Impaired Store-Operated Calcium Entry and STIM1 Loss Lead to Reduced Insulin Secretion and Increased Endoplasmic Reticulum Stress in the Diabetic β cell

Running title: Impaired SOCE in the Diabetic β cell

Tatsuyoshi Kono, Ph.D.\textsuperscript{1,2,*}, Xin Tong, Ph.D.\textsuperscript{3}, Solaema Taleb, M.S.\textsuperscript{1}, Robert N. Bone, Ph.D.\textsuperscript{1}, Hitoshi Iida, M.D. Ph.D.\textsuperscript{1}, Chih-Chun Lee, Ph.D.\textsuperscript{1}, Paul Sohn\textsuperscript{4}, Patrick Gilon, Ph.D.\textsuperscript{5}, Michael W. Roe, Ph.D.\textsuperscript{6}, and Carmella Evans-Molina, M.D. Ph.D.\textsuperscript{1,2,4,7,8,9*}

\textsuperscript{1}Department of Medicine, Indiana University School of Medicine, Indianapolis IN, USA
\textsuperscript{2}Richard L. Roudebush VA Medical Center, Indianapolis IN, USA
\textsuperscript{3}Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville TN, USA
\textsuperscript{4}Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis IN, USA
\textsuperscript{5}Pôle d’endocrinologie, diabète et nutrition, Institut de recherche expérimentale et clinique, Université catholique de Louvain, Brussels, Belgium
\textsuperscript{6}Department of Medicine, SUNY Upstate Medical University, Syracuse, NY, USA
\textsuperscript{7}Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis IN, USA
\textsuperscript{8}Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis IN, USA
\textsuperscript{9}Lead Contact

*Address correspondence and requests for reprints to: Carmella Evans-Molina, MD, PhD (cevansmo@iu.edu) or Tatsuyoshi Kono, PhD (konot@iu.edu), Indiana University School of Medicine, 635 Barnhill Drive, MS 2031A, Indianapolis, IN 46202, Telephone: (317) 274-4145, Fax (317) 274-4107

Word Count: 4503
Number of Figures:  8
Number of Supplemental Tables:  2
Number of Supplemental Figures: 4

KEYWORDS
calcium signaling; calcium imaging, insulin secretion, endoplasmic reticulum, β cell biology
ABSTRACT

Store-operated calcium entry (SOCE) is a dynamic process that leads to refilling of ER Ca\textsuperscript{2+} stores through reversible gating of plasma membrane Ca\textsuperscript{2+} channels by the ER Ca\textsuperscript{2+} sensor, STIM1. Pathogenic reductions in β cell ER Ca\textsuperscript{2+} have been observed in diabetes. However, a role for impaired SOCE in this phenotype has not been tested. We measured expression of SOCE molecular components in human and rodent models of diabetes and found a specific reduction in STIM1 mRNA and protein levels in human islets from donors with type 2 diabetes (T2D), islets from hyperglycemic streptozotocin-treated mice, and INS-1 cells treated with pro-inflammatory cytokines and palmitate. Pharmacologic SOCE inhibitors led to impaired islet Ca\textsuperscript{2+} oscillations and insulin secretion, and these effects were phenocopied by β cell STIM1 deletion. STIM1 deletion also led to reduced ER Ca\textsuperscript{2+} storage and increased ER stress, while STIM1 gain of function rescued β cell survival under pro-inflammatory conditions and improved insulin secretion in human islets from donors with T2D. Taken together, these data suggest that loss of STIM1 and impaired SOCE contribute to ER Ca\textsuperscript{2+} dyshomeostasis under diabetic conditions, while efforts to restore SOCE-mediated Ca\textsuperscript{2+} transients may have potential to improve β cell health and function.
INTRODUCTION

Reductions in β cell endoplasmic reticulum (ER) calcium (Ca\(^{2+}\)) contribute to the pathophysiology of both type 1 and type 2 diabetes (T2D) and lead to decreased insulin secretion, activation of intracellular stress pathways, and β cell death. Steady state ER Ca\(^{2+}\) levels are maintained by the balance of Ca\(^{2+}\) transport into the ER lumen by the sarco-endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) pumps and Ca\(^{2+}\) release via the inositol trisphosphate receptors (IP3Rs) and ryanodine receptors (RyRs) (1-4). ER Ca\(^{2+}\) depletion also triggers a tightly regulated rescue mechanism that serves to replenish ER Ca\(^{2+}\) stores through a family of channels referred to as store-operated or Ca\(^{2+}\) release-activated channels (5-7). This process, known as store-operated Ca\(^{2+}\) entry (SOCE), is initiated by dissociation of Ca\(^{2+}\) from the ER Ca\(^{2+}\) sensor, Stromal Interaction Molecule 1 (STIM1), followed by STIM1 oligomerization and translocation to the ER/plasmalemmal junctional regions (8). Here, STIM1 complexes with selective Orai Ca\(^{2+}\) channels (9) and nonspecific transient receptor potential canonical channel 1 (TRPC1), leading to activation of Ca\(^{2+}\) influx from the extracellular space, with subsequent transfer of Ca\(^{2+}\) into the ER lumen (10; 11). Whereas pathologic reductions in SERCA-mediated ER Ca\(^{2+}\) uptake and dysregulated RyR-mediated ER Ca\(^{2+}\) leak have been described in the diabetic β cell (4; 12; 13), a role for impaired β cell SOCE in this phenotype remains untested.

In other cell types, SOCE Ca\(^{2+}\) transients have been implicated in a number of signaling pathways including those that regulate proliferation, growth, inflammation, apoptosis, and lipogenesis. In addition, defective SOCE has been associated with several clinical syndromes including immunodeficiency, myopathy, Alzheimer’s disease, and vascular disease (14-18). Recently, pharmacologic inhibitors of SOCE or dominant negative forms of either Orai1 or TRPC1 were shown to decrease insulin secretion in rat islets and clonal β cell lines (11), while
STIM1 was also shown to interact with the sulfonylurea receptor 1 (SUR1) subunit of the $K_{ATP}$ channel and regulate β cell $K_{ATP}$ activity (19). Given these recent implications of SOCE in the regulation of insulin secretion, we hypothesized that dysfunctional β cell SOCE may likewise contribute to diabetes pathogenesis. To this end, we profiled SOCE and expression of SOCE molecular components in multiple diabetic models including islets from streptozotocin-treated mice, human and mouse islets and INS-1 cells treated with pro-inflammatory cytokines, INS-1 cells treated with palmitate, and human islets isolated from donors with type 2 diabetes (T2D). Our data revealed a preferential loss of STIM1 expression but preserved expression of Orai1 across these models. Moreover, β cell STIM1 loss as well as STIM1 knockdown led to impaired glucose-stimulated Ca$^{2+}$ oscillations and insulin secretion, and increased β cell susceptibility to ER stress, while STIM1 gain of function rescued these defects. Taken together, these data define a novel role for altered SOCE in diabetes and suggest efforts to restore STIM1 expression and/or SOCE-mediated Ca$^{2+}$ transients have potential to improve β cell function and health.
RESEARCH DESIGN AND METHODS

Reagents

Mouse and human IL-1β, IFN-γ, and TNF-α were obtained from Invitrogen (Carlsbad, CA); 2-aminoethoxydiphenyl borate (2-APB), 1-(5-chloronaphthalene-1-sulphonyl)-1H-hexahydro-1,4-diazepine (ML-9), and tunicamycin (TM) were from Tocris Bioscience (Bristol, United Kingdom). Adenoviruses expressing STIM1 and Cre recombinase were from ViraQuest Inc (North Liberty, IA) (19). siRNAs were obtained from GH healthcare (Lafayette, CO); all other chemicals were from Sigma-Aldrich (St. Louis, MO). Supplemental Table S1 and S2 contain a complete list of PCR primers and antibodies.

Animals and human islets

Male C57BL/6J mice were obtained from Jackson Laboratory (Bar Harbor, ME). At 8 weeks of age, C57BL/6J were injected intraperitoneally with 50 mg/kg of streptozotocin (STZ) or normal saline daily for 5 days. Mice with loxP sites flanking exon 2 of the Stim1 gene were obtained from Jackson Laboratory and backcrossed onto a C57BL/6J background for at least 10 generations. Mice were maintained under protocols approved by the Indiana University Institutional Animal Care and Use Committee. Cages were kept in a standard light-dark cycle with ad libitum access to food and water.

Cadaveric human islets from nondiabetic donors and donors with T2D were obtained from the Integrated Islet Distribution Program. Data presented include analysis of islets from eight nondiabetic donors (5 female; 3 male) and eleven donors with T2D (7 female; 4 male). The average age (± S.E.M) of the non-diabetic donors was 45.9 ± 10.3 years; the mean body mass

Diabetes
index (BMI) was $25.6 \pm 4.7 \text{ kg/m}^2$. The average age of donors with T2D was $50.9 \pm 8.9$ years; the average BMI was $33.2 \pm 7.1 \text{ kg/m}^2$.

**Cell culture and islet treatments**

Rat INS-1 832/13 cells and human and mouse pancreatic islets were cultured as previously described (20; 21). CRISPR/Cas9 genomic editing was used to create a STIM1 knockout (KO) INS-1 832/13 cell line in the Genome Engineering and iPSC Center at Washington University (St. Louis, MO). To mimic pro-inflammatory and diabetic conditions, INS-1 cells were treated with 5 ng/mL IL-1β, and mouse and human islets were treated with 5 ng/ml IL-1β, 100 ng/ml IFN-γ, and 10 ng/ml TNF-α for 24 hrs as previously published (12). To induce ER stress, INS-1 cells were treated with 10 µM TM for 3-24 hrs. For viral transduction studies, 50-100 islets were hand-picked within 1 hour of isolation or receipt, incubated with adenovirus for 16 hrs, followed by an additional 48-72 hrs of incubation in fresh medium without adenovirus. To obtain STIM1 KO islets, isolated islets from STIM1\textsuperscript{flox/flox} mice were transduced with an adenovirus encoding Cre recombinase under control of the CMV promoter. Glucose-stimulated insulin secretion (GSIS), immunoblot, and immunofluorescence in cultured wild-type INS-1 cells, STIM1 KO cells, and isolated mouse and human islets were performed as previously described (4; 12). Islet GSIS was also measured using the Biorep Perifusion System (Biorep, Miami Lakes, FL) (22). Twenty-four hrs after isolation, fifty handpicked islets were loaded into each perifusion chamber; islets were perifused with Krebs buffer containing 2.8 mM glucose for 20 min, followed by 16.7 mM glucose for 40 minutes at a rate of 120 uL/min. Secreted insulin was measured using an ELISA (Mercodia, Uppsala, Sweden); results were normalized to DNA content. Quantitative real-time (qRT)-PCR was performed using previously published primer
sequences (23) or primers outlined in Table S1. To generate transmission electron micrographic images, INS-1 cells were fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1M sodium cacodylate buffer and transferred to the Advanced Electron Microscopy Facility at the University of Chicago (Chicago, IL).

**Live cell imaging**

Cytosolic Ca\(^{2+}\) dynamics in INS-1 cells were measured using the FLIPR Calcium 6 Assay Kit and a FlexStation 3 plate reader (Molecular Devices, Sunnyvale, CA). To measure SOCE, INS-1 832/13 β cells were loaded with Calcium 6 in growth medium containing 11 mM glucose for 2h. Immediately prior to Ca\(^{2+}\) imaging, cells were incubated in Ca\(^{2+}\)-free HBSS with the following composition: 138 mM NaCl, 5.3 mM KCl, 0.34 mM Na\(_2\)HPO\(_4\), 0.44 mM KH\(_2\)PO\(_4\), 4.17 mM NaHCO\(_3\), and 5.5 mM glucose for 4 minutes. Ca\(^{2+}\) imaging experiments were performed according to the strategy shown in Figure 1A. Baseline (F0) fluorescence was measured for a minimum of 10 seconds under Ca\(^{2+}\) free conditions and in the presence of 0.5 mM EGTA (Ca\(^{2+}\) chelator), 10 µM verapamil (L-type voltage-dependent Ca\(^{2+}\) channel [VDCC] blocker), and 200 µM diazoxide (K\(_{ATP}\) channel opener applied to prevent VDCC activation). Next, thapsigargin (TG; a SERCA inhibitor) was used to empty ER Ca\(^{2+}\) stores, followed by supplementation with 2 mM Ca\(^{2+}\) in the media. SOCE was detected as an elevation of Calcium 6 intensity (vertical red arrow; ∆F) in response to Ca\(^{2+}\) addition, which was normalized to the basal F0 (red dotted line), according to the formula ∆F/F0. Cytosolic Ca\(^{2+}\) imaging was performed in isolated islets incubated in HBSS buffer supplemented with 2 mM Ca\(^{2+}\) and the ratiometric Ca\(^{2+}\) indicator fura-2-acetoxymethylester (Fura-2 AM) (Life Technologies) using a Zeiss Z1 microscope as previously described (4; 24).
To directly image ER Ca\(^{2+}\) levels, cells were transfected with an adenovirus encoding the ER-targeted D4ER probe expressed under control of the rat insulin promoter (23). Fluorescence Lifetime Imaging Microscopy (FLIM) was used to monitor steady-state ER Ca\(^{2+}\) levels in accordance with previously published protocols (4; 25). For Förster Resonance Energy Transfer (FRET) experiments, confocal images were acquired with a Leica TCS SP8 confocal/multiphoton imaging system (Leica Microsystems, Inc., Buffalo Grove, IL). Imaging was performed using 448 nm single excitation laser line and fluorescent emission collected with two HyDs set to 460-500 nm and 515-550 nm emission slit widths. Time series images (z-stacks) of 2-3 stage-registered fields were acquired over a period of 20 minutes. Images were analyzed using Leica LAS X software (v.3.3) to calculate the change in FRET ratio over time.

**Statistical analysis**

Differences between groups were examined for significance using either a two-tailed Student’s \(t\) test or one-way ANOVA followed by the Tukey-Kramer post-test using GraphPad Prism statistics software (GraphPad Software, Inc., San Diego, CA). Pearson’s correlations were used to analyze relationships between STIM1 mRNA levels and donor BMI. Unless indicated, results were displayed as means ± S.E.M; a \(p\) value < 0.05 was used to indicate a significant difference between groups.
RESULTS

**Pharmacological inhibition of SOCE impaired glucose-stimulated Ca\(^{2+}\) oscillations and insulin secretion**

To image β cell store-operated Ca\(^{2+}\) entry (SOCE), INS-1 cells were loaded with Calcium 6; Ca\(^{2+}\) imaging experiments were performed according to the schematic shown in Fig. 1A and described in the “Methods”. As shown previously (26; 27), pharmacologic SOCE inhibitors, ML-9 and 2-APB, reduced the ∆F/F0 activated in response to ER Ca\(^{2+}\) depletion (Fig. 1B-C). Moreover, ML-9 and 2-APB reduced GSIS by 61% and 68%, respectively in INS-1 β cells (Fig. 1D). To test whether SOCE inhibitors similarly impacted glucose-stimulated Ca\(^{2+}\) oscillations, islets from wild-type C57BL/6J mice were treated with either ML-9 or 2-APB and then loaded with Fura-2AM for analysis of glucose-stimulated Ca\(^{2+}\) responses (Fig. 1E). While pharmacological inhibition of SOCE had no effect on the Phase 1 ∆F response to glucose (Fig. 1F), ML-9 and 2-APB reduced the average amplitude of the oscillatory response (∆F Phase 2) and increased the oscillatory period (Fig. 1G-H). In islet perifusion experiments, the addition of 2-APB to the high glucose buffer significantly reduced both first and second phase insulin secretion (Fig. 1I-J). Consistent with effects observed on glucose-stimulated Ca\(^{2+}\) oscillations, a more striking effects was observed on second phase insulin secretion.

**β cell STIM1 expression was reduced in mouse and human models of diabetes**

Next, islets were isolated from STZ or saline-treated C57BL/6J mice and glucose-stimulated Ca\(^{2+}\) imaging performed. Islets from STZ-treated mice had reduced amplitude of the ∆F Phase 1 and 2 responses and a decreased oscillatory period (Fig. 2A-E). To test whether observed changes in calcium signaling may be related to changes in expression of the molecular
components of the β cell SOCE complex, first, we compared expression levels of STIM and Orai isoforms in human and mouse islets and INS-1 cells (Fig. S1A-C). In human islets, mouse islets, and INS-1 cells, STIM1 was expressed at higher levels compared to STIM2. Orai2 was the most highly expressed Orai isoform in human islets. In mouse islets, Orai1 and 3 levels were nearly equivalent, and these were the most abundantly expressed Orai isoforms. Orai3 was the mostly highly expressed isoform in INS-1 cells. Analysis of islets from STZ-treated mice revealed a specific reduction in STIM1 gene and protein expression (Fig. 2F-H), while no differences in STIM2, Orai1, Orai2, or Orai3 gene expression were observed between saline and STZ-treated groups (Fig. 2F).

Homozygous STIM1 knockout mice experience perinatal lethality, and STIM2 KO mice die shortly after birth (28; 29). To test whether KO of STIM1 was sufficient to impair glucose-stimulated Ca\(^{2+}\) responses, islets from STIM1\(^{\text{floxed/floxed}}\) mice were transduced with an adenovirus encoding Cre recombinase. STIM1 protein was effectively reduced in Cre-transduced islets from STIM\(^{\text{floxed/floxed}}\) mice (pSTIM1KO) (Fig. 2I). Consistent with results observed in islets isolated from STZ-treated mice, the amplitude of the Phase 1 and 2 responses were reduced in pSTIM1KO islets (Fig. 2J-M). Similar to results obtained in islets from STZ-treated mice, the oscillatory period was also reduced in pSTIM1KO islets (Fig. 2E, N). However, this was noted to be in contrast to the increased oscillatory period observed in islets treated with ML-9 or 2-APB (Fig. 1H).

Next, we tested whether expression of SOCE molecular constituents was altered in islets from human cadaveric donors with T2D. This analysis revealed a significant reduction in STIM1 and Orai2 gene expression and a trend towards reduced Orai1 mRNA expression in islets from donors with T2D (p=0.06) (Fig. 3A). Immunoblot analysis revealed no change in Orai1 or Orai2
protein levels (data not shown), but STIM1 protein expression was reduced by ~40% in islets from donors with T2D (Fig. 3B-C). Next, Pearson’s correlation tests were used to assess relationships between donor BMI and STIM1 mRNA levels. In islets from non-diabetic donors, STIM1 mRNA levels were positively correlated with BMI ($R^2=0.573$; $p=0.048$), whereas this correlation was lost in donors with T2D (Fig. 3D).

**Pro-inflammatory cytokine stress reduced β cell STIM1 mRNA and protein expression and led to impaired SOCE.**

To define whether STIM1 expression was similarly impacted under *in vitro* diabetic stress conditions, human and mouse islets were treated with mixture of pro-inflammatory cytokines, consisting of 5 ng/ml IL-1β, 100 ng/ml IFN-γ, and 10 ng/ml TNF-α for 24 hrs. Cytokine treatment led to a significant reduction in STIM1 protein levels in human and mouse islets (Fig. 4A-B). Because INS-1 cells are known to be more sensitive to pro-inflammatory cytokines (30), INS-1 cells were treated with increasing doses of IL-1β alone for 24 hrs. Consistent with results obtained in human and mouse islets, STIM1 protein and mRNA levels decreased significantly with IL-1β treatment (Fig. 4C-E), while no reductions in STIM2, Orai1, Orai2, or Orai3 mRNA expression were observed (Fig, 4C). Reduced STIM1 expression correlated with a dose-dependent reduction in SOCE (Fig. 4F-G). Quantitation of the ΔF in response to thapsigargin (TG) was reduced in parallel, suggesting impaired ER Ca^{2+} storage in IL-1β-treated INS-1 cells (Fig. 4H).

To test these findings in another model of diabetic stress, INS-1 cells were treated with 0.5 mM palmitate combined with 25 mM glucose to mimic glucolipotoxicity (GLT). In GLT-treated INS-1 cells, both STIM1 and STIM2 mRNA levels were reduced (Fig. 4I), and STIM1
protein was reduced in parallel (Fig. 4J). No significant change in Orai1-3 mRNA levels was observed. SOCE was significantly decreased by GLT-treatment as well as palmitate treatment alone (Fig. 4K-L). Similar to results observed with IL-1β-treatment, the ∆F response to thapsigargin (TG) was reduced in both palmitate and GLT-treated INS-1 cells (Fig. 4K and Fig. S2A-B).

Taken together, our data identified a specific and consistent reduction in STIM1 expression in islets and pancreatic β cells across a variety of diabetes models. Moreover, pharmacological inhibition of SOCE reduced GSIS and impaired glucose-stimulated Ca\(^{2+}\) oscillations, while effects on calcium oscillations were partially phenocopied by STIM1 knockdown in islets.

**STIM1 deletion reduced β cell SOCE, ER Ca\(^{2+}\) storage, and glucose-stimulated insulin secretion**

Off-target effects of chemical SOCE inhibitors have been reported, including an effect of 2-APB on IP3R activity (31). To this end, CRISPR/Cas9 genome editing was employed to create an INS-1 STIM1 knockout cell line (KO cells). Gene expression analysis of KO cells demonstrated the absence of STIM1 mRNA, with no compensatory upregulation of other SOCE constituents including STIM2 and Orai1-3 (Fig. 5A). In addition, no change in Serca2b expression was observed. Similar to findings observed in our in vitro models of cytokine, GLT, and palmitate-stress, the ∆F response to TG was reduced in STIM1 KO cells (Fig. S2C-D). In addition, SOCE measured in response to TG and carbachol treatment was significantly reduced in KO cells (Fig. 5B-D), while no further reduction in SOCE was observed when STIM1 KO cells were treated with IL-1β (Fig. S3A-B), GLT (Fig. S3C-D), ML-9, or 2-APB (Fig. S3E).
To more definitively examine the effect of STIM1 KO on ER Ca\(^{2+}\) levels, WT and KO cells were transduced with the ER-targeted, FRET-based, D4ER Cameleon probe and imaging experiments were performed in Ca\(^{2+}\) containing HBSS buffer. Under basal conditions, STIM1KO cells displayed a lower FRET/donor ratio, indicative of decreased ER Ca\(^{2+}\) levels (Fig. 5E). To confirm these findings, FLIM analysis was performed to measure the lifetime of the D4ER CFP donor. As expected, the lifetime of the D4ER donor was increased in KO cells compared to WT cells (Fig. 5F and S3F). Finally, GSIS was measured in WT and STIM1 KO cells. Similar to results obtained with pharmacologic SOCE inhibition (Fig. 1D), STIM1 deletion significantly reduced GSIS, while no effect on basal insulin secretion was observed (Fig. 5G).

**Loss of STIM1 increased β cell ER stress**

We hypothesized reductions in ER Ca\(^{2+}\) levels arising from impaired SOCE may likewise impact stress responses in STIM1 KO cells. To test this, WT and KO cells were treated with 25 mM glucose + 0.5 mM palmitate (GLT) or 10 uM tunicamycin (TM) for 3 hrs. In response to both GLT and TM, an increase in the spliced/unspliced X-box binding protein-1 (XBP-1) ratio was observed in KO cells (Fig. 6A). Moreover, STIM1 KO cells exhibited increased cleaved caspase-3 activation in response to IL-1β treatment (Fig. 6B-C). Next, to compare acute and chronic STIM1 loss, siRNA-mediated STIM1 knockdown was performed in WT INS-1 cells, resulting in a ~50% reduction in STIM1 gene and protein expression (Fig. 6D-E). Similar to results observed in STIM1KO cells, siRNA-treated cells exhibited significantly higher levels of spliced/unspliced XBP-1 in response to TM treatment (Fig. 6F). Finally, to determine whether these functional changes correlated with changes in ER ultrastructure, electron micrographic
images of WT and KO cells were analyzed. In contrast to regularly spaced stacks of ER observed in WT cells, the ER was swollen and dilated in STIM1 KO cells (Fig. 6G). Taken together, these data indicate that STIM1 loss is associated with changes in ER morphology and increased β cell susceptibility to TM, cytokine, and GLT-mediated stress.

**STIM1 overexpression restored ER Ca\(^{2+}\) levels and improved insulin secretion in human islets from donors with Type 2 diabetes**

To test the effects of STIM1 gain of function, WT and KO cells were transduced with a STIM1 expressing adenovirus, and a dose-dependent increase in STIM1 expression was observed (Fig. 7A). Consistent with this, a dose-dependent increase in SOCE was also observed with STIM1 overexpression (Fig. 7B-C). Next, to test the effect of STIM1 overexpression on ER Ca\(^{2+}\) levels, STIM1 KO cells were transduced with the D4ER expressing adenovirus in combination with either an empty vector or STIM1 expressing adenovirus. Under steady state conditions, STIM1 restoration led to an increase in the FRET/CFP ratio in KO cells (Fig. 7D). This result was confirmed by FLIM analysis, in which the lifetime of the D4ER donor probe was significantly reduced by STIM1 overexpression in KO cells (Fig. 7E).

Next, FRET analysis was performed to dynamically monitor ER Ca\(^{2+}\) storage in WT and STIM1 KO cells transduced with an empty virus or STIM1-expressing adenovirus. No differences in the FRET/CFP ratio were observed at baseline under Ca\(^{2+}\) free conditions. The initial response to carbachol was similar between the groups. In contrast, STIM1 KO cells demonstrated impaired ER Ca\(^{2+}\) restoration following ER Ca\(^{2+}\) depletion when Ca\(^{2+}\) was added to the buffer. Moreover, ER Ca\(^{2+}\) restoration was significantly improved in KO cells by STIM1 overexpression (Fig. 7F-H).
Next, we tested whether STIM1 overexpression was sufficient to protect against IL-1β-mediated activation of cleaved-caspase 3. In both WT and STIM1 KO cells, STIM1 overexpression reduced cleaved-caspase 3 protein levels (Fig. 7I-J). Consistent with this, STIM1 overexpression rescued SOCE under conditions of cytokine stress (Fig. S4A-B). Finally, human cadaveric islets from donors with T2D were transduced with the STIM1 expressing adenovirus or an empty viral control (Fig. 7K). Compared to islets transduced with empty virus, GSIS was significantly improved in STIM1-transduced human islets (Fig. 7L).
DISCUSSION

Recent findings suggest that ER dysfunction triggers a range of chronic human diseases, including Alzheimer’s disease, Parkinson’s disease, atherosclerosis, and diabetes (32-35). In the pancreatic β cell, a key determinant of ER function is the maintenance of robust levels of intraluminal Ca\(^{2+}\), which is required for protein folding and a number of key cellular signaling events. Reductions in β cell ER Ca\(^{2+}\) occur in response to pro-inflammatory and glucolipotoxic stress and contribute to the pathophysiology of both major forms of diabetes. Under normal conditions, ER Ca\(^{2+}\) store depletion triggers a tightly regulated rescue mechanism known as SOCE or Ca\(^{2+}\) release-activated Ca\(^{2+}\) current. This process was first proposed by James Putney in 1986 (36). However, a complete understanding of the SOCE molecular complex remained elusive until 2005, when STIM1 was identified as the long sought-after “ER Ca\(^{2+}\) sensor” (8). Since that time, SOCE has been accepted as a predominant pathway of Ca\(^{2+}\) entry into non-excitatory cells, where it has been shown to regulate a variety of cellular functions, including proliferation, growth, inflammation, apoptosis, and lipogenesis (14; 37). To date, however, a role for SOCE in excitable and secretory cells, including the pancreatic β cell, has remained incompletely explored.

Here, we show impairment of glucose-stimulated Ca\(^{2+}\) oscillations and reduced insulin secretion in response to pharmacological SOCE inhibition and under conditions of STIM1 loss. Across multiple models of diabetes, our results revealed a preferential reduction in STIM1 mRNA and protein levels in pancreatic islets and β cells. Furthermore, we show that genetic loss of STIM1 was sufficient to reduce β cell SOCE, glucose-stimulated Ca\(^{2+}\) oscillations and insulin secretion, while simultaneously increasing susceptibility to ER stress and cell death in response to glucolipotoxic and pro-inflammatory conditions. Previously, STIM1, Orai1, and TRPC1 have
been identified as key constituents of the β cell SOCE complex, while pharmacological SOCE inhibition or a dominant negative form of Orai1 or TRPC1 was shown to reduce insulin secretion in rat islets and clonal β cell lines (11). Our data highlight an expanded role for STIM1 in nucleating this process and implicate loss of STIM1 and impaired SOCE in β cell dysfunction under diabetic conditions.

The impact of altered SOCE on insulin exocytosis is likely to be multifactorial. However, a key finding from our study is that SOCE inhibition and STIM1 loss impaired first phase glucose-stimulated cytosolic Ca\(^{2+}\) responses as well as glucose-induced Ca\(^{2+}\) oscillations. These effects were similar to those observed in a mouse model of SERCA2 haploinsufficiency, where reductions in SERCA2 were shown to reduce β cell ER Ca\(^{2+}\) levels and cytosolic Ca\(^{2+}\) oscillations (4). Thus, our data offer additional support to the notion that ER Ca\(^{2+}\) stores shape the architecture of glucose-induced Ca\(^{2+}\) oscillations as well as the β cell insulin secretory response. Intriguingly, STIM1 was shown recently to interact with the sulfonylurea receptor 1 (SUR1) subunit of the K\(_{ATP}\) channel and regulate β cell K\(_{ATP}\) activity in MIN6 cells (19). In this report, shRNA-mediated STIM1 knockdown reduced K\(_{ATP}\) channel activation, while STIM1 reconstitution restored K\(_{ATP}\) activity, suggesting that reductions in insulin secretion with STIM1 loss/SOCE inhibition may also result from impaired K\(_{ATP}\) activity (19). A hallmark of advanced human type 2 diabetes is the development of an impaired response to sulfonylurea medications, which act to close the K\(_{ATP}\) channel to increase insulin secretion (38-40). In our data, we observed a positive correlation between donor BMI and STIM1 expression levels in islets from nondiabetic humans, but this correlation was lost in donors with T2D. Moreover, our data revealed that STIM1 reconstitution in islets from donors with T2D improved GSIS. These findings imply that STIM1 upregulation may be a compensatory response observed in islets from
obese donors that is responsible for the initial maintenance of insulin secretion in the face of advancing insulin resistance. By corollary, our data suggest that reductions in STIM1 could play an important role in the development of impaired insulin secretion and loss of secretagogue efficacy in T2D.

Pathologic reductions in β cell ER Ca$^{2+}$ have been linked with the development of ER stress. Thus, reduced ER Ca$^{2+}$ levels in STIM1 deficient cells led us to hypothesize an additional role for STIM1 in ER stress signaling. Indeed, we found that genetic loss of STIM1 as well as STIM1 knockdown increased β cell susceptibility to TM-induced ER stress and pro-inflammatory cytokine-induced cell death. In contrast, STIM1 overexpression was able to protect against pro-inflammatory cytokine-induced activation of cleaved-caspase 3. Recent reports have implicated SERCA2 loss (4; 12; 41), as well as RyR-mediated ER Ca$^{2+}$ release, in the activation of ER stress signaling in diabetes (13). Here, we have identified an additional role for SOCE dysfunction and STIM1 loss in this phenotype. These observations are in agreement with findings observed in other cell types, including both excitable cells in the central nervous system as well as non-excitable mouse embryonic fibroblasts (42; 43).

Our data revealed a consistent loss of STIM1 in the β cell across multiple models of diabetes. However, a potential caveat to our observation of reduced STIM1 expression in islets from human donors with type 2 diabetes is that loss of islet β cell number may also accompany T2D (44). In addition, STIM1 did not appear as differentially regulated in a microarray analysis of laser-captured islets from human donors with T2D (45). However, we were able to confirm β cell down-regulation of STIM1 expression using in vitro and ex vivo diabetogenic stress in isolated islets and INS-1 β cells. Amongst these models, we found that STIM1 mRNA and protein levels were reduced by pro-inflammatory cytokines, which are known to be a prominent
component of the pathophysiology of type 1 diabetes (46). Additional studies are needed to define whether β cell SOCE dysregulation may also be present in human type 1 diabetes.

We have not yet explored the intervening pathways that lead to loss of β cell STIM1 expression under diabetic conditions. In other cell types, inhibition of both AKT and mTOR signaling led to a similar loss of STIM1 (47). Notably, both of these signaling pathways play an essential role in the maintenance of β cell health and function (48; 49). In addition, NF-κB has been shown to directly bind and modulate STIM1 gene promoter activity in other cell types (50). We and others have previously identified a deleterious effect of NF-κB-mediated nitric-oxide signaling on ER calcium regulation (41; 51), and this will be tested in follow-up studies. Here, we focused on loss of STIM1 expression as the cause of impaired SOCE. However, in other cell types, mislocalization or impaired formation of the SOCE complex has been observed under disease conditions (52). Finally, STIM2 overexpression has been shown to have a strong negative effect on endogenous SOCE in HEK293, PC12, A7r5 and Jurkat T cells (53). Thus, our observation of impaired SOCE in STIM1 KO cells and a reduced Ca^{2+} oscillatory amplitude in STIM1 deleted islets could also be partly explained by alterations in the STIM1/STIM2 ratio.

Notwithstanding these uncertainties, our findings indicate that loss of STIM1 expression and impaired SOCE in rodent and human models of diabetes resulted in decreased β cell ER Ca^{2+} levels, increased ER stress, abnormal Ca^{2+} oscillation patterns, and decreased insulin secretion (Fig. 8). The identification of this novel role for STIM1 in the pathogenesis of diabetes suggests that restoration of STIM1 expression and/or reconstitution of SOCE has the potential to improve β cell health and function.
Acknowledgements: The authors would like to thank Dr. Richard Day (Indiana University) for his helpful advice and technical discussions. The authors would also like to thank Sukrati Kanojia, Preethi Krishnan, Wataru Yamamoto, Morgan Robertson, Gary Considine, Kara Orr, and Daenique Jengelley (Indiana University) for their expert technical assistance.

Funding: This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases grants R01-DK-093954 and UC4-DK-104166 (to C.E.-M.), R01-DK074966 and R01-DK092616 (to M.W.R.). U.S. Department of Veterans Affairs Merit Award I01BX001733 (to C.E.-M.), and gifts from the Sigma Beta Sorority, the Ball Brothers Foundation, the George and Frances Ball Foundation (to C.E.-M.). X.T. was supported by the Diabetes and Obesity DeVault Fellowship at the Indiana University School of Medicine. R.N.B. was supported by NIH NIAID Training Grant (T32 AI060519) and a JDRF Postdoctoral Research Award (3-PDF-2017-385-A-N). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors acknowledge the support of the Islet and Physiology, Translation, and Imaging Cores of the Indiana Diabetes Research Center (P30-DK-097512).

Duality of Interest: No potential conflicts of interest relevant to this article were reported.

Author Contributions: T.K. directed the conception and design of the study, data analysis and interpretation, collection and assembly of data, and manuscript writing. X.T., S.T., R.N.B., C.L., P.S. and H.I. participated in data collection and critical revision of the manuscript. P.G. and M.R. contributed to data analysis, provided critical reagents, and provided critical revision of the manuscript. C.E.-M.: directed funding acquisition, study conception and design, participated in
the collection and assembly of data, contributed to data analysis, directed manuscript writing, and gave final approval of the manuscript. C.E.-M. is the guarantor of this work, had full access to all of the study data, and takes responsibility for the integrity and accuracy of the data.

**Prior Presentations.** Parts of this study were presented at the 76th and 77th Scientific Sessions of the American Diabetes Association.
REFERENCES

1. Lytton J, Westlin M, Burk SE, Shull GE, MacLennan DH: Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. J Biol Chem 1992;267:14483-14489

2. Johnson JD, Kuang S, Misler S, Polonsky KS: Ryanodine receptors in human pancreatic beta cells: localization and effects on insulin secretion. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2004;18:878-880

3. Gilon P, Chae HY, Rutter GA, Ravier MA: Calcium signaling in pancreatic beta-cells in health and in Type 2 diabetes. Cell calcium 2014;56:340-361

4. Tong X, Kono T, Anderson-Baucum EK, Yamamoto W, Gilon P, Lebeche D, Day RN, Shull GE, Evans-Molina C: SERCA2 Deficiency Impairs Pancreatic beta-Cell Function in Response to Diet-Induced Obesity. Diabetes 2016;65:3039-3052

5. Hogan PG: The STIM1-ORAI1 microdomain. Cell calcium 2015;58:357-367

6. Takemura H, Putney JW, Jr.: Capacitative calcium entry in parotid acinar cells. Biochem J 1989;258:409-412

7. Prakriya M, Lewis RS: Store-Operated Calcium Channels. Physiol Rev 2015;95:1383-1436

8. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA: STIM1, an essential and conserved component of store-operated Ca2+ channel function. J Cell Biol 2005;169:435-445

9. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG: Orai1 is an essential pore subunit of the CRAC channel. Nature 2006;443:230-233

10. Yuan JP, Zeng W, Huang GN, Worley PF, Muallem S: STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. Nat Cell Biol 2007;9:636-645

11. Sabourin J, LLG, Lisa Saurwein§, Jacques-Antoine Haefliger§, Eric Raddatz¶ and Florent Allagnat§: Store-operated Ca2+ Entry Mediated by Orai1 and TRPC1 Participates to Insulin Secretion in Rat β-Cells. JBC 2015;

12. Kono T, Ahn G, Moss DR, Gann L, Zarain-Herzberg A, Nishiki Y, Fueger PT, Ogihara T, Evans-Molina C: PPAR-gamma activation restores pancreatic islet SERCA2 levels and prevents beta-cell dysfunction under conditions of hyperglycemic and cytokine stress. Mol Endocrinol 2012;26:257-271

13. Santulli G, Pagano G, Sardu C, Xie W, Reiken S, D'Ascia SL, Cannone M, Marziliano N, Trimarco B, Guise TA, Lacampagne A, Marks AR: Calcium release channel RyR2 regulates insulin release and glucose homeostasis. J Clin Invest 2015;125:4316
14. Lacruz RS, Feske S: Diseases caused by mutations in ORAI1 and STIM1. Ann N Y Acad Sci 2015;1356:45-79

15. Kraft R: STIM and ORAI proteins in the nervous system. Channels (Austin) 2015;9:245-252

16. Bisaillon JM, Motiani RK, Gonzalez-Cobos JC, Potier M, Halligan KE, Alzawahra WF, Barroso M, Singer HA, Jourd'heuil D, Trebak M: Essential role for STIM1/Orai1-mediated calcium influx in PDGF-induced smooth muscle migration. Am J Physiol Cell Physiol 2010;298:C993-1005

17. Chaudhari S, Ma R: Store-operated calcium entry and diabetic complications. Exp Biol Med (Maywood) 2016;241:343-352

18. Maus M, Cuk M, Patel B, Lian J, Ouimet M, Kaufmann U, Yang J, Horvath R, Hornig-Do HT, Chrzanowska-Lightowlers ZM, Moore KJ, Cuervo AM, Feske S: Store-Operated Ca2+ Entry Controls Induction of Lipolysis and the Transcriptional Reprogramming to Lipid Metabolism. Cell Metab 2017;25:698-712

19. Leech CA, Kopp RF, Nelson HA, Nandi J, Roe MW: Stromal Interaction Molecule 1 (STIM1) Regulates ATP-sensitive Potassium (KATP) and Store-operated Ca2+ Channels in MIN6 beta-Cells. The Journal of biological chemistry 2017;292:2266-2277

20. Ogihara T, Chuang JC, Vestermark GL, Garmey JC, Ketchum RJ, Huang X, Brayman KL, Thorner MO, Repa JJ, Mirmira RG, Evans-Molina C: Liver X receptor agonists augment human islet function through activation of anaplerotic pathways and glycerolipid/free fatty acid cycling. The Journal of biological chemistry 2010;285:53926-5404

21. Evans-Molina C, Robbins RD, Kono T, Tersey SA, Vestermark GL, Nunemaker CS, Garmey JC, Deering TG, Keller SR, Maier B, Mirmira RG: Peroxisome proliferator-activated receptor gamma activation restores islet function in diabetic mice through reduction of endoplasmic reticulum stress and maintenance of euchromatin structure. Mol Cell Biol 2009;29:2053-2067

22. Imai Y, Fink BD, Promes JA, Kulkarni CA, Kerns RJ, Sivitz WI: Effect of a mitochondrial-targeted coenzyme Q analog on pancreatic beta-cell function and energetics in high fat fed obese mice. Pharmacol Res Perspect 2018;6:e00393

23. Johnson JS, Kono T, Tong X, Yamamoto WR, Zarain-Herzberg A, Merrins MJ, Satin LS, Gilon P, Evans-Molina C: Pancreatic and duodenal homeobox protein 1 (Pdx-1) maintains endoplasmic reticulum calcium levels through transcriptional regulation of sarco-endoplasmic reticulum calcium ATPase 2b (SERCA2b) in the islet beta cell. The Journal of biological chemistry 2014;289:32798-32810

24. Carter JD, Dula SB, Corbin KL, Wu R, Nunemaker CS: A practical guide to rodent islet isolation and assessment. Biol Proced Online 2009;11:3-31

25. Hum JM, Siegel AP, Pavalko FM, Day RN: Monitoring biosensor activity in living cells with fluorescence lifetime imaging microscopy. Int J Mol Sci 2012;13:14385-14400
26. Dyachok O, Gylfe E: Store-operated influx of Ca(2+) in pancreatic beta-cells exhibits graded dependence on the filling of the endoplasmic reticulum. Journal of cell science 2001;114:2179-2186

27. Smyth JT, Dehaven WI, Bird GS, Putney JW, Jr.: Ca2+-store-dependent and -independent reversal of Stim1 localization and function. J Cell Sci 2008;121:762-772

28. Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, Prakriya M, Feske S, Rao A: Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. Nature immunology 2008;9:432-443

29. Varga-Szabo D, Braun A, Kleinschnitz C, Bender M, Pleines I, Pham M, Renne T, Stoll G, Nieswandt B: The calcium sensor STIM1 is an essential mediator of arterial thrombosis and ischemic brain infarction. J Exp Med 2008;205:1583-1591

30. Miani M, Colli ML, Ladriere L, Cnop M, Eizirik DL: Mild endoplasmic reticulum stress augments the proinflammatory effect of IL-1beta in pancreatic rat beta-cells via the IRE1alpha/XBP1s pathway. Endocrinology 2012;153:3017-3028

31. Saleem H, Tovey SC, Molinski TF, Taylor CW: Interactions of antagonists with subtypes of inositol 1,4,5-trisphosphate (IP3) receptor. British journal of pharmacology 2014;171:3298-3312

32. Hetz C, Chevet E, Harding HP: Targeting the unfolded protein response in disease. Nat Rev Drug Discov 2013;12:703-719

33. Sammels E, Parys JB, Missiaen L, De Smedt H, Bultynck G: Intracellular Ca2+ storage in health and disease: a dynamic equilibrium. Cell Calcium 2010;47:297-314

34. Ozcan L, Tabas I: Role of endoplasmic reticulum stress in metabolic disease and other disorders. Annu Rev Med 2012;63:317-328

35. Wang S, Kaufman RJ: The impact of the unfolded protein response on human disease. J Cell Biol 2012;197:857-867

36. Putney JW, Jr.: A model for receptor-regulated calcium entry. Cell calcium 1986;7:1-12

37. Soboloff J, Rothberg BS, Madesh M, Gill DL: STIM proteins: dynamic calcium signal transducers. Nat Rev Mol Cell Biol 2012;13:549-565

38. Turner RC, Holman RR: Lessons from UK prospective diabetes study. Diabetes Research and Clinical Practice 1995;28:S151-S157

39. Turner RC, Cull CA, Frighi V, Holman RR: Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. JAMA : the journal of the American Medical Association 1999;281:2005-2012
40. Del Prato S, Nauck M, Duran-Garcia S, Maffei L, Rohwedder K, Theuerkauf A, Parikh S: Long-term glycaemic response and tolerability of dapagliflozin versus a sulphonylurea as add-on therapy to metformin in patients with type 2 diabetes: 4-year data. Diabetes Obes Metab 2015;17:581-590

41. Cardozo AK, Ortis F, Storling J, Feng YM, Rasschaert J, Tonnesen M, Van Eylen F, Mandrup-Poulsen T, Herchuelz A, Eizirik DL: Cytokines downregulate the sarcoendoplasmic reticulum pump Ca2+ ATPase 2b and deplete endoplasmic reticulum Ca2+, leading to induction of endoplasmic reticulum stress in pancreatic beta-cells. Diabetes 2005;54:452-461

42. Selvaraj S, Sun Y, Watt JA, Wang S, Lei S, Birnbaumer L, Singh BB: Neurotoxin-induced ER stress in mouse dopaminergic neurons involves downregulation of TRPC1 and inhibition of AKT/mTOR signaling. J Clin Invest 2012;122:1354-1367

43. Henke N, Albrecht P, Pfeiffer A, Toutzaris D, Zanger K, Methner A: Stromal interaction molecule 1 (STIM1) is involved in the regulation of mitochondrial shape and bioenergetics and plays a role in oxidative stress. J Biol Chem 2012;287:42042-42052

44. Kilimnik G, Zhao B, Jo J, Periwal V, Witkowski P, Misawa R, Harq M: Altered islet composition and disproportionate loss of large islets in patients with type 2 diabetes. PloS one 2011;6:e27445

45. Marselli L, Thorne J, Dahiya S, Sgroi DC, Sharma A, Bonner-Weir S, Marchetti P, Weir GC: Gene expression profiles of beta-cell enriched tissue obtained by laser capture microdissection from subjects with type 2 diabetes. PloS one 2010;5:e11499

46. Eizirik DL, Colli ML, Ortis F: The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. Nature reviews Endocrinology 2009;5:219-226

47. Ogawa A, Firth AL, Smith KA, Maliakal MV, Yuan JX: PDGF enhances store-operated Ca2+ entry by upregulating STIM1/Orai1 via activation of Akt/mTOR in human pulmonary arterial smooth muscle cells. Am J Physiol Cell Physiol 2012;302:C4056-411

48. Prentki M, Nolan CJ: Islet beta cell failure in type 2 diabetes. The Journal of clinical investigation 2006;116:1802-1812

49. Elghazi L, Rachdi L, Weiss AJ, Cras-Meneur C, Bernal-Mizrachi E: Regulation of beta-cell mass and function by the Akt/protein kinase B signalling pathway. Diabetes Obes Metab 2007;9 Suppl 2:147-157

50. DebRoy A, Vogel SM, Soni D, Sundivakkam PC, Malik AB, Tiruppathi C: Cooperative signaling via transcription factors NF-kappaB and AP1/c-Fos mediates endothelial cell STIM1 expression and hyperpermeability in response to endotoxin. The Journal of biological chemistry 2014;289:24188-24201

51. Tong X, Kono T, Evans-Molina C: Nitric oxide stress and activation of AMP-activated protein kinase impair beta-cell sarcoendoplasmic reticulum calcium ATPase 2b activity and protein stability. Cell death & disease 2015;6:e1790
52. Xu S, Nam SM, Kim JH, Das R, Choi SK, Nguyen TT, Quan X, Choi SJ, Chung CH, Lee EY, Lee IK, Wiederkehr A, Wollheim CB, Cha SK, Park KS: Palmitate induces ER calcium depletion and apoptosis in mouse podocytes subsequent to mitochondrial oxidative stress. Cell death & disease 2015;6:e1976

53. Soboloff J, Spassova MA, Hewavitharana T, He LP, Xu W, Johnstone LS, Dziadek MA, Gill DL: STIM2 is an inhibitor of STIM1-mediated store-operated Ca2+ Entry. Curr Biol 2006;16:1465-1470
FIGURE LEGENDS

Figure 1. Pharmacological inhibition of SOCE led to impaired insulin secretion and glucose-induced Ca\(^{2+}\) oscillations. INS-1 832/13 β cells were loaded with Calcium 6, and Ca\(^{2+}\) imaging experiments were performed to measure SOCE, according to the strategy shown in Panel A. (B-C) Ca\(^{2+}\) imaging was performed with or without known SOCE inhibitors, 50 µM ML-9 and 200 µM 2-APB, in the presence of 5.5 mM glucose, 200 µM diazoxide and 10 µM verapamil (Dz + V). ER Ca\(^{2+}\) depletion was induced with 3 µM TG and SOCE was quantified as the cytosolic Ca\(^{2+}\) increase following supplementation with 2 mM Ca\(^{2+}\). Results were displayed as the ∆F/F0 following Ca\(^{2+}\) supplementation. (D) Glucose-stimulated insulin secretion was measured in INS-1 cells pretreated with or without ML-9 and 2-APB and normalized to total protein content. (E-H) Glucose-stimulated Ca\(^{2+}\) imaging was performed using Fura-2 AM in islets isolated from C57BL/6J mice and pretreated with ML-9 or 2-APB. (E) Representative cytosolic Ca\(^{2+}\) recording following stimulation with 15 mM glucose. (F) Quantification of the average phase 1 amplitude; (G) phase 2 (oscillation) amplitude; and (H) oscillation period. (I) Mean insulin concentration profiles during islet perifusion at either basal 2.8 mM glucose concentrations (0–20 minutes) and in response to 16.7 mM glucose stimulation (20–60 minutes). DMSO or 200 µM 2-APB was added to the 16.7 mM glucose buffer. 50 islets were loaded per chamber and data are reported as ng/ml of insulin normalized to islet DNA content, n=3. (J) Relative area under curve (AUC) values for 1st phase (20 – 30 minutes) and 2nd phase (30 - 60 minutes) insulin secretion with DMSO and 2-APB treatment. The numbers of replicates for each experiment are indicated by the open circles. Results are displayed as the means ± S.E.M; *p<0.05 compared to control conditions.
**Figure 2. Loss of STIM1 expression led to impaired islet glucose-stimulated Ca\(^{2+}\) oscillations.**

Islets were isolated from saline and STZ-treated C57BL/6J mice and loaded with Fura-2 AM for Ca\(^{2+}\) imaging experiments in the presence of 5.5 mM glucose. (A-B) Representative cytosolic Ca\(^{2+}\) recording of islets following stimulation with 15 mM glucose. (C) Quantification of the average phase 1 amplitude; (D) phase 2 (oscillation) amplitude; and (E) phase 2 period. (F-H) Islets were isolated from saline and STZ-treated C57BL/6J mice. (F) Total islet RNA was subjected to real-time qRT-PCR for quantification of STIM and Orai isoform mRNA expression levels (normalized to actin mRNA expression). (G-H) Immunoblot was performed using antibodies against STIM1 and actin. Shown is a representative immunoblot and the means ± S.E.M. of STIM1 protein levels from multiple replicates. *p<0.05 compared to islets from saline-treated mice. (I-N) Islets isolated from STIM1\(^{\text{floxflo}}\) mice were transduced with a Cre expressing adenovirus (pSTIM1KO) or empty viral control (WT). (I) Reduced STIM1 protein expression in pSTIM1KO islets was confirmed by immunoblot. (J-N) pSTIM1KO islets were loaded with Fura-2 AM, and Ca\(^{2+}\) imaging was performed. (J-K) Representative cytosolic Ca\(^{2+}\) recording following stimulation with 15 mM glucose. (L) Quantification of the average phase 1 amplitude; (M) phase 2 (oscillation) amplitude, and (N) oscillation period; n=4-5 per group. *p<0.05 compared to WT islets. Numbers of replicates for each experiment are indicated by the open circles.

**Figure 3. STIM1 expression was reduced in human islets from donors with T2D.** Human islets were obtained from 7-9 non-diabetic donors (ND) and 7-11 donors with type 2 diabetes (T2D). (A) Total RNA was isolated and subjected to real-time qRT-PCR for quantification of STIM and Orai isoform expression levels. (B-C) Total islet protein was isolated and immunoblot
performed using antibodies against STIM1 and actin; \(*p<0.05\) compared to ND donors. (D) Correlation analysis between islet STIM1 mRNA levels and donor BMI.

**Figure 4. STIM1 expression was decreased under pro-inflammatory cytokine stress and glucolipotoxicity.** Islets from non-diabetic human donors (A) and C57BL/6J mice (B) were treated with 5 ng/ml IL-1\(\beta\), 100 ng/ml IFN-\(\gamma\), and 10 ng/ml TNF-\(\alpha\) for 24hrs. Immunoblot was performed using antibodies against STIM1 and actin. (C) INS-1 \(\beta\) cells were treated with 5 ng/ml IL-1\(\beta\) for 24hrs; total RNA was isolated and subjected to real-time qRT-PCR for quantification of STIM and Orai isoform mRNA expression levels (normalized to actin mRNA expression). (D-H) INS-1 \(\beta\) cells were treated with 0, 0.05, 0.5, or 5 ng/ml IL-1\(\beta\), for 24hrs. Total RNA and protein were isolated and subjected to real-time qRT-PCR for quantification of STIM mRNA (D) and protein levels (E). (F-G) Cytosolic Ca\(^{2+}\) imaging was performed to quantitate SOCE in IL-1\(\beta\)-treated INS-1 cells in the presence of 5.5 mM glucose, 200 \(\mu\)M diazoxide and 10 \(\mu\)M verapamil (Dz + V). (H) ER Ca\(^{2+}\) levels were indirectly estimated by quantitating the \(\Delta F/F_0\) response to TG. (I-L) INS-1 \(\beta\) cells were treated with a combination of 0.5 mM palmitate + 25 mM glucose (GLT), 0.5 mM palmitate + 11 mM glucose (Palmitate), or vehicle control + 11 mM glucose (Cont) for 24 hrs. (I) Total RNA was isolated from control and GLT-treated INS-1 cells and subjected to real-time qRT-PCR for quantification of STIM and Orai mRNA expression levels. (J) Immunoblot for STIM1 and actin was performed in INS-1 cells treated with GLT for 24 hrs. (K-L) Cytosolic Ca\(^{2+}\) imaging was performed to quantitate SOCE in GLT and palmitate-treated INS-1 cells. The number of replicates for each experiment are indicated by the open circles. \(*p<0.05\) compared to control conditions or for indicated comparisons.
Figure 5. STIM1 deletion led to impaired SOCE, reduced ER Ca\textsuperscript{2+} levels, and decreased glucose-stimulated insulin secretion. CRISPR/Cas-9 genomic editing was used to create an INS-1 832/13 STIM1 knockout (KO) cell line. (A) Total RNA was isolated and subjected to real-time qRT-PCR for quantification of STIM and Orai isoforms and SERCA2b mRNA levels. Numbers of replicates are indicated by the open circles and black squares. Cytosolic Ca\textsuperscript{2+} imaging was performed to quantitate SOCE in STIM1 KO and WT cells in response to (B) 3 µM TG and (C) 200 µM carbachol (CCh), which were used to empty ER Ca\textsuperscript{2+} stores in the presence of 5.5 mM glucose, 200 µM diazoxide and 10 µM verapamil (Dz + V). (D) Quantitative assessment of SOCE in WT and KO cells; numbers of replicates are indicated by the open circles. (E-F) ER Ca\textsuperscript{2+} levels were compared in WT and STIM1 KO cells transduced with a D4ER adenovirus, using a confocal microscope imaging system. (E) FRET/CFP ratios were acquired in HBSS buffer containing 2 mM Ca\textsuperscript{2+} in WT cells (n=65) and KO cells (n=53). (F) Average donor lifetime from FLIM analysis of at least 20 cells from each group. For A, D, E, and F, *p<0.05 compared to control WT cells. (G) GSIS in WT and KO cells was measured and normalized to total protein levels. The number of replicates are indicated by the open circles. *p<0.05 compared to 2.5 mM glucose; #p<0.05 compared to WT cells treated with 16.7 mM glucose.

Figure 6. STIM1 deficient cells exhibited increased susceptibility to ER stress. (A) STIM1 KO and WT INS-1 cells were treated with or without 25 mM glucose + 0.5 mM BSA-conjugated palmitate (GLT) or 10 µM tunicamycin (TM) for 3 hrs. Total RNA was isolated and subjected to real-time qRT-PCR. Shown are the average spliced/unspliced XBP1 ratios. *p<0.05, compared to control conditions; #p<0.05 compared for indicated comparisons. (B-C) STIM1 KO and WT INS-1 cells were treated with or without 5 ng/ml IL-1β for 24 hrs. Immunoblot analysis was
performed using antibodies against STIM1, cleaved caspase-3 and actin. Quantitative protein levels are shown graphically. *p<0.05 compared to control conditions; #p<0.05 compared to IL-1β treated WT group. (D-E) INS-1 cells were transfected with siRNAs against STIM1 (siSTIM1) or an siControl. Total RNA and protein were isolated and subjected to real-time qRT-PCR and immunoblot to confirm reduced STIM1 mRNA and protein expression. (F) siSTIM1 and siControl-transfected cells were treated with 10 µM TM for 3 hrs and the spliced/unspliced XBP-1 mRNA ratios were quantitated using qRT-PCR. *p<0.05 compared to control conditions or for indicated comparisons. (G) STIM1KO and WT INS-1 cells were treated with 10 µM TM for 24 hrs, fixed, and analyzed by electron microscopy. Representative images of the β cell ER ultrastructure, indicated by dotted lines in WT and KO cells (scale bar = 2 µm). For each experiment, numbers of replicates are indicated by the open circles.

**Figure 7. STIM1 overexpression restored ER Ca^{2+} levels and improved insulin secretion in human islets from donors with Type 2 diabetes**

(A) STIM1 KO and WT INS-1 cells were transduced with an adenovirus encoding human STIM1 in increasing concentrations (shown as pfu/ml). Immunoblot was performed using antibodies against STIM1 and actin. (B-C) SOCE was measured in STIM1KO cells and WT INS-1 cells that had been transduced with STIM1 expressing adenovirus (Ad-STIM1) or an empty adenoviral control (EV) in the presence of 5.5 mM glucose, 200 µM diazoxide and 10 µM verapamil (Dz +V). Quantitative results are shown as the ∆F/F0; *p<0.05 compared to WT+EV, #p<0.05 compared to EV-treated STIM1KO cells. (D-H) ER Ca^{2+} analysis using FRET and FLIM was performed in D4ER expressing STIM1KO cells and WT INS-1 cells that had been transduced with Ad-STIM1 or EV. (D) Quantitation of FRET/CFP ratio of the D4ER probe at baseline in 2 mM Ca^{2+} containing HBSS buffer. (E) FLIM analysis for the D4ER donor probe is
shown graphically as the relative average donor lifetime. For D-E, *p<0.05 compared to WT cells. #p<0.05 compared to STIM1 KO cells + EV. (F) FRET analysis was performed in WT and STIM1 KO cells transduced with Ad-STIM1 or EV in the presence of 5.5 mM glucose. (G-H) Carbachol-induced reductions in ER Ca\(^{2+}\) levels and ER Ca\(^{2+}\) refilling following the addition of 2 mM Ca\(^{2+}\); results are shown quantitatively as the change in the FRET ratio (ΔR); *p<0.05 compared to WT cells; #p<0.05 compared to STIM1 KO cells +EV. (I) STIM1KO cells and WT INS-1 cells were transduced with STIM1 expressing adenovirus or EV control and treated with or without 5 ng/ml IL-1β for 24hrs. Immunoblot analysis was performed using antibodies against STIM1, cleaved caspase-3 and actin. Relative expression levels of cleaved-caspase 3 are shown graphically in (J); *p<0.05, compared to control conditions; #p<0.05 compared for indicated comparisons. (K-L) Human islets from two donors with type 2 diabetes (T2D) were transduced with STIM1 expressing adenovirus (3x10\(^6\) pfu/ml) or EV. (K) Immunoblot was performed to verify STIM1 overexpression. (L) Glucose-stimulated insulin secretion from 2 separate experiments of 2 donors with 4 replicates each was measured and normalized to total protein content; p<0.05 compared to high glucose conditions in EV-treated islets. For each experiment, the numbers of replicates are indicated by the open circles.

**Figure 8. Overall model.**

Under normal conditions, reductions in ER Ca\(^{2+}\) levels are sensed by STIM1, leading to STIM1 oligomerization and translocation to the ER/plasmalemmal junctional regions. Here, STIM1 complexes with selective Orai and TRPC1 channels allowing Ca\(^{2+}\) influx from the extracellular space, with subsequent transfer of Ca\(^{2+}\) into the ER lumen, leading to ER Ca\(^{2+}\) restoration. Our study revealed a preferential loss of STIM1 expression under diabetic stress conditions,
including exposure to pro-inflammatory cytokines and elevated free fatty acids. Genetic as well as acquired loss of STIM1 was sufficient to impair β cell SOCE, reduce ER Ca^{2+} levels, increase β cell susceptibility to ER stress and death, and cause abnormal glucose-stimulated calcium oscillations and insulin secretory defects. Moreover, our data revealed that STIM1 reconstitution in human cadaveric islets from diabetic donors was sufficient to improve glucose-stimulated insulin secretion, while also protecting against pro-inflammatory cytokine-induced cell death in INS-1 cells.
Figure 1. Pharmacological inhibition of SOCE led to impaired insulin secretion and glucose-induced Ca2+ oscillations. INS-1 832/13 β cells were loaded with Calcium 6, and Ca2+ imaging experiments were performed to measure SOCE, according to the strategy shown in Panel A. (B-C) Ca2+ imaging was performed with or without known SOCE inhibitors, 50 µM ML-9 and 200 µM 2-APB, in the presence of 5.5 mM glucose, 200 µM diazoxide and 10 µM verapamil (Dz + V). ER Ca2+ depletion was induced with 3 µM TG and SOCE was quantified as the cytosolic Ca2+ increase following supplementation with 2 mM Ca2+. Results were displayed as the ∆F/F0 following Ca2+ supplementation. (D) Glucose-stimulated insulin secretion was measured in INS-1 cells pretreated with or without ML-9 and 2-APB and normalized to total protein content. (E-H) Glucose-stimulated Ca2+ imaging was performed using Fura-2 AM in islets isolated from C57BL/6J mice and pretreated with ML-9 or 2-APB. (E) Representative cytosolic Ca2+ recording following stimulation with 15 mM glucose. (F) Quantification of the average phase 1 amplitude; (G) phase 2 (oscillation) amplitude; and (H) oscillation period. (I) Mean insulin concentration profiles during islet perifusion at either basal 2.8 mM glucose concentrations (0–20 minutes) and in response to 16.7 mM glucose stimulation (20–60 minutes). DMSO or 200 µM 2-APB was added to the 16.7 mM glucose buffer. 50 islets were loaded per chamber and data are reported as ng/ml of insulin normalized to islet DNA content, n=3. (J) Relative area under curve (AUC) values for 1st phase (20 – 30 minutes) and 2nd phase (30 – 60 minutes) insulin secretion with DMSO and 2-APB treatment. The numbers of replicates for each experiment are indicated by the open circles. Results are displayed as the means ± S.E.M.; *p<0.05 compared to control conditions.

398x313mm (300 x 300 DPI)
Figure 2. Loss of STIM1 expression led to impaired islet glucose-stimulated Ca2+ oscillations. Islets were isolated from saline and STZ-treated C57BL/6J mice and loaded with Fura-2 AM for Ca2+ imaging experiments in the presence of 5.5 mM glucose. (A-B) Representative cytosolic Ca2+ recording of islets following stimulation with 15 mM glucose. (C) Quantification of the average phase 1 amplitude; (D) phase 2 (oscillation) amplitude; and (E) phase 2 period. (F-H) Islets were isolated from saline and STZ-treated C57BL/6J mice. (F) Total islet RNA was subjected to real-time qRT-PCR for quantification of STIM and Orai isoform mRNA expression levels (normalized to actin mRNA expression). (G-H) Immunoblot was performed using antibodies against STIM1 and actin. Shown is a representative immunoblot and the means ± S.E.M. of STIM1 protein levels from multiple replicates. *p<0.05 compared to islets from saline-treated mice. (I-N) Islets isolated from STIM1flox/flox mice were transduced with a Cre expressing adenovirus (pSTIM1KO) or empty viral control (WT). (I) Reduced STIM1 protein expression in pSTIM1KO islets was confirmed by immunoblot. (J-N) pSTIM1KO islets were loaded with Fura-2 AM, and Ca2+ imaging was performed. (J-K) Representative cytosolic Ca2+ recording following stimulation with 15 mM glucose. (L) Quantification of the average phase 1 amplitude; (M) phase 2 (oscillation) amplitude, and (N) oscillation period; n=4-5 per group. *p<0.05 compared to WT islets. Numbers of replicates for each experiment are indicated by the open circles.
Figure 3. STIM1 expression was reduced in human islets from donors with T2D. Human islets were obtained from 7-9 non-diabetic donors (ND) and 7-11 donors with type 2 diabetes (T2D). (A) Total RNA was isolated and subjected to real-time qRT-PCR for quantification of STIM and Orai isoform expression levels. (B-C) Total islet protein was isolated and immunoblot performed using antibodies against STIM1 and actin; *p<0.05 compared to ND donors. (D) Correlation analysis between islet STIM1 mRNA levels and donor BMI.

591x429mm (600 x 600 DPI)
Figure 4. STIM1 expression was decreased under pro-inflammatory cytokine stress and glucolipotoxicity. Islets from non-diabetic human donors (A) and C57BL/6J mice (B) were treated with 5 ng/ml IL-1β, 100 ng/ml IFN-γ, and 10 ng/ml TNF-α for 24hrs. Immunoblot was performed using antibodies against STIM1 and actin. (C) INS-1 β cells were treated with 5 ng/ml IL-1β for 24hrs; total RNA was isolated and subjected to real-time qRT-PCR for quantification of STIM and Orai isoform mRNA expression levels (normalized to actin mRNA expression). (D-H) INS-1 β cells were treated with 0, 0.05, 0.5, or 5 ng/ml IL-1β, for 24hrs. Total RNA and protein were isolated and subjected to real-time qRT-PCR for quantification of STIM mRNA (D) and protein levels (E). (F-G) Cytosolic Ca²⁺ imaging was performed to quantitate SOCE in IL-1β-treated INS-1 cells in the presence of 5.5 mM glucose, 200 μM diazoxide and 10 μM verapamil (Dz + V). (H) ER Ca²⁺ levels were indirectly estimated by quantitating the ΔF/F₀ response to TG. (I-L) INS-1 β cells were treated with a combination of 0.5 mM palmitate + 25 mM glucose (GLT), 0.5 mM palmitate + 11 mM glucose (Palmitate), or vehicle control + 11 mM glucose (Cont) for 24 hrs. (I) Total RNA was isolated from control and GLT-treated INS-1 cells and subjected to real-time qRT-PCR for quantification of STIM and Orai mRNA expression levels. (J) Immunoblot for STIM1 and actin was performed in INS-1 cells treated with GLT for 24 hrs. (K-L) Cytosolic Ca²⁺ imaging was performed to quantitate SOCE in GLT and palmitate-treated INS-1 cells. The number of replicates for each experiment are indicated by the open circles. *p<0.05 compared to control conditions or for indicated comparisons.
Figure 5. STIM1 deletion led to impaired SOCE, reduced ER Ca2+ levels, and decreased glucose-stimulated insulin secretion. CRISPR/Cas-9 genomic editing was used to create an INS-1 832/13 STIM1 knockout (KO) cell line. (A) Total RNA was isolated and subjected to real-time qRT-PCR for quantification of STIM and Orai isoforms and SERCA2b mRNA levels. Numbers of replicates are indicated by the open circles and black squares. Cytosolic Ca2+ imaging was performed to quantitate SOCE in STIM1 KO and WT cells in response to (B) 3 µM TG and (C) 200 µM carbachol (CCh), which were used to empty ER Ca2+ stores in the presence of 5.5 mM glucose, 200 µM diazoxide and 10 µM verapamil (Dz + V). (D) Quantitative assessment of SOCE in WT and KO cells; numbers of replicates are indicated by the open circles. (E-F) ER Ca2+ levels were compared in WT and STIM1 KO cells transduced with a D4ER adenovirus, using a confocal microscope imaging system. (E) FRET/CFP ratios were acquired in HBSS buffer containing 2 mM Ca2+ in WT cells (n=65) and KO cells (n=53). (F) Average donor lifetime from FLIM analysis of at least 20 cells from each group. For A, D, E, and F, *p<0.05 compared to control WT cells. (G) GSIS in WT and KO cells was measured and normalized to total protein levels. The number of replicates are indicated by the open circles. *p<0.05 compared to 2.5 mM glucose; #p<0.05 compared to WT cells treated with 16.7 mM glucose.
Figure 6. STIM1 deficient cells exhibited increased susceptibility to ER stress. (A) STIM1 KO and WT INS-1 cells were treated with or without 25 mM glucose + 0.5 mM BSA-conjugated palmitate (GLT) or 10 µM tunicamycin (TM) for 3 hrs. Total RNA was isolated and subjected to real-time qRT-PCR. Shown are the average spliced/unspliced XBP1 ratios. *p<0.05, compared to control conditions; #p<0.05 compared for indicated comparisons. (B-C) STIM1 KO and WT INS-1 cells were treated with or without 5 ng/ml IL-1β for 24 hrs. Immunoblot analysis was performed using antibodies against STIM1, cleaved caspase-3 and actin. Quantitative protein levels are shown graphically. *p<0.05 compared to control conditions; #p<0.05 compared to IL-1β treated WT group. (D-E) INS-1 cells were transfected with siRNAs against STIM1 (siSTIM1) or an siControl. Total RNA and protein were isolated and subjected to real-time qRT-PCR and immunoblot to confirm reduced STIM1 mRNA and protein expression. (F) siSTIM1 and siControl-transfected cells were treated with 10 µM TM for 3 hrs and the spliced/unspliced XBP1 mRNA ratios were quantitated using qRT-PCR. *p<0.05 compared to control conditions or for indicated comparisons. (G) STIM1KO and WT INS-1 cells were treated with 10 µM TM for 24 hrs, fixed, and analyzed by electron microscopy. Representative images of the β cell ER ultrastructure, indicated by dotted lines in WT and KO cells (scale bar = 2 µm). For each experiment, numbers of replicates are indicated by the open circles.
Figure 8. Overall model. Under normal conditions, reductions in ER Ca2+ levels are sensed by STIM1, leading to STIM1 oligomerization and translocation to the ER/plasmalemmal junctional regions. Here, STIM1 complexes with selective Orai and TRPC1 channels allowing Ca2+ influx from the extracellular space, with subsequent transfer of Ca2+ into the ER lumen, leading to ER Ca2+ restoration. Our study revealed a preferential loss of STIM1 expression under diabetic stress conditions, including exposure to pro-inflammatory cytokines and elevated free fatty acids. Genetic as well as acquired loss of STIM1 was sufficient to impair β cell SOCE, reduce ER Ca2+ levels, increase β cell susceptibility to ER stress and death, and cause abnormal glucose-stimulated calcium oscillations and insulin secretory defects. Moreover, our data revealed that STIM1 reconstitution in human cadaveric islets from diabetic donors was sufficient to improve glucose-stimulated insulin secretion, while also protecting against pro-inflammatory cytokine-induced cell death in INS-1 cells.
### Table S1: List of Real-Time Quantitative Reverse Transcription PCR Primers

| Targeted gene     | 5’-sense primer-3’                  | 5’-antisense primer-3’                  |
|-------------------|------------------------------------|----------------------------------------|
| STIM1 (human, rat, mouse) | AGCCTCAGCCATGACACGAGT | TTCCACATCCACATCACCATTG |
| STIM2 (human)     | TGAGGTGTGCTGATCATAATCT             | GTTGCCAGCGAAAAAGTCGT                  |
| STIM2 (mouse)     | ACACTCCCAGGATAGCGT                | GTTATGAGGTGCGTGTCAG                   |
| STIM2 (rat)       | CAACGGCATCCTGGGAGAAGT               | CTGGAGGCTTCCCTGAACTGG                 |
| Orai1 (human)     | GTCACCTACCCGGACTGGAT               | TGGAGGCTTTAAAGCTGGCG                 |
| Orai1 (rat, mouse)| AGGTGATGAGCCTCAGAGGAGG            | CTGATCATGAGGGCAACAG                  |
| Orai2 (human)     | GAGGCGGTGAGCAACATCC               | GGAGGAACCTAGAGATCCAGCAAGA             |
| Orai2 (rat, mouse)| GGCCACAAGGGGATGGAGAAGT            | TGAGGGTACTGGTACTGGTC                 |
| Orai3 (human)     | TGGGTCAAGTTTGTGCCCAG              | AGCTGGACTAAGGGGAGGTAGC               |
| Orai3 (rat, mouse)| GCCTGCACCAAGTGATTGGAATGTA         | TGTTGCTCACGGCTTATATG                 |
| Actin (human, rat, mouse) | AGGTGATCAGATTGGCAACGA        | CACTTCATGAGGAAATTTGAGTTAGTG          |
| Total XBP1 (rat)  | AGCACTCAGCAGCTACGTGCGCTCT         | CCAGAATGCCCCAAAAGGATATCAG             |
| Spliced XBP1 (rat)| CTGAGTCCGCCAGCAGG                 | TGTGAGGTCCCCATCGGAAGA                 |
Table S2: List of Antibodies Employed

| Targeted protein | Species | Manufacturer  | Dilution |
|------------------|---------|---------------|----------|
| STIM1            | Mouse   | Invitrogen    | 1:1000   |
| Caspase-3        | Rabbit  | Cell signaling| 1:500    |
| Actin            | Mouse   | Sigma         | 1:10000  |
Figure S1. Expression analysis of STIM and Orai gene isoforms in human and mouse islets and INS-1 β cells. RNA was isolated from cadaveric human islets (A); 8-weeks-old C57BL/6J mouse islets (B); and INS-1 cells (C). Reverse-transcribed RNA was subjected to qRT-PCR for quantitation of STIM1, STIM2, Orai1, Orai2 and Orai3 transcript levels (left panels) using equally efficient primers (right panels). Results are displayed as the means ± S.E.M; *p<0.05 for indicated comparisons.
Figure S2. Thapsigargin-induced ER Ca\(^{2+}\) release is reduced in STIM1KO cells and wild-type INS-1 \(\beta\) cells treated with palmitate and GLT. (A-B) Cytosolic Ca\(^{2+}\) imaging was performed in the presence of 5.5 mM glucose, 200 µM diazoxide and 10 µM verapamil (Dz+V) in INS-1 cells treated with 0.5 mM palmitate + 25 mM glucose (GLT), 0.5 mM palmitate + 11 mM glucose (Palmitate), or vehicle control + 11 mM glucose (Cont) for 24 hrs. (B) ER Ca\(^{2+}\) levels were indirectly estimated by quantitating the \(\Delta F\) in response to TG. (C-D) Cytosolic Ca\(^{2+}\) imaging was performed in WT and STIM1KO cells in the presence of 5.5 mM glucose, 200 µM diazoxide, and 10 µM verapamil (Dz+V). (D) ER Ca\(^{2+}\) levels were indirectly estimated by quantitating the \(\Delta F\) in response to TG. Graphs were generated from data shown in Figure 5B and 4F. For Panels A and C, results were normalized to control conditions. All data are displayed as means \(\pm\) S.E.M.; *p<0.05 vs control conditions. The number of replicates for experiment are indicated by the open circles.
Figure S3. Calcium imaging analysis of wild type and STIM1 KO INS-1 β cells. WT and STIM1KO cells were treated with or without 5 ng/ml IL-1β (A-B) or the combination of 0.5 mM palmitate + 25 mM glucose (GLT) for 24 hrs (C-D). Cells were loaded with Calcium 6, and Ca²⁺ imaging experiments were performed to measure SOCE in the presence of 5.5 mM glucose, 200 µM diazoxide, and 10 µM verapamil (Dz+V). Representative Ca²⁺ imaging traces are shown in Panels A and C. Quantitative results for the ΔF/F₀ response to extracellular Ca²⁺ supplementation are shown as the means ± S.E.M in Panels B, D, and E; n=4-8 replicates for each condition as indicated by the open circles. *p<0.05 for comparison to WT control or for indicated comparisons. (F) WT and STIM1 KO cells were transduced with a D4ER adenovirus, and FLIM analysis was used to measure ER Ca²⁺ levels. Shown is a representative lifetime map with look-up table indicating donor lifetime in nanoseconds (ns). Quantitated results are shown in Figure 5F. Scale bars = 50 µm.
Figure S4. STIM1 overexpression partially rescued cytokine-induced reductions in SOCE. (A) INS-1 cells were transduced with a human STIM1 expressing adenovirus (Ad-STIM1, 1x10⁷ pfu/ml) or empty virus (EV, 1x10⁷ pfu/ml) followed by IL-1β treatment (5 ng/ml for 24h). Cells were loaded with Calcium 6, and Ca²⁺ imaging experiments were performed to measure SOCE in the presence of 5.5 mM glucose, 200 µM diazoxide, and 10 µM verapamil (Dz+V). ER Ca²⁺ depletion was induced with 3 µM TG, and SOCE was quantified as the cytosolic Ca²⁺ increase following supplementation of 2 mM Ca²⁺ into the buffer. (A) Representative Ca²⁺ imaging trace for each condition. (B) Quantitative results for the calculated ΔF/F₀ following Ca²⁺ supplementation. Results are displayed as the means ± S.E.M.; n=6 replicates for each condition as indicated by the open circles in Panel B. *p<0.05 for indicated comparisons.