PTPN2, a Candidate Gene for Type 1 Diabetes, Modulates Pancreatic β-Cell Apoptosis via Regulation of the BH3-Only Protein Bim

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OBJECTIVE—Genome-wide association studies allowed the identification of several associations between specific loci and type 1 diabetes (T1D). However, the mechanisms by which most candidate genes predispose to T1D remain unclear. We presently evaluated the mechanisms by which PTPN2, a candidate gene for T1D, modulates β-cell apoptosis after exposure to type I and II interferons (IFNs), cytokines that contribute to β-cell loss in early T1D.

RESEARCH DESIGN AND METHODS—Small interfering RNAs were used to inhibit PTPN2, STAT1, Bim, and Jun NH2-terminal kinase 1 (JNK1) expression. Cell death was assessed by Hoechst propidium iodide staining. BAX translocation, Bim phosphorylation, cytochrome c release, and caspases 9 and 3 activation were measured by Western blot or immunofluorescence.

RESULTS—PTPN2 knockdown exacerbated type I IFN-induced apoptosis in INS-1E, primary rat, and human β-cells. PTPN2 silencing and exposure to type I and II IFNs induced BAX translocation to the mitochondria, cytochrome c release, and caspases 9 and 3 activation. There was also an increase in Bim phosphorylation that was at least in part regulated by JNK1. Of note, both Bim and JNK1 knockdown protected β-cells against IFN-induced apoptosis in PTPN2-silenced cells.

CONCLUSIONS—The present findings suggest that local IFN production may interact with a genetic factor (PTPN2) to induce aberrant proapoptotic activity of the BH3-only protein Bim, resulting in increased β-cell apoptosis via JNK activation and the intrinsic apoptotic pathway. This is the first indication of a direct interaction between a candidate gene for T1D and the activation of a specific downstream proapoptotic pathway in β-cells.

Type 1 diabetes (T1D) is a chronic autoimmune disease during which pancreatic β-cells are specifically damaged by an aberrant immune response. Susceptibility to T1D is linked to genetic factors, but T1D-predisposing genes have low penetrance and only a small proportion of individuals genetically at risk will develop the disease. During the past few years, genome-wide association studies allowed the identification of a large number of robust associations between specific chromosomal loci and T1D development (1). Well-known susceptibility genes include HLA-DR, CTLA-4, IFIH1 (MDA5), and PTPN22 (2). However, these genes only account for part of interindividual differences in disease predisposition or phenotypic diversity, and the pathophysiological mechanisms by which most candidate genes predispose to T1D remain unclear.

It is likely that an interplay between T1D susceptibility genes and environmental factors contribute to the triggering and progression of the disease (3). In this context, a better understanding of the functional effects of the susceptibility genes and their interaction with putative environmental causalities would help to understand the pathogenesis of T1D (4). In animal models for other autoimmune diseases (e.g., Crohn’s disease), there is a striking interaction between a mutation in the Atg16L1 candidate gene and the Murine norovirus, resulting in pathologic abnormalities similar to Crohn’s disease (5). In the context of T1D, a single nucleotide polymorphism (SNP) in the susceptibility gene PTPN22 and early introduction of cow’s milk in the diet are associated with the induction of islet autoantibodies and diabetes development in the Finnish population (6).

Approximately 30% of the T1D candidate genes are expressed in β-cells (7) (M.L. Colli, F. Moore, D.L. Eizirik, unpublished data), suggesting that β-cells play a role in their own demise in T1D. The reduction of β-cell mass in T1D is preceded by an inflammatory process (insulitis) driven in part by a “dialog” between β-cells and infiltrating immune cells, mediated by the local release of cytokines and chemokines (8). Viruses are potential environmental factors contributing to the triggering of insulin (9,10). During viral infections, β-cells release several chemokines and cytokines, including type I interferons (IFNs) (IFNα and IFNβ) (7,11), which contribute to T1D pathogenesis (12,13). In this respect, the T1D candidate gene IFIH1 (MDA5) is involved in the recognition of double-stranded RNA (dsRNA), a by-product of viral replication (14), and we previously observed that knocking down MDA5 in pancreatic β-cells prevents dsRNA-induced expression of key cytokines and chemokines (7).

PTPN2 (also known as TC-PTP or PTP-S2) is another candidate gene for T1D (1). Known risk alleles for T1D in the PTPN2 gene are noncoding, and noncoding variants that may affect splicing have been identified by resequencing (1). One T1D risk variant of PTPN2 is associated with decreased PTPN2 expression in CD4+ T cells and transformed B-cell lines (15). The PTPN2 gene encodes a phosphatase that is ubiquitously expressed (16). The cytokine IFNγ and tumor necrosis factor-α increase PTPN2 expression in human colonic intraepithelial cells, and an upregulation of PTPN2 expression has been observed in intestinal biopsies from patients with active celiac disease.
PTPN2 is highly expressed in immune-related cells, and its expression is modified in CD4+ T cells from patients with T1D when compared with CD4+ T cells from healthy controls (15).

PTPN2 is an important negative regulator of the JAK-STAT signaling pathway that is activated downstream of type I (IFNα and IFNβ) and II (IFNγ) IFN receptors. We recently described that this phosphatase is induced by IFNγ and a synthetic dsRNA, polyinosinic-polycytidylic acid (PIC), in β-cells and exacerbates IFNγ- and PIC-induced β-cell apoptosis by modulating STAT1 activation (7,19). However, the mechanisms connecting this candidate gene to actual β-cell death remain unclear.

We presently observed that PTPN2 also regulates type I IFN–induced apoptosis in β-cells. By systematically knocking down genes putatively involved in the apoptosis pathway of β-cells (20), we identified the apoptotic mechanisms by which PTPN2 knockdown exacerbates type I and II IFN–induced β-cell death. This clarifies the interaction between a candidate gene for T1D and the activation of specific proapoptotic pathways in β-cells and broadens our understanding of the molecular mechanisms involved in the gene/environment interactions triggering insulin and β-cell apoptosis.

RESULTS

PTPN2 silencing increases type I IFN–induced apoptosis in INS-1E cells and primary β-cells. INS-1E cells were left untransfected or transfected with a control siRNA (siCTRL) or an siRNA targeting PTPN2 (siPTPN2), previously shown to efficiently inhibit PTPN2 expression (19). Cells were subsequently treated for 24 h with IFNα or IFNβ (Fig. 1A). None of the treatments significantly affected cell viability in nontransfected or siCTRL-transfected cells, whereas PTPN2 inhibition exacerbated apoptotic cell death in all IFN-treated cells (Fig. 1A). Additional experiments using a higher concentration of IFNα and IFNβ (2,000 units/mL) confirmed the proapoptotic activity of siPTPN2 (Supplementary Fig. 1A), and the concentration of 1,000 units/mL was chosen for subsequent experiments in INS-1E cells and primary rat β-cells. Because the effects of IFNα and IFNβ were similar (Fig. 1), most subsequent experiments were performed with IFNα. Similar results were observed in primary rat β-cells (Fig. 1B). Thus, inhibition of PTPN2 by two different siRNAs (siPTPN2 and siPTPN2#1) increased apoptosis after a 48-h treatment with type I or II IFNs. Of note, the necrotic component of cell death was negligible (<2% of cells, data not shown) in all experiments.

We next performed viability experiments using different concentrations of siPTPN2 (1, 5, 10, and 30 nM). PTPN2 expression gradually decreased with increasing concentrations of siPTPN2 (Supplementary Fig. 1B), whereas IFNα- and IFNγ-induced β-cell apoptosis progressively increased (Supplementary Fig. 1C).

During viral infections, β-cells release type I IFNs (7,11) that act in an autocrine/paracrine fashion through the type I IFN receptor (IFNAR). To evaluate the potential role of IFNAR in PIC-induced apoptosis, INS-1E cells were transfected with siRNAs targeting PTPN2, IFNAR1, or PTPN2 and IFNAR1 and subsequently transfected with PIC (Fig. 1C). Inhibition of PTPN2 increased PIC-induced apoptosis. IFNAR silencing decreased PIC-induced cell death, whereas the double knockdown of PTPN2 and IFNAR abolished the exacerbating effect of PTPN2 inhibition on PIC-induced apoptosis (Fig 1C). These data suggest that PIC-induced β-cell apoptosis in PTPN2-silenced cells is at
least partially mediated by type I IFNs that are released by the β-cells themselves in response to the synthetic dsRNA. PTPN2 inhibition increases IFNα- and IFNγ-induced apoptosis via STAT1 activation. Type I IFNs preferentially induce STAT1/2 heterodimers that activate ISREs, whereas type II IFNs favor the activation of STAT1 homodimers that bind to the GAS (30). Inhibition of PTPN2 in INS-1E cells significantly increased IFNα-induced ISRE reporter activity (1.7-fold), whereas IFNγ stimulation led to poor ISRE reporter activation, even after PTPN2 silencing (Fig. 2A). The GAS reporter activity was increased in untransfected and siCTRL-transfected INS-1E cells after IFNγ treatment, and PTPN2 silencing further exacerbated activation of the GAS reporter (twofold induction), whereas IFNα did not modify GAS reporter activity (Fig. 2B). Double transfection of PTPN2- and STAT1-targeting siRNAs potently inhibit both target proteins (19), which was confirmed in the present series of experiments (data not shown). PTPN2 knockdown significantly increased IFNα- and IFNγ-induced apoptosis, whereas double knockdown of PTPN2 and STAT1 prevented the proapoptotic effect of PTPN2 inhibition in INS-1E cells exposed to IFNα or IFNγ (Fig. 2C).

These results suggest that STAT1 activation plays a key role in both type I and II IFN–induced β-cell apoptosis in PTPN2-deficient cells.

**PTPN2 inhibition exacerbarates type I and II IFN–induced β-cell apoptosis through the intrinsic mitochondrial pathway.** To clarify the death pathways by which PTPN2 deficiency exacerbarates type I and II IFN–induced cell death, we first analyzed genes induced by the endoplasmic reticulum stress response, previously shown to be associated with interleukin (IL)-1β- and IFNγ-induced apoptosis of β-cells (31). The mRNA expression of CHOP, BIP, or XBP-1s was not induced in siPTPN2-transfected INS-1E cells after 24 h of treatment with IFNα or IFNγ compared with siCTRL (Supplementary Fig. 2). There were also no changes in BIP expression after 2, 4, 8, and 16 h of treatment with IFNα or IFNγ and siPTPN2 exposure (data not shown), making it unlikely that endoplasmic reticulum stress response contributes to IFN-induced apoptosis after PTPN2 knockdown.

We next examined the intrinsic mitochondrial pathway of apoptosis, because IFNγ activates this cell death pathway when associated with the proinflammatory cytokines IL-1β or tumor necrosis factor-α (25,27). Immunofluorescence analysis of INS-1E cells in which PTPN2 was silenced demonstrated diffuse BAX staining in untreated cells (Fig. 3, top), whereas it colocalized with the mitochondrial marker ATP synthase after 16 h of treatment with IFNα (Fig. 3, middle) or IFNγ (Fig. 3, bottom), demonstrating that BAX translocates to the mitochondria in siPTPN2-silenced cells. In line with our initial viability experiments (Fig. 1), fewer apoptotic cells were observed in untreated or IFN-treated siCTRL-transfected cells (data not shown).

Western blot evaluation of mitochondrial and cytoplasmic fractions from siCTRL- and siPTPN2-transfected INS-1E cells demonstrated that IFNα and IFNγ induced mitochondrial cytochrome c release to the cytoplasm in PTPN2-deficient cells but not in siCTRL-transfected cells (Fig. 4A and B). In line with these findings, PTPN2 knockdown also induced caspase 3 activation after 16 and 24 h of IFNα and IFNγ treatment (Fig. 4C and D). Taken together, the data from Figures 3 and 4 confirm the results observed in viability assays (Fig. 1) and indicate that apoptosis occurs mainly through the mitochondria-driven intrinsic pathway of cell death in IFN-treated PTPN2-deficient cells.

**The BH3-only protein Bim mediates β-cell death in PTPN2-silenced cells.** The members of the B-cell lymphoma 2 (BCL-2) family, death protein 5 (Bcl-2), BCL2 binding component 3 (p58 up-regulated modulator of apoptosis) (27), and BCL2-like 11 (Bim) (32), have been shown to contribute to β-cell apoptosis after exposure to cytokines (IL-1β + IFNγ) or metabolic stress. IFNα or IFNγ treatment
induced death protein 5 and p53 upregulated modulator of apoptosis expressions over time in the treated cells, but there were no significant differences between siCTRL- and siPTPN2-transfected cells (Supplementary Fig. 3). On the other hand, cytokine-induced Bim mRNA upregulation was exacerbated in PTPN2-deficient INS-1E cells after 16–24 h of IFNα or IFNγ treatment compared with their respective controls (Fig. 5A and B). However, there was no significant modulation of Bim protein expression in siCTRL and siPTPN2-transfected cells after treatment with IFNα (Fig. 5C) or IFNγ (Fig. 5D).

Bim activity may be controlled by posttranscriptional phosphorylation at multiple serine and threonine residues (33). PTPN2 inhibition increased Bim phosphorylation at residue 65 in untreated INS-1E cells, and this effect was prolonged until 4 h of treatment with IFNα or 8 h after treatment with IFNγ (Fig. 5F). Similar phosphorylation levels were observed in CTRL and PTPN2-deficient cells after 16 h of treatment of IFNα or IFNγ. These results suggest that Bim phosphorylation is increased in PTPN2-silenced cells, which may render these cells more sensitive to the proapoptotic effects of IFNs.

To assess whether Bim indeed contributes for type I and II IFN–induced β-cell death in PTPN2-inhibited cells, we silenced PTPN2 and Bim in a double-knockdown approach. As previously shown (Fig. 1), inhibition of PTPN2 significantly exacerbated INS-1E cell apoptosis after treatment with IFNα, IFNβ, or IFNγ (Fig. 6A). This effect was counteracted by Bim knockdown, which protected INS-1E cells against IFNα-, IFNβ-, and IFNγ-induced apoptosis in PTPN2-silenced cells by 58, 67, and 78%, respectively (Fig. 6A). These observations were confirmed in FACSpurified rat primary β-cells (Fig. 6B), in which Bim inhibition again reversed the exacerbating effect of PTPN2 knockdown, with a 59 and 63% reduction of cell apoptosis after IFNα and IFNγ treatment, respectively. Similar observations were made in dispersed human islet cells (Fig. 6C). Thus, PTPN2 knockdown exacerbated IFN-induced cell death.
death and Bim knockdown reversed the deleterious effect of PTPN2 knockdown, with a reduction of cell death nearly to basal levels (Fig. 6C).

The protective effects of Bim knockdown were confirmed by Western blot analysis of caspases 9 and 3 activation in INS-1E cells. As shown in Figure 6D–F, PTPN2 knockdown exacerbated caspases 9 and 3 activation in IFNα-, IFNβ-, and IFNγ-treated INS-1E cells, whereas the concomitant inhibition of PTPN2 and Bim abrogated this deleterious effect (Fig. 6E and F). As a whole, these results suggest that Bim plays a central role in the exacerbation of IFN-induced β-cell apoptosis in PTPN2-deficient cells.

To assess whether the effect of PTPN2 on Bim phosphorylation was mediated by Jun NH2-terminal kinase 1 (JNK1), we performed a double-knockdown approach, targeting PTPN2 and JNK1 and evaluating Bim phosphorylation by Western blot in INS-1E cells. As previously shown (Fig. 5E), PTPN2 knockdown increased Bim phosphorylation mainly in the nontreated condition (Fig. 7A and B), although this effect was also evident after 2 h of treatment with IFNα (Fig. 7A) or IFNγ (Fig. 7B). After 24 h of treatment with IFNα (Fig. 7A) or IFNγ (Fig. 7B), the phosphorylation level observed in CTRL and PTPN2-deficient cells was similar. After JNK1 knockdown or double knockdown of PTPN2 and JNK1, Bim phosphorylation was decreased in both the nontreated condition (Fig. 7A and B) and after 2 or 24 h of IFNα (Fig. 7A) or IFNγ (Fig. 7B) treatment. These results indicate that the protein kinase JNK1 is in part responsible for the hyperphosphorylation of Bim at serine 65 (Ser65) in PTPN2-silenced β-cells.

We next silenced PTPN2 and JNK1 in a double-knockdown approach to test whether the observed decrease in Bim phosphorylation inhibited IFN-induced apoptosis in PTPN2-silenced cells. As shown in Figure 7C, JNK1 knockdown counteracted the increase in cell death in PTPN2-silenced INS-1E cells after 24 h of treatment with IFNα or IFNγ. These results were confirmed in FACSpurified rat primary β-cells, in which double knockdown of PTPN2 and JNK1 partially prevented the increase in IFN-induced cell death in the presence of PTPN2 inhibition (Fig. 7D).

The protective effects of JNK1 knockdown were confirmed by Western blot analysis of cleaved caspase 3 in INS-1E cells (Fig. 7A and B): PTPN2 inhibition increased cleaved caspase 3 in IFNα- and IFNγ-treated cells, whereas the double knockdown of PTPN2 and JNK1 revoked this effect.

**DISCUSSION**

The current study shows that inhibition of the T1D candidate gene PTPN2 sensitizes pancreatic β-cells to apoptosis induced by both type I and II IFNs. We further demonstrate that blocking IFNAR abolishes the exacerbation of apoptosis induced by the viral dsRNA analog PIC.
in PTPN2-silenced cells. dsRNA is produced in the cytosol of infected cells as a by-product of viral replication. This suggests that type I IFNs released by β-cells in response to a viral insult play an important role in cell death. This is in line with previous observations suggesting that intracellular dsRNA induces type I IFN release by β-cells, which contributes to cell death (7,11).

Silencing of PTPN2 increases ISRE and GAS reporter activity after IFN treatment, and double knockdown of PTPN2 and STAT1 reverses the proapoptotic effect of PTPN2 inhibition. PTPN2 silencing induces BAX translocation to the mitochondria, cytochrome c release to the cytosol, and caspase 3 activation after exposure to type I and II IFNs, characterizing activation of the intrinsic mitochondrial pathway of cell death. This process might be secondary to hyperphosphorylation of Bim in PTPN2-silenced cells via modulation of the protein kinase JNK1. In line with this hypothesis, both Bim and JNK1 knockdown protects β-cells against IFN-induced apoptosis in PTPN2-silenced cells. The key role of Bim was confirmed in human islet cells. This suggests that local IFN production may interact with a genetic factor (PTPN2) to induce aberrant proapoptotic activity of the BH3-only protein Bim, resulting in increased β-cell apoptosis.

Viruses are one of the putative environmental factors associated with T1D triggering in genetically predisposed individuals; several epidemiologic studies in humans and in animal models support this association (34). Thus, it was recently reported that enterovirus-positive blood samples are more frequent among subjects with T1D than among healthy individuals (35), and histologic studies of pancreas from deceased patients with T1D indicate a higher prevalence of Coxsackievirus B4 than in pancreas from control individuals (9,36). Type I IFNs are important mediators of the immune response against viral infections, and several studies demonstrated expression of type I IFNs in the islets and blood of individuals with T1D (37,38) and their role in the initiation or the acceleration of the autoimmune process in NOD mice (39). The present results indicate a potential link between a candidate gene for T1D (PTPN2) and the effects of type I IFNs in the progressive β-cell loss initiated by viral infections in genetically susceptible individuals. Thus, we suggest that development of T1D in some individuals may require a
combination of infection by a potential diabetogenic virus (e.g., Coxsackievirus B4) (9), a vigorous and probably excessive local production of IFNs and other chemokines/cytokines, and the presence of particular polymorphisms in a candidate gene (i.e., \textit{PTPN2}) that exacerbates IFN signaling (present data).

\textbf{Type I and II IFN–induced \(\beta\)-cell apoptosis in PTPN2–silenced cells is accompanied by the exacerbation of the JAK-STAT signaling pathway (present data). On cytokine treatment, PTPN2 inhibition increases the activity of reporter vectors containing ISRE or GAS consensus sequences, and concomitant knockdown of \textit{STAT1} and PTPN2 significantly decreases the exacerbation of IFN-induced apoptosis. Because PTPN2 is a well-known negative regulator of \textit{STAT1} activation (40), our data suggest a key role for \textit{STAT1} in type I and II IFN–induced \(\beta\)-cell apoptosis. Aberrant activation of the JAK-STAT pathway is associated with both T1D and other autoimmune and inflammatory diseases (41,42). For example, \textit{STAT1} phosphorylation is increased in the intestinal mucosa of patients with celiac disease, suggesting that persistent \textit{STAT1} activation contributes to maintain and expand the local inflammatory response (41).

As mentioned above, the present results demonstrate that PTPN2 knockdown contributes to type I and II IFN–induced \(\beta\)-cell apoptosis via activation of the BH3-only protein Bim and consequent activation of the intrinsic mitochondrial pathway of cell death. This pathway leads to BAX translocation to the mitochondrial membrane, mitochondrial permeabilization, cytochrome \(c\) release to the cytosol, activation of caspase 9, subsequent activation of the effector caspase 3, and cell apoptosis. Mitochondrial integrity is controlled by interactions between pro- and antiapoptotic members of the B-cell lymphoma 2 (Bcl-2) protein family (20). Bim is a Bcl-2 member that mediates apoptosis by activating the proapoptotic Bcl-2 members Bax and Bak (43), and it contributes to \(\beta\)-cell apoptosis induced by chronic exposure to high glucose and the Fas-FasL...
Bim-dependent apoptosis is regulated by both modulation of its expression and phosphorylation at specific serine or threonine residues (33). We presently show that PTPN2 inhibition increases Bim phosphorylation at Ser65 via JNK1 activation, and that Bim inhibition abrogates IFN-induced cell death in PTPN2-deficient cells. Our results are in line with previous reports showing that MAPK-p38- or JNK-induced phosphorylation of Bim at Ser65 increases its proapoptotic activity (44–46). It is interesting that PTPN2 silencing in β-cells (present data) induces hyperphosphorylation of Bim at residue Ser65 even in untreated cells, which is not sufficient to decrease cell viability. However, when this is combined with exposure to IFNs, cell death is triggered. This suggests that phosphorylation at Bim Ser65 is an apoptosis-sensitizing event in β-cells, leading to apoptosis only in the context of a wider proapoptotic environment prompted by local production of proinflammatory cytokines or viral infection.

SNPs in the PTPN2 gene are implicated in susceptibility to several autoimmune disorders and inflammatory diseases, including T1D, Crohn’s disease, and celiac disease (1,47,48). One of the SNPs (rs2542151) in the PTPN2 gene is associated with an earlier onset of T1D, marking a genetic difference between patients with early and late onset of the disease (49). The intronic risk allele (rs1803217-C) in PTPN2 correlates with decreased IL-2R signaling in...
CD4+ T cells, as well as with decreased PTPN2 expression in CD45RO T cells (15). Our present data demonstrate that inhibition of PTPN2 activity in pancreatic β-cells increases IFN-induced cell death, suggesting that SNPs leading to a decreased expression of this gene may sensitize β-cells to apoptosis after a triggering event (e.g., type I IFNs produced by β-cells in response to a viral infection). In line with this possibility, PTPN2 knockdown in a model of Crohn’s disease leads to increased claudin-2 (pore-forming protein) expression, further increasing IFNγ-induced intestinal epithelium permeability, which is an important feature of Crohn’s disease (17). Moreover, PTPN2 knockout mice have abnormal production of IFNγ, tumor necrosis factor-α, and other cytokines, resulting in a systemic inflammatory disease (50). Further studies are now required to define how allelic differences in humans affect PTPN2 activity and function, and the impact of these differences in immune responses and β-cell apoptosis.

The current study suggests that environmental factors (e.g., viral infections and the resulting IFN production) may interact with a genetic predisposition factor (e.g., reduced PTPN2 activity) to induce aberrant proapoptotic activity of the BH3-only protein Bim, resulting in increased β-cell apoptosis via the intrinsic apoptotic pathway. Moreover, it indicates that PTPN2 modulates the apoptotic activity of Bim via regulation of the protein kinase JNK1. These findings provide the first indication of a direct interaction between a candidate gene for T1D and the activation of a specific downstream proapoptotic pathway in pancreatic β-cells. Further studies of the molecular interactions between predisposition genes and environmental triggers for autoimmune diseases should clarify the early triggering of autoimmunity and indicate novel avenues for their prevention.

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FIG. 7. Double knockdown of PTPN2 and JNK1 reduces pBIM-Ser65 phosphorylation and protects INS-1E cells and primary rat β-cells from type I and II IFN-induced apoptosis. INS-1E cells and primary rat β-cells were transfected with an siCTRL, siPTPN2, or siRNA targeting JNK1, or double transfected with both siPTPN2 and siRNA targeting JNK1. After 48 h of recovery, they were left untreated or treated with IFNα or IFNγ as indicated. Expression of pBIM-Ser65, cleaved caspase 3, JNK1, PTPN2, and α-tubulin was evaluated by Western blot in INS-1E cells after IFNα (A) or IFNγ (B) treatment. Results are representative of five independent experiments. Apoptosis was evaluated in INS-1E cells (C) and primary rat β-cells (D) by HO/PI staining. Results are the mean ± SEM of three to four independent experiments; §§§P < 0.001 and §§P < 0.01 vs. untreated (i.e., not treated with cytokines) transfected with the same siRNA; ***P < 0.001 and *P < 0.05 vs. siCTRL treated with the same cytokine; ###P < 0.001 vs. siPTPN2 treated with the same cytokine; ANOVA.
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