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Selective deficiency in endothelial PTP1B protects from diabetes and endoplasmic reticulum stress-associated endothelial dysfunction via preventing endothelial cell apoptosis

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Abstract

Diabetes notably increases the risk for endothelial dysfunction, a main precursor for microvascular complications. While endoplasmic reticulum stress (ERS) and protein tyrosine phosphatase 1B (PTP1B) have been associated with endothelial dysfunction in resistance vessels, whether these mechanisms also contribute to diabetes-mediated endothelial dysfunction in conduit arteries remains unknown. Herein, we tested the hypothesis that diabetes induces macrovascular endothelial dysfunction *via* endothelial ERS-induced, PTP1B-mediated apoptosis. We showed that diabetes concomitantly increased the expression of PTP1B and of markers of ERS, including GRP78, XBP1, splXBP1 and CHOP in human vessels. Exposure of aortic rings from wild-type mice to the ERS inducers tunicamycin and thapsigargin markedly reduced endothelium-dependent relaxation. Global and endothelial-specific deletion of PTP1B as well as pharmacological inhibition protected aortic rings from ERS-mediated endothelial dysfunction. Nitric oxide synthase inhibition with L-NAME abolished relaxation in the presence and absence of ERS, but neither reactive oxygen species scavenging with tempol or peg-catalase, nor cyclooxygenase inhibition with indomethacin prevented ERS-mediated endothelial dysfunction. However, both p38-MAPK and JNK inhibition protected aortic rings from ERS-mediated endothelial dysfunction. In HUVECs, PTP1B deletion prevented ERS-induced PARP cleavage and apoptosis. Lastly,

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

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acute ERS inhibition in aortic rings and selective deficiency of endothelial PTP1B in mice protected mice from diabetes-induced endothelial dysfunction. Altogether, these data support the contribution of the p38/JNK-apoptosis pathway in ERS-mediated endothelial dysfunction and present endothelial PTP1B as a major regulator of endothelial cell viability in conduit vessels and a potential target for the management of macrovascular diseases in diabetes.

Keywords

Endothelial function; Diabetes; Protein tyrosine phosphatase 1B; Endoplasmic reticulum stress; Apoptosis

1. Introduction

Diabetes is a chronic disease characterized by hyperglycemia resulting from either a decrease in insulin sensitivity or a lack of insulin production by the pancreas. Persistence of hyperglycemia has been described as a major risk factor for cardiovascular disease (CVD) and was responsible for 1.6 million deaths in 2016 [1,2]. Diabetes-induced endothelial dysfunction is a critical initiating factor in the genesis of diabetic macrovascular complications, such as atherosclerosis and coronary artery diseases [3]. Although several contributing factors for diabetes-induced endothelial dysfunction in the microvessels have been identified including reduction in NO bioavailability and inflammation, the precise mechanisms whereby hyperglycemia impairs endothelial function in the macrovasculature remain ill-defined [3,4].

The endoplasmic reticulum (ER) is a cellular organelle directly bound to the nuclear membrane playing an essential role in protein assembly, folding and transport. Molecular chaperones such as BiP or GRP78 enable the ER to regulate degradation of misfolded proteins thus contributing to cellular homeostasis [5]. Cell perturbations lead to an excess of unfolded proteins resulting in ER stress (ERS). Under ERS, cells react through an unfolded protein response which increases cellular capacity for protein elimination but can also promote apoptosis *via* activation of the pro-apoptotic molecules C/EBP Homologous Protein (CHOP), c-Jun-N-terminal (JNK) and p38-mitogen-activated protein kinase (MAPK) [6]. ERS activation is implicated in several diseases such as diabetes, cardiovascular disorders and endothelial dysfunction [4,7], and more specifically, promotes hyperglycemia-induced endothelial dysfunction [8,9] Tunicamycin (Tunica), an ERS induction agent, leads to impairment of endothelial function notably *via* decreasing NO bioavailability, however these experiments were performed primarily in resistance vessels [10,11].

Protein tyrosine phosphatase 1B PTP1B is a ubiquitous non-transmembrane protein involved in numerous pathological processes including obesity and diabetes [12–14]. Through its phosphatase activity, PTP1B acts as a negative regulator of the insulin-receptor pathway and participates in glucose metabolism regulation. Genetic deletion of PTP1B restores endothelium-dependent vasorelaxation in microvessels in several animal models of heart failure [15] and diabetes [16,17] but the specific role of PTP1B in the endothelium of the macrovasculature, particularly in diabetes-induced endothelial dysfunction, is unknown. PTP1B has been recently identified as a crucial regulator of ERS, however whether it

plays a role in ERS in endothelial cells has yet to be investigated [18,19]. Therefore, the present study aimed to test the hypothesis that diabetes induces macrovascular endothelial dysfunction *via* endothelial ERS-induced, PTP1B-mediated apoptosis.

2. Materials and methods

2.1. Chemicals and enzymes

Acetylcholine (ACh, #A6625), indomethacin (indo, #I8280), PEG-catalase (cat, #C4963), sodium nitroprusside (SNP, #71778), SP600125 (#S5567), tamoxifen (#T5648) and TCS401 (#SML2140) were purchased from Sigma Aldrich (St Louis, MO, US). L-NAME (N(γ)-nitro-L-arginine methyl ester, #06-651-00), phenylephrine (#18-605-133), SB203580 (#12-021-0), streptozotocin (#57-220-1), thapsigargin (Thapsi, #50-464-293), Tauroursodeoxycholic Acid (TUDCA, #NC1266953), and tunicamycin (Tunica, #35-161-0) were purchased from Fisher Scientific (Waltham, MA, US). Tempol (#ALX-430-081-G001) was purchased from Enzo life sciences (Farmingdale, NY, YS).

2.2. Discarded human blood vessels

Discarded aortic and saphenous vein specimens were obtained from diabetic and non-diabetic human patients undergoing open-heart-surgery at Augusta University Medical Center. Human subject protocol was approved by Institutional Review Board at the Medical College of Georgia at Augusta University (#1200473-1).

2.3. Experimental animals

The Institutional Animal Care and Use Committee of Augusta University approved all protocols involving experimental animals (IACUC protocol #2011-0108). Mice were kept at ambient temperature under 12/12 -h light/dark circadian conditions and fed standard rodent chow *ad libitum*. Male mice were utilized between 8 and 12 weeks of age (Jackson Laboratory, Bar Harbor, ME). PTP1B KO (Balb/C) were a generous gift from Dr. Michel L. Tremblay (Rosalind and Morris Goodman Cancer Research Centre at McGill University, Montreal, QC Canada). Endothelium specific PTP1B KO (C57B16/J) mice (PTP1B ECKO) were a generous gift from Dr. Michael Simons (Yale University, New Haven, Connecticut, USA). The deletion of PTP1B in endothelial cells was induced by a 5-day daily intraperitoneal injection of 2 mg of tamoxifen in corn oil. Mice were treated between 4 to 6-weeks of age and used 2–4 weeks after the last injection as previously described [20].

2.4. Streptozotocin (STZ) treatment

Two weeks after the last tamoxifen injection, type 1 diabetes (T1D) was induced in WT and PTP1B ECKO mice *via* daily streptozotocin (STZ) injections (50 mg/kg/d for 5 days, ip.) as previously described [16,17]. Mice were used 21 days after STZ injection and considered diabetic when glycemia was over 240 mg/dL.

2.5. Vascular reactivity measurement

Thoracic aortas were excised for vascular function measurements by wire myography (DMT©, Ann Arbor, MI), as previously described [21–24]. Vessels were preconstricted with phenylephrine (60–80 % of maximal KCl-induced contraction) and endothelial-dependent and independent relaxation investigated with concentration response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) (logarithmic curves: 0.1 nM - 10 μM concentrations), respectively. Aortic rings were exposed to the ERS inducers tunicamycin (Tunica, 5 μg/mL, 4 h) and thapsigargin (Thapsi, 25 nM, 4 h), in the presence or absence of either the ERS-relieving drug TUDCA (100μM), the selective PTP1B inhibitor TCS401 (25μM), the NOS inhibitor L-NAME (100μM), the non-selective cyclooxygenases inhibitor indomethacin (10 nM), the superoxide dismutase mimetic tempol (0.1μM), peg-catalase (100U/mL), the selective p38 inhibitor SB203580 (10μM) or the selective JNK inhibitor SP600125 (150 mM). Relaxation was expressed as percentage of pre-constriction in all conditions. Doses and incubation times for the experiments involving tunicamycin and thapsigargin were determined *via* dose and time response curves conducted in preliminary studies (data not shown).

2.6. Human Endothelial Cell Culture and siRNA transfection

Human aortic (HAECs) and umbilical (HUVEs) endothelial cells (Lonza®) were cultured in EGM-2 (Lonza®) containing 100 U/mL penicillin, 100 mg/mL streptomycin, 10 % of FBS and incubated at 37 °C with 5 % of CO_2 in a water saturated atmosphere. Cells were used between passages 4–8. PTP1B expression was repressed *via* viral infection of 90 % confluent cells with either a scrambled or PTP1B siRNA (Silencer Select siRNA PTPN1, Invitrogen®, catalogue# 1105) in the presence of Lipofectamine RNAiMAX (Invitrogen®). After 3 days, cells were treated or not for 24 h with tunicamycin (5 μg/mL) and used for MTT or western blotting assay.

2.7. MTT assay

Changes in viability in response to tunicamycin treatment were assessed by MTT assay. In brief, after transfection HAECs were seeded in 96 well plates (10,000 cells/well) and incubated for 24 h. Cells were then treated with tunicamycin 5 μg/mL. After 24 h of treatment, media (200 μL) was replaced and 50 μL of MTT in solution in PBS was added (final MTT concentration: 1 mg/mL). Plate was covered with aluminum foil and incubated at 37 °C for 4 h. After aspiration of the media, 200 μL of DMSO was added to solubilize MTT formazan crystals. Absorbance was read at 570 nm (Biotek Synergy HT). Triton-X100 0.1 % was used as positive control.

2.8. RT-qPCR

Human veins and aortas were homogenized and mRNA isolated with Trizol (Qiagen, Germantown, MD) and cDNA obtained by reverse transcription (ThermoFisher, Waltham, MA). RT-qPCR was performed on cDNA utilizing Sybr Green (Applied Biosystems) with the primer sequences listed in Table 1. Ct values from each sample were normalized to 18 s expression within the sample (ΔCt) followed by normalization to control groups (ΔΔCt) prior to calculation of relative gene expression (2-ΔΔCt) as published previously [21,24,25]

and in accordance with Applied Biosystems guidelines for expression analysis (Applied Biosystems User Bulletin, no. 2, 1997).

2.9. Western blot

Proteins extracted from aortic tissues and HAECs (10–25 μg) were separated *via* SDS-PAGE and transferred to Immobilon-P poly(vinylidene fluoride) membranes. Immunoblots were probed with antibodies for cleaved caspase-3 (Cell Signaling, #9661), ERO1α (Santa Cruz, sc-365526), GAPDH (Santa Cruz, sc-32233) and PTP1B (BD bioscience, #610140).

2.10. Immunofluorescence microscopy

Immunofluorescense microscopy studies were performed as previously described [26–28]. After treatment HUVECs grown on coverslips were washed with PBS three times, fixed in 3.7 % paraformaldehyde for 10 min, and permeabilized with 0.2 % Triton X-100 for 10 min. Cells were blocked with PBS-Tween 20 containing 2 % BSA for 30 min and incubated with primary antibodies for 1 h at 37°. Specimens were washed and exposed to Alexa Fluor 488 conjugated secondary antibodies 1 h at room temperature. Actin was visualized by TRITC-labeled phalloidin (1 mM in PBS, with 0.1 % BSA) placed onto the coverslips for 30 min. Coverslips were mounted with ProLong Gold antifade mounting medium (Molecular Probes, Eugene, OR). To detect chromatin condensation and DNA fragmentation, TUNEL assay was assessed and slides were mounted in ProLong Gold antifade mounting medium with DAPI as previously described [29]. Specimens were analyzed under a ZEISS Axio Imager Observer D1 epifluorescence microscope.

2.11. Statistical analysis

Analyses were performed in GraphPad Prism software (La Jolla, CA). Data are expressed as mean ± SEM and *n* represents the number of independent experiments (biological replicates). One-way non-parametric Kruskal-Wallis test were performed. Tukey or Sidak post hoc test followed all ANOVA. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Diabetes increases PTP1B and ERS markers expression in human blood vessels

ERS is a hallmark of diabetes and both diabetes and ERS are major risk factors for endothelial dysfunction. Quantitative real-time RT-PCR (qPCR) and Western-Blot (WB) analysis revealed that diabetes is associated with significant increases in the expression of PTP1B and of markers of ERS and apoptosis: CHOP, ERO1α, XBP1, spliced XBP1 and GRP78 in saphenous veins (Fig.1 A–E) and aortic biopsies (Fig.1F–J) from human patients.

3.2. ERS impairs endothelium-dependent relaxation

In order to investigate the contribution of ERS to endothelial dysfunction in conduit vessels, we developed a model of ERS-mediated endothelial dysfunction. We exposed aortas from wild-type mice to either Tunica or Thapsi and reported that both Tunica (Fig. 2A) and Thapsi (Fig. 2B) markedly reduced ACh-induced relaxation but did not alter SNP-mediated relaxation (Fig. 2C, D) indicating a dysfunction at the level of the endothelium. The

ERS blocker TUDCA blunted Tunica-mediated endothelial dysfunction confirming the contribution of ERS to endothelial dysfunction (Fig. 2A).

3.3. PTP1B deletion protects from ERS-mediated endothelial dysfunction

To investigate the contribution of PTP1B deletion to ERS-associated endothelial dysfunction, we tested the effects of Tunica and Thapsi in aortas from PTP1B KO mice. As reported in Fig. 3, global deletion as well as pharmacological inhibition of PTP1B with TCS401 preserved endothelial function and remarkably fully protected aortic rings from Tunica- and Thapsi-mediated endothelial dysfunction. In addition, SNP-mediated relaxation remained intact in PTP1B KO mice (Fig. 3C and D).

3.4. Selective deletion of PTP1B in endothelial cells protects from ERS-induced endothelial dysfunction

To investigate the specific contribution of endothelial PTP1B to ERS-mediated endothelial dysfunction, Tunica experiments were repeated in aortic rings from animals with a selective deletion of PTP1B in endothelial cells (PTP1B ECKO). As reported in Fig. 4A and B, selective deletion of PTP1B in endothelial cells protected aortic rings from ERS-mediated endothelial dysfunction.

3.5. ERS induces endothelial dysfunction via activation of the p38/JNK pathway

The respective contribution of ROS and cyclooxygenases (COXs) derivatives to ERS-induced endothelial dysfunction was investigated by exposing aortic rings to Tunica in combination with either the superoxide dismutase mimetic tempol, pegylated-catalase, or indomethacin. Neither ROS scavenging (Fig. 5A) nor cyclooxygenases (COX) blockade (Fig. 5B) protected aortic rings from Tunica-induced endothelial dysfunction, which excludes the contribution of ROS and COX-derivatives to ERS-induced endothelial dysfunction in the macrovasculature. However, the nitric oxide (NO) synthase inhibitor L-NAME completely abolished relaxation in the presence or absence of Tunica (Fig. 5C), suggesting that ERS impairs endothelial function *via* reducing NO bioavailability. ERS promotes apoptosis *via* activation of the IRE1α arm of the unfolded protein response, which leads to activation of the pro-apoptotic molecules c-Jun-N-terminal kinase (JNK) and p38-MAPK [5,30,31]. Therefore, we next determined whether activation of these pathways is important in mediating ERS-induced endothelial dysfunction. As reported in Figs. 5D and 5E, inhibition of both p38 and JNK signaling pathways prevented Tunica-induced endothelial dysfunction.

3.6. PTP1B knockdown protects from ERS-induced endothelial cells apoptosis

To determine whether endothelial cell apoptosis could explain the reduced NO bioavailability and contribute to Tunica-induced impaired relaxation, we evaluated the effects of ERS on endothelial cell viability and expression of the well-accepted marker of apoptosis cleaved-caspase 3. Quantification of cell viability *via* MTT assay revealed that ERS induced a significant reduction in endothelial cell viability, which was prevented by PTP1B knockdown (Fig. 6A and C). PTP1B knockdown also prevented Tunica-mediated increases in cleaved-caspase 3, as well as the formation of apoptotic bodies, nuclear DNA

strand breaks and stress fibers, in HUVECs further supporting the contribution of apoptosis to ERS-induced endothelial dysfunction and the protective effects of PTP1B deletion (Fig. 6).

3.7. ERS blockade and selective deletion of PTP1B in endothelial cells protect from diabetes-induced endothelial dysfunction

Lastly, we analyzed the contribution of ERS and endothelial PTP1B to diabetes-associated endothelial dysfunction. We studied the endothelial function of wild-type (ECWT) and PTP1B ECKO mice submitted to type 1 diabetes (STZ). Consistent with the literature, type 1 diabetes (T1DM) impaired ACh-induced relaxation in ECWT mice without altering smooth muscle cell dependent relaxation (Fig. 7A and B). Remarkably, both acute ERS blockade with TUDCA and selective deletion of PTP1B in endothelial cells provided protection from T1DM-mediated endothelial dysfunction without altering blood glucose levels (WT: 384 ± 20 mg/dL *vs* ECKO PTP1B: 519 ± 41 mg/dL, ns).

4. Discussion

The goal of the present study was to investigate the specific contribution of endothelial PTP1B to ERS-and diabetes-induced endothelial dysfunction in the macrocirculation. Herein we reported that 1) diabetes induces a concomitant increase in the expression of PTP1B and of markers of ERS in human conduit vessels, 2) global deletion and pharmacological inhibition of PTP1B protect from ERS-induced endothelial dysfunction, 3) ERS blockade restores endothelial function in aortic rings from diabetic animals, 4) selective deletion of PTP1B in endothelial cells protects form ERS-and diabetes-associated endothelial dysfunction and 5) ERS-induced endothelial dysfunction involves JNK/p38-mediated apoptosis but not ROS generation in the macrocirculation. Relevant to these observations are the mechanisms of endothelial dysfunction in conduit vessels in the context of diabetes and the contribution of endothelial PTP1B.

While previous work from our group reported that diabetes and hyperglycemia respectively increase the expression of PTP1B in mouse aorta and human endothelial cells in culture [17], no study had investigated the translatability of these observations to humans. Herein, we reported for the first time that diabetes increases PTP1B expression in human arteries and veins, which supports the translatability of our initial findings. We extended our data by demonstrating functional evidence that selective deletion of PTP1B in endothelial cells protects from diabetes-associated endothelial dysfunction. In agreement with previous studies by the group of Vincent Richard [15,18], this study presents PTP1B as a major regulator of endothelial cell integrity and potential therapeutic target for the treatment of hyperglycemia-associated vascular diseases.

In addition to an increase in PTP1B expression, vessels from diabetic patients also exhibited increases in the expression of markers of ERS and apoptosis, suggesting a link between diabetes, PTP1B, ERS and endothelial dysfunction. A limitation to our study is the lack of clear characterization of the human population our samples were obtained from. Per our protocol only information regarding the diabetes status of the patients undergoing cardiovascular surgery was reported. Therefore, additional risk factors such as obesity,

hypertension and/or hyperlipidemia may also have contributed to the elevation in PTP1B and ERS marker expression in human samples. Interestingly, it has been previously shown that the GLP-1 analogue liraglutide ameliorates ERS and JNK activation in endothelial cells from patients with DM [32], suggesting that anti-diabetic treatments restore endothelial function through an ERS-dependent pathway. Consequently, the link between diabetes, PTP1B, and ERS in humans would be strengthened by an extensive clinical study adjusted for clinical characteristics and treatments.

Reduction of ERS through pharmacological inhibition or gene deletion had been reported to prevent diabetic microvascular complications including retinopathy, neuropathy, and nephropathy [33–37] as well as hypertension- and chronic heart failure-associated endothelial dysfunction [15,38]. However, whether these improvements involved direct vascular effects remained unclear. Herein, using an *ex vivo* approach, we provided functional evidence that ERS is upstream of PTP1B and most likely regulates its expression and activity. Indeed, we showed that both global and selective deletion of PTP1B in endothelial cells protect from ERS-mediated endothelial dysfunction. Consistent with previous reports [18,39,40], we showed that ERS-mediated endothelial dysfunction involves reduced NO bioavailability, activation of the p38/JNK-MAPK-dependent pathway and endothelial cell apoptosis. However, in contrary to the work by Galan et al. [39], ERS-mediated endothelial cell apoptosis was ROS-independent. Indeed, neither tempol nor PEG-catalase prevented ERS-mediated endothelial dysfunction in mouse aortic rings. The difference in the approach, *in vivo vs. ex vivo* exposure to the ERS inducer, the dose of Tunica used and the time of exposure, may contribute to the discrepancy between studies. Despite the clear functional evidence that blockade of PTP1B and of the p38/JNK-MAPK pathway protect from ERS-mediated endothelial dysfunction, the molecular mechanisms whereby PTP1B regulates ERS and p38/JNK remain elusive. Thiebault et al., proposed that PTP1B negatively regulates ERS *via* dephosphorylating protein kinase RNA-like endoplasmic reticulum kinase (PERK) in endothelial cells [18], while others showed that PTP1B potentiates IRE1 signaling in fibroblast [41]. Herein, our human data suggest that both PERK and IRE1 signaling are activated with increased PTP1B expression. However additional studies are required to further test this hypothesis.

Numerous studies have linked apoptosis with endothelial dysfunction and cardiovascular diseases, notably in the context of diabetes [42,43]. However, the mechanisms whereby apoptosis reduces NO bioavailability and endothelium-dependent relaxation remain elusive. Theoretically, one could expect that apoptosis would reduce the number of viable endothelial cells and therefore the ability of the vessels to produce NO and relax. Our data showing that endothelial PTP1B deletion as well as P38 and JNK inhibition protects from ERS-mediated apoptosis and endothelial dysfunction support this hypothesis. However, our data reporting that acute ERS inhibition with TUDCA restores endothelial function in vessels from animals with type 1 diabetes may challenge this hypothesis, unless TUDCA exerts vasodilatory properties independent of its action as an ERS inhibitor. Hence, additional experiments are required to determine whether ERS reduces viable endothelial cell numbers in blood vessels and whether TUDCA could act as a vasodilator.

5. Conclusion

This study demonstrates for the first time that diabetes induces parallel increases in the expression of PTP1B and of markers of ERS in discarded human vessels and provides strong functional evidence identifying endothelial PTP1B as a major regulator of endothelial integrity in diabetes. It presents PTP1B of as a new effector in ERS-induced apoptosis which likely regulates the P38/JNK-caspase signaling pathway, in the context of diabetes. Taken together, these findings suggest that strategies aiming at decreasing PTP1B activity and ERS, which are currently being considered and tested for the treatment of diabetes and metabolic disorders [44–46], may represent new therapeutic avenues for the treatment of diabetes-associated vascular disorders and the prevention of cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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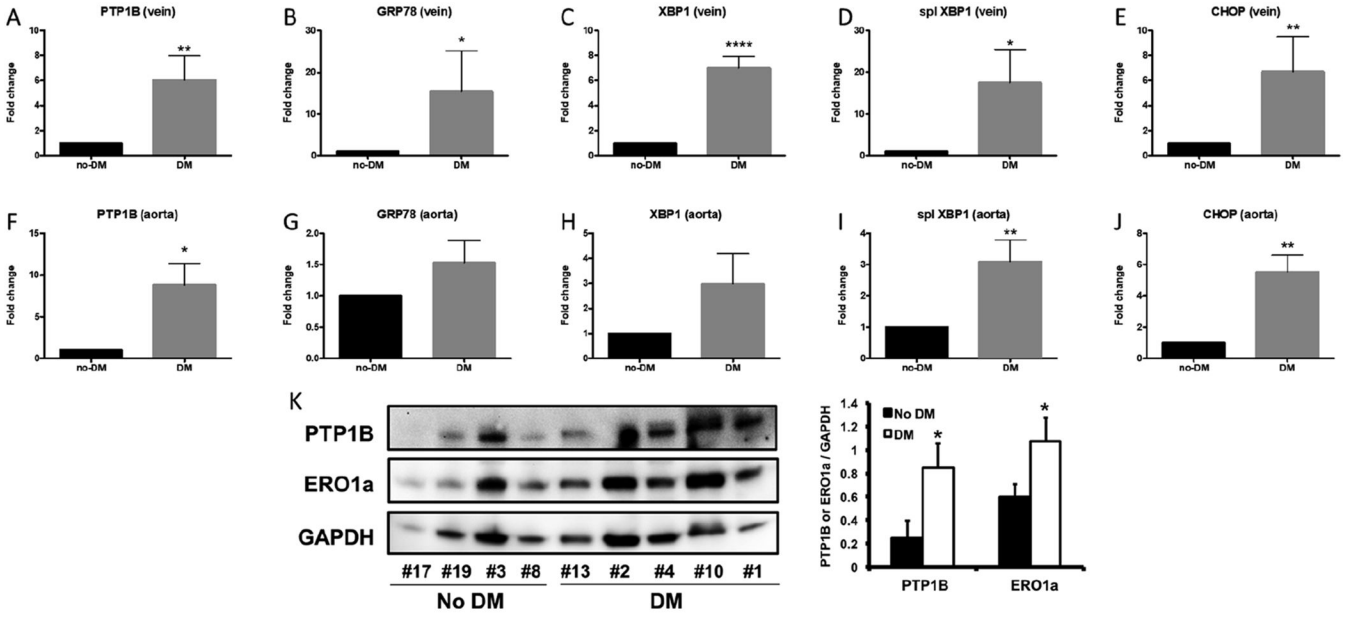

Fig. 1.

Diabetes increases the expression of PTP1B and markers of ERS in human vessels. mRNA levels of PTP1B, GRP78, XBP1, splXBP1 and CHOP in (A-E) veins and (F-J) aortas of diabetic patients (DM) and non-diabetic (no-DM) subjects. (K) Western Blot analysis of PTP1B and the ER stress marker ERO1α in vein of diabetic patients and non-diabetic subjects. Data are Mean ± SEM, n = 3-9. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

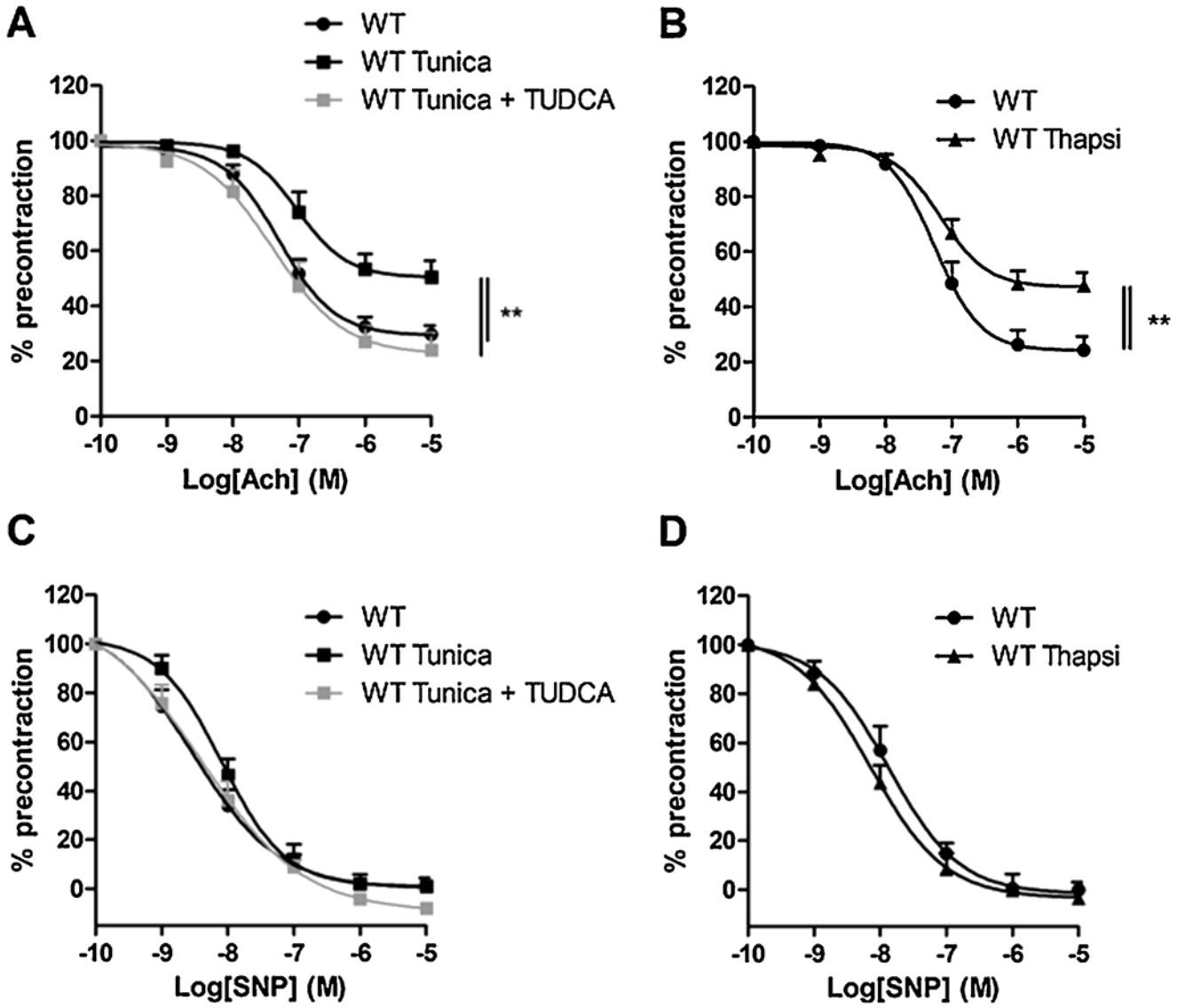

Fig. 2.

ERS impairs endothelial function. Concentration-response curves to (A, B) ACh and (C, D) SNP, in WT mice aorta exposed to either vehicle, tunicamycin (Tunica) or thapsigargin (Thapsi) in the presence or absence of the ERS inhibitor TUDCA. Data are Mean ± SEM. n = 6. **p < 0.01.

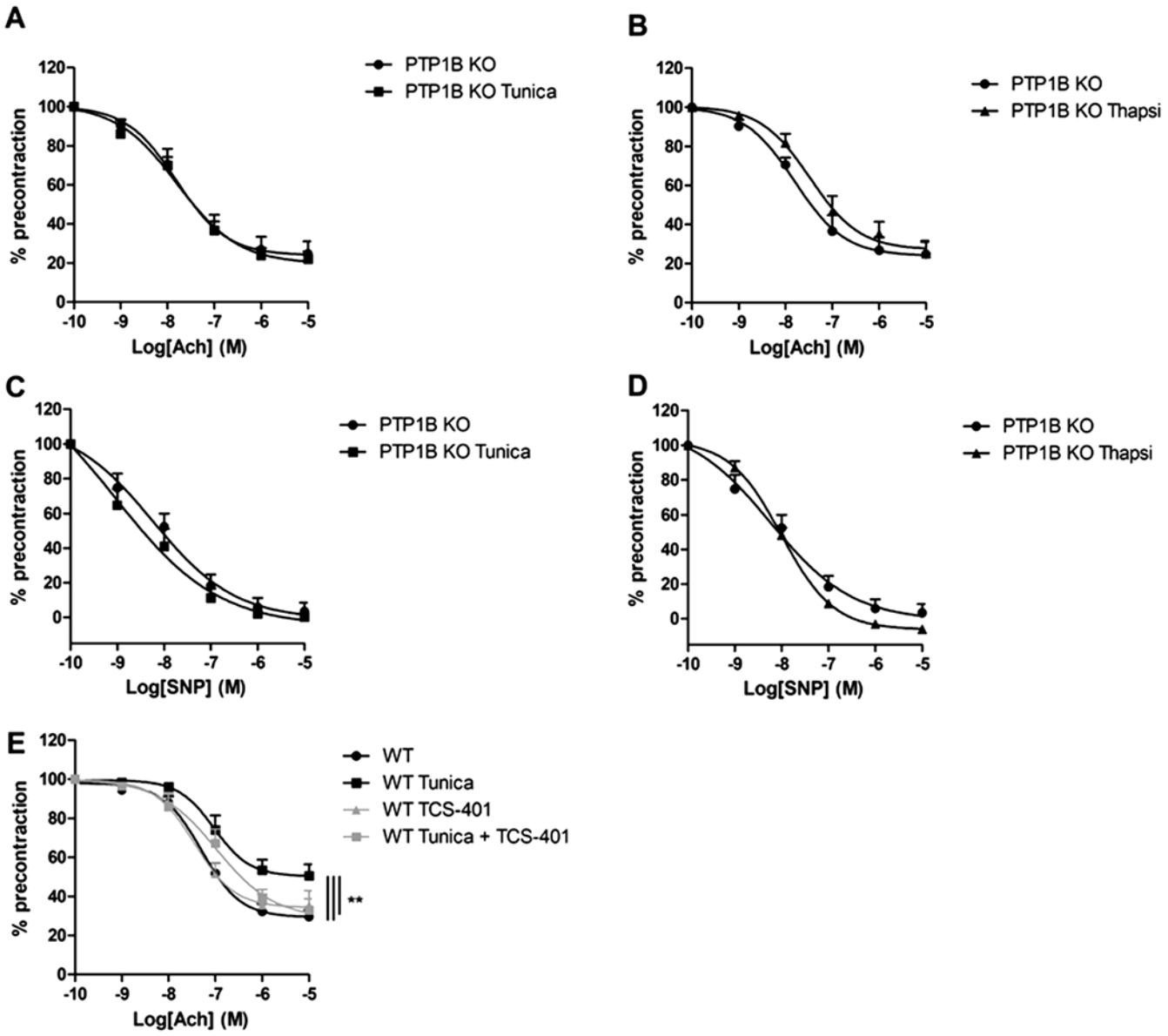

Fig. 3.

PTP1B deletion protects from ERS-mediated endothelial dysfunction.

Concentration-response curves to (A, B) ACh and (C, D) SNP in PTP1B KO mice aorta exposed to either vehicle, tunicamycin (Tunica), thapsigargin (Thapsi), and/or the ERS inhibitor TUDCA. (E) Concentration-response curves to ACh in WT mice aorta exposed to Tunica in the presence or absence of the selective PTP1B inhibitor TCS401. Data are Mean ± SEM, n = 4–10. **p < 0.01.

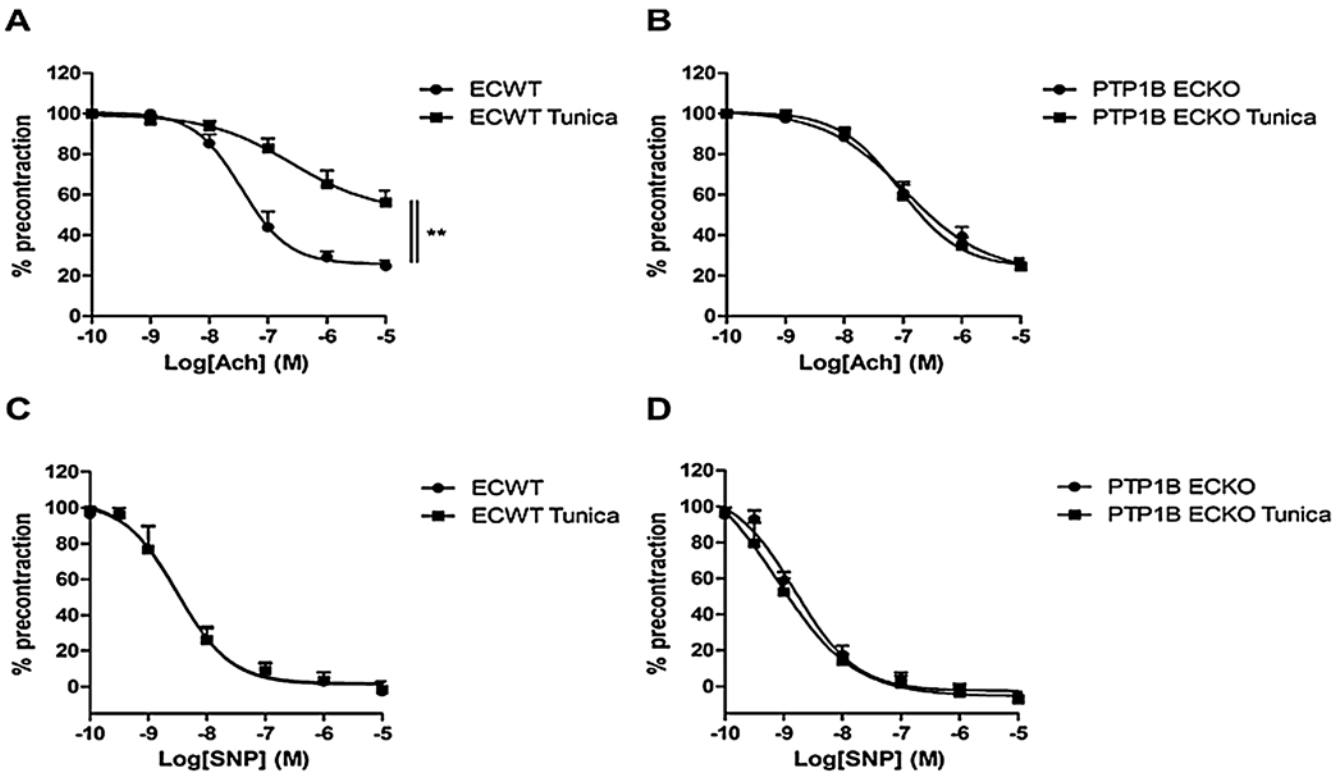

Fig. 4.

Selective deletion of PTP1B in endothelial cells protects from tunicamycin-induced endothelial dysfunction. Concentration-response curves to ACh and SNP in aorta from ECWT (A and C) and ECKO PTP1B (B and D) mice, exposed to either vehicle or tunicamycin (Tunica). Data are Mean ± SEM, n = 3-4. *$p < 0.05$.

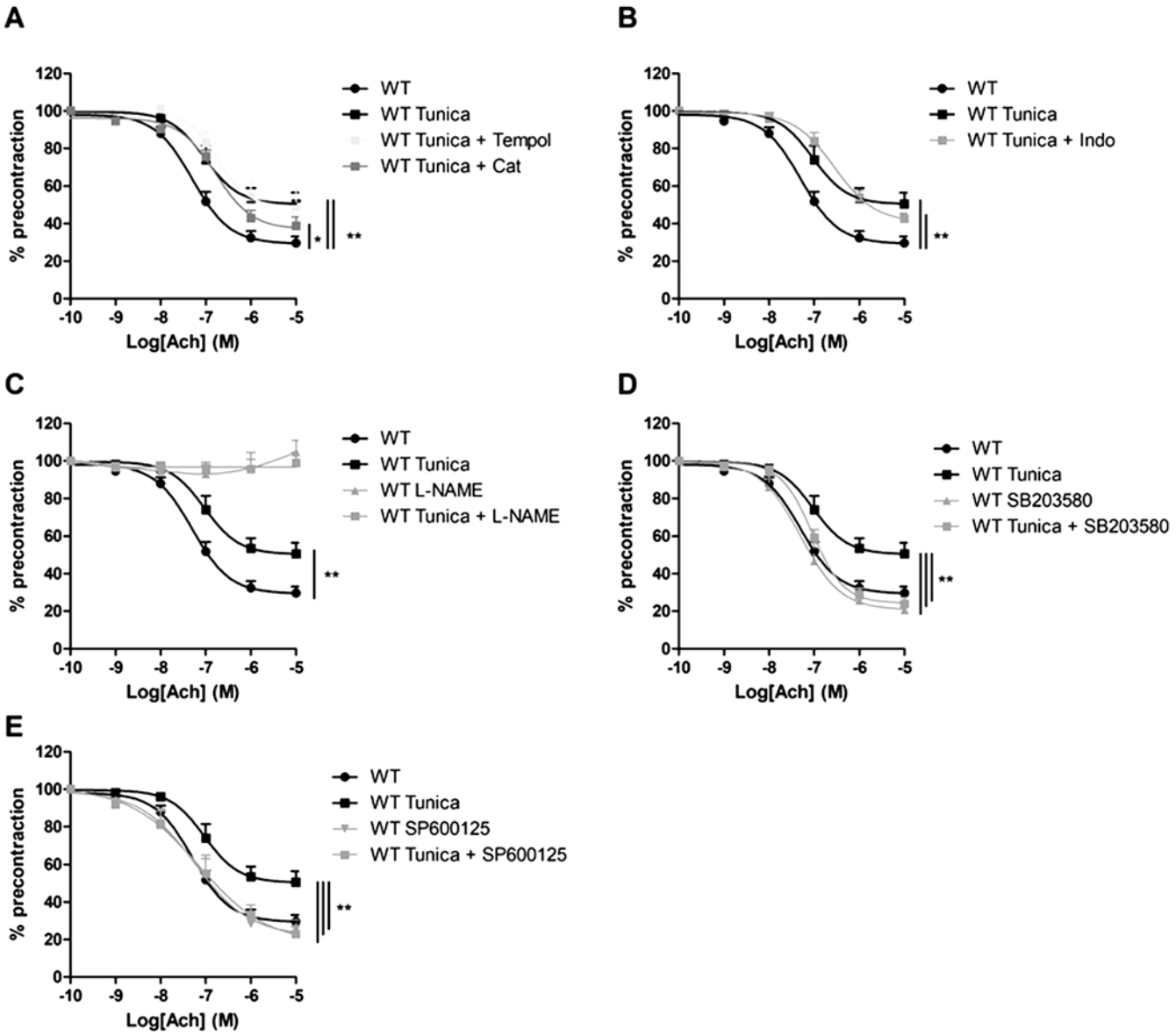

Fig. 5.

ERS induces endothelial dysfunction *via* activation of the p38/JNK pathway. (A-C) Concentration-response curves to ACh in aorta exposed to vehicle or tunicamycin (Tunica) in the presence or absence of the superoxide dismutase mimetic tempol, pegylated-catalase, the non-selective COX inhibitor indomethacin or the NOS inhibitor L-NAME. (D and E) Concentration-response curves to ACh in aorta exposed to vehicle or Tunica in the presence or absence of the selective p38-MAPK inhibitor SB203580 or the selective JNK inhibitor SP600125. Data are Mean ± SEM, n = 4-8. *$p < 0.05$; **$p < 0.01$.

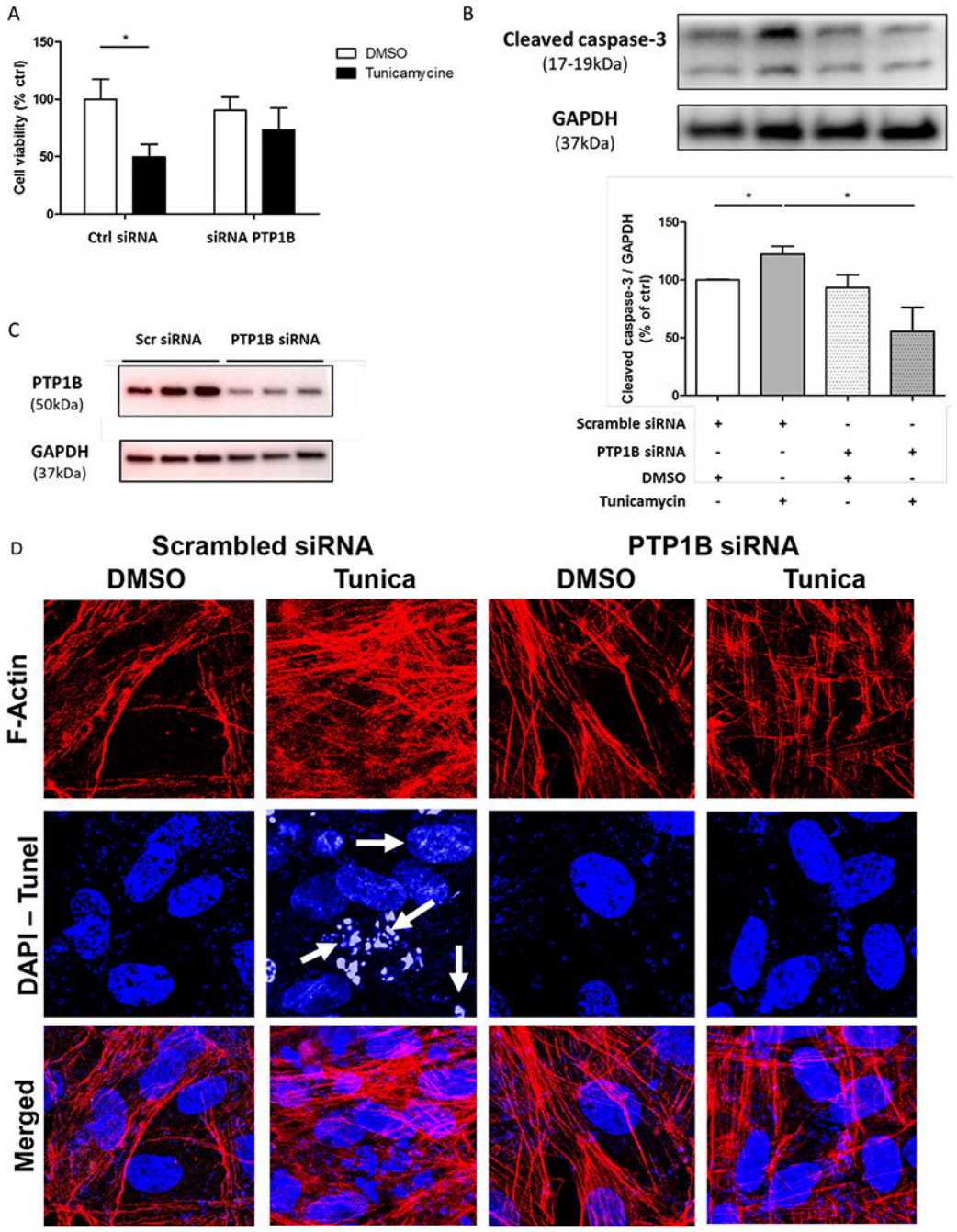

Fig. 6.

Reduction in PTP1B expression *via* gene silencing prevents ERS-induced endothelial cell apoptosis (A) Viability assay (MTT assay) conducted in human aortic endothelial cells (HAECs) exposed to scrambled (Ctrl siRNA) or PTP1B siRNA (siRNA PTP1B) in the presence of vehicle or tunicamycin for 24 h. (B) Western Blot quantification of the pro-apoptotic cleaved caspase-3 in HAECs exposed to scrambled (Ctrl siRNA) or PTP1B siRNA (siRNA PTP1B) in the presence of vehicle or tunicamycin for 24 h. (C) WB confirmation of the efficiency of the PTP1B siRNA at decreasing PTP1B expression in HAECs. (D)

Immunostaining for F-actin, nuclei (DAPI) and DNA strand breaks (TUNEL), of HUVECs expressing PTP1B or nor, in the presence or absence of tunicamycin. Data are Mean ± SEM, n = 3-9. *p < 0.05.

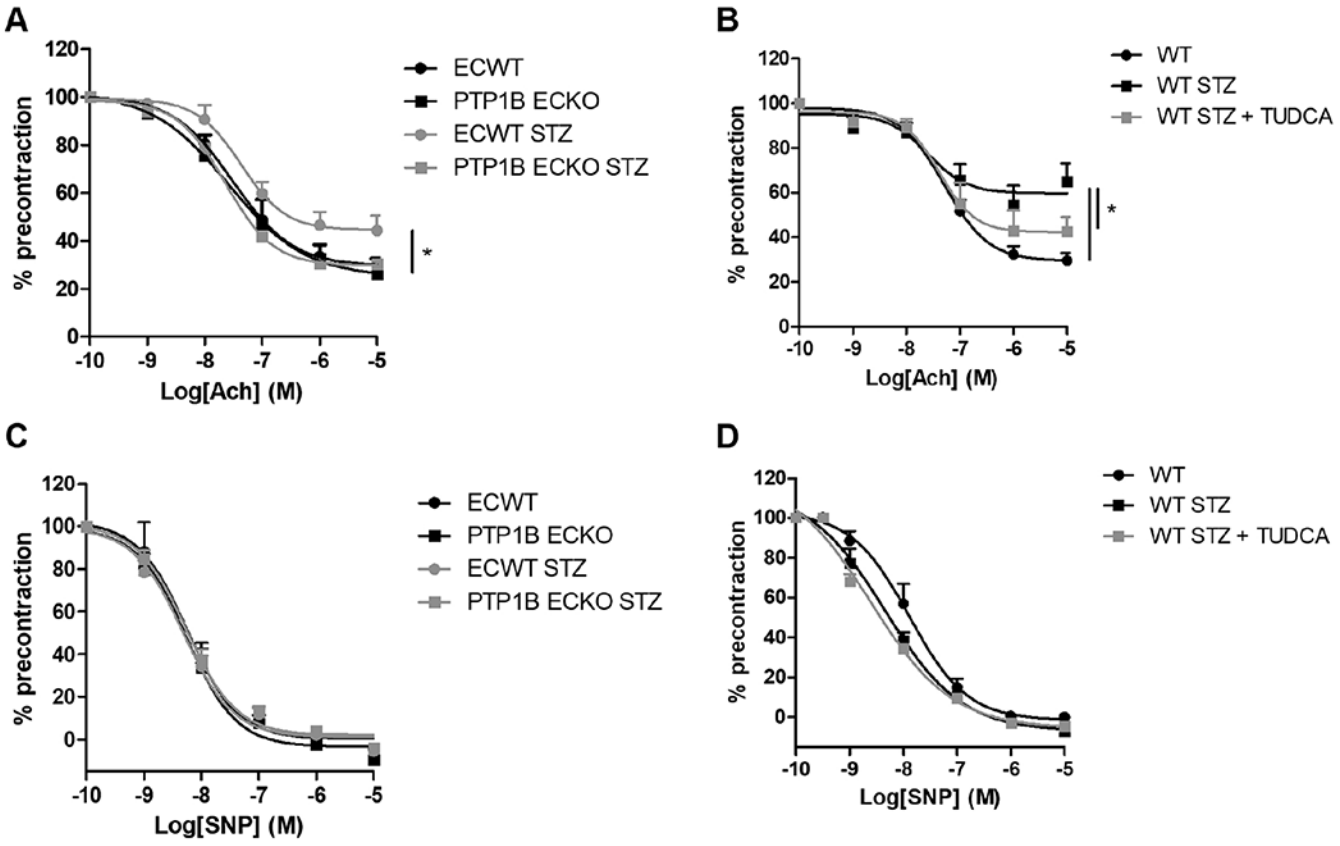

Fig. 7.

Both TUDCA and selective deletion of PTP1B in endothelial cells protect from diabetes-induced endothelial dysfunction. Concentration-response curves to ACh (A) and SNP (C) in aorta from wild-type mice (ECWT) and endothelium specific PTP1B KO (ECKO) mice exposed to vehicle or streptozotocin (STZ). Concentration-response curves to ACh (B) and SNP (D) in aorta from wild-type mice (WT) exposed to vehicle or streptozotocin (STZ) in presence or absence of TUDCA. Data are Mean ± SEM, n = 3-4. *$p < 0.05$.

Table 1

Primer sequences for RT-qPCR.

Gene	Forward	Reverse
Human PTP1B	5′-GCGGCCATTTACCAGTTGAC-3′	5′-ATGACGACACCCCTGCTTTT-3′
Human GRP78	5′-GACGGGCAAAGATGTCAGGA-3′	5′-ATCTGGGTTTATGCCACGGG-3′
Human XBP1	5′-CTGAGTCCGCAGCAGGTG-3′	5′-GGCTGGTAAGGAACTGGGTC-3′
Human spl XBP1	5′-CTGAGTCCGCAGCAGGTG-3′	5′-GGCTGGTAAGGAACTGGGTC-3′
Human CHOP	5′-TCCAACTGCAGAGATGGCAG-3′	5′-TCCTCCTCTTCCTCCTGAGC-3′
Human 18S	5′-TCGAGGCCCTGTAATTGGAA-3′	5′-CCCTCCAATGGATCCTCGTT-3′