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E2F1 transcription factor mediates a link between fat and islets to promote β cell proliferation in response to acute insulin resistance

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SUMMARY

Prevention or amelioration of declining β cell mass is a potential strategy to cure diabetes. Here, we report the pathways utilized by β cells to robustly replicate in response to acute insulin resistance induced by S961, a pharmacological insulin receptor antagonist. Interestingly, pathways that include CENP-A and the transcription factor E2F1 that are independent of insulin signaling and its substrates appeared to mediate S961-induced β cell multiplication. Consistently, pharmacological inhibition of E2F1 blocks β-cell proliferation in S961-injected mice. Serum from S961-treated mice recapitulates replication of β cells in mouse and human islets in an

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AUTHOR CONTRIBUTIONS

J.S. and R.N.K. conceived the idea and contribute to planning the experiments. J.S., Y. Togashi, G.B., T.O., R.I., M.F., M.K., D.F.D.J., N.G., W.Z., T.T., and P.Y., performed the experiments. T.K. and A.M.J.S. contributed to human islet preparation. H.P., J.M.D., and Y. Terauchi contributed to analysis of data and interpretation. J.S. and R.N.K. wrote and edited the manuscript. All authors approved the final version of the manuscript. R.N.K. 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

R.N.K. is on the Scientific Advisory Board of Novo Nordisk, Biomea, REDD, and Inversago.

SUPPLEMENTAL INFORMATION

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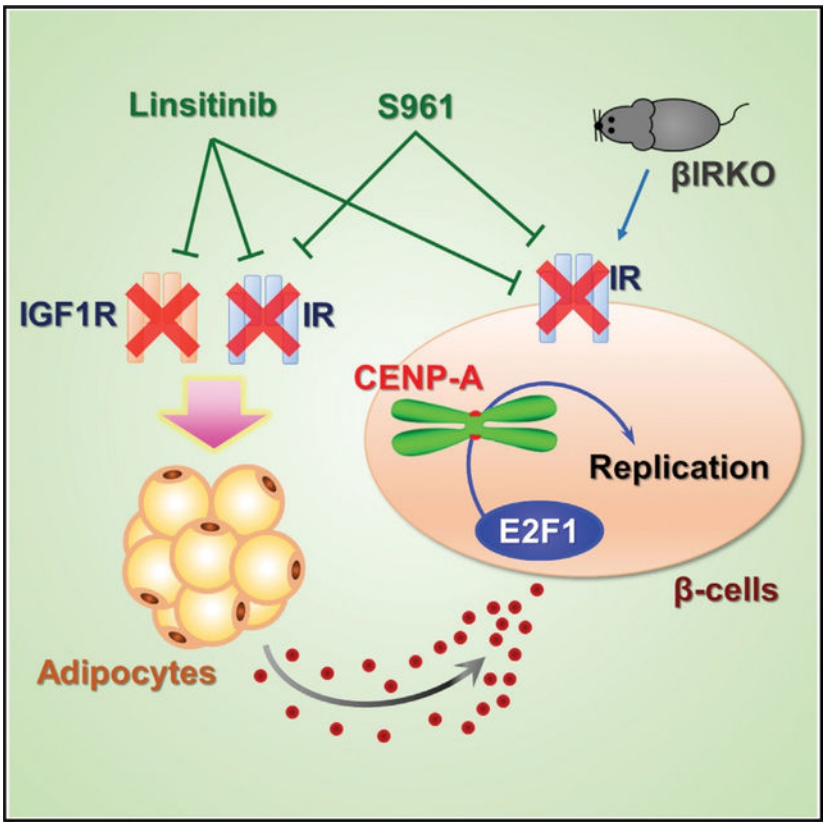
E2F1-dependent manner. Co-culture of islets with adipocytes isolated from S961-treated mice enables β cells to duplicate, while E2F1 inhibition limits their growth even in the presence of adipocytes. These data suggest insulin resistance-induced proliferative signals from adipocytes activate E2F1, a potential therapeutic target, to promote β cell compensation.

In brief

Adaptive β cell proliferation in response to insulin resistance is a compensatory pathway to increase functional β cell mass. Shirakawa et al. reveal that potential circulating factors from adipocytes drive β cell multiplication via insulin signaling-independent E2F1 and CENP-A pathways in response to S961-induced acute insulin resistance.

Graphical Abstract



INTRODUCTION

β cell dysfunction in patients with type 2 diabetes potentially occurs secondary to inappropriate insulin secretory response to insulin resistance and a relative insufficiency in β cell volume. Therefore, enhancing β cell proliferation to increase functional β cell mass to a similar level as in healthy people is a strategy to slow progression and potentially reverse the course of the disease.

The high-fat diet-induced obese (DIO) mouse model is commonly used to dissect the mechanism(s) underlying adaptive β cell proliferation in response to chronic insulin resistance (Golson et al., 2010; Shirakawa and Kulkarni, 2016). In DIO mice, the insulin/insulin receptor substrate (IRS) signaling proteins that activate the downstream pathways involving CENP-A have been reported to be important for β cell replication (Kubota et al., 2004; Shirakawa et al., 2017b; Terauchi et al., 2007). Downstream of insulin signaling via IR/IRS-2, the nuclear exclusion of forkhead box O1 (FoxO1) contributes to the β cell proliferative response to insulin resistance in DIO mice (Takamoto et al., 2008). However, in human type 2 diabetes, the expression of insulin receptor (IR) and its downstream factors, including IRS-2, are attenuated in islets/β cells. Hence, exploring alternative pathway(s) that can drive β cell proliferation independent of the IR or IRS-2 pathway are desirable to identify candidate targets for the therapeutic restoration of β cell volume in patients with type 2 diabetes.

Pharmacological inhibition of IR can induce acute insulin resistance with marked hyperglycemia and hyperinsulinemia (Shirakawa et al., 2014). Previous studies demonstrated that injection for 7 days of S961, an IR antagonist (Schaffer et al., 2008), or OSI-906, a dual inhibitor for IR and insulin-like growth factor receptor (IGF1R) (Mulvihill et al., 2009) can independently facilitate potent β cell proliferation in mice (Tajima et al., 2017). Since S961 and OSI-906 each exert their inhibitory effects systemically, which includes the endocrine pancreas, pathways that are independent of IR signaling in β cells are likely to contribute to the β cell replication in response to the compounds. Thus, these acute insulin resistance models would be useful to identify mechanisms that can increase β cell mass even if the IR-mediated signal is attenuated.

To directly evaluate the pathways that are independent of IR/IRS signaling in the regulation of β cell proliferation induced by acute insulin resistance, we treated β cell-specific IR knockout (βIRKO) or IRS-2-deficient mice either with S961 or OSI-906. Global gene expression analysis of islets from S961-injected mice revealed a transcription factor, E2F transcription factor 1 (E2F1), that mediates β cell proliferation independent of IR/IRS both *in vivo* and *in vitro*. Co-culture of islets with adipocytes suggested that a fat-derived factor contributed to the S961-induced β cell proliferation acting via E2F1. Our data point to a fat-pancreas axis acting via E2F1 in the β cell compensation in response to acute insulin resistance.

RESULTS

Systemic IR inhibition promotes β cell proliferation in βIRKO mice

We used a subcutaneous osmotic pump to inject S961, an IR antagonist, into control (IR-floxed) and βIRKO mice for 9 days, followed by evaluation of glucose homeostasis and analyses of β cell proliferation. No effects on body weight were evident between groups over the duration of the injection (Figure 1A). As expected, S961 induced hyperglycemia within a few days after the injection, equally in both groups, and the glucose levels remained elevated through the 9-day period with no significant differences at any time points (Figure 1B). Evaluation of insulin sensitivity by intraperitoneal injection of insulin on day 9 showed severe resistance that was similar between control and βIRKO mice (Figure 1C). Thus,

treatment with S961 resulted in marked hyperinsulinemia, and there was no difference between the two genotypes (Figure 1D).

We next evaluated the compensatory response by measuring β cell proliferation and mass and observed that both parameters were significantly and equally increased by S961 in both groups (Figures 1E and 1F). These results suggested that the β cell is capable of proliferating and increasing its mass in response to systemic acute insulin resistance by a pathway that is independent of a functional IR. The close homology between the IGF-1 and IRs and the ability of the former to compensate for signaling in the absence of the latter prompted us to treat βIRKO mice with OSI-906, a dual inhibitor for IR and IGF-1 receptors (IGF1Rs) for 8 days. While there were no differences in body weight changes between the two groups treated with OSI-906, we observed a similar level of hyperglycemia and hyperinsulinemia as that observed with S961 in both groups (Figures 1G–1I). Thus, OSI-906 increased β cell mass and proliferation in βIRKO mice to a similar extent as in control mice (Figures 1J and 1K), indicating that IGF1 receptors are unlikely to mediate the proliferation signals.

To examine whether this observation can be generalized to other models lacking proteins in the insulin/IGF-1 signaling pathway, we undertook similar studies by treating IRS-2 KO mice with OSI-906. A similar series of observations on hyperglycemia and increases in β cell mass (1.6- versus 1.5-fold) and proliferation (1.9- versus 2.2-fold) in both wild-type and IRS2KO mice (Figures S1A–S1G) pointed to induction of β cell proliferation by OSI-906 that is independent of insulin/IGF1R/IRS-2 signaling.

IR inhibition with S961 promotes the FoxM1/PLK1/CENP-A pathway in β cells

To begin to examine the pathways activated by acute insulin resistance, we compared the gene expression profiles of freshly isolated islets obtained from mice treated with S961 versus vehicle and focused on the genes that showed a change in expression in response to IR inhibition *in vivo* (Table S1). For example, we observed that mitosis-related genes, such as centromere protein A (Cenpa), PDZ-binding kinase (Pbk), protein regulator of cytokinesis 1 (Prc1), minichromosome maintenance complex component 5 (Mcm5), cell division cycle-associated 3 (Cdca3), cell division cycle 20 (Cdc20), Polo-like kinase 1 (Plk1), cyclin B1 (Ccnb1), or baculoviral IAP repeat containing 5 (Birc5) were all significantly increased in islets from mice treated with S961, while conversely, IR-mediated signaling genes such as cyclin D1 (Ccnd2) and Irs2 were decreased in these islets (Figure 2A; Table 1). Notably, CENP-A showed the lowest false discovery rate (FDR) among all detected genes (FDR Q = 3.98e–6, p = 4.54e–10). Comprehensive pathway analysis of upregulated and downregulated genes suggested that the mitotic G2/M cell-cycle-related pathways were involved in S961-mediated β cell proliferation (Figure 2B). Previously, we have reported that β cell-specific CENP-A KO mice failed to increase β cell proliferation in response to S961 administration (Shirakawa et al., 2017b). The transcription factor forkhead box M1 (FoxM1) regulates CENP-A expression and its deposition to the centromere through PLK1 that is downstream of IR signaling (Shirakawa et al., 2017b). These results prompted us to examine whether S961 is able to engage the FoxM1/PLK1/CENP-A signaling pathway to promote β cell replication even in the absence of functional IR signaling.

Nuclear export of the transcription factor FoxO1 by insulin signaling is evident during adaptive β cell proliferation in DIO mice (Mezza et al., 2016; Terauchi et al., 2007). In contrast, in unstressed wild-type mice, FoxO1 was mainly localized to the cytosol in β cells (Figure S2). The predominant nuclear localization of FoxO1 in β cells observed in vehicle-treated control and βIRKO mice (Figure 2C) was further enhanced by S961 treatment compared with vehicle in both groups (Figure 2C). However, the fluorescence intensity of CENP-A detected by immunostaining was markedly increased in proliferating β cells in both S961-treated control and βIRKO mice (Figure 2D). The expression of Cenpa, Plk1, or Birk5 genes was significantly upregulated in the islets from S961-treated control and βIRKO mice, while the increase in expression of Foxm1 and cyclin-dependent kinase 1 (Cdk1) genes did not reach statistical significance in the latter group (Figure 2E). Expression of Insr, Igf1r, Irs2, or Ccnd2 genes showed no increment in islets of both mice (Figure 2E). These data argue that S961 engages the CENP-A-mediated pathway to induce β cell replication independent of signaling via the IR.

E2F1 plays a crucial role in S961-induced β cell replication

To identify the potential transcription factors that mediate S961-induced IR-independent β cell proliferation based on binding sites, we analyzed the Molecular Signatures Database (MsigDB) transcription factor target (TFT) gene sets (Liberzon et al., 2015). Remarkably, 18 of the top 25 TFT gene sets among the upregulated genes in response to treatment with S961 were related to the E2 factor (E2F) family of transcription factors (Figure 3A). The microarray analysis indicated that the expression of E2F1 and E2F2 were increased in islets following the treatment with S961 (Table 2). Validation studies revealed that the gene expression of E2F1, but not E2F2, E2F3, or E2F4, was significantly upregulated in islets from both S961-treated control and βIRKO mice (Figure 3B). In previous studies, E2F1 deficiency has been reported to reduce β cell mass, and conversely, forced expression of E2F1 has been shown to facilitate β cell proliferation (Fajas et al., 2004; Grouwels et al., 2010). To examine the specificity of the role of E2F1, we concomitantly administered either 2 mg HLM006474 (Ma et al., 2008), an inhibitor for E2F family including E2F1, or vehicle to S961-treated wild-type animals intraperitoneally once a day for 7 days, followed by evaluation of β cell proliferation and mass. Body weights (Figure S3A) and blood glucose levels (Figure S3B) showed no significant differences between vehicle- and HLM006474-treated groups. However, importantly, the enlarged β cell mass secondary to the enhanced β cell proliferation induced by S961 were blunted in the HLM006474-treated group (Figures 3C and 3D), likely due to the inhibition of the E2F family. Furthermore, the expression of Foxm1, Cenpa, Plk1, Cdk1, and Birk5 genes was also attenuated in the islets from mice co-treated with S961 and HLM006474 compared with mice co-treated with S961 and vehicle (Figure 3E).

Circulating factors contribute to β cell replication induced by S961

To explore the source of the factor that promotes an increase in β cell mass in acute insulin resistance, we turned to *in vitro* studies using β cell lines and used the MTT assay to assess cell viability. Treatment of control, IRS1KO, IRS2KO, or βIRKO β cell lines (Assmann et al., 2009; Kulkarni et al., 1999) with 20% serum obtained from mice treated with the S961 compound increased cell viability compared with cells treated with 20% serum

from vehicle-treated animals (Figure 4A). These data suggest that some component in the circulation mediates S961-induced β cell proliferation that is independent of signaling via IRs and its major substrate proteins IRS1 and 2. Because 10% serum had no effects on cell viability and 30% serum demonstrated similar results to those of 20% serum in MTT assay (data not shown), we chose the latter for subsequent experiments. We also confirmed that 20%or 30% mouse serum did not prevent phosphorylation of Akt or ERK, which mediate growth factor signaling, compared with FBS, in mouse islets (Figure S4A). Knockdown of CENP-A (Shirakawa et al., 2017b) also attenuated the increase in β cell viability induced by S961-treated serum (Figure S4B), while treatment with the E2F inhibitor (HLM006474) reduced the S961 serum-induced β cell survival rate (Figure S4C). Finally, as an alternative approach, knocking down E2F1 in β cells by short hairpin RNA (shRNA)-expressing lentivirus (Figure 4B) blunted the serum-mediated S961 induction of β cell viability and proliferation (Figures 4C and 4D).

To examine the physiological relevance of the data in the cell lines, we repeated the studies using freshly isolated islets. Indeed, treatment with 20% serum from S961-treated mice augmented β cell proliferation in both mouse (Figure 4E) and human islets (Figure 4F). Furthermore, E2F inhibition blunted the increase in β cell proliferation induced by serum from S961-treated animals in both mouse and human islets (Figures 4E and 4F). Taken together with the data from the cell lines, these results suggested that circulating factors induced by the systemic effects of S961 enhance the proliferative capacity of both mouse and human β cells that require E2F1.

Adipocyte-derived humoral factors mediate S961-induced β cell proliferation

Among β cell growth factors, serpin family B member 1 (Serpinb1) was identified in the liver-specific IR KO (LIRKO) mouse, a model of chronic insulin resistance (El Ouaamari et al., 2016), while insulin-like growth factor binding protein 1 (Igfbp1) was identified using a genetic screen of zebrafish islets to potentiate *trans*-differentiation of α into β cells (Lu et al., 2016). In the current study, hepatic gene expression of Serpinb1 and Igfbp1 were both increased in response to S961 treatment, suggesting that these two circulating factors could contribute to the increase in β cell mass (Figure S5). However, to specifically examine the contribution of factors from specific metabolic tissues during acute insulin resistance, we examined the liver and adipose tissue, which are known to be associated with enhanced β cell proliferation during states of chronic systemic insulin resistance (Bluher et al., 2002; El Ouaamari et al., 2013; Michael et al., 2000). To this end, we independently co-cultured freshly isolated islets from vehicle-treated mice with either primary hepatocytes or primary adipocytes harvested from S961-treated mice (Figure 5A). Co-culture with hepatocytes increased β cell replication in islets equally between S961-treated mice and vehicle-treated mice (Figure 5B). Inhibition of E2F attenuated the increase in EdU incorporation induced by hepatocyte co-cultivation (Figure 5B). On the other hand, and interestingly, co-culture with adipocytes from S961-treated mice enhanced β cell proliferation even further compared with adipocytes from normal saline (Ns)-treated mice (Figure 5C), and the proliferative effects were attenuated in the presence of the E2F inhibitor HLM006474 (Figure 5C). These results argue for the existence of a humoral factor(s) derived from adipocytes that is able to enhance β cell growth.

Since a recent report implicated the Fabkin complex from fat to reduce β cell mass (Prentice et al., 2021), we explored its expression in adipose tissue. The expression of fatty acid-binding protein 4 (FABP1) and nucleoside diphosphate kinase-a (NDPK-a), but not adenosine kinase (ADK), were increased in adipose tissue from S961-treated mice compared with that from saline-treated mice (Figure S6). Thus, it is unlikely that the Fabkin complex is involved in the increased β cell replication by S961-treated adipose tissue.

DISCUSSION

Pancreatic β cells exhibit striking plasticity *in vivo* in response to both acute and chronic states of insulin resistance. When these compensatory responses are dysregulated, β cell failure ensues and triggers the development of diabetes. It is possible that an increase in functional β cell volume could be achieved by harnessing these adaptive properties of the insulin secreting cells as one potential therapeutic approach to treat diabetes. Pharmacological induction of acute insulin resistance with S961 or OSI-906 has been reported to lead to hyperinsulinemia that is accompanied by a marked increase in β cell numbers (Shirakawa et al., 2014, 2017b), suggesting the feasibility of augmenting functional insulin-secreting β cell mass. Here, we report that a sensing of the reduced insulin signaling in adipocytes acts as a potential trigger to enhance β cell multiplication via humoral factor(s) that requires the transcription factor E2F1.

Our studies suggest pathways that are independent of insulin and glucose, each of which have been reported to promote β cell growth. First, although IRs are involved in the adaptive β cell expansion in mice that are chronically insulin resistant in peripheral issues (Okada et al., 2007), the observation that βIRKO mice have the ability to show a significant increase in β cell mass and proliferation in response to antagonists of the insulin/IGF1Rs (e.g., S961 or OSI-906) in an acute setting suggests activation of reserve pathways that are independent of IR/IGF-1R signaling. This notion is further supported by the comparable β cell growth in OSI-906-treated IRS-2-deficient mice and by accumulation of FoxO1 in the nucleus after treatment with S961 in both control and βIRKO animals and the absence of increase in expression of insulin signaling-related genes including IR, IRS-2, and cyclin D2 in islets obtained from S961-treated mice. Thus, acute insulin resistance facilitated β cell replication in an IR/IGF1R-independent fashion. Second, glucose is known to activate proliferation pathways in β cells (Stamateris et al., 2016). However, some studies argue for effects independent of glucose (Okada et al., 2007; Togashi et al., 2014). For example, we previously demonstrated that β cell proliferation persisted in OSI-906-treated mice despite normalization of blood glucose levels by the sodium glucose co-transporter (SGLT)-2 inhibitor (Shirakawa et al., 2020). In our current study, the downregulation of insulin signaling and nuclear export of FoxO1, which is the opposite of the effects observed in glucose-induced proliferation (Terauchi et al., 2007), indicates that it is unlikely that hyperglycemia principally triggers β cell proliferation.

The CENP-A pathway in conjunction with FoxM1 and PLK1 is essential for the adaptive β cell proliferation downstream of the insulin signaling network (Shirakawa et al., 2017b). We previously reported that β cell-specific CENP-A KO mice exhibit impaired β cell proliferation after injection with S961 (Shirakawa et al., 2017b). However, the upregulation

of CENP-A signaling and G2/M phase-related cell-cycle genes in βIRKO mice treated with S961 reported in the current study suggests IR-independent mechanism(s) can also activate CENP-A. It is notable that the FoxM1/PLK1/CENP-A signaling is known to be activated during β cell proliferation induced by parasympathetic nerve activation (Yamamoto et al., 2017). Whether a similar neuronal relay signaling pathway is activated in β cells in response to S961 treatment requires additional investigation.

The identification of E2F1 in the MsigDB TFT gene datasets as a transcriptional regulator of β cell proliferation was validated by using an inhibitor that abolished further replication induced by S961. Although previous studies have shown that E2F1 plays a crucial role in β cell proliferation (Fajas et al., 2004; Grouwels et al., 2010) and modulates insulin secretion by the transcriptional regulation of Kir 6.2, a KATP channel component, via the insulin signaling pathway (Annicotte et al., 2009), little is known about the relevance of E2F1 in the β cell growth response to insulin resistance. The ability of S961 to induce β cell proliferation in our model suggests that the induction of E2F1 occurs by an insulin signaling-independent mechanism.

Since the E2F inhibitor attenuated the expression of FoxM1, PLK1, and CENP-A in islets from S961-treated mice, E2F1 could be acting upstream of FoxM1 to modulate β cell proliferation. p53 and ataxia telangiectasia mutated (ATM) reportedly increased FoxM1 expression through E2F1 in epirubicin-resistant MCF-7 breast carcinoma cells (Millour et al., 2011). The p38 and mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MAPKAPK2) pathway also enhanced E2F1-induced FoxM1 expression in MCF-7 cells (de Olano et al., 2012). In soft tissue sarcoma tumorigenesis, MAPK-interacting serine/threonine-protein kinase 1 and 2 (MNK1/2) upregulated E2F1, FOXM1, and WEE1 expression (Ke et al., 2021). Considering their roles in regulating cell proliferation and survival, it is possible that p53 or MAPK signaling pathways contribute to the S961-induced E2F1 recruitment to replicate β cells.

We and others have reported the identification of circulating factors contributing to β cell proliferation in diverse models (Dirice et al., 2014; El Ouaamari et al., 2013; Fernandez-Ruiz et al., 2020; Flier et al., 2001; Kondegowda et al., 2015; Shirakawa et al., 2017a, 2020). Furthermore, a combination of different agents, GLP-1 receptor agonists, and DRYK1A inhibitors are able to promote effective β cell replication in human islets (Ackeifi et al., 2020). In the current study, although the expression of SerpinB1 in the liver was increased after S961 injection, the enhanced proliferation observed with co-culture of islets with adipocytes suggests that factors in addition to SerpinB1 likely promote β cell replication in response to the S961-induced acute insulin resistance. It is important to recognize that numerous humoral factors including apolipoproteins, signaling lipids, inflammatory cytokines, or microRNAs (miRNAs) in exosomes that are secreted from adipocytes all have the potential to regulate diverse aspects of β cell biology (Basile et al., 2019; Shirakawa and Kulkarni, 2016).

While the adipokine adipsin has been reported to enhance insulin secretion and protect β cells from apoptosis or dedifferentiation, it did not alter β cell proliferation (Gomez-Banoy et al., 2019; Lo et al., 2014). Among other fat-associated proteins, Fabkin, the FABP4-ADK-

NDPK complex in adipocytes, has been reported to decrease β cell mass, possibly by decreasing proliferation or increasing apoptosis (Prentice et al., 2021). However, in our study, the expression of genes that constitute the Fabkin complex were increased in fat tissue of S961-treated mice despite the increase in β cell mass and proliferation and thus ruling out its direct involvement. Thus, while our studies point to adipokines as part of a cocktail of factors that can promote β cell growth, detailed analyses using independent tissue-specific KOs of SerpinB1, IGFBP1, Fabkin, or their combinations are required to explore their specific contributions.

In summary, we propose a link between fat and islets in the β cell proliferation evident in states of acute insulin resistance. Systemic inhibition of IR signaling by S961 facilitates the secretion of humoral factors from adipose tissue, leading to enhanced E2F1 expression in β cells via an IR/insulin-independent signaling pathway. These observations point to adipocytes and E2F1 signaling in β cells as potential targets to compensate for the β cell loss in patients with diabetes.

Limitations of the study

There are limitations to this study. Since HLM006474 is not a specific inhibitor for E2F1, it is possible that multiple E2F members are involved in the regulation of β cell replication even though a requirement for E2F1 is supported by knockdown experiments. Visceral adipose tissue, used for co-culture in this study, has no direct drainage into the pancreas, and a putative fat-derived factor should engage systemic circulation to reach β cells. Studies comparing the effects of visceral versus subcutaneous adipose tissue are warranted. Furthermore, since most humoral factors in fat tissue are derived from the stromal vascular fraction (SVF), experiments using a co-culture system with adipose tissue explants or SVF using an integrated approach such as lipidomics and/or metabolomics coupled with exosome analyses would be informative.

We used the βIRKO model to allow comparing with our previous results of impaired β cell proliferation in response to chronic insulin resistance (Kulkarni et al., 1999; Okada et al., 2007). However, given that RIP-Cre mice reportedly express a human growth hormone (hGH) minigene in islets with confounding effects on β cell replication (Baan et al., 2015; Brouwers et al., 2014), the use of the Ins1-Cre knockin mouse model will allow clarifying these issues (Thorens et al., 2015). We have used IR-floxed, but not RIP-Cre, mice as controls. Considering potential effects of ectopic Cre expression in the RIP-Cre mouse (Wicksteed et al., 2010), future studies should compare with Cre-only mice to validate our findings. Although we employed *in vitro* co-culture experiments with serum, hepatocytes, or adipocytes to model interactions via humoral factors, one has to also consider non-secretory components in media or islet-derived factors that can impact β cell replication. Parabiosis coupled with transplantation studies might reveal direct effects of circulating factors.

STAR★METHODS

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Rohit N. Kulkarni MD PhD. (rohit.kulkarni@joslin.harvard.edu).

Materials availability—The cell lines generated in this study are available from the lead contact upon request without restriction.

Data and code availability

- Microarray data have been shown in Table S1. Original western blot images are available in Data S1.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice—The βIRKO mice and littermate control insulin-receptor-floxed mice on a C57B6 background (8–11-week-old, male) were obtained as described (Kulkarni et al., 1999) and housed in pathogen-free facilities on a 12 h light–dark cycle at the Animal Care Facility of Joslin Diabetes Center, Boston, MA, USA. The IRS-2 KO mice and littermate control wild type mice on a C57B6 background (8-week-old, male) were obtained as described (Kubota et al., 2000) and housed in pathogen-free facilities on a 12 h light–dark cycle at the Animal Facility of Yokohama City University, Yokohama, Japan. Mice were injected with BrdU intraperitoneally (100 mg/kg body weight) 6 hours prior to animal sacrifice for immunostaining of the pancreas. All protocols were approved by the Institutional Animal Care and Use Committee of the Joslin Diabetes Center and the Yokohama City University Institutional Animal Care and Use Committee (IACUC) (Permit Number: F-A-14–041). This study was conducted in accordance with National Institutes of Health guidelines and the guidelines of the Animal Care Committee of Yokohama City University. All animals were housed in the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited Animal Facility at Joslin Diabetes Center and Yokohama City University.

Cell lines—β-cell lines from control, IRS1KO, IRS2KO, or βIRKO mice were generated in our laboratory as described previously (Assmann et al., 2009; Kulkarni et al., 1999). CENP-A knockdown and control scramble shRNA transduced cells were generated in a previous study of our laboratory (Shirakawa et al., 2017b). All β-cell lines were from male mice. The control cells were used between passages 14 to 26, IRS1KO and IRS2KO cells between passages 11 to 22 and βIRKO cells between passages 9 to 21. Cells were maintained in DMEM media containing 25 mM glucose, supplemented with 10% FBS. Experiments were performed using 80–90% confluent cells.

Human islet studies—Human islets (3 males and 4 females, 22–52 years old) were obtained from the Clinical Islet Laboratory and Clinical Islet Transplant Program of University of Alberta or the Alberta Diabetes Institute IsletCore of the University of Alberta. Ethics approval and informed consent from donors or families were obtained in each institute. Details of human islets are described in Table S2. All studies and protocols used were approved by Yokohama City University Ethics Board (approval B171100025) and the Joslin Diabetes Center's Committee on Human Studies (approval CHS#5–05). Upon receipt, islets were cultured overnight in Miami Media #1A (Cellgro). The islets were embedded in agarose and used for immunostaining studies.

METHOD DETAILS

Mouse studies—S961 was received as a gift from Dr. Lauge Schä ffer from Novo Nordisk (Schaffer et al., 2008). For S961 studies, 8–11-week-old mice (n = 5–6 per group) were anesthetized by intraperitoneal injection 0.3 mg/kg of medetomidine hydrochloride (Kyoritsu Seiyaku Co., Japan), 4.0 mg/kg of midazolam (Maruishi Pharmaceutical Co., Japan), and 5.0 mg/kg of butorphanol tartrate (Meiji Seika Pharma Co., Japan) (5 μL/g body weight) and infused with normal saline (Ns)/PBS alone or Ns/PBS with the insulin receptor antagonist S961 at the dose of 10 nmoles/week (1.43 nmoles/day) for 7–9 days. Infusion was carried out using osmotic pumps (ALZET 2001) implanted subcutaneously. HLM006474 (10 mg/mL) or vehicle (2.5% vol/vol dimethylsulphoxide, 28% wt/vol 2-hydroxypropyl-β-cyclodextrin, 10% vol/vol PEG400 in distilled water) were injected intraperitoneally (5 μL/g body weight) once a day between 08:00 and 09:00 hours. For OSI-906 studies in βIRKO mice, 8–10-week-old bIRKO mice and littermate floxed mice were infused with vehicle (30% PEG400, 0.5%Tween 80, 5% propylene glycol) alone or vehicle with the insulin recptor and IGF1 receptor dual inhibitor OSI-906 at the dose of 10.5 mg/week (1.5 mg/day) using osmotic pumps (ALZET 2001) for 8 days. For OSI-906 studies in IRS2 KO mice, 8-week-old IRS-2 KO mice and littermate wild type mice were given 10 μL/g weight of either the vehicle (30% [wt/vol.] Solutol HS-15; BASF, Ludwigshafen am Rhein, Germany) or OSI-906 (45 mg/kg BW/day or 15 mg/kg BW/day) by gavage for 7 days, as previously described (Shirakawa et al., 2020), between 08:00pm and 09:00pm. Solutol HS-15 was dissolved in water at 30% w/v. The powder of OSI-906 was dissolved in 30% Solutol at a concentration of 4.5 mg/mL. We confirmed that above concentrations of S961 and OSI-906 were appropriate for the assessment of β-cell proliferation without reduction in body weight and enough to suppress IR signaling in the liver, adipose tissue, or skeletal muscle, in 10-week-old C57Bl6 mice. The blood glucose levels were determined using a Contour blood glucose meter (Bayer Health Care) or a Glutest Neo Super (Sanwa Chemical Co., Tokyo, Japan). The plasma insulin levels were measured with an insulin ELISA kit (Crystal Chem Inc. or Morinaga). An insulin tolerance test was performed by intraperitoneal injection with human insulin (0.75 mU/g body weight).

Immunostaining studies—More than five pancreatic tissue sections from each animal were analyzed after fixation and paraffin embedding. The sections were immunostained with antibodies to insulin (Abcam, ab7842), biotinylated secondary antibody with a VECTASTAIN Elite ABC Kit (Vector Laboratories), and a DAB Substrate Kit (Vector Laboratories) to examine β-cell mass using bright-field microscopy. The proportion of the

area of pancreatic tissue occupied by the β-cells was calculated using Image J software. β-cell mass was estimated for each animal by determining the proportion of the β-cell area per animal multiplied by the pancreatic weight. Mouse pancreases were analyzed by immunostaining using anti-insulin (Abcam, ab7842), anti-BrdU (Dako, m0744), anti-mouse-CENP-A (Cell Signaling #2048), or anti-FoxO1 (Cell Signaling #2880) antibodies for immunofluorescence. Cell counting was manually performed in a blinded fashion by a single observer. BrdU+ β-cells were assessed by confocal microscopy (LSM-7 DUO, Carl Zeiss, or Fluo View FV1000-D, Olympus). Insulin+ cells showing nuclear DAPI staining were considered as β-cells. Insulin+ cells showing nuclear colocalized staining for DAPI+ and BrdU+ were counted as proliferating β-cells. At least 1000 β-cells per mouse were analyzed. The fluorescence levels of FoxO1 and CENP-A were determined using Image J software. All images, which were acquired under the same condition, were converted to gray scale. Then, we randomly selected 10 regions of nuclei or cytoplasm of separate islets in each group and measured fluorescence levels. The fluorescent intensity was normalized by the mean background fluorescence levels.

RNA isolation and quantitative RT-PCR—Total RNA was extracted using RNeasy Mini Kit (QIAGEN). One μg RNA was reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed in an ABI 7900HT system, using SYBR Green Supermix (Bio-Rad). GAPDH was used as an internal control. Primers described in Table S2 were used for amplification.

Microarray analysis—10 nM S961 in PBS or PBS was loaded into Alzet osmotic pump 2001 and implanted subcutaneously at the back C57BL/6J male mice (average body weight of 25 gram). All mice were treated with PBS or S961 for 7 days and pancreatic islets were isolated using standard procedure (Shirakawa et al., 2013). Total RNA from pancreatic islets were extracted using TRIzol reagent (Invitrogen) and the contaminating genome DNA was removed using Qiagen RNeasy mini kit. For microarray analysis, the total RNA was amplified and biotin labeled using Illumina TotalPrep RNA Amplication kit (Ambion). The cRNA was analyzed by in house Illumina BeadArray Reader and quantified using Illumina BeadStudio.

Mouse islet studies—Islets were isolated from 8–12 weeks old wild type C57BL/6 male mice using intraductal collagenase technique (Shirakawa et al., 2013). Islets were handpicked and cultured overnight in RPMI 1640 media containing 5mM glucose and 10% fetal bovine serum (FBS).

Analysis of cell viability and proliferation—For a modified MTT assay, cells were plated in 96-well plates @ 10^4 cells in each well. The cell viability was determined using the CellTiter 96 Non-Radioactive Cell Proliferation Assay (Promega, G4001) according to the manufacturer's instructions. For EdU incorporation assay, cells or islets were treated with 10 μm EdU (4h for cells, 24hr for mouse islets, and 48hr for human islets) and stained with Click-iT Plus EdU Alexa Fluor 488 or 594 Imaging Kit (Thermo Fisher, C10637, C10639). Insulin+ cells showing nuclear colocalized staining for DAPI+ and ErdU+ were counted as proliferating β-cells. The proliferating β-cells were measured for 1,000 or more

insulin-positive islet cells per mouse or sample of β-cell lines, or for 12,000 or more β-cells in human islets per donor in each of the groups (1,000–1,200 cells in Figure 4D, 1,200–1,500 β-cells in Figures 4E, 5B, 5C, and 12,000–21,000 β-cells in Figure 4F). Proliferation was determined to be 0% when no EdU-positive β-cells were found in both >100 human islets and >12000 insulin-positive β-cells in >5 independent sections.

Lentiviral transduction—Lentiviral particles for murine E2F1 short hairpin RNA (shRNA) (sc-29297-V) and control scramble shRNA (sc-108080) were purchased from Santa Cruz. Cells were infected by adding the lentiviral particles to the culture with polybrene (sc-134220). For generating stable cell lines, cells were treated with 4 mg/mL of puromycin 48 hours after the transduction and were maintained in selection media for more than 14 days. We generated two separate stable cell lines in each group.

Western blotting—Cells were solubilized in M-PER lysis buffer (Thermo Scientific #78501) with protease inhibitors and phosphatase inhibitors (Sigma P8340, P5726, P0044), and protein concentration was measured using a BCA protein assay kit (Pierce). The extracts were subjected to western blotting with primary antibodies overnight at 4°C. Mouse E2F1 (ab179445) and α-tubulin (ab7291) are from Abcam. Densitometry was performed using Image J software.

Cell and islet culture experiments—The cells or islets were treated with serum from vehicle-, S961-treated mice on day 7 (20% v/v). HLM006474 were added to culture media at the concentration of 10 μM when culture or coculture were started. For hepatocytes isolation, mice were anesthetized with 0.3 mg/kg medetomidine, 4.0 mg/kg midazolam and 5.0 mg/kg butorphanol; the portal vein was cannulated, and the liver was perfused with Liver Perfusion Medium (1X) (Thermo Fisher Scientific, 17703038) and digested with Liver Digest Medium (Thermo Fisher Scientific, 17703034) according to manufacturer instructions. Hepatocytes were washed twice in Hepatocyte Wash Medium (Invitrogen, catalog #17704024). The hepatocytes were seeded in collagen-coated 12-well plates (BD BioCoat) at a density of $3 \times 3\ 10^5$ cells/well in 25 mM glucose-containing DMEM and 10% FBS (v/v). Sixteen hours later, hepatocytes were cultured with 100 islets plated in a Netwell insert with a 74-μm mesh size polyester membrane (Corning, Inc.) for 24 hours in 50% RPMI1640 and 50% DMEM (v/v) medium containing 5.5 mM glucose and 10% FBS. Adipocytes were prepared by collagenase digestion (collagenase type I, Thermo Fisher Scientific, 17100017) of epididymal fat tissue, as described previously (Shirakawa et al., 2011). Epididymal fat tissue from each mouse are washed, minced with dissecting scissors to very fine pieces, and digested with collagenase at 37°C for 60 min. After centrifugation, adipocytes fraction was isolated by flotation from stromal vascular fraction (SVF) pellet fraction. Adipocytes (from 25 mg of epididymal fat) were co-cultured with 100 islets above the co-culture Netwell insert for 24 hours in 50% RPMI1640 and 50% DMEM (v/v) medium containing 5.5 mM glucose and 10% FBS.

Inclusion and exclusion criteria—No inclusion and exclusion criteria were applied to the data collection or the subject selection in this study.

QUANTIFICATION AND STATISTICAL ANALYSIS

All experiments were independently repeated at least three times. Results are shown as means ± SE. Statistical analyses were conducted using Prism 7 software (GraphPad Software). Gaussian distribution was determined by using a D'Agostino-Pearson test. Statistical comparisons between groups were analyzed for significance by an unpaired two-tailed Student's t-test and a one-way analysis of variance (ANOVA) with post-hoc Tukey tests for a parametric test, or a Mann-Whitney U test for a nonparametric test. Differences are considered significant at $p < 0.05$. The exact values of n (depending on the experiment referring to number of animals, donors, or number of independent measurements), statistical measures (mean ± SE) and statistical significance are reported in the figures and in the figure legends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Insulin receptor-independent pathways promote S961-induced β cell multiplication
- β cell proliferation by S961 is mediated via E2F1 and CENP-A
- Serum from S961-treated mice enhance β cell replication in mouse and human islets
- Adipocytes isolated from S961-treated mice enable β cells to duplicate

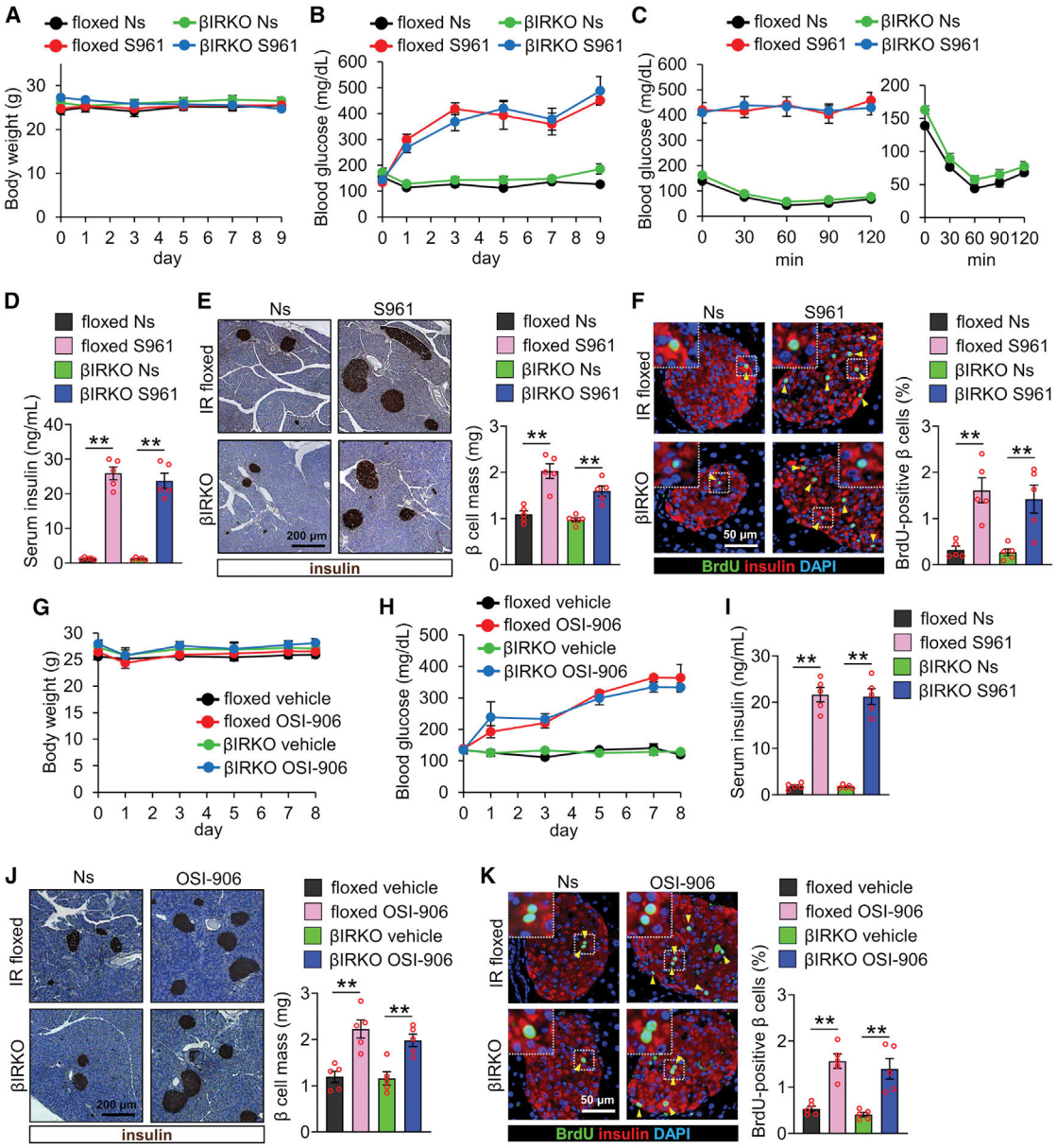

Figure 1. Insulin receptor is dispensable for β cell proliferation induced by S961

(A–E) Experiments were performed on floxed and βIRKO mice administrated S961 (10 nM/week) or normal saline (Ns) by osmotic pump for 9 days.

(A) Body weight gain (n = 5 mice/group).

(B) Fed blood glucose levels (n = 5 mice/group).

(C) Plasma glucose levels during insulin tolerance test (n = 5 mice/group).

(D) Serum insulin levels (n = 5 mice/group).

(E) Left: representative pancreatic sections. Right: β cell mass. **p < 0.01 (n = 5 mice/group).

(F) Left: representative pancreatic sections. Insulin is stained red, nuclei are stained blue (DAPI), and BrdU+ nuclei are stained green. Yellow arrowheads indicate insulin+ and BrdU+ cells. Right: number of BrdU+ β cells in the islets. **p < 0.01 (n = 5 mice/group).

(G–K) Experiments were performed on floxed and βIRKO mice administrated OSI-906 (10.5 mg/week) or vehicle by osmotic pump for 8 days.

(G) Body weight gain (n = 5 mice/group).

(H) Fed blood glucose levels (n = 5 mice/group).

(I) Serum insulin levels (n = 5 mice/group).

(J) Left: representative pancreatic sections. Right: β cell mass. **p < 0.01 (n = 5 mice/group).

(K) Left: representative pancreatic sections. Insulin is stained red, nuclei are stained blue (DAPI), and BrdU+ nuclei are stained green. Yellow arrowheads indicate insulin+ and BrdU+ cells. Right: number of BrdU+ β cells in the islets. **p < 0.01 (n = 5 mice/group). All data from three independent experiments are represented as mean ± SEM. A one-way ANOVA was performed.

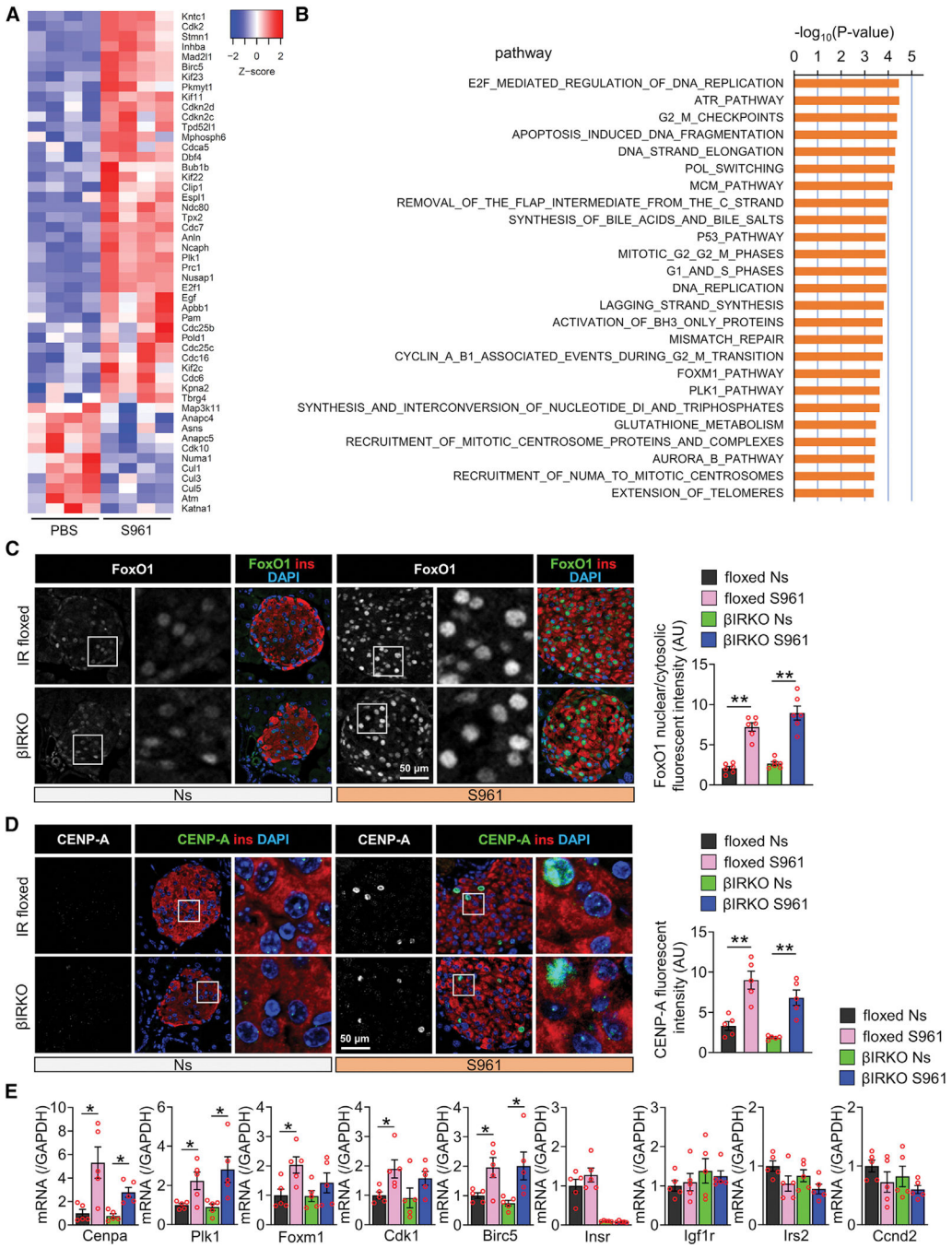

Figure 2. CENP-A signal is activated by S961 injection in islets independent of insulin signal

(A and B) Experiments were performed on C57BL/6J male mice (average body weight of 25 g) administrated S961 (10 nM/week) or vehicle (PBS) by osmotic pump for 7 days.

(A) Heatmap of upregulated and downregulated genes among mitotic cell-cycle genes in islets of S961-treated mice compared with those of PBS-treated mice (n = 4 mice/group).

(B) Pathway analysis of significantly upregulated genes in islets of S961-treated mice compared with those of PBS-treated mice.

(C–E) Experiments were performed on floxed and βIRKO mice administrated S961 (10 nM/week) or vehicle (Ns) by osmotic pump for 9 days.

(C) Left: representative images immunostained for FoxO1, insulin, and DAPI from endocrine pancreas of floxed and βIRKO mice after treatment with S961 or vehicle (Ns) for 9 days. Right: ratio of nuclear/cytosolic FoxO1 fluorescence in insulin+ cells. **$p < 0.01$ ($n = 6$ mice/group).

(D) Left: representative images immunostained for CENP-A, insulin, and DAPI from endocrine pancreas of floxed and βIRKO mice after treatment with S961 or vehicle (Ns) for 9 days. Right: quantification of CENP-A fluorescence in insulin+ cells. **$p < 0.01$ ($n = 5$ mice/group).

(E) Relative mRNA expression levels of indicated genes normalized to GAPDH and plotted as fold change versus control (floxed Ns) in the islets. *$p < 0.05$ ($n = 5$ mice/group).

(C–E) All data from three or four independent experiments are represented as mean ± SEM. A one-way ANOVA was performed.

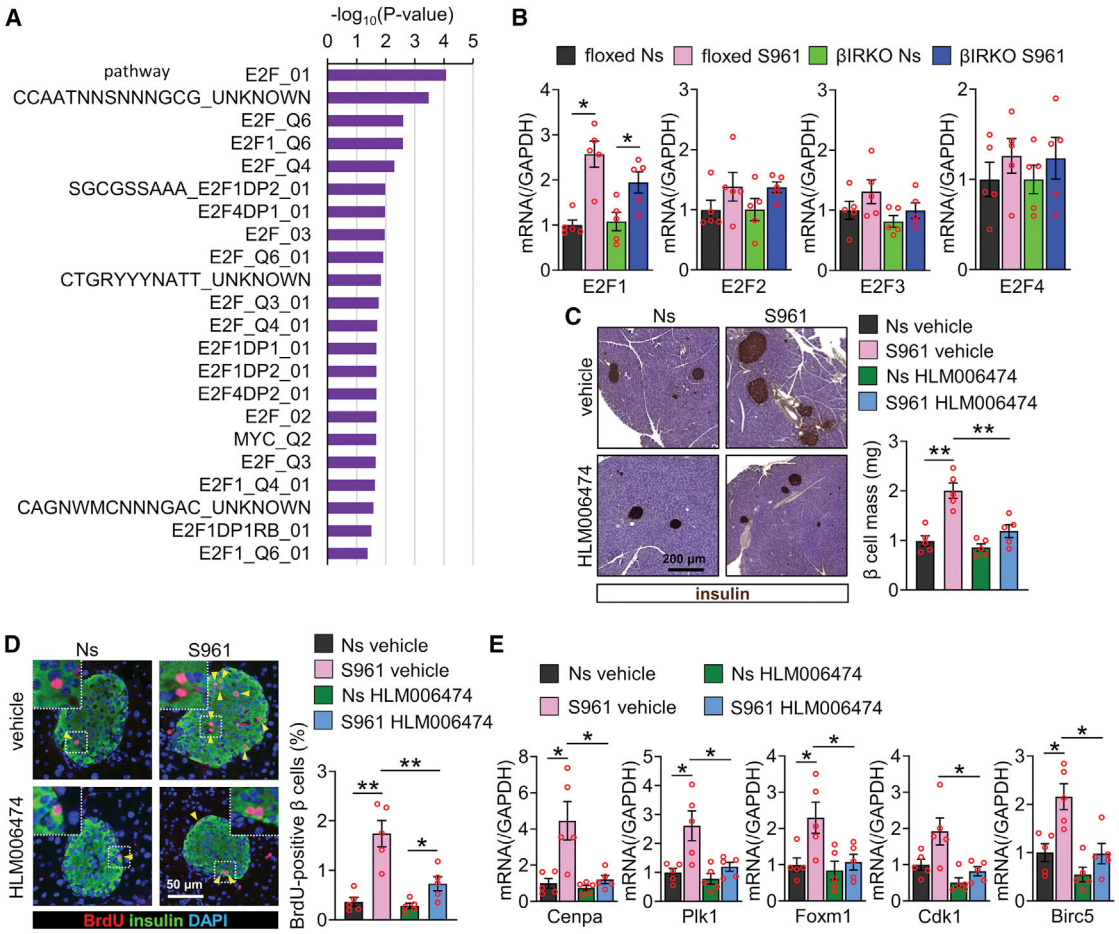

Figure 3. E2F family mediates β cell proliferation induced by IR inhibition with S961

(A and B) Experiments were performed on C57BL/6J male mice (average body weight of 25 g) administrated S961 (10 nM/week) or vehicle (PBS) by osmotic pump for 7 days.

(A) Transcription factor target (TFT) gene sets of upregulated genes in islets from S961-treated mice compared with those from PBS-treated mice in Molecular Signatures Database (MsigDB).

(B) Relative mRNA expression levels of indicated genes normalized to GAPDH and plotted as fold change versus control in the islets. *$p < 0.05$ (n = 5 mice/group).

(C–E) Experiments were performed on C57B6 mice administrated S961 (10 nM/week) or vehicle (Ns) by osmotic pump, concomitantly administered 2 mg of HLM006474 or vehicle intraperitoneally once a day, for 7 days.

(C) Left: representative pancreatic sections. Right: β cell mass. **$p < 0.01$ (n = 5 mice/group).

(D) Left: representative pancreatic sections. Insulin is stained green, nuclei are stained blue (DAPI), and BrdU+ nuclei are stained red. Yellow arrowheads indicate insulin+ and BrdU+ cells. Right: number of BrdU+ β cells in the islets. *$p < 0.05$, **$p < 0.01$ (n = 5 mice/group).

(E) Relative mRNA expression levels of indicated genes normalized to GAPDH and plotted as fold change versus control in the islets. *$p < 0.05$ (n = 5 mice/group).

(B–E) All data from three independent experiments are represented as mean ± SEM. A one-way ANOVA was performed.

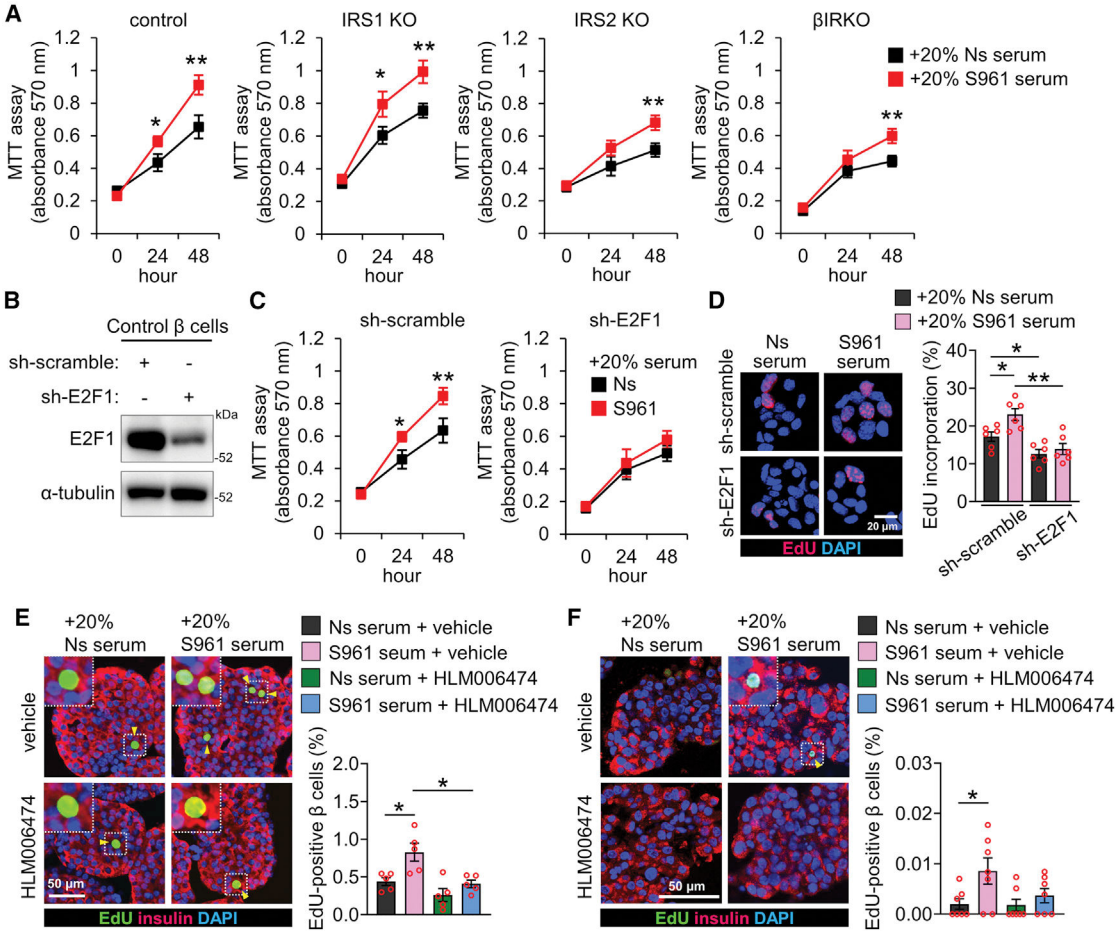

Figure 4. Humoral factors in S961-treated mice evoked β cell replication via E2F1

(A and C–F) The sera were collected from mice at day 7 of treatment. β cells, mouse islets, or human islets were treated with 20% serum from S-961- or Ns-treated C57BL/6J male mice.

(A) MTT assay (absorbance at 570 nm) in control, IRS1KO, IRS2KO, and βIRKO β cells. *p < 0.05, **p < 0.01 (n = 6 biological replicates).

(B) Western blot of indicated proteins in scramble- or E2F1-knockdown control β cells.

(C) MTT assay in scramble- or E2F1-knockdown control β cells. *p < 0.05, **p < 0.01 (n = 6 biological replicates).

(D) Left: representative images of indicated β cells. Nuclei are stained blue, and EdU+ nuclei are stained red. Right: number of ErdU+ β cells. *p < 0.05, **p < 0.01 (n = 6 biological replicates).

(E) Mouse islets were incubated with 20% serum from indicated mice in the presence or absence of HLM006474 for 24 h. Left: representative images of islets. Insulin is stained red, nuclei are stained blue, and EdU+ nuclei are stained green. Yellow arrowheads indicate insulin+ and EdU+ cells. Right: number of ErdU+ β cells in the islets. *p < 0.05 (n = 5 mice/group).

(F) Human islets from non-diabetes donors were incubated with 20% serum from indicated mice in the presence or absence of HLM006474 for 48 h. Yellow arrowheads indicate

insulin+ and EdU+ cells. Left: representative images of islets. Insulin is stained red, nuclei are stained blue, and EdU+ nuclei are stained green. Right: number of ErdU+ β cells in the islets. *$p < 0.05$ ($n = 7$ donors).

(A and C–F) All data from three or more independent experiments are represented as mean ± SEM. An unpaired two-tailed Student's t test (A and C), a one-way ANOVA (D and E), and a Mann-Whitney U test (F) were performed. (B) Data are representative of three independent experiments.

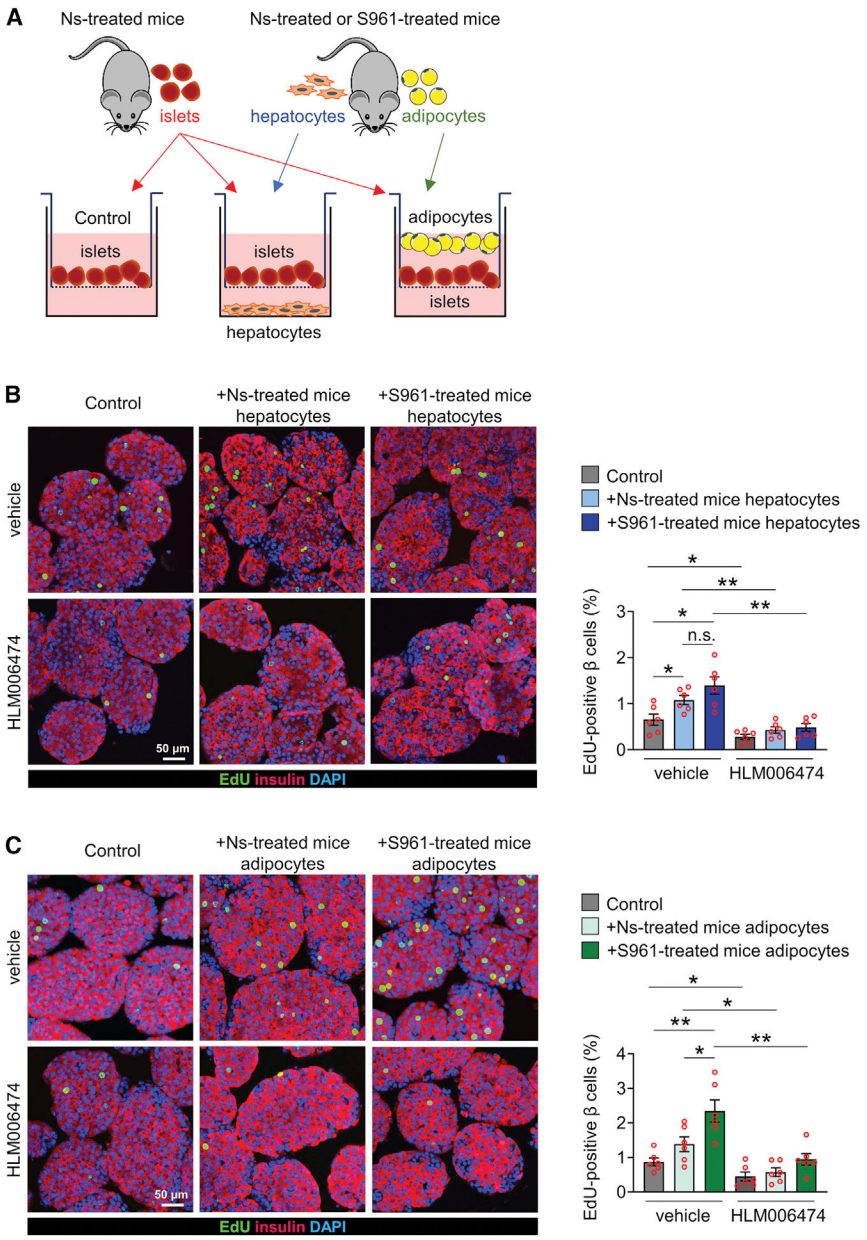

Figure 5. Effects of co-culture of hepatocytes or adipocytes on S961-induced β cell proliferation

(A) Illustration of co-cultured islets and hepatocytes or adipocytes. Primary cells were harvested from S-961- or Ns-treated C57BL/6J male mice.

(B) Left: representative images of islets co-cultured with hepatocytes. Insulin is stained red, nuclei are stained blue (DAPI), and ErdU+ nuclei are stained green. Right: number of ErdU+ β cells in the islets. n.s., not significant, *p < 0.05, **p < 0.01 (n = 5 mice/group).

(C) Left: representative images of islets co-cultured with adipocytes. Insulin is stained red, nuclei are stained blue (DAPI), and ErdU+ nuclei are stained green. Right: number of ErdU+ β cells in the islets. *p < 0.05, **p < 0.01 (n = 5 mice/group).

(B and C) All data from five independent experiments are represented as mean ± SEM. A one-way ANOVA was performed.

Table 1.

Upregulated and downregulated genes in islets from S961-treated mice versus islets from vehicle-treated mice

Symbol	Description	Fold change	p
Cenpa	centromere protein A	5.60	0.0000000005
Pbk	PDZ-binding kinase/MAPKK-like protein kinase	4.70	0.0000000443
Prc1	protein regulator of cytokinesis 1/anaphase spindle elongation 1 homolog	3.35	0.00000000226
Mcm5	minichromosome maintenance complex component 5/CDC46	2.96	0.000000012
Cdca3	cell division cycle-associated 3/trigger of mitotic entry 1 (TOME-1)	2.85	0.0000000223
Cdc20	cell division cycle 20	2.76	0.0000000302
Plk1	polo-like kinase 1	2.73	0.0000000718
Ccnb1	cyclin B1/G2/mitotic-specific cyclin B1	2.30	0.0000000295
Birc5	baculoviral IAP repeat containing 5/survivin variant 3 alpha	2.25	0.000000444
Foxm1	forkhead box M1	1.15	0.0054
Insr	insulin receptor/CD220	1.07	0.113
Igf1r	insulin-like growth factor 1 receptor	−1.18	0.00583
Ccnd2	cyclin D2/G1/S-specific cyclin D2	−1.85	0.001
Irs2	insulin receptor substrate 2	−1.58	0.000466

Table 2.

The expression of E2F family genes in islets from S961-treated mice versus islets from vehicle-treated mice

SYMBOL	Description	Fold change	p
E2f1	E2F transcription factor 1	1.97	0.000000101
E2f2	E2F transcription factor 2	1.76	0.00000128
E2f3	E2F transcription factor 3	1.15	0.0138
E2f7	E2F transcription factor 7	1.14	0.0215
E2f5	E2F transcription factor 5	−1.04	0.306
E2f6	E2F transcription factor 6	−1.07	0.193
E2f4	E2F transcription factor 4	−1.10	0.0285

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Guinea pig anti-insulin polyclonal antibody	Abcam	Cat# ab7842; RRID: AB_306130
Insulin (H-86) antibody	Santa Cruz Biotechnology	Cat# sc-9168; RRID: AB_2126540
anti-bromodeoxyuridine, anti-BrdU antibody	Dako	Cat# M0744; RRID: AB_10013660
Rabbit anti-Mouse CENP-A IF Preferred Monoclonal Antibody	Cell Signaling Technology	Cat# 2048; RRID: AB_1147629
Rabbit anti-Akt Antibody	Cell Signaling Technology	Cat# 9272, RRID:AB_329827
Rabbit anti-Phospho-Akt (Ser473) Antibody	Cell Signaling Technology	Cat# 9271, RRID:AB_329825
Rabbit anti-p44/42 MAPK (Erk1/2) Antibody	Cell Signaling Technology	Cat# 9102; RRID: AB_330744
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP(tm) Rabbit mAb antibody	Cell Signaling Technology	Cat# 4370; RRID: AB_2315112
Rabbit Anti-FoxO1 antibody	Cell Signaling Technology	Cat# 2880; RRID: AB_2106495
Rabbit Anti-E2F1 antibody	Cell Signaling Technology	Cat# 3742; RRID: AB_2096936
Alpha-tubulin antibody [DM1A]	Abcam	Cat# ab7291; RRID: AB_2241126
Donkey Anti-Goat IgG H&L (Alexa Fluor® 488)	Abcam	Cat# ab150129; RRID: AB_2687506
Alexa Fluor® 594 AffiniPure Donkey Anti-Guinea Pig IgG (H+L)	Jackson ImmunoResearch	Cat# 706–585-148; RRID: AB_2340474
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	Cat# A-21206; RRID: AB_141708
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Invitrogen	Cat# A-31572; RRID: AB_162543
Bacterial and virus strains		
Lentiviral particles for control scramble shRNA	Santa Cruz Biotechnology	Cat#sc-108080
Lentiviral particles for murine E2F1 shRNA	Santa Cruz Biotechnology	Cat#sc-34257-v
Biological samples		
Human islets, see Table S2	Alberta Islet Distribution Program, University of Alberta ADI Islet Core	N/A
Chemicals, peptides, and recombinant proteins		
DAPI, dilactate	Sigma-Aldrich	Cat#D9564
S961	Lauge Schaffer (Novo Nordisk) (Schaffer et al., 2008)	N/A
OSI-906	Med Chem express LLC	Cat#HY-10191
5-Bromo-2′-deoxyuridine	Sigma-Aldrich	Cat#B9285
Miami Medium #1A	Cellgro	Cat#98–021-CV
Final Wash/Culture Medium	Cellgro	Cat#99–785-CV
Polybrene	Santa Cruz Biotechnology	Cat#sc-134220
M-PER™ Mammalian Protein Extraction Reagent	Thermo Fisher Scientific	Cat#78501
Protease Inhibitor Cocktail	Sigma-Aldrich	Cat#P8340
Phosphatase Inhibitor Cocktail 2	Sigma-Aldrich	Cat#P5276

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Phosphatase Inhibitor Cocktail 3	Sigma-Aldrich	Cat#P0044
Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific	Cat# 23225
RNeasy Mini Kit	QIAGEN	Cat#74106
High-Capacity cDNA Reverse Transcription Kit	Thermo Fisher Scientific	Cat#4368813
SYBR® Green Supermix	Bio-Rad	Cat#1725274
VECTASTAIN® Elite® ABC HRP Kit	Vector Laboratories	Cat#PK-6101
HLM 006474	Tocris	Cat#5283
Critical commercial assays		
Ultra Sensitive Mouse Insulin ELISA Kit	Crystal Chem	Cat#90080
Contour® Blood Glucose Meter	Bayer Health Care	Cat#9545C
CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT)	Promega	Cat#G4001
Click-iT® Plus EdU Alexa Fluor® 488 Imaging Kit	Thermo Fisher Scientific	Cat#C10637
Click-iT® Plus EdU Alexa Fluor® 594 Imaging Kit	Thermo Fisher Scientific	Cat#C10639
Ultra Sensitive Mouse Insulin ELISA Kit	Morinaga Institute of Biological Science, Yokohama, Japan	Cat#MS303
Glutest Neo Super	Sanwa Chemical Co. Kanagawa, Japan	N/A
Deposited data		
Microarray data, see Table S1	This paper	N/A
Experimental models: Cell lines		
Control β-cells	Laboratory of R. N. Kulkarni (Assmann et al., 2009; Kulkarni et al., 1999)	N/A
IRS1KO β-cells	Laboratory of R. N. Kulkarni (Assmann et al., 2009; Kulkarni et al., 1999)	N/A
IRS2KO β-cells	Laboratory of R. N. Kulkarni (Assmann et al., 2009; Kulkarni et al., 1999)	N/A
β-cell-specific IR KO (βIRKO)-cells	Laboratory of R. N. Kulkarni (Assmann et al., 2009; Kulkarni et al., 1999)	N/A
sh-CENPA Control β-cells	Laboratory of Jun Shirakawa (Shirakawa et al., 2017b)	N/A
sh-E2F1 Control β-cells	This study	N/A
sh-scramble Control β-cells	This study	N/A
Experimental models: Organisms/strains		
βIRKO mouse	Laboratory of R. N. Kulkarni (Kulkarni et al., 1999)	N/A
IR-floxed mouse	Laboratory of R. N. Kulkarni (Kulkarni et al., 1999)	N/A
IRS-2 knockout mouse	Laboratory of Yasuo Terauchi (Kubota et al., 2000)	N/A
Oligonucleotides		
Primers for qPCR, see Table S3	This paper	N/A

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
ImageJ software	NIH	https://imagej.nih.gov/ij/
Prism 8 software	Graph Pad Software	https://www.graphpad.com/scientific-software/prism/
BIOREVO software	KEYENCE	https://www.keyence.co.jp/