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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.

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	FACS Aria, FACS Canto II, LSR II were used to collect FACS Data. Zeiss LSM 880 Rear Port Laser Scanning Confocal and Airyscan FAST Microscope were used for imaging. All open resource and software used for the study were described in the manuscript. Software or program listed below were used for genomic data analysis 1. R studio (https://www.rstudio.com/) 2. Cell Ranger R Kit (https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/rkit) ver 2.0.2 3. Seurat (https://satijalab.org/seurat/) ver 2.0 4. DAVID (https://david.ncifcrf.gov/home.jsp) 5. GOplot (https://wencke.github.io) 6. UCSC genome browser (http://genome.ucsc.edu) 7. Homer (http://homer.ucsd.edu/homer/)
Data analysis	All open resource, software and methods for analysis used for the study were described in the material and methods section of this manuscript. Graph data were analysed by Graphpad Prism 7 or Microsoft Excel. Software or program listed below were used for genomic data analysis 1. R studio (https://www.rstudio.com/) 2. Cell Ranger R Kit (https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/rkit) ver 2.0.2 3. Seurat (https://satijalab.org/seurat/) ver 2.0 4. DAVID (https://david.ncifcrf.gov/home.jsp) 5. GOplot (https://wencke.github.io) 6. UCSC genome browser (http://genome.ucsc.edu) 7. Homer (http://homer.ucsd.edu/homer/)

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. 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

RNA-Seq and ATAC-Seq data reported in this paper have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database, Accession # PRJNA505532.
- Fig. 1a-d; 2d,h,i; 3b
-Ext.Data Fig. 1a,i; 2a,b; 3j; 4c-e; 8a-i; 9a,b; 11a-d
-No restriction for data availability.

Source Data provided for all graph figures

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 were determined empirically based on preliminary experimental findings. For transplantation experiments in C57BL6J and NOD-SCID mice, preliminary experiments were performed with a minimum of 2 mice, and full cohort experiments repeated at least twice. The transplantation experiments in HuPBMC-NSG mice were repeated in more than 2 independent experiments. Experiment in Extended Data Fig.9f was performed once. HILOs generation has been repeated >50 times.
Data exclusions	On principle, data were only excluded for failed experiments. Failed experiments were determined by positive and negative control experiments.
Replication	Replicate experiments were successful. Every experiment was performed multiple times with essentially the same results
Randomization	Sex-matched littermates were randomly assigned to experimental arms. STZ treated mice were grouped based on serum glucose levels, and mice with the highest levels utilized for positive control experiments (i.e. mouse and human islet transplantations in Figures 3d and Extended 1g). Prior to transplantation, HILOs functionality was confirmed based on Insulin, MafA, UCN3, Esrrg and PD-L1 expression, determined by qpcr.
Blinding	No blinding was performed.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	The following antibodies were validated by previous publications as well as in our laboratory using IHC on human islets, mouse

islets and HILOs. All antibodies were used at a 1/100 dilution.

Validation

Antibody	supplier	Cat #
Insulin	abcam	ab7842
c-peptide	abcam	ab30477
glucagon	abcam	ab10988
somatostatin	abcam	ab103790
PD-L1	abcam	ab20592
PDX-1	R&D Systems	AF2419
CHGA	abcam	ab15160
SYNAPTOPHYSIN	Biogenex	MU363-UC
NKX6-1	DSHB	F55A12
Pancreatic Polypeptide	abcam	ab113694
MAFA	abcam	ab26405 (Also confirmed MAFA Novus NB400-1377)
MAFB	abcam	ab66506 (Also confirmed MAFA Bethyl LAb A700-046)

For signal amplification (for MAFA and MAFB) the goat anti-rabbit poly-HRP secondary antibody was used (comes already premixed in goat serum, B40962, Thermo) and developed with TSA-Cy3 reagent (SAT704A001EA, Akoya Biosciences) for 10min. All antibodies were used at a 1/100 dilution and validated by staining human primary islets.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

human induced pluripotent stem cells derived from HUVECs (Salk Stem Cell Core). HuES8 (Harvard University). H1ES (Wicells). hiPSC derived Beta like cells (TAKARA, ChiPSC12&ChiPSC22). HEK293LTV (Cell Biolabs).

Authentication

hiPSC derived Beta like cells from TAKARA are authentificated by TAKARA. HEK293LTV cells are authentificated by Cell Biolabs.

Mycoplasma contamination

The absence of mycoplasma contamination was routinely confirmed at ~2 month intervals.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals

Age- (8 - 20 weeks) and background-matched male C57BL/6J (Stock No 000664), NOD-SCID mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, Stock No 005557), β cell-specific ERRγ knockout mice, hu-PBMC-SGM3 mice (Called as humanized mice. Female NSG™ mice were injected with human peripheral blood mononuclear cells in NSG-SGM3 (Jackson 013062) strain)

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 animal studies were reviewed by Salk IACUC for approval. Human islets studies were reviewed by IIDP for approval.

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

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

Sample preparation is described in Methods.

Instrument

FACS Aria, FACS Canto, LSR II

Software

FACS Diva for collection and FlowJo for analysis

Cell population abundance	Purity was assessed by staining for CD45 and GFP expression
Gating strategy	Relevant gating strategies are shown in Methods

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