SUPPLEMENTAL FILE S1

Gene expression correlations and correlations with other traits: Gene expression data was obtained from (Huang *et al.* 2015). We calculated the Pearson correlation between the expression of candidate genes tested in the validation experiment, as well as the correlation between the expression of those genes and the phenotypes measured in our screen. We also correlated the traits measured in this study against similar or potentially related traits measured in other DGRP studies (Mackay *et al.* 2012; Durham *et al.* 2014; Unckless *et al.* 2015; Garlapow *et al.* 2015). All analysis was done in R (v3.5.3).

We examined whether the expression levels of candidate genes used in the validation were correlated in order to identify suites of co-regulated genes. We examined expression correlations among five of the six candidate genes (*CG8312* did not have available expression data). Using female expression data, we identified that *Ih* expression was positively correlated with *hid* expression and *Rbp* expression, following a multiple test correction (*Ih-hid: r* = 0.25, *p* = 2.5E-3; *Ih-Rbp: r* = 0.24, *p* = 3.1E-3) (Fig. S2a). Using the male expression data and a multiple test correction, we found that *mub* was positively correlated with *hid, Rbp,* and *Ih* and negatively correlated with *Sox21b* (Fig. S2b). We wanted to see whether the number of significant correlations we observed was higher than expected by chance. To this end, we sampled five random genes from the expressed data and calculated the number of significant correlations post-correction. We found that for both male and female expression, we have probably not enriched for a highly-correlated cluster of genes within our validation set (Fig. S2c-

d). Using the same approach on a larger set of 17 candidate genes, we also did not find enrichment for gene expression correlations (Fig. S3).

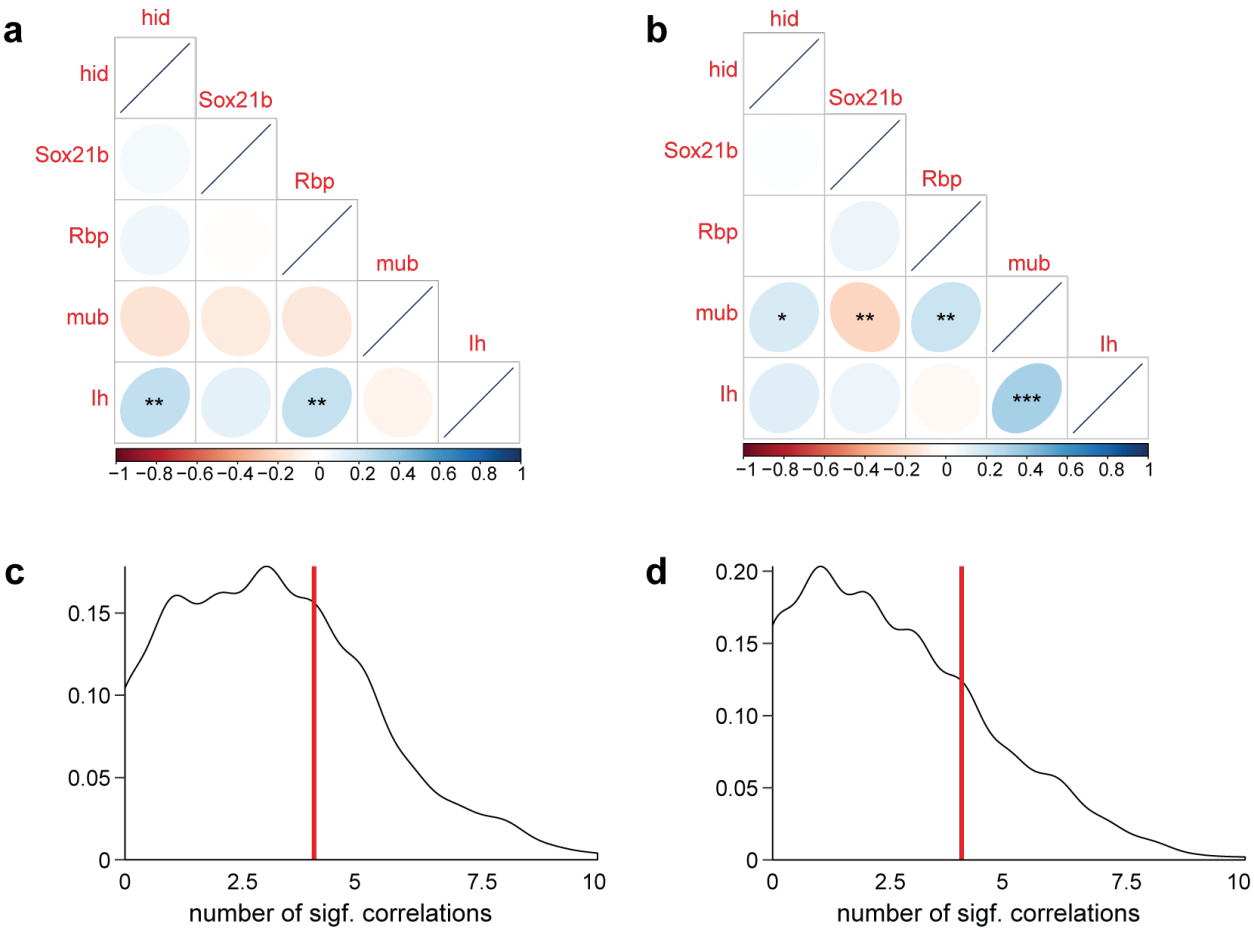

Figure S2. Correlation across DGRP lines of expression of candidate genes used in the validation experiment. a) Female expression data **b)** Male expression data. **c)** Kernel density plots of the number of significant correlations of expression of 5 randomly chosen genes (1000 samples) from female expression data. **d)** As in (c) for male expression data. Red line shows our observed number of significant correlations. Significance levels: * $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$. *P*-values were transformed into *q*-values using the Benjamini-Hochberg method to correct for multiple tests. Here and below, *q*-values refer to the adjusted *p*-values that result from an FDR approach to multiple testing correction.

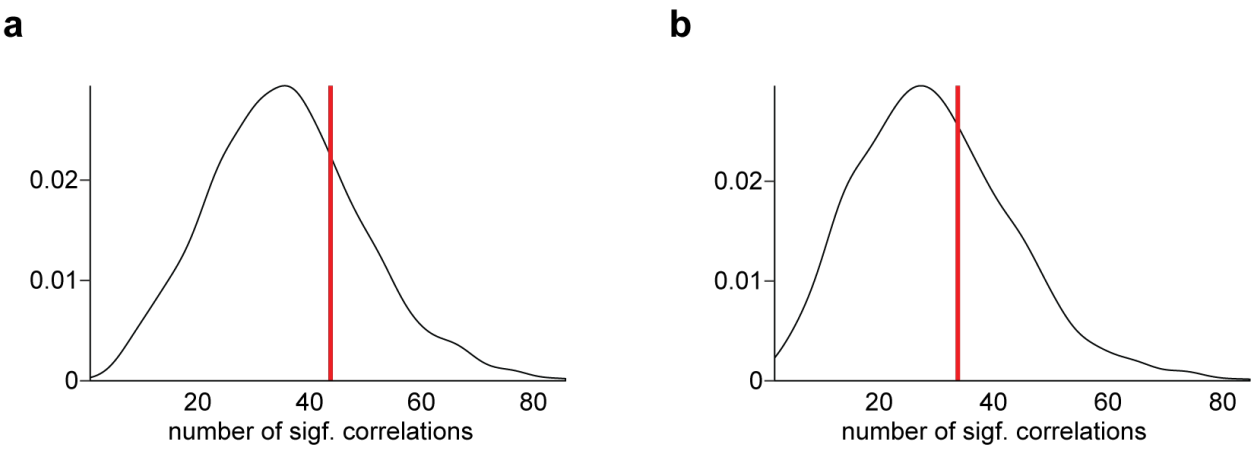

Figure S3. Kernel density plots of the number of significant correlations in expression among 17 randomly chosen genes. 1000 resamples were computed from a) female expression data and b) male expression data. Red line shows our observed number of significant correlations among 17 candidate genes examined. *P*-values were transformed into *q*-values using the Benjamini-Hochberg method to correct for multiple tests prior to determining significance (FDR = 0.05). *Q*-values refer to the adjusted *p*-values that result from an FDR approach to multiple testing correction.

We also examined whether expression of the candidate genes was significantly correlated to the phenotypes measured in this study. We did not find evidence of strong correlations between the gene expression of our candidate genes and the phenotypes measured (Fig. S4).

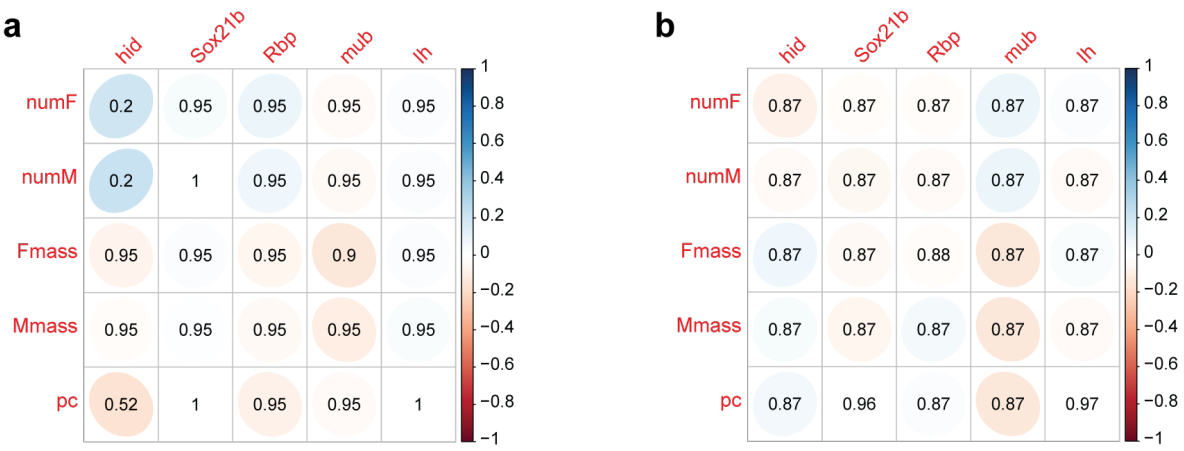

Figure S4. **Correlation of expression of candidate genes to offspring phenotypes . a)** DGRP female expression data **b)** DGRP male expression data. pc = offspring index. *P*-values were transformed into *q*-values using the Benjamini-Hochberg method to correct for multiple tests. *Q*-values refer to the adjusted *p*-values that result from an FDR approach to multiple testing correction.

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