Transcription Factor 7-Like 2 Regulates β-Cell Survival and Function in Human Pancreatic Islets

Luan Shu,[1] Nadine S. Sauter,[1] Fabienne T. Schulthess,[1] Aleksey V. Matveyenko,[1] José Oberholzer,[2] and Kathrin Maedler[1]

OBJECTIVE—Type 2 diabetes is characterized by impaired insulin secretion in response to increased metabolic demand. This defect in β-cell compensation seems to result from the interplay between environmental factors and genetic predisposition. Genome-wide association studies reveal that common variants in transcription factor 7-like 2 (TCF7L2) are associated with increased risk of type 2 diabetes. The aim of the present study was to establish whether TCF7L2 plays a role in β-cell function and/or survival.

RESEARCH DESIGN AND METHODS—To investigate the effects of TCFL7L2 depletion, isolated islets were exposed to TCF7L2 small interfering RNA (siRNA) versus scrambled siRNA, and β-cell survival and function were examined. For TCF7L2 overexpression, islets were cultured in glucose concentrations of 5.5–33.3 mmol/l and the cytokine mix interleukin-1β/γ-interferon with or without overexpression of TCF7L2. Subsequently, glucose-stimulated insulin secretion (GSIS), β-cell apoptosis [by transferase-mediated dUTP nick-end labeling assay and Western blotting for poly(ADP-ribose) polymerase and Caspase-3 cleavage], and β-cell proliferation (by Ki67 immunostaining) were analyzed.

RESULTS—Depleting TCF7L2 by siRNA resulted in a 5.1-fold increase in β-cell apoptosis, 2.2-fold decrease in β-cell proliferation ($P < 0.001$), and 2.6-fold decrease in GSIS ($P < 0.01$) in human islets. Similarly, loss of TCF7L2 resulted in impaired β-cell function in mouse islets. In contrast, overexpression of TCF7L2 protected islets from glucose and cytokine-induced apoptosis and impaired function.

CONCLUSIONS—TCF7L2 is required for maintaining GSIS and β-cell survival. Changes in the level of active TCF7L2 in β-cells from carriers of at-risk allele may be the reason for defective insulin secretion and progression of type 2 diabetes. ***Diabetes*** **57:645–653, 2008**

From the [1]Larry L. Hillblom Islet Research Center, Department of Medicine, University of California, Los Angeles, Los Angeles, California; and the [2]Division of Transplantation, University of Illinois at Chicago, Chicago, Illinois.

Address correspondence and reprint requests to Kathrin Maedler, PhD, Centre for Biomolecular Interactions Bremen, University of Bremen, NW2, Box 33 04 40, D-28334 Bremen, Germany. E-mail: kmaedler@uni-bremen.de.

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N.S.S. and F.T.S. contributed equally to this study.

DAPI, 4,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; GLP-1, glucagon-like peptide 1; GSIS, glucose-stimulated insulin secretion; IFN-γ, γ-interferon; IL, interleukin; KRBB, Krebs-Ringer bicarbonate buffer; PARP, poly(ADP-ribose) polymerase; siRNA, small interfering RNA; siScr, scrambled control siRNA; siTCF7L2, islets depleted for TCF7L2 by siRNA to TCF7L2; SNP, single nucleotide polymorphism; TCF7L2, transcription factor 7-like 2; TUNEL, transferase-mediated dUTP nick-end labeling.

Type 2 diabetes is characterized by impaired insulin secretion and insulin resistance (1). Type 2 diabetes manifests when insulin secretion fails to adaptively increase to increased insulin demand. This might be due to defective β-cell mass and/or impaired β-cell function (2–3). The underlying mechanisms of β-cell failure in type 2 diabetes are still unknown, but recent genome-wide association studies have offered some new targets of interest.

Grant et al. (4) reported linkage between a variant of the transcription factor 7-like 2 (TCF7L2) and type 2 diabetes in Danish and U.S. cohorts (4). This finding has been confirmed by several other genome-wide studies (4–13) and in numerous populations (13). TCF7L2 (previously known as TCF-4) is an important downstream target of the canonical WNT signaling pathway (14). It is highly expressed in most human tissues, including heart, placenta, lung, brain, liver, adipose tissue, kidney, and pancreatic β-cells, but not in the skeletal muscle (10). Interestingly, TCF7L2 expression in adipose tissue is decreased in obese subjects with type 2 diabetes (10).

TCF7L2 has been implicated in glucose homeostasis through the regulation of pro-glucagon gene expression, which encodes glucagon-like peptide 1 (GLP-1) in intestinal cells (15).

The single nucleotide polymorphisms (SNPs) in the TCF7L2 gene that show linkage with type 2 diabetes are all found in the noncoding regions (13). It is unknown whether changes in TCF7L2 expression levels in pancreatic islets directly influence β-cell function or survival. Because type 2 diabetes is characterized by impaired β-cell function and increased β-cell apoptosis, we sought to establish whether islet expression levels of TCF7L2 impact glucose-induced insulin secretion and vulnerability to apoptosis. After manipulating levels of TCF7L2, we examined glucose-induced insulin secretion by perifusion and β-cell survival after exposure to pro-apoptotic glucose concentrations and cytokines.

RESEARCH DESIGN AND METHODS

Islet isolation and culture. Human islets were isolated from pancreata of five healthy organ donors at the University of Illinois at Chicago as described previously (16) and shipped to UCLA directly after isolation. Islet purity of these five preparations was >95%, as judged by dithizone staining. Human islets were cultured in CMRL-1066 medium containing 5.5 mmol/l glucose and 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (Invitrogen, Carlsbad, CA). Mouse islets were isolated using Collagenase type 4 (Worthington, Lakewood, NJ) as described previously (17) and cultured in RPMI 1640 containing 11.1 mmol/l glucose. In culture, a glucose concentration of 11.1 mmol/l accomplishes the lowest frequency of β-cell apoptosis in mouse islets (18–20). Human and mouse islets were precultured for 24 h before the experiment and then plated on matrix-coated plates derived from bovine corneal endothelial cells (Novamed, Jerusalem, Israel) (21). For the perifusion

experiment, islets were maintained in suspension. For experimental treatment of islets, medium was changed to culture medium containing 5.5, 11.1, or 33.3 mmol/l or 5.5 mmol/l plus 2 ng/ml recombinant human interleukin (IL)-1β plus 1,000 units/ml recombinant γ-interferon (IFN-γ) (R&D Systems, Minneapolis, MN) for 96 h.

RNA interference and plasmid transfection. Small interfering RNA (siRNA)-Lipofectamine2000 complexes and DNA-Lipofectamine2000 complexes were prepared according to the manufacturer's instructions (Liptofectamine2000; Invitrogen) using 50 nmol/l siRNA to TCF7L2 (RNAs of 21 nucleotides, designed to target human TCF7L2; Stealth Select RNAi; Invitrogen) and scramble siRNA (Ambion, Austin, TX) or 3 μg/ml DNA of pCMV-TCF7L2 (full-length TCF7L2, from the Full-Length Mammalian Gene Collection; Invitrogen) or an pCMV-empty control plasmid. Islets were precultured for 24 h, medium was changed to OptiMEM (Invitrogen), and siRNA-Lipofectamine2000 complexes or DNA-Lipofectamine2000 was added. After an 8-h incubation, the transfection medium was aspirated and replaced by fresh culture medium with or without elevated glucose or cytokines for additional 4 days. To monitor transfection efficiency of siRNA into the islets, we transfected fluorescein-labeled nontargeted siRNA (Cell Signaling, Beverly, MA) and analyzed transfection under the fluorescent microscope during for 4 days of culture.

β-Cell apoptosis, replication, and TCF7L2 expression. For each independent experiment, 20 islets were plated in a 3-cm culture dish and exposed to the treatment conditions as indicated above. Four dishes per treatment group were used. After washing with PBS, islets were fixed with 4% paraformaldehyde followed by permeabilization with 0.5% Triton X-100. β-Cell apoptosis was analyzed by the transferase-mediated dUTP nick-end labeling (TUNEL) technique (In Situ Cell Death Detection kit, AP; Roche Diagnostics, Indianapolis, IN). For β-cell proliferation, an anti-human Ki67 antibody was used (Zymed, San Francisco, CA), followed by detection using cy3-conjugated donkey anti-mouse antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). For β-cell TCF7L2 expression, islets were incubated with anti-TCF7L2 antibody (Cell Signaling, Beverly, MA), followed by detection using cy3-conjugated donkey anti-rabbit antibody (Jackson). In all experiments, islets were double/triple stained with guinea pig anti-insulin antibody (Dako, Carpinteria, CA), followed by detection using fluorescein-conjugated donkey anti-guinea pig antibody (Jackson). Islets were embedded in glycerol gelatin (Sigma) or Vectashield mounting medium (Vector Laboratories, Burlingame, CA), which visualized all cells by 4,6-diamidino-2-phenylindole (DAPI) staining. Fluorescence was analyzed using a Leica DM6000 microscope, and images were acquired using Openlab software.

Western blot analysis. For Western blot analyses, 100 islets/dish were plated in duplicates for each independent experiment. At the end of the incubation, islets were washed in PBS and lysed as described previously (22). Polyvinylidine fluoride filters were incubated with rabbit anti-TCF7L2 (2566), mouse anti-cleaved poly(ADP-ribose) polymerase (PARP) (9548), rabbit anti-caspase-3 (9961), rabbit anti-actin (4967), anti *p*-AKT (Serin473 9271) (all from Cell Signaling), followed by incubation with horseradish peroxidase–linked IgG peroxidase. The emitted light was captured on X-ray film after adding Immun-Star HRP Substrate (Bio-Rad Laboratories, Hercules, CA). Density of the bands was analyzed using Labworks 4.5 software (BioImaging Systems, Upland, CA).

RNA extraction and RT-PCR. Total RNA was isolated from cultured islets (100 islets/dish) as described previously (23). For quantitative analysis, we used the Light Cycler quantitative PCR system (Roche Diagnostics) with a commercial kit (Light Cycler-DNA Master SYBR Green I; Roche). Primers used were 5′-CTACCTAGTGTGCGGGGAAC-3′ and 5′-GCTGGTAGAGGGAG CAGATG-3′ (insulin), 5′-CTGGATTGGCGTTGTTTGTG-3′ and 5′-CTACAG CACTCCACCTTGGGA-3′ (PDX-1), and 5′-GAAGGAGCGACAGCTTCATA-3′ and 5′-GGGGGAGGCGAATCTAGTAA-3′ (TCF7L2) and compared with the housekeeping gene, 5′-AGAGTCGCGCTGTAAGAAGC-3′ and 5′-TGGTCTTGT CACTTGGCATC-3′ (α-Tubulin) and 5′-TCACCCACACTGTGCCCATCTACGA-3′ and 5′-CAGCGGAACCGCTCATTGCCA ATGG-3′ (β-actin).

Glucose-stimulated insulin secretion, static incubation. For each independent experiment, 20 islets were plated and exposed to the treatment conditions as indicated above. Four dishes per treatment group were used. For acute insulin release, islets were washed and preincubated (30 min) in Krebs-Ringer bicarbonate buffer (KRBB) containing 2.8 mmol/l glucose. The KRBB was then replaced by KRBB containing 2.8 mmol/l glucose for 1 h (basal), followed by an additional 1-h incubation in KRBB containing 16.7 mmol/l glucose. Islets were lysed in lysis buffer, and whole islet protein amount was measured by BCA protein assay (Pierce, Rockford, IL). Insulin was determined using a human insulin ELISA kit (Dako) or a mouse insulin ELISA kit (Alpco, Windham, NH).

Islet perifusion. Human islets plated in suspension dishes (100 islets/dish in triplicates) were precultured for 4 days with scrambled or TCF7L2 siRNA. Twenty islets of similar size from each treatment group were hand-picked and suspended in Bio-Gel P-2 beads (Bio-Rad) and placed in perifusion chambers as described previously (24). The perifusion system (ACUSYST-S; Cellex Biosciences, Minneapolis, MN) consisted of a multichannel peristaltic pump that delivered perifusate through six parallel tubing sets via a heat exchanger and six perfusion chambers at a constant rate of 0.3 ml/min. The perifusion buffers (KRBB) were preheated to 37°C, oxygenized with 95% O_2 and 5% CO_2, and delivered to the perifusion chambers containing the human islets. Islets were perifused for 1 h with KRBB containing 2.8 mmol/l glucose (perifusate was collected during the last 30 min every 5 min) for 40 min with KRBB containing 16.7 mmol/l glucose, for 30 min with KRBB containing 16.7 mmol/l glucose plus 100 nmol/l GLP-1 (fragment 7-37, human; Sigma), for 30 min with KRBB containing 2.8 mmol/l glucose, and for 10 min with 20 mmol/l KCl. The effluent was collected in 5-min (for basal and stimulated insulin secretion) and 2-min intervals (for KCl-induced insulin secretion) for determination of insulin concentrations. At the end of the perifusion, islet were collected by hand-picking and extracted with 0.18 N HCl in 70% ethanol for determination of insulin content. Insulin was determined using a human insulin ELISA kit (Alpco).

Statistical analysis. Immunostainings were evaluated in a randomized manner by a single investigator (L.S.) who was blinded to the treatment conditions. Data are presented as means ± SE and were analyzed by paired Student's *t* test or by ANOVA with a Bonferroni correction for multiple group comparisons.

RESULTS

Depletion in TCF7L2 results in impaired β-cell function. We investigated localization of TCF7L2 in human islets and found its expression in human β-cells, in confirmation with two previous reports (10,12). Triple-staining for TCF7L2, insulin, and DAPI revealed that TCF7L2 is expressed in the nucleus of β-cells (Fig. 1*A*, *1*). Plasmid overexpression of TCF7L2 resulted in increased TCF7L2 expression (Fig. 1*A*, *2*), whereas in islets depleted for TCF7L2 by siRNA to TCF7L2 (siTCF7L2), TCF7L2 was almost undetectable by immunohistochemistry (Fig. 1*A*, *3*).

To establish the effects of TCF7L2 on glucose mediated insulin secretion, we depleted TCF7L2 in pancreatic islets by exposure of mouse islets to three different siRNA sequences to TCF7L2 (siTCF7L2-1, -2, and -3) and human islets to one siRNA (siTCF7L2-2) (or scrambled control siRNA [siScr]) for 4 days. We achieved an average downregulation of TCF7L2 mRNA expression by 58 ± 10% (Fig. 1*F*) and 57 ± 12% protein expression in human (Fig. 3*E*) and in mouse islets (not shown). For evaluating transfection efficiency, islets were transfected with a fluorescein isothiocyanate (FITC)-labeled nontargeted siRNA and green fluorescence monitored over the 4-day culture period. Transfection efficiency was about 5% after 24 h and 75% after 48 h, which was maintained during the 4-day experiment (Fig. 1*A*, *4* and *5*).

We first tested the consequence of such downregulation on β-cell function in mouse islets. After a 4-day culture period with siRNA, we performed static incubation measurement of insulin secretion in mouse and human islets. No significant changes in insulin secretion were observed at basal levels at 2.8 mmol/l glucose. Also, a scrambled control sequence did not change basal or stimulated insulin secretion compared with untreated islets. In contrast, glucose-stimulated insulin secretion (GSIS) (16.7 mmol/l glucose) was decreased in mouse islets (1.6-, 2.3-, and 1.5-fold by siTCF7L2-1, -2, and -3, respectively, $P < 0.05$; Fig. 1*B*) compared with the scrambled control. This resulted in a 2.6-, 3.6-, and 2.6-fold decrease in insulin stimulatory index by siTCF7L2-1, -2, and -3, respectively (Fig. 1*C*). The same analysis was then performed in human islets. Because three different siRNA sequences showed the same results in mice, we performed the experiments in human

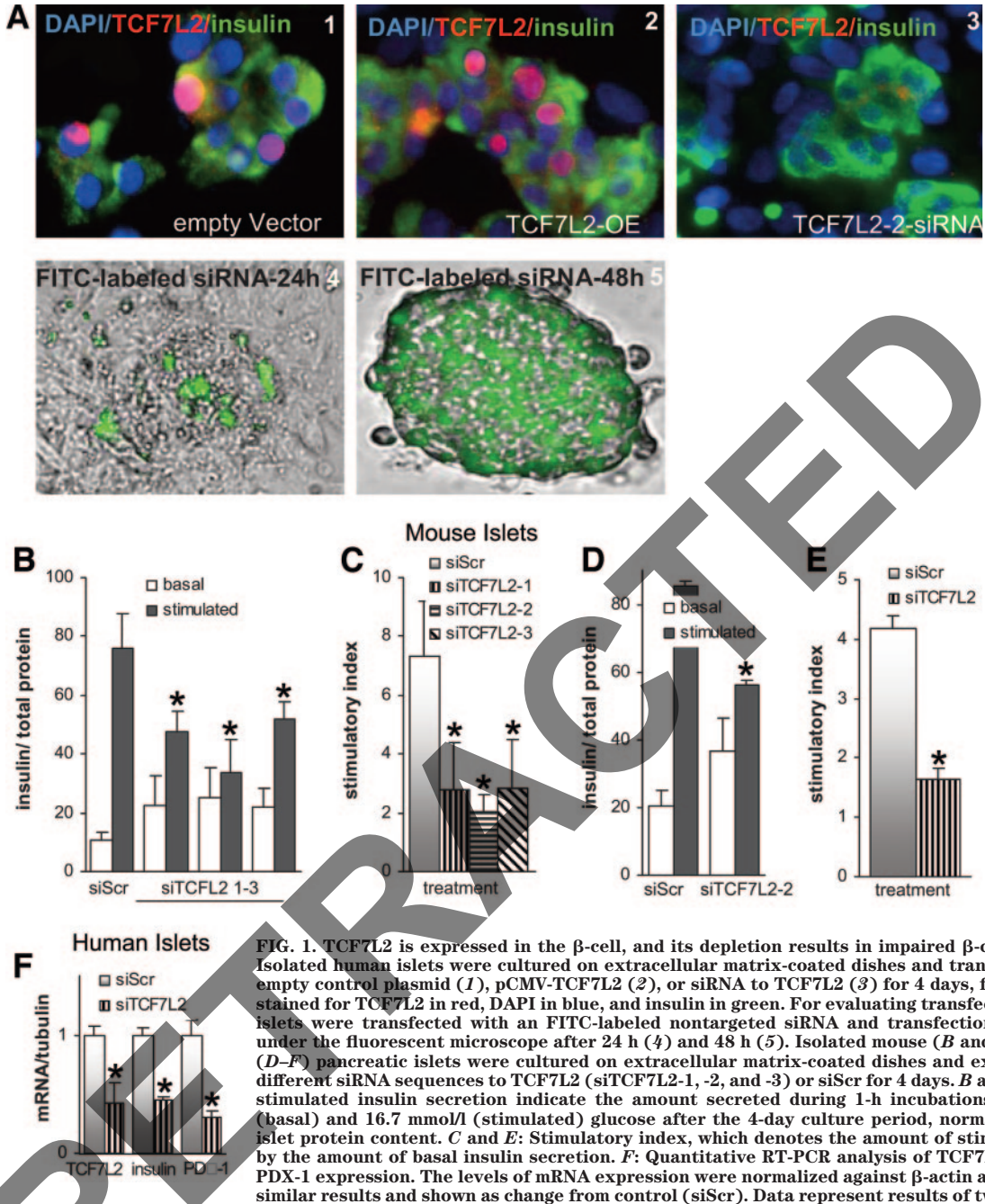

FIG. 1. TCF7L2 is expressed in the β-cell, and its depletion results in impaired β-cell function. *A*: Isolated human islets were cultured on extracellular matrix-coated dishes and transfected with an empty control plasmid (*1*), pCMV-TCF7L2 (*2*), or siRNA to TCF7L2 (*3*) for 4 days, fixed, and triple stained for TCF7L2 in red, DAPI in blue, and insulin in green. For evaluating transfection efficiency, islets were transfected with an FITC-labeled nontargeted siRNA and transfection was analyzed under the fluorescent microscope after 24 h (*4*) and 48 h (*5*). Isolated mouse (*B* and *C*) and human (*D–F*) pancreatic islets were cultured on extracellular matrix-coated dishes and exposed to three different siRNA sequences to TCF7L2 (siTCF7L2-1, -2, and -3) or siScr for 4 days. *B* and *D*: Basal and stimulated insulin secretion indicate the amount secreted during 1-h incubations at 2.8 mmol/l (basal) and 16.7 mmol/l (stimulated) glucose after the 4-day culture period, normalized to whole islet protein content. *C* and *E*: Stimulatory index, which denotes the amount of stimulated divided by the amount of basal insulin secretion. *F*: Quantitative RT-PCR analysis of TCF7L2, insulin, and PDX-1 expression. The levels of mRNA expression were normalized against β-actin and tubulin with similar results and shown as change from control (siScr). Data represent results of two independent experiments from two different organ donors in quadruplicate (human) or from two independent experiments from mouse islets ($n = 8$). Results are means ± SE of siScr-treated controls at 5.5 mmol/l (human islets) or 11.1 mmol/l (mouse islets) glucose, *$P < 0.05$ to scrambled control. (Please see http://dx.doi.org/10.2337/db07-0847 for a high-quality digital representation of this figure.)

islets with siTCF7L2-2 only. Consistent with studies in mouse islets, GSIS was 1.5-fold decreased by siTCF7L2-2 in human islets ($P < 0.05$; Fig. 1*D*), and the stimulatory index was decreased 2.6-fold (Fig. 1*E*). These changes in insulin secretion were accompanied by a 54 and 69% decrease in human islet insulin and PDX-1 mRNA, respectively (Fig. 1*F*) and a 1.4-fold decrease in islet insulin content (17.6 pmol/islet in siScr-treated islets vs. 12.8 pmol/islet in siTCF7L2-treated islets, $P < 0.05$; data not shown).

We also performed islet perifusion studies in human isolated islets, which had been precultured for 4 days with siScr or siTCF7L2. Consistent with our results in the static incubation studies, depletion of TCF7L2 resulted in delayed and quantitatively diminished glucose-mediated insulin secretion ($P < 0.01$ at all time points; Fig. 2*A*). Furthermore, we examined GLP-1–induced stimulation of insulin secretion in control and siTCF7L2-treated islets (Fig. 2*A*). Depletion of TCF7L2 led to 5.4 ± 0.2-fold ($P < 0.001$) decreased ability of GLP-1 to stimulate GSIS in human islets. Finally, we examined β-cell maximal secretory capacity by exposing islets to perifusion medium containing 20 mmol/l KCl. Average KCl-induced insulin levels were 5.2-fold higher in the siScr-treated islets than in the siTCF7L2-treated islets ($P < 0.001$). However, the average stimulatory index from KCl >2.8 mmol/l glucose alone was similar in both treatment groups (2.2 in siScr and 2.3 in siTCF7L2, NS), suggestive of effective KCl stimulation in TCF7L2-depleted islets.

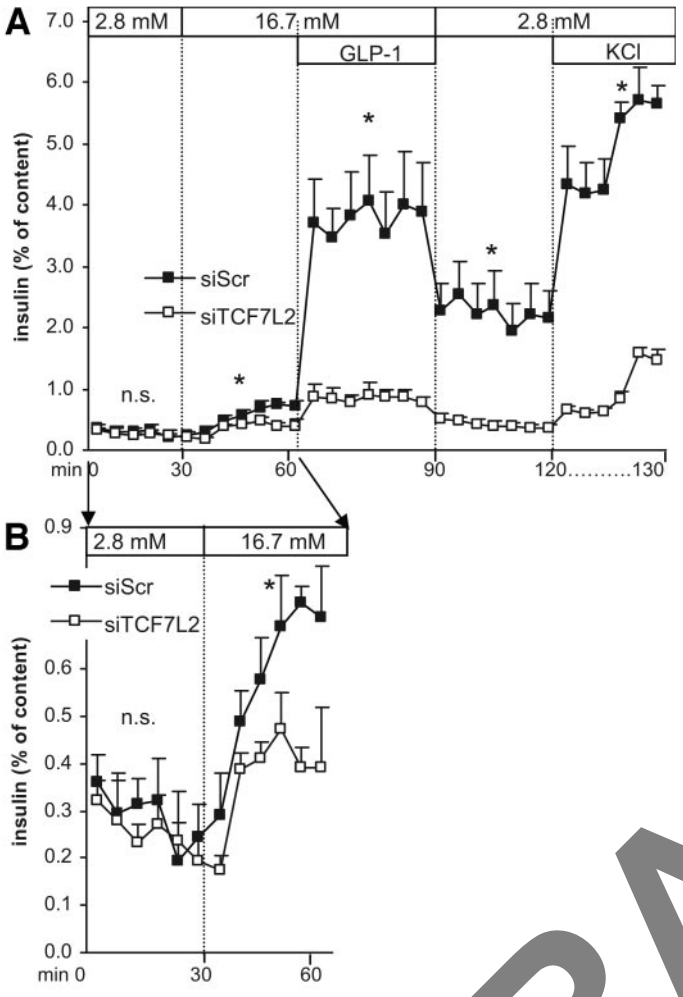

FIG. 2. Loss of TCF7L2 results in impaired glucose and GLP-1 stimulated insulin secretion. Human islets transfected with siScr or siTCF7L2-2 were placed in perifusion chambers in aliquots of 20 of equal size. The perifusate contained 2.8 mmol/l glucose for 1 h, 16.7 mmol/l glucose for 40 min, 16.7 mmol/l glucose and 100 nmol/l GLP-1 for 30 min, 2.8 mmol/l glucose for 30 min (the effluent was collected in 5-min intervals), and 20 mmol/l KCl for 10 min (the effluent was collected in 2-min intervals). Insulin secretion is expressed as percentage of content. One experiment was performed in triplicate from one organ donor. *A* and *B* show results from the same experiment with different *y*-axes scale to visualize changes in GSIS. *$P < 0.05$ to siScr control.

Depletion of TCF7L2 results in impaired β-cell turnover. Because β-cell apoptosis has been recognized as the underlying mechanism of β-cell destruction and consequent decrease in β-cell mass in type 2 diabetes (25), we tested whether depletion of TCF7L2 would also impair β-cell survival. Exposure of human islets to two different siRNA sequences to TCF7L2 resulted in a 2.2- and 2.5-fold decrease in β-cell proliferation, analyzed by double staining for Ki67 and insulin ($P < 0.001$; Fig. 3*A* and *C*) and 5.1- and 3.4-fold increase in β-cell apoptosis, analyzed by double staining for TUNEL and insulin ($P < 0.001$, Fig. 3*B* and *C*). Because the TUNEL assay also recognizes necrosis and DNA repair, we performed Western blot analysis for cleaved Caspase-3 and cleaved PARP, two of the most downstream products of apoptotic signaling. Together with a 2.4-fold downregulation in TCF7L2 protein expression, cleaved Caspase-3 was 5.2-fold and cleaved PARP was 2.1-fold higher expressed in human islets exposed to siTCF7L2 for 4 days (Fig. 3*D* and *E*). AKT is an important survival factor for β-cells. By Western blot analysis, we measured basal AKT phosphorylation in siScr- and siTCF7L2-treated islets. After a 4-day culture of human islets in 10% FCS and 5.5 mmol/l glucose, *p*-AKT was maintained but almost undetectable in the TCF7L2-depleted islets (Fig. 3*F*).

Overexpression of TCF7L2 protects from glucotoxicity and cytokine toxicity. Because the loss of islet TCF7L2 expression resulted in significant impairment of β-cell function and survival, we tested the hypothesis of whether environmental factors that contribute to β-cell failure in diabetes influence TCF7L2 expression levels and whether TCF7L2 overexpression can rescue from β-cell death.

We exposed isolated mouse and human pancreatic islets to increasing glucose concentrations (5.5, 11.1, and 33.3 mmol/l) and the mixture of cytokines (2 ng/ml IL-1β plus 1,000 units/ml IFN-γ) and transfected with either pCMV-TCF7L2 or an empty control plasmid under the same promoter. After the 4-day culture period, we performed GSIS (Fig. 4) and immunostaining for β-cell proliferation and apoptosis (Fig. 5). As we and others have reported before, increased glucose levels and exposure of islets to IL-1β plus IFN-γ impaired β-cell survival and function in mouse and human islets (26). Importantly, islets transfected with TCF7L2 were protected against the deleterious effects of glucose and of cytokines. The stimulatory index was 2.7-fold decreased by 33.3 mmol/l glucose and 7.3-fold by cytokines, compared with control incubations in mouse islets at 11.1 mmol/l alone ($P < 0.01$; Fig. 4*A* and *B*). TCF7L2 overexpression induced a 2.6-fold and 4-fold increase in stimulatory index at 33.3 mmol/l glucose and in islets treated with IL-1β plus IFN-γ, respectively, compared with control, protecting from the deleterious effects of both. Basal insulin secretion levels were increased by 33.3 mmol/l glucose and by cytokines in mouse islets (Fig. 4*A*), possibly as an indicator of the increased β-cell apoptosis. TCF7L2 overexpression reduced such an increase significantly ($P < 0.01$). Additionally, TCF7L2 increased stimulated insulin secretion in cytokine-treated islets, indicating its protective effect on β-cell insulin secretion and survival.

In human islets, similar protective effects were observed. Increased glucose levels (11.1 and 33.3 mmol/l) and the cytokine mixture IL-1β plus IFNγ reduced the islet stimulatory index 3.7-, 4.7-, and 3.2-fold, respectively ($P < 0.01$; Fig. 4*C* and *D*), compared with conditions at 5.5 mmol/l glucose. TCF7L2 overexpression improved β-cell GSIS significantly in all conditions. Interestingly, also at 5.5 mmol/l glucose, TCF7L2 improved stimulated insulin secretion (Fig. 4*C* and *D*). We observed similar protective results of TCF7L2 overexpression when we looked at β-cell apoptosis and proliferation. Elevated glucose concentration dose dependently reduced β-cell proliferation (2.5-, 5.2-, and 7.4-fold reduction by 11.1 and 33.3 mmol/l glucose and by IL-1β plus IFNγ, respectively; $P < 0.001$; Fig. 5*A* and *C*) and induced β-cell apoptosis (1.8-, 2.7-, and 1.6-fold induction by 11.1 and 33.3 mmol/l glucose and by IL-1β plus IFNγ, respectively; $P < 0.001$; Fig. 5*B* and *C*) in isolated human islets, compared with control incubations. This was reversed by TCF7L2 overexpression. Proliferation was 1.8-, 2.5-, and 3.1-fold induced (Fig. 5*A* and *C*) and apoptosis was 1.6-, 1.6-, and 1.5-fold reduced (Fig. 5*B* and *C*) by TCF7L2 at 11.1 and 33.3 mmol/l glucose and at IL-1β plus IFN-γ, respectively ($P < 0.01$). To investigate how chronically elevated glucose levels and cytokines, which

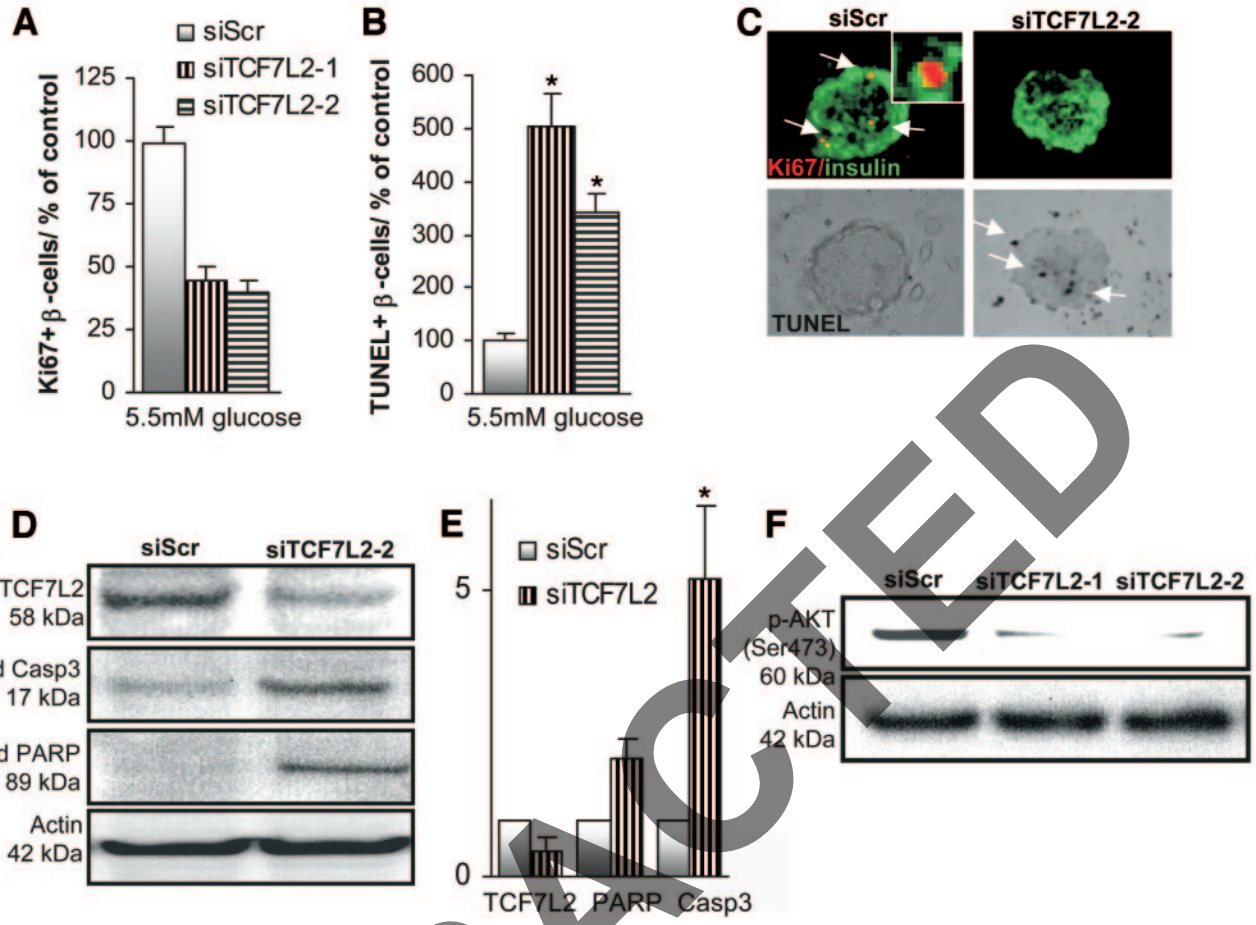

FIG. 3. Depletion in TCF7L2 results in impaired β-cell turnover. Isolated human pancreatic islets were cultured on extracellular matrix-coated dishes and exposed to two different siRNA sequences to TCF7L2 (siTCF7L2-1 and -2) or siScr for 4 days. Proliferation was measured by the Ki67 antibody (*A* and *C*, *top panel*) stained in red (cy3) and apoptosis by the TUNEL assay (*B* and *C*, *bottom panel*) and stained in black (alkaline phosphate). Islets were triple stained for insulin in green (*C*, *top panel*) and counterstained for DAPI in blue (not shown). White arrows point to proliferating and apoptotic β-cells. The insert of higher magnification shows a proliferating β-cell. Results are means ± SE of the percentage of Ki67-positive β-cells (*A*) or TUNEL-positive β-cells (*B*) normalized to control incubations (siScr) at 5.5 mmol/l glucose alone (100%; in absolute value, 0.29% TUNEL-positive and 1.78% Ki67-positive β-cells). The average number of β-cells counted was 26,130 for each treatment group in each of the two separate experiments from two different organ donors in quadruplicate ($n = 8$). *D* and *F*: Western blot analysis of TCF7L2, cleaved Caspase-3, cleaved PARP, Actin (*D*), and *p*-AKT and Actin (*F*) in human islets exposed for 4 days to siScr or siTCF7L2. Actin was used as a loading control on the same membrane after stripping. Blot is representative of three independent experiments from three different organ donors. *E*: The density of expression levels was quantified after scanning, normalized to actin levels, and expressed as change from siScr control, respectively. *$P < 0.05$ to untreated control. (Please see http://dx.doi.org/10.2337/db07-0847 for a high-quality digital representation of this figure.)

contribute to β-cell failure in diabetes, influence TCF7L2 expression levels and to correlate levels of TCF7L2 expression with apoptosis, we performed Western blot analysis from glucose/IL-1β plus IFN-γ–treated human islets transfected with pCMV-TCF7L2 or an empty control plasmid. Elevated glucose and IL-1β plus IFN-γ significantly downregulated TCF7L2 protein expression (Fig. 5*D*). In parallel to the 2.2- and 3.6-fold TCF7L2 downregulation by 11.1 mmol/l glucose or IL-1β plus IFN-γ, cleaved caspase-3 expression was 1.5-fold induced. In islets overexpressing TCF7L2, its protein expression levels were 1.5-, 2.4-, and 3.4-fold induced at 5.5 and 11.1 mmol/l glucose or 5.5 mmol/l glucose with IL-1β plus IFN-γ, compared with the empty vector–treated control, respectively (Fig. 5*D* and *E*; $P < 0.05$), and mRNA levels were 416-, 394-, and 705-fold increased, respectively ($P < 0.01$; data not shown). In contrast, caspase-3 was activated by glucose and IL-1β plus IFN-γ, and TCF7L2 reversed such upregulation. Even at control incubations, the signal for caspase-3, which is mostly seen in isolated human islets, was downregulated by TCF7L2 overexpression, indicating an overall protection from β-cell apoptosis by TCF7L2.

DISCUSSION

The identification of SNPs in the *TCF7L2* gene and its strong association with type 2 diabetes and with impaired insulin secretion (4–13) is an essential breakthrough in the field of genetics of type 2 diabetes.

Our study provides further evidence that TCF7L2 is an important regulator of β-cell function and survival. Chronic hyperglycemia and the cytokine mixture with IL-1β plus IFN-γ decreased TCF7L2 expression in the islets. In turn, TCF7L2 overexpression protected from the effects of chronically elevated glucose and cytokines on β-cell apoptosis and function. In vitro, TCF7L2 depletion in islets reduced proliferation, induced β-cell apoptosis, and decreased GSIS. During islet perifusion, insulin secretion in response to glucose was delayed and impaired in TCF7L2-depleted islets. Interestingly, GLP-1 increased

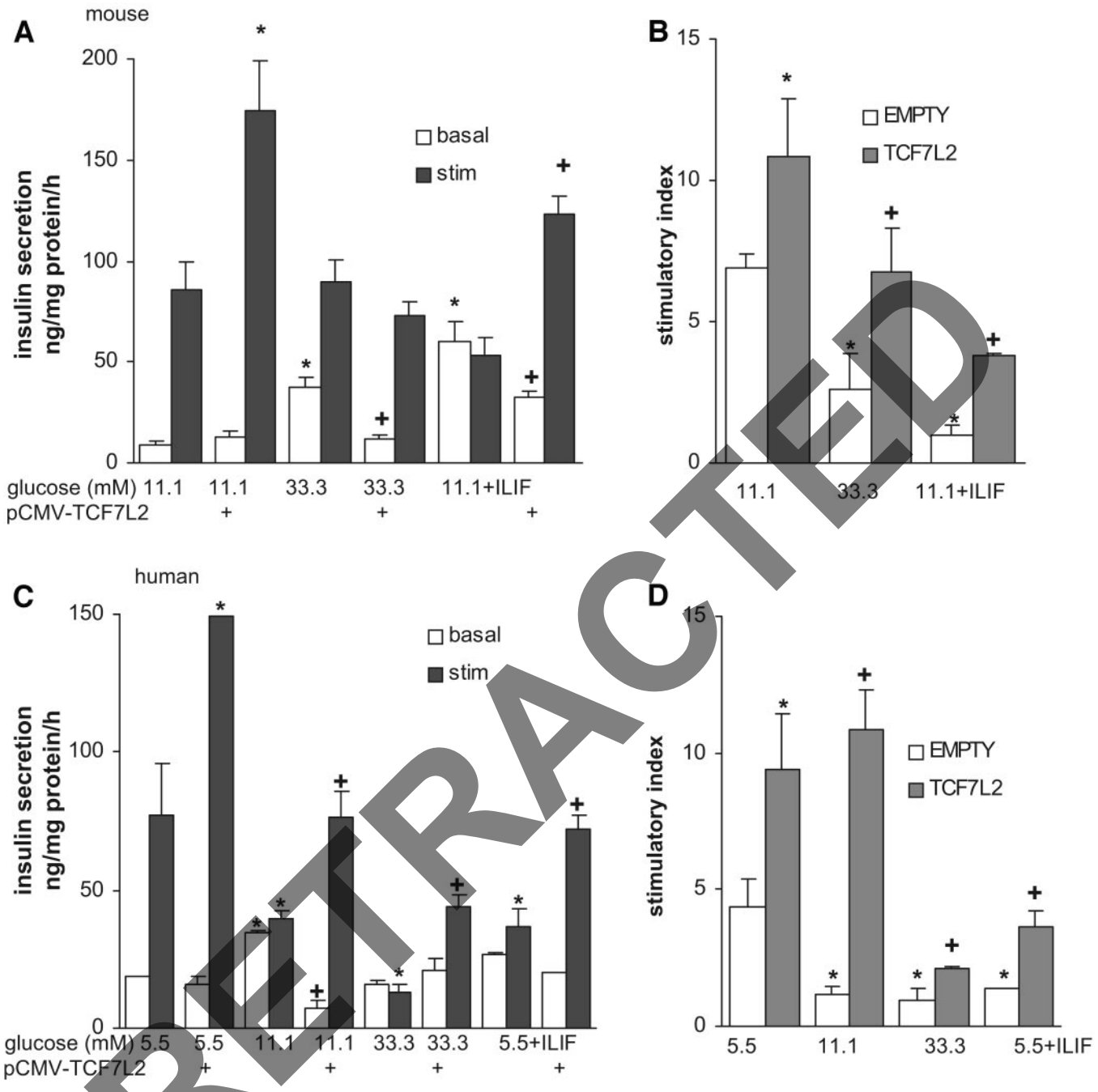

FIG. 4. Overexpression of TCF7L2 protects from glucotoxicity and cytokine toxicity. Isolated mouse (*A* and *B*) and human (*C* and *D*) pancreatic islets were cultured on extracellular matrix-coated dishes and exposed to increasing glucose concentrations (5.5, 11.1, and 33.3 mmol/l) or to the mixture of IL-1β plus IFN-γ (ILIF) and transfected with either pCMV-TCF7L2 or an empty control plasmid under the same promoter. *A* and *C*: Basal and stimulated insulin secretion indicate the amount secreted during 1-h incubations at 2.8 mmol/l (basal) and 16.7 mmol/l (stimulated) glucose after the 4-day culture period, normalized to whole islet protein content. *B* and *D*: Stimulatory index, which denotes the amount of stimulated divided by the amount of basal insulin secretion. Data represent results of two different experiments from two different organ donors in quadruplicate (human) or of two independent experiments from mouse islets ($n = 8$). Results are means ± SE of empty vector–treated control islets at 5.5 mmol/l (human islets) or 11.1 mmol/l (mouse islets) glucose. *$P < 0.05$ to untreated control, +$P < 0.05$ to empty vector–transfected islets at same treatment.

GSIS in control islets to much greater extent than in TCF7L2-depleted islets. These data are in line with a recent study in human patients, which shows that carriers of the TCF7L2 risk allele showed a significant reduction in GLP-1–induced insulin secretion without defects in GLP-1 secretion during an oral glucose tolerance test (27). This supports the hypothesis that changes in TCF7L2 result in a functional defect of GLP-1 signaling in β-cells and may explain the impaired insulin secretion in carriers of the TCF7L2 risk alleles and the increased risk of type 2 diabetes. Cell membrane depolarization by addition of 20 mmol/l KCl raised insulin secretion in both control and TCF7L2-depleted islets. Although the absolute KCl-stimulated insulin secretion was much higher in control islets, the relative increase in insulin levels in response to KCl was comparable in both groups. Calculation of the average stimulatory index in islets in response to KCl showed no differences in control and TCF7L2-depleted islets. One

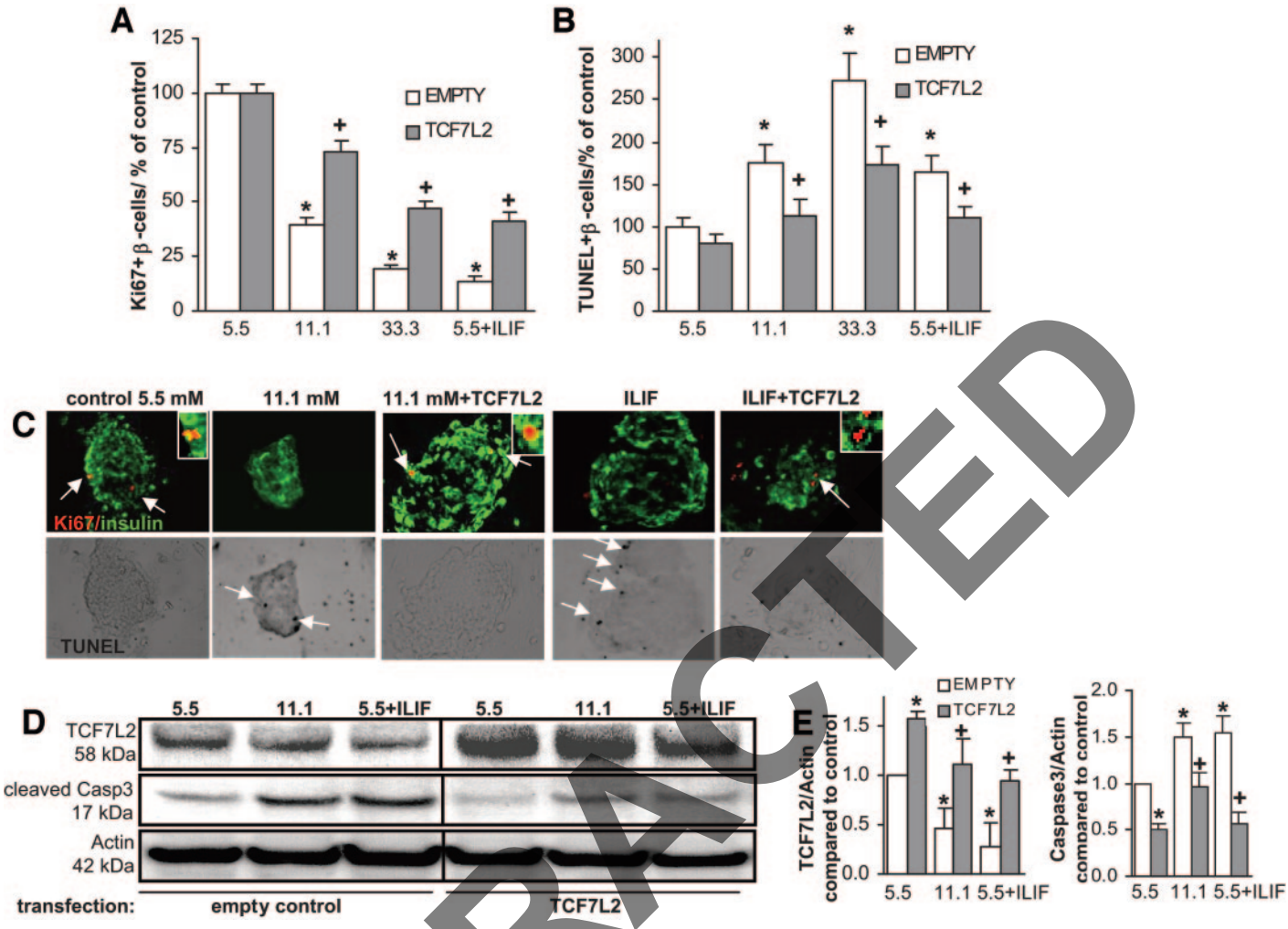

FIG. 5. Overexpression of TCF7L2 protects from glucose- and cytokine-induced β-cell apoptosis and decreased proliferation. Isolated human pancreatic islets were cultured on extracellular matrix-coated dishes and exposed to increasing glucose concentrations (5.5, 11.1, and 33.3 mmol/l) or the mixture of IL-1β plus IFN-γ (ILIF) and transfected with either pCMV-TCF7L2 or an empty control plasmid under the same promoter. Proliferation was measured by the Ki67 antibody (*A* and *C*, *top panel*) stained in red (cy3) and apoptosis by the TUNEL assay (*B* and *C*, *bottom panel*) and stained in black (alkaline phosphate). Islets were triple stained for insulin in green (*C*, *top panel*) and counterstained for DAPI in blue (not shown). White arrows point to proliferating and apoptotic β-cells. The insert of higher magnification shows a proliferating β-cell. Results are means ± SE of the percentage of Ki67-positive β-cells (*A*) or TUNEL-positive β-cells (*B*) normalized to control incubations (pCMV-empty) at 5.5 mmol/l glucose alone (100%; in absolute value, 0.33% TUNEL-positive and 2.09% Ki-67-positive β-cells). The average number of β-cells counted was 20,630 for each treatment group in each of the two separate experiments from two different organ donors in quadruplicate ($n = 8$). *D*: Western blot analysis of TCF7L2, cleaved Caspase-3, and actin in human islets exposed for 4 days to 5.5 or 11.1 mmol/l glucose or 5.5 mmol/l plus IL-1β plus IFN-γ. Islets were transfected with a CMV-TCF7L2 plasmid or an empty control plasmid using the same promoter (pCMV-empty). Actin was used as a loading control on the same membrane after stripping. Blot is representative of three independent experiments from three different organ donors. *E*: The density of expression levels were quantified after scanning, normalized to actin levels and expressed as change from control (5.5 mmol/l glucose). *$P < 0.05$ to untreated control, +$P < 0.05$ to empty vector–transfected islets at same treatment. (Please see http://dx.doi.org/10.2337/db07-0847 for a high-quality digital representation of this figure.)

important mechanism of the potentiation of GSIS by GLP-1 is the increase in the number of insulin secretory granules that dock to the β-cell membrane during stimulation (28). GLP-1 significantly increases the maximum insulin secretion induced by glucose and by KCl (29). Therefore we hypothesize that defective GLP-1 signaling observed in our study is responsible for the observed impaired absolute KCl-induced insulin response.

The protective effect of TCF7L2 overexpression on impaired β-cell function induced by glucotoxicity and cytokine toxicity was mainly a result of a decreased basal insulin secretion. Apoptotic cells have often increased basal insulin levels during the 1-h static incubation, as a result of dying cells, which release their insulin. Especially under conditions of chronic hyperglycemia, basal insulin release is increased (30). These data provide further evidence of the important role of TCF7L2 on β-cell survival.

It has been recognized in the past that isolated islets themselves are highly susceptible to β-cell apoptosis, whereas in vivo, only very few apoptotic cells can be found in pancreatic sections. For analysis of β-cell proliferation in this study, we used islets from young human islet donors (24 and 41 years) and therefore observed a relatively high number of proliferating cells when we plated the islets on extracellular matrix-coated dishes, with the majority being β-cells detected by double staining for Ki67 and insulin. This culture condition allowed us to study long-term islet survival and function, and we also detected some β-cell proliferation (21), whereas in vivo, β-cell proliferation in adult human islets is very limited. Using the same culture conditions, we have recently

shown that there is an age-dependent decline in β-cell proliferation (31), which was consistent with a study by Butler et al. (3) using pancreatic sections from autopsy.

One important regulator of β-cell survival is the protein kinase B/AKT pathway. Also the anti-apoptotic mechanism of GLP-1 is mediated through AKT (32). AKT activation has been reported to mediate survival of isolated human islets through insulin in an autocrine manner (33). Reduced AKT activity has also been associated with defective insulin secretion (34). Moreover, impaired AKT activation was associated with reduced GSK-3 inactivation (35), a gene associated with increased β-cell apoptosis (36) and compound in the WNT signaling pathway, which degrades β-catenin, and thus restricting nuclear activation of TCF7L2 i (37). Almost undetectable *p*-AKT in the TCF7L2-depleted islets therefore may explain the deleterious effects of loss of TCF7L2 on both β-cell survival and insulin secretion.

TCF7L2 is expressed in isolated human islets, shown by Western blot analysis, RT-PCR, and immunostaining. This is in line with recent results from human islets (10,38) and from Zucker diabetic fatty rat islets and their lean controls. In contrast, TCF7L2 was not detectable in mouse pancreatic sections and in the pancreatic InR1-G9 cell line (15).

In our study, we have observed that treatment of isolated islets with siRNA to TCF7L2 resulted in decreased TCF7L2 mRNA and protein expression and induced impaired β-cell survival and function. In contrast, TCF7L2 mRNA is increased in islets isolated from the Zucker diabetic fatty rat (38) and from patients with type 2 diabetes (12). Also, in individuals carrying an increased number of TCF7L2 risk T-alleles, TCF7L2 mRNA expression levels are increased (12). Neither study investigated TCF7L2 protein levels. Because we observed that increased TCF7L2 protein had protective effects on β-cell survival, we hypothesize that the posttranscriptional regulation of TCF7L2 rather than changes in mRNA levels may alter the β-cell.

TCF7L2 mRNA expression was also analyzed in muscle, fat, and lymphocytes in correlation with TCF7L2 variants. In transformed lymphocytes, TCF7L2 was differently regulated in controls and patients with type 2 diabetes; in control individuals, TCF7L2 mRNA expression decreased with the number of T-alleles, whereas in type 2 diabetes, it increased. Interestingly, no changes were observed in muscle or fat tissue (39), although TCF7L2 expression in adipose tissue is decreased in obese individuals with type 2 diabetes (10).

In this study, we report that elevated glucose levels and cytokine in cultured islets decreased TCF7L2 mRNA and protein levels. Therefore, further in vivo studies are needed using TCF7L2 variants in mice to confirm these results and translate them into the pathophysiology of type 2 diabetes.

Our data show that regulation of TCF7L2 plays an important role in the regulation of both β-cell survival and function and that targeting its expression could be a new strategy to maintain β-cell survival in diabetes.

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