Full-length Article

Mir-132 controls beta cell proliferation and survival in mouse model through the PTEN/AKT/FOXO3 signaling

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Abbreviations

miRNA – microRNA; miR-132 – microRNA 132; PTEN – Phosphatase and Tensin homolog; Akt – proteine kinase B; FOXO3 – Forkhead box O3; RT-PCR – Reverse transcription polymerase chain reaction; T2D – type 2 diabetes; BrdU – Bromodeoxyuridine; LCM – laser capture microscopy; IPA – Ingenuity® Pathway Analysis; PI3K – Phosphatidylinositol-4,5-bisphosphate 3-kinase; Ras – Rat sarcoma; Raf – rapidly accelerated fibrosarcoma; MAPK – mitogen-activated protein kinase; SOS1 – Son of sevenless homolog 1; GNB1 – G Protein Subunit Beta 1; NRAS – Neuroblastoma RAS viral oncogene homolog; *PIK3R1* – Phosphoinositide-3- Kinase Regulatory Subunit 1; GNB – G Protein Subunit Beta; FGFR3 – *fibroblast growth factor receptor 3; CREB –* cAMP response element-binding protein

Abstract

Aim and hypothesis:

microRNAs (miRNAs) play an integral role in maintaining beta cell function and identity. However, their diversity as well as the complexity of identifying their endogenous targets and how they are regulated made deciphering their precise role a real challenge. This project was aimed toward the identification of miRNAs and their downstream targets specifically involved in islets beta cells regeneration in mouse model.

Methods:

Using a partially pancreatectomized mouse model to induce beta cell proliferation, we identified several miRNAs that were differentially expressed in relationship to beta cell replication. The further validated by RT-PCR. Transcriptomic analysis and bioinformatic data mining was then performed to identify the downstream target of miR-132 in insulinoma cell line MIN6 cells, which were then further validated at transcriptional and translational levels. Beta cell proliferation was further assessed in pancreatectomized control and miR-132$^{-/-}$ mice to validate the role of miR-132 in vivo.

Results:

Sequence specific inhibition of miR-132 correlated with reduced cell proliferation as well as increased apoptosis, whereas its overexpression resulted in reduced apoptosis. Increased levels of miR-132 correlated with repression of PTEN and thus increased Akt signaling and downstream activation of the transcription factor FOXO3a. Consistent with these findings we found that proliferation of beta cell in miR-132$^{-/-}$ pancreatectomized mice was significantly reduced compared to wild-type littermates.

Conclusions/Interpretations:

Here we have uncovered an exhaustive list of targets and the signaling pathways by which miR-132 controls beta cell proliferation and survival in pancreatic beta cells. The fact that miR-132 controls beta cell proliferation and survival by regulating PTEN activity, the node of Akt/FOXO3 signaling pathway may represent a suitable target to enhance beta cell mass.

Keywords

Micro-RNA132 (miR-132); beta cell function; Islet cell regeneration; apoptosis; PTEN/AKT/FOXO3.

Introduction

miRNAs belong to the class of short non-coding RNAs that regulate gene expression by annealing to 3' untranslated region sequences in the target mRNAs and inducing their post-transcriptional repression. The functional importance of miRNAs has been extensively investigated in recent years and their altered expression has been implicated in wide range of disease including diabetes (Guay and Regazzi for review, 2015), cardiovascular disease (Gupta et al., 2013; Huang et al., 2013), and cancer (Li et al., 2015). In pancreatic beta cells, altered expression of miRNA correlate with profound impairment of glucose metabolism (Dey et al., 2011) and processing of miRNAs appears to be altered by obesity, diabetes, and aging. A number of miRNAs such as miR-7, miR-21, miR-29, miR-34a, miR-212/miR-132, miR-184, miR-200 and miR-375 have been suggested to play an important role in beta cell function (Dalgaard and Eliasson, 2017 for review). RNA sequencing of primary human islets detected 346 miRNAs enriched in beta cells on a total of 366 miRNAs and among them 40 microRNAs were predominately expressed in beta cell in comparison to other tissues (Martijn van de Bunt, 2013). In human and mouse pancreatic islets, miR-375 has been shown to be the miRNA with the highest expression level. Inhibition of miR-375 by morpholino oligonucleotides inhibits pancreatic islet development in *Xenopus laevis* (Kloosterman et al., 2007). Similarly, its global inactivation in mice resulted in decreased beta cell mass and ultimately diabetes (Poy et al., 2009; Latreille et al., 2015). MiR-132 is another miRNA that plays a key role in beta cell function. Its expression is deregulated in different animal models of type 2 diabetes (T2D) (Tattikota, et al., 2014; Nesca et al., 2013; Esguerra et al., 2011; Jacovetti et al., 2012). Overexpression of miR-132 resulted in improved glucose-stimulated insulin release from dissociated rat islet cells (Nesca et al., 2013) as well as triggering beta cell proliferation and cell survival (Tattikota, et al., 2014; Nesca et al., 2013; Jacovetti et al., 2012). MiR-132 is also highly expressed in neural cells where it has been implicated in neural function and development (Remenyi et al., 2010 and Wayman et al., 2008). In primary PC12 cells, miR-132 controls cell survival by direct regulation of PTEN, FOXO3a and p300 signaling. However, in pancreatic islets, the functional relevance of miR-132 in vivo and its downstream targets remain unknown. To identify the major miRNAs as well as their

downstream targets involved in beta cell proliferation, we analyzed the profile of miRNAs differentially expressed after a partial pancreatectomy in comparison to sham-operated mice. We report here that the expression of 14 miRNAs were markedly increased and one miRNA was down-regulated. Moreover, we show in proliferating beta cells that down-regulation of miR-132 expression in insulinoma MIN6 cells correlated with decreased proliferation and increased apoptosis, whereas its up-regulation has a protective effect by reducing apoptosis, presumably by controlling the expression of PTEN and its downstream effectors Akt and FOXO3. We then extended these studies and demonstrated that miR-132 knock out mice have an impaired beta cell proliferation.

Results

miR-132 is upregulated in proliferating beta cells

To identify key miRNAs involved in beta cell proliferation, we used partially pancreatectomized mice (n=3) as a model to induce beta cell replication (Fig. 1A). The removal of 70-80% of the pancreas is a well-established procedure for inducing beta cell proliferation in the remaining pancreas (Bonner-Weir et al., 1993; Mziaut et al., 2008). Proliferating cells were stained with 25µg/hr BrdU continuously delivered by an osmotic mini-pump implanted in the abdomen at the time of pancreatectomy. This approach ensures the labeling of every dividing cell (Mziaut et al., 2008). As controls, 4 mice were similarly implanted with an osmotic mini-pump for BrdU delivery, but only underwent total splenectomy. Seven days post-surgery, all mice were sacrificed and their pancreas excised for serial sectioning (40 sections/mouse). Islets were identified upon cresyl-violoet staining and $BrdU^+$ cells in the islet cores, which in rodents consists mainly of beta cells (Bonner-Weir; Caceido) were counted (Fig. 1B and C) prior to the excision of the islet cores by laser capture microdissection (LCM) (Fig. 1 A and B). As expected, in pancreatectomized mice the fraction of islet core $BrdU^+$ cells/total islet core cells, as determined by nuclear counting, was significantly higher than in sham-operated mice (Fig. 1C). RNA extracted from LCM islet cores was then profiled using Agilent microarrays. Fourteen miRNAs were found to be differently expressed (cut-off values: $p= 0.05$; $FC\geq1.5$) in islet cores of pancreatectomized mice compared to sham operated mice (Table 1). All

these miRNAs were up-regulated, except miR-760, which was reduced by 2.28 fold. Expression levels of all 14 differentially expressed miRNa was further verified by quantitative real time PCR (qRT-PCR). With this analysis only the significant changed expression of miR-132 and miR-141 could be validated (ESM Table 1). Given the postulated role on miR-132 on cell replication of various cell lines (Li et al., 2015; Chen et al., 2016), we focus our attention on the potential involvement of this miR in the regulation of beta cell proliferation.

miR-132 affects proliferation and survival in insulinoma cells

At first, we down-regulated miR-132 expression in mouse insulinoma MIN6 cells using an antagomir approach (Krützfeldt et al., 2005). Reduced expression of miR-132 by 90% (Fig. 2A) correlated with reduced percentage of BrdU+ MIN6 cells in comparison to control cells (27.35 ± 2.23 % vs. 24.48 ± 1.78 % out of 103 fields and a total number of 27, 413 and 25, 246 cells for control and antagomirs treated cells respectively) (Fig. 2B and C). Reduction of miR-132 levels correlated also with increased detection of cleaved Caspase-9 (Fig. 2D and E), while the levels of cleaved Caspase-3 were not significantly changed. Conversely, overexpression of miR-132 with a bicistronic adenovirus vector also encoding for eGFP (Fig. 2F) did not increase further the MIN6 cell proliferation, presumably due to their neoplastic state. (Fig. 2G and H). However, overexpression of miR-132 correlated with reduced levels of pro and cleaved Caspase-9 (Fig. 2I and J), consistent with miR-132 being anti-apoptotic.

miR-132 regulates the expression of *Pten* and *Mapk1.*

Next, we aimed to uncover down-stream targets of miR-132 in MIN6 cells. Microarray gene expression analysis of miR-132 overexpressing MIN6 cells identified 345 unique differentially expressed genes (cut-off values: $p<0.05$, FC ≥ 1.5), with 194 (56.2%) being down regulated and 151 (43.8%) up-regulated (Fig. 3A and ESM Table 2). Querying the TargetScan Mouse 7.1 database 35 of the down- and 2 of the up-regulated genes were predicted to contain highly conserved binding sites for miR-132 (Fig. 3A and ESM Table 3). Further analysis of regulated genes with Ingenuity® Pathway Analysis revealed 8 regulated pathways (Fig. 3B and ESM Table 4) including 26 of the 345 differentially

expressed genes (Fig. 3C). Among the 26 differentially expressed genes, the top 10 most represented genes in the 8 signaling pathways were selected for further validation of their mRNA levels by qRT-PCR (ESM Table 5). As control we also assessed the mRNA expression levels of *RASA1*, an established target of miR-132 (Anand et al., 2010). As shown in Fig. 3D, 6 out of 10 of the selected genes, including *Mapk1/Erk2*, *Pten*, *Nras*, *Pik3r1*, *Gnb1*, *Gnb5*, were confirmed to be down-regulated upon miR-132 overexpression. Three of them, namely *Mapk1, Pten* and *Gnb1,* were also among the 37 predicted targets for miR-132 binding. Notably, *Mapk1,* also known as *Erk2,* is a serine-threonine kinase located downstream of the tumor-suppressor phosphatase *Pten* and both genes play a critical role in control of cell proliferation and survival (Deb et al., 2014; Ya-Chun et al., 2015).

miR-132 regulates Pten signaling in MIN6 cells

Next, we tested whether overexpression of miR-132 affected also the protein levels of the Pten and Mapk1/Erk2. Immunoblotting of MIN6 cells transduced with the miR-132/eGFP viral vector confirmed the down-regulation of Pten in parallel with up-regulation of its targets Akt and phospho-Akt (S473) (Fig. 4A and B), while mRNA levels of Akt were unchanged (ESM Fig. 1). Furthermore, levels of the Akt1 substrate CREB and phospho-CREB (S133) were unchanged, but the phospho-CREB/CREB ratio was also increased. Likewise, overexpression of miR-132 correlated with reduced expression of Mapk1/Erk2, phospho-Mapk1/Erk2 and Rasa1/RasGAP, but not of Erk1 and phospho-Erk1 (Fig. 4C and D).

As down-regulation of miR-132 correlated with elevated cleaved Caspase-9 levels, we further tested whether its overexpression affected the expression of *FOXO3a,* a key mediator of apoptosis. Immunoblotting for FOXO3a showed that its phosphorylation was increased (Fig. 4E-F), although the overall levels of its mRNA (ESM Fig. 1) and protein (Fig. 4E-F) were not changed.

Impaired beta cell proliferation in miR-132 knock out mice

Finally, to verify that miR-132 positively affects beta cell regeneration *in-vivo*, we investigated beta cell proliferation in partially pancreatectomized or sham operated miR-132$^{-/-}$ mice and control littermates (6 mice/group) as described previously. Intraperitoneal glucose tolerance test prior and 6 days after surgery showed no difference between control and miR-132$^{-/-}$ mice (Fig. 5A and B). Daily blood glucose measurements, in particular, showed a comparable slight decrease of glycaemia in partially pancreatectomized wild type and miR-132$^{-/-}$ mice relative to sham operated mice in the first day post-surgery, followed by a complete normalization of glycaemia by the end of the 1-week-long protocol (ESM Fig. 2). Seven days after surgery, the mice were sacrificed, the remnant pancreas excised, and BrdU+/insulin+ beta cells were counted (Fig. 5C-F and Table 2). As assessed by immunostaining for insulin, the average number of beta cells/islet in wt (31,9 beta cells/islet) and miR-132$^{-/-}$ (31,7 beta cells/islet) was increased in partially pancreatectomized mice relative to their sham-operated counterparts (wt: 23,8 beta cells/islet; miR-132$^{-/-}$: 26,9 beta cells/islet). Likewise, the number BrdU+ insulin+ beta cells was increased in both groups of partially pancreatectomized mice compared to sham operated mice (Fig. 5G, Table 2). However, in partially pancreatectomized miR-132$^{-/-}$ mice there were fewer BrdU+/insulin+ cells than in partially pancreatectomized wt mice (Fig. 5G, Table 2). These data provide conclusive evidence for miR-132 exerting a positive role for beta cell regeneration in-vivo.

Discussion

It is well known that miR-132 controls many cellular processes in various tissues including neuronal morphogenesis and regulation of circadian rhythmus. miR-132 altered expression correlated with several neurological disorders, such as Alzheimer's and Huntington's diseases (Wang et al., 2013; Lee et al., 2011). Thus, most of our acknowledge about miR-132 regulation and biological functions emerged from studies performed on neural cells and not much is know about the downstream target of mir-132 in pancreatic beta cells. miR-132 and miR-212 have identical seed sequences and potentially regulate targets in common. Here we have identified miR-132 as one of the mostly up-regulated miRNA with a 5 fold expression change in pancreatectomized mice, a condition

known to induce beta cell proliferation. This finding is in agreement with previous data showing induced miR-132 expression in different T2D models, including db/db mice, high-fat diet-fed mice (Nesca et al., 2013) and in ob/ob mice (Zhao et al., 2009; Poy et al., 2009). Among this list of miRNAs differentially expressed, miR-205 showed the greatest change (5 fold). Interestingly, miR-205 has also been described to be the miRNA with the highest expression change in hepatocytes of mice with obesity induced T2D (Zhao et al., 2009). However, our profiling analysis did not reveal a significant change in the expression of miR-375, a miRNA abundant in pancreatic islets known to regulate insulin secretion and beta cell proliferation (Poy et al., 2009).

Previous work has shown that miR-132 is highly expressed in neurons and may regulate neuronal differentiation (Vo et al. 2005; Wayman et al. 2008). More recent work on primary neurons and PC12 cells showed that miR-132 controls cell survival by direct regulation of PTEN, FOXO3a and P300, key proteins contributing to Alzheimer neuro-degeneration (Wong et al., 2013). To uncover the down stream target of miR-132 in beta cells, we first investigated whether miR-132 has a proliferative role in insulinoma MIN6 cells. We found that miR-132 down-regulation with antagomirs correlated with slight but significant inhibition of MIN6 cells proliferation. The inhibition of the expression of miR-132 showed also an increase in cleaved Caspase-9-mediated apoptosis. Conversely, its up-regulation has a protective effect by reducing Caspase-9 processing. However, the increase in the expression of miR-132 did not correlate with an enhancement in the proliferative rate of MIN6 cells, presumably due to their higher proliferative fate. To uncover the downstream targets of miR-132, we analyzed the expression pattern of differentially expressed genes in cells overexpressing miR-132. PTEN whose expression is known to inversely correlate with cell survival was significantly down-regulated in agreement with our data showing decreased apoptosis upon overexpression of miR-132. The change at transcriptional level of PTEN was further confirmed at the protein level. Importantly, reduced PTEN correlated with increased phosphorylation of Akt (P-Akt) and subsequently to FOXO3 phosphorylation. P-Akt is a major activator of FOXO proteins, transcription factors members of the Forkhead superfamily of winged helix transcription factors which regulates cellular processes including metabolism, stress response, DNA damage repair and cell death. P-FOXO3 is phosphorylated at 3 key residues: Thr32, Ser253 and Ser315, and

associates with 14-3-3 proteins to be exported from the nucleus, where it is transcriptionally inactive (Brunet et al., 1999).

MAPK1/ERK2 (Mitogen-Activated Protein Kinase 1) expression is reduced in cells overexpressing miR-132 and survey of ERK2 activation reveals reduced phosphorylation of ERK2 without change in its activation state (as shown by the ratio of p-ERK2/ERK2) suggesting that the Ras/Raf/ERK1/2 is not preponderant in this biological process.

A considerable number of miRNAs have been associated with pancreatic beta cell development by affecting proliferation or differentiation (e.g., miR-375 (Poy et al., 2009), miR-7 (Wang et al., 2013), miR-124a (Baroukh et al., 2007), miR-24 (Vijayaraghavan et al., 2014), let-7a (Gurung et al., 2014), miR-26a (7Fu et al., 2013), miR-184 (Tattikota et al., 2014), miR-195, miR-15, miR-16 (Joglekar et al., 2007) and miR-132 (Guay et al., 2015 and Zhao 2009). Among those miRNAs, miR-132 was consistently differentially expressed in various T2D models in which beta cells were challenged by increased metabolic demand, condition known to promote beta cell proliferation, including obesity induced diabetes (Regazzi, Zhao et al., 2009). In mouse model, constitutive deletion of miR-132 lead to mice with defect in endocrine development (Ucar et al., 2010). A specific deletion of miR-132/212 locus in adult hippocampus with a retrovirus expressing Cre recombinase caused a dramatic decrease in dendrite length, arborization and spine density, suggesting that miR-132/212 is required for normal dendritic maturation in adult hippocampal neurons (Magill et al., 2010). Here we demonstrate that regeneration of beta cells in pancreatectomized *miR-132* $^{-/-}$ mice is reduced, conceivably through its control of the PTEN/PI3K/AKT signaling.

In conclusion, we have discovered a miRNA which act as sensor of external changes and controls the major signaling pathway regulating cell proliferation and survival at their node. Efforts to develop targeted therapies have not been fully successful, mainly because of extensive networking between pathway suppressors. Thus, our findings suggest that miR-132 could be a suitable candidate for therapeutic intervention to prevent beta cell damages and death as well as in improving beta cell regeneration. Moreover, The identification of a number of targets allowed us to propose mechanisms by which miR-132 is involved in these processes and eventually, could set up the ground toward the identification of the

extracellular signal and the biological processes that favors a signaling pathway over another one in future studies.

Methods

Cloning of mir-132 in pacAd5 Shuttle Vectors

To produce the adenovirus overexpressing mir-132 we used the RAPAd® miRNA Adenoviral Expression System (Cellbiolabs, San Diego, CA, USA). Following the Kit's instructions, the mmu-mir-132 precursor sequence, obtained from www.miRBase.org (GGGCAAC CGTGGCTTTCGATTGTTACTGTGGGAACCGGAGGTAACAGTCTACAGCCATGGTCGC CC), was PCR-amplified from genomic mouse DNA including a ca. 100bp flanking region on each side (Forward: 5'-TCGAGGATCCTCCCTGTGGGTTGCGGTGGG-3'; Reverse: 5'-TCGAGCTAGCACATCG AATGTTGCGTCGCCGC-3') and cloned into the human β-globin Intron of the Kit's pacAd5-miR-GFP-Puro vector via BamHI/NheI (NEB, Ipswich, MA, USA) digestion. This human β-globin Intron, containing the mmu-mir-132 precursor, was then subcloned into the Kit's pacAd5-CMV-eGFP vector via PCR amplification (Forward: 5'-TGCAACCGGTGCCAG AACACAGGTACACATAT-3'; Reverse: 5'-TGCAACCGGTCGTGCTTTGCCAAAGTGATG-3') and AgeI (NEB, Ipswich, MA, USA) digestion to obtain a mir-132 overexpressing shuttle vector with a CMV promoter. The empty pacAd5-CMV-eGFP vector was used for the production of a control virus.

Cell Culture

Mouse MIN6 and rat INS-1 insulinoma cells were kind gifts from Dr. Jun-ichi Miyazaki (Osaka University, Japan), and C. Wollheim (University of Geneva, Switzerland), respectively, and were grown as previously described (Miyazaki et al., 1990; Asfari et al., 1992). MIN6 cells were cultured in 25 mmol/L glucose Dulbecco's modified Eagle's medium (DMEM, high glucose, GlutaMAX(TM), pyruvate) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 15% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin, 100 U/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and 70µM β-Mercaptoethanol (Sigma-Aldrich, St.

Louis, MO, USA), and were incubated at 37°C in a humified atmosphere containing 95% air and 5% CO_2.

Mouse studies

Male C57Bl/6N with an age of 13-19 weeks and a body weight of 28-34 g were used for the experiments. MiR-212/132 loss-of-function mutant mouse line was provided by the group of Chowdhury (Max Planck Institute of Biophysical Chemistry in Goettingen, Germany). This mouse line was backcrossed into C57Bl/6N background for at least seven generations as previously described (Ucar et al., 2010). All animal protocols were approved by the institutional animal care and use committee and all experiments were performed in accordance with relevant guidelines and regulations.

Partial pancreatectomy/sham operation

Mice underwent general anesthesia by mask inhalation of Isoflurane using a small rodents' anesthesia unit (Harvard Apparatus Ltd., Holliston, MA, USA) with an Isoflurane (Baxter Deutschland GmbH, Unterschleißheim) concentration of 4.5–5% for induction and 2–2.5% for maintenance of anesthesia with an airflow rate of 200 ml/min. Perioperative analgesia was accomplished using buprenorphin (0.05 mg/kg bodyweight) administered subcutaneously. The abdomen was opened through an upper midline incision. The spleen and the entire splenic portion of the pancreas were surgically removed while the mesenteric pancreas between the portal vein and the duodenum was left intact. The remnant was defined as the pancreatic tissue within 1–2 mm of the common bile duct that extends from the duct to the first portion of the duodenum. This remnant is the upper portion of the head of the pancreas. This procedure results in a 75% pancreatectomy, confirmed by weighing the removed and remnant portions as previously established (Mziaut et al., 2008). Sham operations were performed by removing the spleen while leaving the pancreas intact. At the end of surgery, Alzet 1007D miniosmotic pumps (Alzet®, Cupertino, CA, USA) were implanted i.p. to deliver 50 $\mu g \cdot \mu l^{-1}$ BrdU (Sigma, St. Louis, MO, USA) in 50% DMSO at a rate of 0.5 $\mu l \cdot h^{-1}$ for 7 days. Blood glucose levels were measured daily from the tail vein with a Glucotrend glucometer (Roche Diagnostics, Basel, Switzerland).

Intraperitoneal Glucose tolerance test (IpGTT)

IpGTT were performed two days before surgery and six days after surgery to assess differences between wildtype and k.o. mice and between pancreatectomized mice and sham-operated animals. After 10 hours overnight fast, mice were injected with 2.0 g/kg body weight of 20% glucose solution. Blood glucose levels were measured from the tail vein at 0, 15, 30, 60, 120 and 180 minutes after glucose injection.

Osmotic BrdU Pump and immunostaining

The pancreatic remnants and the sham-operated pancreata were harvested 7 days post-surgery. Mice were anesthetized with isoflurane as described above and the abdominal incision was re-opened. After fixation by intracardial perfusion with 4% paraformaldehyde, mouse pancreata were removed, further fixed overnight in 10% neutral formalin, and embedded in paraffin. Sections were cut at 5 µm and BrdU Staining in MIN6 Cells was performed as described in (Mziaut et al., 2008).

Quantitative Real time-PCR

cDNA samples were obtained by reverse transcription of 1ug total RNA using the M-MLV Reverse Transcriptase (Promega, USA, WI, Madison). Quantitative real time-PCR was then performed with the GoTaq® qPCR Master Mix (Promega, USA, WI, Madison) according to the manufacturers instruction using the oligonucletides listed in Supplementary table **2**.

Transcriptomic profiling of mouse islets

Total RNA was isolated from the islets of 16 – 19 -week-old wild-type and *mir-132*$^{-/-}$ mice (**6** mice/group) using RNeasy (Qiagen, Hilden, Germany). For microarray analysis, **100** ng of islet RNA was amplified with the Illumina® Total Prep RNA Amplification Kit (Ambion, Inc., Austin, TX, USA) and cRNA was labeled with biotin-UTP as previously described (Mziaut et al., 2016).

Microarray Analysis and data mining

MicroArray data processing and quality control was done with GeneSpring GX 13. After quantile normalization significantly differentially expressed genes were obtained by volcano plot filtering for FC≥|1.5| and $p<0.05$. Functional analysis was done using Ingenuity Pathway Analysis (IPA®, Qiagen, Hilden, Germany).

Statistical analysis

Statistical analyses were performed by using the unpaired Student's *t* test unless otherwise stated. Results are presented as mean SE unless otherwise stated. A value of $P < 0.05$ was considered significant. Error bars show standard deviations from at least three independent experiments unless otherwise stated. Histograms were prepared with Microsoft Excel (Microsoft, Redmont, WA, USA) or GraphPad Prism.

Data Availability:

The data are available on request.

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Contribution statement:

HM and SK conceived the study and the experimental design; GH with the help of KG, JM, and SW performed the gene profiling, validation of the target genes as well data mining; SH and JM under the supervision of SK performed the pancreatectomy, installation of the BrdU pump and immunostaining of pancreatic islets. HM and SK wrote the manuscript. MS contributed with ideas/suggestions as well as with help in editing the manuscript. HM and SK are responsible for the integrity of the study.

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Figure legends:

Figure 1. **miR-132 expression is induced in pancreatectomized mice**

Overview of the experimental design (A). Black6 mice were pancreatectomized and pump was inserted in the abdomen for continous BrdU administration over a week. Pancreatic sections were stained with cresyl-violoet dye to reveal the islet core and labeled with DAPI (pseudogreen) and with anti-BrdU antibody (pseudoblack) (B top panel). The islets core was excised by laser capture microdissection (LCM) and material proceeded for micro-RNAs profiling (B bottom panel). (C) Percentage of $BrdU^+$ in islet core. Experiment included a group of 7 mice in which 4 were shamp operated and 3 were pancreatectomized and 40 slices/group were counted for $BrdU^+$ cells. *, $P < 0.05$

Figure 2. miR-132 regulates proliferation and cell survival in MIN6 cells

Down regulation of mir-132 with antagomires (A). Analysis of the proliferation (B, C) and apoptosis (D, E) of mir-132 depleted cells respectively by quantification of BrdU positive cells and immunoreactivity against cleaved caspase 3 and 9. Adenovirus mediated expression of mir-132 in MIN6 cells (F) and its role on the proliferation (G, H) and apoptosis (I, J) of MIN6 cells. Experiments were performed 3 times for (A), 4 times for (F) and 6 times for (D-E and I-J). *, $P < 0.05$, **<0.01, **<0.001

Figure 3. Identification and validation of mir-132 target genes in MIN6 cells

Venn diagram showing the number of differentially expressed (DE) genes in MIN6 cells over-expressing mir132 (A). Ingenuity Pathway Analysis of significantly altered pathways in cells expressing miR132 (B). Top ranked DE genes with known role in cell proliferation and survival (C). Validation by Q-PCR of DE genes uncovered my microarray analysis (D).

Figure 4. Validation of gene profiling data in MIN6 Cells overexpressing miR132

Immunoblotting with the indicated antibodies on cell extracts from Min6 cells transduced with Adenovirus vector plasmid carrying a CMV promoter driven expression of control or mir-132 in tandem with the reporter protein GFP (A, C and E). (B, D and F) Densitometric

quantification of the indicated proteins as shown in A and C and E. Immunoblotting and quantification were performed from 6 experiments.

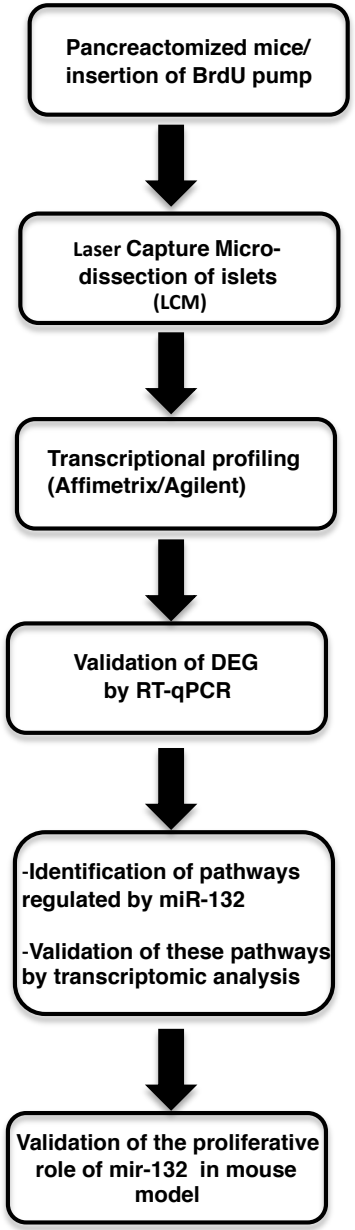
Figure 5. Impaired pancreatic beta cells regeneration in miR-132 knock out mice.

IPGTT in miR-132$^{-/-}$ mice and wild-type mice two days before (A) and six days after (B) surgery in wildtype and miR-132$^{-/-}$ mice, either sham-operated or pancreatectomized. Immunomicroscopy on paraffin sections of the remnant pancreas from sham-operated wild-type (C) or *mir-132* $^{-/-}$ (D) mice, and partially pancreatectomized wild-type (E) or *mir-132* $^{-/-}$ mice (F) after one week of continuous administration of BrdU. Sections were immunolabeled with anti-insulin (pseudored) and anti-BrdU (pseudogreen) antibodies. Nuclei were counterstained with DAPI (pseudoblue). Percentage of insulin^{+} and BrdU^{+} cells (G). Three independent series with two mice per condition were used and at least 50 islets per mouse for a total of 250-300 islets per group were counted. *, $P < 0.05$.

Supplementary Figure 1. Validation of by RT-PCR of DE genes identified by microarray analysis in MIN6 cells over-expressing miR-132

Figure 1. Partial Pancreatectomy as model for beta cell regeneration to uncover DFE micro-RNAs

A



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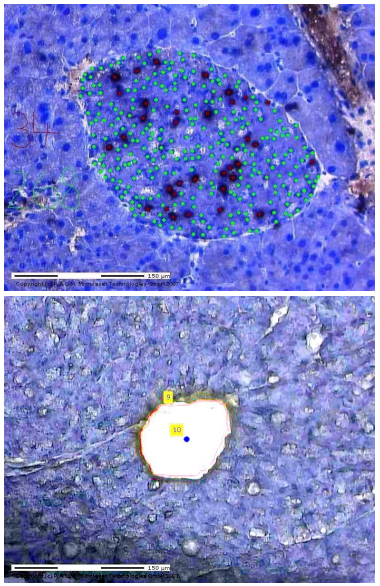

C

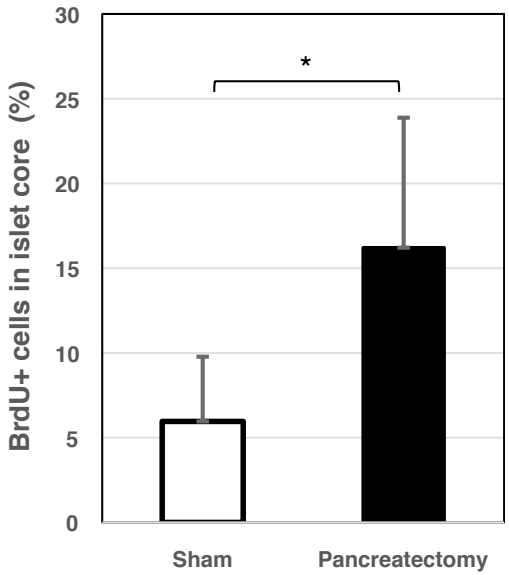

Figure 2. miR-132 regulates proliferation and cell survival in MIN6 cells

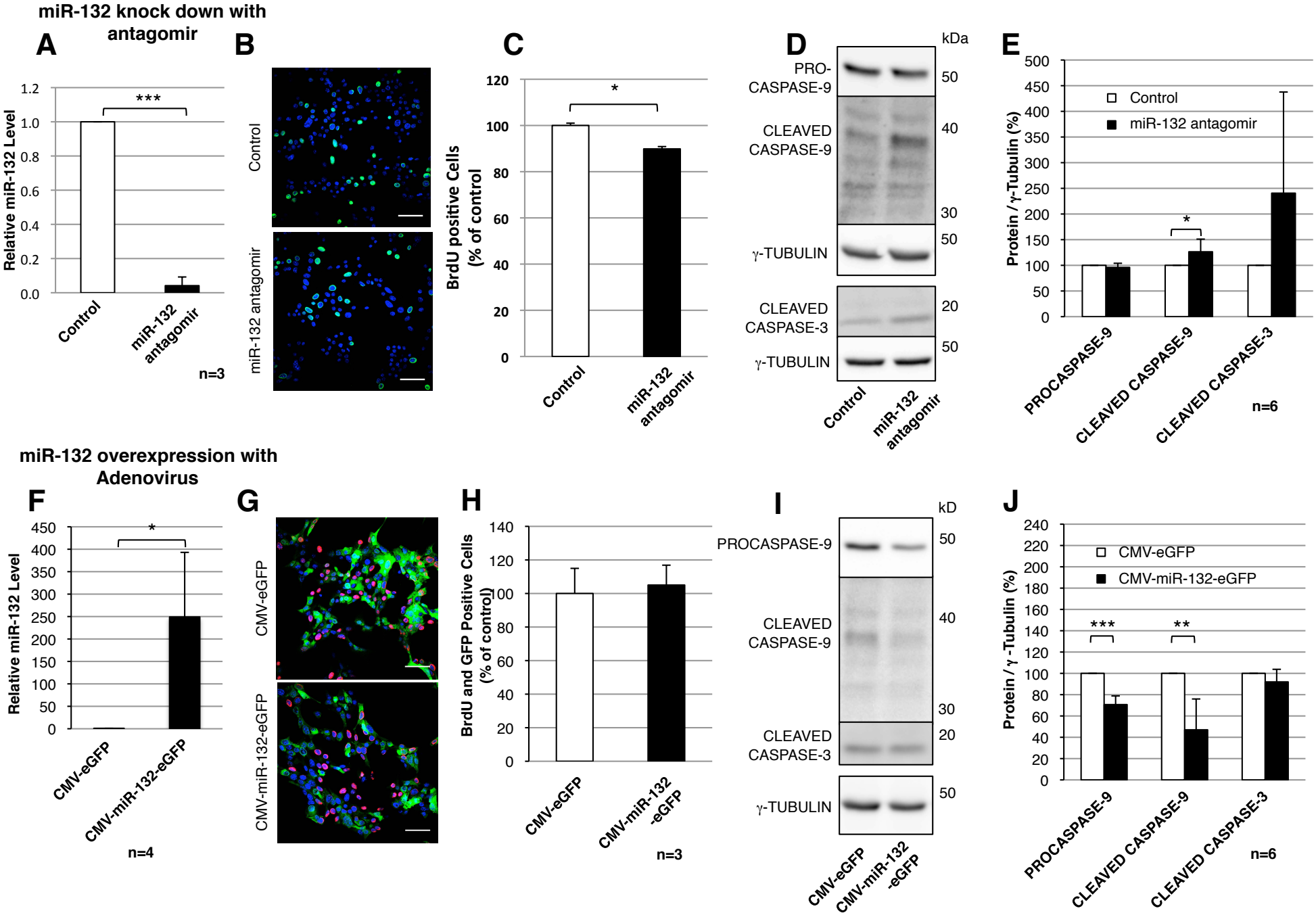

Figure 3. Identification and validation of miR-132 target genes in MIN6 cells

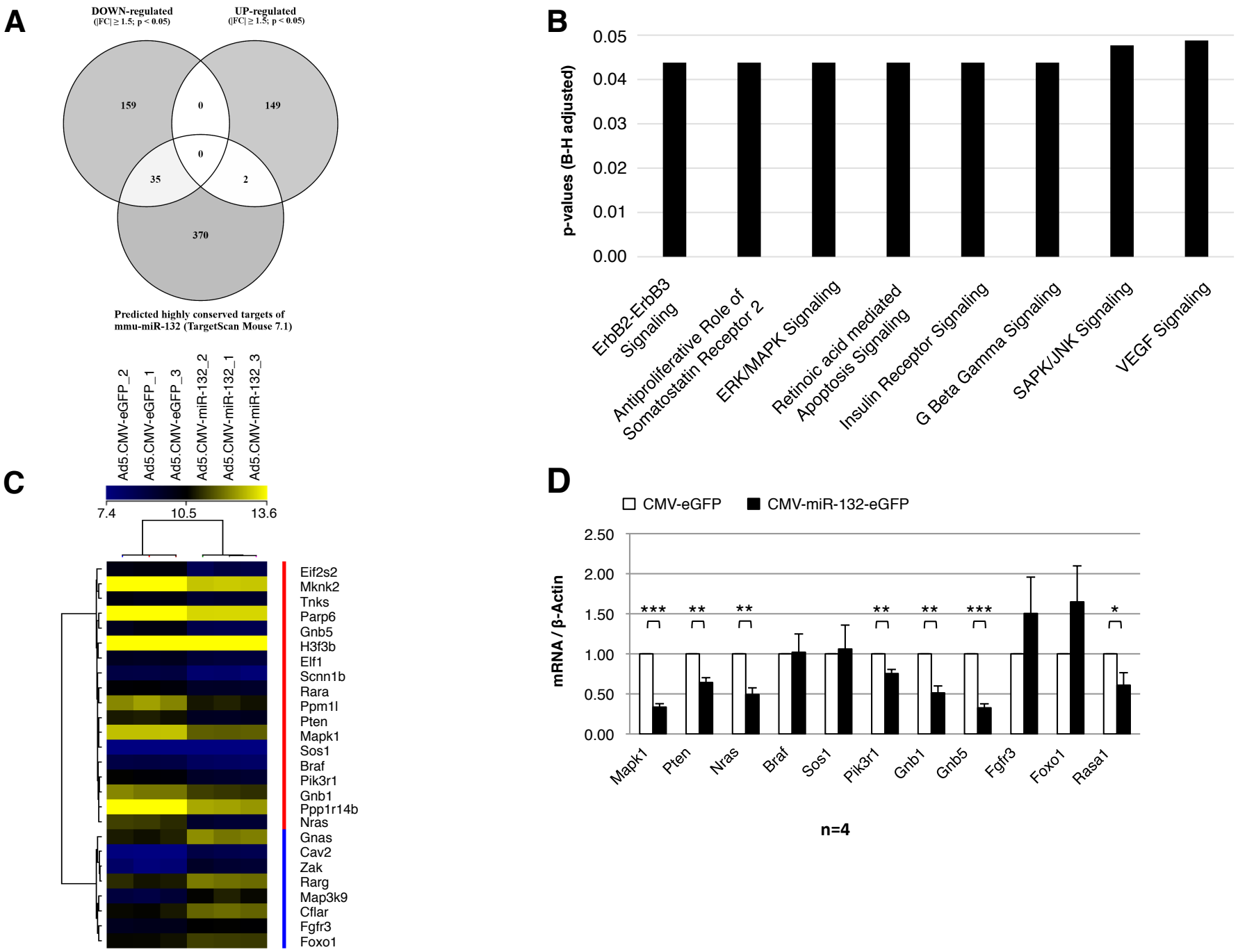

Figure 4. Validation of gene profiling data in MIN6 Cells overexpressing miR-132

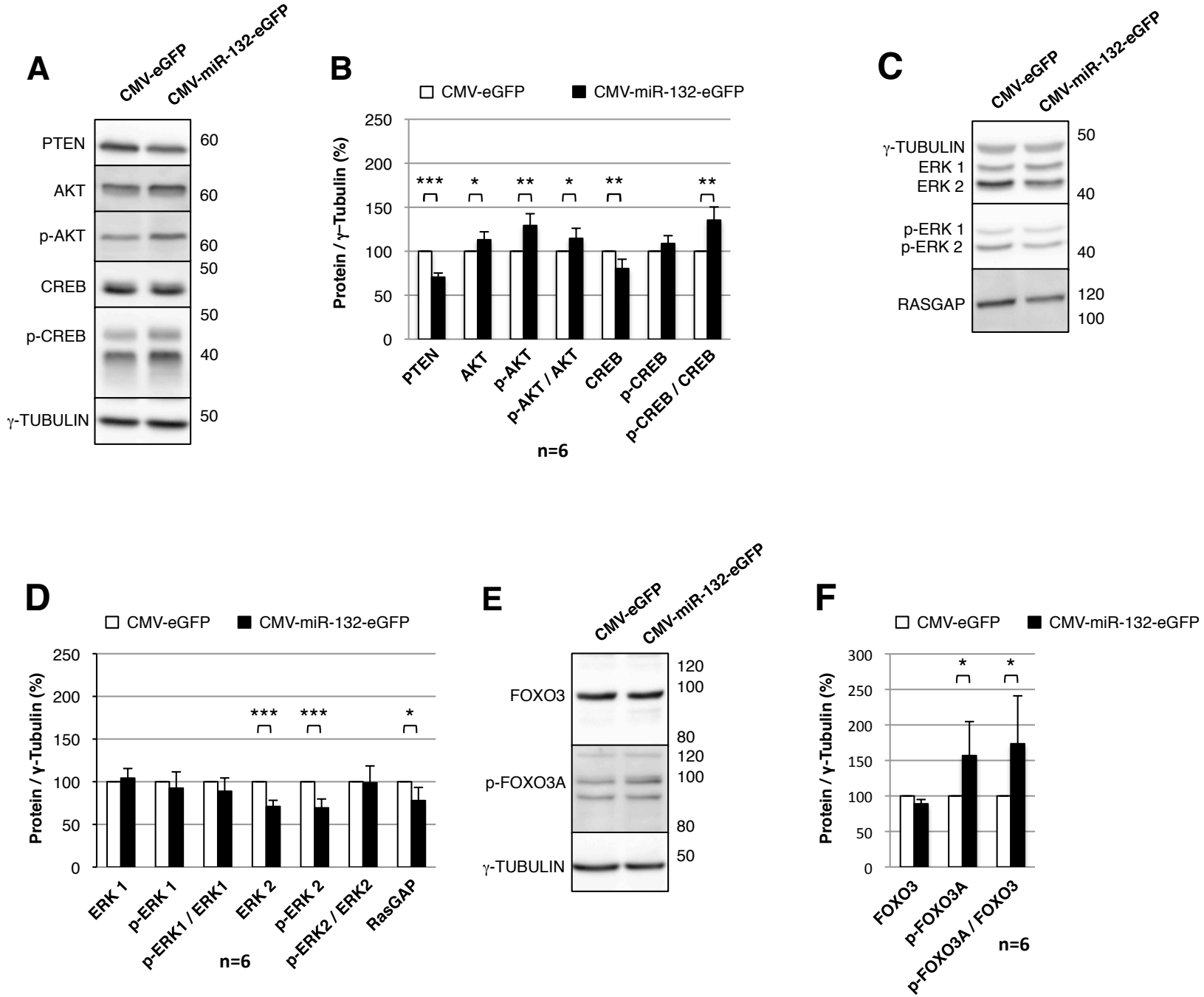

Figure 5. Impaired pancreatic beta cell regeneration in *miR132*$^{-/-}$ mice

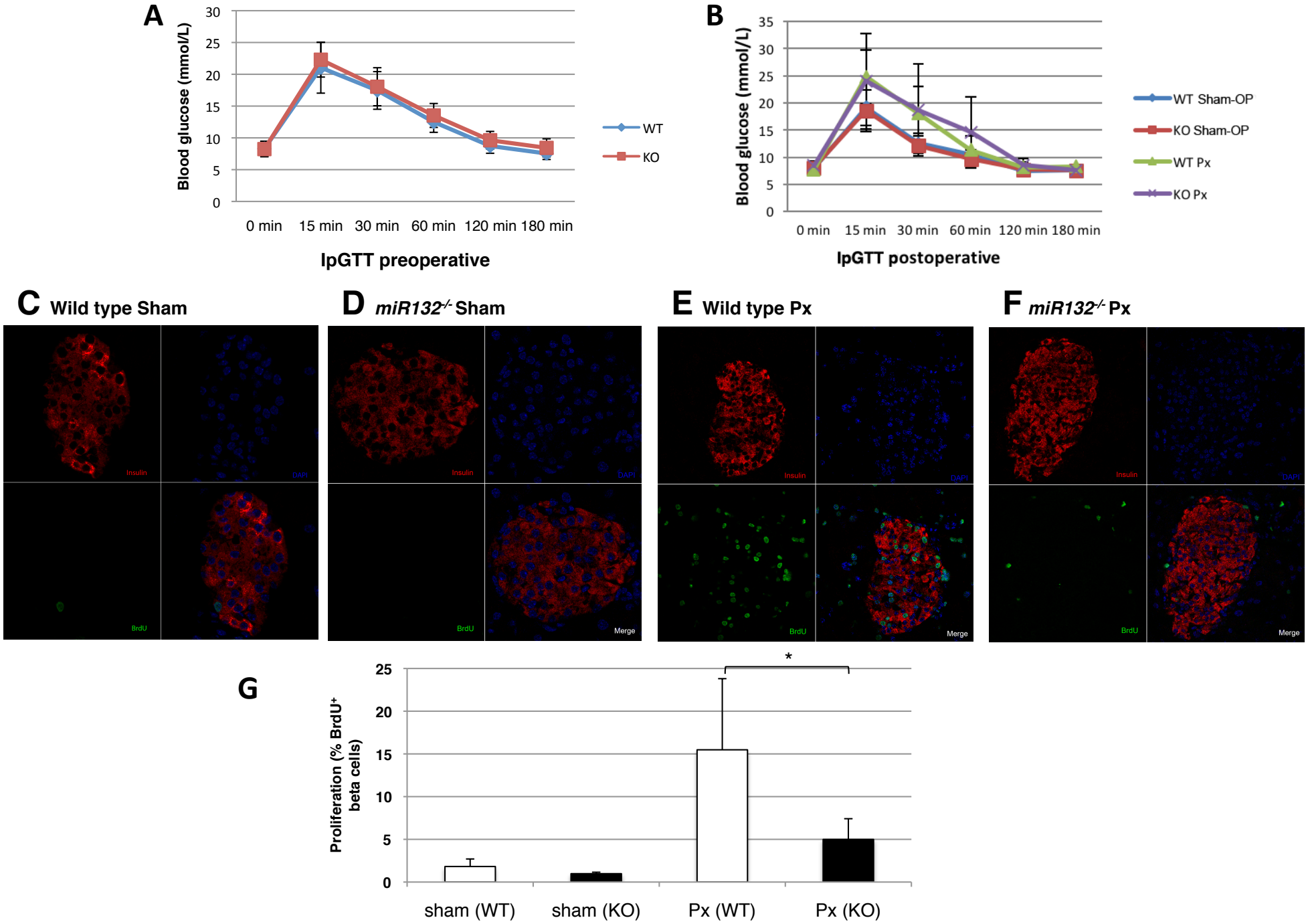

Table 1: miRNAs differentially expressed identified following pancreatectomy

Systematic name	p-value	FC	Px2	Px3	Px4	S1	S2	S3	S4
mmu-mir-205	0.009	4.91	6.5011	12.7521	9.4035	1	5.34742	2.0243	1
mmu-mir-132	0.005	4.63	85.442	90.0032	71.439	26.371	16.9079	22.282	8.6324
mmu-mir-186	0.016	2.5	4.0622	5.34086	3.1434	1.7227	1.1133	1.9388	1.686
mmu-mir-760	0.041	- 2.28	12.088	13.0156	4.6472	12.305	37.794	19.394	17.248
mmu-mir-129-3p	0.022	1.99	213.55	272.406	121.26	118.28	100.958	92.346	68.376
mmu-mir-690	0.03	1.79	49.413	48.5373	30.88	23.957	39.5096	16.126	17.206
mmu-mir-130b	0.014	1.77	42.937	40.5664	29.545	22.405	28.2766	18.247	14.679
mmu-mir-300*	0.045	1.77	2.9997	4.06448	2.006	1.8972	1.78003	1.8814	1
mmu-mir-598	0.049	1.75	18.357	22.9878	14.596	12.314	10.6534	12.703	6.3097
mmu-mir-431	0.013	1.65	23.393	27.3673	17.432	14.414	15.546	13.637	9.7251
mmu-mir-329	0.035	1.64	25.756	29.392	19.037	17.004	16.0322	15.797	9.9605
mmu-mir-154*	0.029	1.63	19.404	19.675	15.306	10.545	11.4188	13.067	8.2187
mmu-mir-141	0.04	1.59	878.04	970.708	655.42	566.11	672.314	515.55	318.15
mmu-mir-200c	0.039	1.58	976.04	997.919	672.69	608.97	681.16	530.91	361.34
mmu-mir-375	0.107	1.48	6291.99	7241.707	4643.13	4778.29	4398.79	4440.34	2461.28

Table 2: Total numbers of counted insulin^{+} and brdU^{+} cells

	Insulin^{+} cells	BrdU^{+} cells	Proliferation rate	Number of counted islets	Average number of Insulin^{+} cells per islet
wildtype sham operation	7127	116	1,82%	300	23,8
mir132$^{-/-}$ sham operation	8066	82	0,98%	300	26,8
wildtype partial pancreatectomy	8572	1277	15,48%	269	31,9
mir132$^{-/-}$ partial pancreatectomy	9524	453	4,99%	300	31,7