A

	18bp	28190	28141	28178	28144	28135	28171
4bp		A	B	C	D	E	F
28240	1	**A1**	**1B**				
28231	2		**B2**	**2C**			
28138	3			**C3**			**3F**
25204	4				**D4**	**4E**	
28211	5	**5A**				**E5**	**5F**
28227	6				**6D**		**F6**
28139	7						**F7**
28122	8			**C8**			

B

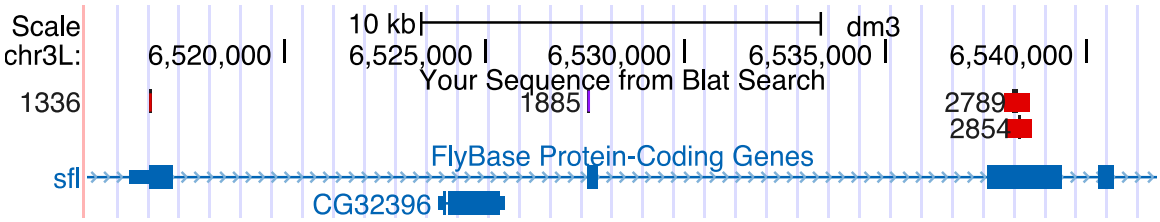

Figure S1 Pyro-sequencing cross and assay design. (A) Cross design for pyro-sequencing. Six 18bp and eight 4bp lines were randomly chosen from the 154 DGRP lines used in GWAS. The Bloomington center stock number is listed. In each cell, the order of the letter/number indicate the direction of the cross. For example, A1 indicates that males of #28240 was crossed to virgin females of #28190. (B) pyro-sequencing assays. Four SNPs were selected within the transcribed regions so as to distinguish alleles associated with the 18/4 bp indel polymorphism.

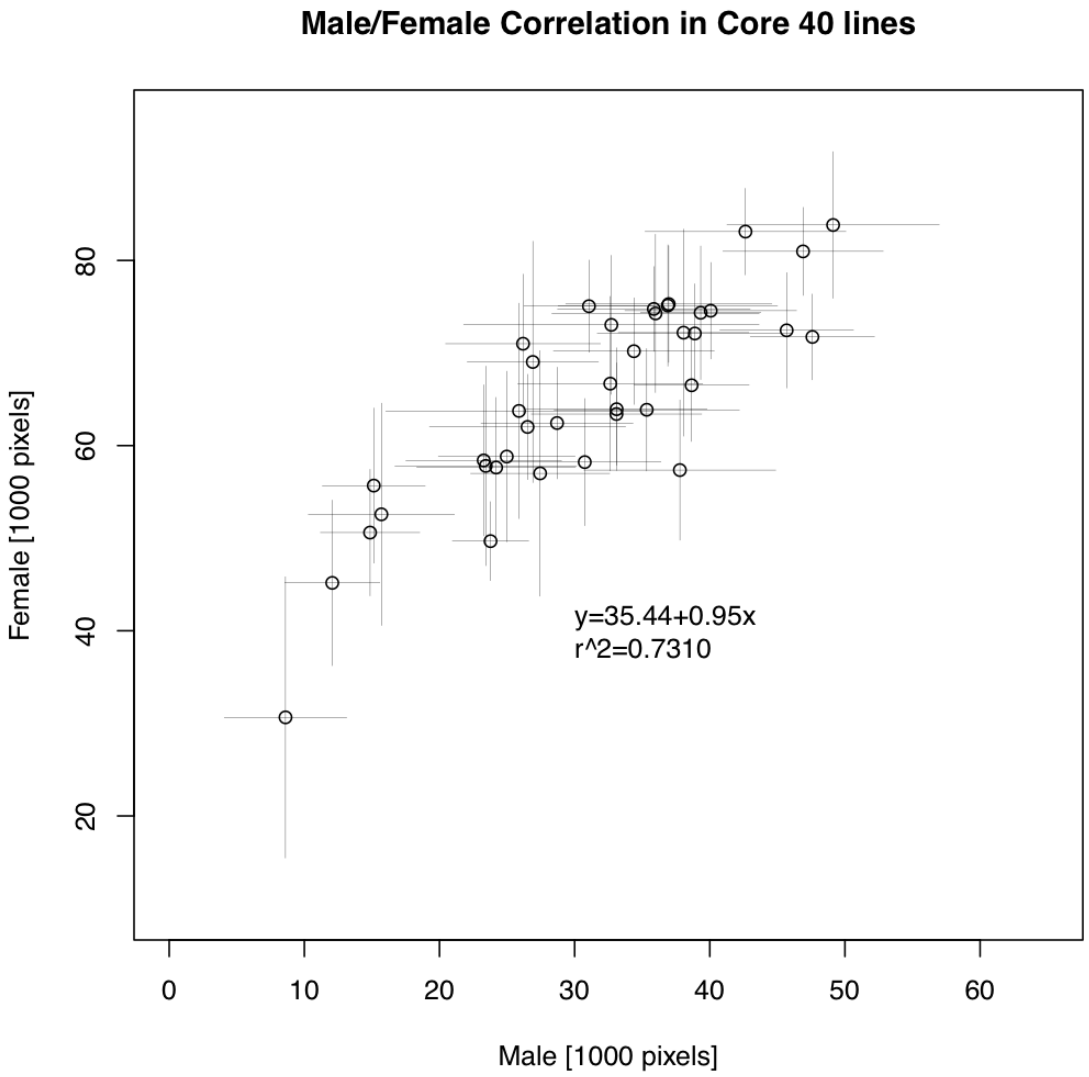

Figure S2 Correlations of eye area between F1 males and females within the same cross. Mean ± 1 s.d. are plotted for a subset of 38 lines. The least square linear fit is indicated.

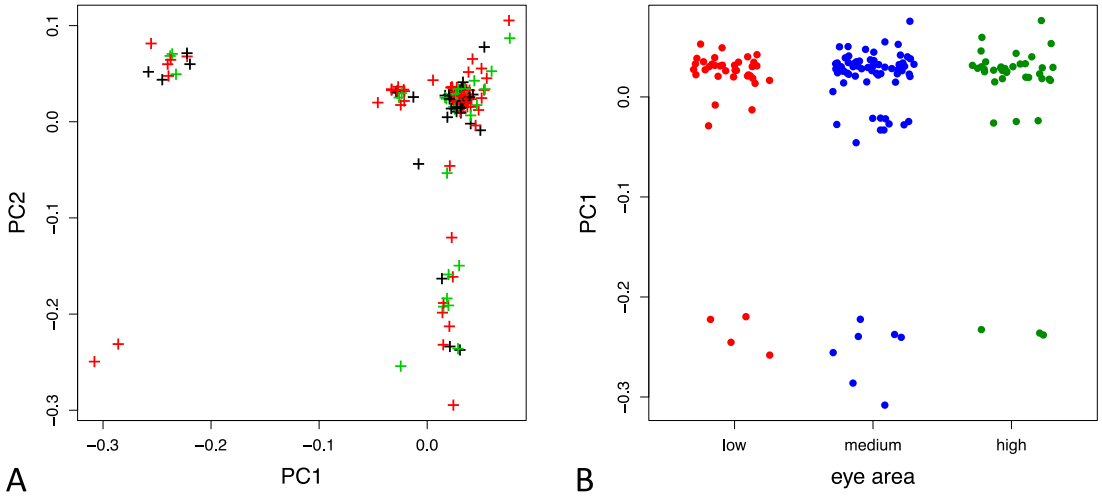

Figure S3 Population structure assessed through principal component analysis (PCA) using 900K autosomal SNPs after LD pruning. (A) 154 DGRP inbred lines projected onto the plane spanned by the first two principal components (PC1, PC2). The points are colored according to the phenotype severity in the hINS^{C96Y} crosses (red: severe, or first 25%; blue: intermediate, 25%-75%; green: mild, 75%-100%, percentiles in eye area distribution from small to large). (B) projection onto PC1 grouped by their phenotype severity showed no correlation between the two.

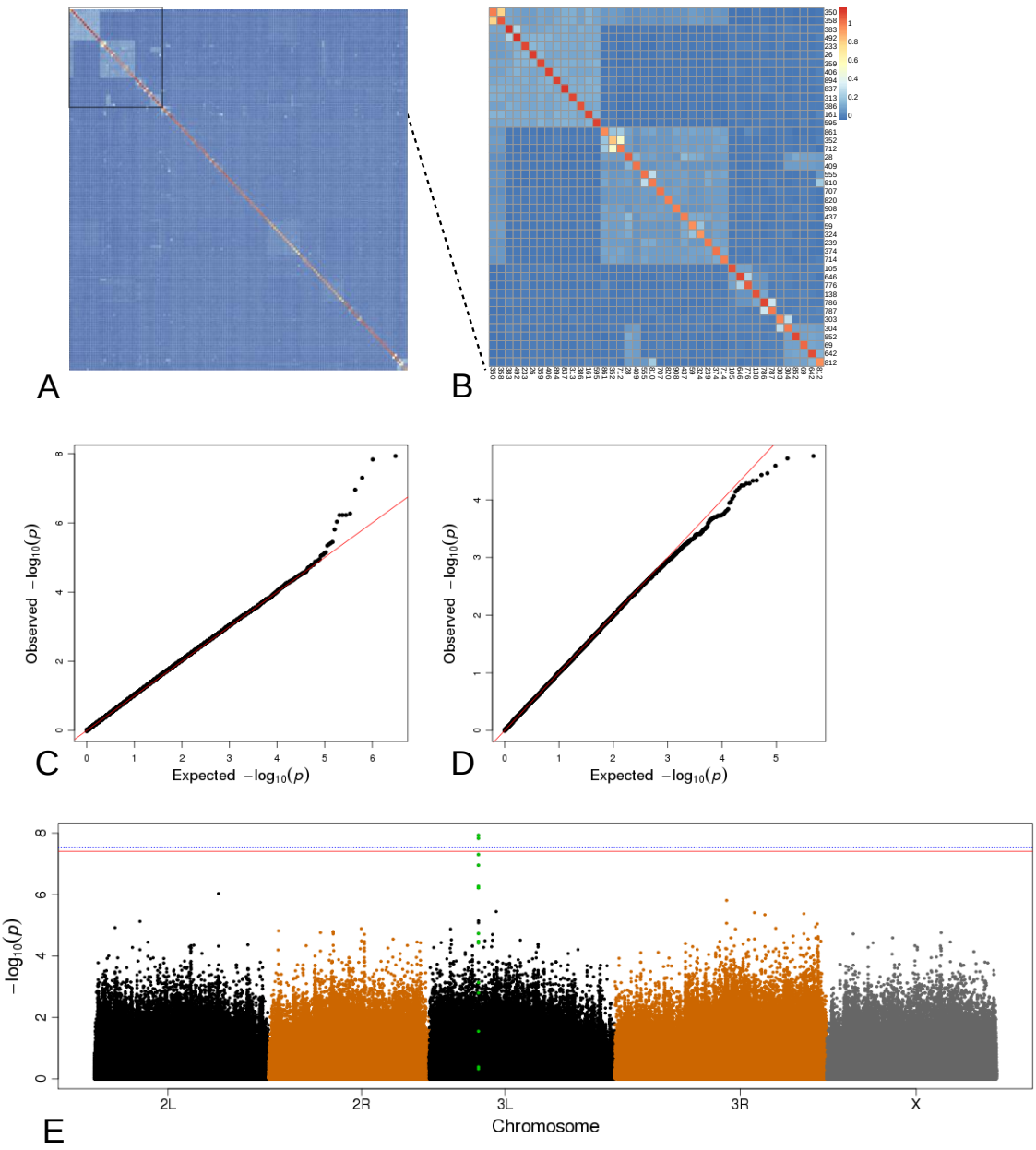

Figure S4 Mixed linear model regression accounting for cryptic relatedness. (A) The heat map shows a 154 x 154 matrix representing the centered genetic relatedness matrix (GRM) estimated using EMMAX. The boxed area is shown in detail in (B), with their line ID (RAL#) indicated on the right and bottom. The GRM was used in a mixed linear model to perform genome wide association in the 154 lines. And the resulting p-values for autosomal and X-linked variants are plotted as Q-Q plot in (C) and (D), with the red line indicating matches between the data and the null (uniform) p-value distribution. (E) Manhattan plot showing the –log10 p-values against the chromosomal coordinates. No association is expected on the X chromosome. The blue dotted line indicates a Bonferroni corrected $P < 0.05$, while the red solid line indicates a 5% genome-wide significant level based on 500 permutations.

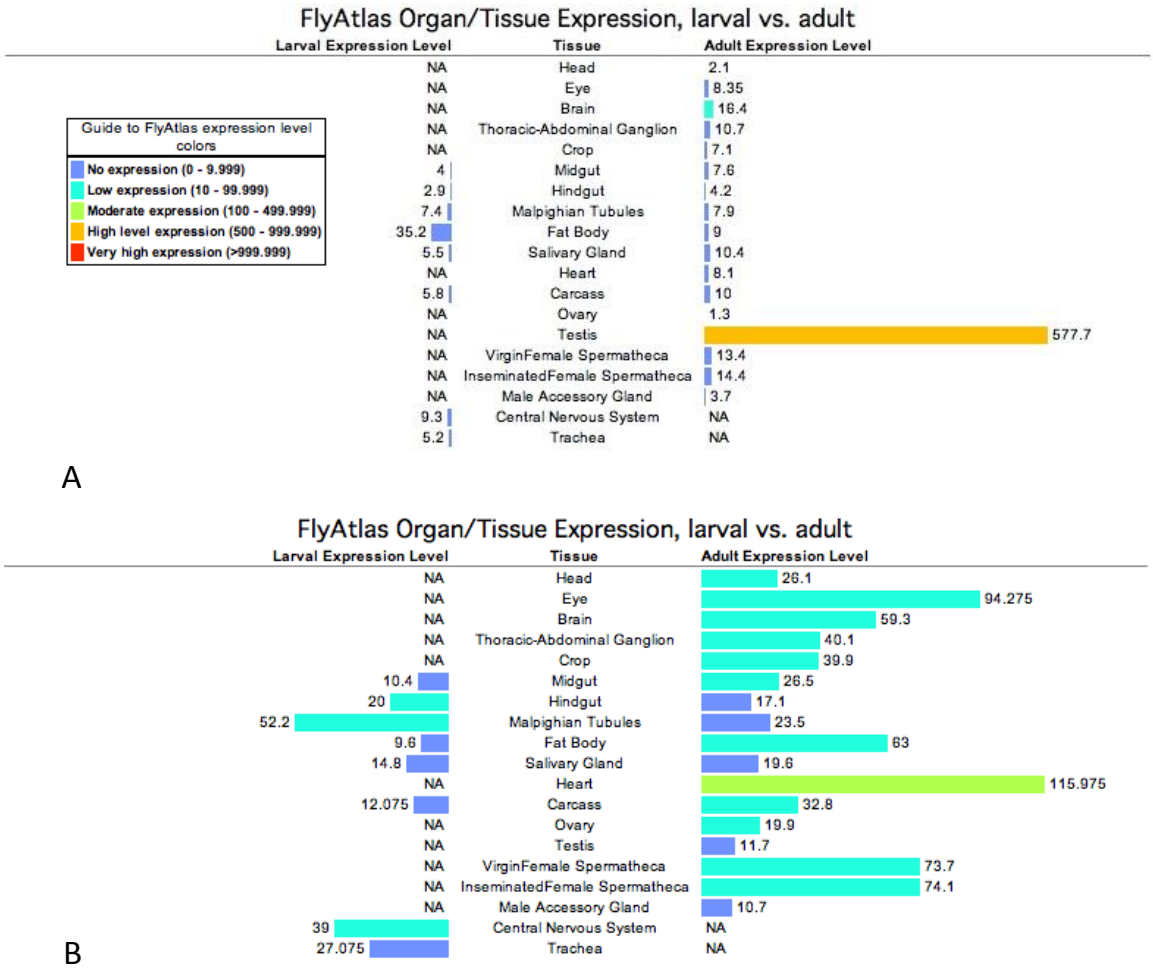

Figure S5 FlyAtlas expression report for CG32396 and sfl. (A) CG32396 (B) sfl. Figure obtained through FlyBase.

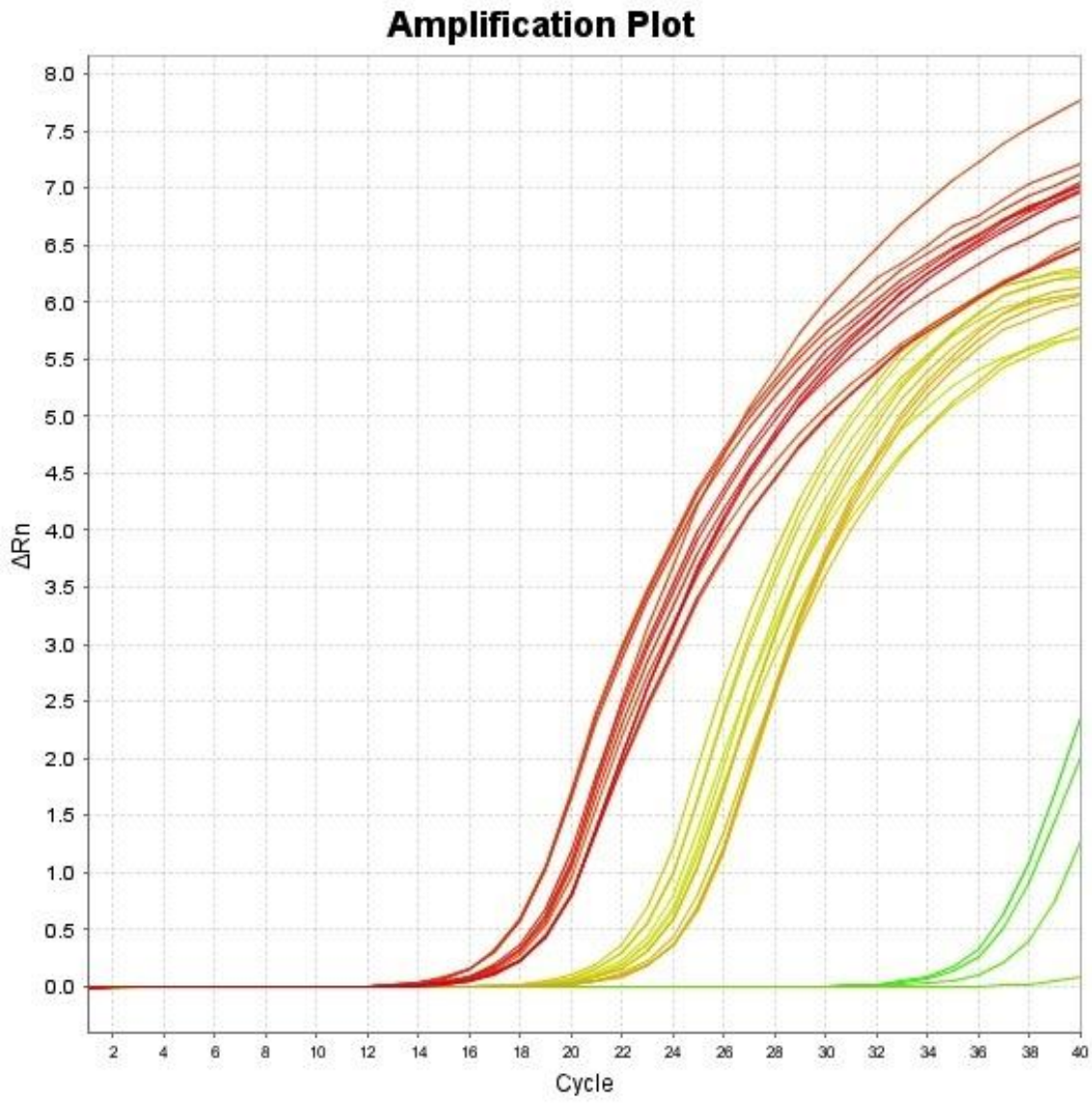

Figure S6 qRT-PCR quantification of mRNA levels for CG32396 and *sfl* in eye imaginal disc samples. Two inbred lines from DGRP were randomly chosen and eye imaginal disc samples were prepared from either 6 male or 6 female larvae, resulting in 4 biological samples. qRT-PCR were performed for each sample and three genes (RP49 -- red curve, *sfl* -- yellow, and CG32396 -- green). Shown is the amplification plot: x-axis -- cycle number; y-axis -- base-line corrected relative fluorescence intensity proportional to the amount of amplicons. Both RP49 and *sfl* were detected starting in the 18-20th cycle, while amplification didn't happen for CG32396 until after 32 cycle. In addition, multiple melting points were detected for CG32396 assays, but not in the other two genes.

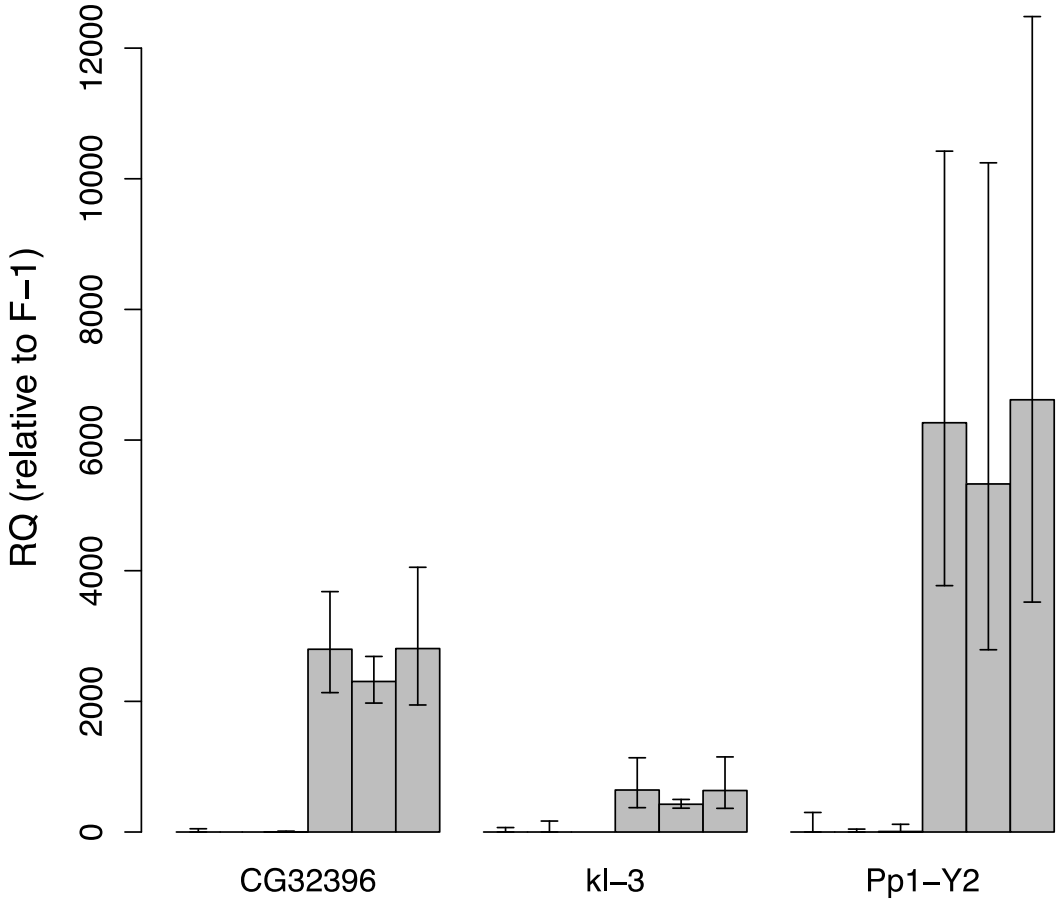

Figure S7 Relative quantity of mRNA quantified by qRT-PCR in male and female larvae. In each category, the first three bars represent three independent female larvae sample (whole larva), each assayed with three technical replicates. The height of the bar represent the mean and the full range of RQ values were indicated by the error bars. The next three bars correspond to three independent male larvae assayed for the same gene. kl-3 and Pp1-Y2 are both located on the Y-chromosome and are known to have a testis-specific expression level. The RQ values were measured using RP49 gene as the internal control, and the first female larva sample (F-1) as the reference, whose RQ is set to one.

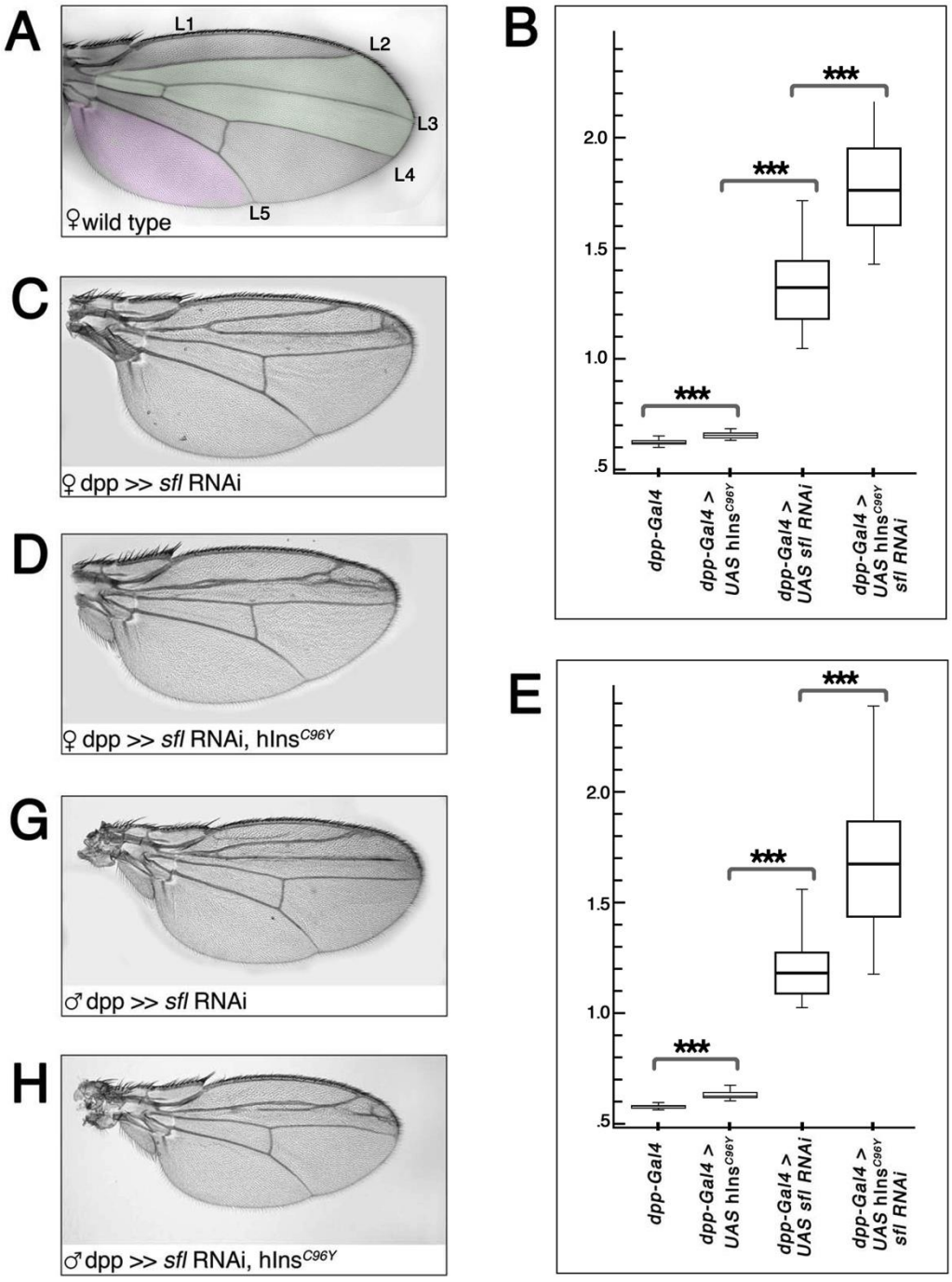

Figure S8 Depletion of *sfl* by RNAi in the developing wing expressing hINS^{C96Y} driven by dpp-Gal4. For both females and males, dpp >> hINS^{C96Y} or Dpp >> *sfl* RNAi expression alone reduces wing area between the L2 and L4 longitudinal veins relative to the posterior-most sector of the wing (bordered by L5). This reduction is more severe in the *sfl* knockdown genotype than in the hINA^{C96Y}-expressing genotype. Co-expression of *sfl* RNAi and hINS ^{C96Y} by dpp-Gal4 results in the obliteration of the L3 vein and further relative reduction of the L2-L4 area.
(A): Wild type wing showing the measured regions of wing used to quantify the effects of both *sfl* RNAi and hINS^{C96Y} expression in dpp-Gal4 domain (L3-L4 intervein sector). Quantification of the (B) female or (E) male wing phenotypes generated by transgenes dpp-Gal4; dpp-Gal4 >UAS-hINS^{C96Y}; (C, G) dpp-Gal4 >> UAS-*sfl* RNAi; and (D, H) dpp-Gal4

>>UAS-*sfl* RNAi; UAS-hINS^{C96Y}. The values represent the ratio of the third posterior cell (in pink color) divided by the L2-L4 intervein sector (in green color) wing area. ***, P < 0.001; Mann-Whitney U test.
Females: dpp-Gal4 (n= 15; Mean= 0.62), dpp-Gal4 >UAS-hINS^{C96Y} (n= 15; Mean=0.65), dpp-Gal4 >> UAS-*sfl* RNAi (n= 23; Mean=1.3) and dpp-Gal4 >>UAS-*sfl* RNAi; UAS-hINS^{C96Y} (n= 22; Mean=1.76).
Males: dpp-Gal4 (n= 15; Mean=0.59), dpp-Gal4 >UAS-hINS^{C96Y} (n= 15; Mean=0.64), dpp-Gal4 >> UAS-*sfl* RNAi (n=23; Mean=1.2) and dpp-Gal4 >>UAS-*sfl* RNAi; UAS-hINS^{C96Y} (n= 29; Mean=1.68).

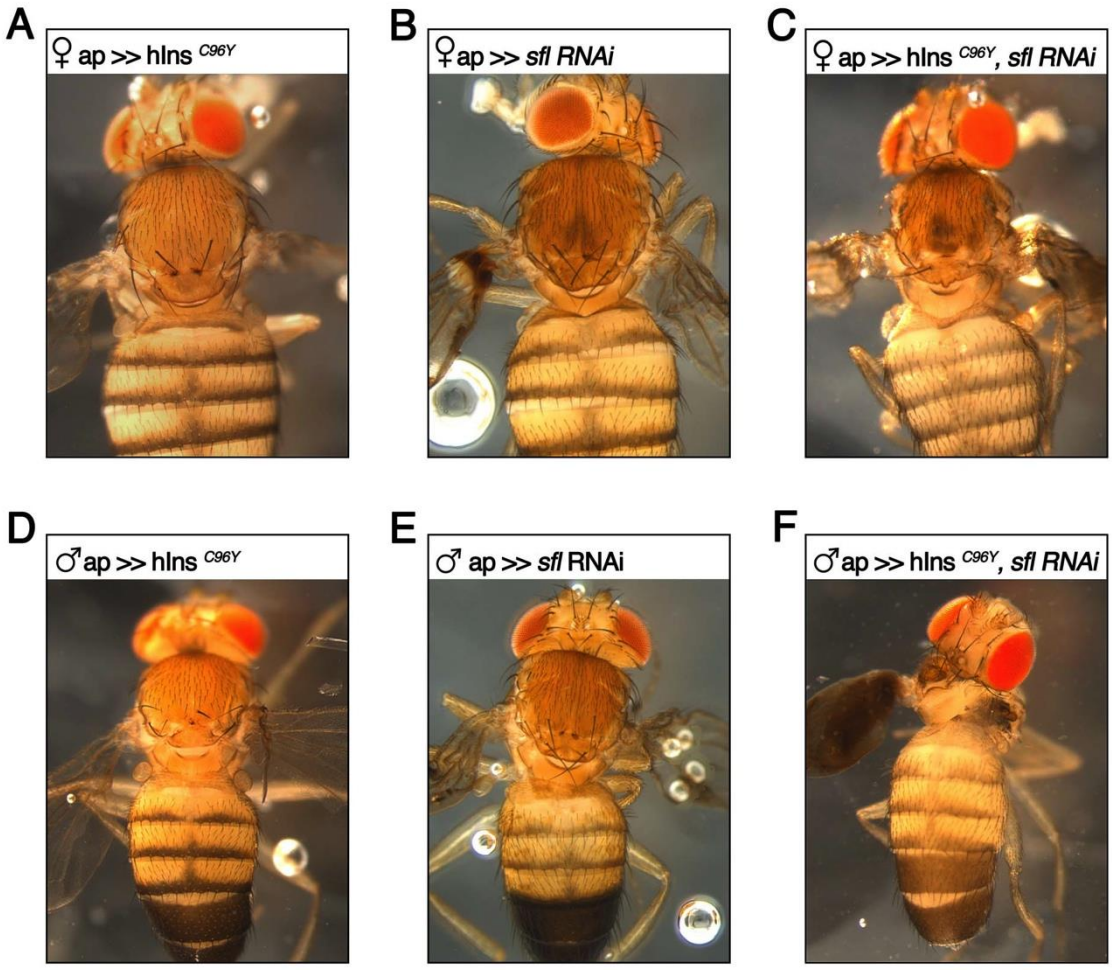

Figure S9 Depletion of *sfl* by RNAi in the developing notum expressing hINS^{C96Y} driven by ap-Gal4. For both females and males, ap > hINS^{C96Y} or ap > *sfl* RNAi expression alone reduces notum area and causes loss of dorsal macrochaetae. Co-expression of *sfl* RNAi and hINS^{C96Y} by ap-Gal4 results in greater destruction of the notum and macrochaetae in both sexes. However, in the male the notum and additional dorsal structures are obliterated and this phenotype is lethal.
ap-Gal4 > hINS^{C96Y} (A) female and (D) male;
ap-Gal4 > *sfl* RNAi (B) female and (E) male;
ap-Gal4>> hINS^{C96Y}, *sfl* RNAi (C) female (F) male

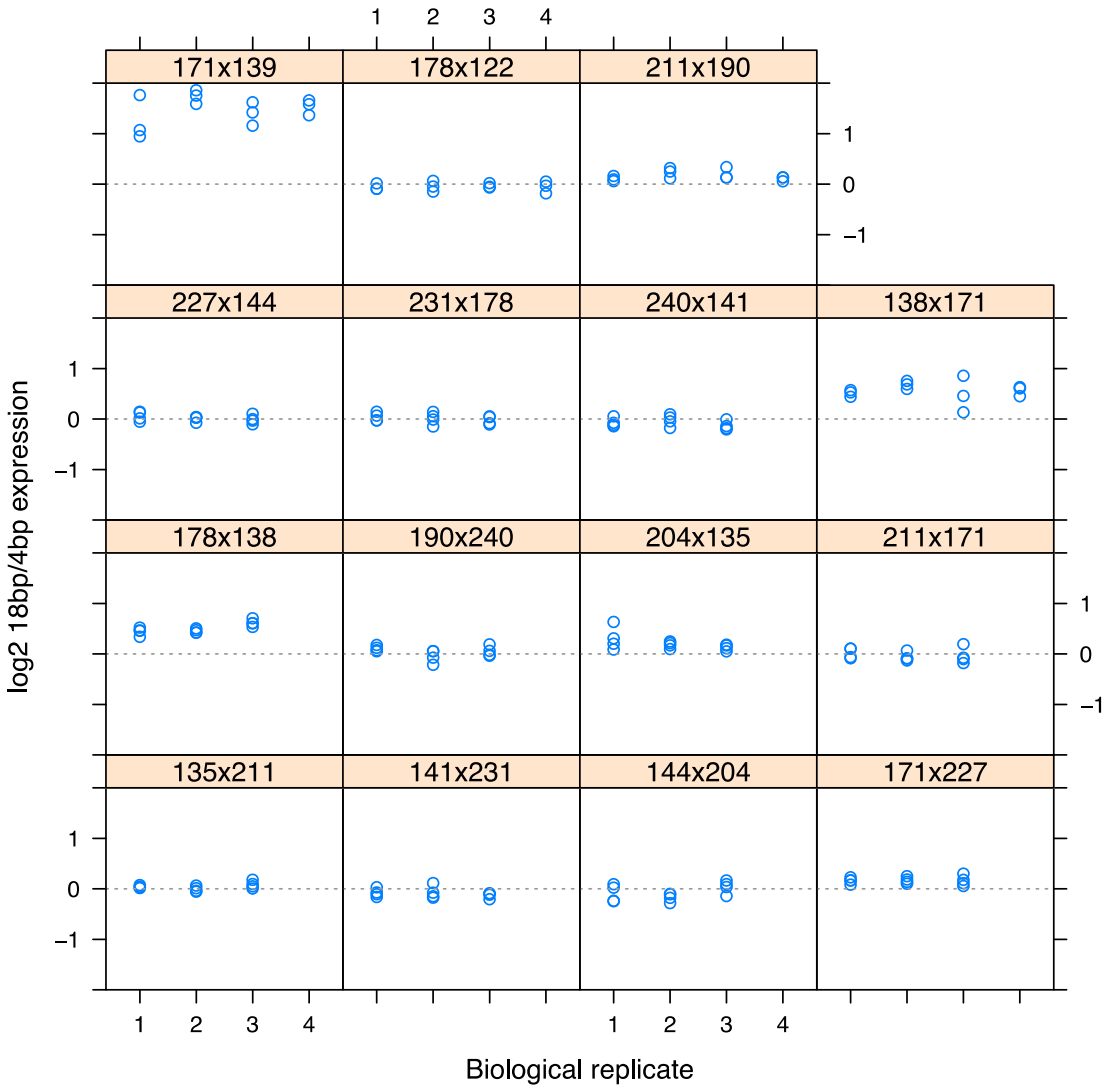

Figure S10 log2 transformed ratios between transcript levels associated with 18bp/4bp alleles. The allele-specific expression ratios were measured in F1 hybrid individuals by pyro-sequencing, with three (or four) biological replicates and four (or three) pyro-technical replicates, to obtain a total of 12 measurements. In each of the 15 crosses, the technical replicates were plotted in a single column, with different columns representing the biological replicates. In the titles of each panel, the last three digits in the stock number were shown for lines used in the cross.

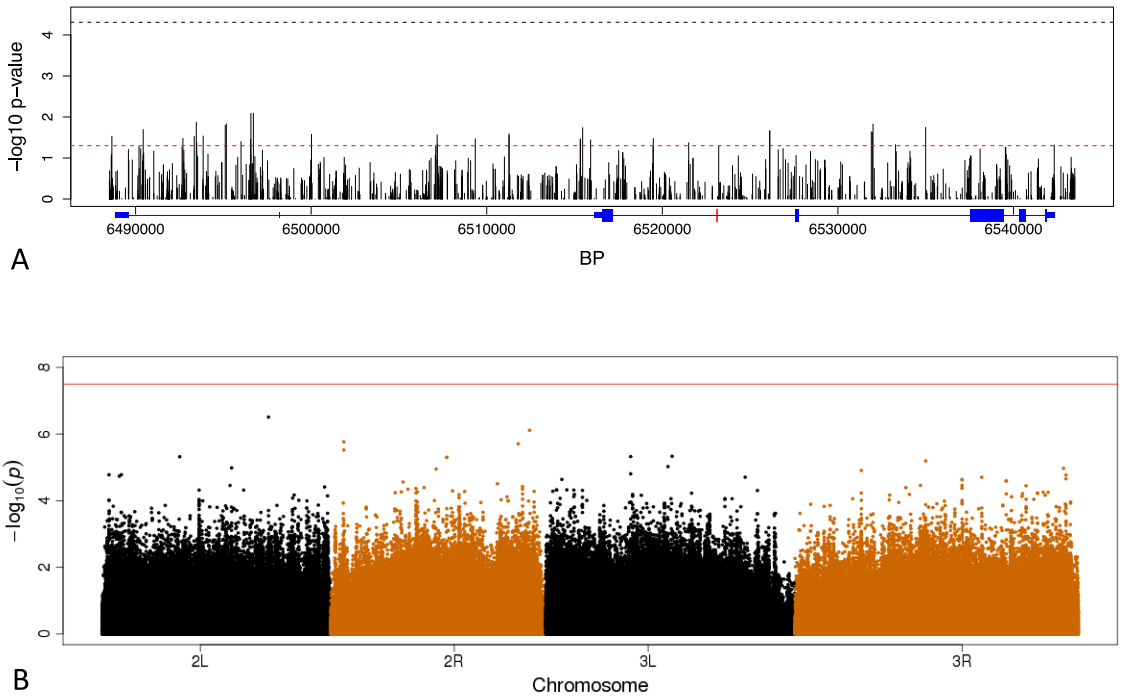

Figure S11 Conditional regression analysis to detect additional SNPs associated with the phenotype of interest. (A) within the *sfl* locus; (B) all chromosomes. The intronic 18/4bp polymorphism in *sfl* is included in the linear model as a covariate. The two dotted lines in (A) correspond to a single test 0.05 level (red) and the multiple testing corrected 0.05 level using Bonferroni's method (blue). The red line in (B) represents the Bonferroni corrected 0.05 level.

File S1

Mixed Model Permutation Test

When (cryptic) relatedness or population structure is present in a sample, then naïve permutation test that randomizes the phenotype values can result in inflated type-1 error (Churchill & Doerge, 2008). To address this concern we employ a permutation scheme that preserves an estimated phenotypic covariance structure as estimated using a mixed model. The idea, which is inspired by (Müller et al., 2011), is to apply a transformation to the phenotypes so that they become (approximately) independent, permute them, and then transform them back. We can show that under the mixed model assumptions, this transformation is the Cholesky decomposed inverse phenotypic covariance matrix, as estimated from using a mixed model. Hence, we transform the phenotypes as follows:

$$Y^* = cholesky(V^{-1})'Y,$$

where *Y* denotes the phenotype vector and the *V* the estimated phenotypic covariance matrix. Under the model, $Var(Y^*) = I$, which allows us to permute those values, and then apply the inverse transformation to obtain permuted phenotypes that preserve the estimated structure as follows:

$$Y_{perm} = cholesky(V)'{Y^*}_{perm}.$$

Interestingly, this approach is similar to the approach of (Aulchenko et al., 2007), where they permuted the residuals after regressing out the genomic BLUP. The difference is that we do not attempt to remove the effects of family and population structure (as inferred by a mixed models) but instead apply a transformation that preserves the (estimated) phenotypic covariance structure. Finally, in the context of mixed model association mapping it is possible to perform the permutation test very efficiently by applying this transformation to the genotypes as well. Then the least square estimate using these transformed quantities (phenotypes and genotypes) is (trivially) identical to the generalized least square estimate as obtained from EMMAX (Kang et al., 2010). For obtaining a 5% genome-wide significance threshold we performed 500 permutations and redid the genome-wide association using the EMMAX algorithm. This permutation test is implemented in the mixmogam software (Segura et al., 2012).

References

Aulchenko, Y. S., de Koning, D.-J., and Haley, C., 2007 Genomewide Rapid Association Using Mixed Model and Regression: A Fast and Simple Method For Genomewide Pedigree-Based Quantitative Trait Loci Association Analysis *Genetics* **177**: 577--585

Churchill, G. A. and Doerge, R. W., 2008 Naive application of permutation testing leads to inflated type I error rates. *Genetics* **178**: 609--610

Kang, H. M. M., Sul, J. H. H., Service, S. K., et al., 2010 Variance component model to account for sample structure in genome-wide association studies. *Nature genetics* **42**: 348--354

Müller, B. U., Stich, B., and Piepho, H.-P. P., 2011 A general method for controlling the genome-wide type I error rate in linkage and association mapping experiments in plants. *Heredity* **106**: 825--831

Segura, V., Vilhjalmsson, B. J., Platt, A., et al., 2012 An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. *Nature genetics* **44**: 825--830

Tables S1-S2
Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.157800/-/DC1

Table S1 Raw data

Table S2 DRRP lines used in this study

Table S3 Sequence primers used in this study

Name	Sequence (5'->3')
	qRT-PCR
sfl_F1	TCGATACGGGCGTGTTTAATGGAC
sfl_R1	TTGATAATGGGTGCGGGATGCG
CG32396_F1	AGCGGAGATTGGGTCGAAATGAG
CG32396_R1	CATGTGAAATCACGTGCCAGAAAG
kl-3F1	ATGGCAAACGTAGACCCACCTC
kl-3R1	GTACCGGCGGACGATTCTTTAG
Pp1-Y2F1	TTTGTTGTCACGGCGGTCTCAG
Pp1-Y2R1	ACGTCACATGGTCGGGCTAATTG
RP49-F1	CGGATCGATATGCTAAGCTGT
RP49-R1	GCGCTTGTTCGATCCGTA
	Pyro-seq
1336F1	CGGGCGGCAATCAACATAA
1336R1	CGGTCACGGAGCTACCAAATT
1336S1	CTCATTAAGCAGCCG
2789F1	GACTGCGACCAGATGATGTGAG
2789R1	CTTCCCTCGTGCCATGATGATA
2789S1	TTCCCGAGAATCCCA
2854F1	CGGGAAAATACTATCATCATGGC
2854R1	GTGCGAAAACCAGTTGAACTC
2854S1	TCCTGAACGTTCTGC
1885F1	TAATGGACTTATTCAACGCGACAC
1885R1	TGTGTTTGCCACCAGAGTTG
1885S1	CGGCAGTTGATAATGG

Table S4 Power calculation for GWAS with 154 lines

Minor Allele Frequency	**Effect Size***				
	0.75	**1**	**1.25**	**1.5**	**2**
0.01	0.00	0.00	0.00	0.00	0.00
0.05	0.00	0.00	0.01	0.03	0.26
0.1	0.00	0.02	0.12	0.39	0.94
0.2	0.02	0.19	0.63	0.94	1.00
0.3	0.06	0.45	0.90	1.00	1.00
0.4	0.11	0.60	0.96	1.00	1.00
0.5	0.13	0.66	0.97	1.00	1.00

* Effect size is measured as the shift in the phenotype mean in units of s.d. for the trait

The calculation is done using the t-distribution. The R-code is attached below:

```
myPower.t <- function(effect.size=1,alpha=0.05,m,n){
  ## Power for GWAS t test
  ## calculate power for a t test comparing two populations with equal variance but unequal sample sizes
  ## m, n: sample size of each allele class, not to be confused with m above
  df = m+n-2
  A = 1/sqrt(1/m+1/n) ## factor for calculating t statistics
  T = qt(1-alpha/2,m+n-2)
  T1 <- T-effect.size*A
  beta <- pt(T1,m+n-2)
  return(1-beta)
}
## plot power of GWAS t test ##
alpha1=.05/1.37e6
power <- NULL
effect.size <- c(0.75,1,1.25,1.5,2)
freq <- c(0.01,0.05,0.1,0.2,0.3,0.4,0.5)
N = 154 # size of GWAS mapping population
for(p in freq){
  m = as.integer(N*p)
  n = N-m
  power <- rbind(power, sapply(effect.size,function(x) myPower.t(x,alpha1,m,n)))
}
dimnames(power) <- list("freq"=freq,"effect.size"=effect.size)
```