

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 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 | Histology: BZ-X710(Keyence), LSM710 NLO(Zeiss), JEM-1400 Flash transmission electron microscope (JEOL)
scRNA-seq: Single Cell 3' Reagent Kits (version 3.1; 10X Genomics). The libraries were then sequenced on a NovaSeq 6000 (Illumina) using paired-end sequencing.
RNA-seq: RNA-seq libraries were generated using the NEBNext Ultra II Directional RNA Library Prep kit for Illumina (NEB). RNA-seq libraries were 684 sequenced on a NextSeq 550 (Illumina) as 86 bp single reads. |
| Data analysis | All statistics were performed using GraphPad Prism 8 ver 8.0(GraphPad Software Inc., La Jolla, CA)
BZ-II analysis software (Keyence)
Genomic data analysis:
R v4.1.1(https://www.r-project.org)
Cell Ranger R kit v3.1.0 and v6.1.1(https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/rkit)
Seurat v3.1.5 and v4.0.2(https://satijalab.org/seurat)
Monocle3 v1.0.0(https://cole-trapnell-lab.github.io/monocle3/)
STARv2.7.8a
cutadapt-3.0(https://cutadapt.readthedocs.io/en/v3.0/)
HTSeq-count v 0.11.2(https://htseq.readthedocs.io/en/master/)
DESeq2 v1.30.0 |

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 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 scRNA-seq data have been submitted to GEO(GSE163947).
The RNA-seq data have been submitted to GEO(GSE178726).

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](https://www.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	No statistical method was used to predetermine sample size. Sample size was chosen based on previously published literatures of the same subject. Biological replicates were at least n = 3 unless otherwise noted. For in vivo study, sample sizes were chosen based on a evaluation of animal ethics versus ability to obtain sufficient samples for statistical analysis. All sample sizes are indicated in the figures legends.
Data exclusions	In mouse scRNA-seq analysis, cells with nfeature > 1,500, nCount between 5,000 and 75,000, and low mitochondrial gene expression (<20%) were analysed. For human scRNA-seq analysis, cells with nfeature > 4,000, nCount between 20,000 and 100,000, and low mitochondrial gene expression (<10%) were utilized.
Replication	All attempts for data replication in in vivo and in vitro conditions were successful with a minimum of three independent biological replicates.
Randomization	For in vivo studies, groups were allocated based on their genotypes. Human and mouse cultures were randomly assigned to treatment groups.
Blinding	The investigators were not blinded to allocation during experiments or analysis, as group allocation was clearly visible in the samples due to phenotypic changes.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Primary antibodies guinea pig polyclonal anti-Insulin (Dako, #IR002)
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mouse monoclonal anti-Glucagon (Santa Cruz Biotechnology, #sc-514592, dilution 1/300)
 rabbit monoclonal anti-Glucagon (Abcam, #ab92517, dilution 1/300)
 mouse monoclonal anti-Somatostatin (Santa Cruz Biotechnology, #sc-55565, dilution 1/300)
 mouse monoclonal anti-Pancreatic polypeptide (Santa Cruz Biotechnology, #sc-514155, dilution 1/300)
 goat polyclonal anti-Pancreatic polypeptide (Abcam, #ab77192, dilution 1/300)
 mouse monoclonal anti-mCherry (Abcam, #sab125096, dilution 1/300)
 rabbit polyclonal anti-mCherry (Abcam, #ab167453, dilution 1/300)
 rabbit monoclonal anti-Ki67 (Abcam, #ab16667, dilution 1/100)
 rabbit polyclonal anti-Cleaved Caspase-3 (Cell Signaling technology, #9661, dilution 1/300)
 Chicken polyclonal anti-GFP (Abcam, #ab13970, dilution 1/300)

Secondary antibodies

CF488A anti-mouse IgG (Biotium, #20014, dilution 1/500)
 CF488 anti-chicken IgG (Biotium, #20166, dilution 1/500)
 CF555 anti-rabbit IgG (Biotium, #20038, dilution 1/500)
 CF555 anti-guinea pig IgG (Biotium, #20276, dilution 1/500)
 CF555 anti-rabbit IgG (Biotium, #20039, dilution 1/500)
 CF647 anti-rabbit IgG (Biotium, #20047, dilution 1/500)
 CF647 anti-mouse IgG (Biotium, #20046, dilution 1/500)
 Histofine Simple Stain Mouse MAX PO (R) (Nichirei Biosciences #414341).

Validation

The antibodies used are commercially available and validated in their websites as well as published peer-review journals.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

ESCs were generated in this study or obtained from Open Biosystems.

MEF were generated in this study.

HEK293T cell line were purchased from RIKEN(RBRC-RCB2202)

Authentication

Cell lines were not authenticated

Mycoplasma contamination

Cell lines were tasted regularly using e-Myco Mycoplasma PCR Detection Kit and were tested negative.

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

The animals were housed under a 12/12 hours dark/light cycle with a temperature of 20-24 °C and a humidity of 45-65%. Aged male C57BL/6J mice (26- to 28 months old) were purchased from CLEA Japan.

Ins1-CreERT2 mice and Rosa-mTmG reporter mice were obtained from Jackson Laboratories.

Myc1 knockout mice were developed by CRISPR/Cas9-mediated zygote genome editing in this laboratory.

Chimeric mice were obtained by injection blastocyst with gene-manipulated ESCs. Briefly, 8-week-old female ICR mice (Japan SLC) were super-ovulated by sequential intraperitoneal administration of 7.5 U eCG (Serotropin, ASKA Animal Health) and 7.5 U hCG (Gonotropin, ASKA Animal Health) 48 hours apart, and then mated with mature male ICR mice (Japan SLC) to obtain embryos. Mated females were sacrificed on the next day after vaginal plugs were observed and two-cell embryos were collected by perfusion of their oviducts with mWM. The collected embryos were cultured in KSOM (Merck-Millipore) for 2 days to permit blastocyst development. Six-to-ten ESCs were injected into a single blastocyst, and the injected blastocysts were transferred into the uteri of 20 pseudopregnant ICR surrogates (20-to-25 blastocysts per individual female) at 2.5 days after coitus to obtain chimeric offspring.

To induce the systemic expression of MYC family genes in vivo, 4-week-old mice were administered 2 mg/mL Dox (Sigma) in drinking water, which was also supplemented with 5 mg/mL sucrose (Nacalai Tesque), for 4 weeks (2-4 weeks for Myc induction). Eight-week-old KH2-Myc1 mice were administered 2 mg/mL Dox (Sigma) in their drinking water without sucrose supplementation to eliminate the effect of sucrose on their blood glucose concentrations.

In this study, only male mice were used for the measurement of islet sizes and blood glucose concentrations. Other experiments including lineage tracing experiments were conducted using both male and female mice.

Wild animals

No wild animals were used in this study.

Field-collected samples

The study did not include field collected samples.

Ethics oversight

All the animal experiments were approved by the Animal Experiment Committee at IMSUT, and animal care was conducted in accordance with institutional guidelines.

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