The nutrient sensor mTORC1 regulates insulin secretion by modulating β-cell autophagy

Tal Israeli†1, Yael Riahi†1, Perla Garzon1, Ruy Andrade Louzada3, Joao Pedro Werneck-de-Castro3, Manuel Blandino-Rosano3, Roni Yeroslaviz-Stolper1, Liat Kadosh1, Sharona Tornovsky-Babeay1, Gilad Hacker1, Nitzan Israeli1, Orly Agmon1, Boaz Tirosh2, Erol Cerasi1, Ernesto Bernal-Mizrachi3, Gil Leibowitz*1

† Diabetes Unit and Endocrine Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; ‡ Stress Signaling Laboratory, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel; § Department of Internal Medicine, Division of Endocrinology, Metabolism and Diabetes, Miller School of Medicine, University of Miami, Miami, FL, USA.

† T.I and Y.R contributed equally to this work.

* Corresponding author and lead contact: Gil Leibowitz MD, Endocrinology and Metabolism Service, Department of Medicine, Hadassah - Hebrew University Medical Center, P.O. Box 12000, Jerusalem 91120, Tel: +972-2-6777951, Fax: +972-2-6437940, E-mail: gleib@hadassah.org.il
Abstract

The dynamic regulation of autophagy in β-cells by cycles of fasting-feeding and its effects on insulin secretion are unknown. In β-cells mTORC1 is inhibited while fasting, and is rapidly stimulated during refeeding by a single amino acid, leucine, and glucose. Stimulation of mTORC1 by nutrients inhibited the autophagy initiator ULK1 and the transcription factor TFEB, thereby preventing autophagy when β-cells are continuously exposed to nutrients. Inhibition of mTORC1 by Raptor knockout mimicked the effects of fasting and stimulated autophagy while inhibiting insulin secretion, whereas moderate inhibition of autophagy under these conditions rescued insulin secretion. These results show that mTORC1 regulates insulin secretion through modulation of autophagy under different nutritional situations. In the fasting state, autophagy is regulated in an mTORC1-dependent manner and its stimulation is required to keep insulin levels low, thereby preventing hypoglycemia. Reciprocally, stimulation of mTORC1 by elevated leucine and glucose, which is common in obesity, may promote hyperinsulinemia by inhibiting autophagy.
Introduction

Autophagy is an mechanism essential for cell survival, especially under stressful conditions\(^1\). Consistently, in β-cells disruption of autophagy induces cellular stress and degeneration, leading to diabetes\(^2, 3, 4, 5, 6, 7, 8\). It is logical to expect that autophagy is important also for physiological control of insulin secretion; however, its housekeeping role has hindered evaluation of its moment-to-moment regulatory effect on β-cell function.

Knockdown of autophagy genes in β-cell lines enhances insulin release, suggesting that autophagy negatively regulates insulin secretion\(^9, 10, 11\); this could help lower insulin in fasting. By contrast, Goginashvili\(^12\) found that in β-cells nutrient deprivation paradoxically inhibits autophagy due to starvation-induced crinophagy (autophagy-independent lysosomal degradation of secretory granules), leading to accumulation of amino acids (AAs) adjacent to lysosomes, thereby stimulating mTORC1.

mTORC1 functions as a hub integrating signals from growth factors, nutrients and energy (ATP) to regulate cellular functions\(^13, 14\). AA sensing by mTORC1 has been extensively studied\(^15, 16, 17\). AAs availability is conveyed to mTORC1 via Rag GTPases, which recruit mTORC1 to the lysosomal surface for activation by Rheb. mTORC1 inhibits autophagy via phosphorylation of ULK1 which initiates autophagosome generation, and TFEB, master regulator of lysosome biogenesis and autophagy\(^18, 19\). The regulation of mTORC1-autophagy signaling in β-cells under different nutritional states, and its impact on insulin secretion is not clear.

Herein, we show that in β-cells mTORC1 is inhibited under fasting, while it is rapidly stimulated by acute exposure to nutrients, mainly leucine and glucose acting in concert to stimulate both mTORC1 and insulin secretion. Nutrient stimulation of mTORC1 is associated with inhibition of ULK1 and TFEB along with decrease in autophagy-regulating genes. Inhibition of mTORC1 by Raptor KO, mimicking the effects of fasting, stimulated autophagy and inhibited nutrient-stimulated insulin secretion, whereas inhibition of autophagy under these conditions rescued insulin release. Thus, mTORC1 is an in vivo physiological regulator of insulin secretion that exerts its action by modulating β-cell autophagy.
Materials and methods

Animals

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Hebrew University of Jerusalem. Mouse strains used in this study included C57BL/6 (Harlan Laboratories, Israel), Tg(Ins1-Cre/ERT)1Lphi (MIP-CreER)\(^{20}\), Rptor\(^{tm1.1Dmsa}\) (Raptor\(^{fl/fl}\)) (Jackson Laboratory, USA)\(^{21}\) and B6.Cg-Atg7<tm1Tchi> (ATG7\(^{fl/fl}\)) (Riken BRC, Japan)\(^{22}\). \(\beta\)-cell-specific KO of Raptor and Atg7 was generated by two consecutive subcutaneous daily injections of 8mg tamoxifen (Sigma; 20mg/ml in corn oil). We performed all the experiments in male mice to avoid leakiness of the system due to activation of Cre by circulating estrogen in females. Mice were housed at the Hebrew University animal care unit under a 12 h light/dark cycle with free access to food and water.

Cell Culture

The \(\beta\)-cell line INS-1 832/13\(^{23}\) was kindly provided by Dr. Ángela M. Valverde (Instituto de Investigaciones Biomédicas "Alberto Sols", Madrid, Spain). Cells were cultured in RPMI-1640 medium containing 11mmol/l glucose and supplemented with 10% heat-inactivated FCS, 100IU/ml penicillin, 100µg/ml streptomycin, 10mmol/l HEPES, 2mmol/l L-glutamine, 1mmol/l sodium pyruvate (Biological Industries, Israel) and 0.05mmol/l 2-mercaptoethanol (Sigma-Aldrich, USA) in a humidified 37°C, 5% CO\(_2\) incubator.

Transfection

mRFP-GFP tandem fluorescent-tagged LC3 (tfLC3) plasmid\(^{24}\) was generously provided by Dr. Adi Kimchi (Weizmann Institute of Science, Israel). pEGFP-N1-TFEB plasmid was a gift from Shawn Ferguson (Addgene plasmid # 38119; http://n2t.net/addgene:38119; RRID:Addgene_38119)\(^{25}\). INS-1 cells were transfected with TransIt LT1 (Mirus Bio LLC, USA).

Metabolic assays
Mice were fasted overnight and then given 2g/kg glucose and/or 0.39g/kg leucine by oral gavage. Blood samples were drawn before and 30 min after gavage. Glucose tolerance was assessed by IPGTT: mice were fasted overnight and then injected IP with 2g/kg glucose. Insulin sensitivity was assessed by ITT: mice were fasted overnight and then given 0.75 U/kg insulin IP. Glucose was monitored and blood samples were obtained at the indicated time points. Plasma insulin was determined using an ultrasensitive mouse insulin ELISA kit (Crystal Chem, USA).

**Islet isolation**
Islets were isolated by collagenase P (Roche Diagnostics GmbH, Germany) injection into the bile duct followed by separation by Histopaque gradient (Sigma-Aldrich). Islets were hand-picked and cultured overnight in complete RPMI-1640 as described above.

**Insulin and proinsulin secretion and content**
Islets or INS-1 cells were pre-incubated for 1 h in Krebs-Ringer Modified Buffer (KRB) containing 3.3mmol/l glucose, then consecutively incubated for 1 h in KRB containing 3.3mmol/l or 16.7mmol/l glucose with or without AAs. Insulin and proinsulin were extracted with GB/NP40 buffer and analyzed by mouse insulin ELISA assays: Crystal Chem Ultra-Sensitive insulin ELISA kit for islet experiments and Mercodia Insulin ELISA kit for experiments in INS-1 cells. Proinsulin was measured in islet extracts using the Mercodia Rat/Mouse Proinsulin ELISA kit.

**Immunofluorescence staining**
Pancreases were fixed with 4% formaldehyde overnight. Paraffin-embedded sections were rehydrated, and antigen retrieval was performed in citrate buffer (pH 6). Sections were blocked with CAS-Block (Thermo Fisher Scientific, USA). Islets were dispersed with trypsin, fixed with 3.7% paraformaldehyde, permeabilized with 0.1% triton-X and blocked with 1% BSA buffer. Primary and secondary antibodies used are listed in Supplemental Table 1. Digital images were obtained with an A1R confocal microscope (Nikon Corporation, Japan) using a x60 or a x40 oil objective. Image analysis was performed with NIS-
Electron microscopy
Mouse islets were fixed using a cold solution of 2.5% glutaraldehyde, 2% formaldehyde in 0.1mol/l cacodylate buffer (pH 7.4) for 2 h at room temperature. Samples were post-fixed and stained with 1% (w/v) osmium tetroxide and 1.5% (w/v) potassium ferricyanide in 0.1mol/l cacodylate buffer for 1 h, followed by dehydration in increasing concentrations of ethanol and embedding in araldite. Ultrathin sections were collected onto copper grids and sequentially stained with uranyl acetate and lead citrate. Sections were imaged with a Tecnai 12 transmission electron microscope (Phillips, Netherlands) equipped with MegaView-II CCD camera and Analysis version 3.0 software (Soft Imaging System GmbH, Germany).

Western blotting
Lysates were separated by electrophoresis and then transferred to a nitrocellulose membrane. Membranes were probed using appropriate primary and secondary antibodies (Supplemental Table 1) and developed with Clarity Western ECL Substrate (Bio-Rad, USA) using Chemidoc Touch Imaging System. Image analysis and quantifications were performed with Image Lab Software v6.1 (Bio-Rad).

Quantitative real-time RT-PCR
RNA was extracted using Bio Tri RNA (Bio-Lab, Israel) and a RNeasy Micro Kit (Qiagen, Netherlands). Samples were reverse transcribed using qScript cDNA Synthesis Kit (Quantabio, USA). qPCR was performed on a StepOnePlus system using Fast SYBR Green Master Mix (Thermo Fisher Scientific). Gene expression was normalized to 18S ribosomal RNA and analyzed with StepOnePlus Software v2.3. The primers used for qPCR are shown in Supplemental Table 2.

Flow Cytometry
Islets were isolated and incubated overnight in complete RPMI-1640 growth medium containing 11mmol/ glucose. The next day islets were pre-incubated for 1 h in starvation medium (RPMI-1640 medium lacking glucose and AAs) and then incubated for 30 min in RPMI-1640 including all AAs supplemented with 2mmol/l L-glutamine and 2.8 or 16.7mmol/l glucose. Islets were then dispersed into single cell suspension using Accutase (STEMCELL Technologies Inc., Canada) and fixed and washed with Cytofix/Cytoperm solution (BD Biosciences, USA), and incubated overnight at 4°C with primary antibodies (Supplemental Table 1). Cells were then washed and incubated for 2 h with species-specific secondary antibodies (Supplemental Table 1). Analysis and quantifications were performed by the LSRFortessa flow cytometer and FlowJo software v10.5.3 (BD Biosciences).

Human islet experiments
Islets were derived from three brain-dead donors (see Human Islet checklist), incubated overnight in RPMI-1640 medium supplemented with 10% FBS at 3.3 or 16.7mmol/l with or without 10nmol/l and 100nmol/l rapamycin and/or 10µmol/l chloroquine (CQ). Static incubations were performed on 15 islets in replicates that were pre-incubated at 3.3 mmol/l glucose for 1 h and then at 3.3 and 16.7mmol/l for 45 min each. Secreted insulin and islet insulin content were analyzed by ELISA (Mercodia).

Statistical analysis
Data is presented as mean ± SEM. Unpaired two-tailed Student's t test with Welch's correction was used to compare differences between two groups. The differences between multiple groups were analyzed by Brown-Forsythe and Welch one-way ANOVA with Dunnet T3 or Games-Howell post-hoc tests or with two-way ANOVA with Dunnet or Šídák post hoc tests. All statistical analyses were performed using Prism 9.0.0 (Graphpad Software, CA, USA). * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

Data availability
All relevant data are available from the authors upon request.
Results

mTORC1 activity during fasting and in response to nutrients

The β-cell line INS-1 823/12 and islets were cultured under starvation conditions (3.3mmol/l glucose in absence of serum and AAs) for 30 min, then at low (3.3mmol/l) or high glucose (HG; 16.7mmol/l) in presence of serum with different AAs for 30 min. Using phosphorylated S6 as readout, detailed analysis of mTORC1 activation in INS-1 β-cells showed that the branched-chain AA (BCAA) leucine rapidly stimulated mTORC1, whereas other AAs known to regulate mTORC1 had either modest or no effect (Supplemental Fig. 1a). In islets, leucine stimulated mTORC1 dose-dependently (Supplemental Fig. 1b). In INS-1 cells, HG failed to activate mTORC1 in absence of AAs; however, it amplified the AA effect. As expected, rapamycin inhibited the stimulation of mTORC1 by AAs (Supplemental Fig. 1c). In islets, HG moderately increased S6 phosphorylation (not significant), whereas AAs increased the number of pS6+ β-cells and fluorescence intensity at both low and high glucose, indicating enhanced mTORC1 activity (Supplemental Fig. 1d). RAPTOR levels remained unchanged by AA starvation or following AA supplementation (Supplemental Fig. 1e). These findings show that in vitro exposure of β-cells to starvation inactivates, rather than stimulating mTORC1; a single AA, leucine, effectively activates mTORC1 and may act in concert with glucose.

We next corroborated the effects of fed-fast states on β-cell mTORC1 activity in vivo. Mice were fasted overnight with free access to water with or without added BCAA (leucine/isoleucine/valine 13.3g/l each). Fasting led to inhibition of mTORC1, evident by decrease in β-cells expressing pS6, and by reduction of total pS6 fluorescence intensity (Fig. 1a). BCAA in drinking water increased mTORC1 activity in fasted animals, proving response to AAs in vivo. We then assessed acute effects of leucine and glucose on mTORC1 activity in β-cells. Leucine (0.39g/kg), glucose (2g/kg) or both were administered by oral gavage after 16 h fast; the pancreases harvested 1 h later and sections or dispersed isolated islets stained for pS6 and insulin. Both leucine and glucose increased β-cell mTORC1 activity in β-cells; feeding them together stimulated mTORC1 in an additive manner (Fig. 1b).
Thus, in β-cells mTORC1 responds to external nutritional cues as expected, with no evidence for paradoxical stimulation by starvation in vitro or in vivo. Leucine is a potent activator of mTORC1, and glucose further amplifies it in β-cells.

**mTORC1 regulation of nutrient-stimulated insulin secretion**

mTORC1 is important in postnatal β-cell development and functional maturation\textsuperscript{26, 27, 28}; however, its role in mature β-cells has less been studied. To this end, we generated MIP-Cre\textit{ER}; Raptor\textit{fl/fl} mice and induced Raptor KO in adult animals by tamoxifen. We assessed insulin secretion early (two weeks) after tamoxifen injection to minimize deleterious effects of prolonged mTORC1 deficiency. At this time point mTORC1 was indeed inhibited (Supplemental Fig. 2a-b); the presence of the MIP-Cre construct and tamoxifen injection did not affect glucose tolerance (Supplemental Fig. 2c, e) or insulin sensitivity (Supplemental Fig. 2d, f). Relevant to the current study, we further show that the MIP-Cre\textit{ER} construct did not affect autophagy in islets (Supplemental Fig. 2h). In agreement with a previous study\textsuperscript{26}, we found that βRaptor KO mice developed mild glucose intolerance (Supplemental Fig. 2g). At 30 min after oral gavage of glucose and leucine, glucose levels were higher in the βRaptor KO than in control mice, along with decreased serum insulin levels and markedly reduced insulinogenic index (Fig. 2a-c).

Leucine stimulated mTORC1 concentration-dependently with maximal effect at 4-10mmol/l (Supplemental Fig. 1b). Consistent with the hypothesis that leucine amplifies insulin secretion in an mTORC1-dependent manner, it dose-dependently increased secretion in islets treated with the metabolic fuels glutamine and glucose (Supplemental Fig 3). Maximal stimulation of mTORC1 and insulin secretion was observed at 10mmol/l leucine, which was used in further in vitro studies. To decipher the role of mTORC1 in mediating the leucine effects on autophagy and insulin secretion, we performed static incubations. The non-metabolizable leucine analog 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) and the AA combination of leucine+glutamine enhance insulin secretion by increasing glutamate oxidation in the TCA cycle\textsuperscript{29}. While at 3.3mmol/l glucose, AA-stimulated insulin secretion was similar in βRaptor
KO and control islets, the response to HG with or without AAs was decreased by ~50% (Fig. 2d). In INS-1 cells, the mTORC1 inhibitor rapamycin inhibited HG+ AAs-stimulated insulin secretion (Fig. 2e). Nutrients regulate insulin secretion by plasma membrane depolarization with subsequent opening of voltage-dependent calcium channels (triggering pathway) and via metabolic signals that amplify secretion independent of KATP channels (amplifying pathway). We studied KATP-independent amplification of secretion by glucose and AAs (leucine+glutamine) in islets and INS-1 cells treated with or without rapamycin. Islets or INS-1 cells were exposed to nutrients in presence of diazoxide (KATP channel opener) together with 30mmol/l KCl (membrane depolarizer) with or without 100 nmol/l rapamycin (Fig. 2f-g). In islets, glucose and AAs (leucine+glutamine) amplified insulin secretion by 2.5-fold and 4-fold, respectively; their combination further augmented secretion. In both islets and INS-1 cells, rapamycin abrogated the amplification of insulin secretion by AAs at HG (Fig. 2f-g). In INS-1 cells, rapamycin also inhibited amplification of insulin secretion by glucose alone (Fig. 2g).

In summary, glucose and AAs act in concert to stimulate mTORC1 and increase insulin secretion in an mTORC1-dependent manner.

**Regulation of β-cell autophagy in the postabsorptive and fed states**

mTORC1 is a central regulator of autophagy; we therefore studied β-cell autophagy in the postabsorptive and fed states. Autophagosome maturation involves lipidation of LC3 recruited to the autophagosome membrane, which is used to quantify autophagosomes. Staining of pancreatic sections of fasted mice showed reduced mTORC1 activity associated with markedly increased autophagosome number, whereas refeeding for 4 h or adding BCAA to drinking water inhibited autophagy (Fig. 3a-b). We then studied the early effects of nutrient administration on autophagy. Surprisingly, gavage of leucine and glucose alone or together markedly increased autophagosomes 1 h following nutrient administration, despite the simultaneous stimulation of mTORC1 (Fig. 3c). Accumulation of autophagosomes may result from increased generation (stimulated autophagy) or from disruption of autophagosome-lysosome fusion (inhibited autophagy). To distinguish, we isolated islets followed by incubation
with glucose and leucine with and without bafilomycin A1, which alkalinizes lysosomes, thereby inhibiting autophagosome-lysosome fusion and degradation. Bafilomycin A1 increased autophagosomes number at both 3.3mmol/l glucose and 16.7mmol/l glucose+leucine, indicating autophagy was indeed enhanced (Fig. 4a). By contrast, prolonged incubation of islets in presence of high glucose and leucine for 16 h inhibited autophagy, as evident by lower number of autophagosomes without and with bafilomycin A1 (Fig. 4b). These findings suggest that glucose and AAs, while rapidly stimulating mTORC1, induce a biphasic response of autophagy: an early burst of autophagosome generation followed by late inhibition of autophagy.

**Mechanisms of nutrient regulation of autophagy in β-cells**

To obtain further insight on nutrient regulation of mTORC1-autophagy interaction, we studied ULK1 and TFEB activities. AA stimulation of mTORC1 in INS-1 cells (Supplemental Fig. 1c) was associated with inhibition of autophagy, evident by decreased LC3-II/I ratio by Western blotting (Fig. 5a) and reduced LC3+ puncta (Fig. 5b), mimicking the autophagy inhibition by prolonged islet exposure to nutrients (Fig. 4b). Accumulation of P62/SQSTM1, which is degraded by lysosomes indicates inhibited autophagic flux. Consistently, P62/SQSTM1 was increased in cells treated with high glucose plus AAs (Fig. 5c). Transfection with an LC3-GFP-RFP construct enables assessing the autophagic flux: high autophagosome turnover in stimulated autophagy leads to enrichment of red RFP+ puncta as GFP fluorescence undergoes quenching in autolysosome; in contrast, inhibition of autophagosome-lysosome fusion is associated with accumulation of yellow RFP+/GFP+ puncta. Incubation of INS-1 cells with AAs and glucose for 4 h increased the number of GFP+/RFP+ puncta (Fig. 5d), suggesting that nutrients inhibited both autophagosome generation and autophagosome-lysosome fusion.

mTORC1 directly phosphorylates ULK1 at serine757, which prevents autophagosome initiation, and TFEB at serine122. We incubated islets and INS-1 cells with glucose and AAs for different periods of time and analyzed mTORC1 activity, and ULK1 and TFEB phosphorylation (Fig. 5e-f). Islet
incubation at HG+leucine increased both total and phosphorylated S6, whereas
the protein levels of TSC1, S6K1 and the housekeeping GAPDH remained
unchanged. S6 is a ribosomal protein involved in protein synthesis, suggesting
that in addition to 4EBP1 and S6 phosphorylation, mTORC1 may promote
protein synthesis by increasing S6 biosynthesis; this was observed in islets, but
not in INS-1 cells (Supplemental Fig. 1a). Incubation of islets with HG or leucine
alone showed that the leucine effect on S6 activity is indeed greater than that
of HG; this was associated with increased ULK1 phosphorylation
(Supplemental Fig. 4). Importantly, incubation of islets and INS-1 cells at
HG+AAs for 1 h increased both ULK1 and TFEB phosphorylation in parallel
with the activation of mTORC1 (Fig. 5e-f).

We studied the effects of nutrients on TFEB localization in INS-1 cells
and islets. We transfected INS-1 cells with TFEB-GFP construct and incubated
the cells at 3.3mmol/l glucose or HG+AAs. Nutrients induced nuclear exclusion
of TFEB (Fig. 5g).

This could also be demonstrated in vivo in pancreases removed 1 h after
gavage from mice administered glucose and leucine. By contrast, in βRaptor
KO pancreases the nuclear localization of TFEB was increased and was not
affected by nutrients (Fig. 6a). Ex vivo exposure of dispersed islets to
HG+leucine showed two patterns of intracellular TFEB distribution: small
puncta diffusely spread in the cytosol and cytolsolic aggregates (Fig. 6b). At low
glucose, majority of cells (~80%) showed diffuse staining, whereas exposure to
HG and leucine resulted in a mixed phenotype, with ~50% containing cytolsolic
aggregates (Fig. 6b). βRaptor KO partially prevented TFEB aggregation in islets
treated with HG+leucine. These alterations in TFEB localization in βRaptor KO
islets were associated with increased expression of TFEB-regulated genes,
including cathepsin D (CtsD), H+ /Cl- exchanger, transporter 7 (Clcn7), WIP11
(Atg18 homolog) along with non-significant increase in CtsA and CtsB (Fig. 6c).
Overnight incubation of wildtype islets with HG+leucine decreased the
expression of TFEB-regulated genes (Fig. 6d). TFEB also regulates lysosome
biogenesis; consistently, we found higher numbers of lysosomes (LAMP1+
vesicles) in βRaptor KO mice treated with or without glucose and leucine
compared to controls (Supplemental Fig. 5).
Regulation of insulin secretion by autophagy

We further assessed how the mTORC1-autophagy crosstalk regulates insulin secretion by inhibiting autophagy in βRaptor KO mice. Induced Raptor KO in β-cells stimulated autophagy along with inhibition of insulin secretion, hence mimicking fasting (Fig. 2). To further assess the role of autophagy in regulating insulin secretion, we generated double-transgenic mice, MIP-CreER; Raptor\textsuperscript{fl/fl}; Atg7\textsuperscript{+/fl}, in which autophagy is moderately inhibited in β-cells in the presence of mTORC1 deficiency. We first tested the effects of βRaptor KO with and without Atg7 haploinsufficiency on autophagy. Indeed, Atg7 haploinsufficiency effectively reduced stimulation of autophagy by βRaptor KO in β-cells (Supplemental Fig. 6a-b). Transmission electron microscopy showed increased autophagosomes and peroxisomes in βRaptor KO mice that was prevented by Atg7 haploinsufficiency (Supplemental Fig. 6c). Secretory granules were reduced in the βRaptor KO mice without affecting the proportion of immature granules (Supplemental Fig. 6d).

Next, we studied the effects of Atg7 haploinsufficiency on insulin secretion in control mice and in βRaptor KO mice. Heterozygous Atg7 KO in mTORC1-competent β-cells (MIP-creER; Atg7\textsuperscript{+/+}) modestly increased glucose-stimulated insulin secretion along with mild improvement in glucose tolerance (Fig 7a-c). Static incubations showed similar glucose-stimulated insulin secretion in control and βAtg7\textsuperscript{+/−} islets (Fig. 7d). Thus, the overall effect of heterozygous Atg7 KO on insulin secretion was relatively small.

We then studied the effects of heterozygous Atg7 KO on insulin secretion in mTORC1 deficient β-cells (MIP-creER; Raptor\textsuperscript{fl/fl}; Atg7\textsuperscript{+/+}). Prevention by Atg7 KO of the autophagy stimulation induced by mTORC1 deficiency was accompanied by notable decrease in postprandial (glucose/leucine gavage) glucose excursions and increase in the insulinogenic index (Fig. 7e-g). Atg7 heterozygous mice failed to correct the reduced islet insulin content of βRaptor KO islets (Fig. 7h); however, it completely prevented the decline in insulin secretion (Fig. 7i). In fact, normalized for the reduced content, insulin secretion was greater than in wildtype islets. The proinsulin/insulin ratio was not modified in the transgenic islets (Fig. 7j). We reiterated these findings by injecting 5 μg/kg
chloroquine (CQ), an inhibitor of lysosomal function, for three days to wildtype mice; this resulted in increased fasting insulin levels along with amplification of glucose-stimulated insulin secretion and improvement of glucose tolerance (Fig. 7k-l). Collectively, these findings show that autophagy restrains insulin secretion in the postabsorptive and fed states. Inhibiting autophagy may enhance insulin secretion in vivo.

We tested whether the above conclusions apply to human islets by incubating at 3.3mmol/l or 16.7mmol/l glucose and 10mmol/l leucine with or without rapamycin and/or CQ. Acute exposure to leucine amplified insulin secretion in islets treated with HG (Supplemental Fig. 7). Rapamycin modestly decreased GSIS (not significant), and significantly reduced exocytosis at HG+leucine. CQ partially prevented the inhibition of insulin secretion by rapamycin (Supplemental Fig. 7). Our studies in mouse islets showed that the inhibition of autophagy by nutrients evolve over time. We therefore tested the effects of overnight treatment with HG+leucine with or without rapamycin and/or CQ on mTORC1-autophagy signaling and insulin secretion. HG+leucine markedly increased mTORC1 activity, including S6 protein level and phosphorylation, 4EBP1, ULK1 and TFEB phosphorylation. Treatment with rapamycin prevented nutrient stimulation of mTORC1, whereas treatment with CQ did not prevent the inhibition of S6 phosphorylation by rapamycin (Figure 8a). As expected, HG increased the accumulation of insulin in the medium without affecting islet insulin content (Fig. 8b-c). Of note, leucine amplified insulin release at HG, whereas rapamycin decreased secretion in islets incubated at HG with and without leucine. Importantly, treatment with CQ completely prevented the inhibition of insulin secretion by rapamycin. We then tested the islet response to glucose after overnight exposure to nutrients and pharmacological inhibitors of mTORC1 and autophagy. Overnight incubation at HG enhanced insulin secretion at low and high glucose concentrations (Fig. 8d). Overnight incubation at HG+leucine further enhanced GSIS, whereas pretreatment with rapamycin including at a low concentration of 10nmol/l, markedly attenuated the acute response to glucose. CQ alone did not affect GSIS in islets that were incubated overnight with HG+leucine, however it completely prevented its inhibition by rapamycin (Fig. 8d).
Collectively, these findings show that exposure to high glucose not only increases the chronic secretion to the medium, but also potentiates glucose-stimulated insulin secretion. Leucine further increased chronic and acute glucose-stimulated insulin secretion, whereas rapamycin markedly inhibited insulin secretion in islets incubated with HG and leucine. Strikingly, treatment with CQ completely prevented the inhibition of insulin secretion by rapamycin. We conclude that leucine amplifies insulin secretion in adult mouse and human islets through modulation of mTORC1 and autophagy (Fig. 8e).

Discussion

mTORC1 governs the functional maturation of β-cells during postnatal development and its adaptation to nutrition. In a seminal paper, Blandino-Rosano et al showed that constitutive disruption of mTORC1 in β-cells by conditional Raptor KO induced apoptosis, decreased proliferation and mass, and impaired function through the S6K and 4EBP2-eIF4E pathways. These alterations were partially prevented by inhibiting lysosomal hydrolases with CQ, which rescued β-cell mass and increased serum insulin. The multiple effects of prolonged mTORC1 deficiency on β-cell mass and function render difficult deciphering the physiological role of mTORC1-autophagy crosstalk in nutrient sensing and regulating insulin secretion. To address this fundamental question, we first studied mTORC1 and autophagy during cycles of fasting and feeding, and performed fine-tuned modulation of mTORC1 activity by short-term inducible Raptor KO in adult β-cells and of autophagy by heterozygous Atg7 KO; with these tools at hand, we tested the effects on glucose and AA stimulation of insulin secretion in vivo and ex vivo.

We show here that mTORC1 is a bona fide nutrient sensor and important regulator of insulin secretion in adult rodent and human β-cells. Furthermore, we demonstrate that the mTORC1 effect on insulin secretion in the post-absorptive period is mediated by autophagy. Nutrient sensors that are well adjusted to cope with fast-feed cycles are expected to exhibit low activity nutrient deprivation and be rapidly and effectively stimulated when food is available. Consistently, we found that β-cell mTORC1 activity is barely detectable under starvation conditions both ex vivo and in vivo fasting, while it
is rapidly stimulated by refeeding or following acute administration of glucose and AAs.

Others and we showed that mTORC1 plays a developmental role in the functional maturation of β-cells, later being downregulated during adulthood. Furthermore, it has been suggested that post-weaning nutrient sensing is switched from mTORC1 to AMPK. However, decline in mTORC1 after weaning, rather than developmental involution, may correspond to change in feeding behavior and diet composition with switch from suckling to periodic feeding, mTORC1 now assuming a new, oscillating role in nutrient sensing. Our findings are in marked disagreement with the suggestion that mTORC1 is activated during fasting through enhanced proteolysis of secretory granules.

In agreement with previous studies, we show here that in β-cells AAs, mainly the BCAA leucine, are potent activators of mTORC1. We further show that in vivo glucose enhances mTORC1 activity and amplifies the leucine effect, perhaps via dihydroxyacetone phosphate (DHAP) as suggested.

It has been recently suggested that in the post-weaning period islets shift from primary AA- to glucose-stimulated insulin secretion. On the contrary, we show here that in adult β-cells mTORC1 exquisitely senses both AAs (leucine) and glucose and that intermittent stimulation of mTORC1 is required for insulin secretion in response to these nutrients.

Leucine acutely stimulate insulin secretion through deamination to α-ketoisocaproic acid (KIC) and enhanced glutaminolysis by allosterically activating glutamate dehydrogenase (GDH) with subsequent glutamate oxidation in the TCA cycle. At HG, GDH promotes nutrients export from the TCA cycle to the cytosol (anaplerosis), rather than glutamate oxidation. We show that under these conditions AAs (leucine) stimulate secretion at least in part through mTORC1. Inhibition of mTORC1 reduced AA-stimulated insulin secretion from depolarized islets and INS-1 cells, suggesting that mTORC1 is required for the amplification of insulin secretion by glucose/AAs downstream to the KATP channel.

Modulation of mTORC1 by the nutritional state was accompanied by inverse modulation of autophagy: fasting was associated with enhanced
autophagy, and this was reversed by refeeding, supplementation of BCAA in drinking water or overnight exposure to high glucose and leucine in vitro. mTORC1 deficiency induced by βRaptor KO also stimulated autophagy, thus mimicking the effects of fasting. Mechanistically, we show that inhibition of mTORC1 either by fasting or by genetic manipulation decreased the inhibitory phosphorylation of ULK1 and TFEB and modulated the intracellular localization of the transcription factor TFEB in islets. This was accompanied by increased expression of the autophagy-regulating gene WIPI1 and of genes involved in lysosomal function and biogenesis. These findings are consistent with recent reports showing that TFEB plays an important role in regulating autophagy in β-cells\textsuperscript{39, 40}.

A striking and unexpected finding was the paradoxical enhancement of autophagy following acute administration of nutrients despite simultaneous stimulation of mTORC1 and inhibition of its downstream targets TFEB and ULK1, suggesting that it is most likely independent of mTORC1-ULK1-TFEB signaling. We speculate that in β-cells nutrient-derived signals may promote a rapid and general mobilization of membranes and granules, including autophagosomes. Further studies are required to clarify the mechanisms for the initial stimulation of autophagy by nutrients and assess whether a similar biphasic response is observed in other metabolic or secretory tissues.

Importantly, we found that the mTORC1-autophagy signaling regulates insulin secretion. Inhibiting autophagy by heterozygous Atg7 KO amplified glucose-stimulated insulin secretion. Short-term treatment with CQ augmented both fasting and stimulated insulin secretion. Furthermore, mTORC1 deficiency impaired insulin secretion in an autophagy-dependent manner, partial inhibition of autophagy being sufficient to rescue secretion. Islet proinsulin and insulin content was reduced in βRaptor KO mice; inhibiting autophagy did not rescue hormone stores, suggesting the restoration of insulin secretion is not due to enhancement of insulin synthesis.

How autophagy regulates insulin secretion is unknown. Based on studies in a β-cell line, we suggested that secretory granules are degraded by autophagy\textsuperscript{10}. However, electron microscopy of βRaptor KO islets showed that secretory granule localization within autophagosomes and lysosomes is
relatively rare (not shown), suggesting that in primary β-cells stimulation of autophagy does not affect exocytosis through major effects on the secretory granule pool. The possibility that autophagy selectively affects a small, readily-releasable pool of secretory granules cannot be excluded. Inhibition of autophagy by Atg7 KO in mice fed on HFD enhanced peroxisome function and modulated lipid composition with increased ether lipids and prevented n-3 polyunsaturated fatty acid depletion; this was associated with a transient increase in insulin secretion\(^4^1\). Interestingly, we found that the number of peroxisomes was modulated by mTORC1 deficiency and autophagy. Ongoing unbiased metabolomic, lipidomic and proteomic analyses will shed light on how autophagy modulates insulin secretion.

Autophagy restrained insulin secretion may have important implications for obesity and diabetes. Elevated serum BCAA along with hyperinsulinemia characterizes obesity-associated insulin resistance\(^4^2\). It is possible that increased β-cell exposure to BCAA inhibits autophagy, which in turn increases basal insulin secretion. We have previously shown that in neonatal diabetes induced by β-cell ER stress, inhibition of mTORC1 precedes the development of diabetes, resulting in marked impairment of β-cell growth and function\(^2^8\). Dysregulation of mTORC1 in response to ER stress might impair insulin secretion in part through sustained stimulation of autophagy.

Finally, our findings may have therapeutic implications. CQ and hydroxychloroquine (HCQ) are FDA-approved drugs. They alkalinize endosomal organelles and inhibit lysosomal cargo degradation. There is a consistent literature, including a randomized placebo-controlled trial on uncontrolled T2D, showing HCQ treatment is associated with improved metabolism by reducing insulin resistance and improving β-cell function\(^4^3, 4^4, 4^5\). Therefore, moderate inhibition of autophagy might become a novel therapeutic approach for the treatment of T2D.

In summary, we show here that in adult β-cells, mTORC1 is a central and dynamic sensor responding to rapid changes in nutrient availability. mTORC1 inhibits autophagy via ULK1 and TFEB with subsequent inhibition of the genetic program regulating autophagy and lysosome biogenesis and activity. As illustrated in Fig. 8e, we propose that the mTORC1-autophagy
crosstalk forms a central node which modulates insulin secretion in accordance with nutrient availability over the diurnal variations. When prevented from oscillating and stimulated continuously by overnutrition, the ensuing inhibition of autophagy and increased insulin secretion constitutes a plausible pathogenic mechanism for the insulin resistance of obesity and the β-cell failure of T2D.

Acknowledgment
This work was supported by grants from the National Institute of Health (NIDDK) Grant: R01-DK073716 to EBM and GL, and Israel Science Foundation (ISF) Grant: ISF-398/20 and GIF Grant: I-429-201.2/2017 to GL. Gil Leibowitz is the guarantor of this work. The authors declare no conflict of interests. Dr. Gil Leibowitz is the guarantor of this work and, as such had full access to all data and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors (Tal Israeli, Yael Riahi, Perla Garzon, Ruy Andrade Louzada, Joao Pedro Werneck-de-Castro, Manuel Blandino-Rosano, Roni Yeroslaviz-Stolper, Liat Kadosh, Sharona Tornovsky-Babeay, Gilad Hacker, Nitzan Israeli, Orly Agmon, Boaz Tiros, Erol Cerasi, Ernesto Bernal-Mizrachi, Gil Leibowitz) declare no conflicts of interest.

Author contributions
G.L., E.C. and E.B.M. conceived the study. G.L., T.I., Y.R., E.C. and E.B.M designed experiments. T.I., Y.R., P.G., R.A.L., J.P.W.-d.-C., M.B.-R., R.Y.-S, L.K., S.T.-B, G.H., N.I. and O.A. performed experiments and analyzed results; G.L. and T.I. wrote the paper.

References
1. Mizushima N. Autophagy in protein and organelle turnover. Cold Spring Harb Symp Quant Biol 76, 397-402 (2011).
2. Jung HS, et al. Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. Cell Metab 8, 318-324 (2008).
3. Ebato C, et al. Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. *Cell Metab* 8, 325-332 (2008).

4. Kim J, et al. Amyloidogenic peptide oligomer accumulation in autophagy-deficient beta cells induces diabetes. *J Clin Invest* 124, 3311-3324 (2014).

5. Rivera JF, Costes S, Gurlo T, Glabe CG, Butler PC. Autophagy defends pancreatic beta cells from human islet amyloid polypeptide-induced toxicity. *J Clin Invest* 124, 3489-3500 (2014).

6. Shigihara N, et al. Human IAPP-induced pancreatic beta cell toxicity and its regulation by autophagy. *J Clin Invest* 124, 3634-3644 (2014).

7. Fujitani Y, Kawamori R, Watada H. The role of autophagy in pancreatic beta-cell and diabetes. *Autophagy* 5, 280-282 (2009).

8. Sheng Q, et al. Autophagy protects pancreatic beta cell mass and function in the setting of a high-fat and high-glucose diet. *Sci Rep* 7, 16348 (2017).

9. Pearson GL, et al. Lysosomal acid lipase and lipophagy are constitutive negative regulators of glucose-stimulated insulin secretion from pancreatic beta cells. *Diabetologia* 57, 129-139 (2014).

10. Riahi Y, et al. Autophagy is a major regulator of beta cell insulin homeostasis. *Diabetologia* 59, 1480-1491 (2016).

11. Israeli T, et al. Opposing effects of intracellular versus extracellular adenine nucleotides on autophagy: implications for beta-cell function. *J Cell Sci* 131, (2018).

12. Goginashvili A, et al. Insulin granules. Insulin secretory granules control autophagy in pancreatic beta cells. *Science* 347, 878-882 (2015).

13. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168, 960-976 (2017).

14. Ardestani A, Lupse B, Kido Y, Leibowitz G, Maedler K. mTORC1 Signaling: A Double-Edged Sword in Diabetic beta Cells. *Cell Metab* 27, 314-331 (2018).

15. Goberdhan DC, Wilson C, Harris AL. Amino Acid Sensing by mTORC1: Intracellular Transporters Mark the Spot. *Cell Metab* 23, 580-589 (2016).

16. Wolfson RL, Sabatini DM. The Dawn of the Age of Amino Acid Sensors for the mTORC1 Pathway. *Cell Metab* 26, 301-309 (2017).
17. Condon KJ, Sabatini DM. Nutrient regulation of mTORC1 at a glance. *J Cell Sci* **132**, (2019).

18. Mizushima N. The role of the Atg1/ULK1 complex in autophagy regulation. *Curr Opin Cell Biol* **22**, 132-139 (2010).

19. Settembre C, *et al.* A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J* **31**, 1095-1108 (2012).

20. Tamarina NA, Roe MW, Philipson L. Characterization of mice expressing Ins1 gene promoter driven CreERT recombinase for conditional gene deletion in pancreatic beta-cells. *Islets* **6**, e27685 (2014).

21. Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM. mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* **468**, 1100-1104 (2010).

22. Komatsu M, *et al.* Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol* **169**, 425-434 (2005).

23. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB. Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes* **49**, 424-430 (2000).

24. Kimura S, Noda T, Yoshimori T. Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. *Autophagy* **3**, 452-460 (2007).

25. Rocznia-Ferguson A, *et al.* The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci Signal* **5**, ra42 (2012).

26. Blandino-Rosano M, *et al.* Loss of mTORC1 signalling impairs beta-cell homeostasis and insulin processing. *Nat Commun* **8**, 16014 (2017).

27. Alejandro EU, *et al.* Maternal diet-induced microRNAs and mTOR underlie β cell dysfunction in offspring. *The Journal of clinical investigation* **124**, 4395-4410 (2014).

28. Riahi Y, *et al.* Inhibition of mTORC1 by ER stress impairs neonatal beta-cell expansion and predisposes to diabetes in the Akita mouse. *Elife* **7**, (2018).

29. Sener A, Malaisse WJ. L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. *Nature* **288**, 187-189 (1980).
30. Ni Q, et al. Raptor regulates functional maturation of murine beta cells. *Nat Commun* **8**, 15755 (2017).

31. Jaafar R, et al. mTORC1 to AMPK switching underlies beta-cell metabolic plasticity during maturation and diabetes. *J Clin Invest* **129**, 4124-4137 (2019).

32. Helman A, et al. A Nutrient-Sensing Transition at Birth Triggers Glucose-Responsive Insulin Secretion. *Cell Metab* **31**, 1004-1016 e1005 (2020).

33. Filiputti E, et al. Augmentation of insulin secretion by leucine supplementation in malnourished rats: possible involvement of the phosphatidylinositol 3-phosphate kinase/mammalian target protein of rapamycin pathway. *Metabolism* **59**, 635-644 (2010).

34. Kwon G, Marshall CA, Pappan KL, Remedi MS, McDaniel ML. Signaling elements involved in the metabolic regulation of mTOR by nutrients, incretins, and growth factors in islets. *Diabetes* **53 Suppl 3**, S225-232 (2004).

35. Xu G, Kwon G, Cruz WS, Marshall CA, McDaniel ML. Metabolic regulation by leucine of translation initiation through the mTOR-signaling pathway by pancreatic beta-cells. *Diabetes* **50**, 353-360 (2001).

36. Orozco JM, et al. Dihydroxyacetone phosphate signals glucose availability to mTORC1. *Nat Metab* **2**, 893-901 (2020).

37. Yang J, Chi Y, Burkhardt BR, Guan Y, Wolf BA. Leucine metabolism in regulation of insulin secretion from pancreatic beta cells. *Nutr Rev* **68**, 270-279 (2010).

38. Li C, et al. Regulation of leucine-stimulated insulin secretion and glutamine metabolism in isolated rat islets. *J Biol Chem* **278**, 2853-2858 (2003).

39. Kim J, et al. An autophagy enhancer ameliorates diabetes of human IAPP-transgenic mice through clearance of amyloidogenic oligomer. *Nat Commun* **12**, 183 (2021).

40. Ji J, et al. Type 2 diabetes is associated with suppression of autophagy and lipid accumulation in beta-cells. *J Cell Mol Med* **23**, 2890-2900 (2019).

41. Chu KY, Mellet N, Thai LM, Meikle PJ, Biden TJ. Short-term inhibition of autophagy benefits pancreatic beta-cells by augmenting ether lipids and peroxisomal function, and by countering depletion of n-3 polyunsaturated fatty acids after fat-feeding. *Mol Metab* **40**, 101023 (2020).
42. Newgard CB, *et al.* A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* **9**, 311-326 (2009).

43. Hage MP, Al-Badri MR, Azar ST. A favorable effect of hydroxychloroquine on glucose and lipid metabolism beyond its anti-inflammatory role. *Ther Adv Endocrinol Metab* **5**, 77-85 (2014).

44. Gerstein HC, Thorpe KE, Taylor DW, Haynes RB. The effectiveness of hydroxychloroquine in patients with type 2 diabetes mellitus who are refractory to sulfonylureas--a randomized trial. *Diabetes Res Clin Pract* **55**, 209-219 (2002).

45. Wasko MC, McClure CK, Kelsey SF, Huber K, Orchard T, Toledo FG. Antidiabetogenic effects of hydroxychloroquine on insulin sensitivity and beta cell function: a randomised trial. *Diabetologia* **58**, 2336-2343 (2015).

**Legend to Figures**

**Figure 1: mTORC1 activity during fasting and in response to nutrients.** *(A)* C57BL/6 mice were fed or fasted overnight with or without branched-chain amino acids (BCAA) added to the drinking water (13.3 g/l each). Islets were isolated and stained for insulin and pS6 (Ser240/244). Scale bar: 20 μm. Left panel: % Ins<sup>+</sup>pS6<sup>+</sup>/Ins<sup>+</sup> cells: n=1500-2000 cells per group; pS6 mean fluorescence intensity (MFI)/cell, n=170-275 cells per group. Right panel: % Ins<sup>+</sup>pS6<sup>+</sup>/Ins<sup>+</sup> cells, n=900-1200 cells per group; pS6 MFI/cell, 400-500 cells per group were counted, n=2 separate experiments. *(B)* Mice were fasted overnight, then received 2 g/kg glucose and/or 0.39 g/kg leucine by oral gavage. Pancreases were removed 1 h following gavage and sections stained for insulin and pS6. S6 phosphorylation in β-cells is expressed as percentage of pS6<sup>+</sup> β-cells. Scale bar: 40 μm. n=3 separate experiments, 2000-3000 cells per treatment were counted.
Figure 2: mTORC1 regulation of insulin secretion. (A-C) Control (Raptor^{fl/fl}) and MIP-CreER; Raptor^{fl/fl} mice were injected tamoxifen. Two weeks later, mice were fasted overnight and then given 2 g/kg glucose + 0.39 g/kg leucine by gavage. Blood samples were obtained before and 30 min following the gavage; (A) plasma glucose measurements, (B) serum insulin levels, and (C) the insulinogenic index calculated as Δinsulin (0-30 min)/Δglucose (0-30 min) ratio. Raptor^{fl/fl}: n=16-21 mice; MIP-CreER; Raptor^{fl/fl} n=16-17 mice. (D) Islets were isolated from βRaptor KO and control mice, pre-incubated in Krebs-Ringer Modified Buffer (KRB) containing 3.3 mmol/l glucose for 40 min, followed by stimulation with high glucose (16.7 mmol/l) and/or amino acids (leucine + glutamine, 10 mmol/l each), or BCH (20 mmol/l) for 1 h, n=5 in triplicates. (E) INS-1 cells were pre-incubated in KRB containing 3.3 mmol/l glucose with or without 100 nmol/l rapamycin for 30 min, followed by incubation in KRB containing 3.3 mmol/l or 16.7 mmol/l glucose, with or without AAs (leucine + glutamine, 10 mmol/l each) with or without rapamycin (100 nmol/l) for 1 h, n=4 in triplicates. (F-G) Islets (F) and INS-1 cells (G) were pre-incubated in KRB containing 3.3 mmol/l glucose with or without 100 nmol/l rapamycin for 1h, followed by incubation in KRB containing 3.3 mmol/l or 16.7 mmol/l glucose with or without AA (leucine + glutamine, 10 mmol/l each) and 100 nmol/l rapamycin in the absence or presence of 250 μmol/l diazoxide and 30 mmol/l KCl for 1 h, n=3 in quadruplicates (islets) or triplicates (INS-1 cells).

Figure 3: Regulation of β-cell autophagy in the postabsorptive and fed states. (A-B) C57BL/6 mice were fasted overnight followed by refeeding for 4 h (A) or treated with BCAA (13.3 g/l each) added to the drinking water (B). Islets were isolated, dispersed and stained for insulin and LC3. n=2 separate experiments, 250-640 cells per group were counted. Scale bar: 5 μm. (C) Mice were fasted overnight and then administered 2 g/kg glucose and/or 0.39 g/kg leucine by gavage, followed 1 h later by removal of pancreases and staining for insulin and LC3. Scale bar: 10 μm. n=3 separate experiments, 250-450 cells per treatment were counted.
Figure 4: Dynamic regulation of β-cell autophagy by nutrients. Islets isolated from C57BL/6 mice were incubated for 1 h (A) or overnight (B) in AA-free RPMI medium supplemented with 10% fetal calf serum and 3.3 mmol/l glucose (LG) or 16.7 mmol/l glucose + 10 mmol/l leucine (HG+leucine) in the presence or absence of 100 nmol/l bafilomycin A1 for 2 h. Islets were dispersed and stained for insulin and LC3. Scale bar: 10 μm. n=3 separate experiments, 120-200 cells per treatment were counted.

Figure 5: mTORC1 regulation of autophagy in β-cells. INS-1 cells were incubated in AA-free RPMI medium supplemented with 3.3 mmol/l or 16.7 mmol/l glucose with or without leucine + glutamine (10 mmol/l each) for 1 h, in the presence or absence of 100 nmol/l bafilomycin A1 for 4 h. Cells were lysed and analyzed by Western blotting for LC3B, P62/SQSTM1 and GAPDH (A, C) or stained for LC3 (B). Scale bar: 10 μm. (A) n=4, LC3II/I ratio was quantified in samples treated with bafilomycin A1, (B) n=2 separate experiments, quantification was performed on 500-900 cells per treatment. (C) n=5. (D) INS-1 cells were transfected with LC3-GFP-RFP plasmid; medium was changed after 24 h to RPMI containing 16.7 mmol/l glucose with or without leucine + glutamine (10 mmol/l each) for 4 h. The cells were imaged by confocal microscope and GFP+ and RFP+ puncta and their colocalization quantified. The fraction of autophagosomes (GFP+/RFP+ puncta, yellow) and autolysosomes (GFP-/RFP+ puncta, red) is shown at the bottom. Scale bar: 5 μm. (E) Islets from 12 mice were isolated, pooled and incubated overnight in complete RPMI. Islets were pre-incubated at 3.3 mmol/l glucose in absence of AAs and serum and then incubated in medium containing 1% serum and 3.3 mmol/l glucose or 16.7 mmol/l glucose + 10 mmol/l leucine for 1 h. Islet extracts were analyzed by Western blotting for pULK1 (Ser757), ULK1, pTFEB (Ser122), TFEB, pS6 (Ser240/244), S6, S6K1, TSC1 and GAPDH. NP-non-phosphorylated, P-phosphorylated. (F) INS-1 cells were incubated at 3.3 mmol/l glucose or 16.7 mmol glucose + leucine and glutamine for different periods of time followed by Western blotting for phospho- and total ULK1, TFEB, S6 and tubulin. Quantifications of pULK1 and pTFEB are shown to the right (n=3). (G) INS-1 cells were transfected with TFEB-GFP construct. After 24 h, the medium was
replaced and the cells were incubated overnight at 3.3 mmol/l or 16.7 mmol/l glucose + leucine and glutamine (10 mmol/l each). TFEB-GFP localization was analyzed by confocal microscope. Quantification of cells with nuclear localization of TFEB is shown to the right, n=2 separate experiments, quantification was performed on 35-50 cells per treatment. Scale bar: 5 μm.

Figure 6: Effects of nutrients on TFEB localization and function in islets. Control (Raptor<sup>fl/fl</sup>) and βRaptor KO (MIP-CreER; Raptor<sup>fl/fl</sup>) mice were fasted overnight and administered water or 2 g/kg glucose + 0.39 g/kg leucine by gavage, followed by removal of the pancreas 1 h later. Pancreatic sections were immunostained for insulin and TFEB. Scale bar: 10 μm. Analysis was performed by confocal microscope. n=2 separate experiments, quantification was performed on 400-700 cells. (B) WT and βRaptor KO islets were incubated in AA-free RPMI supplemented with 1% serum at 3.3 mmol/l glucose or 16.7 mmol/l glucose + 10mmol/l leucine for 24 h. Islets were dispersed and TFEB localization was analyzed by fluorescence microscope. Scale bar: 100 μm. Quantification of the number of cells with cytoplasmic aggregates of TFEB vs diffuse puncta is shown, n=2-3 experiments, 1000-2060 cells were counted for each experimental group. A-Aggregates, DP- diffuse puncta.

(C) Islets were isolated from control and βRaptor KO mice followed by RNA extraction and qPCR for TFEB regulated genes, n=3. (D) Islets isolated from control mice were incubated overnight with AA-free RPMI medium at 3.3 mmol/l glucose or 16.7 mmol/l glucose + 10 mmol/l leucine followed by qPCR for TFEB-regulated genes, n=3.

Figure 7: Regulation of insulin secretion by autophagy. Islets were isolated from control (Raptor<sup>fl/fl</sup> or Atg7<sup>fl/fl</sup>), βAtg7<sup>+/−</sup> (MIP-CreER; Atg7<sup>fl/fl</sup>), βRaptor KO (MIP-CreER; Raptor<sup>fl/fl</sup>) and βRaptor KO; Atg7<sup>+/−</sup> mice (MIP-CreER; Raptor<sup>fl/fl</sup>; Atg7<sup>fl/fl</sup>) were injected with tamoxifen; metabolic tests were performed after 2 weeks. (A) Western blotting for ATG7, P62/SQSTM1 and GAPDH (n=2). (B) IPGTT, (C) glucose-stimulated insulin secretion test, n=15-16 mice per group. (D) Insulin secretion of MIP-CreER, Atg7<sup>fl/fl</sup> compared to control (Atg7<sup>fl/fl</sup>) mice was assessed by static incubations at 3.3 and 16.7 mmol/l glucose for 1 h, n=3.
MIP-CreER; Raptor^fl/fl^ and MIP-CreER; Raptor^fl/fl^; Atg7^fl/+^ mice were injected with tamoxifen. Two weeks later, mice were fasted overnight and received 2 g/kg glucose + 0.39 g/kg leucine by gavage. Blood samples were obtained before and 30 min following the gavage; (E) plasma glucose measurements, (F) serum insulin levels, and (G) the insulinogenic index calculated as \( \Delta \)insulin (0-30 min)/\( \Delta \)glucose (0-30 min) ratio, n=9-16 mice per group. (H-J) Insulin secretion of (Raptor^fl/fl^), \( \beta \)Raptor^-/-^ and \( \beta \)Raptor^-/-^; Atg7^-/-^ KO islets was assessed by static incubations at 3.3 and 16.7 mmol/l glucose for 1 h. (H) Insulin content, (I) secreted insulin (left) and insulin secretion normalized to content (right), (J) proinsulin to insulin ratio in islet extracts, n=3. (K-L) C57BL/6 mice were injected with PBS or 5 \( \mu \)g/kg chloroquine (CQ) for 3 consecutive days, followed by IPGTT. Blood samples were drawn at the indicated time points and analyzed for glucose (K) and insulin (L), n=8.

Figure 8: Effects of nutrients, rapamycin and chloroquine (CQ) on mTORC1 signaling and insulin secretion in human islets. (A-D) Islets were incubated overnight at 3.3 mmol/l glucose, 16.7 mmol/l glucose with or without leucine (10 mmol/l) and/or rapamycin (10 and 100 nmol/l) and/or CQ (10 \( \mu \)mol/l). (A) Western blotting for total and phosphorylated S6, 4EBP1, TFEB and for phosphorylated-ULK1. (B) Cumulative secretion to the medium, (C) insulin content, (D) acute glucose-stimulated insulin secretion assessed by static incubations after overnight exposure to the indicated treatments. Results are the means of three independent experiments on islets from 3 different donors. (E) A model depicting the regulation of insulin secretion by nutrient modulation of mTORC1-autophagy crosstalk. Leucine is an amplifier of insulin secretion in presence of metabolic fuels such as glucose and glutamine. Exposure to high glucose and leucine stimulates mTORC1, which in turn inhibits autophagy with subsequent augmentation of insulin secretion. In the fast state, mTORC1 is inhibited thereby stimulating autophagy which in turn restrains insulin secretion. Periodic feeding leads to parallel transient stimulation of mTORC1 and autophagy, whereas prolonged exposure to nutrients (leucine and glucose) inhibits autophagy with subsequent development of hyperinsulinemia.
Figure 1: mTORC1 activity during fasting and in response to nutrients. (A) C57BL/6 mice were fed or fasted overnight with or without branched-chain amino acids (BCAA) added to the drinking water (13.3 g/l each). Islets were isolated and stained for insulin and pS6 (Ser240/244). Scale bar: 20 μm. Left panel: % Ins+ pS6+/Ins+ cells: n=1500-2000 cells per group; pS6 mean fluorescence intensity (MFI)/cell, n=170-275 cells per group. Right panel: % Ins+ pS6+/Ins+ cells, n=900-1200 cells per group; pS6 MFI/cell, n=2 separate experiments, 400-500 cells per group were counted. (B) Mice were fasted overnight, then received 2 g/kg glucose and/or 0.39 g/kg leucine by oral gavage. Pancreases were removed 1 h following gavage and sections stained for insulin and pS6. S6 phosphorylation in β-cells is expressed as percentage of pS6+ β-cells. Scale bar: 40 μm. n=3 separate experiments, 2000-3000 cells per treatment were counted.
Figure 2: mTORC1 regulation of insulin secretion. (A-C) Control (Raptor\(^{fl/fl}\)) and MIP-CreER; Raptor\(^{fl/fl}\) mice were injected tamoxifen. Two weeks later, mice were fasted overnight and then given 2 g/kg glucose + 0.39 g/kg leucine by gavage. Blood samples were obtained before and 30 min following the gavage; (A) plasma glucose measurements, (B) serum insulin levels, and (C) the insulinogenic index calculated as \(\Delta\text{insulin (0-30 min)} / \Delta\text{glucose (0-30 min)}\) ratio. Raptor\(^{fl/fl}\): n=16-21 mice; MIP-CreER; Raptor\(^{fl/fl}\) n=16-17 mice. (D) Islets were isolated from βRaptor- KO and control mice, pre-incubated in Krebs-Ringer Modified Buffer (KRB) containing 3.3 mmol/l glucose for 40 min, followed by stimulation with high glucose (16.7 mmol/l) and/or amino acids (leucine + glutamine, 10 mmol/l each), or BCH (20 mmol/l) for 1 h, n=5 in triplicates. (E) INS-1 cells were pre-incubated in KRB containing 3.3 mmol/l glucose with or without 100 nmol/l rapamycin for 30 min, followed by incubation in KRB containing 3.3 mmol/l or 16.7 mmol/l glucose, with or without AAs (leucine + glutamine, 10 mmol/l each) with or without rapamycin (100 nmol/l) for 1 h, n=4 in triplicates. (F-G) Islets (F) and INS-1 cells (G) were pre-incubated in KRB containing 3.3 mmol/l glucose with or without 100 nmol/l rapamycin for 1h, followed by incubation in KRB containing 3.3 mmol/l or 16.7 mmol/l glucose.
with or without AA (leucine + glutamine, 10 mmol/l each) and 100 nmol/l rapamycin in the absence or presence of 250 μmol/l diazoxide and 30 mmol/l KCl for 1 h, n=3 in quadruplicates (islets) or triplicates (INS-1 cells).
Figure 3: Regulation of β-cell autophagy in the postabsorptive and fed states. (A-B) C57BL/6 mice were fasted overnight followed by refeeding for 4 h (A) or with BCAA (13.3 g/l each) added to the drinking water (B). Islets were isolated, dispersed and stained for insulin and LC3B. n=2 separate experiments, 250-640 cells per group were counted. Scale bar: 5 μm. (C) Mice were fasted overnight and then administered 2 g/kg glucose and/or 0.39 g/kg leucine by gavage, followed 1 h later by removal of pancreases and staining for insulin and LC3B. Scale bar: 10 μm. n=3 separate experiments, 250-450 cells per treatment were counted.
Figure 4: Dynamic regulation of β-cell autophagy by nutrients. Islets isolated from C57BL/6 mice were incubated for 1 h (A) or overnight (B) in AA-free RPMI medium supplemented with 10% fetal calf serum and 3.3 mmol/l glucose (LG) or 16.7 mmol/l glucose + 10 mmol/l leucine (HG+leucine) in the presence or absence of 100 nmol/l bafilomycin A1 for 2 h. Islets were dispersed and stained for insulin and LC3B. Scale bar: 10 μm. n=3 separate experiments, 120-200 cells per treatment were counted.
Figure 5: mTORC1 regulation of autophagy in β-cells. INS-1 cells were incubated in AA-free RPMI medium supplemented with 3.3 mmol/l or 16.7 mmol/l glucose with or without leucine + glutamine (10 mmol/l each) for 1 h, in the presence or absence of 100 nmol/l bafilomycin A1 for 4 h. Cells were lysed and analyzed by Western blotting for LC3B, P62/SQSTM1 and GAPDH (A, C) or stained for LC3 (B). Scale bar: 10 μm. (A) n=4, LC3II/I ratio was quantified in samples treated with bafilomycin A1. (B) n=2 separate experiments, quantification was performed on 500-900 cells per treatment. (C) n=5. (D) INS-1 cells were transfected with LC3-GFP-RFP plasmid; medium was changed after 24 h to RPMI containing 16.7 mmol/l glucose with or without leucine + glutamine (10 mmol/l each) for 4 h. The cells were imaged by confocal microscope and GFP+ and RFP+ puncta and their colocalization quantified. The fraction of autophagosomes (GFP+/RFP+ puncta, yellow) and autolysosomes (GFP-/RFP+ puncta, red) is shown at the bottom. Scale bar: 5 μm. (E) Islets from 12 mice were isolated, pooled and incubated overnight in complete RPMI. Islets were pre-incubated at 3.3 mmol/l glucose in absence of AAs and serum and then incubated in medium containing 1% serum and 3.3 mmol/l glucose or 16.7 mmol/l glucose + 10 mmol/l leucine for 1 h. Islet extracts were analyzed by Western blotting for pULK1 (Ser757), ULK1, pTFEB (Ser122), TFEB, pS6 (Ser240/244), S6,
S6K1, TSC1 and GAPDH. NP-non-phosphorylated, P-phosphorylated. (F) INS-1 cells were incubated at 3.3 mmol/l glucose or 16.7 mmol glucose + leucine and glutamine for different periods of time followed by Western blotting for phospho- and total ULK1, TFEB, S6 and tubulin. Quantifications of pULK1 and pTFEB are shown to the right (n=3). (G) INS-1 cells were transfected with TFEB-GFP construct. After 24 h, the medium was replaced and the cells were incubated overnight at 3.3 mmol/l or 16.7 mmol/l glucose + leucine and glutamine (10 mmol/l each). TFEB-GFP localization was analyzed by confocal microscope. Quantification of cells with nuclear localization of TFEB is shown to the right, n=2 separate experiments, quantification was performed on 35-50 cells per treatment. Scale bar: 5 μm.
Figure 6: Effects of nutrients on TFEB localization and function in islets. Control (Raptor(fl/fl)) and βRaptor KO (MIP-CreER; Raptorfl/fl) mice were fasted overnight and administered water or 2 g/kg glucose + 0.39 g/kg leucine by gavage, followed by removal of the pancreas 1 h later. Pancreatic sections were immunostained for insulin and TFEB. Scale bar: 10 μm. Analysis was performed by confocal microscope. n=2 separate experiments, quantification was performed on 400-700 cells. (B) WT and βRaptor KO islets were incubated in AA-free RPMI supplemented with 1% serum at 3.3 mmol/l glucose or 16.7 mmol/l glucose + 10mmol/l leucine for 24 h. Islets were dispersed and TFEB localization was analyzed by fluorescence microscope. Scale bar: 100 μm. Quantification of the number of cells with cytoplasmic aggregates of TFEB vs diffuse puncta is shown, n=2-3 experiments, 1000-2060 cells were counted for each experimental group. A - Aggregates, DP-diffuse puncta. (C) Islets were isolated from control and βRaptor- KO mice followed by RNA extraction and qPCR for TFEB regulated genes, n=3. (D) Islets isolated from control mice were incubated overnight with AA-free RPMI medium at 3.3 mmol/l glucose or 16.7 mmol/l glucose + 10 mmol/l leucine followed by qPCR for TFEB-
regulated genes, n=3.
Figure 7: Regulation of insulin secretion by autophagy. Islets were isolated from control (Raptor^fl/fl^), βRaptor KO (MIP-CreER; Raptor^fl/fl^) and βRaptor KO; Atg7^+/−^ mice (MIP-CreER; Raptor^fl/fl^; Atg7^fl/+^) were injected with tamoxifen; metabolic tests were performed after 2 weeks. (A) Western blotting for ATG7, P62/SQSTM1, GFP and GAPDH (n=2). (B-C) Control (Atg7^fl/+^) and MIP-CreER; Atg7^fl/+^ mice were injected with tamoxifen; followed by IPGTT (B) and glucose-stimulated insulin secretion test (C), n=15-16 mice per group. (D) Insulin secretion of MIP-CreER, Atg7^+/fl^ compared to control (Atg7^fl/+^) mice was assessed by static incubations at 3.3 and 16.7 mmol/l glucose for 1 h, n=3. (E-G) MIP-CreER; Raptor^fl/fl^ and MIP-CreER; Raptor^fl/fl^; Atg7^fl/+^ mice were injected with tamoxifen. Two weeks later, mice were fasted overnight and received 2 g/kg glucose + 0.39 g/kg leucine by gavage. Blood samples were obtained before and 30 min following the gavage; (E) plasma glucose measurements, (F) serum insulin levels, and (G) the insulinogenic index calculated as Δinsulin (0-30 min)/Δglucose (0-30 min) ratio. N=9-16 mice per group. (H-J) Insulin secretion of control (Raptor^fl/fl^), βRaptor^−/−^ and βRaptor^−/−^; Atg7^+/−^ KO islets was assessed by static
incubations at 3.3 and 16.7 mmol/l glucose for 1 h. (H) Insulin content, (I) secreted insulin (left) and insulin secretion normalized to content (right), (J) proinsulin to insulin ratio in islet extracts, n=3. (K-L) C57BL/6 mice were injected with PBS or 5 μg/kg chloroquine (CQ) for 3 consecutive days, followed by IPGTT. Blood samples were drawn at the indicated time points and analyzed for glucose (K) and insulin (L), n=8.
Figure 8: Effects of nutrients, rapamycin and chloroquine (CQ) on mTORC1 signaling and insulin secretion in human islets. (A-D) Islets were incubated overnight at 3.3 mmol/l glucose, 16.7 mmol/l glucose with or without leucine (10 mmol/l) and/or rapamycin (10 and 100 nmol/l) and/or CQ (10 μmol/l). (A) Western blotting for total and phosphorylated S6, 4EBP1, TFEB and for phosphorylated-ULK1. (B) Cumulative secretion to the medium, (C) insulin content, (D) acute glucose-stimulated insulin secretion assessed by static incubations after overnight exposure to the indicated treatments. Results are the means of three independent experiments on islets from 3 different donors. (E) A model depicting the regulation of insulin secretion by nutrient modulation of mTORC1-autophagy crosstalk. Leucine is an amplifier of insulin secretion in presence of metabolic fuels such as glucose and glutamine. Exposure to high glucose and leucine stimulates mTORC1, which in turn inhibits autophagy with subsequent augmentation of insulin secretion. In the fast state, mTORC1 is inhibited thereby stimulating autophagy which in turn restrains insulin secretion. Periodic feeding leads to parallel transient stimulation of mTORC1 and autophagy, whereas prolonged exposure to nutrients (leucine and glucose) inhibits autophagy with subsequent development of hyperinsulinemia.
**Supplemental Figure 1: Regulation of mTORC1 by amino acids in INS-1 β-cells and islets.** (A) INS-1 cells were preincubated for 30 min in AA-free RPMI at 3.3 mmol/l glucose without serum and then treated without or with the AAs arginine, leucine, isoleucine, valine, glutamine (10 mmol/l each), or in complete RPMI medium containing a mixture of all amino acids with or without BCAAs for 30 min followed by Western blotting for pS6, (Ser240/244), S6 and tubulin (n=2). (B) Islets were isolated from C57BL/6 mice, preincubated in AA-free RPMI with 3.3 mmol/l glucose with 10% FCS for 40 min and then treated with different concentrations of leucine for 30 min. Islets were dispersed and immunostained for insulin and pS6. Quantification of pS6 activity in β-cells is expressed as percentage of pS6+/insulin+ cells. Scale bar: 20 μm. n=4 separate experiments. (C) INS-1 cells were incubated at 3.3 or 16.7 mmol/l glucose with or without leucine + glutamine (10 mmol/l each) and rapamycin (100 nmol/l) for 1 h. pS6 (Ser240/244) was analyzed by Western blotting. Quantification is shown at the bottom; n=6. (D) Islets were treated at 2.8 or 16.7 mmol/l glucose with or without a mixture of all AAs for 30 min. mTORC1 activity, evident by S6 phosphorylation, was analyzed by flow cytometry, n=4. (E) Western blotting for RAPTOR and pS6 in mouse islets that were cultured in presence or absence of AAs for 2 h; AAs were added to starved cells for different periods of time; n=4.
Supplemental Figure 2: Cre recombinase and tamoxifen did not affect glucose tolerance and insulin sensitivity. Tamoxifen-induced Raptor KO in β-cells leads to mild glucose intolerance. (A-B) Control (Raptor\textsuperscript{fl/fl}) and MIP-CreER; Raptor\textsuperscript{fl/fl} mice were injected with tamoxifen. Two weeks later,
pancreases were harvested, fixed and immunostained for insulin and pS6 (A) or E-cadherin (B). (A) n=2 separate experiments, 1400-3500 cells per group were counted; (B) n=550 cells per group. Scale bar: 40 μm. (C-D) Wildtype (WT) and MIP-CreER mice were injected with tamoxifen, followed by IPGTT (C; 2 g/kg) and insulin tolerance test (D; 1 IU/kg, ITT) two weeks later, n=3 in each group. (E-F) MIP-CreER mice were injected with corn oil (vehicle) or tamoxifen, followed by IPGTT (E), and ITT (F), n=3 mice in each group. (G) Control (Raptor^{fl/fl}) mice and MIP-CreER; Raptor^{fl/fl} mice were injected with tamoxifen, followed by IPGTT two weeks later, n=6-10 mice in each group. (H) Effects of the MIP-CreER construct on β-cell autophagy. Wildtype and MIP-CreER islets were incubated at 3.3 mmol/l glucose and 1% serum with bafilomycin A1 for 2 h and stained for LC3. n=3 mice, 430 to 530 cells were counted in each group, Scale bar: 10 μm
Supplemental Figure 3. Concentration-dependent amplification of insulin secretion by amino acids. Islets were preincubated in KRB medium and then treated with different concentrations of leucine and glutamine at 3.3 and 16.7 mmol/l glucose for 1 h. Insulin secretion and content were analyzed by ELISA. Results are means ± SEM of 3 separate experiments in quadruplicates.
Supplemental Figure 4. Effects of leucine and glucose on the activation of mTORC1 and of autophagy regulators. Islets from 18 mice were isolated, pooled and incubated overnight in complete RPMI. Islets were pre-incubated at 3.3 mmol/l glucose in absence of AAs and serum and then incubated in medium containing 1% serum and 3.3 mmol/l glucose, 16.7 mmol/l glucose or 10 mmol/l leucine for 1 h. Islet extracts were analyzed by Western blotting for pULK1 (Ser757), ULK1, pTFEB (Ser122), TFEB, pS6 (Ser240/244), S6, S6K1, TSC1, GAPDH and tubulin. Quantifications of the blots are shown.
Supplemental Figure 5. Effects of nutrients and mTORC1 on lysosome number in β-cells. Control and βRaptor KO mice were fasted overnight and administered water or 2 g/kg glucose + 0.39 g/kg leucine by gavage, followed by removal of the pancreas 1 h later. Pancreatic sections were immunostained for insulin and LAMP1. The number of LAMP1+ puncta in β-cells was quantified. Scale bar: 20 μm. n=2 separate experiments, 210-375 cells per treatment were counted.
Supplemental Figure 6: Effects of inhibiting mTORC1 and of heterozygous Atg7 KO on autophagy and β-cell ultrastructure. Islets were isolated from control (MIP-CreER), βRaptor KO (MIP-CreER; Raptor<sup>fl/fl</sup>) and βRaptor KO, Atg7<sup>+/−</sup> mice (MIP-CreER; Raptor<sup>fl/fl</sup>; ATG7<sup>fl/+</sup>). (A) Western blotting for ATG7 and GAPDH (n=2). (B) Dispersed islets were stained for insulin and LC3B followed by confocal microscope analysis and quantification of the number of LC3<sup>+</sup> puncta in β-cells. Scale bar: 10 μm. n=3 separate experiments, 350-1050 cells per group were counted. (C-D) TEM analysis on islets from control (Raptor<sup>fl/fl</sup>), MIP-CreER; Raptor<sup>fl/fl</sup> and MIP-CreER; Raptor<sup>fl/fl</sup>; Atg7<sup>fl/+</sup> mice. Scale bar: 1000 nm (C). Quantifications of the number of autophagosomes and peroxisomes per cell and of mature and young secretory granules (D). n= 7-19 cells per pancreas from three mice. AP, autophagosome, P, peroxisome.
Supplemental Figure 7: Effects of acute exposure to nutrients, rapamycin and chloroquine (CQ) on insulin secretion in human islets. Islets were pre-incubated at 3.3 mmol/l glucose for 1 h and then incubated at 3.3 mmol/l glucose for 45 min followed by 16.7 mmol/l glucose with or without 10 mmol/l leucine, 100 nmol/l rapamycin and/or 10 μmol/l CQ for 45 min. Insulin secretion normalized to content is shown above and insulin content below.
### Primary Antibodies

| Antibody Name                  | Source                     | Product Number          |
|--------------------------------|----------------------------|-------------------------|
| Anti insulin                   | Dako                       | A0564-discontinued      |
| Anti insulin                   | Agilent Technologies       | IR0026                  |
| Anti LC3B                      | Cell Signaling Technology  | 2775                    |
| Anti pS6 (Ser240/244) (D68F8)  | Cell Signaling Technology  | 5364                    |
| Anti S6 Ribosomal Protein (5G10) | Cell Signaling Technology | 2217                    |
| Anti S6 Ribosomal Protein (54D2) | Cell Signaling Technology | 2317                    |
| Anti LAMP1                      | Abcam                      | 24170                   |
| Anti pTFEB (Ser122)            | Cell Signaling Technology  | 86843                   |
| Anti TFEB                      | Bethyl Laboratories        | A303-673A               |
| Anti TFEB (D207D)              | Cell Signaling Technology  | 37785                   |
| Anti GFP                       | Abcam                      | 6673                    |
| Anti P62/SQSTM1                | Cell Signaling Technology  | 5114                    |
| Anti ATG7 (D12B11)             | Cell Signaling Technology  | 8558                    |
| Anti pULK1 (Ser757) (D7O6U)    | Cell Signaling Technology  | 14202                   |
| Anti pULK1 (Ser757)            | Cell Signaling Technology  | 6888                    |
| Anti ULK1 (D8H5)               | Cell Signaling Technology  | 8054                    |
| Anti P-4E-BP1 (Thr37/46) (236B4) | Cell Signaling Technology | 2855                    |
| Anti 4E-BP1 (53H11)            | Cell Signaling Technology  | 9644                    |
| Anti Raptor (24C12)            | Cell Signaling Technology  | 2280                    |
| Anti S6K                       | Cell Signaling Technology  | 9202                    |
| Anti TSC1(D43E2)               | Cell Signaling Technology  | 6935                    |
| Anti GAPDH (6C5)               | Abcam                      | 8245                    |
| Anti Tubulin (4D1)             | Abcam                      | 56676                   |
| Anti-aTubulin                  | Sigma                      | T5168                   |

### Secondary Antibodies

| Antibody Name                  | Source                     | Product Number          |
|--------------------------------|----------------------------|-------------------------|
| Cy5-conjugated Affinipure IgG  | Jackson ImmunoResearch     | 711-175-152             |
| Alexa Fluor 647- conjugated AffiniPure | Jackson ImmunoResearch     | 111714                  |
| Cy3-conjugated Affinipure IgG  | Jackson ImmunoResearch     | 715-165-151             |
| Cy3-conjugated Affinitypure IgG | Jackson ImmunoResearch     | 711-165-152             |
| Alexa Fluor 488-Conjugated IgG | Jackson ImmunoResearch     | 706-545-148             |
| Peroxidase-conjugated AffiniPure IgG | Jackson ImmunoResearch     | 111-035-003             |
| Peroxidase-conjugated AffiniPure IgG | Jackson ImmunoResearch     | 115-035-003             |
| IR-Dye 800CW                   | LI-COR                     | 925-32213               |
| IR-Dye 680RD                   | LI-COR                     | 925-68070               |

**IF**: Immunofluorescence  
**WB**: Western blot  
**FC**: Flow cytometry
| Species             | Dilution Factor                                      |
|---------------------|------------------------------------------------------|
| Guinea pig polyclonal | 1/200 (FC)                                          |
| Guinea pig polyclonal | 1/10 (IF)                                           |
| Rabbit polyclonal   | 1/200 (IF)                                          |
| Rabbit monoclonal   | 1/200 (IF), 1/300 (FC) 1/1000-4000 (WB)             |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Mouse monoclonal    | 1/1000 (WB on human samples)                        |
| Rabbit monoclonal   | 1/1000 (WB on human samples)                        |
| Rabbit monoclonal   | 1/200 (IF), 1/1000 (WB)                              |
| Rabbit monoclonal   | 1/500-1000 (WB)                                     |
| Rabbit Polyclonal   | 1/200 (IF), 1/1000 (WB)                              |
| Rabbit monoclonal   | 1/1000 (WB on human samples)                        |
| Goat Polyclonal     | 1/200 (IF)                                          |
| Rabbit polyclonal   | 1/1000 (WB)                                         |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Rabbit monoclonal   | 1/500 (WB)                                          |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Rabbit monoclonal   | 1/1000 (WB)                                         |
| Mouse monoclonal    | 1/1000 (WB)                                         |
| Mouse monoclonal    | 1/1000 (WB)                                         |
| Mouse monoclonal    | 1/4000 (WB on human samples)                        |

| Species             | Dilution Factor                                      |
|---------------------|------------------------------------------------------|
| Donkey anti Rabbit  | 1/200 (IF, FC)                                       |
| Donkey anti Goat    | 1/200 (IF)                                           |
| Donkey anti Mouse   | 1/200 (IF)                                           |
| Donkey anti Rabbit  | 1/200 (IF)                                           |
| Donkey anti Guinea Pig | 1/200 (IF, FC)                                  |
| Goat anti Rabbit    | 1/4000 (WB)                                          |
| Goat anti Mouse     | 1/4000 (WB)                                          |
| Donkey anti Rabbit  | 1/8000 (WB on human samples)                        |
| Goat anti Mouse     | 1/8000 (WB on human samples)                        |
### Supplemental table 2

Primer (5'-3') sequences

| Gene  | Forward                          |
|-------|----------------------------------|
| Becn1 | CAGGAACTCACAGCTCCATTAC          |
| Map1lc3a | GTTAACATGAGCGAGTTGGT        |
| Map1lc3b | CCACCAAGATCCCACTGCATTAG      |
| Sqstm1 | TGGGAACCGCTATAAGTG             |
| Atg5  | CAAAGATGTGCTTCGAGATGTG          |
| Atg7  | GCCAAGATCTCCTACTCAATC           |
| Atg9a | CTGGTTAGCTGTGGAGACTATG          |
| Wipi1 | CTGCCCTGGAAGAATCCATCTAT         |
| Ctsa  | TATCTTCACTCGCTTGCCAC            |
| Ctsb  | CCAATGGCCGAGTCAATGTG            |
| Ctsd  | GGAAAGACGATCTGCTGAG             |
| Ctsf  | ATGTTGGACAAGGATCCCT            |
| Clcn7 | ACCCACCAGGCTCTATAAT             |
| Rn18s | GTAACCCGTTGAACCCATT            |
| Reverse |
|---------|
| CCATCCTGGCGAGTTTCAATA |
| GTTTCTTGGGAGGCATAGACC |
| CAAGCGCGGTCTGATTATCT |
| GGGAAAGATGAGCTTGCTGT |
| GCAAATAGTATGGTCTGCTTCTC |
| GGCATTCACTCCGGAAATA |
| GAATCCTGGCACTACAGACTTG |
| CAGAGACCTGTGGGTGTTGA |
| GAGCCTTCCGAACATATGGG |
| GGAGGGATGGGTAGGGTAAG |
| GCAAAGCCGACCCTATTGTA |
| GGTGATCCATACGTAGCTG |
| GGATGAAGACACCAGCAGATAC |
| CCATCCAATCGGTAGTAGCG |
Checklist for Reporting Human Islet Preparations Used in Research

Adapted from Hart NJ, Powers AC (2018) Progress, challenges, and suggestions for using human islets to understand islet biology and human diabetes. Diabetologia https://doi.org/10.1007/s00125-018-4772-2.

| Manuscript DOI: | https://doi.org/10.2337/[insert manuscript submission number] | (Example, https://doi.org/10.2337/db18-1234) |
|-----------------|-------------------------------------------------------------|-----------------------------------------------|
| Title:          | The nutrient sensor mTORC1 regulates insulin secretion by modulating β-cell autophagy |
| Author list:    | Israeli T., Riahi Y., Garzon P., Louzada RA., Joao Pedro Werneck-de-Castro JP., Blandino-Rosano M., Yeroslaviz-Stolper R., Kadosh L., Tornovsky-Babeay S., Hacker G., Israeli N., Agmon O., Tirosh B., Cerasi E., Bernal-Mizrachi E., Leibowitz G. |
| Corresponding author: | Gil Leibowitz MD |
| Email address:  | gleib@hadassah.org.il |

| Islet preparation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8* |
|-------------------|---|---|---|---|---|---|---|----|

MANDATORY INFORMATION

For Peer Review Only
| Unique identifier | HP-21161-01 | HP-21203-01 | HP-21216-01 |
|-------------------|-------------|-------------|-------------|
| Donor age (years) | 43          | 25          | 54          |
| Donor sex (M/F)   | M           | M           | F           |
| Donor BMI (kg/m^2)| 25.5        | 26.5        | 31.8        |
| Donor HbA_1c or other measure of blood glucose control | 5.4% | 5.8% | 5.8 |
| Origin/source of islets^b | Prodo Labs | Prodo Labs | Prodo Labs |
| Islet isolation centre | Prodo Labs | Prodo Labs | Prodo Labs |
| Donor history of diabetes? Yes/No | No | No | No |

**If Yes, complete the next two lines if this information is available**

| Diabetes duration (years) |  |
| Glucose-lowering therapy at time of death^c |  |
| RECOMMENDED INFORMATION          | Donor cause of death | Head trauma | Head trauma | Stroke |
|----------------------------------|----------------------|-------------|-------------|--------|
| Warm ischaemia time (h)          |                      |             |             |        |
| Cold ischaemia time (h)          |                      |             |             |        |
| Estimated purity (%)             | 90                   | 95          | 95          |        |
| Estimated viability (%)          | 95                   | 95          | 95          |        |
| Total culture time (h)\(^d\)     | 4-5 days             | 4-5 days    | 4-5 days    |        |
| Glucose-stimulated insulin secretion or other functional measurement\(^e\) | GSIS, available in Fig 8 and S6 | GSIS, available in Fig 8 | GSIS, available in Fig 8 |        |
| Handpicked to purity? Yes/No     | Yes                  | Yes         | Yes         |        |
| Additional notes                 |                      |             |             |        |

\(^a\)If you have used more than eight islet preparations, please complete additional forms as necessary

\(^b\)For example, IIDP, ECIT, Alberta IsletCore
Please specify the therapy/therapies

Time of islet culture at the isolation centre, during shipment and at the receiving laboratory

Please specify the test and the results