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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	Moflo Astrios (Beckman Coutler) system on Summit v6.2 (Beckman Coutler) software. Seahorse XFe96 system on Seahorse Wave Controller Software 2.6 (Agilent) Data collection was mainly performed using Microsoft Excel.
Data analysis	Analysis of the FAC-sorting data was done on Kaluza Analysis v 2.0 (Beckman Coulter) software. Analysis of islet respirometry was done on Wave Software 2.6.1 (Agilent). For bluk RNA-seq, reverse transcription and cDNA amplification were performed using the SMARTer Ultra Low RNA kit (Clontech). cDNA libraries were prepared using Nextera XT DNA Sample Preparation kit (Illumina) and sequenced on an Illumina HiSeq4000 platform with single-end 100-bp. All sequencing data were uploaded to and aligned on the Galaxy project (PMID: 27137889) against Ensembl reference genome GRCm38.p6 (release 100) using STAR version 2.7.2b (PMID: 23104886) n 2-pass mapping mode. Aligned data were counted using HTSeq version 0.9.1 (PMID: 25260700) in union mode. Analyses were performed in a pair-wise manner between the four conditions analyzed using DESeq2 version 1.28.1 (PMID: 25516281). For each pairwise comparison, genes were discarded if they had fewer than 5 counts per sample on average. After calculating differential expression between groups, log2 fold changes were shrunken using the normal estimator (PMID: 25516281). Genes were considered to be differentially expressed if the absolute shrunken log2 fold changes were equal to or above 1, and adjusted p-values were equal to or below 0.05. Normalization and analysis of the qPCR data were done with the RT-PCR analysis_macro v1.1 (from the Genomic Platform, University of Geneva) using two normalization genes (Gapdh and β-actin).

In all experiments cells were manually counted and considered positive for a marker when one nucleus was clearly surrounded by the hormone.

Data analysis was performed using Microsoft Excel.

All statistical analyses were performed with GraphPad Prism v9.0.

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

The mouse β-cell bulk RNA-seq generated in this study have been deposited in the NCBI GEO database with the accession number GSE226479 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE226479). All sequencing data were uploaded to and aligned on the Galaxy project80 against Ensembl reference genome GRCm38.p6 (release 100) (https://www.ensembl.org/Mus_musculus/Info/Index). The unfiltered list of differentially expressed genes is provided as Supplementary Information. The β-ID gene list generated by van Gurp et al. 2022 (NCBI GEO database with the accession number: GSE150724) was used for analysis.

Source data for Figs. 1-6 and Extended Data Figures are provided with this paper in Source Data files, availability of associated source data is indicated in each figure legend.
Donor details for human samples are available in Extended Data Supplementary Table 4.

All data and materials used are available from the authors or from commercially available sources. This data is available in the Methods section.

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	We did not discriminate in sex/gender for human islet experiments. All information about human donors are described in Extended Data Table 4.
Reporting on race, ethnicity, or other socially relevant groupings	All information about human donors are described in Extended Data Table 4.
Population characteristics	All information about human donors are described in Extended Data Table 4.
Recruitment	We did not recruit human participants. All human islet samples are isolated from cadaveric donors at isolation centers.
Ethics oversight	All studies involving human samples were approved by ethical committee in University of Geneva.

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 size was chosen to ensure adequate power and to detect a pre-specified effect based on the available literature and protocols in the field. Therefore, sample sizes are comparable with the ones used in the published literature in the field. For multiple transgenics and human samples the numbers were also limited by the availability of the phenotype. No statistical methods were used to predetermine sample size.
Data exclusions	At the beginning of each experiment, mice must be (1) healthy, (2) normoglycemic, (4) bearing all the desired transgenes, (5) for age-matched controls we prefered, when possible, litter mates. These exclusion criteria were pre-established.

Replication	All experiments were performed independently and replicated at least once; except for the human experiments, which could only be performed once due to restricted number of material. All attempts at replication were successful.
Randomization	In all mouse experiments, we randomly selected in each litter the experimental and control animals. Co-variates such as the health status of the mice over the experimental design were considered for the data analysis. Mice were weekly controlled for body weight and glycemia. In the experiments using human donor samples, sample selections were not randomized. Only non-diabetic human donors were selected.
Blinding	Most of the experiments were performed blind. For some experiments (such as S961 treatment), blinding was not possible due to regular control of the hyperglycemia in the treated animals. In vitro experiments, investigators were blinded to group allocation during data collection and analysis.

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 primary antibodies used were: guinea pig anti-insulin (1/400; DAKO, A0564), rabbit anti-insulin (1/3000; Molecular Probes, 701265), chicken anti-insulin (1:200; Abcam ab14042); rabbit anti-insulin (1:300, Cell signaling, 3014), mouse anti-glucagon (1/1000; Sigma, G2654), rabbit anti-glucagon (1/200; DAKO, A0565), mouse anti-somatostatin (1/200; BCBC Ab1985), rabbit anti-somatostatin (1/200; DAKO, A0566), rat anti-somatostatin (1:200; Merck Mab354), mouse anti-Ppy (1/400; Santa Cruz, MAB62971), goat anti-Ppy (1/300; Novus Biologicals, NB 100-1793), goat anti-Glp1 (1:100; Santa Cruz sc-7782), rabbit andti-Ki67 (1:700, Epredia, RM-9106). Sections were also stained with DAPI. Secondary antibodies were coupled to Alexa fluorophores. Goat anti-rabbit Alexa 488 (1/500; Molecular Probes; 1070-03), donkey anti-goat Alexa 568 1/500; Molecular Probes; A-11057), goat anti-guinea pig Alexa 647 (1/500; Molecular Probes; A-21450). Goat anti-mouse TRITC (1/500; Southern Biotech, 1070-03) was also used.
Validation	All antibodies used were validated by the respective commercial source for the application used in this manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Sst-DTR mice were generated under a C57BL/6 background. Then, these Sst-DTR mice were crossed with Gcg-DTR and Ppy-DTR mice, with a mix background. All transgenic mice (non-beta-DTR mice) used in this study had a mixed background. 2-3 months old male and female mice were used for all experiments. Except for long-term following of alpha, delta and gamma-cell ablated animals, which were kept until one-year-old.
Wild animals	No wild animals were used in this study.
Reporting on sex	Most of the experiments were performed on male mice for simplicity in interpreting the results. However, females were also used to confirm the main phenotype observed in the study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	The study follows all ethical regulations regarding animal experimentation, all experiments were performed under the guidelines of the Direction General de la Santé du Canton de Genève (license numbers: GE/111/17, GE/121/17 and GE15820).

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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

Mouse RNA-Seq analyses. Islet cells were obtained from one month after DT treatment of Glucagon-DTR; Somatostatin-DTR; Ppy-DTR mice. Islet isolation and cell isolation by flow cytometry (FACS) were performed using previously described protocols (Chera et al 2014).

Human islet cell sorting. Samples were obtained from isolated islets from cadaveric donors at isolation centers. Samples were prepared using previously described protocols (Furuyama et al 2019)

Instrument

Cell sorting using FACS were performed using a Moflo Astrios (Beckman Coutler) system.

Software

Cell sorting was performed on Summit v 6.2 (Beckman Coulter) software. Kaluza Analysis v 2.0 (Beckman Coulter) software was used for subsequent analysis.

Cell population abundance

For mouse experiments. For validation of the purity, small fractions of sorted cells were FACS-sorted again to confirm the gating strategy.

For human experiments. For evaluation of cell purity, sorted islet cell fractions were immunostained for insulin, glucagon, somatostatin and pancreatic polypeptide. Stained cells were examined with a confocal microscope (Leica TCS SPE). Confirmation of the purity of beta-pseudoislets was performed for each donor by immunostaining and qPCR for each hormone. Only batches with high purity (> 98%) were used in following experiments.

Gating strategy

Single viable islet cells were gated by forward scatter, side scatter and pulse-width parameters and by negative staining for DAPI (Life Technologies) or DRAQ7 (B25595, BD Biosciences) to remove doublets and dead cells.
In mice experiments, boundaries between positive and negative were very clear because of very high expression of reporter proteins.
In human experiments, beta-cell population was gated on HIC1-2B4 and HIC3-2D12. The fraction was further purified based on CD9 and FSC/SSC.

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