SUPPLEMENTAL METHODS

Human islet culture*.* Pancreatic human islets were obtained through the Integrated Islet Distribution Program. Criteria for human donor islet acceptance: receipt within 36 h isolation, and of at least 70% purity and 90% viability (ESM Table 1). Upon receipt, human islets were first allowed to recover in Connaught Medical Research Laboratories (CMRL) medium for 2 h, and then were handpicked using a green gelatin filter to eliminate residual non-islet material. Human islets were treated with either a cytokine mixture (10 ng/ml TNF-α; 100ng/ml IFN-γ, and 5 ng/ml IL-1β) or glucolipotoxic (GLT) mixture (16.7-25 mmol/l glucose plus 0.5 mmol/l palmitate (Sigma, St. Louis, MO, USA), each purchased from ProSpec, East Brunswick, NJ, USA) in glucose-free RPMI 1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% (vol/vol) FBS (HyClone, South Logan, UT, USA) and 1% (vo/vol) penicillin/streptomycin (Gibco) for the times indicated in the text. Islets were washed in PBS then lysed and boiled in Laemmli sample buffer for SDS-PAGE protein resolution and immunoblot analysis. mRNA was quantified from islets by quantified real time PCR as previously described [16]. Primers used for the detection of *Pak1* (h*Pak1* forward: 5'-ggagtttacgggaatgccagag-3' and reverse: 5'-cagcctgcgggtttttcttc-3') and 18SrRNA (cat no.# PPH05666E) were obtained from Integrated DNA Technologies (Coralville, IA, USA) and Qiagen (Valencia, CA, USA), respectively.

INS 832/13 cell culture, transient transfection and adenoviral transduction. INS 832/13 cells (gift from Dr. Christopher Newgard, Duke University, Durham, NC, USA; passage 55-80) were grown in RPMI 1640 medium supplemented with 10 mmol/l HEPES, 10% FBS, 0.5% penicillin/Streptomycin, 2 mmol/l L-glutamine, 1mmol/l sodium pyruvate, and 50 μmol/l β-mercaptoethanol. Cells at 70% confluence were cultured under GLT conditions for 24 h or transfected with small interfering (si) RNA oligonucleotides: si*Pak1*(5'-CCGGTTCTATCGATCCATCTT-3', cat. no.# S103082926) or siCtrl (5'-AATTCTCCGAACGTGTCACGT-3', cat no.# 1027310; Qiagen, Valencia, CA USA) using

RNAiMAX (Invitrogen, Carlsbad, CA, USA). Cells were subsequently treated with GLT or vehicle (fatty acid-free BSA) for 2 h prior to harvest for immunoblotting or cell death assays. Beta cell specific expressed h*Pak1* adenovirus was generated by insertion of the full-length h*Pak1* cDNA from pCMV6M-h*Pak1* plasmid (gifted by Dr. Gunst, Susan, Indiana University) into the *PmeI* site of the Ins2-adenoviral vector (gifted by Drs. Thomas Becker and Chris Newgard, Duke University, Durham, NC, USA). Adenoviruses were packaged with EGFP (to enable visualisation of infection efficiency), and then amplified and purified for use (by Viraquest, North Liberty, IA, USA). Adenovirus rat insulin promoter (AdRIP)-h*Pak1* or AdRIP-Ctrl viruses were incubated with islets or cells for 2 h at a multiplicity of infection (MOI)=100, then cells were washed with PBS and incubated for 46 h.

Immunoblotting. Proteins were resolved on 10-12% SDS-PAGE for transfer to polyvinylidene difluoride for LI-COR fluorescence imaging (PVDF/FL) membranes for immunoblotting. Rabbit anti-phospho PAK1$^{Thr\text{-}423}$ PAK2 ^{Thr402}, PAK1, phospho extracellular signal-related kinase (ERK1/2$^{T202/Y204}$), ERK1/2, B cell lymphoma 2 (Bcl2), and cleaved caspase 3 (Cell Signaling, Danvers, MA, USA), phospho Bcl2^{S70} and rabbit anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Abcam, Cambridge, MA, USA) mouse anti-tubulin (Sigma, St. Louis, MO, USA) antibodies were used at 1:1000. Goat anti-mouse and anti-rabbit horseradish peroxidase secondary antibodies, were obtained from Bio-Rad (Irvine, CA, USA) and used at 1:5000 dilutions. IRDye 680 RD goat anti-mouse and IRDYE 800CW goat anti-rabbit were obtained from (LI-COR, Lincoln, NE, USA). Immunoreactive bands were visualized with enhanced chemiluminescence (ECL) or ECL prime reagents (GE Healthcare, Buckinghamshire, UK) and imaged using a Chemi-Doc Touch gel documentation system (Bio-Rad). Phosphorylated and total ERK1/2 blots were imaged using a Li-COR CLx imaging system (LI-COR). All antibodies were validated for specificity based upon correct molecular weight of

bands detected against molecular weight standards and lysate controls from the antibody manufacturers.

Animals and diets. The male *Pak1* KO mice used in these studies were generated in-house from breeding stock originally obtained from Jonathan Chernoff (Fox Chase Cancer Center, Philadelphia, PA, USA), generated as previously described [18], and are a classic whole-body gene-ablation model on the C57BL/6J strain background. Paired wild-type (WT) littermates, obtained through heterozygous crossings, were used as controls. After weaning at 21 days of age, mice were group housed and allowed free access to water and standard chow (2018SX Teklad Global 18% Protein Extruded Rodent Diet, Harlan Laboratories, IN, USA), under standard housing using paper bedding, on a 12 hr light cycle (7:00 lights on, 19:00 lights off). All animal procedures/protocols were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee (Indianapolis, IN, USA). Male 6-week old WT and *Pak1* KO mice were acclimated to the low-fat diet (LFD) for 2 weeks prior to starting the high-fat "western" diet (WD); at start of the WD all mice were individually housed for the purposes of collecting food intake data. Both the WD (45% energy from fat, cat no.# D01030108) and LFD (10% kcal from fat, cat no.# D01030107) diets are pelleted, semi-purified nutritionally complete experimental diets (Research Diets, Brunswick, NJ, USA). The WD diet contains 23.6 g of fat/100 g of diet (20.7 g of palm oil and 2.9 g of soybean oil; 45% energy from fat); the LFD diet contains 4.3 g of fat/100 g of diet (1.9 g of palm oil and 2.4 g of soybean oil; 10% energy from fat). During the study, both WT and *Pak1* KO mice were provided *ad libitum* access to either the WD or LFD diet for 10 to 13 weeks; the animals were placed into dietary groups based upon comparable starting body weights. Body weight was measured once per week, and energy intake was monitored three times per week. At the completion of the 13-wk feeding period, tissues were harvested; pancreases were fixed for evaluation of beta cell mass. Mice showed equivalent body weight and tissue/organ weight (Table1).

Serum analytes, i.p. glucose tolerance testing and HOMA-IR. Fasting blood glucose, insulin response to an acute glucose challenge, and glucose tolerance were assessed at 10, 11, and 13 weeks, respectively, following the initiation of the WD or LFD diet. Mice were fasted for 6 h prior to i.p. D-glucose (Sigma) injection (2 g/kg body weight) for measurements of serum insulin response and i.p. glucose tolerance tests (IPGTT). Glucose measurements were sampled from the tail vein and assessed with a HemoCue B-Glucose Analyzer (Hemocue, Mission Viejo, CA, USA). Serum insulin was quantified using a sensitive rat insulin radio immunoassay kit (EMD Millipore,). The homeostasis model for insulin resistance (HOMA-IR) was calculated according to Matthews et al [19].

Morphometric assessment of islet cell mass. Mouse islet morphometry was evaluated using anti-insulin immunohistochemical staining of pancreatic sections as previously described [20]. Briefly, pancreases were fixed with formalin, paraffin-embedded, and sectioned longitudinally at 5-µm thickness and 100-µm intervals. The sectioned tissues were deparafinized, rehydrated, blocked in 5% horse serum, and incubated overnight at 4°C with rabbit anti-insulin antibody (Santa Cruz Biotechnology, Dallas, TX, USA). Following PBS washes and incubation with HRP-conjugated secondary antibody, the sections were incubated in peroxidase substrates (Vector Labs, Burlingame, CA, USA) and counterstained with hematoxylin. Digital images were acquired on an AxioObserver Z1 microscope fitted with an AxioCam high resolution color camera (Zeiss, Jena, Germany). Percentage of beta-cell area was calculated using AxioVision Software (www.zeiss.com/microscopy/en_us/downloads/axiovision), version LE4.8; beta cell mass was calculated by multiplying percentage of beta-cell area with pancreas weight.

Proliferation assay and immunofluorescent confocal microscopy. Paraffin-embedded pancreatic tissue sections were deparafinized, rehydrated, blocked with blocking buffer (DAKO, CA, USA), and incubated overnight at 4°C with rabbit anti-insulin antibody (1: 500 dilution, Santa Cruz Biotechnology, Dallas TX, USA), anti-rabbit phospho-Histone 3, Ser10 (pHH3) (1:500 dilution, EMD Millipore, Billerica, MA, USA). Following PBS washes and incubation with Alexa Fluor 488 goat anti-guinea pig IgG (H+L) and Alexa Fluor 568 goat anti-rabbit IgG (H+L) (1: 500, Invitrogen) secondary antibody for 1 h at room temperature. Tissues were washed three times with PBS and added DAPI to stain nuclei. To assess proliferation, three pancreatic sections, each separated by at least 100 μm, from at least three mice per group, immunostained slides that stained positive for insulin, pHH3 and DAPI. Sections were scanned using a fully motorized TiE inverted microscope (Nikon Instruments, Melville, NY, USA) with a Xenon lamp source (Sutter Instruments, Novato, CA, USA) and an ORCA interline CCD (Hamamatsu, Hamamatsu City, Japan) for counting. Whole sections were imaged with 10-20% overlap in tiled images. More than 2,000 total insulin-positive cells were counted per animal. Results were expressed as the percentage of cells positive for all three stains relative to the total number of insulin-positive cells. Guinea pig anti-glucagon (EMD Millipore, , 1:500 dilution) was used for staining of alpha cells (ESM Fig. 1).

Cell death assay. Apoptosis was measured by assessment of DNA fragmentation and histone release from the nucleus during the apoptosis process, using the Cell Death Detection ELISA Plus kit (Roche Applied Science, Indianapolis, IN, USA). In brief, INS 832/13 cells were seeded at $4x10^4$ cells per well. Cells were either treated with GLT for 24 h, or were transfected with siRNA or transduced with adenoviruses and then treated with GLT or vehicle for 2 h. Cells were harvested into lysis buffer for centrifugation and supernatant transferred to the streptavidin-

coated microplate for immunodetection and photometric analysis. Absorbance was measured at 405 nm. All samples were assessed in duplicate in a total of 5-6 independent experiments.

Statistical analysis. Data are expressed as the mean ± SEM. Statistical comparisons were made by two-tailed Student's *t* test or ANOVA, using GraphPad Prism Version 6.0 (GraphPad Software, La Jolla, CA, USA). A value of $p<0.05$ was considered significant.

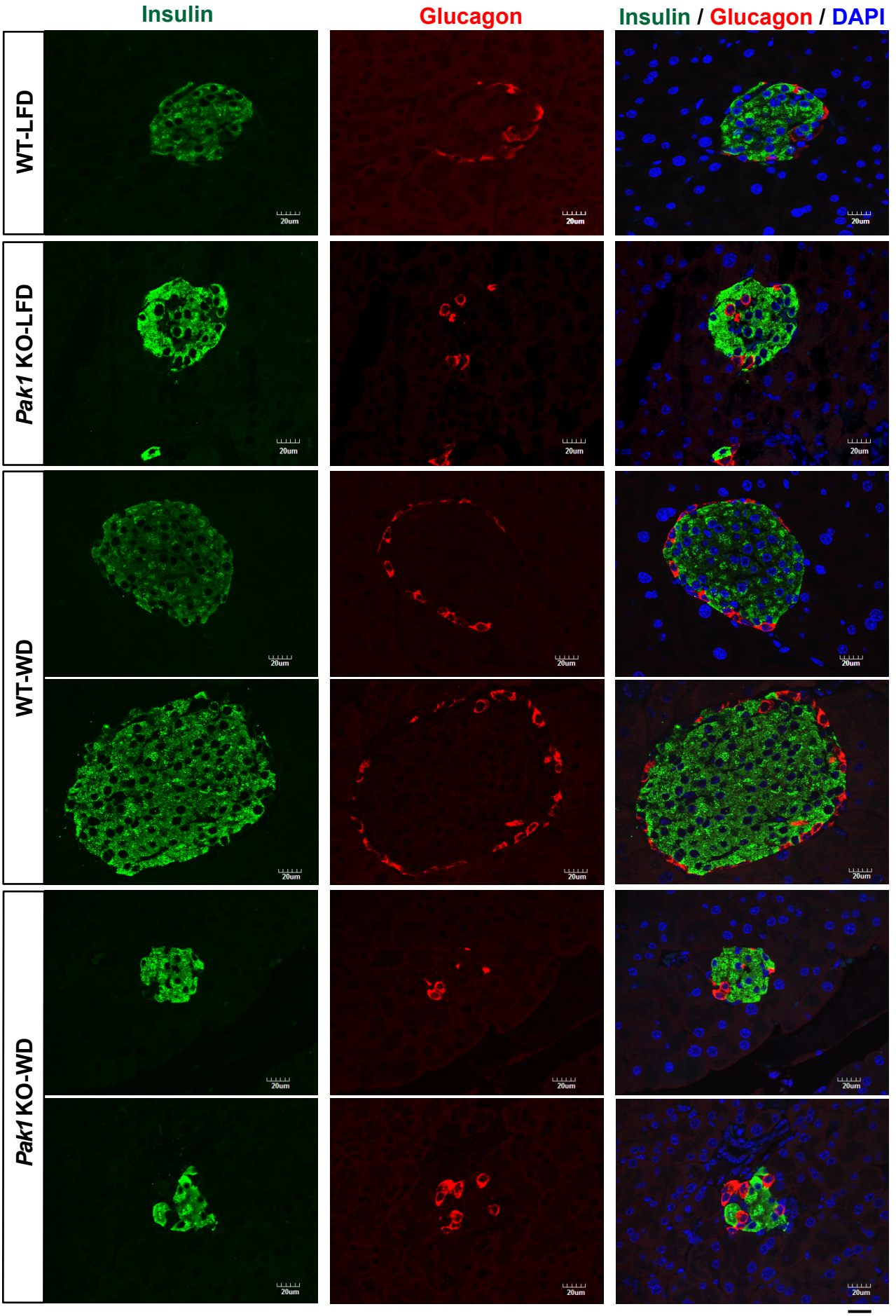
ESM Table 1

Human Islet Donor Profiles

Unos Id No.	Sex	Age	BMI	Race	Islet Purity (%)	Islet Viability (%)	Experimental Use of islets
AAFC089	M	46	28.8	White	75	95	Cytokine
AAIQ315	M	68	30.6	White	90	95	Cytokine
AAJR359	F	54	15.0	White	90	94	Cytokine
ACAM113	M	18	27.9	White	80	97	Cytokine, GLT
AAKD226	F	51	35.6	White	80	94	GLT
AALH177	F	47	34.5	Hispanic	75	90	GLT
ABIT067	F	32	39.4	White	90	98	GLT
ABIT447	M	52	36.7	African American	90	93	GLT
ABJK265	M	52	29.0	White	75	97	GLT
ABJV388	F	48	32.8	White	90	95	GLT
ABKH361	M	48	30.7	White	80	97	GLT
YAB306	M	30	26.2	White	95	98	mRNA
ABFV308	F	45	27.4	White	85	97	mRNA
ABKR234	F	55	22.6	White	90	95	mRNA
ZJM395	F	51	21.2	White	85	92	mRNA
ABFG183	F	44	32.8	White	90	94	mRNA
YBG327	M	52	50.0	White	92	99	mRNA
ABDG032	M	61	42.1	Hispanic	80	95	mRNA
ZBR200	M	58	48.9	White	83	95	mRNA

ESM Supplemental Figure Legend:

ESM Supplemental Fig. 1: Insulin and glucagon staining in pancreases from LFD- and WD-fed WT and *Pak1* KO mice. Whole pancreases were fixed and immunostained for insulin (green), glucagon (red) or DAPI (blue). Merged images of all three stains are shown in the far right column. Images are representative of >3 islet images from two mice per group. Two different islets are shown for the WD-fed WT and *Pak1* KO.


Insulin
Glucagon
Insulin / Glucagon / DAPI
WT-LFD
Pak1 KO-LFD
WT-WD
Pak1 KO-WD
20um
20 µm