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Reporting Summary

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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	FACSDiva v 8.0.1 (BD Bioscience) for cell-sorting on FACSAria2. Summit v 6.2 (Beckman Coulter) for cell-sorting on Moflo Astrios. Confocal image acquisition was performed with Leica LAS AF v 2.7.9723.3.
Data analysis	Data were analysed with GraphPad Prism7 or Microsoft Excel. For omics analyses, FASTQC v.0.11.2, TopHat v2.0.13, RSeQC v2.4, PicardTools v1.80, RStudio v.1.0.153 (with R v.3.4.3), R/Bioconductor package TCC v.1.16.0, cellrangerRkit v.2.1, Seurat v.2.3.2, Monocle 2. ggplot2 v.2.2.1, scatterplot3d v.0.3-41, RColorBrewer v.1.1-2 were used alongside base packages for the statistical analysis and visualisation. For pathway analysis, Ingenuty Pathway Analyses software (Ingenuity Systems, Redwood City, CA), gene set enrichment analysis (GSEA, http://software.broadinstitute.org/gsea/index.jsp) were used. For FACS analysis, Kaluza analysis v.1.2 (Beckman) was used. For live imaging of cultured cells, images were captured manually at culture day 7 using Nikon Eclipse TE300 microscope (Nikon). All information about software is reported in the material and methods section of the manuscript.

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

RNA-Seq data that support the findings of this study have been deposited in NCBI's Gene Expression Omnibus and accessible through GEO series accession code: GSE117454 (bulk RNA-Seq) and GSE123844 (scRNA-Seq). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD011933.

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	No statistical method was used to predetermine 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. However, the sample numbers in the transplantation experiments to rescue diabetic mice were limited by the availability of the human donor samples in the limited time-window. Instead of simple replication, we performed 2 independent experiments using total 9 donors' samples.
Data exclusions	In STZ- or DT-mediated diabetic mouse models, only mice exhibiting hyperglycemia (> 20 mM) were used. We used only pure alpha-, beta- or gamma-cell fractions with over 90 % purity. All purity data are shown in Supplementary Table 2 and Extended Data Fig. 1b. These exclusion criteria were pre-established.
Replication	All the data presented in this study were reproduced, with the exception of gamma-cell sorting. As we mentioned and described all sorting data in Extended Data Fig.1b,c, we were able to obtain gamma-cell fractions (>90%) from only some donors (12/42), probably due to the difference between donor islet preparations which we received (all donor information: Supplementary Table 1). The exact number of replicates for each experiment is indicated in figure legends and Supplementary Tables.
Randomization	For all animal experiments, experimental and control animals were chosen at random from the age-/sex-matched colonies . All experiments for diabetic induction were performed with healthy animal controls. In the experiments using human donor samples, sample selections were not randomized.
Blinding	In vitro experiments, investigators were blinded to group allocation during data collection and analysis. But in some experiments of in vivo transplantation, only one mouse was allocated to alpha-PM grafted group because of limited availability of the human donor samples, which caused the difficulty in blinding.

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	Primary antibodies used were: guinea pig anti-porcine insulin (A0564, DAKO, 1:600), chicken anti-insulin (GW10064F, Sigma, 1:1000), mouse anti-glucagon (G2654, Sigma, 1:1000), rabbit anti-glucagon (A0565, DAKO, 1:600), rabbit anti-somatostatin (A0566, DAKO, 1:600), rabbit anti-pancreatic polypeptide (T-4088, PenLabs, 1:750), goat anti-ghrelin (sc-10368, SantaCruz, 1:200), rabbit anti-GFP (Life Technologies, 1:500), chicken anti-GFP (ab-13970, Abcam, 1:500), guinea pig anti-Pdx1 (gift from C. Wright, 1:1000), rabbit anti-MafA (A300-611A, Bethyl, 1:500), rabbit anti-Nkx6.1 (BCBC, 1:400), rabbit anti-pHH3 (06-570, Upstate, 1:500), rabbit anti-CD31 (ab28364, abcam, 1:50), rabbit anti-Vimentin (ab92547, abcam, 1:100), rabbit anti-Tyrosine hydroxylase (ab152, chemicon, 1:1000), guinea pig anti-ARX(AB2834, BCBC, 1:100), rabbit anti-Synaptophysin (A0010, DAKO, 1:50), anti-GCGR monoclonal antibody (A-9, Eli-Lilly, United States patent US 8158759 B2), biotinylated anti-CD9 (13-0098-80, eBioscience, 1:2), mouse anti-HIC1-2B4 (HPi2) antibody (M.Grompe lab, 1:3), mouse anti-HIC3-2D12 (HPa3) antibody (M.Grompe lab, 1:3) . Secondary antibodies were coupled to Alexa 405, 488, 568, 647 (Life Technologies), FITC, Cy3, Cy5 (Jackson Immunoresearch), TRITC (Southern Biotech), Streptavidin PE (12-4317-87, eBioscience, 1:150), APC-AffiniPure Goat Anti-Ms IgG (115-135-164, Jackson, 1:400).
Validation	All antibodies used were validated by the respective commercial source for the application used in this manuscript.

Animals and other organisms

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

Laboratory animals	As described in method section, NOD.Cg-Prkdc scid Il2rg tm1Wj/SzJ (abbreviated as NSG) mice were obtained from Charles River. NSG RIP-DTR mice were generated in the Jackson Laboratory by backcrossing the RIP-DTR allele from B6-background animals (Hprt tm1(Ins2-HBEGF)Herr). For STZ-diabetic models, male NSG mice (2-4-month-old) were used. For DT-diabetic models in Exp.#3, male NSG-RIP-DTR mice (2-4-month-old) were used, but female mice (2-4-month-old) were used in Exp.#4.
Wild animals	No wild animals were used.
Field-collected samples	No field-collected samples were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics	All information about human donors are described in Supplementary Tables 1 and 22.
Recruitment	We did not recruit human participants. All human islet samples are isolated from cadaveric donors at islet isolation centers.

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	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	Cells were sorted on a FACSAria2 (BD Biosciences) or Moflo Astrios (Beckman Coulter) system.
Software	FACSDiva (BD Biosciences) for sorting on a FACSAria2. Summit (Beckman Coulter) for sorting on a Moflo Astrios. For analyses, Kaluza analysis (Beckman) was used.
Cell population abundance	For evaluation of cell purity, sorted islet cell fractions were immunostained for insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin. Stained cells were examined with a confocal microscope (Leica TCS SPE). Only batches with high purity (> 90%) were used to analyze in following experiments (see Extended Data Fig. 1b and Supplementary Table 2).
Gating strategy	As previously published (Dorrell et al., Nat Commun 2016) and shown in Extended Data Fig. 1a, single viable islet cells were gated by forward scatter (FSC), side scatter (SSC) and pulse-width parameters and by negative staining for DAPI (D1306, Invitrogen) to remove doublets and dead cells. Alpha-/beta-/gamma-cell fractions were gated on HIC1-2B4 and HIC3-2D12. Alpha-cell fraction was further selected based on CD9 and FSC/SSC.

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