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Differential Regulation of Endoplasmic Reticulum Stress by Protein Tyrosine Phosphatase 1B and T Cell Protein Tyrosine Phosphatase*[S]

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Ahmed Bettaieb[‡1], Siming Liu[‡1], Yannan Xi[‡], Naoto Nagata[‡], Kosuke Matsuo[‡], Izumi Matsuo[‡], Samah Chahed[‡], Jesse Bakke[‡], Heike Keilhack[§], Tony Tiganis[¶], and Fawaz G. Haj[‡2]

From the [‡]Department of Nutrition, University of California, Davis, California 95616, [§]Merck Research Laboratories, Boston, Massachusetts 02115, and the [¶]Department of Biochemistry and Molecular Biology, Monash University, Victoria 3800, Australia

Protein-tyrosine phosphatase 1B (PTP1B) and T cell protein-tyrosine phosphatase (TCPTP) are closely related intracellular phosphatases implicated in the control of glucose homeostasis. PTP1B and TCPTP can function coordinately to regulate protein tyrosine kinase signaling, and PTP1B has been implicated previously in the regulation of endoplasmic reticulum (ER) stress. In this study, we assessed the roles of PTP1B and TCPTP in regulating ER stress in the endocrine pancreas. PTP1B and TCPTP expression was determined in pancreases from chow and high fat fed mice and the impact of PTP1B and TCPTP over- or underexpression on palmitate- or tunicamycin-induced ER stress signaling assessed in MIN6 insulinoma β cells. PTP1B expression was increased, and TCPTP expression decreased in pancreases of mice fed a high fat diet, as well as in MIN6 cells treated with palmitate. PTP1B overexpression or TCPTP knockdown in MIN6 cells mitigated palmitate- or tunicamycin-induced PERK/eIF2α ER stress signaling, whereas PTP1B deficiency enhanced ER stress. Moreover, PTP1B deficiency increased ER stress-induced cell death, whereas TCPTP deficiency protected MIN6 cells from ER stress-induced death. ER stress coincided with the inhibition of Src family kinases (SFKs), which was exacerbated by PTP1B overexpression and largely prevented by TCPTP knockdown. Pharmacological inhibition of SFKs ameliorated the protective effect of TCPTP deficiency on ER stress-induced cell death. These results demonstrate that PTP1B and TCPTP play nonredundant roles in modulating ER stress in pancreatic β cells and suggest that changes in PTP1B and TCPTP expression may serve as an adaptive response for the mitigation of chronic ER stress.

Diabetes is a prevalent metabolic disease that is caused by absolute insulin deficiency (type 1 diabetes) or insulin resistance (type 2 diabetes) (1). Insulin is secreted from pancreatic β cells into the portal circulation and acts as a major regulator of glucose homeostasis by means of a complex network of signaling events (2). Pancreatic β cell failure/death is a common pathological component in the progression of both type 1 and type 2 diabetes (3, 4). β cells dynamically respond to fluctuations in blood glucose concentrations with the regulated secretion of insulin (5). Proinsulin undergoes meticulous folding into its correct tertiary structure in the lumen of the endoplasmic reticulum (ER)[3] (6). When the folding capacity of the ER is exceeded, under pathological and/or physiological conditions, unfolded/misfolded proteins accumulate and lead to ER stress (7). β cells use adaptive mechanisms to mitigate ER stress collectively known as the unfolded protein response (UPR) (8). UPR is triggered by transmembrane sensors that detect unfolded proteins in the ER through their luminal domain and convey information through their cytosolic domain. These proteins are protein kinase activated by double-stranded RNA-like endoplasmic reticulum-regulated kinase (PERK), inositol requiring protein 1α (IRE1α), and activating transcription factor 6 (ATF6) (9). UPR can attenuate stress by increasing the folding capacity of the ER, translational attenuation, ER biogenesis, and ER-associated protein degradation (7). For professional secretory β cells, UPR is indispensable for ensuring proper function and survival (8). However, if these compensatory mechanisms fail to restore/preserve homeostasis, ER stress-related apoptosis commences (10).

Protein tyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TCPTP) are intracellular tyrosine-specific phosphatases that share a high degree of structural similarity (11) but have distinct physiological functions. PTP1B KO mice exhibit improved insulin sensitivity and resistance to high fat diet (HFD)-induced obesity (12, 13). On the other hand, TCPTP knock-out mice die a few weeks after birth due to defects in the hematopoietic compartment (14). In addition, TCPTP has been implicated in the regulation of insulin signaling and glucose homeostasis (15, 16). Moreover, PTP1B and TCPTP play nonredundant roles in macrophage development

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[1] Both authors contributed equally to this work.

[2] To whom correspondence should be addressed: University of California, Davis, 3135 Meyer Hall, One Shields Ave., Davis, CA 95616. Tel.: 530-752-3214; E-mail: fghaj@ucdavis.edu.

[3] The abbreviations used are: ER, endoplasmic reticulum; PERK, PKR-like ER-regulated kinase; PTP, protein-tyrosine phosphatase; PTP1B, protein-tyrosine phosphatase 1B; TCPTP, T cell protein-tyrosine phosphatase; KD, knockdown; SFK, Src family kinase; KDR, reconstituted KD cells; UPR, unfolded protein response; IRE1, inositol requiring protein 1; XBP1, X-box binding protein 1; BiP, Binding immunoglobulin protein; sXBP1, spliced X-box binding protein 1; CHOP, C/EBP homologous protein.

and IFN-γ signaling (17) and function in a coordinated and temporally distinct manner to regulate insulin receptor signaling (18).

The roles of TCPTP and PTP1B in modulating ER stress in the endocrine pancreas remain largely unexplored. TCPTP has been recently identified as a candidate gene for type 1 diabetes, and its inhibition exacerbates cytokine-induced pancreatic β cell apoptosis (19). On the other hand, PTP1B deficiency has been shown to spare islets and delay the onset of diabetes in insulin receptor substrate 2 knock-out mice, pointing toward PTP1B having an integral role in regulating β cell homeostasis *in vivo* (20). In addition, PTP1B has been reported to regulate ER stress signaling. Mouse embryonic fibroblasts lacking PTP1B exhibit impaired IRE1α signaling and attenuated ER stress-induced apoptosis (21), whereas liver-specific PTP1B deficiency protects mice against HFD-induced ER stress (22). In this study, we determined the role of PTP1B and TCPTP in ER stress signaling in β cells.

EXPERIMENTAL PROCEDURES

Reagents—DMEM, penicillin/streptomycin, puromycin, FBS, and trypsin were purchased from Invitrogen. Antibodies used in this study (source, species, and conditions for use) are listed in supplemental Table S1. c-Src Y527F-pJ3Ω and c-Src WT-pJ3Ω have been described previously (41). Unless otherwise indicated, chemicals were purchased from Sigma.

Mouse Studies—All mice studied were age-matched males on a mixed 129Sv/J × C57Bl/6J background and were maintained on a 12-h light-dark cycle with free access to water and food. Mice were placed on standard lab chow (Purina lab chow, no. 5001) at weaning or switched to a HFD (60% kcal from fat, no. D12492, Research Diets) at 5–8 weeks of age. All mouse studies were approved by the Institutional Animal Care and Use Committee at University of California, Davis.

Cell Culture and Immunohistochemistry—MIN6 β cells were maintained in DMEM containing 25 millimolar glucose, 15% FBS, 50 units/ml penicillin, and 50 μg/ml streptomycin. PTP1B and TCPTP silencing was achieved by testing five different hairpins for each PTP (Open Biosystems). Packaging (psPAX2) and envelope (pMD2.G) vectors were obtained from Addgene (Boston). Lentiviruses were generated by co-transfection of vectors in HEK293FT cells using Lipofectamine 2000 (Invitrogen) following the manufacturer's guidelines and then used to infect MIN6 cells. Cells were selected using puromycin (2 μg/ml), and drug-resistant pools were propagated. Cells with knockdown (KD) of PTP1B or TCPTP were reconstituted by transient transfection of pWZL human PTP1B (hPTP1B) and pCG mouse TCPTP, respectively. For overexpression studies, MIN6 cells were transfected with hPTP1B and then selected with hygromycin (Sigma) as we described previously (23). To induce ER stress, cells (2.5×10^5 cells/60-mm plate) were treated with 1 mM palmitate or 2 ng/ml tunicamycin for 24 h. Palmitate was freshly prepared in HEPES/DMEM buffer containing 10.5% fatty acid-free BSA.

For immunohistochemistry, pancreases were fixed in Z-fix (Fisher Scientific) and embedded in paraffin. Sections were stained with insulin, mouse PTP1B and TCPTP antibodies, and DAPI (nuclear staining). Detection was performed with appropriate fluorescein-conjugated secondary antibodies and visualized using a Leica DMI3000B inverted microscope.

Biochemical Analyses—Cells were lysed in radioimmunoprecipitation assay buffer (150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 7.4, 5 mM EDTA, 1 mM NaF, 1 mM sodium orthovanadate, and protease inhibitors) as we described previously (24). Lysates were clarified by centrifugation at 13,000 rpm for 10 min, and protein concentrations were determined using a bicinchoninic acid assay kit (Pierce Chemical). Proteins (30–90 μg) were resolved by SDS-PAGE and transferred to PVDF membranes. Immunoblotting of cell lysates was performed with primary antibodies, and after incubation with secondary antibodies, proteins were visualized using enhanced chemiluminescence (Amersham Biosciences). Pixel intensities of immunoreactive bands were quantified using FluorChem Q Imaging software (Alpha Innotech).

Cell Survival and Apoptosis—After treatment with palmitate or tunicamycin for 24 h, MIN6 cells were washed with PBS then stained with 50 μg/ml Hoechst for 15 min. Cells were then washed twice with PBS and visualized using a Leica DMI3000B microscope, and at least 200 cells were counted for each condition.

Statistical Analyses—Data are expressed as means ± S.E. All statistical analyses with the JMP program (SAS Institute) were performed using unpaired, two-tail Student's *t* tests. Differences were considered significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$. A single symbol (such as an asterisk) corresponds to $p \leq 0.05$, whereas a double symbol (such as double asterisks) corresponds to $p \leq 0.01$.

RESULTS

ER Stress Increases PTP1B and Decreases TCPTP Protein Levels in β Cells—To assess PTP1B and TCPTP localization in the endocrine pancreas, we stained pancreatic sections from wild type mice for either PTP1B or TCPTP and insulin as described under "Experimental Procedures." Both phosphatases exhibited stronger expression in insulin-positive islets than in the surrounding exocrine tissue (Fig. 1*A*). PTP1B staining was exclusively cytosolic consistent with its ER localization (25), whereas TCPTP was predominantly nuclear in line with the major murine isoform of TCPTP being the 45-kDa form (26). This isoform of TCPTP is targeted to the nucleus by a bipartite nuclear localization sequence (27) but readily shuttles in and out of the nucleus and has access to both nuclear and cytoplasmic substrates (28). Next, we evaluated PTP1B and TCPTP expression in pancreases of mice fed a normal chow diet (Purina lab chow) *versus* mice fed a HFD (60% kcal from fat) for 22 weeks to induce chronic ER stress. Because PERK is highly expressed in pancreatic islets (29) and the PERK/eIF2α subpathway is important for ER stress signaling in β cells (30, 31), this pathway was used as readout for ER stress. In addition, the status of downstream markers such as XBP1, which regulates the transcription of a number of genes, provided further readout of ER stress signaling. Consistent with published data (32), high fat feeding led to elevated ER stress in the pancreas. Immunoblot analysis of pancreatic lysates indicated that PERK (Thr^{980}) and eIF2α (Ser^{51}) phosphorylation was increased in

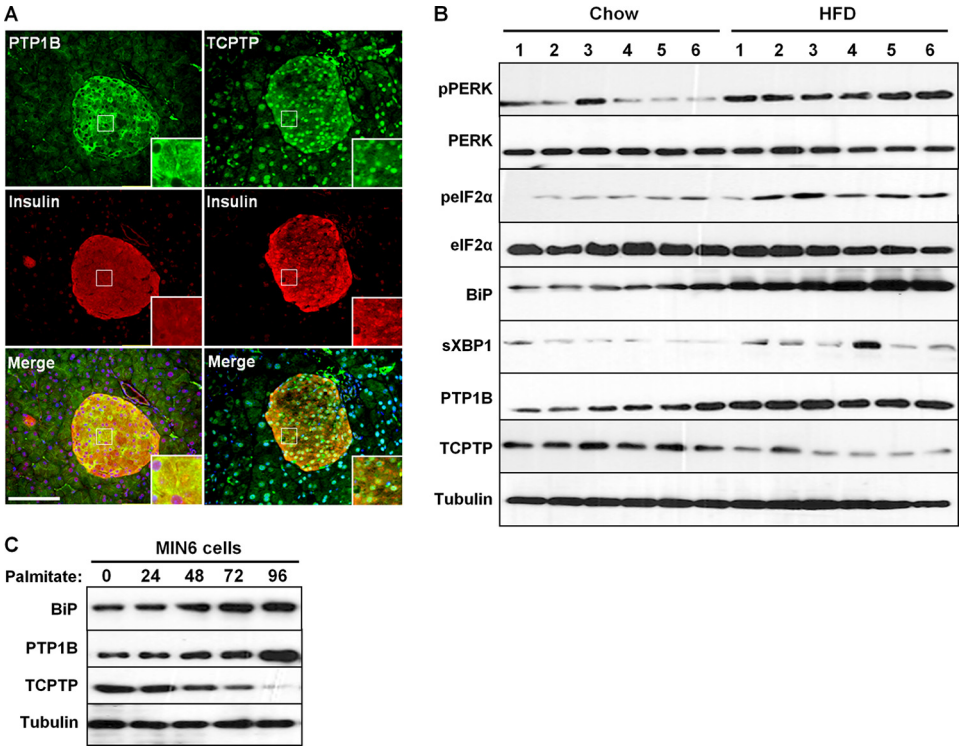
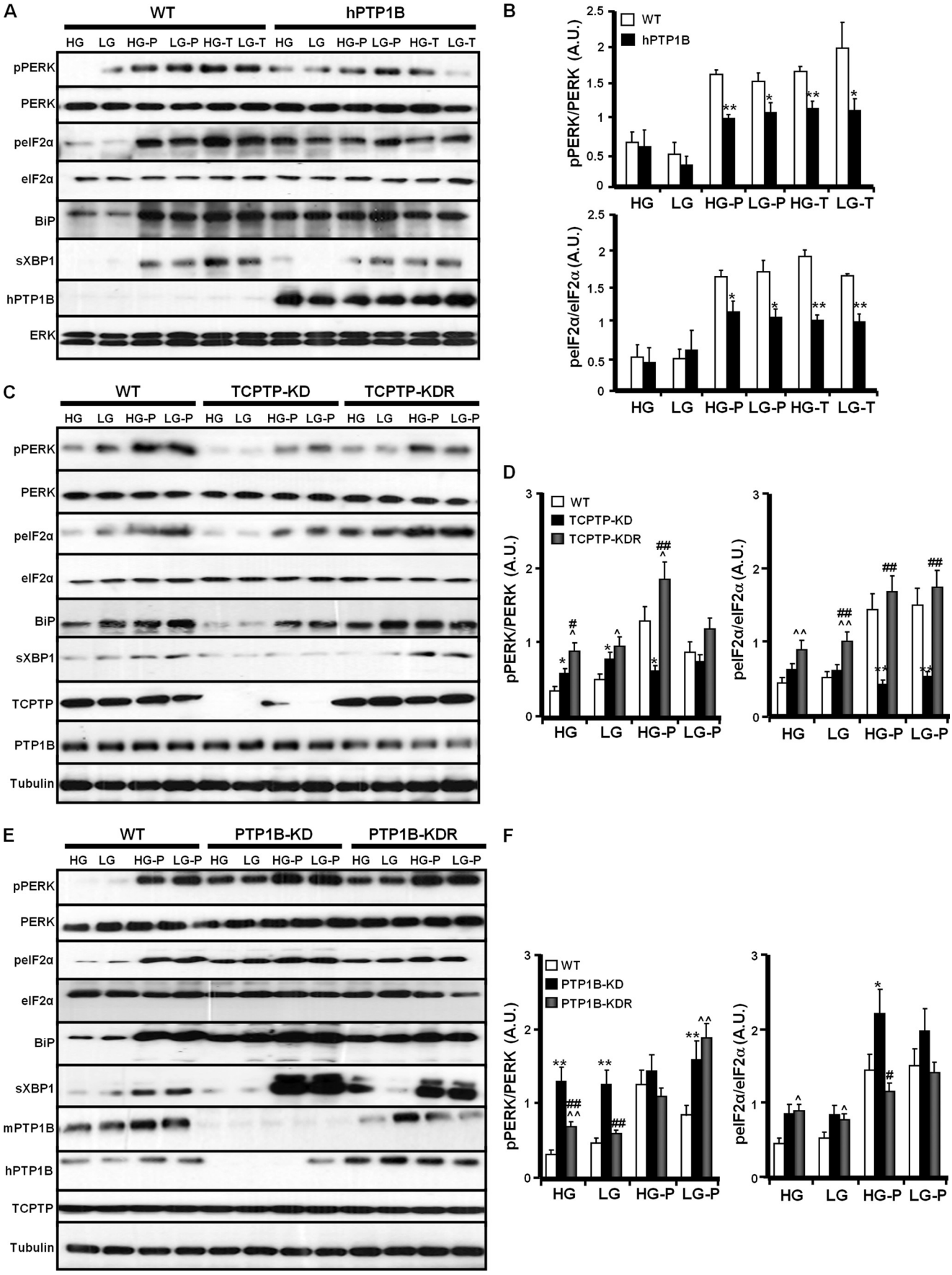


FIGURE 1. **ER stress increases PTP1B and decreases TCPTP protein in β cells.** *A*, pancreas sections of wild type male mice on a HFD for 20 weeks immunostained for PTP1B and TCPTP (*green*), insulin (*red*), and merge (includes DAPI, stained *blue*). *Boxed areas* are magnified in the *bottom right*, and the *scale bar* corresponds to 100 μm. *B*, pancreases were isolated from male mice on regular and a HFD (for 22 weeks), and lysates immunoblotted for pPERK (Thr^{980}), PERK, peIF2α (Ser^{51}), eIF2α, BiP, sXBP1, PTP1B, TCPTP, and tubulin. Each lane represents a sample from a separate animal. *C*, MIN6 cells were treated with 0.5 mM palmitate for the indicated hours and lysates immunoblotted for BiP, PTP1B, TCPTP, and tubulin.

mice on a HFD (Fig. 1*B*). In addition, BiP and sXBP1 expression was increased in pancreases of mice on a HFD compared with those on chow. Importantly, pancreatic PTP1B expression was increased, whereas TCPTP expression was decreased after high fat feeding (Fig. 1*B*). To investigate whether ER stress also leads to comparable alterations in PTP1B and TCPTP expression, we treated the glucose-responsive insulinoma MIN6 β cells (33) with palmitate. This led to increased BiP expression and PERK (Thr^{980}) phosphorylation within 48 h of treatment (Fig. 1*C* and data not shown). Notably, prolonged treatment with palmitate (≥48 h) resulted in increased PTP1B and concomitant decreased TCPTP protein levels. Taken together, these results indicate that ER stress induces alterations in PTP1B and TCPTP expression in β cells *in vivo* and *in vitro*. Moreover, these results identify MIN6 β cells as a model cell system for the assessment of PTP1B and TCPTP function in ER stress.

PTP1B and TCPTP Regulate ER Stress Signaling—The increased PTP1B and decreased TCPTP expression in response to ER stress raised the possibility that these phosphatases may play an important role in regulating the ER stress response in islets. To test this, we either overexpressed PTP1B in MIN6 β cells (Fig. 2*A*) or stably knocked down TCPTP using lentiviral shRNAs (Fig. 2*C*). As a complementary approach for assessing PTP1B's role in ER stress signaling we also knocked down PTP1B expression (Fig. 2*E*); there was no compensatory increase in TCPTP expression after PTP1B KD and *vice versa* (data not shown). To rule out off-target effects, KD cells were reconstituted (KDR) with the respective phosphatase at levels comparable to those in control cells (Fig. 2, *C* and *E*). ER stress was induced using palmitate or tunicamycin as described previously (34). Palmitate induces ER stress through degradation of carboxypeptidase E (35), whereas tunicamycin, an inhibitor of *N*-linked glycosylation, inhibits dolichol pyrophosphate-mediated glycosylation of asparaginyl residues of glycoproteins (36). Because glucose concentration affects ER stress in β cells (37), we performed these studies under low and high glucose conditions. MIN6 cells were treated with palmitate or tunicamycin for 24 h, so that PTP1B and TCPTP levels were not altered and overt cell loss due to cell death was not evident (data not shown).

The effects of PTP1B overexpression in MIN6 cells on the PERK/eIF2α arm of ER stress signaling were evaluated. In accordance with previous studies (10, 38), palmitate and tunicamycin induced ER stress as evidenced by enhanced PERK (Thr^{980}) and eIF2α (Ser^{51}) phosphorylation and increased BiP and sXBP1 expression in control cells after treatment with either drug (Fig. 2 and supplemental Fig. S1). PTP1B overexpression (~4–5-fold) led to significant decrease in palmitate- and tunicamycin-induced PERK and eIF2α phosphorylation (Fig. 2, *A* and *B*). TCPTP knockdown also mitigated palmitate- and tunicamycin-induced ER stress and led to decreased PERK (Thr^{980}) and eIF2α (Ser phosphorylation and decreased BiP and sXBP1 expression when compared with controls, and this could reversed by TCPTP reconstitution (Fig. 2, *C* and *D* and supplemental Fig. S1, *A* and *B*). Conversely, PTP1B deficiency potentiated ER stress as assessed by PERK and eIF2α phosphorylation and BiP and sXBP1 expression, and this was partly reversed by the reconstitution of PTP1B (Fig. 2, *E* and *F*, and supplemental Fig. S1, *C* and *D*). In accordance with these biochemical find-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pPERK
PERK
peIF2α
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BiP
sXBP1
hPTP1B
ERK
B
WT
hPTP1B
pPERK/PERK (A.U.)
peIF2α/eIF2α (A.U.)
HG LG HG-P LG-P HG-T LG-T
C
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TCPTP-KD
TCPTP-KDR
HG LG HG-P LG-P HG LG HG-P LG-P HG LG HG-P LG-P
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PERK
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BiP
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ings, real-time PCR revealed that PTP1B deficiency increased BiP, CHOP, and sXBP1 mRNA expression, whereas TCPTP deficiency decreased expression (data not shown). Taken together, our data provide evidence for PTP1B and TCPTP regulating ER stress signaling in MIN6 cells. Moreover, they suggest that the concomitant increase in PTP1B and decrease in TCPTP protein levels in the pancreases of HFD fed mice may function in concert for the mitigation of ER stress signaling.

Differential Regulation of ER Stress-induced SFK Signaling by PTP1B and TCPTP—Our studies indicate that PTP1B and TCPTP function coordinately to regulate ER stress signaling in β cells. Previous studies have established the capacity for these two phosphatases to differentially contribute to the regulation of Src family kinases (SFKs). PTP1B dephosphorylates the inhibitory C-terminal c-Src Tyr^{527} phosphorylation site to activate c-Src (39, 40), whereas TCPTP dephosphorylates the tyrosine kinase activation loop Tyr^{416} site to inactivate c-Src (41). The numbering in our article refers to the prototypical chicken c-Src sequence (Tyr^{527} in chicken c-Src; Tyr^{529} in mammalian c-Src, and Tyr^{416} in chicken c-Src; and Tyr^{418} in mammalian c-Src). Accordingly, we reasoned that the increased PTP1B and decreased TCPTP protein levels in response to ER stress *in vivo* might serve to activate SFKs. As a first step toward assessing this possibility, we assessed the activation status of SFKs in pancreatic lysates in response to ER stress using SFK Tyr^{527} and Tyr^{416} phosphorylation-specific antibodies. Moreover, we monitored the tyrosine phosphorylation status of the SFK substrate STAT3 (signal transducer and activator of transcription 3) using antibodies specific for the STAT3 Tyr^{705} site as an additional measure of SFK activation. SFK Tyr^{416} and STAT3 Tyr^{705} phosphorylation were dramatically increased in pancreatic lysates of mice on a HFD as compared with those on chow (Fig. 3, *A* and *B*).

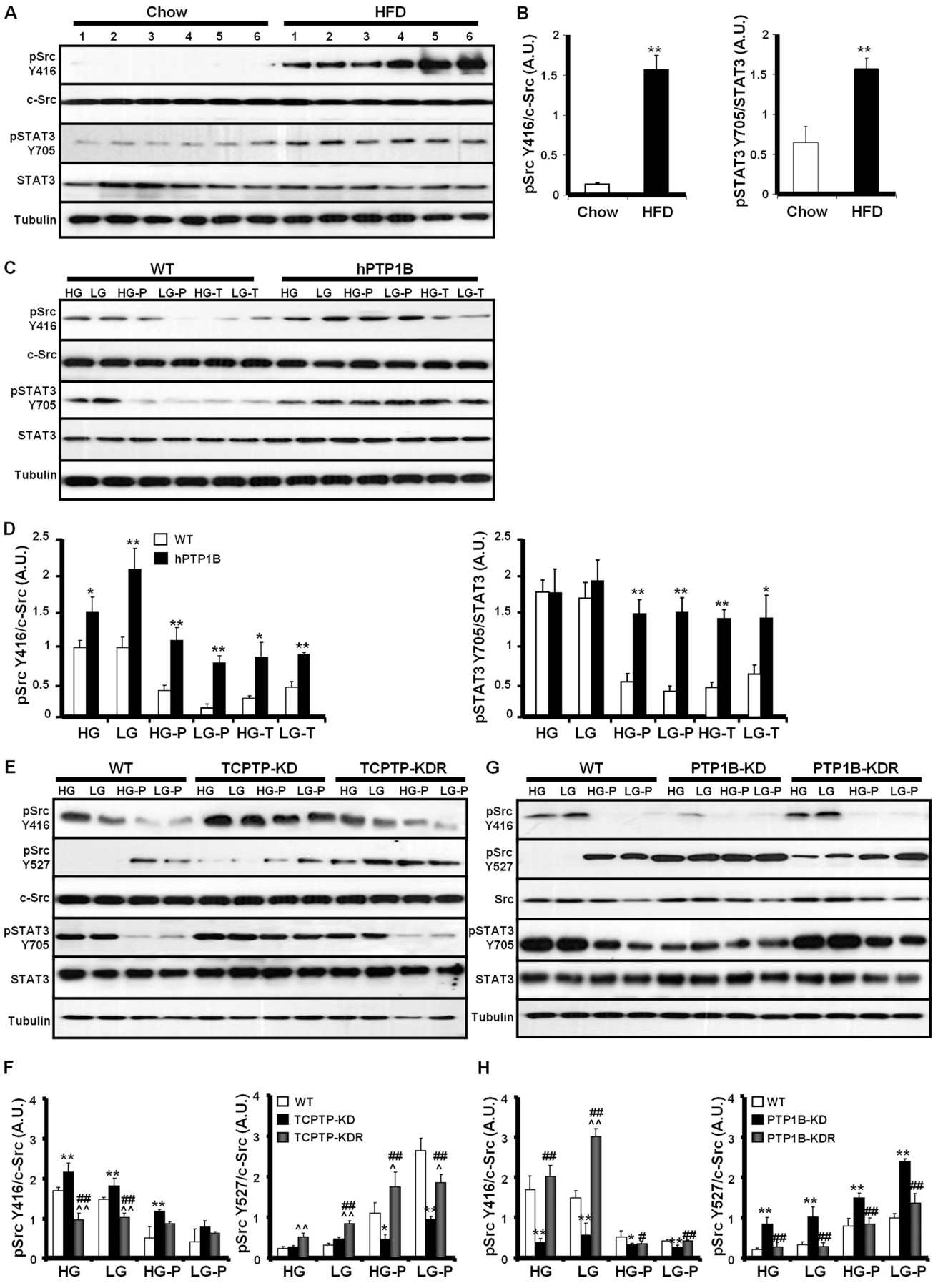
Next, we assessed the impact of overexpressing PTP1B *versus* knocking down TCPTP in MIN6 cells on palmitate-induced SFK signaling. For these studies, MIN6 cells were treated with palmitate for 24 h, a condition that promotes ER stress but does not elicit changes in PTP1B and TCPTP levels. Overexpression of PTP1B enhanced SFK activation, as monitored by Tyr^{416} phosphorylation and the downstream phosphorylation of STAT3 (Fig. 3, *C* and *D*). Similarly, TCPTP knockdown increased SFK and STAT3 activation and TCPTP reconstitution reversed this (Fig. 3, *E* and *F*). On the other hand, PTP1B knockdown decreased SFK and STAT3 activation (Fig. 3, *G* and *H*). Comparable results were observed using tunicamycin (supplemental Fig. S2). Taken together, these results indicate that ER stress-induced changes in PTP1B and TCPTP protein levels may serve to promote SFK signaling.

To determine whether the activation of SFKs associated with changes in PTP1B and TCPTP levels after prolonged ER stress might in turn impact on ER stress signaling, we treated TCPTP KD cells with SFK inhibitors PP2 (42) or SU6656 (43) as described under "Experimental Procedures." SFK activation in TCPTP KD cells, as assessed using Tyr^{416} phosphorylation-specific antibodies, was significantly reduced after treatment with PP2 (Fig. 4). Comparable results were obtained in cells treated with SU6566 (supplemental Fig. S3). Treatment of TCPTP KD cells with PP2 or SU6566 attenuated the protective effect of TCPTP deficiency on palmitate- and tunicamycin-induced ER stress; TCPTP KD cells treated with PP2 or SU6566 exhibited increased PERK and eIF2α phosphorylation and elevated BiP and caspase-3 expression (Fig. 4 and supplemental Fig S3, *A* and *B*). Treatment of PTP1B KD cells with PP2 or SU6566 did not lead to significant alterations in PERK and eIF2α phosphorylation and expression of BiP and caspase-3 consistent with the decreased Src activity in these cells (data not shown). Moreover, expression of constitutively active mutant c-Src Y527F in PTP1B KD cells significantly mitigated palmitate- and tunicamycin-induced ER stress, as well as BiP and caspase-3 expression compared with PTP1B KD cells expressing c-Src wild type (Fig. 4, *C* and *D*). Taken together, these findings indicate that PTP1B and TCPTP differentially regulate SFK activation in the context of ER stress. Moreover, these results indicate that alterations in PTP1B and TCPTP protein levels in response to prolonged or chronic ER stress might serve to activate SFK signaling for the attenuation of pancreatic ER stress.

PTP1B and TCPTP Regulate ER Stress-induced Cell Survival—ER stress is deployed by β cells to cope with nutrient-induced changes in protein synthesis and to balance newly synthesized protein load against the ER protein-folding capacity. However, excessive ER stress leads to cell death through mitochondrial-dependent and -independent pathways. To investigate the regulation of ER stress-induced apoptotic signaling by TCPTP and PTP1B, we evaluated the expression of proteins that mediate cell death in response to ER stress. p53 has been implicated in palmitate- and tunicamycin-induced apoptosis (44, 45). Once activated, p53 can transactivate genes such as Bax, which regulates downstream caspase-mediated apoptosis (46). TCPTP knockdown in MIN6 cells led to decreased expression of p53 and Bax and a decreased Bax/Bcl-xL ratio when compared with controls (Fig. 5*A*), whereas PTP1B knockdown led to increased expression of p53 and Bax and an increased Bax/Bcl-x_L ratio (Fig. 5*B*). Comparable results were observed using tunicamycin (supplemental Fig. S4, *A* and *B*).

Caspase-3, -7, -9, and -12 are involved in ER stress-induced cell death (47). After exposure to apoptotic stimuli, cells activate initiator caspases (such as caspase-8 and -9) that proteolytically cleave and activate effector caspases (such as caspase-3 and -7) to dismantle dying cells (48). Accordingly, we next

FIGURE 2. **Regulation of ER stress signaling by PTP1B and TCPTP.** MIN6 cells overexpressing hPTP1B (*A*) with KD and reconstituted expression of TCPTP (*C*) and PTP1B (*E*) were cultured in high (*HG*) and low (*LG*) glucose media, then treated with palmitate (*HG-P*; *LG-P*) or tunicamycin (*HG-T*; *LG-T*). Total cell lysates were immunoblotted for pPERK (Thr980), PERK, peIF2α (Ser51), eIF2α, BiP, XBP1, TCPTP, mouse (*m*), and human (*h*) PTP1B and tubulin. *Blots* were scanned and quantitated using Fluorchem 8900 (Alpha Innotech). *B*, *D*, and *F*, *bar graphs* represent normalized data expressed as arbitrary units (*A.U.*) for pPERK/PERK and peIF2α/eIF2α from three independent experiments and presented as means $\pm$ S.E. Statistical analysis was performed using two-tailed Student's *t*-test. In *B*, an *asterisk* indicates a significant difference between WT and hPTP1B-expressing cells. In *D* and *F*, an *asterisk* indicates a significant difference between WT and KD cells; # a significant difference between KD and KDR cells; and ^, a significant difference between KDR and WT cells. A single symbol (such as *) corresponds to $p < 0.05$, while double symbol (such as **) corresponds to $p < 0.01$.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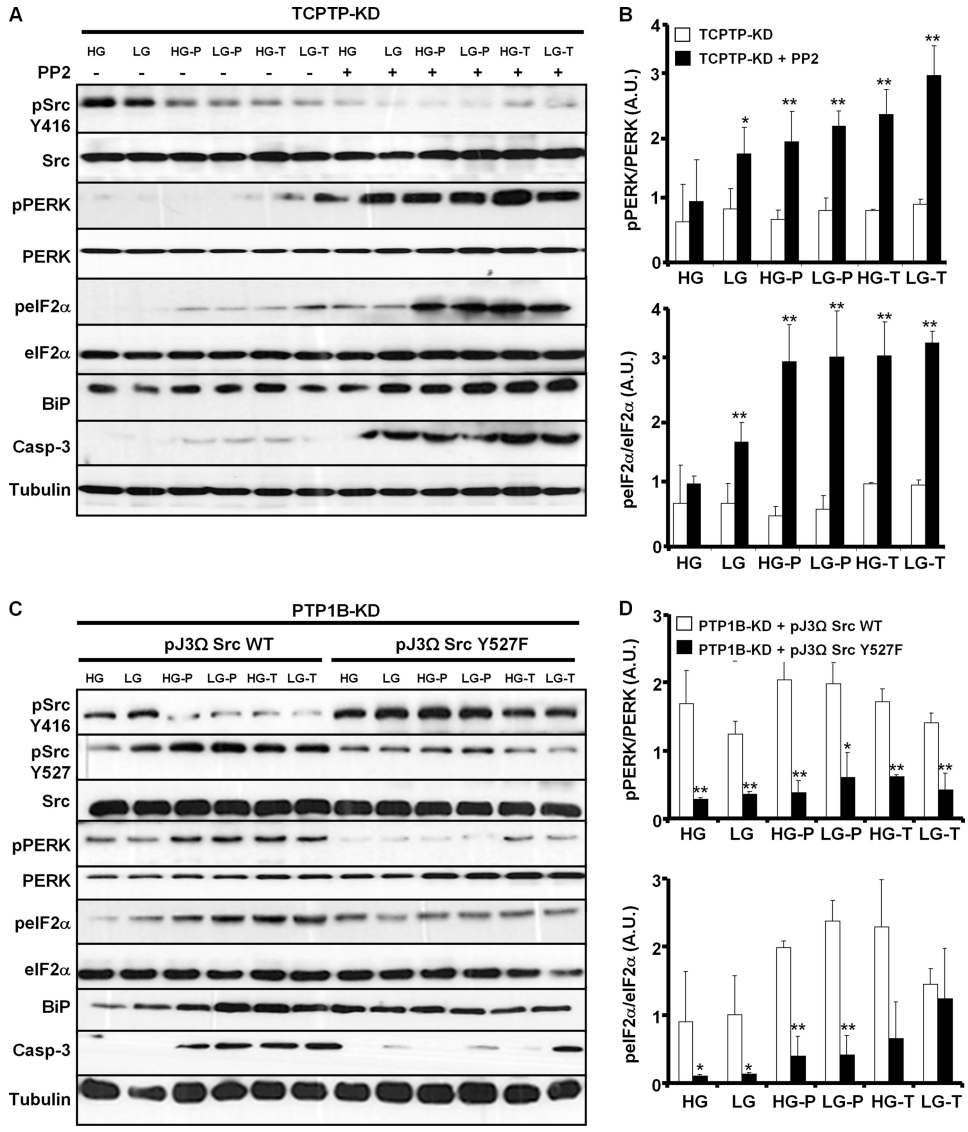
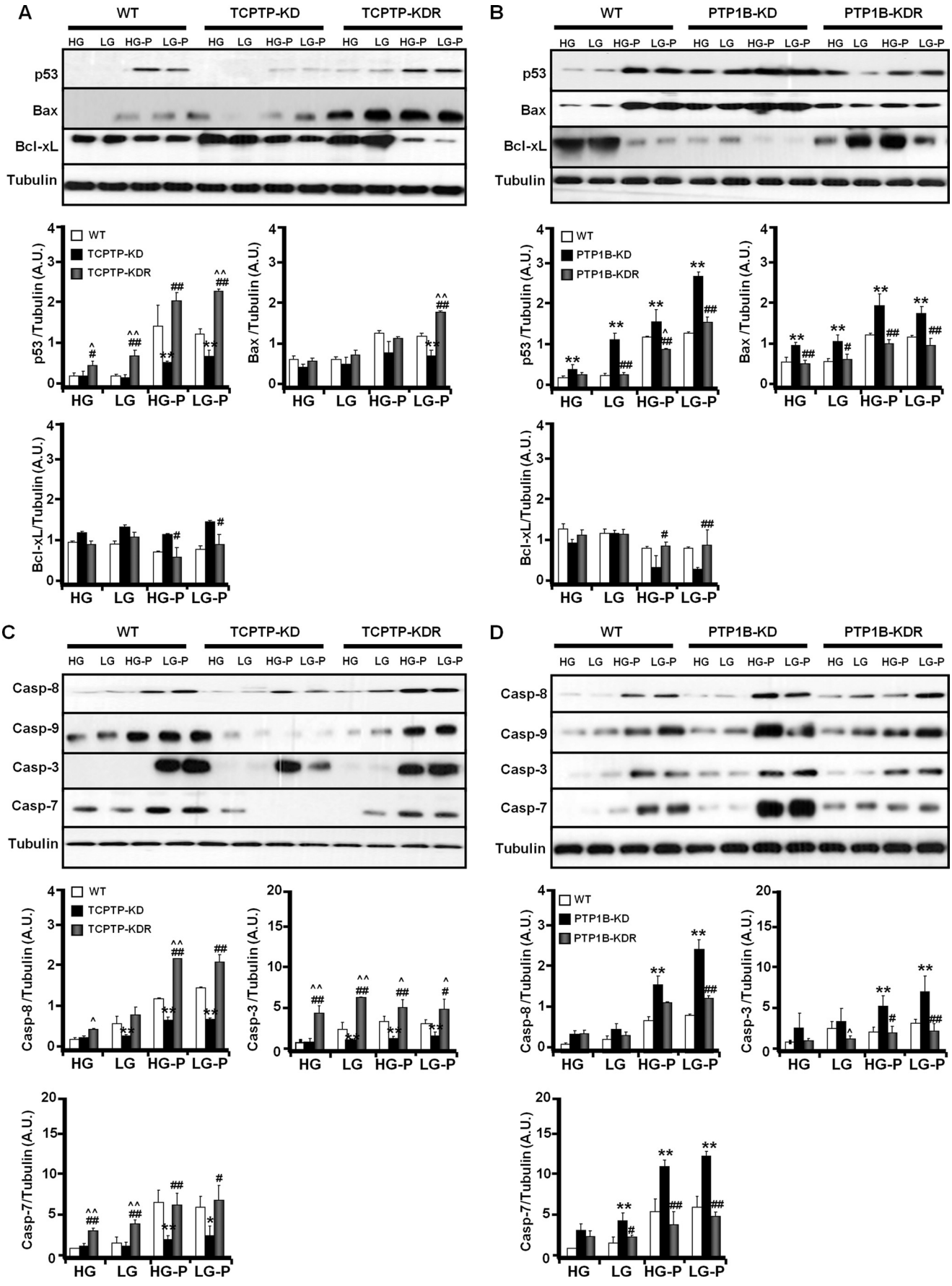


FIGURE 4. **SFK activation regulates plamitate- and tunicamycin-induced ER stress.** *A*, cells with TCPTP KD were treated with the SFK inhibitor PP2. Total cell lysates were immunoblotted for pSrc (Tyr^{416}), Src, pPERK (Thr^{980}), PERK, peIF2α (Ser^{51}), eIF2α, BiP, sXBP1, caspase-3 (*Casp-3*), and tubulin. *C* and *D*, MIN6 cells with PTP1B KD were transiently transfected with WT or constitutively active mutant of Src (Y527F), both in pJ3Ω vector. Cells were cultured in high and low glucose media and then treated with palmitate or tunicamycin. Total cell lysates were immunoblotted for pSrc (Tyr^{416}), c-Src, STAT3, pPERK (Thr^{980}), PERK, peIF2α (Ser^{51}), eIF2α, BiP, caspase-3, and tubulin. *B* and *D*, bar graphs represent normalized data for pPERK (Thr^{980}) and peIF2α (Ser^{51}) from at least two independent experiments and presented as means ± S.E. In *B*, an *asterisk* indicates significant difference between TCPTP KD cells with and without PP2 treatment. In *D*, an *asterisk* indicates significant difference between PTP1B KD expressing c-Src Y527F and c-Src WT.

assessed ER stress-induced expression of initiator and effector Caspases in control *versus* TCPTP or PTP1B KD and their respective KDR cells. TCPTP knockdown significantly decreased the expression of active caspase-8, -9, -3, and -7 in response to palmitate- or tunicamycin-induced ER stress (Fig. 5*C* and supplemental Fig. S4*C*). Conversely, PTP1B deficiency led to significantly increased the expression of active caspase-8, -9, -3, and -7 compared with controls (Fig. 5*D*; supplemental Fig. S4*D*).

To evaluate the contributions of PTP1B and TCPTP on ER stress-induced cell death directly, we monitored palmitate-induced chromatin condensation and DNA fragmentation (using Hoechst staining). In control cells cultured in high and low glucose media, apoptosis increased by 26 and 34%, respectively,

FIGURE 3. **Differential regulation of ER stress-induced SFKs signaling by PTP1B and TCPTP.** *A*, pancreata were isolated from male mice on regular chow and a HFD for 22 weeks and lysates immunoblotted for pSrc (Tyr$_{416}$), Src, pSTAT3 (Tyr$_{705}$), STAT3, and tubulin. Each *lane* represents a sample from a separate animal. MIN6 cells over-expressing hPTP1B (*C*) and with knockdown and reconstituted expression of TCPTP (*E*) and PTP1B (*G*) were cultured in high (*HG*) and low (*LG*) glucose media, then treated with palmitate (*HG-P*; *LG-P*) or tunicamycin (*HG-T*; *LG-T*). All *blots* were scanned and quantitated using Fluorchem 8900. *B*, *D*, *F*, and *H*, *bar graphs* represent normalized data expressed as arbitrary units (*A.U.*) for pSrc (Tyr$_{416}$)/Src, pSrc (Tyr$_{527}$)/Src, and pSTAT3 (Tyr$_{705}$)/STAT3 from three independent experiments and presented as means ± S.E. Statistical analysis was performed using two-tailed Student's *t*-test. In *B*, an *asterisk* indicates a significant difference between chow and HFD fed mice, and in *D*, a significant difference between WT and hPTP1B-expressing cells. In *F* and *H*, an *asterisk* indicates a significant difference between WT and KD cells; **#**, a significant difference between KD and KDR cells, and ^, a significant difference between KDR and WT cells. A single symbol (such as *) corresponds to $p < 0.05$, while a double symbol (such as **) corresponds to $p < 0.01$.


A
WT
TCPTP-KD
TCPTP-KDR
HG LG HG-P LG-P
p53
Bax
Bcl-xL
Tubulin
p53 /Tubulin (A.U.)
Bax /Tubulin (A.U.)
Bcl-xL/Tubulin (A.U.)
B
PTP1B-KD
PTP1B-KDR
C
Casp-8
Casp-9
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Casp-7/Tubulin (A.U.)
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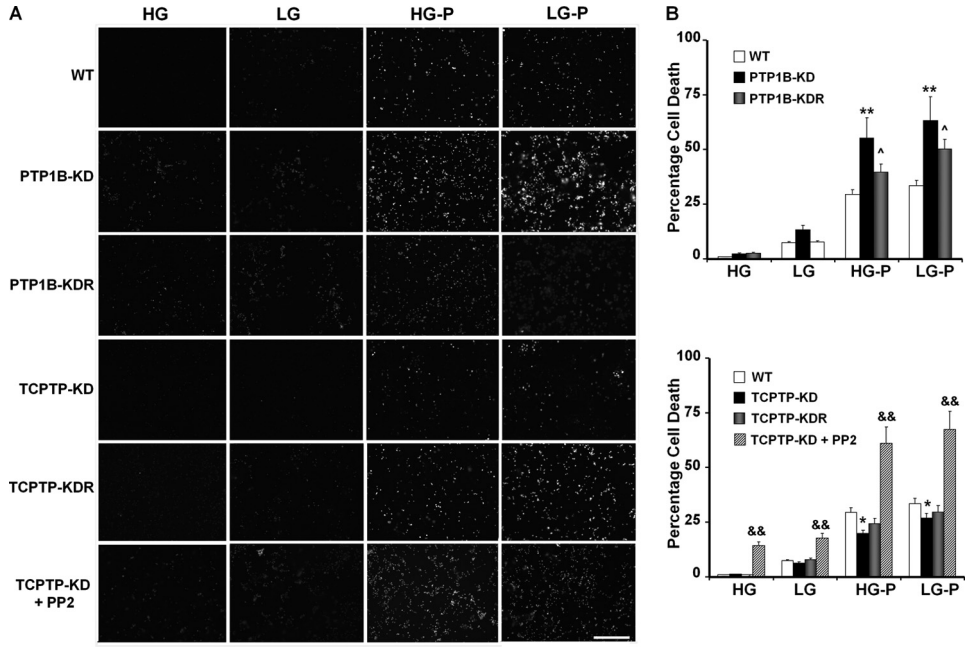

FIGURE 6. **Regulation of ER stress-induced apoptosis by TCPTP and PTP1B.** Cells were cultured in high and low glucose media, with or without SFKs inhibitor, and then ER stress was induced using palmitate. *A*, cells were stained with Hoechst as described under "Methods" and fractions of apoptotic cells, relative to total cell number, were determined after knockdown and reconstitution of PTP1B and TCPTP. *B*, *bar graphs* represent normalized data pooled from three independent experiments and are presented as means ± S.E. Statistical analysis was performed using two-tailed Student's *t*-test. An *asterisk* indicates significant difference between WT and KD cells; ^, a significant difference between KDR and WT cells; and &, a significant difference between KD cells with and without PP2 treatment. A single symbol (such as *) corresponds to $p < 0.05$, while a doble symbol (such as **) corresponds to $p < 0.01$.

after palmitate treatment (Fig. 6, *A* and *B*). In line with our biochemical observations, TCPTP knockdown significantly reduced apoptosis under high (20%) and low (27%) glucose conditions, whereas PTP1B knockdown increased palmitate-induced apoptosis under high (55%) and low (64%) glucose conditions (Fig. 6, *A* and *B*).

Finally, to determine the potential for altered PTP1B/TCPTP levels and elevated SFK signaling to impact on cell survival under conditions of chronic ER stress, we assessed the consequence of inhibiting SFKs on palmitate-induced ER stress signaling and cell death in TCPTP KD cells. The inhibition of SFKs with PP2 in TCPTP KD cells resulted in a significant enhancement in palmitate-induced ER stress signaling as shown previously. This in turn coincided with a significant increase in palmitate-induced effector caspase-3 expression and a significant increase in ER stress-induced cell death (Fig. 6, *A* and *B*). These results indicate that PTP1B-deficient MIN6 cells are susceptible to ER stress-induced apoptosis, whereas TCPTP-deficient cells are resistant. Moreover, these results suggest that activation of SFKs following a period of prolonged ER stress might serve to protect cells against ER stress-induced apoptosis.

DISCUSSION

The role of PTP1B and TCPTP in regulating ER stress signaling in β cells has heretofore remained largely unexplored. We demonstrate expression of these phosphatases in the endocrine pancreas and reveal differential expression in response to ER stress in pancreas and MIN6 β cells. Our findings establish PTP1B and TCPTP as key regulators of PERK/eIF2α arm of ER stress signaling sub-pathway. This arm of ER stress signaling is critical for the UPR in pancreatic islets. Indeed, PERK knockout mice exhibit progressive loss of β cells and develop diabetes a few weeks after birth (30). In addition, homozygous eIF2α S51A (the residue that is phosphorylated by all eIF2α kinases) knock-in mice exhibit more severe dysfunction of β cells compared with PERK knock-outs (31). Together, our findings reveal that the regulated expression of pancreatic PTP1B and TCPTP in response to high fat feeding, and potentially other challenges, modulates PERK/eIF2α signaling and presents an adaptive mechanism to mitigate ER stress.

Differential regulation of ER stress in MIN6 β cells by PTP1B and TCPTP is due, at least in part, to the opposing effects of these phosphatases on SFKs activation. Our observations are in line with previously proposed roles for these phosphatases. PTP1B activates c-Src by dephophorylating Tyr^{527} (39, 40), whereas TCPTP inactivates c-Src by dephosporylating Tyr^{416} (41). Consistent with published observations, knockdown of PTP1B in MIN6 cells led to increased Tyr^{527} phosphorylation (SFK inactivation), whereas TCPTP knockdown led to

FIGURE 5. **Regulation of ER stress-induced apoptosis signaling by TCPTP and PTP1B.** MIN6 cells with KD and reconstituted expression of TCPTP (*A*, *C*) and PTP1B (*B*, *D*) were cultured in high (*HG*) and low (*LG*) glucose media then treated with palmitate (*HG-P*; *LG-P*). Total cell lysates were immunoblotted for p53, Bax, Bcl-xL, and tubulin (*A*, *B*), and Caspases 8, 9, 3, and 7, and tubulin (*C*, *D*). *Blots* are representative of three independent experiments. *Bar graphs* represent normalized data expressed as arbitrary units (*A.U.*) for p53, Bax, Bcl-xL, Caspases 8, 3, and 7 from three independent experiments and presented as means ± S.E. Statistical analysis was performed using two-tailed Student's *t*-test. An *asterisk* indicates a significant difference between WT and KD cells; #, a significant difference between KD and KDR cells; and ^, a significant difference between KDR and WT cells. A single symbol (such as *) corresponds to $p < 0.05$, while a double symbol (such as **) corresponds to $p < 0.01$.

increased Tyr^{416} phosphorylation (SFK activation). Importantly, in TCPTP KD cells, the increased SFK activation correlated with decreased ER stress response and pharmacological inhibition of SFKs (using PP2 and SU6656) resulted in increased ER stress. In addition, expression of constitutively active mutant c-Src in PTP1B KD cells significantly mitigated ER stress. These findings are in line with emerging evidence that implicate SFKs in ER stress. Ulianich *et al.* (49) reveal that ER stress induces dedifferentiation and an epithelial-mesenchymal transition in thyroid cells (PC C13). Notably, SFKs are activated following ER stress in these cells and their inhibition with PP2 prevents epithelial dedifferentiation. In addition, Src has been proposed to regulate ER stress-induced transcriptional activation of BiP (50). In our studies, the phosphorylation of STAT3 and its activation correlated with SFK activation status. STAT3 is an established regulator of cell survival and inhibits apoptosis by up-regulating prosurvival proteins (51). The increased activation of STAT3 in TCPTP KD cells could account for the decreased apoptosis. Together, changes in pancreatic PTP1B/TCPTP expression associated with prolonged ER stress and emulated by TCPTP KD and PTP1B overexpression in MIN6 cells may be a compensatory response to chronic ER stress to activate SFKs and attempt to delay cell death. Overall, our findings provide evidence that regulation of ER stress signaling and responses by PTP1B and TCPTP is mediated through a SFK signaling pathway.

Conceivably, PTP1B and TCPTP can regulate ER stress through additional mechanism(s). One scenario involves direct interaction and dephosphorylation of component(s) in PERK/eIF2α subpathway. Mass spectroscopy studies reveal tyrosine phosphorylation of recombinant PERK (52). In addition, PERK is capable of autophosphorylating on tyrosine residues (53). In particular, Tyr^{615} plays an essential role in PERK kinase activation, and its phosphorylation coincides with phosphorylation at Thr^{980} (53). Additional studies are required to address the potential regulation of PERK activation through tyrosine dephosphorylation by PTP1B and/or TCPTP. In addition, we do not exclude the possibility that these PTPs regulate IRE1α and/or ATF6 arms of the ER stress response. Indeed, the absence of PTP1B in fibroblasts leads to impaired ER stress-induced IRE1α signaling in fibroblasts (21).

TCPTP deletion in pancreatic β cells sensitizes them to IFN-γ-induced apoptosis (19), whereas PTP1B deficiency in fibroblasts (21) and liver (22) attenuates ER stress-induced apoptosis and protects against HFD-induced ER stress, respectively. By contrast, our studies demonstrate that TCPTP deficiency mitigates, whereas PTP1B deficiency enhances ER stress in β cells. The reasons for these differences are not apparent but could be due to different systems used (MIN6, fibroblasts, and liver) and/or inducers of ER stress and apoptosis (plamitate, tunicamycin *versus* Azc, IFN-γ, and HFD). Conceivably, PTP1B and TCPTP have distinct substrates and/or regulate distinct arms of ER stress signaling in different cells/tissues. Alternatively, they may affect the same pathways in different cells/tissues, but the effects of those pathways and/or the feedback regulatory pathways may differ in a tissue-specific manner. Of note, the duration and amplitude of PERK activation could determine the switch from PERK to IRE1α signaling. Extended PERK and IRE1α signaling have opposite effects on cell viability. Sustained PERK signaling impairs cell proliferation and promotes apoptosis in 293 cells, whereas sustained IRE1α signaling enhances cell proliferation without promoting cell death (54).

Our data provide new insights into the regulation of ER stress and reveal that PTP1B and TCPTP function in nonredundant manner to regulate pancreatic ER stress and apoptosis. Modulation of pancreatic PTP1B and TCPTP expression may be an adaptive mechanism that mitigates chronic ER stress. A better understanding of the dynamic regulation of these phosphatases will likely have implications for modulating pancreatic function and metabolic responses.

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