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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	A Zeiss Axio Observer Z.1 microscope and an inverted Zeiss LSM 780 confocal microscope were used for immunofluorescence imaging and iDISCO-cleared small tissues. An ultramicroscope II (LaVision BioTec, Bielefeld, Germany) was used for iDISCO-cleared pancreas. SpectraMax i3X, Molecular Devices, was used for ELISA detection. A Bayer Contour Glucometer was used to obtain blood-glucose values.
Data analysis	GraphPad Prism 8 software R (ver.8.4.2) was used for statistical analysis and graphic plotting. ImageJ (ver 1.52P) was used for transfection analysis (using the 'Analyze Particle function') and dual pancreas/liver transduction (JaCOP plugin). Imaris Software, Bitplane, ver.9.31-6.5.1 was used for 3D imaging segmentation (using 'Surface Tool' and 'Sports Tool'), analysis and video preparation. SoftMax Pro 7.0 was used for ELISA quantification.

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 main data supporting the findings of this study are available within the paper and its Supplementary Information. The raw and analysed datasets generated during the study are too large to be publicly shared, yet they are available for research purposes from the corresponding author on reasonable request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	The study did not involve human research participants.
Population characteristics	–
Recruitment	–
Ethics oversight	–

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	For the mapping of pancreatic innervation, at least 4 or 5 animals were used, on the basis of previously published studies (Fasanella et al. 2008, Quinson et al. 2001, Makhmutova et al. 2019). For AAV serotypes and delivery-route analysis, 5–7 animals were used, on the basis of previously published studies (Mason et al. 2010). For the in vitro Studies, 3 independent replicates were done per condition, as per previously reported studies on cell transfection (such as Takasugi et al. 2013). For in vivo metabolic studies, 7–16 mice were used, to detect statistical differences between groups (statistical power 0.8; effect size = 1.2 standard deviations; statistical significance, 0.05; two tailed) and based on previously published results (Moak et al. 2014, de Leon et al. 2018, Raun et al. 2020). For parasympathetic nerve ablation, 4 or 5 mice were used for the detection of statistical differences on the innervation density. Animal numbers were based on previously reported studies (Machado et al, 2020).
Data exclusions	Animals receiving misplaced injections were not included in the analysis.
Replication	All in vitro experiments were performed at least three times, for independent confirmation of the results. Viral tracing and metabolic animal studies were done at least using two cohorts. Information about replicates is included in the relevant figure legends. All attempts at replication were successful.
Randomization	All samples and all animals from the same litter were randomly allocated into experimental groups.
Blinding	Blinded data collection and analyses were conducted for the liver-de-targeting studies, for the in vivo metabolic analysis (the animals' IDs were decoded during the assays), for the Imaging analysis to compare viral titers, and for pancreatic innervation after targeted ablation. In vitro data acquisition could not be blinded because of the need to label the samples. Nevertheless, acquisition and analysis were performed using the same parameters for all the samples.

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

Methods

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

Antibodies

Antibodies used

For immunofluorescence staining the following primary antibodies were used: mCherry (Abcam, Cambridge, GBR; ab205402, Lot#GR3176028-11 dilution 1:1000), synapsin1 (D12G5) (Cell Signaling, Danvers, MA; 5297S, lot#07/2019, dilution 1:1000), GFP (AVES, Tigard, OR; GFP-1020 , Lot# 697986, at 1:1000 dilution), tyrosine hydroxylase (TH) (Millipore, Burlington, MA; AB152 , Lot#3328928, at 1:500 dilution), CTβ (Abcam, ab34992, Lot# GR3210643-1, at 1:500 dilution), Insulin (R&D, MAB1417, Lot# IDM0218121, at 1:1000 dilution)Vesicular Acetylcholine Transporter, Synaptic Systems, 139103 , Lot# 4-55, at 1:500 dilution). Somatostasin (SST, R210-01 ImmunoStar), Glucagon (SIGMA, G2624, Lot#0000080612), Gastrin Release Peptide, GRP (ImmunoStar, #20073, Lot#1420001), and Vasoactive Intestinal Peptide, VIP (ImmunoStar, #20077, Lot#2003001) at 1:500 dilution; cFOS (Abcam, # ab190289) at 1: 50. The secondary antibodies were: Alexa Fluor 647 anti-rabbit (Jackson ImmunoResearch, West Grove, PA; 711-605-152, Lot#139046), Alexa Fluor 546 anti-rabbit (Thermo Fisher Scientific; A10040, Lot#1946340), Alexa Fluor 647 anti-chicken (Jackson ImmunoResearch; 703-605-155, Lot#138591), Alexa Fluor 594 anti-mouse (ThermoFisher Scientific; A21203, Lot#1820087) ,Alexa Fluor 488 anti-guinea pig (Thermofisher Scientific; A11073, lot#2087691)and/or Alexa Fluor 488 anti-Rat (Thermofisher Scientific; A-21208, Lot#2092264) . All secondaries were diluted at 1:1000, with the exception of cFOS staining (Alexa Fluor 647 anti-rabbit at 1:50 dilution).

Validation

The information on antibody validation was taken from manufacturer's website(s), or through direct communication with the companies, as follows:

mCherry: Recombinant full length protein (His-tag) corresponding to mCherry. The immunogen sequence is from a following reference. Shaner NC et al. Nature Biotechnology 22:1567-1572 (2004).

Synapsin-1: (D12G5) XP® Rabbit mAb detects endogenous levels of total synapsin protein. The antigen is 100% conserved between human synapsin-1a and synapsin-1b. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gln483 of human synapsin-1 protein. Relevant Citations: Wang Q et al 2022, Chen J et al 2021. According to Manufacturer's communication: 'Validation testing can include any of the following or combinations of the following: 1) Analysis of a large panel of cell lines with known target expression levels (high vs low expressors), 2) Treatment of cells with appropriate kinase-specific activators and/or inhibitors, 3) Phosphatase treatment 4) Correct subcellular localization or treatment-induced translocation 5) Comparison of results with antibody and isotype control to ensure acceptable signal-to-background ratio 6) Target-specific signal verified in transfected cells, knockout cells, or siRNA-treated cells 7) Blocking with antigen peptide to confirm elimination of specific signal.

GFP: According to manufacturer's website, Chickens were immunized with purified recombinant green fluorescent protein (GFP) emulsified in Freund's adjuvant. After multiple injections, eggs were collected from the hens, and IgY fractions were prepared from the yolks and then affinity-purified antibodies were prepared using GFP conjugated to an agarose matrix. The final product is a filter-sterilized mixture of both affinity-purified antibodies (30 µg/mL) and purified IgY (10 mg/mL). To validate the product, manufacturer states: Antibodies were analyzed by western blot analysis (1:5000 dilution) and immunohistochemistry (1:500 dilution) using transgenic mice expressing the GFP gene product. Western blots were performed using BlokHen® (Aves Labs) as the blocking reagent, and HRP-labeled goat anti-chicken antibodies (Aves Labs, Cat. #H-1004) as the detection reagent. Immunohistochemistry used tetramethyl rhodamine-labeled anti-chicken IgY. Anti-Tyrosine Hydroxylase Antibody detects level of TH and has been published and validated for use in ELISA, IF, IH, IH(P), IP and WB. Manufacturer's website states that this antobody is routinely evaluated by Western Blot on PC12 lysates.

CTb: Rabbit polyclonal to beta subunit Cholera Toxin. Relevant citations: Song SY et al 2020, Sieveritz Bet al 2020, Lan H et al 2020. Insulin: Immunogen to E. coli-derived recombinant human Insulin. Detects bovine, human, and mouse insulin. Relevant citations: Rui J et al 2021, Elizondo DM et al 2020.

VACHT: According to manufacturer's website, This antibody detects the glycosylated and unglycosylated protein and is an excellent marker for cholinergic axons. Reacts with: human (Q16572), rat (Q62666), mouse (O35304), pig. Other species not tested yet. Specific for VAChT. (K.O. verified). Communication from the manufacturer: 'The antibody has been tested transfected and untransfected cells and only stains VachT transfected cells. It shows a band of expected molecular weight that is enriched in synaptic vesicle fraction. It shows the expected staining patter on cultured neurons and tissue sections.It has been KO validated for WB in this publication: https://pubmed.ncbi.nlm.nih.gov/24027290/'.Relevant citations: Martin-Silva C et al 2011, Teixiera VP et al 2020.

SOMATOSTATIN:According to manufacturer's website, The specificity of the antiserum was examined by soluble preadsorption with the peptides at a final concentration of 106 M. Somatostatin immunolabeling was completely abolished by preadsorption with somatostatin, somatostatin 25, and somatostatin 28. Preadsorption with the following peptides resulted in no reduction of immunostaining: substance P, amylin, glucagon, insulin, neuropeptide Y, and VIP. Relevant citations are: Chiazza F et al, 2021, An J.J et al 2020, Valbuena S et al 2019.

VIP: According to manufacturer's website, The specificity of the antiserum was examined by soluble pre-adsorption with the peptides in question at a final concentration of 10-5 M. VIP immunolabeling was completely abolished by pre-adsorption with VIP. Pre-adsorption with the following peptides resulted in no reduction of immunostaining: Secretin, gastric inhibitory polypeptide, somatostatin, glucagon, insulin, ACTH, gastrin 34, FMRF-amide, rat GHRF, human GHRF, peptide histidine isoleucine 27, rat pancreatic polypeptide, motilin, peptide YY, substance P, neuropeptide Y, and CGRP. Relevant citations are: Mukherjee A et al 2021, Ferraro S et al 2021,

GRP: The ImmunoStar Gastrin Releasing Peptide antiserum was quality control tested using standard immunohistochemical methods. The antiserum demonstrates strongly positive labeling of rat dorsal horn of spinal cord using indirect immunofluorescent and biotin/avidin-HRP techniques. Recommended primary dilution is 1/1,000-1/2,000 in PBS/0.3% Triton X-100 – Bn/Av-HRP Technique. Staining is completely eliminated by pretreatment of 1 mL of diluted antibody with 50 µg of bombesin. Relevant citations: Mou H et al 2021, Hamnett R et al 2021.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human embryonic kidney cells (HEK 293T) (ATCC CRL-3216, mycoplasma testing and STR profiling performed by ATCC). Neuro2A (ATCC CCL-131, mycoplasma testing and STR profiling performed by ATCC). AML12 cells (ATCC CRL-2254, mycoplasma testing and STR profiling performed by ATCC).
Authentication	STR analysis was performed by ATCC on all the human cell lines that we used.
Mycoplasma contamination	We did not test the cell lines for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	Male and female WT C57BL/6J, heterozygous ChAT-IRES-Cre, and Snap25-2A-GCaMP6s-D on a C57BL/6 background (Jackson Laboratories; #000664, #028861 and #025111, respectively) aged 8–12 weeks were used, and maintained with access to food ad-libitum. All mice were housed in a temperature-controlled environment (20–22 Celsius, 50–60% humidity) with twelve hours of light per day at the Center for Comparative Medicine and Surgery (CCMS) at the Icahn School of Medicine at Mount Sinai (New York, NY, USA).
Wild animals	The study did not involve wild animals.
Reporting on sex	Sex was considered in the study design for the initial in vivo metabolic studies. Male and female mice were age-matched and were subjected to the same experimental protocol. Data from chemogenetic stimulation of pancreatic parasympathetic nerves were disaggregated for sex. Only males presented significant differences. Therefore, the remaining of metabolic studies were performed in males.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal studies were performed with the approval of, and in accordance with, guidelines established by the Icahn School of Medicine at Mount Sinai, and principles of laboratory animal care were followed. The IACUC protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai.

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