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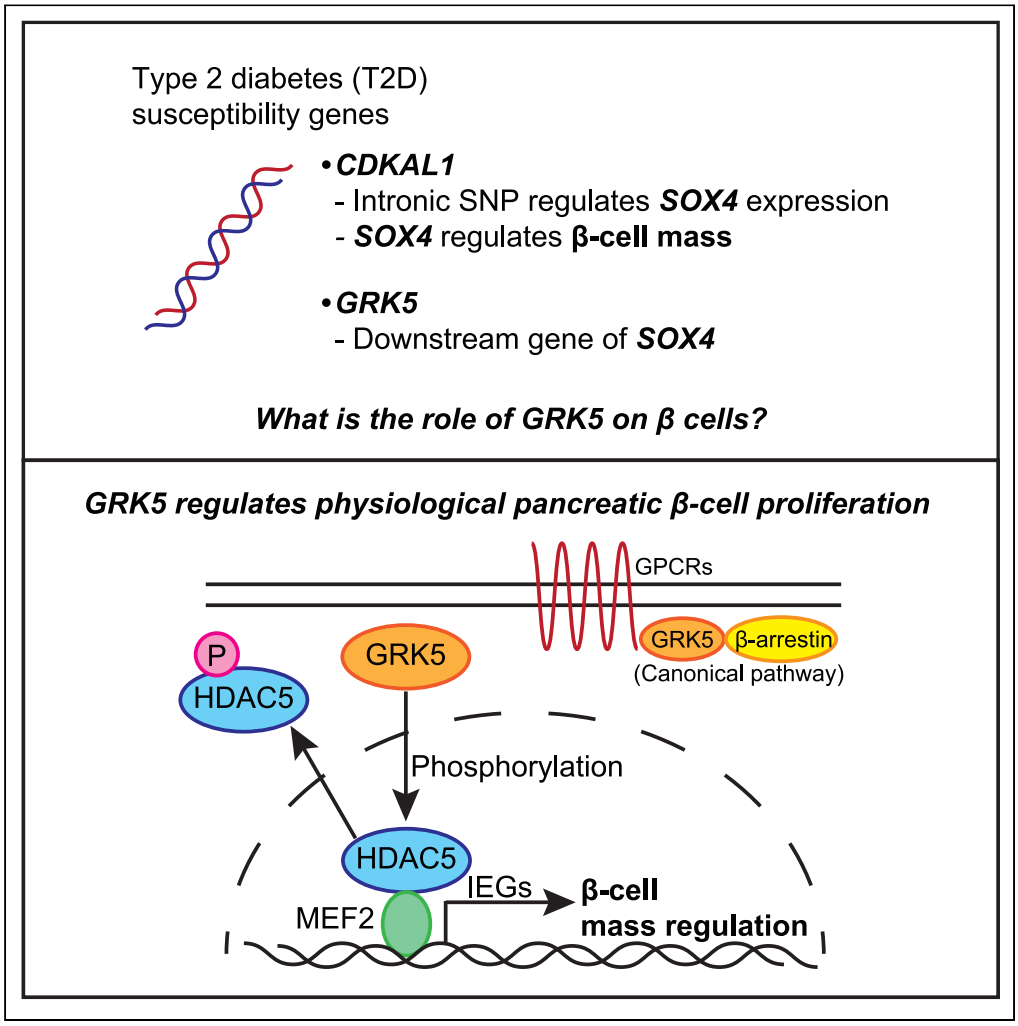

Shugo Sasaki, Cuilan Nian, Eric E. Xu, ..., Sanya Grover, Dan S. Luciani, Francis C. Lynn

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Highlights

SOX4 and G protein receptor kinase GRK5 are potential type 2 diabetes risk genes

SOX4 regulates β cell growth through regulating expression of GRK5

GRK5 knockout mice have reduced β cell mass and glucose intolerance

GRK5 phosphorylates HDAC5 to derepress β cell proliferation

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Type 2 diabetes susceptibility gene GRK5 regulates physiological pancreatic β-cell proliferation via phosphorylation of HDAC5

Shugo Sasaki,[1,2] Cuilan Nian,[1,2,3] Eric E. Xu,[1,3] Daniel J. Pasula,[1,2] Helena Winata,[1,2,3] Sanya Grover,[1] Dan S. Luciani,[1,2] and Francis C. Lynn[1,2,3,4,5,6,*]

SUMMARY

Restoring functional β cell mass is a potential therapy for those with diabetes. However, the pathways regulating β cell mass are not fully understood. Previously, we demonstrated that Sox4 is required for β cell proliferation during prediabetes. Here, we report that Sox4 regulates β cell mass through modulating expression of the type 2 diabetes (T2D) susceptibility gene *GRK5*. β cell-specific Grk5 knockout mice showed impaired glucose tolerance with reduced β cell mass, which was accompanied by upregulation of cell cycle inhibitor gene *Cdkn1a*. Furthermore, we found that Grk5 may drive β cell proliferation through a pathway that includes phosphorylation of HDAC5 and subsequent transcription of immediate-early genes (IEGs) such as *Nr4a1*, *Fosb*, *Junb*, *Arc*, *Egr1*, and *Srf*. Together, these studies suggest GRK5 is linked to T2D through regulation of β cell growth and that it may be a target to preserve β cells during the development of T2D.

INTRODUCTION

Loss of functional insulin-producing β cells in the pancreas is one of the major pathologies of diabetes, and thus restoring functional β cell mass is a promising therapy for those with diabetes. However, the mechanisms of β cell mass regulation are not fully understood. The intronic SNPs of type 2 diabetes (T2D) susceptibility gene, *CDKAL1*, are hypothesized to regulate the expression of the nearby gene, *SOX4*, not *CDKAL1* itself.[1,2] SOX4 is a DNA-binding transcription factor that regulates β cell mass through direct binding to the promoter region of the cell cycle gene, *Cdkn1a/p21*, among other targets.[3,4]

G protein-coupled receptor kinases (GRKs) act to alter G protein coupled receptor (GPCR) signaling by changing the affinity of receptors for arrestins; usually, they facilitate a switch from signaling through G proteins to signaling to downstream of arrestin binding.[5,6] The activity of GRK5 is regulated by cell activity and calcium influx; Ca/CaM binding both increases GRK5 kinase activity and displaces the kinase from the membrane, potentially allowing GRK5 to phosphorylate cytoplasmic and nuclear targets.[7–9] GRK5 has been identified as a T2D susceptibility gene by genome-wide association study (GWAS).[10–14] A variant of GRK5 is associated with the therapeutic efficacy of insulin secretagogue, repaglinide, in those with T2D,[15] and with plasma LDL-cholesterol levels.[16] Germline Grk5 knockout mice displayed mildly impaired glucose tolerance.[17] Despite these observations, the role of GRK5 in β cells has not been well studied.

Here, we report that β cell mass regulation by Sox4 is accompanied by a change of *Grk5* expression in islets. In order to determine the role of Grk5 *in vivo*, β cell specific Grk5 knockout mice (βGRK5 KO) were generated and found to have impaired glucose tolerance with reduced β cell mass. In agreement with this phenotype, cell cycle inhibitor gene, *Cdkn1a* was upregulated in βGRK5 KO islets. As one potential mechanism linking kinase function to β cell proliferation, we found that Grk5 may regulate β cell mass through phosphorylation of HDAC5 and subsequent de-repression of immediate-early genes (IEGs) that are important for β cell growth.

RESULTS

Grk5 is downregulated in β cell specific Sox4 knockout islets

Sox4 ablation in β cells led to reduced β cell mass during prediabetes in mice.[3] To investigate downstream mechanism of Sox4 in the adult mouse islets, we performed RNA-sequencing (RNA-seq) using the islets of

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β cell specific Sox4 knockouts (βSOX4 KO). As a result, several key genes associated with β cell identity as well as T2D susceptibility including *Slc2a2*, *Pclo*, and *Slc30a8* were downregulated in βSOX4 KO islets (Figure S1A). In addition, pathway analysis showed that G-protein signaling was altered, and among the genes listed in that pathway, G protein-coupled receptor kinase 5 (Grk5) gene expression was downregulated in Sox4 KO islets (Figures S1A–S1C). GRK5 has been identified as T2D susceptibility gene in Asian populations by GWAS[10] although its functions in β cells have not been investigated. Thus, we hypothesized that SOX4 regulates β cell mass, at least in part, through regulation of GRK5 expression and tested this hypothesis here.

β cell specific Grk5 knockout mice displayed impaired glucose tolerance

To investigate a role of Grk5 in the islet, βGRK5 KO were generated by crossing *Pdx1-CreER* transgenic[18] with *Grk5 floxed* mice.[19] Tamoxifen was given at 6 weeks of age to induce knockout (Figure 1A). Grk5 mRNA was reduced by >70% and protein expression lost in 8- and 18-week-old βGRK5 KO mice (Figures 1B and 1C). Body weight was not changed while average fasted and non-fasted blood glucose levels across 30 weeks were significantly higher in βGRK5 KO (Figure 1D). Additionally, βGRK5 KO mice became glucose intolerant with aging (Figure 1E). Females exhibited a similar phenotype to males with milder impaired glucose tolerance (Figure S2). As such, males were selected for further studies. By 18 weeks, fasting and fed insulin secretion were reduced in βGRK5 KO mice without changes in insulin sensitivity measured by insulin tolerance test (ITT) (Figures 1F and 1G). Together, these studies suggest that β cell function or β cell mass was impaired in βGRK5 KO.

β cell function was evaluated by glucose-stimulated insulin-secretion (GSIS) using isolated βGRK5 KO mouse islets; no alterations in high glucose- or KCl-stimulated insulin secretion were observed (Figure S3A). Total insulin in the isolated islets was unchanged (Figure S3A). Dynamics of glucose- and KCl-regulated Ca^{2+} influx into the isolated islets were not altered (Figure S3B). As GRKs normally phosphorylate GPCRs to reduce G protein binding and induce β-arrestin binding, we tested if knockout of Grk5 led to inappropriate activation of GLP-1 receptor by stimulating islets with GLP-1 receptor agonist Exendin-4. Neither Exendin-4-induced Ca^{2+} influx or insulin secretion was altered in the βGRK5 KO (Figures S3A and S3B). Together, these studies indicate that GRK5 KO does not markedly influence β cell stimulus-secretion coupling.

Grk5 deletion results in reduced β cell mass with less proliferation and more apoptosis

Next, we tested whether β cell mass was affected by β cell specific Grk5 deletion. βGRK5 KO islets displayed reduced β cell numbers at 18 weeks of age (Figures 2A and 2B). EdU-labelled proliferative β cells were decreased in βGRK5 KO earlier, at 8 weeks of age, 1 week following knockout (Figures 2C and 2D). Apoptotic β cells assessed by TUNEL were increased in βGRK5 KO (Figures 2E and 2F). To uncover mechanisms for decreased proliferation and increased apoptosis in β cells in βGRK5 KO, cell cycle-related gene expression was investigated. Expression levels of cyclin-dependent kinase inhibitor 1a gene *Cdkn1a (p21)* increased in βGRK5 KO, similar to what we previously observed in the βSOX4 KO (Figures 2G and S1A).[3] As such, we conclude that impaired glucose tolerance in the βGRK5 KO results from reduced β cell mass driven by reduced proliferation and increased apoptosis, potentially as a result of *Cdkn1a* induction. Furthermore, the βGRK5 KO phenocopies the βSOX4 KO, suggesting that Grk5 functions downstream of Sox4 in adult β cells.

RNA-sequencing revealed alteration of mass regulation-related gene expressions in βGRK5 KO islets

Next, RNA-seq was conducted to uncover the pathways downstream of GRK5 important for regulation of β cell-mass. Among 17,946 detected transcripts, 601 genes were upregulated, and 398 genes were downregulated in βGRK5 KO islets (Figure 3A). As expected, pathway analysis showed alteration of G-protein-related signaling (Figure 3B). Of note, the most significantly changed pathway was PI3 kinase pathway, in which *Pik3r1*, *Pik3ca*, *Prkca*, *Gsk3b*, and *Sos2* genes were included (Figures 3B and 3C). In addition to the alteration of AKT and ERK signaling pathways, IEGs such as *Arc*, *Fosb*, *Junb*, and *Nr4a1* were downregulated in both βGRK5 KO and βSOX4 KO islets, suggesting that appropriate expression levels of IEGs are necessary for β cell mass regulation in accordance with a previous reports (Figures 3A and 3C).[20] To identify major kinases driving the gene expression change, a kinase inference analyses (eXpression2Kinases;[21]) was performed. The results showed the potential upstream kinases were CK2, ERK1, ERK2, CDK4, and HIK2 (Figure S4A). These kinases, which are known to regulate the cell cycle, have smaller p values than AKT,

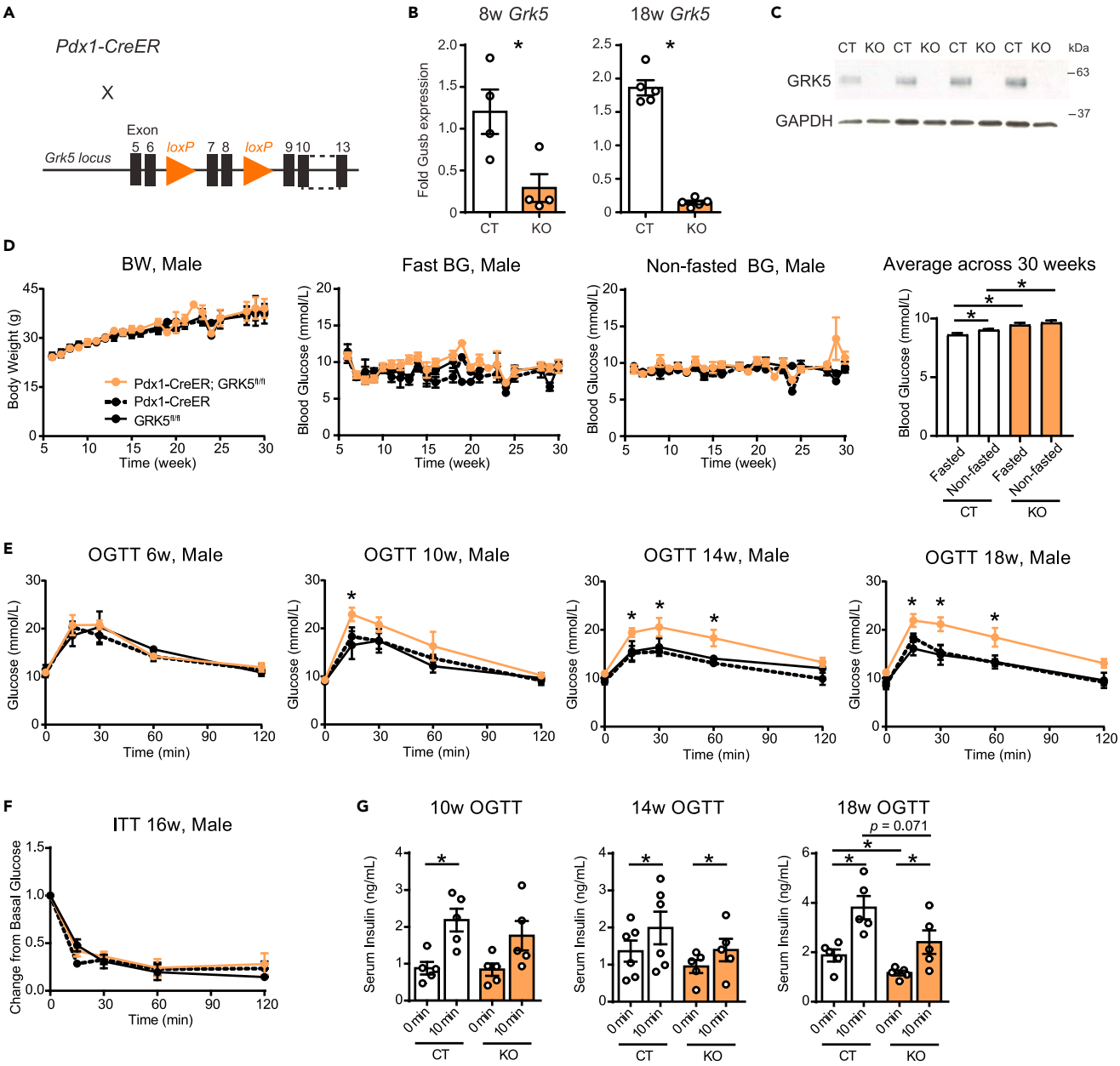

Figure 1. β cell specific GRK5 knockout mice displayed impaired glucose tolerance

(A) Scheme for generation of β cell specific GRK5 knockout mice (Pdx1-CreER; GRK5$^{fl/fl}$).

(B) GRK5 mRNA expression levels in 8- and 18-week-old mice; tamoxifen was injected at 6-week. CT: GRK5$^{fl/fl}$, KO: Pdx1-CreER; GRK5$^{fl/fl}$. n = 4 for 8-week, n = 5 for 18-week. *p < 0.05.

(C) Western blotting for GRK5 and GAPDH. CT: GRK5$^{fl/fl}$, KO: Pdx1-CreER; GRK5$^{fl/fl}$. n = 4.

(D) Body weight, fasting blood glucose, and non-fasted blood glucose levels over time. Average blood glucose levels across 30 weeks. n = 6 for each group. *p < 0.05.

(E) OGTT profiles for 6-, 10-, 14-, and 18- week-old mice. n = 6 for each group. *p < 0.05.

(F) ITT profiles at 16-week. n = 6 for each group.

(G) Serum insulin levels during OGTT. CT: GRK5$^{fl/fl}$, KO: Pdx1-CreER; GRK5$^{fl/fl}$. n = 5–6. *p < 0.05.

GSK3β and other kinases involved in insulin secretion, suggesting that GRK5 is more involved in mass regulation rather than in secretory capacity in β cells (Figure S4B). Furthermore, 15 transcripts affected by Grk5 ablation have been reported as T2D susceptibility genes, indicating their potential involvement in β cell mass regulation (Figures 3 and S5).

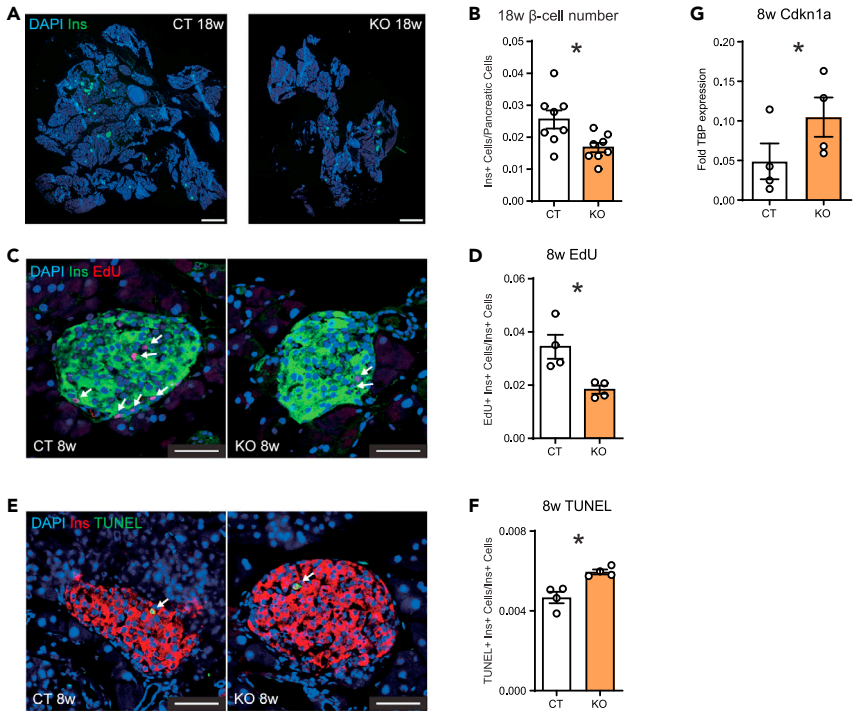

Figure 2. β cell specific GRK5 knockout mice displayed reduced β cell mass with less proliferation and more apoptosis

(A) Immunohistochemical analysis for insulin in the pancreas at 18-week. Scale bar, 1 mm.

(B) Quantification for β cell number. n = 7. *p < 0.05.

(C) Immunohistochemical analysis for insulin and EdU at 8-week. Arrows indicate EdU-positive cells. Scale bar, 50 μm.

(D) Quantification for EdU-positive β cell number. n = 4. *p < 0.05.

(E) Immunohistochemical analysis for insulin and TUNEL at 8-week. Arrows indicate TUNEL-positive cells. Scale bar, 50 μm.

(F) Quantification for TUNEL-positive β cell number. n = 4. *p < 0.05.

(G) Cdkn1a gene expression levels in the islet. CT: GRK5$^{fl/fl}$, KO: Pdx1-CreER; GRK5$^{fl/fl}$. n = 5–6. *p < 0.05.

Phosphorylation of HDAC5, but not ERK and AKT signaling, is altered in βGRK5 KO

In order to test whether Grk5 modulates GPCR signaling in β cells, we investigated the AKT and ERK pathways, in response to activation of the β cell GLP-1R. Grk5 knockout did not significantly change AKT or ERK phosphorylation by multiple GLP-1R agonists that have previously been shown to signal predominantly through the PI3K/AKT (GLP-1, exendin-4) or the β-arrestin/ERK (exendin-4, oxyntomodulin) pathways.[22–24] Notably, phospho-ERK trended toward being elevated in the Grk5 KO (Figure S6) suggesting that GRK5 may indeed be important for regulating β arrestin binding to activated GLP-1 receptors. However, as ERK signaling is thought to drive increases in β cell mass and the βGRK5 KO islets had reduced mass it seems unlikely that GRK5 modulation of GLP-1R signaling contributes to beta cell proliferation.[25]

GRK5 functions by phosphorylation of G-proteins in order to regulate β-arrestin recruitment and ERK signaling but also phosphorylates non-canonical pathways. For example, GRK5 phosphorylation of HDAC5 leads to de-repression of MEF2 transcriptional activity, and cellular proliferation in cardiomyocytes, neurons and other cell types through induction of IEGs.[6,7,26,27]

Among HDAC family members, HDAC5 was most highly expressed in mouse islets (Figure S7A). Thus, we investigated whether GRK5-HDAC5-MEF2-IEG axis is active in β cells. The isolated βGRK5 KO islets displayed less phosphorylation of HDAC5 at baseline (Figure 4A). When the control islets were stimulated by glucose and KCl, phosphorylation of HDAC5 was increased, which was suppressed in βGRK5 KO islets, suggesting GRK5 was necessary for stimulation-induced acute phosphorylation of HDAC5 (Figure 4B). amlexanox is recently identified as a selective GRK5 inhibitor.[28] Amlexanox inhibits GRK5 with 5- to 10-fold

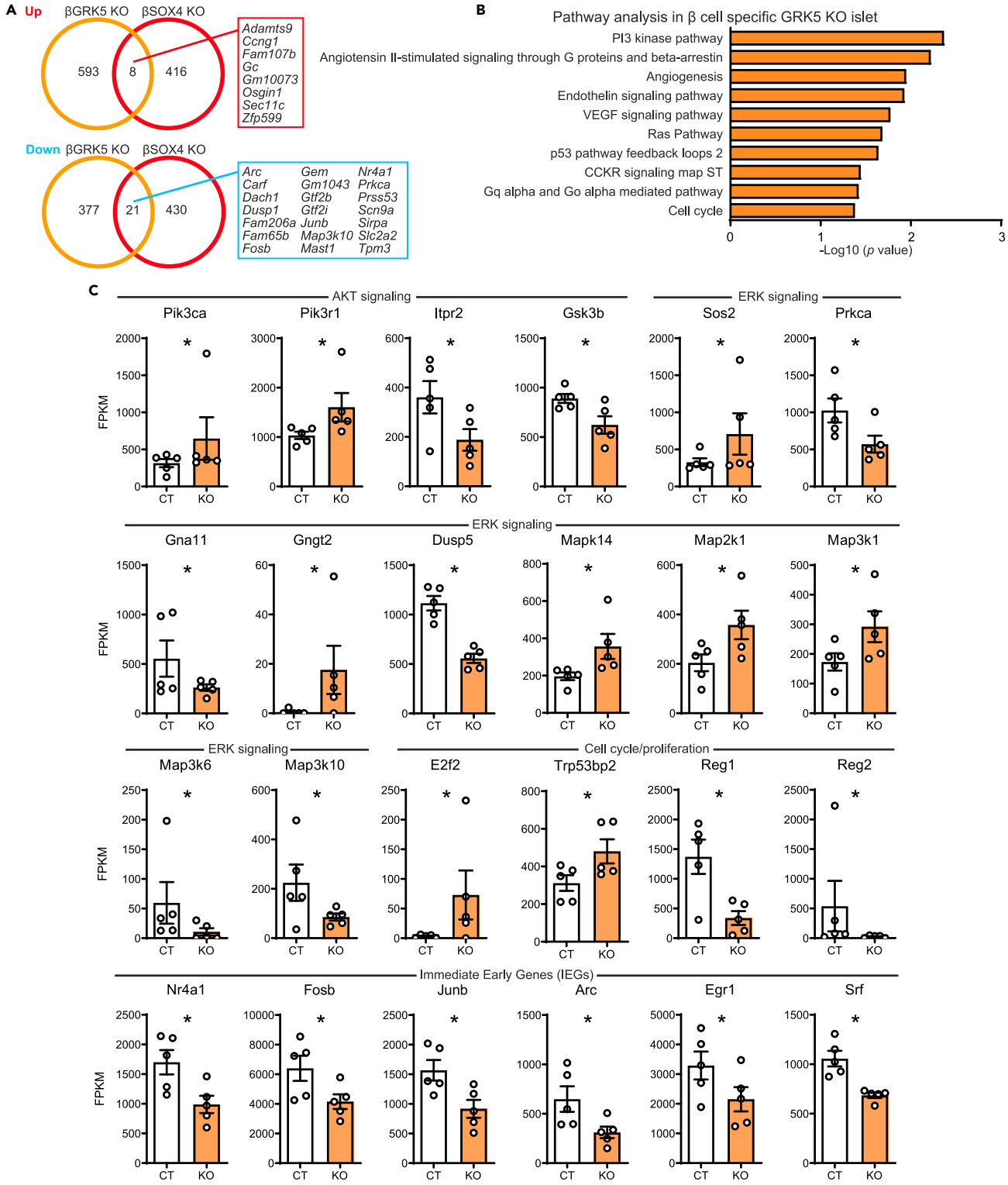

Figure 3. RNA-sequencing revealed alteration of mass regulation-related gene expressions in β cell specific GRK5 knockout mice

(A) The number of differentially expressed genes (DEGs) in GRK5 and SOX4 knockout islets. Overlapped DEGs are shown.

(B) Panther pathway analysis in β cell specific GRK5 KO islets at 18-week. Pathways with $p < 0.01$ are shown.

(C) FPKM for DEGs related to AKT signaling, ERK signaling, cell cycle/proliferation, and IEGs are shown. CT: $GRK5^{fl/fl}$, KO: Pdx1-CreER; $GRK5^{fl/fl}$. n = 5. *$p < 0.05$.

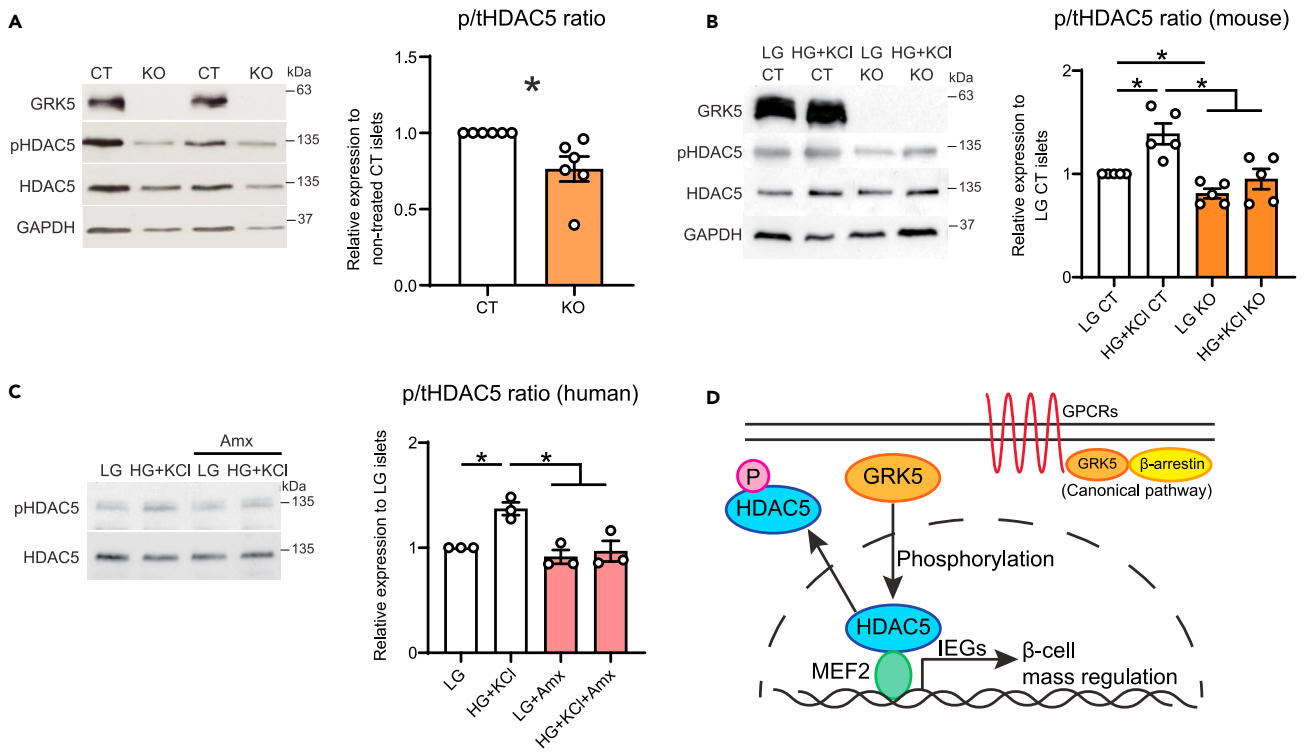

Figure 4. Phosphorylation of HDAC5 is altered in β cell specific GRK5 knockout mice and human islets treated with amlexanox

(A) Western blotting for HDAC5 phosphorylation. 80 mouse islets (18-week) were applied to each lane. CT: GRK5$^{fl/fl}$, KO: Pdx1-CreER; GRK5$^{fl/fl}$. p/tHDAC5 ratio: phospho HDAC5/total HDAC5 ratio. n = 6. *p < 0.05.

(B) Western blotting for HDAC5 phosphorylation status after stimulation. 80 mouse islets (18-week) were applied to each lane. The islets were treated by LG (2.8 mM low glucose) and HG + KCl (16 mM high glucose +30 mM KCl) for 1 h. n = 5. *p < 0.05.

(C) Western blotting for HDAC5 phosphorylation status after stimulation. 100 human islets were applied to each lane. The islets were treated by LG, HG + KCl and Amx (50 μM amlexanox) for 1 h. n = 3. *p < 0.05.

(D) Scheme for β cell mass regulation by GRK5. HDAC5 is suppressing activity of MEF2 that transcribes IEGs and regulates cell mass. GRK5 inactivates HDAC5 by phosphorylation, and de-suppresses MEF2 activity, resulting in β cell mass expansion.

selectivity over the other GRKs, and administration of 50 μM amlexanox abolishes MEF2 activity through GRK5 inhibition.[28] In human islets, stimulation-induced phosphorylation of HDAC5 was blocked by inhibiting Grk5 with amlexanox (Figure 4C). In sum these data, along with the observed downregulation of previously characterized HDAC5 target IEGs revealed by the RNA-seq (Figure 3C), suggest that phosphorylation of HDAC5 contributes to the positive regulation of β cell mass by GRK5.

DISCUSSION

In this study, we found that GRK5 is downstream of SOX4 and is important for regulation of β cell mass in mice. To our knowledge, this is the first report demonstrating the role of the T2D susceptibility gene *GRK5* in β cells. Whereas β cell function was not altered by GRK5 KO, β cell proliferation was decreased, and apoptosis were increased, along with upregulation of cell-cycle arrest genes. Phosphorylation of HDAC5 by GRK5 and subsequent upregulation of IEGs was revealed as a potential mechanism of β cell mass regulation. Further, we found that GRK5 is necessary for phosphorylation of HDAC, which was induced by glucose and KCl-stimulation, of the mouse and human β cell. Although the rationale for focusing on GRK5 included the observation that it was regulated by SOX4 in β cells, only a partial overlap of differentially expressed genes between the GRK5 and SOX4 knockout islets was seen. This suggests that while some GRK5 regulation may be downstream of SOX4, there is much complexity (Table S1). Further mining of the RNA-seq datasets could lead to other diabetes relevant pathways regulated by GRK5.

GRK5 was first identified as T2D susceptibility gene in the Chinese population.[10] The C allele of the GRK5 rs10886471 (the alternative allele is T) was detected as a risk-increasing allele for T2D by the risk allele

frequency of 0.79 in East Asians and 0.48 in Europeans. The C allele of rs10886471 was associated with higher GRK5 mRNA expression levels in the blood, higher fasting insulin, but not with fasting glucose, suggesting that it might impair insulin sensitivity. This is partially supported by the phenotype of mildly impaired glucose tolerance in germline Grk5 knockout mice.[17] Controversially, another study revealed the higher frequency of the GRK5 rs10886471 T allele polymorphism in Chinese with T2D than in healthy subjects.[15] In this study, patients with the TT genotype at rs10886471 were resistant to treatment by insulin secretagogue, repaglinide (a lower reduction of fasting plasma glucose and lower differential values of postprandial serum insulin) compared with those with CC and CT genotypes. This suggests that GRK5 also plays the key role in regulating β cell mass and/or function. In general, East Asians show a higher insulin sensitivity and a lower acute insulin response than other races,[29] which could be partially explained by reductions in GRK5 function and reduced β cell mass, as shown in mice here.

GRK5 has been actively investigated in chronic degenerative diseases such as heart failure, neurological disorders, and cancer.[6,30] Notably, GRK5 promotes pathological cell proliferation in cardiac hypertrophy.[7,8,26] Additionally, GRK5 catalytic activity is essential for preserving cardiomyocyte survival by preventing p53-induced apoptosis.[31] An *in vitro* study demonstrated that knockdown of GRK5 resulted in increased p53 and CDKN1A expression, and showed that GRK5 controlled microtubule nucleation and normal cell cycle progression.[32] In β cells, Cdkn1a was associated with cycle arrest and reductions in proliferation[33]; our previous study revealed that SOX4 regulates Cdkn1a in β cells to modulate proliferation by binding directly to *Cdkn1a* promoter regions.[3] Taken together with our data (e.g., Figures 2 and 3), it appears that GRK5 regulates cell cycle physiologically and pathologically in tissue- and context-specific manner. Currently, the development of small-molecule inhibitors targeting GRK5 for the treatment of heart failure and other conditions has attracted wide attention,[30] however, it is possible that GRK5 inhibition may have long-term unfavorable effects on β cell mass and diabetes risk.

More than 800 GPCRs have been identified in rodents and humans while only seven GRKs work to tune GPCR signaling. Expression analyses show that of the GRK-family transcripts, GRK4-6, are most abundantly expressed GRKs in both mouse and human islets (Figures S7B and S7C). As GLP-1R signaling is important for normal β cell function, we tested whether Grk5 loss altered the AKT or ERK pathways and found no significant effects. However, a slight increase in phosphorylation of ERK was observed in GRK5 KO islets, which seems contrary to the accepted view that phosphorylation of ERK promotes cell growth. One potential explanation for this discrepancy is the complexity of the mechanisms that regulate β cell growth and mass. Other GPCRs such as the M3R and GPR40 could be regulated by Grk5 in the β cell, and we will be interested in testing this in future studies.[34] Interestingly, β cell apoptosis was increased in GRK5 KO islets whereas that was not affected in SOX4 KO, suggesting that GRK5 might control GPCR-mediated cell death. In addition to HDAC5, Grk5 may phosphorylate other immediate-early gene targets in β cells. IEGs regulate cell cycle genes and cell mass in various tissues.[27] It is controversial whether Nr4a1 alone regulates β cell mass[35,36]; however, a set of IEGs including *Srf*, *Junb*, *Fos*, and *Egr1* have been shown to induce β cell proliferation.[20] The role of IEGs in β cell proliferation may change depending on the pathophysiological context and orchestrated control of IEGs through manipulation of GRK5 activity may be another target for diabetes therapy.

In conclusion, we discovered that T2D susceptibility gene *GRK5* regulates physiological pancreatic β cell proliferation, possibly through phosphorylation of HDAC5 and transcription of IEGs. Future studies need to assess whether GRK5 is a tractable β cell drug target and carefully consider the safety of pleiotropic roles of GRK5.

Limitations of the study

There were no clear alterations in insulin secretion by GSIS assay and no change in Ca^{2+} influx in GRK5 KO islets, although slight decrease in high glucose stimulated insulin secretion and in total insulin were observed in KO (Figure S3). This implies that GRK5 impacts not only β cell mass but may also regulate insulin secretion. Future studies will aim to address this as understanding the mechanisms potentially linking GRK5 and insulin secretion will be important to understand how drugs that modify GRK5 activity might impact β cells.

For the *ex vivo* islet phosphorylation analysis of AKT and ERK, the islets were stimulated for 1 h. Typically, major AKT and ERK phosphorylation events occur within 5–20 min. However, 1-h and 5-h stimulations were

applied to examine cell survival related change in pERK, e.g.,[37] Additionally, temporal changes of pERK following GLP-1 stimulation demonstrated a 2-fold increase in pERK with 60 min of GLP-1 stimulation.[38] Since we focused on the mechanisms of β cell mass regulation here, and not acute insulin secretion, a 1-h stimulation time was selected.

It was hard to directly investigate β cell proliferation controlled by GRK5 and HDAC5 phosphorylation using *in vitro* experiments (Figure 4). The experiments were performed to test if glucose/KCl-stimulation could change HDAC5 phosphorylation directly and acutely in β cells, and determine if HDAC5 phosphorylation can be changed by GRK5 KO, not as the chronic effect of GRK5 KO *in vivo*. Further study focusing on cell proliferation/death *in vitro* could reveal the mechanisms of GRK5-HDAC5 regulated cell proliferation in β cells, that could lead cell therapy to treat diabetes.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107311.

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UBC and BC Children's Hospital are situated on the traditional, ancestral, and unceded territories of the Coast Salish peoples, the Sḵwxwú7mesh (Squamish), səlilwətaʔɬ (Tsleil-Waututh), and xʷməθkʷəyəm (Musqueam) Nations.

AUTHOR CONTRIBUTIONS

S.S., C.N., E.E.X., D.J.P., H.W., and S.G. generated and analyzed data. S.S., D.S.L., and F.C.L. designed experiments. S.S. and F.C.L. drafted the manuscript. All the authors approved the version of the manuscript to be published. F.C.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Guinea pig anti-insulin	Dako	Cat#A0564; RRID:AB_10013624
Mouse anti-GRK5 (D-9)	Santa Cruz Biotechnology	Cat#sc-518005
Mouse anti-phospho Erk1/2 (E10)	Cell Signaling Technology	Cat#9106S; RRID:AB_331768
Mouse anti-Erk1/2 (3A7)	Cell Signaling Technology	Cat#9107S; RRID:AB_10695739
Rabbit anti-phospho Akt	Cell Signaling Technology	Cat#9271S; RRID:AB_329825
Rabbit anti-Akt	Cell Signaling Technology	Cat#9272S; RRID:AB_329827
Rabbit anti-phospho HDAC5	Abcam	Cat#ab47283; RRID:AB_880358
Mouse anti-HDAC5 (B-11)	Santa Cruz Biotechnology	Cat#sc-133106; RRID:AB_2116793
Mouse anti-GAPDH	Sigma-Aldrich	Cat#G8795; RRID:AB_1078991
Donkey anti-guinea pig FITC	Jackson Immuno Research Laboratories	Cat#706-096-148; RRID:AB_2340454
Donkey anti-guinea pig Cy3	Jackson Immuno Research Laboratories	Cat#706-166-148; RRID:AB_2340461
Peroxidase AffiniPure Goat Anti-Mouse IgG	Jackson Immuno Research Laboratories	Cat#115-035-174; RRID:AB_2338512
Peroxidase AffiniPure Goat Anti-Rabbit IgG	Jackson Immuno Research Laboratories	Cat#111-035-008; RRID:AB_2337937
Alexa 594-conjugated Azide	Life Technologies	Cat#A10270
Chemicals, peptides, and recombinant proteins		
Exendin-4	Sigma-Aldrich	Cat#E7144
Oxyntomodulin	R&D	Cat#2094/1
SlowFade Diamond Antifade	Invitrogen	Cat# S36972
EdU	Toronto Research Chemicals	Cat# E932175
Fura-2/AM	Invitrogen	Cat#F1221
TRIzol	Thermo Fisher Scientific	Cat# 15596018
Turbo DNAse Free	Thermo Fisher Scientific	Cat# AM1907
Superscript III	Thermo Fisher Scientific	Cat# 18080044
RiboGone Mammalian-Low Input Ribosomal RNA Removal Kit	Takara Bio	Cat# 634847
Agencourt AMPure XP SPRI beads	Beckman Coulter	Cat# B23318
RNA Pico 6000 kit	Agilent	Cat# 5067-1513
SMARTer Stranded RNA-Seq Kit	Takara Bio	Cat# 634836
Qubit 3.0 dsDNA high-sensitivity assay kit	Thermo Fisher Scientific	Cat# Q32854
Critical commercial assays		
OneTouch UltraMini glucometer	Lifescan	
Insulin ELISA kit	Alpco	Cat#80-INSHU-CH01
In Situ Cell Death Detection Kit, TMR red	Roche	Cat# 12156792910
Deposited data		
RNA-seq data	This paper	GSE221716
Experimental models: Organisms/strains		
Mouse: Pdx1-CreER	Gift	Gu et al.[18]
Mouse: Sox4$^{flox/flox}$	Gift	Penzo-Méndez et al.[39]
Mouse: Grk5$^{tm1a(KOMP)Mbp}$	IMPC	BL4363
Human islets	University of Alberta Islet Distribution Program	Checklist attached

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Mouse Grk5	IDT	forward [F], 5′-GCACTCAACGAAAAGCAGATTC-3′; reverse [R], 5′-GTGCATCTTTGGTTTCATAGGC-3′; and probe 5′-AGGTCAACAGCCAGTTTGTGGTCA-3′),
Mouse Cdkn1a	IDT	F, 5′-CTGAGCGGCCTGAAGATT-3′; R, 5′-ATCTGCGCTTGGAGTGATAG-3′; and probe 5′-AAATCTGTCAGGCTGGTCTGCCTC-3′
Mouse Gusb	IDT	F, 5′-TCTAGCTGGAAATGTTCACTGCCCTG-3′; R, 5′-CACCCCTACCACTTACATCG-3′; and probe 5′-ACTTTGCCACCCTCATCC-3′
Human GRK5	IDT	F, 5′-AGGTCTTCACACTGGCTAATG-3′; R, 5′-CAATGGAGCTGGAAAACATCG-3′; and probe 5′-AGGAAAGCGCAAAGGGAAAAGCAAG-3′
Human TBP	IDT	F, 5′-TGGGATTATATTCGGCGTTTCGGGC-3′; R, 5′-GAGAGTTCTGGGATTGTACCG-3′; and probe 5′-ATCCTCATGATTACCGCAGC-3′
Software and algorithms		
GraphPad Prism Version 9.0	GraphPad	https://www.graphpad.com
ImageJ	NIH	https://imagej.nih.gov/ij/
cellSens Dimension software	Olympus	https://www.olympus-lifescience.com/ja/software/cellsens/
CellProfiler 3.0	Broad institute	McQuin et al.[40]
eXpression2Kinases	Ma'ayan Lab	Clarke et al.[21]

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Francis C. Lynn (francis.lynn@ubc.ca).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- RNA-seq data have been deposited at GEO and are publicly available as of the date of publication. The accession number is listed in the key resources table. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- This paper does not report original code.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals and human islet studies

All experiments were approved by the University of British Columbia Animal Care Committee. Animals were housed under a 12-h light/dark cycle, fed *ad libitum* with standard chow diet (5010; Lab Diets). Mouse strains used were on the C57BL/6 background. Pdx1-CreER; $Sox4^{flox/flox}$ mice were generated as described.[3,18,39] $Grk5^{flox/flox}$ mice were generated by crossing FLP mice[41] with $Grk5^{tm1a(KOMP)Mbp}$ mice[19] obtained from International Mouse Phenotyping Consortium. $Grk5^{flox/flox}$ mice were crossed with Pdx1-CreER mice[18] to generate Pdx1-CreER; $Grk5^{flox/flox}$ experimental mice. To control for potential tamoxifen effects on β cell replication, all mice including Pdx1-CreER only controls and a $Grk5^{flox/flox}$ only controls were administered 8 mg tamoxifen in corn oil (60 mg/mL) by oral gavage every other day beginning at 6 weeks of age. Human islet studies were approved by the BC Children's and Women's Hospital Research Ethics Board. Islets were provided by the University of Alberta Islet Distribution Program. Human islet data is provided in the attachment.

Metabolic phenotype analysis

For glucose tolerance tests, mice were weighed and fasting saphenous vein blood glucose levels obtained using a OneTouch UltraMini glucometer (Lifescan) after a 10-h fast during the dark cycle. 2 g/kg D-glucose was delivered via intraperitoneal (i.p.) injection and blood glucose levels determined at 0, 15, 30, 60, and 120 min. Blood was collected at fasting and 10 min following i.p. glucose for determination of serum insulin levels using ELISA (STELLUX; ALPCO Salem, NH). Blood glucose levels greater than the glucometer detection limit were reported as 33.3 mmol. Intraperitoneal insulin tolerance test was performed following a 3-h fast during the light cycle with 1 unit/kg i.p. insulin injection.

METHOD DETAILS

Immunohistochemical analysis

Immunostaining was performed on 5-μm paraffin sections as described.[3] Primary antibodies were applied overnight at 4°C in PBS- 0.3% Triton+ 5% horse serum. After washing with PBS, secondary antibodies and DAPI were applied for 1 h at room temperature, before mounting on slides using SlowFade Diamond Antifade (Invitrogen). A primary antibody was guinea pig anti-insulin (1:1000; A0564; Dako). Secondary antibodies were donkey anti-guinea pig FITC and Cy3 (1:200; Jackson Immuno Research Laboratories, #706-096-148 and # 706-166-148). 5-Ethynyl-2′-deoxyuridine (EdU) staining on sections was performed as described.[3] A total of 0.5 mg EdU was administered (i.p.) twice a day for 7 days, beginning at 7 weeks of age. TdT-mediated dUTP-X nick end labeling (TUNEL) was performed using *In Situ* Cell Death Detection Kit, TMR red (Roche). Twelve sections (250-μm intervals) of each pancreas were imaged using a BX61 microscope and tiled using the cellSens Dimension software (Olympus). CellProfiler 3.0[40] was used to quantify images, and counts were normalized to total pancreatic nuclei. For EdU and TUNEL quantification, sections which contain 20-30 islets per mouse were imaged using confocal microscopy (Leica SP8; Leica Microsystems) and counted as above.

Ex vivo islet assays

Mouse pancreatic islets were isolated and recovered overnight. Human islets were recovered overnight after receipt. Following preincubation in Krebs-Ringer buffer containing 2.8 mM glucose for 1 h at 37°C, 100 islets were incubated in either 2.8, 25 (±50 nM exendin-4), or 2.8 mM glucose with 40 mM KCl for 1 h at 37°C. Supernatants were collected for insulin measurement. Fura-2 calcium imaging was performed as described previously.[42]

Gene expression assays

RNA was isolated with TRIzol, DNase treated (Turbo DNAse Free; Thermo Fisher Scientific), and reverse transcribed with Superscript III (Thermo Fisher Scientific) as previously described.[3] TaqMan quantitative PCR (qPCR) was performed as described.[3] Primers used were: mouse Grk5 (forward [F], 5′-GCACTCAAC GAAAAGCAGATTC-3′; reverse [R], 5′-GTGCATCTTTGGTTTCATAGGC-3′; and probe 5′-AGGTCAA CAGCCAGTTTGTGGTCA-3′), mouse Cdkn1a (F, 5′-CTGAGCGGCCTGAAGATT-3′; R, 5′-ATCTGCGCTT GGAGTGATAG-3′; and probe 5′-AAATCTGTCAGGCTGGTCTGCCTC-3′), mouse Gusb (F, 5′-TCTAGCTG GAAATGTTCACTGCCCTG-3′; R, 5′-CACCCCTACCACTTACATCG-3′; and probe 5′-ACTTTGCCACCCT CATCC-3′), human GRK5 (F, 5′-AGGTCTTCACACTGGCTAATG-3′; R, 5′-CAATGGAGCTGGAAAACAT CG-3′; and probe 5′-AGGAAAGCGCAAAGGGAAAAGCAAG-3′), and human TBP (F, 5′-TGGGATTA TATTCGGCGTTTCGGGC-3′; R, 5′-GAGAGTTCTGGGATTGTACCG-3′; and probe 5′-ATCCTCATGAT TACCGCAGC-3′).

RNA-sequencing

Following total RNA extraction and DNase treatment, samples were rRNA depleted utilizing the RiboGone Mammalian-Low Input Ribosomal RNA Removal Kit (Takara Bio USA Inc., Mountain View, CA, USA), and Agencourt AMPure XP SPRI beads (Beckman Coulter). rRNA depleted total RNA was assessed for rRNA depletion, RNA integrity, size distribution, and concentration prior to library generation with the Agilent RNA Pico 6000 kit on the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). rRNA depleted RNA passing quality control was used in the SMARTer Stranded RNA-Seq Kit (Takara Bio USA). Following RNA incubation with stranded N6 primer, buffer, and water, samples were incubated at 94°C for 4 min, appropriate for samples with RIN values between 4 and 7. Following purification of first-strand cDNA, libraries were PCR amplified in accordance with their input RNA amount and purified. Final adapter-ligated cDNA was quantitated by Qubit 3.0 dsDNA high-sensitivity assay kit (Thermo Fisher

Scientific) together with qPCR amplification using standard curves of a known concentration of adapter-ligated libraries. Sequencing was performed on the NextSeq 500 with the High Output Reagent Cartridge v2 150 cycles (75 bp x 2) (Illumina, San Diego, CA, USA). Linux Ubuntu operating system version 14.04 LTS was used for analyses. Fastq files from each sample were concatenated. ENSEMBL Mus musculus genome GRCm38p.4 fastq and gtf annotation file were used for alignments. STAR v2.4.0.1 230 was utilized to index the genome fastq files, align sample transcriptomes against the genome, and output as sorted by coordinate BAM files. Counts were generated from BAM files with HTSeq[43] against annotated genes from GRCm38p.4. Finally, differential gene analysis was performed between control and experimental samples using DESeq2,[44] with an FPKM criteria of ≥ 5 in two or more samples, applying a Wald test on *p*-values from genes passing filtering, and adjusted for multiple testing via Benjamini and Hochberg procedure. Panther pathway analysis performed for differentially expressed genes.[45]

Western blot

Western blots were performed as described.[46] Primary antibodies used included: mouse anti-GRK5 (D-9, 1:1,000; SCBT, sc-518005), mouse anti-phospho Erk1/2 (E10, 1:1,000; CST, 9106S), mouse anti-Erk1/2 (3A7, 1:1,000; CST, 9107S), rabbit anti-phospho Akt (1:1,000; CST, 9271S), rabbit anti-Akt (1:1,000; CST, 9272S), rabbit anti-phospho HDAC5 (1:1,000; Abcam, ab47283), mouse anti-HDAC5 (B-11; 1:1,000, SCBT, sc-133106), and mouse anti-GAPDH (1; 100,000; Sigma-Aldrich, G8795). Secondary horseradish peroxidase–conjugated antibodies were from Jackson ImmunoResearch Laboratories (#115-035-174 and #111-035-008). ImageJ (NIH) was used for quantification.

Data sources

Type 2 diabetes susceptibility genes are listed using the NHGRI-EBI GWAS Catalog.[12] GRK1-7 gene expression data in human islets and β cells are obtained from the GEO database (accession GSE73433,[47] GSE140403,[48] and GSE57973[49]).

QUANTIFICATION AND STATISTICAL ANALYSIS

Measurements were performed on independent samples unless otherwise specified. Statistical analyses were performed using the Prism 9.0 (GraphPad Software, La Jolla, CA). Multiple groups were analyzed by one-way ANOVA with a multiple comparison test and the Tukey-Kramer's post-hoc test was used to compare different groups. A p-value < 0.05 was considered to indicate a statistically significant difference between two groups. Data are presented as the mean $\pm$ SEM.