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Mettl3 deficiency leads to impaired insulin secretion via regulating Ire1a of mature β-cells in mice

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The modification of N6-methyladenosine (m^6A) influences the translation and stability of transcripts, allowing for the coordination of gene regulation during cell state maintenance and transition. Deregulation of components in the m^6A regulatory network is associated with glucose homeostasis and development of diabetes. In this study, we investigated the functional role of Mettl3, which is the key component of the m^6A methyltransferase complex, in regulating β-cell identity and function in two pancreatic β-cell-specific Mettl3 knockout mouse models. The glucose metabolic phenotype, β-cell proliferation, islet architecture and insulin secretion were analyzed in vivo. We next analyzed the expression levels of genes associated with endoplasmic reticulum (ER) stress in the Mettl3 ablated islets. MeRIP-qPCR was applied to detect the m^6A modification enrichment of Ire1α mRNA. Adenovirus-mediated Mettl3 infection was performed on islets to explore the effect of Mettl3 overexpression on ER stress and insulin secretion. Our results showed that Mettl3 deficiency led to loss of β-cell identity and impaired insulin secretion in mice. Depletion of Mettl3 verified the m^6A modification in Ire1α and consequently induced ER stress in islet cells. Mettl3 overexpression in islets could alleviate ER stress and improve the insulin secretion capacity. Our findings demonstrated that Mettl3 was an important regulator of ER stress and insulin secretion in mouse pancreatic β-cells.

Keywords m^6A methyltransferase, Mettl3, Pancreatic β-cell, Insulin secretion, ER stress, Ire1α

Type 2 Diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by impaired insulin secretion and insulin resistance[1,2]. An inadequate functional β-cell mass, resulted from β-cell exhaustion and apoptosis, contributes to development of T2DM[3–6]. Recently, it has been suggested that the loss of β-cell identity, or β-cell dedifferentiation, may also contribute to the pathogenesis of T2DM[7–9].

N6-methyladenosine (m^6A) RNA methylation is the most predominant modification in eukaryotic messenger RNA (mRNA)[10]. The deposition of m^6A onto RNA is predominantly carried out by methyltransferase complexes consisting of multiple subunits including METTL3, METTL14 and WTAP, commonly referred to as m^6A writers. The m^6A modification of RNA can be reversed by demethylases including FTO and ALKBH5, known as m^6A erasers, rendering m^6A methylation a reversible and dynamic process. Another class of regulatory proteins, the m^6A readers, comprising RNA binding proteins (RBPs), selectively recognize and bind to m^6A sites, dictating the destiny of the target RNA[10,11]. This dynamic and reversible modification of RNA plays a critical role in determining the destiny of modified RNA molecules at the post-transcriptional level, exerting a pervasive impact on a wide range of fundamental biological processes.

Deregulation of m^6A methylation has been linked to the development of a range of metabolic conditions, including obesity, diabetes, and metabolic syndrome[12,13]. Increased expression of the FTO gene may contribute to lower levels of m^6A in individuals with T2DM[14]. Additionally, METTL3, the key component of m^6A writers, is crucial for regulating metabolic disorders triggered by a high-fat diet, such as hepatogenous diabetes[15]. The protein levels of METTL3 and METTL14 were reduced in the islets from T2DM patients, which also exhibited decreased levels of PDX1 in comparison to the control group[16]. Recent studies have shown that m^6A regulatory proteins played significant roles in β-cell maturation, proliferation, survival and function[16–22]. m^6A reader proteins YTHDC1 and IMP2/IGF2BP2 were reported to regulate pancreatic β-cell function in the knockout

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models[17,18]. The key components of m^{6}A writers Mettl3 and Mettl14 have been reported to play an essential role in neonatal β-cell development and functional maturation in a mouse model with Mettl3/14 deleted in Ngn3$^+$ endocrine progenitors[19]. Ablation of Mettl14 in pancreatic β-cells led to glucose intolerance and impaired insulin secretion in mice, and Mettl14 deficiency led to alterations in gene expression responsible for inflammatory response and endoplasmic reticulum (ER) stress[20,21]. Under inflammatory and oxidative stress conditions, Mettl3 expression is downregulated, and deficiency of Mettl3 specifically in islet β-cells leads to β-cell dysfunction and hyperglycemia[22]. However, the mechanism of Mettl3 regulating the function of mature β-cell is not well elucidated. In this study, we generated two mouse models with β-cell-specific ablation of Mettl3 to explore its role and mechanism in regulating insulin secretion and glucose metabolism. Our results demonstrated that Mettl3 deficiency led to loss of β-cell identity and impaired insulin generation in mice. Depletion of Mettl3 verified the m^{6}A modification in Ire1α and consequently induced ER stress in islet cells, while overexpression of Mettl3 in islets could reduce ER stress and ameliorate insulin secretion.

Materials and methods

Animal models

We generated conditional β-cell Mettl3 knockout mice using the Cre/loxP system. Mettl3$^{flox/flox}$ mice, obtained from the laboratory of Dr. Binghua Li, were bred with mice expressing Cre recombinase driven by the mouse Ins1 promoter (MIP-Cre)[23] to generate the MKO-MIP mice. The MKO-RIP mice were generated by crossing Mettl3$^{flox/flox}$ mice with mice expressing Cre recombinase driven by the rat Ins2 promoter (RIP-Cre^{Herr}). Mettl3$^{flox/flox}$ mice were used as the control group. Mouse genotypes were confirmed by PCR using the primers listed in Table 1 (Supplementary Material). If not stated otherwise, the mice used for the experiments were males. All the mice were housed in pathogen-free facilities with a 12 h light/dark cycle. All the animal experiments were performed in accordance with the principles of Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The animal studies were approved by Institutional Animal Review Board.

Glucose tolerance test and insulin tolerance test

Fasting blood glucose levels of the MKO-MIP, MKO-RIP, and control mice were monitored at the indicated ages. Intraperitoneal glucose tolerance test (IPGTT) was performed after 12–14 h fasting with a glucose dose of 2 g/kg body weight. Serum insulin levels were assessed using a mouse insulin ELISA kit (Crystal Chem) by analyzing blood samples taken at 0, 15, and 30 min from mice. Insulin tolerance test (ITT) was performed after 6 h fasting with intraperitoneal injection of 0.75 U insulin/kg body weight. Blood glucose measurements were taken at 0, 15, 30, 60, 90, and 120 min using One-Touch Ultra glucometers (LifeScan).

Isolation and culture of mouse Islets

Isolation of mice islets was performed as previously described[24]. Mice were euthanized by 120 mg/kg sodium pentobarbital (i.p., Sigma-Aldrich). Subsequently, the pancreas was perfused by collagenase XI (Sigma-Aldrich) injection via bile duct. Then the pancreas was removed and dissociated by mechanical pipetting after incubation at 37 ℃ for 17 min. The islets were handpicked under a dissecting microscope after multiple purification steps. The islets were cultured in RPMI 1640 (Invitrogen) culture medium supplemented with 10% fetal bovine serum (Invitrogen) and 100 U/ml penicillin-streptomycin (Invitrogen) for recovery in a sterile incubator at 37 ℃ with 5% CO2 infusion and humidified air.

Islets treatment and adenoviral infection

For induction of ER stress in vitro, the isolated islets were treated with thapsigargin (300 nM) for 24 h after overnight recovery. The adenovirus expressing Mettl3 (Ad-Mettl3) was used for Mettl3 overexpression in islets. Ad-GFP was used as a control. After ER stress induction, the islets were infected with Ad-Mettl3 at 80 multiplicity of infection (MOI) for 72 h and harvested for RNA extraction and further analysis.

Glucose-stimulated insulin secretion test

For in vitro glucose stimulated insulin secretion test (GSIS), the isolated islets were recovered in 1640 RPMI supplemented with 10% serum for overnight, then the islets were preincubated in Krebs-Ringer bicarbonate (KRB) buffer containing 0.25% BSA and 2.8 mM glucose for 30 min. After that, the islets were incubated in pre-warmed KRH buffer containing low glucose (2.8 mM) or high glucose (16.7 mM) as indicated for another 60 min. The incubation mediums were immediately collected for measuring the insulin level. The islets were extracted using 0.18 mM HCl in 70% ethanol to assess insulin content. Insulin levels were quantified using a mouse insulin ELISA kit and normalized by total insulin content.

Immunofluorescence and immunohistochemical staining

The mouse pancreas was harvested and fixed in 4% PFA for at least 24 h, dehydrated and paraffin embedded. Immunofluorescence (IF) and immunohistochemical (IHC) staining were performed according to standard protocols as previously described[24]. The antibodies used in this study were listed in Fig. S1 (Supplementary Material). The images were acquired using a confocal microscope system (LSM 710, ZEISS). Quantification of cell number was analyzed by ImageJ software. The β-cell proliferation was analyzed using the percentage of Ki67$^+$ and insulin$^+$ cells, and at least 5,000 β-cells per animal were counted. The β-cell apoptosis was analyzed using the percentage of TUNEL$^+$ and insulin$^+$ cells and at least 8,000 β-cells per animal were counted.

RNA extraction and quantitative PCR

The total RNA of islets was extracted using the RNeasy mini kit (QIAGEN). The extracted total RNA was reverse transcribed into cDNA using the Primerscript RT master kit. Quantitative PCR (qPCR) was performed using

Applied Biosystem QuantStudio 7 Flex instrument with a SYBR Premix Ex Tap Kit (Takara). The expression levels were normalized to housekeeper gene GAPDH. All the qPCR primers were listed in Fig. S2 (Supplementary Material).

MeRIP quantitative PCR

MeRIP quantitative PCR was performed with the GenSeq™ m^{6}A MeRIP Kit (Cloudseq) by following the manufacturer's instructions. m^{6}A enrichment of both the input sample without immunoprecipitation and the m^{6}A IP samples was analyzed by qPCR with specific primers and data were normalized to input. The mRNA m^{6}A sites of Ire1α were predicted using the sequence-based RNA adenosine methylation site predictor (SRAMP) program. Primer sequences were listed in Fig. S2 (Supplementary Material).

Data quality assessment and statistics analysis

The Animals in Research Reporting In Vivo Experiments 2.0 (ARRIVE 2.0) guidelines were used to evaluate the reporting quality of the studies[25]. Significant differences were analyzed using two-tail unpaired Student's t-test for two independent datasets. For multiple comparisons, one-way or two-way analysis of variance (ANOVA) were used. The error bars in graphs represent standard deviation (SD). $P<0.05$ was considered as statistically significant.

Results

β-cell specific knockout of Mettl3 leads to glucose intolerance and impaired insulin secretion

The RIP-Cre^{Herr} offers high efficiency in inducing Cre-mediated recombination but may result in some leakage in the brain[26]. In contrast, the MIP-Cre provides high specificity for β-cells without leakage[23]. To target the Mettl3 gene in mature pancreatic β-cells for functional analysis in vivo, we breed Mettl3$^{flox/flox}$ mice with either RIP-Cre^{Herr} or MIP-Cre mice to generate the β-cell-specific Mettl3 knockout mouse models (MKO-RIP and MKO-MIP). The mRNA and protein levels of Mettl3 were confirmed to indicate the successful knockout of Mettl3 in half or most of the β-cells of MKO-MIP and MKO-RIP mice (Fig. 1A–D). The knockout efficiency of Mettl3 in β-cells was higher in MKO-RIP than MKO-MIP mice (Fig. 1A–D). We noticed that the MKO-RIP mice showed lower body weight compared to control mice (Mettl3$^{flox/flox}$), and the body weight of MKO-MIP mice was comparable to control mice (data not shown). Considering the different knockout efficiency and specificity of the two mouse models, both models were used for subsequent analysis.

We first characterized the glucose metabolic phenotype of MKO-RIP and MKO-MIP mice. The MKO-RIP mice suffered severe hyperglycemia with increased fasting blood glucose levels at 8 weeks compared to control mice, whereas the fasting blood glucose levels of MKO-MIP were normal (Fig. 1E and F). Both of the two mouse models showed impaired glucose tolerance upon glucose stimulation in vivo (Fig. 1G and H). Insulin tolerance tests suggested no impact of Mettl3 knockout on insulin sensitivity in the two mouse models (Fig. 1I and J). In MKO-RIP mice, there was a significant decrease in insulin secretion in response to glucose challenge (Fig. 2A), while the in vivo insulin secretion was not significantly reduced in MKO-MIP mice in response to glucose challenge (Fig. 2B). The MKO-RIP mice displayed a more pronounced phenotype, with more severe impairments in glucose tolerance and insulin secretion. In vitro glucose stimulated insulin secretion test showed the insulin secretion stimulated by 16.7 mM glucose was significantly decreased in both the MKO-MIP and MKO-RIP islets compared with control islets (Fig. 2C, D). Besides, the insulin content was reduced in the islets of both MKO-MIP and MKO-RIP mice compared to that of control mice (Fig. 2E, F). Furthermore, the mRNA levels of *Ins1* and *Ins2* were decreased in the islets of MKO-RIP and MKO-MIP mice (Fig. 2G and H). Altogether, these results indicated that knockout of Mettl3 in β-cells led to notable glucose intolerance and reduced insulin secretion and production in mice.

Mettl3 ablation led to decreased proliferation and increased apoptosis in β-cells

To investigate the potential impact of Mettl3 ablation on pancreatic β-cell abundance, cell proliferation and apoptosis were evaluated through Ki67 and TUNEL staining in islets of MKO-RIP mice. Our data showed approximately 69% reduction of Ki67$^+$ β-cells in MKO-RIP mice compared to that of control group (Fig. 3A and B). The TUNEL staining analysis identified a significant increase in β-cell apoptosis in MKO-RIP mice (Fig. 3C). These results indicated that Mettl3 deficiency increased β-cell apoptosis and reduced β-cell proliferation in mice. Ki67 and TUNEL staining in the islets of MKO-MIP mice would further strengthen the role of Mettl3 in β-cell proliferation and apoptosis.

Mettl3 knockout caused loss of β-cell identity and abnormal islet architecture

We further analyzed the islet architecture in MKO-RIP mice. The cell type composition was determined by immunostaining of four endocrine hormones including insulin secreted by β-cell, glucagon secreted by α-cell, somatostatin secreted by δ-cell, and pancreatic polypeptide secreted by PP cells. We found that upon Mettl3 knockout there was a dramatic increase of cells expressing glucagon, somatostatin or pancreatic polypeptide. The proportion of cells that were positive for both insulin and glucagon, relative to the total number of insulin-positive cells, was significantly increased in MKO-RIP mice. Similarly, the ratio of cells co-expressing both somatostatin and insulin, compared to insulin-positive cells, showed a significant elevation in MKO-RIP mice. And the ratio of cells positive for both pancreatic polypeptide and insulin to the count of insulin-positive cells was markedly higher in MKO-RIP mice (Fig. 3D and E). Immunostaining of the four endocrine hormones in the islet of MKO-MIP mice would further prove the impact of Mettl3 deficiency on the islet architecture. Besides that, mRNA levels of the critical transcription factors required for β-cell identity and function, including *Pdx1*, *MafA* and *Nkx6.1*, were reduced in the islets of MKO-RIP and MKO-MIP mice (Fig. 3F, G), consistent with previous reports[16,19]. Whereas, loss of Mettl3 increased the level of the marker genes of the other hormone cells

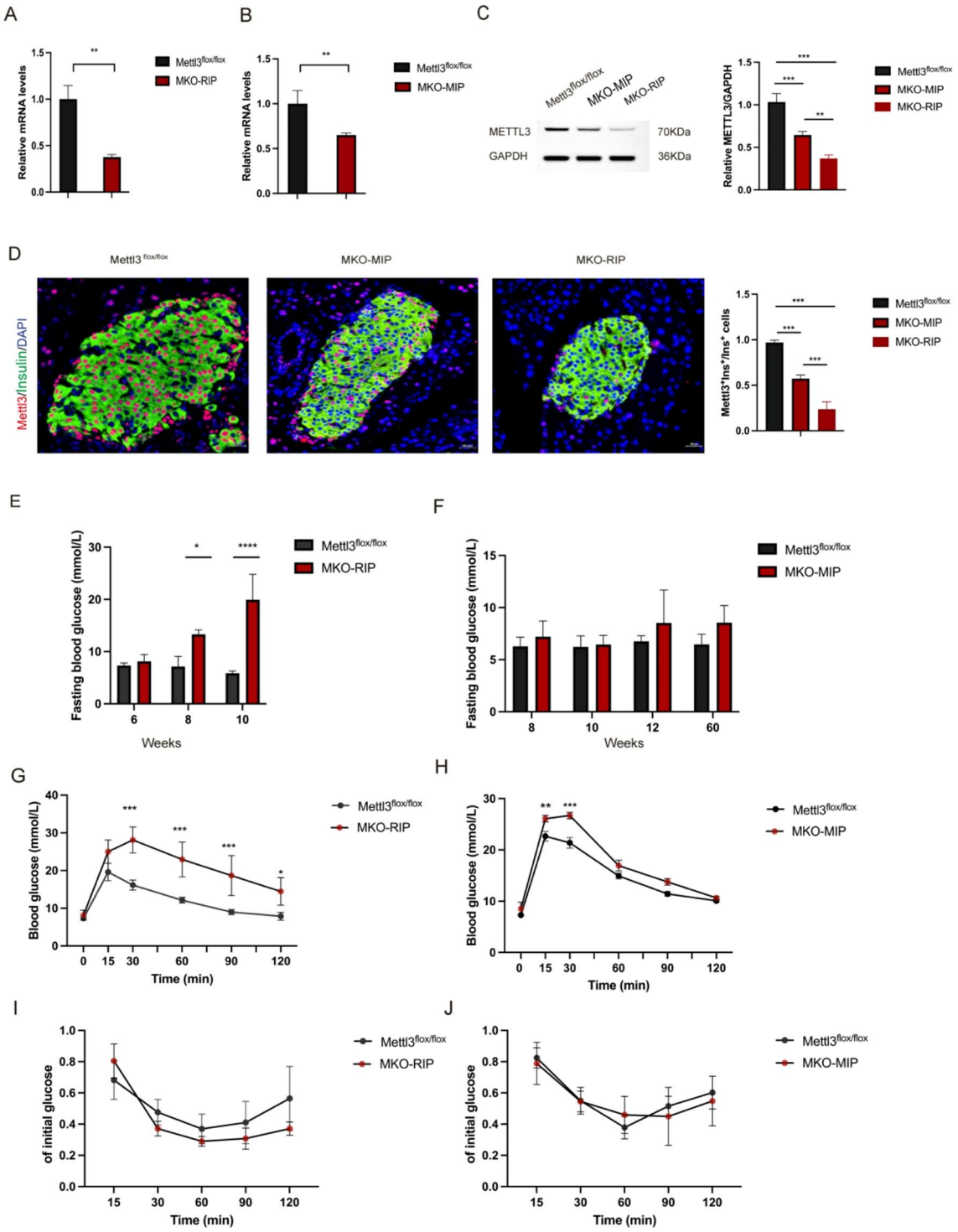

Fig. 1. Pancreatic β-cell specific knockout of Mettl3 led to glucose intolerance in mice. (**A**,**B**) mRNA levels of *Mettl3* in the islets of 8-week-old MKO-RIP, MKO-MIP and control mice ($n = 3$–4). (**C**) Protein levels of METTL3 in the islets of 8-week-old MKO-RIP, MKO-MIP and control mice ($n = 3$ for each group). (**D**) Immunofluorescence staining and analysis of Mettl3 and insulin on the pancreatic sections of 8-week-old control, MKO-MIP and MKO-RIP mice ($n = 3$ for each group). Scale bars, 20 μm. (**E**,**F**) Fasting blood glucose levels of MKO-RIP, MKO-MIP and control mice in the indicated ages ($n = 5$ for each group). (**G**) Intraperitoneal glucose tolerance tests of 8-week-old MKO-RIP and control mice ($n = 5$ for each group). (**H**) Intraperitoneal glucose tolerance tests of 10-week-old MKO-MIP and control mice ($n = 5$ for each group). (**I**,**J**) Intraperitoneal insulin tolerance tests of 10-week-old MKO-RIP, MKO-MIP and control mice, fold of initial glucose level was shown ($n = 5$ for each group). Male mice were used for the experiments. Data represent mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (**A**,**B**) two-tailed Unpaired Student's t-tests. (**C**,**D**) One-way ANOVA. (**E**–**J**) Two-way ANOVA.

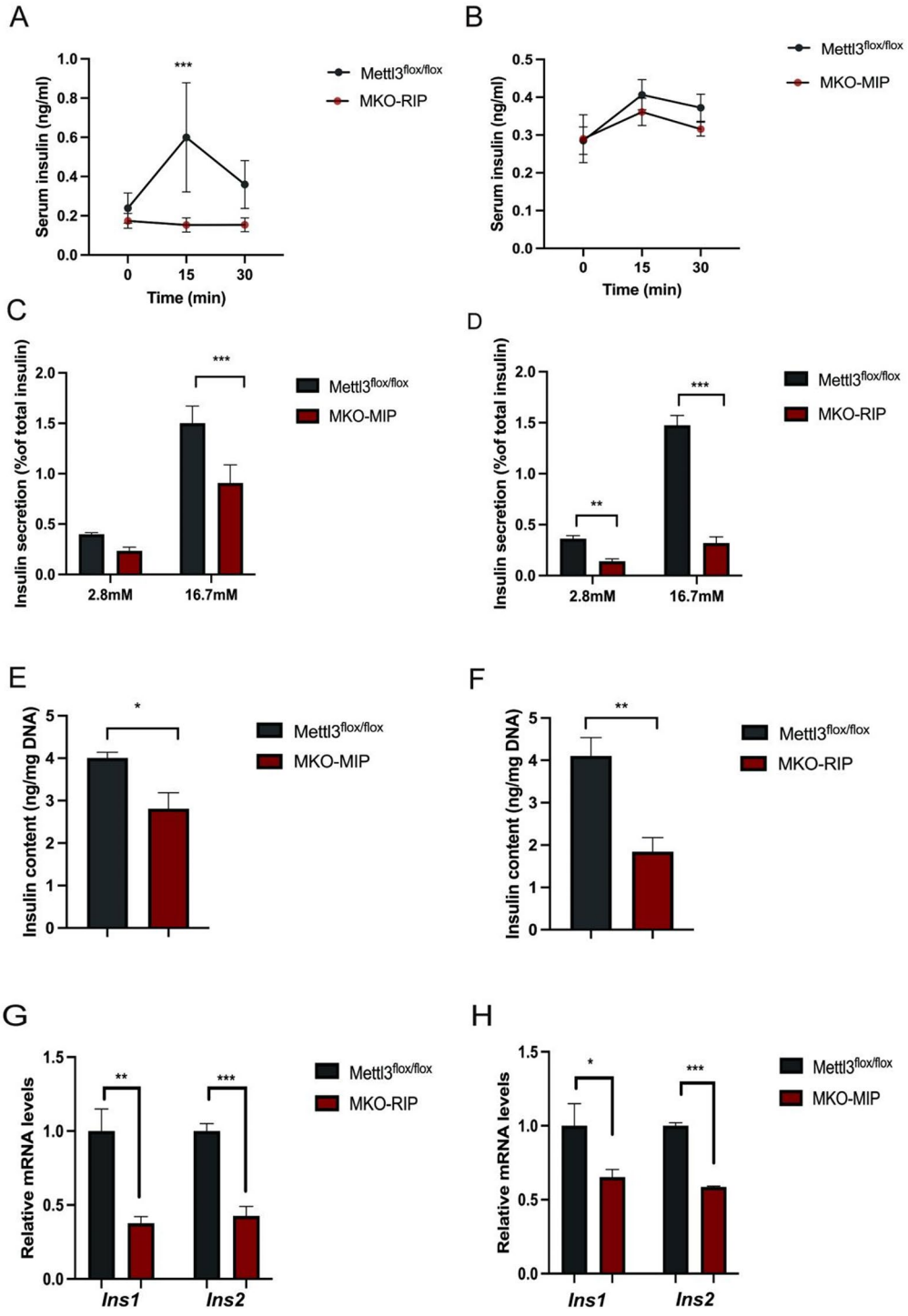

Fig. 2. Loss of Mettl3 impaired insulin production and secretion capacity of pancreatic β-cells in mice. (**A**) Serum insulin levels after glucose stimulation of 8-week-old MKO-RIP and control mice ($n = 5$ for each group). (**B**) Serum insulin levels after glucose stimulation of 12-week-old MKO-MIP and control mice ($n = 5$ for each group). (**C**) Glucose-stimulated insulin secretion (GSIS) in response to 2.8 mM and 16.7 mM glucose of isolated islets from 18-week-old MKO-MIP and control mice ($n = 3$ for each group). Insulin levels were normalized to the total insulin in the islets. (**D**) Glucose-stimulated insulin secretion (GSIS) in response to 2.8 mM and 16.7 mM glucose of isolated islets from 8-week-old MKO-RIP and control mice ($n = 3$ for each group). Insulin levels were normalized to the total insulin in the islets. (**E**) Islet insulin content of 18-week-old MKO-MIP and control mice ($n = 4$ for each group). (**F**) Islet insulin content of 8-week-old MKO-RIP and control mice ($n = 3$ for each group). (**G**,**H**) mRNA levels of *Ins1* and *Ins2* in the islets of MKO-RIP, MKO-MIP and control mice ($n = 3$–4). Male mice were used for the experiments. Data represent mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (**A**–**D**) Two-way ANOVA. (**E**–**H**) two-tailed Unpaired Student's t-tests.

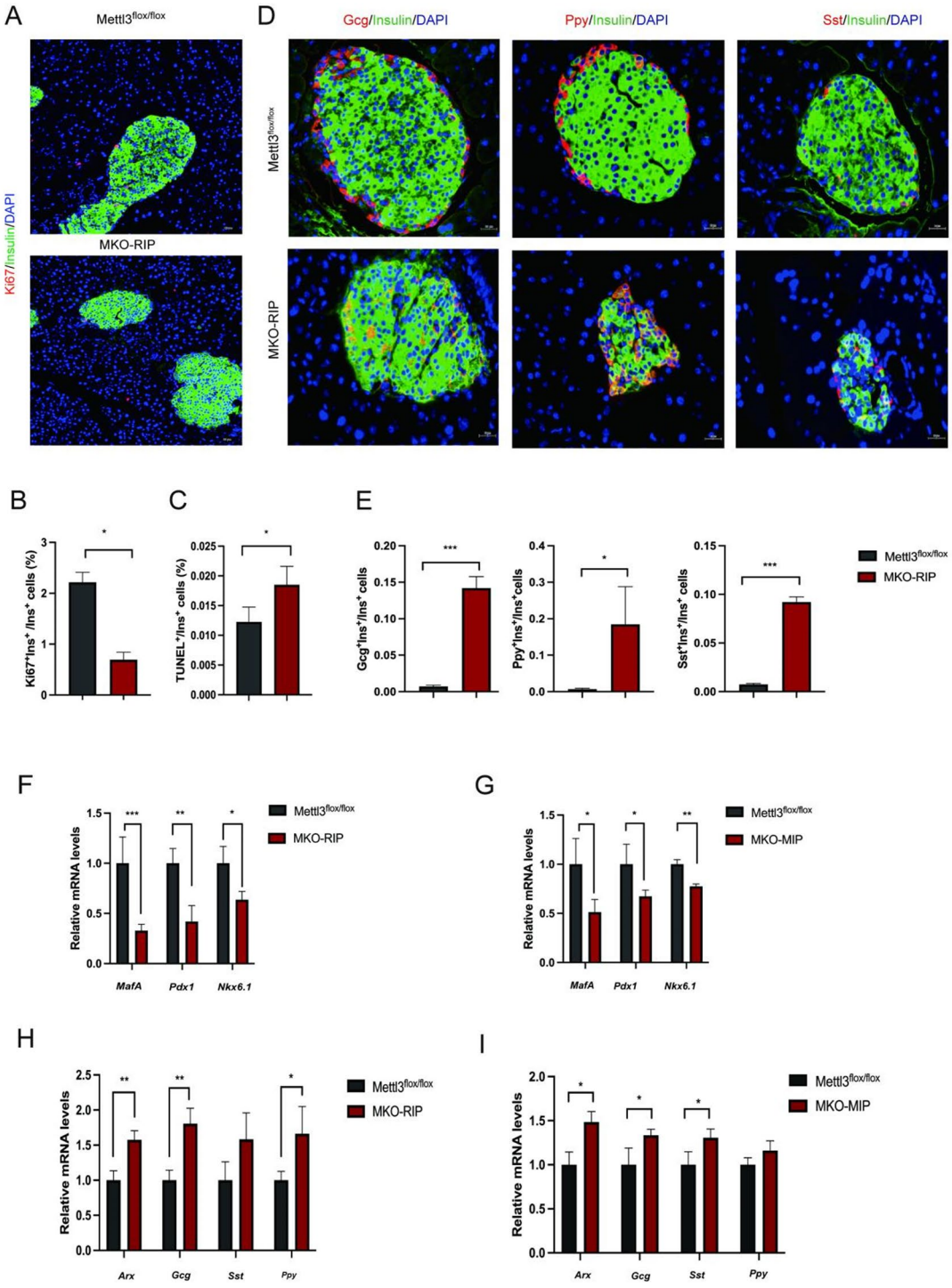

Fig. 3. Pancreatic β-cell specific knockout of Mettl3 reduced β-cell proliferation and led to loss of β-cell identity in mice. (**A**,**B**) Immunofluorescence staining and analysis of ki67 and insulin on the pancreatic sections of MKO-RIP and control mice ($n = 4$ for each group). Scale bars, 50 μm. (**C**) TUNEL analysis of pancreatic β-cells in the islets of MKO-RIP and control mice ($n = 3$ for each group). (**D**) Immunofluorescence staining of insulin, glucagon, somatostatin and pancreatic polypeptide on the pancreatic sections of MKO-RIP and control mice ($n = 3$). Scale bars, 20 μm. (**E**) The proportion of cells that were positive for both insulin and glucagon relative to the total number of insulin-positive cells, the ratio of cells co-expressing both somatostatin and insulin compared to insulin-positive cells, and the ratio of cells positive for both pancreatic polypeptide and insulin to the count of insulin-positive cells in the islets of MKO-RIP and control mice ($n = 3$ for each group). (**F**–**I**) mRNA levels of cell identity genes in the islets of MKO-RIP, MKO-RIP and control mice ($n = 3$ for each group). Male mice were used for the experiments. Data represent mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (**B**–**I**) two-tailed Unpaired Student's t-tests.

in the islets of MKO-RIP and MKO-MIP mice (Fig. 3H, I). It has been reported that the multi-hormone cell increase and loss of β-cell identity might be manifestations of β-cell transdifferentiation[27–29]. Taken together, out results demonstrated that Mettl3 deletion led to change of islet architecture and loss of β-cell identity, which may indicate the transdifferentiation of β-cells.

Mettl3 deletion verified the m^{6}A modification of Ire1α and resulted in ER stress in Islets

Multiple investigations implicate ER stress and inappropriate UPR in the etiology of T1DM and T2DM[30–32]. Specifically, alterations in genes that regulate ER stress responses, including but not limited to the major UPR components PERK, eIF2α, CHOP, XBP1, IRE1α and WSF1, frequently lead to β-cell dysfunction and/or apoptosis in experimental models and humans[32–34]. It was reported that Mettl14 deletion induced excessive ER stress in the regenerating liver[35]. We next analyzed the expression levels of genes associated with ER stress in the islets of the two mouse models. The mRNA and protein levels of Ire1α and Xbp1 were upregulated in the islets upon Mettl3 ablation (Fig. 4A and B). The expression levels of *Ero1b* and *Pdia4* were elevated in the islets of MKO-MIP mice (Fig. 4A). These results suggested that Mettl3 deficiency caused ER stress in mouse islets. It has been reported that Mettl14 deficiency induced the upregulation of IRE1α in mouse islets[21]. We applied MeRIP-qPCR with specific primers in fragmented RNA samples after the m^{6}A modification site prediction using SRAMP. Our data showed relative lower m^{6}A enrichment of Ire1α mRNA in the islets of MKO-MIP mice compared with those from control mice (Fig. 4C). Therefore, our results demonstrated that Mettl3 deletion led to aggravated ER stress and the level of Ire1α was upregulated due to less m^{6}A modification upon Mettl3 ablation in mouse islets.

Mettl3 overexpression could rescue ER stress and β-cell function

To identify whether overexpression of Mettl3 could alter the level of ER stress and rescue the β-cell function, we first generated the in vitro ER stress model by using a ER stress inducer thapsigargin[36,37]. Furthermore, the islets were infected with adenovirus to overexpress Mettl3 after thapsigargin treatment (Fig. 4D). Compared to control group, Mettl3 overexpression decreased the expression of *Ire1α* and *Xbp1* and increased the expression level of insulin (Fig. 4E). In addition, in vitro glucose-stimulated insulin secretion experiment showed that overexpression of Mettl3 could increase insulin secretion ability in mouse islets (Fig. 4F). Altogether, our data indicated that Mettl3 overexpression could alleviate Ire1α mediated ER stress and improve insulin secretion in mouse islets.

Discussion

Abnormal expression of m^{6}A regulators, which leads to the functional dysregulation of m^{6}A modification, is implicated in various diseases including T2DM and T1DM[16,38]. Emerging evidence suggests that m^{6}A regulatory proteins have been implicated in critical processes related to β-cell maturation, proliferation, survival, function and antiviral innate immune response[16–22,38]. As in mature β-cells, conditional knockout of Mettl14 in pancreatic β-cells resulted in glucose intolerance in mice[20,21]. Targeted deletion of Mettl3 in islet β-cells leads to impaired β-cell function and the development of hyperglycemia[22]. In this study, we showed that the specific removal of Mettl3 gene in β-cells resulted in glucose intolerance and impaired insulin secretion. Mettl3 deletion caused a decrease in β-cell proliferation and an increase in apoptosis, which was consistent with previously reported results[22]. It was worth noting that the hyperglycemia and hypoinsulinemia phenotypes were more severe in the knockout mice with RIP-Cre than that with MIP-Cre, which was probably due to the different knockout efficiency between them.

Innovatively, loss of Mettl3 led to the loss of β-cell identity and abnormal structure of the islets. Mettl3 deficiency resulted in decreased expression levels of key genes involved in maintaining mature β-cell function, including Pdx1, MafA, and Nkx6.1, which were responsible for preserving the cell identity. β-cell dedifferentiation or loss of β-cell identity has been proposed as a potential contributor to the development of T2DM[7–9]. In the islets of T2DM patients, there was a reduction in protein levels of METTL3 and METTL14, as well as a decrease in the levels of PDX1 compared to the control group[16]. Thus, Mettl3 is crucial for the maintaining of β-cell identity.

An elevation in glucose levels can result in ER stress within β-cells, as a consequence of the heightened demand for insulin processing and synthesis[39]. The ER stress prompts the activation of the unfolded protein response (UPR) in β-cells which involves various proteins, including PERK, IRE1, ATF6, and XBP1[40]. This concerted action serves to impede protein synthesis to facilitate the refolding or elimination of incorrectly folded proteins. Nonetheless, if the UPR is not resolved, it can lead to the induction of CHOP, a protein implicated in programmed cell death[41,42]. Augmented proinsulin production represents a primary etiology of ER stress in pancreatic β-cells during pre-diabetic and initial stages[43,44]. Elevated levels of β-cell oxidative stress and ER stress, accompanied by increased glucose metabolism and insulin production, were present in mouse and cellular models of both type 1 and type 2 diabetes[45,46]. It was reported human islets pre-exposed to ER stress by thapsigargin treatment did not exhibit upregulation of METTL3 similar to control islets while cytokine treatment led to the upregulation of all three components of the m^{6}A writer complex[38]. In our results, Mettl3 deficiency caused ER stress in mouse islets, and the Mettl3 ablated mice suffered from hyperglycemia and hypoinsulinemia.

It was reported that Mettl3 controlled the maturation, proliferation, survival, and function of pancreatic β-cells via regulating multiple genes as Slc2a2, Igf1r, MafA and Bcl2[16,19]. We also found that the mRNA expression levels of key transcription factors essential for maintaining β-cell identity and function, such as Pdx1, MafA, and Nkx6.1, were downregulated in the islets of MKO-RIP and MKO-MIP mice. Other than that, MeRIP-qPCR confirmed that Ire1α, which was an ER stress sensor, was the direct target gene of Mettl3. In the previously published RNA-seq data Ire1α was also one of the differential genes in Mettl3 or Mettl14 ablated islets[21,22]. IRE1α/XBP1 was the most classical UPR pathway. Sustained overexpression of XBP1 would cause β-cell apoptosis[47]. However, it was also reported XBP1 exhibited the ability to preserve β-cell identity, suppressed the conversion of

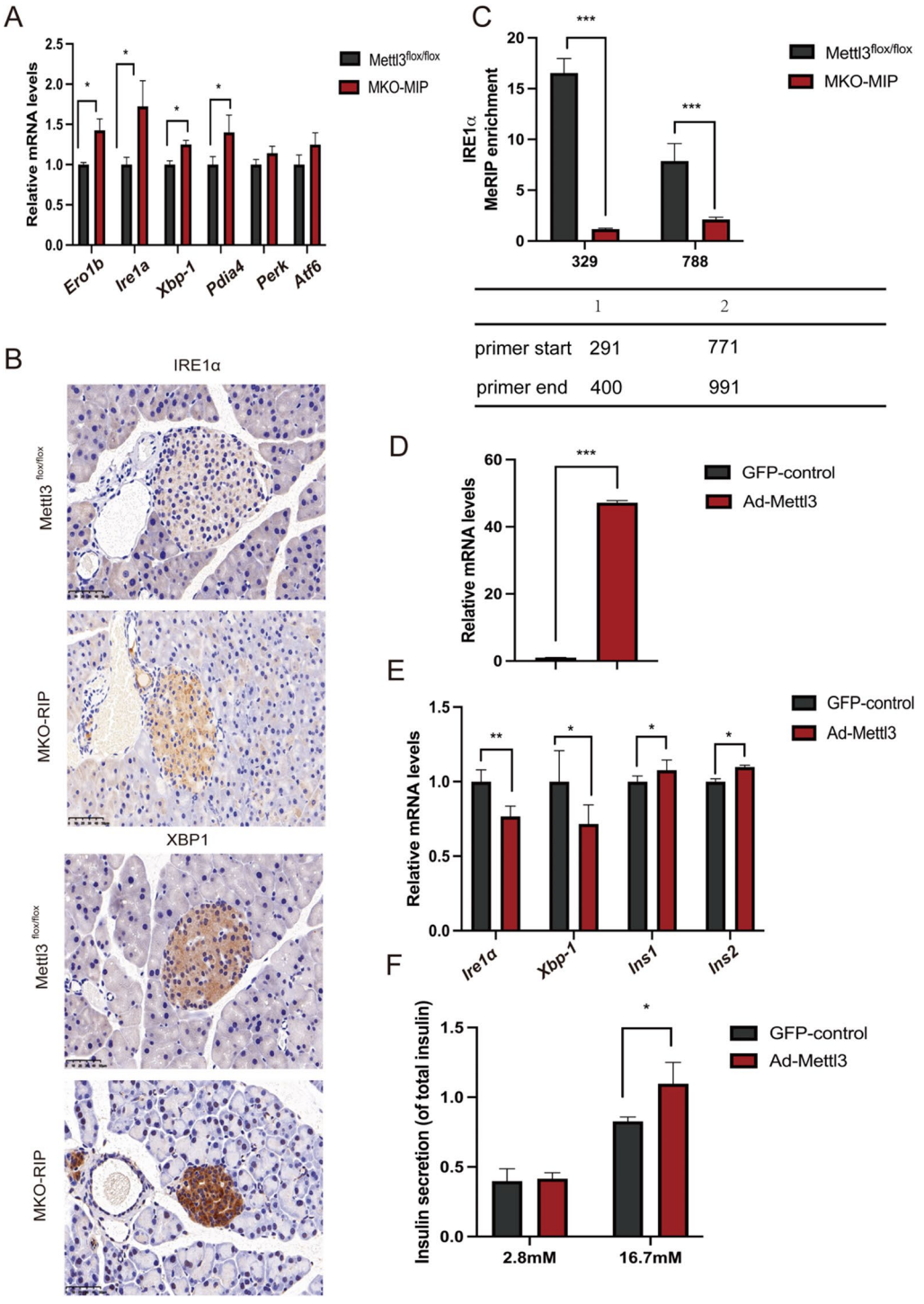

Fig. 4. β-cell specific knockout of Mettl3 caused ER stress while overexpression of Mettl3 relieved ER stress and promoted insulin secretion in the islets. (**A**) mRNA levels of ER stress related genes in the islets of MKO-MIP and control mice ($n = 4$ for each group). (**B**) Immunohistochemistry staining of XBP1 and IRE1α on the pancreatic sections of MKO-RIP and control mice ($n = 3$). Scale bars, 50 μm. (**C**) m^6A modification level of Ire1α in islets of MKO-MIP and control mice by MeRIP-qPCR analysis. (**D**) mRNA levels of *Mettl3* in the control islets infected by adenovirus Ad-Mettl3 and GFP-control virus (MOI = 80, 72 h) after thapsigargin (300 nM, 24 h) treatment ($n = 3$ for each group). (**E**) mRNA levels of *Ins1*, *Ins2* and ER stress related genes in the islets which were treated with thapsigargin (300 nM, 24 h) and infected by Ad-Mettl3 and GFP-control virus (MOI = 80, 72 h) ($n = 3$ for each group). (**F**) Glucose-stimulated insulin secretion (GSIS) in response to 2.8 mM and 16.7 mM glucose of isolated islets which were treated with thapsigargin (300 nM, 24 h) and infected by Ad-Mettl3 and GFP-control virus (MOI = 80, 72 h) ($n = 3$ for each group). Insulin levels were normalized to the total insulin in the islets. Male mice were used for the experiments. Data represent mean ± SD, $^*p<0.05$,$^{**}p<0.01$,$^{* **}p<0.001$. (**A**, **C**, **D** and **E**) two-tailed Unpaired Student's t-tests. (**F**) Two-way ANOVA.

β-cells into alpha cells, and provided protection against metabolic stress-induced β-cell dysfunction in murine models of diabetes[28]. Our data showed that Mettl3 overexpression could alleviate Ire1α mediated ER stress and improve insulin secretion.

Collectively, our findings suggested Mettl3 played a significant role in maintaining mature β-cell identity and function. Ablation of Mettl3 in pancreatic β-cells verified the m^6A modification of Ire1α, resulted in ER stress and deficiencies of insulin production and survival. The findings highlighted the novel functions and mechanisms of Mettl3 in β-cells and diabetes, and potentially elucidated pathways for therapeutic interventions targeting Mettl3 and ER stress in diabetes.

Data availability

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

X.Z. contributed to investigation, validation and original draft preparation. A.S. and J.C. contributed to investigation and software. Y.C. contributed to conceptualization and reviewing. X.J. contributed to conceptualization, manuscript preparation, reviewing and editing. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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