TCF1 links GIPR signaling to the control of beta cell function and survival

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The glucagon-like peptide-1 (GLP-1) receptor and the glucose-dependent insulinotropic polypeptide (GIP) receptor transduce nutrient-stimulated signals to control beta cell function1. Although the GLP-1 receptor (GLP-1R) is a validated drug target for diabetes1, the importance of the GIP receptor (GIPR) for the function of beta cells remains uncertain2–4. We demonstrate that mice with selective ablation of GIPR in beta cells (MIP-Cre:Giprlox/lox, Gipr−/−/βCell) exhibit lower levels of meal-stimulated insulin secretion, decreased expansion of adipose tissue mass and preservation of insulin sensitivity when compared to MIP-Cre controls. Beta cells from Gipr−/−/βCell mice display greater sensitivity to apoptosis and markedly lower islet expression of T cell–specific transcription factor-1 (TCF1, encoded by Tcf7), a protein not previously characterized in beta cells. GIP, but not GLP-1, promotes beta cell Tcf7 expression via a cyclic adenosine monophosphate (cAMP)-independent and extracellular signal–regulated kinase (ERK)-dependent pathway. Tcf7 (in mice) or TCF7 (in humans) levels are lower in islets taken from diabetic mice and in humans with type 2 diabetes; knockdown of TCF7 in human and mouse islets impairs the cytoprotective responsiveness to GIP and enhances the magnitude of apoptotic injury, whereas restoring TCF1 levels in beta cells from Gipr−/−/βCell mice lowers the number of apoptotic cells compared to that seen in MIP-Cre controls. Tcf7−/− mice show impaired insulin secretion, deterioration of glucose tolerance with either aging and/or high-fat feeding and increased sensitivity to beta cell injury relative to wild-type (WT) controls. Hence the GIPR-TCF1 axis represents a potential therapeutic target for preserving both the function and survival of vulnerable, diabetic beta cells.

The ingestion of nutrients triggers the secretion of multiple gut peptides, including GLP-1 and GIP, both of which are incretin hormones that amplify insulin secretion. GLP-1 and GIP also control beta cell growth and survival1, rendering them attractive targets for the treatment of type 2 diabetes (T2D). Indeed, the prevention of incretin degradation by inhibiting dipeptidyl peptidase-4 (DPP4)5 and the augmentation of incretin receptor signaling using GLP-1R agonists or emerging co- and tri-agonists that target this pathway6,7 represent established and investigational strategies for the treatment of T2D.

The receptors for GLP-1 and GIP are highly related in structure; both are regulated by transcription factor-7-like 2 (TCF7L2)8 in islets and control beta cell function and survival through cAMP-dependent pathways. Although genetic variation within the coding region of the GLP1R gene has been linked to differences in fasting glucose levels and in beta cell function in humans9, interpretation of the importance of the GIPR for beta cell function is confounded by genetic and physiological data in humans and in mice and rats that suggest roles for the GIPR in the regulation of adipose-tissue accretion, body weight and insulin sensitivity2–4,10.

GLP-1R agonists are increasingly used for the treatment of T2D and obesity and are under investigation for the treatment of type 1 diabetes1,11. By contrast, there is less interest in the GIPR as a drug target because of reports of defective GIP action in people with diabetes who are severely hyperglycemic12. Nevertheless, a brief period of insulin therapy markedly restores GIP responsiveness in subjects with T2D (ref. 13), and GIPR activation is a key component of the action of several novel co- and tri-agonists that are under investigation as potential treatments for diabetes and obesity6,7. Accordingly, to delineate the importance of GIPR signaling in beta cells independently of its potentially confounding actions in extrapancreatic tissues, we mated MIP-CreERT mice with Giprlox/lox mice to generate mice with conditional and selective inactivation of Gipr in adult beta cells (Gipr−/−/βCell) (Supplementary Fig. 1a). Levels of Gipr mRNA transcripts were 90% lower in the islets of Gipr−/−/βCell mice than in those of MIP-Cre mice, Giprlox/lox mice and WT mice (Fig. 1a and Supplementary Fig. 1b), whereas Gipr expression in adipose tissue was not perturbed (Supplementary Fig. 1b). GIP robustly reduced...
glycemic excursion after glucose challenge in all three lines of control mice but did not lower glycemia in Gipr−/−βCell mice (Supplementary Fig. 1c). GIP also robustly stimulated insulin secretion in perfused islets from control mice, but not in those from Gipr−/−βCell mice (Fig. 1b). Administration of a low dose of a DPP4 inhibitor modestly improved glucose tolerance and enhanced glucose-stimulated insulin secretion in control mice but not in Gipr−/−βCell mice (Fig. 1c).

For subsequent experiments, we used MIP-Cre mice as controls, minimizing any potential confounding effects associated with Cre-driver lines in beta cells14,15. Notably, human growth hormone (hGH) release and expression of tryptophan hydroxylase-1 (Tph1) and -2 (Tph2) were similar in islets from MIP-Cre and from Gipr−/−βCell mice (Supplementary Fig. 1d).

Although mice with whole-body deletion of Gipr exhibit modestly impaired beta cell function16, 8-week-old Gipr−/−βCell mice fed a low-fat diet (LFD) showed normal oral glucose tolerance and glucose-stimulated insulin levels (Supplementary Fig. 1e). Furthermore, insulin sensitivity, weight gain, ambient glycemia, food intake and plasma incretin levels were all comparable in Gipr−/−βCell and MIP-Cre control mice (Supplementary Fig. 1f–h). Unexpectedly, oral and intraperitoneal glucose tolerance and insulin sensitivity were paradoxically better in 18-week-old Gipr−/−βCell mice than in MIP-Cre controls (Fig. 1d and Supplementary Fig. 1i). Gipr−/−βCell mice accumulated less white adipose tissue (WAT) with age and exhibited reduced adipocyte size (Fig. 1e and Supplementary Fig. 2a), despite having both similar expression levels of the genes that regulate adipose-tissue metabolism and comparable lipoprotein lipase activity in adipose tissue (Supplementary Fig. 2b,c).

We reasoned that GIPR-deficient beta cells produce less insulin than do beta cells with normal GIPR levels, which, in turn, limits expansion of WAT deposits, thereby improving insulin sensitivity16. Accordingly, we reassessed these phenotypes by using two independent strategies to restore insulin levels: (i) exposure to a high-fat diet (HFD) and (ii) direct insulin replacement. A HFD stimulates GIP secretion, which will expand adipose tissue mass, increase both resistin expression and secretion and promote insulin resistance, thereby indirectly enhancing insulin secretion even in the absence of GIPR action in beta cells17,18. HFD-fed Gipr−/−βCell mice exhibited weight
gain, plasma incretin levels and glycemia similar to those seen in MIP-Cre controls (Supplementary Fig. 2d), but they no longer exhibited improved glucose tolerance, higher insulin sensitivity or less adipose tissue mass as compared to MIP-Cre controls (Fig. 1f and Supplementary Fig. 2d).

Consistent with an essential role for the beta cell GIPR in nutrient-regulated insulin secretion, insulin levels were lower in LFD-fed Gipr−/−βCell mice than in LFD-fed MIP-Cre mice during the re-feeding period after an overnight fast, despite showing comparable levels of glycemia (Fig. 1g). In contrast, HFD feeding restored insulin profiles in Gipr−/−βCell mice to the levels observed in MIP-Cre mice (Fig. 1g). We next implanted insulin pellets in Gipr−/−βCell mice to reverse the relative hypoinsulinemia detected under LFD conditions (Fig. 1g). The pellets raised insulin levels by ~1 ng per ml (Fig. 1h), an amount similar to the difference in postprandial insulin values detected in MIP-Cre compared to Gipr−/−βCell mice (Fig. 1g); insulin pellets had no effect on body weight, glycemia (Supplementary Fig. 2e) or food intake (data not shown). The normalization of postprandial insulinemia restored adiposity and insulin sensitivity to control levels in Gipr−/−βCell mice (Fig. 1i and Supplementary Fig. 2f).

The relatively modest basal glucose-tolerance phenotype of Gipr−/−βCell mice may reflect the upregulation of related nutrient-sensitive beta cell signaling pathways. Indeed Gipr−/−βCell mice exhibited greater insulin secretion in response to the GLP-1R agonist exendin-4 (Ex4) in vivo and in isolated perfused islets ex vivo relative to MIP-Cre mice (Fig. 1j). Conversely, the selective reduction of GLP-1R signaling impaired glucose tolerance to a greater extent in Gipr−/−βCell mice than in MIP-Cre controls (Supplementary Fig. 2g). Similarly, individual patch-clamped beta cells from Gipr−/−βCell mice did not respond to GIP yet exhibited enhanced sensitivity to Ex4 (Supplementary Fig. 2h), whereas beta cells from Gipr−/−βCell mice demonstrated normal actin-depolymerization responses to high levels of either glucose or Ex4 but did not respond to GIP (Supplementary Fig. 2i). Gipr−/−βCell mice also exhibited enhanced sensitivity to exogenous administration of the insulinotropic G protein–coupled receptor (GPCR) 119 (GPR119) agonist AR231453 compared to control mice (Supplementary Fig. 2j).

Given that class B GPCRs modulate beta cell growth and survival, we analyzed Gipr−/−βCell islets. Loss of the beta cell GIPR reduced beta cell area, but islet size, islet number, granule morphology and granule size were all unaffected (Supplementary Fig. 3a,b). The adaptive increase in beta cell mass after exposure to a HFD was preserved in Gipr−/−βCell mice (Supplementary Fig. 3a), revealing that the beta cell GIPR is dispensable for the adaptive response to insulin resistance. Because GIPR signaling controls beta cell survival and proliferation, we assessed the response to the beta cell toxin streptozotocin (STZ).
More apoptotic beta cells were detected in islets from STZ-treated Gipr−/− mice than in those from MIP-Cre controls (Fig. 2a), whereas levels of mRNA transcripts for genes encoding either pro-survival GPCRs, components of the glucose-sensing machinery, including GLUT2 (Sfrs2a), which transports STZ), and mediators of proliferation or survival were not differentially expressed in Gipr−/− mice (Supplementary Fig. 3c and Supplementary Table 1).

As the Wnt target TCF7L2 regulates both incretin-receptor expression and beta cell survival8,25, we assessed mRNA levels of β-catenin and members of the TCF family. Islet expression levels of catenin (cadherin-associated protein), beta 1 (Ctnnb1), Tcf7l1, Tcf7l2 and Tcf4 were not affected by a loss of GIPR in beta cells; however, mRNA transcripts for Tcf7 and for lymphoid enhancer binding factor 1 (Lef1), both of which encode proteins not previously assigned functional roles in beta cells, were markedly lower in Gipr−/− mouse islets (Fig. 2b). Moreover, GIP directly increased Tcf7 expression in WT mouse islets ex vivo (Fig. 2c). Furthermore, although GLP-1R and GIP share cAMP-dependent pathways26 to regulate beta cell function and survival27, Tcf7 expression was not perturbed in islets from Gp1r−/− mice (Fig. 2d).

Immunohistochemistry localized Tcf1 (the protein product of Tcf7) to mouse islets (Fig. 2e and Supplementary Table 2). Tcf7 has been principally studied in immune cells, wherein alternative promoter utilization and RNA splicing28 gives rise to multiple RNA transcripts, including two major transcripts (~1.3 kb and ~0.9 kb) in the thymus (Fig. 2f). The dominant Tcf7 mRNA transcript detected in mouse islets and beta cell lines was ~0.9 kb (Fig. 2f) and was absent in Tcf7−/− islets (Supplementary Fig. 3d). GIP directly induced Tcf7 expression in the rat insulin-secreting (INS-1) beta cell line, and in human islets (TCF7) (Supplementary Fig. 3e,f). Although GIP lowered glycemia in WT control mice, 12-week-old, obese, diabetic db/db mice displayed significantly lower islet levels of Tcf7 and Gipr mRNA transcripts and did not lower their glucose levels or secrete insulin in response to exogenous GIP (Fig. 2g and Supplementary Fig. 4a–d). By contrast, the levels of Gip1r and Tcf7l2 mRNA transcripts were unchanged in islet RNA from 6- or 12-week-old db/db mice (Supplementary Fig. 4e). Levels of TCF7 mRNA transcripts were lower in islets from obese, non-diabetic subjects and significantly lower in islets from subjects with T2D than in non-obese, non-diabetic control subjects (Fig. 2h and Supplementary Fig. 4f). The GIP-dependent induction of Tcf7 expression was not mimicked by forskolin or by Ex4 and was not sensitive to the cAMP-inhibitor Rp-cAMP (Fig. 2i). By contrast, the inhibition of ERK1/2 phosphorylation with the mitogen-activated protein kinase inhibitor U0126 completely prevented the GIP-stimulated increase in Tcf7 RNA (Fig. 2i) and Tcf7 protein levels (Fig. 2j). Hence signaling via the GIPR, but not the GLP-1R, controls Tcf7 expression through a cAMP-independent and ERK1/2-dependent pathway in beta cells.

To identify a role for Tcf7 in beta cells, we studied Tcf7−/− mice. Body weight and fat mass were similar in WT mice and Tcf7−/− mice maintained on a LFD or HFD (Supplementary Fig. 5a,b). Glucose tolerance and plasma insulin levels were normal in 8-week-old Tcf7−/− mice on a LFD (Supplementary Fig. 5c). However, intraperitoneal glucose tolerance was profoundly impaired, and the insulin response to glucose was markedly attenuated, in older (18-week-old) Tcf7−/− mice on a LFD (Supplementary Fig. 5a). Both oral and intraperitoneal glucose tolerance were severely impaired after only 4 weeks of HFD feeding because of the defective upregulation of glucose-stimulated insulin levels (Fig. 3b and Supplementary Fig. 5d). Moreover, sensitivity to exogenous insulin was comparable in WT and Tcf7−/− mice (Fig. 3c). Isolated islets from Tcf7−/− mice displayed significantly impaired insulin release in response to glucose and GIP (Fig. 3d). Similarly to islets from Gipr−/− mice, Tcf7−/− beta cells failed to respond to GIP yet demonstrated enhanced sensitivity to Ex4 (Supplementary Fig. 5e).
Furthermore, knockdown of TCF7 in human beta cells produced a defective exocytosis response to GIP but not to Ex4 (Fig. 3e). Consistent with findings in Gipr$^{-/-}$ β-cell islets, Tcf7$^{-/-}$ islets showed no significant differences in their expression of genes encoding pro-survival GPCRs, components of the glucose-sensing machinery, mediators of proliferation or survival, or members of the TCF family (Supplementary Fig. 5f). Unlike findings for Gipr$^{-/-}$ β-cell mice, both LFD- and HFD-fed Tcf7$^{-/-}$ mice demonstrated normal glycemia and insulin release in response to refedding after an overnight fast (Supplementary Fig. 5g). However, histological analysis of Tcf7$^{-/-}$ pancreata revealed an increase in beta cell mass after HFD feeding compared to that in WT controls (Supplementary Fig. 5h), comparable to findings in Gipr$^{-/-}$ β-cell mice (Supplementary Fig. 3a). Furthermore, Tcf7$^{-/-}$ mice on either a LFD or a HFD demonstrated reduced glycemia in response to the administration of DPP4 inhibitor Safrin (relative expression) compared to findings in Gipr$^{-/-}$ β-cell mice (Supplementary Fig. 5f). Interestingly, Tcf7$^{-/-}$ islets exhibited a higher number of apoptotic beta cells after STZ administration than did WT islets (Fig. 3f). Furthermore, GIP reduced apoptosis in the islets of STZ-treated WT mice but not in islets from Tcf7$^{-/-}$ mice (Fig. 3g). By contrast, Ex4 significantly reduced islet apoptosis in both STZ-treated WT and STZ-treated Tcf7$^{-/-}$ mice (Fig. 3g).

Knockdown of TCF7 in human islet cells increased the number of apoptotic cells in islets cultured either in low glucose alone or in 25 mM glucose with interleukin 1β (Fig. 3h). Rescue of Tcf1 levels in dispersed islets from Gipr$^{-/-}$ β-cell mice lowered apoptotic rates (after thapsigargin administration) to levels comparable to those detected in WT controls (Fig. 3i and Supplementary Fig. 7).

The induction of apoptosis by administration of thapsigargin enhanced Tcf7 expression in mouse insulina 6 (MIN6) beta cells, which was significantly attenuated by knockdown of the Gipr, but not of the Gipr1r (Fig. 4a,b). The transfection of MIN6 beta cells with cDNAs encoding the single TCF1 protein, Tcf7912 (the isoform expressed in mouse islets), increased the expression of anti-apoptotic genes (Fig. 4c) and reduced thapsigargin-stimulated caspase-3 cleavage and apoptosis (Fig. 4d,e and Supplementary Fig. 8a).

To further elucidate the consequences of TCF1-deficiency in beta cells, we performed RNA-seq analysis on islets from young, metabolically healthy (8-week-old, LFD) mice, older, obese (20-week-old, HFD) Tcf7$^{-/-}$ mice and littermate control mice. Robust changes in genes important for apoptosis and inflammation were detected in RNA from the islets of older Tcf7$^{-/-}$ mice (Fig. 4f). Notably, expression of purine transporters, several cytokine signalling genes, and transcription factors was increased in islets from older Tcf7$^{-/-}$ mice (Fig. 4g). Ptg1 expression was also significantly reduced both in RNA from older Tcf7$^{-/-}$ islets (Fig. 4g) and in islet RNA (Fig. 4g) from 12-week-old, diabetic db/db mice that exhibit reduced expression of Gipr and Tcf7 (Fig. 2g).
These results are consistent with a GIPR-TCF1-PTTG1 axis. Finally, knockdown of Tcf7 mRNA transcripts in MIN6 beta cells (Supplementary Fig. 8b) led to a significant reduction in Pttg1 expression (Fig. 4h), whereas the restoration of Pttg1 expression attenuated the increased susceptibility to apoptosis in MIN6 beta cells seen after Tcf7 knockdown (Fig. 4i).

Taken together, these results greatly extend our understanding of incretin action and beta cell function and have direct translational implications. Previous findings that Gipr−/− mice are protected against diet-induced obesity and insulin resistance, coupled with genetic linkage of variants in the human GIPR to body weight, were attributed to GIP action in adipose tissue. The relatively modest differences in glucose tolerance arising from the selective loss of GIPR signaling in beta cells is ameliorated in part by compensatory increases in the activity of functionally related beta cell GPCRs. Nevertheless, our data demonstrate that the selective attenuation of GIP action in beta cells limits meal-related insulin release, indirectly reducing the expansion of adipose tissue mass and leading to improvements in insulin sensitivity and glucose tolerance. These findings support observations linking variation in the human GIPR gene with reduced insulin secretion and decreased body mass index, reevaluating reconsideration of the importance of direct versus indirect GIP action in beta cells compared to that in adipocytes for the control of insulin sensitivity and adipose tissue mass.

Although GLP-1 and GIP exert their actions through structurally and functionally related incretin receptors, our findings that GIPR, but not GLP-1R, signaling controls Tcf7 expression independently of cAMP signaling identify divergent downstream signaling pathways that link incretin-receptor signaling to beta cell survival. Indeed, the loss of GLP-1R, but not of GIPR, signaling, impairs expression of insulin receptor substrate 2 (Irs2) in mouse islets, whereas beta cells from Gipr−/− mice exhibit greater sensitivity to STZ-induced apoptotic injury than Gipr−/− beta cells. Furthermore, GIP, but not GLP-1, controls osteopontin expression in mouse islets, whereas GLP-1R, but not GIPR, signaling is essential for the adaptive islet response to pregnancy. Our identification of a GIPR-TCF1 axis uncovers a novel mechanistic pathway linking differential incretin-receptor signaling to the control of beta cell mass and function, which has encouraging implications for therapeutic strategies based on GIPR agonism.

Considerable evidence from human genetic and physiological studies has linked variation within the human TCF7L2 gene to impairment of beta cell function and an increased risk of developing T2D. Nevertheless, our understanding of how TCF7L2 controls beta cell function has been challenged by studies demonstrating that genetic inactivation of the Tcf7l2 gene in beta cells does not impair beta cell function, whereas enhanced Tcf7l2 expression (TCF4) in liver activated a metabolic gene-expression program linked to increased hepatic glucose output. This suggests that TCF4 may indirectly affect beta cell function through hepatic Wnt signaling. Tcf1 is deficient in the hepatocytes of diabetic mice, and restoring Tcf7 expression reduces gluconeogenesis, further positioning Tcf7 in the pathophysiology of diabetes. Collectively, our findings demonstrated that Tcf7/Tcf1 is required for maintaining beta cell survival and that the disruption of Tcf7 (loss of Tcf1) unmasks divergent mechanisms regulating the anti-apoptotic actions of GIPR as compared to GLP-1R signaling in mouse islets. In contrast to conflicting data surrounding the role of TCF7L2 in beta cells, our latest data unequivocally reveal the importance of Tcf7/Tcf1, regulated by GIPR signaling, for the direct control of beta cell survival in murine and human islets.

Levels of Tcf7 mRNA transcripts are reduced in the pancreas from HFD-fed rats, and variation in the human TCF7 gene has been linked to an increased risk for development of type 1 diabetes, whether these latter findings reflect TCF1-dependent disturbances of immune regulation, beta cell function or beta cell survival in susceptible individuals requires further investigation. Moreover, the importance of the TCF1 target, PTTG1, for beta cell function and survival has been independently shown in studies of older Pttg1−/− mice, which exhibited reduced beta cell mass, impaired beta cell function and increased beta cell apoptosis.

Our current data establish that TCF1, acting through PTTG1, links GIPR signaling to the control of insulin secretion, the survival of beta cells and the adaptation of these cells to metabolic stress. Given that GIPR, but not GLP-1R, signaling controlled the TCF1-PTTG1 axis in beta cells, and that GIP responsiveness was rapidly restored after a brief period of improved glucose control in human subjects with T2D, our findings highlight the potential of targeting GIPR-TCF1-PTTG1 signaling for the preservation of beta cell mass and the treatment of diabetes. Collectively, our data imply that the development of GIP-based therapies may target novel pathways, independently of GLP-1R signaling, thus linking nutrient-activated signals to the control of beta cell function and survival.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. RNA-seq data has been deposited with accession code GSE65361.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.E.C. and D.J.D. designed and directed the study, analyzed data and wrote the manuscript. J.R.U., E.E.M., L.L.B. and P.E.M. contributed to the study design and the preparation of the manuscript. J.E.C., J.R.U., E.E.M., J.K., L.L.B., X.C., B.J.L. and T.M. performed experiments. J.K. and P.E.M. carried out experiments on human islets. Y.L. and J.L.W. provided assistance with the RNA-seq data. N.T. and L.H.P. provided MIP-Cre mice. C.I.S. performed the electron microscopy. All authors reviewed the manuscript and provided final approval for submission.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.


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D.J.D. has been a consultant to Novo Nordisk Inc. and other companies that develop and/or sell incretin-based therapies, including Arisaph Pharmaceuticals Inc., Intarcia Therapeutics, Merck Research Laboratories, MedImmune, Receptos, Sanofi, Takeda and Transition Pharmaceuticals Inc. Neither D.J.D. nor his family members hold stock directly or indirectly in any of these companies.

EDITORIAL SUMMARY

AOP: The details of the GIP signaling pathway are murky, but new data identify a downstream pathway involving Tcf7 that regulates beta cell survival and activity.
**ONLINE METHODS**

**Animals.** Male mice were used for all mouse studies and were maintained under a 12 h light/12 h dark cycle at constant temperature (23 °C) with free access to food and water. All animal studies were approved by Mt. Sinai Hospital (Toronto) and the Toronto Centre for Phenogenomics animal-care committee. Animals were fed either a low-fat diet (10% kcal from fat; Research Diets, D12450B) or high-fat diet (45% kcal from fat; Research Diets, D12451). To generate Gipr<sup>−/−</sup>–β Cell mice, MPcreER transgenic mice (on a C57BL/6J background) expressing tamoxifen-inducible Cre driven by the mouse insulin promoter were bred with floxed Gipr mice (Gipr<sup>Flox/Flox</sup>, backcrossed 8 times to C57BL/6J background)<sup>41</sup>. Cre-induced inactivation of the Gipr gene was carried out via 5 consecutive daily intraperitoneal (i.p.) injections of tamoxifen (40 mg per kg) in 6-week-old mice. Gipr<sup>+/−</sup>–/− mice (both on C57BL/6J backgrounds) have been previously described<sup>42,43</sup>. Gipr<sup>+/−</sup>–/− mice were generously provided by R. DiMarchi, University of Indiana.

**Peptides and reagents.** Peptides were reconstituted in phosphate-buffered saline (PBS), aliquoted and stored at −80 °C. [D-Ala<sup>2</sup>]-GIP (GIP) was from Chi Scientific, Ex4 (exendin-4) was from California Peptide Research Inc. Plasmid constructs (Tcf7<sup>L</sup> and Potg1) and siRNA (Gipr, Gipr<sub>1</sub>–<sub>1</sub>, Tcf7<sup>L</sup>, Tcf7<sup>F</sup>) were from Origene. The GLP-1R antagonist (JANT-4) was a generous gift from R. DiMarchi, University of Indiana.

**Mouse islet isolation.** Primary mouse islets were isolated as previously described<sup>45</sup>. Briefly, the pancreas was inflated via the pancreatic duct with collagenase type V (0.8 mg per ml), excised and digested for 10–15 min. The digest was washed with cold RPMI (2 mM L-glutamine, 10 mM glucose, 0.25% BSA, 100 U/ml penicillin, and 100 μg/ml streptomycin), and the islets were separated using a Histopaque gradient. Individual islets were handpicked and either immersed in TRI Reagent for subsequent mRNA isolation or allowed to rest in vivo for subsequent mRNA isolation or allowed to recover overnight in RPMI with 10% FBS for experiments ex vivo.

**Primary mouse islet insulin secretion.** After overnight incubation, 75–80 medium-sized islets were handpicked into 0.275 ml chambers containing KRB buffer (135 mM NaCl, 3.6 mM KCl, 1.5 mM CaCl<sub>2</sub>, 0.5 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.5 mM MgSO<sub>4</sub>, 5 mM Hapes, 5 mM Hepes, 0.1% BSA pH 7.5). Islets were perfused for 1 h in KRB with 2.7 mM glucose at a flow rate of 200 μl per min using the Biorep Perfusion system. After this equilibration period, islets were perfused at 8 min intervals in experimental media (KRB plus various conditions), then collected and lysed in acid ethanol for total insulin measurements. Insulin concentrations were determined by radioimmunoassay (Millipore), and insulin secretion was expressed as a percent of initial cell size.

**Islet hGH release.** Isolated islets were incubated in batches of 100 at 37 °C and concentration of libraries were assessed using BioAnalyzer and qPCR, and BioAnalyzer (Agilent), respectively. Ribosomal RNA was removed using the Illumina TruSeq RNA sample preparation kit. The quality and concentration of libraries were assessed using BioAnalyzer and qPCR, respectively. The libraries were loaded as two indexed samples per lane on an Illumina sequencing instrument.

**Insulin supplementation.** 8-week-old mice had a single insulin pellet (7 or 14 mg, LinBit) inserted into the intrascapular region under isoflurane anesthesia by following the manufacturer’s protocol.

**Apoptosis in vivo.** Mice were treated with streptozotocin (Sigma, 50 mg per kg) for 5 consecutive days at 0800. Twenty-four hours after the final treatment, mice were euthanized and the pancreas was excised and immediately immersed in 10% formalin. All histological analysis was performed in a blinded fashion.

**Real time quantitative PCR.** First-strand cDNA was synthesized from total RNA using the SuperScript III synthesis system (Invitrogen). Real-time PCR was carried out with the ABI Prism 7900 Sequence Detection System using TaqMan Gene Expression Assays (Applied Biosystems). Relative mRNA transcript levels were quantified with the 2−ΔΔCT method. PCR primers are shown in [Supplementary Table 1](#)

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**Islet hGH release.** Isolated islets were incubated in batches of 100 at 37 °C in HEPES Krebs buffer containing 20 mM glucose, as previously described<sup>7</sup>. After 1 h, the buffer was removed and assayed for hGH release using a hGH ELISA (Invitrogen).

**Glucose- and insulin-tolerance tests.** Oral and intraperitoneal glucose-tolerance tests (GTTs) were performed in mice fasted for 5 h (0700–1200) using a glucose dose of 1.5 g per kg. During IP GTT, mice were i.p. injected with either GIP (4 nmol per kg), Ex4 (0.3 nmol per kg), or saline (veh), 10 min before glucose administration. For tests using a DPP4 inhibitor, sitagliptin (Merck, 40 μg per mouse) was given orally 30 min before glucose. For both OGTT and IP GTT, blood was collected at 10, 30 and 60 min in capillary tubes coated with 10% (vol/vol) TED (500,000 IU/ml Trasylol; 1.2 mg/ml EDTA; and 0.1 mM diprotin A) and plasma separated by centrifugation at 4 °C and stored at −80 °C. Insulin-tolerance tests (ITTs) were performed in mice fasted for 5 h (0700–1200) using an insulin dose of 0.7 U/kg (Humalog, Lilly).

**Plasma hormone analysis.** Insulin (Alpco Diagnostics) and total GIP (Linco) levels were analyzed by ELISA. Total GLP-1 levels were measured by immunomassay (Mesoscale).

**Capacitance measurement.** Islets were dispersed in calcium-free dissociation buffer in 35 mm dishes and incubated overnight in RPMI containing 11 mM (mouse) or DMEM containing 5.5 mM (human) glucose. GIP (10 nM), Ex4 (1 nM) or vehicle (H<sub>2</sub>O) was added to each dish 1 h before patch clamping, using the standard whole-cell technique with the sine + DC lock-in function of an EPC10 amplifier and Patchmaster software (HEKA Electronics). Experiments were performed as described previously<sup>47</sup> at 32–35 °C using an extracellular bath solution (118 mM NaCl, 20 mM TEA, 5.6 mM KCl, 1.2 mM MgCl<sub>2</sub>, 2.6 mM CaCl<sub>2</sub>, 5 mM glucose, 5 mM Hepes, pH –7.4) and pipette solution (125 mM CsGlutamate, 10 mM CsCl, 10 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.05 mM EGTA, 5 mM Hepes, 3 mM MgATP, 0.1 mM cAMP, pH –7.15). Capacitance responses to a train of 10 depolarizations from −70 to 0 mV at 1 Hz were normalized to initial cell size and expressed as femtofarad per picofarad (ff/pf). β cells were identified using positive insulin immunostaining.

**RNA-seq.** Total RNA from isolated islets was extracted using TRI Reagent. The yield and quality of total RNA was assessed using Nanodrop (Thermo Fisher) and BioAnalyzer (Agilent), respectively. Ribosomal RNA was removed using a bead-based hybridization kit (RiboZero, Epicentre) and cDNA libraries were prepared using the Illumina TruSeq RNA sample preparation kit. The quality and concentration of libraries were assessed using BioAnalyzer and qPCR, respectively. The libraries were loaded as two indexed samples per lane on an Illumina sequencing instrument.
Illumina HiSeq 2000. Raw sequenced reads were obtained in fastq format, and mapped onto the mouse genome (mm9) using Tophat1.4.1, and then analyzed using a custom R-based pipeline to calculate gene-expression profiles using ENSEMBL annotation for coding genes. The number of reads mapped onto the gene was counted regardless of transcription isoform and normalized to total mapped reads to obtain transcript union Read Per Million total reads (truRPMs). The data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE65361.

Cell-line culture. INS1823/32 cells were a generous gift from C. Newgard, Duke University. Cells were grown in RPMI (10% FBS, 1% P/S). For GIP experiments, cells were starved in RPMI (1% FBS, 1% P/S) for 3 h. MIN6 cells (from ATCC) were grown in DMEM (20% FBS, 1% P/S). siRNA knockdown experiments were performed by following the manufacturer's instructions (Origene). All cell lines were previously tested for mycoplasma contamination.

Apoptosis assays. MIN6 beta cells. After 24 h exposure to thapsigargin (5 uM, Sigma) in culture media (DMEM, 20% FBS, 1% P/S), apoptosis was analyzed using a MitoPT assay (ImmunoChemistry Technologies) by following the kit's instructions. Briefly, cells were exposed to 100 nM MitoPT for 10 min, washed 1 × with PBS and then detached from the plate with trypsin. An aliquot of cells was visualized on a microscope slide. Total cell number was counted using bright field and tetracythylrhodamine methyl ester (TMRM) uptake was visualized with a 545 nM filter. Apoptotic cells were calculated as (total cells – TMRM positive cells)/Total cells × 100%.

Mouse islets. Dispersed islets were transduced with Ad-GFP or Ad-Tcf7-GFP at an MOI of 10 to achieve a >90% induction rate. After transduction, cells were exposed to control or thapsigargin (100 uM) for 72 h. Apoptosis was assessed using a MitoPT assay.

Human islets. Dispersed human cells were transfected with human siTCF7 or siScram control duplexes (OriGene, Rockville, MD) and an Alexa488 labeled negative control siRNA (Qiagen, Toronto, ON), using Dharmafect (Thermo Scientific, Ottawa, ON, Canada). 24 h post-transfection culture medium was changed to fresh medium containing glucose and/or human recombinant IL1-β (Sigma, Oakville, ON, Canada), as indicated. Cell-death assays were performed on dispersed human islet cells by use of the In situ Cell Death Detection Kit TMR Red (Roche, Mannheim, Germany), using TUNEL technology, according to the manufacturer's directions. Images were obtained using a Zeiss AxioObserver Z1 with a Zeiss-Colibri light source at 488 nm and 594 nm, a × 40/1.3 NA lens, and an AxioCam HRm camera. Images were acquired in Axiosvision 4.8 software (Carl Zeiss MicroImaging, Göttingen, Germany) and analyzed using ImageJ software (National Institutes of Health). Cell death was determined as ((#TUNEL+/Alexa488+) / (# Alexa488+)) and expressed as a fold increase over control, unstimulated conditions (5.5mM glucose, scrambled siRNA).

Statistical analysis. All values are presented as mean ± s.e.m. Statistical analysis was performed using GraphPad Prism 5.0. The appropriate t-test, one-way analysis of variance (ANOVA), or two-way ANOVA was completed using P < 0.05 to signify significant differences. Bonferroni post-hoc analysis was performed where appropriate. All data was assessed to ensure normal distribution and equal variance between groups, using GraphPad Prism 5.0. Prior to the experiment, it was determined that individual data points would be excluded if their value was greater than 2 × SD from the mean, in an experiment with a sample size greater than seven.


**Article Title:**
TCF1 links GIPR signaling to control of β cell function and survival

**Corresponding Author:**
Daniel J. Drucker, drucker@lunenfeld.ca

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<td>Generation of $Gipr^{-/-}$βCell mice.</td>
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<tr>
<td>Supplementary Table 2</td>
<td>List of antibodies</td>
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Nature Medicine: doi:10.1038/nm.3997
Supplementary Figure 1

(a) MIP promoter → CRE → ER

Glucose (mM)

(b) Gipr mRNA

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)

(c) Glucose (mM)

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)

(d) Glucose (mM)

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)

(e) Glucose (mM)

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)

(f) Glucose (mM)

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)

(g) Glucose (mM)

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)

(h) Glucose (mM)

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)

(i) Glucose (mM)

WT

MIP-Cre

Gipr

Gipr

Glucose (mM)

(absolute expression)
Supplementary Figure 3

a) Graph showing β cell area (% of pancreas) and islet number per unit pancreas area for different genotypes.

b) Images showing granule size for MIP-Cre and Gipr⁻⁻ β cell genotypes.

c) Graphs showing mRNA expression levels for various genes under different conditions.

d) Gel showing the amplification of a 0.9 kb fragment.

e) Gel showing Tcf7 expression with and without GIP for 28 and 34 cycles.

f) Graph showing TCF7 mRNA expression levels in human islets.

Nature Medicine: doi:10.1038/nm.3997
Supplementary Figure 4

**Figure a**
Body weight (g)
- 6 wk WT
- 6 wk db/db
- 12 wk WT
- 12 wk db/db

**Figure b**
Fat Mass (% of body weight)
- 6 wk WT
- 6 wk db/db
- 12 wk WT
- 12 wk db/db

**Figure c**
Glucose (mM) vs Time (min)
- Veh
- GIP

**Figure d**
Glucose and Insulin levels over time
- 6 wk WT
- 6 wk db/db
- 12 wk WT
- 12 wk db/db

**Figure e**
Gene expression for Glp1r and Tcf7l2

**Figure f**
BMI (kg/m²)
- 6 wk WT
- 6 wk db/db
- 12 wk WT
- 12 wk db/db
Supplementary Figure 6

Low-Fat Diet:

(a) Glucose (mM) vs Time (min) for WT and Tcf7l2-/- mice with either H2O or DPP4i treatment. 

(b) Glucose (mM) vs Time (min) for WT and Tcf7l2-/- mice with IPGTT + PBS or GIP treatment. 

High-Fat Diet:

(c) Glucose (mM) vs Time (min) for WT and Tcf7l2-/- mice with H2O, DPP4i, or Ex4 treatment. 

(d) Glucose (mM) vs Time (min) for WT and Tcf7l2-/- mice with PBS, GIP, or Ex4 treatment.

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Supplementary Figure 7

(a) Bright field images of TMRM staining in Ad-Gfp and Ad-Tcf7-Gfp conditions. Bright field and GFP images are shown.

(b) Western blot analysis showing Tcf1 expression under different conditions:
- Ad-Gfp (MOI 10)
- Ad-Tcf7-Gfp (MOI 10)
- Thapsigargin + Ad-Gfp
- Thapsigargin + Ad-Tcf7

(c) MIP-Cre and Gipr−/− β cell conditions:
- PBS + Ad-Gfp
- PBS + Ad-Tcf7
- Thapsigargin + Ad-Gfp
- Thapsigargin + Ad-Tcf7

Nature Medicine: doi:10.1038/nm.3997
Supplementary Figure 8

(a) Bright field vs TMRM images of MIN6 β cells under different conditions:
- Control
- Thapsigargin
- Thapsigargin + TCF1

(b) Bar graph showing the relative expression of Tcf7 mRNA in MIN6 β cells:
- Scrambled
- Tcf7 siRNA with a significant decrease indicated by an asterisk.
## Supplementary Table 1. List of Real-Time Primers

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All Cell signaling technologies antibodies are profiled on antibodypedia.
Supplemental Information

TCF1 links GIPR signaling to the control of β cell function and survival

Jonathan E Campbell1, John R Ussher1,7, Erin E Mulvihill1, Jelena Kolic2,3, Laurie L Baggis1, Xiemen Cao1, Yu Liu1, Benjamin J Lamont1,7, Tsukasa Morii1,7, Catherine J Streutker4, Natalia Tamarina5, Louis H Philipson5, Jeffrey L Wrana1, Patrick E MacDonald2,3 & Daniel J Drucker1,6

Supplemental Figure 1 Generation of Gipr−/−βCell mice. (a) Schematic illustrating the generation of Gipr−/−βCell mice. (b) Gipr expression in mouse islets (left, n=3, 5, 3, and 5) and epididymal adipose tissue (right, n=7). (c) Intraperitoneal glucose tolerance test (IPGTT) in 10-week-old mice fed a LFD (n=8, 4, 7, and 5). (d) hGH release (left) from mouse islets in culture, Tph1 (middle) and Tph2 (right) expression in mouse islets (n=5, 5, and 6). (e) Glycemic (left) and insulin (right) response during an oral glucose tolerance test (OGTT) in 10-week-old mice fed a LFD (n=7). (f) Glycemic response during an intraperitoneal insulin tolerance test in 8-week-old mice fed a LFD (n=4 and 6). (g) Body weight (left), fed glucose values (middle), and average food intake (right) in mice fed a LFD (n=7). (h) Total GIP (left) and GLP-1 (right) plasma concentration during an OGTT in mice fed a LFD (n=7). (i) IPGTT in 18-week-old mice fed a LFD (n=7). *P<0.05 vs control, or as indicated. Data are expressed as Mean± SEM. Statistical tests used: t-test – for g (right) and AUC inset (c, e and i); 1-way analysis of variance (ANOVA) – for b and d; 2-way ANOVA - for c, and e-i. LFD – low fat diet, GIP – glucose-dependent insulinotropic polypeptide, GLP-1 – glucagon-like peptide 1.

Supplemental Figure 2 Phenotype of Gipr−/−βCell mice. (a) Adipose depot weights (left) and epididymal adipocyte size (right) from 20-week-old mice (n=7). (b) qPCR analysis of genes important for adipose tissue metabolism (n=7). (c) Lipoprotein lipase activity in epididymal adipose depots from 20-week-old mice (n=7). (d) (left to right) Body weight (n=9 and 13), plasma total GIP concentrations (n=9), plasma total GLP-1 concentrations (n=9), fed glycemia (n=9 and 13), and intraperitoneal glucose tolerance test (IPGTT) (n=9 and 13) in mice fed a HFD. (e) Body weight (two left panels) and glycemia (two right panels) following sham (blue and red) or insulin pellet (purple and brown) surgery (n=3, 5, 3, and 6; MIP-Cre (sham), Gipr−/−βCell (sham), MIP-Cre (pellet), and Gipr−/−βCell (pellet)). (f) Glycemic response to insulin tolerance test in 18-week-old mice following sham (left) or insulin pellet (right) surgery (n=3, 5, 3, and 6; MIP-Cre (sham), Gipr−/−βCell (sham), MIP-Cre (pellet), and Gipr−/−βCell (pellet)). (g) Glycemic response during an oral glucose tolerance test in 16-week-old mice fed a HFD and given a GLP-1R antagonist (n=9). (h) Cumulative capacitance in isolated β cells determined by whole cell patch clamping, treated with vehicle (left panel), GIP (middle panel) or exendin-4 (right panel) (i) Actin intensity in individual β cells stimulated with low glucose (n=55 and 42, MIP-Cre and Gipr−/−βCell) 16.7 mM glucose (n=48 and 51, MIP-Cre and Gipr−/−βCell), 1 nM Ex4 (n=46 and 44, MIP-Cre and Gipr−/−βCell), 10 nM GIP (n=47 and 44, MIP-Cre and Gipr−/−βCell), or 10 μM latrunculin B (LatB) (n=42 and 44, MIP-Cre and Gipr−/−βCell). (j) IPGTT in 16-week-old mice given a GPR119 agonist (n=8 and 13). *P<0.05 vs control. Data are expressed as Mean± SEM. Statistical tests used: t-test – for a-c and i, and AUC (d, f and...
Supplemental Figure 3 Characterization of \( \text{Gipr}^{-/-} \beta \text{Cell} \) islets. (a) Histological analysis of \( \beta \) cell area (left), islet size (middle), and islet number (right) in samples from mice fed a LFD (red and blue, \( n=7 \) and \( 7 \)) or HFD (pink and light blue, \( n=9 \) and \( 13 \)). (b) Electron microscopy showing insulin granular size in pancreata samples from mice fed a LFD (\( n=7 \)). (c) qPCR analysis of RNA from isolated islets from 20-week-old mice fed a LFD (\( n=7 \)). (d) Representative image of \( \text{Tcf7} \) expression in RNA from thymus and islets from 8-week-old mice fed a LFD. (e) Representative image of \( \text{Tcf7} \) expression in islets treated with Veh (-) or GIP (+) following sequential PCR cycling (\( n=3 \)). (f) TCF7 expression in human islets treated with Veh or GIP (\( n=4 \)). \( *P<0.05 \) vs control. Data are expressed as Mean± SEM. Statistical tests used: t-test – for b, c and f; 2-way analysis of variance (ANOVA) – for a. Veh – vehicle, GIP – glucose-dependent insulinotropic polypeptide. LFD – low fat diet, HFD – high fat diet.

Supplemental Figure 4 Diabetic \( \text{db/db} \) mice fail to lower glucose in response to exogenous GIP. (a) Body weight (\( n=5 \)). (b) Body adiposity determined by MRI (\( n=5 \)). (c) Glycemic response during an intraperitoneal glucose tolerance test in 6-week-old WT (left) and \( \text{db/db} \) (right) mice (\( n=5 \)). (d) Glycemic response to exogenous GIP in random fed mice (\( n=5 \)). (e) \( \text{Glp1r} \) (left) and \( \text{Tcf7l2} \) (right) expression in RNA from mouse islets (\( n=5 \)). (f) Body mass index in human subjects (\( n=5, 5, \) and 4). \( *P<0.05 \) vs control, or as indicated. Data are expressed as Mean± SEM. Statistical tests used: t-test – for b, c and f; 2-way analysis of variance (ANOVA) – for a. Veh – vehicle, GIP – glucose-dependent insulinotropic polypeptide, ND – non-diabetic, T2D – type 2 diabetic.

Supplemental Figure 5 Phenotype of \( \text{Tcf7}^{-/-} \) mice. (a) Body weight in 18-week-old mice fed a LFD (dark blue and dark grey, \( n=7 \) and \( 5 \)) or HFD (light blue and light grey, \( n=10 \) and \( 7 \)). (b) Body adiposity determined by MRI in mice fed a LFD (dark blue and dark grey) or HFD (light blue and light grey) (\( n=7, 5, 10, \) and \( 7 \)). (c) Glycemic (left) and insulin (middle) response during an oral glucose tolerance test (OGTT) and glycemic (right) response during an intraperitoneal glucose test (IPGTT) in 8-week-old mice on a LFD (\( n=7 \) and \( 8 \)). (d) Glycemic response during an IPGTT in 12-week-old mice on a HFD (\( n=10 \) and \( 7 \)). (e) Cumulative capacitance in individual \( \beta \) cells stimulated with Veh (left, \( n=18 \) and \( 20 \)), GIP (middle, \( n=18 \) and \( 18 \)), or Ex4 (right, \( n=15 \) and \( 17 \)). (f) qPCR analysis of RNA from islets from 18-week-old mice fed a LFD (\( n=4 \)). (g) Glycemic and insulin response during a fasting-refeeding test in 10-week-old mice fed a LFD (two left panels, \( n=7 \) and \( 5 \)) or HFD (two right panels, \( n=7 \) and \( 10 \)). (h) Histological analysis of \( \beta \) cell area (left), islet size (middle), and islet number (right) in samples from mice fed a LFD (dark blue and grey, \( n=7 \) and \( 5 \)) or HFD (light blue and light grey, \( n=10 \) and \( 7 \)). \( *P<0.05 \) vs control, or as indicated. Data are expressed as Mean± SEM. Statistical tests used: t-test for f and AUC inset (c and d); 2-way analysis of variance (ANOVA) – for a-e, g, h. LFD- low fat diet, HFD – high fat diet, Veh – vehicle, GIP – glucose-dependent insulinotropic polypeptide, Ex4 – exendin-4.
**Supplemental Figure 6** Glycemic and insulin response to incretin receptor agonists and DPP4 inhibition in Tcf7−/− mice. (a) Glycemic and insulin response during an oral glucose tolerance test (OGTT) in 12-week-old mice fed a LFD (n=5). (b) Glycemic and insulin response during an intraperitoneal glucose tolerance test (IPGTT) in 12-week-old mice fed a LFD (n=7 and 5, WT and Tcf7−/−). (c) Glycemic and insulin response during an OGTT in 12-week-old mice fed a HFD (n=9). (b) Glycemic and insulin response during an intraperitoneal glucose tolerance test (IPGTT) in 12-week-old mice fed a HFD (n=10 and 8 WT and Tcf7−/−). *P<0.05 vs control, or as indicated. Data are expressed as Mean±SEM. Statistical test used: 2-way analysis of variance (ANOVA). LFD- low fat diet, HFD – high fat diet, DPP4i – dipeptidyl peptidase 4 inhibitor, Veh – vehicle, GIP – glucose-dependent insulinotropic polypeptide, Ex4 – exendin-4.

**Supplemental Figure 7** Representative images of adenoviral transduction and apoptotic index. (a) Representative image of bright field and GFP (495 nm) in mouse β cells. (b) Tcf1 protein expression in baby hamster kidney (BHK) fibroblast cells and mouse thymus. (c) Representative images of bright field and TMRM (545 nm) in mouse β cells.

**Supplemental Figure 8** Representative image of apoptotic index and Tcf7 knockdown in MIN6 β cells. (a) Representative images of bright field and TMRM (545 nm) in MIN6 β cells. (b) Tcf7 expression in MIN6 β cells (n=6). *P<0.05 vs control, or as indicated. Data are expressed as Mean±SEM. Statistical test used: t-test – for b.
Online Methods

Animals.

Male mice were used for all mouse studies and were maintained under a 12 h light/12 h dark cycle at constant temperature (23 °C) with free access to food and water. All animal studies were approved by Mt. Sinai Hospital (Toronto) and the Toronto Centre for Phenogenomics animal-care committee. Animals were fed either a low-fat diet (10% kcal from fat; Research Diets, D12450B) or high-fat diet (45% kcal from fat; Research Diets, D12451). To generate \textit{Gipr}^{–/–}\textit{βCell} mice, MIPcreER transgenic mice (on a C57BL/6J background) expressing tamoxifen-inducible Cre driven by the mouse insulin promoter were bred with floxed \textit{Gipr} mice (\textit{Gipr}^{Flox/Flox}), backcrossed 8 times to C57BL/6J background)\textsuperscript{41}. Cre-induced inactivation of the \textit{Gipr} gene was carried out via 5 consecutive daily intraperitoneal (\textit{i.p.}) injections of tamoxifen (40 mg per kg) in 6-week-old mice. \textit{Glp1r}^{–/–} and \textit{Tcf7}^{–/–} mice (both on C57BL/6J backgrounds) have been previously described\textsuperscript{42,43}. \textit{Tcf7}^{–/–} mice were generously provided by H. Clevers, \textit{db/db} mice were purchased from Jackson laboratories (#000697). For all animal experiments, the sample size required to achieve adequate power was estimated on the basis of pilot work or previous experiments. When appropriate, animals were randomly allocated to individual experimental groups.

Peptides and reagents.

Peptides were reconstituted in phosphate-buffered saline (PBS), aliquoted and stored at –80 °C. [D-Ala\textsuperscript{2}]GIP (GIP) was from Chi Scientific, Ex4 (exendin-4) was from California Peptide Research Inc. Plasmid constructs (\textit{Tcf7} and \textit{Pttg1}) and siRNA (\textit{Gipr}, \textit{Glp1r}, \textit{Tcf7}, \textit{TCF7}) were from Origene. The GLP-1R antagonist (JANT-4)\textsuperscript{44} was a generous gift from R. DiMarchi, University of Indiana.

Mouse islet isolation.

Primary mouse islets were isolated as previously described\textsuperscript{45}. Briefly, the pancreas was inflated via the pancreatic duct with collagenase type V (0.8 mg per ml), excised and digested for 10–15 min. The digest was washed with cold RPMI (2 mM L-glutamine, 10 mM glucose, 0.25% BSA, 100 U/ml penicillin, and 100 μg/ml streptomycin), and the islets were separated using a Histopaque gradient. Individual islets were handpicked and either immersed in TRI Reagent for subsequent mRNA isolation or allowed to recover overnight in RPMI with 10% FBS for experiments \textit{ex vivo}.

Primary mouse islet insulin secretion.

After overnight incubation, 75–80 medium-sized islets were handpicked into 0.275 ml chambers containing KRB buffer (135 mM NaCl, 3.6 mM KCl, 1.5 mM CaCl\textsubscript{2}, 0.5 mM NaH\textsubscript{2}PO\textsubscript{4}, 0.5 mM MgSO\textsubscript{4}, 5 mM Heps, 5 mM NaCO\textsubscript{3}, 0.1% BSA pH 7.5). Islets were perifused for 1 h in KRB with 2.7 mM glucose at a flow rate of 200 μl per min using the Biorep Perifusion system. After this equilibration period, islets were perifused at 8 min intervals in experimental media (KRB plus various conditions), then collected and lysed in acid ethanol for total insulin measurements. Insulin concentrations were determined by radioimmunoassay (Millipore), and insulin secretion was expressed as a percent of total insulin.
Islet hGH release.
Isolated islets were incubated in batches of 100 at 37 °C in HEPES Krebs buffer containing 20 mM glucose, as previously described. After 1 h, the buffer was removed and assayed for hGH release using a hGH ELISA (Invitrogen).

Glucose- and insulin-tolerance tests.
Oral and intraperitoneal glucose-tolerance tests (GTTs) were performed in mice fasted for 5 h (0700–1200) using a glucose dose of 1.5 g per kg. During IPGTT, mice were i.p. injected with either GIP (4 nmol per kg), Ex4 (0.3 nmol per kg), or saline (veh), 10 min before glucose administration. For tests using a DPP4 inhibitor, sitagliptin (Merck, 40 μg per mouse) was given orally 30 min before glucose. For both OGTT and IPGTT, blood was collected at 0, 10 and 30 min in capillary tubes coated with 10% (vol/vol) TED (500,000 IU/ml Trasylol; 1.2 mg/ml EDTA; and 0.1 mM diprotin A) and plasma separated by centrifugation at 4 °C and stored at –80 °C. Insulin-tolerance tests (ITTs) were performed in mice fasted for 5 h (0700–1200) using an insulin dose of 0.7 U/kg (Humalog, Lilly).

Plasma hormone analysis.
Insulin (Alpco Diagnostics) and total GIP (Linco) levels were analyzed by ELISA. Total GLP-1 levels were measured by immunoassay (Mesoscale).

Insulin supplementation.
8-week-old mice had a single insulin pellet (7 or 14 mg, LinBit) inserted into the intrascapular region under isoflurane anesthesia by following the manufacturer’s protocol.

Apoptosis in vivo.
Mice were treated with streptozotocin (Sigma, 50 mg per kg) for 5 consecutive days at 0800. Twenty-four hours after the final treatment, mice were euthanized and the pancreas was excised and immediately immersed in 10% formalin. All histological analysis was performed in a blinded fashion.

Real time quantitative PCR.
First-strand cDNA was synthesized from total RNA using the SuperScript III synthesis system (Invitrogen). Real-time PCR was carried out with the ABI Prism 7900 Sequence Detection System using TaqMan Gene Expression Assays (Applied Biosystems). Relative mRNA transcript levels were quantified with the 2^–ΔCt method. PCR primers are shown in Supplementary Table 1.

Qualitative PCR.
Amplification of mouse Gipr cDNA was performed using the primer pairs 5′–CTG CTT CTG CTG TGG T–3′ (forward primer) and 5′–CAC ATG CAG CAT CCC AGA–3′ (reverse primer). PCR was carried out using 35 cycles at an annealing temperature of 50 °C to generate a 1.5 kb product. Amplification of the mouse Tcf7 isoforms was performed using the common reverse primer 5′–CTA GAG CAC TGT CAT CAT CGG–3′and
two different 5’ (forward) primers targeting alternative start codons (1.3 kb product – ATG CCG CAG CTG GAC TCG; 0.9 kb product – ATG TAC AAA GAG ACT GTC TAC T). PCR was carried out using 35 cycles at an annealing temperature of 56 °C.

**Western blot analysis.**

Thirty μg of total protein was separated by SDS-PAGE, transferred to nitrocellulose membranes and blocked in 5% milk in PBS-T for 1 h before incubation in primary antibody overnight at 4 °C. Immunoblots were visualized with the enhanced chemiluminescence Western blot detection kit (Perkin Elmer) and quantified with Carestream Molecular Imaging Software (Kodak). Primary antibodies are shown in **Supplementary Table 2**.

**Human islet isolation.**

Primary human islets were isolated as previously described6 at the Alberta Diabetes Institute Islet Core (www.bcell.org/isletcore.html) and the Clinical Islet Isolation Facility at the University of Alberta and cultured in low glucose (5.5 mM) DMEM with L-glutamine, 110 mg per L sodium pyruvate, 10% FBS and 100 U/ml penicillin/streptomycin. We studied islets from 15 non-diabetic, lean donors (age: 57 ± 11 years; HbA1c: 5.7 ± 1.4 BMI range 19–39) and 4 T2D donors (age: 63 ± 5 years; HbA1c: 6.6 ± 0.9; BMI range- 29–37).

**Capacitance measurement.**

Islets were dispersed in calcium-free dissociation buffer in 35 mm dishes and incubated overnight in RPMI containing 11mM (mouse) or 5.5 mM (human) glucose. GIP (10 nM), Ex4 (1 nM) or vehicle (H2O) was added to each dish 1 h before patch clamping, using the standard whole-cell technique with the sine + DC locking function of an EPC10 amplifier and Patchmaster software (HEKA Electronics). Experiments were performed as described previously7 at 32–35 °C using an extracellular bath solution (118 mM NaCl, 20 mM TEA, 5.6 mM KCl, 1.2 mM MgCl2, 2.6 mM CaCl2, 5 mM glucose, 5 mM Hepes, pH – 7.4) and pipette solution (125 mM CsGlutamate, 10 mM CsCl, 10 mM NaCl, 1 mM MgCl2, 0.05 mM EGTA, 5 mM Hepes, 3 mM MgATP, 0.1 mM cAMP, pH – 7.15). Capacitance responses to a train of 10 depolarizations from –70 to 0 mV at 1 Hz were normalized to initial cell size and expressed as femtofarad per picofarad (fF/pF). β cells were identified using positive insulin immunostaining.

**RNA-seq.**

Total RNA from isolated islets was extracted using TRI Reagent. The yield and quality of total RNA was assessed using Nanodrop (Thermo Fisher) and BioAnalyzer (Agilent), respectively. Ribosomal RNA was removed using a bead-based hybridization kit (RiboZero, Epicentre) and cDNA libraries were prepared using the Illumina TruSeq RNA sample preparation kit. The quality and concentration of libraries were assessed using BioAnalyzer and qPCR, respectively. The libraries were loaded as two indexed samples per lane on an Illumina HiSeq 2000. Raw sequenced reads were obtained in fastq format, and mapped onto the mouse genome (mm9) using Tophat1.4.1, and then analyzed using a custom R-based pipeline to calculate gene-expression profiles using ENSEMBL annotation for coding genes. The number of reads mapped onto the gene was counted.
regardless of transcription isoform and normalized to total mapped reads to obtain transcript union Read Per Million total reads (truRPMs). The data have been deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO Series accession number GSE65361.

Cell-line culture.

INS1823/32 cells were a generous gift from C. Newgard, Duke University. Cells were grown in RPMI (10% FBS, 1% P/S). For GIP experiments, cells were starved in RPMI (1% FBS, 1% P/S) for 3 h. MIN6 cells (from ATCC) were grown in DMEM (20% FBS, 1% P/S). siRNA knockdown experiments were performed by following the manufacturer’s instructions (Origene). All cell lines were previously tested for mycoplasma contamination.

Apoptosis assays.

MIN6 beta cells. After 24 h exposure to thapsigargin (5 uM, Sigma) in culture media (DMEM, 20% FBS, 1% P/S), apoptosis was analyzed using a MitoPT assay (ImmunoChemistry Technologies) by following the kit’s instructions. Briefly, cells were exposed to 100 nM MitoPT for 10 min, washed 1 × with PBS and then detached from the plate with trypsin. An aliquot of cells was visualized on a microscope slide. Total cell number was counted using bright field and tetramethylrhodamine methyl ester (TMRM) uptake was visualized with a 545 nM filter. Apoptotic cells were calculated as (total cells – TMRM positive cells)/Total cells × 100%.

Mouse islets. Dispersed islets were transduced with Ad-GFP or Ad-Tcf7-GFP at an MOI of 10 to achieve a >90% induction rate. After transduction, cells were exposed to control or thapsigargin (100 uM) for 72 h. Apoptosis was assessed using a MitoPT assay.

Human islets. Dispersed human cells were transfected with human siTCF7 or siScram control duplexes (OriGene, Rockville, MD) and an Alexa488 labeled negative control siRNA (Qiagen, Toronto, ON), using Dharmafect (Thermo Scientific, Ottawa, ON, Canada). 24 h post-transfection culture medium was changed to fresh medium containing glucose and/or human recombinant IL1-β (Sigma, Oakville, ON, Canada), as indicated. Cell-death assays were performed on dispersed human islet cells by use of the In situ Cell Death Detection Kit TMR Red (Roche, Mannheim, Germany), using TUNEL technology, according to the manufacturer’s directions. Images were obtained using a Zeiss AxioObserver Z1 with a Zeiss-Colibri light source at 488 nm and 594 nm, a × 40/1.3 NA lens, and an AxioCam HRm camera. Images were acquired in Axiovision 4.8 software (Carl Zeiss MicroImaging, Göttingen, Germany) and analyzed using ImageJ software (National Institutes of Health). Cell death was determined as ((#TUNEL+/Alexa488+) / (#Alexa488+)) and expressed as a fold increase over control, unstimulated conditions (5.5mM glucose, scrambled siRNA).

Statistical analysis.

All values are presented as mean ± s.e.m. Statistical analysis was performed using GraphPad Prism 5.0. The appropriate t-test, one-way analysis of variance (ANOVA), or two-way ANOVA was completed using P < 0.05 to signify significant differences. Bonferroni post-hoc analysis was performed where appropriate. All data was assessed to
ensure normal distribution and equal variance between groups, using GraphPad Prism 5.0. Prior to the experiment, it was determined that individual data points would be excluded if their value was greater than $2 \times$ SD from the mean, in an experiment with a sample size greater than seven.


