

<sup>1</sup> Transcripts with high distal heritability mediate genetic effects on  
<sup>2</sup> complex traits

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<sup>6</sup> **Abstract**

<sup>7</sup> Gene expression is an important mediator of genetic effects on phenotype. Although many genes are subject  
<sup>8</sup> to simple, local regulation, recent evidence suggests that complex distal regulation may be more important  
<sup>9</sup> in mediating trait variability. To investigate this possibility, we combined two large, data sets modeling  
<sup>10</sup> diet-induced obesity and metabolic disease in genetically diverse mice. Using a novel high-dimensional  
<sup>11</sup> mediation analysis, we identified a heritable composite transcript that explained 30% of the variation across  
<sup>12</sup> all metabolic traits. The composite transcript was interpretable in terms of enriched biological processes  
<sup>13</sup> and predicted obesity status in an independent mouse cohort as well as in human cohorts with measured  
<sup>14</sup> gene expression. Transcripts contributing most strongly to this composite mediator tended to have complex,  
<sup>15</sup> distal regulation distributed throughout the genome. These results suggest that trait-relevant variation in  
<sup>16</sup> transcription is largely distally regulated, but is nonetheless identifiable, interpretable, and translatable across  
<sup>17</sup> species.

<sup>18</sup> **Introduction**

<sup>19</sup> In the quest to understand the genetic architecture of complex traits, gene expression is an important mediator  
<sup>20</sup> between genotype and phenotype. There is ample evidence from genome-wide association studies (GWAS)  
<sup>21</sup> that regulation of gene expression accounts for the bulk of the genetic effect on complex traits, as most  
<sup>22</sup> trait-associated variants lie in gene regulatory regions<sup>1–7</sup>. It is widely assumed that these variants influence  
<sup>23</sup> local transcription, and methods such as transcriptome-wide association studies (TWAS)<sup>8–11</sup>, summary  
<sup>24</sup> data-based Mendelian randomization (SMR)<sup>10</sup>, and others capitalize on this idea to identify genes associated  
<sup>25</sup> with multiple disease traits<sup>12–15</sup>

26 Despite the great promise of these methods, explaining trait effects with local gene regulation has been more  
27 difficult than initially assumed<sup>16;17</sup>. Although trait-associated variants tend to lie in non-coding, regulatory  
28 regions, they often do not have detectable effects on gene expression<sup>16</sup> and tend not to co-localize with  
29 expression quantitative trait loci (eQTLs)<sup>17;18</sup>.

30 One possible explanation for these observations is that gene expression is not being measured in the appropriate  
31 cell types and thus local eQTLs influencing traits cannot be detected<sup>16</sup>. An alternative explanation that has  
32 been discussed in recent years is that effects of these variants are mediated not through local regulation of  
33 gene expression, but through distal regulation<sup>18–20;15</sup>.

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

38 To assess the role of wide-spread distal gene regulation on complex traits, we investigated diet-induced obesity  
39 and metabolic disease as an archetypal example. Diet-induced obesity and metabolic disease are genetically  
40 complex with hundreds of variants mapped through GWAS [REFS]. These variants are known to act through  
41 multiple tissues that interact dynamically with each other [REFS], including adipose tissue, pancreatic  
42 islets, liver, and skeletal muscle. The multi-system etiology of metabolic disease complicates mechanistic  
43 dissection of the genetic architecture, requiring large, dedicated data sets that include high-dimensional,  
44 clinically relevant phenotyping, dense genotyping in a highly recombined population, and transcriptome-wide  
45 measurements of gene expression in multiple tissues. Measuring gene expression in multiple tissues is critical  
46 to adequately assess the extent to which local gene regulation varies across multiple tissues and whether such  
47 variability might account for previous failed attempts to identify trait-relevant local eQTL. Such data sets  
48 are extremely difficult to obtain in human populations, particularly in the large numbers of subjects required  
49 for adequate statistical power. Thus, to investigate further the role of local and distal gene regulation on  
50 complex traits, we have generated an appropriate data set in a large population of diversity outbred (DO)  
51 mice<sup>21</sup> in a population model of diet-induced obesity and metabolic disease<sup>12</sup>.

52 The DO mice were derived from eight inbred founder mouse strains, five classical lab strains, and three  
53 strains more recently derived from wild mice<sup>21</sup>. They represent three subspecies of mouse *Mus musculus*  
54 *domesticus*, *Mus musculus musculus*, and *Mus musculus castaneus*, and capture 90% of the known variation  
55 in laboratory mice<sup>22</sup>. They are maintained with a breeding scheme that ensures equal contributions from  
56 each founder across the genome thus rendering almost the whole genome visible to genetic inquiry<sup>21</sup>. We

57 paired clinically relevant metabolic traits from 500 DO mice [REF], including body weight, plasma levels  
58 of insulin and glucose and plasma lipids, with transcriptome-wide gene expression in four tissues related to  
59 metabolic disease: adipose tissue, pancreatic islets, liver, and skeletal muscle. Taken together, these data  
60 enable a comprehensive view into the genetic architecture of metabolic disease.

## 61 Results

### 62 Genetic variation contributed to wide phenotypic variation

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

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

77 The trait correlations (Fig. 1G) shows that most of the metabolic trait pairs were weakly correlated indicating  
78 complex relationships among the measured traits. This low level of redundancy suggests a broad sampling of  
79 multiple heritable aspects of metabolic disease including overall body weight, glucose homeostasis, pancreatic  
80 composition and liver function.

### 81 Distal Heritability Correlated with Phenotype Relevance

82 We performed eQTL analysis using R/qltl2<sup>24</sup> (Methods) and identified both local and distal eQTLs for  
83 transcripts in each of the four tissues (Supp. Fig 1). Significant local eQTLs far outnumbered distal eQTLs  
84 (Supp. Fig. 1F) and tended to be shared across tissues (Supp. Fig. 1G) whereas the few significant distal  
85 eQTLs we identified tended to be tissue-specific (Supp. Fig. 1H)

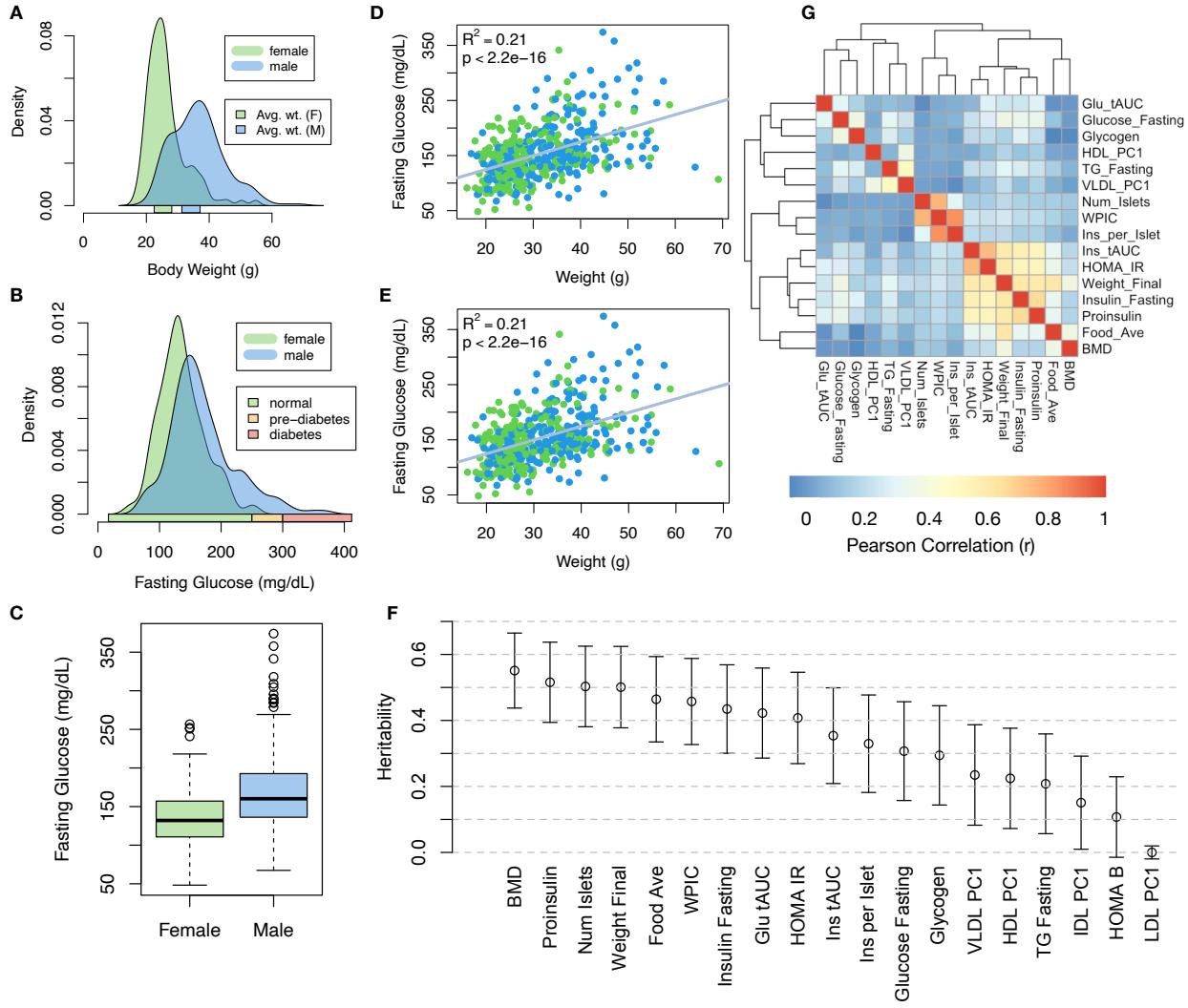


Figure 1: Clinical overview. **A.** Distributions of final body weight in the diversity outbred mice. Sex is indicated by color. The average B6 male and female adult weights at 24 weeks of age are indicated by blue and green bars on the x-axis. **B.** The distribution of final fasting glucose across 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.

We calculated the heritability of each transcript in terms of local and distal genetic factors (Methods). Overall, local and distal genetic factors contributed approximately equally to transcript abundance. In all tissues, both local and distal factors explained between 8 and 18% of the variance in the median transcript (Fig 2A). The local heritability of transcripts was negatively correlated with their trait relevance, defined as the maximum correlation of a transcript across all traits (Fig. 2B). This suggests that the more local genotype influenced transcript abundance, the less effect this variation had on the measured traits. Conversely, the

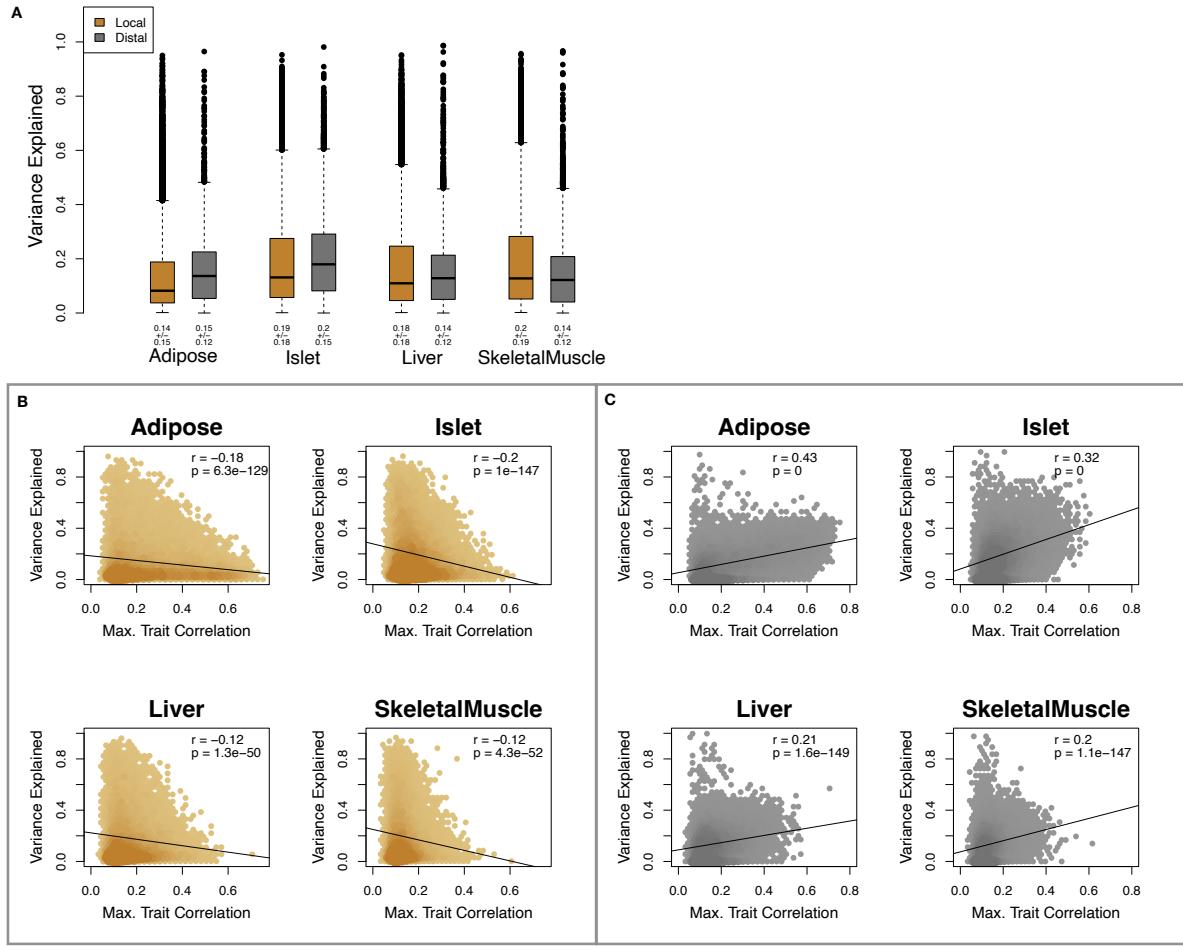


Figure 2: Transcript heritability and trait relevance. **A.** Distributions of distal and local heritability of transcripts across the four tissues. Overall local and distal factors contribute equally to transcript heritability. The relationship between **(B.)** local and **(C.)** distal heritability 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.

92 distal heritability of transcripts was positively correlated with trait relevance (Fig. 2C). That is, transcripts  
 93 that were more highly correlated with the measured traits tended to be distally, rather than locally, heritable.  
 94 Importantly, this pattern was consistent across all tissues, strongly suggesting that this is a generic finding.  
 95 This finding is consistent with previous observations that low-heritability transcripts explain more expression-  
 96 mediated disease heritability than high-heritability transcripts<sup>19</sup>. However, the positive relationship between  
 97 trait correlation and distal heritability demonstrated further that there are diffuse genetic effects throughout  
 98 the genome converging on trait-related transcripts.

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

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

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

114 which corresponds to the path coefficient in the mediation model [REF]. Operationally, HDMA is closely  
115 related to generalized canonical correlation analysis, for which provably convergent algorithms have recently  
116 been developed<sup>25</sup>. Implementation details for HDMA are available in **Supp. Methods XXX**.

117 We used high-dimensioal mediation to identify the major axis of variation in the transcriptome that mediated  
118 the effects of the genome on metabolic traits (Fig 3). Fig. 3A shows the partial correlations ( $\rho$ ) between  
119 the pairs of these composite vectors. The partial correlation between  $G_C$  and  $T_C$  was 0.42, and the partial  
120 correlation between  $T_C$  and  $P_C$  was 0.78. However, when the transcriptome was taken into account, the partial  
121 correlation between  $G_C$  and  $P_C$  was effectively zero (0.039).  $P_C$  captured 30% of the overall trait variance,  
122 and its estimated heritability was  $0.71 \pm 0.084$ , which was higher than any of the measured traits (Fig. 1F).  
123 Thus, HDMA identified a maximally heritable metabolic composite trait that was perfectly mediated by a  
124 highly heritable component of the transcriptome.

125 Standard CCA is prone to over-fitting because in any two large matrices it can be trivial to identify highly  
126 correlated composite vectors [REF]. To assess whether our implementation of HDMA was similarly prone to

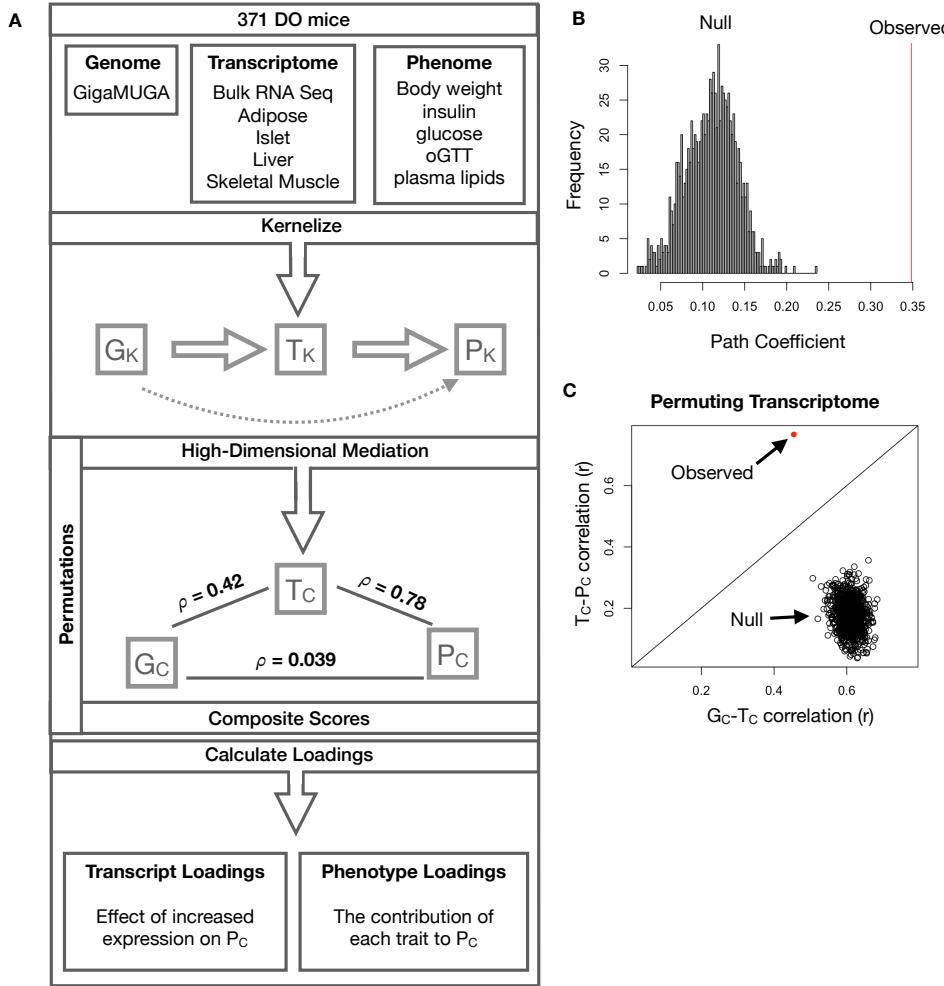


Figure 3: High-dimensional mediation. **A.** Workflow indicating major steps of high-dimensional mediation. The genotype, transcriptome, and phenotype matrices were kernelized to yield single matrices representing the relationships between all individuals for each data modality ( $G_K$  = genome kernel,  $T_K$  = 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  $\rho$  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).

127 over-fitting in a high-dimensional space, we performed permutation testing. We permuted the individual  
 128 labels on the transcriptome matrix 1000 times and recalculated the path coefficient, which is the partial  
 129 correlation of  $G_C$  and  $T_C$  multiplied by the partial correlation of  $T_C$  and  $P_C$ . This represents the path  
 130 from  $G_C$  to  $P_C$  that is mediated through  $T_C$ . The null distribution of the path coefficient is shown in Fig.  
 131 3B, and the observed path coefficient from the original data is indicated by the red line. The observed  
 132 path coefficient was well outside the null distribution generated by permutations ( $p < 10^{-16}$ ). Fig. 3C

133 illustrates this observation in more detail. Although we identified high correlations between  $G_C$  and  $T_C$ , and  
134 modest correlations between  $T_C$  and  $P_C$  in the null data (Fig 3C), these two values could not be maximized  
135 simultaneously in the null data. In contrast, the red dot shows that in the real data both the  $G_C-T_C$   
136 correlation and the  $T_C-P_C$  correlation could be maximized simultaneously suggesting that the path from  
137 genotype to phenotype through transcriptome is highly non-trivial and identifiable in this case. These results  
138 suggest that these composite vectors represent genetically determined variation in phenotype that is mediated  
139 through genetically determined variation in transcription.

140 **Body weight and insulin resistance were highly represented in the expression-mediated composite trait**

142 Each composite score is simply a weighted combination of the measured variables and the magnitude and  
143 sign of the weights, called loadings, correspond the relative importance and directionality of each variable in  
144 the composite score. The loadings of each measured trait onto  $P_C$  indicate how much each contributed to  
145 the composite phenotype. Final body weight contributed the most (Fig. 4), followed by homeostatic insulin  
146 resistance (HOMA\_IR) and fasting plasma insulin levels (Insulin\_Fasting). We can thus interpret  $P_C$  as  
147 an index of metabolic disease (Fig. 4B). Individuals with high values of  $P_C$  have a higher metabolic index  
148 and greater metabolic disease, including higher body weight and higher insulin resistance. We refer to  $P_C$  as  
149 the metabolic index going forward. Traits contributing the least to the metabolic index were measures of  
150 cholesterol and pancreas composition. Thus, when we interpret the transcriptomic signature identified by  
151 HDMA, we are explaining primarily the transcriptional mediation of body weight and insulin resistance, as  
152 opposed to cholesterol measurements.

153 **High-loading transcripts have low local heritability, high distal heritability, and were linked  
154 mechanistically to obesity**

155 We interpreted large loadings onto transcripts as indicating strong mediation of the effect of genetics on  
156 metabolic index. Large positive loadings indicate that higher expression was associated with a higher  
157 metabolic index (i.e. higher risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). Conversely,  
158 large negative loadings indicate that high expression of these transcripts was associated with a lower metabolic  
159 index (i.e. lower risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). We used gene set  
160 enrichment analysis (GSEA)<sup>26;27</sup> to look for biological processes and pathways that were enriched at the top  
161 and bottom of this list (Methods).

162 In adipose tissue, both GO processes and KEGG pathway enrichments pointed to an axis of inflammation

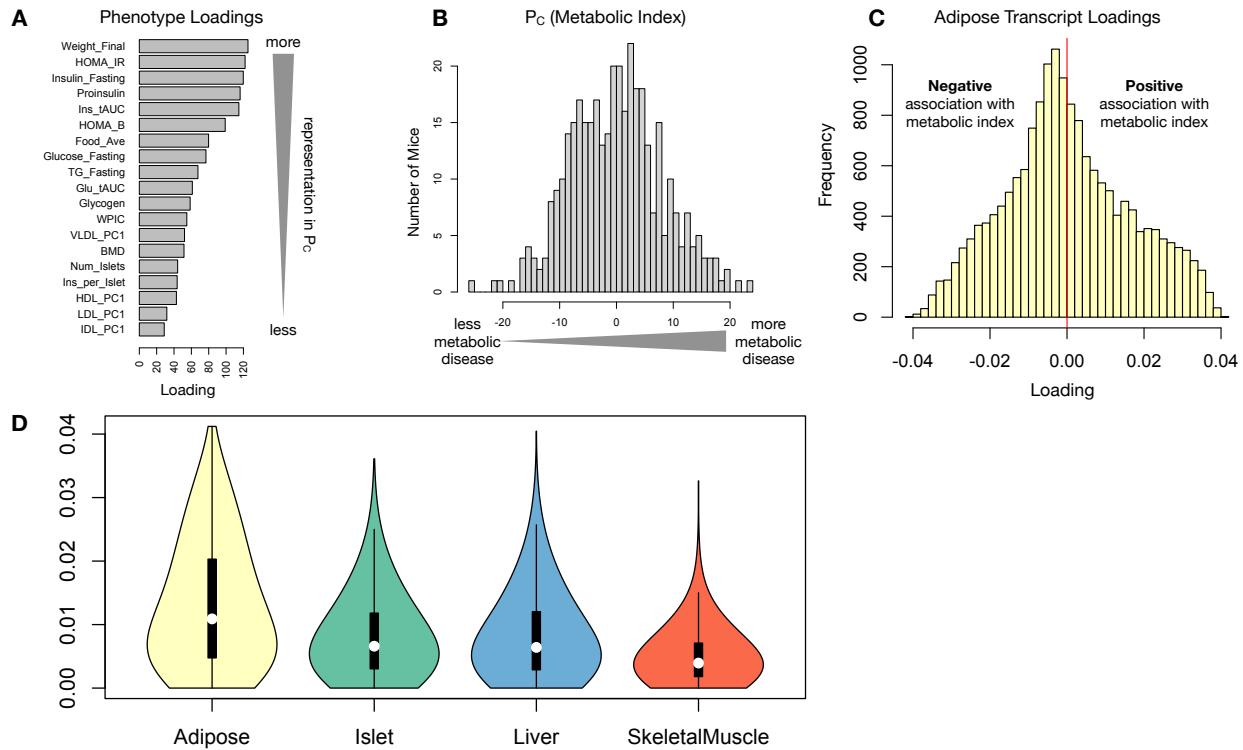


Figure 4: Interpretation of loadings. **A.** Loadings across traits. Body weight and insulin resistance contributed the most to the composite trait. **B.** Phenotype scores across individuals. Individuals with large positive phenotype scores had higher body weight and insulin resistance than average. 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.

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

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

Transcripts associated with the citric acid (TCA) cycle as well as the catabolism of the branched-chain amino acids (BCAA) (valine, leucine, and isoleucine) were strongly enriched with negative loadings in adipose

173 tissue (Supp. Fig. 3). Expression of genes in both pathways (for which there is some overlap) has been  
174 previously associated with insulin sensitivity<sup>12;28;29</sup>, suggesting that heritable variation in regulation of these  
175 pathways may influence risk of insulin resistance.

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

186 We performed a literature search for the genes in each of these groups along with the terms “diabetes”,  
187 “obesity”, and the name of the expressing tissue to determine whether any of these genes had previous  
188 associations with metabolic disease in the literature (Methods). Multiple genes in each group had been  
189 previously associated with obesity and diabetes (Fig. 5 bolded gene names). Genes with high loadings were  
190 most highly enriched for previous literature support. They were 2.375 more likely than TWAS hits and 3.8  
191 times more likely than genes with high local heritability to be previously associated with obesity or diabetes.

## 192 **Tissue-specific transcriptional programs were associated with metabolic traits**

193 Clustering of transcripts with top loadings in each tissue showed tissue-specific functional modules associated  
194 with obesity and insulin resistance (Fig. 6A) (Methods). The clustering highlights the importance of immune  
195 activation particularly in adipose tissue. Except for the “mitosis” cluster, which had large positive loadings  
196 in three of the four tissues, all clusters were strongly loaded in only one or two tissues. For example, the lipid  
197 metabolism cluster was loaded most heavily in liver. The positive loadings suggest that high expression of  
198 these genes particularly in the liver was associated with increased metabolic disease. This cluster included  
199 the gene *Pparg*, whose primary role is in the adipose tissue where it is considered a master regulator of  
200 adipogenesis<sup>30</sup>. Agonists of *Pparg*, such as thiazolidinediones, are FDA-approved to treat type II diabetes,  
201 and reduce inflammation and adipose hypertrophy<sup>30</sup>. Consistent with this role, the loading for *Pparg* in  
202 adipose tissue was negative, suggesting that higher expression was associated with leaner mice (Fig. 6B). In  
203 contrast, *Pparg* had a large positive loading in liver, where it is known to play a role in the development of

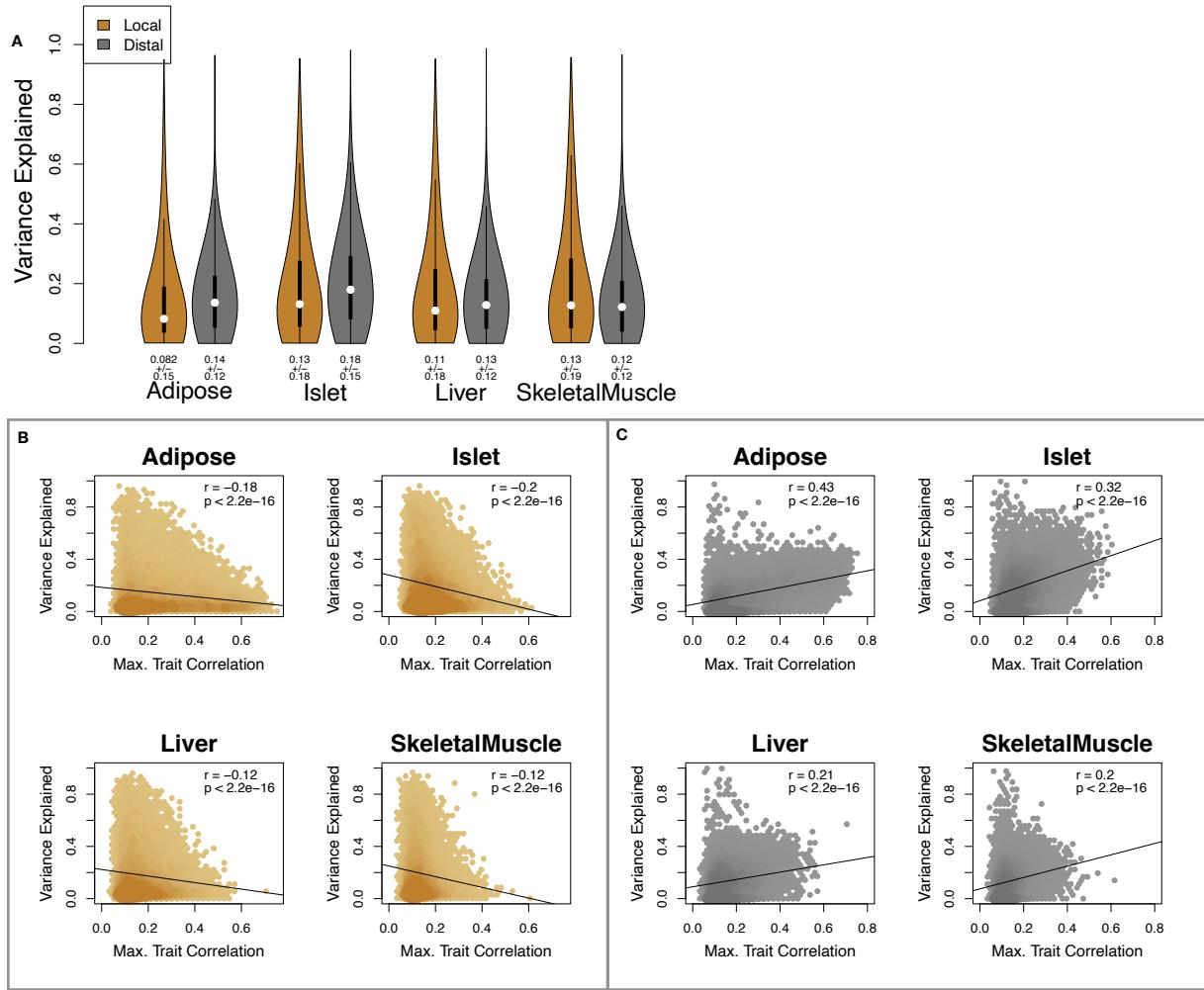


Figure 5: Transcripts with high loadings have high distal heritability and literature support. Each panel has a bar plot showing the loadings of transcripts selected by different criteria. Bar color indicates the tissue of origin. The heat map shows the local (L - left) and distal (D - right) heritability of each transcript. **A.** Loadings for the 10 transcripts with the largest positive loadings and the 10 transcripts with the largest negative loadings for each tissue. **B.** Loadings of TWAS candidates with the 10 largest positive correlations with traits and the largest negative correlations with traits across all four tissues. **C.** The transcripts with the largest local heritability (top 20) across all four tissues.

- 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<sup>31;32</sup>. Overexpression of *Pparg* in the livers of mice with a *Ppara* knockout, causes upregulation of genes involved in adipogenesis<sup>33</sup>. 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<sup>34;35</sup>.
- 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

211 high suggesting it is complexly regulated and has sufficient variation in this population to drive variation in  
 212 phenotype. Both local and distal heritability of *Pparg* in the islet are relatively high, but the loading is low,  
 213 suggesting that variability of expression in the islet does not drive variation in metabolic index. These results  
 214 highlight the importance of tissue context when investigating the role of heritable transcript variability in  
 215 driving phenotype.

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

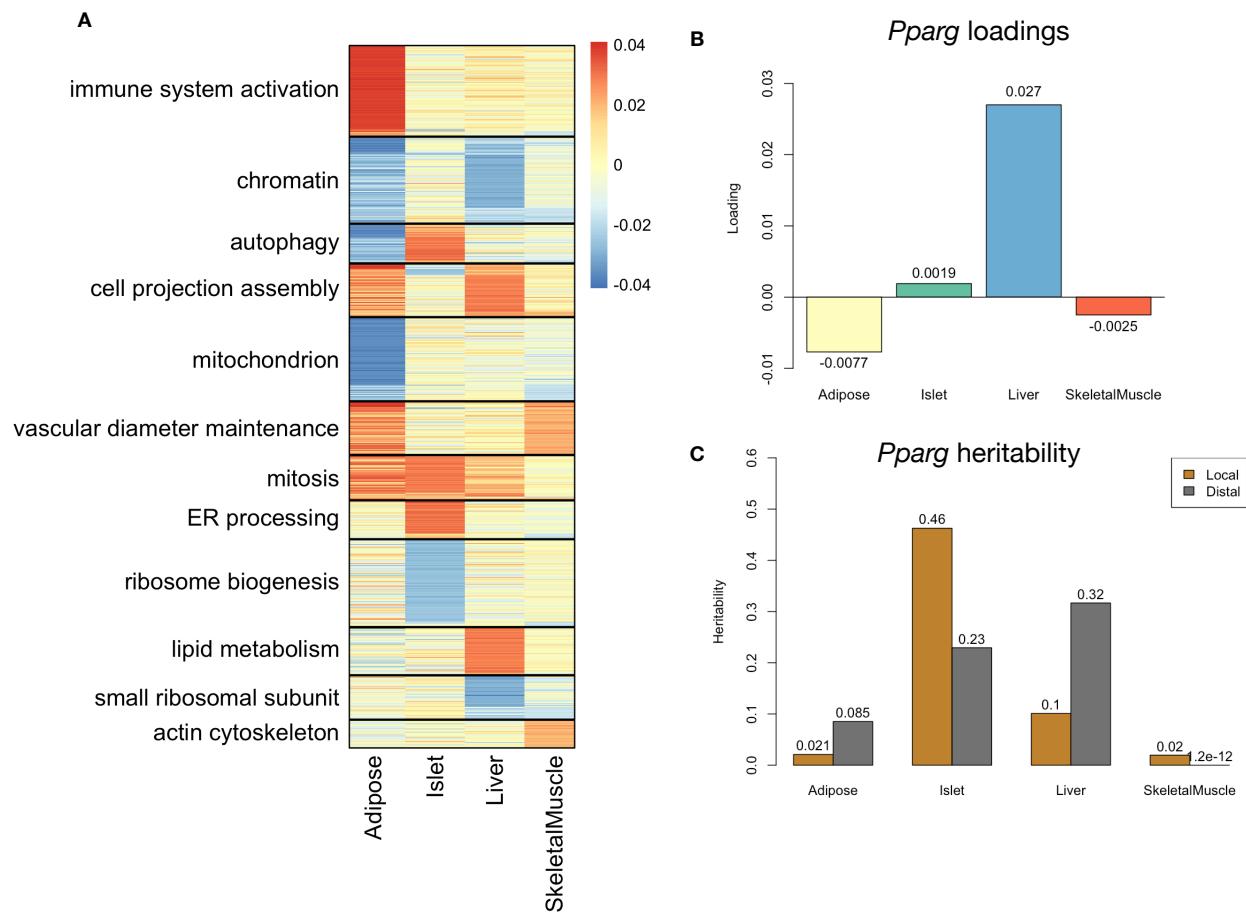


Figure 6: Tissue-specific transcriptional programs were associated with obesity and insulin resistance. **A** Heat map showing the loadings of all transcripts with loadings greater than 2.5 standard deviations from the mean in any tissue. The heat map was clustered using k medoid clustering. Functional 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.

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

218 To test whether the transcript loadings identified in the DO could be translated to another population, we  
 219 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 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.

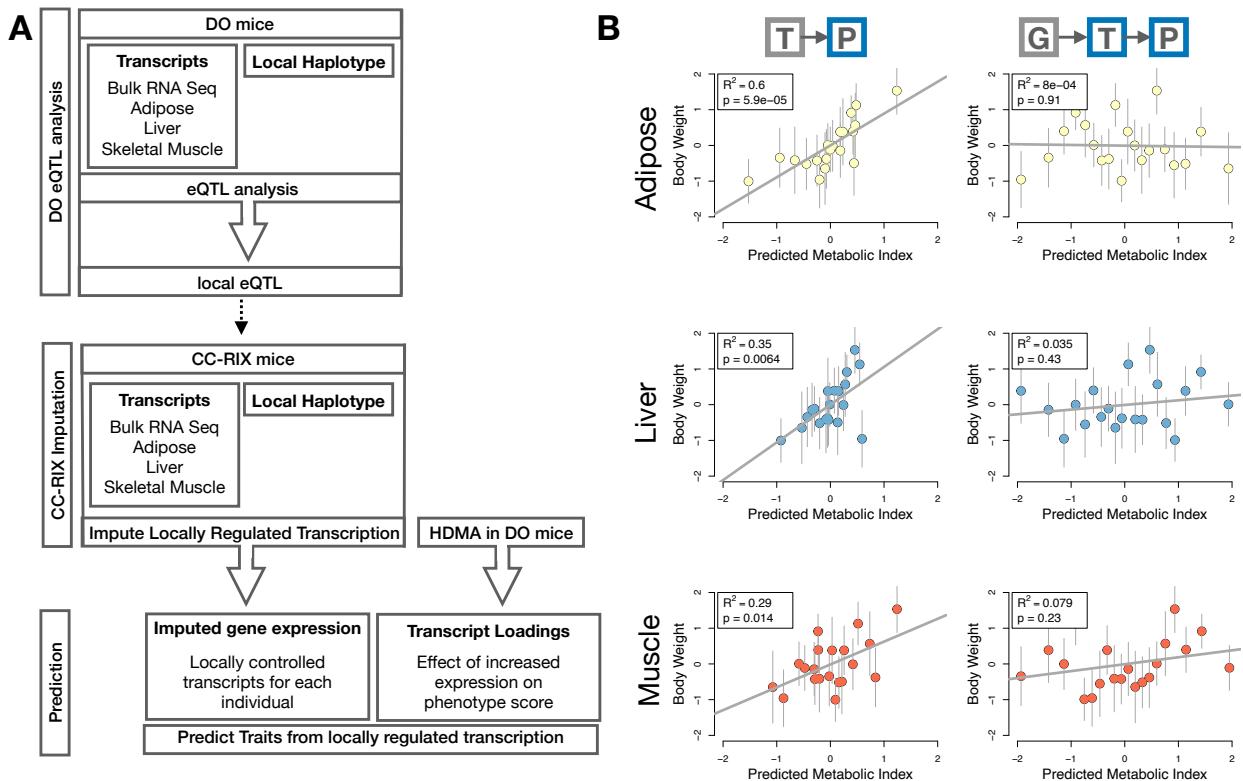


Figure 7: Transcription, but not local genotype, predicts phenotype in the CC-RIX. **A.** Workflow showing procedure for translating HDMA results to an independent population of mice. **B.** Relationships between the predicted metabolic index and measured body weight. The left column shows the predictions 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.

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

232 same eight founder strains and so carry the same alleles throughout the genome. We imputed gene expression  
233 in the CC-RIX using local genotype and were able to estimate variation in gene transcription robustly (Supp.  
234 Fig. 4). However, these imputed values failed to predict body weight in the CC-RIX when weighted with the  
235 loadings from HDMA. (Fig. 7B right column). This result suggests that local regulation of gene expression is  
236 not the primary factor driving heritability of complex traits, consistent with our findings in the DO population  
237 that distal heritability was a major driver of trait-relevant variation and that high-loading transcripts had  
238 comparatively high distal and low local heritability.

239 **Distally heritable transcriptomic signatures reflected variation in composition of adipose tissue  
240 and islets**

241 The interpretation of global genetic influences on gene expression and phenotype is potentially more challenging  
242 than the interpretation and translation of local genetic influences, as genetic effects cannot be localized to  
243 individual gene variants or transcripts. However, there are global patterns across the loadings that can  
244 inform mechanism. For example, heritable variation in cell type composition can be derived from transcript  
245 loadings. We observed above that immune activation in the adipose tissues was an important driver of obesity  
246 in the DO population. To determine whether this is reflected as an increase in macrophages in adipose  
247 tissue, we compared loadings of cell-type specific genes in adipose tissue (Methods). The mean loading  
248 of macrophage-specific genes was significantly greater than 0 (Fig. 8A), indicating that obese mice were  
249 genetically predisposed to have high levels of macrophage infiltration in adipose tissue in response to the  
250 high-fat, high-sugar diet.

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

258 Notably, the loadings for pancreatic beta cell-type specific loadings was not significantly different from zero.  
259 This is not necessarily reflective of the function of the beta cells in the obese mice, but rather suggests that  
260 any variation in the number of beta cells in these mice was unrelated to obesity and insulin resistance. This  
261 is further consistent with the islet composition traits having small loadings in the phenome score (Fig. 4).

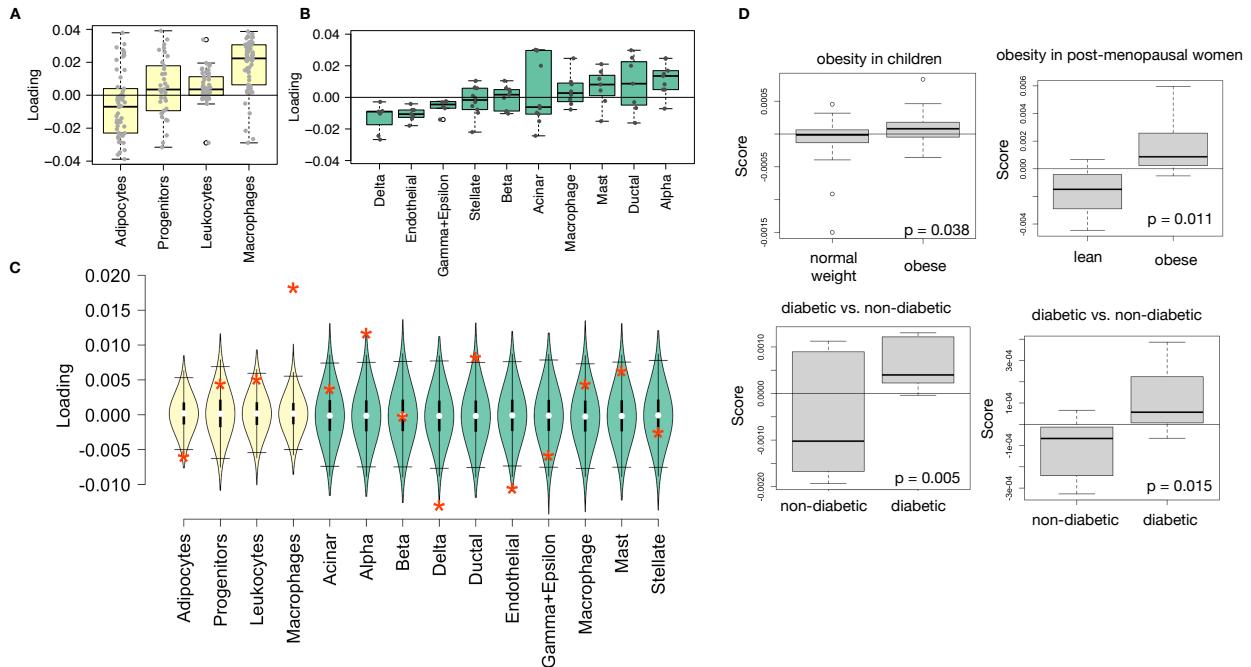


Figure 8: HDMA results translate to humans. **A.** Distribution of loadings for cell-type-specific transcripts in adipose tissue. **B.** Distribution of loadings for cell-type-specific transcripts in pancreatic islets (green). **C.** Null distributions for the mean loading of randomly selected transcripts 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.

## 262 Heritable transcriptomic signatures translated to human disease

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

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

274 **Targeting gene signatures**

275 Another potential application of the transcript loading landscape is in ranking potential drug candidates  
276 for the treatment of metabolic disease. Although high-loading transcripts may be good candidates for  
277 understanding specific biology related to obesity, the transcriptome overall is highly interconnected and  
278 redundant, and focusing on individual transcripts for treatment may be less effective than using broader  
279 transcriptomic signatures that capture the emergent biology [cite or remove]. The ConnectivityMap (CMAP)  
280 database<sup>36</sup> developed by the Broad Institute allows us to query thousands of compounds that reverse or  
281 enhance the extreme ends of transcriptomic signatures in multiple different cell types. By identifying drugs  
282 that reverse pathogenic transcriptomic signatures, we can potentially identify compounds that have favorable  
283 effects on gene expression.

284 To test this hypothesis, we queried the CMAP database through the CLUE online query tool (<https://clue.io/query/>, version 1.1.1.43) (Methods). We identified top anti-correlated hits across all cell types. To  
285 get more tissue-specific results, we also looked at top results in cell types that most closely resembled our  
286 tissues. We looked at results in adipocytes (ASC) as well as pancreatic tumor cells (YAPC) regardless of *p*  
287 value (Supplemental Figure XXX and XXX).

288 Looking broadly across cell types, the notable top hits from the adipose tissue loadings included mTOR  
289 inhibitors and glucocorticoid agonists (Supplemental Figure XXX). It is thought that metformin, which  
290 is commonly used to improve glycemic control, acts, at least in part, by inhibiting mTOR signaling<sup>37;38</sup>.  
291 However, long-term use of other mTOR inhibitors, such as rapamycin, are known to cause insulin resistance  
292 and  $\beta$ -cell toxicity<sup>38–40</sup>. Glucocorticoids are used to reduce inflammation, which was a prominent signature  
293 in the adipose tissues, but these drugs also promote hyperglycemia and diabetes<sup>41;42</sup>. Accute treatment  
294 with glucocorticoids has further been shown to reduce thermogenesis in rodent adipocytes<sup>43–45</sup>, but increase  
295 thermogenesis in human adipocytes<sup>46;47</sup>. Thus, the pathways identified by CMAP across all cell types were  
296 highly related to the transcript loading profiles, but the relationship was not a simple reversal.

297 The top hit for the adipose composite transcript in CMAP adipocytes was a PARP inhibitor (Supplemental  
298 Figure XXXB). PARPs play a role in lipid metabolism and are involved in the development of obesity and  
299 diabetes<sup>48</sup>. PARP1 inhibition increases mitochondrial biogenesis<sup>49</sup>. Inhibition of PARP1 activity can further  
300 prevent necrosis in favor of the less inflammatory apoptosis<sup>50</sup>, thereby potentially reducing inflammation in  
301 stressed adipocytes. Other notable hits among the top 20 were BTK inhibitors, which have been observed  
302 to suppress inflammation and improve insulin resistance<sup>51</sup> as well as to reduce insulin antibodies in type I  
303 diabetes<sup>52</sup>. IKK inhibitors have been shown to improve glucose control in type II diabetes<sup>53;54</sup>.

305 Among the top most significant hits for the transcript loadings from pancreatic islets (Fig. XXX), was  
306 suppression of T cell receptor signaling, which is known to be involved in Type 1 diabetes<sup>55</sup>, as well as  
307 TNFR1, which has been associated with mortality in diabetes patients<sup>56</sup>. Suppression of NOD1/2 signaling  
308 was also among the top hits. NOD1 and 2 sense ER stress<sup>57;58</sup>, which is associated with  $\beta$ -cell death in type  
309 1 and type 2 diabetes<sup>59</sup>. This cell death process is dependent on NOD1/2 signaling<sup>57</sup>, although the specifics  
310 have not yet been worked out.

311 We also looked specifically at hits in pancreatic tumor cells (YAPC) regardless of significance level. Hits  
312 in this list included widely used diabetes drugs, such as sulfonylureas, PPAR receptor agonists, and insulin  
313 sensitizers. Rosiglitazone is a PPAR- $\gamma$  agonist and was one of the most prescribed drugs for type 2 diabetes  
314 before its use was reduced due to cardiac side-effects<sup>60</sup>. Sulfonylureas are another commonly prescribed drug  
315 class for type 2 diabetes, but also have notable side effects including hypoglycemia and accelerated  $\beta$ -cell  
316 death<sup>61</sup>.

## 317 Discussion

318 Here we used a novel high-dimensional mediation analysis (HDMA) to investigate the relative contributions of  
319 local and distal gene regulation to heritable trait variation in a genetically diverse mouse model of diet-induced  
320 obesity and metabolic disease. We identified tissue-specific composite transcripts mediating the effect of  
321 genetic background on metabolic traits. Transcripts contributing most strongly to these composite transcripts  
322 were distally heritable. These composite transcripts, but not local eQTL, were able to predict obesity in  
323 an independent mouse population with divergent allelic structure. Moreover, the composite transcript from  
324 adipose tissue predicted obesity and diabetes status in human cohorts with measured adipose gene expression.  
325 Taken together, these results support the hypothesis that gene expression mediating the effect of genetic  
326 background on phenotype is primarily distally regulated, and that the gene regulatory networks influencing  
327 metabolic disease are conserved across mice and humans.

328 It has frequently been assumed that gene regulation in *cis* is the primary driver of genetically associated  
329 trait variation, but attempts to use local gene regulation to explain phenotypic variation have had limited  
330 success<sup>16;17</sup>. In recent years, evidence has mounted that distal gene regulation may be an important mediator  
331 of trait heritability<sup>19;18;62</sup>. It has been observed that transcripts with high local heritability explain less  
332 expression-mediated disease heritability than transcripts with low local heritability<sup>19</sup>. Consistent with this  
333 observation, genes located near GWAS hits tend to be complexly regulated<sup>18</sup>. They also tend to be enriched  
334 with functional annotations, in contrast to genes with simple local regulation, which tend to be depleted  
335 of functional annotations suggesting they are less likely to be directly involved in disease traits<sup>18</sup>. These

336 observations are consistent with principles of robustness in complex systems<sup>63–65</sup>. If a transcript were both  
337 important to a trait and subject to strong local regulation, a population would be susceptible to extremes  
338 in phenotype that might frequently cross the threshold to disease. Indeed, strong disruption of highly  
339 trait-relevant genes is the cause of Mendelian disease.

340 The composite transcripts we identified here supported the hypothesis that distally regulated gene expression  
341 is the dominant mediator of trait variation. Transcript loadings (the degree to which they contributed to  
342 the composite transcript) were negatively correlated with local heritability and positively correlated with  
343 distal heritability. The most strongly loaded transcripts were enriched for functional annotations associated  
344 with metabolic disease. These distally regulated composite transcripts were highly heritable and explained a  
345 high proportion of disease risk, further supporting their role as mediators. The composite transcripts were  
346 moreover able to predict obesity in an independent cohort of mice whereas models using local eQTL only  
347 could not. Together these observations suggest that distal gene regulation was the dominant mode through  
348 which gene expression mediated the effect of genetic background on complex metabolic traits.

349 Identification of this distally heritable signature depended on the high-dimensional approach we used. Because  
350 HDMA uses a kinship matrix rather than genotypes at individual loci, it allows for arbitrarily complex  
351 gene regulation, as well as the interconnectedness and redundancy of the transcriptome. This feature also  
352 means that HDMA assumes that traits are highly polygenic and distributed across the genome. In contrast,  
353 one-dimensional, univariate approaches assume a large, localized genetic effect. Thus, the HDMA approach  
354 is consistent with the omnigenic model of complex traits which posits that complex traits are massively  
355 polygenic and that their heritability is spread out across the genome<sup>66</sup>. In the omnigenic model, genes  
356 are classified either as “core genes,” which directly impinge on the trait, or “peripheral genes,” which are  
357 not directly trait-related, but influence core genes through the complex gene regulatory network. HDMA  
358 explicitly models a central proposal of the omnigenic model which posits that once the expression of the  
359 core genes (i.e. trait-mediating genes) is accounted for, there should be no residual correlation between the  
360 genome and the phenotype. Here, when the composite transcript was taken into account there was no residual  
361 correlation between the composite genome and composite phenotype (Fig. 3A).

362 Thus, the composite transcript is essentially a weighted vector with larger weights (loadings) indicating higher  
363 “core-ness” of a transcript. There was no clear demarcation between the core and peripheral genes in loading  
364 magnitude, but we do not necessarily expect a clear separation given the complexity of gene regulation and  
365 the genotype-phenotype map<sup>67</sup>. Still, the transcripts with the largest loadings had high distal heritability,  
366 low local heritability, and were enriched for biological processes related to metabolic traits, as we would  
367 predict for core genes.

368 An extension of the omnigenic model proposed that most heritability of complex traits is driven by weak  
369 distal eQTLs<sup>62</sup>. This is consistent with what we observed here. The transcripts with the largest loadings  
370 were strongly distally regulated and only weakly locally regulated, suggesting that distal gene regulation  
371 plays a primary role in driving heritable trait variation. We saw further that the patterns of distal heritability  
372 were complex spread across the genome. Even for transcripts whose expression was strongly regulated by  
373 distal factors, these factors were multiple and spread across the genome. For example, *Nucb2*, was a strongly  
374 mediating transcript in islet and was also strongly distally regulated (66% distal heritability) (Fig. 5). This  
375 gene is expressed in pancreatic  $\beta$  cells and is involved in insulin and glucagon release<sup>68–70</sup>. Although its  
376 transcription was highly heritable in islets, that regulation was distributed across the genome, with no clear  
377 distal eQTL (Supp. Fig. 5). Thus, although distal regulation of some genes may be strong, this regulation is  
378 likely to be highly complex and not easily localized.

379 The composite transcripts identified by HDMA are richly interpretable in both tissue- and gene-specific  
380 manners. The transcripts with the strongest loadings were enriched in biological functions previously known  
381 to be involved in the pathogenesis of metabolic disease, such as inflammation in adipose tissue. That these  
382 processes were identified in this analysis suggests that they have a heritable component, and that some  
383 individuals are genetically susceptible to greater adipose inflammation on a high-fat, high-sugar diet.

384 Individual transcripts also demonstrated biologically interpretable, tissue-specific patterns. We highlighted  
385 *Pparg*, which is known to be protective in adipose tissue<sup>30</sup> where it was negatively loaded, and harmful in the  
386 liver<sup>31–35</sup>, where it was positively loaded. Such granular patterns may be useful in generating hypotheses for  
387 further testing, and prioritizing genes as therapeutic targets. The tissue-specific nature of the loadings also  
388 may provide clues to tissue-specific effects, or side effects, of targeting particular genes system-wide, since  
389 antagonists of *Pparg* may reduce fatty liver disease, but exacerbate adipose tissue inflammation.

390 We showed further that these composite transcripts can be used as weighted vectors in multiple types of  
391 analysis, such as drug prioritization using gene set enrichment analysis (GSEA) and the CMAP database. In  
392 particular, the CMAP analysis identified drugs which have been demonstrated to reverse insulin resistance  
393 and other aspects of metabolic disease. This finding supports the causal role of these gene signatures in  
394 pathogenesis of metabolic disease and thus their utility in prioritizing drugs and gene targets as therapeutics.

395 Another useful application of the composite transcripts is to pair them with cell-type specific genes to generate  
396 hypotheses about cell composition in individual tissues. Combining the multi-tissue, transcriptome-wide  
397 weighted vectors with public databases and data sets thus provides a path for generating a wide range of  
398 testable hypotheses. Moreover, each data set presented here was derived from human tissues or cell lines,

399 thus demonstrating the translatability of these results. That the mouse-derived adipose composite transcript  
400 was able to classify human adipose gene expression in terms of obesity and diabetes status further supports  
401 the direct translatability of these findings, the utility of HDMA, and the continued importance of mouse  
402 models of human disease in which it is possible to obtain complete transcriptomes in multiple tissues across  
403 large numbers of individuals.

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

410 **Data Availability**

411 Here we tell people where to find the data

412 **Acknowledgements**

413 Here we thank people

414 **Supplemental Figures**

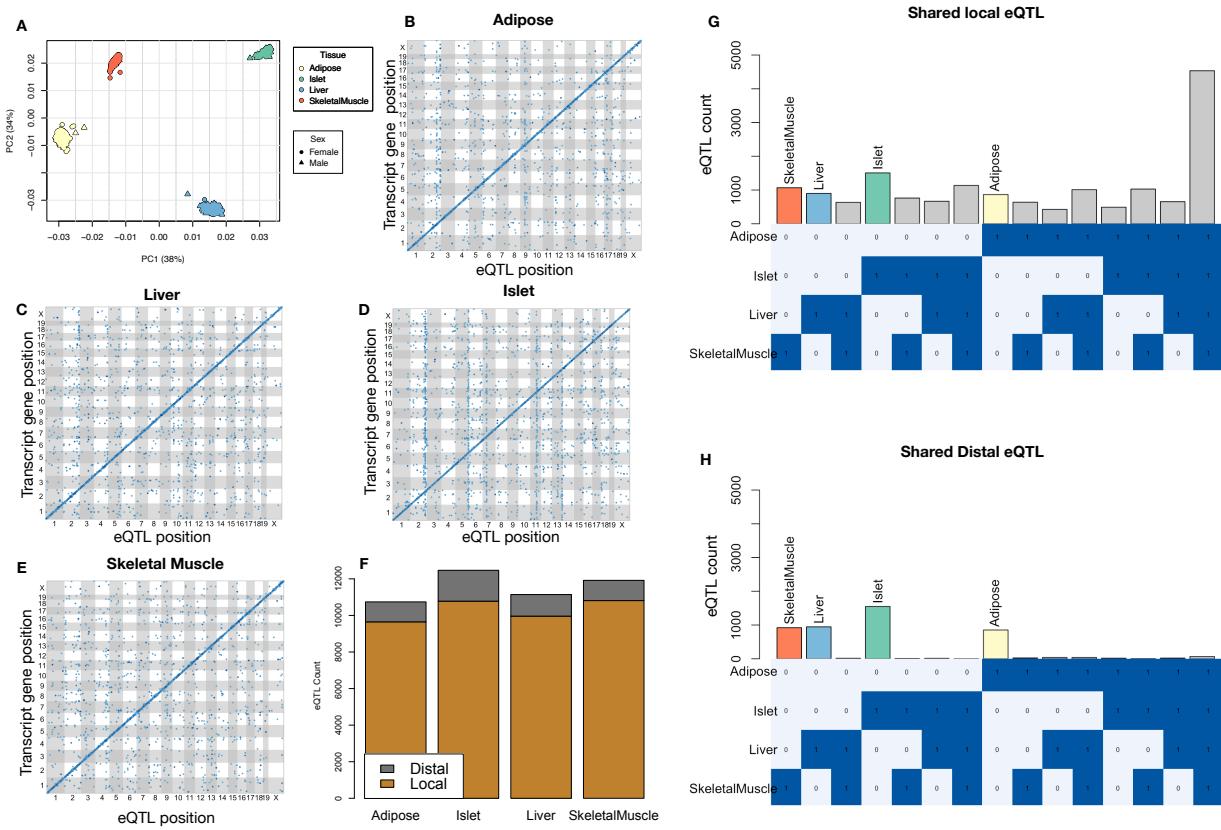


Figure 9: Overview of eQTL analysis in DO mice. **A.** RNA seq samples from the four different tissues clustered by tissue. **B.-E.** eQTL maps are shown for each tissue. The *x*-axis shows the position of the mapped eQTL, and the *y*-axis shows the physical position of the gene encoding each mapped 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

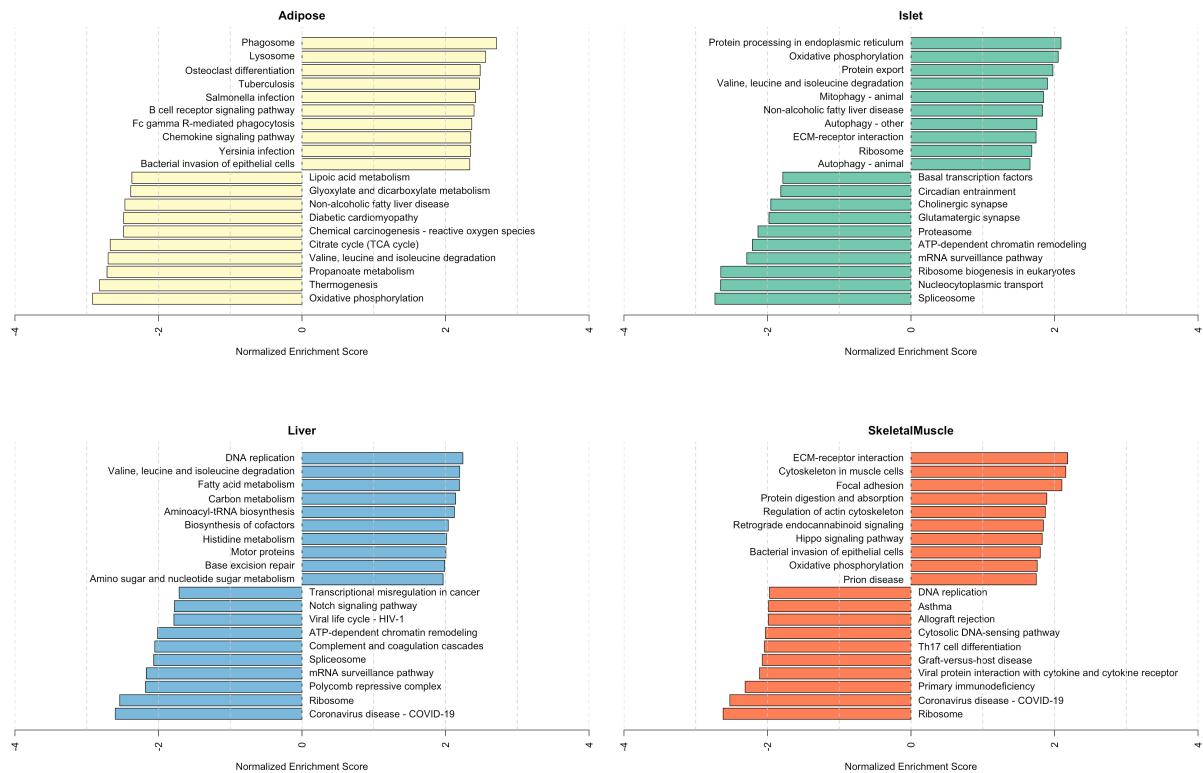


Figure 10: Bar plots showing normalized enrichment scores (NES) for KEGG pathways as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched KEGG pathway.

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## Top GO term enrichments by GSEA

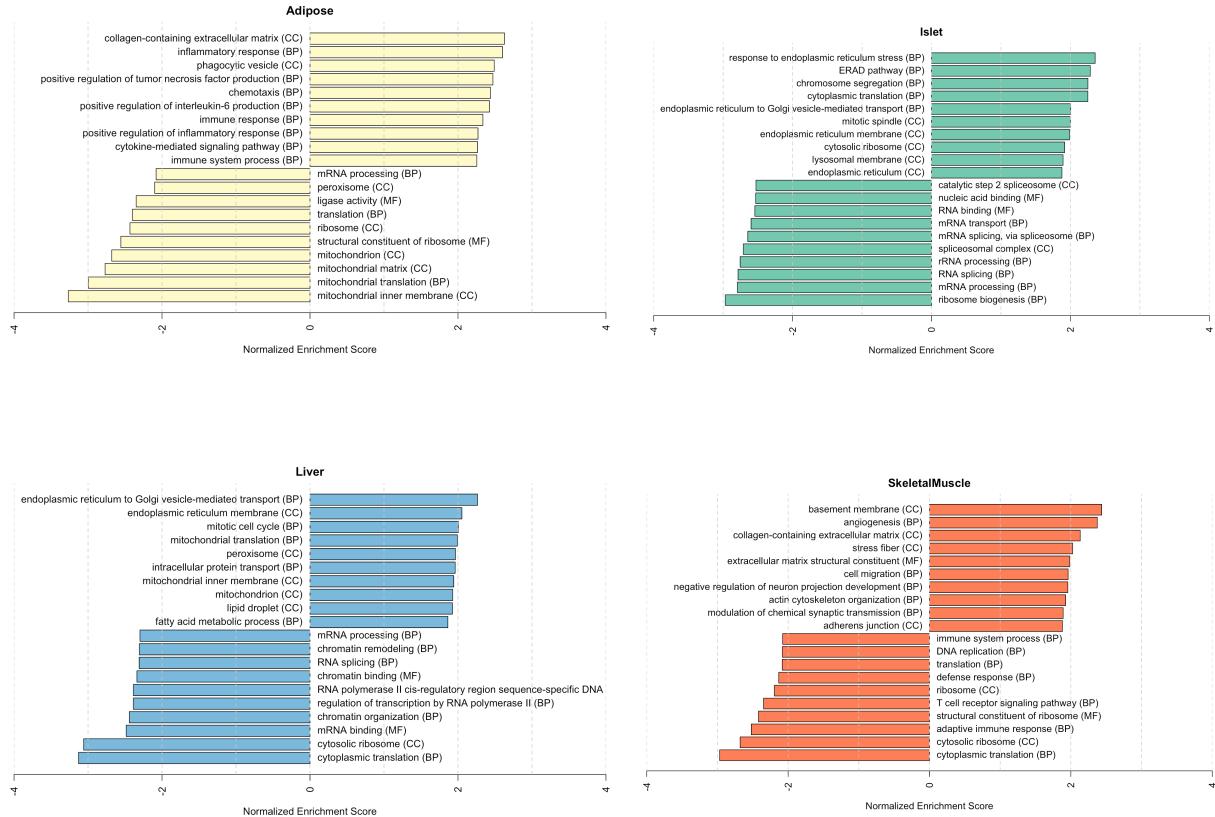


Figure 11: Bar plots showing normalized enrichment scores (NES) for GO terms as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched GO term. The letters 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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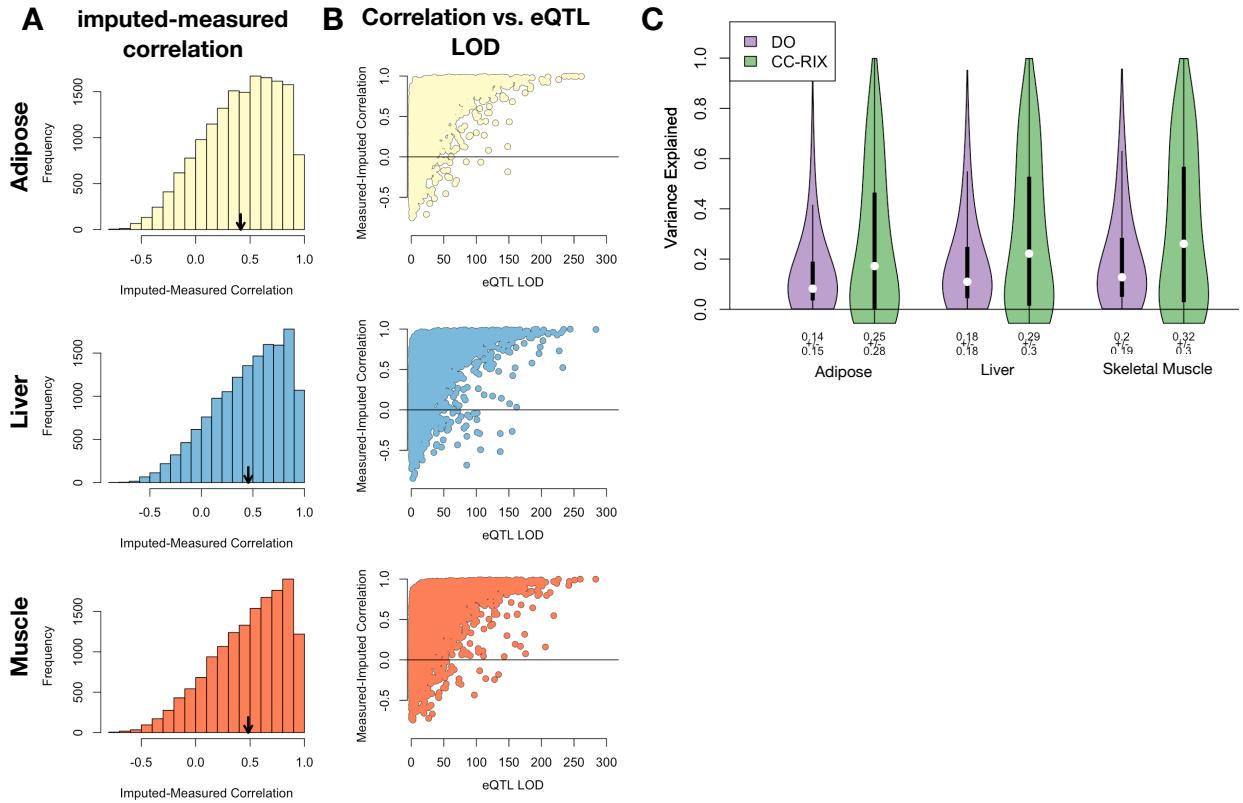


Figure 12: Validation of transcript imputation in the CC-RIX. **A.** Distributions of correlations between imputed and measured transcripts in the CC-RIX. The mean of each distribution is shown by the red line. All distributions were skewed toward positive correlations and had positive means near a Pearson 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.

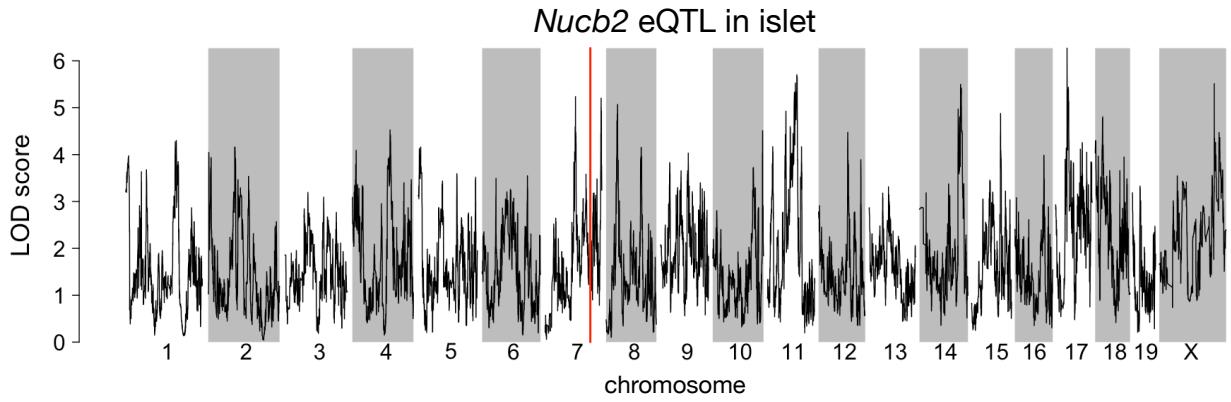


Figure 13: Regulation of *Nucb2* expression in islet. *Nucb2* is encoded on mouse chromosome 7 at 116.5 Mb (red line). In islets the heritability of *Nucb2* expression levels is 69% heritable. This LOD score trace shows that there is no local eQTL at that position, nor any strong distal eQTL anywhere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

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