

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Single-cell sequencing data was collected using the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 and the Illumina NovaSeq 6000. qPCR data was obtained using the Bio-Rad CFX Opus 384 Real-Time PCR System. Western blot data was acquired using the Bio-Rad electrophoresis system and detected with the GE Amersham Imager 680. Calcium flux measurements were conducted using the Zeiss AXIO Observer, and fluorescence images were captured with the Zeiss LSM 900.
Data analysis	All software details are provided in the Methods section. Briefly, raw sequencing files were aligned using Cell Ranger vX or higher with the GRCh38-2020A genome reference. Data analysis was conducted in R v4.4.0 using Seurat v5.1.0, with visualization performed using ggplot2 v3.5.1 and statistical analysis with ggpvr v0.6.0. Pathway enrichment analysis was carried out with escape v2.2.2, referencing msigdb v7.5.1. For basic statistical analysis, dplyr v2.5.0 and tidyverse v2.0.0 were used. Loom file generation was performed with velocity v0.17.16, and RNA velocity analysis was executed in Python v3.12.7 using scanpy v1.10.4 and scvelo v0.3.2. For visualization, seaborn v0.13.2 and matplotlib v3.9.2 were employed. No custom code was used; however, the code for integration, trajectory analysis, and commonality analysis is publicly available at https://github.com/randystyle21?tab=repositories .

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](#)

All raw sequencing reads for the scRNA-seq and snRNA-seq data generated from isolated human islets for this study are compiled and available from NCBI SRA [PRJNA900882] (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE217837>) under the GEO accession code: GSE217837. The publicly available multiome data of snRNA-seq/snATAC-seq datasets on human islets that were used in this manuscript are from Gene Expression Omnibus (GEO; accession code [GSE1996363], samples [GSM5979678], [GSM5979679], [GSM5979680], [GSM5979681], [GSM7096627], [GSM7096628], [GSM7096629], and [GSM7096630]) as part of Augsnornworawat et al.'s study (34). Raw FASTQ data from the Human Pancreas Analysis Program (HPAP) database, consortia under the Human Islet Research Network (HIRN) were obtained from <https://hpap.pmacs.upenn.edu/> with provided bash script from HIRN-HPAP website. All single cell RNA sequencing data were aligned with Cell Ranger 8.0 or higher with GRCh-38-2020A reference genome. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	The dataset included 21 males and 10 females.
Reporting on race, ethnicity, or other socially relevant groupings	In our study, we did not collect or analyze data regarding the race or ethnicity of our donors.
Population characteristics	The characteristics of donors for human islets, pancreas tissue, and serum are provided in Supplementary Table 1.
Recruitment	Human islet samples were acquired from Prodo Laboratories and the IIDP at City of Hope. Human pancreas tissue was obtained from the IIDP at City of Hope. Human serum samples were collected from the IIDP at City of Hope and Medix Biochemica.
Ethics oversight	Our research complies with all relevant ethical regulations. This study was approved by the Institutional Review Board at City of Hope (IRB Protocol #23726, Ref #255725).

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

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 were limited by donor availability. Single-cell and single-nucleus RNA sequencing (sc/sn-RNAseq) were performed on islets from three donors, consistent with a previous publication from our lab (PMID: 37127706). Western blot analysis was conducted on islets from three non-diabetic and three T2D donors. qPCR was performed on islets from 3–5 non-diabetic donors, depending on the experimental design. Electron microscopy was conducted on islets from three non-diabetic donors. Immunofluorescence (IF) imaging was performed on pancreas tissue from four non-diabetic and four T2D donors. Human serum samples were collected from five non-diabetic and four T2D donors.
Data exclusions	For single-cell and single-nucleus RNA sequencing data, we excluded cells with fewer than 500 detected genes, fewer than 250 gene features, a log10 gene per UMI ratio below 0.8, and a mitochondrial gene content of 20% or higher. For doublet removal, we applied a stringent 20% doublet formation ratio as a hyperparameter and excluded cells identified as doublets by the DoubletFinder algorithm. A detailed discussion and specific filtering parameters are provided in the Methods section.
Replication	Replication was ensured by using multiple biological replicates and employing several orthogonal approaches to validate the reproducibility of our findings.
Randomization	Randomization was not applicable to this study, as we assessed differences between non-diabetic and T2D islet donors. No treatments were applied to these samples that would necessitate randomization.
Blinding	Blinding was not implemented in this study, as there were no human participants subject to potential 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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	The antibodies used in this study include SMOC1 (cat# ab313569, Abcam), FOXO1 (cat# 2880, Cell Signaling), C-peptide (cat# GN-ID4, DSHB), glucagon (cat# ab10988, Abcam), and actin (cat# ab0145-200, Origene).
Validation	All antibodies used in this study have been validated as shown on the supplier's website.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	EndoC-βH1 cells were obtained from Human Cell Design (Toulouse, France).
Authentication	No additional authentication was conducted.
Mycoplasma contamination	Mycoplasma testing confirmed negative results in the cell line culture.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A