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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Functional and transcriptomic data from primary islets were analyzed based on the maximum number of risk allele carriers available in the combined collection of islets from study centers in Oxford, UK, and Edmonton, Canada. For cellular data, effect sizes could not be estimated prospectively (precluding power analysis), and standard scientific conventions were followed for these studies. Specifically, for insulin secretion and exocytosis experiments, three independent passages of cells were analyzed in biologically independent replicates. In each case, cells were only taken forward for detailed phenotyping if a satisfactory glucose responsiveness (fold induction > 1.5) could be ascertained. For the quantitative electron microscopy, a total of ten cells were counted per treatment. Tissue expression panels were analyzed in single replicates of pooled tissues (full information available from commercial suppliers, as stated in the methods). In the case of determining CgA amidation status, additional replicates were requested at the revision stage; the estimated effect size derived from the first set of experiments was used to inform the level of replication required in the follow-up analysis.

2. Data exclusions

Describe any data exclusions.

To remove significant outliers among technical replicates for primary islet data, Grubbs outlier test was systematically applied on a per-individual basis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

For the human islet data, replication could not be attempted due to the difficulties in obtaining primary islets from cadaveric donors and the low frequency of the T2D risk alleles in PAM. Several aspects of the cellular data, including insulin secretion defects, have been replicated by parallel studies in the lab, and on-going work has successfully replicated the effects of the PAM variants in kinetic assays. Electron microscopy was independently quantified by two blinded observers, identifying the same overall trends. Western blotting following PAM knockdown or inhibition was successfully reproduced in at least three different passages of the cell line, in experiments performed by two independent researchers.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

PAM variant carriers were matched for age, gender, and BMI to available controls in the same cohort of primary human islets (n = 187). For cellular phenotyping, samples were randomly positioned on microplates to minimize any possibly systematic bias from technical artefacts.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The post-hoc matching of islet samples was performed based on the three listed covariates and sample genotype, with the researcher being blinded to experimental data. All subjective quantifications of cellular data, including the scoring of electron micrographs, were performed by blinded observers.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Data were analysed using:
- Olympus VS120-S6 analysis software,
- OriginPro V8.5.1
- ImageJ 1.52b
- Image Lab 5.0
- R version 3.3.1 using the following standard packages: drc, ggplot2, mass, pwr, scales, stats.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials used are available from the authors upon request or from standard commercial sources (as indicated in relevant sections of the online methods), except the cell line, EndoC-βH1, which is available from Univercell-Biosolutions (formerly EndoCells), subject to an MTA.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies were validated by dilution test in the cell models used either for immunofluorescence (IF), electron microscopy (EM) or western blot (WB).
Primary Ab:
• rabbit anti-PAM (Abcam ab109175, clone EPR2643(2), IF and EM 1:100)
• guinea-pig anti-insulin (Belfast, in-house antibody, IF 1:500), ref doi:10.1038/ncomms5639
• mouse anti-glucagon (Sigma-Aldrich G2654, clone K79bB10, IF 1:500)
• mouse anti-Myc (Millipore, Clone 4A6, WB 1:10000, IF 1:1000)
• rabbit anti-TGN46 (Invitrogen, PA5-23068, IF 1:200)
• rabbit anti-calnexin H-70 (Santa Cruz Biotechnology sc-11397, IF 1:50)
• rabbit anti-CgAGly (Abcam ab52983, clone EP1031Y, IF and WB 1:100)
• rabbit polyclonal anti-total CgA (Abcam, ab15160, IF and WB 1:100)
• guinea pig anti-insulin (Dako, A0564, EM 1:500)

Secondary Ab:
• mouse alexa Fluor 488 (Life Technologies, A11029, IF 1:500)
• goat anti-guinea pig Alexa Fluor 488 (Life technologies, A11073, IF 1:500)
• guinea- pig Alexa Fluor 633 (Life Technologies, A21105, IF 1:250)
• goat anti-rabbit Alexa Fluor 594 (ThermoFisher Technologies, A11037, IF 1:400)
• goat anti-mouse TRITC (Sigma-Aldrich, T7782, lot# 023M4752V, IF 1:250)
• tyramide amplification (IF, thermofisher, TSA Kit T20912)
• anti-rabbit biotin (Vector Laboratories, BA-1000, EM 1:50)
• streptavidin gold 15nm (Biocell, EMSTP15, EM 1:20)
• anti-guinea pig gold 10nm (Biocell, EMGAG10, EM 1:20)

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

• The EndoC-βH1 cell line was acquired from Raphael Scharfmann (EndoCells, Paris) under an MTA.
• HEK293 has been purchased from sigma, ref: 85120602.

b. Describe the method of cell line authentication used.

The EndoC-βH1 has not been authenticated by formal STR profiling, but the cell line is routinely subjected to rigorous testing in a number of different ways, all of which have confirmed the identity as a bona fide human beta cell line. Relevant tests include microarray-based genotyping, RNA-seq, ATAC-seq, and extensive cellular phenotyping, including insulin secretion and content (see Thomsen et al, 2016, Diabetes, PMID: 27554474) and electrophysiology (see Hastoy et al, BiorXiv, doi: 10.1101/226282)

c. Report whether the cell lines were tested for mycoplasma contamination.

Cells were tested for mycoplasma contamination on a quarterly basis, and tested negative on all occasions.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

HEK-293 cells were used exclusively for expression and purification of recombinant PAM protein, and the identity of the cell line is, as such, not relevant to the interpretation of the kinetic data.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Pancreatic islet RNA was extracted from female NMRI mice between 12 and 28 weeks old.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Islet donors analyzed for insulin secretion and content measurements had the following population characteristics (Carriers; Controls): n (16; 16), Age [y] (51.8; 52.6), BMI [kg/m2] (29.6; 29.3), Gender [m/f] (8/8; 8/8).

Islet donors analyzed for exocytosis measurements had the following population characteristics (Carriers; Controls): n (7; 7), Age [y] (49; 58.4), BMI [kg/m2] (29.3; 26.6), Gender [m/f] (5/2; 3/4).