Supplemental Information

Supplemental Experimental Procedures

Intermediate metabolic traits. Body mass index (BMI) is the weight in kilograms divided by the *square* of the height in *meters.* The HOMA2-IR and HOMA2-B indexes were calculated using the HOMA (HOmeostasis Model Assessment) calculator based on fasting glucose and fasting insulin levels in subjects in steady-state situation (available at http://www.dtu.ox.ac.uk/homacalculator). We also calculated the Quantitative Insulin Sensitivity Check Index (QUICKI) using fasting glucose and fasting insulin levels (available at https://sasl.unibas.ch/11calculators-QUICKI.php). The degree of steatosis was defined as the percentage of hepatocytes containing fat droplets. In the control group of normoglycemic samples, the association levels reported in our study cannot be influenced by T2D treatment.

DNA and RNA isolation. The DNA samples were isolated using the Gentra Puregene Tissue Kit (Qiagen, Les Ulis, France). In order to improve isolation, DNA was then resuspended in Tris/Hcl/EDTA buffer and precipitated by adding *chloroform:isoamyl alcohol (4 %).* Purity was determined by using a NanoDrop (NanoDrop Technologies, Wilmington, USA) and concentration was determined by using the Qubit® dsDNA BR Assay Kit (Life Technologies a brand of Thermo Fisher Scientific, Saint Aubin, France). The RNeasy® Lipid Tissue kit (Qiagen) and QIAamp RNA Blood Mini Kit (Qiagen) were used to isolate RNA from liver tissue and blood, respectively. Quality and integrity were tested on 2100 Bioanalyzer Instruments by using a RNA 6000 Nanochip (Agilent Technologies, Les Ulis, France). Concentration was determined by using the Qubit® RNA BR Assay Kit (Life Technologies).

Oil Red O staining. IHH cells were grown in a 24-well plate (at an initial density of 10^5 cells/well) and incubated with 100 nM insulin for 16 hrs and 24 hrs. Cells were then washed three times with iced PBS and fixed with 4% paraformaldehyde for 30 minutes. After fixation, cells were washed three times and stained with Oil Red O solution for 15 min at room temperature. For Oil Red O content levels quantification, DMSO was added to sample; after shaking at room temperature for 5 min, the density of samples were read at 490 nm on a spectrophotometer

Cell proliferation and apoptosis. IHH cells were cultured with 100 nM insulin at different incubation times. Cell number was counted by trypan blue and apoptosis was determined by scoring cells displaying pycnotic or fragmented nuclei (visualized with Hoechst 33342). The counting was performed blind by two different experimenters.

RNA-sequencing. RNA samples with a RNA integrity number higher than 9.0 were extracted from IHH cells treated or not with 200 nM insulin for 24 hours, from at least three independent experiments. RNA libraries were prepared using the TruSeq Stranded mRNA Library Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The libraries were sequenced with the NextSeq 500 (Illumina). A mean of 50 million paired-end reads of 100 bp were generated for each sample. More than 92% of the reads for each library were effectively mapped to the hg19 human genome assembly using TopHat2. Subsequently, both quantification and annotation of the reads were performed using Rsubread. Finally, the differential gene expression analyses (*i.e.* 24 h insulin-treated IHH cells *versus* IHH cells at baseline) were performed using DESeq2. The differentially expressed genes were subjected to Ingenuity Pathway Analysis (Qiagen, Hilden, Germany) to decipher the major biological pathways and diseases emphasized by the significantly deregulated genes (with a p-value < 0.05).

PamChip peptide microarrays for kinome analysis. kinome analysis was achieved usin Serine Threonine Kinases microarrays, which were purchased from PamGene International BV. Each array contained 140 target peptides as well as 4 control peptides. Sample incubation, detection, and analysis were performed in a PamStation 12 according to the manufacturer's instructions. Briefly, extracts from

IHH cells cultured with insulin for 24 hours were made using M-PER mammalian extraction buffer (Thermo Scientific) for 20 minutes on ice. The lysates were then centrifuged at 15,871 *g* for 20 minutes to remove all debris. The supernatant was aliquoted, snap-frozen in liquid nitrogen, and stored at –80°C until further processing. Prior to incubation with the kinase reaction mix, the arrays were blocked with 2% BSA in water for 30 cycles and washed 3 times with PK assay buffer. Kinase reactions were performed for 1 hour with 5 µg of total extract for the mouse experiment or 2.5 µg of total extract for the mature adipocyte and 400 µM ATP at 30°C. Phosphorylated peptides were detected with an anti-rabbit–FITC antibody that recognizes a pool of anti–phospho serine/threonine antibodies. The instrument contains a 12-bit CCD camera suitable for imaging of FITC-labeled arrays. The images obtained from the phosphorylated arrays were quantified using the BioNavigator software (PamGene International BV), and the list of peptides whose phosphorylation was significantly different between control and test conditions was uploaded to GeneGo for pathway analysis.

Animal experiments. The animal welfare committees of the DIfE as well as the local authorities (LUGV, Brandenburg, Germany) approved all animal experiments (reference number V3-2347-37-2011 and 2347-28-2014). All mice were housed in temperature controlled room (22±1 °C) on a 12:12 h light dark cycle (light on at 6:00 am) and had free access to food and water at any time. C57BL/6J breeding pairs (Charles River, Germany) as well as NZO/HIBomDife breeding mice (Dr. Kluge, German Institute of Human Nutrition, Germany) received a standard chow (SD; V153x R/M-H, Ssniff).
After weaning male C57BL/6J mice were fed a HFD (60 kcal% fat, D12492, Research Diets) ad libitum in groups of two to six animals per cage. Weekly body weight measurements were performed in the morning (8-10 am). Classification of mice either prone or resistant to diet-induced obesity was described before [Kammel et al. 2016 - PMID: 27126637]. At the age of 6 weeks, mice were killed after 6 h fasting. All tissues were directly frozen in liquid nitrogen and stored at -80 °C until further processing.
Female NZO mice received a standard chow after weaning and starting with 5 weeks of age, mice were switched to HFD containing 60 kcal% fat (D12492, Research Diets). Classification of diabetes-resistant (DR) and diabetes-prone (DP) mice in week 10 is based on assessment of liver density by computed tomography (LaTheta LCT-200, Hitachi-Aloka) combined with the measurement of blood glucose (CONTOUR® Glucometer, Bayer) [Lubura et al. 2015 - PMID: 26487005]. Liver density <55.2 Hounsfield units and blood glucose values of >8.8 and <16.6 mM both measured in week 10 predict later onset of diabetes with 83% and 84% probability, respectively. Combination of both parameters increased the prediction probability to 90 %.
Expression analysis. Total RNA from liver was isolated using QIAzol Lysis Reagent and RNeasy Mini Kit as recommended. Residual DNA was removed by DNase digestion using the RNase-Free DNase Set (QIAGEN). Subsequently, cDNA synthesis was conducted with 1 µg RNA, random hexamer primer as well as oligo(dT)$_{15}$ primer and M-MLV reverse transcriptase (Promega). qRT-PCR was performed with 12.5 ng cDNA in an Applied Biosystems 7500 Fast Real-time PCR system with PrimeTime® qPCR Probe Assay (IDT) for *Pdgfa* and the GoTaq® Probe qPCR Master Mix (Promega). Data were normalized to the expression of *Eef2* (TaqMan Gene Expression Assay, Life Technologies) as endogenous control. For genome expression profile either 4x44K or 8x60K whole mouse genome microarrays (Agilent Technologies) were used.

In vivo insulin stimulation
Eight week-old male C57Bl/6J mice were fasted overnight and then injected with NaCl 9% or 5 unit of regular human insulin/kg (Actrapid Penfill, NovoNordisk) through peritoneal injection. Ten minutes after the injection of the insulin bolus, livers were removed and snap frozen in liquid nitrogen.

Immunoblots analysis. Proteins (40 µg) were subjected to SDS-PAGE analysis on 10% gels and transferred to nitrocellulose membranes. Rabbit polyclonal for total Akt, phospho-Akt (Ser-473 and Thr-308) were purchased from Cell Signaling. Monoclonal mouse β actin (clone AC-74; Sigma-Aldrich) was used as loading control.

Primer sequences. Primers for human *PDGFA* (sense 5′- GACCAGGACGGTCATTTACG -3′; antisense 5′- CGCACTCCAAATGCTCCT-3′). Primers for mouse *Pdgfa* (sense 5′- CAAGACCAGGACGGTCATTT -3′; antisense 5′- GATGGTCTGGGTTCAGGTTG-3′). Primers for

human *RPLP0* (sense 5′- ACCTCCTTTTTCCAGGCTTT -3′; antisense 5′- CCCACTTTGTCTCCAGTCTTG -3′). Primers for human *IRS1* (sense 5′- ACAGGCTTGGGCACGAGT-3′; antisense 5′- AGACCCTCCTCTGGGTAGGA -3′)

Glycogen measurement

Total glycogen in IHH cells was determined by using Abcam Glycogen assay kit according to the manufacturer's instruction. The glycogen content was determined by using fluorometric assay and the fluorescence was measured using plate reader. Corrections for background glucose were made in all the samples and the corrected fluorescent readings were applied to standard curve and amount of glycogen in each well was determined. The glycogen content of each sample was normalized to their respective protein content and plotted using Graph Pad Prism 5