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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	CSDiva v 8.0.1 (BD Biosciences) for sorting on a FACSAria2. Summit v 6.2 (Beckman Coulter) for sorting on a Moflo Astrios.
Data analysis	All statistical analyses were performed with GraphPad Prism6. Biological quality control and summarization of RNA samples were done with the RSeQC v2.4, the PicardTools v1.92 and the SamTools v0.1.18. Bioinformatic data were analised with the Ingenuty Pathway Analyses software (Ingenuity Systems, Redwood City, CA). Pathway analyses were performed with gene set enrichment analysis (GSEA, http://software.broadinstitute.org/gsea/index.jsp). Gene sets with significant enrichment in GSEA were identified among mm_GO of Gene Set Knowledgebase (GSKB). The normalization and differential expression analysis was performed with the R/Bioconductor package edgeR package v.3.20.9. For live imaging of cultured cells, images were captured manually at culture day 7 using Nikon Eclipse TE300 microscope (Nikon). Sections were examined with a confocal microscope (Leica TCS SPE). In all experiments cells were manually counted and considered bihormonal (glucagon+insulin+; somatostatin +insulin+) or coexpressing markers (i.e. insulin+YFP+) when one nucleus was clearly surrounded by both hormone / reporter staining.

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/reviewers upon request. 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

The RNA-seq data generated in this study have been deposited in the NCBI GEO database under accession number GSE109285. All data and materials used are available from the authors or from commercially available sources.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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 transplantation studies 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. For the analyses of the RNA-seq data sonly only one outliner data point was excluded due to incorrect genotyping (false negative).
Replication	For all experiments, all attempts at replication were successful.
Randomization	In the in vivo experiments, between members of the same litter we randomly selected the experimental animals and controls.
Blinding	No blinding was possible during treatment do to regular glycemia control.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	Antibodies used in this study are the following: Rabbit and guinea pig anti-Pdx1 (C.W. Wright, 1/5000 and 1/750 respectively), Rabbit anti-Nkx6.1 (BCBC AB1069, 1/800), Guinea pig anti-porcine insulin (DAKO, 1/400), Mouse anti-glucagon (Sigma, 1/1000), Mouse anti-somatostatin (BCBC Ab1985, 1/200) or Goat anti-somatostatin (Santa Cruz 7918 1:200), Rabbit anti-GFP (Molecular Probes, 1/400), Mouse anti-mCherry (Abcam ab125096, 1/500), Goat anti-Ihh (Santa Cruz sc-1196 1:50). Secondary antibodies were coupled to Alexa 405, 488, 647 (Molecular Probes), Cy3, Cy5 (Jackson Immunoresearch), or TRITC (Southern Biotech) and all used at a 1/500 dilution.

Validation	All antibodies used were validated by the respective commercial source for the application used in this manuscript.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	No cell lines were used in the manuscript.
Authentication	The cell lines were not authenticated.
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample.

Animals and other organisms

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

Laboratory animals	All transgenic mice used in this studies had a mixed background. 2-3 months old male and female mice were used for all experiments, except for transplantation experiments, where only males were used as hosts.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.

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	RNA-Seq analyses, islet isolation and cell isolation by flow cytometry (FACS) were performed using previously described protocols (Chera et al 2014). Purified cells were obtained as follows: - mature α-cells from 3-month-old Glucagon-Venus mice (whose glucagon-expressing α-cells also express the fluorescent reporter Venus). - α-cells 30 days after DT from Glucagon-Venus; RIP-DTR mice (whose glucagon-expressing α-cells express the fluorescent reporter Venus and β-cells can be ablated by DT administration), injected with DT at 2 months of age. - mature β-cells from 3-month-old Insulin-mCherry mice (whose insulin-expressing β-cells also express the fluorescent reporter mCherry). - α-cells ectopically expressing PDX1 from 3-month-old Glucagon-rtTA; TetO-Cre; R26YFP; CAG-Pdx1 (whose glucagon-expressing α-cells express the transcription factor PDX1 and the fluorescent reporter YFP upon DOX administration). 18 - α-cells expressing PDX1 30 days after DT from Glucagon-rtTA; TetO-Cre; R26YFP; CAG-Pdx1; RIP-DTR mice (in which α-cells express PDX1 and YFP after DOX administration, and β-cells can be ablated with DT).
Instrument	Cells were sorted on a FACSAria2 (BD Biosciences) or Moflo Astrios (Beckman Coulter) system.
Software	FACSDiva v 8.0.1 (BD Biosciences) for sorting on a FACSAria2. Summit v 6.2 (Beckman Coulter) for sorting on a Moflo Astrios. Kaluza Analysis v 2.0 (Beckman Coulter) for analysis.
Cell population abundance	For validation of the purity, small fractions of sorted cells were FACS-sorted again to confirm the gating strategy, and also evaluated by immunostaining, showing more than 99% abundance within the post-sort fractions.
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. Boundaries between positive and negative were very clear because of very high expression of reporter proteins.

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