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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	Confocal microscopy images were acquired with Zeiss Zen v2.3 SP1 (black edition) and Leica Application Suite (LAS) X v3.5.2.18963 software. Fura-2 calcium recordings were acquired with Metafluor software v7.7.3.0 and Zeiss Zen v2.61 (blue edition). Western blots were scanned using LI-COR Image Studio v5.2.
Data analysis	Statistical analysis was performed in Graphpad Prism 8. Images were analyzed with the FIJI distribution of ImageJ (NIH) v1.52p, JACoP (Just Another Colocalization Plugin for ImageJ), v.2.1.1. Single cell RNA-seq analysis was performed in R v3.3.1 and Bioconductor v3.4 with package Scran: methods for single-cell RNA-Seq Analysis v.1.2.2.

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

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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 sizes were chosen based on power analyses taking into account expected variance within and between groups. In other cases (ie. the number of donors tested), the maximum number of (as in all) islet donors we could obtain over the time period of the study were tested. In instances of microscopy image analysis, a power analysis was often not possible as the variance was unknown in advance, thus reasonable sample sizes (value of n is listed on all figure legends) to achieve statistical power were chosen based on the prior experience of the investigators working with this type of data.
Data exclusions	The only data to be excluded from analysis were occasional biosensor cell calcium recordings when there was an insufficient biosensor cell response to allow for data quantification due to ineffective sample preparation. This exclusion criterion was pre-established.
Replication	All attempts at replication were successful. Of note, GABA pulses were replicated by two independent labs using different equipment, islets, and biosensor cells. The glucose-independence of aggregate GABA release was replicated by three different labs. Reproducibility of the experimental findings was verified by three or more independent experiments.
Randomization	Within experiments, islets were aliquoted equally from a stock culture into random individual groups. Coverslips containing monolayer cultures of rat hippocampal neurons from the same tissue preparation were randomly assigned to individual groups.
Blinding	Investigators were blinded to group allocation during data analysis. It was not always possible or practical for investigators to be blinded to donor type (type 2 diabetic, etc) during data collection as human islets were received from one donor at time.

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 utilized for immunofluorescence staining:
insulin | gp | polyclonal | Linco #4011-01 | 1:10000 | lot No not available
insulin | gp | polyclonal | Dako #A0564 | 1:1000| lot No 10123176
insulin | ck | polyclonal | Abcam #ab14042 | 1:2000 | lot No GR177519-2, GR177519-8
glucagon | sh | polyclonal | Abcam #ab36232 | 1:500 | lot No GR288569-3
somatostatin | sh | polyclonal | Abcam #ab35425 | 1:200 | lot No GR-59464-1
pancreatic polypeptide | rb | polyclonal | Abcam #ab113694 | 1:1000| lot No not available
GABA | rb | polyclonal | Sigma-Aldrich #A2052 | 1:500 | lot No 112M4768, O65M4818V
GAD65 C-term (GAD6) | ms | monoclonal | in-house RRID #AB_2314499 | 1:1000 | lot No not available
GAD65 N-term | ms | monoclonal | Christiane Hampe Lab | 1:300 | lot No not available
GAD65 | rb | polyclonal | Synaptic Systems #198102 | 1:2000 | lot No not available
GAD65 | gp | polyclonal |Synaptic Systems #198104 | 1:500 | lot No not available
TauT | rb | polyclonal | Sigma-Aldrich #HPA015028 | 1:200 | lot No A89837
VGAT Oyster 650-labeled | rb | polyclonal | Synaptic Systems #131103C5 | 1:1000 | lot No 131103C5/1
synaptophysin | rb | polyclonal | Abcam #ab14692 | 1:250 | lot No not available
SV2C | rb | polyclonal | Synaptic Systems #119202 | 1:500 | lot No 11920/4
4F2HC (CD98) | rb | polyclonal | Santa Cruz Biotech #sc9160 | 1:250 | lot No i1014

LAT2 (SLC7A8) | ms | monoclonal, clone #UMAB70 | OriGene #UM500058 | 1:100 | lot No not available
Bestrophin 1 | rb | polyclonal | Abcam #ab14928 | 1:250 | lot No not available
GAT1 | rb | polyclonal | Synaptic Systems #274102 | 1:500 | lot No 274102/1
GAT2 | rb | polyclonal | Abcam #ab2896 | 1:200 | lot No GR139542-1
GAT3 | rb | polyclonal | Synaptic Systems #274303 | 1:500 | lot No 274303/1
GCP60 | rb | polyclonal | Novus Biologicals # NBP1-83379 | 1:500 | lot No not available
taurine | rb | polyclonal | Sigma-Aldrich #AB5022 | 1:100 | lot No 2893310

Alexa Fluor conjugated secondary antibodies (Thermo Fisher)
Goat/Donkey anti-gp/ck/ms/rb/sh IgG (H+L) Highly Cross-Adsorbed Secondary Antibodies, Alexa Fluor 405/488/568/647 | 1:200
Thermo Fisher #A-31553, A-31556, A-11029, A-11034, A-11073, A32931, A-11031, A-11036, A-11075, A-11041, A-21236, A-21245, A-21450, A32933, A-21202, A-21206, A-11015, A10037, A10042, A-21099, A-31571, A-31573, A-21448

The following antibodies were utilized for Western blotting:
LRRC8A | rb | polyclonal | Cell Signaling #24979 | 1:500 | lot No not available
beta actin | ms | monoclonal, clone #AC-15| Sigma-Aldrich #A1978 | 1:10000 | lot No not available

IRDye conjugated secondary antibodies (LI-COR, Inc)
IRDye 800CW Goat anti-Rabbit IgG | LI-COR #926-32211 | 1:10000 | lot No not available
IRDye 680RD Goat anti-Mouse IgG | LI-COR # 926-68070 | 1:40000 | lot No not available

gp = guinea pig, ck = chicken, ms = mouse, rb = rabbit, sh = sheep

Validation

All commercial antibodies were purchased from vendors that included validation and characterization statements on the manufacturer's information sheet. For antibodies that were non-commercial, validation was also performed in previous publications, as referenced in the Methods section. We further conducted extensive validation of the antibody for GABA (Extended Data 6). The commercial LRRC8A antibodies were validated using knockout MIN6 cells and knockout mouse islets.

The following antibodies utilized for immunofluorescence staining were validated as follows:
insulin | gp | Linco #4011-01 | colocalization with second known primary antibody specific for same target, cell-type specificity (beta cells only) in conjunction with glucagon and somatostatin, subcellular localization (insulin granules), western blot single band at expected molecular weight
insulin | gp | Dako #A0564 | colocalization with second known primary antibody specific for same target, cell-type specificity (beta cells only) in conjunction with glucagon and somatostatin, subcellular localization (insulin granules)
insulin | ck | Abcam #ab14042 | colocalization with second known primary antibody specific for same target, cell-type specificity (beta cells only) in conjunction with glucagon and somatostatin, subcellular localization (insulin granules)
glucagon | sh | Abcam #ab36232 | morphology, cell-type specificity (alpha cells only), cell-type specificity (alpha cells only) in conjunction with insulin and somatostatin, subcellular localization (glucagon secretory granules)
somatostatin | sh | Abcam #ab35425 | morphology, cell-type specificity (delta cells only) in conjunction with insulin and glucagon
pancreatic polypeptide | rb | Abcam #ab113694 | morphology / cell-type specificity (PP cells only) in conjunction with insulin and glucagon
GABA | rb | Sigma-Aldrich #A2052 | no non-specific binding with dot blot array of 21 essential amino acids and GABA, no staining in knockout models (cells known not to produce GABA), cell treatment with GABA biosynthesis inhibitor allylglycine, competitive inhibition of binding with soluble GABA, colocalization in neurons with known synaptic vesicle markers
GAD65 C-term (GAD6) | ms | in-house RRID #AB_2314499 | colocalization with second known primary antibody specific for same target, knockout models (cells known not to produce GAD65), morphology and subcellular localization (Golgi + vesicles)
GAD65 N-term | ms | Christiane Hampe Lab | colocalization with second known primary antibody specific for same target, morphology and subcellular localization (Golgi + vesicles), western blot band at expected molecular weight
GAD65 | rb | Synaptic Systems #198102 | colocalization with second known primary antibody specific for same target, morphology and subcellular localization (Golgi + vesicles), vendor validation: immunofluorescence and western blot
GAD65 | gp |Synaptic Systems #198104 | colocalization with second known primary antibody specific for same target, morphology and subcellular localization (Golgi + vesicles), vendor validation: immunofluorescence and western blot
TauT | rb | Sigma-Aldrich #HPA015028 | relied on vendor validation
VGAT Oyster 650-labeled | rb | Synaptic Systems #131103C5 | colocalization in neurons with known synaptic vesicle markers, vendor validation: immunofluorescence
synaptophysin | rb | Abcam #ab14692 | colocalization in neurons with known synaptic vesicle markers, vendor validation immunofluorescence and western blot
SV2C | rb | Synaptic Systems #119202 | colocalization in neurons with known synaptic vesicle markers, vendor validation: western blot and immunofluorescence
4F2HC (CD98) | rb | polyclonal | Santa Cruz Biotech #sc9160 | morphology / subcellular localization, colocalization with LAT2
LAT2 (SLC7A8) | ms | monoclonal, clone #UMAB70 | OriGene #UM500058 | morphology / subcellular localization, colocalization with 4F2HC
Bestrophin 1 | rb | polyclonal | Abcam #ab14928 | vendor validation: immunofluorescence
GAT1 | rb | Synaptic Systems #274102 | vendor validation: immunofluorescence and western blot
GAT2 | rb | Abcam #ab2896 | vendor validation: immunofluorescence
GAT3 | rb | Synaptic Systems #274303 | vendor validation: western blot and immunofluorescence
GCP60 | rb | Novus Biologicals #NBP1-83379 | vendor validation: western blot in knockout cells and immunofluorescence
taurine | rb | Sigma-Aldrich #AB5022 | vendor validation: against a spectrum of antigens to assure hapten selectivity and proper affinity. No measurable glutaraldehyde-fixed tissue cross-reactivity (<1:1000) against L-alanine, γ-aminobutyrate, 1-amino-4-guanidobutane (AGB), D/L-arganine, D/L-aspartate, L-citrulline, L-cysteine, D/L-glutamate, D/L-glutamine, glutathione, glycine, L-lysine, L-ornithine, L-serine, L-threonine, L-tryptophan, L-tyrosine.

The following antibodies were utilized for Western blotting:

LRRC8A | rb | Cell Signaling #24979 | Western blotting: Band at expected molecular weight, knockout models show loss of expression, vendor validation: western blot
beta actin | ms | Sigma-Aldrich #A1978 | Western blotting: Single band at expected molecular weight, vendor validation: western blot, immunofluorescence, highly cited loading control antibody.

gp = guinea pig, ck = chicken, ms = mouse, rb = rabbit, sh = sheep

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	GABA Biosensor CHO Cells (Novartis Institute for Biomedical Science) Serotonin Biosensor CHO Cells (Alejandro Caicedo lab, University of Miami) WT and LRRC8A-/- MIN6 cells (Rajan Sah lab, Washington University in St. Louis)
Authentication	Biosensor cells were authenticated in our labs to produce specific Ca2+ responses only to the intended ligand (see Methods section for extensive details). KO MIN6 cells were obtained at low passage and were authenticated by the generating lab (Rajan Sah, Univ. of Iowa) in a recent Nature Communications paper (doi:10.1038/s41467-017-02664-0).
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination
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	Rat islets were isolated from pancreases of male and female P5 Sprague Dawley rats (Charles River). Mouse islets were isolated from the pancreases of 8-14 week old male and female LRRC8A-/- knockout mice and LRRC8A+/- heterozygous littermates.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collect from the field.
Ethics oversight	All experimental protocols using animals were approved by the University of Miami, University of Florida, or EPFL Animal Care and Use Committees.

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