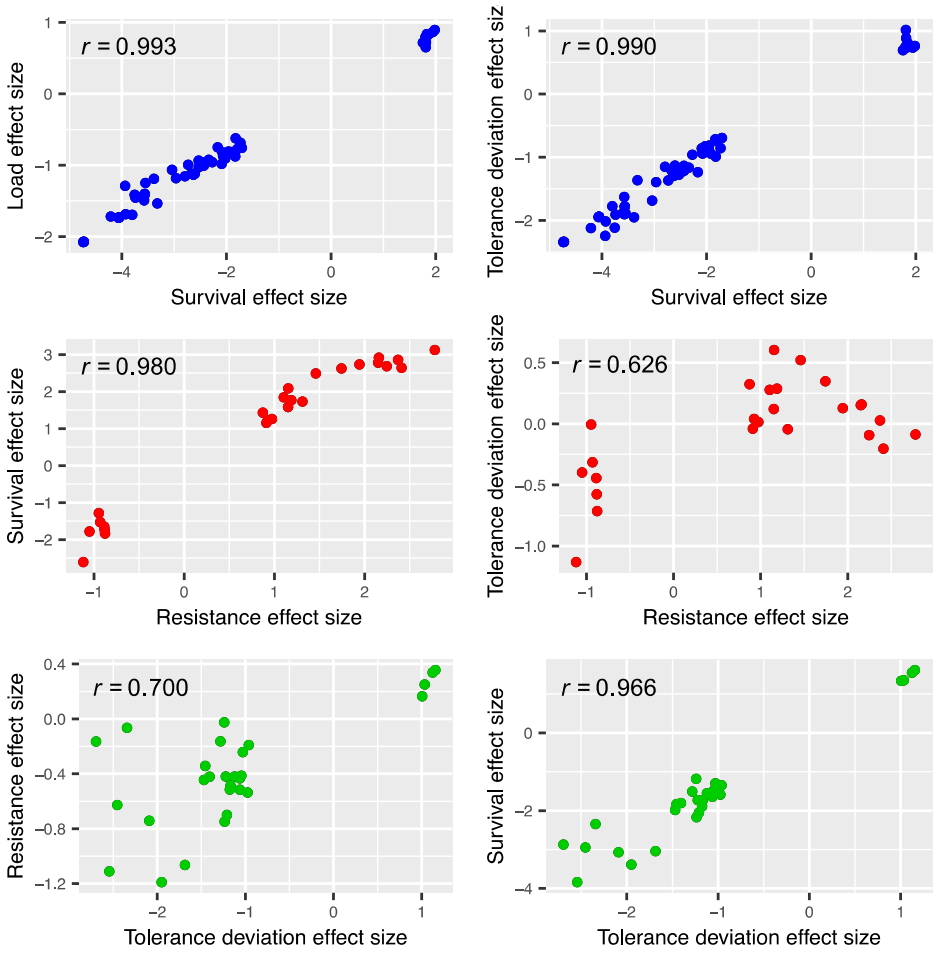


Figure 3. Positive correlation between effect sizes across traits. The effect sizes of each SNP that mapped significantly for each trait are plotted against the absolute value of the effect size of that SNP (regardless of significance level) on the other two mapped traits. The blue points represent SNPs that significantly predicted survival. The red points represent SNPs that significantly predicted resistance. The green points represent SNPs that significantly predicted the genotypic tolerance deviation.

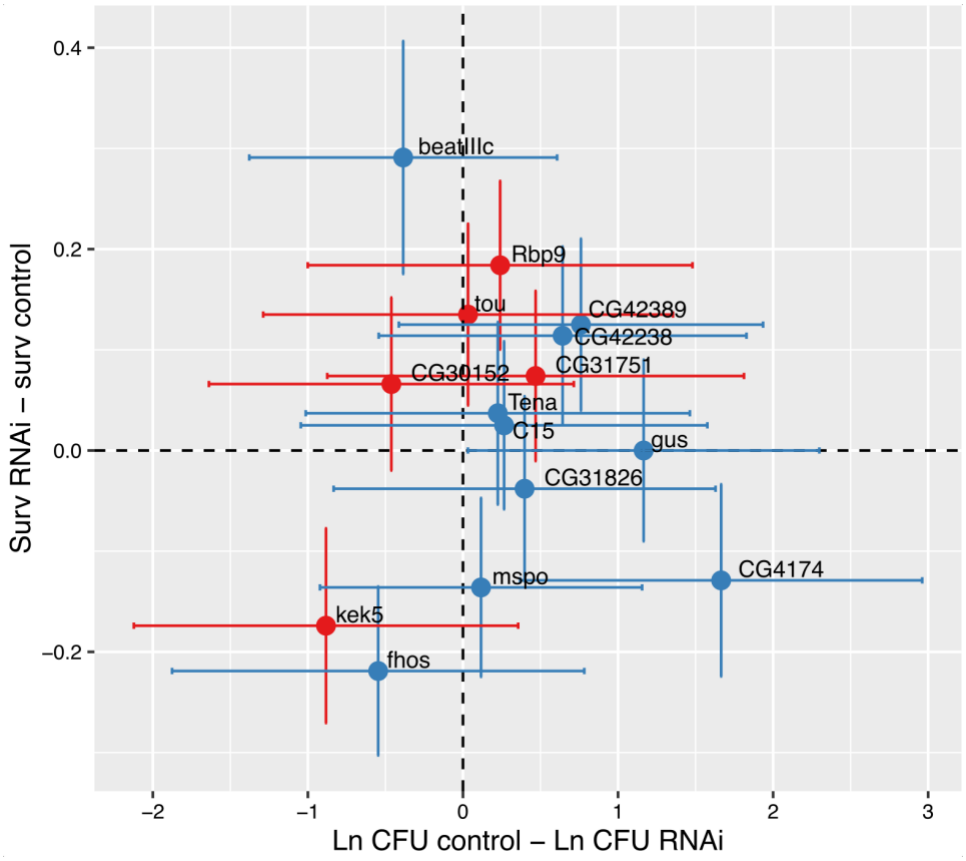


Figure 4

Functional testing of candidate tolerance genes identified in GWAS. Ten genes that had SNPs that explained genetic variation in tolerance to infection were tested to determine whether they functionally altered defense. We found that five of these ten genes (*beat-IIIc*, *CG4174*, *fhos*, *mspo*, and *gus*) altered either survival or bacterial load. One of five randomly selected control genes also altered survival of infection (*Rbp9*). Blue points are the candidate tolerance genes, red points are the control genes. The x-axis is the natural log of the bacterial load from the knock-down line subtracted from the control for that line (driver crossed to background). A higher value represents an increase in resistance with knocked down expression of the targeted gene. The y-axis represents the difference in survival between the knock-down line and the control. A high value represents higher survival in the knockdown flies. The dashed black lines represent the normalized control for each gene tested. The dashed lines represent the level of survival and bacterial load of knock-down control (background genotype crossed to the driver line). The error bars represent one standard error from the mean.

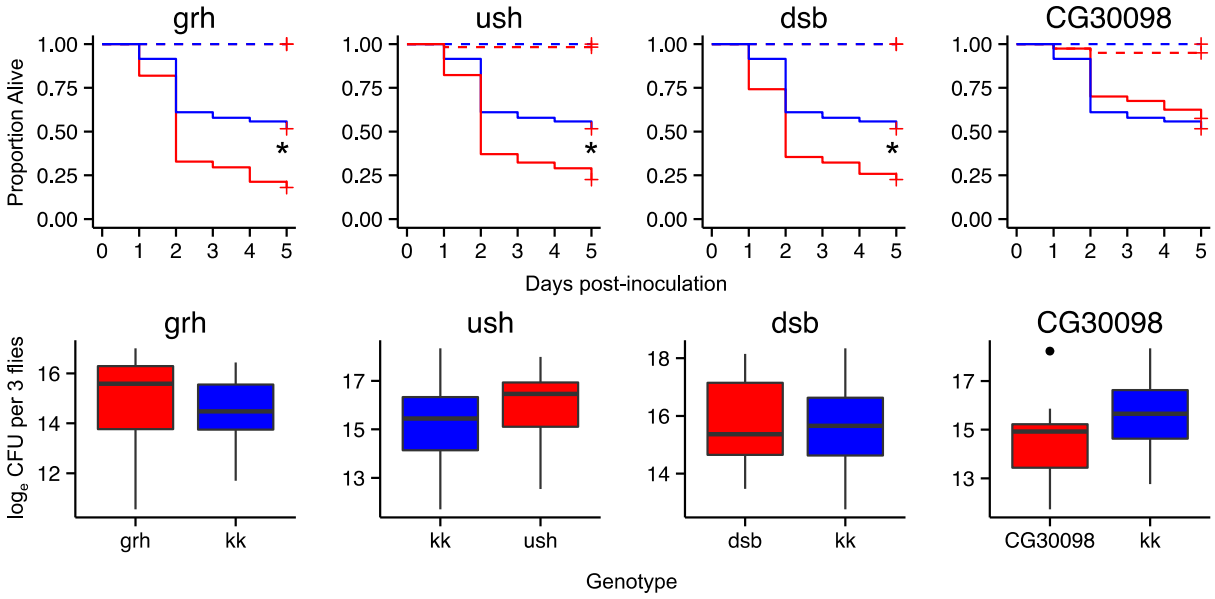


Figure 5

Survival and bacterial load after RNAi knockdown of four genes primarily in the fat body and hemocytes paired with the matched control (genotypic background for the RNAi construct crossed to the knockdown driver). The red line and box represents the knocked-down genotype and the blue represents the control (background genotype crossed to the driver line). A star represents a *p*-value less than 0.05. The dashed lines in the survival plots represent the wounded controls, where the solid lines represent the infected treatments.

Supplemental Figures

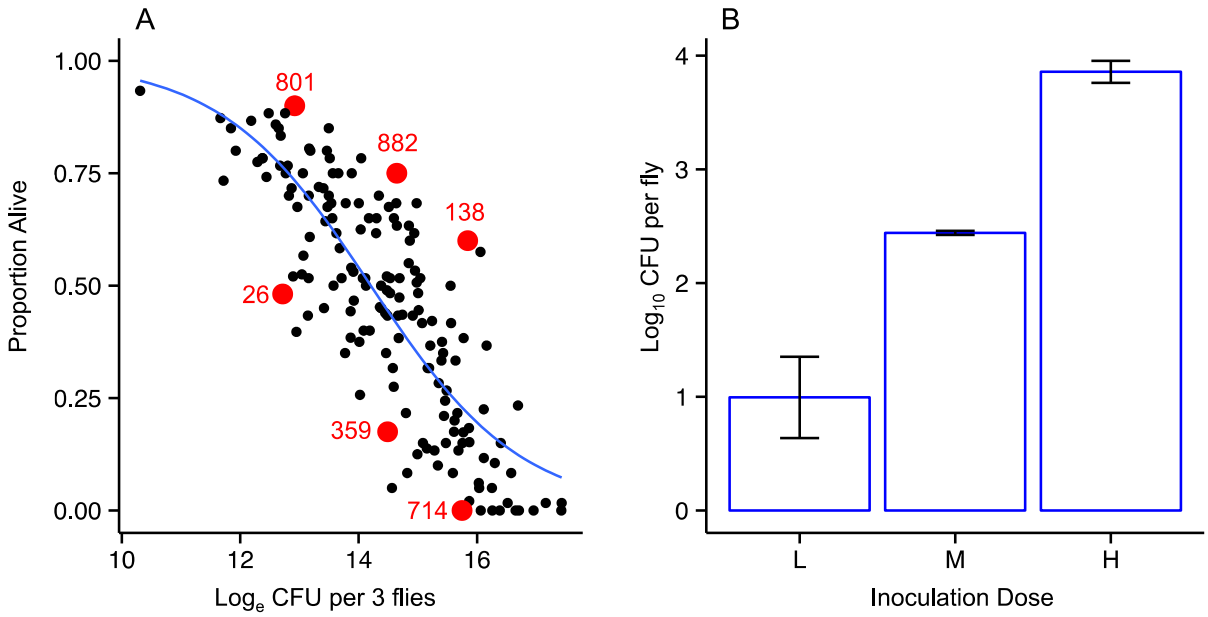


Figure S1

(A) Six lines were chosen that varied in tolerance and resistance in the initial measurements of the DGRP. These lines are represented by large red points. (B) The initial bacterial burden introduced in the low, medium, or high inoculation doses.

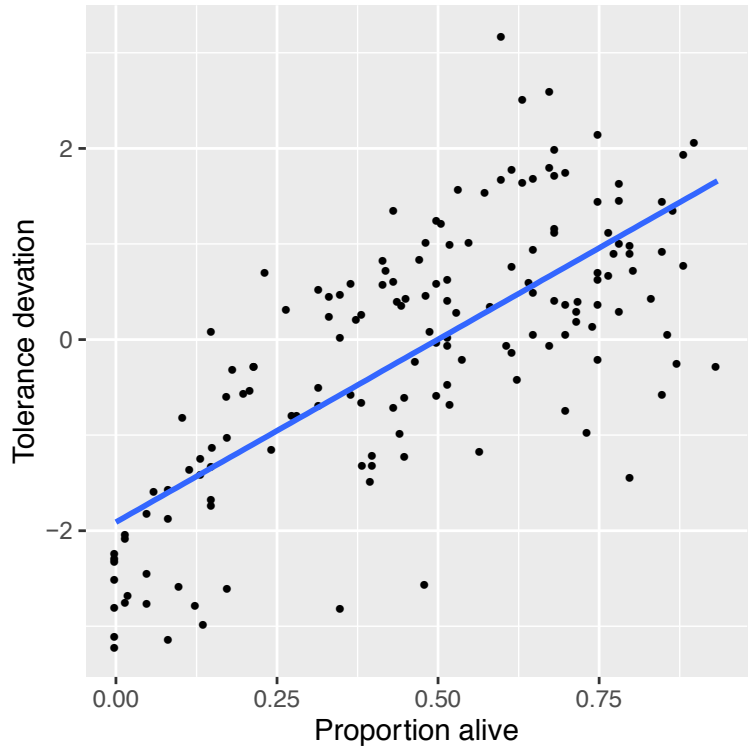


Figure S2. Correlation between genotypic deviation in tolerance and the proportion of flies surviving the infection. There was a strong positive correlation between survival and the genotypic deviation in tolerance ($r = 0.772$, $p < 0.0001$) .

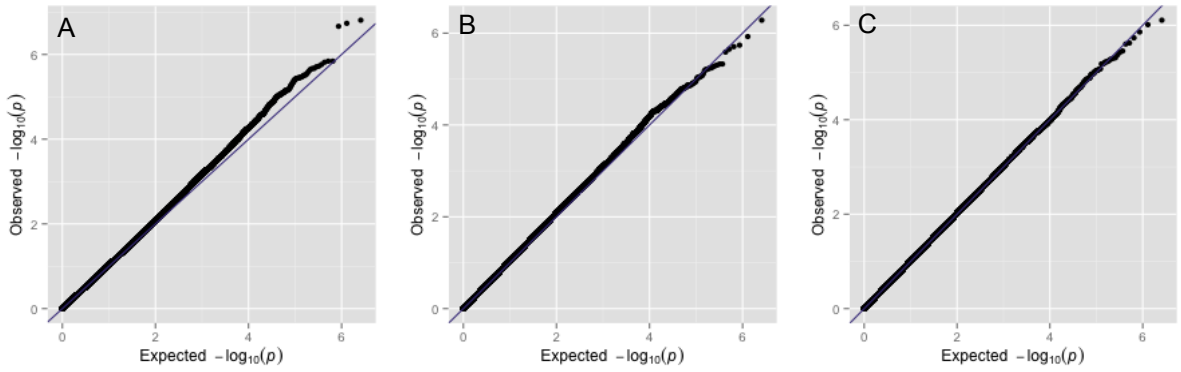
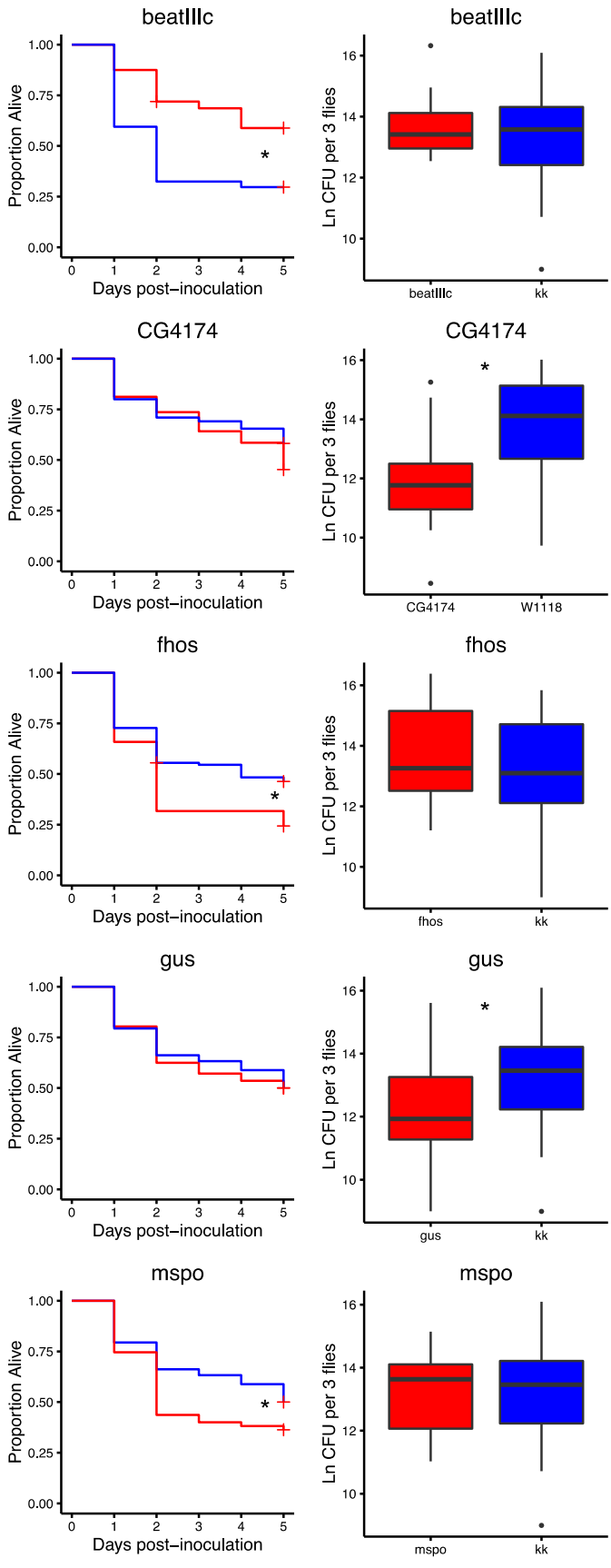


Figure S3

Quantile-quantile plots for survival (A), load (B), and genotypic deviation in tolerance (C). The observed *p*-value is plotted on the y-axis against the expected *p*-value based on a null distribution.


beatIIIc
Proportion Alive
Days post−inoculation
beatIIIc
Ln CFU per 3 flies
beatIIIc
kk
CG4174
Proportion Alive
Days post−inoculation
CG4174
Ln CFU per 3 flies
CG4174
W1118
fhos
Proportion Alive
Days post−inoculation
fhos
Ln CFU per 3 flies
fhos
kk
gus
Proportion Alive
Days post−inoculation
gus
Ln CFU per 3 flies
gus
kk
mspo
Proportion Alive
Days post−inoculation
mspo
Ln CFU per 3 flies
mspo
kk

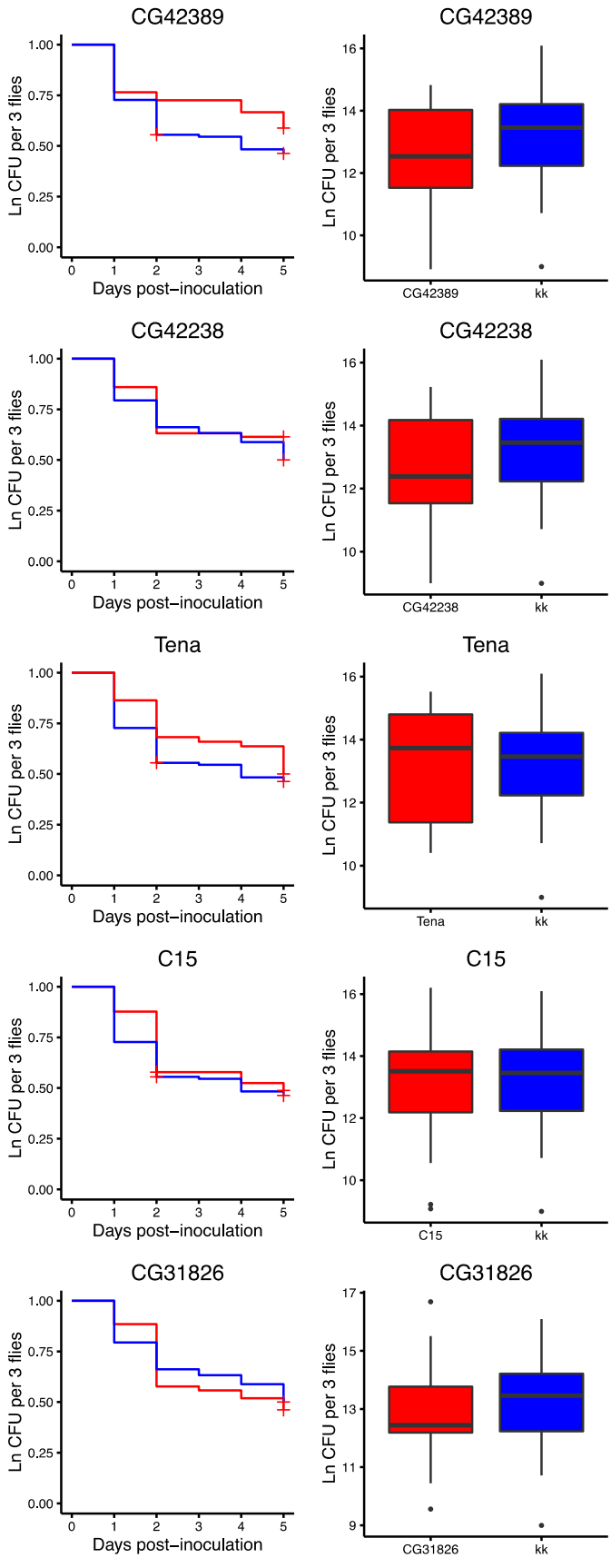

CG42389
Ln CFU per 3 flies
Days post−inoculation
CG42389
kk
CG42238
CG42238
Tena
Tena
C15
C15
CG31826
CG31826

Figure S4 Ubiquitous RNAi knock-down of candidate tolerance genes. Survival and bacterial load plotted with the matched control (genotypic background crossed to the driver). The red line and box represents the knocked-down genotype and the blue represents the control. * Represents $p < 0.05$.

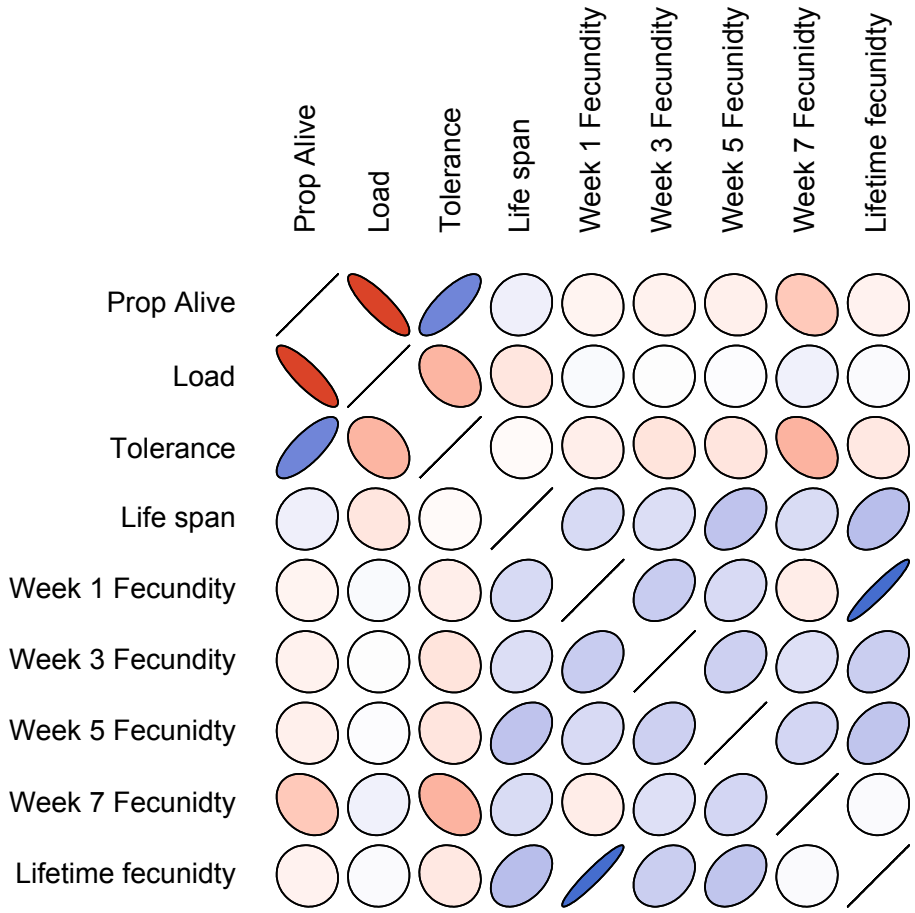


Figure S5. To understand how tolerance and resistance relate to other life-history traits we compared our data with that of Durham (2014), which measured biweekly fecundity at one, three, five, and seven weeks post-eclosion, as well as lifespan. We found no positive correlations between the two studies. We found a weak negative correlation between our estimate of tolerance and late-life fecundity measured at 7 weeks post-eclosion ($p = 0.013$).

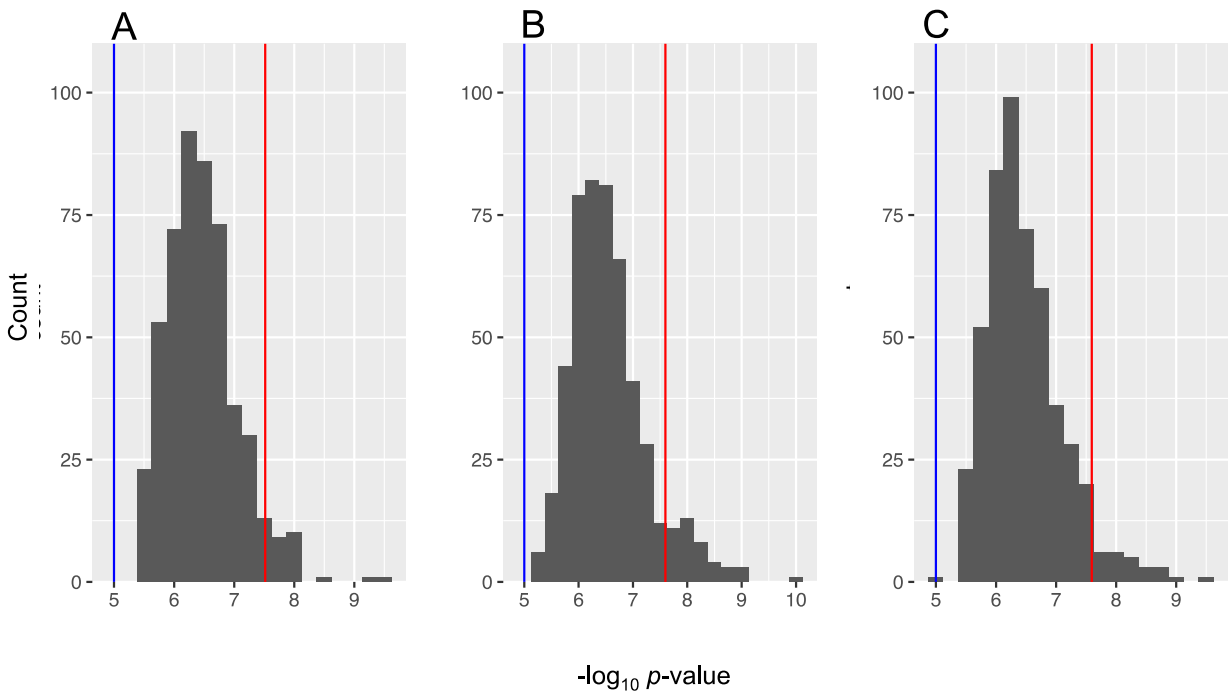


Figure S6. The distribution of the lowest *p*-values from the permutation analysis for (A) survival, (B) bacterial load, and (C) tolerance. A permutation analysis was performed for each trait by randomly assigning the data among the lines and running the association study 500 times. A new *p*-value threshold was reached by extracting the lowest p-value from each permutation and using the 5% quantile as the significance threshold. The blue line represents the significance threshold used in this study and the red line represents the significance threshold determined through the permutation analysis. The permutation based *p*-value cut-offs 1.05×10^{-8} for survival, 3.04×10^{-8} for bacterial load, and 2.55×10^{-8} for tolerance. None of the associated SNPs survived this correction.

Table S1

SNPs that predicted variation in survival of infection

SNP	Gene	Site Class	survival *p*-value	tolerance *p*-value	resistance *p*-value
2R:18,866,084	Dpt	Non-synonymous	1.55E-07	4.53E-05	1.36E-05
2R:18,866,081	Dpt	Synonymous	1.84E-07	0.000105	9.18E-06
X:10,961,811	Myo10A	Intron	2.17E-07	1.10E-05	5.19E-05
2R:19,360,877	CG11961	Synonymous	1.44E-06	0.0005665	4.88E-05
2R:19,360,889	CG11961	Synonymous	1.44E-06	0.0005665	4.88E-05
2R:19,360,892	CG11961	Synonymous	1.52E-06	0.0003159	8.27E-05
2R:18,382,565	CG43202/IM23	Upstream/Downstream	1.73E-06	0.002012	1.37E-05
3L:11,396,932	CG11726	Downstream	1.89E-06	5.60E-05	0.0001657
X:15,722,601	CG8184	Synonymous	1.95E-06	0.000125	2.65E-05
2R:18,159,585	CG10914	Synonymous	2.12E-06	0.000667	6.90E-05
2R:18,865,112	CG43071	Intron	2.14E-06	0.0001323	9.97E-05
3L:16,538,967	CG43373	Intron	2.24E-06	0.0007115	4.46E-05
2L:9,926,696	CG42366	Synonymous	2.46E-06	0.001584	5.94E-06
2R:20,227,263	CG11044/tRNA:G3:56EFa	Upstream	2.73E-06	0.001015	3.30E-05
2R:18,867,233	CG43109/DptB	Downstream/Upstream	3.01E-06	0.0002889	0.0002961
3L:8,003,005	nmo	Intron	3.06E-06	5.13E-05	0.0006615
3L:18,085,826	CG34253/CG43253	Downstream	3.13E-06	4.61E-05	0.0001839
3L:16,539,058	CG43373	Intron	3.32E-06	0.001175	2.06E-05
3L:7,238,058	CG14829	Synonymous	3.40E-06	5.07E-05	0.0007381
2R:14,648,549	CG10139	Downstream	3.47E-06	5.90E-06	0.001528
2R:19,137,295	CG15118	Intron	3.56E-06	5.47E-05	0.0003167
2R:19,137,316	CG15118	Intron	3.56E-06	5.47E-05	0.0003167
2L:580,954	Gsc/Pph13	Downstream	3.64E-06	1.06E-05	0.001957
2L:7,632,178	none annotated	NA	3.68E-06	3.72E-05	8.46E-05
3R:15,004,279	none annotated	NA	3.72E-06	0.001091	7.02E-05
2R:19,361,105	CG11961	Synonymous	4.00E-06	0.001193	3.76E-05
2R:19,361,111	CG11961	Synonymous	4.00E-06	0.001193	3.76E-05
3R:12,876,113	none annotated	NA	4.50E-06	0.0002418	0.000144
3L:12,893,194	CG14118	Intron	4.58E-06	0.0007841	7.33E-05
2R:15,517,660	Khc-73	3' UTR	4.96E-06	6.15E-06	0.001922
X:11,094,476	CG2061	3' UTR	5.35E-06	0.001322	1.29E-05
3L:9,893,290	CG14164/CG6709/CalpB	3' UTR	5.40E-06	9.43E-05	0.0003557
3R:29,648,939	DopR2	Intron	6.06E-06	4.53E-05	0.0002957
X:16,364,296	nonA	Synonymous	6.34E-06	0.002038	0.0002497
2L:6,026,092	CG9109	Intron	6.51E-06	6.99E-05	0.0003556
2R:18,865,898	Dpt	Synonymous	6.81E-06	0.001576	1.97E-05
2R:21,580,570	CG10440/CG30222	Upstream	6.87E-06	0.0001873	0.0001573
2L:6,026,096	CG9109	Intron	7.01E-06	9.78E-05	0.0003566
2R:21,580,510	CG10440/CG30222	Upstream	7.11E-06	0.000198	0.0001476

2R:21,580,514	CG10440/CG30222	Upstream	7.11E-06	0.000198	0.0001476
2R:21,580,577	CG10440/CG30222	Upstream	7.11E-06	0.000198	0.0001476
2R:21,580,580	CG10440/CG30222	Upstream	7.11E-06	0.000198	0.0001476
2R:16,357,001	CG30098/CR43416	Upstream	7.30E-06	0.0002538	0.000402
2L:9,405,390	Shawl	Intron	7.82E-06	0.001746	8.53E-05
2R:15,615,251	CG42524	Non-synonymous	7.84E-06	0.0001332	0.0001457
2R:15,615,256	CG42524	Non-synonymous	7.84E-06	0.0001332	0.0001457
2R:16,198,200	CG8446	Synonymous	8.09E-06	0.0007847	7.50E-05
2R:22,564,958	dnr1	Synonymous	8.17E-06	0.000168	0.0005826
2L:7,619,363	CG6739	Downstream	8.24E-06	0.0001196	0.0001196
2L:7,619,586	CG6739	Downstream	8.24E-06	0.0001196	0.0001196
2R:21,491,413	Fkbp13	Intron	8.67E-06	0.0001657	0.0001881
X:11,773,771	dy	Intron	8.71E-06	1.05E-05	0.001428
2R:18,382,581	CG43202/IM23	Upstream/Downstream	8.75E-06	0.0041	7.63E-05
2R:21,497,707	Fkbp13	Intron	8.84E-06	4.60E-05	0.002199
3R:7,586,053	CG34127	Intron	8.93E-06	9.00E-06	0.002169
2L:7,619,415	CG6739	Downstream	8.96E-06	0.000128	0.0001281
2L:7,619,444	CG6739	Downstream	8.96E-06	0.000128	0.0001281
2L:7,511,417	Rapgap1	Intron	9.42E-06	4.38E-05	0.0004888
2L:20,948,154	none annotated	NA	9.44E-06	1.27E-05	0.002593
3L:8,212,830	CG8006	Non-synonymous	9.44E-06	9.44E-05	0.0006971
2R:14,291,166	Ih	Non-synonymous	9.65E-06	1.34E-05	0.001046
3L:8,898,030	TrpA1	Intron	9.84E-06	0.0001091	0.0003891
2R:20,339,990	CG13870/CG16739	Upstream	9.93E-06	0.0004392	0.0002729

Table S2

SNPs that predicted variation in resistance to infection.

SNP	Gene	Site Class	Load p-value	Tolerance p-value	Survival p-value
X:20,133,406	CG15322	Upstream	5.22E-07	0.8626	0.003466
X:20,133,406	CG42582	Upstream	5.22E-07	0.8626	0.003466
2R:20,102,058	none annotated	NA	1.19E-06	0.1185	5.72E-05
X:20,032,422	D2R	Upstream	1.83E-06	0.7745	0.00249
2R:22,444,306	none annotated	NA	1.97E-06	0.3194	0.0001255
3L:1,909,764	CG1887	Intron	2.23E-06	0.8637	0.002501
2R:21,681,039	CG30263	Synonymous	2.58E-06	0.901	0.009787
X:5,760,226	MAPk-Ak2	3' UTR	4.70E-06	0.7987	0.001575
3L:1,108,730	none annotated	NA	4.70E-06	0.4402	0.0006726
2L:382,411	al	Intron	4.94E-06	0.1607	0.0001008
X:18,923,524	none annotated	NA	5.14E-06	0.8659	0.00451
X:18,923,637	none annotated	NA	5.34E-06	0.9545	0.003872
2R:20,102,018	none annotated	NA	5.71E-06	0.1983	0.0002542
X:20,854,194	shakB	Intron	5.75E-06	0.746	0.01498
2L:9,926,696	CG42366	Synonymous	5.94E-06	0.001584	2.46E-06
2R:20,289,946	CkIIbeta2	Synonymous	6.15E-06	0.05472	3.35E-05
2R:20,289,993	CkIIbeta2	Synonymous	6.17E-06	0.01155	1.01E-05
3R:30,255,259	CG18404	Downstream	6.57E-06	0.04354	4.76E-05
3R:30,255,259	CG9682	Downstream	6.57E-06	0.04354	4.76E-05
X:6,206,890	vanin-like	Upstream	7.67E-06	0.8984	0.003415
X:20,707,400	none annotated	NA	8.35E-06	0.9649	0.008307
2L:10,686,264	CG7296	Downstream	8.67E-06	0.1458	0.0002447
2L:10,686,264	CG7299	Upstream	8.67E-06	0.1458	0.0002447
2L:383,384	al	Intron	8.76E-06	0.7825	0.003203
2R:18,866,081	Dpt	Synonymous	9.18E-06	0.000105	1.84E-07
X:9,230,968	CG12121	Non-synonymous	9.20E-06	0.6918	0.002686
3L:8,732,726	Cp16	Downstream	9.21E-06	0.9827	0.003715
3L:8,732,726	Prm	Downstream	9.21E-06	0.9827	0.003715
3L:15,942,843	Pka-C3	Intron	9.82E-06	0.3589	0.001091

Table S3: Overlapping SNPs between mapped traits

SNP	Gene	Site Class	Tolerance *p*-value	Survival *p*-value	Load *p*-value
2R:18,866,081	Dpt	Synonymous	0.000105	1.84E-07	9.18E-06
2L:9,926,696	CG42366	Synonymous	0.001584	2.46E-06	5.94E-06
2R:15,517,660	Khc-73	3' UTR	6.15E-06	4.96E-06	1.92E-03
2R:14,648,549	CG10139	Downstream	5.90E-06	3.47E-06	1.53E-03
3R:7,586,053	CG34127	Intron	9.00E-06	8.93E-06	2.17E-03

Table S4

Gene ontology for survival of infection using the functional association gene set.

GO ID	Number of genes	Nominal p-value	GO category
GO:0019731	4	0.0025	antibacterial humoral response
GO:0042742	5	0.00451	defense response to bacterium
GO:0031519	2	0.00455	PcG protein complex
GO:0022843	3	0.00557	voltage-gated cation channel activity
GO:0009617	5	0.00565	response to bacterium
GO:0019730	4	0.00599	antimicrobial humoral response
GO:0006952	6	0.00666	defense response
GO:0006959	4	0.00757	humoral immune response
GO:0050830	3	0.00777	defense response to Gram-positive bacterium
GO:0008234	2	0.00835	cysteine-type peptidase activity
GO:0005244	3	0.00836	voltage-gated ion channel activity
GO:0022832	3	0.00836	voltage-gated channel activity
GO:1901265	10	0.01082	nucleoside phosphate binding
GO:0000166	10	0.01082	nucleotide binding
GO:0051704	7	0.01484	multi-organism process
GO:0051707	5	0.01603	response to other organism
GO:0009607	5	0.01618	response to biotic stimulus
GO:0042745	2	0.01628	circadian sleep/wake cycle
GO:0036094	10	0.01699	small molecule binding
GO:0006342	2	0.01955	chromatin silencing
GO:0045814	2	0.01962	negative regulation of gene expression, epigenetic
GO:0044704	2	0.01993	single-organism reproductive behavior
GO:0005249	2	0.02378	voltage-gated potassium channel activity
GO:0042803	3	0.02687	protein homodimerization activity
GO:0030431	2	0.0287	sleep
GO:0019783	1	0.02953	small conjugating protein-specific protease activity
GO:0006955	4	0.03062	immune response
GO:0006950	8	0.03221	response to stress
GO:0051101	1	0.03389	regulation of DNA binding
GO:0042802	3	0.03539	identical protein binding
GO:0070647	2	0.0368	protein modification by small protein conjugation or removal
GO:0016579	1	0.03769	protein deubiquitination
GO:0034605	1	0.0385	cellular response to heat
GO:0043277	1	0.04351	apoptotic cell clearance
GO:0051098	1	0.04444	regulation of binding

GO:0031347	2	0.04495	regulation of defense response
GO:0051172	4	0.04547	negative regulation of nitrogen compound metabolic process
GO:0008144	1	0.04614	drug binding
GO:0003774	2	0.04617	motor activity
GO:0070646	1	0.04843	protein modification by small protein removal
GO:0016461	1	0.04937	unconventional myosin complex

Table S5

Gene ontology for bacterial load using the functional association gene set.

GO ID	Number of genes	Nominal p-value	GO category
GO:0004672	4	0.00295	protein kinase activity
GO:0006468	4	0.00397	protein phosphorylation
GO:0005956	1	0.00433	protein kinase CK2 complex
GO:0005213	1	0.00513	structural constituent of chorion
GO:0016773	4	0.00532	phosphotransferase activity, alcohol group as acceptor
GO:0004674	3	0.00673	protein serine/threonine kinase activity
GO:0016301	4	0.00684	kinase activity
GO:0016310	4	0.00846	phosphorylation
GO:0019731	2	0.01068	antibacterial humoral response
GO:0015277	1	0.01104	kainate selective glutamate receptor activity
GO:0009651	1	0.01277	response to salt stress
GO:0016772	4	0.01536	transferase activity, transferring phosphorus-containing groups
GO:0006970	1	0.01858	response to osmotic stress
GO:0019730	2	0.02656	antimicrobial humoral response
GO:0008010	1	0.02878	structural constituent of chitin-based larval cuticle
GO:0006952	3	0.03085	defense response
GO:0008287	1	0.0322	protein serine/threonine phosphatase complex
GO:0006959	2	0.03267	humoral immune response
GO:0007630	1	0.03269	jump response
GO:0042600	1	0.03555	chorion
GO:0005198	3	0.03728	structural molecule activity
GO:0030312	1	0.03854	external encapsulating structure
GO:0006464	4	0.04143	cellular protein modification process
GO:0036211	4	0.04143	protein modification process
GO:0042742	2	0.04227	defense response to bacterium
GO:0009881	1	0.04279	photoreceptor activity
GO:0010927	2	0.04381	cellular component assembly involved in morphogenesis
GO:0045793	1	0.04396	positive regulation of cell size

GO:0019888	1	0.04441	protein phosphatase regulator activity
GO:0043412	4	0.04526	macromolecule modification
GO:0005044	1	0.0455	scavenger receptor activity
GO:0045089	1	0.04655	positive regulation of innate immune response
GO:0019208	1	0.0477	phosphatase regulator activity
GO:0031349	1	0.04788	positive regulation of defense response
GO:0048800	1	0.04823	antennal morphogenesis

Table S6

Gene ontology for tolerance of infection using the functional association gene set.

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GO ID	Number of genes	Nominal p-value	GO category
GO:0008094	2	0.00097	DNA-dependent ATPase activity
GO:0010629	5	0.00118	negative regulation of gene expression
GO:0045747	2	0.00213	positive regulation of Notch signaling pathway
GO:0045892	4	0.00301	negative regulation of transcription, DNA-dependent
GO:0051253	4	0.00319	negative regulation of RNA metabolic process
GO:0002683	2	0.00335	negative regulation of immune system process
GO:0045934	4	0.00379	negative regulation of nucleobase-containing compound metabolic process
GO:0042803	3	0.00391	protein homodimerization activity
GO:0051172	4	0.00393	negative regulation of nitrogen compound metabolic process
GO:0008595	3	0.00446	anterior/posterior axis specification, embryo
GO:0007351	3	0.00446	tripartite regional subdivision
GO:0010605	5	0.00458	negative regulation of macromolecule metabolic process
GO:0009948	3	0.0051	anterior/posterior axis specification
GO:0000578	3	0.00513	embryonic axis specification
GO:0042802	3	0.0052	identical protein binding
GO:0009892	5	0.00543	negative regulation of metabolic process
GO:0060968	2	0.00665	regulation of gene silencing
GO:0004386	2	0.00759	helicase activity
GO:2000113	4	0.00807	negative regulation of cellular macromolecule biosynthetic process
GO:0010558	4	0.00807	negative regulation of macromolecule biosynthetic process
GO:0009890	4	0.00881	negative regulation of biosynthetic process
GO:0031327	4	0.00881	negative regulation of cellular biosynthetic process
GO:0030097	2	0.01235	hemopoiesis
GO:0009952	3	0.01281	anterior/posterior pattern specification

GO:0033202	1	0.01475	DNA helicase complex
GO:0009798	3	0.01491	axis specification
GO:0008593	2	0.01517	regulation of Notch signaling pathway
GO:0007314	2	0.0152	oocyte anterior/posterior axis specification
GO:0031324	4	0.01526	negative regulation of cellular metabolic process
GO:0016043	10	0.01556	cellular component organization
GO:0008358	2	0.01606	maternal determination of anterior/posterior axis, embryo
GO:0051252	6	0.0163	regulation of RNA metabolic process
GO:0007350	3	0.01716	blastoderm segmentation
GO:0071840	10	0.01735	cellular component organization or biogenesis
GO:0090304	7	0.01847	nucleic acid metabolic process
GO:0046483	8	0.0192	heterocycle metabolic process
GO:0046983	3	0.01923	protein dimerization activity
GO:0002520	2	0.01989	immune system development
GO:0048534	2	0.01989	hematopoietic or lymphoid organ development
GO:0009880	3	0.0213	embryonic pattern specification
GO:0030154	10	0.02288	cell differentiation
GO:0032200	1	0.02355	telomere organization
GO:0000723	1	0.02355	telomere maintenance
GO:0040029	2	0.02383	regulation of gene expression, epigenetic
GO:0003684	1	0.02411	damaged DNA binding
GO:0004003	1	0.02544	ATP-dependent DNA helicase activity
GO:0016458	2	0.02548	gene silencing
GO:0019219	6	0.02662	regulation of nucleobase-containing compound metabolic process
GO:1901360	8	0.02692	organic cyclic compound metabolic process
GO:0048869	10	0.02752	cellular developmental process
GO:0035282	3	0.02768	segmentation
GO:0019511	1	0.03076	peptidyl-proline hydroxylation
GO:0018401	1	0.03076	peptidyl-proline hydroxylation to 4-hydroxy-L-proline
GO:0019471	1	0.03076	4-hydroxyproline metabolic process
GO:0051171	6	0.03083	regulation of nitrogen compound metabolic process
GO:0051294	1	0.03108	establishment of spindle orientation
GO:0007309	2	0.032	oocyte axis specification
GO:0016222	1	0.03206	procollagen-proline 4-dioxygenase complex
GO:0003678	1	0.03263	DNA helicase activity
GO:0007308	2	0.03293	oocyte construction
GO:0044707	13	0.03298	single-multicellular organism process
GO:0035152	2	0.03339	regulation of tube architecture, open tracheal system
GO:0005578	2	0.03343	proteinaceous extracellular matrix

GO:0004656	1	0.0339	procollagen-proline 4-dioxygenase activity
GO:0031545	1	0.0339	peptidyl-proline 4-dioxygenase activity
GO:0019798	1	0.0339	procollagen-proline dioxygenase activity
GO:0031543	1	0.0339	peptidyl-proline dioxygenase activity
GO:0007219	2	0.03538	Notch signaling pathway
GO:0071390	1	0.03573	cellular response to ecdysone
GO:1901655	1	0.03573	cellular response to ketone
GO:0036315	1	0.03573	cellular response to sterol
GO:0031418	1	0.03647	L-ascorbic acid binding
GO:0006302	1	0.03669	double-strand break repair
GO:0048599	2	0.0368	oocyte development
GO:0007009	1	0.03684	plasma membrane organization
GO:0010468	6	0.03755	regulation of gene expression
GO:0097306	1	0.03812	cellular response to alcohol
GO:0005769	1	0.03813	early endosome
GO:0001071	4	0.03846	nucleic acid binding transcription factor activity
GO:0003700	4	0.03846	sequence-specific DNA binding transcription factor activity
GO:0016070	6	0.03882	RNA metabolic process
GO:0022416	2	0.03951	chaeta development
GO:0018208	1	0.03964	peptidyl-proline modification
GO:0007501	1	0.04009	mesodermal cell fate specification
GO:0005634	7	0.04052	nucleus
GO:0016702	1	0.04141	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen
GO:0006807	8	0.04214	nitrogen compound metabolic process
GO:0016701	1	0.04304	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen
GO:2001141	5	0.04354	regulation of RNA biosynthetic process
GO:0006355	5	0.04354	regulation of transcription, DNA-dependent
GO:0048469	2	0.04367	cell maturation
GO:0009994	2	0.0441	oocyte differentiation
GO:0060249	2	0.04452	anatomical structure homeostasis
GO:0060966	1	0.04603	regulation of gene silencing by RNA
GO:0031012	2	0.04657	extracellular matrix
GO:0007392	1	0.0467	initiation of dorsal closure
GO:0006139	7	0.04757	nucleobase-containing compound metabolic process
GO:0006351	5	0.04798	transcription, DNA-dependent
GO:0032774	5	0.04807	RNA biosynthetic process
GO:0001078	1	0.04847	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription
GO:0035195	1	0.04931	gene silencing by miRNA

GO:0031935	1	0.04966	regulation of chromatin silencing
GO:0043231	9	0.0499	intracellular membrane-bounded organelle

Table S7

Proportion surviving, bacterial load and estimates of genotypic deviation in tolerance for DGRP lines used in this study. Adjusted proportion alive, adjusted load, and tolerance were the values used for the GWAS.

DGRP Line	Proportion Alive	Adjusted Prop Alive	Bacterial Load	Adjusted Load	Tolerance
RAL-101	0.866666667	4.112926825	12.18604494	-1.86477172	1.362368299
RAL-105	0.643137255	1.964202267	13.43986165	-0.821253633	0.608697811
RAL-109	0.216666667	-1.322928889	14.79973529	0.747724183	-0.276195227
RAL-129	0.65	1.954399978	13.55604826	-0.445324053	0.064711081
RAL-136	0.75	0.979268277	13.56442947	-0.094005303	0.374416318
RAL-138	0.6	1.823228005	15.84247728	0.877345793	3.17897223
RAL-142	0.483333333	-0.637222646	15.00492148	0.877690141	0.473020215
RAL-149	0.8	2.325120025	13.4644145	-0.58596969	0.991030762
RAL-153	0.421428572	-0.276181613	15.24032023	0.602574101	0.731556802
RAL-158	0.133333333	-2.293830441	15.68569685	0.694233253	-1.235593516
RAL-176	0.516666667	-1.030887154	15.04163411	1.148619607	0.424685291
RAL-177	0.105555556	-2.183817382	16.30033545	1.277395291	-0.806675609
RAL-181	0.173809524	-2.631197103	15.76958752	1.49290015	-0.58362993
RAL-195	0.45	-0.662554108	14.38108779	0.126671184	-0.595229209
RAL-208	0.083333333	-2.772509509	15.59190928	0.538113727	-1.862052819
RAL-217	0.683333333	1.547913407	14.63559401	-0.12852851	1.130330976
RAL-223	0.216666667	-2.334242504	15.66892829	1.40897396	-0.276700837
RAL-227	0.383333333	-0.975532678	15.77288607	0.828899035	0.269996517
RAL-228	0.608333334	1.502585103	13.17495655	-1.074727758	-0.053612342
RAL-229	0.633333333	1.530844107	14.64598075	0.042188121	1.650472151
RAL-233	NA	NA	13.52835969	-0.194246879	NA
RAL-235	0.775	3.698782263	12.2913421	-2.041564118	0.914115677
RAL-237	0.683333333	1.987948693	14.97967156	0.047127802	1.998491224
RAL-239	0.499754902	1.280026793	15.55335982	-0.060162413	1.257819519
RAL-26	0.481372549	0.400617458	12.71687617	-2.098787858	-2.553693995
RAL-28	0.061111111	-3.697717009	16.0294644	1.974903747	-1.579508603
RAL-287	0.275	-1.668156553	14.59331946	0.604934266	-0.784807662
RAL-301	0.530952381	0.008107901	13.91104164	0.214232408	0.298004591
RAL-304	0.05	-3.764391034	16.25188702	1.563303877	-1.813254638
RAL-306	0.683333333	1.244877984	14.00430504	0.134478001	1.169315745
RAL-307	0	-4.176162881	16.38424935	1.820568039	-2.282631912

RAL-309	0.016666667	-4.679621867	17.1606631	2.427142647	-2.028912461
RAL-315	0.5	0.492031987	13.57641863	-0.657454411	-0.573548292
RAL-317	0.483333333	-0.410769938	14.53220093	1.106832148	1.025350033
RAL-318	0.283333333	-1.870725742	15.35370123	0.709022439	-0.787849861
RAL-319	0.85	3.128910691	12.65137255	-1.348042507	0.935325531
RAL-320	0.137878788	-4.139841529	15.15316389	1.157391701	-2.967856094
RAL-321	0.244047619	-1.878982921	15.46065821	0.845591559	-1.134261414
RAL-324	0.452272728	0.246922655	14.36035375	-0.020452453	0.439343934
RAL-332	0.333333333	-0.130529254	15.63697351	0.326909256	0.255884859
RAL-335	0.872727273	2.568141391	11.66674901	-2.196675032	-0.243899253
RAL-336	0.416666667	-0.650594991	15.56208123	1.014642378	0.833243599
RAL-338	0.719444444	2.156248282	13.32916767	-1.304220848	0.40335054
RAL-340	0.783333333	2.968682497	12.37482407	-1.84234297	0.301834359
RAL-350	0.683333333	2.171501073	13.78241365	-0.427489164	1.722008871
RAL-352	0.833333333	2.823264734	12.68413354	-1.627102543	0.444176189
RAL-356	0.489705883	0.572924057	14.47831071	-0.166505275	0.097230453
RAL-357	0.016666667	-4.327074821	17.42931057	2.160149474	-2.072878519
RAL-358	0.4	-0.203256321	14.18844634	-0.717823033	-1.201469833
RAL-359	0.175	-2.288110609	14.48988348	-0.220436811	-2.59151144
RAL-360	0.516666667	0.523174636	14.07133219	-0.29065233	0.035518764
RAL-361	0.65	1.616241045	14.30278342	-0.665253959	0.506744607
RAL-362	0.766666667	2.617197885	12.80300702	-1.288553633	0.676322687
RAL-365	0.8	2.925783246	13.18550633	-1.259164636	0.914233588
RAL-367	0.016666667	-5.16927669	16.51736239	2.138163919	-2.741656024
RAL-370	0.516666667	0.51896716	13.15512632	-0.328517979	-0.049096279
RAL-373	0.575	1.663922411	16.05778501	-0.162572104	1.553824752
RAL-374	0.05	-3.912605442	14.56364116	0.91557208	-2.748596817
RAL-377	0.125	-3.465362044	14.99384304	0.427973307	-2.769206945
RAL-378	0.433333333	NA	14.66536677	NA	NA
RAL-379	0.75	2.902884788	13.05861867	-1.427371502	0.709448803
RAL-38	0.1	-4.227573464	15.33848196	1.466944939	-2.568430999
RAL-380	0.7	1.527397925	13.49714561	-1.174307797	-0.734756353
RAL-381	0.445454545	-0.467100496	15.01350466	0.641328092	0.371551489
RAL-382	0.083333333	-4.118346806	14.82165008	0.72521792	-3.129256934
RAL-383	0.5	0.539104539	14.37961981	-0.022901085	0.598956892
RAL-385	0.133333333	-3.102028588	15.2813129	1.19442215	-1.405316987
RAL-386	0.05	-4.21398806	16.0361834	1.265857815	-2.435991738
RAL-387	0.516666667	NA	14.69190177	NA	NA
RAL-391	NA	NA	14.87986903	0.973021739	NA
RAL-392	0.266666667	-0.586325971	15.48660977	0.026836311	0.320721962
RAL-393	0.225	NA	16.11332658	NA	NA

RAL-398	0.375	NA	15.40888052	NA	NA
RAL-399	0.35	-0.561238975	14.46403985	0.447080459	0.027750708
RAL-40	0.783333333	2.937853954	12.37868045	-1.212405105	1.019550958
RAL-405	0.883333333	3.086408428	12.48217371	-1.874098415	0.784551251
RAL-406	0.366666667	-0.497681521	16.16205749	0.923816638	0.596533255
RAL-409	0.020833333	-4.763978916	15.86868291	1.752374942	-2.671418622
RAL-42	0.366666667	-1.027856264	15.21026025	0.472176548	-0.563807059
RAL-426	0.716666667	2.740054308	12.86803176	-1.718767385	0.299428159
RAL-427	0.397222222	-0.370893831	12.94951566	-1.114933385	-1.473407286
RAL-437	0.520588235	0.972725807	14.47658606	0.043593111	1.004299963
RAL-440	0.75	2.873174795	13.8840852	-0.4727262	2.150405829
RAL-441	0.2	-2.342305677	15.62017276	1.22096907	-0.549960552
RAL-443	0.566666667	1.094878892	13.06739056	-1.401985289	-1.155463365
RAL-45	0.520512821	1.306709008	12.89418136	-1.401320608	-0.667978968
RAL-461	0.675	1.201825223	12.96611512	-0.711023465	-0.055898325
RAL-476	0.435294118	NA	14.74017416	NA	NA
RAL-486	0.384313725	-0.95040243	13.86534526	-0.127835927	-1.30519968
RAL-49	0	-4.802338906	16.25795258	1.848388822	-2.79351116
RAL-491	0.416666667	0.105027477	15.07234009	0.406588054	0.580970673
RAL-492	0.766666667	3.271686574	12.67511902	-1.3902521	1.128058394
RAL-502	0.783333333	2.611552609	14.04384569	-0.660677299	1.468946877
RAL-508	0.433333333	0.455993035	14.91449638	0.524109059	1.361246307
RAL-513	0.516666667	0.25862093	13.71307213	-0.432067377	-0.463344533
RAL-514	0.525	NA	13.04148531	NA	NA
RAL-530	0.438888889	-0.394615182	14.45061574	0.567958158	0.410050385
RAL-535	0.175	-2.686709671	15.61148574	1.296485174	-1.017180339
RAL-554	0.516666667	NA	14.53268957	NA	NA
RAL-555	0.85	3.100343977	11.84401752	-2.783636645	-0.562423808
RAL-57	0.633333333	2.184335623	14.85002893	0.238848113	2.518368007
RAL-584	0.083333333	-3.897300469	16.57733118	1.762595457	-1.556575772
RAL-589	0.75	1.242037784	13.65802689	-0.160592935	0.635750105
RAL-59	0.183333333	-2.042584356	15.86399392	1.320193588	-0.298968487
RAL-595	0.783333333	1.718022079	13.51370327	-0.097400236	1.642458606
RAL-642	0.15	-2.485940722	15.0860575	0.470444641	-1.661916185
RAL-69	0.75	2.263840207	12.76923124	-1.63702862	-0.195579213
RAL-703	0.539705883	1.205167762	13.87555393	-0.979133003	-0.197494403
RAL-707	0.15	-3.390395007	16.40093314	1.639730038	-1.321766073
RAL-712	0.466666667	0.579682989	13.9195565	-0.601281552	-0.219130792
RAL-714	0	-5.255791511	15.74690968	1.952170263	-3.209690812
RAL-716	0.625	1.220918286	14.03523134	-0.799769621	-0.405311828
RAL-721	0.473529412	-0.395787881	14.68871107	0.840430825	0.843297494

RAL-727	0.65	1.594738337	14.17415639	-0.362385152	0.957068509
RAL-73	0.35	-1.669480701	13.77175429	-0.683301792	-2.798036244
RAL-732	0.8	2.481385781	11.92536527	-2.838941544	-1.436979545
RAL-737	0.506862745	0.147515925	14.98144447	0.789631838	1.222117528
RAL-738	0.683333333	1.27525205	13.53872865	-0.316337521	0.42133098
RAL-748	0.741666667	1.692178295	12.44404737	-1.163313038	0.143871546
RAL-75	0.7	1.290909454	14.34194419	0.314880173	1.760097907
RAL-750	0	NA	17.42384034	NA	NA
RAL-761	0.516666667	0.539558307	14.1135381	0.014794457	0.634227355
RAL-765	0.15	-2.3022529	15.75433916	1.780832709	0.096593303
RAL-771	NA	NA	12.70969172	NA	NA
RAL-774	0.316666667	-0.743829856	14.57841825	-0.147207177	-0.679217555
RAL-776	0.45	0.040480899	13.41507499	-0.632297699	-1.213542831
RAL-783	0.583333333	0.595739692	13.68120506	-0.125167182	0.353073358
RAL-786	0.4	-1.792550523	14.08492304	0.582341404	-1.307555581
RAL-787	0.151851852	-3.196791674	15.87064654	1.654861762	-1.117642104
RAL-790	0.933333333	3.22162102	10.30980761	-3.471745406	-0.265701051
RAL-796	0.65	1.584253484	14.59640153	-0.001368671	1.699712464
RAL-80	0.257142857	NA	14.02341242	NA	NA
RAL-801	0.9	3.968001645	12.92067888	-1.251651365	2.07647334
RAL-802	0.6	0.88594661	14.86498299	0.677344117	1.682357884
RAL-804	0.15	-3.16839413	15.47411246	1.022080421	-1.727940796
RAL-805	0.858333334	3.072778343	12.60069262	-2.490635129	0.060375352
RAL-808	0.883333333	4.162673286	12.75797426	-1.649209355	1.941732666
RAL-810	0.616666667	1.495518399	14.29506489	-0.553180623	0.774193051
RAL-812	0.316666667	-1.318502387	15.19095139	1.428618101	0.53258601
RAL-819	0.383333333	-0.897809449	14.67734092	0.257647536	-0.643113422
RAL-820	0.804901961	2.474091749	13.16477704	-1.294852	0.730913901
RAL-821	0.442982456	-0.254752065	13.86521779	-0.333423	-0.969776088
RAL-822	0	-4.79252762	16.70737412	2.350562721	-2.31319037
RAL-83	0.333333333	-0.362597187	15.39899669	0.79974199	0.462523879
RAL-832	0	-4.542612346	16.65240471	2.124933341	-2.502176879
RAL-837	0.5	-0.174560549	14.12488031	0.210974138	-0.020599082
RAL-843	0.233333333	-1.355739541	16.69132581	1.365010925	0.707978347
RAL-849	0.433333333	0.595483597	14.48705156	-0.304362801	0.619244257
RAL-85	0.7	2.497059015	13.15843301	-1.39470165	0.376075507
RAL-850	0	-4.802338906	16.06285156	1.610706016	-3.097828447
RAL-852	0.116666667	-3.633585956	16.11471552	1.630728431	-1.351813229
RAL-853	0.85	2.678756823	13.4964841	-0.557526255	1.459648271
RAL-855	0.7	2.063543257	12.8234777	-1.322716189	0.067681752
RAL-857	0.533333333	1.07267712	14.95302332	0.398948843	1.581985259

RAL-859	0.375	-1.387996595	14.01325258	0.150231075	0.218549595
RAL-879	0.316666667	-1.54143735	15.16601375	0.827748936	-0.490834423
RAL-88	0.675	2.867348101	13.47443523	-0.835975156	2.603619276
RAL-882	0.75	1.713179834	14.64483589	0.035992789	1.457709022
RAL-884	0.21025641	-2.43373507	15.44175604	1.410774209	-0.524695842
RAL-890	0.35	-0.7903367	15.42777508	0.829095382	0.484746509
RAL-892	0.675	2.2806889	14.51067879	-0.033859803	1.813567245
RAL-894	0.433333333	0.726434165	13.14358091	-1.183309308	-0.703675356
RAL-897	0.55	0.078163025	14.84712841	0.643106866	1.026595026
RAL-900	0.616666667	1.24274402	14.93966064	0.385629743	1.787703319
RAL-907	0.716666667	1.975244382	13.40538715	-1.071773036	0.203622517
RAL-908	0.733333333	2.959692029	11.71855334	-2.716276166	-0.963677119
RAL-913	0.616666667	1.175800053	13.62416397	-0.709813208	-0.120916548
RAL-93	0	-4.193378961	16.9549169	2.194522153	-2.230407675

Table S8

List of VDRC RNAi lines tested.

Gene	Trait	Transformant ID	Construct ID	Library	CG Number	Actin5C viable	Tested with C564
psq	tolerance	106404	111691	KK	CG2368	no	no
CG31826	tolerance	100639	104704	KK	CG31826	yes	no
CG42389	tolerance	105154	102530	KK	CG42389	yes	no
beat-IIIc	tolerance	109015	111040	KK	CG15138	yes	no
CG42238	tolerance	104807	109919	KK	CG42238	yes	no
ush	tolerance	104102	104016	KK	CG2762	no	yes
gus	tolerance	101738	108241	KK	CG2944	yes	no
mspo	tolerance	107608	106896	KK	CG10145	yes	no
dpr13	tolerance	107676	112959	KK	CG33996	no	no
Blimp-1	tolerance	108374	107466	KK	CG5249	no	no
Fhos	tolerance	108347	108388	KK	CG42610	yes	no
C15	tolerance	107334	109374	KK	CG7937	yes	no
CG34127	tolerance	100376	106100	KK	CG34127	no	no
Ten-a	tolerance	103298	112809	KK	CG42338	no	no
Ih	tolerance	110274	100190	KK	CG8585	no	no
CadN	tolerance	101642	105304	KK	CG7100	no	no
grh	tolerance	101428	109135	KK	CG42311	no	yes
CG4174	tolerance	41328	6169	GD	CG4174	yes	no
dsb	resistance	100219	107147	KK	CG1887	NA	yes
CG30098	survival	108326	106520	KK	CG30098	NA	yes

CG31751	control	110319	100608	KK	CG31751	yes	no
mbl	control	105486	107778	KK	CG33197	no	no
Rbp9	control	101412	109093	KK	CG3151	yes	no
kek5	control	47770	14493	GD	CG12199	yes	no
AlCR2	control	106146	102954	KK	CG13702	no	no
tou	control	100735	108263	KK	CG10897	yes	no
CG15765	control	101194	107102	KK	CG15765	no	no
Pka-R2	control	101763	109446	KK	CG15862	no	no
CG30152	control	105959	101219	KK	CG30152	yes	no
pyd	control	104159	105581	KK	CG43140	no	no
bap	control	101354	108567	KK	CG7902	no	no