

## Supplementary methods

### Animals

All animal experiments were conducted at the University of Geneva Medical Centre with the approval of the animal care and experimentation authorities of the Canton of Geneva (#GE/128/15). Mice were maintained on a 12-h dark/12-h light cycle with water and food *ad libitum*. All  $\beta$ -Phb2<sup>-/-</sup> and Phb2<sup>fl/fl</sup> mice were generated and genotyped as previously described (1; 2) and maintained on a mixed genetic background (C57BL/6J  $\times$  129/Sv) to avoid inbred strain-specific phenotypes. We used male mice, since the phenotype of  $\beta$ -Phb2<sup>-/-</sup> mice is similar between males and females (1). The age of the mice is specified for each experiment. Upon sample collection, food was withdrawn from cages 3h in advance in order to homogenize glycemia in an early fasting state, i.e. before complete exhaustion of hepatic glycogen stores (3), while starvation mechanisms are not yet operating (4). Blood was collected into EDTA-coated tubes (no. 20.1341; Sarstedt, Inc.) via retro-orbital bleeding and centrifuged at 2,000 rpm at 4°C for plasma separation.

### Human subjects

We analyzed blood fraction samples stored at -80°C collected in the fasting state from participants who were already recruited for studies in Geneva (Switzerland), Rome (Italy), Maastricht (Netherlands), and Amsterdam (Netherlands).

### Rome cohort: participants with partial pancreas resection

Patients (n=9, 6 females; 3 males; mean age 62.8 years  $\pm$  14.8 SD) undergoing pylorus-preserving pancreatoduodenectomy were recruited from the Digestive Surgery Unit and studied at the Centre for Endocrine and Metabolic Diseases unit (both at the Agostino Gemelli University Hospital, Rome, Italy). Clinical characteristics are provided in Table S1. The study protocol (ClinicalTrials.gov Identifier: NCT02175459) was approved by the local ethics

committee (P/656/CE2010 and 22573/14) and all participants provided written informed consent, which was followed by a comprehensive medical evaluation.

Patients were scheduled for pancreatoduodenectomy, which was performed according to the pylorus preserving technique (5). The volume of pancreas removed during the surgery is constant (~50%), as previously reported (6), maintaining almost the same remaining portion of the endocrine pancreas. This surgery is therefore a unique way to examine the effects of a sudden decrease of functional  $\beta$ -cell mass (7; 8). See reference (8) for details on inclusion/exclusion criteria.

A standard 75 g OGTT was performed in all subjects both before and after surgery  $40 \pm 7$  days (8). Matsuda indexes were calculated as indexes of whole body insulin sensitivity based on insulin and glucose values obtained from the OGTT, while  $\beta$ -cell function was evaluated by calculating the insulinogenic index as the change in insulin over the first 30 min divided by the change in glucose over the first 30 min (9). Integrated  $\beta$ -cell function was also measured using the oral disposition index, calculated as the product of the insulinogenic index and the Matsuda index (10), which provides an assessment of insulin secretion in relation to insulin sensitivity.

#### Geneva cohort: participants with established T2D and NGT

Participants were recruited at Geneva University Hospital (Switzerland) and provided informed consent. The designed protocol was reviewed and approved by the institutional medical ethics committee (CER11-015), see reference (8) for further details on inclusion/exclusion criteria and details on blood sampling. Participants without antidiabetic treatments and with HbA1c < 6.0 % (42 mmol/mol) were included in the non-type 2 diabetic control group with normal glucose tolerance (NGT) (n=31). Participants with oral antidiabetic treatment or HbA1c  $\geq$  6.5 % (48 mmol/mol) were included in the T2D group (n=31). All the participants were asked to follow a moderate diet without excess fat or alcohol intake 24 hours prior the blood sampling day. All clinical data are reported in Table S2 and diabetic medications reported in Table S3.

HOMA indexes were calculated in these subjects using the updated HOMA2 method. The disposition index, calculated as the product of HOMA-S% by HOMA-B%, reflects the overall homeostatic ability of an individual using static measurements such as fasting plasma glucose and insulin.

Maastricht cohort: participants at increased risk of developing type 2 diabetes

Patients at high risk of developing type 2 diabetes participated in 3 different studies at Maastricht University: ReFDR study (n=12) (11); ACCT study (n=11) (12), and HIIT study (n=7) (8). Their clinical characteristics are provided in Table S4.

After an overnight fast, blood samples were drawn for measurements of plasma HbA1c, plasma glucose, and serum insulin; and at time points 0, 90, and 120 min for measurement of serum insulin and plasma glucose for calculation of glucose clearance as defined by the oral glucose insulin sensitivity model (13). In/exclusion criteria for the ReFDR (11), ACCT (12), and HIIT (8) studies have been described previously. In these patients, the increased risk for T2D was defined as follows: 1) in participants from the ReFDR study, it was defined as having at least one first-degree relative with T2D, BMI between 27 and 35 kg/m<sup>2</sup>, and disrupted glucose homeostasis defined as glucose clearance calculated using the oral glucose insulin sensitivity (OGIS) model of  $\leq 350$  ml/kg/min; 2) in participants from the ACCT study, it was defined as having plasma glucose level between 7.8 and 11.1 mmol/L 2 h after an OGTT; 3) in participants from the HIIT study, it was defined as having glucose clearance calculated using the OGIS model of  $\leq 350$  ml/kg/min and/or a plasma glucose level between 7.8 and 11.1 mmol/L 2 h after an OGTT. These patients were stratified into two subgroups, according to the ADA criterion for prediabetes (American Diabetes Association Diagnosis and classification of diabetes mellitus) of HbA1c  $>5.7\%$  (14) (HbA1c  $\geq 5.7\%$  n=17, HbA1c  $<5.7\%$  n=13).

Amsterdam cohort: participants with established T2D and NGT

We included four overweight or obese individuals with T2D according to the 2010 American Diabetes Association (ADA) criteria, BMI 25–40 kg/m<sup>2</sup>, male sex, aged 30–75 years old, and using no glucose-lowering drugs other than metformin. Another four NGT control participants were included with BMI  $\leq$ 25 kg/m<sup>2</sup>, male sex and age 30–75 years. Their clinical characteristics are provided in Table S5. The study was approved by the Institutional Review Board of the Amsterdam UMC (location AMC) and performed according to the Declaration of Helsinki of October 2004. The study was registered at the Netherlands Trial Registry (number NTR3234) and was performed between February 2012 and March 2013 at the Department of Endocrinology and Metabolism of the Amsterdam UMC. For a detailed description of the study protocol, please refer to (15; 16).

#### Lipidomic determinations and statistical analysis

Lipid extracts were prepared using a modified MTBE extraction protocol with addition of internal lipid standards, as previously described (17). Mass spectrometry analysis for the quantification of phospho- and sphingolipid species was performed using multiple reaction monitoring on a TSQ Vantage Extended Mass Range Mass Spectrometer (ThermoFisher Scientific), equipped with a robotic nanoflow ion source (Triversa Nanomate, Advion Biosciences) as previously described (17). Optimized fragmentation was generated using appropriate collision energies and s-lens values for each lipid class. Mass spectrometry data were acquired with TSQ Tune 2.6 SP1 and treated with Xcalibur 4.0 QF2 software (ThermoFisher Scientific) Lipid quantification was carried out using an analysis platform for lipidomics data hosted at EPFL (Lausanne, Switzerland, <http://lipidomes.epfl.ch/>). Quantification procedure was described in (18). Dried lipid extracts were resuspended in 250  $\mu$ L MS-grade chloroform/methanol (1:1) and further diluted in either chloroform/methanol (1:2) plus 5 mmol/L ammonium acetate (negative ion mode) or in chloroform:methanol:H<sub>2</sub>O (2:7:1) plus 5 mmol/L ammonium acetate (positive ion mode). Lipid concentrations were

calculated relative to the relevant internal standards and then normalized to the total phosphate content of each total lipid extract and expressed as percentage of total lipids detected. Lipid concentrations were not corrected for class II isotopic overlaps. Unless specified, multiple paired *t* tests were performed for Rome cohort and unpaired for all the rest of cohorts and mouse experiments (Benjamini, Krieger and Yiekutieli). P values and false discovery rates were computed. Lipids were considered significant when  $p$  value  $< 0.05$ .

Hierarchical cluster analysis and data visualization as heatmap were based on Ward Algorithm with Euclidean distance using the Software MetaboAnalyst 5.0 (Quebec, Canada).

#### Pancreatic islets, treatment, and insulin secretion experiments

Mouse pancreatic islets were isolated through collagenase digestion and cultured overnight in complete RPMI 1640 medium, as described previously (19). Human islets were isolated from pancreases of deceased multiorgan donors (NGT  $n=3$ , T2D  $n=1$ ). Islets from the non-diabetic donors, who provided informed consent, were provided by the ECIT consortium (<http://ecit.dri-sanraffaele.org/>), while the islets from the T2D patient were purchased from Tebubio Srl. (Paris, France). Donor information is shown in Table S6. Islets were maintained for standard recovery period of time (between 1 to 4 days) in CMRL-1066 medium containing 5.5 mM glucose supplemented with 10% (vol./vol.) FCS and antibiotics. For secretion experiments, batches of 25 islets were hand-picked and preincubated with Krebs-Ringer bicarbonate HEPES buffer supplemented with 2.8 mmol/L glucose and 0.1% BSA, at 37°C for 1 h. Subsequently, islets were incubated with basal glucose (2.8 mmol/L) with a commercial mixture of bovine liver LysoPIs containing 90% LysoPI18:0, 5% LysoPI18:1, and 5% LysoPI16:0 (Avanti Lipids, AL, USA) at the specified concentration dissolved in DMSO or vehicle, and subsequently at the specified stimulatory glucose concentration for 1 h. At the end of the incubation period, supernatants were collected, and islets were resuspended in Trizol.

Insulin in supernatants was measured using radioimmunoassay (Millipore) or Human insulin ELISA kit (Mercodia, Uppsala, Sweden).

#### Isolation of RNA and quantitative RT-PCR

Total RNA was extracted from isolated islets from prediabetic ( $\beta$ -Phb2<sup>-/-</sup>) and control ( $\beta$ -Phb2<sup>fl/fl</sup>) mice with the Trizol reagent (Invitrogen, Carlsbad, CA, USA) and 2  $\mu$ g were converted into cDNA as described previously (20). See list of primers in Supplementary Table S7. QT-RT-PCR was performed using an StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). PCR products were quantified fluorometrically using the SYBR Green Master kit (Roche, Mannheim, Germany). Experiments were performed in duplicate for the gene *Gpr119* and mean values were normalized to those of the reference mRNA ubiquitin C (*Ubc*).

#### RNA-sequencing and network analysis

Total RNA was extracted from cultured isolated human islets with Trizol reagent (Invitrogen, Carlsbad, CA, USA) (20). RNA sequencing was performed in Susanne Mandrup's laboratory (University of Southern Denmark, Odense, Denmark) as detailed previously (21). Results are expressed in log<sub>2</sub> fold changes (log<sub>2</sub> FC) and individual adjusted *p* values calculated for each donor. For network analysis of RNA-sequencing data, we delineated a functional interaction network of selected genes using the STRING knowledgebase (22; 23), and then, transcriptomic data were added into the functional gene network for visualization of each islet batch corresponding to the individual donors #1–5 (21). Significant changes were considered when two or more independent islet batches (donors) exhibited down or upregulation with a log<sub>2</sub> fold change (log<sub>2</sub> FC) threshold of 0.5 associated with at least one or more adjusted *p* < 0.05; highlighted in bold in the corresponding figure (21).

#### INS-1E cell culture, treatment, and luminesce-based secretion assay

Modified INS-1E  $\beta$ -cells (RRID: CVCL\_0351) expressing a Gaussia luciferase in place of the insulin c-peptide (24; 25) were used for luminescence-based secretion assay. Cells were cultured in RPMI-1640 GlutaMAX™ medium at 11.1 mmol/L glucose supplemented with 10 mM HEPES, 5% (vol./vol.) heat-inactivated FCS, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, 1 mM sodium pyruvate and 50  $\mu$ M  $\beta$ -mercaptoethanol. For secretion experiments, cells were starved for 2 h in glucose-free RPMI-1640 medium, washed and incubated further for 1h with basal glucose (2.8 mmol/L) in KRBH with LysoPIs mix 40  $\mu$ M dissolved in DMSO or vehicle. Five  $\mu$ M of native coelenterazine (Nanolight Technologies, Pinetop, AZ) were added to the wells, incubated for 20 min and luminescence emitted upon 7 and 15 mmol/L glucose online stimulation was monitored for 65 min using the Fluostar plate-reader (20).

#### In vivo administration of lysoPI

C57BL/6J high-fed wild-type control mice were starved for 3 hours and injected in the tail vein with 25  $\mu$ g LysoPI mix dissolved in 100  $\mu$ l saline with 10% DMSO (80  $\mu$ M) (n=3 for insulin, n=2 for glycemia) or vehicle (n=2 for insulin, n=2 for glycemia) or intraperitoneally with 25  $\mu$ g LysoPI mix dissolved in 500  $\mu$ l saline with 10% DMSO (400  $\mu$ M) (n=4 for insulin, n=3 for glycemia) or vehicle (n=5 for insulin, n=2 for glycemia). Glycemia was measured with a glucometer Accu-Check® (Roche, Basel, Switzerland) from a drop of blood collected from the tail vein at times 0, 15, 30, 45, 60, 90, and 120 minutes after the injection. Insulin was measured by ELISA (Mercodia, Uppsala, Sweden) at times 0 and 15 after injection in blood collected from the submandibular vein.

#### Data and resource availability

The data sets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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