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Reporting Summary

Nature Portfolio 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 Portfolio 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	Imaging: CODEX Instrument Manager (CIM) v1.29.
Data analysis	Functional profiling: GraphPad Prism v9.3. Imaging: HALO v3.1, Metamorph v7.10, CODEX Processor v1.7.0.6, CRAN v0.2.7.0, GraphPad Prism v9.3, custom code for cell neighorhood analysis. Bulk RNA-seq: FlowJo v10.1.5, FastQC v0.11.8, fastq-mcf v1.05, cutadapt v2.5, STAR splice-aware aligner v2.5.4b, DESeq2 v1.28.0, RUVseq, RNA-enrich, REVIGO, Cytoscape v3.8.2, Metascape v3.5, gprofiler2, GARFIELD, AME v5.3.2, MEME-Suite. Multiome: HRC-1000G-check-bim.pl v4.2.9, Eagle2, Minimac4, STAR v2.7.3a, starSOLO, cta, BWA-MEM, Picard MarkDuplicates v2.25.1, SAMtoolsview, DropletUtils v1.6.1, Demuxlet, DecontX, Seurat, AMULET v1.0-beta, bedtools bamtobed, MACS2, UCSC bedGraphToBigWig, FIMO v5.0.4, cisBP v2.0, atactk, Single-Cell rEgulatory Network Inference and Clustering (SCENIC). Independent instrumental variables: islet eQTL analysis of InsPIRE (p-value<0.01; LD pruning with 50 SNP windows, 5 SNPs each step, and LD<0.2 by PLINK). Mendelian Randomization: R package MendelianRandomization (Egger, weighted median, debiased inverse-variance weighted methods) and Pleiotropy RESidual Sum and Outlier (PRESSO).

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw genotyping, bulk RNA-seq, and single nucleus multiome data is available via the European Genome-Phenome Archive (EGA; RRID:SCR_004944) as study EGAS00001006273. Raw imaging datasets are available from the authors upon request. Processed imaging data is available via Pancreatlas (RRID:SCR_018567; https://pancreatlas.org) and at https://doi.org/10.5281/zenodo.8125026, weighted gene co-expression network analysis at https://theparkerlab.shinyapps.io/Islet-RNAseq-WGCNA/, and processed pseudoislet multiome data at https://theparkerlab.med.umich.edu/data/public/cellbrowser/?ds=Pseudoislet10XMultiome. In addition, intermediate processed results (expression matrices, etc.) are available via Zenodo (https://doi.org/10.5281/zenodo.6515987). All datasets are linked from http://theparkerlab.org/manuscripts/2021_islet-rfx6/. Packages and algorithms used for transcriptomic and cell neighborhood analyses are published in Github repositories managed by Parker and Liu labs (https://github.com/ParkerLab; https://github.com/liu-bioinfo-lab).

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	Sample sizes for comparisons between nondiabetic (ND) and type 2 diabetes (T2D) groups were driven by availability of organs and tissue that met our predefined collection criteria. This study's sample size is larger than other similar comparisons of diseased human islet and tissue (PMIDs 27133132, 30713109, 30713110, 32739450, 34731614) and thus are sufficient to detect differences. For validation studies, sample size was selected based on previous studies using genetically-altered pseudoislets (PMIDs 32352931, 33446570). UK Biobank is a large nation-wide biobank study including genetic and medical data for over 500,000 participants aged 40-69. Summary statistics from T2D GWAS in UK Biobank performed in 19,119 T2D cases and 423,698 controls of European ancestry were downloaded from http://diagram-consortium.org/. Summary statistics from T2D GWAS in Vujkovic et al. meta-analysis (PMID 32541925) performed in 148,726 T2D cases and 965,732 controls of European ancestry were accessed from dbGAP (pha004945.v1.p1). The InsPIRE (Integrated Network for Systematic analysis of Pancreatic Islet RNA Expression) consortium consists of collected human islet tissue and genetic data from 420 participants. The human islet eQTL results were downloaded from https://zenodo.org/record/3408356#.Yo0B-5PMKCc.
Data exclusions	Functional profiling: in two cases, data was excluded due to evidence of sample degradation during storage; these perifusion traces were shown when normalized by islet volume but not by islet content. Imaging: tissue and islet areas were annotated by hand to exclude out-of-focus regions and poor tissue quality. Bulk RNA-seq: assessment criteria are described in Methods ('RNA isolation and bulk RNA-sequencing') and shown in Supplementary Fig. 2. Multiome: assessment criteria are described in Methods ('Selection of quality nuclei for downstream analysis' and 'Removal of ambient RNA counts') and shown in Supplementary Fig. 6.
Replication	For ND vs. T2D, replication was not possible given the unique nature of tissues; however, we validated the robustness of our findings through the use of a large donor cohort. Additionally, our multimodal approach allowed us to perform orthogonal validation of key findings using other experimental approaches. For validation studies, comparisons are a result of paired analysis across multiple independent nondiabetic donors.
Randomization	De-identified medical histories enabled stratification of human tissue specimens into age- and BMI-matched groups, nondiabetic (ND) and type 2 diabetes (T2D). Detailed information, including sample types and experimental usage for each case, is available in Supplementary Table 1. See also Results ('Identification, collection, and processing of short-duration T2D donor pancreata') and Methods ('Human subjects').
Blinding	Data were generated using quantitative methods that were applied uniformly to all samples regardless of group assignment. In cases where experimenter input was required (e.g., algorithm tuning for image analysis), sample group assignment was hidden in effort to mitigate any experimental bias.

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: All antibodies used in this study are detailed in Supplementary Tables 2-3.

Validation: All antibodies used in this study are commercially available and validated (see Supplementary Tables 2-3 for manufacturer information).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s): *State the source of each cell line used.*

Authentication: *Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*

Mycoplasma contamination: *Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*

Commonly misidentified lines (See ICLAC register): *Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance: *Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*

Specimen deposition: *Indicate where the specimens have been deposited to permit free access by other researchers.*

Dating methods: *If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight: *Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

Animals and other organisms

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

Laboratory animals: Mus musculus; immunodeficient NOD.Cg-PrkdcscidIl2rgtm1Wjl/Sz (NSG) male mice, ages 10 to 12 weeks, were used for human islet transplantations experiments, detailed in Methods ('Human islet transplantation'). All animal procedures were approved by the Vanderbilt Institutional Animal Care and Use Committees.

Wild animals: Study did not involve wild animals.

Field-collected samples: Study did not involve samples collected from the field.

Ethics oversight: Ethical approval and guidance was provided by the Institutional Animal Care and Use Committee at Vanderbilt University Medical Center.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	De-identified donor information is detailed in Supplementary Table 1.
Recruitment	Pancreata from nondiabetic (ND) (n=19) donors and those with type 2 diabetes (T2D) (n=20) were obtained through partnerships with the International Institute for Advancement of Medicine (IIAM), National Disease Research Interchange (NDRI), and local organ procurement organizations. Additional ND human islet preparations (n=27) were obtained through partnerships with the Integrated Islet Distribution Program (IIDP) and Alberta Diabetes Institute (ADI) Isletcore and served as assay-specific controls or were used for pseudoislet studies. De-identified donor information is detailed in Supplementary Table 1.
Ethics oversight	The Vanderbilt University Institutional Review Board declared that studies on de-identified human pancreatic specimens do not qualify as human subject research.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Isolated islets were dispersed with trypsin as detailed in Methods ('Purification of α and β cells by FACS').
Instrument	FACSAria III cell sorter (BD Biosciences)
Software	FlowJo v10.1.5
Cell population abundance	Percent endocrine cell population for each islet preparation used for cell sorting is provided in Supplementary Table 1 ('Estimated purity'). Purity by sorting strategy been established previously (PMID:30449685) and was confirmed by RNA-sequencing.
Gating strategy	Samples were assessed by forward scatter (FSC), side scatter (SSC), SSC and FSC pulse geometry, viability, and then positivity for cell-specific markers, as illustrated in Supplementary Figure 2a.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.