Obesity-induced miR-455 upregulation promotes adaptive pancreatic β-cell proliferation through the CPEB1/CDKN1B pathway

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Abstract

Pancreatic β-cell adapt to compensate for increased metabolic demand during obesity. Although the microRNA (miRNA) pathway has an essential role in β-cell expansion, whether it is involved in adaptive proliferation is largely unknown. First, we report that EGR2 binding to the miR-455 promoter induced miR-455 upregulation in the pancreatic islets of obesity mouse models. Then, in vitro gain- or loss-of-function studies showed that miR-455 overexpression facilitated β-cell proliferation. Knockdown of miR-455 in ob/ob mice via pancreatic intraductal infusion prevented compensatory β-cell expansion. Mechanistically, our results revealed that increased miR-455 expression inhibits the expression of its target cytoplasmic polyadenylation element binding protein 1 (CPEB1), an mRNA binding protein that plays an important role in regulating insulin resistance and cell proliferation. Decreased CPEB1 expression inhibits elongation of the poly-A tail and the subsequent translation of Cdkn1b mRNA, reducing the CDKN1B expression level and finally promoting β-cell proliferation. Taken together, our results show that the miR-455/CPEB1/CDKN1B pathway contributes to adaptive proliferation of β-cells to meet metabolic demand during obesity.
Pancreatic β-cell function and mass are markedly adaptive to compensate for the changes in insulin requirements observed during obesity (1). Clinical results have shown that the β-cell mass in the pancreases of nondiabetic or prediabetic obese individuals is larger than that in lean normoglycemic subjects (2). Evidence for this compensatory process has been consistently provided by obese rodent models and human pancreas necropsies (3, 4). In obesity, β-cell mass increases by 30–40%, whereas insulin secretory output is augmented by 100% (5). Postmortem histology further revealed a 20–65% decrease in β-cell mass in islets from obese individuals with type 2 diabetes (T2D) compared to BMI-matched nondiabetic subjects (6, 7). This adaptive capacity of human islets to obesity has been confirmed in experimental murine models (8, 9). Strategies aimed at improving β-cell function and mass plasticity could be of major interest for designing innovative therapeutics to prevent β-cell decline and restore β-cell functional adaptive ability in diabetes. However, the physiological mechanisms that promote adaptive pancreatic β-cell expansion are still not completely understood.

The adaptive capacity of β-cell mass and function depends on the activity of transcriptional and translational regulators, and miRNAs are extremely important in accomplishing this task (10, 11). In pancreatic β cells, miRNAs regulate insulin production by directly or indirectly affecting the expression of key transcription factors and contribute to fine-tuning of hormone release by modulating the levels of important components of the β-cell secretory machinery (12-14). Our and other studies have reported changes in the expression of islet miRNAs in animal models of diabetes, with detrimental effects on the secretory activity and survival of β cells (15, 16). Moreover, obese mice lacking certain miRNAs failed to compensate for insulin resistance and developed a severe diabetic phenotype (3, 12, 17). These observations prompted us to investigate whether changes in
miRNA expression contribute to compensatory β cell mass expansion during obesity.

In this study, we found that miR-455 was upregulated during obesity to inhibit the expression of CPEB1 in pancreatic β cells. Both in vivo and in vitro experimental results revealed overexpression of miR-455 promoted adaptive pancreatic β-cell proliferation, and knockdown of miR-455 prevented compensatory β-cell expansion. Moreover, we observed that the miR-455/CPEB1 pathway inhibits translation of Cdkn1b mRNA and ultimately leads to lower levels of CDKN1B in β-cells, in turn significantly promoting β-cell adaptive proliferation. These observations show that miR-455 plays an integral role in the β cell compensatory mechanism during obesity.

RESEARCH DESIGN AND METHODS

Animal care

Eight-week-old C57BL/6J mice, ob/ob mice (4-12 weeks), and db/db mice (4-12 weeks) were obtained from GemPharmatech Co., Ltd. (Nanjing, China). All animals were on the C57BL/6 background unless otherwise stated. The db/db mice were on the BKS background. The care of all animals was within institutional animal care committee guidelines, and all procedures were approved by the animal ethics committee of China Pharmaceutical University (Permit Number: 2162326) and were in accordance with international laws and policies (EEC Council Directive 86/609, 1987). Unless otherwise stated, C57BL/6J mice were fed a normal chow diet (NCD, D12450J, 10% calories from fat) and provided water ad libitum. Diet-induced obesity was obtained by feeding a high-fat diet (HFD, D12494, 60% calories from fat) for at least 15 weeks. Male mice were used for all the indicated studies.

Isolation and culture of primary islets
6 human islets were provided by Tianjin First Central Hospital. All human subjects provided informed consent, and all procedures using human islets were approved by the Research Ethics Committee of the Tianjin First Central Hospital (18). High purity islets (>80%) were collected and cultured in CMRL-1066 medium (Corning, Manassas, VA, USA) supplemented with 10% human serum albumin (Baxter, Vienna, Austria), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in 5% CO₂. The islets from 6 human donors were mixed randomly, then mixed islets were used to perform for each type of experiments and performed 3 technical replicates.

Mouse islets were isolated via collagenase digestion and enriched using a Histopaque (Sigma Aldrich) density gradient as described previously (15, 19). To assess the effects of pathophysiological concentrations of palmitate, glucose, and proinflammatory cytokines, islets were incubated in modified medium with 0.5% (weight for volume) BSA and various concentrations of glucose (low glucose: 2.5 mM; high glucose: 25 mM), palmitate (0.5 mM), and IL-1β+TNFα (IL-1β: 5 ng/ml; TNFα: 30 ng/ml).

**MIN6 cell culture**

The mouse pancreatic β cell line MIN6 (passages 15-20) was cultured in DMEM (Gibco) containing 15% FBS (Gibco, Burlington, USA), 100 IU/mL penicillin, 100 μg/ml streptomycin, and 50 μM β-mercaptoethanol (Sigma Aldrich, St. Louis, MO, USA) at 37°C in a humidified atmosphere containing 5% CO₂.

Human EndoC-βH1 cell culture

Human EndoC-βH1 cell were obtained from Univercell Biosolutions (France). Human EndoC-βH1 cells were cultured in ECM/fibronectin-coated plates in low-glucose DMEM with supplements as previously described (20, 21).
Insulin secretion assay

For the glucose-stimulated insulin secretion (GSIS) assay, the MIN6 cells or human islets were preincubated overnight in KRBH balanced buffer containing 0.2% BSA supplemented with 2.5 mM glucose and incubated for 2 h with 2.5 or 16.7 mM glucose. Immediately after incubation, an aliquot of the medium was removed for insulin analysis, and the cells were incubated in acid-ethanol for insulin content determination using a mouse insulin ELISA kit (ExCell Bio, Shanghai, China) according to the manufacturer’s instructions.

miRNA sequencing and analysis

Total RNA was isolated from islets from 8-week-old control mice and 8-week-old ob/ob mice using a RNeasy Plus Universal Mini Kit (Qiagen) following the protocol for total RNA isolation. RNA integrity and concentration were assessed using an RNA Nano 6000 Assay Kit for the Bioanalyzer 2100 system (Agilent Technologies, CA, USA) according to the manufacturer’s instructions. To extract small RNA (18-30 nt), total RNA was separated in 15% agarose gels. After ethanol precipitation and centrifugal enrichment of small-RNA samples, a small-RNA library was prepared using a Small RNA Sample Preparation Kit (Illumina, RS-200-0048) according to the manufacturer’s instructions. Briefly, a 3’ adaptor or 5’ adaptor was attached directionally. Each library was indexed with the Illumina adaptor (6-base barcode). The small-RNA library was size-fractionated in a 6% TBE urea polyacrylamide gel, and the 140- to 160-base-pair (bp) fraction was excised from the gel. The library RNA concentration was measured using a Qubit® RNA Assay Kit in Qubit® 2.0 for preliminary quantification and then diluted to 1 ng/μl. Insert size was assessed using an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA), and after the insert size was confirmed to be consistent with expectations, the qualified insert size was accurately
quantified using a TaqMan fluorescence probe and an AB Step One Plus Real-Time PCR system (valid library concentration > 2 nM). Then, the qualified libraries were sequenced on an Illumina HiSeq 2500 platform. We calculated the FPKM (fragments per kilobase per million mapped reads) for mature miRNA from the mapped reads using a custom Python script. Differentially expressed miRNAs were analyzed using the DESeq package (DESeq version 1.26.0) based on the negative binomial distribution test (22), and the \( p \)-value was corrected for false discovery rate (FDR) analysis for multiple testing (23). Differentially expressed genes with an adjusted \( p \)-value \( \leq 0.05 \) and an absolute value of log 2 (fold change) > 1 were considered significantly differentially expressed genes. The raw data are presented in Table S1 and have been deposited in the NCBI’s Sequence Read Archive (SRA) database (PRJNA731006).

**Serum samples of non-diabetic and type-2 diabetic**

The serum and clinicopathological data were collected from the Zhongda Hospital, Affiliated to southeast University (Nanjing, China). All human subjects provided informed consent. All human studies were conducted according to the principles of the Declaration of Helsinki, were approved by the Ethics Committees of the Department Zhongda Hospital Southeast University.

**Electrophoretic mobility shift assay (EMSA) analysis**

The probe, an approximately 25 bp fragment including the binding size, was biotin end-labeled according to the instructions of a Biotin 3’ End DNA Labeling Kit (Thermo Pierce, Massachusetts, USA) and then annealed to double-stranded probe DNA. Egr2-DNA complexes were generated according to the instructions of a LightShift Chemiluminescent EMSA Kit (Thermo Fisher Scientific, Massachusetts, USA). Probe sequences are listed in Table S2.

**Chromatin immunoprecipitation (ChIP) experiment**
MIN6 cells and human islets were fixed with 37% formaldehyde for 10 min, followed by 30 rounds of sonication (each for 3 s) to fragment the chromatin. Chromatin was incubated with an anti-EGR2 antibody at 4°C overnight and then immunoprecipitated with Proteinase K (Millipore). Purified DNA was amplified via qRT-PCR using PCR and primer pairs that spanned the predicted Egr2 binding sites. Primer sequences are listed in Table S2.

**Plasmid construction**

The coding sequences for Egr2 (NM_001373983.1), Cpeb1 (NM_001252525.1) and Cdkn1b (NM_009875.4) were amplified via PCR from full-length cDNA of MIN6 cells and then cloned into a pcDNA 3.1 vector. The primer sequences for PCR are listed in Table S2.

The sgRNAs for the miR-455 promoter were constructed in a lentiCRISPRv2 puro vector according to the Zhang F libraries(24). The lentiCRISPRv2 puro vector was digested with BsmBI. The sgRNA seed sequences are listed in Table S3.

**Luciferase assays**

MIN6 cells (2×10^5 cells well⁻¹) were transfected with 0.4 μg miR-455 promoter, 0.4 μg Egr2 and 0.1 μg constitutive Renilla expression plasmid, and luciferase activities were measured using a dual-luciferase reporter assay system (Vazyme, Nanjing, China) after transfection for 24 h.

The complete 3'-UTR of murine Cpeb1 containing either the wild-type or mutated miR-455 binding site was cloned behind the stop codon of the firefly luciferase open reading frame using specific primers. MIN6 cells were transfected with pmir-PGLO reporter (100 ng) along with miR-455 or miR-455 inhibitor using Lipofectamine 2000 transfection reagent (Invitrogen). At 24 h posttransfection, dual luciferase reporter assays were performed using a luciferase assay system (Vazyme, Nanjing, China). The wild-type and mutation site primers are listed in Table S2.
Flow cytometric analysis of proliferation

INSULIN and KI-67 double-positive cells were detected following a standard intracellular staining procedure using Cytofix/Cytoperm solution (Becton, Dickinson and Company, USA). INSULIN-APC and KI-67-FITC antibodies were used for staining, and isotype antibodies were used as a negative control. After double staining with INSULIN-APC and KI-67-FITC, the cells were analyzed with a flow cytometer (FACScan®; BD Biosciences) equipped with FlowJo v10 software (BD Biosciences).

β-Cell purification via flow cytometric analysis

Purified islets were incubated with 2 mg/mL collagenase II (Sigma Aldrich, St. Louis, MO, USA) for 10 min, and INSULIN-positive cells were detected following a standard intracellular staining procedure using Cytofix/Cytoperm solution (Becton, Dickinson and Company, USA). After staining with INSULIN-APC, the cells were analyzed via flow cytometry (BD FACS Aria II SORP). Purified β-cell and non β-cell fractions were collected for RNA extraction and qRT-PCR analysis.

Pancreatic intraductal viral infusion in mice

miR-455, miR-455 inhibitor, sh-Cpeb1, and sh-Cdkn1b were inserted into a lentivirus-MIP (mouse insulin 2 promoter)-PGLV3/H1/Puro vector (len-miR-455, len-anti-miR-455, len-shCpeb1, len-shCdkn1b). The indicated lentivirus (1 × 10⁹) was dissolved in 0.15 ml normal saline (NS) or normal saline (NS) and infused into the pancreatic duct at 6 µl per min for 25 min using an R462 perfusion pump (RWD, China, Shengzhen) according to the procedure described by Xiao X et al(25). At 72 h after injection, islets were lysed to extract total RNA or protein to measure the overexpression efficacy. At 1 week after injection, the mice were fed a high-fat diet (HFD; D12494, 60% energy from fat) for 20 weeks.
Mouse metabolic assays

Mouse fasting blood glucose (FBG) levels and fasting serum insulin (FINS) levels were examined using a glucometer (OMRON, Japan) and an ELISA (ExCell Bio, Shanghai, China) after 12 h of fasting treatment. To perform the glucose tolerance tests, 1.5 g/kg glucose (Sigma-Aldrich, St Louis, MO, USA) was intraperitoneally (i.p.) injected into mice, whereas 0.75 U/kg insulin (Novolin R, Novo Nordisk, Bagsvaerd, Denmark) was injected i.p. into mice for insulin tolerance tests.

RNA extraction and qRT-PCR analysis

Total RNA was extracted using TRIzol (Invitrogen) as previously described [17]. Reverse transcription reactions were performed using a PrimeScript™ RT Reagent Kit (Takara, Tokyo, Japan), and diluted cDNA was used for qRT-PCR analysis using a SYBR Premix Ex Taq II Kit (Takara) with the appropriate primers listed in Table S4. The relative expression of genes was determined using a comparative method (2-ΔΔCT). miRNA and mRNA levels were normalized to the expression of small RNAs (sno234 and U6) or mRNA (Gapdh, Hprt and Ppia), respectively. For miR-455 and U6, TaqMan probes (Ambion) were used to confirm our results.

PCR poly (A) tail (PAT) assay

Total cellular RNA was reversed transcribed using MultiScribe reverse transcriptase (Life Technologies) and an oligo (dT) anchor primer (5′-GCGAGCTCCGCAGCCCCGTTTTTTTTTTTTT-3′), and subsequent PCR was conducted with an anchor primer (5′-AAAAACGCGGCGGCGAAGCTCGC-3′) and a specific primer for Cdkn1b (5′-GCCAATTATTGTTACACATT-3′) located near the 3′ end of the Cdkn1b 3′UTR.

RNA immunoprecipitation (RIP)

RNA immunoprecipitation was performed using an EZMagna RIP Kit (Millipore, Billerica, MA,
USA) following the manufacturer’s protocol. Cells were lysed in complete RIP lysis buffer, and then, 100 μl of whole cell extract was incubated with RIP buffer containing magnetic beads conjugated with anti-Ago2 (CST) or anti-CPEB1 (Abcam) antibody or negative control normal mouse IgG (Abcam). Furthermore, purified RNA was subjected to qRT-PCR analysis to demonstrate the presence of the binding targets using the respective primers. The primer sequences are listed in Table S4.

**Immunohistochemistry and immunofluorescence**

Pancreases were fixed in 4% paraformaldehyde and embedded in paraffin, and the antigen in the cut sections was retrieved by boiling in 10 mM Tris/EDTA (pH 9.0). Sections were permeabilized and blocked in PBS buffer containing 0.3% Triton X-100, 1% BSA, and 5% goat serum. Primary antibody (anti-KI67, anti-INSULIN, anti-glucagon) binding was performed overnight at 4°C, and incubation with secondary antibody was performed at room temperature for 1 h. The slides were analyzed via confocal laser scanning microscopy (CLSM; Carl Zeiss LSM700) at ×20 or ×40 magnification. The antibodies are listed in Table S5.

**Fluorescence in situ hybridization (FISH)**

A Cy3-labeled miR-455 probe was designed and synthesized by GenePharma (Shanghai, China). For the FISH assay, pancreases were fixed in 4% formaldehyde, permeabilized with 0.3% Triton X-100 for 15 min, and washed with PBS three times and once in 2× SSC buffer. Hybridization was carried out at 37°C for 16 h using DNA probe sets, followed by incubation with anti-INSULIN antibody overnight at 4°C and incubation with secondary antibody at room temperature for 1 h. Images were obtained with a confocal laser scanning microscope (CLSM, LSM700, Zeiss, Germany) and processed using ZEN imaging software.
Western blot Analysis

Mouse islets (200 islets per group) and MIN6 cells were lysed with RIPA lysis buffer (Beyotime) containing 1% PMSF (Sigma). Then, western blot analyses were conducted according to standard procedures using specific antibodies. The antibodies are listed in Table S5.

Insulin/KI-67 double staining

MIN6 cells and primary islets were fixed in 4% paraformaldehyde and permeabilized and blocked in PBS buffer containing 0.3% Triton X-100 and 1% BSA. Primary INSULIN and KI-67 antibody binding was performed overnight at 4°C, and then, incubation with secondary antibody was performed at room temperature for 1 h. Finally, the nuclei were stained with DAPI. Images were obtained using a confocal laser scanning microscope (CLSM, Carl Zeiss LSM700) at ×40 or ×20 magnification.

β-cell mass

Pancreatic sections were stained with anti-INSULIN antibody and DAPI to determine β-cell mass. The pancreatic sections were scanned entirely using the 10× objective of a Zeiss LSM700 microscope. β-Cell mass was calculated by multiplying the area of insulin-positive cells/pancreas area using ImageJ (26).

Cell counting kit-8 (CCK-8) assay

MIN6 cells were seeded into 96-well plates (4×10^4 cells well^-1) in 100 μl culture medium. A CCK-8 assay (Vazyme, Nanjing, Jiangsu, China) was performed according to the manufacturer’s instructions.

Statistical analysis

All data represent at least 3 independent experiments and are shown as the mean ± SD. Comparisons
between two groups were performed using Student’s *t* test and between multiple groups via ANOVA. Dunn’s multiple comparisons for one-way ANOVA and Fisher’s least significant difference (LSD) for two-way ANOVA were used. The level of significance was set at \( *p < 0.05 \), \( **p < 0.01 \), and \( ***p < 0.001 \). GraphPad Prism 7 software (GraphPad, San Diego, CA, USA) was used for all calculations.

**Data and Resource Availability**

The data sets generated and/or analyzed during the current study are available from the corresponding authors on reasonable request. The RNA-seq raw data that support the findings of this study has been deposited in the NCBI’s Sequence Read Archive (SRA) database (PRJNA731006 [https://submit.ncbi.nlm.nih.gov/subs/sra/SUB9682154/overview]).

**RESULTS**

**miR-455 is elevated in the islets of obese mouse models.**

To identify miRNAs potentially involved in β-cell mass expansion during obesity, we performed small RNA sequencing on total RNA from 8-week-old *ob/ob* mice, which are capable of long-term compensatory insulin hypersecretion(27). The characteristics of the animals used in this study are presented in Supplementary Figure 1a and b. Using an absolute fold change of at least 1.0 and *p* value of less than 0.05, we observed that the expression of 39 miRNAs was significantly upregulated and that the expression of 46 miRNAs was downregulated in *ob/ob* mice compared with wild-type (WT) mice. Among the increased miRNAs identified, the expression of *miR-6932-3p* (*miR-6932*) and *miR-455-5p* (*miR-455*) was the most significantly increased (Figure 1a, Table S1). *miR-6932* expression was significantly lower than *miR-455* expression in human islets, mouse islets and MIN6 cells (Supplementary Figure 1c). Therefore, we chose *miR-455* for further analysis. Then, we
measured the expression of miR-455 in the islets of ob/ob mice aged 4-12 weeks and observed an increase in miR-455 expression at 6 weeks of age with the onset of insulin resistance (Figure 1b). Similarly, the pri-miR-455 transcript was also upregulated in the islets of ob/ob mice (Figure 1c), indicating that miR-455 is regulated at the transcriptional level. We also observed a similar increase in the expression of mature miR-455 in the islets of mice treated with a 15-week HFD, while miR-455 expression was significantly decreased, accompanied by an increase in glucose (Figure 1d). We also found that miR-455 expression was dramatically increased in the islets of young db/db mice (Figure 1e), all of which showed that this observation is not limited to one mouse model of obesity and insulin resistance. In addition, the expression of miR-455 was only significantly increased in islets, white adipose tissue (WAT), brown adipose tissue (BAT) and muscle in ob/ob and HFD mice compared with their respective controls (Figure 1f). Interestingly, we also found that miR-455 levels were significantly increased in Type-2 diabetic donors compared with non-diabetic donors (Figure 1g), and serum miR-455 expression levels were significantly correlated with the BMI and HbA1c of these subjects (Figure 1h-i). Then, we found that miR-455 expression was approximately 4-fold higher than that in exocrine glands, indicating that islets represent the main source of miR-455 in the pancreas (Figure 1j, k). Moreover, we purified β-cells from islets via flow cytometric analysis, and the results revealed that miR-455 was enriched in purified β-cells (Figure 1l). Overall, miR-455 expression in islets is increased in both dietary and genetic mouse models of obesity.

Silencing of Egr2 in pancreatic β-cells suppresses miR-455 expression

To determine the potential reason for the changes in miR-455 expression detected in the islets of obese mice, MIN6 cells, EndoC-βH1 cells, normal mouse islets and human islets were exposed to pathophysiological concentrations of palmitate, glucose, and proinflammatory cytokines. The
expression of miR-455 was increased in the presence of palmitate (0.5 mM) (Figure 2a-d) and a high glucose level (25 mM) (Figure 2e-h), but these changes were not observed in MIN6 cells or mouse islets incubated with proinflammatory cytokines (Supplementary Figure 2a-c).

Next, we explored the potential molecular mechanism of miR-455 upregulation in the islets of obese mice. We predicted a miR-455 promoter region 2 kb upstream of the mouse and human miR-455 sequence and constructed three sgRNAs corresponding to this promoter in a lenti-CRISPRv2 puro vector. Supplementary Figure 2d and e show that miR-455 levels were decreased in MIN6 cells and human islets transfected with sgRNAs. These results revealed that the predicted promoter region can modify miR-455 expression. Then, MIN6 cells were exposed to 0.5 mM palmitate for 48 h, and qRT-PCR was performed to confirm the expression levels of high-score transcription factors (score>15) of the miR-455 promoter, which were predicted by JASPAR. Among these candidates, early growth response 2 (Egr2), which has two potential binding sites in the mus-miR-455 promoter (Supplementary Figure 2f), was mostly upregulated (Supplementary Figure 2g). Interestingly, Egr2 also has one potential binding site in the hsa-miR-455 promoter (Supplementary Figure 2f). Moreover, the mRNA and protein levels of EGR2 were upregulated in obese mouse islets (Figure 2i, g) and MIN6 cells in response to palmitate treatment (Supplementary Figure 2h) or high glucose treatment (Supplementary Figure 2i). The expression levels of EGR2 were also increased in human islets (Supplementary Figure 2j and k) and EndoC-βH1 cells (Figure 2 k and l) exposed to palmitate and high glucose.

Egr2 is known as an important regulator of insulin resistance(28) and can be upregulated by palmitate in MIN6 cells(29). We next focused on studying whether obesity facilitates miR-455 upregulation by increasing Egr2 expression. As expected, in dual luciferase assays, MIN6 cells...
transfected with Egr2 overexpression plasmid exhibited a significantly higher Egr2 binding ability to the miR-455 promoter compared to the control, and after mutation of the R1 binding region, the binding ability was significantly decreased (Supplementary Figure 2I). Moreover, Egr2 in the miR-455 promoter region was increased in the islets of obese mice (Figure 2m and Supplementary Figure 2m). A similar trend was obtained in MIN6 cells (Supplementary Figure 2n, o), in EndoC-βH1 cells (Figure 2n) and human islets (Figure 2o) transfected with oe-Egr2. EMSA results revealed that the signal from the probe-protein-anti-EGR2 complex could be detected using a miR-455 probe in mouse islets (Figure 2p), EndoC-βH1 cells (Figure 2q) and human islets (Figure 2r) and MIN6 cells (Supplementary Figure 2p). Further research showed that palmitate-induced miR-455 upregulation in MIN6 cells and human islets was partially reversed by knockdown of Egr2 (Figure 2s, t). These results indicate that the increased miR-455 expression in the islets of obese mice is mediated by upregulation of Egr2.

**miR-455 regulates β-cell proliferation**

To explore the potential miR-455 function in β-cells, MIN6 cells were transfected with miR-455 mimic (miR-455) or miR-455 inhibitor (anti-miR-455). The knockdown and overexpression efficiencies were approximately 80% and 220-fold, respectively (Supplementary Figure 3a). CCk-8 assay results showed that cell proliferation was reduced by miR-455 silencing (Supplementary Figure 3b). We observed that the upregulation of miR-455 in MIN6 cells, resulted in an increase in the number of INSULIN+/KI-67+ cells (Figure 3a). Similar results were obtained in MIN6 cells (Figure 3b), EndoC-βH1 cells (Figure 3c), human islets (Figure 3d), and mouse islets (Supplementary Figure 3c). Next, we assessed whether miR-455 regulates MIN6 cell proliferation by evaluating cell cycle status. Flow cytometry results indicated that miR-455 reduced the
proportion of MIN6 cells in G1 phase and increased the proportion in S phase (Figure 3e). Since cell cycle progression is tightly regulated by cyclin-related proteins, we next tested the expression levels of cyclin-related proteins in MIN6 cells transfected with miR-455 or anti-miR-455. As shown in Supplementary Figure 3d, miR-455 significantly decreased the expression level of cyclin-dependent kinase inhibitor (CDKN1B), which is an inhibitor of G1-S transition. The CDKN1B protein level was also decreased in MIN6 cells transfected with miR-455 (Figure 3f). However, miR-455 did not significantly affect insulin synthesis, insulin content or insulin secretion (Supplementary Figure 3e-g) in MIN6 cells. Similar results were observed in human islets (Supplementary Figure 3h-j).

In view of these findings, to verify whether ectopic expression of miR-455 also affects β cell proliferation in vivo, 1 × 10⁹ lentivirus-MIP-miR-455 (len-miR-455) cells or 1 × 10⁹ lentivirus-MIP-anti-miR-455 (len-anti-miR-455) cells were injected into 8-week-old male C57BL/6J mice through pancreatic intraductal infusion. We observed an approximately 65-fold miR-455 upregulation in the islets that received len-miR-455 compared to those that received lentivirus-MIP-miR-NC (len-miR-NC) and an approximately 70% decrease in miR-455 expression in the islets of len-anti-miR-455 mice compared to lentivirus-MIP-anti-NC (len-anti-NC) mice (Figure 3g), whereas the expression level of miR-455 was not significantly changed in other organs (Supplementary Figure 3k). len-miR-455 mice exhibited slightly improved glucose tolerance in the IPGTT test (Figure 3h). Similarly, the insulin levels in len-miR-455 mice improved after glucose injection compared with those in control mice (Figure 3i), while miR-455 had no effect on glucagon in vivo (Supplementary Figure 3l). len-miR-455 mice showed a significant increase in both β-cell proliferation (Figure 3j) and β-cell mass (Supplementary Figure 3m). Moreover, the CDKN1B mRNA and protein levels

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were suppressed in the islets of len-miR-455 mice, and vice versa (Supplementary Figure 3n, Figure 3k, l). Furthermore, no change was detected in TUNEL-positive β-cells after ectopic expression of miR-455 (Supplementary Figure 3o), indicating that cell death was not a primary mechanism underlying the effect on β-cell mass.

**Loss of miR-455 expression in obese mice inhibits β-cell proliferation**

To address whether miR-455 mediates the compensatory expansion of β-cells during insulin resistance, $1 \times 10^9$ lentivirus-MIP-anti-miR-455 (len-anti-miR-455) cells were injected into 8-week-old male C57BL/6 mice via pancreatic ductal infusion, and then, the mice were fed an HFD for 20 weeks (Figure 4a). As shown in Figure 4b, miR-455 was downregulated to 70% in the islets of len-anti-miR-455 mice compared to those receiving len-anti-NC even 20 weeks after injection. len-anti-miR-455 treatment had no effect on cumulative energy intake (Supplementary Figure 4a), body weight (Supplementary Figure 4b), or body fat content (Supplementary Figure 4c). However, inhibition of miR-455 slowly increased glycemia in random-fed mice after 20 weeks of high-fat diet treatment (Supplementary Figure 4d). The obesity-associated rise in serum insulin concentrations was slightly lower in HFD animals treated with len-anti-miR-455 (Figure 4c), and HOMA-IR indices in mice with miR-455 repression were increased (Figure 4d). In accordance with this, glucose tolerance tests revealed impairment of glucose tolerance upon miR-455 knockdown (Figure 4e), and insulin sensitivity was damaged upon downregulation of miR-455 (Figure 4f). Counts of KI-67-positive β-cells were lower in len-anti-miR-455 mice than in control animals (Figure 4g). Moreover, we found that miR-455 knockdown markedly decreased β-cell mass compared to that in len-anti-NC mice (Supplementary Figure 4e). These results indicate that the miR-455 inhibitor resulted in damage to β-cell mass in diet-induced obese mice.

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Furthermore, high expression of miR-455 in the islets of ob/ob mice was silenced by len-anti-miR-455 through pancreatic intraductal infusion (Supplementary Figure 4f). Compared to len-anti-NC/ob mice, len-anti-miR-455/ob mice exhibited severe hyperglycemia and reduced systemic insulin levels (Figure 4 h, i) due to loss of β cell mass and compensation (Figure 4j, Supplementary Figure 4g). Collectively, these data show that suppression of miR-455 expression in obesity model mice attenuated adaptive β-cell proliferation.

*miR-455 affects β-cell proliferation by targeting Cpeb1*

Most miRNAs function by suppressing their target gene expression, and we found an inverse expression pattern between miR-455 and Cdkn1b, which prompted us to verify whether Cdkn1b is a target gene of miR-455. Unfortunately, using a double luciferase reporter, we found no targeting relationship between miR-455 and Cdkn1b (Supplementary Figure 5a).

To identify the potential miRNA targets of miR-455, in silico analysis was performed using TargetScan, miRDB, miRWalk, and starBase, which jointly predicted that 2 genes (Cpeb1 and Usp9x) may act as biological targets of miR-455 (Figure 5a). Next, we verified the targeting relationship between the two genes and miR-455. Cpeb1 harbored a miR-455 binding site, which was conserved in humans, mice, and rats (Figure 5b). We cloned a portion of the mouse 3’ UTR of Cpeb1 (1316 nt) into a luciferase reporter construct and observed decreased activity in the presence of a miR-455 mimic (Figure 5c). In addition, mutation of eight nucleotides in the binding site within the mouse UTR (at positions 1289-1296) from GGCACAUA to AATGTGCG abolished the inhibitory effect of miR-455 (Figure 5c). Moreover, we conducted anti-Ago2 RIP in MIN6 cells transiently overexpressing miR-455. Endogenous Cpeb1 pull-down by Ago2 was specifically enriched in miR-455-transfected cells (Figure 5d), and vice versa (Supplementary Figure 5b).
Overexpression of miR-455 decreased CPEB1 expression in the MIN6 cells (Supplementary Figure 5c). The mRNA and protein levels of CPEB1 were also significantly decreased in the islets of len-miR-455 mice but increased in the islets of len-anti-miR-455 mice (Figure 5e, f and Supplementary Figure 5d). Finally, silencing the expression of miR-455 in the pancreas of ob/ob mice increased CPEB1 expression (Figure 5g and Supplementary Figure 5e). Unfortunately, using the same technical means, we did not find a targeted relationship between miR-455 and Usp9x (Supplementary Figure 5f).

To address the relevance of the miR-455: Cpeb1 interaction in the islets of obese mice, we measured the expression of Cpeb1 in pancreatic islets from ob/ob and HFD mice. As shown in Figure 5 h and i, the mRNA and protein levels of CPEB1 were significantly decreased, and similar trends were observed in MIN6 cells incubated with high glucose (Figure 5j) or 0.5 mM palmitate (Figure 5k), indicating a targeted relationship between miR-455 and Cpeb1. We next verified whether miR-455 regulates β cell proliferation by suppressing Cpeb1. We studied the role of Cpeb1 in β-cell proliferation, and the results showed that transfection with Cpeb1 siRNA (si-Cpeb1) markedly promoted MIN6 cell proliferation and that the effects of Cpeb1 knockdown were similar to those of miR-455 overexpression (Figure 5l). Compared to cells transfected with si-Cpeb1, MIN6 cells transfected with si-Cpeb1 and anti-miR-455 exhibited significantly lower β-cell expansion ability (Figure 5l, Supplementary Figure 5g, h). Moreover, we found that len-shCpeb1-treated mice showed a higher positive KI-67 β-cell count and β-cell mass, while len-anti-miR-455 injection restored this effect (Figure 5m, Supplementary Figure 5i), suggesting that restoration of CPEB1 attenuated the β-cell proliferation effect of miR-455. Taken together, these findings indicate that miR-455 promotes β-cell mass expansion in a CPEB1-dependent manner.
CPEB1 regulates Cdkn1b expression by promoting its translation efficiency

As we previously verified, there is no targeting relationship between miR-455 and Cdkn1b, but miR-455 promotes adaptive pancreatic β-cell proliferation during obesity in a CDKN1B-dependent manner. This suggests that the regulatory effect of miR-455 on Cdkn1b is indirect. On the other hand, we confirmed that CPEB1 is a target gene of miR-455, and by controlling mRNA translation efficiency via the 3'UTR, CPEB1 regulates many important biological processes, ranging from cell cycle control(30) to regulation of insulin resistance(31, 32). All these clues prompted us to hypothesize that CPEB1 might regulate Cdkn1b expression.

To better understand the relationship between CPEB1 and CDKN1B, we performed a bioinformatics search for target transcripts that could potentially be modulated by CPEB1. As expected, we found that Cdkn1b was one of the most promising predicted targets (Supplementary Figure 6a). Since CPEB1 acts as a sequence-specific RNA-binding protein(32), we analyzed the possible interaction between CPEB1 and Cdkn1b mRNA using RNA-binding protein-immunoprecipitation (RIP) experiments. The RIP results revealed that the CPEB1 antibody but not IgG successfully pulled down Cdkn1b transcripts (Figure 6a) and that overexpression of Cpeb1 in MIN6 cells enhanced this binding ability (Supplementary Figure 6b). These data further confirmed that Cdkn1b mRNA is in the CPEB1 complex. In an attempt to determine the regulatory mechanisms underlying the interaction between CPEB1 and Cdkn1b, a sequence containing the wild-type or mutated 3'UTR of Cdkn1b (Cdkn1b-WT or Cdkn1b-MUT) was cloned into a pGL3-control vector to construct a reporter system. When we cotransfected MIN6 cells with si-Cpeb1 and Cdkn1b-WT or Cdkn1b-MUT, we observed that overexpression of Cpeb1 strongly increased luciferase expression in the Cdkn1b-WT cotransfected group but not in the Cdkn1b-MUT group (Figure 6b).
These results support CPEB1 regulation of Cdkn1b mRNA through its 3' UTR.

To further clarify the molecular mechanisms of CPEB1 in Cdkn1b regulation, we examined whether Cdkn1b mRNA stability was affected as a consequence of CPEB1 ectopic modulation. MIN6 cells transfected with oe-Cpeb1, si-Cpeb1 or vehicle were treated with actinomycin D (10 µg/ml). Consistent with the results of Galardi et al.(33), the ectopic modulation of CPEB1 did not change the half-life of Cdkn1b mRNA, indicating that CPEB1 action is not exerted through modulation of Cdkn1b mRNA stability (Supplementary Figure 6c). Since CPEB1 was originally identified as a sequence-specific RNA-binding protein that promotes polyadenylation-induced translation(32), we asked whether the polyadenylation status of Cdkn1b mRNA was altered by CPEB1 modulation. We performed a PCR poly-A tail assay on endogenous Cdkn1b mRNA in MIN6 cells transfected with oe-Cpeb1, si-Cpeb1 or vehicle. Overexpression of Cpeb1 enhanced the polyadenylation of Cdkn1b mRNA, while si-Cpeb1-mediated knockdown of Cpeb1 decreased polyadenylation (Figure 6c). As poly-A lengthening is expected to result in more efficient translation, we examined whether the CDKN1B level was altered by CPEB1 ectopic modulation. As shown in Figure 6d and e, ectopic modulation of Cpeb1 changed the protein level of CDKN1B but not the mRNA level of Cdkn1b. Analysis of CDKN1B in the islets of lentivirus-MIP-shCpeb1 (len-shCpeb1) mice revealed a similar trend (Figure 6f, g). All these observations indicate that CPEB1 regulates Cdkn1b expression by promoting elongation of the poly-A tail and translation efficiency.

The miR-455/CPEB1/CDKN1B axis promotes adaptive β-cell expansion during insulin resistance

To verify that miR-455 can indirectly regulate Cdkn1b through CPEB1, we conducted a series
of functional recovery experiments. Through RIP experiments, we found that the interaction between \textit{Cdkn1b} and CPEB1 was significantly regulated by \textit{miR}-455 (Figure 7a, Supplementary Figure 7a). And as shown Figure 7b, CDKN1B protein levels were decreased by \textit{Cpeb1} silencing, while \textit{anti-miR}-455 restored CDKN1B expression. Analysis of CDKN1B in the islets of \textit{lentivirus-MIP-shCpeb1} (\textit{len-shCpeb1}) or \textit{len-shCpeb1} and \textit{len-anti-miR}-455 mice displayed a similar trend (Figure 7c). Next, we found that \textit{si-Cdkn1b} enhanced MIN6 cell proliferation, while \textit{oe-Cpeb1} abrogated this effect (Figure 7d, e and Supplementary Figure 7b). Moreover, we verified that \textit{lentivirus-MIP-shCdkn1b} (\textit{len-shCdkn1b}) promoted \(\beta\)-cell proliferation and expanded \(\beta\)-cell mass, while \textit{len-Cpeb1} partially restore this phenomenon (Figure 7f, g, Supplementary Figure 7c). These results support the role of \textit{miR}-455 in promoting \(\beta\)-cell proliferation by inhibiting the expression of \(Cdkn1b\) via direct targeting of \(Cpeb1\).

\textbf{DISCUSSION}

Obesity is frequently associated with diminished insulin sensitivity, which is normally compensated for by an expansion of the functional \(\beta\)-cell mass that prevents chronic hyperglycemia and the development of T2D. The molecular basis underlying compensatory \(\beta\)-cell mass expansion is largely unknown. In rodents, we found that \(\beta\)-cell mass expansion during obesity is associated with upregulated expression of \textit{miR}-455, which was induced by Egr2. And we observed that overexpression of \textit{miR}-455 increased \(\beta\)-cell proliferation both \textit{in vitro} and \textit{in vivo}. Mechanistically, we observed that the \textit{miR}-455/CPEB1 pathway inhibits translation of \(Cdkn1b\) mRNA and finally leads to lower levels of CDKN1B in \(\beta\)-cells, in turn significantly promoting \(\beta\)-cell adaptive proliferation (Figure 8). These findings point to a major role for \textit{miR}-455 in compensatory \(\beta\)-cell mass expansion during obesity.
Ob/ob mice are a model of increased islet mass that is induced by severe insulin resistance and have been extensively studied as a model of type 2 diabetes (34). In our study, we used RNA-Seq to detect differentially expressed miRNAs in the islets of ob/ob mice. Our results revealed that 85 miRNAs were significantly changed, of which 65 miRNAs had been identified as differentially expressed in previous research (35, 36), including miR-455, miR-184, miR-375, and miR-122a. Yang ZM found that miR-455 expression was increased in the serum of patients with insulin resistance (37). However, the mechanism of obesity-induced miR-455 upregulation and whether miR-455 can regulate β-cell function remain unclear. Therefore, we chose miR-455 for further analysis. First, to identify the possible causes of the variation in miR-455 expression in the islets of obese mice, we used a bioinformatics approach to search for putative transcription factors that could bind to the miR-455 promoter, among which Egr2 was found to be upregulated in response to palmitate treatment in primary islets and in ob/ob mouse islets. Our results are consistent with a report by Hayes MG, in which the expression of Egr2 was found to be associated with T2D via genome-wide association studies (GWASs) (38). Accumulating evidence has revealed that palmitate and glucose can induce increased expression of Egr2 under insulin resistance (39, 40). Here, we showed that the Egr2 expression level was elevated in the pancreatic islets of obese mouse models, in MIN6 cells and in human islets incubated with 0.5 mM palmitate and 25 mM glucose. Luciferase reporter, EMSA and ChIP analyses showed that Egr2 can directly combine with the promoter of mus-miR-455 and hsa-miR-455 to induce an increase in miR-455 expression.

Here, we showed that obesity induced miR-455 upregulation in islets and the levels of miR-455 were increased in the muscle, white adipose tissue (WAT), and brown adipose tissue (BAT) of obese mice. miR-455 has previously been shown to activate AMPKa1 and then promote the brown
adipogenic program and mitochondrial biogenesis, suggesting that miR-455 is a potential therapeutic for treating obesity. miR-455 expression has been evaluated in muscle with induced inflammation. Fang and collaborators confirmed that miR-455 can ameliorate lipid metabolic disorders of the liver in db/db mice by inhibiting SOCS3. All these studies indicate that miR-455 may play a significant role during diabetes. However, despite this body of evidence, thus far, no studies have investigated the role of miR-455 in pancreatic β-cells. In line with previous studies showing that miR-455 plays a critical role in cell proliferation, we observed that miR-455 overexpression induced β-cell proliferation in vivo and in vitro. Furthermore, we demonstrate that β-cell-specific knockdown of miR-455 through pancreatic ductal infusion markedly aggravated HFD-induced insulin resistance resulting from a decrease in β-cell hyperplasia. Moreover, in line with islet expression analysis in insulin-resistant models, β-cell-specific knockdown of miR-455 (len-MIP-anti-miR-455) via pancreatic ductal infusion in ob/ob mice decreased the β-cell mass and KI-67 incorporation rate. The level of miR-455 was simultaneously increased in the islets of different types of obese mouse models characterized by β-cell mass expansion, suggesting a general role for miR-455 in this important compensatory mechanism. Interestingly, our in vivo experimental results revealed that insulin secretion and the blood glucose level did not substantially differ between len-miR-455 and control mice. Although many studies have explored whether miRNAs play an important role in obesity and diabetes, miRNAs simply act as regulators of gene expression via posttranscriptional regulation. Thus, the function of miRNAs might not trigger pleiotropic effects. For example, Tattikota SG found that miR-184 regulates β-cell proliferation but has no effect on insulin secretion, and Jacovetti C showed that miR-338-3p and miR-451 regulate β-cell proliferation but do not significantly affect insulin content or glucose-induced insulin
secretion.

Based on a report by Shoshan E, miR-455 contributes to melanoma growth and metastasis by targeting CPEB1(47). In our study, we verified that miR-455 can directly target CPEB1 in β-cells, which regulate many important biological processes, ranging from cell cycle control to insulin resistance(30, 31), by controlling mRNA translation efficiency via 3′UTR(32, 48). In agreement with these findings, we discovered that CPEB1 promotes Cdkn1b translation efficiency through elongation of the 3′ UTR of Cdkn1b mRNA. Consistent with the results of Galardi et al., CPEB stimulates Cdkn1b expression by promoting elongation of the poly-A tail and translation efficiency(33). Here, our results revealed that Cpeb1 overexpression facilitates Cdkn1b translation, while miR-455 restored this effect. Previous studies have shown that CDKN1B plays a negative role in the proliferation of β-cells, which is consistent with our findings that the expression level of CDKN1B is downregulated in compensatory proliferative β-cells(49, 50). Altogether, these results support a role of miR-455 in promoting β-cell adaptive proliferation by inhibiting the expression of Cdkn1b via direct targeting of Cpeb1.

In summary, we revealed the effects of nutritional stress on miR-455, which is involved in compensatory β-cell mass expansion during obesity. Our results demonstrate that the miR-455/CPEB1/CDKN1B axis is required for obesity-induced adaptive pancreatic β-cell proliferation. These findings provide novel insights into the mechanism of compensatory β-cell expansion in response to increased insulin demand. Detailed knowledge of the mechanisms controlling the level and activity of miR-455 during obesity may pave the way to new therapeutic strategies, with the ultimate goal being prevention of T2D by promoting β cell mass regeneration.

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Figure legends

Figure 1 miR-455 is elevated in the islets of obese mouse models

(a) Comparison of small RNA sequencing analysis of total RNA from islets of 8-week-old ob/ob mice and wild-type (WT) littermates (n = 6 mice per group). qRT-PCR was used to detect the expression levels of miR-455 (b) and pri-miR-455 (c) in the islets of 4-12-week-old ob/ob mice and control mice (n = 3 mice per group). (d) miR-455 levels in the islets of C57BL/6J mice treated with a 15-week high-fat diet. The glucose concentrations indicate blood glucose levels in the mice. NCD: normal chow diet (n = 3 mice per group). (e) miR-455 levels in the islets of 4- to 12-week-old db/db mice (n = 3 mice per group). (f) The expression levels of miR-455 in different tissues from ob/ob mice and HFD mice compared to wild-type mice and NCD mice, respectively (n = 3 mice per group). (g) The expression levels of miR-455 in the serum extracted from non-diabetic (n = 21) and Type-2 diabetic (n = 76), using Ce-miR-39-1 as positive control. Correlation between miR-455 levels and BMI (h) or HbA1c (i). Pearson’s correlation coefficients (R) is shown. (j) miR-455 expression levels in the pancreas, islets and exocrine glands of C57BL/6J mice (n = 3 mice per group). (k) Immunofluorescence was performed to determine the miR-455 expression levels in the pancreas of C57BL/6J mice. Magnification: 20 ×; scale bar, 20 μm. (l) The levels of miR-455 in the islet, purified β-cell and non β-cell fractions (n = 5 mice per group). The data are presented as the mean ± SD. ** p < 0.01, *** p < 0.001.

Figure 2 Silencing of Egr2 in pancreatic β-cells represses miR-455 expression

qRT-PCR was performed to examine the miR-455 expression levels in the MIN6 cells, EndoC-βH1 cells, mouse primary islets and human islets incubated with 0.5 mM palmitate (a-d) or with 25 mM glucose (e-h). The protein and mRNA levels of EGR2 in the islets of HFD (i, n = 5 mice per group)
and ob/ob mice (j, n = 5 mice per group) as well as in the EndoC-βH1 cells incubated with 0.5 mM palmitate (k) or 25 mM glucose (l). (m-o) Enrichment of Egr2 on the miR-455 promoter relative to IgG in the islets of ob/ob mice (m), EndoC-βH1 cells (n) and in human islets (o) transfected with oe-Egr2, pcDNA 3.1 vector, si-Egr2 or si-NC detected by ChIP-qPCR assays. Direct binding of Egr2 to the miR-455 promoter in mouse islets (p, n = 5 mice per group), EndoC-βH1 cells (q) and human islets (r) determined by EMSA. C1 and C2 represent nuclear protein-miR-455 probe complexes and nuclear protein-miR-455 probe-anti-EGR2 complexes, respectively. The miR-455 expression levels in the MIN6 cells (s) and human islets (t) incubated with 0.5 mM palmitate and cotransfected with si-Egr2. The data are presented as the mean ± SD. ** p < 0.01, *** p < 0.001.

**Figure 3 miR-455 regulates β-cell proliferation**

MIN6 cells were transfected with miR-455 mimic or miR-455 inhibitor (anti-miR-455) for 48 h. Then, INSULIN+/KI-67+ cells were counted via flow cytometry (a) and immunofluorescence (b). Magnification: 20 x; scale bar, 20 μm. The INSULIN/KI-67-positive cells in EndoC-βH1 cells (c) and human islets (d) transfected with miR-455 or anti-miR-455 were counted via immunofluorescence. Magnification: 20 x; scale bar, 20 μm. (e) The percentage of cells in G1, S or G2 phase was determined by flow cytometry. (f) The CDKN1B protein level. (g) Lentivirus-MIP-miR-455 (lentivirus-miR-455) or lentivirus-MIP-anti-miR-455 (lentivirus-anti-miR-455) was injected via pancreatic ductal infusion for 72 h, and the miR-455 expression levels in the islets were detected by qRT-PCR (n = 3 mice per group). (h) IPGTT (1.5 g kg⁻¹) in overnight fasted len-miR-455 mice, len-anti-miR-455 mice and control mice (n = 9 mice per group). (i) In vivo insulin excursions of overnight fasted len-miR-455 mice, len-anti-miR-455 mice and control mice after IPGTT exposure (n = 9 mice per group). (j) KI-67-positive β-cells in the islets of len-miR-455 mice
and len-anti-miR-455 mice. Magnification: 20 x; scale bar, 20 μm. (k-l) The CDKN1B protein levels in the islets of len-miR-455 mice and len-anti-miR-455 mice. The data are presented as the mean ± SD. * p < 0.05, *** p < 0.001.

**Figure 4 Loss of miR-455 expression in obese mice inhibits β-cell proliferation**

(a) Flowchart of the in vivo experiments designed for detection of β-cell function after pancreatic ductal infusion. Eight-week-old male len-anti-miR-455 mice and control mice (lentivirus-MIP (mouse insulin2 promoter)-LV3/H1 vector) were exposed to an HFD for 20 weeks. (b) The miR-455 expression levels were examined via qRT-PCR. Fasting insulin (FINS) levels in HFD-fed mice were measured via ELISA (c, n = 9 mice per group), and the homeostatic model assessment of insulin resistance (HOMA-IR) index was measured (d, n = 9 mice per group). The HOMA-IR index was calculated using the equation (FBG (mmol l⁻¹) x FINS (mIU l⁻¹))/22.5. (e-f) Intraperitoneal glucose tolerance tests (IPGTTs) (1.5 g/kg) (e) and intraperitoneal insulin tolerance tests (IPITTs; 0.75 U/kg) (f) were performed in len-anti-miR-455 mice and control mice at the 8th or 10th week of high-fat diet administration, respectively. The corresponding area under the curve (AUC) of the blood glucose level was calculated (n = 9 mice per group). (g) KI-67-positive β-cells were analyzed by immunofluorescence (n = 3 mice per group). Magnification: 20 x; scale bar, 20 μm. (h) Random blood glucose level in 8-week-old ob/ob mice treated with len-anti-miR-455 (n = 7 mice per group). (i) Plasma insulin concentrations in 8-week-old ob/ob mice treated with len-anti-miR-455 (n = 7 mice per group). (j) The number of KI-67-positive β-cells in 8-week-old len-anti-miR-455/ob mice (n = 3 mice per group). The data are presented as the mean ± SD. * p < 0.05, *** p < 0.001.

**Figure 5 miR-455 affects β-cell proliferation by targeting Cpeb1**

(a) Four independent miRNA target prediction algorithms were used to predict the target genes of
miR-455. (b) Graphic representation of the conserved miR-455 binding motif in the Cpeb1 3’UTR of three mammalian species. Binding of the wild-type (top) and mutated (bottom) murine Cpeb1-3’UTR to the miR-455 seed sequence was assessed in reporter gene experiments. (c) Relative luciferase activity of MIN6 cells cotransfected with miR-455 mimic and a luciferase reporter containing either Cpeb1-WT or Cpeb1-MUT. The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. (d) Anti-Ago2 RIP was performed in MIN6 cells transiently overexpressing miR-455, followed by qRT-PCR to detect Cpeb1 associated with Ago2 (nonspecific IgG served as a negative control). (e-g) The CPEB1 protein level in islets of len-miR-455 mice (e, n = 5 mice per group), len-anti-miR-455 mice (f, n = 5 mice per group), and len-anti-miR-455/ob mice (g, n = 5 mice per group). The CPEB1 mRNA and protein levels in islets of ob/ob (h, n = 5 mice per group) and HFD (i, n = 5 mice per group) mice. MIN6 cells were incubated with 2.5 mM or 25 mM glucose (j) or with 0.5 mM palmitate (k) for 48 h. Then, the CPEB1 mRNA and protein levels were measured via qRT-PCR and western blotting. (l) The number of INSULIN+/KI-67+ cells were determined by immunofluorescence. Magnification: 20 x; scale bar, 20 μm. (m) KI-67 positive β-cells were analyzed by immunofluorescence (n = 3 mice per group). Magnification: 20 x; scale bar, 20 μm. The data are presented as the mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure 6 CPEB1 regulates Cdkn1b expression by promoting its translation efficiency

(a) Primary islets and MIN6 cell lysates were subjected to anti-CPEB1 RNA immunoprecipitation (RIP), and Cdkn1b levels were examined via qRT-PCR. Gapdh served as a control to validate the CPEB1–Cdkn1b interaction (nonspecific IgG served as a negative control). (b) The wild-type and mutated murine Cdkn1b-3’UTR were inserted into a pGL3-control vector, and binding to Cpeb1
was assessed. Relative luciferase activity of MIN6 cells cotransfected with oe-Cpeb1 or si-Cpeb1 and a luciferase reporter containing either Cdkn1b-WT or Cdkn1b-MUT. The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. (c) MIN6 cells were transfected with oe-Cpeb1 or si-Cpeb1 for 48 h, and total RNA was isolated and subjected to PAT assays. Western blotting (d) and qRT-PCR (e) were used to assess the CDKN1B expression level in MIN6 cells transfected with oe-Cpeb1 or si-Cpeb1. The protein (f) or mRNA (g) level of CDKN1B in islets of len-shCpeb1 mice (n = 5 mice per group). The data are presented the mean ± SD. ***p < 0.001.

**Figure 7** The miR-455/CPEB1/CDKN1B axis blocks β-cell proliferation during insulin resistance

(a) MIN6 cells were transfected with miR-455 and anti-miR-455 for 48 h. Then, MIN6 cell lysates were subjected to anti-CPEB1 RNA immunoprecipitation (RIP), and Cdkn1b levels were examined via qRT-PCR. Gapdh served as a control to validate the CPEB1–Cdkn1b interaction (nonspecific IgG served as a negative control). (b) The CDKN1B protein level in MIN6 cells transfected with si-Cpeb1 or si-Cpeb1 and anti-miR-455. (c) The CDKN1B protein expression level in islets of len-shCpeb1 mice or len-shCpeb1 and len-anti-miR-455 mice. INSULIN+/KI-67+ cells were counted via flow cytometry (d) and immunofluorescence (e). Magnification: 20 x; scale bar, 20 μm. (f) KI-67-positive β-cells were analyzed via immunofluorescence (n = 3 mice per group). Magnification: 20 x; scale bar, 20 μm. (g) Representative HE-stained pancreas (scale bar, 200 μm, n = 3 mice per group). The data are presented as the mean ± SD. ***p < 0.001.

**Figure 8** Schematic illustration of the mechanism by which the obesity-induced increase in miR-455 improves β-cell proliferation. During obesity, miR-455 is upregulated by Egr2, and miR-455
can directly target *Cpeb1*, suppressing CPEB1 binding to the *Cdkn1b* 3'-UTR, inhibiting CDKN1B expression, and finally facilitating β-cell proliferation.
Figure 1 miR-455 is elevated in the islets of obese mouse models

190x275mm (300 x 300 DPI)
Figure 2 Silencing of Egr2 in pancreatic β-cells represses miR-455 expression

190x275mm (300 x 300 DPI)
Figure 3 miR-455 regulates β-cell proliferation

190x275mm (300 x 300 DPI)
Figure 4 Loss of miR-455 expression in obese mice inhibits β-cell proliferation

190x275mm (300 x 300 DPI)
Figure 5 miR-455 affects β-cell proliferation by targeting Cpeb1

190x275mm (300 x 300 DPI)
Figure 6 CPEB1 regulates Cdkn1b expression by promoting its translation efficiency

190x275mm (300 x 300 DPI)
Figure 7 The miR-455/CPEB1/CDKN1B axis blocks β-cell proliferation during insulin resistance
Figure 8 Schematic illustration of the mechanism by which the obesity-induced increase in miR-455 improves β-cell proliferation. During obesity, miR-455 is upregulated by Egr2, and miR-455 can directly target Cpeb1, suppressing CPEB1 binding to the Cdkn1b 3'-UTR, inhibiting CDKN1B expression, and finally facilitating β-cell proliferation.
Supplementary Files

Obesity-induced miR-455 upregulation promotes adaptive pancreatic β-cell proliferation through the CPEB1/CDKN1B pathway

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Supplementary Figure 1 (a) Blood glucose measurements during a glucose tolerance test in 8-week ob/ob mice and control mice (n = 6 mice per group). (b) The weight of 8-week-old ob/ob mice (n = 6 mice per group). (c) The expression levels of miR-455 and miR-6932 in human islets, mouse islets and MIN6 cells. The data are presented as the mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001.
Supplementary Figure 2 MIN6 cells (a), mouse primary islets (b, n = 3), and human islets (c) were incubated with a combination of interleukin-1β (IL-1β, 5 ng/ml) and tumor necrosis factor-α (TNF-α, 30 ng/ml) for 48 h, and qRT-PCR was performed to examine the miR-455 expression level. (d-e) The expression level of miR-455 in MIN6 cells (d) and in human islets (e) transfected
with miR-455 sgRNA. (f) The binding sites of Egr2 in the mus-miR-455 and human-miR-455 promoters predicted by JASPAR. (g) MIN6 cells were incubated with 0.5 mM palmitate for 48 h, and qRT-PCR was performed to examine the expression levels of transcription factors predicted by JASPAR. MIN6 cells and human islets were incubated with 0.5 mM palmitate or 25 mM glucose for 48 h, and the protein and mRNA levels of EGR2 were determined by western blotting and qRT-PCR (h-k), respectively. (l) Relative luciferase activity in MIN6 cells cotransfected with Egr2 overexpression plasmid and a luciferase reporter containing either miR-455 promoter or miR-455-MUT (Egr2-binding sequence mutated). The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. ChIP experiments were conducted to verify that Egr2 binds to the promoter of miR-455 in islets from obese mice (m). ChIP experiments were conducted to verify that Egr2 binds to the promoter of miR-455 in MIN6 cells transfected with oe-Egr2 or si-Egr2, followed by RT-PCR (n) and qRT-PCR (o) assays. (p) Based on EMSA results, Egr2 can directly bind to the miR-455 promoter in MIN6 cells. C1 and C2 represent nuclear protein-miR-455 probe complexes and nuclear protein-miR-455 probe-anti-EGR2 complexes, respectively. The data are presented as the mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001.
Supplementary Figure 3 MIN6 cells were transfected with miR-455 or anti-miR-455 for 48 h, and qRT-PCR was performed to detect the miR-455 expression level (a). (b) CCK-8 assays were carried out at 24, 48, and 72 h after transfection. (c) INSULIN+/KI-67+ cells were counted via immunofluorescence. Magnification: 20 x; scale bar, 20 μm. (d) The mRNA expression levels of
Cdkn1 and Cdkn2 family genes. The Ins1 and Ins2 expression levels (e), insulin content (f) and insulin secretion (g) in MIN6 cells. INSULIN levels (h), insulin content (i) and insulin secretion (j) in human islets. (k) The miR-455 expression levels in different tissues from len-miR-455 or len-anti-miR-455 mice (n = 3). (l) The glucagon level in serum from len-miR-455 or len-anti-miR-455 mice (n = 9 mice per group). (m) The β cell mass in len-miR-455 or len-anti-miR-455 mice (n = 3). (n) The mRNA levels of Cdkn1b in the islets of len-miR-455 or len-anti-miR-455 mice (n = 3). (o) INSULIN+/TUNEL+ cells were counted via immunofluorescence. Magnification: 20 x; scale bar, 20 μm. The data are presented as the mean ± SD. ** p < 0.01, *** p < 0.001.

Supplementary Figure 4

Supplementary Figure 4 Eight-week-old male len-anti-miR-455 mice and control mice (lentivirus-MIP (mouse insulin2 promoter)-LV3/H1 vector) were exposed to an HFD for 20 weeks.
Then, cumulative energy intake (a, \( n = 9 \) mice per group), changes in body weight (b, \( n = 9 \) mice per group), the white adipose tissue weight per body weight ratio (c, \( n = 9 \) mice per group) and fasting blood glucose level (FBG) (d, \( n = 9 \) mice per group) were measured. (e) The \( \beta \) cell mass of len-anti-miR-455 mice was measured (\( n = 3 \)). (f) miR-455 expression levels in the islets of len-anti-miR-455/ob mice (\( n = 3 \)). (g) The \( \beta \) cell mass in len-anti-miR-455/ob mice was measured (\( n = 3 \)). The data are presented as the mean ± SD. *** \( p < 0.001 \).

**Supplementary Figure 5**

(a) Relative luciferase activity of MIN6 cells cotransfected with miR-455 mimic and a luciferase reporter containing either Cdkn1b-WT or Cdkn1b-MUT. The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. (b)
MIN6 cells were transfected with Cpeb1 overexpression plasmid for 48 h. Then, MIN6 cell lysate was subjected to anti-Ago2 RNA immunoprecipitation (RIP), and the miR-455 and Cpeb1 expression levels were examined via qRT-PCR. Gapdh served as a control (nonspecific IgG served as a negative control). (c) CPEB1 mRNA and protein levels in MIN6 cells transfected with miR-455 or anti-miR-455. (d-e) The Cpeb1 mRNA level in islets from len-miR-455 mice and len-anti-miR-455/ob mice (n = 3). (f) Relative luciferase activity of MIN6 cells cotransfected with miR-455 mimic and a luciferase reporter containing either Usp9x-WT or Usp9x-MUT. The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. (g) CCK-8 assays were carried out at 24, 48, and 72 h after transfection with si-Cpeb1 or si-Cpeb1 and anti-miR-455. (h) The INSULIN+/KI-67+ cells were counted via flow cytometry. (i) The β cell mass in len-shCpeb1 mice was measured (n = 3). The data are presented as the mean ± SD. ** p < 0.01, *** p < 0.001.
Supplementary Figure 6 (a) ctaRAPID and RBPmap were used to predict mRNAs that can bind to CPEB1. (b) MIN6 cells were transfected with oe-Cpeb1 and si-Cpeb1 for 48 h. Then, MIN6 cell lysate was subjected to anti-CPEB1 RNA immunoprecipitation (RIP), and the precipitated RNAs were examined via qRT-PCR. Gapdh served as a control (nonspecific IgG served as a negative control). (c) MIN6 cells transfected with oe-Cpeb1 or si-Cpeb1 were treated with actinomycin D (10 μg/ml). RNA was isolated at various time points after the treatment and analyzed via RT-qPCR. The data are presented as the mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001.
Supplementary Figure 7 (a) RT-PCR-RIP was performed to verify the CPEB1–Cdkn1b interaction. (b) CCk-8 assays were carried out at 24, 48, and 72 h after transfection with si-Cdkn1b or si-Cdkn1b and oe-Cpeb1. (c) The β cell mass in len-shCpeb1 mice was measured (n = 3). The data are presented as the mean ± SD. **p < 0.01.
Supplementary Spreadsheets:

Table S1 RNA isolated from islets of control mice and ob/ob mice, this table shows significantly changed miRNA (Log2 fold change ≥ 2). The mean expression of each group was presented in a log2 scale.

| miR_name          | control-read | ob/ob-read | log2(fold change) | p-value     | FDR          |
|-------------------|--------------|------------|-------------------|-------------|--------------|
| mmu-miR-6932-3p   | 0.00         | 5.20       | 12.34             | 0.000899    | 0.018575     |
| mmu-miR-455-5p    | 1075.12      | 15104.93   | 3.84              | 3.60E-08    | 2.13E-06     |
| mmu-miR-677-3p    | 1.25         | 13.57      | 3.44              | 6.30E-05    | 0.002277     |
| mmu-miR-6546-5p   | 1.29         | 12.19      | 3.25              | 0.000176    | 0.005328     |
| mmu-miR-212-3p    | 815.84       | 7001.88    | 3.10              | 9.37E-20    | 2.44E-17     |
| mmu-miR-132-3p    | 5648.74      | 41049.84   | 3.84              | 3.60E-08    | 2.13E-06     |
| mmu-miR-132-5p    | 1.25         | 13.57      | 3.44              | 6.30E-05    | 0.002277     |
| mmu-miR-501-5p    | 91.11        | 636.67     | 3.28              | 3.60E-08    | 2.13E-06     |
| mmu-miR-212-5p    | 516.13       | 3417.69    | 3.10              | 9.37E-20    | 2.44E-17     |
| mmu-miR-1945      | 2.42         | 14.32      | 3.60              | 0.00368     | 0.007813     |
| mmu-miR-6546-3p   | 2.31         | 11.77      | 3.28              | 0.002614    | 0.038215     |
| mmu-miR-1983      | 38.48        | 185.64     | 2.73              | 4.47E-15    | 5.81E-13     |
| mmu-miR-665-5p    | 5.71         | 25.86      | 2.73              | 4.47E-15    | 5.81E-13     |
| mmu-miR-7093-3p   | 8.78         | 36.52      | 2.06              | 7.71E-05    | 0.002638     |
| mmu-miR-690       | 62.28        | 256.98     | 2.04              | 1.63E-07    | 9.23E-06     |
| mmu-miR-712-5p    | 3.44         | 14.07      | 2.03              | 0.002991    | 0.042759     |
| mmu-miR-150-5p    | 3591.51      | 867.79     | -2.05             | 1.25E-09    | 9.00E-08     |
| mmu-miR-142a-3p   | 4707.08      | 1492.88    | -2.17             | 4.24E-11    | 3.67E-09     |
| mmu-miR-669a-5p   | 18.09        | 3.74       | -2.28             | 0.000317    | 0.00792      |
| mmu-miR-592-5p    | 101.53       | 18.73      | -2.28             | 3.20E-08    | 1.98E-06     |
| mmu-miR-216b-5p   | 5763.70      | 974.22     | -2.56             | 2.46E-14    | 2.91E-12     |
| mmu-miR-216a-5p   | 13489.70     | 2161.92    | -2.64             | 2.81E-16    | 4.58E-14     |
| mmu-miR-217-5p    | 6074.72      | 865.87     | -2.81             | 1.54E-16    | 2.86E-14     |
| mmu-miR-216b-3p   | 741.02       | 102.69     | -2.85             | 5.42E-14    | 5.88E-12     |
| mmu-miR-216a-3p   | 99.84        | 13.45      | -2.89             | 4.48E-10    | 3.65E-08     |
| mmu-miR-6969-5p   | 30.21        | 4.05       | -2.90             | 1.22E-06    | 6.10E-05     |
| mmu-miR-142a-5p   | 12379.31     | 1612.28    | -2.94             | 3.99E-19    | 8.66E-17     |
| mmu-miR-217-3p    | 297.30       | 38.14      | -2.96             | 3.63E-13    | 3.37E-11     |
| mmu-miR-150-3p    | 10.44        | 1.27       | -3.03             | 0.000203    | 0.005812     |
| mmu-miR-6969-3p   | 69.65        | 8.49       | -3.04             | 2.76E-09    | 1.89E-07     |
| mmu-miR-196b-5p   | 13.90        | 1.26       | -3.46             | 1.28E-05    | 0.000522     |
| mmu-miR-184-3p    | 4929.72      | 257.98     | -4.26             | 1.16E-30    | 7.54E-28     |
| mmu-miR-122-3p    | 191.54       | 3.09       | -5.95             | 8.33E-28    | 3.61E-25     |
| mmu-miR-122-5p    | 5805.91      | 84.06      | -6.11             | 4.47E-52    | 5.81E-49     |
| Gene name    | Forward Primer                              | Reverse Primer                              |
|-------------|---------------------------------------------|---------------------------------------------|
| oe-Cpeb1    | ctataggagacaccaactgtATGGCTTTCTCT CTGGAAGAAGC | getgtacagggttttaacTCAGTTCTTTCTG GTTCTCATTAGG |
| oe-Egr2     | ctataggagacaccaactgtATGATGACCCG AAGCC      | getgtacagggttttaacTCACGCTTGTCCT GGGTCCG     |
| Has-EGR2    | ctataggagacaccaactgtATGATGACCGG CAAGGGCC   | getgtacagggttttaacTCACGCTTGTCCT GGGTCCG     |
| oe-Cdkn1b   | ctataggagacaccaactgtATGTCAAACGTG AGAGTGTCTAACG | getgtacagggttttaacTTACGTCTGGCG TCGAAGGC     |
| promoter-miR-455 | gagetttacggtgtcgtgATTTGGCAGAGGA CAAGAACAGG | cagtaccggaatgccaagtcGGCAGGCCCTT ACTTCATG |
| Has-miR-455 | acaacactttagatgcagatGATGACATAGGC CTTTGAGGCA | acaacactttagatgcagatGATGACATAGGC CTTTGAGGCA |
| MUS-EMSA-W  | CGGGAGTGTGTGTGCGGGAGGAGTG               | CACACTCCCCCACACACACTCCCG                 |
| MUS-EMSA-M  | CGGGATGTGTGTGTTATCCTTCTGGT               | CACACTCCCCCACACACACTCCCG                 |
| HAS-EMSA-W  | CTGTCCAGTGTGTGGTGACATTACATC               | CACACTCCCCCACACACACTCCCG                 |
| HAS-EMSA-M  | CTGTCCACTGTGTGGTGACATTACATC               | CACACTCCCCCACACACACTCCCG                 |
| Cpeb1-WT    | TCGAGttgtctctactgttctctTG                | CTAGAcagacaggatccaaatgtggtgggctttacttacttttaacatctcgagaatcttcgccag aacaaaatgggagacactgtcAG |
| Cpeb1-MUT   | TCGAGttgtctctactgttctctTG                | CTAGAcagacaggatccaaatgtggtgggctttacttacttttaacatctcgagaatcttcgccag aacaaaatgggagacactgtcAG |
| Cdkn1b-WT   | TCGAGttgtctctactgttctctTG                | CTAGAcagacaggatccaaatgtggtgggctttacttacttttaacatctcgagaatcttcgccag aacaaaatgggagacactgtcAG |
| Cdkn1b-MUT  | TCGAGttgtctctactgttctctTG                | CTAGAcagacaggatccaaatgtggtgggctttacttacttttaacatctcgagaatcttcgccag aacaaaatgggagacactgtcAG |
| SiRNA/sgRNA name | Forward promer | 
|-----------------|---------------| 
| si-Cpeb1        | GCAGCACACAGUCAGUAUUTT AUAACUGACUGUGUGUCUGCTT | 
| si-Cdkn1b       | CCCGGUCAAUCAUGAGAATT UUCUUAUGAUGAGCAAGTTT | 
| si-Egr2         | AAGGCTATCGCAATATTTGAGT ACTCAACTATTGATGATAACCTT | 
| miR-455-sgRNA-1 | CACCGGGGCTGGCATCCCTCAAGC AG AAACCTGCTTGGGAAGTGAGCC ACC | 
| miR-455-sgRNA-2 | CACCGGGTGGCTACCTCCCAAAGC CCA AACTGGGCTTGCGCGGCGGA CCC | 
| miR-455-sgRNA-3 | CACCGGCTGCATCCCTTGCTGCA TC AAACGACGGCAAGGATGCA GCC | 
| Has-si-EGR2     | AGCUGUCUGACAAACAUUCACC UAGAUGUUUGACAGACUGGG | 
| Has-miR-455 sgRNA-1 | CACCGACTAACAGCGGCTGGA ACC AAACGTCCTCCGCTGTTAG TC | 
| Has-miR-455 sgRNA-1 | CACCGAAGGGCTAACCCTCAGC CCG AAACGGGCGAGGTTACTGCCCT C | 
| Has-miR-455 sgRNA-1 | CACCGGGACAGAAAGGCTCAGC GCT AAACAGCGGCCAGTCCTTCTG CC |
| Gene          | Forward Primer       | Reverse Primer       |
|---------------|----------------------|----------------------|
| Mus-miR-455   | TAATCTGACTATGTGCCCTTTGGACT | TATGTTTTTGACGACTGGTGTTGAT |
| Has-miR-455   | TAATCTGACTATGTGCCCTTTGGACT | TATGTTTTTGACGACTGGTGTTGAT |
| Cpeb1         | AAGGATTGCGCTGGGACAAACCAGA | GGCACCAGGGGAGGATTCTCTTG |
| Gapdh         | AGTGCGGCTGAGTGGGATAGTGGTG | TGTAGACCATGATGTTGAGGTCA |
| Cdkn1b        | TCAACCTGAGAAGCTGTCAGTC | CCGGCGGACGAGATGTTTCTC |
| ChIP-R1       | AGGAGCTGCTGAGGCTGTCAGTC | AACTCAAGGAGGAGGCAGGG |
| ChIP-R2       | GGTCCGAGGCTGAGGCTGTCAGTC | TGGGTCCCCAAGATGTTGACGCT |
| HAS-ChIP      | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Ins1          | CACTTCCCTACCTCTCTCTCTTG | ACCAAAGTGGTGGTGGTCAGCA |
| Ins2          | GCTTCTCTACTACCACTCCCTATG | AGCAGTTGATCTACATGGGG |
| Zfp281        | CTTTTGGGCTGCGCTGTCAGTC | CCCCTTGGGCTGCGCTGTCAGTC |
| Fox1l         | GAGAGCTGCTGAGGCTGTCAGTC | CTTCTGAGGAGGAGGAGGG |
| Tcf3          | CACTTCCCTACCTCTCTCTCTTG | ACCAAAGTGGTGGTGGTCAGCA |
| Meis1         | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Spi1          | ATGGCTAAGGCGCTGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Rhox11        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Fox3          | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Gfi1b         | ATGGCTAAGGCGCTGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Wt1           | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Ecm10         | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Igfbp6        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Tnem160       | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Caz1          | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Gmnn          | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Vangl2        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Cux1          | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Dvl2          | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| GLUT8         | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Ccni          | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Rhb1cc1       | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Tnfrsf12a     | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Rhoc          | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Cdkn1a        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Cdkn1c        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Cdkn2a        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Cdkn2b        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Cdkn2c        | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |
| Has-CPEB1     | GGGGCTGGGTTATGGAGGACTGGTG | GGGGCTGACCCGAGGAGCCACATTT |

Table S4 The primers used in Real-time PCR (5'-3').
| Gene          | Forward Primer | Reverse Primer |
|--------------|----------------|----------------|
| Has-EGR2     | TCAACATTGACATGACTGGAG | AGTGAAGGTCTGGTTTCTAGGT |
| Has-Cdkn1b   | AACGTGCGAGTGTCTAACGG  | CCCTCTAGGGGTCTTGTGATTCT |
| β-Actin      | GGCTGTATTCCCTCCATCG | CCAGTTGGTAAACAATGCCATGT |
Table S5 The antibodies used in Western blot, IHC and IF.

| Protein          | Catalog                      | Dilution for WB | Dilution for IHC and IF | FACS |
|------------------|------------------------------|-----------------|-------------------------|------|
| INSULIN-647      | Cell Signaling (c27c9)       |                 |                         | 1:50 |
| KI-67 FITC       | Biolegend (652409)           |                 |                         | 1:50 |
| EGR2             | Abcam (ab108399)             | 1:1000          |                         |      |
| CPEB1            | Abcam (ab73287)              | 1:1000          |                         |      |
| CDKN1B           | Abcam (193379)               | 1:1000          |                         |      |
| INSULIN          | Servicebio (GB13121)         |                 | 1:500                   |      |
| GLUCAGON         | CST (2760)                   |                 | 1:500                   |      |
| Argonaute 2      | CST (2897s)                  | 1:1000          |                         |      |
| KI-67            | Abcam (ab1667)               |                 | 1:500                   |      |
| GAPDH            | Abcam (ab181602)             | 1:1000          |                         |      |
Checklist for Reporting Human Islet Preparations Used in Research

Adapted from Hart NJ, Powers AC (2018) Progress, challenges, and suggestions for using human islets to understand islet biology and human diabetes. Diabetologia https://doi.org/10.1007/s00125-018-4772-2.

| Manuscript DOI: |  |  |  |  |  |  |  |  |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Title: | Obesity-induced **miR-455** upregulation promotes adaptive pancreatic β-cell proliferation through the CPEB1/CDKN1B pathway |
| Author list: | Qianxing Hu‡, Jinming Mu‡, Yuhong Liu‡, Yue Yang, Yue Liu, Yi Pan, Yanfeng Zhang, Ling Li, Dechen Liu, Jianqiu Chen*, Fangfang Zhang*, Liang Jin* |
| Corresponding author: Liang Jin | Email address: ljstemcell@cpu.edu.cn |

| Islet preparation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8* |
|-------------------|---|---|---|---|---|---|---|----|

**MANDATORY INFORMATION**
| Unique identifier       | Ling wang | Xun Ma | Ling Yin | Chonglan Zhang | Hao Hu | Hua Xu |
|------------------------|-----------|--------|----------|----------------|--------|--------|
| Donor age (years)      | 42        | 38     | 46       | 39             | 32     | 49     |
| Donor sex (M/F)        | M         | M      | M        | F              | F      | F      |
| Donor BMI (kg/m²)      | 23.3      | 24.5   | 25.7     | 23.2           | 24.1   | 26.2   |
| Donor HbA₁c or other measure of blood glucose control | 9.36 | 5.64 | 4.72 | 8.93 | 5.63 | 5.32 |
| Origin/source of islets\textsuperscript{b} | Tianjin First Central Hospital | Tianjin First Central Hospital | Tianjin First Central Hospital | Tianjin First Central Hospital | Tianjin First Central Hospital | Tianjin First Central Hospital |
| Islet isolation centre | Organ Transplant Center, Tianjin First Central Hospital, Nankai University, China | Organ Transplant Center, Tianjin First Central Hospital, Nankai University, China | Organ Transplant Center, Tianjin First Central Hospital, Nankai University, China | Organ Transplant Center, Tianjin First Central Hospital, Nankai University, China | Organ Transplant Center, Tianjin First Central Hospital, Nankai University, China | Organ Transplant Center, Tianjin First Central Hospital, Nankai University, China |
| Donor history of diabetes? Yes/No | No | No | No | No | No | No | No |
|----------------------------------|----|----|----|----|----|----|----|
| If Yes, complete the next two lines if this information is available |
| Diabetes duration (years) |
| Glucose-lowering therapy at time of death |

**RECOMMENDED INFORMATION**

| Donor cause of death | cancer | hematencephalon | hematencephalon | cancer | hematencephalon | hematencephalon |
|----------------------|--------|-----------------|-----------------|--------|-----------------|-----------------|
| Warm ischaemia time (h) | 0      | 0               | 0               | 0      | 0               | 0               |
| Cold ischaemia time (h) | About 5.5 | About 5.5 | About 5.5 | About 5.5 | About 5.5 | About 5.5 |
| Estimated purity (%) | >80 %  | >80 %           | >80 %           | >80 %  | >80 %           | >80 %           |
| Estimated viability (%) | >80 %  | >80 %           | >80 %           | >80 %  | >80 %           | >80 %           |
| Total culture time (h)$c$ | About 6 h | About 6 h | About 6 h | About 6 h | About 6 h | About 6 h |
| Glucose-stimulated insulin secretion or other functional measurement\(\textsuperscript{a}\) | Islets were mixed for qRT-PCR assays, Glucose-stimulated insulin secretion assays; KI-67 staining; EMSA assays and Chip assays; | Islets were mixed for qRT-PCR assays, Glucose-stimulated insulin secretion assays; KI-67 staining; EMSA assays and Chip assays; | Islets were mixed for qRT-PCR assays, Glucose-stimulated insulin secretion assays; KI-67 staining; EMSA assays and Chip assays; | Islets were mixed for qRT-PCR assays, Glucose-stimulated insulin secretion assays; KI-67 staining; EMSA assays and Chip assays; | Islets were mixed for qRT-PCR assays, Glucose-stimulated insulin secretion assays; KI-67 staining; EMSA assays and Chip assays; |
|---|---|---|---|---|---|
| Handpicked to purity? Yes/No | Yes | Yes | Yes | Yes | Yes |
| Additional notes | \(\textsuperscript{a}\)If you have used more than eight islet preparations, please complete additional forms as necessary | \(\textsuperscript{b}\)For example, IIDP, ECIT, Alberta IsletCore | \(\textsuperscript{c}\)Please specify the therapy/therapies | \(\textsuperscript{d}\)Time of islet culture at the isolation centre, during shipment and at the receiving laboratory | \(\textsuperscript{e}\)Please specify the test and the results |