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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Plasma. Ten microliters of lipid extract were injected through SII for Xcalibur by an Ultimate 3000 RSLC autosampler (Thermo Scientific) coupled to a Q Exactive Focus mass spectrometer run by Tune software version 2.5.0.2042 (Thermo Scientific). Liver. One microliter of lipid extract was injected through SII for Xcalibur (Thermo Scientific) by a Vanquish Split Sampler HT autosampler (Thermo Scientific) coupled to a Q Exactive HF mass spectrometer run by Tune software version 2.8.0.2688 (Thermo Scientific). Cells. Ten microliters of lipid extract were injected through SII for Xcalibur (Thermo Scientific) by a Vanquish Split Sampler HT autosampler (Thermo Scientific) coupled to a Q Exactive HF mass spectrometer run by Tune software version 2.9.3.2948 (Thermo Scientific).
Data analysis	The resulting LC-MS lipidomics raw files were converted to mgf files via MSConvertGUI (ProteoWizard, Dr. Parag Mallick, Stanford University) and processed using Compound Discoverer 2.0 (Thermo Fisher Scientific) and an in-house developed open-source software suite, LipiDex. The quantification of the internal standard was obtained through TraceFinder 4.0 (Thermo Fisher Scientific). Data analysis was largely performed using R in RStudio. The lipid metabolite data were adjusted for batch effects using the Combat algorithm as implemented in the R/sva software package. The genome scans were performed using the scan1() function in R/qtl2. Data formatting was performed utilizing R/dplyr_0.8.3, R/tidyr_1.0.0 and R/reshape2_1.4.3 and visualizations were created using R/ggplot2_3.2.1, R/RColorBrewer_1.1-2, and for exploratory analysis, R/plotly_4.9.0. Heatmaps were generated using R/pheatmap_1.0.12 and manhattan plots were generated based on code accessible via the R graph gallery. All boxplots were generated by ggplot2:geom_boxplot. A 95% Bayesian confidence interval (CI) for each QTL was calculated using the function find_peaks() in R/qtl2. Allele effects for each QTL were generated using the scan1blup() function of R/qtl2. SNP associations were performed using the scan1snps() function in R/qtl2_0.20 accessing variants from the database cc_variants.sqlite (available here: https://ndownloader.figshare.com/files/18533342) and genes from mouse_genes_mgi.sqlite (available here: https://ndownloader.figshare.com/files/17609252) via R/RSQLite_2.1.2. The data preparation and QTL mapping analysis are reproducibly documented in UNIX shell and R scripts posted on github (https://github.com/dmgatti/AttieMetabolomics). Code for data analysis and plotting is available at https://github.com/vanilink/DOLipids/ with input from Supplementary Tables S8 and S9. The genome-lipid associations are also accessible through an interactive web-based analysis tool that will allow users to replicate the analyses reported here (http://lipidgenie.com/). The source code for this resource can be found at https://

github.com/coongroup/LipidGenie.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Genotypes and additional phenotype data associated with the DO mouse population have been deposited with Dryad (doi:10.5061/dryad.pj105; data files: Attie Islet eQTL data). In addition, the data reported here are available for download and interactive web-based analysis at https://churchilllab.jax.org/qtlviewer/attie/islets. Genotyping used the Mouse Universal Genotyping Array (GigaMUGA; 143,259 markers).
Mass spectrometry data have been deposited in Chorus (http://chorusproject.org/) under ID 1610 (direct links to cell experiments https://chorusproject.org/anonymous/download/experiment/4984245205453479277, DO liver https://chorusproject.org/anonymous/download/experiment/a639bcc5602c441c9a1df94f4340d626, DO plasma https://chorusproject.org/anonymous/download/experiment/f8b273d222364f2a9d92cfdd0eb601b6, FS liver https://chorusproject.org/anonymous/download/experiment/c930cd419eb34dfebda7f53508c6969e, and FS plasma https://chorusproject.org/anonymous/download/experiment/9d4d025df0114687924d4075f3c927ca).
Human Mouse homologues were obtained from the MGI homology database (available here: http://www.informatics.jax.org/downloads/reports/HOM_MouseHumanSequence.rpt). SNP associations were performed accessing variants from the database cc_variants.sqlite (available here: https://ndownloader.figshare.com/files/18533342) and genes from mouse_genes_mgi.sqlite (available here: https://ndownloader.figshare.com/files/17609252).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	A sample size of four waves of 100 mice was chosen. A total of n=384 mice were sacrificed at ~22 weeks of age. This cohort of mice analyzed for this study existed and was previously described.[32, 33, 72] 32. Keller, M. P. et al. Genetic Drivers of Pancreatic Islet Function. Genetics 209, 335–356 (2018). 33. Keller, M. P. et al. Gene loci associated with insulin secretion in islets from nondiabetic mice. Journal of Clinical Investigation vol. 129 4419–4432 (2019). 72. Mitok, K. A. et al. Islet proteomics reveals genetic variation in dopamine production resulting in altered insulin secretion. Journal of Biological Chemistry vol. 293 5860–5877 (2018). For the cell validation experiments, a sample size of n=12 was chosen (n=3 biological replicates x n=4 technical replicates) for each mutant and control based on previous experience with the minimum samples size required for MS validation experiments. Sample size calculation was not performed, but deemed sufficient for the purpose upon observing statistical significance in data analysis.
Data exclusions	No data were excluded from the analyses.
Replication	"Although there is no biological replication of genetically identical animals in an outcross population, there is replication of genotypes at specific loci. This local genetic replication enables one to link phenotype with genotype, as in a human GWAS or QTL mapping studies in DO mice."[32] 32. Keller, M. P. et al. Genetic Drivers of Pancreatic Islet Function. Genetics 209, 335–356 (2018). In addition, the founder strain (FS) data set represents a way of confirming or supporting the gene-lipid associations found in the DO. Several of such instances are highlighted in the manuscript, while the full FS data set is provided in the supplement to enable others to use the founder strain data for additional examples not discussed in the manuscript. As noted above, cell validation experiments were performed in biological and technical replicate. For all MS experiments, subsets of samples were re-analyzed as technical replicates on the LC-MS platform and the results successfully reproduced (i.e. analysis reproducibility).
Randomization	Mice were allocated by waves of 100 each with an equal number of males and females. Samples were randomized into batches for sample preparation and randomized again for running on the LC-MS system. Cell samples were also randomized for sample preparation and randomized again for running on the LC-MS system.
Blinding	Blinding was not relevant to this study as there were no groups.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Rabbit anti-MYC antibody, CST, 2278 clone 71D10, lot: 5
Goat anti-rabbit-HRP conjugated antibody, CST, 7074S, lot: 24

Validation

The company validated the antibody and expects reactivity against all species since the tag is a humanized MYC that would not otherwise be present in our Hepa1-6 cells. In-house, we further validated by running untransfected control cells, and cells transferred with an untagged control plasmid (GFP) as comparison.

"Myc-tag (71D10) Rabbit mAb detects recombinant proteins containing the Myc epitope tag. The antibody recognizes the Myc-tag fused to either the amino acid or carboxy terminus of targeted proteins in transfected cells. During the production process, side by side comparisons are performed between the new antibody lot and the previous lot. New lots are not released for sale unless the performance is equal to or better than the previous lot in each application. In our tests of the #2278 antibody, we were able to detect Myc-Akt in our transfected cell lines. No signal was observed in the non-transfected controls.

The Myc epitope tag is widely used to detect expression of recombinant proteins in bacteria, yeast, insect and mammalian cell systems. [Munro, S. and Pelham, H.R. (1984) EMBO J 3, 3087-93.]"
See manufacturer's note here: https://www.cellsignal.com/products/primary-antibodies/myc-tag-71d10-rabbit-mab/2278

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Hepa1-6 cells (ATCC® CRL-1830)

Authentication

We did not authenticate in-house. Cells were purchased directly from ATCC by J. Simcox, and propagated for use in our lab.

Mycoplasma contamination

We did save scraped cell RNA to test for mycoplasma but could not run the qPCR due to the lab shutdown.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Equal numbers of male and female Diversity Outbred (DO) mice and the eight founder strains (C57BL/6J (B6), A/J, 129S1/SvImJ (129), NOD/ShiLtJ (NOD), NZO/HILtJ (NZO), PWK/PhJ (PWK), WSB/EiJ (WSB), and CAST/EiJ (CAST)) were all obtained from the Jackson Labs and have been previously described.[32,33,72] Briefly, all mice were housed within the vivarium at the Biochemistry Department, University of Wisconsin-Madison, and maintained on a Western-style high-fat/high-sucrose (HF/HS) diet (44.6% kcal fat, 34% carbohydrate and 17.3% protein) from Envigo Teklad (TD.08811) for 16 weeks. All mice were maintained in a temperature and humidity-controlled room on a 12 hr light/dark cycle (lights on at 6AM and off at 6PM), and provided water ad libitum. At ~22 weeks of age, mice were sacrificed following a 4 hr fast.

32. Keller, M. P. et al. Genetic Drivers of Pancreatic Islet Function. Genetics 209, 335–356 (2018).
33. Keller, M. P. et al. Gene loci associated with insulin secretion in islets from nondiabetic mice. Journal of Clinical Investigation vol. 129 4419–4432 (2019).
72. Mitok, K. A. et al. Islet proteomics reveals genetic variation in dopamine production resulting in altered insulin secretion. Journal of Biological Chemistry vol. 293 5860–5877 (2018).

Wild animals

The study did not involve wild animals.

Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experiments involving mice were preapproved by an AAALAC-accredited institutional Animal Care and Use Committee of the College of Agriculture and Life Sciences (CALS) at the University of Wisconsin-Madison. The CALS Animal Care and Use Protocol number associated with the study is A005821, A. D. Attie, Principal Investigator.

Note that full information on the approval of the study protocol must also be provided in the manuscript.