

1 Transcripts with high distal heritability mediate genetic effects on
2 complex traits

3

4 **Abstract**

5 The transcriptome is increasingly viewed as a bridge between genetic risk factors for complex disease and
6 their associated pathophysiology. Powerful insights into disease mechanism can be made by linking genetic
7 variants affecting gene expression (expression quantitative trait loci - eQTLs) to phenotypes.

8 To assess the role of gene regulation in mediating variation in metabolic traits in this population, we propose
9 a novel high-dimensional mediation analysis (HDMA) that uses the theory of causal modeling to explicitly
10 model the emergent transcriptomic state mediating the distal effects driving trait-relevant gene expression. In
11 univariate approaches, such as TWAS, SMR, and other Mendelian randomization approaches, each transcript
12 is tested independently for mediation of a local variant on a trait. This process requires huge numbers of
13 statistical tests, which is computationally expensive, requires strict corrections for multiple testing, and
14 assumes independence of genetic variants and transcripts. Such methods are therefore limited to detecting
15 only the largest statistical effects and are biased toward local gene regulation. In contrast, HDMA assesses
16 genome-, transcriptome-, and phenotype-wide relationships as a whole and, in particular, identifies a highly
17 heritable trait profile that is perfectly mediated by a transcriptomic signature. We found these transcriptomic
18 signatures are tissue-specific and highly interpretable in terms of biological processes as well as cell type
19 composition. Heritability analysis of the transcripts showed that the strongest transcriptional mediators of
20 metabolic disease had low local heritability and high distal heritability. Finally, we showed that the HDMA
21 transcriptomic signatures predicted obesity in an independent population of Collaborative Cross recombinant
22 inbred (CC-RIX) mice and in human subjects. In contrast, local eQTL were unable to predict obesity in the
23 CC-RIX mice. Together our results suggest that both the tissue used for gene expression analysis as well
24 as distal gene regulation are critically important in identifying transcriptional mediators of the genome on
25 complex traits.

26 Introduction

27 In the quest to understand the genetic architecture of complex traits, gene expression is an important mediator
28 between genotype and phenotype. There is ample evidence from genome-wide association studies (GWAS)
29 that regulation of gene expression accounts for the bulk of the genetic effect on complex traits, as most
30 trait-associated variants lie in gene regulatory regions^{1–7}. It is widely assumed that these variants influence
31 local transcription, and methods such as transcriptome-wide association studies (TWAS)^{8–11}, summary
32 data-based Mendelian randomization (SMR)¹⁰, and others capitalize on this idea to identify genes associated
33 with multiple disease traits^{12–15}

34 Despite the great promise of these methods, their efficacy has been limited. The vast majority of complex
35 trait heritability remains unexplained [REF], implicating a subtler genetic architecture than assumed. Ineed,
36 although trait-associated variants tend to lie in non-coding, regulatory regions, they often do not have
37 detectable effects on gene expression¹⁶ and tend not to co-localize with expression quantitative trait loci
38 (eQTLs)^{17;18}.

39 One possible explanation for these observations is that gene expression is not being measured in the appropriate
40 cell types and thus true eQTLs influencing traits cannot be detected¹⁶. An alternative explanation that has
41 been discussed in recent years is that effects of these variants are mediated not through local regulation of
42 gene expression, but through distal regulation^{18–20;15}.

43 In this model, a gene's expression is influenced by many variants throughout the genome through their
44 cumulative effects on a broader regulatory network. In other words, the heritable component of the
45 transcriptome is an emergent state arising from the myriad molecular interactions defining and constraining
46 gene expression.

47 To assess the role of wide-spread distal gene regulation on complex traits, we have studied diet-induced obesity
48 and metabolic disease as an archetypal example. Diet-induced obesity and metabolic disease are genetically
49 complex with hundreds of variants mapped through GWAS [REFS]. These variants are known to act through
50 multiple tissues that interact dynamically with each other [REFS], including adipose tissue, pancreatic
51 islets, liver, and skeletal muscle. The multi-system etiology of metabolic disease complicates mechanistic
52 dissection of the genetic architecture, requiring large, dedicated data sets that include high-dimensional,
53 clinically relevant phenotyping, dense genotyping in a highly recombined population, and transcriptome-wide
54 measurements of gene expression in multiple tissues. Measuring gene expression in multiple tissues is critical
55 to adequately assess the extent to which local gene regulation varies across multiple tissues and whether such
56 variablilty might account for previous failed attempts to identify trait-relevant local eQTL. Such data sets

57 are extremely difficult to obtain in human populations, particularly in the large numbers of subjects required
58 for adequate statistical power. Thus, to investigate further the role of local and distal gene regulation on
59 complex traits, we have generated an appropriate data set in a large population of diversity outbred (DO)
60 mice²¹ in a population model of diet-induced obesity and metabolic disease¹².

61 The DO mice were derived from eight inbred founder mouse strains, five classical lab strains, and three
62 strains more recently derived from wild mice²¹. They represent three subspecies of mouse *Mus musculus*
63 *domesticus*, *Mus musculus musculus*, and *Mus musculus castaneus*, and capture 90% of the known variation
64 in laboratory mice²². They are maintained with a breeding scheme that ensures equal contributions from
65 each founder across the genome thus rendering almost the whole genome visible to genetic inquiry²¹. We
66 paired clinically relevant metabolic traits from 500 DO mice [REF], including body weight, plasma levels
67 of insulin and glucose and plasma lipids, with transcriptome-wide gene expression in four tissues related to
68 metabolic disease: adipose tissue, pancreatic islets, liver, and skeletal muscle. Taken together, these data
69 enable a comprehensive view into the genetic architecture of metabolic disease.

70 Results

71 Genetic variation contributed to wide phenotypic variation

72 Although the environment was consistent across all animals, the genetic diversity present in this population
73 resulted in widely varying distributions across physiological measurements (Fig. 1). For example, body
74 weights of adult individuals varied from less than the average adult B6 body weight to several times the body
75 weight of a B6 adult in both sexes (Males: 18.5 - 69.1g, Females: 16.0 - 54.8g) (Fig. 1A). Fasting blood
76 glucose (FBG) also varied considerably (Fig. 1B) although few of the animals had FBG levels that would
77 indicate pre-diabetes (19 animals, 3.8%), or diabetes (7 animals, 1.4%) according to previously developed
78 cutoffs (pre-diabetes: FBG \geq 250 mg/dL, diabetes: FBG \geq 300, mg/dL)²³. Males had higher FBG than
79 females on average (Fig. 1C) as has been observed before suggesting either that males were more susceptible
80 to metabolic disease on the high-fat diet, or that males and females may require different thresholds for
81 pre-diabetes and diabetes.

82 Body weight was strongly positively correlated with food consumption (Fig. 1D $R^2 = 0.51, p < 2.2 \times 10^{-16}$)
83 and fasting blood glucose (FBG) (Fig. 1E, $R^2 = 0.21, p < 2.2 \times 10^{-16}$) suggesting a link between behavioral
84 factors and metabolic disease. However, the heritability of this trait and others (Fig. 1F) indicates that
85 background genetics contribute substantially to correlates of metabolic disease in this population.

86 The trait correlations (Fig. 1G) shows that most of the metabolic trait pairs were weakly correlated indicating

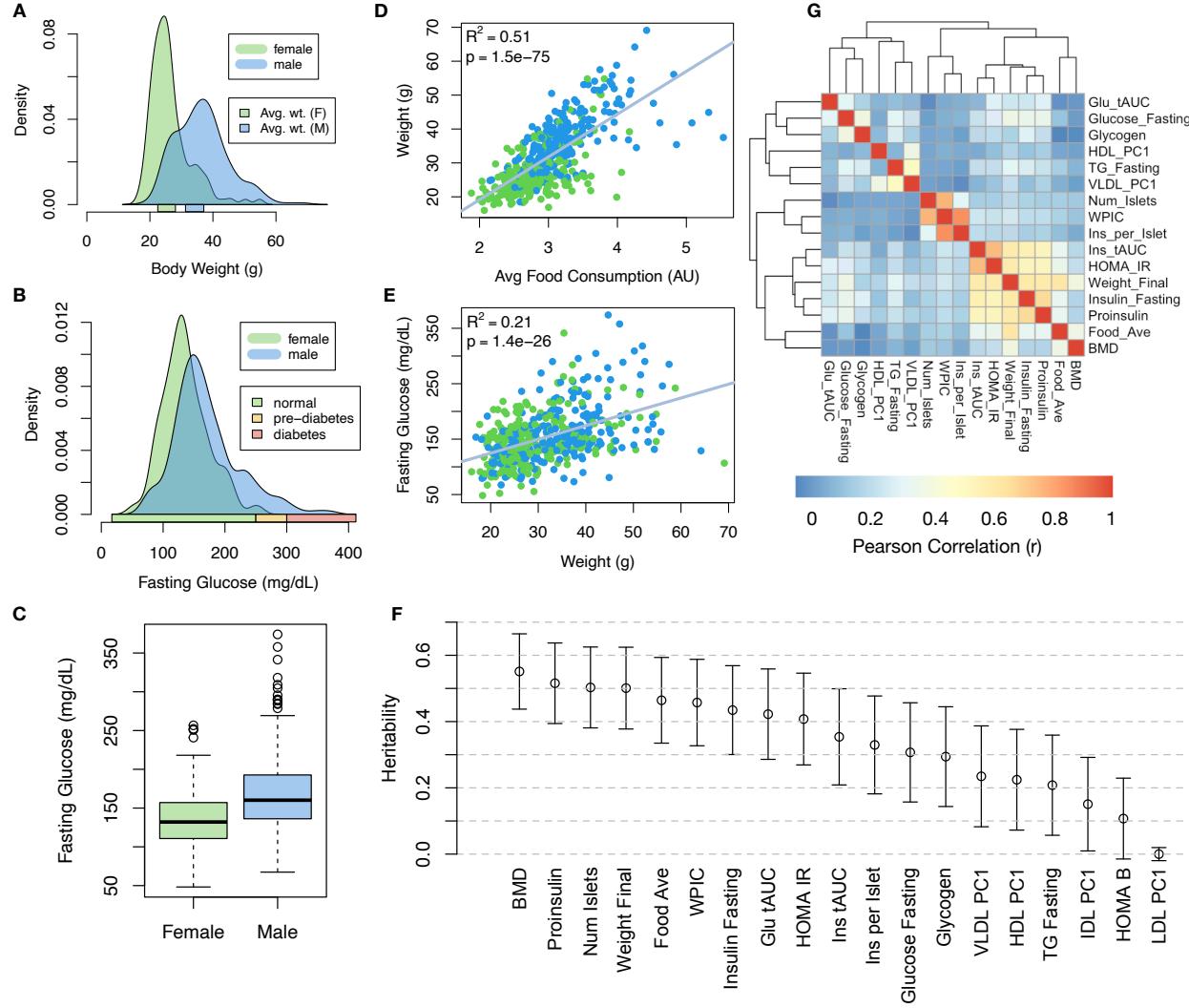


Figure 1: Clinical overview. **A.** Distributions of final body weight in the diversity outbred mice. Sex is indicated by color. The average B6 male and female adult weights at 24 weeks of age are indicated by blue and green bars on the x-axis. **B.** The distribution of final fasting glucose across the population split by sex. Normal, pre-diabetic, and diabetic fasting glucose levels for mice are shown by colored bars along the x-axis. **C.** Males had higher fasting blood glucose on average than females. **D.** The relationship between food consumption and body weight for both sexes. **E.** Relationship between body weight and fasting glucose for both sexes. **F.** Heritability estimates for each physiological trait. Bars show standard error of the estimate. **G.** Correlation structure between pairs of physiological traits.

87 complex relationships among the measured traits. This low level of redundancy suggests a broad sampling of
 88 multiple heritable aspects of metabolic disease including overall body weight, glucose homeostasis, pancreatic
 89 composition and liver function.

90 **Distal Heritability Correlated with Phenotype Relevance**

91 We performed eQTL analysis using R/qtl2²⁴ (Methods) and identified both local and distal eQTLs for
 92 transcripts in each of the four tissues (Supp. Fig 1). Significant local eQTLs far outnumbered distal eQTLs
 93 (Supp. Fig. 1F) and tended to be shared across tissues (Supp. Fig. 1G) whereas the few significant distal
 94 eQTLs we identified tended to be tissue-specific (Supp. Fig. 1H)

95 We calculated the heritability of each transcript in terms of local and distal genetic factors (Methods). Overall,
 96 local and distal genetic factors contributed approximately equally to transcript abundance. In all tissues,
 97 both local and distal factors explained between 8 and 18% of the variance in the median transcript (Fig 2A).

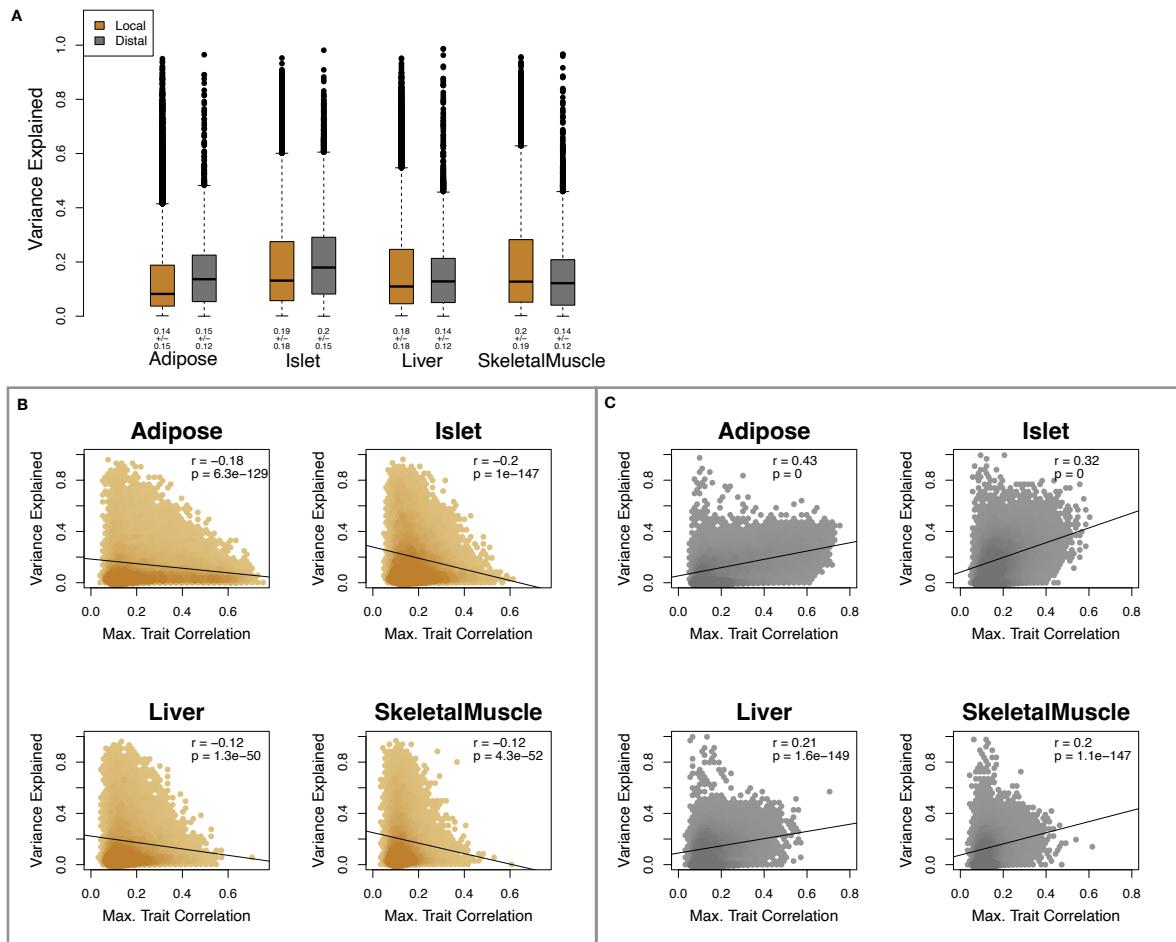


Figure 2: Transcript heritability and trait relevance. **A.** Distributions of distal and local heritability of transcripts across the four tissues. Overall local and distal factors contribute equally to transcript heritability. The relationship between **(B.)** local and **(C.)** distal heritability and trait relevance across all four tissues. Here trait relevance is defined as the maximum correlation between the transcript and all traits. Local heritability was negatively correlated with trait relevance, and distal heritability is positively correlated with trait relevance. Pearson (r) and p values for each correlation are shown in the upper-right of each panel.

98 The local heritability of transcripts was negatively correlated with their trait relevance, defined as the
99 maximum correlation of a transcript across all traits (Fig. 2B). This suggests that the more local genotype
100 influenced transcript abundance, the less effect this variation had on the measured traits. Conversely, the
101 distal heritability of transcripts was positively correlated with trait relevance (Fig. 2C). That is, transcripts
102 that were more highly correlated with the measured traits tended to be distally, rather than locally, heritable.
103 Importantly, this pattern was consistent across all tissues, strongly suggesting that this is a generic finding.
104 This finding is consistent with previous observations that low-heritability transcripts explain more expression-
105 mediated disease heritability than high-heritability transcripts¹⁹. However, the positive relationship between
106 trait correlation and distal heritability demonstrated further that there are diffuse genetic effects throughout
107 the genome converging on trait-related transcripts.

108 **High-Dimensional Mediation identified a high-heritability composite trait that was perfectly
109 mediated by a composite transcript**

110 The above univariate analyses establish the importance of distal heritability for trait-relevant transcripts.
111 However, the number of transcripts dramatically exceeds the number of degrees of freedom of the phenome.
112 Thus, we expect the heritable, trait-relevant transcripts to be highly correlated and organized according
113 to coherent, emergent biological processes representing the mediating endophenotypes driving clinical trait
114 variation. To identify these endophenotypes in a theoretically principled way, we developed a novel dimension-
115 reduction technique, HDMA, that uses the theory of causal graphical models to identify a transcriptomic
116 signature that is simultaneously 1) highly heritable, 2) strongly correlated to the measured phenotypes, and
117 3) conforms to the causal mediation hypothesis (Fig. 3). HDMA projects the high-dimensional scores—a
118 composite genome score (G_C), a composite transcriptome score (T_C), and a composite phenome score
119 (P_C)—and uses the univariate theory of mediation to constrain these projections to satisfy the hypotheses of
120 perfect mediation. Specifically, perfect mediation implies that upon controlling for the transcriptomic score,
121 the genome score is uncorrelated to the phenome score, which can also be viewed as a constraint on the
122 correlation coefficients

$$\text{Corr}(G_C, P_C) = \text{Corr}(G_C, T_C)\text{Corr}(T_C, P_C),$$

123 which corresponds to the path coefficient in the mediation model [REF]. Operationally, HDMA is closely
124 related to generalized canonical correlation analysis, for which provably convergent algorithms have recently
125 been developed²⁵. Implementation details for HDMA are available in **Supp. Methods XXX**.

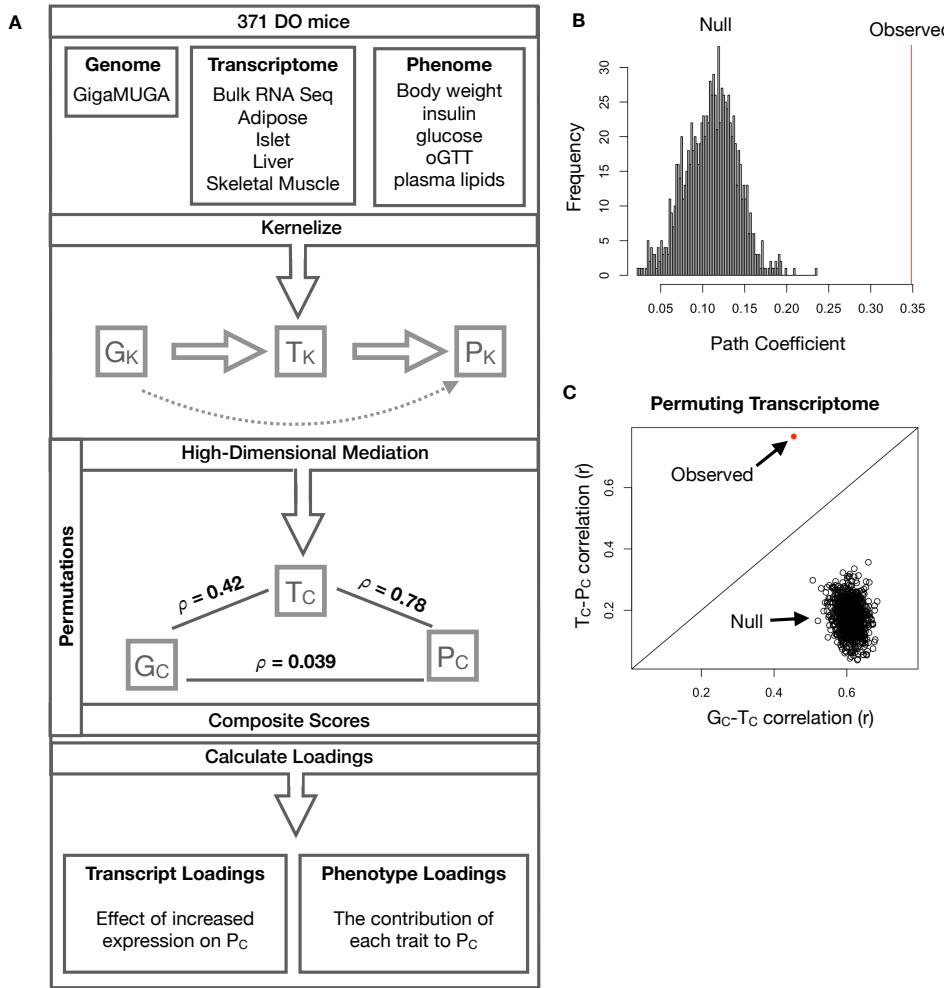


Figure 3: High-dimensional mediation. **A.** Workflow indicating major steps of high-dimensional mediation. The genotype, transcriptome, and phenotype matrices were kernelized to yield single matrices representing the relationships between all individuals for each data modality (G_K = genome kernel, T_K = transcriptome kernel; P_K = phenotype kernel). High-dimensional mediation was applied to these matrices to maximize the direct path $G \rightarrow T \rightarrow P$, the mediating pathway (arrows), while simultaneously minimizing the direct $G \rightarrow P$ pathway (dotted line). The composite vectors that resulted from high-dimensional mediation were G_c , T_c , and P_c . The partial correlations ρ between these vectors indicated perfect mediation. Transcript and trait loadings were calculated as described in the methods. **B.** The null distribution of the path coefficient derived from 10,000 permutations compared to the observed path coefficient (red line). **C.** The null distribution of the G_c-T_c correlation vs. the T_c-P_c correlation compared with the observed value (red dot).

126 We used high-dimensional mediation to identify the major axis of variation in the transcriptome that mediated
 127 the effects of the genome on metabolic traits (Fig 3). Fig. 3A shows the partial correlations (ρ) between
 128 the pairs of these composite vectors. The partial correlation between G_c and T_c was 0.42, and the partial
 129 correlation between T_c and P_c was 0.78. However, when the transcriptome was taken into account, the
 130 partial correlation between G_c and P_c was effectively zero (0.039). P_c captured 30% of the overall trait
 131 variance, and its estimated heritability was $0.71 \pm 0.084\%$, which was higher than any of the measured

132 traits (Fig. 1F). Thus, HDMA identified a maximally heritable metabolic composite trait that was perfectly
133 mediated by a highly heritable component of the transcriptome.

134 Standard CCA is prone to over-fitting because in any two large matrices it can be trivial to identify highly
135 correlated composite vectors [REF]. To assess whether our implementation of HDMA was similarly prone to
136 over-fitting in a high-dimensional space, we performed permutation testing. We permuted the individual
137 labels on the transcriptome matrix 1000 times and recalculated the path coefficient, which is the partial
138 correlation of G_C and T_C multiplied by the partial correlation of T_C and P_C . This represents the path
139 from G_C to P_C that is mediated through T_C . The null distribution of the path coefficient is shown in Fig.
140 3B, and the observed path coefficient from the original data is indicated by the red line. The observed
141 path coefficient was well outside the null distribution generated by permutations ($p < 10^{-16}$). Fig. 3C
142 illustrates this observation in more detail. Although we identified high correlations between G_C and T_C , and
143 modest correlations between T_C and P_C in the null data (Fig 3C), these two values could not be maximized
144 simultaneously in the null data. In contrast, the red dot shows that in the real data both the G_C - T_C
145 correlation and the T_C - P_C correlation could be maximized simultaneously suggesting that the path from
146 genotype to phenotype through transcriptome is highly non-trivial and identifiable in this case. These results
147 suggest that these composite vectors represent genetically determined variation in phenotype that is mediated
148 through genetically determined variation in transcription.

149 **Body weight and insulin resistance were highly represented in the expression-mediated com-**
150 **posite trait**

151 Each composite score is simply a weighted combination of the measured variables and the magnitude and
152 sign of the weights, called loadings, correspond the relative importance and directionality of each variable in
153 the composite score. The loadings of each measured trait onto P_C indicate how much each contributed to
154 the composite phenotype. Final body weight contributed the most (Fig. 4), followed by homeostatic insulin
155 resistance (HOMA_IR) and fasting plasma insulin levels (Insulin_Fasting). We can thus interpret P_C as
156 an index of metabolic disease (Fig. 4B). Individuals with high values of P_C have a higher metabolic index
157 and greater metabolic disease, including higher body weight and higher insulin resistance. We refer to P_C as
158 the metabolic index going forward. Traits contributing the least to the metabolic index were measures of
159 cholesterol and pancreas composition. Thus, when we interpret the transcriptomic signature identified by
160 HDMA, we are explaining primarily the transcriptional mediation of body weight and insulin resistance, as
161 opposed to cholesterol measurements.

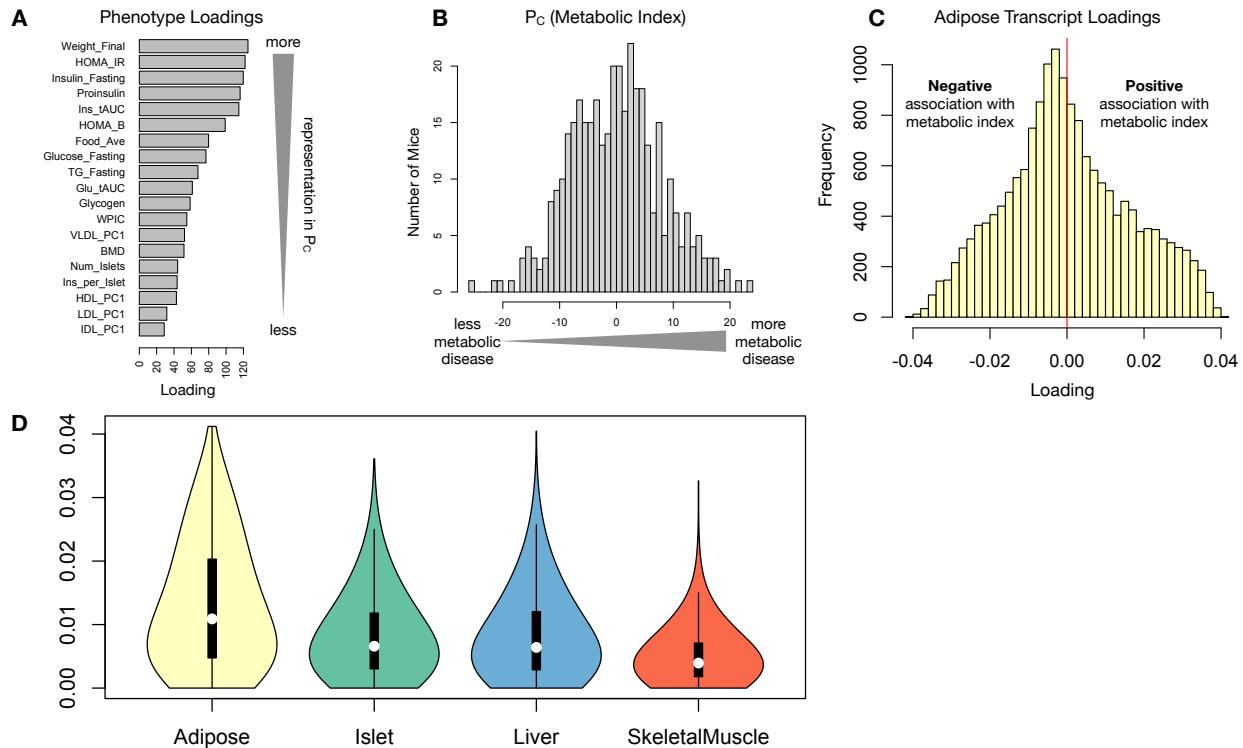


Figure 4: Interpretation of loadings. **A.** Loadings across traits. Body weight and insulin resistance contributed the most to the composite trait. **B.** Phenotype scores across individuals. Individuals with large positive phenotype scores had higher body weight and insulin resistance than average. Individuals with large negative phenotype scores had lower body weight and insulin resistance than average. **C.** Distribution of transcript loadings in adipose tissue. For transcripts with large positive loadings, higher expression was associated with higher phenotype scores. For transcripts with large negative loadings, higher expression was associated with lower phenotype scores. **D.** Distribution of absolute value of transcript loadings across tissues. Transcripts in adipose tissue had the largest loadings indicating that transcripts in adipose tissue were the best mediators of the genetic effects on body weight and insulin resistance.

- 162 **High-loading transcripts have low local heritability, high distal heritability, and were linked**
 163 **mechanistically to obesity**
- 164 We interpreted large loadings onto transcripts as indicating strong mediation of the effect of genetics on
 165 metabolic index. Large positive loadings indicate that higher expression was associated with a higher
 166 metabolic index (i.e. higher risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). Conversely,
 167 large negative loadings indicate that high expression of these transcripts was associated with a lower metabolic
 168 index (i.e. lower risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). We used gene set
 169 enrichment analysis (GSEA)^{26;27} to look for biological processes and pathways that were enriched at the top
 170 and bottom of this list (Methods).
- 171 In adipose tissue, both GO processes and KEGG pathway enrichments pointed to an axis of inflammation

172 and metabolism (Supp. Fig. 2 and Fig. 11). GO terms and KEGG pathways associated with inflammation,
173 particularly macrophage infiltration, were positively associated with metabolic index, indicating that increased
174 expression in inflammatory pathways was associated with a higher metabolic index. It is well established that
175 adipose tissue in obese individuals is inflamed [cite] and infiltrated by macrophages [cite], and the results
176 here suggest that this may be a heritable component of metabolic disease.

177 The strongest negative enrichments in adipose tissue were related to mitochondrial activity in general, and
178 thermogenesis in particular (Supp. Fig. 2 and Fig. 11). It has been shown mouse strains with greater
179 thermogenic potential are also less susceptible to obesity on a high-fat diet [cite].

180 Transcripts associated with the citric acid (TCA) cycle as well as the catabolism of the branched-chain amino
181 acids (BCAA) (valine, leucine, and isoleucine) were strongly enriched with negative loadings in adipose
182 tissue (Supp. Fig. 3). Expression of genes in both pathways (for which there is some overlap) has been
183 previously associated with insulin sensitivity^{12;28;29}, suggesting that heritable variation in regulation of these
184 pathways may influence risk of insulin resistance.

185 Looking at the 10 most positively and negatively loaded transcripts from each tissue, it is apparent that
186 transcripts in the adipose tissue had the largest loadings, both positive and negative, of all tissues (Fig. 5A
187 bar plot). This suggests that much of the effect of genetics on body weight and insulin resistance is mediated
188 through gene expression in adipose tissue. The strongest loadings in liver and pancreas were comparable,
189 and those in skeletal muscle were the weakest (Fig. 5A), suggesting that less of the genetic effects were
190 mediated through transcription in skeletal muscle. Heritability analysis showed that transcripts with the
191 largest loadings had higher distal heritability than local heritability (Fig. 5A heat map and box plot). This
192 pattern contrasts with transcripts nominated by TWAS (Fig. 5B), which tended to have lower loadings,
193 higher local heritability and lower distal heritability. Transcripts with the highest local heritability in each
194 tissue (Fig. 5C) had the lowest loadings.

195 We performed a literature search for the genes in each of these groups along with the terms “diabetes”,
196 “obesity”, and the name of the expressing tissue to determine whether any of these genes had previous
197 associations with metabolic disease in the literature (Methods). Multiple genes in each group had been
198 previously associated with obesity and diabetes (Fig. 5 bolded gene names). Genes with high loadings were
199 most highly enriched for previous literature support. They were 2.25 more likely than TWAS hits and 3.6
200 times more likely than genes with high local heritability to be previously associated with obesity or diabetes.

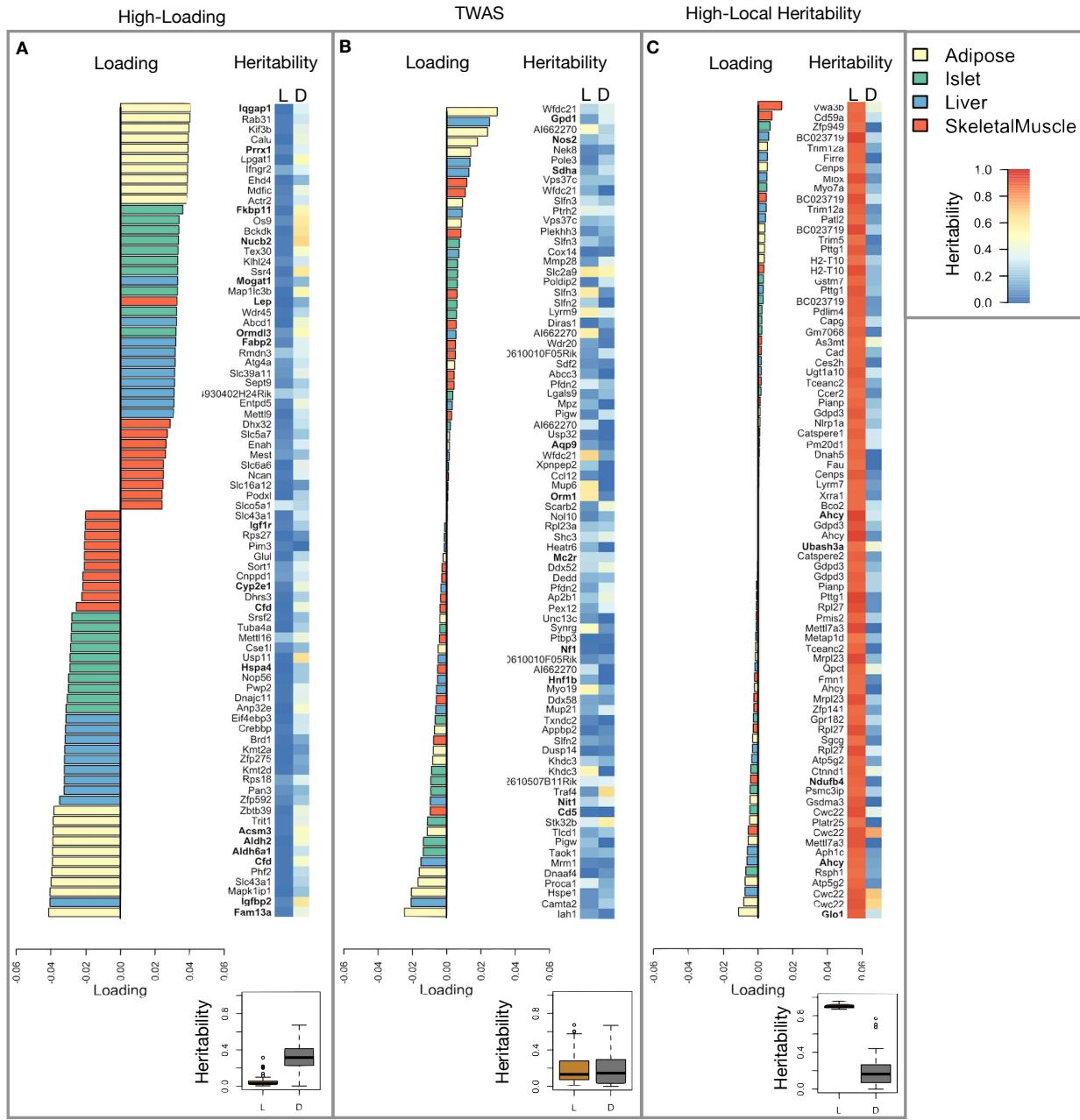


Figure 5: Transcripts with high loadings have high distal heritability and literature support. Each panel has a bar plot showing the loadings of transcripts selected by different criteria. Bar color indicates the tissue of origin. The heat map shows the local (L - left) and distal (D - right) heritability of each transcript.

201 **Tissue-specific transcriptional programs were associated with metabolic traits**

202 Clustering of transcripts with top loadings in each tissue showed tissue-specific functional modules associated
203 with obesity and insulin resistance (Fig. 6A) (Methods). The clustering highlights the importance of immune

activation particularly in adipose tissue. Except for the “mitosis” cluster, which had large positive loadings in three of the four tissues, all clusters were strongly loaded in only one or two tissues. For example, the lipid metabolism cluster was loaded most heavily in liver. The positive loadings suggest that high expression of these genes particularly in the liver was associated with increased metabolic disease. This cluster included the gene *Pparg*, whose primary role is in the adipose tissue where it is considered a master regulator of adipogenesis³⁰. Agonists of *Pparg*, such as thiazolidinediones, which are FDA-approved to treat type II diabetes, reduce inflammation and adipose hypertrophy³⁰. Consistent with this role, the loading for *Pparg* in adipose tissue was negative, suggesting that higher expression was associated with leaner mice (Fig. 6B). In contrast, *Pparg* had a large positive loading in liver, where it is known to play a role in the development of hepatic steatosis, or fatty liver. Mice that lack *Pparg* specifically in the liver, are protected from developing steatosis and show reduced expression of lipogenic genes^{31;32}. Overexpression of *Pparg* in the livers of mice with a *Ppara* knockout, causes upregulation of genes involved in adipogenesis³³. In the livers of both mice and humans high *Pparg* expression is associated with hepatocytes that accumulate large lipid droplets and have gene expression profiles similar to adipocytes^{34;35}.

The local and distal heritability of *Pparg* is low in adipose tissue suggesting its expression in this tissue is highly constrained in the population (Fig. 6B). However, the distal heritability of *Pparg* in liver is relatively high suggesting it is complexly regulated and has sufficient variation in this population to drive variation in phenotype. Both local and distal heritability of *Pparg* in the islet are relatively high, but the loading is low, suggesting that variability of expression in the islet does not drive variation in metabolic index. These results highlight the importance of tissue context when investigating the role of heritable transcript variability in driving phenotype.

Gene lists for all clusters are available in Supplemental File XXX.

Gene expression, but not local eQTLs, predicted body weight in an independent population

To test whether the transcript loadings identified in the DO could be translated to another population, we tested whether they could predict metabolic phenotype in an independent population of CC-RIX mice, which were F1 mice derived from multiple pairings of Collaborative Cross (CC) [cite] strains (Fig. 7) (Methods). We tested two questions. First, we asked whether the loadings identified in the DO mice were relevant to the relationship between the transcriptome and the phenotype in the CC-RIX. We predicted body weight (a surrogate for metabolic index) in each CC-RIX individual using measured gene expression in each tissue and the transcript loadings identified in the DO (Methods). The predicted body weight and actual body weight were highly correlated in all tissues (Fig. 7B left column). The best prediction was achieved for adipose

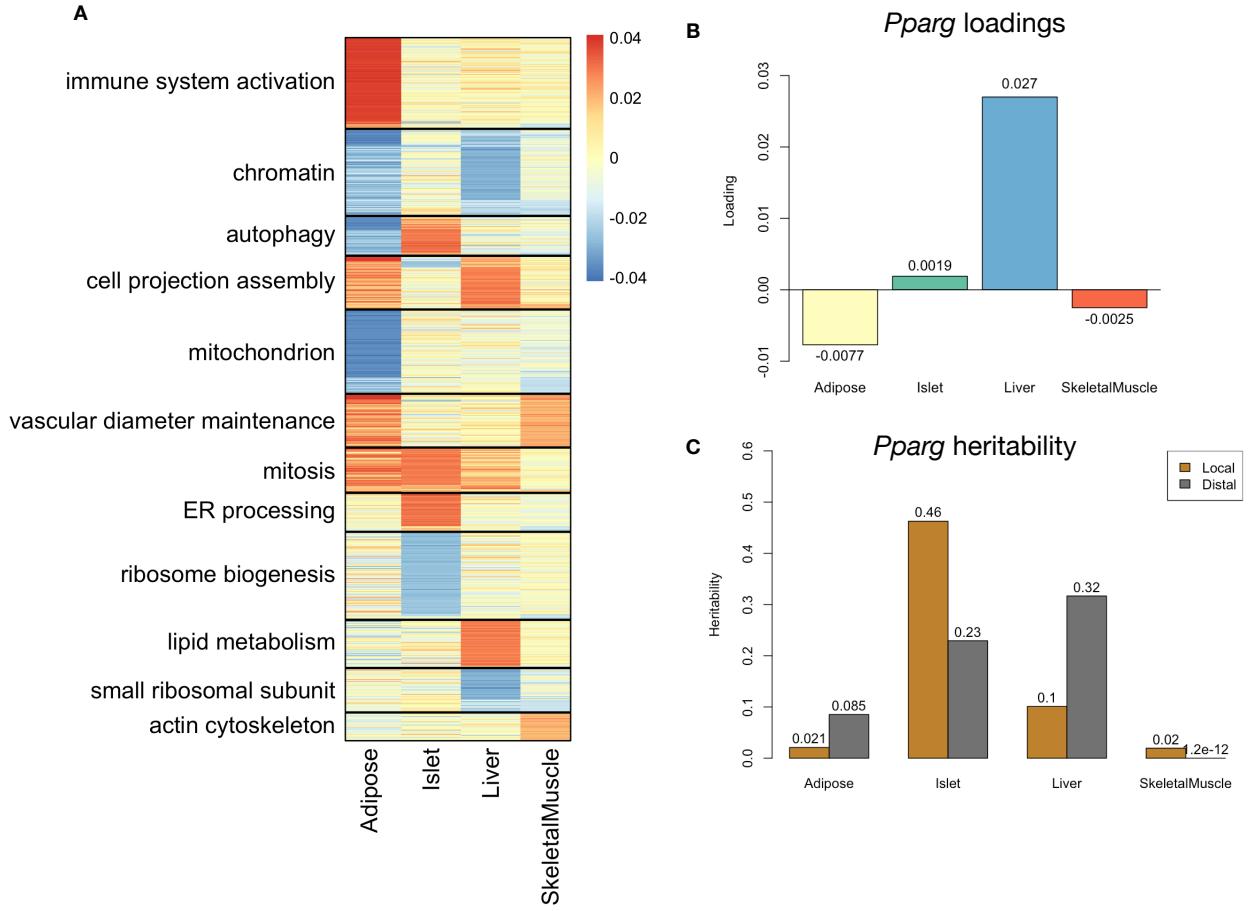


Figure 6: Tissue-specific transcriptional programs were associated with obesity and insulin resistance. **A** Heat map showing the loadings of all transcripts with loadings greater than 2.5 standard deviations from the mean in any tissue. The heat map was clustered using k medoid clustering. Functional enrichments of each cluster are indicated along the left margin. **B** Loadings for *Pparg* in different tissues. **C** Local and distal of *Pparg* expression in different tissues.

tissue, which supports the observation in the DO that adipose expression was the strongest mediator of the genetic effect on metabolic index. This result also confirms the validity and translatability of the transcript loadings and their relationship to metabolic disease.

The second question related to the source of the relevant variation in gene expression. If local regulation was the predominant factor influencing gene expression, we should be able to predict phenotype in the CC-RIX using transcripts imputed from local genotype (Fig. 7A). The DO and the CC-RIX were derived from the same eight founder strains and so carry the same alleles throughout the genome. We imputed gene expression in the CC-RIX using local genotype and were able to estimate variation in gene transcription robustly (Supp. Fig. 4). However, these imputed values failed to predict body weight in the CC-RIX when weighted with the loadings from HDMA. (Fig. 7B right column). This result suggests that local regulation of gene expression is

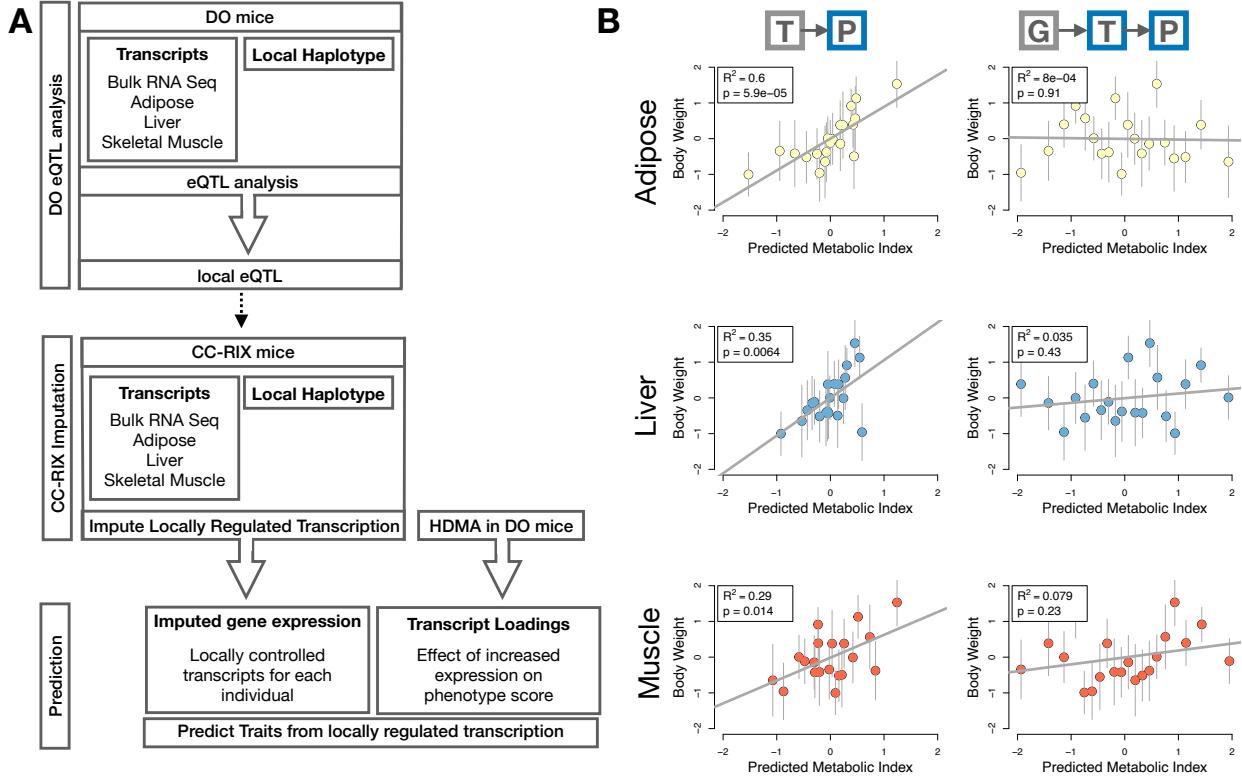


Figure 7: Transcription, but not local genotype, predicts phenotype in the CC-RIX. **A.** Workflow showing procedure for translating HDMA results to an independent population of mice. **B.** Relationships between the predicted metabolic index and measured body weight. The left column shows the predictions using measured transcripts. The right column shows the prediction using transcript levels imputed from local genotype. Gray boxes indicate measured quantities, and blue boxes indicate calculated quantities. The dots in each panel represent individual CC-RIX strains. The gray lines show the standard deviation on body weight for the strain.

not the primary factor driving heritability of complex traits, consistent with our findings in the DO population
 that distal heritability was a major driver of trait-relevant variation and that high-loading transcripts had
 comparatively high distal and low local heritability.

245 **Distally heritable transcriptomic signatures reflected variation in composition of adipose tissue
 246 and islets**

247 The interpretation of global genetic influences on gene expression and phenotype is potentially more challenging
 248 than the interpretation and translation of local genetic influences, as genetic effects cannot be localized to
 249 individual gene variants or transcripts. However, there are global patterns across the loadings that can
 250 inform mechanism. For example, heritable variation in cell type composition can be derived from transcript
 251 loadings. We observed above that immune activation in the adipose tissues was an important driver of obesity
 252 in the DO population. To determine whether this is reflected as an increase in macrophages in adipose
 253 tissue, we analyzed the transcript loadings for genes involved in macrophage activation. We found that
 254 the highest-loading transcripts in the adipose tissue were associated with genes involved in macrophage
 255 activation, such as *Cebpd*, *Cebpd*, and *Cebpd*. These genes have been shown to play a role in macrophage
 256 activation and differentiation. Our results suggest that the variation in cell type composition in the adipose
 257 tissue is reflected in the transcript loadings, which can be used to predict phenotypic variation.

256 tissue, we compared loadings of cell-type specific genes in adipose tissue (Methods). The mean loading
 257 of macrophage-specific genes was significantly greater than 0 (Fig. 8A), indicating that obese mice were
 258 genetically predisposed to have high levels of macrophage infiltration in adipose tissue in response to the
 259 high-fat, high-sugar diet.

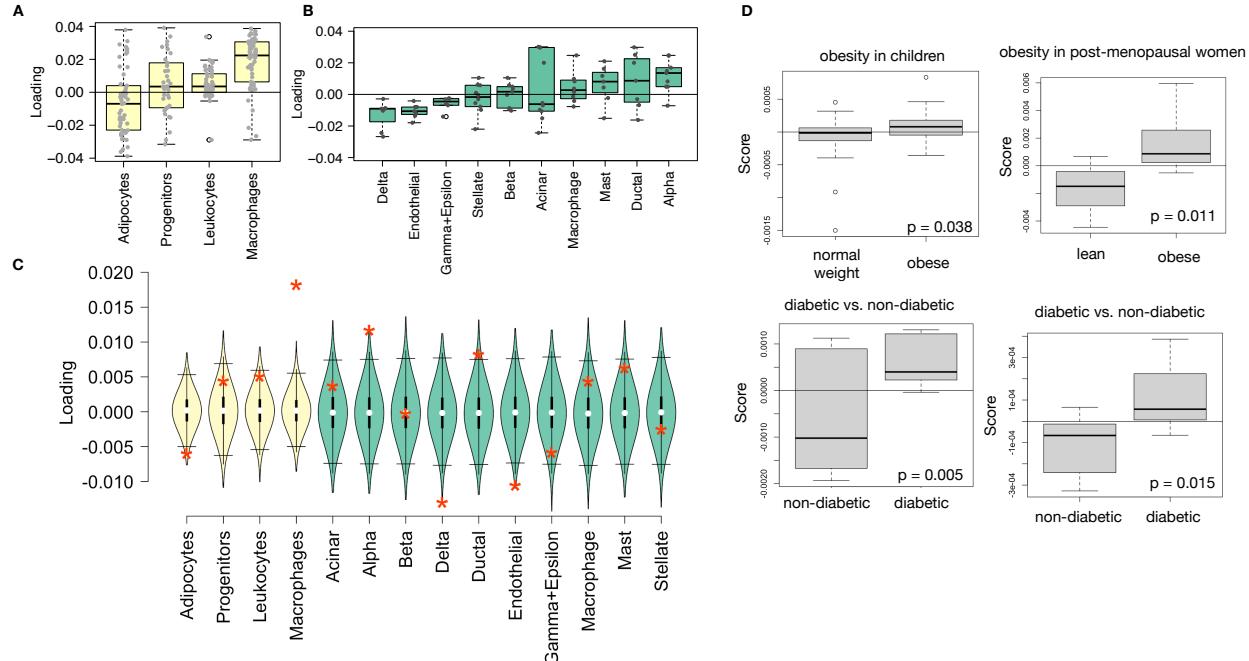


Figure 8: HDMA results translate to humans. **A.** Distribution of loadings for cell-type-specific transcripts in adipose tissue. **B.** Distribution of loadings for cell-type-specific transcripts in pancreatic islets (green). **C.** Null distributions for the mean loading of randomly selected transcripts in each cell type compared with the observed mean loading of each group of transcripts (red asterisk). **D.** Predictions of metabolic phenotypes in four adipose transcription data sets downloaded from GEO. In each study the obese/diabetic patients were predicted to have greater metabolic disease than the lean/non-diabetic patients based on the HDMA results from DO mice.

260 We also compared loadings of cell-type specific transcripts in islet (Methods). The mean loadings for alpha-cell
 261 specific transcripts were significantly greater than 0, while the mean loadings for delta- and endothelial-cell
 262 specific genes were significantly less than 0 (Fig. 8B). These results suggest either that mice with higher
 263 metabolic index had inherited a higher proportions of alpha cells, and lower proportions of endothelial and
 264 delta cells in their pancreatic islets, that such compositional changes were induced by the HFHS diet in a
 265 heritable way, or both. In either case, these results support the hypothesis that alterations in islet composition
 266 drive variation in metabolic index.

267 Notably, the loadings for pancreatic beta cell-type specific loadings was not significantly different from zero.
 268 This is not necessarily reflective of the function of the beta cells in the obese mice, but rather suggests that
 269 any variation in the number of beta cells in these mice was unrelated to obesity and insulin resistance. This

270 is further consistent with the islet composition traits having small loadings in the genome score (Fig. 4).

271 **Heritable transcriptomic signatures translated to human disease**

272 Ultimately, the heritable transcriptomic signatures that we identified in DO mice will be useful if they inform
273 pathogenicity and treatment of human disease. To investigate the potential for translation of the gene
274 signatures identified in DO mice, we compared them to transcriptional profiles in obese and non-obese human
275 subjects (Methods). We limited our analysis to adipose tissue because the adipose tissue signature had the
276 strongest relationship to obesity and insulin resistance in the DO.

277 We calculated a predicted obesity score for each individual in the human studies based on their adipose
278 tissue gene expression (Methods) and compared the predicted scores for obese and non-obese groups as well
279 as diabetic and non-diabetic groups. In all cases, the predicted obesity scores were higher on average for
280 individuals in the obese and diabetic groups compared with the lean and non-diabetic groups (Fig. 8D).
281 This indicates that the distally heritable signature of obesity identified in DO mice is relevant to obesity and
282 diabetes in human subjects.

283 **Targeting gene signatures**

284 Another global view of the transcript loading landscape is in ranking potential drug candidates for the
285 treatment of metabolic disease. Although high-loading transcripts may be good candidates for understanding
286 specific biology related to obesity, the transcriptome overall is highly interconnected and redundant, and
287 focusing on individual transcripts for treatment may be less effective than using broader transcriptomic
288 signatures that capture the emergent biology. The ConnectivityMap (CMAP) database [cite] developed by
289 the Broad Institute allows us to query thousands of compounds that reverse or enhance the extreme ends
290 of transcriptomic signatures in multiple different cell types. By identifying drugs that reverse pathogenic
291 transcriptomic signatures, we can potentially identify compounds that have favorable effects on gene expression.

292 To test this hypothesis, we queried the CMAP database through the CLUE online query tool [cite] (Methods).
293 We identified top anti-correlated hits both across all cell types, as well as in adipocytes and pancreatic tumor
294 cells (Supplemental Figure XXX and XXX).

295 Looking broadly across cell types, the notable top hits from the adipose tissue loadings included mTOR
296 inhibitors and glucocorticoid agonists (Supplemental Figure XXX). It is thought that metformin, which
297 is commonly used to improve glycemic control, acts, at least in part, by inhibiting mTOR signaling^{36:37}.
298 However, long-term use of other mTOR inhibitors, such as rapamycin, are known to cause insulin resistance
299 and β -cell toxicity³⁷⁻³⁹. Glucocorticoids are used to reduce inflammation, which was a prominent signature

300 in the adipose tissues, but these drugs also promote hyperglycemia and diabetes^{40;41}. Accute treatment
301 with glucocorticoids has further been shown to reduce thermogenesis in rodent adipocytes^{42–44}, but increase
302 thermogenesis in human adipocytes^{45;46}. Thus, the pathways identified by CMAP across all cell types were
303 highly related to the transcript loading profiles, but the relationship was not a simple reversal.

304 The top hit in adipocytes was a PARP inhibitor (Supplemental Figure XXXB). PARPs play a role in lipid
305 metabolism and are involved in the development of obesity and diabetes⁴⁷. PARP1 inhibition increases
306 mitochondrial biogenesis⁴⁸. Inhibition of PARP1 activity can further prevent necrosis in favor of the less
307 inflammatory apoptosis⁴⁹, thereby potentially reducing inflammation in stressed adipocytes. Other notable
308 hits in the top 20 were BTK inhibitors, which have been observed to suppress inflammation and improve
309 insulin resistance⁵⁰ as well as to reduce insulin antibodies in type I diabetes⁵¹, and IKK inhibitors have been
310 shown to improve glucose control in type II diabetes^{52;53}.

311 The CMAP database includes assays in multiple cell types. Among the top hits for the query with transcript
312 loadings from pancreatic islets (Fig. XXX), was suppression of T cell receptor signaling, which is known to
313 be involved in Type 1 diabetes⁵⁴, as well as TNFR1, which has been associated with mortality in diabetes
314 patients⁵⁵. Suppression of NOD1/2 signaling was also among the top hits. NOD1 and 2 sense ER stress^{56;57},
315 which is associated with β -cell death in type 1 and type 2 diabetes⁵⁸. This cell death process is dependent
316 on NOD1/2 signaling⁵⁶, although the specifics have not yet been worked out.

317 Among the top hits in pancreatic tumor cells were known diabetes drugs, including sulfonylureas, PPAR
318 receptor agonists, and insulin sensitizers. Rosiglitazone is a PPAR- γ agonist and was one of the most
319 prescribed drugs for type 2 diabetes before its use was reduced due to cardiac side-effects⁵⁹. Sulfonylureas
320 are another commonly prescribed drug class for type 2 diabetes, but also have notable side effects including
321 hypoglycemia and accellerated β -cell death⁶⁰.

322 Discussion

323 It is thought that the bulk of the effect of genomic variation on complex traits is mediated through regulation
324 of gene expression. It has frequently been assumed that this regulation is largely in *cis*, but attempts to use
325 local gene regulation to explain phenotypic variation have yet to explain much trait heritability. In recent
326 years, the discussion has turned to distal gene regulation. Although, distal gene regulation is more complex
327 to identify, evidence suggests that it is an important component of trait heritability.

328 Yao *et al.*¹⁹ observed that in humans, transcripts with low local heritability explained more expression-
329 mediated disease heritability than transcripts with high local heritability. We observed the same trend here

330 in mice in a well-powered study of all major tissues of action simultaneously. This pattern is consistent
331 with principles of robustness in complex systems^{61–63}. If a transcript were both important to a trait and
332 subject to strong local regulation, a population would be susceptible to extremes in phenotype that might
333 frequently cross the threshold to disease. Indeed, strong disruption of highly trait-relevant genes is the cause
334 of Mendelian disease.

335 Rather, studies suggest that genes that are near GWAS hits and have obvious functional relevance to a trait
336 tend to have highly complex regulatory landscapes under strong selection pressures¹⁸. In contrast, genes
337 with strong local regulation tend to be depleted of functional annotations and are under looser selection
338 constraints¹⁸. These observations and others led Liu et al.⁶⁴ to suggest that most heritability of complex
339 traits is driven by weak distal eQTLs. They proposed a framework of understanding heritability of complex
340 traits in which massive polygenicity is distributed across common variants in both functional “core genes”, as
341 well as more peripheral genes that may not seem obviously related to the trait.

342 Here, we used a large, comprehensive, and purpose-built data set to investigate the genetic architecture of
343 complex traits related to metabolic disease in mice as well as the roles of local and distal gene regulation
344 in mediating these traits. We presented a systems-level method called high-dimension mediation analysis
345 (HDMA) to specifically identify the distally regulated transcriptomic signature mediating the effect of the
346 genotype on phenotype. This approach contrasts with traditional univariate approaches in several important
347 respects. First, in contrast to univariate approaches, which assume independence of genetic variants and
348 transcripts, HDMA allows for arbitrarily complex gene regulation, as well as the interconnectedness and
349 redundancy of the transcriptome. Second, rather than assuming a single, large genetic effect as univariate
350 approaches do, HDMA assumes that traits are highly polygenic, and that genetic effects are weak and are
351 distributed across the genome. HDMA does not use statistical thresholds to identify true positive effects, but
352 generates a weighted vector of transcripts that can be analyzed as a whole, or dissected to identify transcripts
353 with stronger and weaker effects. This method explicitly models a central proposal of the omnigenic model
354 which posits that once the expression of the core genes (i.e. trait-mediating genes) is accounted for, there
355 should be no residual correlation between the genome and the phenome.

356 Using HDMA, we identified a highly heritable composite trait ($h^2 = 0.71$) that explained 30% of overall trait
357 variance, and was perfectly mediated by a composite transcript that included expression from four tissues
358 known to be involved in metabolic disease. Gene expression in adipose tissue was the strongest mediator of
359 genetic effects on metabolic disease. Further analysis of the loadings onto transcripts in each tissue revealed
360 that the mediating signatures were tissue-specific transcriptional programs, many of which were previously
361 known to be involved in the pathogenesis of metabolic disease. We showed here that regulation of these

362 programs is heritable and mediated a large proportion of disease risk.

363 The transcripts with the highest loadings are similar to the core genes of the omnigenic model [REF]. These
364 were transcripts of moderate local heritability that were highly functionally related to the traits. Transcripts
365 with small loadings are more peripheral to the traits measured in this experiment. There was no clear
366 demarcation between the core and peripheral genes as far as loading, but a clear separation should not be
367 expected given the complexity of gene regulation and the genotype-phenotype map⁶⁵.

368 The strength of mediation (transcript loading) was negatively correlated with local heritability and positively
369 correlated with distal heritability, suggesting that distal gene regulation was the dominant mode through which
370 gene expression mediated the effect of genotype on phenotype. We saw further that the distal heritability
371 was weak and spread across the genome, consistent with the prediction by Liu *et al.*⁶⁴ that trait heritability
372 is mediated through weak distal eQTLs. Most strongly mediating transcripts had modest distal heritability,
373 and even for those whose expression was strongly regulated by distal factors, these factors were multiple
374 and spread across the genome. For example, *Nucb2*, was a strongly mediating transcript in islet and was
375 also strongly distally regulated (66% distal heritability). This gene is expressed in pancreatic β cells and is
376 involved in insulin and glucagon release^{66–68}. Although its transcription was highly heritable in islets, that
377 regulation was distributed across the genome, with no clear distal eQTL (Supp. Fig. 5). Thus, although
378 distal regulation of some genes may be strong, this regulation is likely to be highly complex and not easily
379 localized.

380 The high complexity of gene regulation combined with a systems-level analysis yields continuous results
381 that do not necessarily implicate individual transcripts or genetic loci in disease pathogenesis. Most studies
382 have focused on pinpointing individual loci whose mechanistic roles can be clearly dissected through further
383 experiments and exploited as therapeutic targets. In this analysis, too, it is possible to focus on individual
384 genes and their context in both tissues and pathways. For example, the loadings on *Pparg* were tissue-specific
385 in a way that comports with known biology, i.e. it is known to be protective in adipose tissue where it was
386 negatively loaded, and harmful in the liver, where it was positively loaded. However, whole transcriptomic
387 signatures can also be informative in their own right. Combined with increasing amounts of high-dimensional
388 data in public databases, weighted vectors can be useful for generating hypotheses and potential drug
389 treatments. We showed that weighted vectors of genes can be analyzed for enriched biological functions
390 and pathways using GSEA. These vectors can also be paired with data about cell-type specific genes to
391 generate hypotheses about cell composition in individual tissues. Gene expression derived from patient
392 biopsies confirmed that the transcriptional signatures we identified in mice predict obesity status in humans,
393 further supporting the translatability of these results. Finally, we used the CMAP database to show that the

394 transcriptomic signatures we identified in mice could be translated into human drug targets, as currently
395 used diabetes drugs and targets were among the top hits for reversing the disease-associated signatures. That
396 these drugs are known to reverse diabetes pathogenesis supports the causal role of these gene signatures in
397 disease risk as modeled by high-dimensional mediation.

398 In conclusion, we have shown that both tissue specificity and distal gene regulation are critically important to
399 understanding the genetic architecture of complex traits. We identified important genes and gene signatures
400 that were heritable, causal of disease, and translatable to other mouse populations and to humans. Finally,
401 we have shown that by directly acknowledging the complexity of both gene regulation and the genotype-to-
402 phenotype map, we can gain a new perspective on disease pathogenesis and develop actionable hypotheses
403 about pathogenic mechanisms and potential treatments.

404 Potentially comment on: 1) Mediating on other endophenotypes 2) Limitation that we only find signatures
405 “consistent with” mediation and that our approach is hypothesis generating 3) Is the kinship matrix the
406 sum of all local QTLs, or is it something more inclusive, including distal regulation, development, etc. 4)
407 endophenotypes don’t need to be gene expression. Can be anything you think is causally related to phenotype
408 and can be manipulated

409 **Data Availability**

410 Here we tell people where to find the data

411 **Acknowledgements**

412 Here we thank people

413 **Supplemental Figures**

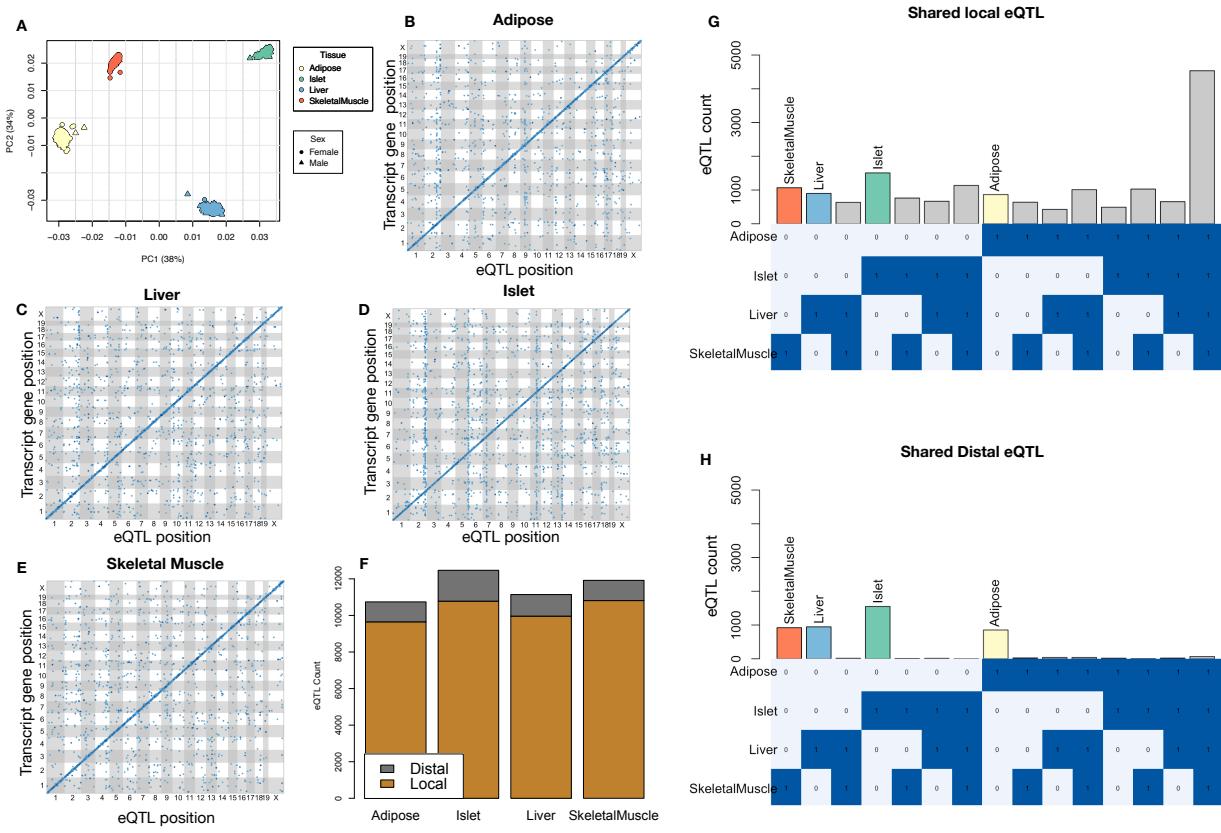


Figure 9: Overview of eQTL analysis in DO mice. **A.** RNA seq samples from the four different tissues clustered by tissue. **B.-E.** eQTL maps are shown for each tissue. The *x*-axis shows the position of the mapped eQTL, and the *y*-axis shows the physical position of the gene encoding each mapped transcript. Each dot represents an eQTL with a minimum LOD score of 8. The dots on the diagonal are locally regulated eQTL for which the mapped eQTL is at the within 4Mb of the encoding gene. Dots off the diagonal are distally regulated eQTL for which the mapped eQTL is distant from the gene encoding the transcript. **F.** Comparison of the total number of local and distal eQTL with a minimum LOD score of 8 in each tissue. All tissues have comparable numbers of eQTL. Local eQTL are much more numerous than distal eQTL. **G.** Counts of transcripts with local eQTL shared across multiple tissues. The majority of local eQTL were shared across all four tissues. **H.** Counts of transcripts with distal eQTL shared across multiple tissues. The majority of distal eQTL were tissue-specific and not shared across multiple tissues. For both G and H, eQTL for a given transcript were considered shared in two tissues if they were within 4Mb of each other. Colored bars indicate the counts for individual tissues for easy of visualization.

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KEGG pathway enrichments by GSEA

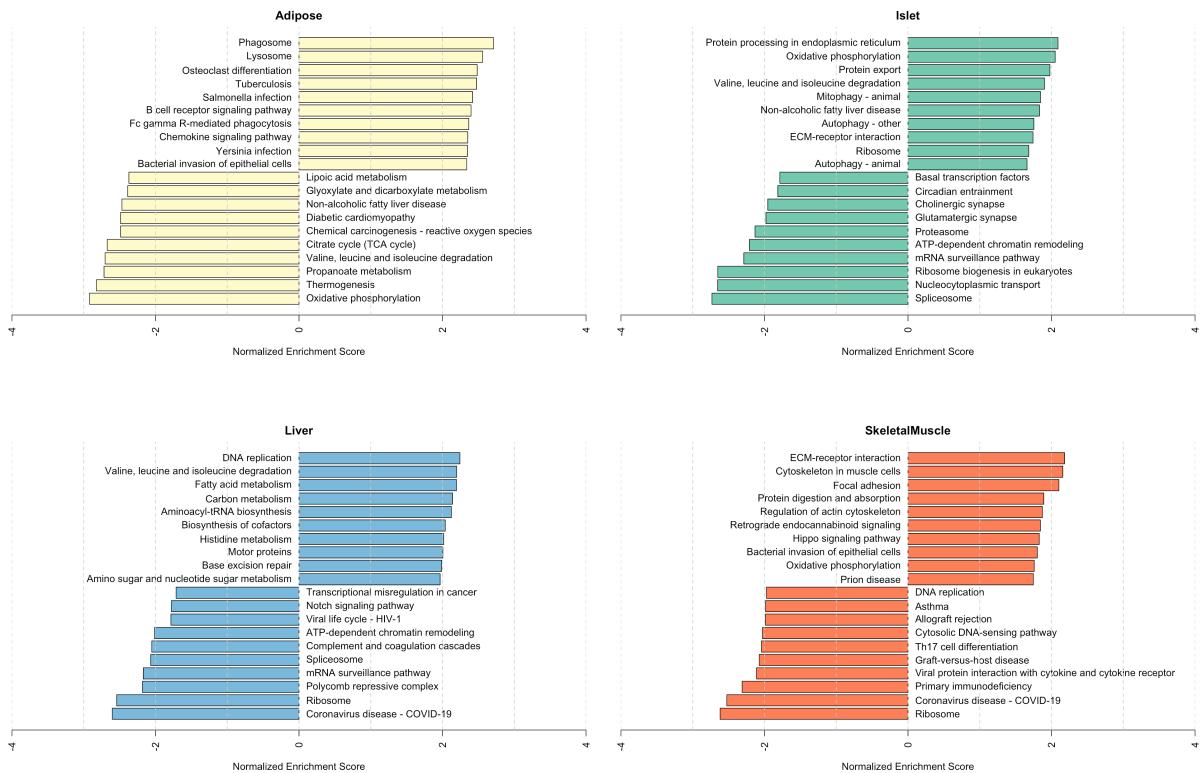


Figure 10: Bar plots showing normalized enrichment scores (NES) for KEGG pathways as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched KEGG pathway.

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Top GO term enrichments by GSEA

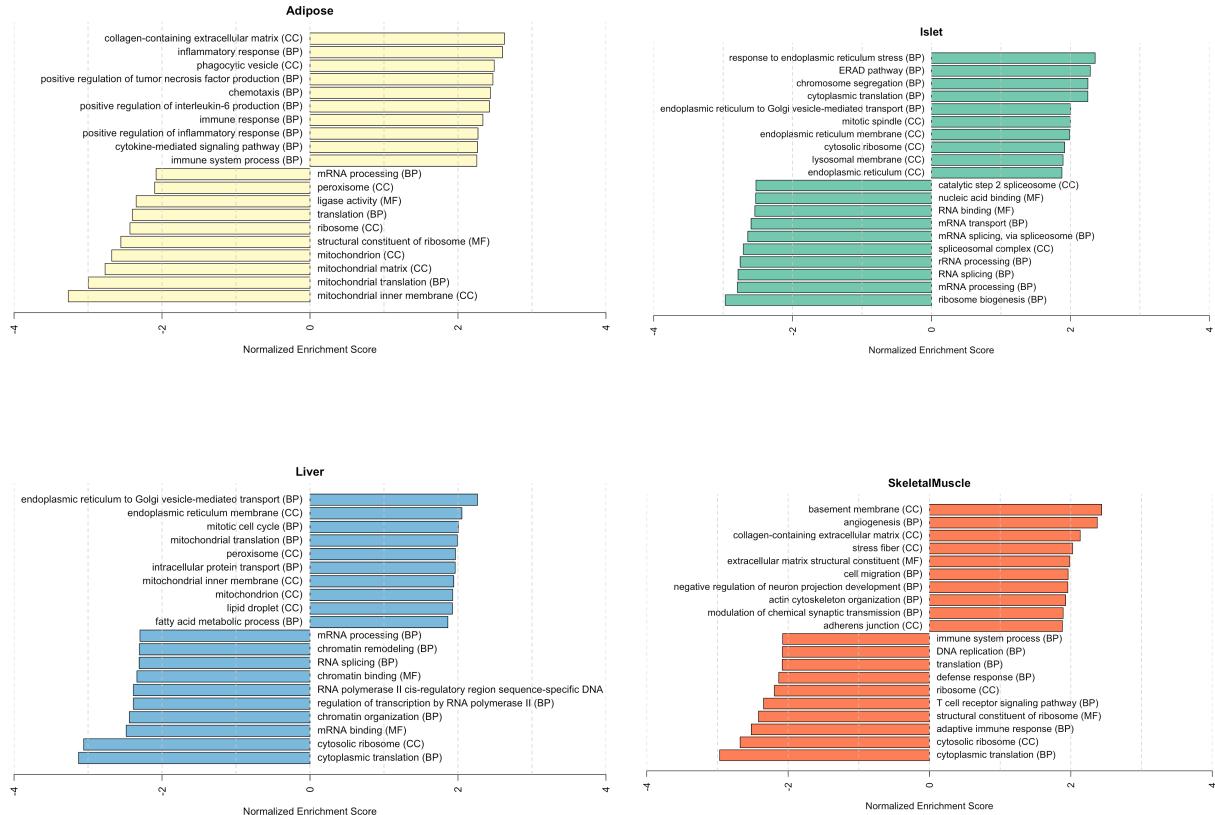


Figure 11: Bar plots showing normalized enrichment scores (NES) for GO terms as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched GO term. The letters in parentheses indicate whether the term is from the biological process ontology (BP), the molecular function ontology (MF), or the cellular compartment ontology (CC).

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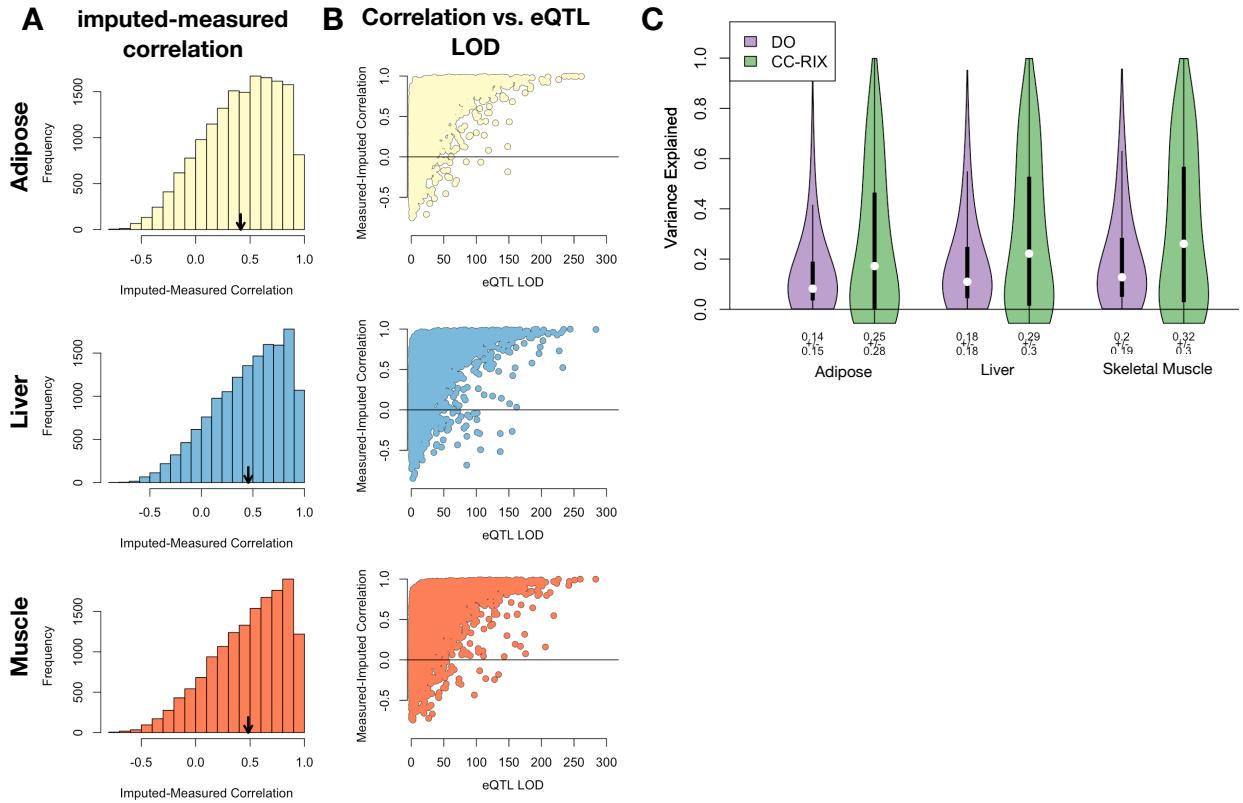


Figure 12: Validation of transcript imputation in the CC-RIX. **A.** Distributions of correlations between imputed and measured transcripts in the CC-RIX. The mean of each distribution is shown by the red line. All distributions were skewed toward positive correlations and had positive means near a Pearson correlation (r) of 0.5. **B.** The relationship between the correlation between measured and imputed expression in the CC-RIX (x-axis) and eQTL LOD score. As expected, imputations are more accurate for transcripts with strong local eQTL. **C.** Variance explained by local genotype in the DO and CC-RIX.

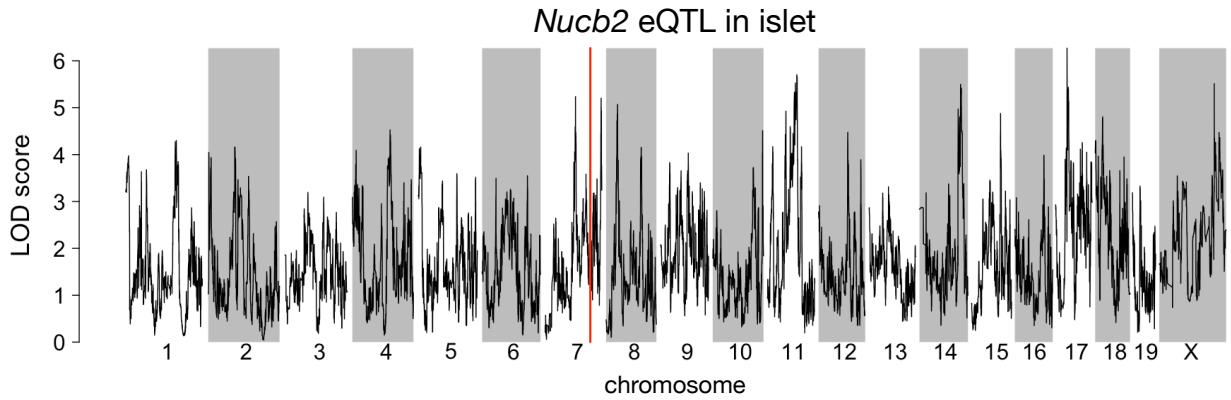


Figure 13: Regulation of *Nucb2* expression in islet. *Nucb2* is encoded on mouse chromosome 7 at 116.5 Mb (red line). In islets the heritability of *Nucb2* expression levels is 69% heritable. This LOD score trace shows that there is no local eQTL at that position, nor any strong distal eQTL anywhere else in the genome.

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