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ARTICLE

Deficiency of WTAP in islet beta cells results in beta cell failure and diabetes in mice

Xinzhi Li[1] · Ying Yang[1] · Zhenzhi Li[1] · Yuqin Wang[1] · Jingting Qiao[2] · Zheng Chen[1]

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Abstract

Aims/hypothesis N^6-methyladenosine (m^6A) mRNA methylation and m^6A-related proteins (methyltransferase-like 3 [METTL3], methyltransferase-like 14 [METTL14] and YTH domain containing 1 [YTHDC1]) have been shown to regulate islet beta cell function and the pathogenesis of diabetes. However, whether Wilms' tumour 1-associating protein (WTAP), a key regulator of the m^6A RNA methyltransferase complex, regulates islet beta cell failure during pathogenesis of diabetes is largely unknown. The present study aimed to investigate the role of WTAP in the regulation of islet beta cell failure and diabetes.

Methods Islet beta cell-specific *Wtap*-knockout and beta cell-specific *Mettl3*-overexpressing mice were generated for this study. Blood glucose, glucose tolerance, serum insulin, glucose-stimulated insulin secretion (both in vivo and in vitro), insulin levels, glucagon levels and beta cell apoptosis were examined. RNA-seq and MeRIP-seq were performed, and the data were well analysed.

Results WTAP was downregulated in islet beta cells in type 2 diabetes, due to lipotoxicity and chronic inflammation, and islet beta cell-specific deletion of *Wtap* (*Wtap*-betaKO) induced beta cell failure and diabetes. *Wtap*-betaKO mice showed severe hyperglycaemia (above 20 mmol/l [360 mg/dl]) from 8 weeks of age onwards. Mechanistically, WTAP deficiency decreased m^6A mRNA modification and reduced the expression of islet beta cell-specific transcription factors and insulin secretion-related genes by reducing METTL3 protein levels. Islet beta cell-specific overexpression of *Mettl3* partially reversed the abnormalities observed in *Wtap*-betaKO mice.

Conclusions/interpretation WTAP plays a key role in maintaining beta cell function by regulating m^6A mRNA modification depending on METTL3, and the downregulation of WTAP leads to beta cell failure and diabetes.

Data availability The RNA-seq and MeRIP-seq datasets generated during the current study are available in the Gene Expression Omnibus database repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE215156; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE215360).

Keywords Hyperglycaemia · Insulin secretion · Islet beta cells · METTL3 · N^6-methyladenosine · WTAP

Abbreviations

BAT	Brown adipose tissue
iBAT	Interscapular brown adipose tissue
ER	Endoplasmic reticulum
GO	Gene Ontology
GSIS	Glucose-stimulated insulin secretion
KEGG	Kyoto Encyclopedia of Genes and Genomes
m^6A	N^6-methyladenosine
MeRIP-seq	Methylated RNA immunoprecipitation sequencing
METTL3	Methyltransferase-like 3
METTL14	Methyltransferase-like 14
Mettl3-betaKO	Beta cell-specific *Mettl3*-knockout
Mettl3-betaOE	Beta cell-specific *Mettl3*-overexpressing
NASH	Non-alcoholic steatohepatitis
NIK	NF-κB-inducing kinase
PA	Palmitic acid
qRT-PCR	Quantitative reverse transcription PCR
RPKM	Reads per kilobase per million mapped reads
WTAP	Wilms' tumour 1-associating protein

Xinzhi Li and Ying Yang contributed equally to this work.

✉ Zheng Chen
chenzheng@hit.edu.cn

[1] HIT Center for Life Sciences, School of Life Science and Technology, Harbin Institute of Technology, Harbin, China

[2] Department of Endocrinology and Metabolism, Tianjin Medical University General Hospital, Tianjin, China

Research in context

What is already known about this subject?

- N^6-methyladenosine (m^6A) mRNA methylation and m^6A-related proteins (METTL3, METTL14 and YTHDC1) have been shown to regulate islet beta cell function and the pathogenesis of diabetes

What is the key question?

- Does Wilms' tumour 1-associating protein (WTAP), a key regulatory protein of the m^6A RNA methyltransferase complex, regulate islet beta cell failure during the pathogenesis of diabetes?

What are the new findings?

- WTAP is downregulated in islet beta cells in type 2 diabetes, owing to lipotoxicity and chronic inflammation, and islet beta cell-specific deletion of *Wtap* induces beta cell failure and diabetes
- *Wtap* deficiency decreases m^6A mRNA modification and reduces the expression of islet beta cell-specific transcription factors and insulin secretion-related genes by reducing METTL3 protein levels
- Islet beta cell-specific overexpression of *Mettl3* partially reverses the abnormalities observed in mice with beta cell-specific *Wtap* knockout

How might this impact on clinical practice in the foreseeable future?

- Our study indicates that maintaining m^6A levels or the expression of m^6A-related proteins (WTAP and METTL3) may be a good strategy for the treatment of beta cell failure and diabetes

Wtap-betaKO	Beta cell-specific *Wtap*-knockout
YTHDC1	YTH domain containing 1

Introduction

Islet beta cell failure is the major cause of diabetes. Islet beta cell-specific transcriptional factors such as MAF bZIP transcription factor A (MafA), NK6 homeobox 1 (NKX6-1), pancreatic and duodenal homeobox 1 (PDX1), neuronal differentiation 1 (NEUROD1) and forkhead box A2 (FOXA2) play an important role in maintaining beta cell function [1–7]. These transcriptional factors control the expression of key insulin secretion-related genes including *Ins1*, *Ins2*, *Gck* and *Glut2* [1–7]. Mutations in any of these transcriptional factors and key insulin secretion-related genes cause islet beta cell failure and diabetes [1–7]. Some of the genes belong to the MODY family, including *GCK* (MODY2), *PDX1* (MODY4), *NEUROD1* (MODY6), *INS* (MODY10) [8]. An abundance of data shows that these transcription factors and key insulin secretion-related genes are regulated at the transcriptional level [1–7]. Whether and how they undergo post-transcriptional regulation by RNA processing are not fully understood. Some evidence shows that RNA processing such as N^6-methyladenosine (m^6A) mRNA modification regulates islet beta cell function [9–11]. However, the detailed molecular mechanisms are not fully understood.

m^6A mRNA methylation is catalysed by m^6A methyltransferase complex. Methyltransferase-like 3 (METTL3) is the key m^6A methyltransferase, and Wilms' tumour 1-associating protein (WTAP) interacts with METTL3 and methyltransferase-like 14 (METTL14) in the nucleus, serving as a regulatory protein of m^6A mRNA modification [12]. m^6A mRNA methylation can be recognised by its reader proteins (YTH domain containing 1/2 [YTHDC1/2] and YTH N^6-methyladenosine RNA binding protein 1–3 [YTHDF1–3]) [13, 14]. m^6A mRNA methylation is reversible and can be demethylated by its eraser proteins (FTO α-ketoglutarate dependent dioxygenase [FTO] and AlkB homolog 5, RNA demethylase [ALKBH5]) [13, 14]. Previous work by our group and others shows that m^6A-related proteins are involved in regulating obesity [15], non-alcoholic steatohepatitis (NASH) [16, 17] and diabetes [9–11, 18, 19]. For example, METTL3 and WTAP play a key role in the postnatal development of interscapular brown adipose tissue (iBAT), which is associated with energy metabolism and obesity [15, 20]. We have also shown that WTAP regulates postnatal development of iBAT by stabilising METTL3 [20] and that both METTL3 and WTAP negatively regulate the pathogenesis of NASH [16, 17]. METTL3 and METTL14 are required for the neonatal maturation of islet beta cells [18]. m^6A modification, *Mettl3*, *Mettl14* and *Ythdc1* are significantly downregulated in the islets of humans with type 2 diabetes, and islet beta cell-specific deletion of either *Mettl3*, *Mettl14* or *Ythdc1* leads to hyperglycaemia due to islet beta cell failure [9–11, 18, 19].

These previous studies have demonstrated that m^6A mRNA methylation and its related proteins (METTL3, METTL14 and YTHDC1) play key roles in maintaining islet beta cell function, and their downregulation during the pathogenesis of diabetes leads to beta cell failure and diabetes. However, whether WTAP regulates beta cell failure and diabetes is largely unknown, and whether WTAP regulates islet beta cell function depending on METTL3 is also unknown. Therefore, building on our previous work and that of others, we investigated these two important questions in this study.

Methods

Animal experiments Animal experiments were approved by the Institutional Animal Care and Use Committee of Harbin Institute of Technology (HIT/IACUC). Mice were housed under controlled light (12h light–dark cycle), temperature (24±2°C) and humidity (50±10%) conditions and fed a normal chow diet with a free access to water. *Wtap*$^{flox/flox}$ mice, in which the exon 4 of the *Wtap* gene was flanked by two loxp sites, were generated using the CRISPR-Cas9 technique as described previously [17, 20]. *Wtap*$^{flox/flox}$ mice were crossed with C57BL/6J mice expressing Cre under the rat insulin-2 promoter (*RIP-Cre* mice) [21] to generate islet beta cell-specific *Wtap*-knockout (*Wtap*-betaKO) mice. The genotype of *Wtap*-betaKO mice was *Wtap*$^{flox/flox}$*RIP-Cre*$^{+/-}$. *Wtap*$^{flox/flox}$ mice served as a control. *STOP-Mettl3* mice were generated using the CRISPR-Cas9 technique to insert a *STOP-FLAG-Mettl3* cassette into the *Rosa26* allele as described previously [16, 20]. To generate islet beta cell-specific *Mettl3*-overexpressing and *Wtap*-knockout (*Wtap*-betaKO/*Mettl3*-betaOE) mice, *STOP-Mettl3* mice were crossed with *Wtap*-betaKO mice. The genotype of the *Wtap*-betaKO/*Mettl3*-betaOE mice is *Wtap*$^{flox/flox}$*STOP-Mettl3*$^{+/-}$*RIP-Cre*$^{+/-}$. The genotype of the *Mettl3*-betaOE mice is *STOP-Mettl3*$^{+/-}$*RIP-Cre*$^{+/-}$. *STOP-Mettl3*$^{+/-}$ mice were used as a control for *Mettl3*-betaOE mice. *Wtap*$^{flox/flox}$, *STOP-Mettl3*$^{+/-}$ and *RIP-Cre*$^{+/-}$ mice were on the C57BL/6J background. Blood glucose levels were measured as described previously [9]. Blood samples were collected from the orbital sinus. Determination of glucose-stimulated insulin secretion (GSIS) in vivo is described in electronic supplementary material (ESM) Methods. Serum glucagon and insulin levels were measured using glucagon ELISA kits (DGCG0; R&D Systems; USA) and insulin ELISA kits (MS100; EZassay, China), respectively. The sex, age, genotype and number of mice are described in the relevant figure legends.

Cell culture INS-1 832/13 cells (RRID: CVCL_7226) were cultured at 37°C and 5% CO_2 in RPMI-1640 medium supplemented with 10% FBS and 50 mmol/l β-mercaptoethanol as described previously [22, 23]. INS-1 832/13 cells were treated with cytokines (TNF-α 20 ng/ml, IL-β 20 ng/ml and IFN-γ 200 ng/ml) or palmitic acid (PA) (0.5 mmol/l) overnight and subjected to quantitative reverse transcription PCR (qRT-PCR) and immunoblotting assays.

Pancreatic islet isolation Pancreases from male mice were harvested, cut into small pieces and incubated at 37°C for 10 min in Hanks' balanced salt solution (HBSS) (pH 7.4) supplemented with 5 mmol/l glucose and 1 mg/ml collagenase P (Roche Diagnostics, Germany). Individual islets were hand-picked and cultured at 37°C and 5% CO_2 in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Determination of GSIS in vitro, and RNA-seq and methylated RNA immunoprecipitation sequencing (MeRIP-seq), were carried out as described in ESM Methods. RNA-seq and MeRIP-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession codes GSE215156 and GSE215360.

Immunoprecipitation and immunoblotting INS-1 832/13 cells were infected with Ad-betaGal, Ad-FLAG-METTL3 or Ad-FLAG-WTAP adenovirus overnight. Total cell lysates were harvested in lysis buffer (R0020; Solarbio, China), immunoprecipitated with anti-FLAG antibody (F1804; Sigma, USA) or IgG1 control, and then immunoblotted with anti-WTAP or anti-METTL3 antibodies. Antibody dilution ratios were as follows: METTL3 (96391; CST, USA; 1:2500 dilution; RRID: AB_2800261); FLAG (F1804; Sigma, USA; 1:5000 dilution; RRID: AB_262044); FLAG (20543-1-AP; Proteintech, China; 1:5000 dilution); β-actin (60008-1-lg; Proteintech, China; 1:5000); and WTAP (10200-1-lg; Proteintech; 1:2500 dilution). The primary antibodies were diluted in PBST containing 3% wt/vol. BSA. Goat anti-mouse IgG (H+L), HRP (ZB-2305; ZSGB-BIO, China; 1:5000 dilution) and Goat anti-rabbit IgG (H+L) secondary antibody, HRP (ZB-2301; ZSGB-BIO; 1:5000 dilution) were diluted in PBST containing 5% wt/vol. milk.

Immunostaining and TUNEL assays Pancreases were fixed in 4% wt/vol. paraformaldehyde for 3 h and then in 30% wt/vol. sucrose overnight. Five frozen pancreatic sections (10 μm, spaced >200 μm apart) were taken from each mouse for staining and analysis. These sections were stained with the indicated antibodies. The dilution ratios were as follows: insulin (A0564; Dako, Denmark; 1:1000 dilution); glucagon (G2654; Sigma; 1:500 dilution; RRID: AB_259852); FLAG (F1804; Sigma; 1:500 dilution). Goat anti-guinea pig IgG (H+L)/AF488 antibody (bs-0358G-AF488; Bioss, China; 1:1000 dilution) and cy3 goat anti-mouse IgG (H+L) antibody (K1207; APExBIO, China; 1:1000

dilution) were secondary antibodies. Primary and secondary antibodies were diluted in PBS containing 5% vol./vol. goat serum and 1% wt/vol. BSA. Islet insulin- and glucagon-positive areas were measured using Image J software version 1.39f (National Institutes of Health, USA) and normalised to pancreatic section areas. TUNEL assays were performed by using cell death detection kits (Roche Diagnostics). The sections were also stained with DAPI to visualise total cells.

Statistical analysis Data are presented as means ±SEM. Differences between two groups were analysed by two-tailed Student's *t* tests. Differences between three groups were analysed by one-factor ANOVA and the least significance difference *t* test. In all analyses, $p<0.05$ was considered statistically significant. The Kolmogorov–Smirnov normality test with $p>0.1$ suggested our samples followed a normal distribution. Statistical analyses and figures were made using GraphPad Prism version 6.02 (GraphPad Software, USA). ANOVA and least significance difference *t* test analyses were performed using SPSS 21.0 (SPSS, USA).

Results

WTAP is downregulated in diabetic islets It has been shown that METTL3, METTL14 and YTHDC1 are required for islet beta cell function [9, 18, 19], and their expression is downregulated in islets from individuals with type 2 diabetes [10, 18, 19]. WTAP interacts with METTL3 and METTL14 to form an m^6A methyltransferase complex [12]. WTAP serves as a regulatory protein [12]. We investigated whether WTAP also contributes to the pathogenesis of beta cell failure and diabetes. We analysed the published islet single-cell RNA-seq data (GSE153855) from individuals with and without type 2 diabetes [24]. As shown in Fig. 1a, the reads per kilobase per million mapped reads (RPKM) values of *Wtap* were significantly decreased in type 2 diabetes. Lipotoxicity, chronic inflammation and hyperglycaemia contribute to the pathogenesis of type 2 diabetes [25]. It has been shown that METTL3 is downregulated by chronic inflammation and oxidative stress in islet beta cells [9]. *Mettl3* was also downregulated by lipotoxicity (PA treatment) in INS-1 832/13 cells (ESM Fig. 1a). We asked whether WTAP is regulated by lipotoxicity, chronic inflammation and hyperglycaemia. As shown in Fig. 1b–e, *Wtap* mRNA was downregulated by treatment with PA or cytokines (TNF-α, IL-1β and IFN-γ) in both primary islets and INS-1 832/13 cells. Cytokines decreased *Wtap* mRNA levels in a time dependent manner (Fig. 1e). Consistently, WTAP protein was also downregulated by treatment with PA or cytokines in both primary islets and INS-1 832/13 cells (Fig. 1f–i). However, high glucose treatment (25 mmol/l) did not change the expression of WTAP (ESM Fig. 1b). These data indicate that WTAP is downregulated in islet beta cells of type 2 diabetes likely due to lipotoxicity and chronic inflammation.

Islet beta cell-specific deletion of *Wtap* causes diabetes To test whether *Wtap* downregulation in pancreatic beta cells contributes to diabetes, we created *Wtap*-betaKO mice by crossing $Wtap^{flox/flox}$ mice with *RIP-Cre* transgenic mice. $RIP\text{-}Cre^{+/-}$ and $Wtap^{flox/flox}$ mice showed similar body weight and blood glucose (ESM Fig. 2a, b). Therefore, we used $Wtap^{flox/flox}$ mice as the control for *Wtap*-betaKO mice. WTAP protein levels were reduced by 62% in the islets of *Wtap*-betaKO mice vs $Wtap^{flox/flox}$ mice, as expected (Fig. 2a,b). Both male and female *Wtap*-betaKO mice had body weights comparable with $Wtap^{flox/flox}$ mice (ESM Fig. 3a, b). However, starting at 4 weeks of age, feeding blood glucose levels in male *Wtap*-betaKO mice gradually increased, reaching approximately 20 mmol/l (360 mg/dl) at 8 weeks of age and remaining above 20 mmol/l after 8 weeks of age, whereas at 3–12 weeks of age, blood glucose levels of male $Wtap^{flox/flox}$ control mice remained constant (Fig. 2c). The fasting blood glucose levels in male *Wtap*-betaKO mice were also significantly higher than those in male $RIP\text{-}Cre^{+/-}$ and $Wtap^{flox/flox}$ mice (ESM Fig. 2b). Consistently, female *Wtap*-betaKO mice also showed hyperglycaemia (Fig. 2d). Furthermore, *Wtap*-betaKO mice demonstrated severe glucose intolerance in both male and female animals (Fig. 2e–h). The glucose AUCs were considerably elevated (by 2.7- and 2.46-fold, respectively) in male and female *Wtap*-betaKO mice (Fig. 2f,h). Male and female *Wtap*-betaKO mice both displayed insulin sensitivity at 8 weeks of age comparable with that shown in $Wtap^{flox/flox}$ mice (ESM Fig. 3c–f). These data indicate that both male and female *Wtap*-betaKO mice displayed hyperglycaemia and glucose intolerance. Therefore, we used male mice for subsequent experiments. Male *Wtap*-betaKO mice at 12 weeks of age had an 87% reduction in serum insulin levels (Fig. 2i), indicating dramatically decreased insulin secretion. Consistently, at 8 weeks of age, male *Wtap*-betaKO mice had severely reduced GSIS (Fig. 2j). We also noted that the basal (fasting) serum insulin levels in male *Wtap*-betaKO mice at 8 weeks of age were also dramatically reduced, by 67.3% (Fig. 2j). GSIS and insulin content were also dramatically reduced in islets isolated from male *Wtap*-betaKO mice at 7 weeks of age (Fig. 2k,l). Consistently, male *Wtap*-betaKO mice at 12 weeks of age showed a 97.2% decrease in pancreatic insulin content (Fig. 2m). These data demonstrate that beta cell-specific deletion *Wtap* leads to beta cell failure and diabetes due to severely impaired insulin secretion.

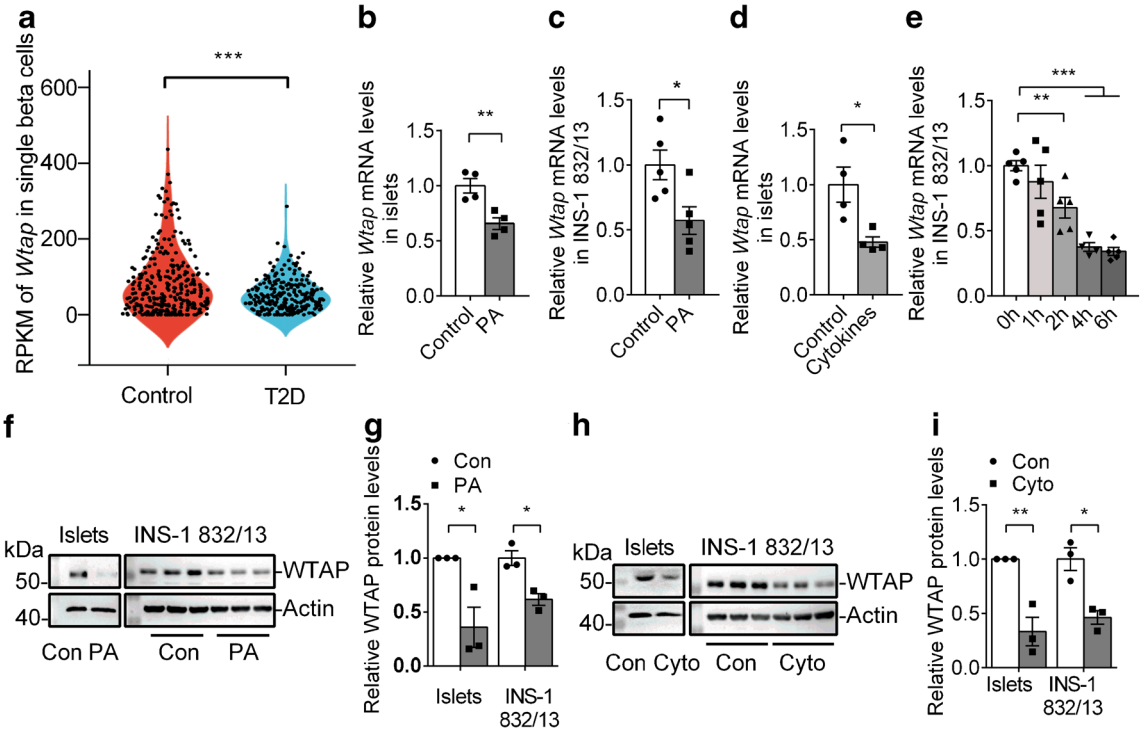


Fig. 1 WTAP is downregulated in diabetic islets. (**a**) *Wtap* RPKM values in single beta cells from a high-quality pancreatic islet single-cell RNA-seq (scRNA-seq) dataset from individuals with and without type 2 diabetes (GSE153855). (**b**, **c**, **f**, **g**) Pancreatic islets were isolated from C57BL/6 mice (100 islets from one or two mice in each sample). These samples underwent an overnight treatment with or without PA (0.5 mmol/l). PA (0.5 mmol/l) was also applied to INS-1 832/13 cells overnight. qRT-PCR was used to determine *Wtap* mRNA levels (**b**, **c**) (n=4 or 5). Relative *Wtap* mRNA levels were normalised to 36B4. Immunoblotting analysis was used to measure WTAP and actin protein levels (**f**) and Image J was used to quantify those levels (**g**) (n=3 independent samples for islets; n=3 separate samples for INS-1 832/13 cells). Relative WTAP protein levels were normalised to actin. (**d**, **e**, **h**, **i**) One hundred pancreatic islets from one or two mice were incubated in each sample. Islet samples were treated with cytokines (TNF-α 20 ng/ml, IL-1β 20 ng/ml and IFN-γ 200 ng/ml) or not for 2 h. Cytokine treatments were also applied to INS-1 832/13 cells for different times (0, 1, 2, 4 and 6 h). qRT-PCR was used to measure *Wtap* mRNA levels (**d**, **e**) (n=4 or 5). Relative *Wtap* mRNA levels were normalised to 36B4. For immunoblotting, INS-1 832/13 cells were treated with or without cytokines overnight. Immunoblotting analysis was used to measure the WTAP and actin protein levels (**h**), and Image J was used to quantify those levels (**i**) (n=3 independent samples for islets; n=3 separate samples for INS-1 832/13 cells). Relative WTAP protein levels were normalised to actin. *p<0.05, **p<0.01 and ***p<0.001. Data represent the mean ± SEM. Con, control (no treatment); Cyto, cytokines; T2D, type 2 diabetes

Islet beta cell-specific deletion of *Wtap* reduces insulin-positive area Reduced pancreatic insulin content may be associated with decreased insulin-positive area. To test this possibility, immunofluorescence analysis was performed. As shown in Fig. 3a,b, the relative insulin-positive area in *Wtap*-betaKO mice at 12 weeks of age was dramatically decreased, by 97.3%, when compared with control $Wtap^{flox/flox}$ mice. Serum glucagon levels, pancreatic glucagon content and the relative glucagon-positive area were similar when comparing male $Wtap^{flox/flox}$ and *Wtap*-betaKO mice at 12 weeks of age (ESM Fig. 3g–i), indicating that glucagon is less likely to contribute to hyperglycaemia in *Wtap*-betaKO mice. To determine whether beta cell death contributed to the observed reduction in insulin-positive area in *Wtap*-betaKO mice, we measured islet beta cell apoptosis by TUNEL staining. Pancreatic sections were co-immunostained with an anti-insulin antibody to visualise beta cells. The number of TUNEL-positive beta cells was much greater in *Wtap*-betaKO islets than in their littermate control (Fig. 3c,d). These data suggest that beta cell-specific *Wtap* deletion leads to beta cell death, causing a significant reduction in insulin-positive area and glucose tolerance in *Wtap*-betaKO mice.

Islet beta cell-specific deletion of *Wtap* changes gene expression profile in islets To further determine the molecular mechanisms by which WTAP regulates beta cell function, we performed RNA-seq analysis in islets isolated from male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice. As shown in Fig. 4a, 2900 genes were upregulated and 3015 genes were downregulated in islets from *Wtap*-betaKO mice. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that protein processing in endoplasmic reticulum (ER), synaptic vesicle cycle, protein export, insulin secretion, tricarboxylic acid (TCA) cycle, type 2 diabetes, MODY, insulin signalling pathway, mammalian target of rapamycin (mTOR)

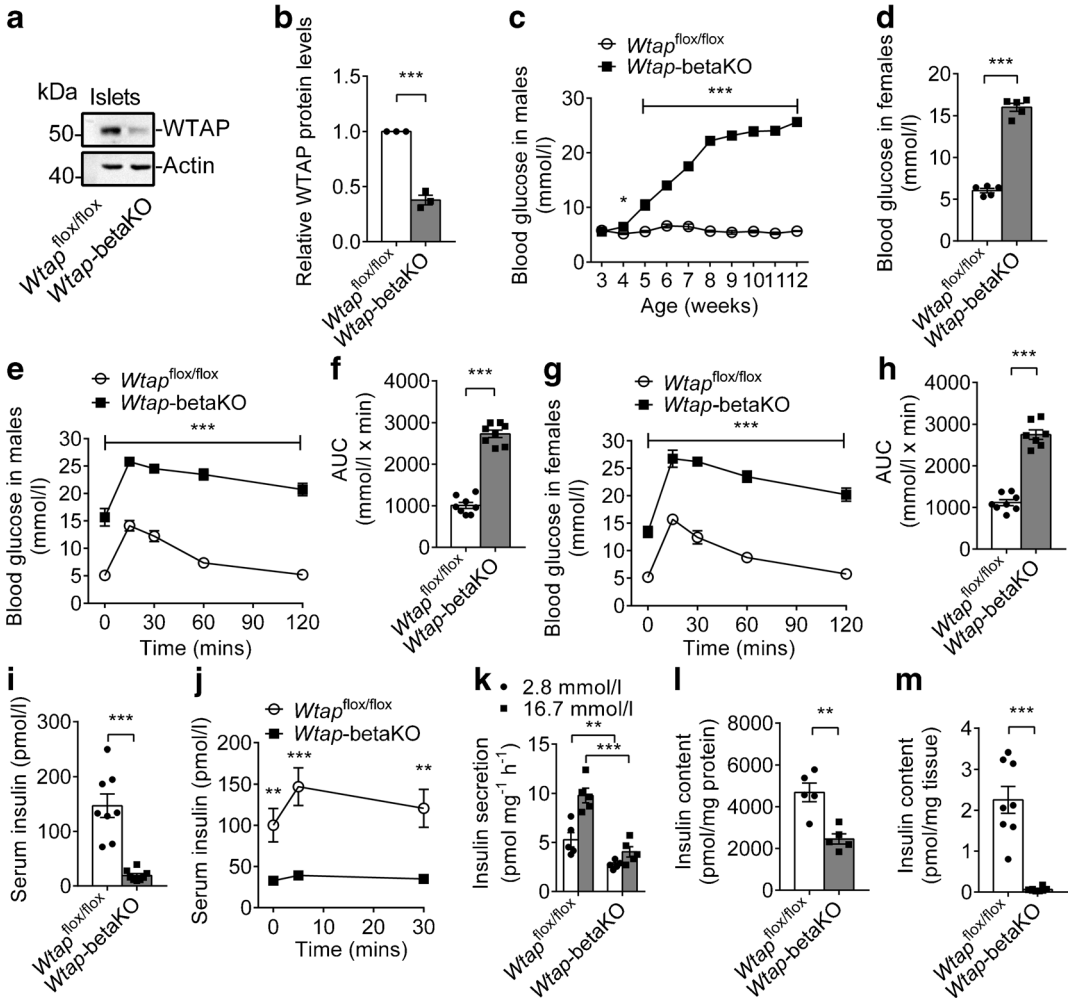


Fig. 2 Islet beta cell-specific deletion of *Wtap* leads to beta cell failure and diabetes. (**a**, **b**) Pancreatic islets were isolated from 7-week-old male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice (100 islets from 4–6 *Wtap*-betaKO mice or 2 or 3 $Wtap^{flox/flox}$ mice were present in each sample). Immunoblotting analysis was used to determine the levels of WTAP and actin proteins (**a**), and Image J was used for quantification (**b**) (n=3 independent samples). Relative WTAP protein levels were normalised to actin. (**c**) From 3 to 12 weeks of age, male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice had their feeding blood glucose levels monitored (*Wtap*-betaKO, n=9 or 10; $Wtap^{flox/flox}$, n=10). (**d**) At 8 weeks of age, female *Wtap*-betaKO and $Wtap^{flox/flox}$ mice had their feeding blood glucose levels measured (*Wtap*-betaKO, n=5; $Wtap^{flox/flox}$, n=5). (**e**, **f**) At 8 weeks of age, male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice underwent GTTs (*Wtap*-betaKO, n=8; $Wtap^{flox/flox}$, n=8). Blood glucose levels (**e**) and AUC (**f**) were determined. (**g**, **h**) At 8 weeks of age, female *Wtap*-betaKO and $Wtap^{flox/flox}$ mice underwent GTTs (*Wtap*-betaKO, n=7; $Wtap^{flox/flox}$, n=8). Blood glucose levels (**g**) and AUC (**h**) were determined. (**i**) At 12 weeks of age, male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice had their serum insulin levels measured (*Wtap*-betaKO, n=8; $Wtap^{flox/flox}$, n=8). (**j**) At 8 weeks of age, male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice had their GSIS tested (*Wtap*-betaKO, n=8; $Wtap^{flox/flox}$, n=8). (**k**, **l**) At 7 weeks of age, isolated islets from male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice were examined for GSIS (basal glucose concentration 2.8 mmol/l; high glucose concentration 16.7 mmol/l) and insulin content (*Wtap*-betaKO, n=5; $Wtap^{flox/flox}$, n=5). (**m**) At 12 weeks of age, male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice had their pancreas insulin content measured (*Wtap*-betaKO, n=8; $Wtap^{flox/flox}$, n=8). *p<0.05, **p<0.01 and ***p<0.001. Data represent the mean ± SEM. n is the number of biologically independent mice

signalling pathway and prolactin signalling pathways were downregulated (Fig. 4b), whereas ribosome, extracellular matrix (ECM)-receptor interaction, cell adhesion molecules, and antigen processing and presentation were upregulated (ESM Fig. 4a). Gene ontology (GO) analysis indicated that the downregulated genes were primarily related to Golgi vesicle transport, vesicle organisation, response to ER stress, ER organisation, cellular response to hormone stimulus, response to insulin, and Golgi organisation (Fig. 4c), whereas the upregulated genes were related to collagen metabolic process, angiogenesis, extracellular structure organisation, cell-substrate adhesion, and response to wounding (ESM Fig. 4b). Among the differentially regulated genes, we noticed that apoptosis-related genes (including *Bcl10*, *Casp6*, *Casp7*, *Cd24a*, *Dedd2*, *Dnase2a*, *Rps3* and *Xkr8*) were upregulated, whereas anti-apoptosis-related genes (including *Acvr1c*, *Akt1*, *Cdk5rap3*,

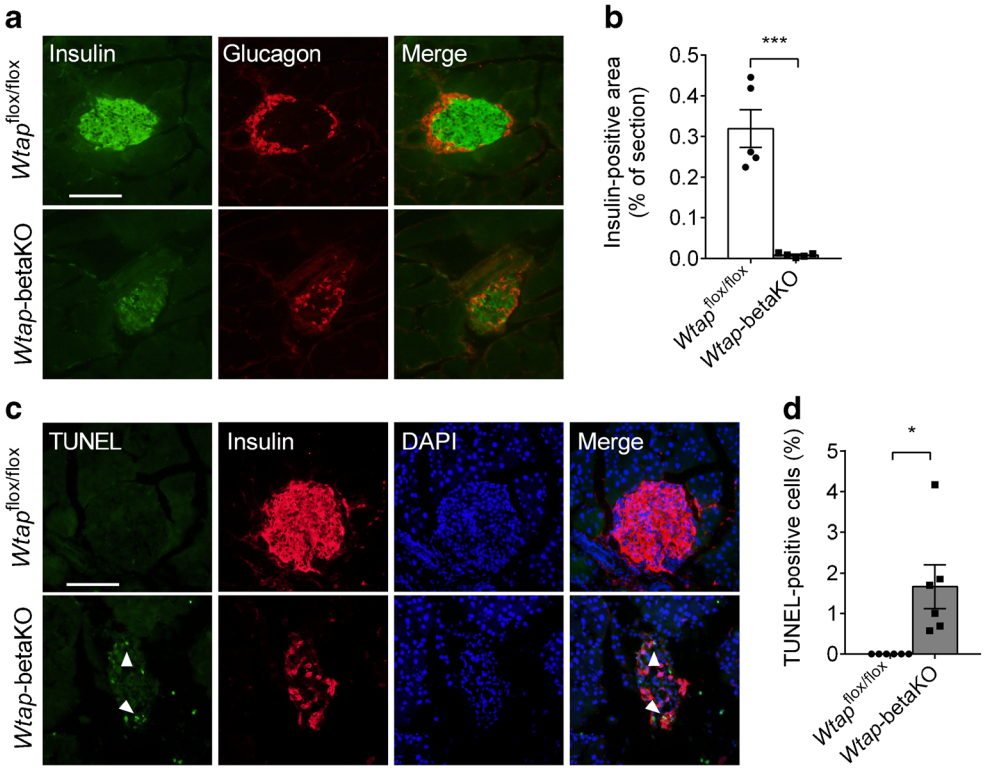
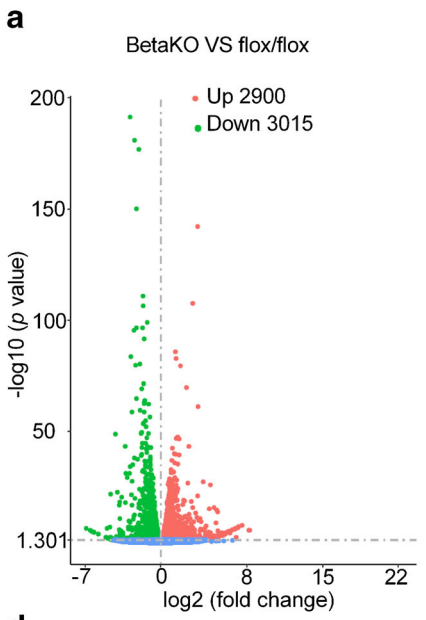


Fig. 3 Islet beta cell-specific deletion of *Wtap* leads to reduced insulin-positive area due to increased beta cell death. (**a**, **b**) At 12 weeks of age, pancreatic sections from male *Wtap*-betaKO and *Wtap*$^{flox/flox}$ mice were immunostained for insulin and glucagon. Representative islet images are shown (**a**). Scale bar, 100 μm. The amount of insulin-positive area per pancreatic section was measured (*Wtap*-betaKO, *n*=5; *Wtap*$^{flox/flox}$, *n*=5) (**b**). (**c**, **d**) TUNEL assays were used to measure the apoptosis of islet beta cells in male *Wtap*-betaKO and *Wtap*$^{flox/flox}$ mice at 8 weeks of age (*Wtap*-betaKO, *n*=6; *Wtap*$^{flox/flox}$, *n*=6). Representative islet images are shown (**c**). Scale bar, 100 μm. TUNEL-positive cells were counted and normalised to total cell number (**d**). **p*<0.05 and ****p*<0.001. Data represent the mean ± SEM. *n* is the number of biologically independent mice

and *Madd*) were downregulated in *Wtap*-betaKO mouse islets (ESM Fig. 4c). This increase in apoptosis-related genes and decrease in anti-apoptosis-related genes may contribute to the increased apoptosis in islet beta cells of *Wtap*-betaKO.

WTAP, METTL3 and METTL14 form a m^{6}A methyltransferase complex [12]. METTL3 serves as a key m^{6}A methyltransferase and WTAP is a regulatory protein [12]. Both *Wtap*-betaKO and beta cell-specific *Mettl3*-knockout (*Mettl3*-betaKO) mice showed beta cell failure and diabetes. It is necessary to determine whether WTAP and METTL3 regulate the same set of genes. Combined analysis of RNA-seq data from *Wtap*-betaKO vs *Wtap*$^{flox/flox}$ and *Mettl3*-betaKO vs *Mettl3*$^{flox/flox}$ mouse islets showed that 1131 genes were downregulated in these two datasets (Fig. 4d). KEGG pathway enrichment analysis showed that insulin secretion, protein processing in ER, MODY, type 2 diabetes, and insulin signalling pathways were downregulated (Fig. 4d). GO analysis indicated that the downregulated genes were primarily related to signal release, Golgi vesicle transport, peptide hormone secretion, hormone secretion, and insulin secretion (Fig. 4d). The downregulated genes included those encoding key transcriptional factors (e.g. *Mafa*, *Nkx6-1*, *Pdx1*, *Neurod1* and *Foxa2*) in islet beta cells, and other important insulin secretion-related genes (e.g. *Ins1*, *Ins2*, *Brsk2*, *Cacna1c*, *Doc2b*, *Ffar1*, *G6pc2*, *Gck*, *Gipr*, *Hadh*, *Ica1*, *Nnat*, *Park7*, *Pclo*, *Selenot*, *Serp1*, *Slc30a8*, *Stxbp51*, *Sytl4*, *Trpm2*, *Ucn3* and *Uqcc2*) (Fig. 4e). These results suggest that both WTAP and METTL3 are required for maintaining the expression of beta cell-specific transcription factors and insulin secretion-related genes.

WTAP is necessary for m^{6}A mRNA modification of insulin secretion-related transcripts The molecular mechanisms by which WTAP controls islet beta cell function were then investigated. It has been demonstrated that WTAP interacts with METTL3 in the nucleus and acts as a METTL3 regulatory protein [12, 20, 26]. We recently reported that METTL3 controls RNA m^{6}A modification, which is necessary for islet beta cell function [9]. It is possible that WTAP regulates islet beta cell function through METTL3. To test this hypothesis, we initially verified that WTAP interacted with METTL3 in beta cells (Fig. 5a–d). WTAP may control the stability of the METTL3 protein, as shown by the reduced METTL3 protein but not mRNA levels in *Wtap*-betaKO mouse islets (Fig. 5e,f). Interestingly, BAT-specific deletion of *Wtap* also resulted

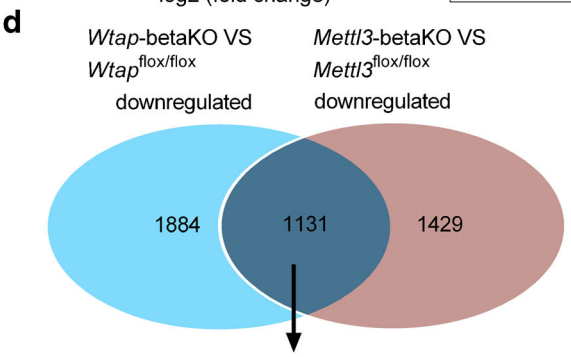


b KEGG analysis of downregulated genes in betaKO islets

Pathway	Count	*p* value
Protein processing in endoplasmic reticulum	66	1.70×10^{-12}
Synaptic vesicle cycle	26	9.08×10^{-7}
Protein export	16	2.02×10^{-6}
Insulin secretion	33	2.54×10^{-6}
Citrate cycle (TCA cycle)	17	4.20×10^{-6}
Type 2 diabetes mellitus	22	5.32×10^{-6}
Maturity onset diabetes of the young	15	6.76×10^{-6}
Insulin signaling pathway	42	9.63×10^{-5}
mTOR signaling pathway	44	1.09×10^{-4}
Prolactin signaling pathway	24	1.31×10^{-4}

c GO analysis of downregulated genes in betaKO islets

GO biological process term (down)	Count	*p* value
Golgi vesicle transport	97	1.49×10^{-19}
Vesicle organization	111	1.29×10^{-18}
Response to endoplasmic reticulum stress	78	3.31×10^{-13}
Endomembrane system organization	107	7.61×10^{-13}
Cellular response to hormone stimulus	131	1.02×10^{-10}
Response to insulin	68	1.87×10^{-10}
Golgi organization	47	5.19×10^{-10}



Pathway	Count	*p* value
Insulin secretion	20	2.31×10^{-8}
Protein processing in endoplasmic reticulum	27	2.34×10^{-7}
Maturity onset diabetes of the young	10	8.71×10^{-7}
Type 2 diabetes mellitus	12	7.93×10^{-6}
Insulin signaling pathway	20	7.20×10^{-5}

GOID	Biological process term	Count	*p* value
GO:0023061	Signal release	71	1.46×10^{-18}
GO:0048193	Golgi vesicle transport	45	6.47×10^{-15}
GO:0030072	Peptide hormone secretion	46	1.15×10^{-14}
GO:0046879	Hormone secretion	53	4.10×10^{-14}
GO:0030073	Insulin secretion	38	8.00×10^{-13}

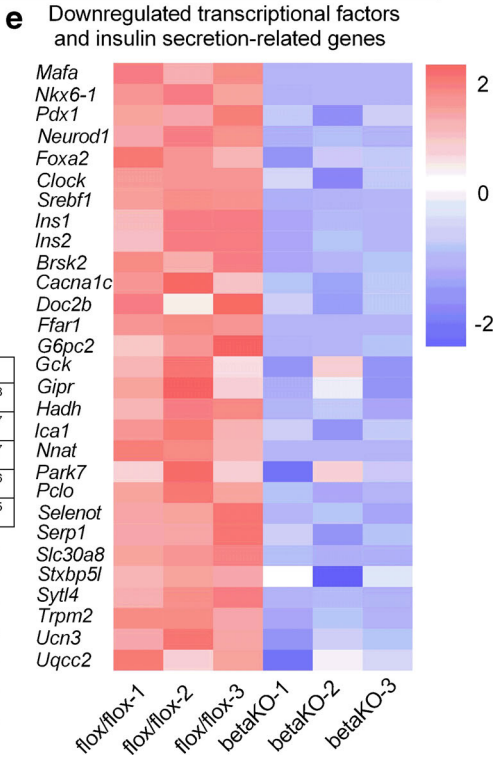
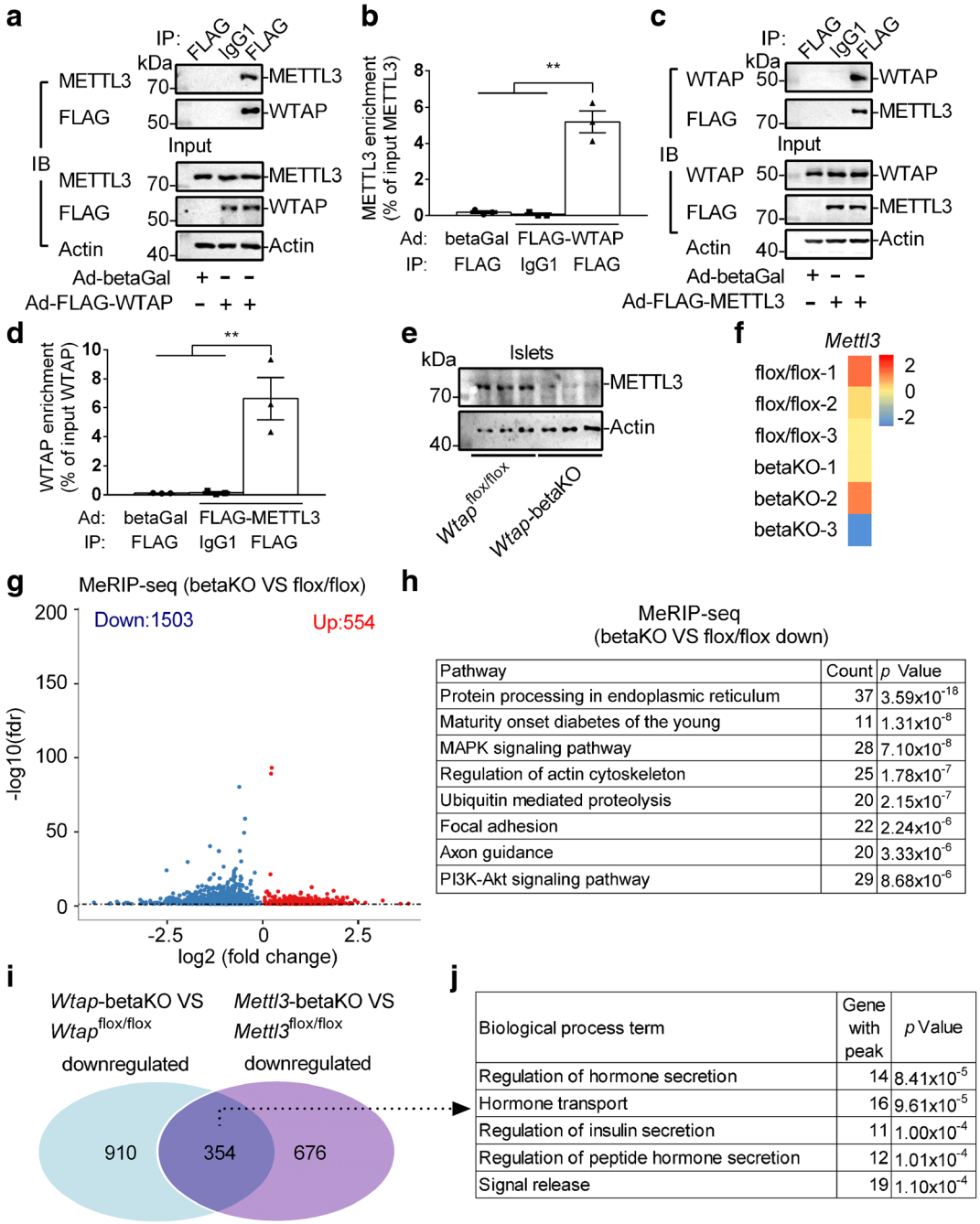


Fig. 4 Islet beta cell-specific deletion of *Wtap* decreases expression of beta cell-specific transcription factors and insulin secretion-related genes. (**a**) At 7 weeks of age, primary islets were extracted from male *Wtap*-betaKO and $Wtap^{flox/flox}$ mice and subjected to RNA-seq analysis (n=3 independent mice). A volcano plot was used to display the differentially expressed genes (DEGs) (*Wtap*-betaKO vs $Wtap^{flox/flox}$), which included 3015 downregulated genes and 2900 upregulated genes ($|\log_2[\text{foldchange}]| > 0$, $p<0.05$). (**b**) Analysis of the downregulated genes' KEGG signalling pathways. (**c**) The enriched GO biological process terms for the genes that were downregulated are displayed. (**d**) A Venn diagram of the RNA-seq data from *Wtap*-betaKO vs $Wtap^{flox/flox}$ and *Mettl3*-betaKO vs $Mettl3^{flox/flox}$ islets revealed that 1131 genes were downregulated in both *Wtap*-betaKO and *Mettl3*-betaKO islets. These 1131 downregulated genes were further analysed by KEGG and GO enrichment analysis, respectively. (**e**) Heatmap results revealed that in *Wtap*-betaKO islets, the beta cell-specific transcription factors and insulin secretion-related genes were downregulated



Pathway	Count	*p* Value
Protein processing in endoplasmic reticulum	37	3.59×10^{-18}
Maturity onset diabetes of the young	11	1.31×10^{-8}
MAPK signaling pathway	28	7.10×10^{-8}
Regulation of actin cytoskeleton	25	1.78×10^{-7}
Ubiquitin mediated proteolysis	20	2.15×10^{-7}
Focal adhesion	22	2.24×10^{-6}
Axon guidance	20	3.33×10^{-6}
PI3K-Akt signaling pathway	29	8.68×10^{-6}

j

Biological process term	Gene with peak	*p* Value
Regulation of hormone secretion	14	8.41×10^{-5}
Hormone transport	16	9.61×10^{-5}
Regulation of insulin secretion	11	1.00×10^{-4}
Regulation of peptide hormone secretion	12	1.01×10^{-4}
Signal release	19	1.10×10^{-4}

Fig. 5 Islet beta cell-specific deletion of *Wtap* leads to beta cell failure due to decreased m^{6}A modification and expression of insulin secretion-related genes. (**a**–**d**) INS-1 832/13 cells were infected with Ad-betaGal, Ad-FLAG-METTL3 or Ad-FLAG-WTAP adenovirus overnight. Total cell lysates were immunoprecipitated (IP) with FLAG beads or IgG1 control. Anti-WTAP or anti-METTL3 antibodies were then used for immunoblotting (IB). Representative immunoblotting images are shown (**a**, **c**). The levels of METTL3 co-IP with FLAG-WTAP or IgG1 were normalised to the input METTL3 (**b**) and the levels of WTAP co-IP with FLAG-METTL3 or IgG1 were normalised to the input WTAP (**d**). n=3 independent results in three independent experiments, with similar results. (**e**) Immunoblotting analysis was used to measure the amounts of METTL3 and actin proteins in islets isolated from male *Wtap*$^{flox/flox}$ and *Wtap*-betaKO mice at 7 weeks old (n=3). (**f**) Heatmap of RNA-seq data showing the relative *Mettl3* mRNA levels in islets of male *Wtap*$^{flox/flox}$ and *Wtap*-betaKO mice at 7 weeks old (n=3). (**g**) At 7 weeks of age, primary islets from male *Wtap*-betaKO and *Wtap*$^{flox/flox}$ mice were subjected to MeRIP-seq analysis. A volcano plot depicts the differential peaks ($|\log_2[\text{foldchange}]| > 1$, $p<0.05$). (**h**) The downregulated genes in *Wtap*-betaKO islets were analysed by KEGG pathway enrichment analysis. (**i**) Venn diagram illustrating 354 transcripts with decreased m^{6}A peaks in both MeRIP-seq datasets from *Wtap*-betaKO vs *Wtap*$^{flox/flox}$ and *Mettl3*-betaKO vs *Mettl3*$^{flox/flox}$ mouse islets. (**j**) These 354 genes were further analysed by GO enrichment analysis. **$p<0.01$. Data represent the mean ± SEM. n is the number of biologically independent cell samples or mice

in the reduction of METTL3 protein levels, through decreasing the stability of METTL3 protein depending on proteasome [20]. These results indicate that WTAP is essential for maintaining METTL3 protein stability.

We carried out an m^{6}A RNA immunoprecipitation sequencing (MeRIP-seq) study in islets of *Wtap*-betaKO and *Wtap*$^{flox/flox}$ mice in order to further ascertain whether m^{6}A modification of mRNA regulated by WTAP was connected to islet beta cell function. The m^{6}A peaks identified in islets of *Wtap*$^{flox/flox}$ mice were enriched at the stop codon and 3′-UTR and were characterised by the canonical GGACU motif (ESM Fig. 5a, b), which is consistent with previously published MeRIP-seq results [9, 20, 27]. Although the m^{6}A peaks identified in islets of *Wtap*-betaKO mice were also enriched at stop codon and 3′-UTR (ESM Fig. 5c), no GGACU motif was characterised. We found around 10,964 significant m^{6}A peaks (false discovery rate <0.05) in roughly 6250 transcripts in the islets of *Wtap*$^{flox/flox}$ mice (ESM Table 1). There was a total of 2057 differential peaks, including 1503 downregulated and 554 upregulated m^{6}A peaks in the islets of *Wtap*-betaKO mice (Fig. 5g and ESM Table 2). The genes with downregulated m^{6}A peaks included beta cell-specific transcription factors and insulin secretion-related genes (Fig. 5g and ESM Table 2), which may contribute to the reduction of their mRNA levels. According to KEGG analysis, genes with downregulated m^{6}A peaks were related to protein processing in ER, MODY, MAPK signalling, regulation of actin cytoskeleton, ubiquitin-mediated proteolysis, focal adhesion, axon guidance, and PI3K–Akt signalling pathways (Fig. 5h). METTL3 serves as a key m^{6}A methyltransferase, and WTAP is a regulatory protein [12]. Both *Wtap*-betaKO and *Mettl3*-betaKO mice showed beta cell failure and diabetes. It is necessary to determine whether WTAP and METTL3 regulate m^{6}A modification of the same set of genes. Combined analysis of MeRIP-seq data from *Wtap*-betaKO vs *Wtap*$^{flox/flox}$ and *Mettl3*-betaKO vs *Mettl3*$^{flox/flox}$ mouse islets showed 354 transcripts exhibiting decreased m^{6}A levels in these two datasets (Fig. 5i). GO analysis showed that transcripts exhibiting decreased m^{6}A levels were associated with regulation of hormone secretion, hormone transport, regulation of insulin secretion, regulation of peptide hormone secretion, and signal release (Fig. 5j). These data suggest that both WTAP and METTL3 regulate m^{6}A modification of insulin secretion-related transcripts.

Islet beta cell-specific overexpression of *Mettl3* partially reverses beta cell failure and diabetes in *Wtap*-betaKO mice

Islet beta cell-specific deletion of *Wtap* resulted in impaired beta cell function, which is likely due to the decreased METTL3-mediated RNA m^{6}A modification. To determine whether METTL3 is really involved in this process, we restored METTL3 expression in islet beta cells of *Wtap*-betaKO mice by crossing islet *Mettl3*-betaOE with *Wtap*-betaKO mice. As shown in Fig. 6a, FLAG-tagged METTL3 was overexpressed in islet beta cells of *Wtap*-betaKO/*Mettl3*-betaOE but not *Wtap*-betaKO or *Wtap*$^{flox/flox}$ mice. Under normal conditions, *Mettl3*-betaOE and *STOP-Mettl3* mice showed similar body weight and blood glucose (ESM Fig. 6a, b). However, islet beta cell-specific overexpression of *Mettl3* in *Wtap*-betaKO mice partially reversed beta cell failure and diabetes in the *Wtap*-betaKO mice, as revealed by decreased hyperglycaemia (Fig. 6b), without changing body weight (Fig. 6c); further findings included improved glucose tolerance (Fig. 6d,e) (without changes in insulin sensitivity; Fig. 6f,g), increased plasma insulin levels (Fig. 6h), elevated insulin content (Fig. 6i) and increased insulin-positive area (Fig. 6j,k). Glucagon-positive areas (Fig. 6j,l) were similar in these three genotypes of mice. These data indicate that WTAP regulates beta cell function partially depending on METTL3.

Discussion

Maintenance of islet beta cell function is required for controlling blood glucose homeostasis. Impaired islet beta cell function leads to hyperglycaemia and diabetes. Elucidating the molecular mechanisms of islet beta cell dysfunction or failure is very important to find novel treatments for diabetes. Some evidence shows that m^{6}A mRNA modification regulates beta cell function [9–11]. In this study, we demonstrated that WTAP is downregulated in islets in type 2 diabetes by lipotoxicity and chronic inflammation, and that beta cell-specific deletion of *Wtap* leads to beta cell failure and diabetes likely due to decreased m^{6}A modification and expression of insulin secretion-related transcripts.

Our previous research and other studies have demonstrated that m^{6}A modification, its key writer proteins (METTL3/METTL14), and its reader protein YTHDC1 are significantly downregulated in the islets of humans with type 2 diabetes likely due to lipotoxicity, oxidative stress and chronic inflammation [9–11, 18, 19]. In this study, we show that WTAP, another key m^{6}A writer protein, is also downregulated in the islet beta cells of humans with type 2 diabetes likely due to lipotoxicity and chronic inflammation. Multiple signalling pathways may contribute to the downregulation of m^{6}A signalling pathway in islets during type 2 diabetes. For example, our previous studies have demonstrated that activation of the non-canonical NF-κB signalling pathway leads to the downregulation of METTL3 and YTHDC1 [9, 19], and overexpression of NF-κB-inducing kinase (NIK) induces beta cell failure and diabetes [28]. These results indicate that NIK activation causes the downregulation of m^{6}A-related proteins and further leads to beta cell failure and diabetes. However, we cannot rule out other possible molecular mechanisms that also contribute to the downregulation of m^{6}A-related proteins in

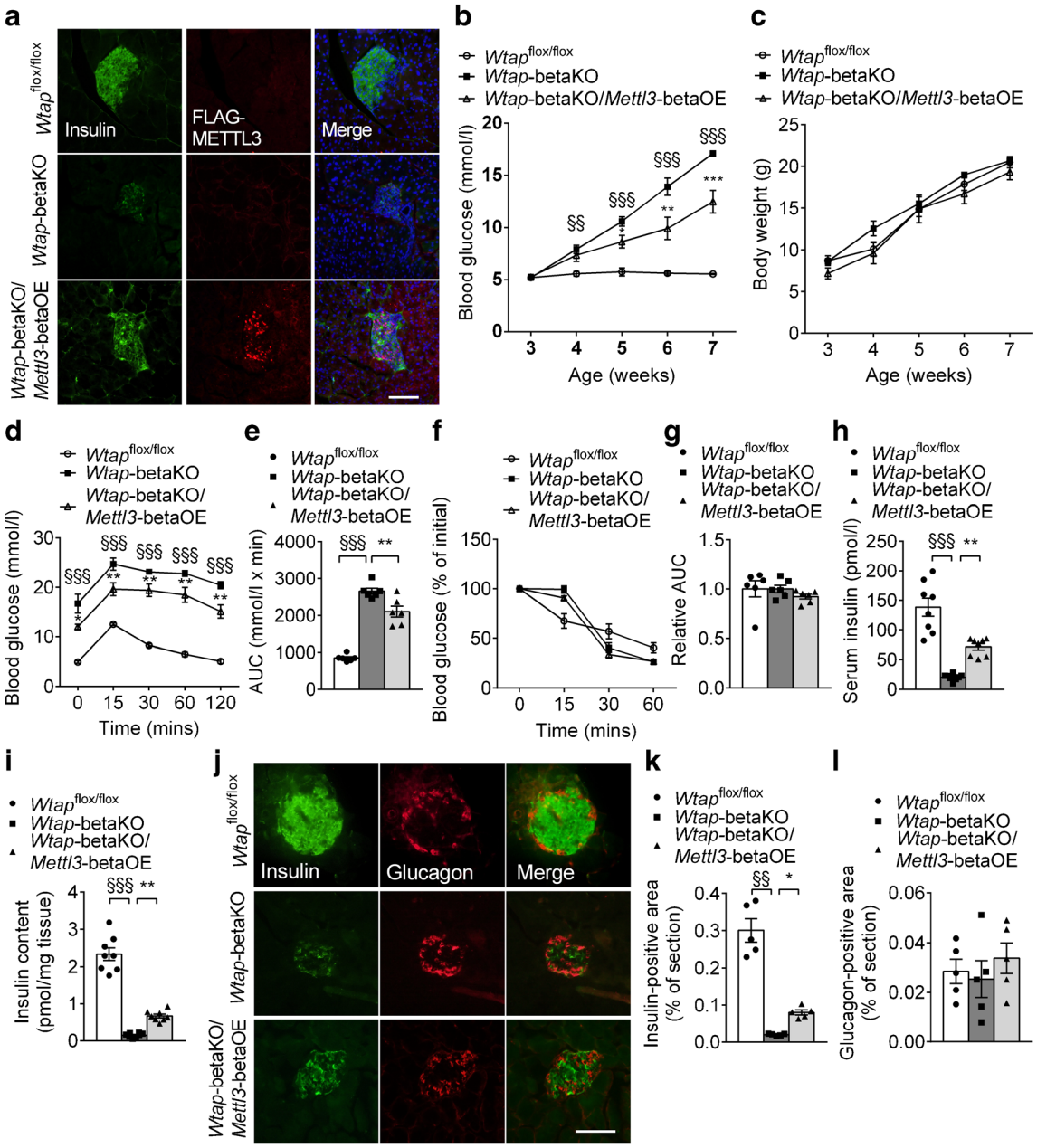


Fig. 6 Islet beta cell-specific overexpression of METTL3 partially reverses beta cell failure and diabetes in *Wtap*-betaKO mice. *STOP-FLAG-Mettl3* transgenic mice were crossed with *Wtap*-betaKO mice to generate *Wtap*-betaKO/*Mettl3*-betaOE mice. (**a**) Co-immunostaining of insulin and FLAG-METTL3 with anti-insulin and anti-FLAG antibodies in pancreatic sections of male *Wtap*$^{flox/flox}$, *Wtap*-betaKO and *Wtap*-betaKO/*Mettl3*-betaOE mice at 8 weeks old. Representative images are shown. Scale bar, 100 μm. (**b**, **c**) Feeding blood glucose levels (**b**) and body weight (**c**) were measured in male *Wtap*$^{flox/flox}$, *Wtap*-betaKO and *Wtap*-betaKO/*Mettl3*-betaOE mice from 3 to 7 weeks of age (n=6 for each group). (**d**–**g**) GTTs (**d**) and ITTs (**f**) were performed in male *Wtap*$^{flox/flox}$, *Wtap*-betaKO and *Wtap*-betaKO/*Mettl3*-betaOE mice at 7 weeks of age (n=6 for each group); AUC for GTTs and relative AUC for ITTs are shown (**e**, **g**). (**h**, **i**) Serum insulin levels and pancreas insulin content were measured in male *Wtap*$^{flox/flox}$, *Wtap*-betaKO and *Wtap*-betaKO/*Mettl3*-betaOE mice at 7 weeks of age (n=8 for each group). (**j**–**l**) Pancreas sections of male *Wtap*$^{flox/flox}$, *Wtap*-betaKO and *Wtap*-betaKO/*Mettl3*-betaOE mice at 8 weeks of age were immunostained for insulin and glucagon. Representative images are shown (**j**). Scale bar, 100 μm. Insulin-positive (**k**) and glucagon-positive (**l**) areas per pancreatic section were quantified (n=5 for each group). $^{§§}p$<0.01 and $^{§§§}p$<0.001, *Wtap*-betaKO vs *Wtap*$^{flox/flox}$ mice; *p<0.05, **p<0.01 and ***p<0.001, *Wtap*-betaKO/*Mettl3*-betaOE vs *Wtap*-betaKO mice. Data represent the mean ± SEM. n is the number of biologically independent mice

islets during type 2 diabetes. Because the access to human pancreatic islets is limited, most of the regulation assays were performed in mouse islets or INS-1 832/13 cells. Developing good human beta cell lines will solve this problem.

Downregulation of m^{6}A and its related proteins in islet beta cells results in beta cell failure and diabetes [9–11, 18, 19]. Islet beta cell-specific deletion of *Mettl3*, *Mettl14*, *Ythdc1* or *Wtap* leads to hyperglycaemia and diabetes due to impaired

insulin secretion at a young age [9–11, 18, 19]. One possible explanation is that m^{6}A and its related proteins are required for the postnatal maturation of islet beta cells [18]. Another tissue-specific knockout mouse model supports this explanation. BAT-specific deletion of either *Mettl3* or *Wtap* impairs the postnatal development of iBAT [15, 20].

Wtap-betaKO mice show very similar phenotypes to the phenotypes observed in *Mettl3*-betaKO mice [9]. Both mice show hyperglycaemia, hypoinsulinaemia and diabetes, with a dramatically reduced insulin-positive islet area and impaired insulin secretion. Significantly decreased expression of beta cell-specific transcription factors and insulin secretion-related genes results in these abnormalities observed in both *Wtap*-betaKO and *Mettl3*-betaKO mice. However, it should be noticed that loss of beta cells in these two knockout mice would naturally reduce the abundance of beta cell-specific genes. The reduction in insulin-positive area may be attributed to beta cell apoptosis. Some apoptosis-related genes are upregulated, whereas some anti-apoptosis-related genes are downregulated in *Wtap*-betaKO mouse islets. m^{6}A peaks in *Cd24a* and *Dedd2* transcripts are differentially regulated in *Wtap*-betaKO mouse islets. Whether WTAP directly regulates these apoptosis- and anti-apoptosis-related transcripts depending on m^{6}A modification is still unknown. Meanwhile, both mRNA levels and m^{6}A peaks of *Pdx1* were significantly decreased in *Wtap*-betaKO mouse islets. Downregulation of *Pdx1* may also contribute to beta cell apoptosis in *Wtap*-betaKO mouse islets because it has been reported that half deletion of *Pdx1* induces beta cell apoptosis and diabetes [29].

Wtap deficiency decreases METTL3 protein levels but does not affect *Mettl3* mRNA levels in islets, and BAT-specific deletion of *Wtap* shows similar results in iBAT [20]. WTAP binds to METTL3 in many cell types including beta cells and iBAT. WTAP is essential for maintaining the protein stability of METTL3 [20]. Many of the transcripts that show a decrease in m^{6}A modification when *Wtap* is specifically deleted from islet beta cells are consistent with those seen in *Mettl3*-betaKO mouse islets. The m^{6}A modifications in many transcripts encoding beta cell-specific transcription factors and insulin secretion-related proteins are decreased in both *Wtap*-betaKO and *Mettl3*-betaKO islets. It is likely that YTHDC1 identifies these m^{6}A modifications and controls the expression of beta cell-specific transcription factors and insulin secretion-related genes [19]. In *Wtap*-betaKO mice, overexpression of METTL3 in islet beta cells partially reverses the hyperglycaemia/diabetes/hypoinsulinaemia. These findings suggest that WTAP controls beta cell function via METTL3. We discovered that overexpressing METTL3 just in islet beta cells does not completely reverse the abnormalities seen in *Wtap*-betaKO mice, suggesting that other molecular processes may also be involved in WTAP function in islet beta cells. Our recent research demonstrates that WTAP and METTL3 can bind to gene promoters and control chromatin accessibility [16, 17]. It is important to find out whether WTAP and METTL3 can control gene transcription directly in islet beta cells.

WTAP and METTL3, as m^{6}A writer proteins, are essential for maintaining islet beta cell function [9]. YTHDC1, as an m^{6}A reader protein, plays a key role in beta cell failure [19]. It is necessary to determine whether m^{6}A eraser proteins (FTO and ALKBH5) promote islet beta cell failure and diabetes in vivo. Maintaining m^{6}A levels or homeostasis might be a good strategy for the treatment of islet beta cell failure and diabetes.

In conclusion, we have demonstrated that WTAP is downregulated in islets in type 2 diabetes by lipotoxicity and chronic inflammation, and that beta cell-specific deletion of *Wtap* leads to beta cell failure and diabetes due to reduced METTL3-mediated m^{6}A modification and decreased expression of beta cell-specific key transcription factors and insulin secretion-related genes. Islet beta cell-specific overexpression of *Mettl3* partially reverses the abnormalities observed in *Wtap*-betaKO mice. These data suggest that WTAP plays an essential role in maintaining beta cell function partially by stabilising METTL3, and also indicate that downregulation of either WTAP or METTL3 contributes to beta cell failure during the pathogenesis of diabetes.

Supplementary Information The online version contains peer-reviewed but unedited supplementary material available at https://doi.org/10.1007/s00125-023-05900-z.

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Data availability The RNA-seq and MeRIP-seq datasets generated during the current study are available in the Gene Expression Omnibus database repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE215156; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE215360).

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Authors' relationships and activities The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Contribution statement XL and YY performed the experiments, collected data, analysed and interpreted data, and drafted the manuscript. ZL, YW and JQ analysed and interpreted data, and revised the manuscript. ZC designed the project and wrote the manuscript. ZC is the guarantor of this work. All authors approved the final version to be published.

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