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ARTICLE

Pancreatic T cell protein–tyrosine phosphatase deficiency affects beta cell function in mice

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Abstract

Aims/hypothesis T cell protein tyrosine phosphatase (TCPTP, encoded by *PTPN2*) regulates cytokine-induced pancreatic beta cell apoptosis and may contribute to the pathogenesis of type 1 diabetes. However, the role of TCPTP in pancreatic endocrine function and insulin secretion remains largely unknown.

Yannan Xi and Siming Liu contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-014-3413-7) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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Methods To investigate the endocrine role of pancreatic TCPTP we generated mice with pancreas *Ptpn2/*TCPTP deletion (panc-TCPTP KO).

Results When fed regular chow, panc-TCPTP KO and control mice exhibited comparable glucose tolerance. However, when challenged with prolonged high fat feeding panc-TCPTP KO mice exhibited impaired glucose tolerance and attenuated glucose-stimulated insulin secretion (GSIS). The defect in GSIS was recapitulated in primary islets ex vivo and after TCPTP pharmacological inhibition or lentiviral-mediated TCPTP knockdown in the glucose-responsive MIN6 beta cells, consistent with this being cell autonomous. Reconstitution of TCPTP in knockdown cells reversed the defect in GSIS demonstrating that the defect was a direct consequence of TCPTP deficiency. The reduced insulin secretion in TCPTP knockdown MIN6 beta cells was associated with decreased insulin content and glucose sensing. Furthermore, TCPTP deficiency led to enhanced tyrosyl phosphorylation of signal transducer and activator of transcription 1 and 3 (STAT 1/3), and substrate trapping studies in MIN6 beta cells identified STAT 1/3 as TCPTP substrates. STAT3 pharmacological inhibition and small interfering RNA-mediated STAT3 knockdown in TCPTP deficient cells restored GSIS to control levels, indicating that the effects of TCPTP deficiency were mediated, at least in part, through enhanced STAT3 phosphorylation and signalling.

Conclusions/interpretation These studies identify a novel role for TCPTP in insulin secretion and uncover STAT3 as a physiologically relevant target for TCPTP in the endocrine pancreas.

Keywords Insulin secretion · *Ptpn2* · STAT1 · STAT3 · T cell protein–tyrosine phosphatase · Type 2 diabetes

Abbreviations

ER	Endoplasmic reticulum

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GK	Glucokinase
GLUT2	Glucose transporter 2
GSIS	Glucose-stimulated insulin secretion
GTT	Glucose tolerance test
HFD	High fat diet
IR	Insulin receptor
ITT	Insulin tolerance test
KO	Knockout
Panc-TCPTP KO	Pancreatic *Ptpn2*/TCPTP knockout
PDX1	Pancreatic and duodenal homeobox 1
PTKs	Protein tyrosine kinases
PTP1B	Protein tyrosine phosphatase 1B
PTPs	Protein tyrosine phosphatases
RIP	Rat insulin promoter
RTKs	Receptor protein tyrosine kinases
SHP2	Src homology phosphatase 2
shRNA	Small hairpin RNA
siRNA	Small interfering RNA
STAT	Signal transducer and activator of transcription
TCPTP	T cell protein tyrosine phosphatase

Introduction

Type 2 diabetes is one of the most prevalent metabolic diseases and is characterised by hyperinsulinaemia, insulin resistance and pancreatic beta cell dysfunction [1]. Beta cells dynamically respond to fluctuations in blood glucose levels by the regulated secretion of insulin to modulate glucose homeostasis [2]. Tyrosyl phosphorylation is an important regulator of insulin secretion and action, and is tightly controlled by the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Pancreas-specific deletion of receptor PTKs (RTKs) implicated the insulin receptor (IR) [3] and insulin growth factor 1 receptor [4] in beta cell function in mice. However, the role of PTPs in beta cell function remains largely unexplored.

T cell protein tyrosine phosphatase (TCPTP; encoded by *PTPN2*) is a ubiquitously expressed PTP [5]. TCPTP has two splice variants: a 48 kDa variant, which is anchored to the endoplasmic reticulum (ER), and a 45 kDa variant that has access to cytosolic and nuclear substrates [6]. TCPTP has several substrates that include: (1) RTKs such as the IR [7] and epidermal growth factor receptor [6]; (2) non-receptor PTKs such as c-Src [8] and Janus family kinases 1/3 [9]; (3) substrates of RTKs such as signal transducer and activator of transcription (STAT) 1, 3, 5 and 6 [10–13]. Accordingly, TCPTP has the capacity to modulate diverse signalling pathways.

Insights into the physiological functions of TCPTP have emerged from studies in mice with global TCPTP deficiency.

TCPTP whole-body knockout (KO) mice exhibit haematopoietic defects and progressive systemic inflammatory disease, and die after birth thus hampering detailed assessment of their metabolic phenotype [14]. More recently, tissue-specific TCPTP deletion has helped define the cell type and tissue-specific functions of this phosphatase. T cell-specific TCPTP deficiency establishes a role for this phosphatase in attenuating T cell signalling [15]. While muscle-specific TCPTP deficiency does not alter insulin signalling and glucose homeostasis [16], brain-specific TCPTP deficiency leads to enhanced leptin sensitivity and resistance to high fat diet (HFD)-induced weight gain [17]. Moreover, liver-specific TCPTP deficiency promotes hepatosteatosis, obesity and insulin resistance [18]. The function of TCPTP in the pancreas remains largely unresolved, but a growing body of evidence suggests a role for this phosphatase in beta cell function. Genome-wide association studies identified *PTPN2* as a susceptibility gene in the pathogenesis of type 1 diabetes [19]. In addition, TCPTP regulates cytokine-induced beta cell apoptosis [19, 20]. Notably, progressive erosion of beta cell function is observed prior to the full manifestation of type 1 diabetes [21], and TCPTP could modulate insulin secretion during pathogenesis of the disease. Previously, we demonstrated that TCPTP modulates ER stress in MIN6 beta cells and that alterations in pancreatic TCPTP expression may serve to mitigate chronic ER stress [22]. More recently, we reported that pancreatic TCPTP deficiency mitigates cerulein-induced acute pancreatitis in mice [23]. In the current study, we investigated the endocrine role of pancreatic TCPTP and its underlying molecular mechanism.

Methods

Mouse studies Details of mouse studies are provided in the electronic supplementary material (ESM) Methods. Briefly, metabolic variables, insulin tolerance tests (ITTs), glucose tolerance tests (GTTs) and glucose-stimulated insulin secretion (GSIS) tests were performed as previously described [24]. All mouse studies were conducted according to federal guidelines and were approved by the Institutional Animal Care and Use Committee at University of California, Davis, CA, USA.

Cellular and biochemical studies MIN6 beta cell culture, knockdown and biochemical studies, pancreas immunostaining and morphometric analysis of islets are described in detail in the ESM Methods. Pharmacological inhibition of TCPTP and STAT3 was achieved by treating MIN6 beta cells with TCPTP inhibitor compound 8 (provided by Z-Y Zhang; 50 nmol/l) [25] and STAT3 inhibitor BP-1-102 (10 μmol/l; Millipore, Temecula, CA, USA) [26], respectively. TCPTP enzymatic activity was determined as described in the ESM Methods. SYBR Green (BioRad, Hercules, CA, USA)

quantitative real time PCR was used to determine mRNA of *CaCna1d*, *Gk*, *Glut2* (also known as *Slc2a2*), *Ins1*, *Ins2*, *Kir6.2* (also known as *Kcnj11*), *Pdx1* and *Ptpn2* using appropriate primers (ESM Table 1).

Statistical analyses Data are expressed as means±SEM. Repeated measures ANOVA was used to analyse multiple comparisons. Single data point comparisons were performed using Tukey–Kramer honest significant difference analyses; both analyses were performed using JMP program (SAS Institute, Cary, NC, USA). Differences were considered significant at $p<0.05$ and highly significant at $p<0.01$.

Results

Generation of pancreatic TCPTP KO mice To determine pancreatic TCPTP endocrine function we generated mice with pancreas *Ptpn2* deletion by crossing *Ptpn2*fl/fl (TCPTPfl/fl) mice to transgenic mice expressing *Cre* recombinase under the control of pancreatic and duodenal homeobox 1 (Pdx1) promoter. Pancreatic *Ptpn2*/TCPTP KO (panc-TCPTP KO) mice survived to adulthood and did not display gross defects in the pancreas. Immunoblot analysis of primary islets lysates revealed loss of TCPTP protein expression in panc-TCPTP KO compared with control mice (Fig. 1a). Similarly, immunoblotting of total pancreas lysates demonstrated significant reduction in TCPTP protein in panc-TCPTP KO compared with control mice (Fig. 1a), consistent with TCPTP deletion occurring in islet and non-islet cells. In addition, expression of protein tyrosine phosphatase 1B (PTP1B) and Src homology

phosphatase 2 (SHP2) was not altered upon TCPTP deletion. Importantly, TCPTP expression was not changed in liver, muscle, adipose tissue or hypothalamus (Fig. 1b). Immunostaining of pancreas sections from control mice revealed abundant TCPTP expression in the pancreas, which was predominantly nuclear, suggesting that the major murine isoform is the 45 kDa variant (Fig. 1c). Consistent with immunoblotting data, TCPTP staining was reduced in panc-TCPTP KO islets confirming its ablation in these mice. Together, these data demonstrate efficient TCPTP deletion in pancreatic islets enabling the determination of its metabolic function in the endocrine pancreas.

Prolonged high fat feeding impairs glucose tolerance in panc-TCPTP KO mice To investigate the endocrine effects of pancreatic TCPTP deletion we assessed metabolic alterations in control and panc-TCPTP KO mice fed a regular chow diet and HFD. Immunoblotting of pancreas lysates revealed decreased TCPTP expression in high fat-fed mice (ESM Fig. 1), consistent with previous studies [22]. Body weights were determined and, as expected, male mice fed HFD gained significantly more weight than their chow-fed counterparts, with control and panc-TCPTP KO mice exhibiting comparable body weights on both diets (Fig. 2a). Fed and fasted glucose concentrations were comparable between panc-TCPTP KO and control mice on either diet (ESM Fig. 2a). In addition, fed glucagon levels were comparable between groups, while fasted concentrations were lower in HFD-fed panc-TCPTP KO mice compared with chow-fed mice (ESM Fig. 2b). To directly assess the effect of pancreatic TCPTP deletion on glucose homeostasis, we determined the ability of mice to dispose of a glucose load by conducting GTTs at different
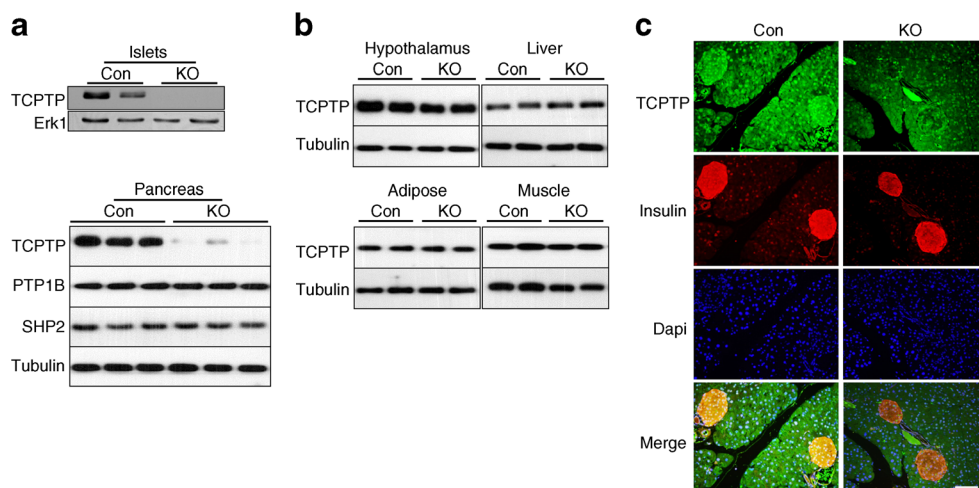

Fig. 1 Pancreatic *Ptpn2* (TCPTP) deletion. (**a**) Islets lysates from TCPTPfl/fl (Con) and panc-TCPTP KO mice immunoblotted for TCPTP and Erk1. Representative immunoblots are shown, *n*=3. Whole pancreas lysates from Con and KO mice immunoblotted for TCPTP, PTP1B, SHP2 and tubulin. (**b**) Representative immunoblots of TCPTP and tubulin in lysates of hypothalamus, liver, adipose and muscle of Con and KO mice, *n*=4. (**c**) Pancreases of Con and KO female mice fed HFD for 36 weeks were sectioned and immunostained for TCPTP (green), insulin (red) and DAPI (blue). Scale bar 100 μm

Fig. 2 Impaired glucose tolerance in HFD-fed panc-TCPTP KO mice. (**a**) Body weights of control (Con; white symbols) and panc-TCPTP KO (black symbols) male mice fed regular chow (full lines) or HFD (dotted lines). GTTs in male mice fed regular chow or HFD at (**b**) 9 weeks of age and (**c**) 45 weeks of age. (**d**) ITT in the same cohort at 51 weeks of age. Data are presented as mean±SEM. *$p<0.05$ control vs panc-TCPTP KO. Numbers of mice were as follows: HFD KO (**a**) 11, (**b**) 7, (**c**) 11, (**d**) 11; HFD Con (**a**) 7, (**b**) 6, (**c**) 5, (**d**) 6; chow KO (**a**) 9, (**b**) 7, (**c**) 7, (**d**) 8; chow Con (**a**) 4, (**b**) 4, (**c**) 3, (**d**) 4
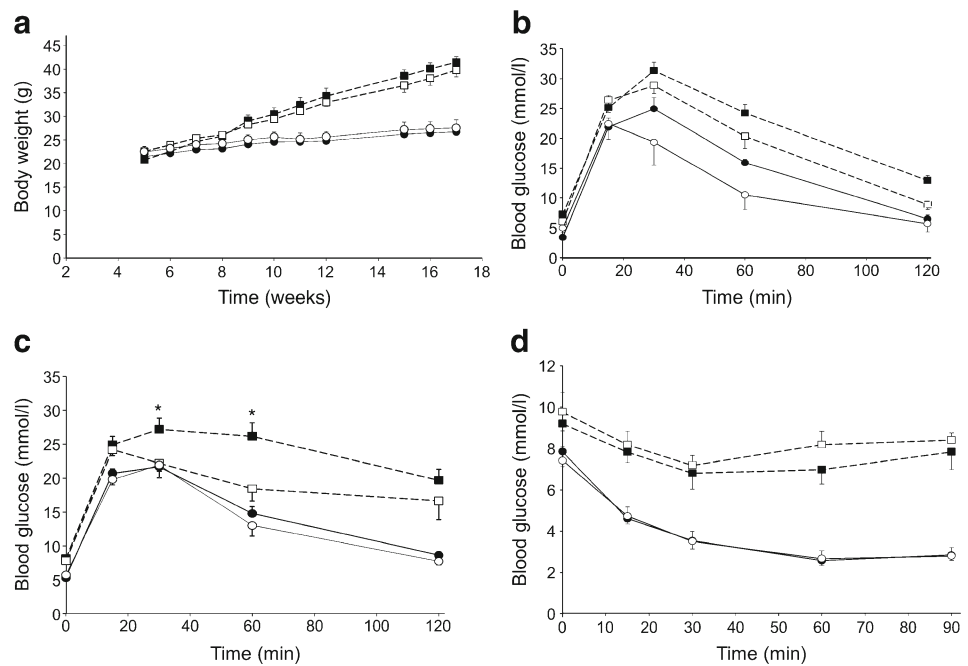
ages. Control and panc-TCPTP KO mice exhibited comparable glucose tolerance when fed regular chow (Fig. 2b, c). However, when fed HFD panc-TCPTP KO mice had a reduced ability to dispose of glucose load compared with controls. This was more pronounced when mice were older and fed HFD for prolonged periods. To determine whether or not differences in peripheral insulin sensitivity contribute to the glucose intolerance of panc-TCPTP KO mice, we performed ITTs. The two groups exhibited comparable insulin sensitivity when fed regular chow or HFD (Fig. 2d). Similar results were obtained in an independent cohort of mice (ESM Fig. 3). Together, these findings indicate that pancreatic TCPTP deficiency impairs glucose tolerance in mice upon prolonged high fat feeding.

Pancreatic TCPTP deficiency attenuates GSIS Impaired glucose tolerance in HFD-fed panc-TCPTP KO mice suggests potential defects in insulin secretion. To test this possibility, we determined concentrations of circulating insulin under basal and glucose-stimulated conditions in control and panc-TCPTP KO mice. Chow-fed panc-TCPTP KO exhibited comparable circulating insulin levels to controls (Fig. 3a). However, HFD-fed control mice exhibited a robust increase in insulin after glucose injection, whereas panc-TCPTP KO mice exhibited lower circulating insulin levels (Fig. 3a, b). To test whether the observed defect in insulin secretion in panc-TCPTP KO mice fed HFD was due to TCPTP deficiency in islets and not in other pancreatic cell types and/or secondary to neural effects, we determined insulin secretion in isolated primary islets ex vivo (Fig. 3c). A glucose dose-dependent increase in insulin secretion was observed in primary islets

from control mice. On the other hand, primary islets from panc-TCPTP KO mice exhibited attenuated GSIS compared with controls. Of note, glucagon-like peptide 1 can partially mitigate the attenuated insulin secretion in panc-TCPTP KO islets. To examine whether or not decreased circulating insulin concentrations in panc-TCPTP KO mice was due to decreased islet mass we immunostained pancreas sections for insulin and quantified islet surface area and number as detailed in the ESM Methods. Islet/pancreas surface area and islet number were comparable between HFD-fed control and panc-TCPTP KO mice (Fig. 3d, e). In addition, islet number/range of islet size was determined in mice fed regular chow and HFD (Fig. 3f). As expected, HFD-fed control and panc-TCPTP KO mice exhibited increased islet number/range of sizes compared with those fed regular chow, but islet number was comparable between HFD-fed control and panc-TCPTP KO mice.

To determine whether or not the defect in insulin secretion was directly caused by TCPTP deficiency, we performed lentiviral small hairpin (shRNA)-mediated TCPTP knockdown in MIN6 beta cells. TCPTP knockdown (~74%) was achieved without compensatory changes in PTP1B and SHP2 expression (Fig. 4a). In addition, knockdown cells were reconstituted with shRNA-resistant TCPTP. As a complementary approach, pharmacological inhibition of TCPTP was achieved using the selective inhibitor compound 8 [25]. TCPTP activity was significantly reduced (~80%) in compound 8-treated control cells (Fig. 4b). Knockdown cells exhibited approximately 60% reduction in TCPTP activity and this was restored to control levels upon TCPTP reconstitution. In line with ex vivo islet studies, TCPTP knockdown
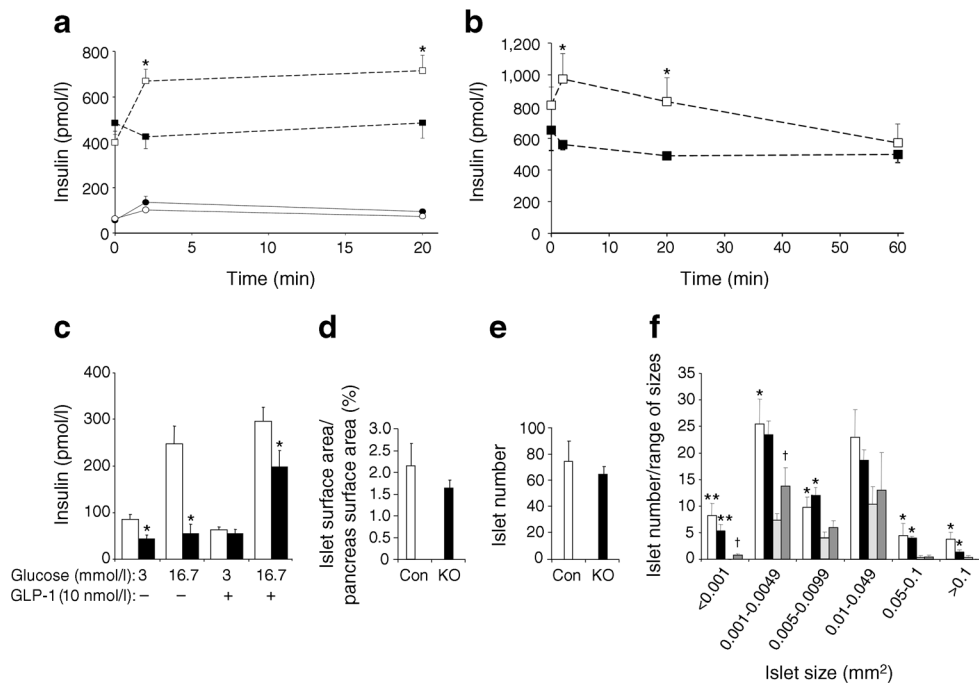
Fig. 3 TCPTP deficiency attenuates GSIS in HFD-fed mice and in islets. Circulating insulin concentrations in control (Con; white symbols) and panc-TCPTP KO (black symbols) male mice fed regular chow (full lines) or HFD (dotted lines) at (**a**) 52 weeks of age and (**b**) 58 weeks of age. (**c**) Primary islets from HFD-fed female control (white bars) and panc-TCPTP KO mice (black bars; 35 weeks old) were treated with glucose with or without glucagon-like peptide 1 (GLP-1). Data are presented as mean±SEM, $n=3$. *$p<0.05$ control vs panc-TCPTP KO. (**d**) Per cent islet surface area/pancreas surface area, (**e**) islet number and (**f**) islet number/range of sizes from HFD control (white bars) and panc-TCPTP KO (black bars) and chow-fed control (light grey bars) and panc-TCPTP KO (dark grey bars) male mice at 60 weeks of age. Data are presented as mean±SEM. *$p<0.05$; **$p<0.01$ chow vs HFD and $^†p<0.05$ control vs panc-TCPTP KO. Numbers of mice were as follows: HFD KO (**a**) 7, (**b**) 5, (**c**) 3, (**d**) 6, (**e**) 6, (**f**) 6; HFD Con (**a**) 5, (**b**) 5, (**c**) 3, (**d**) 4, (**e**) 4, (**f**) 4; chow KO (**a**) 8, (**f**) 4; chow Con (**a**) 3, (**f**) 3

cells exhibited reduced GSIS at 15, 30, 60 and 120 min (Fig. 4c). Importantly, reconstitution of knockdown cells with TCPTP restored insulin secretion to levels comparable with controls. Further, pharmacological inhibition of TCPTP in control cells led to attenuated GSIS that was comparable with knockdown cells (Fig. 4c). This finding was associated with decreased insulin content and glucose sensing in knockdown cells. Indeed, mRNA level of genes involved in beta cell function and differentiation, including *Glut 2* [27], *Pdx1* [28], *Gk* [29], *Ins1* [30], *Ins2* [31], *Kir6.2* [32] and *CaCna1d* [33], was reduced in knockdown cells compared with controls (Fig. 4d). Importantly, expression of these genes was restored to control level upon reconstitution of TCPTP in knockdown cells. Moreover, protein expression of GLUT2, PDX1, GK and KIR6.2 was decreased in TCPTP knockdown cells and restored to control level upon reconstitution (Fig. 4e, f). Collectively, these findings demonstrate that glucose intolerance in HFD-fed panc-TCPTP KO mice was likely to be due to a cell autonomous defect in GSIS that was directly caused by TCPTP deletion.

TCPTP regulates STAT1/3 tyrosyl phosphorylation in beta cells To investigate the molecular basis for decreased GSIS we evaluated tyrosyl phosphorylation of STAT3, a bona fide TCPTP substrate [34]. STAT3 is activated by phosphorylation at Tyr705 leading to relocation to the nucleus to promote gene expression [35]. Primary islets from HFD-fed control and panc-TCPTP KO mice were isolated and stimulated with glucose. Immunoblots revealed elevated STAT3 Tyr705 phosphorylation in panc-TCPTP KO islets compared with controls (Fig. 5a). We used MIN6 beta cells to determine whether or not the enhanced STAT3 tyrosyl phosphorylation in KO islets was cell autonomous and caused by TCPTP deficiency. Glucose stimulation enhanced STAT3 Tyr705 phosphorylation in control cells and this was further enhanced in TCPTP knockdown cells (Fig. 5b). Reconstitution of knockdown cells with TCPTP restored STAT3 phosphorylation to control levels. Similarly, STAT3 immunoprecipitation demonstrated that STAT3 total tyrosyl phosphorylation was enhanced in knockdown cells compared with controls and restored to control levels upon TCPTP reconstitution (Fig. 5c). The elevated STAT3 tyrosine phosphorylation in TCPTP knockdown cells prompted us to test whether or not STAT3 is a direct TCPTP substrate in beta cells. To that end, we used the substrate trapping mutant (wherein the catalytic essential aspartate-182 is mutated to alanine; D/A) [6]. TCPTP D/A was transiently expressed in knockdown cells and TCPTP co-association with STAT3 was determined (Fig. 5d). Modest co-
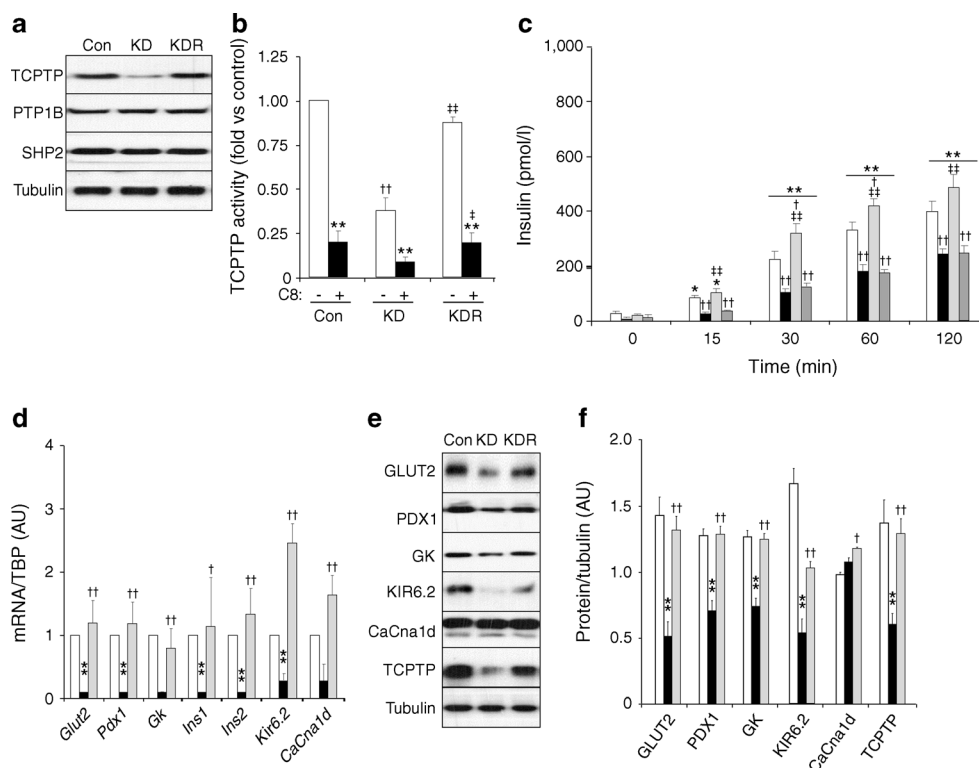
Fig. 4 TCPTP deficiency and pharmacological inhibition attenuate GSIS in MIN6 beta cells. (**a**) Representative immunoblots of TCPTP, PTP1B, SHP2 and tubulin in lysates of MIN6 beta cells (Con), TCPTP knockdown (KD) and reconstituted (KDR) cells, $n=4$. (**b**) TCPTP activity in Con, KD, KDR and TCPTP inhibitor (C8)-treated cells, $n=4$. **$p<0.01$ C8-treated vs non-treated cells; [††]$p<0.01$ KD vs Con; and [‡]$p<0.05$, [‡‡]$p<0.01$ KDR vs KD. (**c**) Basal insulin and GSIS in Con (white bars), KD (black bars), KDR (light grey bars) and C8-treated control (dark grey bars) cells, $n=5$. *$p<0.05$, **$p<0.01$ each cell type at the indicated time vs basal; [†]$p<0.05$, [††]$p<0.01$ each cell type vs Con at indicated time;

[‡‡]$p<0.01$ KDR vs KD at indicated time. (**d**) *Glut2*, *Pdx1*, *Gk*, *Ins1*, *Ins2*, *Kir6.2* and *CaCna1d* mRNA in Con (white bars), KD (black bars) and KDR (light grey bars) cells, $n=3$. **$p<0.01$ KD vs Con; [†]$p<0.05$, [††]$p<0.01$ KDR vs KD. Representative immunoblots (e) and corresponding quantitative bar graph (f) of GLUT2, PDX1, GK, KIR6.2, CaCna1d, TCPTP and tubulin in lysates of Con (white bars), KD (black bars) and KDR (light grey bars) cells, $n=3$. Bar graph represents normalised data for the indicated protein/tubulin as means±SEM. **$p<0.01$ KD vs Con; and [†]$p<0.05$, [††]$p<0.01$ KDR vs KD

immunoprecipitation was observed between TCPTP wild-type and STAT3 upon glucose stimulation and this was disrupted upon lysis in the stringent buffer RIPA (R; Fig. 5d). On the other hand, co-association of TCPTP D/A and STAT3 was detected under basal conditions and was significantly enhanced upon glucose stimulation. Importantly, treatment with pervanadate (V), an inhibitor of PTPs, disrupted TCPTP D/A–STAT3 interactions (Fig. 5d).

Additional members of the STAT family are likely to be regulated by TCPTP in the pancreas. Indeed, STAT1 immunoprecipitation revealed that STAT1 total tyrosyl phosphorylation was enhanced in knockdown cells compared with controls and restored to control levels upon TCPTP reconstitution (Fig. 5e). Moreover, STAT1 co-associated with TCPTP D/A in response to glucose was consistent with STAT1 serving as a TCPTP substrate (Fig. 5f).

STAT3 is a mediator of TCPTP endocrine function To investigate whether or not the effects of TCPTP deficiency on insulin secretion were mediated through STAT3 we

determined the effects of the STAT3 pharmacological inhibitor BP-1-102 on insulin secretion. We reasoned that STAT3 inhibition should mitigate the effects of TCPTP deficiency on GSIS. Indeed, STAT3 inhibitor-treated TCPTP knockdown exhibited significantly improved GSIS and insulin levels were comparable with controls (Fig. 6a). Of note, BP-1-102 treatment inhibited phosphorylation of STAT3 and STAT1 (Fig. 6b). Accordingly, siRNA-mediated knockdown was used to further assess the contributions of STAT3 vs STAT1 to insulin secretion (Fig. 6c, d). Indeed, STAT3 knockdown phenocopied STAT3 pharmacological inhibition. STAT3 knockdown significantly enhanced GSIS at 60 min in control cells, whereas in TCPTP knockdown cells STAT3 deficiency restored GSIS to control levels. By contrast, siRNA-mediated STAT1 knockdown did not significantly alter GSIS in control cells but enhanced GSIS in TCPTP knockdown cells, albeit to a lower extent than that observed for STAT3 knockdown (Fig. 6c). Together, these data demonstrate that TCPTP regulates insulin secretion, at least in part, through STAT3 and that it is a physiologically relevant target of TCPTP in the endocrine pancreas.
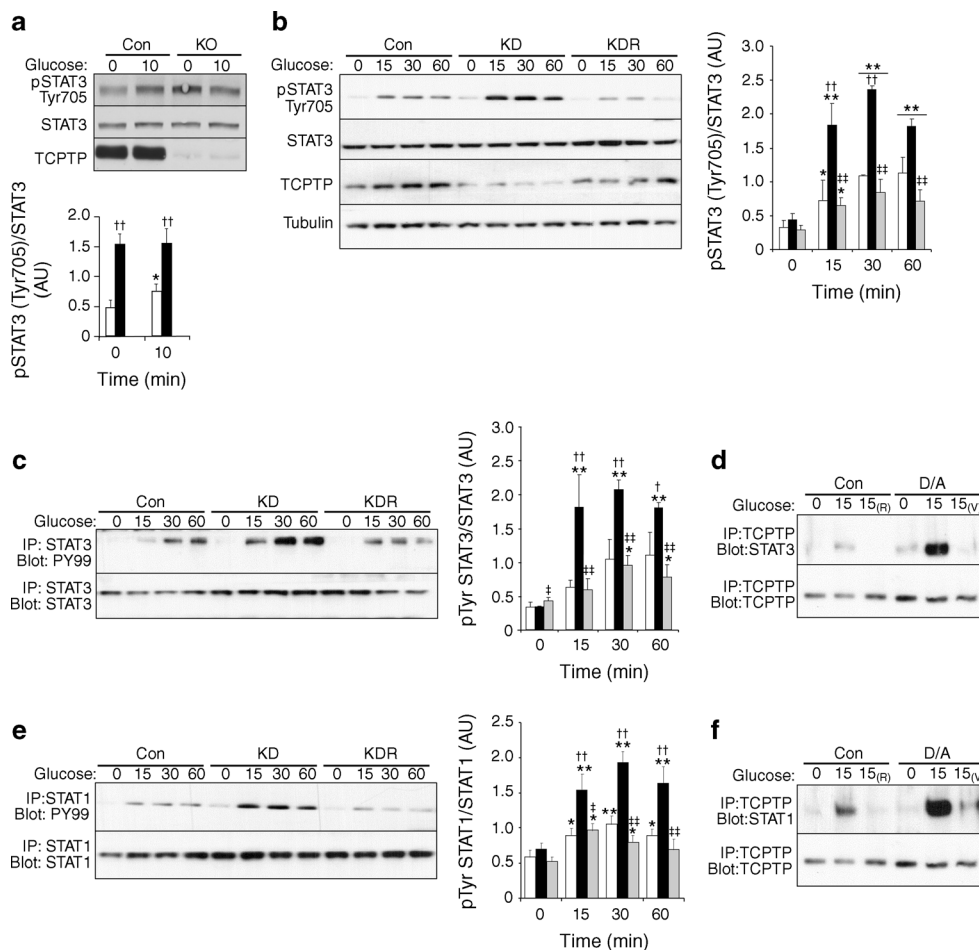
Fig. 5 TCPTP regulates STAT1/3 tyrosyl phosphorylation. (**a**) Representative immunoblots of pSTAT3 (Tyr705), STAT3 and TCPTP in islet lysates from HFD-fed male mice (59 weeks old) under basal and glucose-stimulated (25 mmol/l for 10 min) conditions. Bar graph represents normalised data for pSTAT3 (Tyr705)/STAT3 as means±SEM, $n=3$. *$p<0.05$ glucose-treated vs non-treated islets; ††$p<0.01$ KO (black bars) vs control (Con; white bars). (**b**) Immunoblots of pSTAT3 (Tyr705), STAT3, TCPTP and tubulin in lysates of Con (white bars), KD (black bars) and reconstituted (KDR; grey bars) cells under basal (0) and glucose-stimulated conditions (25 mmol/l for 15, 30 or 60 min), $n=3$. (**c**) STAT3 and **e** STAT1 immunoprecipitates from Con (white bars), KD (black bars) and KDR (grey bars) cells were immunoblotted for

phosphotyrosine (PY99), STAT3 and STAT1 under basal (0) and glucose-stimulated conditions (25 mmol/l for 15, 30 or 60 min). TCPTP immunoprecipitates from Con and TCPTP D/A-transfected cells were immunoblotted for (**d**) STAT3, (**f**) STAT1 and TCPTP under basal (0) and glucose-stimulated conditions (25 mmol/l for 15 min). (R) indicates RIPA lysis and (V) vanadate treatment. In (**b**), (**c**) and (**e**), representative immunoblots are shown from independent repeats and bar graphs represent data for pSTAT3 (Tyr705)/STAT3, pSTAT3/STAT3, and pSTAT1/STAT1 as means±SEM ($n=4$), respectively. For (**b**), (**c**) and (**e**) *$p<0.05$, **$p<0.01$ for each cell type at indicated times vs basal; †$p<0.05$, ††$p<0.01$ for each cell type vs Con at indicated times; ‡$p<0.05$, ‡‡$p<0.01$ KDR vs KD

Discussion

Deciphering the molecular mechanisms that modulate insulin secretion is a prerequisite for repairing beta cell dysfunction in type 2 diabetes. The role of TCPTP in beta cell function and insulin secretion has heretofore remained largely unexplored. The current study demonstrated that pancreatic TCPTP deficiency impaired glucose tolerance and attenuated GSIS in mice with prolonged high fat feeding. At the molecular level, we identified STAT3 as a TCPTP substrate in the endocrine pancreas and demonstrated that TCPTP regulated GSIS, at least in part, through STAT3. Together, these findings provide new insights into the endocrine function of pancreatic TCPTP.

In this study we used Pdx1-*Cre* mice to investigate the role of TCPTP in the endocrine pancreas. Some reports suggest that Pdx1-*Cre* transgenic lines exhibit *Cre* expression in select regions of the brain, including the hypothalamus [36]. This issue was not a factor in our studies since we observed selective TCPTP deletion in the pancreas. Although immunoblotting of hypothalamic lysates is not adequate to detect deletion in select hypothalamic neurons, the comparable gains in body weight of panc-TCPTP KO and controls on chow and HFD suggest this is not a confounding factor in these studies. This is particularly relevant given that TCPTP deletion in the brain has overt effects on body weight and adiposity [17], which was not observed in these studies. When challenged
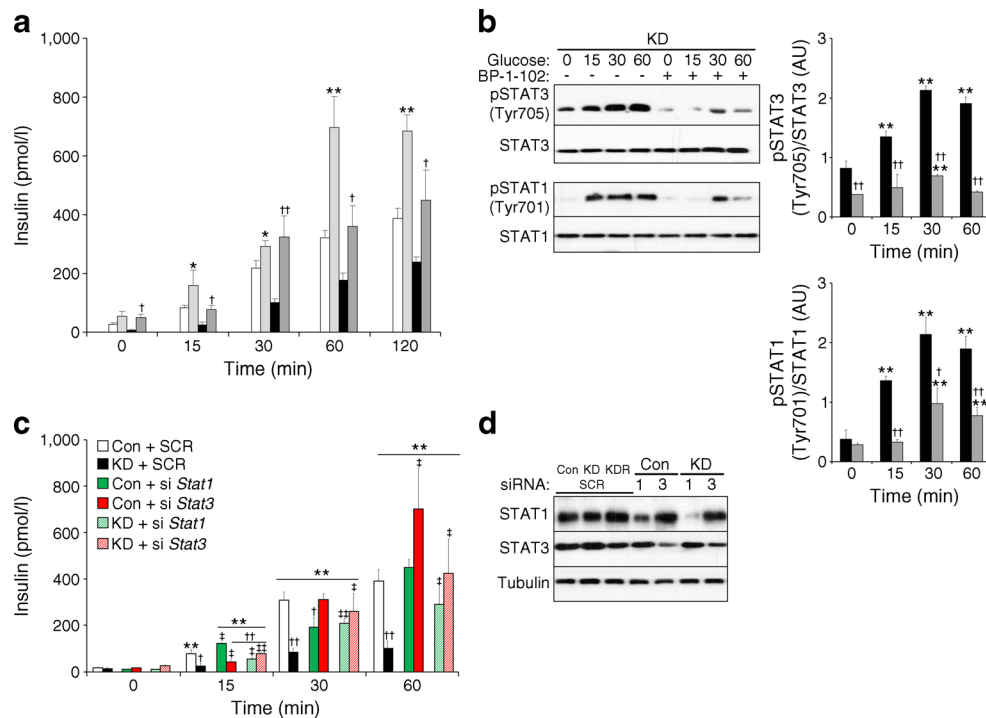
Fig. 6 STAT3 pharmacological inhibition and deficiency restore attenuated GSIS in TCPTP knockdown cells. (**a**) Basal insulin and GSIS in control (Con) and KD cells without (white bars and black bars, respectively) and with (light grey and dark grey bars, respectively) STAT3 inhibitor (BP-1-102) treatment, $n=4$. $*p<0.05$, $**p<0.01$ Con vs Con+BP-1-102 at indicated times; $^{†}p<0.05$, $^{††}p<0.01$ KD vs KD+BP-1-102 at indicated times. (**b**) pSTAT3 (Tyr705), STAT3, pSTAT1 (Tyr701) and STAT1 immunoblots of lysates from KD cells under basal and glucose-stimulated conditions (25 mmol/l for 15, 30 or 60 min), without (black bars) and with (grey bars) BP-1-102 treatment. Bar graph represents normalised data for pSTAT3 (Tyr705)/STAT3 and pSTAT1 (Tyr701)/ STAT1 as means±SEM, $n=4$. $**p<0.01$ glucose-treated vs non-treated cells at indicated times; $^{†}p<0.05$, $^{††}p<0.01$ KD vs KD+BP-1-102 at indicated times. (**c**) Basal insulin and GSIS in Con and KD cells with and without *Stat1* and *Stat3* silencing using siRNA, $n=3$. $**p<0.01$ each cell type at indicated time vs basal; $^{†}p<0.05$, $^{††}p<0.01$ each cell type vs Con at indicated times; $^{‡}p<0.05$, $^{‡‡}p<0.01$ cells with the indicated siRNA vs the same cell transfected with a non-silencing siRNA. (**d**) Representative immunoblots from three independent repeats of STAT1, STAT3 and tubulin in control and knockdown cells treated with non-silencing (SCR), *Stat1* (1) or *Stat3* (3) siRNA

with prolonged high fat feeding panc-TCPTP KO mice exhibited glucose intolerance that was independent of altered insulin sensitivity in the periphery and was due, in large part, to attenuated GSIS. The defect in insulin secretion was cell autonomous since it was recapitulated in primary islets and in MIN6 beta cells after TCPTP knockdown or pharmacological inhibition. However, we cannot completely rule out secondary effects in vivo and contributions from non-beta cells. Indeed, TCPTP inhibition and knockdown in MIN6 beta cells reduced GSIS but this was only observed in vivo after prolonged high fat feeding, implying that non-beta cell autonomous mechanisms modulate the panc-TCPTP KO mice phenotype. The reason for this finding is not clear but may be due to redundancies with other potential STAT3 regulatory phosphatases in chow-fed mice, such as PTP1B [24, 37] or SHP2 [38]. Additionally, we cannot rule out the potential influence of the genetic background. We used the C57Bl/6J strain, which has been reported to have a deletion in *Nnt*, and consequent reduced insulin secretion [39]. Of note, panc-TCPTP KO mice exhibit a phenotype that is comparable with obese glutathione peroxidase 1-deficient mice which display defective insulin

secretion associated with oxidation of PTPs (including TCPTP) and decreased PDX1 and insulin content [40]. Further, we recently demonstrated beneficial effects of pancreatic TCPTP deficiency in mitigating cerulein-induced acute pancreatitis [23], highlighting the diverse functions of TCPTP in the exocrine and endocrine pancreas. Finally, the finding that the metabolic effects of pancreatic TCPTP deficiency are influenced by diet are relevant to human type 2 diabetes, which is known to correlate with old age and obesity. We also observed that the phenotypes in male panc-TCPTP KO mice were more pronounced compared with females. Such a sexual dichotomy in metabolic studies at the whole-animal level has been reported [41, 42], but the underlying reasons are not fully understood.

The current studies identified STAT3 as a TCPTP substrate in the endocrine pancreas. Islets and MIN6 beta cells lacking TCPTP exhibited increased STAT3 phosphorylation, and substrate trapping established a direct association between STAT3 and TCPTP D/A that is consistent with an enzyme-substrate interaction. Pharmacological inhibition of STAT3 in TCPTP knockdown cells mitigated the defect in GSIS demonstrating

that the effects of the phosphatase deficiency are mediated through STAT3. Knockdown of STAT3 phenocopied the effects of STAT3 pharmacological inhibition consistent with the enhanced STAT3 signalling associated with TCPTP deficiency being important in the attenuated GSIS. The precise mechanism is not clear but it is worth noting that we observed increased GLUT2, PDX1 and GK protein expression in TCPTP knockdown cells with STAT3 deficiency (data not shown), consistent with the normalisation of insulin secretion in these cells. Conflicting reports suggest a role for STAT3 in insulin secretion. Pancreatic STAT3 deletion using rat insulin promoter (RIP-*Cre*) leads to glucose intolerance, defects in early phase insulin secretion and mild obesity [43, 44]. However, the RIP-*Cre* transgene has been reported to be expressed in the hypothalamus [45] and STAT3 was deleted in leptin-receptor positive neurons. Other studies aimed at determining the pancreas-specific function of STAT3 using Pdx1-*Cre* have also yielded conflicting data. While STAT3 appeared dispensable for pancreatic beta cell function in one study [46], another demonstrated that the KO mice exhibited impaired insulin secretion [47]. It is important to note that TCPTP can conceivably modulate pancreatic endocrine function through numerous effectors and signalling pathways. Indeed, TCPTP deficiency altered STAT1 phosphorylation, which has been shown to regulate beta cell function, differentiation and apoptosis [48, 49]. In addition, TCPTP is a regulator of insulin [7], leptin [17], c-Src [50] and ER stress [22] signalling pathways all of which play important roles in beta cell function. Previously, we demonstrated that TCPTP deficiency in MIN6 cells mitigated chemically induced ER stress signalling that coincided with increased STAT3 activation [22]. However, our current findings suggest that pancreatic TCPTP deletion coupled with high fat feeding has detrimental effects on insulin secretion. Although we cannot exclude other pathways contributing to the attenuated insulin secretion in HFD-fed panc-TCPTP KO mice, we propose that elevated STAT3 signalling is an important contributing factor.

In summary, pancreatic TCPTP deficiency in mice with prolonged high fat feeding led to delayed glucose clearance and impaired GSIS in a cell autonomous manner. Further, we report that STAT3 is a physiologically relevant mediator of TCPTP actions in islets. Together, these findings provide novel insight into the metabolic actions of TCPTP in the endocrine pancreas.

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