

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

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7 **Abstract**

8 Although many genes are subject to local regulation, recent evidence suggests that complex distal regulation
9 may be more important in mediating phenotypic variability. To assess the role of distal gene regulation in
10 complex traits, we combined multi-tissue transcriptomes with physiological outcomes to model diet-induced
11 obesity and metabolic disease in a population of 371 Diversity Outbred mice. Using a novel high-dimensional
12 mediation analysis, we identified a composite transcriptome signature that summarized genetic effects on
13 gene expression and explained 30% of the variation across all metabolic traits. The signature was heritable,
14 interpretable in biological terms, and predicted obesity status from gene expression in an independently
15 derived mouse cohort and multiple human studies. Transcripts contributing most strongly to this composite
16 mediator frequently had complex, distal regulation distributed throughout the genome. These results suggest
17 that trait-relevant variation in transcription is largely distally regulated, but is nonetheless identifiable,
18 interpretable, and translatable across species.

19 **Introduction**

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

²⁶ with multiple disease traits^{12–15}

²⁷ Despite the great promise of these methods, explaining trait effects with local gene regulation has been more
²⁸ difficult than initially assumed^{16;17}. Although trait-associated variants tend to lie in non-coding, regulatory
²⁹ regions, they often do not have detectable effects on gene expression¹⁶ and tend not to co-localize with
³⁰ expression quantitative trait loci (eQTLs)^{17;18}.

³¹ One possible explanation for these observations is that gene expression is not being measured in the appropriate
³² cell types and thus local eQTLs influencing traits cannot be detected¹⁶. An alternative explanation that has
³³ been discussed in recent years is that effects of these variants are mediated not through local regulation of
³⁴ gene expression, but through distal regulation^{18–20;15}. In this model, a gene's expression is influenced by
³⁵ many variants throughout the genome through their cumulative effects on a broader regulatory network. In
³⁶ other words, the heritable component of the transcriptome is an emergent state arising from the myriad
³⁷ molecular interactions defining and constraining gene expression.

³⁸ To assess the role of wide-spread distal gene regulation on complex traits, we investigated diet-induced
³⁹ obesity and metabolic disease as an archetypal example. Diet-induced obesity and metabolic disease are
⁴⁰ genetically complex with hundreds of variants mapped through GWAS^{21;22}. These variants are known to act
⁴¹ through multiple tissues that interact dynamically with each other^{23;24}, 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.

⁴⁶ Measuring gene expression in multiple tissues is critical to adequately assess the extent to which local gene
⁴⁷ regulation varies across the tissues and whether such variability might account for previous failed attempts to
⁴⁸ identify trait-relevant local eQTL. Such data sets are extremely difficult to obtain in human populations,
⁴⁹ particularly in the large numbers of subjects required for adequate statistical power. Thus, to further
⁵⁰ investigate the role of local and distal gene regulation on complex traits, we generated two complementary
⁵¹ data sets: A discovery data set in a large population of diversity outbred (DO) mice²⁵, and an independent
⁵² validation data set derived by crossing inbred strains from the Collaborative Cross (CC) mice²⁶ to form CC
⁵³ F1 mice (CC-RIX). Both populations modeled diet-induced obesity and metabolic disease¹².

⁵⁴ The DO population and CC recombinant inbred lines were derived from the same eight inbred founder mouse
⁵⁵ strains, five classical lab strains, and three strains more recently derived from wild mice²⁵. They represent
⁵⁶ three subspecies of mouse *Mus musculus domesticus*, *Mus musculus musculus*, and *Mus musculus castaneus*,

57 and capture 90% of the known variation in laboratory mice²⁷. The DO mice are maintained with a breeding
58 scheme that ensures equal contributions from each founder across the genome thus rendering almost the
59 whole genome visible to genetic inquiry²⁵. The CC mice were initially outcrossed to recombine the genomes
60 from all eight founders, and then inbred for at least 20 generations to generate multiple inbred lines. Because
61 these two populations have common ancestral haplotypes, we could directly and unambiguously compare
62 the local genetic effects on gene expression at the whole-transcriptome level while varying the population
63 structure driving distal regulation.

64 In the DO population, we paired clinically relevant metabolic traits from 371 mice¹², including body weight,
65 plasma levels of insulin, glucose and lipids, with transcriptome-wide gene expression in four tissues related to
66 metabolic disease: adipose tissue, pancreatic islets, liver, and skeletal muscle. We measured similar metabolic
67 traits in a CC-RIX population and gene expression from three of the four tissues used in the DO: adipose
68 tissue, liver, and skeletal muscle. Because the CC-RIX carry the same founder alleles as the DO, local gene
69 regulation is expected to match between the populations, but because the alleles are recombined through
70 the genome, distal effects are expected to vary from those in the DO, allowing us to directly assess the
71 role of local gene regulation in driving trait-associated transcript variation. Together, these data enable a
72 comprehensive view into the genetic architecture of metabolic disease.

73 Results

74 To comprehensively assess the genetic control of gene expression in metabolic disease in mice, we assayed
75 metabolic traits and multi-tissue gene expression in DO mice.

76 Genetic variation contributed to wide phenotypic variation

77 Although the environment was consistent across the DO mice, the genetic diversity present in this population
78 resulted in widely varying distributions across physiological measurements (Fig. 1). For example, body
79 weights of adult individuals varied from less than the average adult C57BL/6J (B6) body weight to several
80 times the body weight of a B6 adult in both sexes (Males: 18.5 - 69.1g, Females: 16.0 - 54.8g) (Fig. 1A).
81 Fasting blood glucose (FBG) also varied considerably (Fig. 1B), although few of the animals had FBG levels
82 that would indicate pre-diabetes (19 animals, 3.8%), or diabetes (7 animals, 1.4%) according to previously
83 developed cutoffs (pre-diabetes: $\text{FBG} \geq 250 \text{ mg/dL}$, diabetes: $\text{FBG} \geq 300 \text{ mg/dL}$)²⁸. Males had higher
84 FBG than females on average (Fig. 1C) as has been observed before suggesting either that males were more
85 susceptible to metabolic disease on the high-fat, high-sugar (HFHS) diet, or that males and females may
86 require different thresholds for pre-diabetes and diabetes.

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

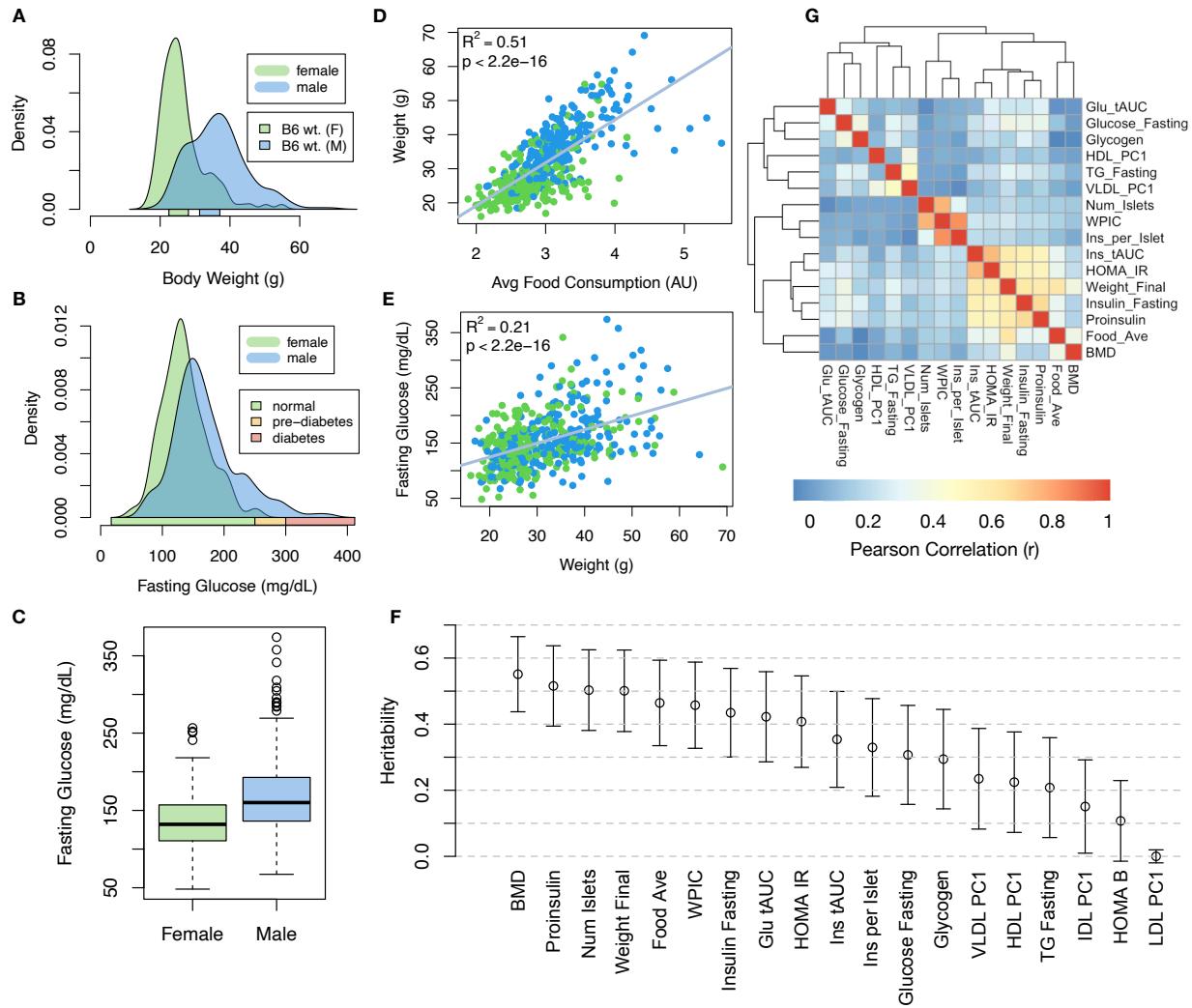


Figure 1: Clinical overview. **A.** Distributions of body weight in the diversity outbred mice. Sex is indicated by color. The average B6 male and female adult weights at 24 weeks of age are indicated by blue and green bars on the x-axis. **B.** The distribution of fasting glucose across the population 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. BMD - bone mineral density, WPIC - whole pancreas insulin content, Glu tAUC - glucose total area under the curve, HOMA IR - homeostatic measurement of insulin resistance, HOMA B - homeostatic measure of beta cell health, VLDL - very low-density lipoprotein, LDL - low-density lipoprotein, IDL - intermediate density lipoprotein, HDL - high-density lipoprotein, TG - triglyceride.

91 **Distal Heritability Correlated with Phenotype Relevance**

92 To comprehensively assess the genetic control of gene expression in metabolic disease we measured overall
93 gene expression via bulk RNA-Seq in adipose, islet, liver, and skeletal muscle in the DO cohort (Supp. Fig.
94 S1A-H). We performed eQTL analysis using R/qltl²⁹ (Methods) and identified both local and distal eQTLs
95 for transcripts in each of the four tissues (Supp. Fig. S1). Significant local eQTLs far outnumbered distal
96 eQTLs (Supp. Fig. S1F) and tended to be shared across tissues (Supp. Fig. S1G) whereas the few significant
97 distal eQTLs we identified tended to be tissue-specific (Supp. Fig. S1H)

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

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

113 **High-Dimensional Mediation identified a high-heritability composite trait that was mediated
114 by a composite transcript**

115 The above univariate analyses establish the importance of distal heritability for trait-relevant transcripts.
116 However, the number of transcripts dramatically exceeds the number of phenotypes. Thus, we expect the
117 heritable, trait-relevant transcripts to be highly correlated and organized according to coherent, biological
118 processes representing the mediating endophenotypes driving clinical trait variation. To identify these
119 endophenotypes in a theoretically principled way, we developed a novel dimension-reduction technique,
120 high-dimension mediation analytis (HDMA), that uses the theory of causal graphical models to identify a
121 transcriptomic signature that is simultaneously 1) highly heritable, 2) strongly correlated to the measured

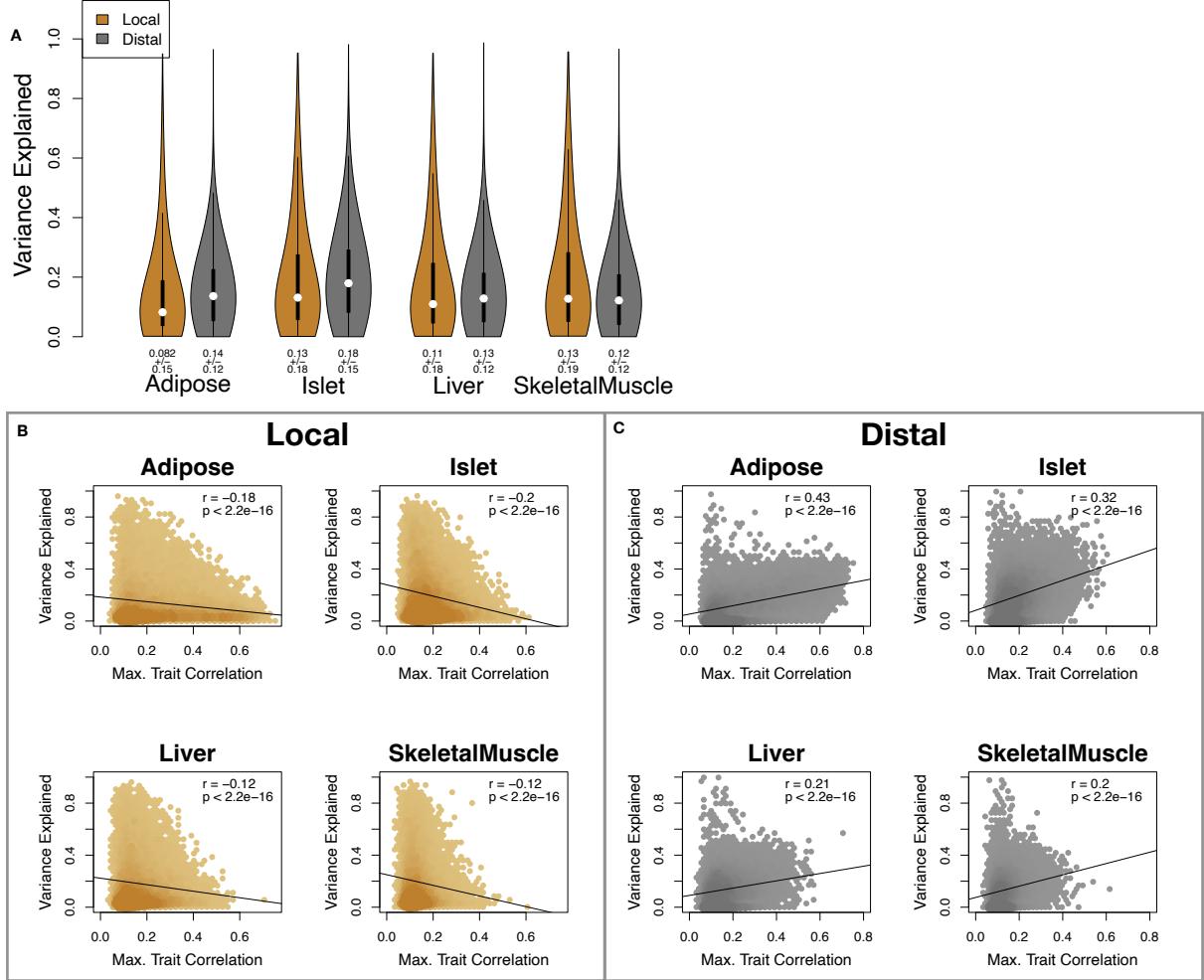


Figure 2: Transcript heritability and trait relevance. **A.** Distributions of distal and local heritability of transcripts across the four tissues. Overall local and distal factors contribute equally to transcript heritability. The relationship between **(B.)** local and **(C.)** distal heritability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

phenotypes, and 3) conforms to the causal mediation hypothesis (Fig. 3). HDMA projects the high-dimensional scores—a composite genome score (G_C), a composite transcriptome score (T_C), and a composite phenome score (P_C)—and uses the univariate theory of mediation to constrain these projections to satisfy the hypotheses of perfect mediation, namely that upon controlling for the transcriptomic score, the genome score is uncorrelated to the phenome score. Formally, perfect mediation implies a constraint on the correlation coefficients among scores as

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

which is equivalent to the partial correlation of G_C and P_C after controlling for T_C being zero. The value $\text{Corr}(G_C, T_C)\text{Corr}(T_C, P_C)$ is called the path coefficient of the mediation model. The projections of the high-dimensional data matrices in HDMA are designed to satisfy this constraint, and thus conform to the perfect mediation hypothesis, as closely as possible. We stress, however, that validating any causal assertion requires direct experimentation and, thus, that the output of HDMA are scores that are consistent with causal mediation. Thus, HDMA is a strategy for causal hypothesis generation, where the causal mediator is a complex endophenotype learned from a high-dimensional readout.

Operationally, HDMA is closely related to generalized canonical correlation analysis (CCA), for which provably convergent algorithms have recently been developed³⁰. A complete mathematical derivation and implementation details for HDMA are available in Supp. Methods.

Using HDMA we identified the major axis of variation in the transcriptome that was consistent with mediating the effects of the genome on metabolic traits (Fig 3). Fig. 3A shows the partial correlations (ρ) between the pairs of these composite vectors. The partial correlation between G_C and T_C was 0.42, and the partial correlation between T_C and P_C was 0.78. However, when the transcriptome was taken into account, the partial correlation between G_C and P_C was effectively zero (0.039). P_C captured 30% of the overall trait variance, and its estimated heritability was 0.71 ± 0.084 , which was higher than any of the measured traits (Fig. 1F). Thus, HDMA identified a maximally heritable metabolic composite trait and a highly heritable component of the transcriptome that are correlated as expected in the perfectly mediated model.

As discussed in Supp. Methods, HDMA is related to a generalized form of CCA. Standard CCA is prone to over-fitting because in any two large matrices it can be trivial to identify highly correlated composite vectors³¹. To assess whether our implementation of HDMA was similarly prone to over-fitting in a high-dimensional space, we performed permutation testing. We permuted the individual labels on the transcriptome matrix 1000 times and recalculated the path coefficient, which is the partial correlation of G_C and T_C multiplied by the partial correlation of T_C and P_C . This represents the strength of the path from G_C to P_C that is putatively mediated through T_C . The null distribution of the path coefficient is shown in Fig. 3B, and the observed path coefficient from the original data is indicated by a red line. The observed path coefficient was well outside the null distribution generated by permutations ($p < 10^{-16}$). Fig. 3C illustrates this observation in more detail. Although we identified high correlations between G_C and T_C , and modest correlations between T_C and P_C in the null data (Fig 3C), these two values could not be maximized simultaneously in the null data. In contrast, the red dot shows that in the real data both the G_C - T_C correlation and the T_C - P_C correlation could be maximized simultaneously suggesting that the path from genotype to phenotype through transcriptome is highly non-trivial and identifiable in this case. These results suggest that these composite vectors represent

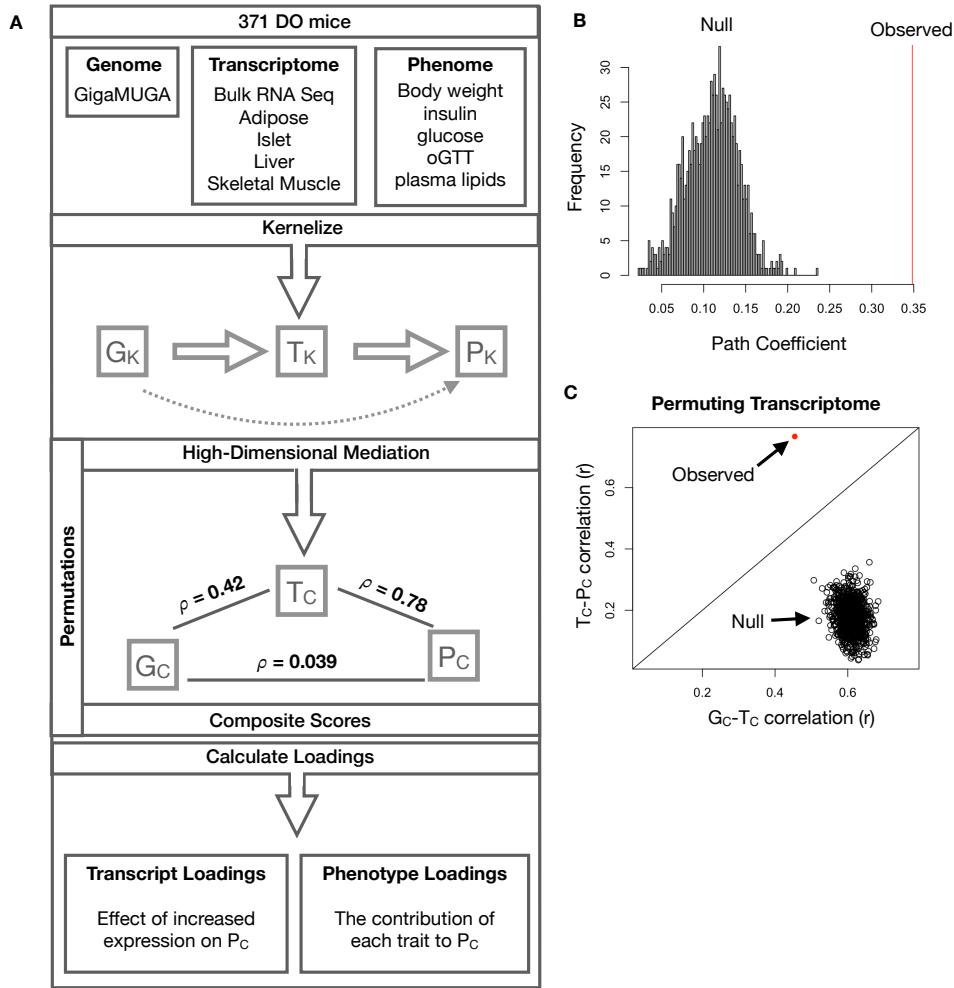


Figure 3: High-dimensional mediation. **A.** Workflow indicating major steps of high-dimensional mediation. The genotype, transcriptome, and phenotype matrices were independently normalized and converted to kernel matrices representing the pairwise relationships between individuals for each data modality (K_G = genome kernel, K_T = transcriptome kernel; K_P = 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).

160 genetically determined variation in phenotype that is mediated through genetically determined variation in
 161 transcription.

¹⁶² **Body weight and insulin resistance were highly represented in the expression-mediated composite trait**

¹⁶⁴ Each composite score is simply a weighted combination of the measured variables and the magnitude and
¹⁶⁵ sign of the weights, called loadings, correspond the relative importance and directionality of each variable
¹⁶⁶ in the composite score. The loadings of each measured trait onto P_C indicate how much each contributed
¹⁶⁷ to the composite phenotype. Body weight contributed the most (Fig. 4), followed by homeostatic insulin
¹⁶⁸ resistance (HOMA_IR) and fasting plasma insulin levels (Insulin_Fasting). We can thus interpret P_C as
¹⁶⁹ an index of metabolic disease (Fig. 4B). Individuals with high values of P_C have a higher metabolic index
¹⁷⁰ and greater metabolic disease, including higher body weight and higher insulin resistance. We refer to P_C
¹⁷¹ as the metabolic index going forward. Traits contributing the least to the metabolic index were measures
¹⁷² of cholesterol and pancreas composition. Thus, when we interpret the transcriptomic signature identified
¹⁷³ by HDMA, we are explaining primarily the putative transcriptional mediation of body weight and insulin
¹⁷⁴ resistance, as opposed to cholesterol measurements.

¹⁷⁵ **High-loading transcripts have low local heritability, high distal heritability, and were linked
176 mechanistically to obesity**

¹⁷⁷ We interpreted large loadings onto transcripts as indicating strong mediation of the effect of genetics on
¹⁷⁸ metabolic index. Large positive loadings indicate that higher expression was associated with a higher
¹⁷⁹ metabolic index (i.e. higher risk of obesity and metabolic disease on the HFHS diet) (Fig. 4C). Conversely,
¹⁸⁰ large negative loadings indicate that high expression of these transcripts was associated with a lower metabolic
¹⁸¹ index (i.e. lower risk of obesity and metabolic disease on the HFHS diet) (Fig. 4C). We used gene set
¹⁸² enrichment analysis (GSEA)^{32;33} to look for biological processes and pathways that were enriched at the top
¹⁸³ and bottom of this list (Methods).

¹⁸⁴ In adipose tissue, both GO processes and KEGG pathway enrichments pointed to an axis of inflammation
¹⁸⁵ and metabolism (Figs. S2 and S3). GO terms and KEGG pathways associated with inflammation were
¹⁸⁶ positively associated with metabolic index, indicating that increased expression in inflammatory pathways
¹⁸⁷ was associated with a higher metabolic index. It is well established that adipose tissue in obese individuals
¹⁸⁸ is inflamed and infiltrated by macrophages^{34–38}, and the results here suggest that this may be a dominant
¹⁸⁹ heritable component of metabolic disease.

¹⁹⁰ The strongest negative enrichments in adipose tissue were related to mitochondrial activity in general, and
¹⁹¹ thermogenesis in particular (Figs. S2 and S2). Genes in the KEGG oxidative phosphorylation pathway in
¹⁹² mice were almost universally negatively loaded in adipose tissue, suggesting that increased expression of these

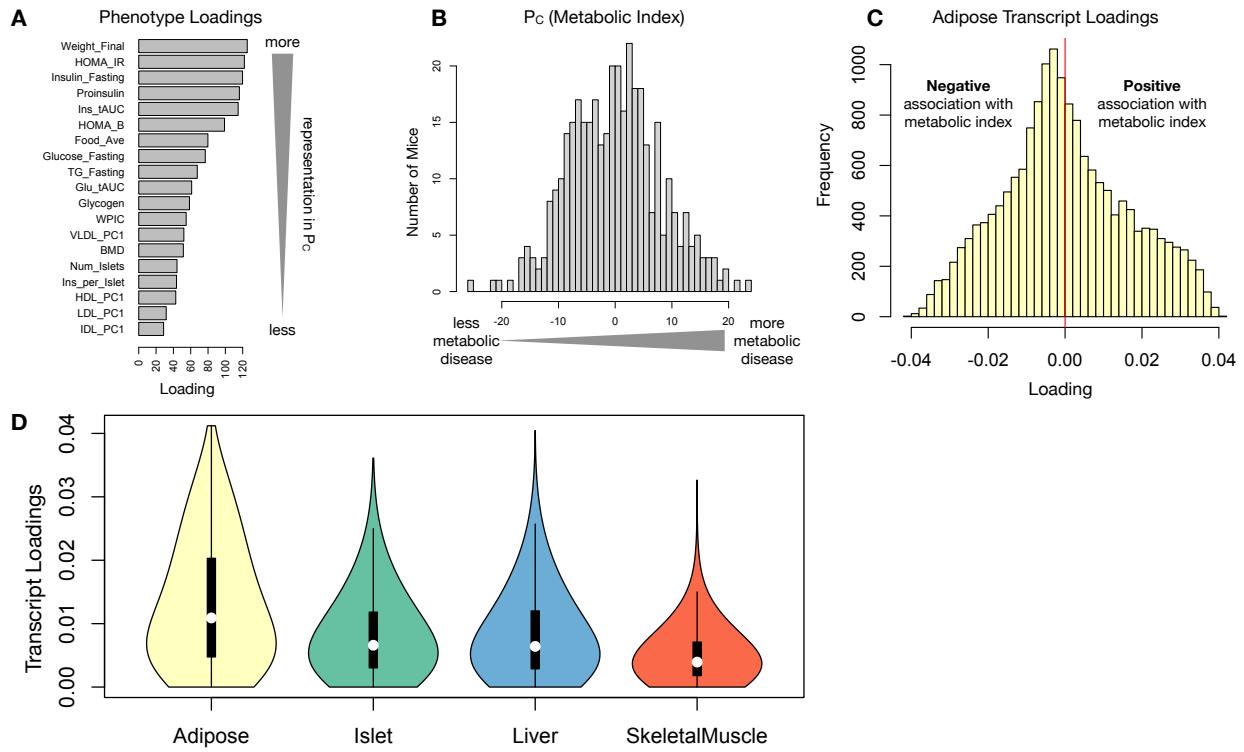


Figure 4: Interpretation of loadings. **A.** Loadings across traits. Body weight and insulin resistance contributed the most to the composite trait. **B.** Phenotype scores across individuals. Individuals with large positive phenotype scores had higher body weight and insulin resistance than average. 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 adipose tissue gene expression was a strong mediator of genotype on body weight and insulin resistance.

193 genes was associated with reduced metabolic index (Supp. Fig. S4). Consistent with this observations, it
 194 has been shown previously that mouse strains with greater thermogenic potential are also less susceptible to
 195 obesity on a HFHS diet³⁹.

196 Transcripts associated with the citric acid (TCA) cycle as well as the catabolism of the branched-chain amino
 197 acids (BCAA) (valine, leucine, and isoleucine) were strongly enriched with negative loadings in adipose
 198 tissue (Supp. Figs. S2, S5 and S6). Expression of genes in both pathways (for which there is some overlap)
 199 has been previously associated with insulin sensitivity^{12;40;41}, suggesting that heritable variation in regulation
 200 of these pathways may influence risk of insulin resistance.

201 Looking a the 10 most positively and negatively loaded transcripts from each tissue, it is apparent that
 202 transcripts in the adipose tissue had the largest loadings, both positive and negative, of all tissues (Fig. 5A

203 bar plot) This suggests that much of the effect of genetics on body weight and insulin resistance is mediated
204 through gene expression in adipose tissue. The strongest loadings in liver and pancreas were comparable,
205 and those in skeletal muscle were the weakest (Fig. 5A), suggesting that less of the genetic effects were
206 mediated through transcription in skeletal muscle. Heritability analysis showed that transcripts with the
207 largest loadings had higher distal heritability than local heritability (Fig. 5A heat map and box plot). This
208 pattern contrasts with transcripts nominated by TWAS (Fig. 5B), which tended to have lower loadings,
209 higher local heritability and lower distal heritability. Transcripts with the highest local heritability in each
210 tissue (Fig. 5C) had the lowest loadings, consistent with our findings above (Fig. 2B).

211 We performed a literature search for the genes in each of these groups along with the terms “diabetes”,
212 “obesity”, and the name of the expressing tissue to determine whether any of these genes had previous
213 associations with metabolic disease in the literature (Methods). Multiple genes in each group had been
214 previously associated with obesity and diabetes (Fig. 5 bolded gene names). Genes with high loadings were
215 most highly enriched for previous literature support. They were 2.4 times more likely than TWAS hits and 3.8
216 times more likely than genes with high local heritability to be previously associated with obesity or diabetes.

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

218 Clustering of transcripts with top loadings in each tissue showed tissue-specific functional modules associated
219 with obesity and insulin resistance (Fig. 6A) (Methods). The clustering highlights the importance of immune
220 activation particularly in adipose tissue. The “mitosis” cluster had large positive loadings in three of the four
221 tissues potentially suggesting system-wide proliferation of immune cells. Otherwise, all clusters were strongly
222 loaded in only one or two tissues. For example, the lipid metabolism cluster was loaded most heavily in liver.
223 The positive loadings suggest that high expression of these genes particularly in the liver was associated with
224 increased metabolic disease. This cluster included the gene *Pparg*, whose primary role is in the adipose tissue
225 where it is considered a master regulator of adipogenesis⁴². Agonists of *Pparg*, such as thiazolidinediones, are
226 FDA-approved to treat type II diabetes, and reduce inflammation and adipose hypertrophy⁴². Consistent
227 with this role, the loading for *Pparg* in adipose tissue was negative, suggesting that higher expression was
228 associated with leaner mice (Fig. 6B). In contrast, *Pparg* had a large positive loading in liver, where it is
229 known to play a role in the development of hepatic steatosis, or fatty liver. Mice that lack *Pparg* specifically
230 in the liver, are protected from developing steatosis and show reduced expression of lipogenic genes^{43;44}.
231 Overexpression of *Pparg* in the livers of mice with a *Ppara* knockout, causes upregulation of genes involved in
232 adipogenesis⁴⁵. In the livers of both mice and humans high *Pparg* expression is associated with hepatocytes
233 that accumulate large lipid droplets and have gene expression profiles similar to that of adipocytes^{46;47}.

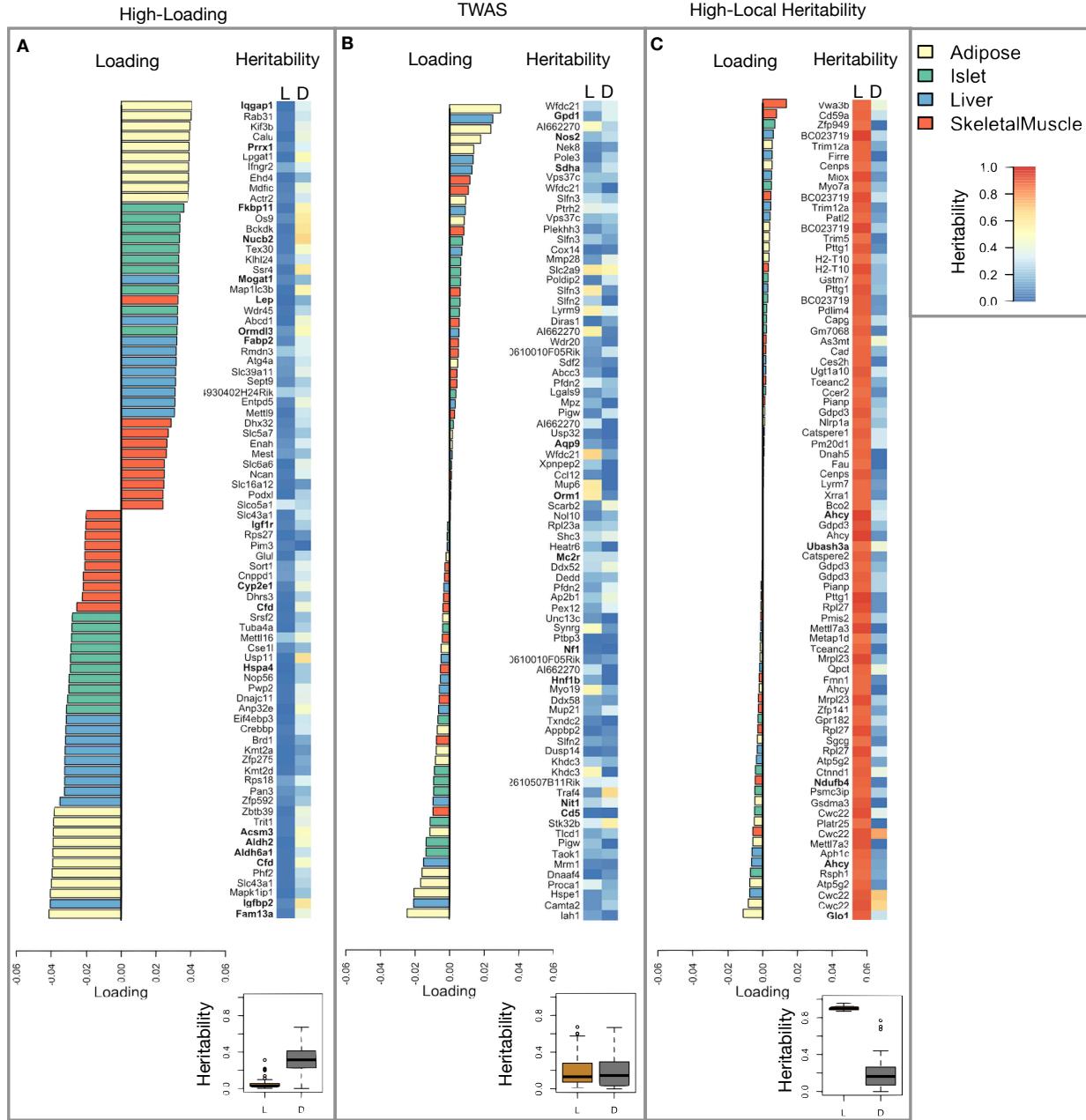


Figure 5: Transcripts with high loadings have high distal heritability and literature support. Each panel has a bar plot showing the loadings of transcripts selected by different criteria. Bar color indicates the tissue of origin. The heat map shows the local (L - left) and distal (D - right) heritability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

234 The local and distal heritability of *Pparg* is low in adipose tissue suggesting its expression in this tissue is
 235 highly constrained in the population (Fig. 6B). However, the distal heritability of *Pparg* in liver is relatively
 236 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 Supp. File 1.

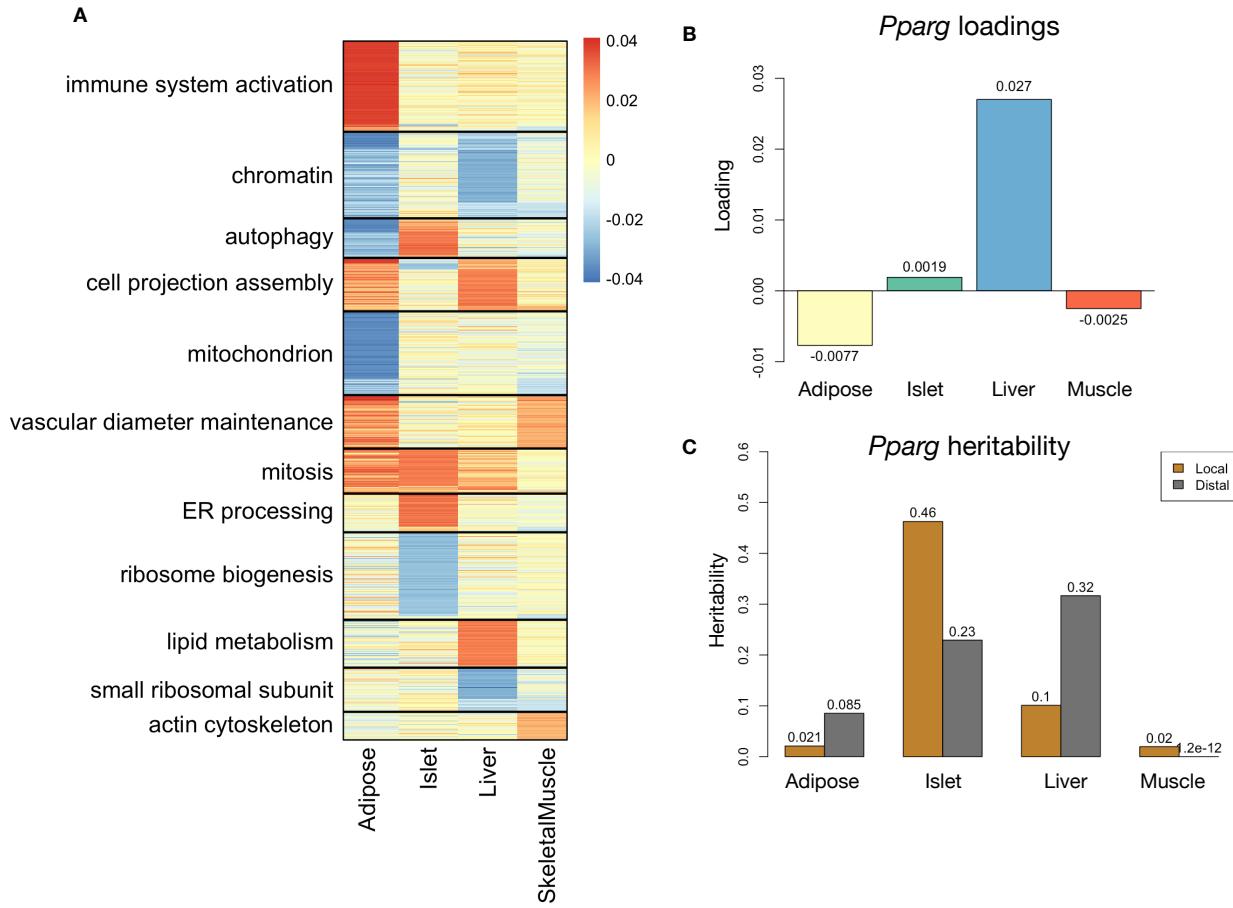


Figure 6: Tissue-specific transcriptional programs were associated with obesity and insulin resistance. **A** Heat map showing the loadings of all transcripts with loadings greater than 2.5 standard deviations from the mean in any tissue. The heat map was clustered using k medoid clustering. Functional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

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)^{48–51} strains (Fig. 7) (Methods). We tested two questions. First, we asked whether the loadings identified in the DO mice were relevant to

247 the relationship between the transcriptome and the phenotype in the CC-RIX. We predicted body weight (a
 248 surrogate for metabolic index) in each CC-RIX individual using measured gene expression in each tissue and
 249 the transcript loadings identified in the DO (Methods). The predicted body weight and actual body weight
 250 were highly correlated in all tissues (Fig. 7B left column). The best prediction was achieved for adipose
 251 tissue, which supports the observation in the DO that adipose expression was the strongest mediator of the
 252 genetic effect on metabolic index. This result also confirms the validity and translatability of the transcript
 253 loadings and their relationship to metabolic disease.

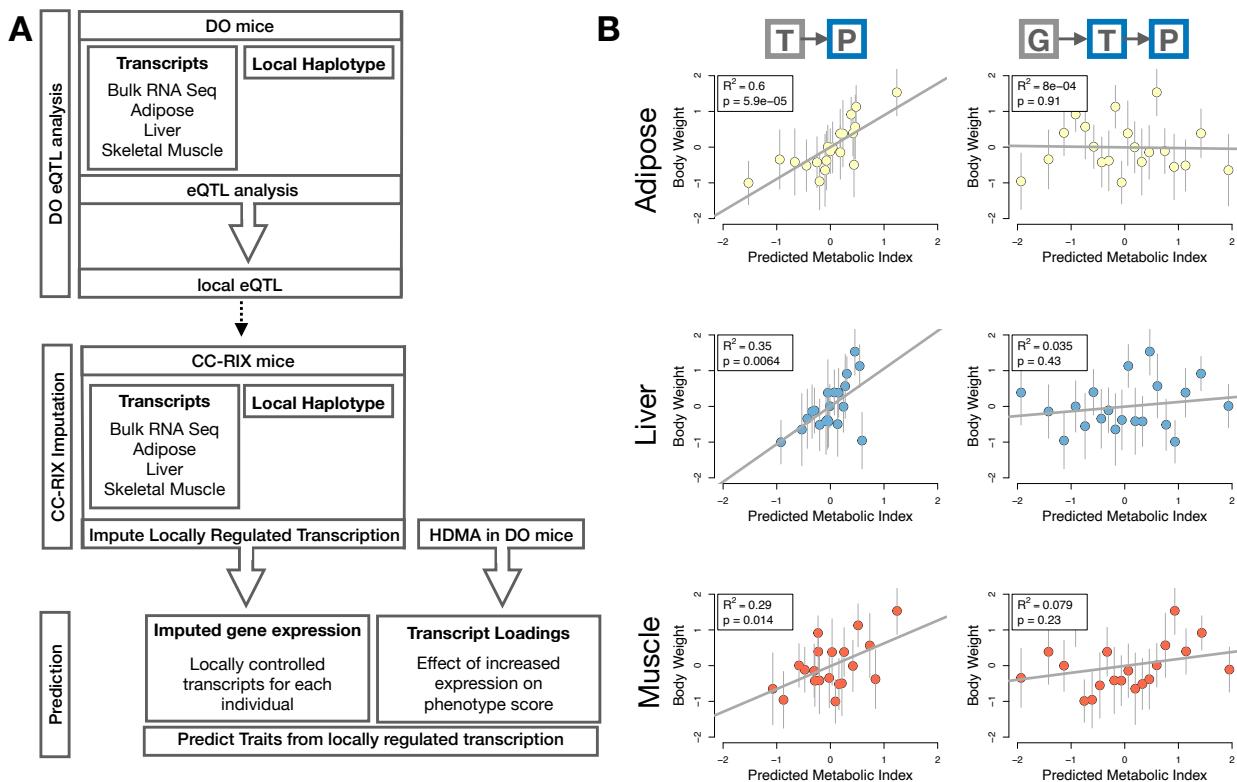


Figure 7: Transcription, but not local genotype, predicts phenotype in the CC-RIX. **A.** Workflow showing procedure for translating HDMA results to an independent population of mice. **B.** Relationships between the predicted metabolic index and measured body weight. The left column shows the predictions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

254 The second question related to the source of the relevant variation in gene expression. If local regulation was
 255 the predominant factor influencing gene expression, we should be able to predict phenotype in the CC-RIX
 256 using transcripts imputed from local genotype (Fig. 7A). The DO and the CC-RIX were derived from the
 257 same eight founder strains and so carry the same alleles throughout the genome. We imputed gene expression
 258 in the CC-RIX using local genotype and were able to estimate variation in gene transcription robustly (Supp.

259 Fig. S7). However, these imputed values failed to predict body weight in the CC-RIX when weighted with the
260 loadings from HDMA. (Fig. 7B right column). This result suggests that local regulation of gene expression is
261 not the primary factor driving heritability of complex traits, consistent with our findings in the DO population
262 that distal heritability was a major driver of trait-relevant variation and that high-loading transcripts had
263 comparatively high distal and low local heritability.

264 **Distally heritable transcriptomic signatures reflected variation in composition of adipose tissue
265 and islets**

266 The interpretation of global genetic influences on gene expression and phenotype is potentially more challenging
267 than the interpretation and translation of local genetic influences, as genetic effects cannot be localized to
268 individual gene variants or transcripts. However, there are global patterns across the loadings that can
269 inform mechanism. For example, heritable variation in cell type composition can be inferred from transcript
270 loadings. We observed above that immune activation in the adipose tissues was a highly enriched process
271 correlating with obesity in the DO population. For example, in humans, it has been extensively observed
272 that macrophage infiltration in adipose tissue is a marker of obesity and metabolic disease⁵². To determine
273 whether the immune activation reflected a heritable change in cell composition in adipose tissue in DO mice,
274 we compared loadings of cell-type specific genes in adipose tissue (Methods). Consistent with human results,
275 the mean loading of macrophage-specific genes was significantly greater than 0 (Fig. 8A), indicating that
276 obese mice were genetically predisposed to have high levels of macrophage infiltration in adipose tissue in
277 response to the HFHS diet. Loading for marker genes for other cell types were not statistically different from
278 zero, indicating that changes in the abundance of those cell types is not a mediator of metabolic index.

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

286 Notably, the loadings for pancreatic beta cell-type specific loadings was not significantly different from zero.
287 We stress that this is not necessarily reflective of the function of the beta cells in the obese mice, but rather
288 suggests that any variation in the number of beta cells in these mice was unrelated to obesity and insulin
289 resistance, the major contributors to metabolic index. This is further consistent with the islet composition

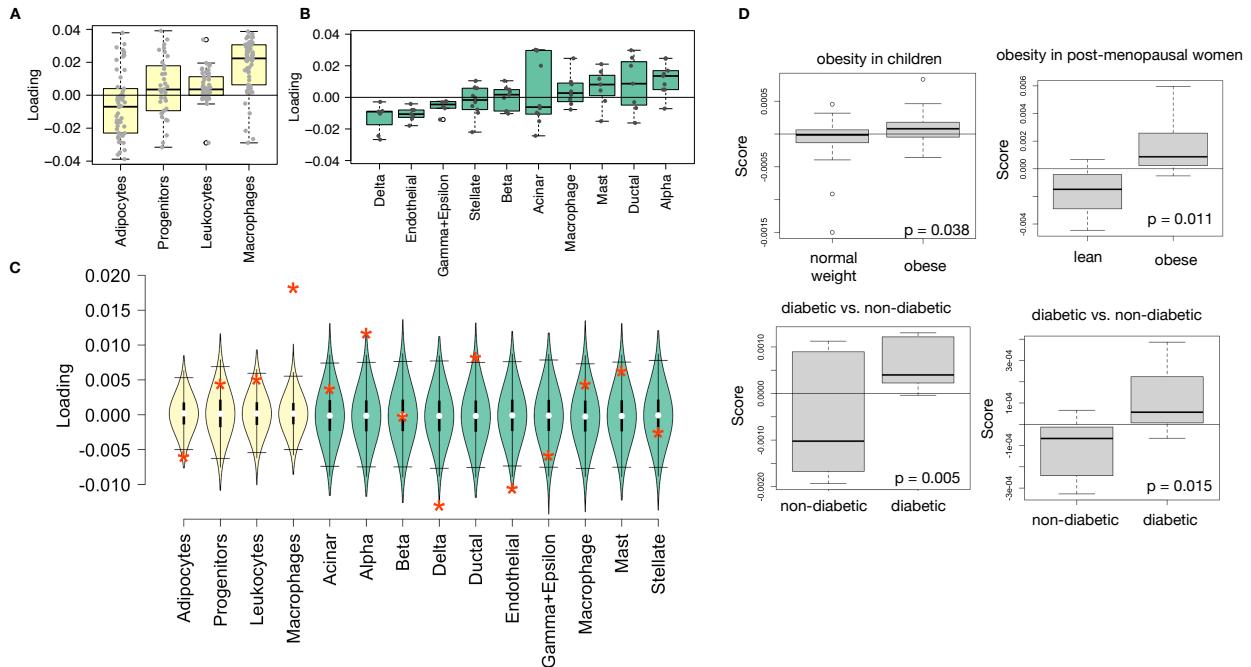


Figure 8: HDMA results translate to humans. **A.** Distribution of loadings for cell-type-specific transcripts in adipose tissue. **B.** Distribution of loadings for cell-type-specific transcripts in pancreatic islets (green). **C.** Null distributions for the mean loading of randomly selected transcripts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

290 traits having small loadings in the phenotype score (Fig. 4).

291 Heritable transcriptomic signatures translated to human disease

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

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

303 **Existing therapies are predicted to target mediator gene signatures**

304 Another potential application of the transcript loading landscape is in ranking potential drug candidates
305 for the treatment of metabolic disease. Although high-loading transcripts may be good candidates for
306 understanding specific biology related to obesity, the transcriptome overall is highly interconnected and
307 redundant. The ConnectivityMap (CMAP) database⁵³ developed by the Broad Institute allows querying
308 thousands of compounds that reverse or enhance the extreme ends of transcriptomic signatures in multiple
309 different cell types. By identifying drugs that reverse pathogenic transcriptomic signatures, we can potentially
310 identify compounds that have favorable effects on gene expression.

311 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
312 (Supp. Figs S8 and S9). To get more tissue-specific results, we also looked at top results in cell types that
313 most closely resembled our tissues. We looked at results in adipocytes (ASC) as well as pancreatic tumor
314 cells (YAPC) regardless of *p* value (Supp. Figs S10 and S11).

316 Looking across all cell types, the notable top hits from the adipose tissue loadings included mTOR inhibitors
317 and glucocorticoid agonists (Supp. Fig. S8). It is thought that metformin, which is commonly used to
318 improve glycemic control, acts, at least in part, by inhibiting mTOR signaling^{54;55}. However, long-term use
319 of other mTOR inhibitors, such as rapamycin, are known to cause insulin resistance and β -cell toxicity^{55–57}.
320 Glucocorticoids are used to reduce inflammation, which was a prominent signature in the adipose tissues,
321 but these drugs also promote hyperglycemia and diabetes^{58;59}. Accute treatment with glucocorticoids has
322 further been shown to reduce thermogenesis in rodent adipocytes^{60–62}, but increase thermogenesis in human
323 adipocytes^{63;64}. Thus, the pathways identified by CMAP across all cell types were highly related to the
324 transcript loading profiles, but the relationship was not a simple reversal.

325 The top hit for the adipose composite transcript in CMAP adipocytes was a PARP inhibitor (Supp. Fig.
326 S10). PARPs play a role in lipid metabolism and are involved in the development of obesity and diabetes⁶⁵.
327 PARP1 inhibition increases mitochondrial biogenesis⁶⁶. Inhibition of PARP1 activity can further prevent
328 necrosis in favor of the less inflammatory apoptosis⁶⁷, thereby potentially reducing inflammation in stressed
329 adipocytes. Other notable hits among the top 20 were BTK inhibitors, which have been observed to suppress
330 inflammation and improve insulin resistance⁶⁸ as well as to reduce insulin antibodies in type I diabetes⁶⁹.
331 IkappaB kinase (IKK) is an enzyme complex involved in regulating cellular responses to inflammation⁷⁰.
332 Inhibitors of IKK have been shown to improve glucose control in type II diabetes^{71;72}.

333 Among the top most significant hits for the transcript loadings from pancreatic islets (Supp. Fig. S9), was

334 suppression of T cell receptor signaling, which is known to be involved in Type 1 diabetes⁷³, as well as
335 TNFR1, which has been associated with mortality in diabetes patients⁷⁴. Suppression of NOD1/2 signaling
336 was also among the top hits. NOD1 and 2 sense ER stress^{75;76}, which is associated with β -cell death in type
337 1 and type 2 diabetes⁷⁷. This cell death process is dependent on NOD1/2 signaling⁷⁵, although the specifics
338 have not yet been worked out.

339 We also looked specifically at hits in pancreatic tumor cells (YAPC) regardless of significance level to get a
340 transcriptional response more specific to the pancreas (Supp. Fig. S11). Hits in this list included widely used
341 diabetes drugs, such as sulfonylureas, PPAR receptor agonists, and insulin sensitizers. Rosiglitazone is a
342 PPAR- γ agonist and was one of the most prescribed drugs for type 2 diabetes before its use was reduced due
343 to cardiac side-effects⁷⁸. Sulfonylureas are another commonly prescribed drug class for type 2 diabetes, but
344 also have notable side effects including hypoglycemia and accelerated β -cell death⁷⁹.

345 In summary, the high-loading transcripts derived from HDMA in mice prioritized of drugs with demonstrated
346 effectiveness in reducing type 2 diabetes phenotypes in humans in a tissue-specific manner. Drugs identified
347 using the islet loadings are known diabetes drugs that act directly on pancreatic function. Drugs identified
348 by the adipose loadings tended to reduce inflammatory responses and have been shown incidentally to reduce
349 obesity-related morbidity.

350 Discussion

351 Here we investigated the relative contributions of local and distal gene regulation in four tissues to heritable
352 variation in traits related to metabolic disease in genetically diverse mice. We found that distal heritability
353 was positively correlated with trait relatedness, whereas high heritability was negatively correlated with
354 trait relatedness. We used a novel high-dimensional mediation analysis (HDMA) to identify tissue-specific
355 composite transcripts that are predicted to mediate the effect of genetic background on metabolic traits. The
356 adipose-derived composite transcript robustly predicted body weight in an independent cohort of diverse
357 mice with disparate population structure. However, gene expression imputed from local genotype failed to
358 predict body weight in the second population. Taken together, these results highlight the complexity of gene
359 expression regulation in relation to trait heritability and suggest that heritable trait variation is mediated
360 primarily through distal gene regulation.

361 Supplemental Discussion

362 Our result that distal regulation accounted for most trait-related gene expression differences is consistent
363 with a complex model of genetic trait determination. It has frequently been assumed that gene regulation in

364 *cis* is the primary driver of genetically associated trait variation, but attempts to use local gene regulation
365 to explain phenotypic variation have had limited success^{16;17}. In recent years, evidence has mounted that
366 distal gene regulation may be an important mediator of trait heritability^{19;18;80}. It has been observed that
367 transcripts with high local heritability explain less expression-mediated disease heritability than those with
368 low local heritability¹⁹. Consistent with this observation, genes located near GWAS hits tend to be complexly
369 regulated¹⁸. They also tend to be enriched with functional annotations, in contrast to genes with simple
370 local regulation, which tend to be depleted of functional annotations suggesting they are less likely to be
371 directly involved in disease traits¹⁸. These observations are consistent with principles of robustness in complex
372 systems in which simple regulation of important elements leads to fragility of the system⁸¹⁻⁸³. Our results
373 are consistent, instead, with a more complex picture where genes whose expression can drive trait variation
374 are buffered from local genetic variation but are extensively influenced indirectly by genetic variation in the
375 regulatory networks converging on those genes.

376 Our results are consistent with the recently proposed omnigenic model, which posits that complex traits are
377 massively polygenic and that their heritability is spread out across the genome⁸⁴. In the omnigenic model,
378 genes are classified either as “core genes,” which directly impinge on the trait, or “peripheral genes,” which
379 are not directly trait-related, but influence core genes through the complex gene regulatory network. HDMA
380 explicitly models a central proposal of the omnigenic model which posits that once the expression of the
381 core genes (i.e. trait-mediating genes) is accounted for, there should be no residual correlation between the
382 genome and the phenotype. Here, when the composite transcript was taken into account there was no residual
383 correlation between the composite genome and composite phenotype (Fig. 3A).

384 Unlike in the omnigenic model, we did not observe a clear demarcation between the core and peripheral
385 genes in loading magnitude, but we do not necessarily expect a clear separation given the complexity of gene
386 regulation and the genotype-phenotype map⁸⁵.

387 An extension of the omnigenic model proposed that most heritability of complex traits is driven by weak
388 distal eQTLs that are potentially below the detection threshold in studies with feasible sample sizes⁸⁰. This
389 is consistent with what we observed here. For example, *Nucb2*, had a high loading in islets and was also
390 strongly distally regulated (66% distal heritability) (Fig. 5). Although its transcription was highly heritable
391 in islets, that regulation was distributed across the genome, with no clear distal eQTL (Supp. Fig. S12).
392 Thus, although distal regulation of some genes may be strong, this regulation is likely to be highly complex
393 and not easily localized.

394 Individual high-loading transcripts also demonstrated biologically interpretable, tissue-specific patterns. We

395 highlighted *Pparg*, which is known to be protective in adipose tissue⁴² where it was negatively loaded, and
396 harmful in the liver^{43–47}, where it was positively loaded. Such granular patterns may be useful in generating
397 hypotheses for further testing, and prioritizing genes as therapeutic targets. The tissue-specific nature of
398 the loadings also may provide clues to tissue-specific effects, or side effects, of targeting particular genes
399 system-wide.

400 In addition to identifying individual transcripts of interest, the composite transcripts can be used as weighted
401 vectors in multiple types of analysis, such as drug prioritization using gene set enrichment analysis (GSEA)
402 and the CMAP database. In particular, the CMAP analysis identified drugs which have been demonstrated
403 to reverse insulin resistance and other aspects of metabolic disease. This finding supports the causal role of
404 these full gene signatures in pathogenesis of metabolic disease and thus their utility in prioritizing drugs and
405 gene targets as therapeutics.

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

412 Data Availability

413 Genotypes: Sequence data for the DO mice used here are available from the Sequence Read Archive
414 <https://www.ncbi.nlm.nih.gov/sra/> (study number SRP125176). Genotype data for the CC mice are available
415 from University of North Carolina Computational Systems Biology (<http://www.csbio.unc.edu/CCstatus/C>
416 CGenomes/).

417 Gene expression: Data can be found at the Gene Expression Omnibus url{<https://www.ncbi.nlm.nih.gov/geo/>}
418 with the following accession numbers: DO adipose tissue - GSE266549; DO liver tissue - GSE266569; DO
419 skeletal muscle - GSE266567; CC-RIX adipose tissue - GSE237737; CC-RIX liver tissue - GSE237743;
420 CC-RIX skeletal muscle - GSE237747. Quantified pancreatic islet gene expression for the DO mice, along
421 with their genotypes and phenotypes can be found on Dryad <https://datadryad.org/stash/dataset/doi:10.5061/dryad.pj105>.

423 Phenotypes: Metabolic phenotypes for the DO mice along with genotypes and quantified gene expression
424 area available from <https://datadryad.org/stash/dataset/doi:10.5061/dryad.pj105>

⁴²⁵ Metabolic phenotypes for the CC-RIX mice are available from XXX

⁴²⁶ Acknowledgements

⁴²⁷ Here we thank people and cite funding sources.

428 Supplemental Figures

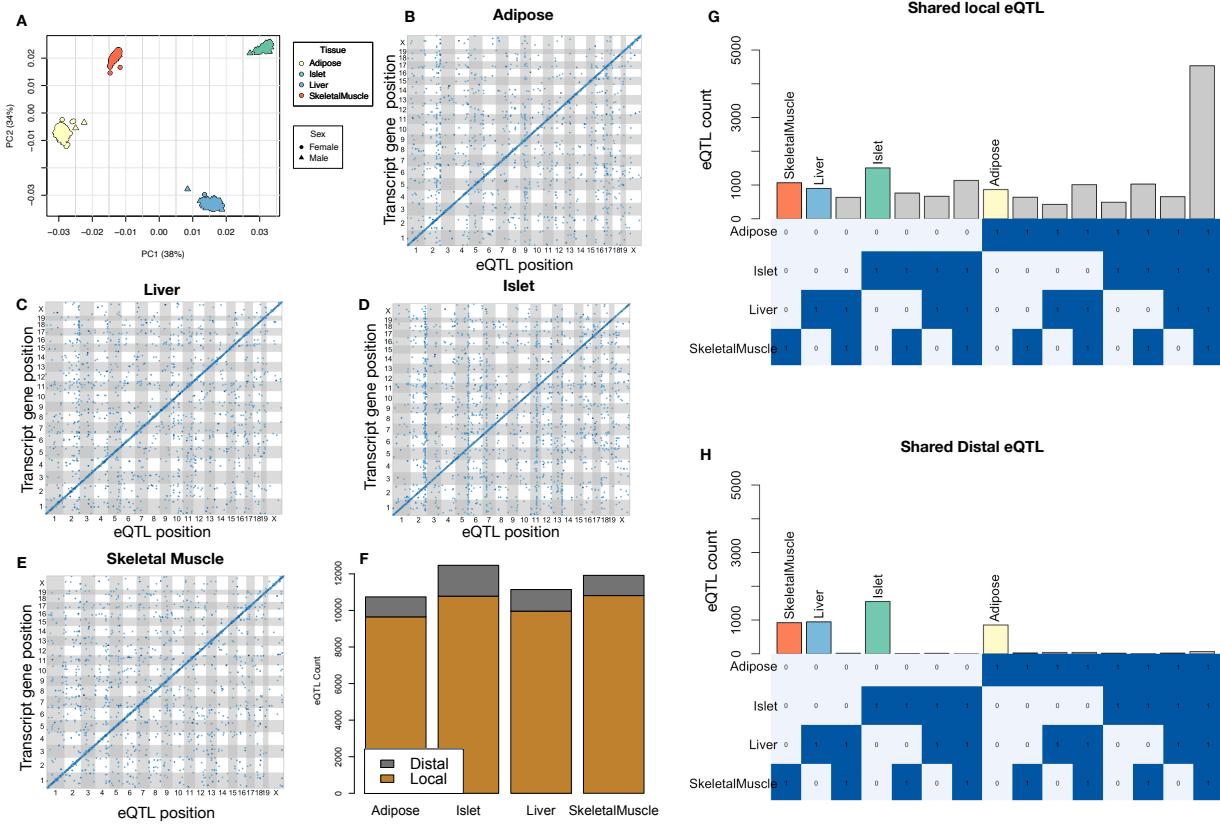


Figure S1: Overview of eQTL analysis in DO mice. **A.** RNA seq samples from the four different tissues clustered by tissue. **B.-E.** eQTL maps are shown for each tissue. The *x*-axis shows the position of the mapped eQTL, and the *y*-axis shows the physical position of the gene encoding each mapped 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.

KEGG pathway enrichments by GSEA

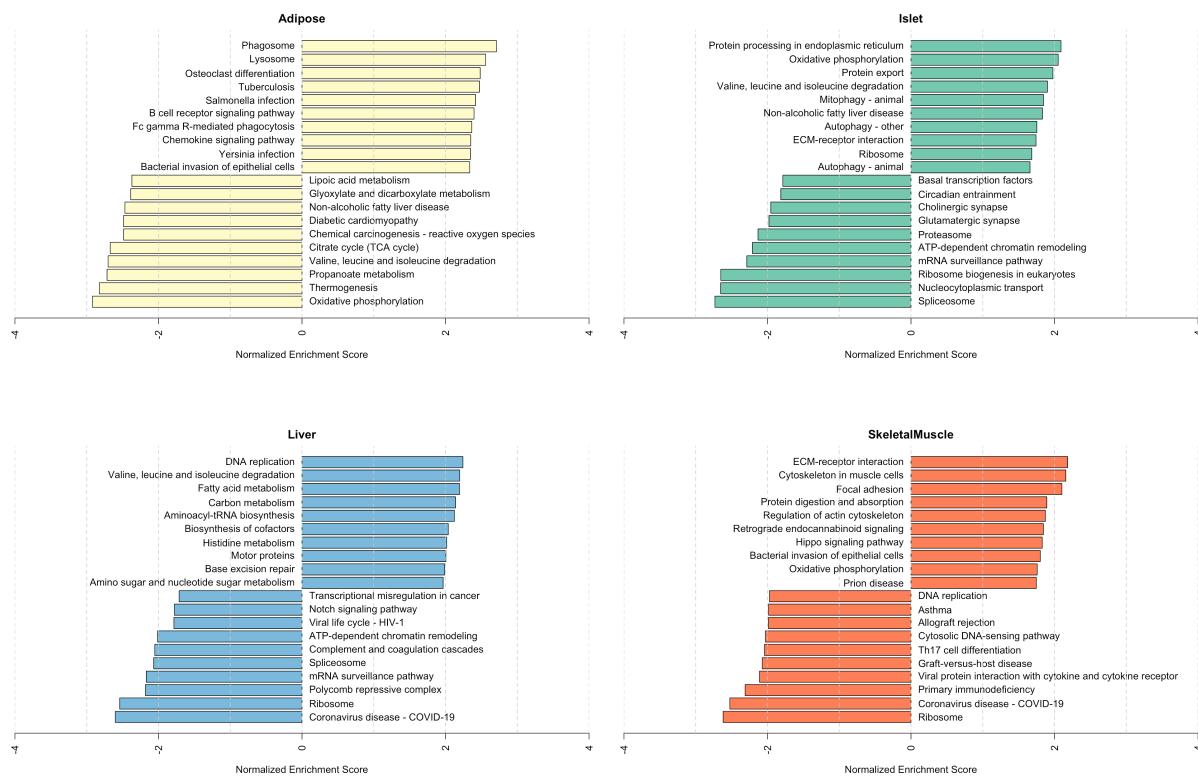


Figure S2: Bar plots showing normalized enrichment scores (NES) for KEGG pathways as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched KEGG pathway.

Top GO term enrichments by GSEA

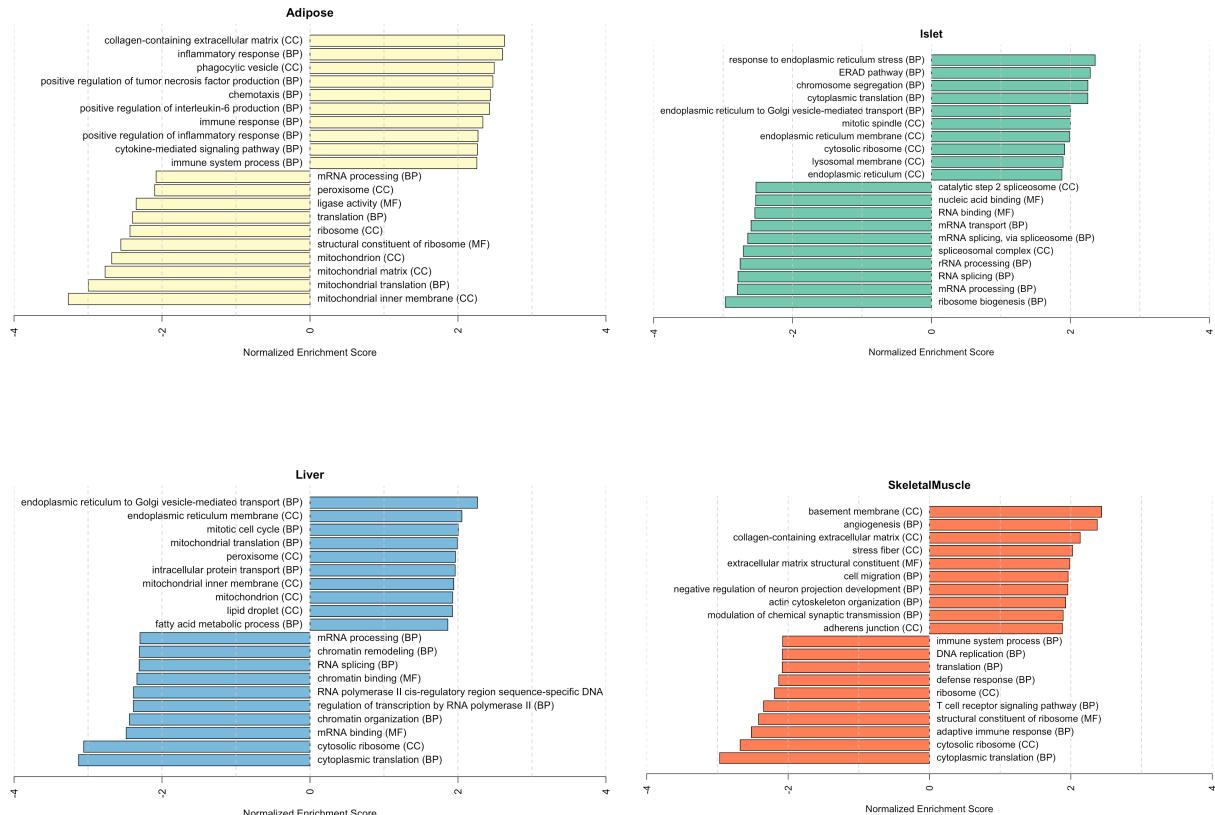


Figure S3: Bar plots showing normalized enrichment scores (NES) for GO terms as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched GO term. The letters in parentheses indicate whether the term is from the biological process ontology (BP), the molecular function ontology (MF), or the cellular compartment ontology (CC).

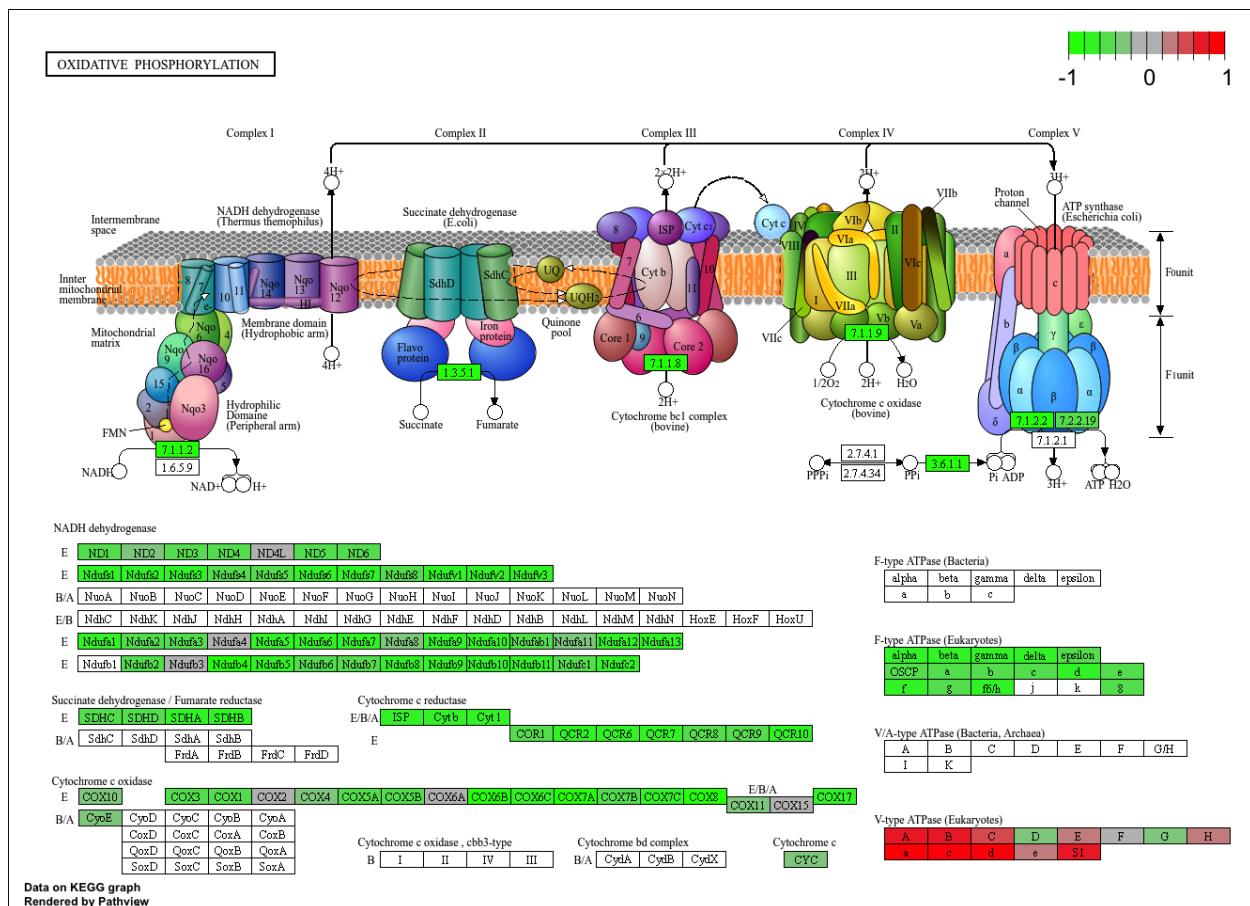


Figure S4: The KEGG pathway for oxidative phosphorylation in mice. Each element is colored based on its HDMA loading from adipose tissue normalized to run from -1 to 1. Genes highlighted in green had negative loadings, and those highlighted in red had positive loadings. Almost the entire pathway was strongly negatively loaded indicating that increased expression of genes involved in oxidative phosphorylation was associated with reduced metabolic index.

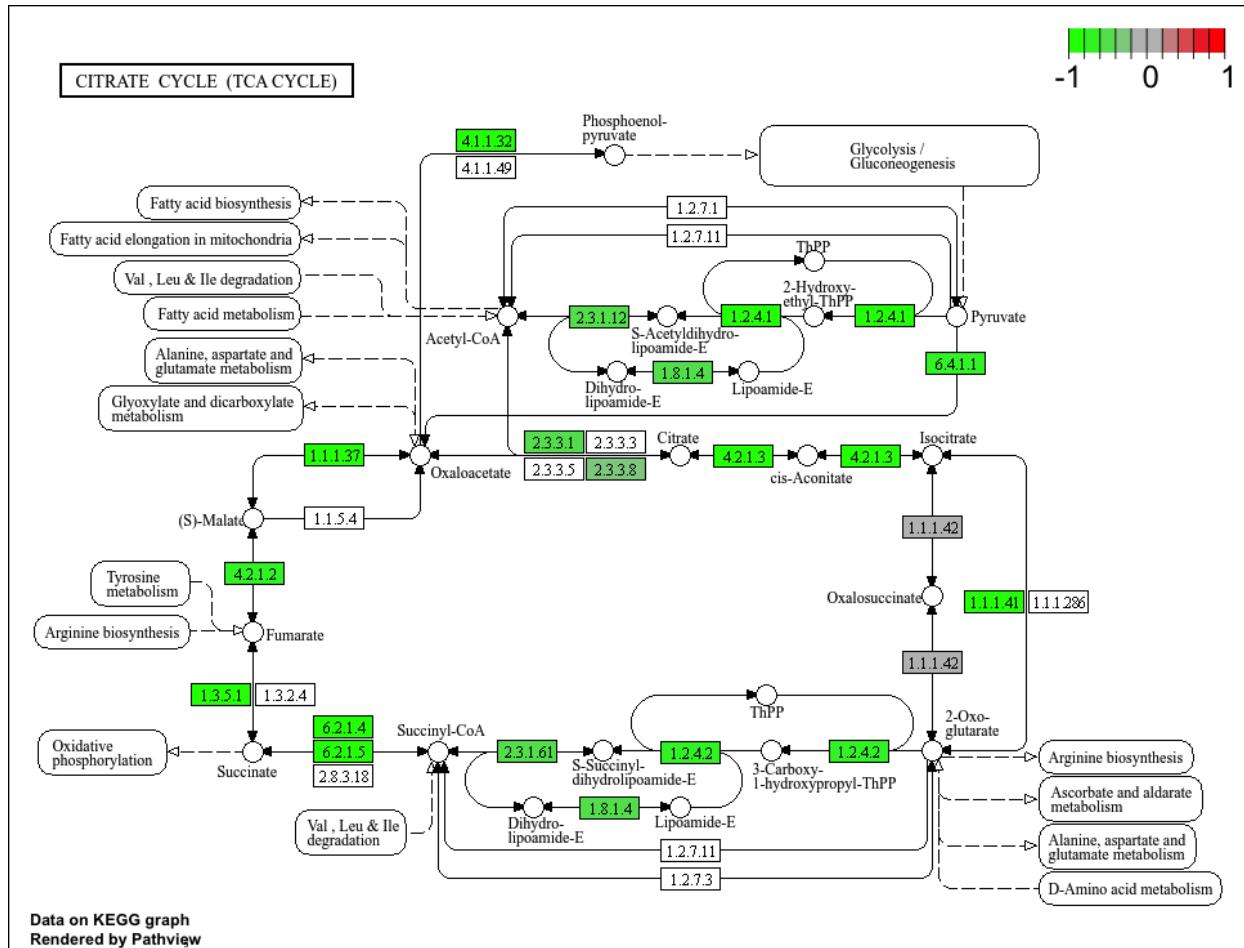


Figure S5: The KEGG pathway for the TCA (citric acid) cycle in mice. Each element is colored based on its HDMA loading from adipose tissue normalized to run from -1 to 1. Genes highlighted in green had negative loadings, and those highlighted in red had positive loadings. Many genes in the cycle were strongly negatively loaded indicating that increased expression of genes involved in branched-chain amino acid degradation was associated with reduced metabolic index.

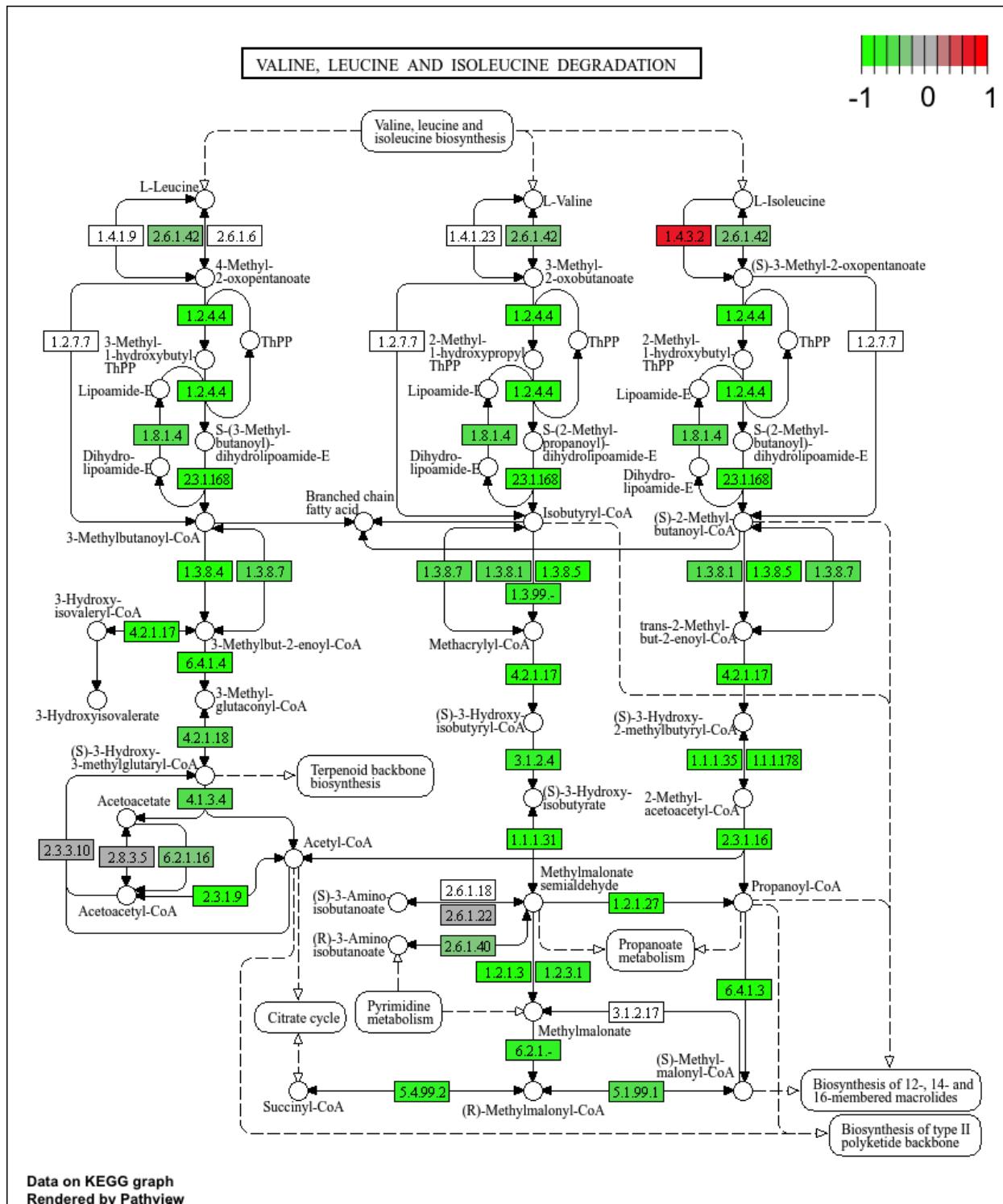


Figure S6: The KEGG pathway for branched-chain amino acid degradation in mice. Each element is colored based on its HDMA loading from adipose tissue normalized to run from -1 to 1. Genes highlighted in green had negative loadings, and those highlighted in red had positive loadings. Almost the entire pathway was strongly negatively loaded indicating that increased expression of genes involved in branched-chain amino acid degradation was associated with reduced metabolic index.

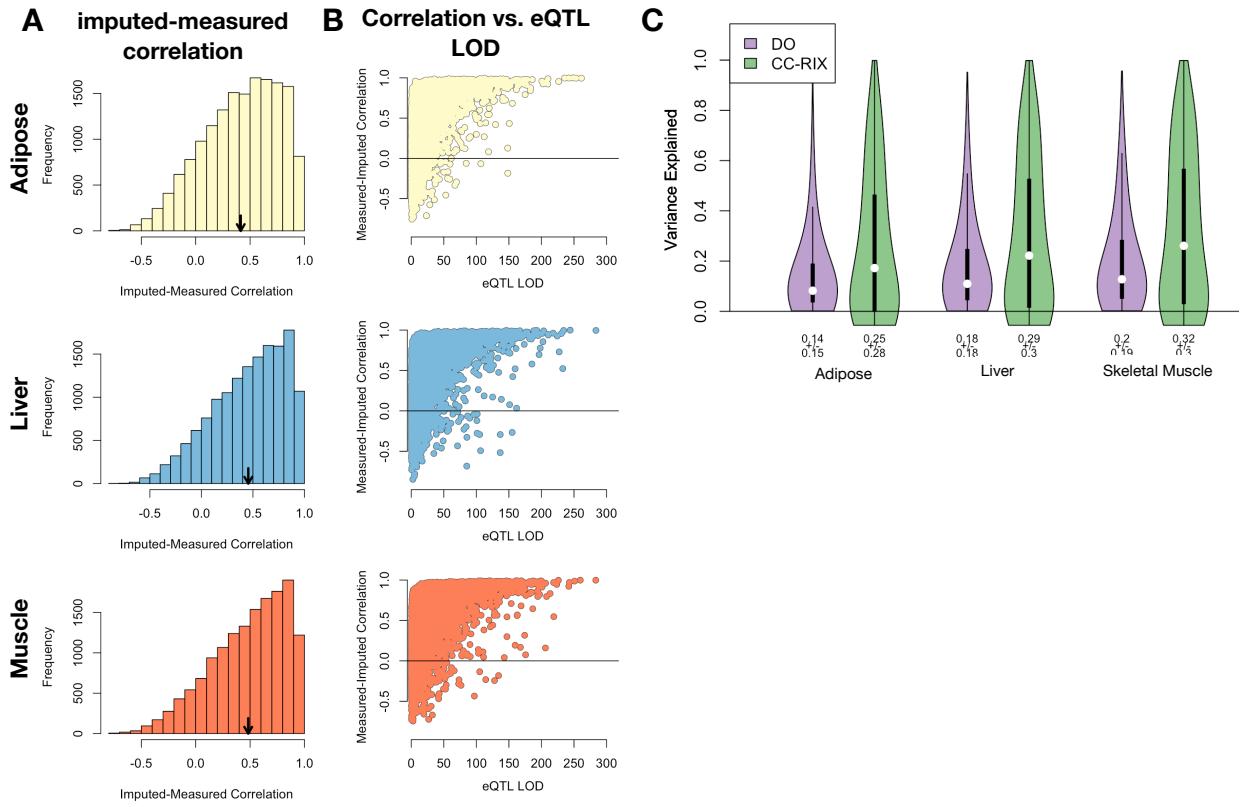


Figure S7: Validation of transcript imputation in the CC-RIX. **A.** Distributions of correlations between imputed and measured transcripts in the CC-RIX. The mean of each distribution is shown by the red line. All distributions were skewed toward positive correlations and had positive means near a Pearson 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.

id	norm_gs	cell_iname	pert_type	raw_cs▲	fdr_q_nlog10	set_type	src_set_id
		HA1E	TRT_CP	-0.97	15.65	PCL	CP_PROTEIN_SYNTHESIS_INHIBITOR
		PC3	TRT_SH.CGS	-0.90	15.65	PATHWAY_SET	BIOCARTA_EIF4_PATHWAY
		A375	TRT_CP	-0.87	15.65	MOA_CLASS	RAF_INHIBITOR
		HCC515	TRT_CP	-0.84	15.65	PCL	CP_TOPOISOMERASE_INHIBITOR
		HEPG2	TRT_SH.CGS	-0.82	15.65	PATHWAY_SET	BIOCARTA_BCR_PATHWAY
		PC3	TRT_CP	-0.77	15.65	MOA_CLASS	MTOR_INHIBITOR
		HCC515	TRT_CP	-0.76	15.65	PCL	CP_GLUCOCORTICOID_RECECTORAGONIST
		HCC515	TRT_CP	-0.76	15.65	MOA_CLASS	GLUCOCORTICOID_RECECTORAGONIST
		A375	TRT_CP	-0.72	15.65	MOA_CLASS	MTOR_INHIBITOR
		-666	TRT_CP	-0.70	15.65	PCL	CP_PROTEIN_SYNTHESIS_INHIBITOR
		-666	TRT_CP	-0.68	15.65	PCL	CP_JAK_INHIBITOR
		A549	TRT_CP	-0.67	15.65	PCL	CP_GLUCOCORTICOID_RECECTORAGONIST
		A549	TRT_CP	-0.67	15.65	MOA_CLASS	GLUCOCORTICOID_RECECTORAGONIST
		-666	TRT_CP	-0.57	15.65	PCL	CP_MTOR_INHIBITOR
		-666	TRT_CP	-0.55	15.65	MOA_CLASS	MTOR_INHIBITOR
		-666	TRT_CP	-0.55	15.65	PCL	CP_PI3K_INHIBITOR
		-666	TRT_CP	0.85	15.65	MOA_CLASS	PKC_ACTIVATOR

Figure S8: CMAP results using the adipose tissue composite transcript as an input. All query results with a $-\log_{10}(q) > 15$ across all cell types are shown.

id	norm_gs	cell_iname	pert_type	raw_cs▲	fdr_q_nlog10	set_type	src_set_id
		VCAP	TRT_SH.CGS	-0.99	15.65	PATHWAY_SET	REACTOME_DOWNSTREAM_TCR_SIGNALING
		VCAP	TRT_SH.CGS	-0.99	15.65	PATHWAY_SET	REACTOME_NOD1_2_SIGNALING_PATHWAY
		A549	TRT_SH.CGS	-0.92	15.65	PATHWAY_SET	BIOCARTA_TNFR1_PATHWAY
		VCAP	TRT_SH.CGS	-0.92	15.65	PATHWAY_SET	HALLMARK_WNT_BETA_CATENIN_SIGNALING
		HT29	TRT_CP	-0.92	15.65	PCL	CP_TUBULIN_INHIBITOR
		-666	TRT_OE	-0.88	15.65	PCL	OE_CELL_CYCLE_INHIBITION
		VCAP	TRT_SH.CGS	-0.87	15.65	PATHWAY_SET	REACTOME_P75_NTR_RECECTOR_MEDiated_SIGNALLING
		HT29	TRT_CP	-0.86	15.65	MOA_CLASS	TUBULIN_INHIBITOR
		MCF7	TRT_CP	-0.85	15.65	PCL	CP_TUBULIN_INHIBITOR
		-666	TRT_CP	-0.81	15.65	PCL	CP_PROTEASOME_INHIBITOR
		-666	TRT_SH.CGS	-0.80	15.65	PATHWAY_SET	REACTOME_DOWNREGULATION_OF_ERBB2_ERBB3_SIGNALING
		HCC515	TRT_CP	-0.80	15.65	PCL	CP_GLUCOCORTICOID_RECECTORAGONIST
		HCC515	TRT_CP	-0.80	15.65	MOA_CLASS	GLUCOCORTICOID_RECECTORAGONIST
		A549	TRT_OE	-0.78	15.65	PATHWAY_SET	REACTOME_RAF_MAP_KINASE CASCADE
		A549	TRT_OE	-0.78	15.65	PATHWAY_SET	PID_RAS_PATHWAY
		-666	TRT_SH.CGS	-0.78	15.65	PCL	KD_RIBOSOMAL_40S_SUBUNIT
		A549	TRT_OE	-0.76	15.65	PATHWAY_SET	REACTOME_SIGNALLING_TO_P38_VIA_RIT_AND_RIN
		A549	TRT_OE	-0.76	15.65	PATHWAY_SET	REACTOME_PROLONGED_ERK_ACTIVATION_EVENTS
		A549	TRT_OE	-0.73	15.65	PATHWAY_SET	PID_TCR_RAS_PATHWAY
		HA1E	TRT_OE	-0.73	15.65	PATHWAY_SET	REACTOME_SHC RELATED EVENTS
		HA1E	TRT_OE	-0.71	15.65	PATHWAY_SET	PID_EPHB_FWD_PATHWAY
		-666	TRT_CP	-0.70	15.65	MOA_CLASS	GLYCOGEN_SYNTHASE_KINASE_INHIBITOR
		HA1E	TRT_OE	-0.70	15.65	PATHWAY_SET	PID_GMCSF_PATHWAY
		A549	TRT_OE	-0.69	15.65	PATHWAY_SET	REACTOME_SIGNALLING_TO_ERKS
		-666	TRT_LIG	-0.69	15.65	PATHWAY_SET	PID_ERBB_NETWORK_PATHWAY
		-666	TRT_CP	-0.67	15.65	MOA_CLASS	PROTEASOME_INHIBITOR
		-666	TRT_CP	-0.66	15.65	PCL	CP_GLYCOGEN_SYNTHASE_KINASE_INHIBITOR
		-666	TRT_CP	0.73	15.65	MOA_CLASS	MTOR_INHIBITOR

Figure S9: CMAP results using the pancreatic islet composite transcript as an input. All query results with a $-\log_{10}(q) > 15$ across all cell types are shown.

id	norm_CS	cell_iname	pert_type	raw_CS ▲	fdr_q_nlog10	set_type	src_set_id
		ASC	TRT_CP	-0.94	0.79	PCL	CP_PARP_INHIBITOR
		ASC	TRT_CP	-0.94	0.79	MOA_CLASS	PROTEIN_TYROSINE_KINASE_INHIBITOR
		ASC	TRT_CP	-0.84	0.45	MOA_CLASS	BTK_INHIBITOR
		ASC	TRT_CP	-0.81	0.39	MOA_CLASS	LEUCINE_RICH_REPEAT_KINASE_INHIBITOR
		ASC	TRT_CP	-0.81	0.79	PCL	CP_HSP_INHIBITOR
		ASC	TRT_CP	-0.80	0.93	PCL	CP_EGFR_INHIBITOR
		ASC	TRT_CP	-0.79	0.32	MOA_CLASS	T-TYPE_CALCIUM_CHANNEL_BLOCKER
		ASC	TRT_CP	-0.79	1.09	PCL	CP_MTOR_INHIBITOR
		ASC	TRT_CP	-0.76	0.97	PCL	CP_PI3K_INHIBITOR
		ASC	TRT_CP	-0.75	0.20	MOA_CLASS	HISTONE_DEMETHYLASE_INHIBITOR
		ASC	TRT_CP	-0.74	0.42	PCL	CP_IKK_INHIBITOR
		ASC	TRT_CP	-0.74	0.83	PCL	CP_AURORA_KINASE_INHIBITOR
		ASC	TRT_CP	-0.74	0.17	PCL	CP_LEUCINE_RICH_REPEAT_KINASE_INHIBITOR
		ASC	TRT_CP	-0.72	0.36	PCL	CP_BROMODOMAIN_INHIBITOR
		ASC	TRT_CP	-0.71	1.09	MOA_CLASS	TYROSINE_KINASE_INHIBITOR
		ASC	TRT_CP	-0.70	0.82	PCL	CP_PROTEIN_SYNTHESIS_INHIBITOR
		ASC	TRT_CP	-0.67	0.69	PCL	CP_SRC_INHIBITOR
		ASC	TRT_CP	-0.67	0.81	MOA_CLASS	AURORA_KINASE_INHIBITOR
		ASC	TRT_CP	-0.65	0.89	MOA_CLASS	FLT3_INHIBITOR
		ASC	TRT_CP	-0.62	0.40	MOA_CLASS	FGFR_INHIBITOR
		ASC	TRT_CP	-0.59	0.66	MOA_CLASS	MEK_INHIBITOR
		ASC	TRT_CP	-0.59	0.13	MOA_CLASS	SYK_INHIBITOR
		ASC	TRT_CP	-0.58	0.01	PCL	CP_PKC_INHIBITOR
		ASC	TRT_CP	-0.58	0.65	PCL	CP_HDAC_INHIBITOR
		ASC	TRT_CP	-0.58	0.65	PCL	CP_ATPASE_INHIBITOR
		ASC	TRT_CP	-0.53	0.09	PCL	CP_FLT3_INHIBITOR
		ASC	TRT_CP	-0.53	0.42	PCL	CP_P38_MAPK_INHIBITOR
		ASC	TRT_CP	-0.53	0.22	MOA_CLASS	IKK_INHIBITOR
		ASC	TRT_CP	-0.52	0.58	PCL	CP_VEGFR_INHIBITOR
		ASC	TRT_CP	-0.51	-0.00	PCL	CP_T_TYPE_CALCIUM_CHANNEL_BLOCKER

Figure S10: CMAP results using the adipose tissue composite transcript as an input. Query results are limited to the 30 most negatively correlated signals from normal adipocytes.

norm_CS						
id	cell_name	pert_type	raw_CS	fdr_q_nlog10	set_type	src_set_id
	YAPC	TRT_CP	-1.00	0.67	MOA_CLASS	ABL_KINASE_INHIBITOR
	YAPC	TRT_CP	-0.99	0.66	PCL	CP_CDK_INHIBITOR
	YAPC	TRT_CP	-0.97	1.41	PCL	CP_TOPOISOMERASE_INHIBITOR
	YAPC	TRT_CP	-0.95	0.70	MOA_CLASS	THYMIDYLATE_SYNTHASE_INHIBITOR
	YAPC	TRT_CP	-0.95	0.62	MOA_CLASS	ADRENERGIC_INHIBITOR
	YAPC	TRT_CP	-0.94	0.50	MOA_CLASS	BENZODIAZEPINE_RECECTOR_ANTAGONIST
	YAPC	TRT_CP	-0.89	0.63	PCL	CP_RIBONUCLEOTIDE_REDUCTASE_INHIBITOR
	YAPC	TRT_CP	-0.88	0.52	MOA_CLASS	VASOPRESSIN_RECECTOR_ANTAGONIST
	YAPC	TRT_CP	-0.85	0.63	MOA_CLASS	ANGIOTENSIN_RECECTOR_ANTAGONIST
	YAPC	TRT_CP	-0.85	0.33	PCL	CP_CANNABINOID_RECECTORAGONIST
	YAPC	TRT_CP	-0.84	0.30	PCL	CP_RETINOID_RECECTORAGONIST
	YAPC	TRT_CP	-0.83	1.19	MOA_CLASS	NFKB_PATHWAY_INHIBITOR
	YAPC	TRT_CP	-0.83	0.54	MOA_CLASS	DNA_ALKYLATING_DRUG
	YAPC	TRT_CP	-0.80	0.50	MOA_CLASS	CHOLESTEROL_INHIBITOR
	YAPC	TRT_CP	-0.79	0.15	MOA_CLASS	SULFONYLUREA
	YAPC	TRT_CP	-0.78	0.52	MOA_CLASS	HIV_INTEGRASE_INHIBITOR
	YAPC	TRT_CP	-0.78	0.13	MOA_CLASS	LEUKOTRIENE_INHIBITOR
	YAPC	TRT_CP	-0.78	0.45	PCL	CP_PPAR_RECECTORAGONIST
	YAPC	TRT_CP	-0.78	0.54	MOA_CLASS	INSULIN_SENSITIZER
	YAPC	TRT_CP	-0.77	0.51	MOA_CLASS	ESTROGEN_RECECTORANTAGONIST
	YAPC	TRT_CP	-0.77	0.76	MOA_CLASS	DNA_SYNTHESIS_INHIBITOR
	YAPC	TRT_XPR	-0.77	0.67	PATHWAY_SET	BIOCARTA_PARKIN_PATHWAY
	YAPC	TRT_CP	-0.77	0.51	PCL	CP_VEGFR_INHIBITOR
	YAPC	TRT_CP	-0.75	0.39	MOA_CLASS	RNA_SYNTHESIS_INHIBITOR
	YAPC	TRT_CP	-0.72	0.60	MOA_CLASS	BCR-ABL_KINASE_INHIBITOR
	YAPC	TRT_XPR	-0.71	0.66	PATHWAY_SET	BIOCARTA_EIF_PATHWAY
	YAPC	TRT_XPR	-0.69	0.54	PATHWAY_SET	PID_CIRCADIAN_PATHWAY
	YAPC	TRT_CP	-0.68	0.77	MOA_CLASS	TOPOISOMERASE_INHIBITOR
	YAPC	TRT_XPR	-0.64	0.49	PATHWAY_SET	BIOCARTA_CBL_PATHWAY
	YAPC	TRT_CP	-0.64	0.53	MOA_CLASS	TUBULIN_INHIBITOR

Figure S11: CMAP results using the pancreatic islet composite transcript as an input. Query results are limited to the 30 most negatively correlated signals from YAPC cells, which were derived from a pancreatic carcinoma cells.

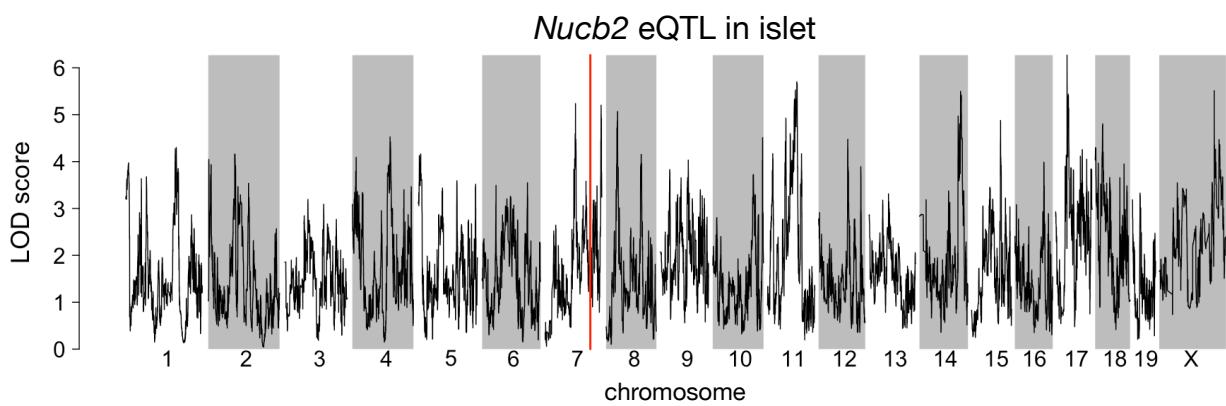


Figure S12: Regulation of *Nucb2* expression in islet. *Nucb2* is encoded on mouse chromosome 7 at 116.5 Mb (red line). In islets the heritability of *Nucb2* expression levels is 69% heritable. This LOD score trace shows that there is no local eQTL at that position, nor any strong distal eQTL anywhere else in the genome.

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