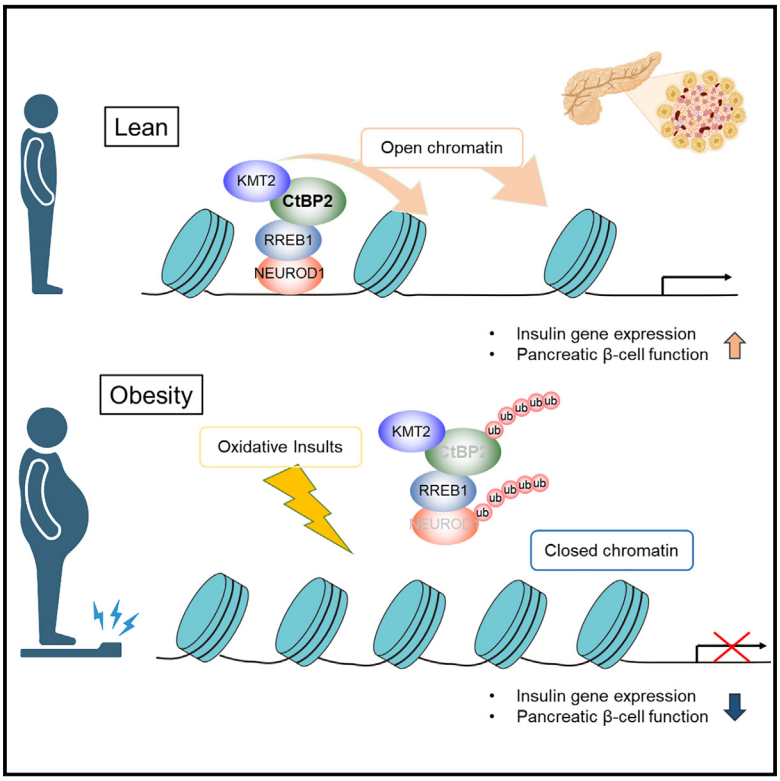
Cell Reports

Article

Loss of CtBP2 may be a mechanistic link between metabolic derangements and progressive impairment of pancreatic β cell function

Graphical abstract



Authors

Motohiro Sekiya, Yang Ma, Kenta Kainoh, ..., Seiya Mizuno, Satoru Takahashi, Hitoshi Shimano

Correspondence

msekiya@md.tsukuba.ac.jp

In brief

Sekiya et al. demonstrated that a transcriptional cofactor, CtBP2, interacts with NEUROD1 to maintain pancreatic β cell functions. CtBP2 is vulnerable to oxidative stress, and its expression is markedly decreased in obesity. Since loss of CtBP2 impairs insulin secretion, this mechanism may underlie the progressive impairment of β cell function in obesity.

Highlights

- CtBP2 coactivates key genes in the pancreatic β cells through an interaction with NEUROD1
- CtBP2 protein expression is markedly reduced in the pancreatic β cells in obesity
- CtBP2 protein undergoes polyubiquitination upon oxidative stress
- Loss of CtBP2 impairs insulin secretion, leading to glucose intolerance

Sekiya et al., 2023, Cell Reports *42*, 112914
August 29, 2023
https://doi.org/10.1016/j.celrep.2023.112914

CellPress

Cell Reports

CellPress
OPEN ACCESS

Article

Loss of CtBP2 may be a mechanistic link between metabolic derangements and progressive impairment of pancreatic β cell function

Motohiro Sekiya,[1,4,5,*] Yang Ma,[1,4] Kenta Kainoh,[1] Kenji Saito,[1] Daichi Yamazaki,[1] Tomomi Tsuyuzaki,[1] Wanpei Chen,[1] Putu Indah Paramita Adi Putri,[1] Hiroshi Ohno,[1] Takafumi Miyamoto,[1] Yoshinori Takeuchi,[1] Yuki Murayama,[1] Yoko Sugano,[1] Yoshinori Osaki,[1] Hitoshi Iwasaki,[1] Naoya Yahagi,[1] Hiroaki Suzuki,[1] Kaori Motomura,[1] Takashi Matsuzaka,[1,2] Kazuya Murata,[3] Seiya Mizuno,[3] Satoru Takahashi,[3] and Hitoshi Shimano[1]

[1]Department of Endocrinology and Metabolism, Institute of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Ibaraki, Japan

[2]Transborder Medical Research Center, University of Tsukuba, Tsukuba 305-8575, Ibaraki, Japan

[3]Laboratory Animal Resource Center in Transborder Medical Research Center, University of Tsukuba, Tsukuba 305-8575, Ibaraki, Japan

[4]These authors contributed equally

[5]Lead contact

*Correspondence: msekiya@md.tsukuba.ac.jp

https://doi.org/10.1016/j.celrep.2023.112914

SUMMARY

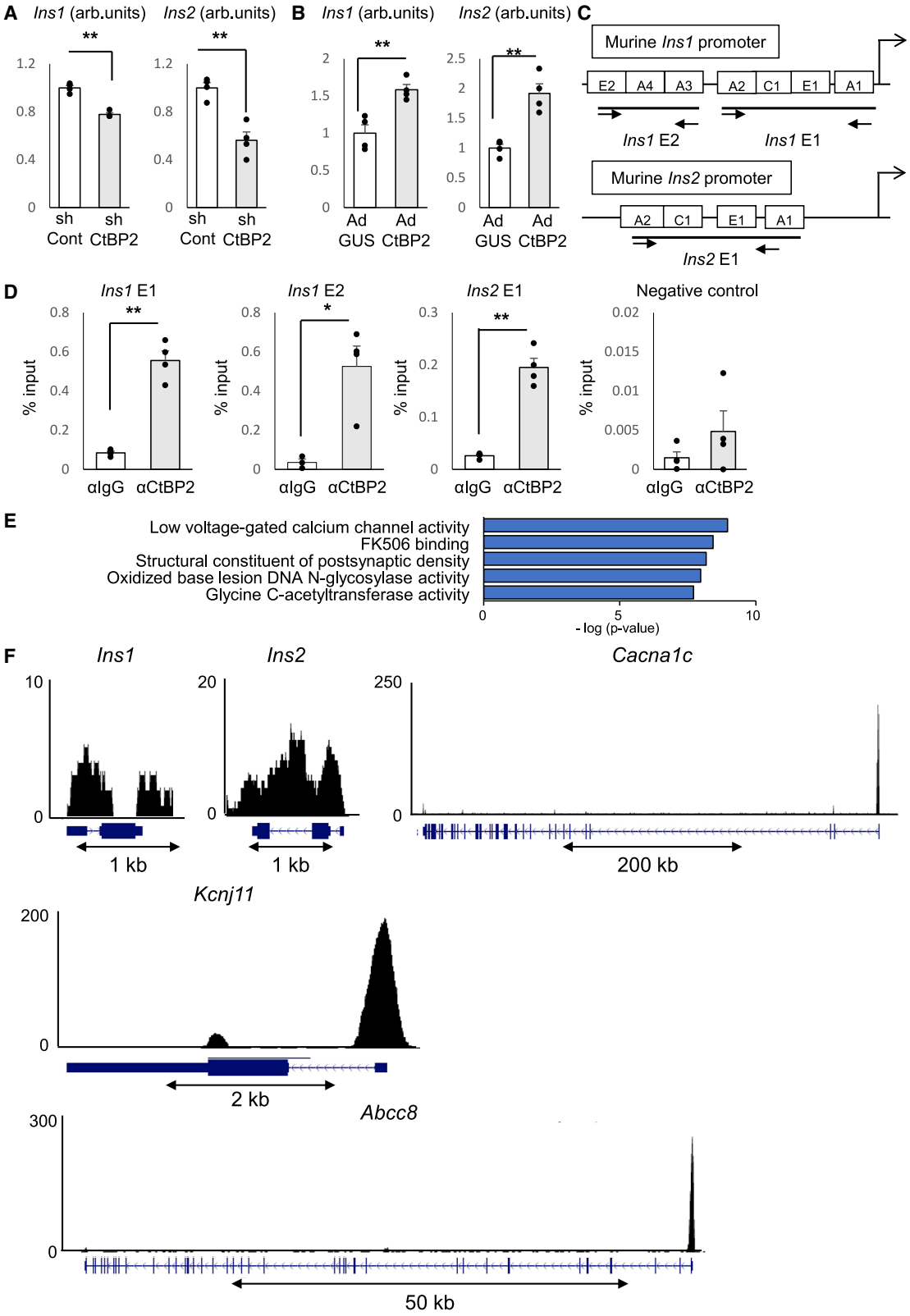
The adaptive increase in insulin secretion in early stages of obesity serves as a safeguard mechanism to maintain glucose homeostasis that cannot be sustained, and the eventual decompensation of β cells is a key event in the pathogenesis of diabetes. Here we describe a crucial system orchestrated by a transcriptional cofactor CtBP2. In cultured β cells, insulin gene expression is coactivated by CtBP2. Global genomic mapping of CtBP2 binding sites identifies a key interaction between CtBP2 and NEUROD1 through which CtBP2 decompacts chromatin in the insulin gene promoter. CtBP2 expression is diminished in pancreatic islets in multiple mouse models of obesity, as well as human obesity. Pancreatic β cell-specific CtBP2-deficient mice manifest glucose intolerance with impaired insulin secretion. Our transcriptome analysis highlights an essential role of CtBP2 in the maintenance of β cell integrity. This system provides clues to the molecular basis in obesity and may be targetable to develop therapeutic approaches.

INTRODUCTION

Despite the broad influence of insulin on a multitude of biological processes, its production and secretion is solely dependent on pancreatic β cells. Together with the fact that these irreplaceable β cells are vulnerable to cell-intrinsic stresses such as endoplasmic reticulum (ER) stress[1,2] and oxidative stress[3] that are evoked by obesity, insulin insufficiency frequently arises as a result of metabolic stresses, leading to diabetes as well as other catabolic illnesses. The complex system that β cells have evolved to exert their pre-eminent role in insulin production has liabilities in this context; however, the molecular bases underlying this obesity-induced β cell dysfunction remain incompletely understood.

Since the insulin gene is specifically expressed in pancreatic β cells, insulin gene expression and β cell differentiation are inseparably regulated at the levels of transcription. We observe significant contributions of key transcription factors to the maintenance of β cell integrity in cases of monogenic diabetes: *HNF4A*/MODY1, *HNF1A*/MODY3, *HNF1B*/MODY5, *PDX1*/*IPF1*/MODY4, and *NEUROD1*/MODY6.[4] Among them, MODY6 is an extremely rare form of monogenic diabetes caused by mutation(s) in *NEUROD1* gene.[5] The low penetrance of MODY6 suggests the susceptibility to environmental factors such as maternal nutritional conditions,[5] implicating that the transcriptional activity of NEUROD1 may also be under the influence of metabolic alterations.[5] Mice lacking the *Neurod1* gene manifest severe diabetes with impaired pancreatic islet morphogenesis that leads to perinatal lethality,[6] providing another line of evidence showing an indispensable role of NEUROD1 in the maintenance of β cell integrity. NEUROD1 is a basic-helix-loop-helix (bHLH) transcription factor that commits neuroendocrine precursor cells to a β cell lineage and is immediately downstream of neurogenin 3 (NEUROG3), another critical bHLH transcription factor.[7] It has been reported that NEUROD1 expressed in specific tissues forms a heterodimer with transcription factor 3 (TCF3, also known as E47), a ubiquitous bHLH factor[8] that can recruit a set of chromatin modifiers.[9]

C-terminal binding protein (CtBP) transcriptional corepressors play critical roles in the regulation of chromatin architecture.[10] Two isoforms exist in mammals, CtBP1 and CtBP2, and only CtBP2 has a nuclear localization signal on its N-terminal region, which specifically designates CtBP2 for regulation of transcription.[11] While CtBP2 is localized exclusively in the nucleus,



(legend on next page)

CtBP1 is distributed throughout cells and also exerts non-transcriptional functions.[12,13] The global deficiency of both isoforms of CtBPs leads to developmental defects, whereas deficiency of only CtBP2 manifests in embryonic lethality, and loss of CtBP1 leads to a milder phenotype.[14] In part because of this lethality, the roles of CtBPs *in vivo* have been underexplored. Recently, we demonstrated that CtBP2 serves as a metabolite sensor with an activation/inactivation equilibrium controlled through binding to NADH/NAD^+ and fatty acyl-CoAs, respectively.[15] While CtBP2 represses FoxO1-mediated gluconeogenesis and SREBP1-mediated lipogenesis to prevent diabetes and hepatic steatosis in healthy liver, fatty acyl-CoAs induced by obesity inactivate CtBP2, resulting in metabolic deterioration.[15] Conversely, CtBP2 activation dramatically ameliorates diabetes as well as hepatic steatosis.[15] Historically, CtBPs have been reported to have preferential binding affinity for NADH compared to NAD^+, which confers CtBPs a unique capability to be activated by increased glycolytic flux,[16,17] albeit with some controversy.[18,19] Interestingly, both glycolysis and fatty acyl-CoA metabolism are tightly coupled with insulin secretory function in β cells.[20]

In this study, we demonstrate that CtBP2 interacts with NEUROD1 to activate insulin gene expression through chromatin remodeling. In mouse models of obesity, the protein expression of CtBP2 in pancreatic islets is markedly decreased, and genetic ablation of CtBP2 in pancreatic β cells results in a diabetic phenotype with reduced insulin secretion. The decreased CtBP2 expression was also observed in human obesity. Our global transcriptome analysis indicates a critical role of CtBP2 in the maintenance of β cell integrity. These data suggest that the loss of CtBP2 may be a mechanistic link between obesity and β cell dysfunction that may be targetable to develop new therapeutic approaches.

RESULTS

CtBP2 is recruited to insulin gene promoters to coactivate the gene expression

Having observed the critical roles of CtBP2 in the liver of obesity,[15] we embarked on an investigation of a potential involvement of CtBP2 in the function of pancreatic β cells. Firstly, we suppressed the expression of endogenous CtBP2 by adenovirus-mediated delivery of shRNA, and we observed decreased expression levels of two mouse insulin genes, *Ins1* and *Ins2,* in MIN6 cells, a mouse β cell line (Figures 1A and S1A). Conversely, when we examined the effects of gain of function of CtBP2, CtBP2 overexpression increased the expression of *Ins1* and *Ins2* compared to the overexpression of control protein β-glucuronidase (GUS) (Figures 1B and S1B). Together, these data suggest a coactivating role of CtBP2 in insulin gene expression. Indeed, we observed robust recruitment of CtBP2 to the promoter regions of insulin genes (Figure 1C) in our chromatin immunoprecipitation (ChIP)-qPCR analysis, suggesting a direct involvement of CtBP2 in insulin gene expression (Figure 1D).

Next, we attempted to gain mechanistic insights into the CtBP2-mediated insulin gene transcriptional activation. CtBP2 lacks a DNA binding domain and binds to specific transcription factors to be recruited to functional DNA elements.[21] Therefore, motifs found in CtBP2 footprints would enable prediction of those key transcription factors. To accomplish this, we deciphered the CtBP2 cistrome in MIN6 cells using ChIP followed by high-throughput DNA sequencing (ChIP-seq). The peak distribution relative to the genomic features was analyzed by the *cis*-regulatory element annotation system (CEAS),[22] and we found that CtBP2 was more frequently recruited onto transcriptional start sites, while peaks were also observed on gene bodies of certain sets of genes (Figures S1C and S1D). Enrichment of gene ontology processes associated with the peaks was analyzed by GREAT (Genomic Regions Enrichment of Annotations Tool), and the top ranking hit in Gene Ontology molecular function terms was related to the voltage-gated calcium channel activity that primarily regulates insulin secretion (Figure 1E), suggesting that CtBP2 may govern a broad array of genes involved in insulin secretion. Indeed, we observed recruitment of CtBP2 to not only insulin (*Ins1* and *Ins2*) and the calcium channel gene (*Cacna1c*) but also other key genes encoding molecules critically involved in the insulin secretory machinery (Figures 1F and S1E). We also observed peaks around the gene bodies of insulin genes, suggesting chromatin rearrangement of insulin genes may be induced not only in the promoters but also in the gene bodies.[23]

CtBP2 forms a transcriptional coactivation complex with NEUROD1 to regulate insulin gene expression

We further extracted consensus motifs from the ChIP-seq peaks and compared these motifs against databases of known motifs of transcription factors[15] (Figure S2). The list of candidate transcription factors contains those reported to directly bind to CtBP2, such as FoxO1[15] and KLFs,[24] ensuring the accuracy of our analysis. Intriguingly, one of the top-ranked motifs (e-value 3.2e-110) suggested a potential interaction with NEUROD1, a master regulator of insulin gene transcription,[25] which was also

Figure 1. CtBP2 is a coactivator for insulin gene expression in pancreatic β cells

(A) The effect of CtBP2 suppression on *Ins1* and *Ins2* gene expression. MIN6 cells were transduced with non-target shRNA (shCont) or shCtBP2 by adenovirus-mediated transduction (n = 4).

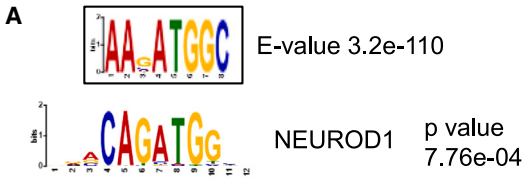
(B) The effect of CtBP2 overexpression on *Ins1* and *Ins2* gene expression. MIN6 cells were transduced with adenoviruses encoding control protein (GUS, AdGUS) or CtBP2 (AdCtBP2) (n = 4).

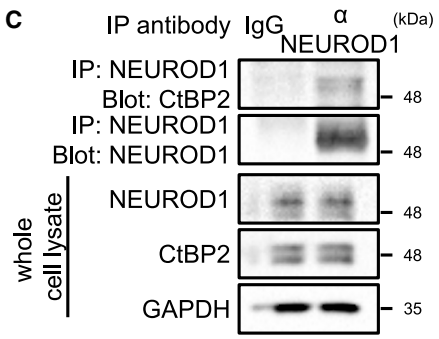
(C) Schematic description of mouse *Ins1* and *Ins2* gene promoters. Elements are termed according to the literature,[48] and the E elements contain typical E-box sequences. Since there are two E-box sequences in the mouse *Ins1* gene promoter, two primer sets were prepared to amplify those sequences (*Ins1* E1 and *Ins1* E2). The primer set to amplify the single mouse *Ins2* gene promoter was designated *Ins2* E1.

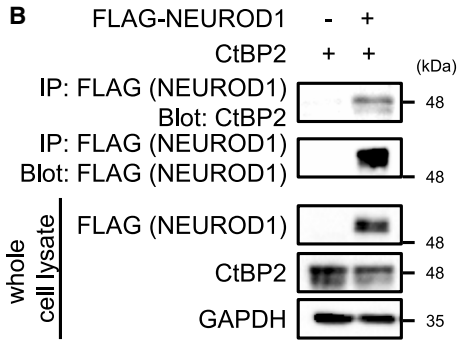
(D) ChIP-qPCR analysis to detect recruitment of CtBP2 to each region of the insulin gene promoters in MIN6 cells (n = 4). The negative control primers were designed to amplify the upstream region of *Alb* gene.

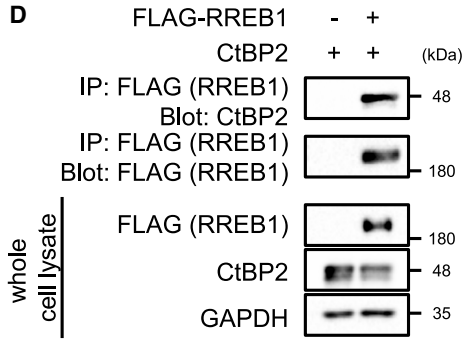
(E) Gene Ontology (GO) analysis of CtBP2 ChIP-seq in MIN6 cells with GREAT tool. Top ranking hits are in GO molecular function terms.

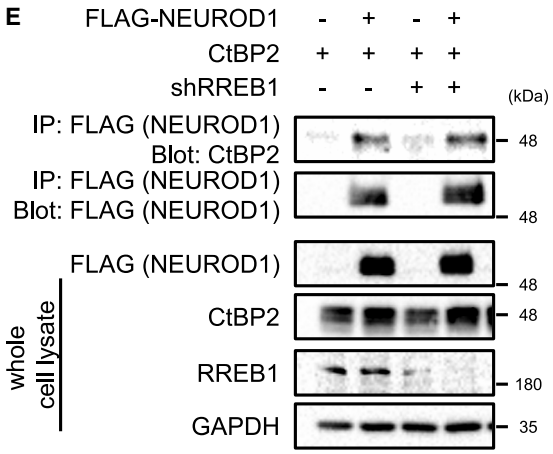
(F) CtBP2 ChIP-seq peaks at representative metabolic gene loci. Data are expressed as the mean ± SEM. *$p < 0.05$ and **$p < 0.01$, determined by Student's t test.

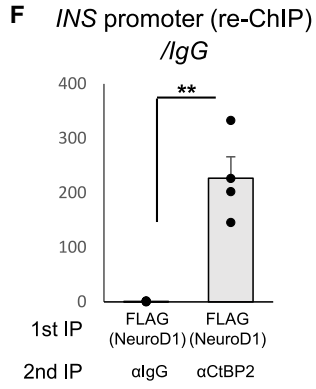


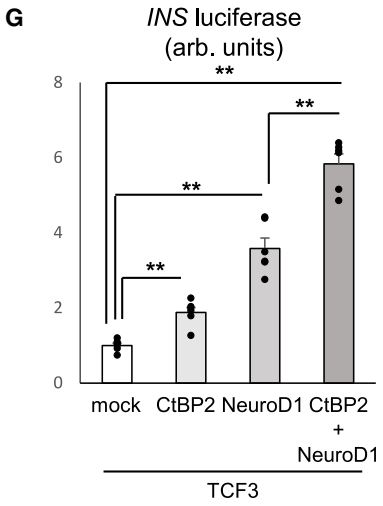












(legend on next page)

supported by a previous report[26] (Figures 2A and S2). Consistent with this finding, we observed an interaction between CtBP2 and NEUROD1 in HEK293 cells exogenously expressing these genes (Figure 2B). Moreover, we were able to detect the endogenous CtBP2-NEUROD1 complex in MIN6 cells (Figure 2C). Despite the fact that the binding partners for CtBP2 share a highly conserved motif Pro-x-Asp-Leu (PxDL) in their primary amino acid sequences,[21] we could not find the putative CtBP interaction site(s) in NEUROD1, implicating the indirect nature of this interaction. Since a previous report suggested that Ras-responsive element binding protein 1 (RREB1) serves as an intermediary molecule between CtBP2 and NEUROD1,[26] we examined the CtBP2-RREB1 interaction. Indeed, we were able to detect this interaction and find the putative CtBP-binding motif in RREB1 (Figures 2D and S3A). However, the CtBP2-NEUROD1 interaction was preserved even after suppressing the expression of RREB1, implicating the existence of a large multi-protein complex that supports the CtBP2-NEUROD1 interaction (Figure 2E).

We further examined the role of this transcriptional complex in the regulation of insulin genes. NEUROD1 has been known to preferentially bind to E-box motifs that are present in the promoters of *Ins1* and *Ins2* genes, with duplication in *Ins1* gene (Figure 1C). As shown in Figure 1D, the recruitment of CtBP2 was detected in all of these E-box sequences. In addition, our re-ChIP experiment demonstrated that the CtBP2-NEUROD1 complex was present in the human insulin gene promoter, which has a single E-box motif (Figure 2F). To observe the functional consequences of this transcriptional complex formation, we performed reporter-based assays and observed that CtBP2 and NEUROD1 synergistically enhanced human insulin promoter activities, substantiating the coactivating role of CtBP2 in insulin gene expression (Figure 2G). Interestingly, we found that the presence of TCF3 (E47), an obligate partner of NEUROD1, was required for this synergistic induction (Figure 2G).

CtBP2 modifies chromatin architecture to coactivate insulin gene promoters

Since CtBP2 has been reported to regulate gene expression through alteration of epigenetic codes,[21] we examined the effects of CtBP2 on histone modifications of the insulin gene promoters. CtBP2 overexpression increased histone marks associated with open chromatin, such as H3K4me3, H3K9ac, and H3K27ac, and it decreased those associated with repressive chromatin, such as H3K27me3[27] (Figure 3A). While the role of H3K4ac in chromatin remodeling remains underexplored,[28,29] we observed that it was decreased by CtBP2 overexpression (Figure S3B). In contrast, suppression of CtBP2 decreased active histone marks and increased repressive ones albeit with some exceptions. Together, these data indicate that CtBP2 maintains an open chromatin architecture at the insulin gene promoters (Figures 3B and S3C). A wide range of histone modifiers have been identified as components of the CtBP2 chromatin-remodeling complex, such as LSD1-KDM1A (lysine-specific demethylase 1, lysine demethylase 1A), EHMT1/2 (euchromatic histone methyltransferase 1/2), PCAF, CBP/p300, and HDAC[26,30] that would be responsible for the dynamic alterations observed in H3K9ac, H3K27ac, and H3K4ac. Since one of the most prominent alterations was methylation of H3K4, we searched *in silico* for H3K4 methyltransferases harboring the PxDL CtBP-binding motif(s) and identified members of the MLL/KMT2 family (Figures S3D and S3E). Indeed, we were able to demonstrate the interactions between CtBP2 and KMT2A/B that were also reported previously (Figure 3C).[31] These data unequivocally demonstrate that CtBP2 activates insulin gene expression through alterations of histone modifications.

CtBP2 protein expression is markedly diminished in the pancreatic β cells in obesity

Having observed an interesting role of CtBP2 in the transcriptional regulation of the insulin genes *in vitro*, we investigated *in vivo* relevance of this novel system. We previously demonstrated that functional alterations of CtBP2 are primarily regulated at the levels of dynamic equilibrium between dimer and monomer in liver, and that CtBP2 is markedly inactivated with a modest decrease in protein expression in the livers of obese mice and humans.[15] To examine the influence of obesity on CtBP2 in pancreatic β cells, we first examined the CtBP2 protein expression levels in multiple animal models of obesity. CtBP2 expression was detected in both exocrine and endocrine pancreas in our immunohistochemical analysis. Notably, CtBP2 protein expression was markedly reduced in pancreatic islets in multiple models of obesity, while expression in exocrine cells was unchanged (Figures S4A–S4C). This finding was further

Figure 2. Analysis of CtBP2 cistrome leads to identification of CtBP2-NEUROD1 transcriptional complex

(A) Motif analysis of CtBP2 binding sites. The motif enclosed by a rectangle is one of the CtBP2-binding motifs enriched in the ChIP-seq. NEUROD1 was predicted to be targeted by CtBP2 based on sequence similarity.

(B) CtBP2-NEUROD1 complex was detected by co-immunoprecipitation. HEK293 cells were transfected with either control plasmid, FLAG-NEUROD1, or CtBP2, and the complex was immunoprecipitated with FLAG magnetic beads.

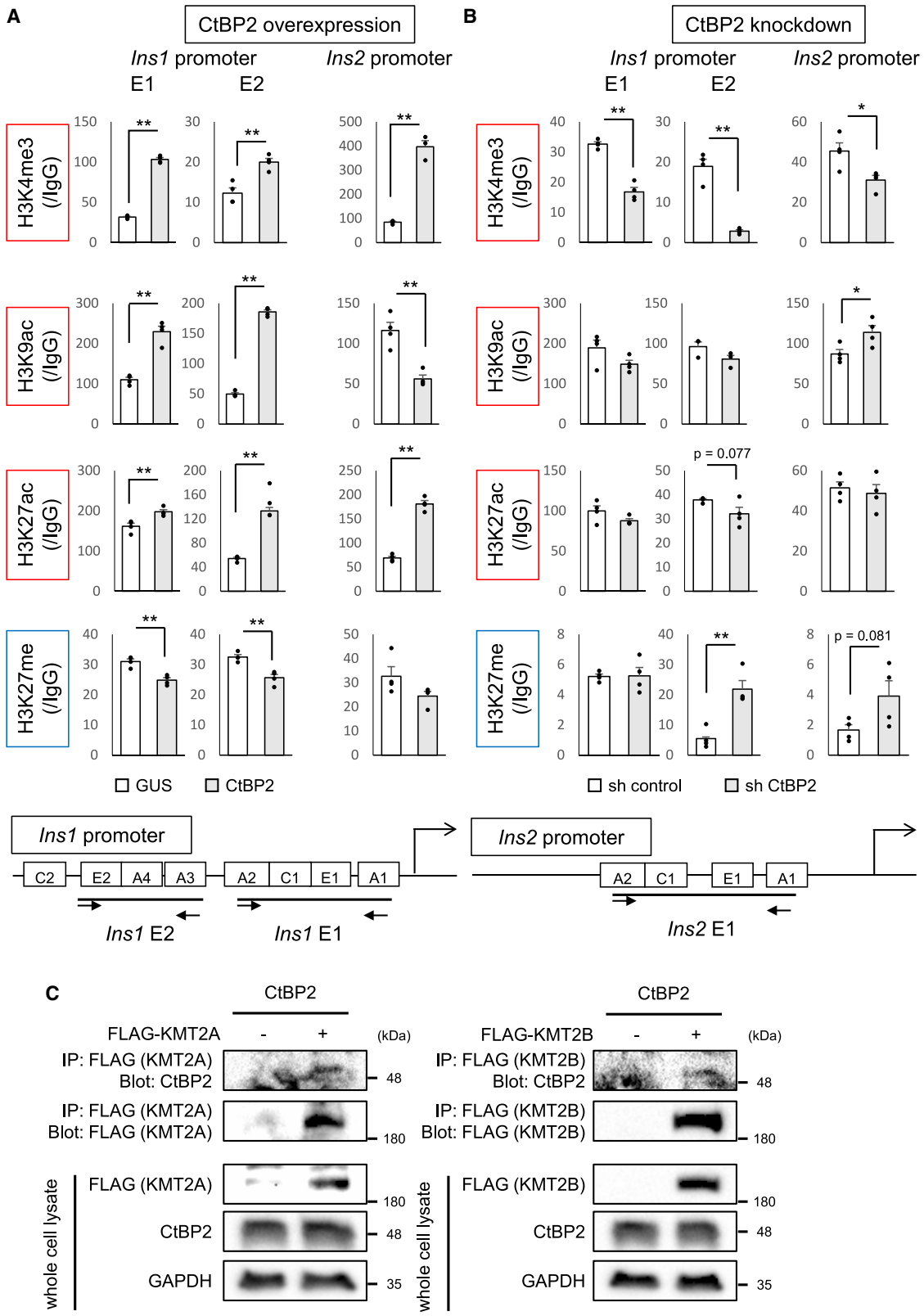
(C) The endogenous CtBP2-NEUROD1 complex was detected by NEUROD1 immunoprecipitation in MIN6 cells.

(D) CtBP2-RREB1 complex was detected by co-immunoprecipitation. HEK293 cells were transfected with either control plasmid, FLAG-RREB1, or CtBP2, and the complex was immunoprecipitated with FLAG magnetic beads.

(E) CtBP2-NEUROD1 complex formation in the presence or absence of RREB1 shRNA. HEK293 cells were transfected with either non-target shRNA or shRREB1 and thereafter transfected with either control plasmid, FLAG-NEUROD1, or CtBP2.

(F) Promoter occupancy of the CtBP2-NEUROD1 complex detected by sequential ChIP (re-ChIP). HEK293 cells were transfected with FLAG-NEUROD1 and CtBP2. The chromatin was first enriched by FLAG immunoprecipitation, and the eluents were further immunoprecipitated with either control IgG or anti-CtBP2 (n = 4).

(G) Insulin gene promoter activities analyzed using a luciferase reporter. HEK293 cells were transfected with CtBP2 and/or NEUROD1 along with TCF3, an obligate partner of NEUROD1, as well as the human *INS* gene promoter luciferase reporter (n = 6). Data are expressed as the mean ± SEM. $^{**}p < 0.01$, determined by Student's t test.



(legend on next page)

validated using isolated islets of obese mouse models, which showed 75%–88% decreases in CtBP2. Conversely, expression of CtBP1, another CtBP isoform, was relatively preserved in islet of obese mice (Figures 4A–4C). These observations led us to examine the mRNA expression of both isoforms of CtBPs. Interestingly, CtBP2 mRNA expression was reduced specifically in leptin-signaling deficient models (*ob/ob* and *db/db*), while it was not affected in diet-induced obesity (DIO, Figure 4D). The reduction of CtBP2 mRNA expression specifically in leptin-signaling deficiency led us to examine the direct effect of leptin on CtBP2 mRNA expression. CtBP2 expression was only rather modestly decreased by leptin treatment in MIN6 cells, implying the existence of indirect mechanism(s) (Figure S5A). Since numerous differences between human and murine β cells have been reported,[32] we further examined human pancreas specimens obtained at autopsy (Figure 4E). Indeed, immunostaining of CtBP2 in islets was also decreased in the obese human specimens (Figure 4E). Overall, decreased CtBP2 protein expression was identified as a feature conserved among all of the obese models including human subjects.

Given that CtBP2 promotes insulin gene transcription, the decreased CtBP2 protein expression in obesity may be the molecular link between the progressive impairment of insulin secretion and chronic hyperglycemia observed during the course of obesity.[33,34] Therefore, we further investigated the molecular mechanisms behind this observation. Obesity evokes several detrimental systems such as oxidative stress and ER stress, and pancreatic β cells are particularly vulnerable to oxidative stresses due to their insufficient anti-oxidative defense system.[33] Intriguingly, hydrogen peroxide (H_2O_2)-induced oxidative stress decreased CtBP2 protein expression in both MIN6 β cells and HEK293 cells that lack characteristics of β cells (Figures 4F and S5B). Reflecting the lack of the anti-oxidative stress response system in β cells, the decrease of CtBP2 protein in MIN6 β cells was more robust (92% after 2-h treatment) compared to that in HEK293 cells (36% after 4-h treatment). Furthermore, cycloheximide chase experiments revealed that this decrease in CtBP2 protein was caused by protein destabilization (Figures 4G and S5C). On the other hand, CtBP1 expression was not influenced in response to the H_2O_2-induced oxidative stress (Figures 4F and 4G). The comparison between the results from β cells and non-β cells highlighted two important points that are consistent with our *in vivo* observations: CtBP2 is sensitive to H_2O_2-induced degradation particularly in β cells, and CtBP1 is insensitive to this oxidative insult. In agreement with these observations, CtBP2 protein underwent modification with polyubiquitin chains that typically destine proteins for degradation upon exposure to H_2O_2 (Figure 4H). We also examined the influence of ER stress on CtBP2 expression. While two classic ER stress inducers, tunicamycin and thapsigargin, increased DNA damage inducible transcript 3 (also known as CHOP) expression, CtBP2 expression was marginally decreased by these stimuli in both HEK293 cells and MIN6 cells, suggesting that the relative contributions of ER stress to the diminished CtBP2 expression in the islets in obesity would be limited (Figures S5D and S5E). We also found that NEUROD1 was polyubiquitinated and degraded by oxidative insults (Figures 4F and S5F). Collectively, these data suggest that CtBP2 protein undergoes targeted degradation particularly upon oxidative stress exposure.

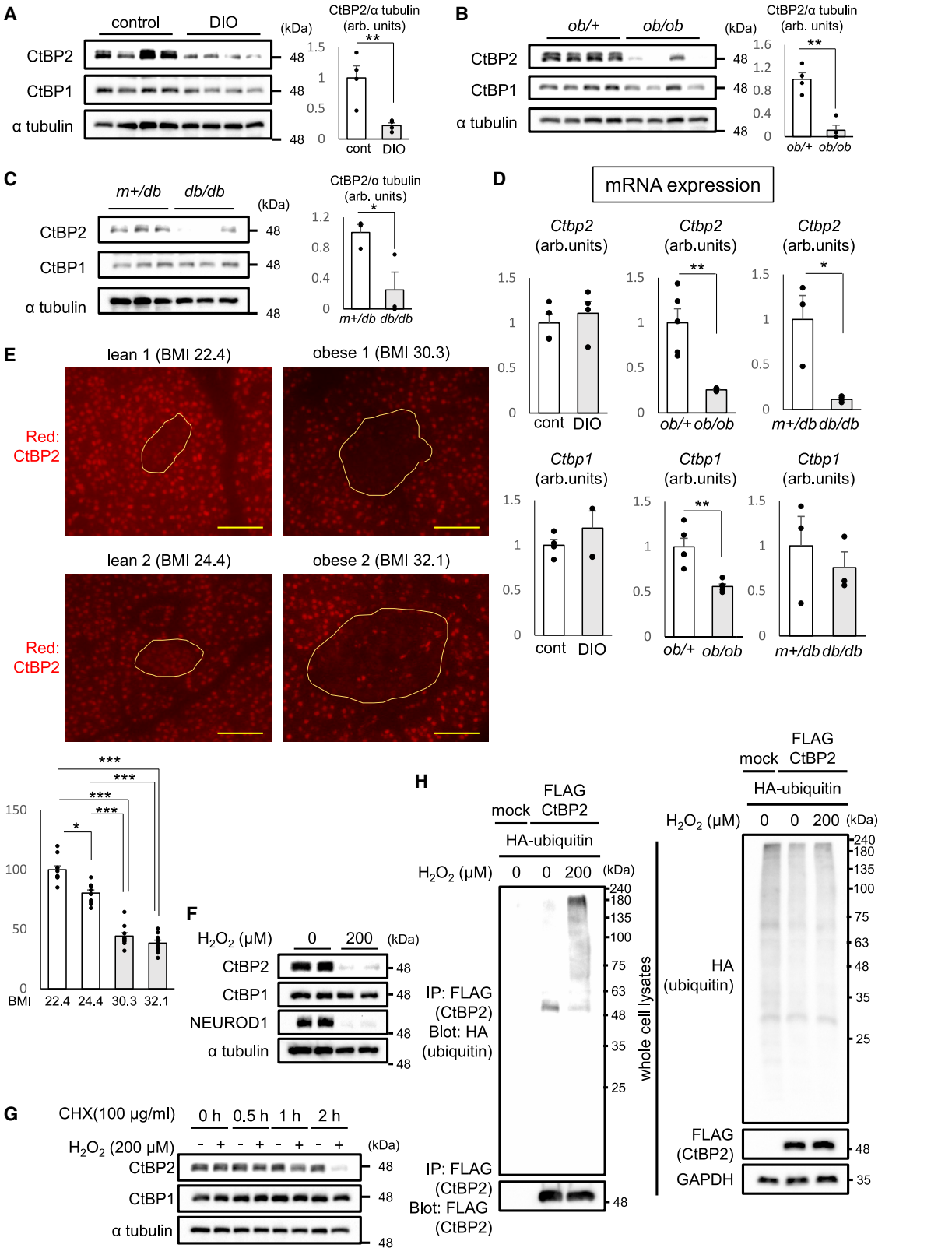
Pancreatic β cell-specific CtBP2 deletion leads to glucose intolerance and impaired insulin secretion

We further addressed the question of whether reduction of CtBP2 leads to β cell dysfunction *in vivo* by generating β cell-specific CtBP2 deletion models. We first created β cell-specific CtBP2 knockout mice by crossing CtBP2 flox mice[15] with Ins1-Cre transgenic mice that exclusively express Cre recombinase in pancreatic β cells[35] (Ins1C2KO, Figure 5A). There were no significant differences in their cumulative body weight gains and tissue weights (Figures 5B and S6A). As expected, the Ins1C2KO mice showed increased hyperglycemia along with reduced plasma insulin levels following a glucose challenge (GTT, Figures 5C–5F). Regarding insulin sensitivity, there was no discernible difference in the insulin tolerance when plotted as "percent of initial value" in insulin tolerance test, although we observed significantly increased blood glucose levels in the Ins1C2KO (Figures 5G, 5H, S6B, and S6C). Histological inspection of the pancreas sections revealed a trend toward decreased size of islets in the Ins1C2KO (Figure 5I). The expression levels of *Ins1* and *Ins2* were decreased in the Ins1C2KO islets, in agreement with the *in vitro* findings (Figure S6D). As expected, the insulin content in the Ins1C2KO islets was also reduced (Figure 5J), and those islets from the Ins1C2KO mice exhibited impaired insulin secretion in response to glucose, as well as depolarizing concentrations of potassium chloride (KCl) *ex vivo* (glucose-stimulated insulin secretion, Figure 5K). Although the specificity of the Cre driver to pancreatic β cells was ensured in the previous report,[35] there may be potential developmental defects. Therefore, we generated an additional β cell-specific CtBP2-deficient model using the tamoxifen-inducible Cre/*loxP* system[36] (ERT2C2KO) to achieve adolescent deletion of CtBP2. This model may more faithfully recapitulate the loss of CtBP2 induced by the metabolic abnormalities, and validation in an independent mouse model would further support our hypothesis and resolve some concerns related to the tissue specificity of pancreas-specific Cre drivers.[37] Similar to the Ins1C2KO, CtBP2 protein expression was depleted in pancreatic islets in this model (Figure 5L). There were no significant differences in their body weights or tissue weights (Figure S6E). Importantly, the ERT2C2KO mice exhibited decreased glucose tolerance with impaired insulin secretion, as observed in Ins1C2KO mice (Figures 5M–5P). Again, the inducible CtBP2 deletion in pancreatic β cells did not affect insulin tolerance (Figures 5Q, 5R, S6F,

Figure 3. CtBP2 regulates insulin gene expression through chromatin remodeling

(A and B) ChIP-qPCR analysis of histone marks induced by CtBP2 overexpression (A) or CtBP2 knockdown (B) in MIN6 cells (n = 4). Histone marks associated with the open chromatin are indicated by red rectangles, and those with closed chromatin are indicated with blue rectangles.

(C) CtBP2-KMT2A and CtBP2-KMT2B complexes were analyzed by co-immunoprecipitation in HEK293 cells. Data are expressed as the mean ± SEM. *$p < 0.05$ and **$p < 0.01$, determined by Student's t test.



(legend on next page)

and S6G). These findings indicate that the sequential events that begin with obesity and lead to impaired insulin secretion and diabetes could be explained at least in part by loss of β cell CtBP2 in obesity. The adaptive response of β cells to obesity is characterized by an initial compensatory increase in insulin production followed by decompensation of β cell function. Since overexpression of CtBP2 increased the insulin gene expression (Figure 1B), we examined the possibility that CtBP2 expression may be increased in the early stage of DIO to contribute to the adaptive response. For this purpose, we isolated the pancreatic islets from mice fed a high-fat diet for 1 week, along with their lean controls. We observed a decrease in CtBP2 protein expression in islets in this early stage of DIO (Figure S6H), implicating obesity-induced alterations of CtBP2 expression may be responsible solely for the progressive decline of β cell function during the course of obesity.

CtBP2 plays an essential role in the maintenance of pancreatic β cell integrity

Next, we attempted to gain broad insights into the transcriptional system regulated by CtBP2 through comprehensive transcriptome analysis of Ins1C2KO islets. While it is well known that pancreatic β cells have a unique expression profile specialized for insulin production, we found that expression levels of numerous β cell genes were decreased in Ins1C2KO islets, suggesting an essential role of CtBP2 in the maintenance of the β cell integrity (Figure 6A). The differentially expressed genes (false discovery rate < 0.05) were further analyzed using ingenuity pathway analysis (IPA) software, and we found that ''Insulin secretion signaling pathway'' was enriched in the canonical pathway analysis, indicating that CtBP2 may govern a broad array of genes involved in insulin secretion (Figure 6B). Several transcription factors with significant contributions to β cell functions were also enriched as predicted upstream regulators through the IPA analysis (Figure 6C), further supporting the key role of CtBP2 in β cells. The close inspection of expression levels of individual genes further supported our idea that CtBP2 coactivates subsets of genes associated with insulin secretion. Interestingly, the *Gcg* gene that encodes the precursor for glucagon and glucagon-like peptide 1 was decreased in Ins1C2KO islets, implicating potential modulation of endocrine cell lineages other than β cells in this system. In line with these findings, immunohistochemical inspection of pancreatic specimens revealed that all of the parameters representing the insulin-producing β cell functions (β cell area, β cell intensity/islet, and β cell intensity/area) were significantly reduced in Ins1C2KO (Figure 6E). Intriguingly, the average α cell area and α cell intensity of single islets were also reduced in Ins1C2KO, although there was no difference in the α cell intensity normalized to unit area (Figure 6F). Indeed, serum glucagon levels were decreased in ERT2C2KO, although the difference was not observed in Ins1C2KO (Figure S6I). These data suggest that CtBP2 deletion in β cells may have some influence on the α cell lineage.

DISCUSSION

In this study, we demonstrated that CtBP2 may play a role in obesity-induced pancreatic β cell dysfunction at least in part through epigenetic mechanisms. CtBP2 forms a large transcriptional complex comprising a wide range of epigenetic modifiers, including KMT2 where an interaction with NEUROD1 is centrally located. We decoded the transcriptional landscape regulated by CtBP2 through comprehensive analyses of ChIP-seq and RNA-seq data, which revealed the critical involvement of CtBP2 in the maintenance of pancreatic β cell integrity.

Studies in humans and laboratory animal models have determined the complicated progression of obesity-induced diabetes where the failure of adaptive β cell responses is the key event triggering its onset.[38] While obesity-induced insulin resistance can be initially compensated by adaptive hyperinsulinemia to maintain systemic glucose levels, this increased workload in the β cells evokes several detrimental pathways, such as ER stress[39] and oxidative stress,[40] consequently resulting in β cell failure and the onset of diabetes. It was demonstrated in a human longitudinal study that this β cell failure occurs early in the development of diabetes,[41] suggesting it is one of the prime targets for the development of therapeutic approaches. The decline in CtBP2 protein expression that starts from the early stage of obesity (Figure S6H) may be responsible for this latent and subclinical β cell dysfunction. In contrast, although we observed that CtBP2 overexpression increased insulin gene expression (Figure 1B), the adaptive increase in insulin secretion in response to weight gain might be explained by other mechanism(s).

In our previous study, we demonstrated a biological system in liver where CtBP2 is inactivated in obesity and activation of CtBP2 ameliorates diabetes as well as hepatic steatosis.[15] This study additionally highlights a potential metabolic benefit

Figure 4. CtBP2 expression is markedly reduced in pancreatic β cells in obesity

(A–C) Expression of CtBPs at the protein levels in pancreatic islets of mouse models of obesity (A, diet-induced obesity [DIO], 14 weeks on high-fat diet) and their control mice on chow diet: (B) genetically obese mice (*ob*/*ob*) and their lean controls (*ob*/+) at 10 weeks of age; (C) genetically obese mice (*db*/*db*) and their lean controls (*m*+/*db*) at 10 weeks of age. The densitometric quantification is shown to the right of each blot.

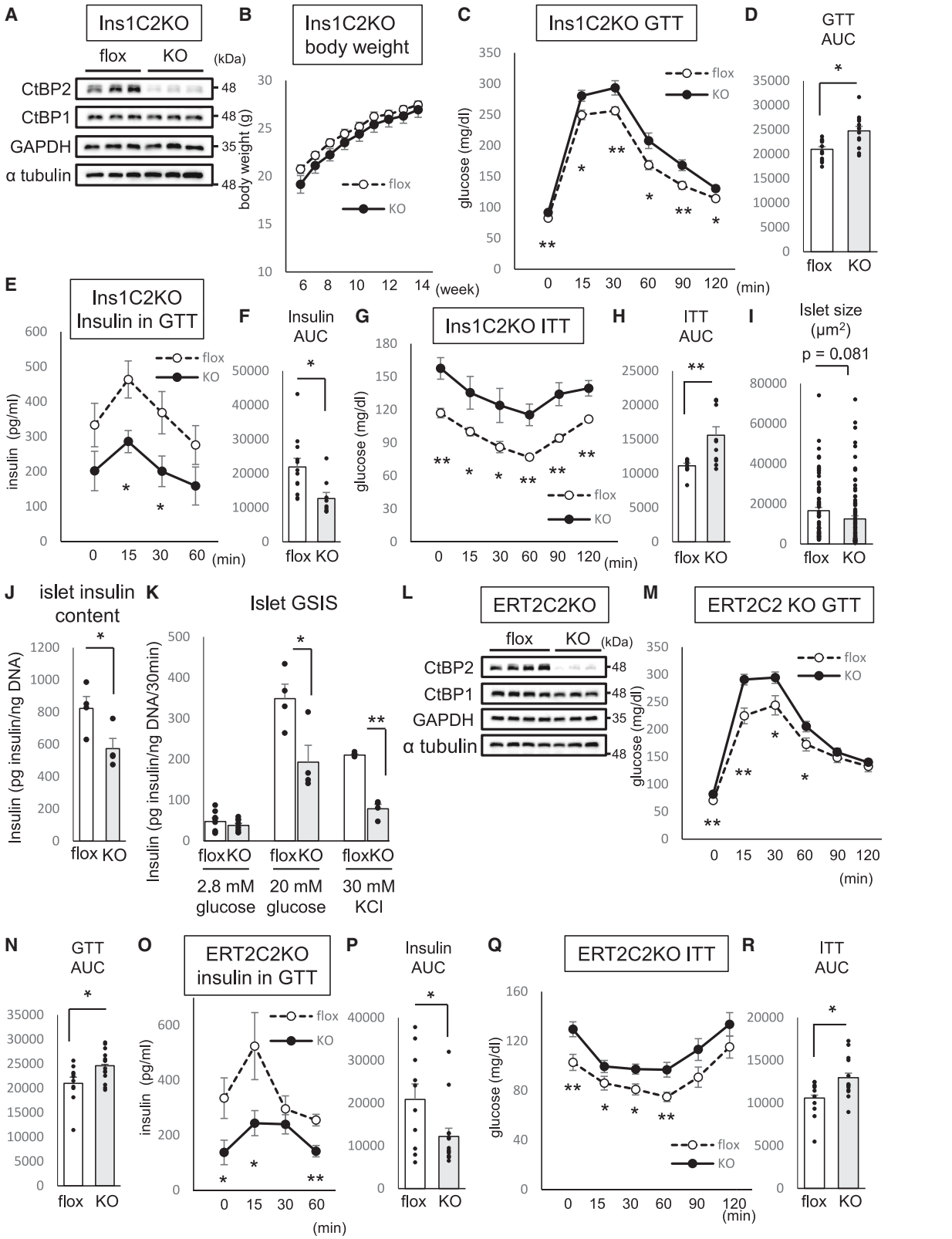
(D) Expression of CtBPs at the mRNA levels in pancreatic islets of mouse models of obesity (n = 3–4).

(E) Immunohistochemical quantification of CtBP2 expression in human pancreas specimens. Samples were obtained at autopsy, and body mass index (BMI) for each subject is indicated above the representative image. The yellow lines indicate islet area, and the yellow scale bars indicate 50 μm. Quantification of the intensity corresponding to CtBP2 expression is shown below the images (two donors for lean and obese, respectively, and 10 islets for each subject).

(F) MIN6 cells were treated with either vehicle or 200 μM H_2O_2 for 2 h to induce oxidative stress. CtBP1 and CtBP2 protein levels were analyzed.

(G) Protein stability was analyzed in a cycloheximide (CHX) chase experiment in MIN6 cells. Protein synthesis was stopped by the addition of cycloheximide, and protein stability was analyzed in the presence or absence of 200 μM H_2O_2.

(H) Polyubiquitination of CtBP2 protein induced by H_2O_2 treatment. HEK293 cells were transfected with FLAG-CtBP2 and HA-ubiquitin and thereafter treated with 200 μM H_2O_2 for 1 h. Ubiquitinated CtBP2 protein was detected by FLAG immunoprecipitation followed by HA immunoblotting. Data are expressed as the mean ± SEM. *p < 0.05 and **p < 0.01, determined by Student's t test.



(legend on next page)

obtained through CtBP2 activation in pancreatic β cells. The activity of CtBP2 is regulated primarily by metabolite-dependent allosteric regulation in liver, while it is regulated at the protein expression levels in pancreatic β cells. Together with the fact that not only the activity of CtBP2 but also its protein stability are regulated by accommodation of metabolites in its pocket structure called the Rossmann fold,[15] pharmacological targeting of this structure to treat obesity-induced diabetes would be attractive and plausible in both liver and pancreatic β cells. We also reported that CtBP2 activation confers protection against oxidative stress in part through interactions with NRF1 and NRF2.[42] This intriguing property of CtBP2 activation would be the most advantageous in β cells since they are vulnerable to oxidative insults compared to other types of cells.[40] In this context, we observed that CtBP2 was also extremely sensitive to oxidative insults, which was in sharp contrast to the insensitivity observed in the other isoform, CtBP1 (Figures 4F–4H, S5B, and S5C). These findings suggest that the expression levels of CtBP2 could be an indicator of β cell capability to manage oxidative stress: as long as the cells can manage oxidative stress through CtBP2-mediated anti-oxidative stress responses[42] and other systems, the cells can maintain CtBP2 protein expression. However, upon overwhelming oxidative insults, cells cannot maintain CtBP2 expression.

In this study, we focused our investigation on the effects of CtBP2 expression. However, the metabolite-dependent alterations of CtBP2 activity would deserve further scrutiny especially under physiological conditions. Glycolysis, a key metabolic process for insulin secretion in response to glucose, regenerates NADH from NAD^+, which allosterically activates CtBP2.[15,21] We reported long-chain fatty acyl-CoAs as inhibitory metabolites for CtBP2,[15] and there have been debates regarding the paradoxical roles of fatty acyl-CoAs in β cell functions. Long-chain fatty acyl-CoAs have been reported to serve as secretagogues for insulin,[43,44] while lipid spillover that generates fatty acyl-CoAs is detrimental to β cells.[45] The fatty acyl-CoAs from endogenous and exogenous origins may also have distinct roles in β cells, and CtBP2 may be able to discriminate the lipids from different sources. Moreover, acyl chain length and saturation largely influence biological activities of fatty acids/fatty acyl-CoAs, which has to be taken into account in the system regulated by CtBP2 as well.

It is of note that expression levels of not only β cell-specific genes but also *Gcg*, an α cell-specific gene, were decreased in the pancreatic islets from Ins1C2KO. Since the expression of Cre recombinase is β cell specific,[35] this finding indicates that CtBP2 deletion in β cells may influence the α cell lineage. This may be due to intercellular communications between α cells and β cells or some defects in cell differentiation processes such as transdifferentiation and dedifferentiation. Indeed, our upstream regulator analysis using IPA identified transcription factors that play key roles in the β cell differentiation as potential contributors to the transcriptional profile in Ins1C2KO (Figure 6C). Although we reported FoxO1 as a key molecule targeted by CtBP2 in liver,[15] β cell-specific FoxO1 deficiency leads to dedifferentiation of β cells accompanied by an increase in α cells.[46] Mice with β cell-specific deletion of NEUROD1 manifest diminished expression of the *Ins1* gene specifically,[47] while the global NEUROD1 deficiency results in a more severe phenotype.[6] To the best of our knowledge, none of the pre-existing animal models faithfully resemble mice with CtBP2 deficiency in β cells, highlighting the uniqueness of the system regulated by CtBP2. Our findings, including data obtained from human autopsy material, may have translational relevance, especially given that the amino acid sequence of CtBP2 is well conserved between humans and rodents. It is also an intriguing possibility that mutation(s) in CtBP2 causally associated with human monogenic diabetes might be identified in the future with the recent advances of genetics and genomics technologies.[4]

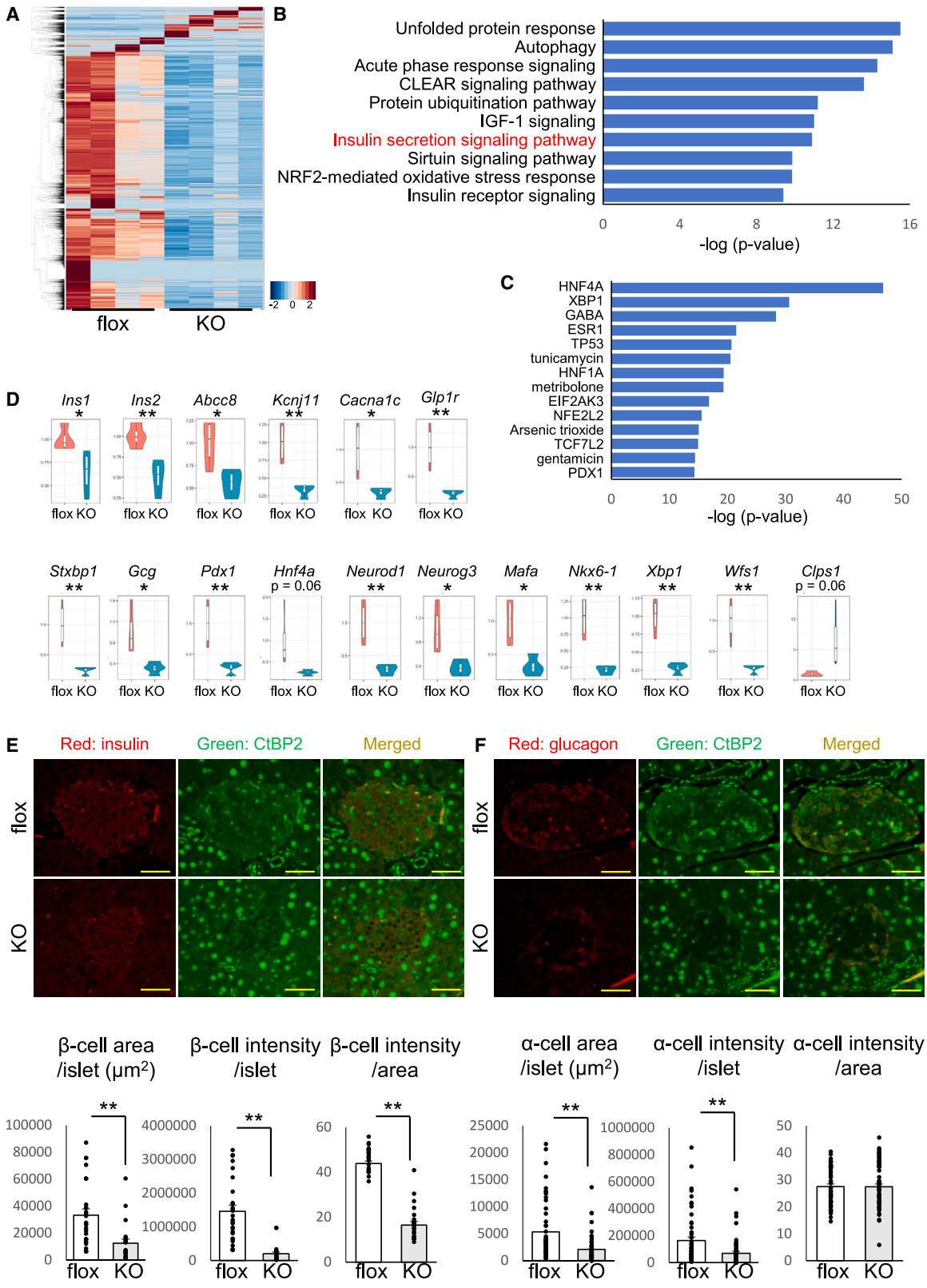
In conclusion, we identified CtBP2 as a key molecule potentially linking obesity to pancreatic β cell dysfunction. Our finding provides a basis for understanding the complex biological systems in pancreatic β cells and could be exploited to develop future therapeutic approaches.

Limitations of the study

We performed our animal studies using only male mice. We performed an RNA-seq analysis using whole islets that contain non-β cells as well, although β cells constitute the predominant population in those islets. CtBP2 may regulate β cell functions not only through modulation of NEUROD1 activity but also by targeting non-NEUROD1 transcriptional system(s). In the analysis of human pancreas sections, we examined a section of pancreatic head from each donor. There are issues that remain to be

Figure 5. Loss of function of CtBP2 in pancreatic β cells of mice leads to glucose intolerance with impaired insulin secretion

(A–K) Ins1 Cre-mediated perinatal deletion of CtBP2 in pancreatic β cells (Ins1C2KO).
(L–R) Tamoxifen-induced adolescent deletion of CtBP2 in pancreatic β cells (ERT2C2KO).
(A) Expression levels of CtBP1 and CtBP2 protein in the islets of Ins1C2KO and their controls (flox).
(B) Growth curves of the Ins1C2KO and their control (flox) mice (n = 22 for flox and 11 for KO).
(C and D) Glucose tolerance test (n = 14 for flox and 13 for KO, 16–20 weeks of age, C) and the areas under the curve (AUC) (D).
(E and F) Plasma insulin levels during the GTT (n = 12 for flox and 9 for KO, E) and AUC (F).
(G and H) Insulin tolerance test (n = 10 for flox and 11 for KO, 17–21 weeks of age, G) and AUC (H).
(I) Quantification of islet size (77 islets for flox and 93 islets for KO, four mice for each genotype).
(J) Insulin content of the isolated islets (n = 4 for each genotype).
(K) Glucose-induced insulin secretion from isolated islets (n = 4 for each genotype).
(L) Expression levels of CtBP1 and CtBP2 protein in the islets of ERT2C2KO and their controls (flox).
(M and N) Glucose tolerance test (n = 14 for flox and 14 for KO, 16–20 weeks of age, M) and AUC (N).
(O and P) Plasma insulin levels during the GTT (n = 11 for flox and 14 for KO, O) and AUC (P).
(Q and R) Insulin tolerance test (n = 11 for flox and 13 for KO, 17–21 week of age, Q) and AUC (R). The reproducibility of the GTT experiments for both models was confirmed by two independent researchers. Data are expressed as the mean ± SEM. *$p < 0.05$ and **$p < 0.01$, determined by Student's t test.



(legend on next page)

considered such as regional differences of pancreatic islet distribution and intersection variability. In addition, we need to demonstrate an improvement of β cell dysfunction by restoration or maintenance of CtBP2 protein levels in obesity to directly link between loss of CtBP2 and obesity-induced β cell dysfunction, which awaits future investigations.

STAR★METHODS

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

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Animals
  - Cell culture
- METHOD DETAILS
  - Quantitative real-time RT-PCR
  - Western blot analysis and co-immunoprecipitation experiments
  - Isolation of mouse pancreatic islets
  - Chromatin immunoprecipitation (ChIP)
  - ChIP-seq analysis and downstream data mining
  - Luciferase assays
  - Immunohistochemistry
  - RNA-seq analysis and downstream data mining
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2023.112914.

ACKNOWLEDGMENTS

We thank the members of the Shimano laboratory for their contributions and invaluable discussions. We thank Dr. Kiyotsugu Yoshida (Jikei University School of Medicine) for providing the RREB1 expression plasmid. We thank Dr. Karen Inouye (Harvard University) for editing our manuscript. We thank Katsuko Ohkubo and Chizuko Fukui (University of Tsukuba) for their technical assistance. M.S. was supported by Japan Promotion of Science (grant number 20K08855), the Japan Agency for Medical Research and Development (AMED) under grant numbers JP18gm5910007 and JP22ek0210175, Takeda Science Foundation, Ono Medical Research Foundation, and Japan Diabetes Foundation.

AUTHOR CONTRIBUTIONS

M.S. and Y.M. performed most of the experiments. K.K., K.S., D.Y., T.T., W.C., and P.I.P.A.P. performed basic experiments required to obtain the final data. H.O., T.Miyamoto, Y.T., K.Motomura, and T.Matsuzaka provided critical expertise. Y.M., Y.S., Y.O., H.I., N.Y., and H.Suzuki provided substantial intellectual advice required for data collection. K.Murata, S.M., and S.T. provided Cre-transgenic mice. H.Shimano. supervised and coordinated the study. M.S. conceived and designed the study and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

Received: March 27, 2023
Revised: June 19, 2023
Accepted: July 16, 2023
Published: August 8, 2023

REFERENCES

1. Harding, H.P., and Ron, D. (2002). Endoplasmic reticulum stress and the development of diabetes: a review. Diabetes *51*, S455–S461. https://doi.org/10.2337/diabetes.51.2007.s455.
2. Fonseca, S.G., Gromada, J., and Urano, F. (2011). Endoplasmic reticulum stress and pancreatic β-cell death. Trends Endocrinol. Metabol. *22*, 266–274. https://doi.org/10.1016/j.tem.2011.02.008.
3. Robertson, R.P., Harmon, J., Tran, P.O.T., and Poitout, V. (2004). Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes *53*, S119–S124. https://doi.org/10.2337/diabetes.53.2007.s119.
4. Murphy, R., Ellard, S., and Hattersley, A.T. (2008). Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nature clinical practice. Endocrinology & metabolism *4*, 200–213. https://doi.org/10.1038/ncpendmet0778.
5. Horikawa, Y. (2018). Maturity-onset diabetes of the young as a model for elucidating the multifactorial origin of type 2 diabetes mellitus. J. Diabetes Investig. *9*, 704–712. https://doi.org/10.1111/jdi.12812.
6. Naya, F.J., Huang, H.P., Qiu, Y., Mutoh, H., DeMayo, F.J., Leiter, A.B., and Tsai, M.J. (1997). Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. Genes Dev. *11*, 2323–2334. https://doi.org/10.1101/gad.11.18.2323.
7. Chakrabarti, S.K., and Mirmira, R.G. (2003). Transcription factors direct the development and function of pancreatic beta cells. Trends Endocrinol. Metabol. *14*, 78–84. https://doi.org/10.1016/s1043-2760(02)00039-5.
8. Naya, F.J., Stellrecht, C.M., and Tsai, M.J. (1995). Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. Genes Dev. *9*, 1009–1019. https://doi.org/10.1101/gad.9.8.1009.

Figure 6. CtBP2 is essential to maintain the pancreatic β cell integrity

(A) RNA-seq analysis of the isolated islets from Ins1C2KO and their controls (flox) (n = 4).

(B) Canonical pathway analysis of the RNA-seq data by IPA.

(C) Analysis of the upstream regulators of the RNA-seq data by IPA.

(D) Expression levels of individual genes critical for the maintenance of β cell integrity. Transcripts per million (TPM) values of genes of interest were normalized to that of *Gapdh*.

(E and F) Immunohistochemical characterization of islets in InsC1KO (KO) and their controls (flox). Characterization of β cells with insulin/CtBP2 double staining (E, n = 28 for flox and n = 22 for KO) and of α cells with glucagon/CtBP2 double staining (F, n = 54 for flox and n = 59 for KO). Representative images along with the quantification results are shown. The yellow scale bars indicate 100 μm β(α) cell area per single islet, fluorescent intensity of β(α) cell per single islet, and fluorescent intensity of β(α) cell per normalized area were calculated. Data are expressed as the mean ± SEM. *$p < 0.05$ and **$p < 0.01$, determined by Student's t test.

9. Pataskar, A., Jung, J., Smialowski, P., Noack, F., Calegari, F., Straub, T., and Tiwari, V.K. (2016). NeuroD1 reprograms chromatin and transcription factor landscapes to induce the neuronal program. EMBO J. *35*, 24–45. https://doi.org/10.15252/embj.201591206.

10. Stankiewicz, T.R., Gray, J.J., Winter, A.N., and Linseman, D.A. (2014). C-terminal binding proteins: central players in development and disease. Biomol. Concepts *5*, 489–511. https://doi.org/10.1515/bmc-2014-0027.

11. Bergman, L.M., Morris, L., Darley, M., Mirnezami, A.H., Gunatilake, S.C., and Blaydes, J.P. (2006). Role of the unique N-terminal domain of CtBP2 in determining the subcellular localisation of CtBP family proteins. BMC Cell Biol. *7*, 35. https://doi.org/10.1186/1471-2121-7-35.

12. Liberali, P., Kakkonen, E., Turacchio, G., Valente, C., Spaar, A., Perinetti, G., Böckmann, R.A., Corda, D., Colanzi, A., Marjomaki, V., and Luini, A. (2008). The closure of Pak1-dependent macropinosomes requires the phosphorylation of CtBP1/BARS. EMBO J. *27*, 970–981. https://doi.org/10.1038/emboj.2008.59.

13. Pagliuso, A., Valente, C., Giordano, L.L., Filograna, A., Li, G., Circolo, D., Turacchio, G., Marzullo, V.M., Mandrich, L., Zhukovsky, M.A., et al. (2016). Golgi membrane fission requires the CtBP1-S/BARS-induced activation of lysophosphatidic acid acyltransferase δ. Nat. Commun. *7*, 12148. https://doi.org/10.1038/ncomms12148.

14. Hildebrand, J.D., and Soriano, P. (2002). Overlapping and unique roles for C-terminal binding protein 1 (CtBP1) and CtBP2 during mouse development. Mol. Cell Biol. *22*, 5296–5307. https://doi.org/10.1128/mcb.22.15.5296-5307.2002.

15. Sekiya, M., Kainoh, K., Sugasawa, T., Yoshino, R., Hirokawa, T., Tokiwa, H., Nakano, S., Nagatoishi, S., Tsumoto, K., Takeuchi, Y., et al. (2021). The transcriptional corepressor CtBP2 serves as a metabolite sensor orchestrating hepatic glucose and lipid homeostasis. Nat. Commun. *12*, 6315. https://doi.org/10.1038/s41467-021-26638-5.

16. Zhang, Q., Piston, D.W., and Goodman, R.H. (2002). Regulation of corepressor function by nuclear NADH. Science (New York, N.Y.) *295*, 1895–1897. https://doi.org/10.1126/science.1069300.

17. Fjeld, C.C., Birdsong, W.T., and Goodman, R.H. (2003). Differential binding of NAD+ and NADH allows the transcriptional corepressor carboxyl-terminal binding protein to serve as a metabolic sensor. Proc. Natl. Acad. Sci. USA *100*, 9202–9207. https://doi.org/10.1073/pnas.1633591100.

18. Madison, D.L., Wirz, J.A., Siess, D., and Lundblad, J.R. (2013). Nicotinamide adenine dinucleotide-induced multimerization of the co-repressor CtBP1 relies on a switching tryptophan. J. Biol. Chem. *288*, 27836–27848. https://doi.org/10.1074/jbc.M113.493569.

19. Bellesis, A.G., Jecrois, A.M., Hayes, J.A., Schiffer, C.A., and Royer, W.E., Jr. (2018). Assembly of human C-terminal binding protein (CtBP) into tetramers. J. Biol. Chem. *293*, 9101–9112. https://doi.org/10.1074/jbc.RA118.002514.

20. Prentki, M., Corkey, B.E., and Madiraju, S.R.M. (2020). Lipid-associated metabolic signalling networks in pancreatic beta cell function. Diabetologia *63*, 10–20. https://doi.org/10.1007/s00125-019-04976-w.

21. Chinnadurai, G. (2007). Transcriptional regulation by C-terminal binding proteins. Int. J. Biochem. Cell Biol. *39*, 1593–1607. https://doi.org/10.1016/j.biocel.2007.01.025.

22. Shin, H., Liu, T., Manrai, A.K., and Liu, X.S. (2009). CEAS: cis-regulatory element annotation system. Bioinformatics *25*, 2605–2606. https://doi.org/10.1093/bioinformatics/btp479.

23. Portela, A., and Esteller, M. (2010). Epigenetic modifications and human disease. Nat. Biotechnol. *28*, 1057–1068. https://doi.org/10.1038/nbt.1685.

24. Lomberk, G., and Urrutia, R. (2005). The family feud: turning off Sp1 by Sp1-like KLF proteins. Biochem. J. *392*, 1–11. https://doi.org/10.1042/bj20051234.

25. van der Meulen, T., and Huising, M.O. (2015). Role of transcription factors in the transdifferentiation of pancreatic islet cells. J. Mol. Endocrinol. *54*, R103–R117. https://doi.org/10.1530/jme-14-0290.

26. Ray, S.K., Li, H.J., Metzger, E., Schüle, R., and Leiter, A.B. (2014). CtBP and associated LSD1 are required for transcriptional activation by NeuroD1 in gastrointestinal endocrine cells. Mol. Cell Biol. *34*, 2308–2317. https://doi.org/10.1128/mcb.01600-13.

27. Zhou, V.W., Goren, A., and Bernstein, B.E. (2011). Charting histone modifications and the functional organization of mammalian genomes. Nat. Rev. Genet. *12*, 7–18. https://doi.org/10.1038/nrg2905.

28. Xhemalce, B., and Kouzarides, T. (2010). A chromodomain switch mediated by histone H3 Lys 4 acetylation regulates heterochromatin assembly. Genes Dev. *24*, 647–652. https://doi.org/10.1101/gad.1881710.

29. Guillemette, B., Drogaris, P., Lin, H.H.S., Armstrong, H., Hiragami-Hamada, K., Imhof, A., Bonneil, E., Thibault, P., Verreault, A., and Festenstein, R.J. (2011). H3 lysine 4 is acetylated at active gene promoters and is regulated by H3 lysine 4 methylation. PLoS Genet. *7*, e1001354. https://doi.org/10.1371/journal.pgen.1001354.

30. Byun, J.S., and Gardner, K. (2013). C-Terminal Binding Protein: A Molecular Link between Metabolic Imbalance and Epigenetic Regulation in Breast Cancer. Int. J. Cell Biol. *2013*, 647975. https://doi.org/10.1155/2013/647975.

31. Xia, Z.B., Anderson, M., Diaz, M.O., and Zeleznik-Le, N.J. (2003). MLL repression domain interacts with histone deacetylases, the polycomb group proteins HPC2 and BMI-1, and the corepressor C-terminal-binding protein. Proc. Natl. Acad. Sci. USA *100*, 8342–8347. https://doi.org/10.1073/pnas.1436338100.

32. Rorsman, P., and Braun, M. (2013). Regulation of insulin secretion in human pancreatic islets. Annu. Rev. Physiol. *75*, 155–179. https://doi.org/10.1146/annurev-physiol-030212-183754.

33. Muoio, D.M., and Newgard, C.B. (2008). Mechanisms of disease:Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat. Rev. Mol. Cell Biol. *9*, 193–205. https://doi.org/10.1038/nrm2327.

34. DeFronzo, R.A., Ferrannini, E., Groop, L., Henry, R.R., Herman, W.H., Holst, J.J., Hu, F.B., Kahn, C.R., Raz, I., Shulman, G.I., et al. (2015). Type 2 diabetes mellitus. Nat. Rev. Dis. Prim. *1*, 15019. https://doi.org/10.1038/nrdp.2015.19.

35. Hasegawa, Y., Daitoku, Y., Mizuno, S., Tanimoto, Y., Mizuno-Iijima, S., Matsuo, M., Kajiwara, N., Ema, M., Oishi, H., Miwa, Y., et al. (2014). Generation and characterization of Ins1-cre-driver C57BL/6N for exclusive pancreatic beta cell-specific Cre-loxP recombination. Exp. Anim. *63*, 183–191. https://doi.org/10.1538/expanim.63.183.

36. Dor, Y., Brown, J., Martinez, O.I., and Melton, D.A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature *429*, 41–46. https://doi.org/10.1038/nature02520.

37. Magnuson, M.A., and Osipovich, A.B. (2013). Pancreas-specific Cre driver lines and considerations for their prudent use. Cell Metabol. *18*, 9–20. https://doi.org/10.1016/j.cmet.2013.06.011.

38. Cavaghan, M.K., Ehrmann, D.A., and Polonsky, K.S. (2000). Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. J. Clin. Invest. *106*, 329–333. https://doi.org/10.1172/jci10761.

39. Yong, J., Johnson, J.D., Arvan, P., Han, J., and Kaufman, R.J. (2021). Therapeutic opportunities for pancreatic β-cell ER stress in diabetes mellitus. Nat. Rev. Endocrinol. *17*, 455–467. https://doi.org/10.1038/s41574-021-00510-4.

40. Gerber, P.A., and Rutter, G.A. (2017). The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus. Antioxidants Redox Signal. *26*, 501–518. https://doi.org/10.1089/ars.2016.6755.

41. Weyer, C., Bogardus, C., Mott, D.M., and Pratley, R.E. (1999). The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J. Clin. Invest. *104*, 787–794. https://doi.org/10.1172/jci7231.

42. Kainoh, K., Takano, R., Sekiya, M., Saito, K., Sugasawa, T., Ma, Y., Murayama, Y., Sugano, Y., Osaki, Y., Iwasaki, H., et al. (2021). CtBP2 confers

protection against oxidative stress through interactions with NRF1 and NRF2. Biochem. Biophys. Res. Commun. *562*, 146–153. https://doi.org/10.1016/j.bbrc.2021.05.069.

43. Sekiya, M., Yahagi, N., Tamura, Y., Okazaki, H., Igarashi, M., Ohta, K., Takanashi, M., Kumagai, M., Takase, S., Nishi, M., et al. (2009). Hormone-sensitive lipase deficiency suppresses insulin secretion from pancreatic islets of Lep ob/ob mice. Biochem. Biophys. Res. Commun. *387*, 511–515. https://doi.org/10.1016/j.bbrc.2009.07.078.
44. Prentki, M., Matschinsky, F.M., and Madiraju, S.R.M. (2013). Metabolic signaling in fuel-induced insulin secretion. Cell Metabol. *18*, 162–185. https://doi.org/10.1016/j.cmet.2013.05.018.
45. Unger, R.H., and Zhou, Y.T. (2001). Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. Diabetes *50*, S118–S121. https://doi.org/10.2337/diabetes.50.2007.s118.
46. Talchai, C., Xuan, S., Lin, H.V., Sussel, L., and Accili, D. (2012). Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell *150*, 1223–1234. https://doi.org/10.1016/j.cell.2012.07.029.
47. Gu, C., Stein, G.H., Pan, N., Goebbels, S., Hörnberg, H., Nave, K.A., Herrera, P., White, P., Kaestner, K.H., Sussel, L., and Lee, J.E. (2010). Pancreatic beta cells require NeuroD to achieve and maintain functional maturity. Cell Metabol. *11*, 298–310. https://doi.org/10.1016/j.cmet.2010.03.006.
48. Odagiri, H., Wang, J., and German, M.S. (1996). Function of the human insulin promoter in primary cultured islet cells. J. Biol. Chem. *271*, 1909–1915. https://doi.org/10.1074/jbc.271.4.1909.
49. Ishikawa, M., Okajima, F., Inoue, N., Motomura, K., Kato, T., Takahashi, A., Oikawa, S., Yamada, N., and Shimano, H. (2006). Distinct effects of pravastatin, atorvastatin, and simvastatin on insulin secretion from a beta-cell line, MIN6 cells. J. Atherosclerosis Thromb. *13*, 329–335. https://doi.org/10.5551/jat.13.329.
50. Liu, H., Hew, H.C., Lu, Z.G., Yamaguchi, T., Miki, Y., and Yoshida, K. (2009). DNA damage signalling recruits RREB-1 to the p53 tumour suppressor promoter. Biochem. J. *422*, 543–551. https://doi.org/10.1042/bj20090342.
51. Nakagawa, Y., Kumagai, K., Han, S.I., Mizunoe, Y., Araki, M., Mizuno, S., Ohno, H., Matsuo, K., Yamada, Y., Kim, J.D., et al. (2021). Starvation-induced transcription factor CREBH negatively governs body growth by controlling GH signaling. FASEB J. *35*, e21663. https://doi.org/10.1096/fj.202002784RR.
52. Amemiya-Kudo, M., Oka, J., Ide, T., Matsuzaka, T., Sone, H., Yoshikawa, T., Yahagi, N., Ishibashi, S., Osuga, J.I., Yamada, N., et al. (2005). Sterol regulatory element-binding proteins activate insulin gene promoter directly and indirectly through synergy with BETA2/E47. J. Biol. Chem. *280*, 34577–34589. https://doi.org/10.1074/jbc.M506718200.
53. Kato, T., Shimano, H., Yamamoto, T., Ishikawa, M., Kumadaki, S., Matsuzaka, T., Nakagawa, Y., Yahagi, N., Nakakuki, M., Hasty, A.H., et al. (2008). Palmitate impairs and eicosapentaenoate restores insulin secretion through regulation of SREBP-1c in pancreatic islets. Diabetes *57*, 2382–2392. https://doi.org/10.2337/db06-1806.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-CtBP1 mAb	BD Biosciences	Cat#: 612042; RRID: AB_399429
Mouse anti-CtBP2 mAb	BD Biosciences	Cat#: 612044; RRID: AB_399431
Mouse anti-FLAG mAb	SIGMA	Cat#: F1804; RRID: AB_262044
Rabbit anti-HA mAb	Cell Signaling	Cat#: 3724; RRID: AB_1549585
Rabbit anti-insulin (C27C9) mAb	Cell Signaling	Cat#: 3014; RRID: AB_2126503
Mouse anti-alpha tubulin mAb	SIGMA	Cat#: T6199; RRID: AB_477583
Mouse anti-RREB1 mAb	Santa Cruz	Cat#: sc-515600; RRID: AB_2277191
Rabbit anti-CHOP (DDIT3) mAb	Cell Signaling	Cat#: D46F1; RRID: AB_10694399
Mouse anti-GAPDH mAb	Santa Cruz	Cat#: sc-32233; RRID: AB_627679
Rabbit anti-NEUROD1 (D35G2) mAb	Cell Signaling	Cat#: 4373; RRID: AB_10545768
Gout anti-CtBP2 (E−16) pAb	Santa Cruz	Cat#: sc-5966; RRID: AB_2086774
Rabbit anti-CtBP2 pAb	Active Motif	Cat#: 61261; RRID: AB_2793573
Normal mouse IgG	Santa Cruz	Cat#: sc-2025; RRID: AB_737182
Normal rabbit IgG	Cell Signaling	Cat#: 2729; RRID: AB_1031062
Rabbit anti-H3K4ac (EPR16596) mAb	Abcam	Cat#: ab176799
Mouse anti-H3K4me2 mAb	Active Motif	Cat#: 39679;RRID: AB_2793302
Rabbit anti-H3K4me3 pAb	Abcam	Cat#: ab8580; RRID: AB_306649
Rabbit anti-H3K9ac pAb	Abcam	Cat#: ab10812; RRID: AB_297491
Rabbit anti-H3K27ac pAb	Active Motif	Cat#: 39133; RRID: AB_2561016
Rabbit anti-H3K27me3 pAb	Active Motif	Cat#: 39155; RRID:AB_2561020
Normal goat IgG	Santa Cruz	Cat#: sc-2028; RRID:AB_737167
Guinea Pig anti-insulin pAb	Abcam	Cat#: ab7842; RRID:AB_306130
Rabbit anti-glucagon pAb	DAKO	Cat#: A0565; RRID:AB_10013726
Goat anti-mouse IgG Fab' Fragment Alexa Fluor 488	Cell Signaling	Cat#: 4408; RRID:AB_10694704
Goat anti-mouse IgG (H + L), F(ab')2 Fragment Alexa Fluor 555	Cell Signaling	Cat#: 4409; RRID:AB_1904022
Goat anti-Guinea pig IgG (H + L), Alexa Fluor 568	Abcam	Cat#: ab175714; RRID:AB_2864763
Bacterial and virus strains		
E coli, DH5alpha (DE3)	This paper	N/A
Adenovirus	This paper	N/A
Biological samples		
Human pancreas from brain dead patients	AnaBios	N/A
Chemicals, peptides, and recombinant proteins		
Sepazol reagent	Nacalai	Cat # 09379-97
Prime Script RT master mix	Takara	Cat # RR036A
Dynabeads protein G	Thermo	Cat # 10004D
Lipofectamine 3000 reagent	Thermo	Cat #L3000008
anti-FLAG magnetic beads	Sigma	Cat #M8823
3x FLAG peptide	Sigma	Cat #F4799
Tamoxifen	Sigma	Cat #T5648
FluorSave Reagent	Calbiochem	Cat # 345789

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
mouse recombinant leptin	Peprotech	Cat # 450-31
Critical commercial assays		
RNeasy Micro Kit	Qiagen	Cat # 74004
Qubit dsDNA HS assay kit	Thermo	Cat #Q32851
Magna ChIP HiSens Chromatin immunoprecipitation system	Merck Millipore	Cat # 17-10460
NEBNext Ultra II DNA Library Prep Kit	New England Biolabs	Cat #E7645
Dual-Luciferase Reporter Assay System	Promega	Cat #E1910
Mouse insulin enzyme-linked immunosorbent assay kit	Wako Pure Chemicals	Cat # 634-01481
Glucagon-HS ELISA kit	Yanaihara Institute Inc	Cat # YK-091
Poly(A) mRNA Magnetic Isolation Module	New England Biolabs	Cat #E7490
NEBNext Ultra II Directional RNA Library Prep Kit	New England Biolabs	Cat# E7765
Deposited data		
Global transcriptional landscape of CtBP2 in pancreatic beta cells (ChIP-seq/RNA-seq)	GEO	GSE221259
Experimental models: Cell lines		
MIN6 pancreatic beta cell line	Ishikawa M et al. J Atheroscler Thromb 2006[49]	N/A
HEK293 embryonic kidney cells	ATCC	Cat # CRL-1573
Experimental models: Organisms/strains		
CtBP2 flox mice	Sekiya M et al. Nat Commun 2021[15]	N/A
Ins1-Cre Tg mice	Hasegawa Y et al. Exp Anim 2016[35]	N/A
Ins2-Cre ERT2 Tg mice	Dor Y et al. Nature 2004[36]	Jackson #008122
Oligonucleotides		
Sequences of primers used in this study are listed in Table S1.		
Recombinant DNA		
RREB1 expression plasmid	Liu H et al. 2009[50] PMID: 19558368	N/A
pcDNA3.1 CtBP2-HA	Sekiya M et al. 2021[15] PMID: 34728642	N/A
pcDNA3.1 FLAG-NeuroD1	This paper	N/A
HA-ubiquitin expression plasmid	Nakagawa Y et al. 2021[51] PMID: 27053096	N/A
pRL-SV40	Kainoh K et al. 2021[42] PMID: 34052660	N/A
pGL2 human insulin gene promoter (−638bp)	This paper	N/A
pCMV7 TCF3 (E47)	Amemiya-Kudo M et al. 2005[52] PMID: 16055439	N/A
Software and algorithms		
BWA	PMID:19451168	RRID: SCR_010910, URL: http://bio-bwa.sourceforge.net/
MACS2	PMID:18798982	RRID: SCR_013291, URL: https://github.com/macs3-project/MACS
CEAS	PMID: 16845068	N/A
MEME Suite	PMID:19458158	RRID: SCR_001783, URL: http://meme-suite.org/
GREAT	PMID:20436461	RRID: SCR_005807, URL: http://great.stanford.edu/public/html/splash.php

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
HISAT2	PMID:25751142	RRID: SCR_015530, URL: http://ccb.jhu.edu/software/hisat2/index.shtml
Samtools	PMID:19505943	RRID: SCR_002105, URL: https://github.com/samtools/samtools
featureCounts	PMID:24227677	RRID: SCR_012919, URL: http://bioinf.wehi.edu.au/featureCounts/
stats, gplots in R packages	PMID:18252159	RRID: SCR_001905, URL: http://www.r-project.org/
IPA	N/A	RRID: SCR_008653, URL: http://www.ingenuity.com/products/pathways_analysis.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Motohiro Sekiya (msekiya@md.tsukuba.ac.jp).

Materials availability

This study did not generate new unique reagents.

Data and code availability

RNA-seq and ChIP-seq data have been deposited at the Gene Expression Omnibus database and are available as of the date of publication. Accession numbers are listed in the key resources table. 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 STUDY PARTICIPANT DETAILS

Animals

The research protocol was approved by the Animal Care Committee, University of Tsukuba, and all experimental procedures involving animals were conducted according to the guidelines. All mice used were males and maintained on a 14-h light and 10-h dark period cycle with free access to water and a standard chow diet. We generated two lines of pancreatic β-cell specific CtBP2-deficient mouse models by crossing the CtBP2 flox mice[15] with either mouse *Ins1* promoter-Cre transgenic mice[35] (Ins1C2KO) or inducible rat *Ins2* promoter-Cre transgenic mice (Jackson #008122)[36] (ERT2C2KO). Tamoxifen (1 mg/mL, Sigma) was administered in the drinking water at the age of ~6 weeks for 7 days to achieve the inducible Cre-mediated recombination. Glucose tolerance tests were performed at the age of 16–20 weeks by intraperitoneal glucose (1 g/kg) administration following an overnight fast, and insulin tolerance tests were performed at the age of 17–21 week by intraperitoneal administration of insulin (0.25 U/kg) following a 3 h food withdrawal. Plasma insulin and glucagon levels were determined with the mouse insulin enzyme-linked immunosorbent assay kit (Wako Chemicals) and glucagon-HS ELISA kit (YK-091, Yanaihara Institute Inc).

Cell culture

MIN6 murine pancreatic β-cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco 11965) containing 25 mM glucose, 100 U/ml penicillin and 100 g/mL streptomycin sulfate supplemented with 15% fetal bovine serum (FBS) and 5 μL/L β-mercaptoethanol. Human embryonic kidney (HEK)293 cells were maintained in DMEM supplemented with 10% FBS.

METHOD DETAILS

Quantitative real-time RT-PCR

Total RNA was isolated using Sepazol Reagent (Nacalai) and cDNA was synthesized with PrimeScript RT Master Mix (Takara Bio). Quantitative real-time PCR analysis was performed using SYBR Green in 7300 Real-Time PCR systems (Applied Biosystems). Data were normalized to 18s ribosomal RNA or acidic ribosomal phosphoprotein P0 (*Rplp0*, 36B4).

Western blot analysis and co-immunoprecipitation experiments

The plasmid encoding RREB1 cDNA is a generous gift from Kiyotsugu Yoshida (Jikei University School of Medicine).[50] Proteins were extracted from cells with buffer A (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 10 mM NaF, 2 mM Na_3VO_4)

with complete protease inhibitors (Sigma-Aldrich) and subjected to SDS-polyacrylamide gel electrophoresis. Membranes were incubated with anti-CtBP1 (BD, 612042), anti-CtBP2 (BD, 612044), anti-FLAG (Sigma, F1804), anti-HA (Cell Signaling, 3724), anti-Insulin (Cell Signaling, C27C9), anti-alpha tubulin (Sigma, T6199) anti-RREB1 (Santa Cruz sc-515600), anti-DDIT3 antibody (Cell Signaling, D46F1) and anti-GAPDH (Santa Cruz, sc-32233). The membranes were incubated with secondary antibody conjugated with horseradish peroxidase (Cell Signaling) and were visualized using an enhanced chemiluminescence system (BioRad). To detect endogenous binding of NEUROD1 and CtBP2, the anti-NEUROD1 (Cell Signaling, D35G2), anti-CtBP2 (Santa Cruz E−16, Active Motif 61261 or BD 612044), control mouse IgG (Santa Cruz) or control goat IgG (Santa Cruz) were cross-linked to Protein G dynabeads (Thermo) with 50 mM dimethyl pimelimidate (Sigma-Aldrich). The protein complex was immunoprecipitated in buffer A with reduced concentration of NP40 (0.5%) (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 10 mM NaF, 2 mM Na_3VO_4) for 4 h at 4°C. The beads were washed with the buffer A with 0.5% NP40 four times, eluted with SDS loading buffer and analyzed by Western blot analysis. FLAG tag co-immunoprecipitation was performed as follows. HEK293 cells were transiently transfected with either control plasmid, FLAG-NEUROD1 along with CtBP2 using lipofectamine3000 (Thermo). Cells were lysed with buffer A with 1% NP40 and immunoprecipitated with FLAG M2 magnetic beads (Sigma) in buffer A with 0.5% NP40 for 4 h at 4°C. The beads were washed four times with buffer A with 0.5% NP40 and eluted with 0.5 mg/mL of 3x FLAG peptide (Sigma). Thereafter, the NEUROD1/CtBP2 complex was eluted and analyzed in the same way.

Isolation of mouse pancreatic islets

Mouse pancreatic Islets were isolated using the collagenase technique from 16 to 20 week old mice, as described previously.[43] Proteins were extracted using the buffer A and subjected to SDS-polyacrylamide gel electrophoresis. RNA was extracted using the RNeasy Micro kit (Qiagen). Glucose stimulated insulin secretion was performed as follows: isolated islets were cultured in RPMI1640 supplemented with 10% FBS and 1% penicillin and streptomycin overnight. Five islets from each mouse were preincubated for 30 min in low glucose buffer (130 mM NaCl, 5.2 mM KCl, 1.3 mM KH_2PO_4, 2.7 mM $CaCl_2$, 1.3 mM $MgSO_4$, 24.8 mM $NaHCO_3$, and 10 mM HEPES at pH 7.4) containing 2.8 mM glucose and 0.5% bovine serum albumin (BSA; Fraction V; Sigma, St. Louis, MO). Thereafter the islets were incubated in the same low glucose buffer for 30 min (low glucose stimulation). The islets were further incubated in the same buffer with either high glucose (20 mM) or KCl (30 mM)/low glucose for 30 min. DNA was isolated with the alkaline lysis method and quantified using Qubit dsDNA HS assay kit (Thermo). Insulin content was measured in acid/ethanol extracts as reported previously.[53] DNA content was measured with the Qubit system (Thermo) for normalization.

Chromatin immunoprecipitation (ChIP)

MIN6 cells were washed and fixed in 1% formaldehyde for 10 min at room temperature. Crosslinking was quenched by adding glycine to a final concentration of 125 mM. Thereafter, ChIP was carried out using Magna ChIP HiSens Chromatin Immunoprecipitation system (EMD Millipore). Chromatin shearing was achieved by sonication (Bronson sonifier 250). Chromatin was immunoprecipitated with either control IgG (Cell Signaling), anti-CtBP2 (Active Motif, 61261), anti-H3K4ac (Abcam, EPR16596), anti-H3K4me2 (Active Motif, 39679), anti-H3K4me3 (Abcam, ab8580), anti-H3K9ac (Abcam, ab10812), anti-H3K9me2 (Active Motif, 39683), anti-H3K27ac (Active Motif, 39133) or anti-H3K27me3 (Active Motif, 39155). Immunoprecipitated DNA and input DNA were quantified by real-time PCR with primers specific for the *Ins1 or Ins2* gene promoter. For sequential ChIP (re-ChIP), HEK293 cells were transiently transfected with FLAG-NEUROD1 and CtBP2 plasmids. The chromatin was first enriched by FLAG magnetic beads (Sigma) and eluted with 3xFLAG peptide (Sigma). Afterward, the second ChIP experiment was performed with either control goat IgG (Santa Cruz) or anti-CtBP2 antibody (Active Motif 61261). The immunoprecipitated DNA was quantified using quantitative real-time PCR with primers specific to the human *INS* gene promoter.

ChIP-seq analysis and downstream data mining

The chromatin immunoprecipitated DNA and input DNA were obtained as described above using the anti-CtBP2 antibody (Active Motif 61261). The sequencing library was constructed with NEBNext Ultra II DNA Library Prep Kit (New England Biolabs). Paired-end sequencing was performed with an Illumina NestSeq500 system. By mapping the sequencing reads to the mouse genome mm9 using BWA (Burrows Wheeler Aligner, version 0.7.5), 38,278,790 final reads were detected. Subsequently, the binding peaks were obtained by model-based analysis of ChIP-seq (MACS2). The peak-related genes were screened based on the number, width and distribution of the peaks. Genomic distribution of CtBP2 binding sites were analyzed using the *cis*-regulatory element annotation system (CEAS). Consensus motifs of CtBP2 binding sites were extracted using MEME (Multiple EM for Motif Elicitation)-ChIP program. To find candidate transcription factors bound to CtBP2, these motifs were compared against databases of known motifs using TOMTOM. Enrichment of gene ontology processes associated with the peaks was analyzed by GREAT (Genomic Regions Enrichment of Annotations Tool). Source: GEO accession No. GSE221259 (superseries number containing both ChIP-seq and RNA-seq data).

Luciferase assays

Each expression plasmid, luciferase reporter plasmid (human insulin promoter containing −638 bp), and pSV40-Renilla plasmid were transiently co-transfected using Lipofectamine 3000 reagent (Thermo) according to the manufacturer's instructions. HEK293 cells were seeded in 24-well plates at a density of 2.5×10^5 cells/well. After 32-h of transfection the media were changed to fresh media,

and after additional 4-h incubation, luciferase assays were carried out using the Dual-Luciferase Reporter Assay System (Promega), and luciferase activity was quantified with a Varioskan LUX multimode microplate reader (Thermo). As an internal standard, SV40 Renilla luciferase control vector was also co-transfected to normalize transfection efficiencies.

Immunohistochemistry

Formalin-fixed pancreatic tissues were embedded in paraffin, and 3-μm-thick sections were used for immunostaining. After dewaxing and rehydration, sections were boiled in citrate buffer (pH 6.0) for 10 min at 121°C for antigen retrieval. After permeabilization with 0.1% Triton X-100 for 10 min, sections were blocked with 5% skim milk for 1 h. Thereafter, the sections were incubated with diluted primary antibodies (anti-CtBP2 (BD 612044) 1:100, anti-insulin (Abcam ab7842) 1:100, and anti-glucagon (Dako A0565)) overnight at 4°C. After washing, the sections were incubated with secondary antibodies (anti-mouse IgG Fab' fragment Alexa Fluor 488 (4408, Cell Signaling, 1:500), anti-mouse IgG Fab' fragment Alexa Fluor 555 (4409, Cell signaling, 1:500) and anti-guinea pig IgG Alexa Fluor 568 (ab175714, Abcam, 1:500) for 1 h at room temperature. Finally, the sections were embedded in FluorSave reagents (Calbiochem) and imaged under a fluorescence microscope (Biozero) at room temperature. Pancreatic specimens from human donors were obtained using the service of AnaBios (San Diego, CA). The samples were obtained at autopsy from male brain dead patients with indicated body mass index (BMI). Approval for this study was granted by the Medical Ethics Committee, University of Tsukuba. Images were captured by means of a BZ-8000 microscopic system with a 10x eyepiece lens and a 10x objective lens (Keyence).

RNA-seq analysis and downstream data mining

Poly(A) mRNA was purified with the poly(A) mRNA Magnetic Isolation Module (New England Biolabs). The sequencing library was constructed with NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs) and sequencing was performed using NovaSeq 6000 (Illumina). The quality of the raw paired-end reads was assessed with FastQC (ver 0.11.7). After trimming, the reads were aligned to the mm10 mouse reference genome using HISAT2 (ver 2.1.0). Sam files were converted into bam files with Samtools (ver 1.9) and the bam files were used to estimate the abundance of uniquely mapped reads with featureCounts (ver 1.6.3). The raw read counts were normalized with transcripts per million (TPM) and clustered with Wald method based on Eucidean distances of the normalized counts using stats (ver 3.6.1) and gplots (ver 3.0.1.1) R packages. The downstream pathway analyses were performed using Ingenuity Pathway Analysis (IPA) software based on the TPM values. Source: GEO accession No. GSE221259 (superseries number containing both ChIP-seq and RNA-seq data).

QUANTIFICATION AND STATISTICAL ANALYSIS

Western blot data were quantified using ImageJ software (NIH). Statistical analyses were carried out with a two-tailed unpaired Student's t-test.