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JunB protects β-cells from lipotoxicity via the XBP1–AKT pathway

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Diets rich in saturated fats may contribute to the loss of pancreatic β-cells in type 2 diabetes. JunB, a member of the activating protein 1 (AP-1) transcription factor family, promotes β-cell survival and mediates part of the beneficial effects of GLP-1 agonists. In this study we interrogated the molecular mechanisms involved in JunB-mediated β-cell protection from lipotoxicity. The saturated fatty acid palmitate decreased JunB expression, and this loss may contribute to β-cell apoptosis, as overexpression of JunB protected cells from lipotoxicity. Array analysis of JunB-deficient β-cells identified a gene expression signature of a downregulated endoplasmic reticulum (ER) stress response and inhibited AKT signaling. JunB stimulates XBP1 expression via the transcription factor c/EBPδ during ER stress, and forced expression of XBP1s rescued the viability of JunB-deficient cells, constituting an important antiapoptotic mechanism. JunB silencing inhibited AKT activation and activated the proapoptotic Bcl-2 protein BAD via its dephosphorylation. BAD knockdown reversed lipotoxic β-cell death potentiated by JunB siRNA. Interestingly, XBP1s links JunB and AKT signaling as XBP1 knockdown also reduced AKT phosphorylation. GLP-1 agonists induced cAMP-dependent AKT phosphorylation leading to β-cell protection against palmitate-induced apoptosis. JunB and XBP1 knockdown or IRE1 inhibition decreased AKT activation by cAMP, leading to β-cell apoptosis. In conclusion, JunB modulates the β-cell ER stress response and AKT signaling via the induction of XBP1s. The activation of the JunB gene network and the crosstalk between the ER stress and AKT pathway constitute a crucial defense mechanism by which GLP-1 agonists protect against lipotoxic β-cell death. These findings elucidate novel β-cell-protective signal transduction in type 2 diabetes.
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The prevalence of type 2 diabetes (T2D) has doubled in the past 30 years and has reached 360 million affected individuals worldwide.[1] In nondiabetic individuals, insulin resistance is compensated by increased insulin secretion by the pancreatic β-cells. Hyperglycemia develops in individuals who lack this compensatory response. This implies that β-cell dysfunction is an essential step in the pathogenesis of T2D.[2,3] In autopsy studies, a decreased β-cell number of ~40% is observed in T2D patients as compared with nondiabetic controls,[4] demonstrating that β-cell loss may also contribute to insulin deficiency.

The mechanisms leading to β-cell dysfunction and death are not well understood. Environmental factors such as diets rich in saturated fats and a sedentary lifestyle combined to an unfavorable genetic background increase T2D risk.[5] Lipid infusion in individuals who are genetically predisposed to T2D induces hepatic insulin resistance and impairs β-cell function.[6] Individuals with high fasting levels of plasma free fatty acids (FFAs) have an elevated risk of developing T2D.[7] Prolonged exposure to FFAs *in vitro*, particularly saturated FFAs such as palmitate, reduces insulin release and induces β-cell apoptosis.[8]

Several studies have suggested that endoplasmic reticulum (ER) stress is a putative mediator of FFA-induced β-cell dysfunction and death in T2D.[9–12] ER stress is defined as an imbalance between the load of proteins in the ER and the capacity of the organelle to handle these. When ER homeostasis is perturbed, signaling pathways are activated, leading to an adaptive response called the unfolded protein response (UPR). Three transmembrane proteins govern the UPR. Protein kinase-like ER kinase (PERK) attenuates protein translation, whereas activating transcription factor (ATF)-6 and inositol-requiring kinase-1 (IRE1) increase ER folding capacity by upregulating ER chaperones and the ER-associated protein degradation machinery. The transcriptional regulation by IRE1 is mediated by its downstream effectors X-box binding protein 1 (XBP1) and JNK.[13,14] The prime function of the UPR is to restore ER homeostasis. The β-cells are particularly sensitive to disturbances in the ER because of the high demand for insulin production.[15] *In vitro*, saturated FFAs induce more pronounced proapoptotic ER stress signaling compared with unsaturated FFAs.[16,17] Rats fed a high-fat diet have increased β-cell PERK phosphorylation and BiP expression.[18]

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Abbreviations: AP-1, activating protein 1; ATF, activating transcription factor; ER, endoplasmic reticulum; FBS, fetal bovine serum; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; IPA, Ingenuity Pathway Analysis; IRE1, inositol-requiring kinase-1; KD, knockdown; PERK, protein kinase-like ER kinase; T2D, type 2 diabetes; UPR, unfolded protein response; XBP1, X-box binding protein 1
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The activating protein 1 (AP-1) transcription factor is formed by several protein families, including Jun (c-Jun, JunB and JunD), Fos (c-Fos, FosB, Fra1 and Fra2) and ATF (ATF2, ATF3, JDP1 and JDP2). AP-1 can be formed of homodimers or heterodimers; these combinatorial possibilities result in diverse outcomes in terms of gene regulation. JunB has been extensively studied in cancer models where it acts as a tumor suppressor.[19] In cellular models of type 1 diabetes (cytokine-treated β-cells), JunB was shown to promote β-cell survival by inhibiting NF-κB activity[20] and regulating ATF3 expression.[21] GLP-1 agonists such as exendin-4, used for the treatment of T2D, have been shown to improve insulin secretion and preserve β-cell mass. Part of their protective effect is mediated by upregulation of JunB expression[22] and consequent protection against FFA-induced β-cell apoptosis.

The mechanism(s) by which JunB protects β-cells from lipotoxicity is unknown. In this study we interrogated the molecular pathways involved in JunB-mediated β-cell protection against palmitate. By array analysis and a series of focused experiments we found that JunB modulates the ER stress response and AKT signaling. JunB-dependent induction of XBP1 mediates the crosstalk between these pathways, constituting a crucial defense mechanism against lipotoxic β-cell death.

Results

JunB modulates lipotoxic β-cell death. Palmitate induced JunB mRNA expression from 8 to 24 h of exposure (Figure 1a). At the protein level, JunB was initially upregulated by palmitate (2 to 8 h) but its expression decreased at later time points (16 to 24 h, Figure 1a), when cell death becomes apparent. JNK has been shown to phosphorylate JunB, leading to its proteasomal degradation.[23] JNK inhibition using the chemical JNK inhibitor SP600125 prevented the downregulation of JunB by palmitate (Figure 1b), demonstrating that palmitate-induced JNK activation leads to JunB degradation. We have previously shown that JunB knockdown (KD) potentiates palmitate-induced apoptosis in INS-1E cells.[22] We confirmed these results here (Figure 1c) and showed increased caspase 3 activation after JunB KD in palmitate-treated INS-1E cells (Figure 1d). JunB deficiency also sensitized primary rat β-cells (Figure 1e) and dispersed human islet cells to lipotoxicity (Figure 1f). Conversely, adenoviral JunB overexpression protected INS-1E cells from palmitate-induced apoptosis and decreased caspase 3 activation (Figures 1g and h and Supplementary Figures S1A and B). Palmitate treatment of human islets also reduced JunB expression and increased JNK phosphorylation (Figure 1i). JunB overexpression in human islet cells decreased caspase 3 cleavage and protected from palmitate toxicity (Figures 1j and k and Supplementary Figures S1C and D), confirming the important role of JunB in both human and rat β-cell survival in the face of metabolic stress.

Identification of JunB-regulated gene networks. To shed light on the underlying protective mechanisms of JunB, we performed a time course microarray analysis of gene expression in INS-1E cells transfected with a specific JunB siRNA.[20] JunB KD modified 776 and 450 probe sets corresponding to 711 and 431 genes after 6 and 14 h of palmitate treatment, respectively. To analyze the array data in an unbiased manner we used DAVID and Ingenuity Pathway Analysis (IPA) software. Using DAVID we found that the top biological function downregulated after 6 h of palmitate treatment in JunB-deficient cells was second messenger-mediated signaling that includes important genes for β-cell maintenance such as PERK (*Eif2ak3*), *CREM* (*cAMP-responsive element modulator*), *insulin-like growth factor 1 receptor* (*Igf1r*) and *phosphoinositide-3-kinase* (*PIK3c2*) (Supplementary Table S1). In JunB KD cells treated for 14 h with palmitate, we observed decreased expression of antiapoptotic and ER stress response genes, whereas genes related to regulation of transcription and dephosphorylation, including the AKT inhibitor *PTEN*, were upregulated (Supplementary Table S1). IPA of this data set showed that cell cycle, RhoGDI signaling and PI3K/AKT signaling were the top canonical pathways modified by JunB KD. In agreement with the changes in the ER stress response pathway, there was an enrichment ($P = 2.8\text{E}-05$) of genes previously shown to be upregulated by XBP1s (Supplementary Table S2). From the 13 XBP1-dependent genes, 12 were downregulated in JunB-deficient cells, pointing to a possible link between JunB and XBP1. Genes were also classified by a previously described manual curation[24] according to their potential role in β-cell dysfunction and death (Figure 2 and Supplementary Table S1). Genes involved in insulin secretion, such as *SNAP25* and *synaptotagmin IV*,[25] were inhibited by JunB depletion in palmitate-treated β-cells (Figure 2 and Supplementary Table S1). There was no change in glucose-stimulated insulin secretion (GSIS) in JunB-depleted cells exposed to palmitate for 16 h (data not shown). We also analyzed GSIS in INS-1E cells exposed for 48 h to a lower, nontoxic (0.4 mM) concentration of palmitate (Supplementary Figure S2A). Palmitate inhibited both insulin content and GSIS (Supplementary Figures S2B and C). JunB KD did not alter basal or GSIS or insulin content (Supplementary Figures S2B and C) in control or palmitate-treated cells. JunB KD did not alter the proinsulin/insulin ratio in the culture medium (data not shown). These analyses suggest that JunB mostly modulates gene networks involved in the regulation of apoptosis and the ER stress response, and thereby promotes β-cell homeostasis.

Role of JunB in the ER stress response. Based on the array analyses we investigated the role of JunB in the ER stress response in β-cells. For this purpose we silenced or overexpressed JunB and evaluated signaling in the three main branches of the UPR, controlled by IRE1, PERK and ATF6. In keeping with the array data, JunB-deficient cells expressed less total and spliced XBP1 mRNA and XBP1 protein (Figures 3a and b). JunB overexpression increased both forms of XBP1 (Figures 3c and d). The changes in XBP1 expression were independent of IRE1 activity, as JunB siRNA or adenovirus did not alter IRE1 phosphorylation levels (Figures 3a and c). PERK and eIF2α phosphorylation, as well as total expression of PERK and its downstream targets CHOP and ATF3, were reduced by JunB KD and augmented in cells with forced JunB expression (Figures 3e

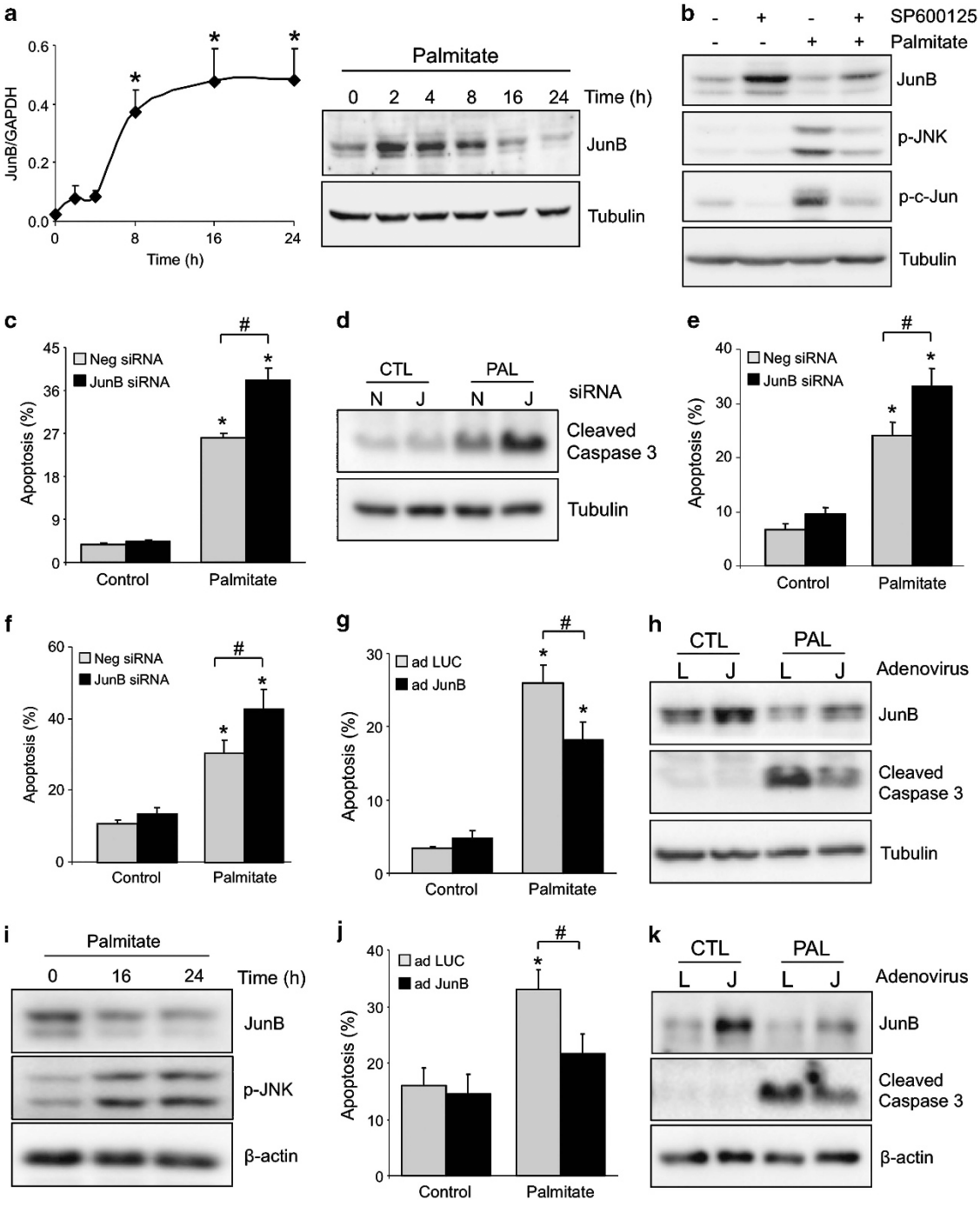

Figure 1 JunB modulates lipotoxic β-cell death. (**a**) JunB mRNA (left panel) and protein expression (right panel) in INS-1E cells treated with 0.5 mM palmitate ($n=3$). (**b**) JunB protein expression and p-JNK and p-c-Jun phosphorylation in INS-1E cells treated with palmitate and/or 10 μM SP600125 for 16 h ($n=3$). (**c**) Apoptosis in INS-1E cells transfected with negative control siRNA (N) or JunB siRNA (J) and then treated or not with palmitate for 16 h ($n=8$). (**d**) Cleaved caspase 3 in INS-1E cells transfected and treated as in (**c**) ($n=3$). (**e**) Apoptosis in primary rat β-cells transfected with negative or JunB siRNA and then treated with palmitate for 24 h ($n=4$). (**f**) Apoptosis in dispersed human islet cells transfected with negative or JunB siRNA and then treated with palmitate for 24 h ($n=4$). (**g**) Apoptosis in INS-1E cells infected with luciferase (LUC)- or JunB-expressing adenovirus (ad) (MOI 10) and then treated with palmitate for 16 h ($n=4$). (**h**) JunB protein expression and cleaved caspase 3 in INS-1E cells infected and treated as in (**g**) ($n=3$). (**i**) JunB protein expression and JNK phosphorylation in human islets treated with palmitate for 16 or 24 h ($n=3$). (**j**) Apoptosis in dispersed human islet cells infected with luciferase or JunB expressing adenovirus (MOI 100) and then treated with palmitate for 24 h ($n=3$). (**k**) JunB protein expression and cleaved caspase 3 in human islet cells infected and treated as in (**j**) ($n=3$). $^*P<0.05$ against the respective controls (untreated cells), $^{\#}P<0.05$ as indicated

and f and Supplementary Figure S3). On the other hand, the ATF6-dependent chaperones BiP and GRP94 were not modified by JunB KD or overexpression (Supplementary Figures S4A–F), suggesting that this branch of the UPR is not a major target of JunB. To test whether the inhibition of ER stress markers by JunB silencing is caused by alterations

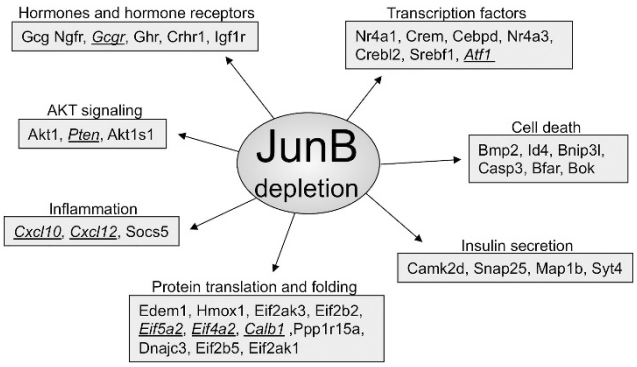

Figure 2 Overview of mRNAs modified by JunB knockdown. The figure summarizes manual curation of JunB-modified mRNAs in INS-1E cells transfected with JunB siRNA and treated with palmitate for 6 or 14 h. Upregulated genes are shown in underlined italic font, and downregulated genes are shown in normal font

in the protein load, we measured the total protein biosynthesis. Negative siRNA and JunB siRNA-transfected cells had similar total protein biosynthesis (Supplementary Figure S4G), arguing for a specific role of JunB in the modulation of the ER stress response.

As XBP1s has a very short half-life,[13] changes in protein stability may influence its steady-state expression levels. We tested whether JunB modulates XBP1 stability by overexpressing XBP1s in JunB-depleted cells treated with the protein synthesis inhibitor cycloheximide. The decay of XBP1s was not altered by JunB silencing (Figure 4a), suggesting that JunB upregulates XBP1s through transcriptional activation. As IRE1 signaling can be pro- or anti-apoptotic in a context-dependent manner,[26,27] we investigated whether this pathway is involved in JunB-mediated β-cell protection. For this purpose, we upregulated JunB expression using an adenoviral vector and silenced XBP1 (Figure 4b and Supplementary Figures S5A and B). The protection from lipotoxicity conferred by JunB overexpression was partially lost following XBP1 silencing (Figures 4b–d and Supplementary Figures S5C and D). Conversely, the sensitization of JunB-deficient cells to palmitate was abolished by adenoviral XBP1 overexpression (Figures 4e–g and Supplementary Figures S5E and F). Together, these observations show that JunB promotes XBP1 expression and that this is a prerequisite for JunB-mediated β-cell protection from lipotoxicity.

As PERK signaling was also decreased in JunB-deficient cells, we took a similar approach to test the involvement of PERK in JunB protection. PERK KD (Supplementary Figure S6A) did not alter β-cell survival in JunB-overexpressing cells (Supplementary Figures S6B and C). To confirm these results, we used GSK2656157, a PERK inhibitor[28] that decreased PERK phosphorylation by 88 ± 1% (Supplementary Figure S6D). In agreement with the siRNA results, PERK inhibition did not change JunB-induced β-cell survival after palmitate treatment (Supplementary Figures S6E and F), showing that the PERK pathway is not a key mediator of JunB protection.

JunB-driven XBP1 expression is c/EBPδ dependent. As the maintenance of XBP1 expression by JunB is crucial for β-cell survival under lipotoxic conditions, we next investigated the molecular mechanisms involved. *In silico* analysis of the XBP1 promoter revealed the presence of a highly conserved c/EBP binding element at -504 bp relative to the transcription start site.[29] From the c/EBP family of transcription factors, only the δ-isoform was induced by palmitate in a JunB-dependent manner (Figure 2 and Supplementary Table S1). We confirmed these results, showing that palmitate induced c/EBPδ mRNA and protein with a peak at 8 h (Figures 5a and b). JunB KD abolished the c/EBPδ upregulation (Figures 5c and d). When c/EBPδ was silenced (Figure 5e), palmitate no longer upregulated total XBP1 mRNA (Figure 5f) or XBP1 protein expression (Figure 5g) and cell death was enhanced (Figure 5h). Conversely, JunB overexpression induced c/EBPδ in parallel with XBP1s (Figure 5i). These results point to c/EBPδ as an important mediator of XBP1 induction by JunB and an integral part of the β-cell-protective response in lipotoxicity.

JunB modulates AKT signaling. In our array analysis, *PTEN*, an inhibitor of AKT, was upregulated in palmitate-treated JunB-deficient cells (Figure 2 and Supplementary Table S1). We therefore examined whether AKT signaling is inhibited following JunB silencing. PTEN phosphorylation or expression was not altered by palmitate (Supplementary Figure S7A). Next, we confirmed that PTEN expression was induced by JunB KD in control and palmitate-treated cells (Figure 6a and Supplementary Figure S7B). PTEN phosphorylation was also increased in JunB KD (Figure 6a and Supplementary Figure S7C) cells but the ratio of p-PTEN/PTEN did not change (Supplementary Figure S7D), suggesting that JunB KD mainly affects total PTEN expression. Importantly, AKT phosphorylation was markedly reduced in JunB-deficient cells (Figure 6a). Under normal conditions, AKT phosphorylates and thereby inhibits BAD, a proapoptotic BH3-only member of the Bcl-2 family. In palmitate-treated, JunB-deficient cells, BAD phosphorylation was decreased (Figure 6a). The proapoptotic Bcl-2 family members DP5 and PUMA, previously shown to play a role in palmitate-induced apoptosis,[11] were not induced by JunB KD (PUMA was actually inhibited), and neither was the expression of the antiapoptotic Bcl-2 and Bcl-XL proteins modified (Supplementary Figures S7E–G). To formally test the role of BAD in JunB-deficient lipotoxic β-cell death, we double knocked down JunB and BAD (Figure 6b). BAD silencing fully protected against JunB KD-dependent apoptosis in palmitate-treated cells (Figure 6c), identifying BAD as a key downstream effector.

As JunB KD decreased AKT phosphorylation, we tested whether JunB induction would potentiate AKT activation. Adenoviral JunB overexpression induced AKT phosphorylation in a dose-dependent manner, supporting the important role for JunB in AKT regulation (Figure 6d). A crosstalk between XBP1 and AKT signaling has been previously demonstrated in other cell types.[30,31] XBP1 KD decreased AKT phosphorylation independently of changes in total AKT or PTEN expression (Figure 6e and Supplementary Figures S7H and I), suggesting that JunB activation of AKT may be via XBP1. XBP1 overexpression did not induce AKT phosphorylation under basal condition (Figure 6f). However, the early

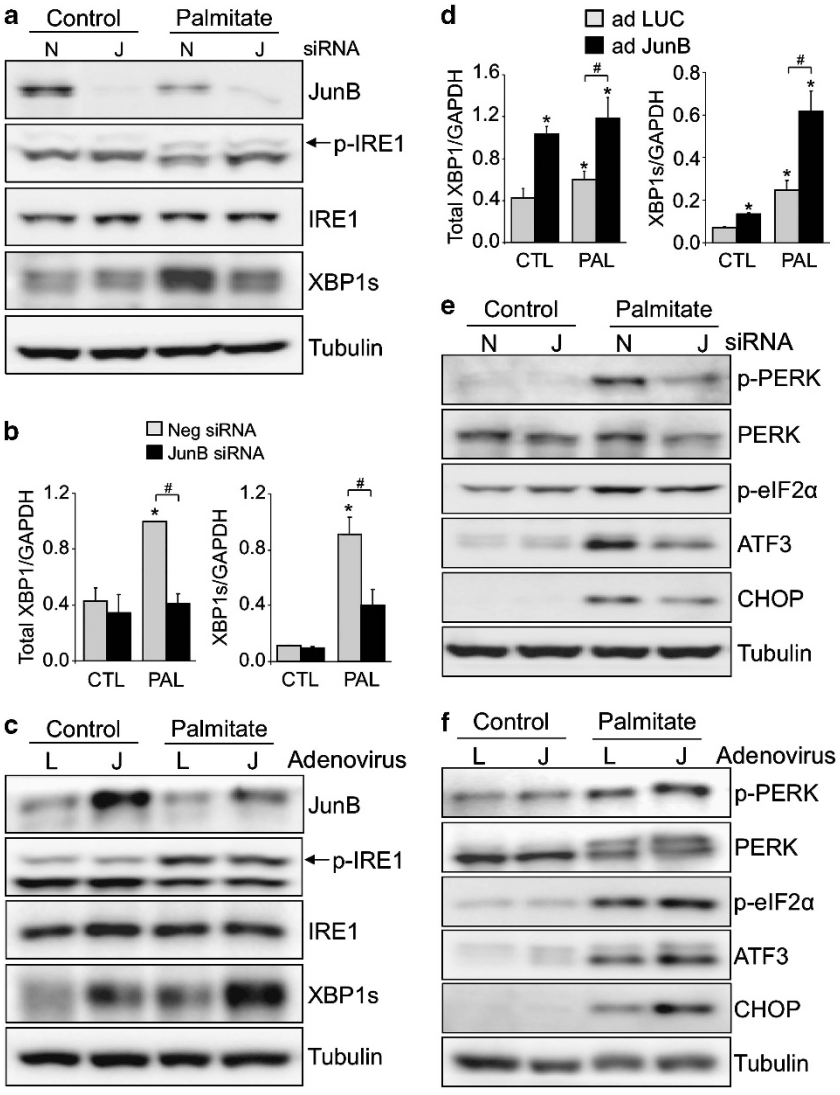

Figure 3 JunB modulates the IRE1 and PERK branches of the ER stress response. (**a**) IRE1 expression and phosphorylation and XBP1 and JunB protein expression in INS-1E cells transfected with negative (N) or JunB siRNA (J) and then treated with palmitate for 16 h ($n=3$). (**b**) Total and spliced XBP1 mRNA expression in INS-1E cells transfected and treated as in (**a**) ($n=3$). (**c**) IRE1 expression and phosphorylation and XBP1 and JunB protein expression in INS-1E cells infected with luciferase (L) or JunB adenovirus (J) and then treated with palmitate for 16 h ($n=3$). (**d**) Total and spliced XBP1 mRNA expression in INS-1E cells infected and treated as in (**c**). (**e**) PERK, eIF2α, ATF3 and CHOP protein expression or phosphorylation in INS-1E cells transfected and treated as in (**a**) ($n=3$). (**f**) PERK, eIF2α, ATF3 and CHOP protein phosphorylation or expression in INS-1E cells transfected and treated as in (**c**) ($n=3$). $^{*}P<0.05$ against untreated cells transfected with negative siRNA or infected with luciferase adenovirus, $^{\#}P<0.05$ as indicated

inhibition of AKT by palmitate (Figure 6f and Supplementary Figure S7A) was prevented in cells overexpressing XBP1s (Figure 6f), demonstrating the important role of XBP1s for AKT phosphorylation. As XBP1 KD *per se* did not alter PTEN protein expression, we examined alternative mechanisms. To test whether PTEN is responsible for the AKT inhibition in JunB-deficient cells we used the PTEN inhibitor Vo-OHPic. Interestingly, AKT inhibition was not reversed by the PTEN inhibitor (Figure 6g). Similar results were obtained using two PTEN siRNAs (data not shown). Thus, the modulation of AKT activation by JunB is dependent on XBP1 but not PTEN. We next evaluated whether insulin could be the link between JunB-XBP1s and the activation of AKT. For this purpose, we inhibited insulin release using diazoxide[32] or we treated cells with a 10-fold excess concentration of insulin as compared with the cumulative insulin released to the medium after 16 h of culture. Diazoxide did not alter AKT phosphorylation in cells transfected with negative, JunB or XBP1 siRNAs (Supplementary Figure S8). In insulin-treated cells, AKT phosphorylation remained inhibited by JunB or XBP1 KD (Supplementary Figures S8A and D). These results are in agreement with the lack of modulation of insulin secretion by JunB (Supplementary Figure S2B) and suggest that JunB and XBP1 do not regulate AKT via modulation of insulin release.

AKT induction by JunB is essential for GLP-1 protection from lipotoxicity. We have previously shown that GLP-1 agonists, widely used to treat T2D, protect β-cells from palmitate via the induction of JunB.[22] We hypothesized that the JunB/XBP1/AKT axis would also operate in this context.

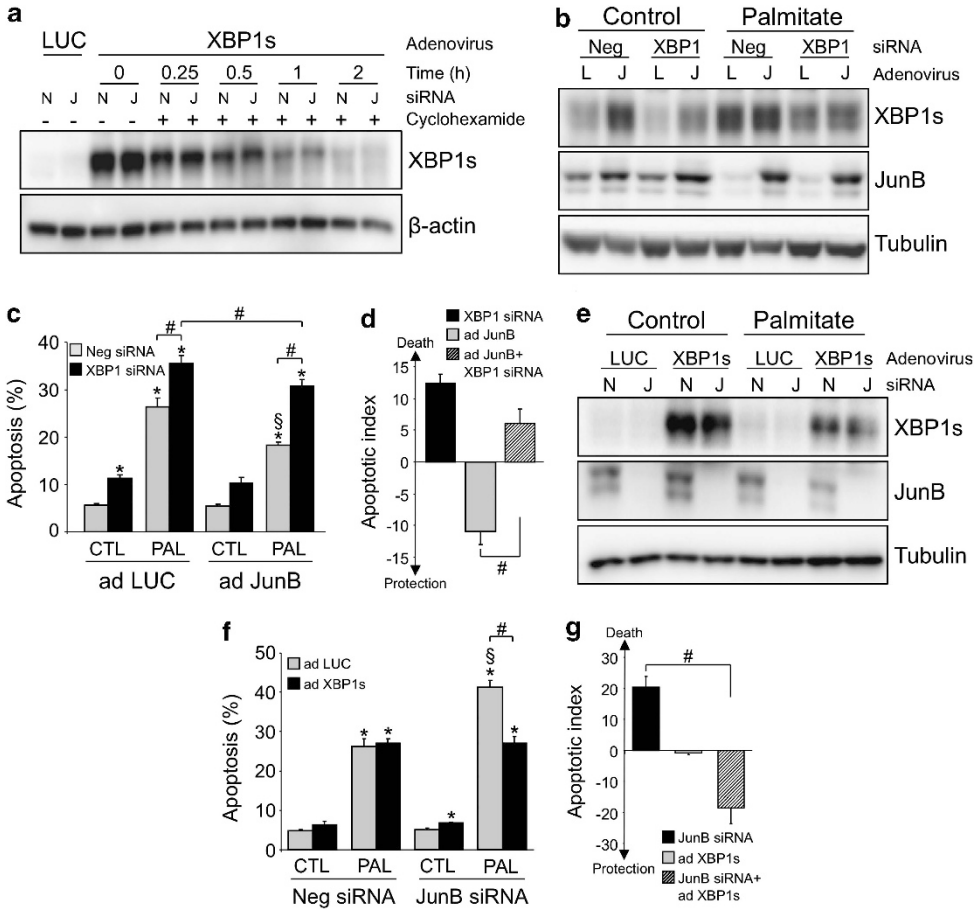

Figure 4 JunB-mediated β-cell protection is XBP1 dependent. (**a**) XBP1 protein expression in INS-1E cells transfected with negative (N) or JunB (J) siRNA, infected with luciferase (LUC)- or XBP1-expressing adenovirus (MOI 1) and then treated with 5 μg/ml cycloheximide for the indicated time points ($n=3$). (**b**) XBP1 and JunB protein expression in INS-1E cells transfected with negative (Neg) or XBP1 siRNA, infected with luciferase (L)- or JunB (J)-expressing adenovirus (ad) (MOI 10) and then treated with palmitate for 16 h ($n=3$). (**c**) Apoptosis in INS-1E cells transfected, infected and treated as in (**b**) ($n=3$). (**d**) Palmitate-induced apoptosis data from (**c**) is presented as apoptotic index. A positive index indicates sensitization to palmitate-induced apoptosis, and a negative index indicates protection against apoptosis by the KD and/or overexpression of the indicated proteins. (**e**) XBP1 and JunB protein expression in INS-1E cells transfected with negative (N) or JunB (J) siRNA, infected with luciferase (LUC)- or XBP1-expressing adenovirus (MOI 1) and then treated with palmitate for 16 h ($n=3$). (**f**) Apoptosis in INS-1E cells transfected, infected and treated as in (**e**) ($n=3$). (**g**) Palmitate-induced apoptosis data from (**f**) is presented as apoptotic index. $^{*}P<0.05$ against untreated cells transfected with negative siRNA alone and/or infected with Luc adenovirus. $^{\S}P<0.05$ against cells transfected with negative siRNA, infected with Luc adenovirus and treated with palmitate, $^{\#}P<0.05$ as indicated

To examine the role of AKT in GLP-1-mediated β-cell survival, we inhibited AKT using the PI3K inhibitor wortmannin (Figure 7a). The GLP-1 agonist exendin-4 or the adenylate cyclase stimulator forskolin, a more potent intracellular cAMP inducer, failed to protect cells from palmitate when used in combination with wortmannin (Figure 7b), showing that AKT is crucial for β-cell protection under lipotoxic conditions. In keeping with our previous results,[22] forskolin stimulated JunB expression and AKT phosphorylation (Figures 7a and c). The latter was partially prevented by JunB KD (Figure 7c).

To investigate the role of XBP1 in the induction of AKT by GLP-1/cAMP, we used the potent IRE1 inhibitor 4μ8C that severely inhibits expression of XBP1s (Figure 7d and Supplementary Figure S9). This XBP1 depletion decreased AKT phosphorylation (Figure 7d). Importantly, the protection against palmitate conferred by forskolin was lost in the absence of XBP1 (Figure 7e). We confirmed that forskolin-induced AKT phosphorylation was markedly decreased and its β-cell-protective effect reduced following XBP1 KD by siRNA (Figures 7f and g). These results demonstrate that AKT activation by GLP-1 agonists is JunB/XBP1 dependent and essential for β-cell survival under lipotoxic conditions.

Discussion

Loss of functional β-cell mass contributes to the development of T2D. Most of the current treatments for T2D focus on lifestyle changes and the enhancement of insulin secretion. At later stages of the disease, these approaches tend to fail and patients need to be placed on insulin therapy. In this context, approaches to preserve or rescue β-cells are important to prevent or treat T2D. Here we show that the transcription factor JunB orchestrates an intricate crosstalk between the UPR and AKT signaling that protects β-cells from lipotoxicity (Figure 8).

The early induction of JunB by palmitate constitutes an important antiapoptotic mechanism operating in rat and

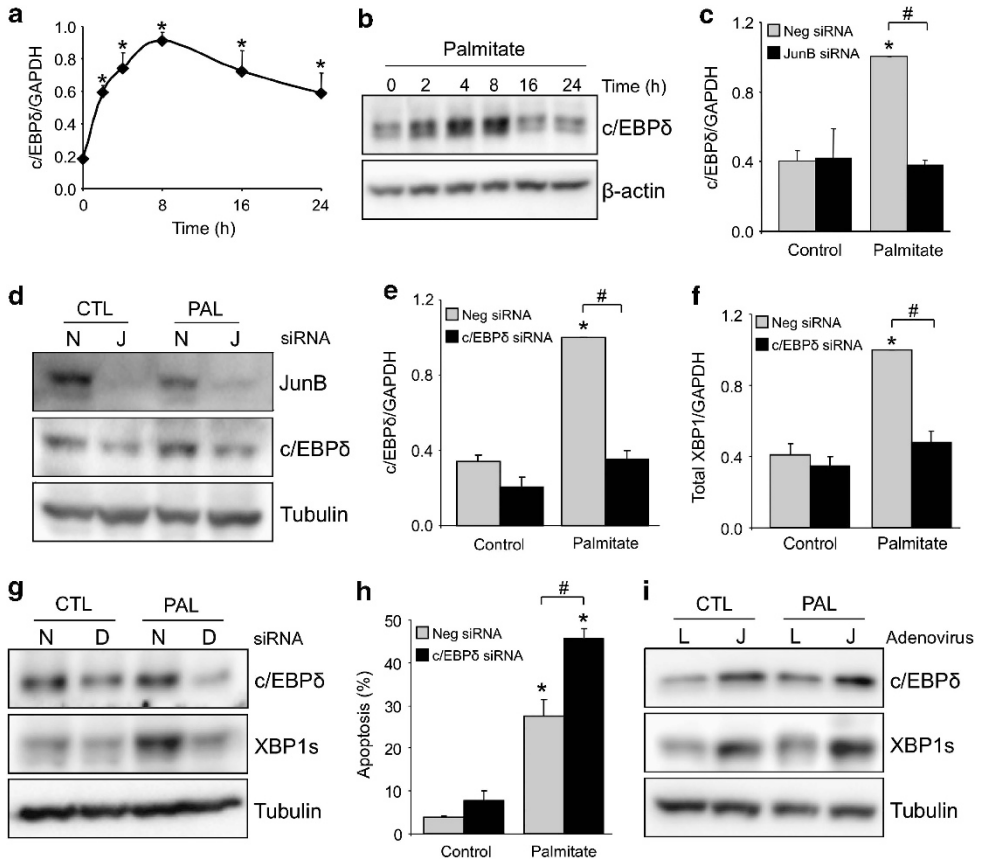

Figure 5 c/EBPδ mediates XBP1 induction by JunB. Time course of c/EBPδ mRNA (**a**) and protein (**b**) expression in INS-1E cells exposed to palmitate ($n=4$). c/EBPδ mRNA (**c**) and protein (**d**) expression in INS-1E cells transfected with negative (N) or JunB (J) siRNA and then treated with palmitate for 16 h ($n=3$). (**e**) c/EBPδ mRNA expression in INS-1E cells transfected with negative or c/EBPδ siRNA and then treated with palmitate for 16 h ($n=3$). (**f**) Total XBP1 mRNA expression in INS-1E cells transfected and treated as in (**e**) ($n=3$). (**g**) XBP1 and c/EBPδ protein expression in INS-1E cells transfected with negative (N) or c/EBPδ (D) siRNA and treated with palmitate for 16 h ($n=3$). (**h**) Apoptosis in INS-1E cells transfected and treated as in (**e**) ($n=3$). (**i**) XBP1 and c/EBPδ protein expression in INS-1E cells infected with luciferase (L) or JunB (J) adenovirus and then treated with palmitate for 16 h ($n=3$). $^{*}P<0.05$ against untreated cells, untreated cells transfected with negative siRNA or infected with luciferase adenovirus, $^{\#}P<0.05$ as indicated

human β-cells. Prolonged exposure to palmitate, however, induces JNK activation and consequent loss of JunB expression, culminating in β-cell death. This is supported by the finding that forced expression of JunB protects β-cells from lipotoxicity. In agreement with the antiapoptotic role of JunB, previous studies have reported improved β-cell viability in JunB-overexpressing cells facing different insults, including proinflammatory cytokines and oxidative stress.[20,33]

Our global analysis of gene expression in JunB-deficient cells pointed to the ER stress response and AKT signaling as possible mechanisms for the JunB-mediated β-cell protection. Indeed, JunB deficiency decreases XBP1 expression, a transcription factor induced by the IRE1-mediated unconventional alternative splicing of its mRNA during ER stress. XBP1s induces ER chaperones and components of the ER-associated protein degradation machinery, improving ER folding capacity and degrading malfolded proteins, essential adaptation steps for cell survival under stress conditions (see Lee *et al*.[34] and present data). Unspliced XBP1 is constitutively expressed and further induced during ER stress by XBP1s and ATF6.[35,36] Our results show a novel pathway in which XBP1 expression is regulated by JunB via the transcription factor c/EBPδ. We cannot exclude that JunB regulates XBP1 directly, but *in silico* analysis of the rat XBP1 promoter did not identify AP-1-binding sites. In addition, c/EBPδ KD reproduces the phenotype of JunB-deficient cells, arguing for the indirect scenario. In adipocytes, XBP1 was shown to be modulated by c/EBPβ to induce adipogenesis.[37]

Induction of XBP1 is crucial for the JunB-mediated defense mechanism against palmitate as XBP1 KD in JunB-overexpressing cells abrogates the JunB protection. In an earlier study, we did not find evidence for a role of XBP1 in GLP-1 protection from lipotoxicity.[22] This may have been because of the inefficient KD ($51\pm14\%$) in the previous study compared with the new data presented here ($74\pm4\%$). We show here that JunB-mediated XBP1 expression protects cells from lipotoxicity through the induction of AKT. In JunB- or XBP1-deficient cells AKT phosphorylation is downregulated whereas JunB overexpression stimulates its phosphorylation. To our knowledge, this is the first time that an AP-1 family member is shown to interact with the ER stress response to promote antiapoptotic signaling. AKT activation is a critical prosurvival signal in β-cells by inhibiting FOXO transcription factors, BAD and GSK3β via direct phosphorylation.

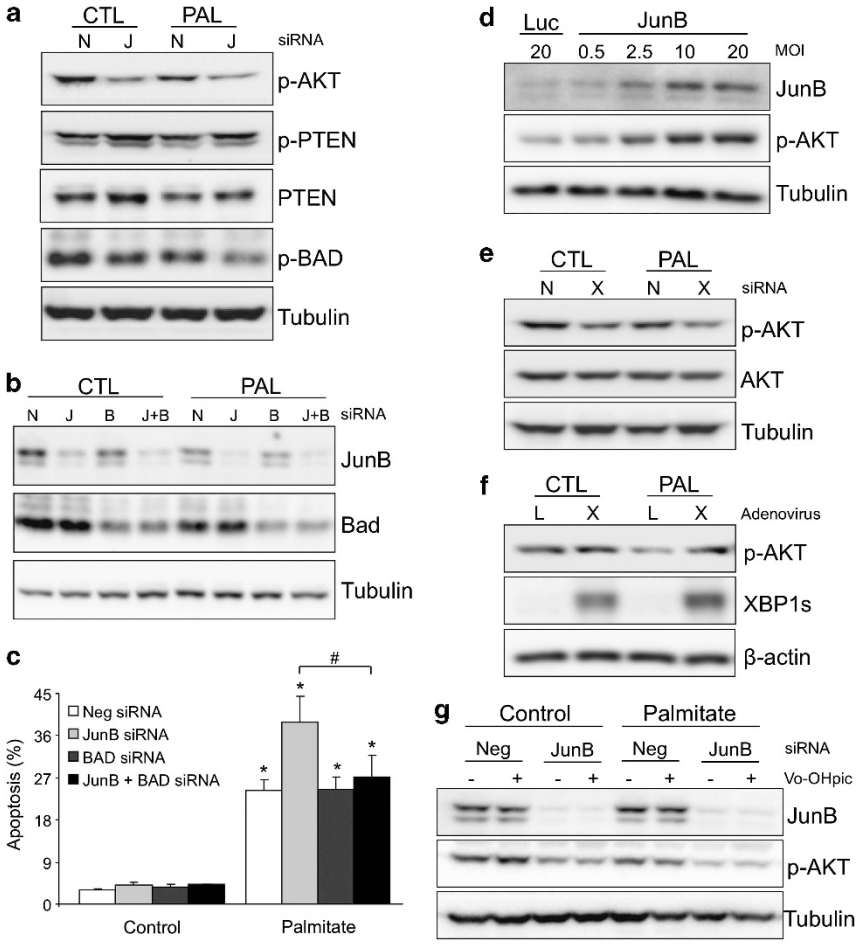

Figure 6 JunB promotes AKT signaling and BAD phosphorylation. (**a**) AKT, PTEN and BAD protein phosphorylation or expression in INS-1E cells transfected with negative (N) or JunB siRNA (J) and then treated with palmitate for 16 h ($n=3$–5). (**b**) JunB and BAD protein expression in INS-1E cells transfected with negative (N), JunB (J) and/or BAD (B) siRNA and then treated with palmitate for 16 h ($n=3$). (**c**) Apoptosis in INS-1E cells transfected and treated as in (**b**) ($n=3$). (**d**) AKT phosphorylation and JunB protein expression in INS-1E cells infected with luciferase (Luc, MOI 20)- or JunB-expressing adenovirus at different MOIs ($n=3$). (**e**) AKT protein expression or phosphorylation in INS-1E cells transfected with negative (N) or XBP1 siRNA (X) and then treated with palmitate for 16 h ($n=3$–4). (**f**) AKT phosphorylation and XBP1 protein expression in INS-1E cells infected with luciferase (L) or XBP1 (X) adenovirus and then treated with palmitate for 2 h ($n=3$). (**g**) AKT phosphorylation and JunB protein expression in INS-1E cells transfected with negative (Neg) or JunB siRNA and then treated with the PTEN inhibitor Vo-OHpic and/or palmitate for 4 h ($n=3$). $^{*}P<0.05$ against untreated cells transfected with negative siRNA, $^{\#}P<0.05$ as indicated

AKT signaling has been shown to protect β-cells from FFA- or ER stress-induced apoptosis[38,39] and to mediate part of the antiapoptotic effects of GLP-1 agonists.[22] This important prosurvival role of AKT is demonstrated here by the increased apoptosis observed in palmitate-treated cells when JunB or XBP1 were knocked down or AKT was inhibited by wortmannin. The reduction of AKT phosphorylation observed under these conditions leads to BAD dephosphorylation and consequent activation of apoptosis. This conclusion is supported by the observation that BAD KD reverts the increased apoptosis observed in JunB-deficient cells treated with palmitate.

PTEN, an inhibitor of PI3K, was upregulated in the absence of JunB in β-cells in the array analysis and western blot. Deletion of PTEN in muscle improves insulin sensitivity in T2D models.[40] In addition, specific PTEN KO in β-cells increased β-cell mass and protected from diabetes induced by high-fat diet.[41] These effects were attributed to increased AKT activity. The decreased AKT phosphorylation in JunB KD β-cells, however, is not mediated by PTEN as its KD or chemical inhibition does not alter AKT phosphorylation. In support of this conclusion, XBP1-deficient cells have reduced AKT phosphorylation without changes in PTEN expression compared with JunB KD cells. XBP1 has been shown to modulate AKT activity in other cell types,[31,42] and XBP1 may directly bind PI3K, facilitating AKT phosphorylation in endothelial cells.[43] JunB may also modulate AKT phosphorylation through a XBP1-dependent improvement of the ER folding capacity and consequent restoration of mTOR or via inhibition of protein phosphatase 2A, two important pathways previously shown to regulate AKT.[44–46]

Incretin analogs such as exendin-4 are used for the treatment of T2D. Exendin-4 binds to the GLP-1 receptor, increasing cAMP production and thereby stimulating the activation of several signaling pathways such as PKA, mTOR and AKT. Activation of these pathways potentiates GSIS, increases β-cell mass in rodents and protects from oxidative stress and proinflammatory cytokines.[47,48] The role of AKT in

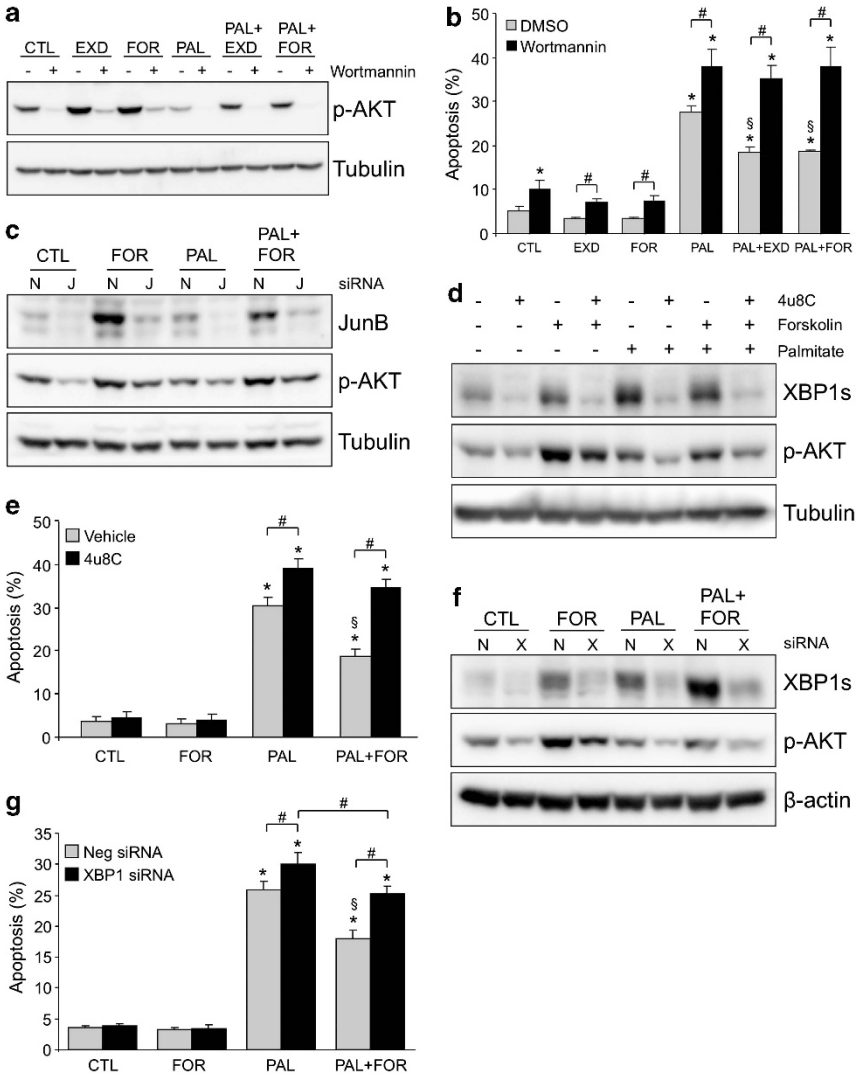

Figure 7 GLP-1 protection against lipotoxicity is AKT dependent. (**a**) AKT phosphorylation in INS-1E cells pretreated or not for 2 h with 100 nM wortmannin and then treated with 0.5 mM palmitate (PAL) and/or 50 nM exendin-4 (EXD) or 20 μM forskolin (FOR) for 16 h. (**b**) Apoptosis in INS-1E cells treated as in (**a**). (**c**) AKT phosphorylation and JunB protein expression in INS-1E cells transfected with negative (N) or JunB (J) siRNA and then treated with 0.5 mM palmitate (PAL) and/or 20 μM forskolin (FOR) for 16 h. (**d**) AKT phosphorylation and XBP1 protein expression in INS-1E cells treated with the IRE1 inhibitor 4μ8C (25 μM), forskolin or palmitate for 16 h ($n=3$). (**e**) Apoptosis in INS-1E cells treated as in (**d**) ($n=3$). (**f**) AKT phosphorylation and XBP1 protein expression in INS-1E cells transfected with negative (N) or XBP1 siRNA (X) and then treated with palmitate and/or forskolin for 16 h ($n=3$). (**g**) Apoptosis in INS-1E cells transfected and treated as in (**f**) ($n=3$). $^{*}P<0.05$ against untreated cells or untreated cells transfected with negative siRNA, $^{\S}P<0.05$ against palmitate-treated cells or palmitate-treated cells transfected with negative siRNA, $^{\#}P<0.05$ as indicated

GLP-1-mediated β-cell survival under lipotoxic conditions has previously been demonstrated,[49] but the mechanisms by which GLP-1-induced cAMP activates AKT are not well established. The present study provides evidence that induction of the JunB–XBP1 axis contributes to increase AKT phosphorylation by exendin-4 and forskolin and protect from lipotoxicity. These novel insights connect the AP-1 and the ER stress response in a complex chain of events that stimulates AKT activation.

In conclusion, we show that JunB is a crucial prosurvival transcription factor for β-cells. JunB promotes XBP1 signaling and AKT activation, thereby preventing β-cell apoptosis. In addition, the JunB–XBP1 pathway contributes to activate AKT signaling by GLP-1 agonists. The crosstalk between these pathways constitutes a crucial defense mechanism against lipotoxic β-cell death and may represent an important mechanism for the prevention/treatment of T2D.

Materials and Methods

Culture of INS-1E, FACS-purified primary rat β-cells and human islet cells. Male Wistar rats (Charles River Laboratories, Brussels, Belgium) were used according to the guidelines of the Belgian Regulations for Animal Care. All experiments were approved by the local Ethical Committee. Islets were separated by collagenase digestion, handpicked and dispersed in single cells. β-Cells were purified by autofluorescence-activated cell sorting (FACSAria, BD Bioscience, San Jose, CA, USA),[50] yielding a purity of $93\pm1\%$ ($n=4$). β-Cells were cultured for 2 days in Ham's F-10 medium containing 10 mM glucose and 5% heat-inactivated fetal bovine serum (FBS) before further experiments. The rat insulin-producing INS-1E cell line (a kind gift from Professor C Wollheim, Centre Medical Universitaire, Geneva, Switzerland) was cultured in RPMI-1640 (with 2 mM GlutaMAX-I) containing 5% FBS[51] and used at passages 59–73.

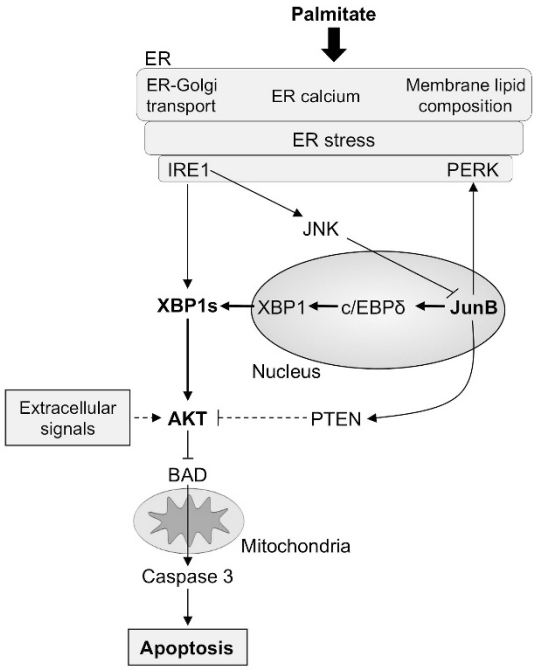

Figure 8 Summary of the crosstalk between JunB and lipotoxic β-cell ER stress and apoptosis. The JunB–AKT pathway that promotes β-cell survival after palmitate treatment is shown in bold. The JunB-independent control of AKT phosphorylation by PTEN and extracellular signals is shown as dashed lines. The protective effect of JunB-c/EBPδ–XBP1–AKT is eventually negated by palmitate-induced JNK activation and consequent JunB degradation. ER, endoplasmic reticulum

Human islets (from 9 donors, age 62 ± 5 years, BMI 25 ± 3 kg/m^2, 6 male and 3 female, cause of death was cerebral hemorrhage (4), cardiovascular disease (3), trauma (1) or unknown (1)) were isolated by collagenase digestion and density gradient purification.[52] The islets were cultured, dispersed and transfected as described previously.[53] The percentage of β-cells, examined by insulin immunofluorescence,[17] was 53 ± 7%.

Treatments. For FFA exposure, primary β-cells were cultured in medium with 1% BSA without FBS and INS-1E cells in medium with 1% FBS and 1% BSA.[54] Palmitate (Sigma, Schnelldorf, Germany) was dissolved in 90% ethanol,[17] and used at a final concentration of 0.5 mM in the presence of 1% BSA, resulting in unbound FFA concentrations in the nM range.[54] The JNK inhibitor SP600125 (Sigma) was used at 10 μM for 2 h before and during FFA exposure.[17] PTEN inhibitor Vo-OHpic (Sigma) was used at 0.5 μM for 2 h before and during FFA exposure. The IRE1 inhibitor 4μ8C[55] was used at 25 μM during FFA exposure. Exendin-4 was diluted in RPMI-1640 and used at 50 nM. Insulin was used at 26 μg/ml (Actrapid, Novo Nordisk, Bagsvaerd, Denmark). Forskolin, cycloheximide, diazoxide and the PI3K inhibitor wortmannin (Sigma) were diluted in DMSO and used at concentrations of 20 μM, 5 μg/ml, 100 μM and 100 nM, respectively. At the concentration used, diazoxide inhibits insulin secretion by $>$80%.[32]

RNAi. Proteins were knocked down using previously validated[11,20,22,56] specific siRNAs against JunB, XBP1, c/EBPδ and BAD (all from Invitrogen, Carlsbad, CA, USA) and PERK (SMARTpool, Dharmacon, Chicago, IL, USA), or a negative control of 21 nucleotide duplex RNA (Qiagen, Hilden, Germany) that does not affect β-cell function, gene expression or viability.[57] Lipid-RNA complexes were formed in Opti-MEM (1 μl Lipofectamine RNAiMAX, Invitrogen, to 150 nM siRNA) and added at a final concentration of 30 nM siRNA.[17] Transfected cells were cultured for 2 to 3 days before treatment.

Microarray analysis. INS-1E cells were transfected with JunB or a negative control siRNA. Two days later, cells were treated or not for 6 or 14 h with 0.5 mM palmitate. RNA was isolated using the RNeasy Mini Kit (Qiagen) and prepared for hybridization as previously described.[24] The array was performed in three independent experiments using the GeneChip Rat Genome 230 2.0 arrays (Affymetrix, Santa Clara, CA, USA) containing $>$31 000 probe sets covering 28 000 rat genes as previously described.[11] Data analysis was performed using the DAVID and IPA 5.5 software. The complete array data will be deposited after publication of this article in the Eurodia database (http://eurodia.vital-it.ch).

Real-time PCR. Poly(A)$^+$-RNA was isolated and reverse transcribed as described previously.[58] The real-time PCR amplification was done using IQ SYBR Green Supermix (BIO-RAD, Hercules, CA, USA) on a MyiQ2 instrument and the PCR product concentration calculated as copies/μl using a standard curve.[59] Gene expression was corrected for the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and normalized to the highest expression value within an experiment. β-Actin was used as the housekeeping gene for human islets.[17] The primer sequences were: for rat total XBP1 forward 5′-GAGCAGCAAGTGGTGGATTT-3′ and reverse 5′-TCTCAATCA CAAGCCCATGA-3′, for rat GRP94 forward 5′-AAGGTCATTGTCACGTCGAAA-3′ and reverse 5′-GTGTTTCCTCTTGGGTCAGC-3′. The other primer sequences have been reported elsewhere.[21,22,60]

Western blot. Western blots were performed using 20 μg whole-cell extract protein as described.[60] IRE1 phosphorylation was detected using Phos-tag-SDS gels. Briefly, cell lysates were run on SDS-PAGE with 10 μM Phos-tag reagent (Wako, Richmond, VA, USA) and 10 μM $MnCl_2$. Gels were then washed for 30 min in transfer buffer containing 1 mM EDTA and proteins transferred to a nitrocellulose membrane. The primary antibodies were anti-p-eIF2α (Ser51), β-actin, Bcl-2, Bcl-XL, p-c-Jun (Ser73), p-JNK (Thr183, Tyr185), PERK, p-PERK (Thr980), IRE1, p-AKT (Ser473), AKT, p-PTEN (Ser380, Thr382/383), PTEN, BiP, p-BAD (Ser112), BAD and cleaved caspase 3 from Cell Signaling (Beverly, MA, USA), JunB, c/EBPδ, XBP1, ATF3 and CHOP from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and anti-α-tubulin (1 : 10 000) from Sigma. For XBP1, the bands correspond to the 54 kDa spliced form of XBP1. Horseradish peroxidase-labeled donkey anti-rabbit or donkey anti-mouse (1 : 10 000, Jackson Immuno Research, Baltimore Pike, PA, USA) antibodies were used as secondary antibodies. Protein signal was visualized using chemiluminescence Supersignal (Pierce, Rockford, IL, USA) and quantified using Scion Image (Scion Corporation, Frederick, MD, USA).

Protein biosynthesis. Protein synthesis was measured as previously reported.[60] Briefly, INS-1E cells were cultured for 2 h in Krebs-Ringer buffer containing 1% BSA, 11 mM glucose and 10 μCi/ml L-(4,5-3H)-leucine (NET135H; PerkinElmer Life Sciences, Waltham, MA, USA) with or without palmitate. The content of 3H-labeled protein was determined by measuring the radioactivity using a liquid scintillation analyzer (Packard, Meriden, CT, USA). Protein biosynthesis was expressed per total protein content.

Infection with recombinant adenoviruses. Cells were infected either with AdLUC (a luciferase expressing control virus), AdJunB (expressing the rat JunB protein, purchased from RIKEN BioResource Center, Tsukuba, Japan) or AdXBP1 (expressing mouse XBP1s[27]) and used at the indicated multiplicity of infection. After 3 h of infection, the medium was changed.

Assessment of β-cell apoptosis. Apoptotic β-cells and INS-1E cells were counted in fluorescence microscopy after staining with the DNA-binding dyes propidium iodide (5 μg/ml) and Hoechst 33342 (10 μg/ml).[60] Apoptosis was confirmed by western blot analysis of cleaved caspase 3.

Glucose-stimulated insulin secretion. INS-1E cells were cultured for 1 h with RPMI-1640 without glucose supplemented with 5% FBS. Cells were then incubated for 30 min in modified Krebs-Ringer bicarbonate HEPES solution[11] without glucose. Insulin secretion was induced by 30 min of incubation with KRBH containing 1.7 or 16.7 mM glucose. Insulin or proinsulin was measured by ELISA in cell-free supernatants and acid-ethanol extracted cell lysates (Mercodia, Uppsala, Sweden).

Statistical analysis. Data are presented as means ± S.E. of the indicated number (n) of independent experiments. Comparisons were performed by ANOVA followed by paired t-test with the Bonferroni correction for multiple comparisons. A P-value of <0.05 was considered statistically significant.

Conflict of Interest

The authors declare no conflict of interest.

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Author contributions

DAC, ENG, DLE and MC contributed to the experimental design of the study. DAC, ENG, FO and NN carried out experiments and/or helped with data analysis. AKC, MB and PM contributed materials and data interpretation. DAC, DLE and MC wrote the manuscript.

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. *Nat Rev Endocrinol* 2012; **8**: 228–236.
2. Cnop M, Vidal J, Hull RL, Utzschneider KM, Carr DB, Schraw T *et al.* Progressive loss of β-cell function leads to worsening glucose tolerance in first-degree relatives of subjects with type 2 diabetes. *Diabetes Care* 2007; **30**: 677–682.
3. Xiang AH, Wang C, Peters RK, Trigo E, Kjos SL, Buchanan TA. Coordinate changes in plasma glucose and pancreatic β-cell function in Latino women at high risk for type 2 diabetes. *Diabetes* 2006; **55**: 1074–1079.
4. Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic β-cell mass in European subjects with type 2 diabetes. *Diabetes Obes Metab* 2008; **10**: 32–42.
5. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; **444**: 840–846.
6. Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W *et al.* A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes* 2003; **52**: 2461–2474.
7. Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr* 2003; **78**: 91–98.
8. Kharroubi I, Ladriere L, Cardozo AK, Dogusan Z, Cnop M, Eizirik DL. Free fatty acids and cytokines induce pancreatic β-cell apoptosis by different mechanisms: role of nuclear factor-κB and endoplasmic reticulum stress. *Endocrinology* 2004; **145**: 5087–5096.
9. Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Busch AK, Biankin AV *et al.* Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* 2007; **50**: 752–763.
10. Marchetti P, Bugliani M, Lupi R, Marselli L, Masini M, Boggi U *et al.* The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. *Diabetologia* 2007; **50**: 2486–2494.
11. Cunha DA, Igoillo-Esteve M, Gurzov EN, Germano CM, Naamane N, Marhfour I *et al.* Death protein 5 and p53-upregulated modulator of apoptosis mediate the endoplasmic reticulum stress-mitochondrial dialog triggering lipotoxic rodent and human β-cell apoptosis. *Diabetes* 2012; **61**: 2763–2775.
12. Cnop M, Abdulkarim B, Bottu G, Cunha DA, Igoillo-Esteve M, Masini M *et al.* RNA-sequencing identifies dysregulation of the human pancreatic islet transcriptome by the saturated fatty acid palmitate. *Diabetes* 2013; e-pub ahead of print 30 December 2013; doi:10.2337/db13-1383.
13. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP *et al.* IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 2002; **415**: 92–96.
14. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP *et al.* Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 2000; **287**: 664–666.
15. Scheuner D, Kaufman RJ. The unfolded protein response: a pathway that links insulin demand with β-cell failure and diabetes. *Endocr Rev* 2008; **29**: 317–333.
16. Karaskov E, Scott C, Zhang L, Teodoro T, Ravazzola M, Volchuk A. Chronic palmitate but not oleate exposure induces endoplasmic reticulum stress, which may contribute to INS-1 pancreatic β-cell apoptosis. *Endocrinology* 2006; **147**: 3398–3407.
17. Cunha DA, Hekerman P, Ladriere L, Bazarra-Castro A, Ortis F, Wakeham MC *et al.* Initiation and execution of lipotoxic ER stress in pancreatic β-cells. *J Cell Sci* 2008; **121**: 2308–2318.
18. Matveyenko AV, Gurlo T, Daval M, Butler AE, Butler PC. Successful versus failed adaptation to high-fat diet-induced insulin resistance: the role of IAPP-induced β-cell endoplasmic reticulum stress. *Diabetes* 2009; **58**: 906–916.
19. Shaulian E. AP-1–the Jun proteins: oncogenes or tumor suppressors in disguise? *Cell Signal* 2010; **22**: 894–899.
20. Gurzov EN, Ortis F, Bakiri L, Wagner EF, Eizirik DL. JunB inhibits ER stress and apoptosis in pancreatic beta cells. *PLoS One* 2008; **3**: e3030.
21. Gurzov EN, Barthson J, Marhfour I, Ortis F, Naamane N, Igoillo-Esteve M *et al.* Pancreatic β-cells activate a JunB/ATF3-dependent survival pathway during inflammation. *Oncogene* 2012; **31**: 1723–1732.
22. Cunha DA, Ladriere L, Ortis F, Igoillo-Esteve M, Gurzov EN, Lupi R *et al.* Glucagon-like peptide-1 agonists protect pancreatic β-cells from lipotoxic endoplasmic reticulum stress through upregulation of BiP and JunB. *Diabetes* 2009; **58**: 2851–2862.
23. Fuchs SY, Xie B, Adler V, Fried VA, Davis RJ, Ronai Z. c-Jun NH2-terminal kinases target the ubiquitination of their associated transcription factors. *J Biol Chem* 1997; **272**: 32163–32168.
24. Ortis F, Naamane N, Flamez D, Ladriere L, Moore F, Cunha DA *et al.* Cytokines interleukin-1β and tumor necrosis factor-α regulate different transcriptional and alternative splicing networks in primary β-cells. *Diabetes* 2010; **59**: 358–374.
25. Cunha DA, Roma LP, Boschero AC. Prolactin modulates the association and phosphorylation of SNARE and kinesin/MAP-2 proteins in neonatal pancreatic rat islets. *Mol Cell Endocrinol* 2007; **273**: 32–41.
26. Yoshida H, Nadanaka S, Sato R, Mori K. XBP1 is critical to protect cells from endoplasmic reticulum stress: evidence from Site-2 protease-deficient Chinese hamster ovary cells. *Cell Struct Funct* 2006; **31**: 117–125.
27. Allagnat F, Christulia F, Ortis F, Pirot P, Lortz S, Lenzen S *et al.* Sustained production of spliced X-box binding protein 1 (XBP1) induces pancreatic beta cell dysfunction and apoptosis. *Diabetologia* 2010; **53**: 1120–1130.
28. Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K *et al.* Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res* 2013; **73**: 1993–2002.
29. Sha H, He Y, Chen H, Wang C, Zenno A, Shi H *et al.* The IRE1α-XBP1 pathway of the unfolded protein response is required for adipogenesis. *Cell Metab.* 2009; **9**: 556–564.
30. Kato H, Nakajima S, Saito Y, Takahashi S, Katoh R, Kitamura M. mTORC1 serves ER stress-triggered apoptosis via selective activation of the IRE1-JNK pathway. *Cell Death Differ* 2011; **19**: 310–320.
31. Hu MC, Gong HY, Lin GH, Hu SY, Chen MH, Huang SJ *et al.* XBP-1, a key regulator of unfolded protein response, activates transcription of IGF1 and Akt phosphorylation in zebrafish embryonic cell line. *Biochem Biophys Res Commun* 2007; **359**: 778–783.
32. Velloso LA, Bjork E, Ballagi AE, Funa K, Andersson A, Kampe O *et al.* Regulation of GAD expression in islets of Langerhans occurs both at the mRNA and protein level. *Mol Cell Endocrinol* 1994; **102**: 31–37.
33. Son YO, Heo JS, Kim TG, Jeon YM, Kim JG, Lee JC. Over-expression of JunB inhibits mitochondrial stress and cytotoxicity in human lymphoma cells exposed to chronic oxidative stress. *BMB Rep* 2010; **43**: 57–61.
34. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* 2003; **23**: 7448–7459.
35. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 2001; **107**: 881–891.
36. Lee K, Tirasophon W, Shen X, Michalak M, Prywes R, Okada T *et al.* IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev* 2002; **16**: 452–466.
37. Sha H, He Y, Chen H, Wang C, Zenno A, Shi H *et al.* The IRE1α-XBP1 pathway of the unfolded protein response is required for adipogenesis. *Cell Metab* 2009; **9**: 556–564.
38. Wrede CE, Dickson LM, Lingohr MK, Briaud I, Rhodes CJ. Fatty acid and phorbol ester-mediated interference of mitogenic signaling via novel protein kinase C isoforms in pancreatic β-cells (INS-1). *J Mol Endocrinol* 2003; **30**: 271–286.
39. Srinivasan S, Ohsugi M, Liu Z, Fatrai S, Bernal-Mizrachi E, Permutt MA. Endoplasmic reticulum stress-induced apoptosis is partly mediated by reduced insulin signaling through phosphatidylinositol 3-kinase/Akt and increased glycogen synthase kinase-3β in mouse insulinoma cells. *Diabetes* 2005; **54**: 968–975.
40. Wijesekara N, Konrad D, Eweida M, Jefferies C, Liadis N, Giacca A *et al.* Muscle-specific Pten deletion protects against insulin resistance and diabetes. *Mol Cell Biol* 2005; **25**: 1135–1145.
41. Wang L, Liu Y, Yan Lu S, Nguyen KT, Schroer SA, Suzuki A *et al.* Deletion of Pten in pancreatic β-cells protects against deficient β-cell mass and function in mouse models of type 2 diabetes. *Diabetes* 2010; **59**: 3117–3126.
42. Akiyama M, Liew CW, Lu S, Hu J, Martinez R, Hambro B *et al.* X-box binding protein 1 is essential for insulin regulation of pancreatic α-cell function. *Diabetes* 2013; **62**: 2439–2449.
43. Zeng L, Xiao Q, Chen M, Margariti A, Martin D, Ivetic A *et al.* Vascular endothelial cell growth-activated XBP1 splicing in endothelial cells is crucial for angiogenesis. *Circulation* 2013; **127**: 1712–1722.
44. Qin L, Wang Z, Tao L, Wang Y. ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy. *Autophagy* 2010; **6**: 239–247.
45. Puthalakath H, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND *et al.* ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* 2007; **129**: 1337–1349.

46. Li L, Ren CH, Tahir SA, Ren C, Thompson TC. Caveolin-1 maintains activated Akt in prostate cancer cells through scaffolding domain binding site interactions with and inhibition of serine/threonine protein phosphatases PP1 and PP2A. *Mol Cell Biol* 2003; **23**: 9389–9404.
47. Padmasekar M, Lingwal N, Samikannu B, Chen C, Sauer H, Linn T. Exendin-4 protects hypoxic islets from oxidative stress and improves islet transplantation outcome. *Endocrinology* 2013; **154**: 1424–1433.
48. Natalicchio A, De Stefano F, Orlando MR, Melchiorre M, Leonardini A, Cignarelli A *et al.* Exendin-4 prevents c-Jun N-terminal protein kinase activation by tumor necrosis factor-α (TNFα) and inhibits TNFα-induced apoptosis in insulin-secreting cells. *Endocrinology* 2010; **151**: 2019–2029.
49. Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M. Glucagon-like peptide-1 prevents beta cell glucolipotoxicity. *Diabetologia* 2004; **47**: 806–815.
50. Rasschaert J, Ladriere L, Urbain M, Dogusan Z, Katabua B, Sato S *et al.* Toll-like receptor 3 and STAT-1 contribute to double-stranded RNA + interferon-γ-induced apoptosis in primary pancreatic β-cells. *J Biol Chem* 2005; **280**: 33984–33991.
51. Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB. Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* 1992; **130**: 167–178.
52. Lupi R, Dotta F, Marselli L, Del Guerra S, Masini M, Santangelo C *et al.* Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that β-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. *Diabetes* 2002; **51**: 1437–1442.
53. Santin I, Moore F, Colli ML, Gurzov EN, Marselli L, Marchetti P *et al.* PTPN2, a candidate gene for type 1 diabetes, modulates pancreatic β-cell apoptosis via regulation of the BH3-only protein Bim. *Diabetes* 2010; **60**: 3279–3288.
54. Cnop M, Hannaert JC, Hoorens A, Eizirik DL, Pipeleers DG. Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. *Diabetes* 2001; **50**: 1771–1777.
55. Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM *et al.* The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. *Proc Natl Acad Sci USA* 2012; **109**: E869–E878.
56. Moore F, Cunha DA, Mulder H, Eizirik DL. Use of RNA interference to investigate cytokine signal transduction in pancreatic β-cells. *Methods Mol Biol* 2012; **820**: 179–194.
57. Moore F, Naamane N, Colli ML, Bouckenooghe T, Ortis F, Gurzov EN *et al.* STAT1 is a master regulator of pancreatic β-cell apoptosis and islet inflammation. *J Biol Chem* 2010; **286**: 929–941.
58. Chen MC, Proost P, Gysemans C, Mathieu C, Eizirik DL. Monocyte chemoattractant protein-1 is expressed in pancreatic islets from prediabetic NOD mice and in interleukin-1β-exposed human and rat islet cells. *Diabetologia* 2001; **44**: 325–332.
59. Overbergh L, Valckx D, Waer M, Mathieu C. Quantification of murine cytokine mRNAs using real time quantitative reverse transcriptase PCR. *Cytokine* 1999; **11**: 305–312.
60. Cnop M, Ladriere L, Hekerman P, Ortis F, Cardozo AK, Dogusan Z *et al.* Selective inhibition of eukaryotic translation initiation factor 2α dephosphorylation potentiates fatty acid-induced endoplasmic reticulum stress and causes pancreatic β-cell dysfunction and apoptosis. *J Biol Chem* 2007; **282**: 3989–3997.

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